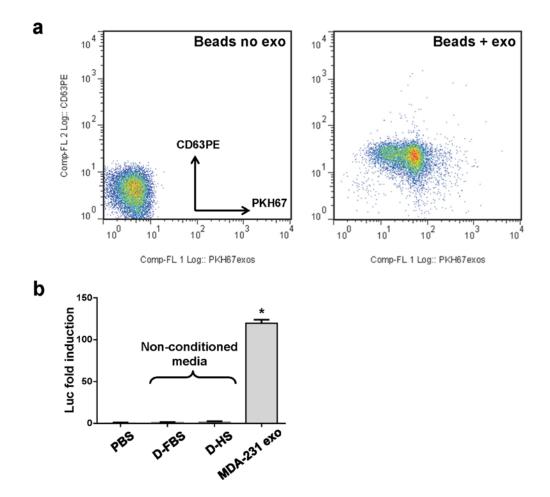
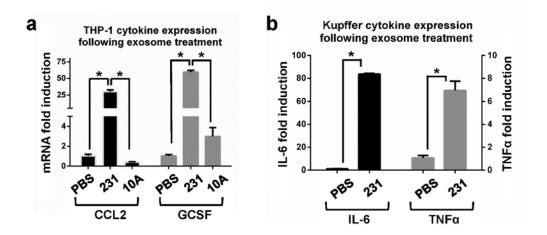
Macrophage immunomodulation by breast cancer-derived exosomes requires Toll-like receptor 2-mediated activation of NF-κB

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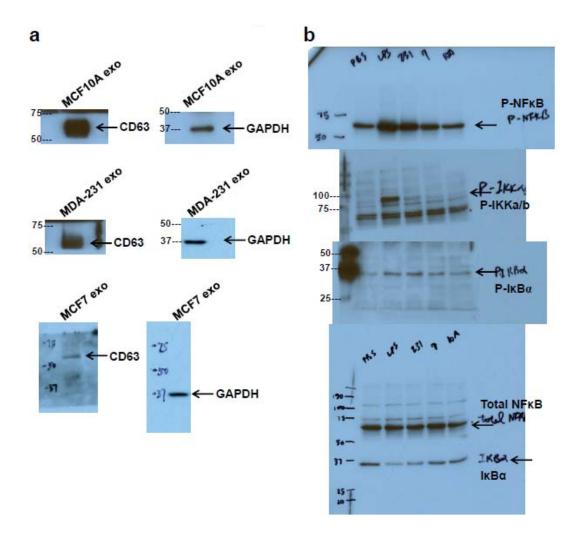
Supplementary Figures and Figure Legends



Supplementary Fig. S1 On-bead flow cytometry analysis of exosomes and effect of non-conditioned media on NF-κB activity. (a) Exosomes were prepared from MDA-231 conditioned media, fluorescently labeled with PKH67, and adsorbed to polymer microspheres. Anti-CD63PE was used to stain adsorbed exosomes prior to flow cytometric analysis. (b) The exosome purification protocol was carried out for non-conditioned MDA-231 and MCF10A media (containing and indicated as exosome-depleted fetal bovine serum/D-FBS and horse serum/D-HS, respectively) and used to treat RawkB cells. Luciferase induction was compared to that obtained by treatment with MDA-231 exosomes. *p < 0.001 compared to the PBS (control) group.



Supplementary Fig. S2 Effects of cancer-derived exosomes on cytokine expression in THP-1 cells and Kupffer cells. Following administration of exosomes derived from indicated cell types, quantitative RT-PCR was used to assess expression of CCL2 and GCSF in THP-1 cells (**a**) and expression of IL-6 and TNF α in Kupffer cells (**b**). *p < 0.001.



Supplementary Fig. S3 Full-length immunoblots for Fig. 1b (a) and Fig. 1e (b). Numbers to the left indicate the positions of size markers (in kDa) and the arrows indicate the band of interest.

Supplementary Table

Supplementary Table S1 Palmitoylated proteins identified in MDA-MB-231 and MCF10A cells

		Number of identified peptides			
Gene name	Protein identity	231 HA+	231 HA-	10A HA+	10A HA-
CD44	CD44 antigen	72	0	28	0
SNAP23	Synaptosomal-associated protein 23	17	0	16	0
LAMTOR1	Ragulator complex protein LAMTOR1	13	0	18	0
RAP2B	Ras-related protein Rap-2b	7	0	8	0
CAV1	Caveolin-1	6	0	16	0
TFRC	Transferrin receptor protein 1	5	0	0	0
IFITM3	Interferon-induced transmembrane protein 3	5	0	0	0
NRAS	GTPase Nras	4	0	1	0
BST2	Bone marrow stromal antigen 2	4	0	0	0
CYB5B	Cytochrome b5 type B	4	0	0	0
CD81	CD81 antigen	3	0	2	0
UBB	Polyubiquitin-B	3	2	2	0
CKAP4	Cytoskeleton-associated protein 4	3	0	0	0
TMX1	Thioredoxin-related transmembrane protein 1	2	0	1	0
THBD	Thrombomodulin	2	0	0	0
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 1	2	0	0	0
S100A14	Protein S100-A14	0	0	81	3
MUC1	Mucin-1	0	0	14	0
DLST	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	0	0	4	0