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	Jackson Labs	C57BL6/J	M/F	IMSR_JAX:000664

Genetically Modified Animals

	Species	Vendor or	Background	Other Information	Persistent ID / URL
		Source	Strain		
Parent - Male	Mice	Jackson Labs	C57BL6/J	CD4-Cre	IMSR_JAX:022071
Parent - Female	Mice	Jackson Labs	C57BL6/J	MyD88 Flox	IMSR_JAX:008888
Parent –	Mice	Jackson Labs	C57BL6/J	MyD88 Knockout	IMSR_JAX:009088
Male/Female					
Parent –	Mice	Jackson Labs	C57BL6/J	TCR alpha Knockout	IMSR_JAX:002116
Male/Female					
Parent –	Mice	Jackson Labs	C57BL6/J	CD45.1	IMSR_JAX:002116
Male/Female					

Antibodies

Target	Vendor or	Catalog #	Working	Persistent ID / URL
antigen	Source		concentration	
CD3	Biolegend	100253	Cell culture: 2.5 μg/mL	https://www.biolegend.com/fr-lu/explore-new-products/ultra- leaf-purified-anti-mouse-cd3-antibody-8078?GroupID=BLG242
CD28	Biolegend	102102	Cell culture: 1.0 μg/mL	https://www.biolegend.com/en-us/products/purified-anti- mouse-cd28-antibody-117
IL-4	Biolegend	504102	Cell culture: 50 ng/mL	https://www.biolegend.com/en-us/products/purified-anti- mouse-il-4-antibody-894
CD49d	Biolegend	103701	Cell culture: 20 μg/mL	https://www.biolegend.com/en-us/products/purified-anti- mouse-cd49d-antibody-194
CD49d	Biolegend	103629	Cell culture: 20 μg/mL	https://www.biolegend.com/en-us/products/ultra-leaf- purified-anti-mouse-cd49d-antibody-18975
CD11a	Biolegend	101101	Cell culture: 20 μg/mL	https://www.biolegend.com/en-us/products/purified-anti- mouse-cd11a-antibody-356
TGFβ 1,2,3	Thermofisher	MA5- 23795	Cell culture: 5 µg/mL	https://www.thermofisher.com/antibody/product/TGF-beta-1- 2-3-Antibody-clone-1D11-Monoclonal/MA5-23795
CD4	Biolegend	100402	IHC 1:200	https://www.biolegend.com/en-us/products/purified-anti- mouse-cd4-antibody-252
CD4 APC- Cy7	Biolegend	100414	FC 1:50	https://www.biolegend.com/en-us/products/apc-cyanine7- anti-mouse-cd4-antibody-1964
CD4 FITC	Biolegend	100406	FC: 1:50	https://www.biolegend.com/en-us/products/fitc-anti-mouse- cd4-antibody-248
CD4 AF594	Biolegend	100446	IF 1:100	https://www.biolegend.com/en-us/products/alexa-fluor-594- anti-mouse-cd4-antibody-9412
CD8 FITC	Biolegend	100705	FC: 1:50	https://www.biolegend.com/en-us/products/fitc-anti-mouse- cd8a-antibody-153
IFNγ APC	Biolegend	505810	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- ifn-gamma-antibody-993

IL-17 PE	Biolegend	506904	FC: 1:50	https://www.biolegend.com/en-us/products/pe-anti-mouse-il- 17a-antibody-1633
Tbx1 PerCP	Biolegend	644806	FC: 1:50	https://www.biolegend.com/en-us/products/percp-cyanine5- 5-anti-t-bet-antibody-5760
TCRβ APC- Cy7	Biolegend	109220	FC: 1:50	https://www.biolegend.com/en-us/products/apc-cyanine7- anti-mouse-tcr-beta-chain-antibody-4137
CD45.2 BV421	Biolegend	109832	FC: 1:50	https://www.biolegend.com/en-us/products/brilliant-violet- 421-anti-mouse-cd45-2-antibody-7328
CD45.2 APC	Biolegend	109813	FC: 1:50	https://www.biolegend.com/en-us/search-results/apc-anti- mouse-cd45-2-antibody-2759
CD45.1 APC	Biolegend	109814	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- cd45-2-antibody-2759
CD45.1 PE	Biolegend	110707	FC: 1:50	https://www.biolegend.com/en-us/products/pe-anti-mouse- cd45-1-antibody-199
CD11a APC	Biolegend	101119	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- cd11a-antibody-9069
CD49d FITC	Biolegend	103606	FC: 1:50	https://www.biolegend.com/en-us/products/fitc-anti-mouse- cd49d-antibody-438
TLR4 PE	Biolegend	145404	FC: 1:50	https://www.biolegend.com/en-us/products/pe-anti-mouse- cd284-tlr4-antibody-8397
IL-1r APC	Biolegend	113509	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- cd121a-il-1-r-type-i-p80-antibody-6371
IL-33r PE- Cy7	Biolegend	145316	FC: 1:50	https://www.biolegend.com/en-us/products/pe-cyanine7-anti- mouse-il-33ralpha-il1rl1-st2-antibody-14675
CD44 APC	Biolegend	103012	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- human-cd44-antibody-312
CD62L PE	Biolegend	104408	FC: 1:50	https://www.biolegend.com/en-us/products/pe-anti-mouse- cd62l-antibody-386
CD25 BV421	Biolegend	102043	FC: 1:50	https://www.biolegend.com/en-us/products/brilliant-violet- 421-anti-mouse-cd25-antibody-7197
CD69 FITC	Biolegend	104506	FC: 1:50	https://www.biolegend.com/en-us/products/fitc-anti-mouse- cd69-antibody-264
CXCR3 APC	Biolegend	126512	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse- cd183-cxcr3-antibody-4683
MHCII APC	Biolegend	107613	FC: 1:50	https://www.biolegend.com/en-us/products/apc-anti-mouse-i- a-i-e-antibody-2488
CD11b APC-Cy7	Biolegend	101225	FC: 1:50	https://www.biolegend.com/en-us/products/apc-cyanine7- anti-mouse-human-cd11b-antibody-3930
CD11c PE	Biolegend	117307	FC: 1:50	https://www.biolegend.com/en-us/products/pe-anti-mouse- cd11c-antibody-1816
Ly6G BV421	Biolegend	127627	FC: 1:50	https://www.biolegend.com/en-us/products/brilliant-violet- 421-anti-mouse-ly-6g-antibody-7161
CCR2 FITC	Biolegend	150607	FC: 1:50	https://www.biolegend.com/en-us/products/fitc-anti-mouse- cd192-ccr2-antibody-13354
Mefsk4 APC	Miltenyi	130-120- 166	FC: 1:50	https://www.miltenyibiotec.com/US-en/products/feeder-cells- antibody-anti-mouse-mef-sk4.html#conjugate=pe:size=30-ug- in-200-ul
Foxp3 PE	Invitrogen	14-5773- 82	FC 1:500	https://www.thermofisher.com/antibody/product/FOXP3- Antibody-clone-FJK-16s-Monoclonal/14-5773-82
IL-1β	CST	12242	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/il- 1b-3a6-mouse-mab/12242
IL-33	R&D	AF3626	WB 1:500	https://www.rndsystems.com/products/mouse-il-33- antibody_af3626
HMGB1	Abcam	79823	WB 1:1000	https://www.abcam.com/hmgb1-antibody-epr3507- ab79823.html

TRIF	NovusBio	NB120- 13810SS	WB 1:500	https://www.novusbio.com/products/trif-ticam1- antibody_nb120-13810
GAPDH	CST	2118	WB 1:2000	https://www.cellsignal.com/products/primary- antibodies/gapdh-14c10-rabbit-mab/2118
MyD88	R&D	AF3109	WB 1:1000 IF 1:100	https://www.rndsystems.com/products/mouse-rat-myd88- antibody_af3109
pERK	CST	9101	WB 1:1000	https://www.cellsignal.com/products/primary- antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204- antibody/9101
ERK	CST	9102	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/p44- 42-mapk-erk1-2-antibody/9102
β-actin	Sigma	A5441	WB 1:2000	https://www.sigmaaldrich.com/US/en/product/sigma/a5441
α-tubulin	CST	3873	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/a- tubulin-dm1a-mouse-mab/3873
α-smooth muscle actin	Sigma	A2547	WB 1:1000 IF 1:100	https://www.sigmaaldrich.com/US/en/product/sigma/a2547
pP38	CST	4511	WB 1:1000	https://www.cellsignal.com/products/primary- antibodies/phospho-p38-mapk-thr180-tyr182-d3f9-xp-rabbit- mab/4511
P38	CST	9212	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/p38- mapk-antibody/9212
pP65	CST	3033	WB 1:1000	https://www.cellsignal.com/products/primary- antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033
P65	CST	4764	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/nf- kb-p65-c22b4-rabbit-mab/4764
рАКТ	CST	4060	WB 1:1000	https://www.cellsignal.com/products/primary- antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060
AKT	CST	4691	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/akt- pan-c67e7-rabbit-mab/4691
pZap70	CST	2717	WB 1:1000 IF 1:200	https://www.cellsignal.com/products/primary- antibodies/phospho-zap-70-tyr319-syk-tyr352-65e4-rabbit- mab/2717
Zap70	CST	3165	WB 1:1000	https://www.cellsignal.com/products/primary-antibodies/zap- 70-d1c10e-xp-rabbit-mab/3165
Collagen 1	Invitrogen	PIPA5295 69	WB 1:1000	https://www.fishersci.com/shop/products/anti-col1a1- polyclonal/PIPA529569
CXCR3	Bios	2209R	WB 1:1000	https://www.biossusa.com/products/bs-2209r
Rabbit IgG	CST	7074	WB 1:2000	https://www.cellsignal.com/products/secondary- antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
Mouse IgG	CST	7076	WB 1:2000	https://www.cellsignal.com/products/secondary- antibodies/anti-mouse-igg-hrp-linked-antibody/7076
Goat IgG	ThermoFishe r	A15999	WB 1:2000	https://www.thermofisher.com/antibody/product/Donkey- anti-Goat-IgG-H-L-Secondary-Antibody-Polyclonal/A15999
Rat IgG Biotin	Jackson	112-065- 062	IHC 1:300	https://www.jacksonimmuno.com/catalog/products/112-065- 062
CD31	Biolegend	102520	IF 1:100	https://www.biolegend.com/en-us/products/alexa-fluor-594- anti-mouse-cd31-antibody-9633?GroupID=BLG10559
PDGFrα	R&D	AF1062	IF 1:100	https://www.rndsystems.com/products/mouse-pdgf-ralpha- antibody_af1062
Ki67	Abcam	Ab15580	IF 1:100	https://www.abcam.com/products/primary-antibodies/ki67- antibody-ab15580.html
aMouse AF488	Thermo	A32766	IF 1:200	https://www.thermofisher.com/antibody/product/Donkey- anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary- Antibody-Polyclonal/A32766
aGoat AF488	Thermo	A11055	IF 1:200	https://www.thermofisher.com/antibody/product/Donkey- anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody- Polyclonal/A-11055

aGoat AF555	Thermo	A21432	IF 1:200	https://www.thermofisher.com/antibody/product/Donkey- anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody- Polyclonal/A-21432
aHamster AF594	Thermo	A78966	IF 1:200	https://www.thermofisher.com/antibody/product/Goat-anti- Armenian-Hamster-IgG-H-L-Highly-Cross-Adsorbed-Secondary- Antibody-Polyclonal/A78966
aRabbit AF555	CST	4413	IF 1:200	https://www.cellsignal.com/products/secondary- antibodies/anti-rabbit-igg-h-l-f-ab-2-fragment-alexa-fluor-555- conjugate/4413

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
Reclustering and analysis of mouse scRNA seq data	Seurat	https://satijalab.org/seurat/
Reclustering and analysis of human citeSeq data	Nebula	https://github.com/lhe17/nebula

Other

Description	Source /	Persistent ID / URL		
	Repository			
CD4 Magnetic Beads	Miltenyi	https://www.miltenyibiotec.com/US-en/products/cd4-I3t4- microbeads-mouse.html#gref		
IL-2	Miltenyi	https://www.miltenyibiotec.com/US-en/products/mouse-il-2- is.html#gref		
IL-12	Peprotech	https://www.peprotech.com/en/recombinant-murine-il-12		
Direct Red 80	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/sial/365548		
Picric Acid	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/sigma/p6744		
AEC Substrate	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/sigma/a5754		
Liberase TL	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/roche/05401020001		
TrypLE	ThermoFisher	https://www.thermofisher.com/order/catalog/product/12605036		
Insulin-Transferrin-Selenium	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/sigma/i3146		
ICAM-1 Fc	Biolegend	https://www.biolegend.com/en-us/products/recombinant-mouse- icam-1-fc-chimera-carrier-free-9893		
VCAM-1 Fc	Biolegend	https://www.biolegend.com/en-us/products/recombinant-humar vcam-1-fc-chimera-carrier-free-9992		
CXCL10	Peprotech	https://www.peprotech.com/en/recombinant-murine-ip-10-cxcl10		
IL-2 ELISA	R & D	https://www.rndsystems.com/products/mouse-il-2-duoset- elisa_dy402		
TNFα ELISA	R & D	https://www.rndsystems.com/products/mouse-tnf-alpha-duoset- elisa_dy410		
IFNY ELISA	R & D	https://www.rndsystems.com/products/mouse-ifn-gamma-duoset- elisa_dy485		
WGA	ThermFisher	https://www.thermofisher.com/order/catalog/product/W11261		
MyD88 Inhibitor Peptide	NovusBio	https://www.novusbio.com/products/myd88-inhibitor_nbp2-29328		
TUNEL Kit	Sigma	https://www.sigmaaldrich.com/US/en/product/roche/12156792910		

ARRIVE GUIDELINES

The ARRIVE guidelines (<u>https://arriveguidelines.org/</u>) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Groups	Sex	Age	Number (prior	Number (after	Littermates	Other description
			to experiment)	termination)	(Yes/No)	

WT Th1	М	8-10	6	6	Yes	Adoptive Transfer 4
Sham		weeks				week time point
WT Th1	M	8-10	9	9	Yes	Adoptive Transfer 4
TAC		weeks				week time point
MyD88 KO	M	8-10	5	5	Yes	Adoptive Transfer 4
Th1 Sham		weeks				week time point
MyD88 KO	M	8-10	9	9	Yes	Adoptive Transfer 4
Th1 TAC		weeks				week time point
Cre- Sham	M/F	8-10	7	7	Yes	4 week time point
		weeks				(histology)
Cre+ Sham	M/F	8-10	8	8	Yes	4 week time point
		weeks				(histology)
Cre- TAC	M/F	8-10	10	10	Yes	4 week time point
		weeks				(histology)
Cre+ TAC	M/F	8-10	12	11	Yes	4 week time point
		weeks				(histology)
Cre- Sham	M/F	8-10	4	4	Yes	Survival
		weeks				
Cre+ Sham	M/F	8-10	4	4	Yes	Survival
		weeks				
Cre- TAC	M/F	8-10	11	10	Yes	Survival
		weeks				
Cre+ TAC	M/F	8-10	10	10	Yes	Survival
		weeks				
Cre- Sham	M/F	8-10	8	8	Yes	4 week time point
		weeks				(Heart Digest / Flow
						cytometry)
Cre+ Sham	M/F	8-10	7	6	Yes	4 week time point
		weeks				(Heart Digest / Flow
						cytometry)
Cre- TAC	M/F	8-10	8	8	Yes	4 week time point
		weeks				(Heart Digest / Flow
						cytometry)
Cre+ TAC	M/F	8-10	8	8	Yes	4 week time point
		weeks				(Heart Digest / Flow
						cytometry)

Sample Size:

Our lab has previously published on multiple occasions using the TAC model (PMID: 36092510, PMID: 33463362, PMID: 33103561, PMID: 30779709), using a minimum sample size based on power calculations for the expected decrease in fractional shortening and increase in T-cell infiltration (http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality), showing a minimum of 6 mice per group is needed to detect statistically significant changes with a power of 90%.

Inclusion Criteria:

Mice underwent TAC surgery between 8 and 10 weeks of age on littermates and/or mice aged matched between litters from the same colony.

Exclusion Criteria:

1 mouse that exhibited no decrease in fractional shortening or well established hallmarks of successful TAC surgery (LV hypertrophy) were excluded from the analysis, and one additional mouse that was determined to have an inconclusive genotype after tissues were harvested. Additionally, mice that exhibited signs of euthanasia criteria post surgery (decrease in body weight, behavioral changes, signs of pain) were euthanized.

Randomization:

Mice were randomized to TAC vs. Sham groups from respective genotypes by a random number generator, as well as randomly received WT or MyD88 KO T-cells by adoptive transfer by a random number generator (https://www.calculator.net/random-number-generator.html)

Blinding:

Echocardiography, tissue collection, microscopy, and flow cytometric analysis were done in a blinded fashion. In short, each animal was assigned an ID number (tail tattoo) prior to surgery by a third party, and the echocardiography, tissue collection, microscopy, and flow cytometry was done only using the ID number. After all data was collected ID numbers were matched with genotypes and surgery conditions.