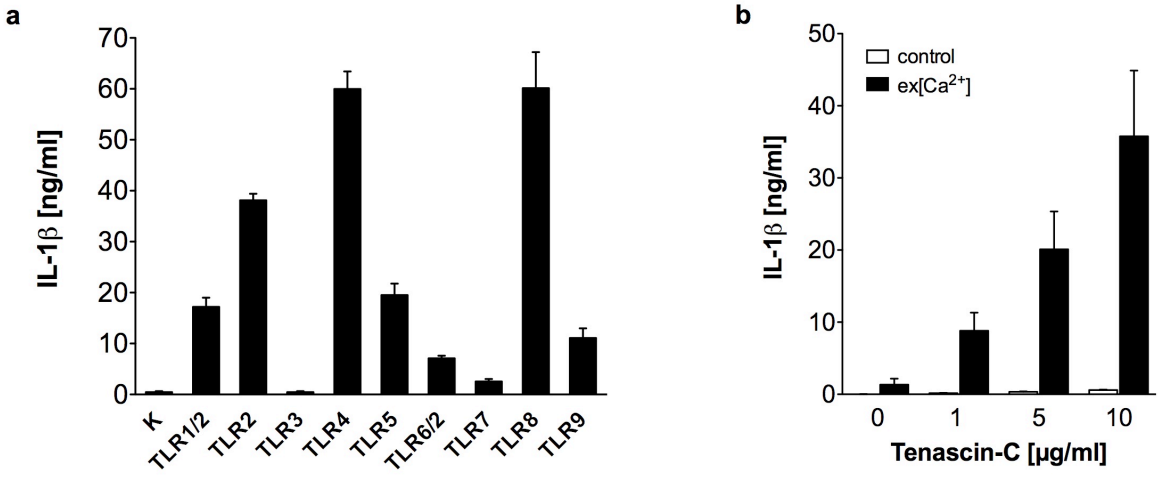
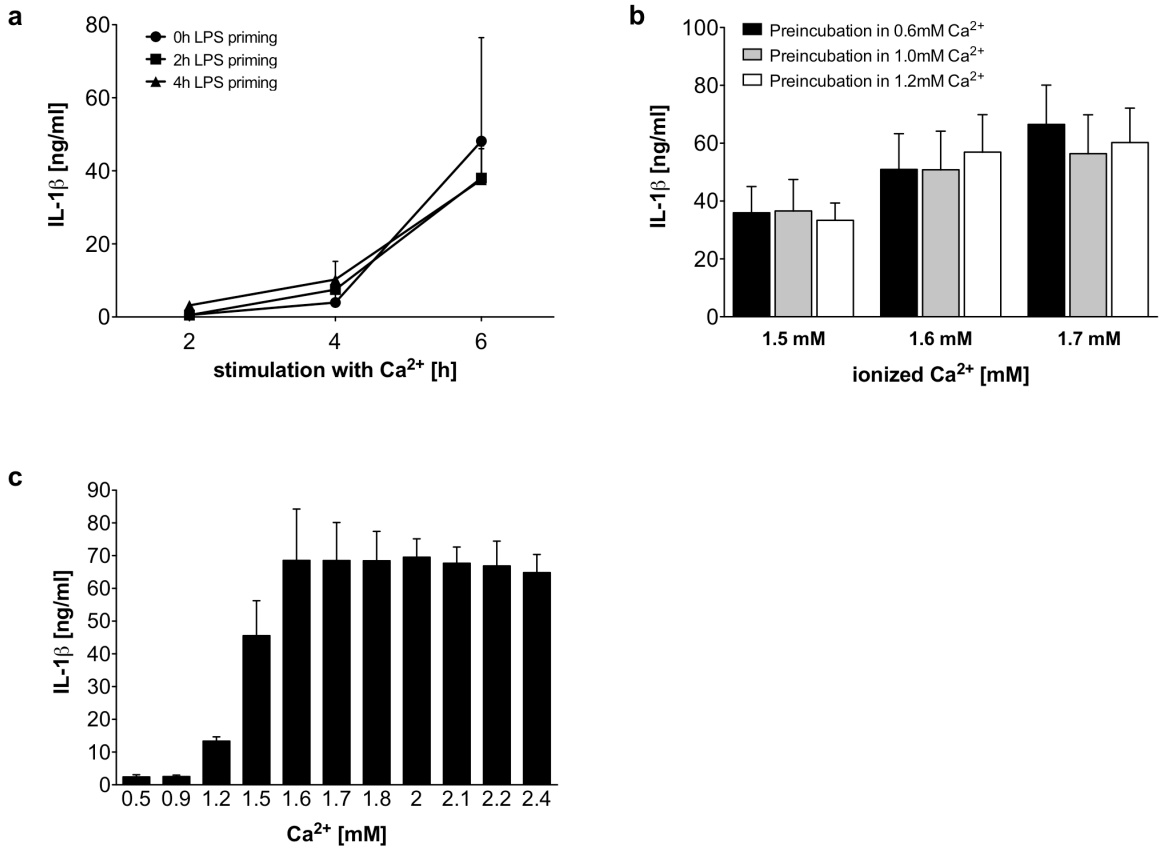
Supplementary information



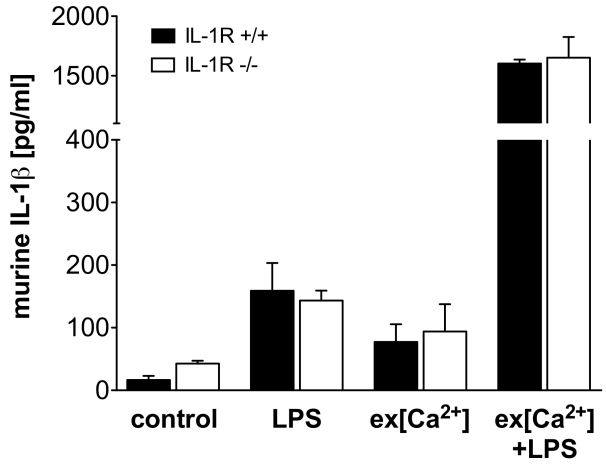
Supplementary Figure S1: Ex[Ca^{2+}]-induced IL-1ß production of monocytes primed with different TLR ligands

IL-1ß release of CD14+ monocytes in response to stimulation for 16 hours with increased ex[Ca^{2+}] (1.7 mM) plus the TLR stimulus indicated. (a) The following TLR ligands were used: Pam3CSK4 (TLR 1/2), heat-killed preparation of *Listeria monocytogenes* (TLR 2), Poly(I:C) (TLR 3), LPS (TLR 4), flagellin (TLR 5), FSL1 (TLR 6/2), Imiquimod (TLR 7), ssRNA40 (TLR 8) and ODN2006 (TLR 9). (b) The endogenous TLR4 ligand Tenascin-C was added in increasing concentrations (n=3). In all experiments, cytokine concentrations were determined in the supernatant by ELISA. All data are expressed as means±SEM.



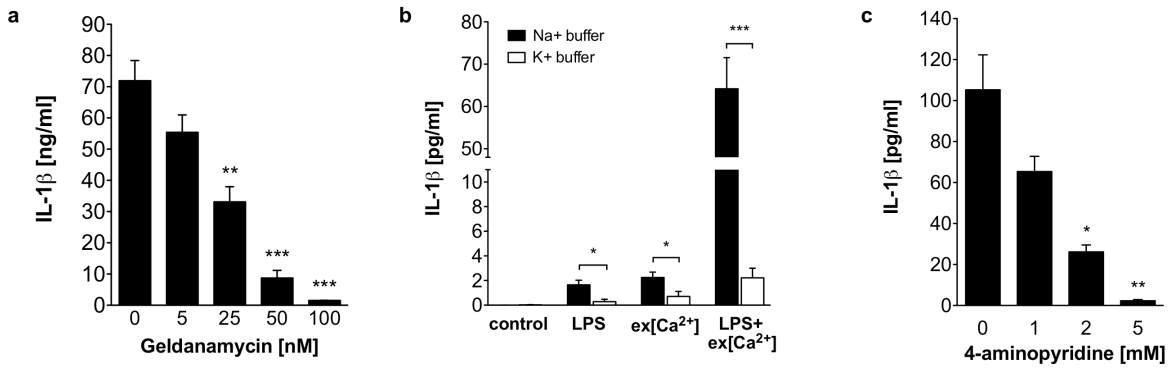
Supplementary Figure S2: Influence of duration of LPS priming, initial extracellular Ca^{2+} concentration and increasing Ca2+ concentration during the culture on IL-1ß release from monocytes

(a) IL-1ß release of CD14+ monocytes in response to stimulation for 2 to 6 hours with increased extracellular calcium concentrations (1.7 mM) for 2 to 6 hours either without LPS priming (full circles) or with LPS priming for 2 or 4h (full squares or full triangles, respectively, n=3). (b) IL-1ß release of CD14+ monocytes in response to stimulation for 16 hours with increased extracellular calcium concentrations (1.5 to 1.7 mM) plus LPS (n=3). Monocytes were preincubated in varying ex[Ca^{2+}] concentrations. (c) IL-1ß release of CD14+ monocytes in response to increasing ex[Ca^{2+}] plus LPS after 16 h of stimulation (n=3). In all experiments, cytokine concentrations were determined in the supernatant by ELISA. All data are expressed as means±SEM.



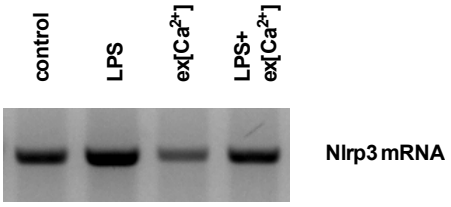
Supplementary Figure S3: IL-1β secretion of monocytes stimulated with ex[Ca^{2+}] plus LPS is independend from the presence of IL-1 receptor.

IL-1β release of CD11b+ peripheral blood monocytes from wildtype mice and IL-1R-/- mice in response to LPS and ex[Ca^{2+}] plus LPS (n=4). All data are expressed as means±SEM.



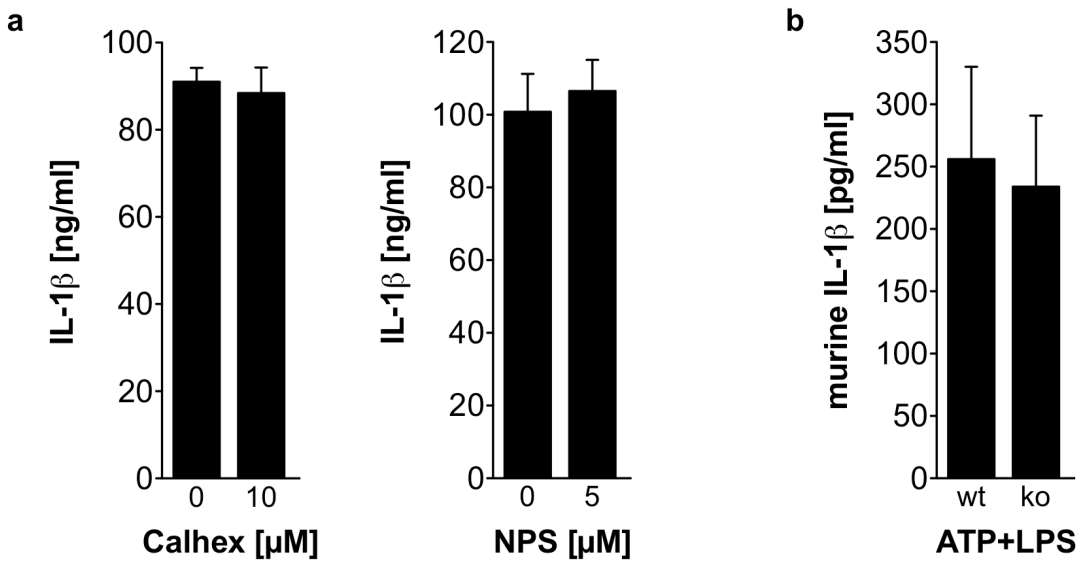
Supplementary Figure S4: Ex[Ca^{2+}]-induced release of IL-1ß is inhibited by Geldanamycin, high extracellular potassium concentrations, and by the potassium channel blocker 4-aminopyridin, indicating K+ efflux-dependent inflammasome activation

(a) IL-1ß release from monocytes in response to ex[Ca^{2+}] (1.7mM) plus LPS in combination with increasing concentrations of the inflammasome inhibitor Geldanamycin (n=3) (b) IL-1ß secretion of unstimulated monocytes (control), monocytes stimulated with LPS, with increased ex[Ca^{2+}] concentration (1.7 mM), or both, in culture media containing high sodium concentrations as control (Na+ buffer) or high extracellular potassium concentrations (K^+ buffer) (n=5). (c) IL-1ß secretion of monocytes stimulated with ex[Ca^{2+}] plus LPS in the absence or presence of the indicated concentrations of 4-aminopyridine (n=3). All data are expressed as means±SEM (*p<0.05, **p<0.01, ***p<0.001).



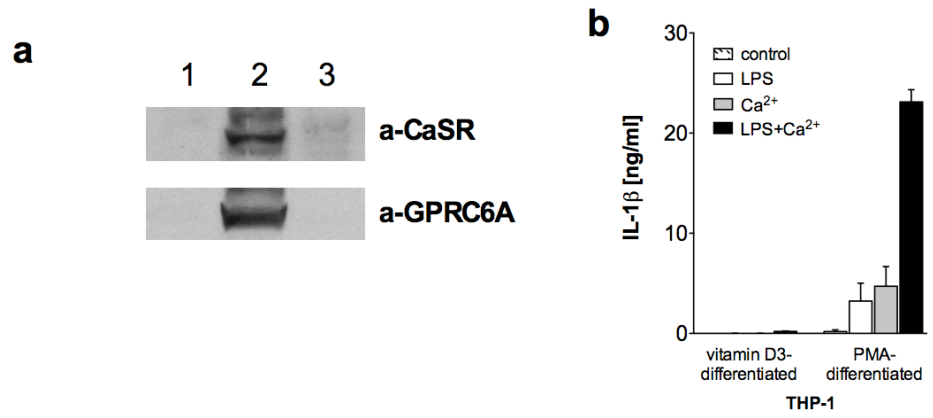
Supplementary Figure S5: No influence of stimulation with ex[Ca^{2+}] on Nlrp3 mRNA expression from LPS-primed monocytes

Nlrp3 mRNA of human monocytes in response to LPS and ex[Ca^{2+}] plus LPS. Shown is one representative experiment out of three.



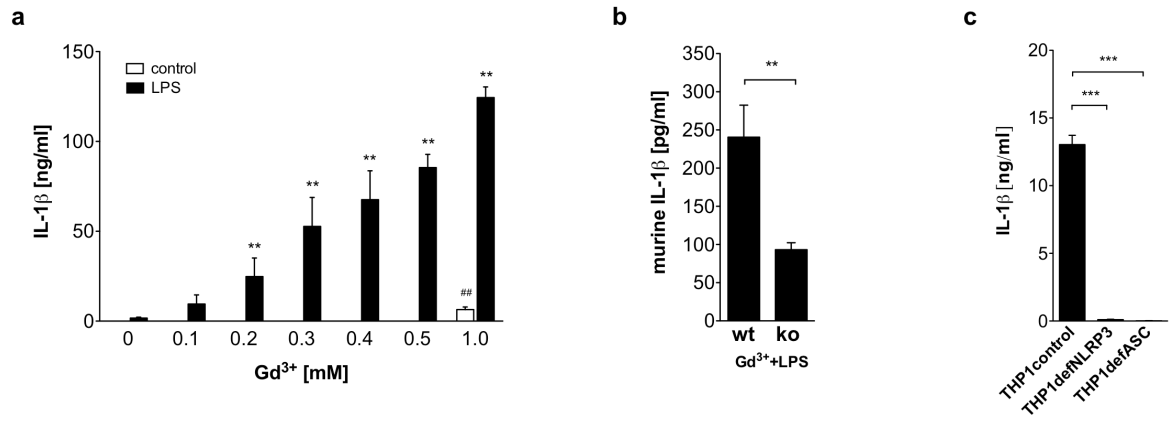
Supplementary Figure S6: No influence of the CaSR/GPRC6A inhibitors Calhex 231 and NPS 2143 on ATP induced inflammasome activation

(a) IL-1ß release from LPS primed monocytes in response to ATP (5mM) in the absence or presence (at the indicated concentrations) of the CaSR/GPRC6A inhibitors Calhex 231 and NPS 2143 (n=3). (b) IL-1ß release from LPS primed murine CD11b+ bone-marrow cells from wildtype mice (wt) and GPRC6A-/- mice (ko) in response to ATP (5mM). All data are expressed as means±SEM.



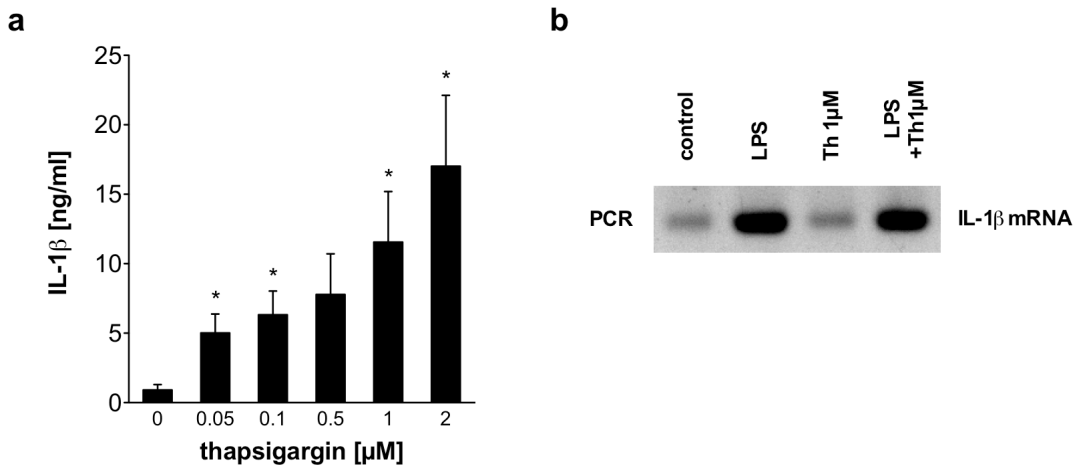
Supplementary Figure S7: THP1 cells differentiated in the presence of PMA express CaSR and GPRC6A and respond to increased ex[Ca^{2+}] with IL-1ß secretion.

(a) Western blot of protein expression of CaSR and GPRC6A in THP1 cells. Lanes were loaded with lysate from undifferentiated (lane 1), PMA differentiated (lane 2) or vitamin D3-differentiated (lane 3) THP1 cells. Shown is one representative experiment out of 3. (b) IL-1ß release of THP1 cells in response to stimulation for 16 hours with ex[Ca^{2+}], with LPS or both (n=3, Mean±SEM).



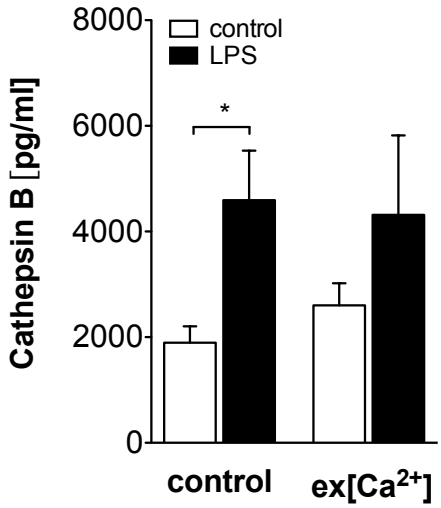
Supplementary Figure S8: The alternative CaSR/GPRC6A ligand Gd^{3+} induces IL-1ß production via the Nlrp3 inflammasome.

(a) IL-1ß secretion of CD14+ monocytes in response to increasing concentration of $Gadolinium^{3+}$ (control) compared to $Gadolinium^{3+}$ plus LPS. Experiments were performed in triplicates. (b) Gd^{3+}-induced IL-1ß secretion of CD11b+ mononuclear cells isolated from bone marrow from $GPRC6A^{+/+}$ (wt) and $GPRC6A^{-/-}$ (ko) mice (n=5). (c) Gd^{3+}-induced IL-1ß secretion of PMA-differentiated control THP-1 cells (THP1 control), Nlrp3-deficient THP-1 cells (THP1defNLRP3) and ASC-deficient THP-1cells (THP1defASC, n=3). All data are expressed as means±SEM (*p<0.05, **p<0.01, ***p<0.001).



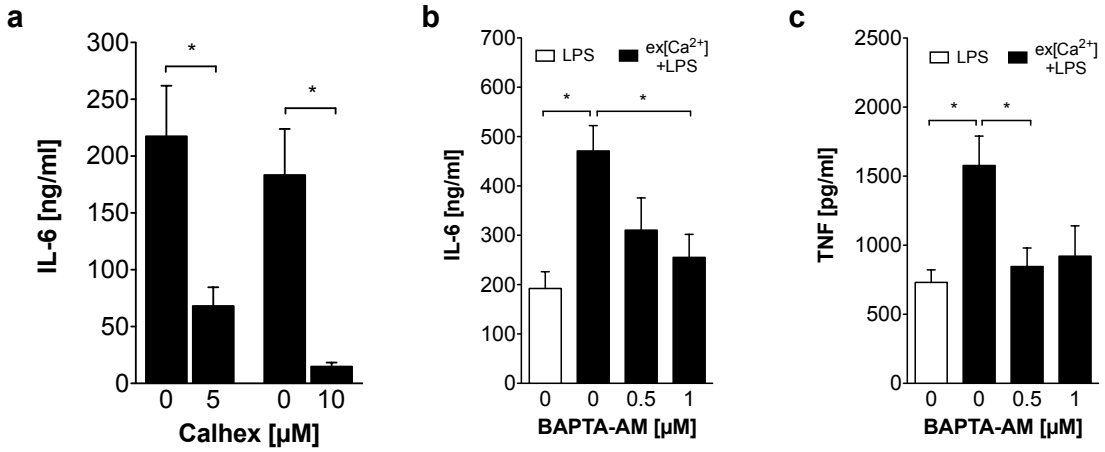
Supplementary Figure S9: Elevation of Ca_i by incubation with Thapsigargin triggers IL-1ß release in LPS primed monocytes

(a) IL-1ß release from monocytes in response to LPS in combination with increasing concentrations of thapsigargin (n=3). All data are expressed as means±SEM (*$p<0.05$). (B) IL-1β mRNA expression in human monocytes was analyzed by semi-quantative PCR in response to LPS with or without thapsigargin (Th). Shown is one representative experiment out of three.



Supplementary Figure S10: No influence of stimulation with ex[Ca^{2+}] on secretion of Cathepsin B from LPS-primed monocytes

Cathepsin B in the supernatant of human monocytes in response to LPS and ex[Ca^{2+}] plus LPS (n=5). All data are expressed as means±SEM (*$p<0.05$).



Supplementary Figure S11: Secretion of IL-6 and TNF is dependent on CaSR and GPRC6A signalling and intracellular calcium accumulation

(a) Influence of the specific inhibitor Calhex231 on the IL-6 secretion of monocytes stimulated with increased ex[Ca2+] (1.7 mM) plus LPS (n=3). (b,c) Secretion of IL-6 (b) and TNF (c) from LPS-stimulated monocytes (clear bars) or from monocytes stimulated with ex[Ca^{2+}] (1.7 mM) plus LPS in the presence of the indicated concentrations of the cell-permeable Ca^{2+}-chelator BAPTA-AM (n=3). All data are expressed as means±SEM (*$p<0.05$).

Supplementary Table S1: Titration of Ca^{2+} concentrations in the culture media

Added $CaCl_2$	**Measured ionized Ca^{2+}**
0	0.6 mM
0.5 mM	0.9 mM
1.0 mM	1.2 mM
1.5 mM	1.5 mM
2.0 mM	1.6 mM
2.5 mM	1.7 mM
3.0 mM	1.8 mM