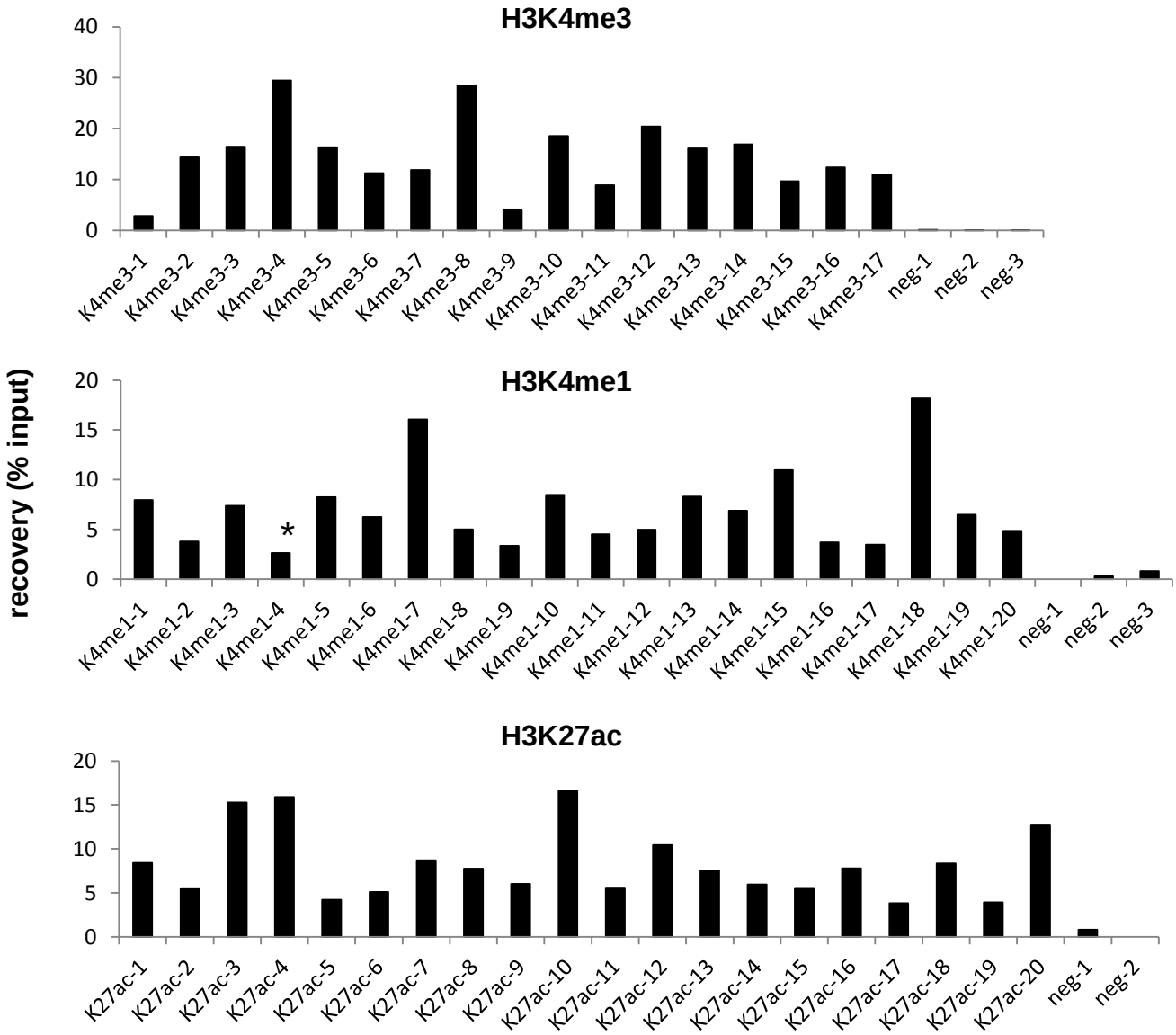
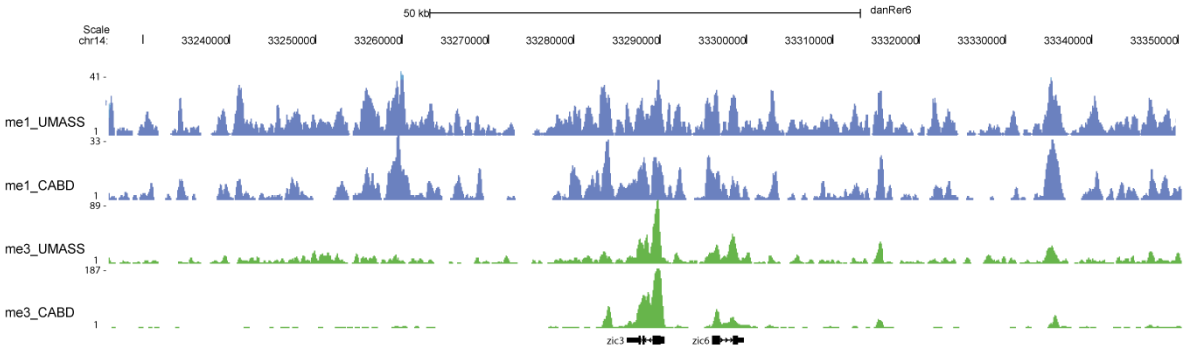
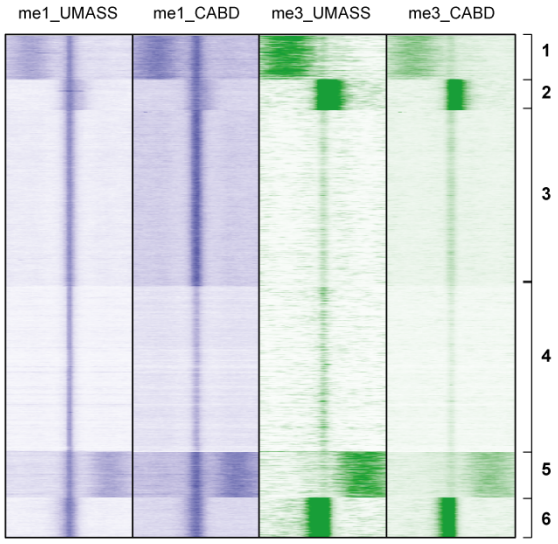


Figure S1. Quantitative PCR validation of genomic peaks of H3K4me3, H3K4me1 and H3K27ac. Chromatin from early embryos (80% epiboly) was immunoprecipitated with antibodies against H3K4me3, H3K4me1 and H3K27ac and amplified in a quantitative PCR reaction with primers corresponding to randomly selected genomic peaks. The negative controls (neg) were used to determine the background level of the ChIP procedure. For H3K4me3 all the tested regions were verified as true positives (> 2.5 times over background, experimental FDR < 0.05) whereas the FDR for H3K4me1 and for H3K27ac were: 0.05 (1 false positive – as indicated by the asterisk sign) and < 0.05 (no false positives), respectively.

A



B



C

	mapped reads	peaks
me1_UMASS	13548123	41501
me1_CABD	4682379	67271
me3_UMASS	7846092	29033
me3_CABD	7961929	21498

Figure S2. Comparison with previously published (Aday et al. 2011) H3K4me3 and H3K4me1 ChIP-seq data at 24hpf of zebrafish development. (A) UCSC genome browser screenshot of the *zic3/zic6* locus. Shown in blue and green are genomic tracks of H3K4me1 and H3K4me3, respectively. The UMASS data corresponds to the study published by Aday et al. (2011) whereas the CABD datasets correspond to the data presented in this study. Both marks show very similar genomic profiles between the two studies. **(B)** K-means clustering (k=6) of H3K4me1 and H3K4me3 UMASS and CABD data, over our 24hpf H3K4me1 peak collection. Again, we observe a good correlation for both H3K4me1 and H3K4me3 between the two studies. **(C)** Mapped reads and peak numbers for H3K4me1 and H3K4me3 data originating from both studies.

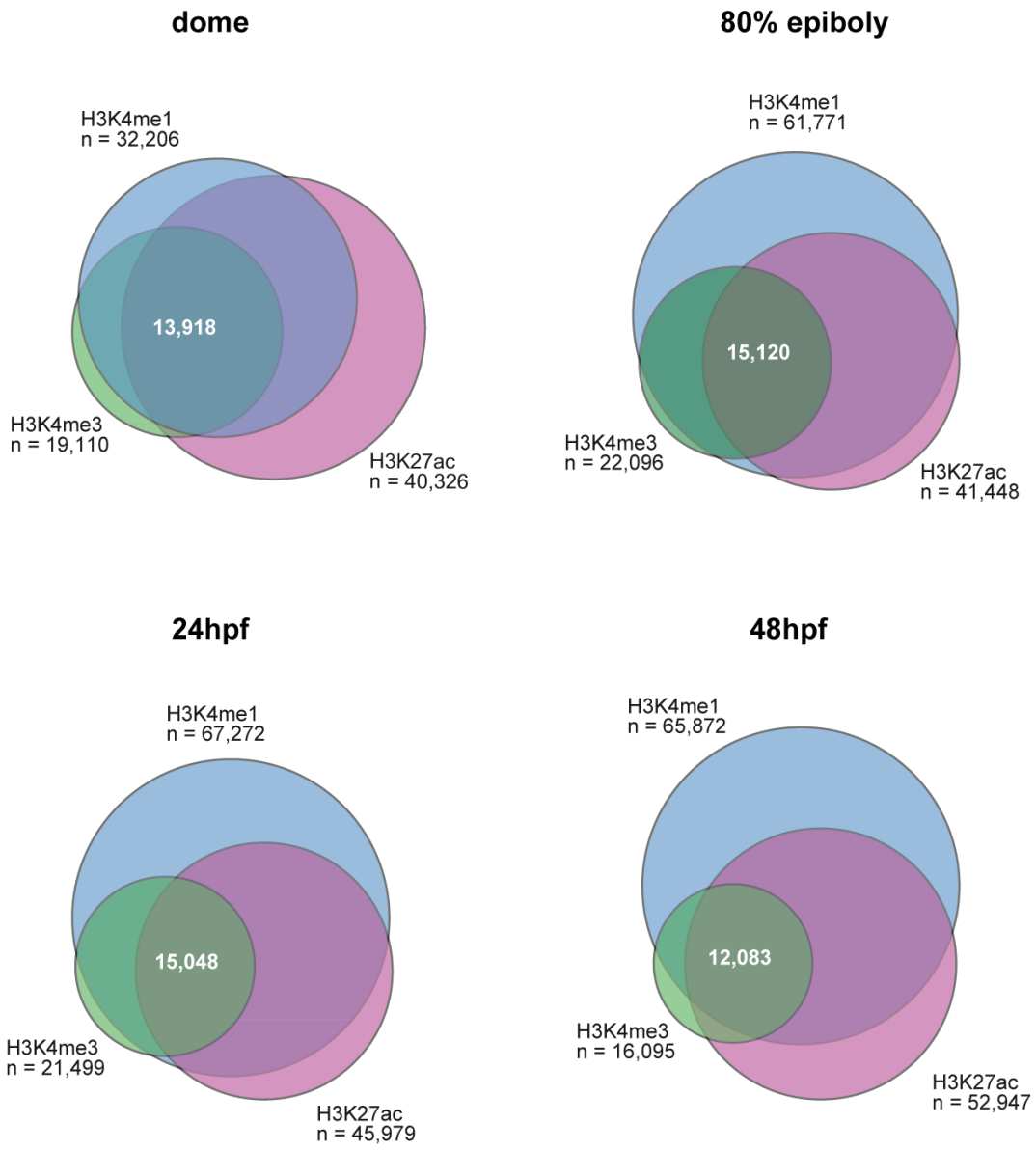


Figure S3. Genomic overlap of H3K4me3, H3K4me1 and H3K27ac peaks. H3K4me1 and H3K27ac peaks overlap (minimum overlap=1bp) on the majority of genomic locations and the majority of H3K4me3 peaks are also marked by H3K4me1 and H3K27ac, as expected from studies in other species (Roy et al. 2010; Heintzman et al. 2009; Creyghton et al. 2010; Rada-Iglesias et al. 2011; Hawkins et al. 2011). The bulk of H3K4me1 in the early embryo corresponds to promoter regions (H3K4me3 mark) whereas in later stage embryos, a robust increase in H3K4me1 peaks is observed outside H3K4me3 peaks. At the dome stage, the number of identified H3K4me1 and H3K27ac peaks is similar whereas at later stages a larger discrepancy in peak numbers corresponding to these two histone marks is observed. In general, there are more H3K4me1 than H3K27ac peaks in line with their functional roles of H3K4me1 being the poised enhancer mark and H3K27ac being the active, more specific, mark.

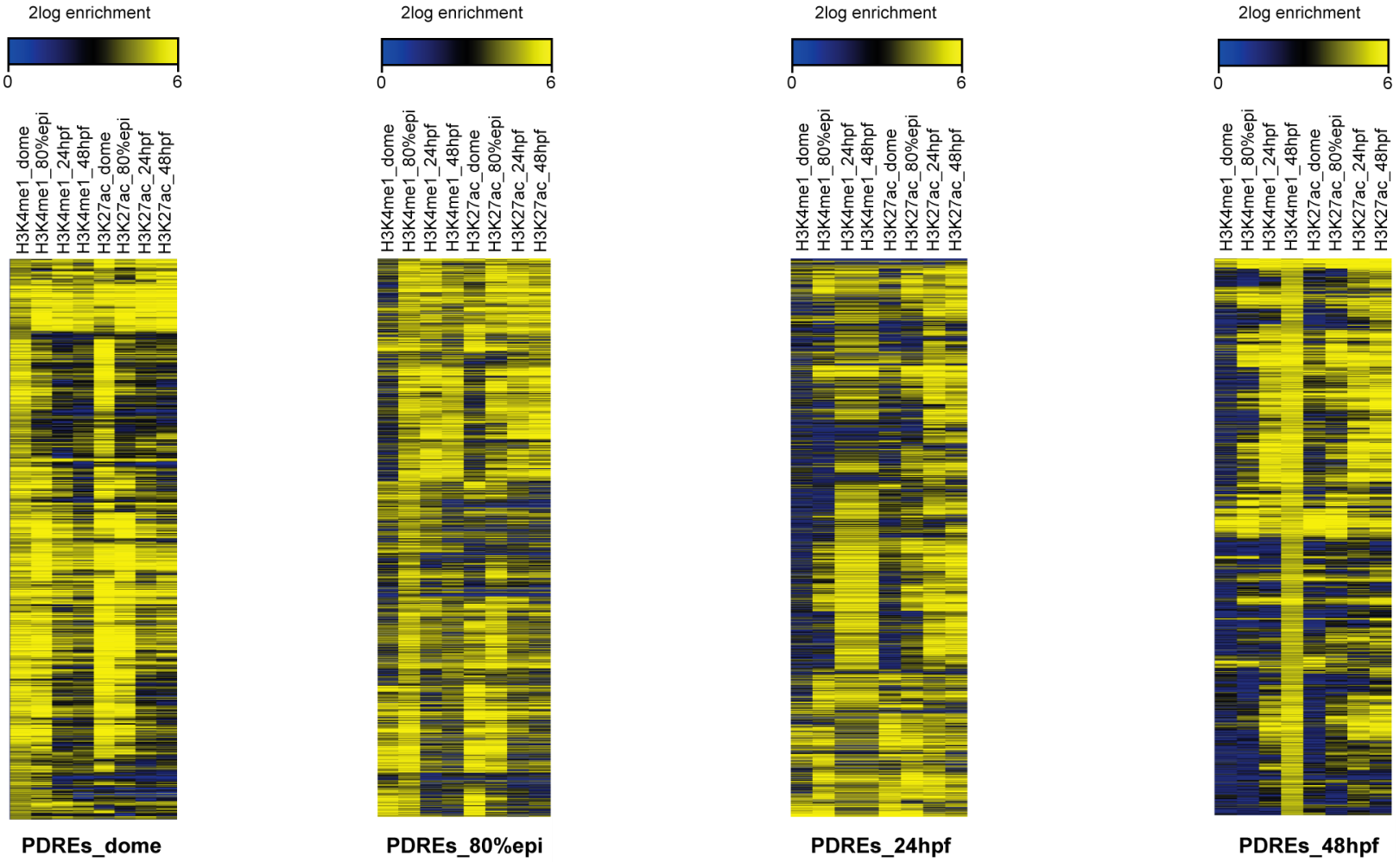


Figure S4. Hierarchical clustering of H3K4me1 and H3K27ac within stage-specific PDREs. H3K4me1 and H3K27ac display similar yet not identical patterns during early development. At the dome stage, three main types of PDREs can be identified: (i) PDREs constitutively marked by H3K4me1 and H3K27ac during early development; (ii) PDREs marked by both marks only at the dome stage; (iii) PDREs marked by both marks only at dome and 80% epiboly. The majority of 80% epiboly PDREs become marked by H3K4me1 at 80% epiboly, even though some PDREs were already H3K4me1 methylated at the dome stage. At the bottom of the figure a cluster of regions can be identified, that have lost H3K27ac at 80% epiboly but still remain H3K4me1 methylated at that stage. The PDREs identified at 24hpf and 48hpf mostly correspond to regions were H3K4me1 was deposited at 24hpf or 48hpf, respectively. The dynamics of histone marks at later stages are more complicated to interpret as 24-48 hpf embryos already display formed tissues and enhancer activity is likely localized to reduced areas of gene expression, making changes in enhancer makeup more difficult to detect in whole embryos.

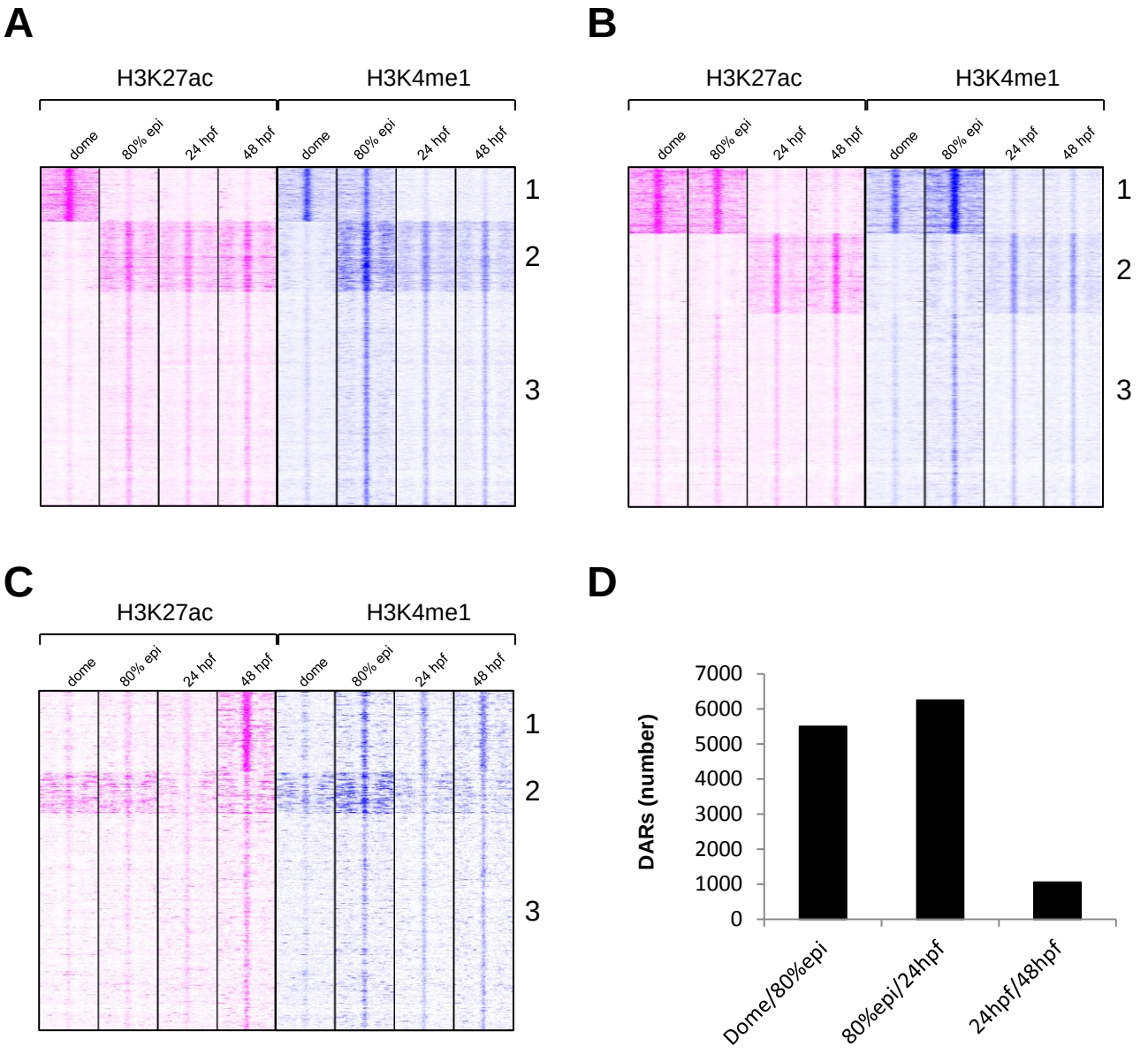


Figure S5. *K*-means clustering of H3K27 Differentially Acetylated Regions (DARs). DARs identified by Fisher's exact test (multiple testing q value cut-off < 0.05) and filtered for > four fold differences in read counts between adjacent stages, were visualized by k-means clustering (k=3, +5kb/-5kb from DAR center). **(A)** Dome VS 80 % epiboly DARs. **(B)** 80% epiboly VS 24 hpf DARs. **(C)** 24 hpf VS 48 hpf DARs. In all three collections of DARs, the first two clusters (1, 2) correspond to regions with substantial temporal changes in read counts whereas cluster 3 corresponds to DARs with low read numbers. **(D)** The highest number of DARs is detected during early development (Dome VS 80% epiboly), whereas that number is considerably lower during at 24 – 48 hpf.

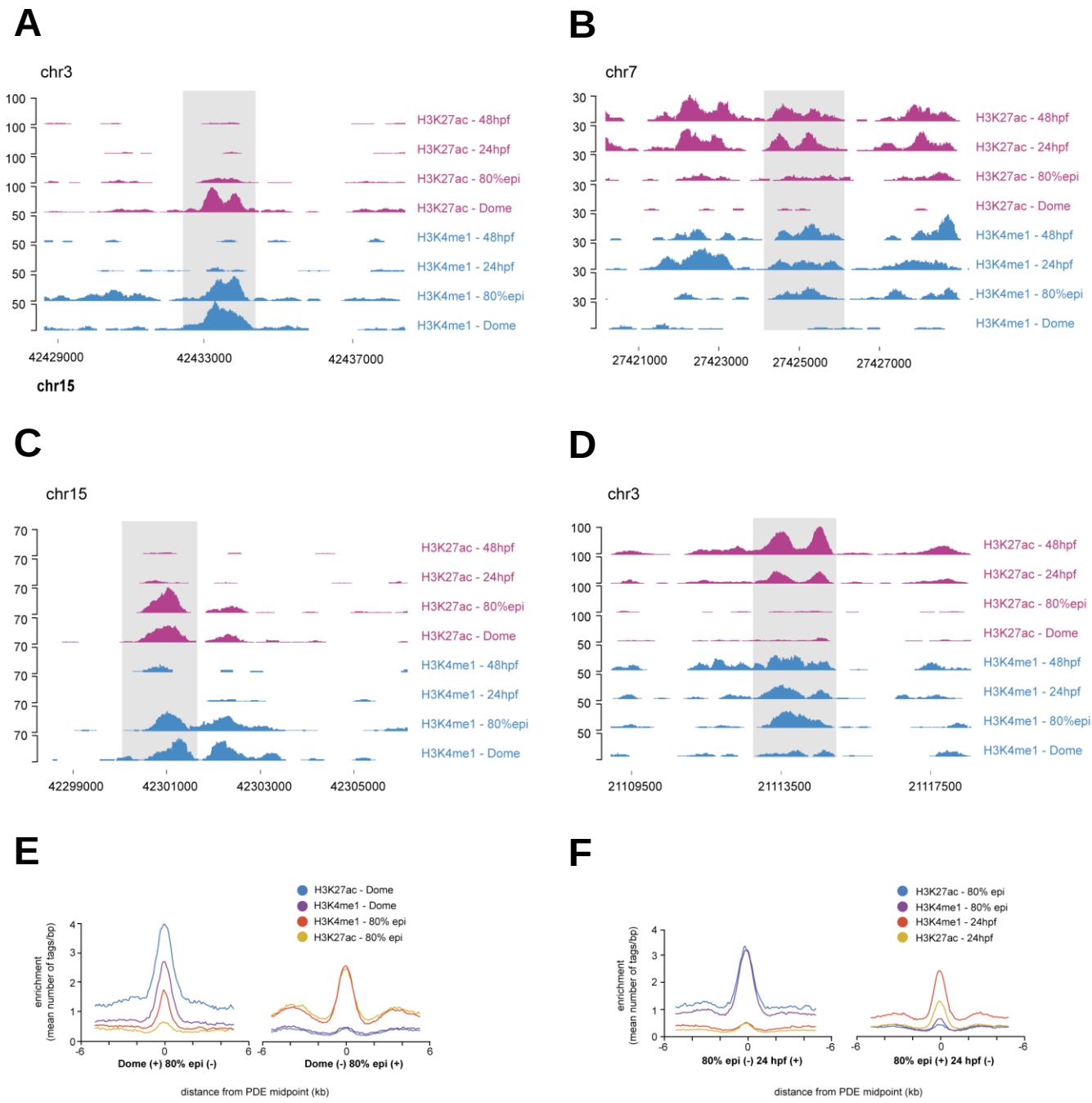


Figure S6. Genomic examples and average histone mark profiles of H3K27 differentially acetylated regions. (A) Example of a Dome(+) 80% epiboly(-) differentially acetylated region. **(B)** Example of a Dome(-) 80% epiboly(+) differentially acetylated region. **(C)** Example of a 80% epiboly(+) 24 hpf (-) differentially acetylated region. **(D)** Example of a 80% epiboly(-) 24 hpf (+) differentially acetylated region. **(E)** Average genomic profiles of dome VS 80% epiboly differentially acetylated regions suggest a synchronized gain of H3K27ac and H3K4me1 at the 80% epiboly stage. (**F)** Similarly to the gain of H3K27ac and H3K4me1 at 80% epiboly, there is a synchronized loss of both H3K27ac and H3K4me1 between 80% epiboly and 24 hpf on these genomic regions

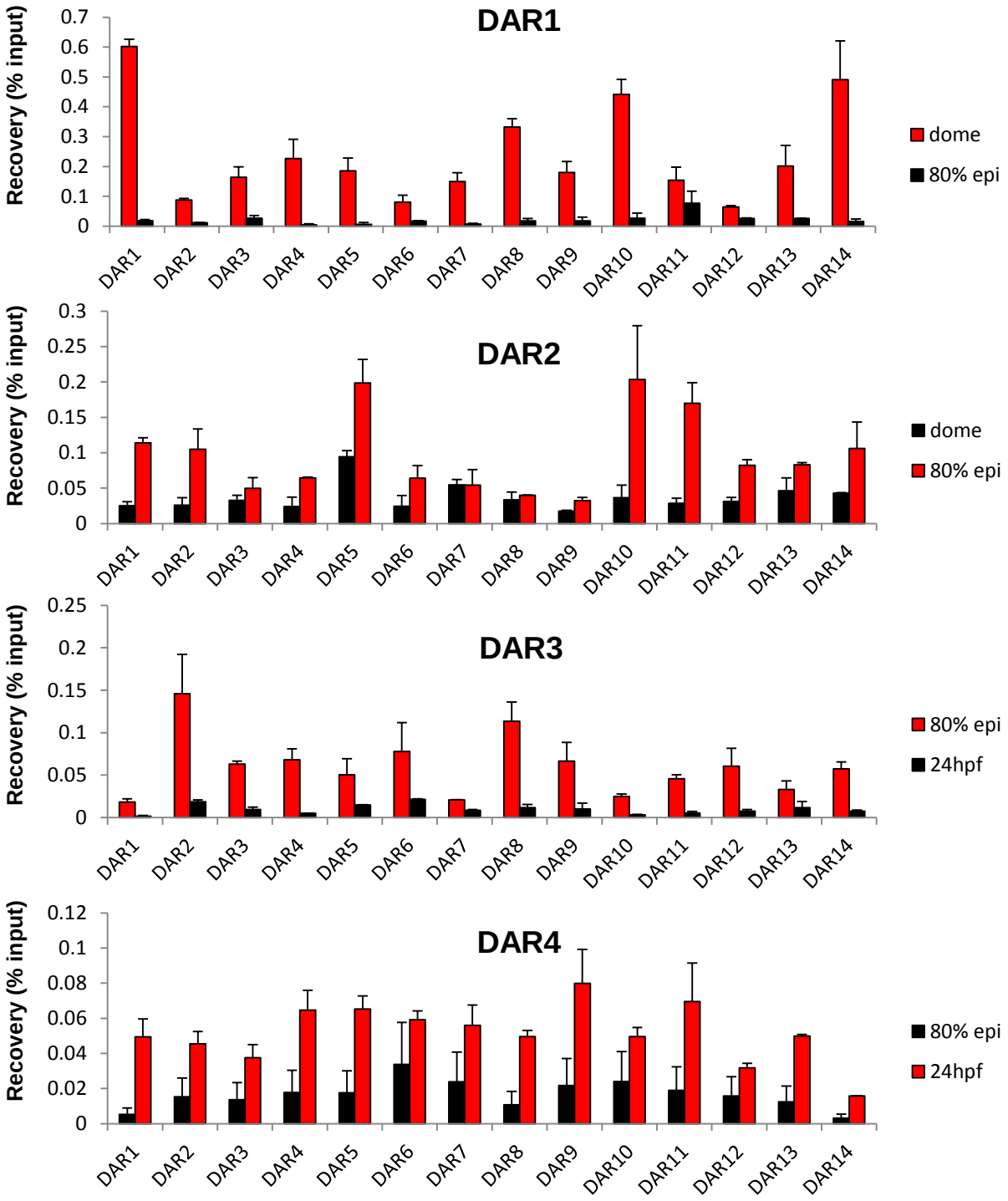


Figure S7. Quantitative PCR validation of Differentially Acetylated Regions (DARs). Differentially H3K27-acetylated regions identified by Fisher's exact test were validated in separate ChIP-QPCR experiments. Each ChIP was performed on 300 embryos corresponding to the described stage. Quantitative PCR reactions were carried out on 1:10 dilutions of ChIP material using the iQ SYBR Green Supermix (Biorad). All four sets of DARs: DAR1 (acetylated at dome, deacetylated at 80% epiboly); DAR2 (deacetylated at dome, acetylated at 80% epiboly); DAR3 (acetylated at 80% epiboly, deacetylated at 24hpf); DAR4 (deacetylated at 80% epiboly, acetylated at 24hpf) were confirmed in this assay. For primer sequences, see Supplemental Table 20.

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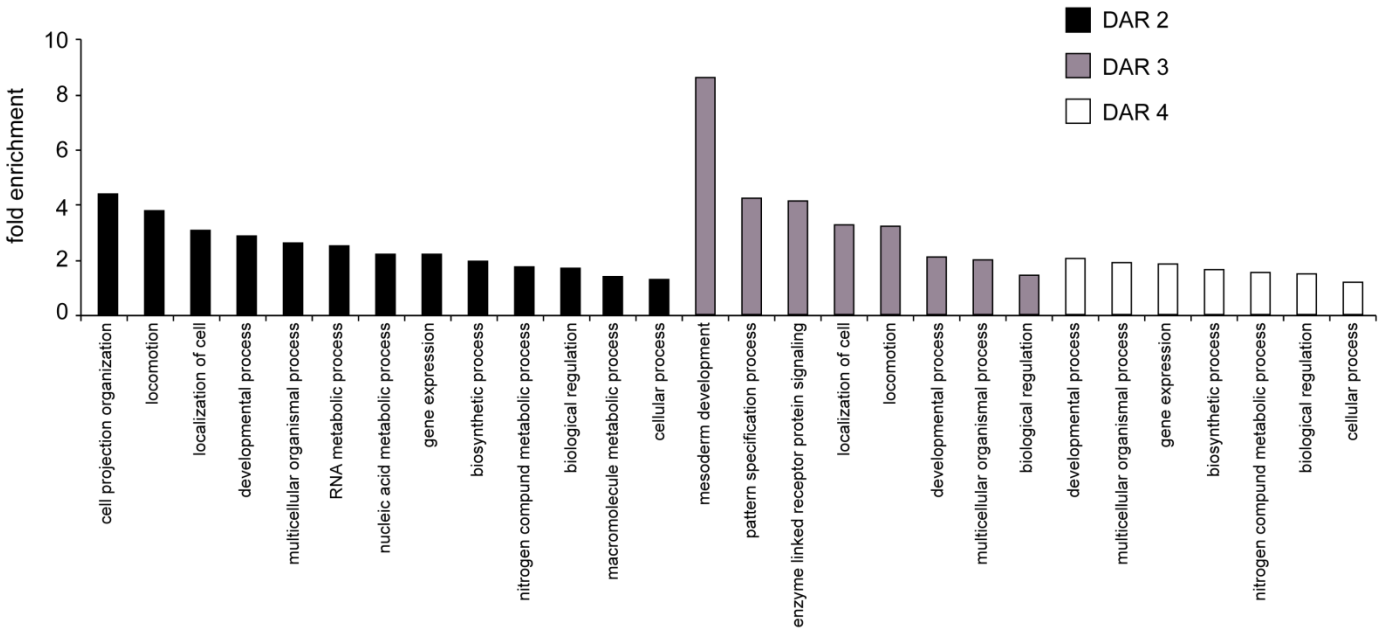

Figure S8. Genes associated with H3K27ac differentially acetylated regions belong to GO categories corresponding to developmental processes. Differentially acetylated regions; DAR2, DAR3 and DAR4 associate with developmental processes. The regions marked with H3K27ac at 80% epiboly but not at 24 hpf (DAR3) are involved in mesoderm development and pattern specification. Only the significantly enriched (*P* < 0.01) GO terms are shown.

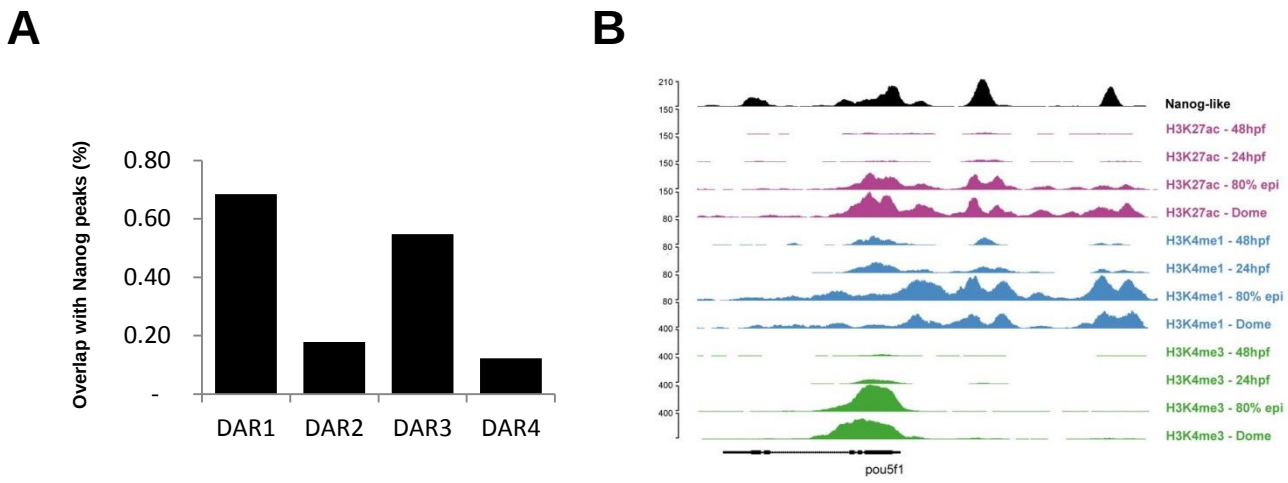

Figure S9. Nanog-like protein associates with early H3K27ac-marked regions and is implicated in pluripotency regulation. (A) Peaks of genomic enrichment of Nanog-like protein at the dome stage, coincide with early DARs (DAR1 and DAR3), regions marked with H3K27ac at dome and 80% epiboly, but not later. Actually, ~60% of early DARs overlap with Nanog-like peaks whereas this number is considerably lower for later DARs. **(B)** Nanog-like protein is enriched in enhancers of pluripotency regulators such as *pou5f1* that are acetylated early (dome and 80% epiboly) but not later.

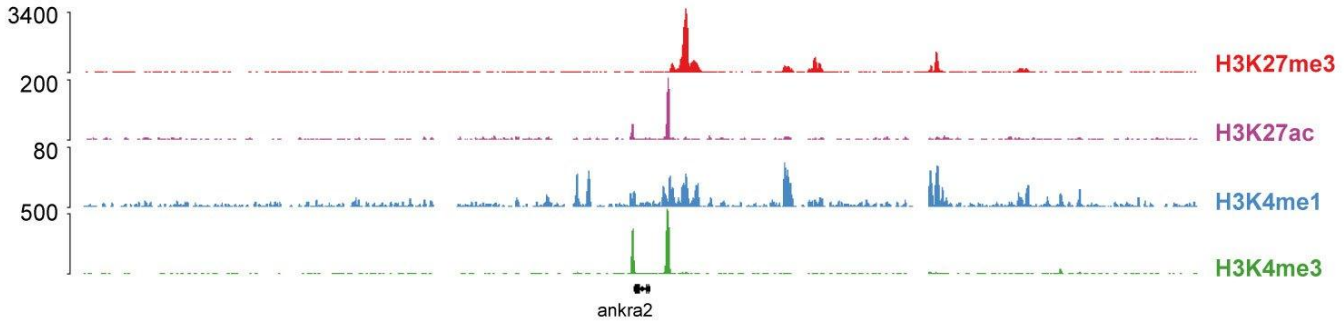

Figure S10. A genomic example of H327me3 peaks. A UCSC genome browser screenshot of H3K37me3, H3K27ac, H3K4me1 and H3K4me3 during gastrulation. As described previously, H3K27me3 and H3K27ac are largely exclusive on the majority of the genomic locations. Regions simultaneously marked with H3K4me1 and H3K27me3 are thought to define either poised or inactive enhancer elements.