Supplemental Material

Online Figures I-XII
Online Table I

Genetic Targeting of Organ-Specific Blood Vessels

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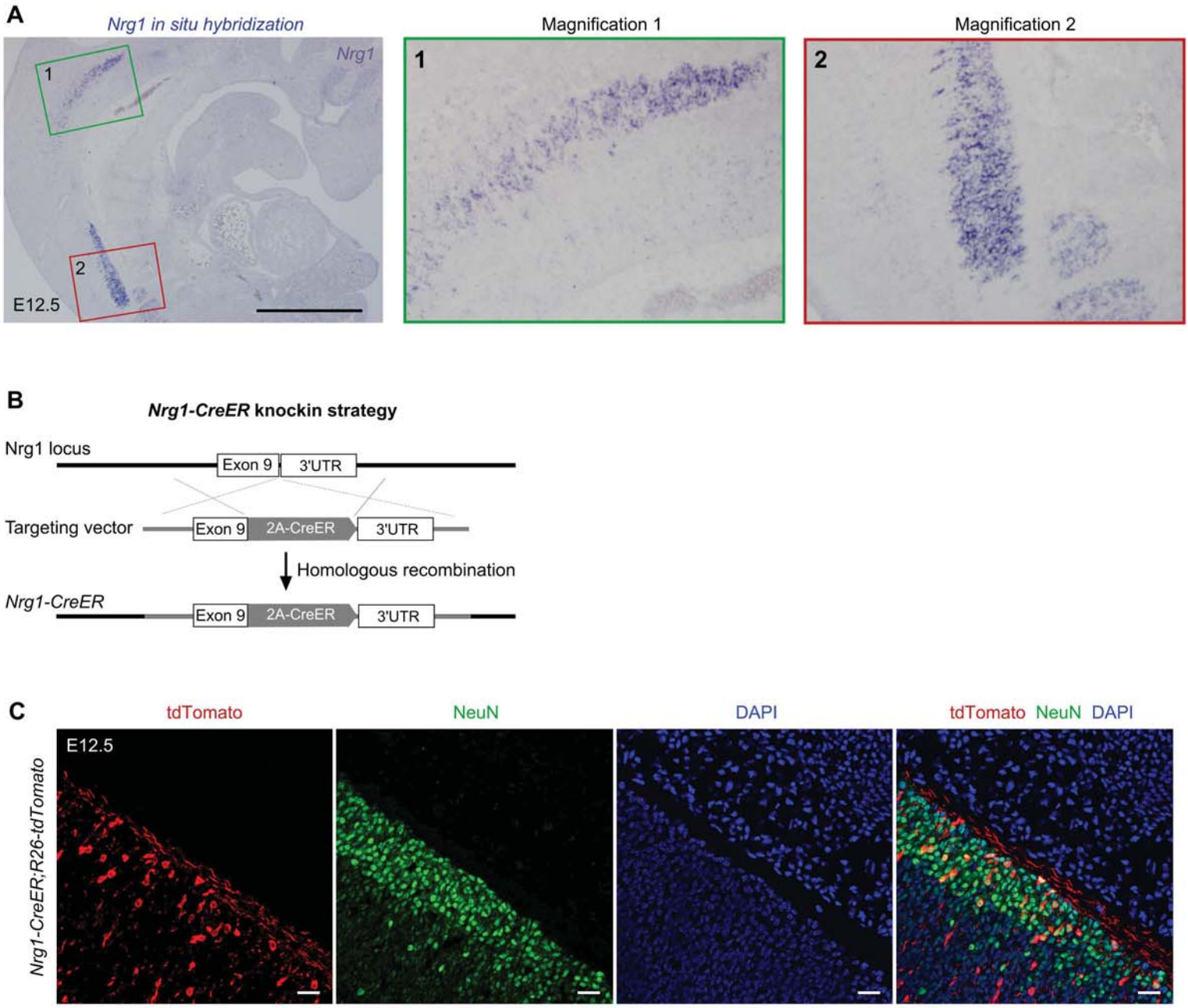
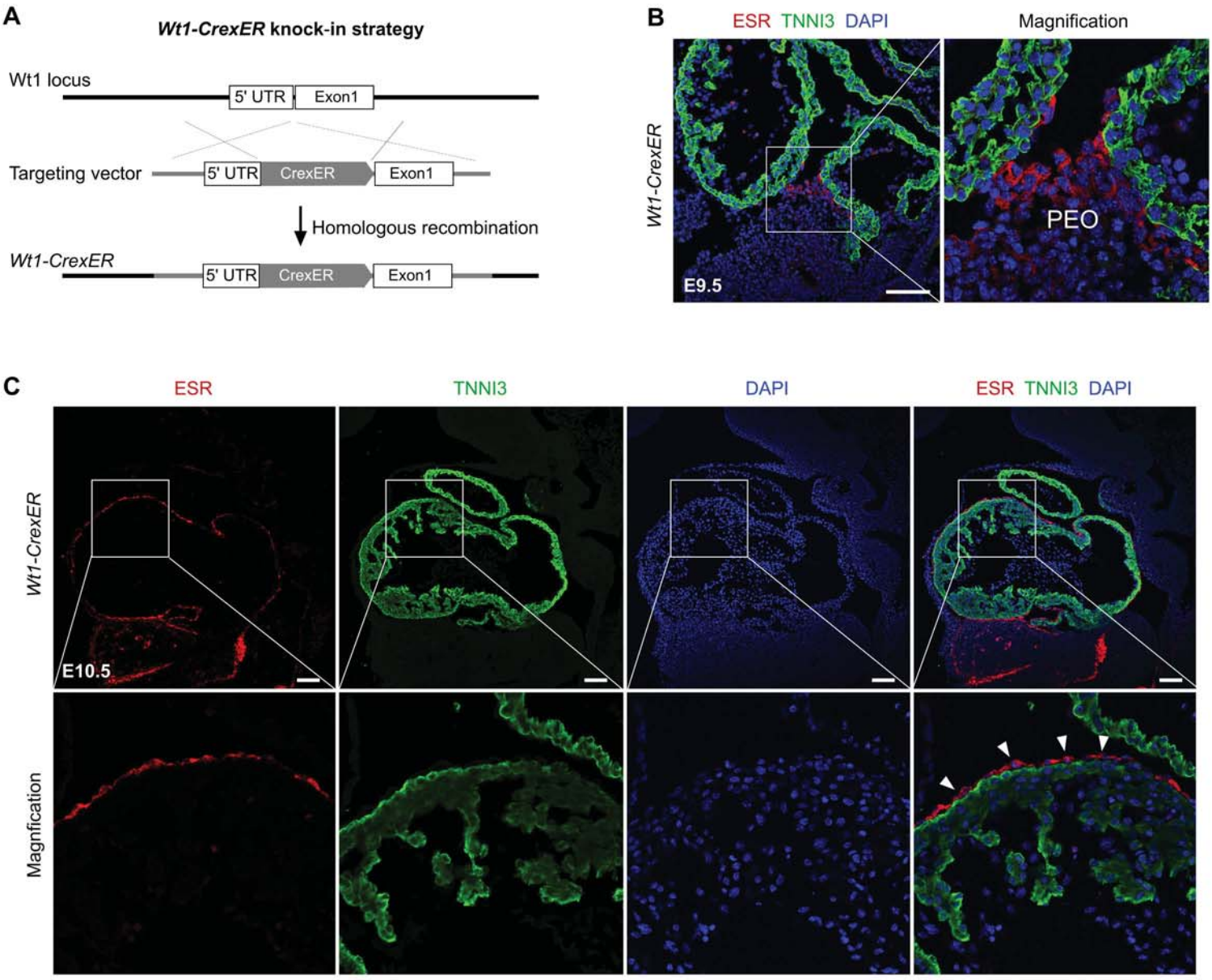
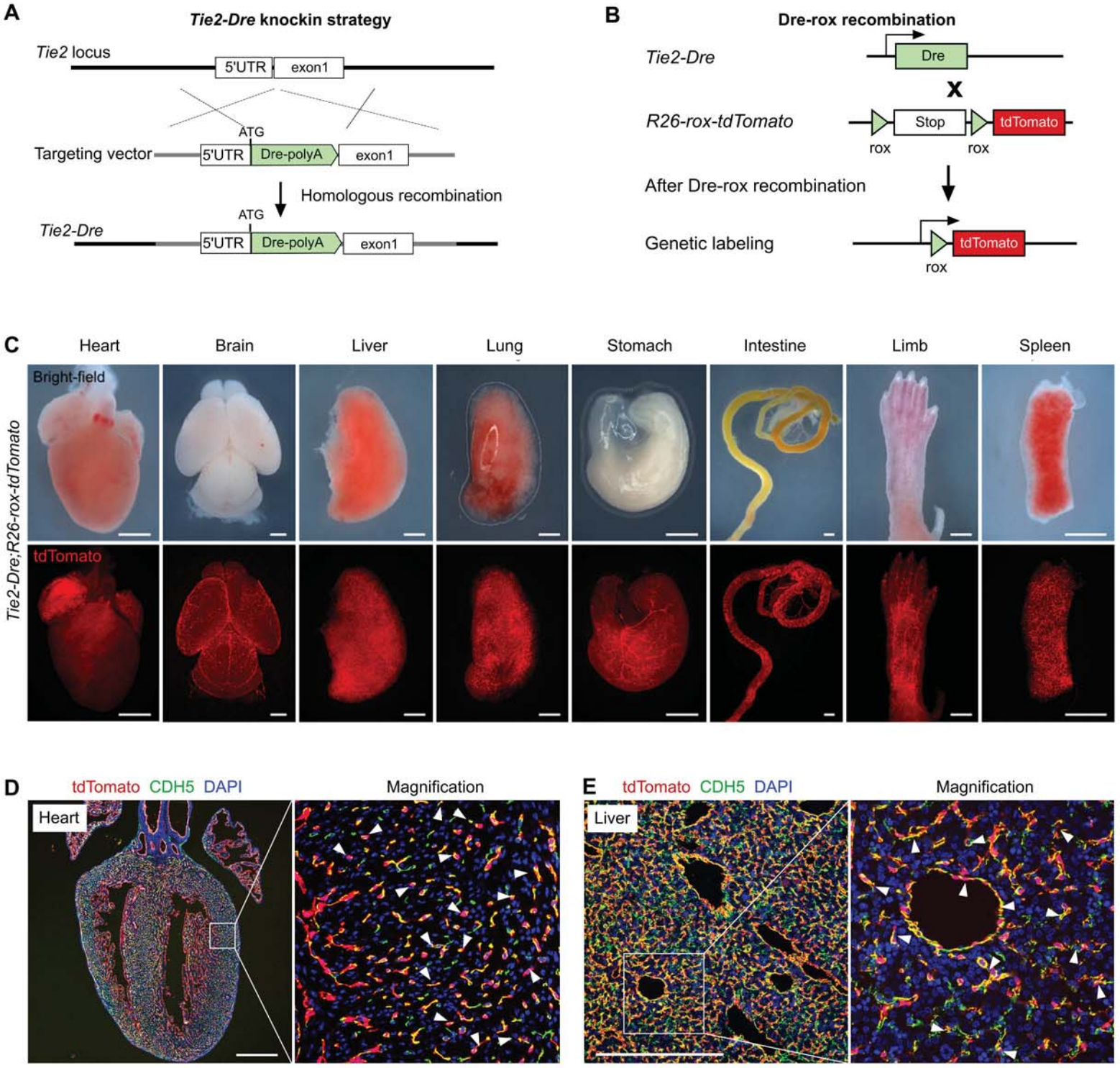


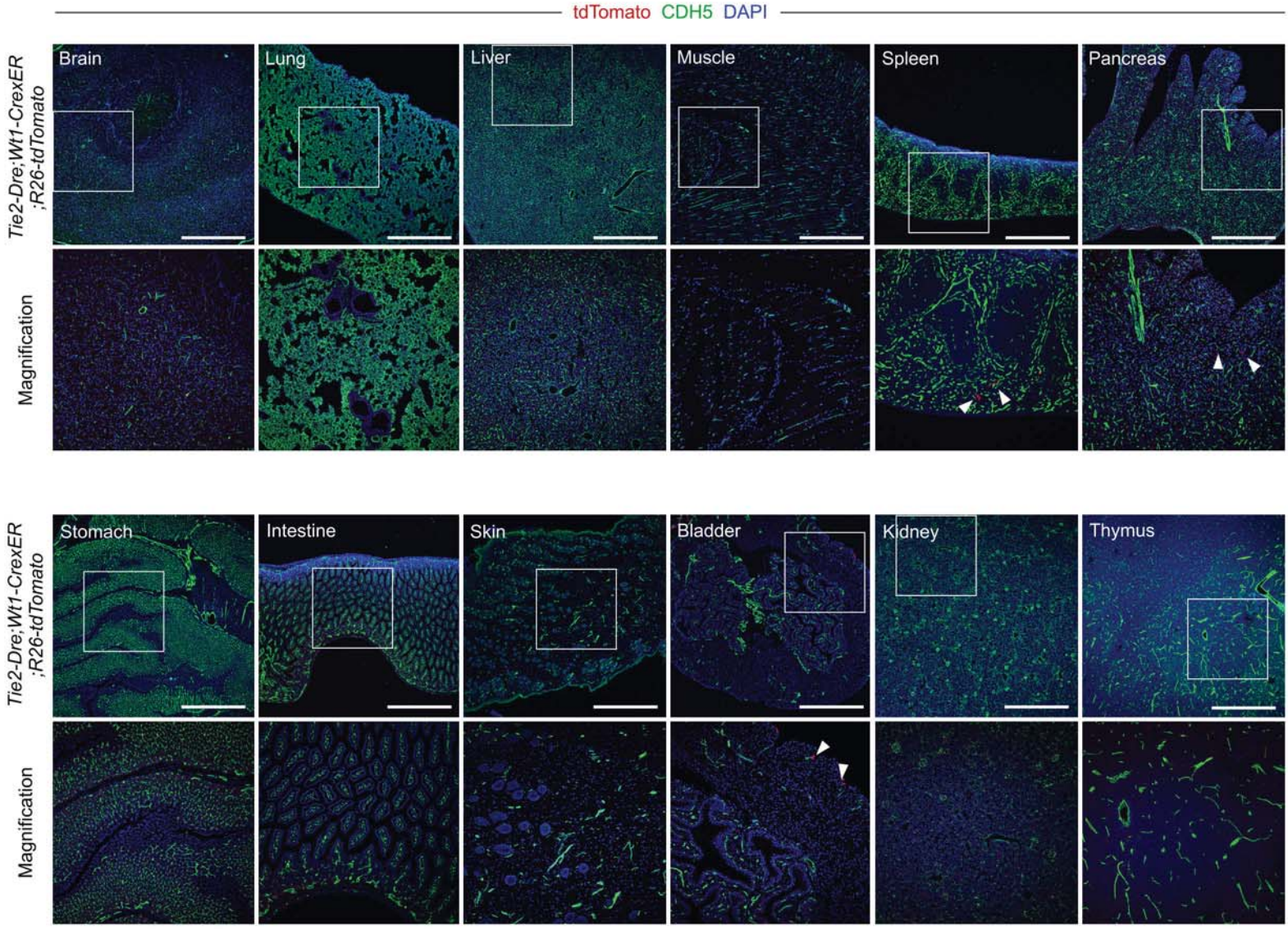
Online Figure I. Generation and characterization of *Nrg1-CreER* line. A, *In situ* hybridization of *Nrg1* on E12.5 wild-type embryonic sections. **B**, Schematic figure showing generation of *Nrg1-CreER* allele by homologous recombination. **C**, Immunostaining for tdTomato and neuron marker NeuN on E12.5 *Nrg1-CreER;R26-tdTomato* embryonic sections. Tamoxifen was induced at E9.5. Scale bars, 100 μm. Each figure is representative of 5 individual samples.



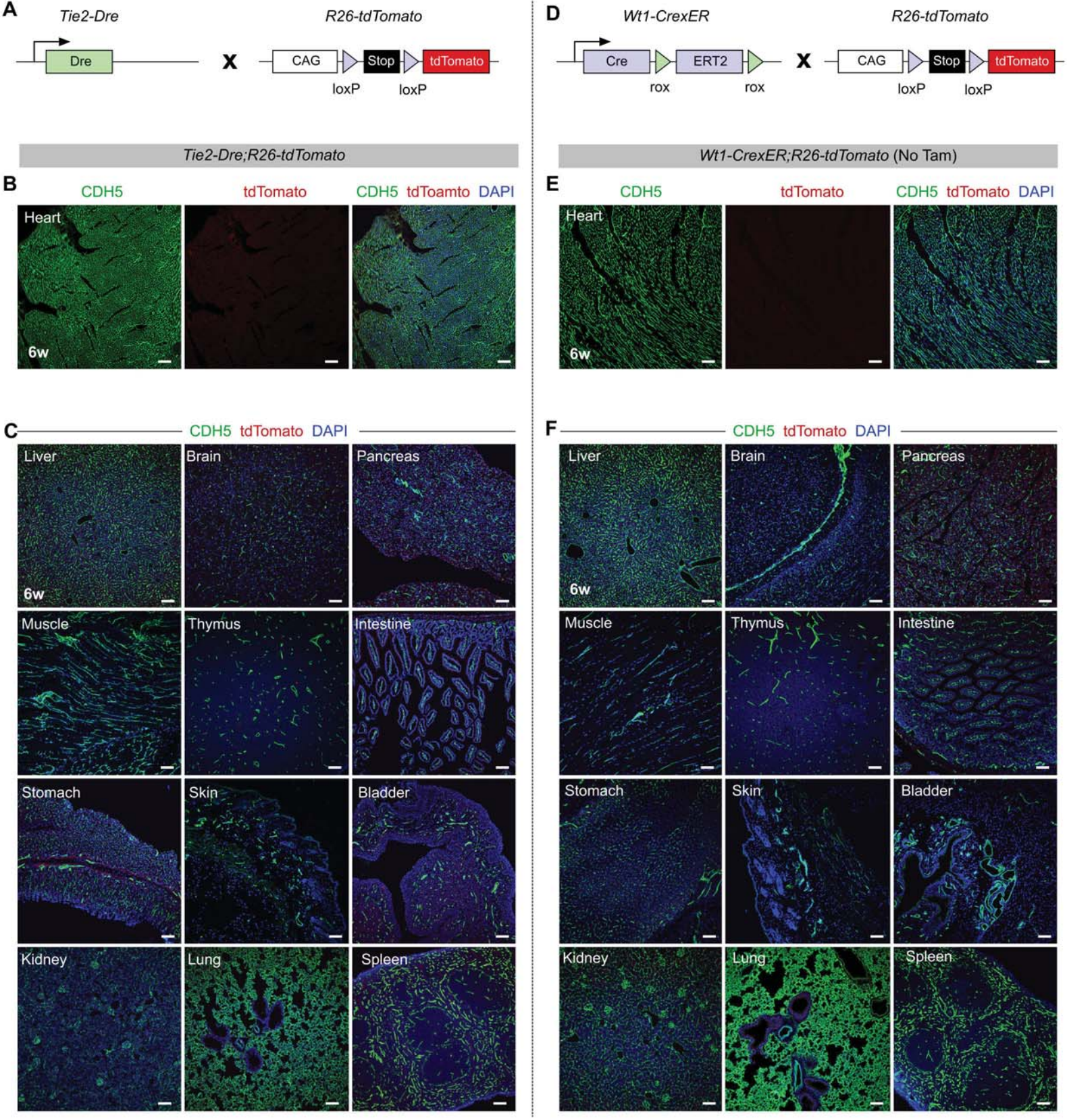
Online Figure II. Generation and characterization of *Wt1-CrexER*. **A**, Schematic figure showing knock-in strategy for *Wt1-CrexER* allele. **B**, Immunostaining for ESR and TNNI3 on E9.5 *Wt1-CrexER* section shows ESR^+ cells in proepicardium (PEO). **C**, Immunostaining for ESR and TNNI3 on E10.5 *Wt1-CrexER* embryonic section shows ESR^+ cells in epicardium (arrowheads). Scale bars, 100 μm. Each image is representative of 5 individual samples.



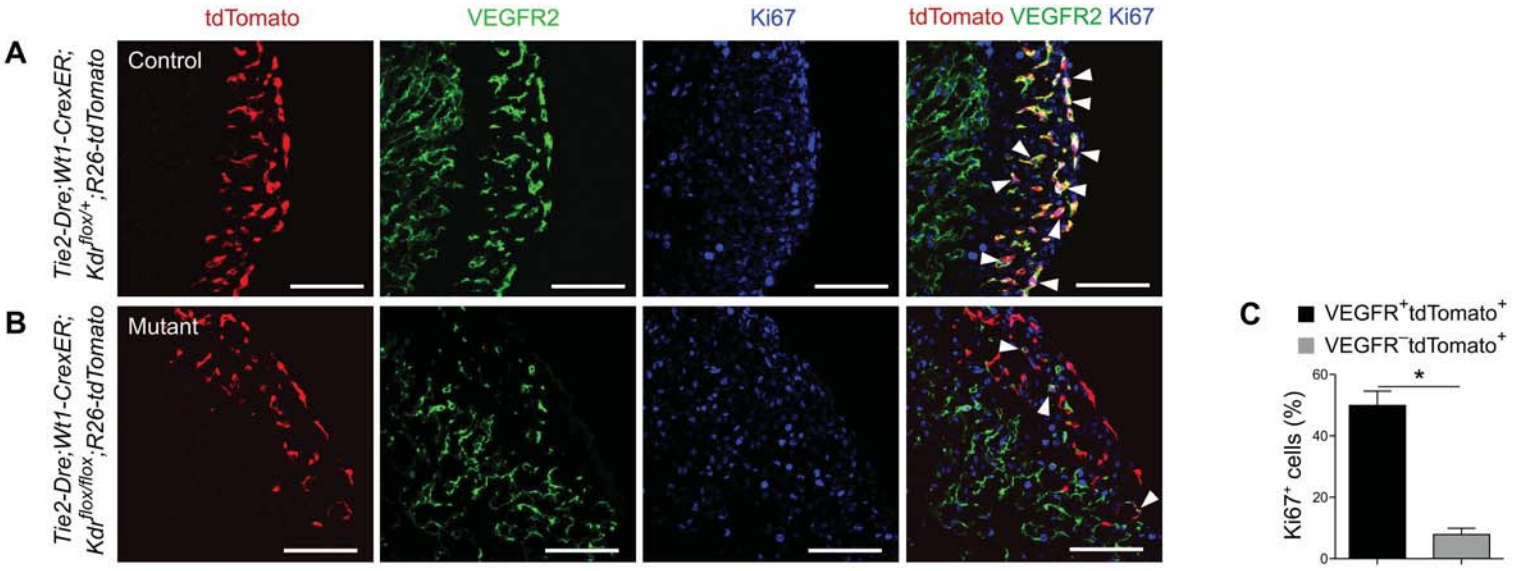
Online Figure III. Generation and characterization of *Tie2-Dre* line. A, Schematic showing strategy for knockin of Tie2-Dre allele by homologous recombination. **B**, Schematic showing *Tie2-Dre* mediated Dre-rox recombination on R26-rox-tdTomato reporter. **C**, Whole-mount view of organs from P0 *Tie2-Dre;R26-rox-tdTomato* mouse. **D,E**, Immunostaining for tdTomato and CDH5 on heart and liver sections. Arrowheads indicate tdTomato$^+$CDH5$^+$ endothelial cells. Scale bars, 1 mm in C; 500 μm in D,E,. Each image is representative of 5 individual samples.



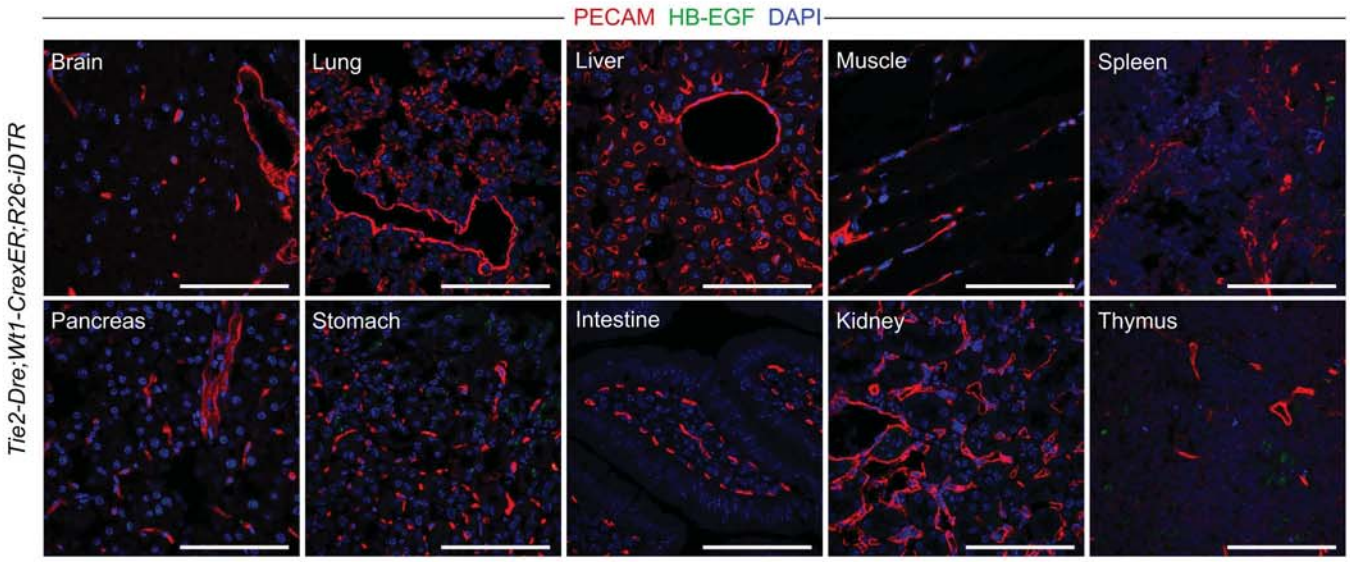
Online Figure IV. Magnification of immunostaining images shows negligible recombination in other organs. Immunostaining for tdTomato and CDH5 on tissue sections collected from different organs of *Tie2-Dre;Wt1-CrexER;R26-tdTomato* mice. Arrowheads indicate tdTomato$^+$ cells. Scale bars, 1 mm. Each image is representative of 5 individual biological samples.



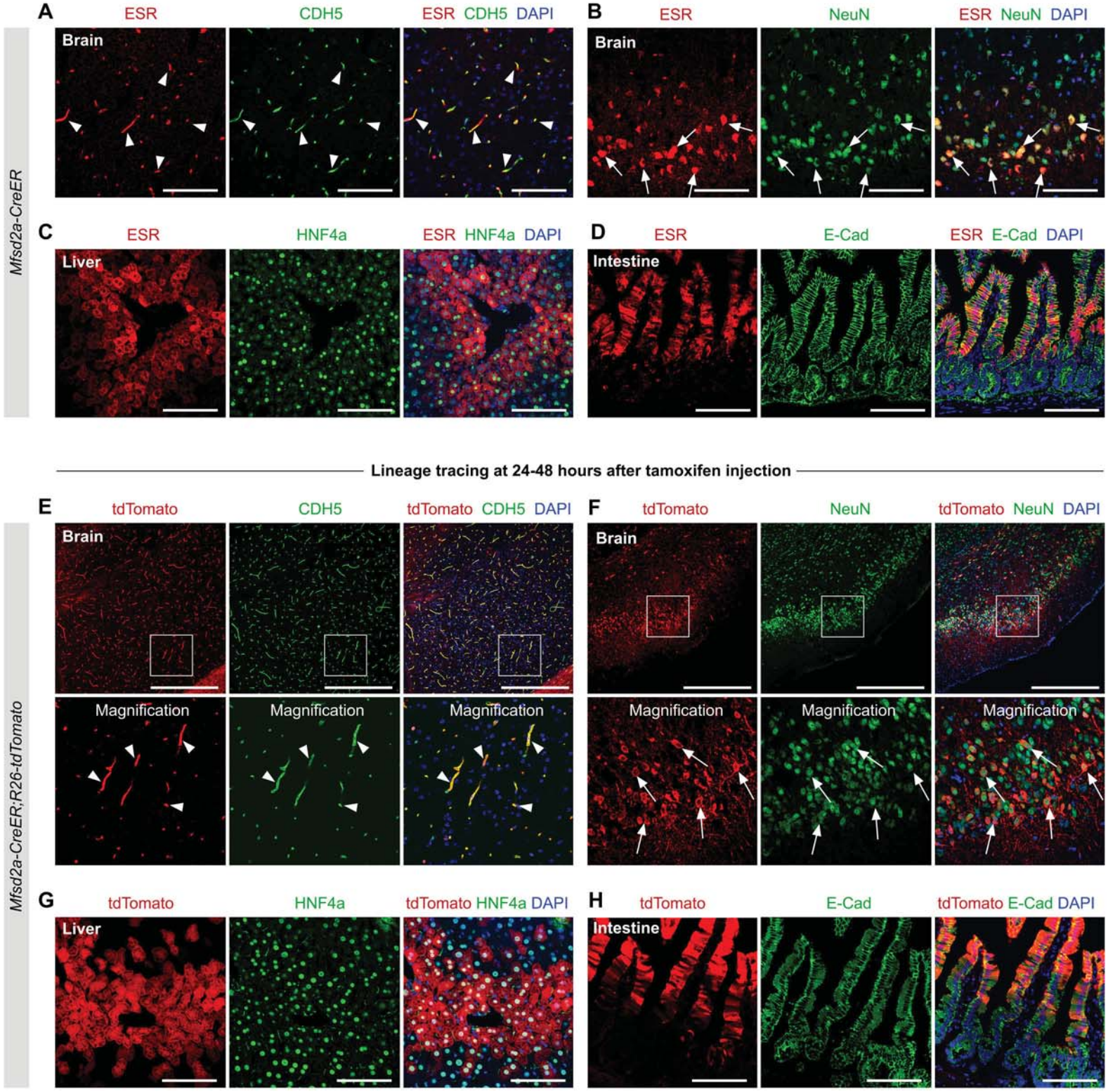
Online Figure V. *Tie2-Dre* and *Wt1-CreXER* does not recombine *R26-tdTomato* reporter. A,**D**, Schematic showing crossing of *Tie2-Dre* with *R26-tdTomato* or *Wt1-CrexER* with *R26-tdTomato*. **B,E**, Immunostaining for tdTomato and CDH5 on heart sections from adult *Tie2-Dre;R26-tdTomato* mice(left panel) or adult *Wt1-CrexER;R26-tdTomato* mice (right panel). **C,F**, Immunostaining for tdTomato and CDH5 on tissue sections from adult *Tie2-Dre;R26-tdTomato* mice (left panel) or adult *Wt1-CrexER;R26-tdTomato* mice (right panel). Scale bars, 100 μm. Each image is representative of 5 individual samples.



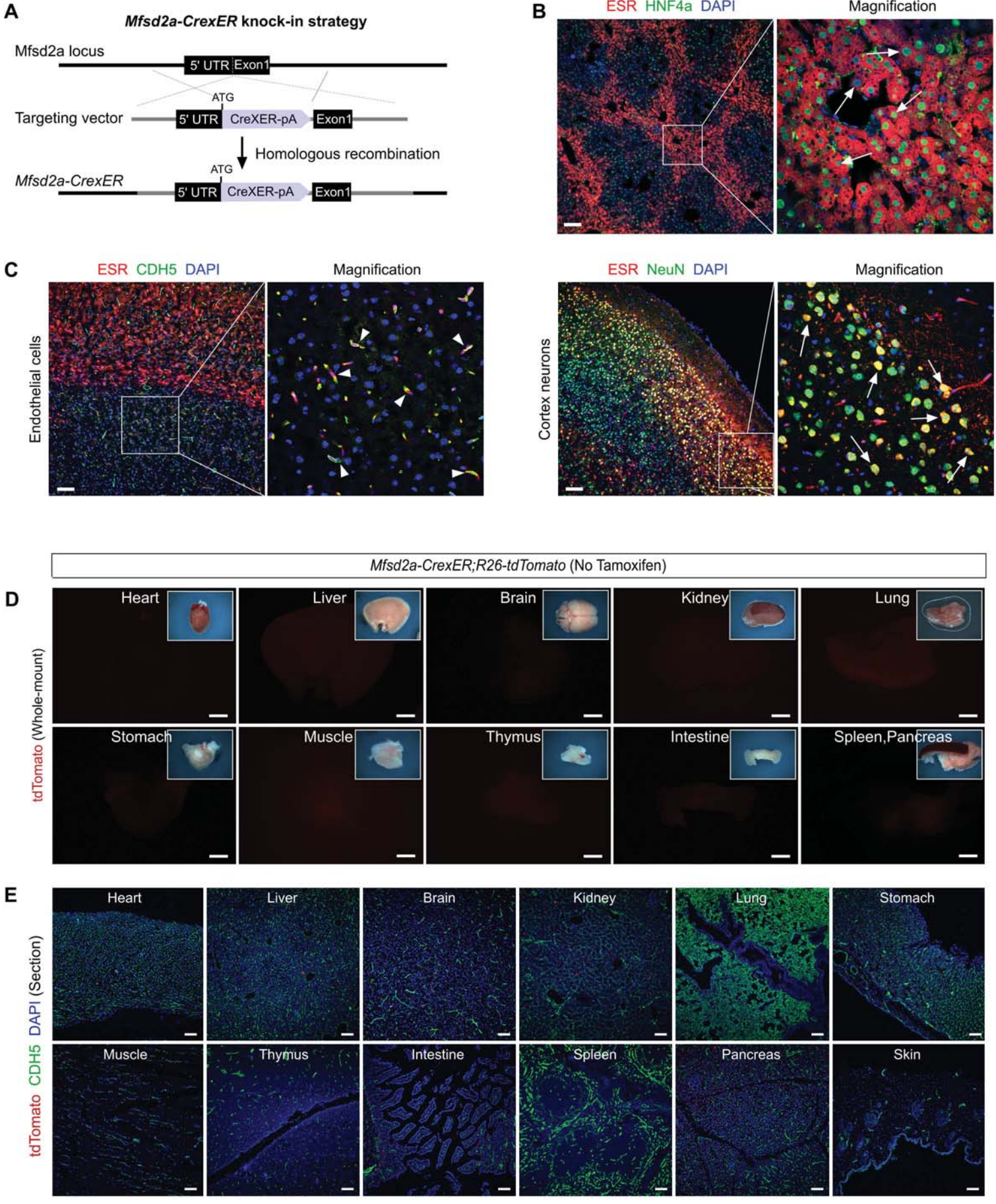
Online Figure VI. Proliferation of *CoEC-Cre* labeled endothelial cells. A,B, Immunostaining for tdTomato, Ki67 and VEGFR2 on E14.5 heart sections of *Tie2-Dre;Wt1-CrexER;Kdr$^{flox/+}$;R2t6-tdTomato* (control) or *Tie2-Dre;Wt1-CrexER;Kdr$^{flox/flox}$;R26-tdTomato* (mutant) embryos. Arrowheads indicate tdTomato^{+}VEGFR2^{+} endothelial cells in the control and mutant hearts. **C**, Quantification of the percentage of Ki67^{+} cells in VEGFR^{+}tdTomato^{+} or VEGFR2^{-}tdTomato^{+} cell populations in compact myocardium. Data are mean ± SEM.; *P < 0.05; n = 5. Scale bars, 100 μm. Each figure is representative of 5 individual samples.



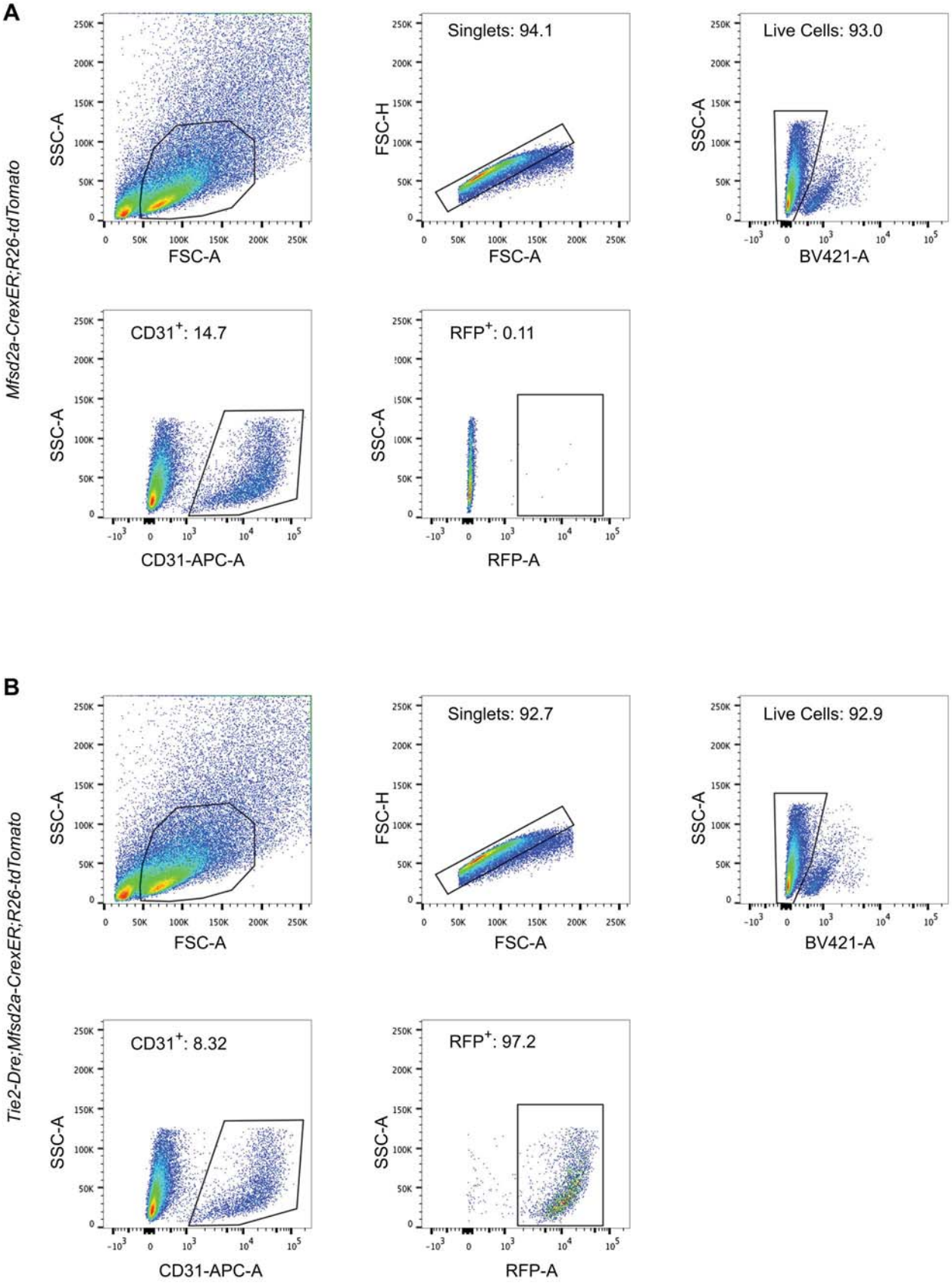
Online Figure VII. *CoEC-Cre* minimally recombines *R26-iDTR* in endothelial cells of other organs or tissues. Immunostaining for PECAM and HB-EGF (DTR) on tissue sections of organs collected from 6 week old *Tie2-Dre;Wt1-CrexER;R26-iDTR* mice. Each image is representative of 5 individual samples. Scale bars, 100 μm.



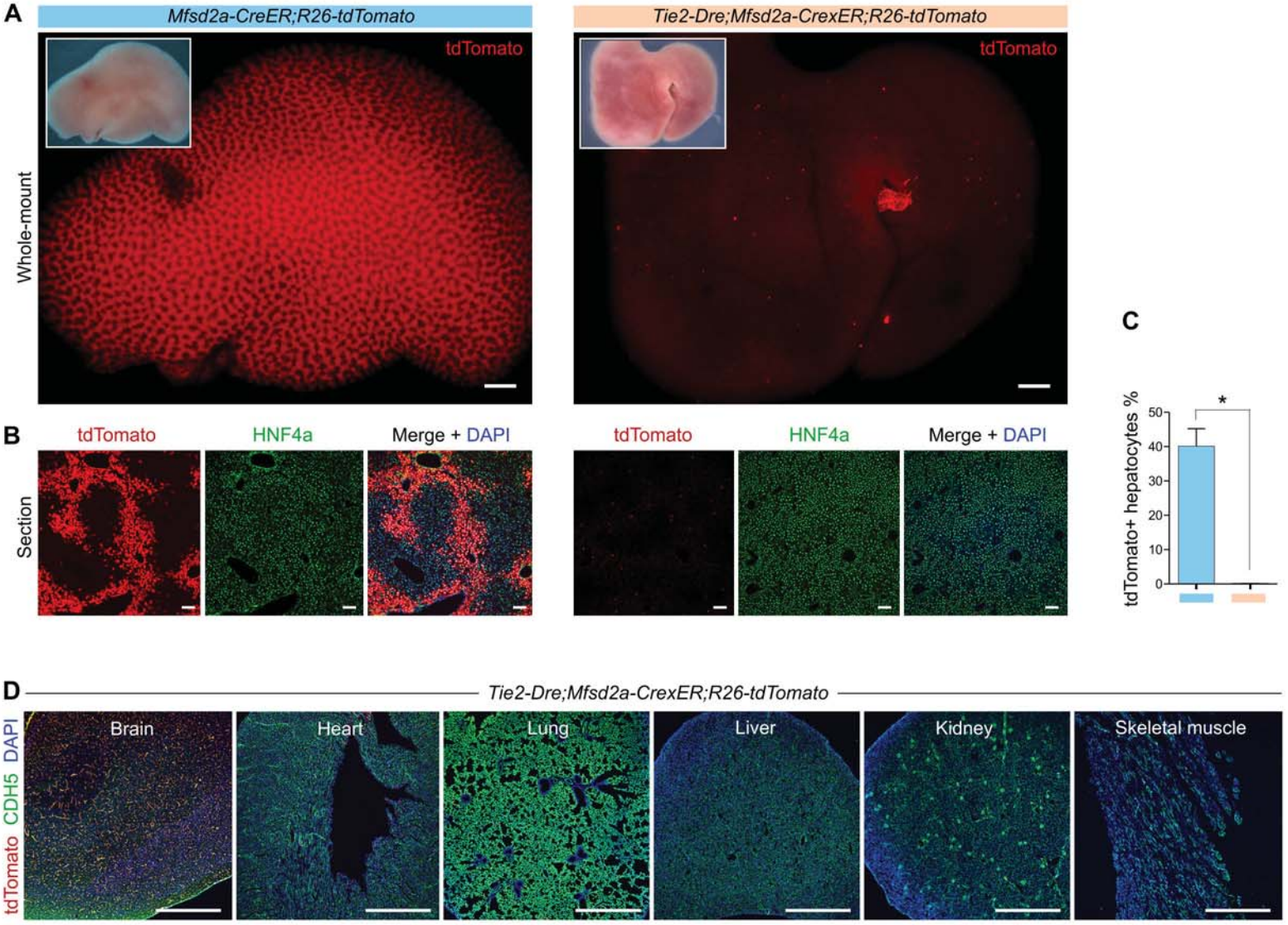
Online Figure VIII. Mfsd2a$^+$ cells detected by *Mfsd2a-CreER* line. A, Immunostaining for ESR (as surrogate for endogenous Mfsd2a) and CDH5 on *Mfsd2a-CreER* brain sections shows ESR$^+$CDH5$^+$ BBB endothelial cells (arrowheads). **B**, Immunostaining for ESR and NeuN on *Mfsd2a-CreER* brain sections shows ESR$^+$NeuN$^+$ neurons (arrows). **C**, Immunostaining for ESR and HNF4a on *Mfsd2a-CreER* liver sections shows ESR$^+$HNF4a$^+$ hepatocytes. **D**, Immunostaining for ESR and E-Cad on small intestine section showed ESR$^+$E-Cad$^+$ cells in villi. **E,F**, Immunostaining for tdTomato and CDH5 (E) or NeuN (F) on brain sections from *Mfsd2a-CreER;R26-tdTomato* mouse at 24-48 hours after tamoxifen injection. Arrowheads indicate Mfsd2a-expressing endothelial cells; arrows indicate Mfsd2a-expressing neurons. Boxed regions are magnified in the lower panels. **G**, Immunostaining for tdTomato and HNF4a on liver section shows Mfsd2a-expressing hepatocytes. **H**, Immunostaining for tdTomato and E-Cad on small intestine section shows Mfsd2a-expressing epithelial cells in villi. Scale bars, 100 μm. Each image is representative of 5 individual samples.



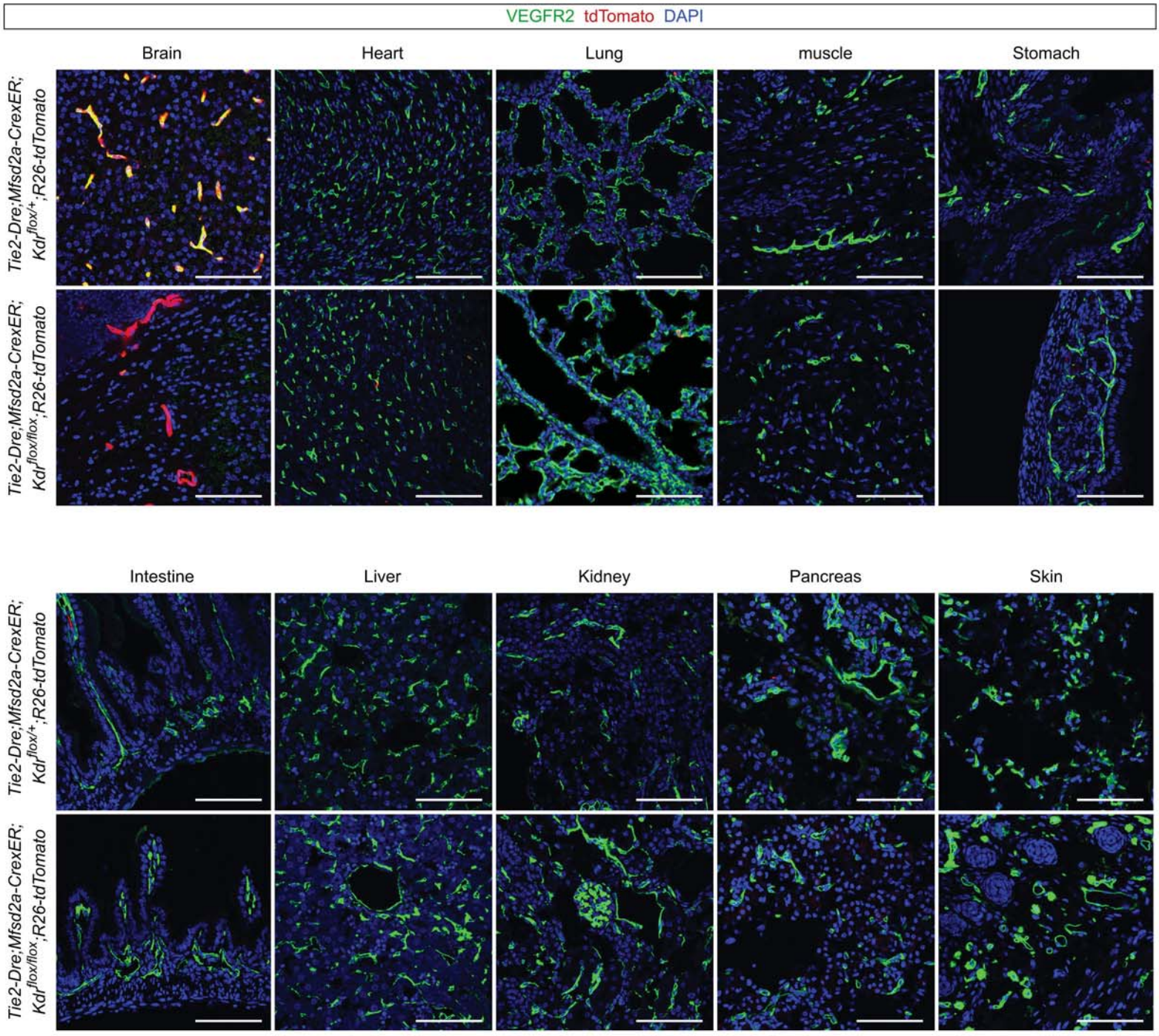
Online Figure IX. Generation and characterization of *Mfsd2a-CrexER* mouse line. A, Schematic showing knock-in strategy for *Mfsd2a-CrexER* allele by homologous recombination. **B**, Immunostaining for ESR (as surrogate for endogenous Mfsd2a) and HNF4a on liver sections of adult mouse. Arrows indicate ESR$^+$HNF4a$^+$ hepatocytes. **C**, Immunorstaining for ESR and CDH5 (left panel) or NeuN (right panel) on brain section. Arrowheads indicate ESR$^+$CDH5$^+$ endothelial cells; arrows indicate ESR$^+$NeuN$^+$ neurons. **D**, Whole-mount fluorescence views of organs collected from *Mfsd2a-CrexER;R26-tdTomato* adult mouse without tamoxifen treatment. Inserts are bright-field images of organs. **E**, Immunostaining for tdTomato and CDH5 on tissues sections from *Mfsd2a-CrexER;R26-tdTomato* adult mouse without tamoxifen treatment. Scale bars, 1 mm in D, 100 μm in B,C,E.



Online Figure X. Flow cytometric analysis of tdTomato⁺ endothelial cells labeled by *BEC-Cre*. Brain of neonatal *Mfsd2a-CrexER;R26-tdTomato* (**A**) and *Tie2-Dre;Mfsd2a-CrexER;R26-tdTomato* (**B**) mice were enzymatically dispersed for flow cyteometric analysis. Single cells were gated by forward scatter. Dead cells were excluded by Violet Dye labelling. Endothelial cells were stained by CD31-APC antibody. RFP⁺ cells were gated to analyze labeling efficiency. Each image is representative of 5 individual biological samples.



Online Figure XI. More precise targeting by intersectional genetics. A,B, Whole-mount fluorescence view of livers and immunostaining for tdTomato and HNF4a on liver sections from *Mfsd2a-CreER;R26-tdTomato* or *Tie2-Dre;Mfsd2a-CrexER;R26-tdTomato* mice. For *Mfsd2a-CreER*, tamoxifen was induced at 6 weeks and samples were collected 2 days later. Inserts are bright-field images of livers. **C**, Quantification of labeled hepatocytes was shown on right panel. Data are mean ± SEM.; **P* < 0.05; n = 5. **D,** Immunostaining for tdTomato and CDH5 on tissue sections from *Tie2-Dre;Mfsd2a-CreXER;R26-tdTomato* mice. Scale bars, 1 mm in A,D; 100 μm in B. Each image is representative of 5 individual samples.



Online Figure XII. Expression of VEGFR2 in multiple organs. Immunostaining for tdTomato and VEGFR2 on sections of multiple organs collected from P0 *Tie2-Dre;Mfsd2a-CrexER;Kdr$^{flox/+}$;R26-tdTomato* (control) or *Tie2-Dre;Mfsd2a-CrexER;Kdr$^{flox/flox}$;R26-tdTomato* (mutant) mice. VEGFR2 is not detected in the brain vessels, but remains in the vessels of other organs or tissues of mutant, in comparison with control. Scale bars, 100 μm. Each image is representative of 5 individual samples.

Online Table I. DNA Sequence for Genotyping Primers

Name	Forward primer	Reverse primer	PCR length
Nrg1-CrexER	TGATTCAGCCTCTTTCAGCCG	GGATAGTTTTTACTGCCAGACCGC	868bp
Nrg1-CreER	TGAAGATACACCATTCCTGGGC	TGCGAACCTCATCACTCGTTG	314bp
CAG-Dre	ACTCCTTGCCGATGTTCCTCAG	TTGTCCCAAATCTGGCGGAG	415bp
WT1-CrexER	ATCTCCTAAAGTGACCCCGCAG	CCAGGTATGCTCAGAAAACGCC	896 bp
Tie2-Dre	TTCTCCTTGCCGCCAACTTG	ACTCCTTGCCGATGTTCCTCAG	207bp
Mfsd2a-CrexER	GGACAGAAGCATTTTCCAGGTATG	TGATGATTGGTCTCGTCTGGCG	593bp
Mfsd2a-CreER	GGACAGAAGCATTTTCCAGGTATG	TGATGATTGGTCTCGTCTGGCG	587bp
Kdr flox	TGGAGAGCAAGGCGCTGCTAGC	CTTTCCACTCCTGCCTACCTAG	439bp
ACTB-Cre	GCCTGCATTACCGGTCGATGC	CAGGGTGTTATAAGCAATCCC	481bp
R26-LacZ	AAAGTCGCTCTGAGTTGTTAT	GGAGCGGGAGAAATGGATAG	330bp
R26-rox-LacZ	TGGAAATGTTACCAAGGAACT	TGACAGGAGATCCTGCCCCGGCACT	716bp
R26-tdTomato	GGCATTAAAGCAGCGTATCC	CTGTTCCTGTACGGCATGG	196bp
R26-rox-tdTomato	ACGGGTGTTGGGTCGTTTGTTC	TTCTTGTAATCGGGGATGTCGGCG	609bp
R26-iDTR	GGCTACTGCTGACTCTCAACATT	TCATGGTGGCGAATTCGAT	700bp

Note: bp, base pair.