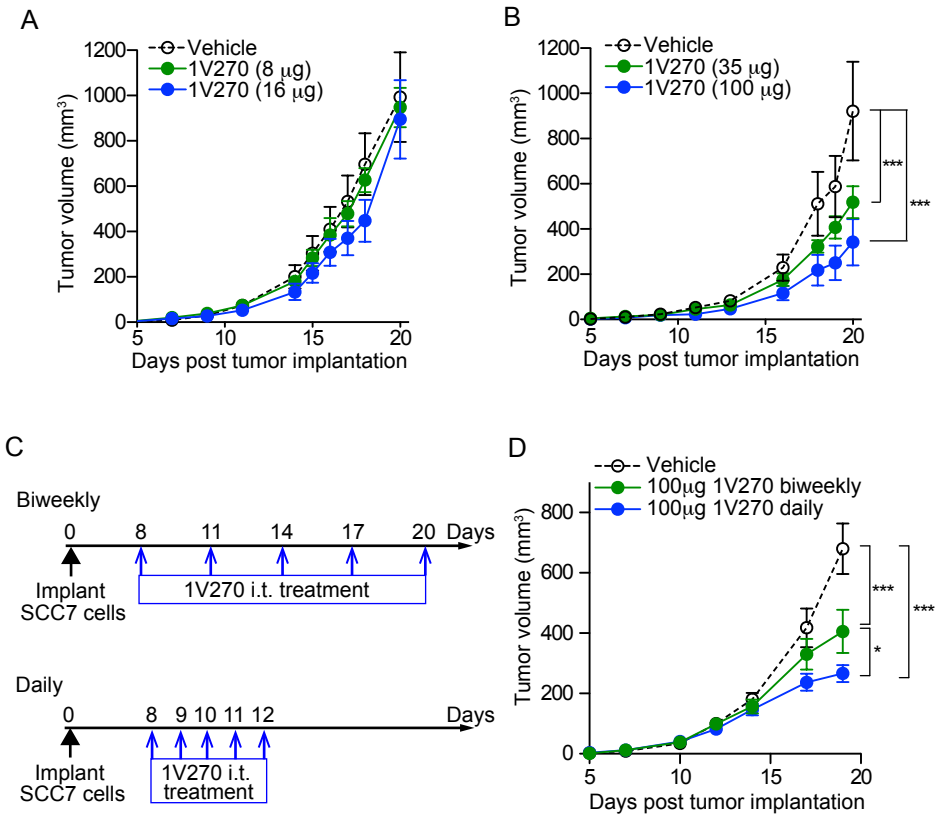
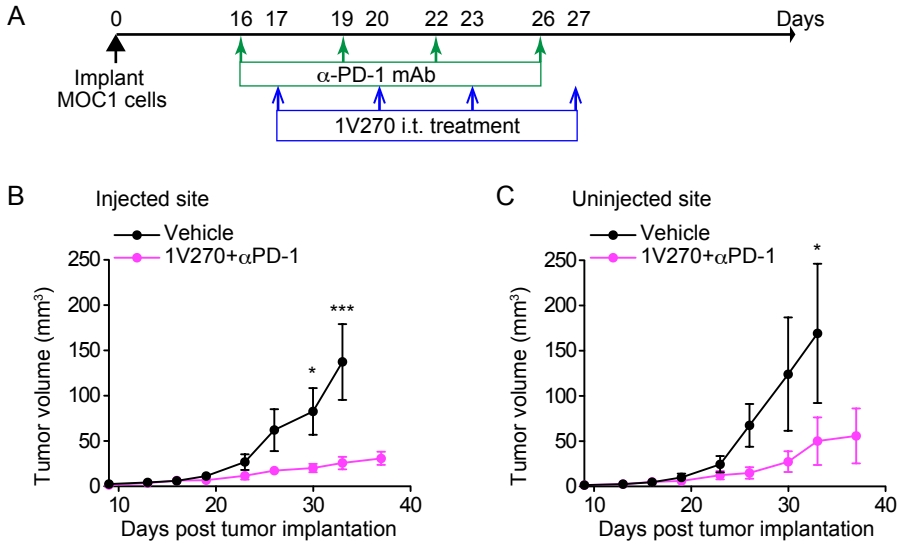
Supplemental Figure 1



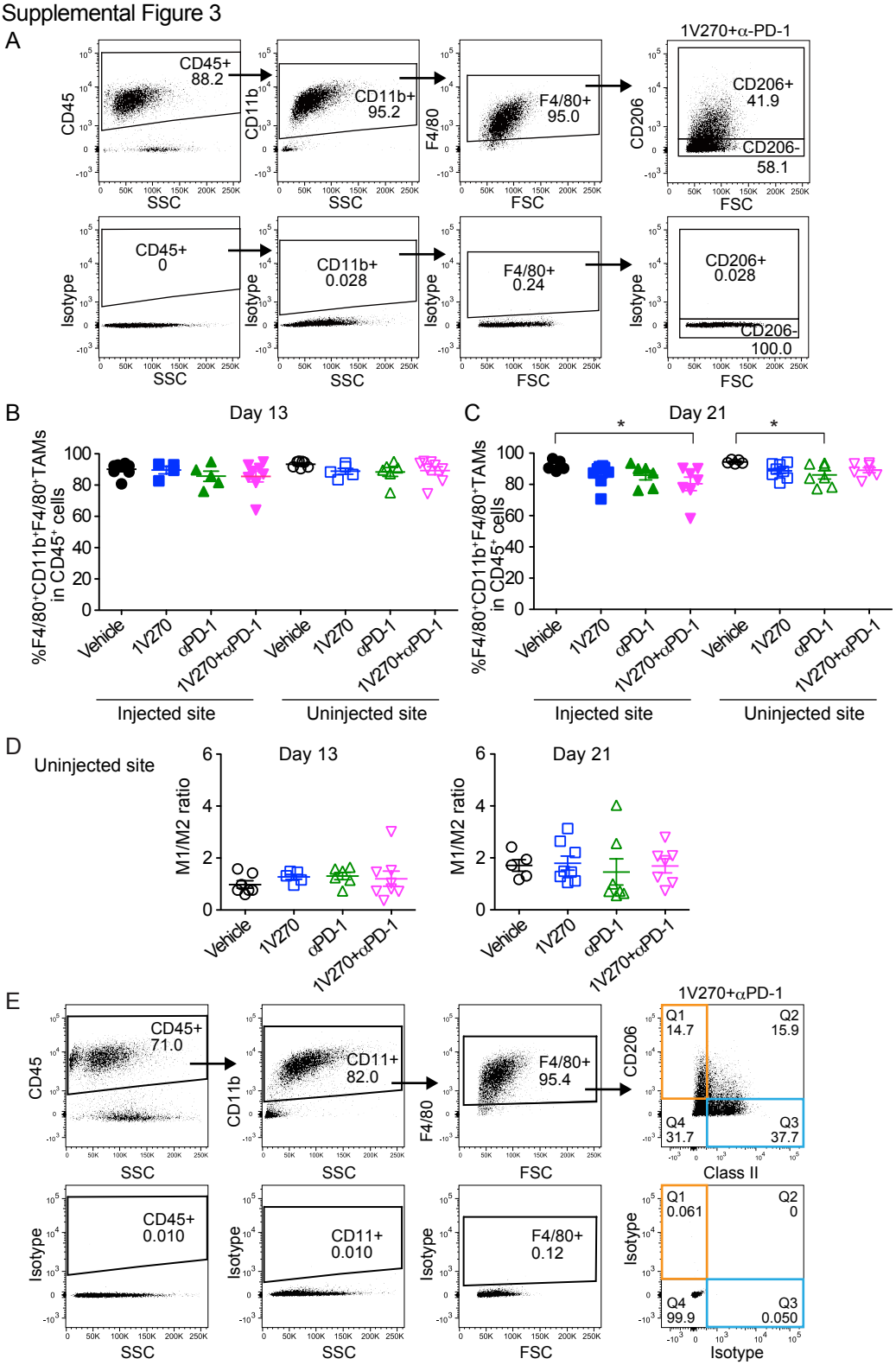
Supplemental Figure 1. Dose and schedule optimization for i.t. administration of 1V270. (**A** and **B**) Dose optimization studies. C3H mice (n=6-9/group) were implanted with 10^5 SCC7 cells. Mice were i.t. treated with 8, 16, 35, or 100 μg/injection of 1V270 starting when tumors reached 2-4 mm diameter, approximately day 8, daily for 5 days. (**C** and **D**) Schedule optimization studies. (**C**) Experimental protocol to determine optimal schedule of i.t. treatment with 1V270. C3H mice (n=14-15/group) were implanted with 10^5 SCC7 cells in both flanks. Intratumoral 1V270 treatment was given daily on days 8, 9, 10, 11, 12, or biweekly (on day 8, 11, 14, 17, and 20). Vehicle (10% DMSO) or isotype antibody administered as a control. (**D**) Tumor volume from mice treated daily (blue solid line) or biweekly (green solid line) with 1V270 or vehicle (black dashed line). Data presented are mean ± SEM **P*<0.05, ***P*<0.01, and ****P*<0.001 (two-way repeated measures ANOVA with Bonferroni *post hoc* test). Data shown are representative of two independent experiments showing similar results.

Supplemental Figure 2



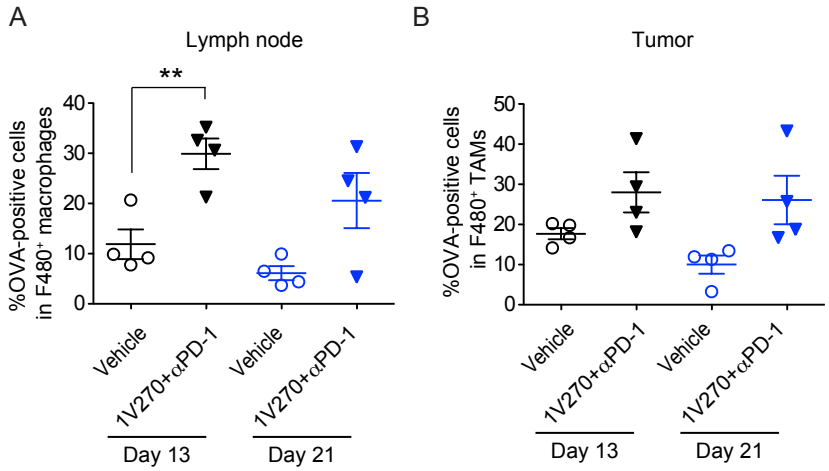
Supplemental Figure 2. The combination therapy inhibits MOC1 tumor progression at both injected and uninjected sites. 2×10^6 MOC1 cells were s.c. implanted in both flanks of C57BL/6 mice (n=5/group). Mice were treated with 1V270 (100 µg/injection) and anti-PD-1 antibody (250 µg/injection).(**A**) Treatment schedule. (**B**) Tumor volume at injected sites. (**C**) Tumor volume at uninjected sites. Data presented are ± SEM **P*<0.05 and ***P*<0.01 (two-way ANOVA with Bonferroni *post hoc* test)

Supplemental Figure 3



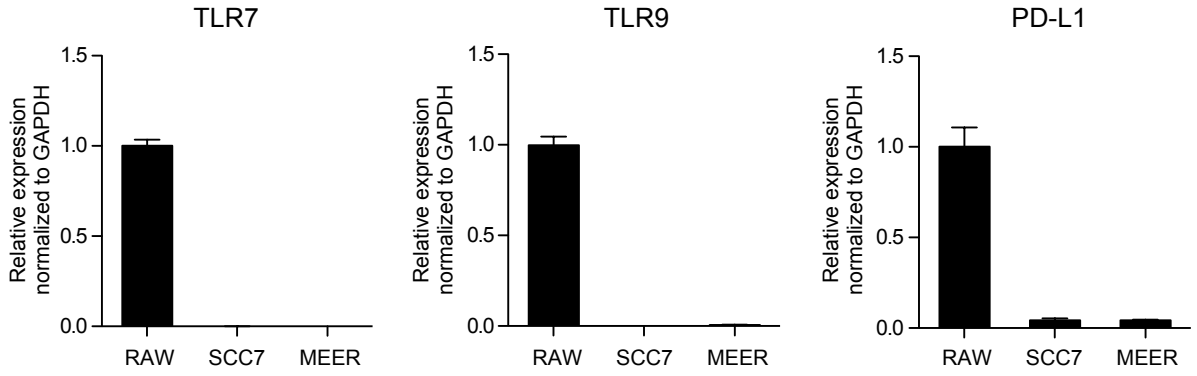
Supplemental Figure 2. CD11b⁺F4/80⁺ macrophages are large populations of tumor filtrating immune cells.
(**A-C**) SCC7 bearing mice were treated as described in Figure 1A. Tumors (injected and uninjected sites) were harvested on days 13 and 21 and TAMs were analyzed by flow cytometry. Single cell suspensions were stained for CD45, CD11b, and F4/80 to identify TAMs. Expression of CD206 was used to identify M2 macrophages. (**A**) Representative flow cytometric plots of tumor infiltrating M1 ($CD206^-$) and M2 ($CD206^+$) macrophages. The plots staining with isotype antibodies are shown on the lower panel. (**B** and **C**) The percentage of macrophage in the gated $CD45^+$ cell population in the tumors harvested on days 13 (B) and 21 (C) was calculated and plotted. Each dot represents a tumor from an individual mouse. The horizontal and vertical bars indicate the mean ± SEM. Data shown are representative of two independent experiments showing similar results. **P*<0.05 (Kruskal-Wallis test with Dunn's *post hoc* test). (**D**) The ratios of M1 to M2 (M1/M2) were calculated as [% M1 ($CD206^-$) population in $CD45^+CD11b^+F4/80^+$]/ [% M2 ($CD206^+$) population in $CD45^+CD11b^+F4/80^+$]. There were no significant differences in M1/M2 ratios among groups at uninjected sites (Kruskal-Wallis test with Dunn's *post hoc* test).
(**E**) Representative flow cytometric plots of $CD206^+$Class II^- and $CD206^-$Class II^+ populations. M1- and M2- like macrophages were identified as $CD45^+CD11b^+F4/80^+CD206^-$Class II^+ and $CD45^+CD11b^+F4/80^+CD206^+$Class II^- populations, respectively.

Supplemental Figure 4



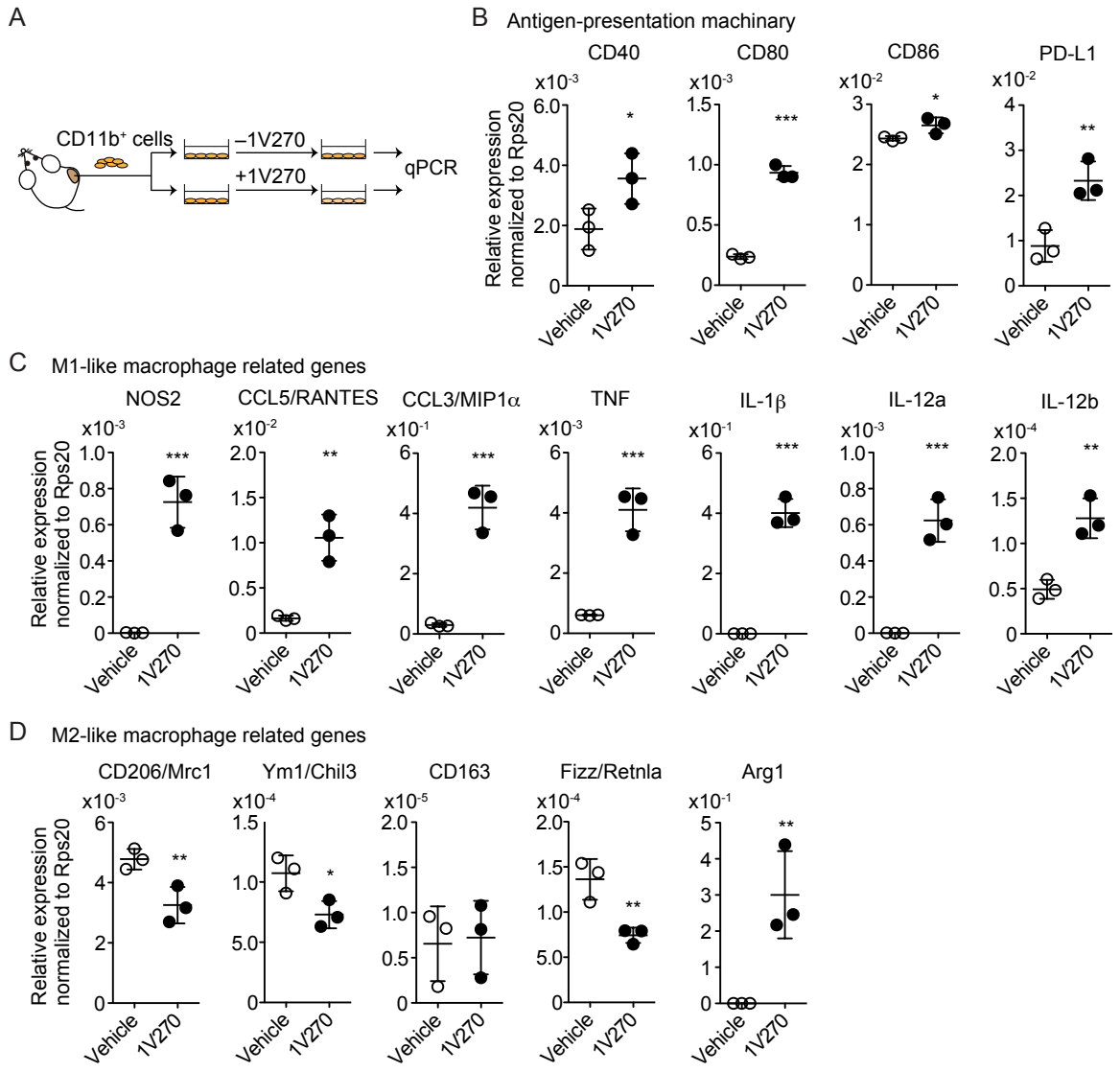
Supplemental Figure 4. Antigen associated macrophages were increased in the dLNs and tumors on days 13 and 21. SCC7-tumor bearing mice were treated with 1V270 and anti-PD-1 antibody as described in Figure 1A and antigen uptake was evaluated on days 13 and 21 (n=4/group). OVA conjugated with Alexa Fluor 488 was i.t. injected one day before the analysis. Single cell suspensions were obtained from tumors at injected sites and draining lymph nodes (dLNs), and stained for CD45, CD11b and F4/80. OVA^+ cells in the gated macrophage population ($CD45^+CD11b^+F4/80^+$) in the dLNs and tumors were analyzed by FACS. (**A**) $\%OVA^+$ cells in $CD45^+CD11b^+F4/80^+$ macrophages. (**B**) $\%OVA^+$ cells in $CD45^+CD11b^+F4/80^+$ TAMs. Data presented are means ± SEM. *P<0.01 (two-tailed Welch' s t test).

Supplemental Figure 5



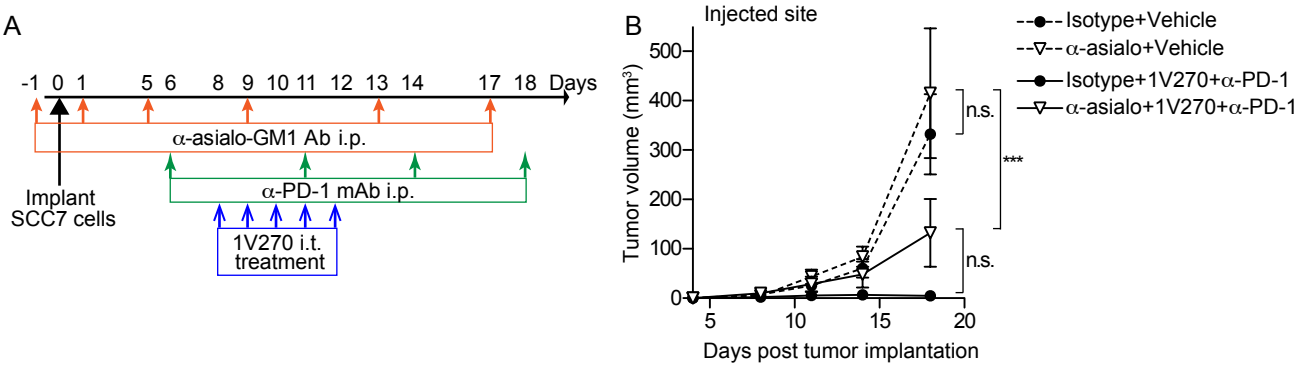
Supplemental Figure 5. TLR7, TLR9 and PD-L1 expression in SCC7 and MEER cells. SCC7 and MEER cells were cultured *in vitro*, harvested, and RNA was isolated. Quantitative RT-PCR was used to compare the expression of TLR7, TLR9, and PD-L1. Gene expression was normalized with GAPDH and then compared to the expression levels in murine macrophage cell line RAW264.7 that was used as a positive control (100%). Data are presented as mean ± SEM for triplicate determinations. Data shown are representative of two independent experiments showing similar results.

Supplemental Figure 6



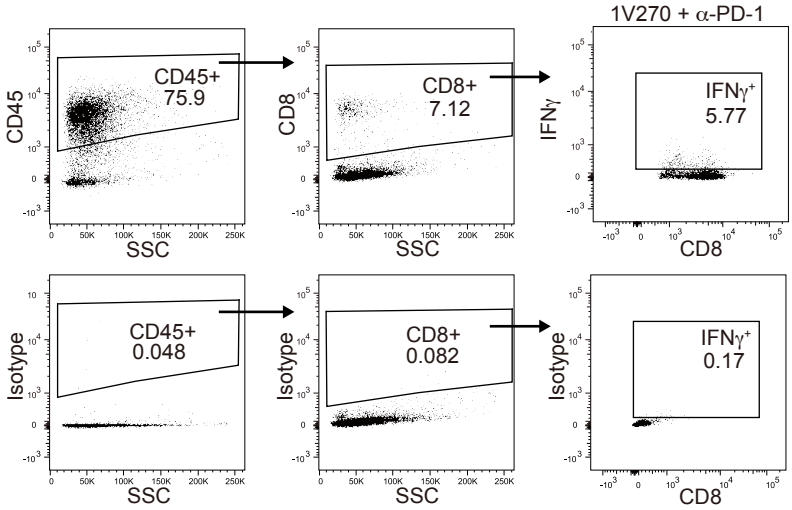
Supplemental Figure 6. Expression of genes related to antigen presenting function and M1 macrophages is upregulated following 1V270 exposure ex vivo. (**A**) Experimental protocol. CD11b+ TAMs were isolated from day 14 SCC7-tumors using CD11b MicroBeads (Miltenyi Biotec) and treated with 1 μM 1V270 or vehicle overnight. Quantitative RT-PCR was performed. Primers and probes, which were purchased from Thermo Fisher Scientific, are listed in Supplemental Table 4. (**B**) Antigen-presentation machinery. (**C**) M1-like macrophage related genes. (**D**) M2-like macrophage related genes. Data are presented as mean ± SD of triplicates. **P*<0.05, ***P*<0.01, and ****P*<0.001 (one-tailed unpaired *t* test).

Supplemental Figure 7



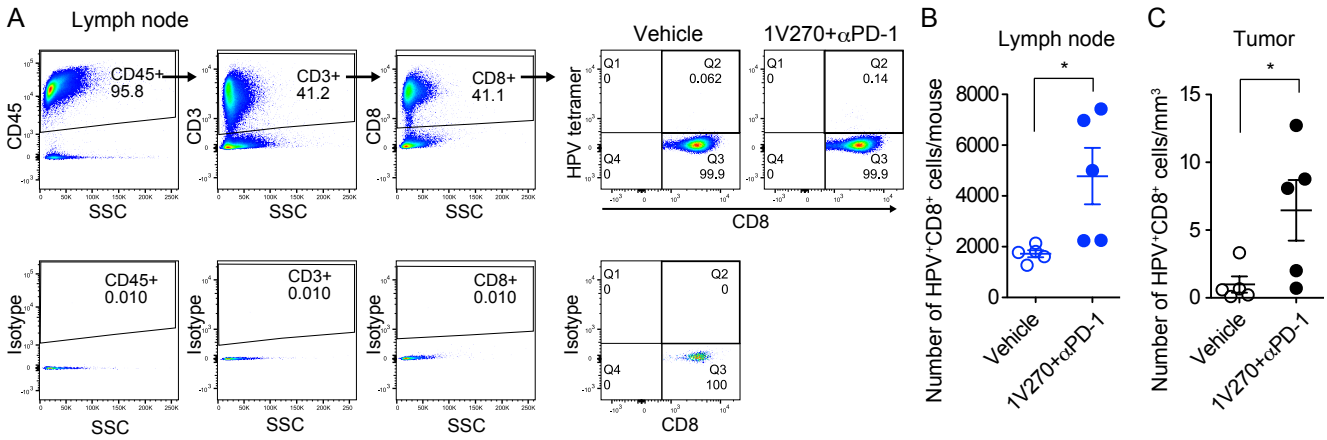
Supplemental Figure 7. NK cells are partially involved in tumor suppression effects by the combination therapy. (**A**) Experimental protocol of NK cell depletion. SCC7-bearing mice were treated with the combination therapy of 1V270 and anti-PD-1 agent. Anti-asialo GM1 rabbit polyclonal antibody (50 μL/injection) or rabbit IgG polyclonal antibody was injected on days -1, 1, 5, 9, 13, and 17. (**B**) Tumor growth at the injected site was monitored. ****P*<0.001; n.s., non-significant by two-way repeated measures ANOVA with Bonferroni *post hoc* test (n=4-5/group).

Supplemental Figure 8



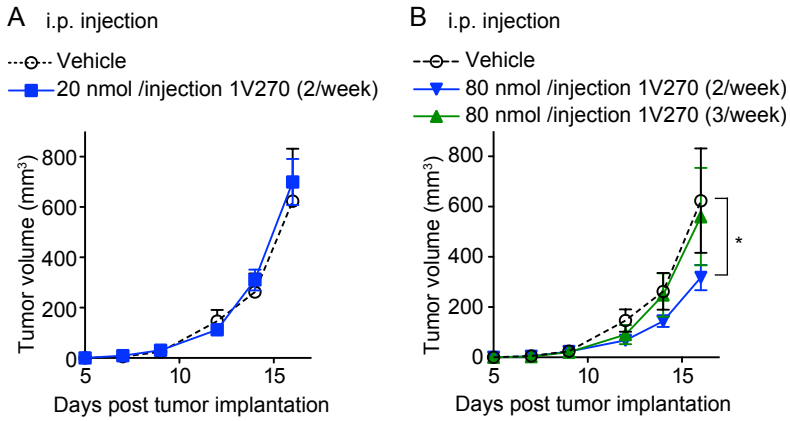
Supplemental Figure 8. Representative flow cytometric plots of tumor infiltrating CD8⁺ T cells. Tumors (injected and uninjected sites) were harvested on day 21 from SCC7-bearing mice treated with 1V270 and/or anti-PD-1 as described in Figure 1A. The single cell suspensions of tumor cells and splenocytes were prepared and stained for CD45, CD8, and intracellular IFNγ to identify tumor infiltrating IFNγ⁺CD8⁺ T cells. Fixation/Permeabilization Solution kit (BD Biosciences) were used for intracellular IFNγ staining. Representative plots of cells after staining with isotype antibodies are shown on the lower panel.

Supplemental Figure 9



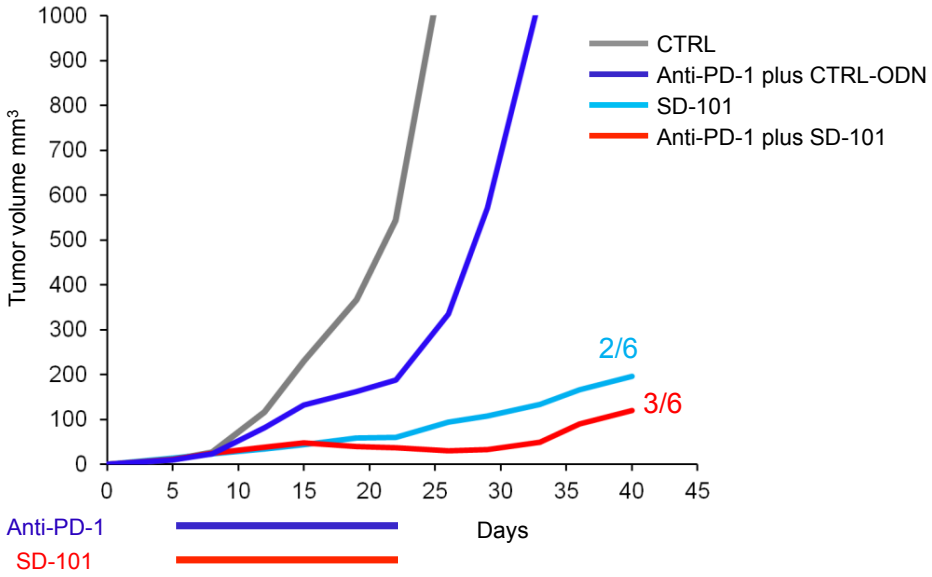
Supplemental Figure 9. The combination treatment increased HPV-tetramer positive CD8⁺ T cells in the dLNs and the tumors. 2.5×10^6 HPV-positive MEER cells were implanted s.c. in both flanks of C57BL/6 mice (n=5/group). The combination treatment with anti-PD-1 and 1V270 was given as described in Figure 2A. One day after the final 1V270 injection, tumors and draining lymph nodes (dLNs) at injected sites were harvested. The single cell suspensions of LN cells and tumor cells were incubated with antibody cocktail (CD45, CD3, CD8 and HPV tetramer). iTAg Tetramer/PE - H-2 Db HPV 16 E7 (RAHYNIVTF) (MBL international Corporation, MA) was used to detect HPV specific CD8⁺ T cells. (**A**) Representative flow cytometric plots. (**B**) Number of CD8⁺HPV⁺ T cells in the dLN. (**C**) Number of CD8⁺HPV⁺ T cells in the tumor at injected sites. Data are presented as mean ± SEM **P*<0.05, one-tailed Welch' s *t* test.

Supplemental Figure 10



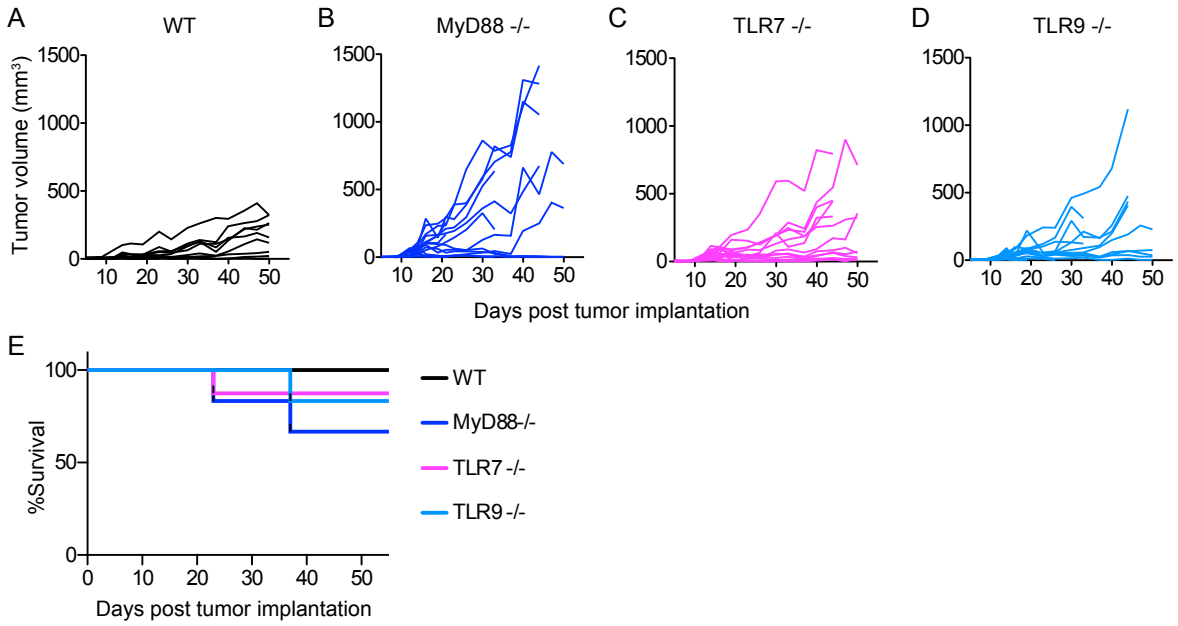
Supplemental Figure 10. Systemic administration of 1V270. (**A** and **B**) 2×10^5 B16 melanoma cells were implanted in the right flank of C57BL/6 mice. Mice were intraperitoneally (i.p.) treated with 1V270 (20 nmol/injection or 80 nmol/injection) twice a week or three times a week. (**A**) 20 nmol/injection of 1V270 twice a week. (**B**) 80 nmol/injection of 1V270 twice a week or three times a week. *P<0.05, and **P<0.01 by two-way ANOVA, Bonferroni *post hoc* test.

Supplemental Figure 11



Supplemental Figure 11. Graphical representation of mean tumor volumes over time. 8×10^4 CT26 tumor cells were injected s.c. in the flank of Balb/c mice on day 0 (n=5-7 mice/group). Anti-PD-1 and SD-101 were both started five days after tumor implantation. Anti-PD-1 and SD-101 were administered every 4 days from day 5 to 22. SD-101 (50 µg/injection) or CTRL-ODN (non-CpG ODN control) was given i.t. and anti-PD-1 (clone RMP1-14, 250 µg/injection) was i.p. administered. CT26 bearing mice received concomitant treatment with anti-PD-1 and SD-101 which led to incomplete tumor rejections (50% rejection). As we previously reported (Wang et al. PNAS 2016, Fig 2A, (30)), anti-PD-1 was given at day 7 and SD-101 at day 19 after tumor implantation which resulted in 100% rejection. Data shown are representative of three independent experiments showing similar results.

Supplemental Figure 12



Supplemental Figure 12. MyD88/TLR signal pathways play roles in anti-tumor immunity. 10^6 HPV$^+$ MEER cells were implanted s.c. in C57BL/6 WT, *Myd88-/-, Tlr7-/- and Tlr9-/-* mice (n=14-20/group) and tumor growth was monitored. (**A**) WT, (**B**) *Myd88-/-*, (**C**) *Tlr7-/-* and (**D**) *Tlr9-/-* mice. (**E**) Kaplan-Meier survival curve.

Supplemental Table 1. M1 and M2 related gene expression in tumor tissues treated with i.t. 1V270 or vehicle (extracted from nCounter® PanCancer Immune Profiling Panel)[a)]

Gene	M1 or M2 related	Control mean (log2)	1V270 mean (log2)	log2 Fold Change	Raw p	Adjusted p (BH)[b)]
Ccl3	M1	5.003286	7.4094	2.406114	2.06E-05	0.001
Ccr7		4.843	6.815	1.972	0.013464	0.022
Cd74		10.87143	12.59	1.718571	0.027306	0.038
Tnf		4.427429	6.039	1.611571	0.000379	0.003
Ccl5		7.489714	8.8728	1.383086	0.002152	0.008
Il1b		5.612714	6.9472	1.334486	0.025593	0.038
Nos2		4.966857	6.2146	1.247743	0.0004	0.003
Il12a		3.618286	4.8412	1.222914	0.012922	0.022
Cxcl10		8.709571	9.7412	1.031629	0.005318	0.015
Cd86		7.018857	7.9256	0.906743	0.001161	0.005
Ido1		3.186571	3.949	0.762429	0.015522	0.024
Ptgs2		6.301714	7.058	0.756286	0.108658	0.129
Il6		3.548429	4.2552	0.706771	0.12615	0.143
Il12b		4.452714	5.1346	0.681886	0.063445	0.083
Ccl2		11.84286	12.064	0.221143	0.51037	0.532
Cxcl13	M2	3.666429	8.0402	4.373771	0.000121	0.002
Pparg		4.385143	6.0906	1.705457	0.006581	0.015
Irf4		3.958714	5.5346	1.575886	0.012448	0.022
Il10		3.186571	4.74	1.553429	0.001085	0.005
Chil3		3.084429	4.299	1.214571	0.005872	0.015
Arg1		9.721571	10.8838	1.162229	0.009092	0.019
Ccl24		2.924714	3.3746	0.449886	0.388212	0.422
Ccl12		9.360143	9.2912	-0.06894	0.847038	0.847
Cd163		6.400571	5.4896	-0.91097	0.073918	0.092
Mrc1/Cd206		10.47571	9.5268	-0.94891	0.003822	0.012

a) The genes were analyzed from nCounter® PanCancer Immune Profiling Panel (NanoString).
b) Adjusted p-values by Benjamini-Hochberg procedure.

Supplemental Table 2. Antibodies used in flow cytometry analysis

Antibody	Clone	Color	Cat#	Source
CD3ε	145-2c11	APC	17-0031	eBioscience
CD4	RM4-5	eFluor 450	48-0042	eBioscience
CD8α	53-6.7	FITC	553030	BD Biosciences
CD8α	53-6.7	eFluor 450	48-0081	eBioscience
CD11b	M1/70	eFluor 450	48-0112	eBioscience
CD45	30-F11	PE/Cy7	103114	BioLegend
F4/80	BM8	APC	17-4801	eBioscience
CD206	C068C2	PE	141706	BioLegend
IFNγ	XMG1.2	APC	17-7311	eBioscience
CD40	1C10	PE	12-0401	eBioscience
CD80	16-10A1	FITC	104706	BioLegend
PD-L1 (CD274, B7-H1)	10F.9G2	PE	124308	BioLegend
MHC Class II (I-Ek)	14-4-4S	FITC	11-5980	eBioscience
CD16/CD32 (FcR)	2.4G2	Purified	553142	BD Biosciences

Supplemental Table 3. Antibodies used for immunofluorescent staining

Primary antibody	**Dilution**	**Cat#**	**Source**
Rat anti-mouse F4/80	25	14-4801	eBiosciences
Rabbit anti-mouse CD206	200	ab64693	abcam
Rat anti-mouse CD8	50	550281	BD bioscience
Secondary antibody			
DyLight 549 Goat anti-Rat IgG	500	112-506-003	Jackson immunoresearch
Alexa 488 Goat anti-Rabbit IgG	1000	111-546-047	Jackson immunoresearch

Supplemental Table 4. Primers and probes used in quantitative RT-PCR analysis

Gene	Primer and probe number[a)]
Nos2	Mm00440502_m1
Ccl5/Rantes	Mm01302427_m1
Ccl3/Mip1α	Mm00441259_m1
Tnf	Mm00443258_m1
Il-1β	Mm00434228_m1
Il-12a	Mm00434165_m1
Il-12b	Mm01288989_m1
Cd206	Mm01329362_m1
Ym1/Chil3	Mm00657889_mH
Cd163	Mm00474091_m1
Fizz/Retnla	Mm00445109_m1
Arg1	Mm00475988_m1
Cd40	Mm00441891_m1
Cd80	Mm00711660_m1
Cd86	Mm00444543_m1
Tlr7	Mm00446590_m1
Tlr9	Mm00446193_m1
Pd-l1	Mm00452054_m1
Rps20	Mm02342828_g1
Gapdh	Mm99999915_g1

a) The primers and probes were purchased from Thermo Fisher Scientific.