SUPPLEMENTAL MATERIAL

Supplemental Methods

Mice

All mice were maintained on a mixed genetic background. *Wnt1-Cre*[1] and *Z/EG*[2] mice were genotyped as previously described. All animal protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Histology and immunohistochemistry

Samples were harvested, fixed overnight in 4% paraformaldehyde and subsequently dehydrated through an ethanol series. All samples were then paraffin embedded and sectioned. The antibody used for SM-22 alpha immunohistochemistry was anti-SM-22 goat polyclonal (Ab10135, Abcam).

Supplemental Tables

Supplemental Table 1: Genomic Location of Jagged1 ECRs

ECR #	**Chromosome2 (chr2) Location**
ECR1	chr2:136942017-136942473
ECR2	chr2:136961395-136961782
ECR3	chr2:136962589-136963124
ECR4	chr2:136936138-136936622
ECR5	chr2:136935754-136935968
ECR6	chr2:136933719-136934335

ECR7	chr2:136932948-136933314
ECR8	chr2:136925118-136925510
ECR9	chr2:136924094-136924282
ECR10	chr2:136917659-136918032
ECR11	chr2:136913540-136914031
ECR12	chr2:136904329-136904753
ECR13	chr2:136903925-136904234
ECR14	chr2:136899727-136900004
ECR15	chr2:136899238-136899515

Supplemental Table 2. Jagged1 ECR, ECR6 deletions and ECR6 mutagenesis Primers

Evolutionarily Conserved Region Primers	
Primer Name	**Primer Sequence (5’ to 3’)**
ECR1-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGGCTCCCAGACCGGCGCTCCGG
ECR1-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCCCGGCTCTAATATACTCCGT
ECR2-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAATAGGGAAGACAAGAGACATG-
ECR2-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTTTCTCCTGTCCCACAGATGCGT
ECR3-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCCAAAAGAAAGGCAAAGACAATCA
ECR3-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTCCCAGAATATTCCAAATACA
ECR4-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTTGCTAGGTTACTGCTTGG
ECR4-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGACATGTCAATAGAGTGTGAT
ECR5-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGAATATTTTATACTCTGGCCA
ECR5-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGTGCCAACACAGCCATTGTACA
ECR6-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGCACCTTCCTTTGCAGTGGT
ECR6-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTAGGCATGAGAGAAAAGCAGGAGGT

ECR7-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCCATGGGTGTGCGGTGCC
ECR7-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGCATATAGGTTTCCCAAAAC
ECR8-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTAATGGGCATAGCTGGCATCT
ECR8-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTCGGAAGGGGAAACAGGCTGGG
ECR9-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTGCTACTGTCATCAGAAT
ECR9-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTAGACTATGCAAATGGCGTGTGG
ECR10-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGGGAATGTTTTTGGAGAGGG
ECR10-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCTGAAAAAAATAAACAAGCCG
ECR11-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTGTGAGTGGCCTCCCCTGAACT
ECR11-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTGTGGGACACATGTATACAGG
ECR12-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCCCACCATTTCCGTTTGGA
ECR12-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTGATGCAACAGGATTGTCCTAATG
ECR13-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTAGGTTGTGGGCAGGTGCTTGT
ECR13-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTACTGGATTTAAGAGCCTTTCC
ECR14-Forward	GGGGACCACTTTGTACAAGAAAGCTGGGTCCAGTTCCTGCAGGAGATTTAC
ECR14-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCAGTTCCTGCAGGAGATTTAC
ECR15-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTACAAGGTCAGTATCTCATCCGA
ECR15-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTACCCTCCTTTAGAAGATGAGCTT
ECR6 Deletion Series Primers	
Primer Name	**Primer Sequence (5’ to 3’)**
D1-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTCAAAACTCTTGGTATCAAT
D1-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTAGGCATGAGAGAAAAGCAGGAGGTTT
D2-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTAAGTGAAGGATGTGGGCCTCAA
D2-Reverse	Same as Deletion1 Reverse
D3-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTAGCCACACATCTGGCCTCATTT
D3-Reverse	Same as Deletion1 Reverse

D4-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCT GAGTCCTATAATTTGTAGGAA
D4-Reverse	Same as Deletion1 Reverse
D5-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAATAAGGTGTAACTTTCACTAA
D5-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTGAGAGAAAAGCAGGAGGTTTCAT
D6-Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGCACCTTCCTTTGCAGTGGTGTT
D6-Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGCCTCTTTAAGTTCCCTA
ECR6 Mutagenesis Primers	
Primer Name	**Primer Sequence (5’ to 3’)**
M1-Forward	CTTTGTGAGCATAATCCCACAGTTATCAGTGAACG
M1-Reverse	CGTTCACTGATAACTGTGGGATTATGCTCACAAAG
M2-Forward	CTTTGTGAGCATTTTCGGACAGTTATCAGTGAACG
M2-Reverse	CGTTCACTGATAACTGTCCGAAAATGCTCACAAAG
M3-Forward	CTTTGTGAGCATGCAGCCACAGTTATCAGTGAACG
M3-Reverse	CGTTCACTGATAACTGTGGCTGCATGCTCACAAAG

Supplemental Table 3: Genotype distribution resulting from *Pax3*$^{Cre/+}$; *Jag1*$^{flox/+}$ x *Jag1*$^{flox/flox}$

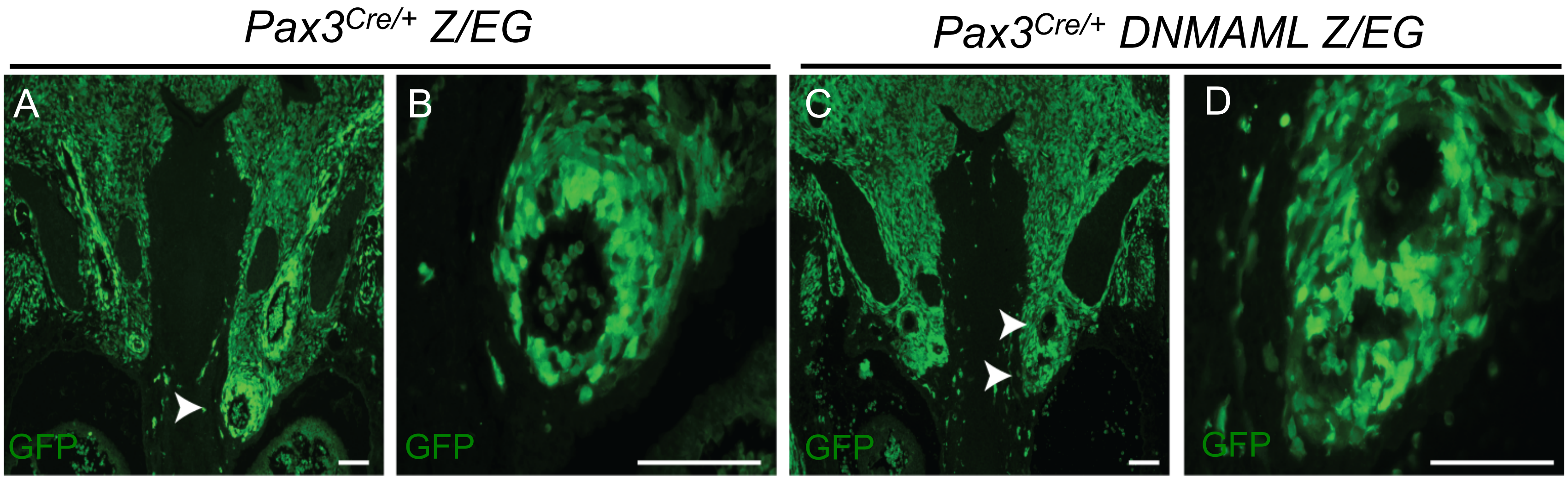
	Genotype				
Age	*Jag1*$^{flox/+}$	*Jag1*$^{flox/flox}$	*Pax3*$^{Cre/+}$ *Jag1*$^{flox/+}$	*Pax3*$^{Cre/+}$ *Jag1*$^{flox/flox}$	Total
E9.5-14.5	25	23	21	14	83
P0	31	35	34	38	138
P10-P12	14*	12*	11*	14*	51*
3 weeks	14*	12*	11*	5*	42*

4 weeks	14*	12*	11*	0*	37*

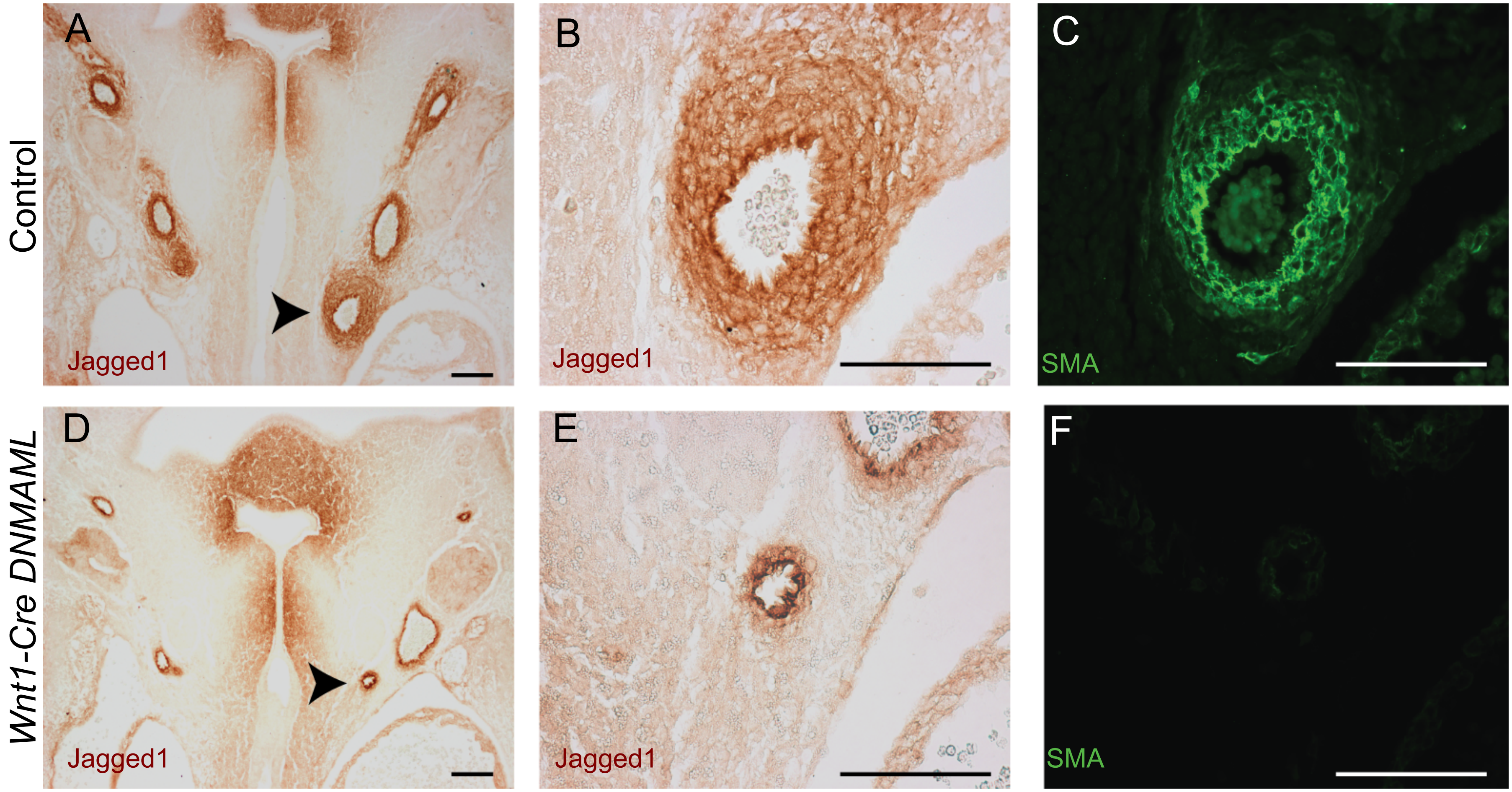
* These offspring (51 total) were followed from the time of genotyping (P10-P12) until 4 weeks of age to determine post-natal lethality of *Pax3*$^{Cre/+}$ *Jag1*$^{flox/flox}$ mutants compared with their littermates.

Supplemental Table 4: Aortic arch phenotypes in *Pax3*$^{Cre/+}$; *Jag1*$^{flox/flox}$ neonates and littermates

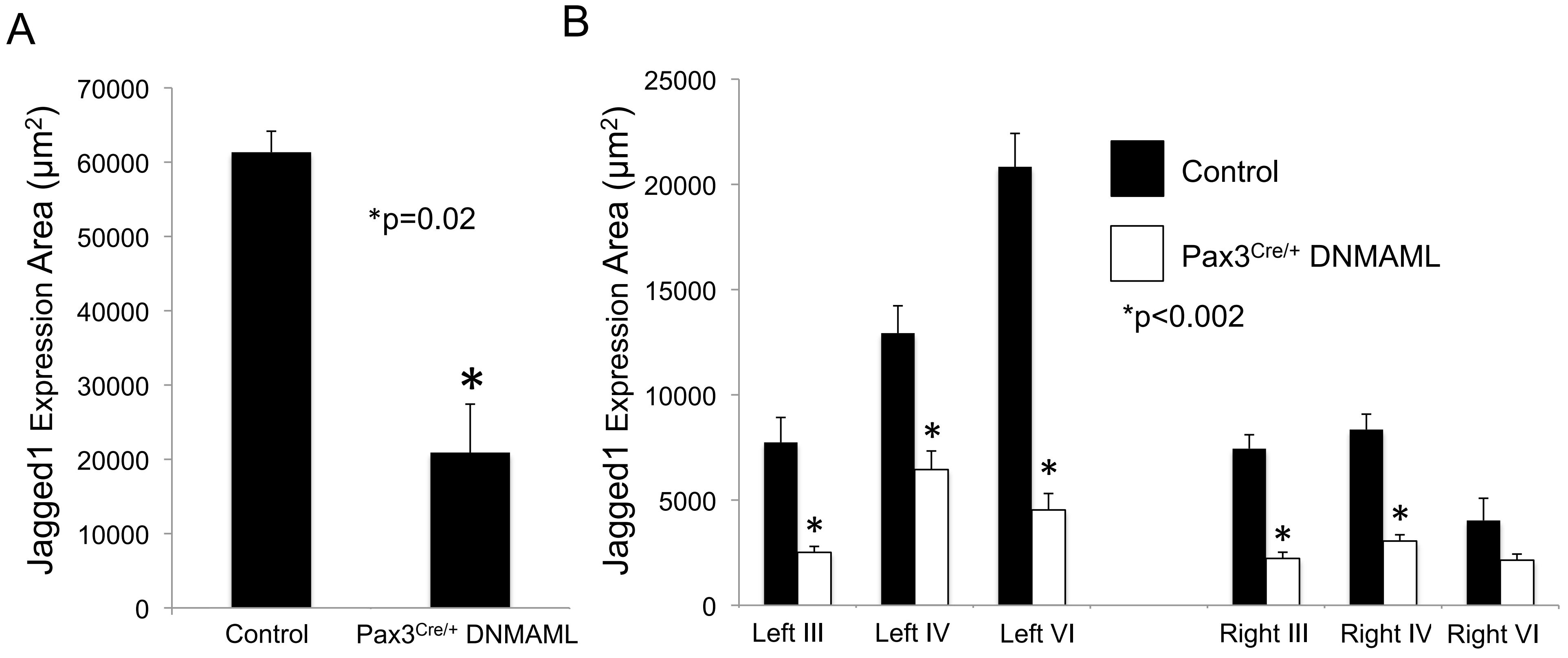
	Genotype					
	Wildtype	***Jag1***$^{flox/+}$	***Jag1***$^{flox/flox}$	***Pax3***$^{Cre/+}$	***Pax3***$^{Cre/+}$ ***Jag1***$^{flox/+}$	***Pax3***$^{Cre/+}$ ***Jag1***$^{flox/flox}$
Total analyzed	**6**	**32**	**27**	**8**	**36**	**29**
Normal	6	31	26	8	29	6
Short or absent brachiocephalic artery	0	1	1	0	6	18
Common carotid trunk	0	0	0	0	1	2
Aberrant right subclavian artery	0	0	0	0	0	3



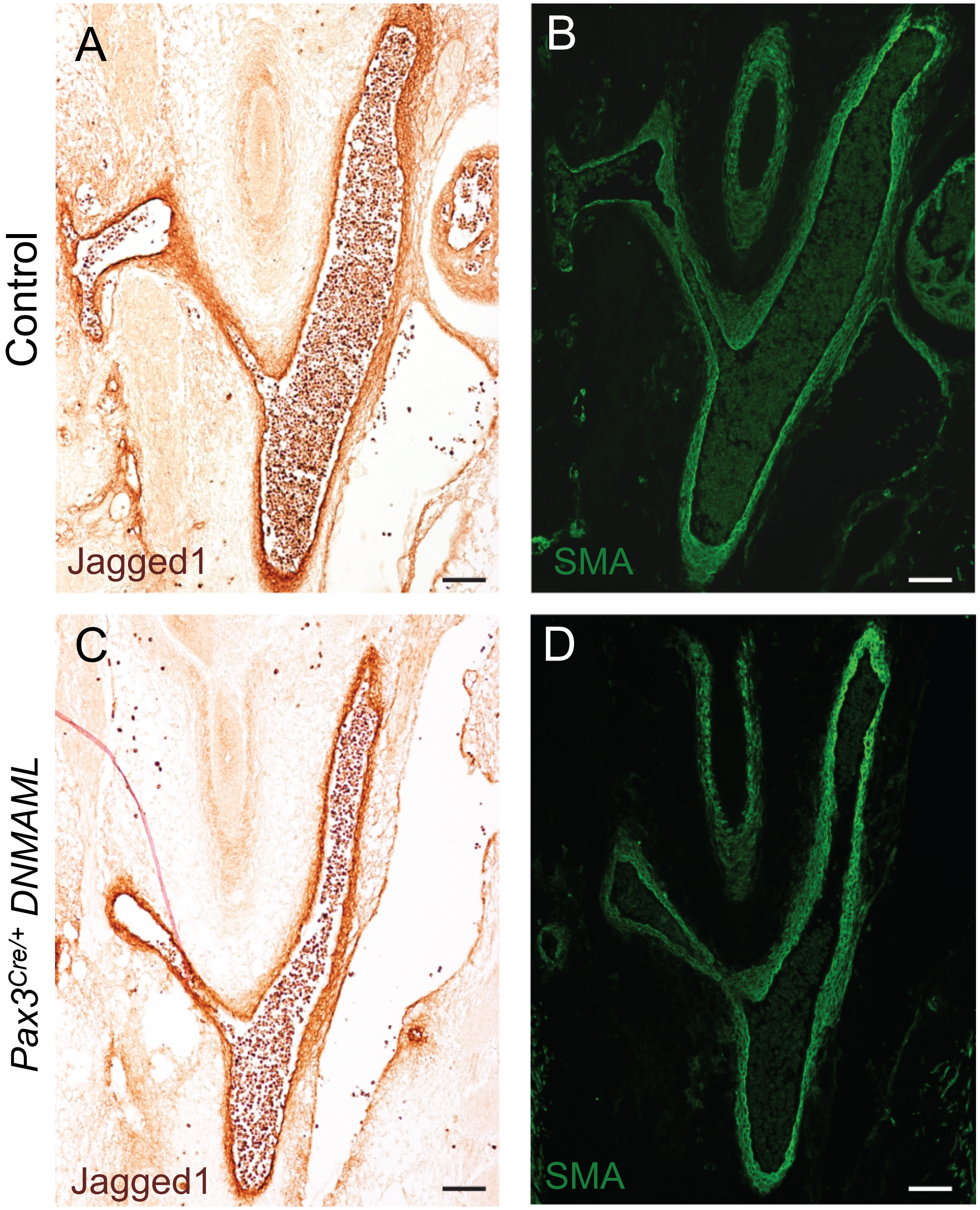
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



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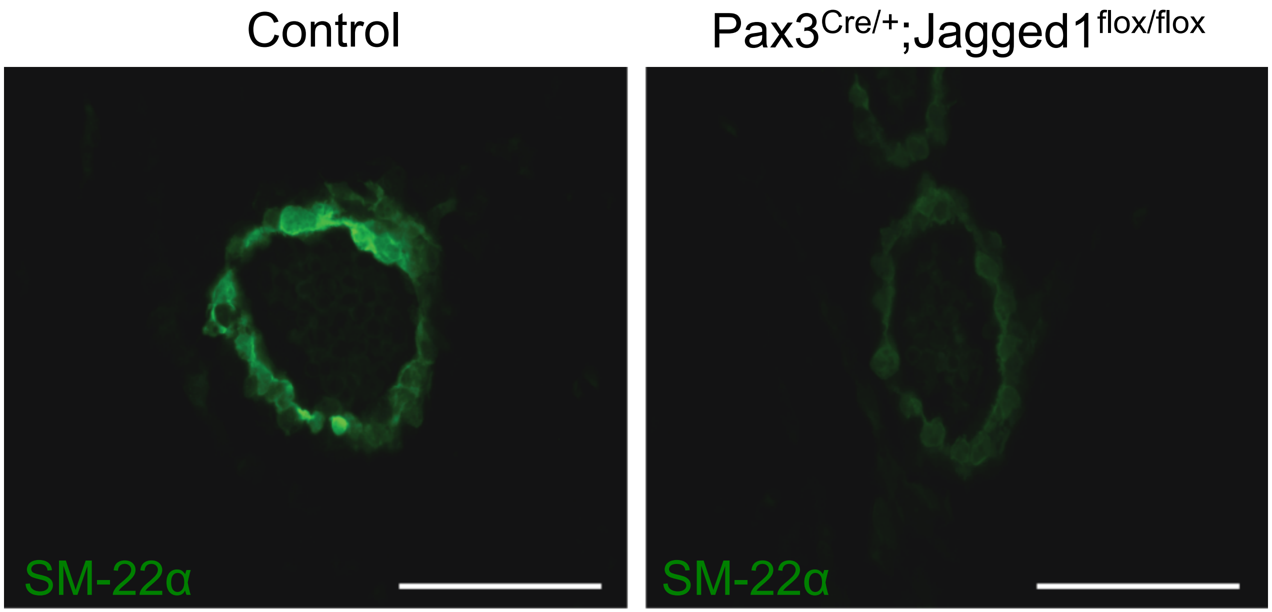

Supplemental Figure S5

Supplemental Figure Legends

Supplemental Figure S1. Inhibition of Notch in the neural crest does not alter neural crest cell number. (A-B) Frontal sections of E12.5 *Pax3*$^{Cre/+}$ *Z/EG* embryos. (C-D) Frontal sections of E12.5 *Pax3*$^{Cre/+}$ *DNMAML Z/EG* embryos. (A-D) Immunostaining of GFP in control (A-B) and Notch inhibited mutants (C-D). No alteration of neural crest cell localization or number was observed in animals expressing the DNMAML allele. Scale bars: 100μm.

Supplemental Figure S2. Inhibition of Notch in neural crest impairs Jagged1 expression. (A-C) Frontal sections of E12.5 control embryos. (D-F) Frontal sections of E12.5 *Wnt1-Cre DNMAML* embryos. (A, B, D and E) Immunostaining of Jagged1 in control (A and B) and Notch inhibited mutants (D and E). Notch inhibited animals display decreased Jagged1 protein expression. The most marked decrease in Jagged1 protein is observed at the left sixth arch artery (arrowheads in A, D and shown in higher magnification in B, E, respectively). (C, F) Adjacent sections to those shown in B, E, respectively, stained for smooth muscle actin (SMA). *Wnt1-Cre DNMAML* embryos display a decrease in SMA expression (F) indicating decreased smooth muscle differentiation as compared to control embryos (C). Scale bars: 100μm.

Supplemental Figure S3. Quantification of Jagged1 expression following Inhibition of Notch in neural crest. (A) Overall Jagged1 expression surrounding the six aortic arch arteries in control and *Pax3*$^{Cre/+}$ *DNMAML* embryos. Jagged1 protein expression area was calculated using ImageJ software (National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997-2011). Jagged1 positive area was determined based upon the presence of brown stain at the cell membranes. Seven pairs of control and *Pax3*$^{Cre/+}$ *DNMAML* embryos from 3 independent litters were included in the analysis. From each embryo, 4 non-consecutive sections were stained for Jagged1 and areas were calculated covering ~50μm of each artery. Overall Jagged1 protein expression is significantly decreased in *Pax3*$^{Cre/+}$ *DNMAML* embryos. Data represent the mean + standard error of the mean (SEM). (B) Jagged1 protein expression area at each individual arch artery is indicated. Jagged1 protein expression is significantly decreased at all arch arteries, except the right sixth. Data represent the mean + standard error of the mean (SEM). Asterisks denote statistically significant differences between the wildtype and *Pax3*$^{Cre/+}$ *DNMAML* embryos, as assessed a two-tailed, paired *t* test.

Supplemental Figure S4. Jagged1 expression is not affected in all vascular smooth muscle when Notch is inhibited in the neural crest. (A-D) Frontal sections of E12.5 embryos. Examination of Jagged1 protein and smooth muscle actin expression along the descending aorta in control and *Pax3*$^{Cre/+}$ *DNMAML* embryos. The vascular smooth muscle surrounding the descending aorta is not derived from neural crest and is not affected by inhibition of Notch by DNMAML in neural crest indicating that smooth muscle defects are cell autonomous and not generalized in these embryos. Scale bars: 100μm.

Supplemental Figure S5. Inactivation of Jagged1 in neural crest results in decreased smooth muscle differentiation. (A-B) Frontal sections of E12.5 mouse embryos. (A, B)

Left sixth aortic arch arteries of control (A) and *Pax3*$^{Cre/+}$*; Jagged1*$^{flox/flox}$ (B) embryos stained for SM-22 alpha (SM-22α). Scale bars: 100μm.

Supplemental References

1. Jiang X, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cardiac neural crest. *Development*. 2000;127:1607-1616.

2. Novak A, Guo C, Yang W, Nagy A, Lobe CG. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. Genesis. 2000; 28: 147-155.

Supplemental Movie 1. Optical projection tomography of E11.5 ECR6-lacZ embryo shows extent of X-gal staining.