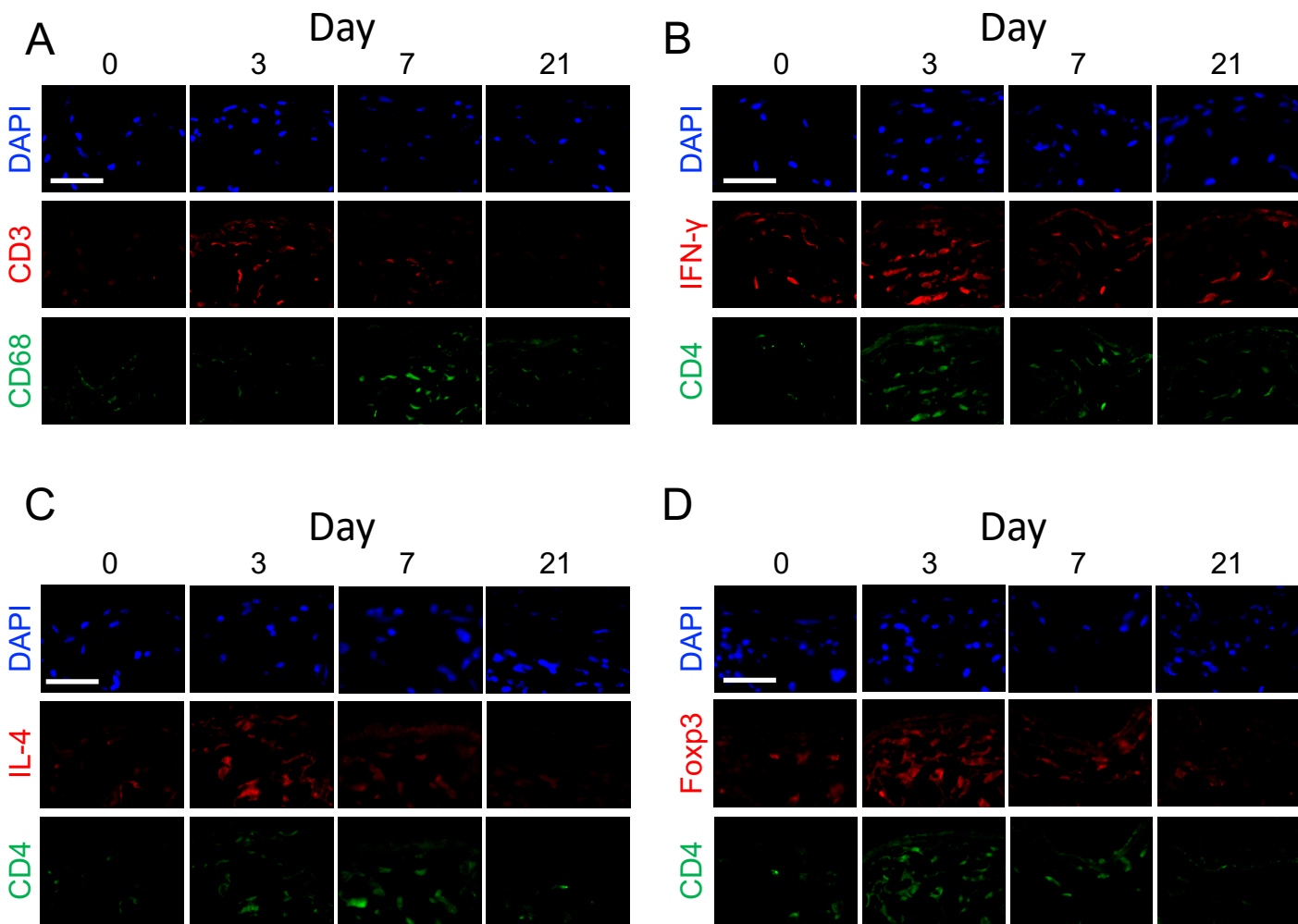


# SUPPLEMENTAL MATERIALS

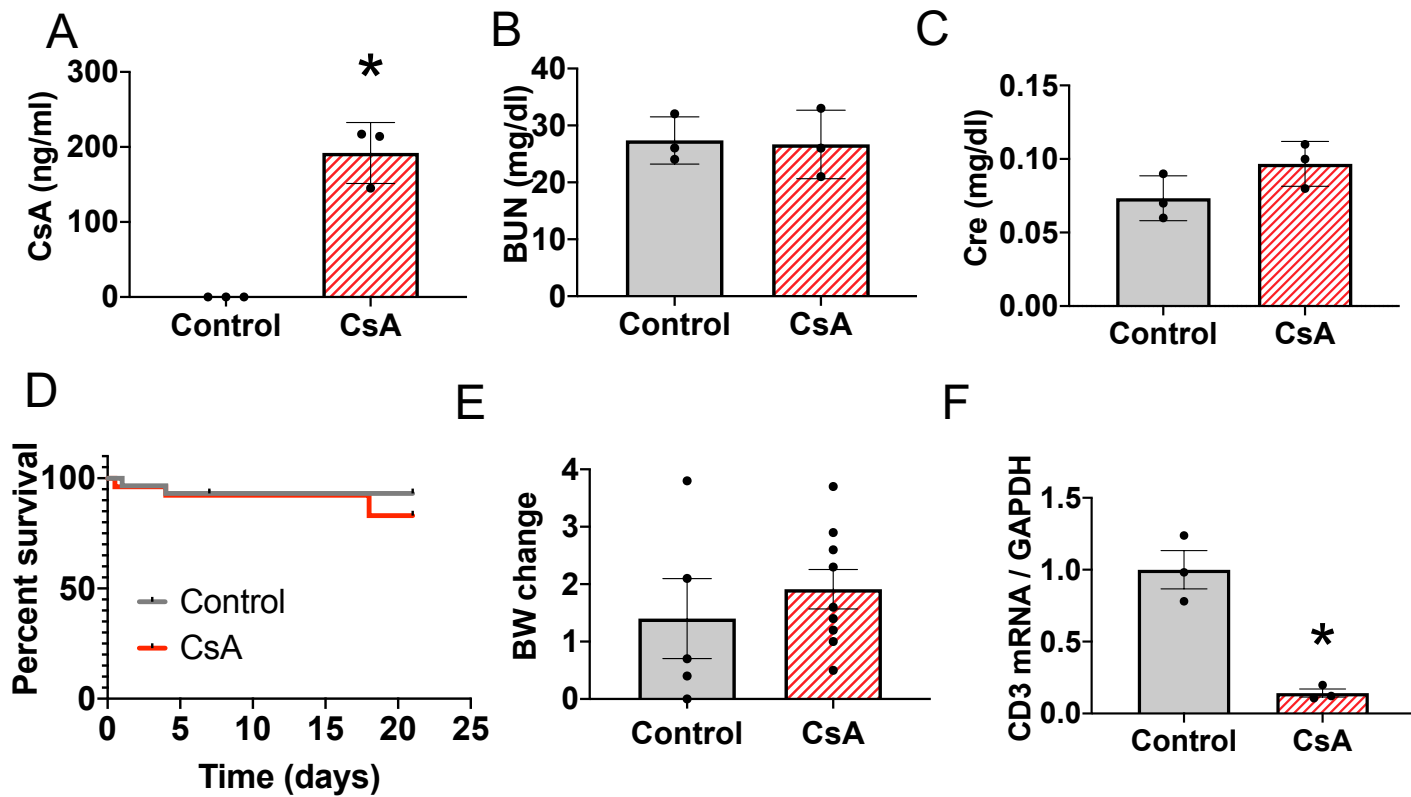
## Inhibition of T-cells by cyclosporine A reduces macrophage accumulation to regulate venous adaptive remodeling and increase arteriovenous maturation

Yutaka Matsubara<sup>1,2</sup>, Gathe Kiwan<sup>1</sup>, Jia Liu<sup>1</sup>, Luis Gonzalez<sup>1</sup>, John Langford<sup>1</sup>, Mingjie Gao<sup>1</sup>, Xixiang Gao<sup>1</sup>, Ryosuke Taniguchi<sup>1,3</sup>, Bogdan Yatsula<sup>1</sup>, Tadashi Furuyama<sup>2</sup>, Takuya Matsumoto<sup>4</sup>, Kimihiro Komori<sup>5</sup> and \*Alan Dardik<sup>1,6,7</sup>

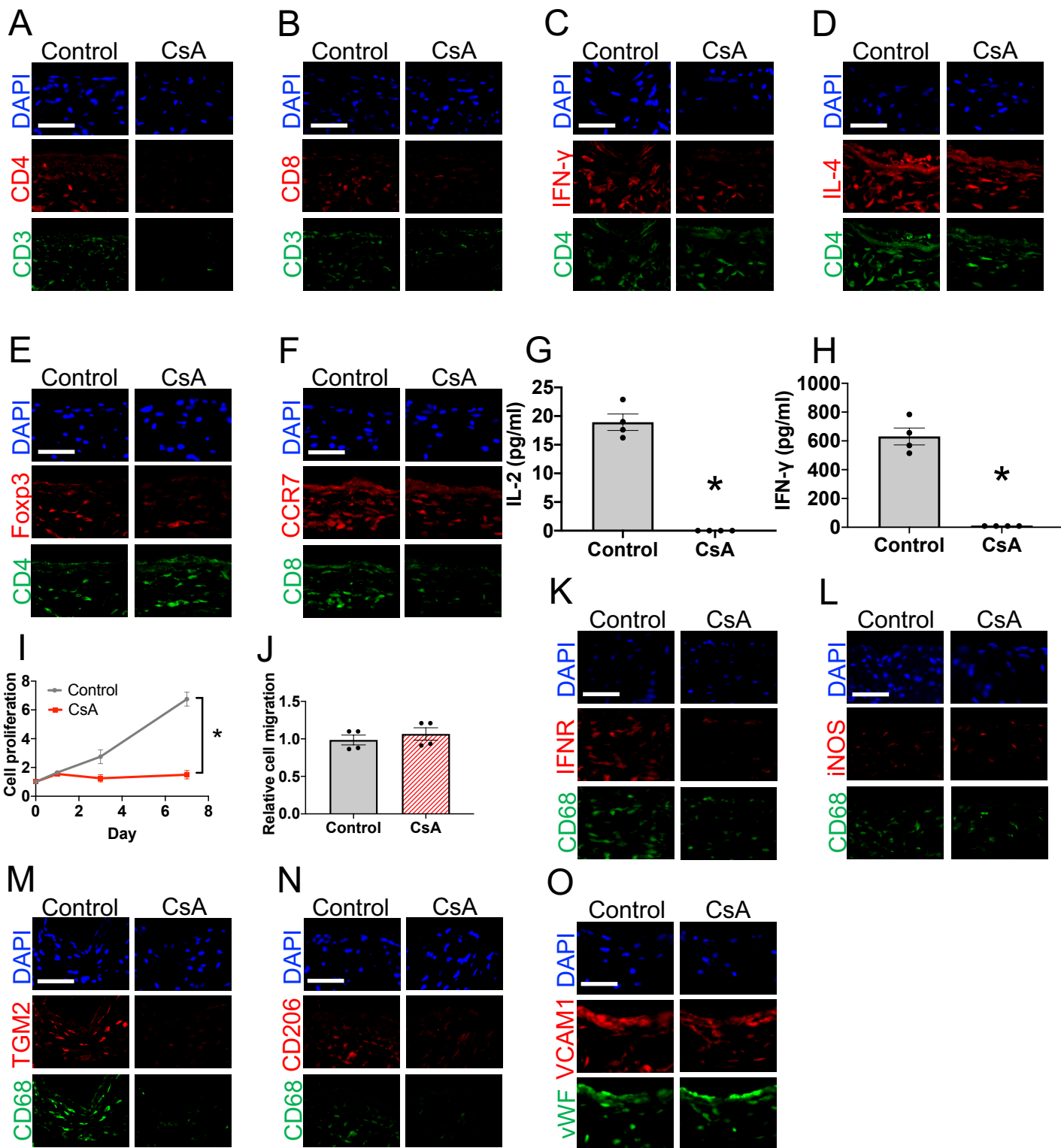
1. Vascular Biology and Therapeutics Program, Yale School of Medicine, New Haven, CT
2. Department of Surgery and Sciences, Kyushu University, Fukuoka, Japan
3. Division of Vascular Surgery, The University of Tokyo, Tokyo, Japan
4. Department of Vascular Surgery, Kyushu Central Hospital, Fukuoka, Japan
5. Division of Vascular Surgery, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan
6. Division of Vascular and Endovascular Surgery, Department of Surgery, Yale School of Medicine, New Haven, CT
7. Department of Surgery, VA Connecticut Healthcare Systems, West Haven, CT



**Supplemental Figure I. Individual channels of immunofluorescence shown in Figure 2. (A)** Representative photomicrographs showing CD3 (red) and CD68 (green). **(B)** Representative photomicrographs showing CD4 (green) and IFN- $\gamma$  (red). **(C)** Representative photomicrographs showing CD4 (green) and IL-4 (red). **(D)** Representative photomicrographs showing CD4 (green) and Foxp3 (red). Wild type mouse AVF wall at days 0, 3, 7 or 21. Scale bar, 50  $\mu$ m.

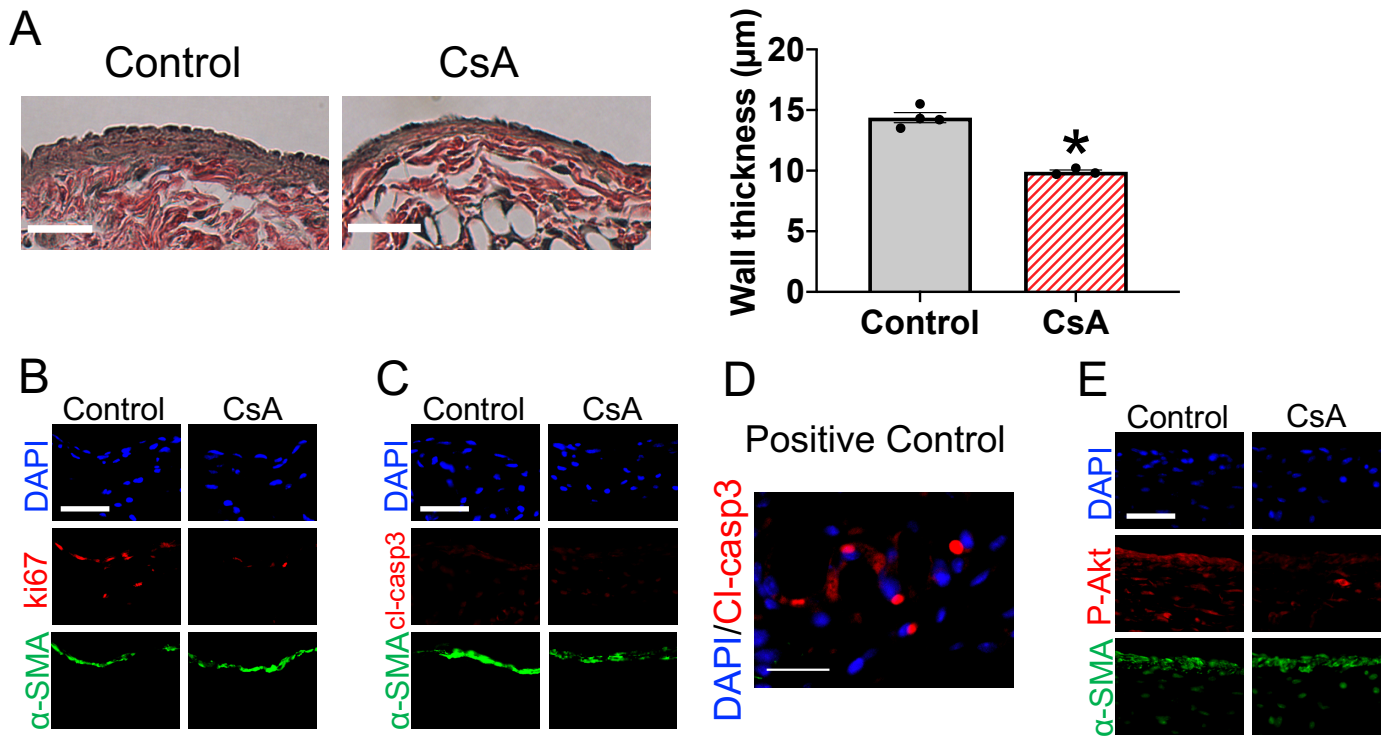


**Supplemental Figure II. Lack of cyclosporine toxicity in vivo.** (A) Bar graph shows serum CsA concentration in C57BL6/J mice treated with vehicle (control) or CsA. n=3. \*P=0.021 (t-test), P=0.1000 (Mann-Whitney U test). (B) Bar graph shows blood urea nitrogen (BUN) level in C57BL6/J mice treated with vehicle (control) or CsA. n=3. P=0.8824 (t-test), P=0.9999 (Mann-Whitney U test). (C) Bar graph shows blood creatine (Cre) level in C57BL6/J mice treated with vehicle (control) or CsA. n=3. P=0.1347 (t-test), P=0.2000 (Mann-Whitney U test). (D) Survival curves after AVF creation in C57BL6/J mice treated with vehicle (control) or CsA. n=26-29. P=0.6795 (log-rank test). (E) Bar graph shows body weight changes (day 21) in C57BL6/J mice treated with vehicle (control) or CsA. n=5-9. P=0.4710 (t-test). (F) Bar graph shows relative number of CD3 mRNA transcripts (day 7) in the spleen of C57BL6/J mice treated with vehicle (control) or CsA. n=3, \*P=0.0032 (t-test), P=0.1000 (Mann-Whitney U test).

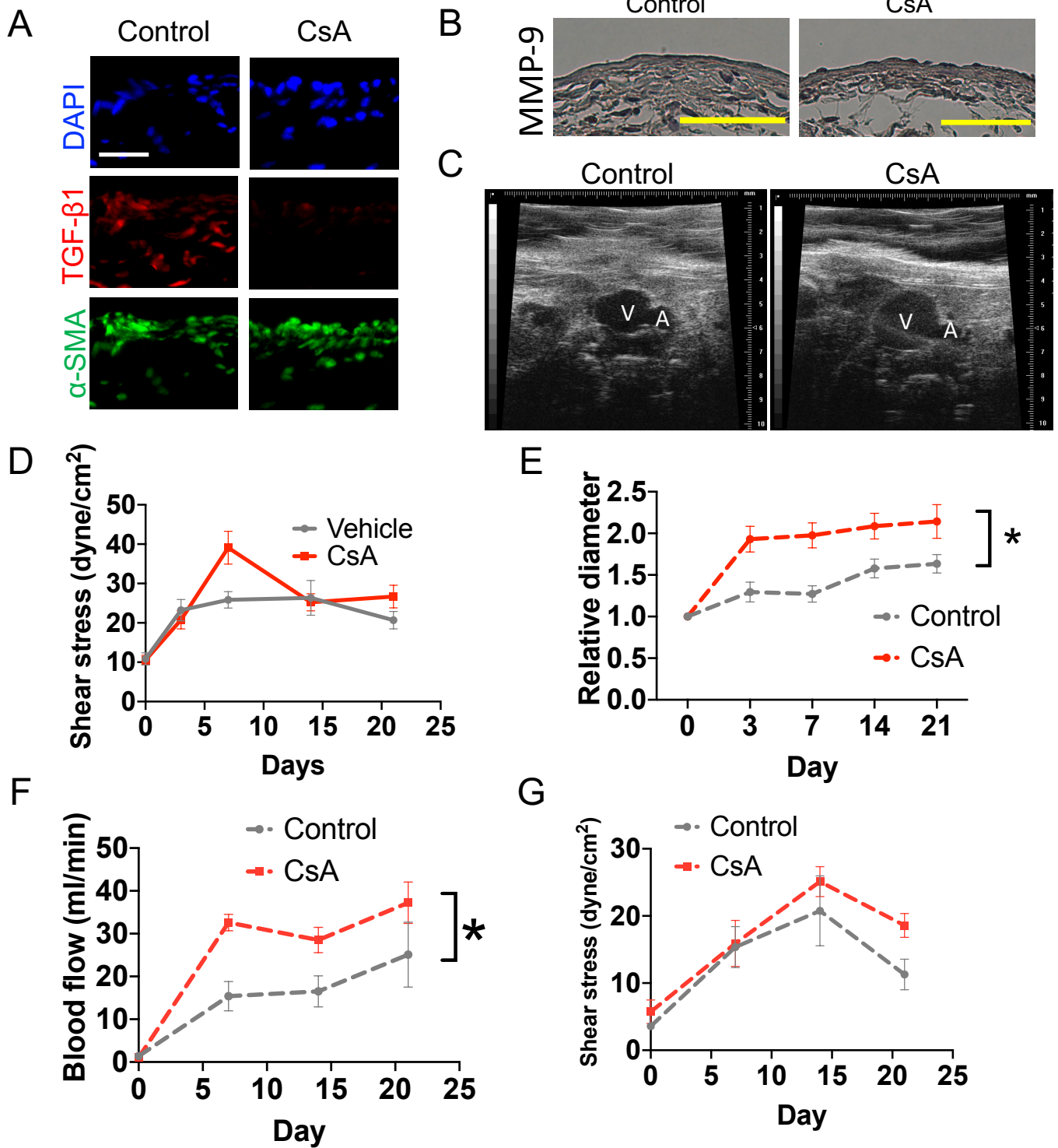


**Supplemental Figure III. CsA is associated with reduced T-cells and macrophages during venous remodeling. (A)** Representative photomicrographs showing CD3 (green) and CD4 (red). Scale bar, 50  $\mu$ m. **(B)** Representative photomicrographs showing CD3 (green) and CD8 (red). **(C)** Representative photomicrographs showing CD4 (green) and IFN- $\gamma$  (red). **(D)** Representative photomicrographs showing CD4 (green) and IL-4 (red). **(E)** Representative photomicrographs showing CD4 (green) and Foxp3 (red). **(F)** Representative photomicrographs showing CD8 (green) and CCR7 (red). **(G)** Bar graph shows IL-2 concentration in cell culture supernatants of mouse lymphocytes treated with vehicle (control) or CsA.  $n=4$ . \* $P<0.0001$  (t-test),  $P=0.0286$  (Mann-Whitney U test). **(H)** Bar graph shows IFN- $\gamma$  concentration in cell culture supernatants of mouse lymphocytes treated with vehicle (control) or CsA.  $n=4$ . \* $P<0.0001$  (t-test),  $P=0.0286$  (Mann-Whitney U test). **(I)** Line graph shows relative cell numbers of mouse lymphocytes treated with vehicle (control) or CsA.  $n=4$ .  $P=0.0002$  (ANOVA). The values are normalized to day 0. Day 1,  $P=0.8624$  (post hoc). Day 3,  $P=0.0542$  (post hoc). Day 7, \* $P=0.0009$  (post hoc). **(J)** Bar graph shows relative numbers of migrating mouse lymphocytes treated with vehicle (control) or CsA. The values are normalized to the control group.  $n=4$ .  $P=0.2366$  (t-test),  $P=0.1429$  (Mann-Whitney U test). **(K)** Representative photomicrographs showing CD68 (green) and IFN- $\gamma$  (red). **(L)** Representative photomicrographs showing CD68 (green) and iNOS (red). **(M)** Representative photomicrographs showing CD68 (green) and TGM2 (red). **(N)** Representative photomicrographs showing CD68 (green) and CD206 (red). **(O)** Representative photomicrographs showing vWF (green) and VCAM1 (red). Wild type mouse AVF wall at day 7 treated with vehicle (control) or cyclosporine (CsA). Scale bar, 50  $\mu$ m.

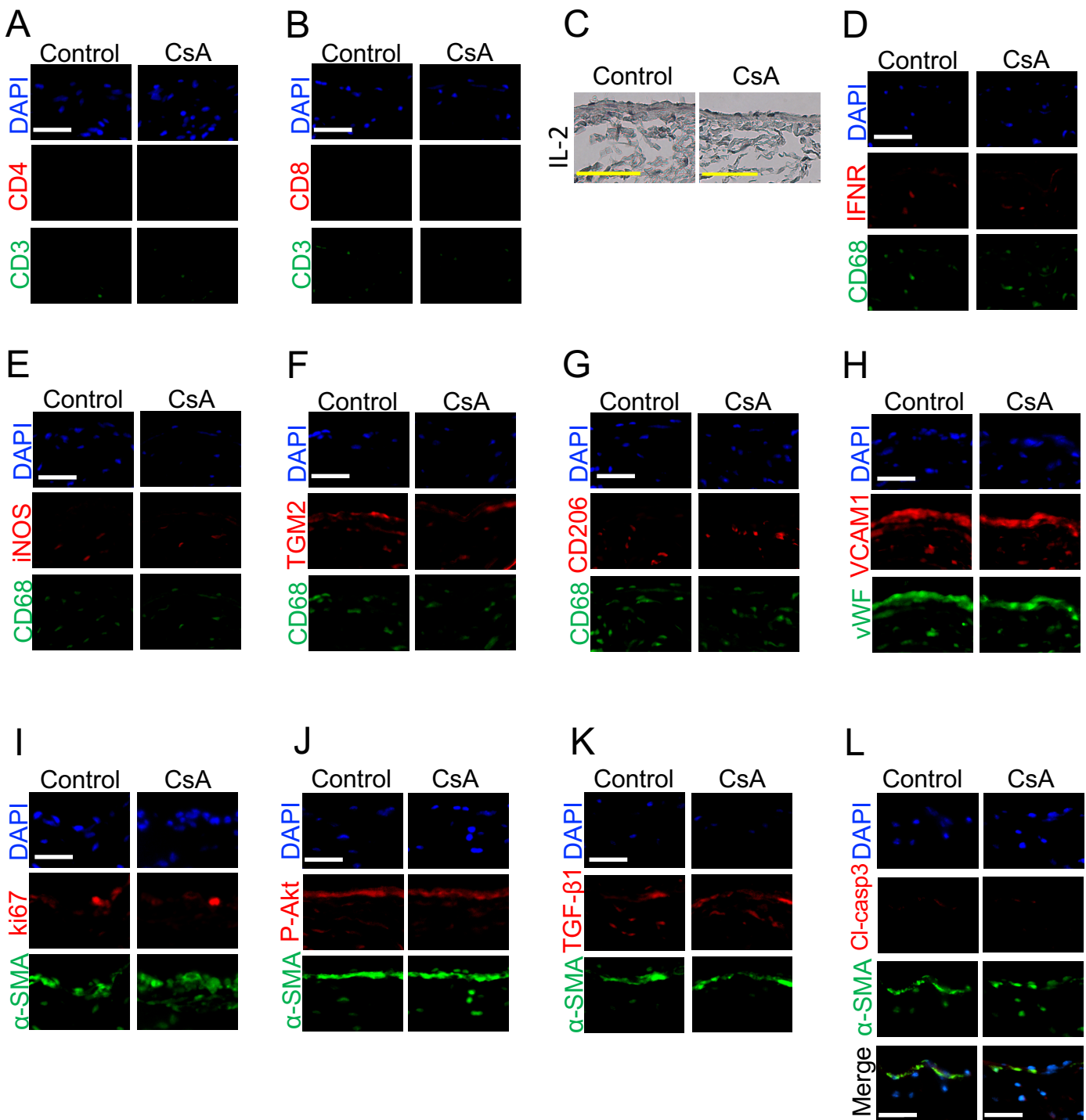




**Supplemental Figure IV. CsA reduces AVF wall thickening and SMC proliferation in wild type female mice.** (A) Representative photomicrographs of the wild type female mouse AVF wall treated with vehicle (control) or Cyclosporine (CsA), stained with Elastin van Gieson (EVG); day 21. Scale bar, 25 μm. Bar graph shows intima-media thickness of the AVF wall in mice treated with control or CsA. n=3-4. \*P=0.0571 (Mann-Whitney U test). (B) Representative photomicrographs showing α-SMA (green) and ki67 (red) (C) Representative photomicrographs showing α-SMA (green) and cleaved caspase (Cl-casp) 3 (red) (D) Representative photomicrographs showing positive control of Cl-casp3 (red) (E) Representative photomicrographs showing α-SMA (green) and P-Akt (red). Wild type mouse AVF wall at day 7 treated with vehicle (control) or cyclosporine (CsA). Scale bar, 50 μm.



**Supplemental Figure V. CsA reduces TGF- $\beta$  expression and collagen density to promote AVF outward remodeling.** (A) Representative photomicrographs showing individual channels of Figure 5G.  $\alpha$ -SMA (green) and TGF- $\beta$ 1 (red). Wild type mouse AVF wall at day 7 treated with vehicle (control) or cyclosporine (CsA). Scale bar, 50  $\mu$ m. (B) Representative photomicrographs showing immunohistochemistry of MMP-9 in the AVF of wild type male mice treated with control or CsA. Scale bar, 50  $\mu$ m. (C) Representative ultrasound images showing IVC (V) and Aorta (A) treated with control or CsA. (D) Line graph shows wall shear stress in AVF of wild type male mice treated with control or CsA.  $P = 0.1541$  (ANOVA).  $n = 5-20$ . (E) Line graph shows relative AVF diameter of wild type female mice treated with control or CsA, normalized to day 0.  $n = 3-10$ .  $*P = 0.0172$  (ANOVA). (F) Line graph shows blood flow of wild type female mouse AVF treated with control or CsA.  $n = 3-10$ .  $*P = 0.0412$  (ANOVA). (G) Line graph shows wall shear stress in AVF of wild type female mice treated with control or CsA.  $P = 0.1541$  (ANOVA).  $n = 5-20$ .



**Supplemental Figure VI. Individual channels of immunofluorescence and immunohistochemistry of IL-2 in AVF in nude mice.** (A) Representative photomicrographs showing CD3 (green) and CD4 (red) (B) Representative photomicrographs showing CD3 (green) and CD8 (red) (C) Representative photomicrographs showing immunohistochemistry of IL-2 in nude mouse AVF at day 7. Scale bar, 50  $\mu$ m. (D) Representative photomicrographs showing CD68 (green) and IFNR (red). (E) Representative photomicrographs showing CD68 (green) and iNOS (red). (F) Representative photomicrographs showing CD68 (green) and TGM2 (red). (G) Representative photomicrographs showing CD68 (green) and CD206 (red). (H) Representative photomicrographs showing vWF (green) and VCAM1 (red). (I) Representative photomicrographs showing  $\alpha$ -SMA (green) and ki67 (red). (J) Representative photomicrographs showing  $\alpha$ -SMA (green) and P-Akt (red). (K) Representative photomicrographs showing  $\alpha$ -SMA (green) and TGF- $\beta$ 1 (red). (L) Representative photomicrographs showing  $\alpha$ -SMA (green) and cleaved caspase (Cl-casp) 3 (red). Nude mouse AVF wall at day 7 treated with vehicle (control) or cyclosporine (CsA). Scale bar, 50  $\mu$ m.

## Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

### Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mice	The Jackson Laboratory	C57BL6/J	Male	000664
Mice	The Jackson Laboratory	C57BL6/J	Female	000664
Mice	The Jackson Laboratory	NU/J	Male	002019

### Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
vWF	Abcam	ab11713	5µg/ml	<a href="https://www.abcam.com/von-willebrand-factor-antibody-ab11713.html">https://www.abcam.com/von-willebrand-factor-antibody-ab11713.html</a>
IFN-γ	R&D systems	AF485	5µg/ml	<a href="https://www.rndsystems.com/products/mouse-ifn-gamma-antibody_af-485-na">https://www.rndsystems.com/products/mouse-ifn-gamma-antibody_af-485-na</a>
IL-4	R&D systems	AF404	5µg/ml	<a href="https://www.rndsystems.com/products/mouse-il-4-antibody_af-404-na">https://www.rndsystems.com/products/mouse-il-4-antibody_af-404-na</a>
IFNγ-R	Abcam	ab77246	10µg/ml	<a href="https://www.abcam.com/tnf-alpha-antibody-ab6671.html">https://www.abcam.com/tnf-alpha-antibody-ab6671.html</a>
iNOS	Abcam	ab15323	10µg/ml	<a href="https://www.abcam.com/inos-antibody-ab15323.html">https://www.abcam.com/inos-antibody-ab15323.html</a>
TGM2	Cell signaling technology	3557	10µg/ml	<a href="https://www.cellsignal.co.uk/products/primary-antibodies/tgm2-d11a6-xp-rabbit-mab/3557?N=4294967254+4294956287&amp;Nrpp=200&amp;No=600&amp;fromPage=plp">https://www.cellsignal.co.uk/products/primary-antibodies/tgm2-d11a6-xp-rabbit-mab/3557?N=4294967254+4294956287&amp;Nrpp=200&amp;No=600&amp;fromPage=plp</a>
CD206	Abcam	ab64693	5µg/ml	<a href="https://www.abcam.com/mannose-receptor-antibody-ab64693.html">https://www.abcam.com/mannose-receptor-antibody-ab64693.html</a>
CD3	R&D systems	MAB4841	25µg/ml (IF, IHC) 1µg/ml (WB)	<a href="https://www.rndsystems.com/products/mouse-cd3-antibody-17a2_mab4841">https://www.rndsystems.com/products/mouse-cd3-antibody-17a2_mab4841</a>
CD4	Abcam	ab183685	10µg/ml	<a href="https://www.abcam.com/cd4-antibody-epr19514-ab183685.html">https://www.abcam.com/cd4-antibody-epr19514-ab183685.html</a>
CD8	Abcam	ab203035	10µg/ml	<a href="https://www.abcam.com/cd8-antibody-ab203035.html">https://www.abcam.com/cd8-antibody-ab203035.html</a>
CD68	Bio-Rad	MCA1957	10µg/ml (IF, IHC) 1µg/ml (WB)	<a href="https://www.bio-rad-antibodies.com/monoclonal/mouse-cd68-antibody-fa-11-mca1957.html?f=purified">https://www.bio-rad-antibodies.com/monoclonal/mouse-cd68-antibody-fa-11-mca1957.html?f=purified</a>
α-SMA	Abcam	ab7817	5µg/ml	<a href="https://www.abcam.com/alpha-smooth-muscle-actin-antibody-1a4-ab7817.html">https://www.abcam.com/alpha-smooth-muscle-actin-antibody-1a4-ab7817.html</a>
ki67	Abcam	ab15580	5µg/ml	<a href="https://www.abcam.com/ki67-antibody-ab15580.html">https://www.abcam.com/ki67-antibody-ab15580.html</a>
IL-2	Abcam	ab180780	10 µg/ml	<a href="https://www.abcam.com/il-2-antibody-ab180780.html">https://www.abcam.com/il-2-antibody-ab180780.html</a>
TGF-β1	Abcam	ab92486	10 µg/ml	<a href="https://www.abcam.com/tgf-beta-1-antibody-ab92486.html">https://www.abcam.com/tgf-beta-1-antibody-ab92486.html</a>
MCP-1	Abcam	ab25124	10 µg/ml	<a href="https://www.abcam.com/mcp1-antibody-ab25124.html">https://www.abcam.com/mcp1-antibody-ab25124.html</a>
LIX-1	Abcam	ab235524	10 µg/ml	<a href="https://www.abcam.com/lix1-antibody-ab235524.html">https://www.abcam.com/lix1-antibody-ab235524.html</a>
collagen I	Abcam	ab34710	10 µg/ml	<a href="https://www.abcam.com/collagen-i-antibody-ab34710.html">https://www.abcam.com/collagen-i-antibody-ab34710.html</a>
collagen III	Abcam	ab7778	10 µg/ml	<a href="https://www.abcam.com/collagen-iii-antibody-ab7778.html">https://www.abcam.com/collagen-iii-antibody-ab7778.html</a>
Foxp 3	Abcam	ab212700	5µg/ml	<a href="https://www.abcam.com/foxp3-antibody-3g3-bsa-and-azide-free-ab212700.html">https://www.abcam.com/foxp3-antibody-3g3-bsa-and-azide-free-ab212700.html</a>
cleaved-caspase 3	Cell signaling technology	9664	0.2µg/ml	<a href="https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664?country=USA">https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664?country=USA</a>
CCR7	Novus Biologicals	NB100-712	10 µg/ml	<a href="https://www.novusbio.com/products/ccr7-antibody_nb100-712">https://www.novusbio.com/products/ccr7-antibody_nb100-712</a>
VCAM-1	Abcam	ab106777	10 µg/ml	<a href="https://www.abcam.com/vcam1-antibody-ab106777.html">https://www.abcam.com/vcam1-antibody-ab106777.html</a>

DOI [to be added]

Akt-1	Cell signaling technology	2967	0.5 µg/ml	<a href="https://www.cellsignal.com/products/primary-antibodies/akt1-2h10-mouse-mab/2967">https://www.cellsignal.com/products/primary-antibodies/akt1-2h10-mouse-mab/2967</a>
Phospho-Akt	Cell signaling technology	9018	10 µg/m (IF) 0.5 µg/ml (WB)	<a href="https://www.cellsignal.com/products/primary-antibodies/phospho-akt1-ser473-d7f10-xp-rabbit-mab-akt1-specific/9018">https://www.cellsignal.com/products/primary-antibodies/phospho-akt1-ser473-d7f10-xp-rabbit-mab-akt1-specific/9018</a>
GAPDH	Cell signaling technology	2118	0.01µg/ml	<a href="https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118?country=USA">https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118?country=USA</a>
Anti-sheep Alexa-Fluor-488	Invitrogen	A-11015	5µg/ml	<a href="https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11015">https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11015</a>
Rabbit IgG control	Santa-Cruz	sc-3888	Same as primary Antibodies	<a href="https://datasheets.scbt.com/sc-3888.pdf">https://datasheets.scbt.com/sc-3888.pdf</a>
Mouse IgG control	Santa-Cruz	sc-2025	Same as primary Antibodies	<a href="https://datasheets.scbt.com/sc-2025.pdf">https://datasheets.scbt.com/sc-2025.pdf</a>
Rat IgG control	BD Bioscience	559478	Same as primary Antibodies	<a href="https://www.bdbiosciences.com/us/applications/research/b-cell-research/immunoassays/elisa/pairs-and-standards/other-species/purified-rat-igg2b-isotype-control-a95-1/p/559478">https://www.bdbiosciences.com/us/applications/research/b-cell-research/immunoassays/elisa/pairs-and-standards/other-species/purified-rat-igg2b-isotype-control-a95-1/p/559478</a>
Goat IgG control	R&D systems	AB-108-C	Same as primary Antibodies	<a href="https://www.rndsystems.com/products/normal-goat-igg-control_ab-108-c">https://www.rndsystems.com/products/normal-goat-igg-control_ab-108-c</a>
Anti-goat Alexa-Fluor-568	Invitrogen	A-11057	5µg/ml	<a href="https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057">https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057</a>
Anti-rabbit Alexa-Fluor-568	Invitrogen	A10042	5µg/ml	<a href="https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10042">https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10042</a>
Anti-rat Alexa-Fluor-488	Invitrogen	A-21208	5µg/ml	<a href="https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208">https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208</a>
Anti-mouse Alexa-Fluor-488	Invitrogen	A-21202	5µg/ml	<a href="https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202">https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202</a>
HRP conjugated anti-rat IgG	Cell signaling technology	7077	0.5 µg/ml (WB)	<a href="https://www.cellsignal.com/products/secondary-antibodies/anti-rat-igg-hrp-linked-antibody/7077">https://www.cellsignal.com/products/secondary-antibodies/anti-rat-igg-hrp-linked-antibody/7077</a>
HRP conjugated anti-rat IgG	Abcam	ab97057	5 µg/ml (IF)	<a href="https://www.abcam.com/goat-rat-igg-hl-hrp-ab97057.html">https://www.abcam.com/goat-rat-igg-hl-hrp-ab97057.html</a>
HRP conjugated anti-rabbit IgG	Cell signaling technology	7074	0.5 µg/ml (WB)	<a href="https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074">https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074</a>
Dako EnVision+ Dual link	Dako	K4061	no dilution	<a href="https://www.agilent.com/en/product/immunohistochemistry/visualization-systems/envision-systems/envision-dual-link-single-reagents-(hrp-rabbit-mouse)-76787">https://www.agilent.com/en/product/immunohistochemistry/visualization-systems/envision-systems/envision-dual-link-single-reagents-(hrp-rabbit-mouse)-76787</a>

## Other

Description	Source / Repository	Persistent ID / URL
Western Lightning Plus ECL reagent	PerkinElmer	<a href="https://www.perkinelmer.com/product/western-lightning-plus-ecl-680ml-nel105001ea">https://www.perkinelmer.com/product/western-lightning-plus-ecl-680ml-nel105001ea</a>
RNeasy Mini Kit	Qiagen	<a href="https://www.qiagen.com/us/shop/pcr/rneasy-mini-kit/">https://www.qiagen.com/us/shop/pcr/rneasy-mini-kit/</a>

SuperScript III First-Strand Synthesis Supermix	Invitrogen	<a href="https://www.thermofisher.com/order/catalog/product/11752050#/11752050">https://www.thermofisher.com/order/catalog/product/11752050#/11752050</a>
iQ SYBR Green Supermix	Bio-Rad	<a href="https://www.bio-rad.com/en-us/sku/1708880-iq-sybr-green-supermix-100-x-50-ul-rxns-2-5-ml-2-x-1-25-ml?ID=1708880">https://www.bio-rad.com/en-us/sku/1708880-iq-sybr-green-supermix-100-x-50-ul-rxns-2-5-ml-2-x-1-25-ml?ID=1708880</a>

**Quantitative polymerase chain reaction primer sequences.**

Primers	Forward	Reverse
GAPDH	AATGTGTCCGTCGTGGATCTGA	AGTGTAGCCCAAGATGCCCTTC
CD3	TCAGAAATGAAGTAATGAGCTGGC	CGTCACTGTCTAGAGGGCAC
CD68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA