

## 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
Mouse	The Jackson Laboratory	C57BL/6J	Male/Female	<a href="https://www.jax.org/strain/000664">https://www.jax.org/strain/000664</a>

### Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
<b>Parent – Male/Female</b>	Mouse	UT MD Anderson, Abe lab	C57BL/6J	ERK5S496A mutation knock in mouse, S496Aki	

### Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration (Western blots)	Lot # (preferred but not required)	Persistent ID / URL
Phospho p90RSK	Cell signaling	9341	1:1000		RRID: ab_330753
Total RSK	R&D Biotech	MAB 2056	1:2000		N/A
Phospho ERK5S496	Biorbyt	Orb5183	1:1000		RRID:AB_10934187
PhosphoERK5TEY	Cell signaling	3371	1:1000		RRID:AB_2140424
Total ERK5	Cell signaling	3372	1:1000		RRID:AB_330491
Thioredoxin 1	Proteintech	14999-1 AP20	1:2000		RRID:AB_2272597
HO-1	abcam	ab13248	1:3000		RRID:AB_2118663
Gas6	Bioss	BS-7549R	1:1000		N/A
TNF alpha	Novus Biologicals	NBP1-19532	1:1000		RRID:AB_1643202
CD36	Cayman chemicals	100011	1:1000		RRID:AB_327790
AHR	Invitrogen	MA1-514	1:2000		RRID:AB_2273723
NRF2	Gentex	GTX103322	1:1000		RRID:AB_1950993
PCNA	Invitrogen	13-3900	1:1000		RRID:AB_86593
Lamin b1	LSBio	LS-C82208	1:1000		RRID:AB_1655944
P53	Santacruz	SC-98	1:500		RRID:AB_628085

DOI [to be added]

P21	Cell signaling	2947	1:1000		RRID:AB_823586
P16	Proteintech	10883-1-AP	1:1000		N/A
DNMT3a	Novus Biologicals	NB12013888	1:500		RRID:AB_789607
Phospho ERK1/2	Cell signaling	9101	1:1000		RRID:AB_331646
Total ERK1/2	Cell signaling	9194	1:1000		N/A
P53bp1	Novus Biologicals	NB100-304	1:1000		RRID:AB_10003037
KLF2	Novus Biologicals	NBP261812	1:2000		N/A
SUMO2/3	MBL Lifesciences	M114-3	1:2000		RRID:AB_592769
Alpha tubulin	Sigma	T5168	1:4000		RRID:AB_477579
Ki67	abcam	Ab15580	1:300		RRID:AB_10562976
goat anti-rabbit	Thermofisher scientific	A-21038	1:5000		RRID:AB_2535709
goat anti-mouse	Thermofisher scientific	A-21237	1:5000		RRID:AB_2535806
HRP - GAR-	Novus Biologicals	NB7160	1:5000		RRID:AB_524669
HRP - GAM-	Novus Biologicals	NB7539	1:5000		RRID:AB_10126266
Alexa 488 anti-rabbit secondary antibody	Molecular probes	A11055	1:200		RRID: AB_2534102
Alexa 488 anti-mouse secondary antibody	Molecular probes	A11029	1:200		RRID: AB_2534088
Alexa 546 anti-mouse secondary antibody	Molecular probes	A11081	1:200		RRID: AB_141738
F4/80	BD biosciences	565410	1:100		RRID:AB_10526888
CD11b	BD biosciences	553310	1:100		RRID:AB_394774

<b>IMC antibodies</b>					
CD31	R&D	AF3628	10 µg/mL		RRID:AB_2161028
p-Tyrosine	Fluidigm	3144024D	10 µg/mL		N/A
CD107b	Invitrogen	14-1072-85	20 ug/mL		RRID:AB_657562
TOP2b	R&D	MAB6348	20 ug/mL		RRID:AB_10889629
a-SMA	Fluidigm	3141017D	2.5 µg/mL		RRID:AB_2890139
Trx	Proteintech	14999-1-AP	10 ug/mL		RRID:AB_2272597
CD11b	Fluidigm	3149028D	5 µg/mL		RRID:AB_2891189
p-PKCzeta-T410	Abclonal	AP0520	30 ug/mL		RRID:AB_2771419
p-TERF2IP-S205	(Home made)		30 ug/mL		N/A
p-AMPK alpha (Thr172)	Affinity Biosciences	AF3423	10 ug/mL		RRID:AB_2834865
CCR7	Biorbyt	orb547290	10 ug/mL		N/A
Tyro3	R&D	MAB759	30 ug/mL		RRID:AB_2210988
Gas6	Abclonal	A8545	20 ug/mL		RRID:AB_2769575
p90RSK1	StressMarq	SPC-147F	10 ug/mL		RRID:AB_2703590
p-p90RSK (S380)	Bioworld	BS4870	20 ug/mL		N/A
ERG	Biorbyt	orb192164	10 ug/mL		N/A
p16	Abcam	ab54210	20 ug/mL		RRID:AB_881819
IL-6	R&D	AF-406-NA	10 ug/mL		RRID:AB_354478
Arginase-1	Fluidigm	3164027D	10 µg/mL		RRID:AB_2891145
p53	Abcam	ab131442	30 ug/mL		RRID:AB_11155283
vWF	Millipore	AB7356	10 ug/mL		RRID:AB_92216
Ki67	Fluidigm	3168022D	10 µg/mL		RRID:AB_2811061
DNMT3a	Novus	NB120-13888	10 ug/mL		RRID:AB_2261802
p-ERK	Fluidigm	3171021D	10 µg/mL		RRID:AB_2927686
p-JAK2	AbCam	ab219728	30 ug/mL		N/A
YAP	Abcam	ab56701	10 ug/mL		RRID:AB_2219140

p-YAP (Y357)	Abcam	ab62751	10 ug/mL		RRID:AB_956486
<b>IF antibodies</b>					
p53	abcam	ab131442	1:50		RRID:AB_11155283
TRX	Proteintech	14999-1-AP	1:40		RRID:AB_2272597
DNMT3a	Novus	NB120-13888	1:25		RRID:AB_2261802
Mac3	BD Biosciences	550292	1:150		RRID:AB_393587

### DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
ERK5b	Gene ID: AA288345	Yan et al., J Biol Chem.276(140):10870-8. (2001)	N/A
ERK5TEY mutant	Gene ID: AA288345	This paper	
ERK5S496A	Gene ID: AA288345	Le et al., Circ Res110(4):536-50 (2012).	N/A
CA-MEK5		Cameron et al., J Biol Chem. 278 (20):18682-18688 (2003).	N/A
ERK5WT	Gene ID: AA288345	Le et al., Circ Res110(4):536-50 (2012).	N/A
pNF-kB Luc	Gene ID: JQ513377	Stratagene	N/A
NRF2WT	Gene ID: NM_001313902.1	GeneCopoeia	EX-I4333-M29
NRF2K518R	Gen ID: NM_001313902.1Lys518Arg	GeneCopoeia	CS-14333-M29-01
pAAV/D377Y-mPCSK9	Gene ID: 100102	Add gene	RRID_58736
pRL-TK	Gene ID: AF025846	Promega	RRID_11313
Gal4-ERK5		Nigro et al., 116(11):1971-1979	N/A

### Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Bone marrow derived macrophages	In house	Male/female	N/A
L929 Cells	ATCC	Unknown	RRID: CVCL_0462

### Data & Code Availability

DOI [to be added]

Description	Source / Repository	Persistent ID / URL
RNA seq data	NCBI Ominibus	GSE210949

### Other

Description	Source / Repository	Persistent ID / URL
<b>Protein&amp;Lipids</b>		
GM-CSF	R&D Biosciences	415-ML-020
Ox-LDL	Dr. Henry J. Pownall Lab, Houston Methodist Research INstitute	
<b>Kits</b>		
Mito SOX	Invitrogen	M36008
ARE Reporter kit	BPS Biosciences	60514
Efferocytosis kit	Essen Biosciences	4649
Agilent Seahorse XF cell Mito stress kit	Agilent Technologies	103015-100
Seahorse XF Glycolysis stress kit	Agilent Technologies	103020-100
ATP kit	abcam	ab83355
NAD kit	Promega	G9071
Dual Luciferase reporter assay system	Promega	E1910
iScript cDNA synthesis kit	Bio-rad	1708891
iQSYBR Green Super mix	Bio-rad	170-8884
ApoTag peroxidase <i>in situ</i> apoptosis detection kit	Millipore	S7100
Cholesterol assay kit	Bioassay systems	EHDL-100
Mouse Inflammatory array kit	Ray Biotech, Inc	AAM-INF-1-4
HE staining kit		
<b>Drug and Reagent</b>		
XMD8-92	Selleck chemicals	S7525
FMK-MEA	MCE	HY-52101C
NRF2 Activator	Calbiochem	5303510001

DOI [to be added]

AX-15836	Tocris	5843
Mito-Tempol	abcam	Ab144644
MitoNeoD	MedKoo Biosciences	563761
IMDM	Gibco	12440053
Protein A dyna beads	Invitrogen	10001D
Protein G dyna beads	Invitrogen	10003D
Opti-MEM	Invitrogen	31985062
Laemmli buffer	Bio-Rad	1610747
NCM membrane	Bio-Rad	1620115
Lipofectamine 2000	Invitrogen	11668019
<b>Instrument and Software</b>		
GraphPad prism version 9.0.1	GraphPad Software	RRID:SCR_002798
Flowjo™ v10	FLOWJO	RRID:SCR_008520
Incucyte	Essen Biosciences	RRID:SCR_019874
Flow cytometer-Cytoflex	Beckman coulter	RRID:SCR_019627
VEVO-2100 System	Visual sonics	RRID:SCR_015816
Hyperion imaging system	Standard Biotools	SCR_023195
Seahorse XF24 analyzer	Agilent technologies	RRID:SCR_019539
Fluorometer	BMG Lab Tech	N/A
TD-20/20 Luminometer	N/A	Turner Biosystems
Image J	NIH	RRID:SCR_003070
Cuttdiff	N/A	RRID:scr_001647
GSEA	N/A	RRID:SCR_005724
Gene expression omnibus	N/A	RRID:SCR_005012
Tissue processor- leica-TP1020	Leica	N/A



## ARRIVE GUIDELINES

The ARRIVE guidelines (<https://arriveguidelines.org/>) 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 to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
<b>Fig1A &amp;1SA</b>						
Group 1 (WT)	M	8 weeks	6	6	No	BMDM's were isolated from mice treated with or without oxLDL
Group 2 (ERK5S496A)	M	8 weeks	6	6	No	Same as above
<b>Figure 1C-D &amp; 2S (1) A-E</b>						
Group 1 (WT)	M	8 weeks	20	15	No	rAAV8-D377Y-mPCSK9 injected into WT mice; had been fed an adjusted-calorie (high-fat) diet (HFD) for 16 weeks. Five mice were excluded due to the LDL cholesterol level less than 300mg/dL after 16 weeks of HFD
Group 2 (ERK5S496A)	M	8 weeks	20	20	No	Same as above
<b>Figure 1E-F &amp;2A-O</b>						
Group 1 (WT)	M	8 weeks	10	8	No	rAAV8-D377Y-mPCSK9 injected into WT mice; had been fed an adjusted-calorie (high-fat) diet (HFD) for 16 weeks. Two mice were excluded due to the LDL cholesterol level less than 300mg/dL after 16 weeks of HFD
Group 2 (ERK5S496A)	M	8 weeks	10	10	No	Same as above



<b>Figure 1I &amp; 7A-F&amp; S2-1 F</b>						
Group 1 (WT)	M	8 weeks	15	15	No	BMDM's were isolated from mice treated with or without oxLDL
Group 2 (ERK5S496A)	M	8 weeks	15	15	No	Same as above
<b>Figure 3F-O&amp; 4A-K &amp; S1H&amp; S8A</b>						
Group 1 (WT)	M	8 weeks	20	20	No	rAAV8-D377Y-mPCSK9 injected into WT mice; had been fed a chow diet (CD) for 16 weeks. After that isolated BMDM's
Group 2 (ERK5S496A)	M	8 weeks	20	20	No	Same as above
Group 3 (WT)	M	8 weeks	20	20	No	rAAV8-D377Y-mPCSK9 injected into WT mice; had been fed an adjusted-calorie (high-fat) diet (HFD) for 16 weeks. After that isolated BMDM's
Group 4 (ERK5S496A)	M	8 weeks	20	20	No	Same as above
<b>Figure 5 &amp; S1F,G and I</b>						
WT	M	8 weeks	10	10	No	BMDM's were isolated from mice treated with or without Indicated treatments
<b>Figure 6 &amp; 7 G-L</b>						
Group 1 (WT)	M	8 weeks	15	15	No	BMDM's were isolated from WT mice. Transfected with NRF2 WT or NRF2K518R
<b>Figure S1D-E &amp;S3J&amp; S (2) G-K</b>						

Group 1 (WT)	M	8 weeks	10	7	No	rAAV8-D377Y- mPCSK9 injected into WT mice; had been fed an adjusted-calorie (high-fat) diet (HFD) for 16 weeks. Three mice were excluded due to the LDL cholesterol level less than 300mg/dL after 16 weeks of HFD
Group 2 (ERK5S496A)	M	8 weeks	10	7	No	Same as above

**Sample Size:** Please explain how the sample size was decided Please provide details of any a *prior* sample size calculation, if done.

Our preliminary studies showed that the coefficient of covariation was about 30%. A sample size of 15 mice in each group was expected to have 90% power to detect an effect size of 1.226-SD at a two-sided significance level of 0.05 using the two-sample t-test. The effect size of 1.226 corresponds to about 36% mean change between the two groups, for example, atherosclerosis lesion size between wild-type and ERK5 S496A KI groups.

#### **Inclusion Criteria**

Wild-type or ERK5 S496A knock-in (C57BL/6 background) mice at the age of 8-10 weeks will be included.

#### **Exclusion Criteria**

In the AAV-PCSK9 injection model, mice with LDL cholesterol levels less than 300mg/dL after 16 weeks of HFD will be excluded.

#### **Randomization**

All animals were randomly assigned to different experimental groups.

#### **Blinding**

All animal experiments were performed and analyzed in a blinded manner. All data were collected and analyzed by more than one observer, unaware of the group assignment or treatment of the mice.