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Supplementary Materials for

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

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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. 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 F480+ 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 F480+ 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. 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 μ m.

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 μ m.



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- SAR405treated B16-F10 tumor-bearing mice. Mice were injected intraperitoneally (i.p.) with 250 μ g of either control isotype or α NK1.1 or α CD8 antibody on the indicated days (black arrows). B16-F10 tumor cells (0.2x106) 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. 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. 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 uM) 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 ± 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 pSTAT1Tyr701 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. 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. 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. 0.2x10⁶ B16-F10 cells or 10⁶ 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 200µg of α PD-L1, 100µg α PD1 or control isotype on day 9, 11, 13 and 15 (black arrows).



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	Surviva	months	#	Patient ID	Vital Stat	us	Survival	months	#	Patient ID	Vital Status	Survival	months
1		dead 1	DFS	33.0	1	TCG4-D2-451V	alivo 0	#	DFS tN/A	05 #N/A	1	TCGA-\\/F- ^9714	alive 0	DFS	05 101 25
2	TCGA-DA-A3FZ	alive 0	38.24	38.24	2	TCGA-DS-ASIK	alive 0	# #	tN/A	#N/A	2	TCGA-WE-A82IM	alive 0	0.53	45.27
3	TCGA-D3-A3C8	alive 0	5.78	46.29	3	TCGA-LB-A401	alive 0	#	tN/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	#	ŧN/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	#	, ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN∕A	#N/A	10	TCGA-EB-A431	alive 0	18.66	18.66
11	TCGA-EB-A5SG	alive 0	45.07	68.2	11	TCGA-FW-A3TU	dead 1	#	ŧN/A	#N/A	11	TCGA-DA-A1I4	dead 1	29.2	35.91
12	TCGA-ER-A19S	alive 0	49.44	49.44	12	TCGA-EE-A182	dead 1	#	ŧN/A	#N/A	12	TCGA-WE-A8K6	alive 0	8.51	17.94
13	TCGA-W3-AATW	alive U	25 16 26	218.99	13	TCGA-DA-ATIU	dead 1	#	FN/A FNI/A	#N/A #N/A	13	TCGA-BF-A3DM	alive U	19.74	19.74
14	TCGA-EE-A2108	dead 1	18.50	23 75	14	TCGA-DS-A2JD	dead 1	2	4N/A 26.25	#IN/A 28.81	14	TCGA-D9-A422	alive 0	2.05	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-A42K	dead 1	6.77	12.94
17	TCGA-HR-A2OH	dead 1	NA	65.83	17	TCGA-D9-A6EC	alive 0	7	7.5	77.5	17	TCGA-FS-A1ZB	dead 1	48	48.82
18	TCGA-WE-AAA4	alive 0	24.97	24.97	18	TCGA-FS-A4F5	dead 1	N	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	1	.3.7	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	1	4.36	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	#	ŧN/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	#	ŧN/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-A3J4	dead 1	#	ŧN∕A	#N/A	23	TCGA-BF-AAP1	alive 0	13.44	13.44
24	TCGA-EE-A2ME	dead 1	99.11	103.19	24	ICGA-OD-A75X	dead 1	#	FN/A	#N/A	24	ICGA-FS-A1ZE	dead 1	25	46.42
25	TCGA EE ADAR	alive 0	100.39	124.2	25	TCGA PD ACOT	dead 1	#	fiN/A fni/a	#IN/A #NI/A	25	TCGA HP ATNC	uead 1	10.66	27.2
20 27	TCGA-GN-A26C	dead 1	123.95 24 R	134.3 26.97	20 27	TCGA-RP-A095	alive 0	# #	tN/A tN/Δ	#IN/A #N/Δ	20 27	TCGA-RE-AAPE	alive 0	10.68	32.85 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-A475	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	#	, ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/A	#N/A	34	TCGA-XV-AAZW	dead 1	NA	12.91
35	TCGA-EB-A85I	alive 0	11.89	11.89	35	TCGA-FS-A4FC	dead 1	#	ŧN∕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	#	ŧN/A	#N/A	36	TCGA-FS-A1ZS	alive 0	148.69	148.69
37	TCGA-EB-A4IS	alive 0	25.43	25.43	3/	TCGA-ER-A198	dead 1	#	fN/A	#N/A	37	TCGA-ER-A19L	dead 1	121.19	131.41
38	TCGA-EB-A44Q	dood 1	13.80	13.80	38	TCGA-W3-AAIV	alivo 0	#	4N/A	#IN/A	38	TCGA-GN-A403	dood 1	90.54 NA	121.81
40	TCGA-D3-A3CF	dead 1	51 48	60.18	40	TCGA-EB-A51R	alive 0		1.39 ±N/Δ	22.0 #N/Δ	40	TCGA-D3-A5GI	alive 0	35.87	125 69
41	TCGA-GN-A4U8	alive 0	48.85	48.85	41	TCGA-DA-A112	dead 1	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN/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	#	ŧN∕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	#	ŧN/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	1	22.63	122.63	49	TCGA-GN-A268	dead 1	59.33	62.75
50	TCGA-D3-AIQ/	alive U	35.78	133.15	50	TCGA-DA-AII7	alive 0	#	FIN/A	#N/A	50	TCGA-GN-A264	dead 1	105.81	117.84
52	TCGA-FB-A551	alive 0	10 38	10 38	52	TCGA-EB-ASSH	dood 1	5	4.99 π/Δ	33.96 #N/A	51	TCGA-EE-AZ9C	dead 1	12.07	7 7 9 1
53	TCGA-3N-A9WC	alive 0	56.01	66.43	53	TCGA-ER-A19T	dead 1	ד ז	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	#	ŧN/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	#	ŧN/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-A8GO	dead 1	#	ŧN/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	#	ŧN/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	#	ŧN∕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	#	ŧN∕A	#N/A	59	TCGA-EE-A2GO	alive 0	122.08	126.71
60	TCGA-DA-A1IB	dead 1	7.98	40.57	60	TCGA-EE-A3AF	dead 1	#	ŧN/A	#N/A	60	TCGA-EE-A3AH	dead 1	137.45	138.7
61	TCGA EE ADAG	alive 0	00.03	00.03	61 62	TCGA EF ADDU	alive 0	#	fiN/A fni/a	#IN/A #NI/A	61 62	TCGA VV ADVZ	uead 1	8.9/ 0.26	10.02
62		L D69U	35.01 37.00	01.47 18 05	62	TCGA-EE-A29H	deed 1	#	hN/A hN/A	#IN/A #NI/A	62	TCGA EE A 210	diive U dood 1	0.30 34	U.30 3/1 2
64	TCGA-D3-4511	alive 0	65 97	145 01	64	TCGA-D3-A3MU	alive 0	#	τN/Α	#N/A	64	TCGA-DA-A95V	dead 1	11 1	14.13
65	TCGA-D3-A3MR	alive 0	102.1	103.52	65	TCGA-BF-A1PZ	alive 0	#	ŧΝ/Α	#N/A	65	TCGA-FS-A1YY	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	#	ŧN/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	#	ŧN∕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	#	ŧN/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	#	ŧN∕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	#	ŧN∕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	#	ŧN/A	#N/A	72	TCGA-IH-A3EA	alive 0	10.32	17.21
73	TCGA-D3-A51H	alive 0	27.53	56.31	73	ICGA-YG-AA30	dead 1	#	FN/A	#N/A	73	TCGA-BF-A9VF	alive 0	14.45	14.45
/4 75	TCGA EE ASIE	dive U	36 52	36.02	74 75	TCGA ES A17	dead 1	#	FIN/A FNI/A	#N/A #N/A	/4 75	TCGA EP A2UV	alive 0	80.35 1.20	85.02 1.29
76	TCGA-LE-ASJS	dead 1	18 96	32.72	76	TCGA-XV-A9W5	alive 0	#	τι η / Α έΝ / Α	#N/A	76	TCGA-DA-A1IA	alive 1	1.20 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	#	ŧN/A	#N/A	79	TCGA-BF-AAOU	alive 0	15.64	15.64
80	TCGA-ER-A195	dead 1	NA	35.41	80	TCGA-WE-AAA0	alive 0	#	ŧN/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	#	ŧN∕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	1	29.17	129.17	82	TCGA-DA-A3F5	alive 1	224.74	225.79

		i	i		i .	i i	1	1	1		i .	г. т	1	I	1		1	i
	83	TCGA-D9-A1JX	dead	1	6.41	7.1	83	TCGA-EE-A2GB	alive	0	#N/A	#N/A	83	TCGA-YD-A89C	alive	0	6.9	6.9
	84	TCGA-ER-A199	dead	1	3.91	9.17	84	TCGA-WE-A8JZ	alive	0	#N/A	#N/A	84	TCGA-D3-A2JE	alive	1	25.62	27.63
	85	TCGA-BF-AAP4	alive	0	11.01	11.01	85	TCGA-EE-A2MT	alive	0	#N/A	#N/A	85	TCGA-EE-A29W	alive	0	159	194.88
	86	TCGA-EE-A2A6	alive	0	49.44	86.07	86	TCGA-EB-A42Z	alive	0	14.49	14.49	86	TCGA-EB-A5KH	alive	1	NA	20.34
	87	TCGA-0B-AA90	dead	1	NΔ	18 04	87	TCGA-ES-A17E	dead	1	NΔ	15 44	87	TCGA-D3-A51R	alive	0	33 38	63 76
	00		dood	1	12.25	10.04	00		alivo	<u>^</u>	12.01	12.01	00		alivo	1	NIA	26 71
	00	TCGA-D3-A2JU	ueau	1	42.33	43.4	00	TCGA-DA-A332	alive	0	13.01	13.01	00	TCGA-F3-A4FB	alive	1	0.20	20.71
	89	ICGA-EB-A5UL	alive	0	29.27	29.27	89	ICGA-ER-A3PL	alive	0	#N/A	#N/A	89	TCGA-D3-A3IVIO	alive	1	8.28	9.33
	90	TCGA-FS-A1ZM	alive	0	101.18	101.18	90	TCGA-EE-A2GP	dead	1	#N/A	#N/A	90	TCGA-FR-A3YO	alive	0	#N/A	#N/A
	91	TCGA-D9-A3Z1	dead	1	11.01	15.37	91	TCGA-EB-A24D	alive	0	16.43	21.19	91	TCGA-DA-A960	alive	0	26.41	26.41
	92	TCGA-WE-A8ZX	alive	0	10.71	35.78	92	TCGA-GN-A4U9	dead	1	12.61	22.11	92	TCGA-EE-A29V	alive	1	23.03	25.85
	93	TCGA-D3-A2JB	dead	1	105.75	167.87	93	TCGA-EB-A3XC	alive	0	#N/A	#N/A	93	TCGA-EB-A4IQ	alive	1	NA	20.89
	94	TCGA-FR-4421	alive	0	125 23	148 92	94	TCGA-FR-A19F	dead	1	, #N/Δ	, #N/Δ	94	TCGA-D3-A8GK	alive	0	51.08	170.07
	05		dood	1	2 61	12 02	05		dood	1	1 50	71 75	05		alivo	1	40.22	E0 07
	95 06	TCGA-LK-A157	deau	1	0.01	13.55	35	TCGA-D3-AIQ0	dead	1	1.50	12.04	55	TCGA-GIN-AOLK	alive	1	40.23	12.42
	96	ICGA-D3-A8GS	dead	1	93.56	117.08	96	ICGA-ER-A19E	dead	1	2.1	13.01	96	ICGA-EB-A55F	alive	1	NA	12.12
	97	TCGA-ER-A1A1	alive	0	64.75	104.99	97	TCGA-EB-A1NK	alive	0	34.13	34.13	97	TCGA-GF-A2C7	alive	0	0.69	0.69
	98	TCGA-QB-A6FS	alive	0	6.27	7.23	98	TCGA-EB-A6QY	alive	0	1.18	12.55	98	TCGA-BF-A1PU	alive	0	15.9	12.71
	99	TCGA-ER-A19O	dead	1	#N/A	#N/A	99	TCGA-D3-A1Q4	alive	0	111.96	111.96	99	TCGA-D9-A1X3	alive	0	18.1	18.1
	100	TCGA-ER-A194	dead	1	NA	44.48	100	TCGA-EB-A42Y	dead	1	#N/A	#N/A	100	TCGA-ER-A2NE	alive	1	NA	20.14
	101	TCGA-Z2-AA3V	alive	0	15.97	15.97	101	TCGA-W3-A828	dead	1	NA	120.99	101	TCGA-EE-A185	alive	1	2.79	4.96
	102	TCGA-FF-A2GK	alive	0	49 21	54.7	102	TCGA-FB-A5SF	dead	1	12.06	13 17	102	TCGA-FB-A24C	alive	0	15 28	20.76
	102		dood	1	15.21	20 1E	102		dood	1	12.00	216 75	102		alivo	0	77.76	20.70
	105	TCGA-ER-AZIND	ueau	1	15.14	20.15	103	TCGA-FS-ALTW	ueau	1		210.75	105	TCGA-F5-A4F0	anve	0	//./0	//./0
	104	ICGA-EB-A44R	dead	1	NA	10.35	104	1CGA-D9-A4Z6	dead	1	/	18.43	104	ICGA-EE-AZGR	alive	1	NA	42.74
	105	TCGA-FR-A3R1	alive	0	22.27	22.5	105	TCGA-EB-A44O	alive	0	1.97	2.66	105	TCGA-EE-A3AD	alive	1	NA	28.75
	106	TCGA-GF-A3OT	alive	0	#N/A	#N/A	106	TCGA-WE-A8ZY	dead	1	NA	49.47	106	TCGA-ER-A42H	alive	1	8.51	13.99
	107	TCGA-EE-A2M5	dead	1	#N/A	#N/A	107	TCGA-EE-A2GD	dead	1	333.21	339.88	107	TCGA-EE-A29A	alive	1	58.97	63.3
	108	TCGA-FS-A1ZW	alive	0	#N/A	#N/A	108	TCGA-D3-A8GC	dead	1	75.16	79.53	108	TCGA-WE-A8ZQ	alive	0	63.17	63.17
	109	TCGA-FF-A2A0	dead	1	#N/A	#N/A	109	TCGA-D3-A8GP	alive	0	17 58	152 63	109	TCGA-D9-A6FG	alive	1	13 07	22.93
	110	TCGA-EE-A3IA	head	1	#N/Δ	#NI/A	110	TCGA-D9-A473	alivo	0	3 20	16 59	110	TCGA-FE-A29E	alivo	0	63 73	63 73
	114	TCCA ED ACIA	alter	- 0	26 42	26 42	110		der d	1	J.23	±0.33	110	TCCA FC A452	alive	1	NA	50.75
	111	TCGA-EB-A6L9	alive	0	36.43	36.43	111	TCGA-EB-A57IVI	dead	1	#N/A	#N/A	111	TCGA-FS-A4FZ	alive	1	NA	50.1
	112	TCGA-DA-A95V	alive	0	#N/A	#N/A	112	TCGA-D3-A1Q8	dead	1	25.59	28.06	112	TCGA-EE-A29L	alive	1	1.81	2.6
	113	TCGA-ER-A2NF	dead	1	12.48	28.81	113	TCGA-EB-A41A	alive	0	8.61	8.61	113	TCGA-FR-A2OS	alive	1	10.38	12.09
	114	TCGA-DA-A1I5	alive	0	134.92	134.92	114	TCGA-FS-A1YX	dead	1	NA	48.55	114	TCGA-FS-A1ZK	alive	1	12.06	23.92
	115	TCGA-FS-A1ZT	alive	0	53.12	53.12	115	TCGA-EE-A2GN	dead	1	79.57	102.04	115	TCGA-3N-A9WB	alive	1	16	17.02
	116	TCGA-FF-A29M	alive	0	56.8	56.8	116	TCGA-FR-A69P	alive	0	9	15.7	116	TCGA-FB-A5FP	alive	1	NA	14 91
	117		alivo	0	96.7	96.7	117		dood	1	107 72	112 44	117		alivo	1	6.01	10.41
	117	TCGA-D3-A2JC	alive	0	00.7	00.7	11/	TCGA-D3-A2JG	deau	1	107.72	113.44	11/	TCGA-GIN-A407	alive	1	0.01	10.41
	118	TCGA-EE-AZ91	alive	0	#IN/A	#IN/A	511	TCGA-GIN-AZ6D	dead	1	32.92	47.96	118	TCGA-ER-A3ES	alive	1	NA 	240.85
	119	TCGA-D3-A1Q9	dead	1	16.52	31.57	119	TCGA-EE-A20H	dead	1	163.93	168.13	119	TCGA-EE-A20C	alive	1	145.89	151.15
	120	TCGA-D3-A2J7	dead	1	71.02	103.02	120	TCGA-BF-AAP8	alive	0	14.68	14.68	120	TCGA-D3-A8GL	alive	1	84.63	89.06
	121	TCGA-GF-A6C8	alive	0	2.04	2.04	121	TCGA-DA-A1IC	dead	1	#N/A	#N/A	121	TCGA-EE-A29D	alive	1	NA	13.96
	122	TCGA-D3-A5GN	alive	0	#N/A	#N/A	122	TCGA-FS-A1ZP	dead	1	#N/A	#N/A	122	TCGA-BF-A1PV	alive	0	0.46	0.46
	123	TCGA-3N-A9WD	dead	1	#N/A	#N/A	123	TCGA-ER-A2NC	dead	1	38.01	43.79	123	TCGA-FS-A1ZU	alive	1	22.31	26.54
	124	TCGA-RP-A693	alive	0	033	0 33	124	TCGA-GN-A4U4	alive	0	7 13	39 32	124	TCGA-D3-A3MI	alive	1	12 68	13.86
	124		alivo	0	01.40	0.33	127		alivo	0	17 44	17 44	124		alivo	1	12.00	27.07
	125	TCGA-EE-AZUF	dive	0	91.49	91.49	123	TCGA-FW-ASIS	alive	0	17.44	17.44	125	TCGA-FS-A121	alive	1	NA 4.57	27.07
	126	ICGA-D3-A3CF	dead	1	21.52	24.51	126	ICGA-WE-A8ZR	dead	1	NA	9	126	TCGA-EE-A17Z	alive	1	4.57	8.64
	127	TCGA-WE-A8ZO	alive	0	58.97	70.47	127	TCGA-ER-A19J	dead	1	3.22	6.44	127	TCGA-FS-A1ZC	alive	1	339.49	357.1
	128	TCGA-FS-A1Z4	dead	1	NA	28.06	128	TCGA-ER-A3ET	dead	1	#N/A	#N/A	128	TCGA-ER-A19T	alive	1	3.88	8.87
	129	TCGA-EE-A3AC	alive	0	63.99	63.99	129	TCGA-DA-A3F3	dead	1	#N/A	#N/A	129	TCGA-FS-A1ZD	alive	1	49.18	53.48
	130	TCGA-EE-A2GJ	dead	1	86.2	107.29	130	TCGA-EE-A180	dead	1	89.55	94.91	130	TCGA-GN-A262	alive	0	120.34	139.78
	131	TCGA-D9-A149	alive	0	42.38	54.63	131	TCGA-EB-A550	dead	1	NA	8.67	131	TCGA-D3-A3C6	alive	1	53.32	58.02
	132	TCGA_FB_A82B	alivo	0	12.81	12.81	<u> </u>											
	122	TCCA ER A100	dood	1	10 50	12.01 E0.0E												
	133		dead	1	40.35	10.05												
	134	ICGA-EE-AZ9N	dead	1	17.97	18.59												
	135	TCGA-Z2-A8RT	alive	0	27.56	27.56												
	136	TCGA-D3-A5GR	alive	0	#N/A	#N/A												
	137	TCGA-EE-A2MI	dead	1	#N/A	#N/A												
	138	TCGA-RP-A6K9	alive	0	#N/A	#N/A												
	139	TCGA-FW-A5DY	alive	0	#N/A	#N/A												
	140	TCGA-EE-A2GL	alive	0	#N/A	#N/A												
	141	TCGA-EE-A2A1	alive	0	114.59	115.87												
	142	TCGA-D9-A1 IM	alive	0	0 32	3 65												
	1/2		alive	0	21.20	21.20												
	143	TCGA-WE-AAA3	aiive	0	21.39	21.39												
	144	TCGA-FR-A3YN	alive	U	92.9	92.9												
	145	TCGA-ER-A2NF	dead	1	12.48	28.81												
	146	TCGA-EE-A2ML	dead	1	195.93	216.49												
	147	TCGA-EE-A2MP	alive	0	246.58	248.46												
	148	TCGA-EE-A2MN	dead	1	44.65	47.5												
	149	TCGA-EE-A2MG	dead	1	101.74	103.12												
	150	TCGA-ES-A4E8	head	1	#N/A	#NI/A												
	151	TCCA EE ADAM	alina	<u>`</u>	190.26	190.26												
	152	TCGA-EE-AZIVIK	anve	0	100.20	100.20												
	127	TCGA-GN-A265	alive	U	3.25	30.85												
	153	ICGA-D3-A2JF	alive	0	43.86	62.02												
	154	TCGA-DA-A1I1	alive	0	183.67	222.34												
	155	TCGA-GN-A266	dead	1	3.58	10.12												
	156	TCGA-EE-A2GC	alive	0	67.38	67.38												
	157	TCGA-D3-A5GS	alive	0	5.29	18.17												
	158	TCGA-FR-A19H	dead	1	NA	152.23												
	150		alivo	0	174 7	17/ 7												
	160		doord	1	100 70	202 5												
	100	TCGA-FS-AIZU	uead	T	100.18	202.5												
	161	ILGA-FR-A7U9	alive	0	#N/A	#N/A												
	162	ICGA-HR-A2OG	alive	0	#N/A	#N/A												
	163	TCGA-D3-A2JA	alive	0	1.31	115.44												
- 1			-	-														
	164	TCGA-EE-A3AA	alive	0	#N/A	#N/A												
	164 165	TCGA-EE-A3AA TCGA-D3-A8GE	alive alive	0 0	#N/A #N/A	#N/A #N/A												

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

<u>Table S1</u>: 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.