**Supplementary Materials**

**Generation of null mice**

A targeting vector was made to delete exon 3 that encodes the N-terminus, including a portion of the extracellular ligand binding domain. The genomic structure of *Tgfbr3* was determined by searching the Celera database® using the published mouse cDNA. Sequence fragments were assembled using DNASTAR software to generate a single, contiguous genomic sequence that spanned all 17 *Tgfbr3* exons. Oligonucleotides were designed to PCR amplify 5' and 3' arms of homology and *Tgfbr3* exon 3 from 129 SvEvTac mouse genomic DNA. Primers were as follows: Long Arm: Forward 5' GTCGACTTATAAAAGTTTCTGTGAGGA3', Reverse 5' GTCGACGTCAAGGAAACCTCCCAATGG; Short Arm: Forward 5'CTCGAGTAGTTCCTTATTGAGTTACCA3', Reverse 5'CTCGAGACCCTACCTCCTTCTTCTATCT3'; exon3: forward 5' GGATCCACACATAAATAAAGAGAAATCA3', Reverse 5'CTCGAGTATTGAAGCATATTACATACGATATGCTTCAATATCCAGGAGCAA TGTGTCTTCT3'. Amplification products were subcloned into pLOX-TKneo after removal of the HSV-TK cassette. The construct was linearized by digestion with Notl (Fig. S1A). ES cells from 129/SvEvTac blastocysts were electroporated with the linearized targeting construct. Seven-hundred G418 resistant clones were screened by Southern blot. One positive clone was expanded and injected into C57BL/6 blastocysts implanted into pseudopregnant female mice. Chimeric mice were mated to C57BL/6 mice and germ line transmission of the targeted allele
was confirmed by PCR analysis. 3loxP Tgfbr3 heterozygotes were mated to Ella-Cre transgenic mice. Progeny were screened by PCR for null alleles produced by Cre mediated recombination (Fig. S1C). Heterozygous null mice were mated to generate homozygous null mice.

**Southern blot and hybridization**

Three $^{32}$P-labeled DNA probes were used to identify ES cells that underwent homologous recombination. Probe templates external to the 5' and 3' regions of recombination were generated by PCR and an internal probe was generated by releasing the neomycin cassette from pLOX-TKneo with SpeI and BamHI (Fig. 1A). (5' probe template forward 5'TCGAGTAGATATGAAAACACCTT3', reverse 5'TCGAGTAGATATGAAAACACCTT3'; 3' probe template forward 5' TTACAGAAATACTGCATA3', reverse 5’ GCCAGGCATGCTCAGACG3’). Genomic DNA from 700 expanded ES cell clones was restriction digested with BgIII and SpeI and probed by standard methods (Fig S1B).

**Generation of MEFs**

E13.5 embryos were harvested in sterile phosphate buffered saline (PBS), minced, and incubated in trypsin EDTA for 30 minutes. The trypsin solution was transferred to culture dishes containing DMEM, 10% FBS, and 1:100 Pen/Strep. This process was repeated until there was no remaining tissue.
RT-PCR

Total RNA was harvested from mouse embryonic fibroblasts (MEFs) (Trizol Reagent-Invitrogen) and further purified with RNeasy Mini Kit (Qiagen). RNA was reverse-transcribed and the resultant DNA was PCR amplified using Titan One Tube RT-PCR kit (Roche). Oligonucleotides were positioned in exons 2 and 5 to determine the presence or absence of exon 3 across genotypes. (forward 5’GCTACACCCGACTTGCCACACT3’; reverse 5’GACCACAGAACCCTCCGAAACC3’). Products were electrophoresed on a 1% agarose/TAE gel (Fig. S1D).

Affinity Labeling with $^{125}$I TGFβ

MEFs were cultured to confluence in six well dishes in duplicate. Crosslinking performed as described previously ¹ (Fig. S1E).

Histology and Wholemount immunohistochemistry

Embryonic tissue used for hematoxylin and eosin (H&E) staining was processed by standard methods. For wholemounts, tissue was fixed in 4:1 methanol:DMSO overnight at 4°C and stained by standard methods ² (primary antibody to platelet endothelial cells adhesion molecule (PECAM) was at 1:100 (BD).

β-galactosidase staining of transgenic reporter mice

$Tgfbr3$ +/- mice were crossed to mice harboring the SM22alpha lacZ tranogene ³ to produce double heterozygotes. Embryos generated by mating double
heterozygotes were harvested in PBS, fixed in 2% PFA for two hours, and stained by standard methods. Embryos were washed in PBS and photographed.
**Figure S1. Targeting of Tgfbr3.** A-E. Strategy to generate and confirm Tgfbr3 targeting. A. loxP sites flanking exon 3 and the neomycin resistance cassette were introduced into the Tgfbr3 locus by homologous recombination. Exon 3 and the neomycin cassette were removed from the targeted allele by Cre-mediated recombination in mice to generate a null allele. B. Southern blot of ES cell DNA digested with BglII and SpeI. Positions of 5', neo and 3' probes are indicated by the patterned bars (A). 5' and 3' probes bind an 8.5 kb and 4.3 kb targeted allele, respectively and an 11 kb wild-type allele. An internal neo probe binds a 4.3 kb fragment. C. PCR amplification of genomic DNA from wt (+/+), heterozygous null (+/-) and null (-/-) mice using oligonucleotides depicted as arrowheads (A). D. RT-PCR using total RNA from +/-, +/- and -/- mouse embryonic fibroblasts (MEFs) demonstrating the absence of the 184 bp exon 3 in the Tgfbr3 transcript. Forward and reverse oligonucleotides were positioned in exons 2 and 5, respectively, as depicted in the partial map of the cDNA. E. Ligand crosslinking with $^{125}$I-TGFβ to MEFs confirms loss of protein expression in nulls.

**Figure S2. Smooth muscle recruitment to the coronary ostia occurs in null embryos.** A-B. LacZ-positive cells are present in the epicardial layer and subepicardium in all genotypes. A. lacZ-positive cells are prominently associated with dysmorphic vascular structures in nulls. B. lacZ-positive cells are found in association with blood islands in the subepicardium at the apex of the heart (arrowheads). C. At E15.0 lacZ-positive cells are associated with large vessels in wild-type hearts.
Table S1. Genotypes of harvested embryos

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Number in parentheses indicates embryos dead at harvest
References


Figure S1