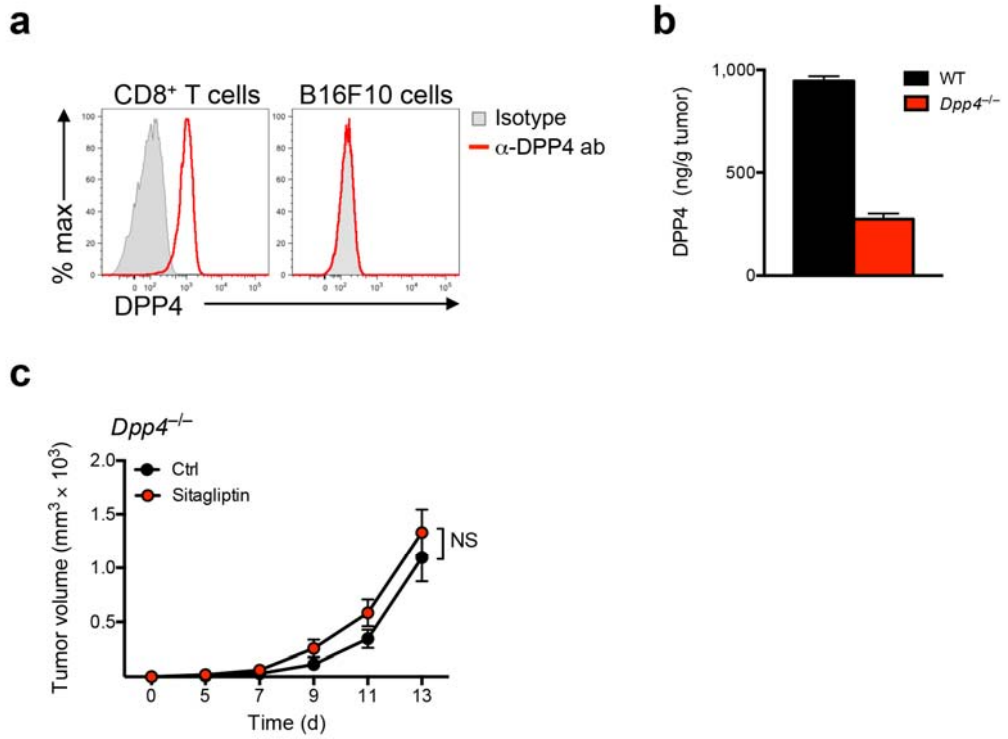
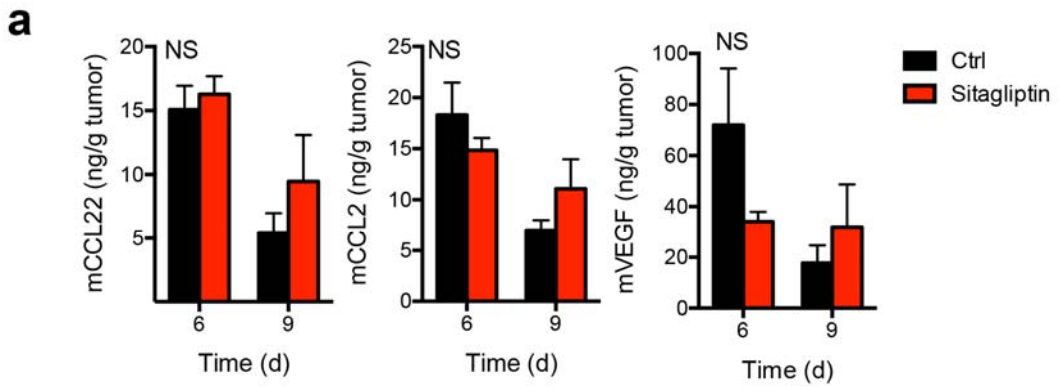
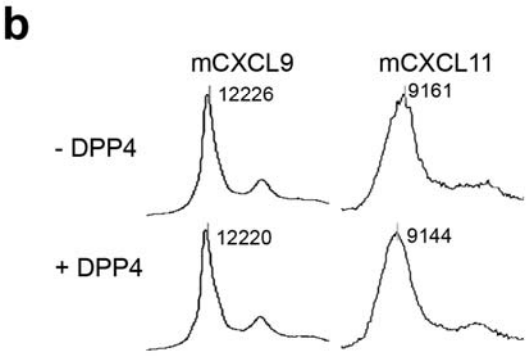


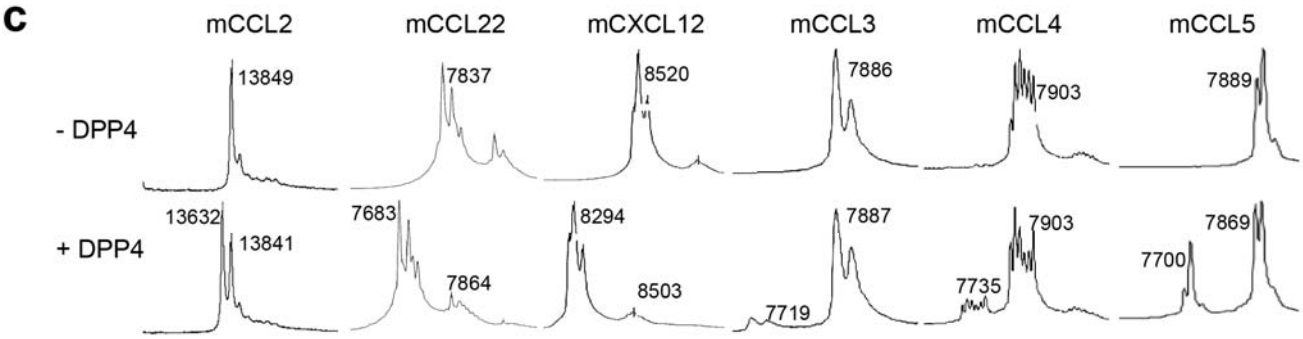
Supplementary Figure 1

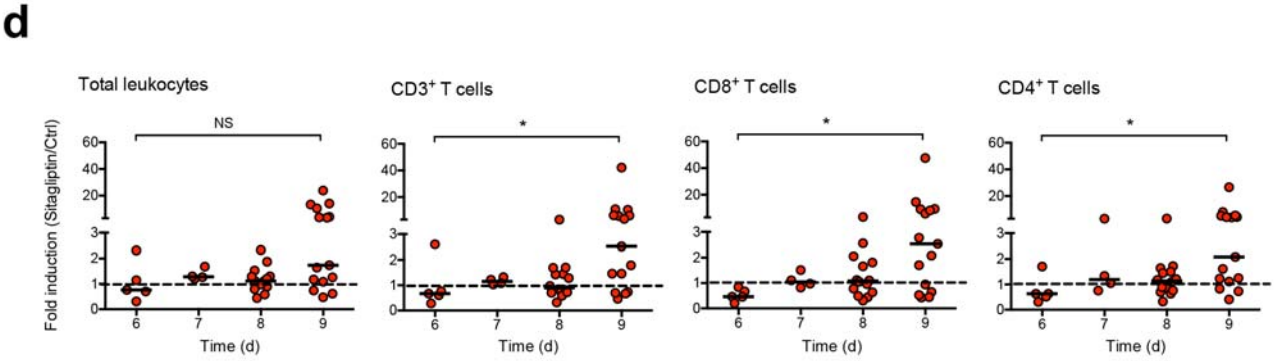
Expression of DPP4 in B16F10 cells and tumors.

*(**a**) C57BL/6 splenocytes and B16F10 melanoma cells were incubated with fluorochrome-conjugated anti-mDPP4 or with an isotype control. $CD8^+$ T cells on splenocytes were gated and shown as positive control for DPP4 expression. (**b**) C57BL/6 wild-type (WT) and $Dpp4^{-/-}$ mice were subcutaneously injected with B16F10 cells. Eight days after injection, tumor homogenates were prepared and the DPP4 concentration was determined (bars represent mean ± s.e.m.; n = 3 (WT) and 4 ($Dpp4^{-/-}$) mice). (**c**) $Dpp4^{-/-}$ mice were fed with control (ctrl) or sitagliptin chow prior to subcutaneous injection of B16F10 tumor cells. Tumor volumes are shown (data represent mean ± s.e.m.; n = 5 mice per group). Significance was determined using two-way ANOVA. Data are representative of 3 (**a**) and 2 (**b**,**c**) independent experiments.*


a
NS
mCCL22 (ng/g tumor)
Time (d)
NS
mCCL2 (ng/g tumor)
Time (d)
NS
mVEGF (ng/g tumor)
Time (d)
Ctrl
Sitagliptin


b
mCXCL9
mCXCL11
12226
9161
- DPP4
12220
9144
+ DPP4

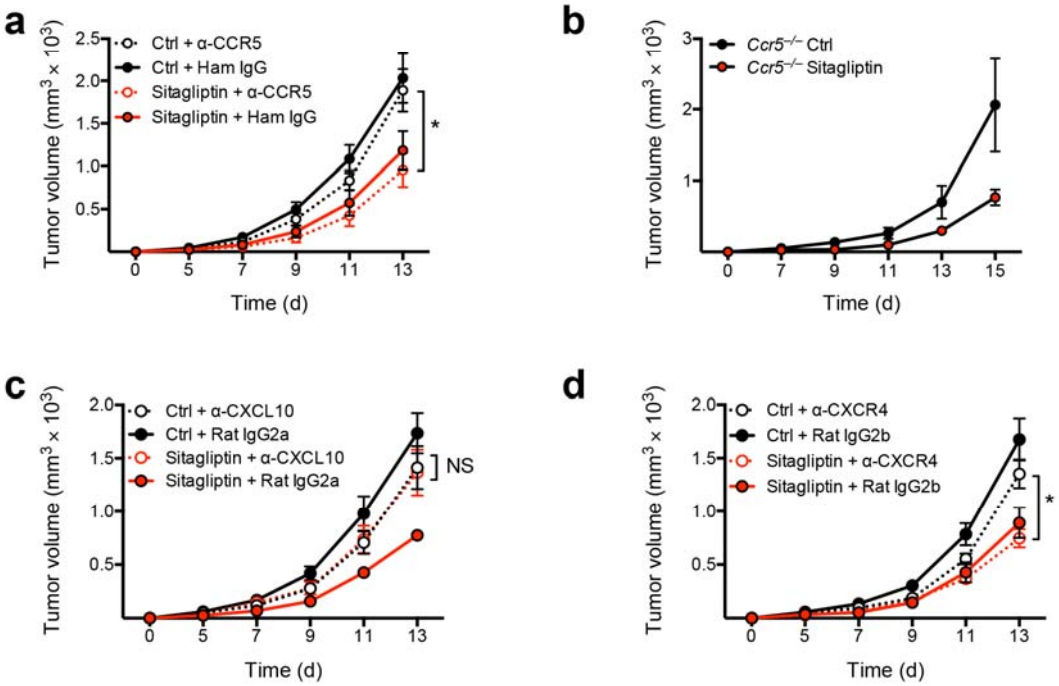

c
mCCL2
mCCL22
mCXCL12
mCCL3
mCCL4
mCCL5
13849
7837
8520
7886
7903
7889
- DPP4
13632
13841
7683
7864
8294
8503
7887
7719
7903
7735
7869
7700
+ DPP4


d
Total leukocytes
CD3+ T cells
CD8+ T cells
CD4+ T cells
NS
*
Fold induction (Sitagliptin/Ctrl)
Time (d)

Supplementary Figure 2

Susceptibility of mouse chemokines to DPP4-mediated processing.

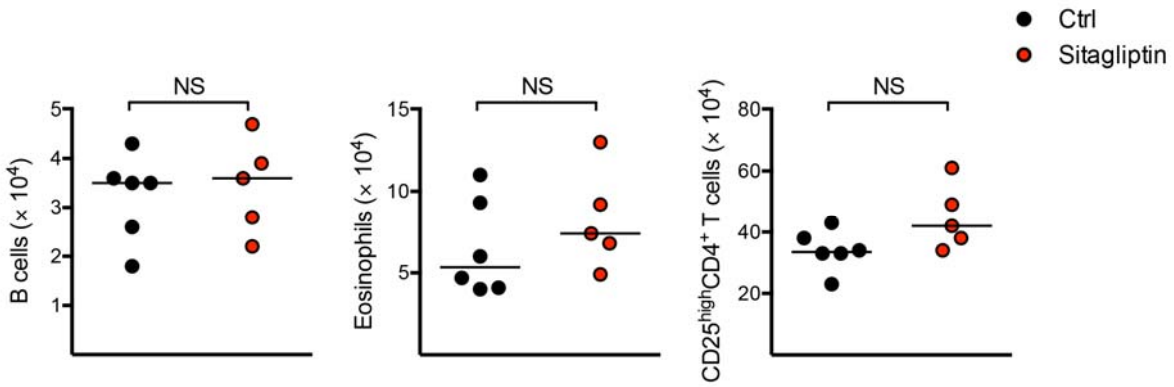
*(**a**) WT mice fed with control (ctrl) or sitagliptin chow were subcutaneously injected with B16F10 cells. Tumors were dissected at the indicated time points after tumor cell injection, and mCCL22, mCCL2 and mVEGF expression in tumor homogenates was evaluated (data represent mean ± s.e.m.; n = 4 mice per group). (**b**,**c**) Recombinant mCXCL9 and mCXCL11 (**b**) and mCCL2, mCCL22, mCXCL12, mCCL3, mCCL4 and mCCL5 (**c**) were incubated in the absence (–) or presence (+) of recombinant DPP4 and analyzed by SELDI-TOF mass spectrometry. (**d**) WT mice were treated as described in **a**. Tumors were dissected at the indicated time points, and the number of infiltrating leukocytes was determined. Graphs represent fold change in tumor infiltrates upon sitagliptin treatment when compared with ctrl treatment (dashed line). Each circle represents a single mouse; *P < 0.05. P values were generated via Mann-Whitney test. Data are representative of 2 independent experiments (**a**) or are pooled from 2–3 independent experiments (**d**).*



Supplementary Figure 3

DPP4 inhibition enhances CXCL10-mediated antitumor responses to B16F10 melanoma.

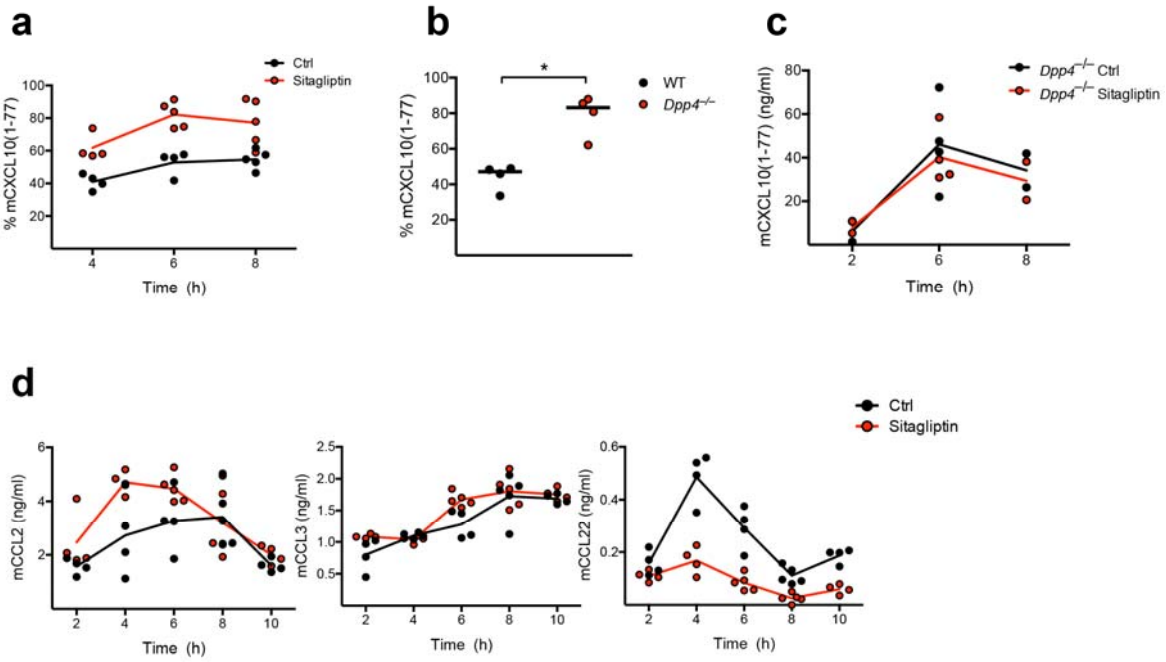
*(**a**–**d**) WT (**a, c** and **d**) and Ccr5$^{-/-}$ (**b**) mice fed with ctrl or sitagliptin chow were subcutaneously injected with B16F10 cells. Mice were treated with blocking antibodies to (**a**) mCCR5, (**c**) mCXCR4 or (**d**) mCXCL10 and compared to their respective isotype ctrl–treated animals. Tumor volumes are shown (data represent mean ± s.e.m.; n = 12 (**a**), 4 (**b**) and 6 (**c, d**) mice per group, *P < 0.05). Significance was determined using two-way ANOVA. Data are from 1 experiment (**c,d**), are representative of 2 independent experiments (**b**) or are pooled from 2 independent experiments (**a**).*



Supplementary Figure 4

DPP4 inhibition does not induce recruitment of B cells, eosinophils or regulatory T cells into CT26 tumors.

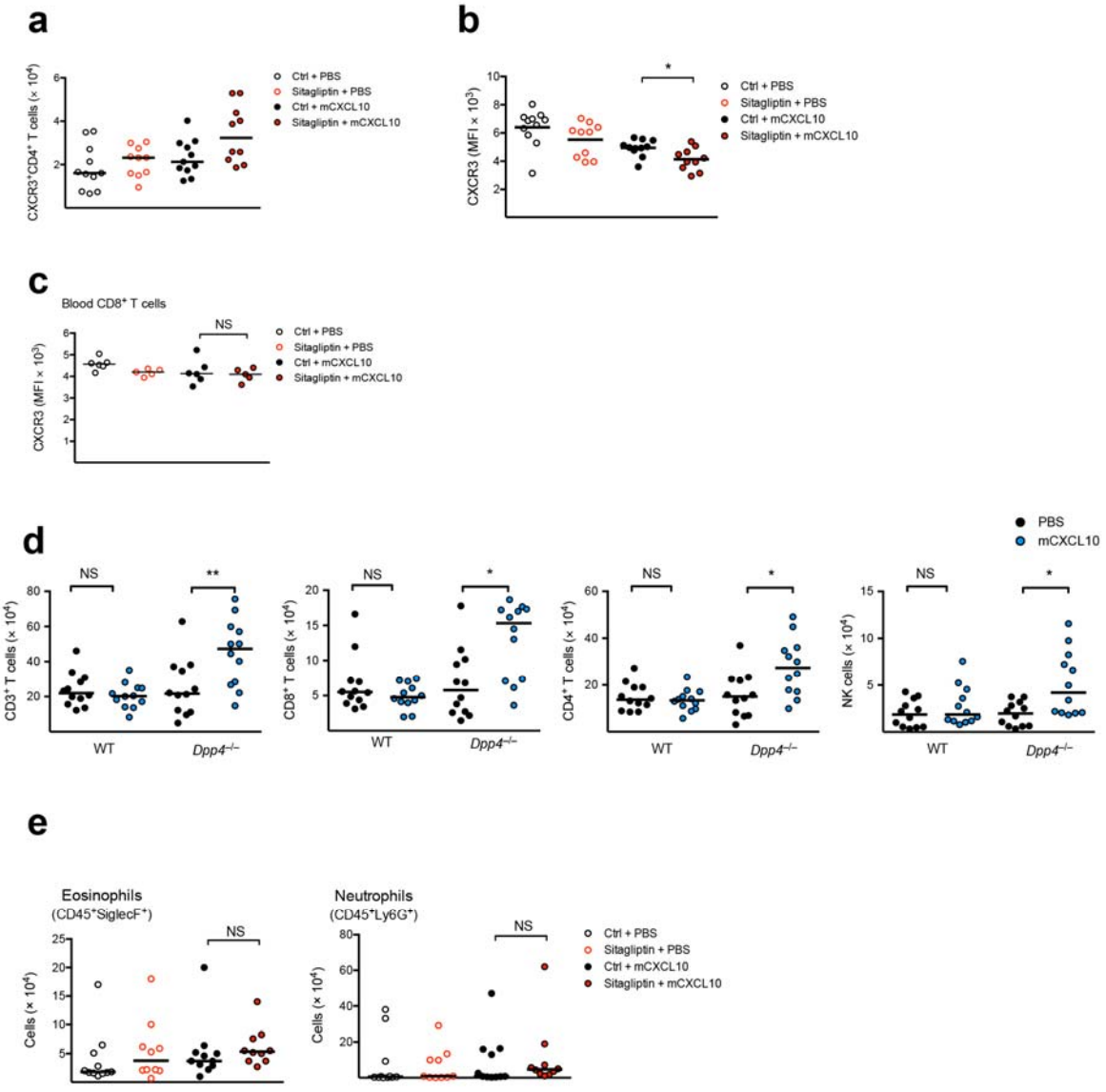
BALB/c WT mice fed with ctrl or sitagliptin chow were subcutaneously injected with CT26 cells. Tumors were dissociated on day 11, and the number of infiltrating leukocytes was analyzed. Significance was determined via Mann-Whitney test. Data are representative of 2 independent experiments.



Supplementary Figure 5

DPP4 expression modulates chemokine expression *in vivo*.

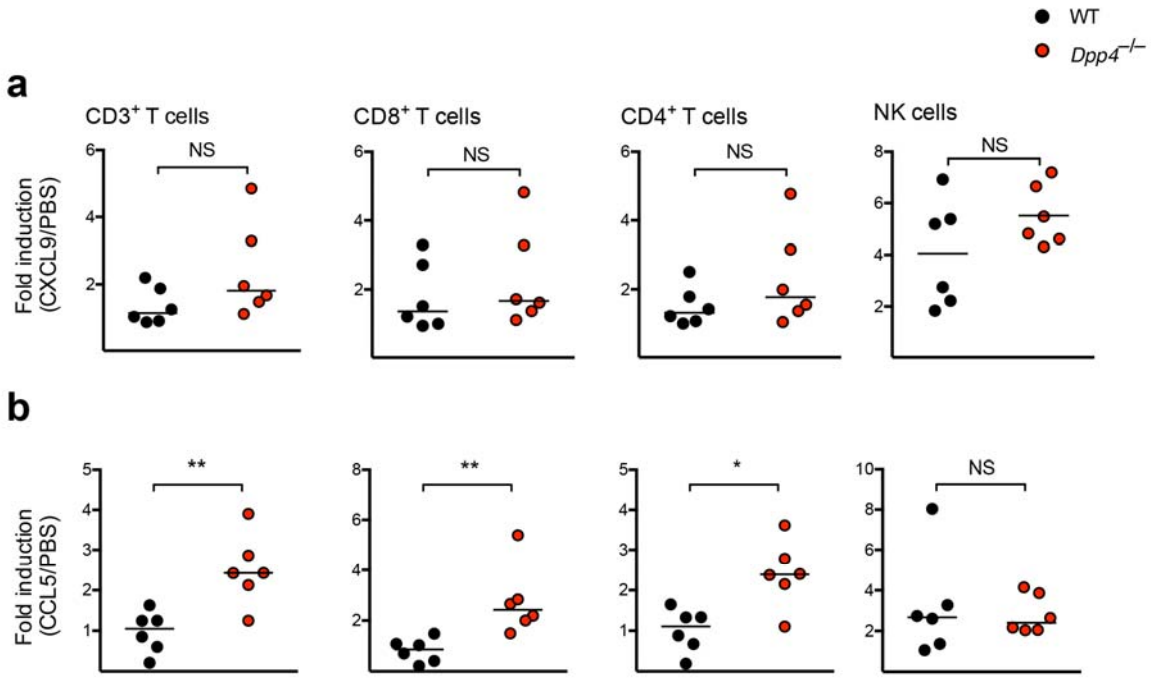
*WT (***a**,**d***) or Dpp4*$^{-/-}$ *(***c***) mice fed with sitagliptin or ctrl chow were injected intravenously with 5 μg of CpG-A. Graph represents percentage of mCXCL10(1–77) among total mCXCL10 determined in plasma samples at the indicated time points (6 h after CpG injection in* **b***). (***c***) Dpp4*$^{-/-}$ *mice fed with ctrl or sitagliptin chow were treated as described in* **a***. Quantification of mCXCL10(1–77) in plasma samples is shown. (***d***) Expression of mCCL2, mCCL3 and mCCL22 was determined in WT mice treated as described in* **a***. Each circle on the graphs represents a single mouse. Significance was determined using the Mann-Whitney test (*P < 0.05). Data are representative of 2 independent experiments.*



Supplementary Figure 6

DPP4 inhibition induces CXCL10-mediated leukocyte trafficking *in vivo*.

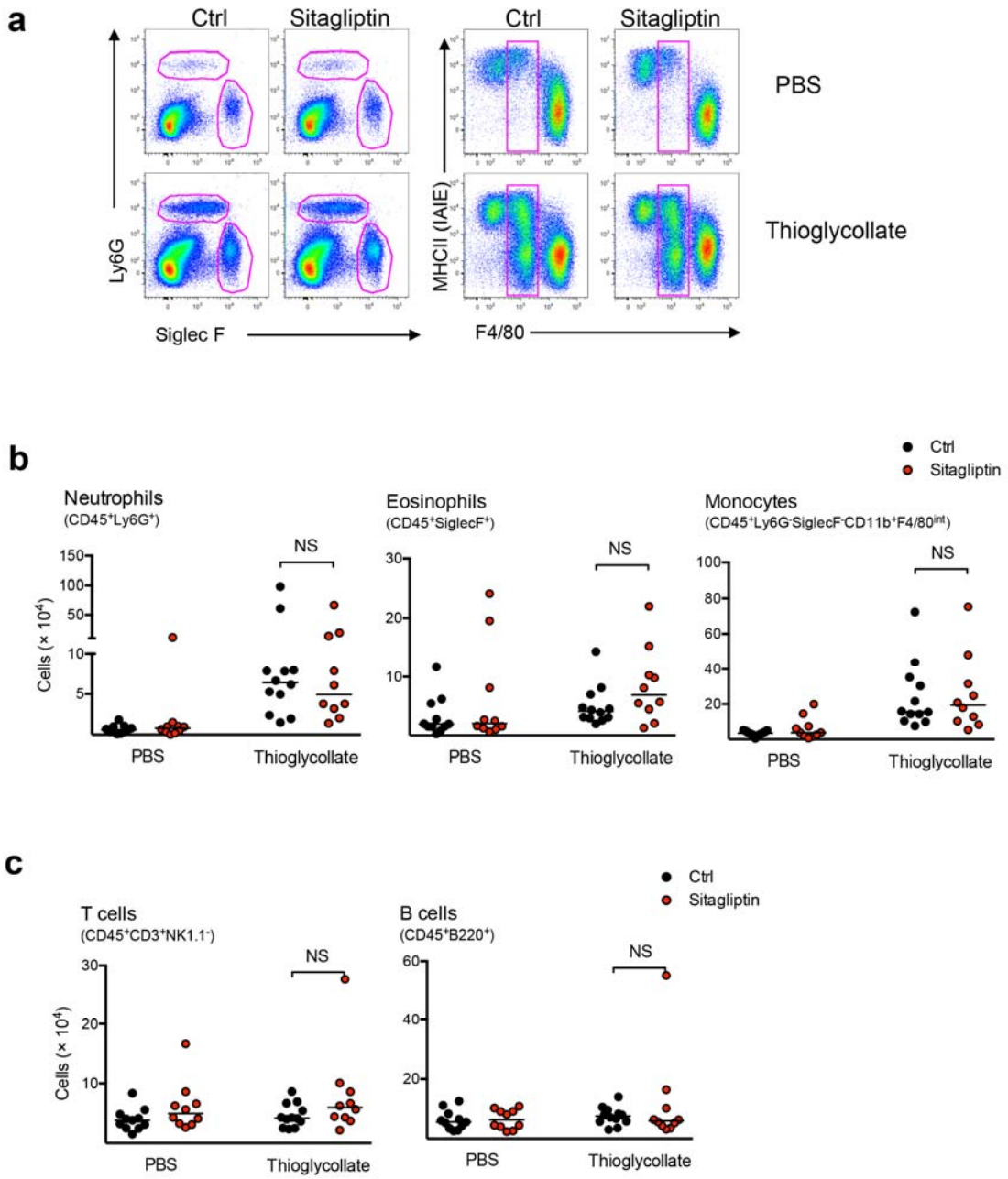
*WT mice fed with sitagliptin or ctrl chow were injected intraperitoneally with mCXCL10 or PBS (***a**–**c**, **e**). *Cellular contents in the peritoneal cavity were analyzed 12–14 h after injection. The number (**a**) and CXCR3 MFI (**b**) on CXCR3-expressing CD4$^+$ T cells were analyzed (*P < 0.05). (**c**) CXCR3 MFI of peripheral blood CXCR3$^+$CD8$^+$ T cells. (**d**) WT and Dpp4$^{-/-}$ mice were treated as described in **a**. The number of leukocytes in the peritoneal cavity was evaluated 6 h after mCXCL10 injection (*P < 0.05, **P < 0.01). (**e**) WT mice were treated as described in **a**, and the number of eosinophils and neutrophils in the peritoneal cavity was evaluated. Each circle represents one mouse; data were combined from 2 independent experiments. P values were generated via Mann-Whitney test; data are from 1 experiment (**c**) or are pooled from 2 (**a**,**b**,**e**) or 3 (**d**) independent experiments.*



Supplementary Figure 7

DPP4 regulates the chemotactic activity of DPP4-sensitive chemokines *in vivo*.

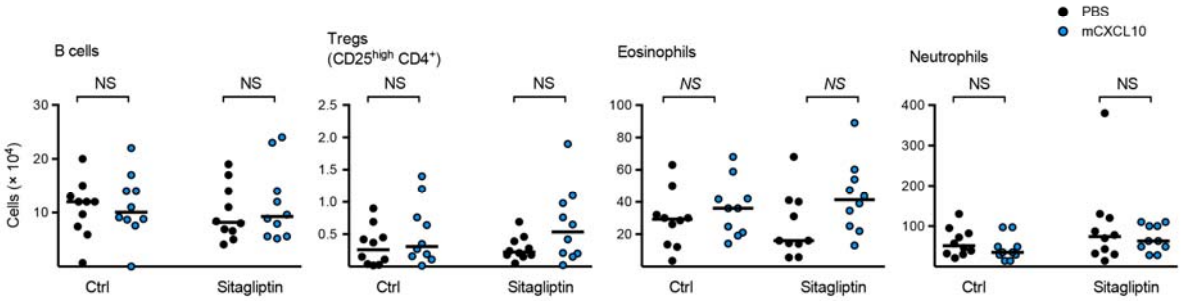
*WT and Dpp4$^{-/-}$ mice were injected via the intraperitoneal (IP) route with PBS, (***a***) 1 μg of mCXCL9 or (***b***) 1 μg of mCCL5. IP washes were collected 6 h after injection, and the number of leukocyte populations was analyzed. Fold induction of chemokine-mediated over PBS-mediated leukocyte trafficking was calculated. *P < 0.05, **P < 0.005. Each circle represents a single mouse. P values from Mann-Whitney test. Data are combined from 2 independent experiments.*



Supplementary Figure 8

DPP4 inhibition does not affect thioglycollate-mediated peritonitis.

*WT mice fed with sitagliptin or ctrl chow were injected intraperitoneally with thioglycollate. Cellular contents in the peritoneal cavity were analyzed 24 h after injection. (***a***) The gating strategy used for the identification of neutrophils, eosinophils and monocytes among peritoneal leukocytes is shown. (***b***,***c***) The cell number of infiltrating myeloid (***b***) and lymphoid (***c***) populations is indicated. P values from Mann-Whitney test. Data are combined from 2 independent experiments.*



Supplementary Figure 9

DPP4 inhibition does not affect the recruitment of myeloid cells in B16F10 tumors.

WT mice fed with ctrl or sitagliptin chow were subcutaneously injected with B16F10 tumor cells. Mice were given an intratumoral injection of PBS or mCXCL10 7 d after tumor-cell implant. The number of endogenous leukocytes was analyzed 12–14 h after intratumoral injection. Each circle represents a single mouse. P values from Mann-Whitney test. Data are combined from 2 independent experiments.