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Supplemental information

Gene signature reveals decreased SOX10-dependent transcripts in malignant cells from immune checkpoint inhibitor-resistant cutaneous melanomas

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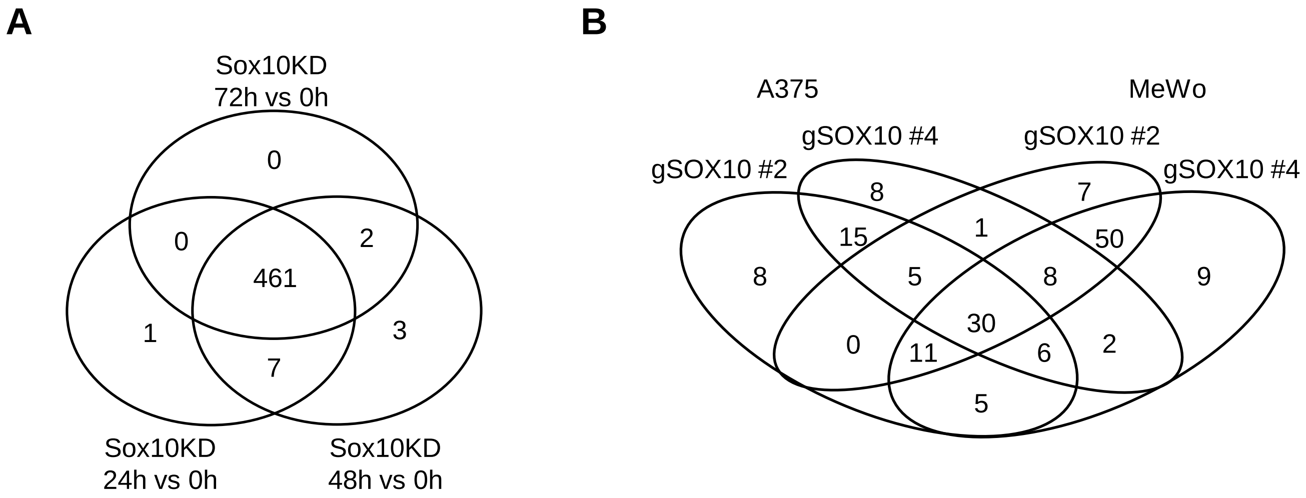
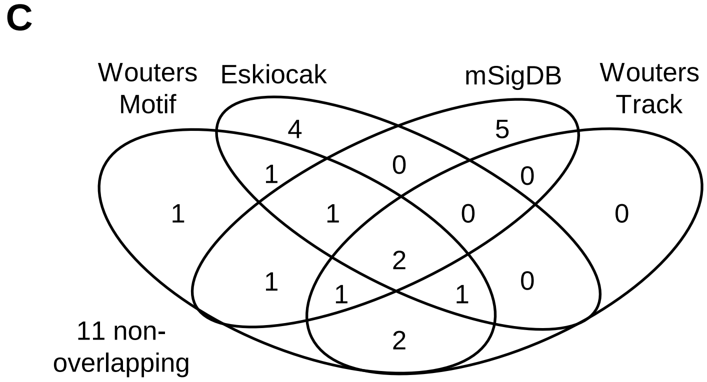




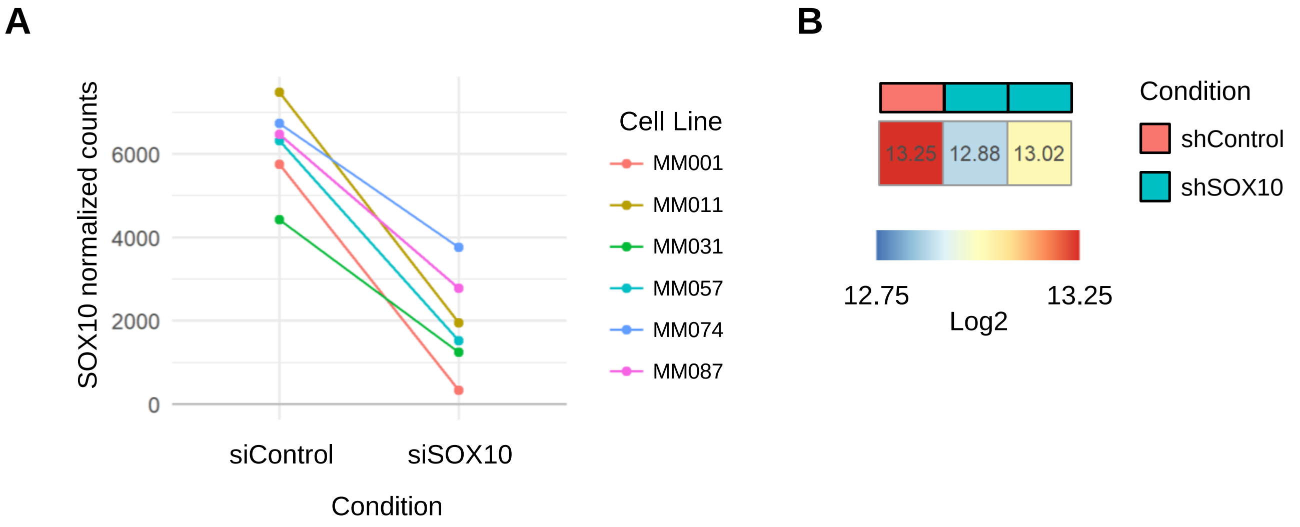
D

Term	*p*-val.	Fold Enrich.	BHFDR
GO:0030036 actin cytoskeleton organization	0.0016	16.43	0.373
GO:0014037 Schwann cell differentiation	0.0090	212.45	1
GO:0046847 filopodium assembly	0.0306	61.96	1

E

Group vs. parental	PMEL		MLANA	
	p-value	Log2 Fold Change	*p*-value	Log2 Fold Change
MeWo gSOX10 #2	< 1.0 e-250	-11.678	1.676 e-30	-13.660
MeWo gSOX10 #4	< 1.0 e-250	-10.706	3.598 e-31	-11.536
A375 gSOX10 #2	0.064	-0.758	No Expr.	No Expr.
A375 gSOX10 #4	0.001	-1.145	No Expr.	No Expr.

Figure S1. Generation of a SOX10 gene signature, Related to Figure 1. A) A Venn diagram showing the number of genes overlapping between the different SOX10 knockdown ATAC-seq time-points data. **B**) Venn diagram plot showing the genes overlapping between gSOX10 RNA-seq results. **C**) Venn diagram showing the count of genes from the refined SOX10 signature that overlaps signatures from other sources. **D**) A table showing enriched gene sets using DAVID for our SOX10 gene signature. **E**) A table showing statistical results for the tumor-associated-antigen genes PMEL and MLANA following SOX10 knockout.



C

Wouters SOX10 KD Bulk

Gene Set	NES	p-val
ESKIOCAK_siSOX10	-2.451	0
ESKIOCAK_25gene	-2.019	0
WOUTERS_TRACK	-2.008	0
WOUTERS_MOTIF	-1.832	0
MSIGDB	-1.603	0

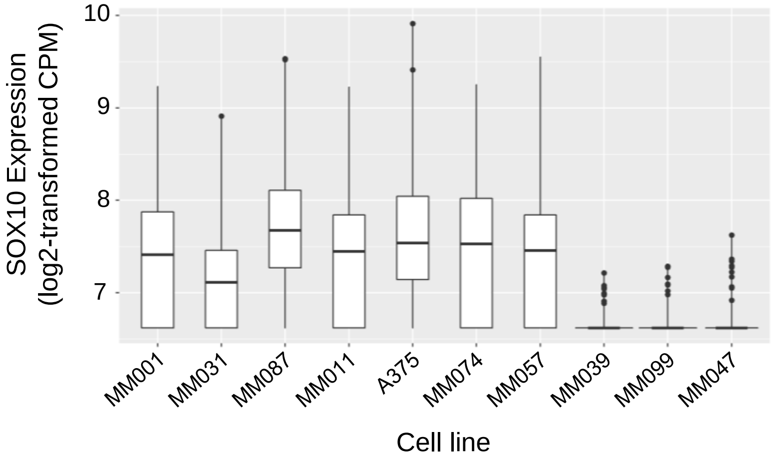
D

Sun SOX10 KD Bulk

Gene Set	NES	p-val
ESKIOCAK_siSOX10	-1.792	0
WOUTERS_TRACK	-1.685	0
WOUTERS_MOTIF	-1.522	0
MSIGDB	-1.397	0
ESKIOCAK_25gene	-1.082	0.358

Figure S2. SOX10 expression in knockdown bulk sequencing datasets, Related to Figure 2. A) A scatter plot showing the changes in expression of SOX10 before and after SOX10 knockdown for samples used in Fig. 2A. **B**) A heatmap showing the log2-transformed normalized SOX10 expression data in a parental and two SOX10 knockdown samples used in Fig. 2B. **C**) A table showing GSEA results for the Wouters Bulk RNA-seq SOX10 KD data using the SOX10 gene sets described in Fig. 1A, B for sequencing data used in Fig 2A. **D**) A table showing GSEA results for the Sun Bulk RNA-seq SOX10 KD data using the SOX10 gene sets described in Fig. 1A, B for sequencing data used in Fig 2B.

A



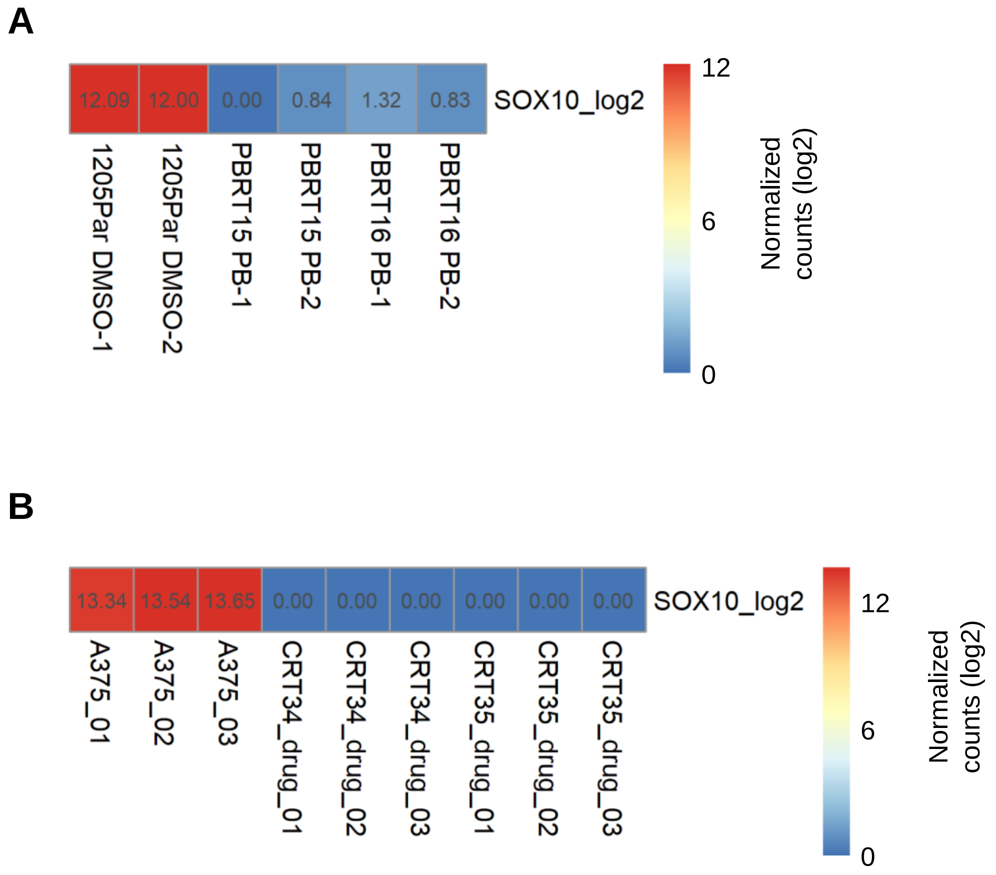
B

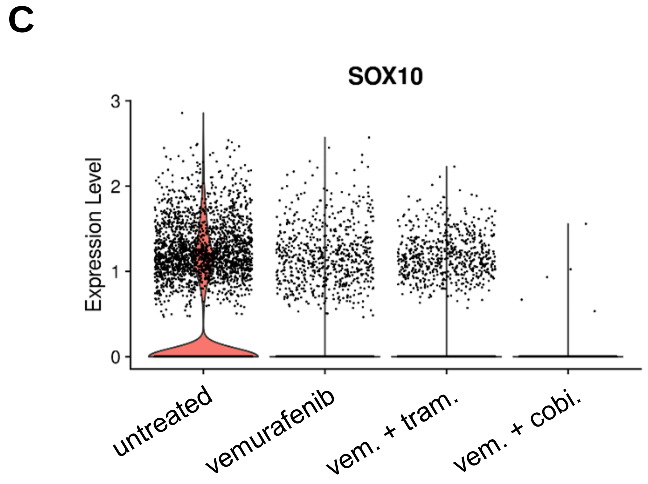
SOX10 null cell line	SOX10 expressing cell line	p-value	Mann-Whitney statistic	95% CI low	95% CI high
MM099	A375	2.13E-112	24293	-0.070	-0.060
MM099	MM057	2.88E-142	4682	-0.094	-0.086
MM099	MM087	3.93E-50	45127.5	-0.035	-0.028
MM099	MM074	1.45E-111	9317.5	-0.085	-0.076
MM099	MM031	2.57E-54	43770.5	-0.034	-0.027
MM099	MM011	2.42E-66	20389.5	-0.046	-0.038
MM099	MM001	1.42E-38	49218.5	-0.028	-0.021
MM047	A375	3.72E-89	17564	-0.068	-0.058
MM047	MM057	4.01E-117	3101.5	-0.092	-0.083
MM047	MM087	2.25E-38	32966.5	-0.033	-0.025
MM047	MM074	7.09E-93	6599.5	-0.084	-0.073
MM047	MM031	8.41E-42	31948.5	-0.031	-0.024
MM047	MM011	6.68E-55	14700.5	-0.044	-0.035
MM047	MM001	1.30E-28	36216.5	-0.026	-0.018
MM029	A375	1.98E-132	14181	-0.080	-0.071
MM029	MM057	3.45E-148	1966	-0.104	-0.096
MM029	MM087	4.13E-78	29022	-0.046	-0.038
MM029	MM074	1.45E-121	5345	-0.096	-0.086
MM029	MM031	2.07E-85	26467.5	-0.044	-0.037
MM029	MM011	4.66E-88	11625.5	-0.056	-0.048
MM029	MM001	2.77E-68	31219.5	-0.038	-0.031

C

SOX10 protein vs	Pearson Rho	Pearson *p*-value	Spearman's Rho	Spearman's *p*-value
SOX10 RNA-seq	0.082	0.672	0.125	0.516
Signature score	0.170	0.377	0.193	0.315

Figure S3. Signature scores in cell line data, Related to Figure 3. A) Box-plots showing SOX10 expression levels in scRNA-seq for cell lines displayed in Fig. 3E, F. **B**) Pairwise comparison results showing statistically significant differences in SOX10 signature scores between SOX10-low and SOX10-expressing cell lines from scRNA-seq data. **C**) Correlation results for SOX10 proteomics data with SOX10 RNA-seq gene expression and SOX10 signature scores calculated using RNA-seq data. Proteomics and RNA-seq data were obtained from DepMap.[S1, 2]





D

Sample	SOX10 expressed (% cells)
Untreated	33.339%
Vemurafenib	16.602%
vem + tram.	7.356%
vem. + cobi.	0.067%

Figure S4. SOX10 expression in targeted inhibitor resistant datasets, Related to Figure 4. A) SOX10 expression from RNA-seq data for samples in Fig. 4A. **B**) SOX10 expression from RNA-seq data for samples in Fig. 4B. **C**) SOX10 expression from scRNA-seq data for samples in Fig. 4C. **D**) Percent of malignant cells with SOX10 expression for samples in Fig. 4C.

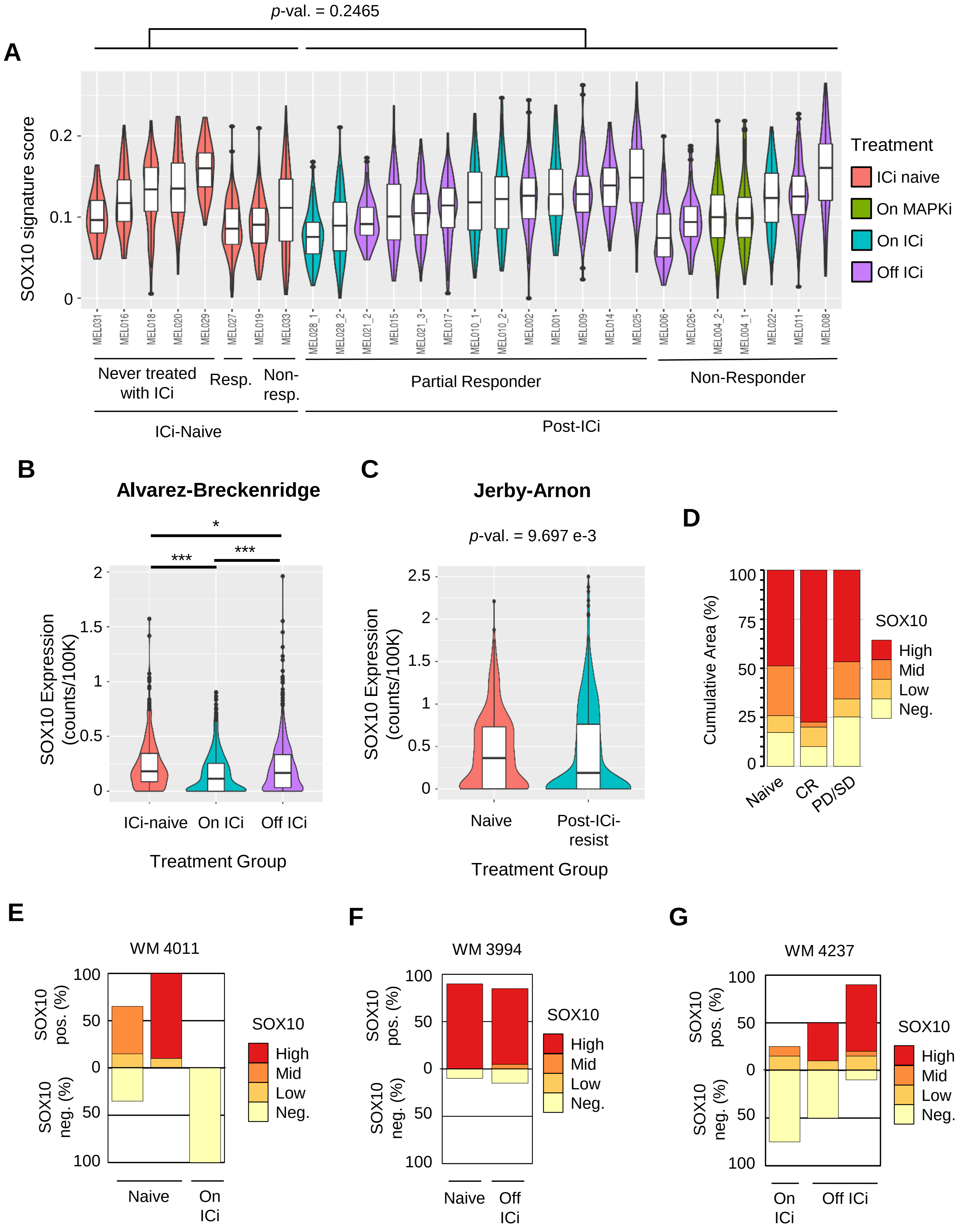

A
p-val. = 0.2465
SOX10 signature score
0
0.1
0.2
MEL031
MEL016
MEL018
MEL020
MEL029
MEL027
MEL019
MEL033
MEL028_1
MEL028_2
MEL021_2
MEL015
MEL021_3
MEL017
MEL010_1
MEL010_2
MEL002
MEL001
MEL009
MEL014
MEL025
MEL006
MEL026
MEL004_2
MEL004_1
MEL022
MEL011
MEL008
Never treated with ICi
Resp.
Non-resp.
Partial Responder
Non-Responder
ICi-Naive
Post-ICi
Treatment
ICi naive
On MAPKi
On ICi
Off ICi
B
Alvarez-Breckenridge
*
***
***
SOX10 Expression (counts/100K)
0
0.5
1
1.5
2
ICi-naive
On ICi
Off ICi
Treatment Group
C
Jerby-Arnon
p-val. = 9.697 e-3
SOX10 Expression (counts/100K)
0
0.5
1
1.5
2
2.5
Naive
Post-ICi-resist
Treatment Group
D
Cumulative Area (%)
0
25
50
75
100
Naive
CR
PD/SD
SOX10
High
Mid
Low
Neg.
E
WM 4011
SOX10 pos. (%)
SOX10 neg. (%)
100
50
0
50
100
Naive
On ICi
SOX10
High
Mid
Low
Neg.
F
WM 3994
SOX10 pos. (%)
SOX10 neg. (%)
100
50
0
50
100
Naive
Off ICi
SOX10
High
Mid
Low
Neg.
G
WM 4237
SOX10 pos. (%)
SOX10 neg. (%)
100
50
0
50
100
On ICi
Off ICi
SOX10
High
Mid
Low
Neg.

Figure S5. SOX10 expression and patient-matched SOX10 IHC results, Related to Figure 5. A) Violin plots of SOX10 signature score values for malignant cells from resected brain metastases from scRNA-seq data in Alvarez-Breckenridge et al.[S3] Samples are grouped by classifications in Alvarez-Breckenridge et al.,[S3] ordered by SOX10 signature score values and colored by systemic treatment at the time of tumor resection. **B**) Violin plots showing lower normalized SOX10 expression values in malignant cells from On ICi tumors compared to Off ICi and ICi-naïve tumors from Alvarez-Breckenridge et al.[S3] dataset. **C**) Violin plots showing lower normalized SOX10 expression values in malignant cells from ICi-resistant tumors compared to ICi-naïve tumors from Jerby-Arnon et al.[S4] dataset. **D**) Bar plot displaying a higher percentage of SOX10 negative malignant cells in PD/SD ICi-treated tumors compared to treatment-naïve and CR ICi-treated tumors. (**E-G**) Waterfall plots depicting the percent of malignant cells staining positive (low, mid, and high) or negative for SOX10 in treatment-naïve and ICi-treated tumors from patients WM4011 (**E**), WM3994 (**F**), and WM4237 (**G**). Significance was assessed by the Mann-Whitney, $*p<0.05$, $**p<0.01$, $***p<0.001$. NS: Not Significant.

Supplemental References

S1. Ghandi, M., et al., *Next-generation characterization of the Cancer Cell Line Encyclopedia.* Nature, 2019. **569**(7757): p. 503-508.

S2. Nusinow, D.P., et al., *Quantitative Proteomics of the Cancer Cell Line Encyclopedia.* Cell, 2020. **180**(2): p. 387-402 e16.

S3. Alvarez-Breckenridge, C., et al., *Microenvironmental Landscape of Human Melanoma Brain Metastases in Response to Immune Checkpoint Inhibition.* Cancer Immunol Res, 2022. **10**(8): p. 996-1012.

S4. Jerby-Arnon, L., et al., *A Cancer Cell Program Promotes T Cell Exclusion and Resistance to Checkpoint Blockade.* Cell, 2018. **175**(4): p. 984-997 e24.