Figure S1

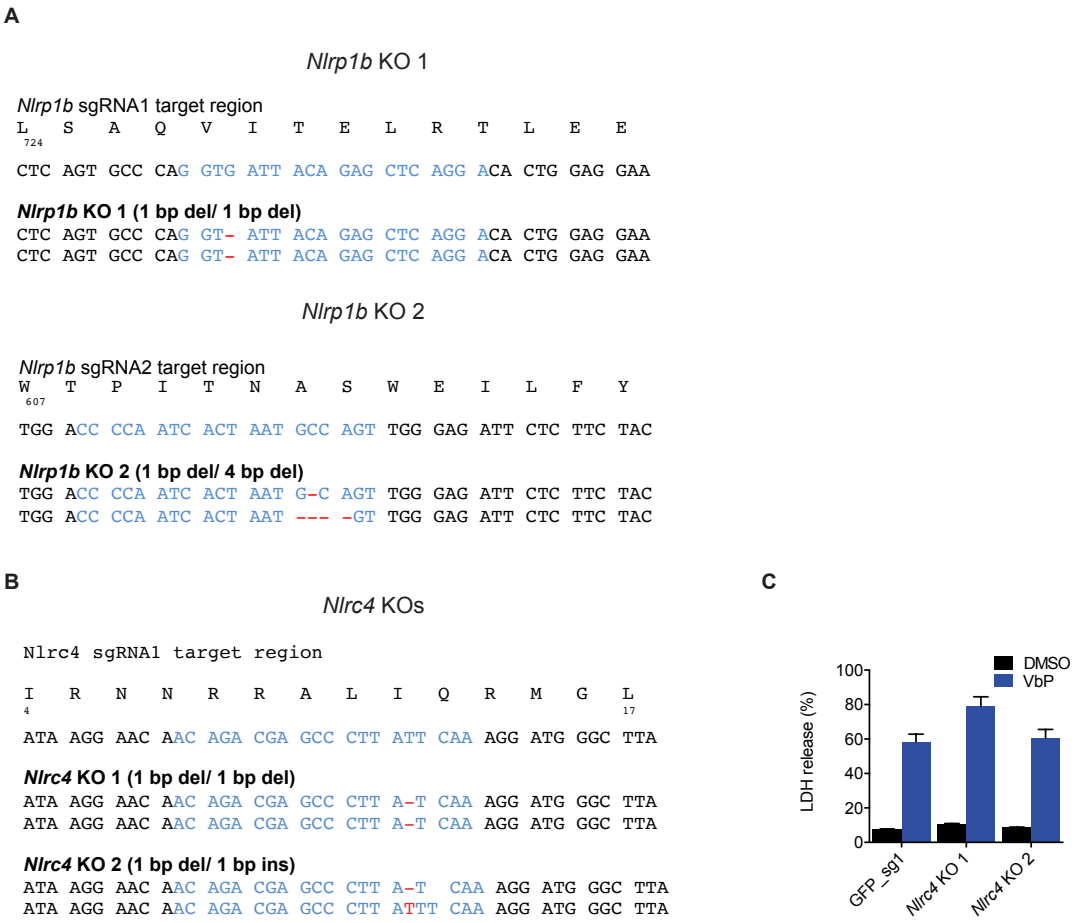

Figure S1. *Nlrp1b* knockout, but not *Nlrc4* knockout, gives resistance to VbP. Related to Figure 1. (A,B) RAW 264.7 *Nlrp1b* KO1 and KO2 (A) and *Nlrc4* KO1 and KO2 (B) cell lines were confirmed by DNA sequencing. The sgRNA target region is shown in blue. (C) RAW 264.7 *Nlrc4* KO1 and KO2 cell lines release LDH after treatment with Val-boroPro (2 μM, 24h). Data are means ± SEM of three biological replicates.

Figure S2

A

Nlrp1b allele	Strains	Anthrax LT MΦ pyroptosis	Anthrax LT Nlrp1b Cleavage	Dpp8/9 Inhibitor MΦ pyroptosis
1	129S1/SvimJ, BALB/cJ, C3H/HeJ, CBA/J, FVB/NJ, NON/LtJ, NZO/HILtJ, SWR/J	Yes	Yes	Yes
2	A/J, C57BL/6J, I/LnJ	No	No	Yes
3	AKR/J, NOD/LtJ, SJL/J	No	Yes	Yes
4	DBA/2J, P/J, SM/J	No	NT	No
5	CAST/EiJ	Yes	NT	No

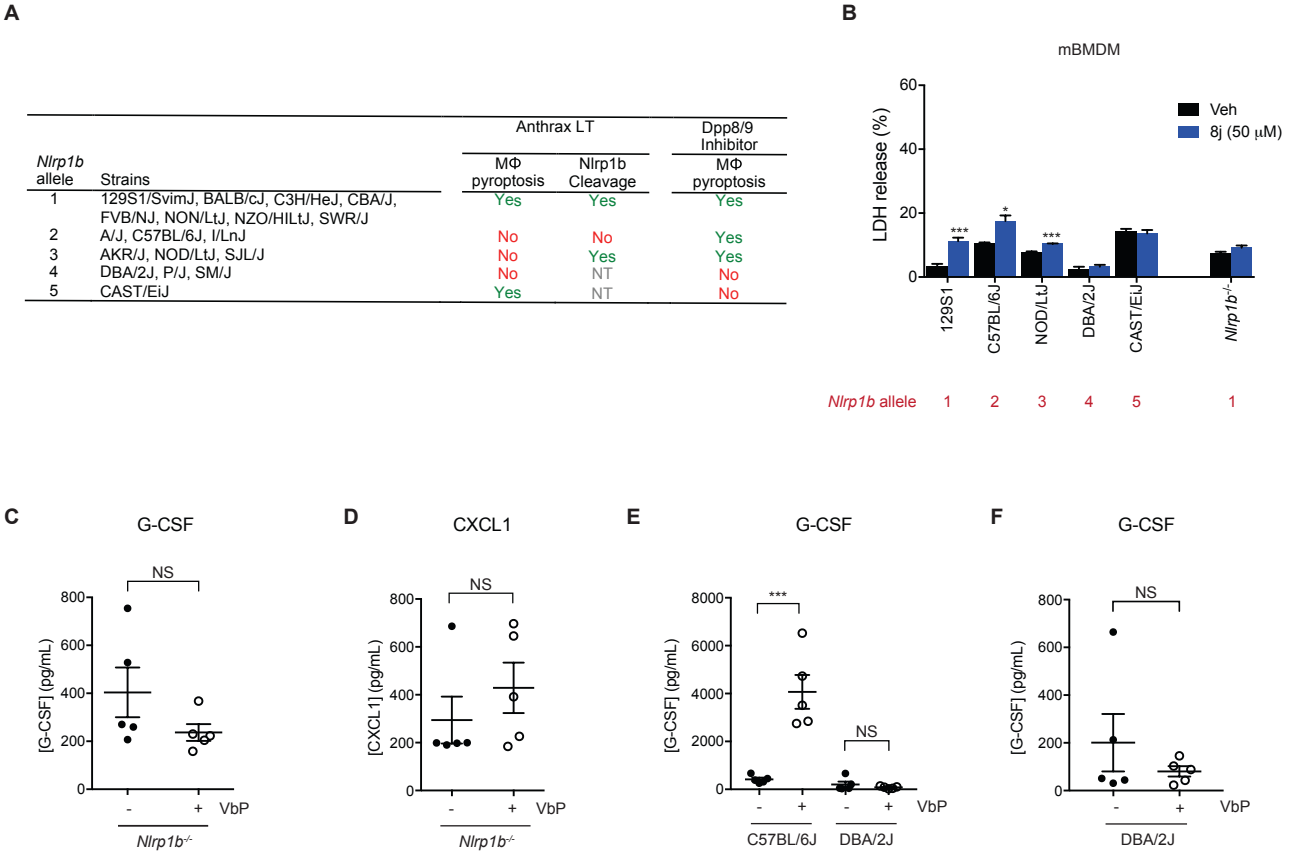

Figure S2. The *Nlrp1* allele controls primary mouse macrophage sensitivity to Dpp8/9 inhibitors. Related to Figure 2. (A) Summary of inbred mouse strain sensitivity to anthrax LT and Val-boroPro. LT data from references: (Boyden and Dietrich, 2006; Chavarria-Smith et al., 2016; Chavarria-Smith and Vance, 2013; Hellmich et al., 2012). Strains tested for VbP sensitivity are: 129S, C57BL/6J, NOD/LtJ, DBA/2J, and CAST/EiJ. NT, not tested. (B) mBMDMs from the indicated inbred mouse strains were treated with compound 8j and LDH release was assessed after 24 h. The macrophage sensitivity to 8j matches Val-boroPro. Data are means ± SEM of three biological replicates. *p <0.05, ***p < 0.001 by two-sided Student's *t*-test for DMSO versus compound-treated cells. (C,D) Val-boroPro (100 μg/mouse) does not induce serum G-CSF (C) and CXCL1/KC (D) after 6 h in *Nlrp1b*$^{-/-}$ mice as measured by ELISA. This data is another representation of Figure 2C,D. (E,F) Val-boroPro (20 μg/mouse, i.p.) does not induce serum G-CSF after 6 h in DBA/2J mice as measured by ELISA. The data in F is another representation of panel E. Data are means ± SEM, n = 5 mice/group. NS, not significant; p <0.001 by two-sided Student's *t*-test for vehicle versus Val-boroPro-treated mice.

Figure S3

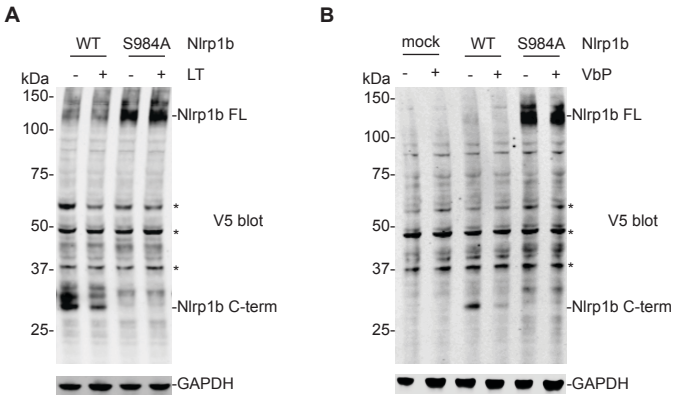

Figure S3. Confirmation of Nlrp1b expression in RAW 264.7 cells. Related to Figure 3. (A,B) Plasmids encoding WT or autoproteolysis-deficient mutant (S984A) Nlrp1b containing a C-terminal V5 tag were nucleofected into *Nlrp1b*$^{-/-}$ RAW 264.7 cells, which were then treated with LT (1 μg/mL, 6 h) (A) or Val-boroPro (10 μM, 24 h) (B). Nlrp1b protein expression was assessed by immunoblotting. It should be noted that less WT Nlrp1b is expressed likely due to the toxicity of its expression and that the LT cleavage product is too small to be observed by SDS-PAGE.