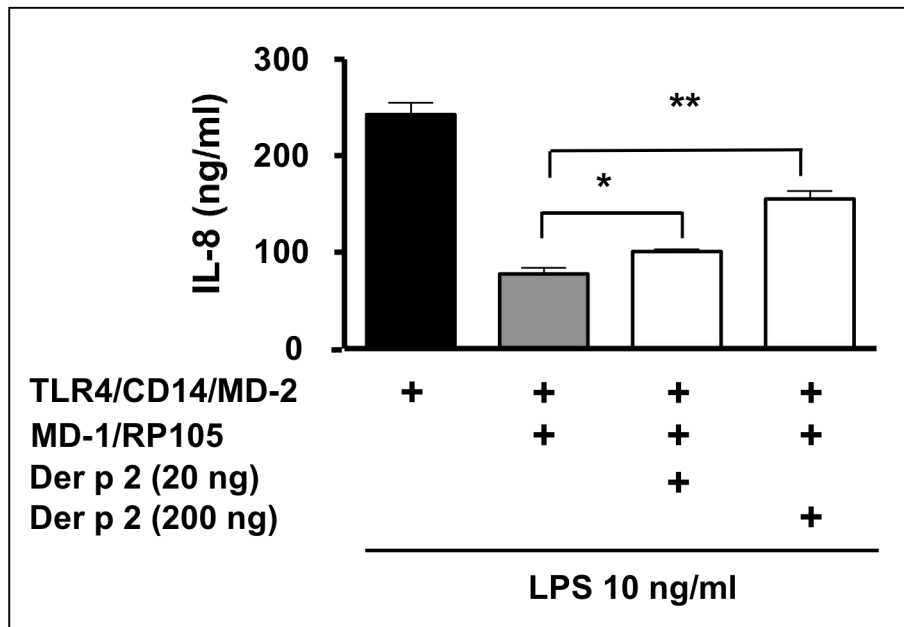


## SUPPLEMENTARY INFORMATION



**Supplementary Figure 1. Der p 2 blunts RP105/MD-1-associated inhibition of LPS-driven IL-8 production in HEK293 cells expressing TLR4/MD-2.** HEK293 cells stably expressing CD14 and TLR4 were transiently transfected with MD-2, or MD-2 plus MD-1 and RP105, and subsequently stimulated with LPS (or left unstimulated), in the absence or presence of immunoaffinity-purified natural Der p 2 at the indicated concentrations. Data represent means  $\pm$  S.E. of cultures (stimulated - mock-stimulated) in a single experiment, representative of an experimental  $n$  of 3. \* $P$  < 0.05; \*\* $P$  < 0.005; unpaired  $t$ -test.

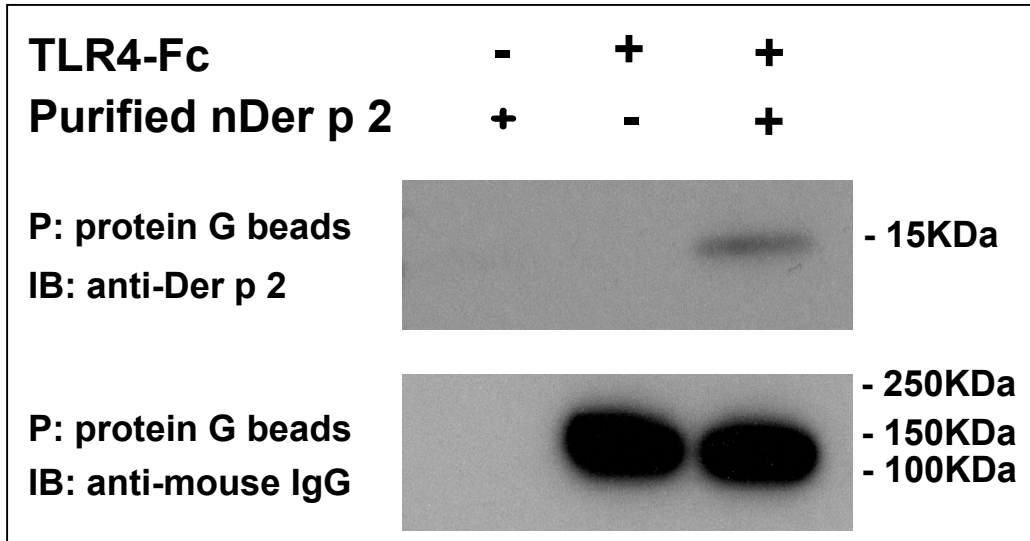
Amino acid 91 in Der p 2 sequence,  
aa102 in MD-2 sequences



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Human MD-2      88 PKRKEVICRGSDDDYSFCRALKGETVNTTISFSFKGIKFSKGKYKCVV 135
Chimpanzee MD-2 88 PKRKEVICRGSDDDYSFCRALKGETVNTTISFSFKGIKFSKGKYKCVV 135
Macaca MD-2     88 PKRKEVICRGSDDDYSFCRALKGETVNTTVSFSFKGIKFSKGKYKCVV 135
Murine MD-2     88 PKRKEVICRGSDDDYSFCRALKGETVNTSIPFSFEGILFPKGHYRCVA 135
Rat MD-2        88 PKRKEIVCHGYDDDYSFCRALKGEAVNTAIPFSFDGILFPKGHRCVA 135
Hamster MD-2    88 PTRKEIICHGYDDNYSFCRALKGETVNTVVPFSFKGILFPKGQYRCVA 135
Rabbit MD-2     88 PKRKEIICKGSDDVYSFCRALKGETVNTTVPFSGIRLSKGQYRCVV 135
Pig MD-2        88 PMRKEVICREYGGDYSFCGALKGETVNTTIPFSFQIRFSPGQYHCVV 135
Bovine MD-2     88 PMRKEVICREYGGDYSFCGALKGETVNTTIPFSFQIRFSPGQYHCVV 135
Equine MD-2     88 PMRKEVICRGSDDDYSFCRALKGETVNTTVSFSFRGMRFPKGRYSCIA 135
Human MD-1      95 LNFSYPICEAALPKFSFCGRRKGEQIYYAGPVNNPEFTIPQGEYQVLL 142
Chimpanzee MD-1 95 LNFSYPICEAALPKFSFCGRRKGEHCYFAGPSPRVSSFVHQGEYQVLL 142
Macaca MD-1     95 LNFSYPICEAALPKFSFCGRRKGEQIYYAGPVNNPEFTIPQGEYQVLL 142
Murine MD-1     95 LNYSYPICEEDQPKFSFCGRRKGEQIYYAGPVNNPGLDVPQGEYQLLL 142
Pig MD-1        92 LNLSYPICEADLPKFSFCGRRKGEQIYYAGPVNNLGFEPPTGEYQVLL 139
Chicken MD-1    90 LSYSETICGPGLSKLIFCGKKKGEHLYYEGPITLGIKEIPQGDYTITA 137

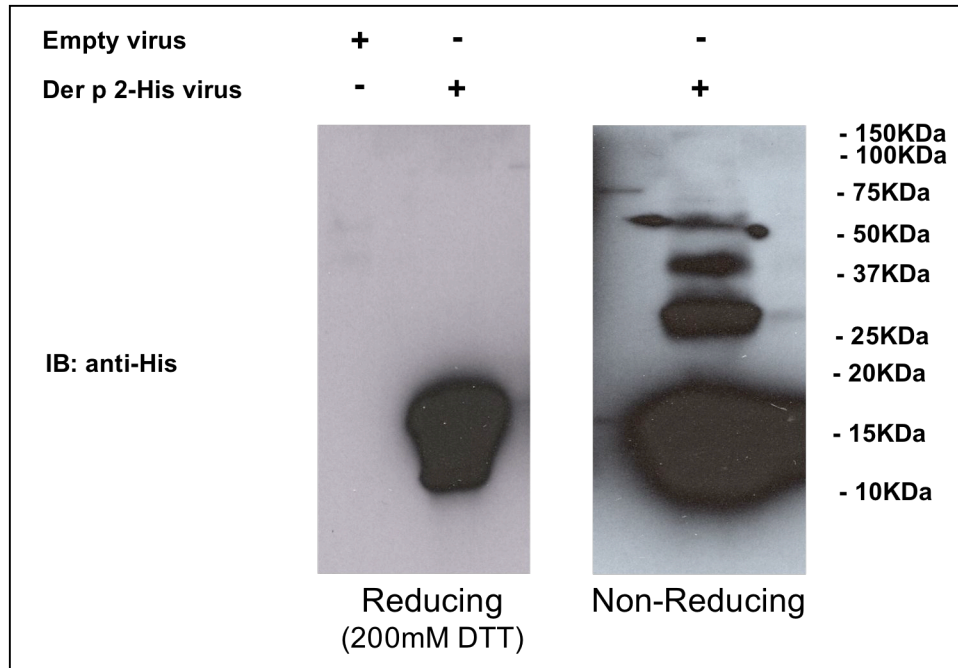
Der p 2         82 PGIDPNACH-----YMKCPLVKGQQYDIKYTWNVP-KIAPKSE-NVVV 122
gi#9280543
```

**Supplementary Figure 2. Alignment of Der p 2 with MD-2 and MD-1.** Conserved cysteines are in blue; Conserved tyrosine 91 (Der p 2)/102 (MD-2) is in red. Sequence alignments were performed using MacVector 7.2.2 software.



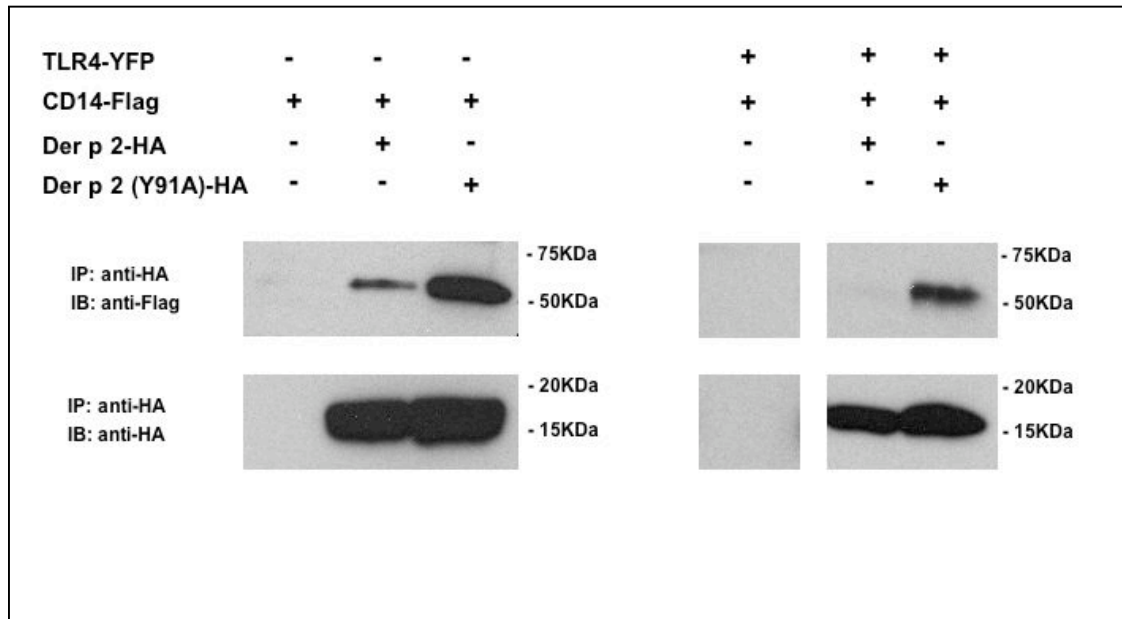
**Supplementary Figure 3. Der p 2 interacts directly with the TLR4 ectodomain.**

Protein G beads were used to precipitate a TLR4 ectodomain-F<sub>c</sub> fusion protein in the absence or presence of immunoaffinity-purified Der p 2, followed by immunoblotting of precipitates with mAb to Der p 2.

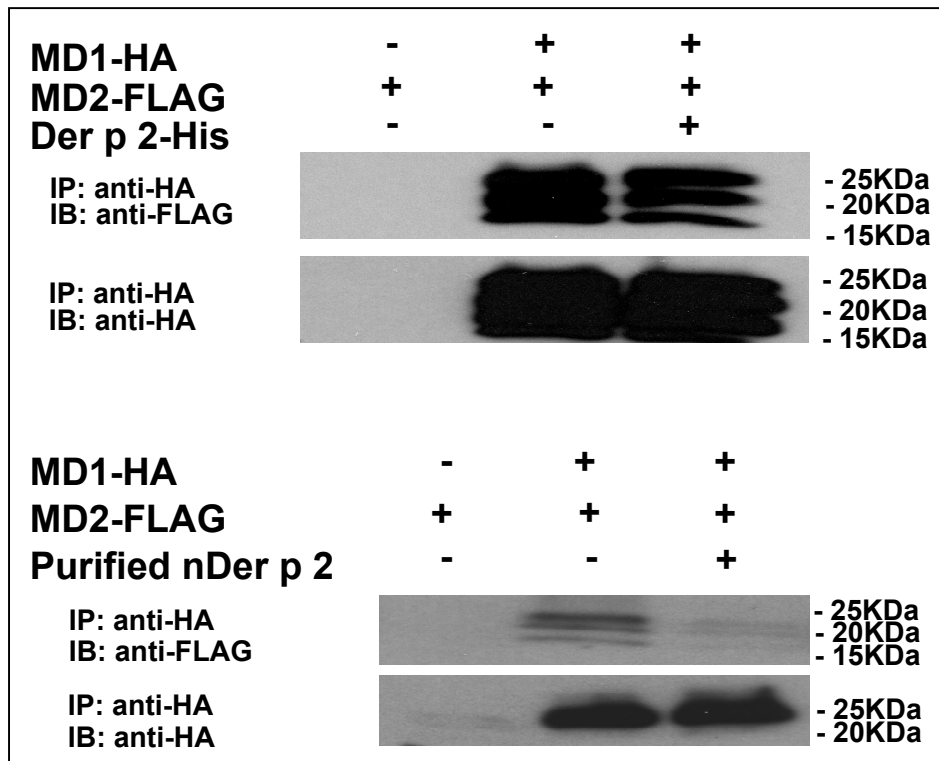


**Supplementary Figure 4. Like MD-2, Der p 2 forms disulfide-linked aggregates.**

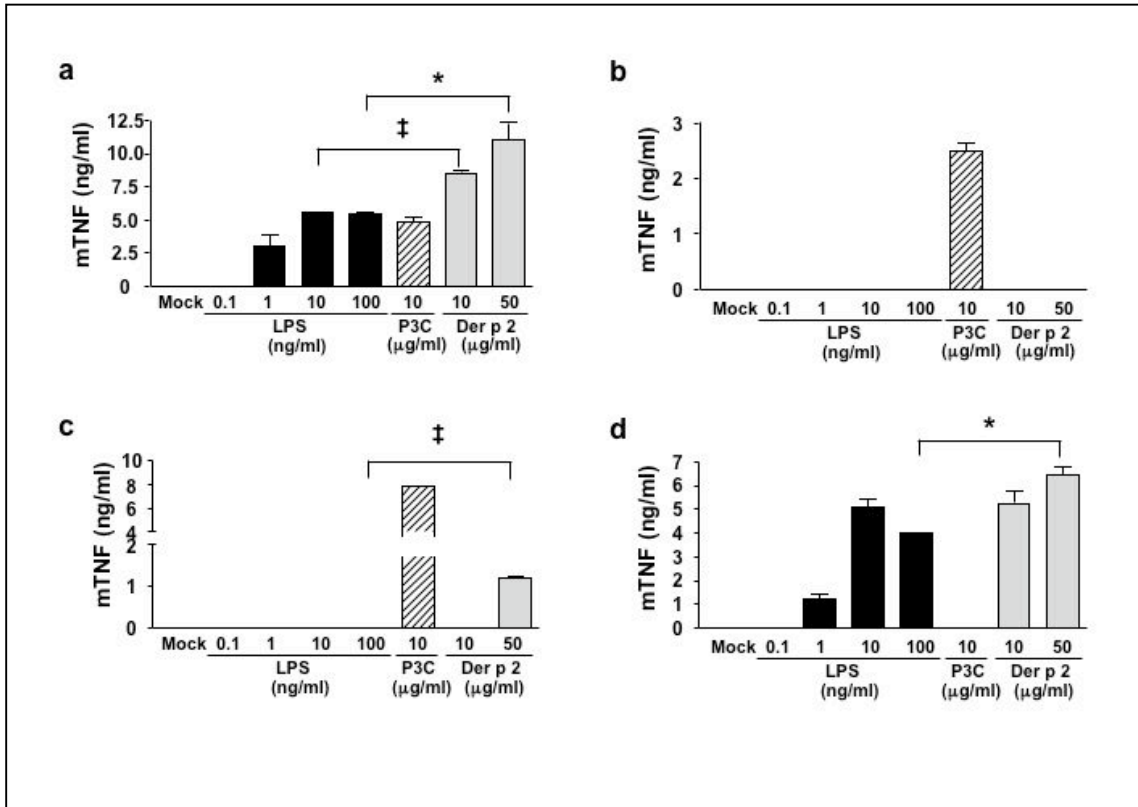
Recombinant His-tagged Der p 2, generated in baculovirus systems (or control supernatants from infection with baculovirus lacking Der p 2) was subjected to SDS-PAGE under non-reducing or reducing conditions, followed by immunoblotting with anti-His antibody. Data are representative of an  $n = 7$ . Similar results were seen with Der p 2 expressed in mammalian cells (data not shown).



**Supplementary Figure 5. Comparative immunoprecipitation analysis of Der p 2 and Der p 2 (Y91A): increased binding of the latter to CD14.** Lysates of HEK293FT cells transiently transfected with the indicated constructs, or EV controls (-), were immunoprecipitated with Ab to HA. The association of HA-tagged Der p 2 or HA-tagged Der p 2 (Y91A) with Flag-tagged CD14 was analyzed by immunoblotting with anti-Flag Ab, in absence (left panel;  $n = 6$ ) or presence (right panel;  $n = 4$ ) of YFP-tagged TLR4. Co-immunoprecipitation analysis suggested similar binding of Der p 2 and Der p 2 (Y91A) to TLR4 and MD-2 (data not shown).



**Supplementary Figure 6. Der p 2 reduces co-immunoprecipitation of MD-2 with MD-1.** Lysates of HEK293FT cells were transiently transfected with the indicated constructs, or EV controls (-). Co-immunoprecipitation techniques similar to those employed in **Figure 2**, were used to analyze the effect of Der p 2 (delivered as a transgene [top panel] or as an immunoaffinity-purified protein [bottom panel]) on the association of HA-tagged MD-1 with FLAG-tagged MD-2.



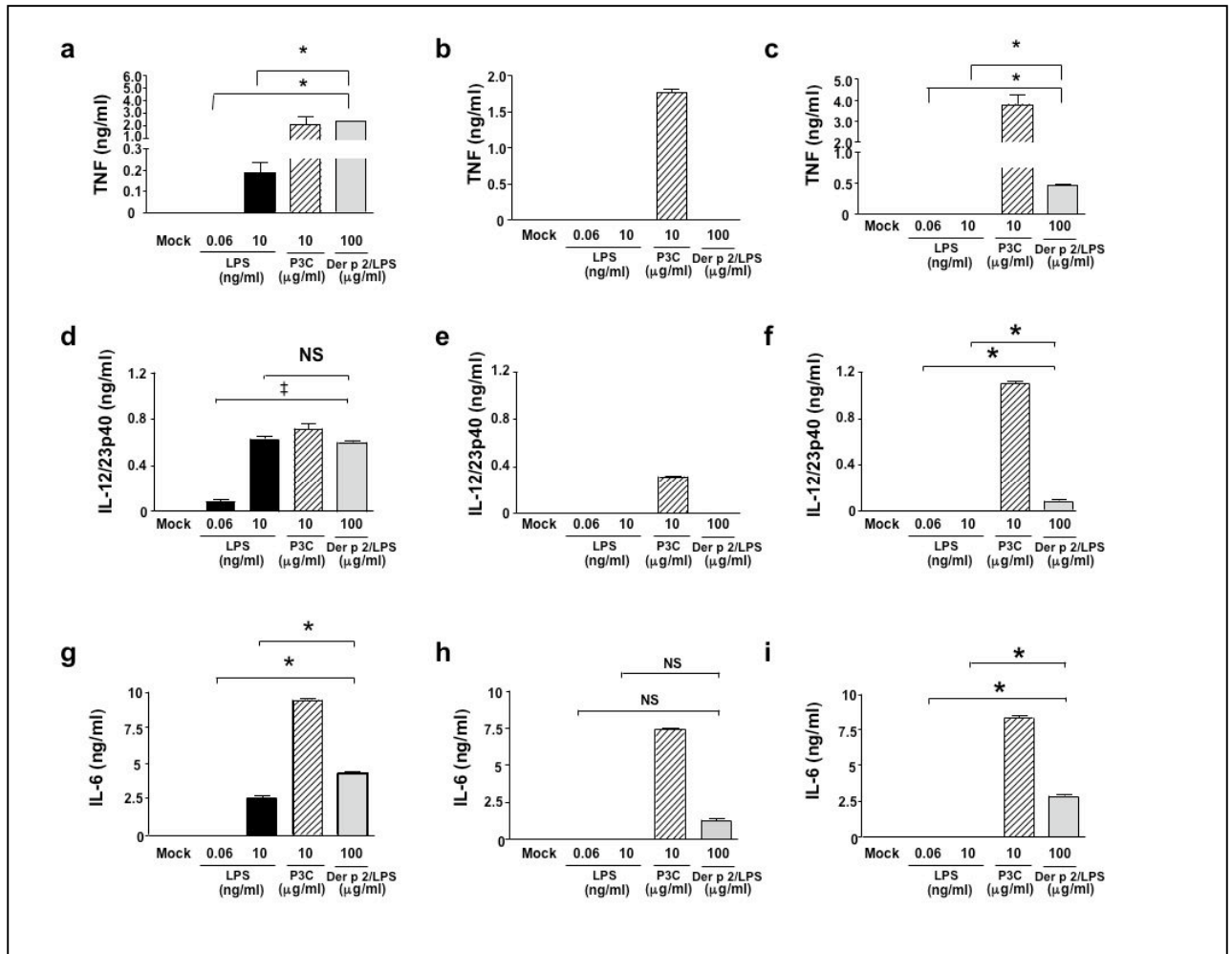
**Supplementary Figure 7. Immunoaffinity-purified Der p 2 drives TLR4 signalling in bone marrow-derived DCs, reconstituting TLR4 signalling in the absence of MD-2.**

DCs from (a) wild type, (b) TLR4<sup>-/-</sup>, (c) MD-2<sup>-/-</sup>, and (d) TLR2<sup>-/-</sup> mice were stimulated for 24 h with *E. coli* K235 LPS (0.1-100 ng/ml), Pam3Cys (P3C; 10 μg/ml), or immunoaffinity purified Der p 2 (10-50 μg/ml). TNF-α was quantified by ELISA.

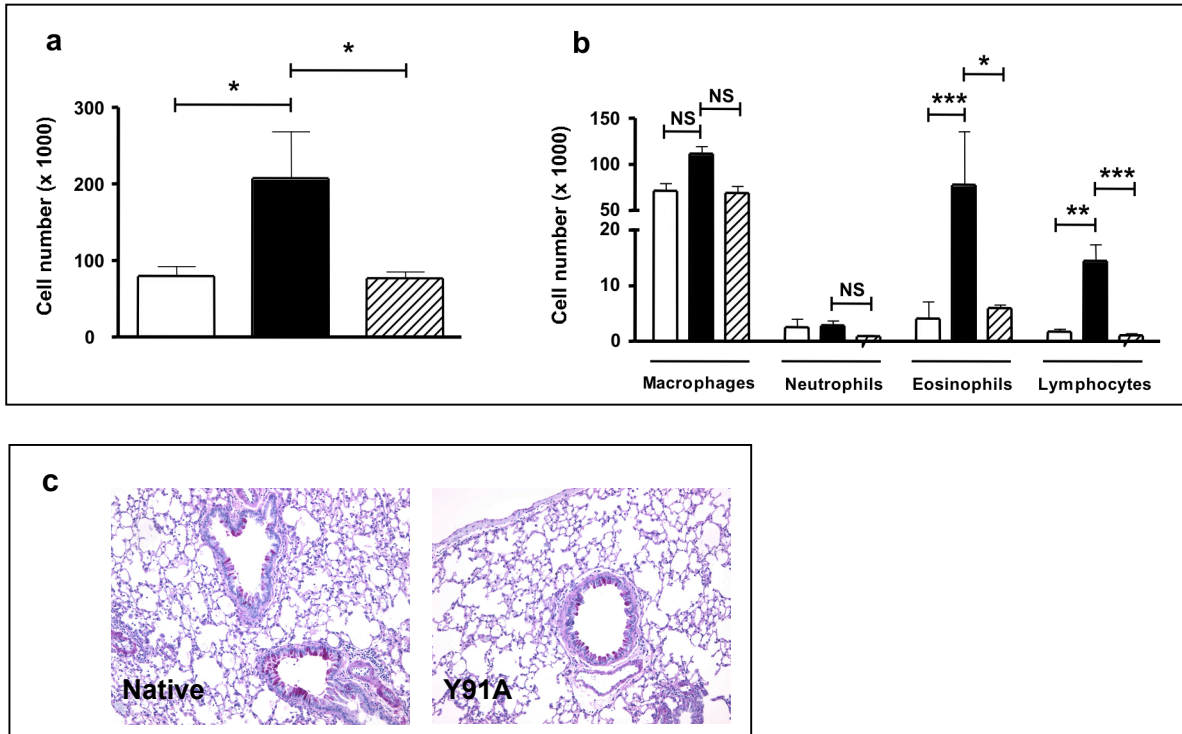
Immunoaffinity-purified Der p 2 contained endotoxin-like activity corresponding to 0.4 ng *E. coli* LPS/μg Der p 2, as determined by the *Limulus* amoebocyte lysate assay. Data represent means ± S.E from a single experiment, representative of an experimental  $n = 4$ .

Mock, mock stimulated. \* $P < 0.05$ ; †  $P < 0.005$ ; unpaired  $t$ -test.





**Supplementary Figure 8. Der p 2 drives TLR4 signalling in primary macrophages, reconstituting TLR4 signalling in the absence of MD-2.** Elicited peritoneal macrophages from (a, d, g) wild type, (b, e, h)  $TLR4^{-/-}$ , and (c, f, i)  $MD-2^{-/-}$  mice were stimulated for 24 h with *E. coli* K235 LPS (0.06-10 ng/ml), Pam<sub>3</sub>Cys (P3C; 10 μg/ml), or recombinant Der p 2 (100 μg/ml) co-purified with *E. coli* K235 LPS (final concentration of 60 pg LPS/100 μg Der p 2, as measured by the *Limulus* ameobocyte lysate assay). TNF- $\alpha$  (a-c), IL-12/23p40 (d-f) and IL-6 (g-i) were quantified by ELISA. Data represent means  $\pm$  S.E. from a single experiment, and are representative of an experimental  $n = 3-7$ . Mock, mock stimulated. \* $P < 0.001$ ; unpaired  $t$ -test.



**Supplementary Figure 9. Der p 2 (Y91A) fails to induce experimental allergic asthma.** Wild type mice were sensitized, challenged and analyzed as outlined in **Figure 3**. Sensitization was with PBS/LPS (white bars), rDer p 2/LPS (black bars), or rDer p 2 (Y91A)/LPS (hatched bars). **a, b** Inflammatory cell composition of bronchoalveolar lavage (BAL) fluids. **a**, total cell numbers. **b**, differential cell counts. **c**, Representative lung sections stained with periodic acid-Schiff. Data (**a-b**) represent means  $\pm$  S.E. of 4-7 animals/group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; ANOVA on log-transformed data.