SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 RP105 phylogeny. Phylogenetic relationships among the human TLRs as inferred by sequence alignment using ClustalW software. Similar results were obtained with PileUp software. Horizontal branch lengths are proportional to the degree of inferred evolutionary change.

Supplementary Figure 2 MD-1 expression is necessary for RP105-mediated suppression of TLR4 signaling. (**a**) HEK293 cells stably expressing CD14 and TLR4 were transiently transfected with MD-2; along with EV, MD-1 and/or RP105 as indicated. Cells were subsequently stimulated with purified *E. coli* K235 LPS (10 ng/ml). Means +/- SE of triplicate cultures in a single experiment are depicted, representative of an experimental $n = 4$. *$p < 0.008$. (**b**) HEK293 cells were analyzed for surface and intracellular RP105 expression by FACS (representative of 2 separate experiments).

Supplementary Figure 3 RP105 fails to inhibit NF-κB transactivation driven by overexpression of TLR4 signaling molecules. HEK293FT cells were co-transfected with an NF-κB-firefly luciferase reporter plasmid (pELAM; 0.5 μg), along with plasmids encoding an I-κB super-repressor, RP105 (100 ng) plus MD-1 (50 ng), Mal (100ng), IRAK-1 (100 ng), Myd88 (100 ng), TRIF (100 ng) and/or empty vector (EV; 100 ng). After 48 h, cells were lysed and luciferase activity was quantified. Means +/- SE of triplicate cultures in a single experiment are depicted, representative of an experimental $n = 3$.

Supplementary Figure 4 MD-1 and MD-2 can interact directly with each other.

HEK293T cells were transiently transfected with MD-2 and/or MD-1 constructs, as indicated. Lysates were immunoprecipated with antibodies to FLAG or HA, and the association between MD-1 and MD-2 was examined by immunoblotting using the antibodies indicated.

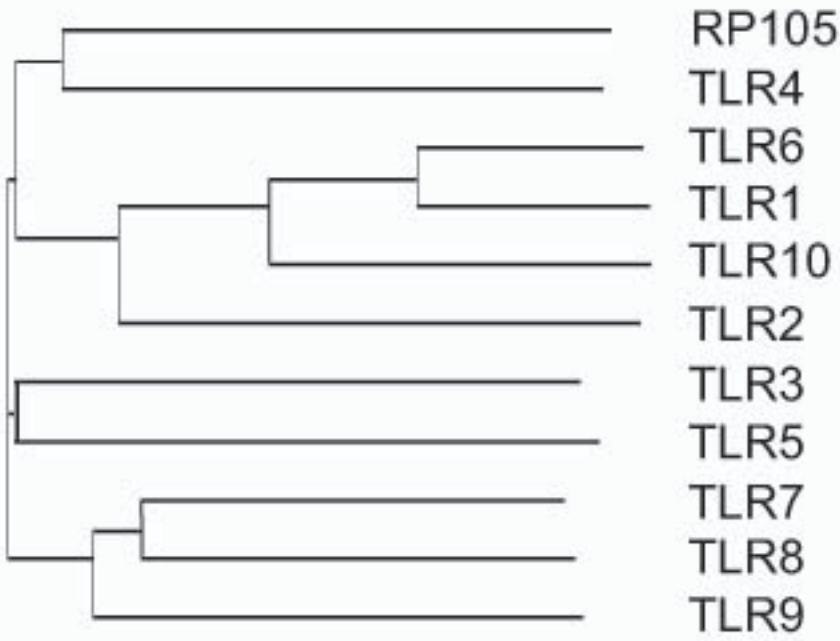
Supplementary Figure 5 Similar expression of molecules regulating TLR signaling in bone marrow-derived dendritic cells from RP105-deficient and wild-type mice. mRNA expression was analyzed by quantitative RT-PCR in bone marrow-derived DC from wild type (open symbols) or $RP105^{-/-}$ (filled symbols) mice prior to and following stimulation with 10 ng of purified *E. coli* K235 LPS. (**a**) IRAK-M, (**b**) Tollip, (**c**) ST2, (**d**) SIGIRR.

Supplementary Figure 6 Altered TLR4-induced cytokine production by resident peritoneal macrophages from RP105-deficient mice. Resident peritoneal macrophages from wild type (open symbols) or RP105-deficient (filled symbols) mice were stimulated with purified *E. coli* K235 LPS. Supernatants were harvested after 24 h. * $p < 0.005$, ** $p < 0.05$.

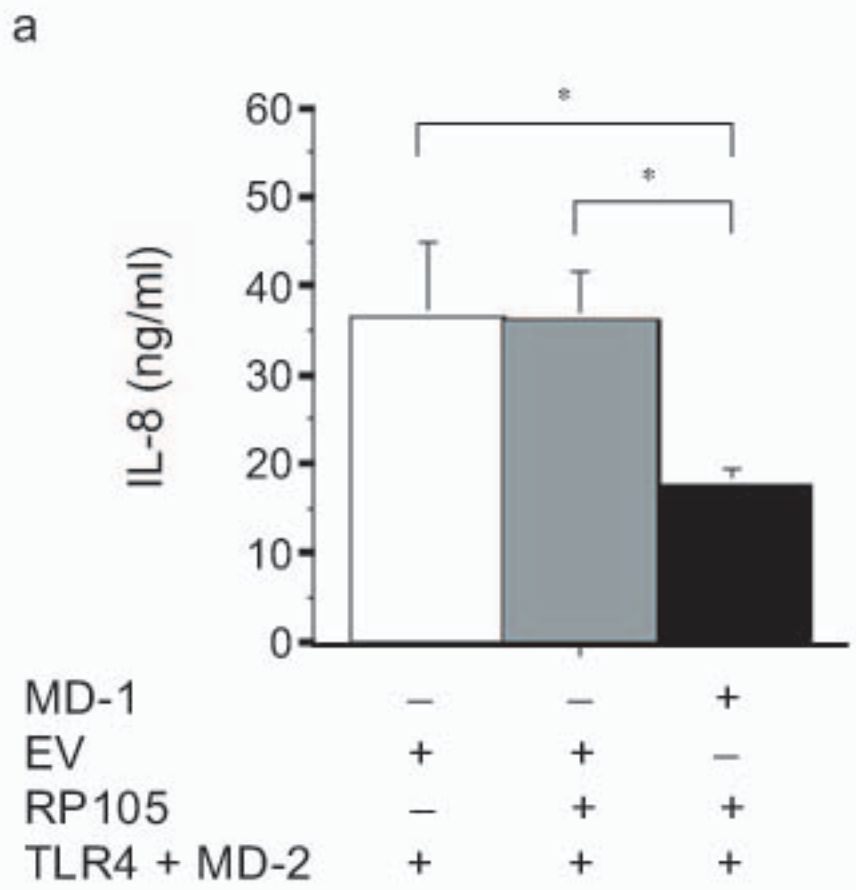
Supplementary Figure 7 Regulation of RP105 expression by LPS. DC were stimulated with purified *E. coli* K235 LPS. Kinetic analysis of RP105 and TLR4 mRNA expression was performed by quantitative RT-PCR. (**a**) Regulation of TLR4 and RP105 expression in mouse bone marrow-derived DC. Means + SE of duplicate PCR reactions in a single experiment, representative of 3 different experiments. (**b**) Regulation of TLR4 and RP105 expression in human monocyte-derived DC. Means + SE of duplicate PCR

reactions from 3 different donors. RP105 expression: square symbols; TLR4 expresssion: round symbols. RP105-deficient mice: filled symbols.

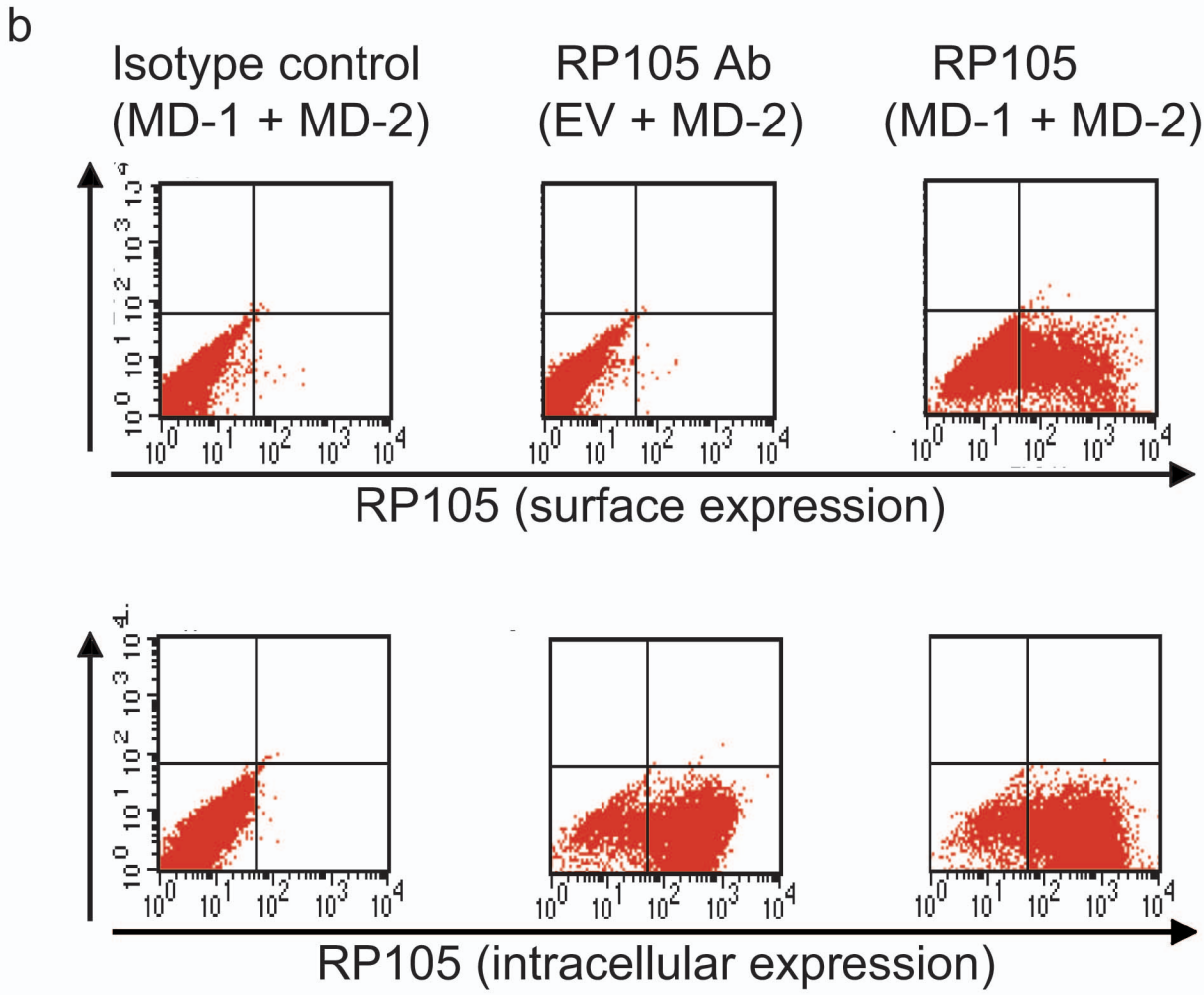
Supplementary Figure 8 Genotyping and phenotyping of RP105-deficient mice. (**a**) Genotyping via standard techniques. (**b**) FACS analysis of peripheral blood cells.



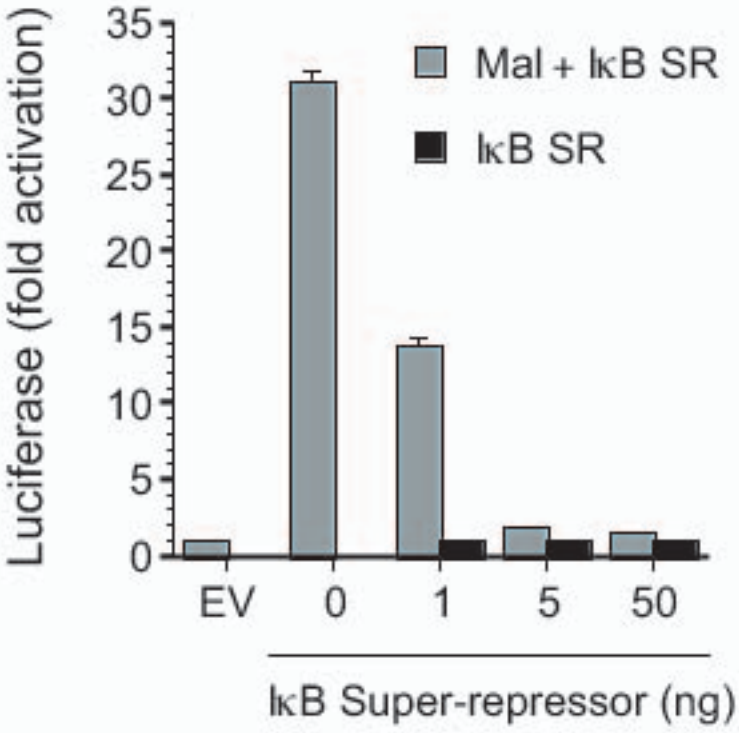
Supplementary Figure 1

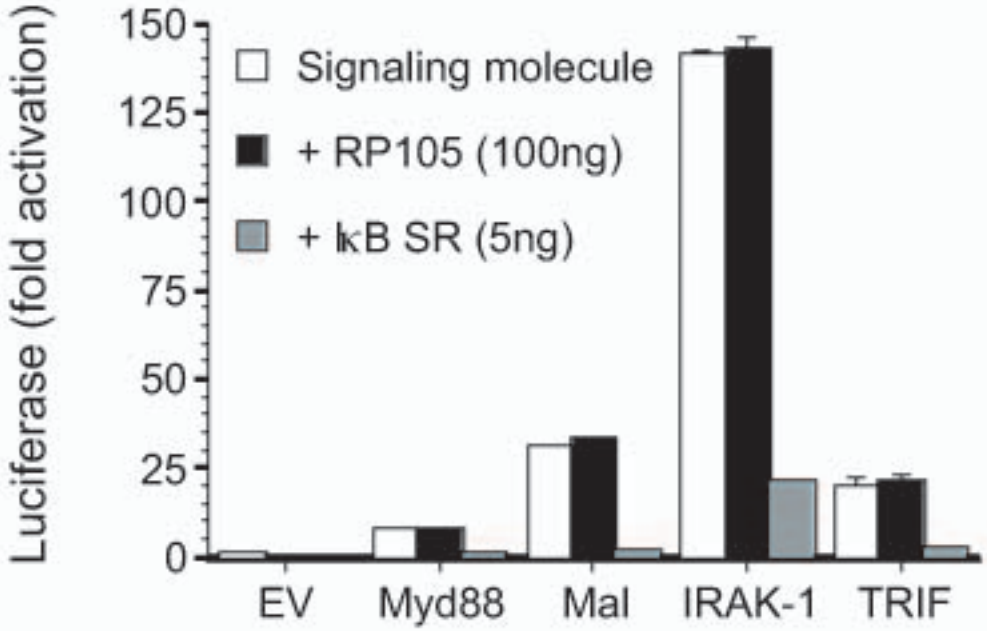


Supplementary Figure 2

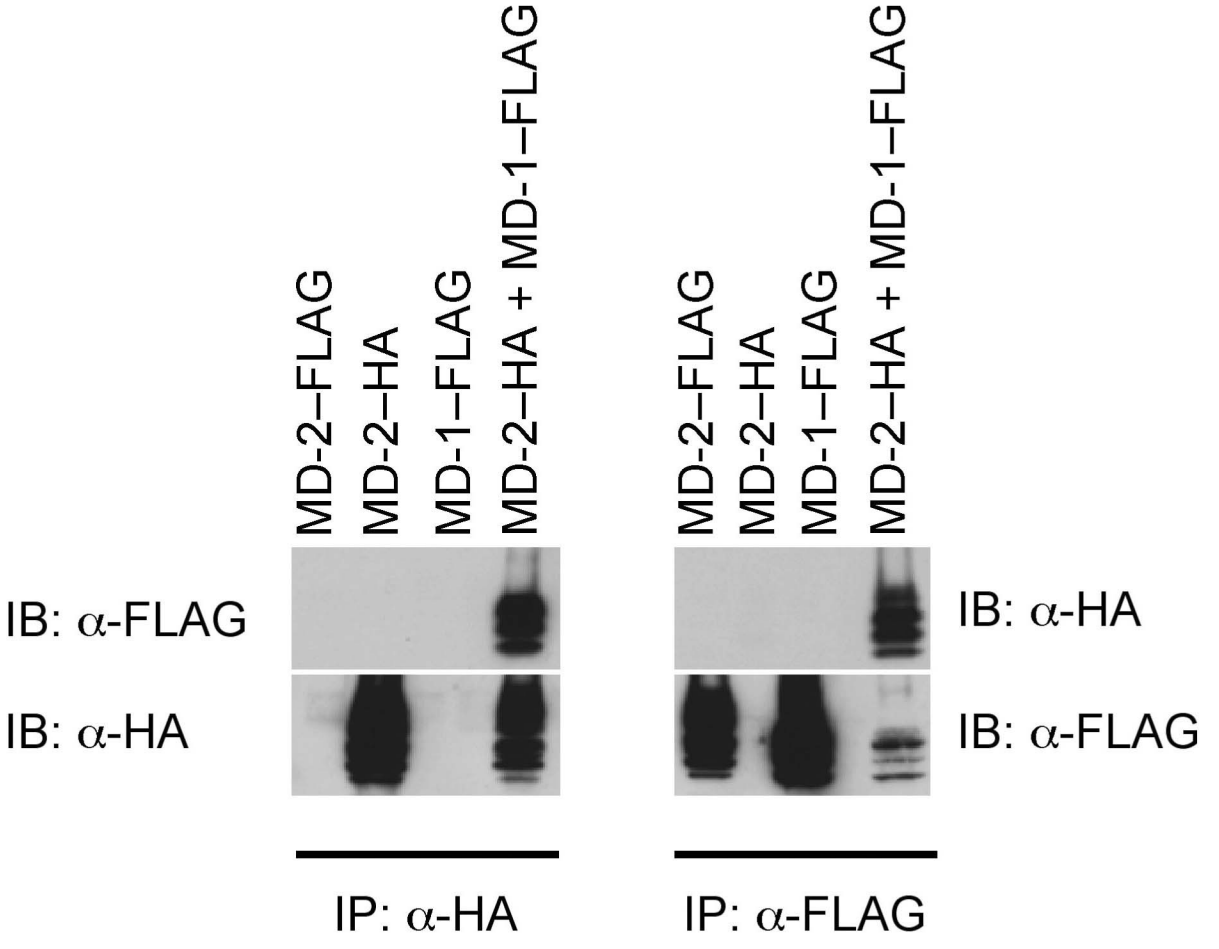


Supplementary Figure 2

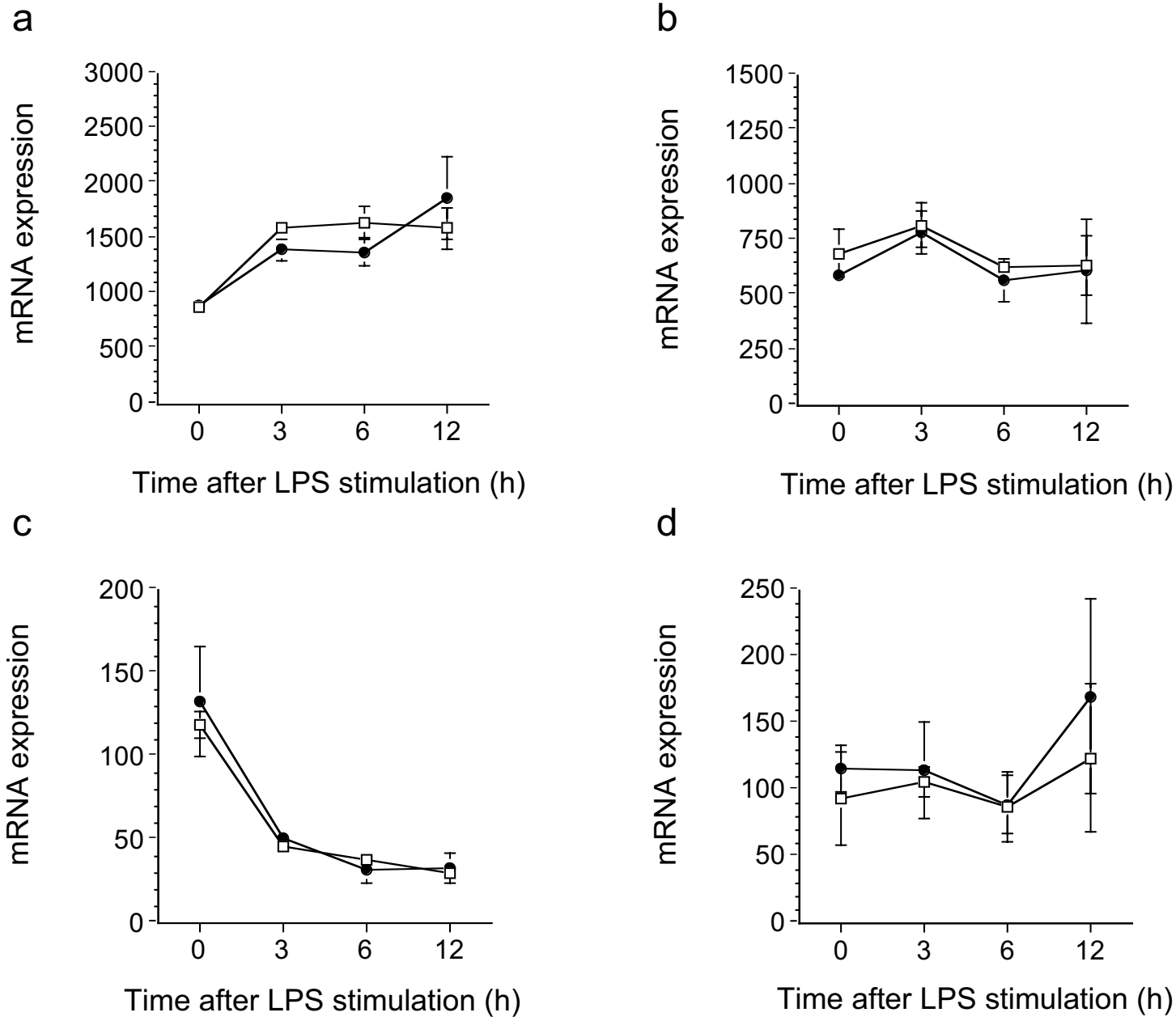




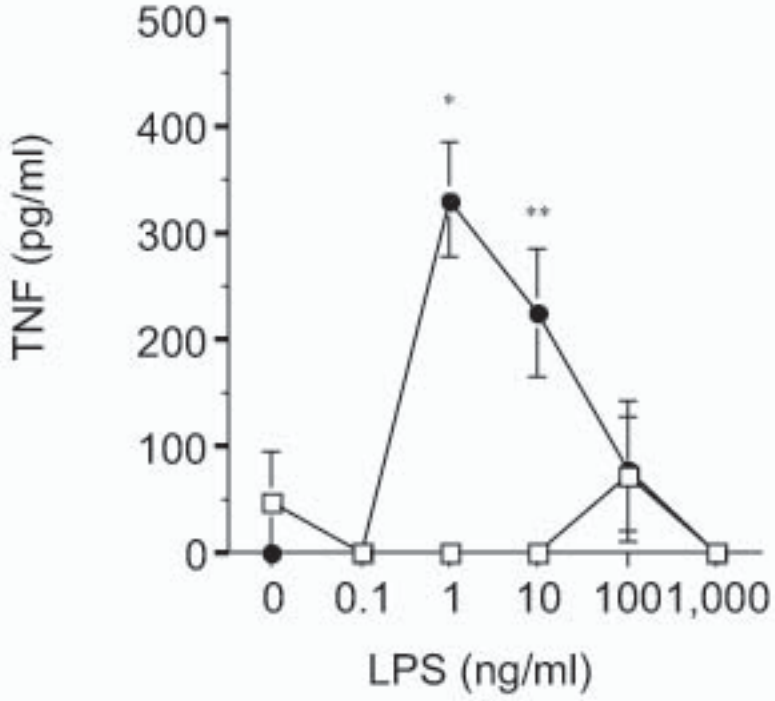
Supplementary Figure 3



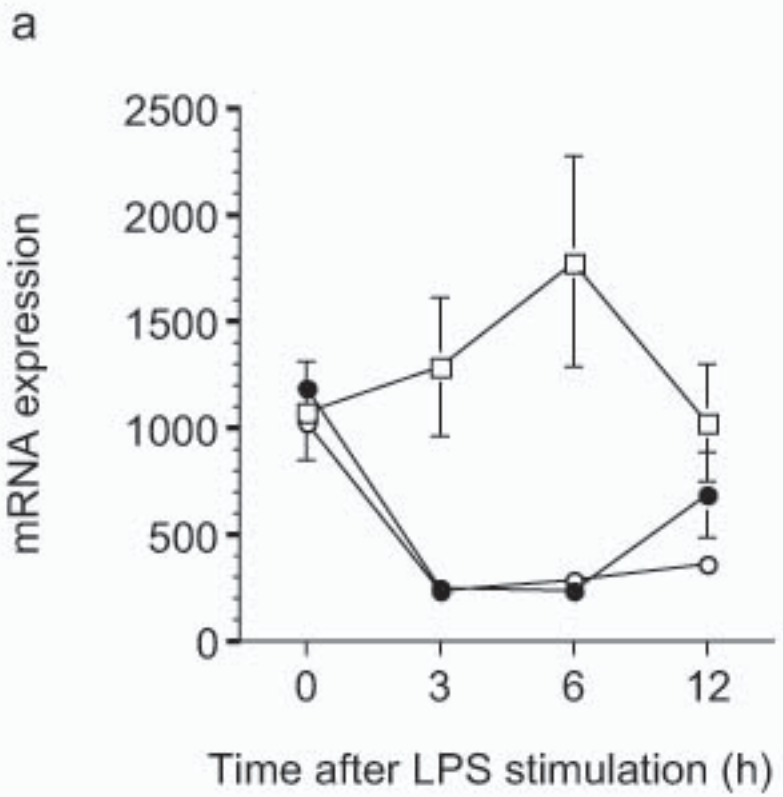
Supplementary Figure 4

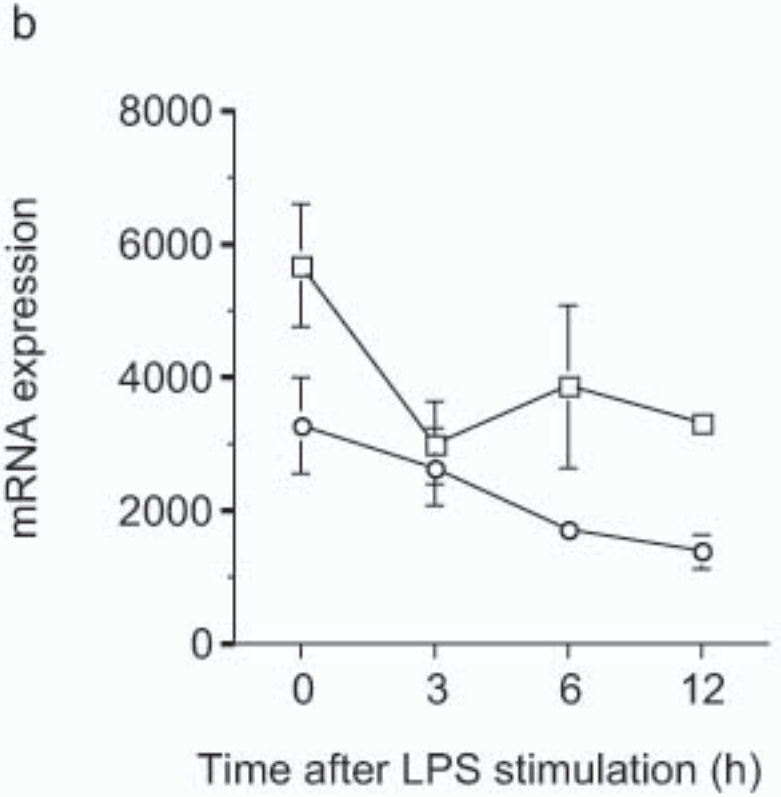


Supplementary Figure 5

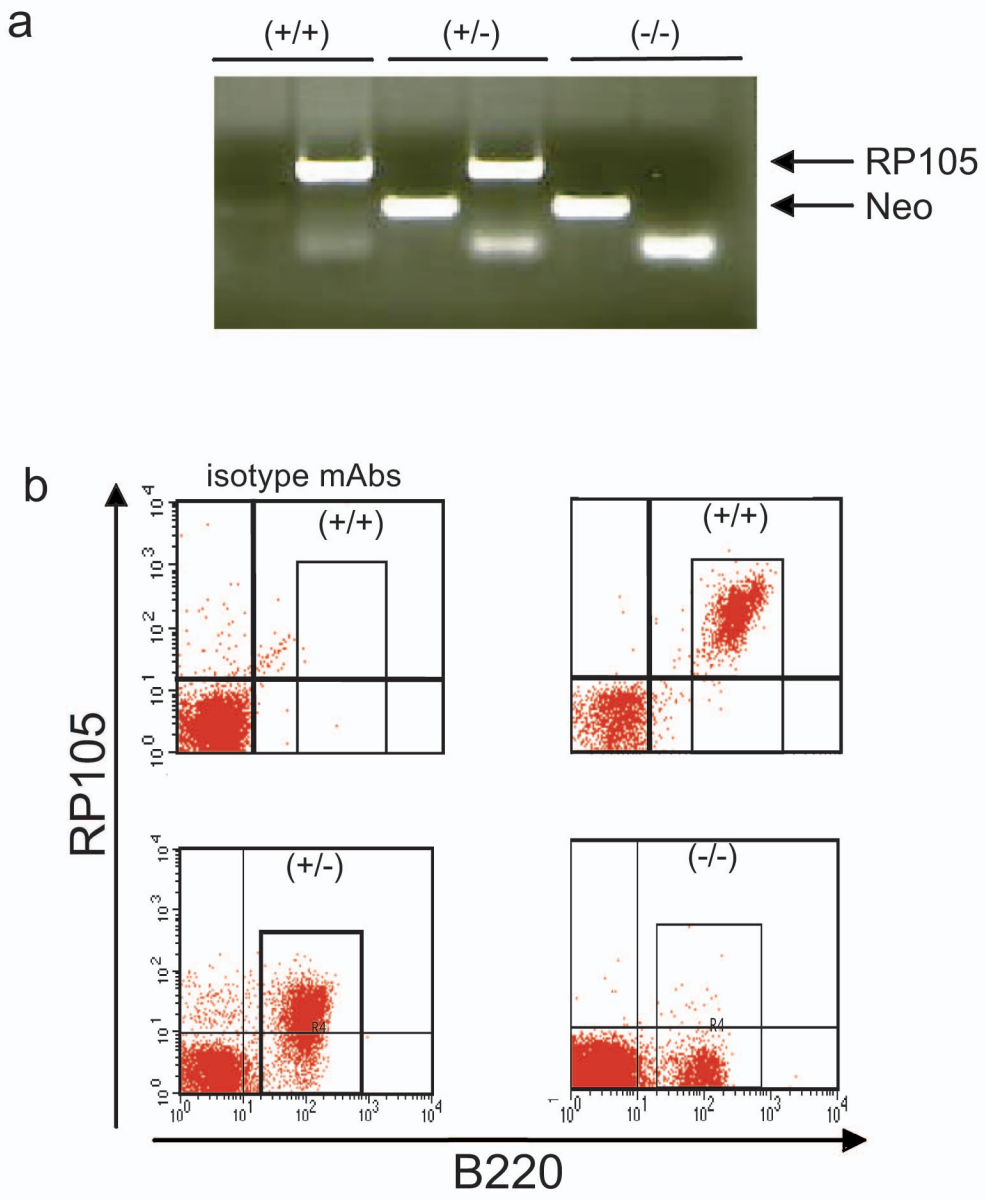


Supplementary Figure 6





Supplementary Figure 7



Supplementary Figure 8