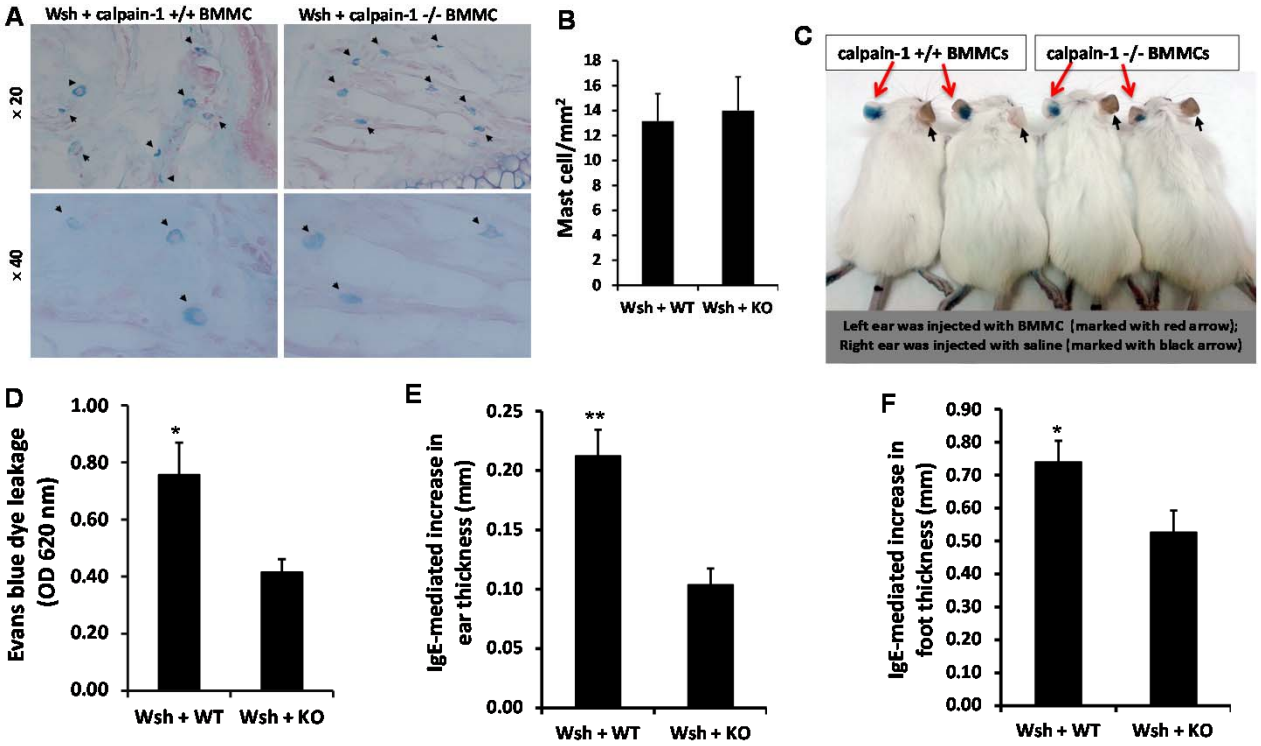
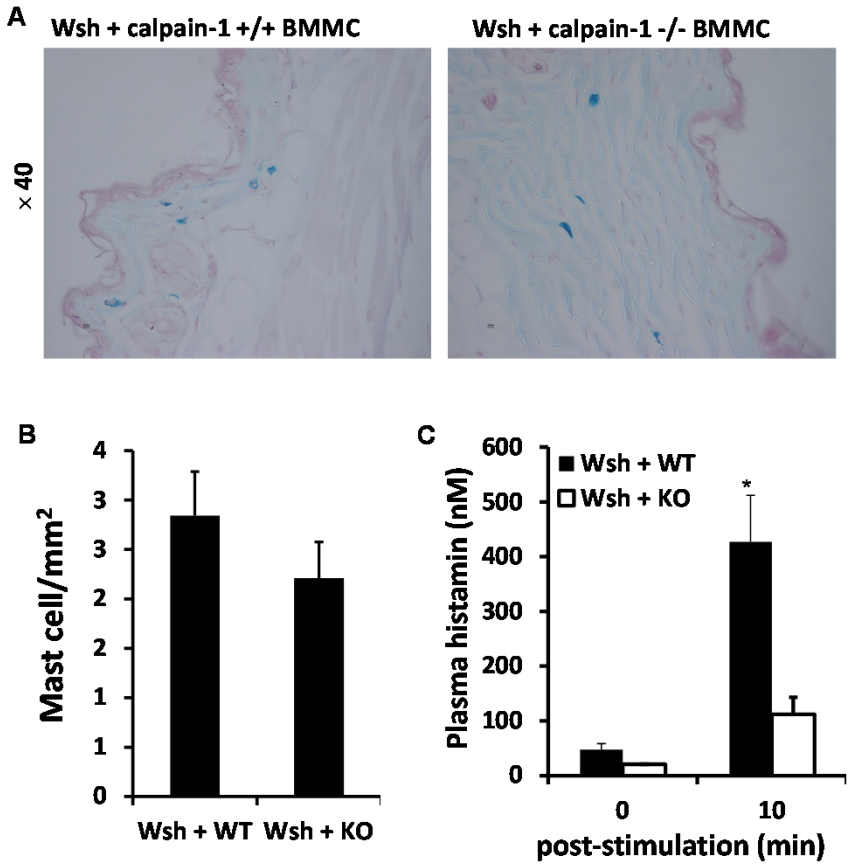
Supplemental Fig. S1



Supplemental Fig S1. Reduced passive cutaneous anaphylaxis in the absence of calpain-1. W-sh mice were reconstituted with mast cells by injection of 20 μl wild-type or calpain-1 null bone marrow-derived mast cells (BMMCs) at a density of 2.5×10^7 cells/ml in RPMI 1640 (5×10^5 cells/injection) into left ear and footpad, whereas right ear and footpad were injected with 20 μl saline as control. Five weeks later, mast cell-reconstituted W-sh mice were used for the following experiments. ***A***, Ear tissues were collected and fixed in Carnoy's fixative. Specimens were embedded in paraffin and sectioned onto microscope slides, and stained with 1% alcian blue and 1% safranin O. All images were taken using equivalent setting on a Nikon E600 microscope equipped with a 20× and 40× objective lens using ACT-1 software (Nikon). A representative photomicrograph is shown from three different mice of each group. Mast cells were labeled by arrowhead. ***B***, The number of mast cells/mm^2 of ear tissue from mast cell-reconstituted Wsh mice was quantitated by counting cells stained with alcian blue and safranin O (*n* = 10 of 20× fields from each of 3 mice per group). ***C***, Upon IgE sensitization and dinitrophenyl (DNP)-bovine serum albumin (BSA) challenge, leakage of injected Evans blue dye into the ear is visible in the area where mast cells were reconstituted. Representative results from eight mice of each group are shown. ***D***, Vascular leakage assayed by Evans blue dye extravasation into ear tissues was impaired in mice reconstituted with calpain-1 null BMMCs. Data are mean (OD_{620nm} of left ear – OD_{620nm} of right ear) ± SEM. *n* = 8 mice, *p< 0.05. ***E-F,*** Mice were sensitized by intravenous injection of anti-DNP IgE. Twenty-four hours later, a cutaneous reaction was elicited by the application of a solution (20 μl) of 0.3% DNFB in acetone/olive oil (4:1) to both side ears and footpads. After another 24 h, the cutaneous reaction was assessed by comparing the thickness of the left and right ear (***E***) or footpad (***F***). Data are mean (thickness of left side – thickness of right side) ± SEM. *n* = 8 mice, *p< 0.05, **p< 0.01.

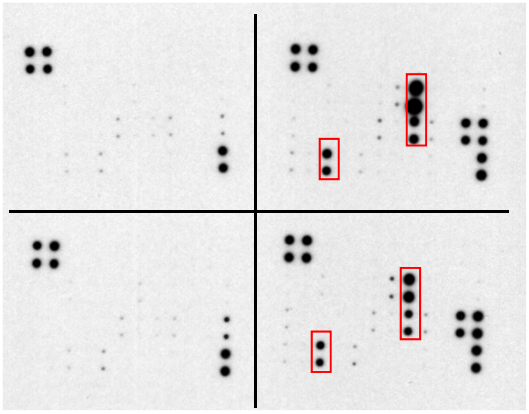
Supplemental Fig. S2



Supplemental Fig S2. Reduced passive systemic anaphylaxis in the absence of calpain-1. Five million wild-type or calpain-1 null BMMCs in 200 μl of PBS were injected i.v. via tail vein into 5-6 week old W-sh mice for five weeks. ***A***, Ear tissues were collected, fixed, and stained with alcian blue and safranin O. A representative photomicrograph is shown from three different mice of each group. ***B***, The number of mast cells/mm^2 of ear tissue from mast cell-reconstituted W-sh mice was quantitated by counting alcian blue and safranin O stained cells (*n* = 10 of 20× fields from 3 mice per group). ***C***, Passive systemic anaphylaxis (PSA) in wild-type or calpain-1 null BMMC-reconstituted W-sh mice were performed and monitored by measuring plasma histamine level. About 200 μl of blood were collected from each mouse. Then, mice were sensitized with 10 μg anti-DNP IgE in 100 μl saline by i.v. injection. Twenty four hours after anti-DNP IgE injection, mice were injected (i.v.) with 1.5 mg DNP in 100 μl saline. Blood were collected at 10 min post DNP injection. Histamine levels in the plasma were measured by ELISA (Beckman Coulter, Brea, CA, USA). Data are mean ± SE, *n* = 5 mice per group, *p < 0.05.

Supplemental Fig. S3

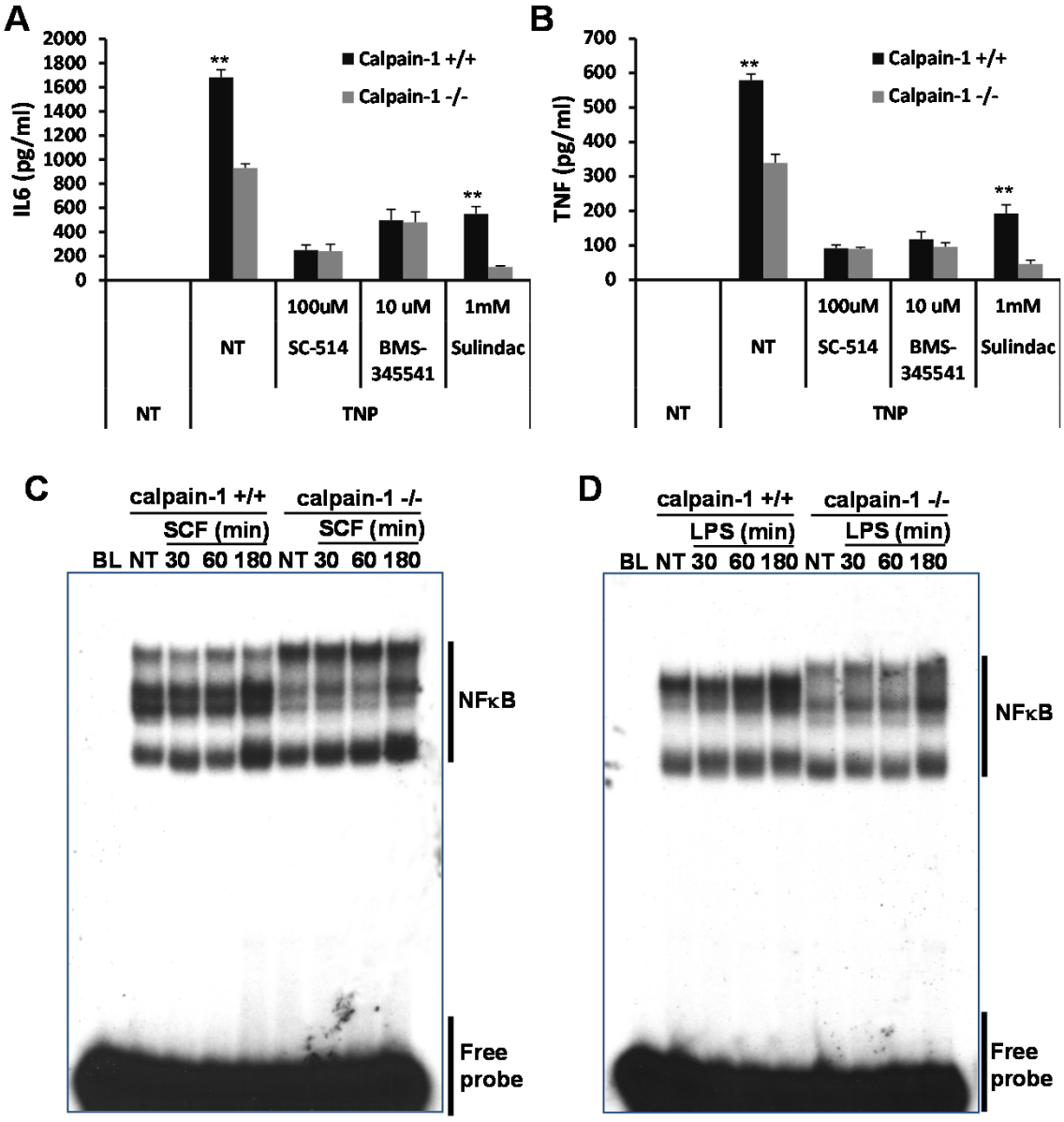
WT, NT	WT, TNP 6 h
KO, NT	KO, TNP 6 h



POS	POS	NEG	NEG	Blank	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
POS	POS	NEG	NEG	Blank	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
GM-CSF	IFNγ	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
GM-CSF	IFNγ	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	CCL2	MCSF	MIG	MIP-1α	MIP-1γ
IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	CCL2	MCSF	MIG	MIP-1α	MIP-1γ
RANTES	SDF-1	CCL1	TECK	TIMP-1	TIMP-2	TNF	sTNF RI	sTNF R II	Blank	Blank	POS
RANTES	SDF-1	CCL1	TECK	TIMP-1	TIMP-2	TNF	sTNF RI	sTNF R II	Blank	Blank	POS

Supplemental Fig S3. Calpain-1 deficiency affects IL-6, CCL1, and CCL2 production by mast cells. Cell-free culture supernatants from wild-type and calpain-1 null BMMCs were analyzed for production of 40 cytokines by the protein array. Samples were collected at 6 hours following IgE-dependent mast cell activation. Out of 40 cytokines tested, a reduction in IL-6, CCL1, and CCL2 production was observed.

Supplemental Fig. S4



Supplemental Fig S4. IKK inhibitors reduced IgE-mediated cytokine production in the presence and absence of calpain-1. ***A & B***, BMMCs were pre-treated with IKK inhibitors (100 μM SC-514, 10 μM BMS-345541, or 1 mM sulindac) for 2 hours, and then stimulated with TNP-BSA for 4 hours. Cell free supernatants were analyzed for IL6 (***A***) and TNF (***B***) production. IL6 and TNF production were reduced by IKK inhibitors in both wild-type and calpain-1 null BMMCs, suggesting that both calpain-1 dependent and independent cytokine production was inhibited by IKK-IkB-NF-kB pathway blockade. *n* = 4 cultures of BMMCs. ***C & D***, BMMCs were stimulated with 100 ng/ml SCF (***C***) or 3 μg/ml LPS (***D***), and then nuclear proteins were extracted and subjected to EMSA analysis to detect NF-kB activity. NF-kB activation was reduced in calpain-1 null BMMCs as compared to wild-type BMMCs.