

Supplementary Materials for

Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy

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Figs. S1 to S9
Table S1

Supplementary materials – Manuscript # aax7881

Fig. S1

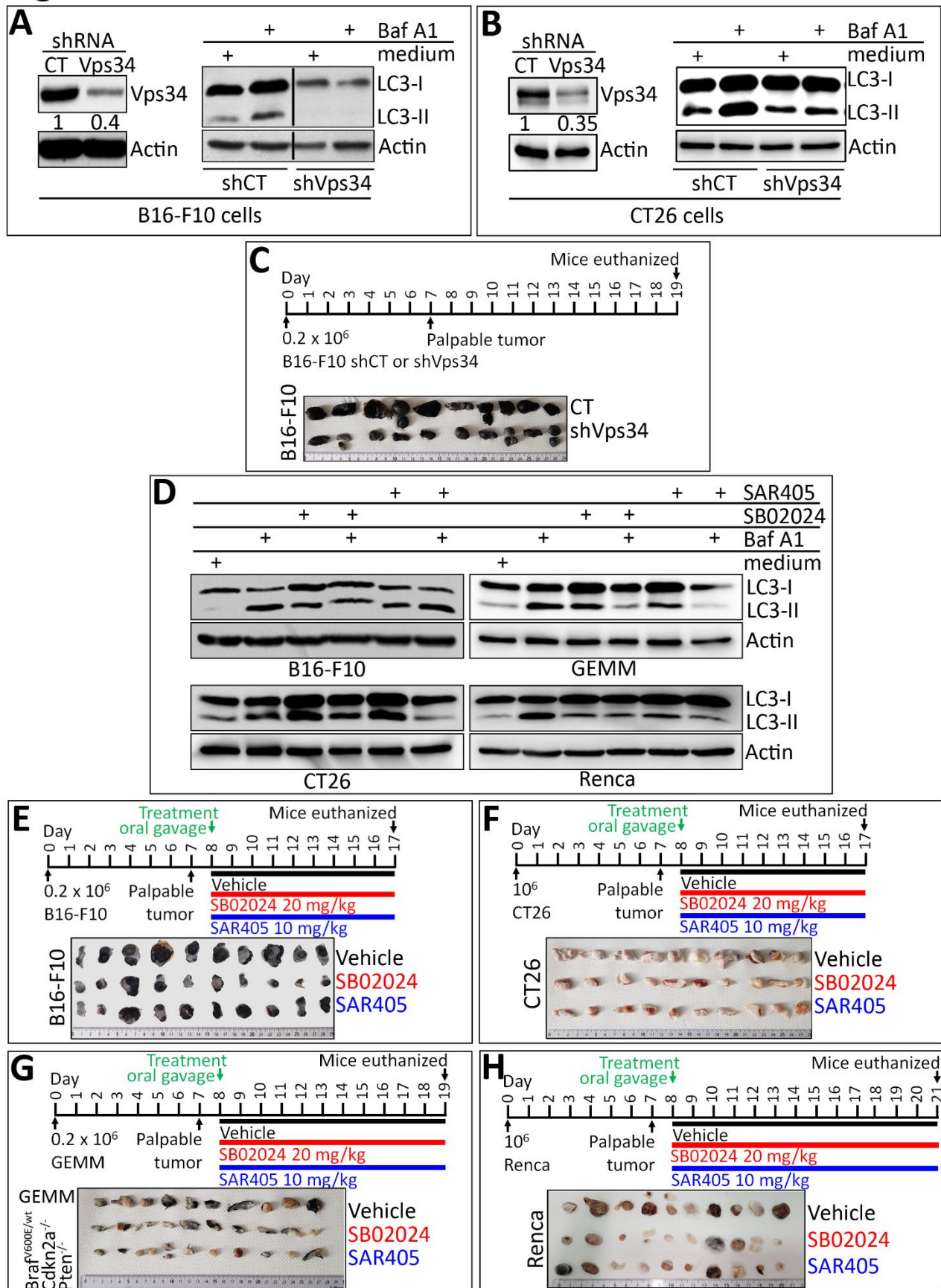


Fig. S1. Genetic or pharmacological targeting of Vps34 inhibits autophagy flux in multiple tumor cells in vitro.

A and B: B16-F10 melanoma (A) and CT26 colorectal cancer (B) cells were transfected with control (shRNA-CT) or Vps34 specific shRNA (shRNA-Vps34). The protein expression of Vps34 was shown in the left of each panel. Actin was used as loading control and the Vps34/actin ratio is reported in the Figure. The expression of LC3-I and -II protein in shRNA-CT and shRNA-Vps34 B16-F10 and CT26 cells cultured in either control medium (+) or in medium containing Bafilomycin A1 (+) is reported in the right panels of A and B.

C: Experimental schedule and representative tumor images of control (shCT) or Vps34-targeted (shVps34) of B16-F10 melanoma.

D: The expression of LC3-I and -II in B16-F10, CT26, GEMM and Renca cells cultured in: control medium (+), medium containing Bafilomycin A1 (+), medium containing Vps34i SB02024 (+) or SAR405 (+), or medium containing both Bafilomycin A1 and Vps34i (SB02024 or SAR405). Actin was used as loading control. The experiments in A, B and C were repeated three times with the same results.

E, F, G and H: Experimental schedule and representative tumor images of B16-F10 melanoma (E); CT26 colorectal (F); GEMM (YUMM) melanoma (G); or Renca renal carcinoma (H) tumors in mice treated with control vehicle (vehicle) or Vps34i (SB02024 or SAR405).

Fig. S2

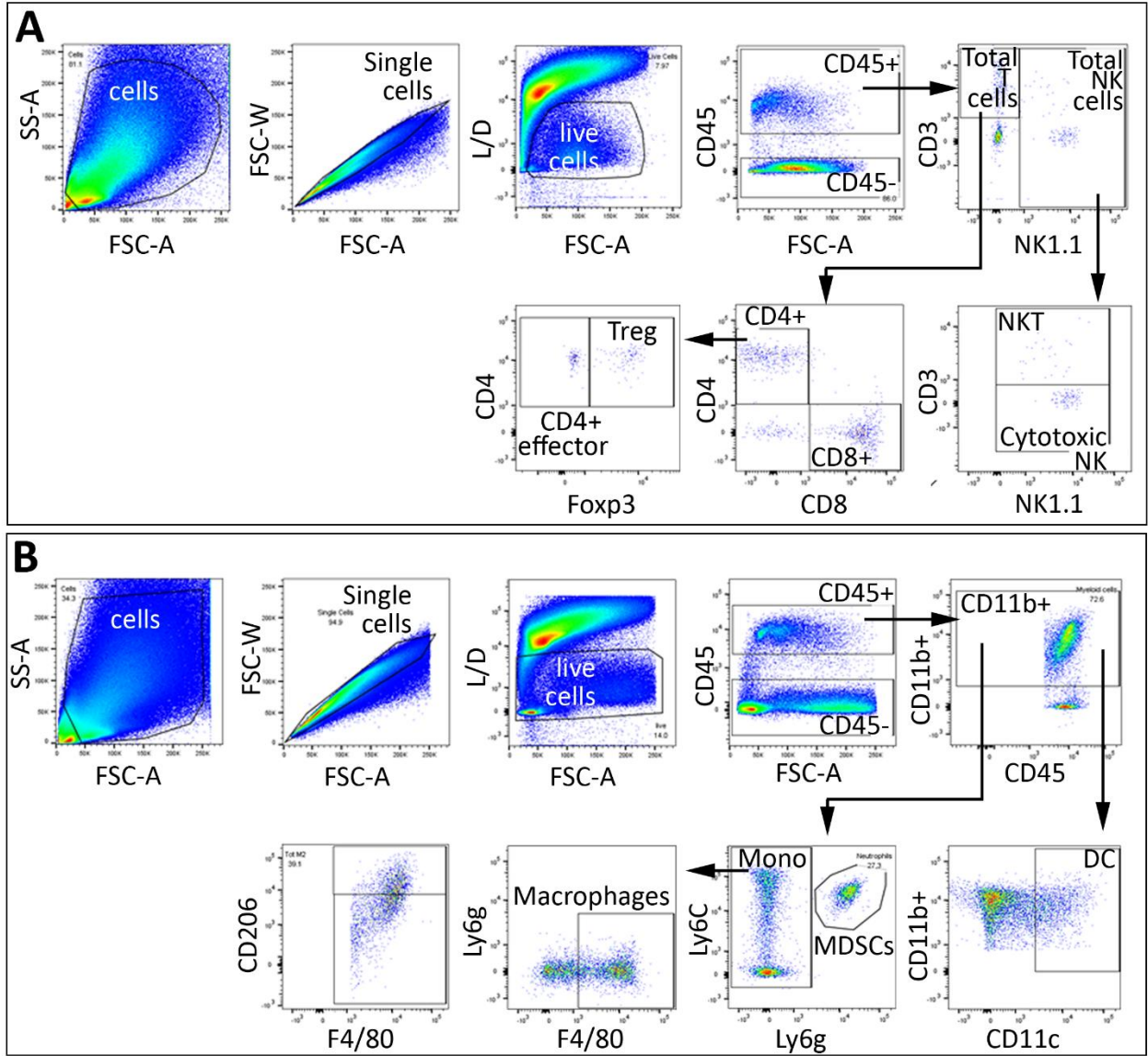


Fig. S2. Gating strategy for lymphoid and myeloid population in tumors

A: A Live/Dead dye was used to select only live cells. CD45+ CD3- NK1.1+ cells were defined as NK cells. Lymphocytes were defined as the CD3+ subpopulation of the CD45+ NK1.1- gate. CD4+ and CD8+ T lymphocytes were derived from the CD3+ subpopulation. Tregs were subdivided from CD4+ T lymphocytes and were defined as Foxp3+ and CD4+ Foxp3- cells were considered to be CD4+ T effector cells population.

B: CD45+ CD11b+ cells were defined as a subset of live cells. DC were defined as the CD11c+ subpopulation of the CD45+ CD11b+ subset. MDSC were defined as the Ly6G+ Ly6C- subpopulation of the CD45+ CD11b+ subset. The Ly6C+ Ly6G- subpopulation was defined as the monocytic subpopulation. Total macrophages were defined as the F4/80+ subpopulation of the CD45+ CD11b+ subset. Inflammatory anti-tumoral macrophages (M1) were defined as F4/80+ CD206-, and pro-tumoral macrophages (M2) were defined as F4/80+ CD206+ subpopulations of the F4/80+ CD45+ CD11b+ cells. The percentages of the different immune cell populations defined above were calculated by reporting back to the total CD45+ live cells.

Fig. S3

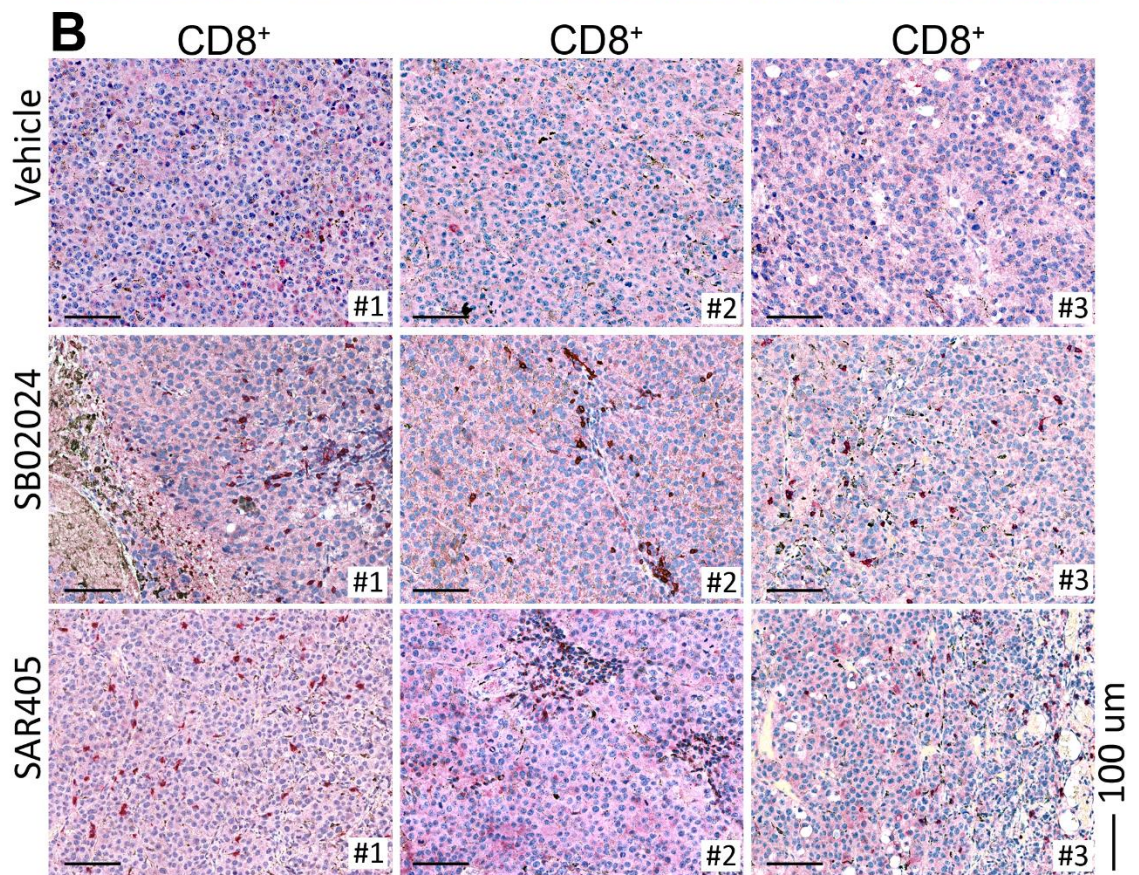
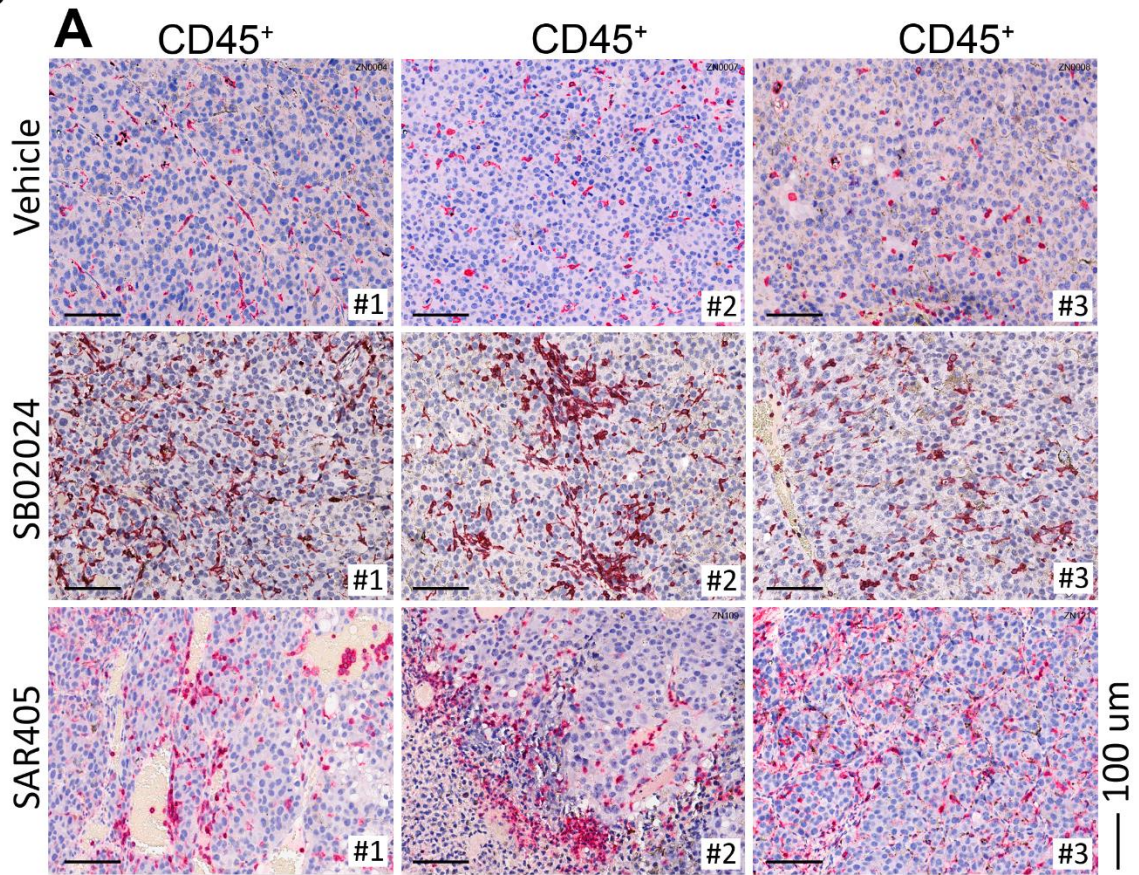


Fig. S3. Immunohistochemical staining of CD45+ and CD3+ cells on tumors.

A: Immunohistochemical staining of CD45 on vehicle (upper panels), SB02024 (middle panels), and SAR405 (lower panels) treated B16-F10 tumors (n=3 tumors/group #1, #2 and #3). Scale bar: 100 μm .

B: Immunohistochemical staining of CD8 on vehicle (upper panels), SB02024 (middle panels), and SAR405 (lower panels) treated B16-F10 tumors (n=3 tumors/group #1, #2 and #3). Scale bar: 100 μm .

Fig. S4

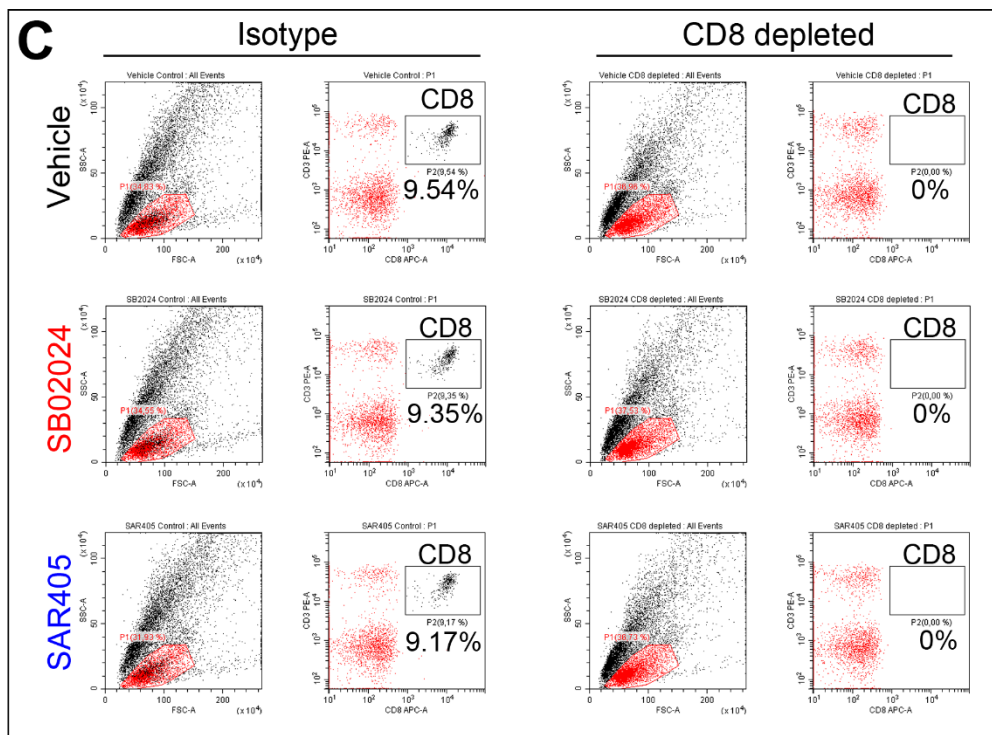
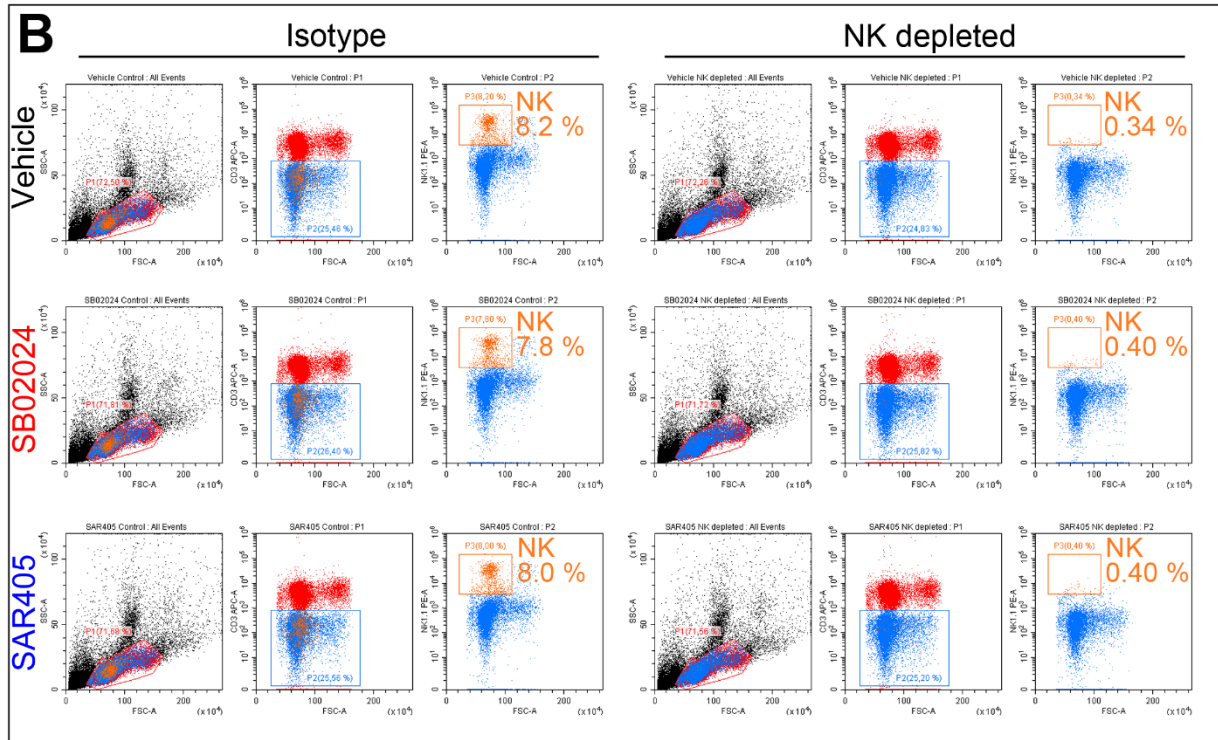
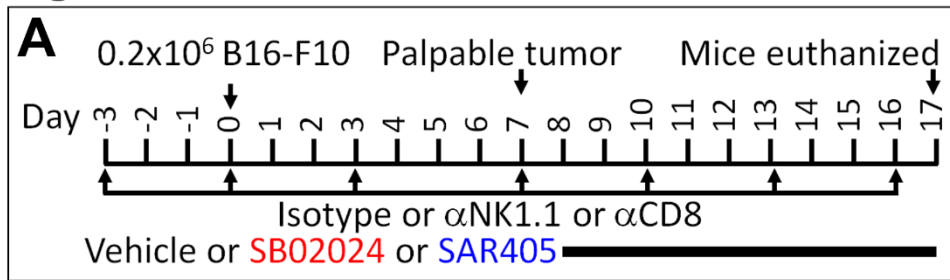


Fig. S4. Depletion of NK or CD8 cells in C57BL/6 mice

A: Schematic representation of the NK cell or CD8 depletion strategy in vehicle, SB02024- SAR405-treated B16-F10 tumor-bearing mice. Mice were injected intraperitoneally (i.p.) with 250 µg of either control isotype or αNK1.1 or αCD8 antibody on the indicated days (black arrows). B16-F10 tumor cells (0.2×10^6) were injected into syngeneic C57BL/6 mice and palpable tumors appeared on day 7. Tumor bearing mice were treated with either vehicle, SB02024 or SAR405 daily from day 8 to day 17. Three and 11 days post-injection of the first depletion antibodies (days 0 and 8, respectively), the efficiency of the NK and CD8 depletion was determined by FACS staining on blood samples of mice chosen randomly.

B: Flow cytometry dot plot analysis of NK cell population in representative blood samples from B16-F10 tumor bearing mice treated with either vehicle, SB02024 or SAR405 and previously injected with control isotype or NK-depleted antibody. NK1.1+ subset was defined in the selected cell population as CD3- NK1.1+ live cells.

C: Flow cytometry dot plot analysis of CD8 cell population in representative blood samples from B16-F10 tumor bearing mice treated with either vehicle, SB02024 or SAR405 and previously injected with control isotype or CD8-depleted antibody. CD8+ subset was defined in the selected cell population as CD3+ CD8+ live cells.

Fig. S5

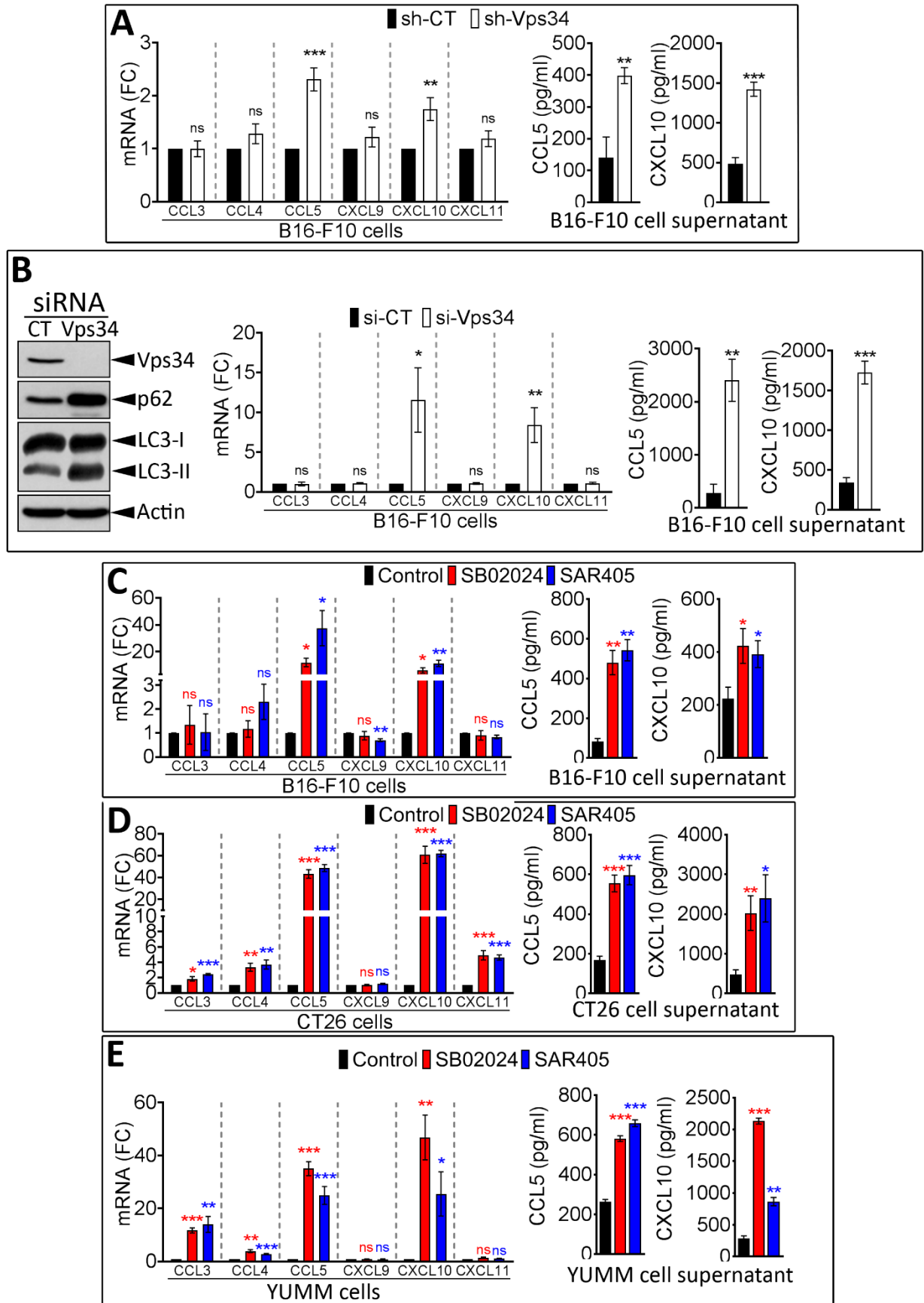
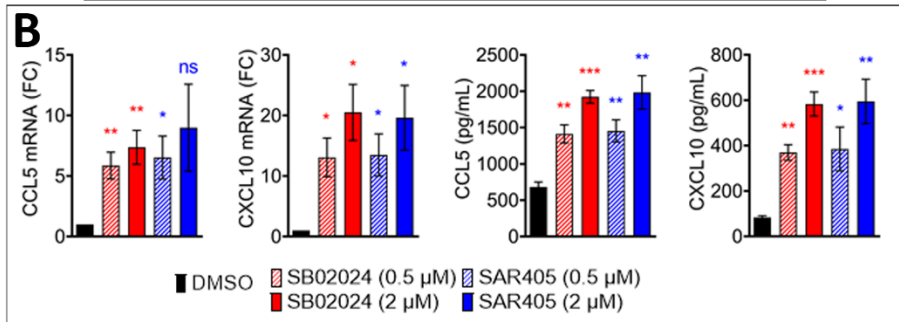
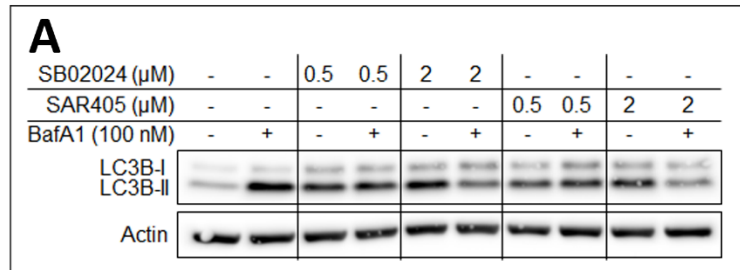


Fig. S5. Impact of genetic and pharmacological inhibition of Vps34 on the expression and the release of CCL5 and CXCL10 in tumor cells.

A, B, C, D and E: The mRNA expression of CCL3, CCL4, CCL5, CXCL9, CXCL10 and CXCL11 in the following cells: control (shCT) or Vps34-targeted (shVps34) B16-F10 melanoma (**A**); siRNA (CT) or siRNA targeting Vps34 (Vps34) B16-F10 melanoma (**B**); B16-F10 melanoma (**C**); CT26 colorectal (**D**) and YUMM GEMM melanoma cells (**E**) treated with control DMSO (control) or Vps34i (SB02024 or SAR405). Results are reported as fold change (FC). The quantification of the secreted CCL5 and CXCL10 protein levels by ELISA in cells described in A, B, C, D and E is shown in the right of each panel. Results are reported in pg/ml of cell supernatant. Data in A, B, C, D and E are reported as the average of 3 or 6 independent experiments (performed in triplicate). Results are shown as mean \pm SEM (error bars). Statistically significant differences (indicated by asterisks) calculated compared to control conditions using an unpaired two-tailed Student's t-test are shown (ns= not significant, * = $p < 0.05$, ** = $p < 0.005$ and ***= $p < 0.0005$).

Fig. S6



C

| Cancer type | CRC | m CRC | CAC | NSCLC | TNBC | AML | ML | CLL | ML | | |
|---------------|------------------|---------------|-------------|-------------|------------|--------------|-------------|-----------------|--------------|---------------|-------------|
| Vps34i | Cell line | HCT116 | MC38 | DLD1 | PC9 | H1299 | T47D | Kasumi-1 | THP-1 | OCI-M1 | U937 |
| SB02024 | CCL5 | 2.80 | 3.00 | 1.23 | 2.24 | 2.66 | 2.34 | 0.87 | 12.30 | 9.64 | 3.80 |
| | CXCL10 | 8.00 | 3.00 | 1.48 | 0.94 | 1.66 | 1.72 | 4.51 | 30.00 | 42.88 | 10.49 |
| SAR405 | CCL5 | 2.90 | 3.20 | 1.15 | 1.69 | 2.09 | 2.18 | 1.12 | 10.00 | 8.10 | 3.88 |
| | CXCL10 | 3.04 | 4.00 | 1.45 | 1.08 | 1.26 | 2.24 | 6.04 | 25.00 | 42.30 | 11.34 |

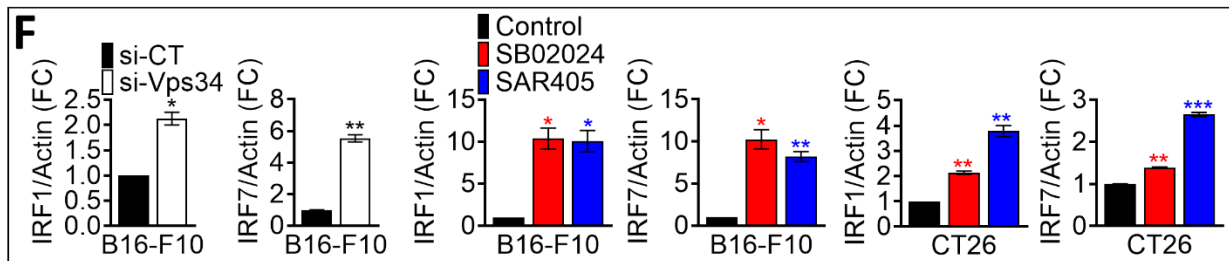
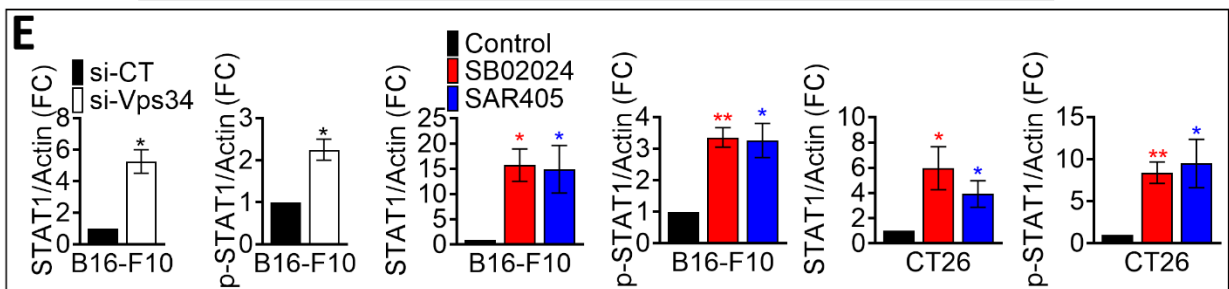
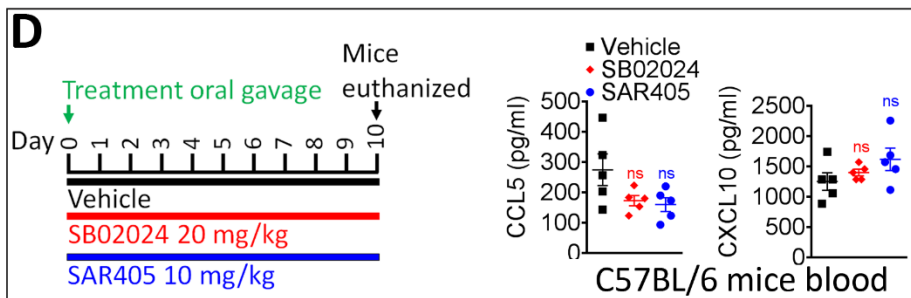


Fig. S6. Effect of Vps34i treatment on the release of CCL5 and CXCL10 in a panel of mouse and human cell lines and on the expression of STAT and IRF7 in B16-F10 and CT26 cells.

A: Expression of LC3-I and LC3-II proteins in Human Me30966 melanoma cells treated with SB02024 or SAR405 (0.5 and 2 μ M) for 24 hours, in absence (-) or presence (+) of 100 nM Bafilomycin A1 (BafA1) during the last 4 hours incubation. The experiment was repeated three times with the same results.

B: The mRNA expression levels of indicated chemokines in Me30966 cells treated with either control (DMSO), SB02024 or SAR405 (left panels). Results are reported as fold change (FC) compared to control conditions and represent the average of three independent RT-qPCR experiments, each condition performed in duplicate. Quantification of the secreted CCL5 and CXCL10 protein levels by ELISA in the supernatant of Me30966 melanoma cells treated with either control (DMSO), SB02024 or SAR405 (right panels). Results are reported in pg/ml and represent the average of three independent experiments. Results are shown as mean \pm SEM (error bars). Statistically significant differences (indicated by asterisks) are calculated using an unpaired two-tailed Student's t-test (ns= not significant, * = $p < 0.05$, ** = $p < 0.005$ and ***= $p < 0.0005$).

C: Effect of Vps34i (SB02024 and SAR405) on the secretion of CCL5 and CXCL10 protein in human and mouse cancer cell lines. Results are shown as ratio of Vps34i treated cells versus untreated (DMSO control) cells. CRC : Colorectal carcinoma ; m CRC : mouse Colorectal carcinoma ; CAC : Colon adenocarcinoma ; NSCLC : Non-small-cell lung carcinoma ; TNBC : Triple negative breast cancer ; AML : Acute myeloblastic leukemia ; ML : Monocytic leukemia ; CLL : Chronic lymphocytic leukemia ; ML : Myeloid leukaemia U937.

D: Left panel: Schematic representation of the treatment strategy with either vehicle, SB02024 (20 mg/kg) or SAR405 (10 mg/kg) of naïve tumor free C57BL/6 mice. Mice were treated daily by oral gavage for day 0 to day 10. **Right panel:** Quantification of the secreted CCL5 and CXCL10 protein levels by ELISA in the blood samples of mice treated according the strategy described in left panel. Results are reported in pg/ml and represent the average of five mice per group. Results are shown as mean \pm SEM (error bars). Statistically significant differences (indicated by asterisks) are calculated using an unpaired two-tailed Student's t-test (ns= not significant).

E: Western blot densitometry quantification of the STAT1 or pSTAT1 protein level in control (siCT) or Vps34-targeted (siVps34) B16-F10 melanoma cells (left panels); B16-F10 melanoma cells (middle panels) and CT26 colorectal cancer cells (right panels) treated with control (DMSO) or Vps34i (SB02024 or SAR405). Results are reported as fold change (FC) of STAT1 or pSTAT1^{Tyr701} signals to actin signal ratio compared to control conditions and represent the average of 3 independent western blot experiments.

F: Western blot densitometry quantification of the IRF1 or IRF7 protein level in control (siCT) or Vps34-targeted (siVps34) B16-F10 melanoma cells (left panels); B16-F10 melanoma cells (middle panels) and CT26 colorectal cancer cells (right panels) treated with control (DMSO) or Vps34i (SB02024 or SAR405). Results are reported as fold change (FC) of IRF1 or IRF7 signals to actin signal ratio compared to control conditions and represent the average of 3 independent western blot experiments.

Fig. S7

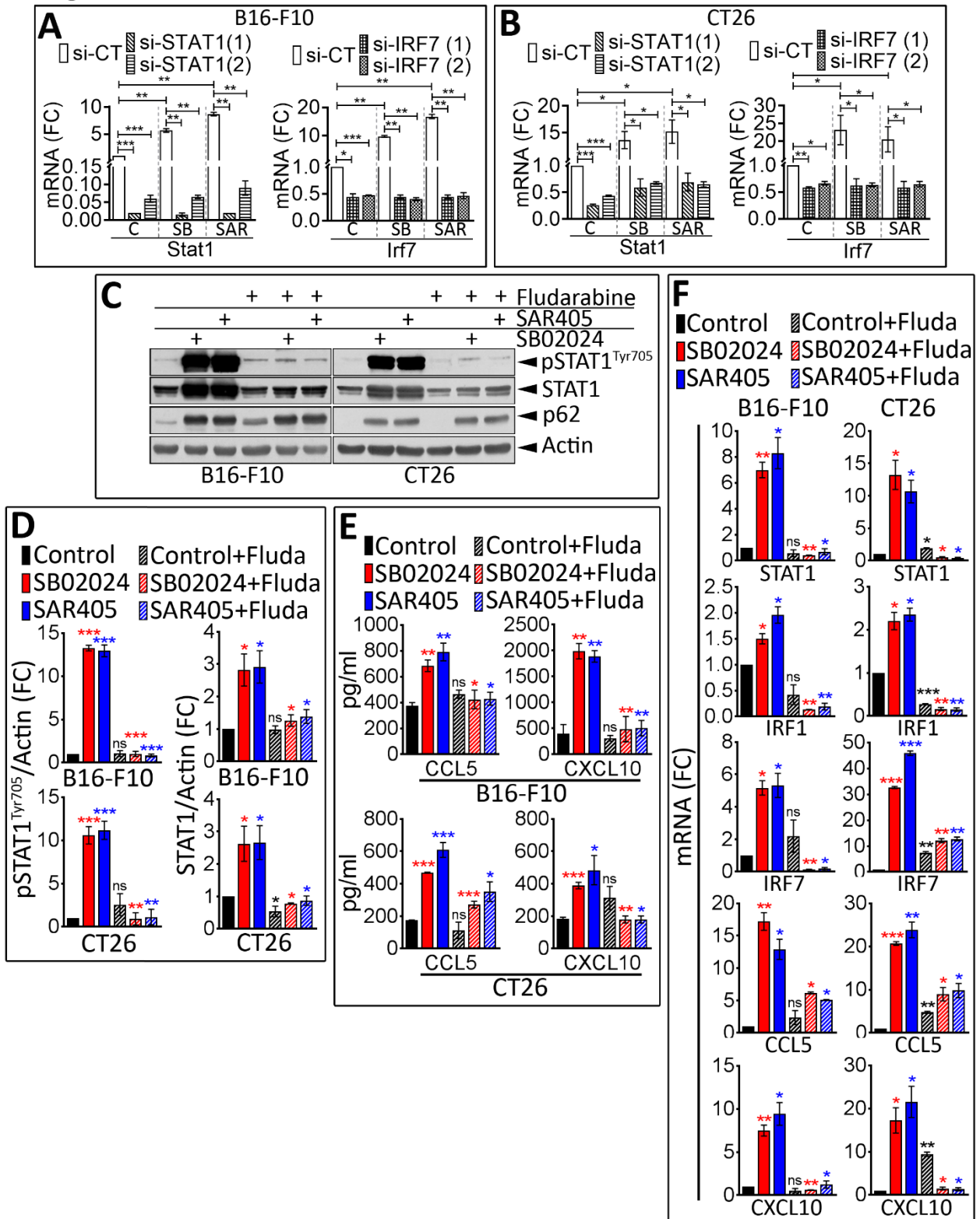


Fig. S7. STAT1/IRF7 axis is involved in the upregulation of CCL5 and CXCL10 in Vps34i (SB02024 or SAR405)-treated tumor cells

A and B: The expression of STAT1 and IRF7 mRNA by RT-qPCR in control (C), SB02024 (SB) or SAR405 (SAR) treated B16-F10 melanoma (A) cells and CT26 colorectal cancer cells (B) transfected with control siRNA (si-CT) or two different siRNA sequences (1 or 2) targeting either STAT1 or IRF7. Results are reported as fold change (FC) compared to control conditions and represent the average of 2 independent RT-qPCR experiments, each condition performed in triplicate.

C: The expression of pSTAT1Tyr701 and total STAT1 protein levels by western blot in B16-F10 melanoma cells (left panels) and CT26 cells (right panels) untreated (-) or treated with SB02024 or SAR405 alone or in combination with STAT1 inhibitor Fludarabine. The expression of p62 was used to show the inhibition of autophagy by SB02024 or SAR405 treatment and the expression of β -actin used as a loading control.

D: Western blot densitometry quantification of pSTAT1 (left panels) and STAT1 (right panels) protein levels in B16-F10 melanoma cells (upper panels) and CT26 colorectal cancer cells (lower panels) treated with DMSO control (Control) or Vps34i (SB02024 or SAR405) in the absence or presence (+Fluda) of Fludarabine. Results are reported as fold change (FC) of pSTAT1Tyr705 or STAT1 signals to actin signal ratio compared to control conditions and represent the average of 3 independent western blot experiments.

E: ELISA quantification of CCL5 and CXCL10 protein levels in the supernatants B16-F10 and CT26 cells treated with control (DMSO) or Vps34i (SB02024 or SAR405) in the absence or presence (+Fluda) of Fludarabine. Results are reported in pg/ml and represent the average of 3 independent ELISA experiments, each condition performed in triplicate.

F: The expression of STAT1, IRF1, IRF7, CCL5 and CXCL10 mRNA by RT-qPCR in B16-F10 melanoma cells (left panel) and CT26 colorectal cancer cells (right panel) treated with control (DMSO) or Vps34i (SB02024 or SAR405) in the absence or presence (+Fluda) of Fludarabine. Results are reported as fold change (FC) compared to control conditions and represent the average of 3 independent RT-qPCR experiments, each condition performed in triplicate.

Results in A, B, D, E and F are shown as mean \pm SEM (error bars). Statistically significant differences (indicated by asterisks) were calculated using an unpaired two-tailed Student's t-test (ns= not significant; * = $p < 0.05$, ** = $p < 0.005$ and ***= $p < 0.0005$).

Fig. S8

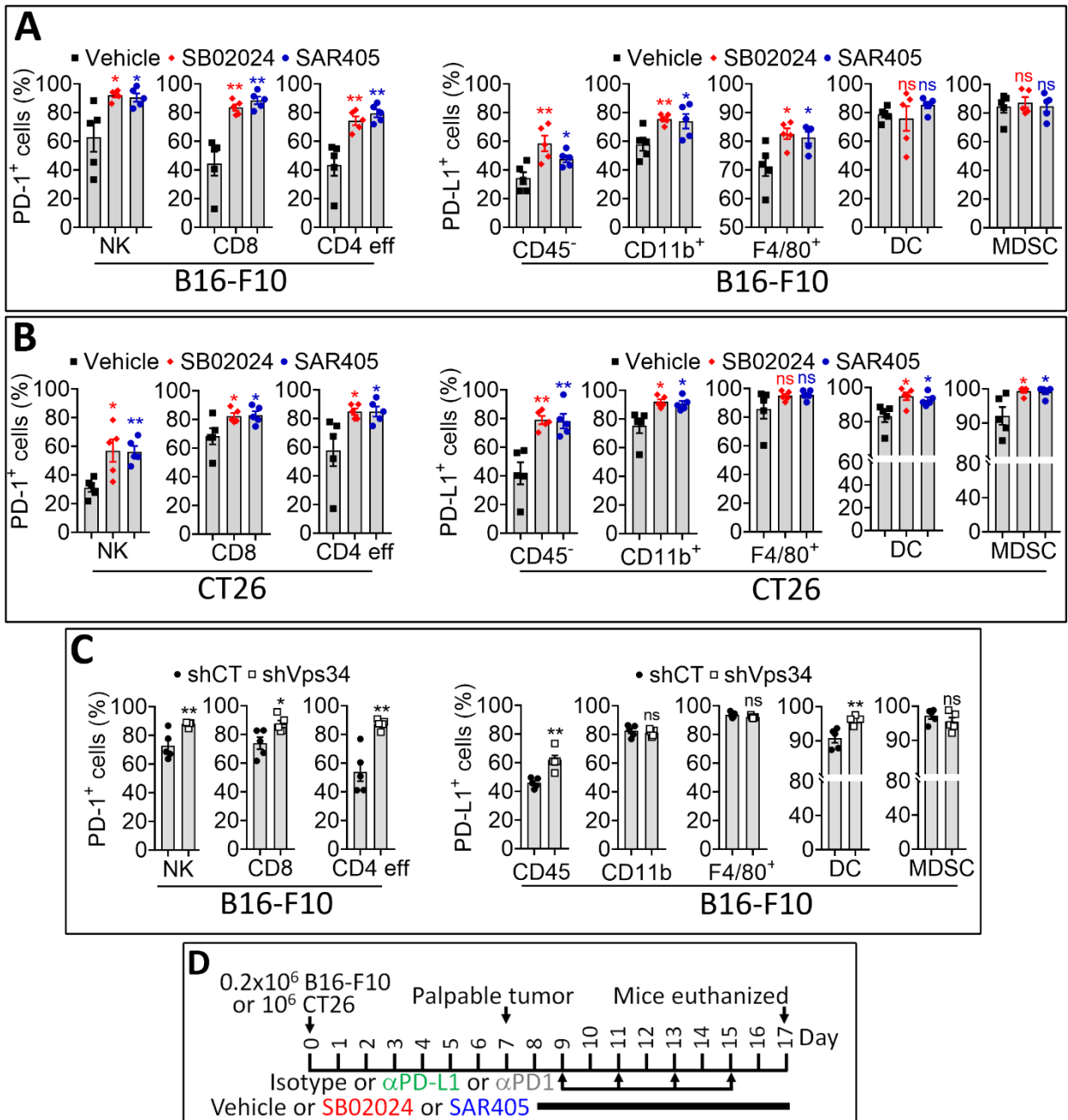


Fig. S8. CCL5 and CXCL10 protein levels in the blood of naïve tumor free mice and the expression of PD-1 and PD-L1 on various immune cells in Vps34-targeted tumors.

A, B and C: Left panels: Quantification of the percent of PD1+ NK, CD8 and CD4 eff T cells in control (shCT) or Vps34-targeted (sh-Vps34) B16-F10 melanoma (A); B16-F10 melanoma (B) and CT26 colorectal (C) tumors treated with control vehicle (vehicle) or Vps34i (SB02024 or SAR405).

Right panels: Quantification of the percent of PD-L1+ cells in CD45- tumor cells, CD11b+ cells, F480+ cells, DC and MDSC on tumor described in left panels. All of the experiments were conducted on well-established tumors on day 15. In all of the panels, the defined subpopulations were gated and quantified in live CD45+ cells. Each dot represents one tumor. The data are reported as the average of 5 mice per group. Results are shown as mean \pm SEM (error bars). Statistically significant differences (indicated by asterisks) calculated using an unpaired two-tailed Student's t-test are shown (ns= not significant, * = $P < 0.05$, ** = $P < 0.005$ and ***= $P < 0.0005$).

D: Schematic representation of the treatment strategy with a combination of Vps34i (SB02024 or SAR405) and anti-PD-L1 (α PD-L1) or anti-PD-1 (α PD1) in B16-F10 and CT26 tumor-bearing mice. 0.2×10^6 B16-F10 cells or 10^6 CT26 cells were subcutaneously injected into the right flank of syngeneic host C57BL/6 or Balb/C mice respectively at day 0. After the development of palpable tumors (typically at day 7), mice were treated with vehicle or Vps34i (SB02024 or SAR405) daily by oral gavage for 10 days (from day 8 to day 17). Mice were intraperitoneally (i.p.) injected with 200 μ g of α PD-L1, 100 μ g α PD1 or control isotype on day 9, 11, 13 and 15 (black arrows).

Fig. S9

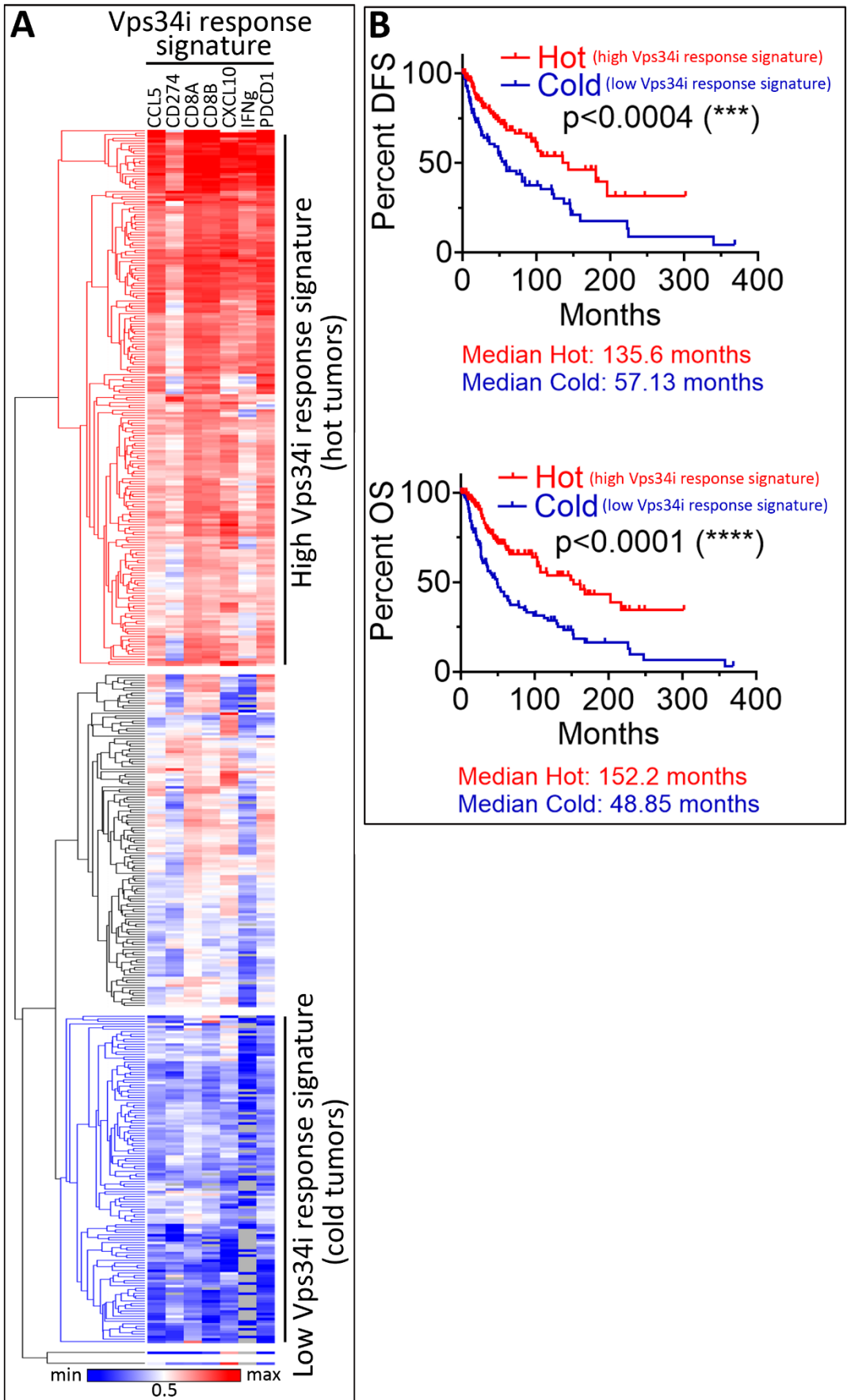


Fig. S9. Clustering melanoma patients into hot and cold tumors according to Vps34i response signature and defining their overall and disease free survivals.

A: Clustering of 473 melanoma samples from TCGA database according to Vps34i response signature (CCL5/CXCL10/IFN γ /PD-1/PD-L1/CD8A/CD8B). Melanoma patients were segregated into three well-defined clusters comprising tumors with high Vps34i signature (hot, n=211 patients) and tumors with low Vps34i response signature (cold, n=131).

B: Kaplan–Meier overall survival and disease free survival curves of the hot and cold clusters reported in A. Patients displaying immune hot and high Vps34i response signature have significantly improved both disease free survival (DFS), upper panel, (median: 135.6 months) and overall survival (OS), lower panel, (median: 152.2 months), than those showing immune cold and low Vps34i response signature.

Table S1

| Hot tumors (High Vps34i response signature) | | | | | Moderate tumors | | | | | Cold tumors (Low Vps34i response signature) | | | | |
|---|--------------|--------------|-----------------|--------|-----------------|--------------|--------------|-----------------|--------|---|--------------|--------------|-----------------|--------|
| # | Patient ID | Vital Status | Survival months | | # | Patient ID | Vital Status | Survival months | | # | Patient ID | Vital Status | Survival months | |
| | | | DFS | OS | | | | DFS | OS | | | | DFS | OS |
| 1 | TCGA-DA-A3F2 | dead 1 | 14.13 | 33.9 | 1 | TCGA-D3-A51K | alive 0 | #N/A | #N/A | 1 | TCGA-WE-A8ZM | alive 0 | 101.25 | 101.25 |
| 2 | TCGA-FR-A7UA | alive 0 | 38.24 | 38.24 | 2 | TCGA-EB-A4OY | alive 0 | #N/A | #N/A | 2 | TCGA-D3-A3MV | alive 0 | 0.53 | 45.27 |
| 3 | TCGA-D3-A3C8 | alive 0 | 5.78 | 46.29 | 3 | TCGA-XV-AB01 | alive 0 | #N/A | #N/A | 3 | TCGA-EE-A3AG | dead 1 | 24.8 | 41.56 |
| 4 | TCGA-D9-A6E9 | alive 0 | 9.89 | 9.89 | 4 | TCGA-XV-AAZY | alive 0 | #N/A | #N/A | 4 | TCGA-ER-A196 | alive 0 | 58.64 | 58.64 |
| 5 | TCGA-D3-A51F | alive 0 | 55.68 | 55.68 | 5 | TCGA-FS-A4F4 | dead 1 | #N/A | #N/A | 5 | TCGA-YG-AA3P | alive 0 | 14.42 | 14.42 |
| 6 | TCGA-EE-A29P | alive 0 | 54.8 | 56.37 | 6 | TCGA-EE-A2GT | alive 0 | #N/A | #N/A | 6 | TCGA-D3-A1Q5 | dead 1 | 80.55 | 112.48 |
| 7 | TCGA-D3-A8GJ | alive 0 | 47.4 | 241.2 | 7 | TCGA-EE-A183 | dead 1 | #N/A | #N/A | 7 | TCGA-BF-A3DN | alive 0 | 23.55 | 23.55 |
| 8 | TCGA-FR-A44A | alive 0 | 174.08 | 174.08 | 8 | TCGA-WE-AA9Y | alive 0 | #N/A | #N/A | 8 | TCGA-D9-A6EA | alive 0 | 25.16 | 25.16 |
| 9 | TCGA-EE-A3JE | alive 0 | 6.37 | 51.31 | 9 | TCGA-D3-A3C1 | alive 0 | #N/A | #N/A | 9 | TCGA-FS-A1ZZ | dead 1 | NA | 27 |
| 10 | TCGA-ER-A19W | dead 1 | 135.55 | 148.06 | 10 | TCGA-GN-A8LL | dead 1 | #N/A | #N/A | 10 | TCGA-EB-A431 | alive 0 | 18.66 | 18.66 |
| 11 | TCGA-EB-A55G | alive 0 | 45.07 | 68.2 | 11 | TCGA-FW-A3TU | dead 1 | #N/A | #N/A | 11 | TCGA-DA-A114 | dead 1 | 29.2 | 35.91 |
| 12 | TCGA-ER-A19S | alive 0 | 49.44 | 49.44 | 12 | TCGA-EE-A182 | dead 1 | #N/A | #N/A | 12 | TCGA-WE-A8K6 | alive 0 | 8.51 | 17.94 |
| 13 | TCGA-W3-AA1W | alive 0 | 25 | 218.99 | 13 | TCGA-DA-A110 | dead 1 | #N/A | #N/A | 13 | TCGA-BF-A3DM | alive 0 | 19.74 | 19.74 |
| 14 | TCGA-EE-A2M8 | dead 1 | 16.36 | 19.74 | 14 | TCGA-D3-A2JD | dead 1 | #N/A | #N/A | 14 | TCGA-D9-A4Z2 | dead 1 | 2.63 | 6.24 |
| 15 | TCGA-D3-A2J9 | dead 1 | 18.1 | 23.75 | 15 | TCGA-EE-A2M7 | dead 1 | 26.25 | 28.81 | 15 | TCGA-LH-A9QB | alive 0 | 368.5 | 368.5 |
| 16 | TCGA-DA-A3F8 | alive 0 | 43.33 | 43.33 | 16 | TCGA-EB-A3XF | alive 0 | #N/A | #N/A | 16 | TCGA-ER-A4ZK | dead 1 | 6.77 | 12.94 |
| 17 | TCGA-HR-A2OH | dead 1 | NA | 65.83 | 17 | TCGA-D9-A6EC | alive 0 | 77.5 | 77.5 | 17 | TCGA-FS-A1ZB | dead 1 | 48 | 48.82 |
| 18 | TCGA-WE-AA4A | alive 0 | 24.97 | 24.97 | 18 | TCGA-FS-A4F5 | dead 1 | NA | 28.71 | 18 | TCGA-EE-A3JI | dead 1 | 146.09 | 152.69 |
| 19 | TCGA-D3-A2JH | alive 0 | 18.69 | 42.05 | 19 | TCGA-XV-A9W2 | alive 0 | 13.7 | 13.7 | 19 | TCGA-D3-A3CC | alive 0 | 84.13 | 86.86 |
| 20 | TCGA-D3-A1QB | alive 0 | 30.98 | 95.66 | 20 | TCGA-GN-A8LN | alive 0 | 14.36 | 25.36 | 20 | TCGA-FS-A4F9 | alive 0 | 12.35 | 34 |
| 21 | TCGA-ER-A2NH | alive 0 | 41.52 | 41.52 | 21 | TCGA-EE-A2MM | dead 1 | #N/A | #N/A | 21 | TCGA-FR-A7U8 | alive 0 | 18.63 | 27.83 |
| 22 | TCGA-ER-A19P | dead 1 | 142.97 | 161.96 | 22 | TCGA-EE-A3JB | alive 0 | #N/A | #N/A | 22 | TCGA-D3-A1Q1 | dead 1 | 15.41 | 16.56 |
| 23 | TCGA-GF-A4EO | alive 0 | 19.42 | 19.42 | 23 | TCGA-EE-A3JA | dead 1 | #N/A | #N/A | 23 | TCGA-BF-AAP1 | alive 0 | 13.44 | 13.44 |
| 24 | TCGA-EE-A2ME | dead 1 | 99.11 | 103.19 | 24 | TCGA-OD-A75X | dead 1 | #N/A | #N/A | 24 | TCGA-FS-A1ZE | dead 1 | 25 | 46.42 |
| 25 | TCGA-D3-A3CB | alive 0 | 166.39 | 166.39 | 25 | TCGA-GN-A263 | dead 1 | #N/A | #N/A | 25 | TCGA-EE-A17Y | dead 1 | 16.66 | 27.2 |
| 26 | TCGA-EE-A2MR | alive 0 | 123.95 | 134.3 | 26 | TCGA-RP-A695 | alive 0 | #N/A | #N/A | 26 | TCGA-HR-A5NC | alive 0 | 5.06 | 32.85 |
| 27 | TCGA-GN-A26C | dead 1 | 24.8 | 26.97 | 27 | TCGA-EB-A5UN | alive 0 | #N/A | #N/A | 27 | TCGA-BF-AAP6 | alive 0 | 10.68 | 10.68 |
| 28 | TCGA-ER-A19M | dead 1 | 56.83 | 61.01 | 28 | TCGA-EB-A44P | alive 0 | #N/A | #N/A | 28 | TCGA-D9-A4Z5 | alive 0 | 7.16 | 7.16 |
| 29 | TCGA-EE-A2GH | alive 0 | 220.07 | 220.07 | 29 | TCGA-EB-A3XB | alive 0 | #N/A | #N/A | 29 | TCGA-DA-A118 | dead 1 | 42.97 | 53.88 |
| 30 | TCGA-D3-A8GB | dead 1 | 15.14 | 30.81 | 30 | TCGA-D3-A1QA | alive 0 | #N/A | #N/A | 30 | TCGA-EB-A82C | alive 0 | 0.56 | 0.56 |
| 31 | TCGA-W3-A824 | alive 0 | 79.04 | 227.99 | 31 | TCGA-BF-A1Q0 | alive 0 | #N/A | #N/A | 31 | TCGA-EB-A6QZ | dead 1 | NA | 11.56 |
| 32 | TCGA-EE-A2MU | alive 0 | 53.22 | 53.22 | 32 | TCGA-DA-A95X | alive 0 | #N/A | #N/A | 32 | TCGA-D3-A8GI | dead 1 | 57.13 | 58.48 |
| 33 | TCGA-ER-A193 | dead 1 | 26.48 | 31.37 | 33 | TCGA-EE-A2MQ | dead 1 | #N/A | #N/A | 33 | TCGA-FS-A1ZG | dead 1 | 4.6 | 9.69 |
| 34 | TCGA-WE-A8K1 | alive 0 | 49.01 | 49.01 | 34 | TCGA-BF-A3DJ | alive 0 | #N/A | #N/A | 34 | TCGA-XV-AAZW | dead 1 | NA | 12.91 |
| 35 | TCGA-EB-A851 | alive 0 | 11.89 | 11.89 | 35 | TCGA-FS-A4FC | dead 1 | #N/A | #N/A | 35 | TCGA-RP-A690 | alive 0 | 0.2 | 0.2 |
| 36 | TCGA-EE-A2GI | alive 0 | 48.69 | 48.69 | 36 | TCGA-ER-A19K | dead 1 | #N/A | #N/A | 36 | TCGA-FS-A1ZS | alive 0 | 148.69 | 148.69 |
| 37 | TCGA-EB-A4IS | alive 0 | 25.43 | 25.43 | 37 | TCGA-ER-A198 | dead 1 | #N/A | #N/A | 37 | TCGA-ER-A19L | dead 1 | 121.19 | 131.41 |
| 38 | TCGA-EB-A44Q | alive 0 | 13.86 | 13.86 | 38 | TCGA-W3-AA1V | dead 1 | #N/A | #N/A | 38 | TCGA-GN-A4U3 | alive 0 | 90.54 | 121.81 |
| 39 | TCGA-EE-A181 | dead 1 | 25.89 | 33.71 | 39 | TCGA-D3-A51N | alive 0 | 0.59 | 22.6 | 39 | TCGA-EE-A29Q | dead 1 | NA | 66.69 |
| 40 | TCGA-D3-A3CE | dead 1 | 51.48 | 60.18 | 40 | TCGA-EB-A51B | alive 0 | #N/A | #N/A | 40 | TCGA-D3-A5GL | alive 0 | 35.87 | 125.69 |
| 41 | TCGA-GN-A4U8 | alive 0 | 48.85 | 48.85 | 41 | TCGA-DA-A112 | dead 1 | #N/A | #N/A | 41 | TCGA-D3-A8GR | dead 1 | 124.01 | 129.53 |
| 42 | TCGA-EB-A85J | alive 0 | 4.53 | 11.83 | 42 | TCGA-YD-A9TB | alive 0 | #N/A | #N/A | 42 | TCGA-ER-A19C | dead 1 | NA | 48.85 |
| 43 | TCGA-DA-A1HW | dead 1 | 27.99 | 36.01 | 43 | TCGA-EE-A2GS | dead 1 | #N/A | #N/A | 43 | TCGA-W3-AA1O | dead 1 | NA | 4.01 |
| 44 | TCGA-EE-A3JD | dead 1 | 13.07 | 27.33 | 44 | TCGA-FS-A1ZR | dead 1 | #N/A | #N/A | 44 | TCGA-BF-A3DL | alive 0 | 25.26 | 25.26 |
| 45 | TCGA-FR-A729 | alive 0 | 206.93 | 220.63 | 45 | TCGA-EB-A3XE | alive 0 | #N/A | #N/A | 45 | TCGA-EE-A17X | dead 1 | 17.44 | 29.8 |
| 46 | TCGA-DA-A1HV | alive 0 | 28.45 | 76.51 | 46 | TCGA-EB-A3XD | alive 0 | #N/A | #N/A | 46 | TCGA-EE-A29R | alive 0 | 14.03 | 14.45 |
| 47 | TCGA-EE-A2MJ | dead 1 | 41.23 | 96.16 | 47 | TCGA-ER-A3EV | dead 1 | #N/A | #N/A | 47 | TCGA-BF-A5EP | alive 0 | 11.01 | 11.01 |
| 48 | TCGA-GN-A4U5 | alive 0 | 37.98 | 37.98 | 48 | TCGA-EB-A553 | alive 0 | #N/A | #N/A | 48 | TCGA-EE-A2A5 | dead 1 | 36.7 | 39.26 |
| 49 | TCGA-ER-A19A | alive 0 | 53.12 | 77.69 | 49 | TCGA-EE-A3AB | alive 0 | 122.63 | 122.63 | 49 | TCGA-GN-A268 | dead 1 | 59.33 | 62.75 |
| 50 | TCGA-D3-A1Q7 | alive 0 | 35.78 | 133.15 | 50 | TCGA-DA-A117 | alive 0 | #N/A | #N/A | 50 | TCGA-GN-A264 | dead 1 | 105.81 | 117.84 |
| 51 | TCGA-D3-A2J8 | dead 1 | 59.59 | 65.44 | 51 | TCGA-EB-A55H | alive 0 | 34.99 | 53.98 | 51 | TCGA-EE-A29C | dead 1 | 72.67 | 78.91 |
| 52 | TCGA-EB-A551 | alive 0 | 19.38 | 19.38 | 52 | TCGA-W3-AA21 | dead 1 | #N/A | #N/A | 52 | TCGA-FS-A1Z7 | dead 1 | 4.47 | 7.79 |
| 53 | TCGA-3N-A9WC | alive 0 | 56.01 | 66.43 | 53 | TCGA-ER-A19T | dead 1 | 3.88 | 8.87 | 53 | TCGA-FW-A5DX | alive 0 | 21.02 | 21.02 |
| 54 | TCGA-EE-A3JH | alive 0 | 108.71 | 134.23 | 54 | TCGA-Z2-AA3S | alive 0 | #N/A | #N/A | 54 | TCGA-FS-A1Z3 | dead 1 | 9.07 | 20.89 |
| 55 | TCGA-GN-A4U8 | alive 0 | 48.85 | 48.85 | 55 | TCGA-FW-A3TV | alive 0 | #N/A | #N/A | 55 | TCGA-EB-A5VU | dead 1 | 7.13 | 10.55 |
| 56 | TCGA-BF-AAP0 | alive 0 | 14.91 | 14.91 | 56 | TCGA-D3-A8G0 | dead 1 | #N/A | #N/A | 56 | TCGA-DA-A1HY | alive 0 | 144.78 | 144.78 |
| 57 | TCGA-WE-A8K4 | alive 0 | 20.17 | 20.17 | 57 | TCGA-BF-A5EO | alive 0 | #N/A | #N/A | 57 | TCGA-GF-A769 | dead 1 | NA | 35.15 |
| 58 | TCGA-D3-A8GD | alive 0 | 4.83 | 23.59 | 58 | TCGA-FR-A8YC | dead 1 | #N/A | #N/A | 58 | TCGA-FS-A1ZN | dead 1 | 11.86 | 23.98 |
| 59 | TCGA-EE-A2GE | alive 0 | 173.65 | 173.65 | 59 | TCGA-EE-A29S | dead 1 | #N/A | #N/A | 59 | TCGA-EE-A2G0 | alive 0 | 122.08 | 126.71 |
| 60 | TCGA-DA-A1IB | dead 1 | 7.98 | 40.57 | 60 | TCGA-EE-A3AF | dead 1 | #N/A | #N/A | 60 | TCGA-EE-A3AH | dead 1 | 137.45 | 138.7 |
| 61 | TCGA-D3-A2JO | alive 0 | 66.03 | 66.03 | 61 | TCGA-BF-AAP2 | alive 0 | #N/A | #N/A | 61 | TCGA-FR-A726 | dead 1 | 8.97 | 10.02 |
| 62 | TCGA-EE-A2MC | dead 1 | 35.61 | 61.47 | 62 | TCGA-EE-A29H | alive 0 | #N/A | #N/A | 62 | TCGA-XV-A9VZ | alive 0 | 0.36 | 0.36 |
| 63 | TCGA-ER-A2NG | dead 1 | 37.88 | 48.95 | 63 | TCGA-EE-A2MD | dead 1 | #N/A | #N/A | 63 | TCGA-EE-A3J8 | dead 1 | 34 | 34.3 |
| 64 | TCGA-D3-A51J | alive 0 | 65.97 | 145.01 | 64 | TCGA-D3-A3MU | alive 0 | #N/A | #N/A | 64 | TCGA-DA-A95Y | dead 1 | 11.1 | 14.13 |
| 65 | TCGA-D3-A3MR | alive 0 | 102.1 | 103.52 | 65 | TCGA-BF-A1PZ | alive 0 | #N/A | #N/A | 65 | TCGA-FS-A1Y1 | dead 1 | 222.63 | 228.42 |
| 66 | TCGA-EB-A5VV | alive 0 | 7.03 | 7.03 | 66 | TCGA-BF-A5ES | alive 0 | #N/A | #N/A | 66 | TCGA-BF-A5ER | alive 0 | 10.74 | 10.74 |
| 67 | TCGA-BF-A1PX | dead 1 | NA | 9.26 | 67 | TCGA-EE-A29G | dead 1 | #N/A | #N/A | 67 | TCGA-D3-A5GT | alive 0 | 16 | 16 |
| 68 | TCGA-YG-AA3N | alive 0 | 10.05 | 10.05 | 68 | TCGA-D3-A51T | alive 0 | #N/A | #N/A | 68 | TCGA-EB-A3Y7 | dead 1 | NA | 10.71 |
| 69 | TCGA-WE-A8ZN | alive 0 | 47.6 | 58.94 | 69 | TCGA-EB-A44N | dead 1 | #N/A | #N/A | 69 | TCGA-D3-A2JK | dead 1 | 8.8 | 12.09 |
| 70 | TCGA-EB-A4OZ | alive 0 | 20.37 | 20.37 | 70 | TCGA-EE-A2MF | dead 1 | #N/A | #N/A | 70 | TCGA-WE-A8ZT | alive 0 | 2.27 | 11.79 |
| 71 | TCGA-BF-AAP7 | alive 0 | 10.45 | 10.45 | 71 | TCGA-EE-A3J3 | dead 1 | #N/A | #N/A | 71 | TCGA-ER-A19B | alive 1 | 52.07 | 98.32 |
| 72 | TCGA-D3-A2JL | alive 0 | 98.39 | 171.45 | 72 | TCGA-EE-A2GM | alive 0 | #N/A | #N/A | 72 | TCGA-IH-A3EA | alive 0 | 10.32 | 17.21 |
| 73 | TCGA-D3-A51H | alive 0 | 27.53 | 56.31 | 73 | TCGA-YG-AA3O | dead 1 | #N/A | #N/A | 73 | TCGA-BF-A9VF | alive 0 | 14.45 | 14.45 |
| 74 | TCGA-EE-A3AE | alive 0 | 54.47 | 54.47 | 74 | TCGA-EE-A3J7 | alive 0 | #N/A | #N/A | 74 | TCGA-EE-A29B | alive 1 | 80.35 | 85.02 |
| 75 | TCGA-EE-A3J5 | dead 1 | 36.53 | 36.93 | 75 | TCGA-FS-A1ZJ | dead 1 | #N/A | #N/A | 75 | TCGA-EB-A3HV | alive 0 | 1.28 | 1.28 |
| 76 | TCGA-FS-A1ZH | dead 1 | 18.96 | 32.72 | 76 | TCGA-XV-A9W5 | alive 0 | #N/A | #N/A | 76 | TCGA-DA-A11A | alive 1 | NA | 65.87 |
| 77 | TCGA-FR-A728 | alive 0 | 2.1 | 19.15 | 77 | TCGA-EB-A4P0 | dead 1 | #N/A | #N/A | 77 | TCGA-BF-AAOX | alive 0 | 14.59 | 14.59 |
| 78 | TCGA-D3-A3BZ | alive 0 | 76.87 | 130.62 | 78 | TCGA-ER-A2ND | dead 1 | #N/A | #N/A | 78 | TCGA-D3-A8GV | alive 1 | 158.94 | 167.58 |
| 79 | TCGA-D3-A8GN | alive 0 | 25.85 | 160.87 | 79 | TCGA-D3-A8GQ | dead 1 | #N/A | #N/A | 79 | TCGA-BF-AAOU | alive 0 | 15.64 | 15.64 |
| 80 | TCGA-ER-A19S | dead 1 | NA | 35.41 | 80 | TCGA-WE-AAAO | alive 0 | #N/A | #N/A | 80 | TCGA-FS-A1ZQ | alive 1 | #N/A | #N/A |
| 81 | TCGA-ER-A19N | dead 1 | 0 | 44.05 | 81 | TCGA-FW-A3R5 | alive 0 | #N/A | #N/A | 81 | TCGA-FR-A8YD | alive 1 | NA | 36.24 |
| 82 | TCGA-EE-A2GU | alive 0 | 94.74 | 94.74 | 82 | TCGA-EE-A2M6 | alive 0 | 129.17 | 129.17 | 82 | TCGA-DA-A3F5 | alive 1 | 224.74 | 225.79 |

| | | | | | |
|-----|--------------|-------|---|--------|--------|
| 166 | TCGA-EE-A2MS | alive | 0 | #N/A | #N/A |
| 167 | TCGA-EB-A430 | alive | 0 | #N/A | #N/A |
| 168 | TCGA-DA-A95W | alive | 0 | #N/A | #N/A |
| 169 | TCGA-D3-A51G | alive | 0 | #N/A | #N/A |
| 170 | TCGA-EE-A20B | alive | 0 | #N/A | #N/A |
| 171 | TCGA-D3-A3C3 | alive | 0 | #N/A | #N/A |
| 172 | TCGA-BF-A5EQ | alive | 0 | #N/A | #N/A |
| 173 | TCGA-XV-AAZV | alive | 0 | #N/A | #N/A |
| 174 | TCGA-ER-A19D | dead | 1 | #N/A | #N/A |
| 175 | TCGA-RP-A694 | alive | 0 | #N/A | #N/A |
| 176 | TCGA-EB-A299 | alive | 0 | #N/A | #N/A |
| 177 | TCGA-EB-A3Y6 | alive | 0 | #N/A | #N/A |
| 178 | TCGA-EE-A2A2 | alive | 0 | #N/A | #N/A |
| 179 | TCGA-D3-A1QA | alive | 0 | #N/A | #N/A |
| 180 | TCGA-FS-A4FD | dead | 1 | #N/A | #N/A |
| 181 | TCGA-EB-A41B | alive | 0 | #N/A | #N/A |
| 182 | TCGA-W3-AA1R | dead | 1 | #N/A | #N/A |
| 183 | TCGA-FS-A1ZA | dead | 1 | #N/A | #N/A |
| 184 | TCGA-YD-A9TA | alive | 0 | #N/A | #N/A |
| 185 | TCGA-GN-A26A | dead | 1 | #N/A | #N/A |
| 186 | TCGA-D3-A8GM | dead | 1 | NA | 107.06 |
| 187 | TCGA-D3-A5GO | alive | 0 | 79.37 | 137.81 |
| 188 | TCGA-EB-A4XL | alive | 0 | #N/A | #N/A |
| 189 | TCGA-WE-A8K5 | dead | 1 | #N/A | #N/A |
| 190 | TCGA-D9-A3Z4 | dead | 1 | #N/A | #N/A |
| 191 | TCGA-EB-A97M | alive | 0 | #N/A | #N/A |
| 192 | TCGA-D9-A148 | alive | 0 | #N/A | #N/A |
| 193 | TCGA-EE-A20I | dead | 1 | #N/A | #N/A |
| 194 | TCGA-FR-A8YE | alive | 0 | #N/A | #N/A |
| 195 | TCGA-D3-A1Q3 | dead | 1 | #N/A | #N/A |
| 196 | TCGA-W3-AA1Q | dead | 1 | #N/A | #N/A |
| 197 | TCGA-D9-A3Z3 | alive | 0 | #N/A | #N/A |
| 198 | TCGA-EE-A29X | dead | 1 | #N/A | #N/A |
| 199 | TCGA-EE-A184 | dead | 1 | #N/A | #N/A |
| 200 | TCGA-GF-A6C9 | alive | 0 | 15.77 | 15.77 |
| 201 | TCGA-EB-A5UM | alive | 0 | #N/A | #N/A |
| 202 | TCGA-D3-A3C7 | alive | 0 | #N/A | #N/A |
| 203 | TCGA-D3-A2JP | alive | 0 | #N/A | #N/A |
| 204 | TCGA-W3-A825 | dead | 1 | #N/A | #N/A |
| 205 | TCGA-D3-A5GU | alive | 0 | #N/A | #N/A |
| 206 | TCGA-EE-A2MH | dead | 1 | #N/A | #N/A |
| 207 | TCGA-GN-A267 | dead | 1 | #N/A | #N/A |
| 208 | TCGA-GN-A9SD | dead | 1 | #N/A | #N/A |
| 209 | TCGA-D3-A2JN | dead | 1 | #N/A | #N/A |
| 210 | TCGA-EB-A6R0 | dead | 1 | NA | 19.97 |
| 211 | TCGA-ER-A19G | alive | 0 | 301.84 | 301.84 |

Table S1: Information about TCGA melanoma patient described in Fig. S8. The table shows TCGA patient ID, vital status, and both disease free survival (DFS) and overall survivals (OS) reported in months. Missing values are annotated as #N/A.