Supplementary Materials and Methods

siRNA sequences

The sequences of the siRNAs used are as follows: Satb1 siRNA 1– AAGGTGGTACAAACATTTCAA, Satb1 siRNA 2– CAGGAAATGAAGCGTGCTAAA, Satb1 siRNA 3 – CCCGAAGTACACCATCATCAA, Satb2 siRNA 1 – CCGAAGGACTAGACTGTGAA, Satb2 siRNA 2-ATGGCCCATCTGATAAACCAA, Satb2 siRNA 3–CAGGGATTATTGTCAGAGATA.

Immunofluorescence protocol and intensity measurements and antibody details

ICM cells were identified through sequential scanning through embryo z-stacks by their position as well as through the use of lineage markers. Nanog expressing cells in the ICM that did not express PE markers were identified as EPI. Inside and outside cells were identified by careful scanning through the z-stack. Only in cases when outer (with nuclei that were not surrounded by other cells and adjacent to the outside of the embryo) and inner (with nuclei that were entirely surrounded by other cells) cells could be unambiguously identified where they used for analyses. Fluorescence intensity was quantified by normalising to DAPI and layer-normalising using the built-in IMAGEJ function. Intensity measurements were done on the normalised sections using the IMAGEJ measure function**.** For antibody details see Supplementary Materials and Methods. Primary antibodies used: goat anti-Sox17 (1:200; R&D Systems, AF1924), goat anti-Pdgfrα (1:200; Santa Cruz, sc-31178), goat anti-Gata6 (1:200; R&D Systems, AF1700), rabbit anti-Nanog (1:200; Abcam, AB80892), rabbit anti-Sox2 (1:200; Abcam, ab59776), rabbit anti-Sox2 (1:200; Millipore, AB5603) mouse anti-Cdx2 (1:200; Biogenex, AM392), rabbit anti-Satb1 (1:50; Abcam, AB49061), rabbit anti-Satb2 (1:200; Abcam, AB34735), rat anti-Nanog (1:200; Ebiosciences, 14-5761-80). Secondary antibodies used: Alexa Fluor 647 donkey anti-mouse IgG, Alexa Fluor 568 donkey anti-goat IgG, Alexa Fluor 568 donkey anti-rabbit IgG, Alexa Fluor 647 donkey anti-rabbit IgG, Alexa Fluor 647 donkey anti-goat IgG, Alexa Fluor 647 phalloidin.

Primer details

The following primers were used all written 5'-3': Gapdh Forward, AGAGACGGCCGCATCTTC, Reverse, CCCAATACGGCCAAATCCGT'; Histone H2A.Z Forward, CGTCAGAGAGACGCTTACCG, Reverse, AAGCCTCCAACTTGCTCAAA; Satb1 Forward, AGTGCCCCCTTTCACAGAG, Reverse, TGCTGCTGAGACATTTGCAT; Satb2

Forward, ATGAACCCCAATGTGAGCAT, Reverse, GTTGTCGGTGTCGAGGTTTT; Cdx2 Forward, AAACCTGTGCGAGTGGATG, Reverse, TCTGTGTACACCACCCGGTA; Nanog Forward, GGTTGAAGACTAGCAATGGTCTGA, Reverse, TGCAATGGATGCTGGGATACT; Oct3 Forward, TTGGGCTAGAGAAGGATGTGGTT, Reverse, GGAAAAGGGACTGAGTAGAG TGTGG; Sox2 primer set 1 Forward, GCGGAGTGGAAACTTTTGTCC, Reverse, CGGGAAGCGTGTACTTATCCTT; Sox2 primer set 2 Forward, GCGGAGTGGAAACTTTTGTCC Reverse, GGGAAGCGTGTACTTATCCTTCT; Sox17 Forward, GATGCGGGATACGCCAGTG, Reverse, CCACCACCTCGCCTTTCAC; Id2 Forward, ATGAAAGCCTTCAGTCCGGTG, Reverse, AGCAGACTCATCGGGTCGT; Gata6 Forward, TTGCTCCGGTAACAGCAGTG, Reverse, GTGGTCGCTTGTGTAGAAGGA

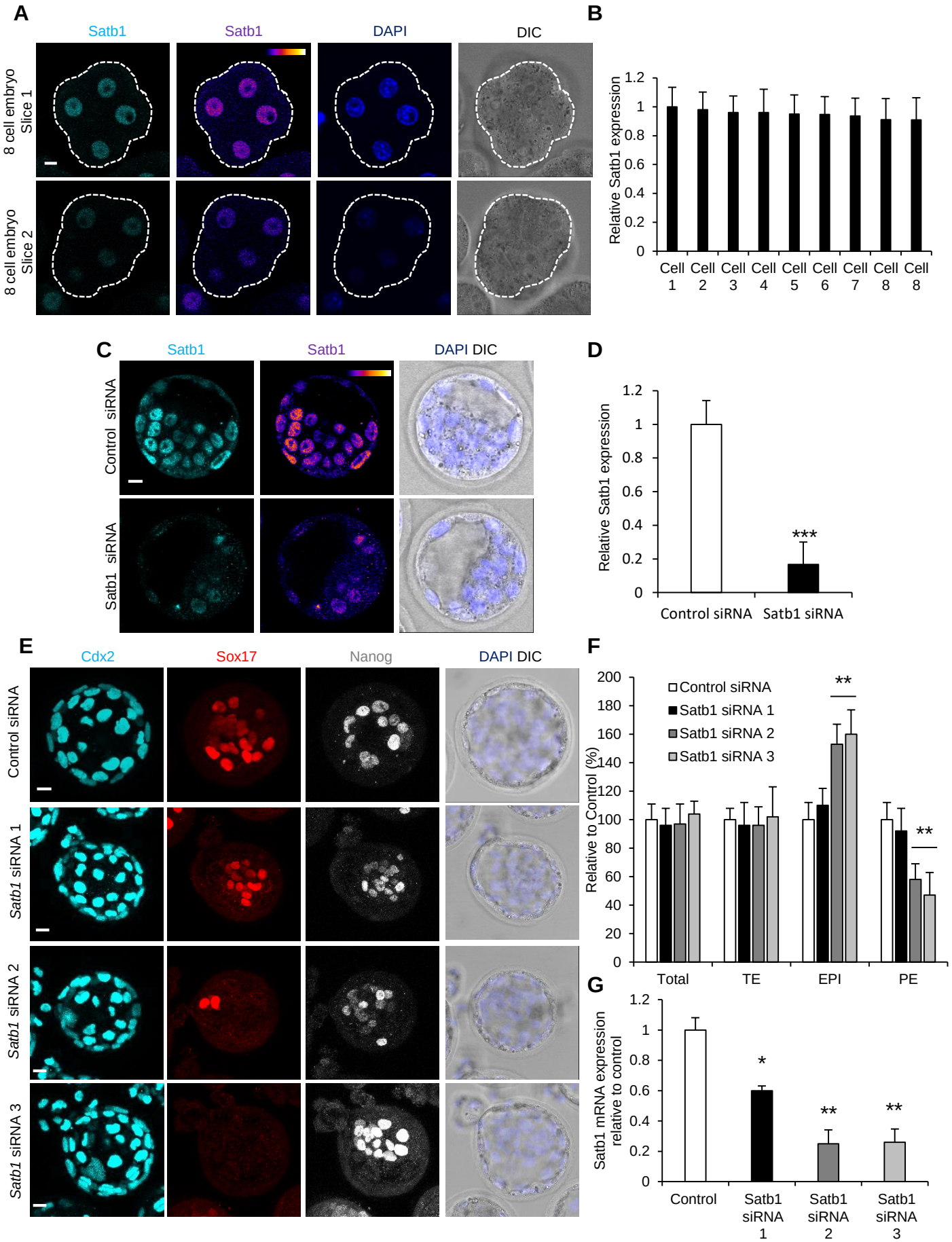

Figure S1. Confirmation of *Satb1* siRNA persistence and specificity and Satb1 embryo staining. A) Immunofluorescence of Satb1 in 8-cell embryos (n=25). Embryo boundary is outlined in white. B) Quantification of immunofluorescence represented in A. Fluorescence quantified and normalized to the nucleus with the strongest staining per individual embryo. C) Immunofluorescence of Satb1 in early blastocysts after being injected with control (n=14) or Satb1 siRNA (n=18). D) Quantification of relative fluorescent intensity of Satb1 staining from C. E) Confocal images of control and *Satb1* siRNA 1, *Satb1* siRNA 2 and *Satb1* siRNA 3 injected embryos. Nanog, (EPI), Sox17 (PE) and Cdx2 (TE) were used as lineage markers. Quantification of this experiment shown in F.F) Contribution of control (n=17) and *Satb1* siRNA 1 (n=19),2 (n=21), and 3 (n=23) injected embryos to EPI, PE, and TE. G) qRT–PCR of control (n=47 embryos, three biological repeats), *Satb1* siRNA 1 (n=58 embryos, three biological repeats), *Satb1* siRNA 2 (n=53 embryos, three biological repeats), *Satb1* siRNA 3 (n=49 embryos, three biological repeats) injected embryos to investigate *Satb1* mRNA levels.
Student's t-test was used to test significance *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$. Error bars represent s.e.m. Scale bars, 10 μm.

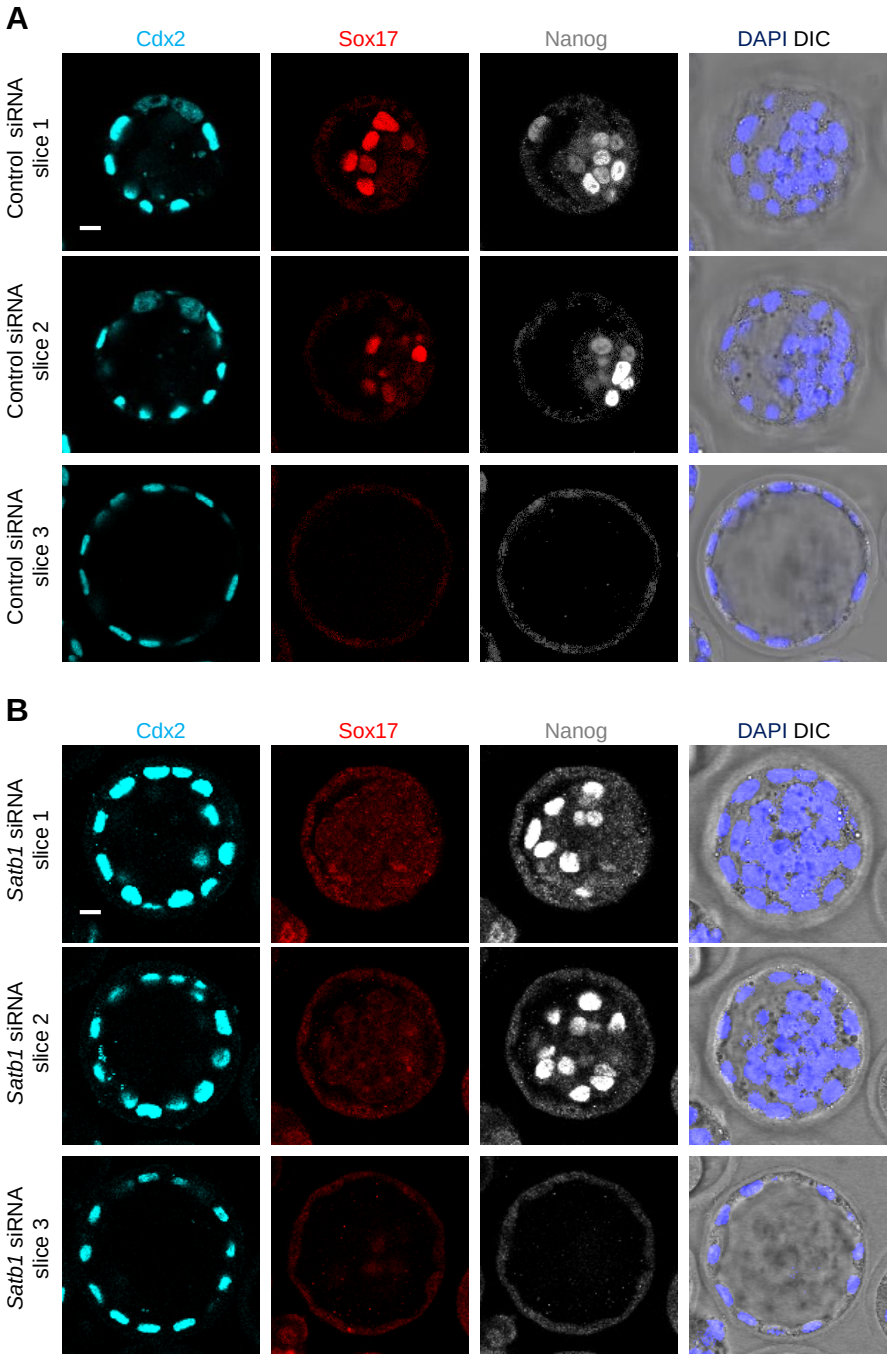

Figure S2. Z-stack slices of confocal images from Figure 2E. A) Slices of the confocal z-stack of the control siRNA injected embryo presented in Fig 2 E. Nanog, (EPI), Sox17 (PE) and Cdx2 (TE) were used as lineage markers B) Slices of the confocal Z-stack of the Satb1 siRNA injected embryo presented in Fig 2 E (Embryo 1). Nanog, (EPI), Sox17 (PE) and Cdx2 (TE) were used as lineage markers.
Scale bars, 10 μm.

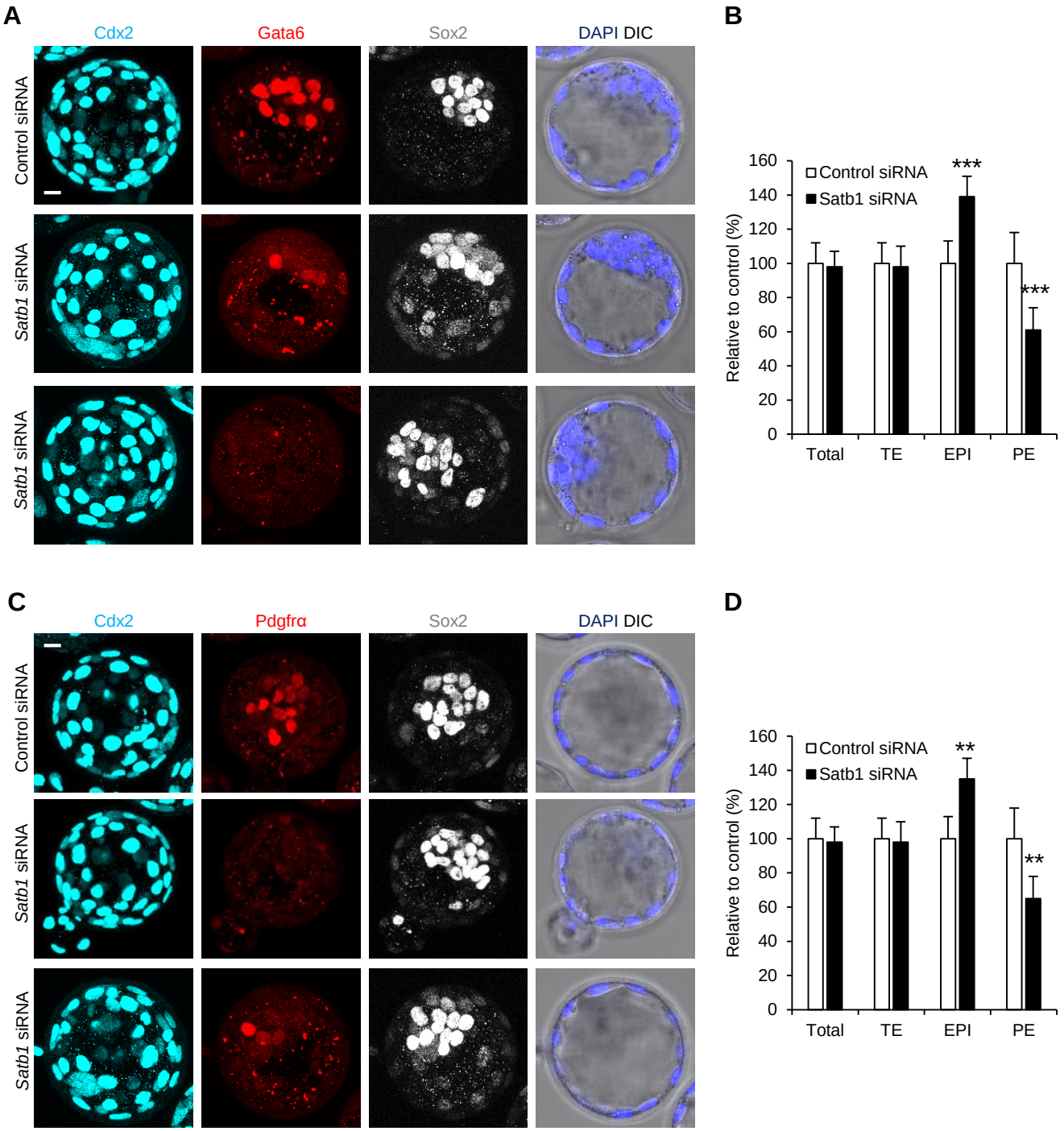

Figure S3. *Satb1* siRNA phenotype assessed by Sox2, Gata6 and Pdgfrα. A) Confocal images of control (n=31) and Satb1 siRNA (n=30) injected embryos. Sox2, (EPI), Gata6 (PE) and Cdx2 (TE) were used as lineage markers. B) Contribution of control and Satb1 siRNA injected embryos represented in A to EPI, PE, and TE. C) Confocal images of control (n=27) and Satb1 siRNA (n=29) injected embryos. Sox2, (EPI), Pdgfrα (PE) and Cdx2 (TE) were used as lineage markers. D) Contribution of control and Satb1 siRNA injected embryos represented in C to EPI, PE, and TE.

Student's t-test was used to test significance *= $p<0.05$, **= $p<0.01$. Error bars represent s.e.m. Scale bars, 10 μm.

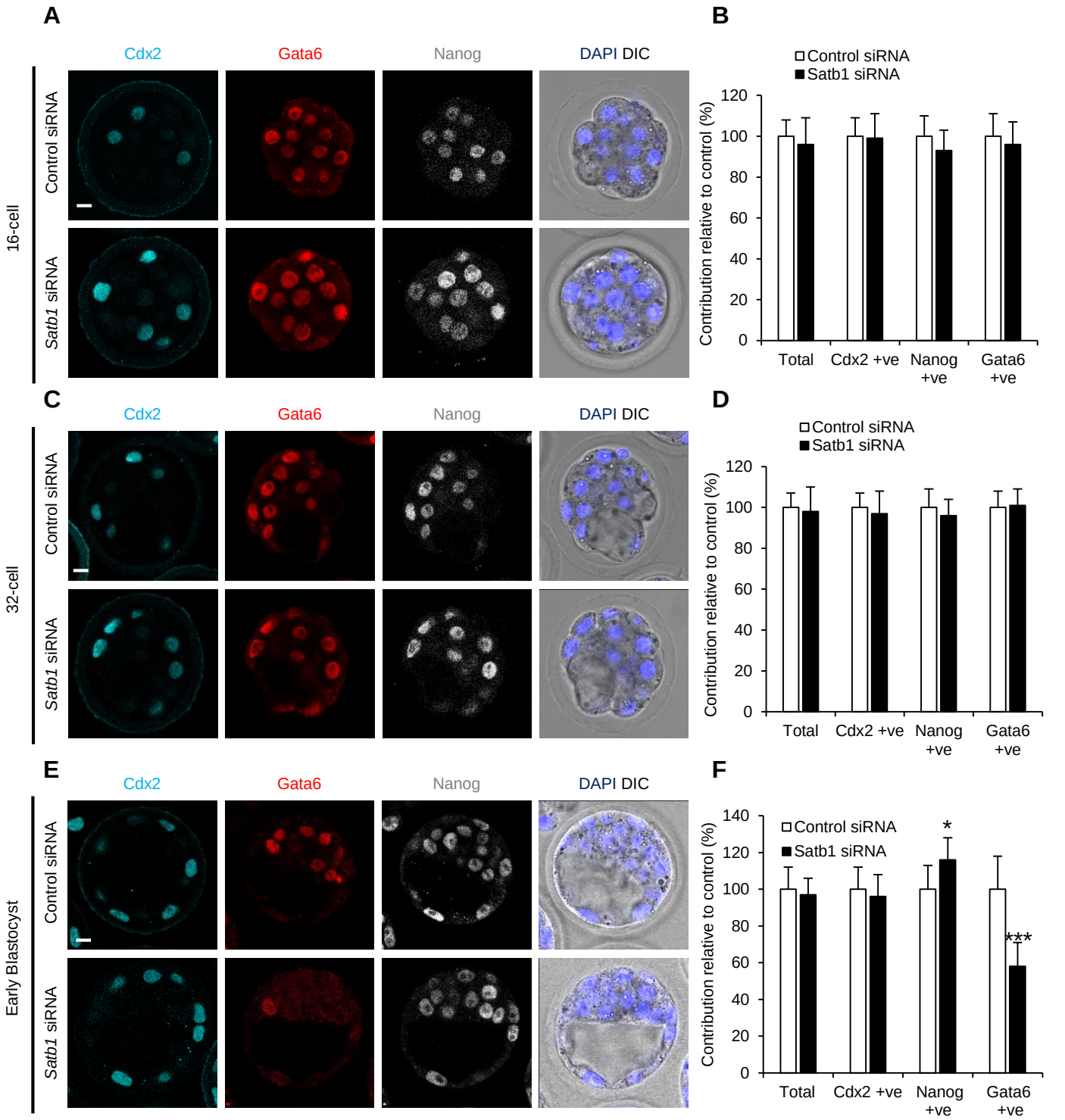

Figure S4. Timing of effect of *Satb1* RNAi. A) Confocal images of control (n=15) and Satb1 siRNA (n=21) injected embryos at the 16-cell stage showing the localisation and distribution of Cdx2, Gata6 and Nanog. B) Relative number of blastomeres that are positive for Cdx2, Nanog and Gata6 from control and Satb1 siRNA injected embryos represented in A. C) Confocal images of control (n=17) and Satb1 siRNA (n=19) injected embryos at the 32-cell stage showing the localisation and distribution of Cdx2, Gata6 and Nanog. D) Relative number of blastomeres that are positive for Cdx2, Nanog and Gata6 from control and Satb1 siRNA injected embryos represented in C. E) Confocal images of control (n=19) and Satb1 siRNA (n=28) injected embryos at the early blastocyst stage showing the localisation and distribution of Cdx2, Gata6 and Nanog. F) Relative number of blastomeres that are positive for Cdx2, Nanog and Gata6 from control and Satb1 siRNA injected embryos represented in E.
Student's t-test was used to test significance *= p<0.005,***= p<0.001. Error bars represent s.e.m. Scale bars, 10 μm.

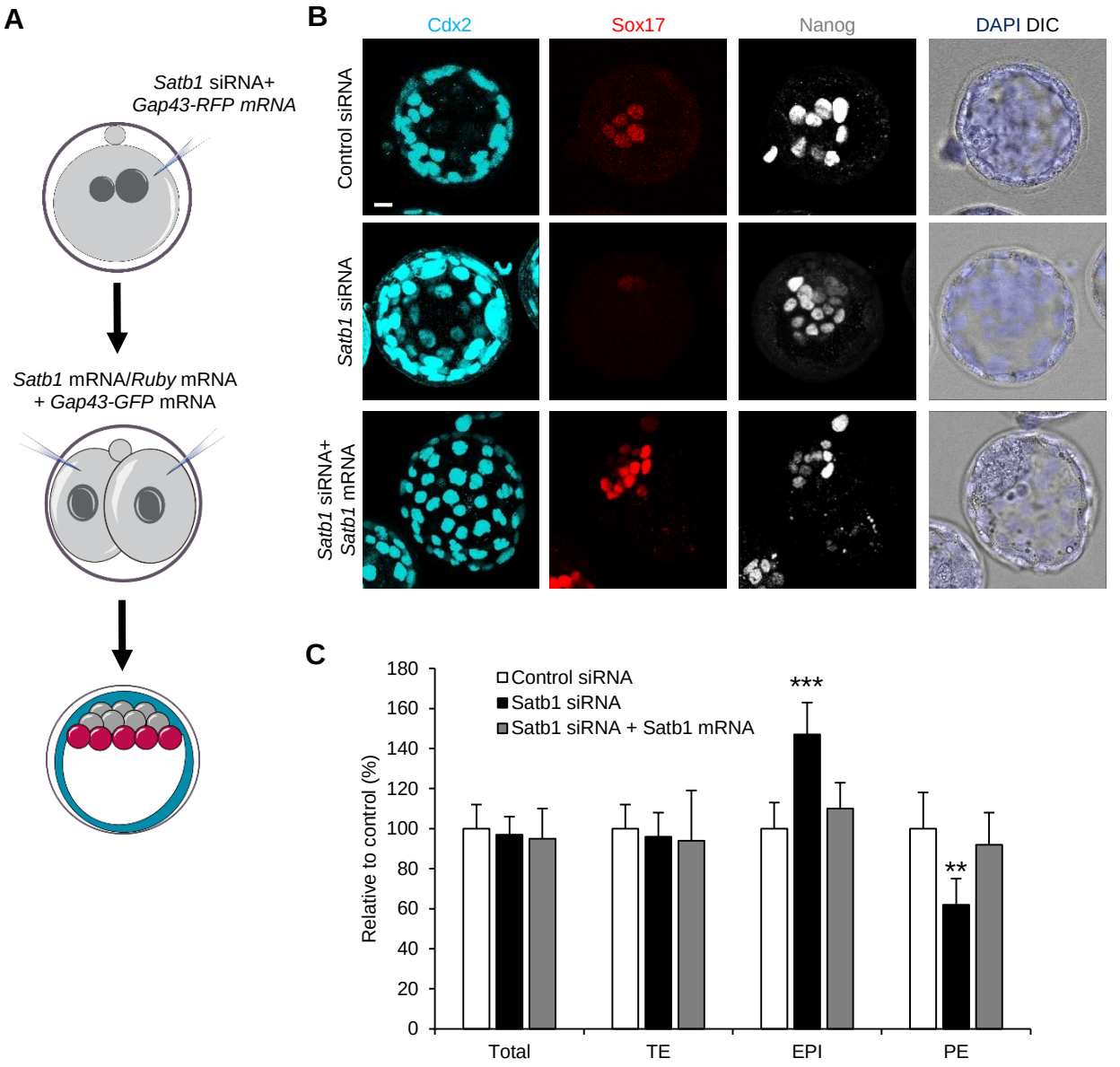

Figure S5. Rescue of *Satb1* siRNA phenotype by *Satb1* mRNA. A) Scheme of Satb1 rescue experiment shown in B and C. Zygotes were injected with *Satb1* siRNA or control siRNA. At the 2-cell stage both blastomeres were then injected with either *Satb1* mRNA or *Ruby* mRNA as a control and then cultured until E4.5. B) Confocal images of control siRNA (n=12), *Satb1* siRNA (n=21) and *Satb1* siRNA + *Satb1* mRNA (n=26) injected embryos. Nanog (EPI), Sox17 (PE) and Cdx2 (TE) were used as lineage markers. C) Contribution of *Satb1* siRNA and *Satb1* siRNA + *Satb1* mRNA injected embryos to TE, PE and EPI, relative to control siRNA injected cells from experiment shown in B.
Student's t-test was used to test significance **= $p<0.01$, ***= $p<0.001$. Error bars represent s.e.m. Scale bar, 10 μm.

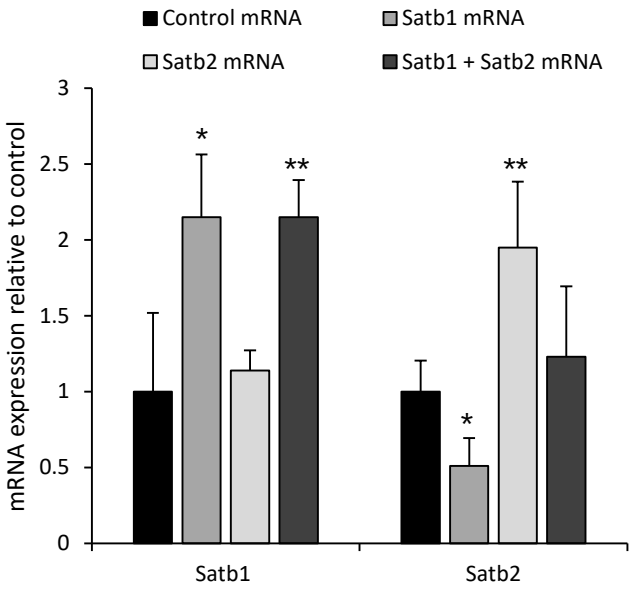

Figure S6. Effect of Satb1 and Satb2 overexpression on Satb1 and Satb2 mRNA levels. qRT–PCR of control (n=63 embryos, three biological repeats), Satb1 (n=71 embryos, three biological repeats), Satb2 (n=58 embryos, three biological repeats), Satb1 + Satb2 (n=47 embryos, three biological repeats) mRNA injected embryos to investigate Satb1 and Satb2 mRNA levels.
Student's t-test was used to test significance **= p<0.01, ***= p<0.001. Error bars represent s.e.m. Scale bar, 10 µm.

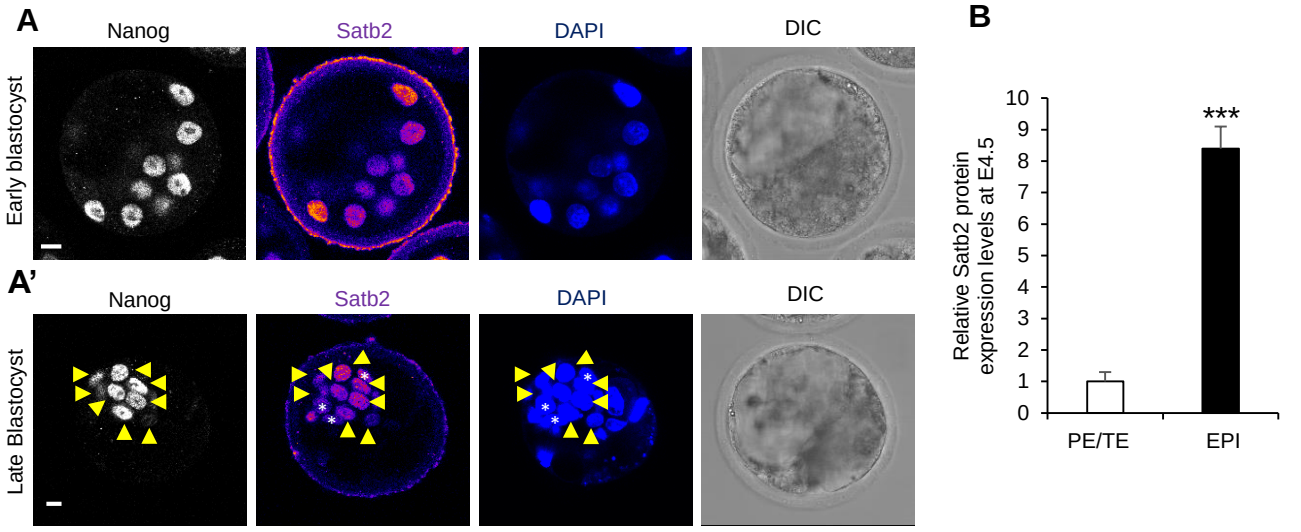

Figure S7. Satb1 expression pattern in blastocysts. A) Confocal images of Satb2 and Nanog staining in early blastocysts (n=12). A') Confocal images of Satb2 and Nanog staining in late blastocysts (n=16). Yellow arrows indicate EPI cells positive for Satb2 . White asterisks indicate PE cells positive for Satb2. B) Quantification of relative fluorescent intensity of Satb2 staining in EPI cells compared to PE/TE cells in late blastocysts as shown in A'. EPI cells were identified by the expression of Nanog.
Scale bars, 10 μm.