SUPPLEMENTAL MATERIAL

Supplemental Methods

Femoral artery endothelial denudation:

Femoral artery endothelial denudation was performed as previously described[28, 45-46] on littermate control and TET2-deficient male mice of at least 16 weeks of age. Briefly, the femoral artery was temporarily ligated proximally and distally to the deep muscular branch. The muscular branch was permanently ligated distal to the branch point. An arteriotomy was created between the ligature and the branch point, and a 0.015" guidewire (Cook Medical, C-SF-15-15) was inserted 1cm proximally. The wire was left in place for 60 seconds. Upon wire removal the muscular branch was permanently ligated proximal to the arteriotomy. Injured right femoral arteries and contralateral controls were harvested 21days after surgery.

In vitro cell culture:

Primary mouse aortic vascular smooth muscle cells (mVSMCs) were isolated by enzymatic dissociation as described[45] (See Supplemental Methods for additional detail). Briefly, aortas were incubated with 175U/ml collagenase II (Thermo, 17101015), 1.25U/ml elastase (Worthington, LS002274) in HBSS (Gibco, 14025092) for 10 minutes at room temperature to facilitate stripping the adventitia. Stripped vessels were then minced with a sterile blade and incubated with 400U/ml collagenase II, 2.5U/ml elastase, and 0.2mg/ml soybean trypsin inhibitor (Cayman, 14502) in HBSS for 1.5 hours in a 37°C water bath with periodic manual vortexing. The cell suspension was triturated, washed with complete medium, filtered through a 75um filter, and allowed to adhere for 3 days before replacing the medium. mVSMCs were propagated in DMEM (Gibco, 11965-092) supplemented with 20% FBS, 100U/ml each penicillin-streptomycin, and 1mM sodium pyruvate (Gibco, 11360070).

Primary human aortic vascular smooth muscle cells (hVSMCs, Lonza) were propagated in 199 medium (Thermo, 11150-067) supplemented with 10% FBS, 100U/ml each penicillin-streptomycin, 2.7ng/ml rhEGF (Biolegend, 713008), and 2ng/ml rhFGF (Biolegend, 71034). hVSMCs were used for experiments at passages 5-7. Cultured VSMCs were treated with recombinant IFNγ from the same species (recombinant human IFNγ (rh-IFNγ): R&D Systems, 285-IF-100, recombinant mouse IFNγ (rm-IFNγ): Biolegend, 714006). In TNFα treatment experiments, mVSMCs were treated with 15ng/ml recombinant murine TNFα (rm-TNFα; Biolegend, 718004).

Control (++; homozygous wildtype) or *Tet2-deficient* (FF; homozygous floxed) mouse VSMCs were prepared from female littermate mice that harbored one copy of the Rosa26-mTmG mutant allele. Recombination of the floxed region of the *Tet2* gene, and the Rosa locus, was accomplished by treating cultured cells with 200MOI of Cre adenovirus (see Supplemental Methods) + 10ug/ml polybrene for at least two days. Recombination efficiency was monitored microscopically by observing RFP to GFP transition. *TET2* knockdown was accomplished by transfecting hVSMCs with a pool of 4 *TET2*-targeting siRNAs (Dharmacon, L-013776-03-0005), versus a non-targeting siRNA pool (Dharmacon, D-001810-10-5), as per the protocol for Dharmafect 2 (Dharmacon, T-2002-02). In ascorbic acid (AA) *in vitro* experiments, cells were treated with 250uM ascorbic acid dissolved in DPBS and filter sterilized.

ELISA for genomic 5-hmC content (Epigentek, P-1032-96) from cultured cells was performed on 10-20ng/sample denatured genomic DNA. gDNA was isolated using Qiagen or Zymo Research Kits (69504 or D7001, respectively).

Adenovirus preparation:

Adenoviruses encoding GFP (Vector Biolabs, 1060), TET2b-IRES-GFP (Welgen custom, NM_017628.4), sh-TET2-IRES-GFP (Welgen custom, shRNA: CAGGTGAAAGCAGTCAACCAAATGTCTCC), and Cre adenovirus as described elsewhere[47], were amplified in HEK-293A cells (Invitrogen, R70507) as described in the Adeno-X Expression system manual (Clonetech, protocol #PT3414-1). Viral titer was determined by Adeno-X Rapid Titer Kit (Clontech, 632250). VSMCs were infected with 200MOI adenovirus in the presence of 10ug/ml polybrene.

Cell viability assay

Viability was determined by crystal violet staining. Cells were fixed with 4% paraformaldehyde (PFA) in PBS at the indicated time points. 24hours after plating 10-15k cells/well in 24-well cluster plates, cells were collected as a Time=0 control for plating efficiency. Cells were stained with 0.2% crystal violet solution in 90% ethanol, destained with water, dried, solubilized in 10% acetic acid, and the absorbance at 570nm was assessed by spectrophotometer. For each assay type, cells were quiesced in 0.5% FBS medium for 24hr ± AA prior to IFNγ or conditioned media (CM) stimulation.

Tissue staining

Coronary artery samples from age-matched control (patients who died from non-cardiovascular diseases) or patients who had received heart transplants (CAV; or kidney transplant (GA)) were preserved in neutral-buffered formalin. Tissues were paraffin embedded and microtome-sectioned at 5µm. Sections were de-paraffinized with Histoclear (National Diagnostics, HS-200) and re-hydrated to PBS prior to immunostaining. Samples were obtained from the Yale Pathology Tissue Service.

Murine graft tissues were prepared by perfusion of the recipient vasculature with PBS containing 40µg/ml sodium nitroprusside, and then with 4% paraformaldehyde (PFA)-PBS. Tissues were excised and further fixed in 4% PFA-PBS at 4°C for 24hours. Tissues were cryopreserved in 30% sucrose-PBS for at least 48hours, then in 50% cryosectioning medium (TissueTek, 4583) plus 15% sucrose-PBS for at least 24hours.

Elastin Van Geison (EVG) staining was performed on 5µm cryosections as described elsewhere[48]. Briefly, sections were stained with Verhoeff's Hematoxylin for 30min, destained with 2% ferric chloride solution, counterstained with Van Gieson's Solution (0.05% acid fuchsin in picric acid) for 65sec, washed with ethanol, cleared with Histoclear, and mounted with DPX mountant (Electron Microscopy Sciences, 13510).

Immunohistochemical staining was performed after heat-induced epitope retrieval in pH6.0 10mM sodium citrate buffer containing 0.05% tween-20 at 95°C for 20-40min. Endogenous peroxidases were quenched with 1-3% H_20_2 prior to blocking in 4% normal goat serum-PBS. Sections were incubated with primary antibodies (see Sup. Table 1) for 30min at room temperature. Sections were incubated with biotinylated secondary antibody, and streptavidin conjugated to horseradish peroxidase (HRP) (Vector Laboratories, PK-4001). 3,3'-diaminobenzidine (DAB) was used for chromogenic staining (Vector Labs, SK-4100) and fluorescent tyramides were used for fluorescent staining (Akoya Biosciences, NEL701A001KT/ NEL704A001KT/ NEL705A001KT). Serial staining with multiple rabbit-derived primary antibodies was accomplished by stripping first-round primary antibodies in pH2 glycine-SDS buffer at 50°C[49]. In-direct immunofluorescent staining was performed by incubating sections with primary antibodies at room temperature for 2hr or overnight at 4°C. Fluorescent moiety-conjugated secondary antibodies (see Sup. Table 1) were incubated with sections for 2hr at room temperature. 2ug/ml 4',6-diamidino-2-phenylindole (DAPI) staining was used to visualize nuclei. Slides were mounted with 1x PBS in 90% glycerol containing 0.2% *n*-propyl-gallate as an anti-fade agent. Micrographs of stained tissues were taken on a Nikon 80i epifluorescence microscope, a Nikon spinning disk confocal microscope, or a Leica SP8 laser scanning confocal microscope. Quantification of micrographs was performed in ImageJ. Image quantitation was automated, when possible, and data was performed singly blinded.

Western blotting:

Whole cell lysates were prepared using RIPA lysis buffer (Cell Signaling Technologies, 9806) containing 1x protease inhibitor cocktail (Roche, 4693124001) and phosphatase inhibitor cocktail (Roche, 4906845001). Lysates were cleared by centrifugation at ≥14k*g. SDS-PAGE was performed by standard methods; equal amounts of protein were loaded in each lane. Bicinchronicic acid assay (Pierce, 23225) was used to quantitate sample protein content. Electrophoresed proteins were transferred to nitrocellulose membranes and blocked with 5% bovine serum albumin or 5% non-fat dry milk in tris-buffered saline. Membranes were probed with primary antibodies, and subsequently with horseradish peroxidase-conjugated secondary antibodies. SuperSignal West chemiluminescent substrate (Thermo, 37069 or 34094) was used as the HRP substrate for detection.

Condition medium was concentrated prior to western blotting for extracellular cytochrome C release by trichloroacetic acid (TCA)/deoxycholic acid (DOC) precipitation[49]. Briefly, one volume 50% TCA and one volume 0.1% DOC were added to 6 volumes of conditioned medium. Samples were incubated at 4°C overnight, pelleted at 14,000*g for 10min at 4°C, and solubilized in 1x Laemmli buffer containing 12% of 2M tris base and 25% β-mercaptoethanol at 95°C for 5min prior to SDS-page and western blotting.

Apoptosis antibody array:

Murine VSMC lysates were prepared from ++ or FF mice and treated with Cre virus (see above section on *in vitro* cell culture). Cell lysates were collected 5days after IFNγ stimulation as described in the R&D Systems Proteome Profiler manual. 100μg of protein was assayed for each condition by mouse-specific apoptosis antibody array (R&D Systems, ARY031). Immobilon Forte (Millipore, WBLUF0100) was used as the HRP substrate for detection. Densitometry of spots was performed in ImageJ.

Quantitative Real Time PCR (qPCR):

Total RNAs were isolated from tissues or cultured cells with one of the following kits: RNeasy (Qiagen,74106) with Qiashredders (79656) for homogenization, MiRNeasy (Qiagen, 217004), or Quick-DNA/RNA (Zymo Research, D7001). Reverse transcription of mRNAs was achieved with All-In-One RT

MasterMix (Applied Biological Materials, G490). qPCR was performed with BrightGreen qPCR MasterMix (Applied Biological Materials, MasterMix-S). qPCR was performed with 300nM primers (see Sup. Table 2) following the manufacturer's thermocycler protocol.

Hydroxymethylated DNA Immunoprecipitation (hMe-DIP) qPCR:

Genomic DNA was isolated from cultured control (++) and TET2-deficient (FF) mVSMCs 48hours after treatment with 50nM rapamycin, or vehicle control. hMe-DIP was performed as described previously[23]. Briefly, DNA decorated with 5-hmC was immunoprecipitated with the hydroxymethylated DNA IP kit (Diagenode, C02010031) as per the manufacturer's instructions. Precipitated genomic DNA was quantified by qPCR using SsoFast Supermix (BioRad, 1725200) and primers specific to the proximal promoters of *Cnn1*, and *Myh11* (see Supplemental Table 2 for primer sequences.)

RNA sequencing and data analysis:

Total RNAs were isolated from cultured control (++) and TET2-deficient (FF) mTmG-labeled mVSMCs 5days after treatment with 100ng/ml rm-IFNy. RNA sequencing (RNA-seq) was performed at the Yale Center for Genome Analysis. RNA-seq reads were aligned to the *mus musculus* genome build mm10 (Refseq transcripts version 94) using the STAR aligner. Differential expression quantification was performed in Partek Flow by quantifying to a pre-built annotation model (mm10 v94). mRNA reads counts were normalized by fragments per kilobase of transcript per million mapped reads (FPKM) and expressed as FPKM plus 1, Log base 2. Gene set analysis (GSA) and principal component analysis (PCA) were performed on normalized read counts in Partek Flow. Histograms, PCA plot, and differential gene expression filtering were performed in Partek Flow. A gene list was generated based on genes that were upregulated (fold change >2, $P<0.01$, FDR<0.1) in FF cells, but not similarly upregulated in ++ cells. This gene list was used to perform downstream Ingenuity Pathway Analysis (IPA).

Supplemental Tables

Primary Antibodies			
Target	Supplier, Catalogue #	Target	Supplier, Catalogue #
5-hmC	Active Motif, 39769	MKI67	Thermo Scientific, MA5-14520
ACTA2	Sigma, A2547	MYH11	Biomedical Technologies, BT-562
ACTA2-660	eBiosciences, 50-9760-82	PDGFRA	CST, 3174
ACTA2-cy3	Sigma, C6198	PDGFRB	CST, 3169
ACTB	Sigma, A2228	phospho-STAT1	CST, 7649
CASP3	CST, 9662	STAT1	CST, 14994
cleaved CASP3	CST, 9661	TAGLN	Abcam, ab10135
CD3	Abcam, ab16669	Mouse-specific TET2	Abcam, ab124297
CNN1	Thermo Scientific, RM-2102-S0	TET1	Genetex, GTX123207
CYCS	Abcam, ab90529	TET2	Abcam, ab94580
GFP	Life Technologies, A11122	TET3	Abcam, ab139805
GFP	Abcam, ab13970		
* CST = Cell Signaling Technologies			
Secondary Antibodies			
Target	Supplier, Catalogue #	Target	Supplier, Catalogue #
α-mouse-HRP	Thermo Scientific, 31450	α-rabbit-alexa546	Invitrogen, A11010
α-rabbit-HRP	Thermo Scientific, 31460	α-rabbit-alexa488	Invitrogen, A11008
α-goat-HRP	Thermo Scientific, 31402	α-chicken-alexa488	Jackson Immuno., 103-545-155
		α-rabbit-HRP IHC kit	Vector Labs, PK-4001

Supplemental Table I: Primary and secondary antibodies used in this study.

Human mRNAs		
Target	Forward	Reverse
RNA18SN5	TAACGAACGAGACTCTGGCAT	CGGACATCTAAGGGCATCACAG
TET2	TGGCAAACATTCAGCAGCAC	TTGCCCTCAACATGGTTGGT
MYOCD	CCACAGCACATCAGTTTGCC	ACTTTGGCCCCACCTTATCA
SRF	TCACCTACCAGGTGTCGGAGTC	GTGCTGTTTGGATGGTGGAGGT
MYH11	CCTTGAGGAGAGGATTAGTGA	TTCCTTCTTTAGCCGCACTTC
KLF4	CATCTCAAGGCACACCTGCGAA	TCGGTCGCATTTTTGGCACTGG
PDGFRA	AACATCGGAGGAGAAGTTTCCCA	GCTCACTTCACTCTCCCCAA
PDGFRB	CAAGGACACCATGCGGCTTC	AGCAGGTCAGAACGAAGGTG
CNN1	ATGTCCTCTGCTCACTTC	ATACTTCTGGGCCAGCTTGTT
ACTA2	CTATGCCTCTGGACGCACAACT	CAGATCCAGACGCATGATGGCA
Mouse mRNAs		
Target	Forward	Reverse
Hprt	TGGATACAGGCCAGACTTTGTT	CAGATTCAACTTGCGCTCATC
Tet2	GGCTGCCCTGTAGGATTTGT	AATGAATCCAGCAGCACCGT
Myocd	GCTGGGCTCAACCCTTGTCCC	CGTTGGCCCCACCTTGTCAGA
Srf	GCCGCGTGAAGATCAAGATG	GTCAGCGTGGACAGCTCATA
Myh11	GCAACTACAGGCTGAGAGGAAG	TCAGCCGTGACCTTCTCTAGCT
Klf4	CTATGCAGGCTGTGGCAAAACC	TTGCGGTAGTGCCTGGTCAGTT
hMe-DIP		
Target	Forward	Reverse
Cnn1	AAGGGGTGGGCTGTATGAGA	CACCCCACACATACTCTGGC
Myh11	GCTGGGATGGTCCAAATCTC	CCTCCCCTTTCCACTCTCAG

Supplemental Table II: Oligonucleotide primers used in this study.

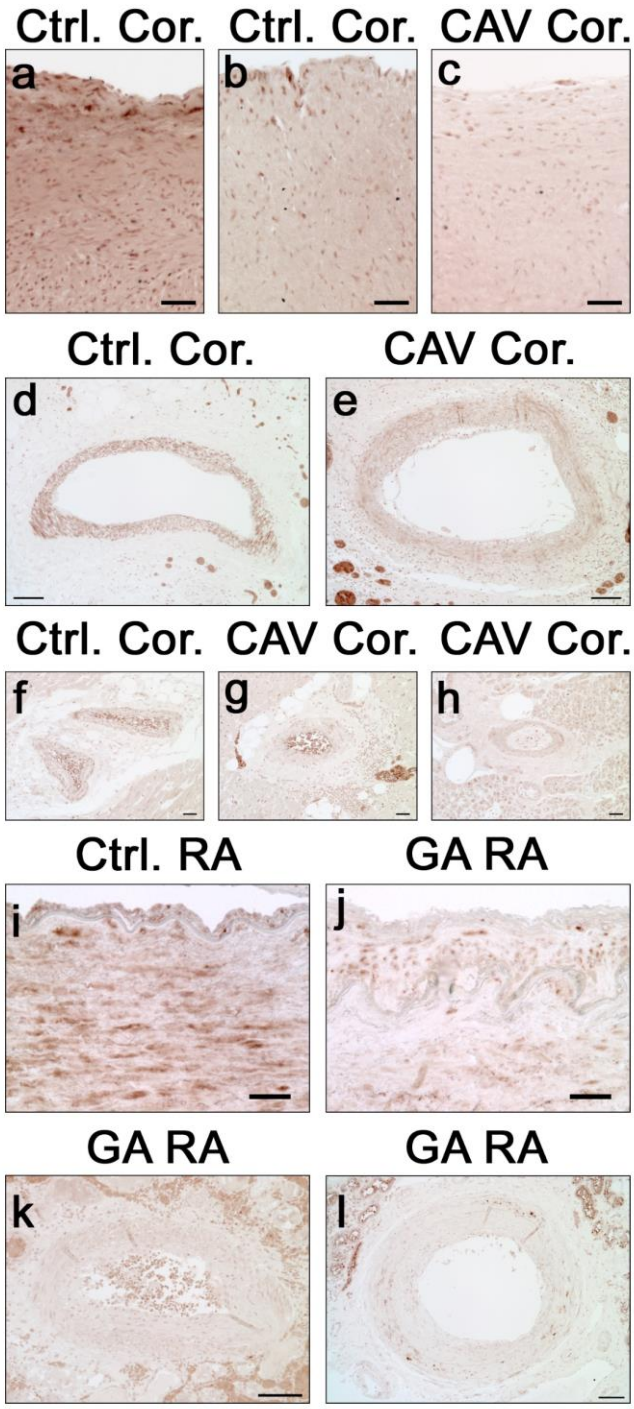
Gene symbol	Transcript ID	Fold change (1+ Log2)	P-value	FDR step up
Ifi27l2a	NM_029803	4.84	2.78E-04	7.86E-03
Tnfaip3	NM_009397	3.25	1.04E-05	5.89E-04
Apod	NM_001301353	2.79	1.57E-04	5.06E-03
Cxcl1	NM_008176	2.67	2.05E-03	3.17E-02
Epha3	NM_010140	2.60	4.13E-05	1.79E-03
Luc7l	NM_025881	2.56	5.23E-03	5.94E-02
Ifi206	NM_001372436	2.54	5.86E-05	2.34E-03
Trim25	NM_009546	2.50	8.81E-06	5.05E-04
Foxf1	NM_010426	2.45	1.08E-05	6.06E-04
Gdf15	NM_011819	2.39	2.33E-03	3.44E-02
Il15	NM_008357	2.39	7.63E-05	2.89E-03
Fosl1	NM_010235	2.38	3.08E-04	8.46E-03
4933412E12Rik	NR_038025	2.35	3.05E-04	8.41E-03
Slc25a25	NM_001290558	2.34	9.87E-03	9.00E-02
Ptgs2	NM_011198	2.34	5.83E-05	2.33E-03
Zc3hav1	NM_001347122	2.33	4.55E-06	2.93E-04
Pml	NM_178087	2.32	4.38E-06	2.83E-04
Hmga2	NM_010441	2.32	2.04E-06	1.49E-04
H2-DMb2	NM_010388	2.32	9.95E-05	3.57E-03
Serpina3f	NM_001168295	2.29	2.00E-06	1.47E-04
Parp3	NM_001311150	2.29	5.57E-05	2.24E-03
Nos2	NM_010927	2.28	2.00E-06	1.47E-04
Prrg4	NM_178695	2.27	6.01E-04	1.38E-02
Tgm2	NM_009373	2.23	1.50E-05	7.90E-04
Cavin2	NM_138741	2.23	1.97E-04	6.02E-03
Tcirg1	NM_016921	2.23	6.14E-06	3.75E-04
Itpr3	NM_080553	2.22	2.51E-07	2.65E-05
Slc2a6	NM_172659	2.20	1.89E-05	9.38E-04
P2ry10b	NM_001122596	2.20	9.59E-04	1.90E-02
9930111J21Rik1	NM_001114679	2.19	1.07E-06	8.90E-05
Tmcc3	NM_172051	2.17	3.10E-04	8.49E-03
Lgals3	NM_001145953	2.15	8.53E-04	1.76E-02
Emx2	NM_010132	2.13	2.46E-05	1.18E-03
Jun	NM_010591	2.13	7.11E-06	4.19E-04
Rnf14	NM_001361265	2.13	2.67E-03	3.78E-02
Wipf1	NM_001289722	2.12	2.27E-03	3.39E-02
Csrnp1	NM_001357561	2.12	3.51E-03	4.52E-02
Daxx	NM_007829	2.11	6.15E-06	3.75E-04
Slco3a1	NM_001038643	2.09	3.72E-05	1.64E-03
Acot2	NM_134188	2.09	1.84E-05	9.15E-04
Gdf15	NM_001330687	2.08	4.28E-05	1.83E-03
Usf1	NM_001305678	2.08	1.61E-04	5.15E-03
Pnp2	NM_001123371	2.07	3.07E-05	1.40E-03
Naip2	NM_001126182	2.06	3.33E-05	1.50E-03
Ido1	NM_001293690	2.06	9.04E-07	7.77E-05
Csf1	NM_001113529	2.05	1.53E-04	4.97E-03
Nfkbia	NM_010907	2.05	5.89E-05	2.34E-03
Tut7	NM_001373964	2.04	2.81E-05	1.31E-03
Lif	NM_008501	2.04	2.91E-03	4.00E-02
Prr3	NM_001282013	2.04	6.33E-05	2.49E-03
Slco3a1	NM_023908	2.04	1.66E-06	1.27E-04
G3bp2	NM_011816	2.04	2.64E-05	1.24E-03
Sp100	NM_013673	2.03	1.43E-04	4.71E-03
Trim26	NM_001286727	2.03	3.85E-05	1.69E-03
Isoc1	NM_025478	2.02	1.86E-05	9.25E-04
Zfp429	NM_001080941	2.01	6.61E-05	2.58E-03
Gna13	NM_010303	2.01	9.02E-08	1.17E-05
Cyren	NM_001135611	2.01	4.04E-05	1.75E-03
Ntn1	NM_008744	2.00	1.42E-06	1.13E-04
E330013P04Rik	NR_026942	2.00	1.56E-06	1.21E-04

IPA Pathway Genes
TNFR2 Signaling
Jun
Naip
Nfkbia
Tnfaip3
MIF Regulation of Innate Immunity
Jun
Nfkbia
Nos2
Ptgs2
TNFR1 Signaling
Jun
Naip
Nfkbia
Tnfaip3
CD40 Signaling
Jun
Nfkbia
Ptgs2
Tnfaip3
Role of NFAT in Regulation of the Immune Response
Gna13
H2-DMb2
Itpr3
Jun
Nfkpia
Death Receptor Signaling
Daxx
Naip
Nfkbia
Parp3
CD28 Signaling in T Helper Cells
H2-DMb2
Itpr3
Jun
Nfkbia
Induction of Apoptosis by HIV1
Daxx
Naip
Nfkbia

Supplemental Table III. Gene Lists from RNA Sequencing. Left) 60 genes found to be induced in iKO mVSMCs by IFNy that were not similarly induced by IFNy in control mVSMCs. Parameters shown include Gene symbols, Ensembl transcript IDs, fold change (+1 log 2), P value, false discovery rate (FDR) step up. Right) Regulated genes from leftward list contributing to significantly regulated pathways in Ingenuity Pathway Analysis (IPA).

Supplemental Figures and Figure Legends:

SF-I-A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Ctrl. Cor.
CAV Cor.
a
b
c
Ctrl. Cor.
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d
e
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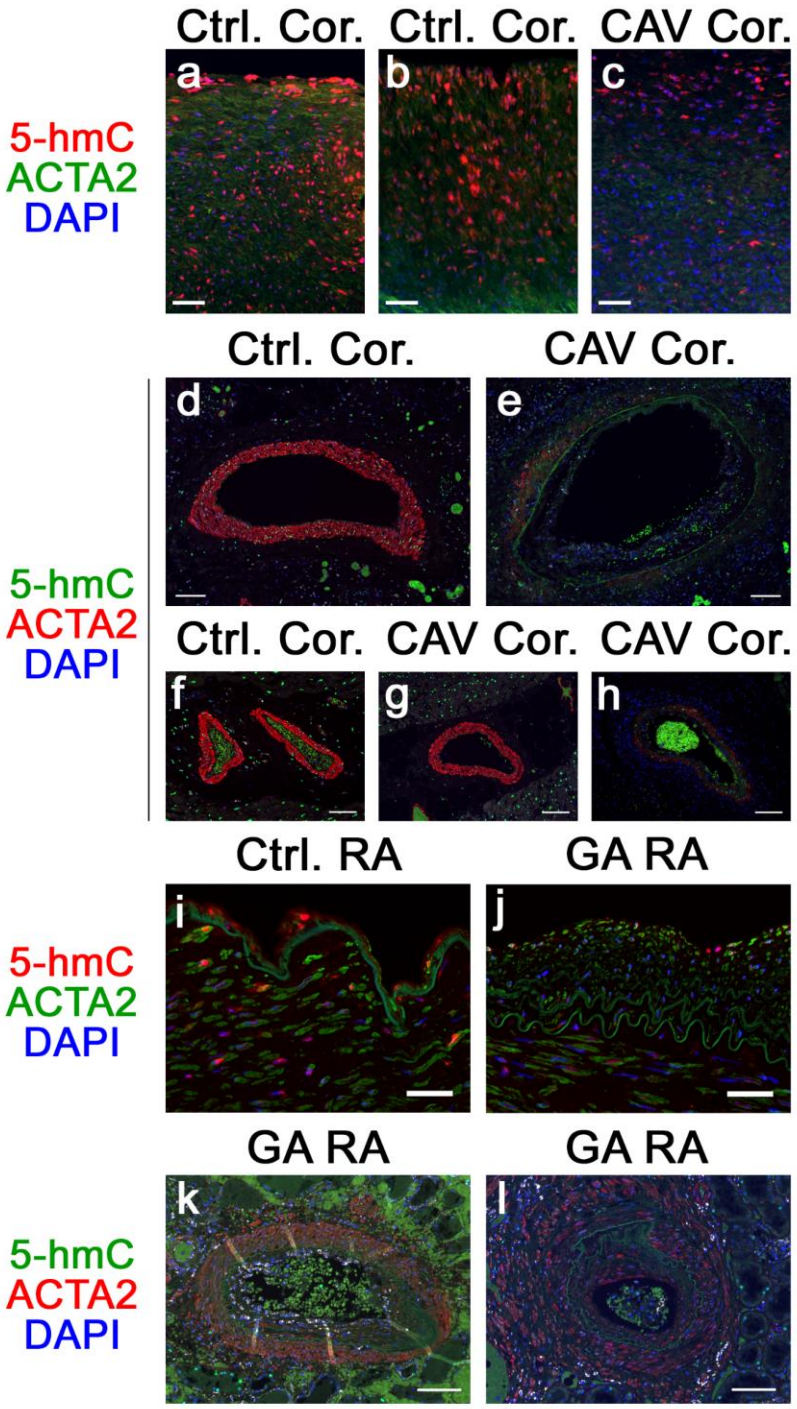
i
j
GA RA
GA RA
k
l

SF-I-B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CAV Cor.
5-hmC
ACTA2
DAPI
a
b
c
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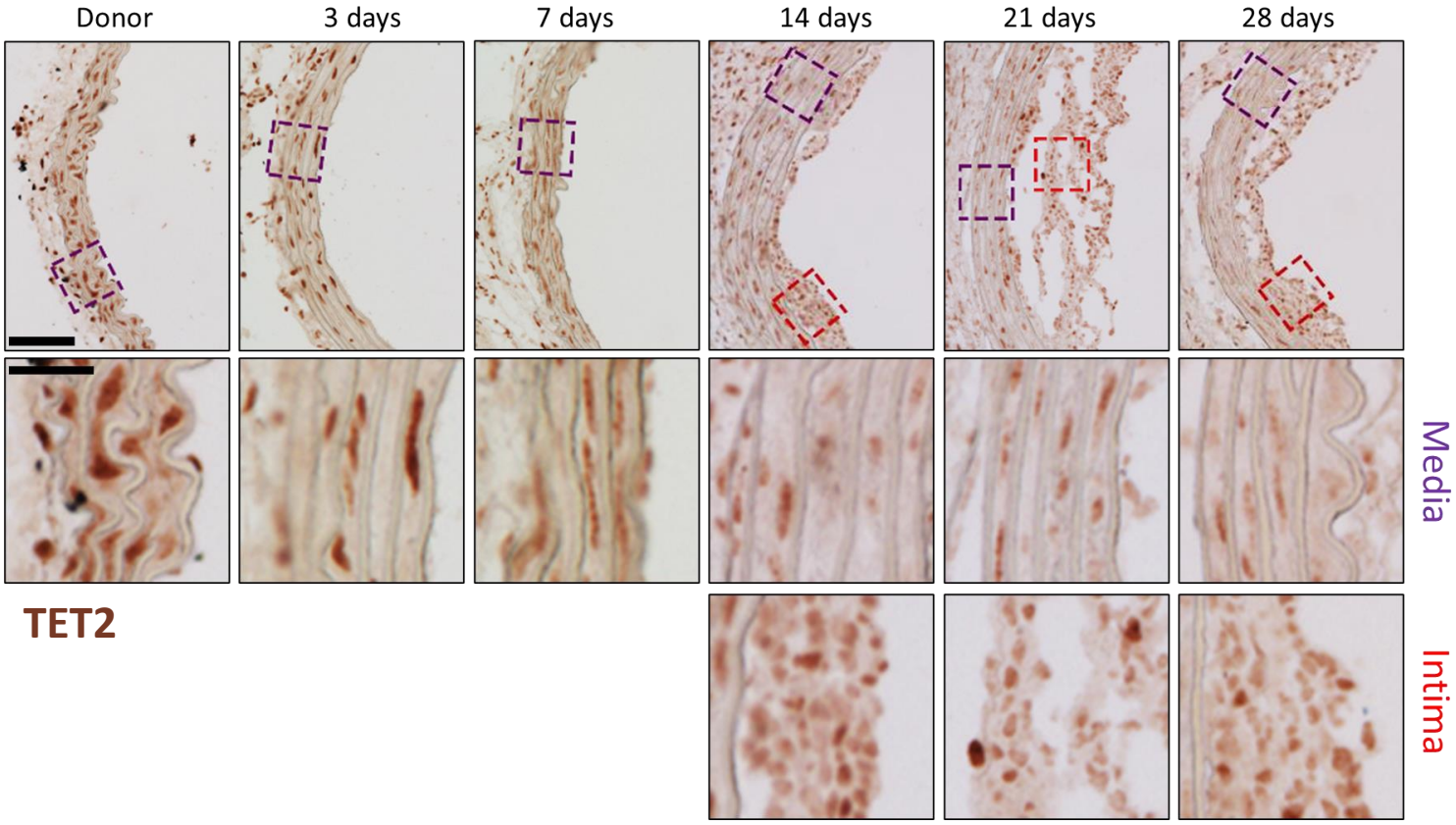
ACTA2
DAPI
d
e
Ctrl. Cor.
CAV Cor.
CAV Cor.
f
g
h
Ctrl. RA
GA RA
5-hmC
ACTA2
DAPI
i
j
GA RA
GA RA
5-hmC
ACTA2
DAPI
k
l

Supplemental Figure I: Analysis of TET2 and 5-hmC in human control and transplant artery tissues. Formalin-fixed artery tissue was obtained from autopsy specimens from similarly aged patients who had received heart transplants (coronary arteries with cardiac allograft vasculopathy (CAV Cor.), N=7), kidney transplants (graft arteriopathy renal arteries (GA RA) N=3), or who died from non-cardiovascular diseases and had mild atherosclerotic intimal thickening (control coronaries (Ctrl. Cor.) N=10, normal renal artery (Ctrl. RA) N=1)). 5µm serial sections were immunostained for SF-I-A) TET2, or S1B) 5-hmC and ACTA2 (panels a-c, i-j: 5-hmC in red and ACTA2 in green; panels d-h, k-l: 5-hmC in green and ACTA2 in red, DAPI nuclear stain in blue). SF-I-A, B) a,b,d,f: control coronaries; c,e,g,h: CAV coronaries; i: normal renal artery; j-l: GA renal arteries. Representative images shown. Scale Bars: a-c, f-l: 50µm, d-e: 100µm.

SF-II-A


Donor
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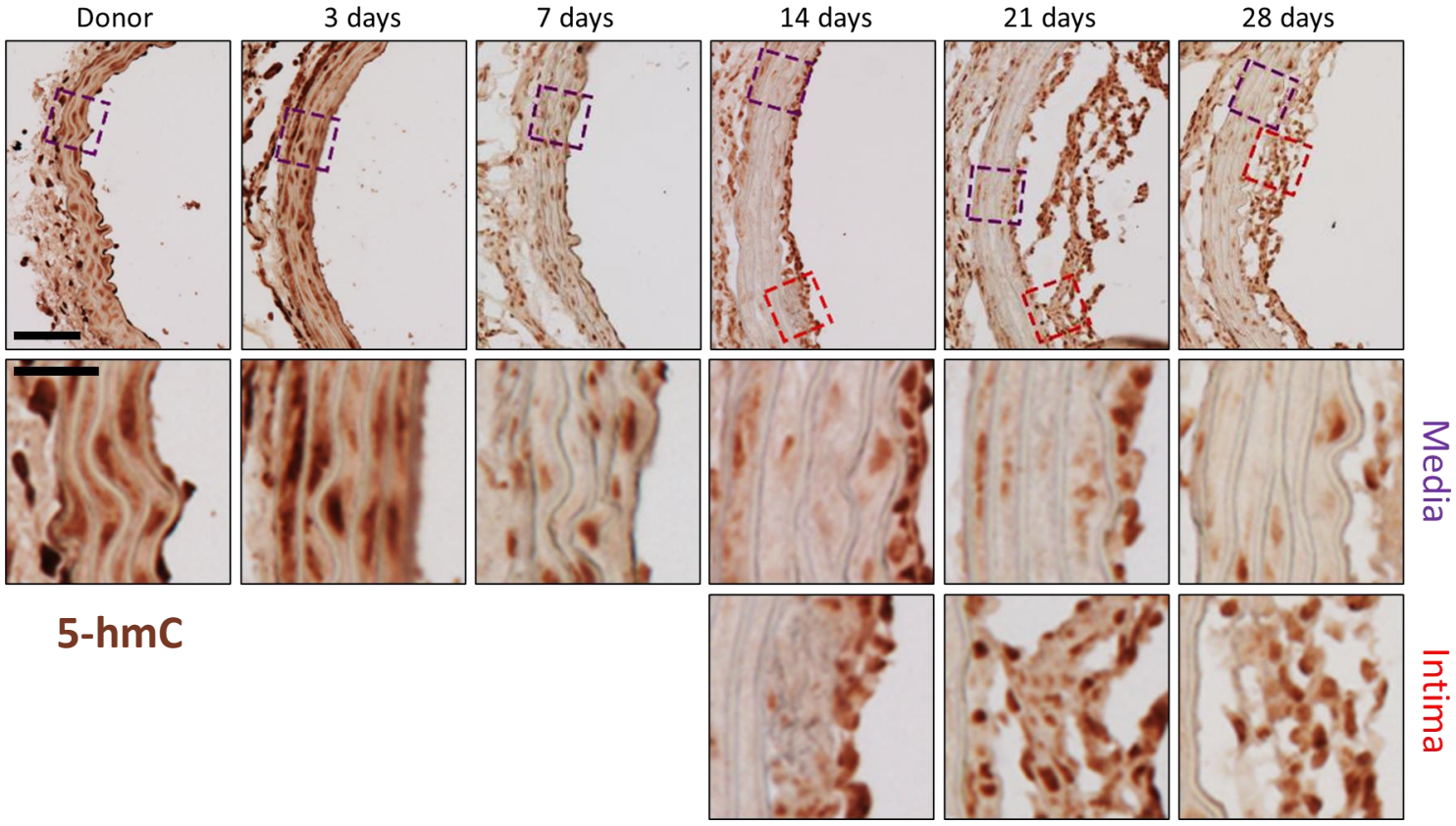
7 days
14 days
21 days
28 days
Media
Intima
TET2

SF-II-B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7 days
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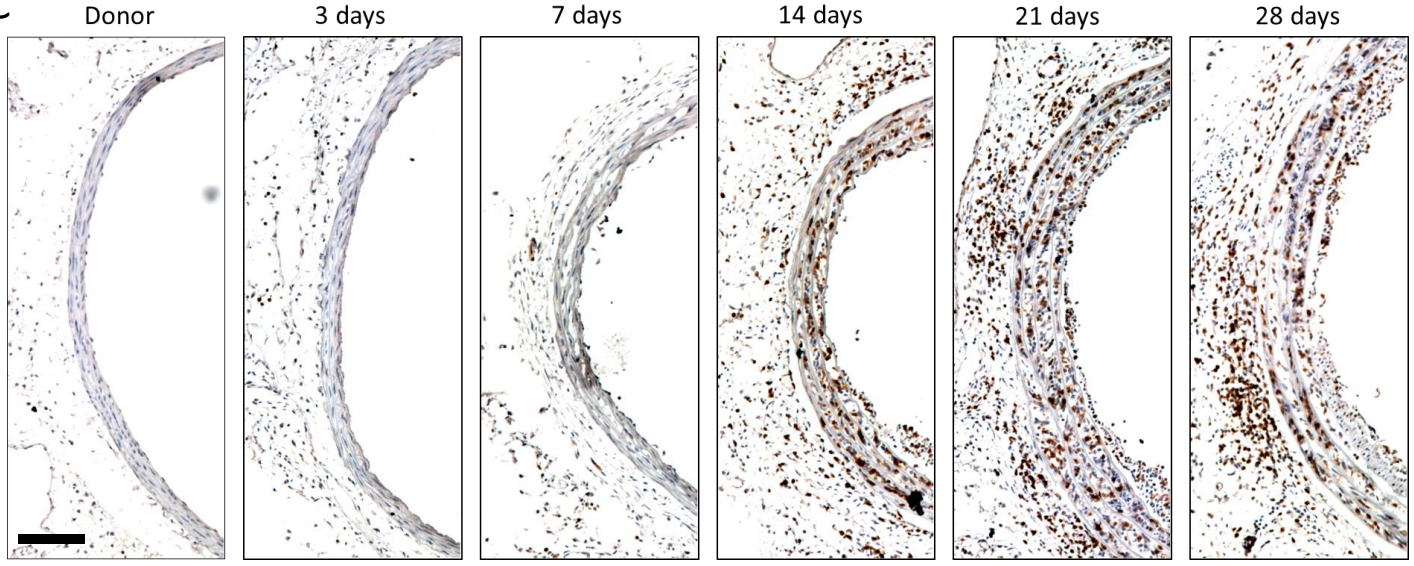
28 days
Media
Intima
5-hmC

SF-II-C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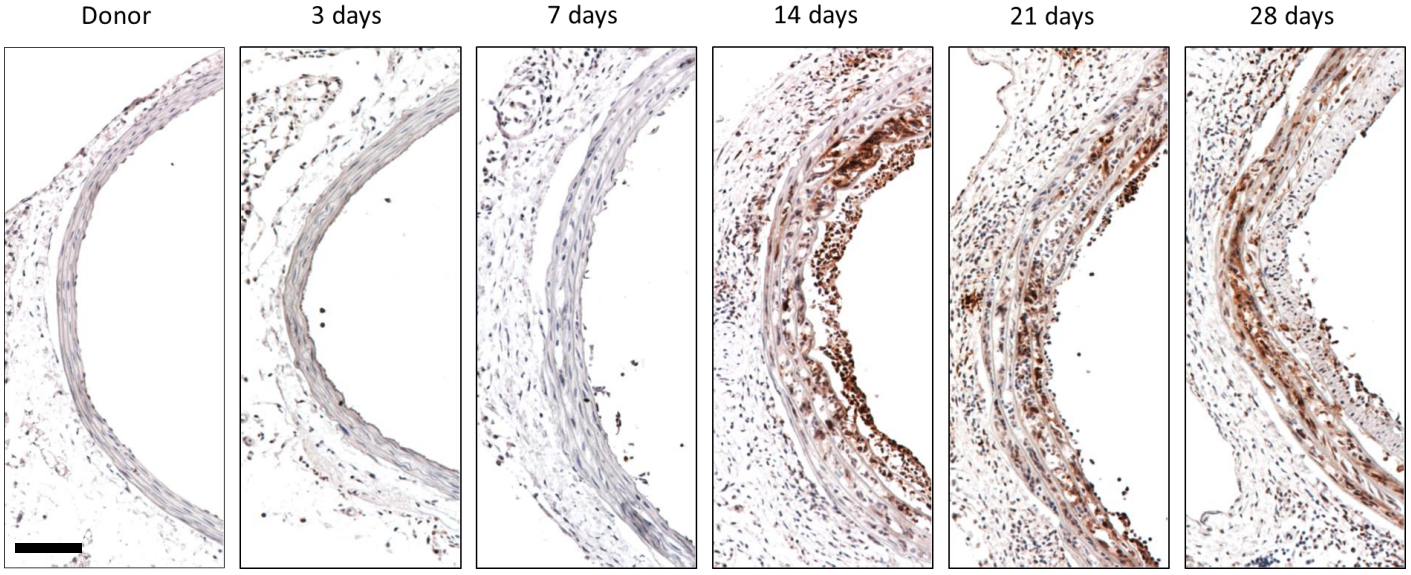
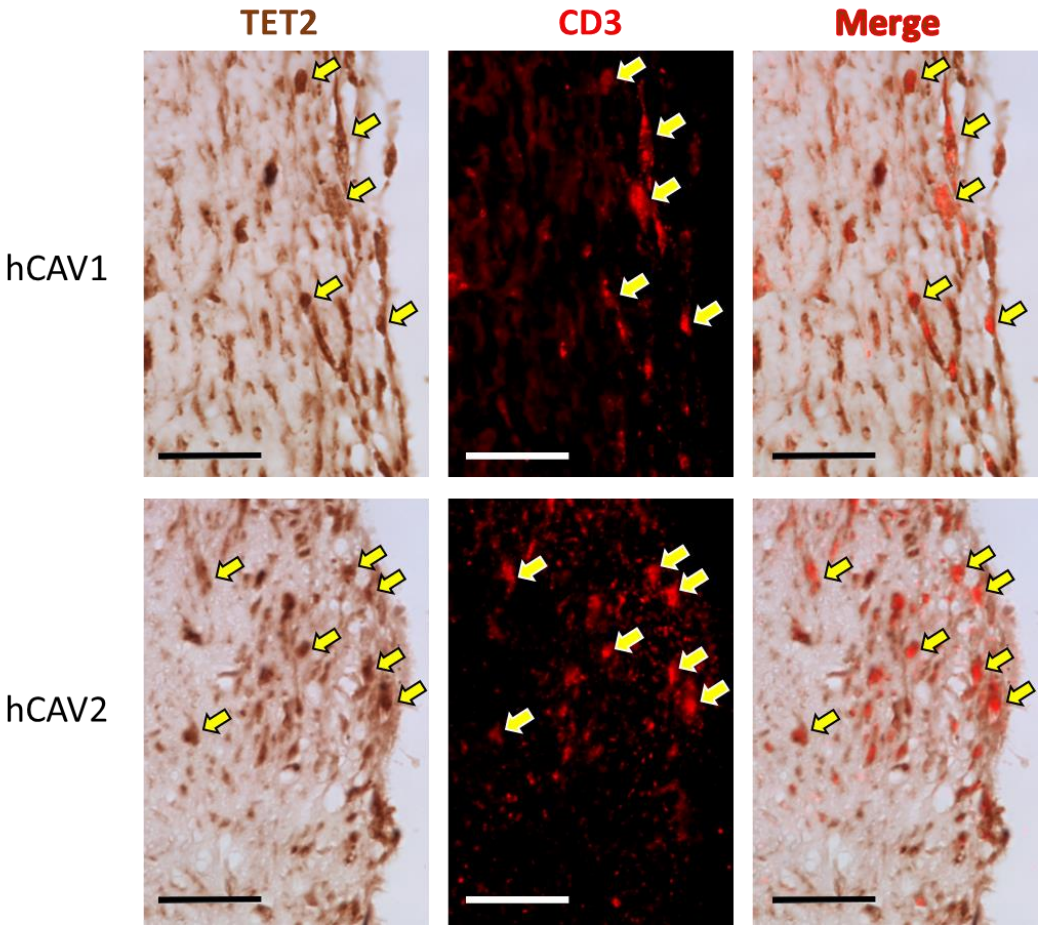
7 days
14 days
21 days
28 days
CD3

SF-II-D


Donor
3 days
7 days
14 days
21 days
28 days
pSTAT1

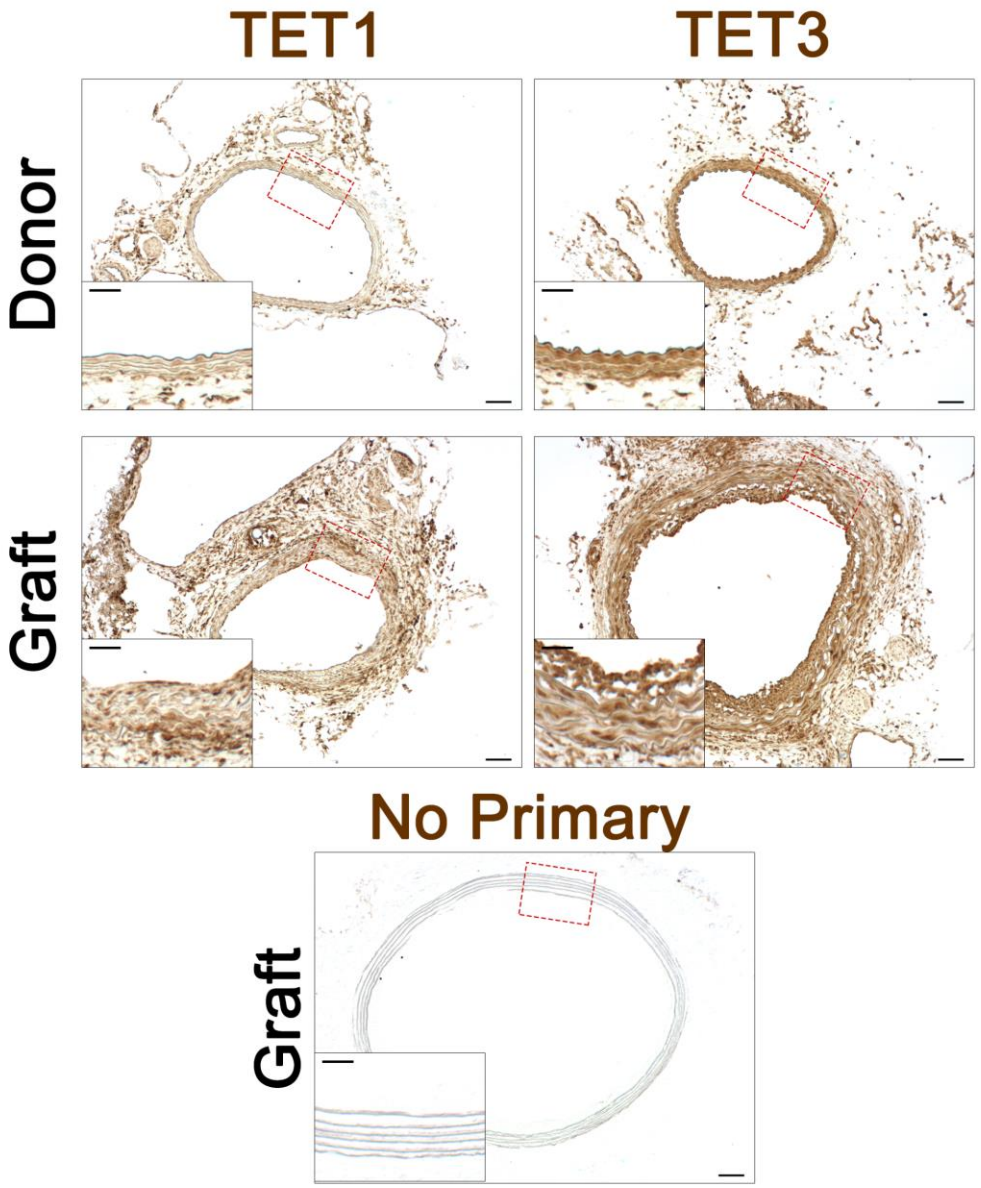
Supplemental Figure II: Time course analysis of TET2 and 5-hmC, T cell infiltration, and IFNy signaling in sex-mismatched aorta grafts by immunohistochemistry. Tissues were harvested from mice that had received aorta transplants at 3, 7, 14, 21, and 28 days after surgery. Graft sections were immunostained for SF-II-A) TET2, SF-II-B) 5-hmC, S2C) CD3, and S2D) pSTAT1. Purple dashed boxes indicate regions of higher magnification from the medial layer. Red dashed boxes indicate regions of higher magnification from the (neo)intimal layer. Insets shown below the low magnification images in SF-II-A-B. Representative images are shown of 4 individual experiments. Scale bars: SF-II-A, B) upper panels 100µm, insets 25µm; S2C, D) 100µm.

SF-III

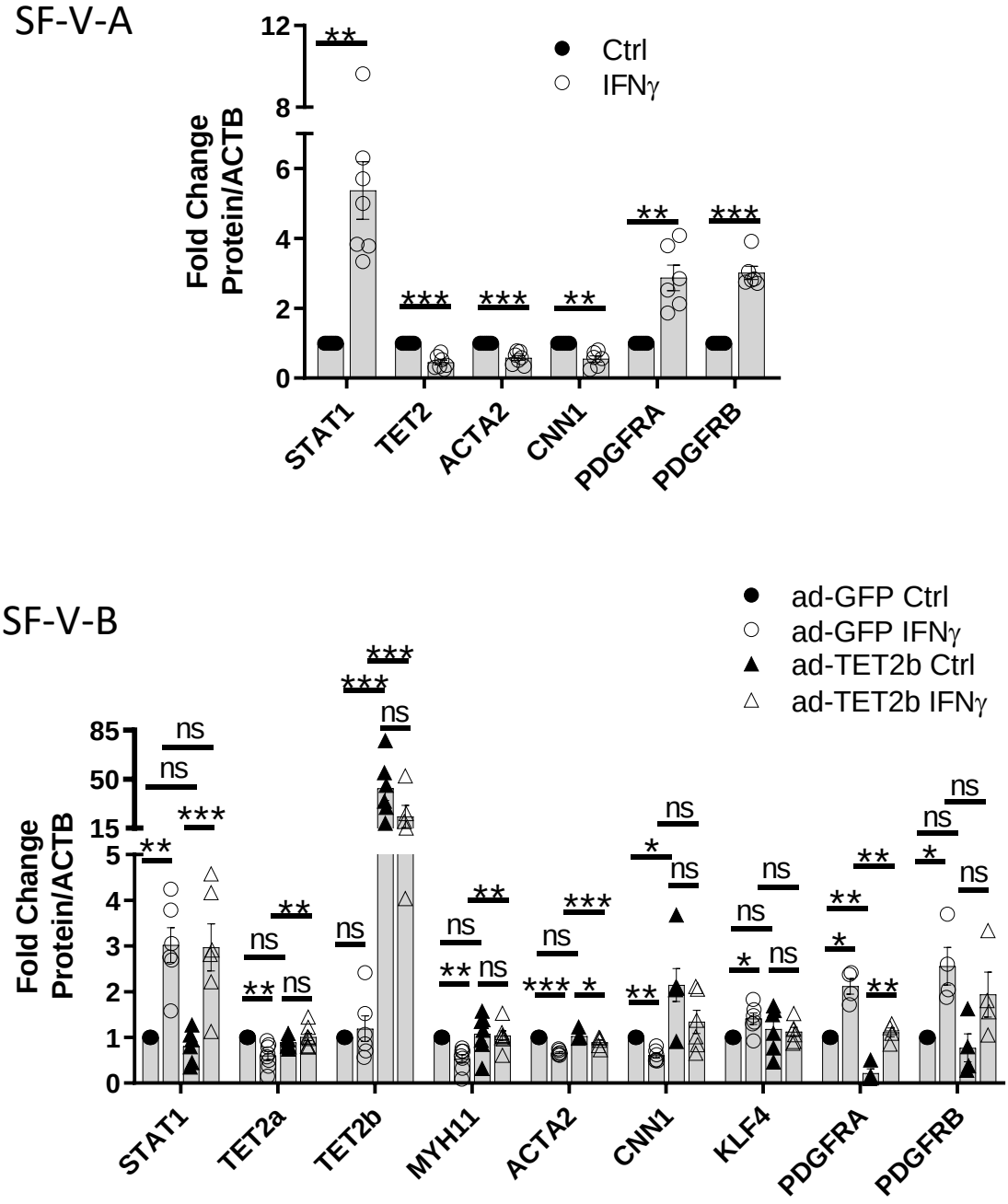


Supplemental Figure III: Many cells expressing high levels of TET2 in hCAV tissues are CD3 positive. Sections of coronary arteries from transplanted hearts (N=2) were immunostained for TET2 (brown) and CD3 (red fluorescent overlay). Yellow arrows indicate $TET2^{high}$ cells that also stain positive for the T cell marker CD3. Scale bars 33.3µm.

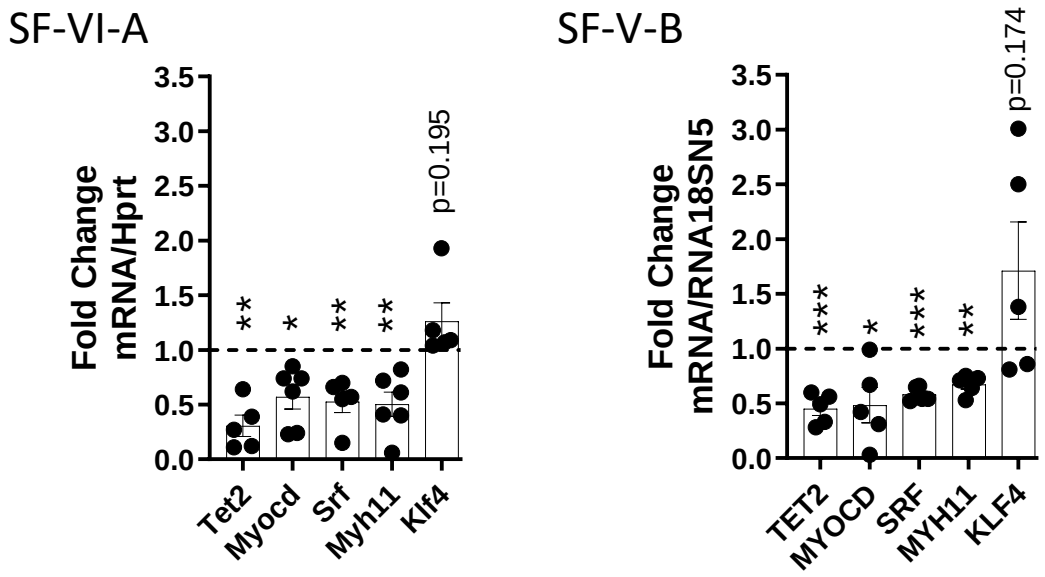
SF-IV



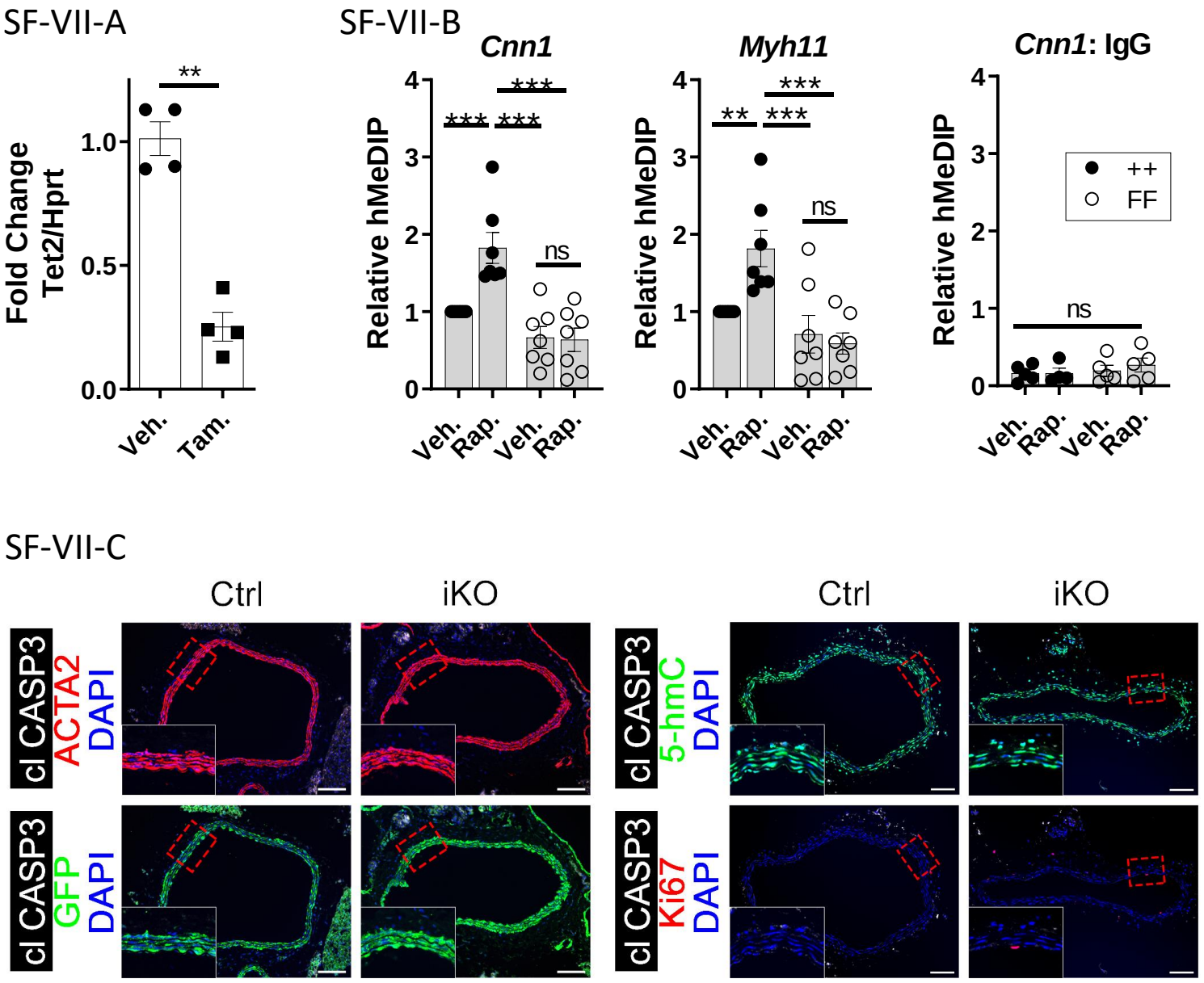
Supplemental Figure IV: Neither TET1 nor TET3 are repressed in murine aortic graft tissues 28d days after transplant. Tissues were harvested from mice that had received aorta transplants at 28 days after surgery. Graft sections were immunostained for TET1 and TET3, or no primary antibody control. Representative images shown of N=4 donor samples and N=6 graft samples. Scale bars: 100µm.



Supplemental Figure V: Quantification of western blots shown in Figure 2. Densitometry analysis was performed on western blot data shown in Figures 2A (SF-V-A) and 2C (SF-V-B). Fold change in protein relative to ACTB protein shown. A&B) N=6-8. SF-V-A: One sample t test, SF-V-B: Two-way ANOVA: *P<0.05, **P<0.01, ***P<0.001.

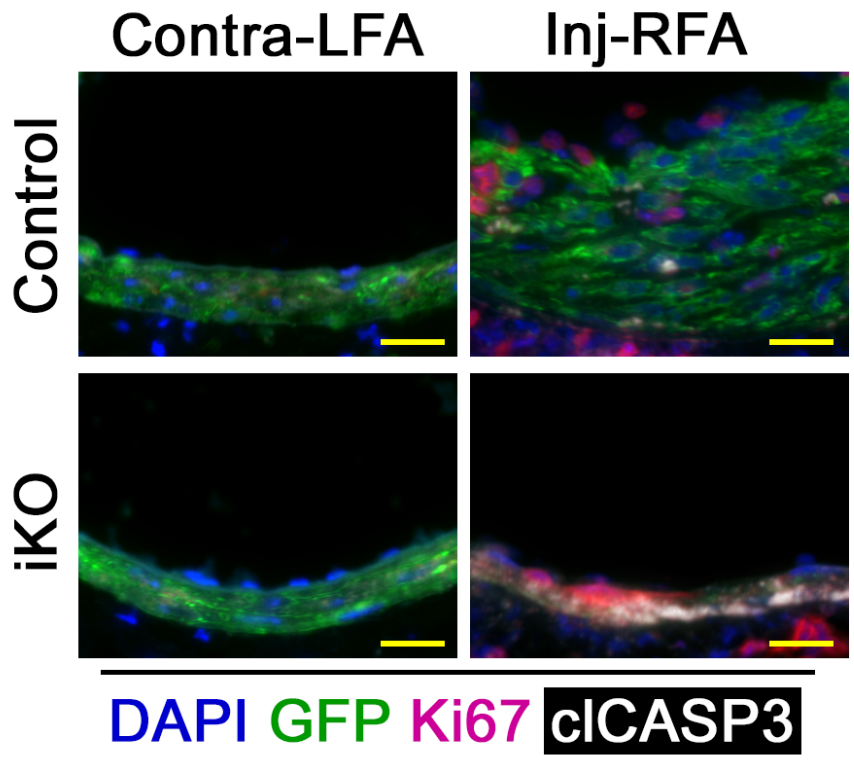


Supplemental Figure VI: IFNγ causes TET2 repression, and an activated phenotype in murine VSMCs; *TET2* knockdown recapitulates much of the IFNγ-induced phenotype in VSMCs. SF-VI-A) Primary mouse vascular smooth muscle cells (mVSMCs) were cultured under control conditions (denoted by dotted line) or treated with 100ng/ml recombinant mouse IFNγ (Bars) for 48hrs. mRNA levels for indicated genes determined by qPCR normalized to *Hprt,* expressed as fold change relative to control (dotted line) (N=5-6). SF-VI-B) Human VSMCs were transfected with *TET2* siRNA or non-targeting siRNA (NT). mRNA levels for indicated genes determined by qPCR normalized to *RNA18SN5,* expressed as fold change relative to control (dotted line) (N=5). One sample test: *P<0.05, ***P<0.001.



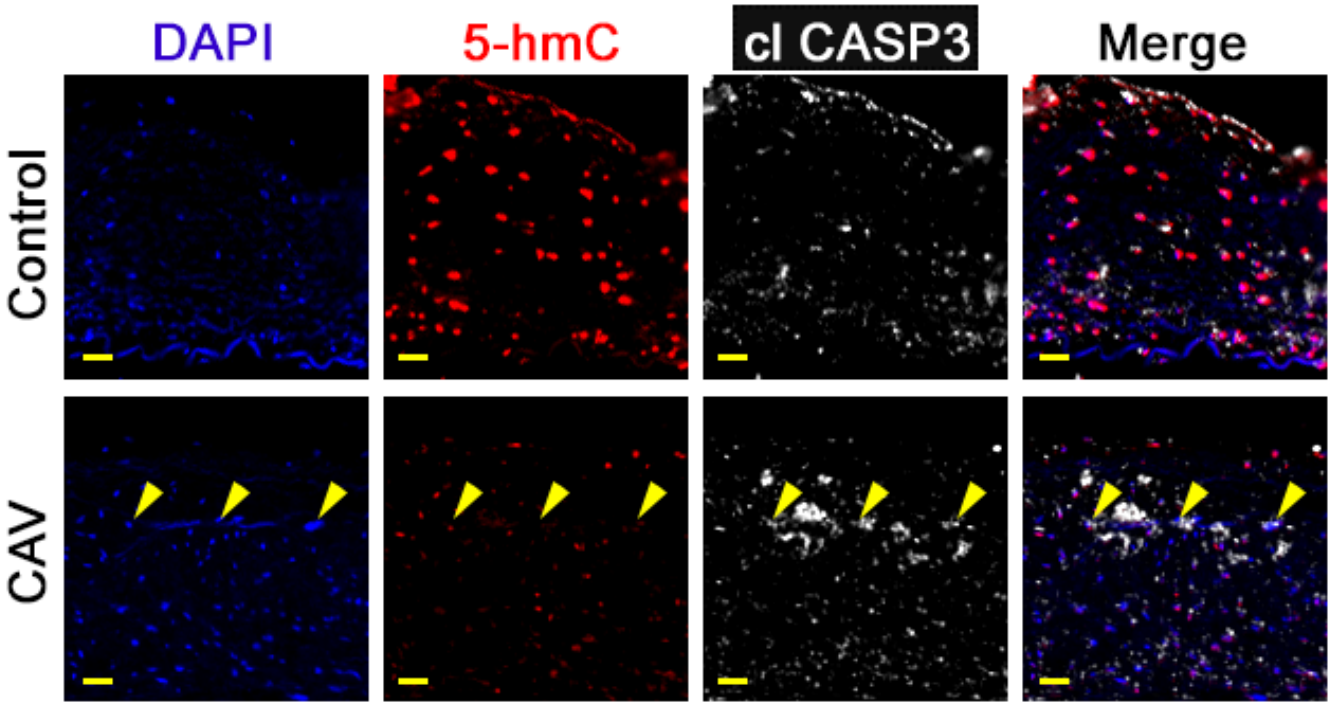
Supplemental Figure VII: Validation of inducible *Tet2*-deficiency *in vivo*. *Tet2* homozygous floxed mice harboring the *Myh11-Cre* transgene (on the Y chromosome) were treated with vehicle or 1mg/mouse/day tamoxifen for 10days. SF-VII-A) Aortas were collected, enzymatically stripped of the adventitial layer, and total RNA was isolated from the remaining media/intima. qPCR analysis of *Tet2* mRNA at the floxed region relative to *Hprt* (N=4). Student's t test: **P<0.01. SF-VII-B) To confirm enzymatic loss-of-function in TET2-deficient cells, hydroxymethylated DNA immunoprecipitation (hMe-DIP) qPCR was performed 48hrs after cell differentiation with 50nM rapamycin (Rap.) versus vehicle (Veh.) 5-hmC was assessed at the proximal promoters of *Cnn1* and *Myh11* genes (N=7). IgG IP samples were assayed for *Cnn1* (N=5). Two-way ANOVA: **P<0.01., ***P<0.001. SF-VII-C) 5µm sections of abdominal aorta segments of donor mice (harboring the Rosa26-mTmG reporter transgene to inducibly label VSMCs with GFP) used for aorta graft studies were assessed for expression of cleaved caspase 3 (cl CASP3), ACTA2, 5hmC, and the proliferation marker Ki67 by immunofluorescence. 5hmC did not significantly differ between control and TET2-deficient vessels, likely because the deletion was induced in adult mice, where the highly stable 5hmC mark[23] persists due to the very low levels of proliferation in VSMC. Representative images shown of N=8 homozygous wild type (Ctrl) samples and N=6 homozygous floxed (iKO) samples. Scale bars: 100µm.

SF-VIII

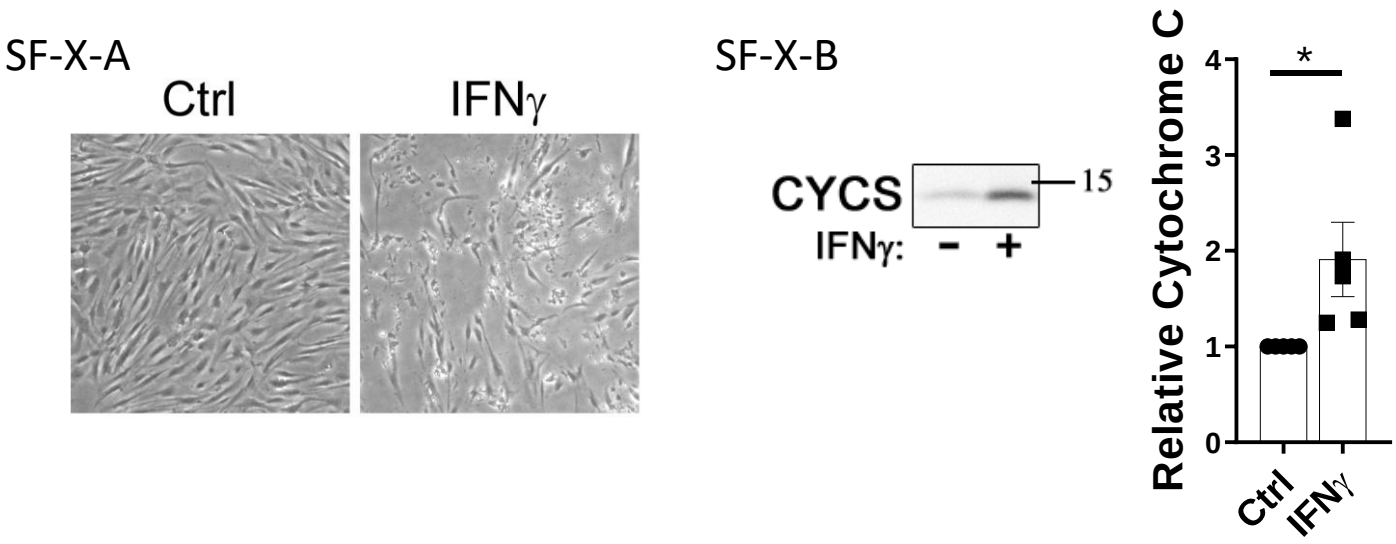


Supplemental Figure VIII: Pronounced apoptosis is observed in injured TET2-deficient femoral arteries. Guidewire-injured right femoral arteries (Inj-RFA), and contralateral control left femoral arteries (Contra-LFA), of littermate *Tet2*$^{++}$*Myh11*-Cre (Control mTmG-labeled N=6) and *Tet2*^{FF}*Myh11*-Cre (iKO mTmG-labeled N=6) were harvested 21days after surgery. 5µm sections were immunostained for the VSMC lineage marker GFP, Ki67, and cleaved caspase 3 (clCASP3). Representative micrographs are shown. Scale bar= 25µm.

SF-IX

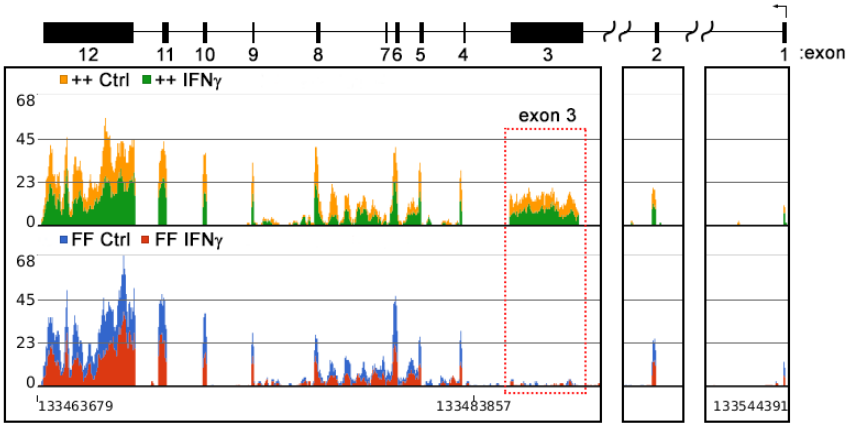


Supplemental Figure IX: 5-hmC and apoptosis are inversely correlated in CAV. Sections of coronary arteries from patients with coronary allograft vasculopathy (CAV; N=2) and age-matched controls (Control; N=4) were immunostained for cleaved caspase 3 (clCASP3), and 5-hmC with DAPI nuclear counterstain and demonstrate evidence of a negative association between apoptosis (clCASP3) and TET activity (5-hmC). Yellow arrowheads indicate cells exhibiting low 5-hmC and high clCASP3. Representative images shown. Scale Bar 25µm.

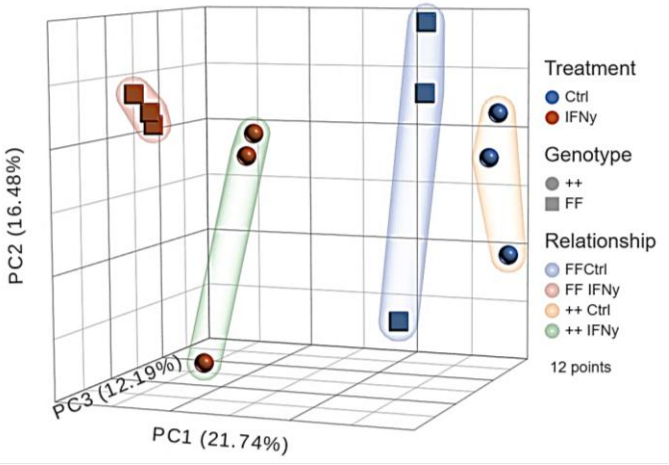


Supplemental Figure X: IFNy is sufficient to induce apoptosis in TET2-competent human VSMCs. Human VSMCs were cultured under control conditions (Ctrl) or treated with 100ng/ml IFNy for 14 days (IFNy). SF-X-A) Representative phase contrast micrographs of Ctrl and IFNy-treated cells. SF-X-B) Left) Representative western blot of conditioned medium isolated from Ctrl and IFNy-treated hVSMCs and Right) quantitation of 5 independent experiments. One sample t test: *P<0.05.

SF-XI-A



SF-XI-B



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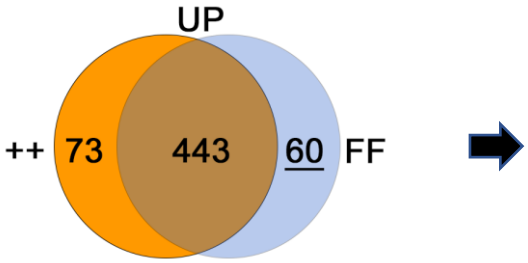
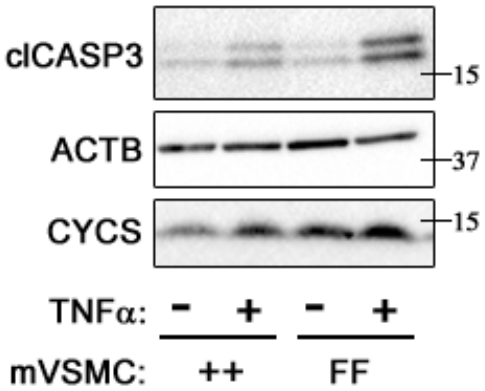
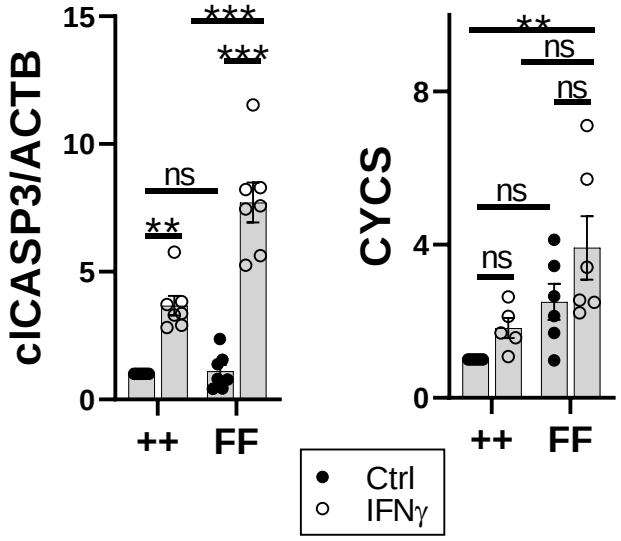


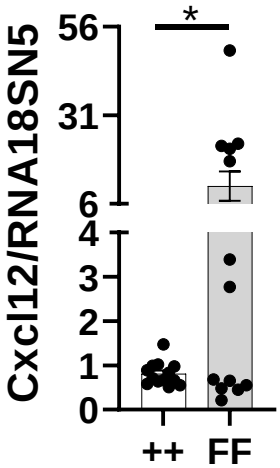
IPA Canonical Pathways	-log(p-value)
TNFR2 Signaling	6.26
MIF Regulation of Innate Immunity	5.66
TNFR1 Signaling	5.35
CD40 Signaling	4.9
Role of NFAT in Regulation of the Immune Response	4.32
Death Receptor Signaling	4.32
CD28 Signaling in T Helper Cells	3.85
Induction of Apoptosis by HIV1	3.48

SF-XI-D



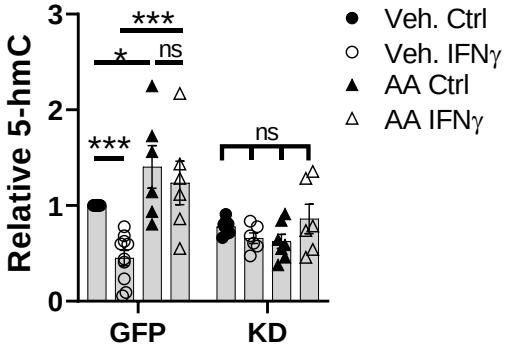


SF-XI-E

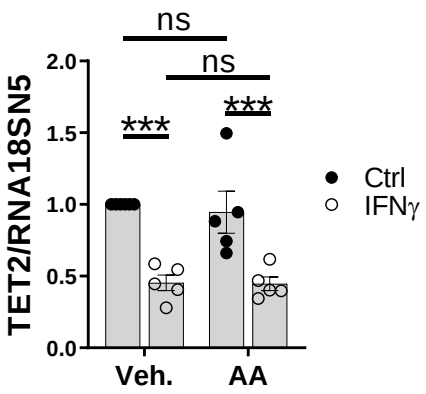


Supplemental Figure XI: Bulk RNA sequencing of control and TET2-deficient cells revealed a signature of increased TNFR signaling that reflects increased sensitivity of TET2-deficient cells to TNFα-induced apoptosis. mVSMCs isolated from *Tet2*$^{++}$mTmG^{mut+}*Myh11*-Cre (++) and *Tet2*^{FF}mTmG^{mut+}*Myh11*-Cre (FF) mice were treated with Cre recombinase-encoding adenovirus *in vitro*. Homozygous control (++) and TET2-deficient (FF) mVSMCs were then treated with 100ng/ml IFNy for 5days (N=3/group). mRNAs were isolated and differential expression analysis was performed by RNA sequencing. SF-XI-A) Histogram of the TET2 gene showing efficient deletion of the floxed exon 3. Yellow: control mVSMCs cultured under control conditions (++ Ctrl). Green: control mVSMCs treated with IFNy (++ IFNy). Blue: TET2-deficient mVSMCs cultured under control conditions (FF Ctrl). Red: TET2-deficient mVSMCs treated with IFNy (FF IFNy). Black bars indicate antisense gene structure. SF-XI-B) Principal component analysis revealed that genotype and IFNy treatment resulted in four distinct clusters. SF-XI-C) Differentially regulated genes were filtered based on those significantly upregulated in iKO cells by IFNy that were not significantly upregulated in control cells by IFNy (See Sup. Table III). Ingenuity Pathway Analysis was performed on the resulting gene list. SF-XI-D) Control (++) and TET2-deficient (FF) mVSMCs were cultured under control conditions (-) or treated with 15ng/ml TNFα for 48hrs (+). Left) Western blots of whole cell lysates for cleaved caspase 3 (clCASP3), and ACTB; and of precipitated conditioned medium for cytochrome c (CYCS). Right) quantification of whole cell lysate and conditioned medium blots. N= 7. Two-way ANOVA: ** P<0.01, *** P<0.001. SF-XI-E) Control (++) and TET2-deficient (FF) cells were serum-restricted for 24hr in 0.5% serum medium, total RNA was isolated, and Cxcl12 mRNA abundance assessed by qPCR relative to 18s (RNA18SN5). N=13. Welch's t test: ** P<0.01.

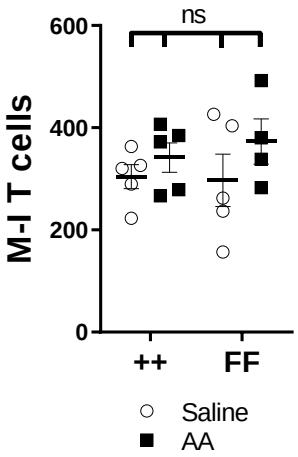
SF-XII-A



SF-XII-B



SF-XII-C



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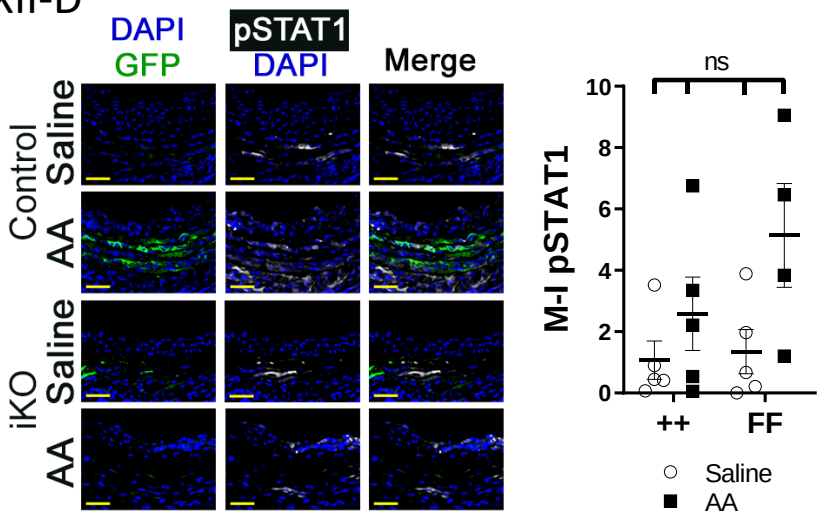
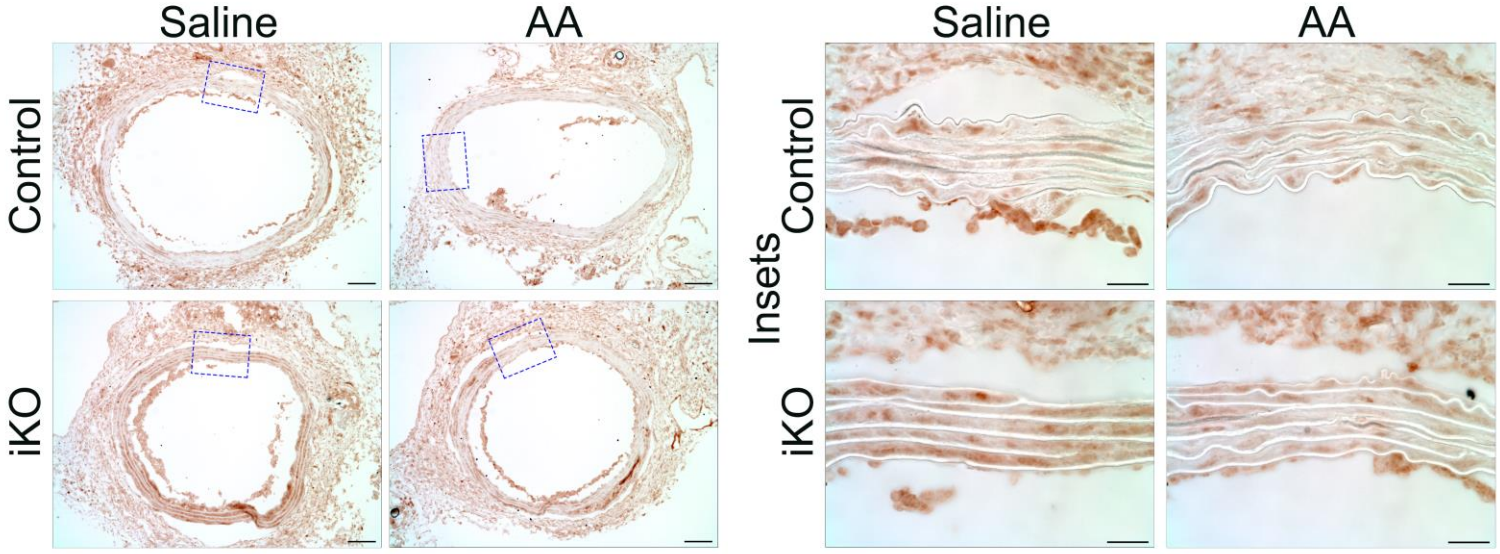


SF-XII-E



Supplemental Figure XII) AA prevents repression of TET2 activity, but not *TET2* mRNA level by IFNy; AA does not significantly reduce T cell recruitment or IFNy signaling *in vivo*. SF-XII-A) Human VSMCs were infected with GFP control adenovirus (GFP) or *TET2*-shRNA-expressing adenovirus (KD; knockdown), serum-restricted in the presence (AA) or absence (Veh) of 250μM ascorbic acid (AA) for 24 hr, and then cultured under control conditions or treated with 100 ng/ml IFNy for 48hrs. SF-XII-A) Genomic 5-hmC content was assessed by ELISA. N=6-12. SF-XII-B) hVMSCs were treated with AA and IFNy as in SF-XII-A. *TET2* mRNA level determined by qPCR normalized to *RNA18SN5* (N=5). SF-XII-C, D) Female C57bl/6J recipients received littermate *Tet2*$^{++}$mTmG^{mut+}*Myh11*-Cre (Control) or *Tet2*^{FF}mTmG^{mut+}*Myh11*-Cre (iKO) grafts. Recipient animals were treated with saline or 4mg/kg/day ascorbic acid (AA), and graft tissue was harvested 28 days after surgery. Quantification of S12C) T cells (CD3^{+}) and S12D) nuclear pSTAT1 intensity in the media - intima combined area ("M-I") as assessed by immunofluorescent staining. Micrographs of CD3 staining shown in Figure 7A. ++ saline N=5, ++ AA N=5, FF saline N=5, FF AA N=4. SF-XII-E) TET2 immunostaining (ab124298[50]) of 10-day control and iKO graft tissue sections from mice administered IP saline or 4mg/kg/day ascorbic acid (AA). TET2 staining in both control and TET2-deficient grafts is fairly diffuse due to downregulation of TET2 at this 10day time point. The antibody employed detects a C-terminal epitope, mRNA for which is present in both WT and TET2-deficient strains (the TET2-deficient strain is an exon 3 deletion loss of function mutant (Sup. Fig. VII-B and[26])). Representative images shown: ++ saline N=6, ++ AA N=5, FF saline N=5, FF AA N=4. S12A: Two-way ANOVA: *P<0.05, *** P<0.001.