Supplemental Information

Figure S1, related to Figure 1.

Figure S2, related to Figure 2.

Figure S3, related to Figure 3.

Figure S4, related to Figure 4 and 5.

Figure S1, related to Figure 1.

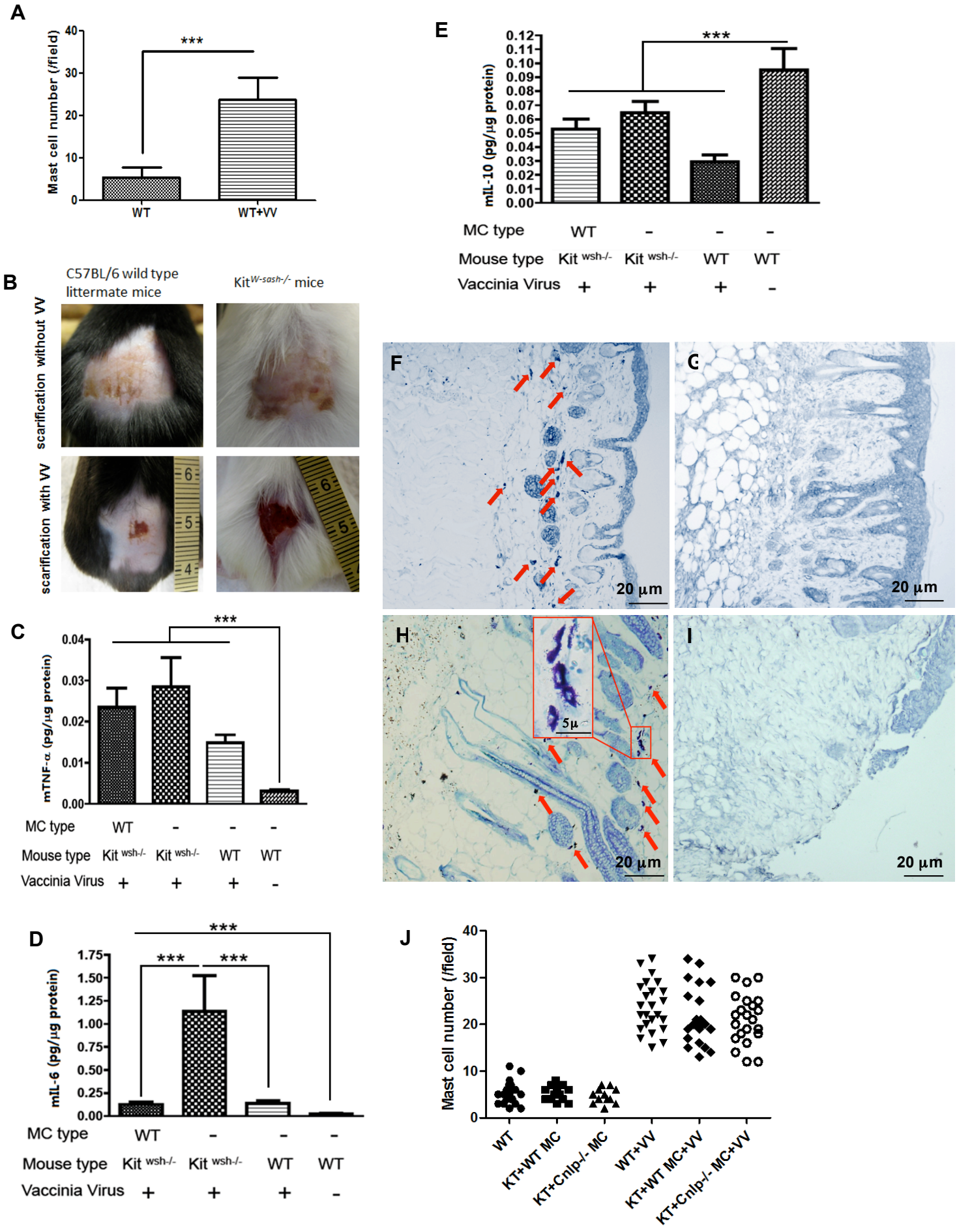
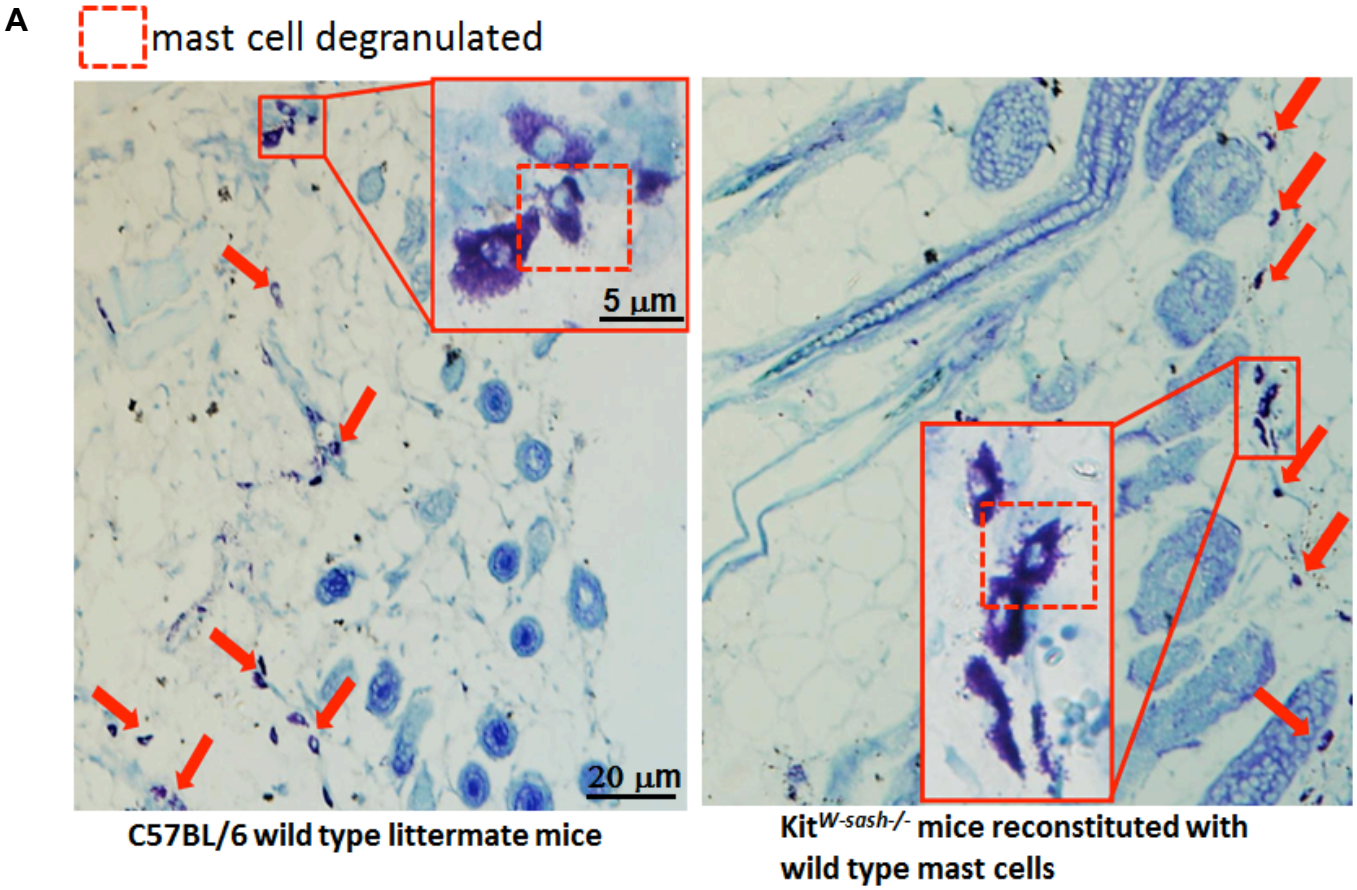
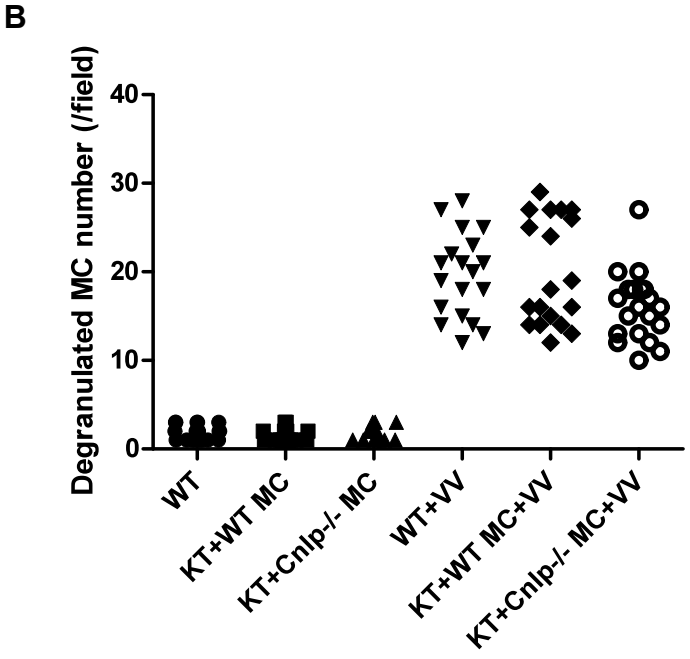


Figure S2, related to Figure 2.





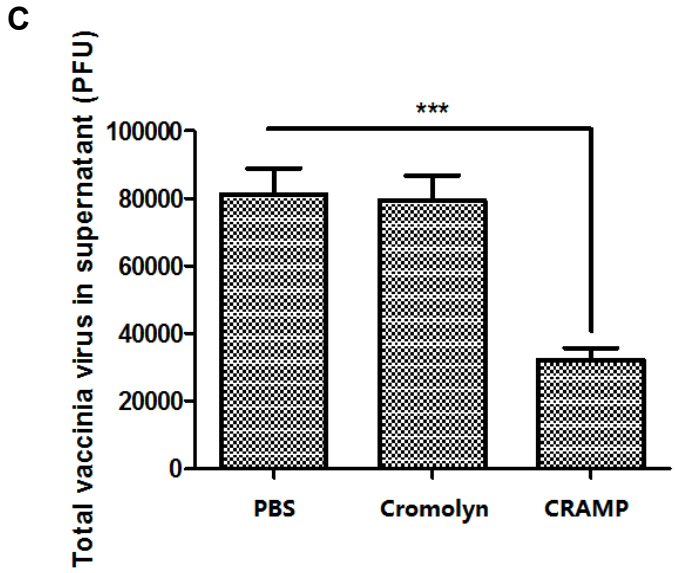


Figure S3, related to Figure 3.

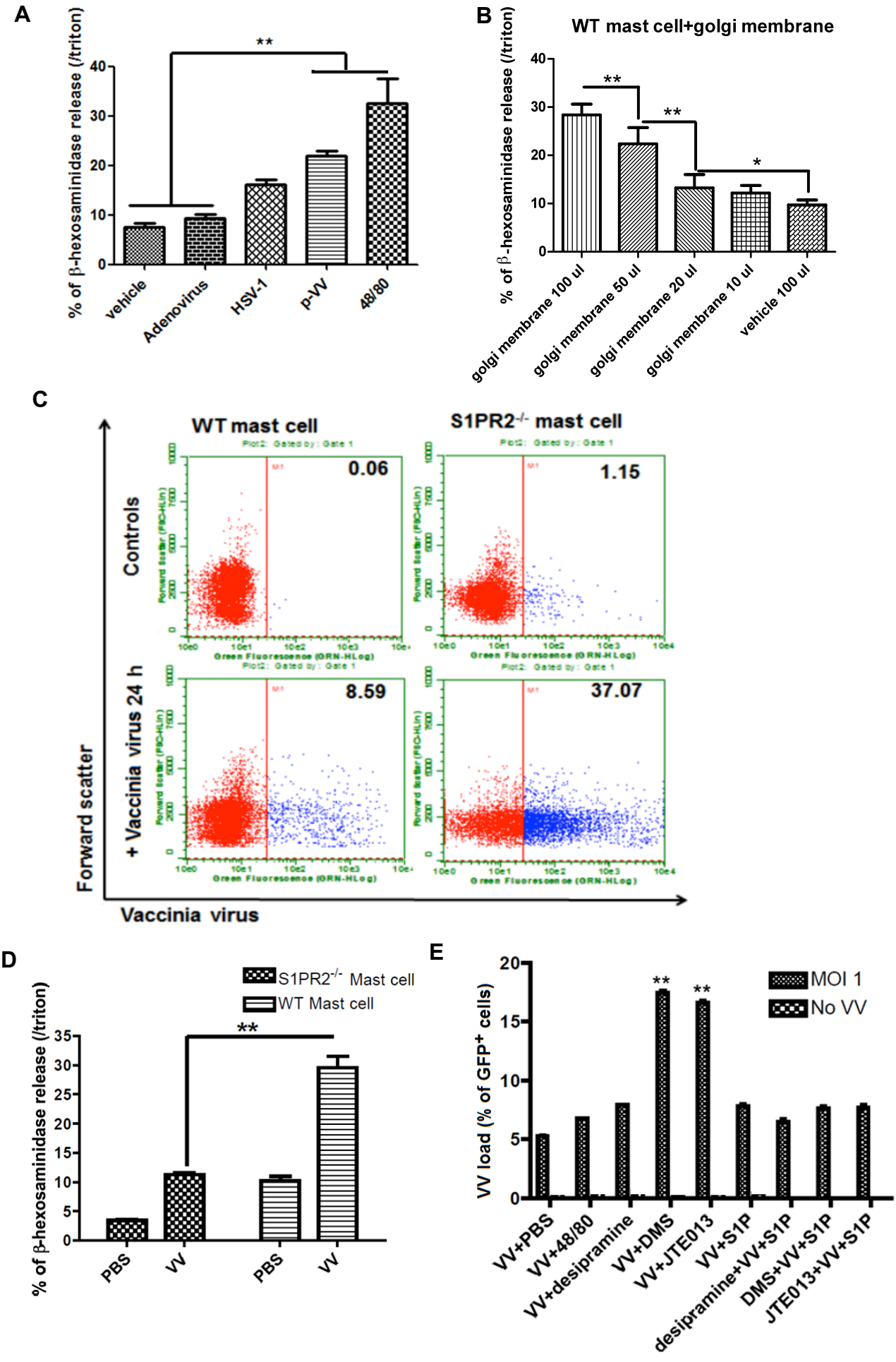


Figure S4, related to Figure 4 and 5.

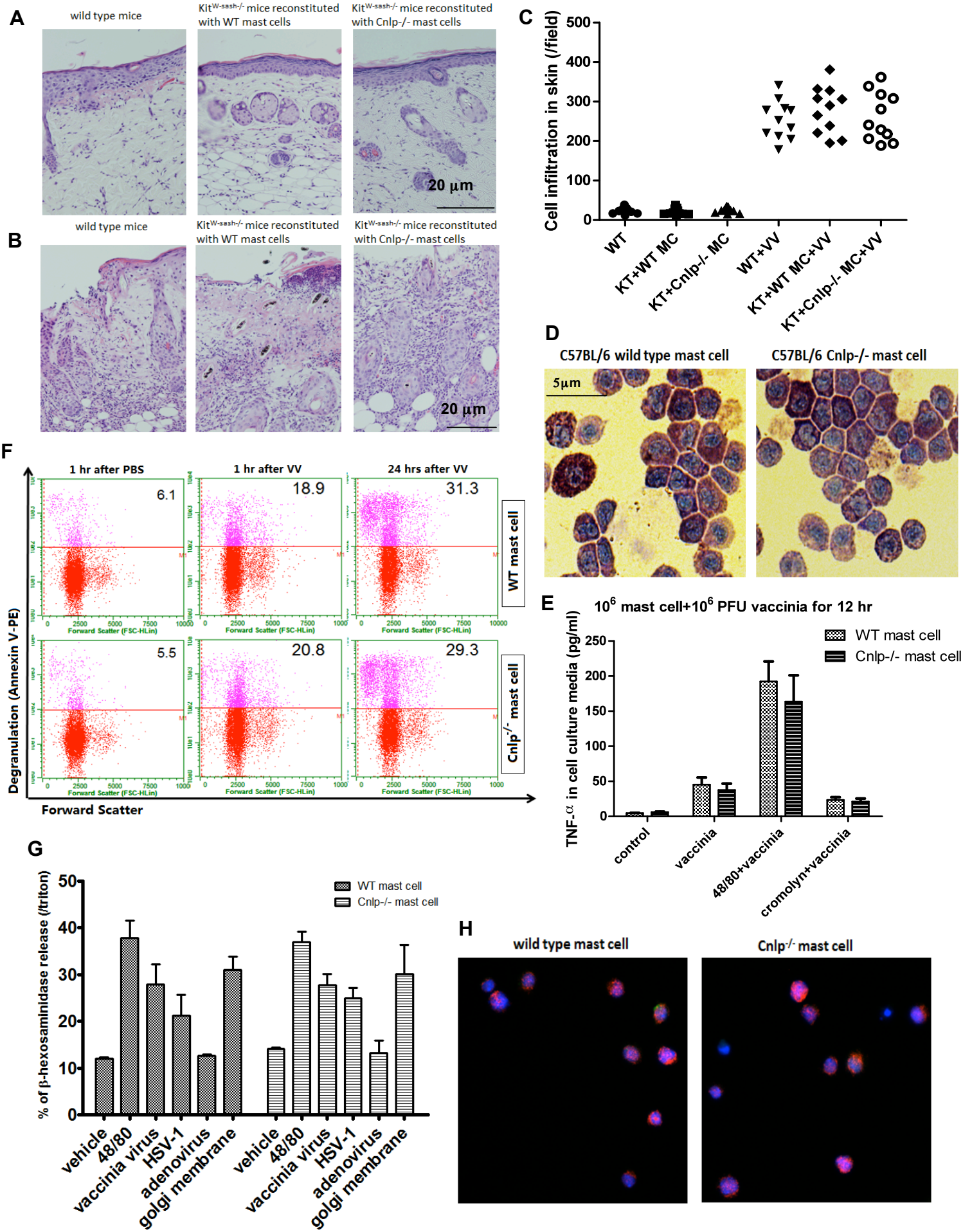


Figure S1, related to Figure 1. Mast cell reconstitution of Kit $^{wsh-/-}$ mice. (A) Mast cell was counted in randomly selected fields (X200) of baseline control and 72 hours after VV inoculation. ***P<0.001. **(B)** Wild type and *Kit* $^{wsh-/-}$ mouse skin present no difference after scarification without VV**.** Our protocol requires superficial scarification. Baseline control was wild type and *Kit* $^{wsh-/-}$ mice scarified without VV application. As visible in the image of *Kit* $^{wsh-/-}$ mouse scarified without VV do not differ from wild type mouse and do not develop spontaneous wound in the absence of VV. The figure shows the scarification area after 72 hours for wild type and of *Kit* $^{wsh-/-}$ mouse.

Cytokines expression in the tissue homogenate from skin lesions of wild-type mice, *Kit* $^{wsh-/-}$ mice and *Kit* $^{wsh-/-}$ mice reconstituted with wild-type mast cells 3 days post-infection with 10^6 PFU VV. **(C)** Mouse TNF-α ELISA quantification. Infected tissue showed no difference in TNF-α levels, while TNF-α was significantly increased in all of VV infected mice without regard to their mast cell status compared to non-infected mice. ***P<0.001. **(D)** Mouse IL-6 ELISA quantification. IL-6 was dramatically increased in the mast cell deficient non-reconstituted mice; and it was also significantly increased in all of infected mice when compared to non-infected mice meaning that its increase is related to the severity of the infection. ***P<0.001. **(E)** Mouse IL-10 ELISA quantification. IL-10 was significantly decreased in all of infected mice when compared to non-infected mice. ***P<0.001. Please note the unit of cytokines protein is pg/μg total protein, not pg/ml as usually used. So the value of

cytokines protein is much smaller, but more accurate for measuring cytokines protein in solid tissue samples such as skin (Lira et al., 2009). **(F)** C57BL/6 wild type littermate mouse (mast cells in dermis are indicated by arrows). **(G)** *Kit* $^{wsh-/-}$ mouse skin section (there are no mast cell in the dermis). **(H)** *Kit* $^{wsh-/-}$ mouse reconstituted with mast cells stained with toluidine blue 2 weeks after injection into the skin (mast cells in dermis indicated by arrows). **(I)** *Kit* $^{wsh-/-}$ mouse skin section 72 h after vaccinia virus infection. Still mast cells are not visible in the section. **(J)** Mast cell was counted in randomly selected fields (X200) in the dermis of the scarification area in baseline control without VV infection and mice 72 h after VV inoculation in Wild type and *Kit* $^{wsh-/-}$ mice reconstituted with wild type MCs and *Cnlp-/-* MCs. There is no difference in the mast cell number in skin of WT mice and *Kit* $^{wsh-/-}$ mice reconstituted with wild type or *Cnlp-/-* mast cells before and after VV infection. Three independent experiments with 5 mice per group were performed for each experiment

Figure S2, related to Figure 2. *In vivo* Mast cell degranulation. (A) Mast cells were determined by morphometry (high power 400X, mast cells stain metachromatically with toluidine blue). Mast cells were defined as "degranulated" when more than eight metachromatic extracellular mast cell granules were detected by light microscopy (Paus et al., 1995). **(B)** Mast cell was counted in randomly selected fields (X200) of baseline control and 72

hours after VV inoculation. No significant difference in mast cell degranulation *in situ* in the skin between C57BL/6 wild type littermate mice and *Kit*$^{wsh-/-}$ mice reconstituted with mast cells. **(C)** Cromolyn does not directly affect VV. The graph shows the VV survival in PFU. ***P<0.001. Three independent experiments with 5 mice per group were performed for each experiment

Figure S3, related to Figure 3. Mast cells degranulate in contact with viral envelopes. (A) β-hexosaminidase release of mast cells upon contact with purified live VV (p-VV), HSV-1 and adenovirus at MOI=1 for 1h. 48/80 is the positive control. NS: no significant difference between vehicle and Adenovirus treatment. **P<0.01 compared to other treatments except for treatment with Adenovirus. **(B)** Dose-dependent mast cell degranulation by golgi membrane. Different dilution of separated golgi membranes induce degranulation proportionally to the relative concentration. *P<0.05, **P<0.01. **(C)** FACS analysis of S1PR2$^{-/-}$ MCs generated from bone marrow of Balb/C ByJ background S1PR2$^{-/-}$ mice and their wild-type littermate mice. Mast cells were inoculated with GFP^{+} VV at MOI=1 for 24 hours. Left quadrants show the percentage of MCs infected by VV. **(D) b**-hexosaminidase analysis of granule release was determined in mast cells generated from bone marrow of S1PR2$^{-/-}$ Balb/C ByJ mice and their wild-type littermate mice. **P<0.01. **(E)** FACS analysis of GFP^{+} MCs infected by GFP^{+} VV. **P<0.01 compared to other

groups. Each experiment has been performed in triplicate

Figure S4, related to Figure 4 and 5. (A-C) Hematoxylin and eosin staining (H&E) of skin section of WT mice and *Kit*$^{wsh-/-}$ mice reconstituted with wild type MCs and *Cnlp-/-* mast cells of baseline control without VV infection and 72 hours after VV inoculation at 200X. **(A)** Baseline control **(B)** 72 hours after VV inoculation. **(C)** The number of infiltrated cells was counted in randomly selected high power fields (×400). No difference in cell infiltration was noted between wild type and reconstituted mice. Three independent experiments with 5 mice per group were performed for each experiment

Cnlp*$^{-/-}$ mast cell characterization*: **(D-H) (D)** *Cnlp*$^{-/-}$ mast cell metachromasia is similar to wild type MCs. **(E)** *Cnlp*$^{-/-}$ mast cell TNF-α response to stimula is similar to wild type MCs. **(F)** FACS analysis of mast cell degranulation by annexin-V Phycoerythrin (PE) staining (Demo et al., 1999; Suzuki and Verma, 2008). The ability of mast cell degranulation in response to VV was evaluated by adding VV at MOI=1 to the cell cultures and evaluated at 1 and 24 hours. Upper quadrants show the percentage of cells that degranulated in response to VV. **(G)** β-hexosaminidase analysis of granule release was determined in wild-type C57BL/6 MCs and *Cnlp*$^{-/-}$ MCs in the presence or absence of different degranulation conditions. The release of β-hexosaminadase of *Cnlp*$^{-/-}$ MCs was similar to wild type cells. **(H)** Autophagy in mast cells. LC3B antibody (red) was

used to detect signals of autophagy in the presence of VV at MOI=1. Mast cells counterstaining was done with DAPI (blue). No difference were noticed between *Cnlp*$^{-/-}$ and wild type mast cells. Each experiment has been performed in triplicate.