

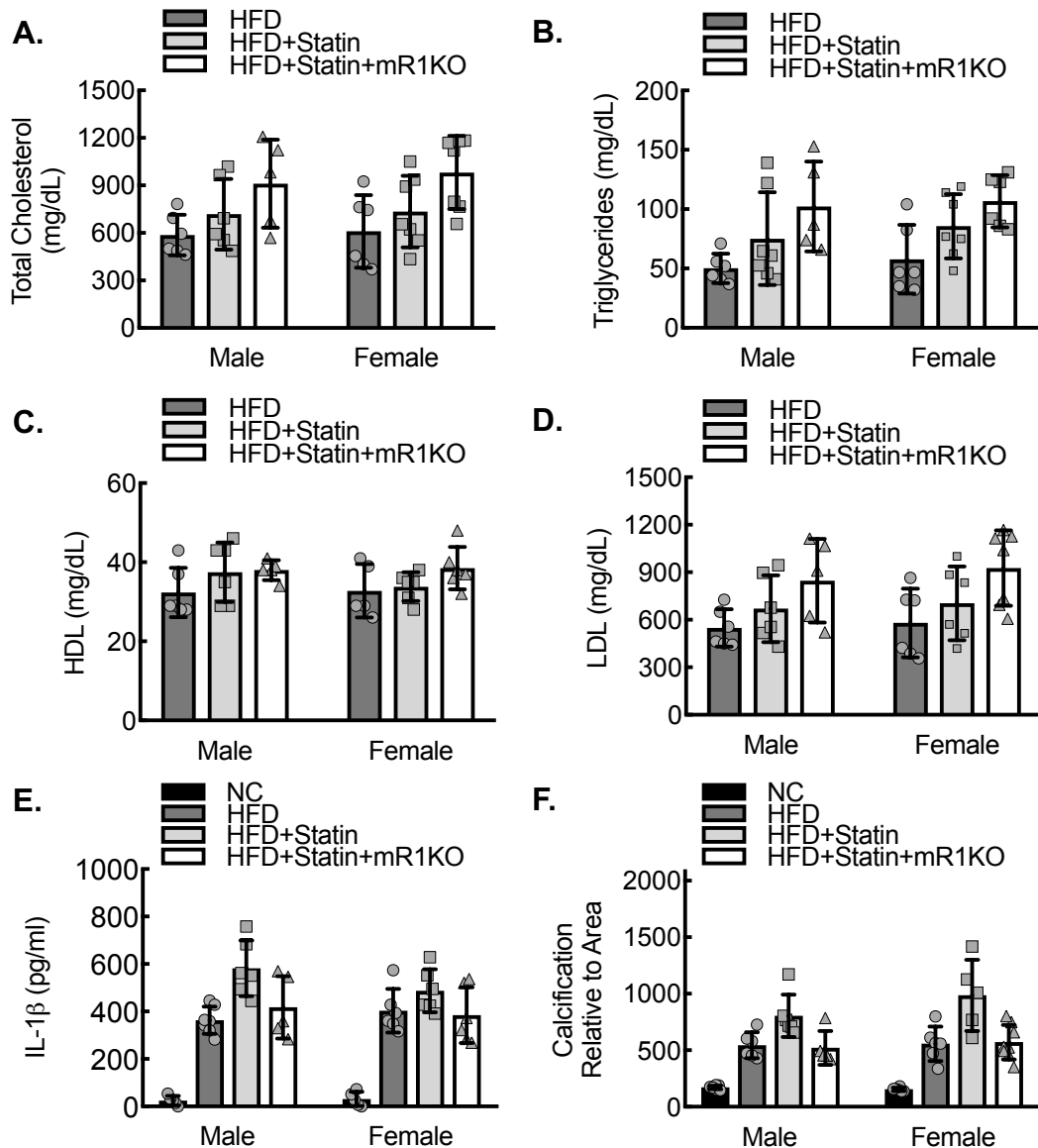
SUPPLEMENTAL MATERIALS

Statins Disrupt Macrophage Rac1 Regulation Leading to Increased Atherosclerotic Plaque
Calcification

Abigail Healy, Joshua M. Berus, Jared L. Christensen, Cadence Lee, Chris Mantsounga, Willie Dong, Jerome P. Watts Jr., Maen Assali, Nicolle Ceneri, Rachael Nilson, Jade Neverson, Wen-Chih Wu, Gaurav Choudhary, and Alan R. Morrison*

Providence VA Medical Center, Providence, Rhode Island 02908, USA.

Department of Internal Medicine (Section of Cardiovascular Medicine), Alpert Medical School at Brown University, Providence, Rhode Island, 02903 USA.



Supplemental Figure 1. *ApoE*^{-/-}*CSF1R*^{mcm}*Rac1*^{fl/fl} mice fed a HFD supplemented with atorvastatin do not demonstrate significant sex differences in lipid metabolism, serum IL-1 β expression or atherosclerotic calcification. Serum total cholesterol (A), triglycerides (B), HDL (C), and LDL (D) concentrations after 18 weeks of HFD, HFD+Statin or HFD+Statin+mR1KO (no significance between male and female by ANOVA; $n=6$ male and 6 female). (E) Serum IL-1 β concentrations by ELISA from *ApoE*^{-/-}*CSF1R*^{mcm}*Rac1*^{fl/fl} mice after 18 weeks of normal chow, HFD, HFD+Statin or HFD+Statin+mR1KO (no significance between male and female by ANOVA; $n=6$ male and 6 female). (F) Quantification *ex vivo* near-infrared calcium imaging from mice on normal chow, HFD, HFD+Statin or HFD+Statin+mR1KO for 18 weeks (no significance between male and female by ANOVA; $n=6$ male and 6 female).

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J (ApoE ^{-/-}) #002052	F, M
Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J (ApoE ^{-/-}) #002052, Tg(Csf1r-Mer-iCre-Mer)1Jwp (CSF1R ^{mcm}) #019098, Rac1 ^{tm1Djk} /J (Rac1 ^{fl/fl}) #005550	F, M

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent 1.1 - Male	Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J #002052	
Parent 1.2 - Female	Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J #002052	
Parent 2.1 - Male	Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J #002052, Tg(Csf1r-Mer-iCre-Mer)1Jwp #019098, Rac1 ^{tm1Djk} /J #005550	
Parent 2.2 - Female	Mouse	The Jackson Laboratory	B6.129P2-ApoE ^{tm1Unc} /J #002052, Tg(Csf1r-Mer-iCre-Mer)1Jwp #019098, Rac1 ^{tm1Djk} /J #005550	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
Activated Rac1-GTP	New East Biosciences	26903	1:100 (1mg/mL)	

Anti-Actin, Alpha-Smooth Muscle-cy3 Mouse Monoclonal	Sigma-Aldrich	C6198	1:200 (1mg/mL)	
Anti-CD68 Rat Monoclonal	BioLegend	137002	1:100 (0.5mg/mL)	
Anti-Alkaline Phosphatase, Tissue Non-Specific Antibody	Abcam	Ab65834	1:500 (0.5mg/mL)	
Anti-Rac1 Antibody	Abcam	Ab97732	1:100 (1mg/mL)	
Goat Anti-Rat IgG H&L (Cy5)	Abcam	Ab6565	1:500 (1mg/mL)	
Goat Anti-Mouse IgG, IgM (H+L), Alexa Fluor 488	Invitrogen	A10680	1:500 (2mg/mL)	
Anti-RUNX2 Antibody	Abcam	Ab76956	1:100 (1mg/mL)	
Goat Anti-Mouse IgG2b, Alexa Fluor 488	Invitrogen	A21141	1:500 (2mg/mL)	
Goat Anti-Rabbit IgG, Alexa Fluor 488	Invitrogen	A27034	1:500 (1mg/mL)	
Goat Anti-Mouse IgM, Alexa Fluor 488	Invitrogen	A21042	1:500 (2mg/mL)	

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Primary Bone Marrow Derived Macrophages	Above animals	F, M