ECM transcriptome dynamics during aging

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Bulk RNA-seq Analysis (reproducing the main results reported in [1])

[1]. https://doi.org/10.3390/life14050593

Data Source & Metadata

- RNA-seq data for analysis were obtained from https://www.gtexportal.org/home/downloads/adult-gtex/bulk_tissue_expression (the v8 subsection)
- Sample and donor metadata were obtained from https://storage.googleapis.com/adult-gtex/annotations/v8/metadata-files
- Genes were classified by type (*core matrisome, matrisome associated, other*) and category as per [1]

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Categories

Core matrisome
- Collagens
- ECM Glycoproteins
- Proteoglycans

Matrisome associated
- ECM-affiliated Proteins
- ECM Regulators
- Secreted Factors

	[,1]
Blood	755
Heart	705
Muscle	641
Brain	432
Lung	431
Liver	177
Kidney	73

[1]. MatrisomeDB: 2023 updates of the ECM protein knowledge database. Shao X, Gomez CD, Kapoor N, Considine JM, Gao Y, Naba A. *Nucleic Acids Research*, 2022, gkac1009. doi.org/10.1093/nar/gkac1009

Data Filtering & Analysis Setup

- Gene expression analyzed only for samples collected from the ***blood, brain, heart, kidney, liver, lungs, muscle***
- Analysis performed separately for samples collected from males and females
- No detailed information provided by the authors on the filters used (or the source data was modified lately) => discrepancies in the cohort sizes between our analysis and the one reported.

Our filtering result

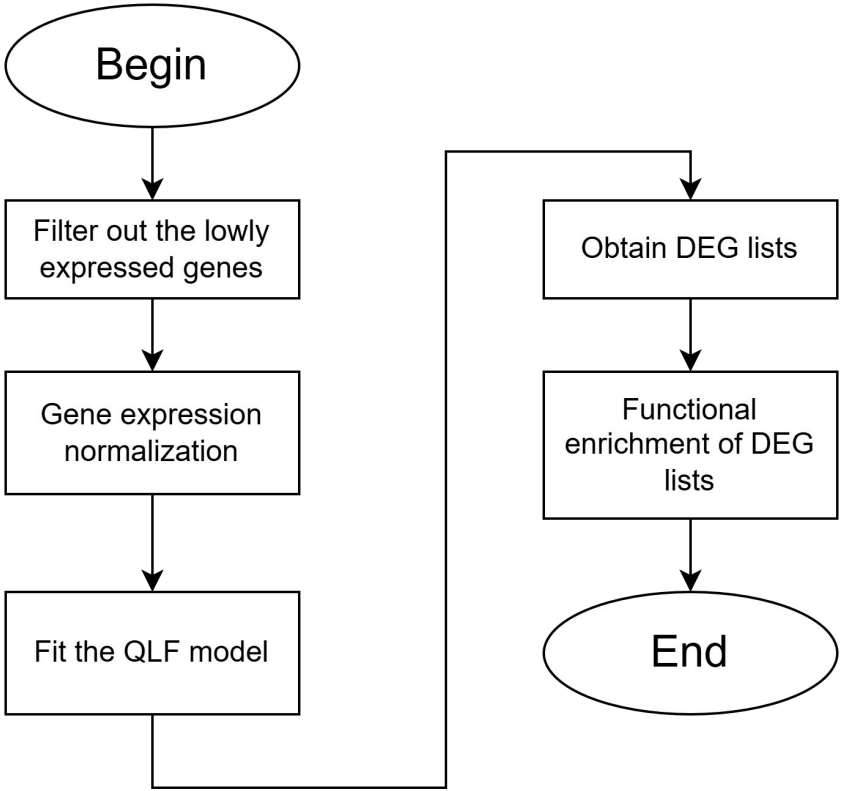
Sex	Age Group	Blood	Brain	Heart	Kidney	Liver	Lungs	Muscle
Male	20-39	92	14	29	4	12	35	69
	40-59	226	130	229	24	64	143	191
	60-79	183	175	221	28	51	123	180
Female	20-39	44	6	24	1	4	15	28
	40-59	121	43	110	8	25	59	102
	60-79	89	64	92	8	21	56	71

Supplementary Table S1. GTEx cohort characteristics by tissue.

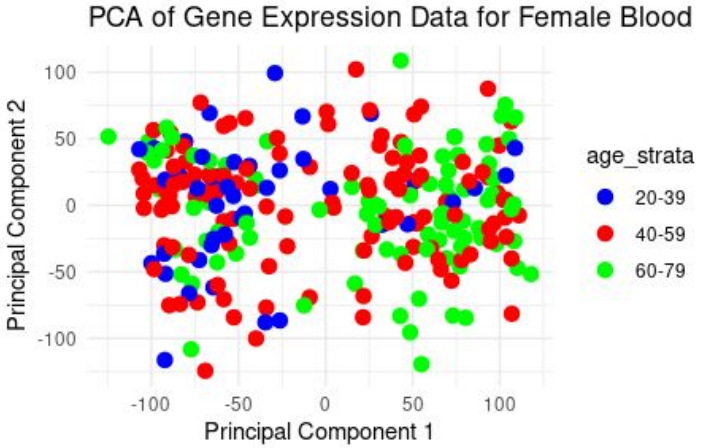
Sex	Age group	Blood	Brain	Heart	Kidney	Liver	Lungs	Muscle
Male	20-39	92	2	17	7	18	50	93
	40-59	226	20	103	29	84	105	245
	60-79	183	27	83	30	59	140	205
Female	20-39	44	3	11	2	6	23	39
	40-59	121	10	52	8	34	88	134
	60-79	89	8	34	9	25	72	87

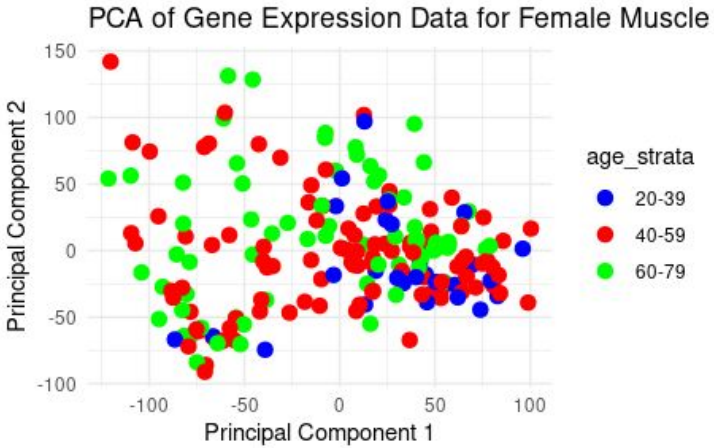
Data Analysis Tools & Pipeline

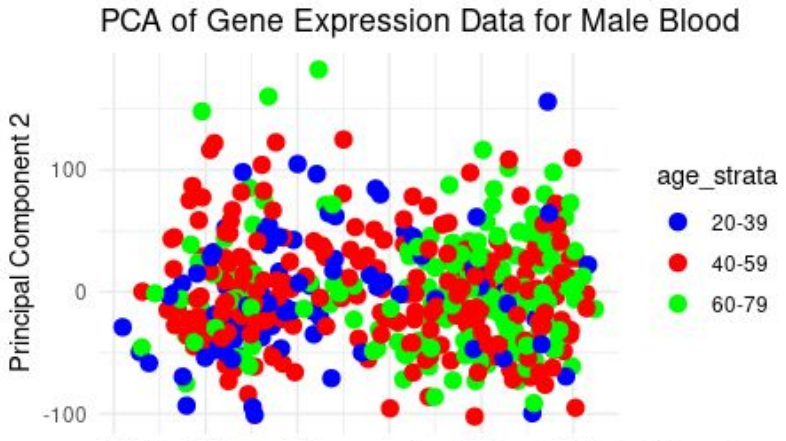
- EdgeR used for RNA-seq analysis: determining the DEGs between age 2 and 3 age groups + PCA analysis
- StringDB used for functional enrichment and exploring interprotein interactions

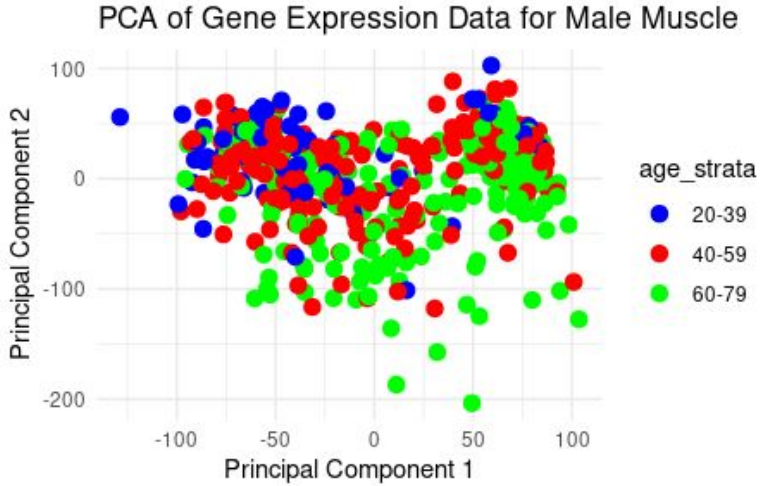


PCA analysis

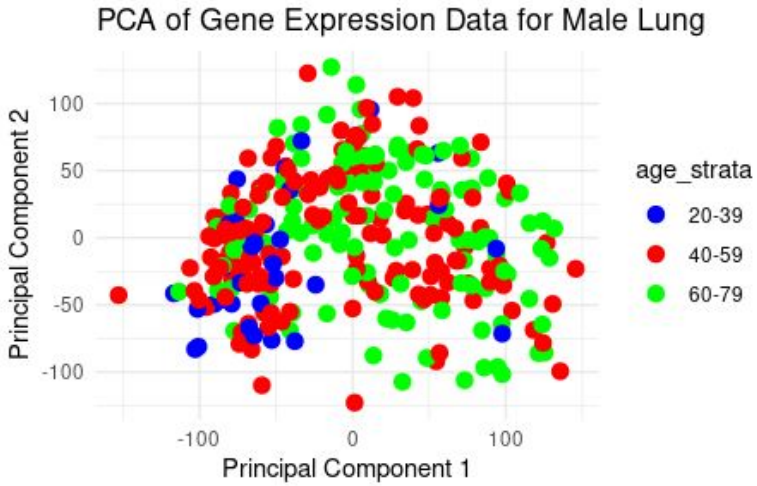


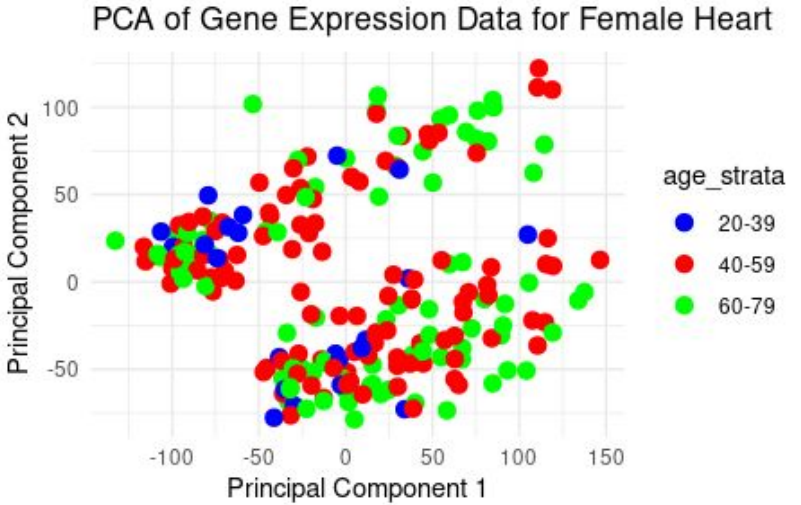


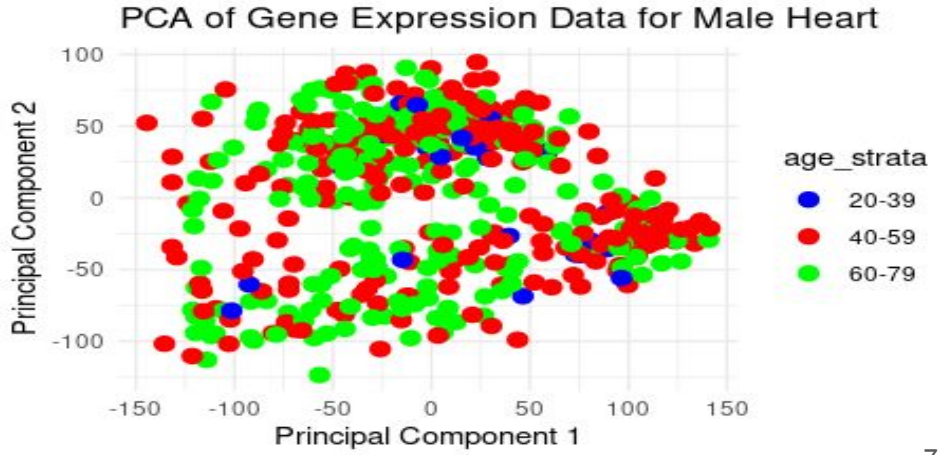




PCA analysis (all genes)







DEG Analysis Results

Tissue/Organ	Female				Male			
	Upregulated		Downregulated		Upregulated		Downregulated	
	C	MA	C	MA	C	MA	C	MA
Blood	32	98	16	73	34	119	22	92
Heart	54	71	1	16	53	129	11	47
Brain	15	37	10	17	19	77	1	19
Liver	6	16	5	22	4	11	9	11
Kidneys	0	2	8	23	6	25	5	17
Lung	57	85	2	26	87	172	11	93
Muscle	46	74	3	33	100	169	4	36

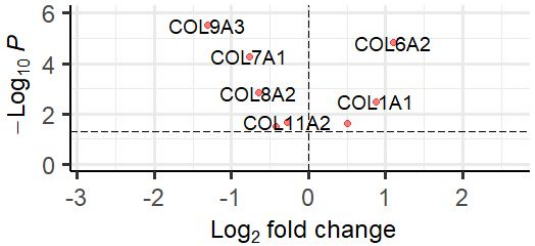
Our analysis,
logCPM > 0,
p-value < 0.05

Tissue/Organ	Female				Male			
	Upregulated		Downregulated		Upregulated		Downregulated	
	C	MA	C	MA	C	MA	C	MA
Blood	29	99	17	79	33	129	27	98
Heart	27	21	0	15	43	87	10	50
Brain	19	35	6	25	7	19	13	25
Liver	8	23	7	36	4	15	16	55
Kidneys	0	15	1	8	11	26	10	18
Lung	78	99	7	49	106	194	10	107
Muscle	54	84	4	34	106	164	19	38

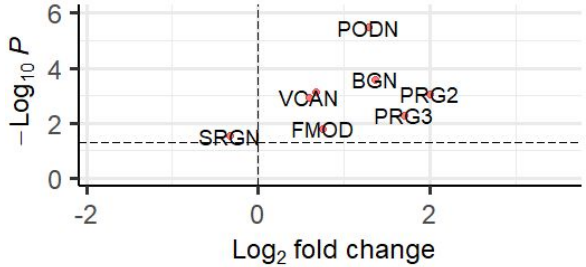
Reported results,
logCPM > 0,
p-value < 0.05

DEG Analysis Results. ECM genes in Female Blood

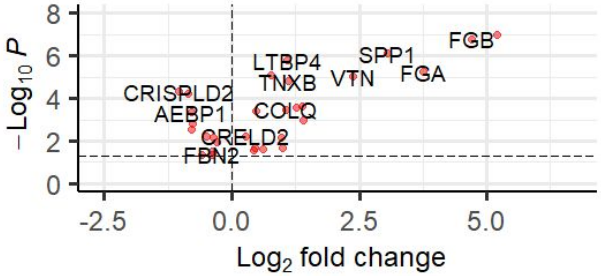
Collagens



Proteoglycans



ECM glycoproteins



collagens	*COL6A2*	*COL9A3* *COL7A1*
ECM glycoproteins	*FGG* *FGB* *FGA* *SPP1* *SPON2* *THBS1* *LTBP4* *COLQ* *TNXB* *LAMB3*	*CRISPLD2* *TNFAIP6* *AEBP1* *MFAP3* *LRG1* *FGL2* *FBN2* *LAMC1* *THBS3* *MFAP1*
proteoglycans	*PRG2* *PRG3* *SPOCK2* *VCAN*	*SRGN*

logCPM>2, p-value < 0.05

DEG Analysis Results. ECM genes in Male Blood

Description	logFC	FDR	gene_subtype
COL6A2	1.0972975	1.716062e-04	Collagen
COL1A1	0.8768284	1.041375e-02	Collagen
COL18A1	-0.4165368	6.516823e-02	Collagen
COL7A1	-0.7600833	4.349153e-04	Collagen
COL9A3	-1.3141332	5.426705e-05	Collagen

Description	logFC	FDR	gene_subtype
PRG3	2.6961919	5.752495e-08	Proteoglycans
PRG2	2.3969168	6.535625e-07	Proteoglycans
SPOCK2	0.7392273	2.755983e-06	Proteoglycans
BGN	0.5864127	2.288502e-02	Proteoglycans
VCAN	0.5314022	1.212506e-04	Proteoglycans
SRGN	-0.4705252	2.628872e-05	Proteoglycans

logCPM > 2, p-value < 0.05

Description	logFC	FDR	gene_subtype
FGG	2.4200640	9.467534e-07	ECM_glycoprotein
FGA	2.1350490	4.471059e-06	ECM_glycoprotein
FGB	1.8538164	6.640801e-05	ECM_glycoprotein
VWCE	1.7856452	5.893204e-12	ECM_glycoprotein
THBS1	1.3948098	7.517501e-08	ECM_glycoprotein
COLQ	0.9301354	6.332205e-08	ECM_glycoprotein
LTBP4	0.8758652	4.399225e-10	ECM_glycoprotein
SPP1	0.6301921	6.311077e-02	ECM_glycoprotein
FN1	0.6219741	1.167423e-02	ECM_glycoprotein
LTBP3	0.4362056	4.965415e-04	ECM_glycoprotein
CRISPLD2	-1.0885172	4.868042e-10	ECM_glycoprotein
PCOLCE2	-0.8799335	7.845014e-05	ECM_glycoprotein
LRG1	-0.7308963	3.713276e-04	ECM_glycoprotein
VWA5A	-0.7120060	6.903215e-08	ECM_glycoprotein
EFEMP2	-0.5037302	7.614181e-06	ECM_glycoprotein
MFAP1	-0.4678938	1.090727e-07	ECM_glycoprotein
THBS3	-0.4138677	1.444837e-06	ECM_glycoprotein

Reported DEG Analysis Results. ECM genes in Blood

		Upregulated		Downregulated	
Tissue/Organ	Category	Female	Male	Female	Male
Blood	collagens	*COL6A2*	*COL6A2*	*COL9A3* *COL7A1*	*COL9A3* *COL7A1* *COL9A2* *COL18A1*
	ECM glycoproteins	*FGG* *FGB* *FGA* *SPP1* *SPON2* *THBS1* *LTBP4* *COLQ* *TNXB* *LAMB3*	*FGG* *VWCE* *THBS1* *FGA* *SPON2* *FGB* *COLQ* *LTBP4* *IGFBP4* *LTBP3*	*CRISPLD2* *TNFAIP6* *AEBP1* *MFAP3* *LRG1* *FGL2* *FBN2* *LAMC1* *THBS3* *MFAP1*	*TNFAIP6* *CRISPLD2* *PCOLCE2* *LRG1* *MFAP3* *VWA5A* *EFEMP2* *MFAP1* *THBS3* *GAS6*
	proteoglycans	*PRG2* *PRG3* *SPOCK2* *VCAN*	*PRG3* *PRG2* *SPOCK2* *VCAN*	*SRGN*	*SRGN*
	collagens	*COL6A3* *COL1A1* *COL8A2* *COL1A2* *COL16A1*		*COL27A1*	*COL26A1*

logCPM > 2, p-value < 0.05

DEG Analysis Results. Matr. Assoc. genes in Male Blood

Description	logFC	FDR	gene_subtype
IGF2	2.5008682	1.748333e-10	Secreted factors
CCL3	2.2004194	2.864790e-09	Secreted factors
CCL4L2	2.0848667	2.345728e-10	Secreted factors
TNFSF9	1.9090413	1.854429e-08	Secreted factors
AREG	1.6918613	2.689206e-08	Secreted factors
LIF	1.5909113	3.477499e-04	Secreted factors
VEGFA	1.4370014	8.894309e-08	Secreted factors
HBEGF	1.2931964	6.832705e-06	Secreted factors
IL1RN	-0.8175009	4.412686e-08	Secreted factors
MEGF9	-0.8207596	6.262274e-08	Secreted factors
S100A4	-0.8472878	3.715264e-09	Secreted factors
TNFSF14	-0.8759828	1.521736e-07	Secreted factors
S100A8	-0.8784909	6.325390e-05	Secreted factors
TGFA	-0.8944810	2.519802e-07	Secreted factors
S100A9	-0.9054147	6.723969e-07	Secreted factors
S100A12	-0.9662648	1.434318e-05	Secreted factors
PDGFC	-1.0365963	3.546775e-06	Secreted factors
TNFSF10	-1.2624050	4.963350e-09	Secreted factors

Description	logFC	FDR	gene_subtype
HRG	3.3727695	1.965179e-09	ECM regulators
AMBP	2.5797060	3.084958e-07	ECM regulators
SERPINH1	1.7772279	4.964479e-08	ECM regulators
SERPINA3	1.6466851	7.128530e-06	ECM regulators
PLAU	1.5911112	7.769653e-06	ECM regulators
TGM3	1.3852067	5.509492e-06	ECM regulators
CTSW	1.3239998	1.749443e-09	ECM regulators
SERPINB2	1.2371238	1.491554e-06	ECM regulators
CTSG	1.1614011	1.022408e-03	ECM regulators
SERPINE1	1.1533815	2.503985e-05	ECM regulators
SERPINB8	-0.7100229	9.279917e-12	ECM regulators
MMP8	-0.7228314	7.458185e-03	ECM regulators
MMP25	-0.7379472	7.561588e-05	ECM regulators
CTSK	-0.7429314	2.519705e-13	ECM regulators
CSTA	-0.7500154	7.244520e-08	ECM regulators
ADAMTS2	-0.7505984	1.789789e-02	ECM regulators
ADAM17	-0.7508632	1.283152e-08	ECM regulators
EGLN1	-0.7649804	8.623554e-08	ECM regulators
SERPINB1	-0.8477462	1.678013e-06	ECM regulators
HPSE	-1.0798299	3.854136e-14	ECM regulators

Description	logFC	FDR	gene_subtype
SDC2	1.1559916	1.356018e-06	ECM affiliated
ITLN1	1.0621543	1.678314e-03	ECM affiliated
PLXDC1	1.0332113	3.926363e-08	ECM affiliated
SDC4	0.9070199	1.058836e-07	ECM affiliated
PLXNA3	0.8694726	2.365615e-08	ECM affiliated
SEMA4C	0.8080364	4.646054e-09	ECM affiliated
CLEC2B	0.7605047	1.233481e-08	ECM affiliated
CLEC11A	0.7513596	6.527319e-05	ECM affiliated
C1QC	0.7459376	4.566718e-03	ECM affiliated
C1QA	0.7339886	9.782455e-04	ECM affiliated
SEMA4B	-0.6299925	1.895395e-07	ECM affiliated
PLXNC1	-0.7496036	7.207472e-07	ECM affiliated
CLEC1B	-0.7811976	2.100740e-05	ECM affiliated
ANXA1	-0.7982271	1.364615e-07	ECM affiliated
CLEC4A	-0.8516600	4.720722e-11	ECM affiliated
ANXA3	-0.9369413	6.802901e-06	ECM affiliated
CLEC12B	-0.9895588	2.282027e-07	ECM affiliated
CLEC4D	-1.0608045	9.194342e-06	ECM affiliated
ANXA9	-1.0711524	2.281853e-12	ECM affiliated
LGALSL	-1.2334179	1.097280e-12	ECM affiliated

Reported DEG Analysis Results. Matr. Assoc. genes in Blood

		Upregulated ↑		Downregulated ↓	
Tissue/ Organ	**Category**	**Female**	**Male**	**Female**	**Male**
Blood	ECM Regulators	AMBP (1.79)	HRG (1.47)	PRSS2 (-0.9)	HPSE (-0.55)
		SERPINA3 (1.5)	SERPINH1 (0.91)	HPSE (-0.43)	HYAL2 (-0.46)
		HRG (1.34)	AMBP (0.89)	MMP25 (-0.41)	SERPINB1 (-0.43)
		SERPINH1 (0.97)	PLAU (0.82)	EGLN1 (-0.38)	ADAMTS2 (-0.42)
		CTSW (0.81)	TGM3 (0.7)	HYAL2 (-0.31)	MMP25 (-0.41)
		SERPINB2 (0.77)	CTSW (0.66)	ST14 (-0.27)	EGLN1 (-0.4)
		PLAU (0.62)	CTSG (0.64)	CTSK (-0.26)	CTSK (-0.37)
		P4HA1 (0.57)	SERPINB2 (0.63)	ADAM17 (-0.26)	ADAM17 (-0.37)
		CTSG (0.57)	ELANE (0.53)	SERPINB8 (-0.25)	CSTA (-0.37)
		ADAMTS10 (0.51)	MMP19 (0.52)	ADAM19 (-0.24)	SERPINB8 (-0.36)
	ECM-affiliated Proteins	SDC2 (0.86)	CLC (0.71)	SFTPB (-1.02)	CLEC4E (-0.67)
		C1QC (0.54)	SDC2 (0.59)	ANXA9 (-0.49)	ANXA9 (-0.54)
		C1QB (0.47)	PLXDC1 (0.54)	CLEC4A (-0.36)	CLEC4D (-0.53)
		C1QA (0.46)	SDC4 (0.46)	CLEC4E (-0.34)	CLEC12A (-0.49)
		CLC (0.44)	PLXNA3 (0.45)	SEMA4B (-0.34)	ANXA3 (-0.48)
		PLXNA3 (0.43)	CLEC11A (0.42)	ANXA3 (-0.32)	CLEC12B (-0.48)
		CLEC2B (0.42)	SEMA4C (0.4)	PLXNA2 (-0.29)	ANXA1 (-0.41)
		CLEC11A (0.42)	C1QC (0.36)	CLEC12A (-0.29)	CLEC4A (-0.41)
		SEMA4C (0.34)	CLEC2B (0.36)	CLEC4D (-0.28)	CLEC1B (-0.39)
		ANXA6 (0.31)	C1QA (0.35)	PLXNC1 (-0.27)	PLXNC1 (-0.38)
	Secreted Factors	IGF2 (1.29)	CCL3L3 (1.13)	TNFSF10 (-0.53)	TNFSF10 (-0.66)
		IFNG (1.24)	IGF2 (1.12)	INHBB (-0.46)	PDGFC (-0.53)
		CCL3 (1.15)	CCL3 (1.08)	IL1B (-0.44)	S100A12 (-0.5)
		CCL4 (1.03)	CCL4L2 (1.05)	IL1RN (-0.43)	S100A9 (-0.47)
		LEP (1.02)	CCL4 (0.93)	TNFSF14 (-0.42)	S100A8 (-0.47)
		LIF (0.98)	TNFSF9 (0.93)	S100A4 (-0.34)	TNFSF14 (-0.46)
		CCL4L2 (0.92)	EREG (0.84)	INSL3 (-0.34)	TGFA (-0.45)
		CCL5 (0.81)	XCL2 (0.81)	TGFA (-0.33)	S100A4 (-0.44)
		AREG (0.8)	AREG (0.81)	MEGF9 (-0.32)	MEGF9 (-0.41)
		VEGFA (0.8)	PRL (0.76)	PPBP (-0.29)	IL1RN (-0.4)

logCPM > 2, p-value < 0.05

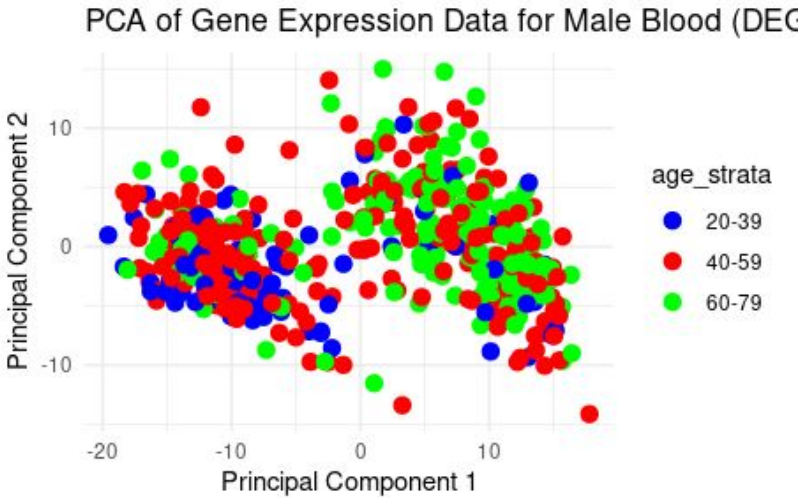
DEG Analysis Results. Matr. Assoc. genes in Female Blood

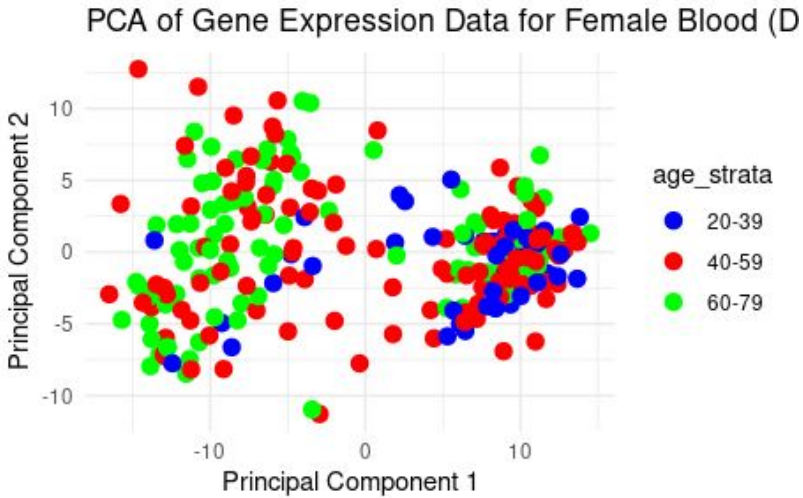
Description	logFC	FDR	gene_subtype
IGF2	2.9887340	3.676168e-06	Secreted factors
CCL3	2.4949868	7.974830e-05	Secreted factors
LIF	2.3596570	1.129358e-03	Secreted factors
CCL4L2	2.2200385	4.594902e-05	Secreted factors
VEGFA	1.7803708	6.561193e-05	Secreted factors
AREG	1.7383353	3.792461e-04	Secreted factors
CCL5	1.7306954	5.979227e-08	Secreted factors
HBEGF	1.7295252	2.556154e-04	Secreted factors
FGFBP2	1.6402980	9.036047e-06	Secreted factors
PDGFC	-0.6153285	6.792712e-02	Secreted factors
TGFA	-0.6706868	6.760612e-03	Secreted factors
S100A4	-0.7102221	5.548460e-04	Secreted factors
INSL3	-0.7392880	1.989358e-03	Secreted factors
IL1B	-0.8025321	8.238446e-04	Secreted factors
IL1RN	-0.8415156	9.158334e-05	Secreted factors
TNFSF14	-0.8460055	1.233192e-03	Secreted factors
INHBB	-0.9576619	1.542016e-02	Secreted factors
TNFSF10	-1.0690030	5.946457e-04	Secreted factors

Description	logFC	FDR	gene_subtype
AMBP	3.9791284	4.581579e-06	ECM regulators
HRG	3.5054283	7.480164e-05	ECM regulators
SERPINA3	3.2553723	1.468284e-06	ECM regulators
SERPINH1	2.2025614	5.365416e-05	ECM regulators
SERPINB2	1.7660984	1.968972e-05	ECM regulators
CTSW	1.7081052	8.097528e-06	ECM regulators
PLAU	1.4278838	1.310080e-02	ECM regulators
P4HA1	1.1968426	3.604021e-06	ECM regulators
SERPING1	1.1942335	2.012673e-03	ECM regulators
CTSL	1.1668899	5.928092e-04	ECM regulators
SERPINE1	1.1324895	2.495722e-03	ECM regulators
ADAM19	-0.4911141	2.557666e-03	ECM regulators
SERPINB8	-0.5129647	5.525374e-04	ECM regulators
CSTA	-0.5133750	1.127372e-02	ECM regulators
ST14	-0.5266343	3.521507e-03	ECM regulators
ADAM17	-0.5399849	6.760612e-03	ECM regulators
CTSK	-0.5618765	2.341715e-04	ECM regulators
EGLN1	-0.7575184	2.983619e-04	ECM regulators
HPSE	-0.8573201	1.243439e-04	ECM regulators
MMP25	-0.8637453	1.507610e-03	ECM regulators
PRSS2	-1.9613242	1.125871e-03	ECM regulators

Description	logFC	FDR	gene_subtype
SDC2	1.9488576	2.030401e-06	ECM affiliated
C1QC	1.2959030	2.269876e-03	ECM affiliated
C1QB	1.1283732	4.069469e-03	ECM affiliated
C1QA	1.0231171	4.390234e-03	ECM affiliated
PLXNA3	0.9019938	2.174400e-04	ECM affiliated
CLEC2B	0.8322105	2.463298e-04	ECM affiliated
CLEC11A	0.7701702	7.428039e-03	ECM affiliated
SEMA4C	0.7365973	6.572554e-04	ECM affiliated
PLXNC1	-0.5425531	1.533909e-02	ECM affiliated
CLEC1B	-0.5433505	4.078531e-02	ECM affiliated
CLEC4D	-0.6085946	5.951738e-02	ECM affiliated
PLXNA2	-0.6269029	9.240206e-04	ECM affiliated
SEMA4B	-0.6615662	1.728616e-04	ECM affiliated
ANXA3	-0.6796452	1.479927e-02	ECM affiliated
CLEC4A	-0.7433130	4.170954e-05	ECM affiliated
ANXA9	-0.9741165	4.829923e-05	ECM affiliated
LGALSL	-1.1418744	4.343717e-05	ECM affiliated
SFTPB	-1.9793875	5.309454e-04	ECM affiliated

PCA Analysis of the DEGs. Samples can be split by the difference in the DEG expression profile





Functional Enrichment of the upregulated DEGs list in all Female Tissues

color	cluster Id	gene count	description
	Cluster 1	92	+ Extracellular matrix organization
	Cluster 2	7	Semaphorin-plexin signaling pathway
	Cluster 3	7	+ Molecules associated with elastic fibres
	Cluster 4	6	+ Complement activation, classical pathway
	Cluster 5	5	+ Canonical Wnt signaling pathway
	Cluster 6	3	+ LGI-ADAM interactions
	Cluster 7	3	+ Antagonism of Activin by Follistatin
	Cluster 8	3	+ Antigen processing and presentation of exogenous peptide antigen via MHC clas...
	Cluster 9	3	Defective GALNT3 causes HFTC
	Cluster 10	2	CTSG, SERPINB9
	Cluster 11	2	Mixed, incl. Cytolysis, and Regulation of extrathymic T cell differentiation
	Cluster 12	2	Von Willebrand factor (vWF) type C domain
	Cluster 13	2	Alcoholic pancreatitis, and Typhus
	Cluster 14	2	Extracellular matrix structural constituent conferring compression resistance
	Cluster 15	2	S100/CaBP-9k-type, calcium binding, subdomain, and Annexin
	Cluster 16	2	S-100/ICaBP type calcium binding domain
	Cluster 17	2	Platelet-derived and vascular endothelial growth factors (PDGF, VEGF) family

Network Stats

number of nodes: 167
number of edges: 1086
average node degree: 13
avg. local clustering coefficient: 0.537

expected number of edges: 173
PPI enrichment p-value: < 1.0e-16

Interaction Enrichment

This means that your proteins have more interactions among themselves than what would be expected for a random set of proteins of the same size and degree distribution drawn from the genome. Such an enrichment indicates that the proteins are at least partially biologically connected, as a group.

KEGG Pathways

description	*count in network*	*strength*	*signal*	*false discovery rate*
ECM-receptor interaction	18 of 88	1.38	3.28	2.96e-16
Complement and coagulation cascades	15 of 82	1.33	2.72	2.80e-13
PI3K-Akt signaling pathway	28 of 349	0.98	2.35	2.96e-16
Focal adhesion	20 of 195	1.08	2.31	1.98e-13
Proteoglycans in cancer	17 of 194	1.01	1.84	1.79e-10
AGE-RAGE signaling pathway in diabetic complications	12 of 96	1.17	1.8	5.57e-09
Amoebiasis	12 of 101	1.15	1.75	8.33e-09
Human papillomavirus infection	20 of 324	0.86	1.56	6.75e-10
Protein digestion and absorption	11 of 100	1.11	1.55	9.10e-08
Chagas disease	10 of 97	1.08	1.37	7.87e-07
Malaria	7 of 46	1.25	1.25	8.89e-06
Cytokine-cytokine receptor interaction	13 of 282	0.74	0.88	3.44e-05
Rheumatoid arthritis	7 of 83	1.0	0.86	0.00024
Pathways in cancer	18 of 515	0.62	0.84	1.67e-05
MAPK signaling pathway	12 of 286	0.69	0.75	0.00021
Pertussis	6 of 73	0.99	0.72	0.0011
Relaxin signaling pathway	7 of 126	0.82	0.61	0.0026
Small cell lung cancer	6 of 92	0.89	0.61	0.0031
TGF-beta signaling pathway	6 of 91	0.89	0.61	0.0031
Viral protein interaction with cytokine and cytokine receptor	6 of 96	0.87	0.59	0.0034
Toll-like receptor signaling pathway	6 of 100	0.85	0.58	0.0040

Functional Enrichment of the Upregulated DEGs List in all Male Tissues

color	cluster Id	gene count	description
	Cluster 1	189	+ Extracellular matrix organization
	Cluster 2	43	+ Viral protein interaction with cytokine and cytokine receptor
	Cluster 3	30	+ Semaphorin-plexin signaling pathway
	Cluster 4	13	+ Lysosome
	Cluster 5	12	+ Molecules associated with elastic fibres
	Cluster 6	9	+ Canonical Wnt signaling pathway
	Cluster 7	9	Complement activation
	Cluster 8	8	+ Calcium-dependent protein binding
	Cluster 9	8	Regulation of pathway-restricted SMAD protein phosphorylation, and Signaling by B...
	Cluster 10	5	+ Regulation of pathway-restricted SMAD protein phosphorylation, and Signaling b...
	Cluster 11	5	+ LGI-ADAM interactions
	Cluster 12	5	+ Myeloid leukocyte mediated immunity
	Cluster 13	5	+ Osteogenesis
	Cluster 14	5	+ Basement membrane
	Cluster 15	4	Defective B3GALTL causes PpS
	Cluster 16	4	Defective GALNT3 causes HFTC
	Cluster 17	3	Transforming growth factor beta complex
	Cluster 18	3	Galactoside-binding lectin

Network Stats

number of nodes: 454
number of edges: 6479
average node degree: 28.5
avg. local clustering coefficient: 0.508

expected number of edges: 947
PPI enrichment p-value: < 1.0e-16
your network has significantly more interactions

Interaction Enrichment

This means that your proteins have more interactions among themselves than what would be expected for a random set of proteins of the same size and degree distribution drawn from the genome. Such an enrichment indicates that the proteins are at least partially biologically connected, as a group.

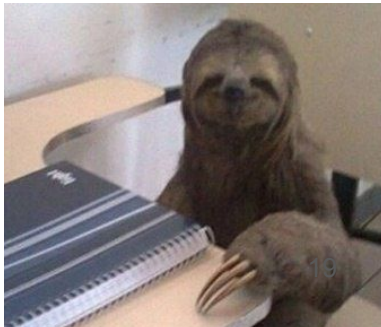
KEGG Pathways

description	*count in network*	*strength*	*signal*	*false discovery rate*
ECM-receptor interaction	34 of 88	1.22	4.11	2.83e-25
Protein digestion and absorption	29 of 100	1.1	3.08	4.45e-19
Cytokine-cytokine receptor interaction	49 of 282	0.88	2.72	6.27e-24
Amoebiasis	26 of 101	1.05	2.64	3.98e-16
PI3K-Akt signaling pathway	55 of 349	0.83	2.62	5.53e-25
Focal adhesion	37 of 195	0.92	2.56	4.45e-19
TGF-beta signaling pathway	24 of 91	1.06	2.55	4.04e-15
Axon guidance	30 of 176	0.87	2.09	1.73e-14
Malaria	14 of 46	1.12	1.89	1.15e-09
Complement and coagulation cascades	18 of 82	0.98	1.83	2.51e-10
Viral protein interaction with cytokine and cytokine receptor	19 of 96	0.93	1.76	2.97e-10
Proteoglycans in cancer	28 of 194	0.8	1.73	6.10e-12
Rheumatoid arthritis	16 of 83	0.92	1.53	1.45e-08
Human papillomavirus infection	35 of 324	0.67	1.49	1.20e-11
AGE-RAGE signaling pathway in diabetic complications	17 of 96	0.89	1.49	1.43e-08
Pathways in cancer	42 of 515	0.55	1.21	2.48e-10
Chagas disease	14 of 97	0.8	1.09	3.42e-06
Hippo signaling pathway	18 of 154	0.71	1.07	1.26e-06
MAPK signaling pathway	26 of 286	0.6	1.05	2.28e-07
Ras signaling pathway	22 of 225	0.63	1.02	9.00e-07
Rap1 signaling pathway	19 of 201	0.61	0.89	1.02e-05
EGFR tyrosine kinase inhibitor resistance	11 of 77	0.79	0.89	6.60e-05
IL-17 signaling pathway	12 of 91	0.76	0.88	5.41e-05

Conclusions

- The reported results were reproduced using the same bioinformatic tools as employed in the considered analysis
- The results for the blood samples are almost fully consistent with the ones reported in the article
- The rest of the results were reproduced *grosso modo*, with the causes of imperfection lying in the lack of detailed information on the used filtering and/or the possibly changed data annotations
- The results for DEG list functional enrichment and network analysis of the corresponding proteins interactions are consistent with the ones reported and indicate the importance of studying ECM involvement in aging.

Weighted Gene Co-expression Network Analysis (WGCNA)



Workflow: Heart, Liver, Lung

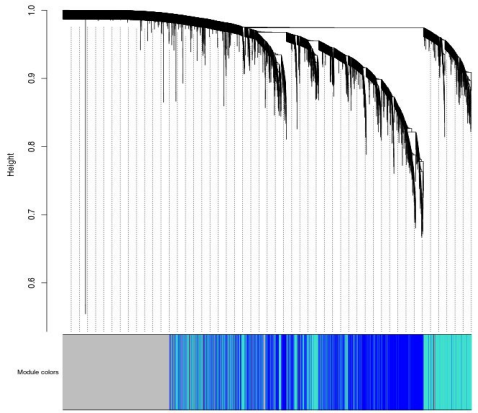
Data for 7 tissues (blood, brain, heart, kidneys, liver, lungs, and skeletal muscle) were used

Data were preprocessed (metadata, gene filtration, normalization, variance stabilizing transformation)

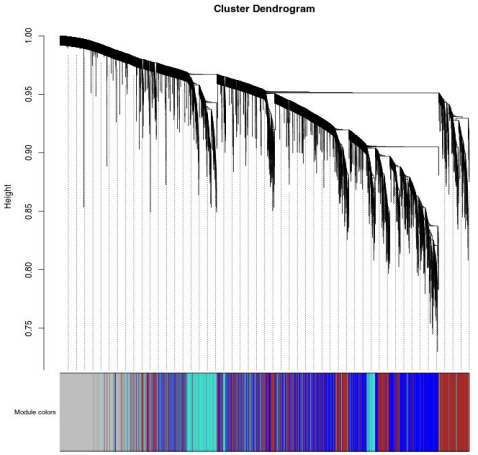
Power of correlation was chosen

Gene modules were identified [due to technical reasons] only for Heart, Liver and Lung samples; corr < 0.7

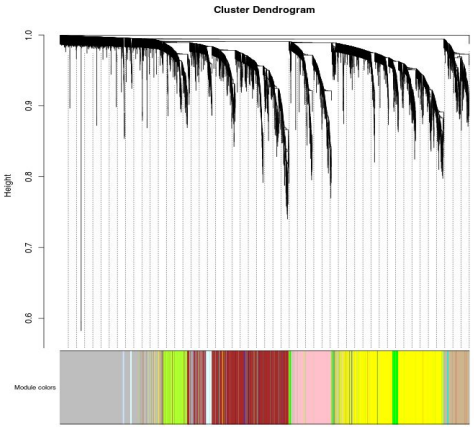
Analysis of genes in the most significant modules



Heart

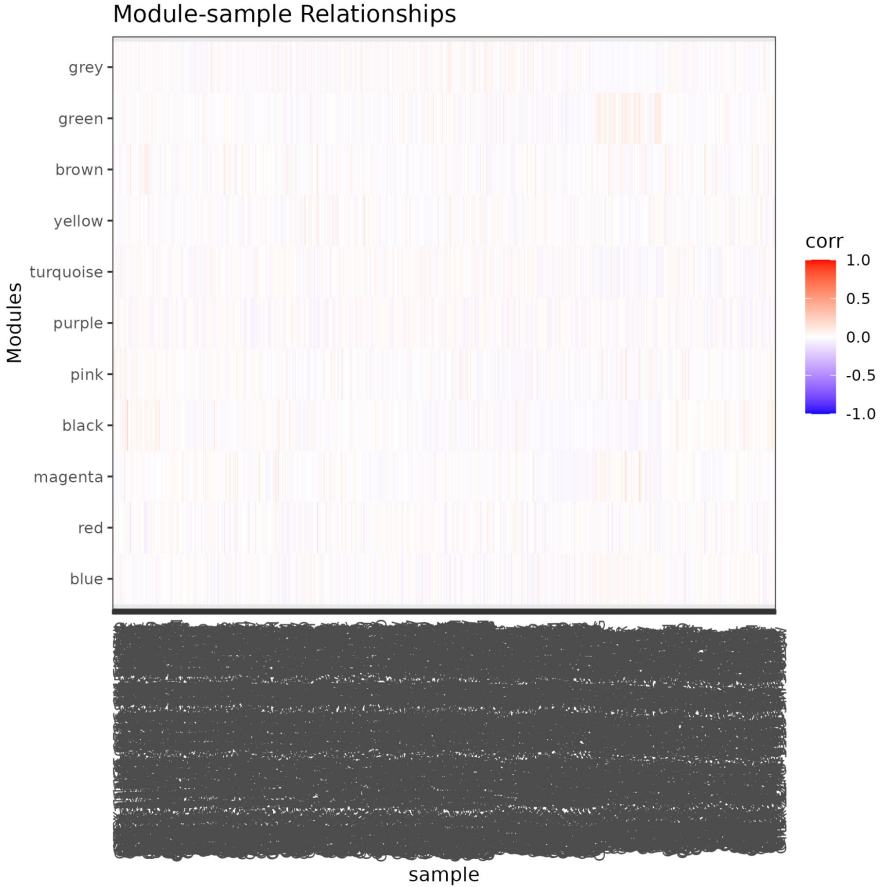


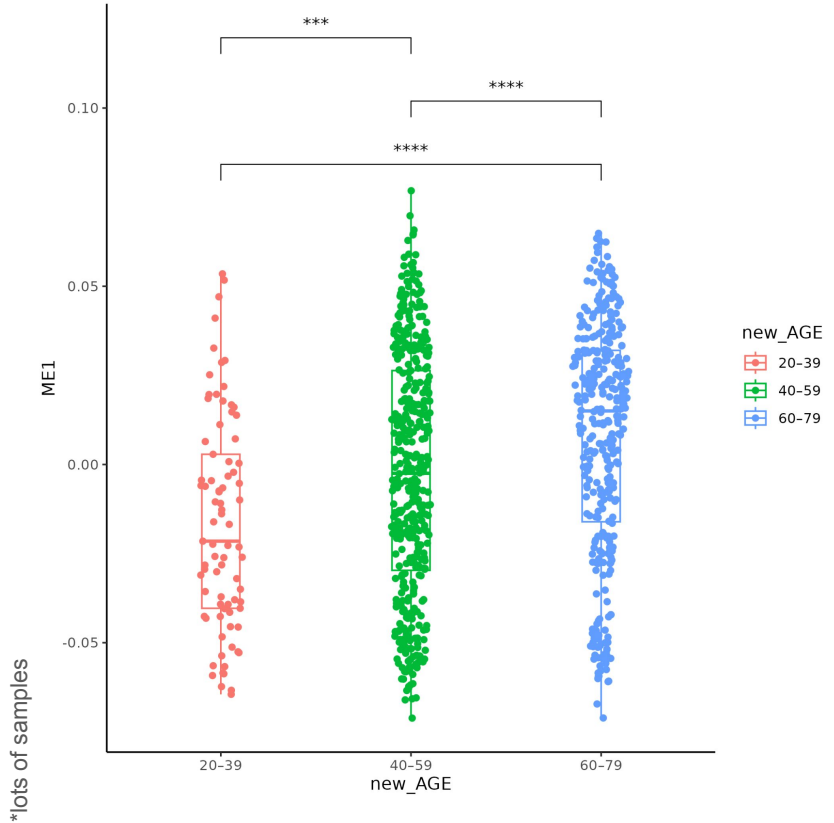
Lung



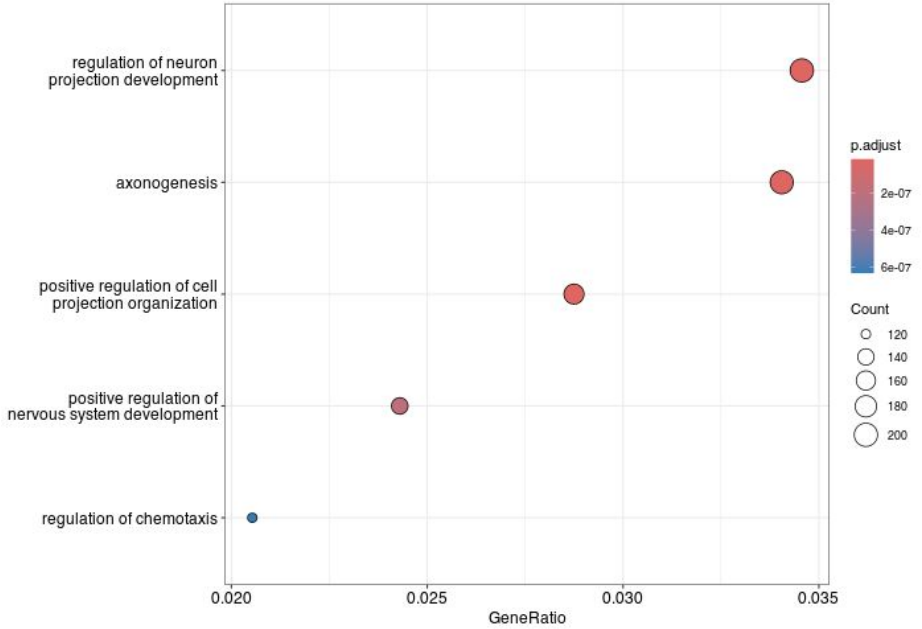
Liver

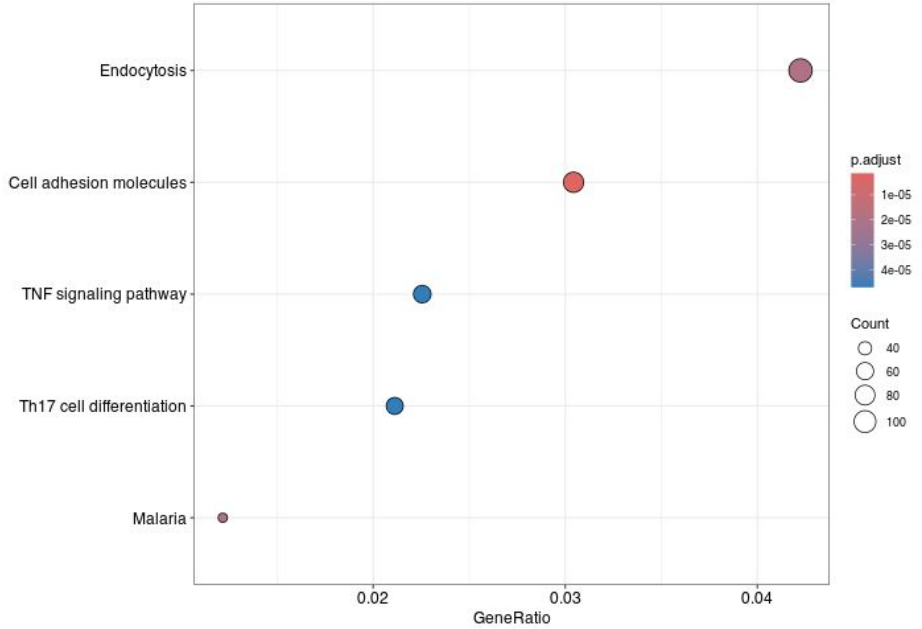
Heart: significant but not really bright





GO- and KEGG-enrichment for Heart





As it was expected or not?

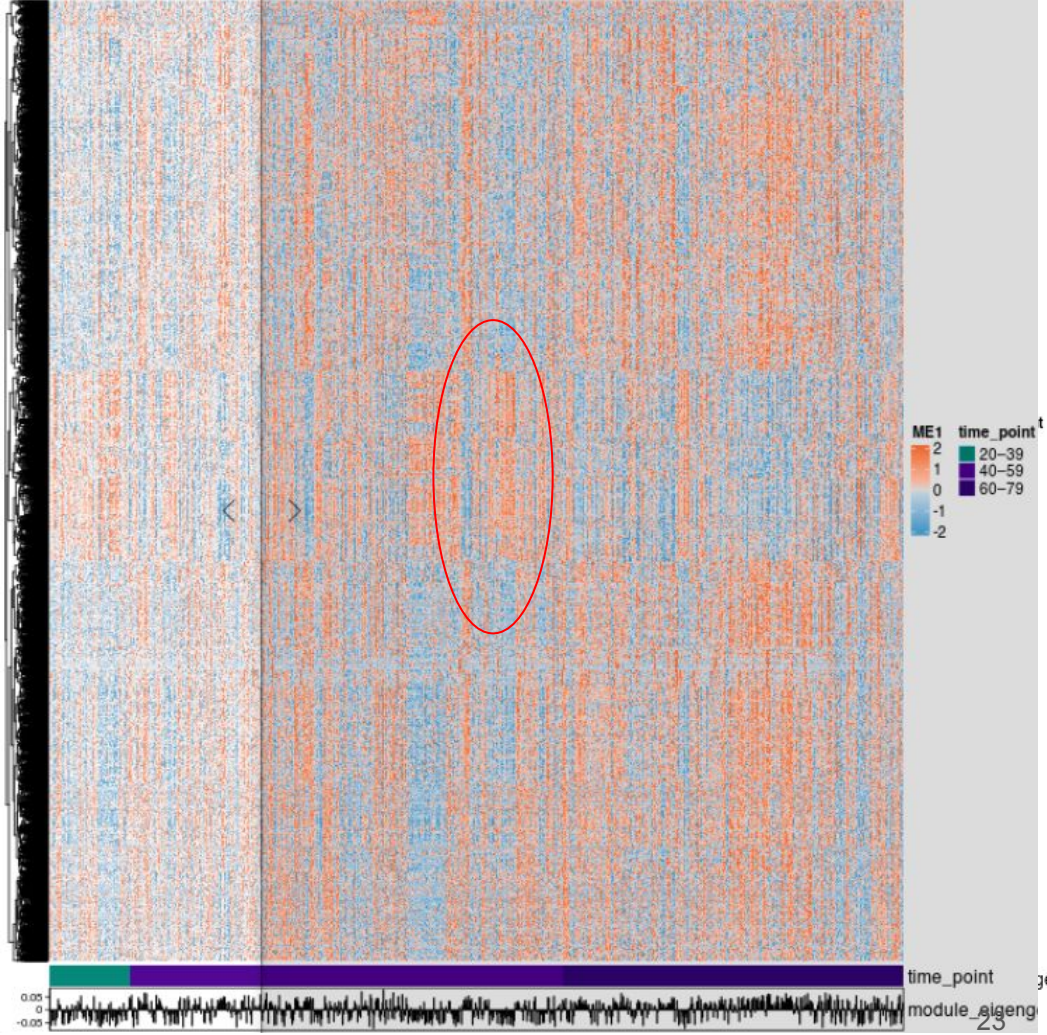
Heart

Are samples homogeneous?

Heart samples consist of left ventricle and atrial appendage tissues meaning cardiomyocytes, blood vessels, autonomic nerves, and connective tissues.

What is about donors?

They’re post-mortem. Did they have any illnesses?



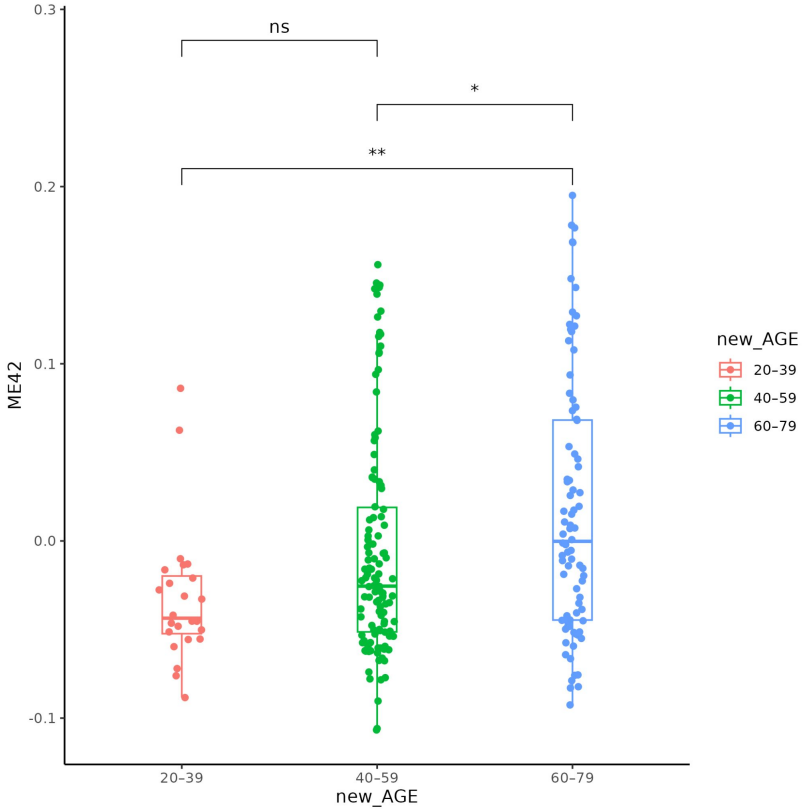
The most significant Heart module genes ∩ matrisome genes

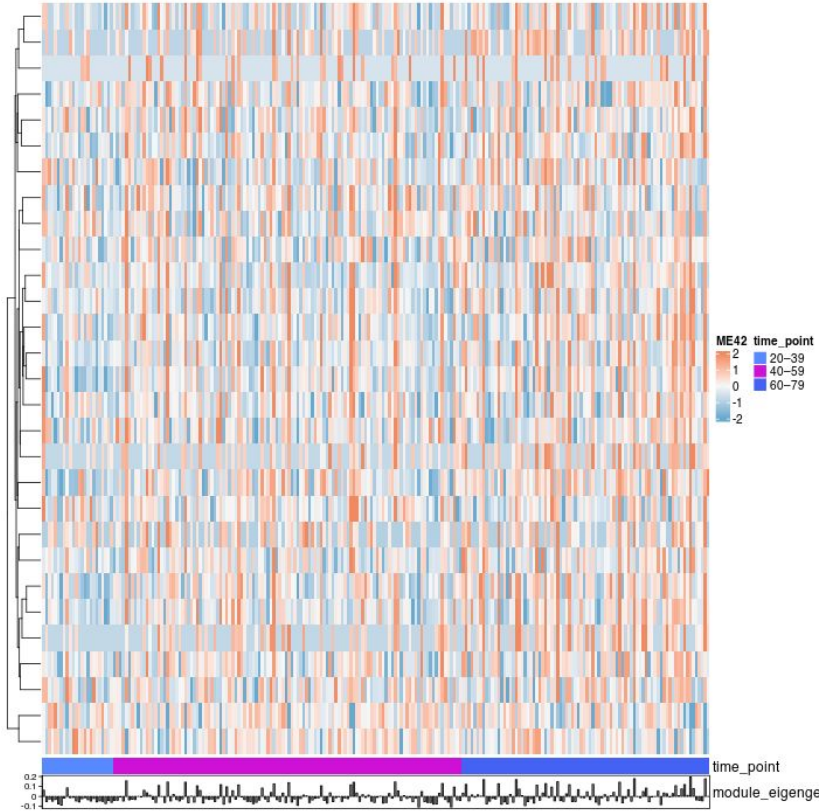
DCN, VCAN, COL9A2, ELN, COL11A1, NTN1, FBLN1, COL4A4, EPYC, COL16A1, LTBP4, COL9A3, PAPLN, MXRA5, SRPX, COMP, PCOLCE, AEBP1, OGN, ASPN, ECM2, LGI1, COL1A1, TECTA, VWA5A, MGP, COL12A1, COL9A1, SMOC2, IMPG1, SPARC, THBS4, EFEMP1, FN1, PRG4, MFAP2, LTBP2, TNN, TGFBI, CRISPLD1, FMOD, SRGN, TNFAIP6, MATN4, COL21A1, FGL2, COL5A1, LAMA5, EMILIN2, MATN2, POSTN, LAMC1, CHAD, IGFBPL1, EMILIN1, CILP, MMRN1, FRAS1, LUM, KERA, FBLN5, MFAP1, MFGE8, IGFBP4, COL8A1, NTN5, TINAGL1, FNDC7, DPT, HMCN1, ECM1, FBLN7, COL8A1, SLIT2, VWA5B2, HAPLN1, RSPO3, VWDE, IGFBP3, IGFBP1, RSPO2, CRIM1, SPOCK1, IGSF10, LGI2, LGI4, ABI3BP, ACAN, SPON2, CILP2, NTN3, MATN1, NTNG1,SNED1,COL6A3 ,IGFBP7 ,FBLN2 ,PCOLCE2 , ESM1 , BMPER, COL1A2 ,FNDC1 ,SBSPON ,CTHRC1 ,SVEP1 , FBN1 , MFAP4, IGFBP6 ,LTBP3 ,TNXB ,COL3A1 ,COL4A3 ,RSPO1, THBS3 ,COL22A1 ,COL24A1 ,COL8A2 ,MMRN2 ,PODN ,BGN , TSKU ,COL18A1 ,SLIT3 ,NELL2 ,THBS2 ,EMID1 ,HAPLN4 , COL14A1 ,COL4A5 ,AGRN ,PRELP ,RELN ,NTNG2 ,COL27A1 , COL13A1 ,MFAP5 ,SMOC1 ,COL5A2 ,COL15A1 ,COL6A6 , COL28A1 ,BGLAP ,SPON1

143

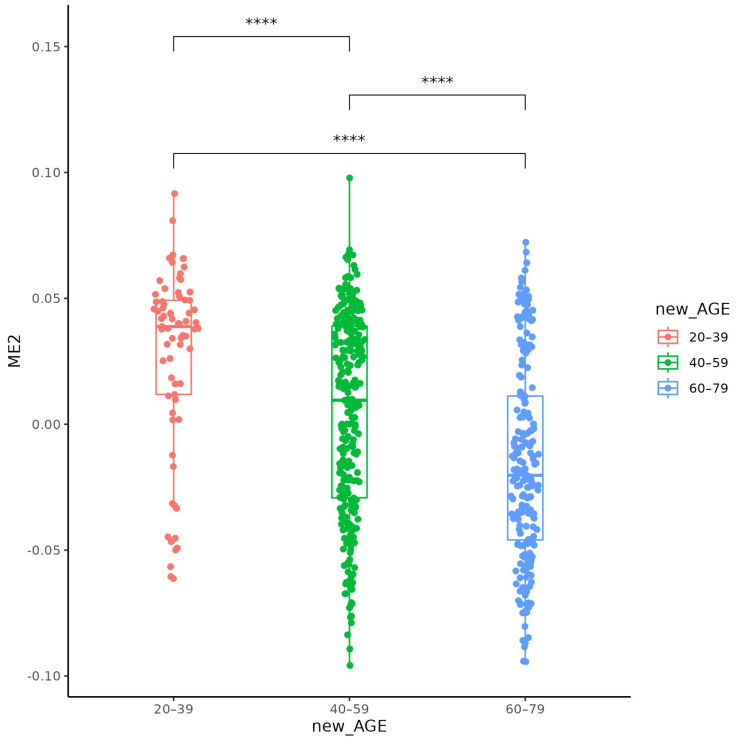
Size of a module is huge (9127 genes).
Intersection is significant at $p < 0.05$ (Chi-Square)

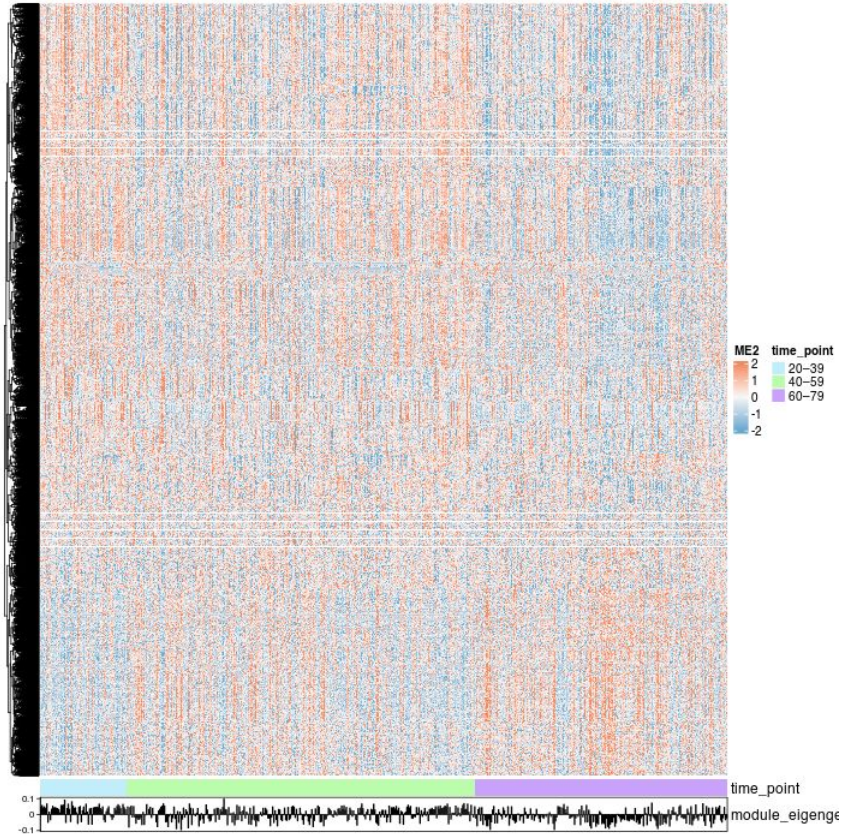
Liver: no GO and KEGG, no intersection



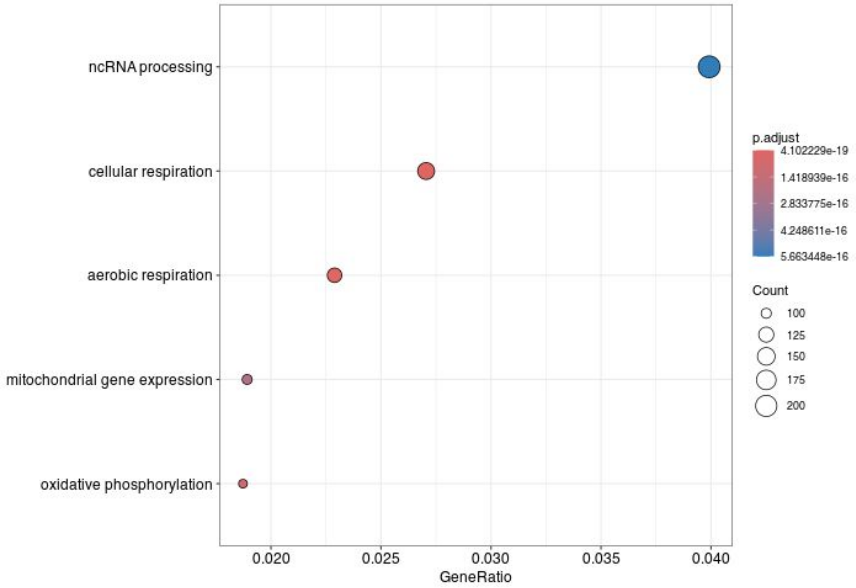


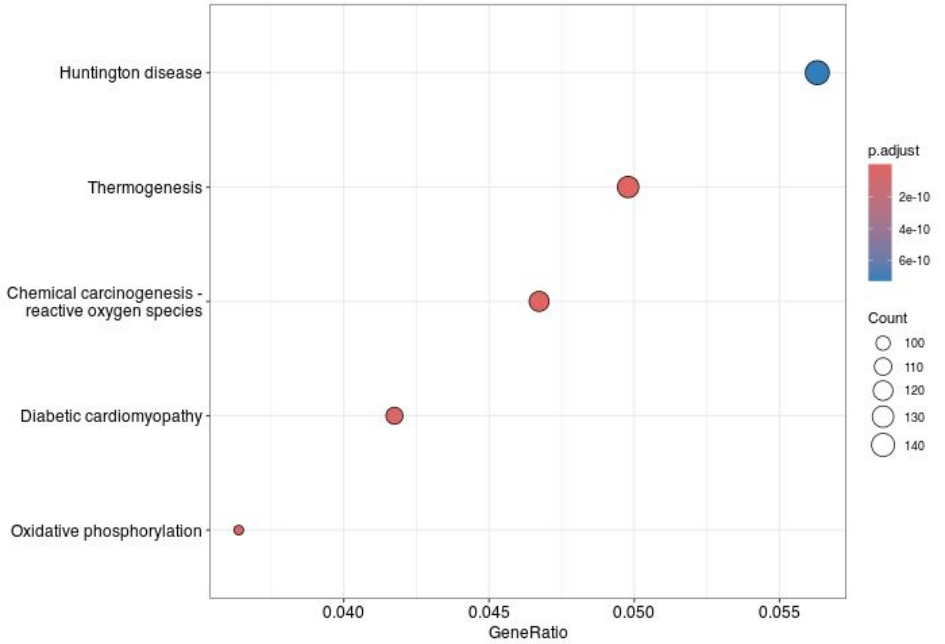
Lung: the same idea of homogeneity





Lung: GO and KEGG





Intersection: 40 matrisome genes

What can be done further?

Check for other modules and for other samples from this database

Repeat of the same workflow with more homogeneous samples (filtration by expression patterns?)

Intersect matrisome genes with categories in GO/KEGG enrichment

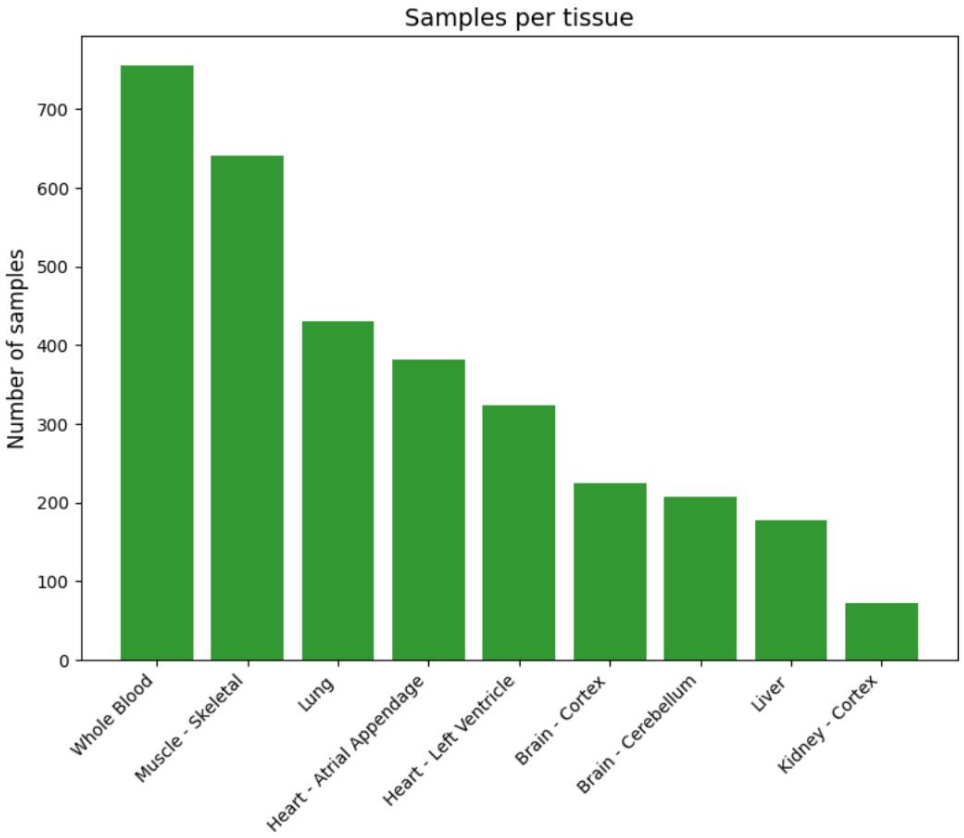
Use GO/KEGG groups to compare particular gene expression dynamics through different age (for now it’s not really informative)

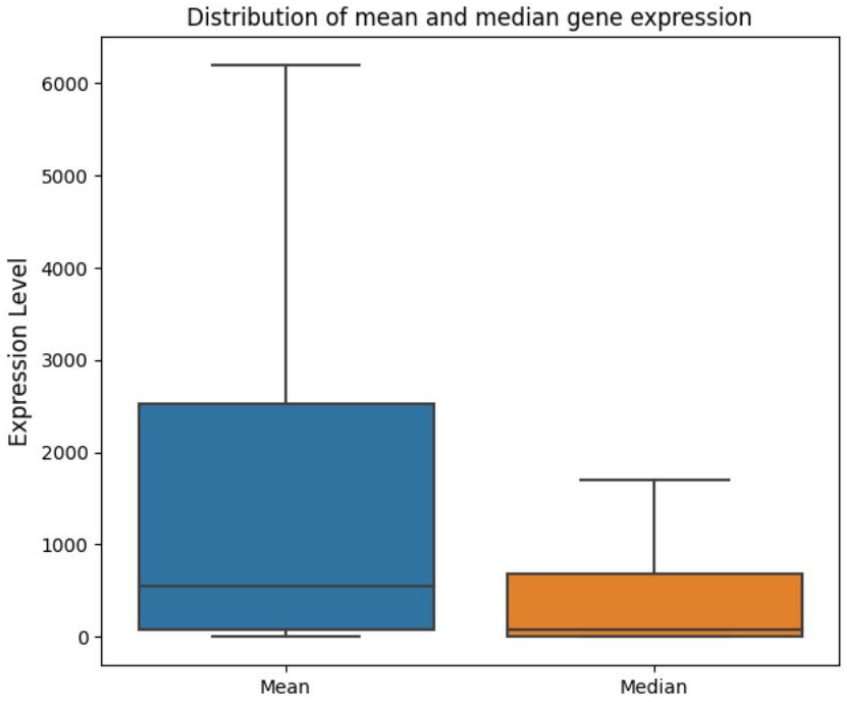
Construct regulatory networks to restore molecular mechanisms of agents interactions in modules

Check for found matrisome gene functions

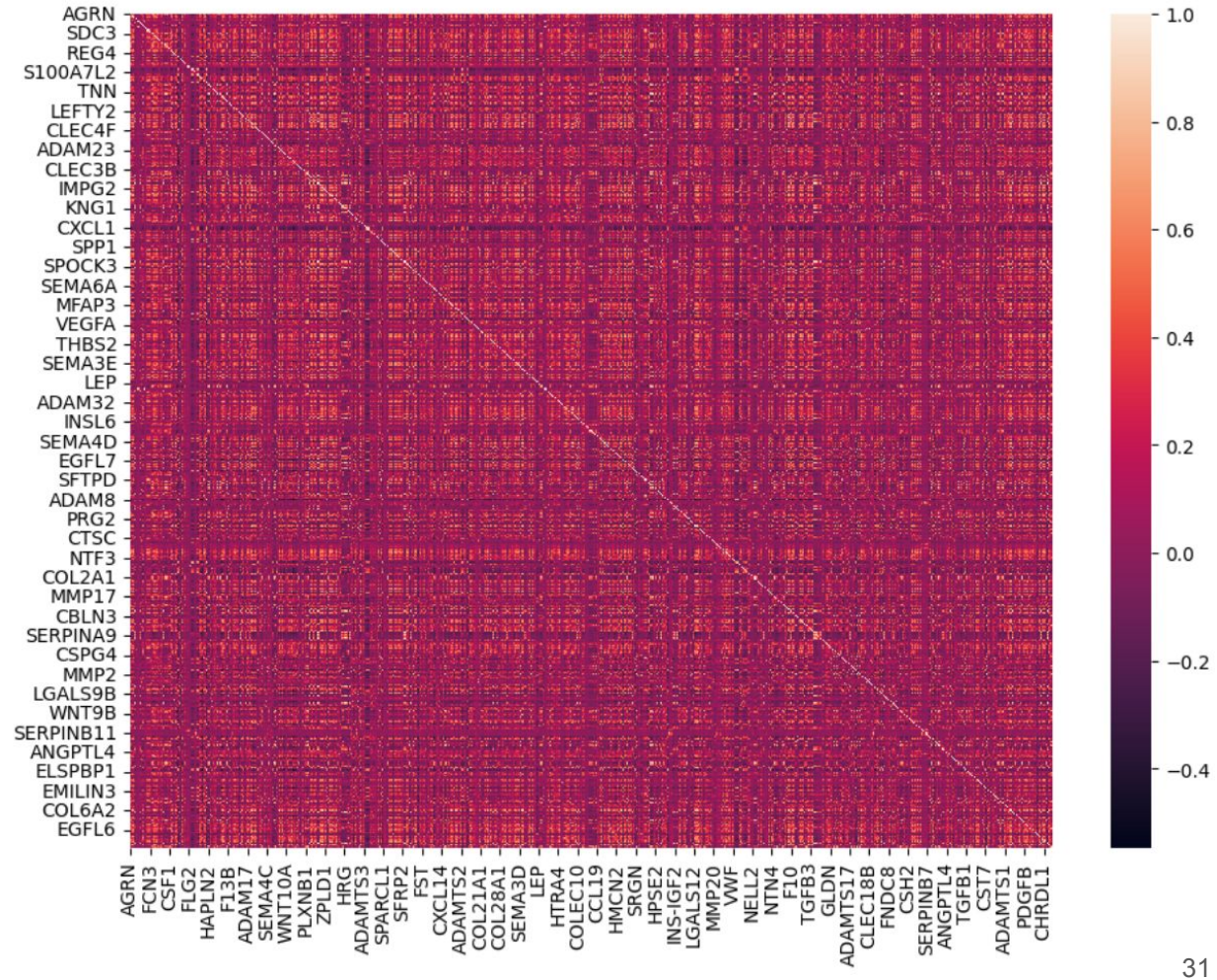
Matrisome gene expression prediction

3415 samples and 902 matrisome genes



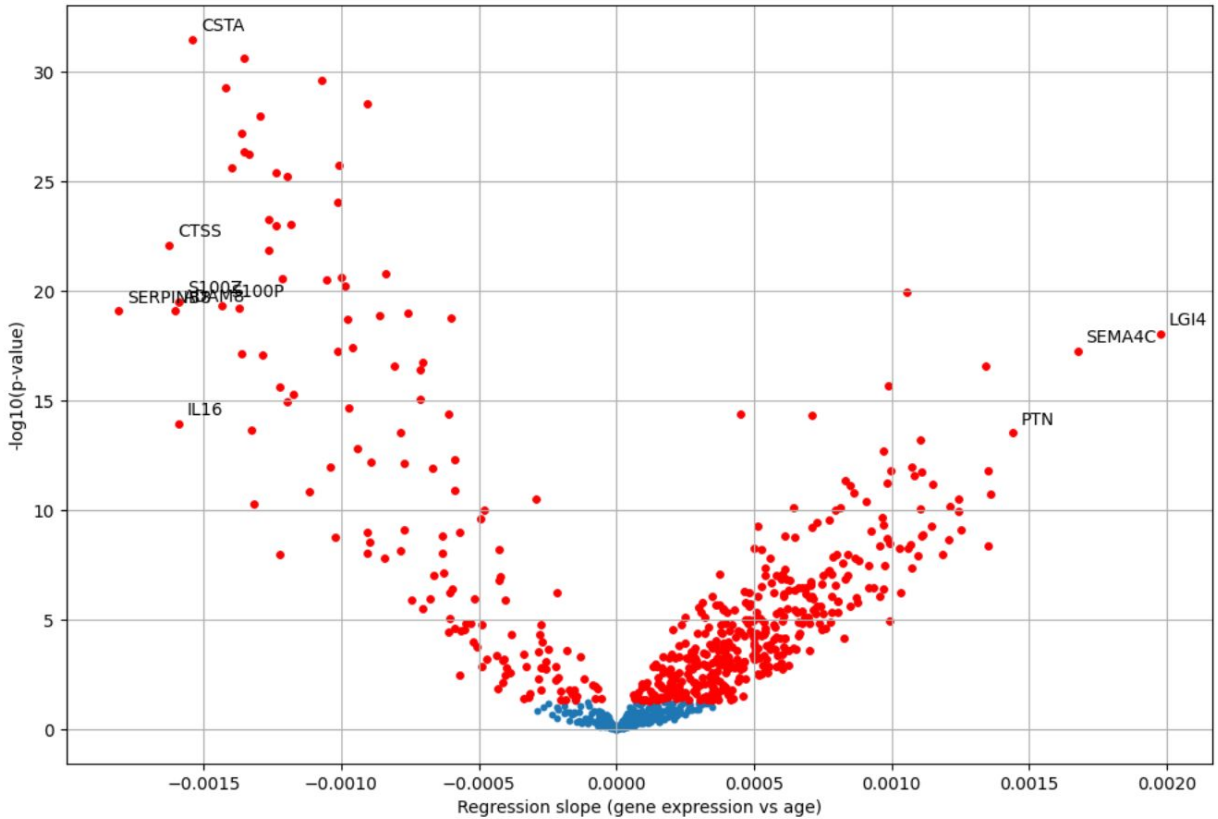


5808 unique gene pairs with:
0.7 < correlation < 1

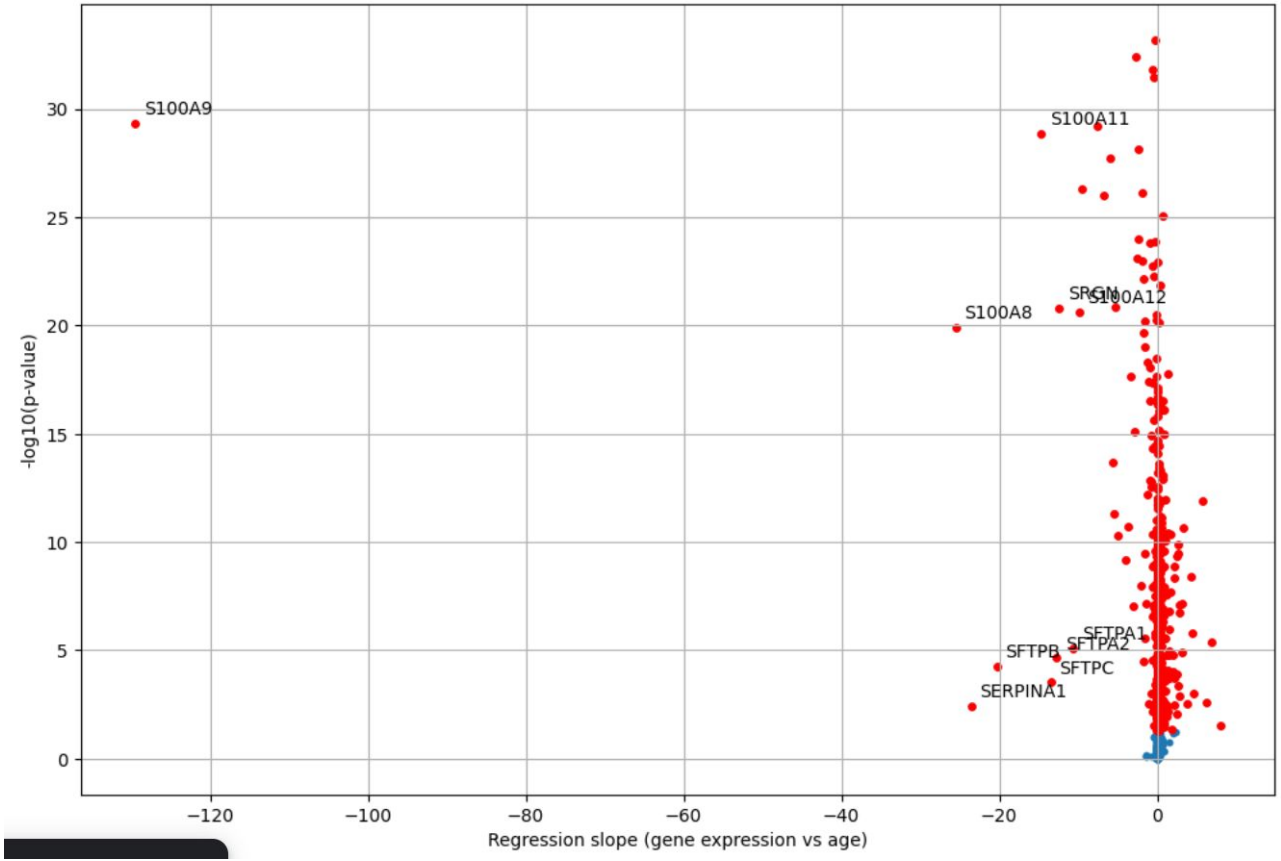


LGI4
SERPINB8
SEMA4C
CTSS
ADAM8
IL16
S100Z
CSTA
PTN
S100P

Regression slope (gene expression vs age) and p-value

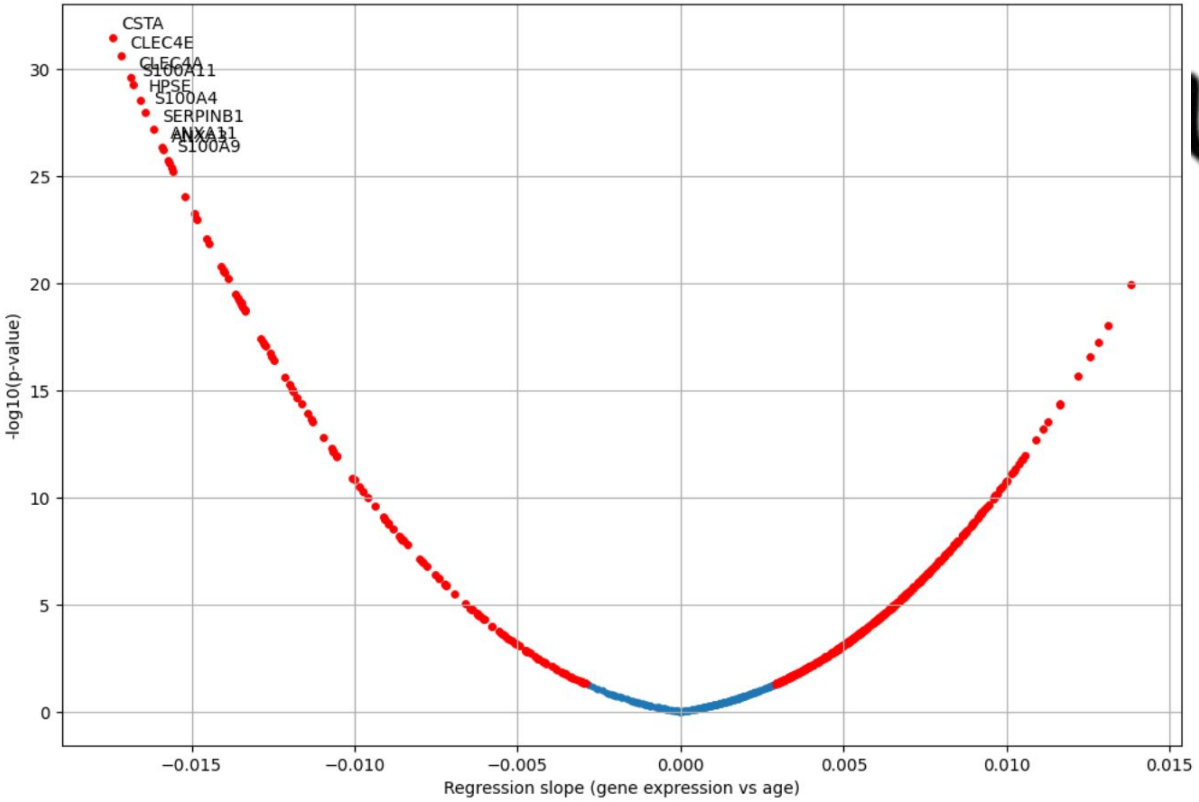


CPM normalisation



CSTA
CLEC4E
CLEC4A
S100A11
HPSE
S100A4
SERPINB1
ANXA11
ANXA3
S100A9

StandartScaler



Workflow

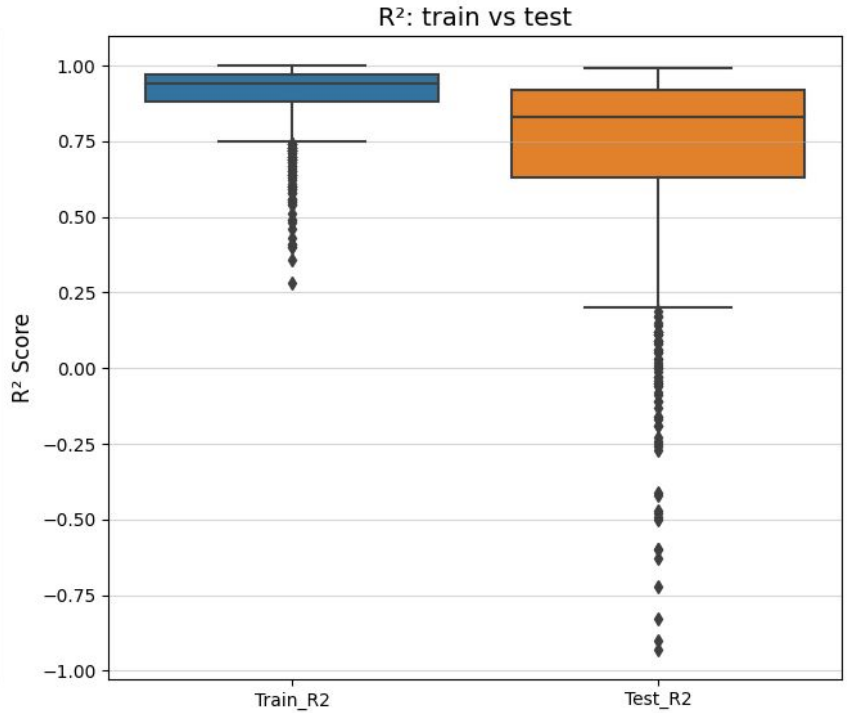
1) Keep only **matrisome genes** from the gene expression table (**902 genes**)
2) Encode categorical features (Age, Sex, Tissue)
3) **Divide** dataset on **train and test** sets
4) On train set obtain **correlations**
5) Choose model and go through all 902 genes:
   - remove redundant genes (correlation > 0.7)
   - optimise hyperparameters with optuna (if needed)
   - make prediction on train and test sets

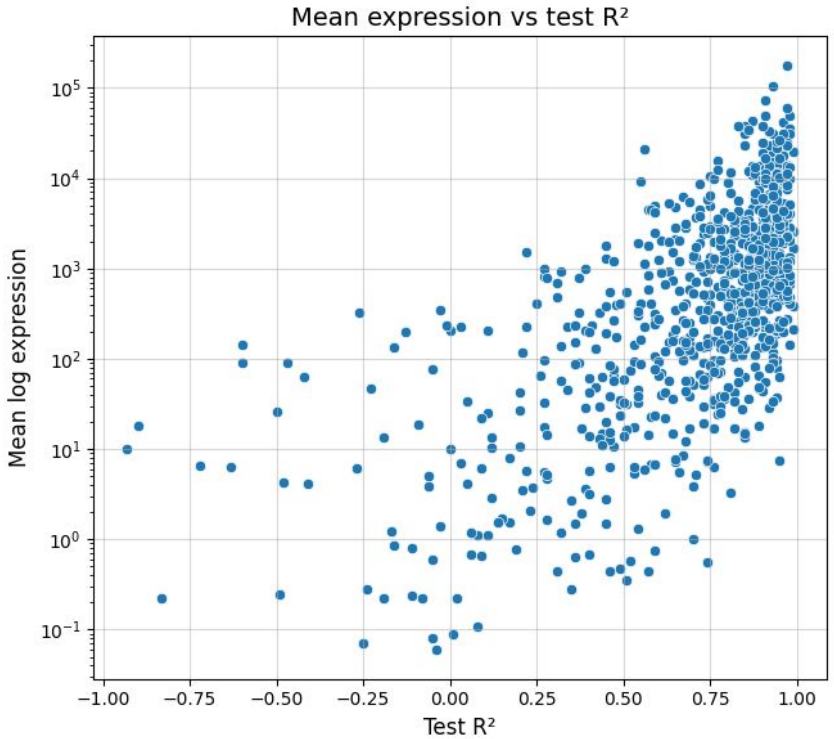
Results

	Linear Regression	Linear Regression (without corr genes)	Ridge (alpha = 100)	Ridge (optuna)	Lasso (optuna)	**Elasticnet (optuna)**	**KNN**	DecisionTree
Mean R2 test	-0,21	0,09	0,32	0,39	0,41	0,57	**0,59**	-0,46
Median R2 test	0,72	0,74	**0,82**	0,79	0,79	0,72	0,7	0,6
Smallest R2	-217,72	-182,19	-102,48	-91,46	-88,87	-36,48	-17,63	-390,82
Number of R2<0	126	89	64	34	22	17	23	101

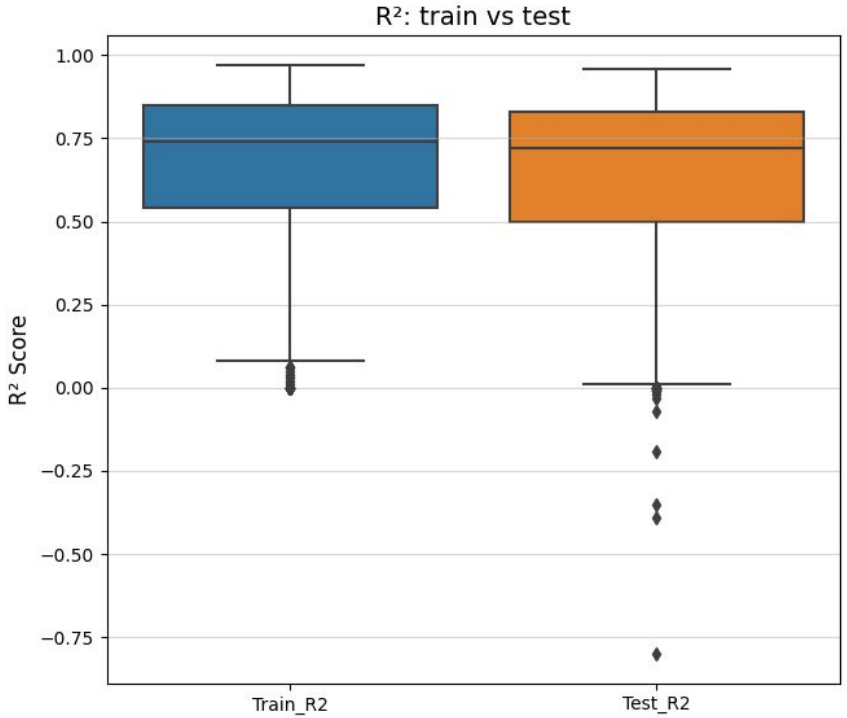
*XGBoost and random forest were computed for 500+ minutes each, so I stopped them

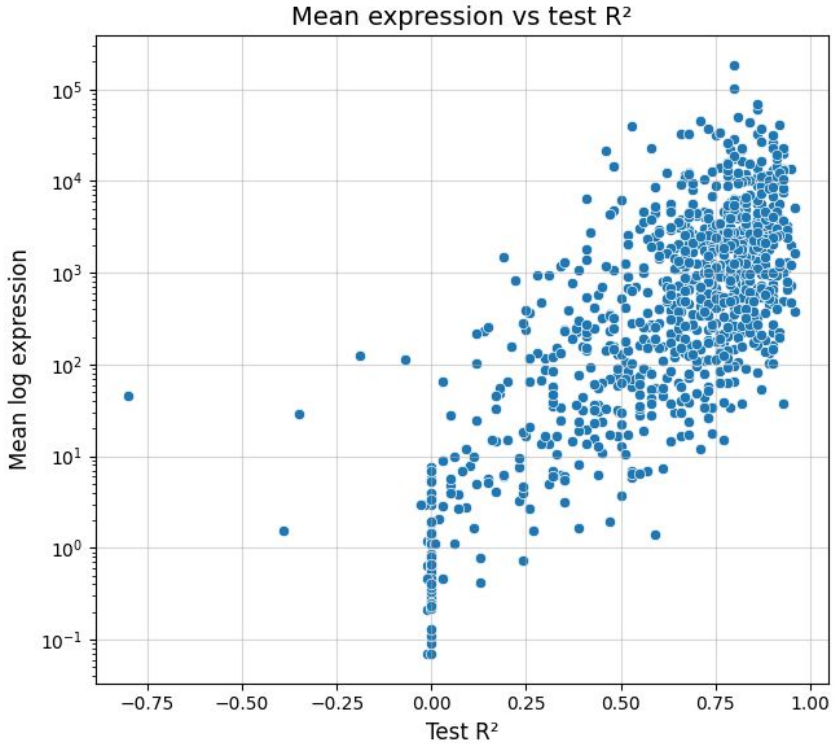
Ridge (alpha = 100)





ElasticNet


R²: train vs test
R² Score
1.00
0.75
0.50
0.25
0.00
−0.25
−0.50
−0.75
Train_R2
Test_R2

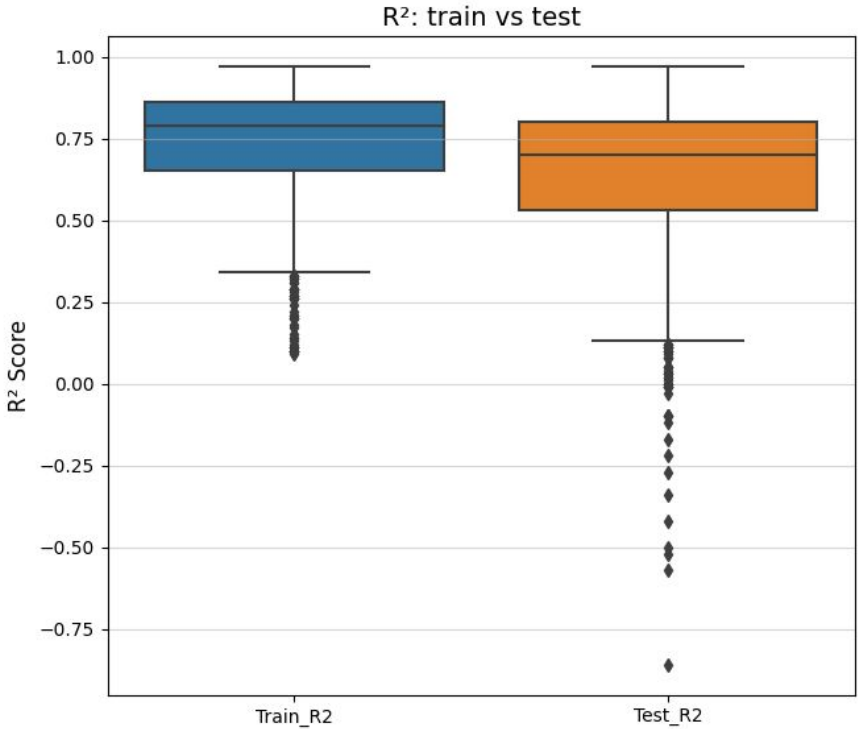

Mean expression vs test R²
Mean log expression
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
−0.75
−0.50
−0.25
0.00
0.25
0.50
0.75
1.00
Test R²

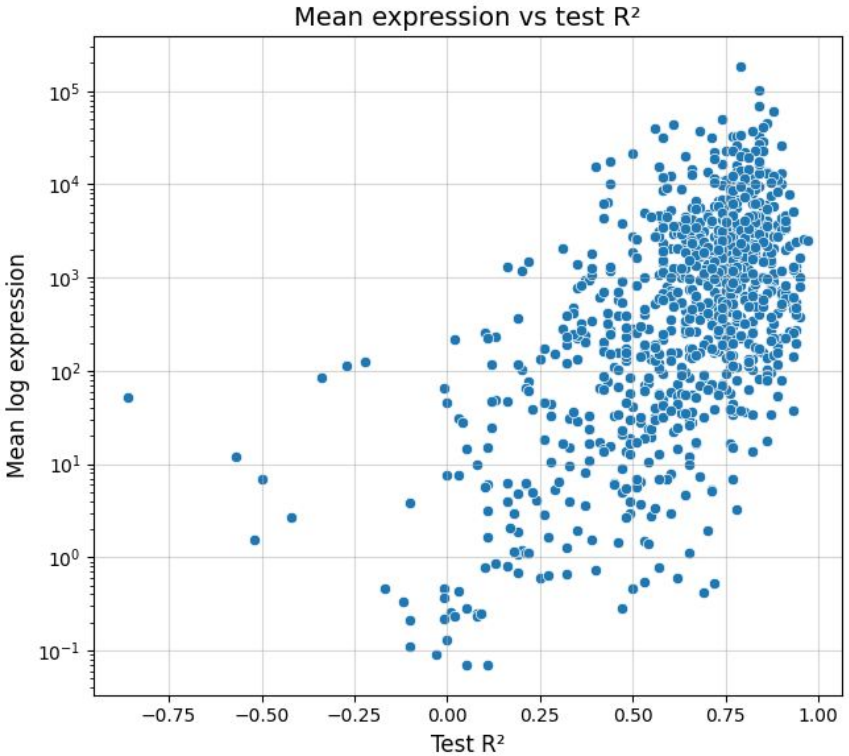
What else can be done?

1. Optimize code
2. Maybe normalise data with another approach
3. Check other regression models from sklearn
4. Use more data (not only tissues from article)
5. Combine with WGCNA analysis
6. Take age coefficients from regression and check, for which genes they are the highest + compare them with volcano plot results

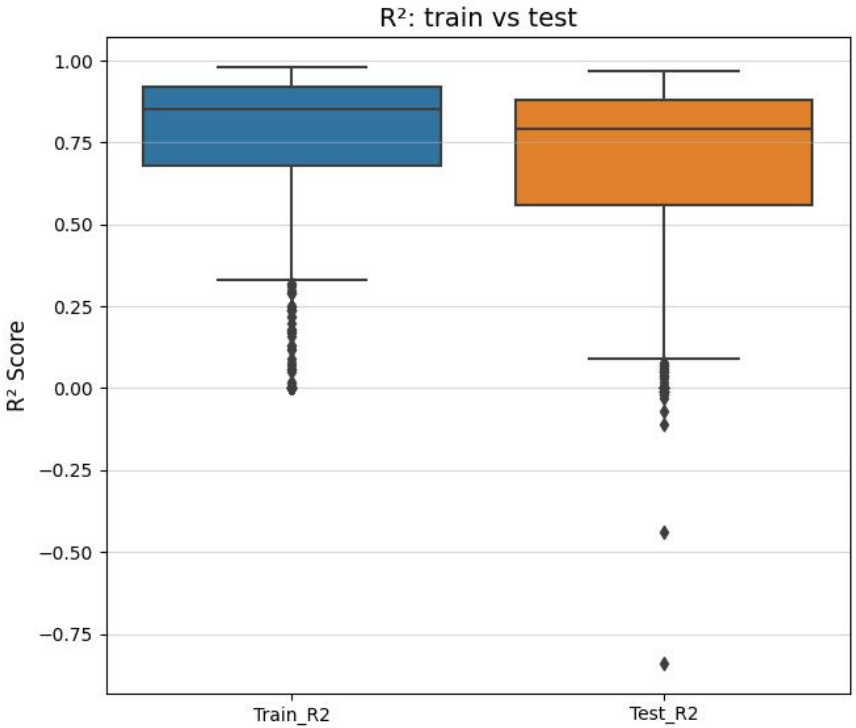

THANK YOU

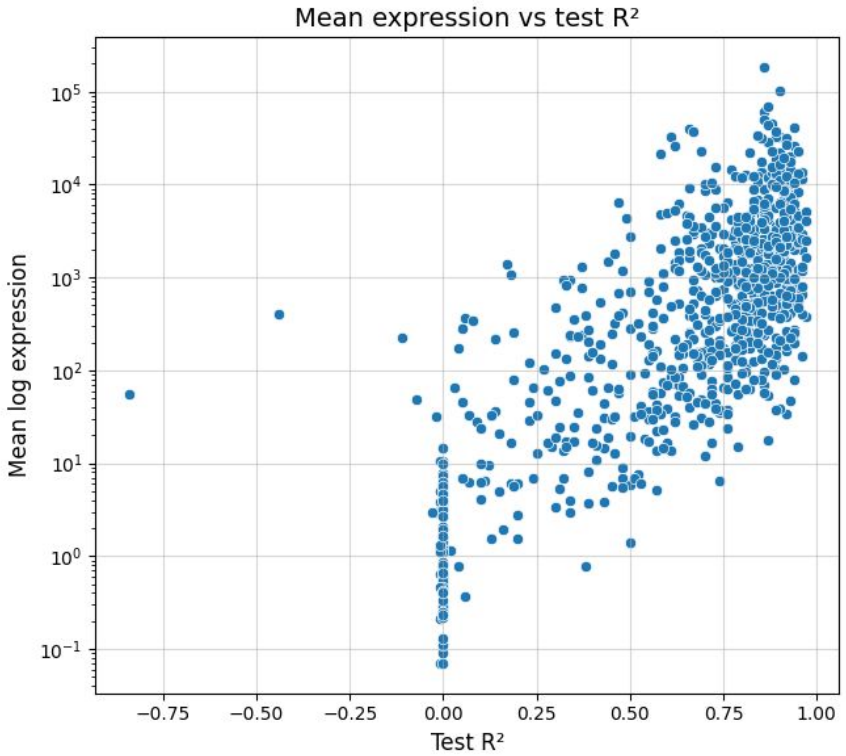
KNN Regressor



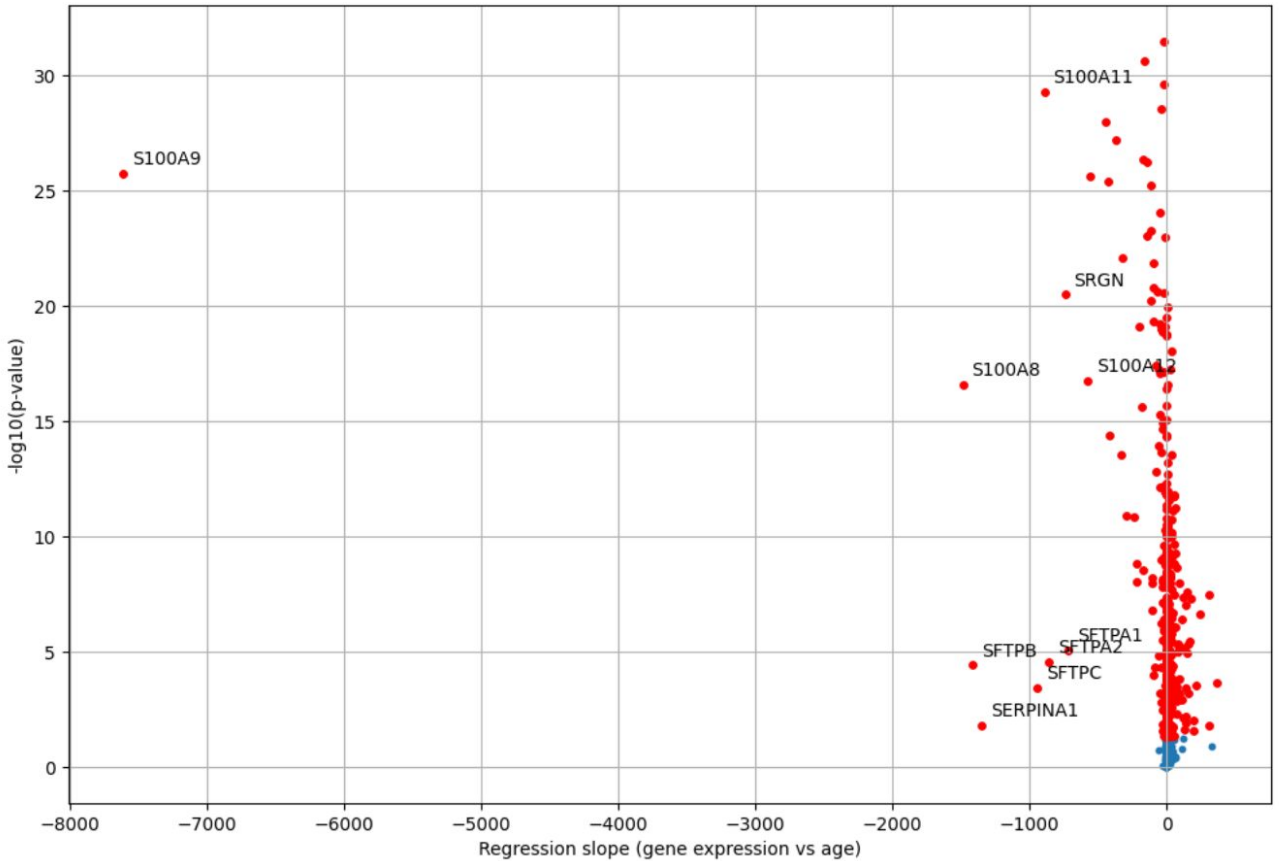


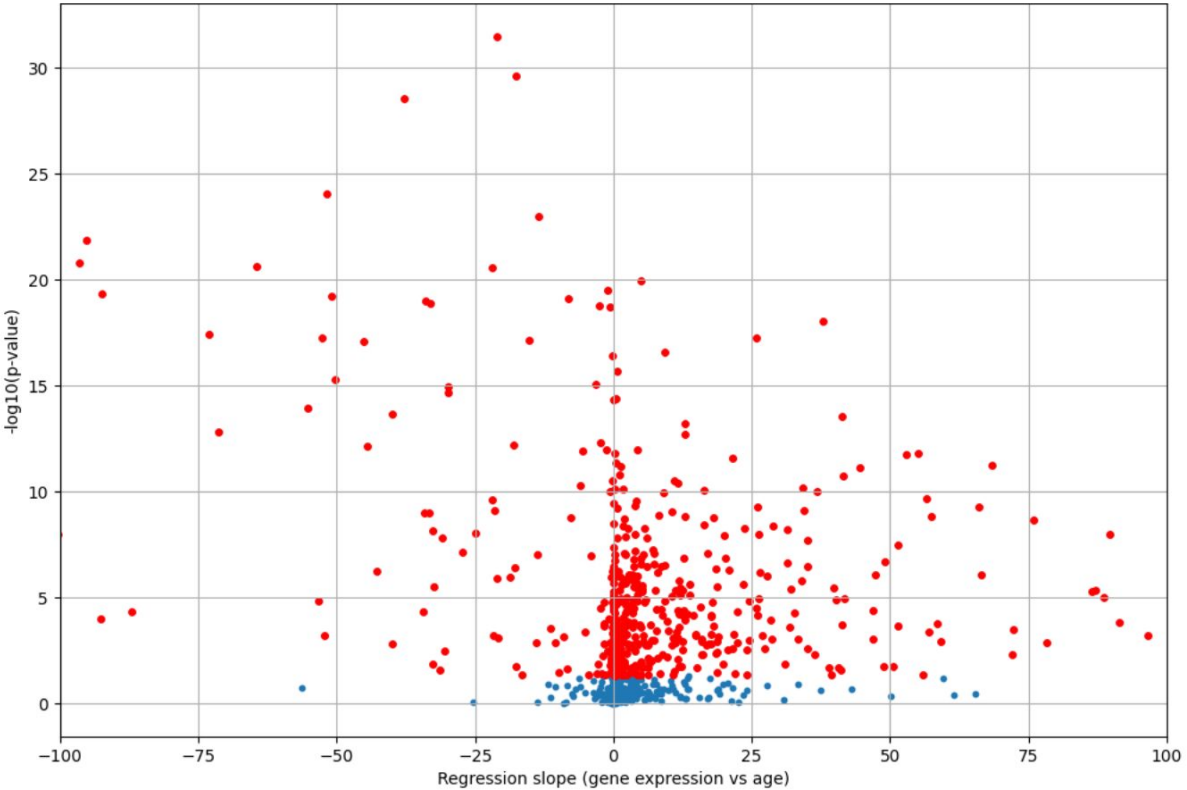
Lasso

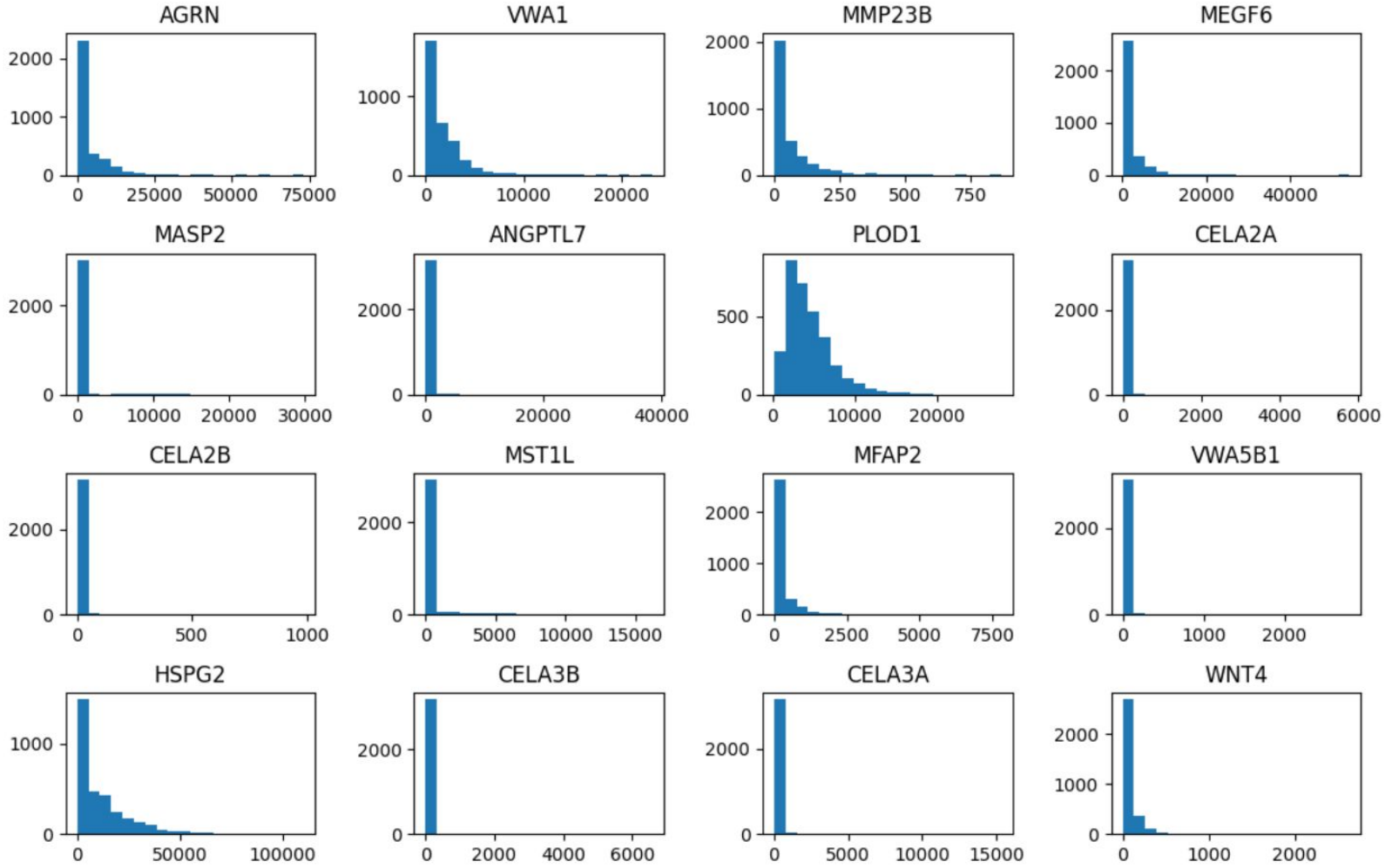

R²: train vs test
R² Score
1.00
0.75
0.50
0.25
0.00
−0.25
−0.50
−0.75
Train_R2
Test_R2


Mean expression vs test R²
Mean log expression
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
−0.75
−0.50
−0.25
0.00
0.25
0.50
0.75
1.00
Test R²

Without normalisation




-log10(p-value)
30
25
20
15
10
5
0
−100
−75
−50
−25
0
25
50
75
100
Regression slope (gene expression vs age)


AGRN
VWA1
MMP23B
MEGF6
MASP2
ANGPTL7
PLOD1
CELA2A
CELA2B
MST1L
MFAP2
VWA5B1
HSPG2
CELA3B
CELA3A
WNT4

$$R^2 = 1 - \frac{\sum_{1=1}^{N} (y_i - \hat{y}_i)^2}{\sum_{1=1}^{N} (y_i - \bar{y})^2}$$