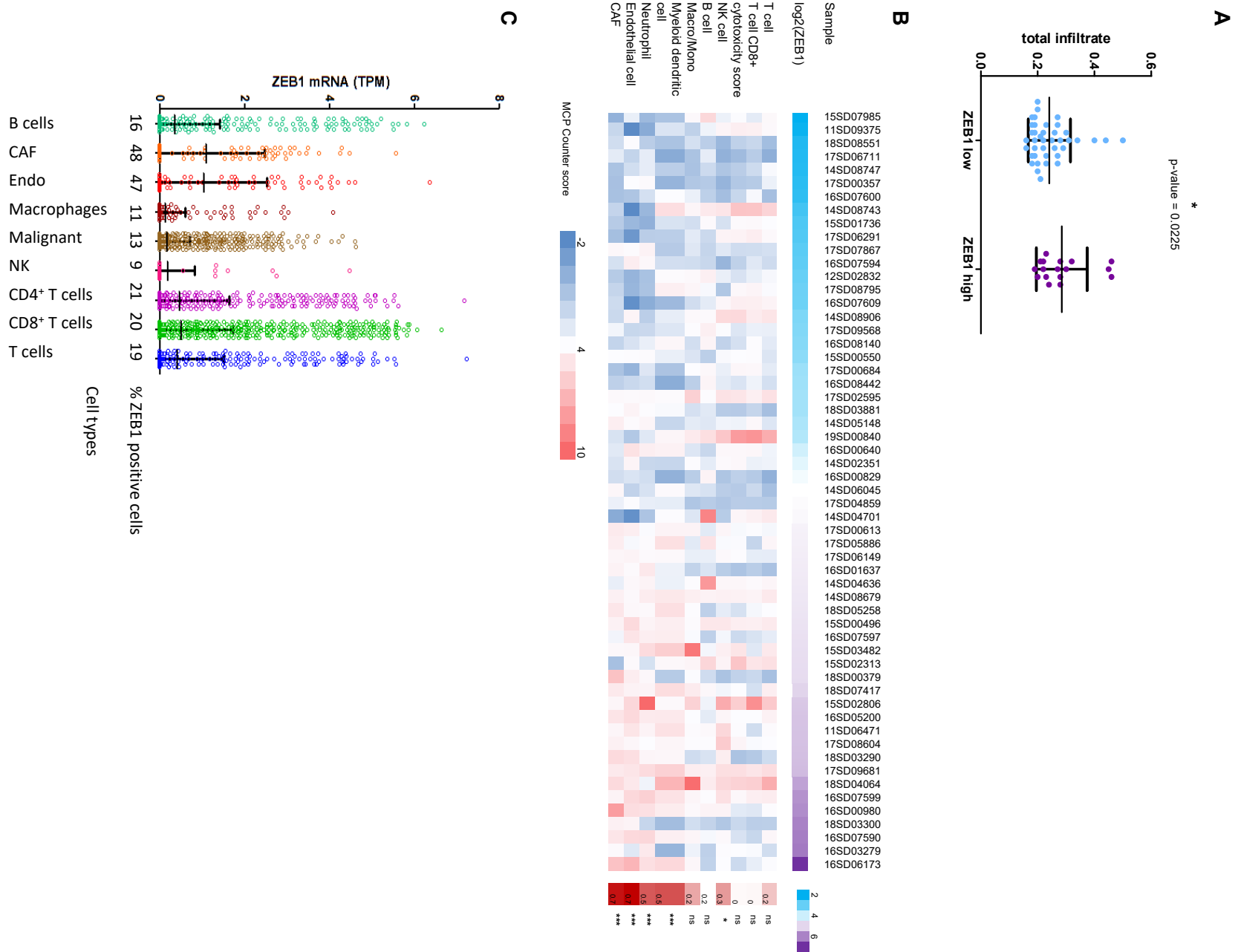


Supplementary Table1: Detailed clinical parameters of melanoma patients

Patient ID	Sex	Age	Cutaneous melanoma	Breslow (mm)	T stage	BRAF/NRAS mutation status	Other mutations	RNA-Seq	Spatial multi-IF	CD8 spatial localization
11SD06471	M	76	primary	1.4	T2a	WT	none	available	available	infiltrated
11SD09375	M	55	primary	3.5	T3b	WT	NRAS Exon2	available	available	excluded
12SD02832	F	76	metastasis	NA	NA	NRASQ61	none	available	available	infiltrated
14SD02351	M	57	metastasis	NA	NA	WT	none	available	available	infiltrated
14SD04636	M	69	primary	16	T4b	WT	none	available	available	infiltrated
14SD04701	F	82	primary	2.5	T3a	WT	none	available	available	infiltrated
14SD05148	M	60	primary	12	T4b	WT	none	available	available	infiltrated
14SD06045	M	73	metastasis	NA	NA	BRAFV600	none	available	available	excluded
14SD08679	M	79	primary	4.7	T4b	WT	none	available	available	infiltrated
14SD08743	M	60	metastasis	NA	NA	WT	MAP2K1	available	available	infiltrated
14SD08747	F	73	primary	17	T4a	WT	none	available	NA	NA
14SD08906	F	66	metastasis	NA	NA	WT	HRAS	available	available	infiltrated
15SD00496	M	51	primary	4.5	T4b	BRAFV600	none	available	available	desert
15SD00550	F	37	primary	8.5	T4b	BRAFV600	none	available	available	infiltrated
15SD01736	M	67	metastasis	NA	NA	BRAFV600	none	available	available	infiltrated
15SD02313	M	71	metastasis	NA	NA	BRAFV600	none	available	NA	NA
15SD03482	M	85	metastasis	NA	NA	BRAFV600	none	available	NA	NA
15SD06183	F	83	metastasis	NA	NA	WT	none	NA	available	excluded
15SD06506	M	75	primary	14	T4b	NRASQ61	none	available	available	excluded
15SD07985	M	85	metastasis	NA	NA	NRASQ61	none	available	available	desert
16SD00640	F	67	primary	2.7	T3a	NRASQ61	none	available	available	infiltrated
16SD00829	F	80	metastasis	NA	NA	BRAFV600	MET	available	available	infiltrated
16SD00980	F	37	metastasis	NA	NA	BRAFV600	none	available	available	desert
16SD01637	F	74	metastasis	NA	NA	WT	none	available	available	desert
16SD03279	M	36	primary	4.2	T4b	BRAFV600	none	available	available	excluded
16SD05200	M	70	primary	0.8	T1a	WT	none	available	available	infiltrated
16SD06173	F	71	metastasis	NA	NA	WT	none	available	available	excluded
16SD06937	F	46	metastasis	NA	NA	BRAFV600	none	NA	available	infiltrated
16SD07590	F	66	primary	1.5	T2a	WT	ckIT	available	available	excluded
16SD07594	M	49	primary	4.2	T4b	BRAFV601	none	available	NA	NA
16SD07597	F	58	primary	0.86	T1b	BRAFV600	none	available	available	infiltrated
16SD07599	M	50	primary	0.7	T1a	BRAFV600	CDKN2A, ARID2, FBXW7, RAC1, pTERT	available	NA	NA
16SD07600	M	45	primary	4	T3b	BRAFV600	none	available	available	desert
16SD07609	M	52	primary	25.4	T4b	BRAFV600	none	available	available	infiltrated
16SD08140	M	54	metastasis	NA	NA	NRASQ61	PDGFRA	available	available	desert
16SD08442	M	24	primary	6.8	T4b	BRAFV600	none	available	available	excluded
17SD00357	M	80	primary	3.5	T3a	WT	none	available	available	desert
17SD00613	M	43	metastasis	NA	NA	WT	MAP2K1, PTEN	available	available	excluded
17SD00684	M	78	primary	20	T4b	NRASQ61	none	available	available	desert
17SD02595	M	53	primary	10	T4b	WT	KIT, KRAS	available	NA	NA
17SD04859	F	43	primary	3.1	T3a	NRASQ61	IDH1	available	available	desert
17SD05886	F	43	primary	6.5	T4a	NRASQ61	RAC1	available	available	excluded
17SD06149	M	69	metastasis	NA	NA	NRASQ61	none	available	available	excluded
17SD06291	M	78	metastasis	NA	NA	BRAFV600	NF1, NRAS G12A, TERT	available	available	infiltrated
17SD06711	M	55	metastasis	NA	NA	WT	EGFR	available	available	desert
17SD07867	M	63	primary	1.6	T2b	WT	NRAS G12C, TERT, CDKN2A	available	available	desert
17SD08604	F	64	metastasis	NA	NA	BRAFV600	none	available	available	excluded
17SD08795	F	60	metastasis	NA	NA	BRAFV600	MET	available	available	desert
17SD09568	M	60	metastasis	NA	NA	BRAFV600	RAC1, TERT, CARD11, ARID1A	available	available	excluded
17SD09681	M	66	primary	1.3	T2a	NRASQ61	TERT	available	available	infiltrated
18SD00379	M	74	primary	4	T3b	WT	none	available	available	excluded
18SD03290	M	58	metastasis	NA	NA	WT	none	available	available	desert
18SD03300	M	46	metastasis	NA	NA	NRASQ61	none	available	available	desert
18SD03881	M	58	primary	9	T4b	BRAFV600	CDKN2A	available	available	desert
18SD04064	F	69	metastasis	NA	NA	WT	none	available	available	infiltrated
18SD05258	F	61	metastasis	NA	NA	BRAFV600	none	available	available	excluded
18SD07417	M	84	primary	6	T4a	WT	NF1, ALK, CDKN2A, TP53, TSC2, TERT	available	available	infiltrated
18SD08551	F	58	metastasis	NA	NA	WT	PDGFRA, CDKN2A	available	available	excluded
19SD00840	F	45	metastasis	NA	NA	BRAFV600	CDKN2A, PTEN, TERT	available	available	infiltrated
19SD07475	M	33	metastasis	NA	NA	WT	ERBB4	NA	available	desert

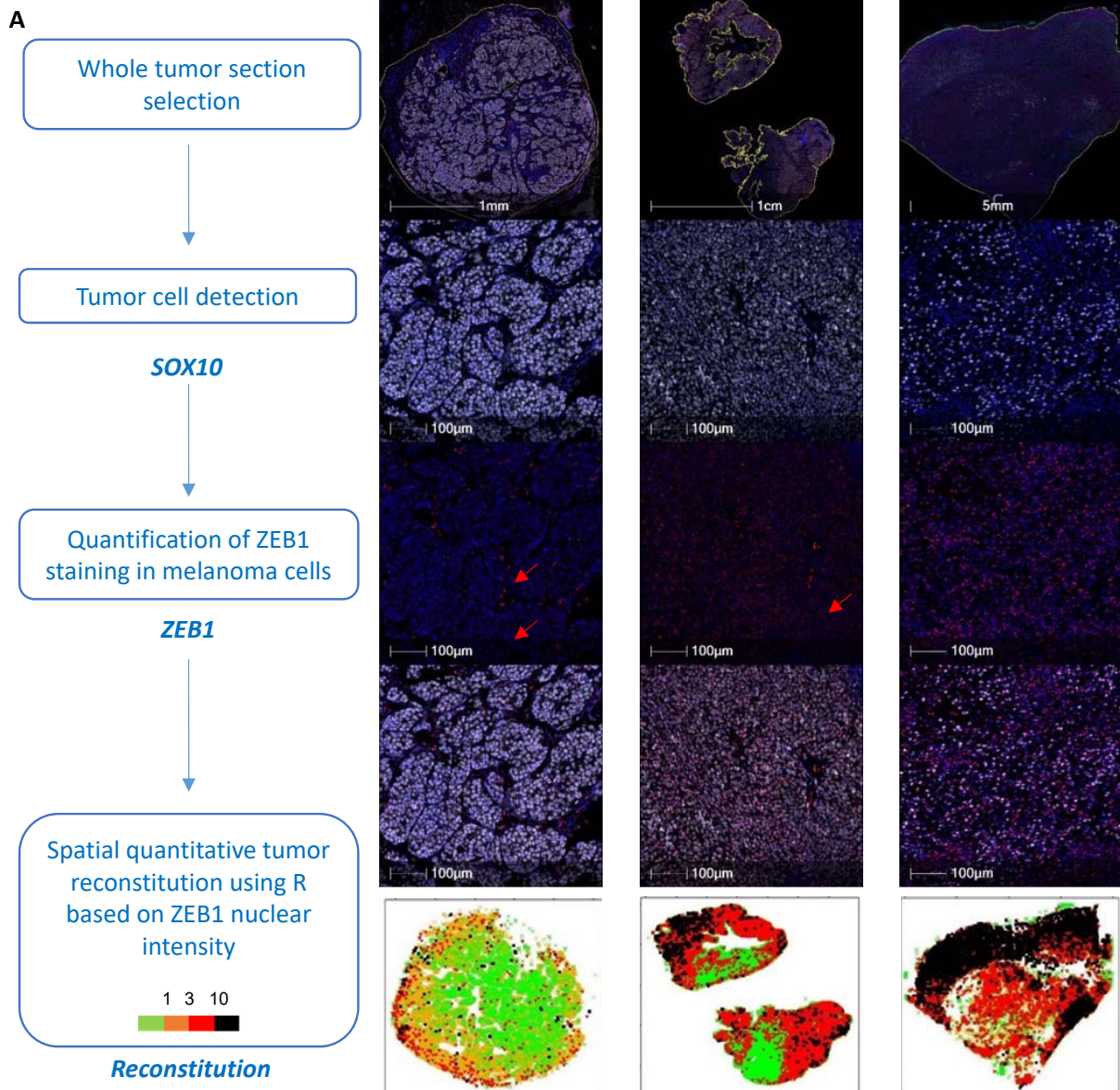
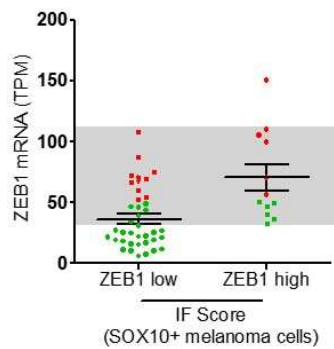
Supplementary Figure 1



**Supplementary Figure 1: Melanoma bulk RNA-Seq analyses and scRNA-Seq re-analyses**

**A**, Proportions of total immune infiltrate determined using QuantiSeq in ZEB1 low and ZEB1 high tumors. ZEB1 low tumors are defined as the 70% lowest ZEB1 TPM values, and ZEB1 high melanomas display the 30% highest ZEB1 TPM values. (Mean with SD, Mann-Whitney test). **B**, Heatmap of RNA-Seq data on 57 cutaneous melanoma samples. Patients are presented in columns. First line: ZEB1 expression ( $\log_2(\text{TPM})$ ). Following lines: MCP Counter scores of T cells, CD8<sup>+</sup> T cells, cytotoxicity score, NK cells, B cells, macrophages and monocytes (Macro/Mono), myeloid dendritic cells, neutrophils, endothelial cells and cancer associated fibroblasts (CAFs). On the right, regression coefficient and p-values calculated using Pearson's correlation between ZEB1 expression and MCP Counter scores are represented (\*: p-value < 0.05; \*\*: p-value < 0.005; \*\*\*: p-value < 0.0005). **C**, Analyses of the melanoma single cell RNA-Seq data set from (Jerby-Arnon et al.). ZEB1 mRNA expression (TPM) within cell types: B cells (n = 818), Cancer Associated Fibroblast (CAFs, n = 106), endothelial cells (Endo, n = 104), macrophages (n = 420), malignant cells (Mal, n = 2018), NK cells (n = 92), CD4 T cells (n = 856), CD8 T cells (n = 1759) and T cells (n = 706). Mean with SD. Percentages of ZEB1 expressing cells (non-zero ZEB1 expression) are calculated for each cell type.

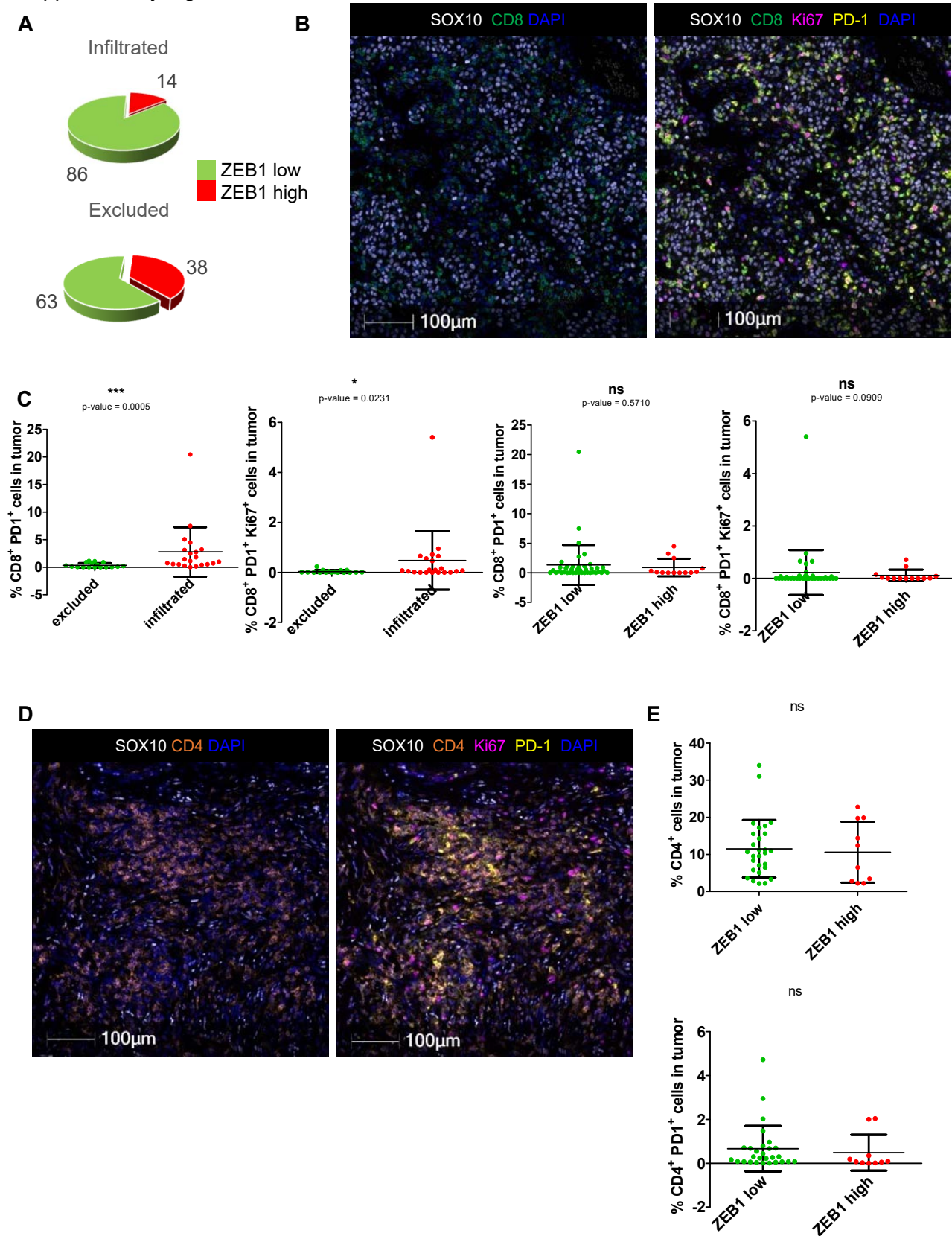
## Supplementary Figure 2

**B**

**Supplementary Figure 2: Immunofluorescence analyses quantification and spatial reconstitution**

**A**, ZEB1 immunofluorescence quantification in melanoma cells. Example of ZEB1 nuclear quantification specifically in melanoma cells in a ZEB1 low and two ZEB1 high melanoma tumors. Melanoma cells are stained using SOX10 (white, nuclear staining). ZEB1 appears in red and nucleus are stained with Dapi (blue). First line: overview of the entire tumor. Second line: SOX10 and DAPI. Third line: ZEB1 and DAPI. Arrows show ZEB1 staining in stromal cells. Forth line: merge ZEB1, SOX10, DAPI. Below: Tumor spatial reconstitution using the R software: melanoma cells are represented according to their nuclear expression level of ZEB1. In green: ZEB1 intensity < 1. In orange: ZEB1 intensity in between 1 and 3. In red: ZEB1 intensity in between 3 and 10. In black ZEB1 intensity > 10. Melanoma composed of at least 15 percent of ZEB1 high cells (intensity > 3) were defined as ZEB1 high melanoma. **B**, Correlation between ZEB1 IF and RNA-seq score. ZEB1 expression level (RNA Seq, TPM) in ZEB1 low and ZEB1 high melanoma samples, as assessed by IF analysis (SOX10+). In green: tumor classified as ZEB1 low using the RNA-Seq approach (the 70% of lowest ZEB1 TPM values). In red: tumors classified as ZEB1 high using the RNA-Seq approach (the 30% of the ZEB1 top values). Grey area: the range of ZEB1 expression levels where tumors can be misclassified. 16 tumors / 50 (32%) are misclassified.

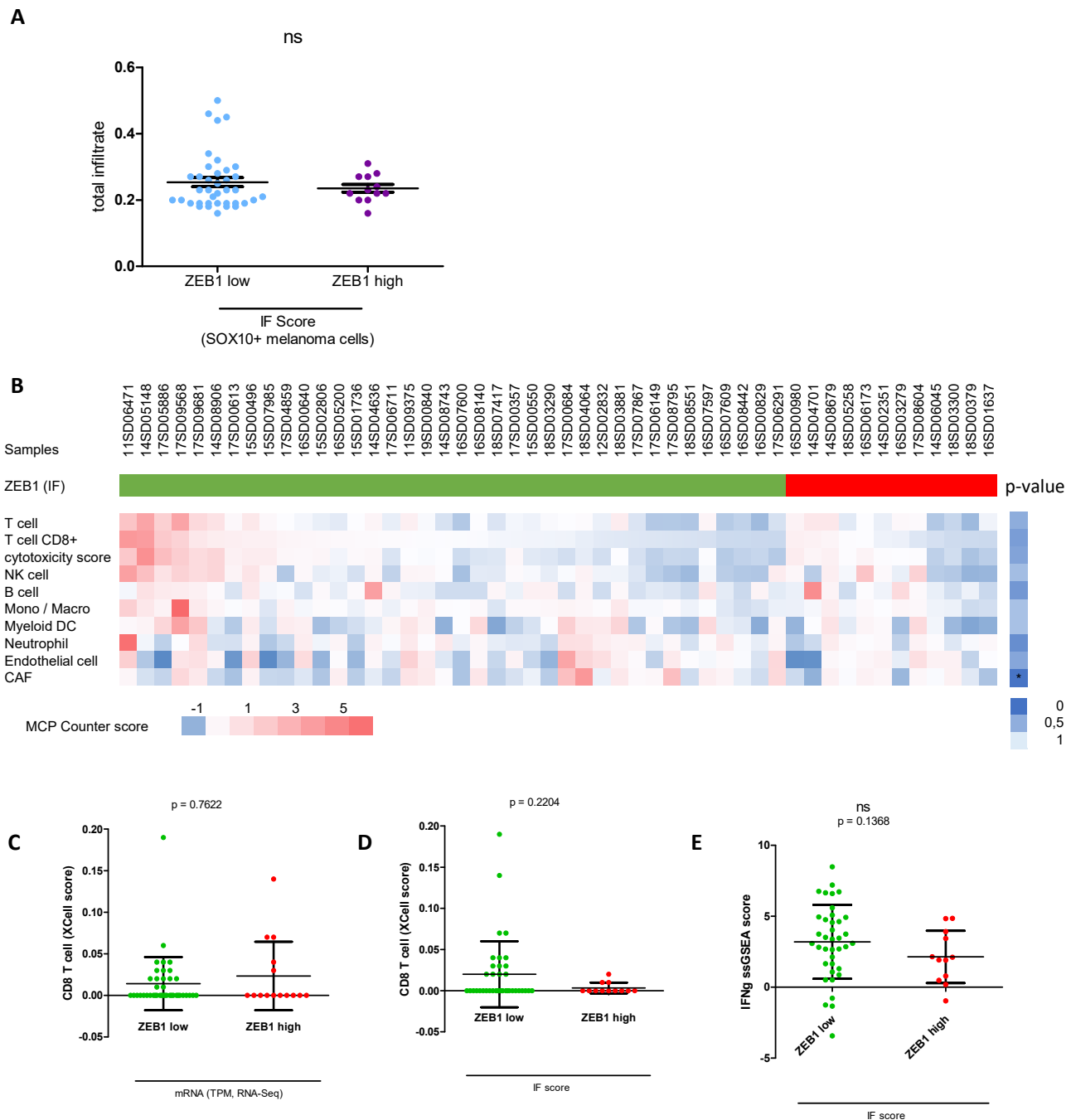
## Supplementary Figure 3



**Supplementary Figure 3: Multi-immunofluorescence analyses of PD-1 and Ki67 activation markers in CD8<sup>+</sup> and CD4<sup>+</sup> T cells**

**A**, Pie charts representing the proportion of ZEB1 low (green) and ZEB1 high (red) tumors within CD8 excluded (n = 19) and CD8 infiltrated (n = 21) tumors. **B**, Representative CD8 staining (in green) (left) and additional markers PD-1 (in yellow) and Ki67 (in magenta). Melanoma cells are stained using SOX10 (in white) and nuclei are stained using DAPI (in blue). Scale bar = 100  $\mu$ m. **C**, Percentage of CD8 cells expressing PD-1 (left, CD8<sup>+</sup> PD-1<sup>+</sup>) and CD8 cells co-expressing PD-1 and Ki67 (right, CD8<sup>+</sup> PD-1+Ki67<sup>+</sup>) in excluded (in green) versus infiltrated (in red) tumors. (Mean with SD, Mann Whitney test). **D**, Representative CD4 staining (in orange, left) and additional markers PD-1 (in yellow) and Ki67 (in magenta). Melanoma cells area stained using SOX10 (in white) and nuclei are stained using DAPI (in blue). Scale bar = 100  $\mu$ m. **E**, Percentage of CD4 T cells (on the left) and CD4 expressing PD-1 (on the right) in ZEB1 low (in green) versus ZEB1 high (in red) tumors. (Mean with SD, Mann Whitney test, ns).

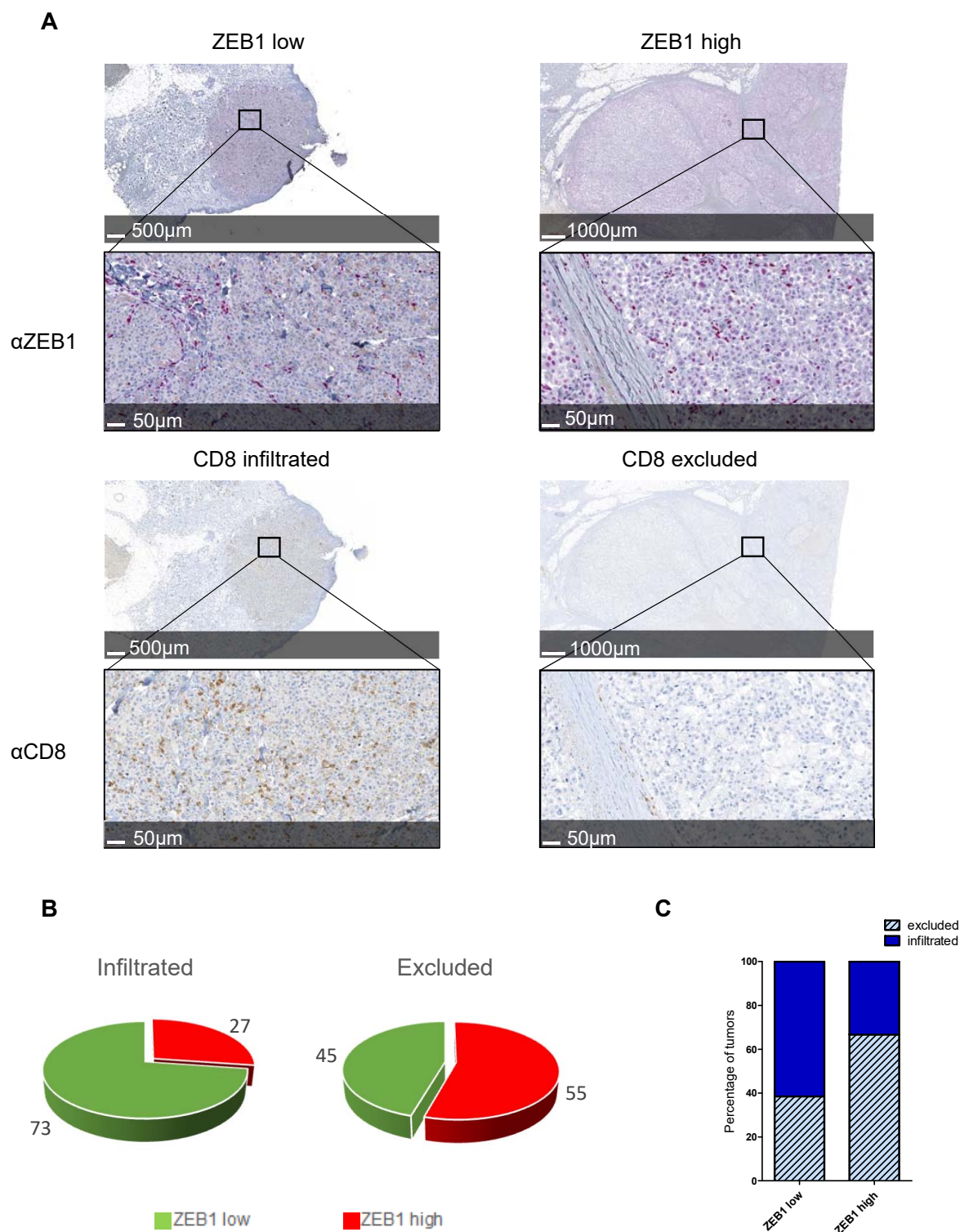
## Supplementary Figure 4

**Supplementary Figure 4: Immune signature analyses from RNA-Seq data**

**A**, Proportions of total immune infiltrate determined using QuantiSeq in ZEB1 low and ZEB1 high tumors. Tumors were segregated in ZEB1 high or low tumors based on IF analysis. (Mean with SD, Mann-Whitney test). **B**, Heatmap of RNA-Seq data on the 50 cutaneous melanoma samples with both IF and RNA-Seq data available. Patients are presented in columns. First line: ZEB1 classification following IF (ZEB1 low in green and ZEB1 high in red). Following lines: MCP Counter scores of T cells, CD8 T cells, cytotoxicity, NK cells, B cells, macrophages and monocytes (Macro/Mono), myeloid dendritic cells, neutrophils, endothelial cells and cancer associated fibroblasts (CAF). Mann-Whitney test between ZEB1 low and high tumors for each population. **C**, Tumors were segregated in ZEB1 high or low tumors based on ZEB1 TPM expression. ZEB1 high represent the 30% of the ZEB1 top values. CD8 T cell Xcell score in ZEB1 low (in green,  $n = 41$ ) and ZEB1 high (in red,  $n = 15$ ) tumors is represented (Mean with SD, Mann Whitney test). **D-E**, Tumors were segregated in ZEB1 high or low tumors based on IF analysis. CD8 T cell Xcell score (**D**) and IFN- $\gamma$  ssGSEA score (**E**) in ZEB1 low (in green,  $n = 38$ ) and high (in red,  $n = 12$ ) tumors (Mean with SD, Mann Whitney test).

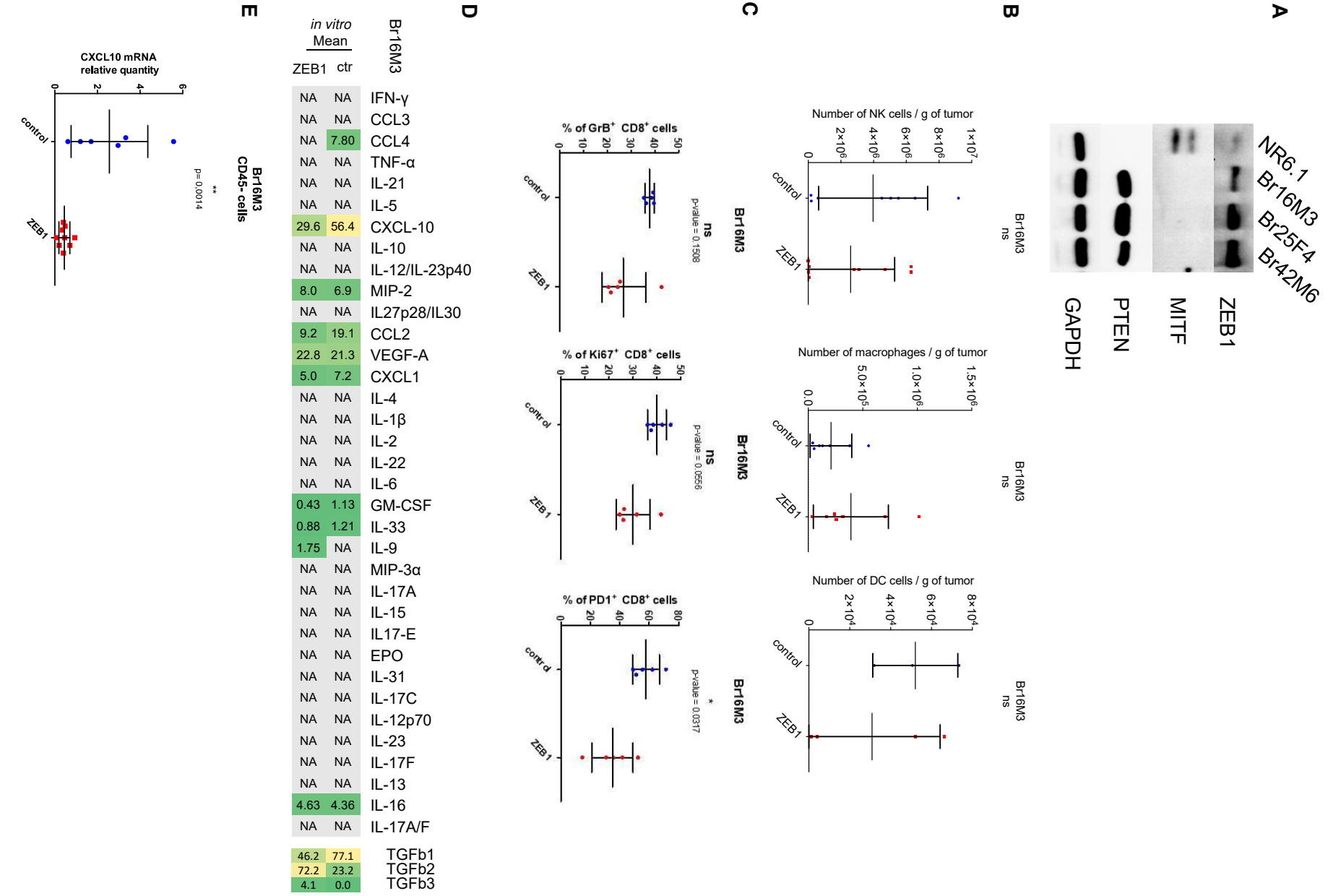


## Supplementary Figure 5



**Supplementary Figure 5: ZEB1 and CD8 immunohistochemical staining in a validation cohort of n=22 cutaneous melanoma samples.** **A**, Representative pictures of ZEB1 (red) and CD8 (brown DAB) immunostaining in a ZEB1 low / CD8 infiltrated and a ZEB1 high / CD8 excluded tumor. **B**, Pie charts representing the proportion of ZEB1 low (green) and ZEB1 high (red) tumors within CD8 excluded (n = 11) and CD8 infiltrated (n = 11) tumors. **C**, Stacked bar representing the percentage of CD8 excluded (light blue) and CD8 infiltrated (dark blue) tumors within ZEB1 low (n = 13) and ZEB1 high (n = 9) tumors.

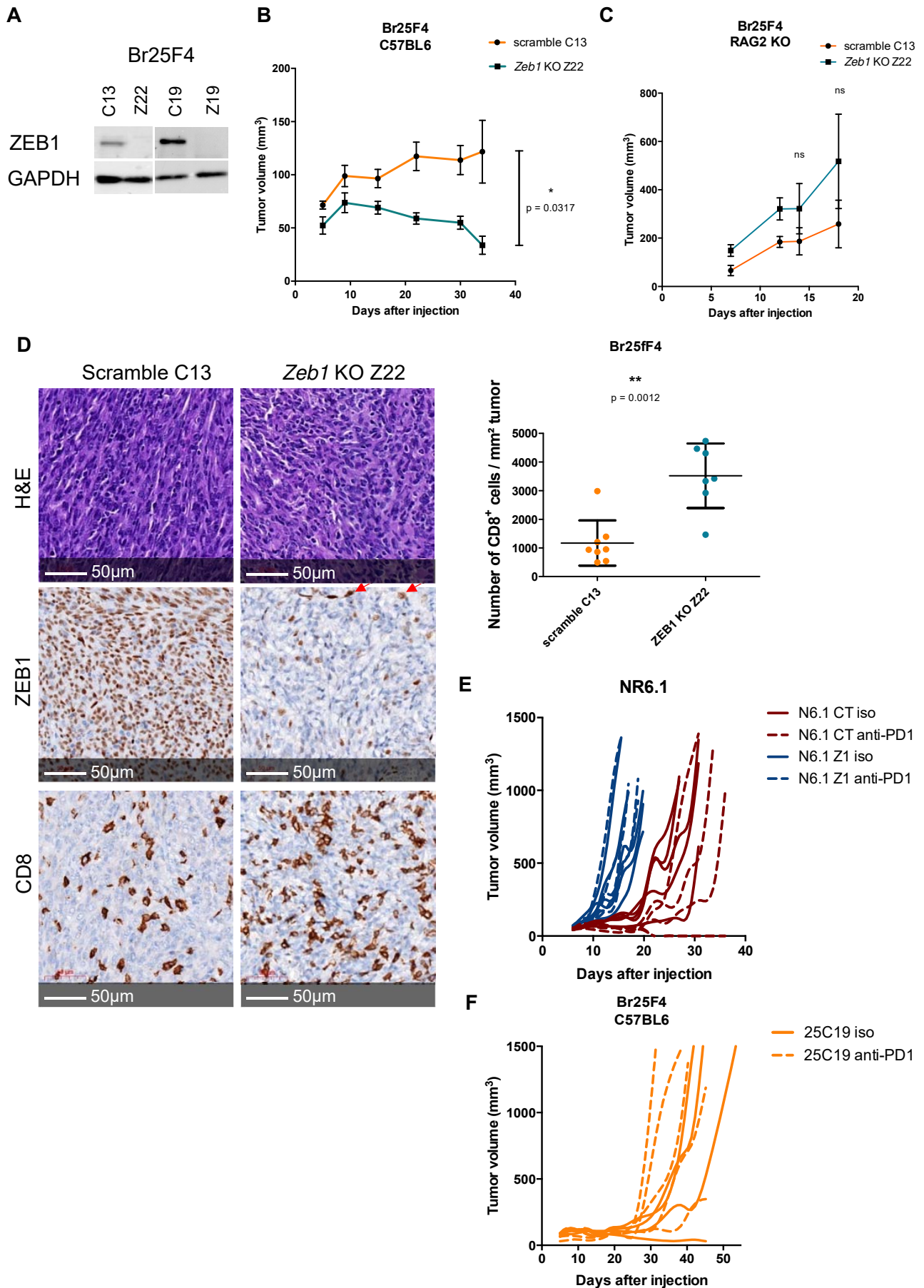
Supplementary Figure 6



**Supplementary Figure 6: Characterization of ZEB1 ectopically expressing mouse models**

**A**, Murine melanoma cell line characterization: Western blot analyses of ZEB1, MITF and PTEN in murine melanoma cell lines. GAPDH was used as a loading control. PTEN expression is maintained in the *BRAFV600* cell lines but not in *NRAS* mutated cells. **B**, FACS analyses of NK cells (NKp46+) (n = 8 and 9), macrophages (CD11b+, F4/80+) (n = 6 per group), and dendritic cells (DC) (IA/IE+, CD11c+) (n = 3 and 4) infiltration in Br16M3 control and ZEB1-overexpressing tumors. Bar charts representing the number of cells per gram of tumor. **C**, Percentage of GrB+, Ki67+ and PD-1+ CD8+ T cells in control (green, n = 5) and ZEB1-overexpressing (red, n = 5) Br16M3 tumors. (Mean with SD, Mann Whitney test). **D**, Relative quantity of 35 analytes measured using the Meso Scale Diagnostics (MSD) technology in *in vitro* supernatants of Br16M3 control (n = 2) and Br16M3 overexpressing ZEB1 (n = 2) cells (Mean in pg/mL). Grey represents values under detection threshold. **E**, CD45<sup>-</sup> cells were sorted from Br16M3 control (n = 6, green) or ZEB1-overexpressing (n = 9, red) tumors and RNA were extracted. Bar chart representing the relative expression of CXCL10 (Mean with SD, Mann Whitney test).

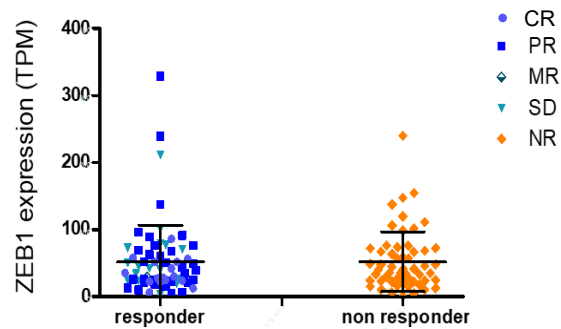
## Supplementary Figure 7



**Supplementary Figure 7 : Knock-out of *Zeb1* in melanoma cells strongly reduces tumor growth, favors CD8<sup>+</sup> T cell infiltration and improves the response to immune checkpoint blockade**

**A**, Br25F4 murine melanoma cells were infected with lentiviruses expressing the Cas9 and guide RNA (sgRNA) targeting *Zeb1* or scramble sgRNA. 25C13 and 25C19: scramble clones. 25Z22 and 25Z19: *Zeb1* KO clones. Western blot analyses of ZEB1. GAPDH was used as a loading control. **B**,  $2.5 \times 10^6$  25C13 (orange) or 25Z22 (blue) cells were injected subcutaneously into immunocompetent mice. The mean tumor volume for 5 mice is represented (+/- SD, Mann Whitney test). **C**,  $2.5 \times 10^6$  25C13 (orange) or 25Z22 (blue) cells were injected subcutaneously into immunocompetent C57BL6 (n = 4-5) or RAG KO (n = 3) mice. The mean tumor volume is represented (+/- SD, Mann Whitney test). **D**, Representative pictures of ZEB1 and CD8 immunostaining in 25C13 (scramble) and 25Z22 (*Zeb1* KO) tumors. Scale bars = 50  $\mu$ m. Arrows show stromal and endothelial cells expressing ZEB1, while tumor cells are *Zeb1* KO. Right panel: Quantification of CD8 infiltration in 25C13 (scramble) and 25Z22 (*Zeb1* KO) tumors. Bar chart representing the number of CD8<sup>+</sup> cells per mm<sup>2</sup> of tumor (Mean with SD, Mann Whitney test). **E-F**, Individual growth curves are depicted for each control (red) or ZEB1 overexpressing (blue) tumor treated with either anti-PD-1 (dashed lines) or control isotype (iso, solid lines) in NR6.1 murine melanoma (**E**) or Br25F4 murine melanoma 25C19 control cells (**F**).

## Supplementary Figure 8



**Supplementary Figure 8 : ZEB1 mRNA expression analyses in the RNA-Seq data from the immunotherapy-treated melanoma cohort from Liu et al. (n = 144).** ZEB1 expression (TPM) in melanoma cells +/- SD is plotted according to the response to anti-PD-1 immunotherapies: responders include complete response (CR), partial response (PR), mixed response (MR), and stable disease (SD); NR: non responders. Mann Whitney test, ns, p-value = 0,7650.