Expanded View Figures

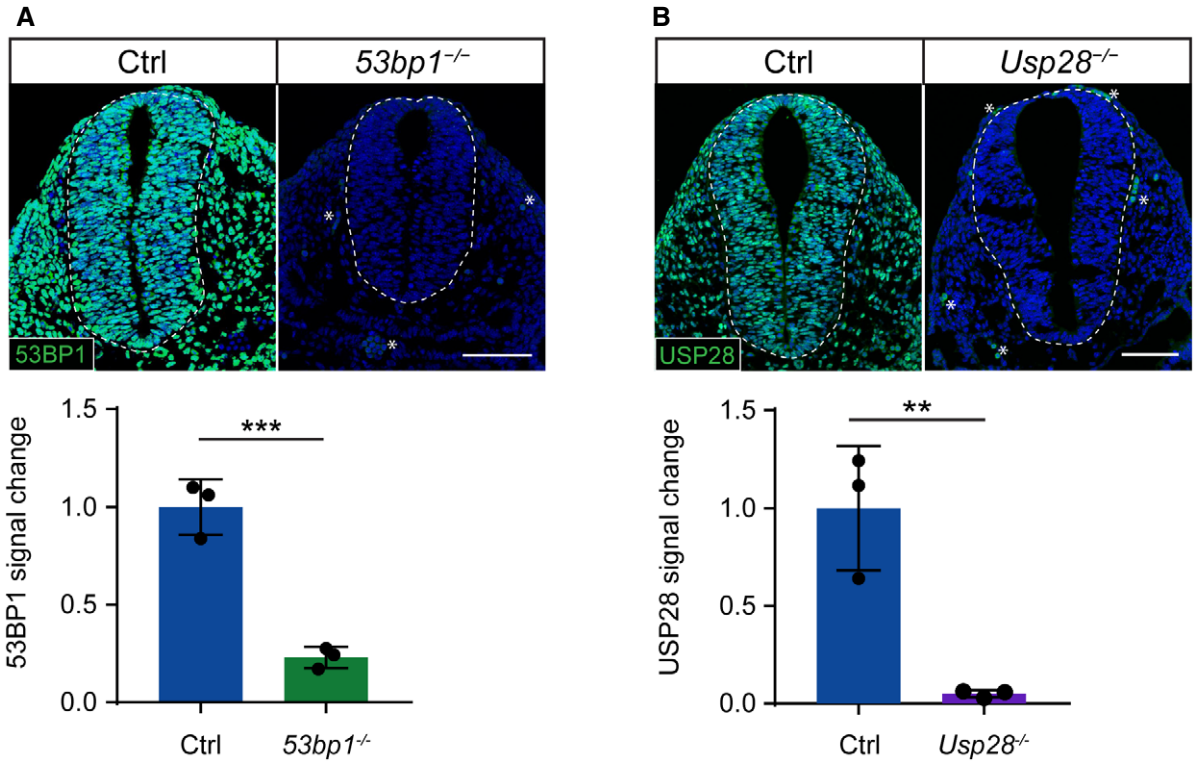

Figure EV1. *53bp1*$^{-/-}$ and *Usp28*$^{-/-}$ are null alleles.

A, B Immunostaining and quantifications for 53BP1 (A) or USP28 (B) on sagittal sections of control (Ctrl) and *Sas-4*$^{-/-}$*53bp1*$^{-/-}$ (A) or *Usp28*$^{-/-}$ (B) embryos at E9.5. The signals for the corresponding proteins in the mutants compared to controls are not detectable and close to background staining of secondary antibody-only negative controls, which were used as baseline for quantification. The cervical or brachial neural tube (the dotted quantified area) and underlying mesenchyme are shown. Asterisks indicate non-specific staining in blood cells. Three embryos per genotype were used for the quantifications. ***$P < 0.001$, **$P < 0.01$ (two-tailed Student's *t*-test). Bars represent mean ± s.d. Scale bars = 100 μm. (A) Ctrl: 1.00 ± 0.12; *53bp1*$^{-/-}$: 0.23 ± 0.04 (B) Ctrl: 1.00 ± 0.26; *Usp28*$^{-/-}$: 0.05 ± 0.02.

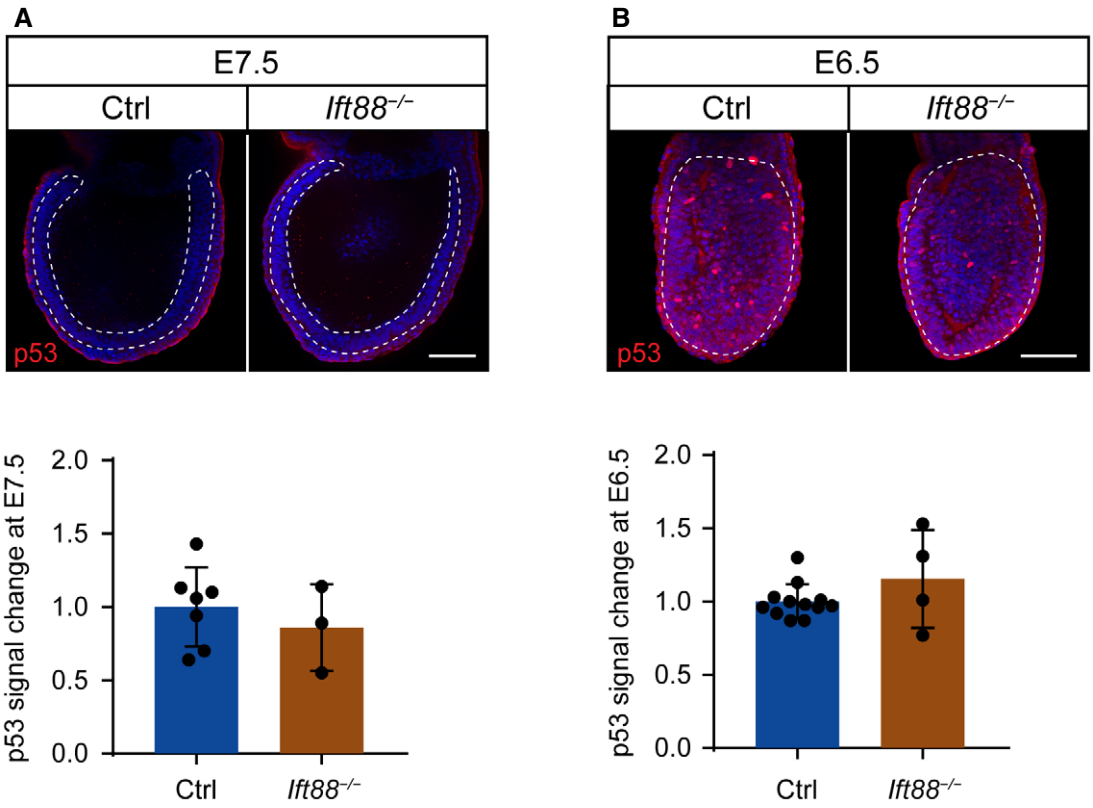

Figure EV2. *Ift88*$^{-/-}$ cilia mutants do not upregulate p53.

A, B Immunostaining and quantification for p53 on whole-mount Ctrl and *Ift88*$^{-/-}$ embryos at E7.5 (A) and E6.5 (B). Mid-sagittal planes are shown with the dotted lines demarcating the epiblast. Scale bars = 100 μm. The quantification of p53 nuclear fluorescence intensity in the epiblast was normalized to Ctrl embryos in the same batch at E7.5 (A) and E6.5 (B). Bars represent mean ± s.d. (two-tailed Student's *t*-test). (A) Ctrl: 1.00 ± 0.25 (n = 11,695 cells from 8 embryos); Ift88$^{-/-}$: 0.86 ± 0.24 (n = 40,815 from 3 embryos) (B) Ctrl: 1.00 ± 0.11 (n = 8,895 from 14 embryos); Ift88$^{-/-}$: 1.16 ± 0.29 (n = 3,470 from 4 embryos).

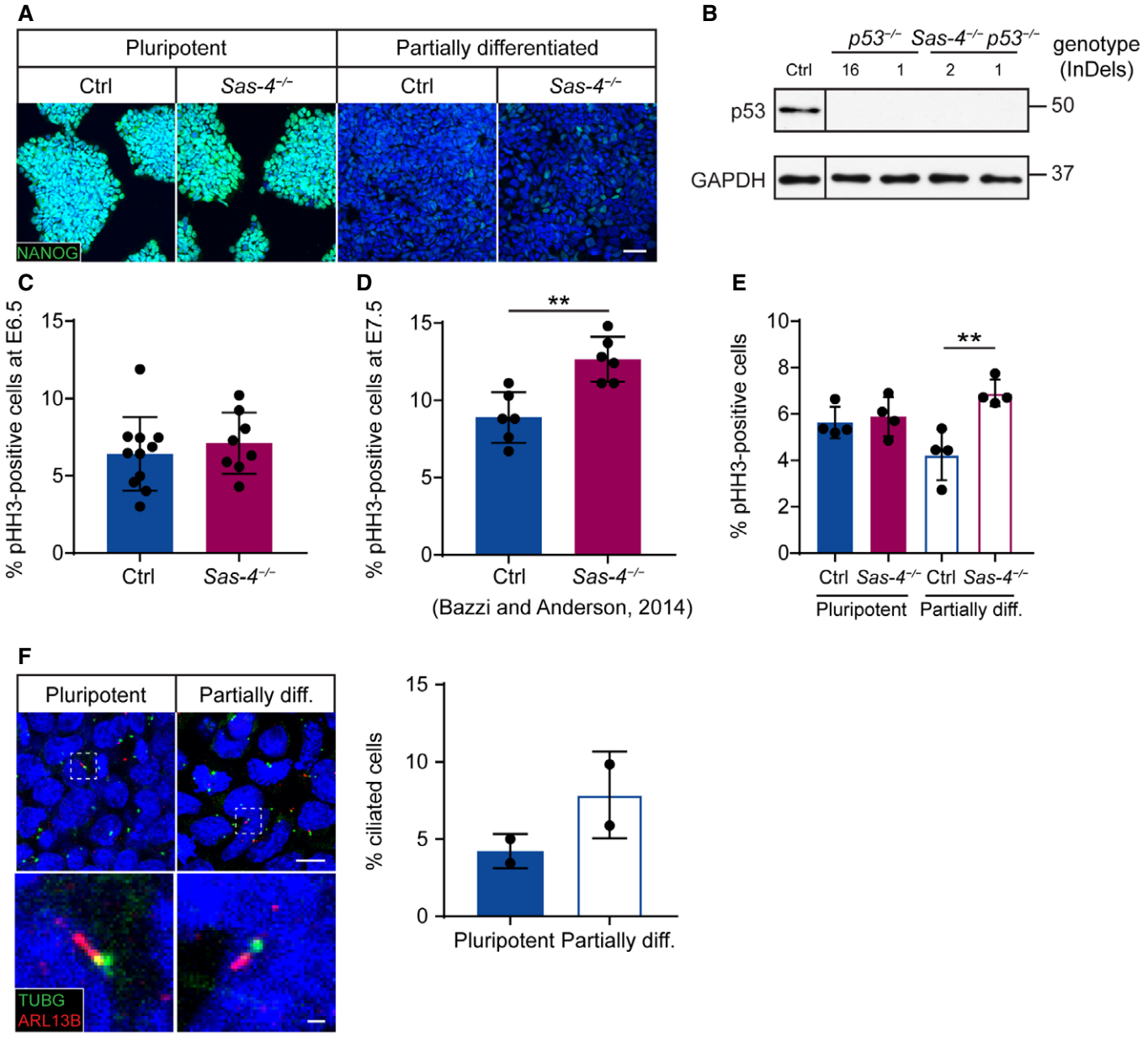

Figure EV3. Pluripotency, *p53*$^{-/-}$ null alleles and the mitotic indices in controls and *Sas-4*$^{-/-}$ mutants.

A Immunostaining for NANOG on Ctrl and *Sas-4*$^{-/-}$ primary mESCs in pluripotent and partially differentiated conditions. Scale bar = 50 μm.

B Western blot analysis for p53 and GAPDH loading control on Ctrl, *p53*$^{-/-}$, and *Sas-4*$^{-/-}$*p53*$^{-/-}$ mESC lysates. The numbers below *p53*$^{-/-}$ and *Sas-4*$^{-/-}$*p53*$^{-/-}$ indicate the number of base pairs deleted.

C–F The mitotic index, or percentage of pHH3-positive cells, of Ctrl and *Sas-4*$^{-/-}$ embryo epiblast at E6.5 (n = 8, (C)) and E7.5 (n = 6, (D)) or mESCs (Four independent experiments) in pluripotent or partially differentiated (diff.) conditions (E). The graph in (D) represents our previously published data (Bazzi & Anderson, 2014). (F) Immunostaining and quantification of the cilia marker ARL13B, together with the basal body marker TUBG, in Ctrl mESCs in pluripotent and partially diff. conditions (Two independent experiments). The selected areas in the top panels are magnified in the bottom panels. Scale bars = 10 μm (top) and 1 μm (bottom). **$P < 0.01$ (two-tailed Student's t-test or one-way ANOVA with Tukey's multiple comparisons tests for (E)). Bars represent mean ± s.d. (C) Ctrl: 6 ± 2 (n = 9,545 cells); *Sas-4*$^{-/-}$: 7 ± 2 (n = 5,875). (D) Ctrl: 9 ± 2 (n = 5,150), *Sas-4*$^{-/-}$: 13 ± 1 (n = 4,990); (E) Ctrl pluripotent: 6 ± 1 (n = 2,445), *Sas-4*$^{-/-}$ pluripotent: 6 ± 1 (n = 2,330); Ctrl partially diff.: 4 ± 1 (n = 3,015); *Sas-4*$^{-/-}$ partially diff.: 7 ± 0 (n = 2,280). (F) Pluripotent mESCs: 4 ± 1 (n = 1,553) Partially diff. mESCs: 8 ± 2 (n = 1,538).