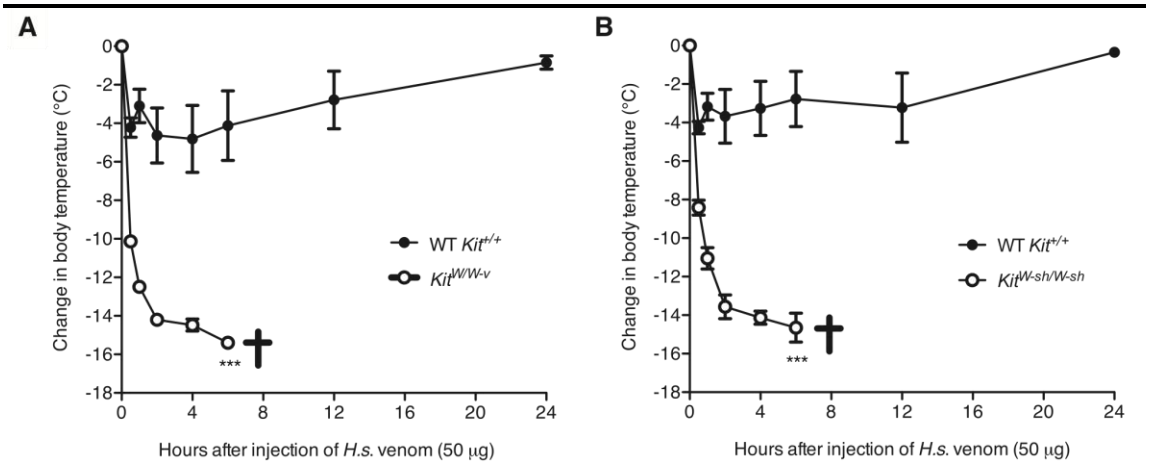
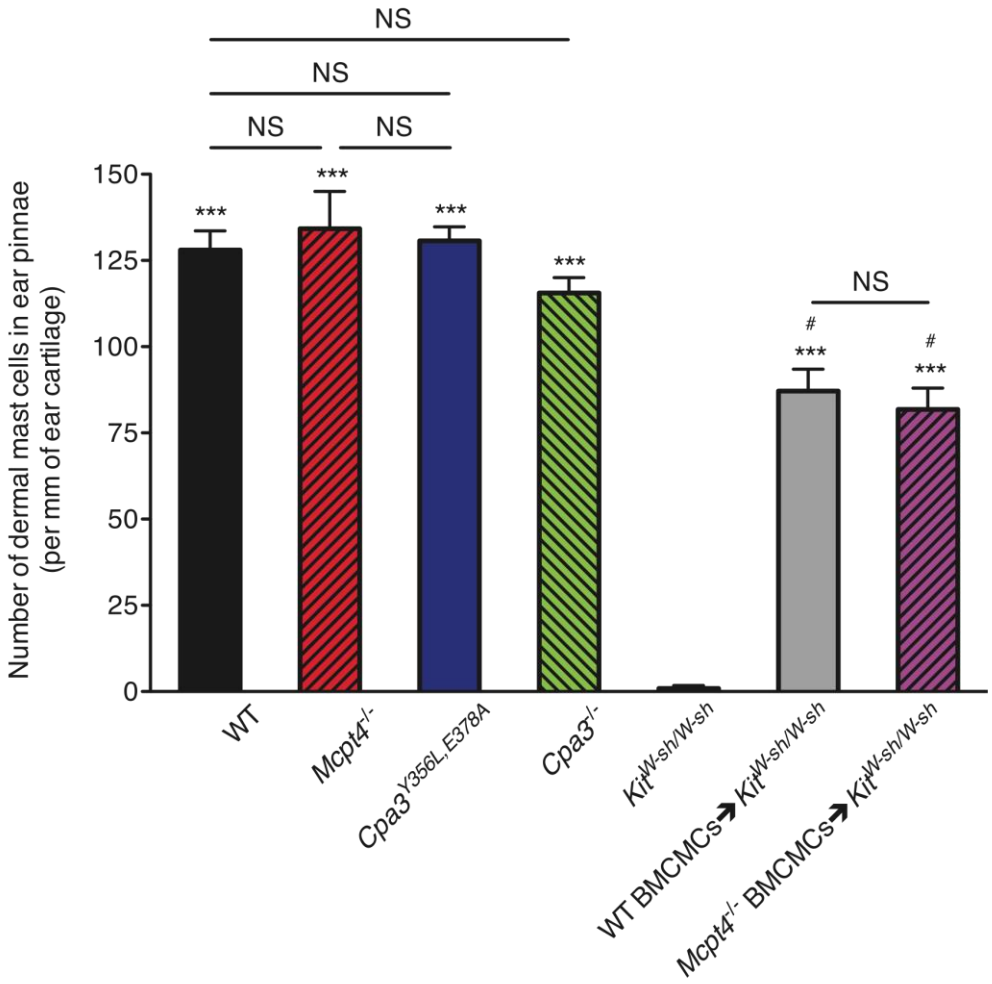
Supplemental figures 1-6 for: Mast cell chymase reduces toxicity of Gila monster and scorpion venoms, and VIP, in mice, by Mitsuteru Akahoshi, Chang Ho Song, Adrian M. Piliponsky, Martin Metz, Andrew Guzzetta, Magnus Åbrink, Susan M. Schlenner, Thorsten B. Feyerabend, Hans-Reimer Rodewald, Gunnar Pejler, Mindy Tsai, and Stephen J. Galli



H.s. venom i.d.	Dead/Total (%) 5 μg	Dead/Total (%) 50 μg	*P* vs WT (50 μg)	Drop in temperature > 10°C/Total (%) 5 μg	Drop in temperature > 10°C/Total (%) 50 μg	*P* vs WT (50 μg)
WT WBB6F$_1$-*Kit*$^{+/+}$	0/7 (0)	2/9 (22)	-	0/7 (0)	2/9 (22)	-
WBB6F$_1$-*Kit*$^{W/W-v}$	0/5 (0)	9/9 (100)	0.001	0/5 (0)	9/9 (100)	0.001
WT C57BL/6-*Kit*$^{+/+}$	0/7 (0)	2/8 (25)	-	0/7 (0)	1/8 (13)	-
C57BL/6-*Kit*$^{W-sh/W-sh}$	0/4 (0)	6/6 (100)	0.009	0/4 (0)	6/6 (100)	0.002

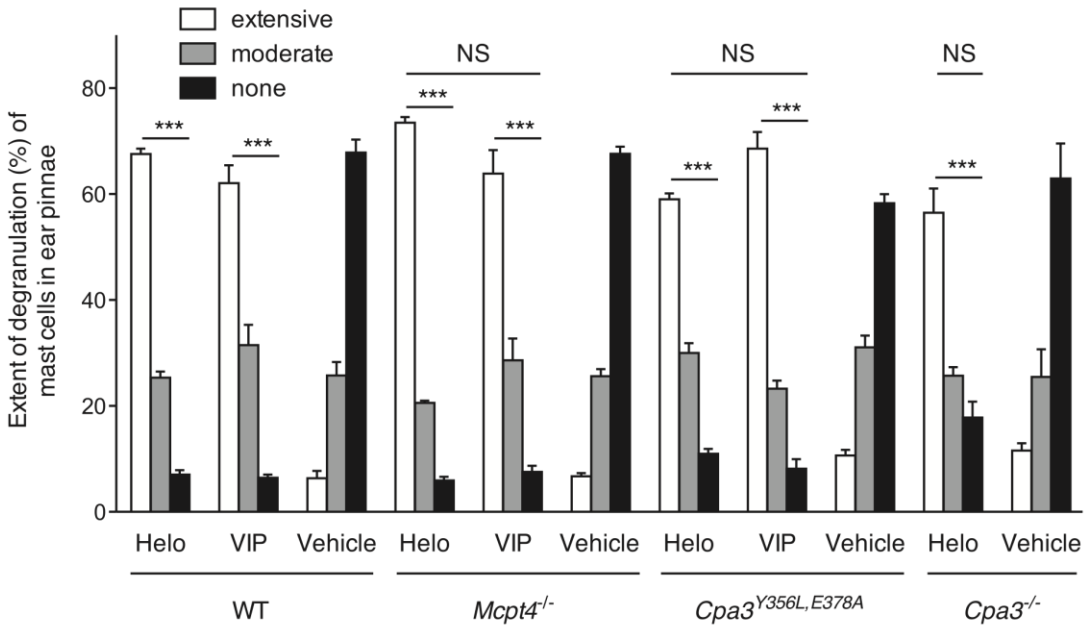
Supplemental Figure 1

Changes in rectal temperatures (in degrees C) (**A, B**), and rates of death and drop in rectal temperature of over 10 degrees within 24 h (**Table**), after i.d. injection of *H.s.*v. (5 μg [**Table**] or 50 μg [**Table** and **A, B**] in 20 μl DMEM solution) into the ear pinnae (one ear pinna of each mouse) of (**A**) WT WBB6F$_1$-*Kit*$^{+/+}$ mice and mast cell-deficient WBB6F$_1$-*Kit*$^{W/W-v}$ mice, and (**B**) WT C57BL/6-*Kit*$^{+/+}$ mice and mast cell-deficient C57BL/6-*Kit*$^{W-sh/W-sh}$ mice. ***$P < 0.001$ versus WT *Kit*$^{+/+}$ mice injected with 50 μg of *H.s.*v.; † = all mice died.



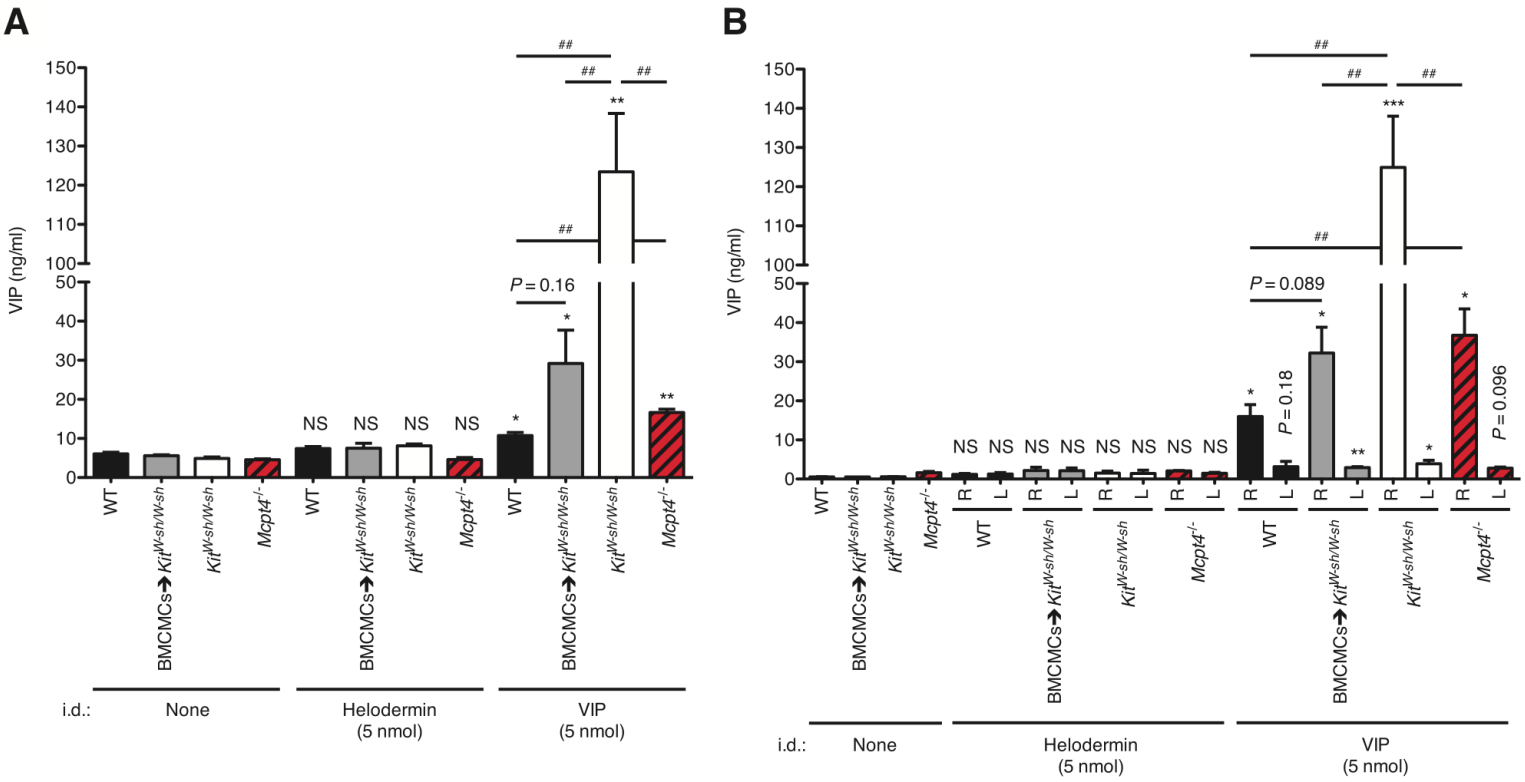
Supplemental Figure 2

Numbers of mast cells in the dermis of ear pinnae 1 h after injection of vehicle-(DMEM-) in WT C57BL/6 mice, *Mcpt4*$^{-/-}$ mice, *Cpa3*^{Y356L,E378A} mice, *Cpa3*$^{-/-}$ mice, mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, WT BMCMCs→*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, and *Mcpt4*$^{-/-}$ BMCMCs→*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (n = 3/group). ***$P < 0.001$ versus C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice; $^{\#}P < 0.05$ versus WT *Kit*$^{+/+}$ mice; NS = not significant ($P > 0.05$).



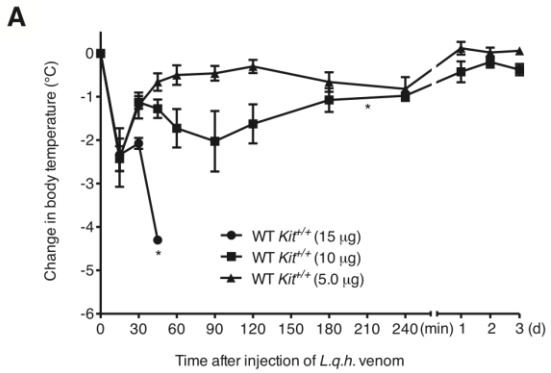
Supplemental Figure 3

Extent of degranulation of dermal mast cells 1 h after i.d. injection of helodermin (Helo; 5 nmol in 20 μl DMEM), VIP (5 nmol in 20 μl DMEM) or vehicle (DMEM) alone into a single ear pinna of C57BL/6 WT, *Mcpt4*$^{-/-}$, *Cpa3*^{Y356L,E378A}, or *Cpa3*$^{-/-}$ mice. ***P < 0.001 versus corresponding vehicle-injected groups; NS = not significant (P > 0.05) versus corresponding values for WT mice; n = 3/group.

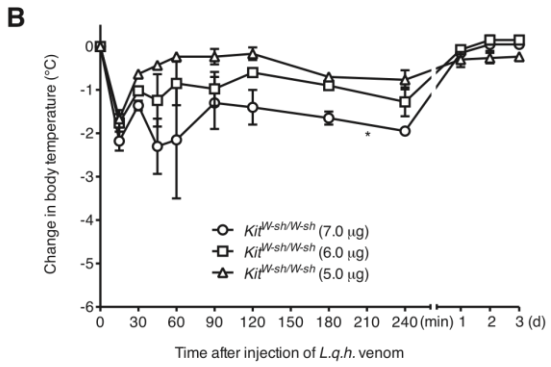


Supplemental Figure 4

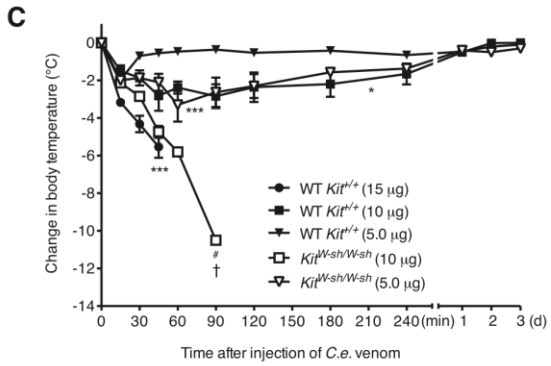
MCPT4 contributes to mast cell-dependent degradation of VIP *in vivo*. Concentrations of VIP in (**A**) blood and (**B**) ear pinnae before (i.d.: none) or at 30 min after i.d. injection with 5 nmol of helodermin or VIP into the right ear pinnae of WT, *Kit*$^{W\text{-}sh/W\text{-}sh}$, WT BMCMCs→*Kit*$^{W\text{-}sh/W\text{-}sh}$, or *Mcpt4*$^{-/-}$ mice. R: right ear (injected with helodermin or VIP); L: left ear (not injected); **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS = not significant (*P* > 0.05) versus the corresponding not injected groups; $^{\#\#}P < 0.01$ for the comparisons shown; n = 3/group, from the 2-3 independent experiments we performed, each of which gave similar results (n = 1-2 mice per group per experiment).



C57BL/6 mice	Dead/Total (%)
WT *Kit*$^{+/+}$ (15 μg)	5/5 (100)
WT *Kit*$^{+/+}$ (10 μg)	0/4 (0)
WT *Kit*$^{+/+}$ (5.0 μg)	0/5 (0)



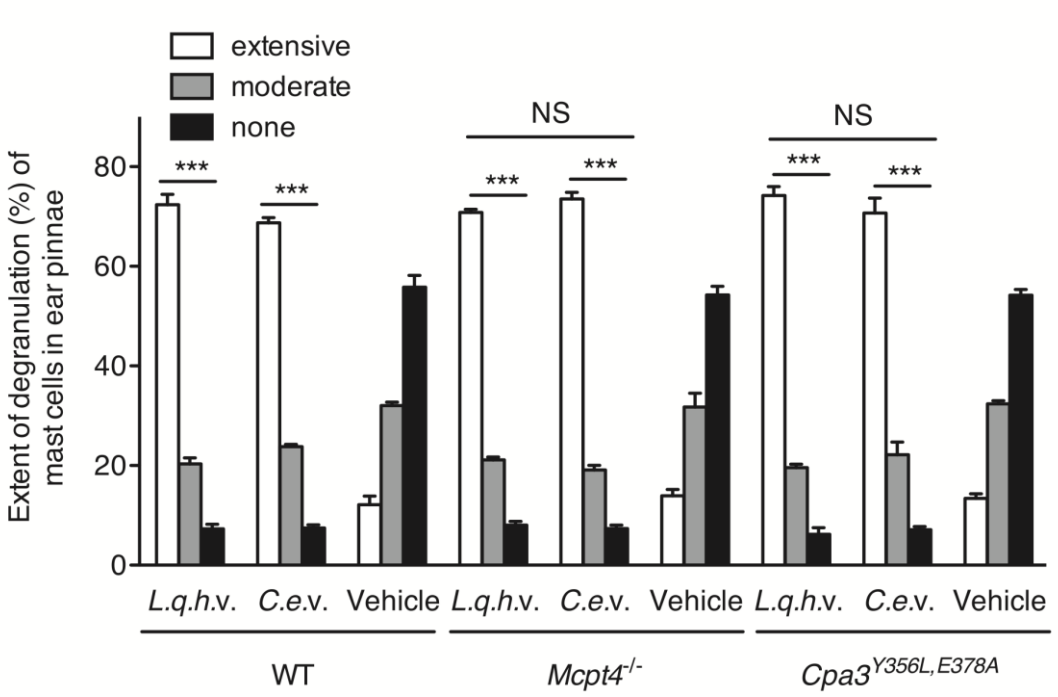
C57BL/6 mice	Dead/Total (%)
Kit$^{W\text{-}sh/W\text{-}sh}$ (7.0 μg)	3/5 (60)
Kit$^{W\text{-}sh/W\text{-}sh}$ (6.0 μg)	1/5 (20)
Kit$^{W\text{-}sh/W\text{-}sh}$ (5.0 μg)	0/3 (0)



C57BL/6 mice	Dead/Total (%)
WT *Kit*$^{+/+}$ (15 μg)	6/6 (100)
WT *Kit*$^{+/+}$ (10 μg)	1/6 (17)
WT *Kit*$^{+/+}$ (5.0 μg)	0/6 (0)
Kit$^{W\text{-}sh/W\text{-}sh}$ (10 μg)	6/6 (100)
Kit$^{W\text{-}sh/W\text{-}sh}$ (5.0 μg)	0/3 (0)

Supplemental Figure 5

Changes in rectal temperature (in degrees C) and 24 h survival after i.d. injection of different amounts of *Leiurus quinquestriatus hebraeus* venom (*L.q.h.* venom, in 50 μl of PBS) or *Centruroides exilicauda* venom (*C.e.* venom, in 50 μl of PBS) into a single ear pinna of: (**A**) WT C57BL/6-*Kit*$^{+/+}$ mice; (**B**) mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice; or (**C**) WT C57BL/6-*Kit*$^{+/+}$ mice and mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. In **A** and **B**: $^{*}P < 0.05$ versus corresponding values for mice injected with 5.0 μg of *L.q.h.* venom; in (**C**): $^{*}P < 0.05$, $^{***}P < 0.001$ versus WT C57BL/6-*Kit*$^{+/+}$ mice injected with 5.0 μg of *C.e.* venom, $^{\#}P < 0.05$ versus WT C57BL/6-*Kit*$^{+/+}$ mice injected with 10 μg of *C.e.* venom, and $^{\dagger}P < 0.01$ versus mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice injected with 5.0 μg of *C.e.* venom.



Supplemental Figure 6

Extent of degranulation of dermal mast cells 1 h after i.d. injection of *L.q.h.*v. (7.5 μg in 50 μl of PBS), *C.e.*v. (7.5 μg in 50 μl of PBS) or vehicle (PBS) alone into a single ear pinna of WT C57BL/6, *Mcpt4*$^{-/-}$, or *Cpa3*^{Y356L,E378A} mice. ***P < 0.001 versus corresponding vehicle-injected groups; NS = not significant (P > 0.05) versus corresponding values for WT mice; n = 3/group.