



**Supplemental Figure 6.** The regulatory effects of Nanog occupancy. (A) Line plots showing the z-score-transformed expression level (Supplemental Table S3) (White et al. 2017) of early ZGA genes (Heyn et al. 2014) targeted by Nanog (pre-MBT target, MBT new target, post-MBT target and nontarget) at different developmental stages. Pre-MBT target genes reached their expression peak (the dome stage) earlier than MBT new target genes (50%-epiboly) and post-MBT new target genes (50%-epiboly). (B) Bar plots showing the percentage of early ZGA genes (Heyn et al. 2014) downregulated at 4hpf and 6 hpf upon *nanog* MO KD compared to that of the wild-type samples. The earlier the genes were targeted by Nanog, the larger the proportion with a downregulation status, indicating that Nanog pre-MBT binding is critical for zebrafish ZGA. (C) Stacked bar plots showing the expression level (White et al. 2017) and distribution of Nanog pre-MBT target genes in the dome stage (left) and 50%-epiboly stage (right). The NBC target genes showed a higher expression level than other target genes, indicating that the binding of NBC genes can ensure a higher expression level. (D) Box plots showing the normalized Nanog signal at the dome stage in three groups of peaks within NBCs, i.e., emerged from 256-cell stage, 1k-cell stage and dome stage. Peaks that emerged earlier show a greater binding strength.