SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Genotyping LysM-Cre Nox2$^{fl/fl}$ mice – Tail DNA was isolated using REDExtract-N-Amp Tissue Prep (Sigma) and the following primers were used to genotype the mice by PCR: *Nox2* forward: GTAAATTCACTGTTCTGGGTC and *Nox2* reverse: ACATGTTCTTCTCACAGGCTC. *LysM-cre*: CCCAGAAATGCCAGATTACG, *LysM* Common: CTTGGGCTGCCAGAATTTCTC and *LysM* WT: TTACAGTCGGCCAGGCTGAC. To confirm excision of the *Nox2* allele, DNA was isolated from macrophages using REDExtract-N-Amp Tissue Prep and subjected to PCR using the following primers. *Nox2 KO*: GGAATTGAGTTGTAAGAATCAAATGAC, *Nox2 Common*: ATGATGTGTCCCAAATGTGC, and *Nox2* WT: GGGGCTGAATGTCTTCCTCT.

Macrophage clearance of apoptotic adipocytes – Briefly, lysosomes of wild-type or knockout BMDMs were loaded with Biotin-FITC-fluorescein, and plasma membranes of adipocytes were labeled with streptavidin. For lysosomal exocytosis, BMDMs (wild-type or knockout) were incubated with live or TNFα-induced apoptotic 3T3-L1 adipocytes for 90min. The extent of lysosomal exocytosis was quantified by the amount of fluorescein fluorescence captured by adipocytes. For macrophage lipid accumulation, BMDMs were incubated with live or TNFα-induced apoptotic 3T3-L1 adipocytes for 24h and the extent of macrophage lipid accumulation was quantified as the amount of LipidTOX Red staining within the Alexa4888-CtB stained macrophages. For both assays, images were acquired using a Zeiss LSM 880 (Thronwood, NY) laser scanning confocal microscope using a 40x 1.3 NA objective, and quantification was based on 25 fields/experiment with an average of 5 macrophages/field. In some cases, *Gp91-/-* macrophages were treated with TNFα (0.1-10ng/mL) for 16h prior to and during the incubation with live or apoptotic adipocytes.

SUPPLEMENTAL FIGURES

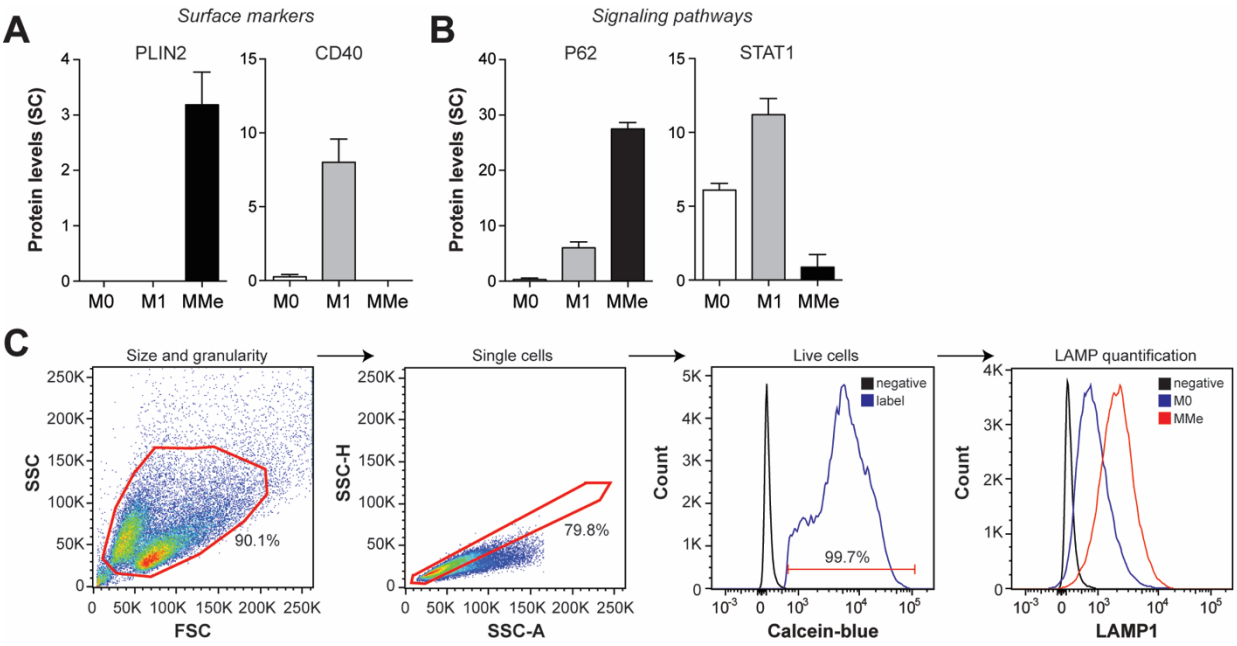

Fig. S1. Analysis of MMe and M1 macrophages *in vitro*. Related to Fig. 1. Bone marrow-derived macrophages were incubated with vehicle control (M0), MMe (palmitate), or M1 (LPS + IFNγ) polarizing stimuli and the plasma membrane proteome was interrogated by mass spectrometry. *Panels A-B*: Levels of proteins associated with the MMe (PLIN2, P62) and M1 (CD40, STAT1) phenotypes were quantified by spectral counting (SC). *Panel C*: Strategy for interrogating cell surface LAMP1 and LAMP2 levels in M0, MMe, and M1 macrophages. Gates (red) represent cells that were selected for further analysis. Quantification of surface LAMP1 levels in M0 and MMe macrophages is shown as an example. Results are mean ± SEM. n=6.

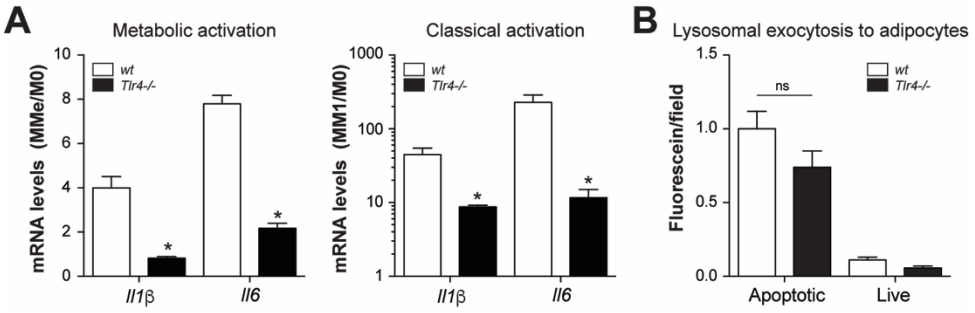

Fig. S2. TLR4 controls inflammatory cytokine expression but not lysosomal exocytosis in MMe macrophages. Related to Fig. 2. *Panel A*: Inflammatory cytokine expression in metabolically activated (MMe) and classically activated macrophages (M1) BMDMs made from wild-type (*wt*) or TLR4-deficient (*Tlr4-/-*) mice. Levels are expressed relative to M0 for each genotype. *Panel B:* Quantification of macrophage lysosomal exocytosis from live or apoptotic 3T3-L1 adipocytes. Results are mean ± SEM. n=6, *, $p<0.05$ Student's *t*-test.

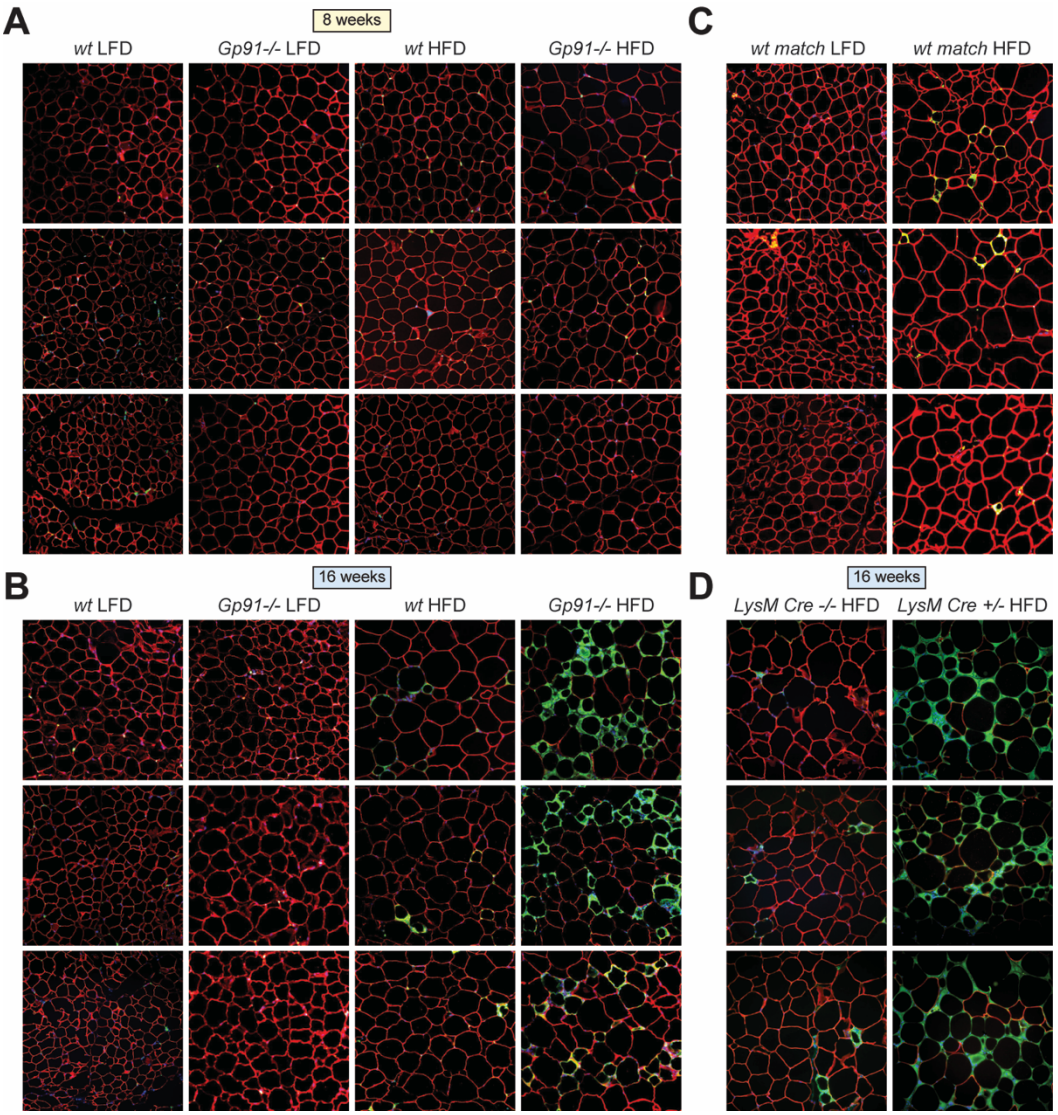

Fig. S3. Representative immunofluorescence images of epididymal fat from mice. Related to Figs. 3-5,7. Epididymal fat was stained with anti-PLIN2 (adipocyte, red), anti-MAC2 (macrophages, green) and DAPI (nuclei, blue) and visualized by microscopy. Panels A-B: *Gp91-/-* and wild type (wt) mice fed a low-fat diet (LFD) or high-fat diet (HFD) for 8 weeks (*Panel A*) or 16 weeks (*Panel B*). Panel C: Wild type mice fed a HFD until their body weight matched *Gp91-/-* mice at 16 weeks (*wt match*). Panel D: *LysM-cre*$^{-/-}$ *Nox2*$^{fl/fl}$ and *LysM-cre*$^{+/-}$ *Nox2*$^{fl/fl}$ mice fed the HFD for 16 weeks.

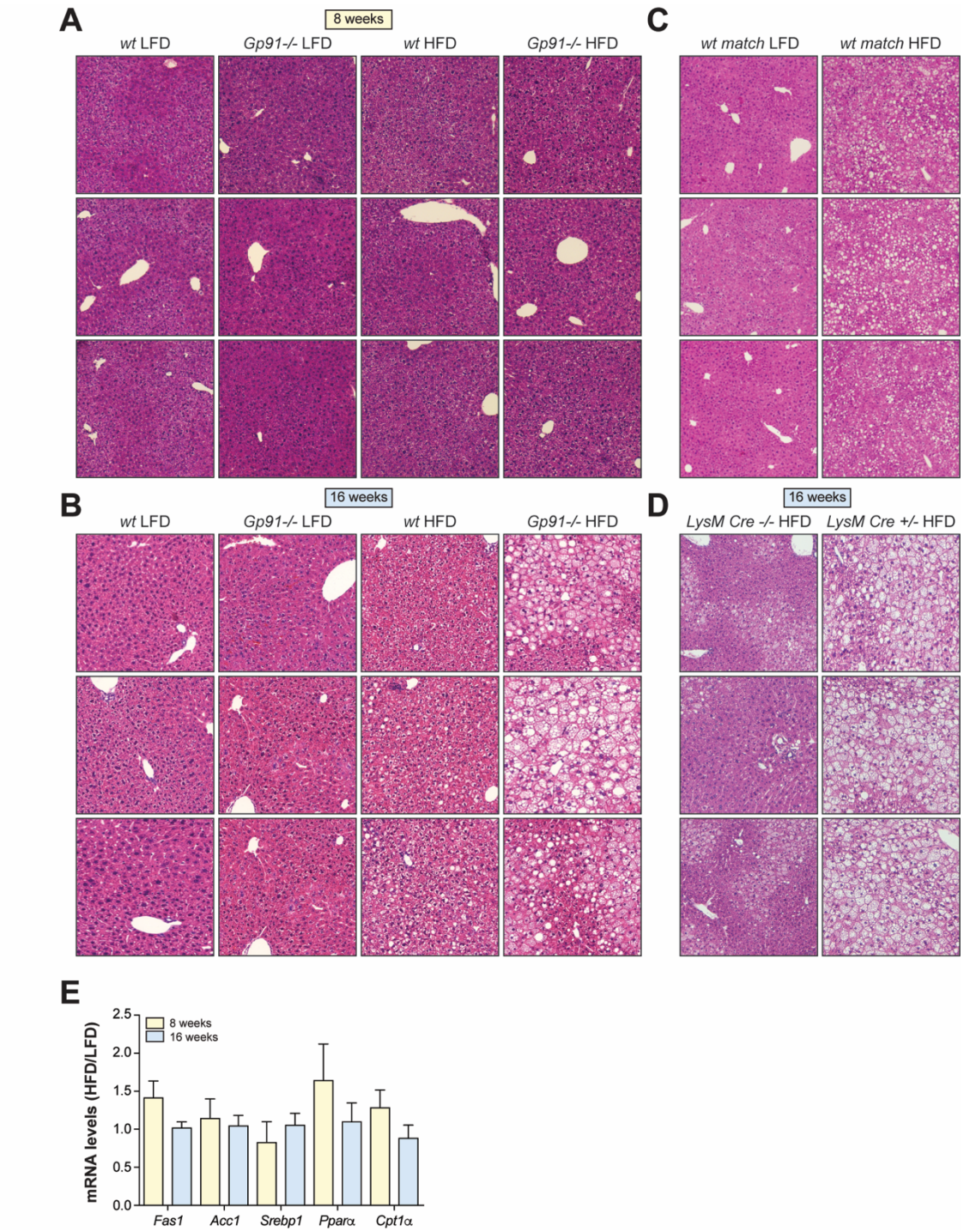

Fig. S4. Representative H&E images of liver and liver gene expression in lean and obese mice. Related to Figs. 3-5,7. Livers were stained with H&E and visualized by microscopy. Panels A-B: *Gp91-/-* and wild type (wt) mice fed a low-fat diet (LFD) or high-fat diet (HFD) for 8 weeks (*Panel A*) or 16 weeks (*Panel B*). Panel C: Wild type mice fed a HFD until their body weight matched *Gp91-/-* mice at 16 weeks (*wt match*). Panel D: *LysM-cre*$^{-/-}$ *Nox2*$^{fl/fl}$ and *LysM-cre*$^{+/-}$ *Nox2*$^{fl/fl}$ mice fed the HFD for 16 weeks. Panel E: Liver mRNA levels for genes involved in triglyceride synthesis (*Fas1*, *Acc1*, *Srebp1*) and metabolism (*Ppar*α, *Cpt1*α). Data are presented as a ratio of HFD/LFD. Results are mean ± SEM. n=5.

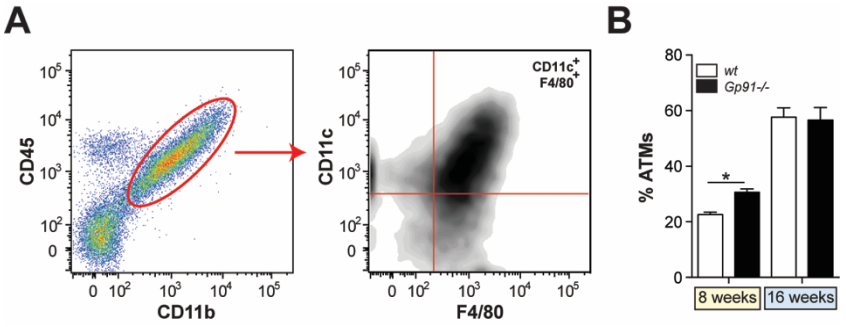

Fig. S5. ATM quantification in epididymal fat. Related to Figs. 4-5. Wild type (*wt*) and *Gp91-/-* mice were placed on a high-fat diet for 8 or 16 weeks. *Panel A*: ATMs were quantified based on the percentage of $CD11b^{+}CD45^{+}$ cells in the stromal vascular fraction, and ATM expression levels of CD11c and F4/80 were determined by flow cytometry. *Panel B*: Quantification of $CD45^{+}Cd11b^{+}$ ATMs. Results are mean ± SEM. n=5, *, $p<0.05$ Student's *t*-test.

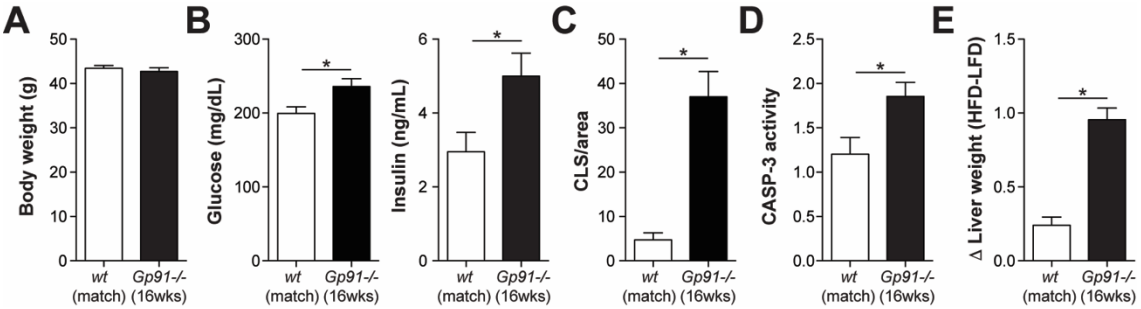

Fig. S6. Increased adiposity does not explain the late-onset metabolic dysfunction in *Nox2-/-* mice fed the high-fat diet. Related to Fig. 5. Wild type (*wt*) mice were placed on the HFD until their average body weight matched that for *Gp91-/-* fed the HFD for 16 weeks (*wt* match, n=10). *Panel A*: Body weight. *Panel B*: Fasting glucose and insulin levels. *Panel C*: Macrophages crown-like structures (CLS) in epididymal fat were quantified by microscopy and standardized per unit area. *Panel D*: Relative caspase-3 (CASP-3) activity in epididymal fat. *Panel E*: Change in liver weight from LFD fed controls (Δ liver weight). Results are mean ± SEM. n=5-10 *, $p<0.05$ Student's *t*-test.

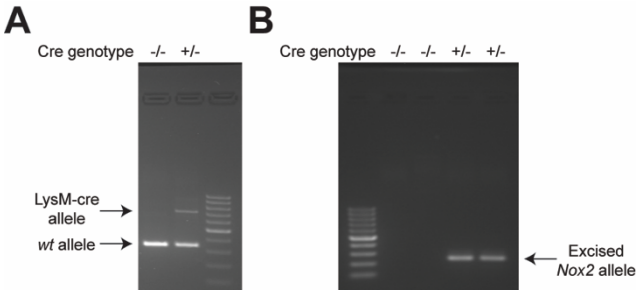

Fig. S7. Genotyping of *LysM-cre*$^{+/-}$ *Nox2*$^{fl/fl}$ mice. Related to Fig. 7. *Panel A*: Genotyping on tail vein DNA from *LysM-cre*$^{-/-}$ *Nox2*$^{fl/fl}$ and *LysM-cre*$^{+/-}$ *Nox2*$^{fl/fl}$ mice. *Panel B*: *LysM-cre*$^{-/-}$ *Nox2*$^{fl/fl}$ and *LysM-cre*$^{+/-}$ *Nox2*$^{fl/fl}$ mice were placed on a 45% high-fat diet for 16 weeks. Adipose tissue macrophages (ATMs) were isolated, and assessed for cre-mediated excision of the floxed region of the *Nox2* gene.

SUPPLEMENTAL TABLE

Protein	*In vitro*: MMe or M1 vs M0 macrophages							*In vivo:* ATMs from *wt* mice fed HFD or LFD				*In vivo:* ATMs from *wt* or *Gp91-/-* mice fed HFD		
	M0 Avg	M1 Avg	MMe Avg	*G*-test (M1:M0)	*t*-test (M1:M0)	*G*-test (MMe:M0)	*t*-test (MMe:M0)	LFD Avg	HFD Avg	*G*-test (HFD:LFD)	*t*-test (HFD:LFD)	*wt* HFD Avg	*Gp91-/-* HFD Avg	*G*-test (*Gp91-/-* :wt)
LAMP2	1.4	1.4	6.7	0.00	9.7E-01	3.8	6.4E-04	0.0	3.7	5.1	3.9E-04	2.4	0.4	-1.58
NPC1	0.4	2.1	4.2	1.17	2.2E-03	3.5	5.7E-03	0.0	1.3	1.8	1.6E-02	*nd*	*nd*	*nd*
AHNAK	13.4	9.2	49.9	-0.79	2.0E-01	22.5	3.3E-05	38.7	83.7	16.9	9.7E-03	15.3	5.8	-4.49
ANXA2	0.9	3.0	22.7	1.25	4.5E-02	25.2	1.3E-07	25.7	53.0	9.7	8.2E-03	52.9	29.5	-6.76
ATP6v1b2	1.3	2.7	17.3	0.45	1.9E-01	16.3	1.1E-03	0.3	8.7	9.6	2.6E-02	4.0	0.6	-2.88
ATP6v1a	4.9	5.2	11.8	0.01	8.4E-01	2.9	4.7E-03	1.0	16.3	16.4	7.1E-03	*nd*	*nd*	*nd*
HSP90ab1	3.8	6.9	10.6	0.92	1.9E-02	3.3	1.1E-02	13.0	21.7	2.2	1.5E-01	6.2	4.4	-0.31
TCIRG1	0.0	0.0	3.7	0.00	1.0E+00	5.2	6.5E-07	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
ATP6v0d1	1.4	1.8	5.2	0.06	4.9E-01	2.3	2.0E-03	*nd*	*nd*	*nd*	*nd*	2.6	2.0	-0.08
LAMP1	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	0.0	1.3	1.8	1.6E-02	4.3	0.8	-2.60
NSF	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	0.0	1.7	2.3	7.5E-03	1.5	0.0	-2.05
UBA1	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	0.0	18.7	25.9	4.5E-04	*nd*	*nd*	*nd*

Table S1. Plasma membrane proteomics of macrophages *in vitro* and *in vivo*. Related to Figs. 1, 6. Plasma membrane proteomics of unstimulated (M0), metabolically activated (MMe) or classically activated (M1) BMDMs, or ATMs isolated from wild-type (*wt*) or *Gp91-/-* mice fed the low-fat diet (LFD) or high-fat diet (HFD). Proteins were quantified by spectral counting and statistical significance was assessed by the *t*-test (*p*-value) and *G*-test (*G*-statistic). Negative *G*-test values reflect down-regulation of protein abundance in the first sample relative to the second sample. Avg = average spectral count; *nd* = not detected.