SUPPLEMENTAL MATERIALS

Regulation of Stress Granule Formation by Inflammation, Vascular Injury, and Atherosclerosis

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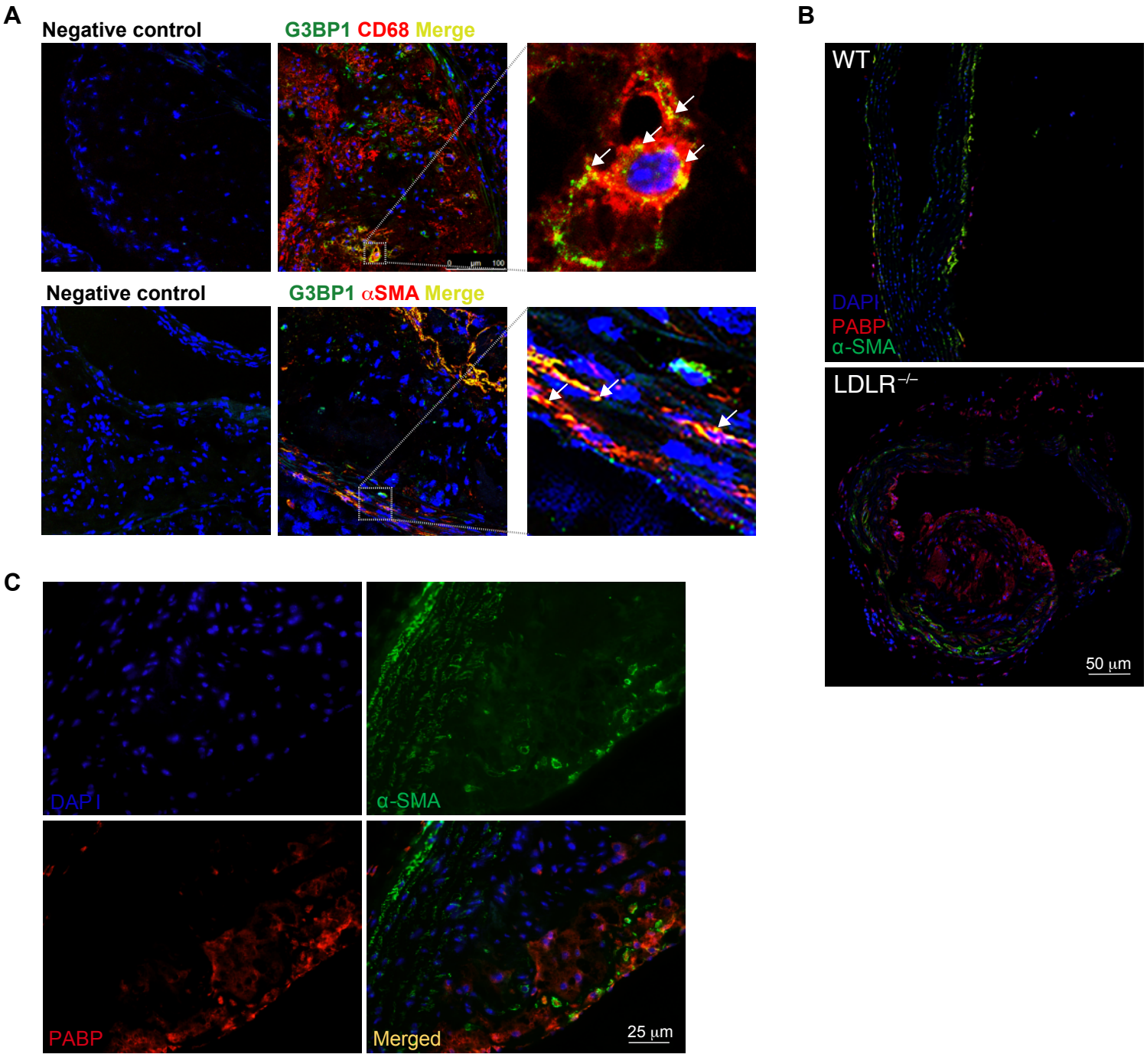
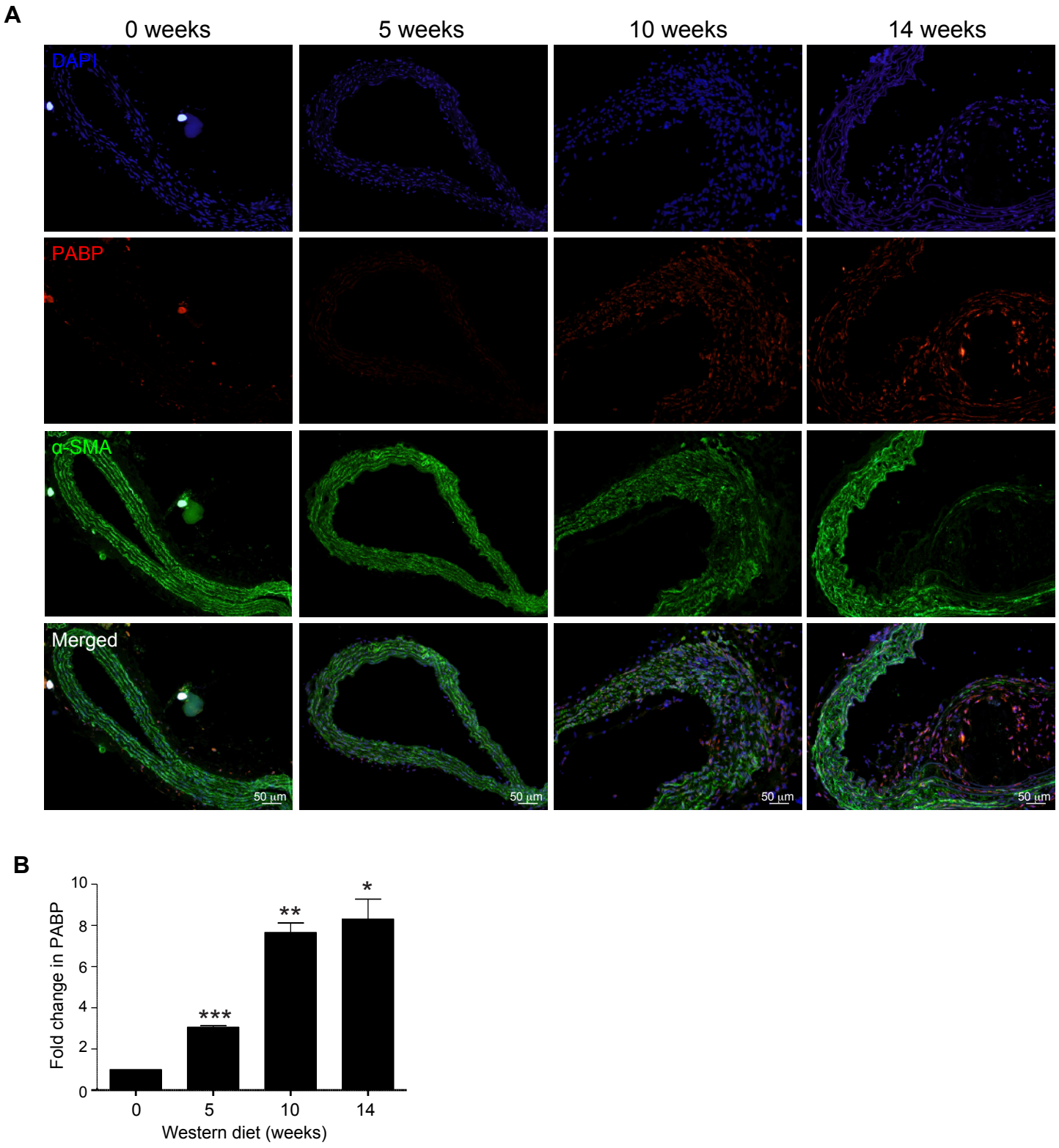
Supplementary Figure I



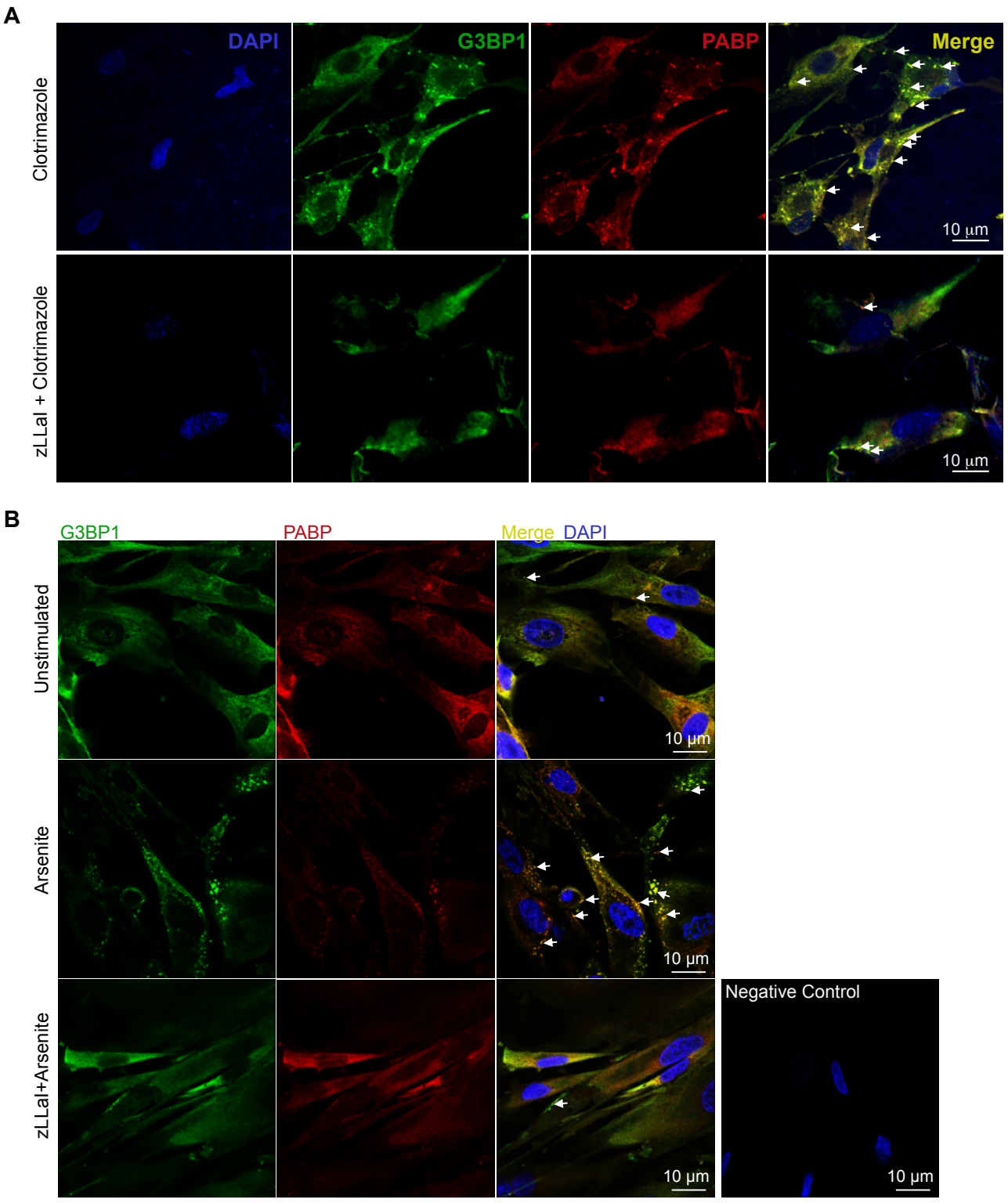
Supplemental Figure I. Expression of SG markers in atherosclerotic plaque. (A) G3BP1 costaining with macrophage and VSMC markers in plaque from Ldlr-/- mice fed a western diet for 14 weeks. (B) Poly-A Binding Protein (PABP) expression in atherosclerotic plaque. Very little PABP was detected in Ldlr-/- mice fed a normal laboratory diet, but large amount of PABP is detected in plaqe from Ldlr-/- mice fed a western diet for 14 weeks. Magnification 400X. (C) Colocalization PABP and a-smooth muscle actin in atherosclerotic plaque from Ldlr-/- mice fed a western diet for 14 weeks. Co-localization of PABP and smooth muscle cells showed that PABP expression was increased specifically in the synthetic intima and cap layer of smooth muscle cells juxtaposed to the plaque, as compared to the quiescent and contractile medial smooth muscle cells in these lesions. Magnification 600X.

Supplementary Figure II



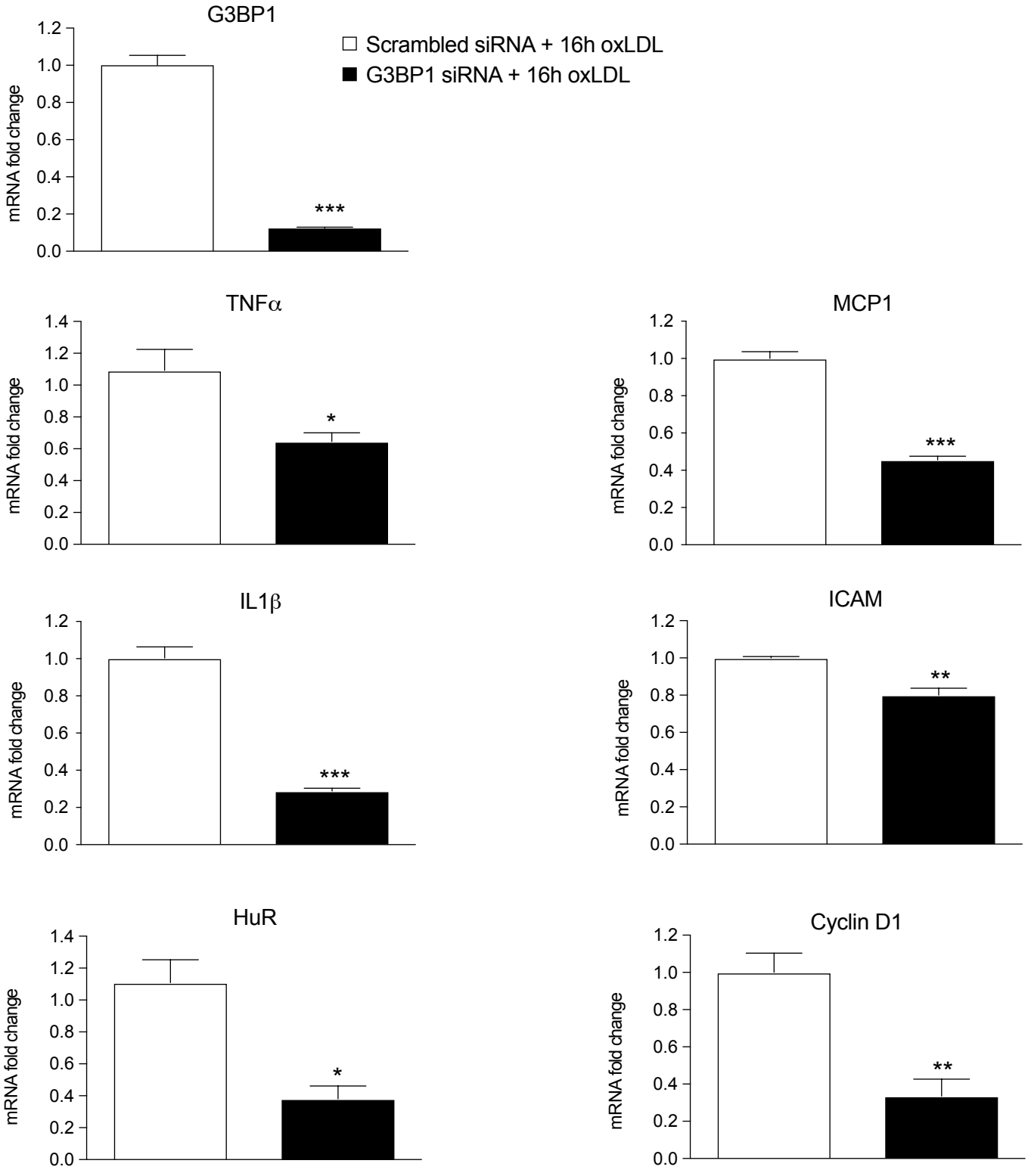
Supplemental Figure II. PABP expression correlates with atherogenesis in LDLR–/– mice. Cumulative staining of the stress granule-specific marker Poly-A Binding Protein (PABP) with atherosclerosis progression. (A) Representative images of aortic arch sections from Ldlr–/– mice fed western diet for 0, 5, 10, and 14 weeks that were immunostained for PABP (red), alpha smooth muscle actin (green), and DAPI (blue). (B) Quantification of PABP staining of aortic arch sections by corrected total cell fluorescence (CTCF). *P≤0.05, ** P≤0.01, or *** P≤0.001.

Supplementary Figure III



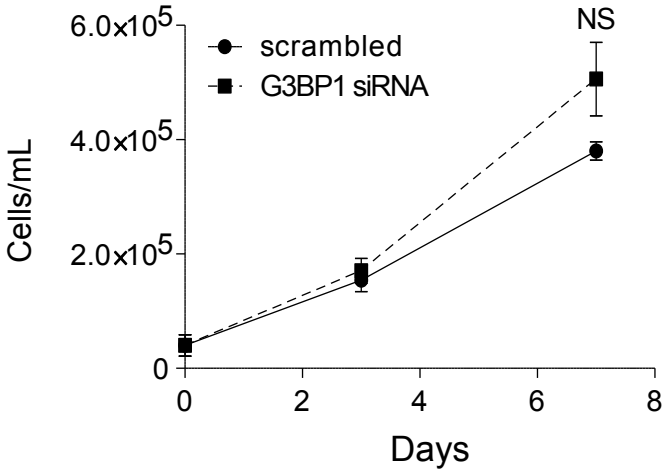
Supplemental Figure III. Immunostaining for PABP (red), G3BP (green) and DAPI (blue) in human VSMCs untreated or pretreated with the calpain inhibitor zLLaI prior to stimulation with clotrimazole (A), and Arsenite (B). Data are representative of 3 independent experiments.

Supplementary Figure IV



Supplemental Figure IV. HVSMCs transfected with siRNA targeting G3BP1 or scrambled control were serum-starved and stimulated with oxLDL for 16 hours, RNA collected, and expression of atherosclerosis-related transcripts were quantitated by PCR. *P≤0.05, ** P≤0.01, or *** P≤0.001.

Supplementary Figure V



Supplemental Figure V. G3BP1 knockdown does not affect VSMC proliferation. HVSMCs were transfected with G3BP1 siRNA or scrambled control siRNA for 48h. Cells were seeded at 10,000 cells/well and counted at days 3 and 7.

Supplemental Methods

Primers used for quantitative reverse transcription PCR were obtained from Integrated DNA Technologies, Inc., Coralville, Iowa.

hGAPDH:	F: CGAGAGTCAGCCGCATCTT,	R: CCCCATGGTGTCTGAGCG
hIL-1β:	F: TCCCCAGCCCTTTTGTTGA,	R: TTAGAACCAAATGTGGCCGTG
hICAM1:	F: CTCCAATGTGCCAGGCTTG,	R: GAGTGGGAAAGTGCCATCCT
hHuR:	F: CCGTCA CAAATGTGAAAGTG,	R: TCGCGGCTTCTTCATAGTTT
hTNFα:	F: GGTCTACTTTGGGATCATTGC,	R: GAAGAGGTTGAGGGTGTCTG
hMCP1:	F: AGCAGAAGTGGGTTCAGGATT,	R: TGTGGAGTGAGTGTTCAAGTCT
hG3BP1:	F: AGTTATGGAAACGTGGTGGAG ,	R: GACATTCAGACGGACCTCAC
hcyclin D1:	F: TATTGCGCTGCTACCGTTGA,	R: CCAATAGCAGCAAACAATGTGAAA

Table 1. Primer pairs used for quantitative RT-PCR.

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mouse	The Jackson Laboratory	C57BL/6 LDLR-/-	M/F
Mouse	The Jackson Laboratory	C57BL/6	M/F

Animal breeding. Not applicable

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
G3BP1	Santa Cruz	Sc365338	WB 6ug/ml, IF 1ug/ul	
PABP	Abcam	Ab21060	WB 6ug/ul, IHC 1ug/ul (cells), 2ug/ml IHC (tissue)	
FXR1	AbCam	Ab50841	5ug/ml	
HuR	AbCam	Ab200342	1ug/ml	
p-eIF2alpha	AbCam	Ab32157	6ug/ml	
Total eIF2alpha	AbCam	Ab5369	6ug/ml	
HSC70	Santa Cruz	Sc7298	0.2ug/ml	
Alpha tubulin	Sigma Aldrich	T6074-200UL	1:1000 of 200ug/ml stock	
HSP90	BD Transduction Laboratories	610419	0.25ug/ml	
Alpha smooth muscle actin	AbCam	Ab21027	2.5ug/ul	
G3BP1	AbCam	181150	0.7ug/ml	
CD68	Bio-Rad	MCA1957	0.25ug/ml	
Smooth muscle cell alpha actin	ThermoFisher	53-9760-82	1.0ug/ml	
G3BP	AbCam	Ab181150	1ug/ml (staining)	

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Human Vascular Smooth Muscle cells	LifeLine	Male
bone marrow derived macrophages	From tibiae and femorae of C57Bl/6 mice	Male and Female