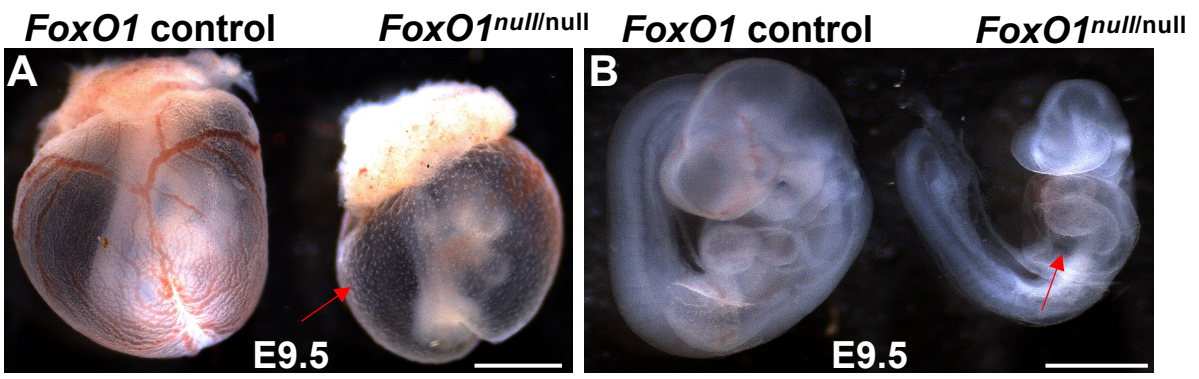


Fig. S1. *FoxO1*^{null} embryos show vascular remodeling defects. (A and B) Bright field images of E9.5 littermate control and *FoxO1*^{null} embryos in and out of the YS. (C) qRT-PCR for FoxO1 expression in control and *FoxO1*^{null} YSs.

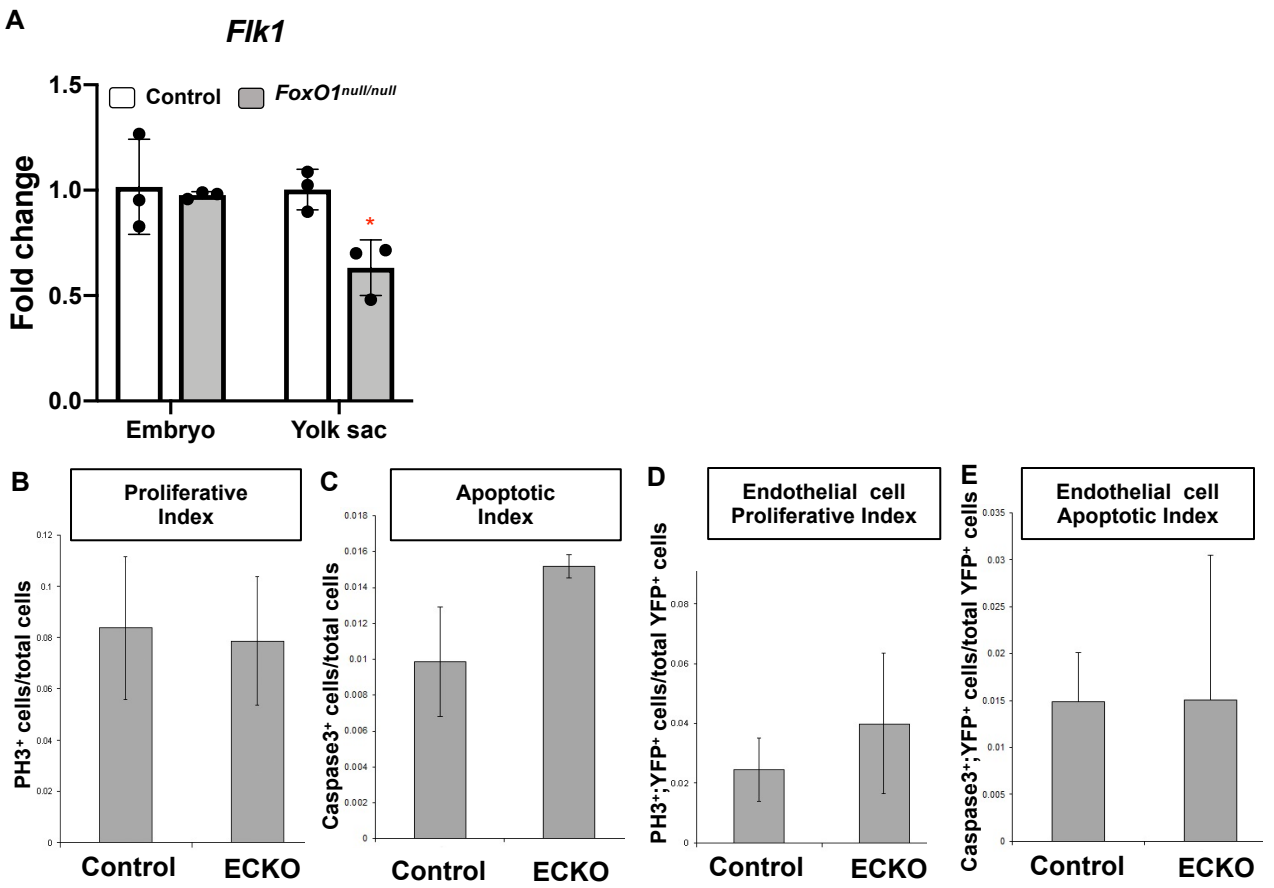
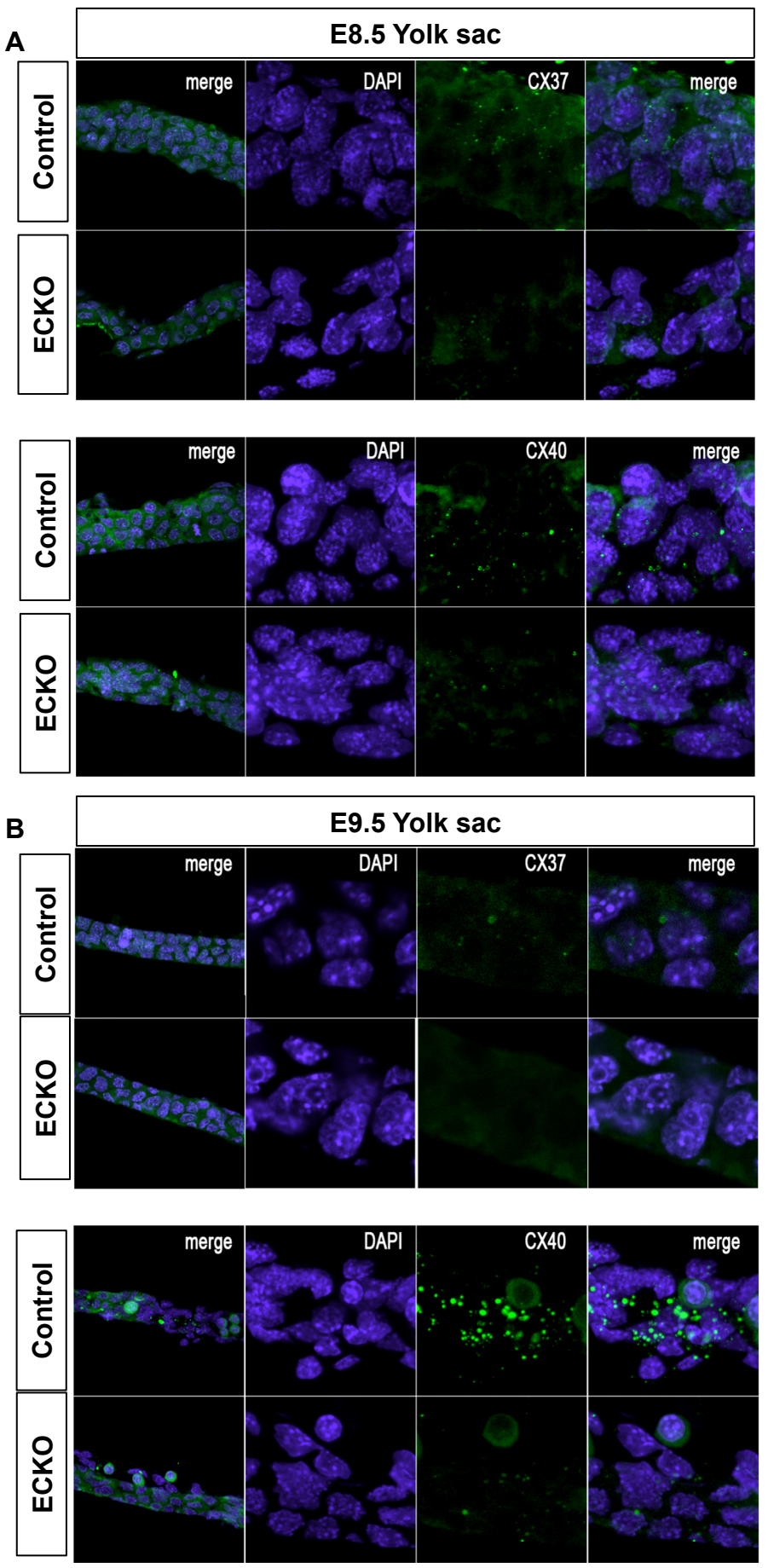
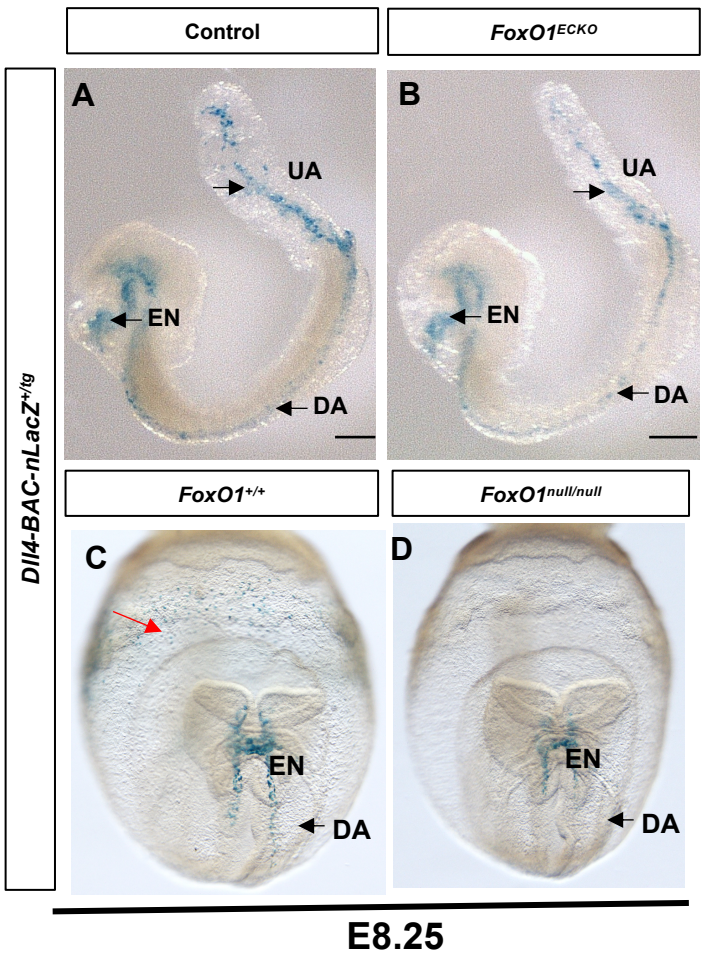


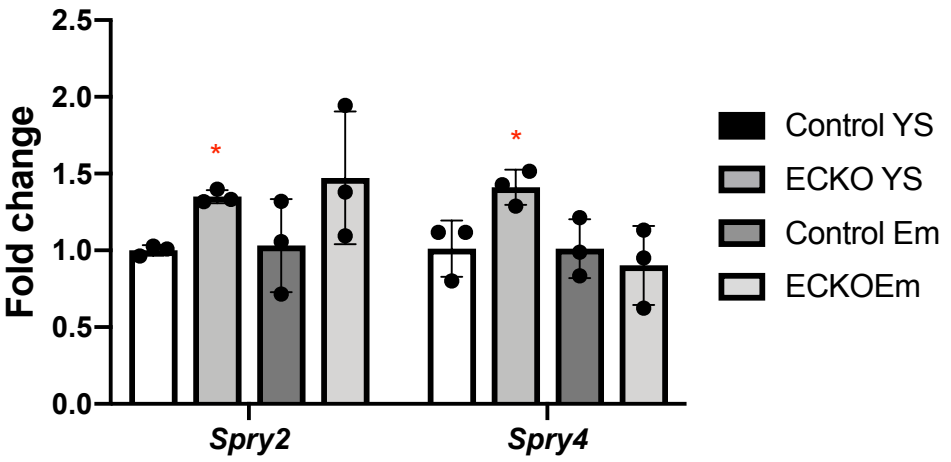
Fig. S2. (A) Flk1 expression in littermate control and *FoxO1*^{null} embryos and YSs by qRT-PCR. (B and C) Quantification of proliferation and apoptosis in littermate control and *FoxO1*^{ECKO} YSs. (D and E) Quantification of proliferation and apoptosis in littermate control and *FoxO1*^{ECKO} YS YFP+ ECs.



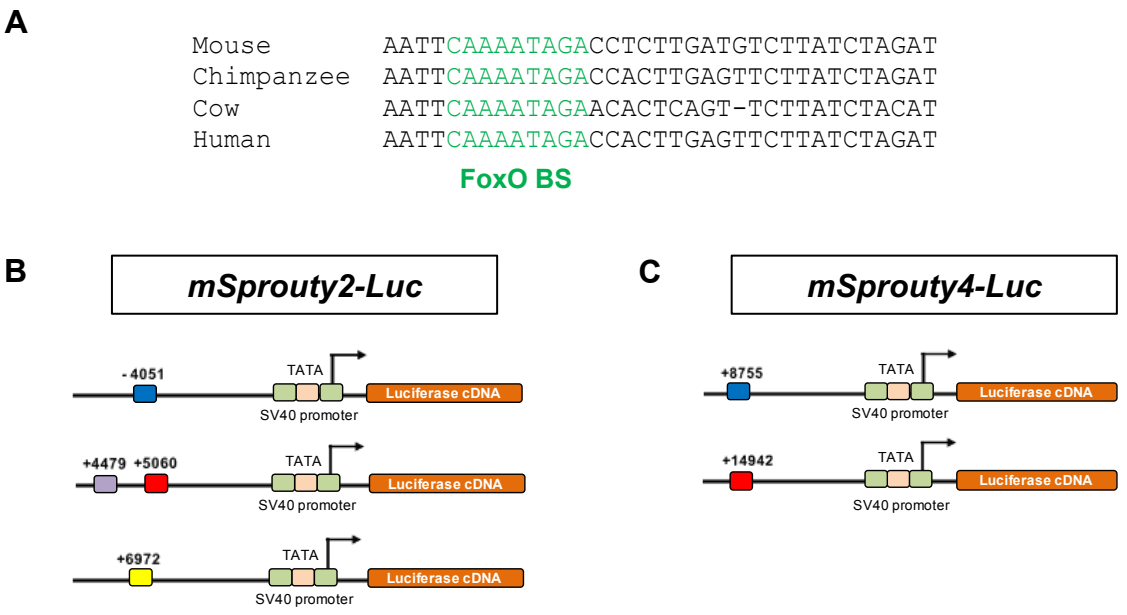
• **Fig. S3.** Co-immuno labeling of CX37, CX40and DAPI in control and *FoxO1*^{ECKO} YSs at E8.25 (A) and E9.5 (B).



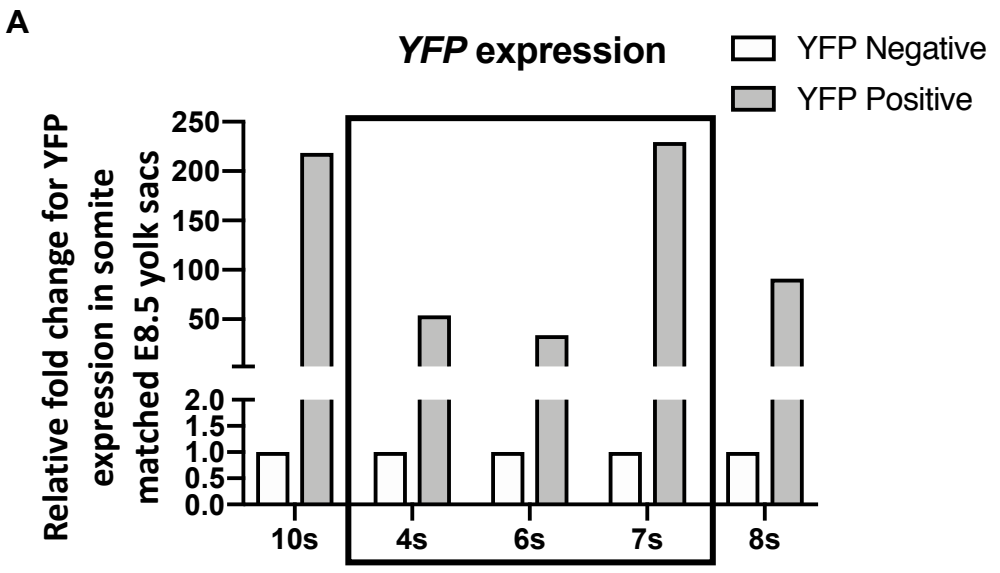
• **Fig. S4.** (A and B) *nlacZ* reporter activity in E8.25 littermate control and *FoxO1*^{ECKO} embryos dissected out of the yolk sac. (C and D) Anterior views of *nlacZ* reporter activity in E8.25 littermate control and *FoxO1*^{null} embryos. UA = umbilical artery, EN = endocardium, DA = dorsal aorta.

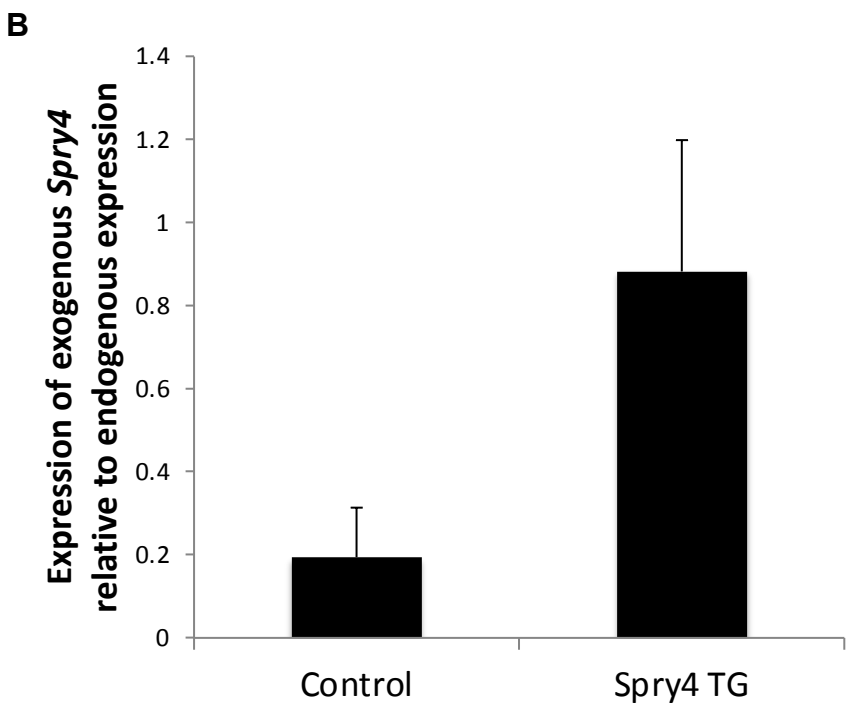


• **Fig. S5.** qRT-PCR for Sprouty2/4 in littermate control and *FoxO1*^{ECKO} YSs and embryos. Bars in graph are means± standard deviation.



- **Fig. S6.** (A) FOXO1 conserved binding site in Mouse, Chimpanzee, Cow, and Human. (B and C) Luciferase constructs containing FOXO1 binding sites for *Sprouty2* and *Sprouty4*.





- **Fig. S7.** (A) qRT-PCR for *YFP* expression in somite matched non-trangenic (control) and transgenic embryos. Black box indicates samples that fall withing E8.25 somite stage and used for further gene analysis.
  (B) qRT-PCR for relative expression of exogenous *Sprouty4* to endogenous *Sprouty4* for all *YFP*+ transgenic embryos and somite matched controls. Bars in graph are means ± standard deviation.

Table S1. Primer sequences used for genotyping, ChIP-qPCR, and cloning

Gene/allele		Primer sequences (5'-3')	Purpose
ChIP *mSprouty2*	(-4051)	TTCCAGTCCTCCAAGCAATCTAG AGTGCCTCCAGGAAGGGAAT	ChIP-qPCR
ChIP *mSprouty2*	(4479)	AATTAGCAAATGGCTCCCGG TTTGTGACTGTGCCATGAAGC	ChIP-qPCR
ChIP *mSprouty2*	(5060)	TAGGGCGACTCAGTGGCTATC GACCGGAGTCAAAGGACCTTC	ChIP-qPCR
ChIP *mSprouty2*	(6972)	CATTTGTGTGTTTTGGGGAGAGAT CGGCAGTTGGGTTGGAATTA	ChIP-qPCR
ChIP *mSprouty4*	(8755)	GATCTCCATCCGAATTCCAAATG CTTGGTTCGGCAAAGGCGAGAAAC	ChIP-qPCR
ChIP *mSprouty4*	(14942)	CCACCACAAAAGTTACCACAGAAG GATATCTTCTAGATCAGTAC	ChIP-qPCR
ChIP negative control		GAAACCCGAATCTACATTCCGTTCC CTGGATTAACCCGATTATACACC	ChIP-qPCR
Luc *mSprouty2*	(-4051)	GTGTACACAGGTATACTCTAGTCACCAACCC GGGACTCGATGTTGCAATGAGATACTCAACTC	PCR cloning
Luc m *Sprouty2*	(4479/5060)	GATCTGTGACAAGCAGTGCCTCTGCTCAG GCCACAAGGTGACTAATGTTGTCAAGATGG	PCR cloning
Luc *mSprouty2*	(6972)	CATTCAGACCTAGCACTGTGATTCATGC CAGTGTTCAGCCAAACCAGGTAGGCCTTGA	PCR cloning
Luc *mSprouty4*	(8755)	CAGCGGTTCACTTGAAGCTGCCTTGACAAG CTCTGCCTCCCAACTGCTGGGATTAAAG	PCR cloning
Luc *mSprouty4*	(14942)	CTGTAGCTGTTTCTGACTTCTTGGCTAGC GGCTGAAGACTCATTGTAGAATGGGTCATG	PCR cloning
Endogenous	*mSpry4 cDNA*	GAAGCCTGTCCCTTGGTGCAGTTCAG CTGGTCAATGGGTAAGATGGTGAGTG	qRT-PCR
Exogenous	*mSpry4 cDNA*	GCGAGGTGCAGGAATTCGTTAAGCTCTCCC CTGGTCAATGGGTAAGATGGTGAGTG	qRT-PCR

* pGL3-Promoter

Table S2. Taqman assays for Gene expression analysis

FoxO1	Mm00490672_m1	*Hey1*	Mm00468865_m1
FoxO3a	Mm01185722_m1	*Hey2*	Mm00468865_m1
FoxO4	Mm00840140_g1	*Jagged 1*	Mm00496902_m1
Flk1 (Kdr)	Mm00840140_g1	*Nrp1*	Mm00435379_m1
PECAM1	Mm01242584_m1	*Nrp2*	Mm00803099_m1
Tie2 (Tek)	Mm01242584_m1	*CoupTFII*	Mm00772789_m1
Flt1	Mm00438980_m1	*EphB4*	Mm01201157_m1
Connexin 43	Mm00438980_m1	*AFP*	Mm00431715_m1
eNOS	Mm00435217_m1	*ADM*	Mm00437438_g1
Connexin 37	Mm00433610_s1	*BMPER*	Mm01175806_m1
EphrinB2	Mm01215897_m1	*Sprouty2*	Mm00442344_m1
Notch1	Mm00435249_m1	*Sprouty4*	Mm00442345_m1
Dll4	Mm00444619_m1		