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Supplemental Data

ER71 Acts Downstream of BMP, Notch, and Wnt Signaling in Blood and Vessel Progenitor Specification

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Figure S1. (A) Sequence of the promoter of *Flk1* used for the luciferase assay. Nine boxes indicate potential Ets binding sites (4 red in sense and 5 blue in anti-sense). T in green box indicates transcriptional start site (based on the numbering by Ronicke et al., 1996). (B) ER71 can activate *Flk1* gene in a dosage dependent manner. RNA from MS1 endothelial cells transfected with control or ER71 were analysed by RT-PCR with the indicated oligos. Numbers indicate the relative ratio of band intensity. *Er71*: forward-5'-GGGGGATTCCAGGACTGCAGCATCCCTTTCG-3', reverse-5'-GGGGAATTCGAAGCGGTATGTGTACTTGCG-3', *Vegfr2* (*Flk1*): forward-5'-TACACAATTCAGAGCGATGTGTGGT-3', reverse-5'-CTGGTTCCTCCAATGGGATATCTTC-3' (Hamada et al., 2005), *β-actin*: forward-5'-ATGAAG ATCCTGACCGAGCG-3', reverse-5'-TACTTGCGCTCAGGAGGAGC-3' (Kubo et al., 2004).

Figure S2. (A) H & E staining of transverse section of E9.0 and E9.5. Mutant embryos show lack of endocardium and disorganized myocardium. The boxed area is shown at higher magnification. DA, dorsal aorta; He, heart; NT, neural tube. Scale bar; 50 μm (B) H & E staining of sagittal section of E9.0. SM; somites. Scale bars; 50 μm. (C) Whole-

mount staining with anti-SM actin of E9.5 embryos. V; ventricle, A; Atrium. Scale bars; 200 μm. (D) Thickness of labyrinth layer. (E) Tunel assay of sagittal sections of E9.5. Fb; Fore-brain, Mb; Mid-brain. Scale bars; 200 μm

Figure S3. (A) Microarray analysis of *Er71*$^{-/-}$ embryos at E8.5 and E9.5. Aliquots of 1μg of total RNA extracted from whole embryos were used as a template for reverse transcriptase with oligo-dT primers (Intron). All the microarray experiments were done with 20K mouse cDNA chips from Digital Genomics (Seoul, South Korea). Genes showing significant expression changes (at least 1.5 fold) were selected. Data represent increase (red; b) or decreased (green; a) gene expression that was changed at least 1.5-fold in *Er71*$^{-/-}$ embryos. 1, E8.5 *Er71*$^{+/+}$ vs. *Er71*$^{+/-;}$ 2, E8.5 *Er71*$^{+/-}$ vs. *Er71*$^{-/-}$; 3, E8.5 *Er71*$^{+/+}$ vs. *Er71*$^{-/-}$; 4, E9.5 *Er71*$^{+/+}$ vs. *Er71*$^{-/-}$. (B) Gene expression of *Er71*$^{+/+}$ and *Er71*$^{-/-}$ embryonic hearts at E9.0 (Abu-Issa and Kirby, 2007). Genes were normalized against *Gapdh* and then the ratio of the gene quantity (WT) to gene quantity (MT) was determined to yield normalized fold change.

Supplementary References

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Wang, X. and Seed, B. (2003). A PCR primer bank for quantitative gene expression analysis. Nucleic Acids Research 31, e154;1-8.

A

-492 **ACGGGGCGGGGGATGCGGTGGCCAAAGCACCATAAAACAAAACTTCCAAG**
TACTGACCAACTCACTGCAAGTTTGTGCCCCGAGTACATCTAGGTTCAGGG
GTTCTTGTCTTCATGCTCCCAACTGCGGGGGGATTTTTGGTCCCTTGGGACT
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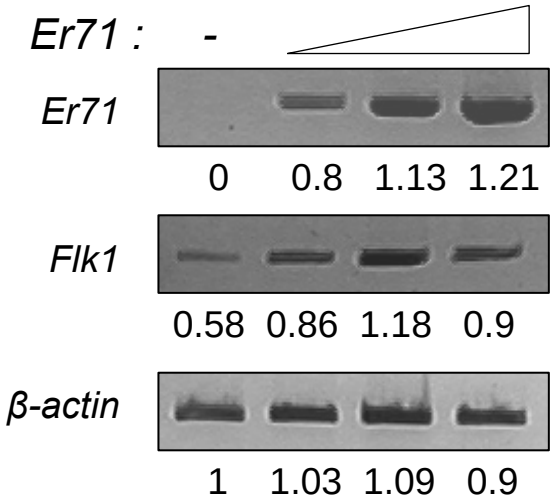
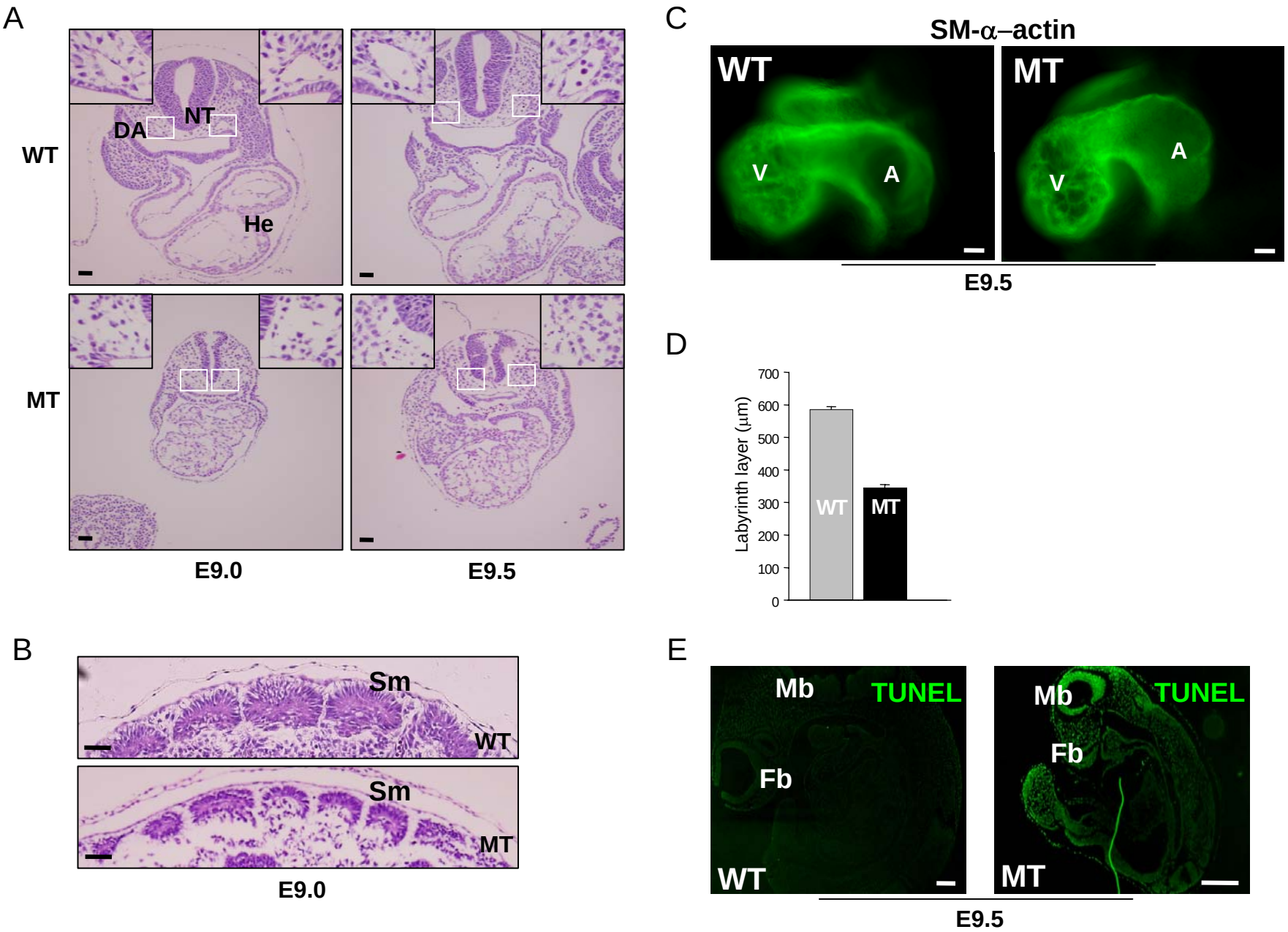
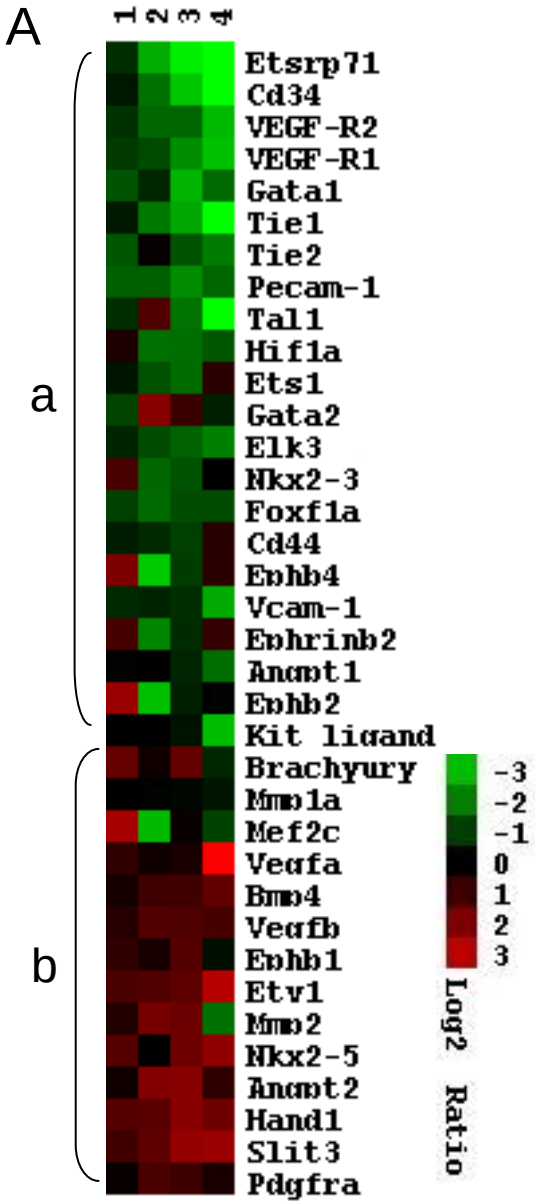
B

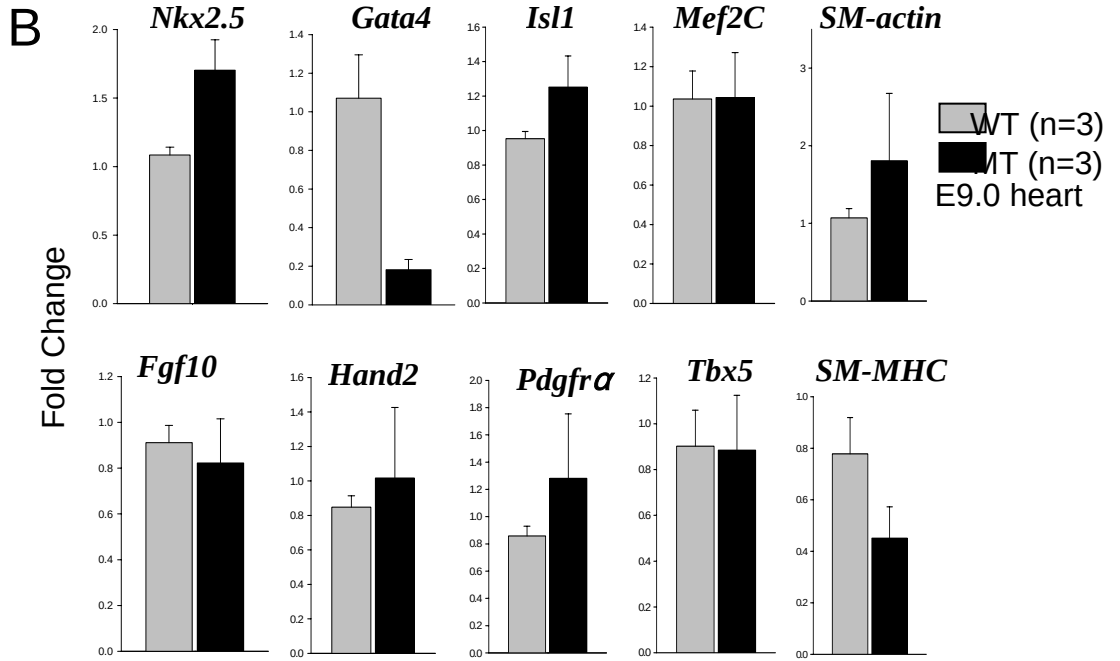


Supplementary Figure 1



Supplementary Figure 2





Supplementary Figure 3

Table S1. Genotypes of progeny from ER71 heterozygous intercrosses

Age (dpc)	No.(%) of mice with indicated genotype			No.(%) of resorptions	Total
	+/+	+/-	-/-		
E8.5	10(23.3)	20(46.5)	12(27.9)	1(2.3)	43
E9.0	20(19.4)	59(57.3)	24(23.3)		103
E9.5	103(34.3)	129(43)	55(18.3)	13(4.3)	300
E11.0-11.5	8(53.3)	7(46.7)	0(0)		15
P21	145(34.8)	272(65.2)	0		417
Total	286(32.6)	487(55.4)	91(10.4)	14(1.6)	878

Table S2. Primer sequences for qRT-PCR

Genes		Sequences (5'-3')	References
Fgf10	Forward	TTTGGTGTCTTCGTTCCCTGT	Primer Bank‡ ID 7106313a1
	Reverse	TAGCTCCGCACATGCCTTC	
Isl1	Forward	ATGATGGTGGTTTACAGGCTAAC	Primer Bank ID 31543007a1
	Reverse	TCGATGCTACTTCACTGCCAG	
Mef2C	Forward	ATGCCATCAGTGAATCAAAGGAT	Primer Bank ID 13384624a1
	Reverse	GTGGTACGGTCTCCCAACT	
Tbx5	Forward	ATGGCCGATACAGATGAGGG	Primer Bank ID 34098933a1
	Reverse	TTCGTGGAACTTCAGCCACAG	
Brachyury	Forward	CTCCCCTGCACATTACACAC	
	Reverse	GAGGCTATGAGGAGGCTTTG	
Cerberus1	Forward	ATCACCTCTACAGGAGGAAGC	Primer Bank ID 6753410a3
	Reverse	GGTCTCCCAGTGTACTTCGTG	
Er71	Forward	CAGAGTCCAGCATTCACCAC	
	Reverse	AGGAATTGCCACAGCTGAAT	
Fgf8	Forward	CCGAGGAGGGATCTAAGGAAC	Primer Bank ID 22094093a1
	Reverse	CTTCCAAAAGTATCGGTCTCCAC	
Foxa2	Forward	ACATACCGACGCAGCTACAC	
	Reverse	CCGGTAGAAAGGGAAGAGGT	
α-FP	Forward	AACTCTGGCGATGGGTGTTTA	Chase et al., 2007
	Reverse	ACACTGATGTCTTTCCACTCCA	
Goosecoid	Forward	CAGATGCTGCCCTACATGAAC	Primer Bank ID 6754076a1
	Reverse	TCTGGGTACTTCGTCTCCTGG	
Mesp1	Forward	GTCACTCGGTCCTGGTTTAAG	Primer Bank ID 33469091a1
	Reverse	ACGATGGGTCCCACGATTCT	
Mesp2	Forward	CGGCGTTCTCTCACCGATG	Primer Bank ID 6678864a1
	Reverse	CACCCCACTACTCATGGCTG	
Pax6	Forward	GCAGATGCAAAAGTCCAGGTG	Primer Bank ID 18138024a3
	Reverse	CAGGTTGCGAAGAACTCTGTTT	
Sox17	Forward	GATGCGGGATACGCCAGTG	Primer Bank ID 6755604a1
	Reverse	CCACCACCTCGCCTTTCAC	
Tbx6	Forward	ATGTACCATCCACGAGAGTTGT	Primer Bank ID 6755722a1
	Reverse	GGTAGCGGTAACCCTCTGTC	

‡ Wang and Seed, 2003.

Table S3. Primer sequences for ChIP and Luciferase Assay Construct

ChIP Primers		Sequences (5'-3')	References
-501~-323	Forward	AAACATTCAGACGGGGCG	Ronicke et al., 1996
	Reverse	TTCGCCGCTGCACTGAAAGTCC	
-321~-155	Forward	AGAGTTCTGCACTTGCAGGC	Ronicke et al., 1996
	Reverse	CAAGTCGCATCTGAGAGCAAAT	
-174~-81	Forward	TTGCTCTCAGATGCGACTTG	Ronicke et al., 1996
	Reverse	CCACTGGATACCAGGTTTGG	
+20~+239	Forward	CAGGACCCCAAGAGAGTAAG	Ronicke et al., 1996
	Reverse	GCCCGCAAAGAAGTCACAG	
-50.4kb	Forward	GAGGGGAAATTGAGCAGGTA	Ronicke et al., 1996
	Reverse	GTGTGACAGTTCCCCTGCTT	
Flk1 promoter		Sequences (5'-3')	
-492~+39	Forward	AATTCTCGAGGACGGGGCGGGGGATGCGGT	Ronicke et al., 1996
	Reverse	AATTAAGCTTCTTACTCTCTTGGGGTCCTG	