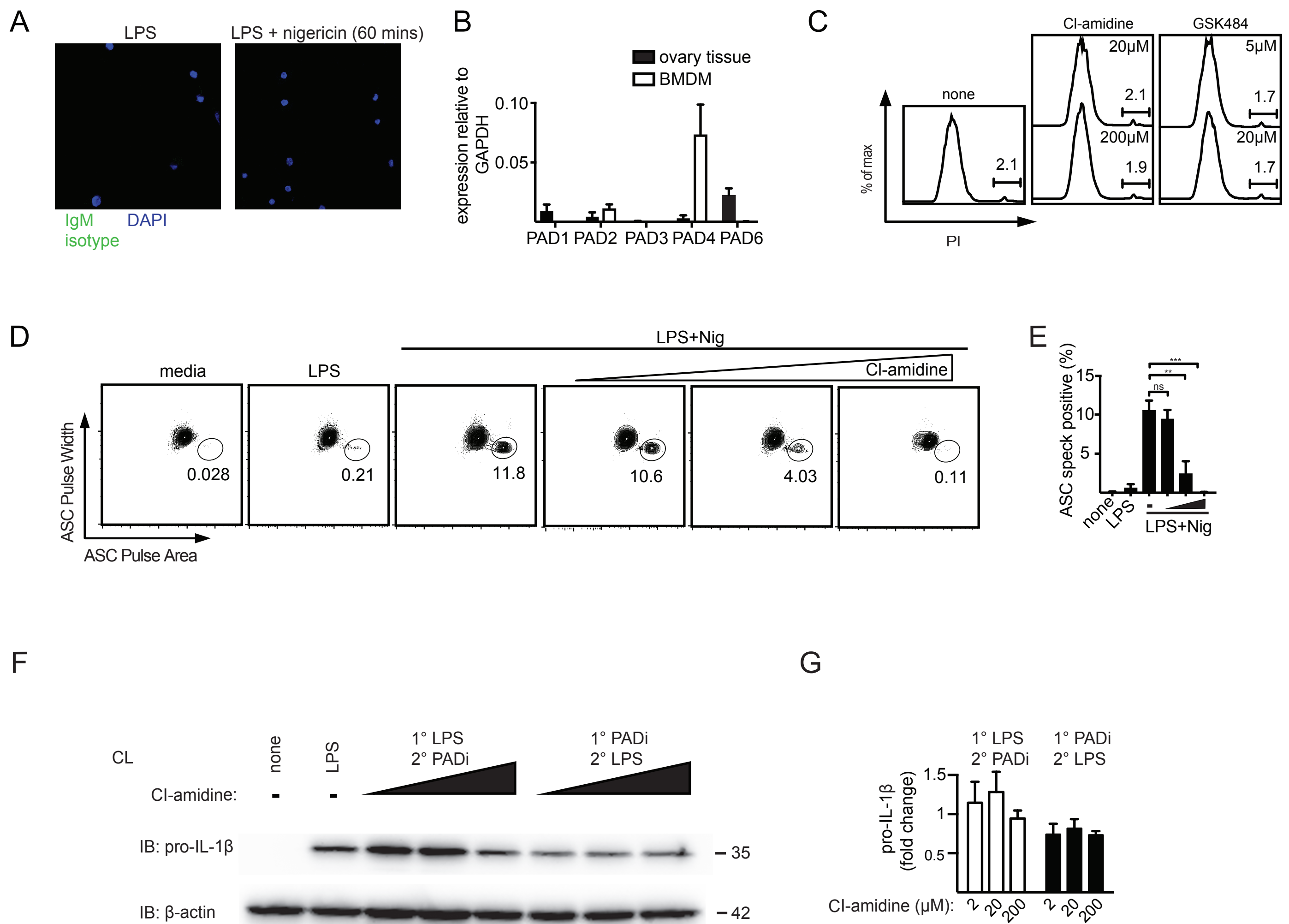
Supplemental Figure 1



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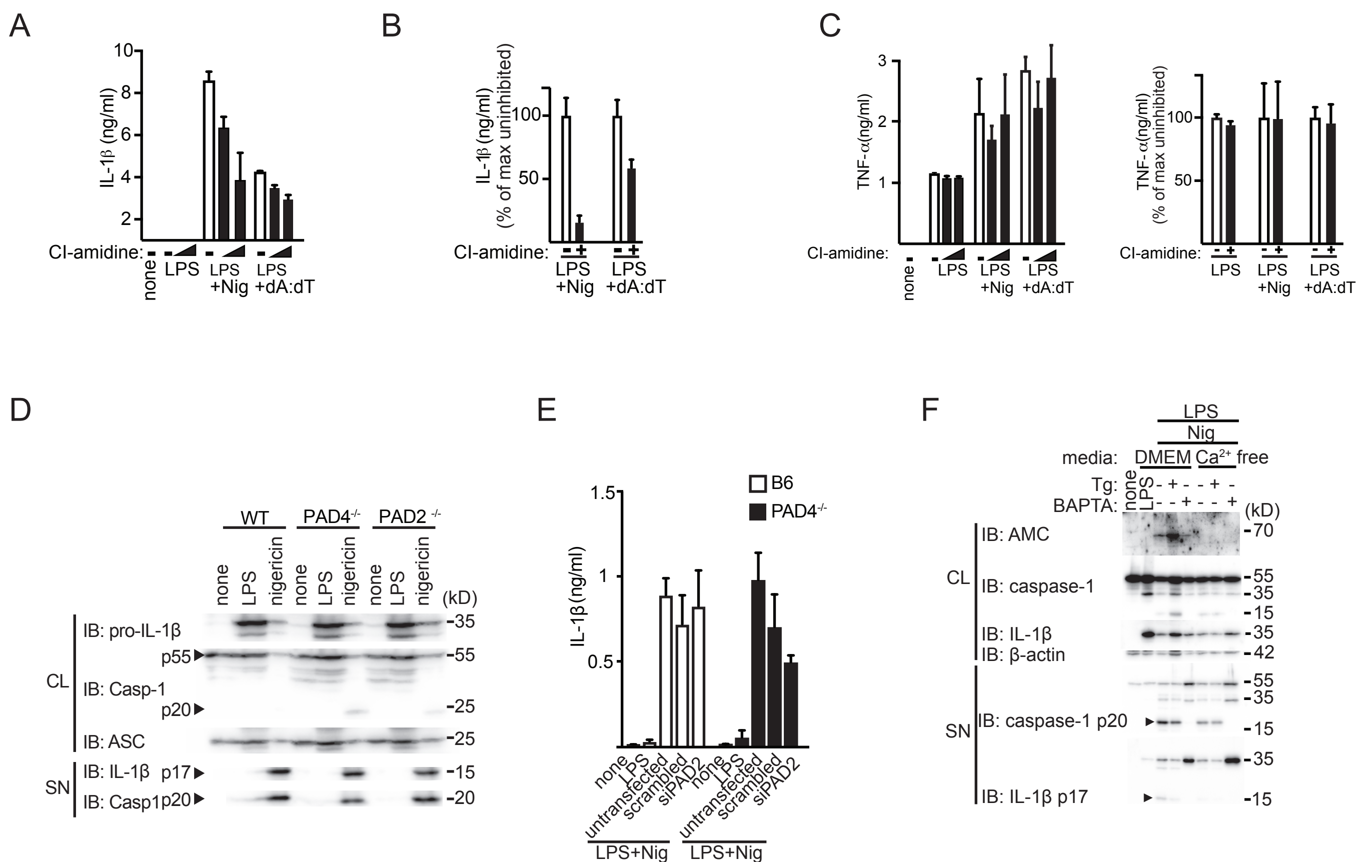
Supplemental Figure 1. Reduction of ASC speck formation by pan-PAD inhibition in NLRP3 activated human monocytes and effect of pan-PAD inhibition on pro-IL-1β synthesis in TLR4 stimulated BMDMs.

(A) Confocal images of BMDMs treated with LPS or LPS and nigericin and stained for IgM isotype control (green) and DAPI (blue). (B) PAD enzyme expression of murine BMDMs. Murine ovary tissue served as positive control for PAD1/3/6, which are not expressed in BMDMs. Data are expressed as mean ± SEM of 4 experiments. (C) Histogram blots of BMDMs left untreated (none) or treated with Cl-amidine and GSK 484 at indicated doses. Numbers within the histograms represent the % of propidium iodide positive cells. (D) Representative contour blots of gated CD14+CD16- human monocytes. Displayed is the intracellular ASC pulse width (y-axis) versus the ASC pulse area (x-axis). Percentages of ASC speck forming monocytes are indicated within the contour blots. (E) Bar graphs represent the same data as in (D) and are expressed as mean ± SEM of 2 individual experiments. (F) Immunoblot for IL-1β from CL of BMDMs that were either primed first with LPS for 2hr and then treated with increasing concentrations of Cl-amidine (1° LPS, 2° PADi) or vice versa (1° PADi, 2° LPS) for 1hr. β-actin served as a loading control. (G) Bar graphs of the band densitometry data showing the fold change in pro-IL-1β protein expression of LPS compared to LPS and Cl-amidine treated BMDMs (fold changes were calculated based on the LPS only condition). Data are expressed as the mean ± SEM of two independent experiments.

Supplemental Figure 2



Supplemental Figure 2. Effect of PAD enzyme inhibition for NLRP3 and AIM2 inflammasome activation:

(A) ELISA for IL-1β from SN of NLRP3 and AIM2 activated BMDMs in presence of PAD inhibitor as indicated. The bar graphs in (B) show the effect of Cl- amidine (200µM) on IL-1β production in % of the uninhibited condition (pooled data of 5 mice, mean ± SEM of duplicate samples). (C) ELISA for TNF-α from SNs of BMDMs stimulated as in (A). (D) Immunoblot for IL-1β, caspase-1 and ASC from CL and SNs of LPS primed BMDMs of WT, PAD2$^{-/-}$ or PAD4$^{-/-}$ (all FVB background) treated with the indicated NLRP3 stimuli. (E) ELISA of IL-1β from SNs of PAD2 siRNA transfected WT and PAD4$^{-/-}$ BMDMs stimulated with LPS and nigericin (wt n=6, PAD4$^{-/-}$ n=2, data pooled from 2 independent experiments (mean ± SEM for duplicate samples). (F) Immunoblot for IL-1β, caspase-1 and ASC from CL and SN of BMDMs stimulated with LPS and nigericin alone or in the presence of thapsigargin or BAPTA. BMDMs were stimulated in the presence or absence of extracellular calcium as indicated. The data is representative of two independent experiments.

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