Systems level analysis of histone H3 post-translational modifications reveals features of PTM crosstalk in chromatin regulation

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Running title: Conserved crosstalk patterns of post-translational histone modifications

Supplementary figure captions

Supplementary Figure 1: Left: Morphology of wild type and Dnmt TKO cells grown in 2i medium. Right: Western blots of Dnmt1, Dnmt3a and Dnmt3b for wild type and Dnmt TKO cells grown in 2i medium and serum.

Supplementary Figure 2: Extracted ion chromatogram of histone H3 polypeptides in the four analyzed conditions. (A) Comparison of four representative runs of the four model systems investigated. The chromatogram was extracted using diagnostic fragment ions of the peptide H3, which were found to be always present in MS/MS spectra of the N-terminal tail independently from the modified form. (B) Three replicates of Suz12 knock out and Ring1A/B knock out runs, showing high reproducibility between technical replicates.

Supplementary Figure 3: Comparison of co-frequencies of 30 most abundant binary marks sorted for WT co-frequencies.

Supplementary Figure 4: Gene ontology (GO slim terms) and KEGG pathway enrichment for genes clustered by the fuzzy c-means algorithm (see Fig. 3).

Supplementary Figure 5: Comparison of co-frequency and interplay value histograms for cell lines and randomized data sets. Medians of co-frequencies of the real data vary between 0.00098 and 0.0017 while the median for the randomized sets increase to 0.0064 and 0.0036. Distribution of interplay values becomes bi-modal after shuffling PTM codes.

Supplementary Figure 6: Correlation between co-frequency of ES datasets. Randomization of intensities does not lead to higher correlation.

Supplementary Figure 7: For each PTM combination, interplay scores versus distance from the central residue are shown (thin lines). The thick line denotes mean values taken over all interplay scores at a distance. The panels show general trends within PTM types, such as positive crosstalk between nearby arginine and lysine methylations.

Supplementary Figure 8: Summarized crosstalk within PTM types arginine methylation and lysine methylation and acetylation on histone H3.3. For each PTM, interplay scores versus distance from its residue are shown (thin lines). The thick line denotes mean values taken over all interplay scores at a distance. The panels show general trends within PTM types, such as positive crosstalk within nearby mono-methylations and acetylations.

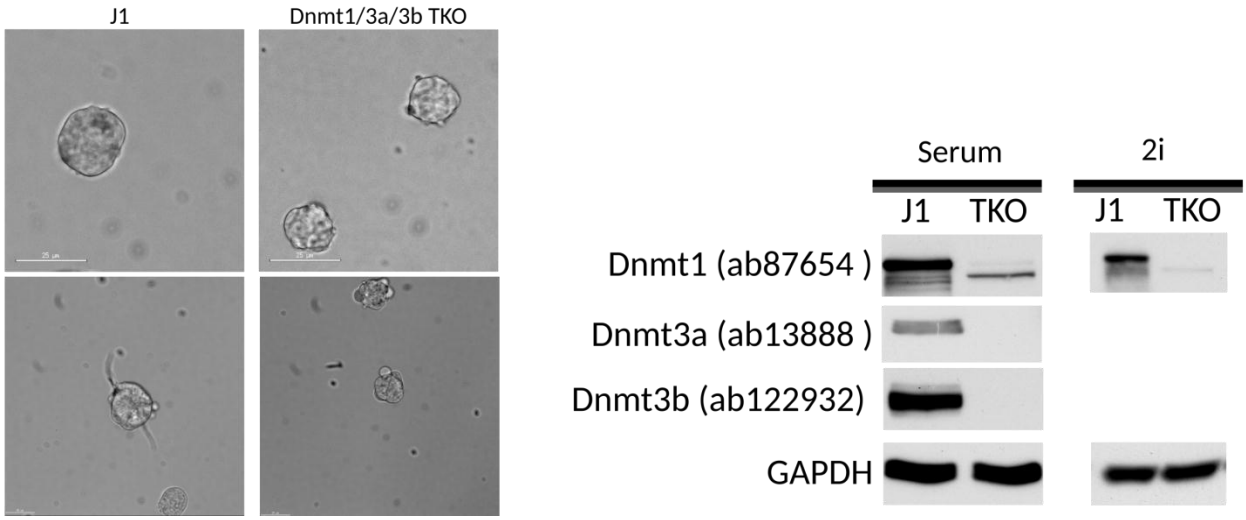
Supplementary Figure 9: Distribution of interplay values of the PTM for Suz12-/-, Dnmt TKO and Ring1A/B-/- cells. Red and green colors denote negative and positive interplay values. Darker colors correspond to interplay values that are conserved in the 4 cell lines. R2me1 and R8 methylations show highly negative crosstalk.

Supplementary Figure 10-18: Word clouds and distribution plots for selected PTMs.

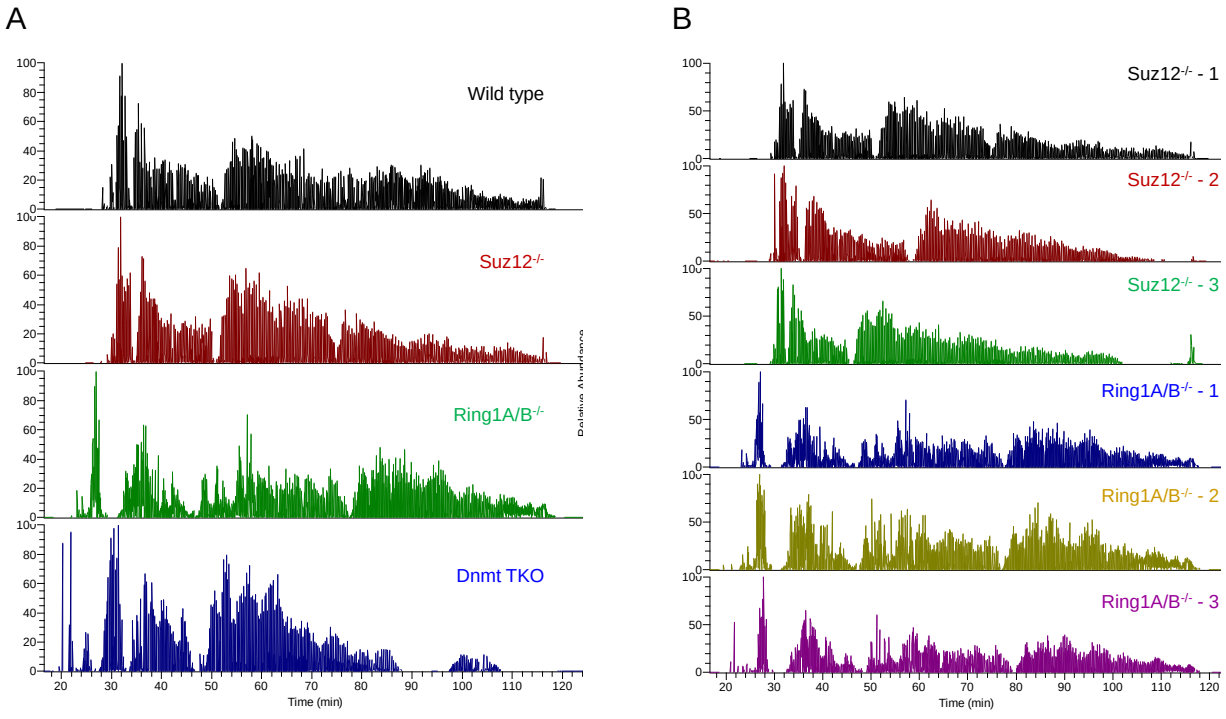
Supplementary Figure 19: Transcriptome analysis of SAM cycle. The top panel displays the extracted KEGG pathway of production and consumption of SAM through, e.g., DNA methyltransferases (although also lysine methyltransferases adopt the same precursor, not displayed in the KEGG pathway). In bold, enzymes that were present in the transcriptomics dataset. Lower panel: Clustering analysis of mRNA expression levels of selected enzymes involved in SAM production and recycling. Brighter colors indicate relative higher expression as compared to the other cell lines. No consistent trends were identified, even though the data suggests that Dnmt TKO and Ring1A/B$^{-/-}$ cell lines have on average higher expression of the enzymes of the SAM pathway.

Supplementary Table 1: list of MS results from the middle-down analysis of histone H3 N-terminal tails. The table is divided into four sheets; (i) 'Combinatorial PTMs' represents the different peptides identified and quantified from the MS analysis (average of all technical replicates). The relative quantification of the various modified peptides is estimated considering the total sum as 100%. (ii) 'Single modifications' is the deconvoluted relative abundance of the individual PTMs. The value was obtained by summing all peptides in the column 'Combinatorial codes' containing the given mark. (iii) 'Binary PTMs' is the calculated relative abundance of binary marks, quantified using the same approach as the single marks. (iv) 'Interplays' is the interplay score between binary marks, calculated as described in the main text. This value represents the interdependency of two PTMs, and it was used to estimate which marks are more likely to co-exist or be mutually exclusive.

Supplementary figure 1

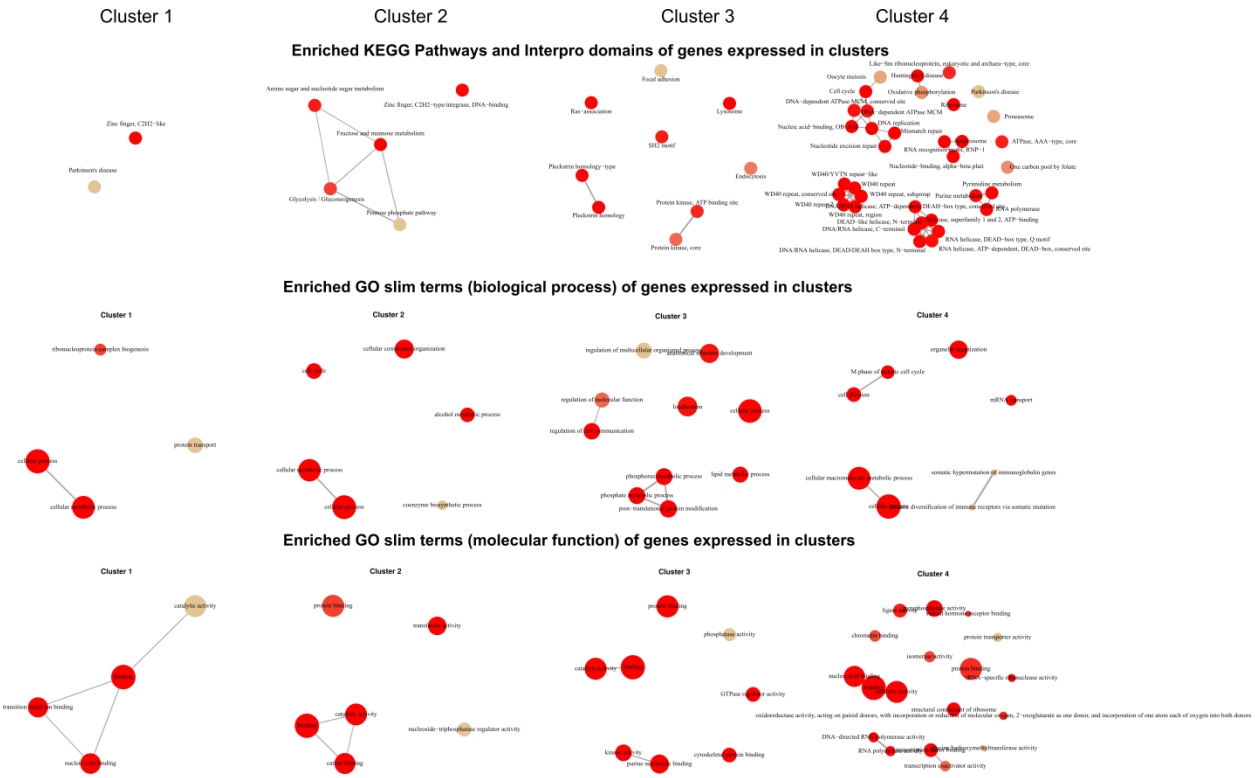


Supplementary figure 2



Supplementary figure 3

Supplementary figure 4



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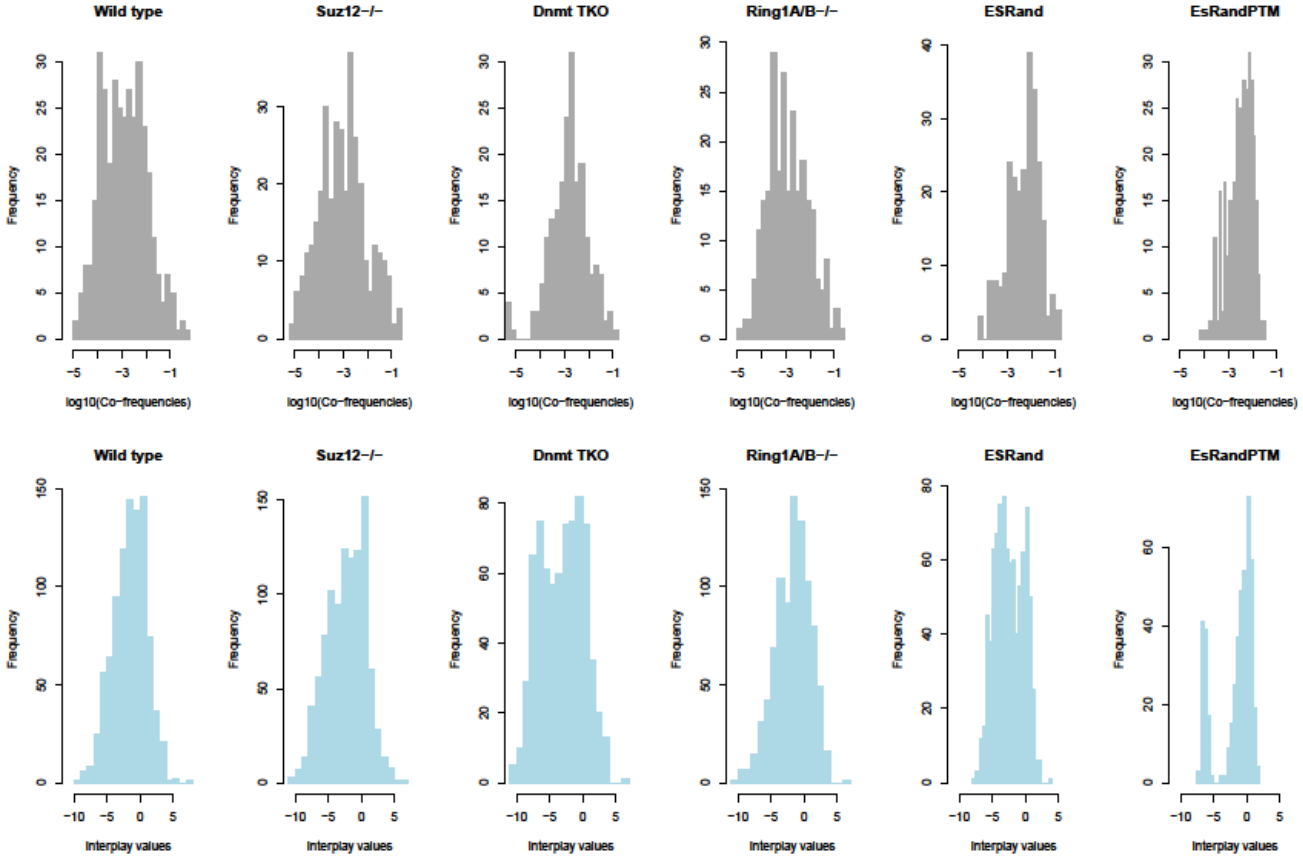

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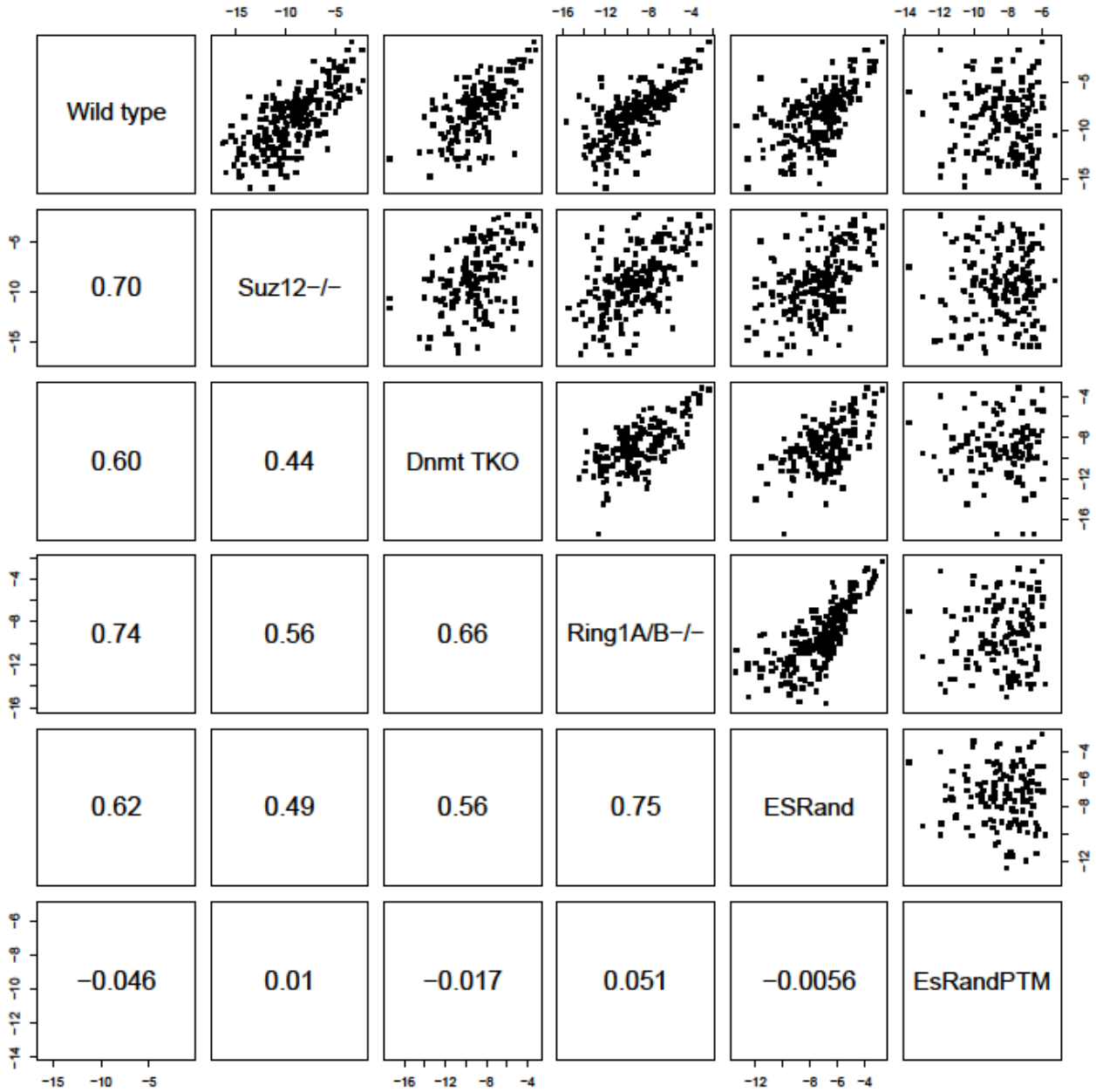

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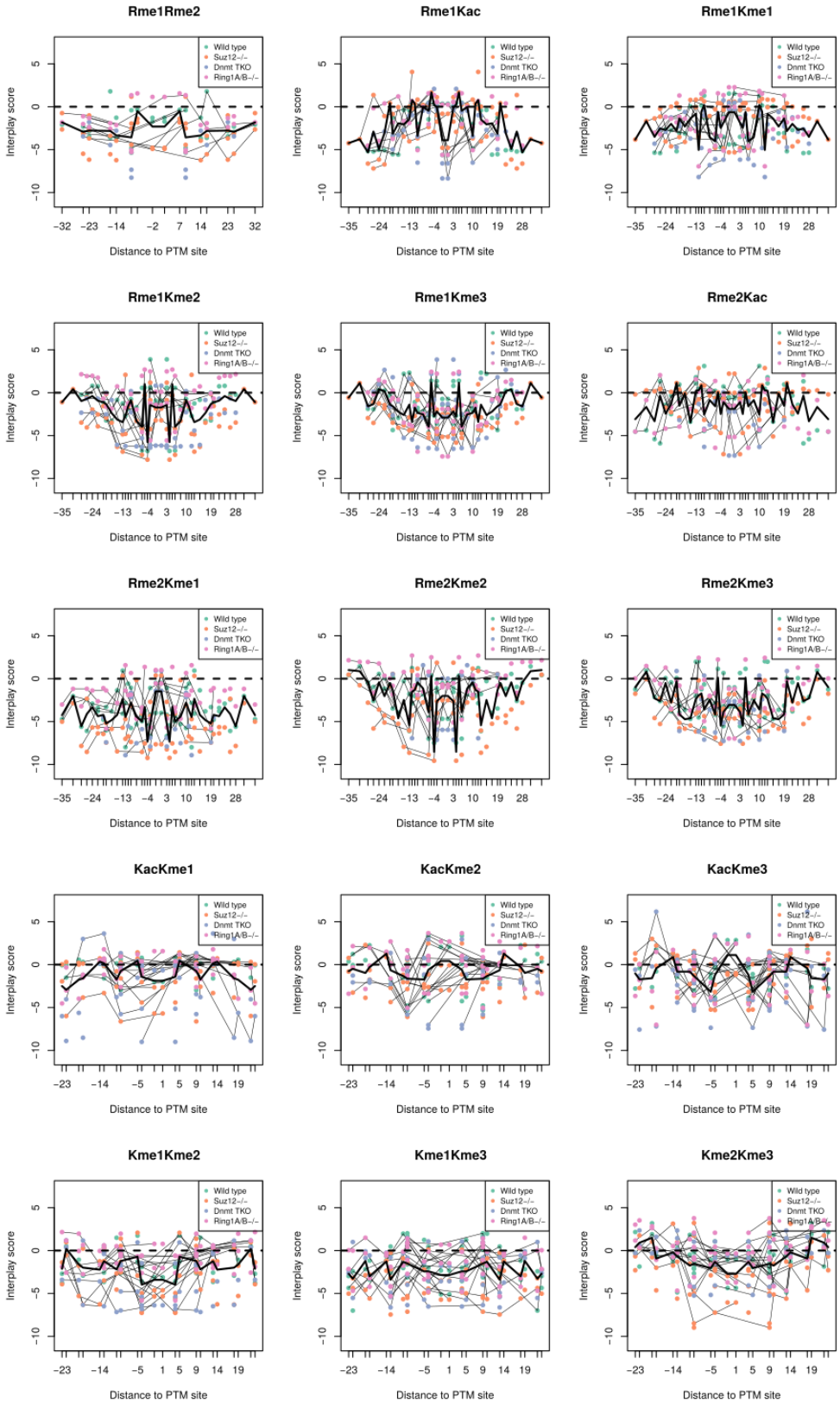

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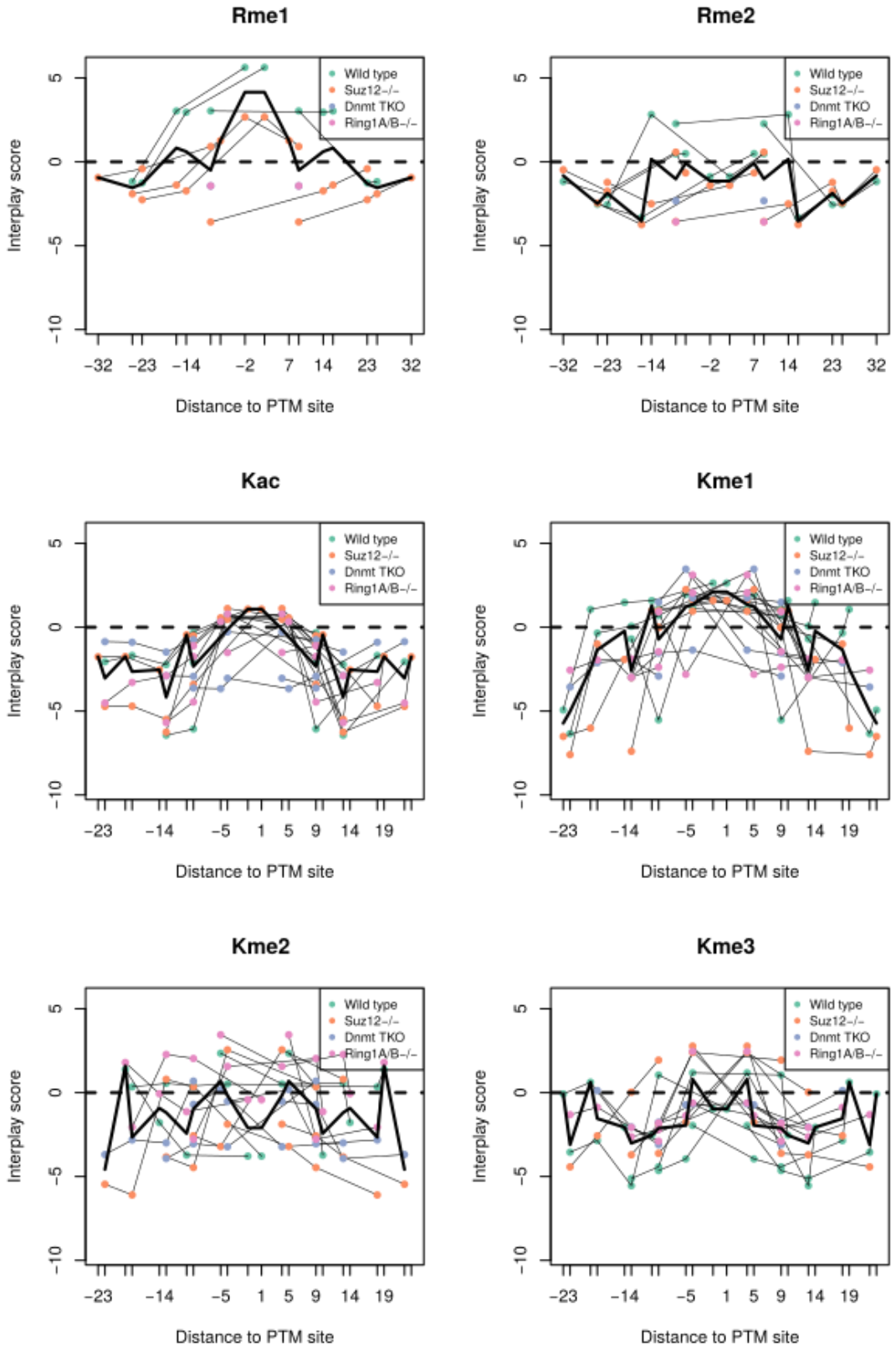

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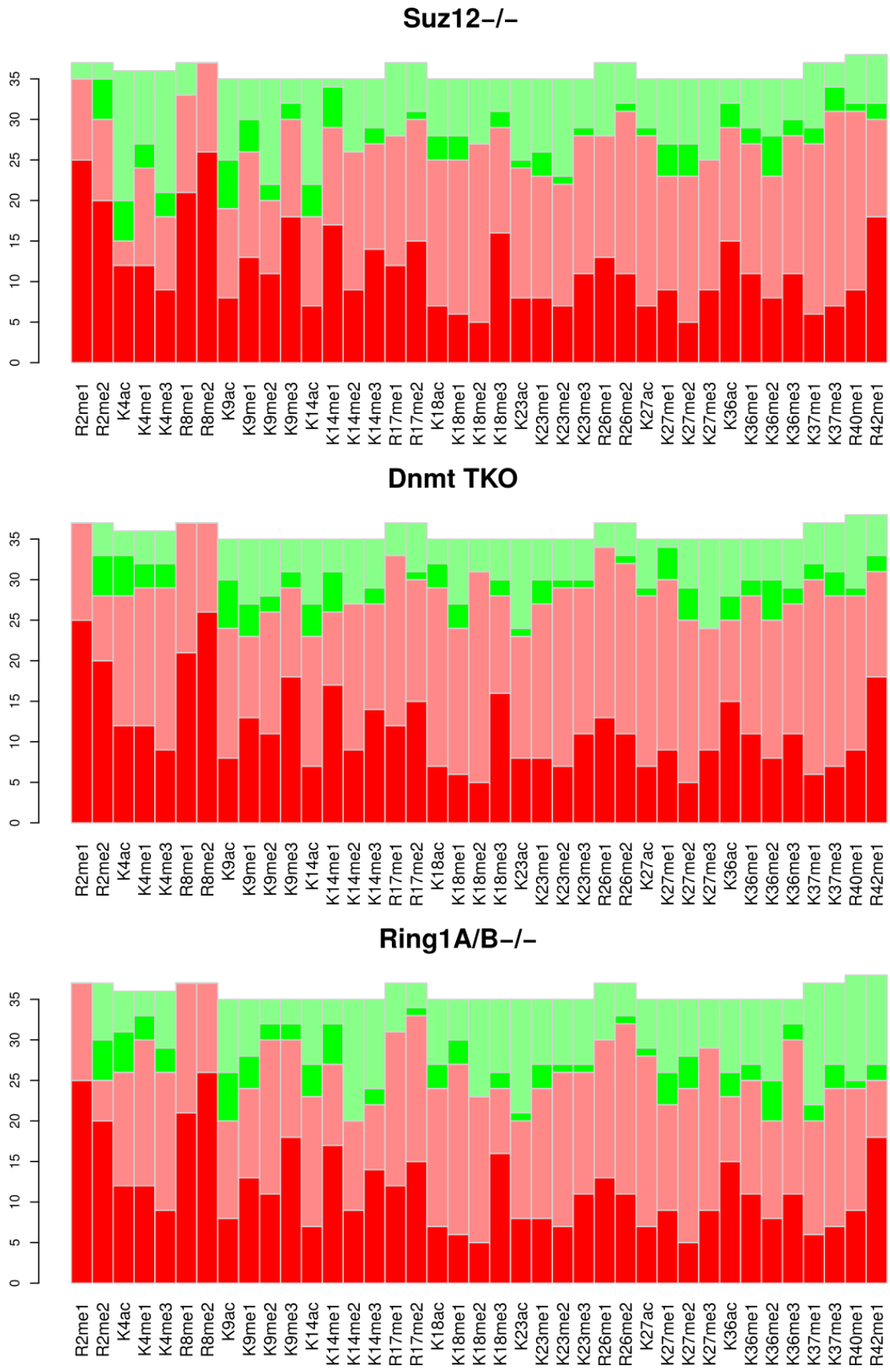

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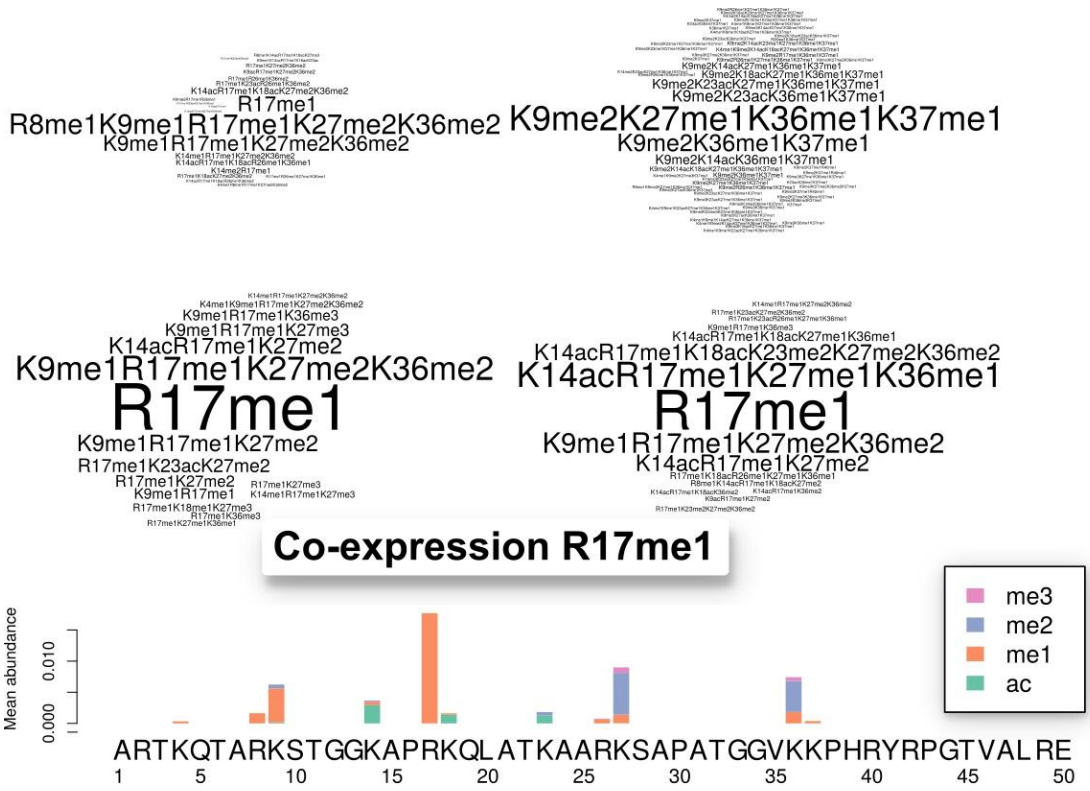

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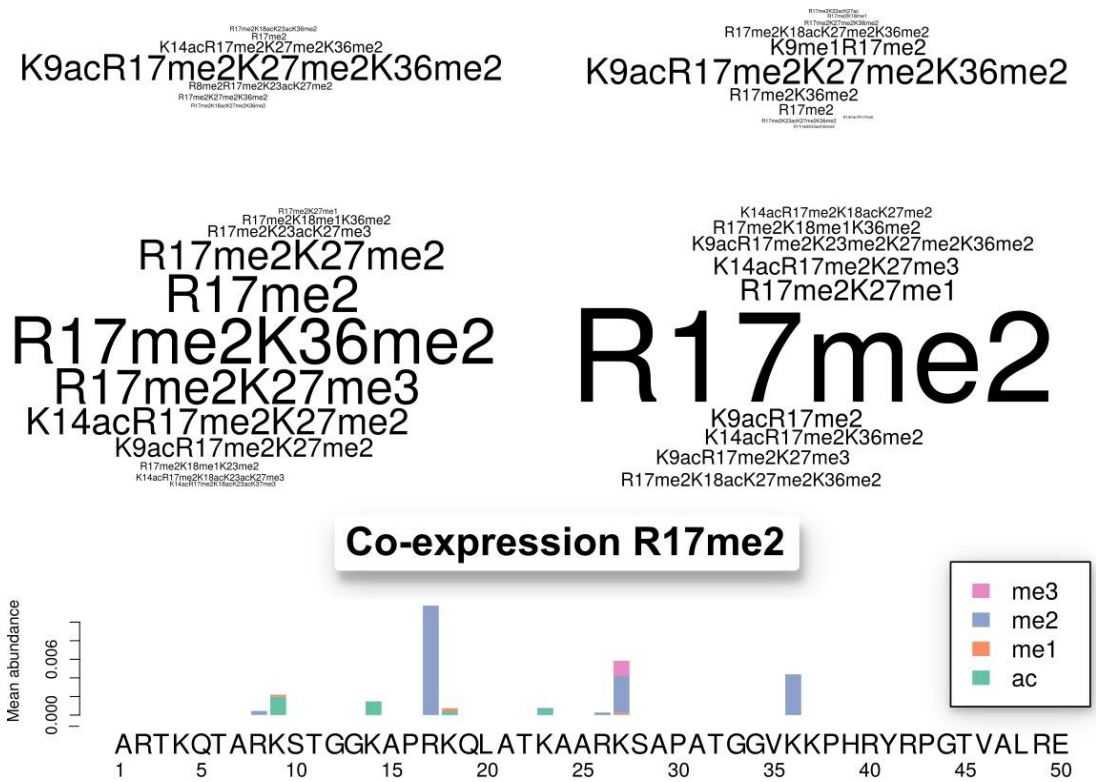

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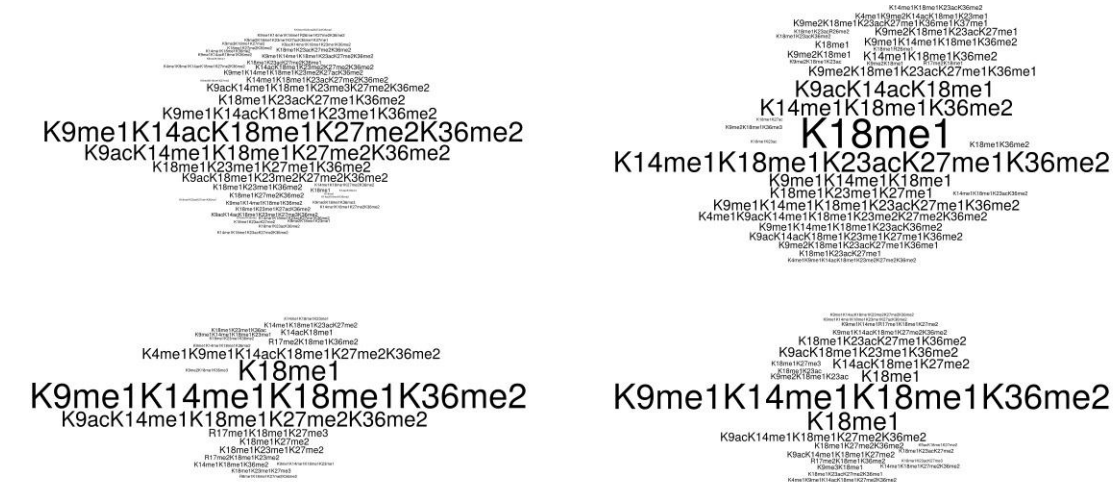

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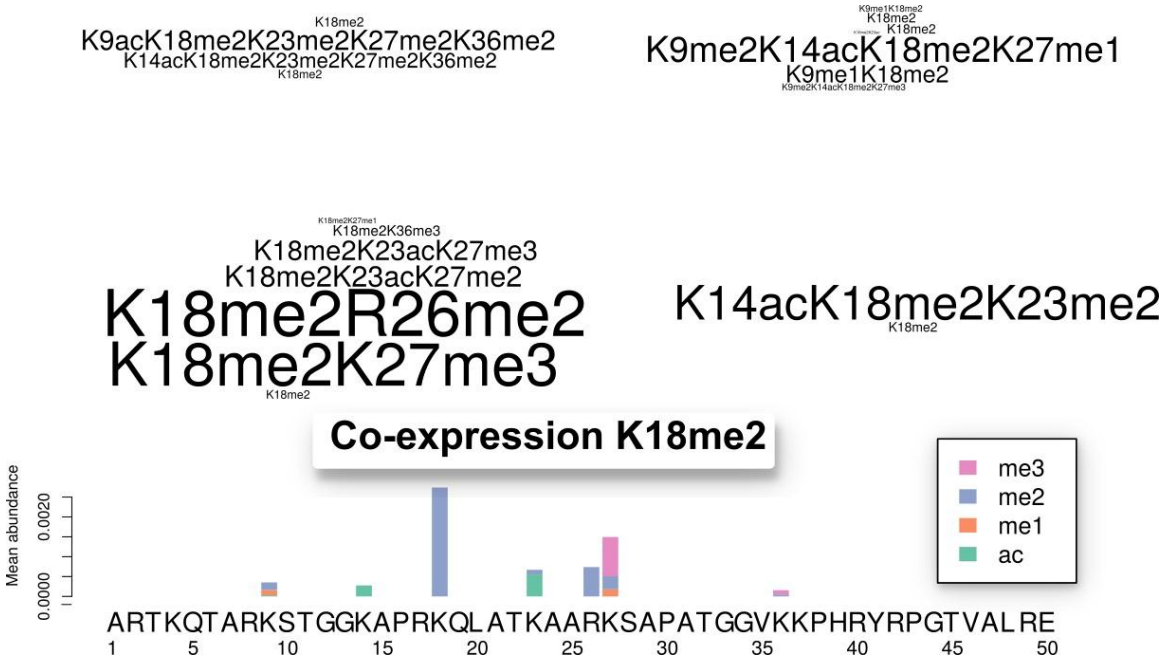

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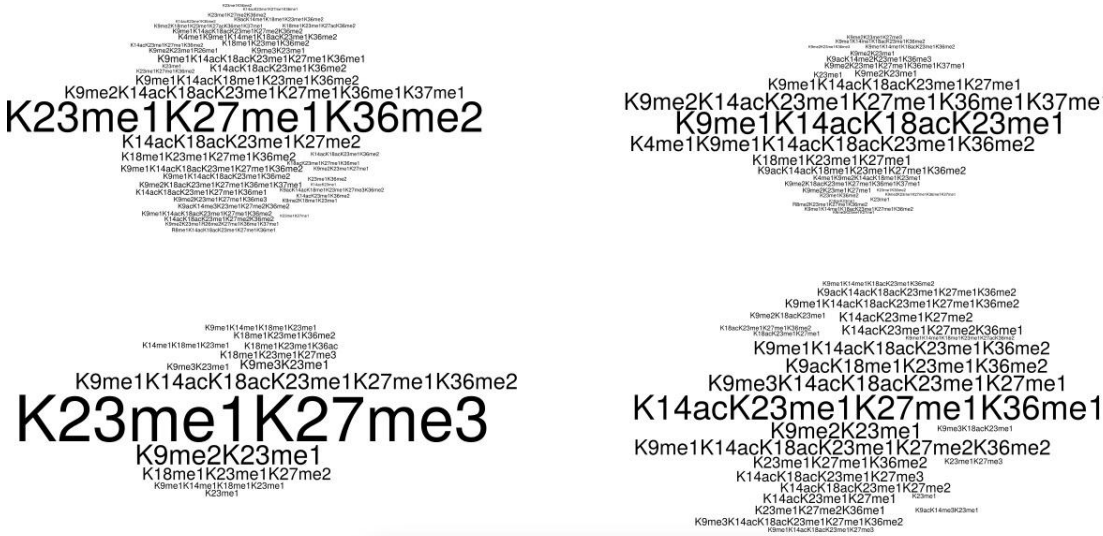
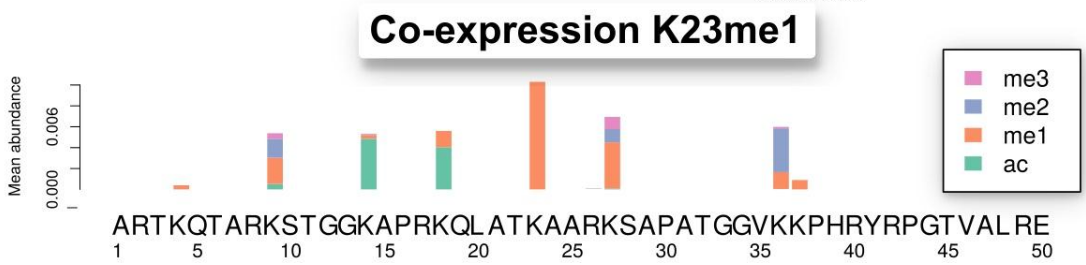
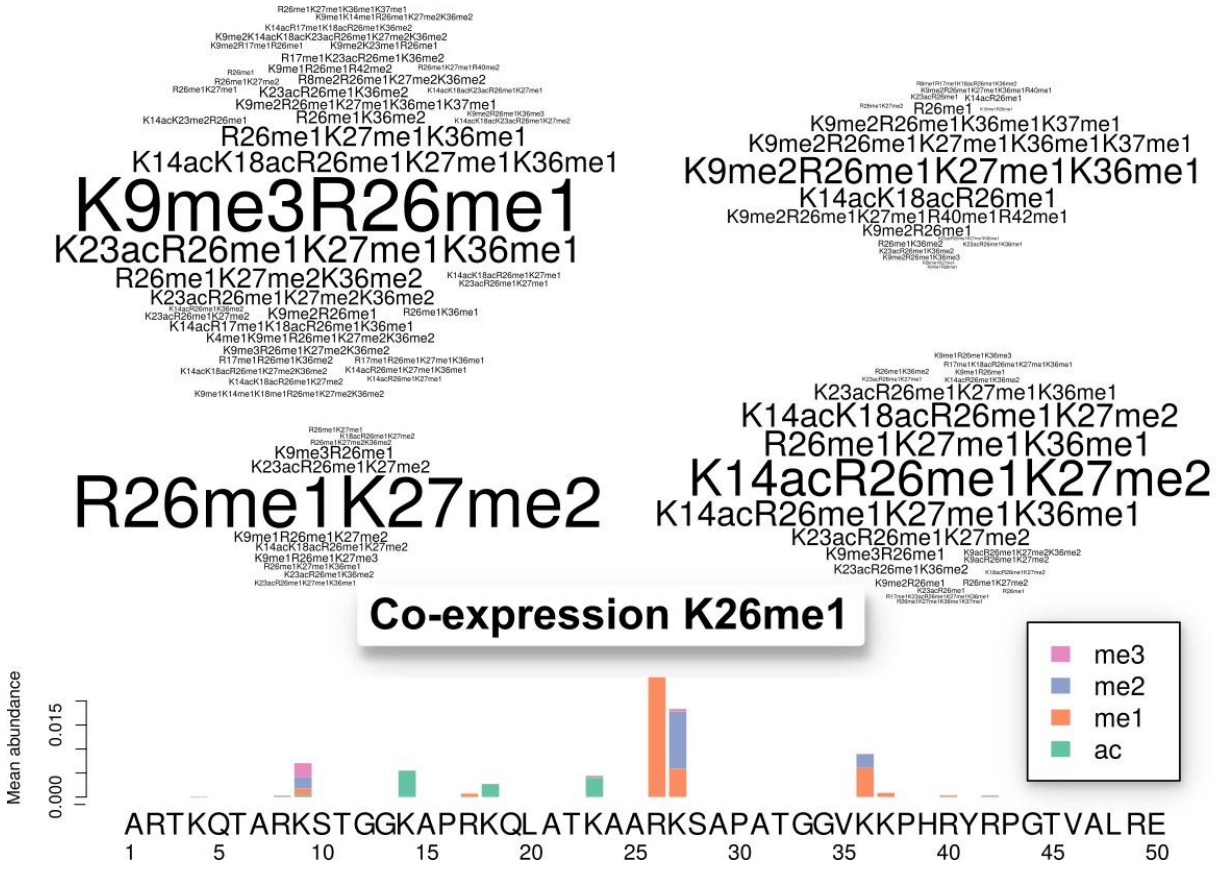




Supplementary figure 15

Supplementary figure 16



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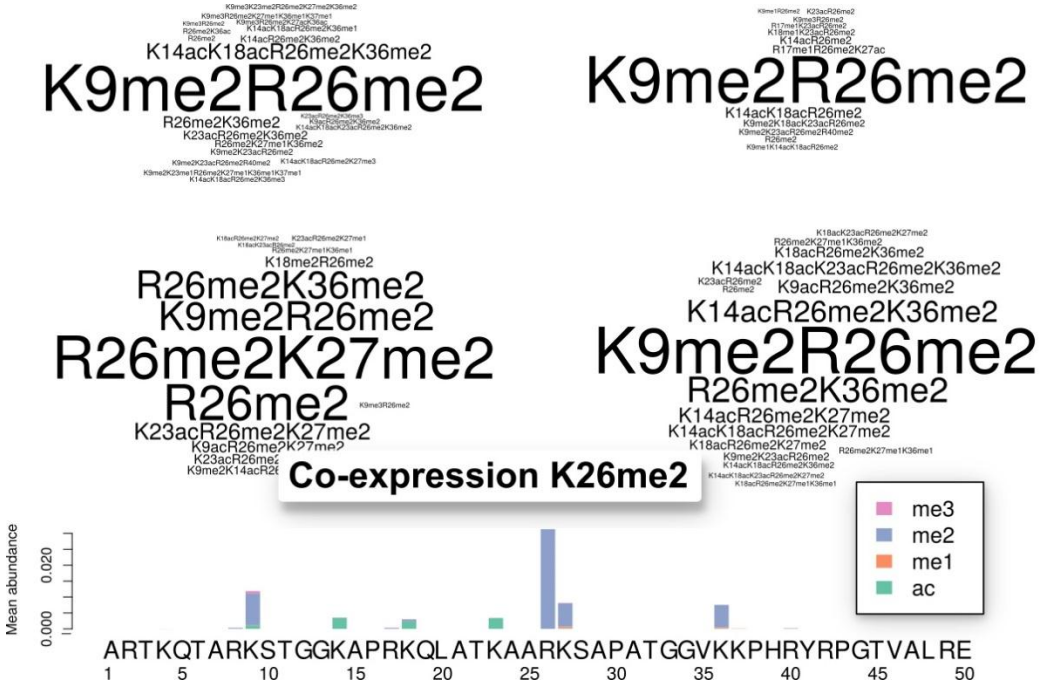

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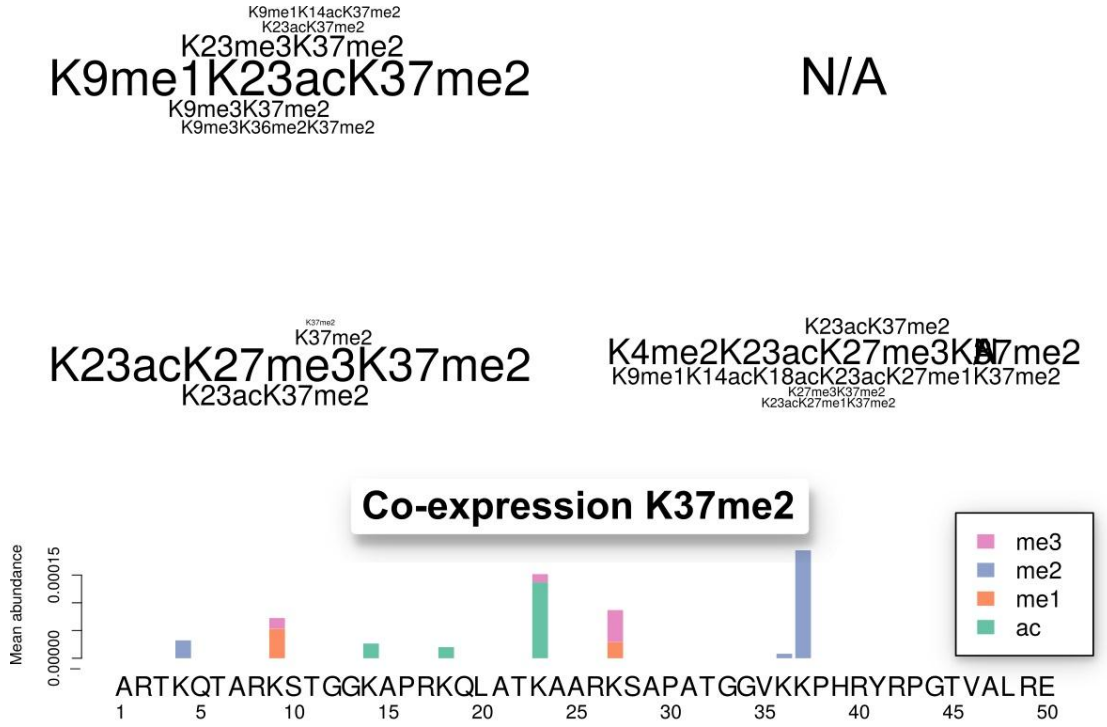

Supplementary figure 19