Figure S1

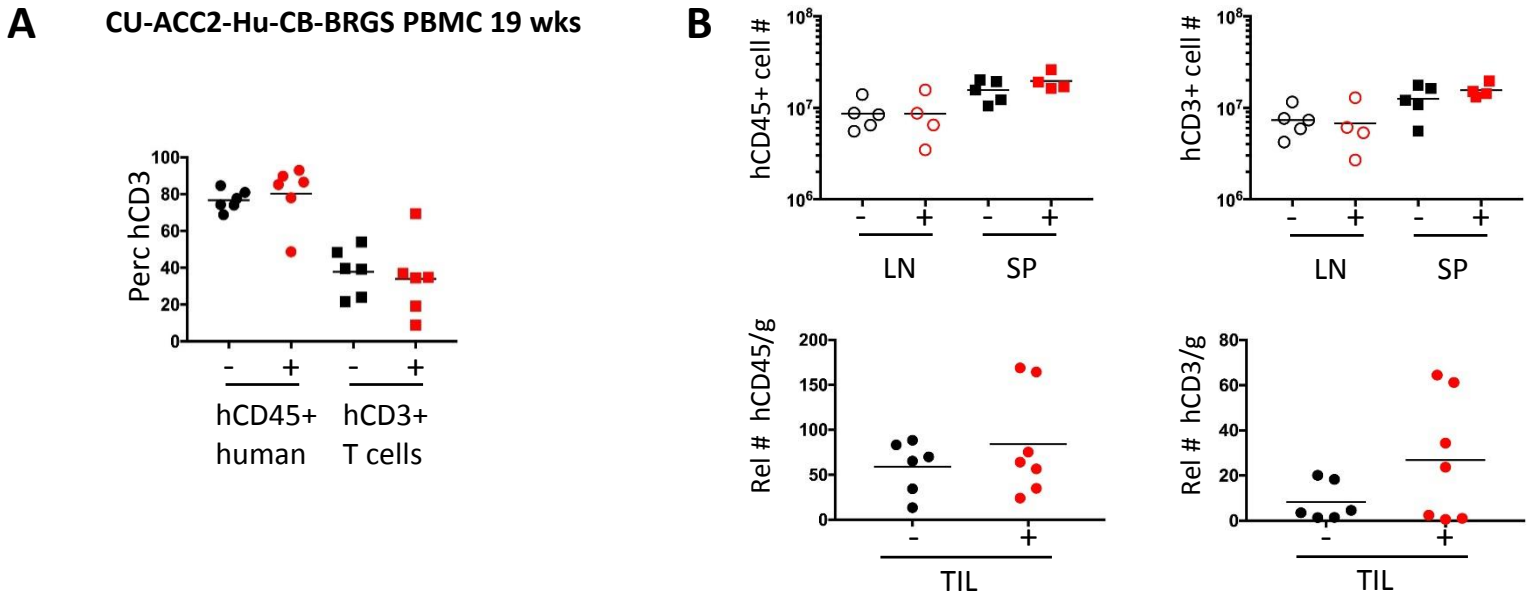

Figure S1: Human chimerism in CU-ACC2-hu-CB-BRGS mice. **A,** Mice were bled at 19 weeks, analyzed for overall human (hCD45+) and T cell (CD3+) chimerism by flow cytometry and sorted into equivalent groups. **B,** Human CD45+ and T (CD3+) cell numbers in LN and SP (top panels) or TILs (bottom panels) of CU-ACC2-hu-CB-BRGS mice. Cell counts in TILs are presented as per gram of tumor. Each dot represents data from an individual mouse, or tumor within a mouse, that was either untreated (-) or treated with pembrolizumab (+), and lines are arithmetic means. SP-spleen, LN-lymph nodes, TIL-tumor infiltrating lymphocytes

A

Figure S2

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	Penta D	Penta E	TH01	TPOX	vWA
Human tissue CU-ACC2-M2B	X	12	12,13	11, 12	15,19, 20	28,29, 30	14, 15	11,12	8, 9	13	23, 24	12, 14	7, 11	6, 9.3	8, 12	16, 18, 19
Human tissue CU-ACC2	x	11,12	13	11,12	19,20	29	14	11,12	8	13,14	24	14	11	9.3	8	16,18,21
CU-ACC2-M2B nude PDX	X	11, 12	13	11, 12	19	29, 30	14	11,12	8	13	24	14	11	9.3	8	16, 18, 19,
CU-ACC2-Hu-CB-BRGS	X	11, 12	12,13	11, 12	19	29,30	14	11, 12	8	13, 14	24,25	14	11	9.3	8	16, 18, 19

Sample name	Reference name	Total # Shared Alleles	Total # Sample Alleles	Total # Reference Alleles	Match
CU-ACC2-Hu-CB-BRGS	Human tissue CU-ACC2	21	25	23	88%
CU-ACC2-Hu-CB-BRGS	CU-ACC2-M2B nude PDX	20	25	22	94%
CU-ACC2-M2B nude PDX	Human tissue CU-ACC2	20	22	23	89%
Human tissue CU-ACC2	Human tissue CU-ACC2-M2B	20	23	32	73%

B

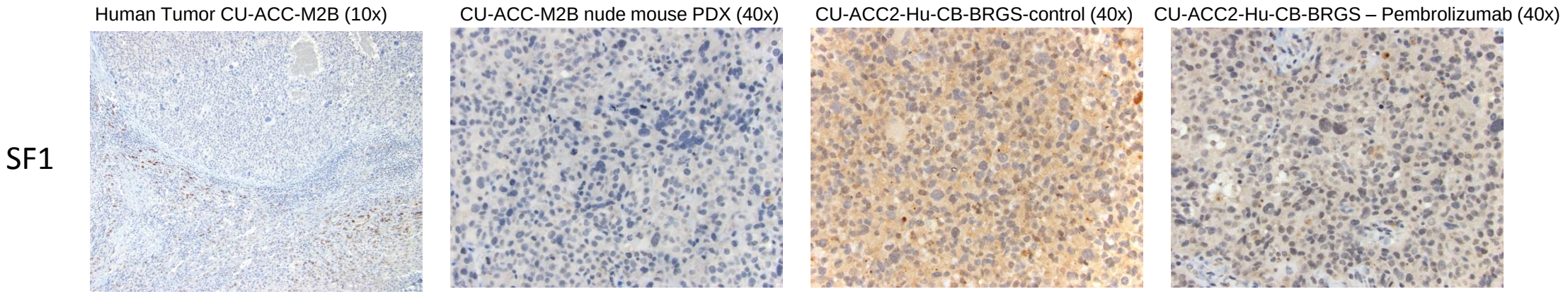

Figure S2: Complete short tandem repeat (STR) profiling and SF1 staining. **A.** STR of human CU-ACC-M2B (liver metastasis), human CU-ACC2 tumor tissue (metastasis), CU-ACC-M2B nude PDX and CU-ACC2-Hu-CB-BRGS (top table) and respective tissue match analysis (bottom table). **B.** SF1 immunohistochemistry in human, nude mouse and humanized mouse PDX show SF1 positivity (brown) as a markers of adrenocortical carcinoma.

Figure S3

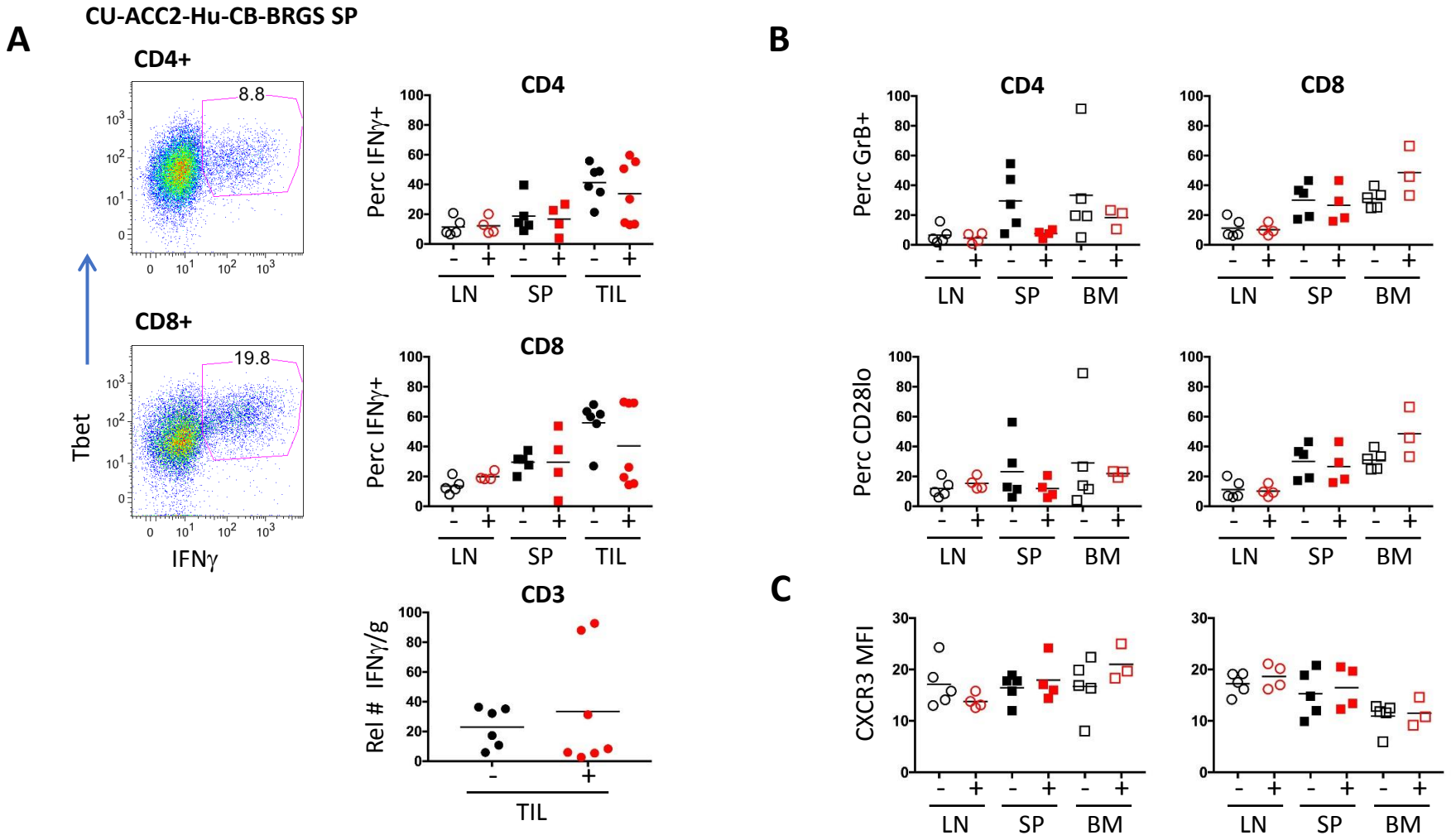

Figure S3: Functional analysis of T cells in CU-ACC2-hu-CB-BRGS mice. **A.** Representative flow plots illustrating gating strategy for expression Tbet and IFNg among CD4 (top) or CD8 (bottom) T cells (hCD45+CD3+) in the spleen of an untreated CU-ACC2-hu-CB-BRGS mouse. The graphs on the right show the percentages of IFNg+ for CD4+ (top) or CD8+ (middle) T cells from peripheral organs. The lower right graph depicts the relative number of IFNg+ T cells in the TILs. **B.** Percentages of GrB+ (top) or CD28^{lo} (bottom) among CD4+ (left) or CD8+ (middle) T cells in lymph organs. **C.** Expression (MFI) of CXCR3 for CD4+ (left) or CD8+ (right) T cells in lymph organs. **A-C.** For all graphs, each dot represents data from the indicated organ from an individual CU-ACC2-hu-CB-BRGS mouse that was either untreated (-) or treated with pembrolizumab (+). Lines are arithmetic means.

Figure S4

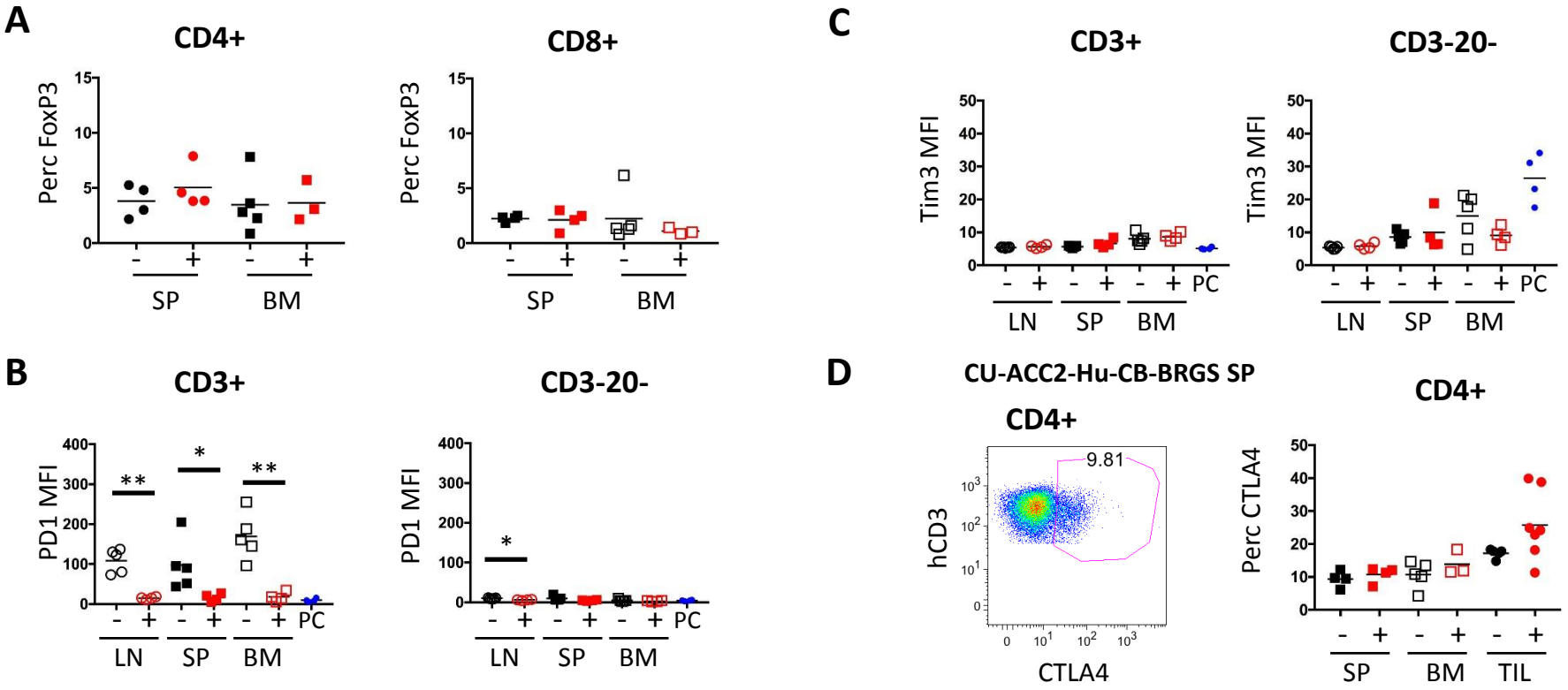

Figure S4: Regulatory T cells and inhibitory receptors in CU-ACC2-hu-CB-BRGS mice. **A.** Percentage FoxP3+ cells among CD4+ (left) and CD8+ (right) T cells from spleen (SP) or bone marrow of CU-ACC2-hu-CB-BRGS mice. **B-C.** Expression (MFI) of PD-1 **(B)** and Tim3 **(C)** inhibitory receptors on human T (CD3+) or myeloid (CD3-CD20-) cells. **D.** Representative flow cytometry plot showing gating strategy for expression of CTLA4 on CD4+ T cells (hCD45+hCD3+). **A-D.** For all graphs, each dot represents data from the indicated organ from an individual CU-ACC2-hu-CB-BRGS mouse that was either untreated (-) or treated with pembrolizumab (+). Lines are arithmetic means. P-values: *<0.05, **<0.01.

Figure S5

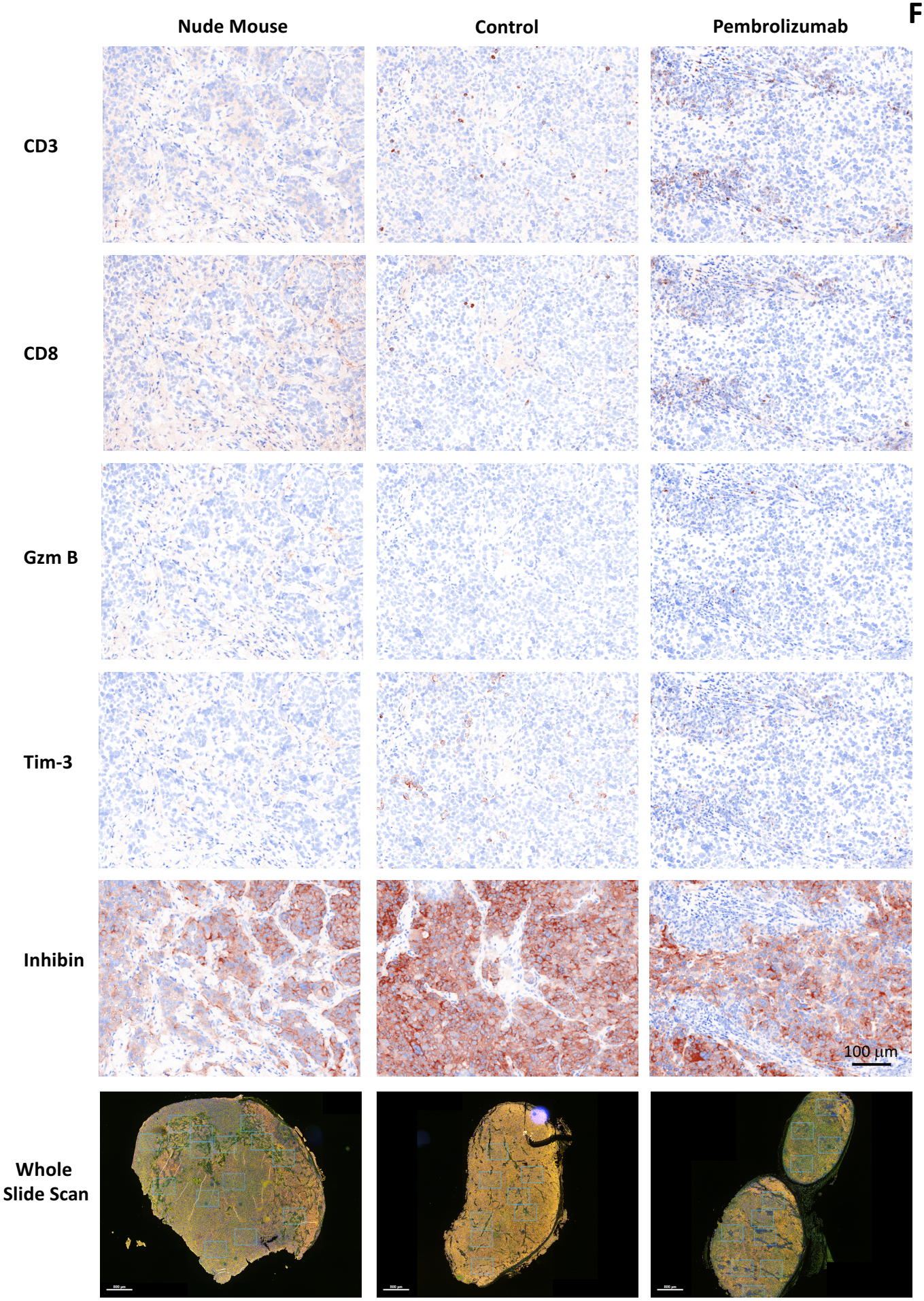

Figure S5: Visualization of a multi-color IHC panel analyzed using inForm software in which each indicated antibody stain was visualized as a pseudo-brightfield image. Tumor sections from a nude mouse bearing a CU-ACC2 tumor, an untreated control CU-ACC2-hu-CB-BRGS tumor, and a pembrolizumab-treated CU-ACC2-hu-CB-BRGS tumor are shown. Locations of IHC representative locations are shown on whole slide scans at the bottom

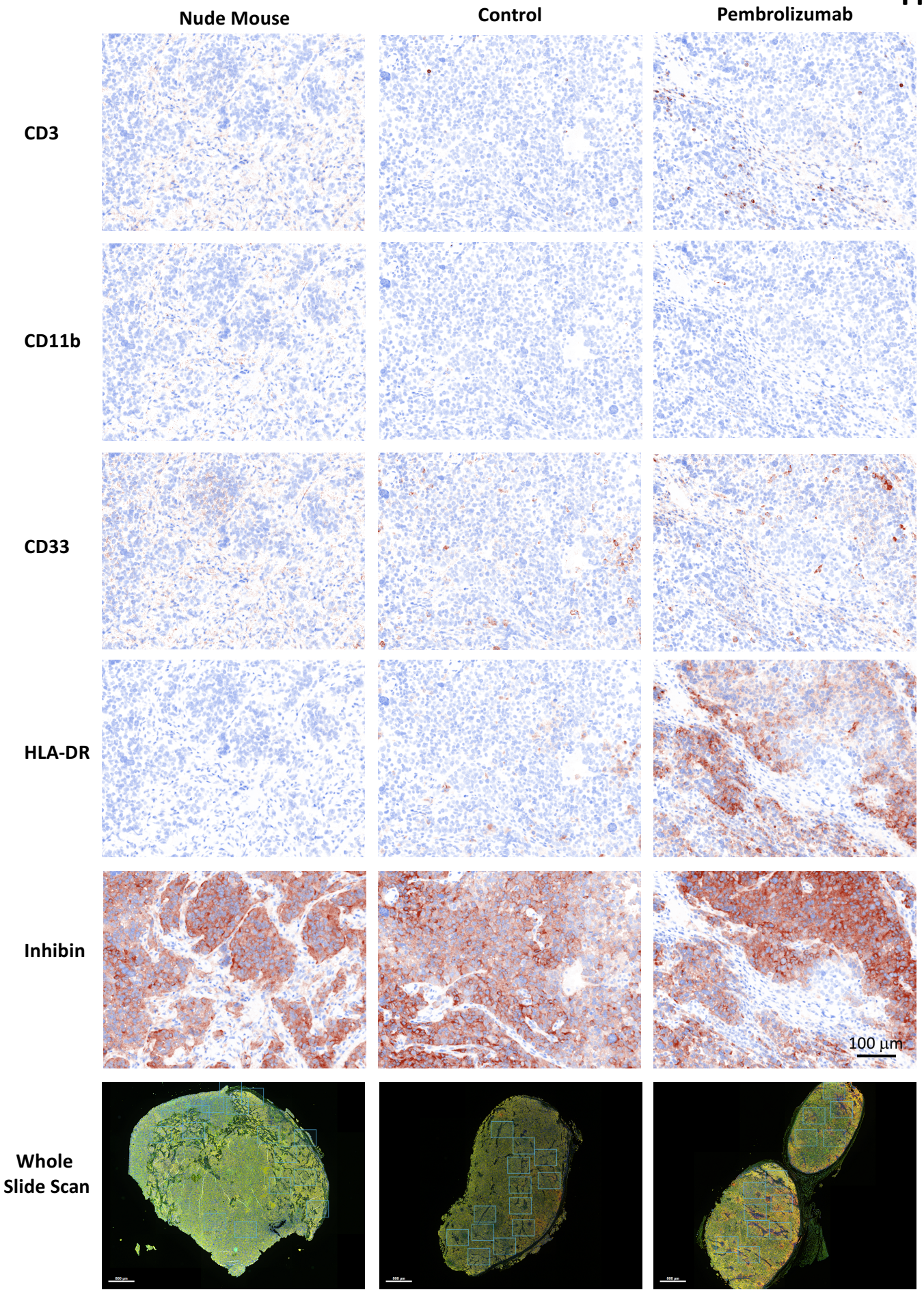

Figure S6: Visualization of a multi-color IHC panel analyzed using inForm software in which each indicated antibody stain was visualized as a pseudo-brightfield image. Tumor sections from a nude mouse bearing a CU-ACC2 tumor, an untreated control CU-ACC2-hu-CB-BRGS tumor, and a pembrolizumab-treated CU-ACC2-hu-CB-BRGS tumor are shown. Locations of IHC representative locations are shown on whole slide scans at the bottom

Figure S7

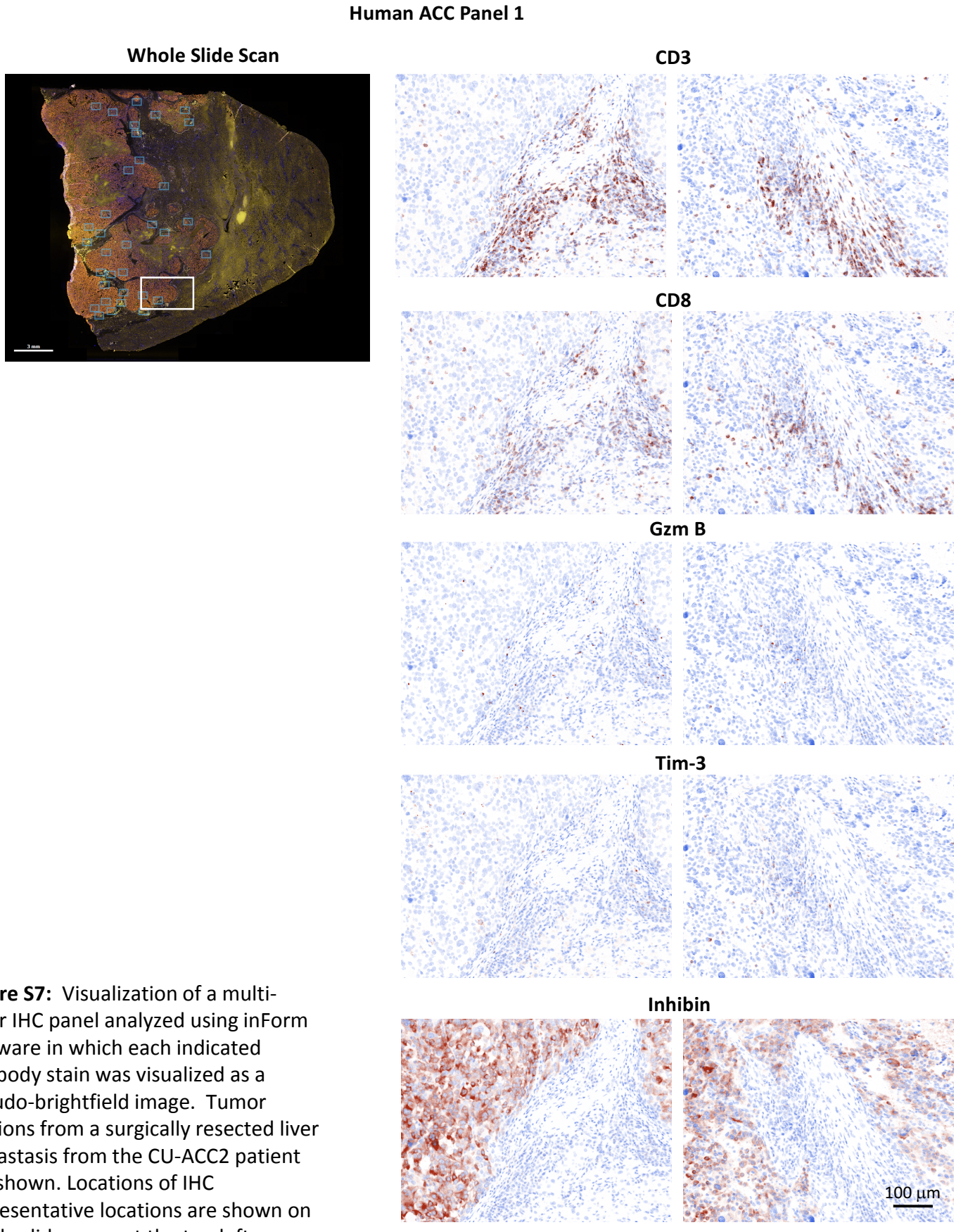

Figure S7: Visualization of a multi-color IHC panel analyzed using inForm software in which each indicated antibody stain was visualized as a pseudo-brightfield image. Tumor sections from a surgically resected liver metastasis from the CU-ACC2 patient are shown. Locations of IHC representative locations are shown on whole slide scans at the top left

Figure S8

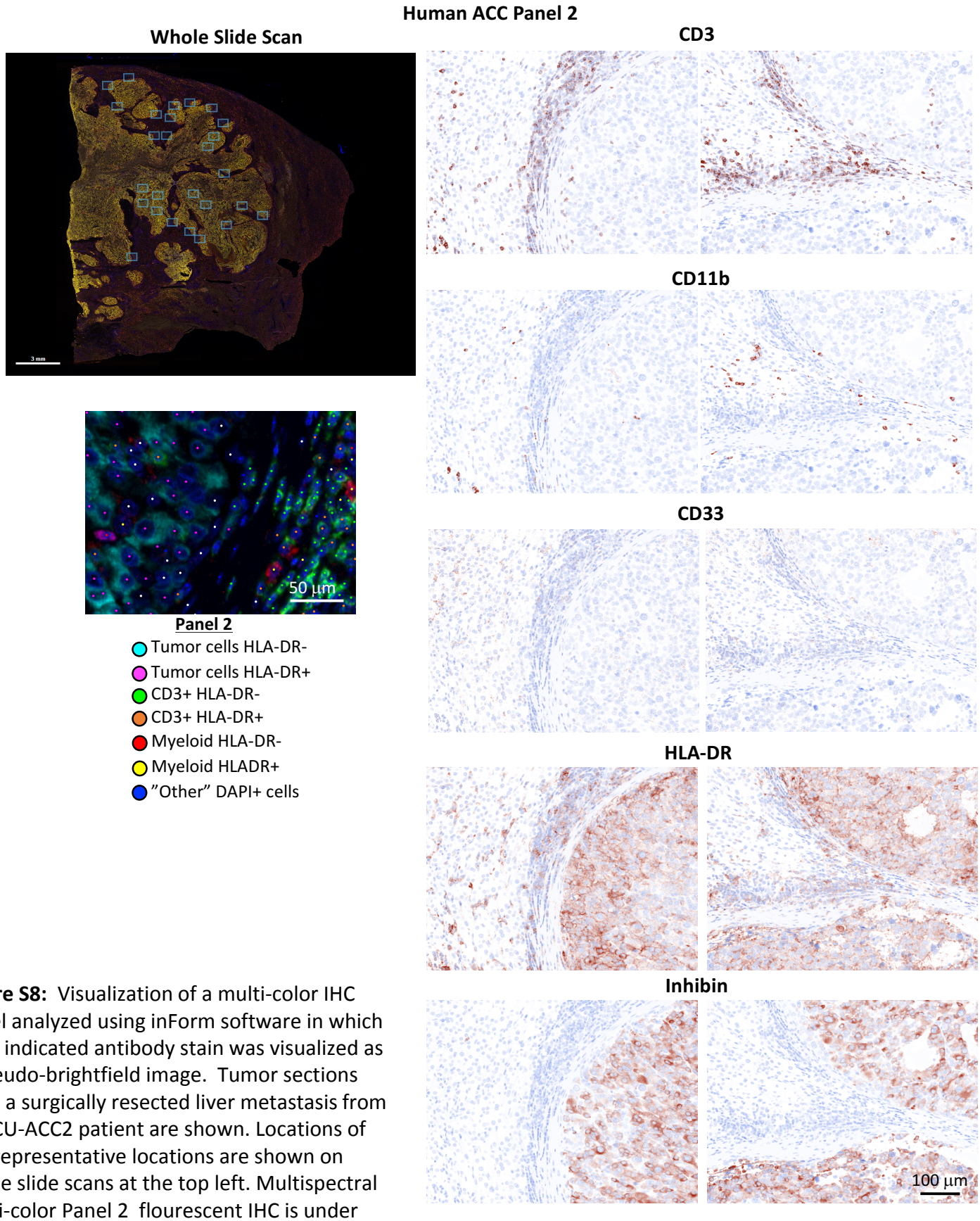

Figure S8: Visualization of a multi-color IHC panel analyzed using inForm software in which each indicated antibody stain was visualized as a pseudo-brightfield image. Tumor sections from a surgically resected liver metastasis from the CU-ACC2 patient are shown. Locations of IHC representative locations are shown on whole slide scans at the top left. Multispectral multi-color Panel 2 flourescent IHC is under whole slide scan

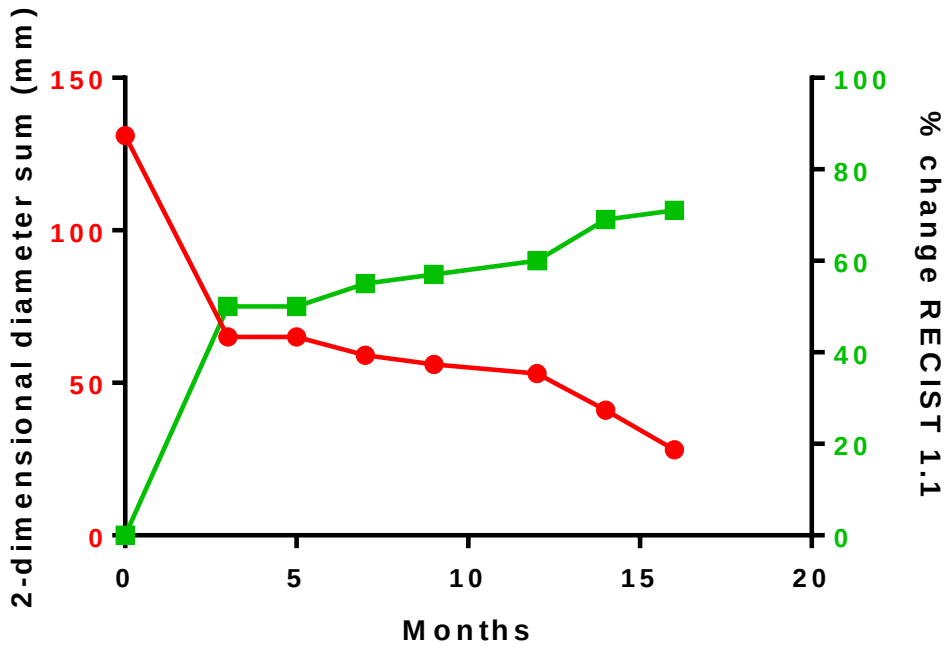

Figure S9: Two target lesions were followed from the time that the CU-ACC2 patient began therapy with pembrolizumab and showed an interval decrease of the total sum of two lesions (red line) with partial remission over period of 16 months by RECIST 1.1 (green line) on successive contrast-enhanced CTs per RECIST 1.1 criteria.