

## Supplementary Text

### Materials and Methods

**Mice.** C57BL/6 wild-type (WT) (obtained from Walter and Eliza Hall Institute, Melbourne, Australia), C57BL/6 TCR $\delta$ -deficient (TCR $\delta^{-/-}$ ), C57BL/6 Ja281-deficient (Ja281 $^{-/-}$ ), C57BL/6 RAG-1-deficient (RAG-1 $^{-/-}$ ) and C57BL6-Ighm deficient (mMT) mice were bred and maintained as previously described<sup>1,2</sup>. Mice 6-14 weeks of age were used in all experiments that were performed according to Peter Mac animal experimental ethics committee guidelines. Some mice were depleted of T cell subsets with anti-CD4 (GK1.5) and anti-CD8 $\beta$  (53.5.8) as previously described<sup>3</sup>.

### Cell lines

MALME-3M, A375, SK-MEL-2, SK-MEL-24, SK-MEL-28, WM266.4, MeWo and HS294T were obtained from the American Tissue Culture Collection and D04-M1 cells were obtained from the Queensland Institute for Medical Research. The BRAF and NRAS mutation status of these cell lines has been reported previously<sup>4,5</sup>. All cell lines were maintained in RPMI 1640 containing 10% FBS, 2 mM L-alanyl-L-glutamine, 1% penicillin/streptomycin and 250 ng/ml amphotericin B in a 37°C humidified, 5% CO<sub>2</sub> incubator.

**Statistical analysis.** Data were analyzed with GraphPad Prism (version 5) software (GraphPad Software, La Jolla, CA). Significant differences between groups were assessed by a two-tailed *t* test, Mann-Whitney *U* test, or Kruskal Wallis test as indicated. Values of *P* < 0.05 were considered significant.

## References

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2. Paget C, Chow MT, Duret H, Mattarollo SR, Smyth MJ. Role of gammadelta T cells in alpha-galactosylceramide-mediated immunity. *J Immunol.* Apr 15 2012;188(8):3928-3939.
3. Koebel CM, Vermi W, Swann JB, et al. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature.* Dec 6 2007;450(7171):903-907.
4. Ikediobi ON, Davies H, Bignell G, et al. Mutation analysis of 24 known cancer genes in the NCI-60 cell line set. *Mol Cancer Ther.* Nov 2006;5(11):2606-2612.
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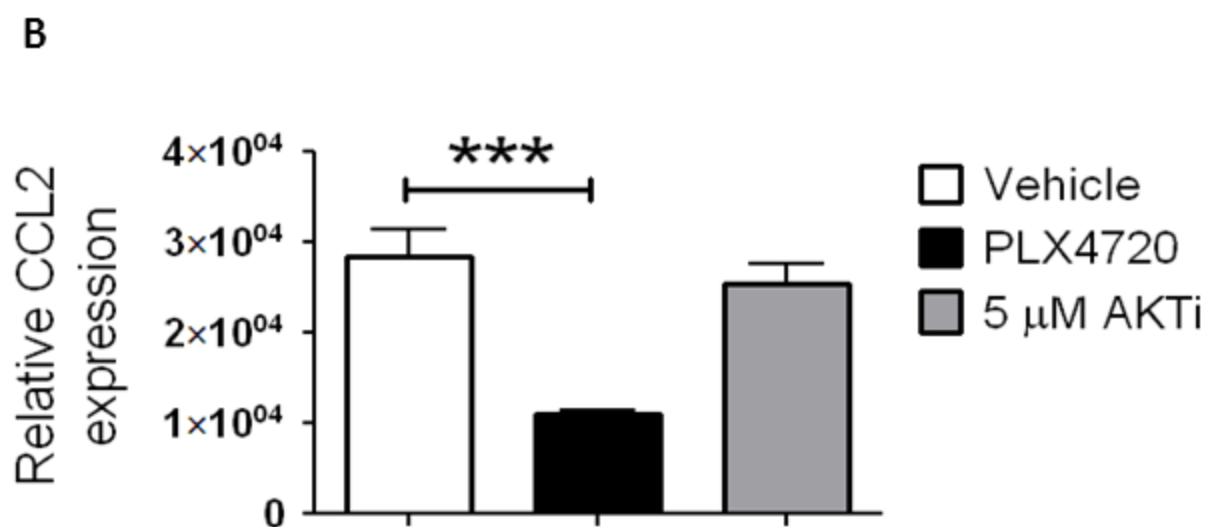
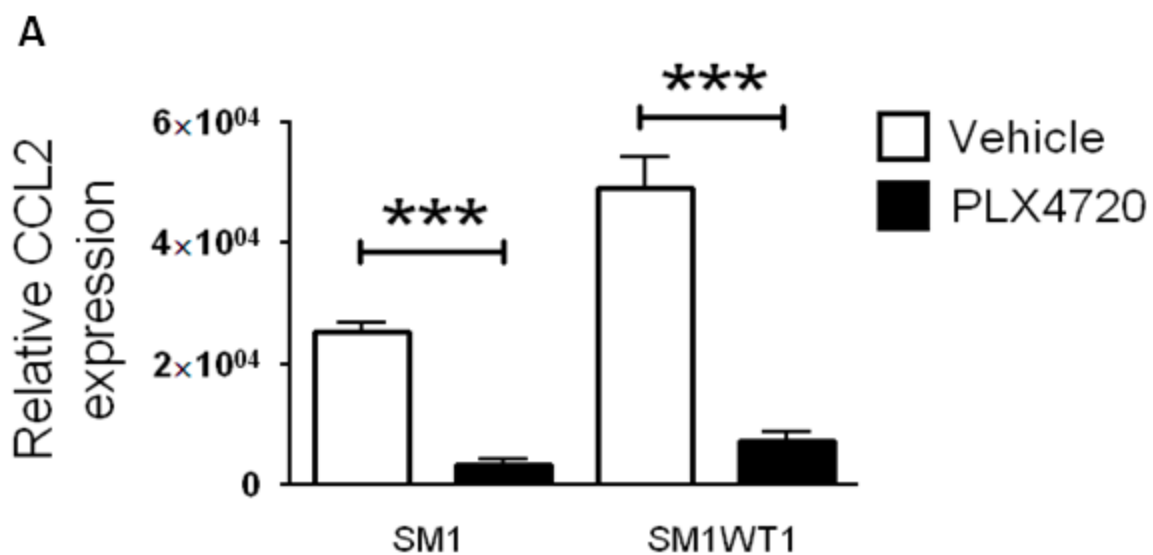
## Supplementary Figures

### Supplementary Table 1. PLX4720 inhibits the proliferation of human BRAF<sup>V600E</sup>

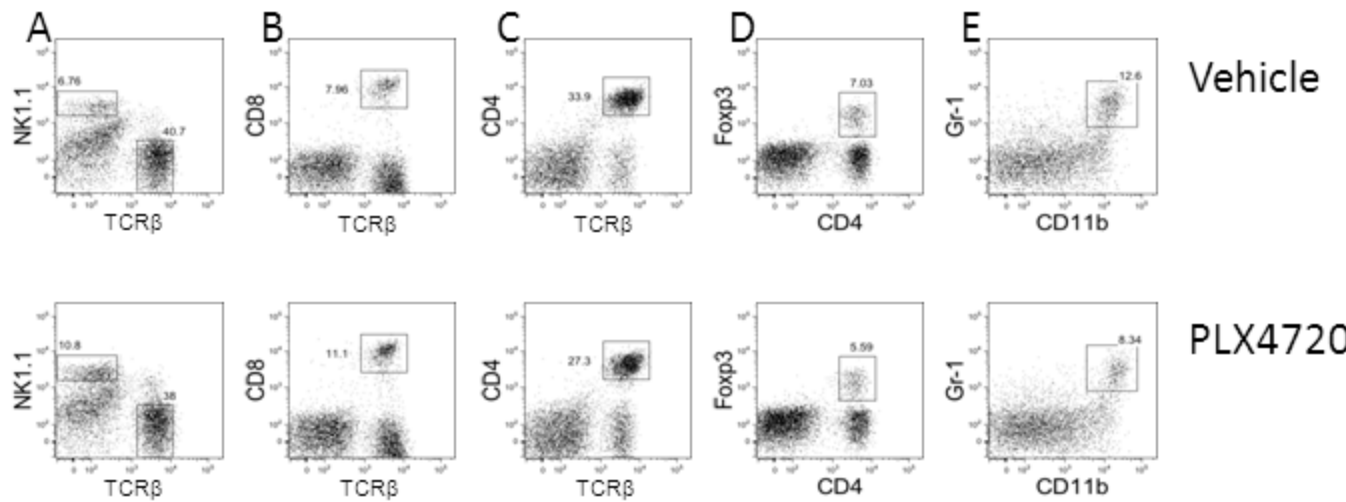
**melanoma cells *in vitro*.** To determine proliferation IC50, various human melanoma cell lines were seeded in 96-well plates and allowed to proliferate for 48 h. Cells were then treated with a range of PLX4720 concentrations (n=3). After 72 h, cell number was determined using the SRB method. IC50 concentrations were determined using non-linear regression. BRAF and NRAS status is indicated.

Cell line	PLX4720 IC50	BRAF status	NRAS status
MALME-3M	12.3 nM	V600E	WT
A375	16.0 nM	V600E	WT
SK-MEL-28	21 nM	V600E	WT
SK-MEL-24	42 nM	V600E	WT
WM266.4	53 nM	V600E	WT
HS294T	2 $\mu$ M	V600E	WT
MeWo	7.7 $\mu$ M	WT	WT
SK-MEL-2	6.9 $\mu$ M	WT	Q61R
D04-M1	1.7 $\mu$ M	WT	Q61L

**Supplementary Fig. S1. PLX4720 suppresses tumor CCL2 expression.** Relative expression of CCL2 mRNA (A) in SM1 and SM1WT1 cell lines treated with vehicle control or 10  $\mu$ M PLX4720, or (B) in SM1WT1 cell line treated with vehicle, 10  $\mu$ M PLX4720 or 5  $\mu$ M AKT inhibitor (AKTi-1/2, Calbiochem Cat No. 124017) at 24 h. Statistical differences in relative CCL2 expression of (A) SM1 and SM1WT1 cell lines treated with vehicle or PLX4720, and (B) SM1WT1 cell line treated with vehicle, PLX4720, or AKT inhibitor for each group were determined by a unpaired t test (\*\*\*)  $P < 0.001$ )

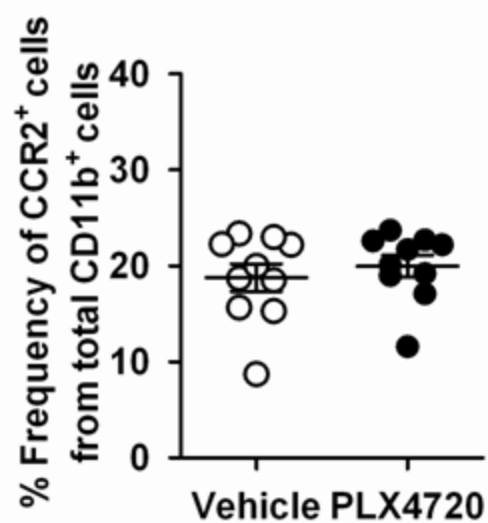
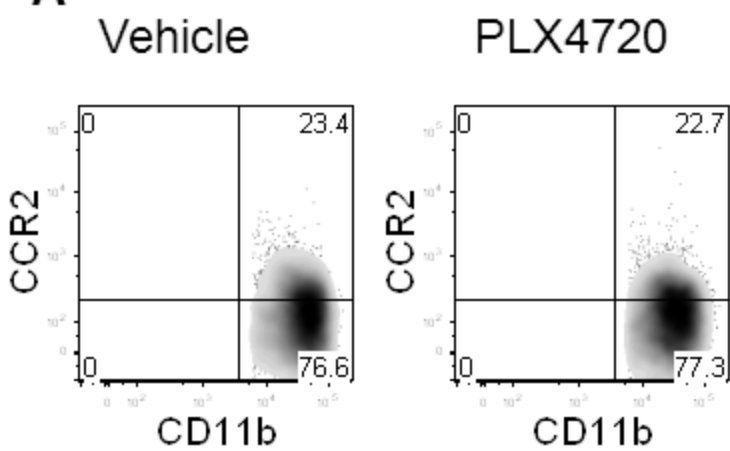
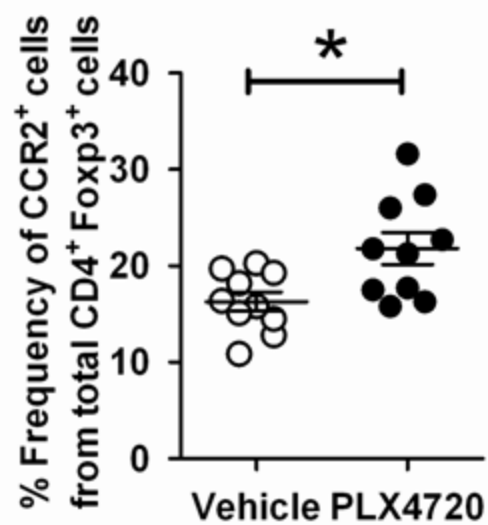
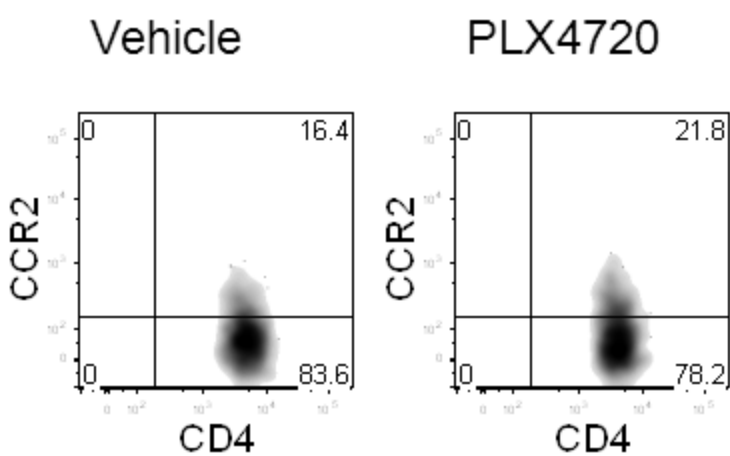


**Supplementary Fig. S2. Representative flow analysis of SM1WT1 TILs post PLX4720 treatment.** Groups of B6 WT mice (n=5-6) were inoculated with  $1 \times 10^6$  SM1WT1 cells. Mice received vehicle or PLX4720 (20 mg/kg i.p.) daily from day 12-20 post tumor inoculation. At day 21, tumors were excised and FACS analyses on TILs were performed. Representative FACS plots for frequencies of (A) NK cells and total T cells, (B) CD8<sup>+</sup> T cells, (C) CD4<sup>+</sup> T cells, (D) Tregs, (E) CD11b<sup>+</sup> Gr-1<sup>+</sup> cells, gated from (A-D) CD45.2<sup>+</sup> or (E) CD45.2<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> TILs from vehicle- or PLX4720-treated mice are shown.





**Supplementary Fig. S3. Intratumor CCR2 expression on CD11b<sup>+</sup> cells and FoxP3<sup>+</sup> Treg.** Groups of B6 WT mice (n=10) were inoculated with  $1 \times 10^6$  SM1WT1 cells. Mice received vehicle or PLX4720 (20 mg/kg i.p.) daily from day 12-20 post tumor inoculation. At day 21, tumors were excised and FACS analyses on TILs were performed. Frequencies of CCR2<sup>+</sup> cells in (A) CD11b<sup>+</sup> cells, (B) CD4<sup>+</sup>Foxp3<sup>+</sup> T cells TILs from vehicle- or PLX4720-treated mice are shown. Representative FACS plots for frequencies of CCR2<sup>+</sup> cells in (A) CD11b<sup>+</sup> cells, (B) CD4<sup>+</sup>Foxp3<sup>+</sup> TILs from vehicle- or PLX4720- treated mice are shown.

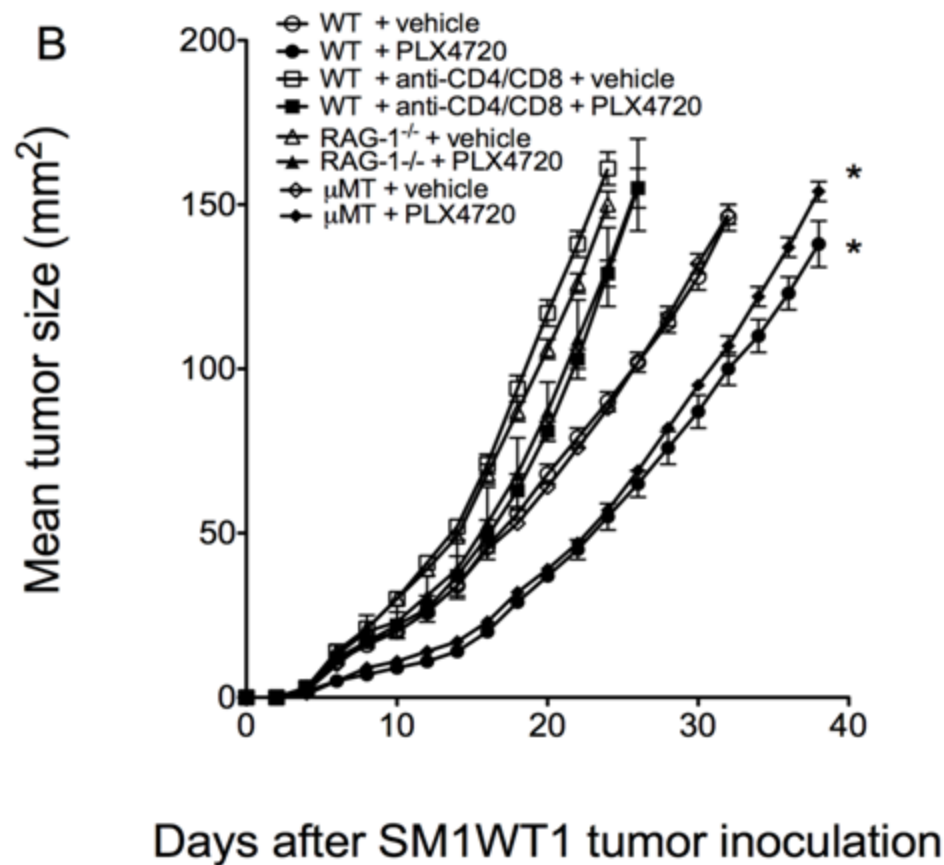
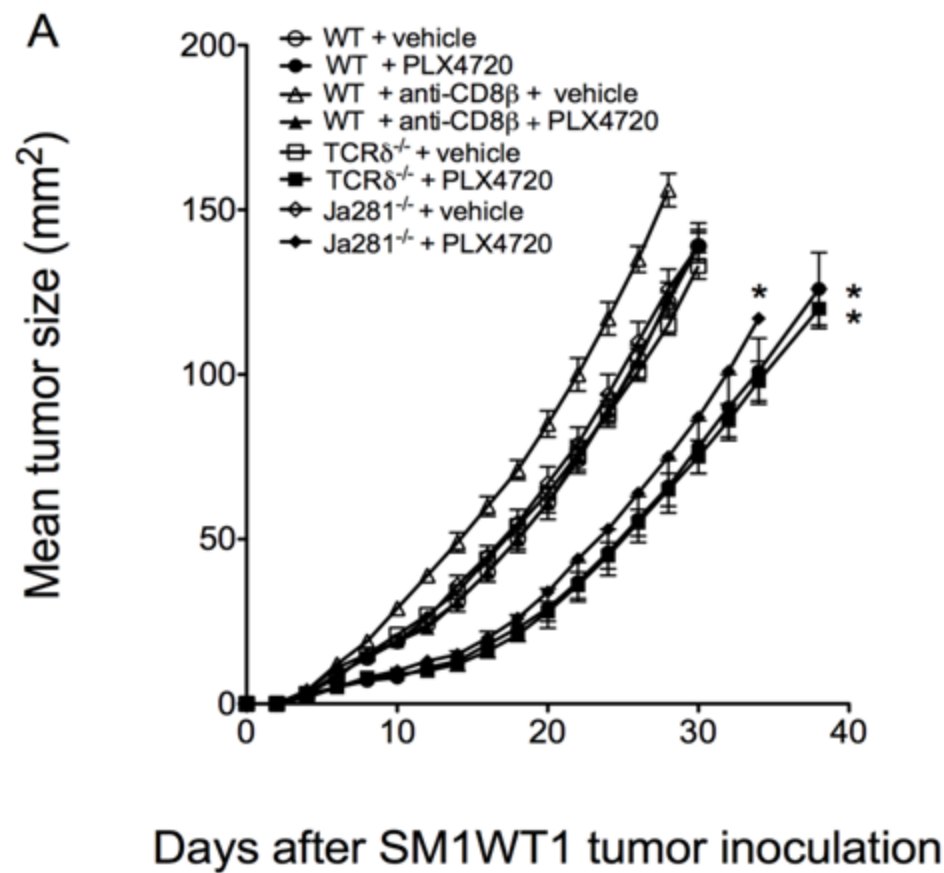
**A****B**

**Supplementary Fig. S4. PLX4720 does not increase the immunogenicity of SM1 or SM1WT1 melanomas.** SM1 and SM1WT1 cells ( $5 \times 10^5$ ) cells were seeded into three medium size flasks per line. Cells were incubated for 24 h, then replaced with culture media containing 12 ml 10% FCS complete RPMI plus with 0  $\mu$ M (1:1000 diluted DMSO), 5.0  $\mu$ M and 10  $\mu$ M PLX4720. After 24 h incubation, cells were collected, washed once with FACS buffer, and stained with the isotype control (grey shaded) or corresponding mAbs as indicated (solid line).

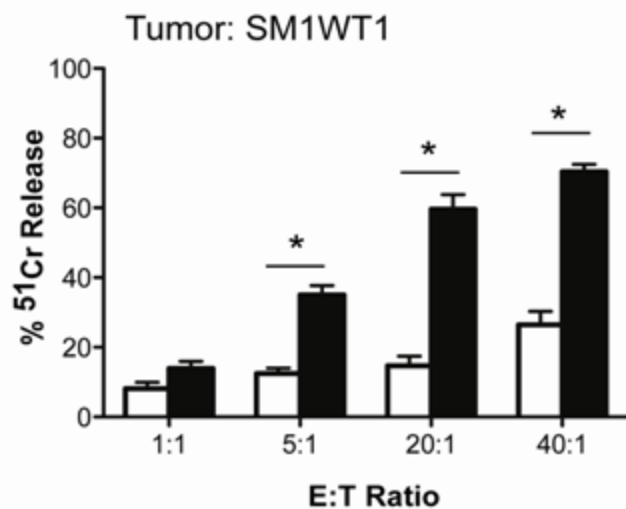
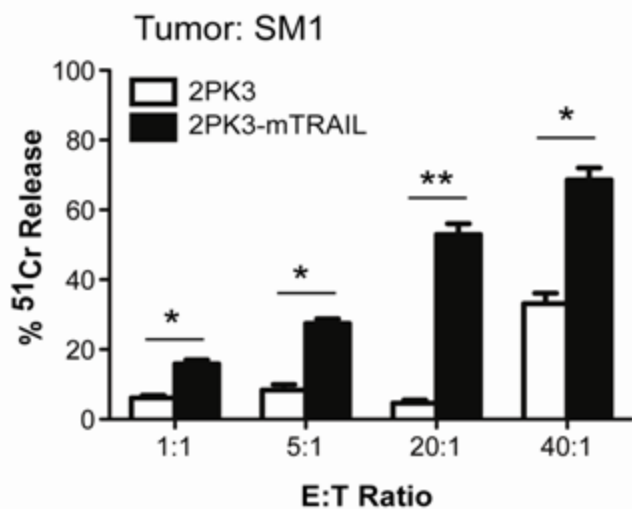
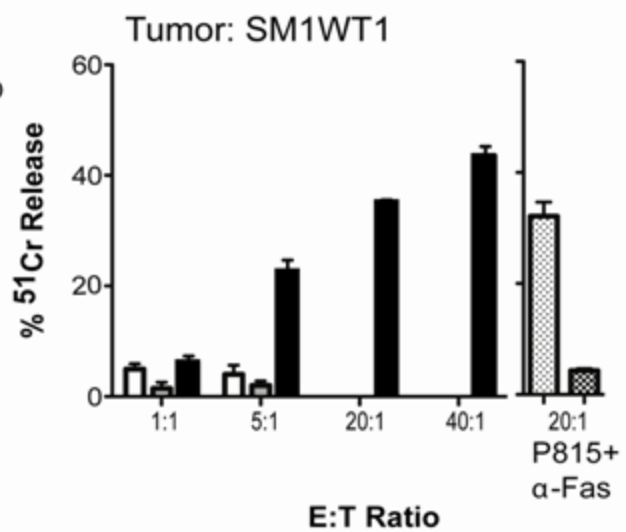
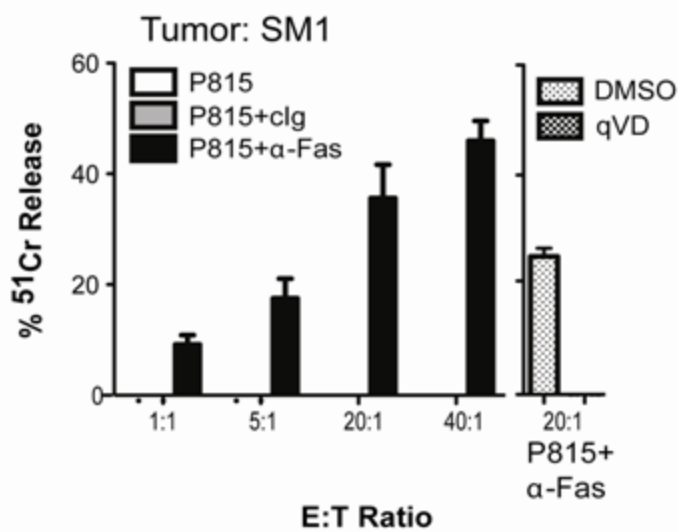


**Supplementary Fig. S5. PLX4720 anti-tumor activity is CD8<sup>+</sup> T cell-dependent.**

**(a)** Groups of 5 WT, TCR $\delta^{-/-}$  or Ja281 $^{-/-}$  mice were inoculated with  $5 \times 10^5$  SM1WT1 cells. Mice received vehicle or PLX4720 (20 mg/kg i.p.) daily from day 3-10 post tumor inoculation. Some groups of WT mice were additionally treated with anti-CD8 $\beta$  (100  $\mu$ g i.p.) on days 2,3,10, 17 and 24 after tumor inoculation. **(b)** Groups of 5 WT, RAG-1 $^{-/-}$ , and  $\mu$ MT mice were inoculated with  $5 \times 10^5$  SM1WT1 cells. Mice received vehicle or PLX4720 (20 mg/kg i.p.) daily from day 3-10 post tumor inoculation. Some groups of WT mice were additionally treated with anti-CD4 and anti-CD8 $\beta$  (100  $\mu$ g i.p. each) on days 2,3,10, 17 and 24 after tumor inoculation. Tumor sizes are represented as the mean  $\pm$  SEM. Statistical differences in tumor sizes between mice treated with vehicle versus PLX4720 for each group were determined by a Mann-Whitney test (\*  $P < 0.05$ ).

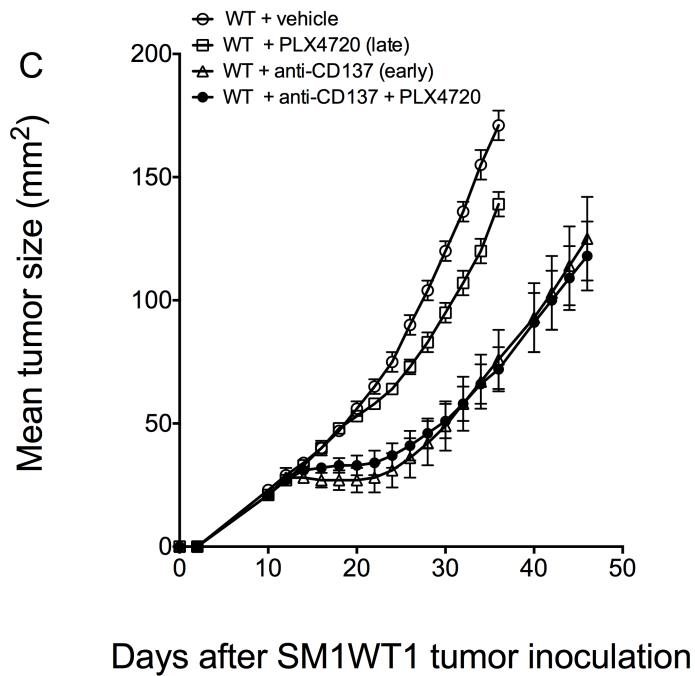
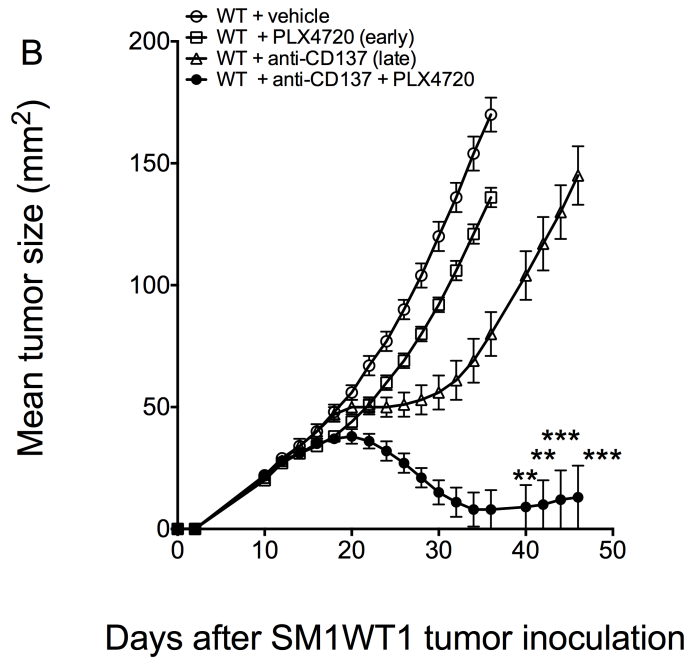
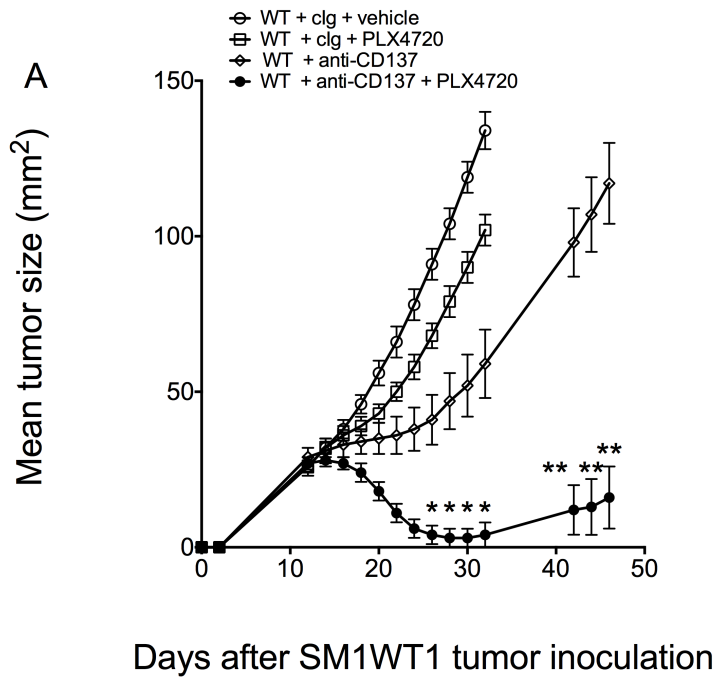


**Supplementary Fig. S6. Sensitivity of the SM1 and SM1-WT1 melanoma cell lines to TRAIL and Fas mediated apoptosis.** Twenty hour <sup>51</sup>Cr release assays were used to assess the sensitivity of the SM1 and SM1WT1 tumor lines to 2PK3-mTRAIL effectors (A) or an anti-Fas (CD95; BD Pharmingen) antibody (10 µg/ml) cross-linked by FcR<sup>+</sup> P815 effectors (B) at the indicated effector/target tumor cell ratios (solid bars). Levels of <sup>51</sup>Cr detection in control wells containing mock-transfected 2PK3 cells (A) or UC8-1B9 control Ig (cIg) (B) are shown (white bars). The apoptotic activity of the anti-Fas antibody was abrogated by pre-treatment of the SM1 and SM1WT1 cells with the pan-caspase inhibitor, qVD (10 µmol/L, InSolution Q-VD-OPh; Calbiochem) (B). DMSO treatment did not disrupt the apoptotic effects of the α-Fas antibody. Each group was performed in triplicate. Results are representative of two experiments (n=6). Statistical differences in <sup>51</sup>Cr release between the 2PK3-mTRAIL and 2PK3 treated SM1 and SM1-WT1 tumor lines were determined by the Mann-Whitney *U* test. \*, P<0.003 and \*\*P<0.0001.

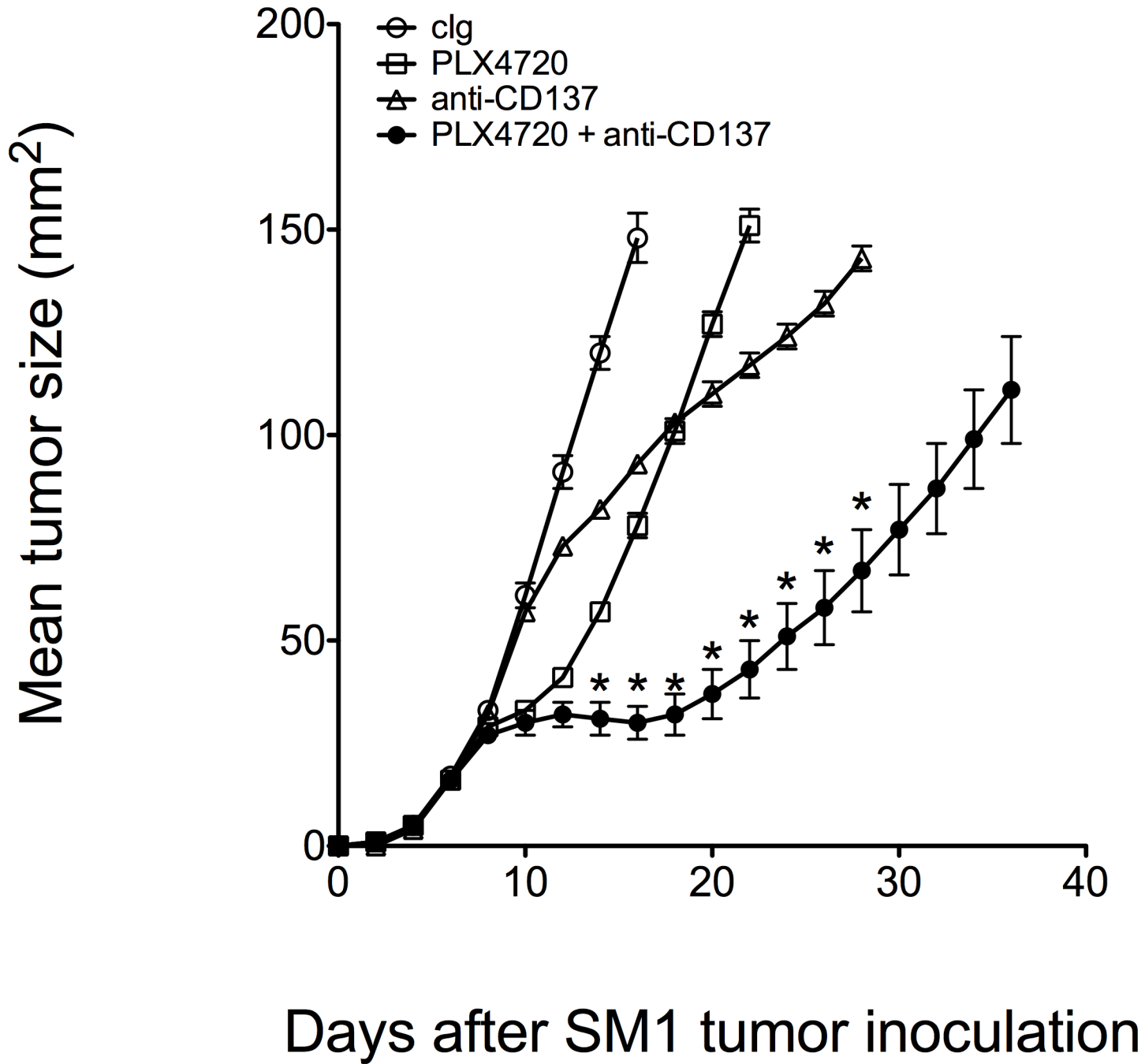
**A****B**



**Supplementary Fig. S7. Anti-tumor activity of coincident PLX4720 and anti-CD137 in combination.** Groups of 5 WT mice were inoculated with  $5 \times 10^5$  SM1WT1 cells. Mice received (A) vehicle or PLX4720 (20 mg/kg i.p.) daily from day 12-16 post tumor inoculation. Some groups of mice were additionally treated with cIg or anti-CD137 (100  $\mu$ g i.p. each) on days 12, 14, 16 and 18 after tumor inoculation. (B) vehicle or PLX4720 (20 mg/kg i.p.) daily from day 12-16 post tumor inoculation (early). Some groups of mice were additionally treated with cIg or anti-CD137 (100  $\mu$ g i.p. each) on days 17, 19, and 21 after tumor inoculation (late). (C) vehicle or PLX4720 (20 mg/kg i.p.) daily from day 17-21 post tumor inoculation (late). Some groups of mice were additionally treated with cIg or anti-CD137 (100  $\mu$ g i.p. each) on days 12, 14, and 16 after tumor inoculation (early). Tumor sizes are represented as the mean  $\pm$  SEM. (A) Statistical differences in tumor sizes between mice treated with anti-CD137 versus combination therapy for each group were determined by a Kruskal-Wallis test with Dunn's correction (\*  $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). The WT + cIg + vehicle and WT + cIg + PLX4720 groups shown in panel A are also presented again in Figure 9. All of the samples in Figure 9 and panel A, or panel B and C, respectively, were derived from the same experiment.



**Supplementary Fig. S8. Synergistic anti-tumor activity of PLX4720 and anti-CD137.** Groups of 5 WT mice were inoculated with  $5 \times 10^5$  SM1 cells. Mice received vehicle or PLX4720 (20 mg/kg i.p.) daily from day 7-10 after tumor inoculation and cIg or anti-CD137 (100  $\mu$ g i.p.) on days 11, 12, 13 and 14 after tumor inoculation. Tumor sizes are represented as the mean  $\pm$  SEM. Statistical differences in tumor sizes between mice treated with single versus combination therapy were determined by a Mann-Whitney test (\*  $P < 0.05$ ).



**Supplementary Fig. S9. PLX4720 dose effect on the anti-tumor activity of the PLX4720 and anti-CD137 combination.** Groups of 5 WT mice were inoculated with  $5 \times 10^5$  SM1WT1 cells. Mice received (A) vehicle or PLX4720 (10 mg/kg i.p.) or (B) vehicle or PLX4720 (5 mg/kg i.p.) daily from day 12-16 post tumor inoculation. Some groups of mice were additionally treated with cIg or anti-CD137 (100  $\mu$ g i.p. each) on days 17, 19, 21 and 23 after tumor inoculation. Tumor sizes are represented as the mean  $\pm$  SEM. The WT + vehicle and WT + anti-CD137 + vehicle groups in panels A and B are shared. All of the samples in Supplemental Figure 9A and 9B were derived from the same experiment. Statistical differences in tumor sizes between mice treated with anti-CD137 + vehicle versus combination therapy was determined by a Kruskal-Wallis test with Dunn's correction (\*P < 0.05).

