Vandersmissen et al, http://www.jcb.org/cgi/content/full/jcb.201502003/DC1

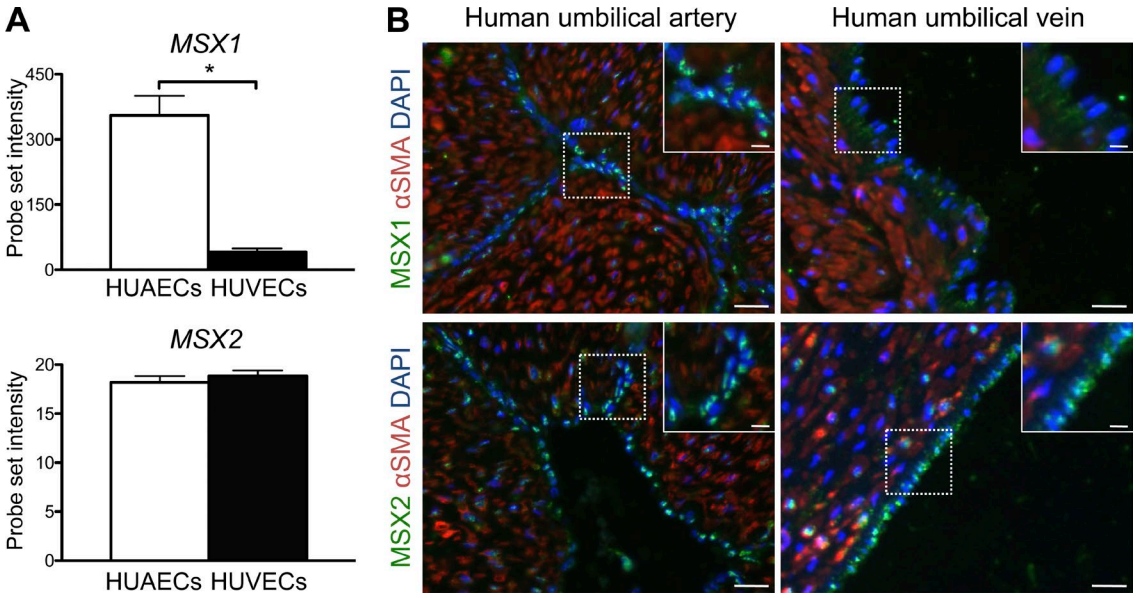

Figure S1. **MSX1 exhibits an arterial-specific expression profile in human umbilical cords.** (A) Microarray probe set intensities in freshly isolated HUAECs and HUVECs. Error bars represent the SEM. $n = 4$. *, $P < 0.05$ versus HUAECs. (B) IF staining on cross sections of human umbilical cords for MSX1 or MSX2 (green), αSMA (red), and DAPI (blue). Bars: 25 µm; 20 µm (magnifications).

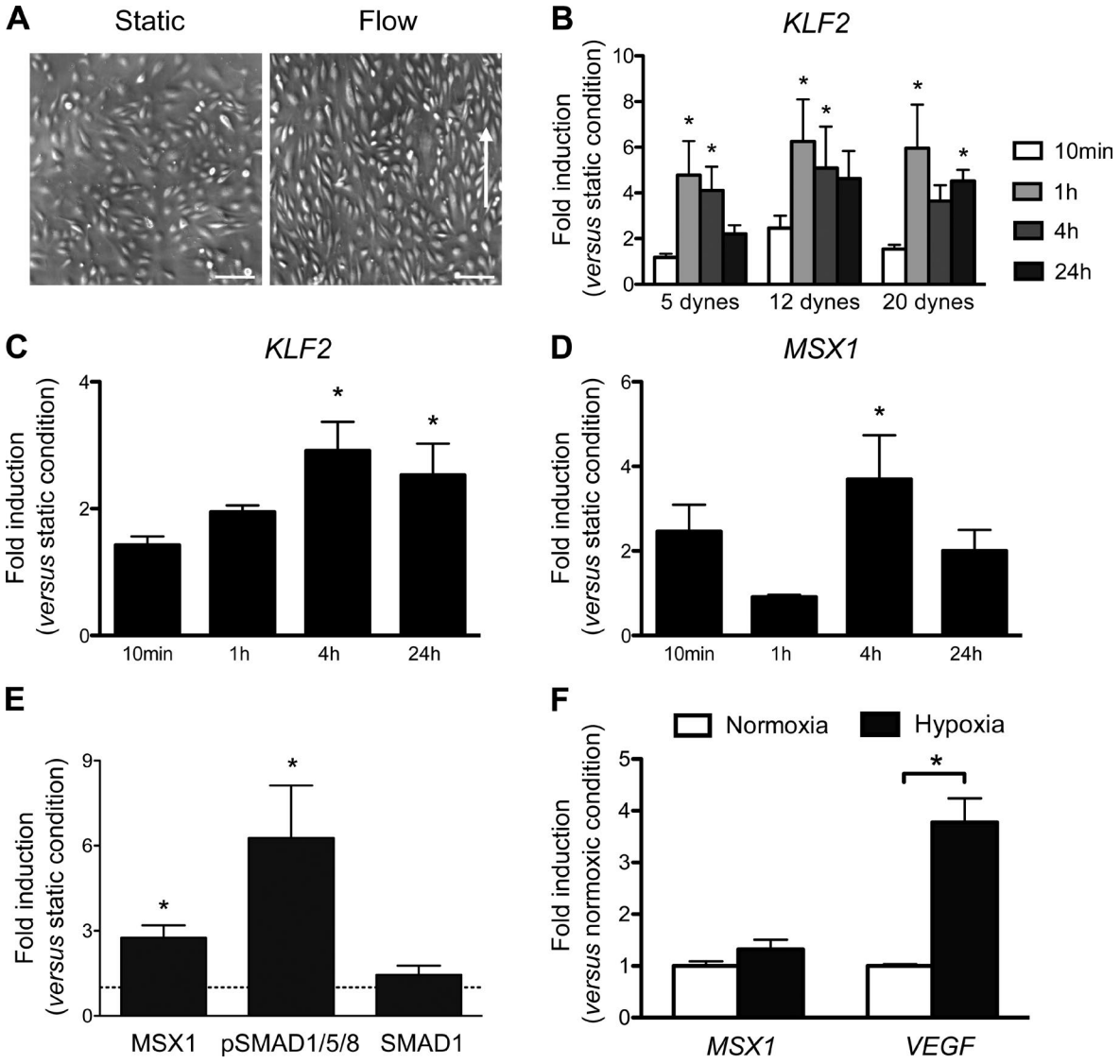

Figure S2. **Triggering cues of *MSX1* expression in ECs.** (A) Brightfield pictures of HUAECs with and without exposure to LSS of 20 dynes/cm^2 for 24 h. The arrow represents the flow direction. Bars, 100 µm. (B) *KLF2* expression analysis by qRT-PCR of HUAECs seeded on fibronectin-coated chambers kept in static conditions or exposed to different LSS levels over time. mRNA expression is represented relative to the static condition (n = 3 for 5 dynes and n = 8 for 12 and 20 dynes). *, P < 0.05 versus static condition. (C and D) Expression analysis by qRT-PCR of HIAECs seeded on fibronectin-coated chambers kept in static conditions or exposed to 20 dynes/cm^2 over time. mRNA expression is represented relative to the static condition for *KLF2* and *MSX1*. n = 4. *, P < 0.05 versus static condition. (E) Quantification of the Western blot of total cell lysates of HUAECs kept in static conditions or exposed to LSS of 20 dynes/cm^2 for 1 h for MSX1, pSMAD1/5/8, and SMAD1. Expression was normalized for the housekeeping protein and represented as fold induction versus the corresponding static condition. n = 3–6. *, P < 0.05 versus the static condition. (F) HUAECs were cultured in 21% (normoxia) or 1% oxygen (hypoxia) conditions. After 3 d, cells were lysed, and mRNA expression was quantified by qRT-PCR and represented relative to the normoxic condition for *MSX1* and *VEGF* as the positive control. n = 3. *, P < 0.05 versus normoxic condition. Error bars represent the SEM.

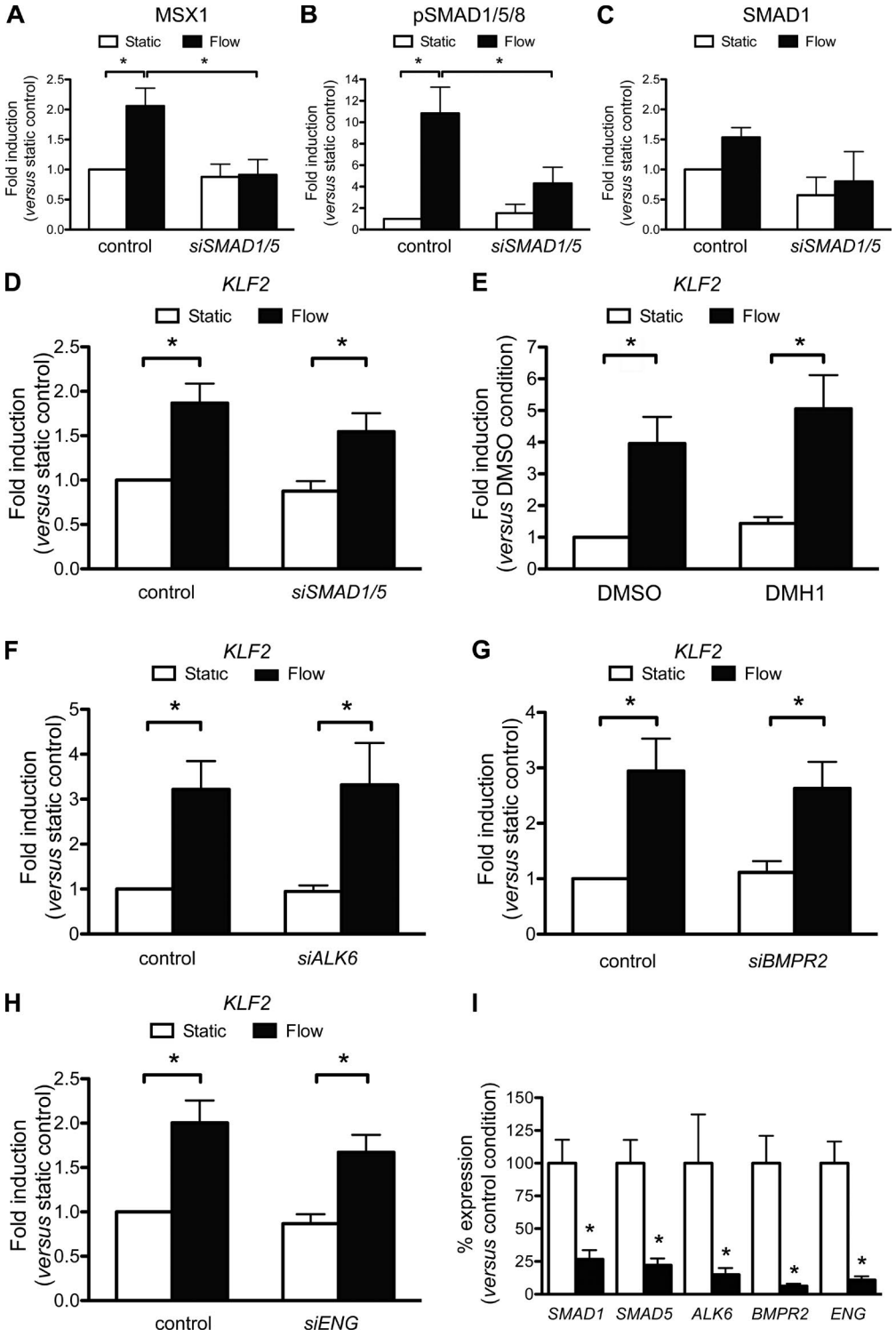

Figure S3. **Assessment of shear induction and siRNA-mediated knockdown efficiency.** (A–C) Quantification of the Western blot of HUAECs transfected with control siRNA or siRNA against *SMAD1* and *SMAD5* seeded in fibronectin-coated chambers and subsequently kept under static conditions or subjected for 1 h to an LSS of 20 dynes/cm^2. MSX1, pSMAD158, and SMAD1 expression was normalized for the housekeeping protein and represented as fold induction versus the nonsilencing control static condition. $n = 3$. *, $P < 0.05$ versus the indicated condition. (D–H) *KLF2* expression analysis by qRT-PCR of HUAECs seeded on fibronectin-coated chambers kept in static conditions or exposed to LSS (20 dynes/cm^2) for 1 h with cells transfected with control siRNA or siRNA against *SMAD1* and *SMAD5* ($n = 7$; D), *ALK6* ($n = 5$; F), *BMPR2* ($n = 6$; G), or *ENG* ($n = 4$; H) or cells pretreated for 1 h with and exposed to flow in the presence of DMSO or 2.5-μM DMH1 ($n = 4$; E). *, $P < 0.05$ versus the indicated condition. (I) siRNA-mediated knockdown efficiency was assessed by means of qRT-PCR for the knockdown of *SMAD1* ($n = 7$), *SMAD5* ($n = 7$), *ALK6* ($n = 5$), *BMPR2* ($n = 6$), and *ENG* ($n = 4$) during each conducted siRNA experiment in static conditions. *, $P < 0.05$ versus control condition (white bars). Error bars represent the SEM.

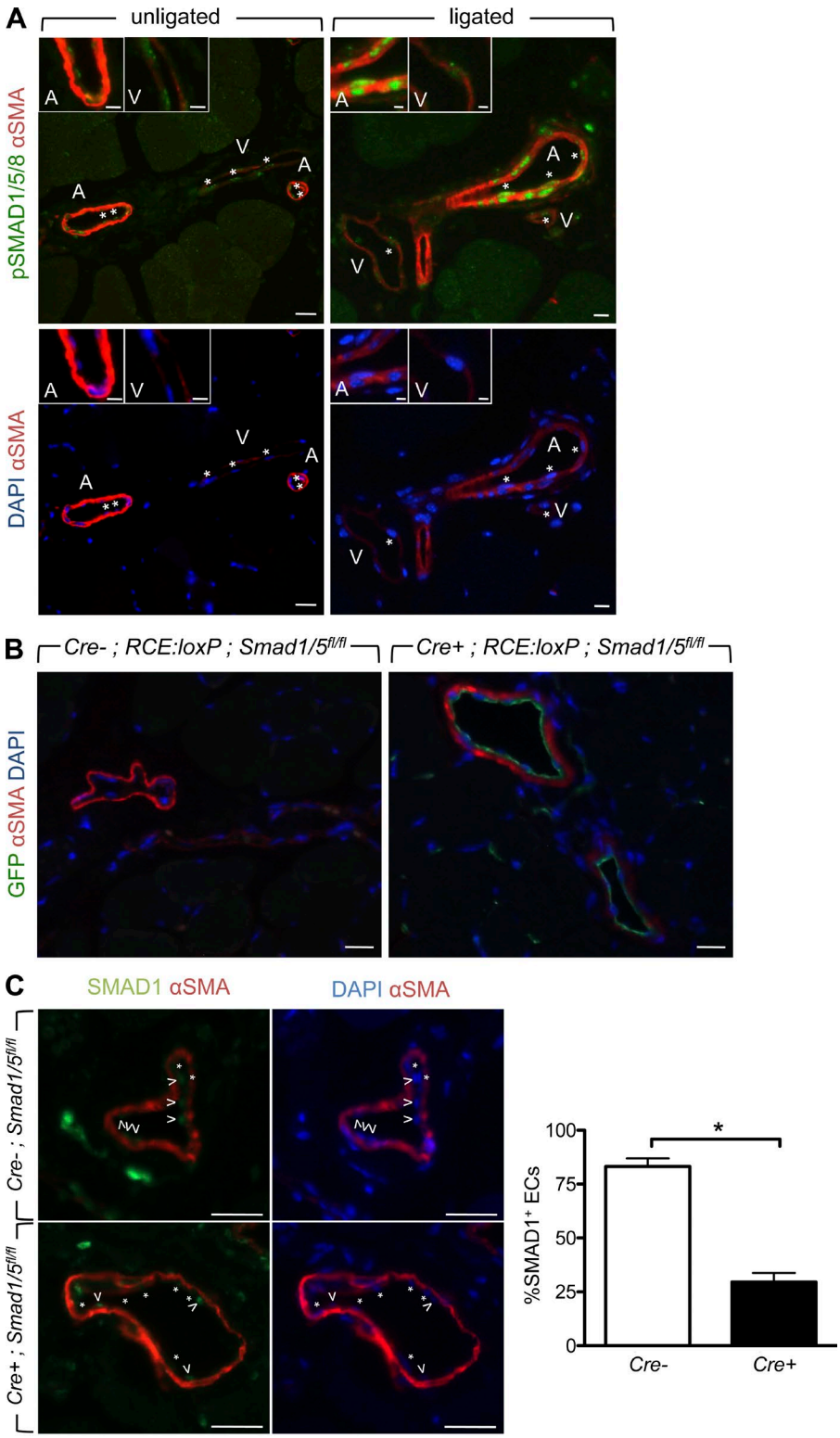

Figure S4. **Asymmetric pSMAD1/5/8 activity induction and validation of Cre recombination in *Cdh5-CreERT2;RCE:loxP;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* and *Cdh5-CreERT2;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice.** (A) pSMAD1/5/8 is ubiquitously expressed in arterial and venous ECs but becomes specifically induced in remodeling arterial collaterals upon ligation. Staining for pSMAD1/5/8 (green), αSMA (red), and DAPI (blue) in unligated and 7-d ligated adductors, with asterisks indicating positive ECs. A, artery; V, vein. Bars, 10 µm and 5 µm (magnifications). (B and C) Mice were treated for five consecutive days with 2 mg tamoxifen. After 2 d of rest, the femoral artery was ligated. (B) Staining for GFP (green), αSMA (red), and DAPI (blue) on transversal sections of the adductor 7 d after ligation indicate the EC-specific recombination upon tamoxifen treatment in *Cdh5-CreERT2;RCE:loxP;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice. Bars, 15 µm. (C) Staining for SMAD1 (green), αSMA (red), and DAPI (blue) on transversal sections of the adductor 7 d after ligation in *Cdh5-CreERT2;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice. The arrowheads and asterisks indicate ECs positive and negative for SMAD1, respectively. Quantification is represented as percent SMAD1-positive ECs. Error bars represent the SEM. *n* = 7 *Cdh5-Cre−;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice and *n* = 9 *Cdh5-Cre+;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice. *, P < 0.05 versus *Cdh5-Cre−;Smad1$^{fl/fl}$:Smad5$^{fl/fl}$* mice. Bars, 25 µm.

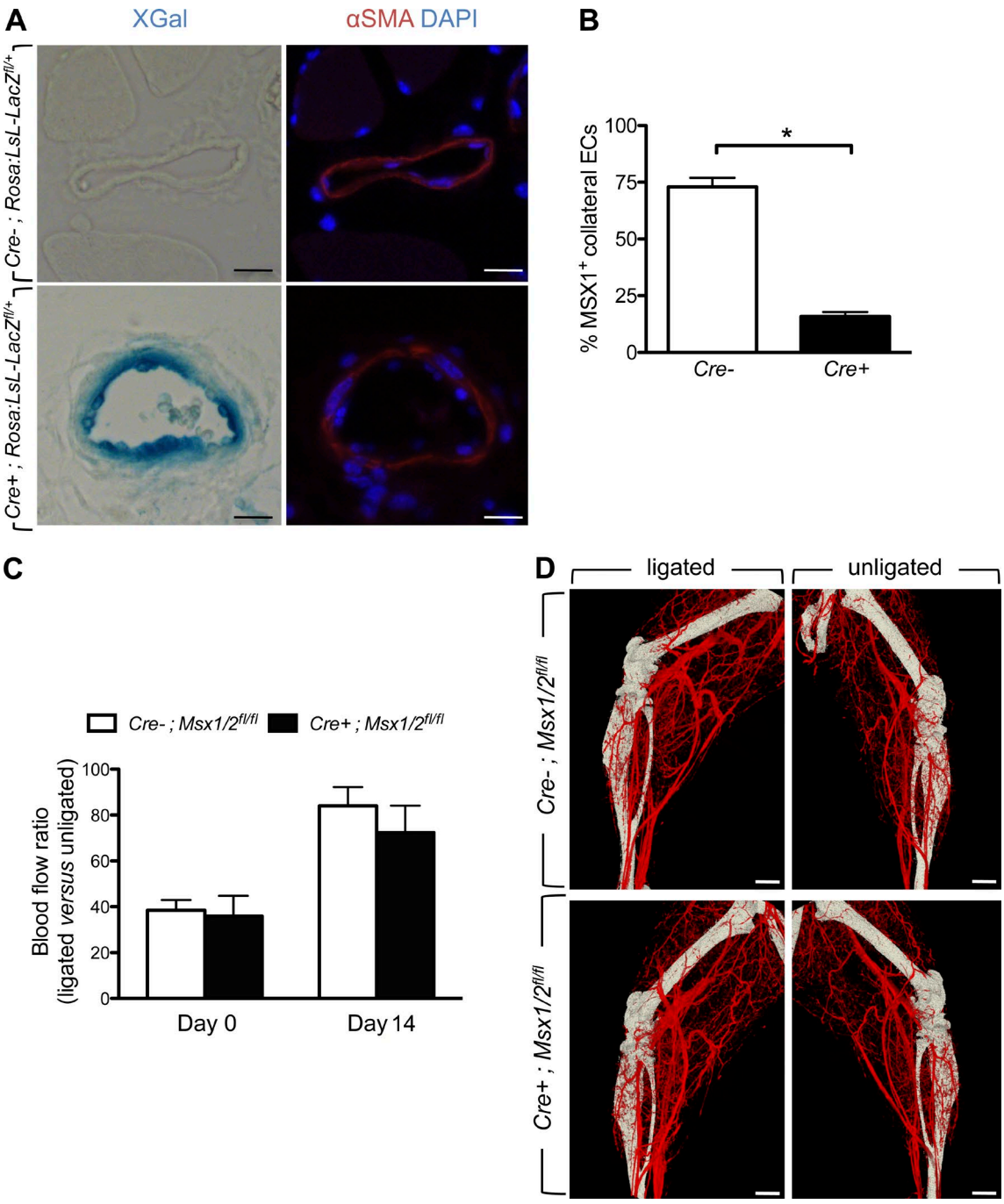

Figure S5. **Validation of Cre recombination in *Cdh5-CreERT2;Rosa:LsL-LacZ*$^{fl/+}$ mice and assessment of Cre recombination, flow, and collateral remodeling in *Cdh5-CreERT2;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ mice.** (A) *Cdh5-CreERT2;Rosa:LsL-LacZ*$^{fl/+}$ mice were treated for five consecutive days with 2 mg tamoxifen. After 2 d of rest, the femoral artery was ligated, and after 7 d the adductor region of Cre+ and Cre– littermates was whole-mount stained for Xgal and paraffin embedded. Transversal sections were subsequently stained for αSMA (red) and DAPI (blue). Bars, 15 µm. (B) In transversal sections of the ligated adductor of *Cdh5-Cre;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ mice after tamoxifen treatment, the number of collateral ECs positive for MSX1 were quantified relative to the total number of collateral ECs. $n = 4$ *Cdh5-Cre–;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ and $n = 5$ *Cdh5-Cre+;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ littermates. *, $P < 0.05$ versus *Cdh5-Cre–;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ mice. (C) High-resolution PET measurements after intravenous injection of ^{13}[N]ammonia on days 0 and 14 after femoral artery ligation in *Cdh5-Cre;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ mice. Blood flow was quantified as a ratio in the ligated versus the unligated leg, represented as a percentage for $n = 4$ *Cdh5-Cre–;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ and $n = 4$ *Cdh5-Cre+;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ littermates. Error bars represent the SEM. (D) Micro-CT angiograms of the ligated and unligated hind limbs of *Cdh5-Cre–;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ (top) and *Cdh5-Cre+;Msx1*$^{fl/fl}$*:Msx2*$^{fl/fl}$ (bottom) mice 7 d after femoral artery ligation. Bars, 2 mm.