Table S3. Ubiquitylated proteins and their intensities identified in Halo-TAB2 TUBE pull-downs from phagosomes of M2(IL-4) macrophages

Gene name	Protein	Intensity TAB2-mut	Intensity TAB2	Ubiquitylated position
Ubc	Polyubiquitin-C	2.7×10^{4}	3.9×10^{5}	63;11;48;1;29;6;27;33
Msr1	**Macrophage scavenger receptor 1**	9.7×10^{4}	1.3×10^{7}	**27**
Slc43a2	Large neutral amino acids transporter small subunit 4	2.2×10^{6}	1.2×10^{7}	283;293;402;557
Ifitm3	Interferon-induced transmembrane protein 3	6.8×10^{4}	3.9×10^{6}	24
Rnf13	E3 ubiquitin-protein ligase RNF13	1.3×10^{5}	7.7×10^{6}	233
Csf1r	Macrophage colony-stimulating factor 1 receptor	3.3×10^{6}	8.0×10^{7}	572;810;625;584
C5ar1	Complement component 5a receptor 1	5.0×10^{6}	1.8×10^{7}	334
Slc38a2	Sodium-coupled neutral amino acid transporter 2	3.5×10^{5}	5.0×10^{6}	38;33
Serinc3	Serine incorporator 3	8.6×10^{4}	5.0×10^{5}	363
Slc7a2	Isoform 2 of Low affinity cationic amino acid transporter 2	0	3.5×10^{6}	449;632;654
Tyrobp	TYRO protein tyrosine kinase-binding protein	0	5.4×10^{5}	82
Lyn	Tyrosine-protein kinase Lyn	8.6×10^{6}	2.0×10^{7}	20
Unc93b1	Protein unc-93 homolog B1	4.1×10^{6}	1.4×10^{7}	197;582
Itm2b	Integral membrane protein 2B	1.6×10^{6}	1.1×10^{7}	13
Slc6a6	Sodium- and chloride-dependent taurine transporter	6.7×10^{5}	3.1×10^{7}	611;15;37
Lat2	Linker for activation of T-cells family member 2	0	1.0×10^{6}	39;84
Tfrc	Transferrin receptor protein 1	7.8×10^{6}	2.2×10^{7}	53;39
Abcg1	ATP-binding cassette sub-family G member 1	1.3×10^{6}	9.9×10^{6}	55
Slc40a1	Solute carrier family 40 member 1	1.9×10^{4}	2.2×10^{5}	269
Itch	E3 ubiquitin-protein ligase Itchy	1.9×10^{5}	8.1×10^{6}	192
Piezo1	Piezo-type mechanosensitive ion channel component 1	3.9×10^{4}	5.3×10^{6}	1914
Ednrb	Endothelin B receptor	3.4×10^{6}	9.5×10^{6}	417
Vamp8	Vesicle-associated membrane protein 8	1.0×10^{5}	2.7×10^{6}	47
Mgl2	Macrophage galactose N-acetyl-galactosamine	1.0×10^{7}	8.7×10^{7}	16
Hspa8	Heat shock 70 kDa protein 8	1.3×10^{7}	6.1×10^{7}	524
Slc23a2	Solute carrier family 23 member 2	7.9×10^{4}	3.2×10^{6}	11
Evi2a	Protein EVI2A	0	1.1×10^{6}	192;221
Itfg3	Protein ITFG3	5.8×10^{4}	1.8×10^{6}	36;38
Emr1	EGF-like module-containing mucin-like hormone receptor-like 1	3.6×10^{6}	1.9×10^{7}	743
Slc12a4	Solute carrier family 12 member 4	8.4×10^{4}	4.3×10^{6}	988
Slc3a2	4F2 cell-surface antigen heavy chain	3.5×10^{6}	3.2×10^{7}	42
Ttyh3	Protein tweety homolog 3	0	1.3×10^{7}	508
Clec4a3	C-type lectin domain family 4 member a3	0	1.6×10^{5}	13
Colec12	Collectin-12	0	2.3×10^{5}	2;17
Ms4a6d	Membrane-spanning 4-domains subfamily A member 6D	0	2.4×10^{5}	174
Slc5a3	Sodium/myo-inositol cotransporter	1.6×10^{5}	1.7×10^{6}	596
Trpv2	Transient receptor potential cation channel subfamily V member 2	3.5×10^{6}	2.3×10^{7}	51
Slc43a3	Solute carrier family 43 member 3	0	2.6×10^{5}	244

Supplementary Figure Legends

Figure S1

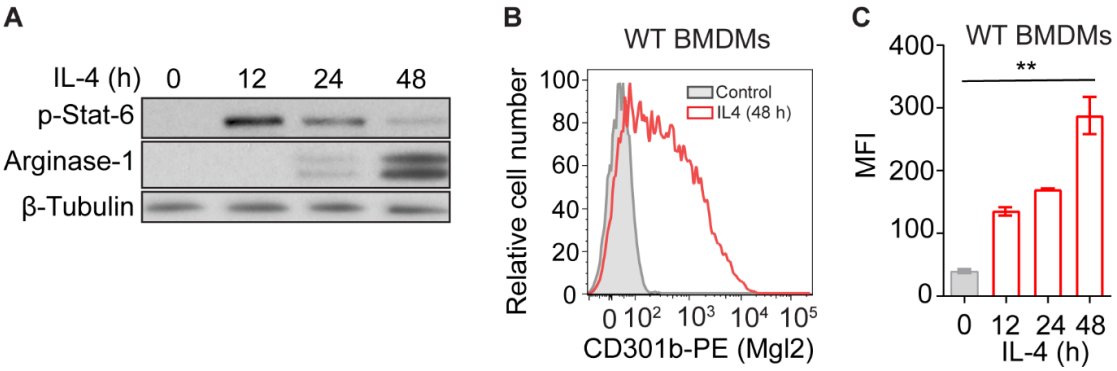

Figure S1: Validation of IL4-activation.

(A) Immunoblot analysis (IB) of IL-4- induced M2 markers including pStat-6 and Arginase-1. Macrophages were fully activated after 48 h as shown by strong induction of Arginase-1. Tubulin serves as loading control. (B) The cell surface levels of CD301b/Mgl2 in response to IL-4 stimulation. (C) Quantification of mean fluorescence intensity (MFI) of cell surface levels of CD301b/Mgl2 in response to IL-4 stimulation.

Figure S2

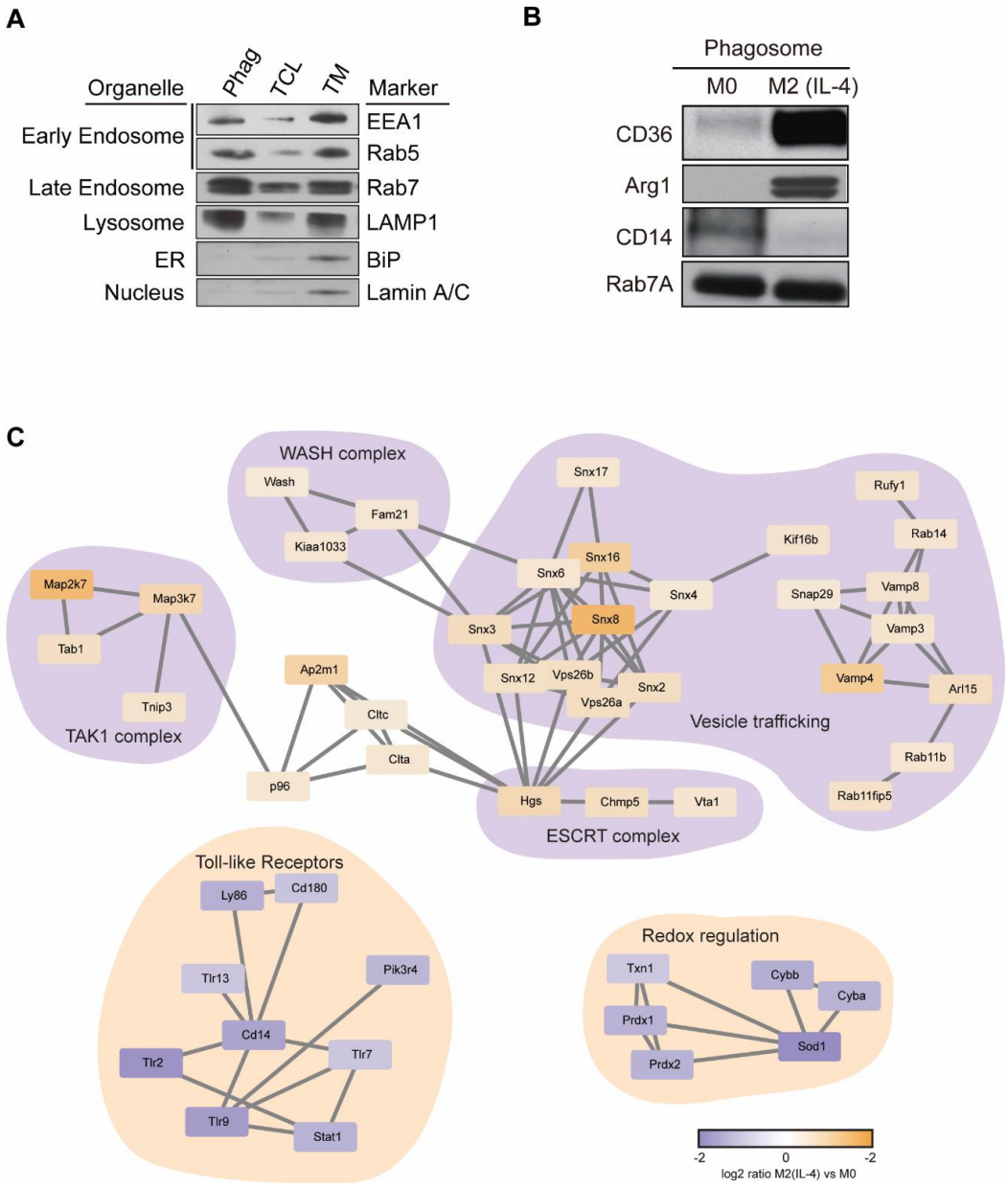

Figure S2: Validation of phagosome proteomics data and specific regulated phagosome protein complexes.

(A) IB of markers of early and late endosomes (EEA1, Rab5a and enriched on phagosomes, while nuclear and ER markers (Lamin A/C and BiP) are more abundant in total cell lysates (TCL) and total membrane (TM), respectively. (B) IB analysis validating selected proteins changing in proteomics data. (C) Protein–protein interaction network of phagosome proteome using the STRING database v10 (Szklarczyk D, et al, Nucleic Acid Res, 2015). Subnetworks positively affected by phagocytosis in M2(IL-4) macrophages were vesicular trafficking and signalling, while proteins associated with innate immune response and ROS were down-regulated. Proteins involved in vesicular trafficking and signalling, Redox regulation and Toll like receptor complexes are displayed. (A-B) Representative blots of two biological replicates.

Figure S3

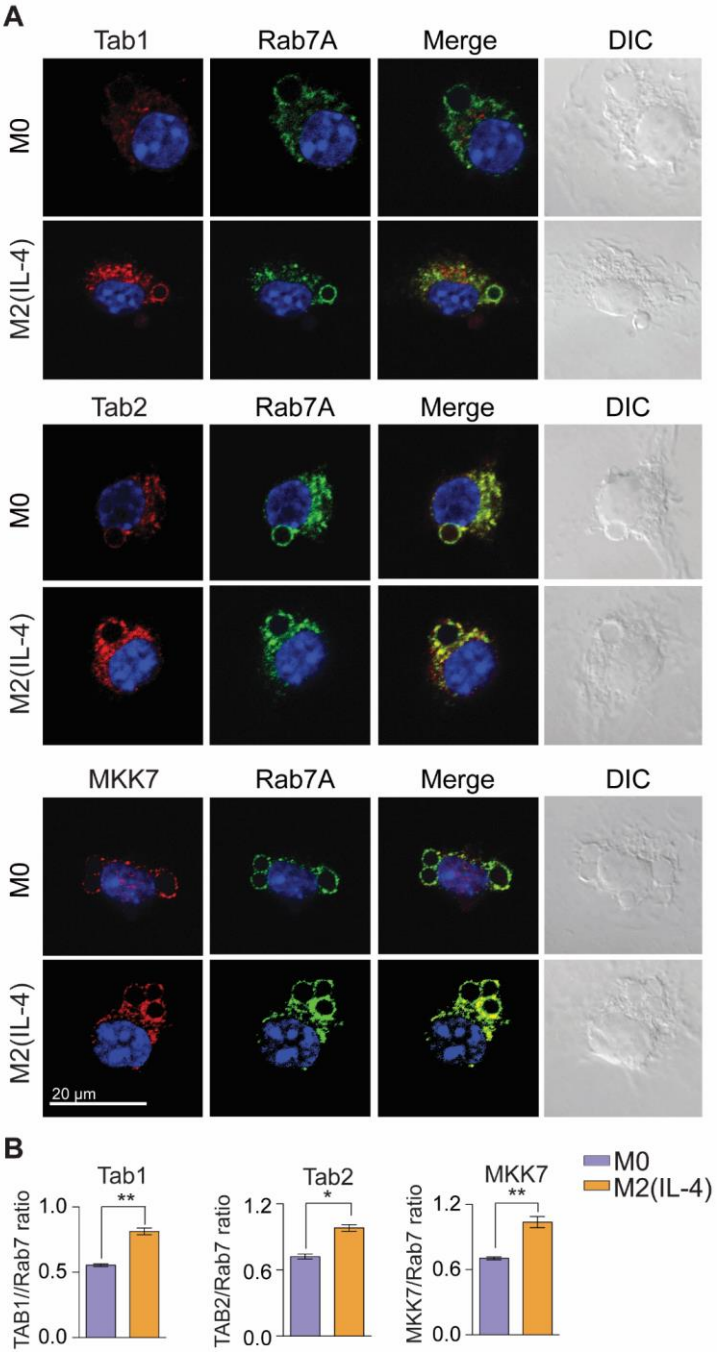

Figure S3: Immunofluorescence of TAB1, TAB2 and MKK7 on M0 and M2 phagosomes.

(A) Immunofluorescence micrographs showing co-localisation of TAB1, TAB2 and MKK7 proteins with Rab7a, a phagosomal marker, around 30 min phagosomes in M0 and M2(IL-4) macrophages. (B) Corresponding quantification of co-localisation of TAB1, TAB2 and MKK7 with Rab7a in M0 and M2(IL-4) macrophage phagosomes, plotted as a ratio of the fluorescence of the target protein to Rab7a fluorescence localized to phagosomes. Scale bar is 20 µm. Data are shown as mean ± SEM from three independent experiments. **p < 0.01, ***p < 0.001; (Student's T-test).

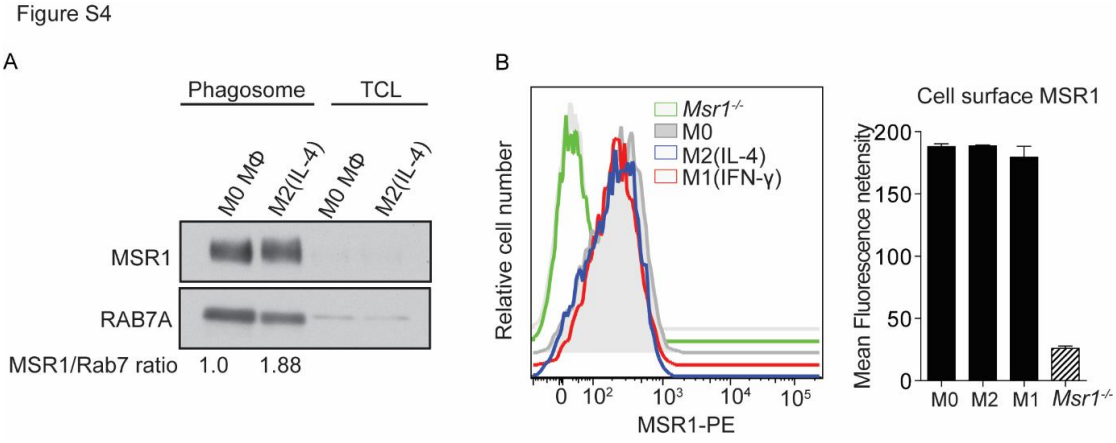

Figure S4: Identification of MSR1 expression and K63 polyubiquitylation on phagosomes in activated macrophages.

(A) IB of MSR1 showing light enrichment of non-ubiquitylated form of the protein on phagosomes in M2(IL-4) macrophages compared to M0 macrophages. (B) Activation does not affect MSR1 surface expression. Flow cytometry analysis and corresponding quantification shows equal cell surface expression of MSR1 in M0, M2(IL-4) and M1(IFN-γ) macrophages. Data are shown as mean ± SEM from three independent experiments. MFI (Median Fluorescence Intensity).

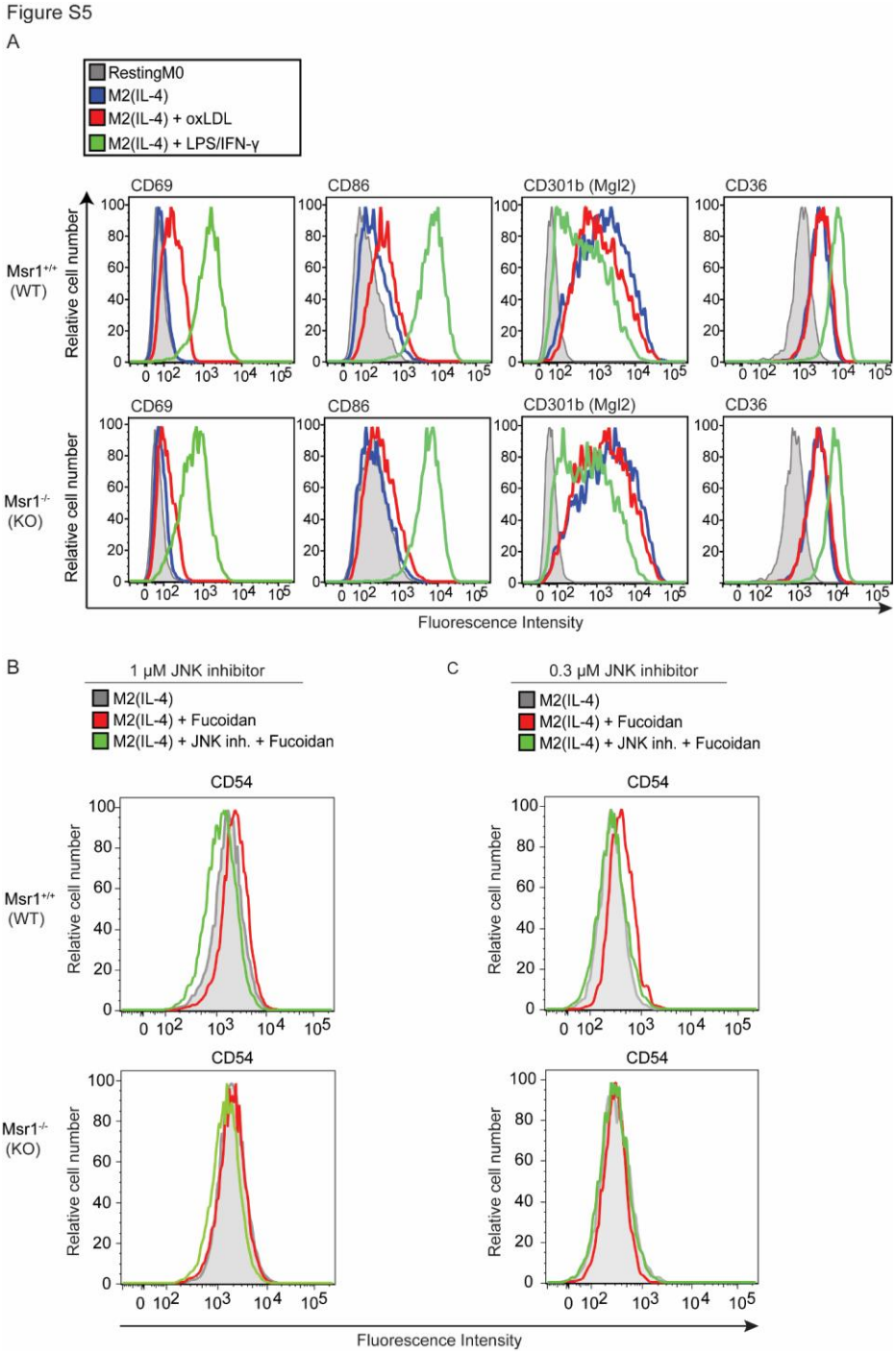

Figure S5: oxLDL induces a MSR1-dependent pro-inflammatory switch and JNK inhibition reverses MSR1-dependent pro-inflammatory activation (related to Figure 5).

(A) Flow cytometry analysis of cell surface expression of CD69, CD86, CD301b and CD36 of M2(IL-4) followed or not by either oxLDL or LPS/IFN-γ treatment. (B-C) Inhibition of JNK by 1 and 0.3 μM (C) of JNK-IN8 reverses the increase of proinflammatory activation of M2(IL-4) macrophages upon fucoidan treatment. Data representative of two independent experiments.