Supplementary Information

Synergy of radiotherapy and PD-1 blockade in *Kras*-mutant lung cancer

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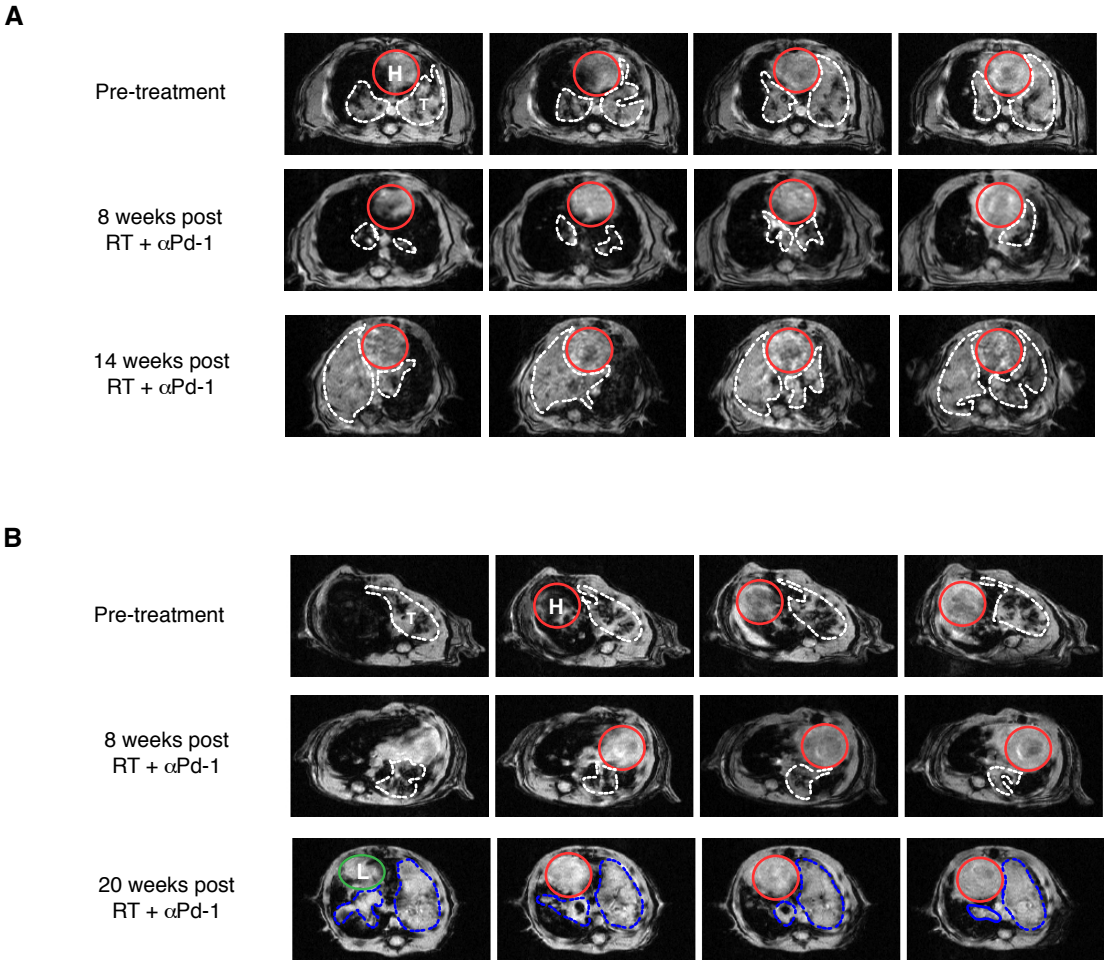
Supplementary Figure 1 – 5

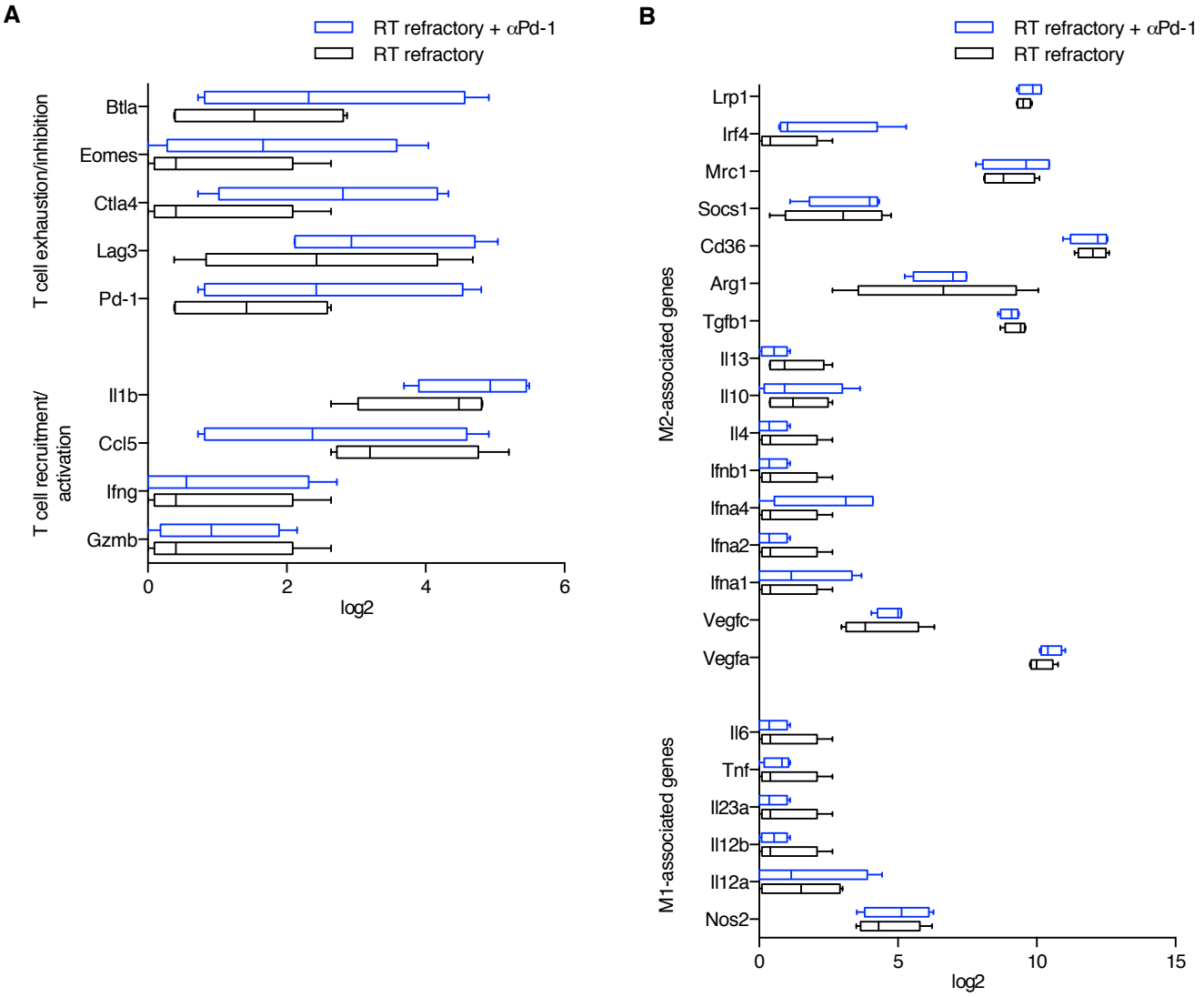
Supplementary Tables

Supplementary Figure 1



Supplementary Figure 1
Tumor growth kinetics in response to RT and αPd-1 treatment in *Kras*-mutant murine NSCLC. (**A**) Representative MR images of a responsive (left lung) *Kras*-driven tumor at different time points (baseline, 8, and 14 weeks post treatment initiation). Contralateral tumor growth in the right lung (n=7). (**B**) Representative MR images of a resistant (left lung) *Kras*-driven tumor at different time points (baseline, 8, and 20 weeks post treatment initiation) (n=3). H (heart) circled in red. L (liver) circled in green. T (tumor) circled with white/blue dotted line.

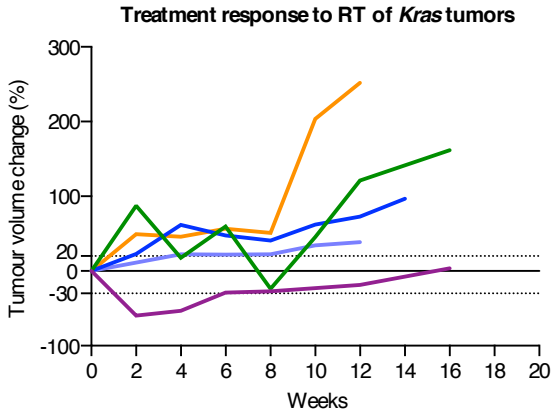
Supplementary Figure 2



Supplementary Figure 2

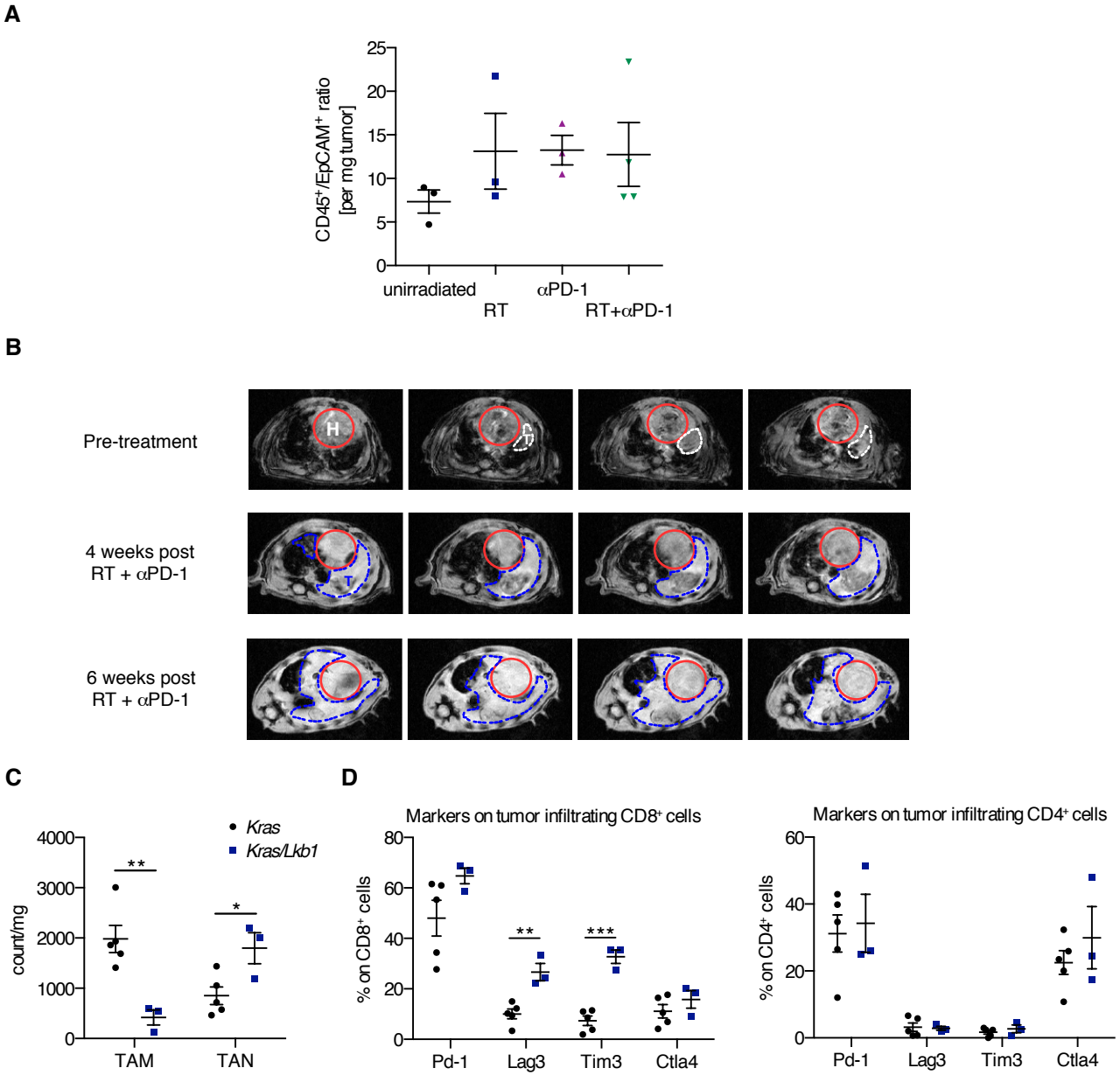
Treatment of RT-refractory *K* tumors with αPd-1 induces marker of T cell inhibition. (**A**) Expression of selected T cell-associated genes in RT-relapsed and RT-relapsed αPd-1-treated tumors. (**B**) Expression of selected macrophage-associated genes in RT-relapsed and RT-relapsed αPd-1-treated tumors. Representative data are shown from mouse nCounter PanCancer Immune Profiling Panel conducted with RNA from 4 RT-treated and 4 RT-refractory αPd-1-treated mice (**A**, and **B**). Data are represented as mean ± SEM. Btla, B- and T-lymphocyte attenuator; Eomes, Eomesodermin; Il1b, Interleukin 1 beta; Ccl5, Chemokine (C-C motif) ligand 5; Ifng, Interferon gamma; Gzmb, granzyme B; Lrp1, Low density lipoprotein receptor-related protein 1; Irf4, Interferon regulatory factor 4; Mrc1, Mannose receptor, C Type 1; Socs1, Suppressor of cytokine signaling 1; CD36, CD36 Molecule/Thrombospondin receptor; Arg1, Arginase 1; Tgfb1, transforming growth factor beta-1; Il13, Interleukin 13; Il10, Interleukin 10; Il4, Interleukin 4; Ifnb1, Interferon beta 1; Ifna4, Interferon alpha 4; Ifna2, Interferon alpha 2; Ifna1, Interferon alpha 1; Vegfc, Vascular endothelial growth factor C; Vegfa, Vascular endothelial growth factor A; Il6, Interleukin 6; Tnf, Tumor necrosis factor; Il23a, Interleukin 23 alpha; Il12b, Interleukin 12 beta; Il12a, Interleukin 12 alpha; Nos2, Nitric oxide synthase 2.

Supplementary Figure 3



Supplementary Figure 3
Tumor growth kinetics in response to RT in *Kras*-mutant murine NSCLC. Tumor volume kinetics of RT-treated tumors. Each line represents one mouse (n=5). Data of this cohort was previously published (Herter-Sprie et al., 2014).

Supplementary Figure 4

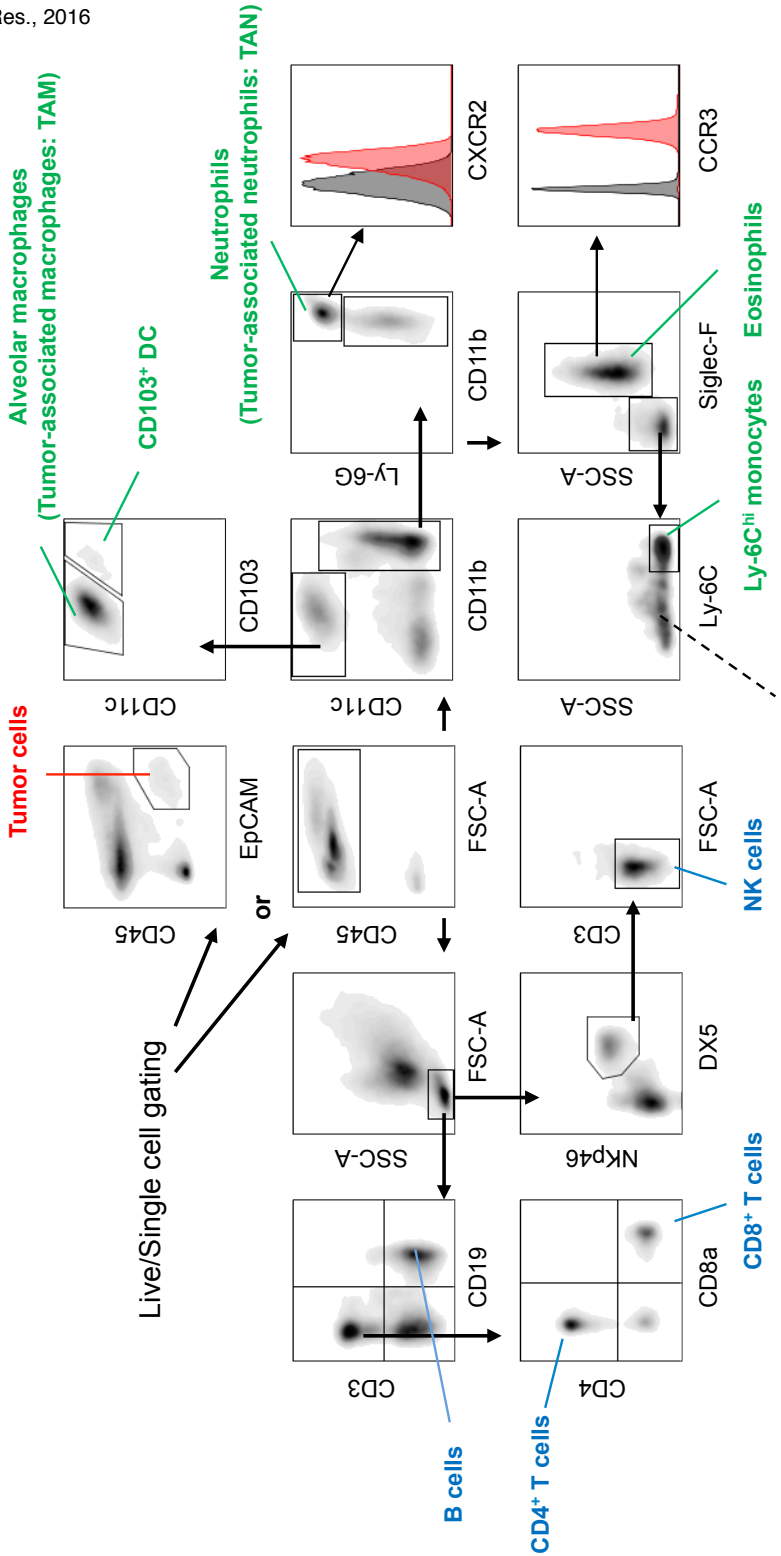


Supplementary Figure 4

Tumor growth kinetics in response to RT and αPD-1 treatment in *Kras/Lkb1*-mutant murine NSCLC and differences in tumor-associated immune cell populations compared to *Kras*-mutant murine NSCLC. (**A**) CD45⁺/EpCAM⁺ ratio calculated from Figure 5B (3 unirradiated, 3 RT-treated, 3 αPD-1-treated, and 4 RT+αPD-1-treated mice). (**B**) MR images of a progressive *Kras/Lkb1* tumor at different time points (baseline, 4, and 6 weeks post treatment initiation) (n=2). H (heart) circled in red. T (tumor) circled with white/blue dotted line. (**C**) Representative flow cytometry data (live/single/total CD45⁺ cells). Total numbers of tumor-infiltrating myeloid cells of unirradiated *Kras* and *Kras/Lkb1* tumors (taken from Figure 2B and Figure 5B). (**D**) Expression of inhibitory T cell markers on CD8⁺ and CD4⁺ T cells of unirradiated *Kras* and *Kras/Lkb1* tumors (taken from Figure 2E and Figure 5D). Representative data are shown conducted with 5 unirradiated *Kras*, and 3 unirradiated *Kras/Lkb1* mice (**C** and **D**). Data are represented as mean ± SEM. *P* values were calculated using two-tailed Student's *t*-test. $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$.

Supplementary Figure 5

adapted from Koyama et al., Cancer Res., 2016



Supplementary Table 1

Antigen	Clone	Source	Antigen	Clone	Source
CD45	30-F11	BioLegend	Siglec F	E50-2440	BD Biosciences
CD3ε	145-2C11	BioLegend	PD-1	29F.1A12	BioLegend
CD4	RM4-5	BioLegend	TIM-3	RMT3-23	BioLegend
CD8a	53-6.7	BioLegend	LAG-3	C9B7W	BioLegend
CD19	6D5	BioLegend	CD103	2E7	BioLegend
DX5	DX5	BioLegend	PD-L1	10F.9G2	BioLegend
NKp46	29A1.4	BioLegend	EpCAM	G8.8	BioLegend
CD11c	N418	BioLegend	CD16/32	2.4G2	BioLegend
CD11b	M1/70	BioLegend			
Ly-6G	1A8	BioLegend	FOXP3	FJK-16s	eBioscience
Ly-6C	HK1.4	BioLegend	CTLA-4	UC10-4B9	eBioscience

Supplementary Table 1

List of murine antibodies used for flow cytometry analysis.