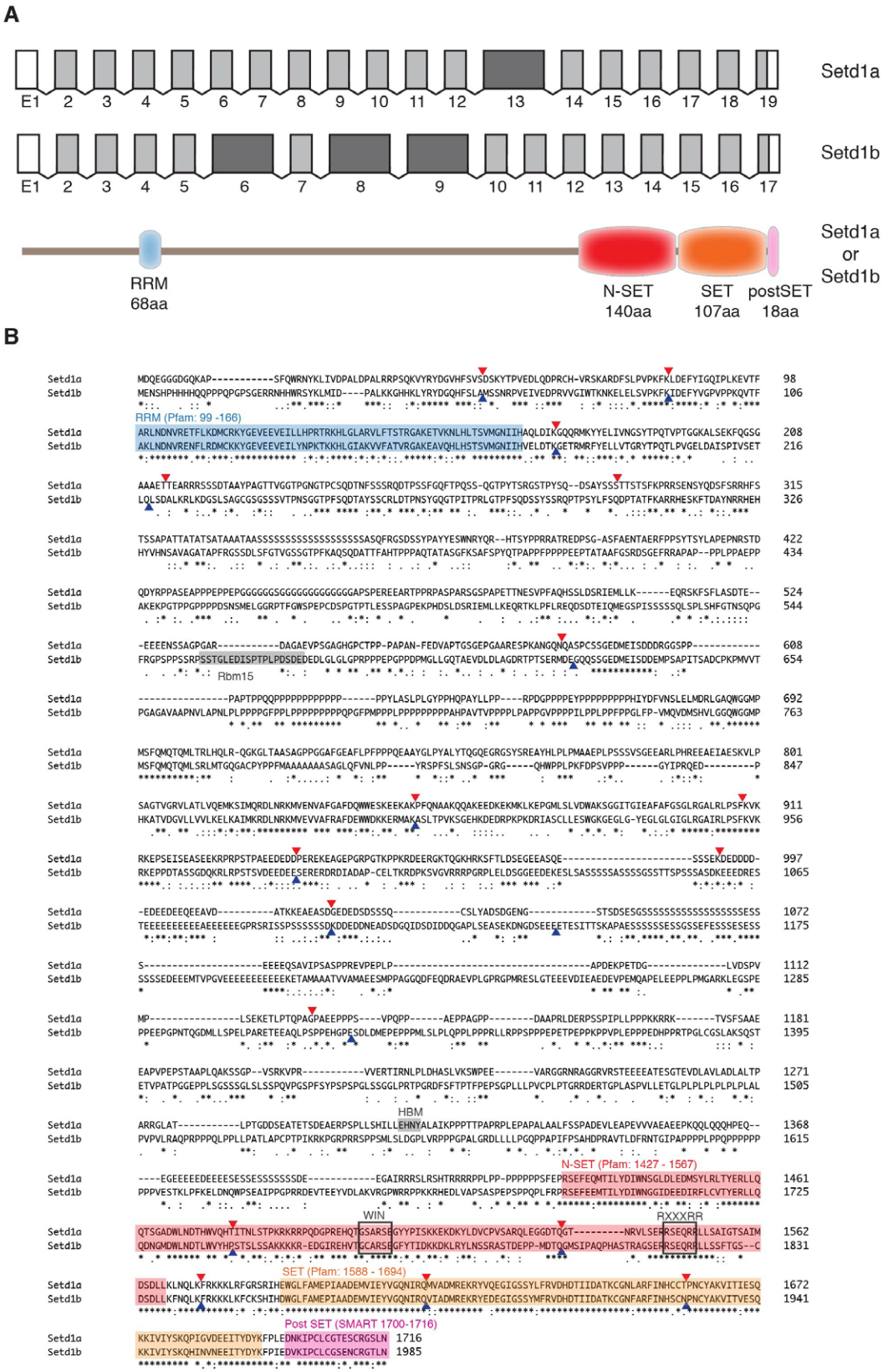

Fig. S1. Setd1a and Setd1b are highly conserved paralogs. (**A**) Schematic representation of the *Setd1a* and *Setd1b* genes. The exon/intron boundaries are conserved between the two genes. For simplicity the exons are displayed in the same size. White boxes represent non-coding and grey boxes coding exons. Black boxes represent a single exon in one gene whereas in the sister gene the same region is split into two exons. Conserved domains in the Setd1 proteins are presented in the cartoon below. The number of the corresponding amino acids is calculated for Setd1a. (RRM = RNA Recognition Motif; E = exon). (**B**) Setd1a and Setd1b protein alignment using ClustalW. Red triangles mark exon/intron junctions in Setd1a and blue triangles in Setd1b. The region highlighted in blue marks the RRM, in red the N-SET, in orange the SET and in pink the postSET domain. The grey regions mark the Host cell factor 1 (Hcf1) binding motif (HBM) in Setd1a and Rbm15 binding site in Setd1b. Black squares indicate the WIN (Wdr5 interaction) and RXXXRR motifs in the N-SET domain.

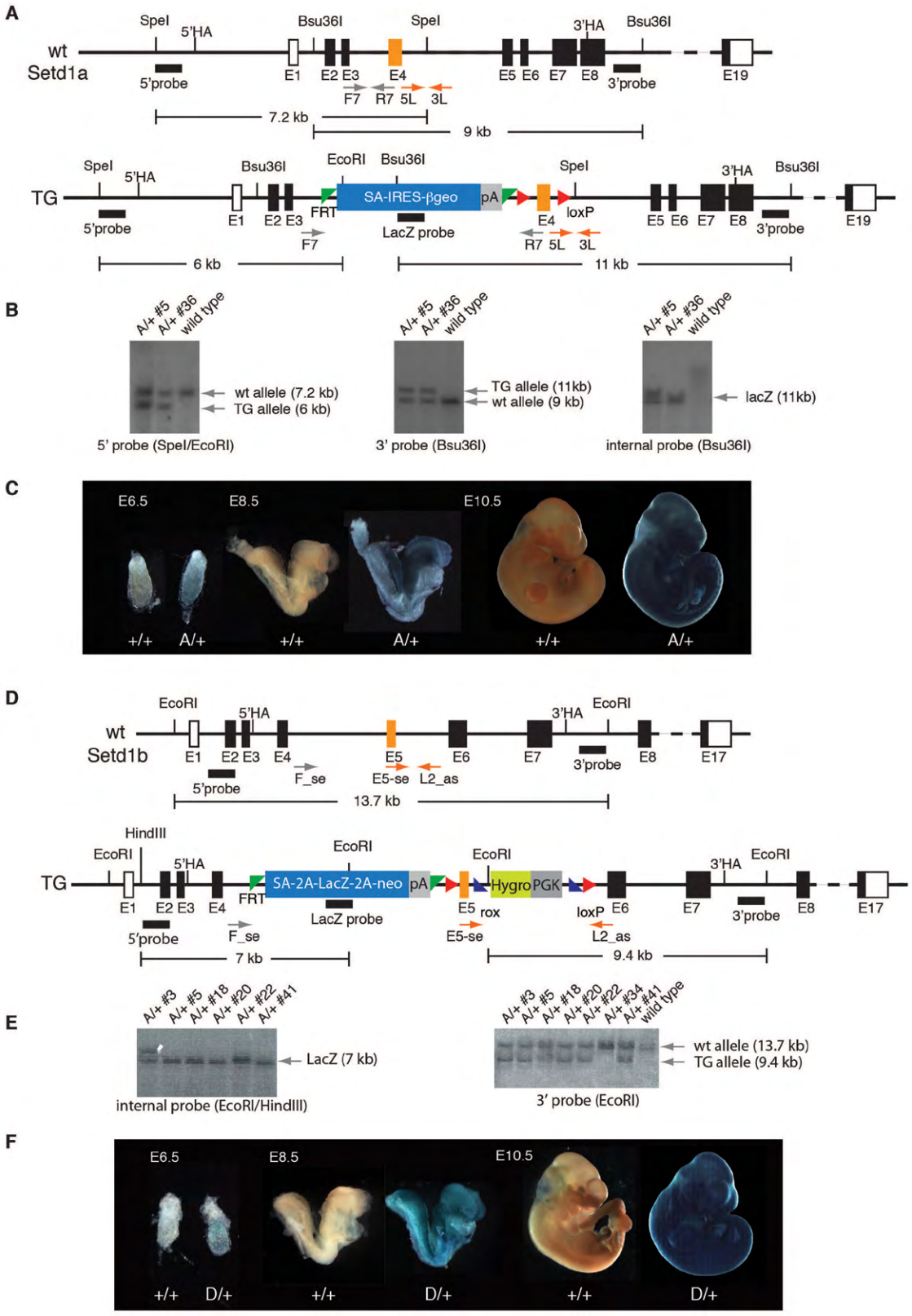

Fig. S2. Gene targeting strategy for *Setd1a* and *Setd1b.* (**A**) Schematic representation of the Southern strategy for identifying correct targeted events in the *Setd1a* locus (horizontal arrows represent primers for the downstream loxP site (5L – 3L) and for detecting Flp recombination (F7 – R7), E = exon, HA = homology arm, SA = splice acceptor, IRES = internal ribosomal entry site, bgeo = fusion between β-galactosidase and neomycin resistance gene, pA = poly adenylation signal, red triangles = loxP sites, green triangles = FRT sites). (**B**) Southern blot analysis using 5' and 3' external probes and LacZ as internal probe. Note that clone #5 contained an additional integration. (**C**) LacZ staining of heterozygous *Setd1a*$^{A/+}$ embryos at different developmental stages. (**D**) Schematic representation of the Southern strategy for identifying correct targeted events in Setd1b (2A = self-cleaving peptide, PGK = phosphoglycerate kinase 1 promoter, Hygro = hygromycin resistance gene, blue triangles = rox sites). (**E**) Southern blot analysis using 3' external and LacZ internal probes. Note that clone #3 contained an additional integration. (**F**) LacZ staining of heterozygous *Setd1b*$^{D/+}$ embryos at different developmental stages.

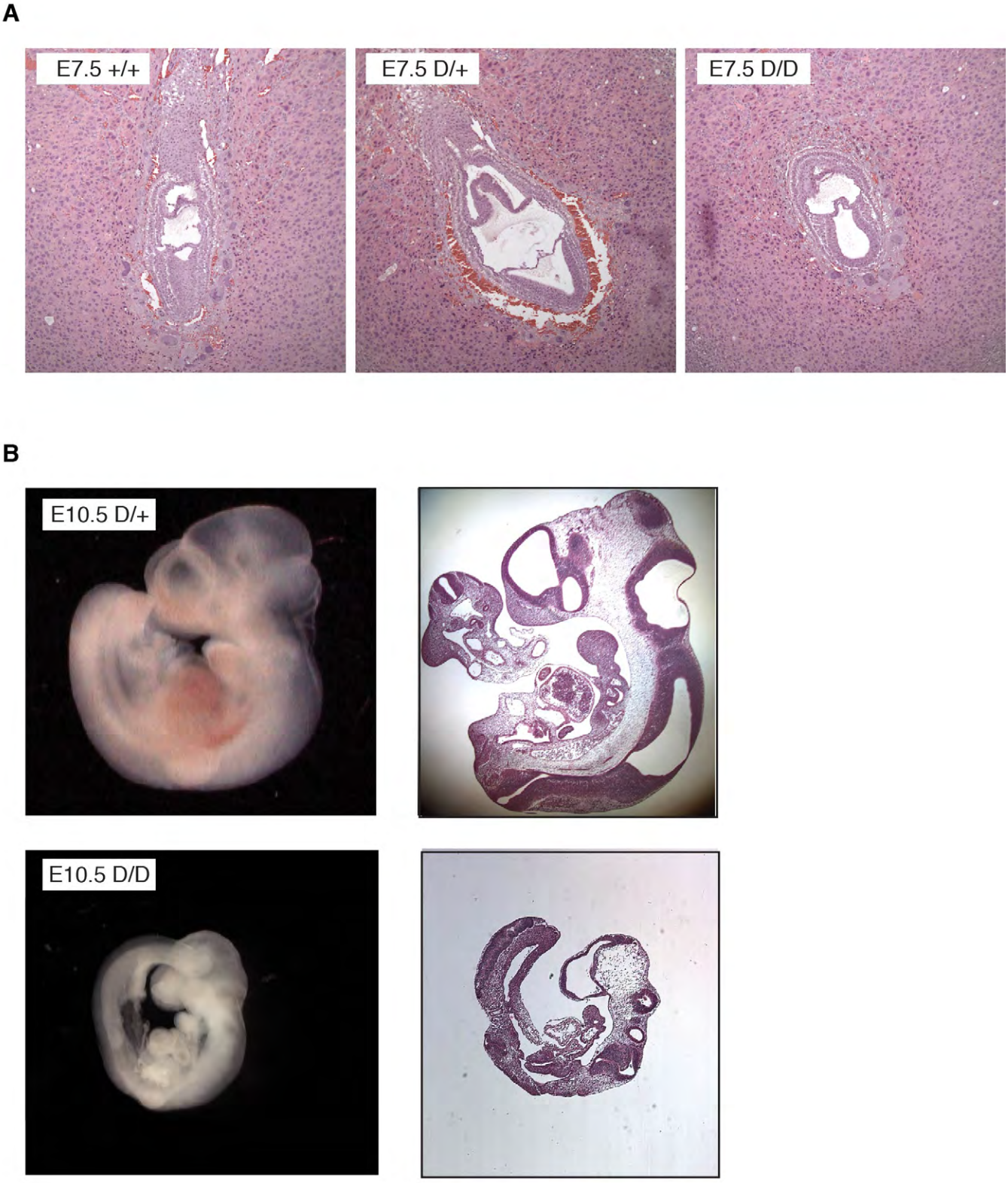

Fig. S3. *Setd1b* knockout embryos form all three germ layers. (**A**) Sagittal sections of E7.5 embryos stained with hematoxylin & eosin (H&E). *Setd1b*$^{D/D}$ embryos are growth retarded but develop all embryonic structures. (**B**) H&E stained sections of E10.5 embryos.

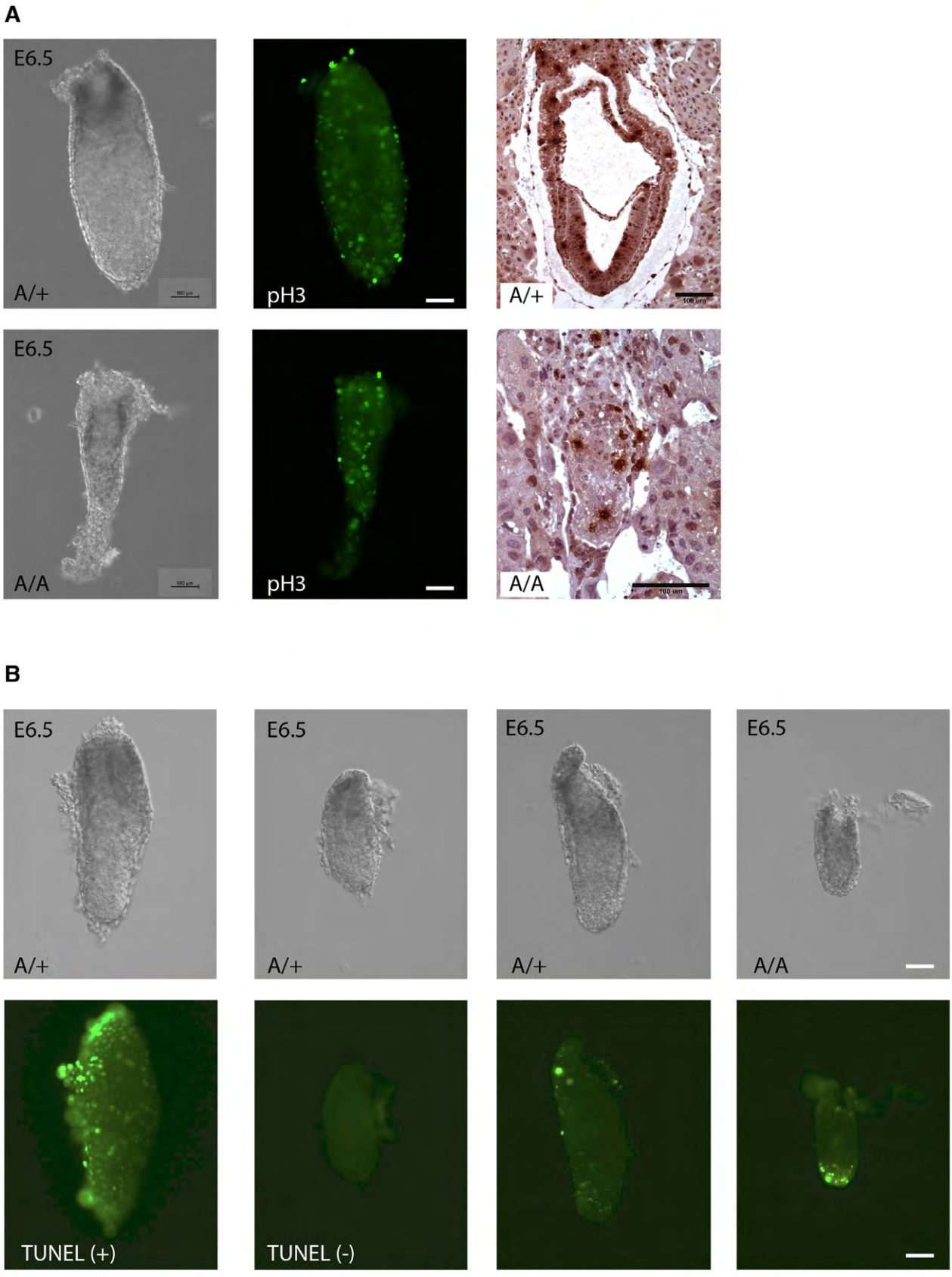

Fig. S4. *Setd1a* mutant embryos retain mitotic activity. (**A**) Whole mount immunostaining with the mitotic marker phospo-H3S10 (pH3). Upper row shows *Setd1a*$^{A/+}$ and lower row *Setd1a*$^{A/A}$ E6.5 embryos. Right panels in both rows show sections stained with pH3. (**B**) Whole mount TUNEL assay. Upper row shows DIC images and lower row fluorescent images. Left column shows the positive control and the second column the negative control for the TUNEL assay. The 3rd and 4th column show images of *Setd1a*$^{A/+}$ and *Setd1a*$^{A/A}$ E6.5 embryos. Scale bar: 100 µm.

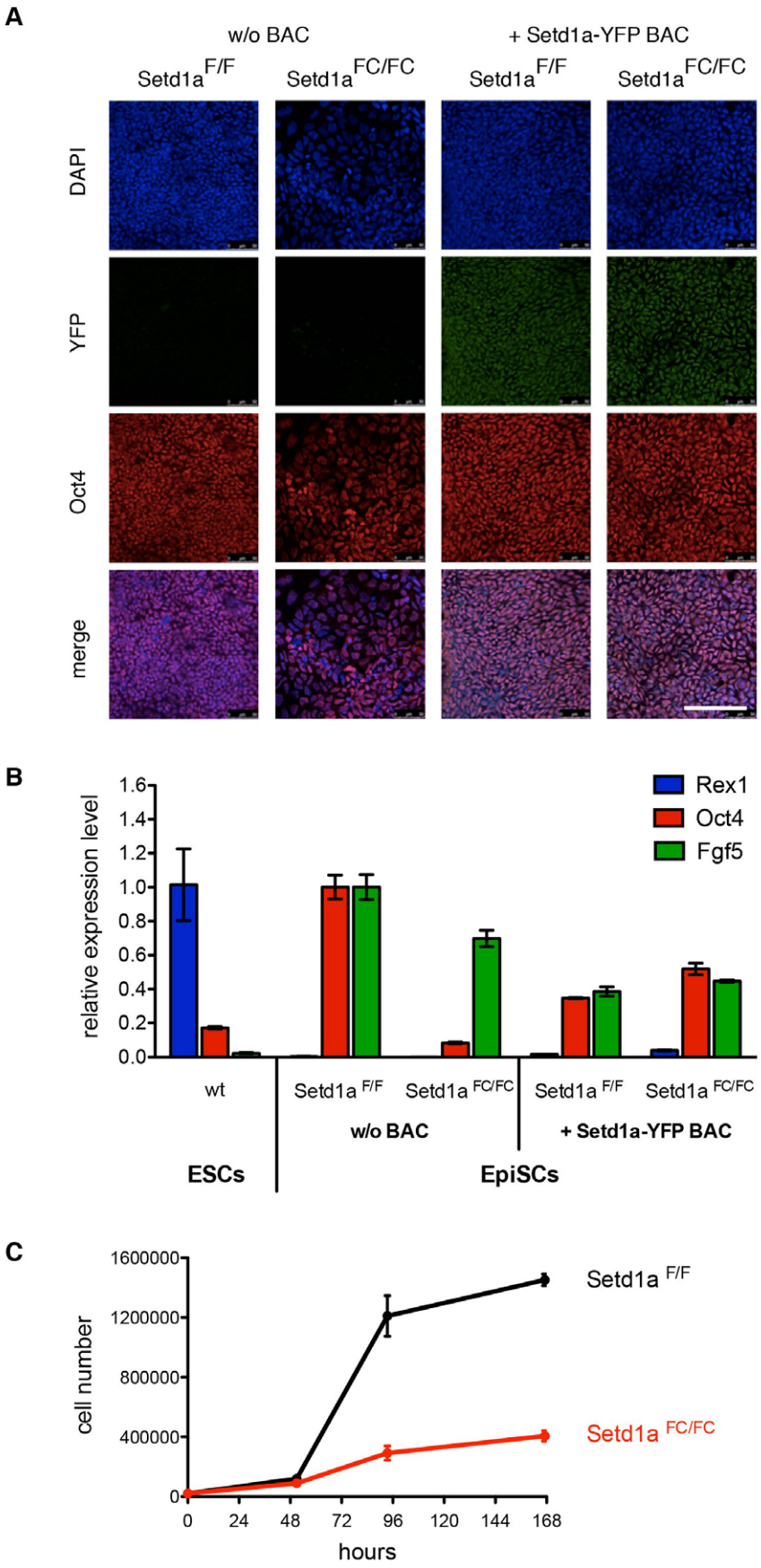

Fig. S5. *Setd1a* is required for EpiSC proliferation. (**A**) Micrographs of EpiSCs generated from conditional *Setd1a*$^{F/F}$ ESCs and of those carrying a *Setd1a-Venus* tagged BAC, which rescues the proliferation defect observed upon 4OHT treatment. EpiSCs are immunostained with anti-Oct4 (3rd row) and the ones that carry the *Setd1a* BAC express Venus (2nd row). (**B**) Relative expression levels detected by qRT-PCR of the ESC marker *Rex1*, the epiblast marker *Fgf5* and *Oct4* in ESC and EpiSC lines. (**C**) Proliferation curve of *Setd1a*$^{F/F}$ conditional EpiSCs after 4OHT treatment. Scale bar: 100 μm.

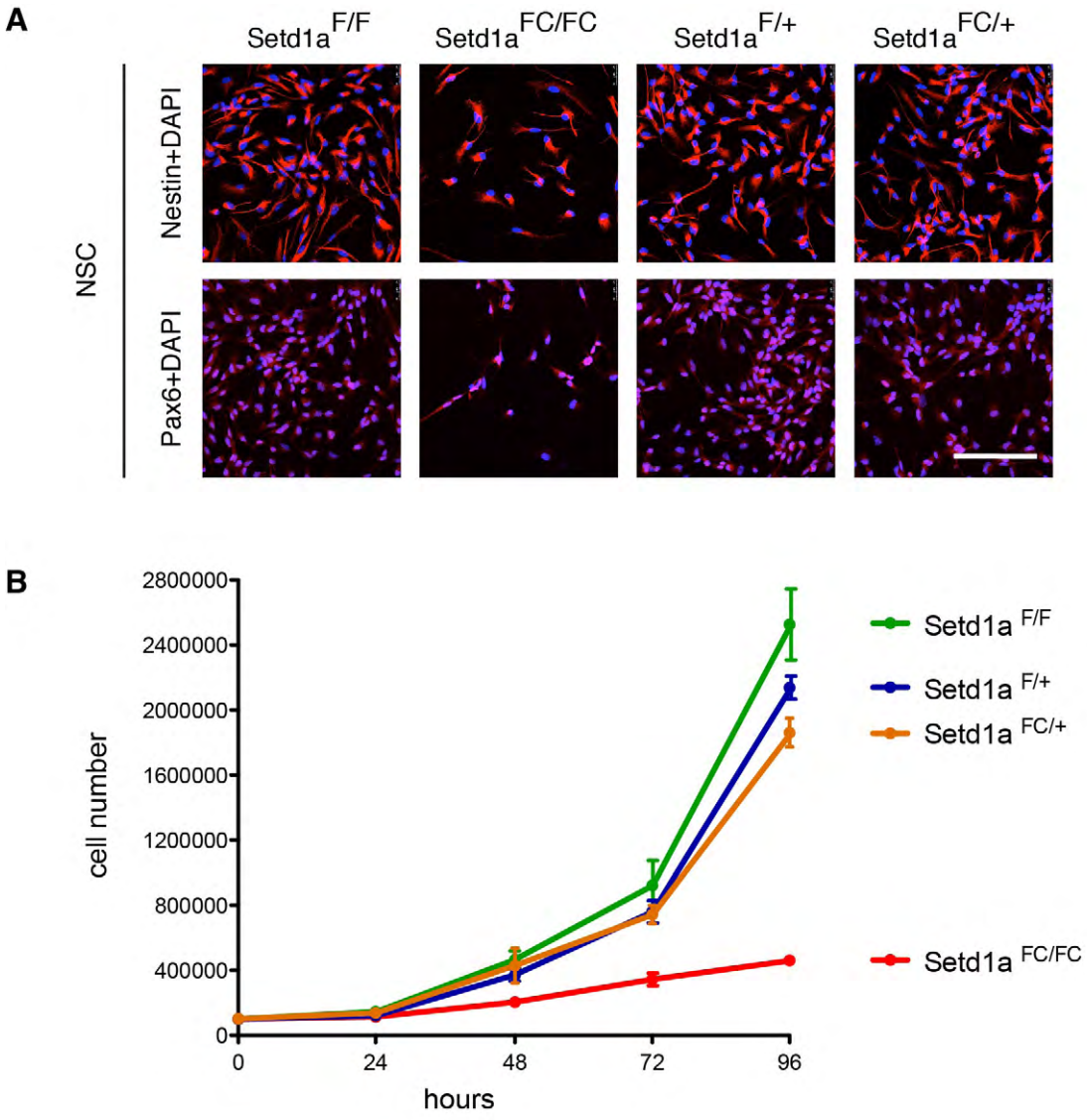

Fig. S6. ***Setd1a*** **is required for NSC proliferation.** (**A**) Micrographs of NSCs immunostained with Nestin or Pax6 and DAPI to visualize the nuclei at day 4 after 4OHT induction. (**B**) Proliferation curve of *Setd1a* conditional NSCs starting 96 hours after 4OHT induction. The proliferation rate of *Setd1a*$^{FC/FC}$ NSCs is reduced as compared to heterozygous and uninduced controls. Scale bar: 100 µm.

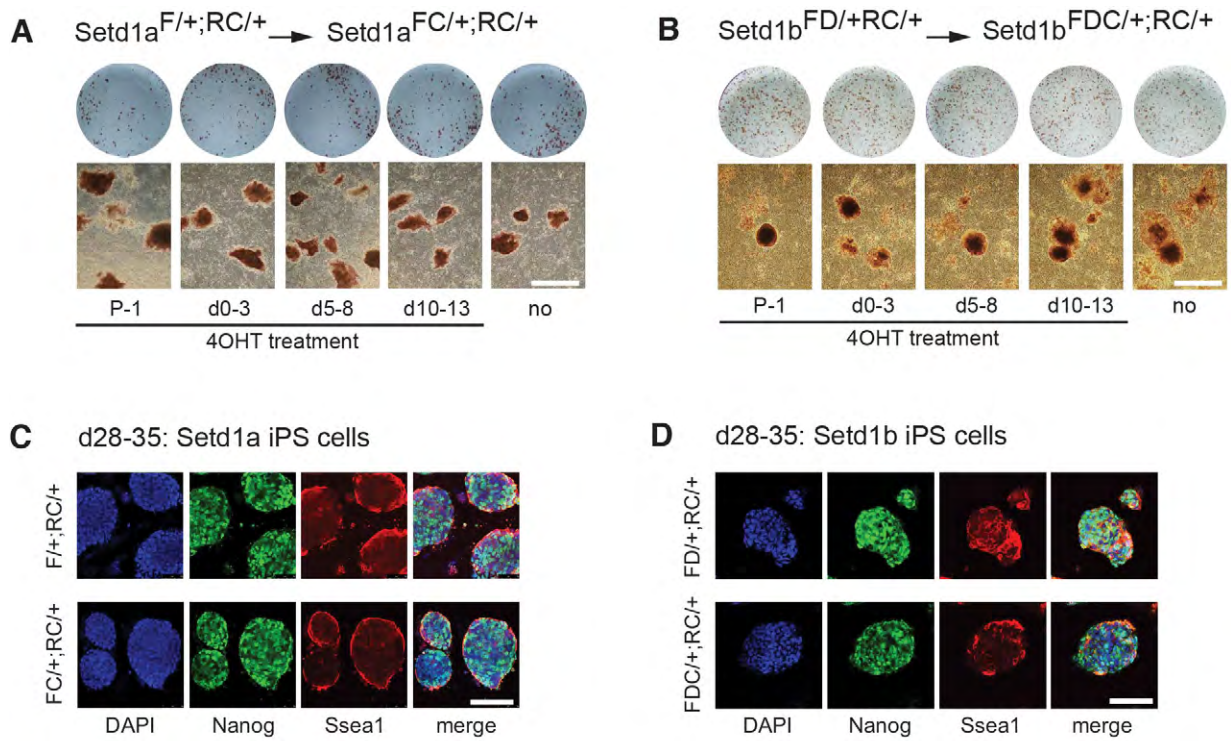

Fig. S7. Heterozygous *Setd1a* or *Setd1b* conditional knockout NSCs can generate iPSCs. (**A-B**) Micrographs of AP-stained iPSC colonies from *Setd1a*$^{F/+;RC/+}$ and *Setd1b*$^{FD/+;RC/+}$ lines. (**C-D**) iPSCs from *Setd1a*$^{F/+;RC/+}$ and *Setd1b*$^{FD/+;RC/+}$ generated without or after 4OHT treatment and immunostained with Nanog and SSEA1. Scale bars: A, B = 1 mm; C, D = 100 μm.

Table S1. Primers for genotyping and qRT-PCR

Primer pairs	Sequence (5′-3′)	Product size (bp)
For genotyping Setd1a knockout		
Setd1a_5loxd Set1d1a_3loxd	GTGGGTGCTGGGAATTGAACTCC TGTGGTTTTGGCAGGCCGTGACC	218 [wt]; 252 [A]
For testing Flp recombination in Setd1a		
Setd1a_F7 Set1d1a_R7.1	CAAGTACTCCCTAGGTAGCCGC GGGCTCATGAGAGGTACATTAGC	314 [wt]; 403 [F]
For testing Cre recombination in Setd1a		
Setd1a_F7 Set1a_3loxd_2	As above CTCCCTGAGCTGGAGAGATG	1088 [wt]; 1211 [F]; 191 [FC]
Nested PCR for constitutive [A] allele followed by Setd1a_5loxd and Setd1a_3loxd		
Setd1a_5loxd_Nest Setd1a_3loxd_Nest	GGCTCAGCCAAGTAATCAGG CTCCCAAGCACAAGGATGAT	527 [wt]; 561 [A]
Nested PCR for conditional [F] allele followed by Setd1a_F7 and Setd1a_3loxd		
Setd1a_5FRT-up Setd1a_3loxd_Nest	AGGTTGTCAGGCTTGGTGAC As above	1638 [wt]; 1761 [F]
For testing Dre recombination in Setd1b		
Setd1b_ex5 (se)	GAAACTCGCATGCGCTTCTAC	507 [wt]; 696 [D]
Setd1b_loxP2 (as)	AGTTCATACTGTGGCTGAATGG	
For testing Cre recombination in Setd1b		
Setd1b_flp (se)	GGGTGGAGAGGGAAAGAAAAG	1305 [wt]; 1695 [FD]; 390 [FDC]
Setd1b_loxP2 (as)	As above	
For qRT-PCR		
Gapdh (se) Gapdh (as)	TCACCACCATGGAGAAGGC GCTAAGCAGTTGGTGGTGCA	169
Rpl19 (se) Rpl19 (as)	CTGATCAAGGATGGGCTGATC CTTCTCAGGCATCCGAGCATT	147
Setd1a-E3 (se) Setd1a-E4 (as)	CTGTCATGTCAGGTCCAAAGCC TTCCCTCACATTGTCATTGAGCC	130
Setd1a-E17 (se) Setd1a-E18 (as)	GTTTGCCATGGAACCCATTGC GTGCAGCAGTGGTTGATGAAC	204
Setd1b-E5 (se) Setd1b-E6 (as)	CTGTTGGTGAGCTGGATGCTA CTGGAGTAAGCTGTGTCTTGG	172
Oct4 (se-1) Oct4 (as-1)	TGAGGCTACAGGGACACCTT GAAGTGGGGGCTTCCATAG	94
Oct4 (se-2) Oct4 (as-2)	CGAGGCCTTGCAGCTCAGCC AGGGAGGGCTTCGGGCACTT	210
Nanog (se) Nanog (as)	TTAGAAGCGTGGGTCTTGGT TCCTCGAGAGTAGCCACCAT	96
Klf4 (se) Klf4 (as)	TGCCAGAGGAGCCCAAGCCA GGCCGGTGCCCTGTGTGTTT	241
Gata4 (se) Gata4 (as)	TTCAAACCAGAAAACGGAA TAGTGGCATTGCTGGAGT	120
T (se) T (as)	TGTCCTCCCTTGTTGCCTTA ATGTTCCAAGGGCAGAACAG	100
Fgf5 (se-1) Fgf5 (as-1)	AATTCGGGAATGTGATGAGC AACCGTCTGTGGTTTCTGTTG	81

Fgf5 (se-2) Fgf5 (as-2)	TGCTGTGTCTCAGGGGATTGTAGGA TTCTGTGGATCGCGGACGCA	170
Rex1 (se) Rex1 (as)	AAGAGCTGGGACACGTGGCAA GGCAGCACAGTGAGGCGATCC	116

Oct4 and Fgf5 primer set (1) was used in ESCs (Fig. 4) and set (2) in EpiSCs (Fig. S5).