Supporting Information

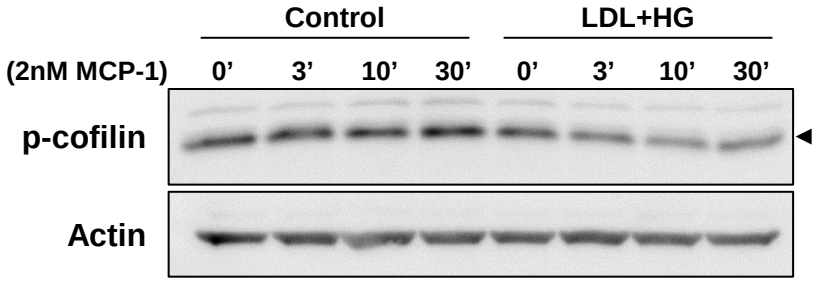


Fig. SI. MCP-1 induced cofilin phosphorylation is suppressed in metabolically primed (LDL+HG) monocytes. Arrow head indicates phosphorylated (Ser 3) cofilin.

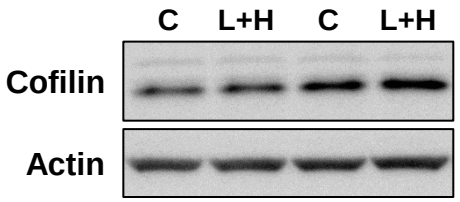


Fig. SII. Cofilin levels do not change in response to metabolic stress (LDL+HG).

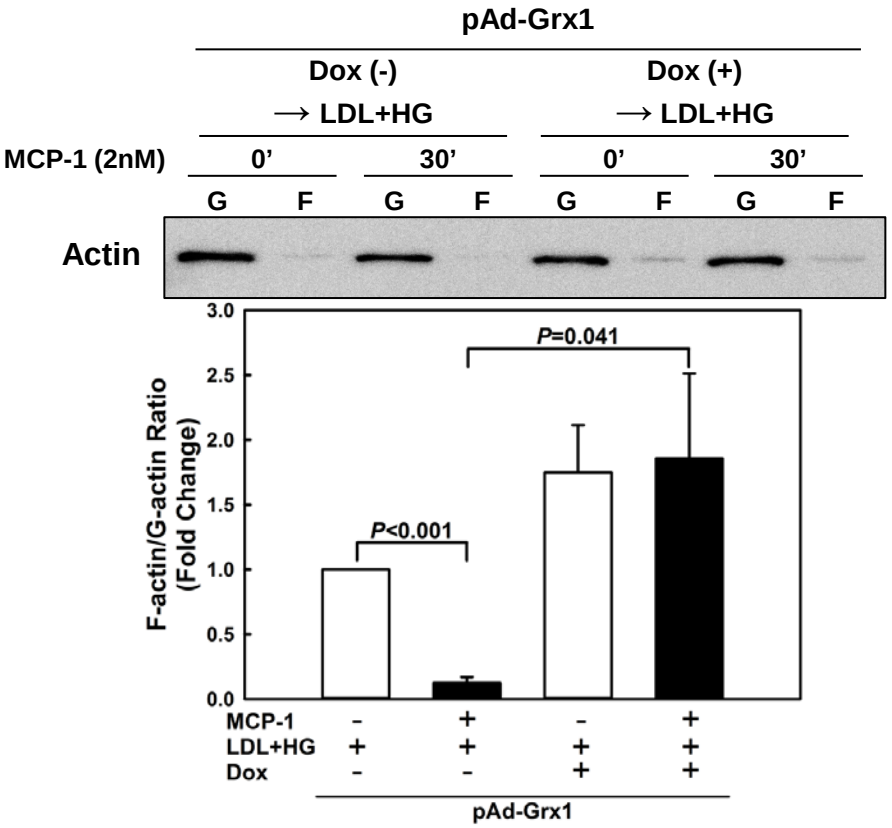


Fig. SIII. Overexpression of glutaredoxin 1 protects metabolically stressed monocytes against accelerated MCP-1-stimulated actin turnover. THP-1 monocytes were infected with a doxycycline (Dox)-inducible adenoviral vector carrying the sequence for a Grx1-EGFP fusion protein (pAd-Grx1). Grx1 expression was induced by adding doxycycline (Dox; 1 μg/ml). THP-1 monocytes were then primed for 24 h with culture medium supplemented with LDL+HG, and stimulated with MCP-1 (2 nM, solid bars) for 30 min. F- and G-actin levels were measured as described under Methods. Results are shown as F-actin/G-actin ratios and are means ± SE of 4 independent experiments.

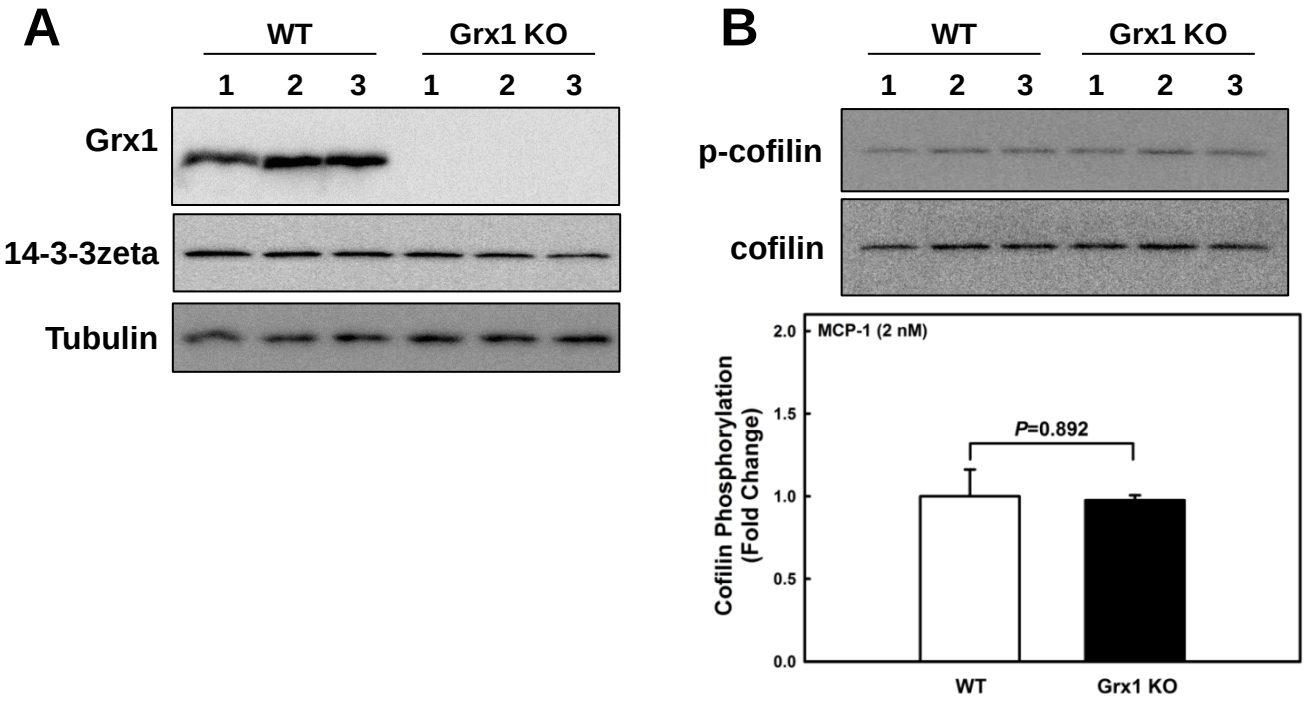


Fig. SIV. Grx1 deficiency alone does not induce a decrease in 14-3-3zeta or cofilin activation. Blood monocytes from wildtype C57BL/6 and Grx1 null mice (n=3 per group) were isolated and 14-3-3zeta protein levels and MCP-1-induced cofilin phosphorylation assessed by Western blot analysis.

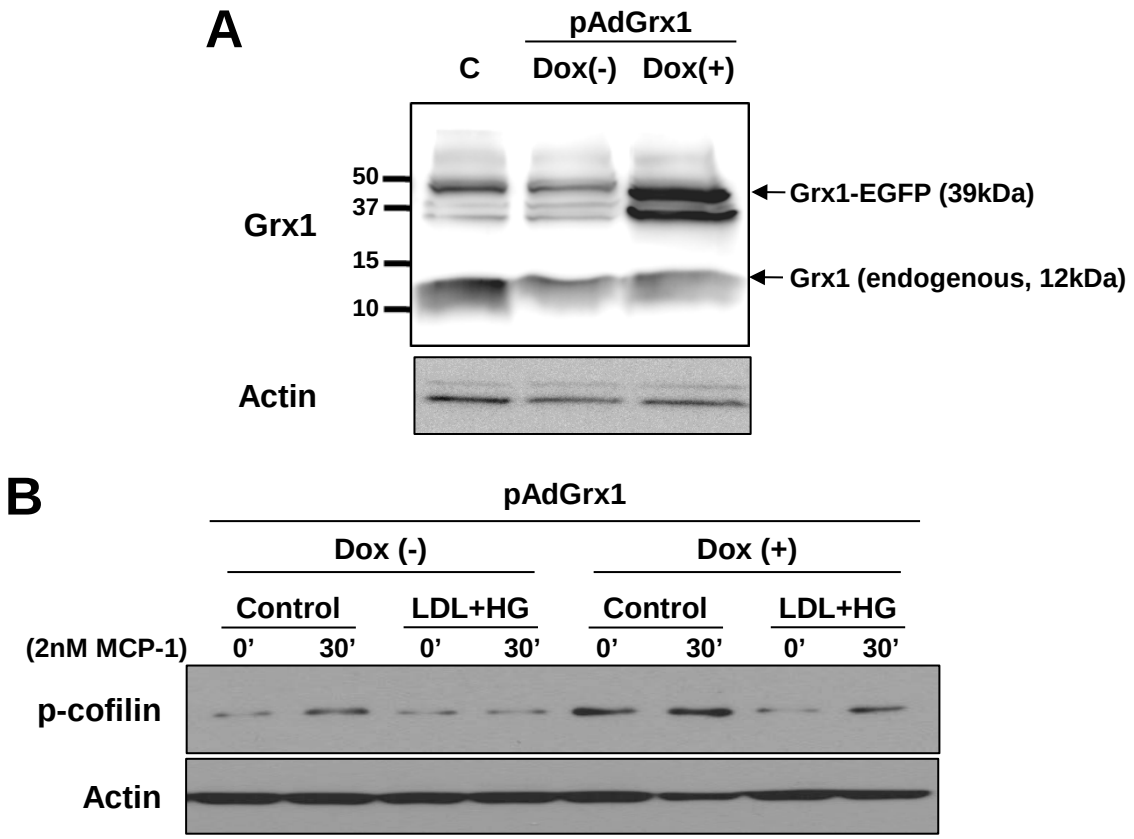


Fig. SV. Grx1 overexpression restores MCP-1-induced cofilin phosphorylation in metabolically primed (LDL+HG) monocytes. THP-1 monocytes were infected with doxycycline-inducible adenoviruses (pAd) carrying a Grx1-EGFP (12kDa + 27kDa = 39kDa) fusion construct. Transgene expression was induced by adding doxycycline (Dox; 1 μg/ml) to the culture medium for 24 h.

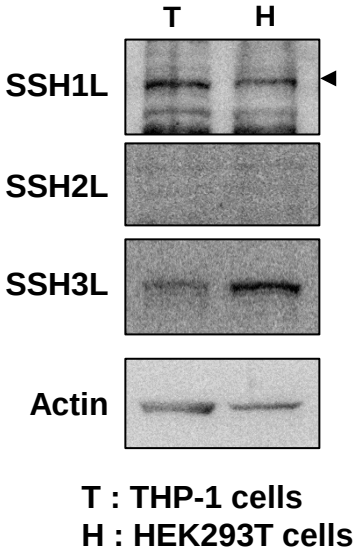


Fig. SVI. Protein expression levels of Slingshot phosphatases (SSH) isoforms in THP-1 monocytes (T) and HEK293T (H) cells (Arrowhead indicates SSH1L band).

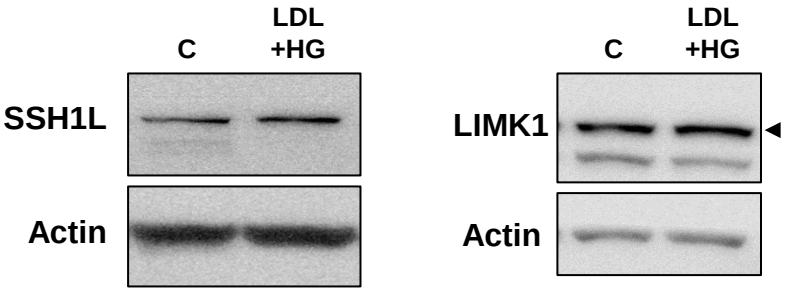


Fig. SVII. Metabolic priming does not change protein levels of SSH1L or LIMK1 in THP-1 monocytes. Protein levels of cofilin phosphatase (Slingshot-1L, SSH1L), cofilin kinase (LIM domain kinase 1, LIMK1) were assessed by Western blot analysis in unprimed (control, **C**) and metabolically primed THP-1 monocytes (**LDL+HG**).

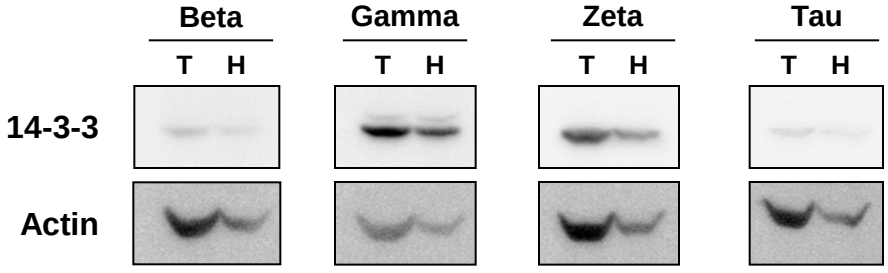


Fig. SVIII. Basal protein expression levels of 14-3-3 isoforms in THP-1 monocytes (T) and HEK293T (H) cells.

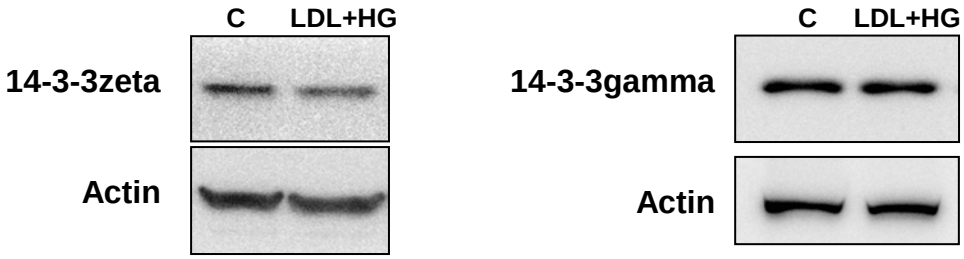


Fig. SIX. 14-3-3zeta protein levels are decreased in LDL+HG-primed THP-1 monocytes.

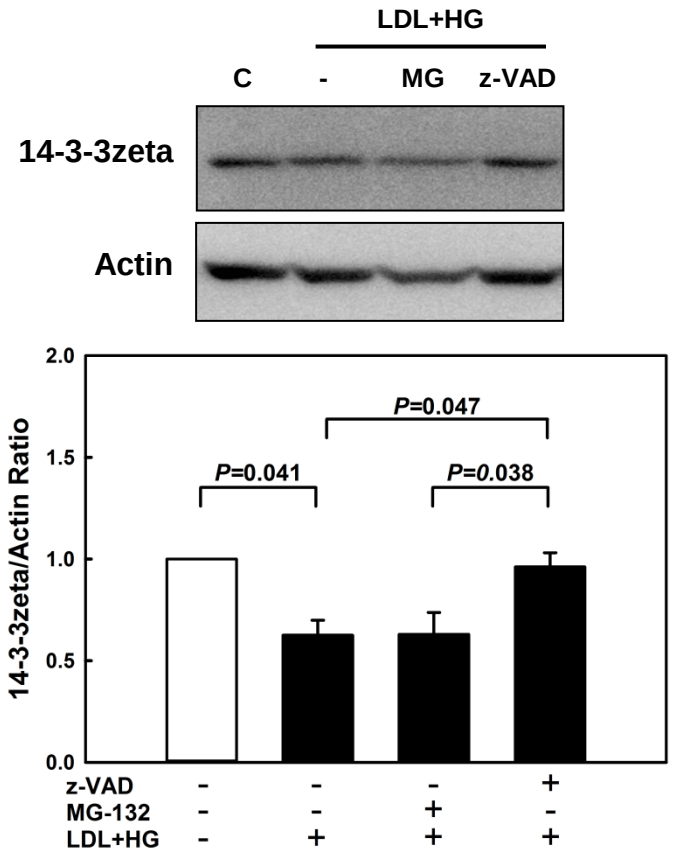


Fig. SX. Metabolic stress induces caspase-dependent degradation of 14-3-3zeta. THP-1 monocytes were primed for 24 h with either vehicle (open bar) or LDL+HG (black bars). Proteasomal inhibitor MG132 (25 µM) or pan caspase inhibitor z-VAD-FMK (10 µM) were added where indicated.

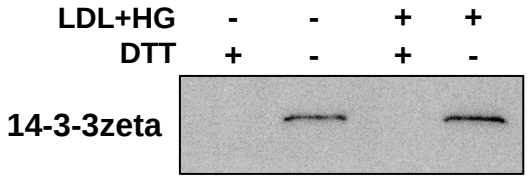


Fig. SXI. *S*-Glutathionylated 14-3-3zeta is increased in metabolically primed (LDL+HG) monocytes. THP-1 monocytes were preincubated with BIOGEE (250 µmol/L) and subsequently primed for 24 h with either vehicle or LDL+HG. Before precipitating biotin-labeled proteins with streptavidin-agarose, lysates were preincubated with either PBS or dithiothreitol (DTT, 10 mmol/L). Precipitates of biotin-labeled proteins were probed for 14-3-3zeta and analyzed by Western blot using an antibody recognizing 14-3-3zeta.

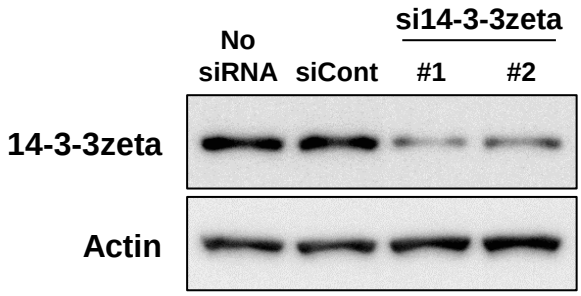


Fig. SXII. Representative Western blot of 14-3-3zeta knockdown in monocytes after siRNA transfection.

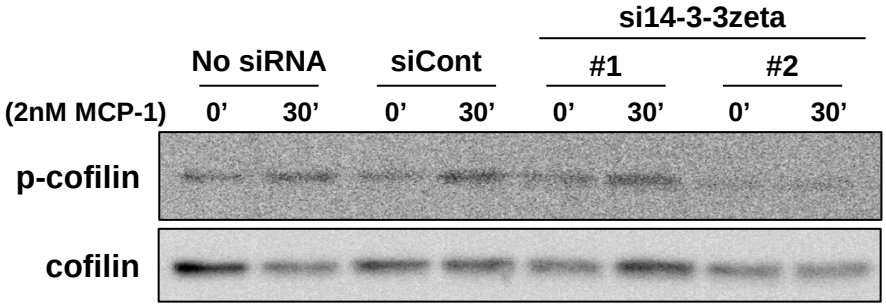


Fig. SXIII. Cofilin phosphorylation is suppressed in monocytes by 14-3-3zeta knockdown after MCP-1 stimulation. (Representative Western blot)

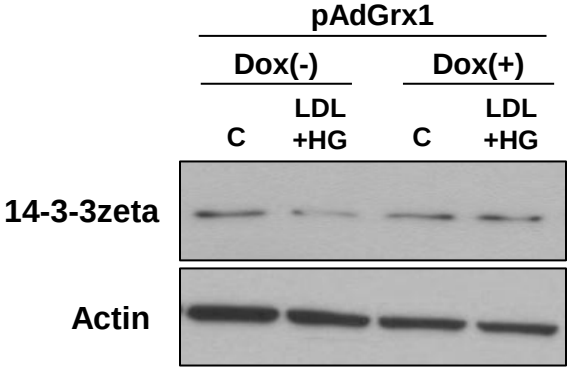


Fig. SXIV. Grx1 overexpression prevents the loss of 14-3-3zeta induced by metabolic priming in THP-1 monocytes. (Representative Western blot)

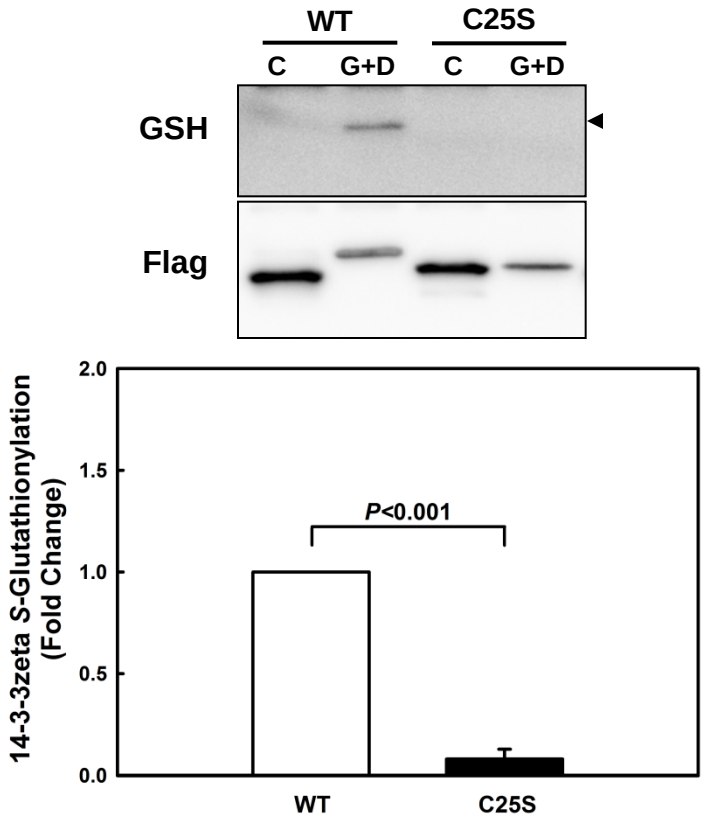


Fig. SXV. Cys[25] is the only *S*-glutathionylated residue in 14-3-3zeta. After HEK 293T cells were transfected for 24 h with either wild type (WT) or mutant (C25S) Flag-14-3-3zeta, cells were subjected to immunoprecipitation with anti-Flag antibodies and *in vitro* *S*-glutathionylation as described in as described in "Materials and Methods.". Arrow head indicates *S*-glutathionylated 14-3-3zeta.

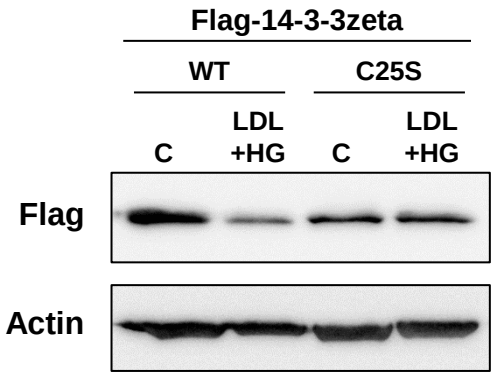


Fig. SXVI. C25S mutant of 14-3-3zeta is resistant to degradation by metabolic stress. THP-1 monocytes were transduced with lentiviral vector carrying the sequence for a Flag-14-3-3zeta (WT) or mutated Flag-14-3-3zeta (C25S). THP-1 monocytes were then treated for 24 h with vehicle or primed with LDL+HG, and Flag-14-3-3zeta levels were measured. (Representative Western blot)

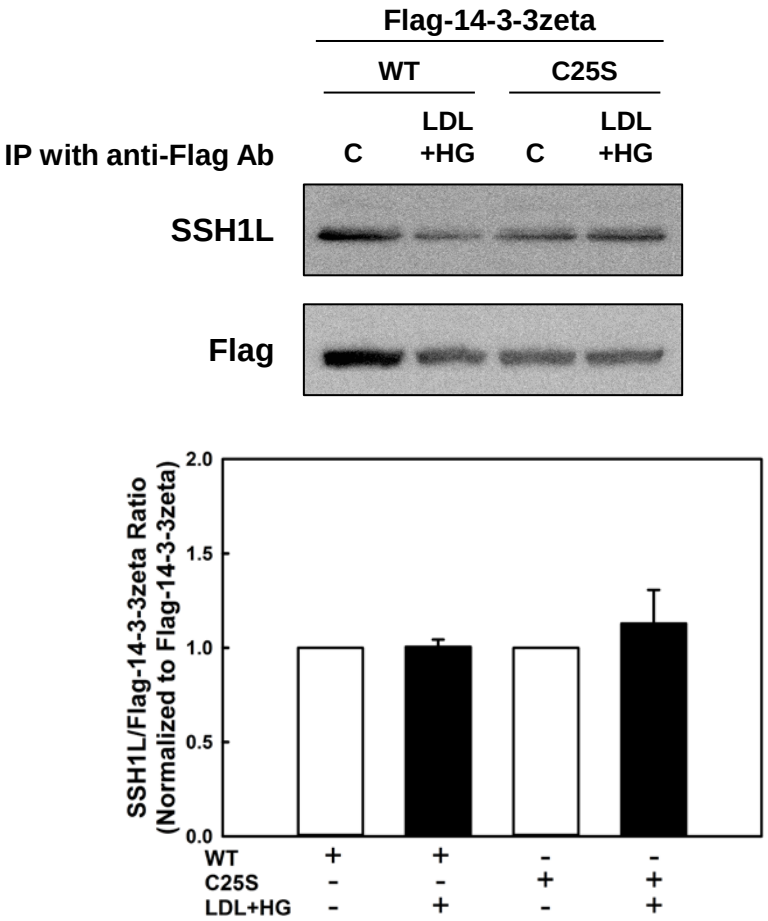


Fig. SXVII. *S*-Glutathionylation of 14-3-3zeta does not change the binding activity to SSH1L. THP-1 monocytes were transduced with lentiviral vectors carrying the sequence for either wild type (WT) or mutant (C25S) Flag-14-3-3zeta. THP-1 monocytes were then treated for 24 h with vehicle or primed with LDL+HG, and subjected to immunoprecipitation (IP) with anti-Flag antibodies. SSH1L bound to 14-3-3zeta and pulled down with anti-Flag antibodies was assessed by Western blot analysis.

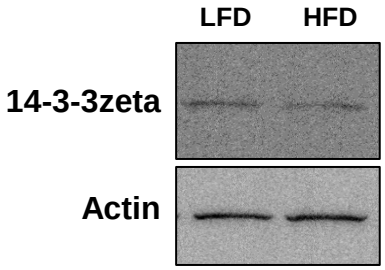


Fig. SXVIII. 14-3-3zeta levels are decreased in blood monocytes of mice suffering from metabolic disorders. Blood monocytes were isolated and purified from LDL receptor-deficient mice that were fed for 10 weeks either a low fat diet (LFD) or a high fat diet (HFD), and monocyte 14-3-3zeta levels were determined by Western blot analysis.

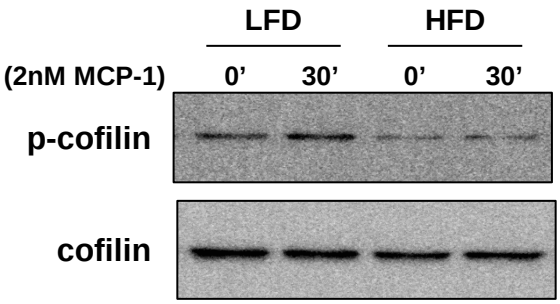
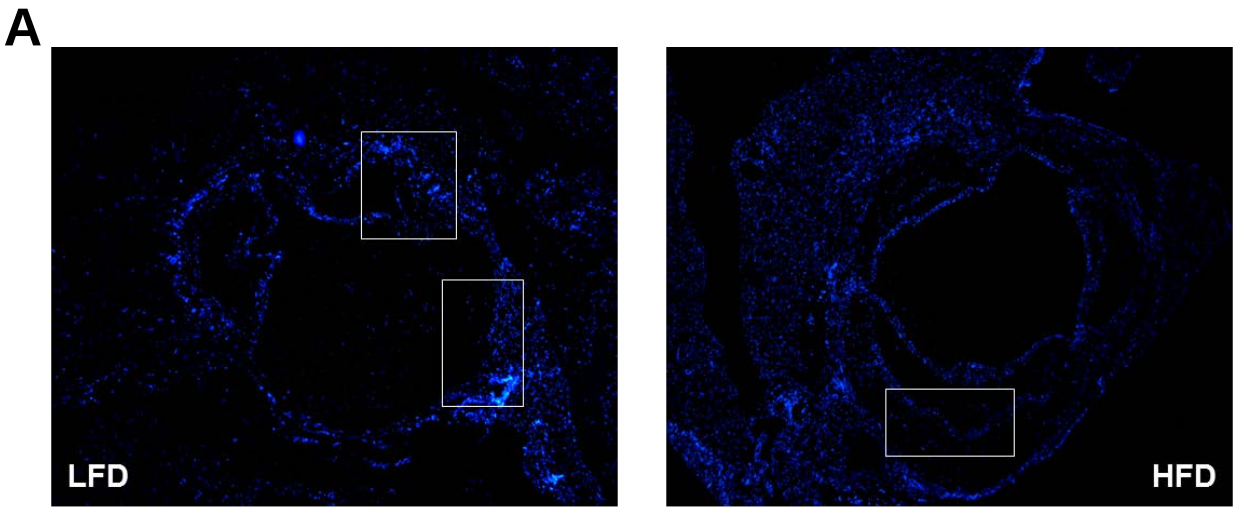
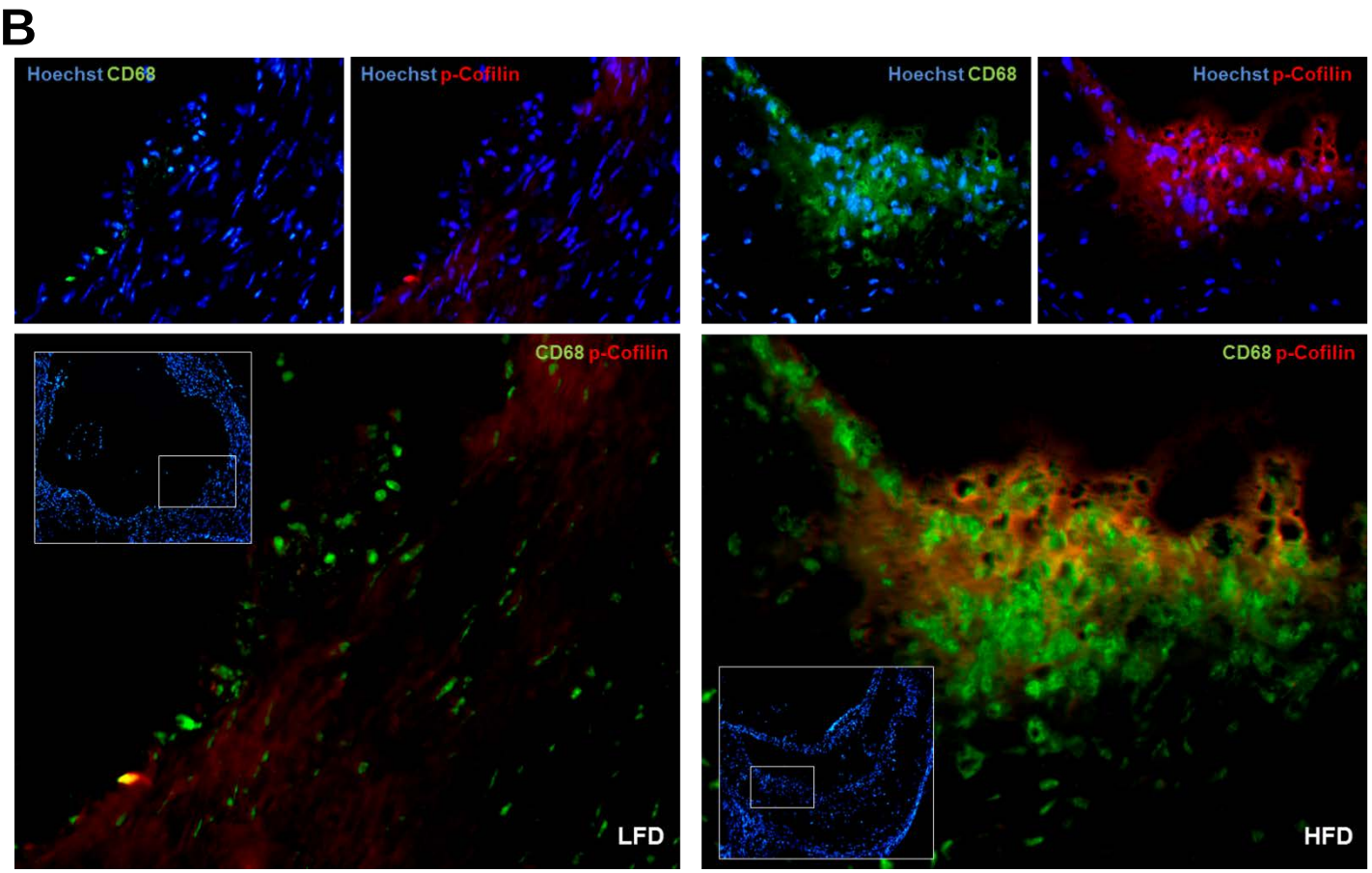


Fig. SXIX. Cofilin phosphorylation is suppressed in blood monocytes of mice suffering from metabolic disorders. (Representative Western blot)



LDL-R$^{-/-}$ mice, low fat diet **LDL-R$^{-/-}$ mice, high fat diet**



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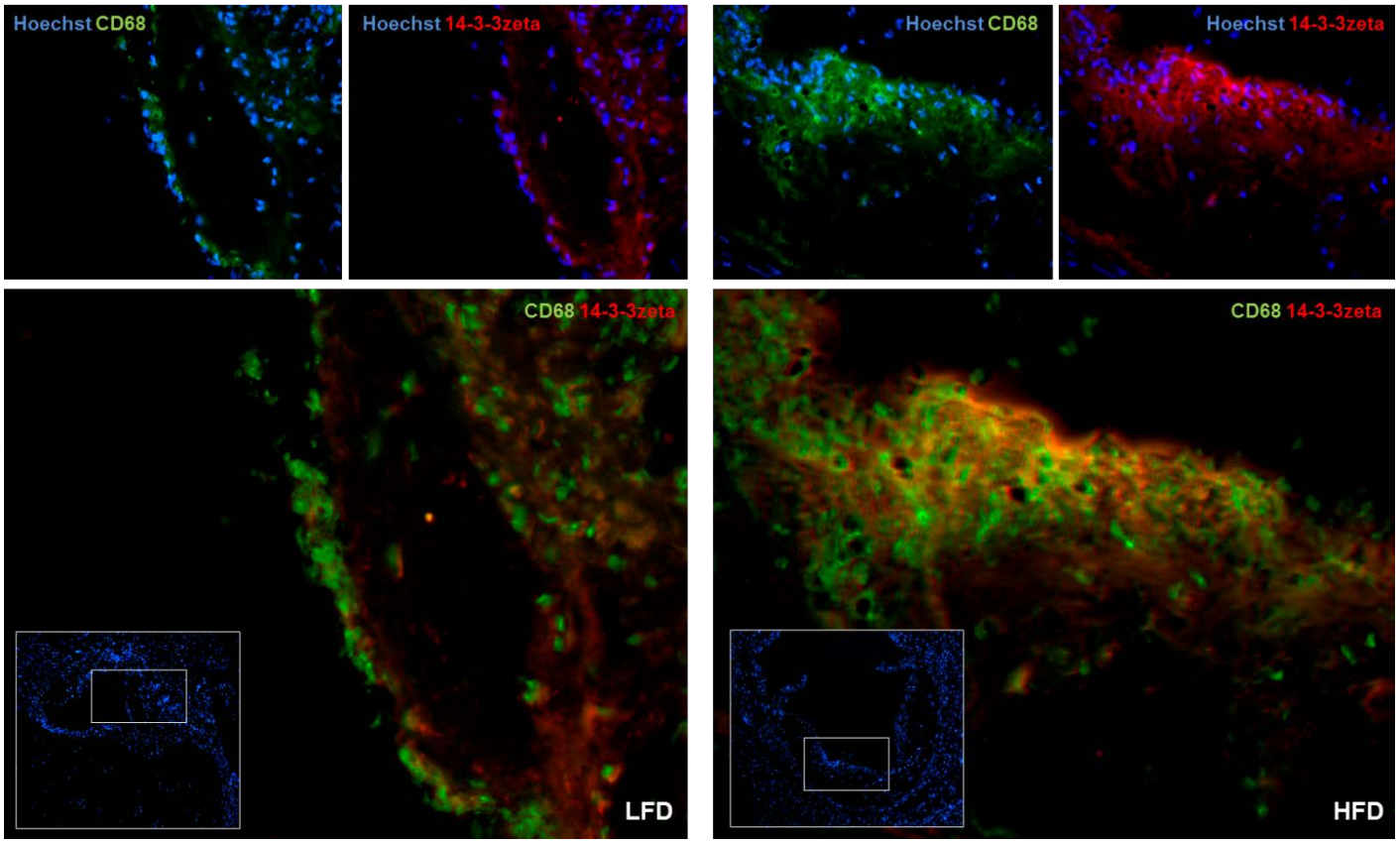

Fig. SXX. 14-3-3zeta levels and phospho-cofilin are localized in macrophage-rich area of atherosclerotic lesions. (A) Aortic roots from LFD fed LDL-R-/- mice and HFD fed LDL-R-/- mice stained Hoechst dye (blue). Magnification ×4. **(B)** Immunostaining images of atherosclerotic lesions with anti-CD68 (green) and anti-p-cofilin (red) antibodies. **(C)** Immunofluorescence images of atherosclerotic lesions stained with anti-CD68 (green) and anti-14-3-3zeta (red) antibodies. Nuclei were stained with Hoechst dye (blue). Enlarged images represent ×40 magnification of the atherosclerotic lesion area enclosed in the white square (×10 magnification).