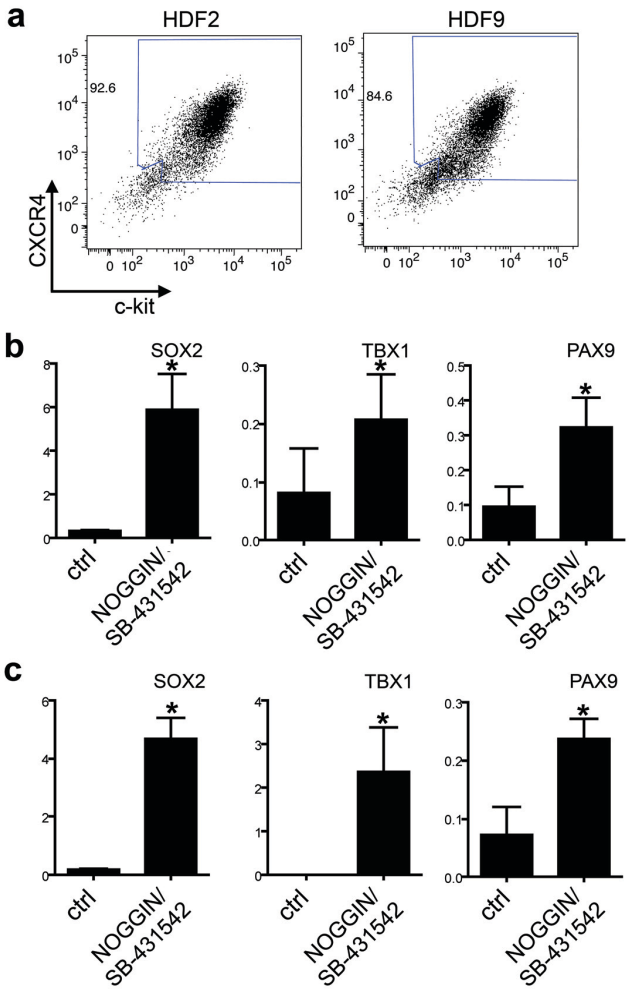
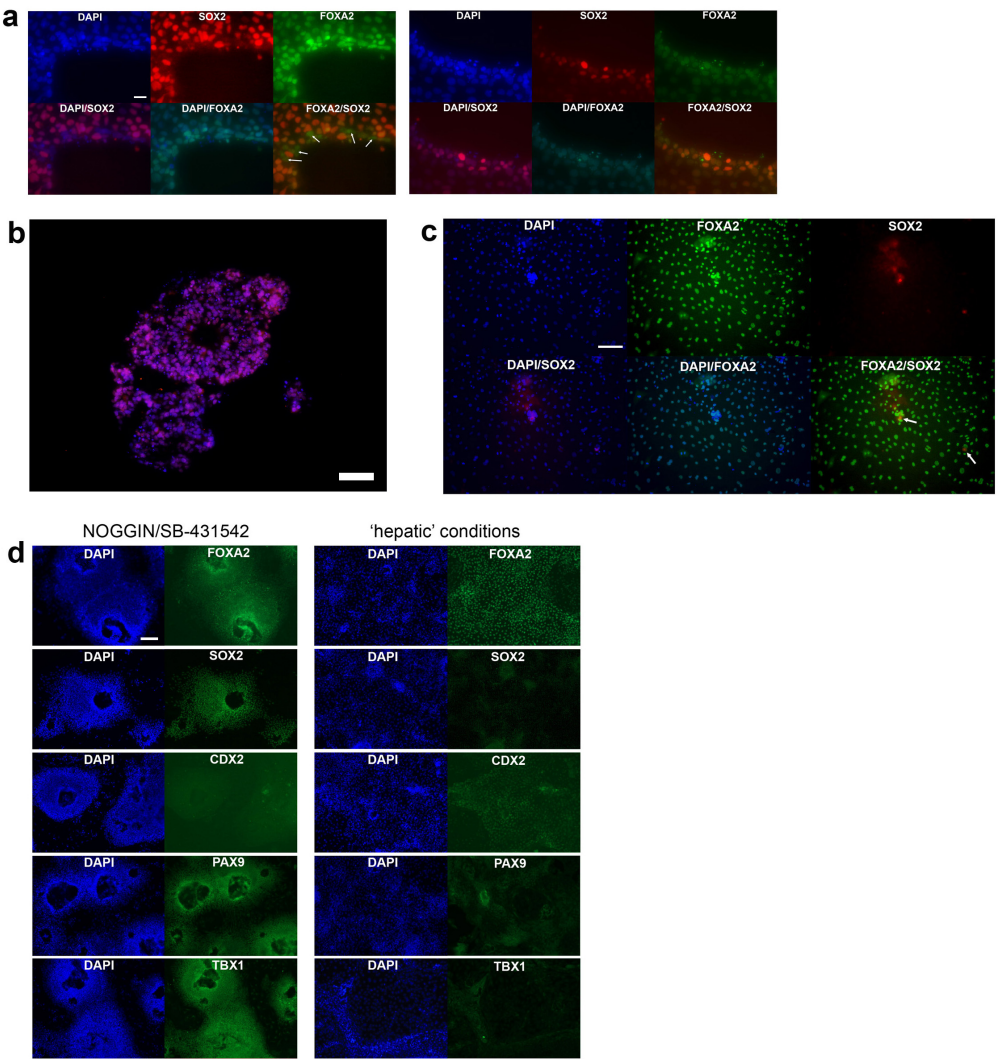
Supplementary figures:



Supplementary Figure 1: Induction of AFE markers in NOGGIN/SB-431542-treated definitive endoderm in hiPS cells. (a) Representative flow cytometric analysis of definitive endodermal markers CXCR4 and C-KIT at day 5 of Activin A induction in HDF2 and HDF9 hiPS cells. **(b)** Expression of *FOXA2, SOX2, and PAX9* mRNA in HFGD2 and HDF9 hiPS cells on day 9 in cultures treated from day 5, after induction of definitive endoderm (see upper left panel), with either serum-free differentiation media (ctrl) or NOGGIN/SB-431542 (n=3 biological triplicate replicates, *significantly different from media ctrl).

Nature Biotechnology: doi:10.1038/nbt.1788



Supplementary Figure 2. Immunofluorescence analysis of NOGGIN/SB-431542-treated definitive endoderm. (a) Expression of FOXA2 and SOX2 at day 9 in cultures of HDF2 (left panel) and HFD9 (right panels) iPS cells treated on day 5, after induction of definitive endoderm, with NOGGIN/SB-431542. Scale bar, 25 μ. **(b)** Staining for SOX2 (red) and DAPI (blue) in colony generated in a Matrigel cultures (see Methods). Scale bar, 25 μ. **(c)** Expression of FOXA2 and SOX2 at day 9 in cultures of HFD9 treated on day 5, after induction of definitive endoderm, with serum-free differentiation media, without addition of NOGGIN/SB-431542 (10x, arrows: $FOXA2^{+}SOX2^{+}$ cells). Scale bar, 100 μ. **(d)** Expression of FOXA2, SOX2, CDX2, PAX9 and TBX1 in day 9 cultures of HES2 cells treated in parallel with either NOGGIN/SB431542 or cultured in 'hepatic' conditions after induction of definitive endoderm until day 5. Scale bar, 100 μ.

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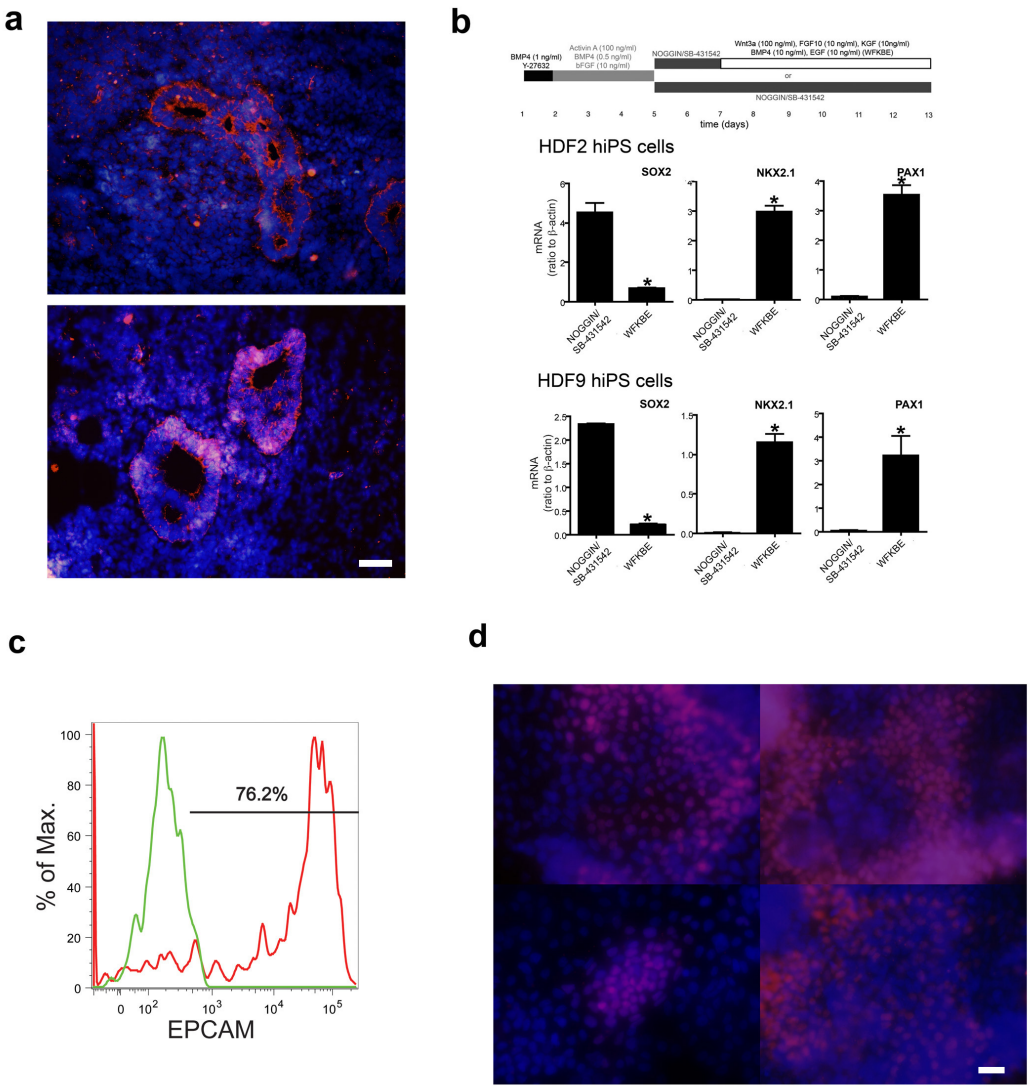

Supplementary Figure 3: Function of AFE generated from hES and hIPS cells. (a) Staining of growths arising 5 weeks after transplantation of NOGGIN/SB-431542-induced AFE cells (day 9) under the kidney capsule of NSG mice for SP-C. Scale bar, 100 μ. **(b)** Expression of *SOX1, NKX2.1*, and *PAX1* mRNA in HDF2 (upper) and HFD9 (lower) hiPS cells differentiated into AFE the conditions schematically represented on top of the panel (n=4 to 6 culture wells from 2 independent experiments, *significantly different from NOGGIN/SB-431542 conditions). **(c)** Expression of EPCAM in HES2 cells differentiated into putative ventral AFE using NOGGIN/SB-431542 followed by WKFBE (green: isotype control, red: EPCAM). **(d)** High magnification views of NKX2.1 immunofluorescence of HDF2 cells differentiated into putative ventral AFE using NOGGIN/SB-431542 followed by WKFBE. Scale bar, 25 μ.

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Supplemental Table 1: Quantitative PCR Primer Sequences

Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
SOX2 hqPCR	GCACATGAAGGAGCACCCGGATTA	CGGGCAGCGTGTACTTATCCTTCTT
GCM2 hqPCR	CAGAGTGGGTCCCTTCTTTACCTACAAC	TGCCTTTCACATTTCCCTGCCT
PAX1 hqPCR	TTAGACTGCCGTACCCTCCTCACAA	AGGAAGGGAAAGAGAAAGGGAAGGGA
SFTPC hqPCR	CCTTCTTATCGTGGTGGTGGTGGT	TCTCCGTGTGTTTCTGGCTCATGT
ACTB hqPCR	TAAGTCCTGCCCTCATTTCCCTCT	TTTGCGGATGTCCACGTCACACTT
GATA6 hqPCR	AGTTCCTACGCTTCGCATCCCTTC	TGAACAGCAGCAAGTCCTCCCA
TBX1 hqPCR	CGGCTCCTACGACTATTGCCC	GGAACGTATTCCTTGCTTGCCCT
SOX17 hqPCR	CTGTTGAATCATAAGCTTGACCTGCCC	ATCTTAAACCCAGCGATGCTTGCC
PAX6 hqPCR	GGGATGAGGATGCATTGTGGTTGT	GAGGAAGAAGAGGGAGAAGAAGGAAGAGG
PAX9 hqPCR	TGGTTATGTTGCTGGACATGGGTG	GGAAGCCGTGACAGAATGACTACCT
CDX2 hqPCR	TAAATGCCAGAGCCAACCTGACTTCC	CAGCAGCAACAACAACACAAACTCCC
FOXP2 hqPCR	TCAGCAAATGCAGCAGATCCTTCAG	ACAGCCTGCTGTTGTTGGAGAAG
MIXL1 hqPCR	CTGTGCTCCTGGAACTGAAACGAA	TGACCTTGGGAGCTAGAGTCAGAGATG
BRACHYURY hqPCR	CAGTGGCAGTCTCAGGTTAAGAAGGA	CGCTACTGCAGGTGTGAGCAA
NXK2.5 hqPCR	TGGAGAAGACAGAGGCGGACAA	ATAGACCTGCGCCTGCGAGAA
NKX2.1 hqPCR	CGGCATGAACATGAGCGGCAT	GCCGACAGGTACTTCTGTTGCTTG
ODD1 hqPCR	CAGCTCACCAACTACTCCTTCCTTCA	TGCAACGCGCTGAAACCATACA
CREB3l3 hqPCR	TCTCCAGAACTTTGCACAACGATGC	TCCTCCGTCGAATTGGTCAGGTT
CEBPA hqPCR	AGAAGTCGGTGGACAAGAACAGCA	ATTGTCACTGGTCAGCTCCAGCA