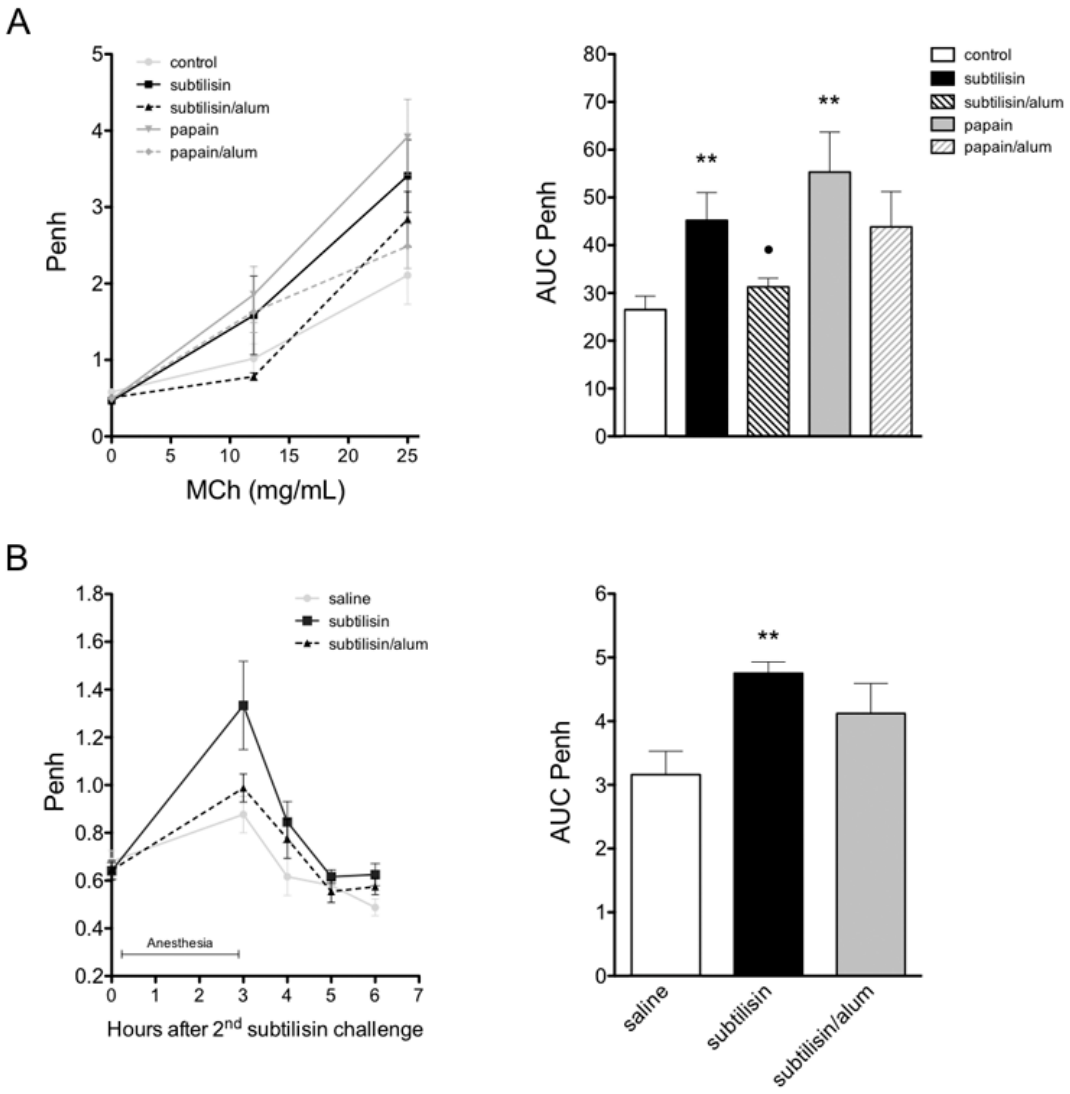
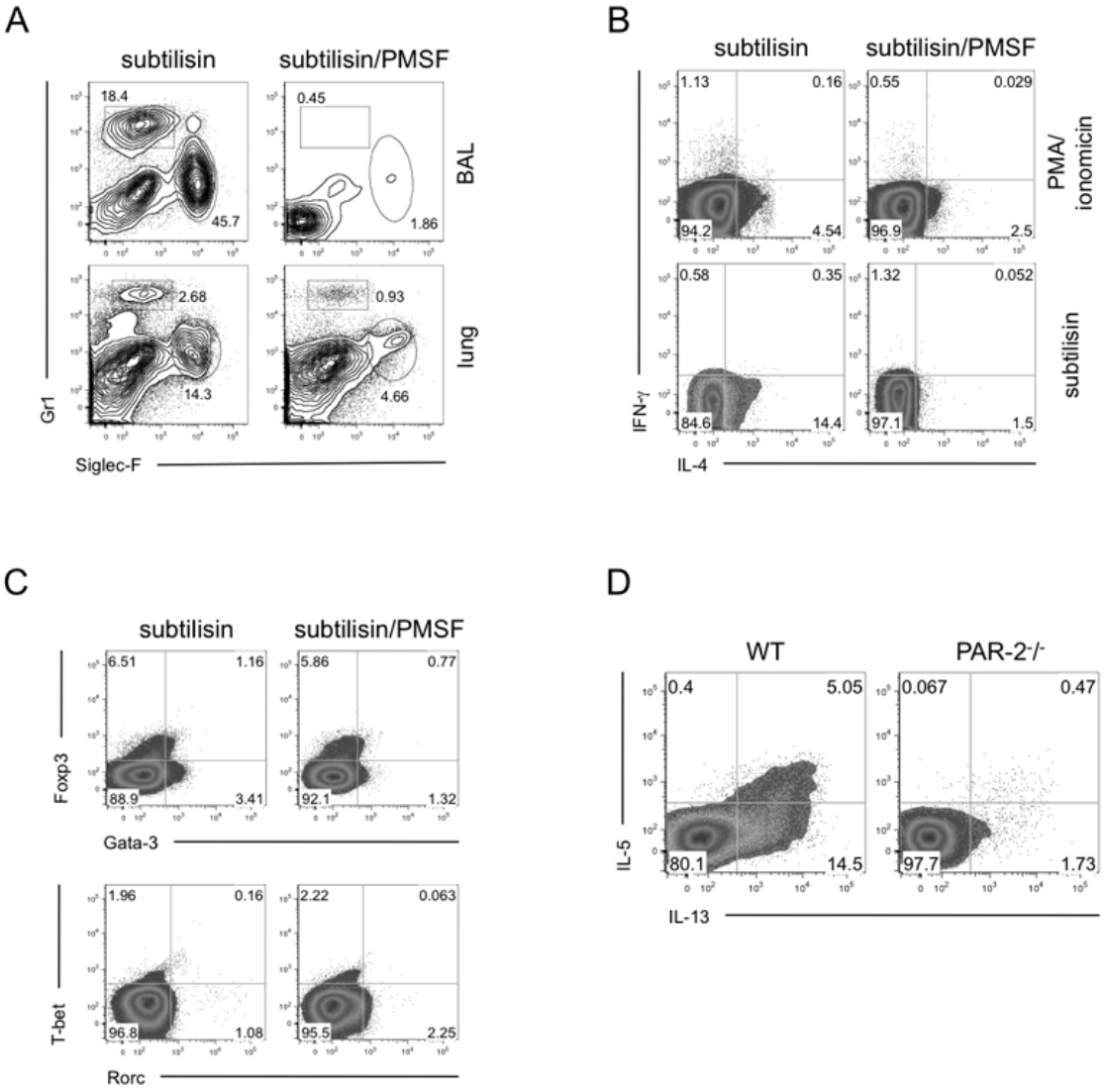
Supplementary Fig. 1: Subtilisin induces airway hyperreactivity and allergen-specific late phase response.



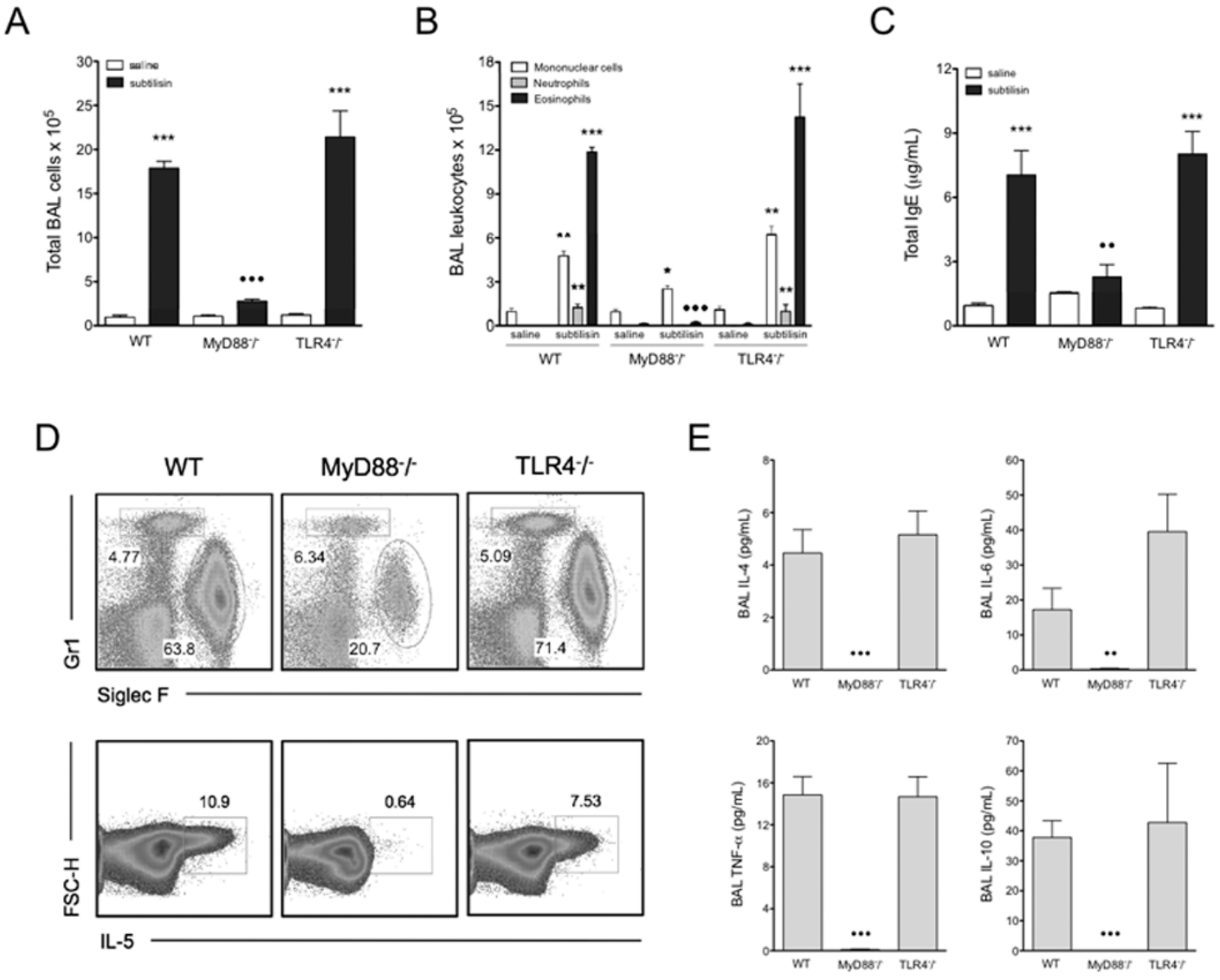
Subtilisin induces airway hyperreactivity and allergen-specific late phase response. C57BL/6 mice were s.c. sensitized with subtilisin or papain with or without alum on days 0 and 7 and i.n. challenged on days 14 and 21 with the respective enzyme. (A) Respiratory pattern in terms of Penh value (enhanced pause) in response to inhalation of crescent doses of methacholine (MCh) on day 15 (A, left). Same data is shown as area under the curve (AUC) on Penh values (A, right). (B) Late phase response to subtilisin was analyzed for 4 h right after the second i.n. challenge, on day 22. Same data is shown as AUC on Penh values (B, right). Data are mean ± SEM and are representative of three independent experiments (n=5). Error bars show **, $P \leq 0.001$; *, $P \leq 0.01$ for significant differences to control group or • to protease without alum group.

Supplementary Fig. 2: Serine protease activity and expression of PAR-2 are essential for Th2 polarization induced by subtilisin.



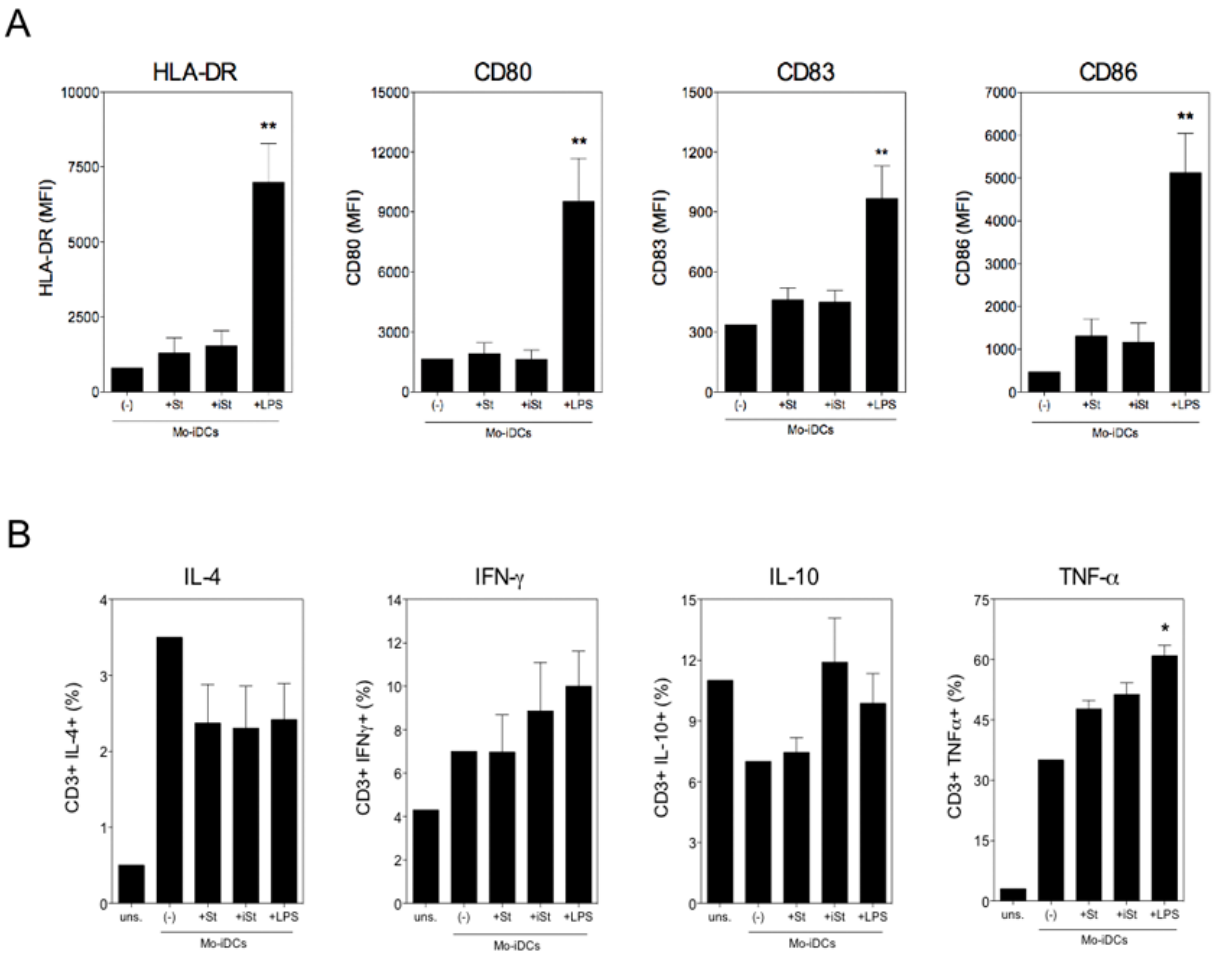
Serine protease activity and expression of PAR-2 are essential for Th2 polarization induced by subtilisin. C57BL/6 mice were sensitized s.c. with active or 10 µM PMSF-inactivated subtilisin on days 0 and 7, and challenged with active subtilisin on days 14 and 21. Lungs were collected on day 22 and were digested with collagenase IV and DNase I. (A) Flow cytometry analysis of BAL (top) and lung (bottom) $CD45^+$ $MHCII^-$ cells. (B) Intracelular staining of lung $CD3^+$ $CD4^+$ cells stimulated with PMA and ionomycin for 5 h (top plots) or with 1 µg/mL subtilisin for 18 h (bottom plots). (C) Intracellular staining for transcription factors of lung $CD3^+$ $CD4^+$ cells stimulated with PMA and ionomycin for 5 h. (D) C57BL/6 WT or PAR-$2^{-/-}$ mice were sensitized with s.c. subtilisin on days 0 and 7 and i.n. challenged on days 14 and 21. On day 22, lungs were perfused and digested for intracellular staining of $CD45^+$ $CD3^+$ $CD4^+$ cells stimulated with PMA and ionomycin for 5 h. Data are representative of two independent experiments (n=5).

Supplementary Fig. 3. Allergic sensitization to subtilisin is dependent on MyD88, but not on TLR4.



Allergic sensitization to subtilisin is dependent on MyD88, but not TLR4. C57BL/6 WT, MyD88$^{-/-}$ or TLR4$^{-/-}$ mice were sensitized with s.c. subtilisin on days 0 and 7 and i.n. challenged on days 14 and 21. (A) Total number of cells in BAL recovered on day 22. (B) Differential count from BAL cells. (C) Total serum IgE. (D, top) Siglec-F and Gr1 staining on gated CD45^{+} MHCII^{-} BAL cells from subtilisin-administered mice. (D, bottom) Intracellular staining for IL-5 of CD45^{+} CD3^{+} CD4^{+} lung cells stimulated with PMA and ionomycin for 5 h. (E) Cytokine production analyzed by CBA from BAL samples of subtilisin-administered groups. Data are mean ± SEM and are representative of two independent experiments (n=5). Error bars show ***, P ≤ 0.0001; **, P ≤ 0.001; *, P ≤ 0.01 for significant differences to control (saline) group or • to subtilisin-sensitized WT group.

Supplementary Fig. 4: Subtilisin-pulsed human DCs are not classically activated nor induce Th2 polarization.



Subtilisin-pulsed DCs are not classically activated nor induce Th2 polarization. DCs generated from healthy donor monocytes were incubated with 1µg/mL active subtilisin (St), heat-inactivated subtilisin (iSt), 50 ng/mL LPS or media (-) for 18 h. (A) Mean fluorescence intensity (MFI) of surface markers associated with DC activation. (B) Cytokine analysis by intracellular staining of $CD3^+$ cells restimulated with PMA and ionomycin for 12 h after co-culture with DCs for 5 days. Data are mean ± SEM and are representative of two independent experiments, each with triplicate samples. Error bars show **, $P \leq 0.001$; *, $P \leq 0.01$ for significant differences to media-stimulated Mo-iDCs (-).