SP140 inhibits STAT1 signaling, induces IFN-gamma in tumor-associated macrophages, and is a predictive biomarker of immunotherapy response

Running title: SP140 and immunotherapy

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Supplementary Materials

Supplementary table 1- List of primers used in this study

Supplementary table 2- Top GSEA gene sets (hallmark and GO: biological pathways) with FWER < 0.05

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Supplementary Figure 2- Relative delta delta Ct expression value for SP140 gene transcript expression in CAL27, FaDu and THP1-derivd naïve macrophages. Results is presented as average of three independent experiment.

Supplementary Figure 3- Disease specific survival of patients with high levels of SP140 (n=261) and low levels of SP140 (n=261) in TCGA HNSCC. The p-value was calculated based on the log-rank test.

Supplementary Figure 4- Correlation of SP140 expression in HPV positive and HPV negative HNSCC with M1 macrophage (A) and CD8 T cell infiltrations levels (B). P-values are calculated after FDR corrections of Spearman's Rho.

Supplementary Figure 5- A, B) SP140 was downregulated using siRNA in naïve (undifferentiated) macrophages and cells were collected for RNA expression analysis by qPCR for IL6 and STAT5a, after 24h. 18s was used as endogenous normalizer. Delta-delta Ct method was used to identify the relative gene expression. Data is presented as mean and SD of fold change compared to the control. ** indicates $p < 0.01$

Supplementary Figure 6- SP140 binding to STAT1 promoter sites by ChIP assays. The ChIP assays showed a binding of SP140 to STAT1 promoter sites in THP-1-derived macrophages treated with SP140 siRNA or control siRNA. Data is presented as means ± SD.

Supplementary Figure 7- A-D) Patients with metastatic melanoma who underwent anti-PD-1 therapy were dichotomized to high expression and low expression group based on median expression of SP140. Tumors with high expression of SP140 (n=13) versus tumors with low levels of SP140 (n=12) showed higher infiltration of M1 macrophages, CD8 T cells, CD4 memory activated T cells, and overall immune score. The Wilcoxon test was used for statistical analysis and p-value was corrected for multiple comparison. * indicates $p < 0.05$

Supplementary Figure 8- A) The levels of SP140 in thymic carcinoma tumors were significantly higher in anti-PD1 treatment responders versus non-responders (n=9). B) The expression levels of SP140 in recurrent glioblastoma patients who received neoadjuvant anti-PD-1 were significantly higher in treatment responders versus non-responders (n=17). C) Patients with recurrent glioblastoma were dichotomized to high expression (n=8) and low expression (n=9) groups based on median expression of SP140, deconvolution of immune cells showed higher infiltration of CD8 T-cells and M1 macrophages in the SP140 high group. * indicates $p < 0.05$

Supplementary table 1- List of primers used in this study

Gene	Forward Primer	Reverse Primer
SP140	CCAGGTGGGGGAGTGTCCTGT	TCTCCCCTGGTGCTGTGCTGT
STAT1	ACGCCCAGAGATTTAATCAGG	CACTCTTTGCCACACCATTG
IL-1RA	GAATGACGCCCTCAATCAAAGT	TCATCTTGGGCAGTCACATACA
Arginase	ACAGTTTGGCAATTGGAAGCA	CACCCAGATGACTCCAAGATCAG
IL6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
STAT5a	TTACTGAAGATCAAGCTGGGG	TCATTGTACAGAATGTGCCGG
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
STAT1 promotor	AGCCCCTTAAGAGTAGGCGA	CCCCATGCCTTCTCAGTTGT

Supplementary table 2- Top GSEA gene sets (hallmark and GO: biological pathways) with FWER < 0.05

HALLMARK						
Name	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
Positive						
HALLMARK_ALLOGRAFT_REJECTION	156	0.72	2.38	0	0	0
HALLMARK_INTERFERON_GAMMA_RESPONSE	169	0.64	2.10	0	0	0
HALLMARK_INFLAMMATORY_RESPONSE	139	0.62	2.01	0	0	0
HALLMARK_IL6_JAK_STAT3_SIGNALING	57	0.62	1.94	0	0	0
HALLMARK_INTERFERON_ALPHA_RESPONSE	88	0.60	1.92	0	0	0
HALLMARK_COMPLEMENT	123	0.55	1.79	0	6.86E-04	0.006
HALLMARK_IL2_STAT5_SIGNALING	113	0.51	1.66	0	0.0049	0.05
Negative						
HALLMARK_MYC_TARGETS_V1	53	-0.516	-3.25	0	0	0
HALLMARK_P53_PATHWAY	99	-0.29	-1.97	0	0.01	0.003
HALLMARK_HYPOXIA	82	-0.28	-1.96	0	0.009	0.003
HALLMARK_GLYCOLYSIS	83	-0.31	-1.80	0	0.034	0.01
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	85	-0.29	-1.69	0	0.043	0.018
GO: BIOLOGICAL PATHWAY						
Positive						
GOBP_IMMUNE_RESPONSE_REGULATING_CELL_SURFACE_RECEPTOR_SIGNALING_PATHWAY	178	0.72	2.39	0	0	0
GOBP_ADAPTIVE_IMMUNE_RESPONSE	299	0.71	2.39	0	0	0
GOBP_ADAPTIVE_IMMUNE_RESPONSE	299	0.71	2.39	0	0	0
GOBP_LYMPHOCYTE_ACTIVATION	417	0.69	2.35	0	0	0
GOBP_POSITIVE_T_CELL_SELECTION	25	0.83	2.35	0	0	0
GOBP_LYMPHOCYTE_ACTIVATION_INVOLVED_IN_IMMUNE_RESPONSE	113	0.73	2.35	0	0	0
GOBP_REGULATION_OF_LYMPHOCYTE_ACTIVATION	271	0.70	2.34	0	0	0
GOBP_NEGATIVE_REGULATION_OF_LEUKOCYTE_CELL_CELL_ADHESION	73	0.73	2.34	0	0	0
GOBP_B_CELL_MEDIATED_IMMUNITY	97	0.71	2.33	0	0	0
GOBP_ANTIGEN_RECEPTOR_MEDIATED_SIGNALING_PATHWAY	120	0.72	2.33	0	0	0
GOBP_DENDRITIC_CELL_MIGRATION	22	0.84	2.33	0	0	0
GOBP_T_CELL_ACTIVATION	297	0.70	2.33	0	0	0
GOBP_REGULATION_OF_LEUKOCYTE_PROLIFERATION	164	0.70	2.32	0	0	0
GOBP_LYMPHOCYTE_MEDIATED_IMMUNITY	195	0.70	2.31	0	0	0
GOBP_REGULATION_OF_T_CELL_ACTIVATION	206	0.70	2.31	0	0	0
GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_EXOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_II	0.83	2.31	0	0	0	0
Negative						
GOBP_KERATINIZATION	27	-0.91	-4.17	0	0	0
GOBP_SKIN_DEVELOPMENT	108	-0.54	-4.11	0	0	0
GOBP_KERATINOCYTE_DIFFERENTIATION	58	-0.68	-3.87	0	0	0
GOBP_EPIDERMAL_CELL_DIFFERENTIATION	78	-0.56	-3.73	0	0	0
GOBP_RIBOSOMAL_LARGE_SUBUNIT_BIOGENESIS	30	-0.62	-2.85	0	0	0
GOBP_KERATINOCYTE_PROLIFERATION	21	-0.62	-2.69	0	0	0
GOBP_INTERMEDIATE_FILAMENT_BASED_PROCES	31	-0.51	-2.52	0	2.11E-	0.002

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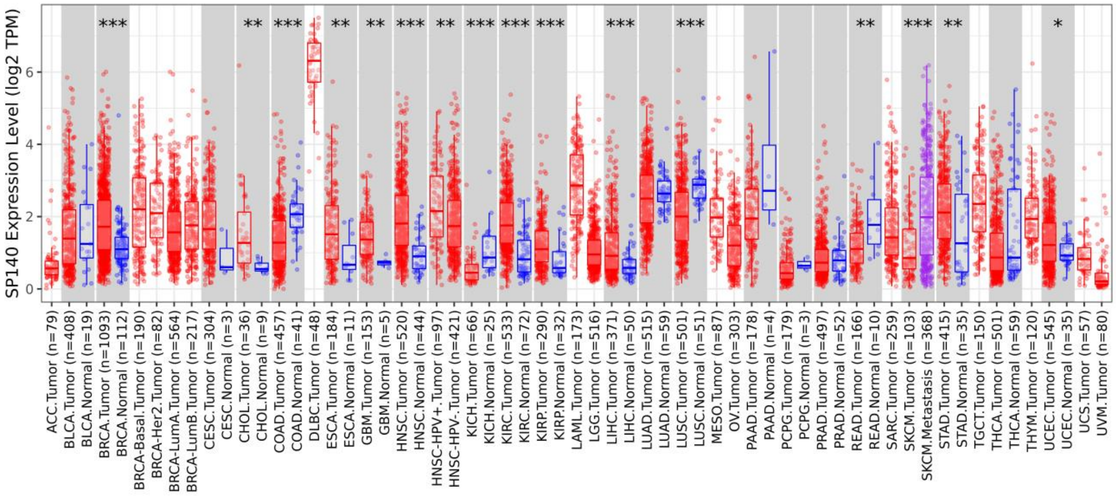
GOBP_RIBOSOME_ASSEMBLY	22	-0.57	-2.50	0	2.03E-04	0.002
GOBP_MOLTING_CYCLE	44	-0.46	-2.47	0	2.92E-04	0.003
GOBP_CYTOPLASMIC_TRANSLATION	39	-0.45	-2.40	0	7.08E-04	0.007

ES: enrichment score; NES: normalized enrichment score; NOM-p-val: nominal p-value; FDR q-val: false discovery rate corrected p-value; FWER p-val: Familywise-error rate p-value



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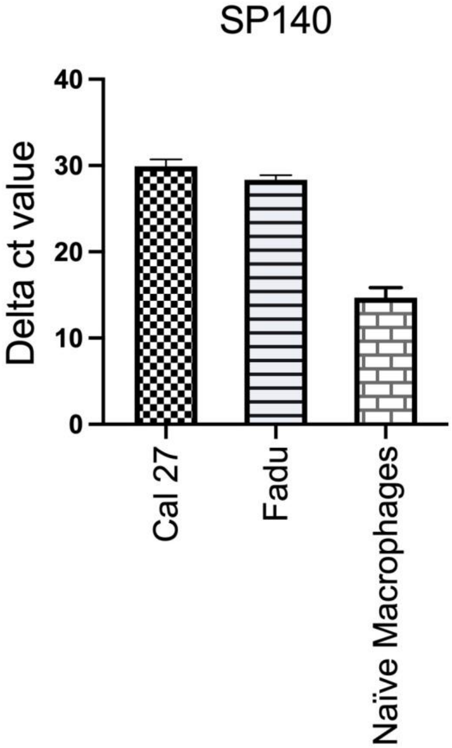

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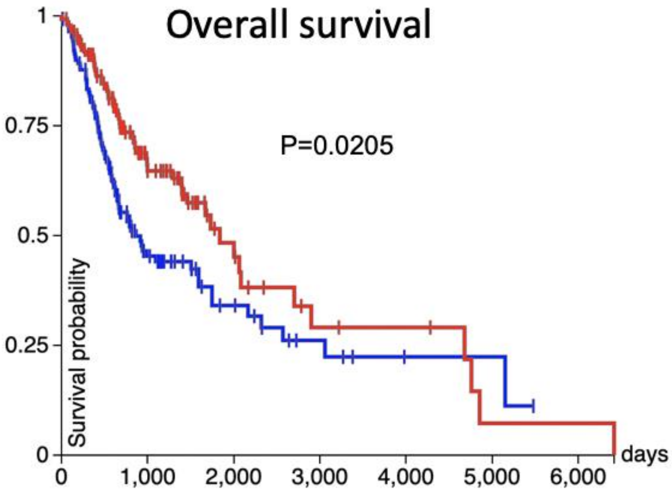

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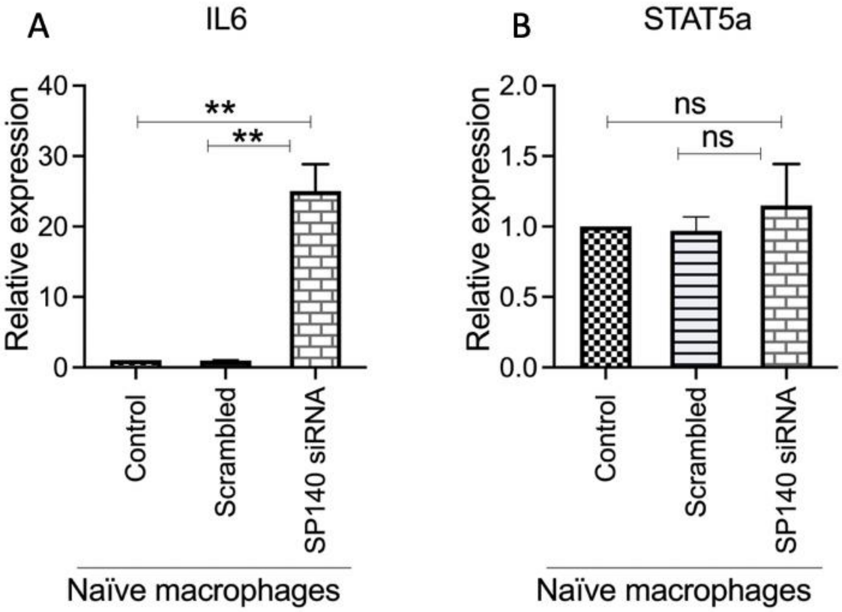

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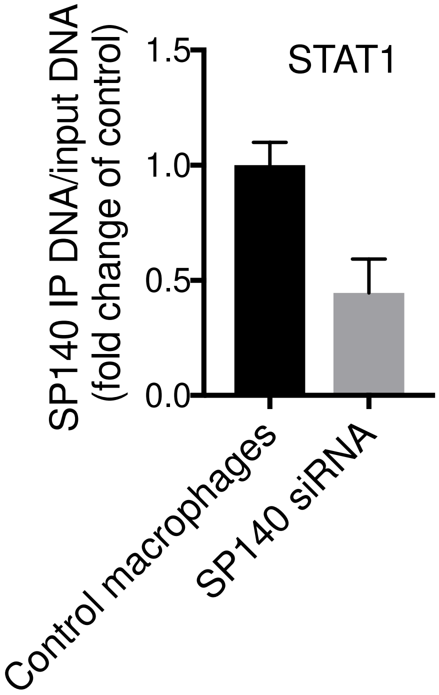

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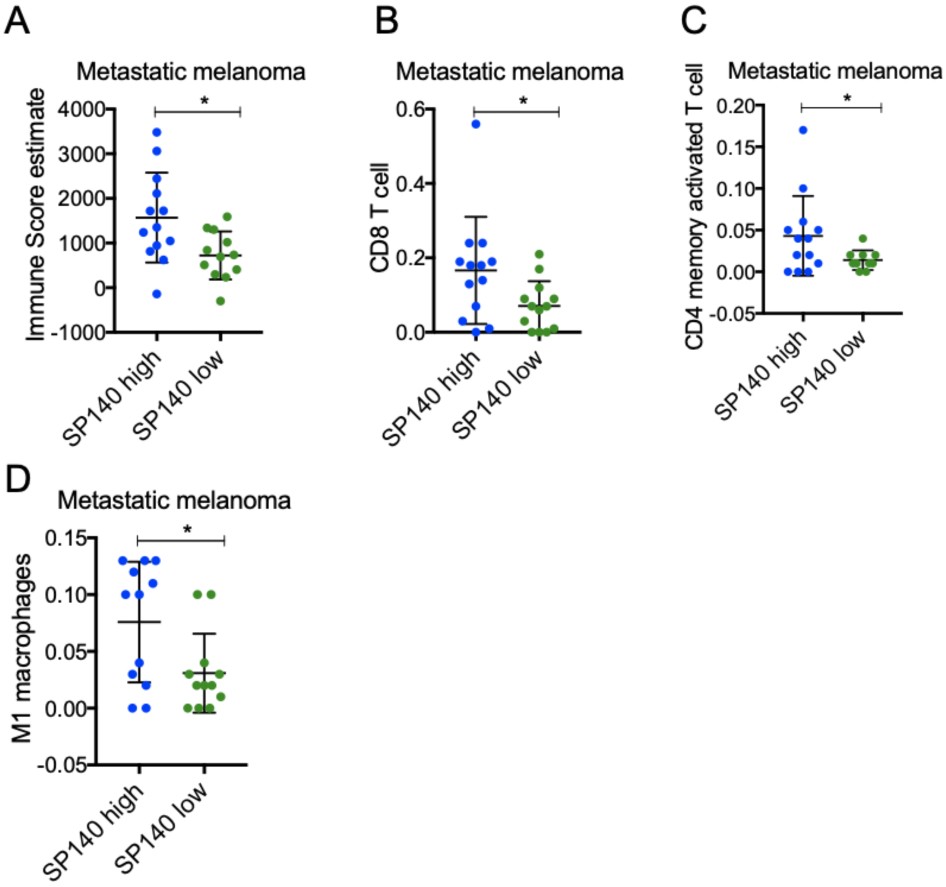

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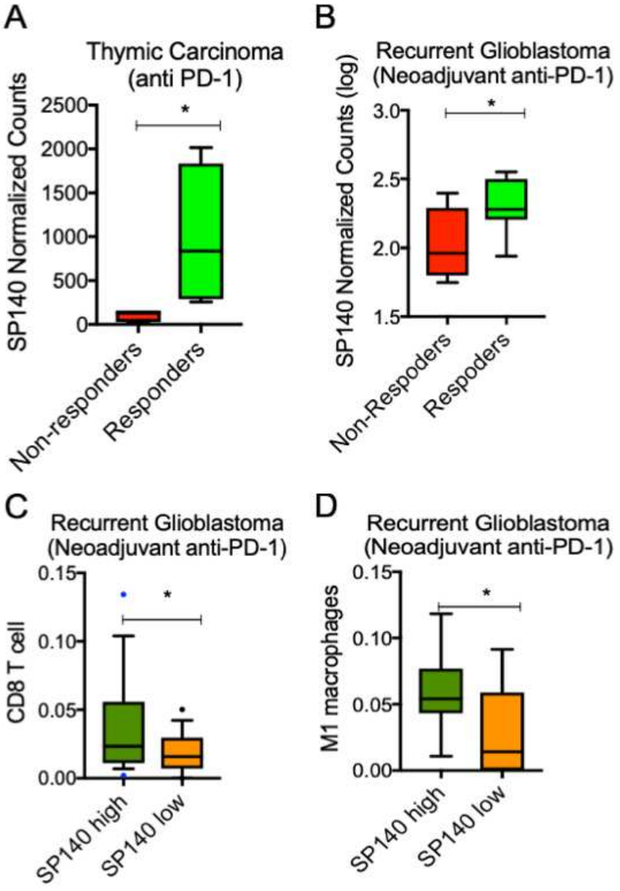

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