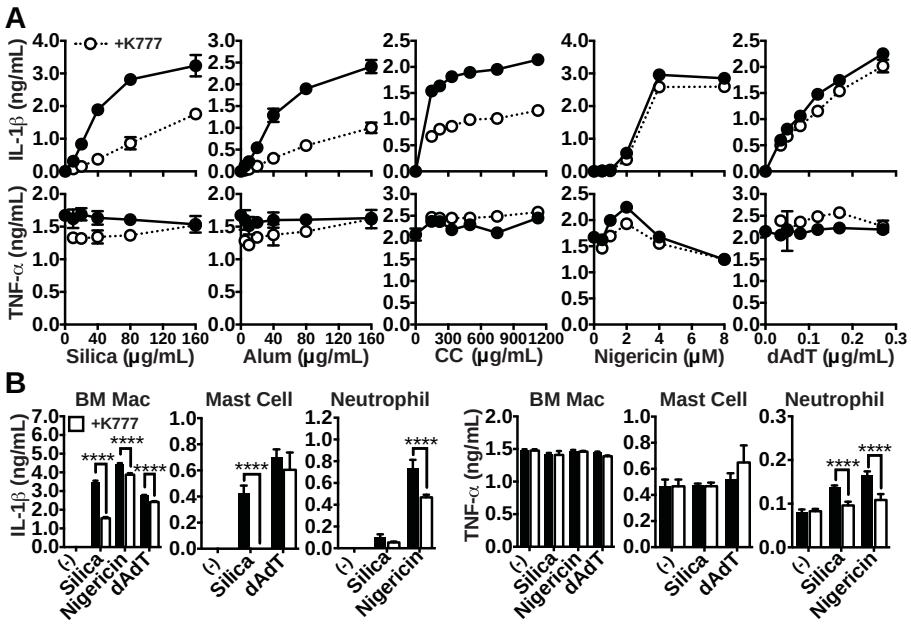
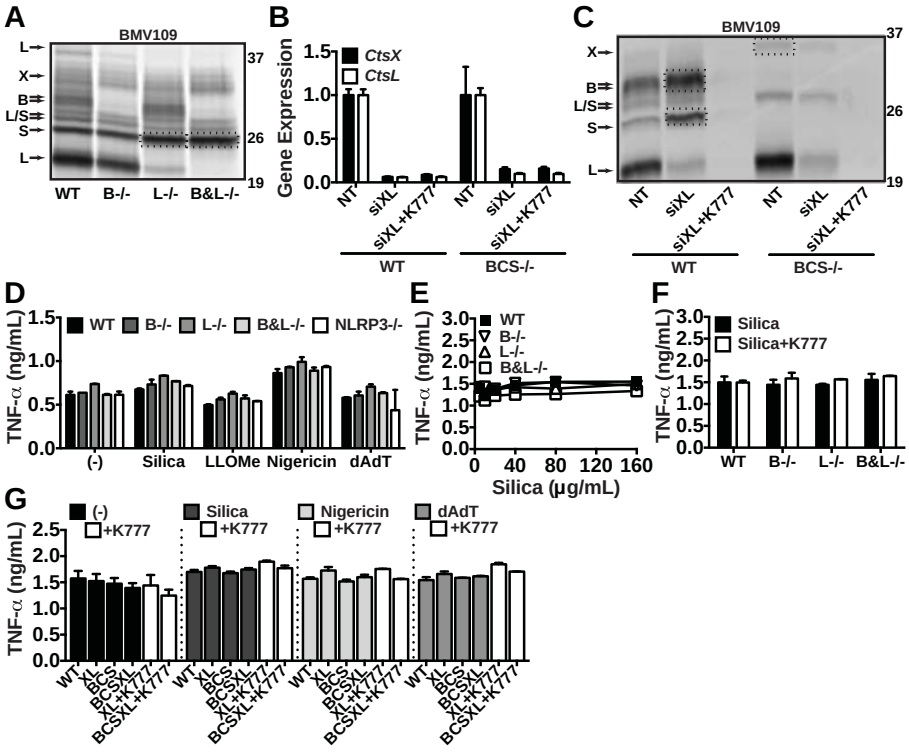


Supplemental Figure 1

Supplemental Figure 1

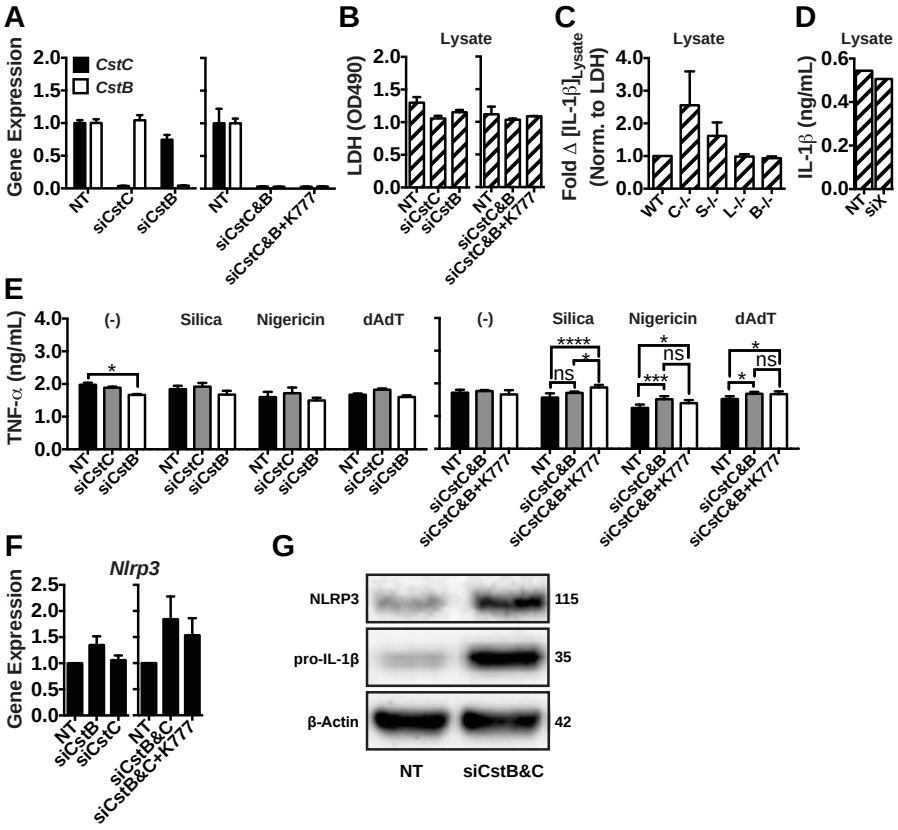
(A) LPS-primed PMs were treated with media control (solid line) or K777 (+K777/dashed line; 15-20 μM) and then stimulated with the indicated concentrations of silica, alum, CC, nigericin or dAdT; IL-1β (upper graphs) and TNF-α (lower graphs) were measured in supernatants. **(B)** LPS-primed bone marrow-derived macrophages (BM Mac), mast cells, or neutrophils were treated with media control (black bars) or K777 (+K777/white bars; 15 μM) and then stimulated with media control (-), silica (40 μg/mL; 100 μg/mL for neutrophils), nigericin (2 μM for BM Macs; 1 μM for neutrophils) and/or dAdT (0.3 μg/mL); IL-1β (graphs on left) and TNF-α (graphs on right) were measured in supernatants. Error bars represent **(A)** S.D. from technical triplicates (CC and dAdT) or range bars of technical duplicates (silica, alum and nigericin), **(B)** S.D. from technical triplicates (BM Mac for silica or nigericin; mast cells and neutrophils) or range bars from technical duplicates (BM Mac for dAdT). Statistical analysis was performed by **(B)** Two-way ANOVA and Sidak's multiple comparisons test; ****P<0.0001. All data are representative of at least three independent experiments.



Supplemental Figure 2

Supplemental Figure 2

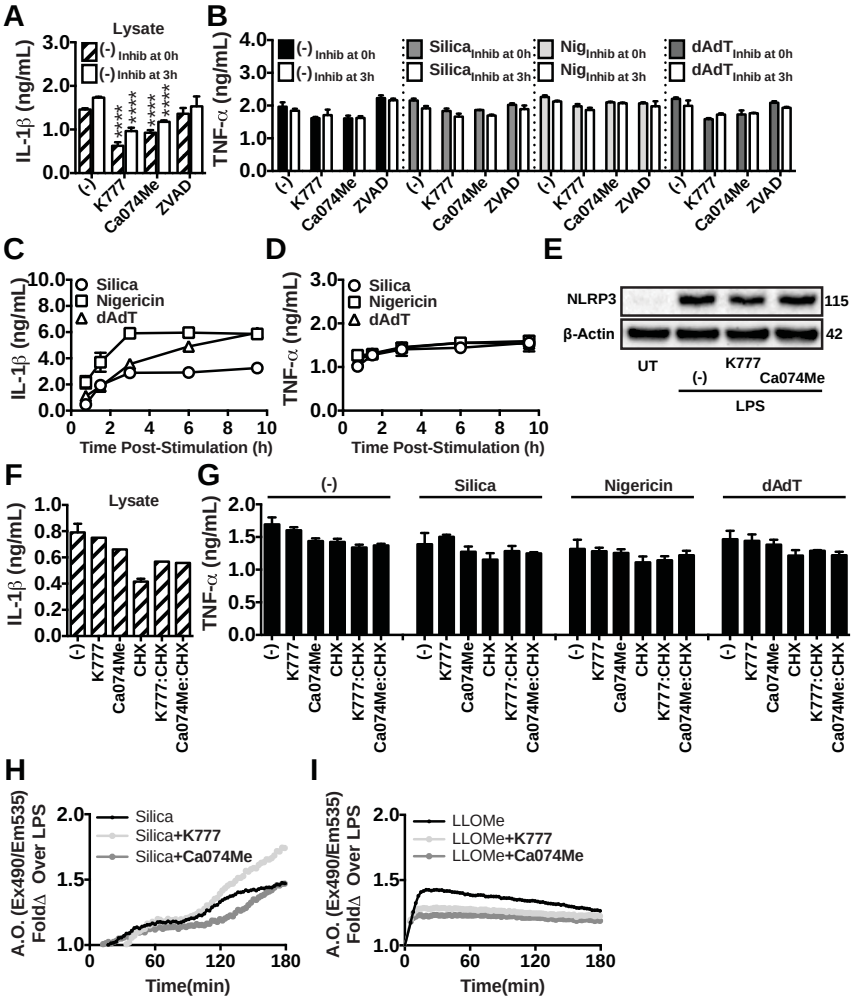
(A,D-F) Lethally irradiated WT mice were reconstituted with bone marrow from WT, cathepsin B (B-/-), L (L-/-), B and L (B&L-/-), or NLRP3 (NLRP3-/-)–deficient donor mice. PMs elicited from these mice were LPS-primed and **(A)** probed for cathepsin activity with BMV109 in live cells; lysates were processed by SDS-PAGE and phosphor imaged; dashed boxes highlight upregulated cathepsin S activity for L-/- & B&L-/- and m.w. markers are on the right in kDa, **(D)** stimulated with media control (-), silica (40 μg/mL), LLOMe (0.75 mM), nigericin (2 μM), or dAdT (0.4 μg/mL), **(E)** stimulated with a range of silica concentrations, or **(F)** stimulated with silica plus media (black bars) or silica plus K777 (white bars; 20 μM). **(B,C,G)** PMs elicited from WT or mice deficient in the three cathepsins B, C and S (BCS) were treated with non-targeting siRNA (WT) or siRNA targeting both cathepsins X and L ("XL" when given to WT, or "BCSXL" when given to BCS) and stimulated with media control (-), silica (80 μg/mL), nigericin (1.5 μM), or dAdT (0.5 μg/mL). XL and BCSXL macrophages were also treated with K777 (XL+K777 and BCSXL+K777; white bars; 15 μM). Knockdown (siXL) was verified by **(B)** cathepsin X (CtsX) and L (CtsL) expression analysis by qPCR; data are normalized to GAPDH expression and plotted relative to non-targeting siRNA (NT), and **(C)** Labeling of cathepsin activity with BMV109 in live cells, as done in "A"; dashed boxes highlight upregulation of cathepsins B & S for siXL and cathepsin X for BCS-/- treated with NT; m.w. markers are on the right in kDa. **(D-G)** TNF-α was measured in supernatants. Error bars represent **(A,D-F)** range bars of technical duplicates **(G)** S.E. of means of either five (WT, XL, BCS, BCSXL) or three (+K777) independent experiments. Statistical analysis was performed by **(A,D-F)** Two-way ANOVA and Sidak's multiple comparisons test or **(G)** Two-tailed Student's t-test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All data are representative of at least three independent experiments.



Supplemental Figure 3

Supplemental Figure 3

In all experiments, PMs were LPS-primed and treated with media control or K777 (+K777; white bars; 15 μM) prior to stimulation or analysis. **(A,B,E,F,G)** PMs were transfected with non-targeting (NT), cystatin C (siCstC), cystatin B (siCstB), or both cystatin C and B (siCstC&B) siRNA. **(A)** Cystatin C (CstC; black bars) or cystatin B (CstB; white bars) expression was analyzed by qPCR; data are normalized to GAPDH expression and plotted relative to non-targeting (NT) siRNA. **(B)** After priming, PMs were treated with media control for 6h and LDH (OD490; hatched bars) was measured in the lysates with a plate reader. **(E)** PMs were stimulated with media control (-), silica (80 μg/mL), nigericin (1.5 μM) or dAdT (0.5 μg/mL) and TNF-α was measured in supernatants. **(C)** LPS-primed PMs from WT mice or mice deficient in either cathepsin C (C-/-), S (S-/-), L (L-/-), or B (B-/-) were treated with media for 6h after LPS priming and IL-1β (hatched bars) was measured in lysates by ELISA; data are normalized to LDH (OD490), measured with a plate reader, and plotted as fold-change in IL-1β relative to WT controls. **(D)** PMs were transfected with NT siRNA or siRNA targeting cathepsin X (siX), then primed with LPS, treated with media for 6h, and IL-1β (hatched bars) was measured in lysates by ELISA. **(F)** *Nlrp3* expression was analyzed by qPCR; data are normalized to GAPDH expression and plotted relative to non-targeting (NT) siRNA. **(G)** Lysates were processed by SDS-PAGE and analyzed for pro-IL-1β, NLRP3 and β-Actin by western blot; m.w. markers are on the right in kDa. Error bars represent **(A)** S.D. of technical triplicates, **(B)** range bars of technical duplicates, **(C)** S.E. of means from three (C-/- and S-/-) or twelve (L-/- and B-/-) independent experiments, **(E)** S.D. of technical quadruplicates, or **(F)** S.E. of averages from three (siCstB, siCstB, siCstB&C+K777) or four (siCstB&C) independent experiments. Statistical analysis was performed by **(E)** Two-way or **(F)** One-way ANOVA and Dunnett's multiple comparisons test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Data are representative of at least three or **(G)** two independent experiments.



Supplemental Figure 4

Supplemental Figure 4

(A,B) LPS-primed PMs were treated with media control (-), K777 (15 μM), Ca074Me (15 μM), or ZVAD (10 μM), which were added at the same time as LPS (Inhib at 0h; hatched or filled bars) or 3h after LPS (Inhib at 3h; white bars) prior to stimulation with media control (-), silica (80 μg/mL), nigericin (1.5 μM) or dAdT (0.5 μg/mL) for an additional 6h. **(A)** IL-1β was measured in lysates, or **(B)** TNF-α was measured in supernatants by ELISA. **(C,D)** LPS-primed PMs were stimulated with silica (40 μg/mL; circle), nigericin (2 μM; square) or dAdT (0.3 μg/mL; triangle); IL-1β & TNF-α were measured in supernatants after 0.75, 1.5, 3, 6, or 9h of stimulation. **(E)** PMs were either untreated (UT) or LPS-primed (indicated by bar) before treatment 2h later with media control (-), K777 (15 μM) or Ca074Me (15 μM) for 1h, as in the Standard Protocol, then cells were treated with media for 4h; lysates were processed by SDS-PAGE and then analyzed for NLRP3 and β-Actin by western blot; m.w. markers are on the right. **(F,G)** PMs were primed with LPS for 5.5h and treated with either media control (-), K777 (15 μM), Ca074Me (15 μM), CHX (1 μM), K777 combined with CHX, or Ca074Me combined with CHX for another 0.5h, and then treated with media control (-), silica (80 μg/mL), nigericin (1.5 μM), or dAdT (0.5 μg/mL) for another 3h. **(F)** IL-1β (hatched bars) was measured in lysates, or **(G)** TNF-α (filled bars) was measured in supernatants by ELISA. **(H,I)** PMs stained with acridine orange (A.O.; increasing values = disruption of lysosomes/pH gradient) were primed with LPS before treatment 2h later with media control, K777 (15 μM) or Ca074Me (15 μM) for 1h prior to stimulation with **(H)** silica (80 μg/mL) or **(I)** LLOMe (1.25 mM); fluorescence traces were monitored by plate reader at short intervals; traces indicate fold change over LPS-treated samples. Error bars represent **(A,B)** S.D. of technical triplicates (-), **(B)** duplicates (silica, nigericin, dAdT), **(C,D)** S.D. of technical duplicates, **(F)** S.D. of technical triplicates, **(G)** S.D. of technical triplicates (media or CHX), duplicates (K777 & Ca074Me ±CHX), sextuplicates (silica, nigericin, dAdT ±CHX), or triplicates (silica, nigericin, dAdT with K777 & Ca074Me ±CHX). Statistical analysis was performed by **(A)** Two-way ANOVA and Dunnett's multiple comparisons test; ****P<0.0001. Data are representative of **(A-D)** at least three independent experiments, or **(E-I)** two independent experiments.