Supplementary Files:

WAVE-1 mediates suppression of phagocytosis by phospholipid-derived DAMPs

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Supplemental Table 1

Sequences of nucleotides used for generation of shRNA or scrambled control plasmids (sense orientation only).

	Nucleotide sequence sense (5' → 3')
PKAc shRNA	ACCGGTGGAAGCTCCCTTCATATTCAAGAGATATGAAGGGAGCTTCCACCTTTTTC
WAVE-1shRNA	ACCGGACCGATTGTCTGTTAGTTTCAAGAGAACTAACAGACAATCGGTCCTTTTTC
PKAc SCR	ACCGACGGCAGTGCTTCACATTTTCAAGAGAAATGTGAAGCACTGCCGTCTTTTTC
WAVE1 SCR	ACCGTCTGGCAACGATTTTGGTTTCAAGAGAACCAAAATCGTTGCCAGACTTTTTC

Supplemental Table 2

Sequences of primers used for RT-PCR

Gene	Sense (5' → 3')	Anti-sense (5' → 3')
WAVE-1	GGAAGCGCCGTCCTCTTG	CTGGGCAGTGCTGTGTGG
AKAP-Lbc	CGCACGTGTCCTGGGTCAT	TGCAGTGATAGAGGGTAGAGCCAGA
Gravin	CGTCGGGAGCAGCTGGAGAT	TGCCCATCCTGGCTTTCCTC
HPRT	GTTAAGCAGTACAGCCCCAAAAG	AAATCCAACAAAGTCTGGCCTGTA

Supplementary Figure Legends:

Supplementary Figure 1: OxPAPCs induces cell spreading and not retraction

(**A**) RAW 264.7 cells were treated with vehicle, DMPC or OxPAPC (10µg/mL) for 30min. Cells were subsequently stained for F-actin (phalloidin; green) and nuclei (DAPI; blue). Nucleic and cytoplasm overlays were automatically generated by the CellProfiler cell image analysis software. (**B**) Calculations were done as described in the methods section to quantify changes in cell shape and size: the form factor, as a mean to express the change in cell shape, the perimeter and the cell area to assess the expansion of the cells. Representative images of the analysis are shown. Data are shown as mean ± SEM; ** indicates $p < 0.01$, and *** $p < 0.001$ versus carrier.

Supplementary Figure 2: OxPAPC induced cell spread and inhibition of phagocytosis is not mediated by CD36

(**A**) Primary peritoneal macrophages of WT and $CD36^{obl}$ mice were incubated with carrier, DMPC, or OxPAPC at 20µg/ml for 30 min. Cells were subsequently fixed and stained for F-actin using Alexa Fluor 488-labelled phalloidin (green) and propidium-iodide (PI) for nuclei (red). Cells were visualized using a LSM 510 confocal laserscanning microscope. (**B**) WT and $CD36^{obl}$ peritoneal macrophages were incubated with carrier, DMPC or OxPAPC at 5µg/ml for 15 min, and phagocytosis of FITC-labeled *E. coli* was assayed after 60 min. Uptake is expressed relative to carrier. Data are representative of two independent experiments performed in triplicates; mean ± SEM; * $p < 0.05$ versus carrier.

Supplementary Figure 3: OxPAPC-mediated inhibition of phagocytosis is reversible

RAW 264.7 cells were incubated with DMPC or OxPAPC at 50µg/ml for 15 min. In indicated samples OxPAPC was removed after 15min by thoroughly washing cells with PBS (OxPAPC washed). Cells were then incubated with FITC-labeled *E. coli* and uptake was analyzed by FACS after 60, 120 and 180 min, respectively. Data are presented as phagocytosis-index as described in the Methods section. Depicted data are in duplicate and expressed as mean ± SEM; * $p < 0.05$ compared to DMPC.

Supplementary Figure 4: OxPL-mediated effects are independent of Clostridium difficile Toxin B

(**A**) RAW 264.7 cells were incubated with 100ng/ml of Clostridium difficile Toxin B (CdTB) for 2 h, followed by incubation with OxPAPC or DMPC at 10µg/ml for 30 min and stained for F-actin (phalloidin; green) and nuclei (DAPI; blue). (**B**) RAW 264.7 cells were incubated with CdTB for 30 min or 2 h, then DMPC or OxPAPC was added at 5µg/ml for 15 min, and subsequently phagocytosis of FITC-labeled *E. coli* was assayed after 60 min. Data are shown as mean ± SEM; * indicates $p < 0.05$, and *** $p < 0.001$ versus control DMPC.

Supplementary Figure 5: OxPAPC activates PKA distinctly, and thereby induces actin polymerization

(**A**) Confocal microscopy images showing RAW 264.7 cells treated with carrier, 6-Bnz-cAMP (specific PKA activator; 100µM), forskolin (100µM), or OxPAPC (10µg/ml) for 30 min. Cells were subsequently stained for F-actin using Alexa Fluor 488-labelled phalloidin (green) and PI for nuclei (red). (**B**) Phagocytosis assays were performed using RAW 264.7 cells that were preincubated with carrier (control) or indicated phospholipids (5µg/ml; for 15 min), forskolin or 6-Bnz-cAMP at 100µM. Uptake of FITC-labelled *E. coli* was evaluated after 60 min and is expressed relative

to control (carrier only) (data are representative of 3 independent experiments). Data are duplicates and shown as mean ± SEM * indicates $p < 0.05$, and ** $p < 0.01$ versus corresponding control.

Supplementary Figure 6: Gravin is expressed in MEF cells but not RAW cells.

RNA was extracted from unstimulated mouse embryonic fibroblasts (MEFs) or RAW 264.7 macrophages and RT-PCR (40 cycles) was conducted for AKAP-Lbc, WAVE-1 and Gravin. Specificity for the Gravin primer was evaluated as a single band at 145bp was observed in MEFs, but not in RAW 264.7 macrophages. GAPDH was included as a loading control and water served as a negative control for the specificity for the RT-PCR.

Supplementary Figure 7: Reconstitution of resident peritoneal macrophages

Quantitative WAVE-1 mRNA transcript levels in resident peritoneal macrophages of chimeric mice reconstituted with WT or WAVE1$^{-/-}$ bone marrow cells. Mice were randomly picked (n=3 mice/group) 9 weeks after transplantation; data depicted are mean +/- SEM.

Supplementary Figure 8: Ig-deficient peritoneal fluid inhibits phagocytosis via the AKAP WAVE-1

(**A**) Primary peritoneal WT macrophages were pre-incubated with medium (control) or IgG-depleted-PDF from one representative patient (shown is patient B) for 15 min, before phagocytosis of *E. coli* was assessed after 60 min by FACS. (**B**) Primary peritoneal WT or WAVE-1$^{-/-}$ macrophages were pre-incubated with medium (co) or IgG-depleted-PDF from patient B for 15 min before phagocytosis of *E. coli* was

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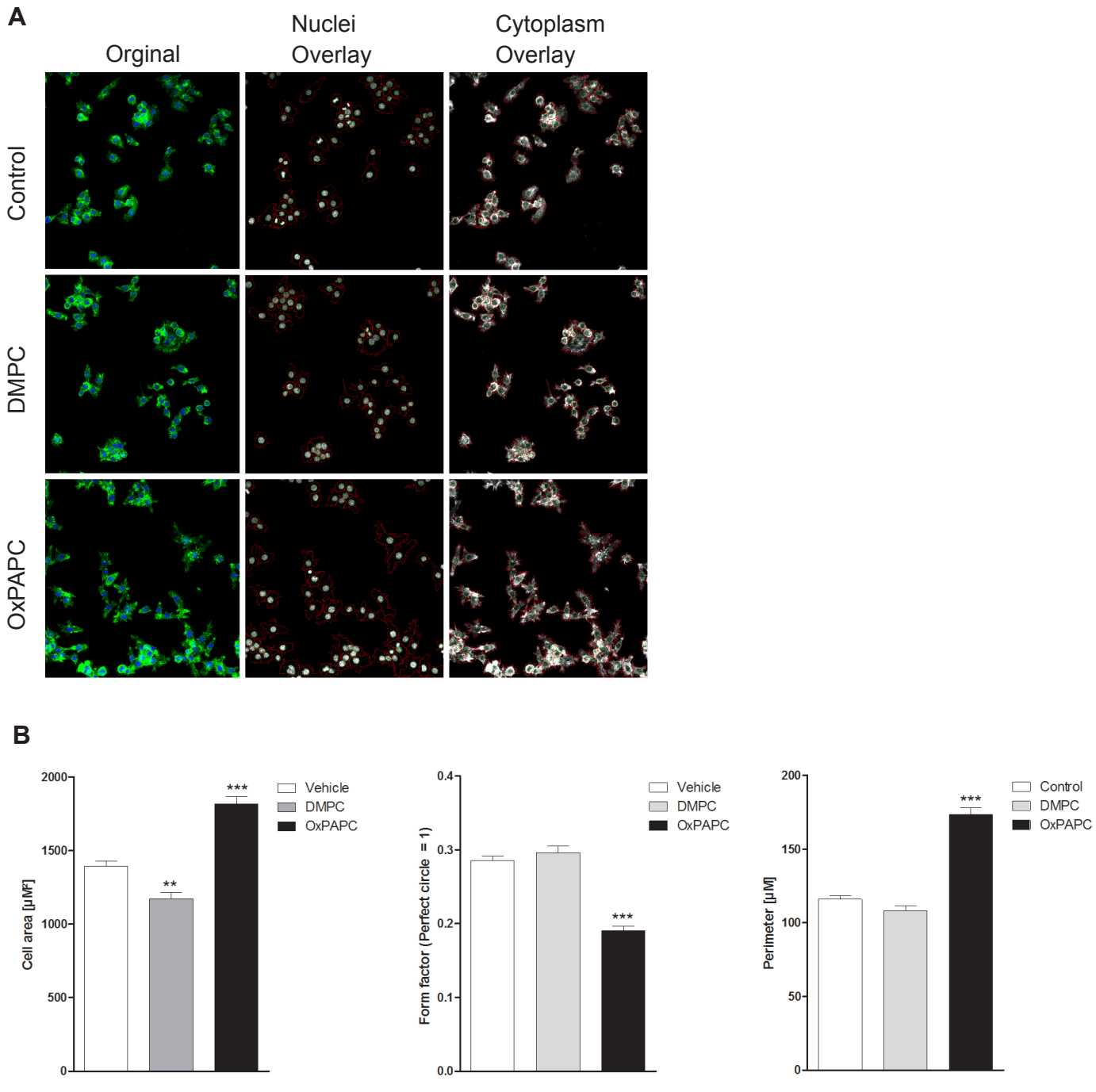

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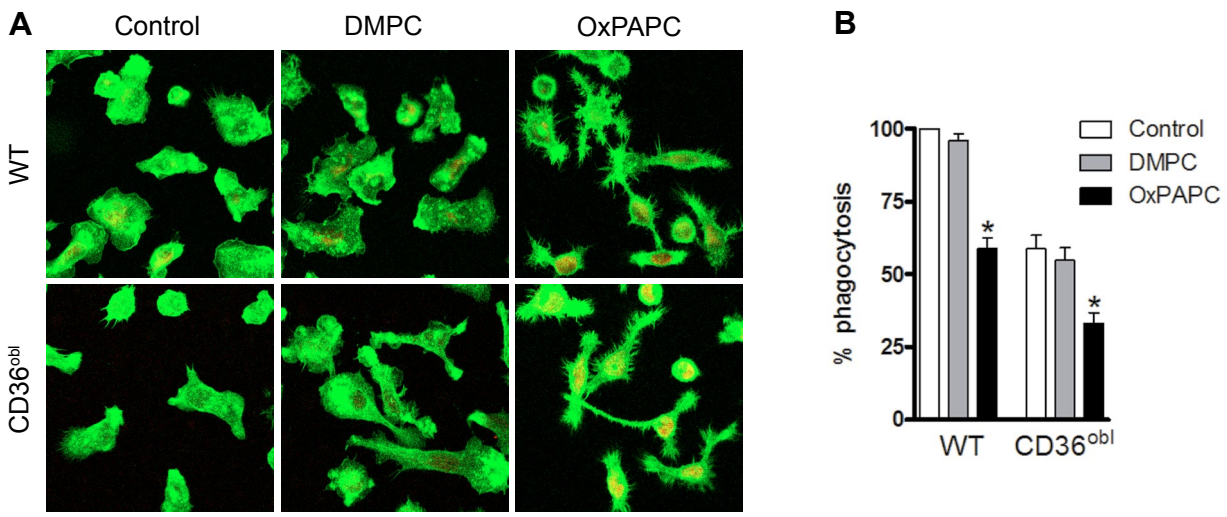

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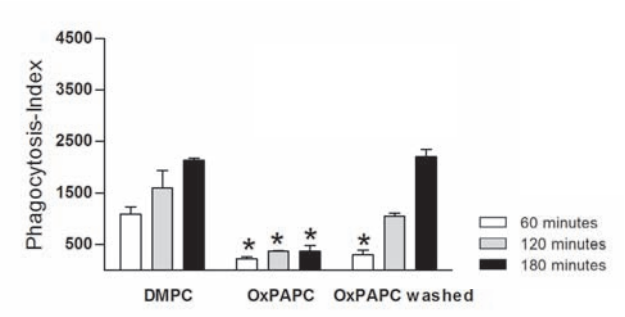

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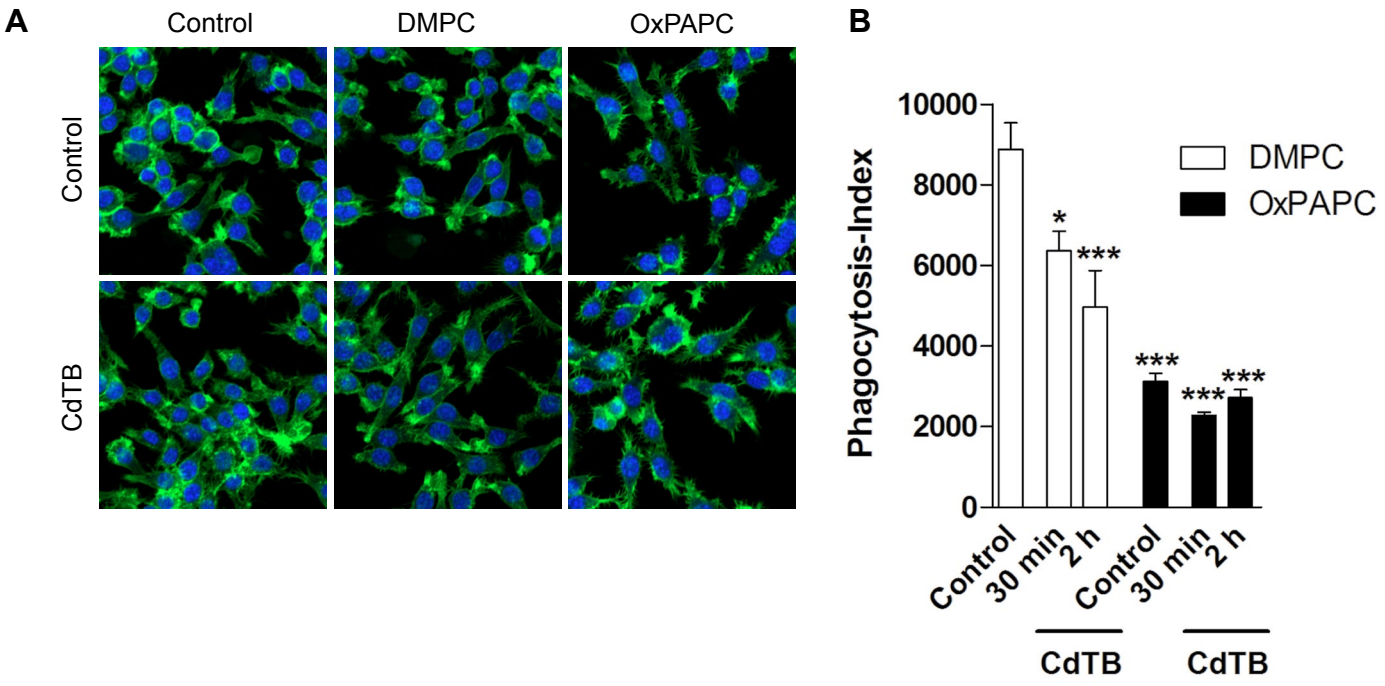

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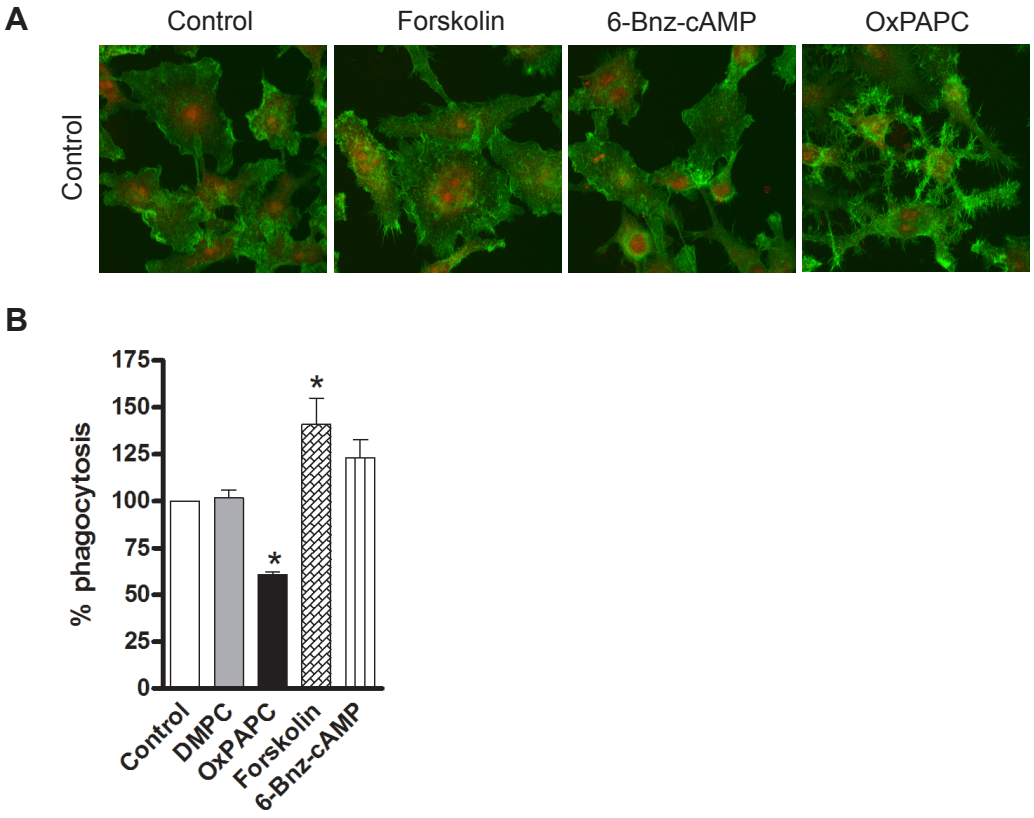

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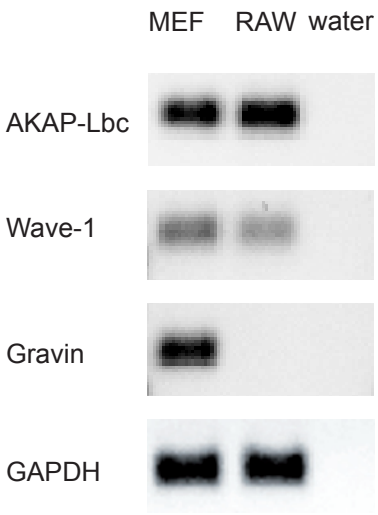

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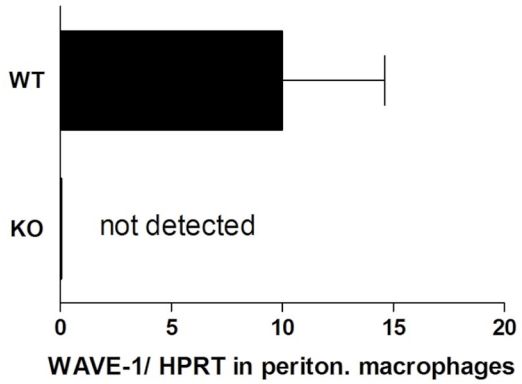

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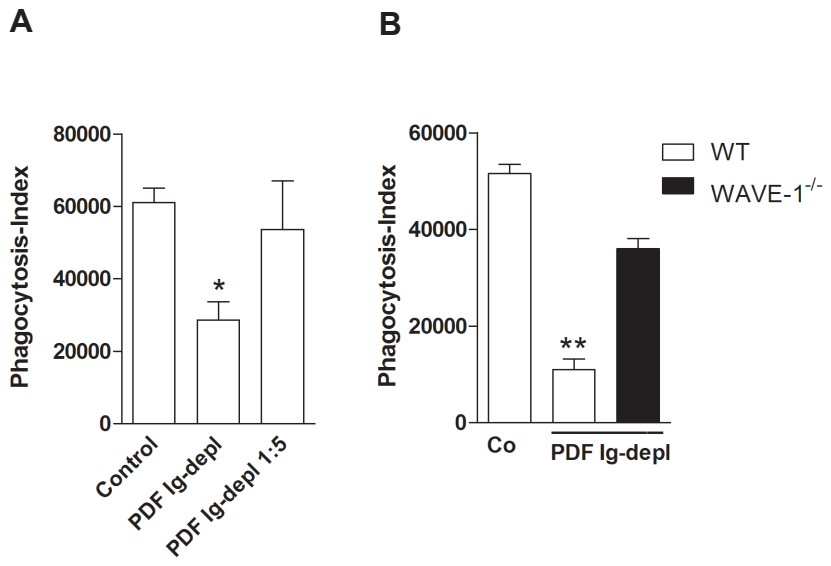

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