**Generation of the SM22α-Cre knock-in (SM22α-CreKI) mouse**

SM22α-CreKI mice were generated by knocking-in the Cre-recombinase coding sequence into the endogenous SM22α gene locus via homologous recombination in ES cells. The targeting vector was generated in the pKO plasmid, which contains the PGK-neo-poly(A) and pol2-DTA-poly(A) cassettes for positive and negative selection, respectively. The targeting vector contains a genomic fragment that includes 3 kb of the SM22α 5'-flanking sequence and the exon 1 sequence through the initiation codon. The Cre-recombinase coding sequence was inserted in-frame at the SM22α initiation site, which allows the Cre-recombinase driven by the endogenous SM22α promoter. The targeting vector also includes the 2.8 kb SM22α genomic fragment that spans exon 1 through intron 1 sequence for homologous recombination. The targeting vector linearized by *Sal I* was transfected into 129S6SvEv/Tac embryonic stem cells through electroporation. G418-resistant colonies were analyzed for homologous recombination by Southern blot analysis after *EcoR I* digestion with a 5' genomic probe that contained sequences that reside outside the targeting vector. Two targeted clones were injected into C57BL/6 blastocysts to produce chimeras, which were crossed with C57BL/6 female mice to generate heterozygous SM22α-CreKI mice. The chimera mice produced from both targeted ES clones show germline transmission. We analyzed two independent SM22α-CreKI mice lines: both of them show similar expression patterns in embryonic stages and in adult. The heterozygous mice were inbred for getting homozygous mice and further characterization. Genotyping was performed using a combination of three-primers as shown in Figure S1a-S1c: GGCCCAGGGGTTGTCAAAATAGTC (common forward primer, 5' to the SM22α initiation site); CTCCTCCAGCTCCTCGTCATACTTC (wild

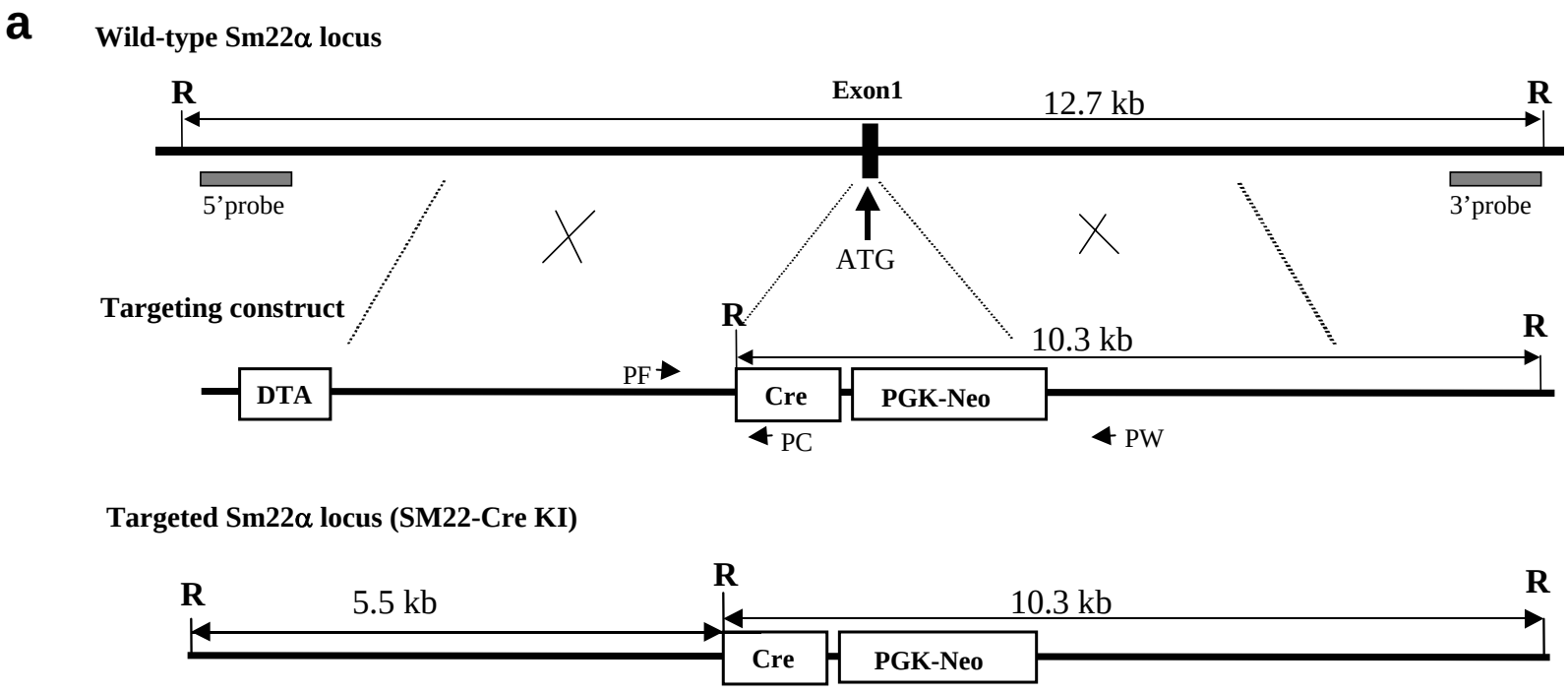
type-specific); and CGCCGCATAACCAGTGAAACAG (knock-in specific). Reaction products of 480 bp, 758 bp or both, represent the wild type, homozygous SM22α-CreKI or heterozygous SM22α-CreKI mice, respectively. As the fist step to characterize SM22α-CreKI mice, we showed that Cre was expressed in SMC-rich tissues such as aorta in both heterozygous and homozygous SM22α-CreKI mice, but not in wild type mice (Fig. S1d).

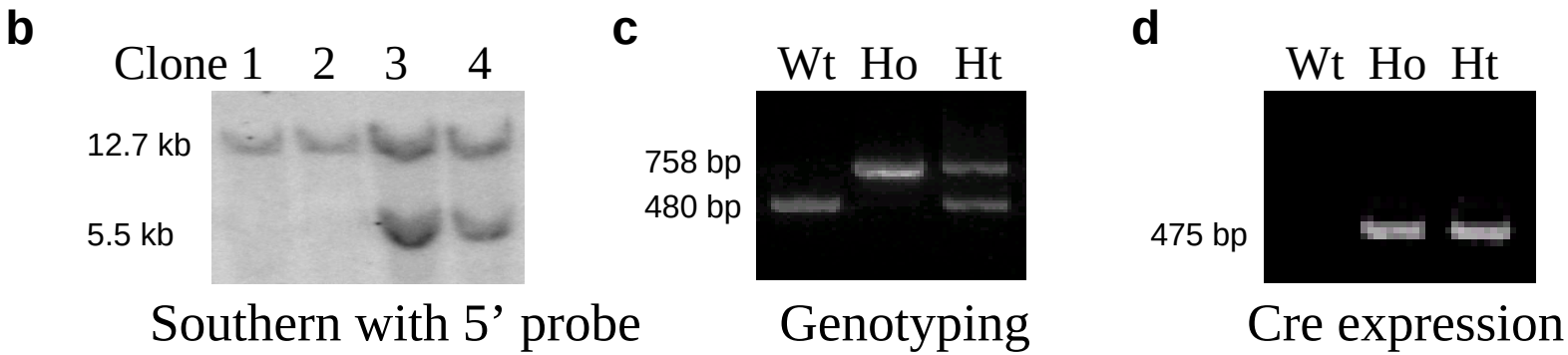
## FIGURE LEGENDS

**Figure S1. Generation of the SM22α-CreKI mouse by in frame targeted integration of Cre-recombinase into mouse SM22α locus.** ***a:*** Strategies used to direct Cre expression to smooth muscle cells. The structures of the wild-type SM22α locus, targeting construct, and targeted SM22α locus are shown. The filled boxes denote the first exon of the SM22α gene. The open boxes represent the Cre coding sequence followed by a BGH polyadenylation signal, a neomycin-resistance gene expression cassette (PGK-neo), and a DTA cassette for negative selection. Also shown is the locations of the probes used to detect a 12.7 kb and a 5.5 or 10.3 kb *EcoR* I (R) fragment of the wild-type and targeted SM22α locus, respectively. ***b:*** Southern blot analyses of *EcoR*I-digested genomic DNA from ES cell clones demonstrates that clone 3 and 4 carry the targeted allele. Both of them were used for generating chimeras. ***c:*** Genomic PCR shows the genotype the SM22α-CreKI mice. Genotyping primers PF and PW generated a 480 bp wild type band. Primers PF and PC generated a 758 bp knock-in band. ***d:*** RT-PCR results indicated that Cre was expressed in aorta of heterozygous and homozygous SM22α-CreKI mice at 8 weeks. Wt: wild type; Ht: heterozygote; Ho: homozygote.

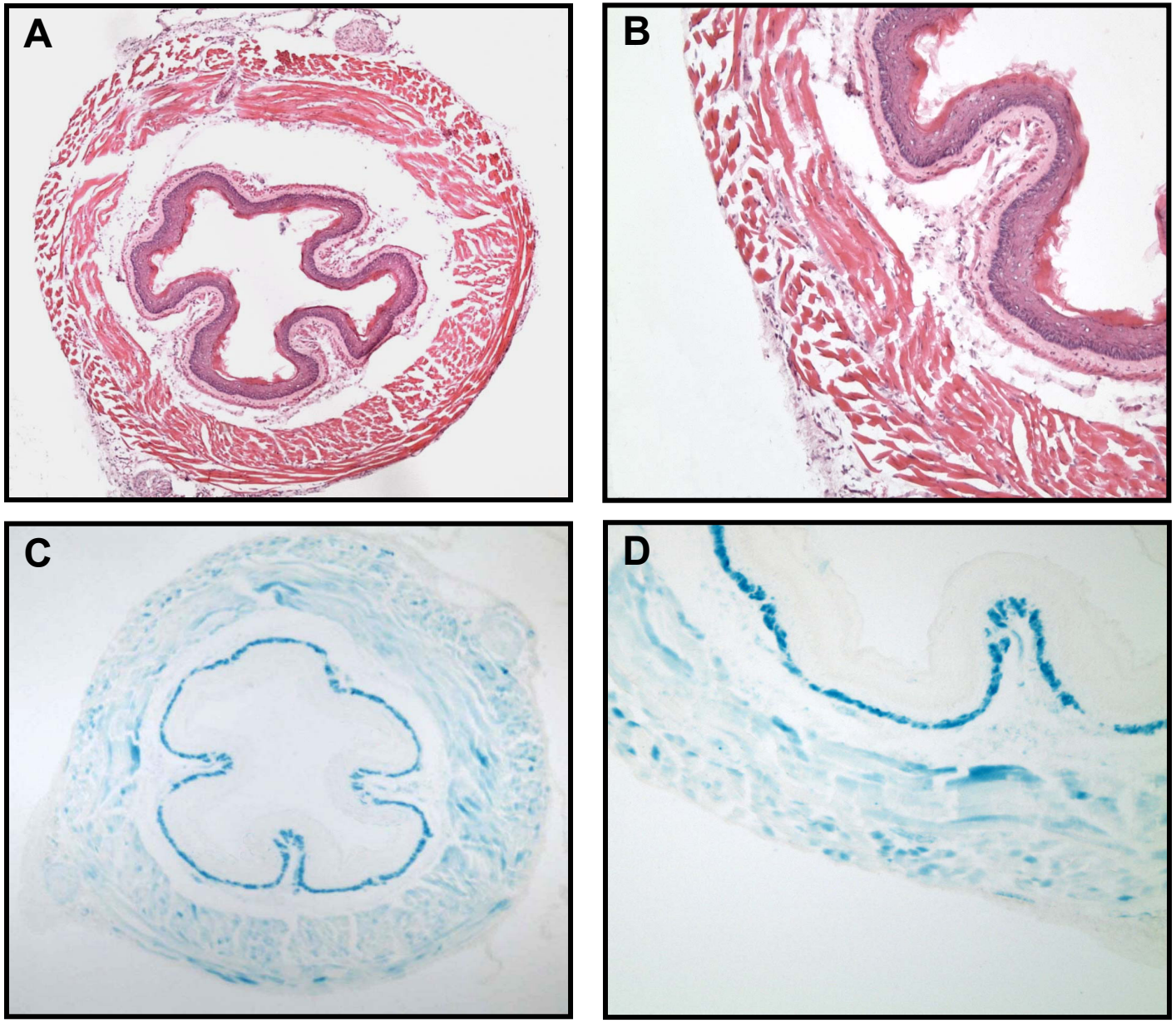
**Figure S2. Characterization of SM22a-CreKI mice.** X-Gal-stained sections were prepared from eight-week-old SM22a-CreKI and *Gt(Rosa)26Sor* double heterozygous mice. **A and B:** HE staining of the esophagus. **C and D:** X-Gal positive staining in smooth muscle cells of esophagus. Magnification: 40X (A and C) and 200X (B and D). **E:** X-Gal positive staining in smooth muscle cells of small intestine. Magnification: 200X. **F:** X-Gal positive staining in vessels of the liver. Magnification: 400X.

**Figure S3. The comparison of LacZ expression by X-gal staining.** (A) The *LacZ* gene driven by a SM22α promoter is highly expressed in the arterial vasculature and the heart at E13 (a positive control for X-gal staining). (B) and (C): The Cre-induced lacZ in a SM22α-CreKI/*Gt(Rosa)26Sor* embryo at E13.5 (B) or E15 (C) is not detectable in the cardiovascular system.

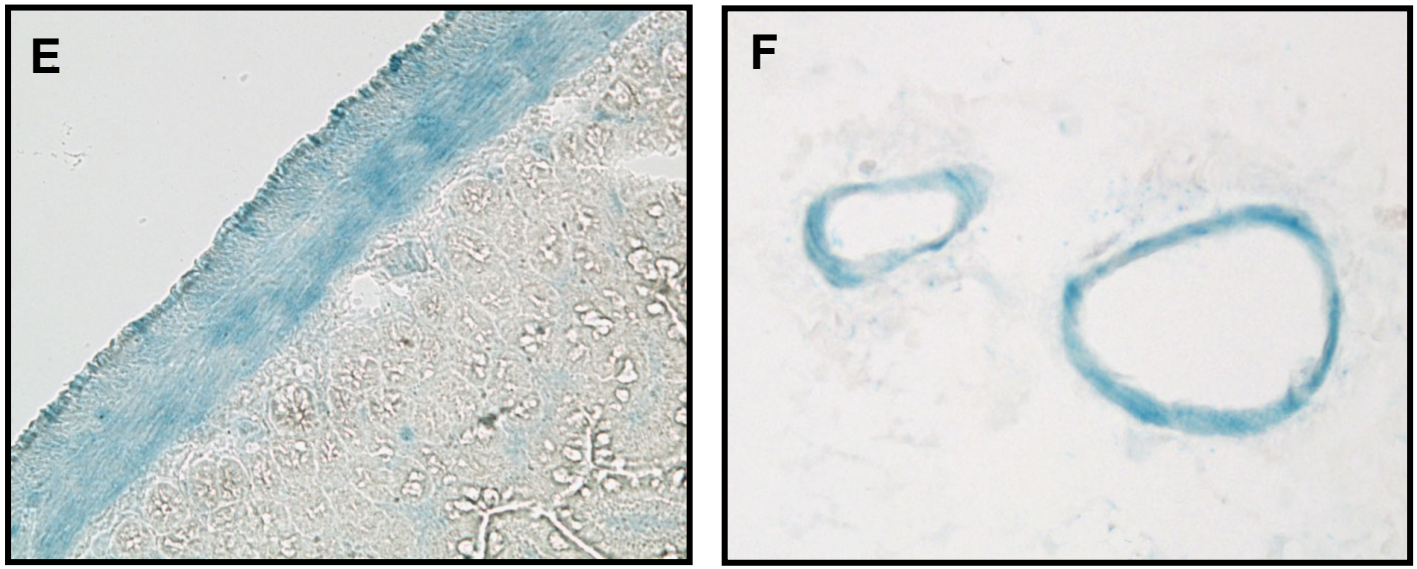


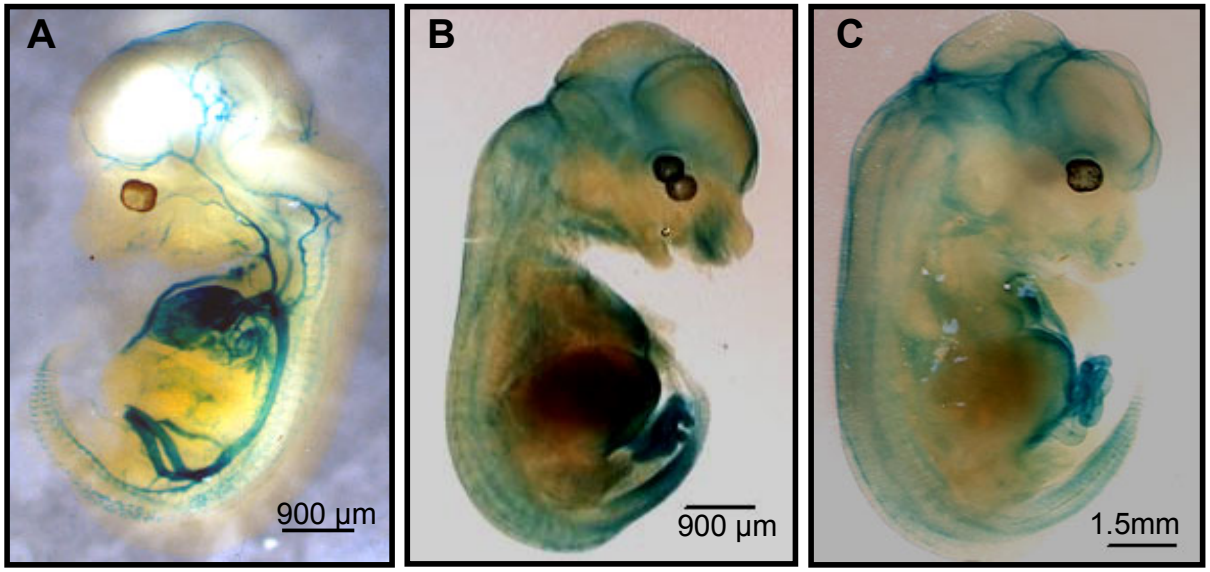

Zhang et al., Fig. S1




Zhang et al., Fig. S2A - D




Zhang et al., Fig. S2E & F




**Zhang et al., Fig. S3**