



Fig S1. *Nelfb*^{-/-} embryos display defects in pluripotent epiblast state transitions, related to

Figure 1.

- (A) Schematic of the *Nelfb* knockout (*Nelfb*⁻) allele.
- (B) Example of embryo genotyping at the *Nelfb* locus.
- (C) Total cell count per blastocyst per genotype per stage.
- (D) Percentage of double positive nuclei: NANOG⁺ GATA6⁺ of the ICM in each blastocyst per genotype. Each dot represents an embryo. Loess line fit is shown.
- (E) Brightfield images of heterozygous and null embryos at E7.5. Scale bar 100μm
- (F) Epiblast layer area in E5.75 embryos.
- (G) Ratio (count/area) of dividing nuclei in the epiblast cup region. Dividing nuclei were visualized via immunostaining for phosphorylated H3 (pH3). Statistical testing was performed using a t-test.
- (H – K) Immunofluorescence of E6.5 embryos. Nuclei are labeled with Hoechst. Single Z slices are shown. Scale bar 100μm.