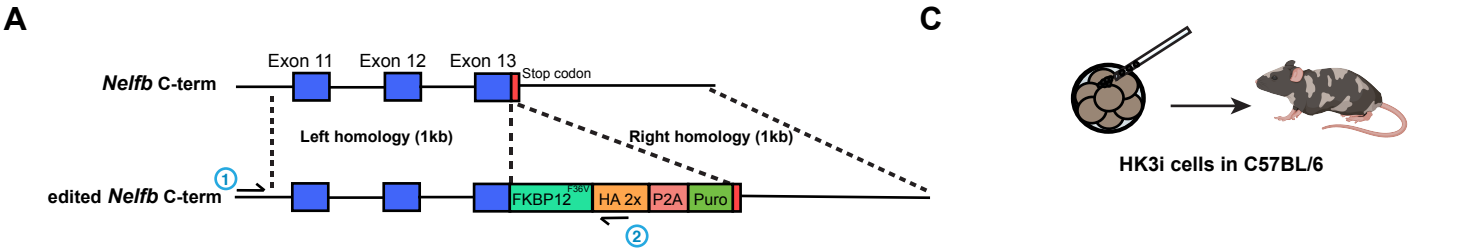
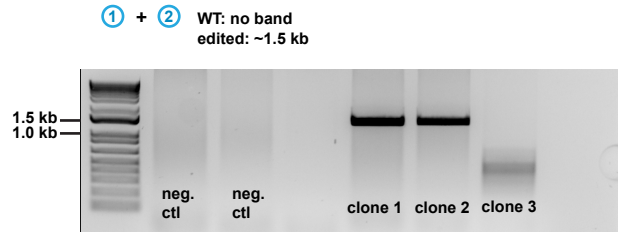
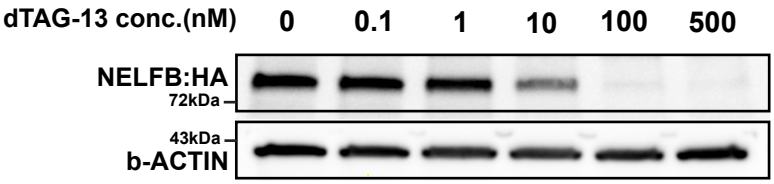
Figure S1

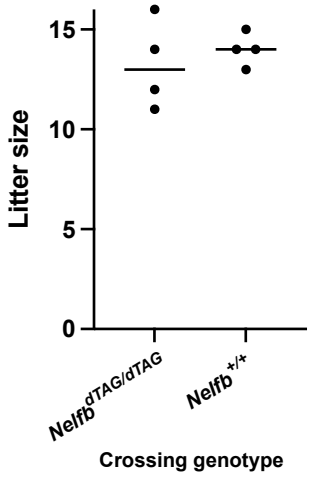


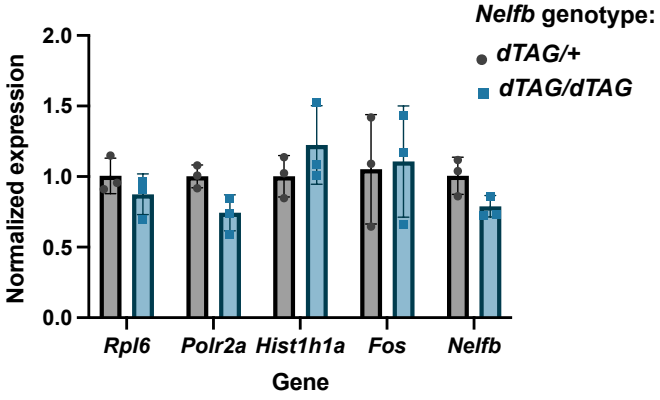


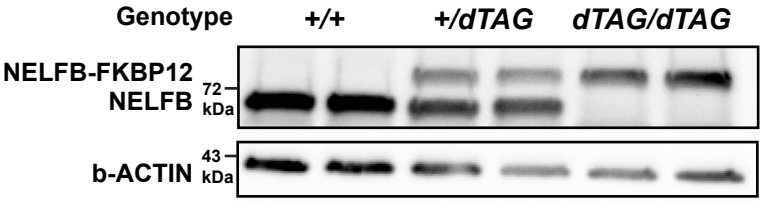


E

Genotype	number (%)
Nelfb$^{+/+}$	20 (24%)
Nelfb$^{dTAG/+}$	39 (48%)
Nelfb$^{dTAG/dTAG}$	23 (28%)
Total	82 (100%)







I

Genotype	number (%)
Nelfb$^{+/+}$	2 (12.5%)
Nelfb$^{dTAG/+}$	4 (25%)
Nelfb$^{-/+}$	5 (31%)
Nelfb$^{dTAG/-}$	5 (31%)
Total	16 (100%)

Figure S1. Genetic targeting and mouse generation strategies, related to Figure 1.

(A) *Nelfb*^{dTAG} knock-in strategy. CRISPR-Cas9 was used to generate a double-stranded break a few bases before the stop codon. The HDR template incorporates the insert immediately before the stop codon.
(B) PCR validation of targeted and expanded mESC clones. Primers targets are shown in A and sequence is available in Table S2.
(C) Strategy of chimeric mice generation. Further details are available in the methods section.
(D) Resulting genotypes from heterozygous *Nelfb*$^{dTAG/+}$ crossing show mendelian ratios of expected genotypes. The data suggests that the edited allele is functional.
(E) Litter sizes from homozygous *Nelfb*$^{dTAG/dTAG}$ crossings are normal.
(F) RT-qPCR of *Nelfb* and several gene targets in RNA extracted from *Nelfb*$^{dTAG/+}$ and *Nelfb*$^{dTAG/dTAG}$ mice livers. The expression is normalized to *Actb* then to the expression of heterozygous samples. N=3 biological replicates per genotype.
(G) Western blot analysis of NELFB and NELFB-FKBP12^{F36V} in liver samples.
(H) Western blot analysis of NELFB:HA in tagged mESCs following 30 mins of degradation with varying concentrations of dTAG-13.
(I) Resulting genotypes from heterozygous *Nelfb*$^{dTAG/+}$ x *Nelfb*$^{+/-}$ mouse suggest that the edited allele complements a null *Nelfb* allele.

Figure S2

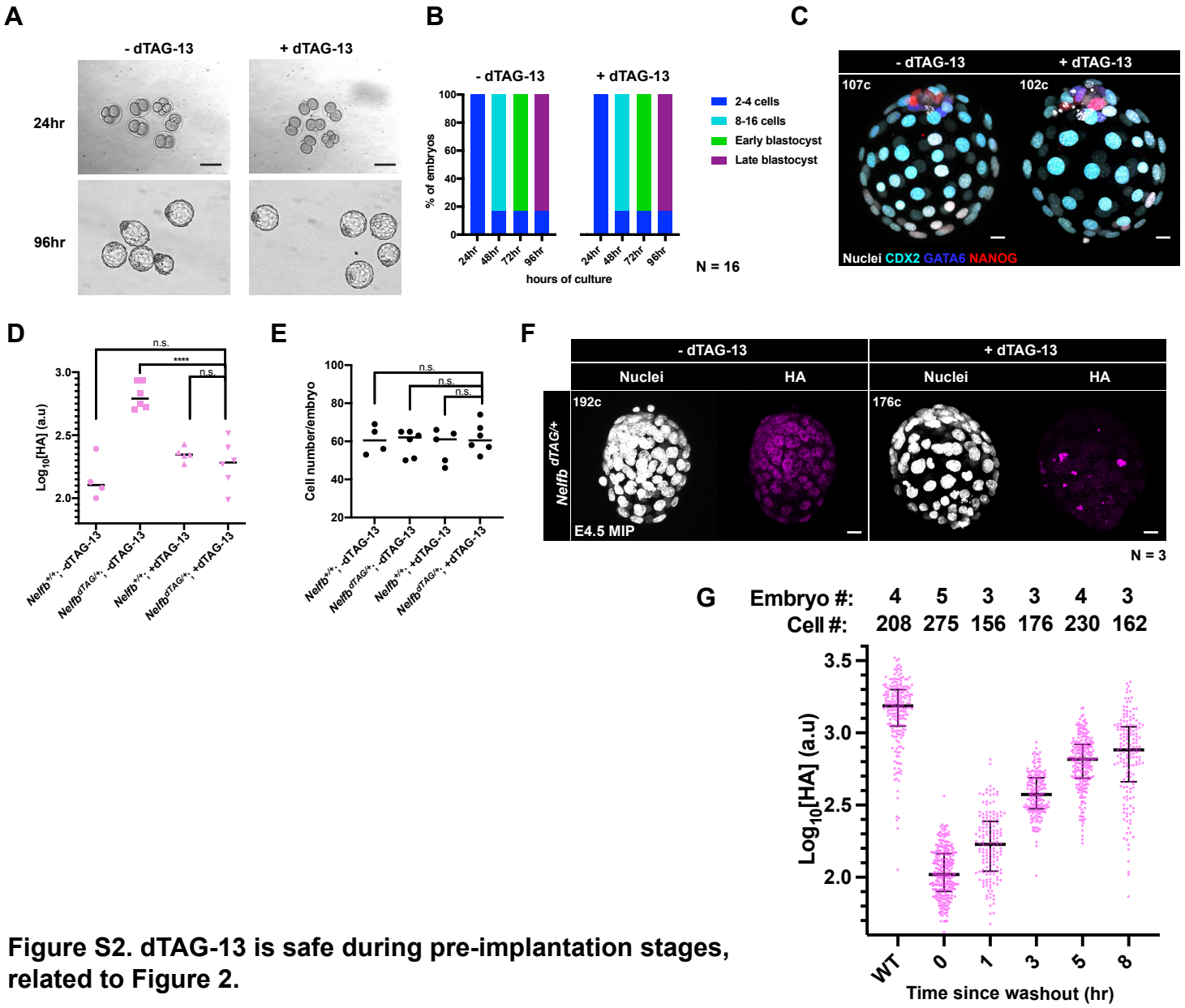


Figure S2. dTAG-13 is safe during pre-implantation stages, related to Figure 2.

(A) Widefield images of zygotes *ex utero* culture +/- dTAG-13. Scale bar, 50μm.
(B) Percentages of cultured zygotes passing each developmental stage +/- dTAG-13.
(C) Immunofluorescence of 96hrs blastocysts post zygote culture +/- dTAG-13. NANOG marks the epiblast, GATA6 marks the primitive endoderm, and CDX2 marks the trophectoderm lineage. Nuclei were labeled with Hoechst. Scale bar, 50μm.
(D) Quantification of mean HA signal of nuclei/embryo. Same data presented in Figure 2C but averaged per embryo.
(E) Average number of nuclei/embryo post culture +/-dTAG-13 for 1hr. Cell number was determined by counting nuclei stained with Hoechst.
(F) Immunofluorescence of E4.5 embryos after 1hr culture +/- dTAG-13. Nuclei are labeled with Hoechst. Total cell count per embryo is shown in the top left corner. Scale bar, 15μm.
(G) Quantification of E3.5 immunofluorescence of HA to determine recovery dynamics following degradation using 50nM dTAG-13 instead of 500nM, related to Figure 2D.

For all experiments, maximum intensity projection (MIP) is shown in images. Plots show each data point with groups means. Student t-test was used to determine significance. Statistical significance is classified based on p-value as: n.s. > 0.05, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

Figure S3

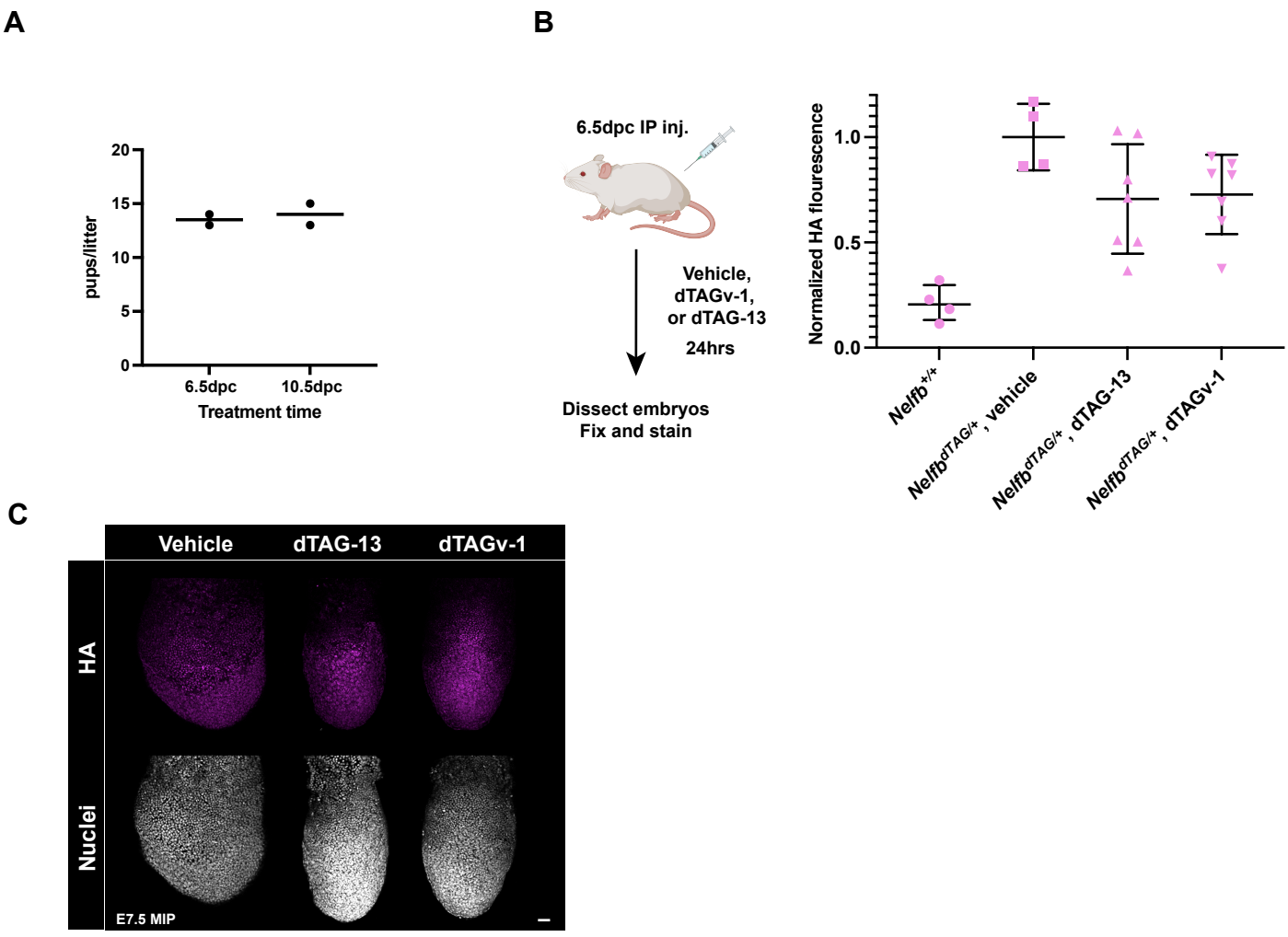


Figure S3. Safety and reversibility of dTAG-13 and dTAGv-1 *in vivo*, related to Figure 3.

(A) Number of pups delivered by pregnant females after 35mg kg-1 dTAG-13 injection at the indicated time points. Bar represents average of two litters.
(B) (Left) Schematic of the experiment. *Nelfb*$^{dTAG/+}$ pregnant females were injected with dTAGv-1 or dTAG-13 6.5dpc. Embryos were collected for analysis 24hrs later. Quantification of mean HA signal intensity in recovered embryos 24hrs post dTAGv-1 or dTAG-13 inj.
(C) Immunofluorescence images of E7.5 embryos 24hrs post dTAGv-1 or dTAG-13 injection to 6.5dpc pregnant females. Nuclei are labeled with Hoechst. Scale bar, 50μm.
For all experiments, maximum intensity projection (MIP) is shown in images. Plots show each data point with groups means and interquartile range.

Figure S4

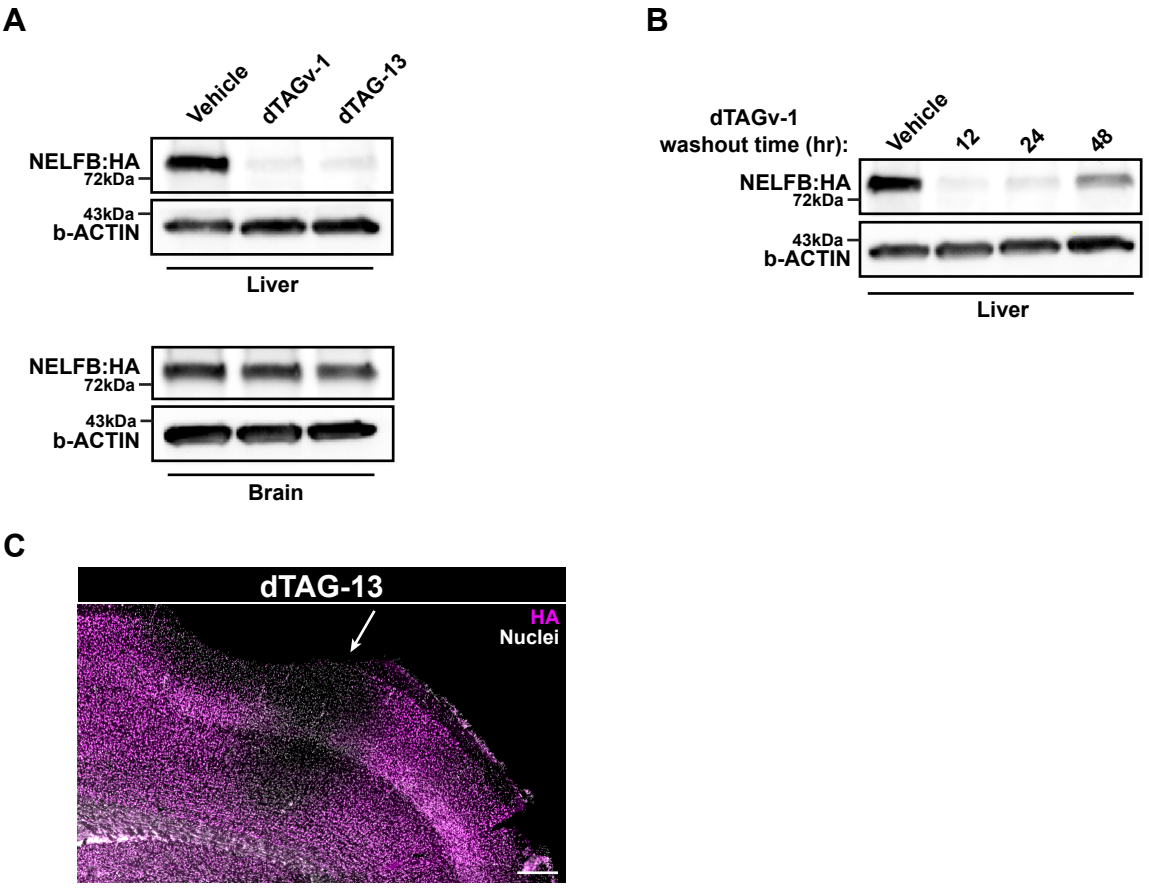


Figure S4. Establishing efficiency of the dTAG system in adult mouse tissues, related to Figure 4.

(A) Western blot analysis of NELFB-FKBP12^{F36V} degradation post 6hrs dTAGv-1 or dTAG-13 IP injection in adult liver and brain.
(B) Western blot analysis of NELFB-FKBP12^{F36V} recovery following a single injection of dTAGv-1.
(C) Immunofluorescence of brain coronal section showing the cortex (outer layer) post dTAG-13 intracerebral injection. Low magnification of dTAG-13 image in Figure 4B. Arrow points to the injection region. Nuclei were labeled by Hoechst. Scale bar, 250μm.

Figure S5

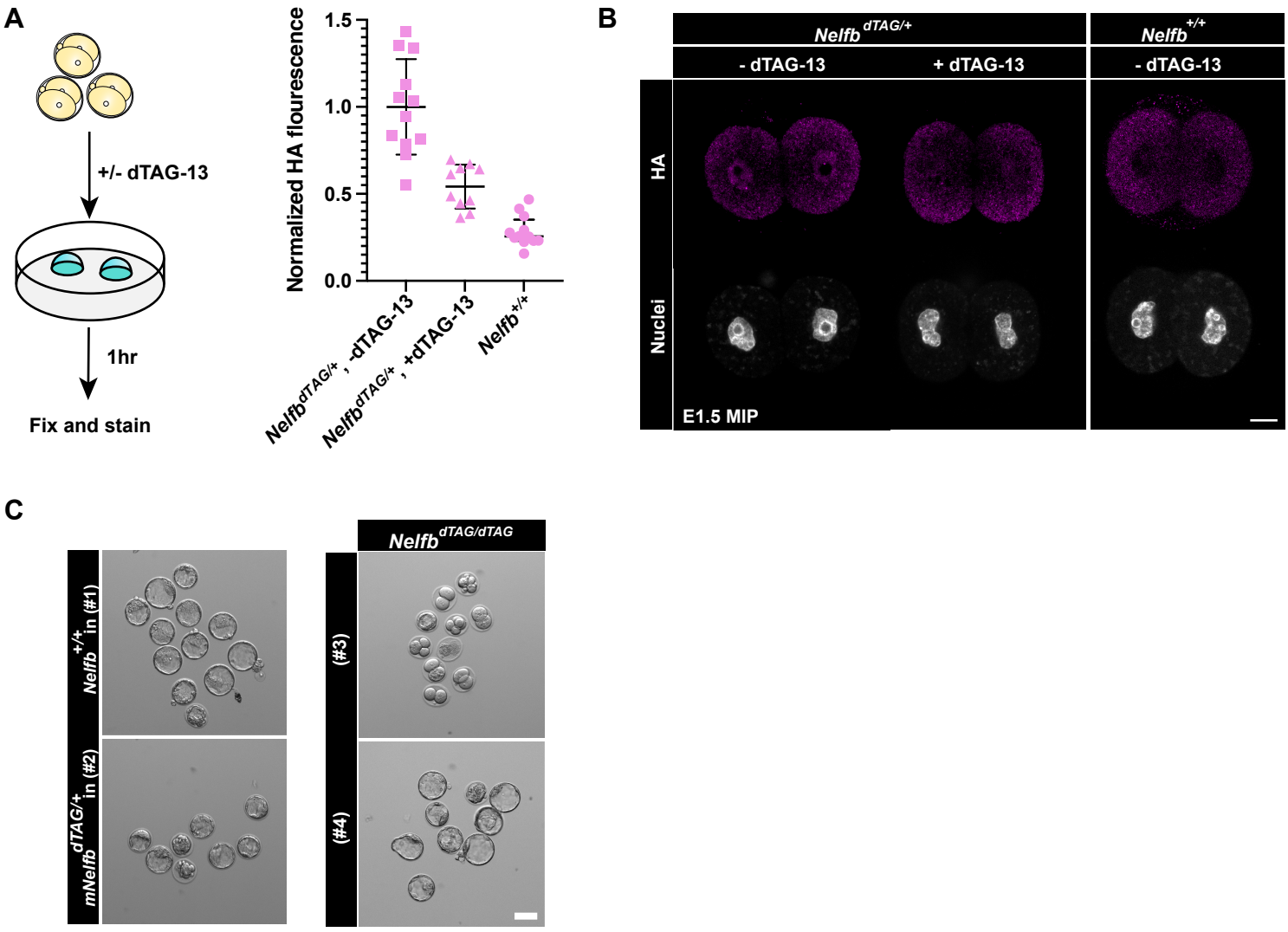


Figure S5. NELFB is required for pre-implantation development, related to Figure 5.

(A) (Left) Schematic of the experiment. *Nelfb*$^{dTAG/+}$ 2-cells stage embryos were culture +/- dTAG-13 for 1hr followed by immunostaining analysis. Quantification of mean HA signal in 2-cell stage embryos post 1hr culture +/- dTAG-13. Data points are single nuclei.

(B) Immunofluorescence images of 2-cell stage embryos +/- dTAG-13 for 1hr. Nuclei are labeled with Hoechst. Scale bar, 10μm.

(C) Additional representative images of zygote to blastocyst stage culture in Figure 4D and 4E. The treatment numbers: (#1): no dTAG-13, (#2) constant dTAG-13, (#3) 36hrs dTAG-13 from zygote to 4-cell stage followed by washing, (#4) 60hrs dTAG-13 from 4-cell stage to late blastocyst stage. Scale bar, 50μm.

For all experiments, Hoechst labels nuclei. Maximum intensity projection (MIP) is shown. Plots show each data point with means.

Figure S6

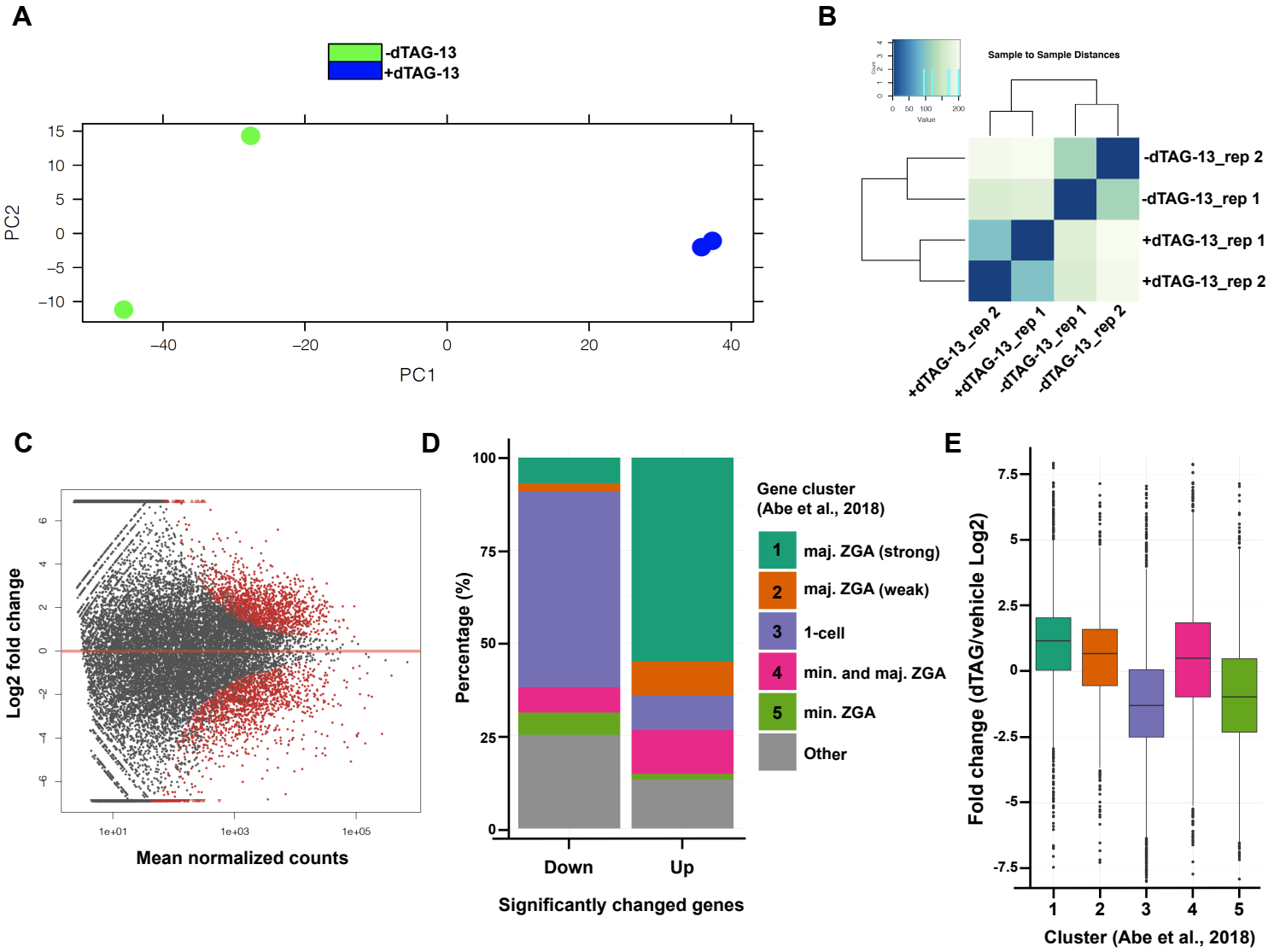


Figure S6. Clustering of RNA-seq samples and identity of differentially expressed genes, related to Figure 6.

(A) PCA plot of RNA-seq replicates.
(B) Sample-to-sample distance analysis of RNA-seq replicates.
(C) MA plot of gene expression levels (+dTAG-13/-dTAG-13) from DEseq2.
(D) Classes of up and downregulated genes using Abe et al., 2018 clustering.
(E) Overall expression changes in clusters in Abe et al., 2018.