SUPPLEMENTAL MATERIALS

Drug Targeting of Plasminogen Activator Inhibitor-1 Inhibits Metabolic Dysfunction and Atherosclerosis in a Murine Model of Metabolic Syndrome

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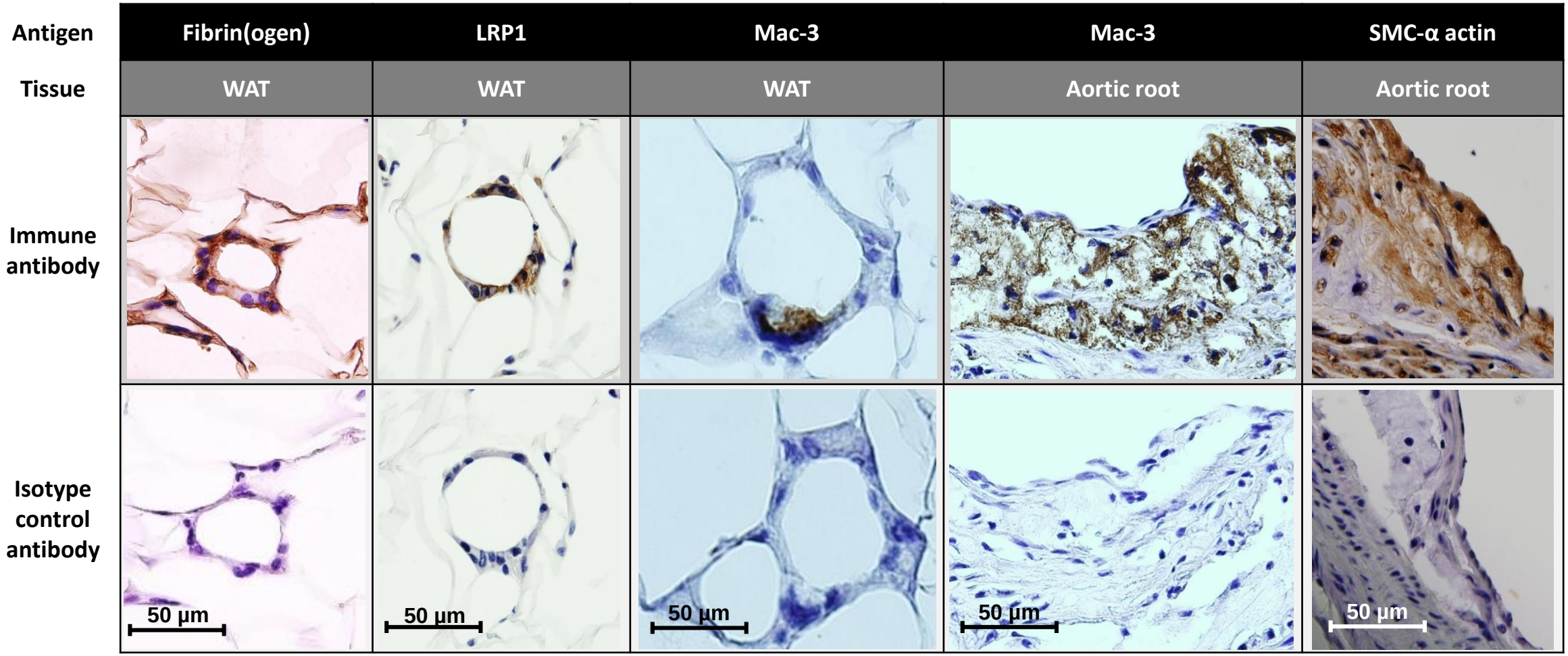
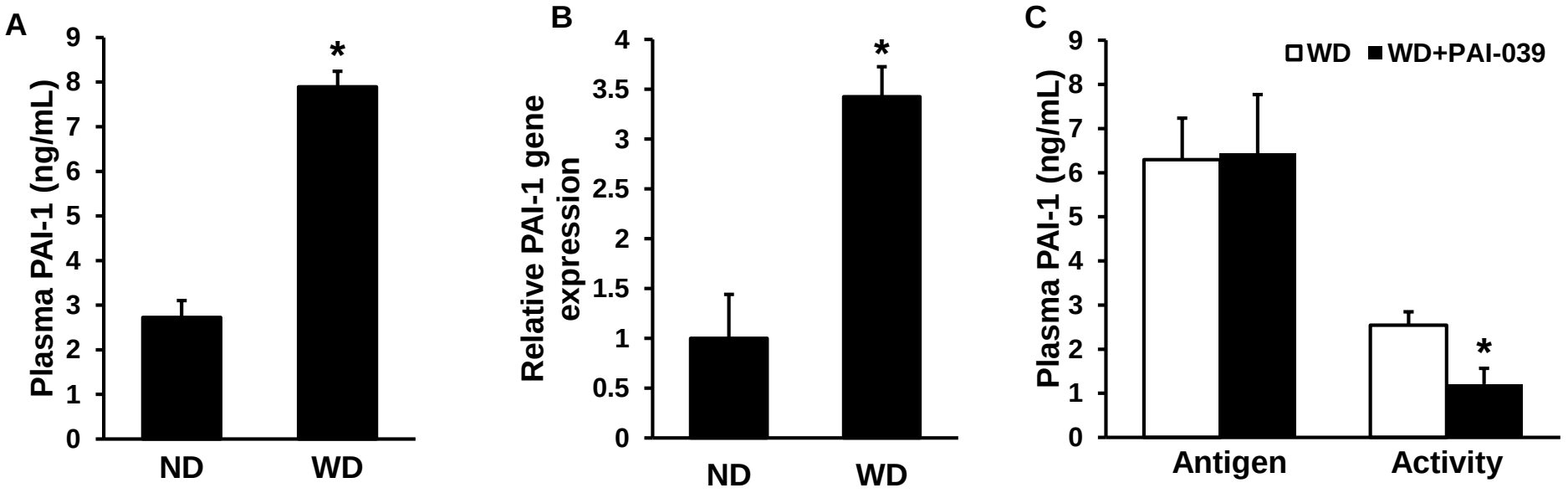
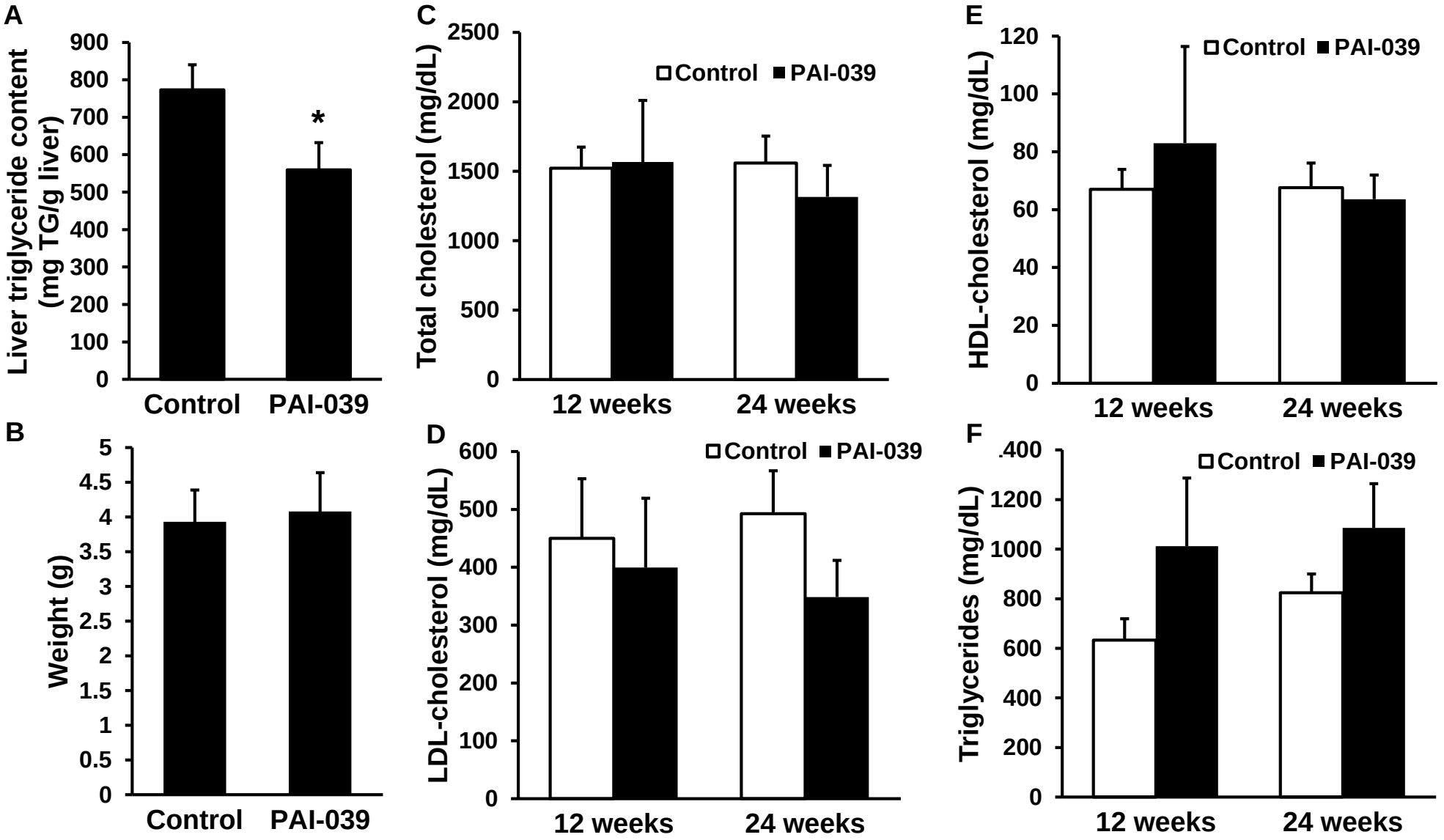


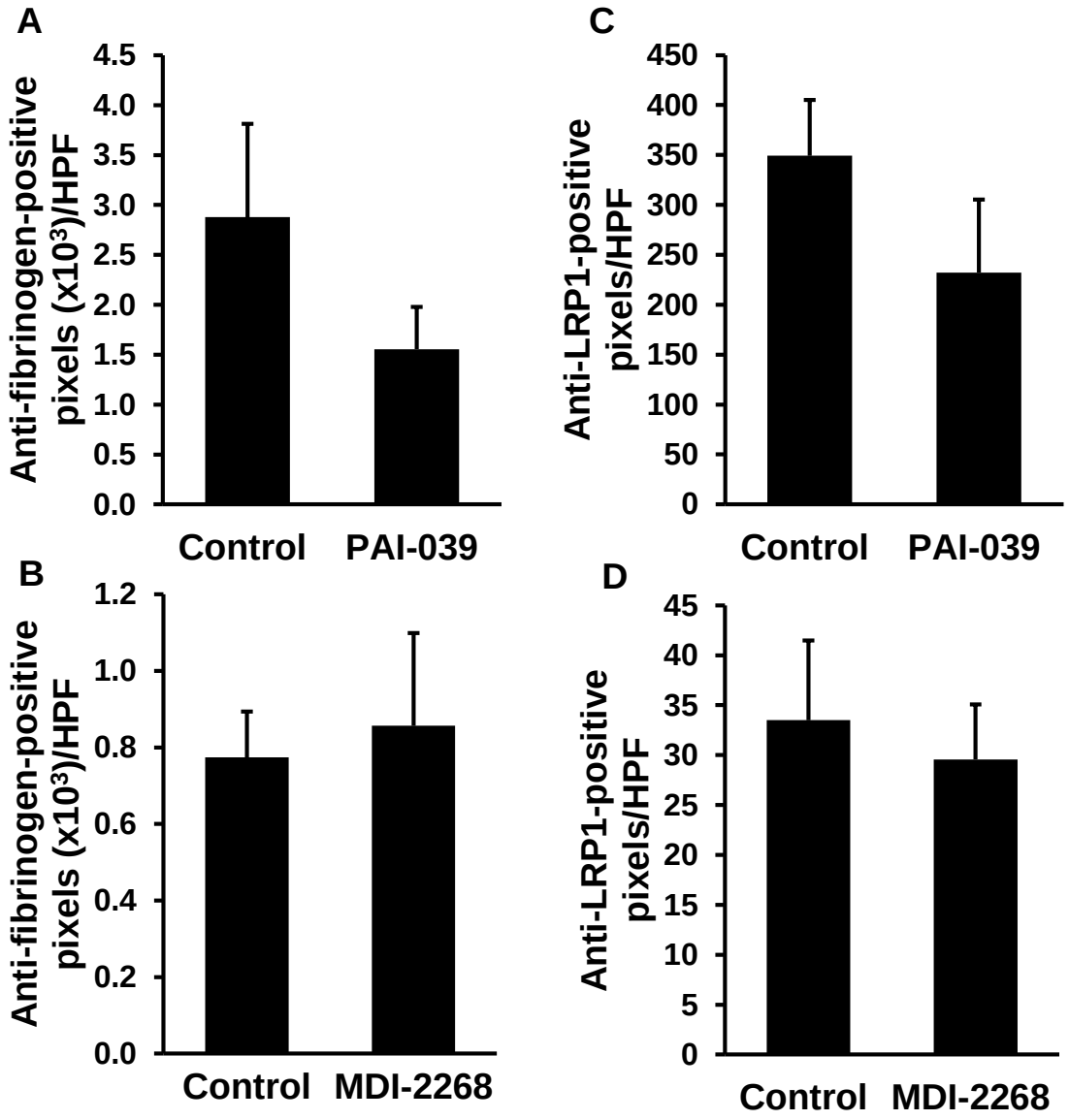
Supplementary Figure I. Demonstration of specificity of antibodies used in immunohistochemical (IHC) analyses. For each antigen shown, consecutive cross-sections (thickness 5-10 µm) were subjected to IHC analysis, as described in methods, using either immune or isotype control primary antibodies, as shown. Positive immunostaining (brown color) is observed with immune antibody, but not with isotype control antibody. SMC, smooth muscle cell; WAT, white adipose tissue.



Supplementary Figure II. Effects of diet and PAI-039 on PAI-1 expression. (A) Plasma PAI-1 antigen is significantly increased in male *ldlr*$^{-/-}$ mice fed western diet (WD) for 4 weeks compared to sex- and age-matched *ldlr*$^{-/-}$ mice maintained on a normal laboratory diet (ND); *P<0.001, n=6/group. **(B)** PAI-1 gene expression is significantly increased in visceral white adipose tissue of *ldlr*$^{-/-}$ mice fed WD compared to those maintained on ND; *P<0.01, n=6/group. **(C)** PAI-039 inhibits plasma PAI-1 activity in *ldlr*$^{-/-}$ mice, assessed after 12 weeks of WD; but does not significantly change PAI-1 antigen (n=4-5/group; *P<0.05).



Supplementary Figure III. PAI-039 decreases steatohepatosis without significantly altering plasma lipid profile. Livers (A-B) and plasma samples (C-F) of *ldlr*$^{-/-}$ mice were assessed after 12 and 24 weeks of WD with or without PAI-039. **(A)** Liver triglyceride content assessed after 12 weeks of WD and normalized to organ's weight; n=7/group; *P<0.05. TG, triglyceride. **(B)** Total liver weight assessed after 24 weeks of WD; n=4-5/group; P>0.8. **(C)** Total cholesterol, **(D)** LDL-cholesterol, **(E)** HDL-cholesterol, and **(F)** Triglycerides levels. In panels C-F, differences between groups did not achieve statistical significance (P>0.1; n=4-5/group). TG, triglyceride.



Supplementary Figure IV. Pharmacologic PAI-1 inhibition does not significantly alter visceral adipose tissue fibrin(ogen) or LRP1 content. Epididymal white adipose tissue of *ldlr*$^{-/-}$ mice was harvested after 12 weeks of WD with or without a PAI-1 inhibitor. **(A)** PAI-039 does not significantly affect fibrin(ogen) deposition in epididymal white adipose tissue; n=4-5/group; P>0.2. **(B)** MDI-2268 does not significantly affect fibrin(ogen) deposition in epididymal white adipose tissue; n=6-7/group; P>0.7. **(C)** PAI-039 does not significantly affect LRP1 content in epididymal white adipose tissue; n=4-5/group; P>0.6. **(D)** MDI-2268 does not significantly affect LRP1 content in epididymal white adipose tissue; n=6-7/group; P>0.2. HPF, high-power field.

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mouse	The Jackson Laboratory	B6.129S7Ldlr<tm1Her>/J HOM Homozygous for Ldlr<tm1Her>	Male
Mouse	The Jackson Laboratory	C57BL/6J	Female

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	mouse	The Jackson Laboratory	B6.129S7Ldlr<tm1Her>/J HOM Homozygous for Ldlr<tm1Her>	-
Parent - Female	mouse	The Jackson Laboratory	C57BL/6J	-

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot #
Mac 3	BD Pharmingen	553322	2 µg/mL	-
LDL receptor-related protein 1	Dudley Strickland, PhD, University of Maryland		0.01 mg/mL	-
LDL receptor-related protein 1	Abcam	Ab92544	4.6µg/mL	-
Alpha-actin	Santa Cruz Biotechonology	Sc-32251	1µg/mL	-
Fibrin(ogen)	Abcam	Ab34269	Dilution 1:200	-
Mouse PAI-1 (capture antibody)	Molecular innovations	H34G6	1µg/mL	#ASMAPAI-GF-BIO-819
Mouse PAI-1 (biotinylated primary antibody)	Molecular innovations	ASMPAI-GF-BIO	1µg/mL	#H34G6-119

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Human coronary artery smooth muscle cells	ThermoFisher Scientific, Lot #1689414	Female