

<sup>1</sup> **A comprehensive proteomic and  
2 bioinformatics analysis of human  
3 spinal cord injury plasma identifies  
4 proteins associated with the  
5 complement cascade as potential  
6 prognostic indicators of neurological  
7 outcome**

<sup>8</sup> **1 Abstract**

<sup>9</sup> Introduction

<sup>10</sup> Spinal Cord Injury (SCI) is a major cause of disability, with complications post-injury often leading  
<sup>11</sup> to life-long health issues with need of extensive treatment. Neurological outcome post-SCI can be  
<sup>12</sup> variable and difficult to predict, particularly in incomplete injured patients. The identification of  
<sup>13</sup> specific SCI biomarkers in blood, may be able to improve prognostics in the field. This study has  
<sup>14</sup> utilised proteomic and bioinformatics methodologies to investigate differentially expressed pro-  
<sup>15</sup> teins in plasma samples across human SCI cohorts with the aim of identifying prognostic biomark-  
<sup>16</sup> ers and biological pathway alterations that relate to neurological outcome.

<sup>17</sup> Methods and Materials

<sup>18</sup> Blood samples were taken, following informed consent, from ASIA impairment scale (AIS) grade C  
<sup>19</sup> "Improvers" (AIS grade improvement) and "Non-Improvers" (No AIS change), and AIS grade A and D  
<sup>20</sup> at <2 weeks ("Acute") and approx. 3 months ("Sub-acute") post-injury. The total protein concentra-  
<sup>21</sup> tion from each sample was extracted, with pooled samples being labelled and non-pooled samples  
<sup>22</sup> treated with ProteoMiner™ beads. Samples were then analysed using two 4-plex isobaric tag for  
<sup>23</sup> relative and absolute quantification (iTRAQ) analyses and a label-free experiment for comparison,  
<sup>24</sup> before quantifying with mass spectrometry. Proteomic datasets were analysed using **bioinfor-**  
<sup>25</sup> **matics...**

<sup>26</sup> Proteins of interest identified from this analysis were further validated by enzyme-linked im-  
<sup>27</sup> munosorbent assay (ELISA). OpenMS (version 2.6.0) was used to process the raw spectra data. R  
<sup>28</sup> (version 4.1.4) and in particular, the R packages MSstats (version 4.0.1), STRINGdb (version 2.4.2)  
<sup>29</sup> and pathview (version 1.32.0) were used for downstream analysis.

<sup>30</sup> Results

<sup>31</sup> The data demonstrated proteomic differences between the cohorts, with the results from the  
<sup>32</sup> iTRAQ approach supporting those of the label-free analysis. A total of 79 and 87 differentially  
<sup>33</sup> abundant proteins across AIS and longitudinal groups were identified from the iTRAQ and label-  
<sup>34</sup> free analyses, respectively. Alpha-2-macroglobulin (A2M), retinol binding protein 4 (RBP4), serum  
<sup>35</sup> amyloid A1 (SAA1), Peroxiredoxin 2, alipoprotein A1 (ApoA1) and several immunoglobulins were

36 identified as biologically relevant and differentially abundant, with potential as individual prognos-  
37 tic biomarkers of neurological outcome. Bioinformatics analyses revealed that the majority of dif-  
38 ferentially abundant proteins were components of the complement cascade and most interacted  
39 directly with the liver.

40 **Conclusions**

41 Many of the proteins of interest identified using proteomics were detected only in a single group  
42 and therefore have potential as a binary (present or absent) biomarkers. Additional investigations  
43 into the chronology of these proteins, and their levels in other tissues (cerebrospinal fluid in par-  
44 ticular) are needed to better understand the underlying pathophysiology, including any potentially  
45 modifiable targets. **The complement cascadde was confirmed using pathway analysis as...**

46 **2 Introduction**

47 Spinal cord injury (SCI) is the transient or permanent loss of normal spinal sensory, motor or au-  
48 tonomic function, and is a major cause of disability. Globally, SCI affects around 500,000 people  
49 each year and is most commonly the result of road traffic accidents or falls.(Crozier-Shaw, Den-  
50 ton, and Morris 2020) Patients typically require extensive medical, rehabilitative and social care at  
51 high financial cost to healthcare providers. The lifetime cost of care in the UK is estimated to be  
52 £1.12 million (mean value) per SCI, with the total cost of SCI in the UK to the NHS being £1.43 bil-  
53 lion in 2016.(McDaid et al. 2019) Individuals with SCI show markedly higher rates of mental illness  
54 relative to the general population.(Furlan, Gulasingam, and Craven 2017) Complications arising  
55 post-SCI can be long-lasting and often include pain, spasticity and cardiovascular disease, where  
56 the systemic inflammatory response that follows SCI can frequently result in organ complications,  
57 particularly in the liver and kidneys.(Gris, Hamilton, and Weaver 2008; X. Sun et al. 2016)

58 The recovery of neurological function post-SCI is highly variable, requiring any clinical trials to have  
59 an impractically large sample size to prove efficacy, hence the translation of novel efficacious ther-  
60 apies is challenging and expensive.(Spiess et al. 2009) Being able to more accurately predict patient  
61 outcomes would aid clinical decisions and facilitate future clinical trials. Therefore, novel biomark-  
62 ers that allow for stratification of injury severity and capacity for neurological recovery would be  
63 of high value to the field.

64 Biomarkers studies in SCI often investigate protein changes in cerebral spinal fluid (CSF) as the  
65 closer proximity of this medium is thought to be more reflective of the parenchymal injury.(Brian  
66 K. Kwon et al. 2019; Hulme et al. 2017) Whilst this makes CSF potentially more informative for  
67 elucidating the pathology of SCI, the repeated use of CSF for routine analysis presents challenges  
68 in clinical care due to the risk and expense associated with the invasiveness of the collection proce-  
69 dure. In contrast, systemic biomarkers measurable in the blood represent a source of information  
70 that can be accessed and interpreted both a lower cost and risk. Studies of traumatic brain injury  
71 have demonstrated that protein markers identified in CSF are also detectable in both plasma and  
72 serum.(Wang et al. 2018) More recently, circulating white blood cell populations have also been  
73 identified as potential SCI injury biomarkers, with a 2021 study showing that elevated levels of neu-  
74 trrophils were associated with no AIS grade conversion, while conversely an increase in lymphocytes  
75 during the first week post-SCI were associated with an AIS grade improvement.

76 A number of individual proteins have been shown to be altered in the bloods post-SCI, including  
77 multiple interleukins (IL), tumour necrosis factor alpha (TNF- $\alpha$ ) and C-reactive protein (CRP).(Segal  
78 et al. 1997; Hayes et al. 2002; Frost et al. 2005)

79 Further, changes in inflammatory marker levels detected in acute SCI patients were found to  
80 be mirrored in donor-matched blood and CSF, albeit at lower absolute concentrations systemi-

<sup>81</sup> cally.(Brian K. Kwon et al. 2010)

<sup>82</sup> Previously, we have shown that routinely collected blood measures associated with liver function  
<sup>83</sup> and inflammation added predictive value to AIS motor and sensor outcomes at discharge and 12-  
<sup>84</sup> months post-injury.(Bernardo Harrington et al. 2020; Brown et al. 2019) The current study uses  
<sup>85</sup> an unbiased shotgun proteomic approach to investigate differentially expressed proteins in SCI  
<sup>86</sup> patients, coupled with bioinformatics pathway and network analyses.

### <sup>87</sup> 3 Methods and Materials

**Table 1.** Patient demographics. ± denotes interquartile range

	n	Percent
<b>Polytrauma</b>		
Yes	16	41
No	23	59
<b>Gender</b>		
F	13	33
M	26	67
<b>Diabetes</b>		
Yes	7	18
No	32	82
<b>Neurological level</b>		
C	26	67
L	4	10
T	9	23
<b>AIS change</b>		
A	11	28
C	7	18
C->D	10	26
D	11	28
Age at injury (Median years±IQR)	53±26	-

#### <sup>88</sup> 3.1 Patients

<sup>89</sup> Blood samples were taken from SCI patients who had provided informed consent and in accordance  
<sup>90</sup> to ethical provided by the National Research Ethics Service [NRES] Committee North West  
<sup>91</sup> Liverpool East [11/NW/0876]. “Improvers” were defined as individuals who experienced an AIS  
<sup>92</sup> grade improvement from admission to a year post-injury, whereas “non-improvers” were defined  
<sup>93</sup> as patients who saw no change in AIS grade in the same period.

#### <sup>94</sup> 3.2 Plasma collection and storage

<sup>95</sup> Plasma samples were collected within 2 weeks of injury (acute) and at approximately 3 months  
<sup>96</sup> post-injury (subacute). Upon collection in EDTA (ethylenediaminetetraacetic acid) coated tubes  
<sup>97</sup> samples were centrifuged at 600g for 15 minutes, to pellet erythrocytes and the resultant plasma  
<sup>98</sup> fraction was aspirated and divided into aliquots for long-term storage in -80°C briefly and liquid  
<sup>99</sup> nitrogen in the longer term.

100 **3.3 Sample preparation and analysis using iTRAQ proteomics**

101 Thawed plasma samples ( $2\mu L$ ) each were diluted with distilled water ( $98\mu L$ ). Total protein was  
102 quantified using a Pierce™ 660nm Protein Assay (Thermo Fisher Scientific, Hemel Hempstead,  
103 UK)(Stoscheck 1987).

104 In brief,  $10\mu L$  of the diluted sample was added to  $150\mu L$  of Thermo Pierce 660nm protein assay  
105 reagent in triplicate and the optical density was read at 660nm.

106 A total of  $100mg$  of plasma protein was taken from each sample and pooled equally to form a  
107 patient test group. For example, the AIS C improver group was pooled from 10 separate patient  
108 samples,  $10mg$  of protein per patient.

109 The pooled plasma samples were then precipitated by incubation of the sample in 6 times the vol-  
110 ume of chilled acetone for 1 hour at  $-20^{\circ}C$ . The samples were then centrifuged at 6,000G for 10  
111 minutes at  $4^{\circ}C$ , and re-suspended in  $200\mu L$  of triethylammonium bicarbonate buffer. Sequencing  
112 Grade Modified Trypsin ( $10\mu g\text{--}85\mu g$  of protein; Promega, Madison, WI, USA) was then added to the  
113 samples for overnight digestion at  $37^{\circ}C$ . Proteins then underwent reduction and alkylation (ac-  
114 cording to the manufacturer's instructions; Applied Biosystems, Bleiswijk, The Netherlands). Tryp-  
115 tic digests were labelled with iTRAQ tags (again according to the manufacturer's instructions for the  
116 iTRAQ kit), before being pooled into test groups and dried in a vacuum centrifuge. The following  
117 tags were used for each group of patient samples 114 tag - acute improvers, 115 tag - sub-acute  
118 improvers, 116 tag - acute non-improvers and 117 tag - sub-acute non-improvers for run 1 and 114  
119 tag - acute improvers, 115 tag - acute non-improvers, 116 tag - AIS grade A and 117 tag - AIS grade  
120 D for run 2.

121 **3.3.0.1 iTraq mass spectrometry analysis** The samples were analysed at the BSRC St. An-  
122 drews University Mass Spectrometry and Proteomics Facility using methods previously described.

123 A total of 12 SCX fractions were analysed by nano-electrospray ionisation-liquid chromatogra-  
124 phy/tandem mass spectrometry (LC-MS/MS) using a TripleTOF 5600 tandem mass spectrometer  
125 (AB Sciex, Framingham, MA, USA) as described previously.(Fuller et al. 2015)

126 **SECTION TO BE REWRITTEN** Each fraction ( $10\mu L$ ) was then analysed by nanoflow LC-ESI-MSMS. The  
127 peptides were separated using a nanoLC Ultra 2D plus loading pump and nanoLC AS-2 autosampler  
128 chromatography system (Eksigent, Redwood City, CA, USA), using a PepMap RSLC column ( $75\mu L \times$   
129  $15cm$ ) and an Acclaim PepMap100 trap ( $100\mu m \times 2cm$ ) (ThermoFisher Scientific, Waltham, MA, USA).  
130 After washing the peptides on the trap column for 20 minutes at  $5\mu L \text{ min}^{-1}$ , the trap was switched  
131 in line with the column and the peptides eluted with a gradient of increasing MeCN from 95% buffer  
132 A (98% H<sub>2</sub>O, 2% MeCN, 0.1% FA), 5% buffer B (2% H<sub>2</sub>O, 98% MeCN, 0.1% FA) to 65% buffer A, 35%  
133 buffer B over 60 minutes, then to 50% buffer A, 50% buffer B over a further 20 minutes, before  
134 increasing the concentration of buffer B to 95% over a further 10 minutes. The column was then  
135 washed with 95% buffer B before re-equilibration in 95% buffer A. A flow rate of  $300nL \text{ min}^{-1}$  was  
136 employed. The eluent was sprayed into a TripleTOF 5600 tandem mass spectrometer (ABSciex,  
137 Foster City, CA, USA), using a NANOSpray III source, and analyzed in Information Dependent Ac-  
138 quisition (IDA) mode, performing  $250ms$  of MS followed by  $100ms$  MSMS analyses on the 20 most  
139 intense peaks with a charge state of +2 to +5. Parent (MS) ions were accepted with a mass toler-  
140 ance of 50 mDa and MSMS was conducted with a rolling collision energy (CE) inclusive of preset  
141 iTRAQ CE adjustments. Analyzed parent ions were then excluded from analysis for 13 s after 3  
142 occurrences.

143    **3.3.1 Sample preparation and analysis using label-free proteomics**

144    No sample pooling was used, and so each of the 73 samples were maintained separately through-  
145    out protein equalisation, mass spectrometry, and label-free quantification steps. Thus, protein  
146    abundance was quantified for each sample, whereupon mean protein abundance across experi-  
147    mental groups was calculated to assess protein changes.

148    To reduce the dynamic range of proteins, ProteoMiner™ beads (BioRad, Hemel Hempstead, UK)  
149    were used. Firstly, plasma was treated with  $1 \mu\text{g}\cdot\text{mL}^{-1}$  of hyaluronidase. Digestion was confirmed  
150    with Coomassie stained 1D-SDS PAGE gel. The supernatant was centrifuged through a  $0.22 \mu\text{m}$  cel-  
151    lulose acetate membrane (Costar Spin-X, Corning, Tokyo, Japan) tube filter (5000g for 15 minutes)  
152    to remove insoluble material. Total protein was quantitated with a Pierce™ 660nm Protein Assay  
153    (Thermo Fisher Scientific, Hemel Hempstead, UK), whereupon 5 mg of total protein was applied to  
154    ProteoMiner™ beads, and processed as described previously.(Stoscheck 1987)

155    **3.3.1.1 Label free mass spectrometry analysis** Tryptic peptides were subjected to LC-MC/MC  
156    via a 2-h gradient on a NanoAcuity™ ultraperformance LC (Waters, Manchester, UK) connected  
157    to a Q-Exactive Quadrupole-Orbitrap instrument (Thermo-Fisher Scientific Hemel Hempstead, UK)  
158    as described **previously**.

159    **REWRITE IN BRIEF** The Q-Exactive was operated in a data dependent positive electrospray ion-  
160    isation mode, automatically switching between full scan MS and MS/MS acquisition. Survey full  
161    scan MS spectra ( $m/z$  300–2000) were acquired in the Orbitrap with 70,000 resolution ( $m/z$  200) fol-  
162    lowing accumulation of ions to  $1 \times 10^6$  target value based on the predictive automatic gain control  
163    values from the previous full scan. Dynamic exclusion was set to 20s, the 10 most intense multiply  
164    charged ions ( $z \geq 2$ ) were sequentially isolated and fragmented in the octopole collision cell by  
165    higher energy collisional dissociation (HCD), with a fixed injection time of 100ms and 35,000 res-  
166    olution. The following mass spectrometric conditions were used: spray voltage, 1.9kV, no sheath  
167    or axillary gas flow; normalised HCD collision energy 30%; heated capillary temperature, 250°C.  
168    MS/MS ion selection threshold was set to  $1 \times 10^4$  count and 2Da isolation width was set.

169    **3.3.2 iTRAQ OpenMS analysis**

170    TripleTOF 5600 tandem mass spectrometer output files produced in the ABSciex proprietary .wiff  
171    file format were converted to an open file format, .mzML for analysis with OpenMS (version 2.6.0).  
172    The docker image of ProteoWizard version 3.0.20287 was used for conversion, and peak picking  
173    was applied on conversion (Chambers et al. 2012). OpenMS version 2.6.0 was used for further anal-  
174    ysis.(Röst et al. 2016) Unless otherwise stated, default arguments were used. The 12 fraction files  
175    were merged and sorted by retention time. A decoy database was generated with DecoyDatabase  
176    and the -enzyme flag set to Trypsin, the human reference proteome was taken from Uniprot (Pro-  
177    teome ID: UP000005640, downloaded: 2020-10-01), as was the .fasta for porcine trypsin (Entry:  
178    P00761, downloaded: 2020-10-01).(The UniProt Consortium 2021)

179    The MSFQPlusAdapter was used to run the search. For the -fixed\_modifications "Methylthio (C)"  
180    and "iTRAQ4plex (N-term)" were passed due to the alkylating agent used in sample preperation  
181    and to account for the N-terminus modifications made by iTRAQ tags. "Oxidation (M)" was passed  
182    to -variable\_modifications to reflect the likely occurrence of methionine oxidation. To reflect the  
183    instrument the following flags were also set: -precursor\_mass\_tolerance 20 -enzyme Trypsin/P  
184    -protocol iTRAQ -instrument high\_res.

185    To annotate the search results PeptideIndexer and PSMFeatureExtractor were used. For peptide  
186    level score estimation and filtering PercolatorAdapter was used with the following arguments:

187    -score\_type q-value -enzyme trypsinp. IDFfilter was used to filter to a peptide score of 0.05  
188    with -score:pep 0.05

189    IsobaricAnalyzer with -type itraq4plex was used with the merged .mzML files to assign protein-  
190    peptide identifications to features or consensus features with IDMapper. The files for each run  
191    output by IDMapper were then merged with FileMerger. Bayesian score estimation and protein  
192    inference was performed with Epifany and the following flags: -greedy\_group\_resolution  
193    remove\_proteins\_wo\_evidence -algorithm:keep\_best\_PSM\_only false Decoys were removed  
194    and 0.05 FDR filtering was done via IDFfilter with -score:protgroup 0.05 -remove\_decoys.  
195    Finally, IDConflictResolver was used to resolve ambiguous annotations of features with peptide  
196    identifications, before quantification with ProteinQuantifier.

197    **3.3.3 Label free OpenMS analysis**

198    For quantification, the raw spectra files were analysed via OpenMS (version 2.6.0) command line  
199    tools, with the workflow from the prior section (3.3.2) adapted to suit a label-free analysis. The  
200    files were first converted from the proprietary .Raw format to the open .mzML standard with the  
201    FileConverter tool via the open-source ThermoRawFileParser.(Röst et al. 2016; Hulstaert et al.  
202    2020) Unless otherwise stated, default arguments were used throughout.

203    The decoy database generated in the prior section (iTRAQ OpenMS analysis) was also re-used. The  
204    CometAdapter was used to run the search.(Eng, Jahan, and Hoopmann 2013) Fixed modifications  
205    were set to "Carbamidomethyl (C)" and "Oxidation (M)" was set as a variable modification. To reflect  
206    the instrument the following flags were also set: -precursor\_mass\_tolerance 20 -isotope\_error  
207    0/1.

208    To annotate the identified peptides with proteins the PeptideIndexer tool was used. PeptideIndexer  
209    and PSMFeatureExtractor were used for annotation. For peptide level score estimation and fil-  
210    tering PercolatorAdapter was used with the following flags: -score\_type q-value -enzyme  
211    trypsin. IDFfilter was used to filter to a peptide score of 0.01 with -score:pep 0.01 followed  
212    by IDScoreSwitcher with the following flags: -new\_score "MS:1001493" -new\_score\_orientation  
213    lower\_better -new\_score\_type "pep" -old\_score "q-value". The ProteomicsLFQ was used for  
214    subsequent processing with the flags: -proteinFDR 0.05 -targeted\_only true. The -out\_msstats  
215    flag was also used to produce quantitative data for downstream statistical analysis with the R  
216    package MSstats.(Choi et al. 2014)

217 **3.3.4 Enzyme-linked immunosorbent assays**

218 Four proteins identified by the iTRAQ analysis were measured by enzyme-linked immunoab-  
219 sorbent assay (ELISA) from non-pooled samples to validate the iTRAQ findings.

220 These proteins were alpha-2-macroglobulin (A2M), retinol binding protein 4 (RBP4), serum amy-  
221 loid A1 (SAA1) and apolipoprotein A1 (ApoA1). They were selected for their biological relevance  
222 and differential abundance between AIS C improvers and non-improvers, implying potential as  
223 biomarkers of neurological outcome prediction. A2M, RBP4 and SAA1 were assessed using a hu-  
224 man DuoSet® ELISAs (R&D Systems, Abingdon, UK). ApoA1 was assessed using a human Quan-  
225 tikine® ELISA (R&D Systems, Abingdon, UK). Samples were diluted 1:600,000 for A2M and RBP4,  
226 1:100 for SAA1 and 1:20,000 for ApoA1 in the respective assay kit diluent. Samples that were above  
227 the assay detection limit were rerun at 1:300 and 1:40,000 for SAA1 and ApoA1 respectively. All  
228 ELISAs were carried out according to the manufacturer's protocol. Protein concentrations were  
229 normalised to the sample dilution factor. Statistical analysis was performed using the statistical  
230 programming language R version 4.1.3 (2022-03-10). Pairwise t tests with bonferroni adjusted P-  
231 values with the R `rstatix` package were used to assess differential abundance.

232 **3.3.5 Network and pathway analysis**

233 Protein interation networks were created using the Bioconductor package STRINGdb which pro-  
234 vides an R interface to STRING version 11.(Szklarczyk et al. 2019) Instantiation of the STRINGdb  
235 reference class was done with `species` and `score_threshold` set to 9606, for *Homo sapiens*, and  
236 400 respectively. Clustering of networks with STRINGdb used the “fastgreedy” algorithm from the  
237 `iGraph` package.

238 The Bioconductor package ReactomePA, which employs the open-source, open access, manually  
239 curated and peer-reviewed pathway database Reactome was used for network analysis.(G. Yu and  
240 He 2016; Jassal et al. 2020)

241 **4 Results**

242 **4.1 Results**

243 Plasma from American Spinal Injury Association (ASIA) grade C SCI patients (total n=17) contrasting  
244 those who experienced an ASIA grade conversion (n=10), and those who did not (n=7) collected  
245 within 2 weeks, and at approximately 3 months post-injury (Improvers n=9 vs Non-improvers n=6).  
246 Relative protein expression in AIS grade A (n=10) and grade D (n=11) patients was also examined.

247 In the interest of brevity, only the plots of acute and subactue AIS C improvers VS non-improvers  
248 are included here, please see the supplemental data for the other comparisons (section 5.2.2).

249 **4.1.1 Comparing OpenMS and ProteinPilot**

250 The AIS A group had 56 and 26 more abundant and 9 and 6 less abundant proteins respectively.  
251 Acutely, AIS C improvers relative to AIS A and D had 21 and 53 more abundant and 46 and 12 less  
252 abundant for OpenMS, whereas ProteinPilot had 5 and 19 more abundant proteins, and 18 and 6  
253 less abundant.

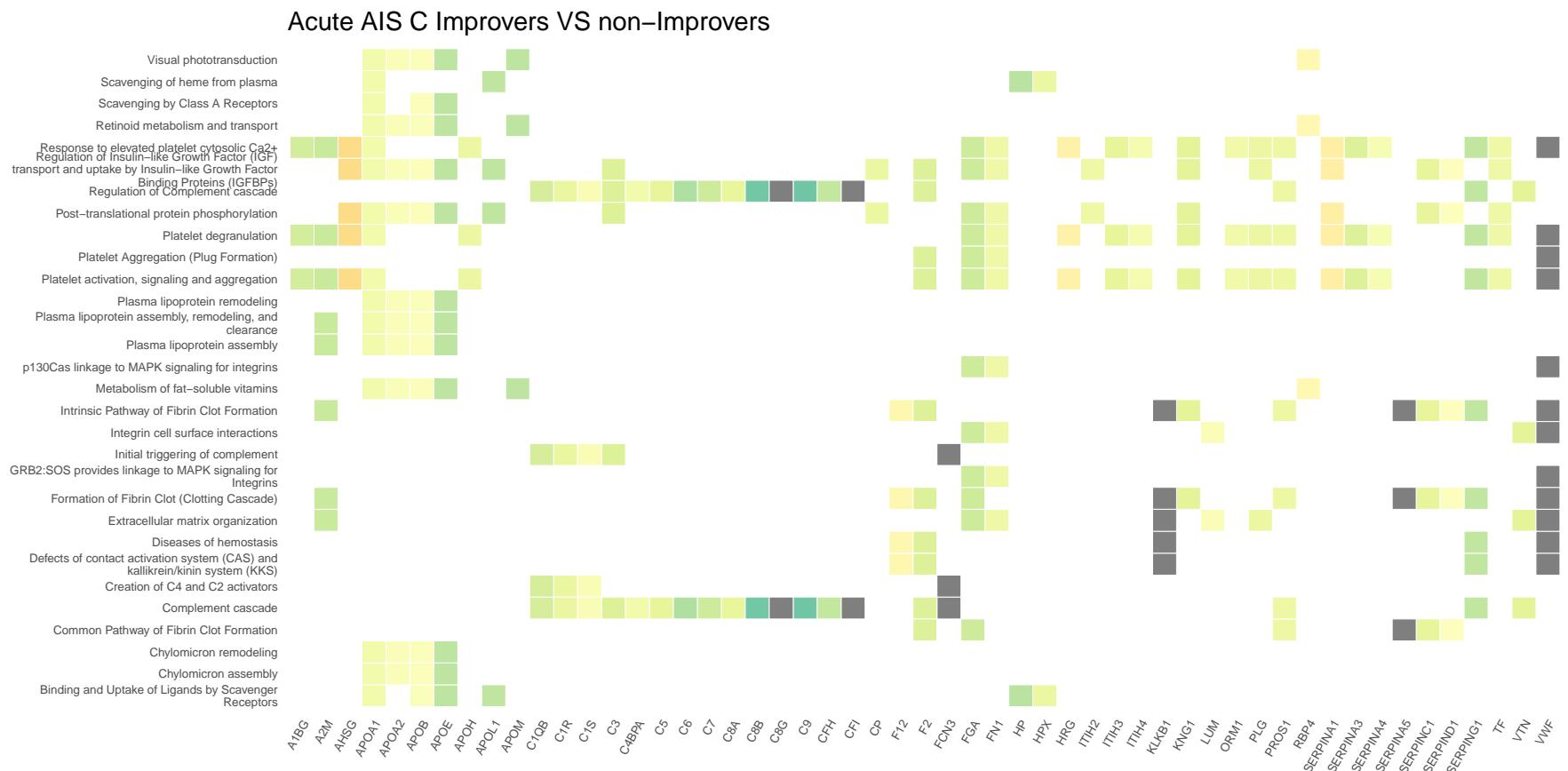
254 **4.1.2 iTRAQ analyses**

255 **4.1.3 Differential protein abundances**

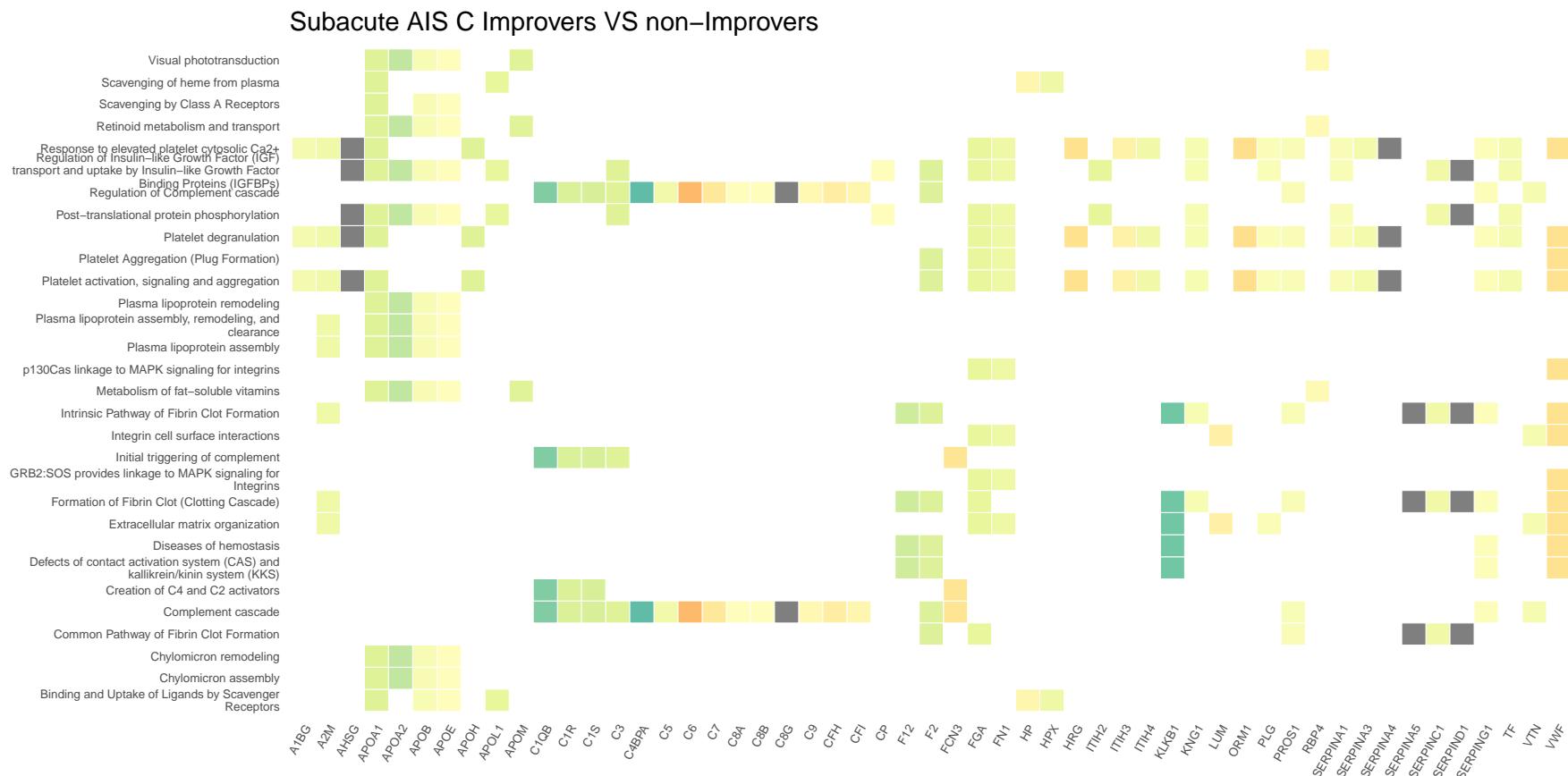
256 AIS C improvers had 18 more abundant proteins and 49 less abundant proteins at the acute phase,  
257 at the subacute phase, AIS C improvers had 34 more abundant proteins and 34 less abundant pro-  
258 teins. The AIS A group had 56 more abundant and 9 less abundant proteins respectively. Acutely,  
259 AIS C improvers relative to AIS A and D had 21 and 53 more abundant and 46 and 12 less abundant  
260 proteins. Please see the appendix for a full list of protein changes.

261 **4.1.4 Heatmaps**

262 The majority of the pathways associated with the proteins identified by these iTRAQ experiments  
263 are related to the complement cascade and platelet activity (Figure 1, 2, S1, S2, S3, S4, S5, S6, S7, S8).  
264 There are also several pathways implicated in metabolic processes, particularly with apolipopro-  
265 teins and retinoids.



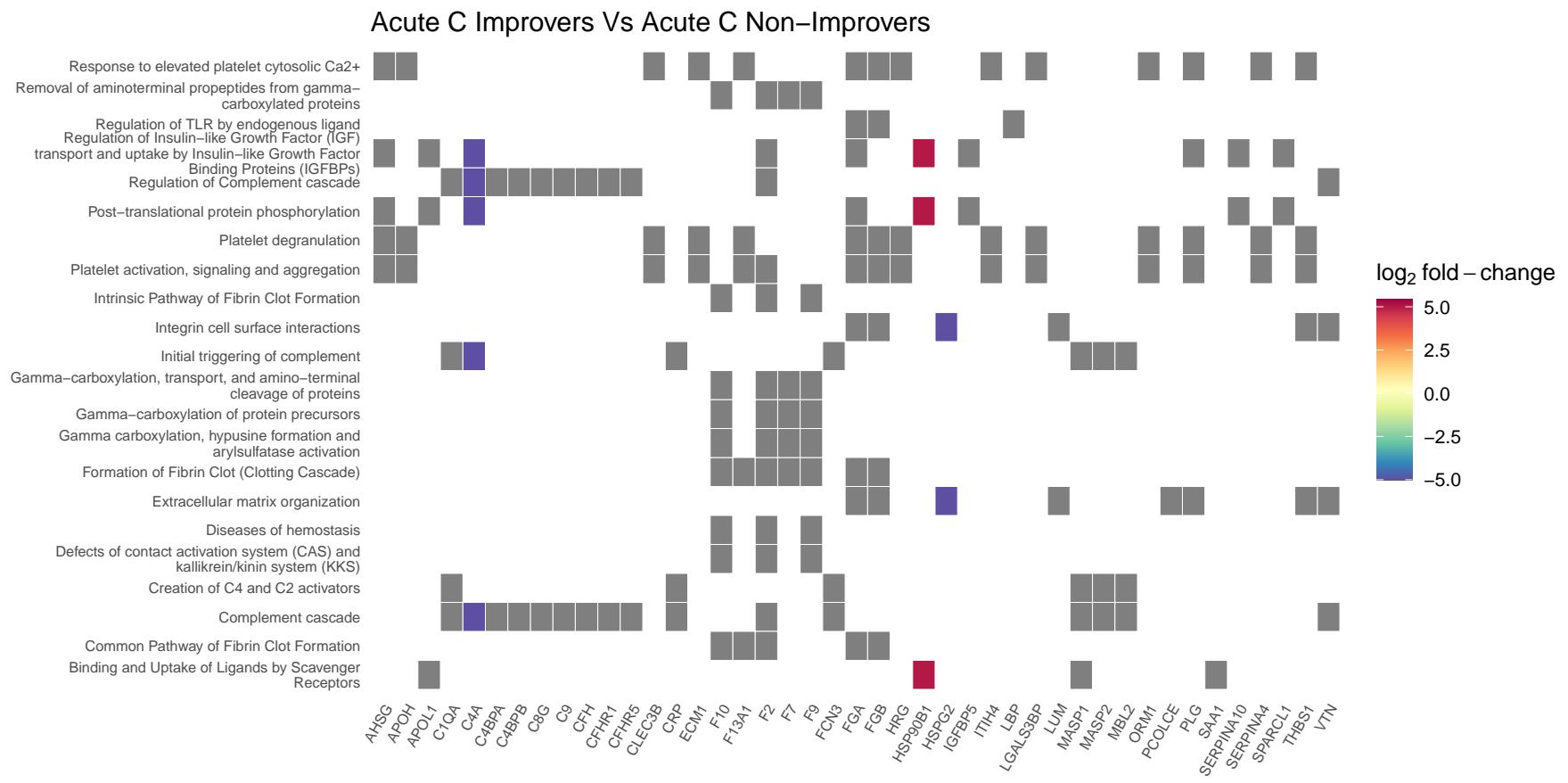
**Figure 1.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.



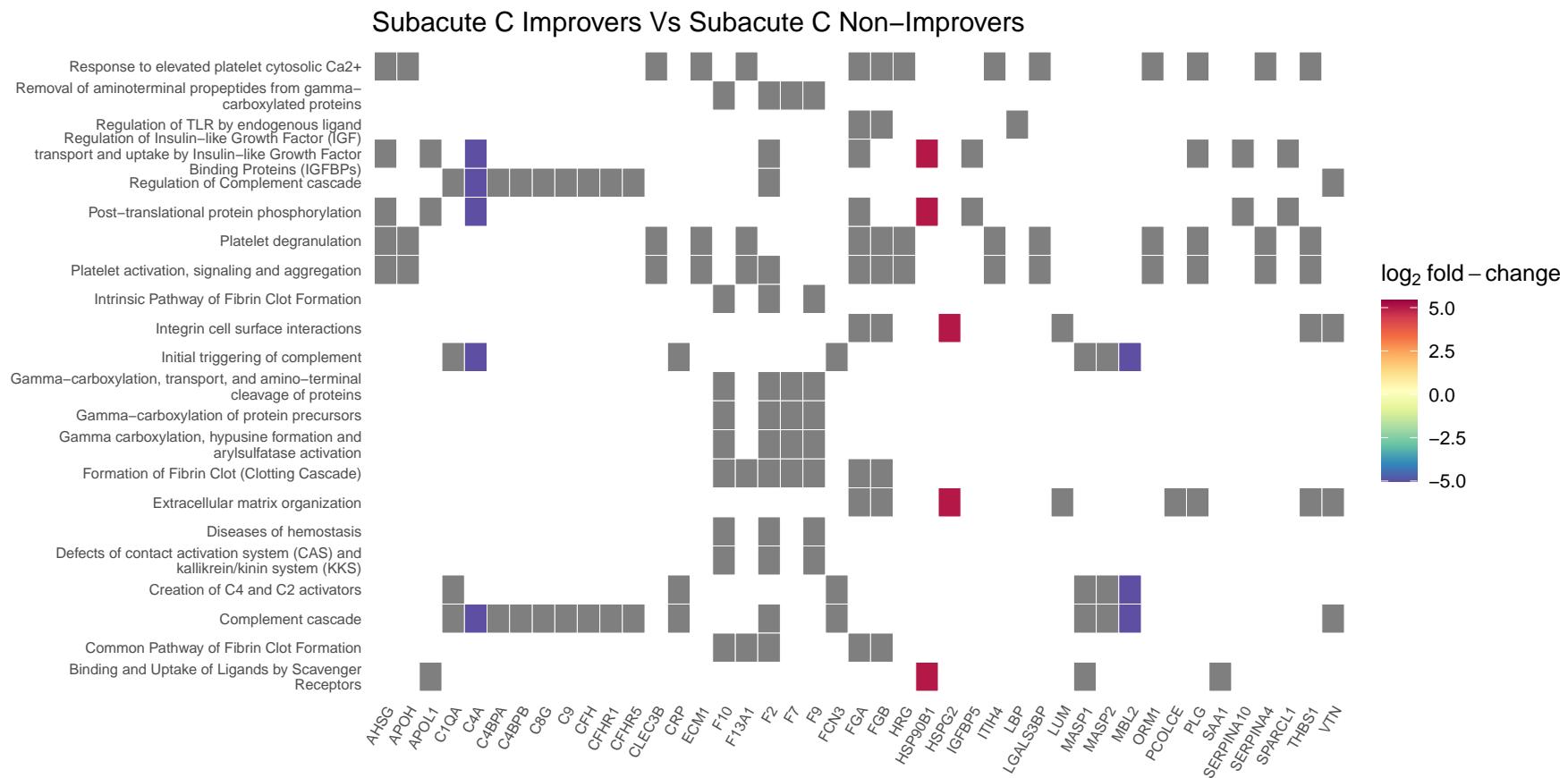
**Figure 2.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

<sup>266</sup> Similarly to the iTRAQ data, many of the Reactome pathways are associated with the complement cascade and platelets activation (Figures 3, 4, S9, S10, S11, S12, S13, S14, S15).

<sup>268</sup> Please see appendix section 5.5 for additional plots.



**Figure 3.** Heatmap denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not. Grey blocks denote proteins not present in the comparison.

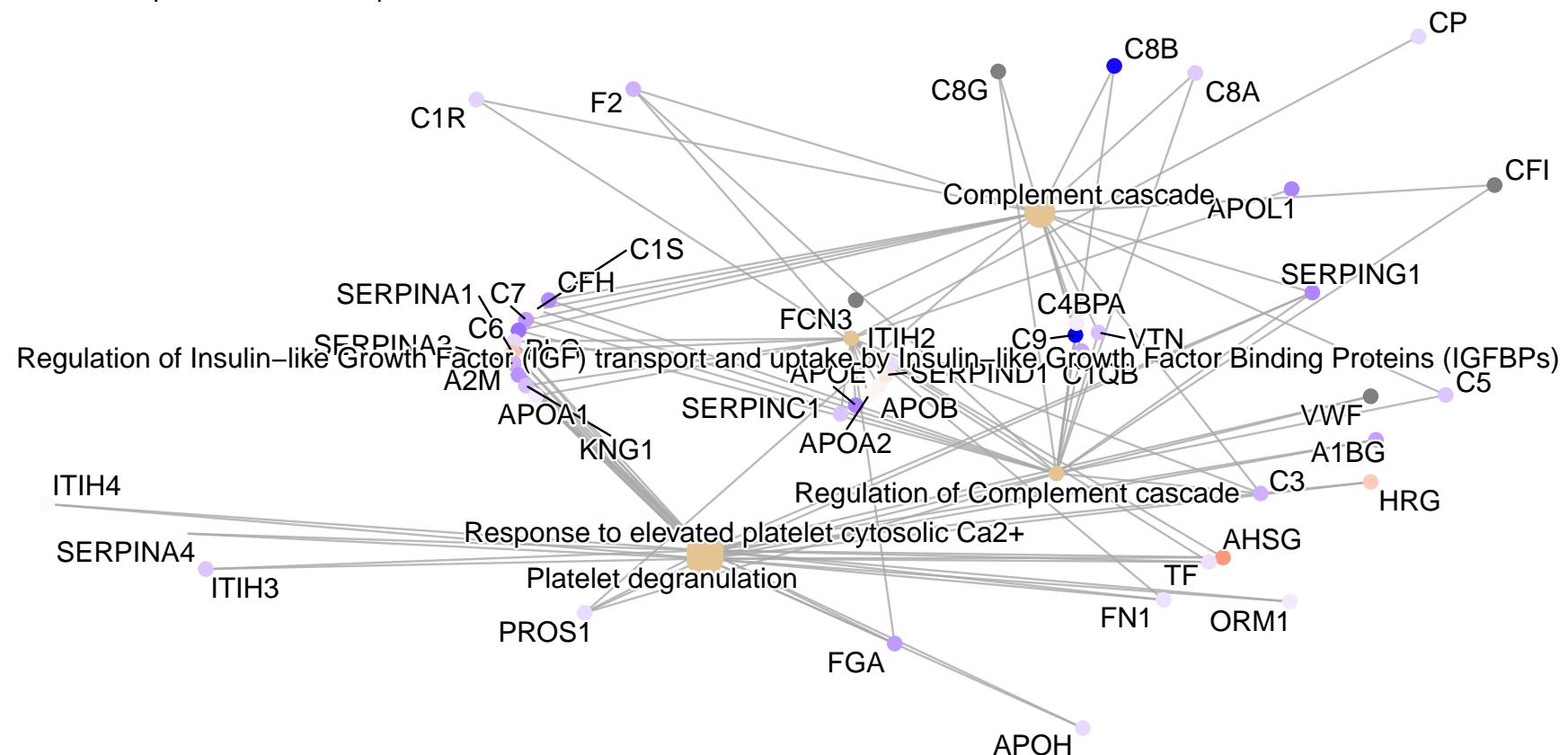


**Figure 4.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not. Grey blocks denote proteins not present in the comparison.

<sup>269</sup> **4.1.5 Cnetplots**

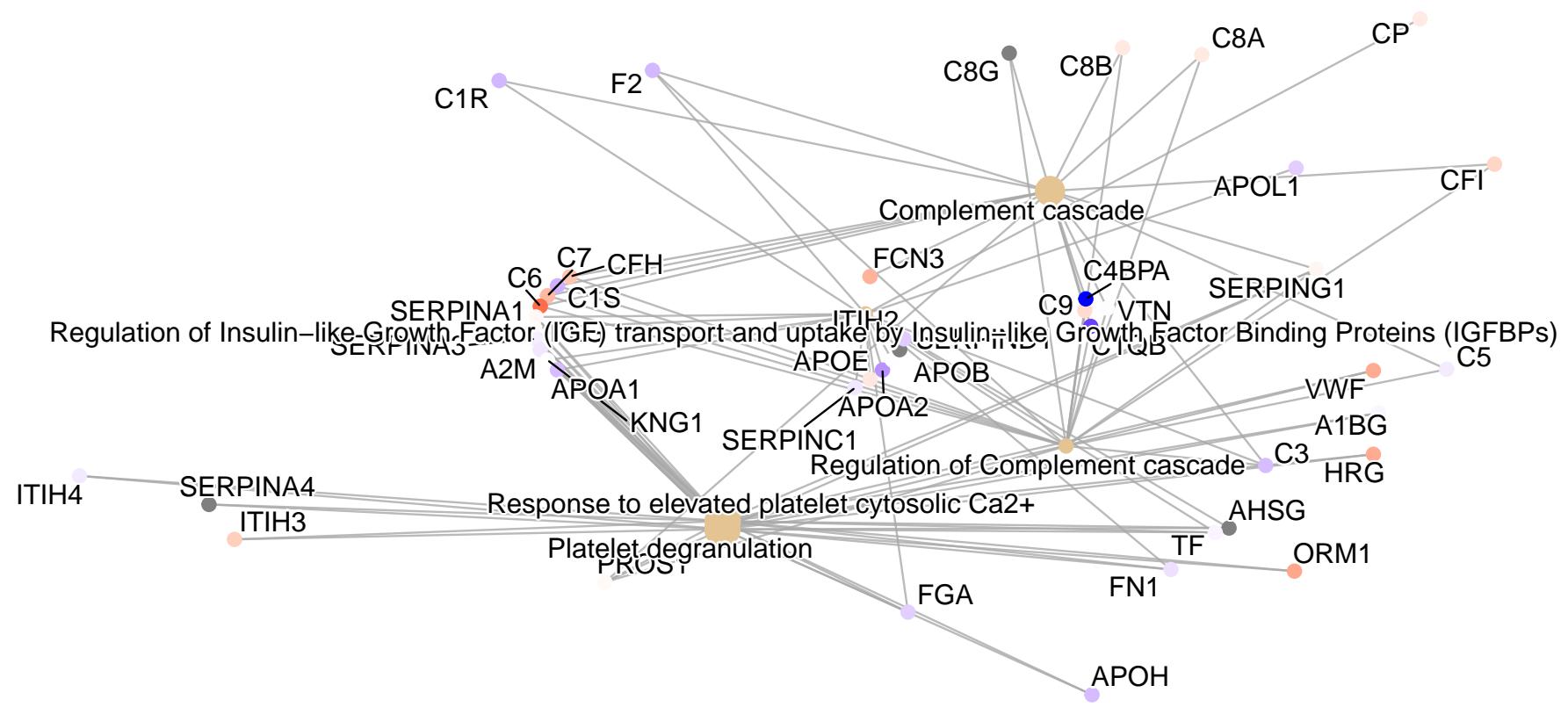
<sup>270</sup> Similar to the heatmaps, network plots highlighted that the majority of proteins changes were  
<sup>271</sup> associated with the complement cascade and pathways linked to platelet activity (Figure 5, 6, S16,  
<sup>272</sup> S17, S18, S19, S20, S21, S22, S23). Several proteins were also associated with the regulation of  
<sup>273</sup> insulin-like growth factor.

## Acute AIS C Improvers VS non-Improvers



**Figure 5.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

Subacute AIS C Improvers VS non-Improvers

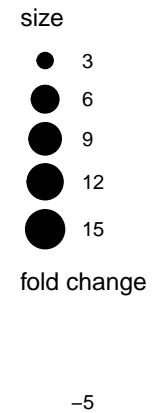
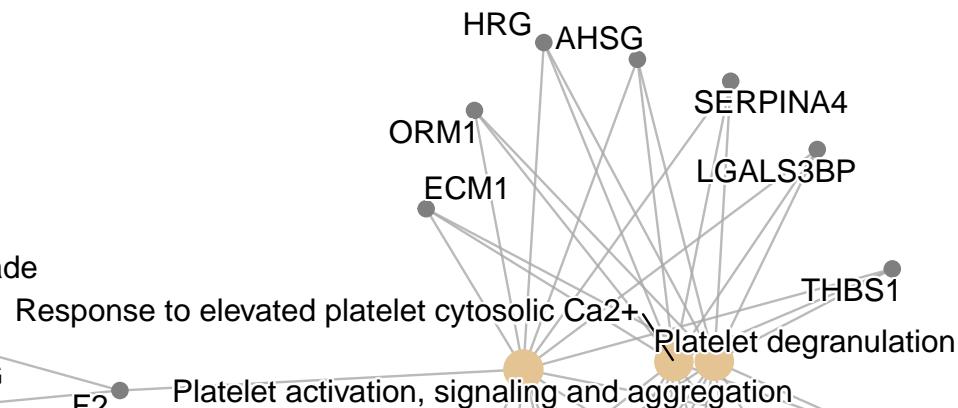
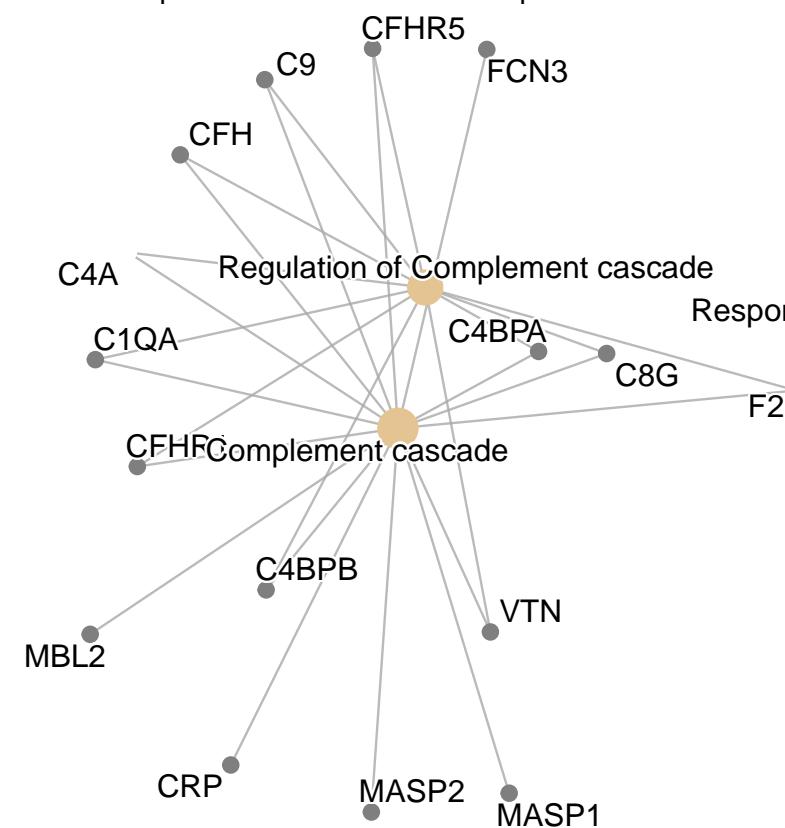


**Figure 6.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

<sup>274</sup> Similarly to the heatmaps and the iTRAQ data, network plots highlight the majority of differential  
<sup>275</sup> proteins are associated with the complement cascade and pathways linked to platelets (Figures 7,  
<sup>276</sup> 8, S24, S25, S26, S27, S28, S29, S30).

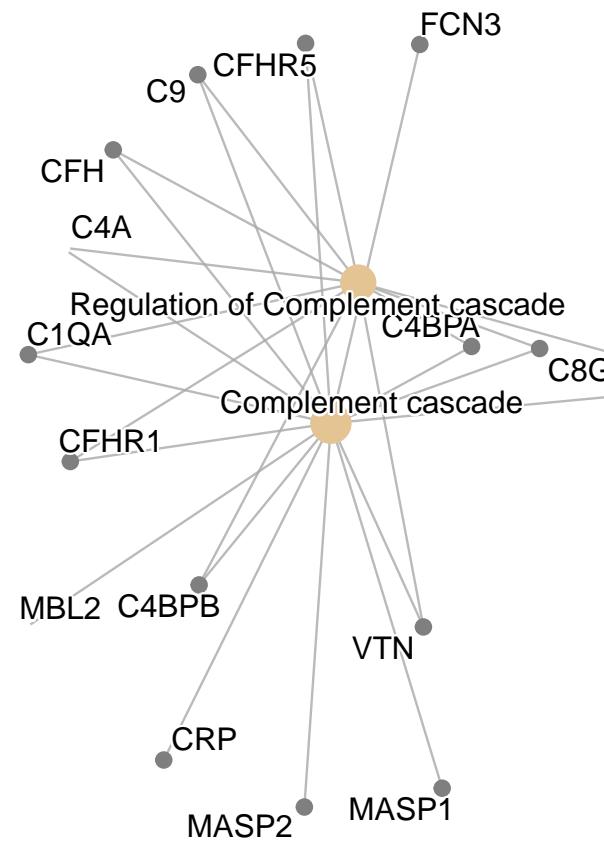
<sup>277</sup> Please see appendix section 5.6 for additional plots.

Acute C Improvers Vs Acute C Non-Improvers

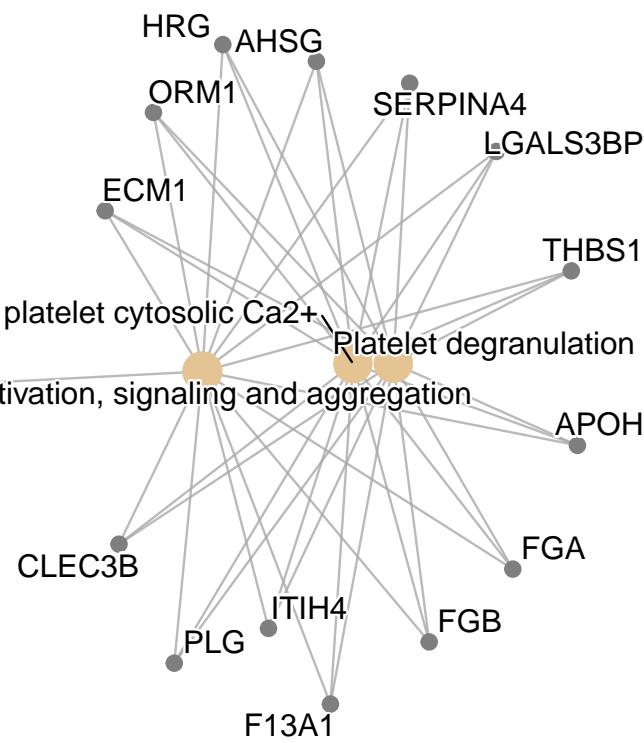


**Figure 7.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

Subacute C Improvers Vs Subacute C Non-Improvers



Regulation of Complement cascade  
Complement cascade

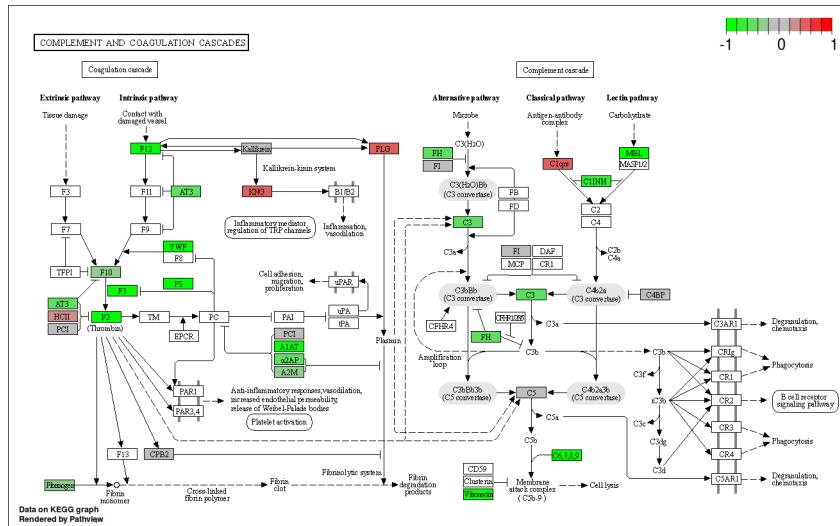


size  
● 3  
● 6  
● 9  
● 12  
● 15  
fold change  
-5

**Figure 8.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

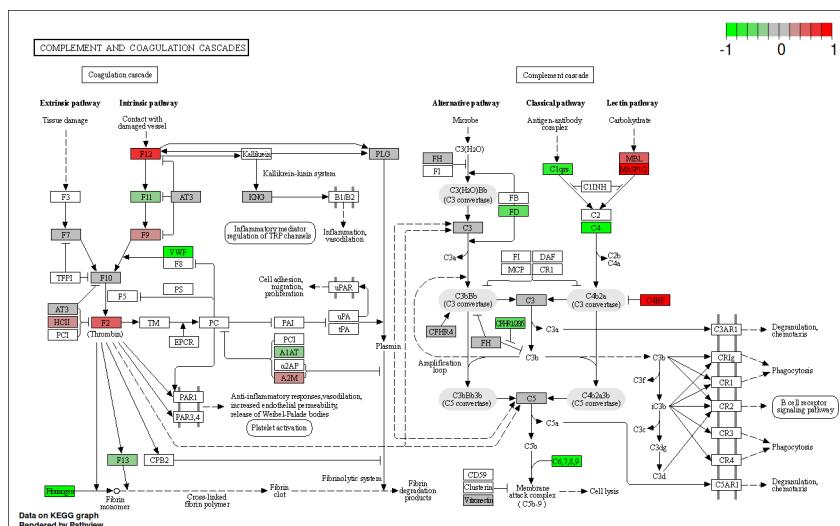
## 278 4.1.6 Pathway analysis

Pathway analysis via the pathview R package returned the complement and coagulation cascade to be the sole significant KEGG pathway to derive from the OpenMS analysed data. The majority of the proteins present in this pathway were less abundant in the 2-week post-injury plasma of AIS C patients who experienced an AIS grade conversion and those who did not (Figure 9).



**Figure 9.** KEGG complement cascade pathway annotated with log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

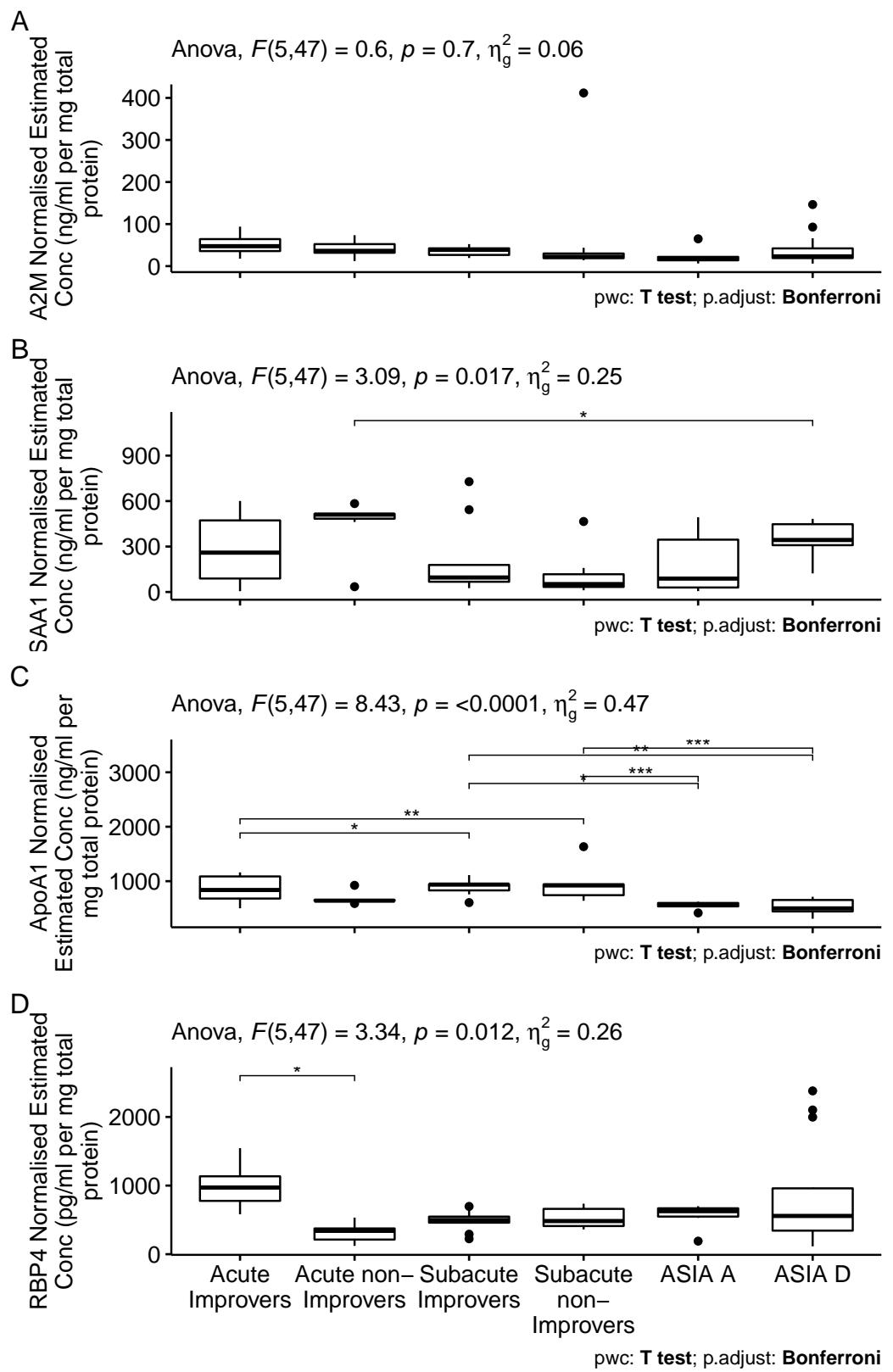
Similarly to the iTRAQ pathway analysis, the label free data analysed via the pathview R package returned the complement and coagulation cascade to be the sole significant KEGG pathway derived from the OpenMS analysed data. The majority of the proteins present in this pathway were less abundant 2-weeks post-injury in the plasma of AIS C patients who experienced an AIS grade conversion than those who did not (Figure 10).



**Figure 10.** KEGG complement cascade pathway annotated with  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not.

288 **4.1.7 ELISAs**

289 No statistically significant difference between groups for A2M abundance in plasma via Du-  
290 oSet&reg ELISAs, though there were outliers in the AIS A and D groups, and particularly in the AIS  
291 C patients at 3-months who did not experience an AIS grade conversion (Figure 11). A significant  
292 difference was found between AIS C non-improvers at 2-weeks and AIS D for SAA1, with outliers  
293 in AIS C non-improvers at 2-weeks, and both AIS C improvers and non-improvers at 3-months  
294 post-injury (Figure 11). For ApoA1 plasma abundance estimated via Quantikine&reg ELISAs,  
295 statistically significant differences were found between AIS C improvers at 2-weeks and both AIS C  
296 improvers and non-improvers at 3-months, AIS C 3-month improvers and AIS A and D, and AIS C  
297 3-month non-improvers and AIS A and D (Figure 11). A statistically significant difference was also  
298 found between AIS C improvers and non-improvers at 2-weeks post-injury for RBP4 (Figure 11).



**Figure 11.** Normalised estimated concentration of  $\alpha$ -2-macroglobulin (A), serum amyloid A1 (B), apolipoprotein A1 (C) and retinol binding protein 4 (D). Estimates were calculated from the optical density of a standard curve produced via a DuoSet® ELISA. Plasma from each patient that made up the pooled iTRAQ samples was assayed and pairwise t-tests with bonferroni adjusted P-values were performed to assess differential abundance.

299 **4.1.8 STRINGdb plots**

300 Network interaction plots generated from the OpenMS processed data via STRINGdb revealed that  
301 all test groups contained similar proteins, albeit with different abundances, with no distinct group-  
302 specific networks observed (Figures S31, S32, S33, S34, S35, S36, S37, S38 and S39).

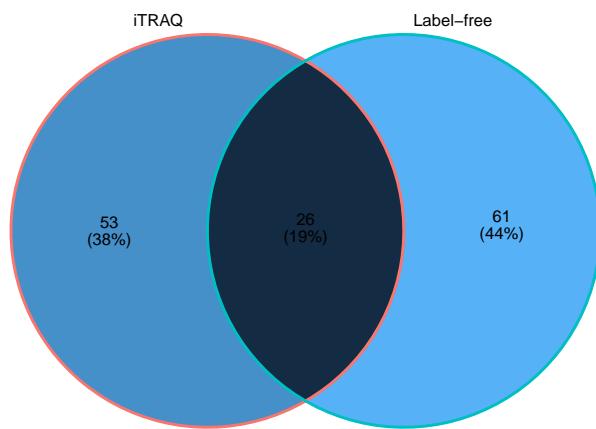
303 Network interaction plots generated of the significant proteins via STRINGdb revealed that all groups  
304 contained similarly smaller networks, with many proteins with no known interactions in the STRING  
305 database (Figures S40, S41, S42, S43, S44, S45, S46, S47, S48).

306 **4.1.9 Volcano plots**

307 The mean number of down-regulated and up-regulated significant proteins in each group is 10.6,  
308 and 6.8. Between AIS C improvers and non-improvers, 8 and 4 proteins were up- and down-  
309 regulated acutely, whereas 6 and 6 were up- and down-regulated subacutely (Figures S49 and  
310 S50). Longitudinally, AIS C acute improvers had 10 up-regulated and 7 down-regulated proteins  
311 relative to subacute improvers, while for non-improvers 6 and 12 were up- and down-regulated  
312 respectively (Figures S51 and S52).

313 **4.1.10 Comparing iTRAQ and label-free proteins**

314 A total of 87 and 79 unique proteins were identified across the label-free and iTRAQ experiments  
315 respectively, with a modest overlap of 26 proteins found using both techniques (Figure 12).



**Figure 12.** Venn diagram of the overlap in unique proteins identified from iTRAQ and label-free proteomic experiments analysed via OpenMS.

316 **5 Discussion**

317 **5.1 thesis iTRAQ discussion**

318 This work builds on the previous chapters (??) modelling of routine bloods by analysing the plasma  
319 proteome of SCI patients grouped by injury severity and improver status. In addition to continuing  
320 the pursuit of novel biomarkers of SCI, the link between the liver and neurological recovery hinted  
321 at in the aforementioned chapter is examined here.

322    **5.1.1 ProteinPilot and OpenMS**

323    Mass spectrometry is a major technique used in several fields, including metabolomics, lipidomics,  
324    interactomics and proteomics, each of which demands a variety of differing approaches to data  
325    acquisition and analysis. Multiple separation methods (liquid chromatography, gas chromatog-  
326    raphy), fragmentation methods (electron-capture dissociation, electron-transfer dissociation,  
327    collision-induced dissociation, etc.) and acquisition strategies (targeted, data-dependent and  
328    data-independent) are used in any combination. With quantification there are different label-  
329    free, isotopic or isobaric labelling approaches to employ. Finally the data analysis may require  
330    a database search, as in proteomics and metabolomics, spectral library search or a targeted  
331    analysis, depending on the experiment. This complexity necessitates a multi-interdependent-step  
332    workflow tailored to the given experiment.

333    The manufacturers of mass spectrometers often offer software tailored to their instruments which  
334    is often used in the literature. However, the source code for these software suits is not pub-  
335    licly available, and indeed manufacturers often boast of their particular inscrutable proprietary  
336    algorithms, often related to peak picking. This combination of completeness and opacity in analy-  
337    sis methodology can make it extremely difficult to reproduce results from other labs, or even  
338    analysis from one's own lab. ("Devil in the Details" 2011)

339    To address this issue many open-source (meaning the source code is publicly available) software  
340    packages which may perform one or several steps of a complex analysis workflow have been devel-  
341    oped. This issue here is that incorporating multiple software packages together can be both time-  
342    consuming and error-prone, and require significant maintenance and documentation to maintain  
343    reproducibility.

344    The OpenMS project aims to address these challenges by providing a flexible software environ-  
345    ment, with both pre-assembled workflows that aim to provide best-practices, and allow for more  
346    granular control with both command line and Python scripting interfaces. OpenMS is also inte-  
347    grated with graphical workflow systems such as KNIME and Galaxy, increasing the accessibility of  
348    the platform. (Berthold et al. 2009; Goecks et al. 2010)

349    Here we used both the vendor provided proprietary ProteinPilot and OpenMS to analyze two 4-  
350   plex iTRAQ experiments. We observe that both approaches produce similar results, with a similar  
351    number of total proteins identified, a large degree of overlap in the specific proteins identified,  
352    and similar fold changes (Figures ?? and ??). As the results are similar we choose to focus on the  
353    OpenMS results due to aforementioned superior reproducibility.

354    **5.1.2 Proteins identified**

355    A total of 79 proteins were identified across both runs for OpenMS, many of which are related in  
356    function. (Figure ??). Here we explore the potential these proteins have as biomarkers of SCI.

357    **5.1.2.1 Alpha-2-macroglobulin** A2M is an inhibitor of an unusually diverse array of proteinases  
358    by a unique 'trapping' mechanism. The protein achieves this with a peptide stretch, called the  
359    "bait region", which contains specific cleavage sites for different proteinases. When a proteinase  
360    cleaves the bait region, a conformational change is induced whereby A2M traps the proteinase.  
361    The entrapped enzyme retains activity against low molecular weight substrates, whereas activity  
362    against high molecular weight substrates is greatly reduced. Following cleavage in the bait region, a  
363    thioester bond is hydrolysed and mediates the covalent binding of the protein to the proteinase. (P.  
364    K. Hall et al. 1981; Sottrup-Jensen et al. 1984) A2M is unique in its ability to inhibit virtually any  
365    protease regardless of its specificity, origin or catalytic mechanism. (Khan 2004; Lin et al. 2012)

366 Alpha macroglobulins are an integral part of innate immunity and thus are evolutionarily con-  
367 served.(Buresova et al. 2009) Alpha macroglobulins have significant primary sequence homology  
368 with complement components C3, C4 and C5. The A2M-proteinase complex is cleared from circu-  
369 lation primarily by receptors on hepatocytes.(Bond, Cianciolo, and Pizzo 2007; Travis and Salvesen  
370 1983) The mammalian receptor for proteinase-reacted A2M is a low-density lipoprotein receptor  
371 related protein.(Fujiyoshi et al. 2011; Larios and Marzolo 2012; Wyatt and Wilson 2013)

372 A2Ms definitive function is the delivery of proteinase to an endocytotic proteinase clearance path-  
373 way. A2Ms trap the proteinases released by granulocytes and other cells during inflammation and  
374 also regulate the extracellular proteolytic activity resulting from clotting and fibrinolysis. A2M can  
375 also help protect against pathogens as it can trap proteinases from non-human origins as well.  
376 A2M can be recognised and phagocytosed by macrophages and hepatocytes, and it has been pro-  
377 posed to aid in the clearance of defensins and other peptide mediators in inflamed tissues, thus  
378 contributing to the regulation and containment of inflammation.(Rehman, Ahsan, and Khan 2013)

379 Myelin basic protein is released into the circulation following traumatic injury and A2M has been  
380 seen to be the only major myelin basic protein-binding protein in human plasma, suggesting A2M  
381 protects the immunogenic protein from degradation by proteases and help in its clearance from  
382 circulation.(Gunnarsson and Jensen 1998) A study looking at male infertility after SCI with pro-  
383 teomics found A2M to be elevated approximately 3-fold in the sperm plasma of SCI patients relative  
384 to normal controls.(Silva et al. 2016)

385 We observe A2M to be less abundant in AIS C improvers, within 2-weeks post injury and at 3-  
386 months, albeit to a lesser extent (Tables S2 and S3). Similarly, A2M was more abundant in AIS As  
387 relative to all groups, and whilst A2M was less abundant in AIS C improvers at 2-weeks compared  
388 to AIS Ds, AIS C non-improvers had more A2M than AIS Ds. (Table S2). With less A2M there would  
389 be more protease activity in these individuals, which may aid in the clearance of damaged tissue,  
390 and in particular may lessen the development of an astroglial scar, thus aiding repair. However,  
391 glial scaring is not entirely negative, the primary benefit it offerers is minimising the extent of sec-  
392 ondary damage to neighbouring areas by functioning as a barrier around the injury site. Animal  
393 studies have demonstrated that prevention of astroglial scar formation following CNS injury leads  
394 to greater lesion size and poorer function outcomes.(Anderson et al. 2016; Wilhelmsson et al.  
395 2006) Interestingly, a rat study using quantitative liquid chromatography-mass spectrometry with  
396 CSF, found A2M to be more abundant in moderately injured animals compared to more severe  
397 injuries.(Lubieniecka et al. 2011)

398 **5.1.2.2 Apolipoproteins** We found ApoA1, ApoA2, ApoH, ApoL1 and ApoM to be less abundant  
399 in AIC improvers at both time points, whereas ApoA4 was more abundant at both time points (Ta-  
400 bles S2 and S3). ApoA1 is the main protein component of high-density lipoproteins (HDL). Plasma  
401 HDL include two main apolipoproteins, these being ApoA1 and ApoA2 (~70% and ~20% of total  
402 HDL protein content respectively), but some HDL particles can also contain small amounts of other  
403 apolipoproteins, including ApoA4, ApoA5, ApoC, ApoD, ApoE, ApoJ and ApoL. The primary function  
404 of HDL in plasma is the transport of cholesterol, which can have dietary origins, but also be pro-  
405 duced endogenously in the liver.

406 **5.1.2.2.1 HDL Activity** HDLs have serve a wide range of functions, including contributing to  
407 anti-inflammatory activity. They can limit chemokine secretion from multiple cells types including  
408 endothelial cells and monocytes.(Cockerill Gillian W. et al. 1995; Vorst et al. 2013; Bursill Christina  
409 A. et al. 2010) Rats injected with ApoA1 showed significant reduction in expression of CCR2 and  
410 CX<sub>3</sub>CR1, the receptors for chemokines of the same name, which play a role in leukocyte migration.  
411 (Bursill Christina A. et al. 2010)

412 HDL is also associated with protection from oxidative damage, also inhibiting the potentially  
413 atherogenic oxidised LDL formation.(Anatol, Sandrine, and John 2003) The exact mechanisms of  
414 these antioxidant effect is still actively researched, the enzyme paraoxonase-1, which is present on  
415 HDL particles are likely important.(Mackness, Durrington, and Mackness 2004) Apolipoproteins,  
416 including ApoA4 and ApoAE also have antioxidant properties, for example phospholipid hydroper-  
417 oxidase can be reduced by methionine residues of ApoA1, forming redox-inactive phospholipid  
418 hydroxides.(Christison, Rye, and Stocker 1995; Zerrad-Saadi Amal et al. 2009)

419 HDLs can also suppress proliferation of haematopoietic stem cells, thus reducing leucocytosis and  
420 monocytosis.(Yvan-Charvet et al. 2010) Furthermore, HDLs are implicated in the transport of mi-  
421 croRNAs, though the mechanisms of loading the microRNAs and their biological significance is still  
422 under study.(Vickers et al. 2011)

423 ApoE was less abundant in AIS C improvers within 2-weeks and more abundant at 3-months, and  
424 more abundant in more severe injury, such as AIS A relative to D or C and in AIS C relative to D  
425 (Table S2). ApoE is primarily produced by hepatocytes in the liver, but second-most in the brain,  
426 synthesised in and secreted by astrocytes, and has been found to an important determinant in  
427 response to types of CNS injuries in both animal and human studies.(Teasdale et al. 1997; Poirier  
428 1994) A key function of ApoE is as a ligand for the LDL receptor family of proteins, which mediate  
429 trafficking of cholesterol to neurons, which is vital for axonal growth, and for synapse formation  
430 and remodelling.(Xu, Finkelstein, and Adlard 2014) Additionally, ApoE is implicated in the clearance  
431 of neuronal apoptotic bodies.(Elliott et al. 2007) In humans there are three variants/alleles of ApoE:  
432 ApoE2, ApoE3 and ApoE4, which have a frequency of 8.4%, 77.9% and 13.7% globally.(C.-C. Liu et  
433 al. 2013) The variant proteins differ by one or two amino acids and have been found to result in  
434 substantial physiological alterations.(Mahley and Rall 2000; Jha et al. 2008) The presence of the  
435 ApoE4 variant has been linked to worse outcomes in SCI and TBI.(Jha et al. 2008; C. Sun et al. 2011;  
436 Smith et al. 2006; Friedman et al. 1999) More specifically, the SCI study reported significantly lower  
437 change in the median AIS motor score compared the individuals without the ApoE4 allele during  
438 rehabilitation.(Jha et al. 2008)

439 Prior *in vivo* rodent studies have demonstrated up-regulation of ApoE following SCI and TBI, though  
440 ApoE is not observed in neurons of rodents under normal neuropathology, and they only posses  
441 a single ApoE allele.(Iwata et al. 2005; Seitz et al. 2003; Mahley, Weisgraber, and Huang 2006) A  
442 separate rodent study reported ApoE levels decreased for the first 3 days post-injury, and then in-  
443 creased peak expression at 7 days post-injury, a similar pattern to our results.(X. Yang et al. 2018)  
444 Furthermore, mouse studies have demonstrated replacement of ApoE in neurons with human  
445 ApoE4 have impaired neurite outgrowth compared to replacement with ApoE2 or ApoE3, suggest-  
446 ing ApoE4 interferes with neuroplasticity.(Seitz et al. 2003; White et al. 2001) The underlying mech-  
447 anism/s by which ApoE and its alleles effect neuroplasticity is not currently known, but proposals  
448 have been made. One possibility is reduced lipid transport from astrocytes to neurons, poten-  
449 tially impeding the membrane generation required to support axon growth or dendrite sprouting.  
450 ApoE has anti-oxidant properties, so others have suggested impaired anti-oxidant activity may con-  
451 tribute. ApoE4 has been found to be both secreted less than ApoE2 or ApoE3, and to have inferior  
452 anti-oxidant abilities, lending some credence to this idea.(Mishra and Brinton 2018; Miyata and  
453 Smith 1996) Knowing this, whilst ApoE may make for a useful biomarker for SCI, it will be impor-  
454 tant that particular variants of ApoE a given patient has could be just as important, if not more so,  
455 than simple abundance.

456 **5.1.2.3 Serum Amyloid A1** SAA1 was less abundant in AIS C improvers at 2-weeks relative to  
457 non-improvers, but more abundance in plasma at 3-months (Table S2. SAA1 was also more abun-  
458 dant in AIS A relative to less severe injuries, and in AIS Cs relative to Ds (Table S2. SAA1 is a major  
459 acute-phase protein mainly produced in the liver by hepatocytes in response to infection, tissue

injury and malignancy.(L. Sun and Ye 2016) SAA1 is a precursor of amyloid A (AA), the aberrant deposition of which leads to inflammatory amyloidosis.(Tape et al. 1988) There are 5 known SAA1 variants, though currently, no indication of substantial functional differences have been identified.(J. Lu et al. 2014) However, some alleles have been linked to disease, including increased amyloidogenesis and tumour suppression.[van der Hilst et al. (2008); lung\_saa1\_2015]

During the APR, plasma levels of SAA increase up to 1000-fold, and so serves as a well-established clinical biomarker for inflammatory disorders.(Gabay and Kushner 1999) SAA isoforms produced by hepatocytes during an APR are swiftly released into the blood where they associate with HDL, displacing ApoA1 and becoming an apolipoprotein of HDL.(Banka et al. 1995; Benditt and Eriksson 1977) Reverse cholesterol transport, whereby cholesterol in non-hepatic tissues is transported back to the liver, is conducted via plasma components such as HDL, ABCA1 and ABCG1. ApoA1 acts as an acceptor for cholesterol in this process, and studies have found that SAA in lipid-free form can similarly function as a cholesterol acceptor for ABCA1. Whilst SAA is thought to be an important facet of lipid metabolism, its role is likely complex as mice knockout studies which eliminate SAA1 and SAA1 have shown little effect on cholesterol transport, HDL levels and ApoA1 clearance.(de Beer et al. 2010, 2011) These studies indicate that the *in vivo* functions of SAA related to lipid metabolism are more complex than prior *in vitro* studies implied.

SAA1 can both induce anti-inflammatory interleukin 10 (IL-10)-secreting neutrophils, but also promotes the interaction of invariant natural killer T cells with those neutrophils, which limits their suppressive activity by diminishing the production of IL-10 and enhancing the production of IL-12, indicating that SAA1 can have both pro- and anti-inflammatory effects.(Santo et al. 2010) There has however been conflicting results reported of SAA's cytokine induction abilities, and some studies have suggested that recombinant human SAA1 provided by some vendors may have additional cytokine-inducing activity due to the altered amino acid sequence.(M.-H. Kim et al. 2013)

Macrophages are a major source of SAA in inflammatory tissues, and elevated SAA production has been observed in rheumatoid arthritis, Crohn's disease, Type 2 diabetes and atherosclerosis.(Marzi et al. 2013; Dong et al. 2011; Vallon et al. 2001; C, F, and B 1997; Meek, Urieli-Shoval, and Benditt 1994) SAA binding to HDL was reported to increase affinity for macrophages whilst decreasing affinity for hepatocytes.(R. Kisilevsky and Subrahmanyam 1992) This change is thought to favour the removal of cholesterol from site of inflammation.(R. Kisilevsky 1991) SAA inhibits the binding of the scavenger receptor SR-BI and cholesterol efflux is enhanced in a SR-BI-dependent manner.(Cai et al. 2005; van der Westhuyzen et al. 2005) It has been suggested that the SR-BI-mediated re-uptake of cholesterol underpins the role of SAA in cholesterol recycling during tissue repair, where a great deal of cholesterol is required.(Robert Kisilevsky and Manley 2012)

In blood circulation SAA1 may also function as an immune opsonin for increased neutrophil uptake of Gram-negative bacteria.(Shah, Hari-Dass, and Raynes 2006) Both human and mouse SAA proteins have been found to bind retinol with nanomolar affinity that limits bacterial burden in tissues after acute infection.(Derebe et al. 2014) Retinol is important to the body's response to microbial infection, so SAA may also have a role in limiting bacterial burden, particularly in the liver, spleen and intestine. The aforementioned study demonstrated that mice lacking in both SAA1 and SAA2 have a higher bacterial burden in the liver and spleen following infection.(Derebe et al. 2014) All 3 SAA isoforms are found in intestinal epithelium, which is exposed to the gut microbiome, in mice. The anti-bacterial properties of SAA isoforms may therefore explain the role of SAA as an acute-phase protein that protects the host in tissues and organs exposed to bacteria.

**5.1.2.4 Retinol-binding protein 4 (RBP4)** In plasma within 2-weeks post-injury, RBP4 was less abundant in AIS C improvers relative to AIS D and A, and more abundant in AIS C non-improvers again, relative to AIS D and A (Table S2. Similarly, AIS A plasma had more RBP4 compared to AIS

507 D, and AIS C improvers were also more abundant in RBP4 compared to non-improvers at both  
508 2-weeks and 3-months post-injury (Table S2).

509 Vitamin A is a collective term for a group of fat-soluble compounds with a range of essential bio-  
510 logical activities including aspects of growth, vision and metabolism.(Blomhoff and Blomhoff 2006)  
511 Following dietary absorption, vitamin A is ferried from the intestine, with chylomicrons as retinyl  
512 esters, to tissues for immediate use or the liver for storage in hepatic stellate cells. A subsequent  
513 dietary deficiency of vitamin A will result in these liver stores being mobilised by hydrolysing the  
514 retinyl esters to release retinol. The retinol is then bound by RBP4, which is also mainly synthesised  
515 in the liver, and secreted into circulation from hepatocytes, whereupon it is bound by an additional  
516 transport protein, transthyretin.(P. A. Peterson 1971) The membrane plasma protein STRA6 facil-  
517 itates retinol transport from RBPs across the cell membrane.(Berry et al. 2012) Once delivered  
518 to target cells, retinol can either be converted to retinaldehyde, which is required for functional  
519 vision, or oxidised to retinoic acid, which is a ligand for nuclear receptors, thus regulating gene  
520 expression.(Lane and Bailey 2005; J. E. Balmer and Blomhoff 2002)

521 RBPs are localised in the ventral region, associated with motor neurons, in the mammalian de-  
522 veloping neural tube.(Pierani et al. 1999; Maden, Ong, and Chytil 1990) The role of retinoid sig-  
523 nalling in spinal cord and motor neuron differentiation, including development of regions of the  
524 spinal cord has been outlined, and implies a possible involvement in maintaining motor neuron  
525 integrity.(Colbert et al. 1995; Sockanathan and Jessell 1998)

526 The mRNA of a rodent homologue of RBP, named cytosolic retinol binding protein, was found to  
527 be up-regulated at 24 hours post-SCI and may promote cell proliferation and regeneration by in-  
528 creasing retinoid metabolism.(Song et al. 2001; Hurst et al. 1999) Another study of amyotrophic  
529 lateral sclerosis (ALS), a neurodegenerative disease, comparing gene expression between post-  
530 mortem spinal cord samples of ALS and controls also observed up-regulation of RBP1 in ALS spinal  
531 cord.(Malaspina, Kaushik, and Belleroche 2001) Furthermore, a transgenic mouse study reported  
532 retinoid signalling may contribute to the retained plasticity and regenerative potential of the ma-  
533 ture spinal cord.(Haskell et al. 2002)

534 The results found here support these findings for AIS C improvers relative to non-improvers as  
535 improver had increased levels of RBP4. Whether this is due to increased expression or due to  
536 higher vitamin A intake is unclear from this data, though at 3-months post-injury this is still that  
537 case even though patients diets could be more similar throughout hospital admission.

### 538 5.1.3 Metabolism and SCI

539 **5.1.3.1 Acute phase response** The bodies first response to injury or infections, including SCI,  
540 is often referred to as the “acute phase response” (APR), which is non-specific, innate reaction  
541 that precedes more specific and situational immune reactions.(Gordon and Koj 1985; Gruys et  
542 al. 2005) This systemic response is largely coordinated by factors released from the liver, but the  
543 APRs effects extend to multiple peripheral organs including the kidneys, lungs and spleen.(Bao et  
544 al. 2012; S. J. Campbell, Zahid, et al. 2008; Fleming et al. 2012; Gris, Hamilton, and Weaver 2008)  
545 This hepatic response is typically transient and quickly fades, but prolonged liver inflammation and  
546 pathology has been observed in rodent SCI models.(Goodus et al. 2018; Sauerbeck et al. 2014)

547 Basic liver functions are chronically impaired by SCI, including metabolising carbohydrates, fats  
548 and proteins, storage of minerals vitamins and glycogen and filtering blood from the digestive  
549 tract.(García-López et al. 2007; DeLeve 2007; Farkas and Gater 2018; Chow et al. 2012; Sauerbeck  
550 et al. 2014) This is likely related to the elevated incidence of metabolic disease in the SCI cohort,  
551 including insulin resistance, impaired glucose tolerance and cardiovascular disease.(Bauman and  
552 Spungen 2001; Maruyama et al. 2008; Lee et al. 2004; J. Myers, Lee, and Kiratli 2007) Long-term

survival is noticeably lower relative to the general population and, whilst mortality in the first 2 year following SCI has decreased in recent decades, long-term survival has not.(Strauss et al. 2006; Shavelle et al. 2015) More recently, a longitudinal study found SCI patients had a significantly higher incidence of acute pancreatitis relative to a matched healthy cohort.(Ho, Yeh, and Pan 2021)

The acute (1-7 days) liver response to SCI is well documented; the inflammatory cytokines including TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$  and IL-6, released at the injury site, reach the liver through the bloodstream.(Fleming et al. 2012; Hundt et al. 2011) This provokes the liver to enter the APR and produce acute phase proteins (APPs) thus stimulating a greater immune response.(Anthony and Couch 2014; Fleming et al. 2012) The hepatocytes that make up the majority of the liver biomass, express receptors that bind the aforementioned inflammatory cytokines; similarly the hepatic macrophage Kupffer cells also bind these cytokines, complement proteins and lipopolysaccharide (LPS) and swiftly remove microorganisms, endotoxins and other debris from the blood.(C.-Y. Yang et al. 2013; Szalai et al. 2000; Crispe 2016; S. J. Campbell et al. 2005) Hepatic stellate cells act as sensors of tissue integrity by exposure to signals of oxidative stress, danger/pathogen associated molecular patterns (DAMPs/PAMPs), chemokines/cytokines and factors secreted from neighbour hepatic cells, and can stimulate innate immunity by releasing cytokines and as antigen presenting cells during the APR. (Weiskirchen and Tacke 2014; Fujita and Narumiya 2016)

SCI studies in rodent and canine models have found the APPs serum amyloid (SA) A, SAP, CRP, fibrinogen, haptoglobin and a1-antichymotrypsin are elevated 4-24 hours post-injury in blood.(Pepys and Baltz 1983; Gabay and Kushner 1999; J. C. E. Hall et al. 2012; Steel and Whitehead 1994) In rodents, hepatic CD68 mRNA is observed to be elevated within 24 hours post-SCI and CD68+ Kupffer cell numbers increase during the first 7 days post-SCI.(Sauerbeck et al. 2014)

Furthermore, it has been suggested that liver inflammation and Kupffer cells activity promote recruitment of leukocytes to the injury site in brain or spinal trauma, potentially enhancing CNS injury.(Anthony and Couch 2014; S. J. Campbell et al. 2005) For example, a rodent study demonstrated depletion of Kupffer cells prior to injury resulted in few neutrophils infiltrating the injury site.(S. J. Campbell, Zahid, et al. 2008; S. J. Campbell, Anthony, et al. 2008)

#### 5.1.4 Microbiome & SCI

Circulating factors from the injury site are not the only potential driver of hepatic inflammation. Within 24 hours post-SCI in rodents tight junctions between epithelial cells become more permeable, thus allowing gut bacteria and the endotoxins they can produce to enter the bloodstream.(J. Liu et al. 2004) This will reach the liver through the portal vein where Kupffer cells function as a "first line of defence".(Jenne and Kubes 2013; M. L. Balmer et al. 2014) It has been proposed that elevated LPS+ endotoxins caused by the post-SCI "leaky gut" causes acute liver inflammation by overloading hepatic filtrations capacity, allowing microbes to bypass the liver and elicit systemic inflammation.(J. Liu et al. 2004; O'Connor et al. 2018) The binding of LPS to Kupffer cells results in the production of a range of growth factors, including TNF- $\alpha$ , multiple interleukins and reactive oxygen species (ROS), stimulating bone-marrow-derived monocytes and neutrophils to infiltrate the liver.(S. A. Myers et al. 2019; Milosevic et al. 2019; Kazankov et al. 2019) A rodent study found transcription factors for tight junctions down-regulated following SCI, and that application of probiotics improved neurological outcomes.(Kigerl, Mostacada, and Popovich 2018; Kigerl et al. 2016) Human studies of the microbiome post-SCI have also demonstrated dysbiosis, both chronically and more acutely post-injury.(Zhang et al. 2018; Gungor et al. 2016; Bazzocchi et al. 2021)

#### 5.1.5 Drivers of liver steatosis

Steatosis, the abnormal retention of lipids within cells or organs, most commonly associated with the liver, has been observed to increase in rodents during the first week post-injury.(Sauerbeck

599 et al. 2014) The liver takes up circulating fatty acids, and when levels exceed the oxidative and  
600 secretory limits of the liver, hepatocytes store the excess as triglycerides.(Diraison and Beylot 1998)  
601 Adipose tissue lipolysis during elevated sympathetic activity leading to spikes in circulating fatty  
602 acids has been reported in human subjects following SCI.(Karlsson 1999)

603 *De novo* lipogenesis occurring within the liver can also drive hepatic steatosis.(Lavoie and Gau-  
604 thier 2006) Ceramides are lipid signalling molecules and regulators of apoptosis and inflamma-  
605 tion; they can contribute to insulin resistance, oxidative stress and inflammation-induce liver adi-  
606 posity through sustained Toll-like-receptor(TRL)-4 activation.(Schilling et al. 2013; Bhargava and  
607 Lee 2012; Pagadala et al. 2012) If released into the circulatory system, ceramides can cause CNS  
608 toxicity, including oxidative damage and changes to the aggregation of proteins associated with  
609 diseases such as Parkinson's, Huntington's and Alzheimer's.(Pagadala et al. 2012; Vidaurre et al.  
610 2014; Czubowicz et al. 2019) Mature and precursors of hepatic ceramides and enzymes which  
611 contribute to ceramide synthesis are elevated by 1 day post-injury.(Sauerbeck et al. 2014) Endo-  
612 toxins can also stimulate the synthesis of ceramides and so the aforementioned "leaky gut" may  
613 also contribute to this elevation.(Chang et al. 2011) Ceramide synthesis and lipogenesis genes are  
614 also stimulated by TNF- $\alpha$ , which, as touched on in the general introduction (??), has been found  
615 to be elevated post-SCI, and associated with differential neurological recovery.(Davies, Hayes, and  
616 Dekaban 2007; Hasturk et al. 2009; Biglari et al. 2015; Sauerbeck et al. 2014; Bikman 2012)

### 617 5.1.6 Chronic liver inflammation in SCI

618 The hepatic APR and associated inflammation that typically follows bodily trauma, subsequently  
619 rapidly subsides, whereas post-SCI this hepatic inflammation persists chronically. This chronic  
620 phase may be due in part to long-term changes in intestinal permeability via fewer tight junc-  
621 tions in intestinal epithelial cells, resulting in gut dysbiosis.(Milosevic et al. 2019; O'Connor et al.  
622 2018; Kigerl, Mostacada, and Popovich 2018; Kigerl et al. 2016) Bacterial translocation and gut  
623 dysbiosis can be the result of non-mechanical intestinal obstruction, impaired intestinal motility  
624 and systemic immune suppression, all of which are potential complications of SCI.(Balzan et al.  
625 2007) Specifically, butyrate-producing bacteria have been found to be reduced in SCI relative to a  
626 healthy cohort.(Gungor et al. 2016) Butyrate is known to modulate epithelial differentiation and  
627 cell growth, and suppress macrophages, including CNS inflammation, thus the reduction in bu-  
628 tyrate from bacteria may contribute to recovery post-SCI, though links to the liver specifically have  
629 not yet been studied.(H.J. Kim et al. 2007; Arpaia et al. 2013; Park et al. 2005; P. S. Chen et al. 2007)

630 LPS is another potential modulator of post-SCI chronic liver physiology. Kupffer cells, hepatic en-  
631 dothelial cells and hepatocytes all participate in the clearance of LPS via CD14- and TLR4-dependent  
632 mechanisms.(Mimura et al. 1995; van Oosten et al. 2001; Vodovotz et al. 2001) LPS induced the  
633 release of factors such as TNF- $\alpha$

### 634 5.1.7 Longitudinal metabolic health

635 Prior work has found at least 25% of acute SCI patients to be obese, which is well known to induce  
636 low-level systemic inflammation, and that this cohort has significantly worse outcomes compared  
637 to non-obese SCI patients (Stenson et al. 2011). Alcohol abuse has also been associated with  
638 poorer SCI neurological outcomes (Elliot et al. 2002). Furthermore, advancing age is associated with  
639 increased liver inflammation and the SCI population has followed the general populations ageing  
640 trend (Bertolotti et al. 2014; Y. Chen, He, and DeVivo 2016). Taken together, it is not unreasonable  
641 to assume that a large number of SCI patients may have pre-existing liver inflammation at injury.  
642 This may be an important differentiator that contributes to the degree of neurological recovery  
643 a given patient may experience. Future experiments investigating neurological outcomes of SCI  
644 may benefit from establishing parameters of metabolic health, including the composition of the

645 microbiome, as close to injury as possible, and potentially monitoring changes in these parameters  
646 longitudinally.

#### 647 **5.1.8 Validation of results**

648 The ELISAs used to validate the proteomic data often did not demonstrate significant differences  
649 between the groups (Figures ??, ??, ?? and ??). This may be in part to the individual variability of  
650 the samples. However, the trends of the data do largely reflect those found in the iTRAQ data, sug-  
651 gesting that with greater statistical power there may be a more robust validation. Furthermore,  
652 the ApoA1 ELISAs resulted in the most significant differences, and was the only Quantikine® kit  
653 used (Figure ??). As the Quantikine® kits are highly optimised, including for use with plasma,  
654 whereas the DuoSet®, which were used for the other proteins, are not. Future studies should  
655 therefore consider either simply using Quantikine® kits, or ensure good optimisation of the Du-  
656 oSet® kits in advance. These results are also corroborated by a recent label-free proteomic SCI  
657 study, using a rodent model, which reported similar proteins associated with complement cascade,  
658 including A2M and C3.(Yao et al. 2021)

#### 659 **5.1.9 Conclusion**

660 This work shows that proteins associated with the complement cascade, and apolipoproteins in  
661 particular, have potential as prognostic biomarkers for SCI. For some of these biomarkers, ApoE  
662 in particular, it may not be pure abundance, but also the particular allele of the patient that may  
663 provide valuable insight. However, the relatively small number of proteins identified here is a lim-  
664 itation, likely due to highly abundant proteins impacting the dynamic range of the samples. The  
665 pooling of samples also obscures individual variability in protein abundance. Subsequent pro-  
666 teomics experiments using label-free techniques, and depletion of highly abundant proteins may  
667 allow for more in-depth pathway analysis. These results, in concert with the prior chapters find-  
668 ings (??), provide further evidence of a link between metabolic function and functional neurological  
669 recovery post-SCI. Further work is needed elucidate the precise biochemistry at play, and perhaps  
670 more importantly, whether modulation of these pathways has the potential to improve outcomes.  
671 Experiments that closely monitor the liver, modify diet and analyse metabolites, particularly longi-  
672 tudinally post-injury, would all give further insight into this relationship.

### 673 **5.2 thesis label-free discussion**

674 As outlined previously (5.1.9), two key limitations of the iTRAQ experiments were the pooling of  
675 samples, which prevents statistically robust group-wise comparisons, and the high dynamic range  
676 of protein abundances in plasma potentially obscuring less abundant proteins. This work seeks  
677 to address these factors by a combination of Proteominer™ beads to shrink the dynamic range of  
678 protein abundances, and by not pooling samples.

#### 679 **5.2.1 Proteins identified**

680 A total of 87 proteins were identified, many of which were only detected in one group. Proteins  
681 only present in limited groups could be highly suited for use as biomarkers as binary indicators are  
682 much simpler to test for, and suggest more dramatic biological differences. Here we explore the  
683 potential these proteins have as biomarkers of SCI.

684 **5.2.1.0.1 Peroxiredoxins** Peroxiredoxins are a large and highly conserved family of enzymes  
685 that reduce peroxides. Peroxiredoxin 2 (PRX-2) is highly abundant in RBCs and intracellularly serves  
686 as an important anti-oxidant role in various cell types, including neurons.(Low, Hampton, and

687 Winterbourn 2008) By contrast, extracellular PRX-2 has been suggested to act as an inflamma-  
688 tory DAMP, leading microglia and macrophages to release a plethora of pro-inflammatory fac-  
689 tors.(Salzano et al. 2014; Garcia-Bonilla and Iadecola 2012; Shichita et al. 2012) An *in vitro* primary  
690 neurons and microglia co-culture study reported PRX-2 activating microglia via TLR-4, potentially  
691 leading to neuronal apoptosis.(Y. Lu et al. 2018) A mouse study found over-expression of PRX-2 at-  
692 tenuated oxidative stress and neuronal apoptosis following subarachnoid haemorrhage.(Y. Lu et al.  
693 2019) Over-expression of PRX-2 is speculated to protect again ischaemic neuronal injury by mod-  
694 ulating the redox-sensitive thioredoxin-apoptosis signal-regulating kinase (ASK) 1 signalling com-  
695 plex.(Gan et al. 2012) Several molecular chaperones can interact with ASK1, including thioredoxin  
696 and TNF receptor-associated factor 6.(Matsuzawa et al. 2005) The dissociation of the thioredoxin-  
697 ASK1 complex activates ASK1. PRX-2 is oxidised after scavenging free radicals, whereupon its an-  
698 tioxidantive activity is reduced. This inactivation can be reversed by the thioredoxin-thioredoxin  
699 reductase system, whereby oxidised PRX-2 can regain its activity by reducing thioredoxin, leading  
700 to the dissociation of the thioredoxin-ASK1 complex.(Rhee and Woo 2011) Additionally, oxidised  
701 PRX-1 can inhibit ASK1-induced apoptosis via the thioredoxin-binding domain on ASK1.(S. Y. Kim,  
702 Kim, and Lee 2008)

703 PRX-2 was found to be present in AIS C improvers and AIS D patients acutely, and in AIS A and  
704 D patients subacutely. The differences in abundance between these groups was not statistically  
705 significant, though acute AIS D had less PRX-2 relative to subacute AIS D ( $\log_2$  fold change -1.9) and  
706 subacute AIS A also had less abundant PRX-2 relative to subacute AIS D ( $\log_2$  fold change -1.7). The  
707 presence of PRX-2 in acute AIS C improvers and absence in acute C non-improvers suggests the  
708 protein could indicate a more protective action against oxidative stress, and implies the protein  
709 has potential value as a biomarker of functional outcomes. Similarly, PRX-2 may be acting as a  
710 healthy response to trauma-induced oxidative stress in both acute AIS D, although the persistence  
711 to the subacute time-point is less clear. Likewise, the presence of PRX-2 in AIS A subacutely, but not  
712 acutely is more perplexing. It should be noted that as plasma was used and cells lysed, so there  
713 is no distinguishing between intracellular and extracellular PRX-2. Perhaps in the more severe AIS  
714 A injury, secondary injuries, including oxidative stress, are greater and so persist to the subacute  
715 time-point. The acute absence may be a result of an overwhelmed physiology unable to respond  
716 or prioritise managing oxidative stress.

717 **5.2.1.1 Neuroinflammation post-SCI** The neuro-inflammatory response begins immediately  
718 post-trauma, and involves a complex series of events that can persist well into the chronic phase.  
719 The sudden emergence of necrotic cell debris and associated DAMPs lead surviving CNS-resident  
720 cells to produce cytokines, complement factors and ROS. Within minutes CNS cells at the lesion site  
721 have been found to secrete several pro-inflammatory mediators, including TNF- $\alpha$  and interleukins,  
722 in both rodent models and human patients with SCI.(Pineau and Lacroix 2006; Chandrasekar et al.  
723 2017; Dalgard et al. 2012; Bastien et al. 2015) The resulting inflammatory response occurs in  
724 parallel to the mechanical destruction of the blood-spinal cord barrier, and the development of  
725 tissue oedema and ischaemia combine to propagate damage to parts of the cord spared by the  
726 initial trauma.(Maikos and Shreiber 2007; Ahuja et al. 2017)

727 The microglial population at the lesion site have been observed to be significantly depleted  
728 immediately post-injury, due to death via both the apoptosis and mechanical injury in a rodent  
729 model.(Bellver-Landete et al. 2019) Surviving microglia change in shape and migration patterns,  
730 and begin to produce ROS, oxidative metabolites and pro-inflammatory cytokines.(Pineau and  
731 Lacroix 2006; Bastien and Lacroix 2014) These cells can associate with damaged axons rapidly  
732 post-injury, but are thought to not actively phagocytose these cells until approximately 4 days  
733 post-trauma.(Bellver-Landete et al. 2019; Pineau and Lacroix 2006; Greenhalgh and David 2014)

734 The following hours and days post-injury are characterised by a substantive complement sys-

735 tem activation and sequential leukocyte migration from the periphery into the injured neural  
736 parenchyma.(Brennan et al. 2015; S. L. Peterson and Anderson 2014; Qiao et al. 2006) Curiously,  
737 though the breakdown of the BSCB would presumably allow unrestricted access of circulating  
738 leukocytes into the injured cord segment, recruitment of these cells remains a highly controlled  
739 process.(Beck et al. 2010; Brennan et al. 2019) A mouse study reported lymphocytes, which  
740 account for approximately 80% of circulating leukocytes, only enter the cord in substantial  
741 numbers at least several weeks to months post-injury.(Beck et al. 2010) Early infiltrate is instead  
742 largely comprised of myeloid cells, predominantly neutrophils, which are a minority of circulating  
743 cells but are the swiftest peripheral responders to SCI, with studies detecting them at the lesion  
744 site within 4 hours of injury.(Wright et al. 2010) Neutrophil numbers have been reported to peak  
745 at 1 day post-trauma, but also to remain at the site for a minimum of 42 days post-injury.(Okada  
746 2016; Kigerl, McGaughy, and Popovich 2006)

747 This neutrophil recruitment is often viewed as principally detrimental to recovery following SCI, but  
748 also wound healing more generally. A recent study found circulating neutrophil numbers in ad-  
749 mission bloods from human SCI patients were negatively correlated with patient outcomes at dis-  
750 charge.(Brennan et al. 2019) The same study utilising a contusive SCI mouse model, showed the ex-  
751 tent of neutrophil presence at the lesion site inversely correlated with neurological outcomes, and  
752 depletion of said cells with an antibody against Ly6G improver recovery of motor function.(Brennan  
753 et al. 2019) However, other studies have suggested neutrophil activity which potentially benefits  
754 SCI recovery. A transgenic mouse contusion model study showed over-expression of secretory  
755 leukocyte protease inhibitor, which can arise from neutrophils and activated macrophages, im-  
756 proved locomotive functional outcomes, and reduced markers of secondary injury.(Ghasemlou  
757 et al. 2010) Another study, using a peripheral nerve injury mouse model, reported neutrophil  
758 infiltration and associated cytokine/chemokine production was vital for clearance of myelin de-  
759 bris.(Lindborg, Mack, and Zigmond 2017) Additionally, another study using a mouse contusion  
760 model found increased lesion sizes and impaired neurological outcomes following neutrophil de-  
761 pleition, though the Gr-1 antibody used also depletes inflammatory monocytes, muddying the pic-  
762 ture somewhat.(Stirling et al. 2009) Regardless, it is clear that the complexity of the role neutrophils  
763 play in the SCI response extends beyond any simple binary beneficial/harmful distinction.

764 Moving forward in the SCI pathology, newly proliferated and recruited microglia begin ac-  
765 tively phagocytosing necrotic cell debris, and begin accumulating around the lesion epicen-  
766 tre.(Greenhalgh and David 2014; Bellver-Landete et al. 2019; Pineau and Lacroix 2006) The  
767 presence of microglia appears to be vital, particularly during the first week post-SCI, as depletion  
768 via the colony stimulating factor-1 inhibitor PLX5622 has been linked to substantially worsened  
769 functional outcomes.(Bellver-Landete et al. 2019; Brennan et al. 2018) Relatedly, another  
770 mouse SCI model study found early enhancement of microglial activation can reduce secondary  
771 pathology.(Stirling et al. 2014)

772 Circulating inflammatory monocytes are also recruited during the first days post-trauma. Adop-  
773 tive transfer experiments have shown recruitment to pick up at approximately 3 days post-injury,  
774 and peak at 7 days.(Blomster et al. 2013) Whilst monocyte turnover at the lesion appears to be  
775 high, infiltrating monocyte-derived macrophages remain at the site of weeks to months post-  
776 trauma.(Blomster et al. 2013; Shechter et al. 2009) Interestingly, the timing of monocyte recruit-  
777 ment appears to be delayed relative to non-neurological tissue injury. For instance, monocytes  
778 are reported to be rapidly recruited to the heart following a myocardial infarction, as early as 1 day  
779 post-injury, and their numbers return to baseline by roughly 16 days post-injury.(Nahrendorf et al.  
780 2007)

781 Owing to the diversity of monocyte subsets and macrophage phenotypes, a complete un-  
782 derstanding of their role with respect to SCI pathology is still lacking, and requires under-  
783 active research.(David and Kroner 2011) Some polarisation states associated with recruited

784 macrophages are thought to be implicated in propagating secondary injury via fibrotic scar  
785 formation and demyelination of axons.(Kigerl et al. 2009; Popovich et al. 1999; Zhu et al. 2015)  
786 Similarly, several studies have reported a reduction in infiltration of monocytes/macrophages  
787 is associated with better SCI outcomes.(Kigerl et al. 2009; Zhu et al. 2015; Horn et al. 2008)  
788 Conversely, others have found depletion o circulating monocytes/macrophages significantly  
789 increased lesion size and results in worse function outcome, with restoration of blood monocyte  
790 numbers attenuating this phenotype.(Shechter et al. 2009) More recent *in vitro* studies suggested  
791 blood-derived macrophages can suppress microglial phagocytosis without reducing microglial  
792 proliferation and extension of processes.(Greenhalgh and David 2014; Greenhalgh et al. 2018)  
793 This literature represents and ongoing controversy over the role of monocytes/macrophages in  
794 relation to recovery post-SCI. Importantly, many of these studies are based on somewhat crude  
795 depletion of cell types, with little discrimination paid toward any potential subpopulations and/or  
796 cell polarisation status. Given the shear complexity of the pathology at play, more nuanced  
797 approaches will likely be needed in future studies to paint a more complete picture.

798 B cell recruitment is yet wave of immune cell infiltration, thought to occur several days post-injury.  
799 These cells can form follicle-like structures in combination with T cells, microglia and macrophages  
800 from roughly 28 days post-trauma, and remain present and the lesion well into the chronic phase  
801 of SCI.(Ankeny, Guan, and Popovich 2009) Whilst the extent of B cell presence has been reported  
802 to vary between animals, they have been correlated with self-reactive antibodies that recognise  
803 epitopes within protein homogenates of the spinal cord.(G. Sun et al. 2017) Adoptive transfer  
804 experiments in a mouse model isolated antibodies from SCI mice, and found injected them into  
805 the neural parenchyma of naïve animals induced significant damage, whereas mice lacking B cells  
806 have improved recovery post-SCI.(Ankeny, Guan, and Popovich 2009)

807 More evidence is needed to establish whether these self-reactive antibodies precede an autoim-  
808 mune event, or signify a autoimmune disease. Alternatively, they may serve as a mechanism for  
809 opsonisation and debris clearance from the lesion site.(Nagele et al. 2013) Naturally occurring  
810 autoantibodies with well-established role in tissue regeneration and repair have been found to  
811 be elevated following SCI.(Palmers et al. 2016; Arevalo-Martin et al. 2018) Much like the afore-  
812 mentioned monocyte/macrophage controversy, it should be pointed out that any positive effects  
813 of these autoantibodies does not preclude any simultaneous negative impacts which could be  
814 modulated. For instance, another study reported naturally occurring IgM antibodies contribute to  
815 secondary injury during the more acute phase post-SCI.(Narang et al. 2017)

816 Neuro-inflammation is less understood at the chronic phase of SCI, as most studies focus on the  
817 first hours and days post-injury. By this stage, the glial scar has established a well-defined border  
818 between the lesion core and the health tissue flanking it.(Sofroniew and Vinters 2010) Infiltrating  
819 immune cells are largely restricted to within the lesion itself, as opposed to the surrounding spared  
820 tissue. B and T cells, macrophages and neutrophils have all been detected here many months post-  
821 trauma.(Beck et al. 2010; Ankeny, Guan, and Popovich 2009; Prüss et al. 2011) The chronic phase is  
822 also marked by substantial metabolic dysfunction, characterised by reduced lipid metabolites and  
823 increased oxidative stress, in addition to elevated pro-inflammatory mediators.(Dulin et al. 2013)

824 There are fewer studies that attempt to elucidate the underlying mechanisms driving this non-  
825 resolving inflammatory response in the chronic phase of SCI. One study suggested communica-  
826 tion with infiltrating monocytes suppresses chronic microglial activation and inflammation after  
827 SCI.(Greenhalgh et al. 2018) Interruption of this communication was linked to worsened func-  
828 tion outcomes, implying the initial microglial response to trauma may be beneficial, their pro-  
829 tracted activation can eventually become detrimental.(Bellver-Landete et al. 2019; Greenhalgh et  
830 al. 2018) Furthermore, a rodent model study of chronic SCI, found use of the anti-inflammatory  
831 drug licoferone, applied daily for 1 month at 8 months post-injury, observed some improvement  
832 to metabolic functions, but no benefit to locomotor function.(Dulin et al. 2013) To summarise, un-

833 derstanding of persistent inflammation during the chronic phase of SCI is lacking, and particularly  
834 complicated by the plateaus in locomotive recovery that typically occurs well before the chronic  
835 SCI phase is reached. Thus, there is a need for further studies to uncover the role of the various  
836 immune cell populations with respect to ongoing neurological dysfunction and pathology during  
837 the chronic phase of SCI.

838 **5.2.1.1.1 Intravenous immunoglobulin** Intravenous immunoglobulin (IVIG) is increasingly  
839 used as an immunomodulatory strategy for managing acute neurological conditions, including  
840 neurotrauma. Originally developed as an antibody replacement therapy for immunodeficiency  
841 disorders, IVIG is a product comprised primarily of immunoglobulin G (IgG) taken from the blood  
842 plasma of healthy donors.(Bayry, Negi, and Kaveri 2011; Schwab and Nimmerjahn 2013) IVIG  
843 therapy was found to increase platelet number in idiopathic thrombocytopenic purpura (ITP)  
844 patients, which lead to an interest in using it as an immunomodulatory therapy.(Imbach et al.  
845 1981) Its potent effects and limited side effects have lead high-dose IVIG therapy to be commonly  
846 used in a plethora of inflammatory and autoimmune disorders, including ITP, arthritis, Kawasaki's  
847 syndrome and Guillain-Barré syndrome.(Lünemann, Nimmerjahn, and Dalakas 2015; Stangel et  
848 al. 1998)

849 Some recent research using a contusive SCI mouse model has reported promising results of high-  
850 dose IVIG as a therapeutic for SCI.(Brennan et al. 2016) The study found that a clinical dose of  
851 IVIG (0.5-2g/kg body weight) lead to a 30-40% reduction in lesion size, and reductions in demyeli-  
852 nation, central canal dilation, and axonal degeneration, though doses below 0.5g/kg were ineffec-  
853 tive.(Brennan et al. 2016) The same study also found albumin treatment did not produce the same  
854 effects as IVIG, suggesting simple protein loading is not the causative mechanism. Likewise, rodent  
855 studies utilising purified human IgG in a high-level (C7-T1) clip aneurysm model, and another lower-  
856 level (T9) contusion SCI study, reported similar improvements.(Nguyen et al. 2012; Chio et al. 2019;  
857 Gok et al. 2009) Additionally, a Phase I/IIa clinical trial aiming to explore the safety and efficacy of  
858 IVIG therapy in human SCI patients is approved and underway (ACTRN12616001385437). How-  
859 ever, whilst there are several pre-clinical studies reporting IVIG treatment can benefit outcomes in  
860 CNS injury from a range of neurological conditions, the exact mechanism/s behind any potential  
861 neuroprotective effects of IVIG for SCI are currently unclear.(Tzekou and Fehlings 2014)

862 In TBI mouse models, animals treated with IVIG were shown to have improved neurobehavioural  
863 outcomes, and a reduction in neuronal degeneration both acutely and chronically, relative to  
864 vehicle-treated controls in rotarod and Morris water maze experiments.(Jeong et al. 2014) Further  
865 mouse studies using cerebral artery occlusion, a model of stroke, reported high-dose IVIG signif-  
866 icantly reduced infarct volumes, neurological impairment and mortality rates.(Arumugam et al.  
867 2007; Widiapradja et al. 2012) Under condition of BBB/BSCB compromise, IVIG has been found to  
868 enter the neural parenchyma within hours of injury.(Brennan et al. 2016; Arumugam et al. 2007)  
869 SCI studies have found IVIG to localise to oligodendrocytes, astrocytes, neurons, macrophages,  
870 microglia, pericytes and blood vessels.(Brennan et al. 2016; Chio et al. 2019) Additionally, reduc-  
871 tions in immune cells, as indicated by F4/80<sup>+</sup> microglia/macrophages and polymorphonuclear  
872 cells in brain and spinal injury models respectively, have also been reported.(Jeong et al. 2014;  
873 Nguyen et al. 2012; Chio et al. 2019) Relatedly, the aforementioned SCI IVIG mouse study found  
874 reduced CD68<sup>+</sup> macrophages at and surrounding the lesion 35 days post-injury.(Brennan et al.  
875 2016) Importantly, these studies do not differentiate between resident microglial and infiltrating  
876 monocytes/macrophages. Thus, further research is needed to understand the influence of IVIG  
877 on both recruitment and activation states of these cell subsets.

878 **5.2.1.1.2 Speculative mechanisms of action for IVIG in SCI** As IVIG is made from pooled anti-  
879 bodies taken from thousands of donors, it includes a vast repertoire of antibodies specific against

880 millions of unique antigens, allowing for a diverse variety of effects in differing disease contexts.  
881 Whilst there is extensive research of IVIG and autoimmune disorders, such as Guillain-Barré syn-  
882 drome, the immune pathology found in the acute phase of CNS injury is not typically considered  
883 to be driven by autoimmune processes.(Lünemann, Nimmerjahn, and Dalakas 2015; Stangel et  
884 al. 1998) There may be some overlap in therapeutic mechanism, but it seems more likely any  
885 benefits are conferred through modulation of the innate rather than adaptive immune responses.  
886 The potential mechanisms of IVIG can be split between those mediated via the IgG constant (Fc)  
887 fragment, which binds the Fc receptors, and the F(ab)'<sub>2</sub> fragment, which governs antigen recogni-  
888 tion.(Schwab and Nimmerjahn 2013) In the context of neurological diseases, mechanisms related  
889 to F(ab)'<sub>2</sub> are thought to potentially bind and therefore neutralise cell surface receptors, comple-  
890 ment, cytokines and autoantibodies. By contrast, Fc-dependent mechanisms are speculated to in-  
891 clude regulation of Fc receptor expression, saturation of the neonatal Fc receptor, block activation  
892 of Fc receptors, and modulate T cells.(Schwab and Nimmerjahn 2013; Lünemann, Nimmerjahn,  
893 and Dalakas 2015; Dalakas 2014) Furthermore, models of neurological injury suggest both F(ab)'<sub>2</sub>  
894 and Fc-dependent signalling cascades could be involved in the modulation of several chemokines  
895 and cytokines.(Dalakas 2014)

896 Modulation via the variable F(ab)'<sub>2</sub> region

897 Self-reactive antibodies have been found circulating in both chronic rodent SCI models and hu-  
898 man patients 1 year post-injury.(Ankeny, Guan, and Popovich 2009; Hayes et al. 2002) Whilst some  
899 studies have suggested potential relevance of naturally occurring autoantibodies (germline en-  
900 coded and produced by B1 cells) in acute SCI, it remains unclear whether IVIG treatment may have  
901 any impact on them.(Palmers et al. 2016; Narang et al. 2017) The impact or lack thereof of IVIG on  
902 chronic phase SCI autoimmunity also remains to be seen.

903 A separate potential F(ab)'<sub>2</sub>-dependent mechanism involves the neutralisation of the cell death  
904 mediator Fas (AKA CD95). Studies of Lyell's syndrome, a disorder whereby active Fas ligand binds  
905 Fas present on keratinocytes, inducing apoptosis, reported IVIG therapy completely inhibited Fas  
906 ligand-induced cell death both *in vitro* and in human patients.(Viard et al. 1998; Altnauer et al.  
907 2003) Importantly, IVIG blocked Fas, as opposed to Fas ligand, in these studies, as this result was  
908 only observed with cells pre-treated with IVIG. Incubation of IVIG with soluble Fas ligand did not  
909 attenuate cell death, implying IVIG contains antibodies specific to Fas.(Viard et al. 1998; Altnauer  
910 et al. 2003) This modulatory effect of the Fas-Fas ligand pathway may have relevance in SCI, as a  
911 study using knock-out mice lacking Fas showed a reduction in both apoptosis at the lesion site and  
912 glial scarring, and improved motor function post-SCI.(Sobrido-Cameán and Barreiro-Iglesias 2018;  
913 W. R. Yu and Fehlings 2011) Neurons and glial cells from post-mortem human patients were found  
914 to be more Fas- and Fas ligand-positive, but this was limited to the acute phase of SCI, and not  
915 observed chronically, suggesting this pathway is more significant immediately post-injury.(W. R. Yu  
916 and Fehlings 2011) Therefore, acute IVIG treatment could act by attenuating secondary cell death  
917 by blocking Fas, thus disrupting this pathway.

918 Conversely, agonistic anti-Fas antibodies have also been reported with IVIG prepara-  
919 tions.(Altnauer et al. 2003) Whilst it remains unknown how these agents may act in SCI,  
920 one could postulate a benefit if they induce apoptosis in circulating leukocytes, which could  
921 otherwise do harm.(Schneider et al. 2017) Supporting this, papers have found reductions in poly-  
922 morphonuclear cell populations within the lesion at 1 day post-injury in rodent models.(Nguyen  
923 et al. 2012; Chio et al. 2019; Gok et al. 2009) However, IVIG-induced apoptosis has only been  
924 observed in human leukocytes, not in rodents, casting doubt on this idea.(Altnauer et al. 2003;  
925 Schneider et al. 2017) Alternatively, the reduced recruitment could be a result of IVIG regulating  
926 the expression of adhesion molecules or molecules involved in leukocytes trafficking. A feline  
927 ischaemia-reperfusion injury model study found IVIG to down-regulate expression of integrins  
928 on leukocyte cell surfaces, inhibiting adhesion and subsequent extravasation of the cells into the

damaged site.(Gill et al. 2005) Again however, these finding are contradicted by an experimental stroke study where IVIG was found to increase leukocyte and platelet trafficking to the injury, leading to formation of aggregates within cerebral vasculature.(Lapointe et al. 2004)

Finally,  $F(ab)'_2$  may act by complement scavenging. Both *in vitro* and *in vivo* studies have found the non-antigen-binding regions of  $F(ab)'_2$  can bind and neutralise the complement activation products C3a and C5a, thus preventing complement-mediated tissue damage.(Milan Basta et al. 2003; M. Basta et al. 1989) Multiple studies utilising various models of CNS injury have reported IVIG attenuating complement.(Brennan et al. 2016; Arumugam et al. 2007) Specifically in SCI, IVIG was found to reduce levels of the complement activation products C3b and C5a within the damaged cord.(Brennan et al. 2016) Similarly, an experimental stroke study reported IVIG reducing C3b levels in the infarct area.(Arumugam et al. 2007) Interestingly, whilst this study found IgG able to bind mouse C3b, supporting the hypothetical neutralisation of complement activation products, they also found IVIG able to attenuate oxygen deprivation-induced production of C3 itself in primary neuron cultures. This seems to suggest IVIG is able to scavenge both secreted complement activation products, and their local production.(Arumugam et al. 2007)

#### Modulation via the constant Fc region

With respect to the Fc region, this portion normally binds to Fc $\gamma$  receptors (Fc $\gamma$ Rs), which are present on most leukocytes and resident CNS cells. Many Fc $\gamma$ Rs act as activating receptors, such as inducing phagocytosis in response to opsonised targets, or as an inhibitory receptor that dampens effector cell responses.(Schwab and Nimmerjahn 2013) A given cells response to an immunoglobulin isotype is determined by the combination of which Fc $\gamma$ Rs are expressed by said cell. Myeloid cell all express come combination of these activating Fc $\gamma$ Rs, as do some innate lymphoid cells which do not express more classical antigen receptors, such as natural killer cells, whereas T and B cells do not.(Perussia et al. 1989) The inhibitory Fc $\gamma$ RIIb receptor is also expressed on myeloid cells, in addition to B cells, but not natural killer cells or resting T cells.(Bruhns and Jönsson 2015) Whilst there is debate over the expression and function of Fc $\gamma$ Rs in neurons, *in vitro* work with neuronal cultures has detected mRNA for all Fc $\gamma$ Rs.(Thom et al. 2017) Astrocytes, microglia and oligodendrocyte precursors have also be found to express Fc $\gamma$ R, and up-regulate them under some disease states.(Thom et al. 2017)

Studies utilising just the Fc fragment have been found to be equally effective as normal IVIG in several non-neurological autoimmune diseases, including nephrotoxic nephritis, ITP and K/BxN arthritis models, suggesting Fc $\gamma$ Rs play a key role in the mechanism of IVIG.(Samuelsson, Towers, and Ravetch 2001; I. K. Campbell et al. 2014; Kaneko et al. 2006) With respect to CNS injury, some evidence suggesting a role of Fc $\gamma$ Rs comes from a mouse study with animals lacking the common  $\gamma$ -chain, and thus no functional Fc $\gamma$ Rs, which were found to be protected from experimental stroke and SCI.(Ankeny, Guan, and Popovich 2009; Komine-Kobayashi et al. 2004)

Within the context of antibody-mediated autoimmune disorders, high-does IVIG may saturate Fc receptor and reduce the half-life of pathogenic endogenous IgG.(Schwab and Nimmerjahn 2013)

**5.2.1.1.3 Immunoglobulins** Several immunoglobulin components were identified here, including 3  $\lambda$  variable precursors (3-19, 3-10 and 2-18), 3 heavy variable precursors (3-15, 1-69 and 1-24) and 2 heavy constant gamma regions (2 and 4). For the  $\lambda$  variable precursors, acute AIS C improvers the precursors 3-19 and 3-10 were detected, whereas 3-10 and 2-18 were detected in acute C non-improvers. That acute C non-improvers expressed the 2-18 precursor whilst the improvers did not, suggests potential as a biomarker of poorer functional outcomes. It is difficult to comment on the biological mechanisms that may be a play here from this data, but one could infer that it is indicative of either a more robust, or a more maladaptive, immune response to the trauma. Given that the injuries are of the same severity by AIS grade, the latter seems more likely, though

976 again, further research is needed to highlight the precise nature of this difference. Interestingly,  
977 whilst the acute C improvers do not express precursor 2-18, both the subacute C improvers and  
978 non-improvers, and subacute As do, whereas acute or subacute Ds do not, seemingly implying this  
979 precursor is also indicative of more severe injury in the latter phases of SCI.

980 In addition of acute C improvers, subacute As and acute Ds also express the 3-19 precursor, with  
981 subacute As possessing the greatest abundance. Again, this would seem to suggest this marker  
982 is indicative of positive outcomes or less severe injury in the acute phase, but may be more detri-  
983 mental in the latter phases. The final  $\lambda$  precursor, 3-10, is present in acute As, subacute As and  
984 both subacute C groups as well as the aforementioned acute C improvers. The curious absence  
985 of 3-10 in both AIS D groups and C non-improvers groups suggests the marker is implicated in a  
986 more beneficial response, but perhaps this is limited to more severe injuries.

987 With respect to the immunoglobulin heavy variable precursors, 3-15 was present in all groups  
988 except acute As and acute C non-improvers, though there was insufficient power to confidently  
989 compare the fold change of groups expressing 3-15. Another heavy variable precursor, 1-69, was  
990 expressed in subacute As, both acute and subacute C improvers, and both acute and subacute  
991 Ds. The final heavy variable precursor, 1-24, was found in all groups except acute C improvers and  
992 non-improvers.

993 For the two immunoglobulin heavy constant  $\gamma$ s, 4 was significant in acute C improvers and non-  
994 improvers, relative to subacute As, whereas  $\gamma$  2 was only significant in acute C improvers relative to  
995 subacute Ds. Both acute C improvers and non-improvers had a lower abundance of  $\gamma$  4 relative to  
996 subacute As (-2.2 and -2.7 respectively), whilst  $\gamma$  2 had a -1.8 fold change between acute C improvers  
997 and subacute Ds.

998 **5.2.2 Conclusion**

999 Much like the iTRAQ experiments (5.1.9), the majority of proteins identified are functionally asso-  
1000 ciated with the complement cascade. Unlike the iTRAQ however, many of the proteins where only  
1001 detected in one group of the pairwise comparisons, suggesting greater suitability as biomarkers.  
1002 PRX-2, a protein associated with oxidative stress, is of particular interest, both as a biomarker for  
1003 improvement in acute AIS C patients, but also mechanistically in relation to functional recovery.  
1004 Furthermore, several immunoglobulins were identified as differentially abundant, though further  
1005 *in vitro/vivo* work is needed to elucidate the pathophysiological relevance of each precursor. The  
1006  $\lambda$  2-18 and 3-10 precursors are of particular relevance to acute and subacute AIS C improvement  
1007 respectively, and both are of interest longitudinally in AIS As, with 2-18 potentially being linked to  
1008 severity of injury.

1009 The small number of statistically significant proteins speaks to the variability of human samples,  
1010 and is likely exacerbated by the inconstant timing of sample collection relative to injury. Post-hoc  
1011 power analysis of the data reveals that to identify a 2.5 fold change with an FDR of 0.5 and a power  
1012 of 0.9, 14 biological replicates would be needed, in contrast to the 7-11 replicates used across  
1013 groups here. Thus, a repeat of this experiment with a larger sample size will likely reveal many  
1014 more proteins of potential interest. Furthermore, a metabolomic analysis with a similar sample  
1015 size would greatly compliment this work, particularly with regards to investigating further links to  
1016 the liver.

1017 **Supplementary material**

1018 **5.3 Session Information**

```
1019 ## -  
1020 ## platform      aarch64-apple-darwin20  
1021 ## arch          aarch64  
1022 ## os            darwin20  
1023 ## system        aarch64, darwin20  
1024 ## status  
1025 ## major         4  
1026 ## minor         1.3  
1027 ## year          2022  
1028 ## month         03  
1029 ## day           10  
1030 ## svn rev       81868  
1031 ## language      R  
1032 ## version.string R version 4.1.3 (2022-03-10)  
1033 ## nickname      One Push-Up
```

**Table S1.** Packages Used

package	version	date
base	4.1.3	2022-03-18
MSstats	4.2.0	2021-05-31
STRINGdb	2.6.5	2020-01-10
ReactomePA	1.38.0	2021-10-26
rlang	1.0.2	2022-03-04
bookdown	0.25	2022-03-16
lime	0.5.2	2021-02-24
RColorBrewer	1.1.3	2022-04-03
ggVennDiagram	1.2.0	2021-10-19
DiagrammeR	1.0.9	2022-03-04
lubridate	1.8.0	2021-10-03
patchwork	1.1.1	2020-12-15
cowplot	1.1.1	2020-12-15
readxl	1.4.0	2022-03-28
BiocManager	1.30.16	2021-06-15
knitr	1.38	2022-03-25
rmarkdown	2.13	2022-03-09
data.table	1.14.2	2021-09-23
naniar	0.6.1	2021-05-14
psych	2.2.3	2022-03-17
Hmisc	4.6.0	2021-10-05
Formula	1.2.4	2020-10-16
survival	3.2.13	2021-08-23
lattice	0.20.45	2021-09-18
bibtex	0.4.2.3	2020-09-19
captioner	2.2.3	2015-07-15

forcats	0.5.1	2021-01-27
stringr	1.4.0	2019-02-09
dplyr	1.0.8	2022-02-07
purrr	0.3.4	2020-04-16
readr	2.1.2	2022-01-30
tidyr	1.2.0	2022-01-27
tibble	3.1.6	2021-10-25
ggplot2	3.3.5	2021-06-24
tidyverse	1.3.1	2021-04-15
kableExtra	1.3.4	2021-02-19



**Table S2.** OpenMS log<sub>2</sub> fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively.

gene	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C improvers acute vs subacute	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS A vs D	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D
A1BG	-0.9031824	-0.1017534	-0.6087849	0.1926441	0.2252650	0.7937347	-0.3497633	0.4439714	-0.5750284	0.2187064
A2M	-1.0385788	-0.2464392	-0.6760613	0.1160783	-1.2300968	1.4247538	-1.6029796	-0.1782258	-0.3728828	1.0518710
AFM	-0.3788476	-1.2248641	0.4815192	-0.3644973	0.5517904	1.1923601	-1.2566085	-0.0642484	-1.8083989	-0.6160388
AHSG	1.1794532	NA	-0.5545288	NA	NA	NA	NA	NA	NA	NA
AMBP	0.6562004	-0.3433433	0.8606588	-0.1388849	-0.9023293	NA	1.2037841	NA	2.1061134	NA
APCS	0.1498290	0.2108936	-0.0114011	0.0496636	NA	0.3557242	NA	NA	-0.0494567	0.3062675
APOA1	-0.1816744	-0.6923621	-0.2337557	-0.7444434	-0.7677301	0.6941282	-1.3172834	-0.6231553	-0.5495533	0.1445749
APOA2	0.0900143	-1.1461360	-0.6667620	-1.9029124	NA	NA	NA	NA	NA	NA
APOA4	0.1295961	0.9636781	-1.2312803	-0.3971983	-1.3254088	0.7876011	-1.3346720	-0.5470709	-0.0092632	0.7783379
APOB	0.1379231	-0.0164100	-0.6332751	-0.7876082	-0.8570393	0.5260041	-1.2345864	-0.7085823	-0.3775471	0.1484570
APOE	-1.2133754	0.2930673	-0.6884490	0.8179937	-0.9078302	0.7746514	-1.5477490	-0.7730977	-0.6399188	0.1347326
APOH	-0.3600286	-0.7024687	-0.6444887	-0.9867188	-0.9996639	2.8143614	-1.0091799	1.8051815	-0.0095159	2.8048455
APOL1	-1.1790763	-0.5193515	-1.0440264	-0.3843015	-0.1152769	0.5652696	0.1299333	0.6952029	0.2452102	0.8104799
APOM	-1.2167971	-0.6819883	0.6934807	1.2282895	NA	0.6561807	NA	NA	0.6664954	1.3226762
ATRN	NA	NA	-1.0062957	NA	NA	NA	NA	NA	NA	NA
AZGP1	1.2191679	1.0251503	0.0811400	-0.1128776	-3.3889514	-3.6440501	0.3702887	-3.2737614	3.7592401	0.1151900
C1QB	-0.8410072	-2.0020393	0.7071113	-0.4539208	-1.9729191	1.3563310	-2.0066282	-0.6502972	-0.0337090	1.3226219
C1R	-0.4335115	-0.7632158	0.0366498	-0.2930545	-0.1467491	0.7976066	0.3564300	1.1540366	0.5031791	1.3007857
C1S	0.0295224	-0.8193739	0.1679558	-0.6809404	NA	NA	NA	NA	NA	NA
C2	NA	NA	NA	NA	-2.5581036	2.5640965	-2.5952702	-0.0311737	-0.0371665	2.5269300
C3	-0.7440620	-0.6968585	0.0652375	0.1124410	-1.0730763	1.2388421	-2.1616420	-0.9227999	-1.0885657	0.1502764
C4BPA	-0.1810388	-2.4454980	1.6627662	-0.6016930	-1.2378707	1.5489731	-1.8448914	-0.2959183	-0.6070207	0.9419523
C5	-0.5447843	-0.2031226	0.9230001	1.2646617	-0.7200022	1.2710496	-1.6768797	-0.4058301	-0.9568775	0.3141721
C6	-1.3936214	1.7817023	-1.3097108	1.8656129	-3.0451914	1.7642372	-3.2550019	-1.4907647	-0.2098105	1.5544267
C7	-0.9642124	0.8848082	-0.7827165	1.0663041	0.9970185	0.0708650	-1.1136320	-1.0427670	-2.1106505	-2.0397855
C8A	-0.51117891	0.2736564	-0.7630145	0.0224310	-2.8108340	0.1731241	-2.1285385	-1.9554144	0.6822955	0.8554196
C8B	-2.1950427	0.2789045	-1.5954883	0.8784589	-1.8943958	-0.4802611	-0.9597537	-1.4400148	0.9346421	0.4543810
C8G	NA	NA	-1.6304866	NA	NA	NA	NA	NA	NA	NA
C9	-2.2199059	0.4534093	-1.9249790	0.7483361	-0.7345863	0.6495872	-3.2424254	-2.5928382	-2.5078391	-1.8582519
CD5L	-0.9293248	-0.6204735	-0.7145571	-0.4057058	-2.4642871	0.4482534	-2.3260120	-1.8777586	0.1382751	0.5865285
CFH	-1.1239737	0.7406948	-1.6480885	0.2165801	-1.0358708	0.1380093	-1.3260484	-1.1880391	-0.2901776	-0.1521683
CFI	NA	0.5359696	NA	1.2578110	NA	NA	NA	NA	NA	NA
CLU	-1.1958984	-0.8681850	-0.1721921	0.1555214	-1.3664377	0.8251962	-2.1976184	-1.3724222	-0.8311807	-0.0059845
CP	-0.3892064	0.2565411	-0.4537277	0.1920199	-0.6657547	0.4235353	-0.2695812	0.1539541	0.3961736	0.8197089
F12	0.4852010	-0.9397905	0.6702925	-0.7546990	-0.8534307	0.5549559	-1.3145850	-0.7596291	-0.4611543	0.0938016
F2	-0.7493082	-0.7563593	0.0982877	0.0912367	-0.5408805	1.1677146	-1.5476188	-0.3799042	-1.0067383	0.1609763
FCN3	NA	0.9644778	NA	NA	NA	NA	NA	NA	NA	NA

**Table S2.** OpenMS log<sub>2</sub> fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively. (continued)

gene	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C improvers acute vs subacute	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS A vs D	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D
FGA	-0.9591400	-0.5109050	0.4841704	0.9324054	-1.0155684	1.0486717	-1.4707952	-0.4221236	-0.4552268	0.5934449
FGB	-0.8339088	-0.1253771	0.0684287	0.7769604	-0.8343143	1.0951087	-1.4646547	-0.3695460	-0.6303405	0.4647683
FGG	-1.1432907	-0.0247316	-0.2978078	0.8207513	-0.7191139	0.7606622	-1.0780014	-0.3173392	-0.3588876	0.4017746
FN1	-0.2795610	-0.3153249	0.2899102	0.2541463	-0.5777631	1.1462731	-1.2550759	-0.1088028	-0.6773129	0.4689602
GC	-0.5583474	0.4050629	-0.7950103	0.1684001	-1.8700166	-0.2961353	-1.2641016	-1.5602369	0.6059149	0.3097797
GSN	0.0704855	0.0479440	-0.6709561	-0.6934976	NA	NA	NA	NA	NA	NA
HABP2	NA	NA	NA	NA	-0.5367242	1.4445961	-0.7070902	0.7375059	-0.1703660	1.2742301
HP	-1.2468596	0.5276209	-0.3488061	1.4256744	-0.6393503	0.9683391	-1.2963281	-0.3279890	-0.6569779	0.3113613
HPX	-0.4104644	-0.2880781	-0.7114901	-0.5891038	-0.3597680	0.9360243	-1.1034368	-0.1674125	-0.7436687	0.1923556
HRG	0.5979026	1.0672891	0.0321566	0.5015431	-0.7300739	0.6893699	-0.8231701	-0.1338002	-0.0930962	0.5962737
IGHA1	1.7635882	1.3476620	0.3628909	-0.0530353	-2.0152404	0.4328016	-2.2081140	-1.7753124	-0.1928737	0.2399280
IGHD	NA	NA	NA	NA	-2.4499647	0.4182281	-3.4284738	-3.0102457	-0.9785091	-0.5602810
IGHG1	-0.0855309	0.9292134	-0.4962961	0.5184482	-0.0970233	-1.8091062	0.4814333	-1.3276728	0.5784566	-1.2306496
IGHG2	0.9720422	0.3501681	0.4607992	-0.1610748	-0.6249433	-1.5106734	0.2705475	-1.2401258	0.8954908	-0.6151826
IGHG3	-0.1941508	1.4323226	-0.9309878	0.6954857	-1.8543540	-0.3927284	-1.8870246	-2.2797530	-0.0326705	-0.4253990
IGHM	-0.6318126	-0.8967300	-0.4174693	-0.6823867	-1.1741740	1.7915993	-2.3508710	-0.5592717	-1.1766971	0.6149023
IGKC	-0.0697458	0.0420359	-0.1150304	-0.0032487	-1.1868447	-0.2875492	-1.1765257	-1.4640749	0.0103190	-0.2772302
IGKV3D- 20	NA	NA	NA	NA	-0.3699302	-0.0536821	0.2114801	0.1577980	0.5814103	0.5277282
ITIH1	-0.9766570	0.7057133	-0.5211753	1.1611951	-0.6149247	0.5495684	-0.5039432	0.0456252	0.1109815	0.6605499
ITIH2	-0.3142692	-0.5283214	-0.2363320	-0.4503842	-0.7431549	0.6757214	-1.2136587	-0.5379373	-0.4705037	0.2052177
ITIH3	-0.5456033	0.6138901	0.3512683	1.5107617	-2.0564371	1.2902341	-1.8743188	-0.5840847	0.1821183	1.4723525
ITIH4	-0.0669542	-0.2189363	0.3808668	0.2288847	-1.0843698	0.9773070	-1.8198452	-0.8425382	-0.7354753	0.2418317
KLKB1	NA	-2.2093082	NA	-0.2713600	NA	NA	NA	NA	NA	NA
KNG1	-0.6198162	-0.0025326	-0.0676278	0.5496558	-0.6644071	0.8052877	0.0312278	0.8365155	0.6956349	1.5009226
LRG1	-0.7988007	0.2565104	0.1402188	1.1955298	-0.9515964	1.7017682	-2.1951046	-0.4933364	-1.2435082	0.4582600
LUM	0.0832323	0.6580097	-1.2635566	-0.6887792	NA	NA	NA	NA	NA	NA
ORM1	-0.1974770	1.1178187	-0.2240143	1.0912814	-1.9126407	1.6761382	-1.3025982	0.3735400	0.6100425	2.2861806
PGLYRP2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PLG	-0.3680073	0.0880557	-0.8410370	-0.3849741	-1.0701631	2.7112467	-2.8493306	-0.1380838	-1.7791675	0.9320793
PROS1	-0.3300860	0.0623958	-0.7963440	-0.4038621	-0.5089636	1.5349629	-3.8745298	-2.3395668	-3.3655662	-1.8306032
RBP4	0.4505693	0.4185795	-0.0211740	-0.0531638	-4.0971240	1.4352287	-2.9877294	-1.5525007	1.1093946	2.5446233
SAA1	-2.7778116	2.3463574	-0.5151865	4.6089825	-1.3858800	2.4855048	-2.5593861	-0.0738814	-1.1735062	1.3119986
SERPINA1	0.6825593	0.0481996	1.7824248	1.1480651	-0.0999129	-0.1558972	-1.3635079	-1.5194051	-1.2635950	-1.4194922
SERPINA3	-0.7582369	-0.1617666	0.1836958	0.7801661	-0.7417534	2.2311097	-2.0353461	0.1957637	-1.2935927	0.9375171
SERPINA4	0.0099121	NA	-1.0180116	NA	-1.4473701	NA	-0.6571525	NA	0.7902176	NA
SERPINAS	NA	NA	NA	0.2757029	NA	NA	NA	NA	NA	NA

**Table S2.** OpenMS log<sub>2</sub> fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively. (continued)

gene	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C improvers acute vs subacute	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS A vs D	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D
SERPINC1	-0.5553486	-0.2339361	-0.5421237	-0.2207112	-0.7720265	1.1066666	-1.3464506	-0.2397839	-0.5744241	0.5322425
SERPIND1	0.2536120	NA	0.0459257	NA	0.3050057	2.3844297	-1.6468854	0.7375442	-1.9518911	0.4325386
SERPING1	-1.1614755	0.1191571	-1.3510892	-0.0704566	-0.9301893	1.0766804	-1.0904641	-0.0137837	-0.1602748	0.9164056
TF	-0.2823635	-0.1105094	-0.4843676	-0.3125135	-0.7681926	0.5875721	-0.9945649	-0.4069929	-0.2263723	0.3611997
VTN	-0.6186100	-0.0323770	-0.2690009	0.3172321	-1.7234623	1.4918535	-2.1517604	-0.6599069	-0.4282982	1.0635554
VWF	NA	1.0585752	NA	1.3917877	-2.5662912	0.5161630	-1.9774026	-1.4612396	0.5888885	1.1050516

**Table S3.** ProteinPilot fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively.

Protein	AIS C improvers acute vs subacute	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D	AIS C	AIS A vs D
A1BG	-1.644372	-1.472312	NA	NA	NA	NA	NA	NA	NA	NA	NA
A2M	-6.137620	-9.908319	NA	1.380384	-5.861382	-3.467369	NA	1.659587	5.861382	3.564511	
AFM	NA	2.511886	NA	-4.055085	NA	NA	NA	NA	NA	NA	-3.499452
AHSG	NA	NA	NA	-2.249055	NA	NA	NA	NA	NA	NA	NA
APCS	NA	1.870682	NA	NA	NA	4.207266	1.721869	NA	NA	NA	NA
APOA1	-11.803206	-3.698282	NA	-3.250873	-2.884031	-2.884031	-3.801894	NA	-1.406047	NA	
APOA2	-14.321879	NA	NA	-4.965923	NA	NA	NA	NA	NA	NA	NA
APOA4	-11.587774	-5.915616	NA	-2.108628	-2.964831	-1.555966	-2.488857	1.870682	NA	NA	-1.629296
APOB	-2.443430	3.019952	NA	-6.025596	3.732502	-1.282331	1.367729	-4.742420	-2.805434	1.721869	
APOC1	NA	NA	NA	-4.528976	NA	NA	NA	NA	NA	NA	NA
APOC4	NA	NA	NA	NA	NA	1.318257	NA	4.920395	NA	-4.528976	
APOE	NA	NA	-1.527566	-1.753880	NA	-1.836538	-3.019952	-1.803018	-3.019952	NA	
AZGP1	2.269865	2.630268	3.597493	NA	1.819701	4.446313	NA	NA	NA	NA	-4.130475
C1QB	NA	NA	NA	NA	NA	-1.513561	NA	NA	NA	NA	NA
C1R	NA	NA	NA	NA	NA	-4.446313	NA	NA	NA	NA	NA
C3	2.754229	-1.940886	NA	3.981072	-2.398833	-4.365158	1.614359	-1.976970	3.597493	6.546362	
C4B	2.269865	-2.147830	-1.940886	2.654606	NA	NA	NA	NA	NA	NA	NA
C4BPA	NA	-1.419058	NA	NA	NA	NA	1.659587	-2.013724	NA	3.250873	
C5	1.737801	NA	NA	2.228435	NA	-2.333458	NA	-1.770109	NA	2.167704	
C6	1.887991	NA	NA	NA	NA	-2.070141	-2.805434	NA	NA	NA	NA
C9	NA	-2.421029	NA	9.908319	NA	-4.055085	NA	-1.499685	7.177943	9.375620	
CD5L	NA	-2.831392	-3.280953	NA	-1.819701	-1.819701	NA	NA	NA	NA	NA
CFB	NA	-1.674943	2.535129	4.285485	NA	-2.128139	2.032357	-1.690441	2.511886	4.055085	
CFH	NA	NA	NA	2.558586	NA	NA	NA	NA	2.333458	1.803018	
CFI	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.269865	
CLU	NA	NA	NA	NA	NA	NA	NA	-2.582260	NA	NA	
CP	NA	NA	2.582260	3.019952	NA	NA	2.187762	NA	2.779713	NA	
F2	NA	NA	NA	NA	NA	NA	1.674943	NA	NA	1.527566	
FGA	3.467369	-1.644372	NA	12.133888	-3.531832	-2.654606	NA	NA	5.199960	4.092606	
FGB	3.280953	NA	2.443431	9.204495	-2.187762	-1.330454	2.654606	NA	5.248075	3.133286	
FGG	2.032357	-1.958845	NA	9.638290	-2.312065	-1.644372	4.325138	NA	9.204495	6.367955	

**Table S3.** ProteinPilot fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively. (continued)

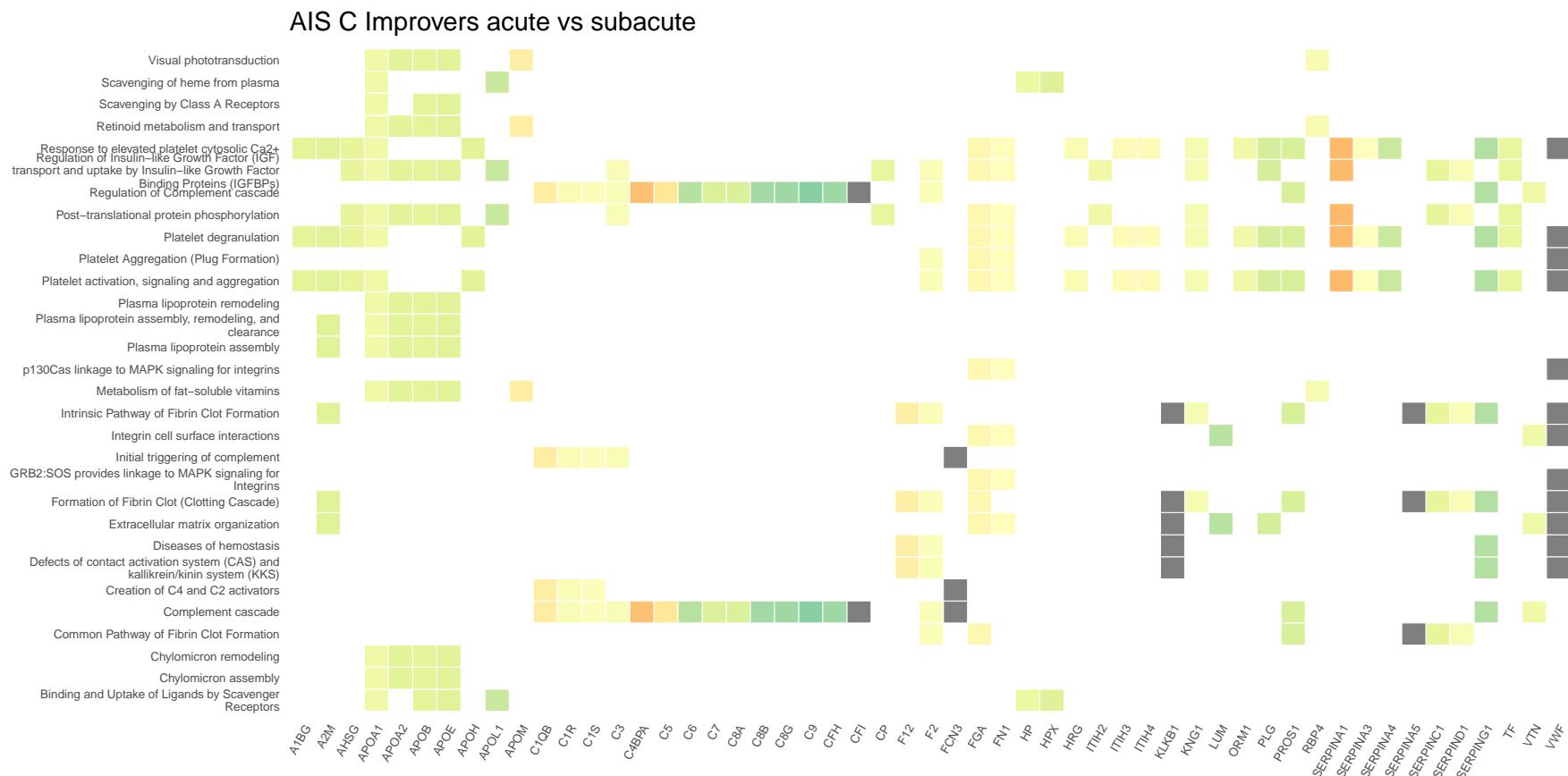
Protein	AIS C improvers acute vs subacute	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D	AIS C	AIS A vs D
FN1	2.582260	2.228435	NA	NA	1.940886	-2.466039	1.472312	-4.875285	NA	3.404082	
GC	NA	NA	NA	NA	NA	NA	1.541700	NA	2.606154	2.398833	
GSN	-2.312065	NA	NA	-4.055085	-3.019952	NA	-4.365158	NA	NA	NA	
HBA1	NA	3.133286	NA	-4.017908	NA	NA	NA	NA	-2.654606	-2.535129	
HBB	NA	10.000000	NA	-15.995580	5.058247	2.167704	NA	NA	-6.137620	-2.558586	
HP	3.499452	NA	2.511886	13.427649	NA	-2.964831	NA	NA	4.092606	4.786301	
HPX	NA	-2.147830	NA	NA	NA	NA	1.995262	NA	2.208005	NA	
HRG	NA	NA	NA	NA	NA	3.531832	NA	3.908409	NA	NA	
IGHM	NA	-5.152286	-3.664376	NA	-5.199960	-4.655861	NA	NA	3.221069	2.937650	
IGKC	NA	NA	NA	NA	NA	1.753880	5.649370	1.786488	5.807644	NA	
ITIH1	NA	NA	NA	NA	NA	NA	NA	-3.597493	NA	NA	
ITIH2	NA	NA	NA	-1.629296	NA	-2.089296	-2.208005	-2.070141	-2.208005	NA	
ITIH3	NA	-2.051162	NA	2.466039	NA	NA	NA	NA	2.108628	2.630268	
ITIH4	1.819701	-2.312065	NA	3.104560	-1.836538	-3.104560	NA	-1.737801	2.376840	4.092606	
JCHAIN	NA	NA	-4.130475	NA	-5.011872	NA	NA	NA	NA	NA	
KNG1	NA	NA	NA	NA	NA	NA	2.754229	NA	NA	NA	
LPA	NA	NA	10.764652	14.723126	NA	NA	NA	NA	NA	NA	
LRG1	NA	-2.167704	NA	3.047895	-6.367955	-9.727472	NA	-1.629296	NA	3.311311	
LUM	-4.405549	NA	NA	-3.250873	NA	NA	NA	NA	NA	NA	
ORM1	NA	NA	16.904409	NA	NA	NA	3.630781	NA	NA	2.992265	
PLG	1.555966	NA	NA	NA	2.312065	1.870682	2.937650	NA	NA	NA	
RBP4	NA	5.495408	NA	NA	NA	NA	NA	NA	NA	NA	
SAA1	NA	NA	28.054337	51.522865	NA	NA	NA	NA	NA	NA	
SAA4	NA	NA	NA	NA	NA	-2.805434	NA	NA	NA	1.905461	
SERPINA1	NA	-2.333458	NA	7.585776	-2.754229	-5.597576	NA	-2.187762	3.221069	7.112135	
SERPINA3	2.108628	-1.737801	3.837072	12.705741	-1.976970	-5.915616	NA	-3.250873	4.325138	12.246162	
SERPIN C1	NA	NA	NA	NA	NA	NA	NA	-2.070141	NA	NA	
SERPIN D1	1.770109	NA	NA	NA	2.032357	NA	NA	NA	NA	NA	
SERPIN F1	NA	NA	NA	NA	NA	-4.365158	-5.248075	NA	NA	NA	
SERPIN F2	NA	NA	NA	NA	NA	-4.207266	NA	-3.467369	NA	NA	
SERPIN G1	NA	-2.535129	NA	2.964831	-1.836538	-4.365158	NA	-2.488857	2.187762	5.248075	

**Table S3.** ProteinPilot fold changes in the plasma proteome of SCI patients. 'Acute' and 'Subacute' samples collected within 2 week and approximately 3-months post-injury respectively. (continued)

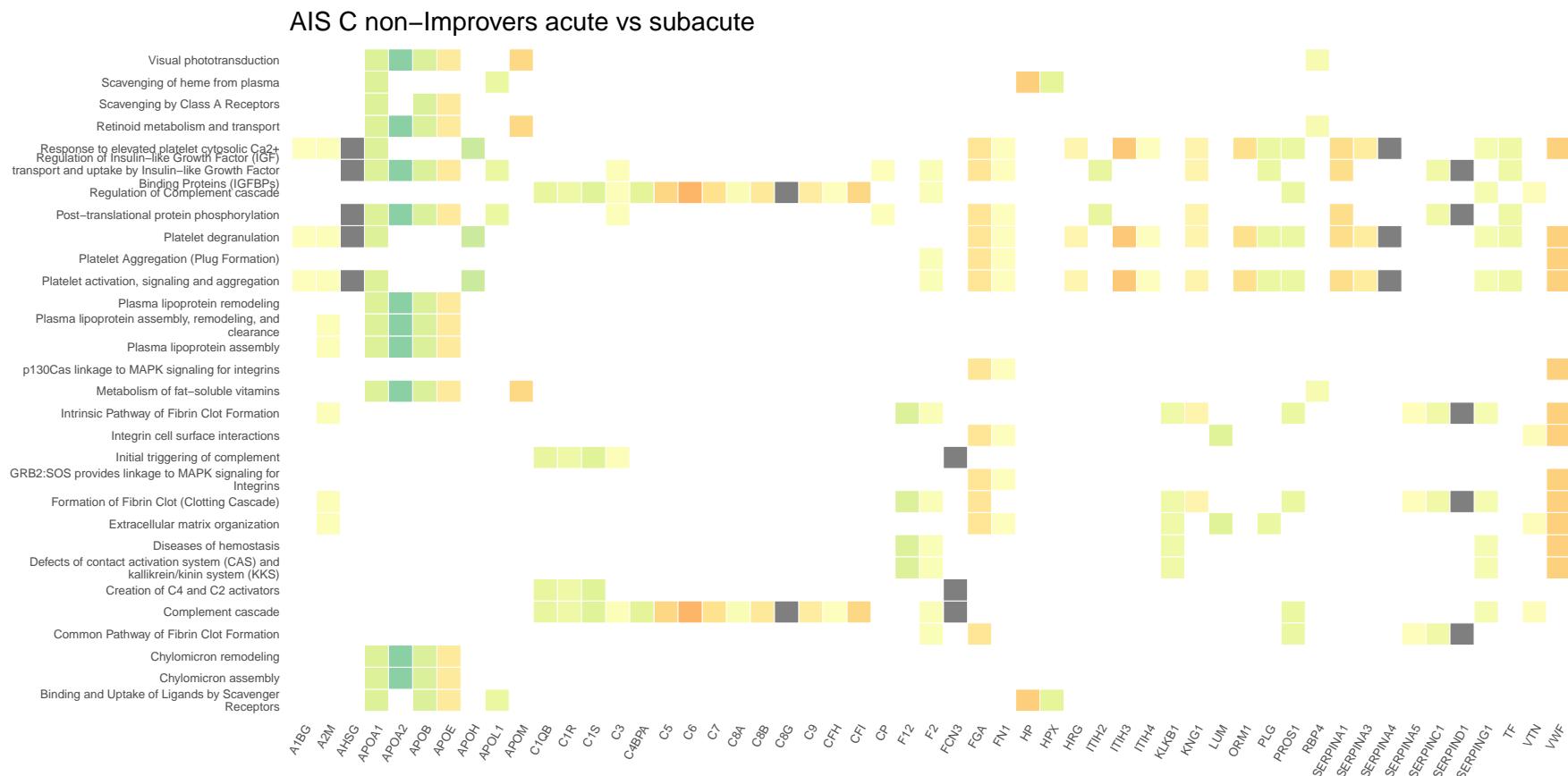
Protein	AIS C improvers acute vs subacute	Acute AIS C improvers vs non-improvers	Subacute AIS C improvers vs non-improvers	AIS C non-improvers acute vs subacute	AIS C improvers vs non-improvers	AIS C improvers vs A	AIS C improvers vs D	AIS C non-improvers vs A	AIS C non-improvers vs D	AIS C	AIS A vs D
TF	-2.728978	NA	-1.527566	-5.445027	NA	NA	1.721869	NA	NA	NA	NA
TTN	NA	NA	NA	NA	NA	-1.706082	-2.208005	-1.770109	NA	NA	1.258925

1035 **5.5 Heatmaps**

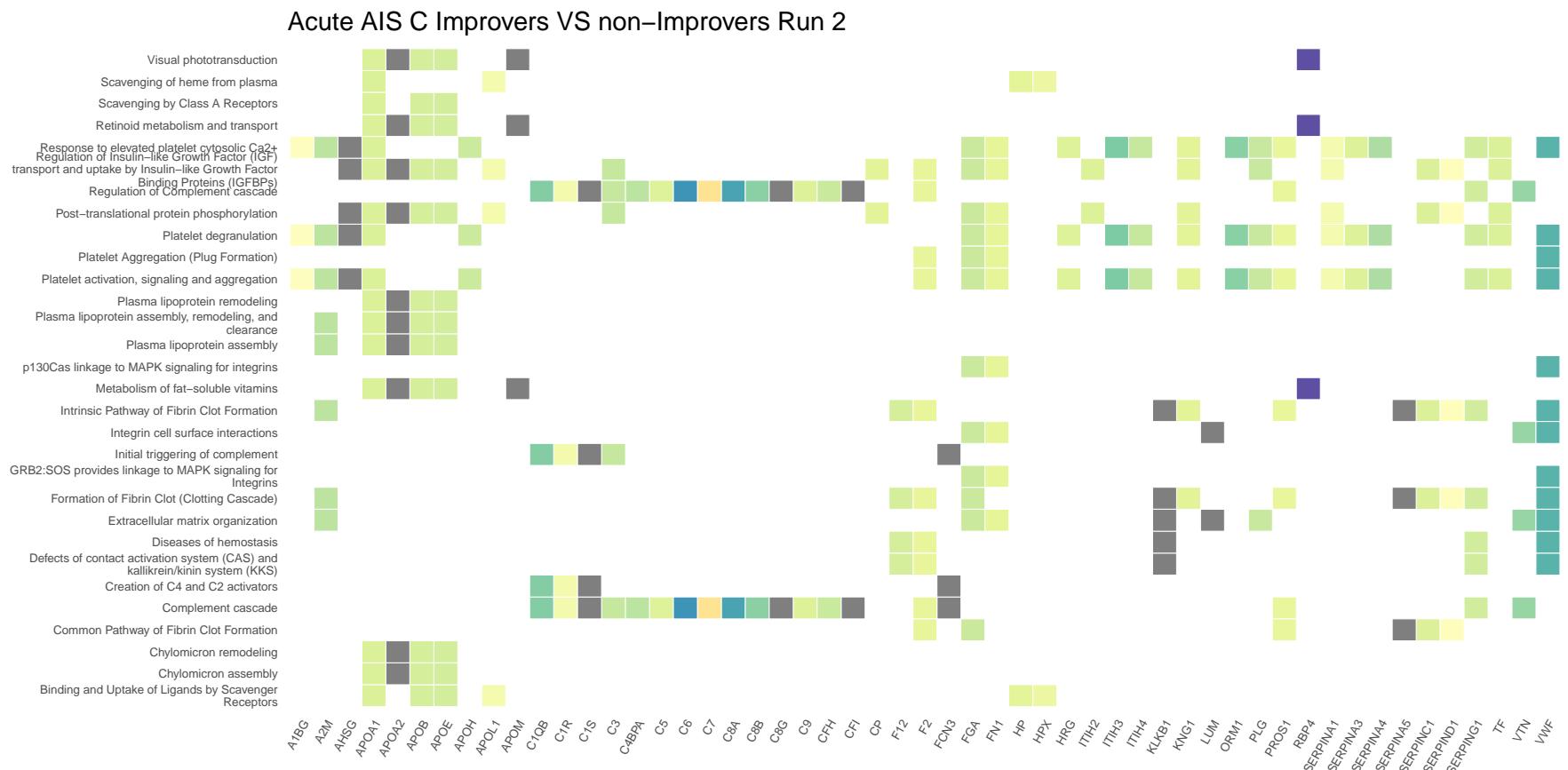
1036 **5.5.1 iTRAQ data**



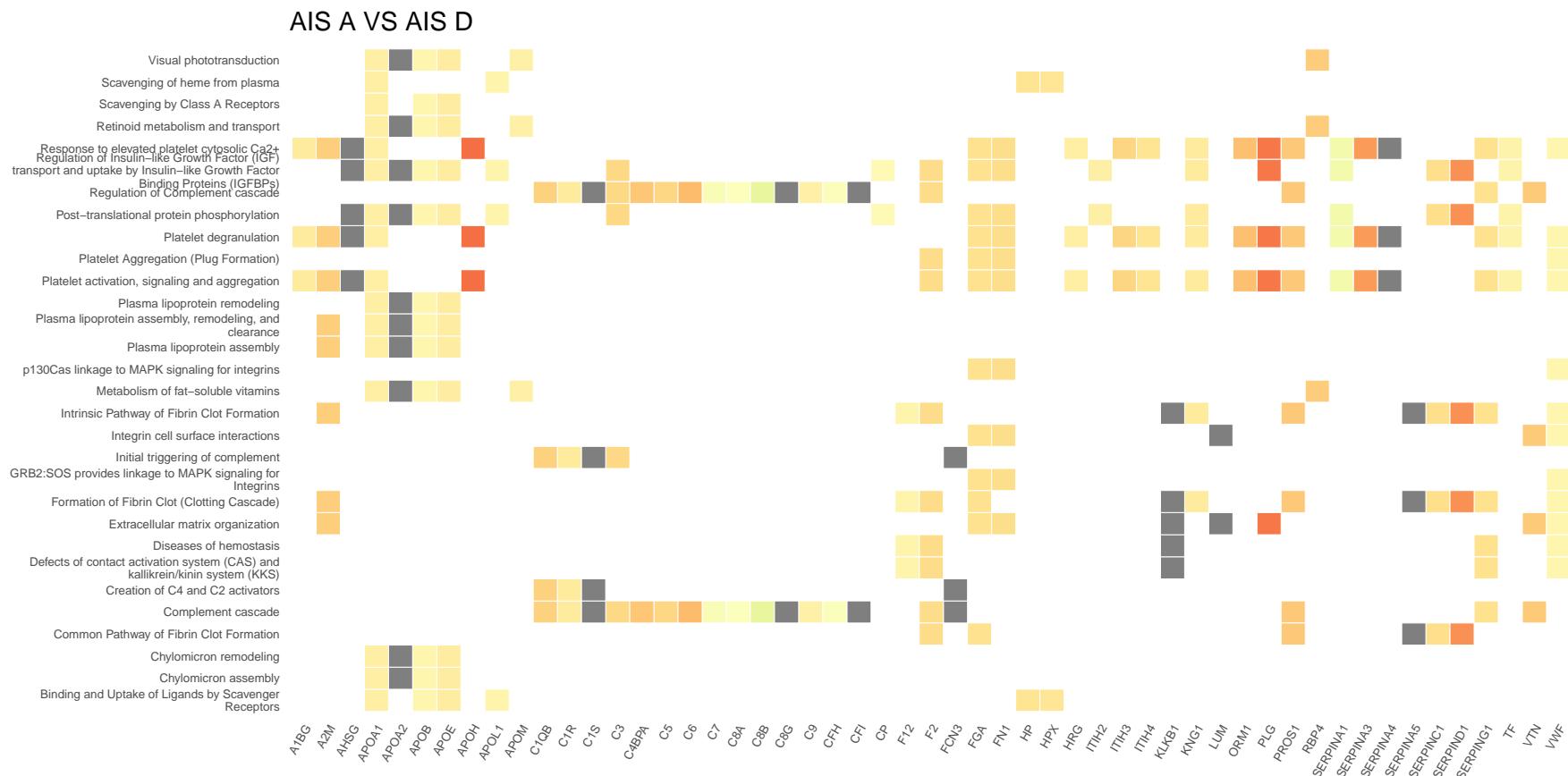
**Figure S1.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement at 2-weeks and 3-months post-injury.



**Figure S2.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement at 2-weeks and 3-months post-injury.



**Figure S3.** Heatmap denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not from the second 4-plex iTRAQ experiment.

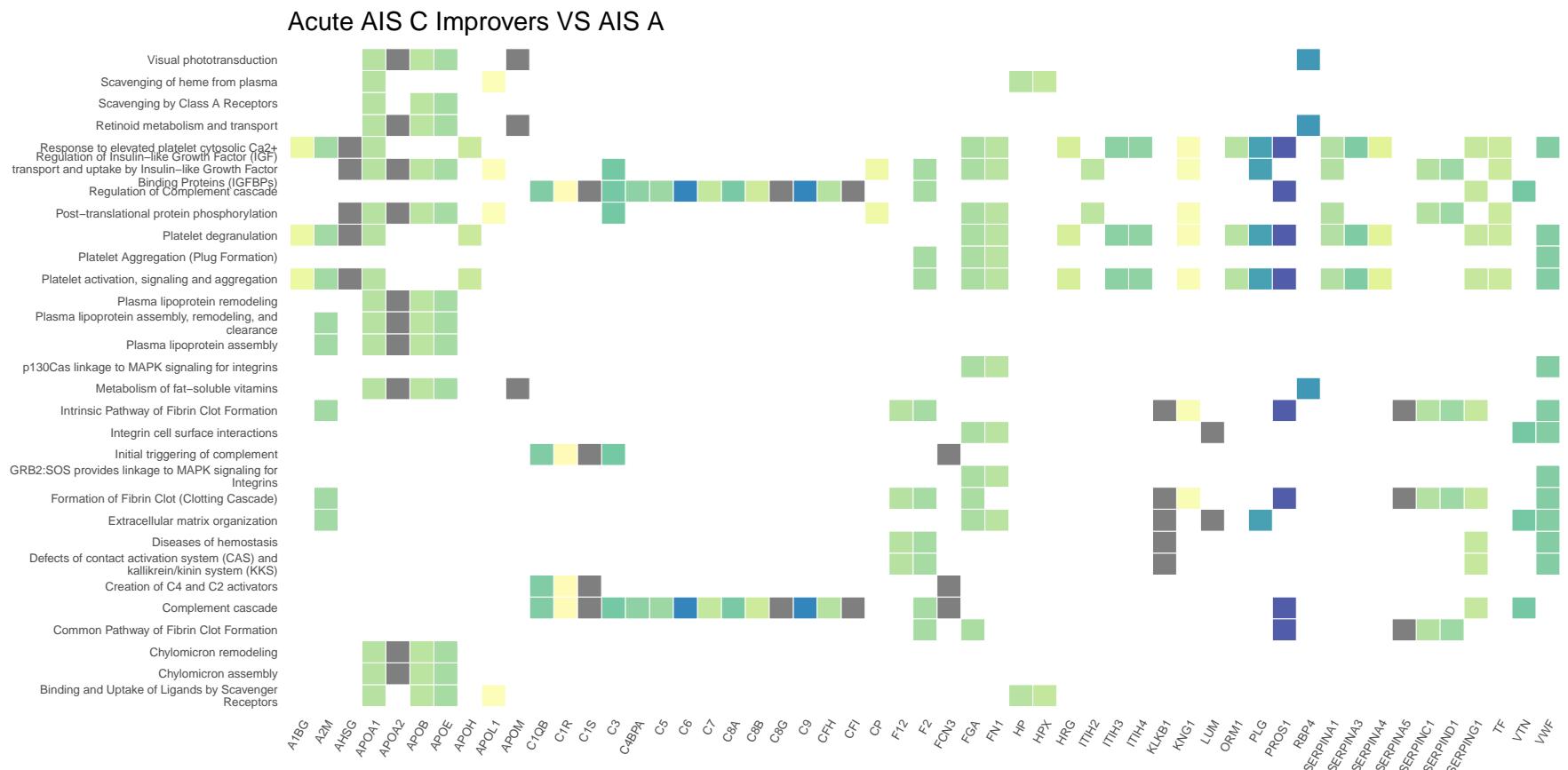


**Figure S4.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS A and AIS D SCI patients.

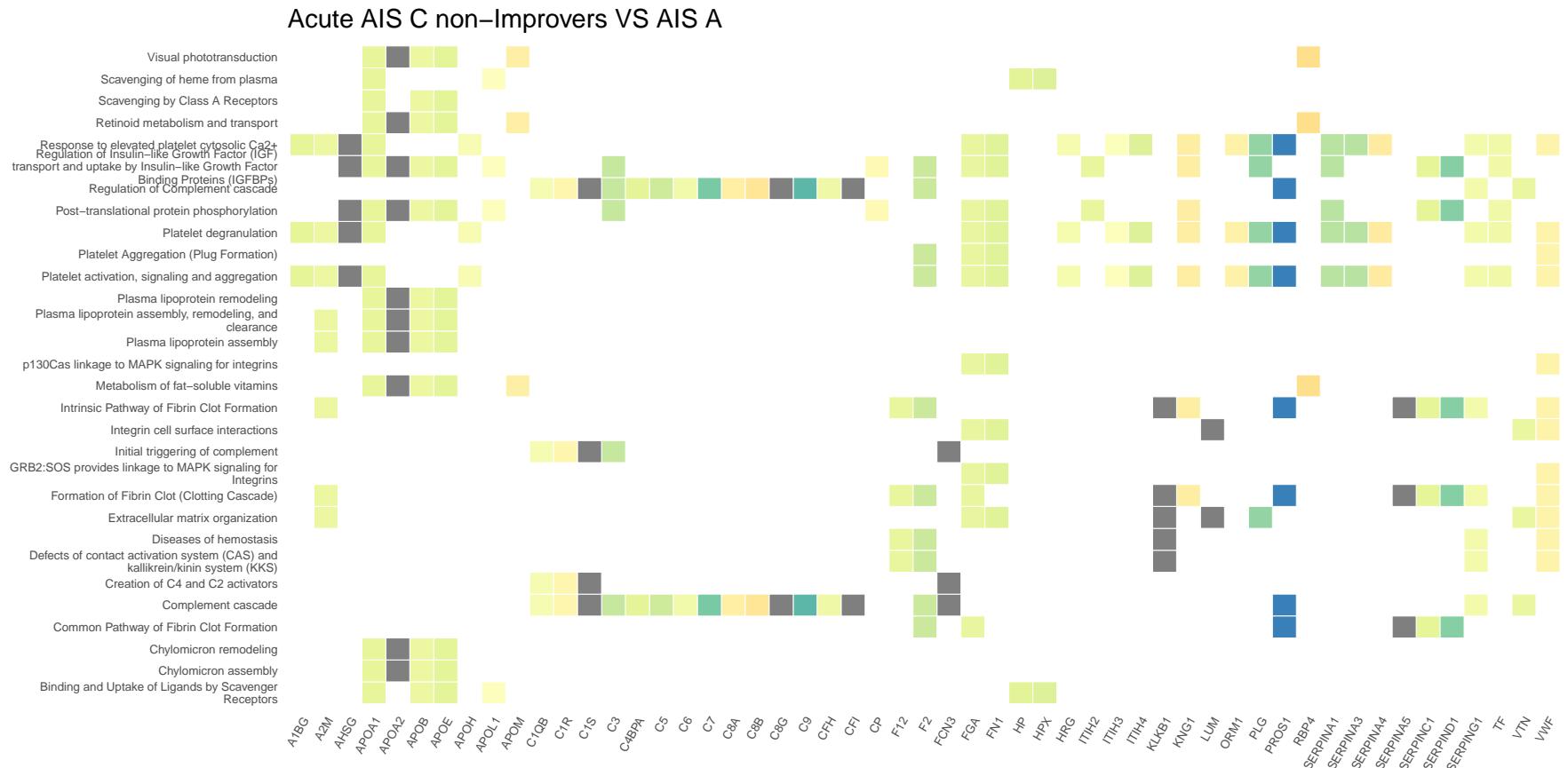
### Acute AIS C Improvers VS AIS D



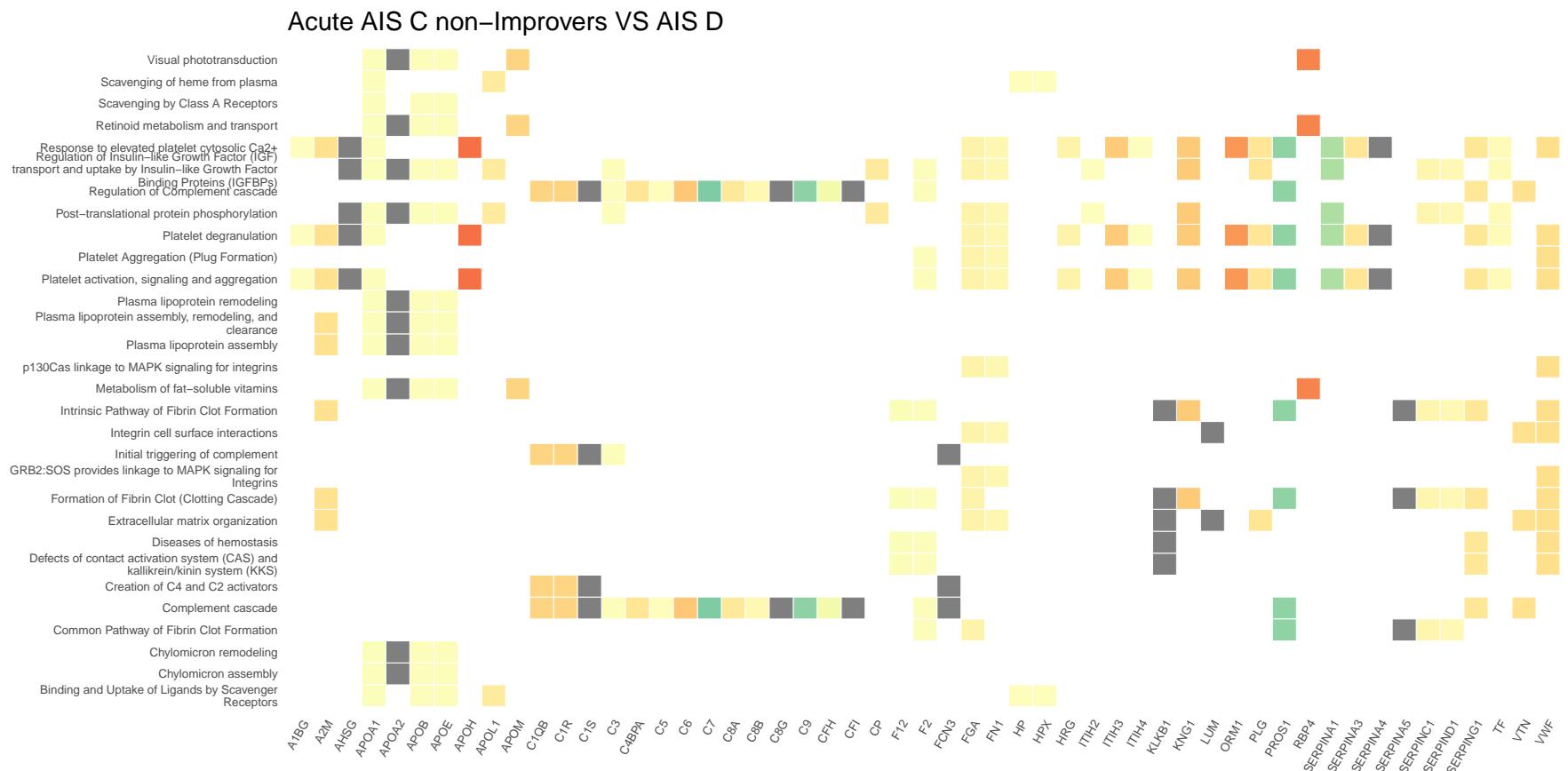
**Figure S5.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS D patients.



**Figure S6.** Heatmap denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS A patients.

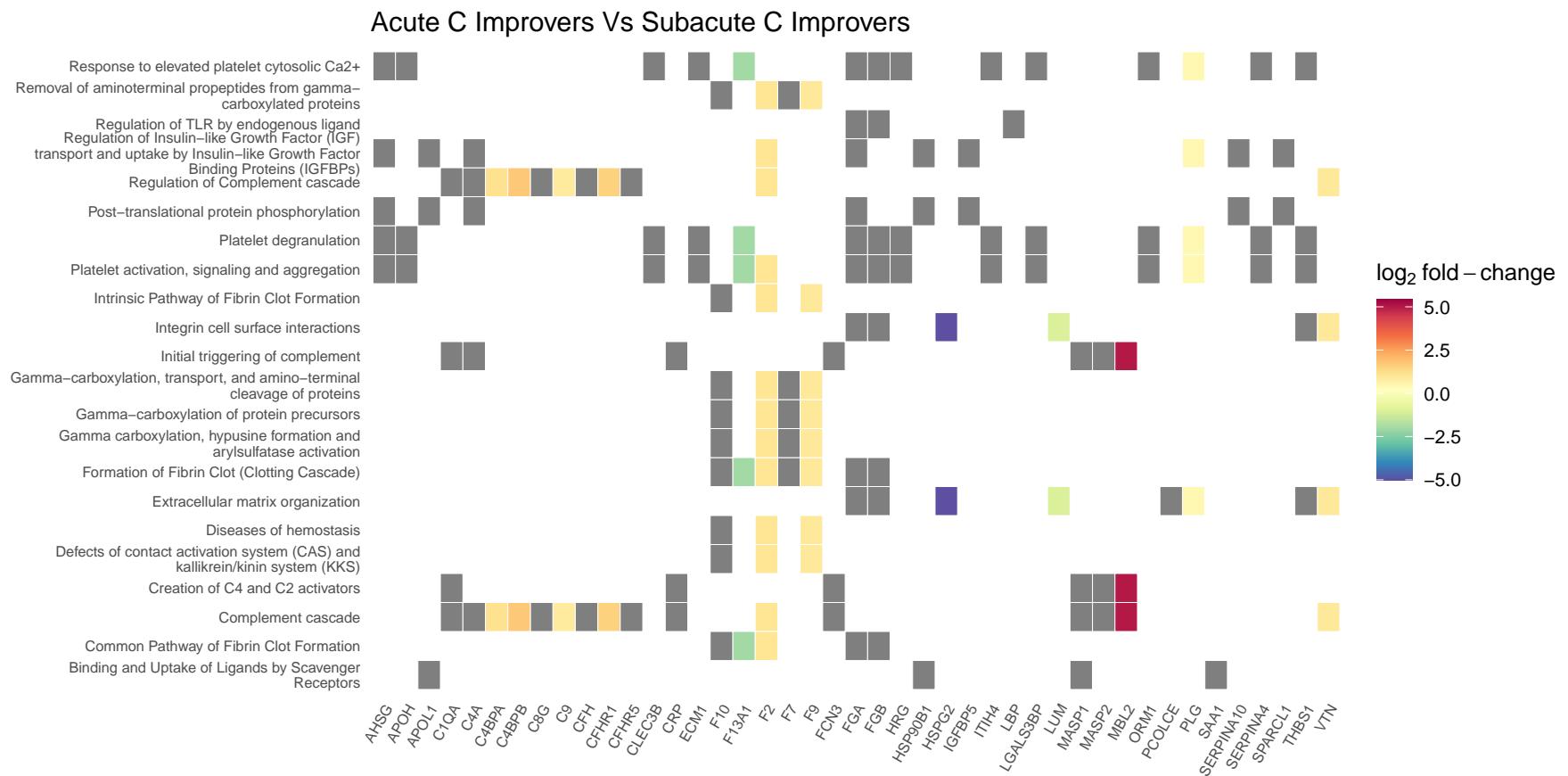


**Figure S7.** Heatmap denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS A patients.

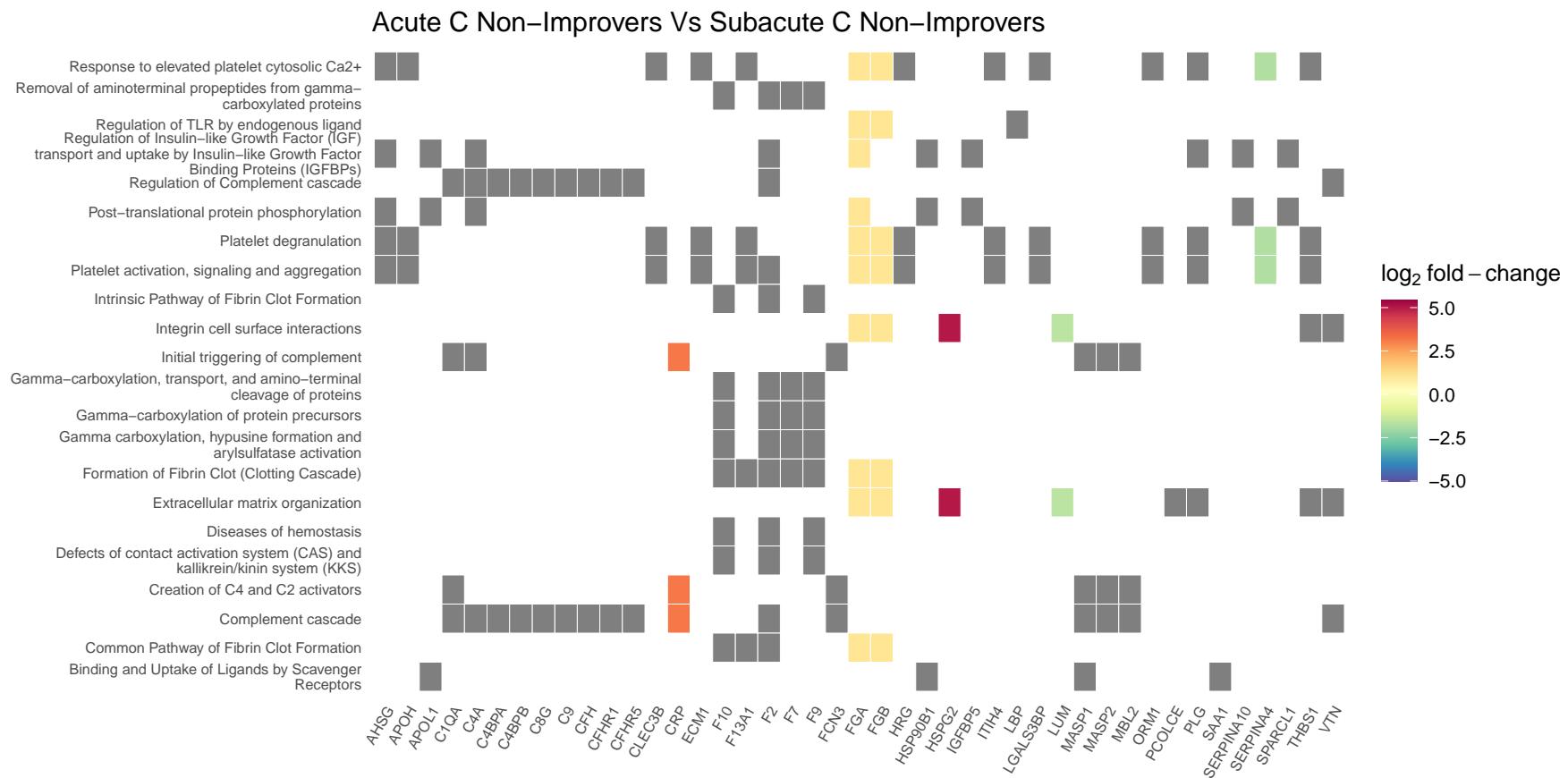


**Figure S8.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS D patients.

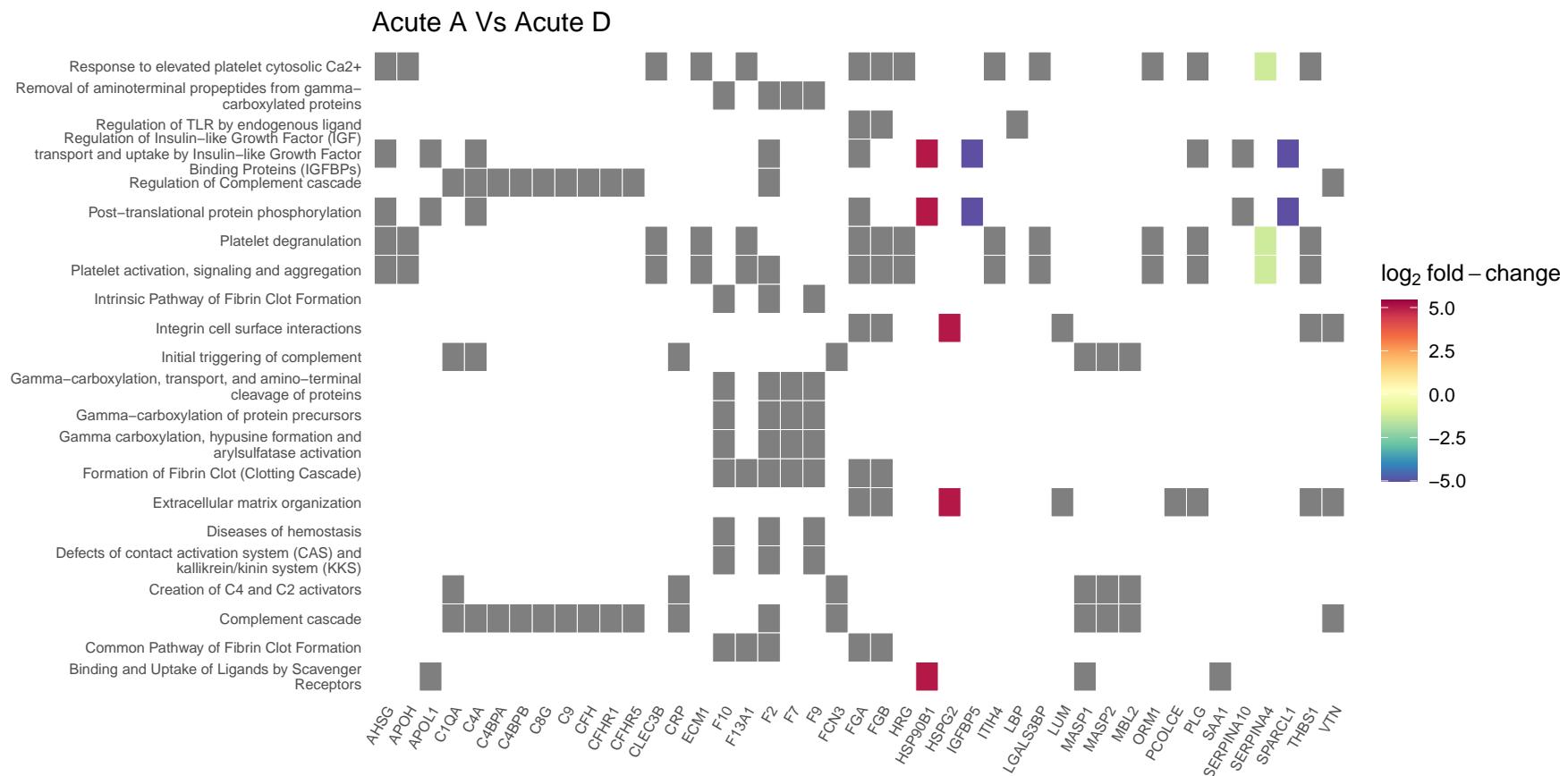




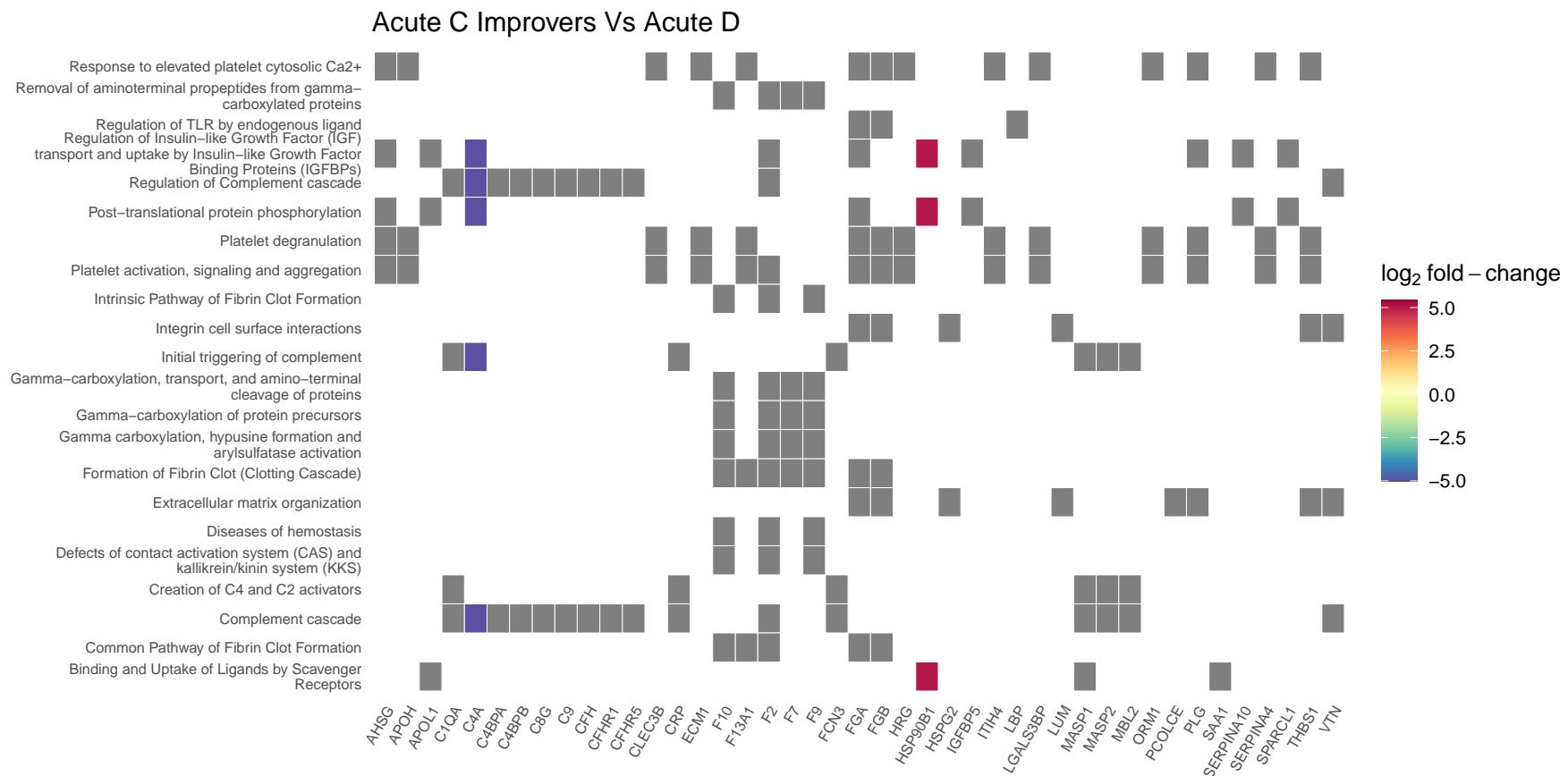
**Figure S9.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement at 2-weeks and 3-months post-injury. Grey blocks denote proteins not present in the comparison.



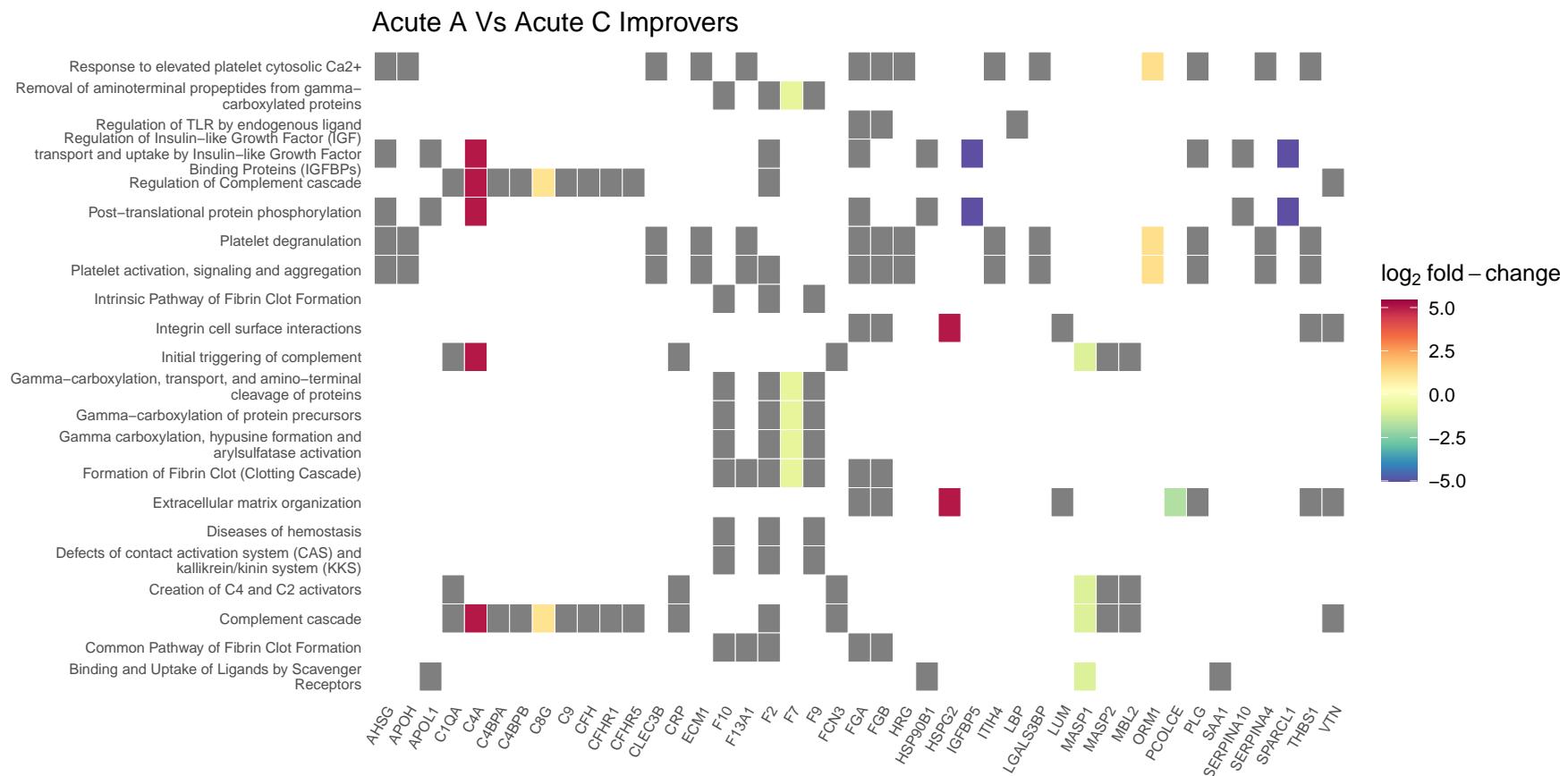
**Figure S10.** Heatmap denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement at 2-weeks and 3-months post-injury. Grey blocks denote proteins not present in the comparison.



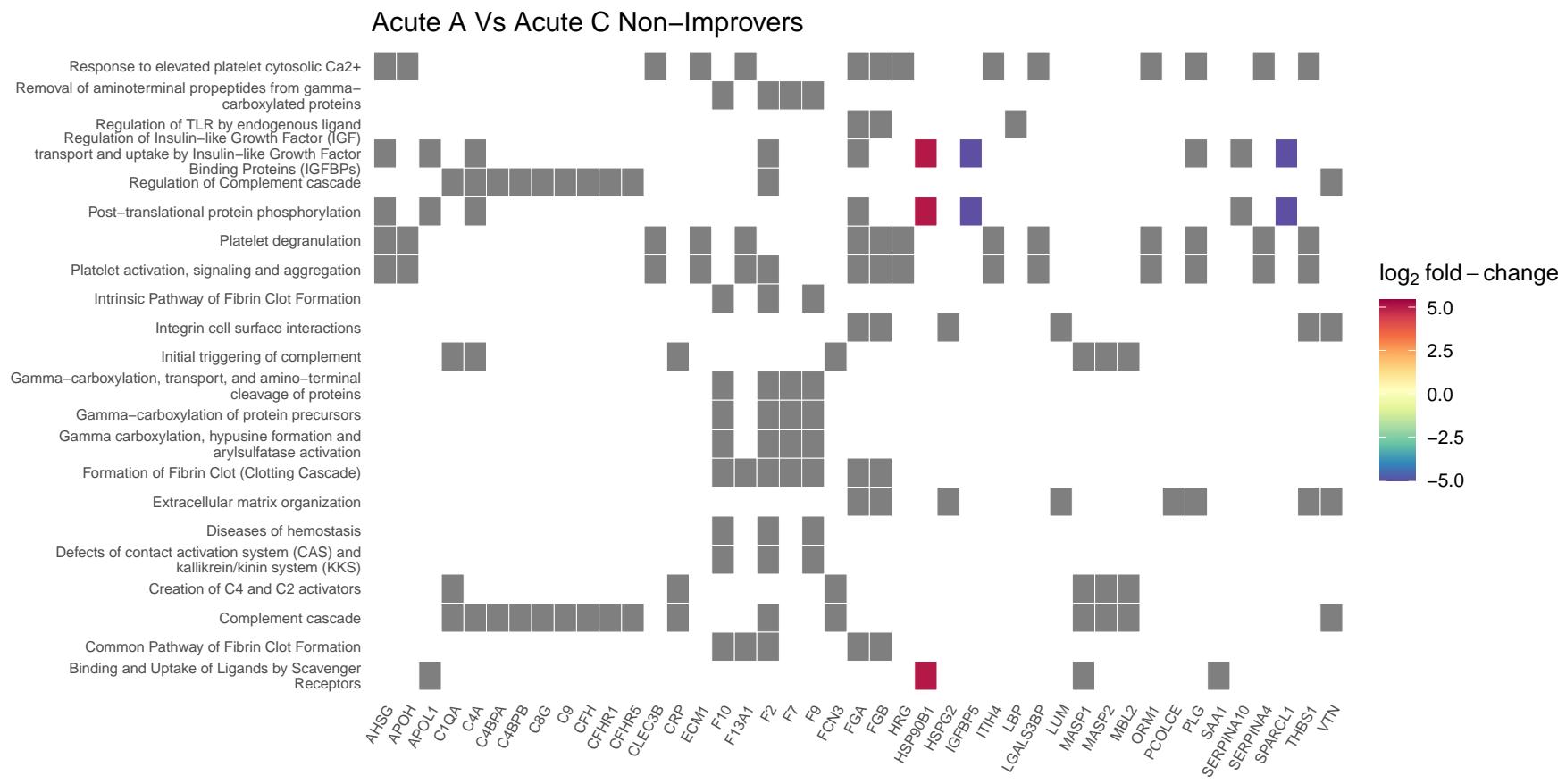
**Figure S11.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS A and AIS D SCI patients. Grey blocks denote proteins not present in the comparison.



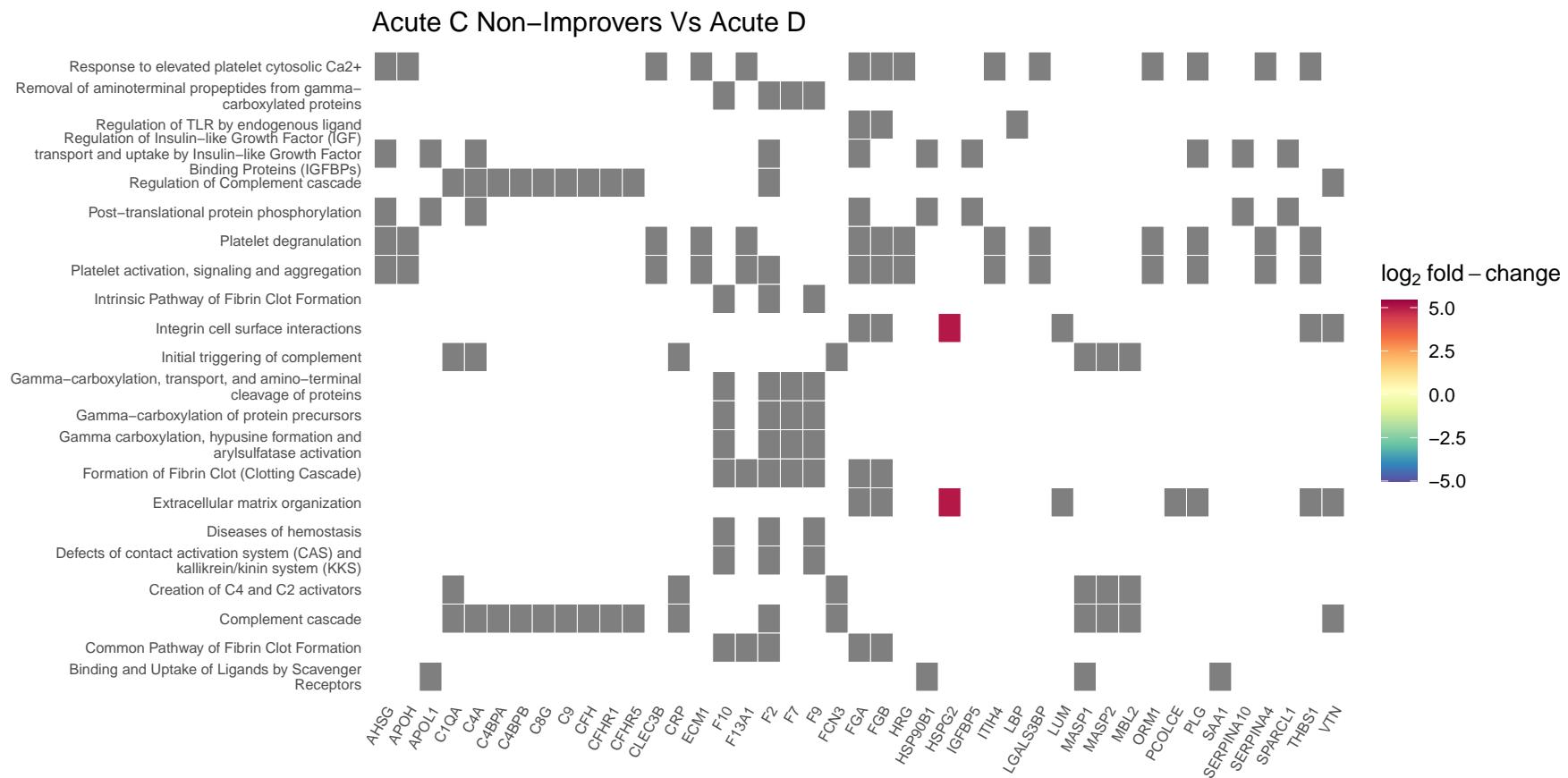
**Figure S12.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS D patients. Grey blocks denote proteins not present in the comparison.



**Figure S13.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS A patients. Grey blocks denote proteins not present in the comparison.



**Figure S14.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS A patients. Grey blocks denote proteins not present in the comparison.

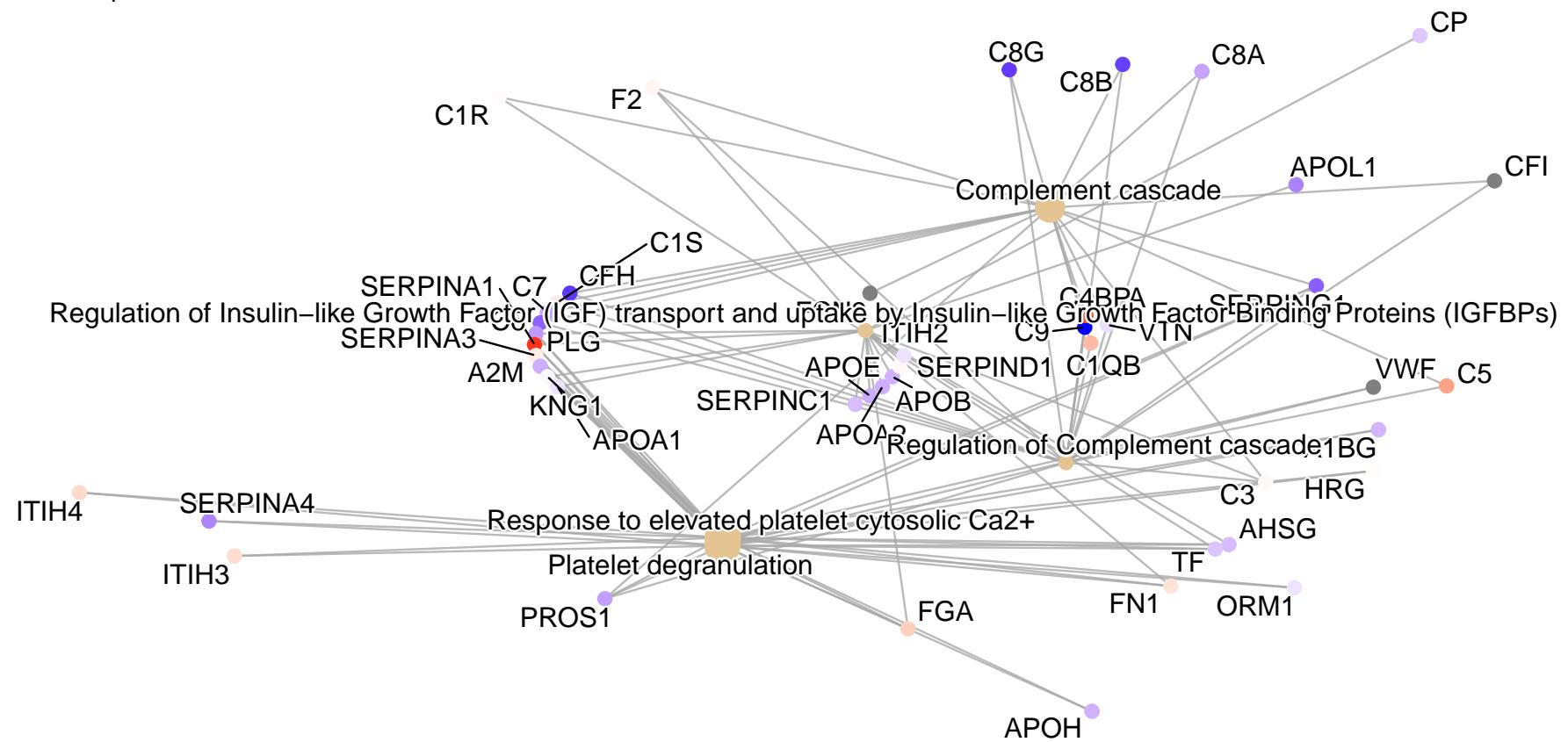


**Figure S15.** Heatmap denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS D patients. Grey blocks denote proteins not present in the comparison.

<sup>1038</sup> **5.6 Cnetplots**

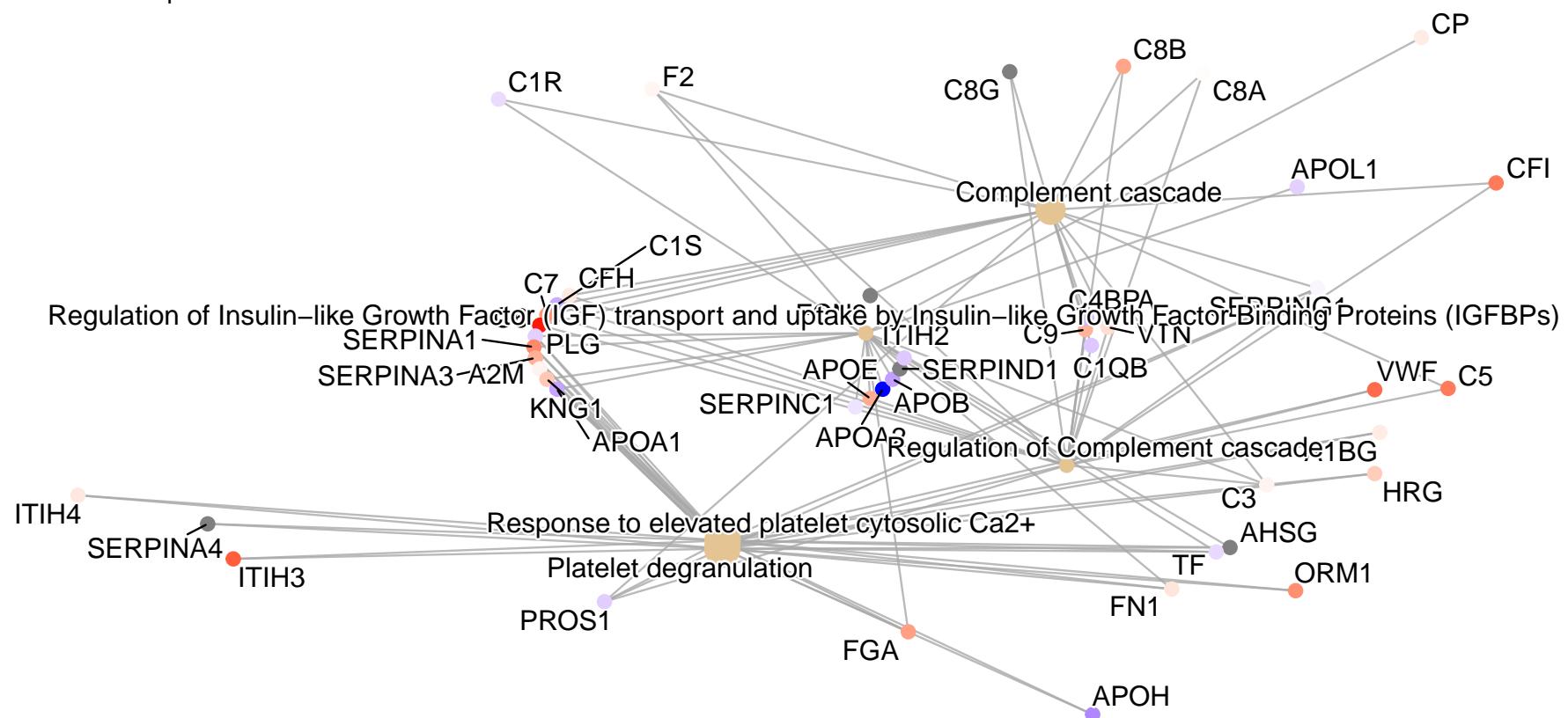
<sup>1039</sup> **5.6.1 iTRAQ data**

AIS C Improvers acute vs subacute



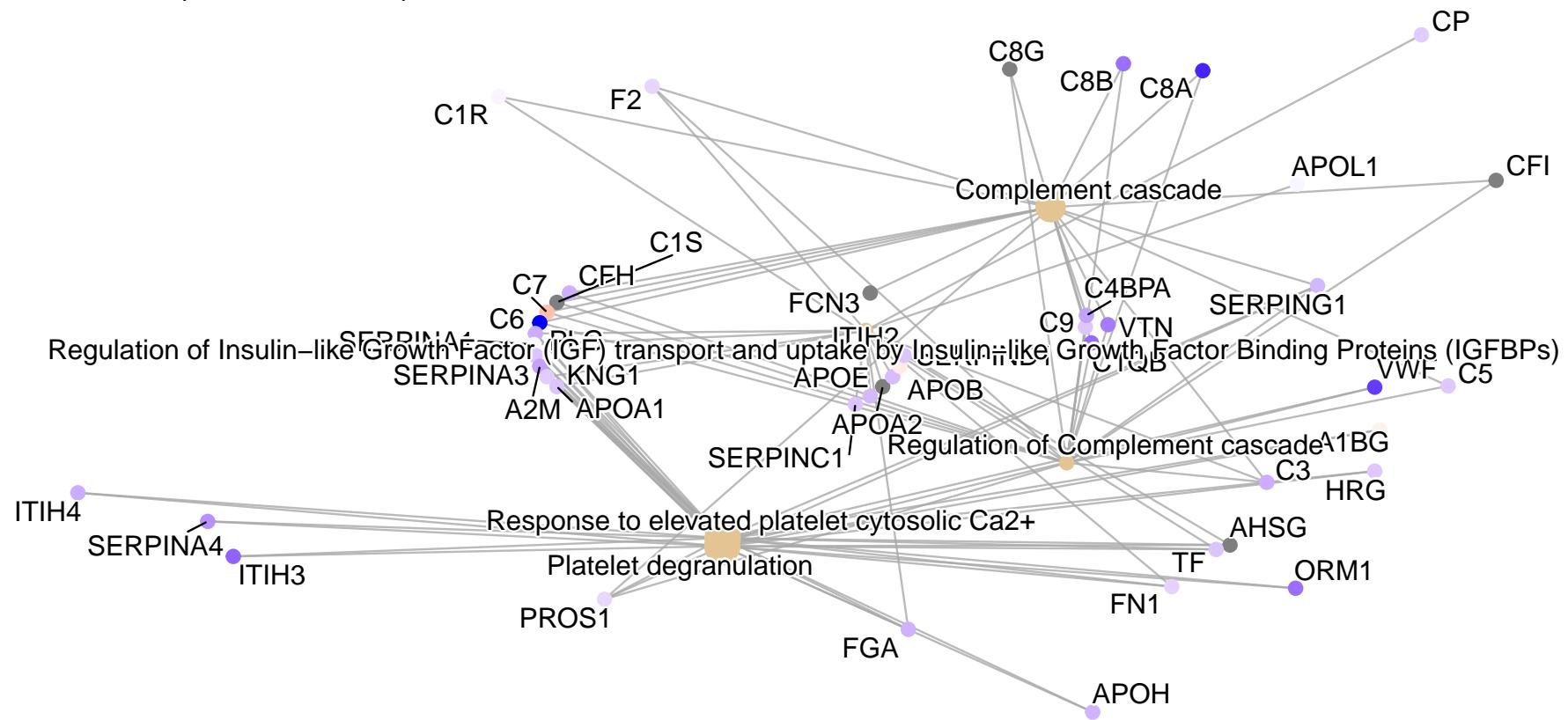
**Figure S16.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement at 2-weeks and 3-months post-injury.

AIS C non-Improvers acute vs subacute



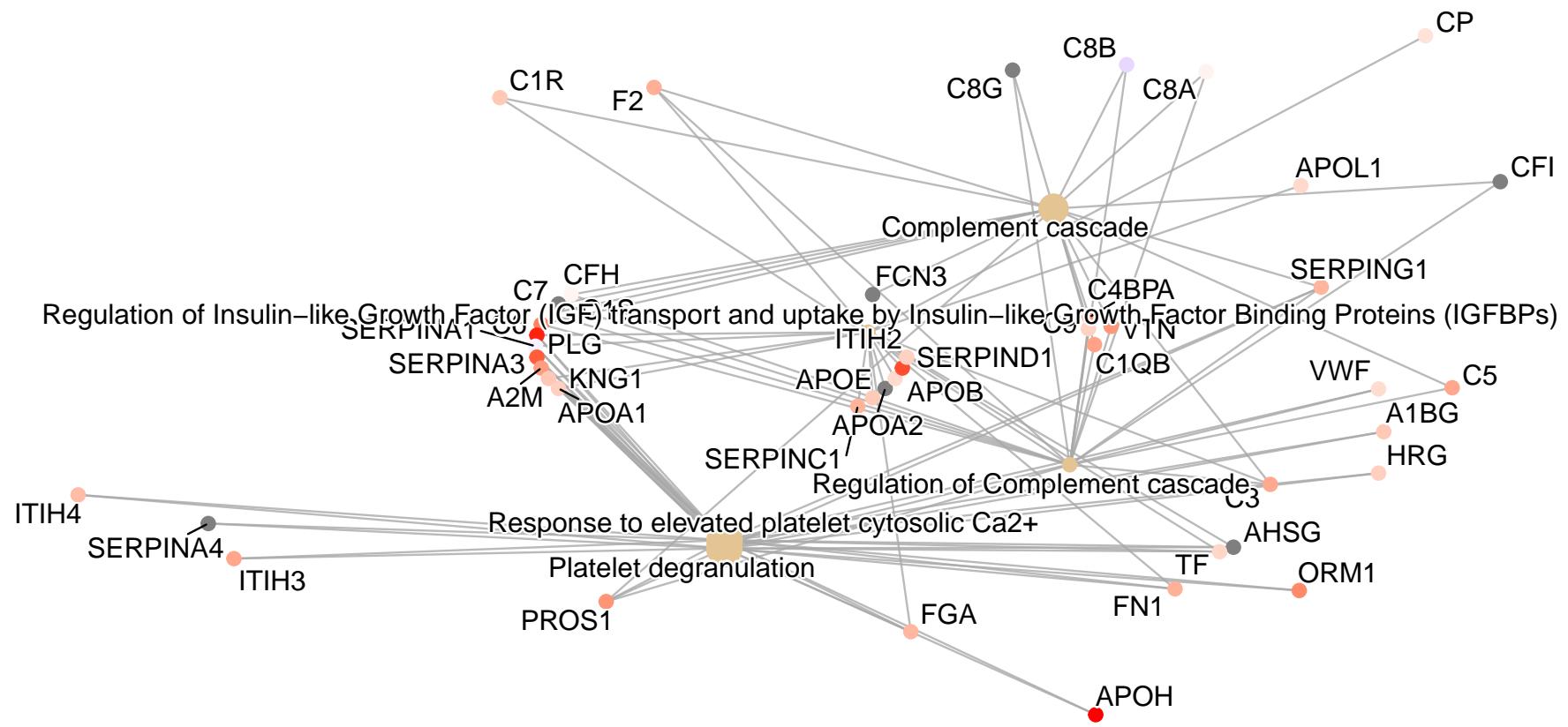
**Figure S17.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement at 2-weeks and 3-months post-injury.

Acute AIS C Improvers VS non-Improvers Run 2



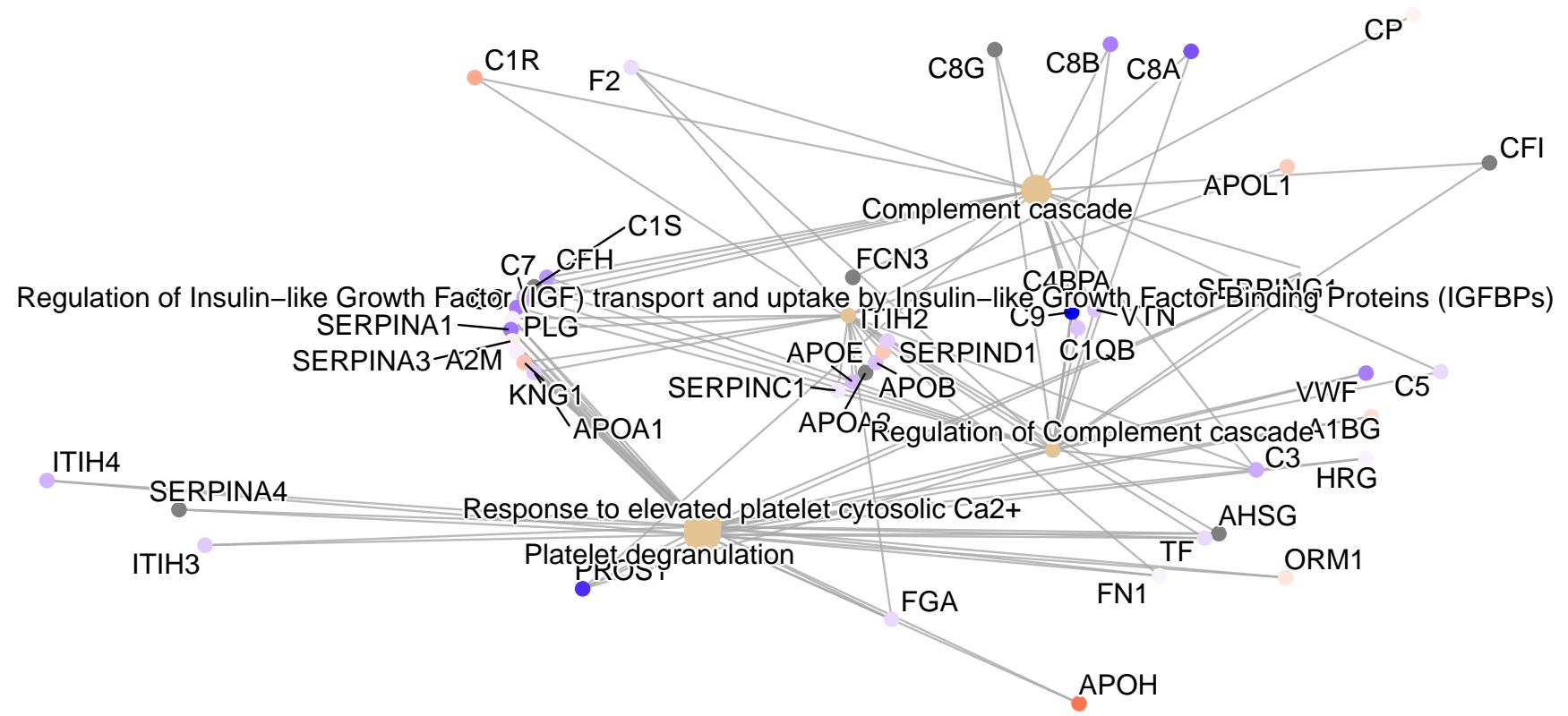
**Figure S18.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and those who did not from the second 4-plex iTRAQ experiment.

AIS A VS AIS D



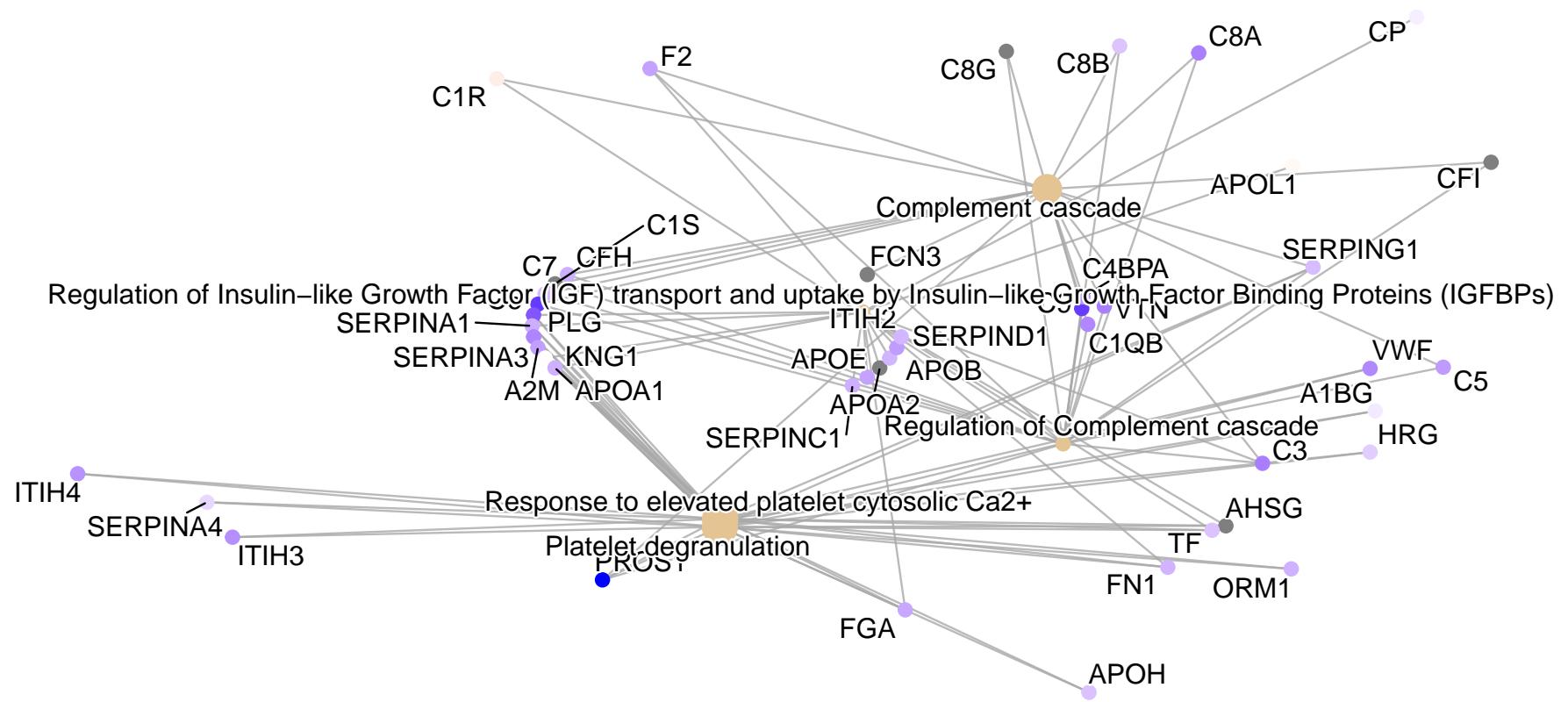
**Figure S19.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS A and AIS D SCI patients.

### Acute AIS C Improvers VS AIS D



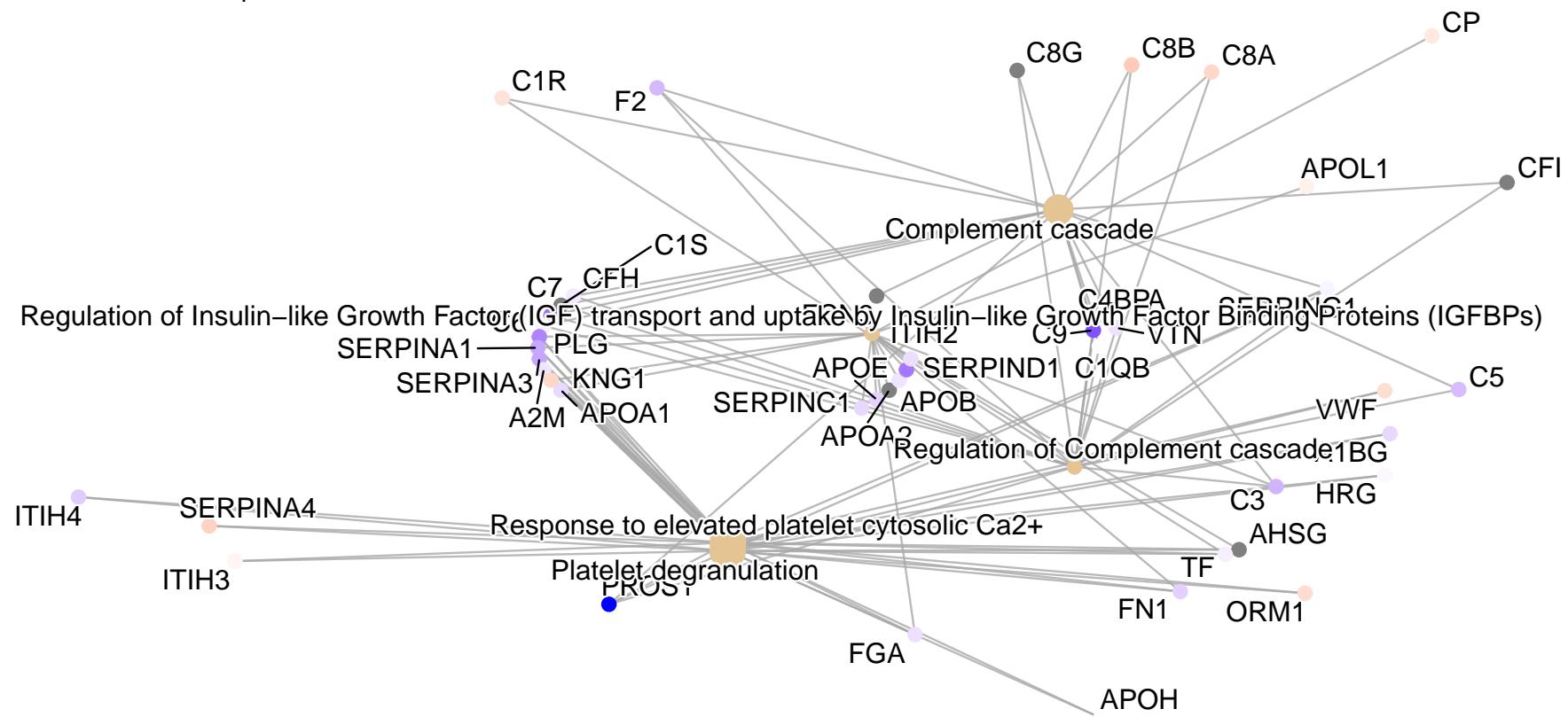
**Figure S20.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS D patients.

### Acute AIS C Improvers VS AIS A



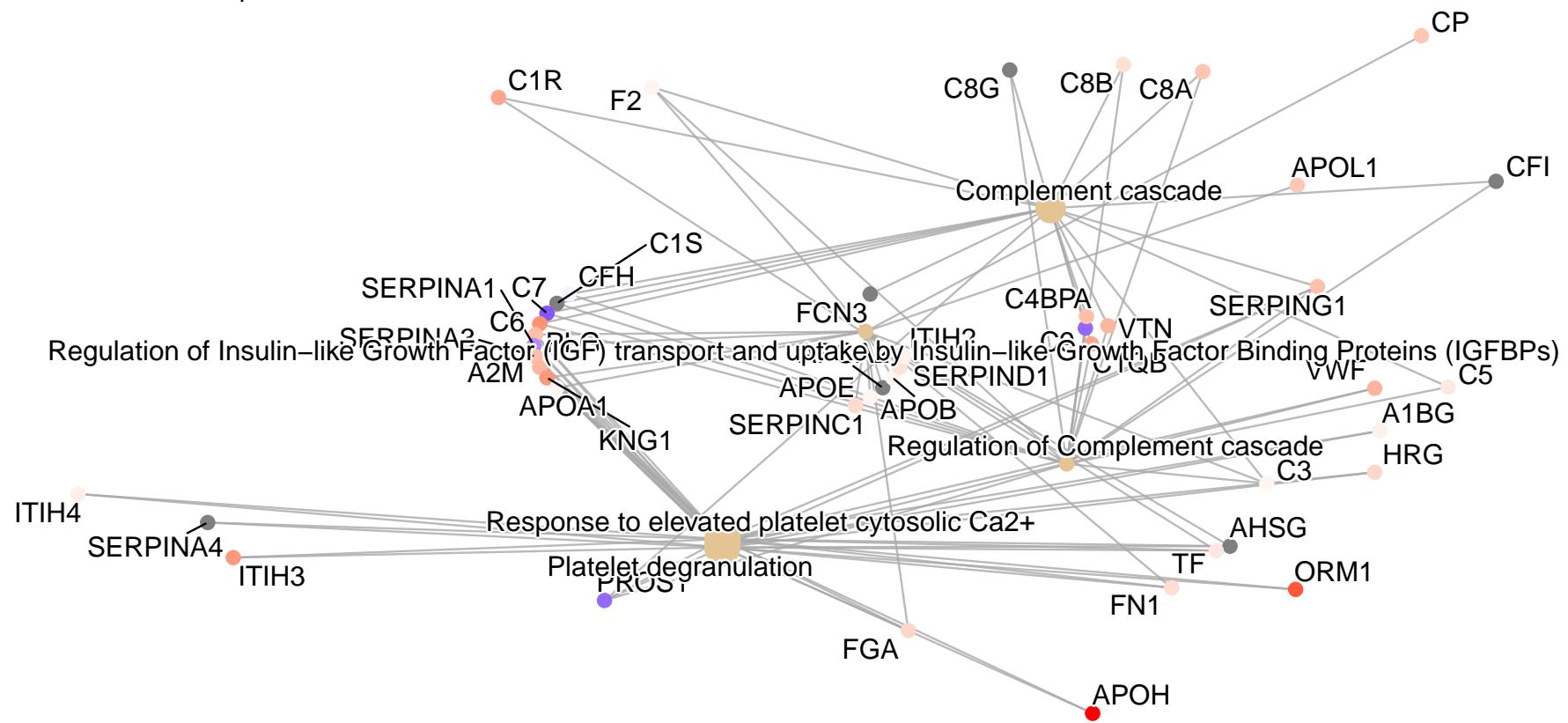
**Figure S21.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS A patients.

Acute AIS C non-Improvers VS AIS A



**Figure S22.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS A patients.

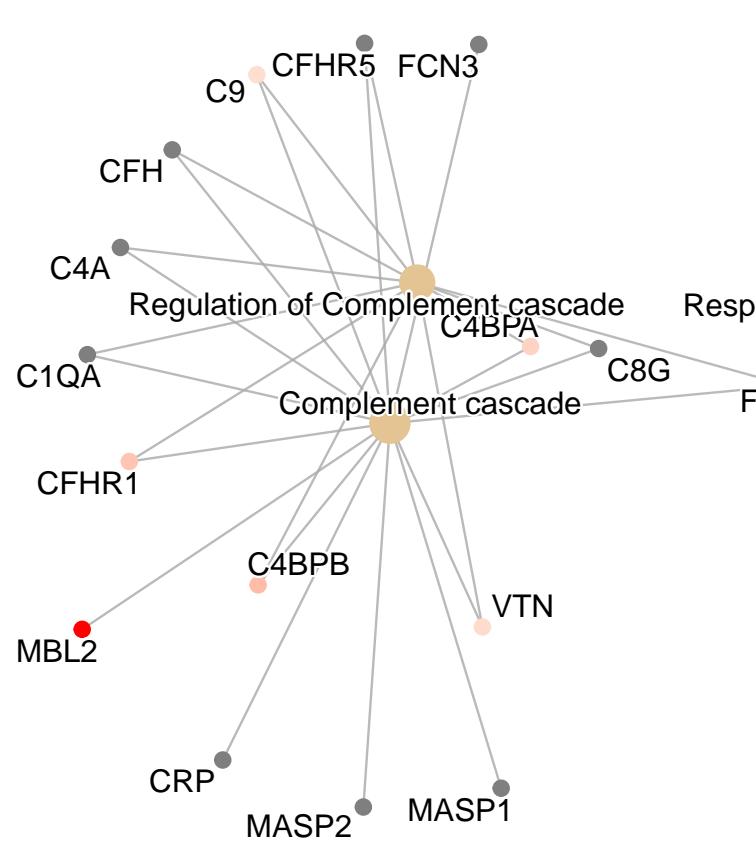
Acute AIS C non-Improvers VS AIS D



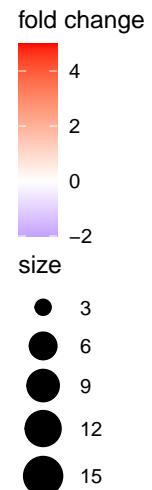
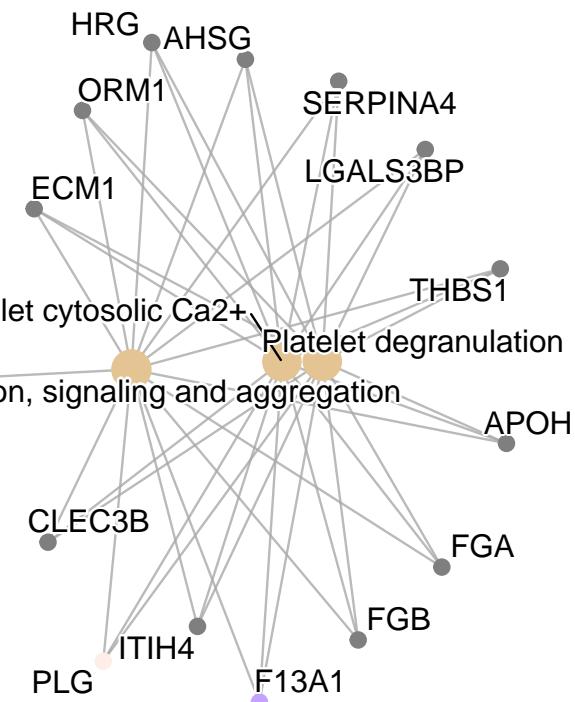
**Figure S23.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS D patients.



### Acute C Improvers Vs Subacute C Improvers

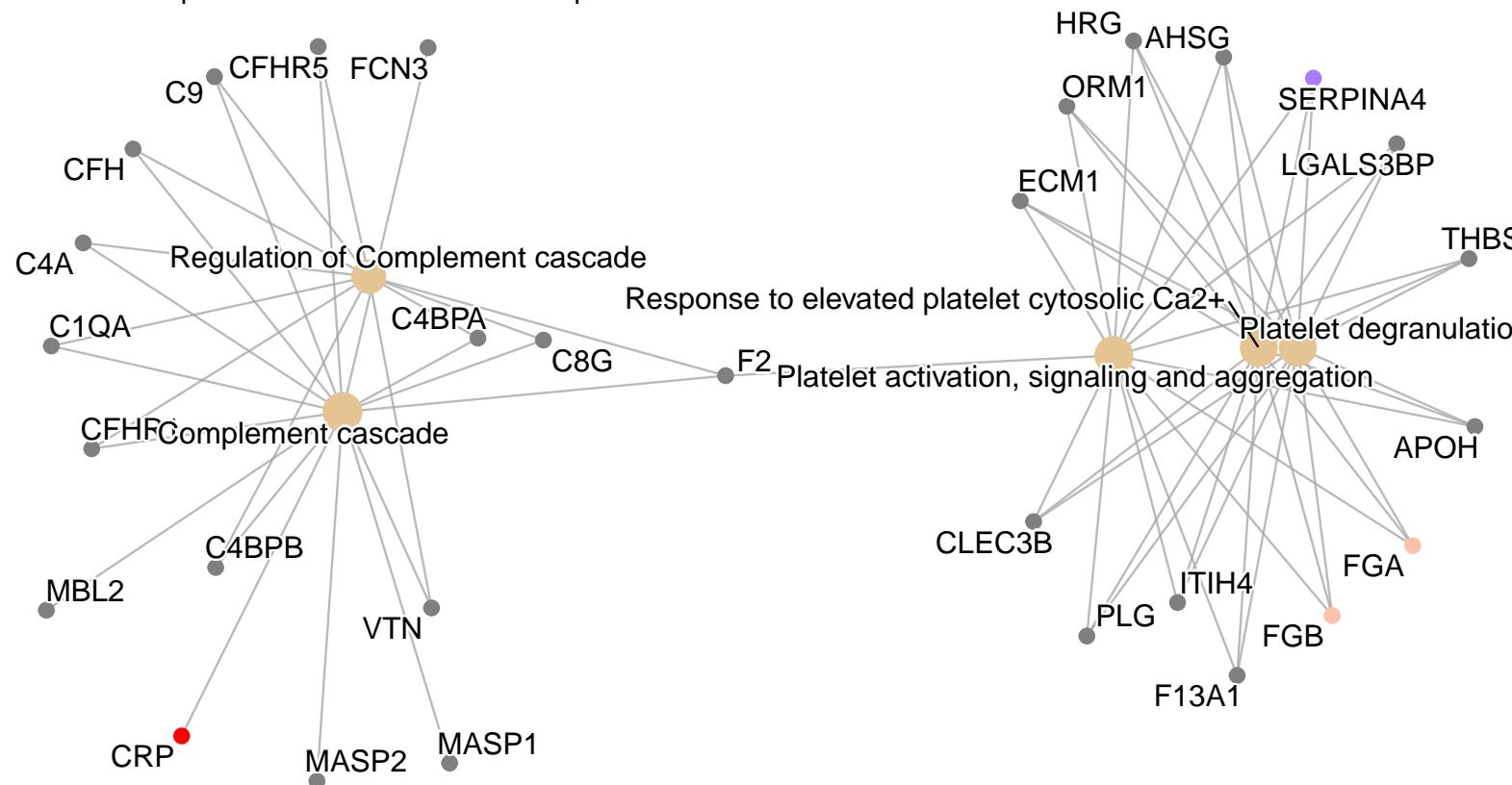


Regulation of Complement cascade  
Complement cascade  
Response to elevated platelet cytosolic Ca<sup>2+</sup>  
Platelet activation, signaling and aggregation



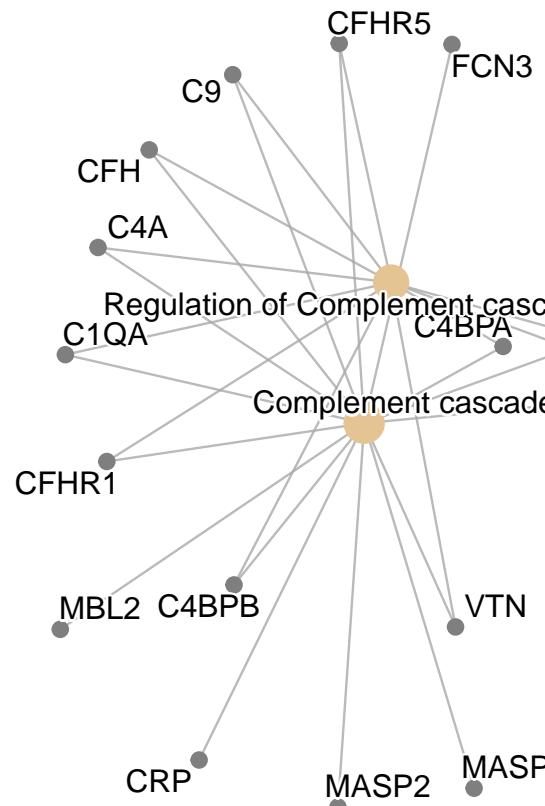
**Figure S24.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement at 2-weeks and 3-months post-injury.

### Acute C Non-Improvers Vs Subacute C Non-Improvers



**Figure S25.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks and 3-months post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement at 2-weeks and 3-months post-injury.

Acute A Vs Acute D



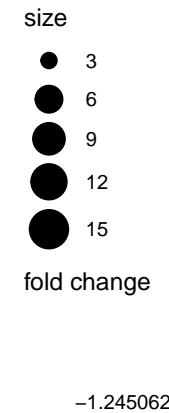
Complement cascade

Regulation of Complement cascade

Response to elevated platelet cytosolic Ca<sup>2+</sup>

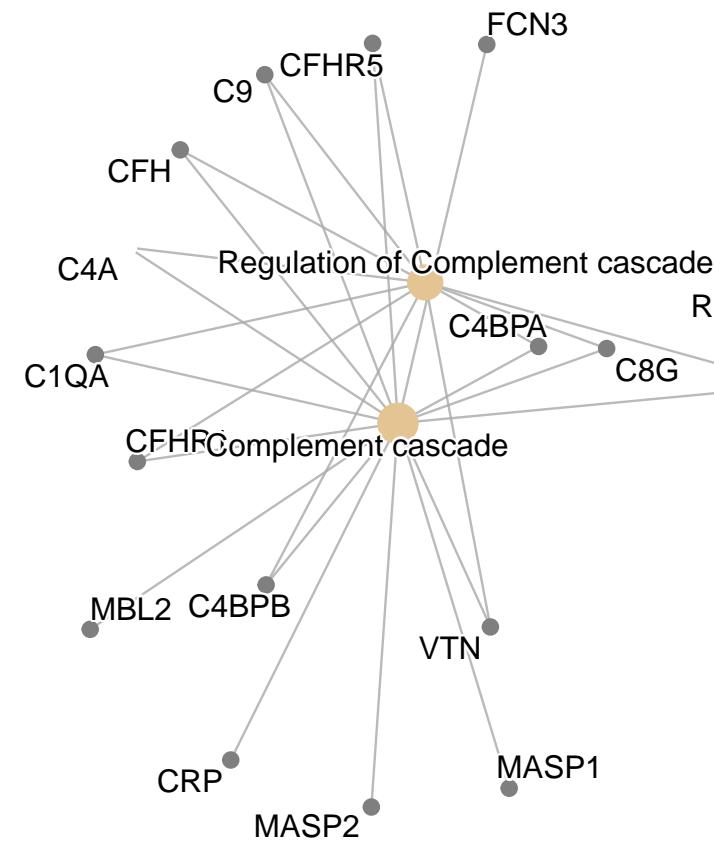
Platelet activation, signalling and aggregation

Platelet degranulation

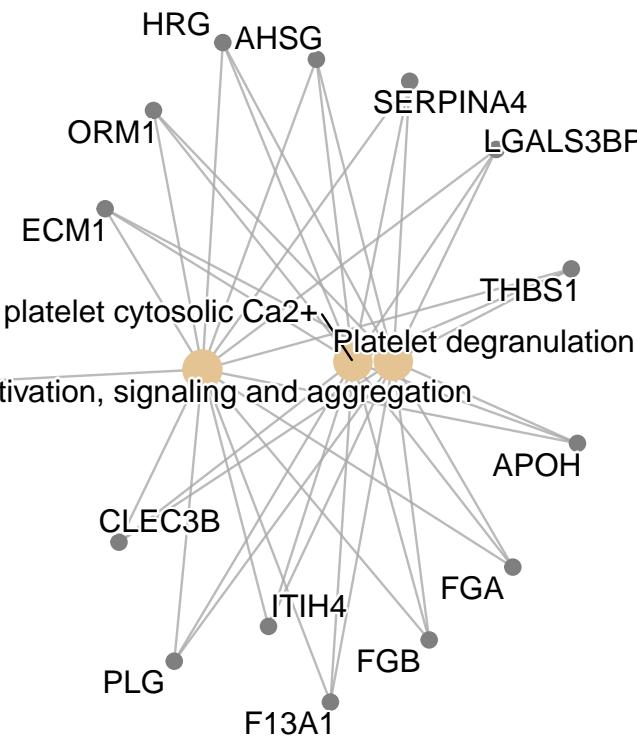


**Figure S26.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS A and AIS D SCI patients.

Acute C Improvers Vs Acute D

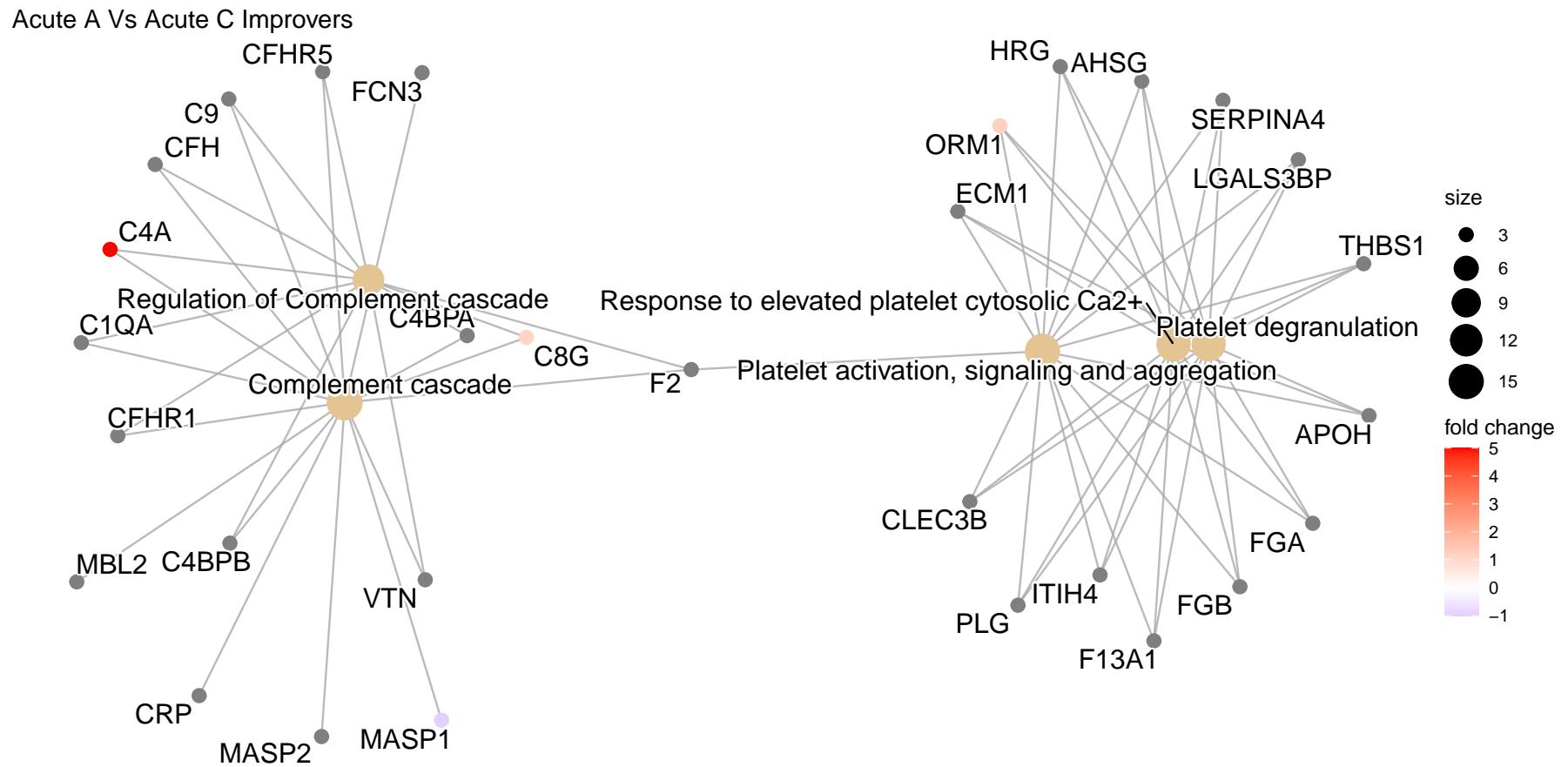


Regulation of Complement cascade  
Complement cascade



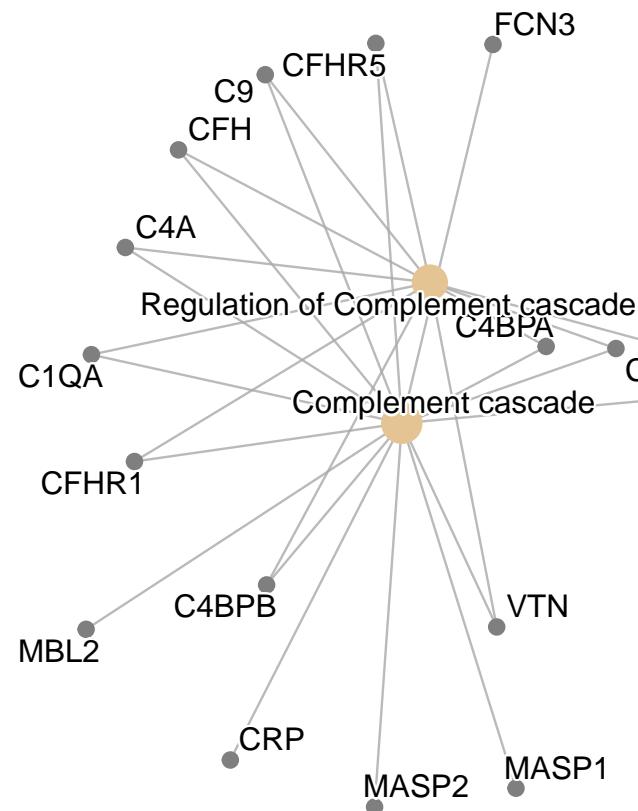
size  
● 3  
● 6  
● 9  
● 12  
● 15  
fold change  
-5

**Figure S27.** Network plot denoting the  $\log_2$  fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS D patients.

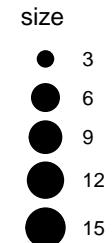
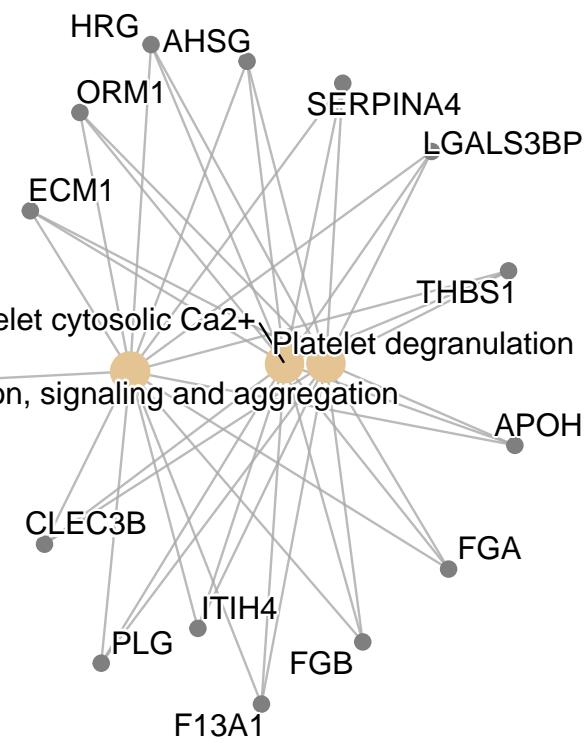


**Figure S28.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who experienced an AIS grade improvement and AIS A patients.

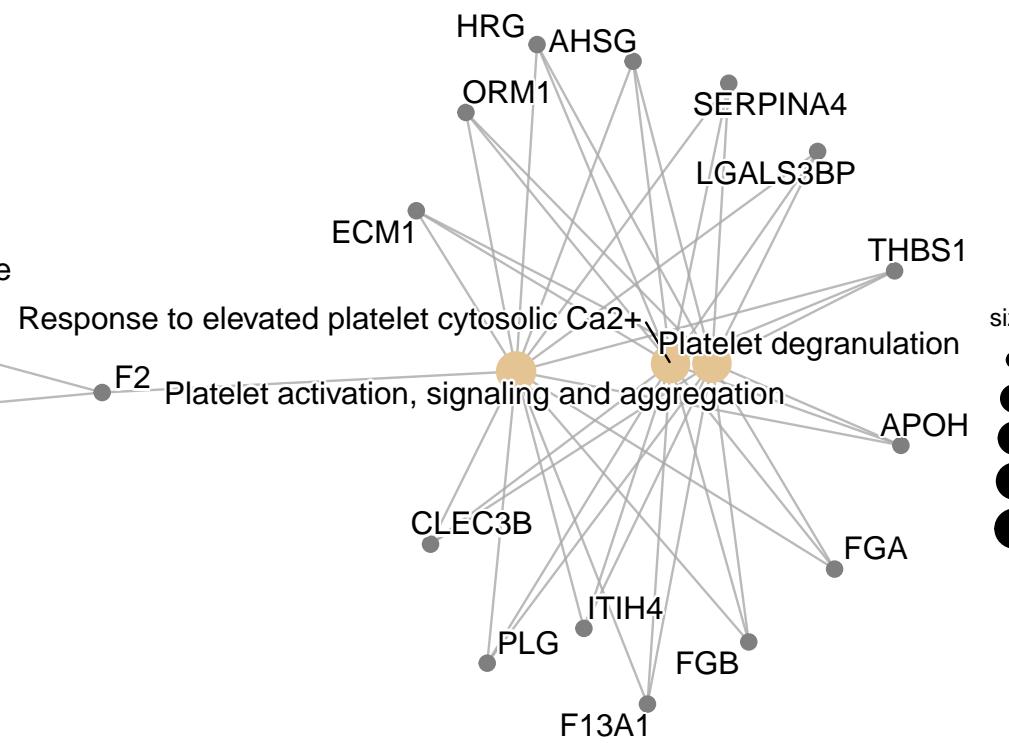
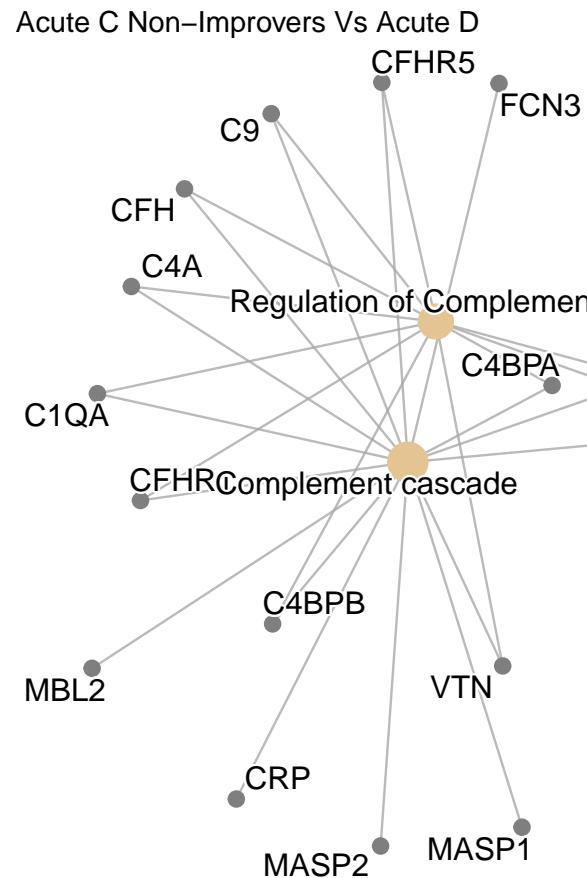
Acute A Vs Acute C Non-Improvers



Regulation of Complement cascade  
Complement cascade  
Response to elevated platelet cytosolic Ca<sup>2+</sup>  
Platelet activation, signaling and aggregation



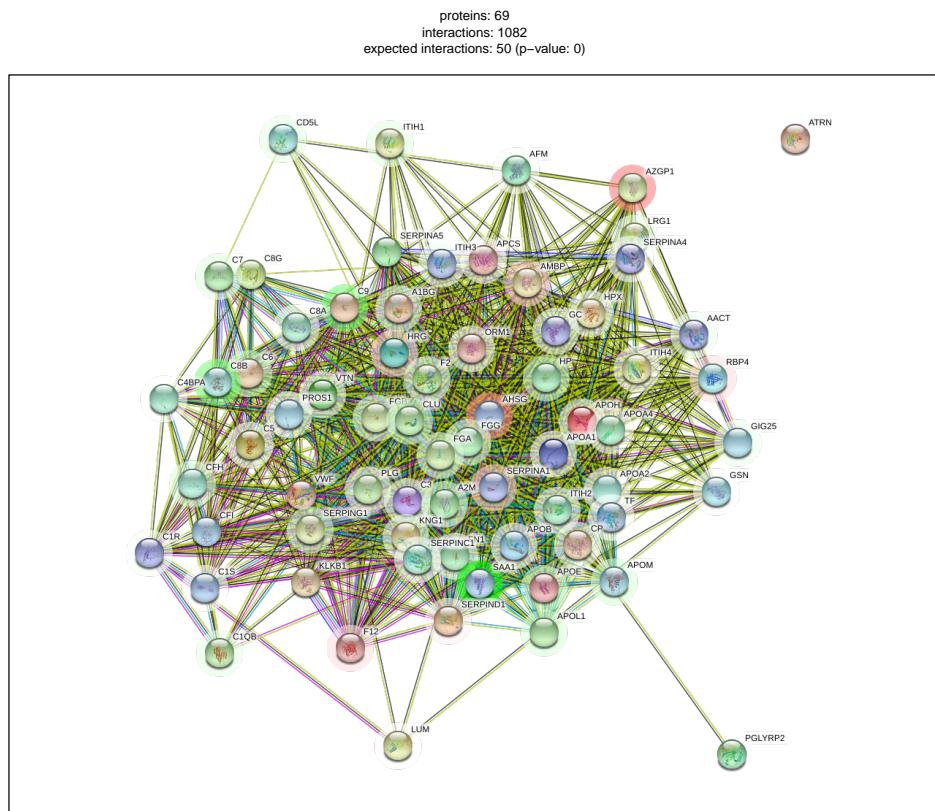
**Figure S29.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS A patients.



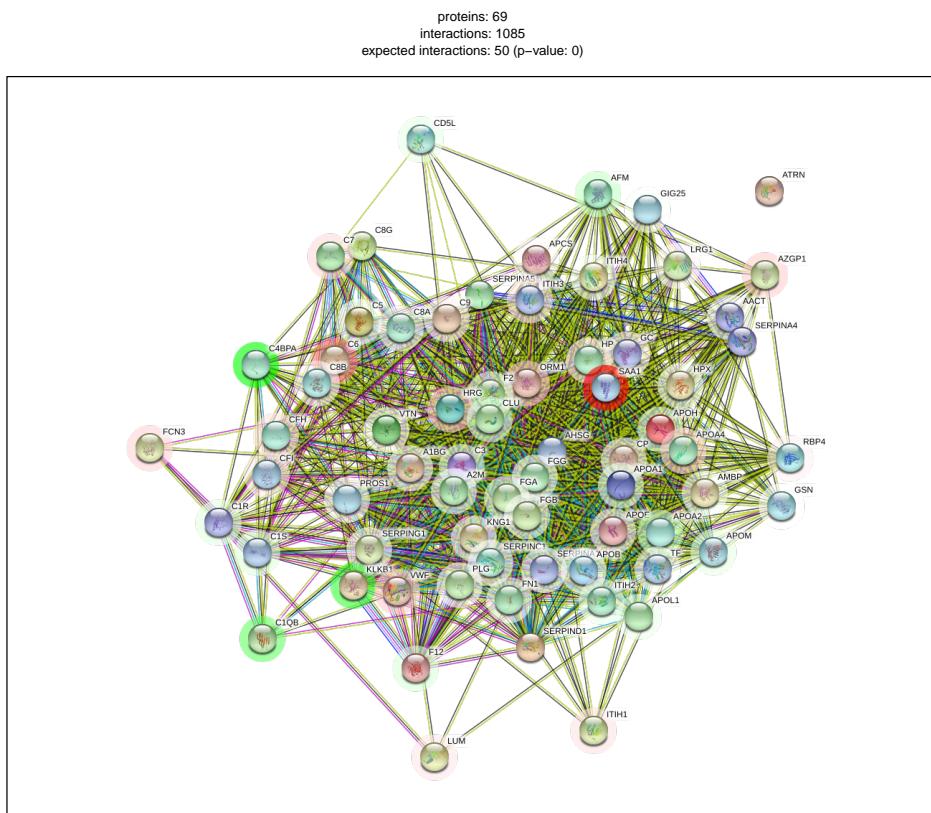
**Figure S30.** Network plot denoting the log<sub>2</sub> fold change of proteins in plasma collected 2-weeks post-injury, and the biological pathways these proteins are associated with on Reactome. This compares AIS C SCI patients who did not experience an AIS grade improvement and AIS D patients.

## 1041 5.7 STRINGdb network plots

## 1042 5.7.1 iTRAQ data

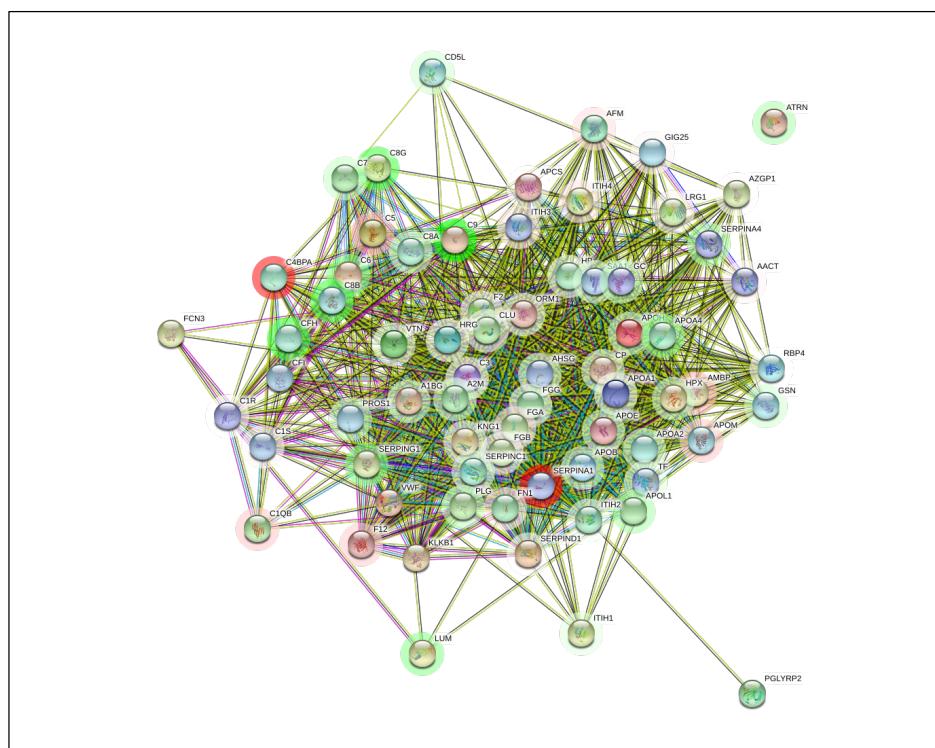


**Figure S31.** The interaction network of differentially abundant proteins found in plasma 2-weeks post-injury, between AIS C patients who experienced an AIS grade conversion and those who did not. The coloured "halo" denotes fold change whereby green indicates that protein is less abundant and red indicates greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood; Others are from gene co-expression; text-mining and protein homology.

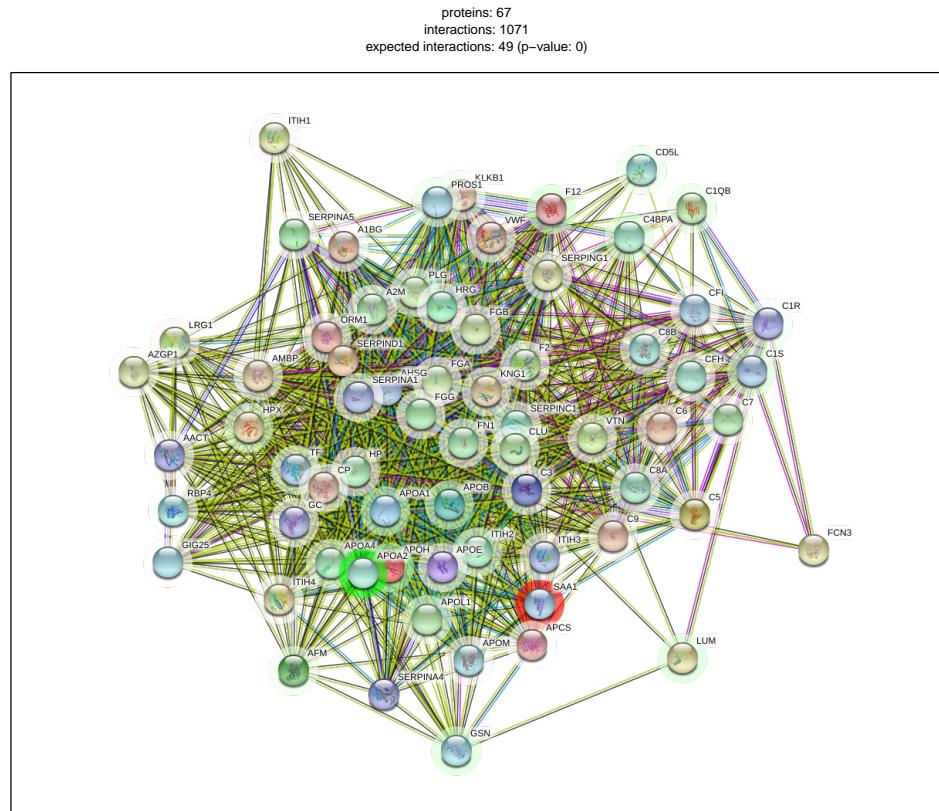


**Figure S32.** Interaction network of differentially abundant proteins from plasma 3-months post-injury, between AIS C patients who experienced an AIS grade conversion and those who did not. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases  and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining  and protein homology .

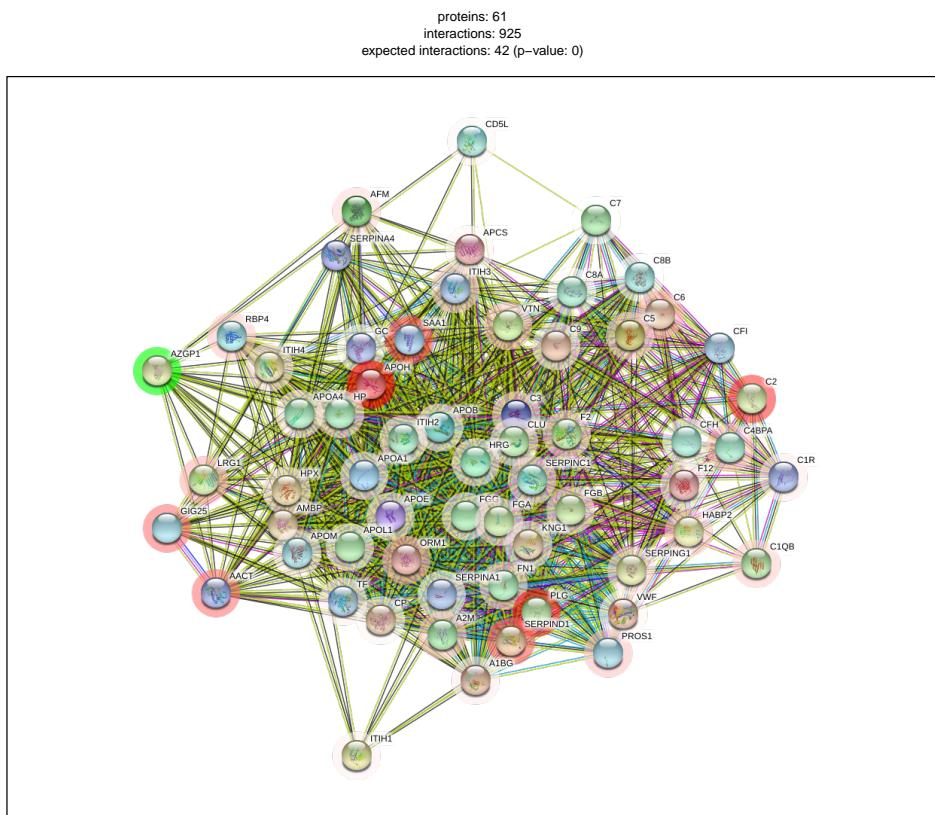
proteins: 69  
interactions: 1064  
expected interactions: 50 (p-value: 0)



**Figure S33.** Interaction network of differentially abundant proteins from plasma 2-weeks and 3-months post-injury for AIS C patients who experienced an AIS grade conversion. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

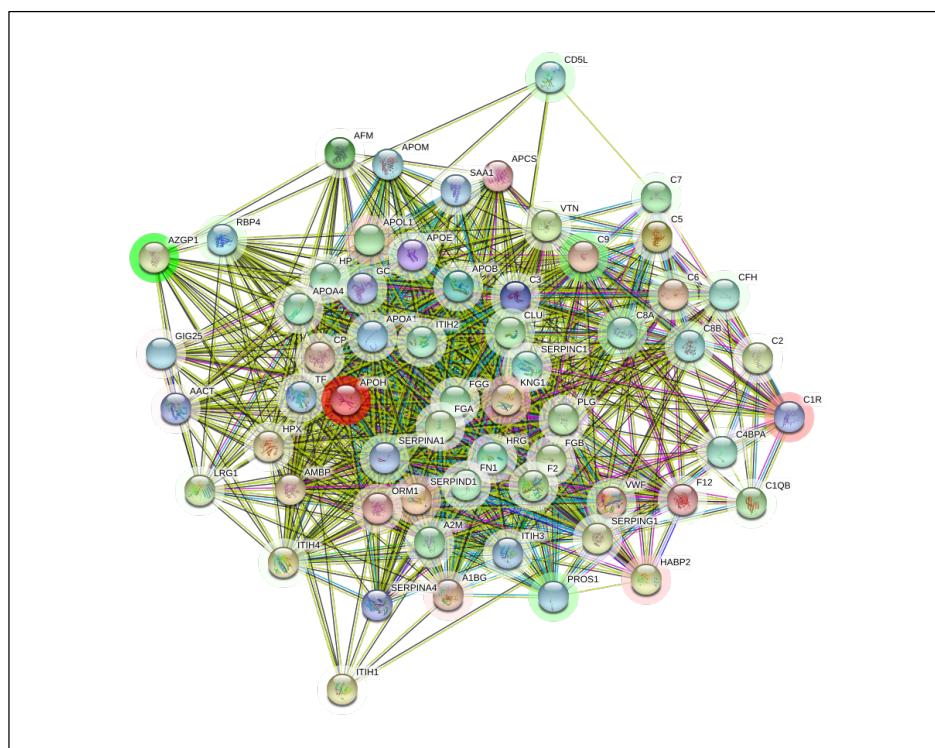


**Figure S34.** Interaction network of differentially abundant proteins from plasma 2-weeks and 3-months post-injury for AIS C patients who did not experience an AIS grade conversion. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood. Others are from gene co-expression; text-mining and protein homology.



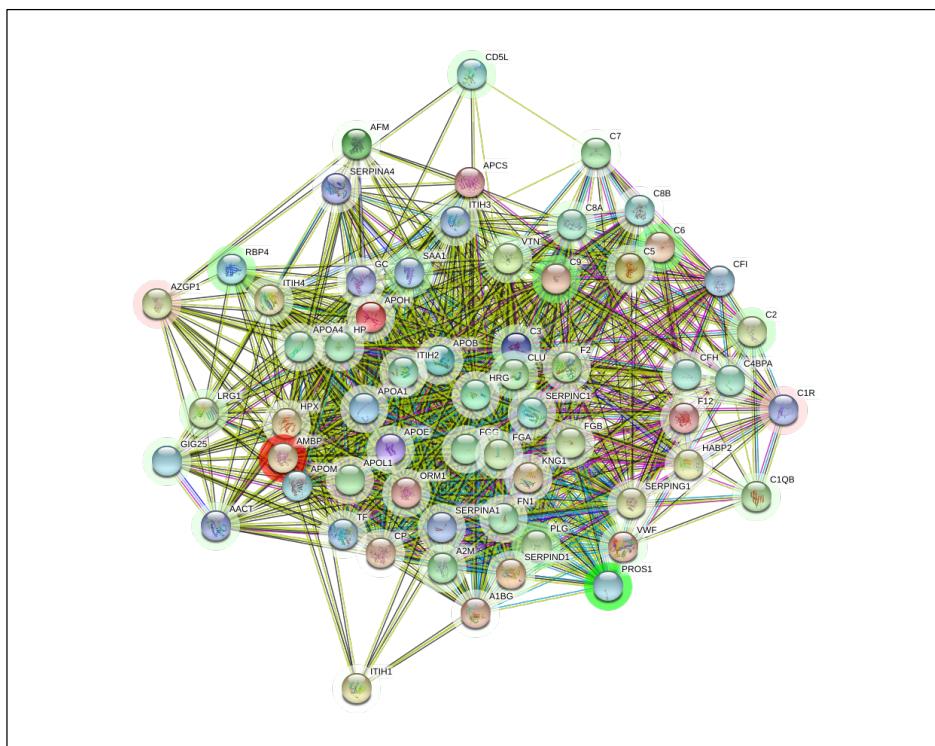
**Figure S35.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS A and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases  and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining  and protein homology .

proteins: 60  
interactions: 903  
expected interactions: 41 (p-value: 0)



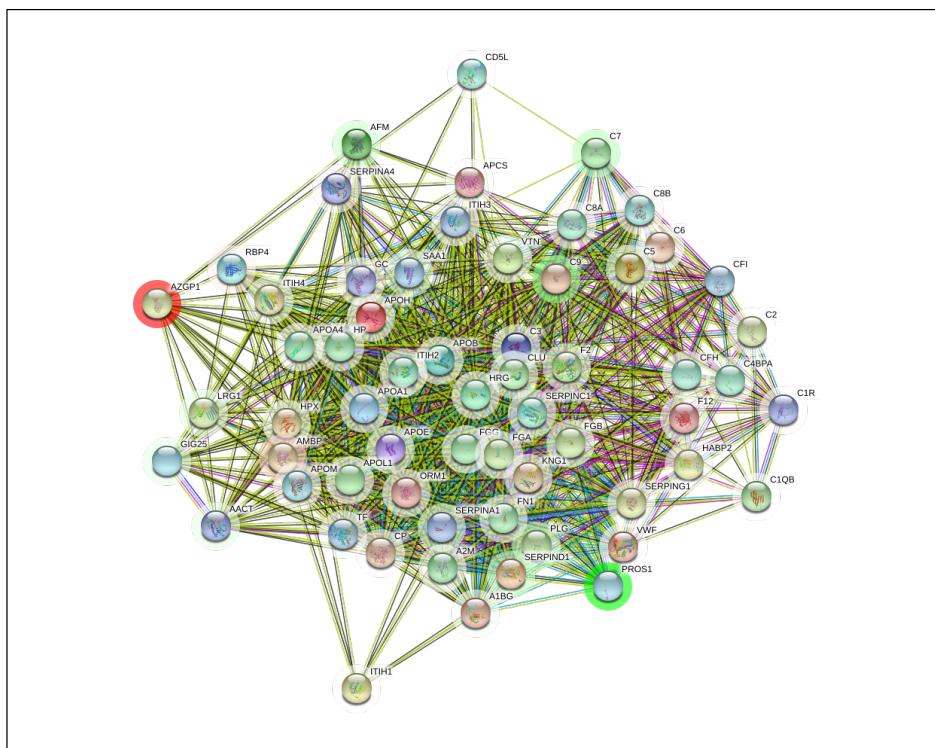
**Figure S36.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C improvers and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

proteins: 61  
interactions: 925  
expected interactions: 42 (p-value: 0)



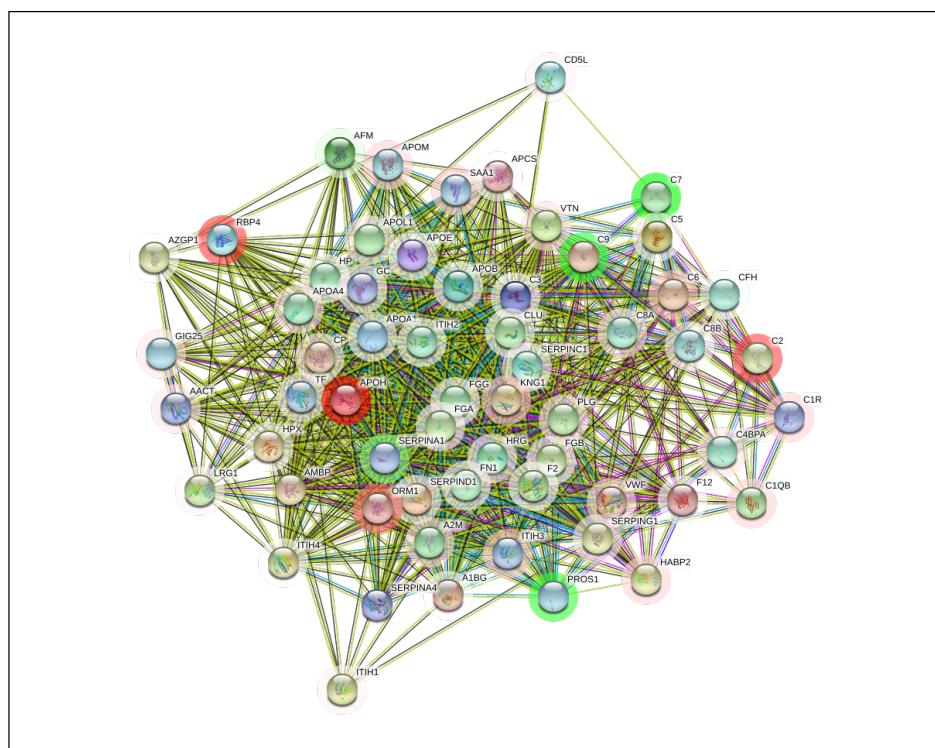
**Figure S37.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C improvers and A. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

proteins: 61  
interactions: 925  
expected interactions: 42 (p-value: 0)



**Figure S38.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C non-improvers and A. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

proteins: 60  
interactions: 903  
expected interactions: 41 (p-value: 0)

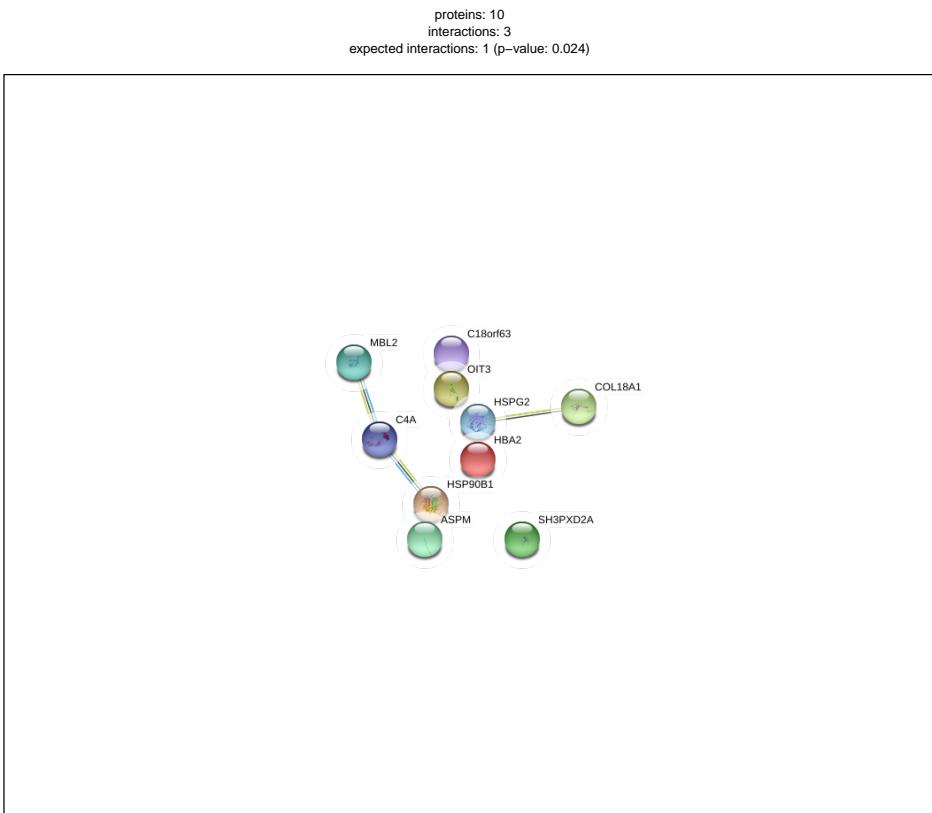


**Figure S39.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C non-improvers and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

1043 5.7.2 Label-free data

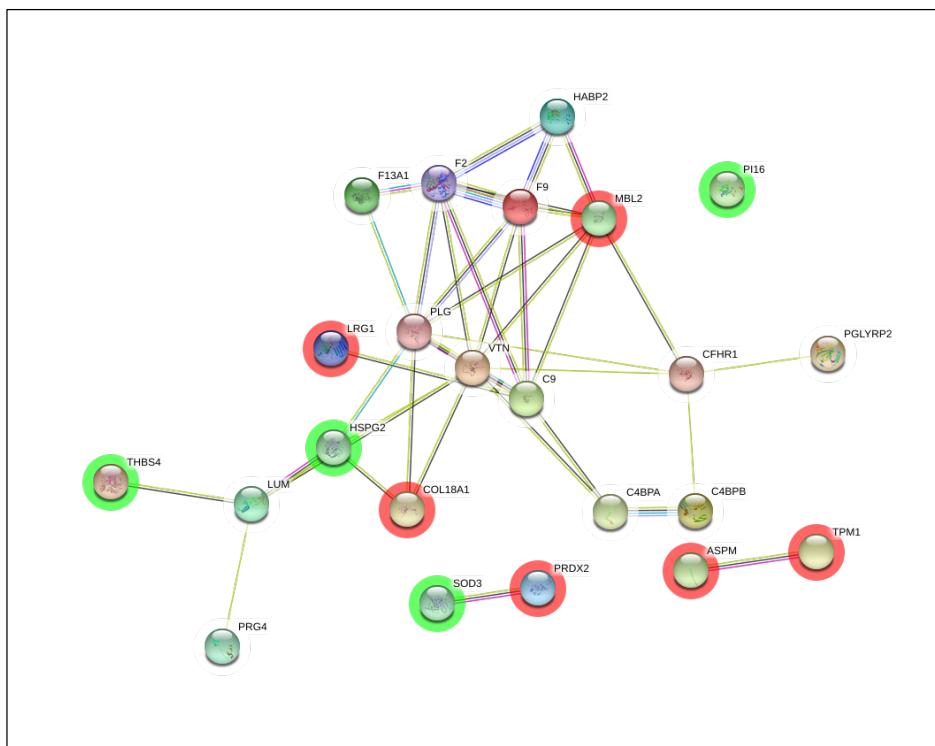


**Figure S40.** The interaction network of differentially abundant proteins found in plasma 2-weeks post-injury, between AIS C patients who experienced an AIS grade conversion and those who did not. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red indicates greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood. Others are from gene co-expression; text-mining and protein homology.

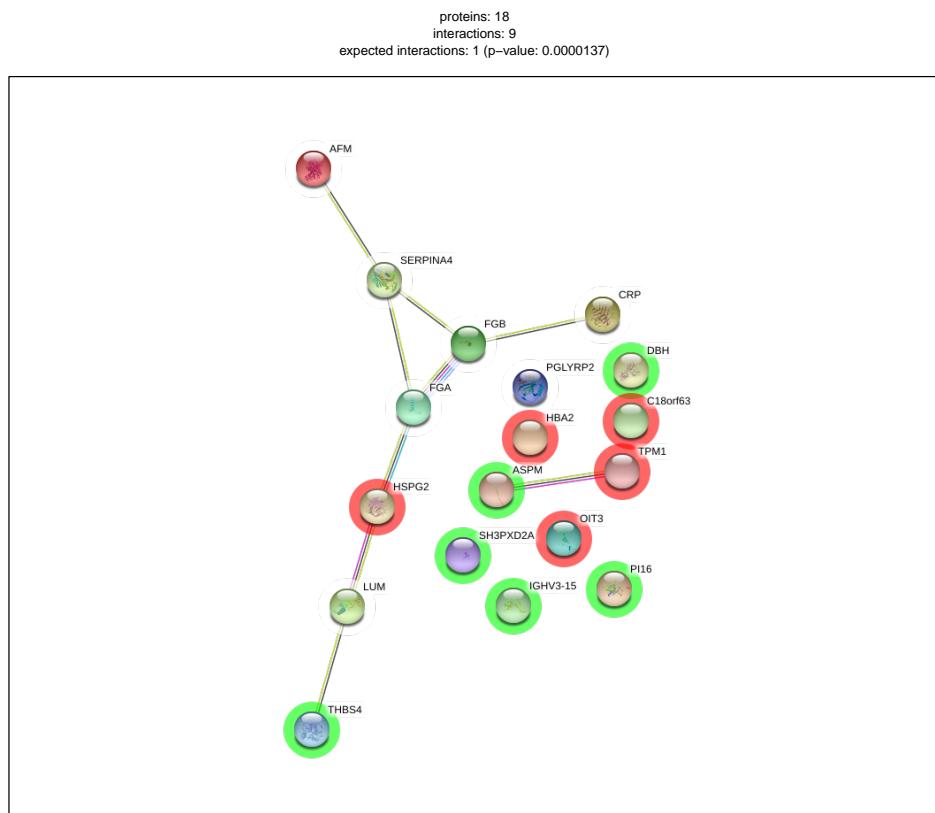


**Figure S41.** Interaction network of differentially abundant proteins from plasma 3-months post-injury, between AIS C patients who experienced an AIS grade conversion and those who did not. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood. Others are from gene co-expression; text-mining and protein homology.

proteins: 23  
interactions: 40  
expected interactions: 3 (p-value: 0)

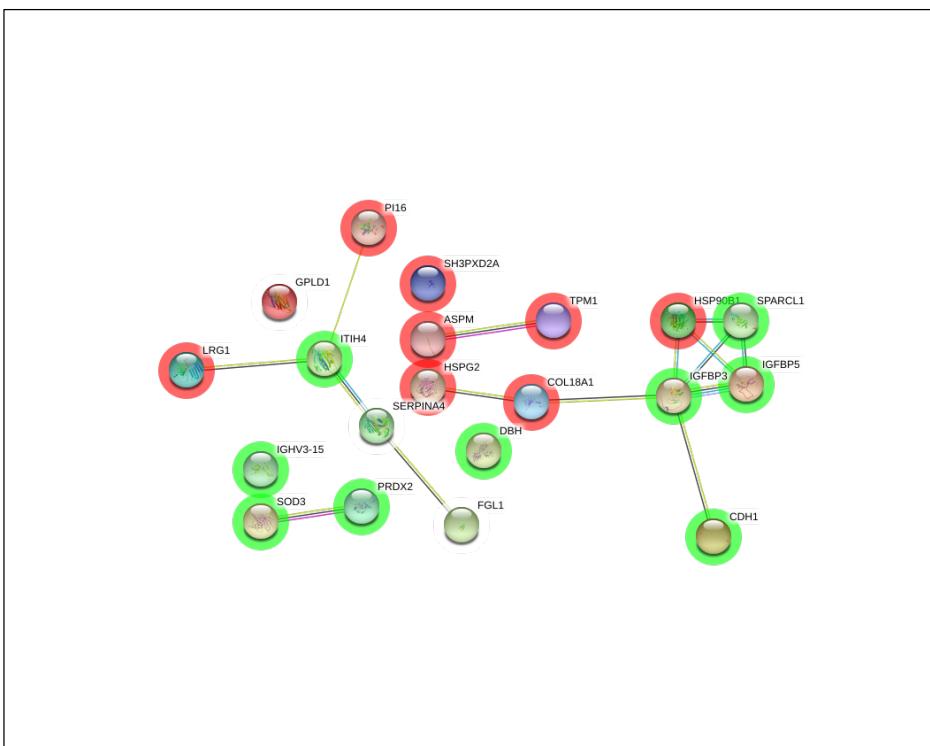


**Figure S42.** Interaction network of differentially abundant proteins from plasma 2-weeks and 3-months post-injury for AIS C patients who experienced an AIS grade conversion. The coloured "halo" denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .



**Figure S43.** Interaction network of differentially abundant proteins from plasma 2-weeks and 3-months post-injury for AIS C patients who did not experience an AIS grade conversion. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood. Others are from gene co-expression; text-mining and protein homology.

proteins: 20  
interactions: 15  
expected interactions: 3 (p-value: 0.00000243)

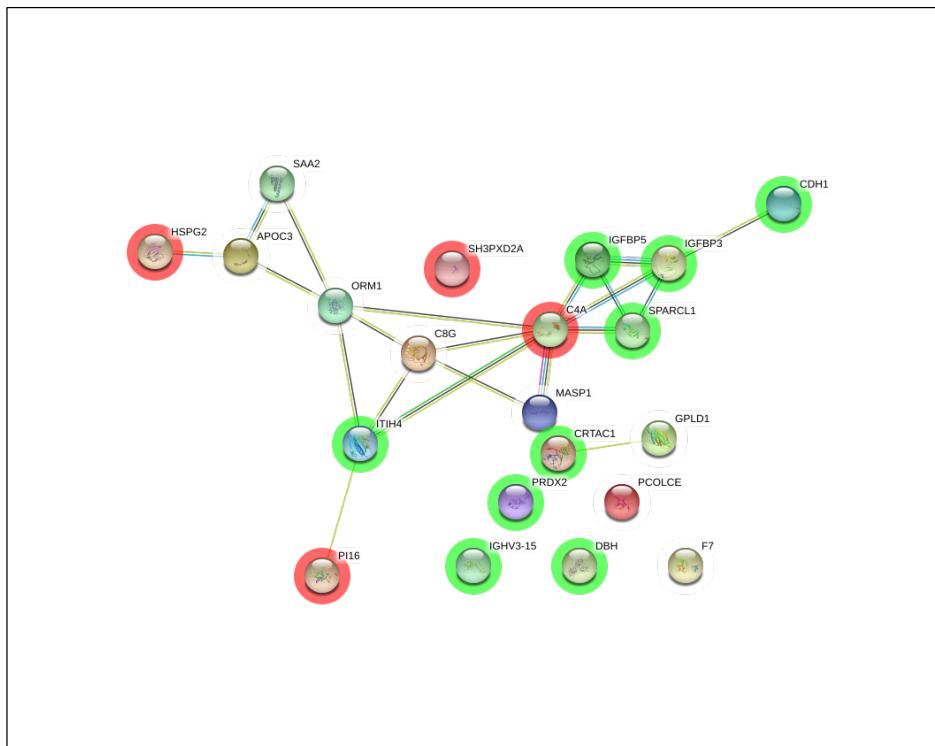


**Figure S44.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS A and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence; gene fusions; gene neighbourhood. Others are from gene co-expression; text-mining and protein homology.

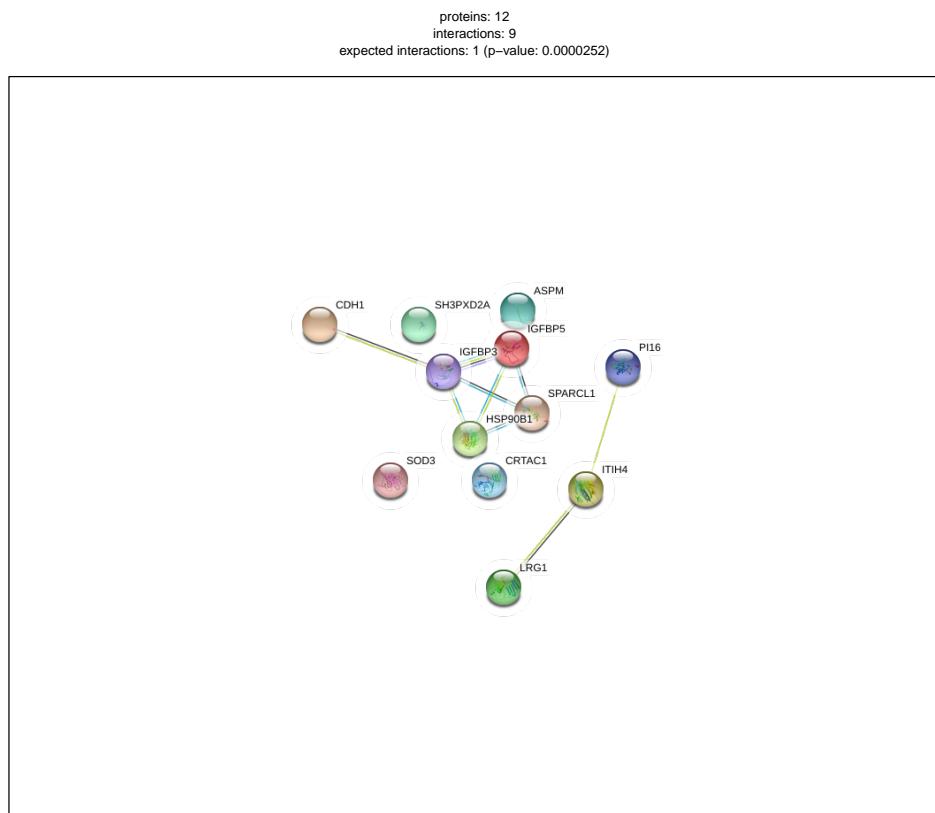


**Figure S45.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C improvers and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

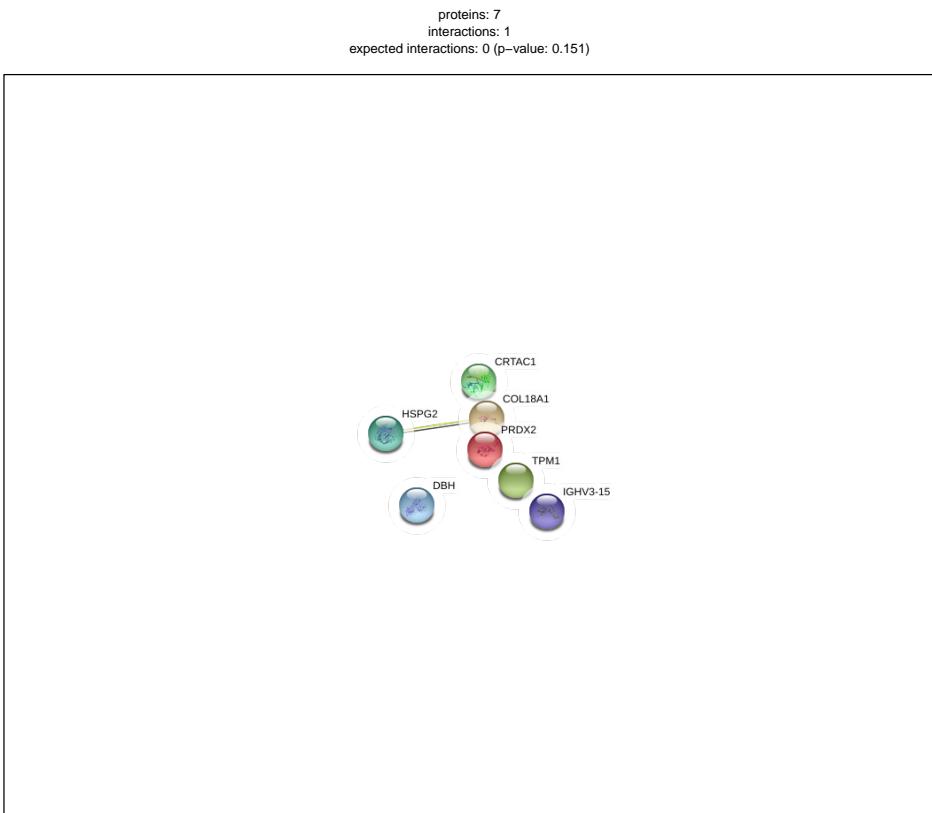
proteins: 21  
interactions: 21  
expected interactions: 2 (p-value: 1.64e-13)



**Figure S46.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C improvers and A. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined. Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

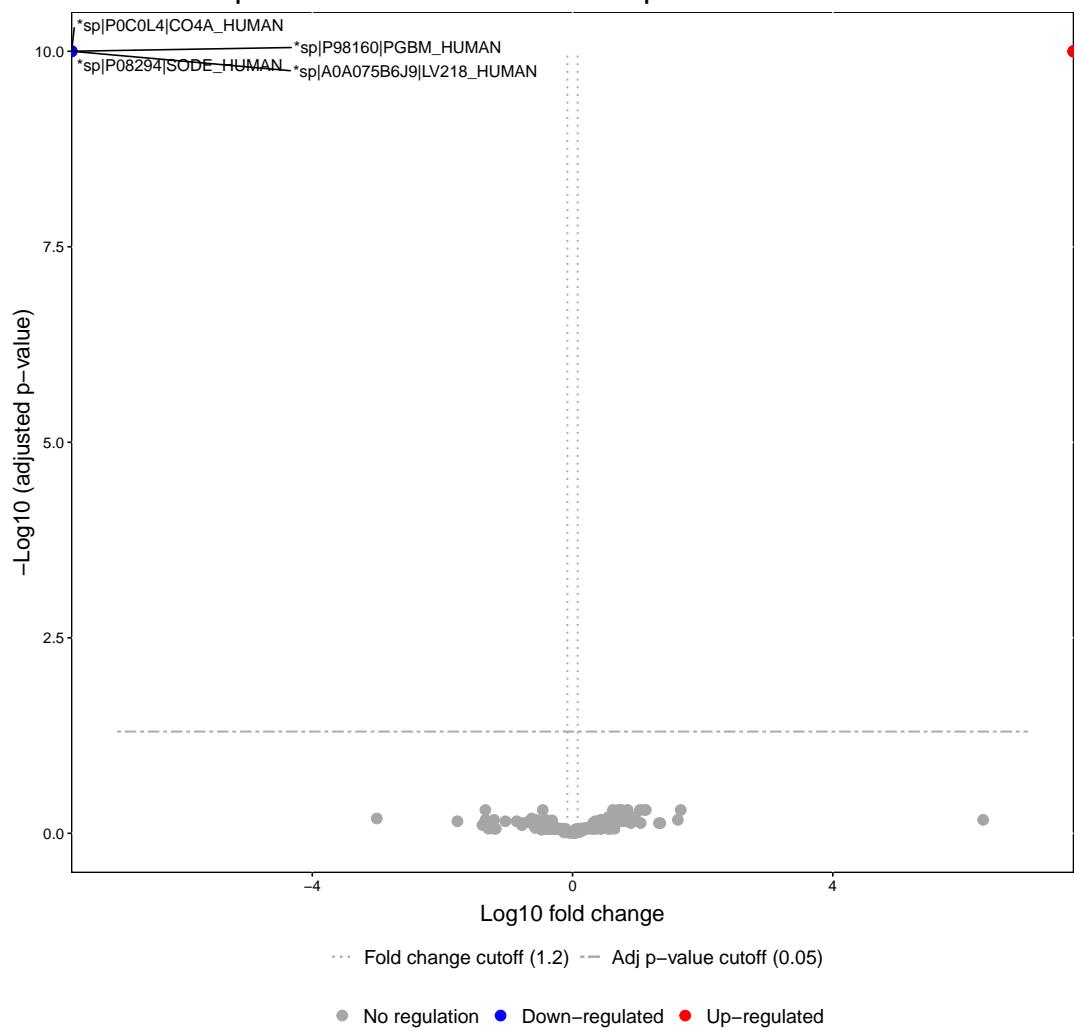


**Figure S47.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C non-improvers and A. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

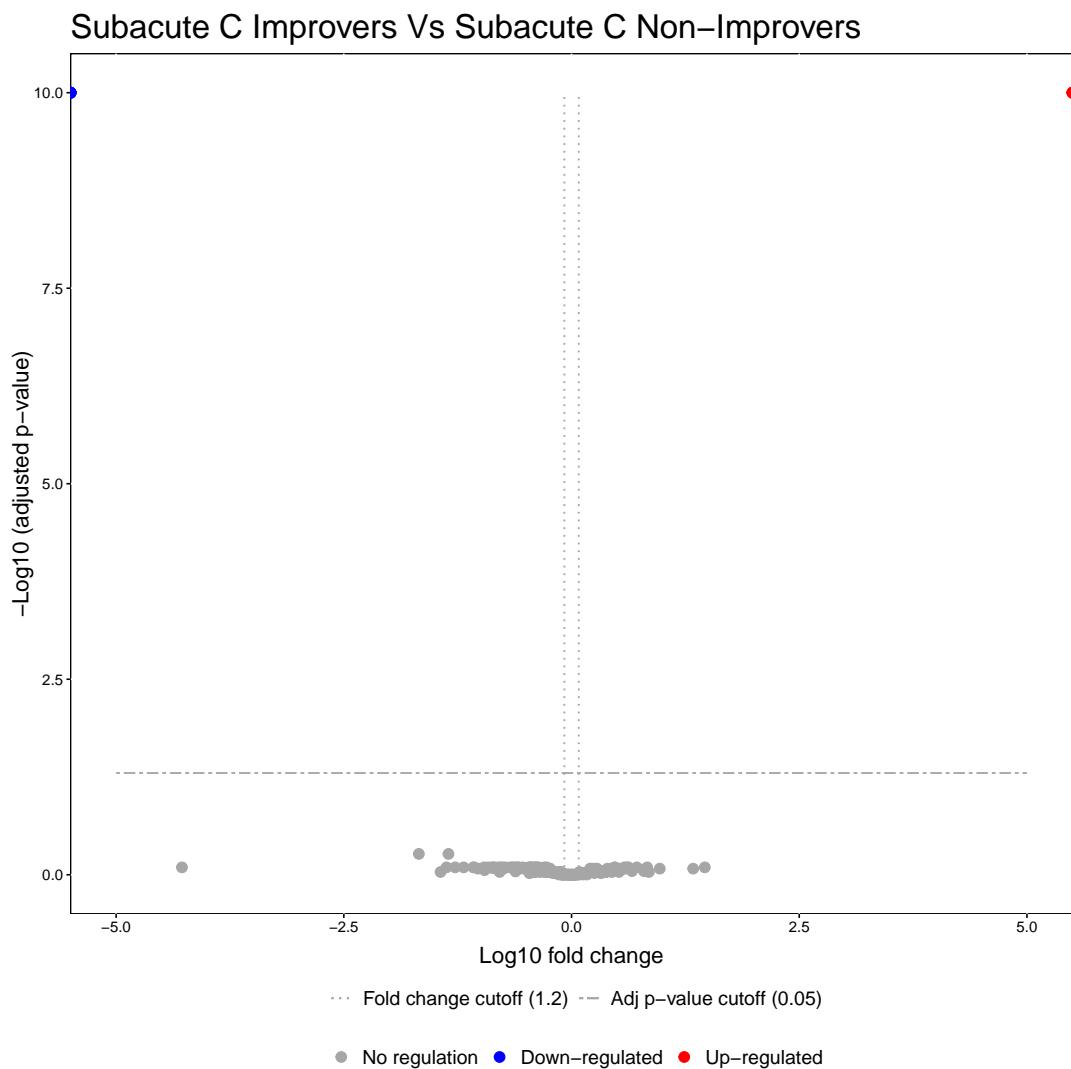


**Figure S48.** Interaction network of differentially abundant proteins from plasma 2-week post-injury, between AIS C non-improvers and D. The coloured “halo” denotes fold change whereby green indicates that protein is less abundant and red that there is greater abundance. Edges represent protein-protein associations; these are known interactions from: curated databases and those that are experimentally determined . Predicted interactions from: gene co-occurrence ; gene fusions ; gene neighbourhood . Others are from gene co-expression ; text-mining and protein homology .

### Acute C Improvers Vs Acute C Non-Improvers

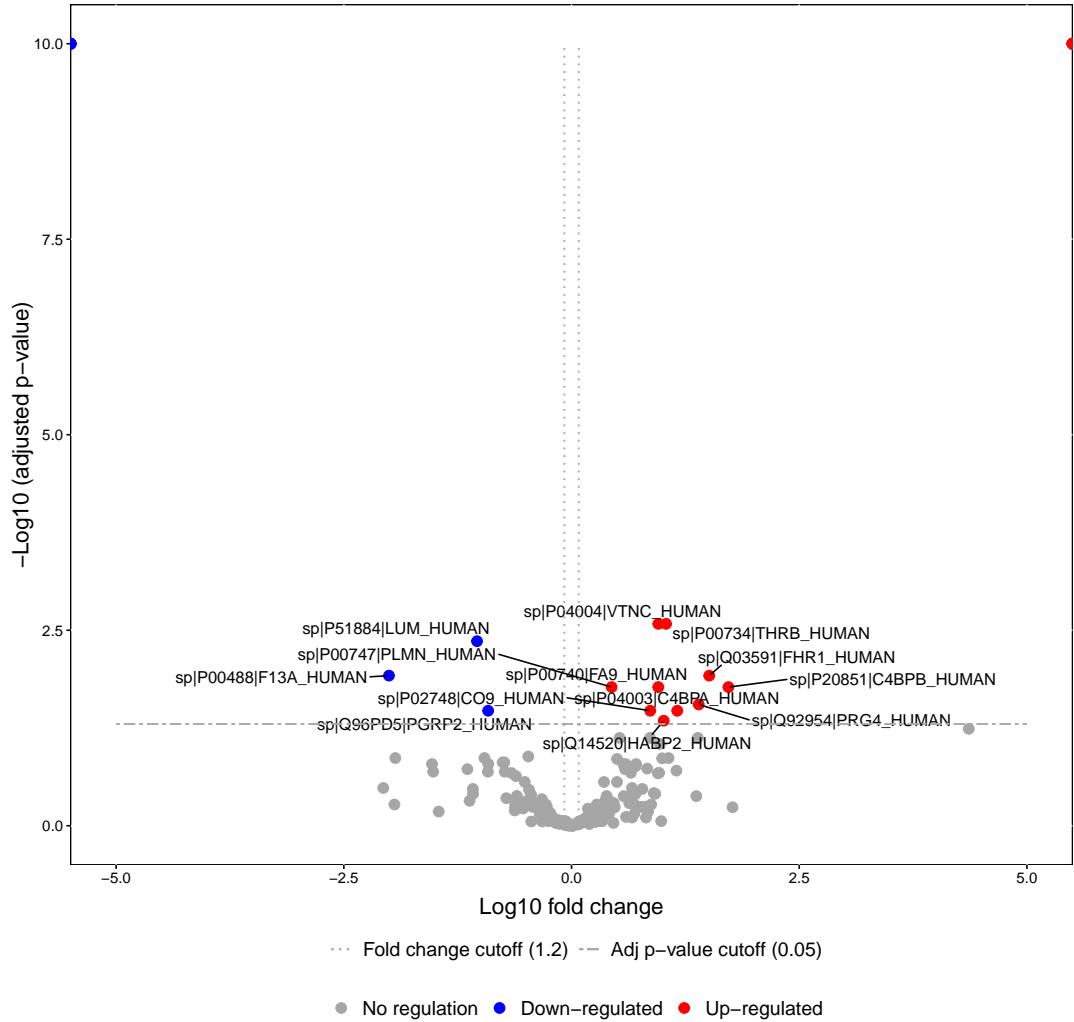


**Figure S49.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks post-injury between AIS C patients who experienced an AIS grade conversion and those who did not. Proteins with a fold changes beyond  $>1.2$  and an adjusted p-value less than 0.05 are labelled.



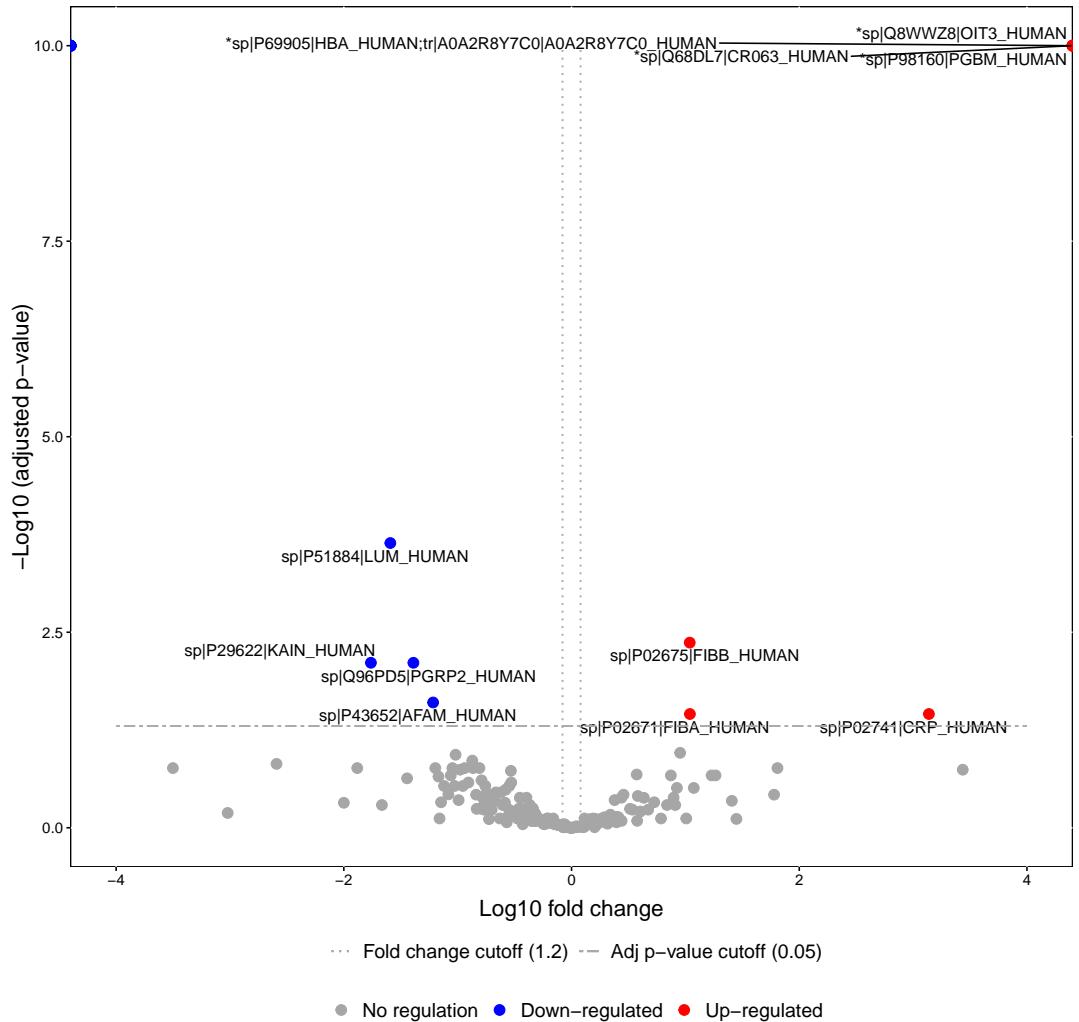
**Figure S50.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 3-months post-injury between AIS C patients who experienced an AIS grade conversion and those who did not. Proteins with a fold changes beyond  $>1.2$  and an adjusted p-value less than 0.05 are labelled.

### Acute C Improvers Vs Subacute C Improvers



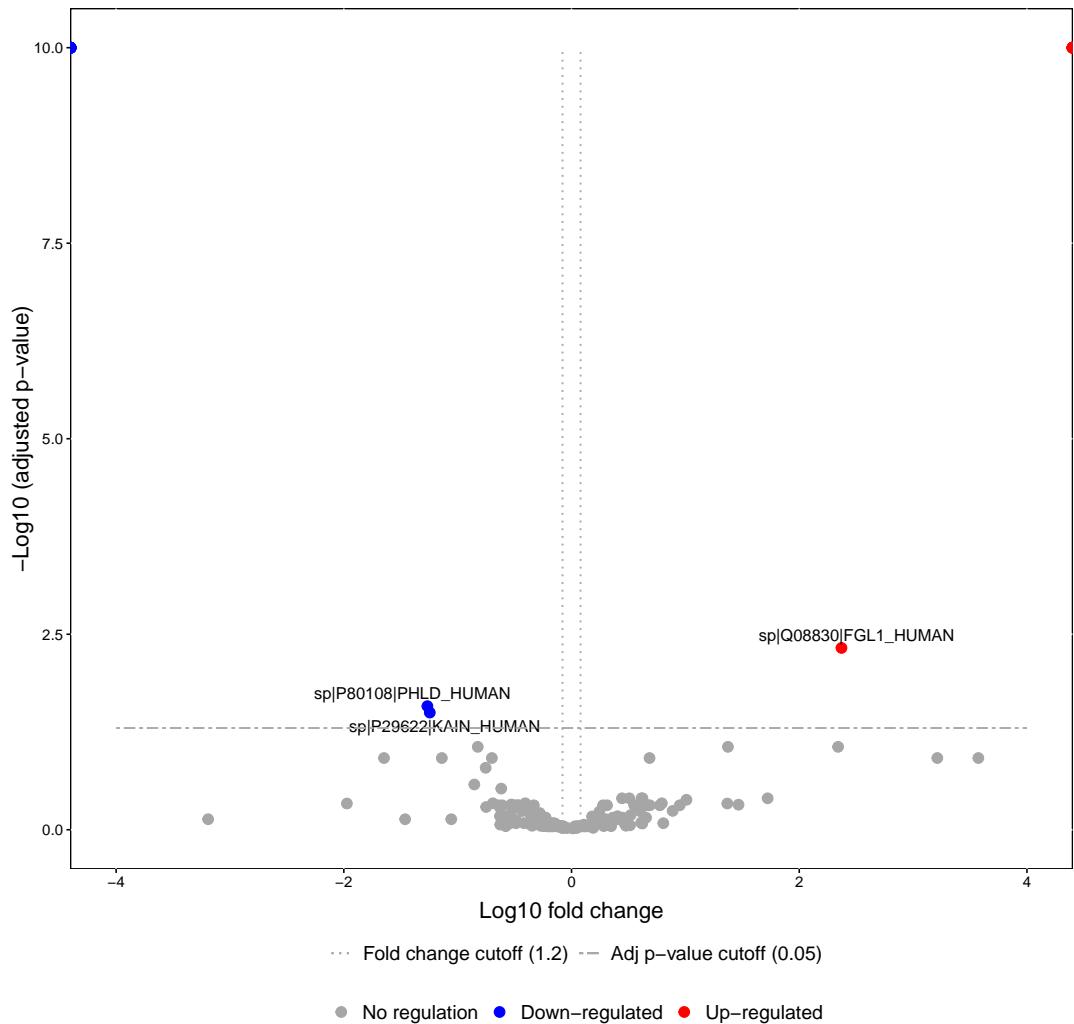
**Figure S51.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks and 3-months post-injury from AIS C patients who experienced an AIS grade conversion. Proteins with a fold changes beyond 1.2 and an adjusted p-value less than 0.05 are labelled.

### Acute C Non-Improvers Vs Subacute C Non-Improvers



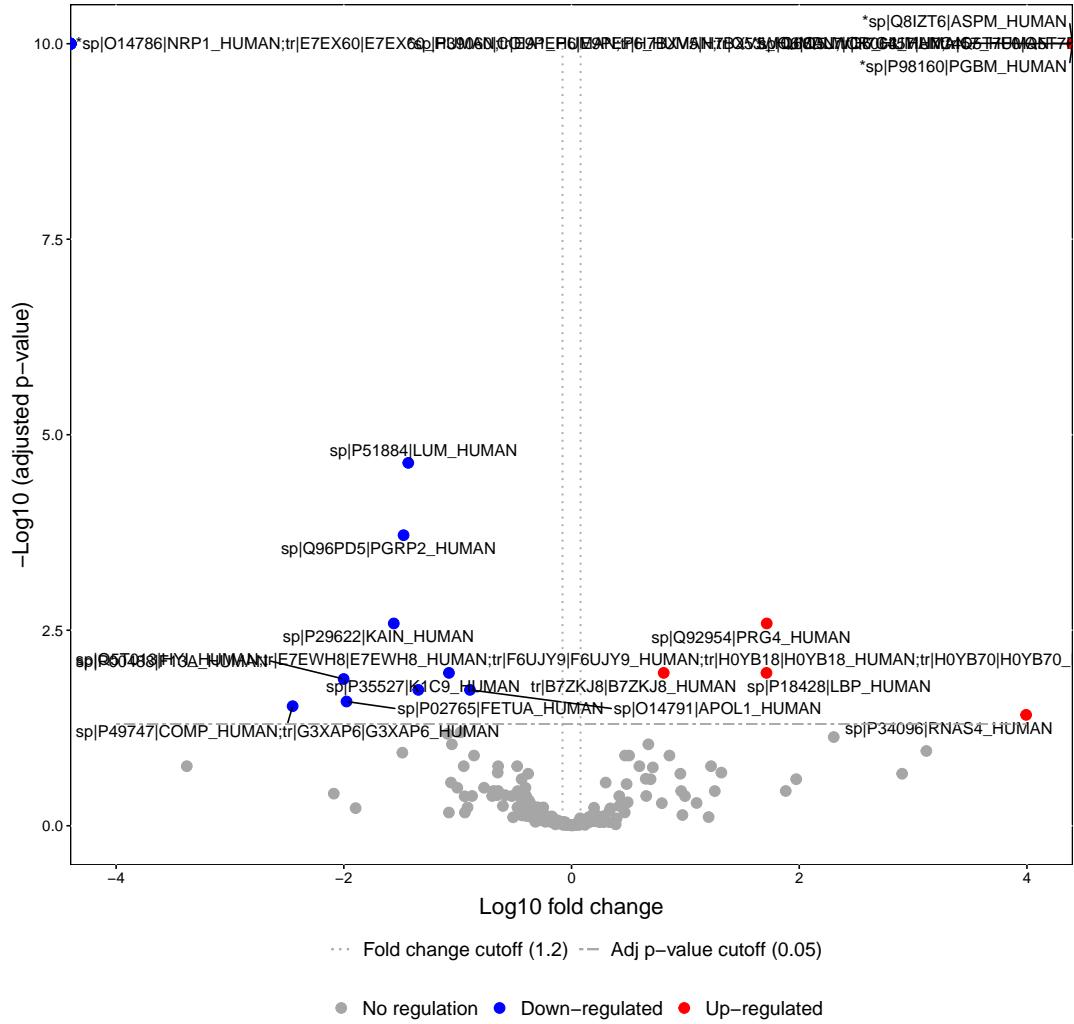
**Figure S52.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks and 3-months post-injury from AIS C patients who did not experience an AIS grade conversion. Proteins with a fold changes beyond  $>1.2$  and an adjusted p-value less than 0.05 are labelled.

### Acute A Vs Acute D



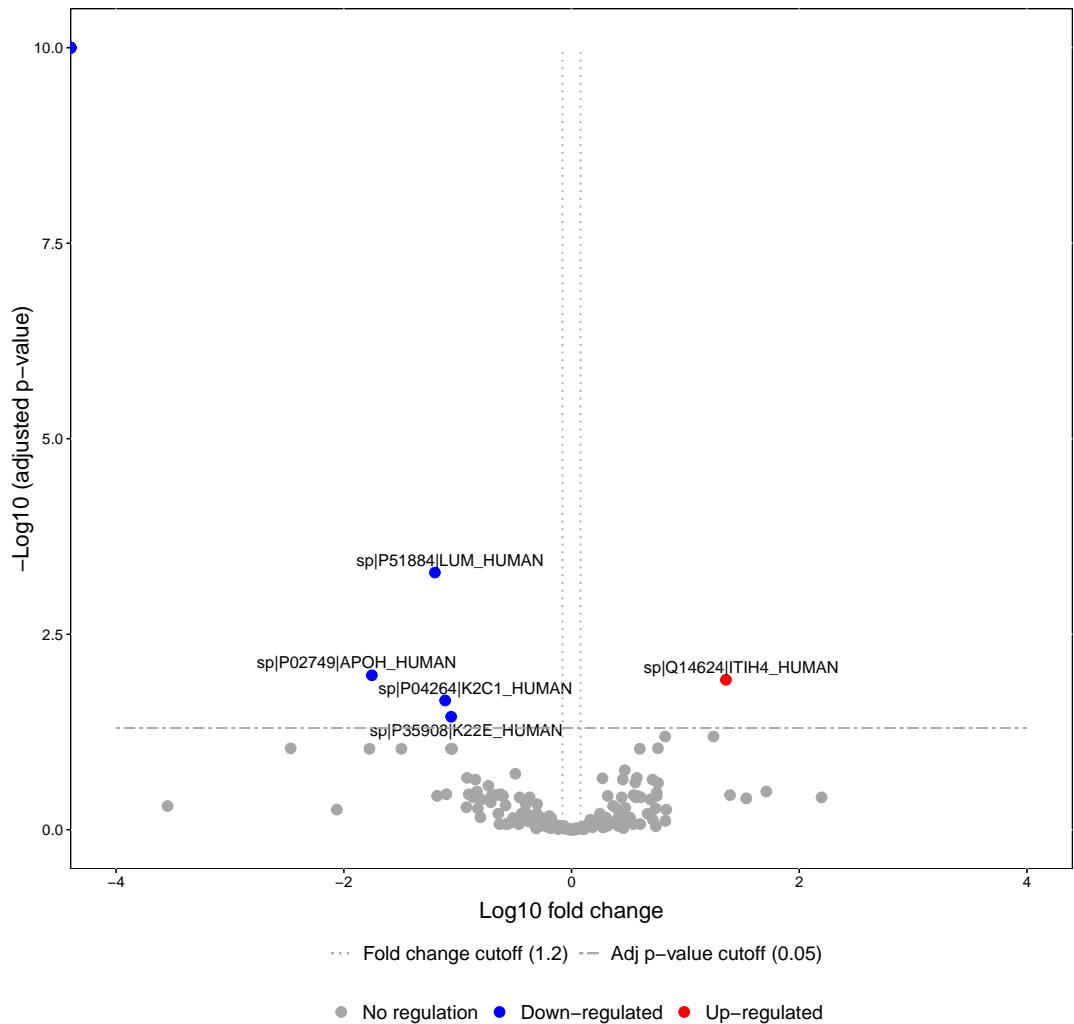
**Figure S53.** Volcano plot of log<sub>10</sub> fold change and log<sub>10</sub> adjusted p-value for plasma proteins from 2-weeks post-injury between AIS A and AIS D patients. Proteins with a fold changes beyond ,1.2 and an adjusted p-value less than 0.05 are labelled.

## Acute A Vs Subacute A



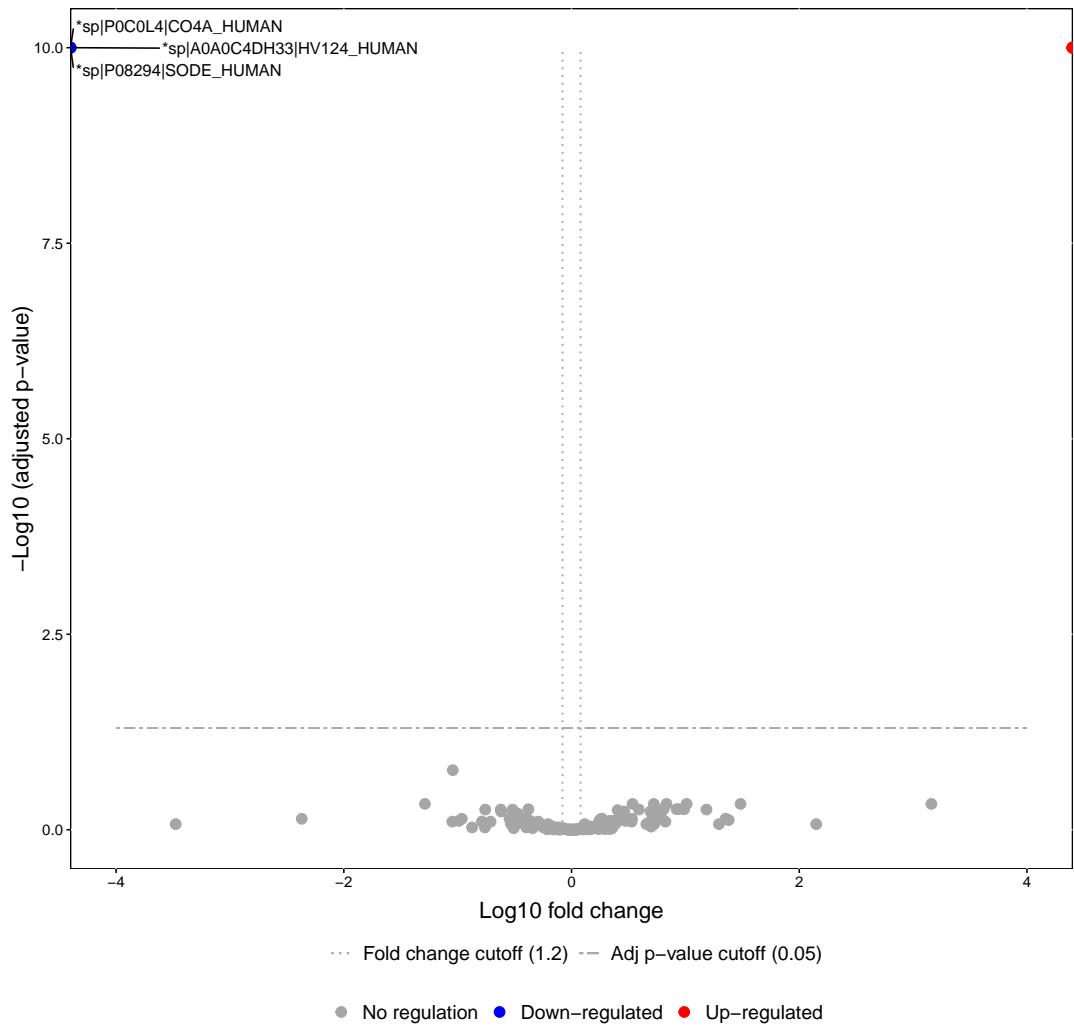
**Figure S54.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks and 3-months post-injury from AIS A patients. Proteins with a fold changes beyond  $>1.2$  and an adjusted p-value less than 0.05 are labelled.

### Acute D Vs Subacute D



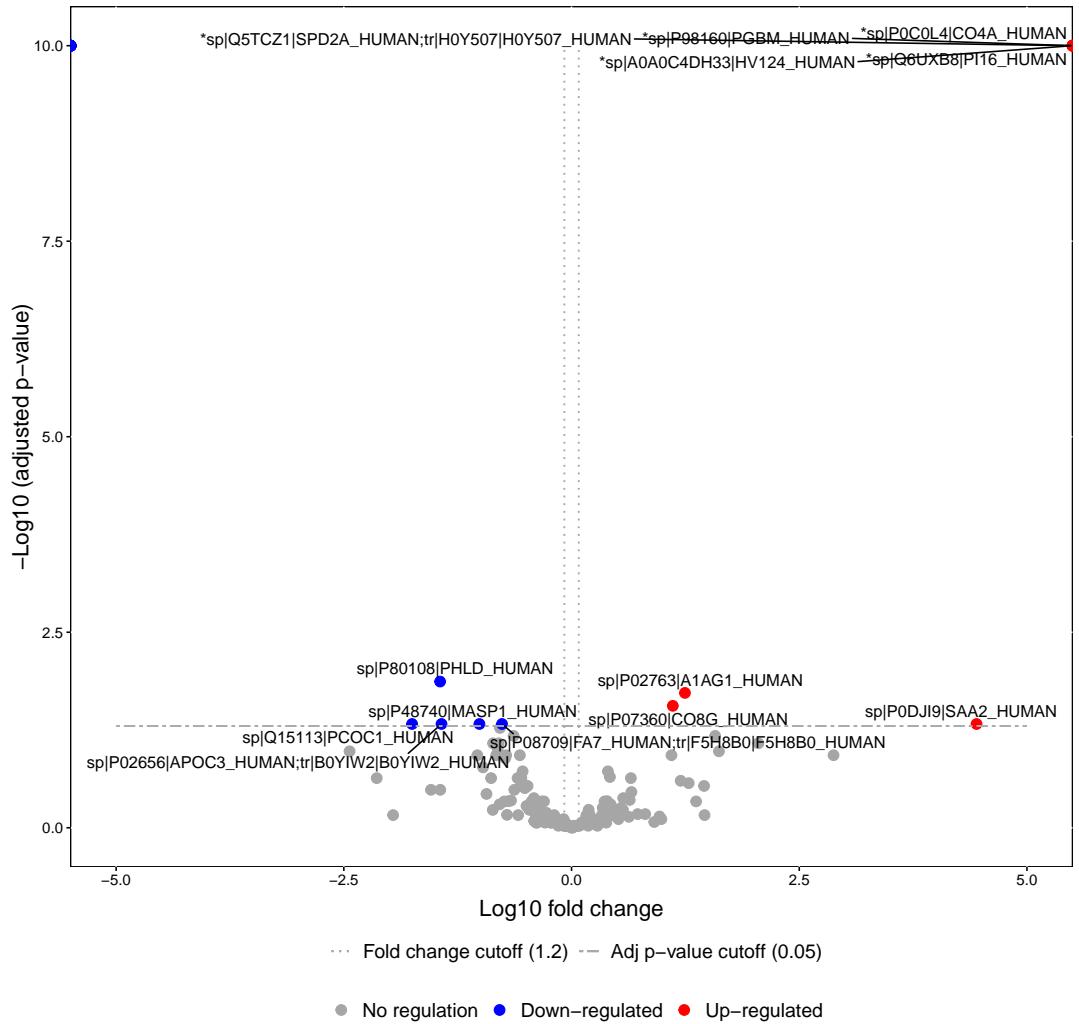
**Figure S55.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks and 3-months post-injury from AIS D patients. Proteins with a fold changes beyond  $>1.2$  and an adjusted p-value less than 0.05 are labelled.

### Acute C Improvers Vs Acute D



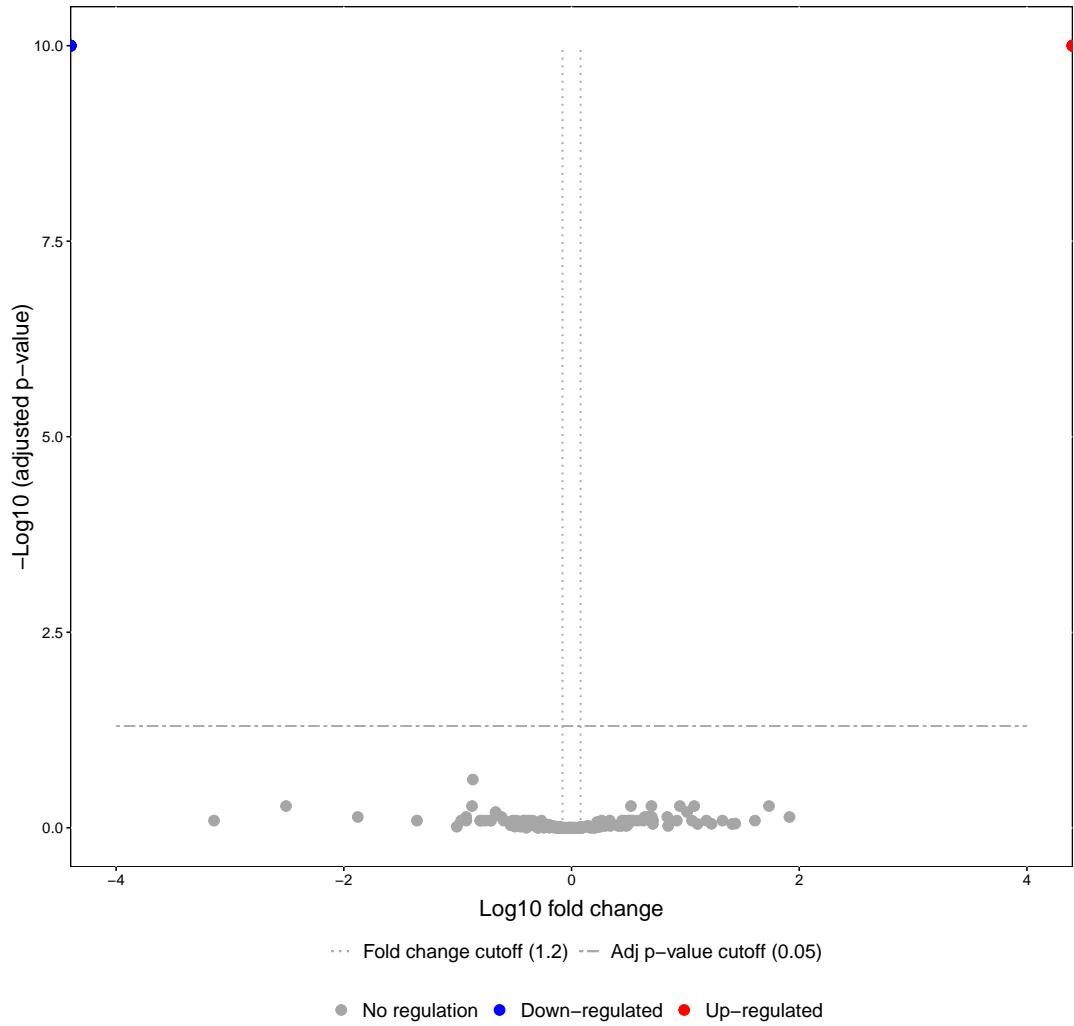
**Figure S56.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks post-injury between AIS C patients who experienced an AIS grade conversion and AIS D patients. Proteins with a fold changes beyond 1.2 and an adjusted p-value less than 0.05 are labelled.

### Acute A Vs Acute C Improvers

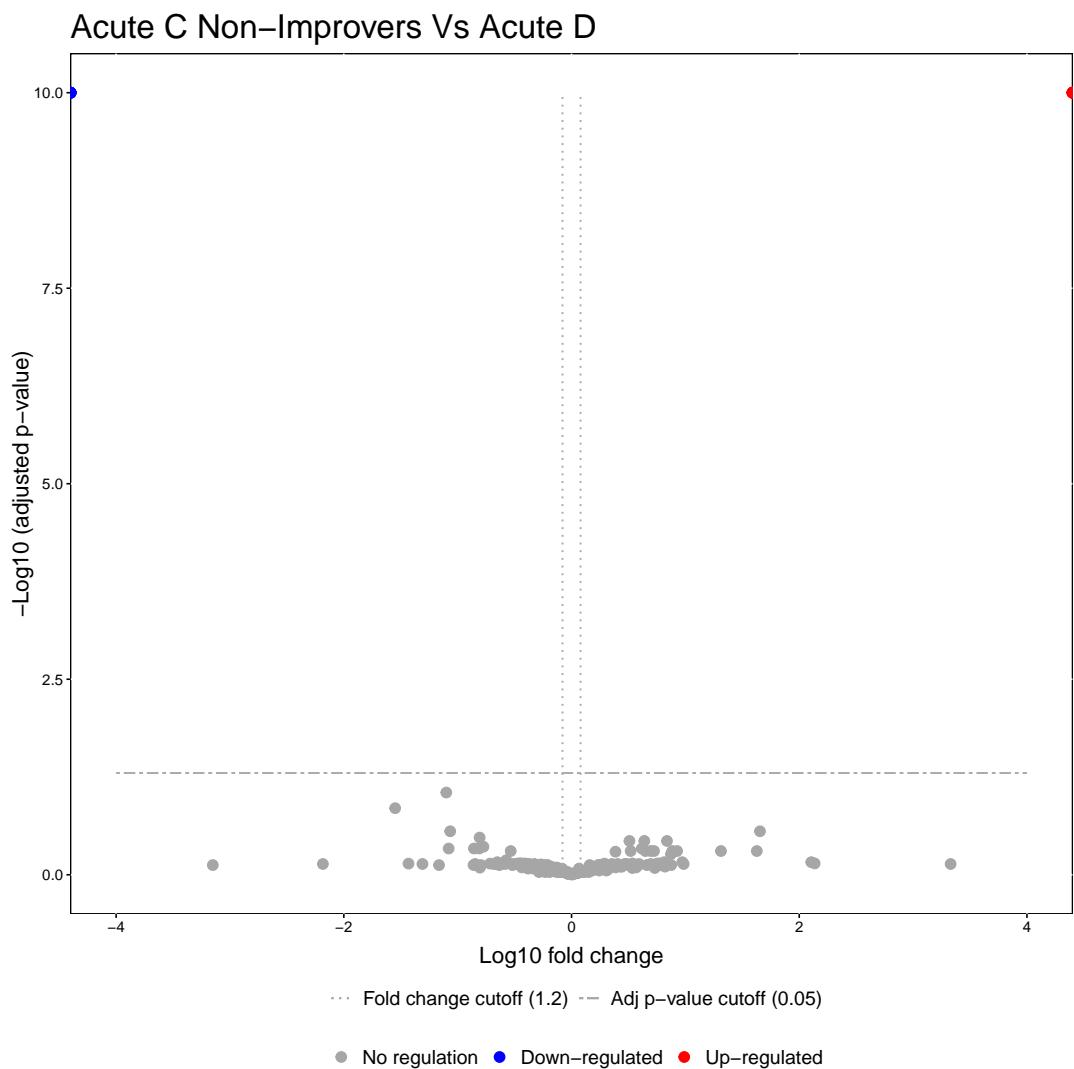


**Figure S57.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks post-injury between AIS A patients and AIS C patients who experienced an AIS grade conversion. Proteins with a fold changes beyond ,1.2 and an adjusted p-value less than 0.05 are labelled.

### Acute A Vs Acute C Non-Improvers



**Figure S58.** Volcano plot of  $\log_{10}$  fold change and  $\log_{10}$  adjusted p-value for plasma proteins from 2-weeks post-injury between AIS A patients and AIS C patients who did not experience an AIS grade conversion. Proteins with a fold changes beyond ,1.2 and an adjusted p-value less than 0.05 are labelled.



**Figure S59.** Volcano plot of log<sub>10</sub> fold change and log<sub>10</sub> adjusted p-value for plasma proteins from 2-weeks post-injury between AIS C patients who did not experience an AIS grade conversion and AIS D patients. Proteins with a fold changes beyond ,1.2 and an adjusted p-value less than 0.05 are labelled.

## 1045 References

- 1046 Ahuja, Christopher S, Jefferson R Wilson, Satoshi Nori, Mark R N Kotter, Claudia Druschel, Armin  
1047 Curt, and Michael G Fehlings. 2017. "Traumatic Spinal Cord Injury." *Nat Rev Dis Primers* 3: 17018.  
1048 <https://doi.org/10.1038/nrdp.2017.18>.
- 1049 Altznauer, Frank, Stephan von Gunten, Peter Späth, and Hans-Uwe Simon. 2003. "Concurrent Pres-  
1050 ence of Agonistic and Antagonistic Anti-Cd95 Autoantibodies in Intravenous Ig Preparations."  
1051 *Journal of Allergy and Clinical Immunology* 112 (6): 1185–90. <https://doi.org/10.1016/j.jaci.2003.09.045>.
- 1052 Anatol, Kontush, Chantepie Sandrine, and Chapman M. John. 2003. "Small, Dense HDL Particles Ex-  
1053 erter Potent Protection of Atherogenic LDL Against Oxidative Stress." *Arteriosclerosis, Thrombosis,  
1054 and Vascular Biology* 23 (10): 1881–88. <https://doi.org/10.1161/01.ATV.0000091338.93223.E8>.
- 1055 Anderson, Mark A., Joshua E. Burda, Yilong Ren, Yan Ao, Timothy M. O'Shea, Riki Kawaguchi, Gio-  
1056 vanni Coppola, Baljit S. Khakh, Timothy J. Deming, and Michael V. Sofroniew. 2016. "Astrocyte  
1057 Scar Formation Aids Central Nervous System Axon Regeneration." *Nature* 532 (7598): 195–200.  
1058

- 1059 https://doi.org/10.1038/nature17623.
- 1060 Ankeny, Daniel P., Zhen Guan, and Phillip G. Popovich. 2009. "B Cells Produce Pathogenic Antibodies and Impair Recovery After Spinal Cord Injury in Mice." *The Journal of Clinical Investigation* 119 (10): 2990–99. https://doi.org/10.1172/JCI39780.
- 1063 Anthony, Daniel C., and Yvonne Couch. 2014. "The Systemic Response to CNS Injury." *Experimental Neurology*, Special Issue: Neuroimmunology of spinal cord injury, 258 (August): 105–11. https://doi.org/10.1016/j.expneurol.2014.03.013.
- 1066 Arevalo-Martin, Angel, Lukas Grassner, Daniel Garcia-Ovejero, Beatriz Paniagua-Torija, Gemma Barroso-Garcia, Alba G. Arandilla, Orpheus Mach, et al. 2018. "Elevated Autoantibodies in Subacute Human Spinal Cord Injury Are Naturally Occurring Antibodies." *Frontiers in Immunology* 9: 2365. https://doi.org/10.3389/fimmu.2018.02365.
- 1070 Arpaia, Nicholas, Clarissa Campbell, Xiyi Fan, Stanislav Dikiy, Joris van der Veeken, Paul deRoos, Hui Liu, et al. 2013. "Metabolites Produced by Commensal Bacteria Promote Peripheral Regulatory T-cell Generation." *Nature* 504 (7480): 451–55. https://doi.org/10.1038/nature12726.
- 1073 Arumugam, Thiruma V., Sung-Chun Tang, Justin D. Lathia, Aiwu Cheng, Mohamed R. Mughal, Srinivasulu Chigurupati, Tim Magnus, et al. 2007. "Intravenous Immunoglobulin (IVIG) Protects the Brain Against Experimental Stroke by Preventing Complement-Mediated Neuronal Cell Death." *Proceedings of the National Academy of Sciences of the United States of America* 104 (35): 14104–9. https://doi.org/10.1073/pnas.0700506104.
- 1078 Balmer, James E., and Rune Blomhoff. 2002. "Gene Expression Regulation by Retinoic Acid." *Journal of Lipid Research* 43 (11): 1773–1808. https://doi.org/10.1194/jlr.R100015-JLR200.
- 1080 Balmer, Maria L., Emma Slack, Andrea de Gottardi, Melissa A. E. Lawson, Siegfried Hapfelmeier, Luca Miele, Antonio Grieco, et al. 2014. "The Liver May Act as a Firewall Mediating Mutualism Between the Host and Its Gut Commensal Microbiota." *Science Translational Medicine* 6 (237): 237ra66–66. https://doi.org/10.1126/scitranslmed.3008618.
- 1084 Balzan, Silvio, Claudio De Almeida Quadros, Roberto De Cleva, Bruno Zilberstein, and Ivan Cecconello. 2007. "Bacterial Translocation: Overview of Mechanisms and Clinical Impact." *Journal of Gastroenterology and Hepatology* 22 (4): 464–71. https://doi.org/10.1111/j.1440-1746.2007.04933.x.
- 1088 Banka, C. L., T. Yuan, M. C. de Beer, M. Kindy, L. K. Curtiss, and F. C. de Beer. 1995. "Serum Amyloid A (SAA): Influence on HDL-mediated Cellular Cholesterol Efflux." *Journal of Lipid Research* 36 (5): 1058–65.
- 1091 Bao, Feng, Vanessa Omana, Arthur Brown, and Lynne C. Weaver. 2012. "The Systemic Inflammatory Response After Spinal Cord Injury in the Rat Is Decreased by a4B1 Integrin Blockade." *Journal of Neurotrauma* 29 (8): 1626–37. https://doi.org/10.1089/neu.2011.2190.
- 1094 Basta, Milan, Fredric Van Goor, Stefano Luccioli, Eric M. Billings, Alexander O. Vortmeyer, Lajos Baranyi, Janos Szebeni, et al. 2003. "F(ab)<sup>1</sup>2-Mediated Neutralization of C3a and C5a Anaphylatoxins: A Novel Effector Function of Immunoglobulins." *Nature Medicine* 9 (4): 431–38. https://doi.org/10.1038/nm836.
- 1098 Basta, M, P Kirshbom, M M Frank, and L F Fries. 1989. "Mechanism of Therapeutic Effect of High-Dose Intravenous Immunoglobulin. Attenuation of Acute, Complement-Dependent Immune Damage in a Guinea Pig Model." *Journal of Clinical Investigation* 84 (6): 1974–81. https://doi.org/10.1172/JCI114387.
- 1102 Bastien, Dominic, Victor Bellver Landete, Martine Lessard, Nicolas Vallières, Mathieu Champagne, Akira Takashima, Marie-Ève Tremblay, Yannick Doyon, and Steve Lacroix. 2015. "IL-1 $\alpha$  Gene Deletion Protects Oligodendrocytes After Spinal Cord Injury Through Upregulation of the Survival Factor Tox3." *The Journal of Neuroscience* 35 (30): 10715–30. https://doi.org/10.1523/JNEUROSCI.0498-15.2015.
- 1107 Bastien, Dominic, and Steve Lacroix. 2014. "Cytokine Pathways Regulating Glial and Leukocyte Function After Spinal Cord and Peripheral Nerve Injury." *Experimental Neurology*, Special Issue: Neuroimmunology of spinal cord injury, 258 (August): 62–77. https://doi.org/10.1016/j.expneu

- 1110       rol.2014.04.006.
- 1111       Bauman, William A., and Ann M. Spungen. 2001. "Carbohydrate And Lipid Metabolism In Chronic  
1112       Spinal Cord Injury." *The Journal of Spinal Cord Medicine* 24 (4): 266-77. <https://doi.org/10.1080/10790268.2001.11753584>.
- 1113
- 1114       Bayry, Jagadeesh, Vir Singh Negi, and Srinivasa Kaveri. 2011. "Intravenous Immunoglobulin Therapy  
1115       in Rheumatic Diseases." *Nature Reviews Rheumatology* 7 (6): 349-59. <https://doi.org/10.1038/nrheum.2011.61>.
- 1116
- 1117       Bazzocchi, Gabriele, Silvia Turroni, Maria Chiara Bulzamini, Federica D'Amico, Angelica Bava, Mirco  
1118       Castiglioni, Valentina Cagnetta, et al. 2021. "Changes in Gut Microbiota in the Acute Phase  
1119       After Spinal Cord Injury Correlate with Severity of the Lesion." *Scientific Reports* 11 (1): 12743.  
1120       <https://doi.org/10.1038/s41598-021-92027-z>.
- 1121       Beck, Kevin D., Hal X. Nguyen, Manuel D. Galvan, Desirée L. Salazar, Trent M. Woodruff, and Aileen  
1122       J. Anderson. 2010. "Quantitative Analysis of Cellular Inflammation After Traumatic Spinal Cord  
1123       Injury: Evidence for a Multiphasic Inflammatory Response in the Acute to Chronic Environment."  
1124       *Brain* 133 (2): 433-47. <https://doi.org/10.1093/brain/awp322>.
- 1125       Bellver-Landete, Victor, Floriane Bretheau, Benoit Mailhot, Nicolas Vallières, Martine Lessard,  
1126       Marie-Eve Janelle, Nathalie Vernoux, et al. 2019. "Microglia Are an Essential Component of the  
1127       Neuroprotective Scar That Forms After Spinal Cord Injury." *Nature Communications* 10 (1): 518.  
1128       <https://doi.org/10.1038/s41467-019-10844-0>.
- 1129       Benditt, E. P., and N. Eriksen. 1977. "Amyloid Protein SAA Is Associated with High Density Lipoprotein  
1130       from Human Serum." *Proceedings of the National Academy of Sciences* 74 (9): 4025-28.  
1131       <https://doi.org/10.1073/pnas.74.9.4025>.
- 1132       Bernardo Harrington, Gabriel Mateus, Paul Cool, Charlotte Hulme, Aheed Osman, Joy Chowdhury,  
1133       Naveen Kumar, Srinivasa Budithi, and Karina Wright. 2020. "Routinely Measured Haematological  
1134       Markers Can Help to Predict AIS Scores Following Spinal Cord Injury." *Journal of Neurotrauma*,  
1135       July. <https://doi.org/10.1089/neu.2020.7144>.
- 1136       Berry, Daniel C., Sheila M. O'Byrne, Amanda C. Vreeland, William S. Blaner, and Noa Noy. 2012.  
1137       "Cross Talk Between Signaling and Vitamin A Transport by the Retinol-Binding Protein Receptor  
1138       Stra6." *Molecular and Cellular Biology* 32 (15): 3164-75. <https://doi.org/10.1128/MCB.00505-12>.
- 1139       Berthold, Michael R., Nicolas Cebron, Fabian Dill, Thomas R. Gabriel, Tobias Kötter, Thorsten Meinl,  
1140       Peter Ohl, Kilian Thiel, and Bernd Wiswedel. 2009. "KNIME - the Konstanz Information Miner:  
1141       Version 2.0 and Beyond." *ACM SIGKDD Explorations Newsletter* 11 (1): 26-31. <https://doi.org/10.1145/1656274.1656280>.
- 1142
- 1143       Bertolotti, Marco, Amedeo Lonardo, Chiara Mussi, Enrica Baldelli, Elisa Pellegrini, Stefano Ballestri,  
1144       Dante Romagnoli, and Paola Loria. 2014. "Nonalcoholic Fatty Liver Disease and Aging: Epidemiology  
1145       to Management." *World Journal of Gastroenterology : WJG* 20 (39): 14185-204. <https://doi.org/10.3748/wjg.v20.i39.14185>.
- 1146
- 1147       Bhargava, Prerna, and Chih-Hao Lee. 2012. "Role and Function of Macrophages in the Metabolic  
1148       Syndrome." *Biochemical Journal* 442 (2): 253-62. <https://doi.org/10.1042/BJ20111708>.
- 1149       Biglari, B., T. Swing, C. Child, A. Büchler, F. Westhauser, T. Bruckner, T. Ferbert, H. Jürgen Gerner,  
1150       and A. Moghaddam. 2015. "A Pilot Study on Temporal Changes in IL-1b and TNF-a Serum Levels  
1151       After Spinal Cord Injury: The Serum Level of TNF-a in Acute SCI Patients as a Possible Marker  
1152       for Neurological Remission." *Spinal Cord* 53 (7): 510-14. <https://doi.org/10.1038/sc.2015.28>.
- 1153       Bikman, Benjamin T. 2012. "A Role for Sphingolipids in the Pathophysiology of Obesity-Induced  
1154       Inflammation." *Cellular and Molecular Life Sciences* 69 (13): 2135-46. <https://doi.org/10.1007/s0018-012-0917-5>.
- 1155
- 1156       Blomhoff, Rune, and Heidi Kiil Blomhoff. 2006. "Overview of Retinoid Metabolism and Function."  
1157       *Journal of Neurobiology* 66 (7): 606-30. <https://doi.org/10.1002/neu.20242>.
- 1158       Blomster, Linda V., Faith H. Brennan, Hong W. Lao, David W. Harle, Alan R. Harvey, and Marc  
1159       J. Ruitenberg. 2013. "Mobilisation of the Splenic Monocyte Reservoir and Peripheral Cx3cr1  
1160       Deficiency Adversely Affects Recovery from Spinal Cord Injury." *Experimental Neurology* 247

- 1161 (September): 226–40. <https://doi.org/10.1016/j.expneurol.2013.05.002>.
- 1162 Bond, Jennifer E., George J. Cianciolo, and Salvatore V. Pizzo. 2007. "Incorporation of Low Molecular  
1163 Weight Molecules into Alpha2-Macroglobulin by Nucleophilic Exchange." *Biochemical and Bio-  
1164 physical Research Communications* 357 (2): 433–38. <https://doi.org/10.1016/j.bbrc.2007.03.151>.
- 1165 Brennan, Faith H., Richard Gordon, Hong W. Lao, Patrick J. Biggins, Stephen M. Taylor, Robin J.  
1166 M. Franklin, Trent M. Woodruff, and Marc J. Ruitenberg. 2015. "The Complement Receptor  
1167 C5aR Controls Acute Inflammation and Astrogliosis Following Spinal Cord Injury." *Journal of  
1168 Neuroscience* 35 (16): 6517–31. <https://doi.org/10.1523/JNEUROSCI.5218-14.2015>.
- 1169 Brennan, Faith H., Jodie C. E. Hall, Zhen Guan, and Phillip G. Popovich. 2018. "Microglia Limit Le-  
1170 sion Expansion and Promote Functional Recovery After Spinal Cord Injury in Mice," September,  
1171 410258. <https://doi.org/10.1101/410258>.
- 1172 Brennan, Faith H., Trisha Jogia, Ellen R. Gillespie, Linda V. Blomster, Xaria X. Li, Bianca Nowlan,  
1173 Gail M. Williams, et al. 2019. "Complement Receptor C3aR1 Controls Neutrophil Mobilization  
1174 Following Spinal Cord Injury Through Physiological Antagonism of Cxcr2." *JCI Insight* 4 (9). <https://doi.org/10.1172/jci.insight.98254>.
- 1175 Brennan, Faith H., Nyoman D. Kurniawan, Jana Vukovic, Perry F. Bartlett, Fabian Käsermann,  
1176 Thiruma V. Arumugam, Milan Basta, and Marc J. Ruitenberg. 2016. "IVIg Attenuates Comple-  
1177 ment and Improves Spinal Cord Injury Outcomes in Mice." *Annals of Clinical and Translational  
1178 Neurology* 3 (7): 495–511. <https://doi.org/10.1002/acn3.318>.
- 1179 Brown, Sharon J., Gabriel M. B. Harrington, Charlotte H. Hulme, Rachel Morris, Anna Bennett, Wai-  
1180 Hung Tsang, Aheed Osman, Joy Chowdhury, Naveen Kumar, and Karina T. Wright. 2019. "A  
1181 Preliminary Cohort Study Assessing Routine Blood Analyte Levels and Neurological Outcome  
1182 After Spinal Cord Injury." *Journal of Neurotrauma*, July. <https://doi.org/10.1089/neu.2019.6495>.
- 1183 Bruhns, Pierre, and Friederike Jönsson. 2015. "Mouse and Human FcR Effector Functions." *Im-  
1184 munological Reviews* 268 (1): 25–51. <https://doi.org/10.1111/imr.12350>.
- 1185 Buresova, Veronika, Ondrej Hajdusek, Zdenek Franta, Daniel Sojka, and Petr Kopacek. 2009.  
1186 "IrAM—An A2-Macroglobulin from the Hard Tick Ixodes Ricinus: Characterization and Function  
1187 in Phagocytosis of a Potential Pathogen Chryseobacterium Indologenes." *Developmental &  
1188 Comparative Immunology* 33 (4): 489–98. <https://doi.org/10.1016/j.dci.2008.09.011>.
- 1189 Bursill Christina A., Castro Maria L., Beattie Douglas T., Nakhla Shirley, van der Vorst Emiel, Heather  
1190 Alison K., Barter Philip J., and Rye Kerry-Anne. 2010. "High-Density Lipoproteins Suppress  
1191 Chemokines and Chemokine Receptors In Vitro and In Vivo." *Arteriosclerosis, Thrombosis, and  
1192 Vascular Biology* 30 (9): 1773–78. <https://doi.org/10.1161/ATVBAHA.110.211342>.
- 1193 C, Niederau, Backmerhoff F, and Schumacher B. 1997. "Inflammatory Mediators and Acute Phase  
1194 Proteins in Patients with Crohn's Disease and Ulcerative Colitis." *Hepato-Gastroenterology* 44  
1195 (13): 90–107.
- 1196 Cai, Lei, Maria C. de Beer, Frederick C. de Beer, and Deneys R. van der Westhuyzen. 2005. "Serum  
1197 Amyloid A Is a Ligand for Scavenger Receptor Class B Type I and Inhibits High Density Lipopro-  
1198 tein Binding and Selective Lipid Uptake\*." *Journal of Biological Chemistry* 280 (4): 2954–61.  
1199 <https://doi.org/10.1074/jbc.M411555200>.
- 1200 Campbell, Ian K, Sylvia Miescher, Donald R Branch, Patrick J Mott, Alan H Lazarus, Dongji Han, Eu-  
1201 gene Maraskovsky, et al. 2014. "Therapeutic Effect of IVIG on Inflammatory Arthritis in Mice Is  
1202 Dependent on the Fc Portion and Independent of Sialylation or Basophils." *Journal of Immunol-  
1203 ogy (Baltimore, Md.* 192 (11): 5031–38. <https://doi.org/10.4049/jimmunol.1301611>.
- 1204 Campbell, Sandra J., Daniel C. Anthony, Fiona Oakley, Harald Carlsen, Ahmed M. Elsharkawy, Rune  
1205 Blomhoff, and Derek A. Mann. 2008. "Hepatic Nuclear Factor  $\kappa$ B Regulates Neutrophil Recruit-  
1206 ment to the Injured Brain." *Journal of Neuropathology & Experimental Neurology* 67 (3): 223–30.  
1207 <https://doi.org/10.1097/NEN.0b013e3181654957>.
- 1208 Campbell, Sandra J., V. Hugh Perry, Fernando J. Pitossi, Angus G. Butchart, Mariela Chertoff, Sara  
1209 Waters, Robert Dempster, and Daniel C. Anthony. 2005. "Central Nervous System Injury Trig-  
1210 gers Hepatic CC and CXC Chemokine Expression That Is Associated with Leukocyte Mobilization

- 1212 and Recruitment to Both the Central Nervous System and the Liver." *The American Journal of*  
1213 *Pathology* 166 (5): 1487–97. [https://doi.org/10.1016/S0002-9440\(10\)62365-6](https://doi.org/10.1016/S0002-9440(10)62365-6).
- 1214 Campbell, Sandra J., Imran Zahid, Patrick Losey, Shing Law, Yanyan Jiang, Mehmet Bilgen, Nico van  
1215 Rooijen, Damineh Morsali, Andrew E. M. Davis, and Daniel C. Anthony. 2008. "Liver Kupffer Cells  
1216 Control the Magnitude of the Inflammatory Response in the Injured Brain and Spinal Cord." *Neuropharmacology* 55 (5): 780–87. <https://doi.org/10.1016/j.neuropharm.2008.06.074>.
- 1217 Chambers, Matthew C., Brendan Maclean, Robert Burke, Dario Amodei, Daniel L. Ruderman, Stef-  
1218 fen Neumann, Laurent Gatto, et al. 2012. "A Cross-Platform Toolkit for Mass Spectrometry and  
1219 Proteomics." *Nature Biotechnology* 30 (10): 918–20. <https://doi.org/10.1038/nbt.2377>.
- 1220 Chandrasekar, Akila, Florian olde Heuvel, Annette Palmer, Birgit Linkus, Albert C. Ludolph, Tobias  
1221 M. Boeckers, Borna Relja, Markus Huber-Lang, and Francesco Roselli. 2017. "Acute Ethanol  
1222 Administration Results in a Protective Cytokine and Neuroinflammatory Profile in Traumatic  
1223 Brain Injury." *International Immunopharmacology* 51 (October): 66–75. <https://doi.org/10.1016/j.intimp.2017.08.002>.
- 1224 Chang, Zhi-Qiang, Su-Yeon Lee, Hye-Jin Kim, Jung Ran Kim, Su-Jung Kim, In-Kyung Hong, Byung-  
1225 Chul Oh, Cheol-Soo Choi, Ira J. Goldberg, and Tae-Sik Park. 2011. "Endotoxin Activates de  
1226 Novo Sphingolipid Biosynthesis via Nuclear Factor Kappa B-mediated Upregulation of Sptlc2." *Prostaglandins & Other Lipid Mediators* 94 (1): 44–52. <https://doi.org/10.1016/j.prostaglandins.2010.12.003>.
- 1227 Chen, P. S., C. -C. Wang, C. D. Bortner, G. -S. Peng, X. Wu, H. Pang, R. -B. Lu, P. -W. Gean, D. -M.  
1228 Chuang, and J. -S. Hong. 2007. "Valproic Acid and Other Histone Deacetylase Inhibitors Induce  
1229 Microglial Apoptosis and Attenuate Lipopolysaccharide-Induced Dopaminergic Neurotoxicity." *Neuroscience* 149 (1): 203–12. <https://doi.org/10.1016/j.neuroscience.2007.06.053>.
- 1230 Chen, Yuying, Yin He, and Michael J. DeVivo. 2016. "Changing Demographics and Injury Profile  
1231 of New Traumatic Spinal Cord Injuries in the United States, 1972–2014." *Archives of Physical  
1232 Medicine and Rehabilitation* 97 (10): 1610–19. <https://doi.org/10.1016/j.apmr.2016.03.017>.
- 1233 Chio, Jonathon Chon Teng, Jian Wang, Anna Badner, James Hong, Vithushan Surendran, and  
1234 Michael G. Fehlings. 2019. "The Effects of Human Immunoglobulin G on Enhancing Tis-  
1235 sue Protection and Neurobehavioral Recovery After Traumatic Cervical Spinal Cord Injury  
1236 Are Mediated Through the Neurovascular Unit." *Journal of Neuroinflammation* 16 (1): 141.  
1237 <https://doi.org/10.1186/s12974-019-1518-0>.
- 1238 Choi, Meena, Ching-Yun Chang, Timothy Clough, Daniel Brody, Trevor Killeen, Brendan MacLean,  
1239 and Olga Vitek. 2014. "MSstats: An R Package for Statistical Analysis of Quantitative Mass  
1240 Spectrometry-Based Proteomic Experiments." *Bioinformatics* 30 (17): 2524–26. <https://doi.org/10.1093/bioinformatics/btu305>.
- 1241 Chow, Diana S. L., Yang Teng, Elizabeth G. Toups, Bizhan Aarabi, James S. Harrop, Christopher I.  
1242 Shaffrey, Michele M. Johnson, et al. 2012. "Pharmacology of Riluzole in Acute Spinal Cord Injury." *Journal of Neurosurgery: Spine* 17 (Suppl1): 129–40. <https://doi.org/10.3171/2012.5.AOSPINE12112>.
- 1243 Christison, J K, K A Rye, and R Stocker. 1995. "Exchange of Oxidized Cholesteryl Linoleate Between  
1244 LDL and HDL Mediated by Cholesteryl Ester Transfer Protein." *Journal of Lipid Research* 36 (9):  
1245 2017–26. [https://doi.org/10.1016/S0022-2275\(20\)41119-8](https://doi.org/10.1016/S0022-2275(20)41119-8).
- 1246 Cockerill Gillian W., Rye Kerry-Anne, Gamble Jennifer R., Vadas Mathew A., and Barter Philip J. 1995.  
1247 "High-Density Lipoproteins Inhibit Cytokine-Induced Expression of Endothelial Cell Adhesion  
1248 Molecules." *Arteriosclerosis, Thrombosis, and Vascular Biology* 15 (11): 1987–94. <https://doi.org/10.1161/01.ATV.15.11.1987>.
- 1249 Colbert, Melissa C., William W. Rubin, Elwood Linney, and Anthony-Samuel LaMantia. 1995.  
1250 "Retinoid signaling and the generation of regional and cellular diversity in the embryonic mouse  
1251 spinal cord." *Developmental Dynamics* 204 (1): 1–12. <https://doi.org/10.1002/aja.1002040102>.
- 1252 Crispe, Ian N. 2016. "Hepatocytes as Immunological Agents." *The Journal of Immunology* 196 (1):  
1253 17–21. <https://doi.org/10.4049/jimmunol.1501668>.

- 1263 Crozier-Shaw, Geoff, Hazel Denton, and Seamus Morris. 2020. "Management Strategies in Acute  
1264 Traumatic Spinal Cord Injury: A Narrative Review." *Neuroimmunology and Neuroinflammation* 7  
1265 (September). <https://doi.org/10.20517/2347-8659.2019.005>.
- 1266 Czubowicz, Kinga, Henryk Jęśko, Przemysław Wencel, Walter J. Lukiw, and Robert P. Strosznajder.  
1267 2019. "The Role of Ceramide and Sphingosine-1-Phosphate in Alzheimer's Disease and Other  
1268 Neurodegenerative Disorders." *Molecular Neurobiology* 56 (8): 5436–55. <https://doi.org/10.1007/s12035-018-1448-3>.
- 1269 Dalakas, Marinos C. 2014. "Mechanistic Effects of IVIg in Neuroinflammatory Diseases: Conclusions  
1270 Based on Clinicopathologic Correlations." *Journal of Clinical Immunology* 34 (1): 120–26. <https://doi.org/10.1007/s10875-014-0024-5>.
- 1271 Dalgard, Clifton, Jeffrey Cole, William Kean, Jessica Lucky, Gauthaman Sukumar, David McMullen,  
1272 Harvey Pollard, and William Watson. 2012. "The Cytokine Temporal Profile in Rat Cortex After  
1273 Controlled Cortical Impact." *Frontiers in Molecular Neuroscience* 5: 6. <https://doi.org/10.3389/fnmol.2012.00006>.
- 1274 David, Samuel, and Antje Kroner. 2011. "Repertoire of Microglial and Macrophage Responses After  
1275 Spinal Cord Injury." *Nature Reviews Neuroscience* 12 (7): 388–99. <https://doi.org/10.1038/nrn3053>.
- 1276 Davies, Andrew L., Keith C. Hayes, and Gregory A. Dekaban. 2007. "Clinical Correlates of Elevated  
1277 Serum Concentrations of Cytokines and Autoantibodies in Patients With Spinal Cord Injury."  
1278 *Archives of Physical Medicine and Rehabilitation* 88 (11): 1384–93. <https://doi.org/10.1016/j.apmr.2007.08.004>.
- 1279 de Beer, Maria C., Ailing Ji, Anisa Jahangiri, Ashley M. Vaughan, Frederick C. de Beer, Deneys R. van  
1280 der Westhuyzen, and Nancy R. Webb. 2011. "ATP Binding Cassette G1-dependent Cholesterol  
1281 Efflux During Inflammation1." *Journal of Lipid Research* 52 (2): 345–53. <https://doi.org/10.1194/jlr.M012328>.
- 1282 de Beer, Maria C., Nancy R. Webb, Joanne M. Wroblewski, Victoria P. Noffsinger, Debra L. Rateri,  
1283 Ailing Ji, Deneys R. van der Westhuyzen, and Frederick C. de Beer. 2010. "Impact of Serum  
1284 Amyloid A on High Density Lipoprotein Composition and Levels." *Journal of Lipid Research* 51  
1285 (11): 3117–25. <https://doi.org/10.1194/jlr.M005413>.
- 1286 DeLeve, Laurie D. 2007. "Hepatic Microvasculature in Liver Injury." *Seminars in Liver Disease* 27 (04):  
1287 390–400. <https://doi.org/10.1055/s-2007-991515>.
- 1288 Derebe, Mehabaw G, Clare M Zlatkov, Sureka Gattu, Kelly A Ruhn, Shipra Vaishnava, Gretchen E  
1289 Diehl, John B MacMillan, Noelle S Williams, and Lora V Hooper. 2014. "Serum Amyloid A Is a  
1290 Retinol Binding Protein That Transports Retinol During Bacterial Infection." Edited by Fiona M  
1291 Powrie. *eLife* 3 (July): e03206. <https://doi.org/10.7554/elife.03206>.
- 1292 "Devil in the Details." 2011. *Nature* 470 (7334): 305–6. <https://doi.org/10.1038/470305b>.
- 1293 Diraison, Frederique, and Michel Beylot. 1998. "Role of Human Liver Lipogenesis and Reesterification  
1294 in Triglycerides Secretion and in FFA Reesterification." *American Journal of Physiology-Endocrinology and Metabolism* 274 (2): E321–27. <https://doi.org/10.1152/ajpendo.1998.274.2.E321>.
- 1295 Dong, Zhe, Tingting Wu, Weidong Qin, Chuankai An, Zhihao Wang, Mingxiang Zhang, Yun Zhang,  
1296 Cheng Zhang, and Fengshuang An. 2011. "Serum Amyloid A Directly Accelerates the Progression  
1297 of Atherosclerosis in Apolipoprotein E-Deficient Mice." *Molecular Medicine* 17 (11): 1357–64.  
1298 <https://doi.org/10.2119/molmed.2011.00186>.
- 1299 Dulin, Jennifer N., Edward D. Karoly, Ying Wang, Henry W. Strobel, and Raymond J. Grill. 2013.  
1300 "Licofelone Modulates Neuroinflammation and Attenuates Mechanical Hypersensitivity in the  
1301 Chronic Phase of Spinal Cord Injury." *Journal of Neuroscience* 33 (2): 652–64. <https://doi.org/10.1523/JNEUROSCI.6128-11.2013>.
- 1302 Elliot, T. R., M. Kurylo, Y. Chen, and B. Hicken. 2002. "Alcohol Abuse History and Adjustment Following  
1303 Spinal Cord Injury." *Rehabilitation Psychology* 47 (3): 278–90. <https://doi.org/10.1037/0090-5550.47.3.278>.

- 1314 Elliott, David A., Woojin S. Kim, David A. Jans, and Brett Garner. 2007. "Apoptosis Induces Neuronal  
1315 Apolipoprotein-E Synthesis and Localization in Apoptotic Bodies." *Neuroscience Letters* 416 (2):  
1316 206–10. <https://doi.org/10.1016/j.neulet.2007.02.014>.
- 1317 Eng, Jimmy K., Tahmina A. Jahan, and Michael R. Hoopmann. 2013. "Comet: An Open-Source  
1318 MS/MS Sequence Database Search Tool." *PROTEOMICS* 13 (1): 22–24. <https://doi.org/10.1002/pmic.201200439>.
- 1320 Farkas, Gary J., and David R. Gater. 2018. "Neurogenic Obesity and Systemic Inflammation Fol-  
1321 lowing Spinal Cord Injury: A Review." *The Journal of Spinal Cord Medicine* 41 (4): 378–87. <https://doi.org/10.1080/10790268.2017.1357104>.
- 1323 Fleming, Jennifer C., Christopher S. Bailey, Hans Hundt, Kevin R. Gurr, Stewart I. Bailey, Gediminas  
1324 Cepinskas, Abdel-rahman Lawandy, and Amit Badhwar. 2012. "Remote Inflammatory Response  
1325 in Liver Is Dependent on the Segmental Level of Spinal Cord Injury." *Journal of Trauma and Acute  
1326 Care Surgery* 72 (5): 1194–1201. <https://doi.org/10.1097/ta.0b013e31824d68bd>.
- 1327 Friedman, G., P. Froom, L. Sazbon, I. Grinblatt, M. Shochina, J. Tsenter, S. Babaey, A. Ben Yehuda,  
1328 and Z. Groswasser. 1999. "Apolipoprotein E-ε4 Genotype Predicts a Poor Outcome in Survivors  
1329 of Traumatic Brain Injury." *Neurology* 52 (2): 244–44. <https://doi.org/10.1212/WNL.52.2.244>.
- 1330 Frost, Frederick, Mary Jo Roach, Irving Kushner, and Peter Schreiber. 2005. "Inflammatory C-  
1331 reactive Protein and Cytokine Levels in Asymptomatic People with Chronic Spinal Cord Injury."  
1332 *Archives of Physical Medicine and Rehabilitation* 86 (2): 312–17. <https://doi.org/10.1016/j.apmr.2004.02.009>.
- 1334 Fujita, Tomoko, and Shuh Narumiya. 2016. "Roles of Hepatic Stellate Cells in Liver Inflammation: A  
1335 New Perspective." *Inflammation and Regeneration* 36 (1). <https://doi.org/10.1186/s41232-016-0005-6>.
- 1337 Fujiyoshi, Masachika, Masanori Tachikawa, Sumio Ohtsuki, Shingo Ito, Yasuo Uchida, Shin-ichi  
1338 Akanuma, Junichi Kamiie, et al. 2011. "Amyloid-β Peptide(1-40) Elimination from Cerebrospinal  
1339 Fluid Involves Low-Density Lipoprotein Receptor-Related Protein 1 at the Blood-Cerebrospinal  
1340 Fluid Barrier." *Journal of Neurochemistry* 118 (3): 407–15. <https://doi.org/10.1111/j.1471-4159.2011.07311.x>.
- 1342 Fuller, Heidi R., Robert Slade, Nataša Jovanov-Milošević, Mirjana Babić, Goran Sedmak, Goran Šimić,  
1343 Matthew A. Fuszard, Sally L. Shirran, Catherine H. Botting, and Monte A. Gates. 2015. "Stath-  
1344 min Is Enriched in the Developing Corticospinal Tract." *Molecular and Cellular Neuroscience* 69  
1345 (November): 12–21. <https://doi.org/10.1016/j.mcn.2015.09.003>.
- 1346 Furlan, Julio C, Sivakumar Gulasingam, and B Catharine Craven. 2017. "The Health Economics of  
1347 the Spinal Cord Injury or Disease Among Veterans of War : A Systematic Review." *The Journal of  
1348 Spinal Cord Medicine* 40 (6): 649–64. <https://doi.org/10.1080/10790268.2017.1368267>.
- 1349 Gabay, Cem, and Irving Kushner. 1999. "Acute-Phase Proteins and Other Systemic Responses to  
1350 Inflammation." Edited by Franklin H. Epstein. *New England Journal of Medicine* 340 (6): 448–54.  
1351 <https://doi.org/10.1056/NEJM199902113400607>.
- 1352 Gan, Yu, Xunming Ji, Xiaoming Hu, Yumin Luo, Lili Zhang, Peiying Li, Xiangrong Liu, et al. 2012.  
1353 "Transgenic Overexpression of Peroxiredoxin-2 Attenuates Ischemic Neuronal Injury Via Sup-  
1354 pression of a Redox-Sensitive Pro-Death Signaling Pathway." *Antioxidants & Redox Signaling* 17  
1355 (5): 719–32. <https://doi.org/10.1089/ars.2011.4298>.
- 1356 Garcia-Bonilla, Lidia, and Costantino Iadecola. 2012. "Peroxiredoxin Sets the Brain on Fire After  
1357 Stroke." *Nature Medicine* 18 (6): 858–59. <https://doi.org/10.1038/nm.2797>.
- 1358 García-López, P., A. Martínez-Cruz, G. Guízar-Sahagún, and G. Castañeda-Hernández. 2007. "Acute  
1359 Spinal Cord Injury Changes the Disposition of Some, but Not All Drugs Given Intravenously."  
1360 *Spinal Cord* 45 (9): 603–8. <https://doi.org/10.1038/sj.sc.3102001>.
- 1361 Ghasemlou, Nader, Delphine Bouhy, Jingxuan Yang, Rubén López-Vales, Michael Haber, Thusanth  
1362 Thuraisingam, Guoan He, Danuta Radzioch, Aihao Ding, and Samuel David. 2010. "Beneficial  
1363 Effects of Secretory Leukocyte Protease Inhibitor After Spinal Cord Injury." *Brain* 133 (1): 126–38.  
1364 <https://doi.org/10.1093/brain/awp304>.

- 1365 Gill, Varinder, Christopher Doig, Derrice Knight, Emma Love, and Paul Kubes. 2005. "Targeting  
1366 Adhesion Molecules as a Potential Mechanism of Action for Intravenous Immunoglobulin." *Circulation* 112 (13): 2031–39. <https://doi.org/10.1161/CIRCULATIONAHA.105.546150>.
- 1368 Goecks, Jeremy, Anton Nekrutenko, James Taylor, and The Galaxy Team. 2010. "Galaxy: A Com-  
1369 prehensive Approach for Supporting Accessible, Reproducible, and Transparent Computational  
1370 Research in the Life Sciences." *Genome Biology* 11 (8): R86. <https://doi.org/10.1186/gb-2010-11-8-r86>.
- 1372 Gok, Beril, Daniel M. Sciubba, Ozerk Okutan, Etem Beskonakli, Selcuk Palaoglu, Husamettin Er-  
1373 damar, and Mustafa F. Sargon. 2009. "Immunomodulation of Acute Experimental Spinal Cord  
1374 Injury with Human Immunoglobulin G." *Journal of Clinical Neuroscience* 16 (4): 549–53. <https://doi.org/10.1016/j.jocn.2008.04.024>.
- 1376 Goodus, Matthew T., Andrew D. Sauerbeck, Phillip G. Popovich, Richard S. Bruno, and Dana M.  
1377 McTigue. 2018. "Dietary Green Tea Extract Prior to Spinal Cord Injury Prevents Hepatic Iron  
1378 Overload but Does Not Improve Chronic Hepatic and Spinal Cord Pathology in Rats." *Journal of  
1379 Neurotrauma* 35 (24): 2872–82. <https://doi.org/10.1089/neu.2018.5771>.
- 1380 Gordon, A. H., and A. Koj, eds. 1985. *The Acute-phase Response to Injury and Infection: The Roles  
1381 of Interleukin 1 and Other Mediators*. Research Monographs in Cell and Tissue Physiology, v. 10.  
1382 Amsterdam ; New York : New York, NY, USA: Elsevier ; Sole distributors for the USA and Canada,  
1383 Elsevier Science Pub. Co.
- 1384 Greenhalgh, Andrew D., and Samuel David. 2014. "Differences in the Phagocytic Response of Mi-  
1385 croglia and Peripheral Macrophages After Spinal Cord Injury and Its Effects on Cell Death." *The  
1386 Journal of Neuroscience* 34 (18): 6316–22. <https://doi.org/10.1523/JNEUROSCI.4912-13.2014>.
- 1387 Greenhalgh, Andrew D., Juan G. Zarruk, Luke M. Healy, Sam J. Baskar Jesudasan, Priya Jhelum,  
1388 Christopher K. Salmon, Albert Formanek, et al. 2018. "Peripherally Derived Macrophages  
1389 Modulate Microglial Function to Reduce Inflammation After CNS Injury." *PLOS Biology* 16 (10):  
1390 e2005264. <https://doi.org/10.1371/journal.pbio.2005264>.
- 1391 Gris, Denis, Ellis F. Hamilton, and Lynne C. Weaver. 2008. "The Systemic Inflammatory Response  
1392 After Spinal Cord Injury Damages Lungs and Kidneys." *Experimental Neurology* 211 (1): 259–70.  
1393 <https://doi.org/10.1016/j.expneurol.2008.01.033>.
- 1394 Gruys, E., M. J. M. Toussaint, T. A. Niewold, and S. J. Koopmans. 2005. "Acute Phase Reaction  
1395 and Acute Phase Proteins." *Journal of Zhejiang University. Science. B* 6 (11): 1045–56. <https://doi.org/10.1631/jzus.2005.B1045>.
- 1397 Gungor, Bilgi, Emre Adiguzel, Ihsan Gursel, Bilge Yilmaz, and Mayda Gursel. 2016. "Intestinal Mi-  
1398 crobiota in Patients with Spinal Cord Injury." *PLOS ONE* 11 (1): e0145878. <https://doi.org/10.1371/journal.pone.0145878>.
- 1400 Gunnarsson, Martin, and Poul Erik H. Jensen. 1998. "Binding of Soluble Myelin Basic Protein to  
1401 Various Conformational Forms of A2-Macroglobulin." *Archives of Biochemistry and Biophysics*  
1402 359 (2): 192–98. <https://doi.org/10.1006/abbi.1998.0902>.
- 1403 Hall, Jodie C. E., John V. Priestley, V. Hugh Perry, and Adina T. Michael-Titus. 2012. "Docosahex-  
1404 aenoic Acid, but Not Eicosapentaenoic Acid, Reduces the Early Inflammatory Response Fol-  
1405 lowing Compression Spinal Cord Injury in the Rat." *Journal of Neurochemistry* 121 (5): 738–50.  
1406 <https://doi.org/10.1111/j.1471-4159.2012.07726.x>.
- 1407 Hall, Philip K., Lynn P. Nelles, James Travis, and Ronald C. Roberts. 1981. "Proteolytic Cleavage Sites  
1408 on A2-Macroglobulin Resulting in Proteinase Binding Are Different for Trypsin and Staphylococ-  
1409 cus Aureus V-8 Proteinase." *Biochemical and Biophysical Research Communications* 100 (1): 8–16.  
1410 [https://doi.org/10.1016/S0006-291X\(81\)80055-1](https://doi.org/10.1016/S0006-291X(81)80055-1).
- 1411 Haskell, Gloria Thompson, Thomas Michael Maynard, Ron Andrew Shatzmiller, and Anthony-  
1412 Samuel Lamantia. 2002. "Retinoic Acid Signaling at Sites of Plasticity in the Mature  
1413 Central Nervous System." *Journal of Comparative Neurology* 452 (3): 228–41. <https://doi.org/10.1002/cne.10369>.
- 1415 Hasturk, Askin, Basar Atalay, Tarkan Calisaneller, Ozgur Ozdemir, Hakan Oruckaptan, and Nur Al-

- tinors. 2009. "Analysis of Serum Levels After Rat Spinal Cord Ischemia/Reperfusion Injury and Correlation with Tissue Damage." *Turkish Neurosurgery* 19: 353–59.
- Hayes, K.c., T.c.l. Hull, G.a. Delaney, P.j. Potter, K.a.j. Sequeira, K. Campbell, and P.g. Popovich. 2002. "Elevated Serum Titers of Proinflammatory Cytokines and CNS Autoantibodies in Patients with Chronic Spinal Cord Injury." *Journal of Neurotrauma* 19 (6): 753–61. <https://doi.org/10.1089/08977150260139129>.
- Ho, Wei-Te, Kuo-Cheng Yeh, and Shin-Liang Pan. 2021. "Increased Risk of Acute Pancreatitis in Persons with Spinal Cord Injury: A Population-Based, Propensity Score-Matched Longitudinal Follow-up Study." *Spinal Cord*, June, 1–7. <https://doi.org/10.1038/s41393-021-00643-3>.
- Horn, Kevin P., Sarah A. Busch, Alicia L. Hawthorne, Nico van Rooijen, and Jerry Silver. 2008. "Another Barrier to Regeneration in the CNS: Activated Macrophages Induce Extensive Retraction of Dystrophic Axons Through Direct Physical Interactions." *The Journal of Neuroscience* 28 (38): 9330–41. <https://doi.org/10.1523/JNEUROSCI.2488-08.2008>.
- Hulme, C. H., S. J. Brown, H. R. Fuller, J. Riddell, A. Osman, J. Chowdhury, N. Kumar, W. E. Johnson, and K. T. Wright. 2017. "The Developing Landscape of Diagnostic and Prognostic Biomarkers for Spinal Cord Injury in Cerebrospinal Fluid and Blood." *Spinal Cord* 55 (2): 114–25. <https://doi.org/10.1038/sc.2016.174>.
- Hulstaert, Niels, Jim Shofstahl, Timo Sachsenberg, Mathias Walzer, Harald Barsnes, Lennart Martens, and Yasset Perez-Riverol. 2020. "ThermoRawFileParser: Modular, Scalable, and Cross-Platform RAW File Conversion." *Journal of Proteome Research* 19 (1): 537–42. <https://doi.org/10.1021/acs.jproteome.9b00328>.
- Hundt, H., J. C. Fleming, J. T. Phillips, A. Lawendy, K. R. Gurr, S. I. Bailey, D. Sanders, et al. 2011. "Assessment of Hepatic Inflammation After Spinal Cord Injury Using Intravital Microscopy." *Injury* 42 (7): 691–96. <https://doi.org/10.1016/j.injury.2010.12.013>.
- Hurst, Robert E., Przemyslaw Waliszewski, Miroslawa Waliszewska, Rebecca B. Bonner, Doris M. Benbrook, Arindam Dar, and George P. Hemstreet. 1999. "Complexity, Retinoid-Responsive Gene Networks, and Bladder Carcinogenesis." In *Advances in Bladder Research*, edited by Laurence S. Baskin and Simon W. Hayward, 462:449–67. Advances in Experimental Medicine and Biology. Boston, MA: Springer US. [https://doi.org/10.1007/978-1-4615-4737-2\\_35](https://doi.org/10.1007/978-1-4615-4737-2_35).
- Imbach, P., V. d'APUZZO, A. Hirt, E. Rossi, M. Vest, S. Barandun, C. Baumgartner, A. Morell, M. Schöni, and H. P. Wagner. 1981. "HIGH-DOSE INTRAVENOUS GAMMAGLOBULIN FOR IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD." *The Lancet* 317 (8232): 1228–31. [https://doi.org/10.1016/S0140-6736\(81\)92400-4](https://doi.org/10.1016/S0140-6736(81)92400-4).
- Iwata, Akira, Kevin D. Browne, Xiao-Han Chen, Takamichi Yuguchi, and Douglas H. Smith. 2005. "Traumatic Brain Injury Induces Biphasic Upregulation of ApoE and ApoJ Protein in Rats." *Journal of Neuroscience Research* 82 (1): 103–14. <https://doi.org/10.1002/jnr.20607>.
- Jassal, Bijay, Lisa Matthews, Guilherme Viteri, Chuqiao Gong, Pascual Lorente, Antonio Fabregat, Konstantinos Sidiroopoulos, et al. 2020. "The Reactome Pathway Knowledgebase." *Nucleic Acids Research* 48 (D1): D498–503. <https://doi.org/10.1093/nar/gkz1031>.
- Jenne, Craig N., and Paul Kubes. 2013. "Immune Surveillance by the Liver." *Nature Immunology* 14 (10): 996–1006. <https://doi.org/10.1038/ni.2691>.
- Jeong, Seongtae, Beilei Lei, Haichen Wang, Hana N. Dawson, and Michael L. James. 2014. "Intravenous Immunoglobulin G Improves Neurobehavioral and Histological Outcomes After Traumatic Brain Injury in Mice." *Journal of Neuroimmunology* 276 (1): 112–18. <https://doi.org/10.1016/j.jneuroim.2014.08.626>.
- Jha, Amitabh, Daniel P Lammertse, Joseph R Coll, Susan Charlifue, Christopher T Coughlin, Gale G Whiteneck, and Gordon Worley. 2008. "Apolipoprotein E E4 Allele and Outcomes of Traumatic Spinal Cord Injury." *The Journal of Spinal Cord Medicine* 31 (2): 171–76.
- Kaneko, Yoshikatsu, Falk Nimmerjahn, Michael P. Madaio, and Jeffrey V. Ravetch. 2006. "Pathology and Protection in Nephrotoxic Nephritis Is Determined by Selective Engagement of Specific Fc Receptors." *Journal of Experimental Medicine* 203 (3): 789–97. <https://doi.org/10.1084/jem.2005>

- 1467 1900.
- 1468 Karlsson, A.-K. 1999. "Insulin Resistance and Sympathetic Function in High Spinal Cord Injury." *Spinal Cord* 37 (7): 494–500. <https://doi.org/10.1038/sj.sc.3100844>.
- 1469 Kazankov, Konstantin, Simon Mark Dahl Jørgensen, Karen Louise Thomsen, Holger Jon Møller, Hendrik Vilstrup, Jacob George, Detlef Schuppan, and Henning Grønbæk. 2019. "The Role of Macrophages in Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis." *Nature Reviews Gastroenterology & Hepatology* 16 (3): 145–59. <https://doi.org/10.1038/s41575-018-0082-x>.
- 1470 Khan, S. 2004. "Oxidized Caprine Alpha-2-Macroglobulin: Damaged but Not Completely Dysfunctional." *Biochimica Et Biophysica Acta (BBA) - General Subjects*, July. <https://doi.org/10.1016/j.bbagen.2004.06.008>.
- 1471 Kigerl, Kristina A., John C. Gensel, Daniel P. Ankeny, Jessica K. Alexander, Dustin J. Donnelly, and Phillip G. Popovich. 2009. "Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing Either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord." *Journal of Neuroscience* 29 (43): 13435–44. <https://doi.org/10.1523/JNEUROSCI.3257-09.2009>.
- 1472 Kigerl, Kristina A., Jodie C. E. Hall, Lingling Wang, Xiaokui Mo, Zhongtang Yu, and Phillip G. Popovich. 2016. "Gut Dysbiosis Impairs Recovery After Spinal Cord Injury." *Journal of Experimental Medicine* 213 (12): 2603–20. <https://doi.org/10.1084/jem.20151345>.
- 1473 Kigerl, Kristina A., Violeta M. McGaughy, and Phillip G. Popovich. 2006. "Comparative Analysis of Lesion Development and Intraspinal Inflammation in Four Strains of Mice Following Spinal Contusion Injury." *Journal of Comparative Neurology* 494 (4): 578–94. <https://doi.org/10.1002/cn.e.20827>.
- 1474 Kigerl, Kristina A., Klauss Mostacada, and Phillip G. Popovich. 2018. "Gut Microbiota Are Disease-Modifying Factors After Traumatic Spinal Cord Injury." *Neurotherapeutics* 15 (1): 60–67. <https://doi.org/10.1007/s13311-017-0583-2>.
- 1475 Kim, Hyeon Ju, Michael Rowe, Ming Ren, Jau-Shyong Hong, Po-See Chen, and De-Maw Chuang. 2007. "Histone Deacetylase Inhibitors Exhibit Anti-Inflammatory and Neuroprotective Effects in a Rat Permanent Ischemic Model of Stroke: Multiple Mechanisms of Action." *Journal of Pharmacology and Experimental Therapeutics* 321 (3): 892–901. <https://doi.org/10.1124/jpet.107.120188>.
- 1476 Kim, Myung-Hee, Maria C. de Beer, Joanne M. Wroblewski, Nancy R. Webb, and Frederick C. de Beer. 2013. "SAA Does Not Induce Cytokine Production in Physiological Conditions." *Cytokine* 61 (2): 506–12. <https://doi.org/10.1016/j.cyto.2012.10.019>.
- 1477 Kim, So Yong, Tae Jin Kim, and Ki-Young Lee. 2008. "A Novel Function of Peroxiredoxin 1 (Prx-1) in Apoptosis Signal-Regulating Kinase 1 (Ask1)-Mediated Signaling Pathway." *FEBS Letters* 582 (13): 1913–18. <https://doi.org/10.1016/j.febslet.2008.05.015>.
- 1478 Kisilevsky, R. 1991. "Serum Amyloid A (SAA), a Protein Without a Function: Some Suggestions with Reference to Cholesterol Metabolism." *Medical Hypotheses* 35 (4): 337–41. [https://doi.org/10.1016/0306-9877\(91\)90280-C](https://doi.org/10.1016/0306-9877(91)90280-C).
- 1479 Kisilevsky, Robert, and Paul N. Manley. 2012. "Acute-Phase Serum Amyloid A: Perspectives on Its Physiological and Pathological Roles." *Amyloid* 19 (1): 5–14. <https://doi.org/10.3109/13506129.2011.654294>.
- 1480 Kisilevsky, R., and L. Subrahmanyam. 1992. "Serum Amyloid A Changes High Density Lipoprotein's Cellular Affinity. A Clue to Serum Amyloid A's Principal Function." *Laboratory Investigation; a Journal of Technical Methods and Pathology* 66 (6): 778–85.
- 1481 Komine-Kobayashi, Miki, Nei Chou, Hideki Mochizuki, Atsuhito Nakao, Yoshikuni Mizuno, and Takao Urabe. 2004. "Dual Role of Fc $\gamma$  Receptor in Transient Focal Cerebral Ischemia in Mice." *Stroke* 35 (4): 958–63. <https://doi.org/10.1161/01.STR.0000120321.30916.8E>.
- 1482 Kwon, Brian K., Ona Bloom, Ina-Beate Wanner, Armin Curt, Jan M. Schwab, James Fawcett, and Kevin K. Wang. 2019. "Neurochemical Biomarkers in Spinal Cord Injury." *Spinal Cord* 57 (10): 819–31. <https://doi.org/10.1038/s41393-019-0319-8>.
- 1483 Kwon, Brian K, Anthea M T Stammers, Lise M Belanger, Arlene Bernardo, Donna Chan, Carole M Bishop, Gerard P Slobogean, et al. 2010. "Cerebrospinal Fluid Inflammatory Cytokines and

- 1518 Biomarkers of Injury Severity in Acute Human Spinal Cord Injury." *Journal of Neurotrauma* 27  
1519 (4): 669–82. <https://doi.org/10.1089/neu.2009.1080>.
- 1520 Lane, Michelle A., and Sarah J. Bailey. 2005. "Role of Retinoid Signalling in the Adult Brain." *Progress*  
1521 *in Neurobiology* 75 (4): 275–93. <https://doi.org/10.1016/j.pneurobio.2005.03.002>.
- 1522 Lapointe, Benoît M., Leonie M. Herx, Varinder Gill, Luanne M. Metz, and Paul Kubes. 2004. "IVIg  
1523 Therapy in Brain Inflammation: Etiology-Dependent Differential Effects on Leucocyte Recruit-  
1524 ment." *Brain* 127 (12): 2649–56. <https://doi.org/10.1093/brain/awh297>.
- 1525 Larios, Jorge A., and Maria-Paz Marzolo. 2012. "Novel Aspects of the Apolipoprotein-E Receptor  
1526 Family: Regulation and Functional Role of Their Proteolytic Processing." *Frontiers in Biology* 7  
1527 (2): 113–43. <https://doi.org/10.1007/s11515-011-1186-7>.
- 1528 Lavoie, J. -M., and M. -S. Gauthier. 2006. "Regulation of Fat Metabolism in the Liver: Link to Non-  
1529 Alcoholic Hepatic Steatosis and Impact of Physical Exercise." *Cellular and Molecular Life Sciences*  
1530 *CMLS* 63 (12): 1393–1409. <https://doi.org/10.1007/s00018-006-6600-y>.
- 1531 Lee, Matthew, Jonathan Myers, Amy Hayes, Sherna Madan, Victor F. Froelicher, Inder Perkash, and  
1532 B. Jenny Kiratli. 2004. "C-Reactive Protein, Metabolic Syndrome, and Insulin Resistance in In-  
1533 dividuals With Spinal Cord Injury." *The Journal of Spinal Cord Medicine* 28 (1): 20–25. <https://doi.org/10.1080/10790268.2005.11753794>.
- 1535 Lin, Zhenxin, Andy Lo, Diane M. Simeone, Mack T. Ruffin, and David M. Lubman. 2012. "An N-  
1536 glycosylation Analysis of Human Alpha-2-Macroglobulin Using an Integrated Approach." *Journal*  
1537 *of Proteomics & Bioinformatics* 5 (May): 127–34. <https://doi.org/10.4172/jpb.1000224>.
- 1538 Lindborg, Jane A., Matthias Mack, and Richard E. Zigmond. 2017. "Neutrophils Are Critical for  
1539 Myelin Removal in a Peripheral Nerve Injury Model of Wallerian Degeneration." *Journal of Neu-  
1540 roscience* 37 (43): 10258–77. <https://doi.org/10.1523/JNEUROSCI.2085-17.2017>.
- 1541 Liu, Chia-Chen, Takahisa Kanekiyo, Huaxi Xu, and Guojun Bu. 2013. "Apolipoprotein E and  
1542 Alzheimer Disease: Risk, Mechanisms, and Therapy." *Nature Reviews. Neurology* 9 (2): 106–18.  
1543 <https://doi.org/10.1038/nrneurol.2012.263>.
- 1544 Liu, JianWei, Hong An, DianMing Jiang, Wei Huang, HaiBo Zou, ChunYang Meng, and HongYu Li.  
1545 2004. "Study of Bacterial Translocation From Gut After Paraplegia Caused by Spinal Cord Injury  
1546 in Rats." *Spine* 29 (2): 164–69. <https://doi.org/10.1097/01.BRS.0000107234.74249.CD>.
- 1547 Low, Felicia M., Mark B. Hampton, and Christine C. Winterbourn. 2008. "Peroxiredoxin 2 and Per-  
1548 oxide Metabolism in the Erythrocyte." *Antioxidants & Redox Signaling* 10 (9): 1621–30. <https://doi.org/10.1089/ars.2008.2081>.
- 1550 Lu, Jinghua, Yadong Yu, Iowis Zhu, Yifan Cheng, and Peter D. Sun. 2014. "Structural Mechanism of  
1551 Serum Amyloid A-mediated Inflammatory Amyloidosis." *Proceedings of the National Academy of*  
1552 *Sciences* 111 (14): 5189–94. <https://doi.org/10.1073/pnas.1322357111>.
- 1553 Lu, Yue, Xiang-Sheng Zhang, Zi-Huan Zhang, Xiao-Ming Zhou, Yong-Yue Gao, Guang-Jie Liu, Han  
1554 Wang, Ling-Yun Wu, Wei Li, and Chun-Hua Hang. 2018. "Peroxiredoxin 2 Activates Microglia by  
1555 Interacting with Toll-like Receptor 4 After Subarachnoid Hemorrhage." *Journal of Neuroinflam-  
1556 mation* 15 (1): 87. <https://doi.org/10.1186/s12974-018-1118-4>.
- 1557 Lu, Yue, Xiang-Sheng Zhang, Xiao-Ming Zhou, Yong-Yue Gao, Chun-Lei Chen, Jing-Peng Liu, Zhen-  
1558 Nan Ye, et al. 2019. "Peroxiredoxin 1/2 Protects Brain Against H2O2-induced Apoptosis After  
1559 Subarachnoid Hemorrhage." *The FASEB Journal* 33 (2): 3051–62. [https://doi.org/10.1096/fj.201801150R](https://doi.org/10.1096/fj.201<br/>1560 801150R).
- 1561 Lubieniecka, Joanna M., Femke Streijger, Jae H. T. Lee, Nikolay Stoynov, Jie Liu, Randy Mottus, Tom  
1562 Pfeifer, et al. 2011. "Biomarkers for Severity of Spinal Cord Injury in the Cerebrospinal Fluid of  
1563 Rats." *PLOS ONE* 6 (4): e19247. <https://doi.org/10.1371/journal.pone.0019247>.
- 1564 Lünemann, Jan D., Falk Nimmerjahn, and Marinos C. Dalakas. 2015. "Intravenous Immunoglobulin  
1565 in Neurology—mode of Action and Clinical Efficacy." *Nature Reviews Neurology* 11 (2): 80–89.  
1566 <https://doi.org/10.1038/nrneurol.2014.253>.
- 1567 Mackness, Michael I., Paul N. Durrington, and Bharti Mackness. 2004. "The Role of Paraoxonase  
1568 1 Activity in Cardiovascular Disease." *American Journal of Cardiovascular Drugs* 4 (4): 211–17.

- 1569 https://doi.org/10.2165/00129784-200404040-00002.
- 1570 Maden, M., D. E. Ong, and F. Chytil. 1990. "Retinoid-Binding Protein Distribution in the Developing  
1571 Mammalian Nervous System." *Development* 109 (1): 75–80.
- 1572 Mahley, Robert W., and Stanley C. Rall. 2000. "Apolipoprotein E: Far More Than a Lipid Transport  
1573 Protein." *Annual Review of Genomics and Human Genetics* 1 (1): 507–37. https://doi.org/10.1146/  
1574 annurev.genom.1.1.507.
- 1575 Mahley, Robert W., Karl H. Weisgraber, and Yadong Huang. 2006. "Apolipoprotein E4: A Causative  
1576 Factor and Therapeutic Target in Neuropathology, Including Alzheimer's Disease." *Proceedings  
1577 of the National Academy of Sciences* 103 (15): 5644–51. https://doi.org/10.1073/pnas.060054910  
1578 3.
- 1579 Maikos, Jason T., and David I. Shreiber. 2007. "Immediate Damage to The Blood-Spinal Cord Barrier  
1580 Due to Mechanical Trauma." *Journal of Neurotrauma* 24 (3): 492–507. https://doi.org/10.1089/  
1581 neu.2006.0149.
- 1582 Malaspina, Andrea, Narendra Kaushik, and Jackie De Belleroche. 2001. "Differential Expression of  
1583 14 Genes in Amyotrophic Lateral Sclerosis Spinal Cord Detected Using Gridded cDNA Arrays."  
1584 *Journal of Neurochemistry* 77 (1): 132–45. https://doi.org/10.1046/j.1471-4159.2001.00231.x.
- 1585 Maruyama, Y., M. Mizuguchi, T. Yaginuma, M. Kusaka, H. Yoshida, K. Yokoyama, Y. Kasahara, and  
1586 T. Hosoya. 2008. "Serum Leptin, Abdominal Obesity and the Metabolic Syndrome in Individuals  
1587 with Chronic Spinal Cord Injury." *Spinal Cord* 46 (7): 494–99. https://doi.org/10.1038/sj.sc.3102  
1588 171.
- 1589 Marzi, Carola, Cornelia Huth, Christian Herder, Jens Baumert, Barbara Thorand, Wolfgang Rath-  
1590 man, Christa Meisinger, et al. 2013. "Acute-Phase Serum Amyloid A Protein and Its Implication  
1591 in the Development of Type 2 Diabetes in the KORA S4/F4 Study." *Diabetes Care* 36 (5): 1321–26.  
1592 https://doi.org/10.2337/dc12-1514.
- 1593 Matsuzawa, Atsushi, Kaoru Saegusa, Takuwa Noguchi, Chiharu Sadamitsu, Hideki Nishi-  
1594 toh, Shigenori Nagai, Shigeo Koyasu, Kunihiro Matsumoto, Kohsuke Takeda, and Hide-  
1595 nori Ichijo. 2005. "ROS-dependent Activation of the TRAF6-ASK1-p38 Pathway Is Selec-  
1596 tively Required for TLR4-mediated Innate Immunity." *Nature Immunology* 6 (6): 587–92.  
1597 https://doi.org/10.1038/ni1200.
- 1598 McDaid, David, A.-La Park, Angela Gall, Mariel Purcell, and Mark Bacon. 2019. "Understanding and  
1599 Modelling the Economic Impact of Spinal Cord Injuries in the United Kingdom." *Spinal Cord* 57  
1600 (9): 778–88. https://doi.org/10.1038/s41393-019-0285-1.
- 1601 Meek, R. L., S. Urieli-Shoval, and E. P. Benditt. 1994. "Expression of Apolipoprotein Serum Amyloid  
1602 A mRNA in Human Atherosclerotic Lesions and Cultured Vascular Cells: Implications for Serum  
1603 Amyloid A Function." *Proceedings of the National Academy of Sciences* 91 (8): 3186–90. https:  
1604 //doi.org/10.1073/pnas.91.8.3186.
- 1605 Milosevic, Ivana, Ankica Vujoovic, Aleksandra Barac, Marina Djelic, Milos Korac, Aleksandra  
1606 Radovanovic Spurnic, Ivana Gmizic, et al. 2019. "Gut-Liver Axis, Gut Microbiota, and Its  
1607 Modulation in the Management of Liver Diseases: A Review of the Literature." *International  
1608 Journal of Molecular Sciences* 20 (2): 395. https://doi.org/10.3390/ijms20020395.
- 1609 Mimura, Yoshihiro, Shotaro Sakisaka, Masaru Harada, Michio Sata, and Kyuichi Tanikawa. 1995.  
1610 "Role of Hepatocytes in Direct Clearance of Lipopolysaccharide in Rats." *Gastroenterology* 109  
1611 (6): 1969–76. https://doi.org/10.1016/0016-5085(95)90765-3.
- 1612 Mishra, Aarti, and Roberta D. Brinton. 2018. "Inflammation: Bridging Age, Menopause and Apoe4  
1613 Genotype to Alzheimer's Disease." *Frontiers in Aging Neuroscience* 10. https://doi.org/10.3389/  
1614 fnagi.2018.00312.
- 1615 Miyata, M., and J. D. Smith. 1996. "Apolipoprotein E Allele-Specific Antioxidant Activity and Effects  
1616 on Cytotoxicity by Oxidative Insults and Beta-Amyloid Peptides." *Nature Genetics* 14 (1): 55–61.  
1617 https://doi.org/10.1038/ng0996-55.
- 1618 Myers, Jonathan, Matthew Lee, and Jenny Kiratli. 2007. "Cardiovascular Disease in Spinal Cord  
1619 Injury: An Overview of Prevalence, Risk, Evaluation, and Management." *American Journal of*

- 1620        *Physical Medicine & Rehabilitation* 86 (2): 142–52. <https://doi.org/10.1097/PHM.0b013e31802f0247>.
- 1621        Myers, Scott A., Leila Gobejishvili, Sujata Saraswat Ohri, C. Garrett Wilson, Kariena R. Andres, Am-  
1622        berly S. Riegler, Hridgandh Donde, Swati Joshi-Barve, Shirish Barve, and Scott R. Whittemore.  
1623        2019. “Following Spinal Cord Injury, Pde4b Drives an Acute, Local Inflammatory Response and  
1624        a Chronic, Systemic Response Exacerbated by Gut Dysbiosis and Endotoxemia.” *Neurobiology of Disease* 124 (April): 353–63. <https://doi.org/10.1016/j.nbd.2018.12.008>.
- 1625        Nagele, Eric P., Min Han, Nimish K. Acharya, Cassandra DeMarshall, Mary C. Kosciuk, and Robert  
1626        G. Nagele. 2013. “Natural IgG Autoantibodies Are Abundant and Ubiquitous in Human Sera,  
1627        and Their Number Is Influenced By Age, Gender, and Disease.” *PLOS ONE* 8 (4): e60726. <https://doi.org/10.1371/journal.pone.0060726>.
- 1628        Nahrendorf, Matthias, Filip K. Swirski, Elena Aikawa, Lars Stangenberg, Thomas Wurdinger, Jose-  
1629        Luiz Figueiredo, Peter Libby, Ralph Weissleder, and Mikael J. Pittet. 2007. “The Healing My-  
1630        ocardium Sequentially Mobilizes Two Monocyte Subsets with Divergent and Complementary  
1631        Functions.” *The Journal of Experimental Medicine* 204 (12): 3037–47. <https://doi.org/10.1084/jem.20070885>.
- 1632        Narang, Aarti, Fei Qiao, Carl Atkinson, Hong Zhu, Xiaofeng Yang, Liudmila Kulik, V. Michael Holers,  
1633        and Stephen Tomlinson. 2017. “Natural IgM Antibodies That Bind Neoepitopes Exposed as  
1634        a Result of Spinal Cord Injury , Drive Secondary Injury by Activating Complement.” *Journal of Neuroinflammation* 14 (1): 120. <https://doi.org/10.1186/s12974-017-0894-6>.
- 1635        Nguyen, Dung Hoang, Newton Cho, Kajana Satkunendarajah, James W Austin, Jian Wang, and Michael G Fehlings. 2012. “Immunoglobulin G (IgG) Attenuates Neuroinflammation and Im-  
1636        proves Neurobehavioral Recovery After Cervical Spinal Cord Injury.” *Journal of Neuroinflamma-  
1637        tion* 9 (September): 224. <https://doi.org/10.1186/1742-2094-9-224>.
- 1638        O’Connor, Gregory, Elisabeth Jeffrey, Derik Madorma, Alexander Marcillo, Maria T. Abreu, Sapna K.  
1639        Deo, W. Dalton Dietrich, and Sylvia Daunert. 2018. “Investigation of Microbiota Alterations and  
1640        Intestinal Inflammation Post-Spinal Cord Injury in Rat Model.” *Journal of Neurotrauma* 35 (18):  
1641        2159–66. <https://doi.org/10.1089/neu.2017.5349>.
- 1642        Okada, Seiji. 2016. “The Pathophysiological Role of Acute Inflammation After Spinal Cord Injury.”  
1643        *Inflammation and Regeneration* 36 (October): 20. <https://doi.org/10.1186/s41232-016-0026-1>.
- 1644        Pagadala, Mangesh, Takhar Kasumov, Arthur J. McCullough, Nizar N. Zein, and John P. Kirwan.  
1645        2012. “Role of Ceramides in Nonalcoholic Fatty Liver Disease.” *Trends in Endocrinology &  
1646        Metabolism* 23 (8): 365–71. <https://doi.org/10.1016/j.tem.2012.04.005>.
- 1647        Palmers, Ilse, Elke Ydens, Eric Put, Bart Depreitere, Helma Bongers-Janssen, Peter Pickkers, Sven  
1648        Hendrix, and Veerle Somers. 2016. “Antibody Profiling Identifies Novel Antigenic Targets in  
1649        Spinal Cord Injury Patients.” *Journal of Neuroinflammation* 13 (1): 243. <https://doi.org/10.1186/s12974-016-0713-5>.
- 1650        Park, Jin-Sun, Moon-Sook Woo, So-Young Kim, Won-Ki Kim, and Hee-Sun Kim. 2005. “Repression  
1651        of Interferon- $\gamma$ -Induced Inducible Nitric Oxide Synthase (iNOS) Gene Expression in Microglia by  
1652        Sodium Butyrate Is Mediated Through Specific Inhibition of ERK Signaling Pathways.” *Journal of Neuroimmunology* 168 (1): 56–64. <https://doi.org/10.1016/j.jneuroim.2005.07.003>.
- 1653        Pepys, M. B., and Marilyn L. Baltz. 1983. “Acute Phase Proteins with Special Reference to C-  
1654        Reactive Protein and Related Proteins (Pentaxins) and Serum Amyloid A Protein.” In *Advances  
1655        in Immunology*, edited by Frank J. Dixon and Henry G. Kunkel, 34:141–212. Academic Press.  
1656        [https://doi.org/10.1016/S0065-2776\(08\)60379-X](https://doi.org/10.1016/S0065-2776(08)60379-X).
- 1657        Perussia, B, M M Tutt, W Q Qiu, W A Kuziel, P W Tucker, G Trinchieri, M Bennett, J V Ravetch, and  
1658        V Kumar. 1989. “Murine Natural Killer Cells Express Functional Fc Gamma Receptor II Encoded  
1659        by the Fc Gamma R Alpha Gene.” *Journal of Experimental Medicine* 170 (1): 73–86. <https://doi.org/10.1084/jem.170.1.73>.
- 1660        Peterson, P. A. 1971. “Studies on the Interaction Between Prealbumin, Retinol-Binding Protein, and  
1661        Vitamin A.” *The Journal of Biological Chemistry* 246 (1): 44–49.
- 1662

- 1671 Peterson, Sheri L., and Aileen J. Anderson. 2014. "Complement and Spinal Cord Injury: Traditional  
1672 and Non-Traditional Aspects of Complement Cascade Function in the Injured Spinal Cord Microenvironment." *Experimental Neurology*, Special Issue: Neuroimmunology of spinal cord injury, 258 (August): 35–47. <https://doi.org/10.1016/j.expneurol.2014.04.028>.
- 1673 Pierani, Alessandra, Susan Brenner-Morton, Chin Chiang, and Thomas M Jessell. 1999. "A Sonic Hedgehog-Independent, Retinoid-Activated Pathway of Neurogenesis in the Ventral Spinal Cord." *Cell* 97 (7): 903–15. [https://doi.org/10.1016/S0092-8674\(00\)80802-8](https://doi.org/10.1016/S0092-8674(00)80802-8).
- 1674 Pineau, Isabelle, and Steve Lacroix. 2006. "Proinflammatory Cytokine Synthesis in the Injured Mouse Spinal Cord : Multiphasic Expression Pattern and Identification of the Cell Types Involved." *Journal of Comparative Neurology* 285 (2): 267–85. <https://doi.org/10.1002/cne.21149>.
- 1675 Poirier, Judes. 1994. "Apolipoprotein E in Animal Models of CNS Injury and in Alzheimer's Disease." *Trends in Neurosciences* 17 (12): 525–30. [https://doi.org/10.1016/0166-2236\(94\)90156-2](https://doi.org/10.1016/0166-2236(94)90156-2).
- 1676 Popovich, Phillip G., Zhen Guan, Ping Wei, Inge Huitinga, Nico van Rooijen, and Bradford T. Stokes. 1999. "Depletion of Hematogenous Macrophages Promotes Partial Hindlimb Recovery and Neuroanatomical Repair After Experimental Spinal Cord Injury." *Experimental Neurology* 158 (2): 351–65. <https://doi.org/10.1006/exnr.1999.7118>.
- 1677 Prüss, Harald, Marcel A Kopp, Benedikt Brommer, Nicole Gatzemeier, Ines Laginha, Ulrich Dirnagl, and Jan M Schwab. 2011. "Non-Resolving Aspects of Acute Inflammation After Spinal Cord Injury (SCI): Indices and Resolution Plateau." *Brain Pathology (Zurich, Switzerland)* 21 (6): 652–60. <https://doi.org/10.1111/j.1750-3639.2011.00488.x>.
- 1678 Qiao, Fei, Carl Atkinson, Hongbin Song, Ravinder Pannu, Inderjit Singh, and Stephen Tomlinson. 2006. "Complement Plays an Important Role in Spinal Cord Injury and Represents a Therapeutic Target for Improving Recovery Following Trauma." *The American Journal of Pathology* 169 (3): 1039–47. <https://doi.org/10.2353/ajpath.2006.060248>.
- 1679 Rehman, Ahmed A., Haseeb Ahsan, and Fahim H. Khan. 2013. "Alpha-2-Macroglobulin: A Physiological Guardian." *Journal of Cellular Physiology* 228 (8): 1665–75. <https://doi.org/10.1002/jcp.24266>.
- 1680 Rhee, Sue Goo, and Hyun Ae Woo. 2011. "Multiple Functions of Peroxiredoxins: Peroxidases, Sensors and Regulators of the Intracellular Messenger H<sub>2</sub>O<sub>2</sub>, and Protein Chaperones." *Antioxidants & Redox Signaling* 15 (3): 781–94. <https://doi.org/10.1089/ars.2010.3393>.
- 1681 Röst, Hannes L., Timo Sachsenberg, Stephan Aiche, Chris Bielow, Hendrik Weisser, Fabian Aicheler, Sandro Andreotti, et al. 2016. "OpenMS: A Flexible Open-Source Software Platform for Mass Spectrometry Data Analysis." *Nature Methods* 13 (9): 741–48. <https://doi.org/10.1038/nmeth.3959>.
- 1682 Salzano, Sonia, Paola Checconi, Eva-Maria Hanschmann, Christopher Horst Lillig, Lucas D. Bowler, Philippe Chan, David Vaudry, et al. 2014. "Linkage of Inflammation and Oxidative Stress via Release of Glutathionylated Peroxiredoxin-2, Which Acts as a Danger Signal." *Proceedings of the National Academy of Sciences* 111 (33): 12157–62. <https://doi.org/10.1073/pnas.1401712111>.
- 1683 Samuelsson, Astrid, Terri L. Towers, and Jeffrey V. Ravetch. 2001. "Anti-Inflammatory Activity of IVIG Mediated Through the Inhibitory Fc Receptor." *Science* 291 (5503): 484–86. <https://doi.org/10.1126/science.291.5503.484>.
- 1684 Santo, Carmela De, Ramon Arscott, Sarah Booth, Ioannis Karydis, Margaret Jones, Ruth Asher, Mariolina Salio, Mark Middleton, and Vincenzo Cerundolo. 2010. "Invariant NKT Cells Modulate the Suppressive Activity of IL-10-secreting Neutrophils Differentiated with Serum Amyloid A." *Nature Immunology* 11 (11): 1039–46. <https://doi.org/10.1038/ni.1942>.
- 1685 Sauerbeck, Andrew D., J. Lukas Laws, Veera V. R. Bandaru, Phillip G. Popovich, Norman J. Haughey, and Dana M. McTigue. 2014. "Spinal Cord Injury Causes Chronic Liver Pathology in Rats." *Journal of Neurotrauma* 32 (3): 159–69. <https://doi.org/10.1089/neu.2014.3497>.
- 1686 Schilling, Joel D., Heather M. Machkovech, Li He, Rohini Sidhu, Hideji Fujiwara, Kassandra Weber, Daniel S. Ory, and Jean E. Schaffer. 2013. "Palmitate and Lipopolysaccharide Trigger Synergistic Ceramide Production in Primary Macrophages \*." *Journal of Biological Chemistry* 288 (5): 2923–

- 1722 32. <https://doi.org/10.1074/jbc.M112.419978>.
- 1723 Schneider, Christoph, Simone Wicki, Stefanie Graeter, Tankica M. Timcheva, Christian W. Keller,  
1724 Isaak Quast, Danila Leontyev, et al. 2017. "IVIG Regulates the Survival of Human but Not Mouse  
1725 Neutrophils." *Scientific Reports* 7 (1): 1296. <https://doi.org/10.1038/s41598-017-01404-0>.
- 1726 Schwab, Inessa, and Falk Nimmerjahn. 2013. "Intravenous Immunoglobulin Therapy: How Does  
1727 IgG Modulate the Immune System?" *Nature Reviews Immunology* 13 (3): 176–89. [g/10.1038/nri3401](https://doi.or<br/>1728 g/10.1038/nri3401).
- 1729 Segal, J. L., E. Gonzales, S. Yousefi, L. Jamshidipour, and S. R. Brunnemann. 1997. "Circulating Levels  
1730 of IL-2r, ICAM-1, and IL-6 in Spinal Cord Injuries." *Archives of Physical Medicine and Rehabilitation*  
1731 78 (1): 44–47. [https://doi.org/10.1016/s0003-9993\(97\)90008-3](https://doi.org/10.1016/s0003-9993(97)90008-3).
- 1732 Seitz, Alexander, Maja Kragol, Elsa Aglow, Louise Showe, and Ellen Heber-Katz. 2003. "Apolipoprotein  
1733 E Expression After Spinal Cord Injury in the Mouse." *Journal of Neuroscience Research* 71 (3):  
1734 417–26. <https://doi.org/10.1002/jnr.10482>.
- 1735 Shah, Chandrabala, Ranjeeta Hari-Dass, and John G. Raynes. 2006. "Serum Amyloid A Is an Innate  
1736 Immune Opsonin for Gram-negative Bacteria." *Blood* 108 (5): 1751–57. [https://doi.org/10.1182/blood-2005-11-011932](https://doi.org/10.118<br/>1737 2/blood-2005-11-011932).
- 1738 Shavelle, Robert M., Michael J. DeVivo, Jordan C. Brooks, David J. Strauss, and David R. Paculdo.  
1739 2015. "Improvements in Long-Term Survival After Spinal Cord Injury?" *Archives of Physical  
1740 Medicine and Rehabilitation* 96 (4): 645–51. <https://doi.org/10.1016/j.apmr.2014.11.003>.
- 1741 Shechter, Ravid, Anat London, Chen Varol, Catarina Raposo, Melania Cusimano, Gili Yovel, Asya  
1742 Rolls, et al. 2009. "Infiltrating Blood-Derived Macrophages Are Vital Cells Playing an Anti-  
1743 inflammatory Role in Recovery from Spinal Cord Injury in Mice." *PLOS Medicine* 6 (7): e1000113.  
1744 <https://doi.org/10.1371/journal.pmed.1000113>.
- 1745 Shichita, Takashi, Eiichi Hasegawa, Akihiro Kimura, Rimpei Morita, Ryota Sakaguchi, Ichiro Takada,  
1746 Takashi Sekiya, et al. 2012. "Peroxiredoxin Family Proteins Are Key Initiators of Post-Ischemic  
1747 Inflammation in the Brain." *Nature Medicine* 18 (6): 911–17. <https://doi.org/10.1038/nm.2749>.
- 1748 Silva, Barbara Ferreira da, Chen Meng, Dominic Helm, Fiona Pachl, Jürgen Schiller, Emad Ibrahim,  
1749 Charles M. Lynne, Nancy L. Brackett, Ricardo Pimenta Bertolla, and Bernhard Kuster. 2016. "To-  
1750 wards Understanding Male Infertility After Spinal Cord Injury Using Quantitative Proteomics."  
1751 *Molecular & Cellular Proteomics* 15 (4): 1424–34. <https://doi.org/10.1074/mcp.M115.052175>.
- 1752 Smith, C, D I Graham, L S Murray, J Stewart, and J A R Nicoll. 2006. "Association of APOE E4 and  
1753 Cerebrovascular Pathology in Traumatic Brain Injury." *Journal of Neurology, Neurosurgery, and  
1754 Psychiatry* 77 (3): 363–66. <https://doi.org/10.1136/jnnp.2005.074617>.
- 1755 Sobrido-Cameán, Daniel, and Antón Barreiro-Iglesias. 2018. "Role of Caspase-8 and Fas in Cell  
1756 Death After Spinal Cord Injury." *Frontiers in Molecular Neuroscience* 11: 101. [https://doi.org/10.3389/fnmol.2018.00101](https://doi.org/10<br/>1757 .3389/fnmol.2018.00101).
- 1758 Sockanathan, Shanthini, and Thomas M Jessell. 1998. "Motor Neuron-Derived Retinoid Signaling  
1759 Specifies the Subtype Identity of Spinal Motor Neurons." *Cell* 94 (4): 503–14. [https://doi.org/10.1016/S0092-8674\(00\)81591-3](https://doi.org/10<br/>1760 .1016/S0092-8674(00)81591-3).
- 1761 Sofroniew, Michael V., and Harry V. Vinters. 2010. "Astrocytes: Biology and Pathology." *Acta Neu-  
1762 ropathologica* 119 (1): 7–35. <https://doi.org/10.1007/s00401-009-0619-8>.
- 1763 Song, Guoqing, Cate Cechvala, Daniel K. Resnick, Robert J. Dempsey, and Vemuganti L. Raghaven-  
1764 dra Rao. 2001. "GeneChip Analysis After Acute Spinal Cord Injury in Rat." *Journal of Neurochem-  
1765 istry* 79 (4): 804–15. <https://doi.org/10.1046/j.1471-4159.2001.00626.x>.
- 1766 Sottrup-Jensen, L., T. M. Stepanik, T. Kristensen, D. M. Wierzbicki, C. M. Jones, P. B. Lønblad, S.  
1767 Magnusson, and T. E. Petersen. 1984. "Primary Structure of Human Alpha 2-Macroglobulin. V.  
1768 The Complete Structure." *The Journal of Biological Chemistry* 259 (13): 8318–27.
- 1769 Spiess, Martina R., Roland M. Müller, Rüdiger Rupp, Christian Schuld, and Hubertus J. A. van Hedel.  
1770 2009. "Conversion in ASIA Impairment Scale During the First Year After Traumatic Spinal Cord  
1771 Injury." *Journal of Neurotrauma* 26 (11): 2027–36. <https://doi.org/10.1089/neu.2008.0760>.
- 1772 Stangel, Martin, Hans-Peter Hartung, Peter Marx, and Ralf Gold. 1998. "Intravenous Immunoglob-

- 1773 ulin Treatment of Neurological Autoimmune Diseases." *Journal of the Neurological Sciences* 153  
1774 (2): 203–14. [https://doi.org/10.1016/S0022-510X\(97\)00292-X](https://doi.org/10.1016/S0022-510X(97)00292-X).
- 1775 Steel, Diana M., and Alexander S. Whitehead. 1994. "The Major Acute Phase Reactants: C-reactive  
1776 Protein, Serum Amyloid P Component and Serum Amyloid A Protein." *Immunology Today* 15 (2):  
1777 81–88. [https://doi.org/10.1016/0167-5699\(94\)90138-4](https://doi.org/10.1016/0167-5699(94)90138-4).
- 1778 Stenson, Katherine W., Anne Deutsch, Allen W. Heinemann, and David Chen. 2011. "Obesity and  
1779 Inpatient Rehabilitation Outcomes for Patients With a Traumatic Spinal Cord Injury." *Archives of  
1780 Physical Medicine and Rehabilitation* 92 (3): 384–90. <https://doi.org/10.1016/j.apmr.2010.07.235>.
- 1781 Stirling, David P., Karen Cummins, Manoj Mishra, Wulin Teo, V. Wee Yong, and Peter Stys. 2014.  
1782 "Toll-Like Receptor 2-Mediated Alternative Activation of Microglia Is Protective After Spinal Cord  
1783 Injury." *Brain* 137 (3): 707–23. <https://doi.org/10.1093/brain/awt341>.
- 1784 Stirling, David P., Shuhong Liu, Paul Kubes, and V. Wee Yong. 2009. "Depletion of Ly6G/Gr-1 Leuko-  
1785 cytes After Spinal Cord Injury in Mice Alters Wound Healing and Worsens Neurological Out-  
1786 come." *Journal of Neuroscience* 29 (3): 753–64. <https://doi.org/10.1523/JNEUROSCI.4918-08.2009>.
- 1787 Stoscheck, Christa M. 1987. "Protein Assay Sensitive at Nanogram Levels." *Analytical Biochemistry*  
1788 160 (2): 301–5. [https://doi.org/10.1016/0003-2697\(87\)90051-0](https://doi.org/10.1016/0003-2697(87)90051-0).
- 1789 Strauss, David J., Michael J. DeVivo, David R. Paculdo, and Robert M. Shavelle. 2006. "Trends in  
1790 Life Expectancy After Spinal Cord Injury." *Archives of Physical Medicine and Rehabilitation* 87 (8):  
1791 1079–85. <https://doi.org/10.1016/j.apmr.2006.04.022>.
- 1792 Sun, ChongYi, GuangRong Ji, QingPeng Liu, and Meng Yao. 2011. "Apolipoprotein E Epsilon 4 Allele  
1793 and Outcomes of Traumatic Spinal Cord Injury in a Chinese Han Population." *Molecular Biology  
1794 Reports* 38 (7): 4793–96. <https://doi.org/10.1007/s11033-010-0620-2>.
- 1795 Sun, Guodong, Shuxian Yang, Guangchao Cao, Qianghua Wang, Jianlei Hao, Qiong Wen, Zhizhong  
1796 Li, et al. 2017. "Gammadelta T Cells Provide the Early Source of IFN-gamma to Aggravate Lesions  
1797 in Spinal Cord Injury." *Journal of Experimental Medicine* 215 (2): 521–35. <https://doi.org/10.1084/jem.20170686>.
- 1798 Sun, Lei, and Richard D. Ye. 2016. "Serum Amyloid A1: Structure, Function and Gene Polymor-  
1799 phism." *Gene* 583 (1): 48–57. <https://doi.org/10.1016/j.gene.2016.02.044>.
- 1800 Sun, Xin, Zachary B. Jones, Xiao-ming Chen, Libing Zhou, Kwok-Fai So, and Yi Ren. 2016. "Multiple  
1801 Organ Dysfunction and Systemic Inflammation After Spinal Cord Injury: A Complex Relation-  
1802 ship." *Journal of Neuroinflammation* 13 (1): 260. <https://doi.org/10.1186/s12974-016-0736-y>.
- 1803 Szalai, Alexander J., Frederik W. van Ginkel, Yue Wang, Jerry R. McGhee, and John E. Volanakis. 2000.  
1804 "Complement-Dependent Acute-Phase Expression of C-Reactive Protein and Serum Amyloid P-  
1805 Component." *The Journal of Immunology* 165 (2): 1030–35. <https://doi.org/10.4049/jimmunol.165.2.1030>.
- 1806 Szklarczyk, Damian, Annika L. Gable, David Lyon, Alexander Junge, Stefan Wyder, Jaime Huerta-  
1807 Cepas, Milan Simonovic, et al. 2019. "STRING V11: Protein-Protein Association Networks with  
1808 Increased Coverage, Supporting Functional Discovery in Genome-Wide Experimental Datasets."  
1809 *Nucleic Acids Research* 47 (D1): D607–13. <https://doi.org/10.1093/nar/gky1131>.
- 1810 Tape, C., R. Tan, M. Neshejm, and R. Kisilevsky. 1988. "Direct Evidence for Circulating apoSAA as the  
1811 Precursor of Tissue AA Amyloid Deposits." *Scandinavian Journal of Immunology* 28 (3): 317–24.  
1812 <https://doi.org/10.1111/j.1365-3083.1988.tb01455.x>.
- 1813 Teasdale, Graham M., James AR Nicoll, Gordon Murray, and Matilda Fiddes. 1997. "Association  
1814 of Apolipoprotein E Polymorphism with Outcome After Head Injury." *The Lancet* 350 (9084):  
1815 1069–71. [https://doi.org/10.1016/S0140-6736\(97\)04318-3](https://doi.org/10.1016/S0140-6736(97)04318-3).
- 1816 The UniProt Consortium. 2021. "UniProt: The Universal Protein Knowledgebase in 2021." *Nucleic  
1817 Acids Research* 49 (D1): D480–89. <https://doi.org/10.1093/nar/gkaa1100>.
- 1818 Thom, Vivien, Thiruma V. Arumugam, Tim Magnus, and Mathias Gelderblom. 2017. "Therapeutic  
1819 Potential of Intravenous Immunoglobulin in Acute Brain Injury." *Frontiers in Immunology* 8: 875.  
1820 <https://doi.org/10.3389/fimmu.2017.00875>.

- 1824 Travis, J., and G. S. Salvesen. 1983. "Human Plasma Proteinase Inhibitors." *Annual Review of Bio-*  
1825 *chemistry* 52 (1): 655–709. <https://doi.org/10.1146/annurev.bi.52.070183.003255>.
- 1826 Tzekou, Apostolia, and Michael G. Fehlings. 2014. "Treatment of Spinal Cord Injury with Intra-  
1827 venous Immunoglobulin G: Preliminary Evidence and Future Perspectives." *Journal of Clinical*  
1828 *Immunology* 34 (1): 132–38. <https://doi.org/10.1007/s10875-014-0021-8>.
- 1829 Vallon, Rüdiger, Felix Freuler, Netsanet Desta-Tsedu, Anna Robeva, Janet Dawson, Peter Wen-  
1830 ner, Petra Engelhardt, et al. 2001. "Serum Amyloid A (apoSAA) Expression Is Up-Regulated in  
1831 Rheumatoid Arthritis and Induces Transcription of Matrix Metalloproteinases." *The Journal of*  
1832 *Immunology* 166 (4): 2801–7. <https://doi.org/10.4049/jimmunol.166.4.2801>.
- 1833 van der Hilst, J. C. H., T. Yamada, H. J. M. Op den Camp, J. W. M. van der Meer, J. P. H. Drenth, and  
1834 A. Simon. 2008. "Increased Susceptibility of Serum Amyloid A 1.1 to Degradation by MMP-1:  
1835 Potential Explanation for Higher Risk of Type AA Amyloidosis." *Rheumatology* 47 (11): 1651–54.  
1836 <https://doi.org/10.1093/rheumatology/ken371>.
- 1837 van der Westhuyzen, Deneys R., Lei Cai, Maria C. de Beer, and Frederick C. de Beer. 2005. "Serum  
1838 Amyloid A Promotes Cholesterol Efflux Mediated by Scavenger Receptor B-I\*." *Journal of Biolog-*  
1839 *ical Chemistry* 280 (43): 35890–95. <https://doi.org/10.1074/jbc.M505685200>.
- 1840 van Oosten, Marijke, Edwin S. van Amersfoort, Theo J. C. van Berk, and Johan Kuiper. 2001.  
1841 "Scavenger Receptor-Like Receptors for the Binding of Lipopolysaccharide and Lipoteichoic Acid  
1842 to Liver Endothelial and Kupffer Cells." *Journal of Endotoxin Research* 7 (5): 381–84. <https://doi.org/10.1177/09680519010070050601>.
- 1843 Viard, Isabelle, Philippe Wehrli, Roberto Bullani, Pascal Schneider, Nils Holler, Denis Salomon,  
1844 Thomas Hunziker, Jean-Hilaire Saurat, Jürg Tschopp, and Lars E. French. 1998. "Inhibition of  
1845 Toxic Epidermal Necrolysis by Blockade of Cd95 with Human Intravenous Immunoglobulin." *Science*  
1846 282 (5388): 490–93. <https://doi.org/10.1126/science.282.5388.490>.
- 1847 Vickers, Kasey C., Brian T. Palmisano, Bassem M. Shoucri, Robert D. Shamburek, and Alan T. Re-  
1848 maley. 2011. "MicroRNAs Are Transported in Plasma and Delivered to Recipient Cells by High-  
1849 Density Lipoproteins." *Nature Cell Biology* 13 (4): 423–33. <https://doi.org/10.1038/ncb2210>.
- 1850 Vidaurre, Oscar G., Jeffery D. Haines, Ilana Katz Sand, Kadidia P. Adula, Jimmy L. Huynh, Corey A.  
1851 McGraw, Fan Zhang, et al. 2014. "Cerebrospinal Fluid Ceramides from Patients with Multiple  
1852 Sclerosis Impair Neuronal Bioenergetics." *Brain* 137 (8): 2271–86. <https://doi.org/10.1093/brain/awu139>.
- 1853 Vodovotz, Yoram, Shubing Liu, Carol McCloskey, Richard Shapiro, Angela Green, and Timothy R.  
1854 Billiar. 2001. "The Hepatocyte as a Microbial Product-Responsive Cell." *Journal of Endotoxin*  
1855 *Research* 7 (5): 365–73. <https://doi.org/10.1177/09680519010070050401>.
- 1856 Vorst, Emiel P. C. van der, Laura Z. Vanags, Louise L. Dunn, Hamish C. Prosser, Kerry-Anne Rye,  
1857 and Christina A. Bursill. 2013. "High-Density Lipoproteins Suppress Chemokine Expression and  
1858 Proliferation in Human Vascular Smooth Muscle Cells." *The FASEB Journal* 27 (4): 1413–25. <https://doi.org/10.1096/fj.12-212753>.
- 1859 Wang, Kevin K., Zhihui Yang, Tian Zhu, Yuan Shi, Richard Rubenstein, J. Adrian Tyndall, and Geoff T.  
1860 Manley. 2018. "An Update on Diagnostic and Prognostic Biomarkers for Traumatic Brain Injury." *Expert*  
1861 *Review of Molecular Diagnostics* 18 (2): 165–80. <https://doi.org/10.1080/14737159.2018.1428089>.
- 1862 Weiskirchen, Ralf, and Frank Tacke. 2014. "Cellular and Molecular Functions of Hepatic Stellate  
1863 Cells in Inflammatory Responses and Liver Immunology." *Hepatobiliary Surgery and Nutrition* 3  
1864 (6): 34463–363. <https://doi.org/10.3978/j.issn.2304-3881.2014.11.03>.
- 1865 White, F., J. A. R. Nicoll, A. D. Roses, and K. Horsburgh. 2001. "Impaired Neuronal Plasticity in  
1866 Transgenic Mice Expressing Human Apolipoprotein E4 Compared to E3 in a Model of Entorhinal  
1867 Cortex Lesion." *Neurobiology of Disease* 8 (4): 611–25. <https://doi.org/10.1006/nbdi.2001.0401>.
- 1868 Widiapradja, Alexander, Viktor Vegh, Ker Zhing Lok, Silvia Manzanero, John Thundyil, Mathias  
1869 Gelderblom, Yi-Lin Cheng, et al. 2012. "Intravenous Immunoglobulin Protects Neurons  
1870 Against Amyloid Beta-Peptide Toxicity and Ischemic Stroke by Attenuating Multiple Cell Death
- 1871

- 1875 Pathways." *Journal of Neurochemistry* 122 (2): 321–32. <https://doi.org/10.1111/j.1471-4159.2012.07754.x>.
- 1876
- 1877 Wilhelmsson, Ulrika, Eric A. Bushong, Diana L. Price, Benjamin L. Smarr, Van Phung, Masako Terada, Mark H. Ellisman, and Milos Pekny. 2006. "Redefining the Concept of Reactive Astrocytes as Cells That Remain Within Their Unique Domains Upon Reaction to Injury." *Proceedings of the National Academy of Sciences of the United States of America* 103 (46): 17513–18. <https://doi.org/10.1073/pnas.0602841103>.
- 1878
- 1879
- 1880
- 1881
- 1882 Wright, Helen L., Robert J. Moots, Roger C. Bucknall, and Steven W. Edwards. 2010. "Neutrophil Function in Inflammation and Inflammatory Diseases." *Rheumatology* 49 (9): 1618–31. <https://doi.org/10.1093/rheumatology/keq045>.
- 1883
- 1884
- 1885 Wyatt, Amy R., and Mark R. Wilson. 2013. "Acute Phase Proteins Are Major Clients for the Chaperone Action of A2-Macroglobulin in Human Plasma." *Cell Stress & Chaperones* 18 (2): 161–70. <https://doi.org/10.1007/s12192-012-0365-z>.
- 1886
- 1887
- 1888 Xu, He, David I. Finkelstein, and Paul A. Adlard. 2014. "Interactions of Metals and Apolipoprotein E in Alzheimer's Disease." *Frontiers in Aging Neuroscience* 6. <https://doi.org/10.3389/fnagi.2014.00121>.
- 1889
- 1890
- 1891 Yang, Chih-Ya, Jiun-Bo Chen, Ting-Fen Tsai, Yi-Chen Tsai, Ching-Yen Tsai, Pi-Hui Liang, Tsui-Ling Hsu, et al. 2013. "Clec4f Is an Inducible C-Type Lectin in F4/80-Positive Cells and Is Involved in Alpha-Galactosylceramide Presentation in Liver." *PLOS ONE* 8 (6): e65070. <https://doi.org/10.1371/journal.pone.0065070>.
- 1892
- 1893
- 1894
- 1895 Yang, Xuan, Shurui Chen, Zhenya Shao, Yuanlong Li, He Wu, Xian Li, Liang Mao, et al. 2018. "Apolipoprotein E Deficiency Exacerbates Spinal Cord Injury in Mice: Inflammatory Response and Oxidative Stress Mediated by NF- $\kappa$ B Signaling Pathway." *Frontiers in Cellular Neuroscience* 12 (May): 142. <https://doi.org/10.3389/fncel.2018.00142>.
- 1896
- 1897
- 1898
- 1899 Yao, Xin-Qiang, Zhong-Yuan Liu, Jia-Ying Chen, Zu-Cheng Huang, Jun-Hao Liu, Bai-Hui Sun, Qing-An Zhu, Ruo-Ting Ding, and Jian-Ting Chen. 2021. "Proteomics and Bioinformatics Reveal Insights into Neuroinflammation in the Acute to Subacute Phases in Rat Models of Spinal Cord Contusion Injury." *The FASEB Journal* 35 (7): e21735. <https://doi.org/10.1096/fj.202100081RR>.
- 1900
- 1901
- 1902
- 1903 Yu, Guangchuang, and Qing-Yu He. 2016. "ReactomePA: An R/Bioconductor Package for Reactome Pathway Analysis and Visualization." *Molecular BioSystems* 12 (2): 477–79. <https://doi.org/10.1039/c5mb00663e>.
- 1904
- 1905
- 1906 Yu, Wen Ru, and Michael G. Fehlings. 2011. "Fas/FasL-mediated Apoptosis and Inflammation Are Key Features of Acute Human Spinal Cord Injury: Implications for Translational, Clinical Application." *Acta Neuropathologica* 122 (6): 747–61. <https://doi.org/10.1007/s00401-011-0882-3>.
- 1907
- 1908
- 1909 Yvan-Charvet, Laurent, Tamara Pagler, Emmanuel L. Gautier, Serine Avagyan, Read L. Siry, Seon-gah Han, Carrie L. Welch, et al. 2010. "ATP-Binding Cassette Transporters and HDL Suppress Hematopoietic Stem Cell Proliferation." *Science* 328 (5986): 1689–93. <https://doi.org/10.1126/science.1189731>.
- 1910
- 1911
- 1912
- 1913 Zerrad-Saadi Amal, Therond Patrice, Chantepie Sandrine, Couturier Martine, Rye Kerry-Anne, Chapman M. John, and Kontush Anatol. 2009. "Hdl3-Mediated Inactivation of LDL-Associated Phospholipid Hydroperoxides Is Determined by the Redox Status of Apolipoprotein A-I and HDL Particle Surface Lipid Rigidity." *Arteriosclerosis, Thrombosis, and Vascular Biology* 29 (12): 2169–75. <https://doi.org/10.1161/ATVBAHA.109.194555>.
- 1914
- 1915
- 1916
- 1917
- 1918 Zhang, Chao, Wenhao Zhang, Jie Zhang, Yingli Jing, Mingliang Yang, Liangjie Du, Feng Gao, et al. 2018. "Gut Microbiota Dysbiosis in Male Patients with Chronic Traumatic Complete Spinal Cord Injury." *Journal of Translational Medicine* 16 (1): 353. <https://doi.org/10.1186/s12967-018-1735-9>.
- 1919
- 1920
- 1921
- 1922 Zhu, Y., C. Soderblom, V. Krishnan, J. Ashbaugh, J. R. Bethea, and J. K. Lee. 2015. "Hematogenous Macrophage Depletion Reduces the Fibrotic Scar and Increases Axonal Growth After Spinal Cord Injury." *Neurobiology of Disease* 74 (February): 114–25. <https://doi.org/10.1016/j.nbd.2014.10.024>.
- 1923
- 1924
- 1925