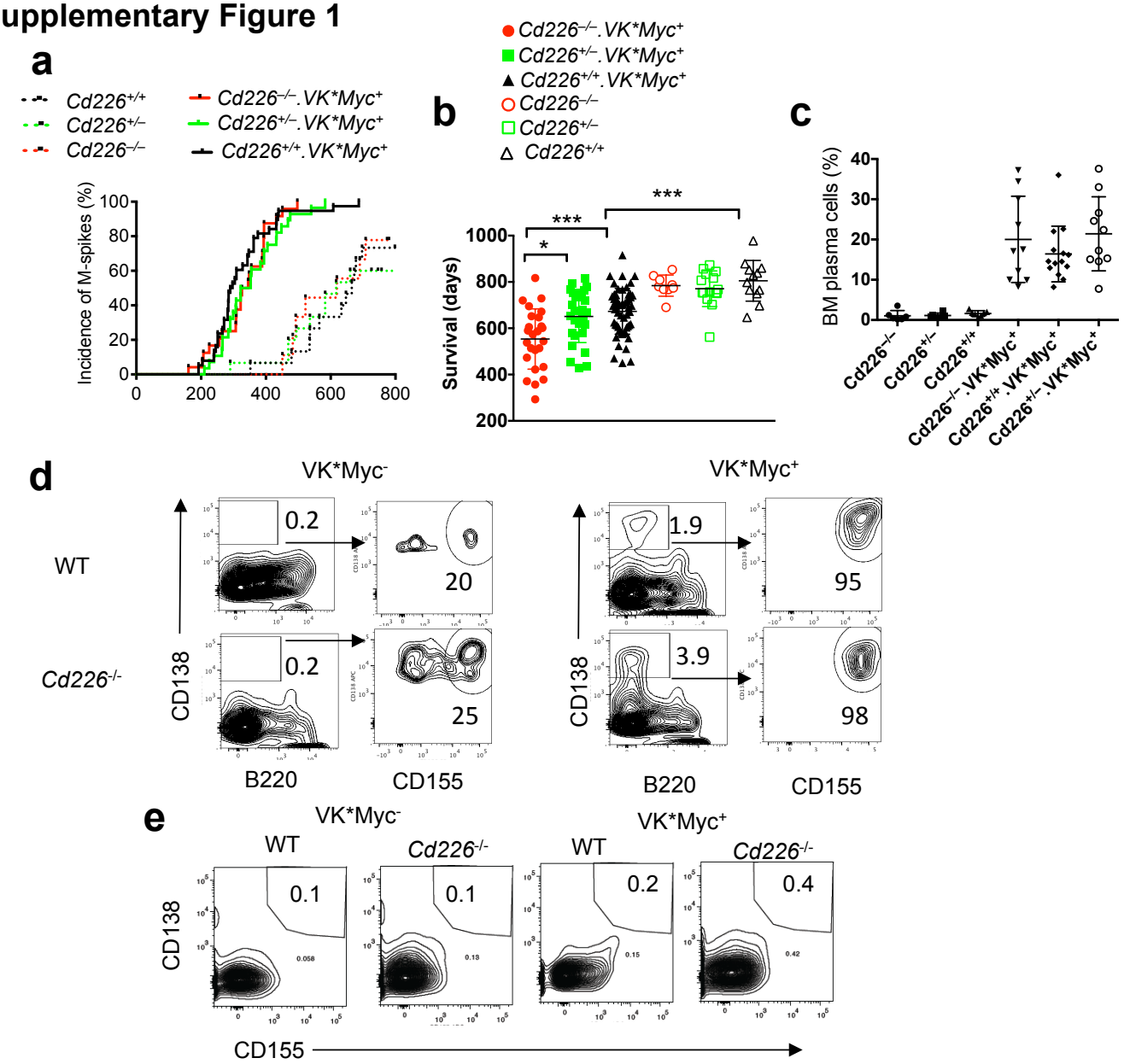
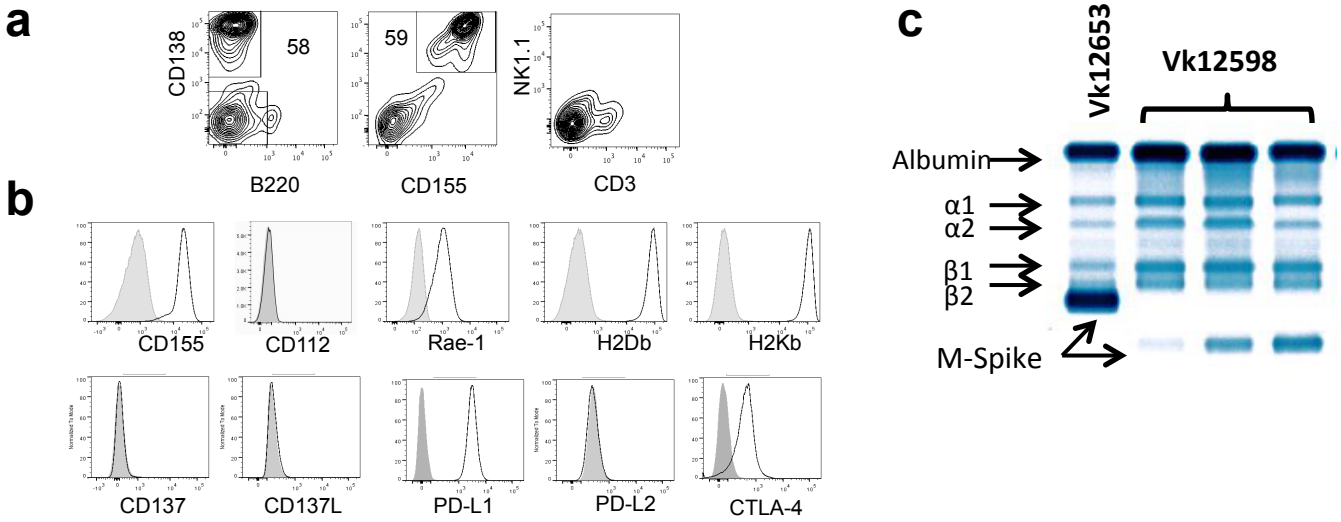
Supplementary Figure 1



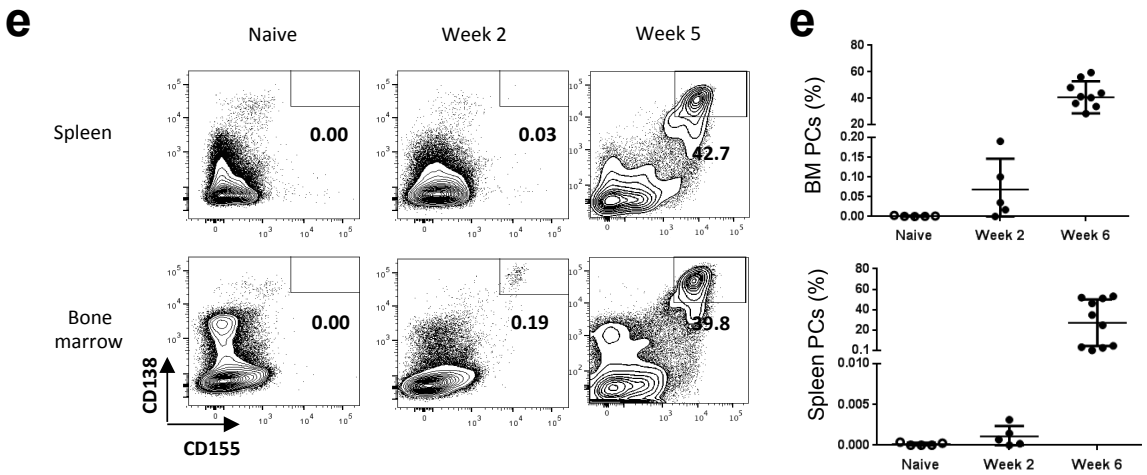
Supplementary Figure 1. a,b. C57BL/6 *Cd226*$^{+/+}$ (n=55), *Cd226*$^{–/+}$ (n=27) and *Cd226*$^{–/–}$ (n=25) Vk*MYC transgenic mice and *Cd226*$^{+/+}$ (n=12), *Cd226*$^{–/+}$ (n=15) and *Cd226*$^{–/–}$ (n=9) non-transgenic littermate controls were monitored for MM development (**a**) and survival (**b**). **a**. The absence of CD226 is not impacting spontaneous MM emergence in Vk*MYC transgenic mice. Graph showing the percentage of the indicated strains of mice with serum paraproteinemia over time. **b.** Histogram showing a significant decrease in the survival of *Cd226*$^{–/–}$ Vk*MYC mice as compared to WT and *Cd226*$^{–/+}$ Vk*MYC littermates. *p<0.05, ***p<0.001, Mann Whitney test. **c.** Graph showing the percentage of CD138^{+}B220^{-} PCs analysed by flow cytometry in the BM of the indicated group of mice at necropsy. **d**. The expansion of plasma cells CD138^{+}B220^{-} expressing CD155^{+} is mainly restricted to the BM in Vk*MYC mice. Representative FACS plots showing the percentage of CD138^{+}B220^{-} PCs and their level of CD155 expression in the bone marrow of *Cd226*$^{+/+}$ (n=15) and *Cd226*$^{–/–}$ (n=14) Vk*MYC transgenic mice and in *Cd226*$^{+/+}$ (n=6) and *Cd226*$^{–/–}$ (n=10) non-transgenic littermate controls at 450 days of age. **e.** Representative FACS plot showing the frequency of CD138^{+}CD155^{+} malignant plasma cells in the spleen of the same strains of mice at 450 days of age.

Supplementary Figure 2



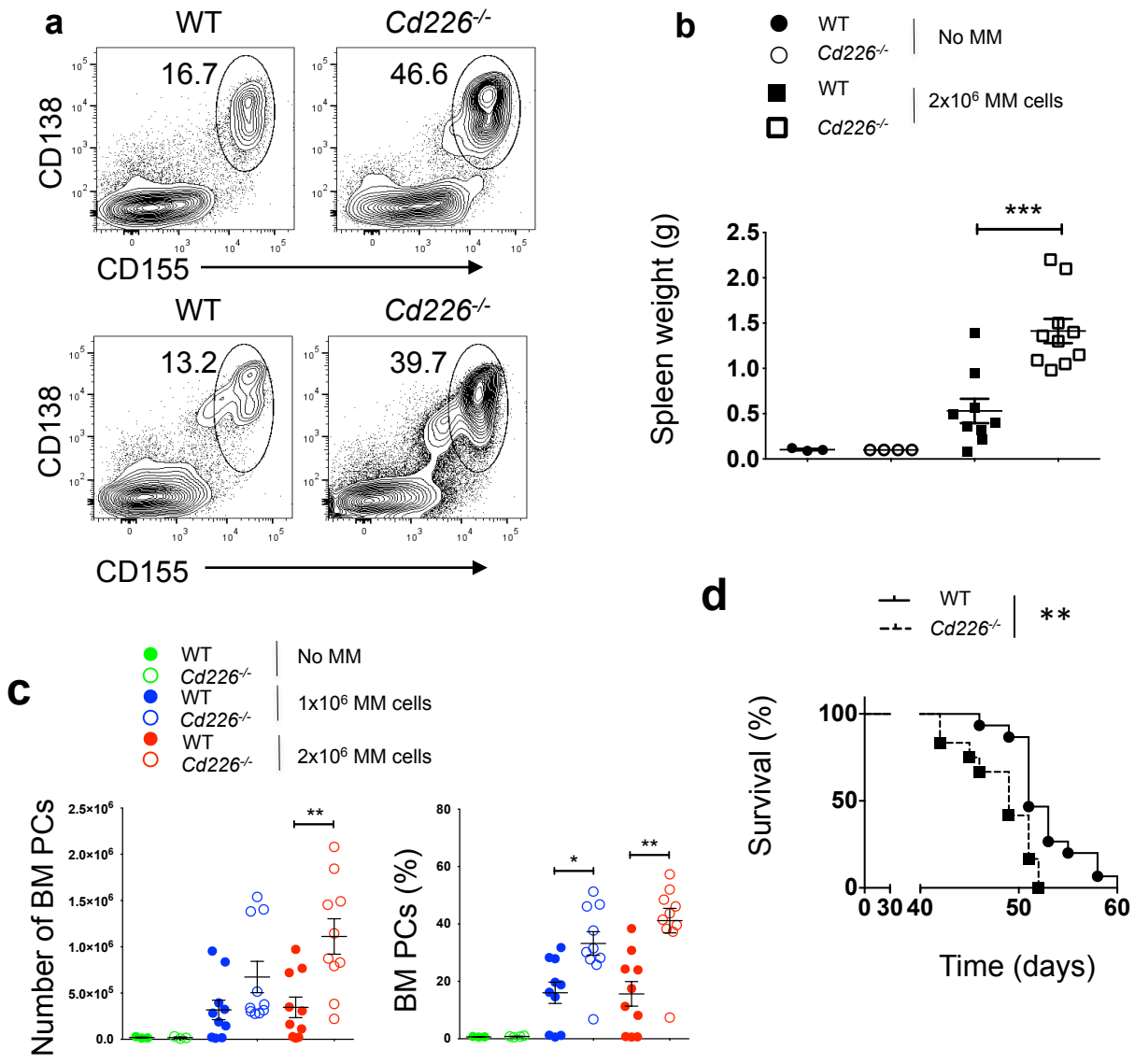
d

alias	chr	start	stop	strand	VkMYC 1	VkMYC 2	VkMYC 3	Vk12598
CD155	7	20488927	20506493	-	7.98	9.43	9.37	6.57
CD112	7	20301993	20334923	-	2.35	0.06	1.32	1.73
CD137L	17	57244808	57247181	+	0.14	0.71	0.07	0.56
CD137	4	150294264	150320212	+	0.05	0.32	0.00	0.05
CTLA-4	1	60965869	60972675	+	0.11	6.44	0.20	0.00
DNAM1	18	89366819	89439720	+	0.06	0.01	0.00	0.00
PD-1	1	95934882	95949131	-	0.07	0.27	0.02	0.42
PD-L2	19	29485409	29547418	+	0.14	0.66	0.05	0.00
PD-L1	19	29441928	29462585	+	7.59	18.59	22.99	8.21



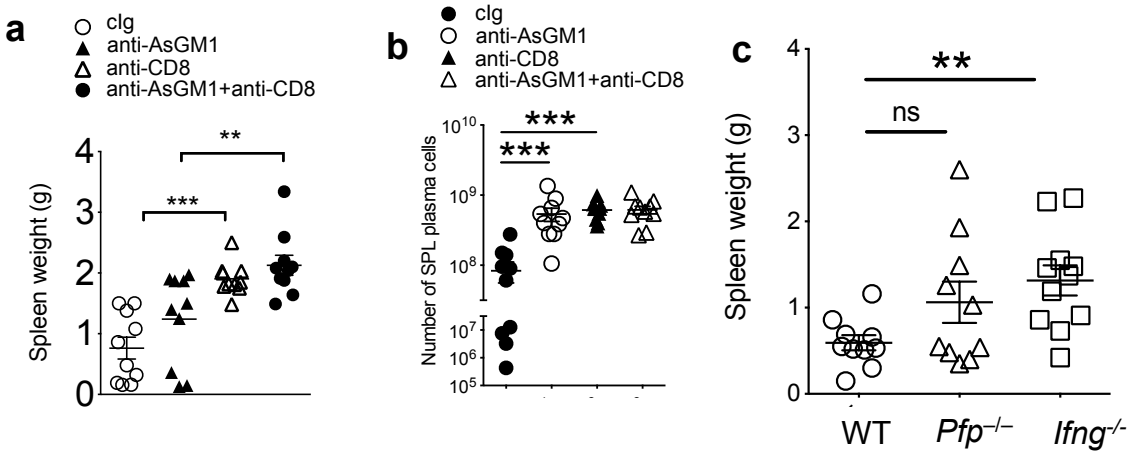
Supplementary Figure 2 : Transplantable VKMYC model.
a-b. 2 x 10^6 Vk12653 cells were injected i.v into 10 *Rag2*$^{-/-}$*ilr2g*$^{-/-}$ mice. **a.** Representative FACS plot showing the percentages and the phenotype of malignant plasma cells in the spleen from *Rag2*$^{-/-}$*ilr2g*$^{-/-}$ mice 5 weeks after injection. **b.** Representative FACS plot showing the expression of the indicated markers (open line) on VK12653 CD138^{+}B220^{-} plasma cells as compared to isotype control staining (filled line). **c.** Representative m-spikes obtained with the Vk12653 and Vk12598 cell lines **d.** Table showing the total expression value of the indicated genes analyzed by RNA sequencing in CD138^{+}B220^{-} plasma cells purified from 3 different MM bearing VK*MYC transgenic mice (VKMYC1-3) or VK12598 MM cells. **e.** Representative FACS plot and graphs showing the percentages of malignant plasma cells in the spleen and the BM of WT mice 2 and 6 weeks after Vk12653 MM cell injection.

Supplementary Figure 3



Supplementary Figure 3 : CD226 and tumor CD155 is required for the immune-control of MM. **a-c.** WT or *Cd226*$^{-/-}$ mice were challenged i.v with 2x10^6 (**a, b, c**) or 1x10^6 **(c)** Vk12653 MM cells and the number and the percentage of malignant PCs was determined by flow cytometry in the spleen and in the BM 5 weeks after injection. **a**. Representative FACS plots showing the frequency of CD138$^+$ CD155$^+$ malignant PCs in the bone marrow (*bottom*) and the spleen (*top*) of the indicated strains of mice. **b.** Spleen weight of the indicated groups of mice as in **a**. Representative experiment out of 4 involving groups of n=10 mice. **c.** Graphs showing the mean percentage and number of B220$^-$CD138$^+$ PCs in the bone marrow of the indicated strains of mice. Each symbol represents one individual mouse. **d.** WT or *Cd226*$^{-/-}$ mice were challenged i.v with or 1x10^6 Vk12653 MM cells and the survival was monitored. Representative experiment involving groups of n=10 mice. *p<0.05, **p<0.01, ***p<0.001. Mann Whitney test (**b, c**) and Mantel Cox test (**d**) .

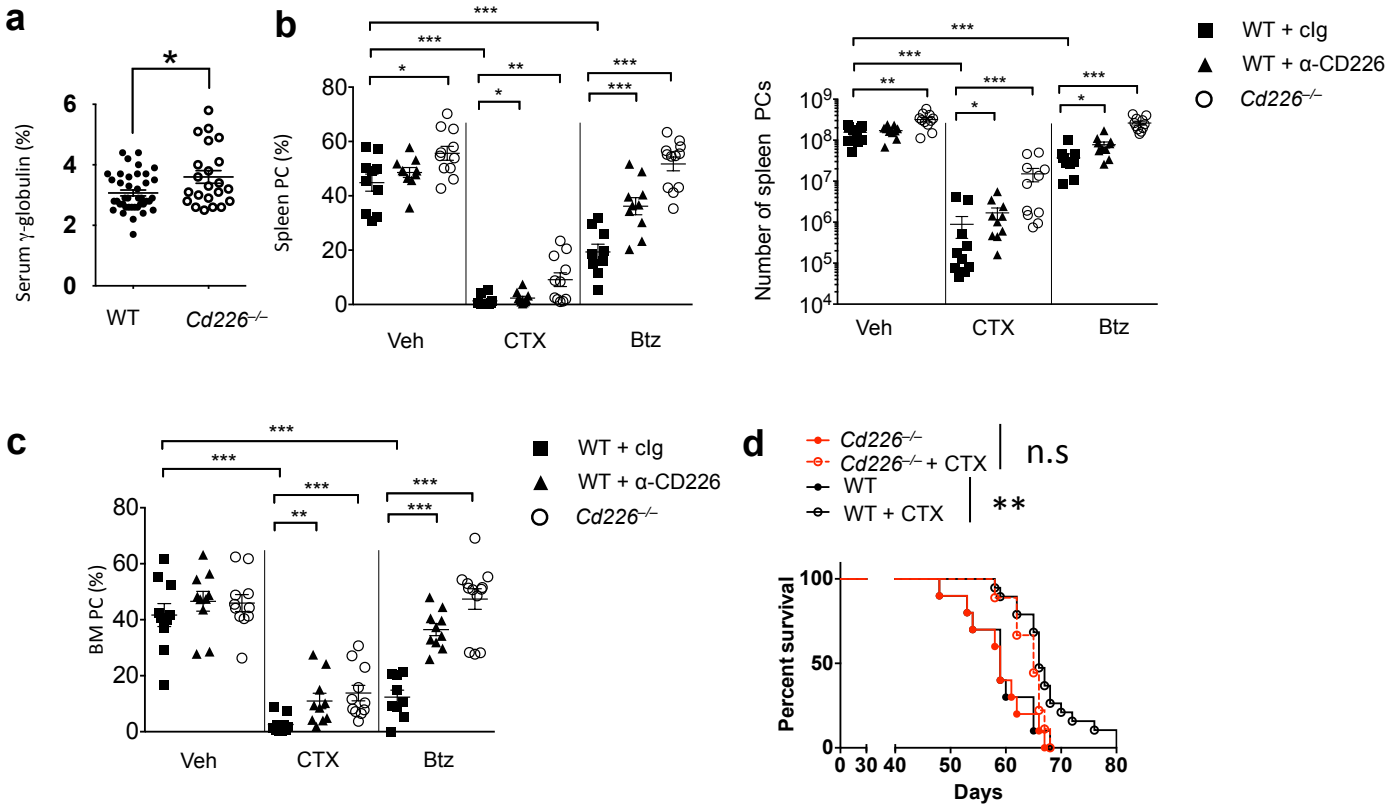
Supplementary Figure 4



Supplementary Figure 4: NK cells and CD8⁺ T cells have non-redundant anti-myeloma activity through perforin and IFN-γ pathways.

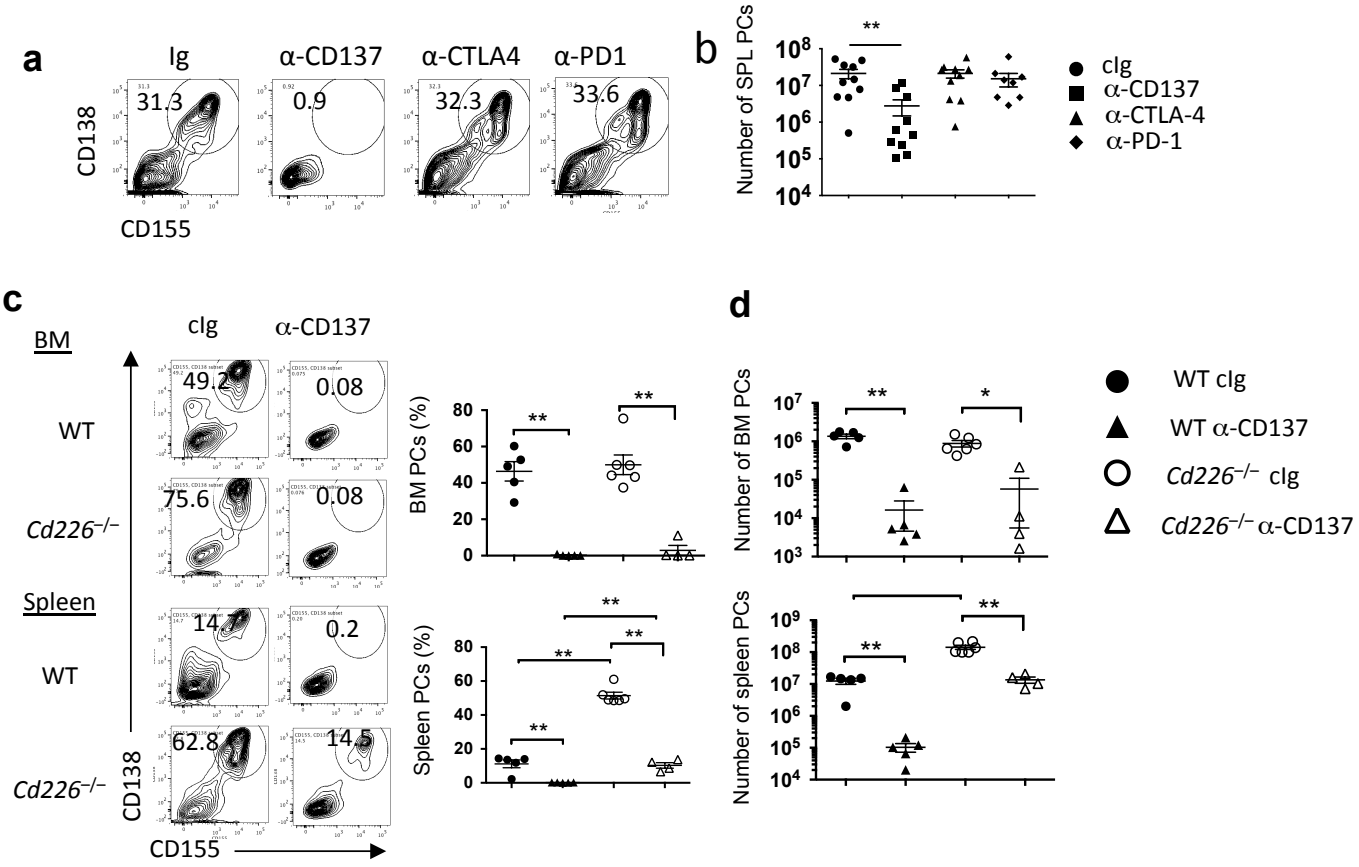
a-b. WT mice were injected with control IgG, anti-AsGM1 and/or anti-CD8 to deplete NK cells and/or CD8⁺ T cells, and were challenged with Vk12653 cells. The spleen weight (**a**) and the number of PCs in the spleen (**b**) were measured 5 weeks after injection. Representative experiments out of 2 involving groups of n=10 mice. Each symbol represents one individual mouse. **c.** WT, *Pfp*$^{-/-}$ and *Ifng*$^{-/-}$ mice were injected i.v with 2 x 10^6 Vk12653 cells. The spleen weight was measured 5 weeks after injection. Representative experiments out of 2 involving groups of n=10 mice. Each symbol represents one individual mouse. ns $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$. Mann Whitney test.

Supplementary Figure 5



Supplementary Figure 5 : Optimal anti-myeloma therapy requires CD226.
(a-c) WT and *Cd226*$^{-/-}$ mice were injected i.v with 2 x 10^6 Vk12653 MM cells. After 3 weeks, mice were injected either with clg or anti-CD226 mAbs and were subsequently treated with PBS, Btz (i.p; 0.5 mg/kg) or CTX (i.p; 50 mg/kg) twice a week. **a**. γ-globulin levels in the serum of WT and *Cd226*$^{-/-}$ mice before therapy 3 weeks after MM injection. **b-c**. graphs showing the percentages and the numbers of CD138$^+$CD155$^+$ PCs in the spleen (**b**) and in the BM (**c**) of the indicated groups of mice at the end of the treatment. Data are pooled from two experiments. Each symbol represents one individual mouse. **d**. WT and *Cd226*$^{-/-}$ mice were injected i.v with 2 x 10^6 Vk12653 MM cells. After 3 weeks, mice were treated with PBS or CTX (i.p; 20 mg/kg) twice a week and the survival of the indicated groups of mice was monitored. Experiment involving groups of n=10 mice. *p<0.05, **p<0.01. Mann Whitney test. The vehicle groups displayed in part c have been presented in Figure 6H and are derived from the same set of experiments.

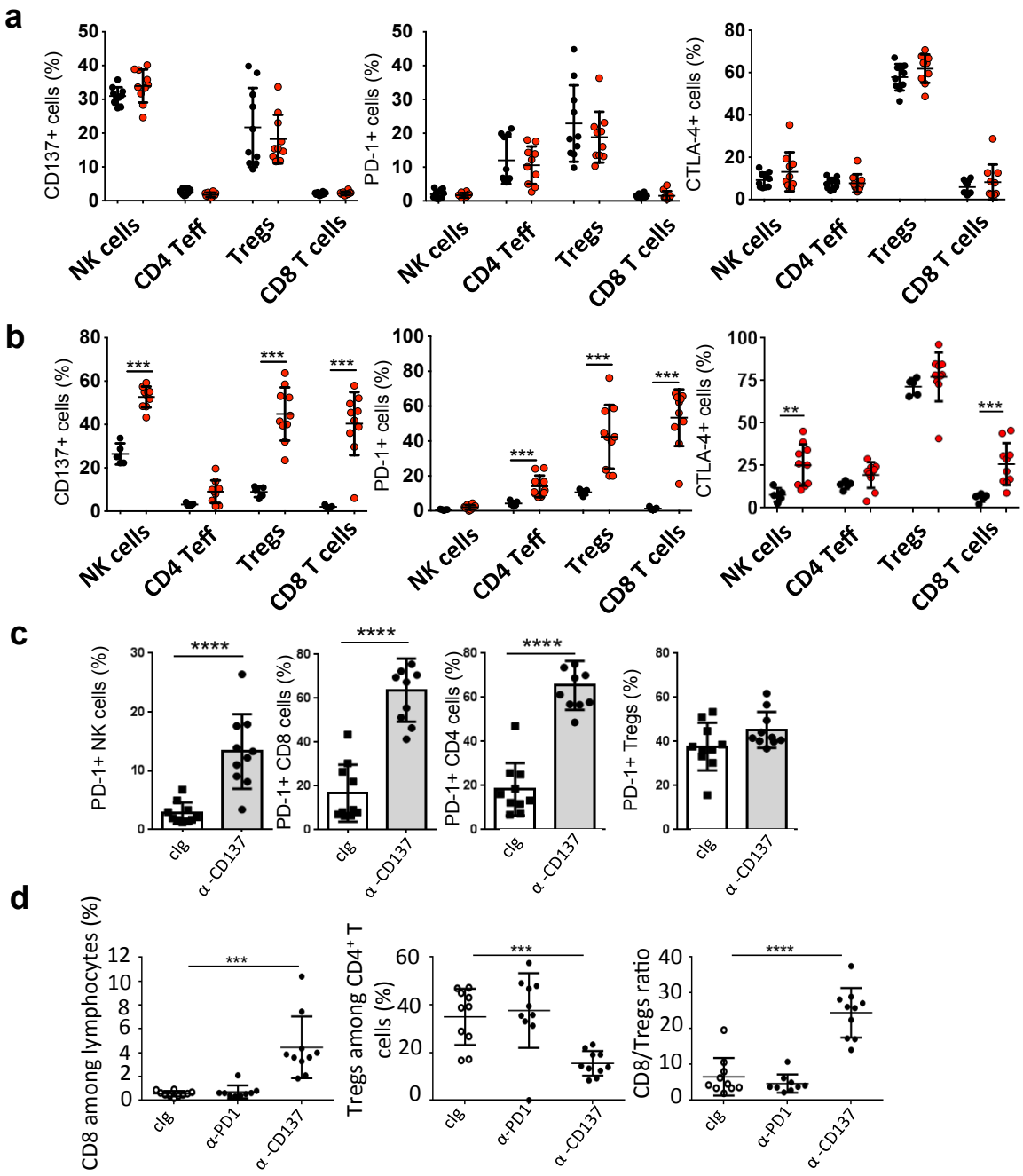
Supplementary Figure 6



Supplementary Figure 6. Therapeutic efficacy of anti-CD137 immunotherapy against myeloma.

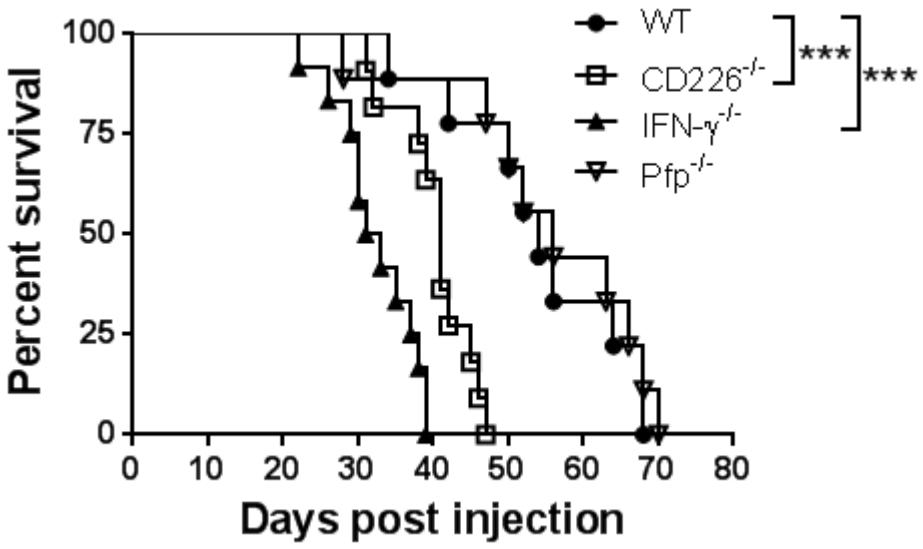
(a-b) WT mice were injected with control Ig, anti-CD137, anti -PD-1 or anti-CTLA-4 as described in the Material and Methods and subsequently challenged with 2 x 10^6 Vk12653 MM cells. Representative FACS plot showing the percentages (**a**) and the numbers (**b**) of malignant $CD138^+CD155^+$ PCs in the spleen of the indicated groups of mice at the end of the treatment. Data are representative of two independent experiments involving groups of n=10 mice. Each symbol represents one individual mouse. **c-d** WT or *Cd226*$^{-/-}$ mice were injected with cIg or anti-CD137 and then challenged with 2 x 10^6 Vk12653 MM cells. **c,d**. Representative FACS plot and graph showing the percentages (**c**), the mean numbers (**d**) ± SEM of malignant $CD138^+CD155^+$ PCs in the spleen and in the BM of the indicated groups of mice at the end of the treatment. Data are representative of two independent experiments involving groups of n=5 mice. *p<0.05, **p<0.01, ***p<0.001. Mann Whitney test.

Supplementary Figure 7



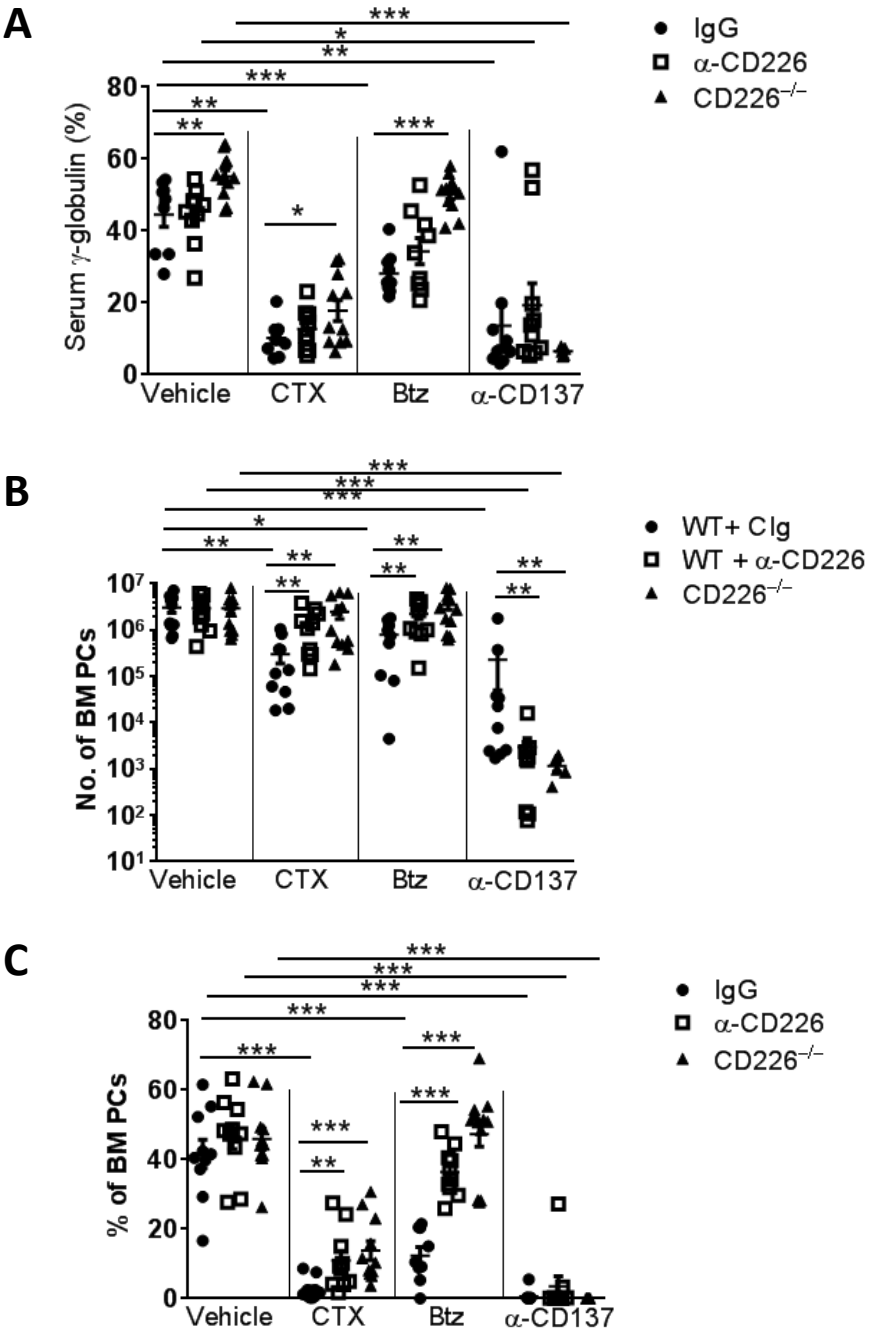
Supplementary Figure 7. Mechanism of anti-CD137 immunotherapy against myeloma. **(a-b)** WT mice were injected with with 2 x 10^6 Vk12653 MM cells. Graphs are showing the percentages of CD137$^+$, PD-1$^+$ or CTLA-4$^+$ cells among the indicated populations of lymphocytes within the BM of WT mice 2 weeks (**a)** or 6 weeks after MM cells injection (**b**). Data are representative of two independent experiments involving groups of n=5-10 mice. Each symbol represents one individual mouse. **c-d.** Mice were injected i.v with 2 x 10^6 Vk12653 MM cells. After 3 weeks, mice with established MM were injected either with IgG, anti-CD137 or anti-PD-1 mAbs. **c**. Graphs are showing the percentages ± SEM of PD-1$^+$ cells among the indicated populations of lymphocytes within the BM of WT mice 2 weeks after the beginning of the indicated treatment. Experiment involving groups of n=10 mice. **d.** Graphs are showing the percentage of the indicated populations of immune cells at the end of the indicated treatment. Data are pooled from two independent experiments. *p<0.05, **p<0.01, ***p<0.001. Mann Whitney test.

Supplementary Figure 8



Supplementary Figure 8. WT mice, CD226$^{-/-}$ mice, IFN-γ$^{-/-}$ mice and pfp$^{-/-}$ mice were injected with 4x10^5 Vk12598 cells and survival was monitored over time. Data displayed here have already been shown in Figures 2E (WT and CD226$^{-/-}$ mice) and 3F (WT and IFN-γ$^{-/-}$ and pfp$^{-/-}$ mice) and are from one experiment with n=9-12 mice per group. *** p<0.001; Mantel-Cox test.

Supplementary Figure 9



Supplementary Figure 9. WT mice and CD226$^{-/-}$ were injected with 2x10^6 Vk12653 cells. After 3 weeks, mice were treated with control IgG (CIg) or α-CD226 blocking antibodies and subsequently treated with PBS (vehicle), CTX, Btz or α-CD137. Graphs show the % of serum γ-globulin (A), the numbers (B) and the % (C) of malignant CD138$^+$CD155$^+$ PCs in the BM of the indicated groups after 3 weeks of treatment. Data are pooled from 2 independent experiments. Data were already presented in Figures 5B and 6D (A), Figures 5D, 6E and 6H (B) and Figures 6H and Supp S5C (C). All the groups from the same set of experiments are shown together here. *P<0.05, **P<0.01, ***P<0.001; Mann-Whitney U test.