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Supplemental information

Shigella OspC3 suppresses murine cytosolic LPS sensing

Changhoon Oh, Ambika Verma, Mohib Hafeez, Brandon Hogland, and Youssef Aachoui

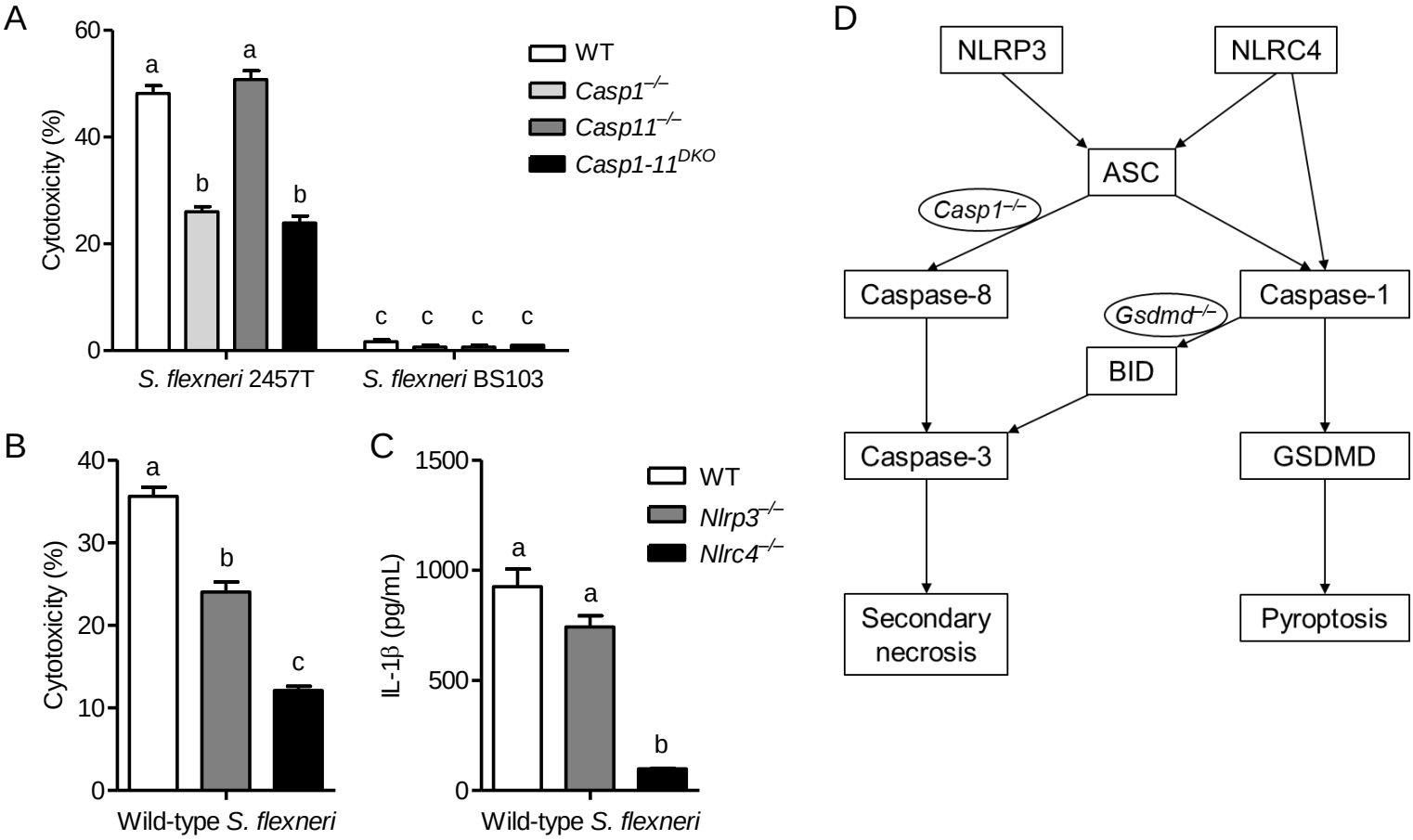

Figure S1. *S. flexneri* is detected by caspase-1 inflammasome but evades caspase-11, Related to figure 1

(A-C) The indicated LPS-primed BMMs were infected with wild-type *S. flexneri* or virulence plasmid-cured strain BS103 at MOI 50 for 4 hr. Cytotoxicity was determined by LDH release assay (A, B), and IL-1β release was determined by ELISA (C). (D) A diagram of the bypass pathways of canonical inflammasomes. Bar represents mean ± SEM. Data are representative of three independent experiments. Cytotoxicity and IL-1β levels were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test (A) or one-way ANOVA followed by Tukey's multiple comparison test (B, C) with a statistical threshold of $p \leq 0.05$. Groups that do not share a letter are significantly different from each other, $p \leq 0.05$.

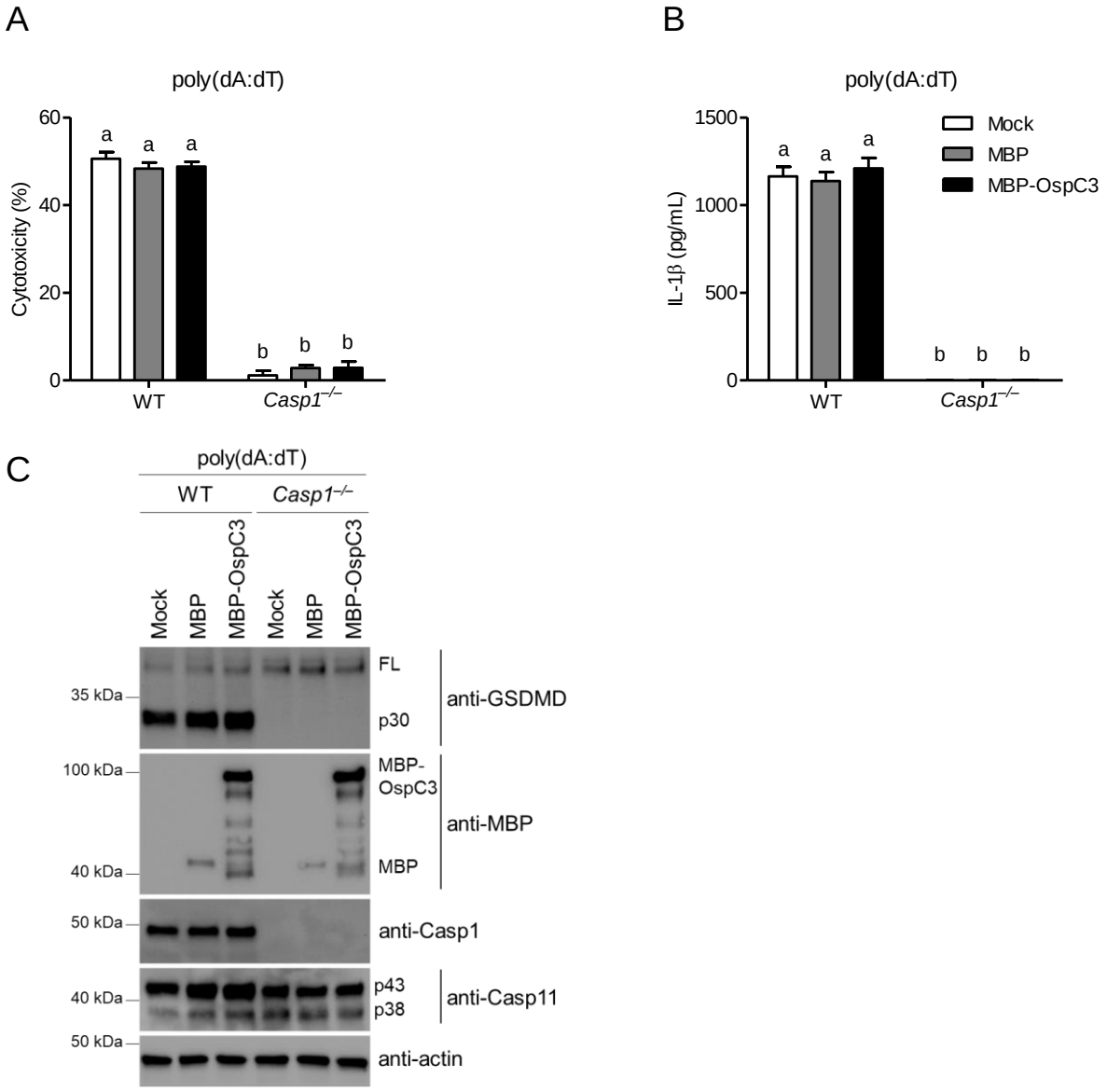

Figure S2. *Shigella* OspC3 effector does not affect caspase-1 inflammasome activation, Related to figure 2

(A-C) LPS-primed BMMs were transfected or not with either MBP or MBP-OspC3 for 4 hr, followed by stimulation with poly(dA:dT) for 4 hr. Cytotoxicity was determined by LDH release assay (A), and IL-1β release was determined by ELISA (B). Gasdermin D cleavage, caspase-1, caspase-11 and MBP or MBP-OspC3 expression were determined by western blot (C). Actin was loaded as a control. Bar represents mean ± SEM. Data are representative of three independent experiments. Cytotoxicity and IL-1β levels were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test with a statistical threshold of $p \leq 0.05$. Groups that do not share a letter are significantly different from each other, $p \leq 0.05$.

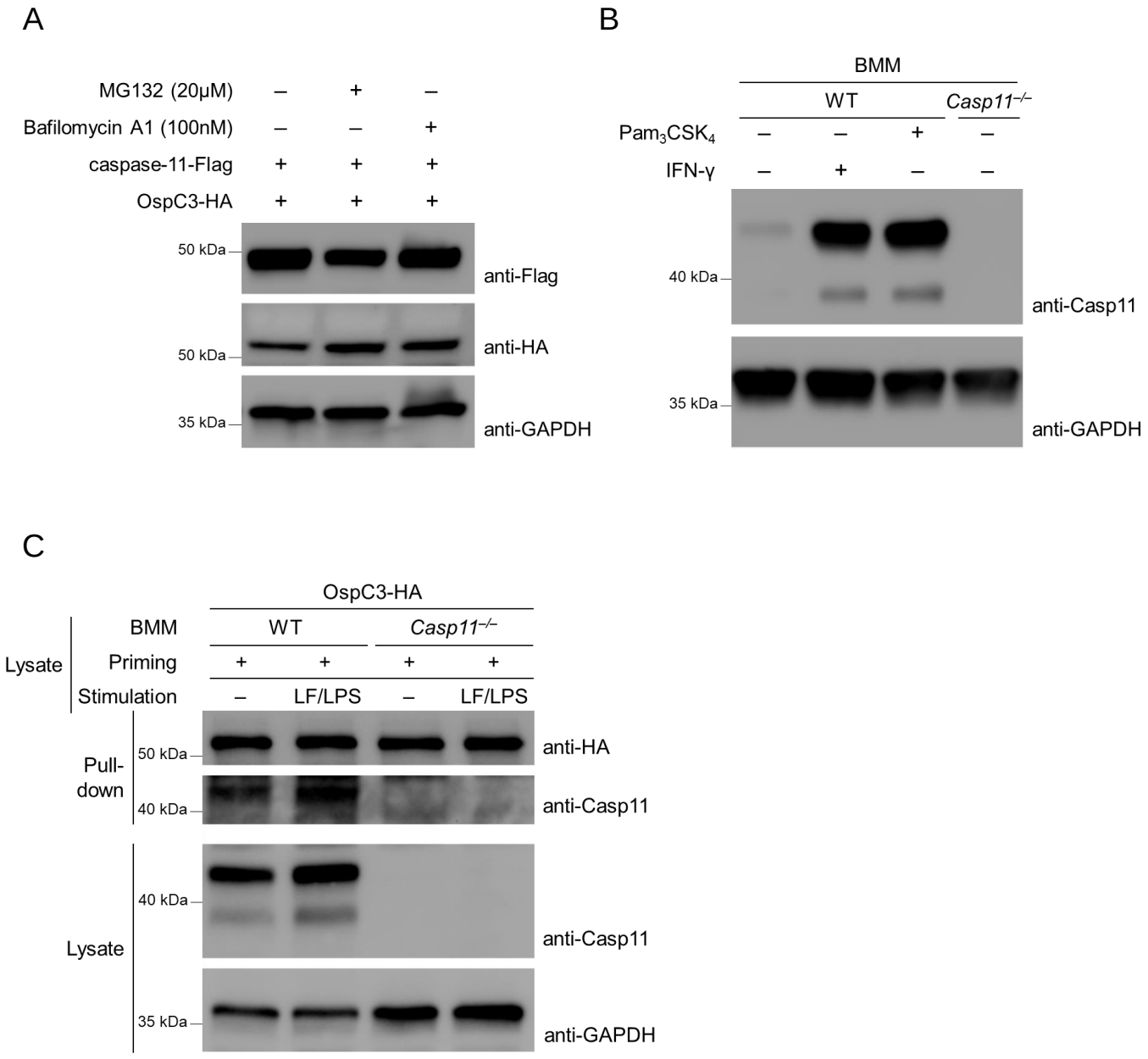

Figure S3. *Shigella* OspC3 effector interacts with primed caspase-11, Related to figure 3

(A) 293T cells expressing caspase-11-Flag and OspC3-HA were treated with MG132 (20 μM), Bafilomyin A1 (100 nM) or not for 4 hr, respectively. The stability of caspase-11 was determined by western blot. (B) BMMs were primed with Pam3CSK4 (1 μg/ml) or IFN-γ (50 ng/ml) for 4hr. Caspase-11 expression were detected by western blot. (C) HA-conjugated beads bound to OspC3-HA were mixed with BMM lysates transfected by mock or LPS to activate non-canonical inflammasome following LPS priming. Western blots were probed with the indicated antibodies. GAPDH was loaded as a control.

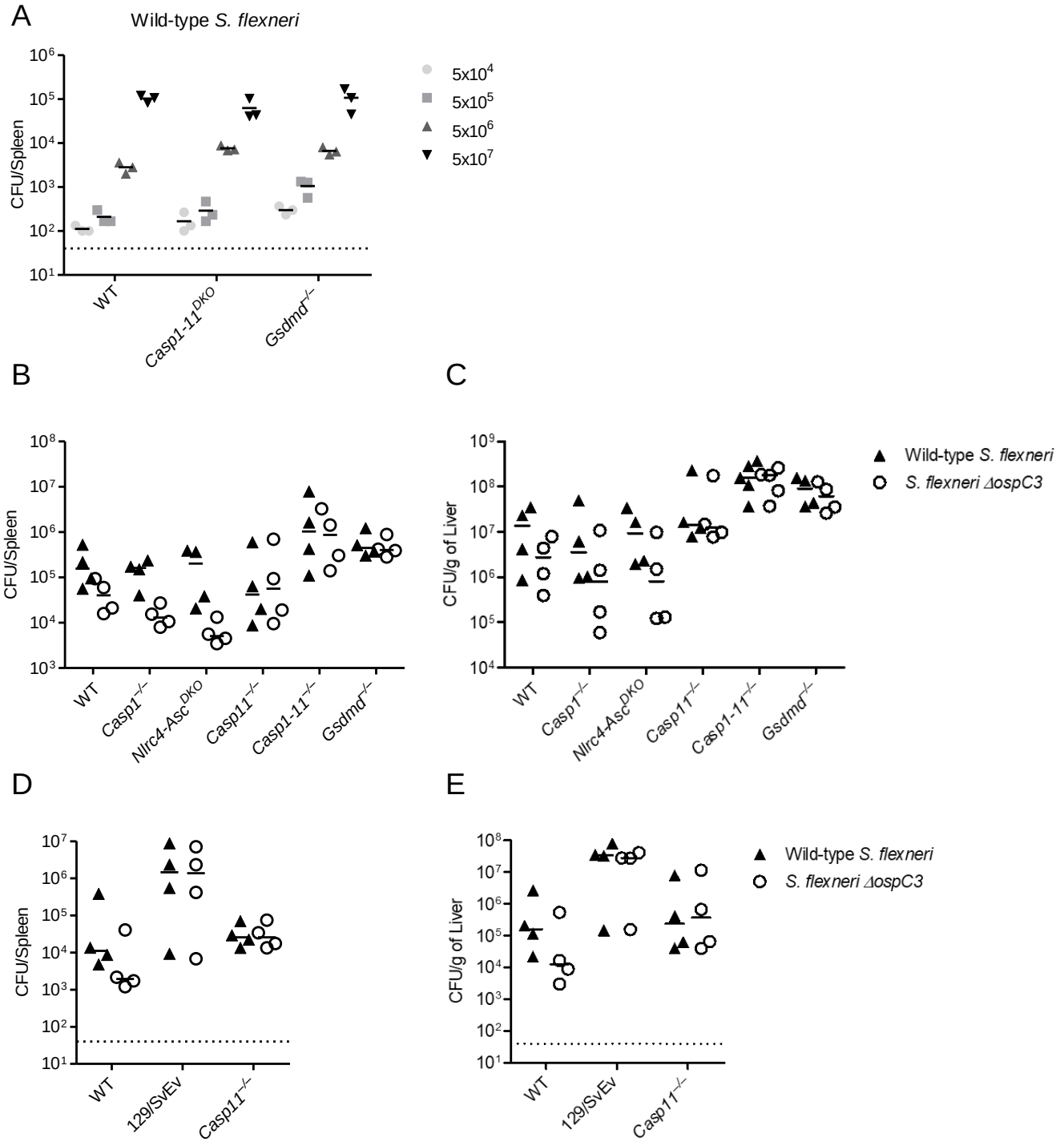

Figure S4. Caspase-11 is essential in defense against *S. flexneri* lacking the T3SS effector *ospC3 in vivo*, Related to figure 4

(A) The indicated mice were infected i.p. with indicated dose of wild-type *S. flexneri*. Bacterial loads from spleen were determined 24 hr later. (B-E) The indicated mice were co-infected i.p. with 2.5×10^7 CFU of both wild-type *S. flexneri* and *S. flexneri* Δ*ospC3* marked with ampicillin resistant or kanamycin resistant respectively. Bacterial loads from spleen (B, D) and liver (C, E) were determined 24 hr later. Dashed line indicates limit of detection (A and D-E).

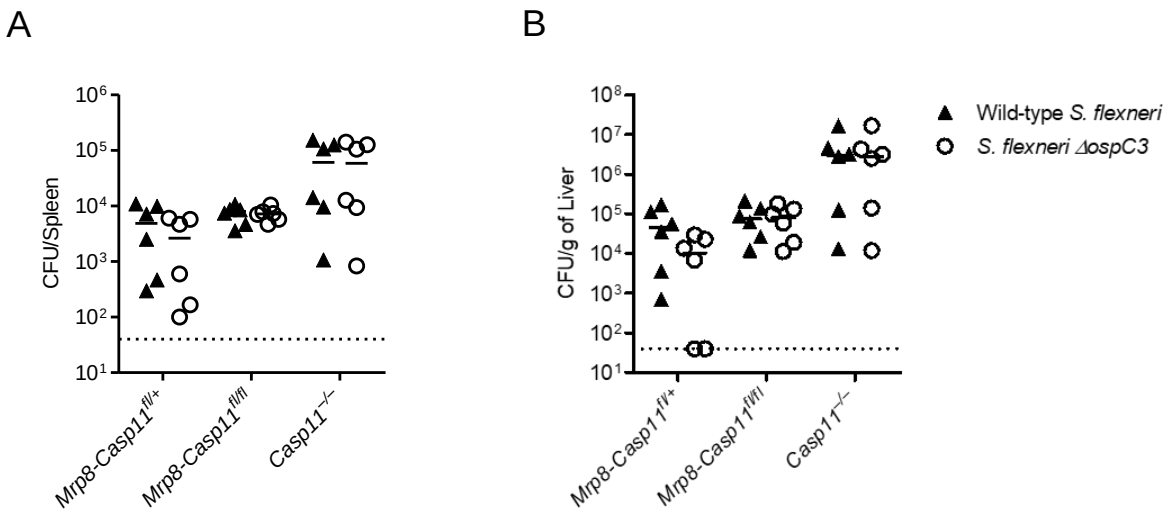

Figure S5. Neutrophils caspase-11 is critical in defense against *S. flexneri ΔospC3*, Related to figure 5

(A and B) The indicated mice were co-infected i.p. with 2.5x10^7 CFU of both wild-type *S. flexneri* and *S. flexneri* Δ*ospC3* marked with ampicillin resistant or kanamycin resistant respectively. Bacterial loads from spleen (A) and liver (B) were determined 24 hr later. Dashed line indicates limit of detection.

Table S1. Numbers of mice used in lethal challenges experiments, Related to Figure 4E-4F and 5F

Figure	Mice genotype	Number of mice
Figure 4E and 4F	Wild-type C57BL/6	14
Figure 4E and 4F	*Casp1*$^{-/-}$	12
Figure 4E and 4F	*Casp11*$^{-/-}$	12
Figure 4E and 4F	*Casp1-11*^{DKO}	12
Figure 4E and 4F	*Nlrc4-Asc*^{DKO}	15
Figure 4E and 4F	*Gsdmd*$^{-/-}$	12
Figure 5F	*Casp11*$^{fl/fl}$*Mrp8-cre*	18
Figure 5F	*Casp11*$^{fl/+}$*Mrp-cre*	9