

## Supplemental Information

### UVB-Induced Tumor Heterogeneity Diminishes

#### Immune Response in Melanoma

**Yochai Wolf, Osnat Bartok, Sushant Patkar, Gitit Bar Eli, Sapir Cohen, Kevin Litchfield, Ronen Levy, Alejandro Jiménez-Sánchez, Sophie Trabish, Joo Sang Lee, Hiren Karathia, Eilon Barnea, Chi-Ping Day, Einat Cinnamon, Ilan Stein, Adam Solomon, Lital Bitton, Eva Pérez-Guijarro, Tania Dubovik, Shai S. Shen-Orr, Martin L. Miller, Glenn Merlino, Yishai Levin, Eli Pikarsky, Lea Eisenbach, Arie Admon, Charles Swanton, Eytan Ruppin, and Yardena Samuels**

## TABLES WITH TITLES AND LEGENDS

**Supplementary Table 1. WES bioinformatics analysis for all cell lines.  
Related to Figures 2 and 5.**

Description	Mutation Load (SNV genomic mutations)	Clonal SNV Fraction	Number of predicted neoantigens, affinity<500nM (non-redundant)	Average affinity	Average Hydrophobicity (KyteDoolittle scale)
Parental B2905	2745	0.079	296	233.637	0.712
UVB B2905	5479	0.063	302	228.673	0.672
UVB.2	9602	0.053	607	2086.41	0.621
UVB.3	7977	0.031	417	2196.95	0.645
SCC 1	3600	0.846	383	231.004	0.637
SCC 2	3720	0.887	137	219.252	0.604
SCC 3	9810	0.901	329	242.262	0.702
SCC 4	4721	0.824	163	244.195	0.762
SCC 5	9999	0.893	348	238.34	0.687
SCC 6	10405	0.874	346	239.534	0.754
SCC 7	13082	0.553	500	237.742	0.674
SCC 8	5652	0.798	177	214.352	0.534
SCC 9	11919	0.294	500	230.336	0.699
SCC 10	9804	0.877	350	234.973	0.678
SCC 11	4796	0.811	172	229.577	0.560
SCC 12	10510	0.877	408	240.755	0.771
SCC 13	10126	0.843	391	236.153	0.739
SCC 14	10103	0.894	334	246.734	0.868
SCC 15	12401	0.866	431	236.538	0.693
SCC 16	4208	0.848	144	235.044	0.704
SCC 17	10502	0.869	378	226.87	0.695
SCC 18	13134	0.657	498	239.086	0.705
SCC 19	11126	0.830	376	243.846	0.732
SCC 20	9497	0.886	361	236.804	0.730
SCC 21	11931	0.882	425	236.145	0.661
SCC 22	16977	0.496	643	233.478	0.665

**Supplementary Table 2. Exonic mutations added to B2905 cell line following UVB irradiation, Related to Figure 2.**

<b>Mutation Type</b>	<b>Parental B2905</b>	<b>UVB B2905</b>	<b>Mutations added to UVB line</b>
T>G	243	234	189
T>C	1235	695	451
T>A	197	580	538
C>A	212	436	405
C>G	76	100	88
C>T	782	3434	3212

**Supplementary Table 3. Peptide information, clone numbers in which they were found, MHC binding information. Related to Figure 5.**

Gene name	MS peptide hit	DNA Change	Protein change	Clone number	Binding score	RMA-S binding
DNM2	<b>FAIFNTEQM</b>	g.chr9:21499367G>T	p.R594M	SCC14	263.91	H2-Db
SGTA	<b>AGLLNSPHFI</b>	g.chr10:81048399T>C	p.N228S	SCC12	146.88	H2-Db
PAPD5	<b>QAFDFAYV</b>	g.chr8:88252012A>T	p.Y447F	SCC17	119.57	H2-Kb
NSD3	<b>KGVGNKTEI</b>	g.chr8:25666033A>G	p.I539V	SCC3, 5,14,15,18,1 9, 22	118.91	Not tested
IFI44	<b>ASLTHVDSL</b>	g.chr3:151738305T>C	p.L338S	SCC2	114.45	H2-Db
POLK	<b>LANETISTL</b>	g.chr13:96483724T>G	p.E736A	SCC3, 4, 5, 9, 15, 16, 19	111.29	Weak binder
NUP98	<b>VINENYDYL</b>	g.chr7:102123687T>C	p.I1658V	SCC17	107.01	H2-Db
RPTOR	<b>SAYEKLYSL</b>	g.chr11:119846903C>T	p.H180Y	SCC2	95.04	H2-Db
CHRD2	<b>IGIYHLVQI</b>	g.chr7:100029349A>T	p.K376I	SCC9	91.9	Not tested
VPS35	<b>HQNVNVENI</b>	g.chr8:85281264T>C	p.K296E	SCC9	86.136	H2-Db
KCNH2	<b>VSLNTNSEKI</b>	g.chr5:24325544G>A	p.P634L	SCC11	80.755	Not tested
KEAP1	<b>FGVMNELHL</b>	g.chr9:21237498C>T	p.R71H	SCC5	80.165	Not tested
SMC4	<b>SAITMAQV</b>	g.chr3:69030402A>T	p.K920M	SCC9, 17	78.616	Not tested
OLFR204	<b>SVTLFYGTL</b>	g.chr16:59314664A>T	p.S248T	SCC15	73.26	Not tested
PA2G4	<b>VAHIFVIGV</b>	g.chr10:128563088G>A	p.T124I	SCC13,18	70.912	H2-Kb
TMEM135	<b>SMYLVSKL</b>	g.chr7:89147986G>A	p.A354V	SCC5	49.715	Non binder
VWA8	<b>FAALLRYSI</b>	g.chr14:78949545G>A	p.G464R	SCC9	42.311	Non binder
TMEM147	<b>VIYDFIGEF</b>	g.chr7:30728676C>A	p.G63V	SCC17	40.103	Not tested

**Supplementary Table 4. Multivariate cox regression analysis to test if ITH remains associated with overall survival in immune checkpoint inhibitor treated cohorts, while controlling for tumor purity as a confounding factor. Related to Figure 7.**

ITH					
$\lambda(t) = \lambda(t) \exp(\beta_1 * ITH + \beta_2 * purity )$					
Study	feature	exp(coefficient) (Hazard Ratio)	standard error	Wald's statistic	p-value
Snyder	ITH	9.464	0.8258	2.722	0.0065
Snyder	purity	4.9392	0.8798	1.815	0.0695
Study	feature	exp(coefficient) (Hazard Ratio)	standard error	Wald's statistic	p-value
Riaz	ITH	2.1505	0.5166	1.482	0.138
Riaz	purity	1.1262	0.7208	0.165	0.869
Study	feature	exp(coefficient) (Hazard Ratio)	standard error	Wald's statistic	p-value
Hugo	ITH	3.976	0.8822	1.565	0.118
Hugo	purity	0.5031	1.3864	-0.495	0.620
Study	feature	exp(coefficient) (Hazard Ratio)	standard error	Wald's statistic	p-value
Van Allen	ITH	0.7551	0.4365	-0.644	0.5199
Van Allen	purity	5.3141	0.9066	1.842	0.0654
Study	feature	exp(coefficient) (Hazard Ratio)	standard error	Wald's statistic	Pmeta-value
Meta-Analysis	ITH	-	-	-	0.01189
Meta-Analysis	purity	-	-	-	0.15013

**Supplementary Table 5. Multivariate cox regression analysis to test if ITH remains associated with overall survival in immune checkpoint inhibitor treated cohorts, while controlling for tumor mutation burden as a confounding factor. Related to Figure 7.**

<b>Study</b>	<b>feature</b>	<b>exp(coefficient) (Hazard Ratio)</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
Snyder	ITH	6.3696061	0.8762292	2.113	0.0346
Snyder	TMB	0.9998089	0.0002168	-0.882	0.378
<b>Study</b>	<b>feature</b>	<b>exp(coefficient) (Hazard Ratio)</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
Riaz	ITH	1.9905849	0.4660073	1.477	0.14
Riaz	TMB	0.9994318	0.0005463	-1.04	0.298
<b>Study</b>	<b>feature</b>	<b>exp(coefficient) (Hazard Ratio)</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
Hugo	ITH	5.0016966	0.8787699	1.832	0.067
Hugo	TMB	0.9991061	0.0006954	-1.286	0.198
<b>Study</b>	<b>feature</b>	<b>exp(coefficient) (Hazard Ratio)</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
Van Allen	ITH	0.9569098	0.382274	-0.115	0.908
Van Allen	TMB	0.9998826	0.0001625	-0.723	0.47
<b>Study</b>	<b>feature</b>	<b>exp(coefficient) (Hazard Ratio)</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>Pmeta-value</b>
Meta-Analysis	ITH	-	-	-	0.03881
Meta-Analysis	TMB	-	-	-	0.33259

**Supplementary Table 6. Multivariate cox regression analysis to test if ITH, Mutation load and CNV load are each associated with survival while controlling for confounding factors in Melanoma TCGA dataset. Related to STAR Methods.**

<b>ITH</b>				
$\lambda(t) = \lambda_0(t) \exp(\beta_1 * age + \beta_2 * stage + \beta_3 * purity + \beta_4 * ITH)$				
<b>feature</b>	<b>regression coefficient</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
<b>stage</b>	1.100826	0.026915	3.569	0.000358
<b>age</b>	1.021558	0.006257	3.409	0.000653
<b>purity</b>	1.150923	0.52276	0.269	0.788015
<b>ITH</b>	1.150773	0.072552	1.936	0.052915
<b>Mutation Load</b>				
$\lambda(t) = \lambda_0(t) \exp(\beta_1 * age + \beta_2 * stage + \beta_3 * load_{mut})$				
<b>feature</b>	<b>regression coefficient</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
<b>stage</b>	1.097798927	0.0295909	3.153	0.001615
<b>age</b>	1.025529947	0.0070752	3.563	0.000367
<b>mutation load</b>	0.999850011	0.0001737	-0.863	0.388043
<b>CNV load</b>				
$\lambda(t) = \lambda_0(t) \exp(\beta_1 * age + \beta_2 * stage + \beta_3 * load_{cnv})$				
<b>feature</b>	<b>regression coefficient</b>	<b>standard error</b>	<b>Wald's statistic</b>	<b>p-value</b>
<b>stage</b>	1.099066299	0.026886	3.513	0.000443
<b>age</b>	1.024565889	0.006162	3.613	0.000303
<b>CNV load</b>	0.837393657	0.329976	-0.538	0.590715
<b>likelihood ratio analysis to test if ITH contributes to survival differences while controlling for tumor purity</b>				
<b>model</b>	<b>log likelihood</b>		P-value associated with increase in log-likelihood = 0.0257	
<b>Survival ~ purity</b>	-683.71			
<b>Survival ~ purity + ITH</b>	-681.11			

**Supplementary Table 7. Related to Figure 6. Percentage of Clonal (shared between all SCCs), Subclonal (shared among a subset of the SCCs), and Private mutations among the 6 and 12 SCC mixes described in Figure 6C-F.**

	<b>6WB</b>	<b>6AB</b>	<b>12WB</b>	<b>12AB</b>
<b>Clonal</b>	<b>12.31</b>	<b>2.82</b>	<b>6.44</b>	<b>1.55</b>
<b>Subclonal</b>	<b>6.62</b>	<b>15.84</b>	<b>8.90</b>	<b>26.48</b>
<b>Private</b>	<b>81.07</b>	<b>81.34</b>	<b>84.67</b>	<b>71.97</b>
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
$\% \left( \frac{\textit{Clonal}}{\textit{Clonal} + \textit{Subclonal}} \right)$	65	15	42	5
$\% \left( \frac{\textit{Subclonal}}{\textit{Clonal} + \textit{Subclonal}} \right)$	35	85	58	95