LF97 - Hugo Germain - Nuclear envelope proteins

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Samples

- 4 groups of Nuclear envelope proteins (CTRL, IFN, NL and IFNNL (VI)) x 3 replicates = 12 samples

Comparisons

- 3 IFN vs 3 CTRL
- 3 NL vs 3 CTRL
- 3 IFNNL (VI) vs 3 CTRL
- 3 IFN vs 3 NL
- 3 IFN vs 3 IFNNL (VI)
- 3 NL vs 3 IFNNL (VI)

Sample preparation and analysis

- Proteins were resuspended in Ammonium bicarbonate (50mM)/Deoxycholate(1%)
- S-S bridges were reduced by DTT and alkylated with Iodoacetamide
- Proteins were digested with Trypsin (1:50) overnight at 37 degree celsius
- Resulting peptides were purified using StageTips C18 and Speed Vac dried
- Samples were resuspended in LC loading solvant and eq. 1µg was injected
- Samples were analyzed by LC-MSMS on an Orbitrap Fusion Tribrid system (Thermo)
- 120 min LC runs (90 min grdients) were used and the MS operated in DDA mode
- Identification and LFQ quantification was done using MaxQuant software v1.6.10.43
- Database : Ref homo sapiens (74485 entries)
- Modifications: Fixed : Carbamidometyl (Cys), Variable : Oxidation(M), Acetyl (Prot N-term)

DATA TREATMENT (R software)

1 - Import & filtering data

- Input file : “proteinGroups.txt” from the MaxQuant software
- Decoy proteins are removed
- For data processing, only the LFQ intensity columns are considered

2 - Data normalization

- For each sample, a median intensity is calculated

- For each sample, an intensity normalization factor is calculated by dividing the median intensity of said sample by the median of the median intensity of all samples.
- Each protein intensity is normalized by dividing their intensity by the normalization factor

3 - Missing data imputation

- For each sample, a noise value corresponding to the 0.01 percentile of the intensities of said sample is calculated
- This noise value is imputated for each samples when the intensity value is missing

4 - Comparisons

- Only proteins which present 100% of intensity values (not noise imputed value) in at least one group are considered. These proteins correspond to *quantifiable proteins*.
- Only proteins identified with at least 2 peptides are considered. They correspond to *quantified proteins*.

5 - Statistics

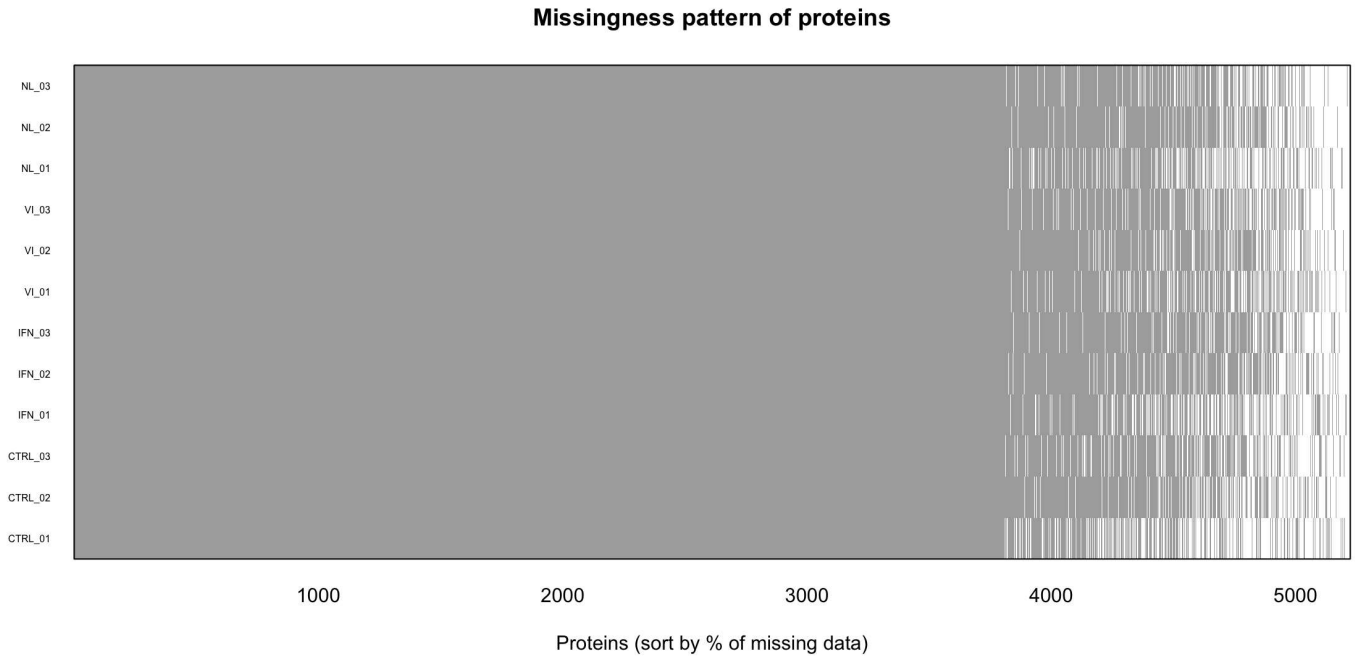
For each protein in each comparison, the following values are calculated :

- the mean intensity in each group (“mean_g1” and “mean_g2”),
- the intensity ratio between each group (“Ratio g1/g2””),
- the log2 of g1/g2 ratio (“log2Ratio””)
- the z-score z = x – mean / standard deviation (“zscore””)
- Limma *p-value* and *q-value* (Benjamini Hochberg adjusted *p-value*) (“pval_Limma” and “qval_Limma”)
- The following filters were applied to define variant proteins : q-value < 0.05, |z-score| > 1.96 (“Sign_zscore_qval_Limma”)

DATA REPRESENTATION

1 - Check missing values

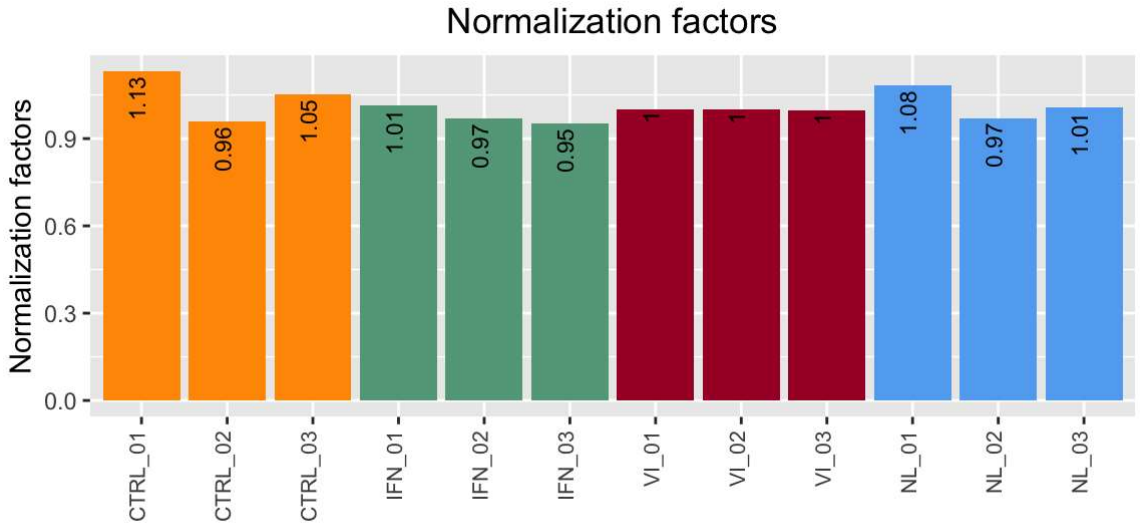
Low abundance proteins signal is usually not extracted in all samples. This graph shows the proportions of intensity values (grey) and missing values (white) for each sample of the analysis. We expect to observe low proportion of missing values (<15-20%) and a similar profile for all samples to compare.



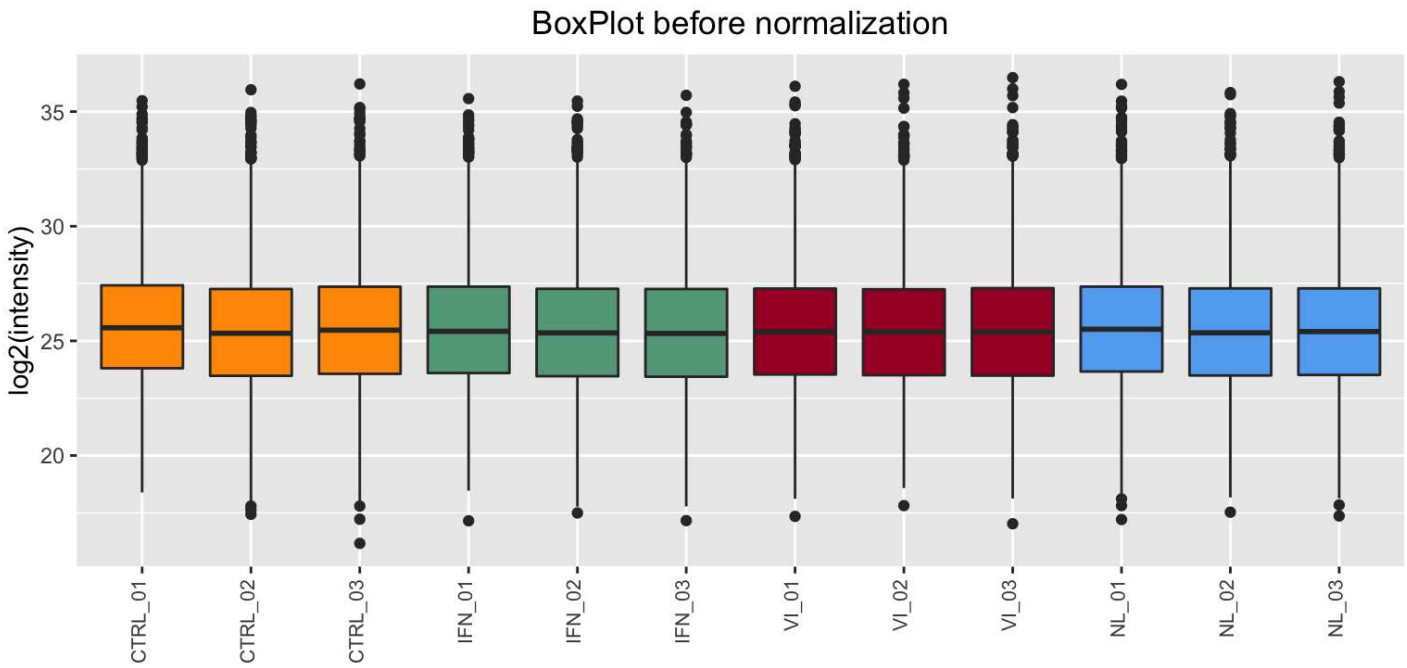
- Between 7.24% and 14.05% of missing values between the samples
- 9.29% of missing values for the whole data set

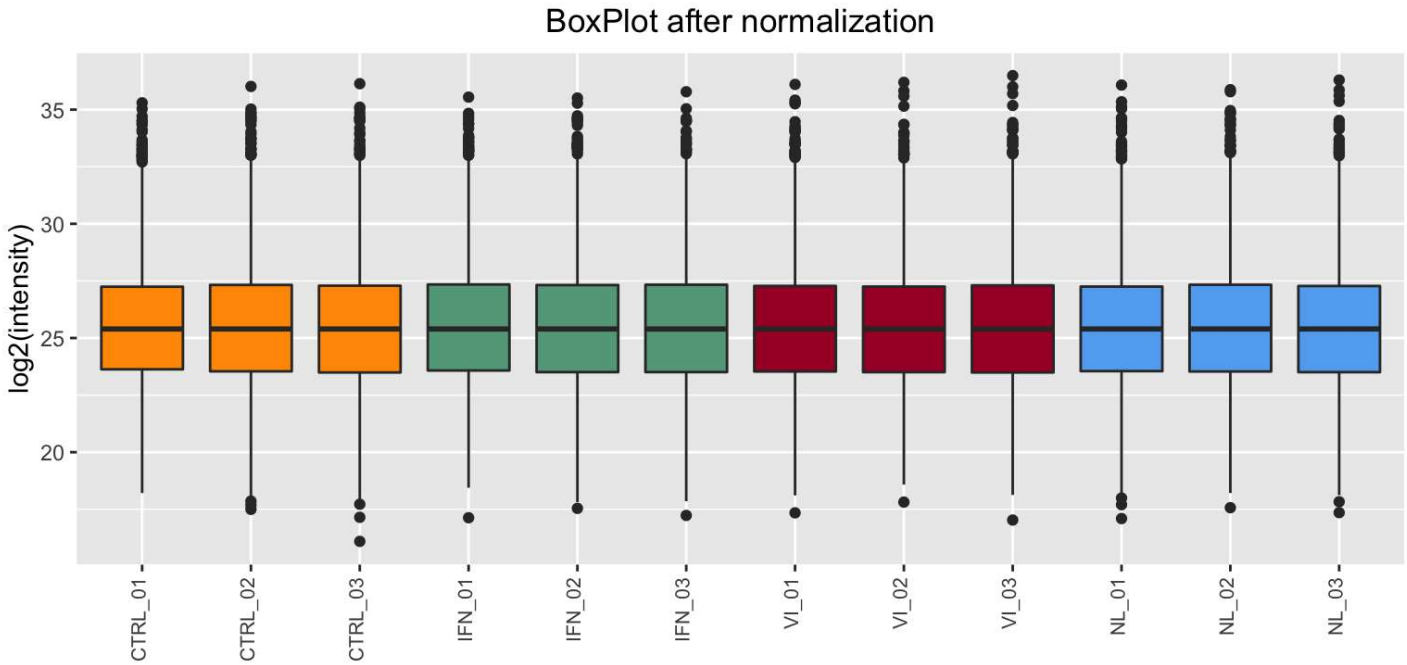
2 - Data normalization

Normalization factors are expected to be close as possible to 1. After normalization, the median of intensities is identical for all samples and the box plots should display a similar repratition of the intensities across all samples.



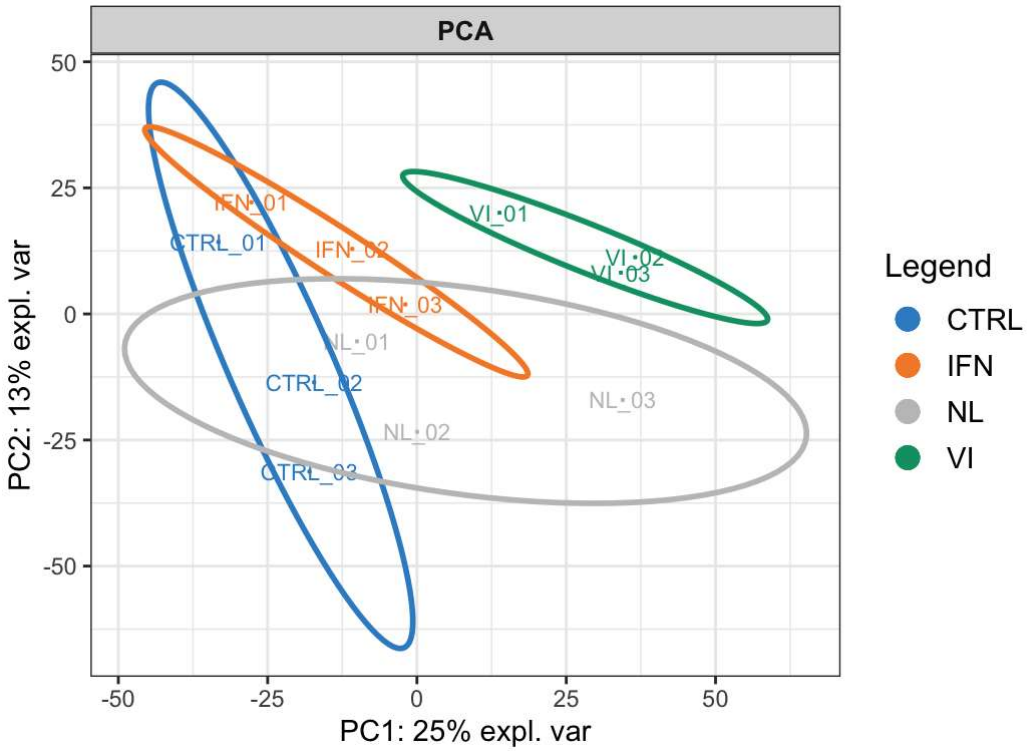
- Normalization factor are between a value of 0.95 and 1.13
- The mean normalization factor is 1.01





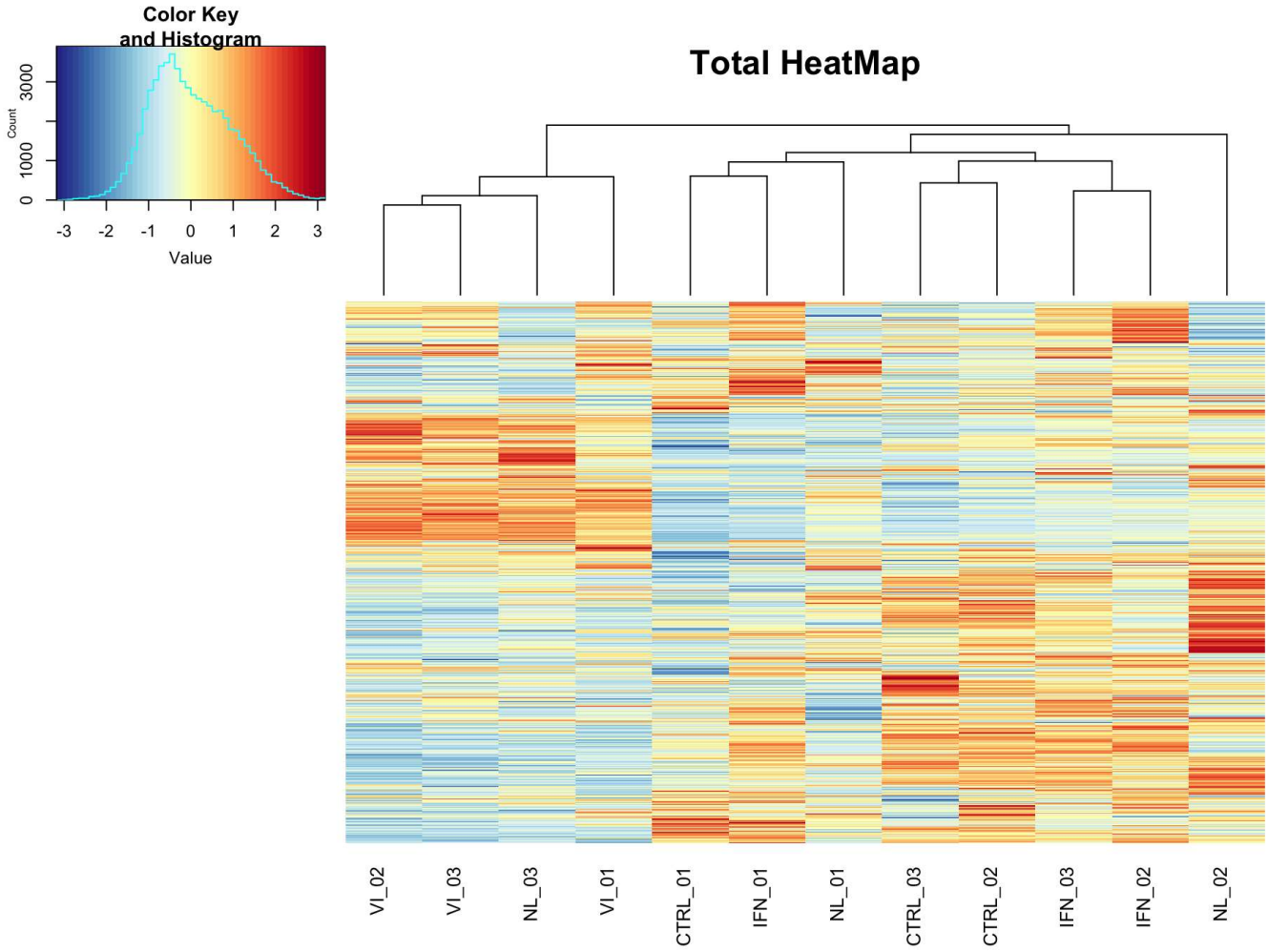
3 - PCA - Principal Component Analysis (proteins)

This representation uses an orthogonal transformation to convert all variables (here proteins) into new variables (principal component) explaining as much as possible the variability across the samples. The first component (PC1) always as the highest variance. Samples having similar proteomic profiles will appear close on the PCA plots. When the ellipses of two groups are not overlapping, we can consider that the sample groups can be distinguished based on their proteomic profiles.



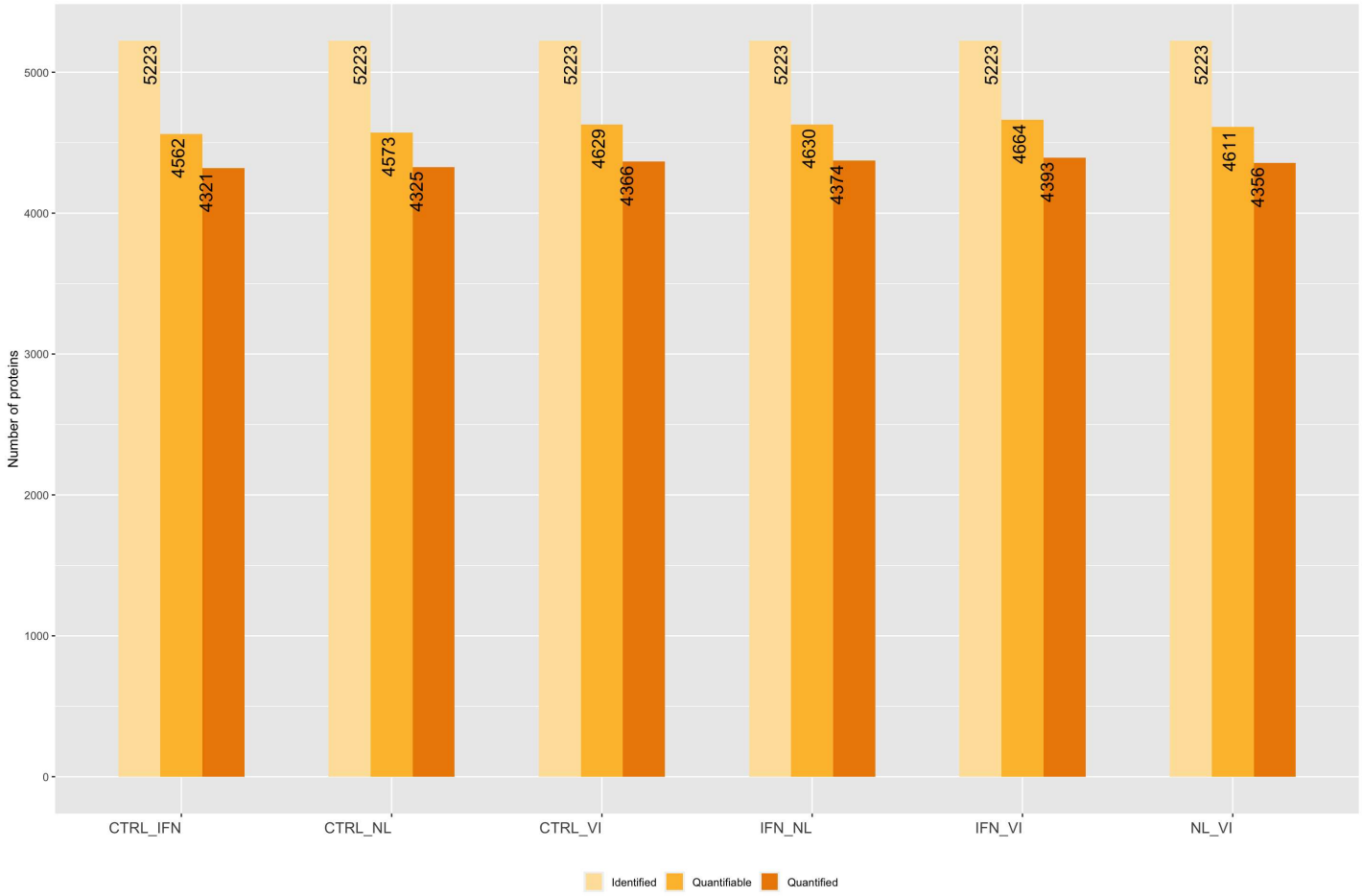
4 - Proteomic profiles heatmap

This heatmap represents the intensity values in all samples of all proteins of the analysis. A hierachical clustering is applied both on rows and columns meaning that samples or proteins having similar profiles appear close to each other



4 - Number of quantifications for each comparison

Not all proteins of an analysis can be quantified, the signal of low abundance proteins might be difficult to extract resulting in missing values for certain sample. Proteins with a sufficient number of not missing values (100) are considered as quantifiable. Moreover, to add more confidence in the quantification value, we only retain proteins quantified with at least 2 peptides.



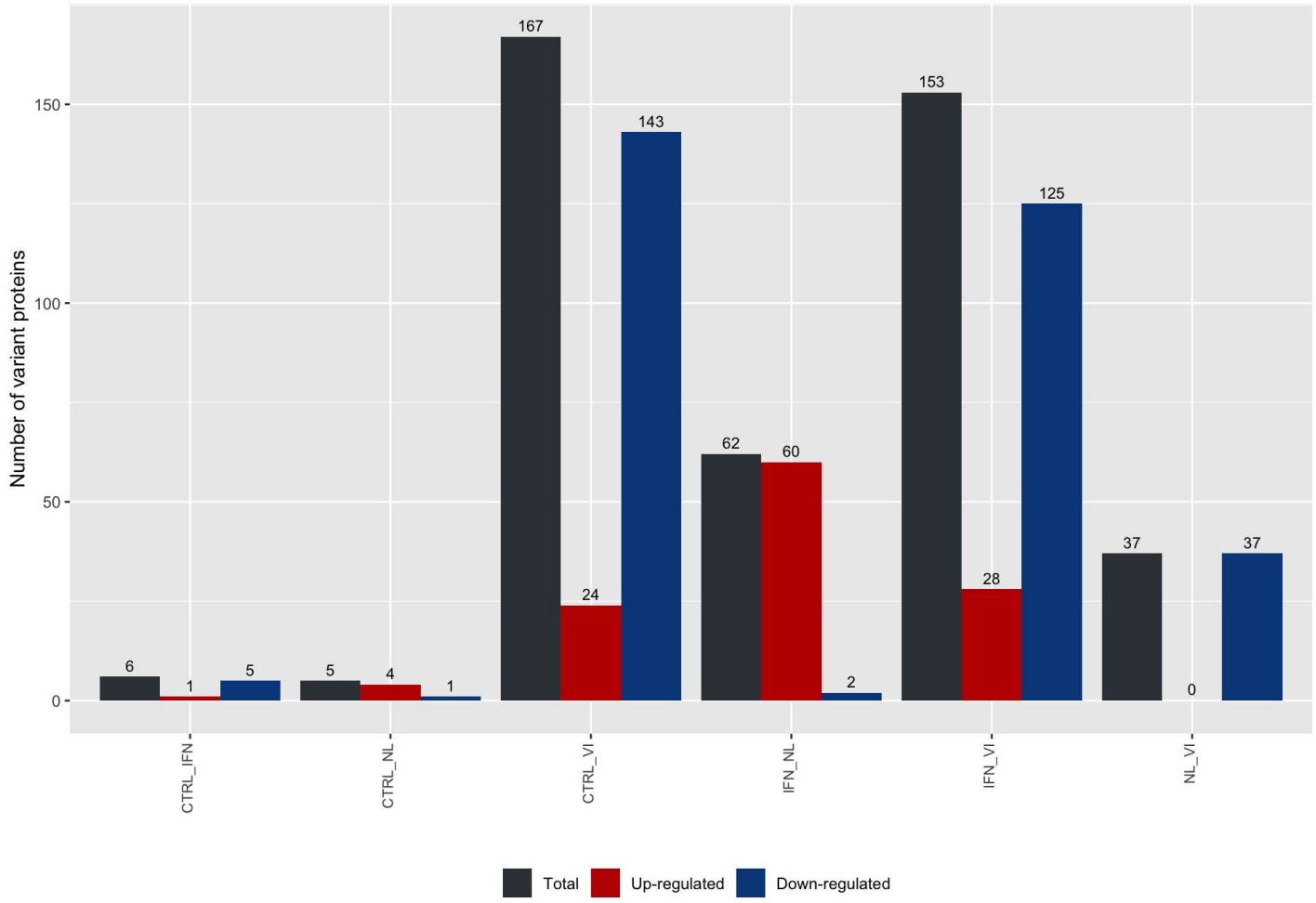
Identified proteins :
Total number of proteins identified in the analysis
5223 proteins identified in this analysis

Quantifiable proteins :
Proteins with at least 100% of observed intensities in each replicates in one of the two groups.
4612 quantifiable proteins on average for the whole analysis

Quantified proteins :
Proteins identified with at least 2 peptides.
4356 quantified proteins on average for the whole analysis

5 - Number of variant proteins for each comparison

Proteins are considered as “variant” between two conditions/groups if they fulfill these criteria : q-value < 0.05 and |z-score| > 1.96 In a conmparison annotated “group1_group2”: the proteins “up-regulated” (red) are more abundant in “group1” than in “group2”, the proteins “down-regulated” (blue) are more abundant in “group2” than in “group1”,

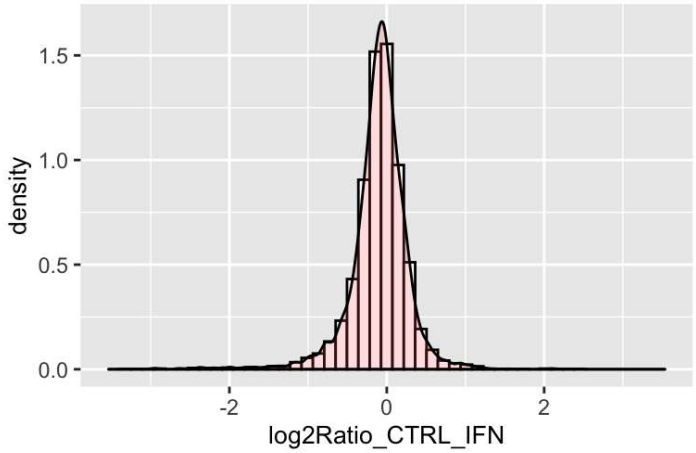


6 - Plots for each comparison

Repartition Histograms and Volcano plots

- The repartition histogram of all protein ratios (log2(ratio)), expected to be centered as much as possible to 0 (ratio g1/g2 = 1)
- The repartition histogram of z-scores (centering), must be centered to 0
- The volcano plot, where x-axis represents the protein ratio (log2(ratio)) and the y-axis the probability (-log10(p-value))
  The proteins are variant if they have a high ratio (in absolute value) and a good probability, i.e proteins in the upper right and upper left corners of the graph.

CTRL_IFN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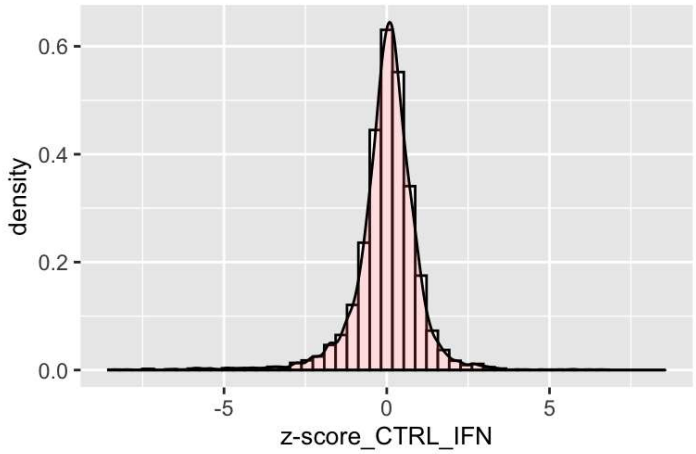
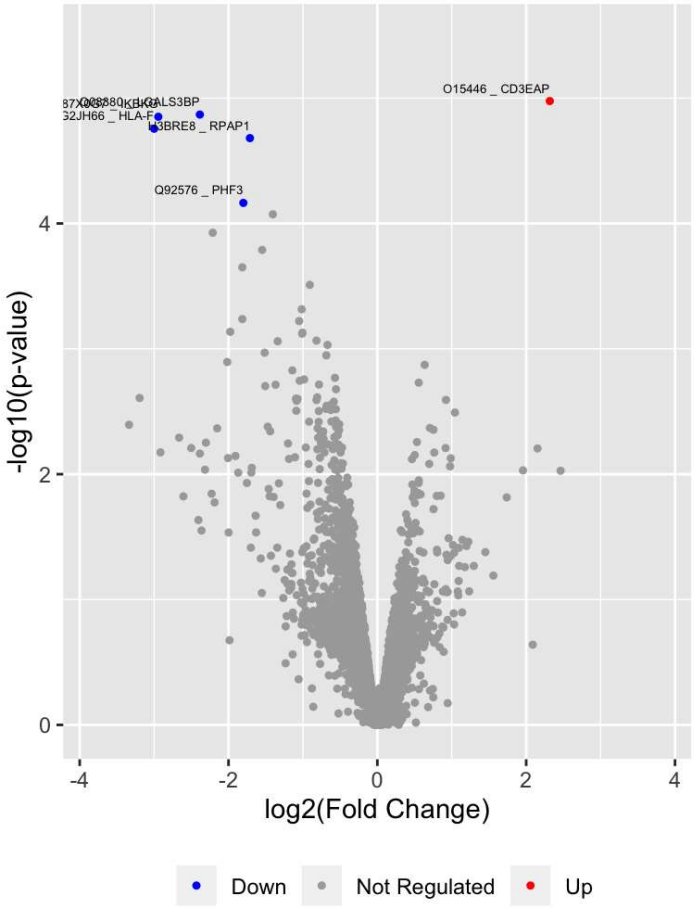
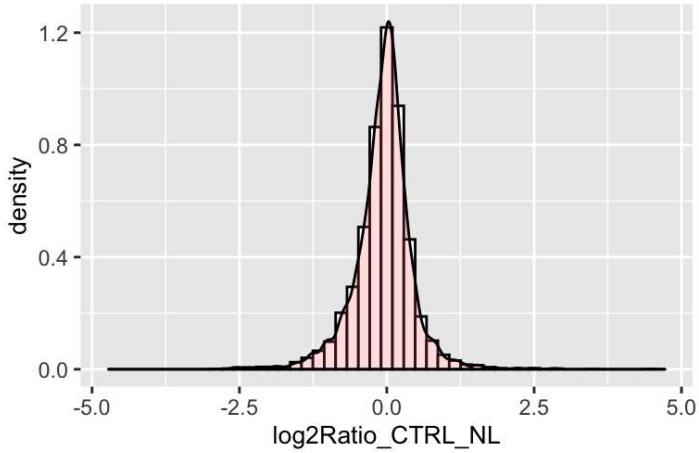
log2Ratio_CTRL_IFN


density
z-score_CTRL_IFN


O15446 _ CD3EAP
LGALS3BP
HLA-F
RPAP1
Q92576 _ PHF3
-log10(p-value)
log2(Fold Change)
Down
Not Regulated
Up

CTRL_NL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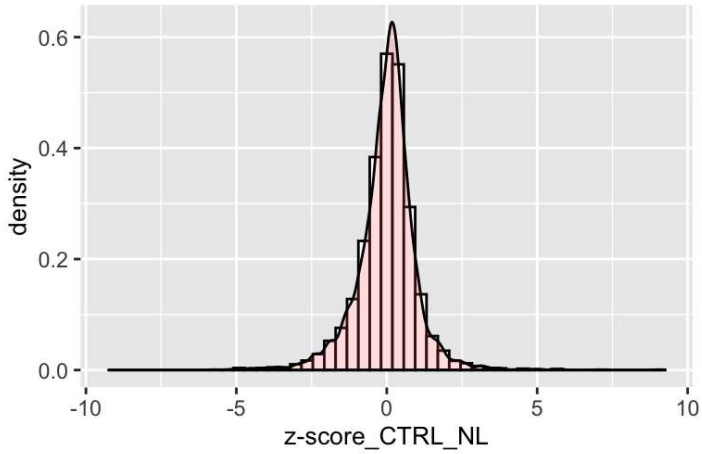
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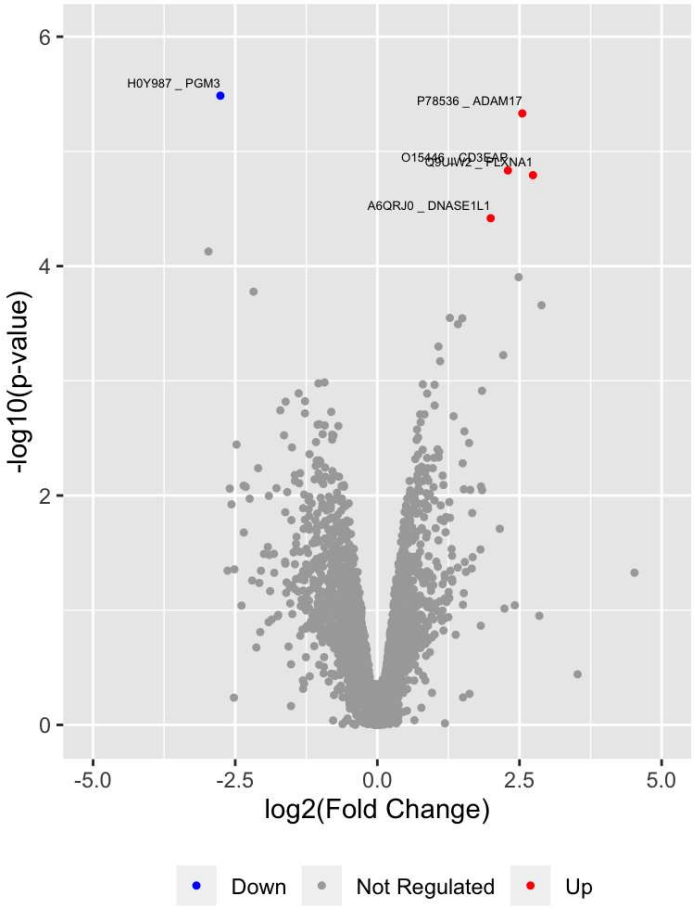
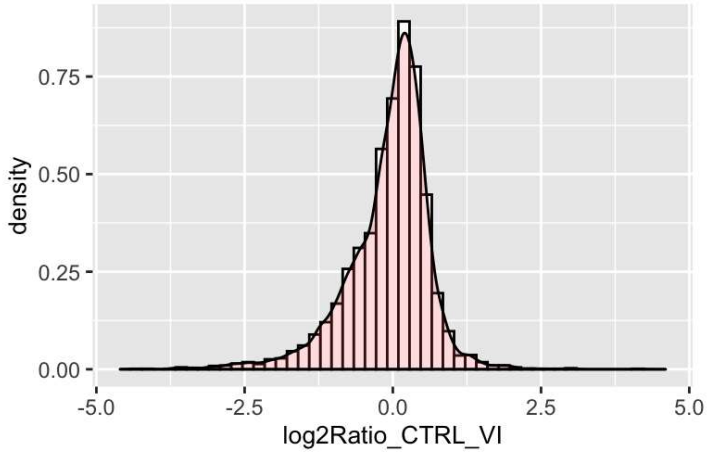

H0Y987 _ PGM3
P78536 _ ADAM17
O15446 _ CD3EAP
Q9UIW2 _ PLXNA1
A6QRJ0 _ DNASE1L1
-log10(p-value)
log2(Fold Change)
Down
Not Regulated
Up

CTRL_VI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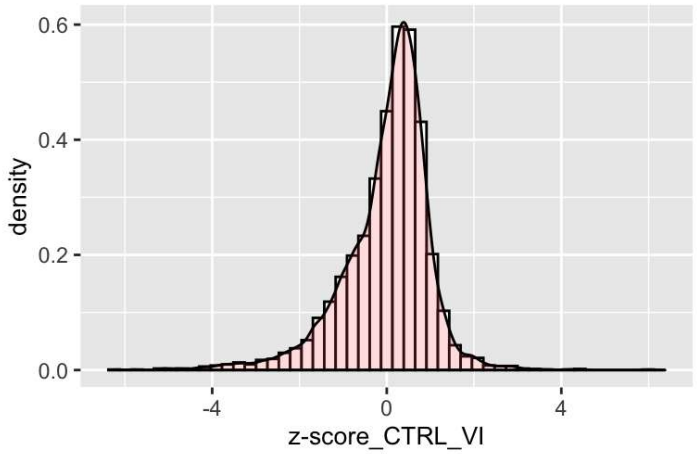
0.0
2.5
5.0
log2Ratio_CTRL_VI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0.0
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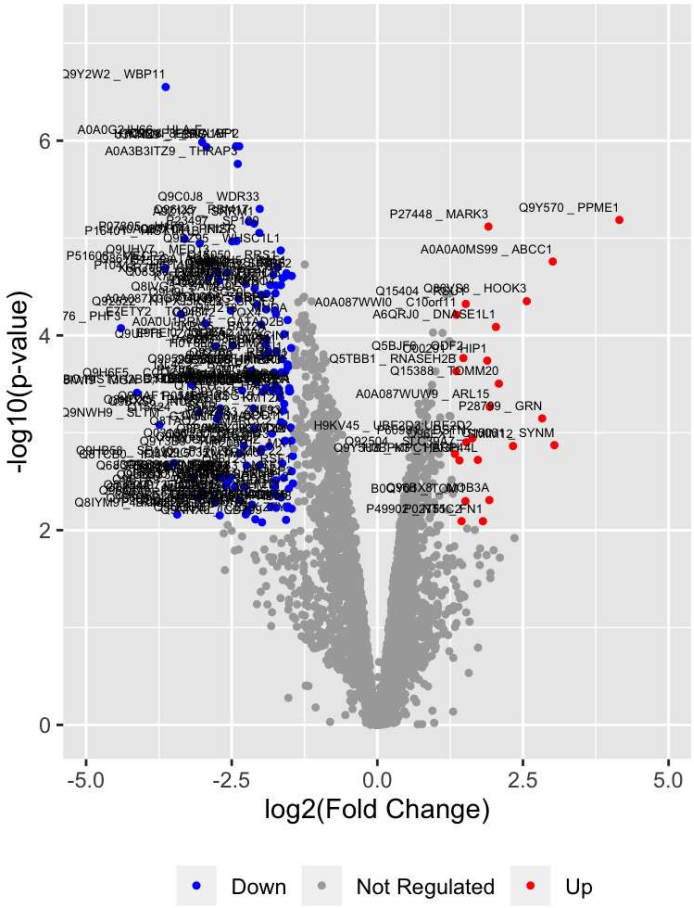
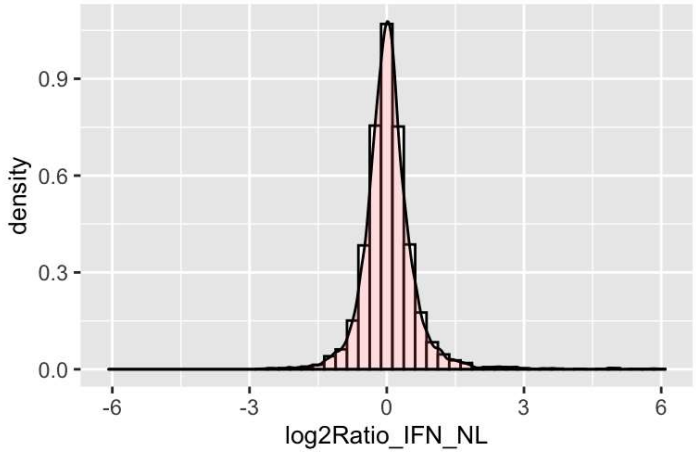
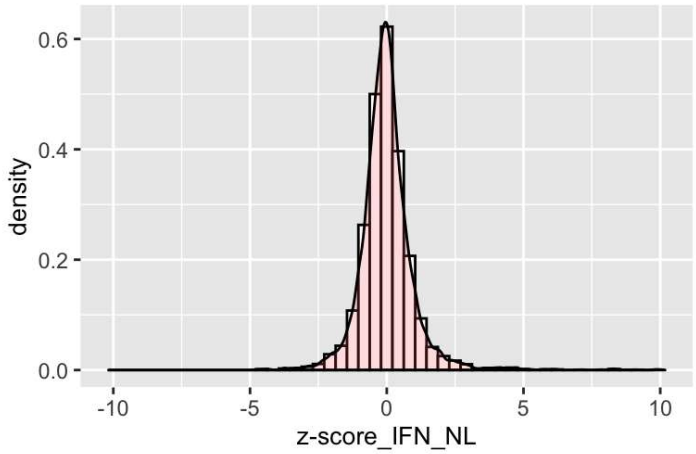
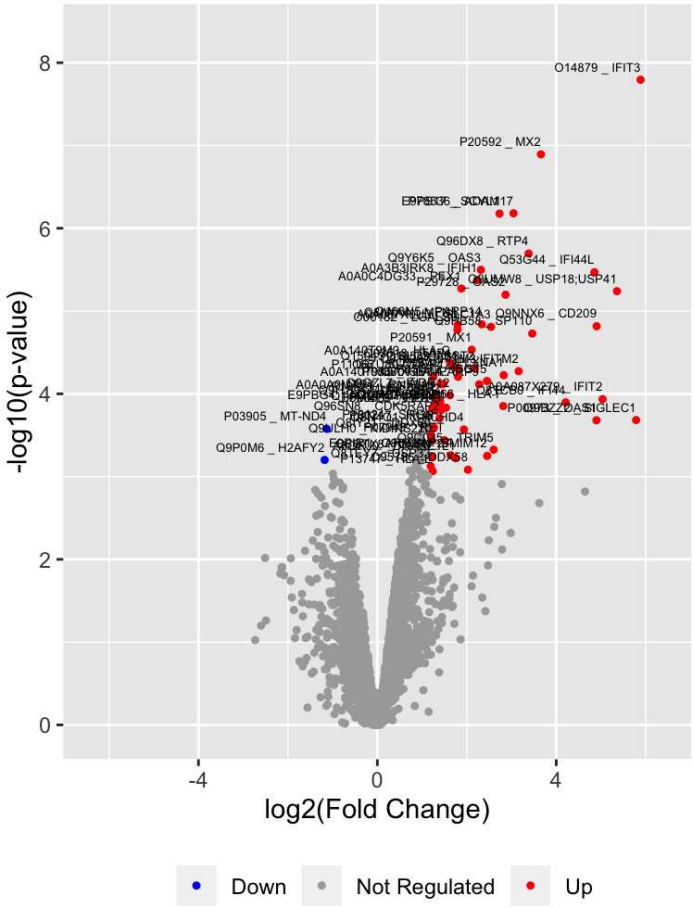
0
4
z-score_CTRL_VI


-log10(p-value)
6
4
2
0
-5.0
-2.5
0.0
2.5
5.0
log2(Fold Change)
Down
Not Regulated
Up

IFN_NL


density
log2Ratio_IFN_NL


density
z-score_IFN_NL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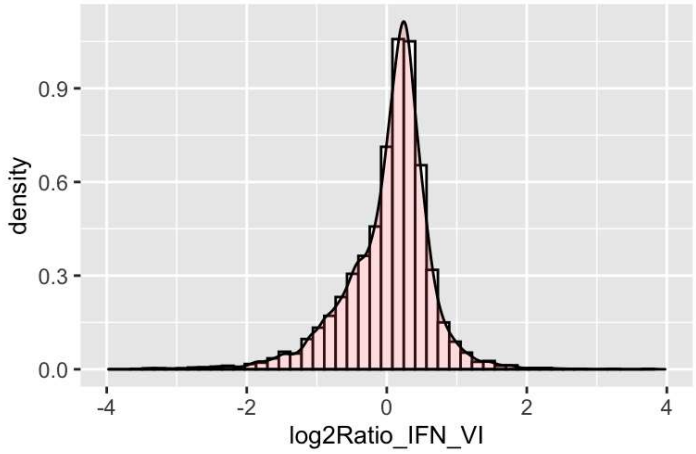
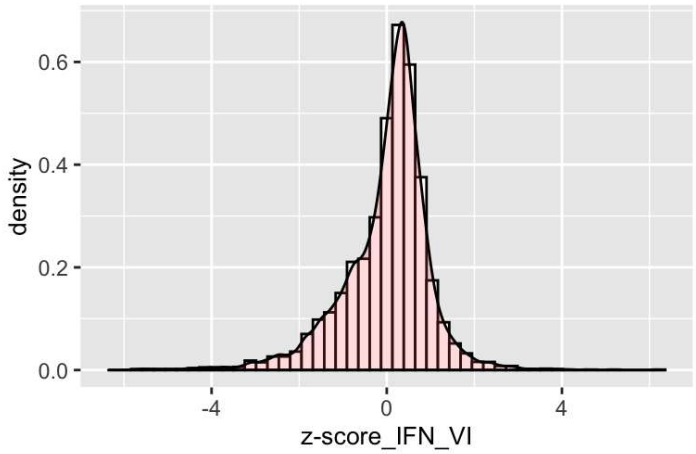
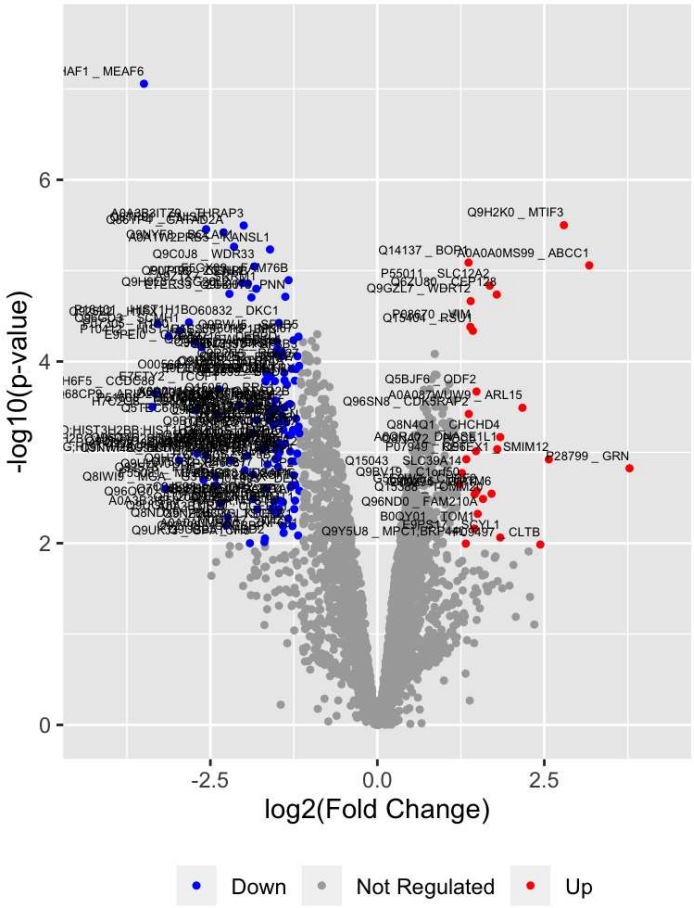
log2(Fold Change)
O14879 _ IFIT3
P20592 _ MX2
Q96DX8 _ RTP4
Q9Y6K5 _ OAS3
Q53G44 _ IFI44L
P20591 _ MX1
Down
Not Regulated
Up

IFN_VI


density
log2Ratio_IFN_VI


density
z-score_IFN_VI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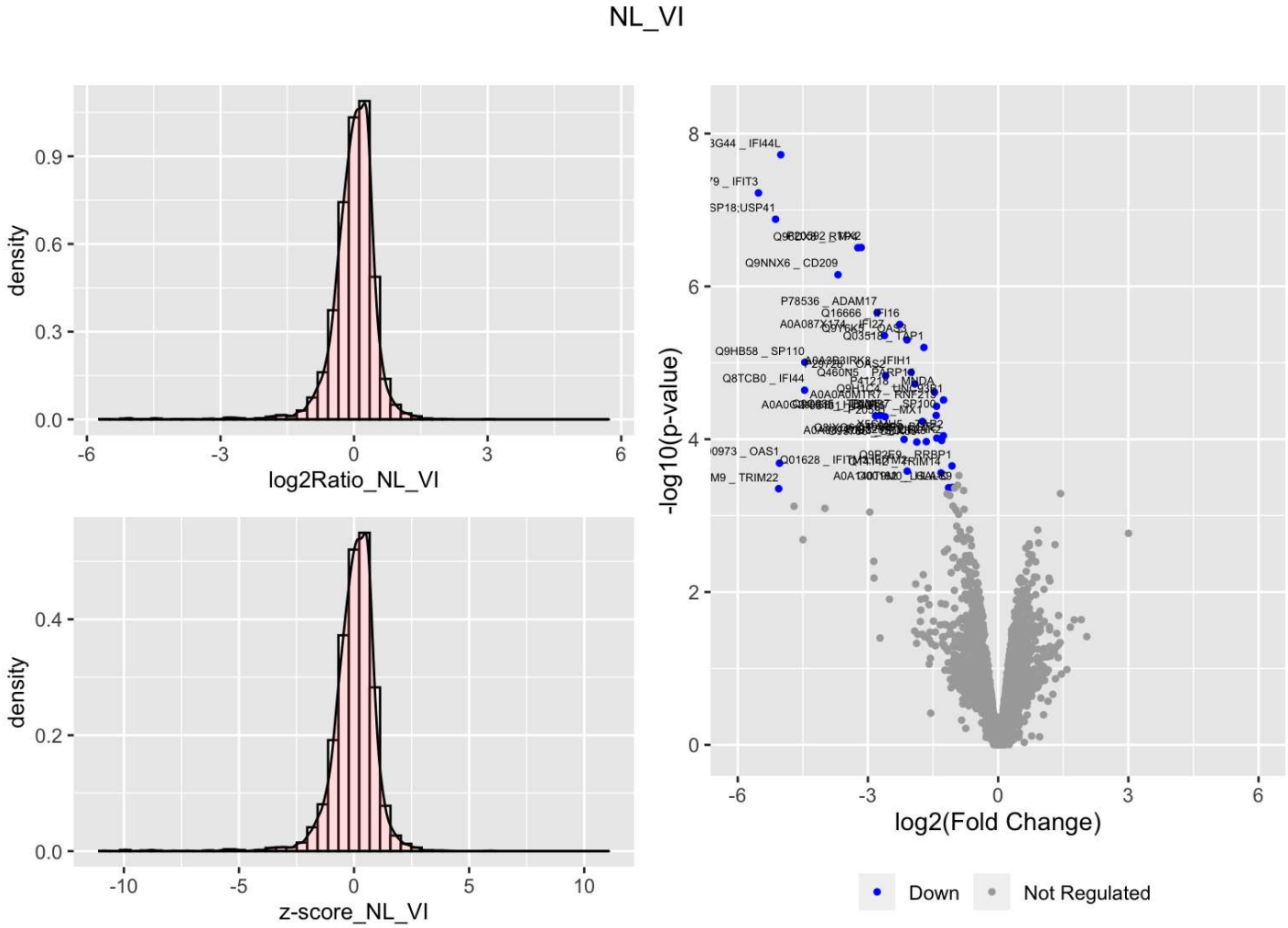
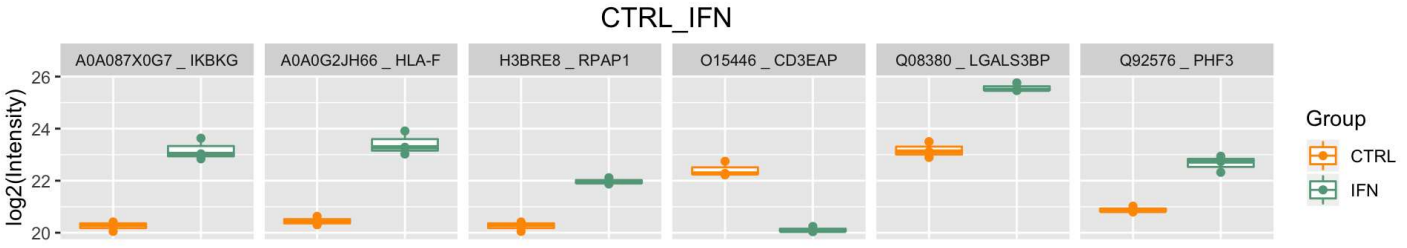
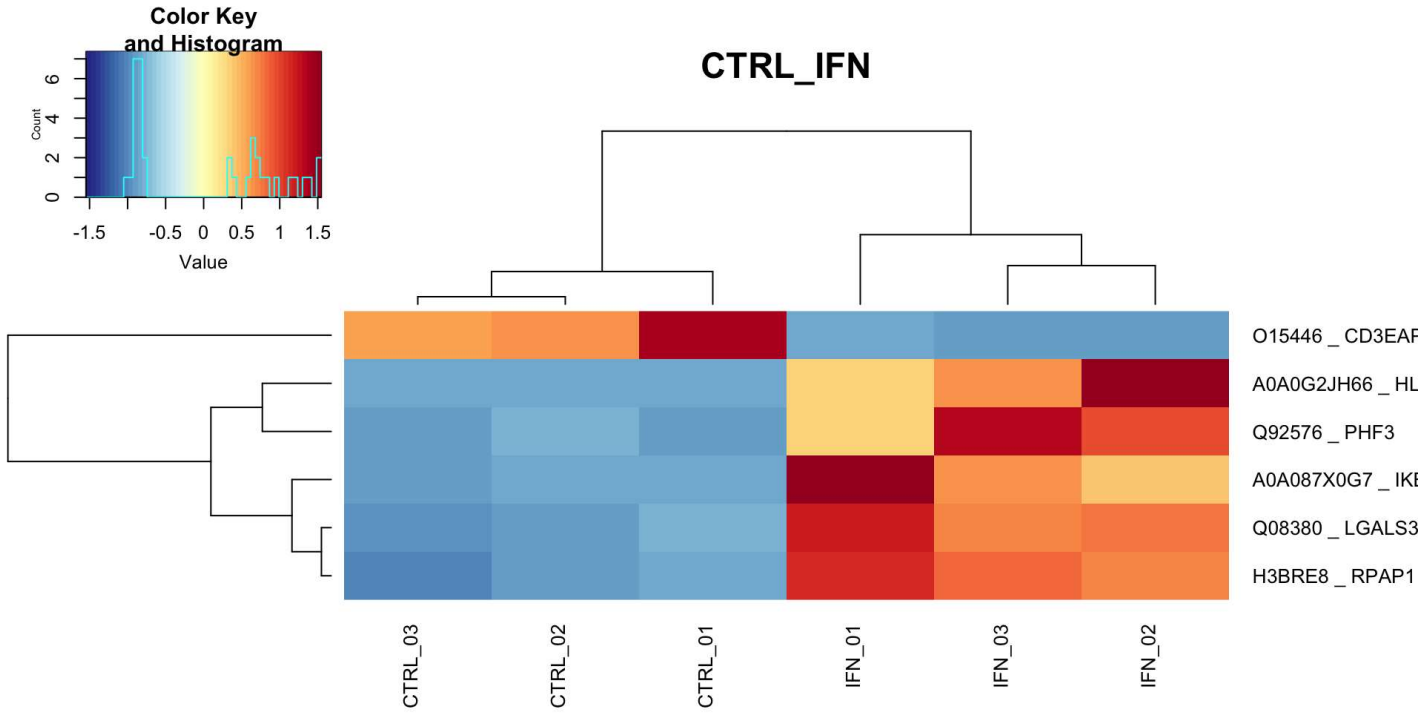
log2(Fold Change)
Q9H2K0 _ MTIF3
Q14137 _ BOP1
A0A0A0MS99 _ ABCC1
P55011 _ SLC12A2
Q9GZL7 _ WDR12
Q15404 _ RSU1
Q5BJF6 _ QDF2
A0A087WUW9 _ ARL15
Q96SN8 _ CDK5RAP2
Q8N4Q1 _ CHCHD4
P07949 _ RET
SMIM12
P28799 _ GRN
Q15043 _ SLC39A14
Q96ND0 _ FAM210A
B0QY01 _ TOM1
Q9Y5U8 _ MPC1
CLTB
Down
Not Regulated
Up

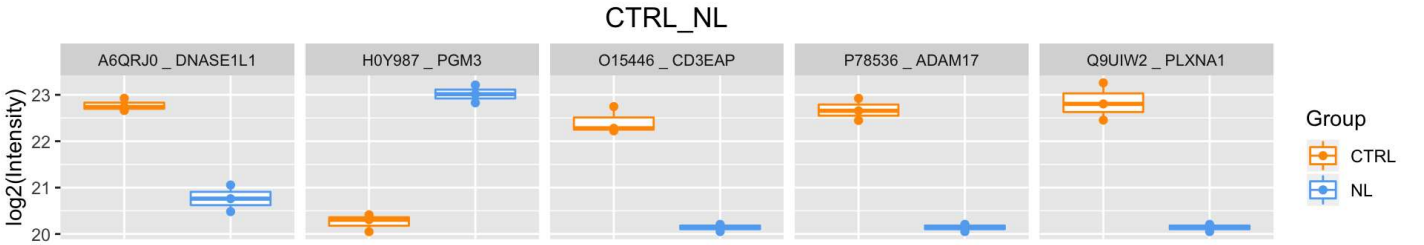


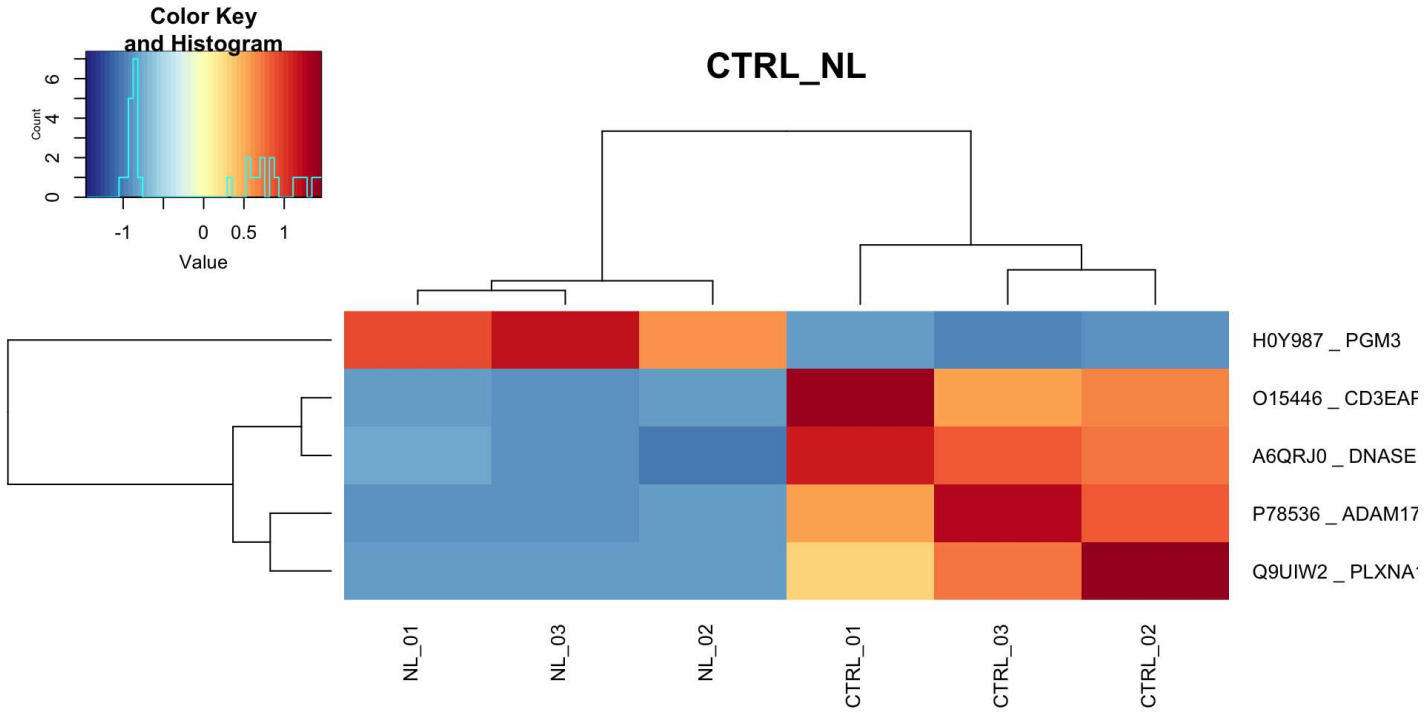
Box-plots and HeatMap of variant proteins

- The box-plots shows the proteins intensity value for all replicates of each group
- The heatmap shows the scaled intensity values for all variant proteins in all replicates, a hierachical clustering is applied both on rows and columns meaning that samples or proteins having similar profiles appear close to each other.

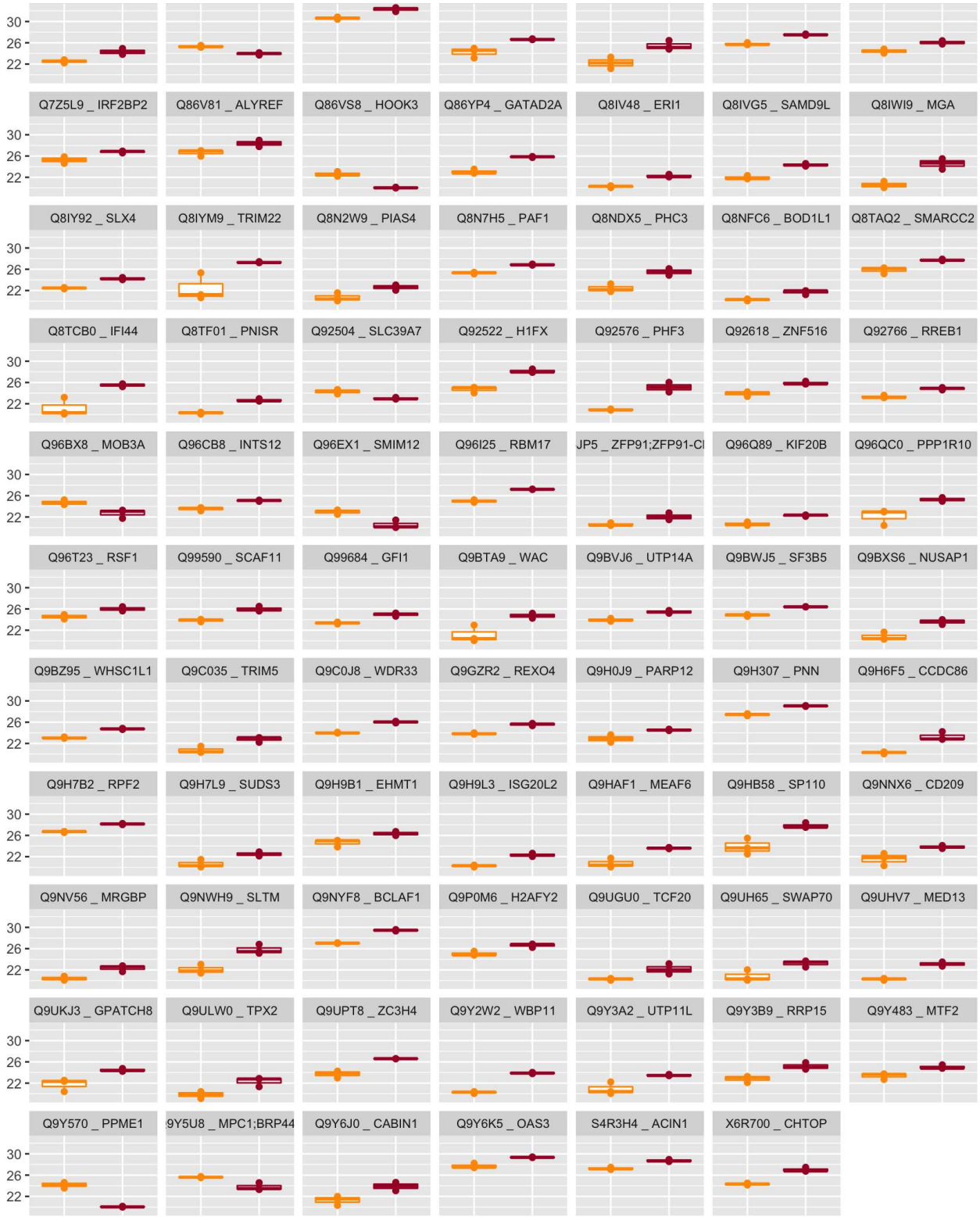



Color Key and Histogram
Count
0
2
4
6
-1.5
-0.5
0
0.5
1
1.5
Value
CTRL_IFN
O15446 _ CD3EAF
A0A0G2JH66 _ HL
Q92576 _ PHF3
A0A087X0G7 _ IKE
Q08380 _ LGALS3
H3BRE8 _ RPAP1
CTRL_03
CTRL_02
CTRL_01
IFN_01
IFN_03
IFN_02


CTRL_NL
A6QRJ0 _ DNASE1L1
H0Y987 _ PGM3
O15446 _ CD3EAP
P78536 _ ADAM17
Q9UIW2 _ PLXNA1
log2(Intensity)
20
21
22
23
Group
CTRL
NL


Color Key and Histogram
Count
0
2
4
6
-1
0
0.5
1
Value
CTRL_NL
H0Y987 _ PGM3
O15446 _ CD3EAF
A6QRJ0 _ DNASE
P78536 _ ADAM17
Q9UIW2 _ PLXNA
NL_01
NL_03
NL_02
CTRL_01
CTRL_03
CTRL_02


CTRL_VI
A0A087WUW9 _ ARL15
A0A087WWI0 _ C10orf1
A0A087X0G7 _ IKBKG
A0A087X174 _ IFI27
A0A0A0MS99 _ ABCC1
A0A0G2JH66 _ HLA-F
A0A0U1RRM1 _ GATAD2
A0A2R8Y7Z3 _ RUNX2
A0A3B3ITZ9 _ THRAP3
A6QRJ0 _ DNASE1L1
A9Z1X7 _ SRRM1
B0QY01 _ TOM1
C9JE98 _ NCOR2
E7ERS3 _ ZC3H18
E7ETY2 _ TCOF1
E9PEH0 _ PRDM8
E9PEI0 _ CDCA2
E9PEP7 _ ZFX;ZFY
G3V5L1 _ MAX
H0Y6E7 _ RBMX
H0Y993 _ DEK
H3BPK3 _ HAGH
H3BV80 _ RNPS1
H7C2Q8 _ EBNA1BP2
KV45 _ UBE2D3;UBE2
I3L2Z5 _ MAZ
I3L4C2 _ BAIAP2
I3VM54 _ KDM2A
J3KNZ9 _ FBRS
J3KPG5 _ BAZ2A
J3KTL2 _ SRSF1
J3QL05 _ SRSF2
J3QRS9 _ ZNF207
K7EQ03 _ RBM42
M0QXA7 _ WIZ
O00291 _ HIP1
O00566 _ MPHOSPH10
O15061 _ SYNM
O43290 _ SART1
O43395 _ PRPF3
O43660 _ PLRG1
O60832 _ DKC1
O75528 _ TADA3
O94842 _ TOX4
O95786 _ DDX58
P00973 _ OAS1
P02751 _ FN1
P05161 _ ISG15
P06748 _ NPM1
P07305 _ H1F0
P10412 _ HIST1H1E
P11387 _ TOP1
P12270 _ TPR
P16401 _ HIST1H1B
P17026 _ ZNF22
P17096 _ HMGA1
P20591 _ MX1
P23497 _ SP100
P27448 _ MARK3
P28799 _ GRN
P29728 _ OAS2
P35789 _ ZNF93
P41218 _ MNDA
P42696 _ RBM34
P46087 _ NOP2
P49716 _ CEBPD
P49902 _ NT5C2
P51608 _ MECP2
P54198 _ HIRA
P55081 _ MFAP1
P60981 _ DSTN
P82979 _ SARNP
Q01831 _ XPC
Q03164 _ KMT2A
Q03188 _ CENPC
Q08380 _ LGALS3BP
Q13111 _ CHAF1A
Q13435 _ SF3B2
Q14814 _ MEF2D
Q15050 _ RRS1
Q15059 _ BRD3
Q15388 _ TOMM20
Q15404 _ RSU1
Q15424 _ SAFB
15695 _ ZRSR1;ZRSR
Q16666 _ IFI16
2BB;HIST1H2BO;HIST3
Q2KHR3 _ QSER1
Q53G44 _ IFI44L
Q5BJF6 _ ODF2
Q5SSJ5 _ HP1BP3
Q5T200 _ ZC3H13
Q5TBB1 _ RNASEH2B
5TEC6 _ HIST2H3PS2
Q5VZL5 _ ZMYM4
Q68CP9 _ ARID2
Q6UN15 _ FIP1L1
Q7Z333 _ SETX
30
26
22
log2(Intensity)
Group
CTRL
VI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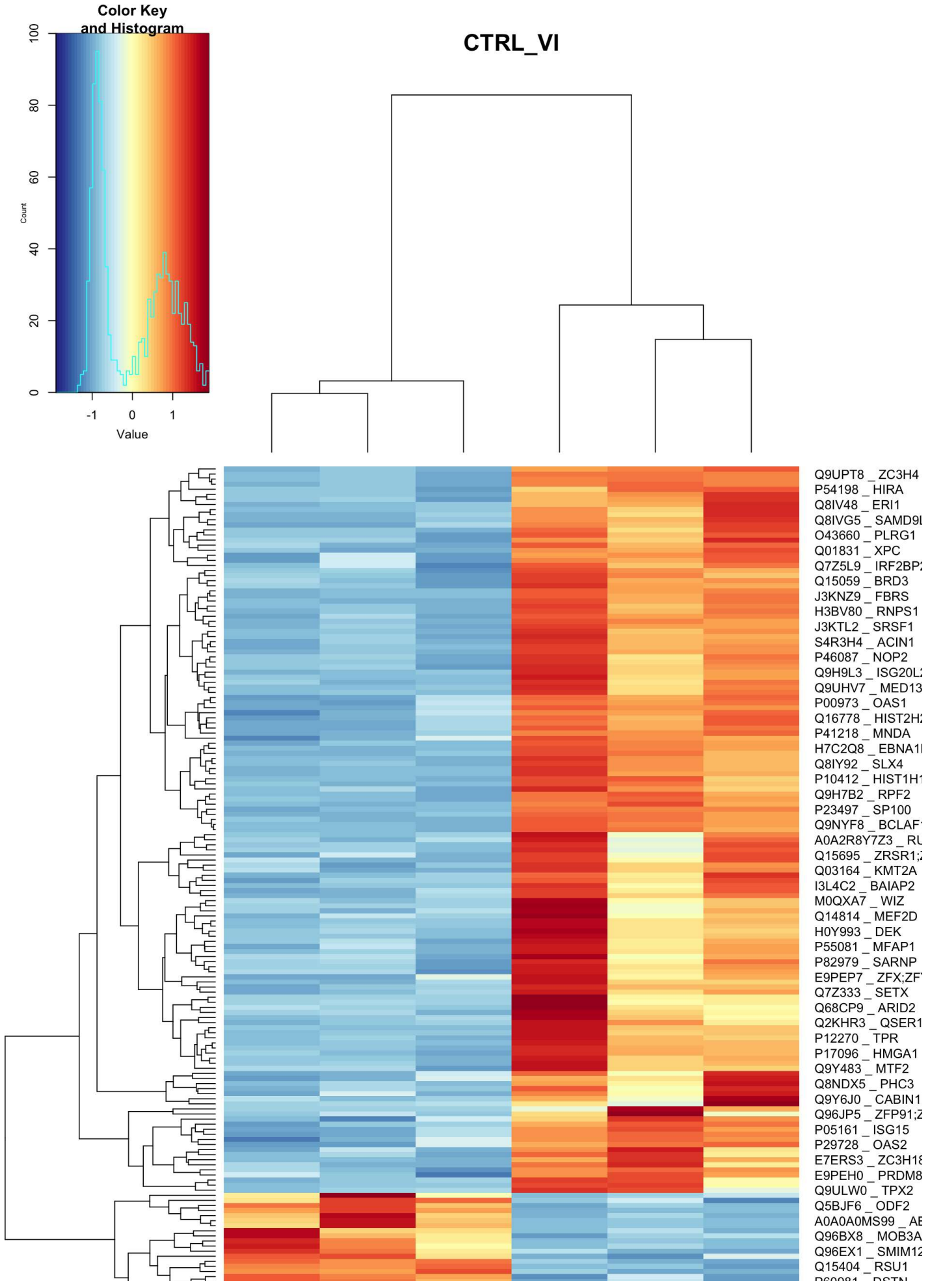
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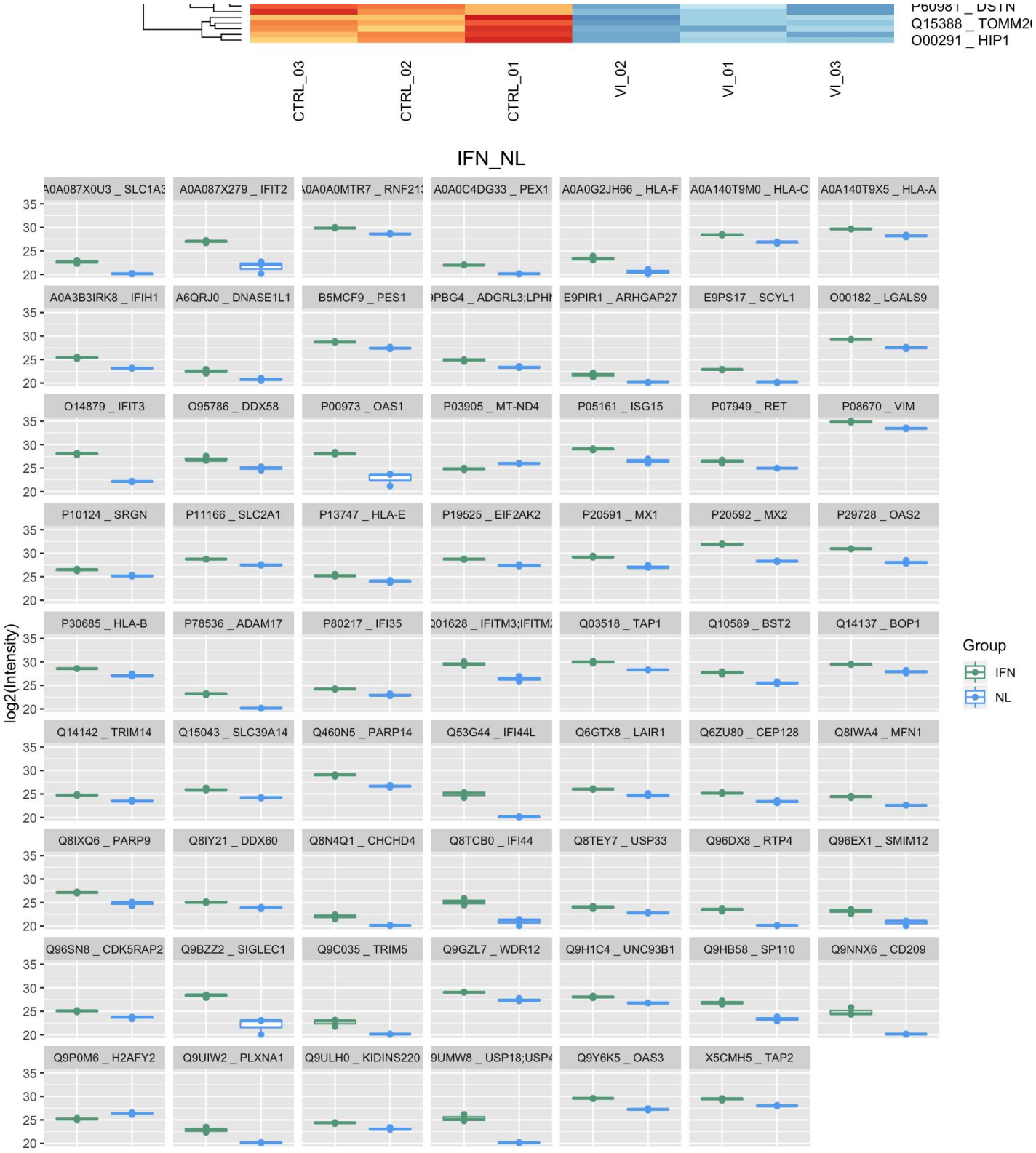
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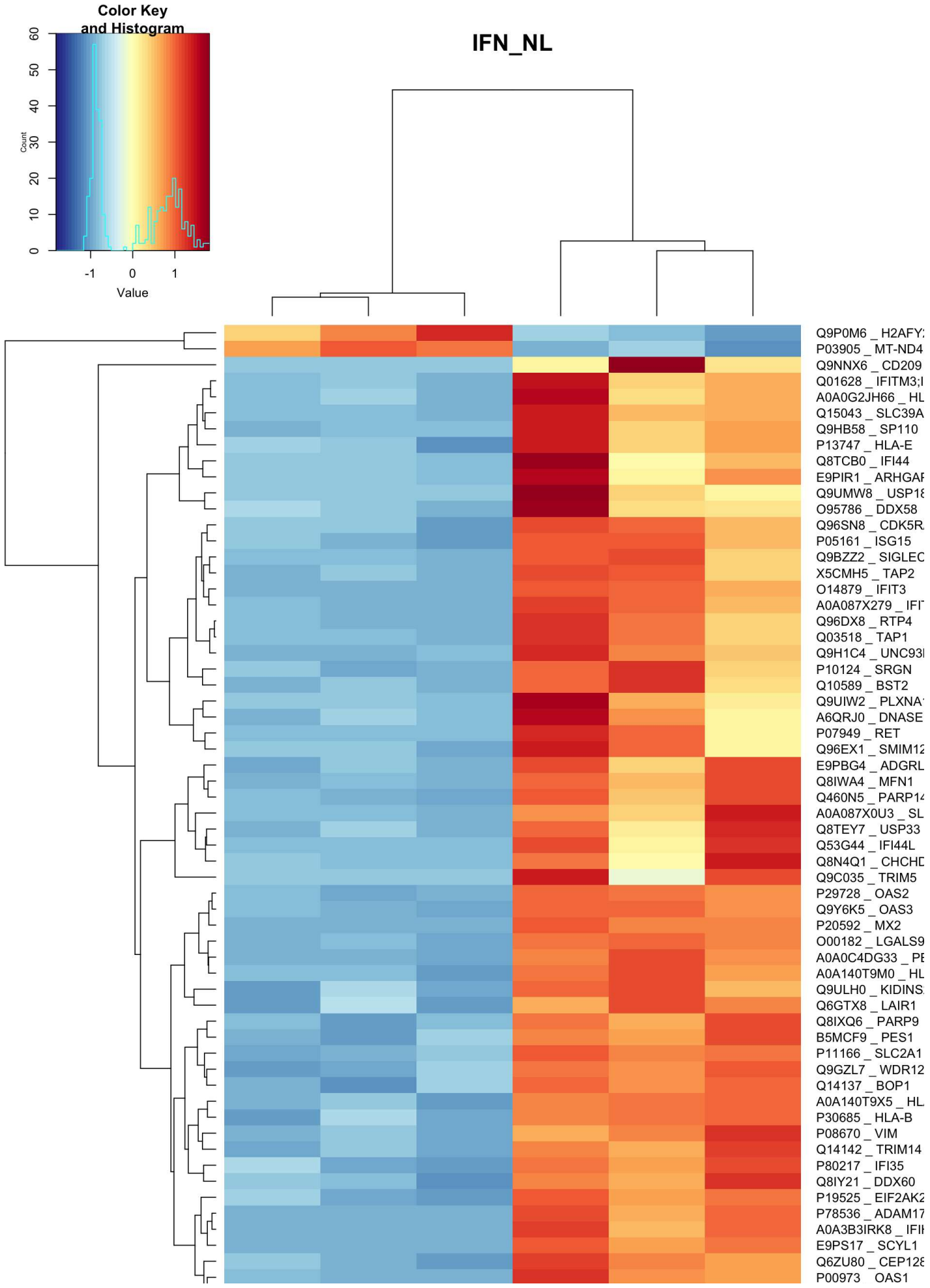
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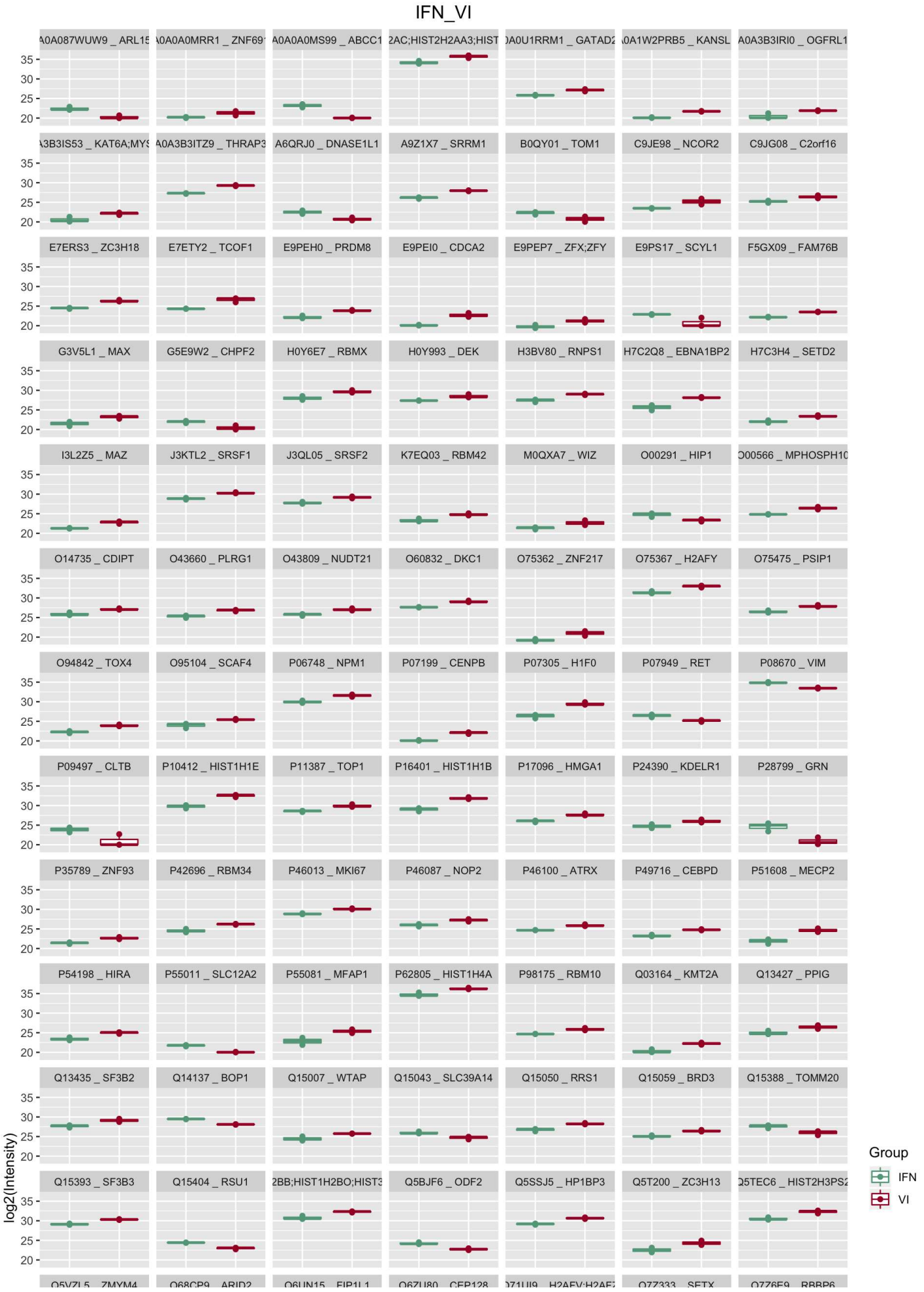
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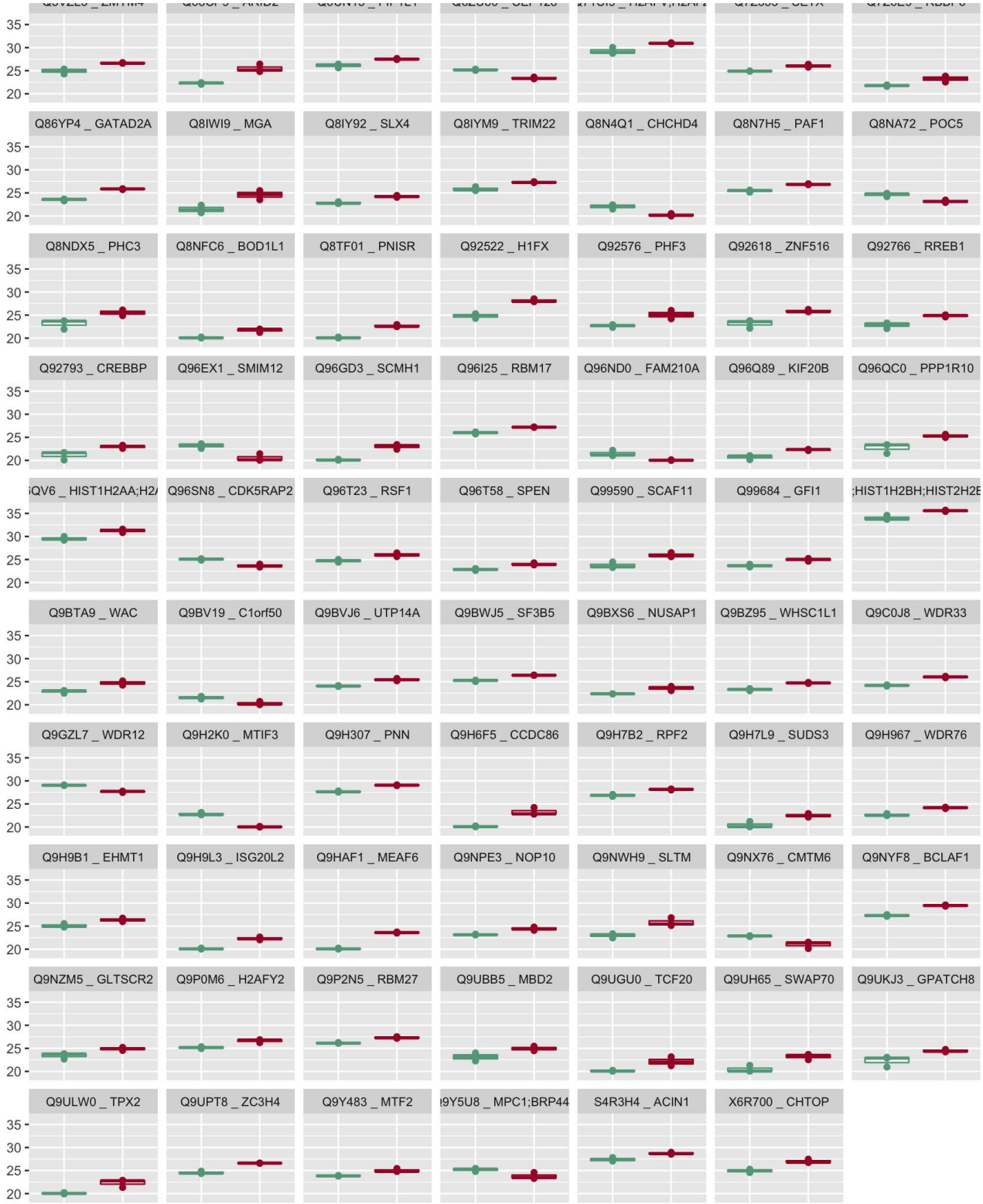
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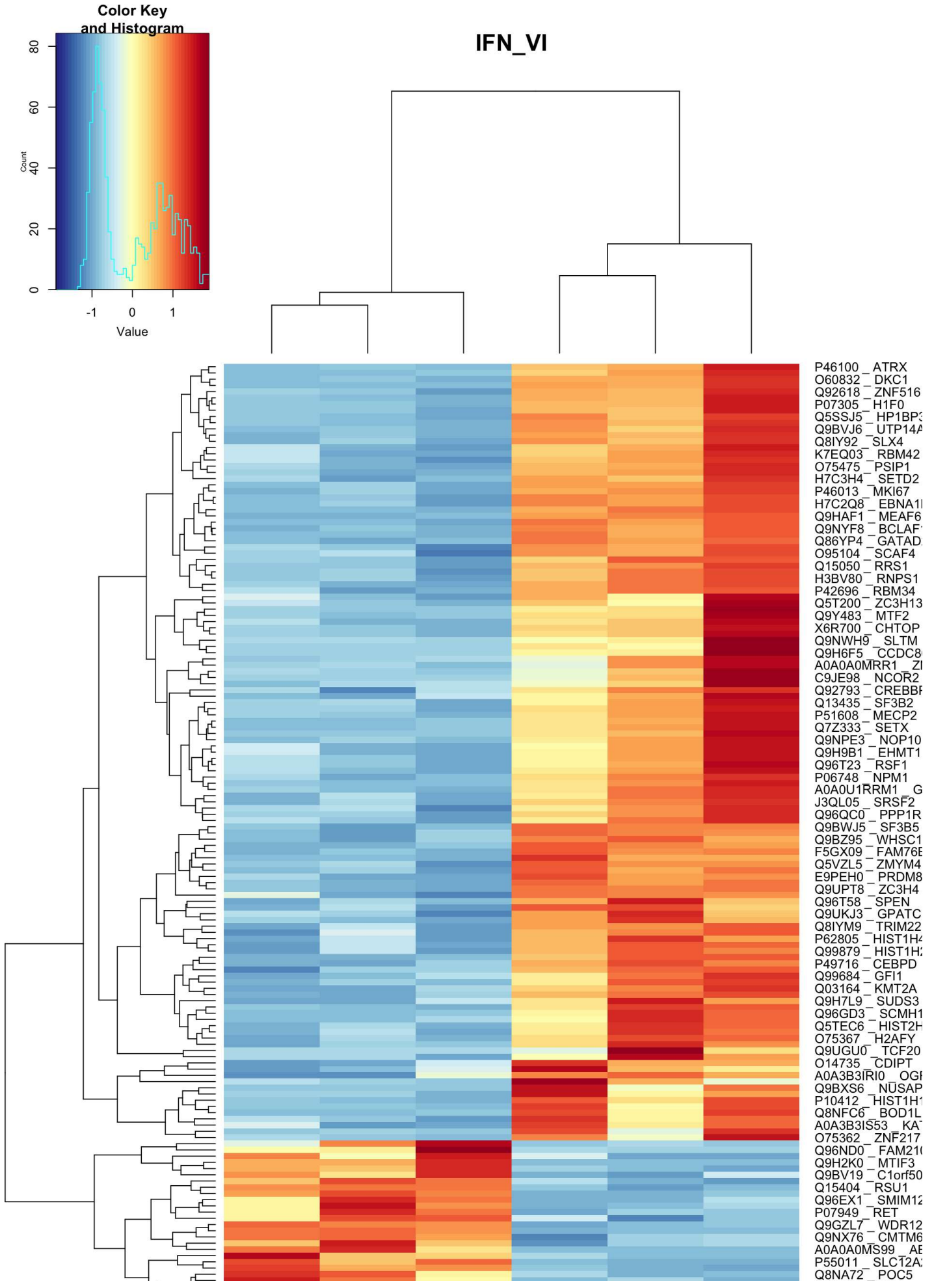
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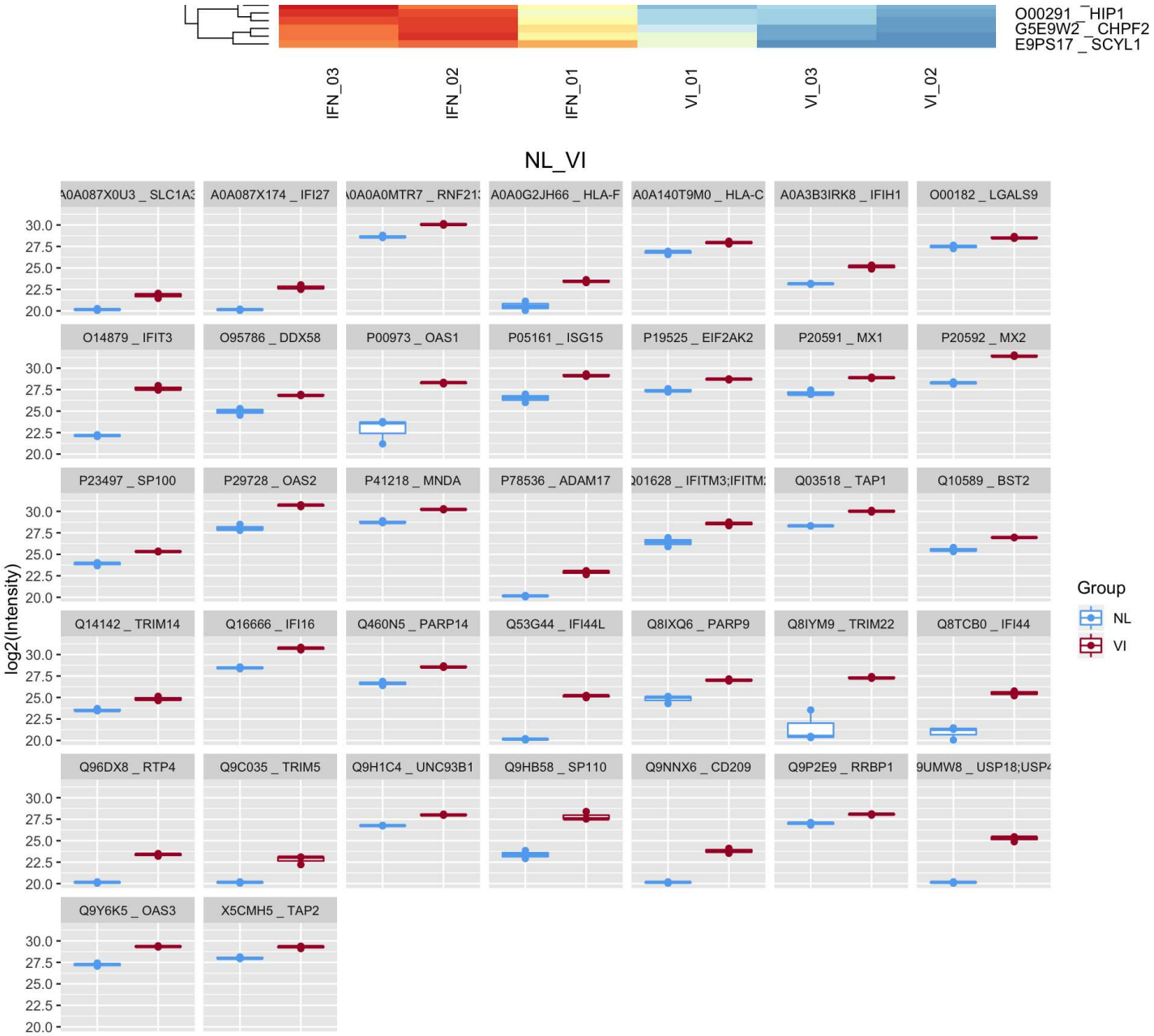
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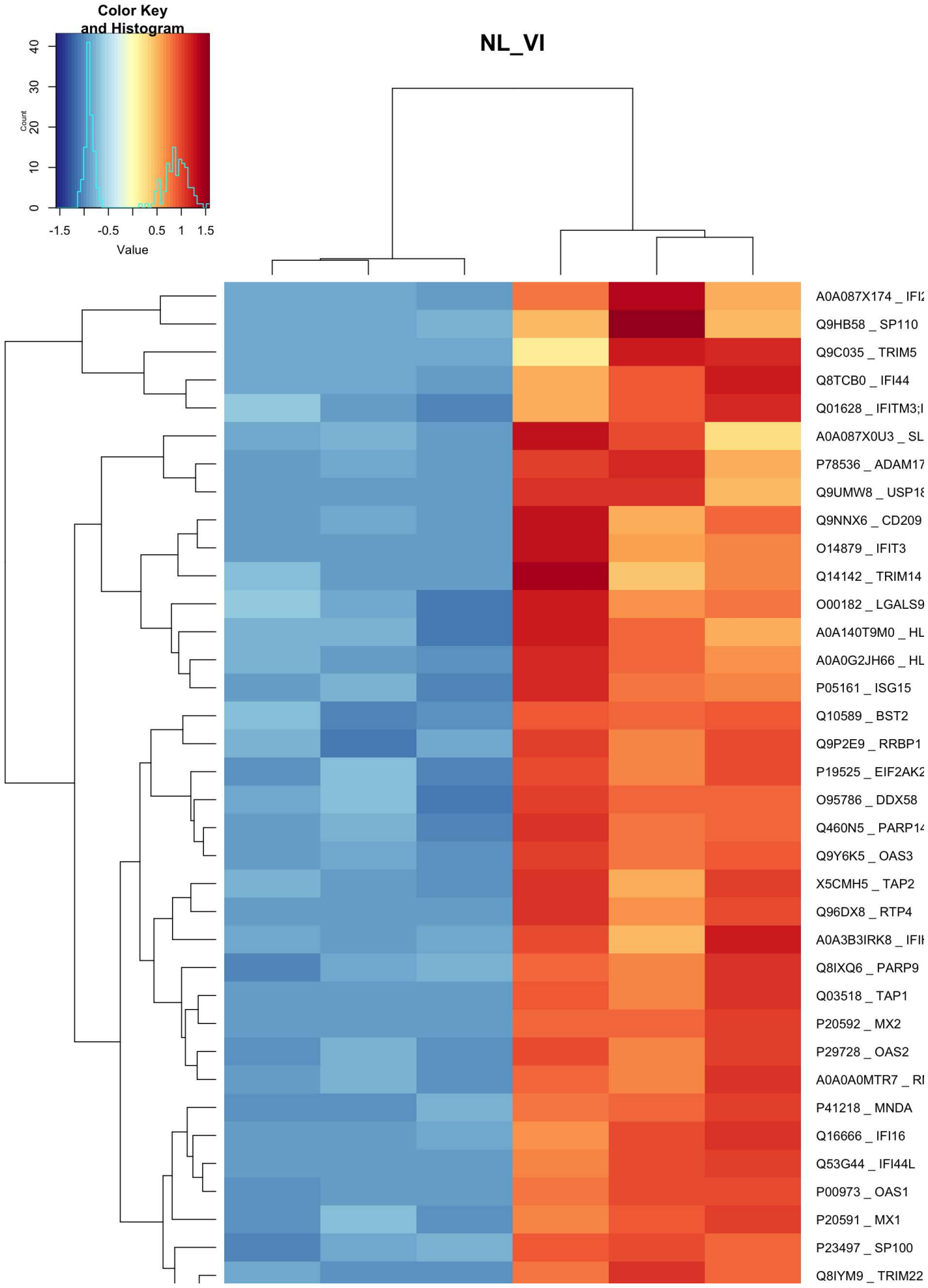
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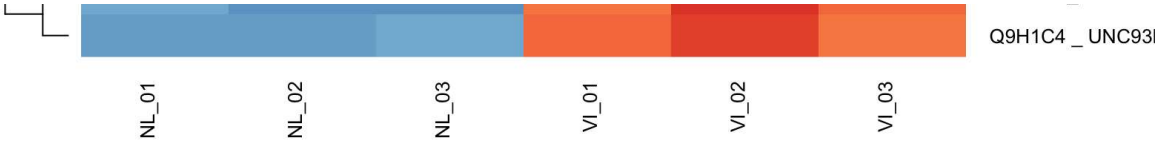
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