

Additional file 1

Supplementary figures and tables with legends

S1: Active chromatin marks overlap with repressed RTSS in all four cell lines.

S2: Chromatin profiles for all chromatin marks around the RTSS center position in GM12878, HeLaS3 and HepG2.

S3: Principal Component Analysis (PCA) of H3K3me2 profiles around expressed RTSS in GM12878 displays a continuum of cluster profiles, rather than discrete, separable sets of clusters.

S4: Average subprofiles for 12 chromatin marks in all cell lines. Note that the profiles of the repressive mark H3K27me3 in HepG2 are different from H3K27me3 in the other cell lines, show resemblance to active marks in HepG2. This observation was not confirmed when we downloaded a second dataset for H3K27me3 in HepG2 from ENCODE. We thus concluded that this dataset was probably suspect, and excluded it from the analysis.

S5: Heatmap of subprofile enrichment in each metacluster for all cell lines.

S6: Average subprofile over all RTSS in each metacluster for each chromatin mark in all cell lines.

S7: Metaclusters have different average RTSS expression level. A) Expression levels for metaclusters can be divided into high (green), intermediate (blue) and low (red). Expression levels displayed in the boxplot have been log₂ transformed. B) Metacluster size and average expression level of metaclusters are correlated. Color codes are for the different cell lines K562 (green), GM12878 (red), HeLaS3 (blue) and HepG2 (black).

S8: Number of unique GREAT terms for each metacluster differs in within- and between-cell comparisons. Generally metacluster contain more unique terms compared to metaclusters in the same cell type compared to metaclusters in other cell types. However, there are also considerable cluster-specific variations. A) Permissive GREAT analysis. B) Conservative GREAT analysis.

S9: PCA of metacluster terms from GREAT identifies groups with different functional associations. The two first components from a Principal Component Analysis (PCA) on the functional terms from GREAT for all clusters in all cell lines. Cell line abbreviations are K5 for K562, Gm for GM12878, He for HeLa-S3 and Hp for HepG2, and c1-c10 are cluster indexes as used previously. A) PCA plot for all cluster scores. Clusters with scores in the same quadrant of the PCA plot have related functional terms, and the distance between the two clusters corresponds to the degree of similarity. Clusters with scores close to origo (the crossing of the axes arrows) have few functional terms associated with them, while those with scores further away from origo are enriched for several terms. B) Interpretations of groupings in the PCA plot, with additional cluster properties also taken into consideration. Three main groups are apparent. The terms proximal, normal and distal refers to the localization of RTSS enrichment relative to genes as calculated by GREAT (Figure 6). The more distal clusters with low or intermediate RTSS expression show the strongest functional associations. Displayed PCA plot are from the conservative GREAT analysis.

S10: See Table caption

S11: Average pooled profile of all active chromatin marks for expressed RTSS and the selected subset of poised RTSS in all cell lines.

S12: Average Pol II profile enrichment around expressed RTSS, the subset of selected poised RTSS and all repressed RTSS in all cell lines.

S13: Description if IFN α and IFN γ stimulation

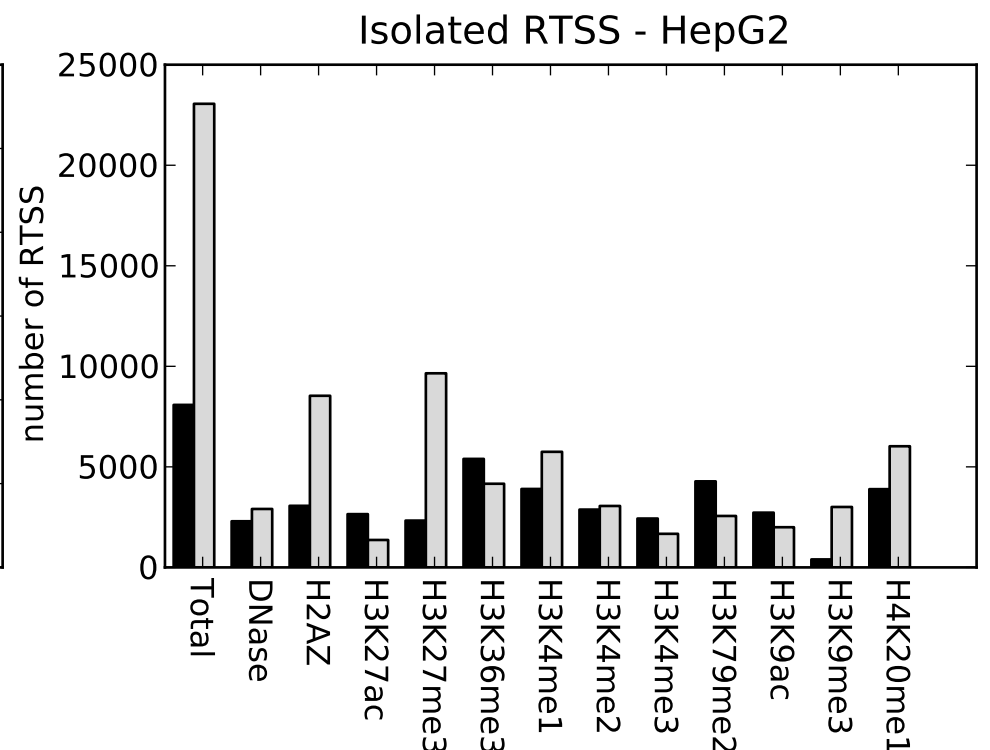
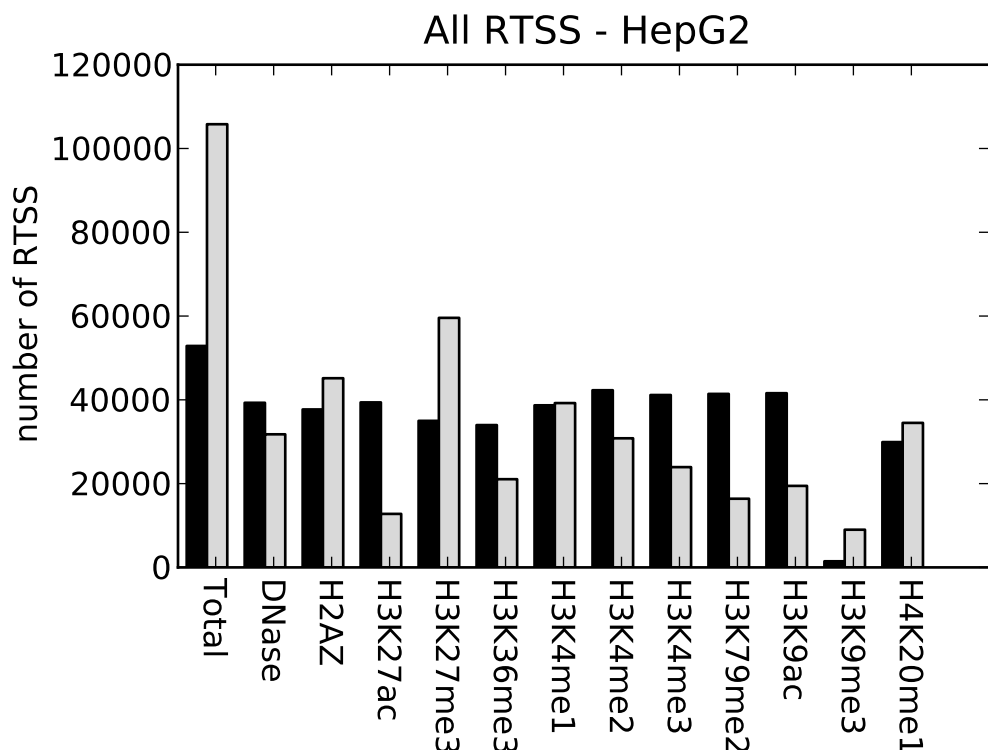
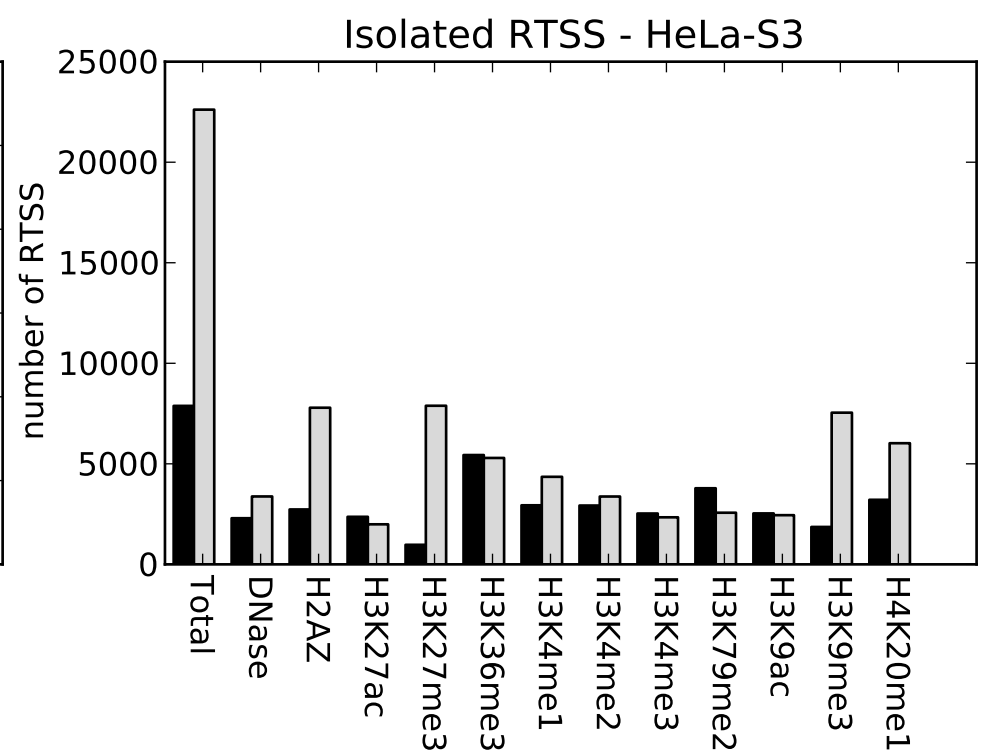
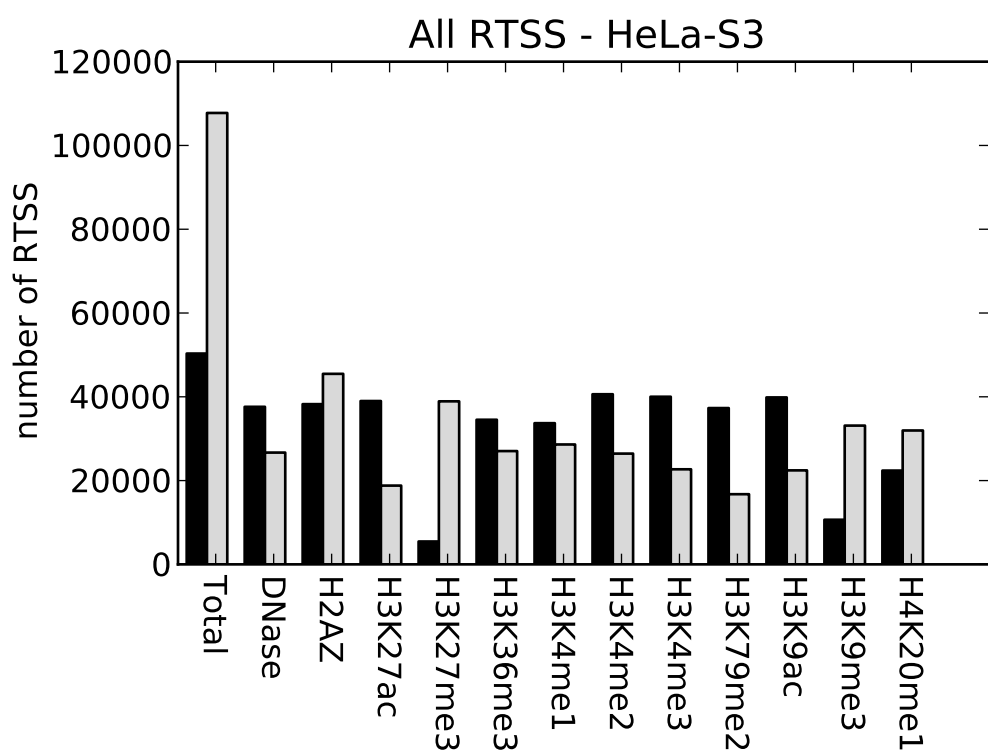
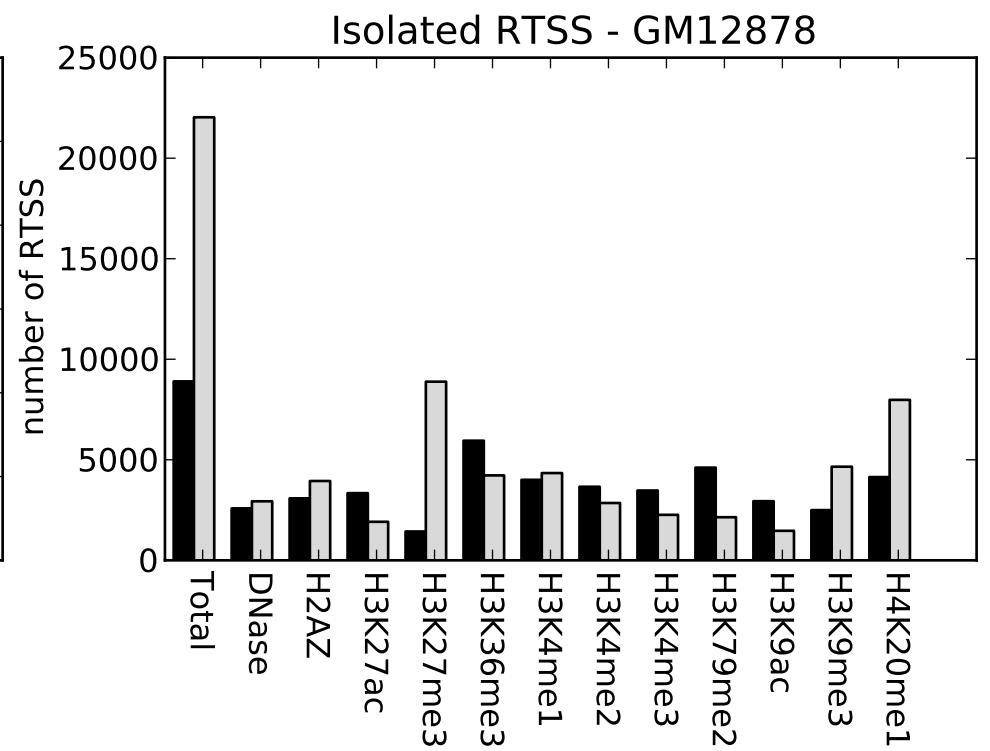
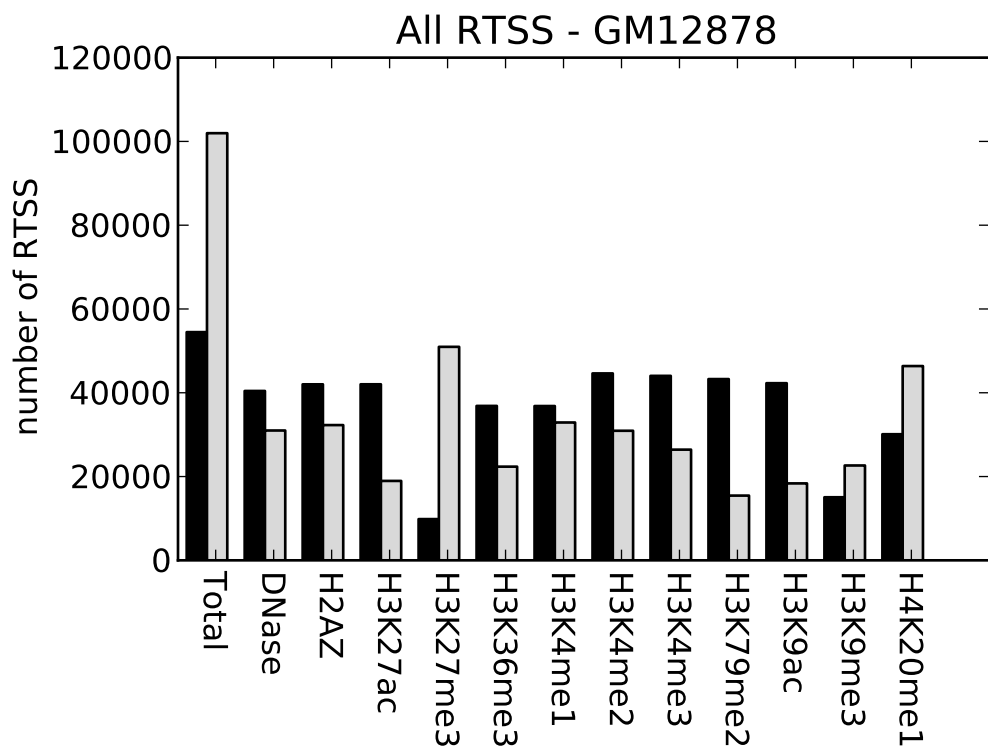
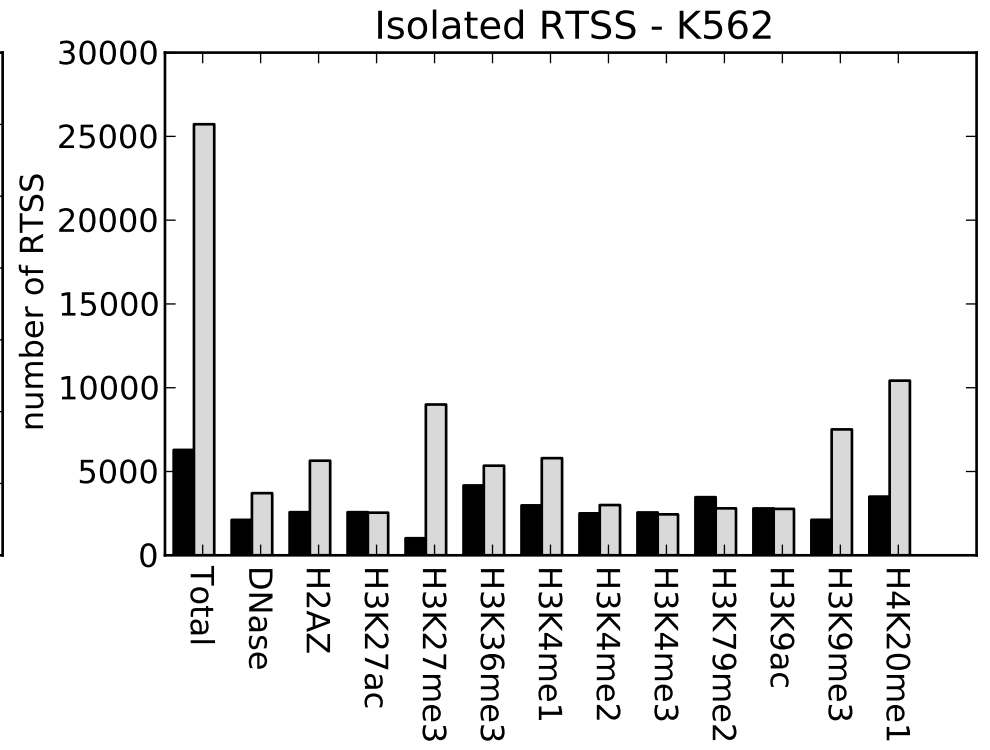
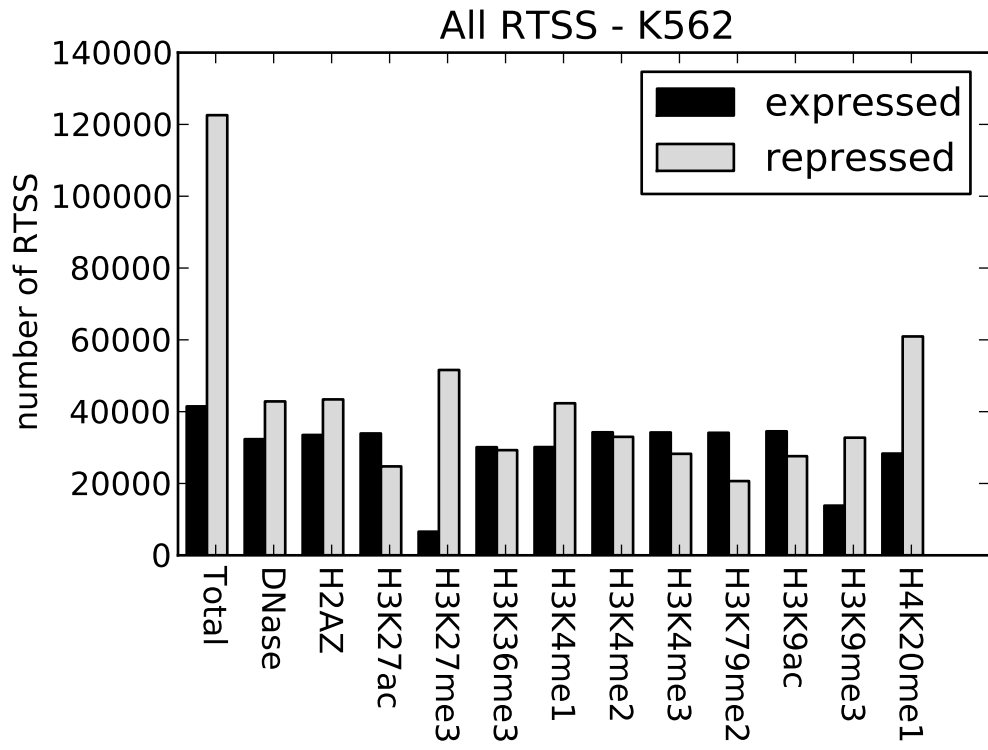
S14: General Pol II levels do not change at poised RTSS in K562 after stimulation with IFN α and IFN γ .

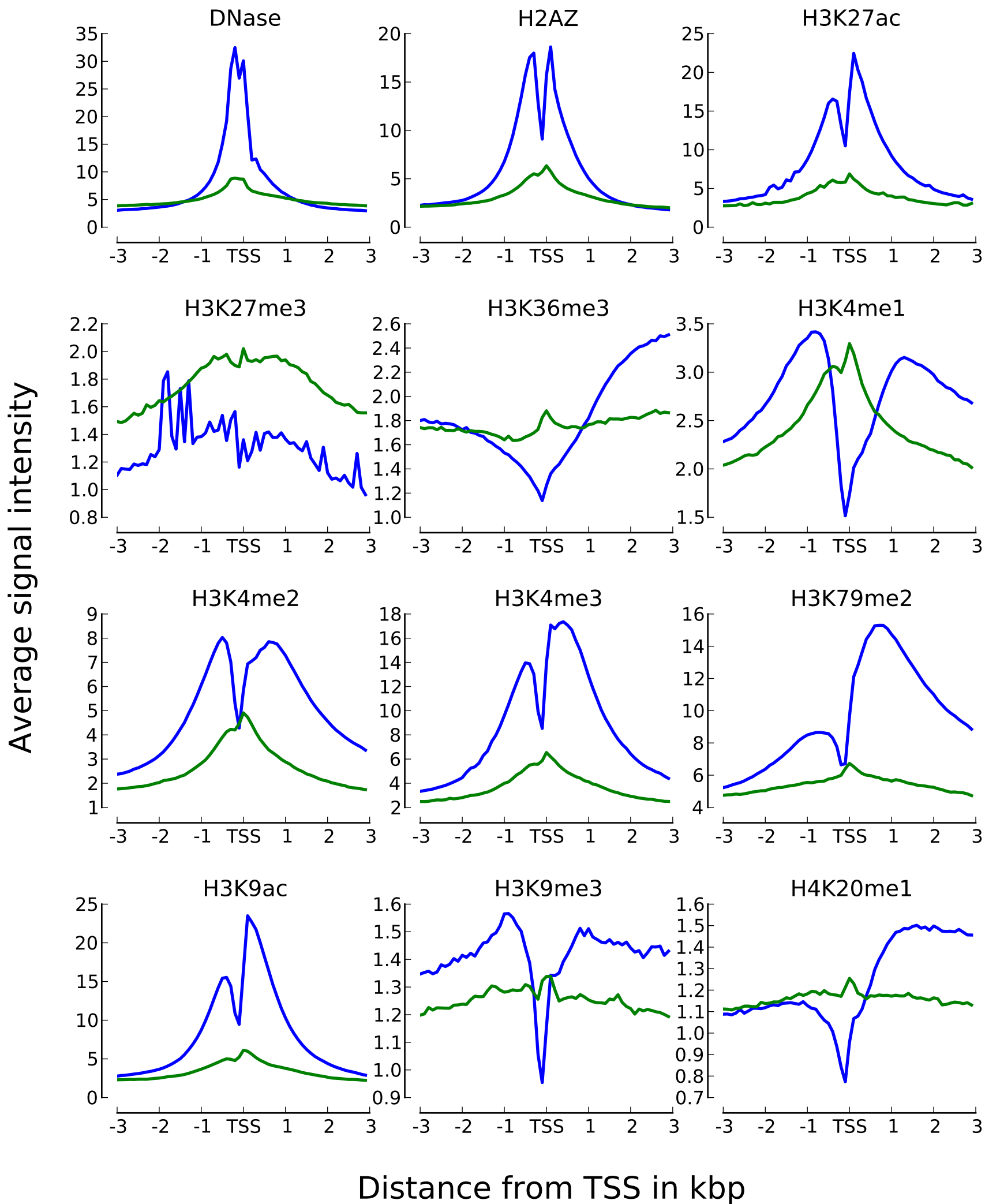
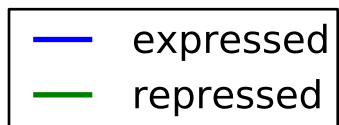
S15: RTSS with increased Pol II signal 30 hours after stimulation by IFN α and IFN γ are depleted for active chromatin marks in the unstimulated state, while some enrichment of active marks are observed at unstimulated RTSS with increased Pol II signal 6 hours after stimulation by IFN γ , but not by IFN α .

S16: Repressed genes associated with poised RTSS by GREAT are enriched for active chromatin marks compared to generally repressed genes, but less enriched than the poised RTSS. The curves are combined averages over RTSS and genes in all four cell lines.

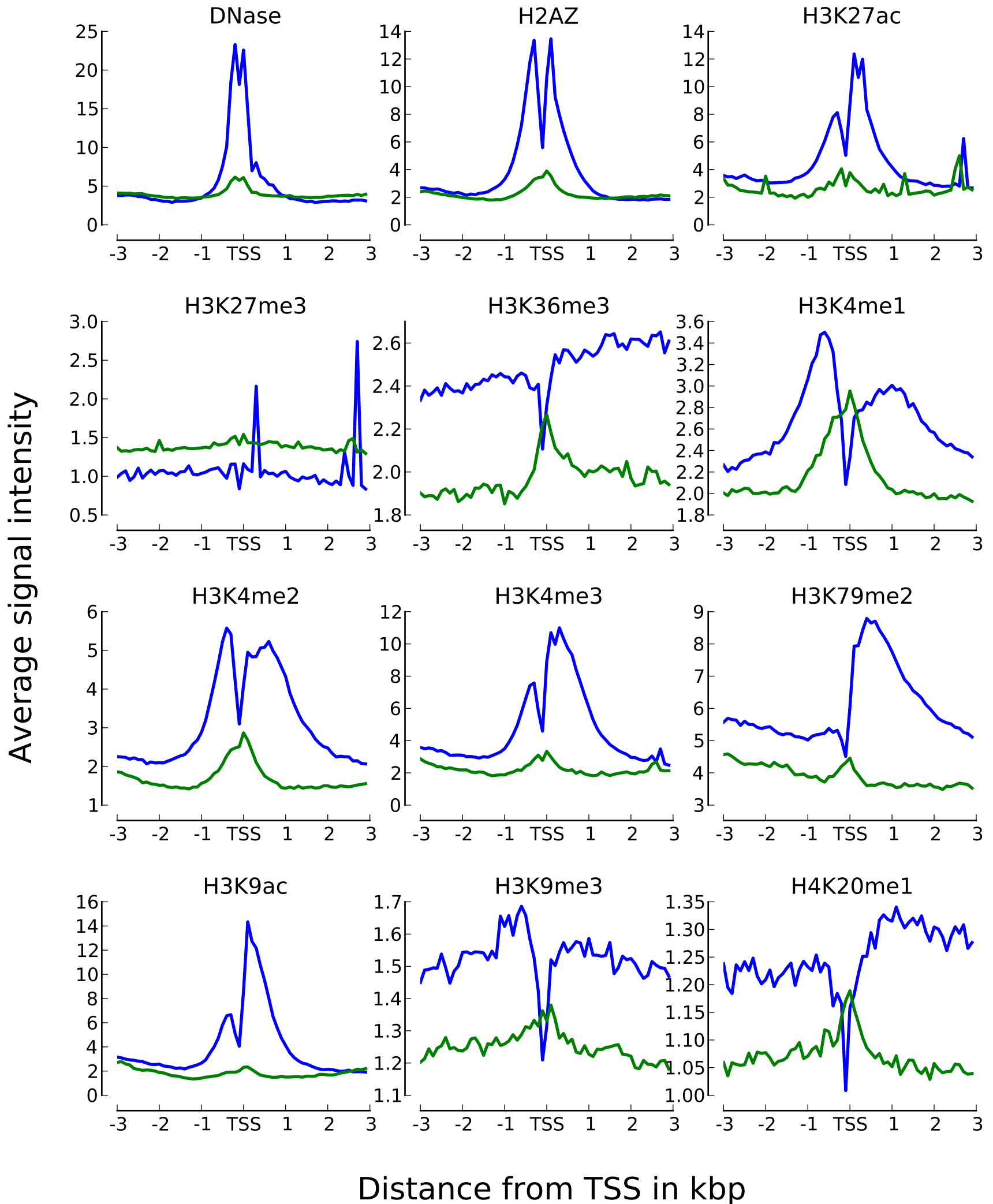
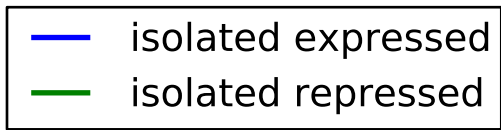
S17: Overview of all datasets from ENCODE downloaded and used for the analysis.

S18: P-values calculated for the overlaps in Figure 1 and S1 using the Genomic Hyperbrowser. Sampling method is either *uniform*, indicating a random shuffling of all RTSS regions within the chromosome arms, or *interseg* which also preserves the distance distribution between RTSS regions in the randomisations.

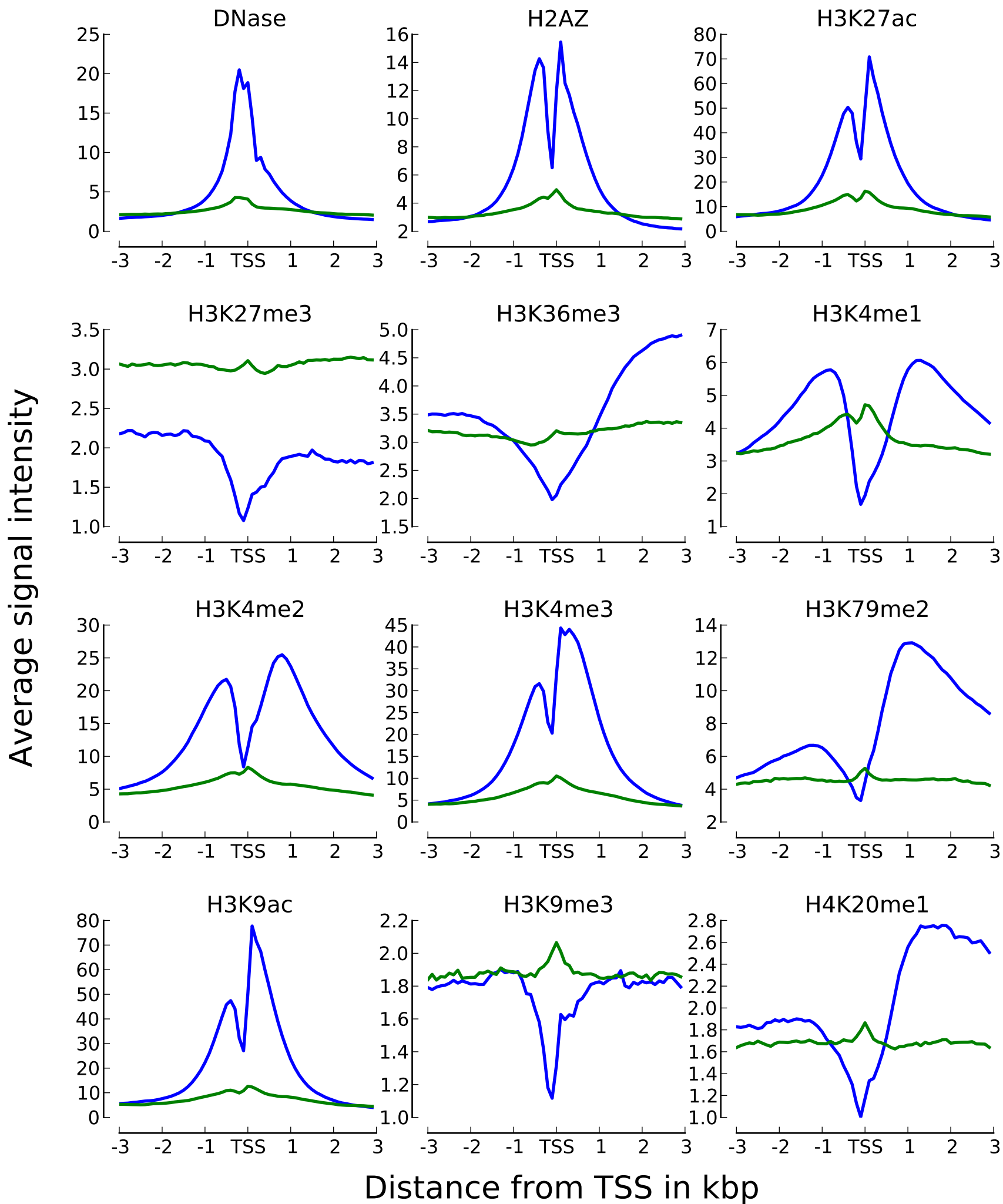
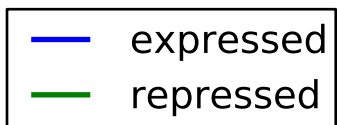




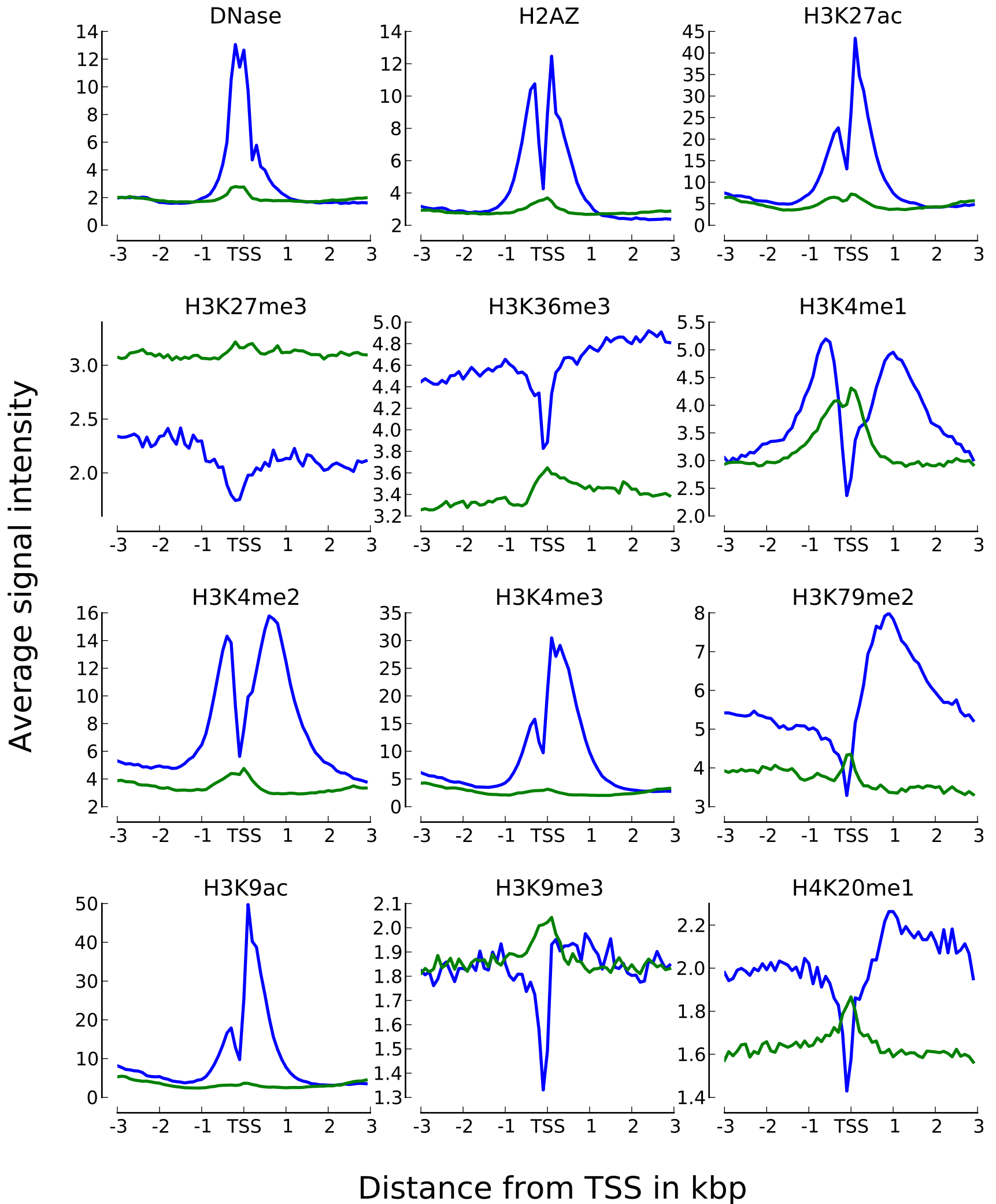
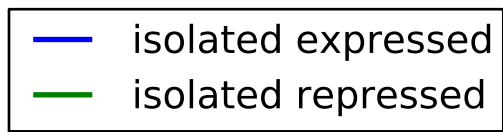
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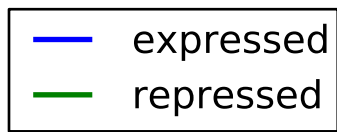


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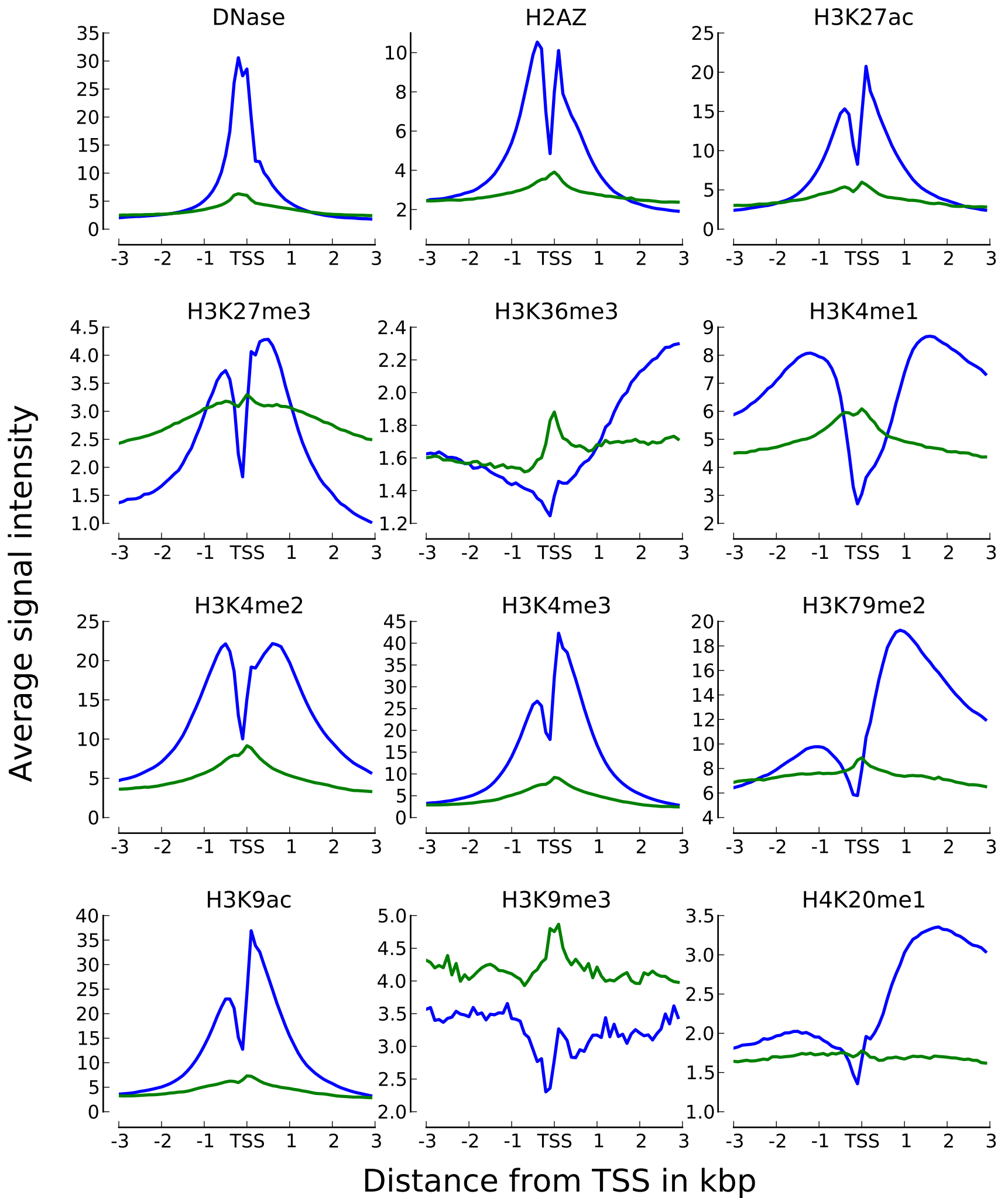


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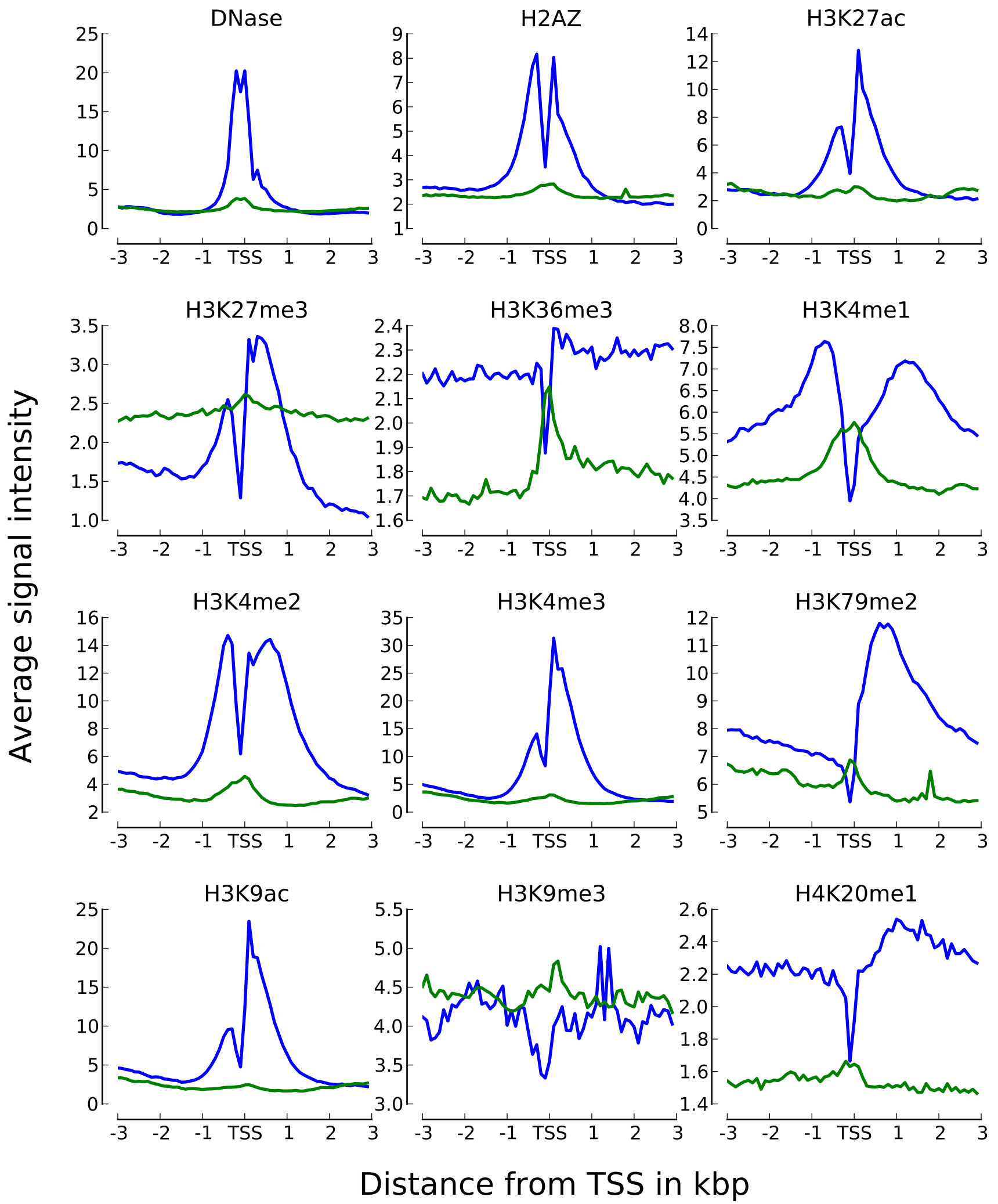
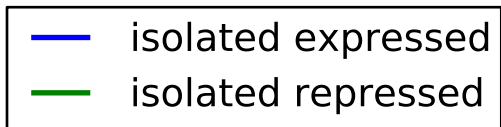




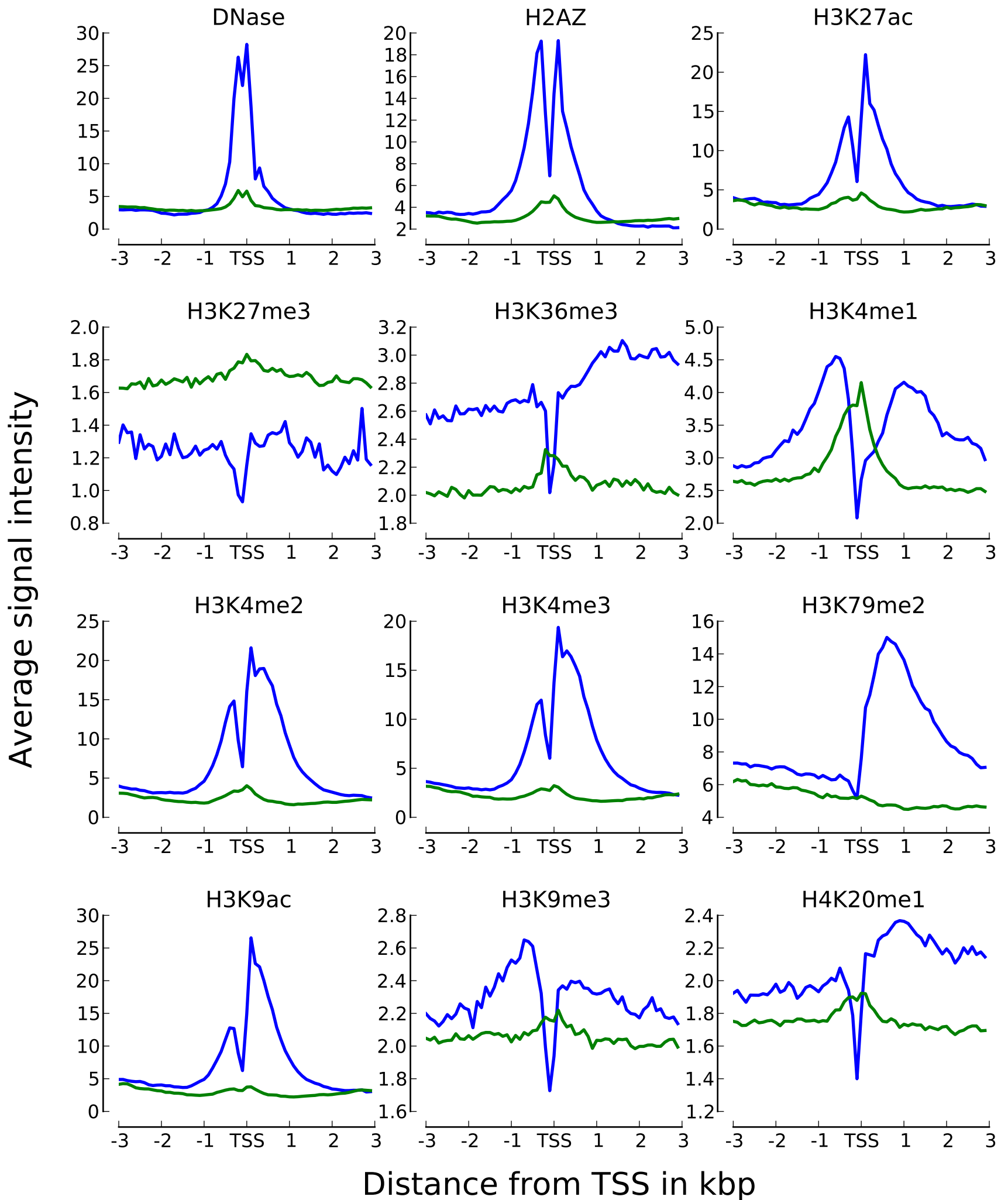
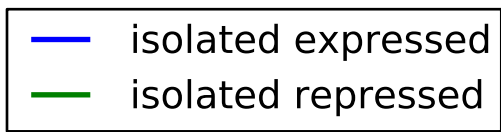
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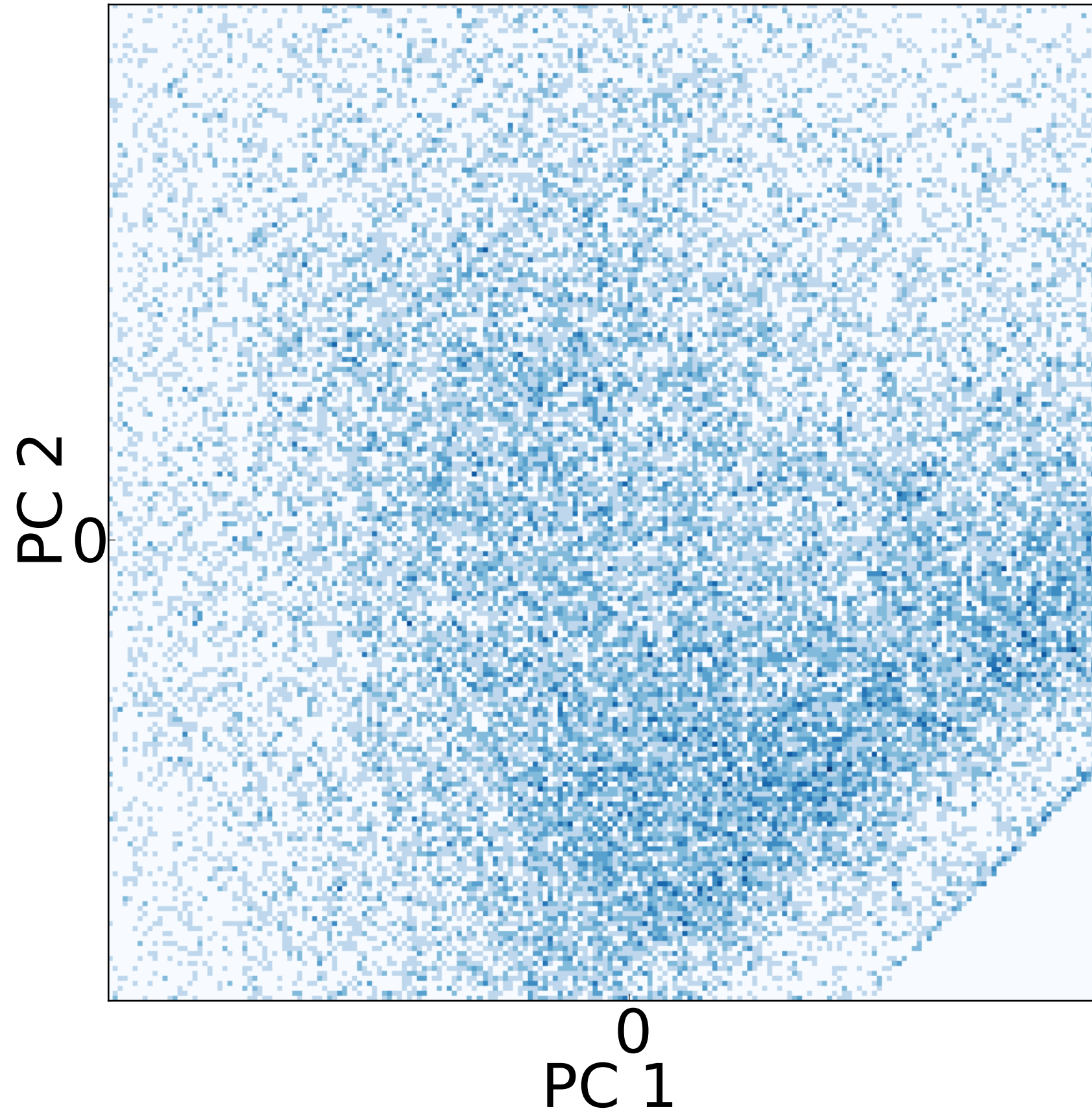
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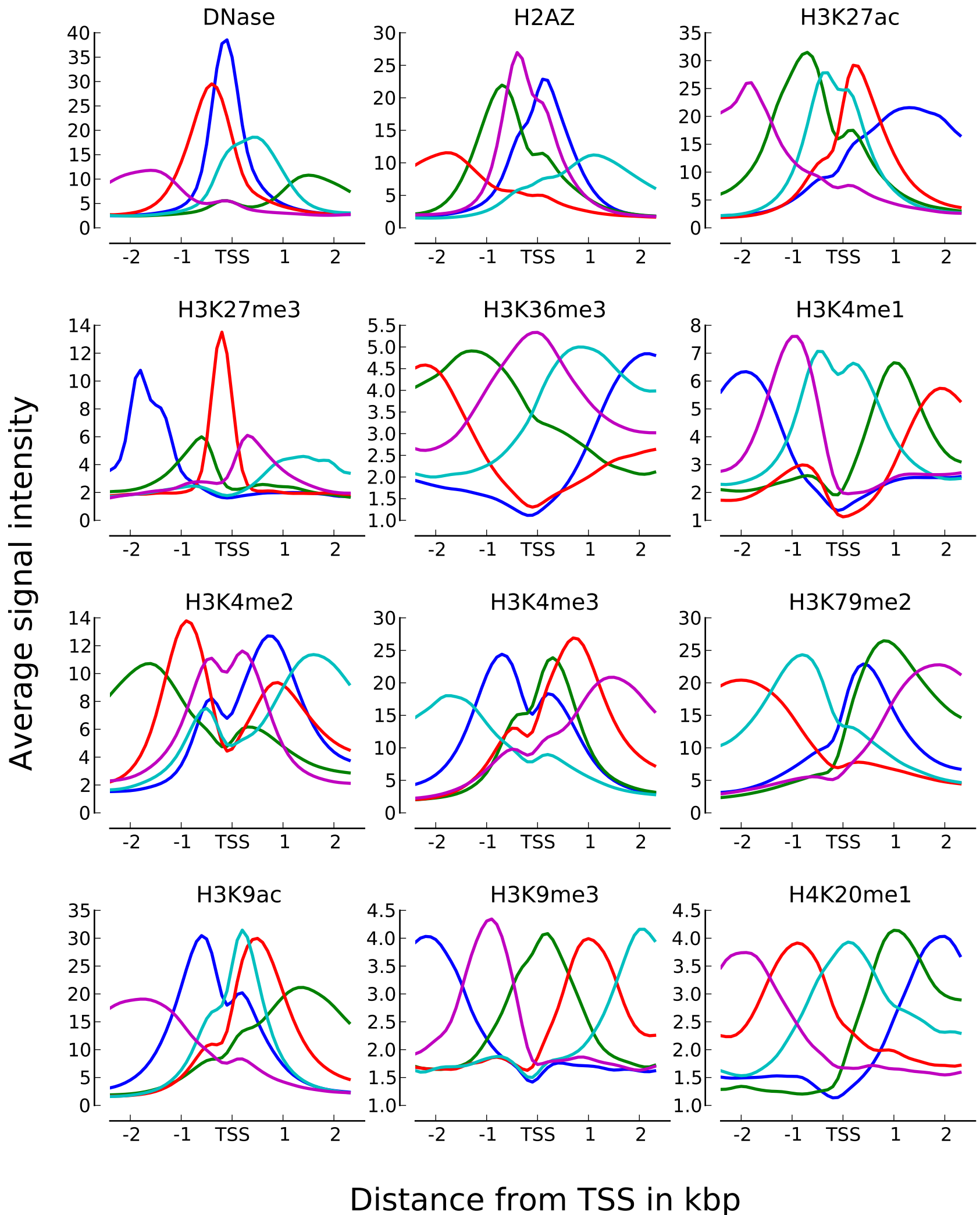


K562

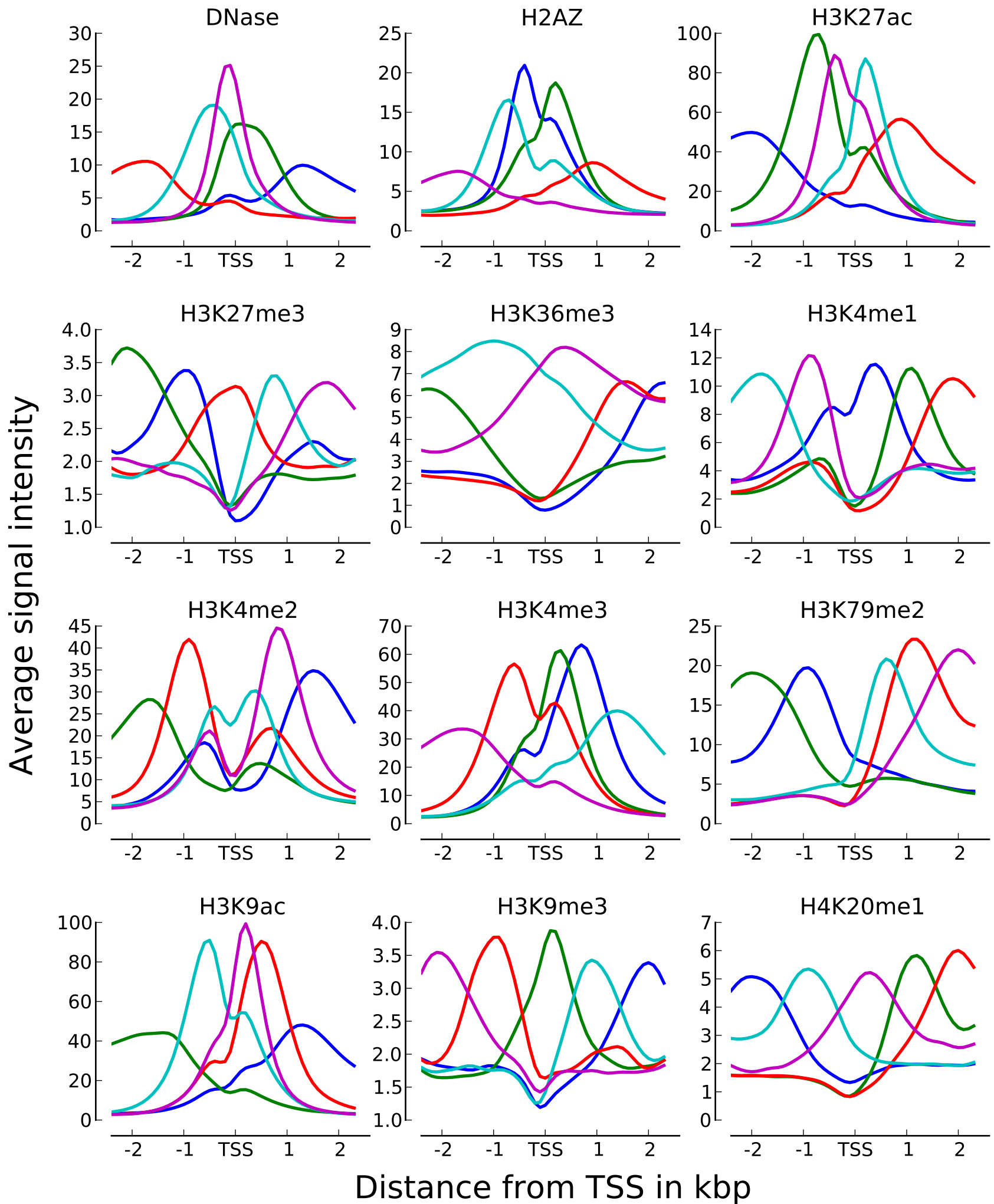


H3K4me2 in GM12878

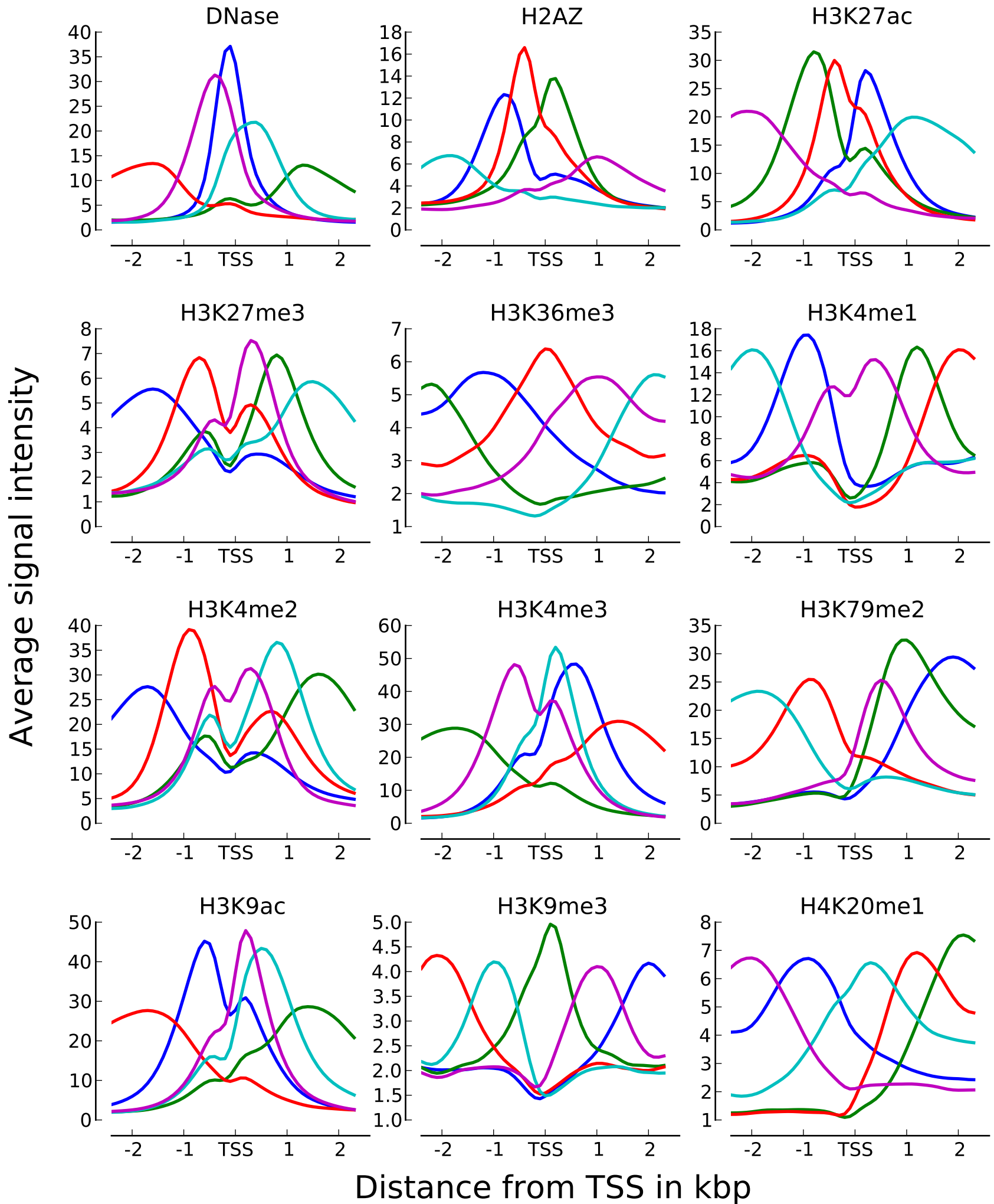




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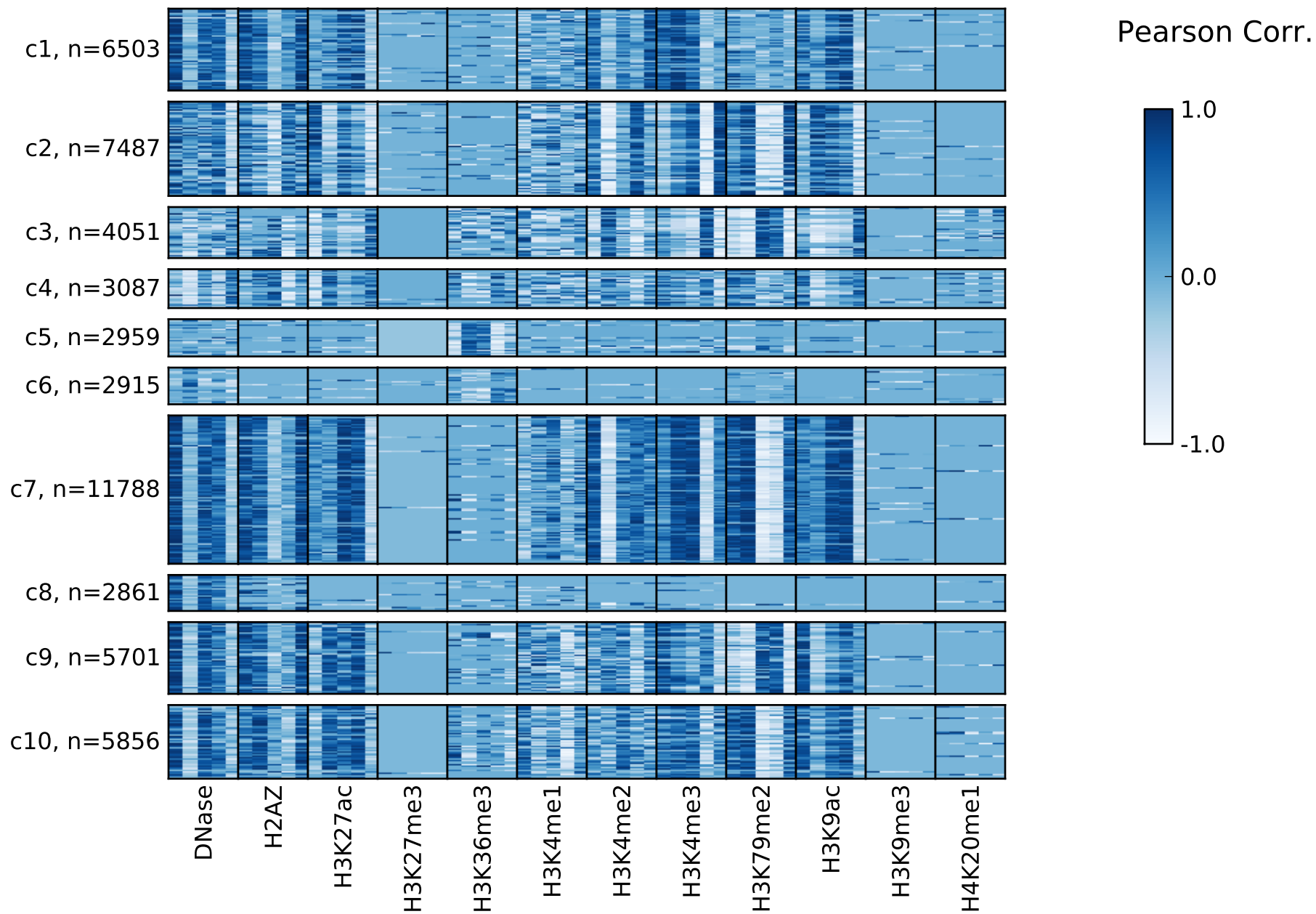


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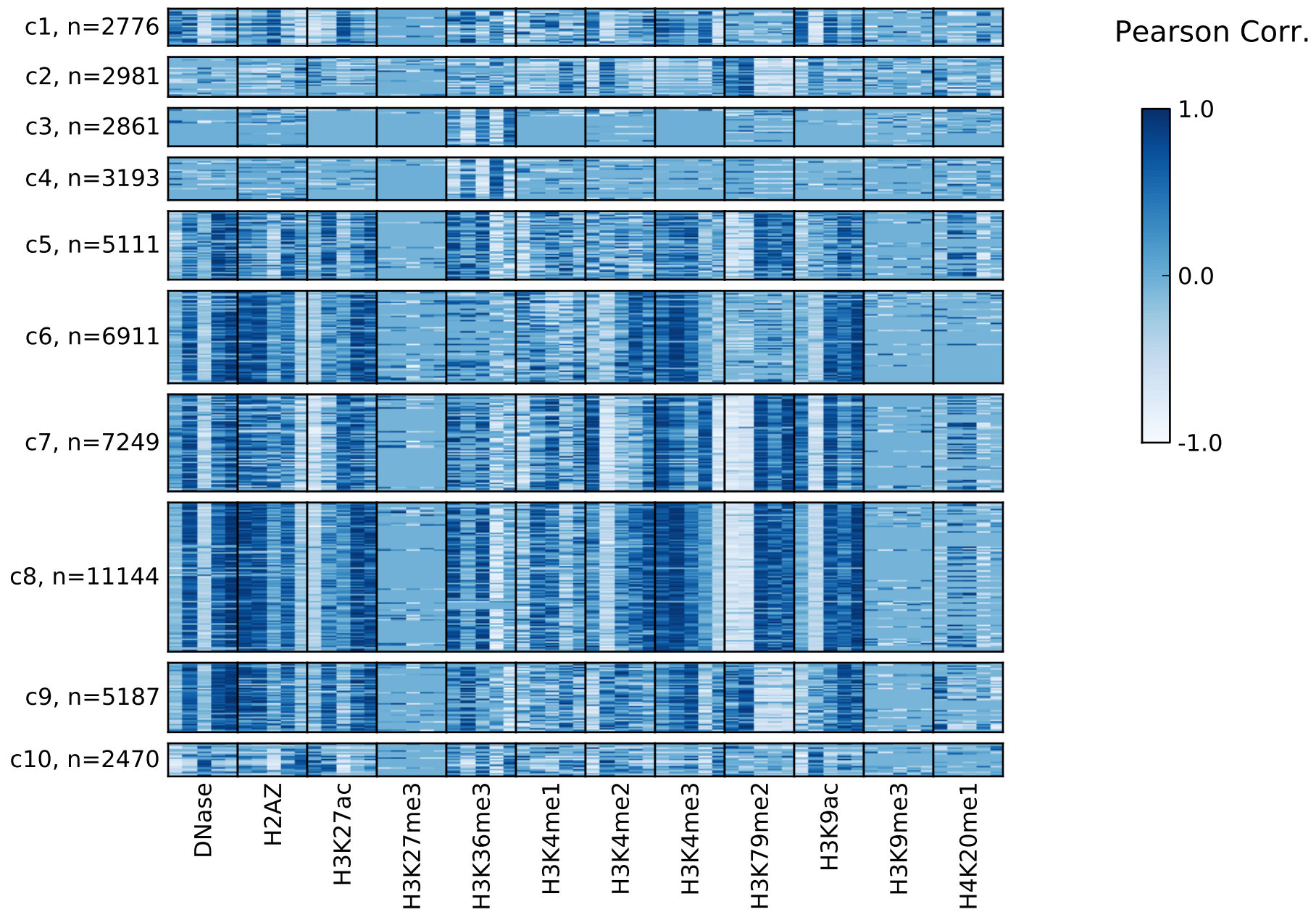


GM12878

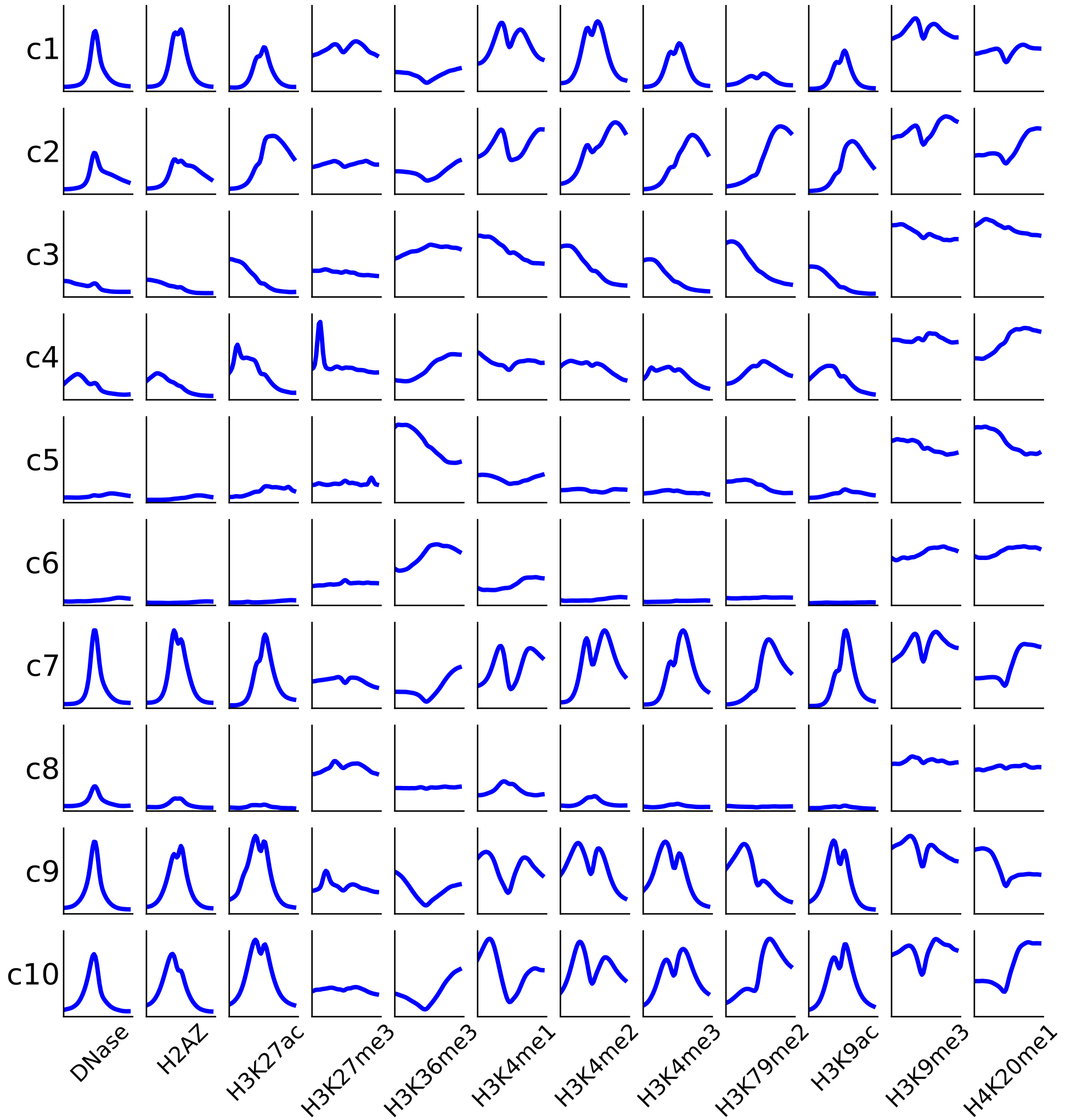
S5



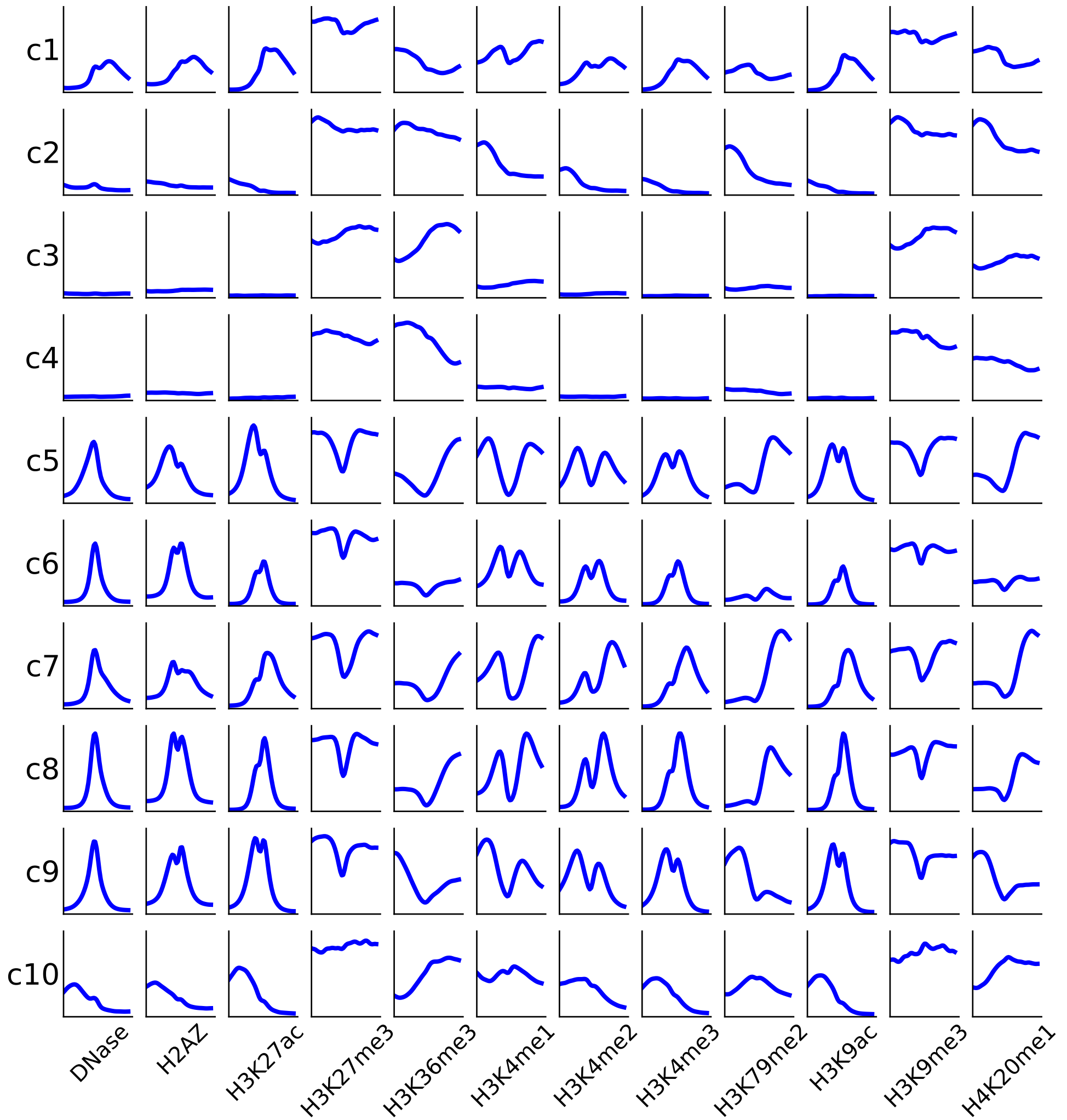
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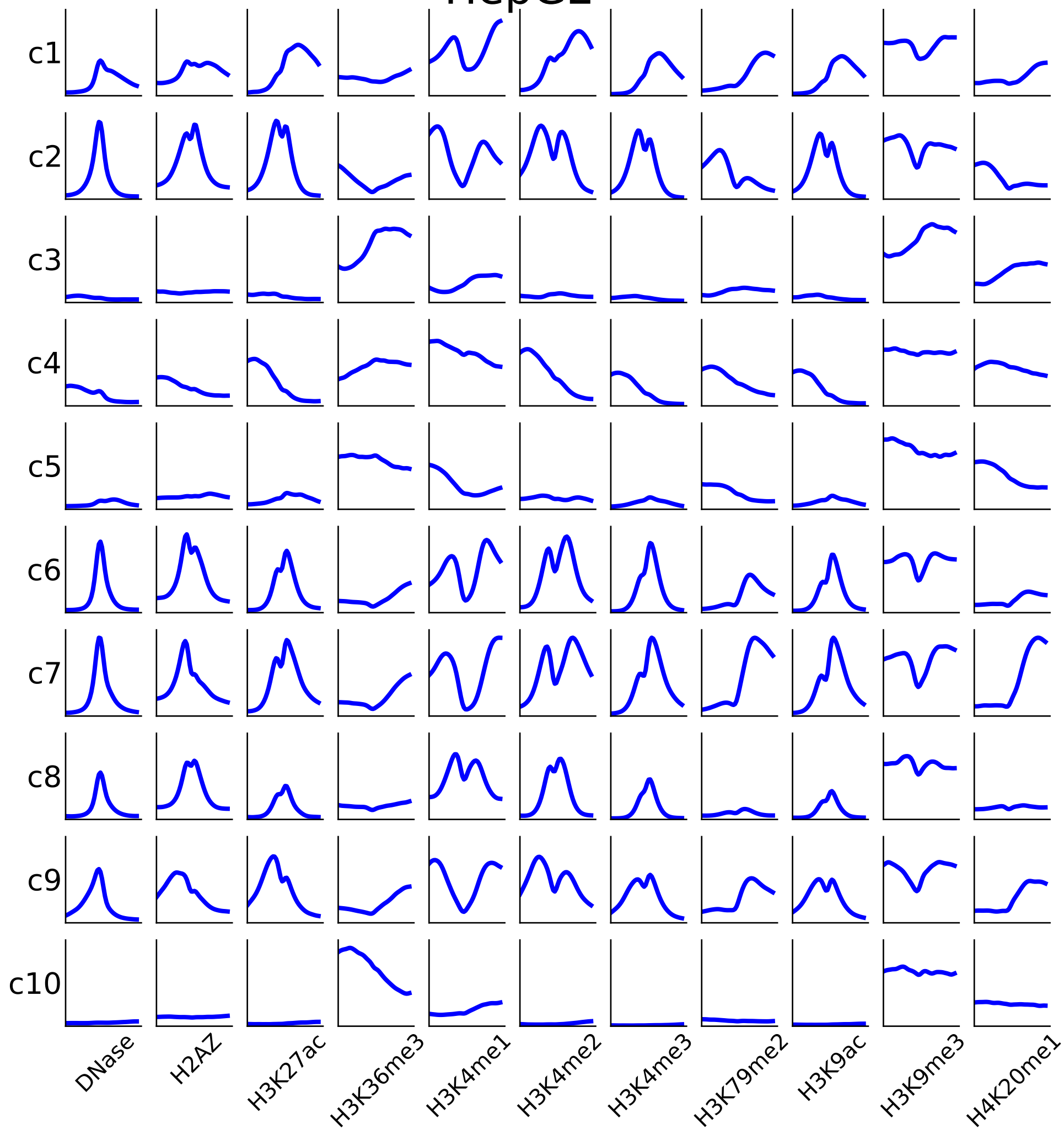
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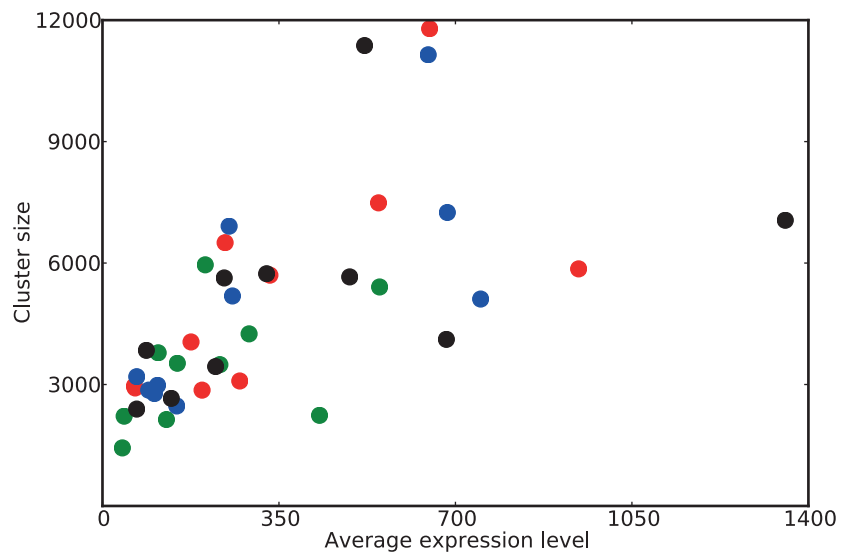
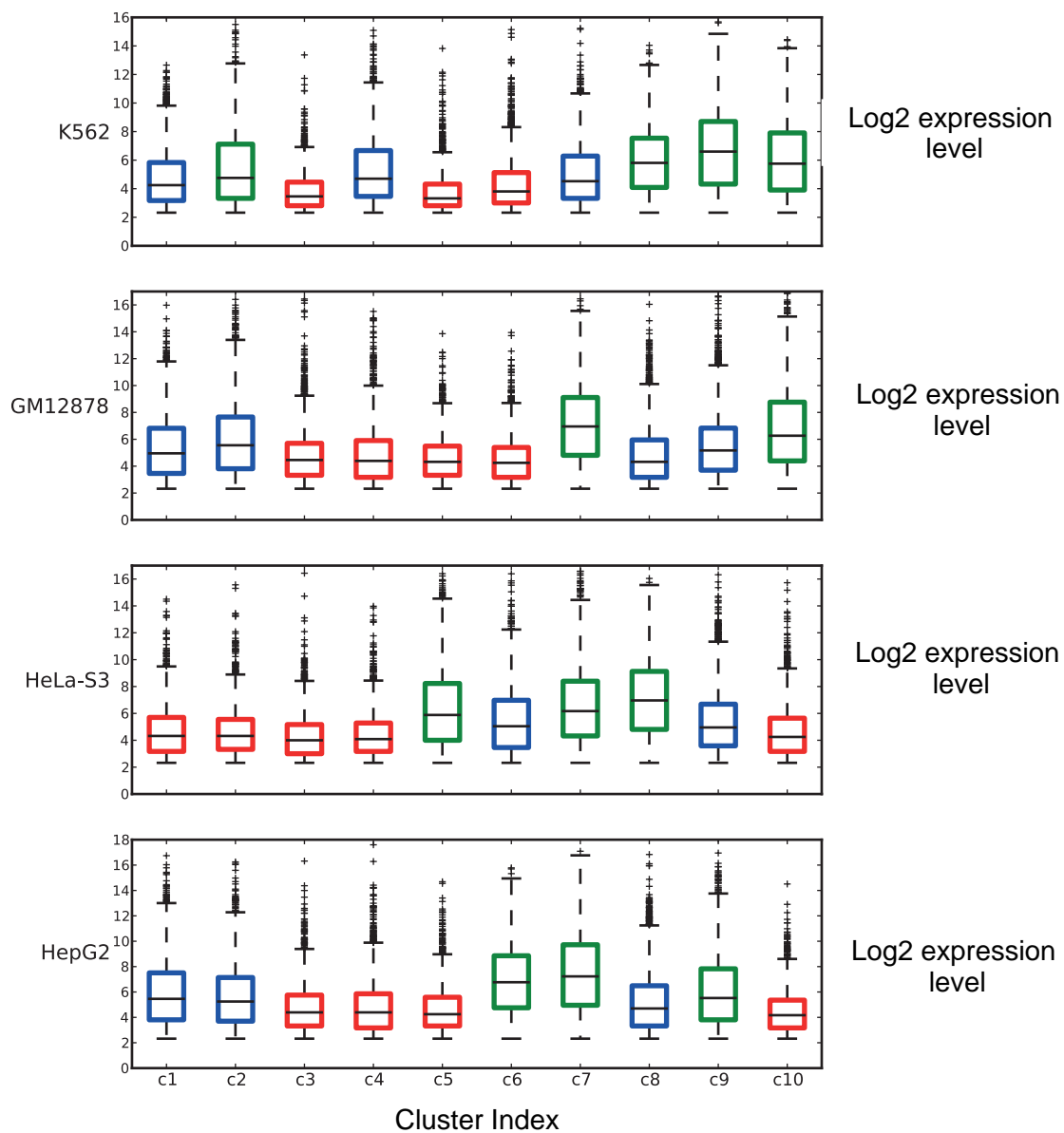


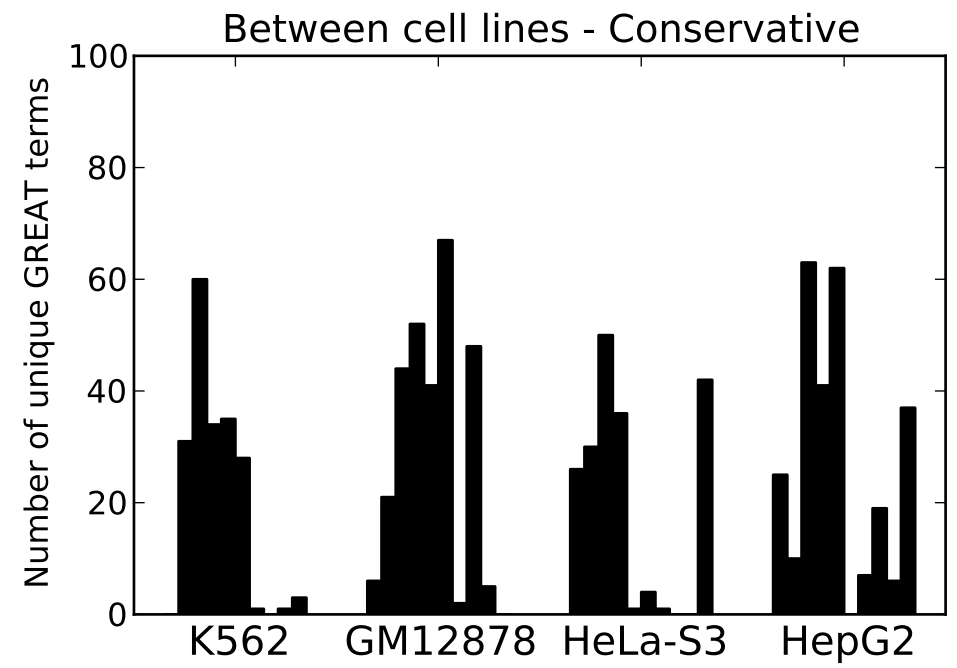
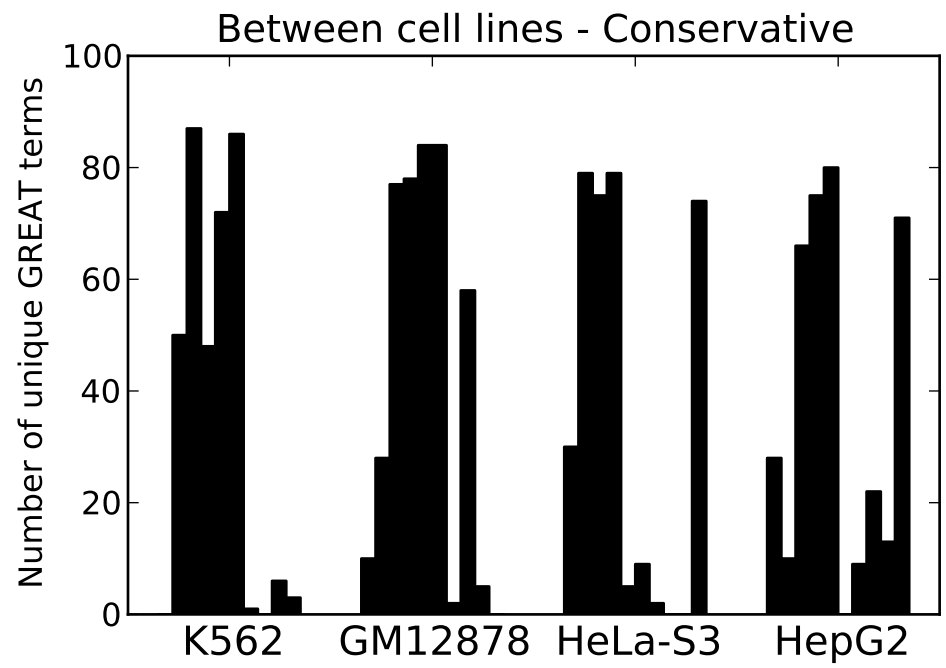
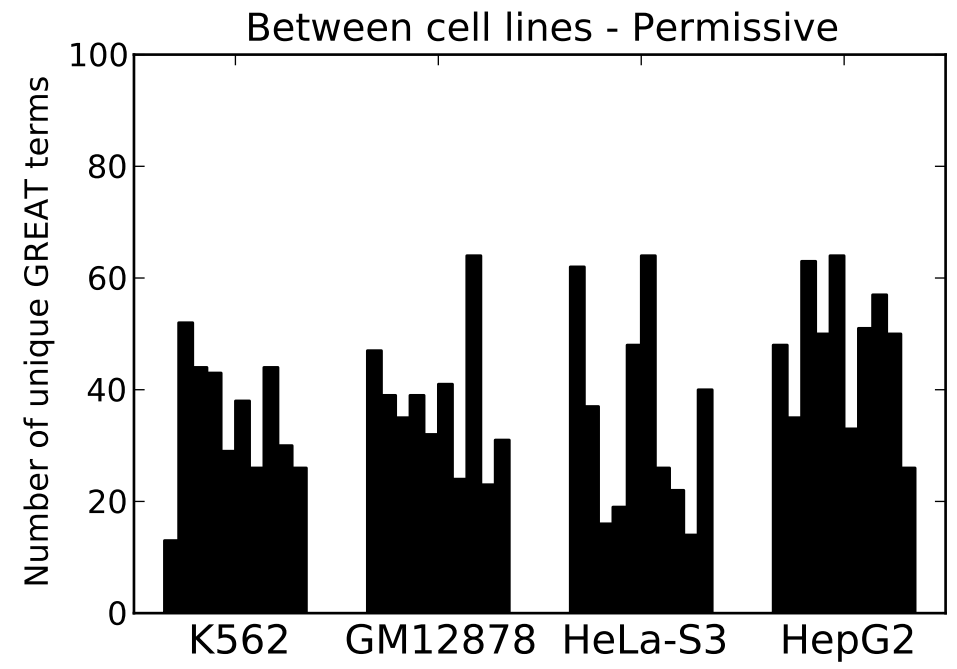
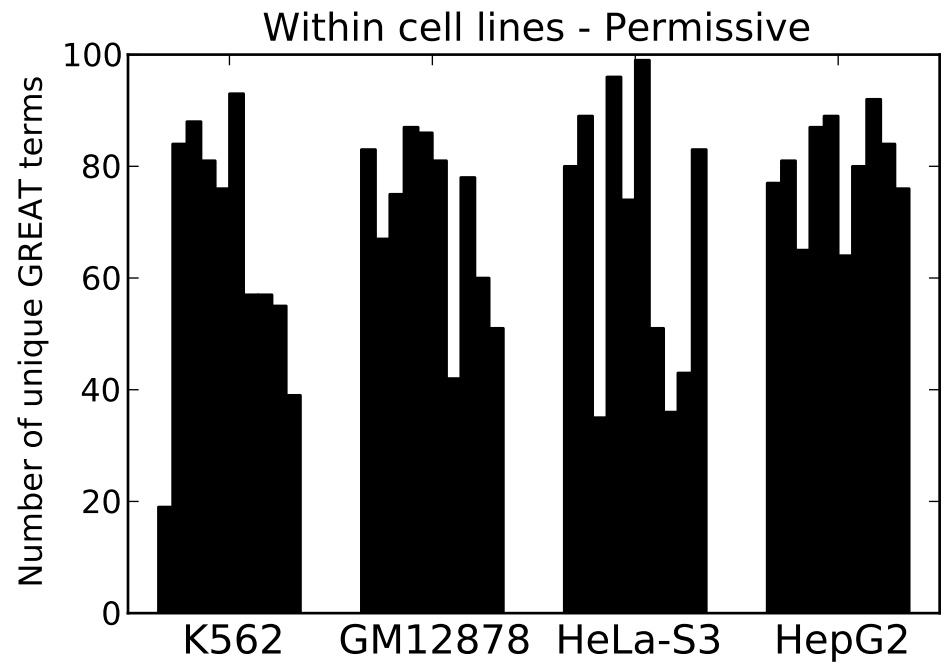
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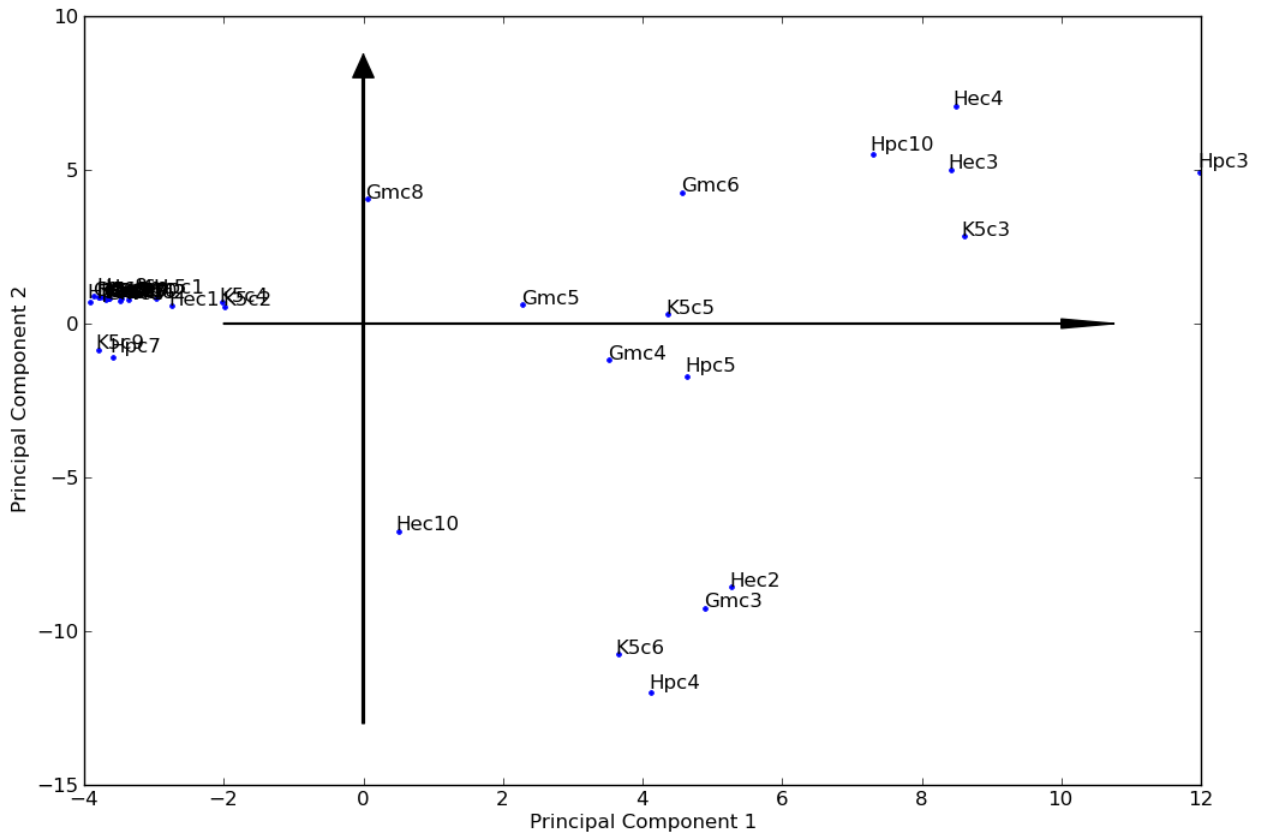
HepG2



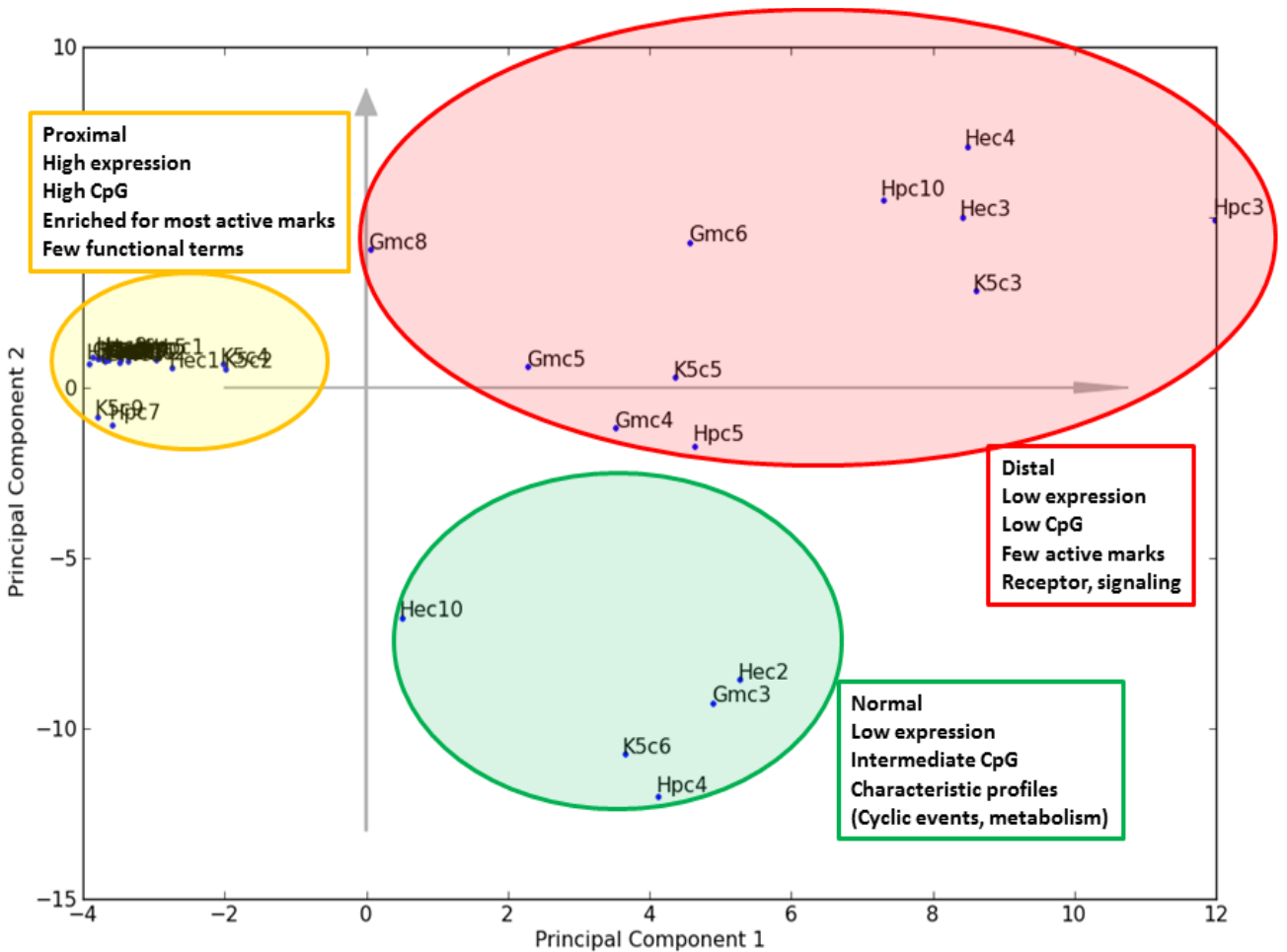




A

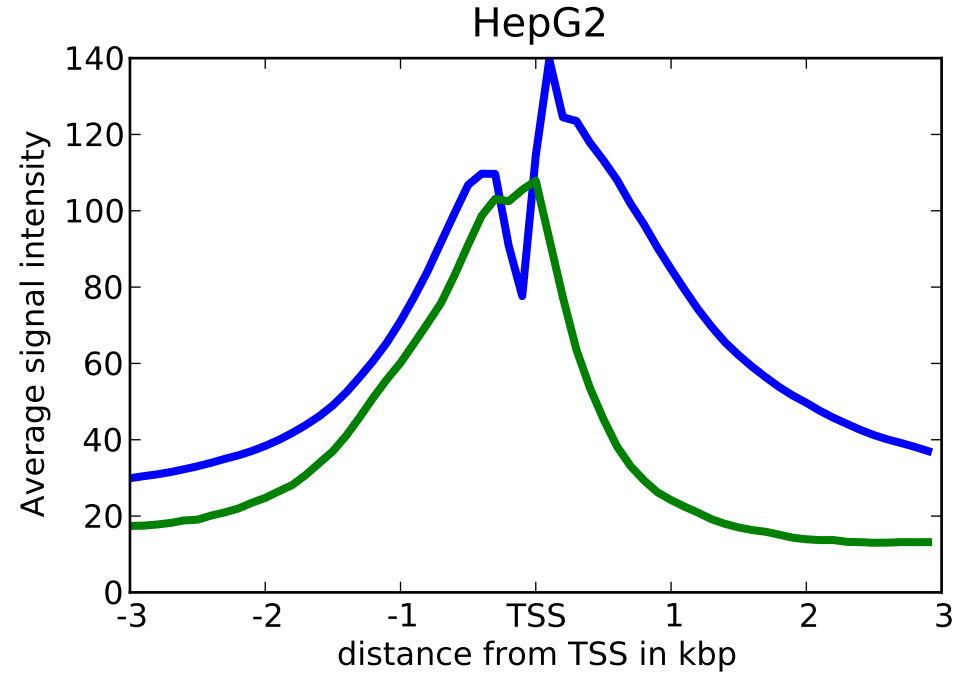
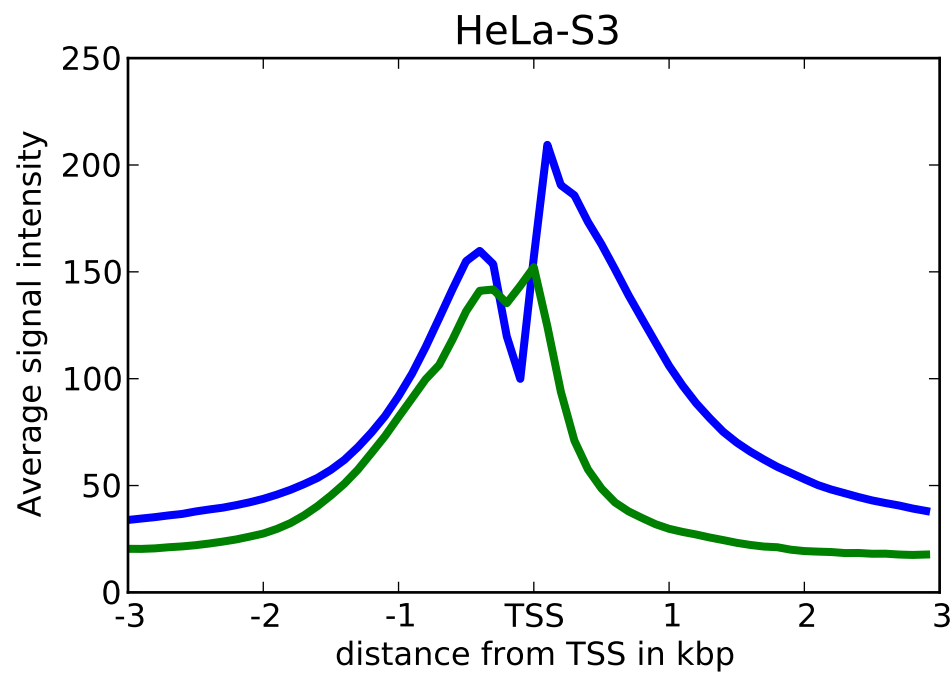
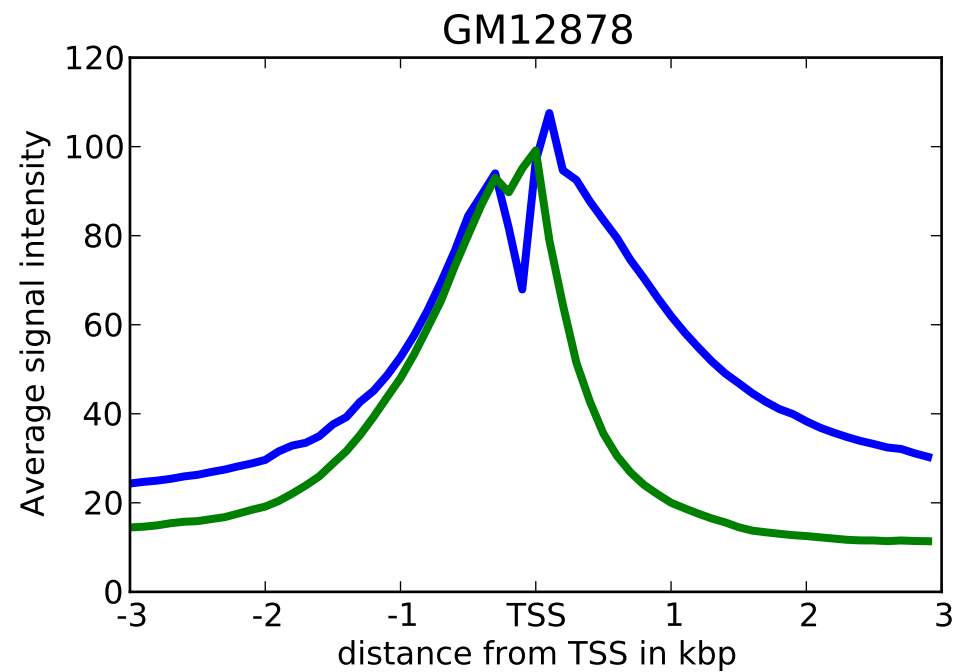
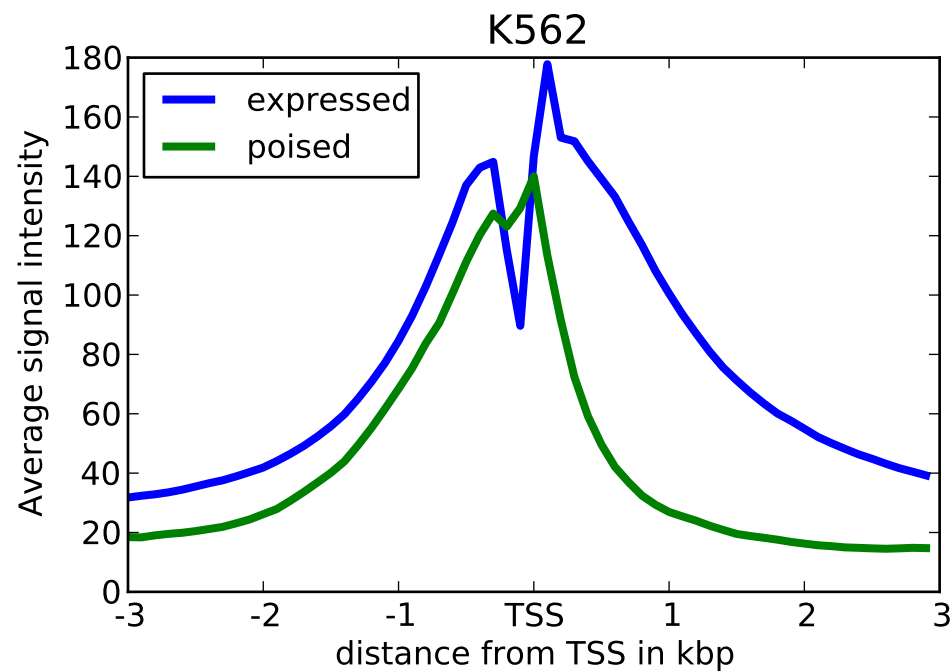


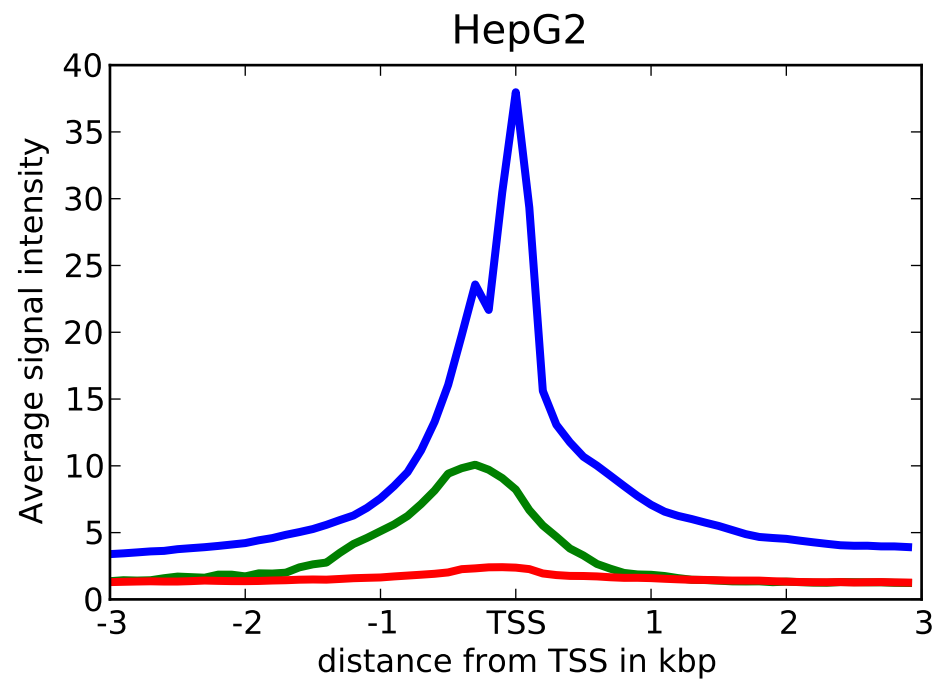
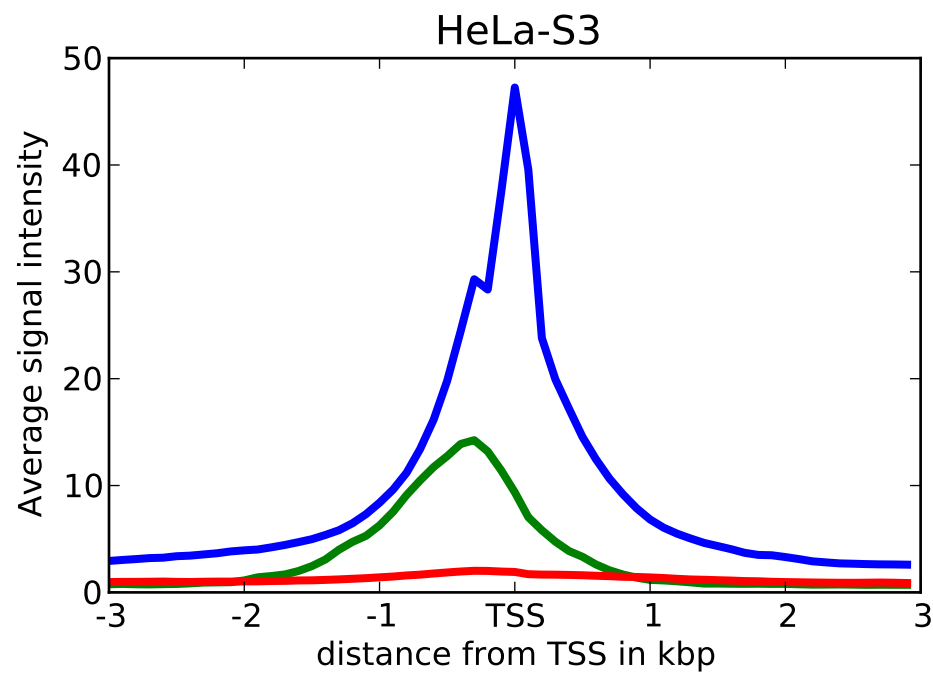
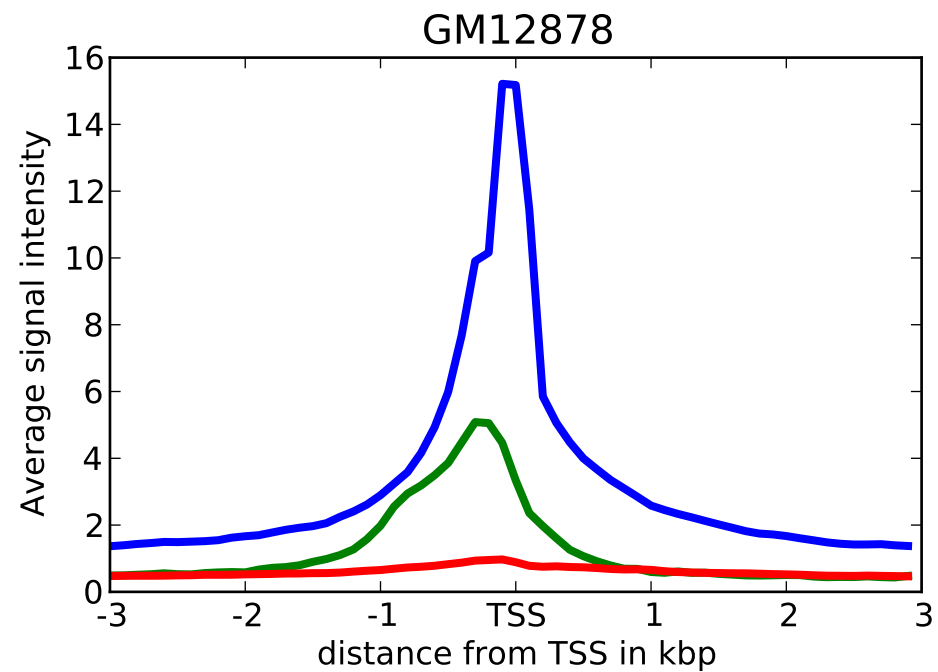
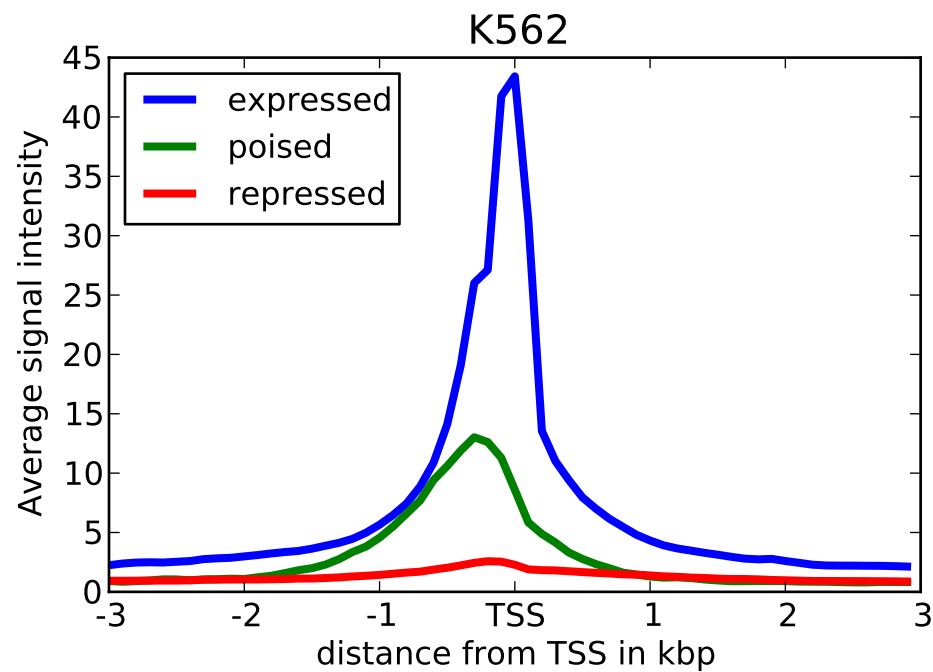
B



Supplementary Table S10: Filtering of repressed RTSS with active marks.

	Total repressed	Peak-overlap	Signal > 1000	Corr. > 0.5	p-value \leq 0.05	Filtered subset
K562	122 575	99 100	34 279	20 948	26 018	6 184
GM12878	101 964	80 594	24 625	17 775	19 297	3 813
HeLaS3	107 756	90 982	34 900	11 946	18 249	4 345
HepG2	105 793	91 323	28 950	18 325	25 541	4 303





Supplementary S13

Poised RTSS and response to stimulation by IFN α and IFN γ

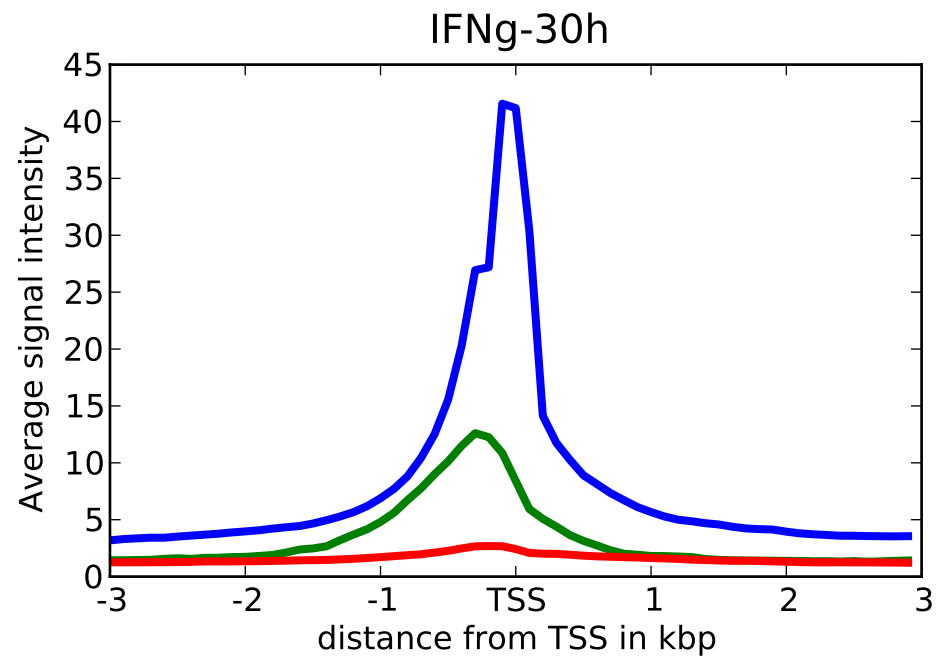
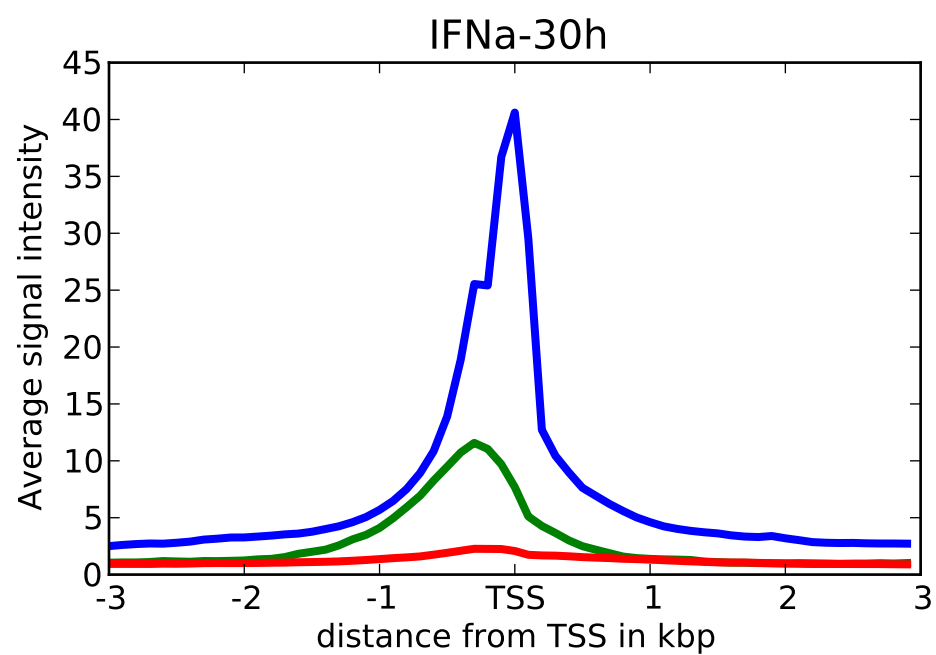
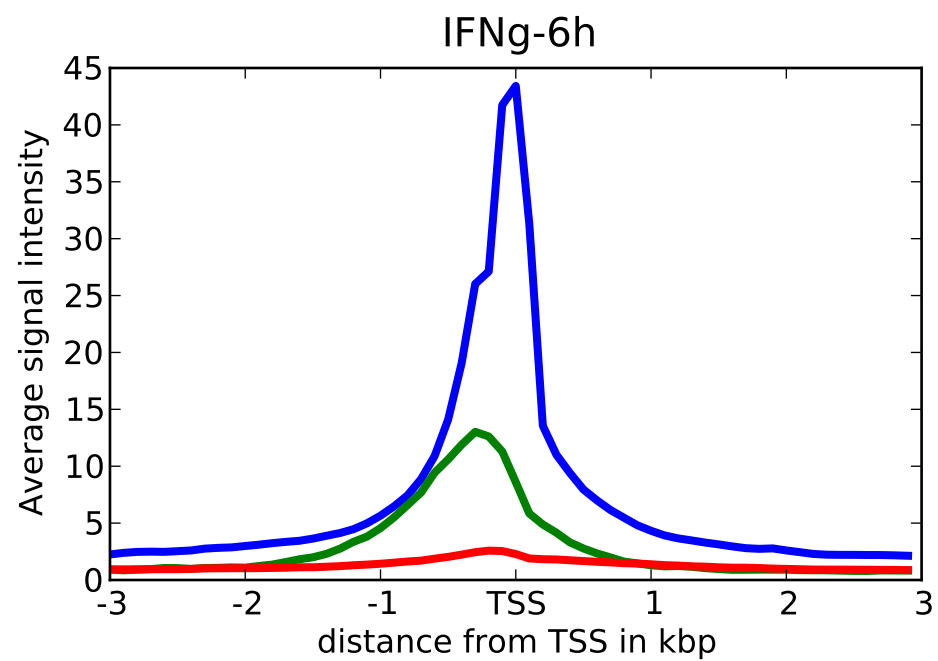
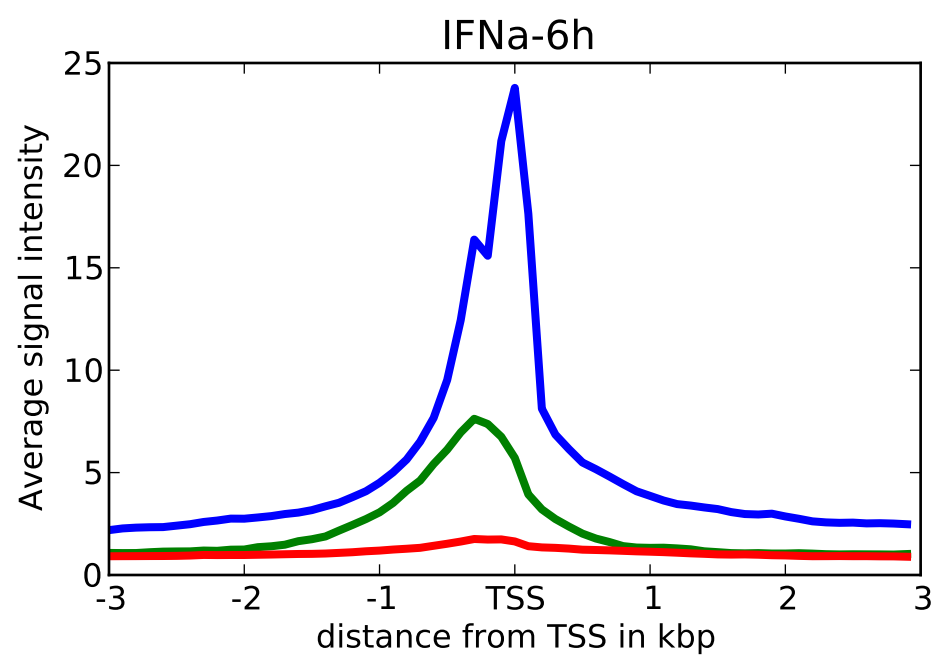
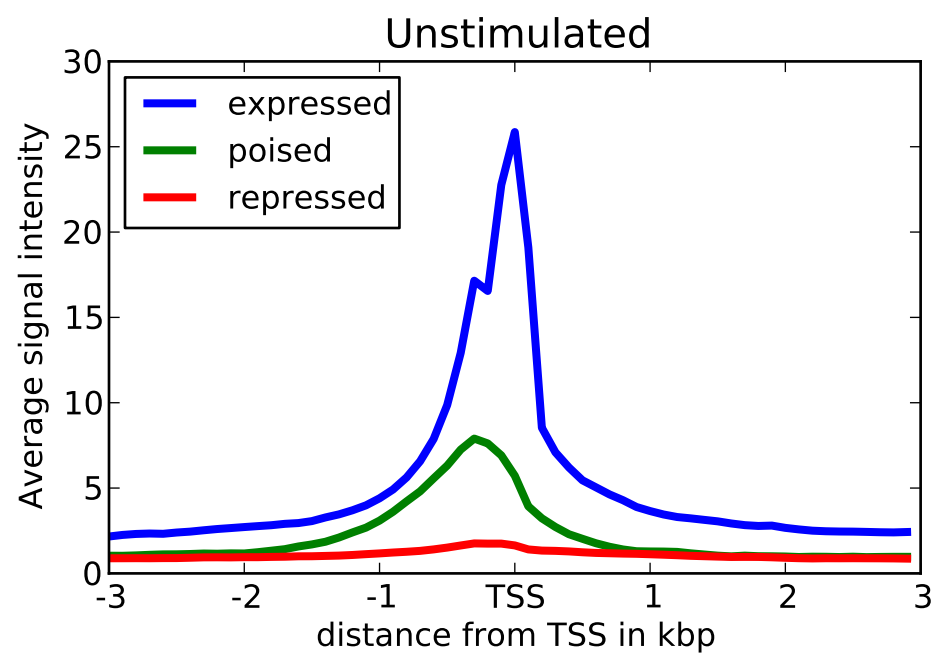
In this analysis we wanted to investigate whether our selected poised RTSS would respond to external stimuli. For this analysis, we could only find one relevant dataset from ENCODE. This was ChIP-Seq data for Pol II in unstimulated cells, and for Pol II in cells stimulated by IFN α and IFN γ (ENCODE, SYDH TFBS, Stanford/Yale/USC/Harvard), both sampled after 6 and 30 hours. We observed similar levels of Pol II for our selected RTSS relative to Pol II levels for expressed RTSS before and after stimulation of both IFN α and IFN γ (Supplementary S13). This was in contrast to the previous studies which reported a general increase in Pol II levels after stimulation (Hargreaves et al. 2009).

Because of this inconsistency we further investigated RTSS that were actually stimulated by IFN α and IFN γ . To identify the RTSS most strongly stimulated in the four datasets, the absolute difference and the ratio of Pol II levels between stimulated and unstimulated cells were calculated for each of 179 369 globally defined RTSS in a ± 300 bp window around the RTSS center. In each stimulated dataset, the RTSS absolute difference and ratio values were sorted independently in descending order, and the total enrichment score for each RTSS calculated as the sum of the ranks in the two sorted lists. The 5000 RTSS with the highest rank for each of the four stimulated datasets were used in the analysis.

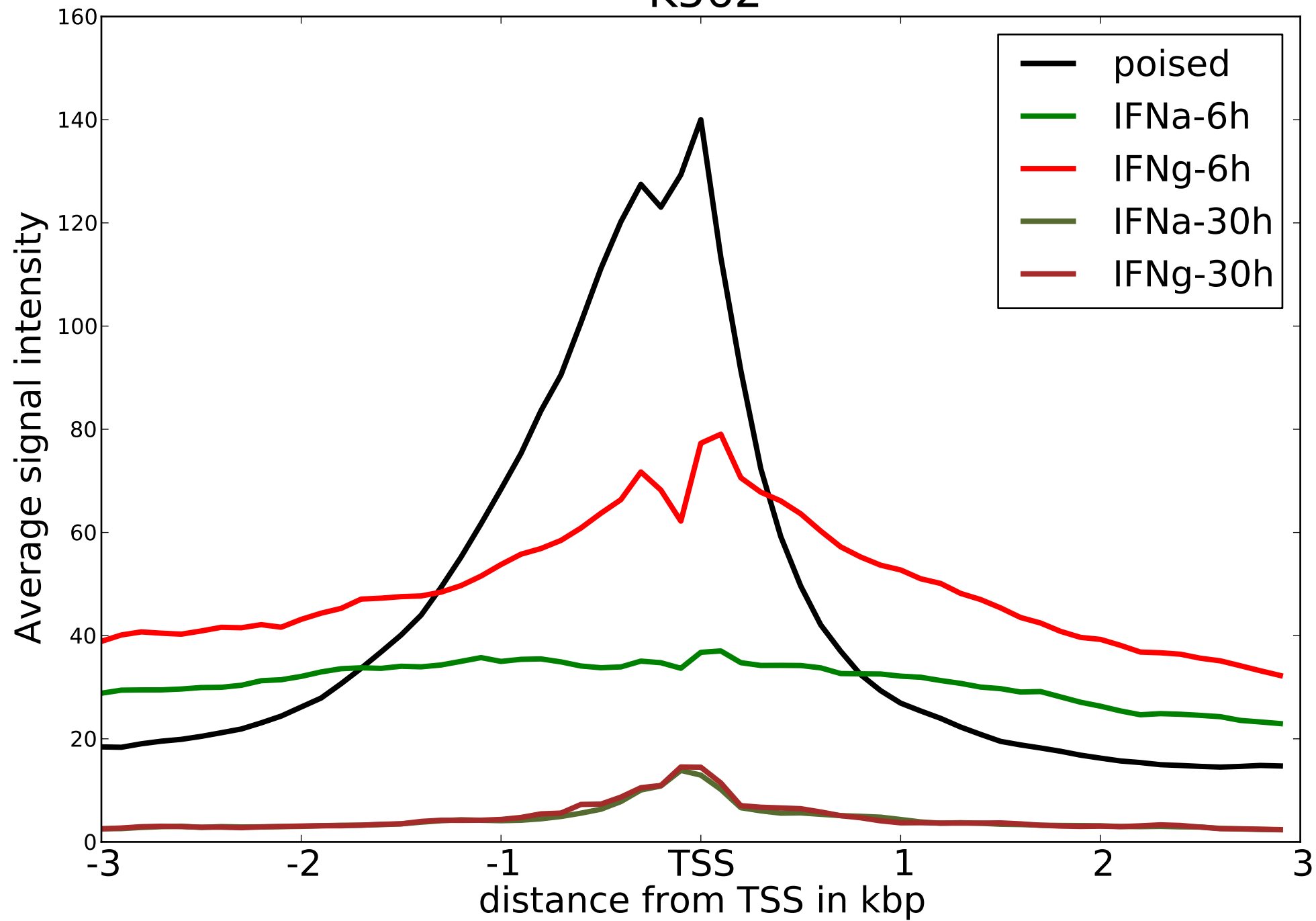
The top 5000 RTSS with increased Pol II signal after IFN α and IFN γ stimulation showed a poor overlap with our poised RTSS (123 (2%) and 194 (3%) RTSS overlap, respectively), while the overlap between IFN α and IFN γ RTSS with increased Pol II was 33%. We observed enrichment of active chromatin marks in IFN γ stimulated RTSS, but this enrichment was less than for our selected poised RTSS. For IFN α stimulated RTSS we observed little enrichment for active chromatin marks (Supplementary S14). We also compared the enrichment of active chromatin marks in IFN α and IFN γ stimulated RTSS after 6 and 30 hours of stimulation, calculating the enrichment over the top 5000 RTSS with the highest increase in Pol II for each dataset (Supplementary S14). The RTSS with the strongest increase in Pol II levels after 30 hours showed a general depletion of active chromatin marks in the unstimulated state.

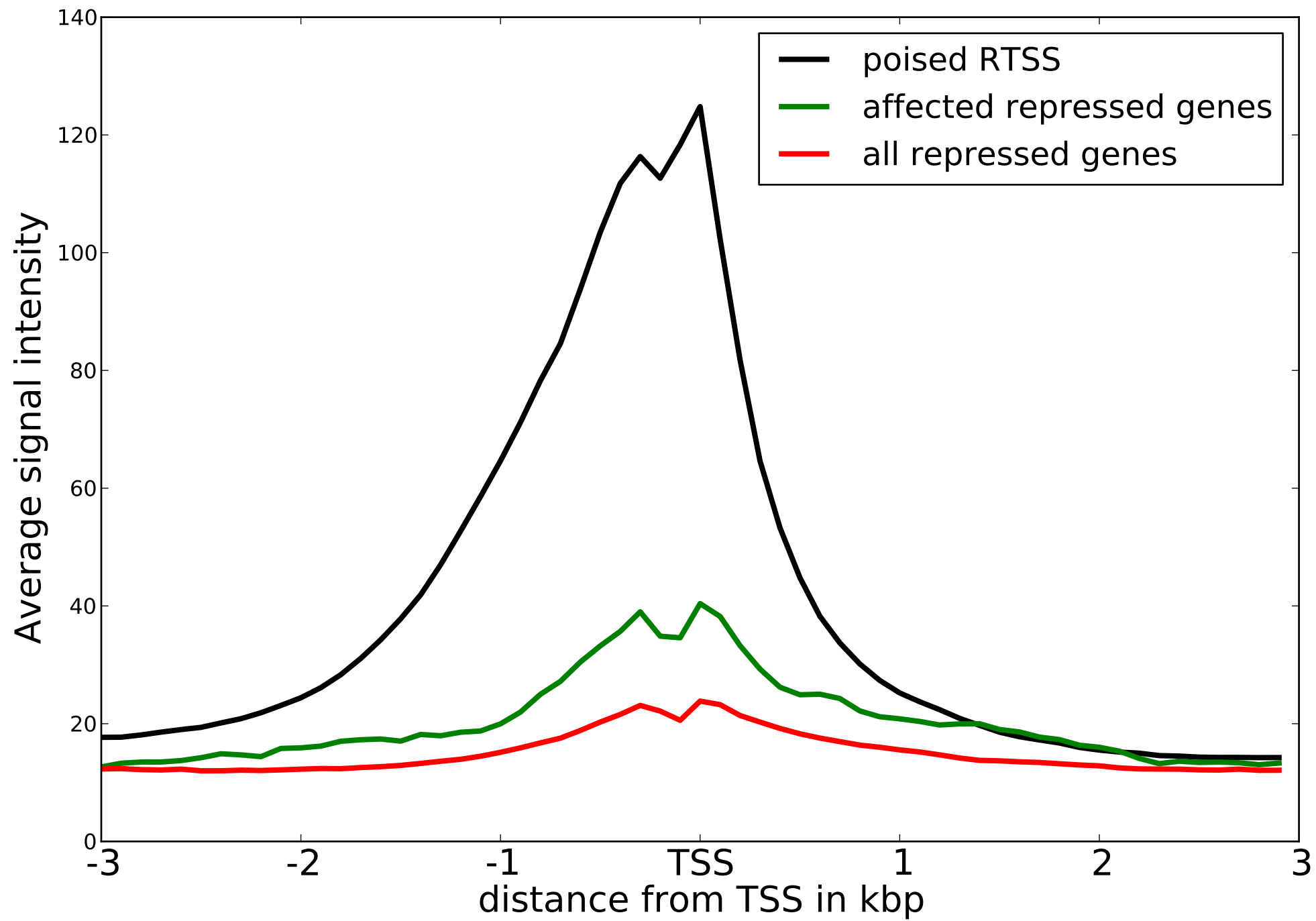
We see two likely reasons for the absent changes in Pol II levels at our poised RTSS after IFN α and IFN γ stimulation. First, we observe that both the poised RTSS and the genes associated with the RTSS are highly cell type specific, with most RTSS and genes found in only one cell type. In addition we observed that the RTSS and genes affected by IFN α and IFN γ have a similar degree of overlap as the RTSS and genes in general between the cell lines, but that both the IFN α and IFN γ stimulated RTSS overlap poorly with our set of poised RTSS. One can picture a complex situation where similar stimuli invoke different responses depending on the cell line, and also that different stimuli invoke different responses in the same cell line. Thus stimulation by IFN α and IFN γ may simply initiate a response that is different from, or with just minor overlap with, the one represented by our poised RTSS. Second, Pol II changes at RTSS were measured 6 and 30 hours after stimulation with IFN α and IFN γ , which may be long enough for the earliest response to attenuate, and what we observe is actually secondary and further downstream responses. The lower level of active chromatin marks in the unstimulated cells for the regions with the highest change in Pol II after 6h, and the absence of active chromatin marks in regions with the highest change after 30h are indications of this. Overall we find it likely that our set of poised RTSS represent

regulatory elements that rapidly produce transcripts in response to environmental stimuli. Though some of the regulatory elements coincide with annotated gene TSS, many are distal to genes and are likely to produce non-coding transcripts. It is tempting to speculate whether rapid responses initiate at the distal elements and then propagate a signal to nearby genes. Which responses that trigger these elements and how the elements relate to the genes they regulate are interesting candidates for further studies.



K562





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SYDH	Gm12878	Polymerase 2	Enrichment Region	wgEncodeSydhTfbsGm12878Pol2StdPk.narrowPeak
SYDH	Helas3	Polymerase 2	Enrichment Region	wgEncodeSydhTfbsHelas3Pol2StdPk.narrowPeak
SYDH	Hepg2	Polymerase 2	Enrichment Region	wgEncodeSydhTfbsHepg2Pol2IlggrabPk.narrowPeak
SYDH	K562	Polymerase 2	Enrichment Region	wgEncodeSydhTfbsK562Pol2StdPk.narrowPeak
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifna30StdAlnRep1.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifna30StdAlnRep2.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifna6StdAlnRep1.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifna6StdAlnRep2.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifng30StdAlnRep1.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifng30StdAlnRep2.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifng6StdAlnRep1.bam
SYDH	K562	IFN-stimulated	Rawdata	wgEncodeSydhTfbsK562Pol2Ifng6StdAlnRep2.bam
SYDH	K562	IFN-stimulated	Enrichment Region	wgEncodeSydhTfbsK562Pol2Ifna30StdPk.narrowPeak
SYDH	K562	IFN-stimulated	Enrichment Region	wgEncodeSydhTfbsK562Pol2Ifna6StdPk.narrowPeak
SYDH	K562	IFN-stimulated	Enrichment Region	wgEncodeSydhTfbsK562Pol2Ifng30StdPk.narrowPeak
SYDH	K562	IFN-stimulated	Enrichment Region	wgEncodeSydhTfbsK562Pol2Ifng6StdPk.narrowPeak
SYDH	Gm12878	Nucleosome	bigWig track	wgEncodeSydhNsomeGm12878Sig.bigWig
SYDH	K562	Nucleosome	bigWig track	wgEncodeSydhNsomeK562Sig.bigWig
Stanford	All	Genomic regions	Unmappable regions	wgEncodeDacMapabilityConsensusExcludable.bed
Duke	All	Genomic regions	Unmappable regions	wgEncodeDukeMapabilityRegionsExcludable.bed

Permutation	Cell type	RTSS track	DNase	H2AZ	H3K27ac	H3K27me3	H3K36me3	H3K4me1	H3K4me2	H3K4me3	H3K79me2	H3K9ac	H3K9me3	H4K20me1
uniform	gm12878	all expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
uniform	gm12878	all repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,045908184	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
uniform	gm12878	isolated expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
uniform	gm12878	isolated repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,712871287	9,999E-05	9,999E-05	9,999E-05	0,900990099	9,999E-05	0,012345679	9,999E-05
interseg	gm12878	all expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
interseg	gm12878	all repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,174129353	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
interseg	gm12878	isolated expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05
interseg	gm12878	isolated repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,673267327	9,999E-05	9,999E-05	9,999E-05	0,920792079	9,999E-05	0,019981835	9,999E-05
interseg	Helas3	all expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Helas3	all repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,174129353	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Helas3	isolated expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Helas3	isolated repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,109634551	9,999E-05	9,999E-05	9,999E-05	0,034941764	9,999E-05	0,821782178	9,999E-05
interseg	Hepg2	all expressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Hepg2	all repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,782178218	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Hepg2	isolated expressed	9,999E-05	9,999E-05	9,999E-05	9,99989E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	Hepg2	isolated repressed	9,999E-05	9,999E-05	0,035663338	9,999E-05	0,326732673	9,999E-05	9,999E-05	9,999E-05	0,405940594	9,999E-05	1	9,999E-05
interseg	K562	all expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	K562	all repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05
interseg	K562	isolated expressed	9,999E-05	9,999E-05	9,999E-05	1	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,841584158	9,999E-05
interseg	K562	isolated repressed	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	9,999E-05	0,000405398	9,999E-05	1	9,999E-05