Supplemental material

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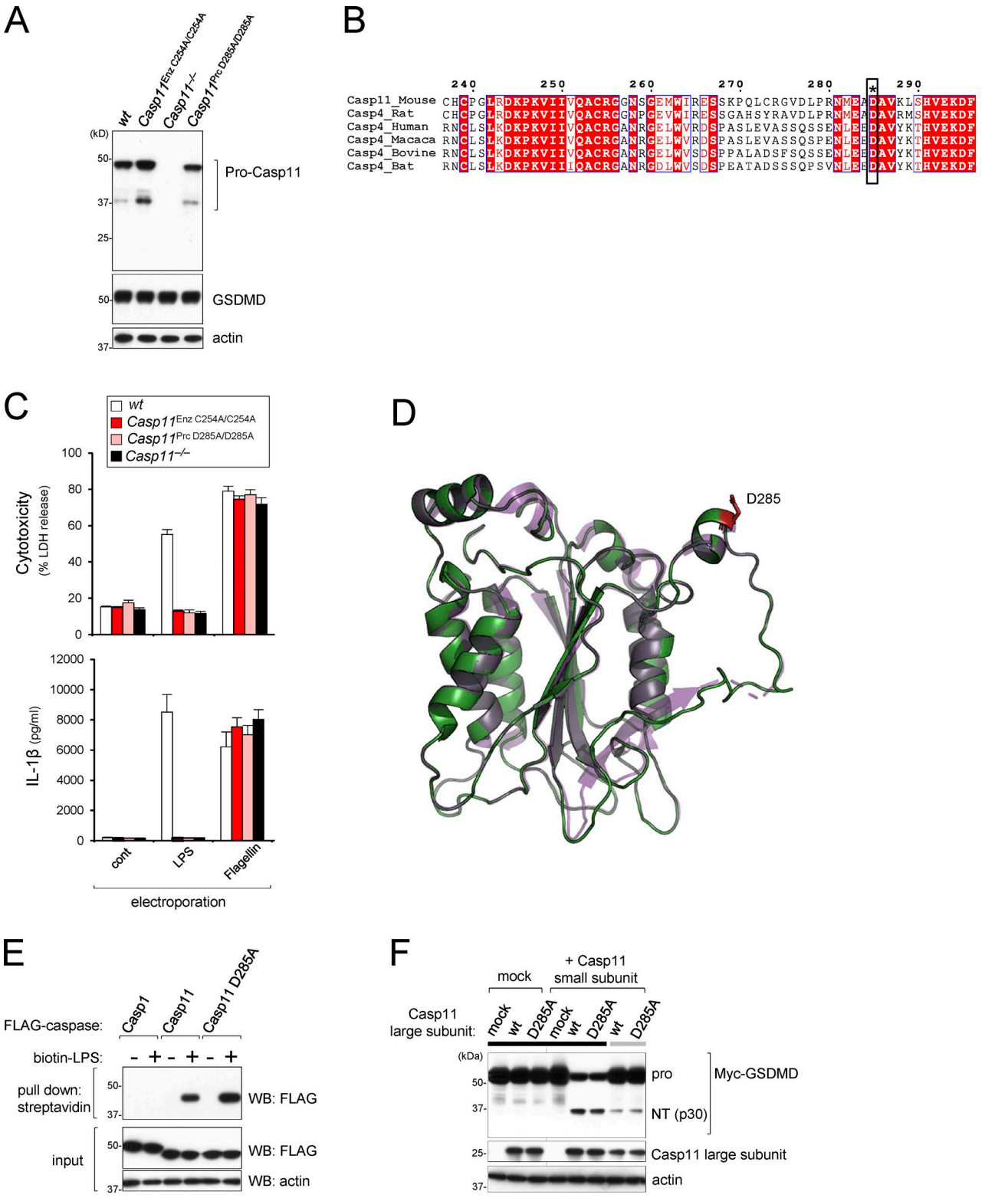

Figure S1. **Characterization of caspase-11 mutants. (A)** Immunoblot of caspase-11 cell extracts from WT, *Casp11*$^{-/-}$, *Casp11*$^{Enz\ C254A/C254A}$, and *Casp11*$^{Prc\ D285A/D285A}$ BMDMs incubated with 1 µg/ml Pam3CSK4 for 5 h. **(B)** Multiple sequence alignment of caspase-4/11. Conserved P_1 residue (Asp_{285} in mouse) is boxed and marked with an asterisk. **(C)** LDH and IL-1β release measured from supernatants of BMDMs (prestimulated with 1 µg/ml Pam3CSK4 for 5 h) following electroporation by AMAXA 4D with LPS or flagellin after 16 h. **(D)** Molecular model of caspase-11. Caspase-11 is shown in green, the Asp_{285} residue in red, and caspase-1 template outlined in purple. **(E)** Immunoblots of streptavidin pull-downs in the presence or absence of biotin-LPS with HEK293T lysates expressing indicated constructs. **(F)** Immunoblots of HEK293T cells cotransfected with 20 ng of Myc-tagged GSDMD together with 20 ng (black bar) or 5 ng (gray bar) each of indicated plasmids. Mock: vector alone control. Data are representative of at least two independent experiments (A, C, E, and F) and presented as mean ± SD (n = 3) in C.

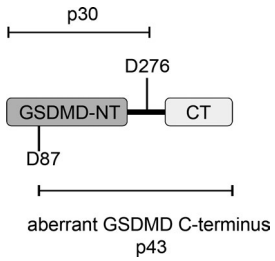

Figure S2. **Schematic of GSDMD.** Mouse GSDMD N-terminus (NT) and C-terminus (CT) are represented along with predicted cleavage sites at D87 (caspase-3) and D276 (caspase-1/4/5/11).

A

Group	-Group	Adjusted p-value
Wt	*Casp11*$^{-/-}$	0.0003
Wt	*Casp11*$^{Enz\ C254A/C254A}$	0.0003
Wt	*Casp11*$^{Prc\ D285A/D285A}$	0.0003
Casp11$^{-/-}$	*Casp11*$^{Enz\ C254A/C254A}$	0.4422
Casp11$^{-/-}$	*Casp11*$^{Prc\ D285A/D285A}$	1
Casp11$^{Enz\ C254A/C254A}$	*Casp11*$^{Prc\ D285A/D285A}$	0.4422

B

Group	-Group	Adjusted p-value
Wt	*Gsdmd*$^{-/-}$	0.0009
Wt	*Gsdmd*$^{D276A/D276A}$	0.0003
Gsdmd$^{-/-}$	*Gsdmd*$^{D276A/D276A}$	0.7434

C

	gRNA target sequence	Donor oligo
Casp11$^{Enz\ C254A/C254A}$	ATCATTGTGCAGGCCTGCAG	AGATGTGCTACAGTATGATACCATCTATCAGATATTCAACAATTGCCACTGTCCAGGTCTACGAGACAAACCCAAgGTaATaATaGTcCAGGCCgccAGAGGTGGTAAGTTCTTTGGTGGAACCACGAACTCCCACGCTGCTGGCTATGCAGGTGCTGGCAGGGTAATGC
Gsdmd$^{D276A/D276A}$	CTCATCAATCCCATCTGGA	CTGCTCCCTGGATCTACCCGCTCCTAACTCCAGTTCCCCAAGACCTCACAGGCTGCCTGCCCTCTGTCCCTCCAGcgGGcATTGATGAGGAGGAATTAATTGAGGCGGCAGACTTCCAGGGCCTGTATGCTGAGGTGAAGGCTTGCTCCTCAGAA

Figure S3. **Statistical analysis for *in vivo* mouse studies and additional KI mouse details. (A and B)** Adjusted P values of Fig. 4 A (A) and Fig. 4 C (B). Statistical analysis was performed with log-rank (Mantel-Cox) tests, and P values were adjusted to account for multiple comparisons using Bonferroni's correction. **(C)** gRNA target sequences and donor oligos for KI mice.