

Antibody Responses to Different Treatments in nmCSPC Prostate Cancer Patients

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Contents

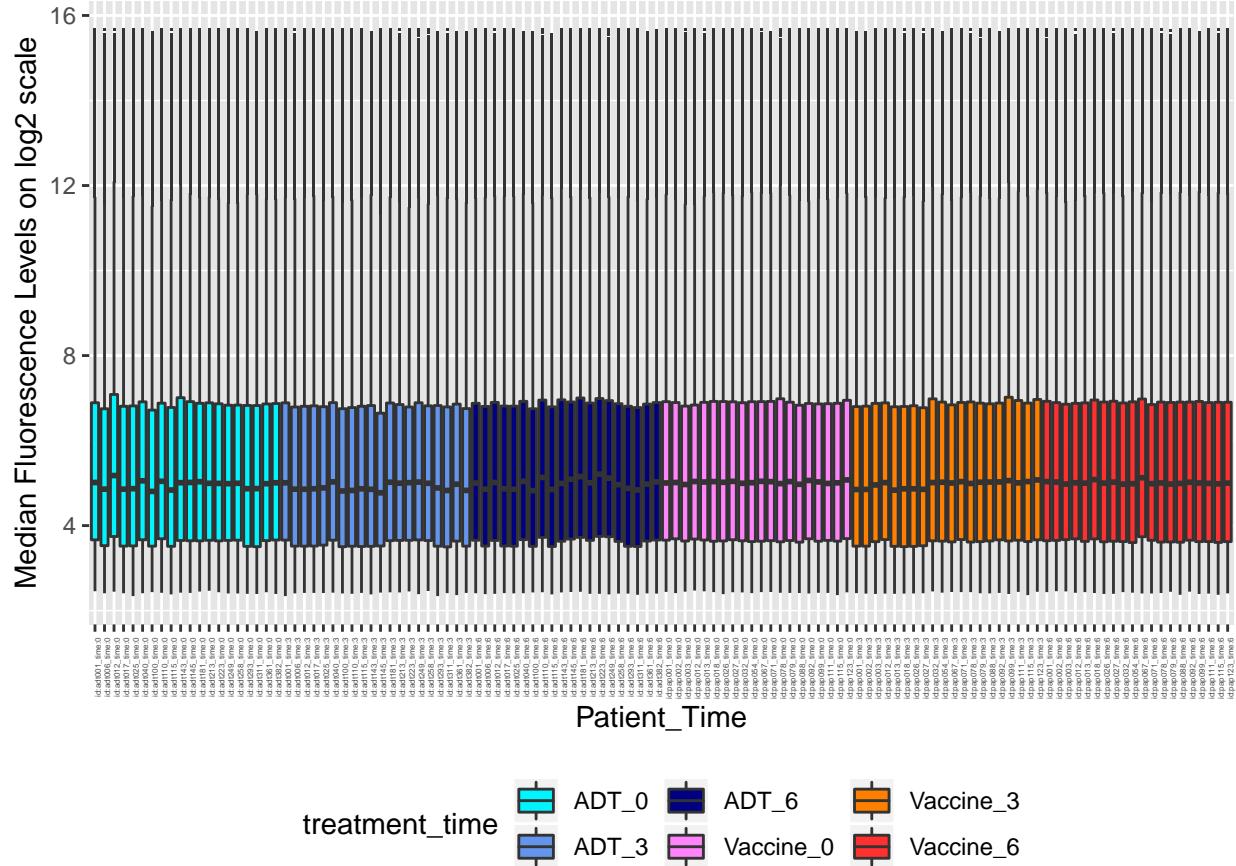
1	Introduction	1
2	Statistical Test for Treatment Effects	2
3	Visualization of Treatment Effects (Higher Antibody Response in PAP)	5
3.1	Treatment Effects at 3 months	6
3.2	Treatment Effects at 6 months	9
3.3	Boxplots of a few Significant Peptides	11
4	Visualization of Treatment Effects (Higher Antibody Response in ADT)	13
4.1	Treatment Effects at 3 months	13
4.2	Treatment Effects at 6 months	16
4.3	Boxplots of a few Significant Peptides	18
5	Gene-Set Analysis (Genes Significant for Treatment Effects)	20
6	Concluding Remarks	28

1 Introduction

We would like to investigate whether different treatment induces different changes in antibody repertoires in individuals over time. To address this question, we used serum samples available from the 40 patients with nmCSPC who were treated with one of two therapies. 20 patients received standard androgen deprivation therapy (ADT; GnRh analogue given every 3 months), and 20 patients received a DNA vaccine encoding prostatic-acid phosphatase (PAP; pTVG-HP given every 14 days for 6 administrations). Samples were collected (3 replicates) from each of these patients at baseline, 3 months, and 6 months following initiation of treatment.

We take \log_2 transformation on the fluorescence levels prior to subsequent steps in our analysis. In order to verify normalization of the fluorescence level, we also plot the boxplots of median (across replicates) \log_2 fluorescence level of all peptides for each patient.

Boxplots of Peptide Fluorescence Levels for Patients at 3 time points



It appears that the fluorescence levels of the peptides are normalized.

2 Statistical Test for Treatment Effects

For each of the peptides, we test the following:

H_0 : Different treatments (regardless of whether ADT or PAP) do not result in different antibody responses over time.

H_1 : Different treatments lead to different antibody responses over time.

We deploy the following nested linear mixed-models using R package *lme4*:

Null model:

pseudo-syntax: `lmer(fluorescence ~ time + (1 + time | patient))`

In mathematical notation, let $y_{j\tau}$ be the median fluorescence level on \log_2 scale for the j^{th} patient at time τ ,

$$y_{j\tau} = \beta_0 + \beta_1 \tau + b_{0j} + b_{1j}\tau + \epsilon_j,$$

where

- β_0 = the baseline antibody response level for all patients
- $\tau = 0, 3$ or 6 months
- $j = 1, \dots, 20$ indexes the patients at time τ
- b_{0j} is the random intercept of the j^{th} patient

- b_{1j} is the random slope of the j^{th} patient
- $\begin{pmatrix} b_{0j} \\ b_{1j} \\ \epsilon_{ij} \end{pmatrix} \sim N_3 \left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \Sigma = \begin{bmatrix} \sigma_0^2 & \rho\sigma_0\sigma_1 & 0 \\ \rho\sigma_0\sigma_1 & \sigma_1^2 & 0 \\ 0 & 0 & \sigma_\epsilon^2 \end{bmatrix} \right)$

Alternative model:

pseudo-syntax: lmer(fluorescence ~ treatment + time + treatment:time + (1 + time | patient))

In mathematical notation,

$$y_{ij\tau} = \beta_0 + \beta_1\tau + \beta_2x_i + \beta_3x_i\tau + b_{0j} + b_{1j}\tau + \epsilon_{ij},$$

where

- β_0 = the baseline antibody response level for patients administered with ADT.
- $\tau = 0, 3$ or 6 months
- $x_i = 1$ if patient is administered with PAP vaccine and 0 otherwise
- $\beta_2 + \beta_3\tau$ represents the difference in antibody response level due to the PAP vaccine compared to ADT at time τ
- $j = 1, \dots, 10$ indexes the patients at time τ for each of the 2 treatment types
- b_{0j} is the random intercept of the j^{th} patient
- b_{1j} is the random slope of the j^{th} patient
- $\begin{pmatrix} b_{0j} \\ b_{1j} \\ \epsilon_{ij} \end{pmatrix} \sim N_3 \left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \Sigma = \begin{bmatrix} \sigma_0^2 & \rho\sigma_0\sigma_1 & 0 \\ \rho\sigma_0\sigma_1 & \sigma_1^2 & 0 \\ 0 & 0 & \sigma_\epsilon^2 \end{bmatrix} \right)$

In other words, we are testing

$$H_0: \beta_2 = \beta_3 = 0.$$

$$H_1: \text{Not both } \beta_2 \text{ and } \beta_3 \text{ are zeroes.}$$

Rationale of the models: Since each patient has multiple measurements, the random effects of the mixed model allow us to capture the within-subject interdependencies. Every patient's antibody response is unique and possibly changes across time due to individual circumstances, so we want our model to include random intercept (representing patient-specific randomness) and random slope (of time). Since measurements were taken across only 3 time points, we refrain from considering more complicated random effects. Where fixed effects are concerned, both types of treatments could lead to a change in antibody response *over time across all patients*, which would be captured in the *time fixed effect*. The alternative model has the additional terms of treatment main effect and treatment-time interaction effects. If different treatments (regardless of whether ADT or PAP) do not result in different antibody responses over time, then these two additional terms could be dropped.

Model-fitting and Test Statistics: Hypothesis testing in linear mixed-models is still an active area of research. Due to the large number of peptides, any non-parametric tests like permutation tests (shuffling treatment identifiers among patients by respecting time blocks) are prohibitively expensive in terms of computation. There are three usual parametric approximate tests for fixed effects in linear mixed models:

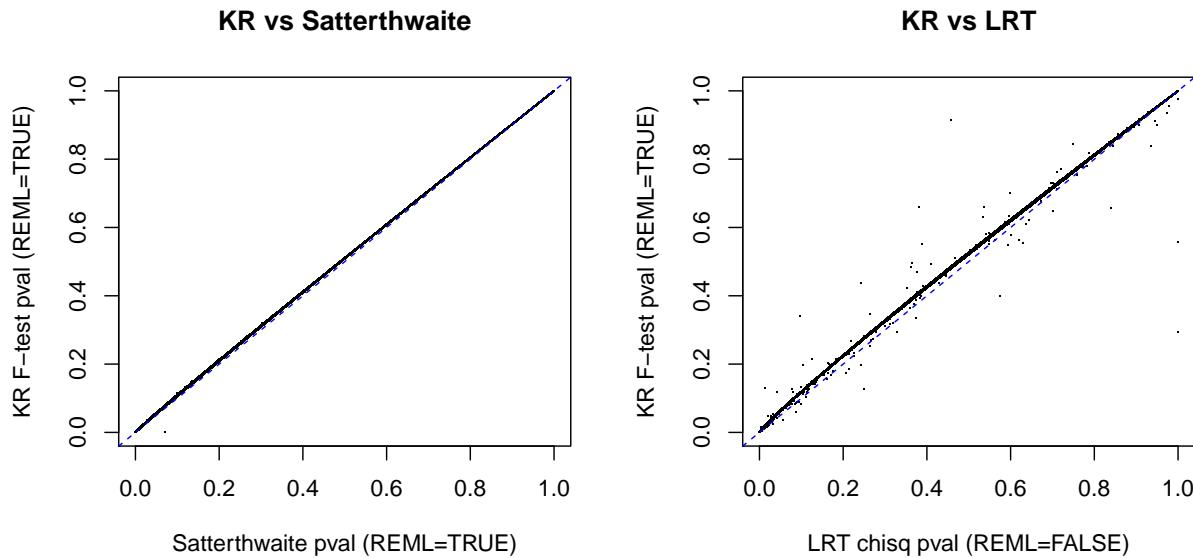
- Kenward-Roger (KR) approximate F-test, with model estimates fitted using the Restricted Maximum Likelihood (REML) approach,
- Satterthwaite approximate F-test, with model estimates also fitted with REML, and
- likelihood ratio test (LRT), with model estimates fitted using the usual Maximum Likelihood (ML) approach.

Roughly, unlike the ML approach, the REML method gives unbiased estimate of $\widehat{\Sigma}$. This is imperative, since $\widehat{\Sigma}$ feeds into the F-test calculations. Both Kenward_Roger and Satterthwaite approximations aim to adjust the degrees-of-freedom in the F-test to account for the additional estimation of covariance terms in the random effects of mixed models, as compared to a vanilla F-test in basic linear models. Likelihood ratio test is only meaningful when parameter estimates are fitted with ML, otherwise the likelihood ratio test statistic may even end up as a negative value.

None of the three tests are uniformly “better” (depends on your own metric) than the others. The consensus is that likelihood ratio test (LRT) could be slightly more liberal than the other two methods. KR and Satterthwaite approximations usually give comparable results, and the Satterthwaite method is also the default linear-mixed-model setting in SAS and in the R package *lmerTest*. (Need to fill in citations for this paragraph)

Statistical Results

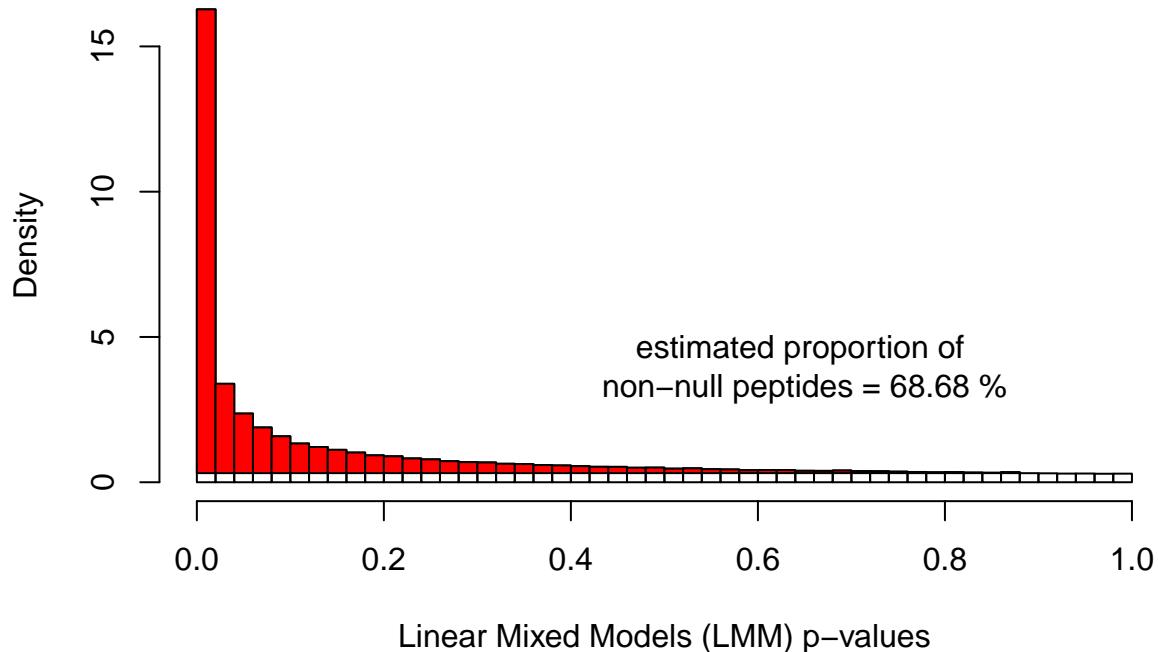
We applied all three methods to obtain the p-values for each of the 177,604 peptides.



It appears that in our case, the Kenward-Roger approximate F-tests give the most conservative p-values out of the 3 methods. Our subsequent analysis will be based on the Kenward-Roger(KR) p-values.

First, we obtain the p-values histogram.

p-values distribution for 177604 peptides



The red-shaded regions of the histogram represents the estimated proportion of non-null peptides in the dataset based on Storey's q-values calculation obtained via the R package `fdrtool`. The q-value is similar to the well known p-value, except that it is a measure of significance in terms of the false discovery rate rather than the false positive rate.

We apply the Benjamini-Hochberg (BH) method on the KR p-values to control for false discovery rate (FDR). The peptide counts at various BH FDR thresholds are tabulated below.

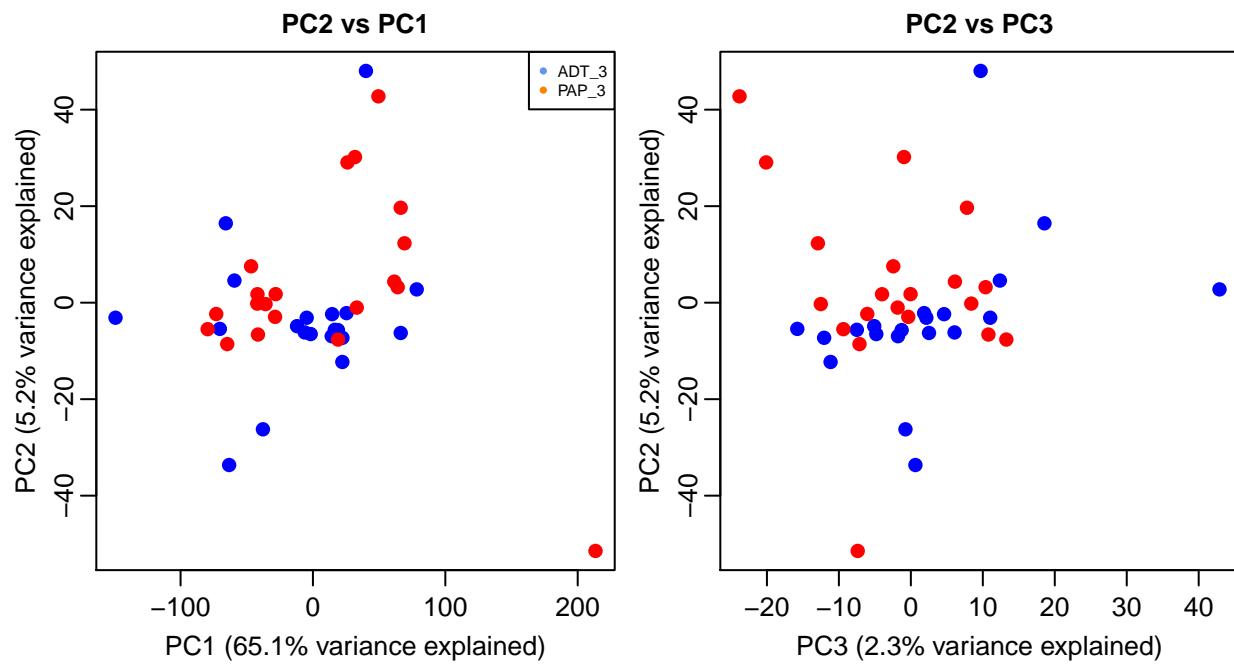
FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	29650	38191	44407	49300	53587	57293	60612	63748	66633	69388

3 Visualization of Treatment Effects (Higher Antibody Response in PAP)

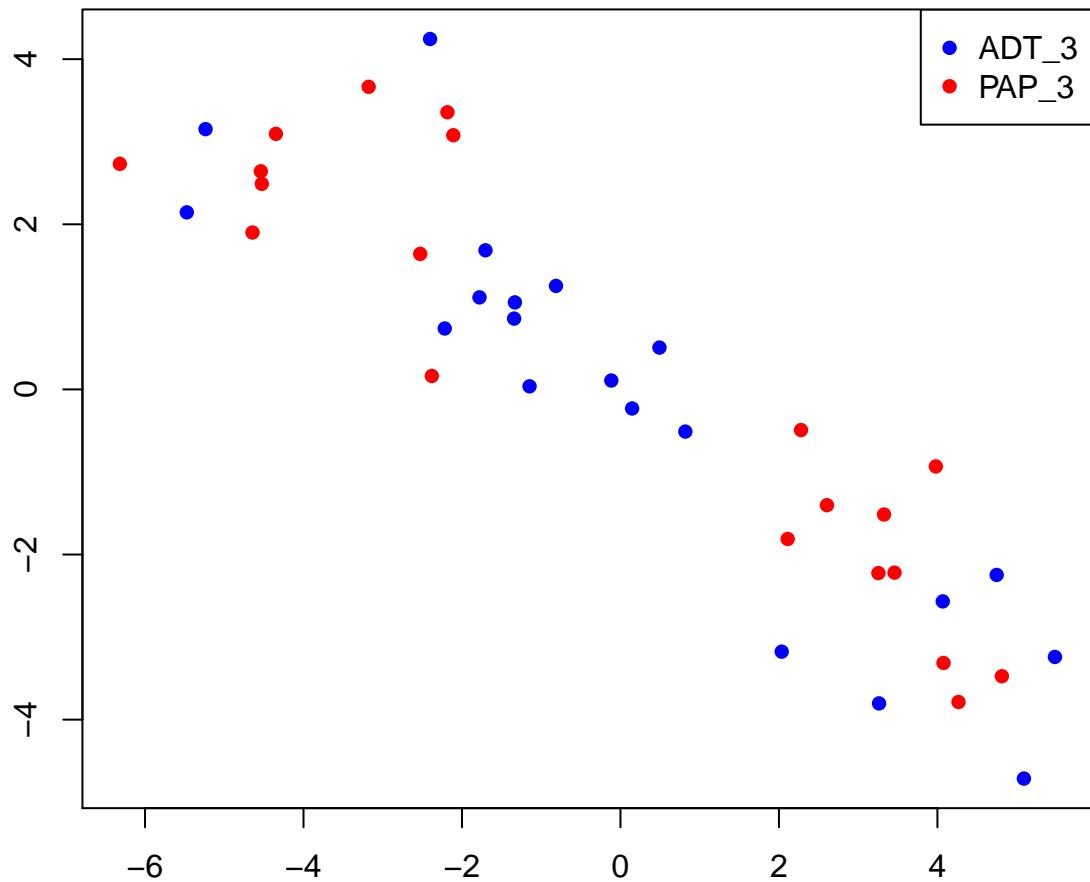
It appears that treatment effects are prominent among many peptides. For the purpose of visualizing more prominent treatment effects brought about by PAP vaccine as compared to ADT, we will focus on the significant peptides at 1 % FDR and that the PAP vaccine leads to higher antibody response by at least two-fold as compared to ADT at times 3-months and 6-months, ie. $(\hat{\beta}_2 + 3 \times \hat{\beta}_3) \geq 1$ and $(\hat{\beta}_2 + 6 \times \hat{\beta}_3) \geq 1$. There are 7351 peptides that fulfill these criteria. We exported the list of these peptides to the Sheet "Higher_in_PAP" in the Excel file *05_Longitudinal_SignifPeptides.xlsx*.

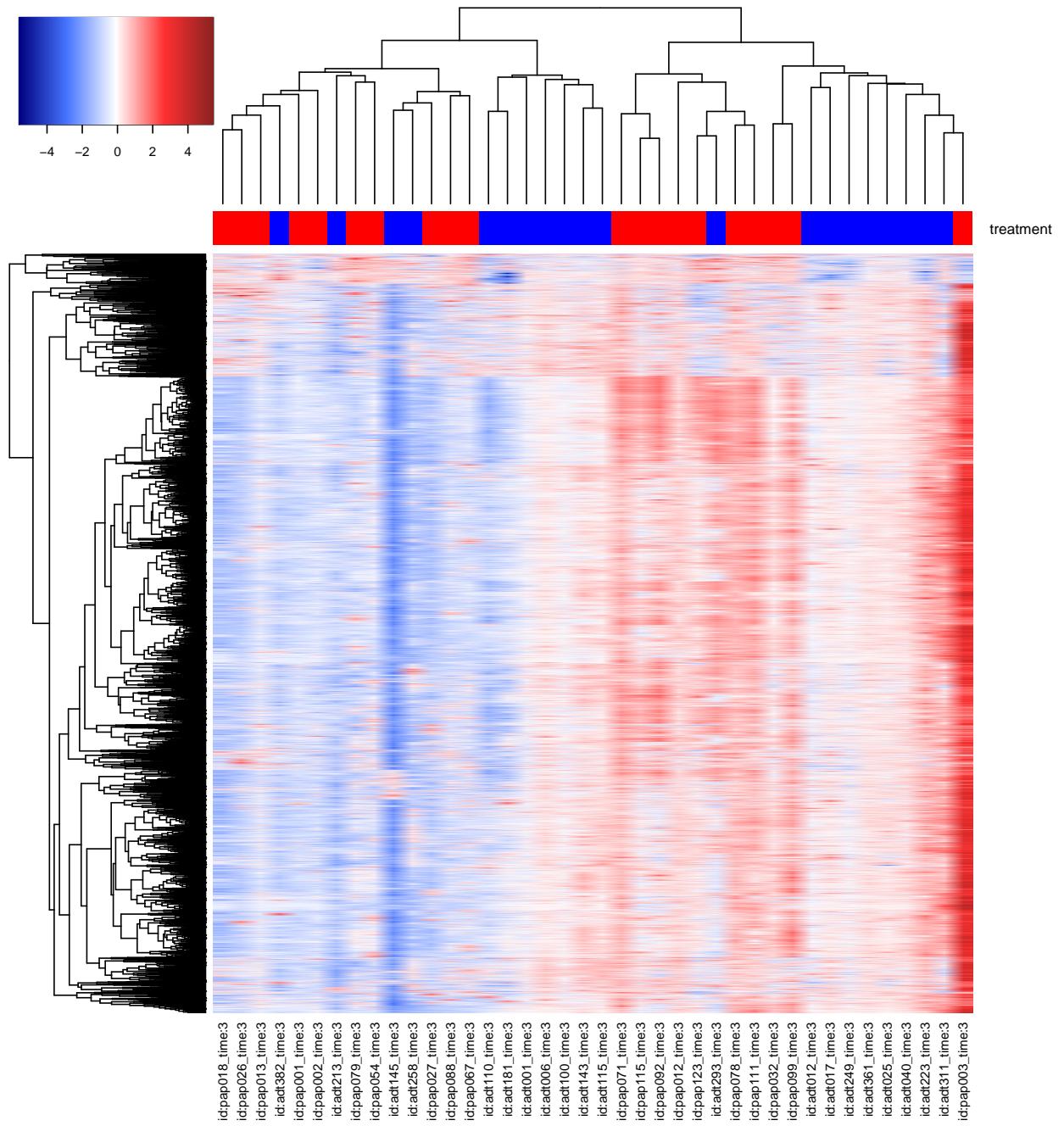
We obtain the residuals of the null model at times 3-months and 6-months, and plot these residuals using the PCA, t-SNE and heatmap approaches. Any observed clustering patterns based on treatment types would reveal the treatment effects not captured in the null model. We find that the clustering patterns are more obvious at 6-months' time than at 3-months' time.

3.1 Treatment Effects at 3 months

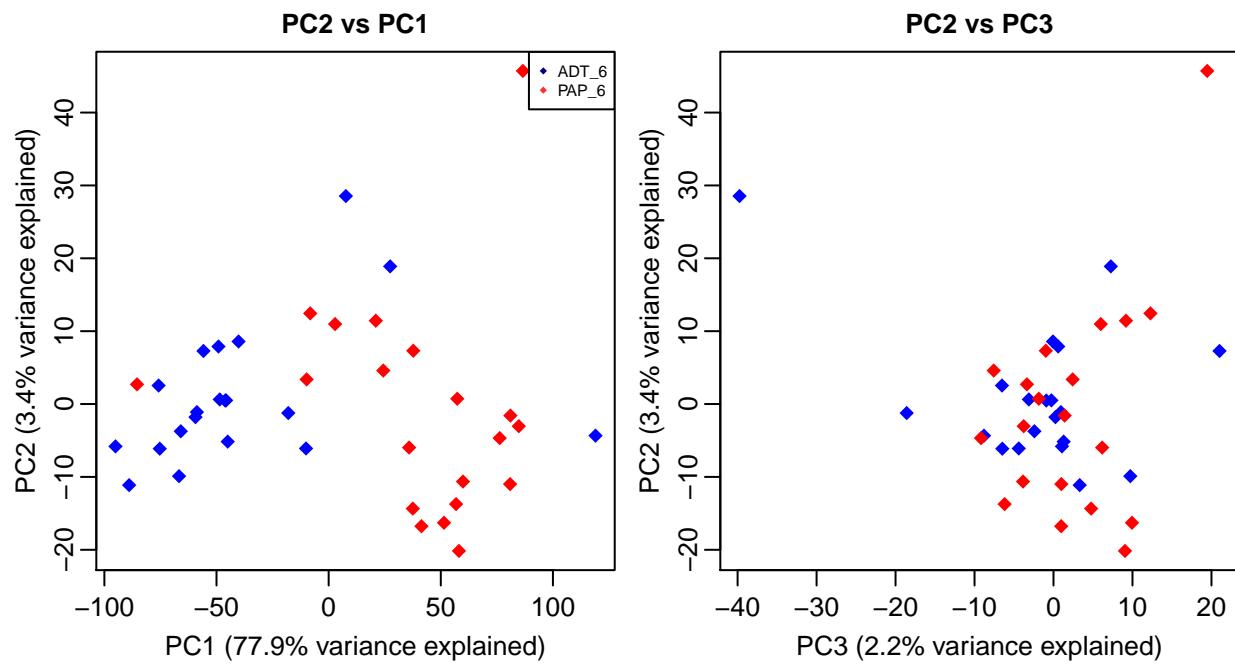


t-SNE plot

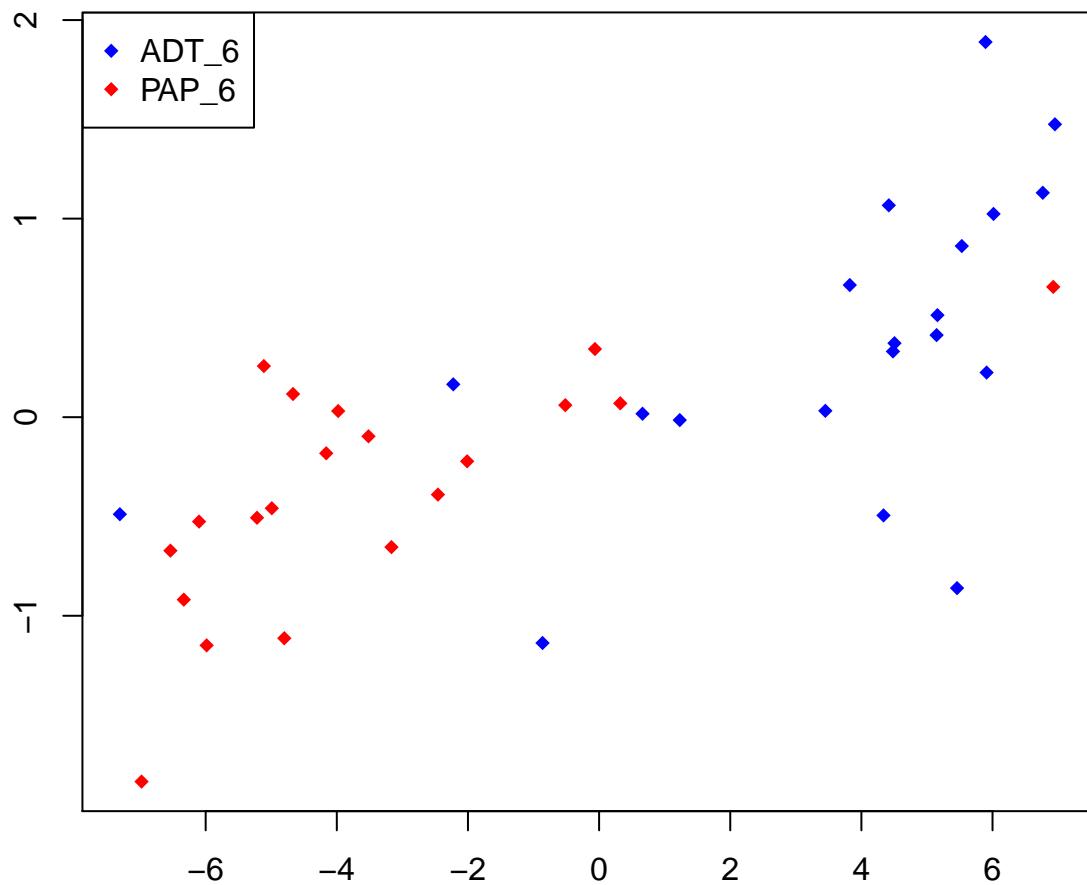


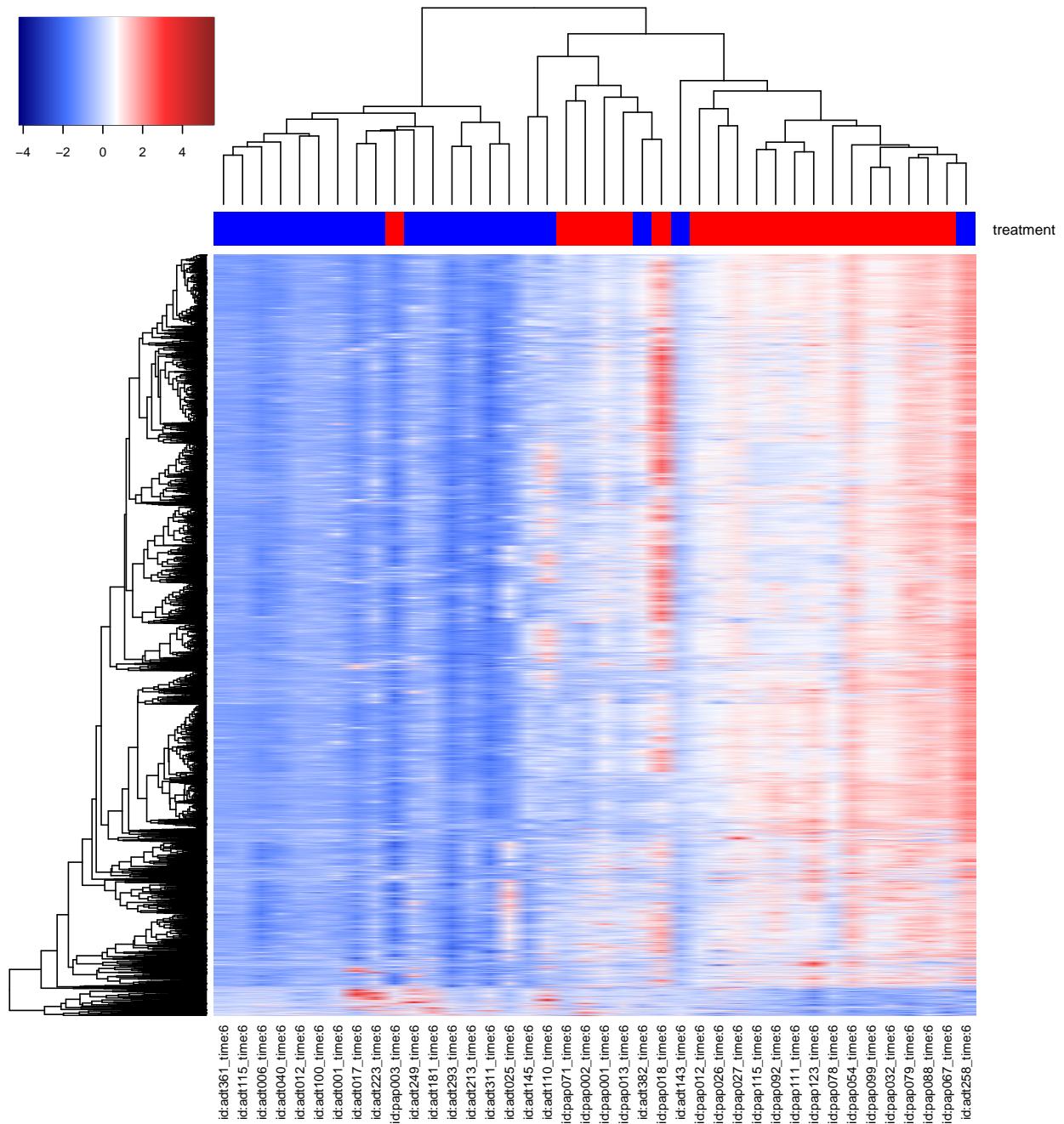


3.2 Treatment Effects at 6 months



t-SNE plot

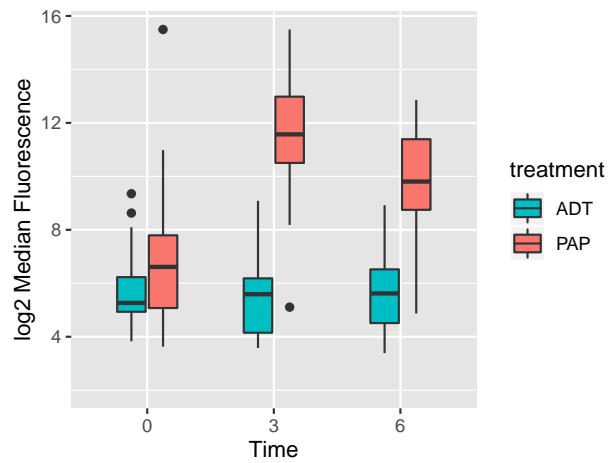




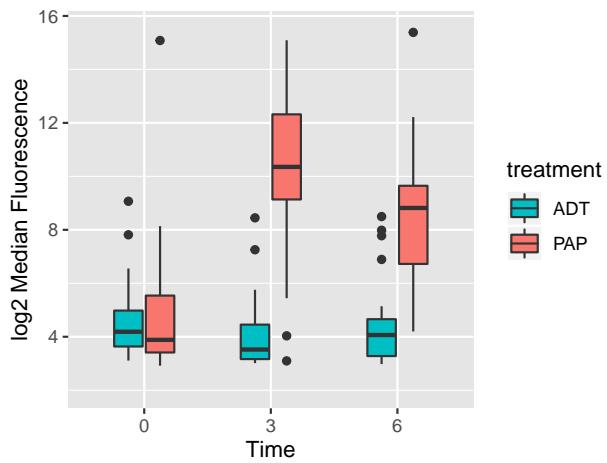
3.3 Boxplots of a few Significant Peptides

We also show the boxplots of fluorescence levels for a few significant peptides that have higher antibody response in PAP compared to ADT

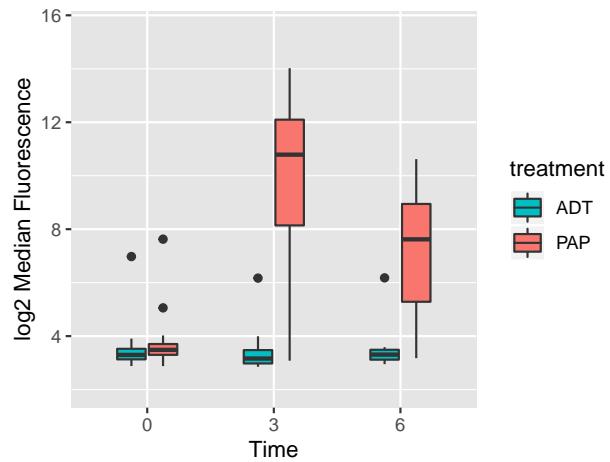
Boxplots of Fluorescence Levels for Signif Peptide: 677_ZNF598_90850;641



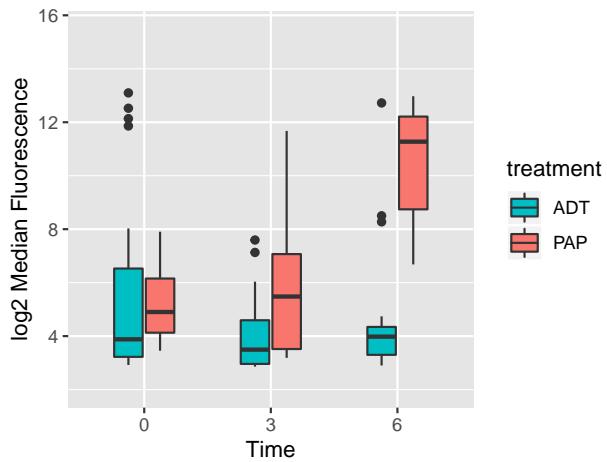
Boxplots of Fluorescence Levels for Signif Peptide: 976_FOXP1_27086;621



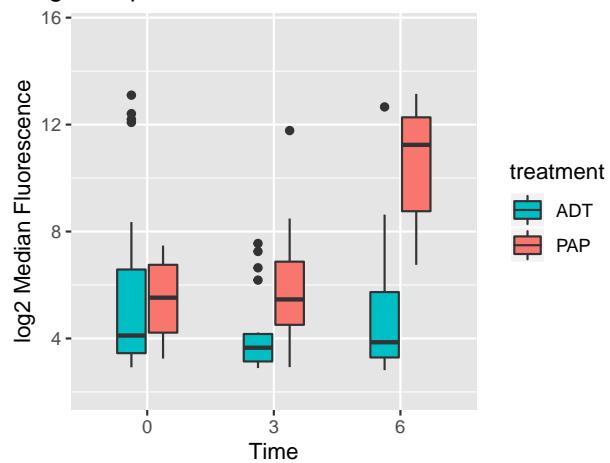
Boxplots of Fluorescence Levels for Signif Peptide: 278_SS2_6746;9



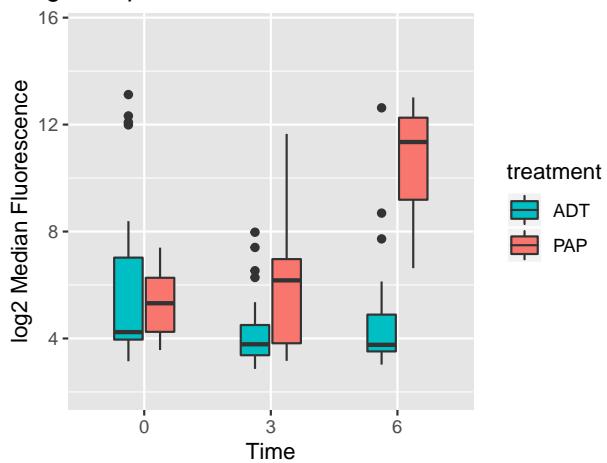
Boxplots of Fluorescence Levels for Signif Peptide: 1052_ARL6IP4_51329;289



Boxplots of Fluorescence Levels for Signif Peptide: 1052_ARL6IP4_51329;293



Boxplots of Fluorescence Levels for Signif Peptide: 1052_ARL6IP4_51329;285

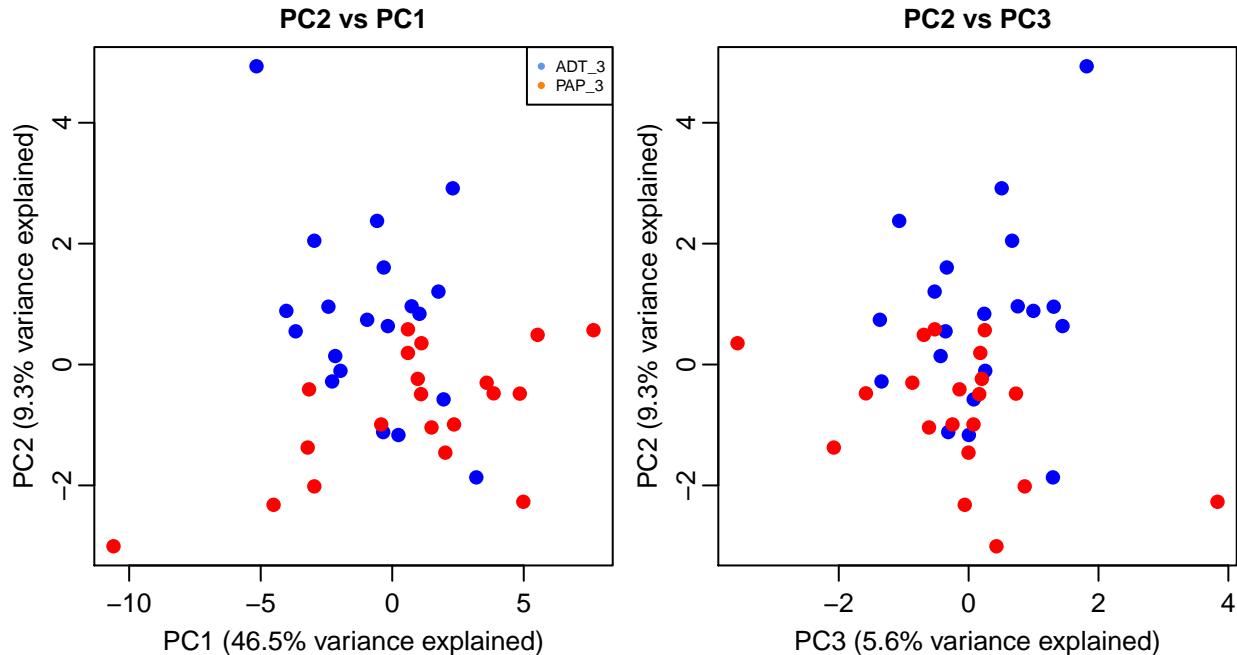


4 Visualization of Treatment Effects (Higher Antibody Response in ADT)

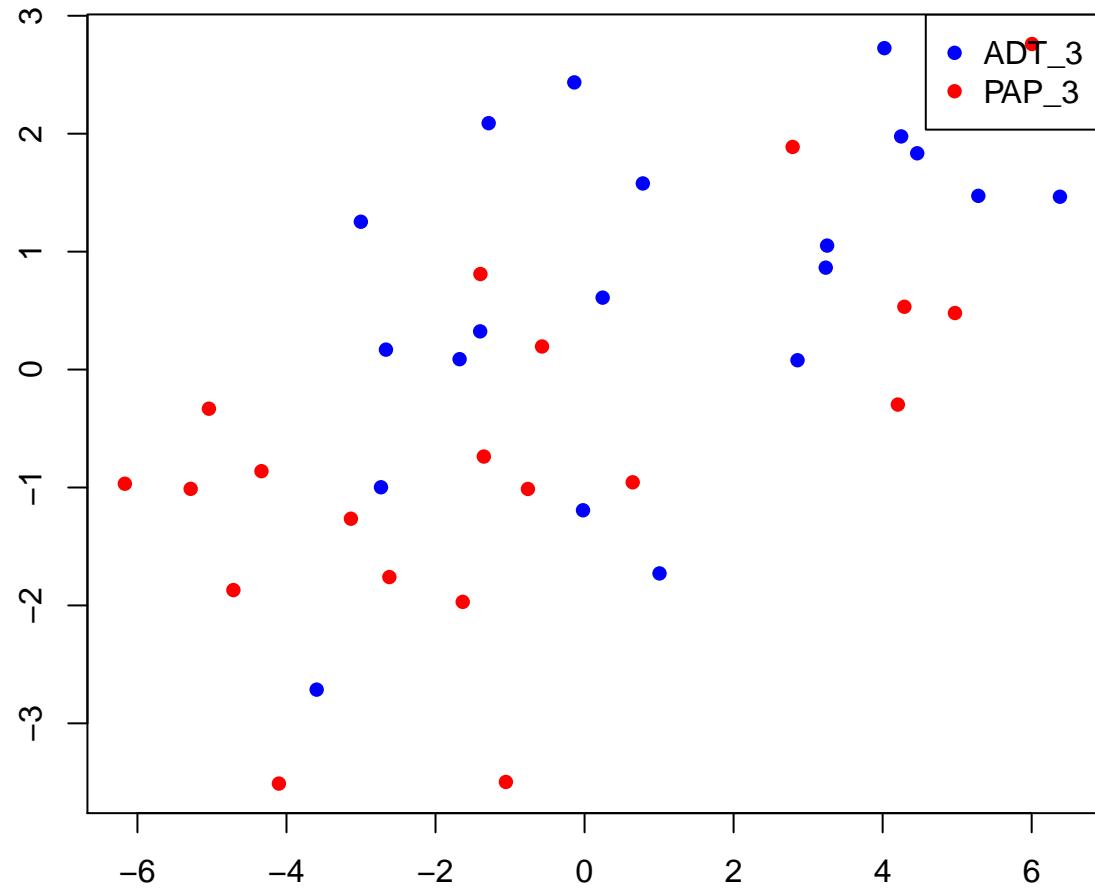
We would also like to find out about the peptides for which ADT causes higher fluorescence levels as compared to the PAP vaccine. This time, we will focus on the significant peptides at 1 % FDR and that the ADT leads to higher antibody response by at least two-fold as compared to PAP at times 3-months and 6-months, ie. $(\hat{\beta}_2 + 3 \times \hat{\beta}_3) \leq -1$ and $(\hat{\beta}_2 + 6 \times \hat{\beta}_3) \leq -1$. There are 114 peptides that fulfill these criteria. We exported the list of these peptides to the Sheet “*Higher_in_ADT*” in the Excel file *05_Longitudinal_SignifPeptides.xlsx*.

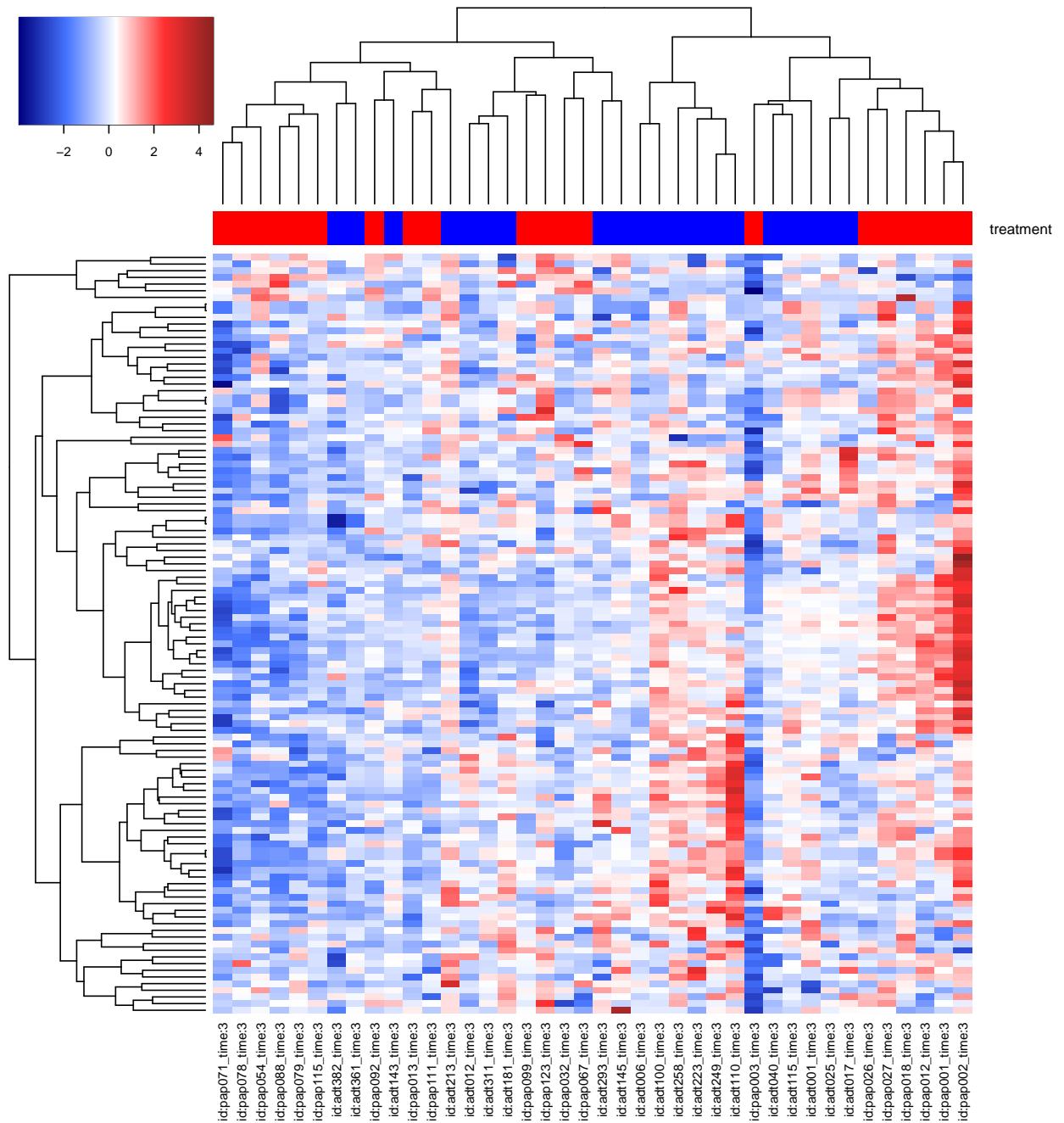
We obtain the residuals of the null model at times 3-months and 6-months, and plot these residuals using the PCA, t-SNE and heatmap approaches. Any observed clustering patterns based on treatment types would reveal the treatment effects not captured in the null model. The clustering patterns observed here are less obvious than the ones we observe for the peptides that exhibit higher antibody responses in PAP.

4.1 Treatment Effects at 3 months

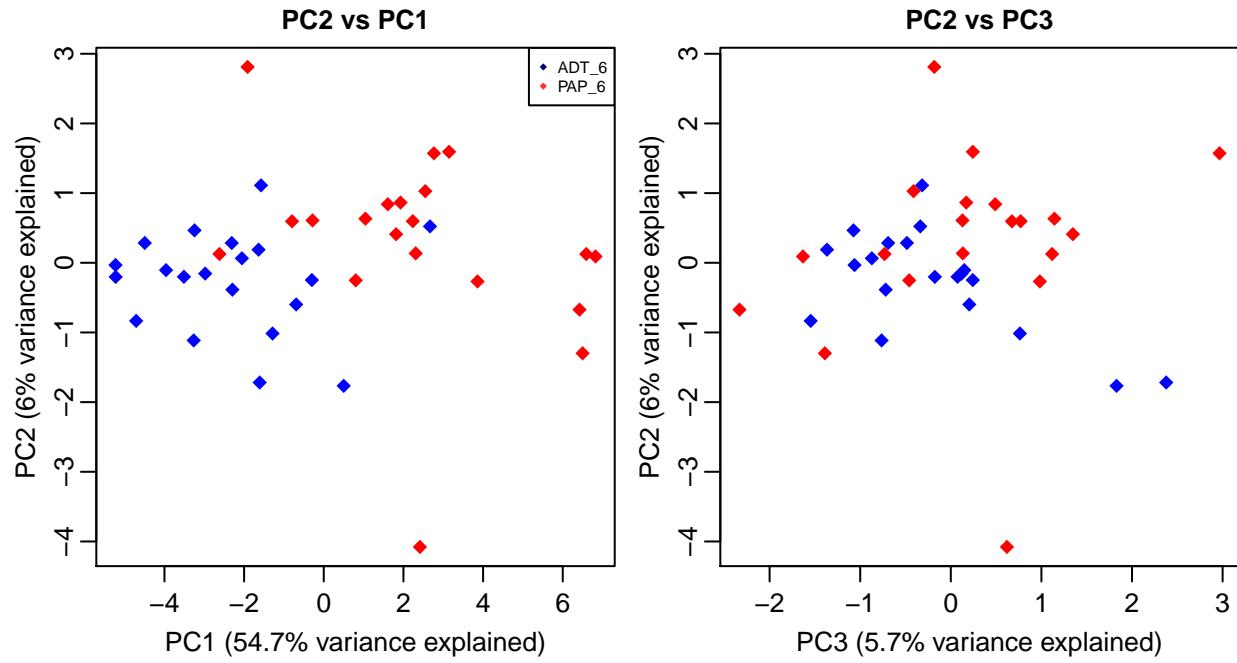


t-SNE plot

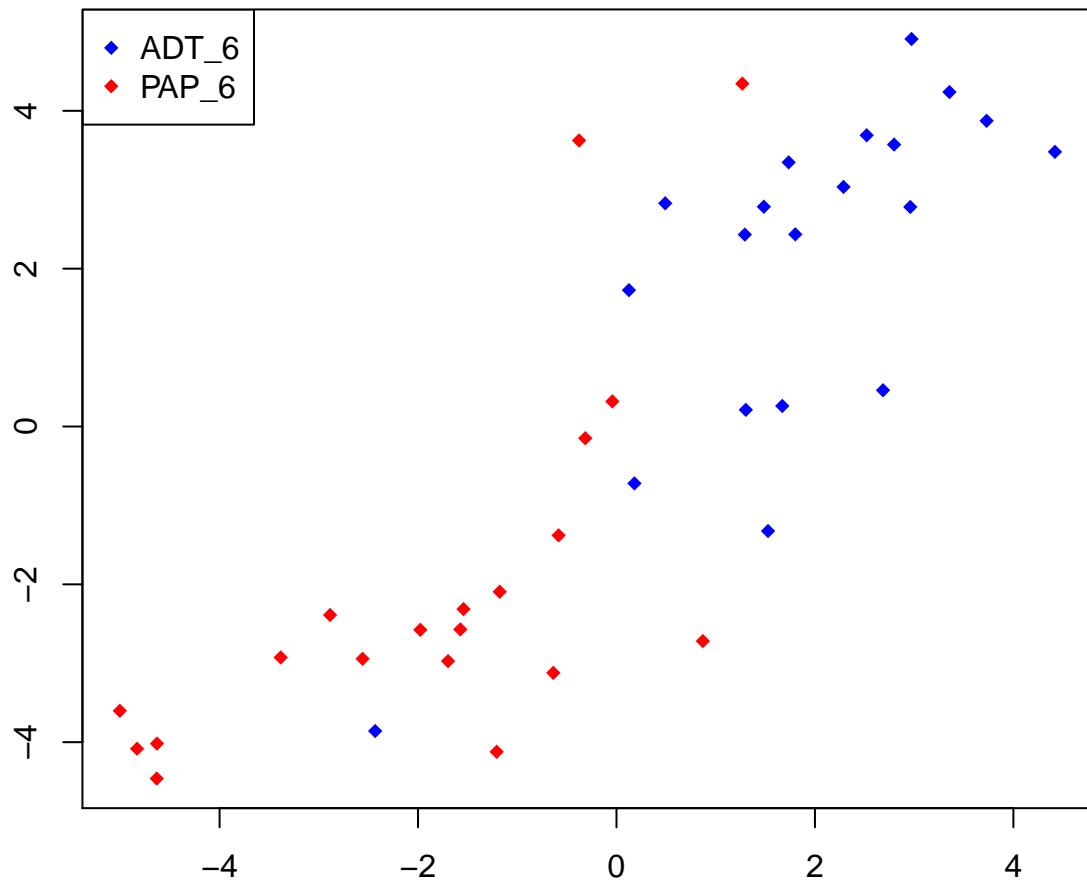


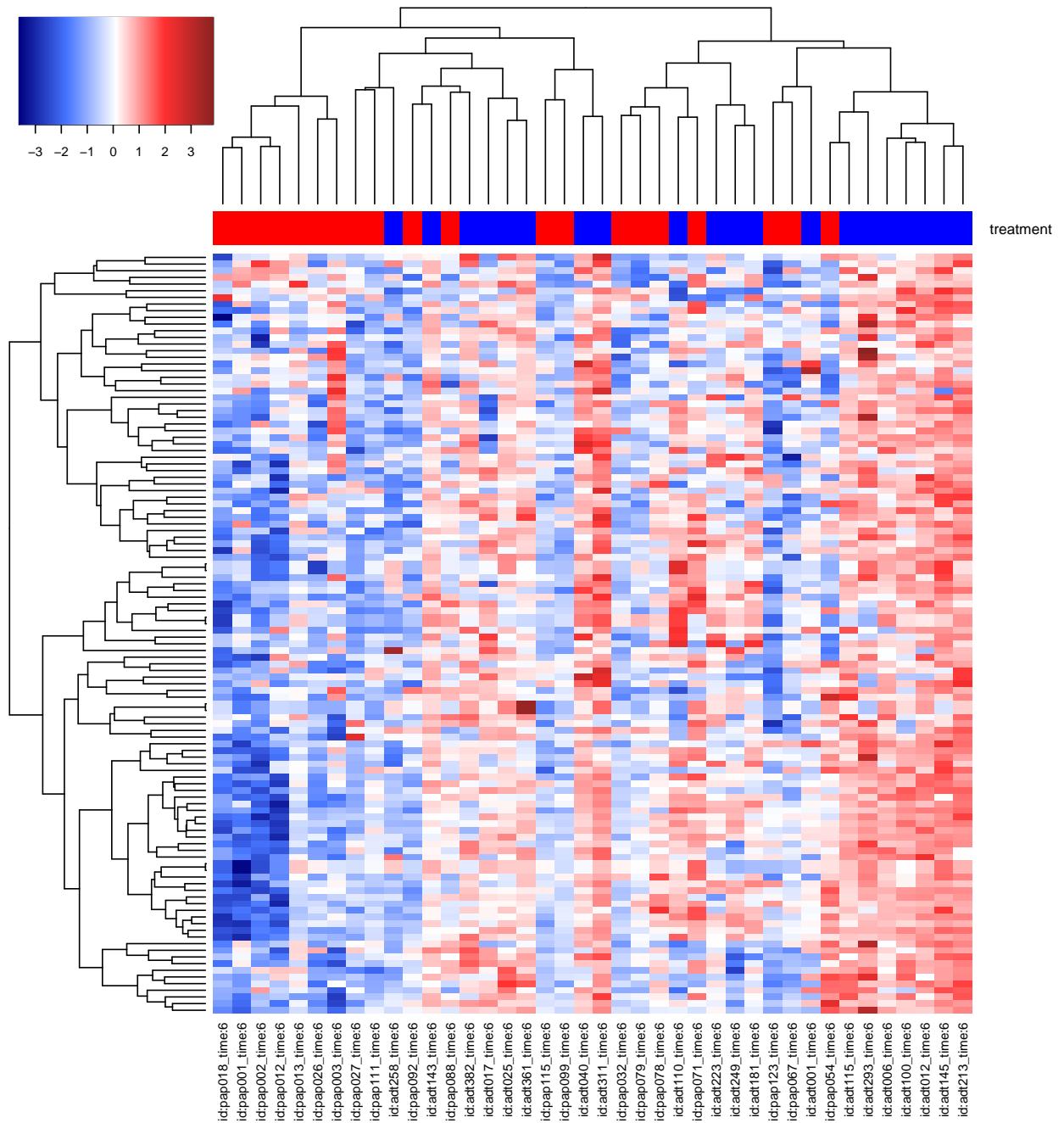


4.2 Treatment Effects at 6 months



t-SNE plot

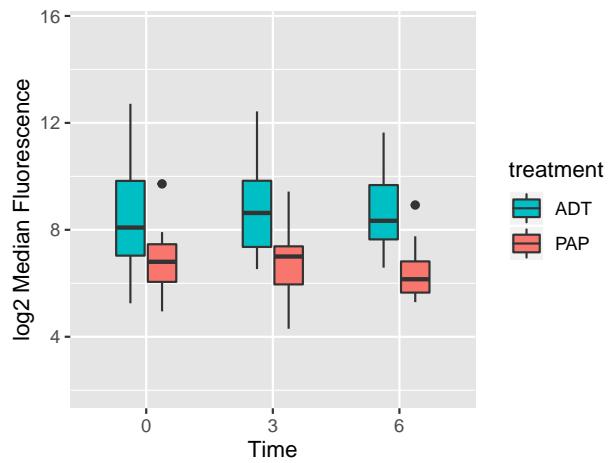




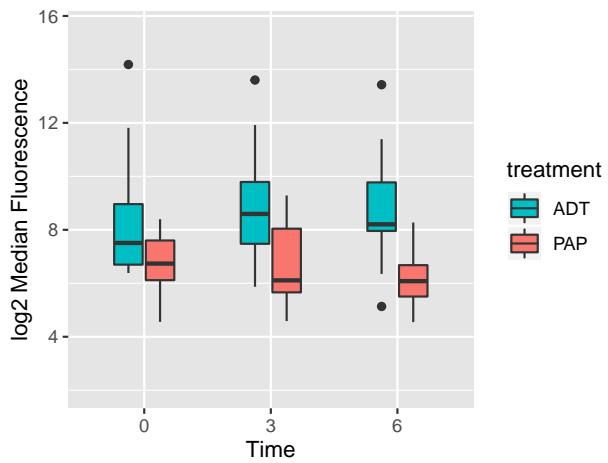
4.3 Boxplots of a few Significant Peptides

We also show the boxplots of fluorescence levels for a few significant peptides that have higher antibody response in ADT compared to PAP.

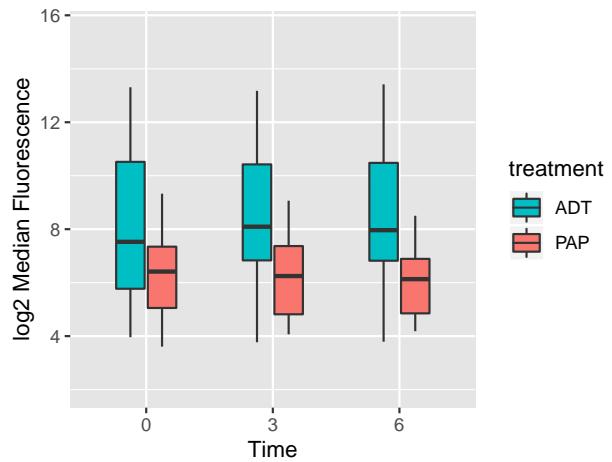
Boxplots of Fluorescence Levels for Signif Peptide: 539_MAML1_9794;681



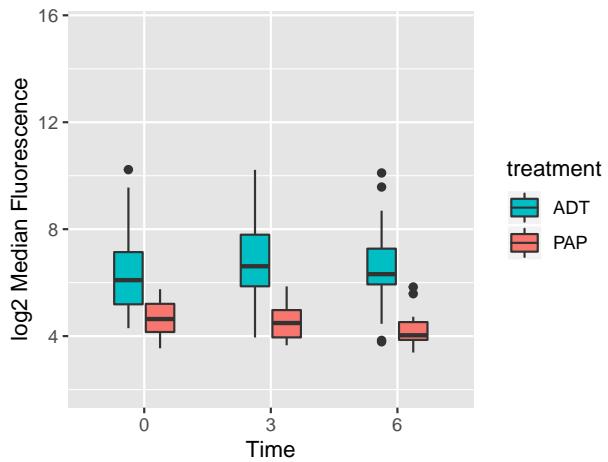
Boxplots of Fluorescence Levels for Signif Peptide: PCA6;445



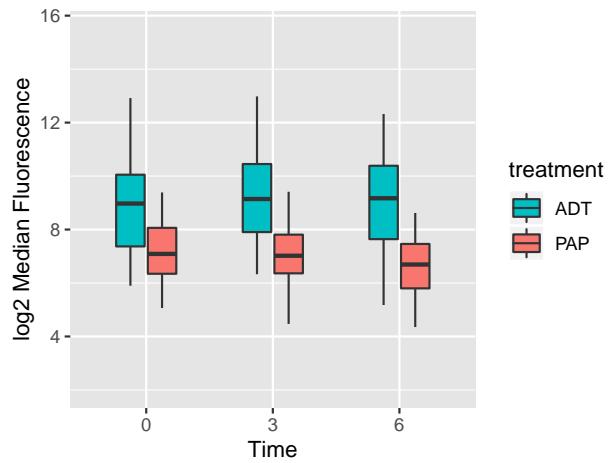
Boxplots of Fluorescence Levels for Signif Peptide: 1099_WDR90_197335;1241



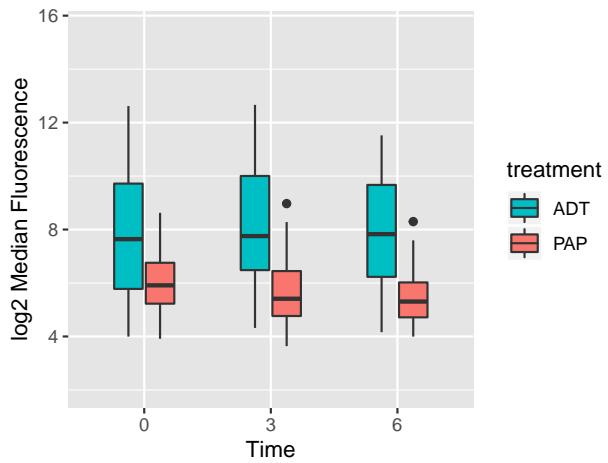
Boxplots of Fluorescence Levels for Signif Peptide: 737_DDX3X_1654;589



Boxplots of Fluorescence Levels for Signif Peptide: 632_NOS1_4842;1137



Boxplots of Fluorescence Levels for Signif Peptide: 1096_ANKHD1_54882;2453



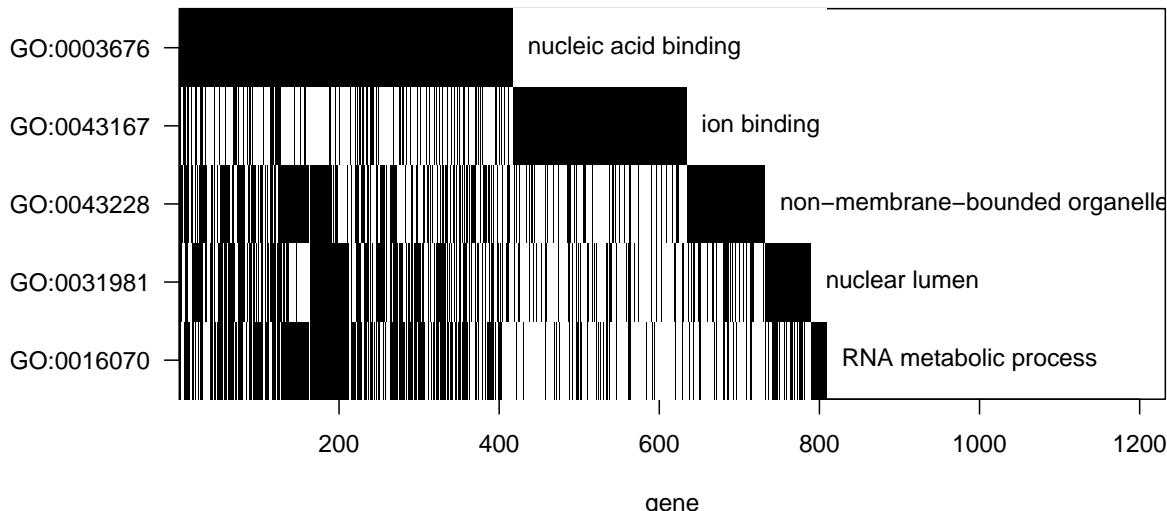
5 Gene-Set Analysis (Genes Significant for Treatment Effects)

Recall that the 177604 peptides correspond to 1611 proteins, and 1462 of these proteins have matching genes in *uniprot_gene_entrez.csv*. The 29650 significant peptides at 1% BH-FDR are associated with 1073 proteins with matching genes. In this analysis, we deem a protein to be significant if it has at least one significant associated peptide. We shall utilize this information to perform gene-set enrichment analysis.

Specifically, we investigate if there are any pre-specified gene-sets that are enriched for the genes associated with the list of significant peptides. These pre-determined gene-sets are defined based on their functional categories or biological properties, such as the Gene Ontology (GO) annotations. Enriched gene-sets could reflect the biological signals in the peptide microarray data.

The gene-set-analysis is performed with the R package **allez**. We shall consider gene-sets containing at least 2 genes with Bonferroni-corrected enrichment p-values not exceeding 5% .

We display the waterfall plot of the gene-set-analysis results.



The waterfall plot was constructed by finding the significant (Bonferroni-corrected p-value < 0.05) GO term having the largest overlap with genes associated with significant peptides (nucleic acid binding GO:0003676) and placing it in the top row of the figure. We next removed these genes from the list and found the significant GO term having the highest overlap with the remainder of the list (ion binding GO: 0043167). This process is repeated, and genes identified by this sequential process are counted along the x-axis, and the overlap between the GO terms can be visually assessed. Shading under the ‘waterfall’ component of the graph indicates genes that were annotated to previously named categories.

We also tabulate the enriched/overrepresented GO terms.

Term	Ontology	set.mean	set.size	z.score	in.genes
nucleolus	CC	0.9098	111/122	4.4789	ACADVL; ADAR; PARP1; CAPG; KLF6; CSTB; CTSB; DDX5; EEF1A1; EEF1D; FBL; XRCC6; GOLGA3; HMGB2; HMOX1; FOXA1; HSPA8; DNAJB1; RBPJ; ILF2; ILF3; KRT18; MARS; MYC; MYO1C; HNRNPM; NCL; NFIB; NFIC; NONO; NPM1; PA2G4; PDHA1; PHF2; PPP1CC; RAN; RBBP6; RPL3; RPL5; RPL7; RPL7A; RPL11; RPL12; RPL13; RPL18; RPL23A; RPL26; RPS3; RPS3A; RPS6; RPS7; RPS10; RPS11; RPS13; RPS14; RPS19; RPS25; RPS27A; SRSF5; SMARCA4; SNRNPB2; SSRP1; TBCA; TCEA1; TSG101; EZR; KAT6A; FXR1; STK24; USO1; EIF3A; EDF1; SELENBP1; TAF1C; COX7A2L; RPL23; H2AFY; MORF4L2; RBM14; NOP56; SUB1; RPL35; PHF8; SUZ12; RPL13A; GTPBP4; ORC6; RPL36; DCAF13; RE XO2; SPATS2L; GNL3; TIMM13; NOP53; JPT1; DDX47; ARL6IP4; MAGEC2; IP6K2; TDP2; NOP58; NSUN2; NOP10; CHD7; TCIM; FAM111A; NUCKS1; MEAF6; DDX50; TSEN34; LAS1L
DNA binding	MF	0.8757	162/185	4.5201	ADAR; PARP1; APLP2; APP; AR; ATF4; BCL6; ZFP36L1; CEBPB; CEBPD; KLF6; CUX1; DDB1; DDX1; DDX3X; DHX9; EEF1D; EPAS1; EZH2; XRCC6; GOLGB1; MSH6; GTF2I; GTF3A; H1F0; HIST1H1C; H3F3A; H3F3B; HDAC1; HMGB1; HMGB2; HMGN1; HMGN2; FOXA1; HNRNPC; HNRNPD; HES1; HSF1; HSPD1; RBPJ; ILF2; ILF3; JUN; JUNB; JUND; LBR; MCM3; KMT2A; MYC; NACA; NCL; NFIA; NFE2L1; NFIB; NFIC; NFIL3; NKX3-1; NONO; NPAS2; NPM1; NUCB2; PA2G4; PNN; POLR2E; PRM2; PURB; UPF1; RPA1; RPL6; RPL7; RPS3; RPS15; RPS27; SET; SMARCA1; SMARCA4; SMARCC2; SON; SOX4; SP3; SP100; SREBF1; SSRP1; TAF7; TCEA1; TCF3; TDG; NR2F2; TSG101; ZNF24; ZKSCAN1; VEZF1; ZFAND5; KAT6A; TAF15; HIST1H2BL; HIST1H2BF; HIST1H2BH; HIST1H4C; HIST1H4L; DDX3Y; EDF1; EED; TAF1C; MTA1; H2AFY; IER2; BCLAF1; THRAP3; DNAJB6; AKAP9; RBM5; SRRM1; ZMPSTE24; BASP1; HOXB13; KHDRBS1; RAI1; ZNF275; SUB1; FOXJ3; TCF25; SMG1; RYBP; TARDBP; SUZ12; LSM14A; NUPR1; FOXP1; REPIN1; IRX4; HP1BP3; SIDT2; LEF1; CXXC5; TDP2; SRRT; XRN1; ZFAND6; BANP; IFT57; STRBP; CHD7; ZNF395; SLC2A4RG; BBX; SCYL1; CHD8; ZNF350; NUCKS1; GPBP1; IRX3; TBL1XR1; RAX2; ZNF587; HIST3H2A; GTF3C6; TOP1MT; ZNF664; CREB3L4; ZMAT2; H3F3C

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
nucleic acid binding	MF	0.8616	417/484	7.5305	ACTN1; ADAR; ADD1; PARP1; SLC25A5; APLP2; APP; AR; ARF1; ATF4; BCL6; ZFP36L1; ETF3; CANX; CAST; CCT6A; CEBPB; CEBPD; CLTC; CNP; KLF6; CSNK1E; CSTB; CTNNA1; CUX1; DDB1; DDX1; DDX3X; DDX5; DHX9; DIAPH1; DSP; EEF1A1; EEF1B2; EEF1D; EEF1G; EIF1AX; EIF2B1; EIF5; EPAS1; FBL; EZH2; FAU; FDPS; XRCC6; GOLGB1; PDIA3; GRSF1; MSH6; GTF2I; GTF3A; H1F0; HIST1H1C; H3F3A; H3F3B; HADHB; HDAC1; HMGB1; HMGB2; HMGN1; HMGN2; FOXA1; HNRNPC; HNRNPD; HNRNPH1; HES1; HSF1; HSPA1A; HSPA8; HSP90AA1; HSPD1; IARS; MRPL58; RBPJ; ILF2; ILF3; EIF3E; JUN; JUNB; JUND; KPNB1; KRT18; KTN1; LBR; LGALS3; LTA4H; CAPRIN1; MARS; MCM3; KMT2A; MYC; MYH9; NACA; HNRNPM; NAP1L1; NCL; RPL10A; NFIA; NFE2L1; NFIB; NFIC; NFIL3; NKX3-1; NONO; NPAS2; NPM1; NUCB2; P4HB; PA2G4; PEBP1; PABPC3; PNN; POLR2E; PP1A; PPP1CC; PKN2; PRM2; PSMA6; PSMC1; PSMD4; PURB; RAN; RANBP2; RARS; RBBP6; RBBP7; UPF1; RPA1; RPL3; RPL5; RPL6; RPL7; RPL7A; RPL8; RPL9; RPL11; RPL12; RPL13; RPL15; RPL17; RPL18; RPL18A; RPL19; RPL21; RPL22; RPL23A; RPL24; RPL26; RPL27; RPL30; RPL27A; RPL28; RPL29; RPL31; RPL32; RPL34; RPL35A; RPL37; RPL37A; RPL38; RPL39; RPL41; RPL36A; RPN1; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; ATXN2; SET; SRSF4; SRSF5; TRA2B; SMARCA1; SMARCA4; SMARCC2; SMN1; SUMO2; SNRNP70; SNRCP1; SNRPD1; SNRPD2; SNRPD3; SNRPE; SNRPF; SNRPG; SON; SOX4; SP3; SP100; SREBF1; SRP14; SRPRA; SSB; SSRP1; SSX1; SSX2; SSX4; TAF7; TARS; TBCA; TCEA1; TCF3; TCP1; TDG; NR2F2; TMSB4X; TPT1; HSP90B1; CCT3; TSG101; TXN; U2AF1; UBE2I; VCP; EZR; YWHAG; SF1; ZNF24; ZKSCAN1; VEZF1; ZFAND5; CSDE1; DAP3; MANF; KAT6A; FXR1; TAF15; AKAP1; AKAP17A; HIST1H2BL; HIST1H2BF; HIST1H2BH; HIST1H4C; HIST1H4L; USO1; RNASET2; DDX3Y; EIF3A; EIF3C; EIF3D; EIF3H; EIF3J; EDF1; EED; TAF1C; RPL14; MTA1; RPL23; ZRANB2; H2AFY; RBM39; IER2; PDIA4; BZW1; BCLAF1; EIF4A3; ZC3H11A; RBM8A; THRAP3; DNAJB6; FARSB; TSFM; AKAP9; RBM5; ALYREF; EIF1; HNRNPR; SRRM1; ZMPSTE24; SAP18; BASP1; RBM14; EIF3M; HOXB13; SYNCRIP; CHERP; NOP56; CCT4; HEXIM1; KHDRBS1; CELF1; RAI1; AHCYL1; SRSF10; ZNF275; SUB1; GCN1; IMMT; NUDT4; WDR6; MRPL3; RPL35; DUSP12; ATXN2L; CASC8; FOXJ3; MAPRE1; TCF25; SNRNP200; SMG1; GANAB; RYBP; TARDBP; SF3B1; SUZ12; MORC3; RPL13A; SRRM2; GTPBP4; RPL36; DCAF13; PNISR; VIRMA; REXO2; SPATS2L; LSM14A; SERBP1; GNL3; NUPR1; DAZAP1; PABPC1; SND1; FOXP1; PDCD4; MCTS1; REPIN1; NOP53; IRX4; HP1BP3; RPS27L; SIDT2; LEF1; DDX47; ARL6IP4; EIF3L; YTHDF2; RTCB; CXXC5; TDP2; SRRM1; NOP58; MRPS21; XRN1; ZFAND6; ANKHD1; NSUN2; YTHDF1; BANP; PTCD3; MRPL20; IFT57; STRBP; NOP10; CHD7; ZNF395; NXP2; SLC2A4RG; BBX; CCDC47; SCYL1; NUFIP2; KIAA1324; CHD8; ZNF350; MRPS35; MRPS14; NUCKS1; MRPL41; MRPL32; GPBP1; RSRC2; DDX50; AHNAK; TSEN34; SECISBP2; IRX3; TBL1XR1; ESRP2; LAS1L; SLIRP; RAX2; ZNF587; ZNF598; HIST3H2A; GTF3C6; TOP1MT; RPS4Y2; ZNF664; CREB3L4; ZMAT2; NSUN5P2; H3F3C; ZFP62; NBPF10

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Term	Ontology	set.mean	set.size	z.score	in.genes
RNA binding	MF	0.8528	307/360	5.6619	ACTN1; ADAR; ADD1; PARP1; SLC25A5; ARF1; ZFP36L1; BTF3; CANX; CAST; CCT6A; CLTC; CNP; CSNK1E; CSTB; CTNNAA1; DDX1; DDX3X; DDX5; DHX9; DIAPH1; DSP; EEF1A1; EEF1B2; EEF1D; EEF1G; EIF1AX; EIF2B1; EIF5; FBL; EZH2; FAU; FDPS; XRCC6; GOLGB1; PDIA3; GRSF1; GTF3A; H1F0; HIST1H1C; HADHB; HMGB1; HMGB2; HMGN2; HNRNPC; HNRNPD; HNRNPH1; HSPA1A; HSPA8; HSP90AA1; HSPD1; IARS; MRPL58; ILF2; ILF3; EIF3E; JUN; KPNB1; KRT18; KTN1; LBR; LGALS3; LTA4H; CAPRIN1; MARS; MYH9; HNRNPM; NAP1L1; NCL; RPL10A; NONO; NPM1; P4HB; PA2G4; PEBP1; PABPC3; PNN; PPIA; PPP1CC; PKN2; PSMA6; PSMC1; PSMD4; PURB; RAN; RANBP2; RARS; RBBP6; RBBP7; UPF1; RPL3; RPL5; RPL6; RPL7; RPL7A; RPL8; RPL9; RPL11; RPL12; RPL13; RPL15; RPL17; RPL18; RPL18A; RPL19; RPL21; RPL22; RPL23A; RPL24; RPL26; RPL27; RPL30; RPL27A; RPL28; RPL29; RPL31; RPL32; RPL34; RPL35A; RPL37; RPL37A; RPL38; RPL39; RPL41; RPL36A; RPN1; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; ATXN2; SRSF4; SRSF5; TRA2B; SMARCA4; SMN1; SUMO2; SNRNP70; SNRPC; SNRPD1; SNRPD2; SNRPD3; SNRPE; SNRPF; SNRPG; SON; SRP14; SRPRA; SSB; SSRP1; TARS; TBCA; TCP1; TMSB4X; TPT1; HSP90B1; CCT3; TXN; U2AF1; UBE2I; VCP; EZR; YWHAG; SF1; CSDE1; DAP3; MANF; FXR1; TAF15; AKAP1; AKAP17A; HIST1H4C; HIST1H4L; USO1; RNASET2; DDX3Y; EIF3A; EIF3C; EIF3D; EIF3H; EIF3J; EDF1; RPL14; RPL23; ZRANB2; RBM39; PDIA4; BZW1; BCLAF1; EIF4A3; ZC3H11A; RBM8A; THRAP3; FARSB; TSFM; RBM5; ALYREF; EIF1; HNRNPR; SRM1; SAP18; RBM14; EIF3M; SYNCRIP; CHERP; NOP56; CCT4; HEXIM1; KHDRBS1; CELF1; AHCYL1; SRSF10; SUB1; GCN1; IMMT; NUDT4; WDR6; MRPL3; RPL35; ATXN2L; CASC3; MAPRE1; SNRNP200; SMG1; GANAB; TARDBP; SF3B1; SUZ12; MORC3; RPL13A; SRRM2; GTBPB4; RPL36; DCAPF13; PNISR; VIRMA; SPATS2L; LSM14A; SERBP1; GNL3; DAZAP1; PABC1; SND1; PDCD4; MCTS1; REPIN1; NOP53; RPS27L; SIDT2; DDX47; ARL6IP4; EIF3L; YTHDF2; RTCB; SRRT; NOP58; MRPS21; XRN1; ANKHD1; NSUN2; YTHDF1; PTCD3; MRPL20; STRBP; NOP10; NXF2; CCDC47; NUFI2; KIAA1324; MRPS35; MRPS14; NUCKS1; MRPL41; MRPL32; RSRC2; DDX50; AHNAK; SECISBP2; ESRP2; LAS1L; SLIRP; ZNF598; RPS4Y2; NSUN5P2; NBPF10

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
ion binding	MF	0.8248	339/411	4.6693	ABAT; ACADVL; ACAT1; ACO2; ACTN1; ACTN2; ADAR; PARP1; AK4; AKT2; ALDH1A3; ANXA1; APLP2; APP; AR; ARF1; ASNS; ATP1A1; ATP1B1; ATP6V1A; BCL6; BMPR1B; ZFP36L1; CA4; CANX; CBS; CCT6A; CDC42; CENPE; CHKA; CKB; KLF6; CSNK1D; CSNK1E; CYB561; DBI; DDX1; DDX3X; DDX5; DHX9; DHCR24; CYB5R3; DLD; DYNC1LI2; DPYSL3; EEF1A1; EIF5; F5; FABP5; FDPS; FOLH1; FPGS; FTL; XRCC6; B4GALT1; GNAS; MKNK2; MSH6; GTF3A; GUCY1A1; HADH; HBB; HK2; HMGB1; HMOX1; HPGD; HPRT1; DNAJA1; HSPA1A; HSPA8; HSP90AA1; HSPD1; IARS; IDH1; IGF1R; ILF2; IMPA1; ITGAV; ITGB1; ITPK1; KIF5C; KPNB1; KRAS; LBR; LDLR; ABILIM1; LIMS1; LRPAP1; LTA4H; NBR1; MARS; MCM3; MDK; MAP3K5; MGP; KMT2A; MT1E; MT1G; MT1H; MT1X; MT2A; COX2; MMUT; MYH9; MYH11; MYL6; MYO1C; MYO6; SEPTIN2; NME3; NOS1; NOTCH2; NPAS2; NRDC; NUCB2; PEBP1; PAFAH1B1; PDPK1; PFKM; PHF2; PPA1; PPP1CC; PPP3R1; PPP6C; PRKAR2A; PKN2; MAP2K3; PRM2; PRNP; PSMA6; PSMC1; PTPRF; PXN; RAB5A; RAN; RANBP2; RAP1B; RARS; RBBP6; UPF1; RPA1; RPL22; RPL29; RPL37; RPL37A; RPS27; RPS27A; CLIP1; S100A10; SGK1; SMARCA1; SMARCA4; SNRPC; SORD; SP3; SPARC; SPOCK1; SQLE; SRPRA; TARS; TCEA1; TCP1; TDG; NR2F2; TPD52; TPT1; HSP90B1; CCT3; U2AF1; UBE2I; UQCRC2; UTRN; VCP; SF1; ZNF24; ZKSCAN1; VEZF1; ZFAND5; DAP3; RNF103; KAT6A; TAF15; PIP4K2B; ULK1; STK24; OGT; DGKD; DDX3Y; TAX1BP1; MTMR4; MTA1; HGS; ZNHIT3; ZRANB2; ITM2B; PMPCB; MICAL2; EIF4A3; EFCAB14; ZC3H11A; MFN2; THRAP3; FARSB; ABCC5; ATP9A; CTDSP2; HIPK3; RBM5; ZMPSTE24; MAEA; MARCH6; PCGF3; ARIH2; ECI2; ZNHIT1; UBE2E3; CCT7; CCT4; CCT2; PAICS; PDLIM5; MYL12A; CAMKK2; PMVK; RRAGA; RAI1; SEPTIN9; HSPH1; ZNF275; RAB10; MMP24; LIAS; RAB35; ADAMTS5; NUDT4; AKAP13; DUSP12; CHSY1; UBOX5; RAB18; LIMCH1; SNRNP200; SMG1; TAB2; POGZ; PHF8; KIF13B; TRIM2; RYBP; ISCU; SUZ12; MORC3; GTPBP4; CBLC; BRD1; HECTD1; WIPI2; FBXL5; ARFGAP3; GNL3; TIMM13; STEAP1; BEX3; ATP2C1; FOXP1; EIF2AK1; ARFIP1; ACAD9; HIPK2; REPIN1; SNX12; CYHRI; RPS27L; CRYL1; ZC2HC1A; ZNF706; SAR1B; HSD17B12; SDF4; DDX47; GLRX5; GMPR2; IP6K2; RTCB; CXXC5; TDP2; CHMP3; RASD1; GALNT7; RIPK4; ZFAND6; NEURL1B; RNF220; PHF10; LAPT4B; DNAJA4; ENOSF1; UBE2Q1; CHD7; RNF130; ZNF395; NSFL1C; WDR45B; SLC2A4RG; MCCC1; ZMIZ1; ATP8B2; SCYL1; SH3RF1; CHD8; RHOU; ZNF462; ZNF350; GALNT11; GOLPH3; XYLT2; WNK1; DDX50; MLPH; ATP13A3; CARS2; MYO19; ALPK1; RUFY1; ING5; ACSS1; ZNF587; ZNF598; NEK9; MYL12B; BBS5; ZNF664; ZMAT2; ANKK1; STEAP2; RNF149; NRBP2; IRF2BP2; ZFP62

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
non-membrane-bounded organelle	CC	0.8201	392/478	4.9226	ACADVL; ACTN1; ACTN2; ADAR; ADD1; PARP1; ANK3; SLC25A5; ANXA1; APP; AR; ARF1; ATF4; BCL6; ZFP36L1; CALD1; CANX; CAPG; CCT6A; CDC42; CDH1; CEBPB; CENPE; CLTA; CLTC; KLF6; CSNK1D; CSTB; CTNNNA1; CTSB; DBB1; DDX1; DDX3X; DDX5; DHX9; DHCR24; CYB5R3; DIAPH1; DYNC1LI2; DPYSL3; DSP; EEF1A1; EEF1D; FBL; EZH2; XRCC6; GOLGA3; GRSF1; MSH6; H1F0; HIST1H1C; HIST1H2AD; H3F3A; H3F3B; HADHB; HDAC1; HMGB1; HMGB2; HMGN1; HMGN2; HMOX1; FOXA1; HNRNPC; HSF1; DNAJA1; HSPA1A; HSPA8; DNAJB1; MRPL58; RBPJ; ILF2; ILF3; EIF3E; JUN; JUNB; JUND; KIF5C; KPNB1; KRT8; KRT18; STMN1; ABLIM1; CAPRIN1; NBR1; MARCKS; MARS; MCM3; MYC; MYH9; MYH11; MYL6; MYO1C; MYO6; HNRNPM; NCL; NDUFAT7; SEPTIN2; RPL10A; NEFH; NFIB; NFIC; NKX3-1; NONO; NOS1; NPM1; PA2G4; PAFAH1B1; PDHA1; PHF2; PNN; PPP1CC; PRKAR2A; PKN2; PRM2; PSMA6; PURB; PXN; RAB5A; RAD21; RAN; RAP1B; RBBP6; RBBP7; UPF1; RPA1; RPL3; RPL5; RPL6; RPL7; RPL7A; RPL8; RPL9; RPL11; RPL12; RPL13; RPL15; RPL17; RPL18; RPL18A; RPL19; RPL21; RPL22; RPL23A; RPL24; RPL26; RPL27; RPL30; RPL27A; RPL28; RPL29; RPL31; RPL32; RPL34; RPL35A; RPL37; RPL37A; RPL38; RPL39; RPL41; RPL36A; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; CLIP1; ATXN2; SEC13; SET; SRSF5; SMARCA1; SMARCA4; SMARCC2; SMN1; SNRPB2; SP100; SSB; SSRP1; TARS; TBCA; TBCD; TCEA1; TCF3; TCP1; TMSB4X; TPM1; TPM3; TPT1; CCT3; TSG101; UBA52; UBE2I; UTRN; VCP; VDAC1; EZR; SF1; PTP4A1; DAP3; KAT6A; CCDC6; FXR1; USP11; HIST3H3; HIST1H2AK; HIST1H2AM; HIST2H2AC; HIST1H2BL; HIST1H2BF; HIST1H2BH; HIST1H4C; HIST1H4L; STK24; USO1; EIF3A; PEA15; EDF1; EED; HIST1H2AG; SELENBP1; TAF1C; RPL14; MTA1; COXA2L; RPL23; MAGED1; MRPL33; H2AFY; RBM39; NCOR2; MORF4L2; IST1; MFN2; PDCD6IP; RANBP9; DNAJB6; ARPC3; BCAP31; AKAP9; WASF2; ALYREF; PLIN3; MAAE; PCGF3; BCAP31; RBL14; TADA3; NOP56; ARL6IP5; CCT7; CCT4; CCT2; PDLIM5; IVNS1ABP; MYL12A; PTGES3; SEPTIN9; HSPH1; SUB1; MORF4L1; CBX1; GCN1; DSTN; AKAP13; MRPL3; RPL35; ATXN2L; CBX3; CASC3; MAPRE1; LIMCH1; DAAM1; POGZ; PHF8; PDS5A; EXOC7; TARDBP; SUZ12; SPIDR; RPL13A; GTPBP4; ORC6; NUP62; RABGAP1; HYPK; METTL7A; RPL36; DCAF13; REXO2; SPATS2L; AUTS2; LSM14A; GNL3; TIMM13; PAPC1; SERP1; EML4; MRPS18B; MCTS1; REPIN1; NOP53; BICRA; CTAG2; HP1BP3; SCCPDH; JPT1; DDX47; MRPL51; ARL6IP4; MAGEC2; YTHDF2; IP6K2; TDP2; NOP58; MRPS21; XRN1; FNBP1L; NSUN2; TTC19; PTCD3; MRPL20; IFT57; PHF10; STRBP; NOP10; CHD7; SYBU; YEATS2; ENAH; H2AFJ; SELENOS; NSFL1C; CFAP298; TCIM; THOC2; SCYL1; NUFIP2; SHROOM3; RHOU; MRPS35; FAM111A; MRPS14; NUCKS1; MEAF6; MRPS6; MRPL41; MRPL32; DDX50; AHNAK; TSEN34; TBL1XR1; MZT2B; MYO19; LAS1L; HIST1H2AH; HIST1H2BK; CEP95; NEK9; HIST3H2A; H2AFV; MYL12B; TOP1MT; RAB3IP; BBS5; RPL10L; KLC3; REEP3; RASSF3; HIST2H2AB; H3F3C; HIST2H2AA4

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
intracellular non-membrane-bounded organelle	CC	0.8201	392/478	4.9226	ACADVL; ACTN1; ACTN2; ADAR; ADD1; PARP1; ANK3; SLC25A5; ANXA1; APP; AR; ARF1; ATF4; BCL6; ZFP36L1; CALD1; CANX; CAPG; CCT6A; CDC42; CDH1; CEBPB; CENPE; CLTA; CLTC; KLF6; CSNK1D; CSTB; CTNNA1; CTSB; DDB1; DDX1; DDX3X; DDX5; DHX9; DHCR24; CYB5R3; DIAPH1; DYNC1LI2; DPYSL3; DSP; EEF1A1; EEF1D; FBL; EZH2; XRCC6; GOLGA3; GRSF1; MSH6; H1F0; HIST1H1C; HIST1H2AD; H3F3A; H3F3B; HADHB; HDAC1; HMGB1; HMGB2; HMGN1; HMGN2; HMOX1; FOXA1; HNRNPC; HSF1; DNAJA1; HSPA1A; HSPA8; DNAJB1; MRPL58; RBPJ; ILF2; ILF3; EIF3E; JUN; JUNB; JUND; KIF5C; KPNB1; KRT8; KRT18; STMN1; ABLIM1; CAPRIN1; NBR1; MARCKS; MARS; MCM3; MYC; MYH9; MYH11; MYL6; MYO1C; MYO6; HNRNPM; NCL; NDUFA7; SEPTIN2; RPL10A; NEFH; NFIB; NFIC; NKX3-1; NONO; NOS1; NPM1; PA2G4; PAFAH1B1; PDHA1; PHF2; PNN; PPP1CC; PRKAR2A; PKN2; PRM2; PSMA6; PURB; PXN; RAB5A; RAD21; RAN; RAP1B; RBBP6; RBBP7; UPF1; RPA1; RPL3; RPL5; RPL6; RPL7; RPL7A; RPL8; RPL9; RPL11; RPL12; RPL13; RPL15; RPL17; RPL18; RPL18A; RPL19; RPL21; RPL22; RPL23A; RPL24; RPL26; RPL27; RPL30; RPL27A; RPL28; RPL29; RPL31; RPL32; RPL34; RPL35A; RPL37; RPL37A; RPL38; RPL39; RPL41; RPL36A; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; CLIP1; ATXN2; SEC13; SET; SRSF5; SMARCA1; SMARCA4; SMARCC2; SMN1; SNRNP2; SP100; SSB; SSRP1; TARS; TBCA; TBCD; TCEA1; TCF3; TCP1; TMSB4X; TPM1; TPM3; TPT1; CCT3; TSG101; UBA52; UBE2I; UTRN; VCP; VDAC1; EZR; SF1; PTP4A1; DAP3; KAT6A; CCDC6; FXR1; USP11; HIST3H3; HIST1H2AK; HIST1H2AM; HIST2H2AC; HIST1H2BL; HIST1H2BF; HIST1H2BH; HIST1H4C; HIST1H4L; STK24; USO1; EIF3A; PEA15; EDF1; EED; HIST1H2AG; SELENBP1; TAF1C; RPL14; MTA1; COX7A2L; RPL23; MAGED1; MRPL33; H2AFY; RBM39; NCOR2; MORF4L2; IST1; MFN2; PDCD6IP; RANBP9; DNAJB6; ARPC3; BCAP31; AKAP9; WASF2; ALYREF; PLIN3; MAEA; PCGF3; BASP1; RBM14; TADA3; NOP56; ARL6IP5; CCT7; CCT4; CCT2; PDLM5; IVNS1ABP; MYL12A; PTGES3; SEPTIN9; HSPH1; SUB1; MORF4L1; CBX1; GCN1; DSTN; AKAP13; MRPL3; RPL35; ATXN2L; CBX3; CASC3; MAPRE1; LIMCH1; DAAM1; POGZ; PHF8; PDS5A; EXOC7; TARDBP; SUZ12; SPIDR; RPL13A; GTPBP4; ORC6; NUP62; RABGAP1; HYPK; METTL7A; RPL36; DCAF13; REXO2; SPATS2L; AUTS2; LSM14A; GNL3; TIMM13; PABC1; SERP1; EML4; MRPS18B; MCTS1; REPIN1; NOP53; BICRA; CTAG2; HP1BP3; SCCPDH; JPT1; DDX47; MRPL51; ARL6IP4; MAGEC2; YTHDF2; IP6K2; TDP2; NOP58; MRPS21; XRN1; FNBP1L; NSUN2; TTC19; PTCD3; MRPL20; IFT57; PHF10; STRBP; NOP10; CHD7; SYBU; YEATS2; ENAH; H2AFJ; SELENOS; NSFL1C; CFAP298; TCIM; THOC2; SCYL1; NUFIP2; SHROOM3; RHOU; MRPS35; FAM111A; MRPS14; NUCKS1; MEAF6; MRPS6; MRPL41; MRPL32; DDX50; AHNAK; TSEN34; TBL1XR1; MZT2B; MYO19; LAS1L; HIST1H2AH; HIST1H2BK; CEP95; NEK9; HIST3H2A; H2AFV; MYL12B; TOP1MT; RAB3IP; BBS5; RPL10L; KLC3; REEP3; RASSF3; HIST2H2AB; H3F3C; HIST2H2AA4

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
nuclear lumen	CC	0.8174	403/493	4.8744	ACADVL; ADAR; ADD1; PARP1; AKT2; ANXA1; AR; ATF4; BCL6; CAPG; CEBPB; CEBPD; CENPE; CKS1B; CNP; KLF6; CSNK1D; CSNK1E; CSTB; CTSB; CUX1; DDB1; DDX1; DDX3X; DDX5; DHX9; DLD; EEF1A1; EEF1D; EPAS1; FBL; EZH2; FABP5; FDPS; FKBP5; XRCC6; GOLGA3; GPI; MKNK2; MSH6; GTF2I; GTF3A; H1F0; HIST1H1C; H3F3A; H3F3B; HADH; HDAC1; HMGB1; HMGB2; HMGN1; HMOX1; FOXA1; HNRNPC; HNRNPD; HNRNPH1; HPGD; HES1; HSF1; HSPA1A; HSPA8; HSP90AA1; DNAJB1; RBPJ; ILF2; ILF3; EIF3E; JUN; JUNB; JUND; KPNB1; KPNA4; KRT8; KRT18; LTA4H; NBR1; MAFG; MARS; MCL1; MCM3; KMT2A; AFDN; MYC; MYO1C; MYO6; HNRNPM; NCL; NFIA; NFIB; NFIC; NKTR; NONO; NOS1; NOTCH2; NPAS2; NPM1; PA2G4; PDHA1; PHF2; PNN; POLR2E; PPP1CC; PPP3R1; PKN2; MAP2K3; PRM2; PSMA6; PSMB1; PSMB4; PSMB7; PSMC1; PSMD1; PSMD3; PSMD4; PSMD7; PSME1; PTMA; PTPN11; PURB; RAD21; RAN; RARS; RBBP6; RBBP7; UPF1; BRD2; RPA1; RPL3; RPL5; RPL7; RPL7A; RPL11; RPL12; RPL13; RPL18; RPL23A; RPL26; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; SEC13; SET; SRSF4; SRSF5; TRA2B; SGK1; SMARCA1; SMARCA4; SMARCC2; SMN1; SUMO2; SNRNP70; SNRPB2; SNRPC; SNRPD1; SNRPD2; SNRPD3; SNRPE; SNRPF; SNRPG; SON; SOX4; SP3; SP100; SPARC; SREBF1; SSB; SSRP1; TAF7; TALDO1; TBCA; TCEA1; TCF3; TCP1; TDG; NR2F2; TPP2; TSG101; TXN; U2AF1; UBA52; UBE2I; UQCRC2; UTRN; VCP; EZR; SF1; VEZF1; DAP3; KAT6A; FXR1; CDK2AP1; TAF15; AKAP17A; USP11; HIST3H3; HIST1H2BL; HIST1H2BF; HIST1H2BH; HIST1H4C; HIST1H4L; PIP4K2B; STK24; OGT; COPS3; USO1; EIF3A; EDF1; EED; SELENBP1; TAF1C; MTA1; COX7A2L; NPIPA1; TRIP12; RPL23; ZRANB2; H2AFY; RBM39; IER2; NCOR2; MORF4L2; BCLAF1; EIF4A3; MAML1; ZC3H11A; POM121; RBM8A; THRAP3; DNAJB6; PDCD7; TSPAN1; CTDSP2; HIPK3; NAMPT; RBM5; ALYREF; HNRNPR; SRRM1; SAP18; MAEA; PCGF3; BASP1; ARIH2; RBM14; EC12; ZNHIT1; TADA3; ATP5PD; UBE2E3; HOXB13; SYNCRIP; CHERP; NOP56; CCT4; HEXIM1; TGOLN2; IVNS1ABP; CAMKK2; KHDRBS1; CELF1; PTGES3; RAI1; SRSF10; HSPH1; TXNL4A; SUB1; MORF4L1; CBX1; PKP3; RPL35; ATXN2L; PHB2; CBX3; CASC3; UBOX5; ELL2; KLHDC10; SNRNP200; SMG1; NPIP8; TAB2; POGZ; PHF8; SEL1L3; PDS5A; NEDD4L; FNBP4; RYBP; TARDBP; SF3B1; SUZ12; SPIDR; MORC3; RPL13A; SRRM2; GTPBP4; ORC6; BRD1; HYPK; RPL36; DCAF13; PNISR; VIRMA; REXO2; SPATS2L; GGA1; WIPI2; GNL3; TIMM13; DAZAP1; FOXP1; MRPS18B; HIPK2; ANKRD11; REPIN1; NOP53; BICRA; CYHR1; HP1BP3; GMNN; JPT1; LEF1; DDX47; WAC; ARL6IP4; ANAPC5; MAGEC2; IP6K2; RTCB; CXXC5; TDP2; PCF11; SRRT; NOP58; UIMC1; ARID4B; PRR13; NSUN2; BANP; PTCD3; ARGLU1; ELP2; PHF10; NOP10; CHD7; YEATS2; NSFL1C; NXF2; SLC2A4RG; TCIM; ENY2; BBX; ZMIZ1; THOC2; NUFIP2; TBC1D14; CHD8; ZNF350; FAM111A; NUCKS1; MEAF6; NIBAN2; DDX50; TSEN34; PAGR1; TBL1XR1; ESRP2; MZT2B; RUFY1; DUSP16; LAS1L; ING5; HIST1H2BK; HIST3H2A; GTF3C6; VSIG10L; CREB3L4; IRF2BP2; H3F3C

(continued)

Term	Ontology	set.mean	set.size	z.score	in.genes
RNA metabolic process	BP	0.8174	394/482	4.7902	ACTN1; ACTN2; ADAR; PARP1; APP; AR; ATF4; AZGP1; BCL6; BMPR1B; ZFP36L1; BTF3; CDH1; CDKN1C; CEBPB; CEBPD; CKS1B; KLF6; CUX1; DDX1; DDX3X; DDX5; DHX9; EPAS1; FBL; EZH2; XRCC6; GOLGB1; GRSF1; GTF2I; GTF3A; H1F0; HIST1H1C; H3F3A; H3F3B; HDAC1; HMGB1; HMGB2; HMGN1; HMOX1; FOXA1; HNRNPC; HNRNPD; HNRNPH1; HES1; HSF1; HSPA1A; HSPA8; HSPD1; DNAJB1; IARS; RBPJ; ILF2; ILF3; EIF3E; JUN; JUNB; JUND; KRAS; LGALS3; LIMS1; EPCAM; MAFG; MAGEA1; MARS; MDK; MAP3K5; KMT2A; MYC; MYO6; HNRNPM; NCL; RPL10A; NFIA; NFE2L1; NFIB; NFIC; NFIL3; NKX3-1; NONO; NOS1; CNOT2; NOTCH2; NPAS2; NPM1; PA2G4; PABPC3; PFKM; PHF2; PNN; POLR2E; FPA1; PPP3R1; MAP2K3; PRNP; PSMA6; PSMB1; PSMB4; PSMB7; PSMC1; PSMD1; PSMD3; PSMD4; PSMD7; PSME1; PTMA; PURB; RAD21; RAN; RARS; RBBP6; RBBP7; UPF1; BRD2; RPL3; RPL5; RPL6; RPL7; RPL7A; RPL8; RPL9; RPL11; RPL12; RPL13; RPL15; RPL17; RPL18; RPL18A; RPL19; RPL21; RPL22; RPL23A; RPL24; RPL26; RPL27; RPL30; RPL27A; RPL28; RPL29; RPL31; RPL32; RPL34; RPL35A; RPL37; RPL37A; RPL38; RPL39; RPL41; RPL36A; RPS2; RPS3; RPS3A; RPS4X; RPS4Y1; RPS6; RPS7; RPS8; RPS10; RPS11; RPS12; RPS13; RPS14; RPS15; RPS15A; RPS16; RPS17; RPS18; RPS19; RPS20; RPS23; RPS24; RPS25; RPS26; RPS27; RPS27A; ATXN2; SET; SRSF4; SRSF5; TRA2B; SGK1; SMARCA1; SMARCA4; SMARCC2; SMN1; SUMO2; SNRNP70; SNRPB2; SNRPC; SNRPD1; SNRPD2; SNRPD3; SNRPE; SNRPF; SNRPG; SON; SOX4; SP3; SP100; SREBF1; SSB; SSRP1; SSX1; SSX2; SSX4; TAF7; TARS; TCEA1; TCF3; TDG; TMBIM6; NR2F2; TMSB4X; TSG101; TXN; U2AF1; UBA52; UBE2I; EZR; YWHAB; SF1; ZNF24; ZKSCAN1; VEZF1; CSDE1; KAT6A; TAF15; AKAP17A; USP9X; FZD4; HIST1H4C; HIST1H4L; OGT; RNASET2; EDF1; EED; TSC22D1; TAX1BP1; TAF1C; RPL14; MTA1; ZNHIT3; RPL23; ZRANB2; MAGED1; H2AFY; RBM39; PRDX6; IER2; NCOR2; MORFAL2; MICAL2; BCLAF1; EIF4A3; MAML1; ZC3H11A; RBM8A; THRAP3; DNAJB6; FARSB; PDCD7; TSFM; CTDSP2; HIPK3; NAMPT; RBM5; ALYREF; HNRNPR; SRRM1; ZMPSTE24; SAP18; PCGF3; BASP1; RBM14; N4BP2L2; TADA3; HOXB13; SYNCRI; CHERP; NOP56; HEXIM1; IVNS1ABP; TXNIP; CAMKK2; KHDRBS1; CELF1; RAI1; AHCYL1; SRSF10; ZNF275; TXNL4A; SUB1; CBX1; YWHAQ; GCN1; PKP3; RPL35; PHB2; CBX3; CASC3; FOXJ3; ELL2; TCF25; SNRNP200; SMG1; TAB2; POGZ; PHF8; WWC1; NEDD4L; RYBP; TARDBP; SF3B1; SUZ12; RPL13A; SRRM2; GTPBP4; NUP62; RPL36; DCAF13; VIRMA; REXO2; AUTS2; SERBP1; GNL3; NUPR1; PABC1; SND1; FOXP1; PDCD4; HIPK2; NOP53; BICRA; IRX4; HP1BP3; GMNN; SIDT2; ZNF706; LEF1; DDX47; WAC; ARL6IP4; YTHDF2; RTCB; CXXC5; TDP2; PCF11; SRRT; NOP58; RASD1; UIMC1; ARID4B; RIPK4; XRN1; NSUN2; BANP; ELP2; NOP10; CHD7; YEATS2; ZNF395; SLC2A4RG; TCIM; ENY2; BBX; ZMIZ1; THOC2; MRTFB; TRMT5; CHD8; ZNF350; SAV1; NUCKS1; NIBAN2; GPBP1; AHNAK; TSEN34; SECISBP2; IRX3; PAGR1; TBL1XR1; ESRP2; LAS1L; ING5; CARD11; RAX2; ZNF587; CTF3C6; ZNF664; HNRNPA1L2; CREB3L4; ZMAT2; IRF2BP2; ZFP62

6 Concluding Remarks

- Counts of significant peptides: 53587 at 5% FDR and 29650 at 1% FDR.
- Out of the 29650 peptides at 1% FDR, 7351 of them also exhibit higher fluorescence levels by at least two-fold due to the PAP vaccine effect as compared to ADT at both times 3-months and 6-months.
- These 7351 peptides demonstrate more obvious clustering effect (based on treatment types) in the PCA, t-SNE and heatmap at 6 month's time rather than at 3 month's time. This also suggests presence of *time* fixed effect in the data set.
- Meanwhile, out of the 29650 peptides at 1% FDR, 114 of them also exhibit higher fluorescence levels by at least two-fold due to ADT as compared to PAP at both times 3-months and 6-months.
- The gene-sets found to be enriched by the genes associated with the significant peptides are comparable to the gene-sets identified in (Figure 5 of) the main manuscript.