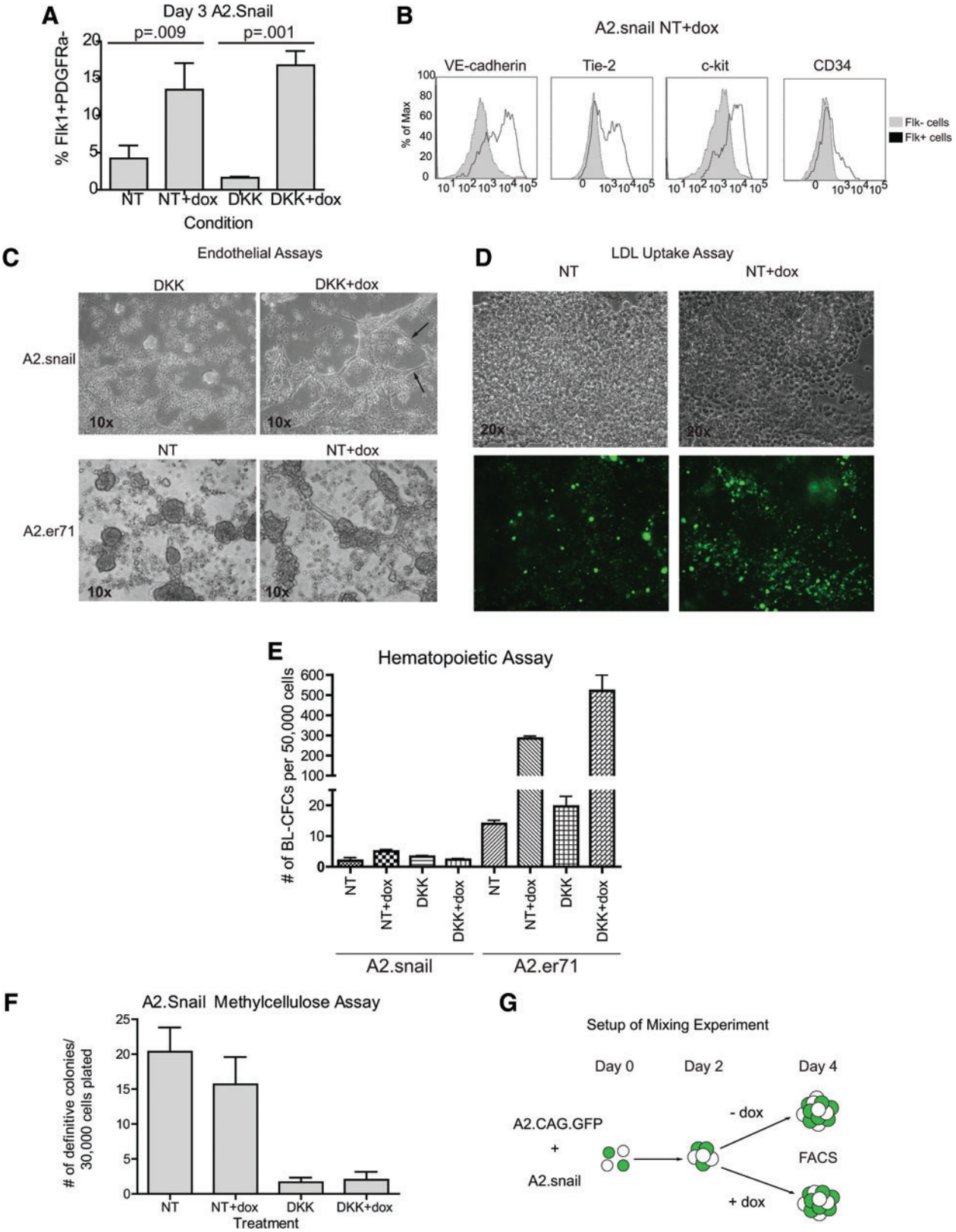
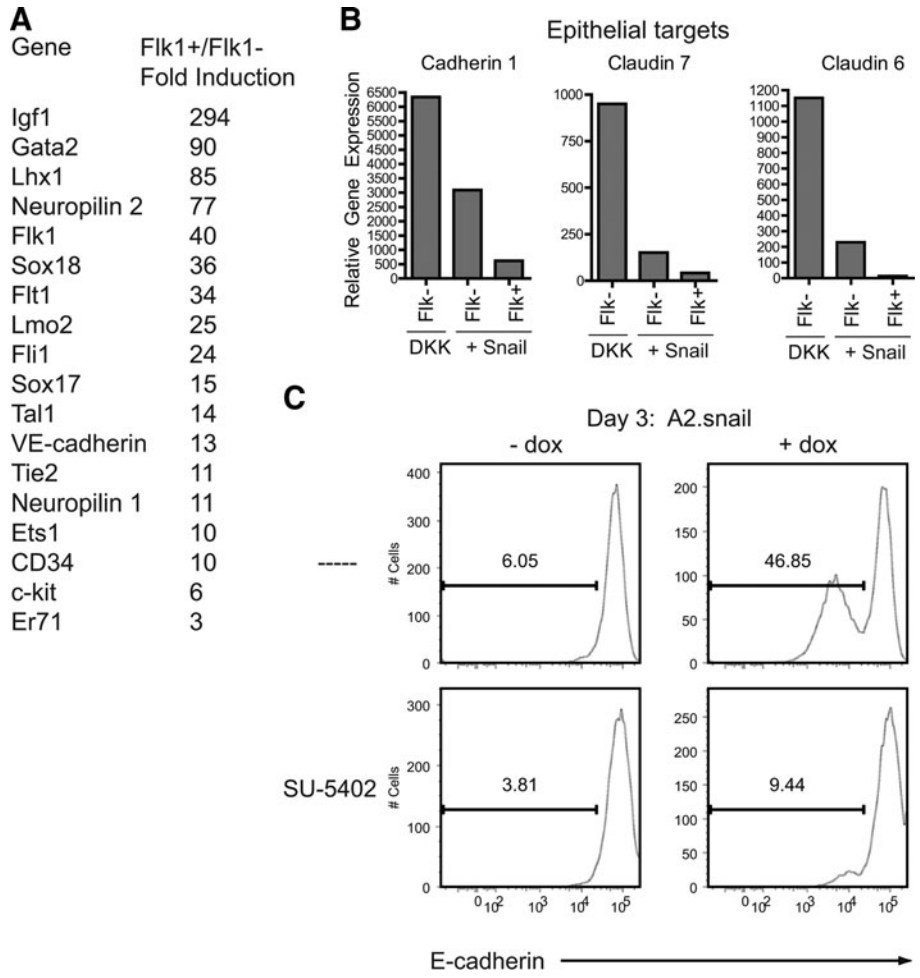
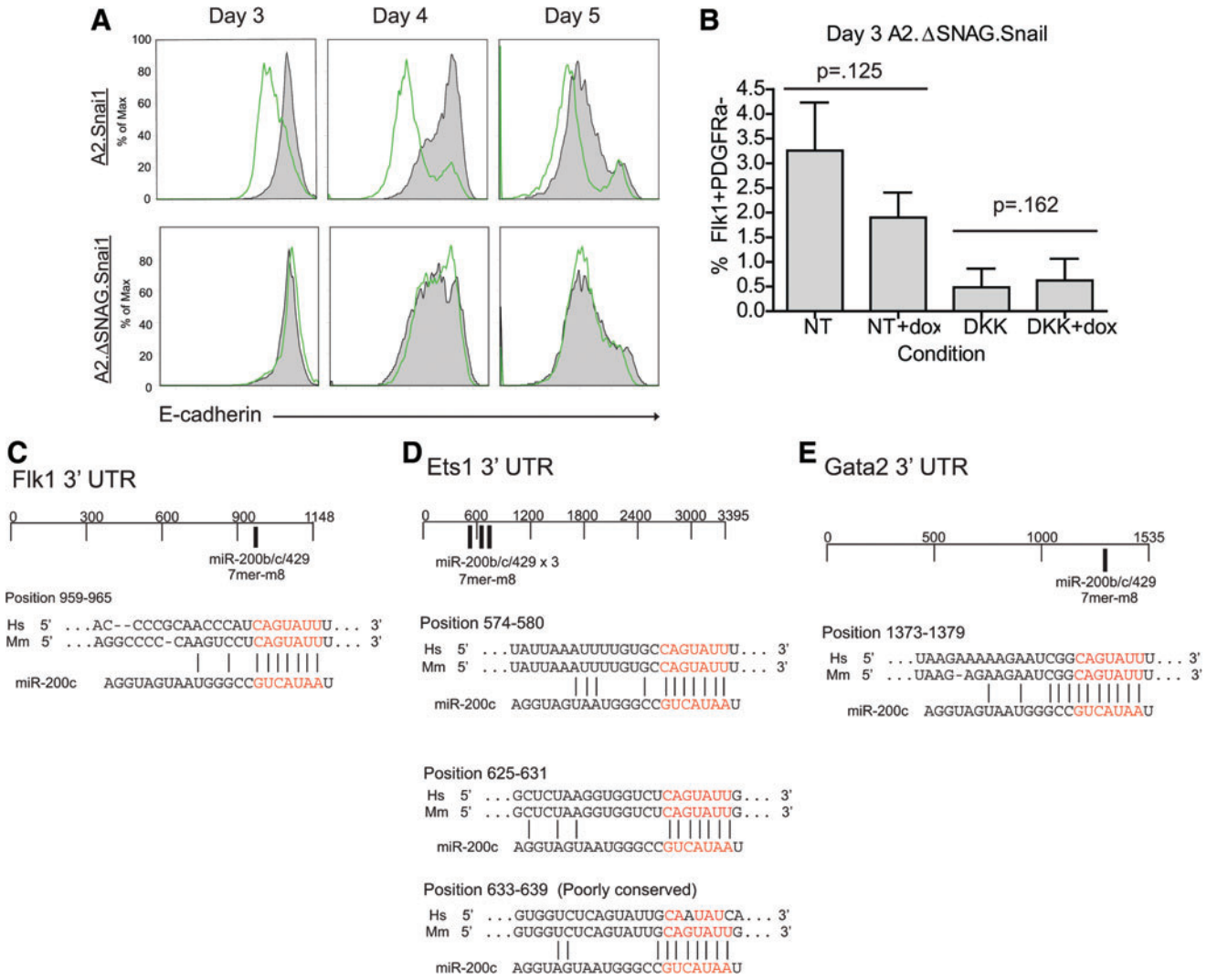
Supplementary Data



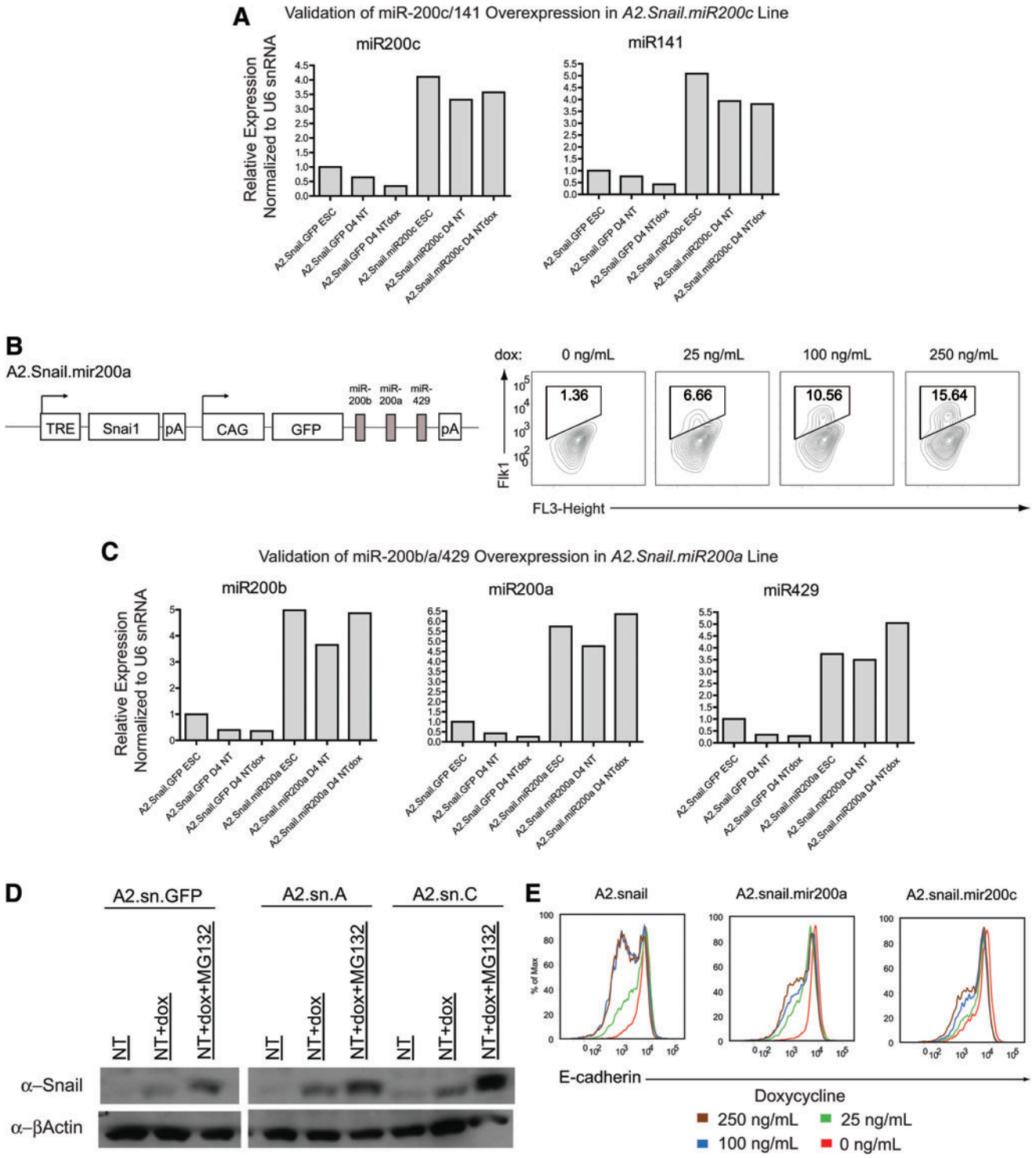
SUPPLEMENTARY FIG. S1. *Snail* promotes the cell-intrinsic induction of endothelial progenitors. **(A)** A2.Snail cells were differentiated in the presence and absence of doxycycline and/or DKK. Shown is the average percentage of Flk1+ PDGFRα- cells from 5 experiments. Error bars indicate SEM, and *P* values were calculated by using a paired student's *t*-test. **(B)** *A2.Snail* cells were differentiated as in Fig. 1B except in the absence of DKK. Cells were analyzed by FACS on day 3 of differentiation. Histograms for VE-cadherin, Tie-2, c-kit, and CD34 are shown for Flk1+ and Flk1- gated populations. **(C)** A2.*Snail* and A2.*Er71* cells were differentiated with or without doxycycline addition on day 1. On day 3, cells were trypsinized and plated in triplicate Matrigel-coated dishes as described in the Materials and Methods section. Shown are images of tubule formation in the indicated cultures after 1 day in culture. **(D)** *A2.Snail* cells were treated with or without doxycyline on day 2. On day 4, embryoid bodies were transferred to Type I collagen-coated dishes and incubated with media containing 10 μg/mL A488-labeled Ac-LDL. Shown are images of the cultures 6 h later. **(E)** A2.*Snail* and A2.*Er71* cells were differentiated in the presence or absence of DKK with doxycycline addition on day 1. On day 3, cells were trypsinized and plated in triplicate methylcellulose dishes with defined cytokines. On day 8, BL-CFC colonies were counted for all conditions. Shown is the average # of BL-CFCs counted per 50,000 cells plated. Error bars indicate SEM. **(F)** *A2.Snail* cells differentiated in the designated conditions just described were plated in methylcellulose blood cultures on day 6. Shown is the # of definitive colonies/30,000 cells counted on day 12. Experiments were done in triplicate, and error bars indicate SEM. **(G)** Diagram of mixing experiment setup. FACS, fluorescence-activated cell sorting; BL-CFC, blast-colony forming cells; Ac-LDL, acetylated-low density lipoprotein; SEM, standard error of the mean.



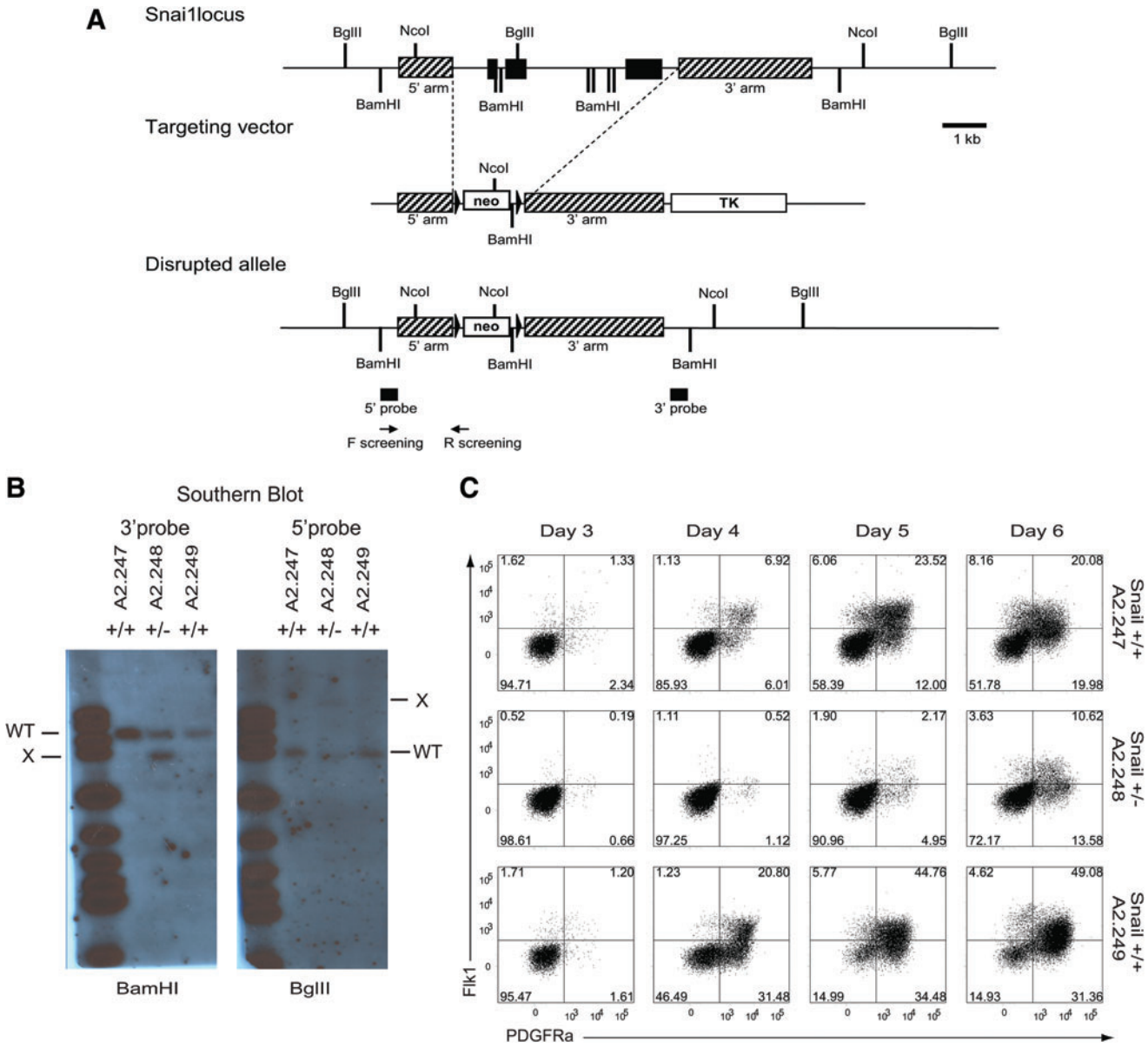
SUPPLEMENTARY FIG. S2. *Snail*-induced Flk1+ progenitors appear to arise from a Gata6+subset of ES cells in an FGF-dependent manner. **(A)** Selected list of highly induced endothelial/blood genes induced in Flk1+ cells 24 h after *Snail* induction. **(B)** Relative gene expression of *Snail*'s direct target genes in Flk1- and Flk1+ subsets. **(C)** *A2.Snail* ES cells were differentiated with and without the addition of doxycycline and the FGF-inhibitor SU-5402 on day 1. FACS analysis was performed on day 3 for E-cadherin. Shown are gated live cells. ES, embryonic stem; FGF, fibroblast growth factor.



SUPPLEMENTARY FIG. S3. Although *Snail* induction of Flk1 is indirect, its downstream target, the miR-200 family, directly targets Flk1 and Ets1 3′ UTRs. **(A)** *A2.Snail* and *A2.ΔSNAG.Snail* cells were differentiated as described in the Methods section. Doxycycline was added on day 2 of differentiation, and FACS analysis was performed on day 4. Shown is an FACS plot of live cells, examining E-cadherin expression. **(B)** A2.ΔSNAG.Snail ES cells were differentiated as described in Figure 3A. Shown is the average percentage of Flk1+ PDGFRα cells from 3 separate experiments (error bars indicate SEM, and *P* values were calculated by using a paired student's *t*-test). **(C)** Diagram of Flk1 3′ UTR with predicted miR-200c target site (shown in red) and alignment. **(D)** Diagram of Ets1 3′ UTR with predicted miR-200c target sites and alignment. **(E)** Diagram of Gata2 3′ UTR with predicted miR-200c target site and alignment.



SUPPLEMENTARY FIG. S4. *Snail* requires the down-regulation of the miR-200 family for efficient generation of Flk1+ endothelial cells. **(A)** Validation of miR-200c/141 overexpression in the *A2.Snail.miR200c* line. RNA was harvested from ES cells as well as day 4 NT and NT+dox cultures of *A2.Snail.GFP* and A2.Snail.CAG.miR200c lines. Taqman miRNA assays were performed to detect miR-200c and miR-141 levels normalized to U6 snRNA. **(B)** (*left*) Diagram of *A2.Snail.miR200a* cell line generated with inducible Snail expression with or without constitutive expression of miR-200b/a/429. (*right*) *A2.Snail.GFP* and *A2.Snail.miR200c* ES cells were differentiated with or without various concentrations of doxycycline induction on day 2. FACS analysis was performed for Flk1 expression on day 4. Shown are gated live cells. **(C)** Validation of miR-200b/a/429 overexpression in the *A2.Snail.miR200a* line. RNA was harvested from ES cells as well as day 4 NT and NT+dox cultures of *A2.Snail.GFP* and the *A2.Snail.miR200a* line. Taqman miRNA assays were performed to detect miR-200b, miR-200a, and miR-429 levels normalized to U6 snRNA. **(D)** Western blot examining *Snail* and β-actin expression in the indicated differentiating ES cell lines on day 3, 24 h after doxycycline induction on day 2. **(E)** *A2.Snail.GFP, A2.Snail.miR200a,* and *A2.Snail.miR200c* ES cells were differentiated with or without various concentrations of doxycycline induction on day 2 (See Fig. 4A). FACS analysis was performed for E-cadherin expression on day 4. Shown are gated live cells.



SUPPLEMENTARY FIG. S5. *Snail* haploinsufficiency leads to decreased differentiation of Flk1 and PDGFRα expressing cells. **(A)** Diagram of the *Snail* locus, the targeting vector used, and the final disrupted allele generated after appropriate homologous recombination. **(B)** Southern blot showing 3 clones picked after targeting A2lox cells. A2.247 and A2.249 are non-targeted *Snail* + / + ES cells, whereas A2.248 is a properly targeted *Snail* + / − ES cell. **(C)** A2.247, A2.248 (*Snail* + / −), and A2.249 were differentiated. On days 3, 4, 5, and 6, cells were harvested, and flow cytometry was performed to assess for Flk1/PDGFRα expression.

Supplementary Table S1. List of Oligonucleotide Primers Used in This Study

ATW ΔSNAG.Snail F	GGCCAACATGAAGCCGTCCGACCCCCGCCGG
ATW ΔSNAG.Snail R	GGACGGCTTCATGTTGGCCAGAGCGACCTA
Flk1 3′UTR F	AATGGAAGTGGTCCTGTCCC
Flk1 3′UTR R	AACATAAGCACACAGGCAGAAACC
ATW Flk1 3′UTR MUT F	CCAAGTCCT**ATCTGAA**TTAGCTTTGTGGCTTCCTGATGGCAG
ATW Flk1 3′UTR MUT R	GCTAA**TTCAGAT**AGGACTTGGGGGCCTGACAGGAGTGGA
Ets1 3′UTR F	TCATGGACAGACGCGCAGAAG
Ets1 3′UTR R	TTATGAATGAATTTCTTTGTTTCTTTAATTTCAC
ATW Ets1 3′UTR MUT F1	GTGC**ATCTGAA**TTTTTTTCCTTAAAAAAATATCGTCTTAAGCTC
ATW Ets1 3′UTR MUT R1	TAAGGAAAAAAA**TTCAGAT**GCACAAAATTTAATAAAATAAACTTCAACAT
ATW Ets1 3′UTR MUT F2	GGTCT**ATCTGAA**G**ATCTGAA**GTGAGTTTGTTGTTATTTGCTGGC
ATW Ets1 3′UTR MUT R2	AC**TTCAGAT**C**TTCAGAT**AGACCACCTTAGAGCTTAAGACGATAT
Gata2 3′UTR F	CAGGACTAGTGCAAGCCTCCCACTGGACAGACA
Gata2 3′UTR R	CAGGTTCGAACAACAGGCGACAGCATTCACAAAAAGTATT
ATW Gata2 3′UTR F	GAAGAATCGG**ATCTGAA**TTCTGTTTTTATGTTTTGGGCTTGTTTTA
ATW Gata2 3′UTR R	CAGAA**TTCAGAT**CCGATTCTTCTCTTATGCGGGTACTAGCAC
miR200c/141 F	GCTGGATCCCCTTTCTTACGAAGACCGAGTCTCCA
miR200c/141 R	CTAGATATCCCTCAAGAGGAGGTGCCCAGG
miR200b/a/429 F	GCTGGATCCCTCCTTGGTTCCATGACCTGAGAA
miR200b/a/429 R	CTAGATATCGACTGGACCTGTTGTCTAGGCTATTCTG

Nucleotides in bold indicate mutation sites (in ATW primers), whereas underlined nucleotides indicate restriction sites.