

Expanded View Figures

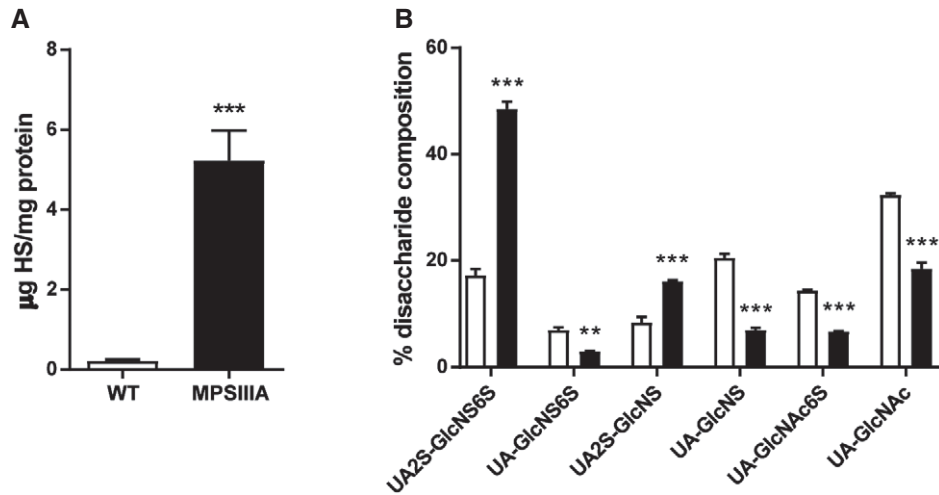


Figure EV1. Heparan sulphate analysis.

A Total relative amounts of HS. Amounts are expressed as µg HS per mg of liver protein and calculated from AMAC fluorescent reads compared to known amounts of HS standards ($n = 3$ GAG samples). Data are expressed as mean $\pm$ STDEV, and data for each cytokine were tested by unpaired t -test; WT versus MPSIIIA *** $P < 0.0001$.

B Compositional disaccharide analysis for HS from WT and MPSIIIA ($n = 3$ GAG samples). Data are expressed as mean $\pm$ STDEV and were tested by one-way ANOVA with Tukey's post-test; ** $P < 0.01$, *** $P < 0.001$. Symbols above bars are versus WT. Exact P -values are indicated in Appendix Table S7. NAc, N -acetylated glucosamine; NS, N -sulphated glucosamine; 2S, 2- O -sulphate group; 6S, 6- O -sulphate group.

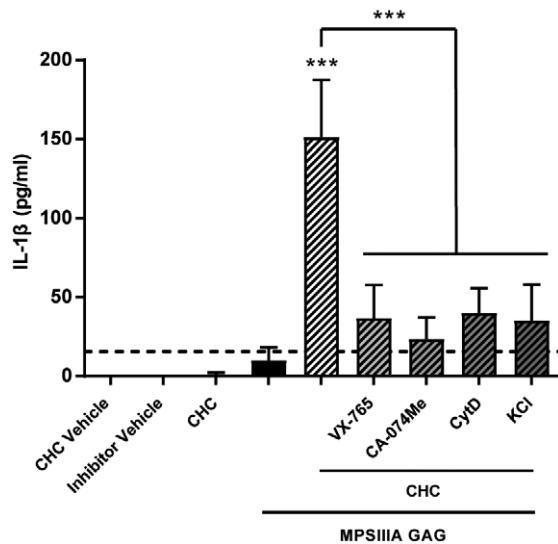


Figure EV2. MPSIIIA secondary storage substrates induce secretion of IL-1β via the NLRP3 inflammasome.

WT-mixed glia were incubated with cholesterol crystals in the presence or absence of caspase-1 inhibitor VX-765 (50 µg/ml), cytochalasin D (2 µM), KCl (130 mM) or cathepsin B inhibitor CA-074Me (10 µM). After the incubation, cell culture supernatants were analysed for IL-1β ($n = 3$ independent experiments each with three intra experimental replicates). Data are expressed as mean $\pm$ STDEV and were tested by one-way ANOVA with Tukey's post-test; *** $P < 0.001$. Symbols above bars are versus MPSIIIA GAG alone. Exact P -values are indicated in Appendix Table S7.

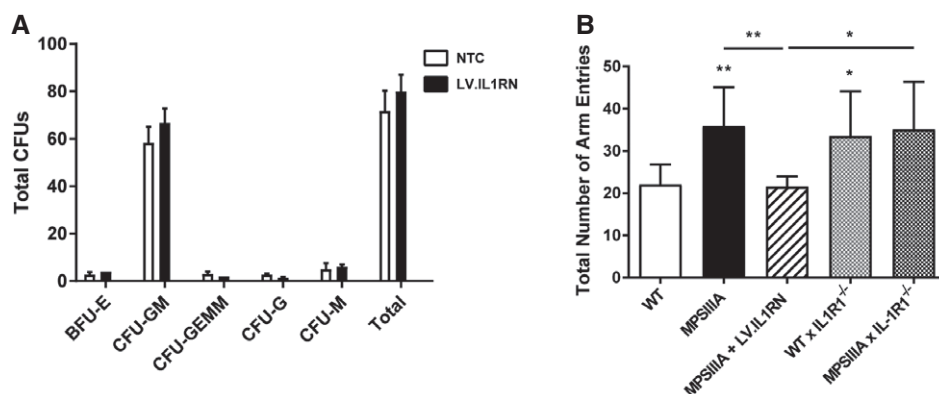


Figure EV3. Further evaluation of LV.IL1RN HSCT safety and efficacy.

A Colony-forming units from lineage-negative-enriched haematopoietic stem cells. Lineage-negative-enriched stem cells were either transduced with LV.CD11b.IL1RN or mock transduced and plated for 14 days in methylcellulose culture medium yielding committed haematopoietic progenitor colonies ($n = 6$ CFU assays). Data are expressed as mean $\pm$ STDEV and were tested by one-way ANOVA with Tukey's post-test.

B Locomotor activity in the Y-maze. The total number of arm entries was assessed as an indicator of explorative behaviour ($n = 10$ mice per group). Data are expressed as mean $\pm$ STDEV and were tested by one-way ANOVA with Tukey's post-test; * $P < 0.05$, ** $P < 0.01$. Symbols above bars are versus WT. Exact P -values are indicated in Appendix Table S7.

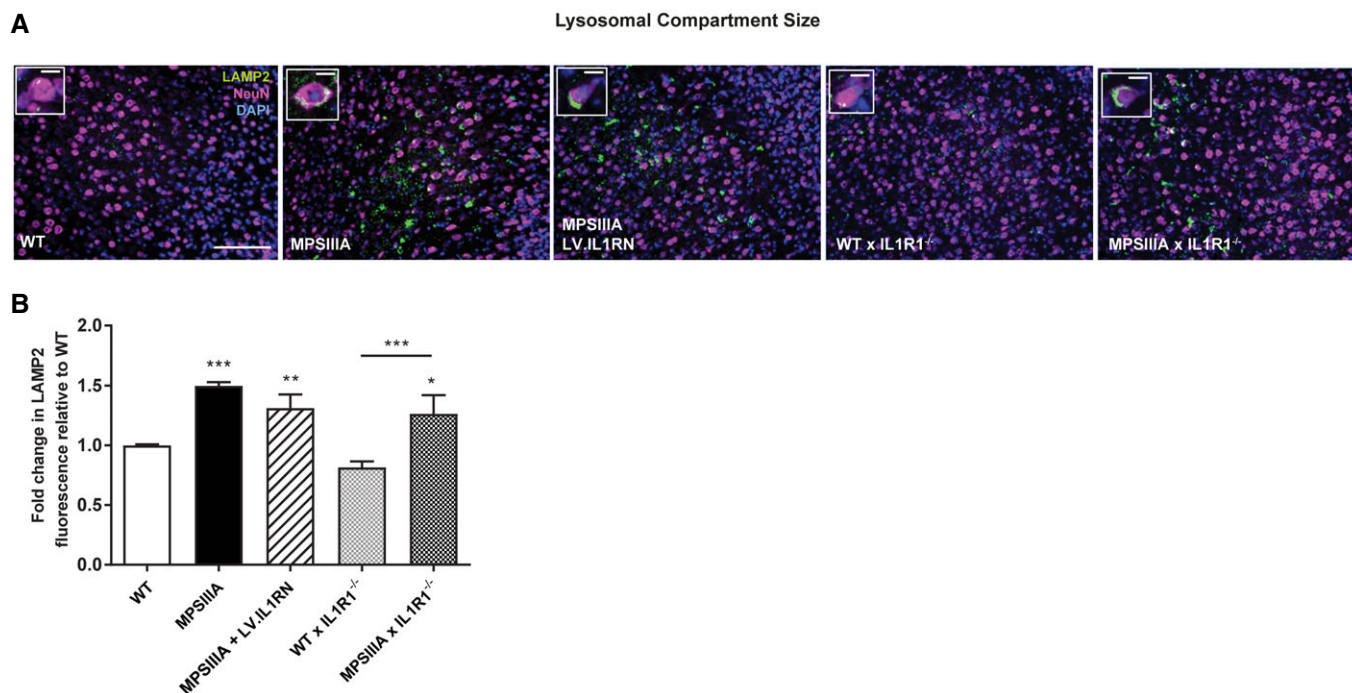


Figure EV4. IL-1 signalling inhibition in MPSIIIA mice has no effect on lysosomal storage.

A Representative images of the cerebral cortex (layers II-V) from control and treated mice stained with LAMP2 (lysosomal size, green)/NeuN (neuronal marker, magenta)/DAPI (nuclear, blue), 20 $\times$, scale bar: 50 μ m. Inserts, 100 $\times$, scale bar: 10 μ m.

B Quantification of fluorescence intensity using ImageJ software. Non-linear adjustments were made equally in fluorescent images to reduce background ($n = 4$ mice per group, average of three fields of view per mouse). Data are expressed as mean $\pm$ STDEV and were tested by one-way ANOVA with Tukey's post-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Symbols above bars are versus WT. Exact P -values are indicated in Appendix Table S7.