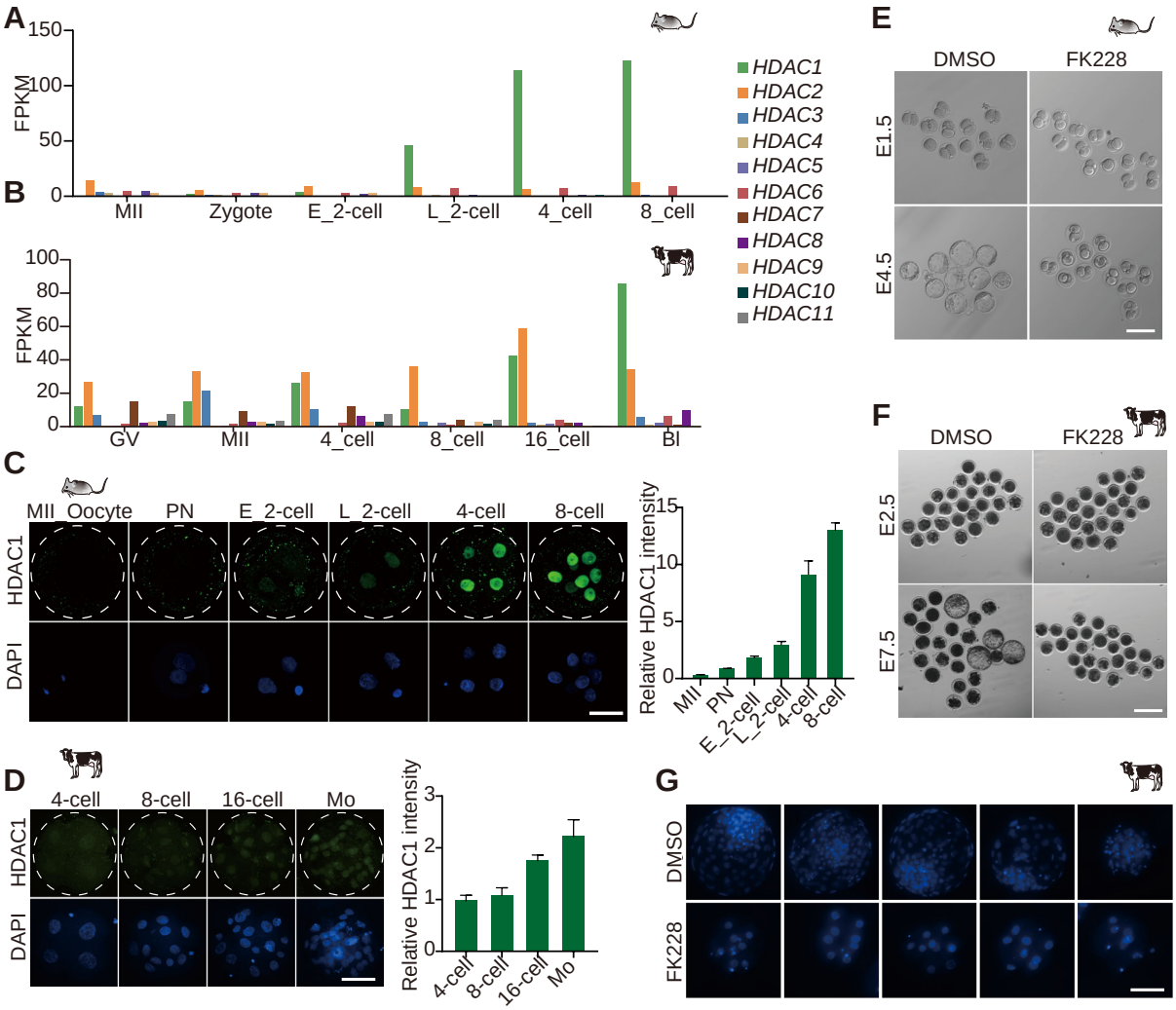

Fig. S1. HDAC1/2 is essential for ZGA in mouse and bovine embryos. A and B, Relative expression of *Hdacs* in mouse (A) and bovine (B) oocytes and preimplantation embryos. **C,** Immunofluorescence staining of HDAC1 for mouse oocytes and preimplantation embryos. Left: representative images, scale bar, 25 μm, right: HDAC1 intensity relative to MII oocytes. Data shown as Means ± s.e.m. (n = 3; 3-7 embryos per group per replicate). **D,** Immunofluorescence staining of HDAC1 for bovine preimplantation embryos. Left: representative images, scale bar, 50 μm, right: HDAC1 intensity relative to 4-cell embryos. Data shown as Means ± s.e.m. (n=3; 3-7 embryos per group per replicate). **E,** Representative images in bright field at day 1.5 and day 4.5 after fertilization of mouse embryos treated with DMSO or FK228. **F,** Representative images in bright field at day 2.5 and day 7.5 after fertilization of bovine embryos treated with DMSO or FK228. **G,** DNA staining with DAPI at day 7.5 after fertilization for bovine embryos treated with DMSO or FK228, scale bar, 50 μm.

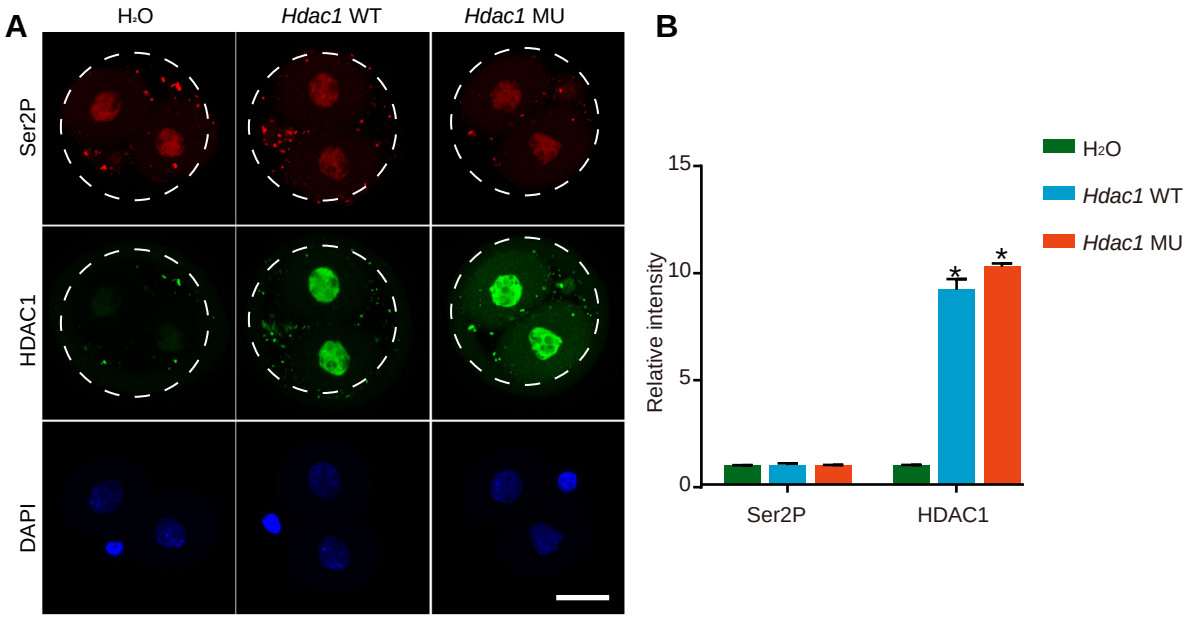

Fig. S2. Global transcriptional activity of mouse embryos. A, Representative images of HDAC1 and Phospho-RNA pol II C-terminal domain (Ser2P) immunofluorescence staining in mouse late 2-cell embryos, scale bar, 25 μm, **B,** HDAC1 and Ser2P intensity relative to H_2O-injected embryos. Data shown as Means ± s.e.m. (n = 3; 3-7 embryos per group per replicate, *$P < 0.05$ (MU or WT vs H_2O))

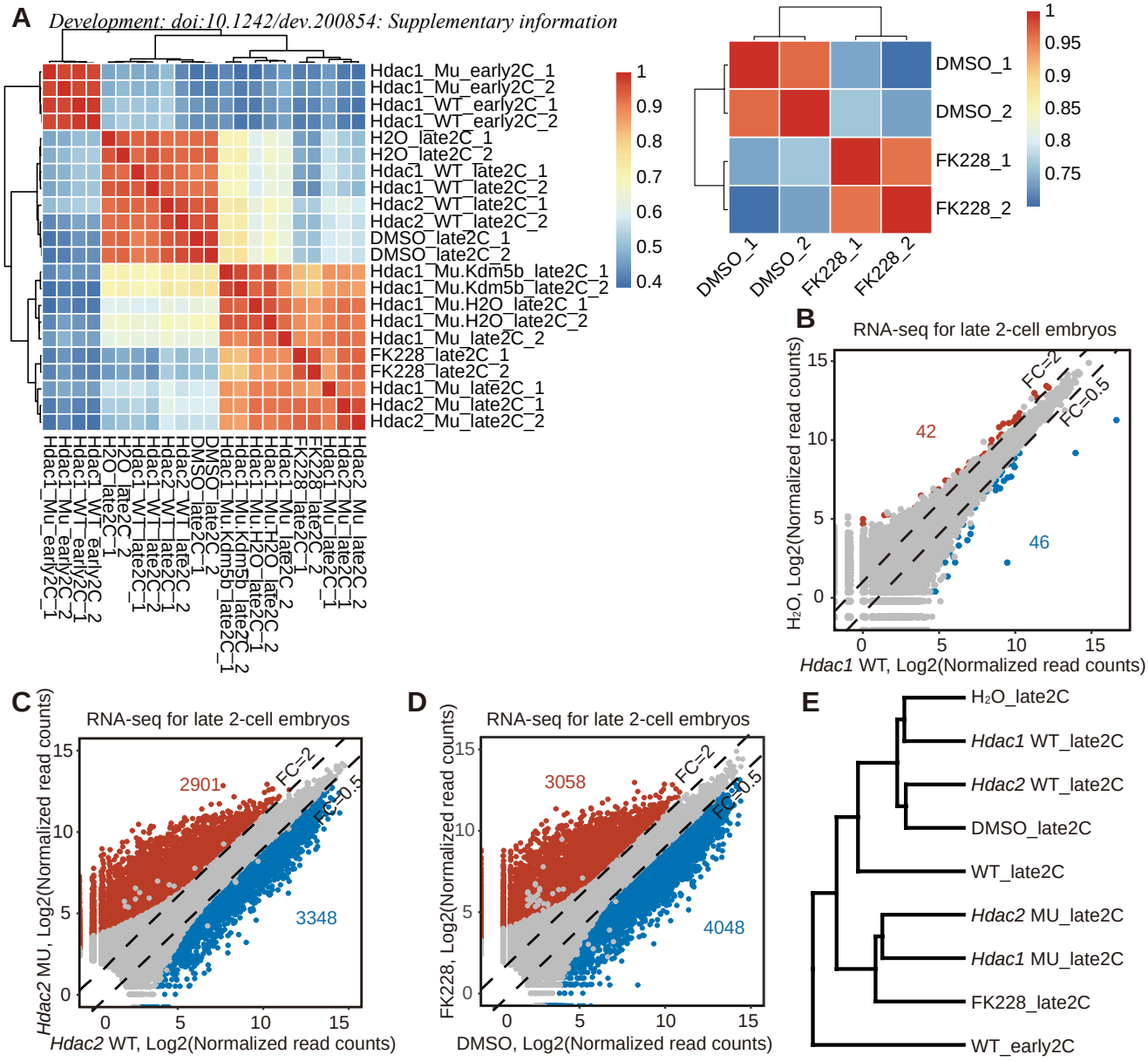
A
Hdac1_Mu_early2C_1
Hdac1_Mu_early2C_2
Hdac1_WT_early2C_1
Hdac1_WT_early2C_2
H2O_late2C_1
H2O_late2C_2
Hdac1_WT_late2C_1
Hdac1_WT_late2C_2
Hdac2_WT_late2C_1
Hdac2_WT_late2C_2
DMSO_late2C_1
DMSO_late2C_2
Hdac1_Mu.Kdm5b_late2C_1
Hdac1_Mu.Kdm5b_late2C_2
Hdac1_Mu.H2O_late2C_1
Hdac1_Mu.H2O_late2C_2
Hdac1_Mu_late2C_2
FK228_late2C_1
FK228_late2C_2
Hdac1_Mu_late2C_1
Hdac2_Mu_late2C_1
Hdac2_Mu_late2C_2
1
0.9
0.8
0.7
0.6
0.5
0.4
DMSO_1
DMSO_2
FK228_1
FK228_2
1
0.95
0.9
0.85
0.8
0.75
B
RNA-seq for late 2-cell embryos
H2O, Log2(Normalized read counts)
Hdac1 WT, Log2(Normalized read counts)
FC=2
FC=0.5
42
46
C
RNA-seq for late 2-cell embryos
Hdac2 MU, Log2(Normalized read counts)
Hdac2 WT, Log2(Normalized read counts)
2901
3348
D
RNA-seq for late 2-cell embryos
FK228, Log2(Normalized read counts)
DMSO, Log2(Normalized read counts)
3058
4048
E
H2O_late2C
Hdac1 WT_late2C
Hdac2 WT_late2C
DMSO_late2C
WT_late2C
Hdac2 MU_late2C
Hdac1 MU_late2C
FK228_late2C
WT_early2C
F
Differential Expressed Genes
(Hdac2 MU vs Hdac2 WT, padj <= 0.05)
UP (FC > 2)
DOWN (FC < 0.5)
Maternal
Minor ZGA
Major ZGA
Developmental
Log2 (observed/expected)
-Log10(pvalue)
< 3
> 15
G
Differential Expressed Genes
(FK228 vs DMSO, padj <= 0.05)
UP (FC > 2)
DOWN (FC < 0.5)
Maternal
Minor ZGA
Major ZGA
Developmental
Log2 (observed/expected)
-Log10(pvalue)
< 3
> 15
H
Hdac2 MU1
Hdac2 MU2
Hdac2 WT1
Hdac2 WT2
FK228_2
FK228_1
DMSO_2
DMSO_1

Fig. S3. Repression of HDAC1/2 dysregulates gene expression during mouse ZGA. A, Heatmaps showing spearman correlation coefficient of two biological replicates for mouse (left) and cattle (right) RNA-seq in this study. **B-D,** Scatter plots showing expression levels of genes in late 2-cell mouse embryos injected with *Hdac1* WT mRNA or H_2O (B) and injected with *Hdac2* WT mRNA or *Hdac2* mutant mRNA (C) and treated with DMSO or FK228 (D). Two RNA-seq replicates are generated for differential expression analysis, and the read counts are normalized by DESeq2. Dash lines indicate the threshold of fold change, and grey dots refer to genes with *Padj* > 0.05, while dots in red and blue refer to genes with *Padj* <= 0.05. Numbers of up- and down- regulated genes are also indicated in the figures. **E,** Hierarchical clustering of mouse late 2-cell embryos in different groups and wildtype embryos (WT_late2C, WT_early2C(Wu et al., 2016)) based on the FPKM in RNA-seq data. **F and G,** Overlap of all differentially expressed genes in late 2-cell embryos (F, *Hdac2* MU vs *Hdac2* WT, G, FK228 vs DMSO) with different gene sets. The gene sets are generated with *k-means* clustering of RNA-seq data(Wu et al., 2016) for mouse oocytes and zygotes, early 2-cell, late 2-cell, 8-cell embryos and ICM. The color of bubbles refers to $\log_2$ ratio of number of observed genes in the gene set to randomly expected frequencies, and the size of bubbles shows $-\log_{10}$ of Fisher exact test *p* value. **H,** Heatmaps showing expression of all major ZGA genes at late 2-cell stage.

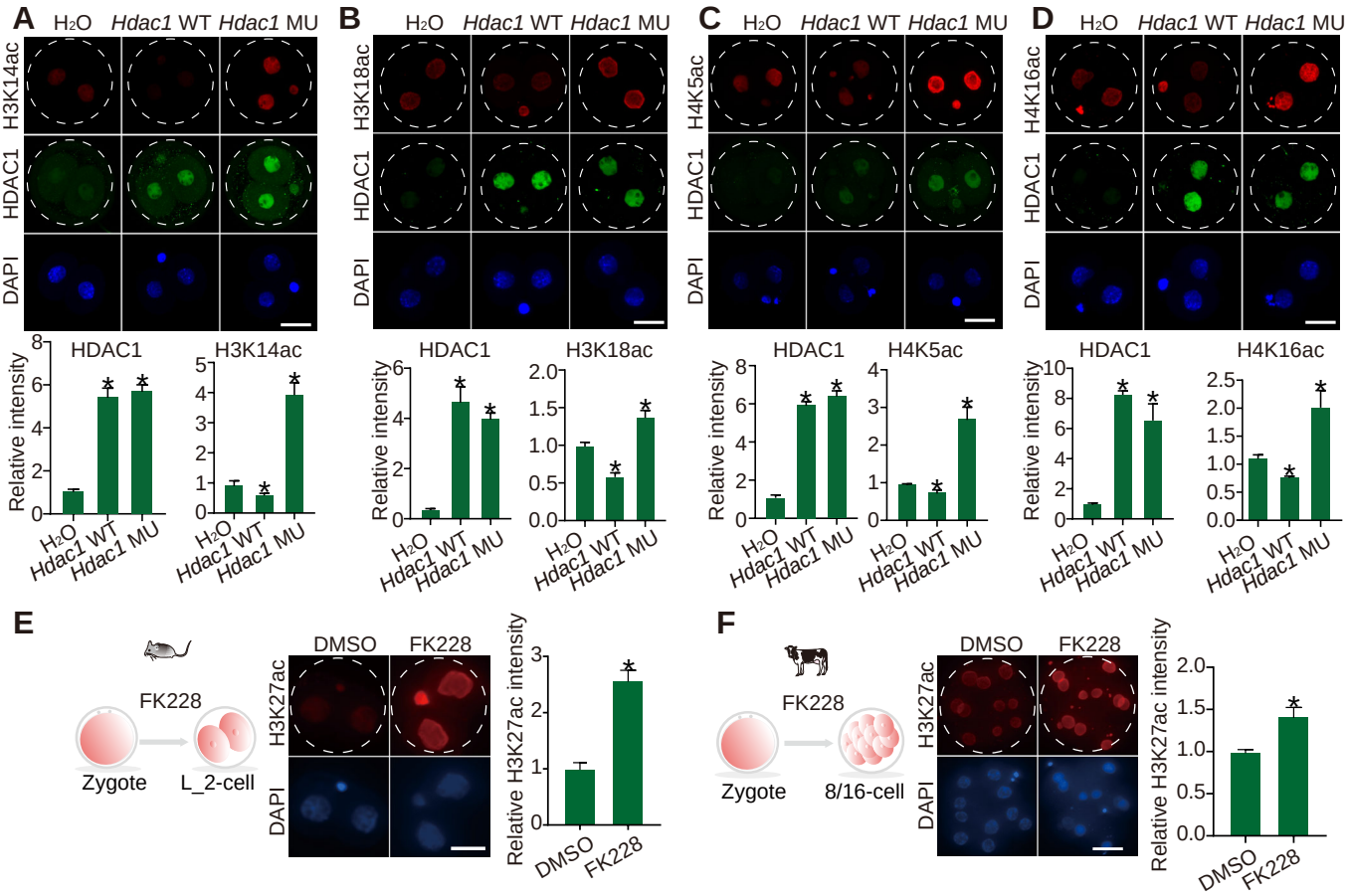

Fig. S4. The histone deacetylase activity of HDAC1/2 is required for reprogramming of histone acetylation during ZGA. A, Immunofluorescence staining (top) of HDAC1 and H3K14ac in late 2-cell embryos, scale bar: 25 μm. HDAC1 and H3K14ac (bottom) intensity relative to embryos injected with H_2O. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$ (*Hdac1* WT or MU vs H_2O)). **B,** Immunofluorescence staining (top) of HDAC1 and H3K18ac in late 2-cell embryos, scale bar: 25 μm. HDAC1 and H3K18ac (bottom) intensity relative to embryos injected with H_2O. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$ (*Hdac1* WT or MU vs H_2O)). **C,** Immunofluorescence staining (top) of HDAC1 and H4K5ac in late 2-cell embryos, scale bar: 25 μm. HDAC1 and H4K5ac (bottom) intensity relative to embryos injected with H_2O. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$ (*Hdac1* WT or MU vs H_2O)). **D,** Immunofluorescence staining (top) of HDAC1 and H4K16ac in late 2-cell embryos, scale bar: 25 μm. HDAC1 and H4K16ac (bottom) intensity relative to embryos injected with H_2O. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$ (*Hdac1* WT or MU vs H_2O)). **E,** Experimental scheme (left) showing H3K27ac immunofluorescence staining at mouse late 2-cell stage (mid), scale bar, 25 μm, H3K27ac intensity relative to DMSO-treated embryos is showed in the right panel. **F,** Experimental scheme (left) showing H3K27ac immunofluorescence staining (mid) at bovine 8/16-cell stage, scale bar, 50 μm, H3K27ac intensity relative to DMSO-treated embryos is showed in the right panel.

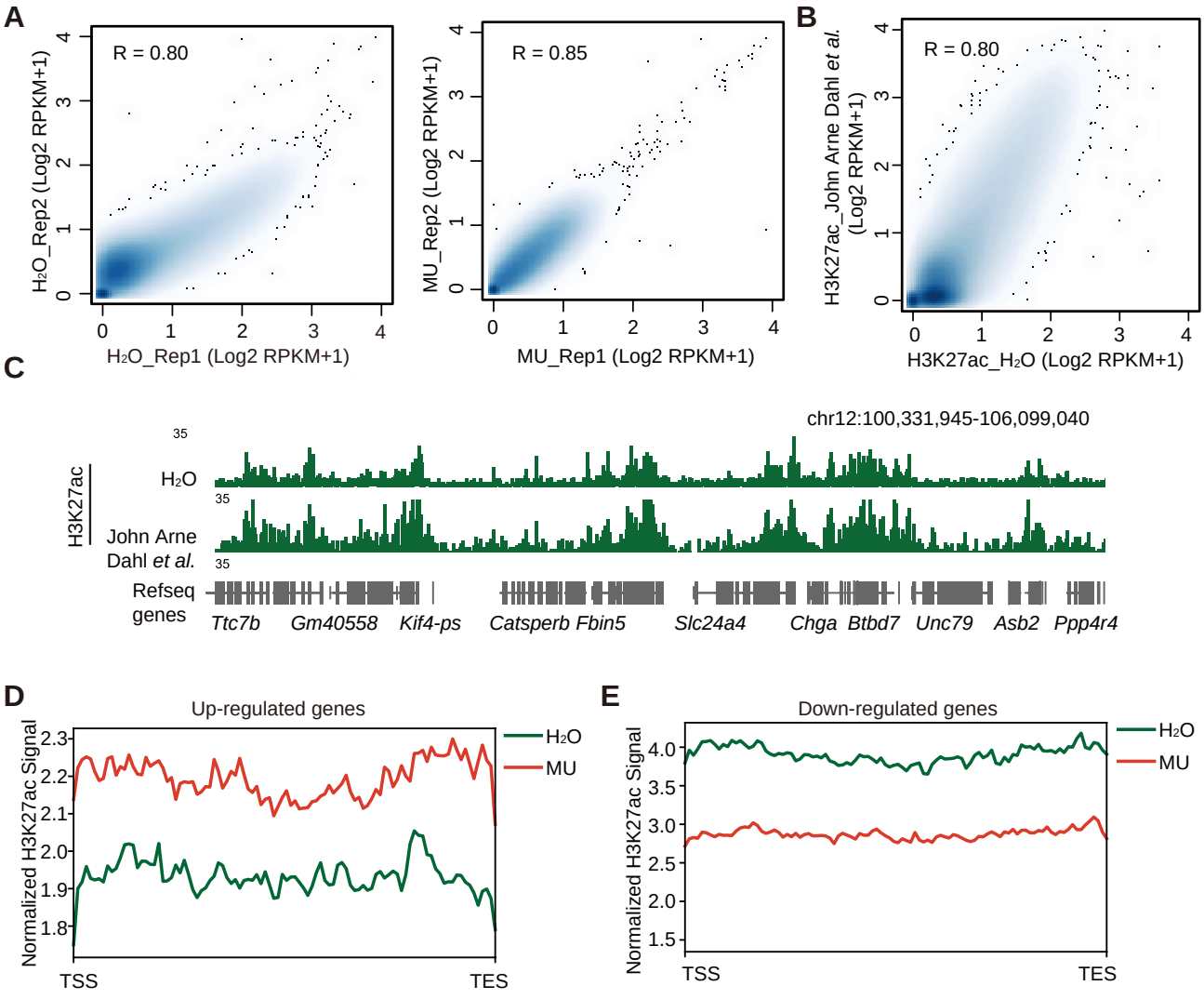

Fig. S5. HDAC1 regulates the distribution of H3K27ac during mouse ZGA. A, Scatter plots showing the H3K27ac signal (RPKM for 2-kb bin across the genome) between two biological replicates. **B,** Scatter plots showing the H3K27ac signal (RPKM for 2-kb bin across the genome) in late 2-cell embryos between data sets of this study and John Arne Dahl *et al*., R refers to Pearson correlation coefficient. **C,** Browser snapshots of H3K27ac in late 2-cell embryos injected with H_2O and published ChIP-seq data. **D and E,** H3K27ac signal in gene body regions of up-regulated (*Hdac1* MU/WT, D) and down-regulated (*Hdac1* MU/WT, E) genes.

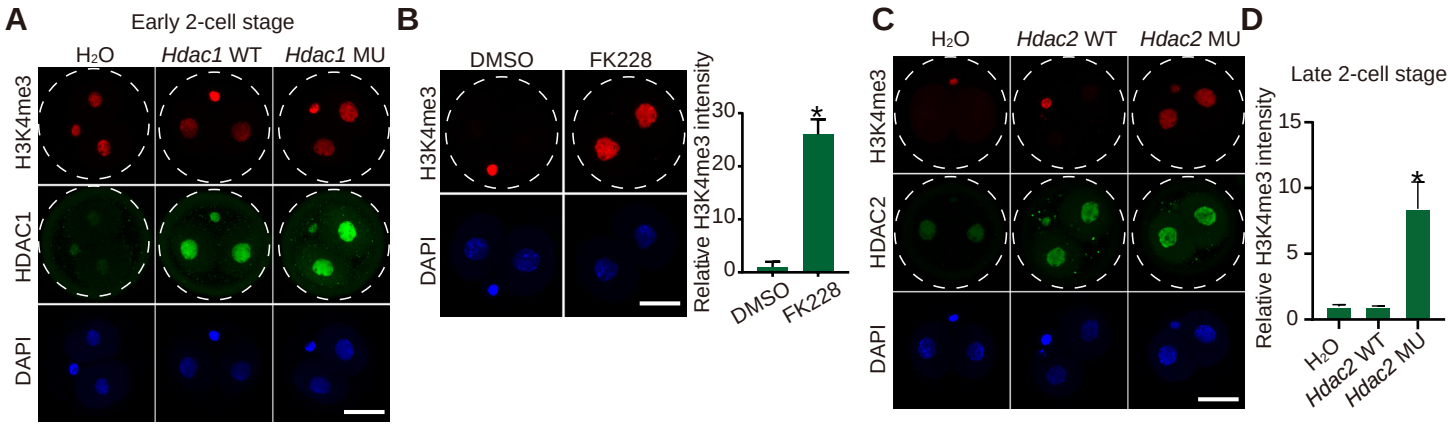

Fig. S6. HDAC1/2 is required for removal of H3K4me3 during ZGA. A, Immunofluorescence staining of H3K4me3 and HDAC1 in mouse embryos at early 2-cell stage. **B,** Immunofluorescence staining of H3K4me3 embryos at late 2-cell stage. Left, representative images, scale bar, 25 μm. right, H3K4me3 intensity relative to DMSO-treated embryos. Data shown as Means ± s.e.m. (n = 3; 3-7 embryos per group per replicate, *$P < 0.05$). **C,** Immunofluorescence staining of H3K4me3 and HDAC2 in mouse embryos at late 2-cell stage. **D,** H3K4me3 intensity relative to H_2O injection embryos. Data shown as Means ± s.e.m. (n = 3; 3-7 embryos per group per replicate, *$P < 0.05$ (MU vs WT or H_2O)).

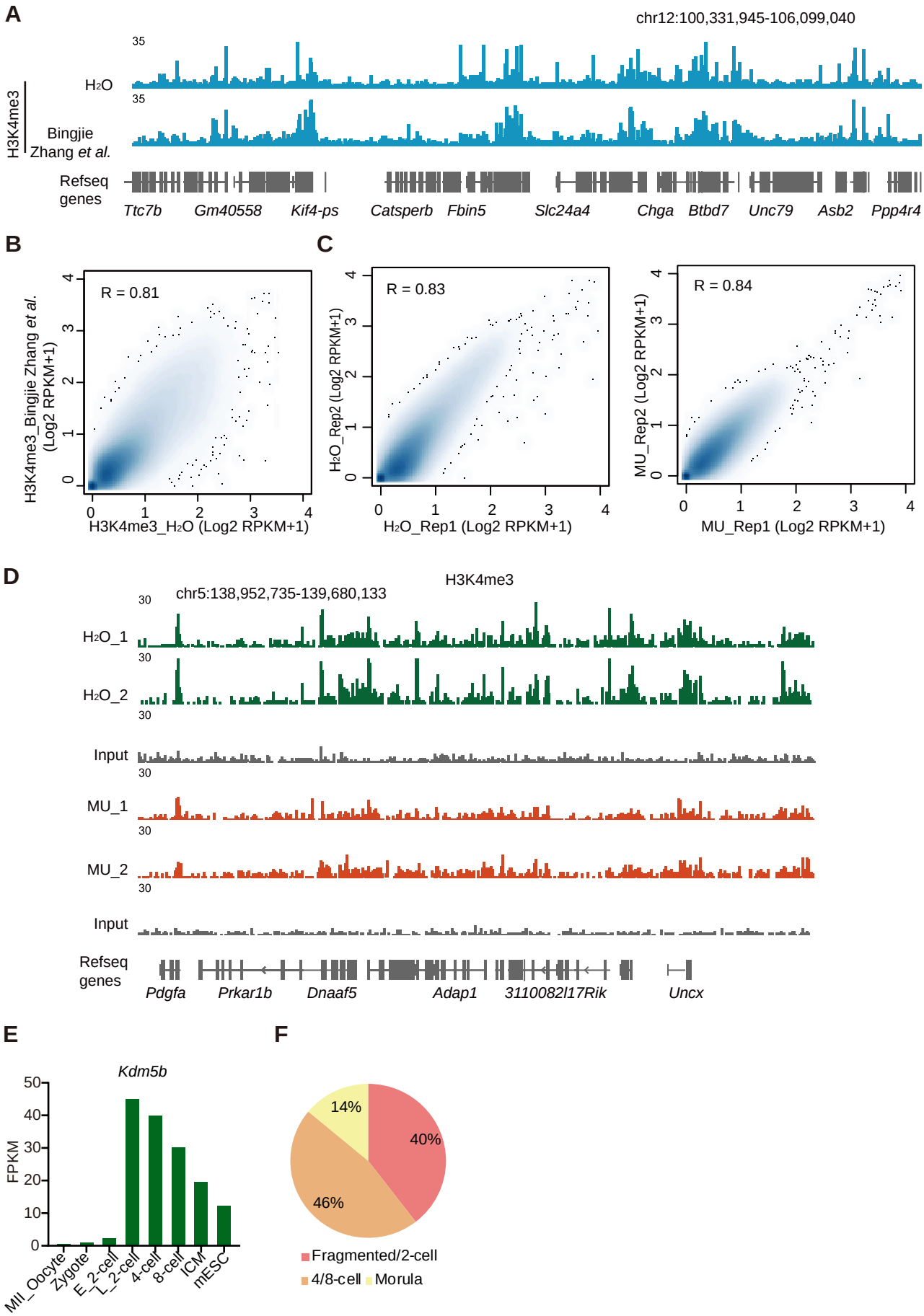

Fig. S7. H3K4me3 ULI-NChIP- seq validation. A, Browser snapshots of H3K4me3 in late 2-cell embryos injected with H_2O and published ChIP-seq data. **B,** Scatter plots showing the H3K4me3 signal (RPKM for 2-kb bin across the genome) in late 2-cell embryos between data sets in this study and Bingjie Zhang *et al*., R refers to Pearson correlation coefficient. **C,** Scatter plots showing the H3K4me3 signal (RPKM for 2-kb bin across the genome) between two biological replicates. **D,** Browser snapshots showing H3K4me3 signals in two biological replicates of embryos injected with H_2O or *Hdac1* mutant mRNA. **E,** Relative expression of *Kdm5b* in mouse oocytes, preimplantation embryos and mouse embryonic stem cells (mESCs). **F,** Distribution of cleavage embryos found in embryos injected with *Hdac1* mutant and *Kdm5b* mRNA at day 3.75 after fertilization.

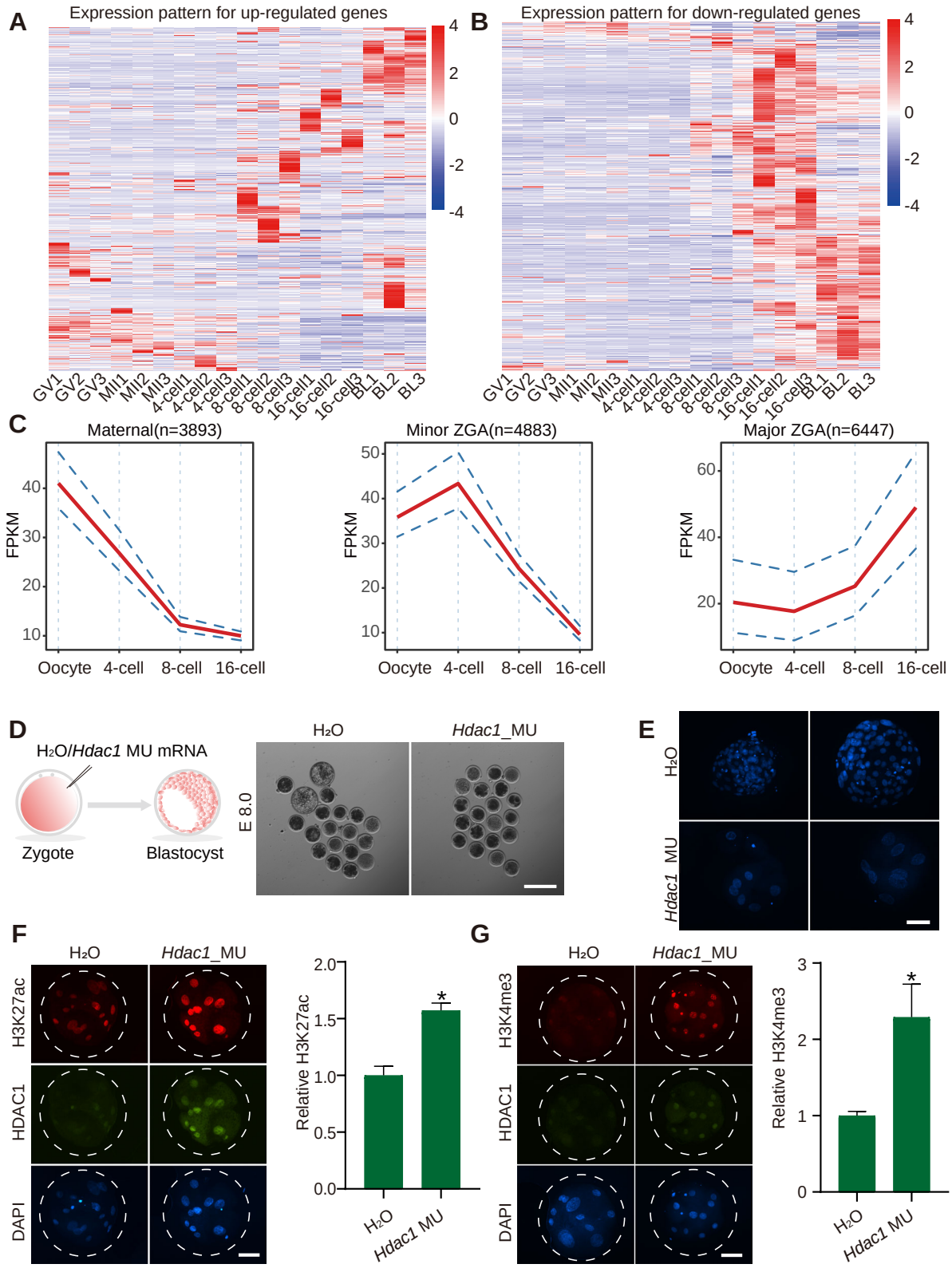

Fig. S8. HDAC1/2 is crucial for bovine ZGA. A and B, Expression pattern of all up-regulated genes (FK228/DMSO, A) and down-regulated genes (FK228/DMSO, B) in bovine oocytes and preimplantation embryos using published RNA-seq data(Graf et al., 2014). **C,** Three distinct gene expression patterns during bovine embryo development. The three gene categories are generated by *k-means* clustering of RNA-seq data(Graf et al., 2014) for bovine MII oocytes and 4-cell, 8-cell, 16-cell embryos and blastocysts. Red lines showing the average FPKM, and dashed blue lines showing 95% confidence interval around the mean. The number of genes in each cluster is also indicated in the brackets. **D**, Experimental scheme (left) for *Hdac1* MU mRNA injection and representative images in bright field (right) on day 8.0 after fertilization of bovine embryos. **E,** DNA staining with DAPI at day 8.0 after fertilization for bovine embryos. **F,** H3K27ac and HDAC1 immunofluorescence staining for bovine 8/16-cell embryos. Left: representative images, scale bar, 50 μm, right: H3K27ac intensity relative to H_2O-injected embryos. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$). **G,** H3K4me3 and HDAC1 immunofluorescence staining for bovine 8/16-cell embryos. Left: representative images, scale bar, 50 μm, right: H3K4me3 intensity relative to H_2O-injected embryos. Data shown as Means ± s.e.m. (n = 3; 3-10 embryos per group per replicate, $*P < 0.05$).

Table S1. Antibody information

Name	Host	Company	Catalog Number	Application
HDAC1	Mouse	Cell Signaling Technology	#5356	IF (1:200)
HDAC2	Mouse	Cell Signaling Technology	#5113	IF (1:200)
H3K27ac	Rabbit	Active Motif	39133	IF (1:200)
H3K4me3	Rabbit	Cell Signaling Technology	#9751	IF (1:200)
Ser2P	Rabbit	Abcam	Ab5095	IF (1:200)
Donkey anti-Rabbit 594		Invitrogen	A21207	IF (1:100)
Goat anti-Mouse 488		Invitrogen	A11001	IF (1:100)

Table S2. Differentially expressed genes identified by RNA-seq between Hdac1 MU and Hdac1 WT mRNA injection group in mice late 2-cell embryos (ranked by adjusted padj). Threshold: padj <= 0.05 & FoldChange >= 2 or FoldChange <= 0.5.

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Table S3. GO enrichment of up-regulated differentially expressed genes.

Click here to download Table S3

Table S4. GO enrichment of down-regulated differentially expressed genes.

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