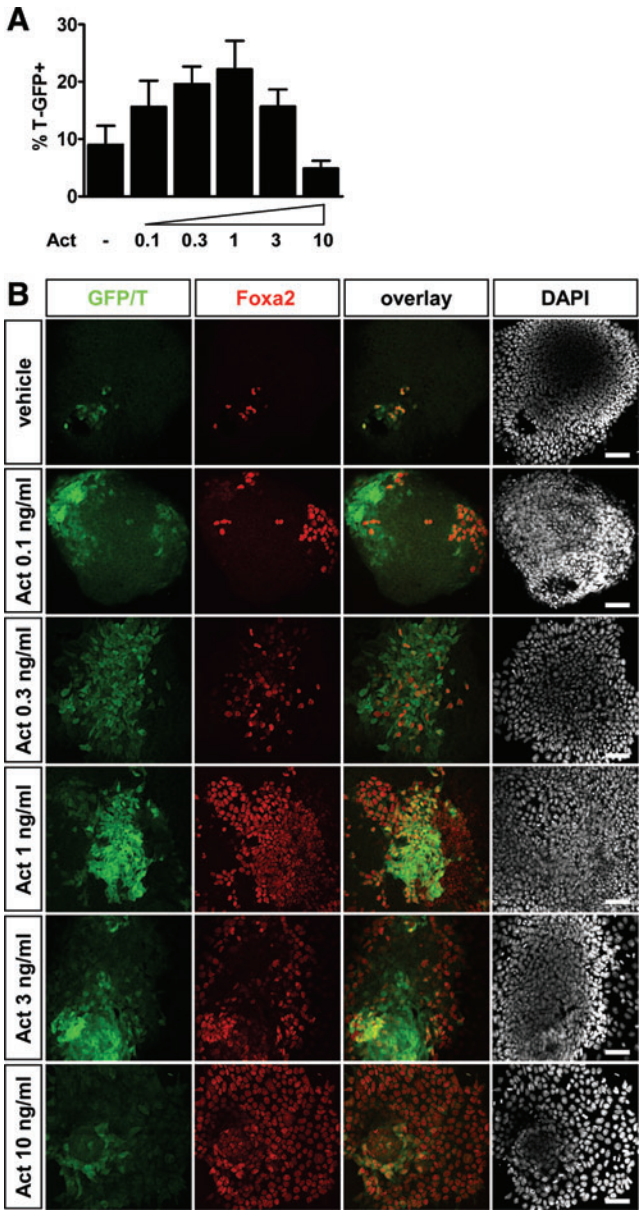
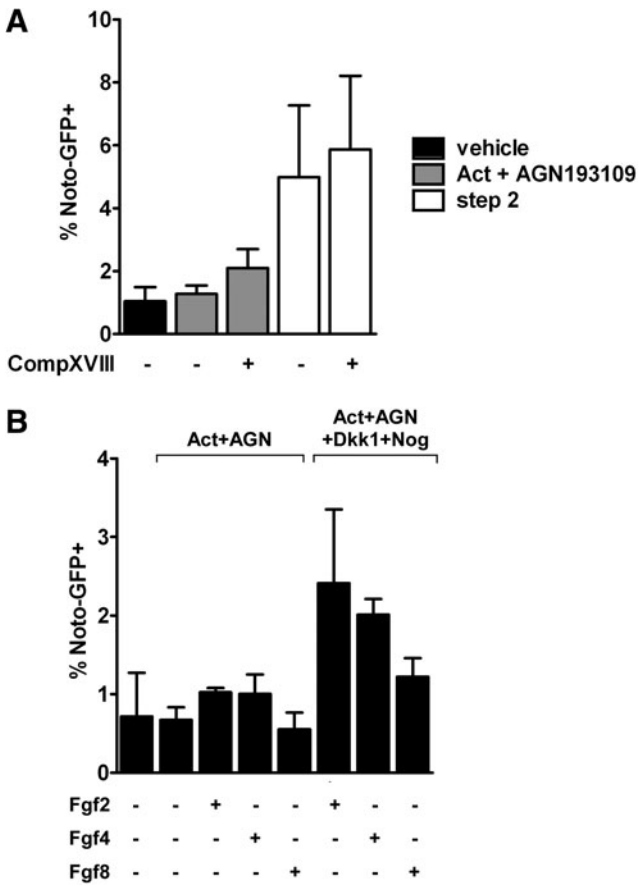
Supplementary Data

Supplementary Table S1. Primer Sequences for Quantitative Reverse Transcriptase–Polymerase Chain Reaction Analysis

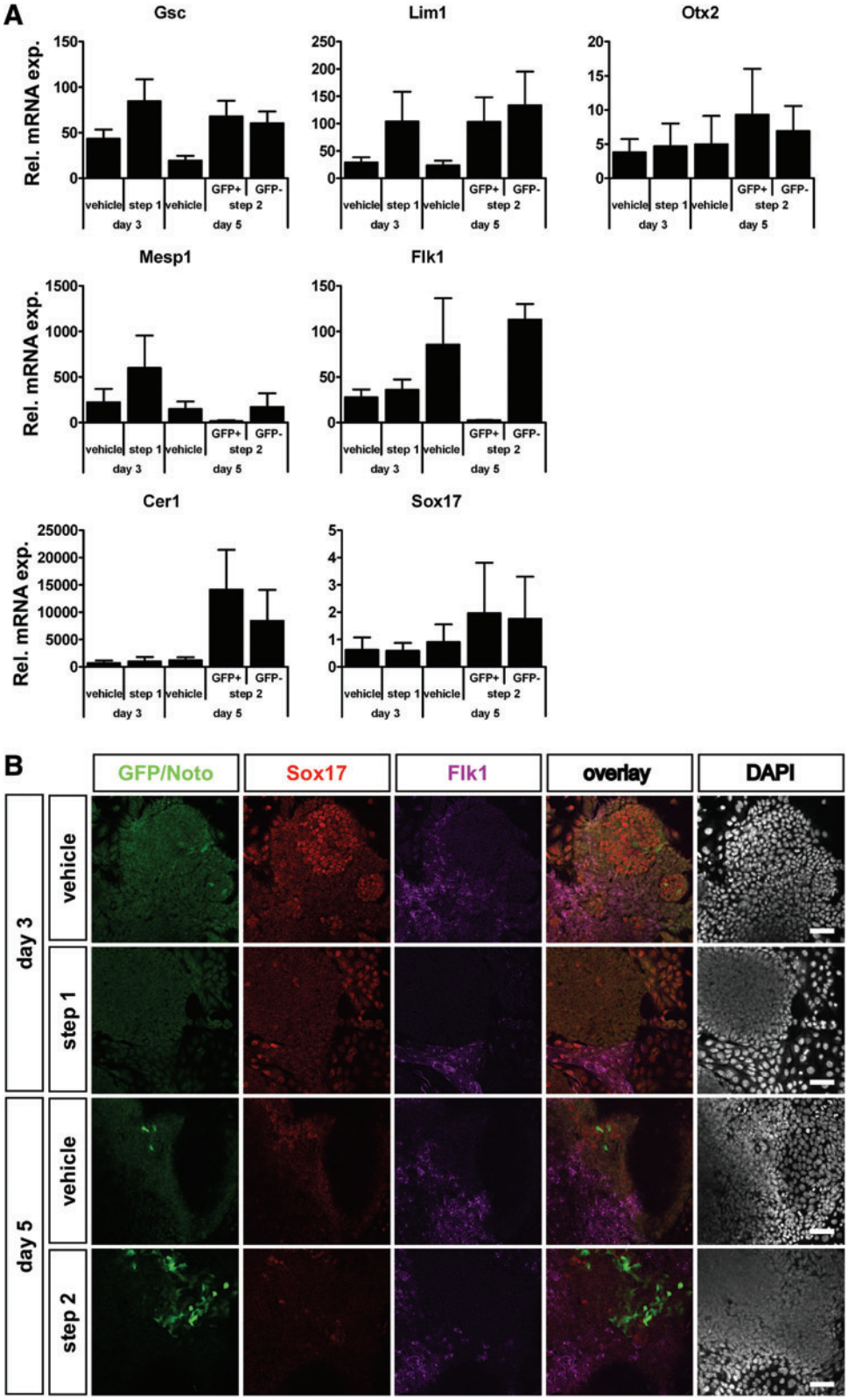
Gene symbol	*Accession no.*	*Forward primer*	*Reverse primer*
Arx	NM_007492.3	CCAACTCAAAGTCCACTCTCCCGT	GTTTAGCCGGGCCGCGCTTA
Cer1	NM_009887.2	CTTTCTTTTAGGCCCGTCCATC	CCAGAGGCAAGAGAACAAGCAG
Chordin	NM_009893	GGCGTGGTAAAAGATCTGGA	CACTCCTTCTGAGGCCAGAC
Flk1	NM_010612.2	CTGACTACACTACCCCAGAAA	ACTTCCTCTTCCTCCATACAG
Foxa2	NM_010446.1	GCTGCAGACACTTCCTACTAC	GGACACAGACAGGTGAGACT
Foxj1	NM_008240.3	GCCGGCACATCAACTGCCCT	TGCTGTAGGAAGGATGTGCCCA
Gsc	NM_010351.1	GGAGAAGGTGGAGGTCTGGTTT	CACTTCTCGGCGTTTTCTGACT
Lim1	NM_008498.2	ACGGAGATTACCAGAGTGAGT	ATGTGCCAGGATGTCAGTA
Mesp1	NM_008588.1	CGTTCCAGTACGCAGAAACA	CAATCATCCGTTGCATTGTC
Nodal	NM_013611.3	AGGAGGGTCAAGTTCCAGGT	GCTCTGGATGTAGGCATGGT
Noggin	NM_008711.2	CGGCCAGCACTATCTACACA	GCGTCTCGTTCAGATCCTTC
Noto	NM_001007472.1	GCTGCAAGAGTTGGAGAAGG	TTTGGAACCAGATCCTCACC
Otx2	NM_144841	GTCCCAACCSTTGCCAGCAGCAGT	CCAGCCATCTCAATCAGTCCCCAGC
Shh	NM_009170.3	AGAAACTCCGAACGATTTAAG	GCCAGCATGCCATACTTGCTG
Sox17	NM_011441.2	GGAGGGTCACCACTGCTTTA	TCAGATGTCTGGAGGTGCTG
T	NM_009309.2	GAACAGCTCTCCAACCTATG	AGACTGGGATACTGGCTAGAG



SUPPLEMENTARY FIG. S1. Low levels of Activin A induce a Foxa2^{+}/T^{+} population. **(A)** FACS analysis of T-GFP expression after 4 days of culture in the presence of different Activin A concentrations ranging from 0.1 to 10 ng/mL. The data represent the mean ± standard deviation of 3 experiments. **(B)** Immunocytochemistry of Foxa2 (*red*) and GFP (*green*) in T$^{Gfp/+}$ cells cultured for 4 days in the presence of different Activin A concentrations. The nuclei were stained with DAPI (*white*). Scale bar = 50 μm. FACS, fluorescence-activated cell sorting.



SUPPLEMENTARY FIG. S2. Notch, Fgf4, and Fgf8 do not increase differentiation into Noto-GFP^{+} cells. FACS analysis of Noto-GFP expression at day 5 of differentiation. After Noto$^{Gfp/+}$ ES cells were cultured for 3 days in 1 ng/mL Activin A, the medium was additionally supplemented with different growth factor combinations **(A)** in the presence or absence of the Notch inhibitor: 10 μM Compound XVIII and **(B)** in the presence of different types of Fgfs (100 ng/mL Fgf2, 100 ng/mL Fgf4, and 100 ng/mL Fgf8). The data represent the mean ± standard deviation of at least 3 independent experiments. ES, embryonic stem.



SUPPLEMENTARY FIG. S3. Characterization of the differentiated ES cell populations. **(A)** mRNA expression level of different markers during differentiation of Noto$^{Gfp/+}$ ES cells with the 2-step protocol or control medium (vehicle). The expression levels of the genes were measured by real-time quantitative reverse transcriptase–polymerase chain reaction. The ratio of gene expression against undifferentiated Noto$^{Gfp/+}$ ES cells is shown. The data represent the mean ± standard error of the mean of at least 3 independent experiments. **(B)** Immunocytochemistry for GFP (*green*), Sox17 (*red*), and Flk1 (*purple*) in differentiated Noto$^{Gfp/+}$ ES cells. Cell nuclei were stained with DAPI (*white*). Scale bar = 50 μm.