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SUPPLEMENTARY INFORMATION

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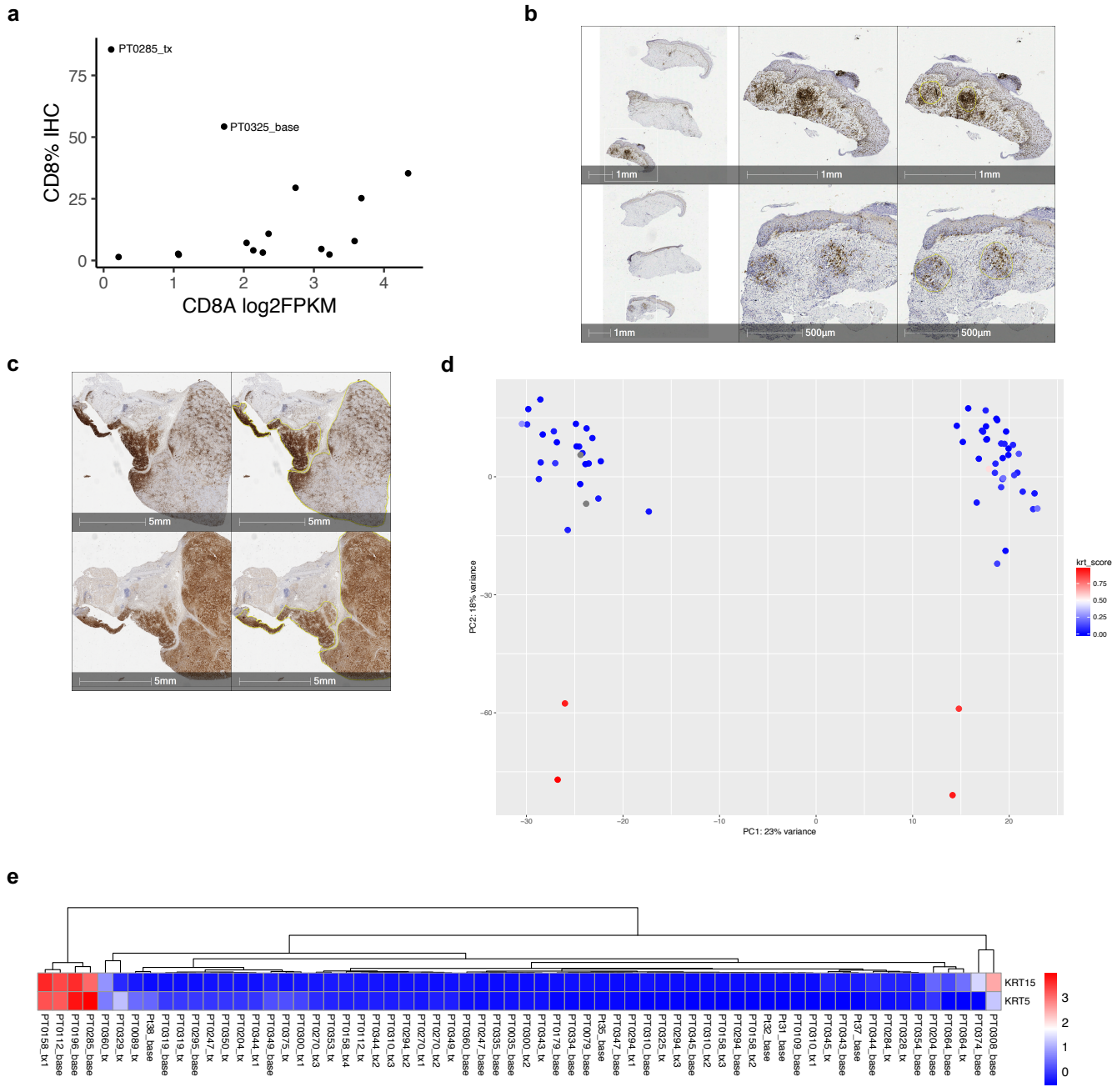
PAK4 inhibition improves PD-1 blockade immunotherapy

Gabriel Abril-Rodriguez[1,2], Davis Y. Torrejon[1], Wei Liu[3,4], Jesse M. Zaretsky[1], Theodore S. Nowicki[5], Jennifer Tsoi[1], Cristina Puig-Saus[1], Ignacio Baselga-Carretero[1], Egmidio Medina[1], Michael J. Quist[1], Alejandro J. Garcia[1], William Senapedis[6], Erkan Baloglu[6], Anusha Kalbasi[7,8,9], Gardenia Cheung-Lau[1], Beata Berent-Maoz[1], Begoña Comin-Anduix[8,9], Siwen Hu-Lieskovan[1,9], Cun-Yu Wang[3,4], Catherine S. Grasso[1,11] and Antoni Ribas[1,2,8,9,10,11]*

[1]Department of Medicine, Division of Hematology and Oncology, University of California, Los Angeles, Los Angeles, CA, USA. [2]Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA, USA. [3]Laboratory of Molecular Signaling, Division of Oral Biology and Medicine, School of Dentistry, University of California, Los Angeles, Los Angeles, CA, USA. [4]Department of Bioengineering, Henry Samueli School of Engineering and Applied Science, University of California, Los Angeles, Los Angeles, CA, USA. [5]Department of Pediatrics, Division of Pediatric Hematology and Oncology, University of California, Los Angeles, Los Angeles, USA. [6]Karyopharm Therapeutics, Newton, MA, USA. [7]Department of Radiation Oncology, University of California, Los Angeles, Los Angeles, CA, USA. [8]Department of Surgery, Division of Surgical Oncology, University of California, Los Angeles, Los Angeles, CA, USA. [9]Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA. [10]Parker Institute for Cancer Immunotherapy, San Francisco, CA, USA. [11]These authors contributed equally: Catherine S. Grasso, Antoni Ribas. *e-mail: aribas@mednet.ucla.edu

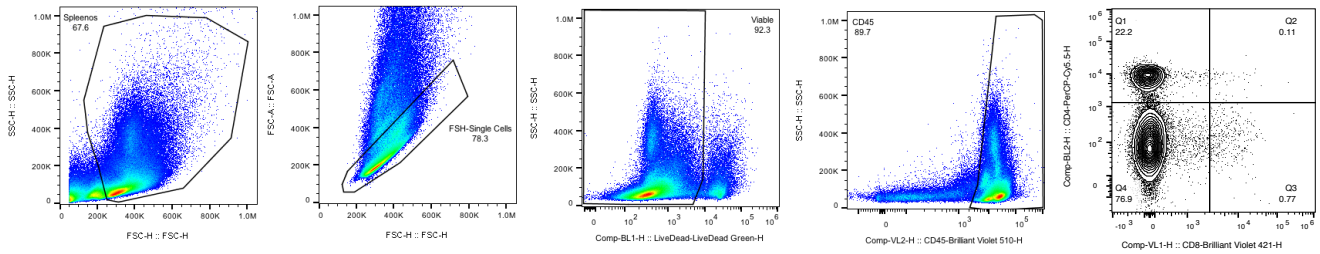
Supplementary Figure 1



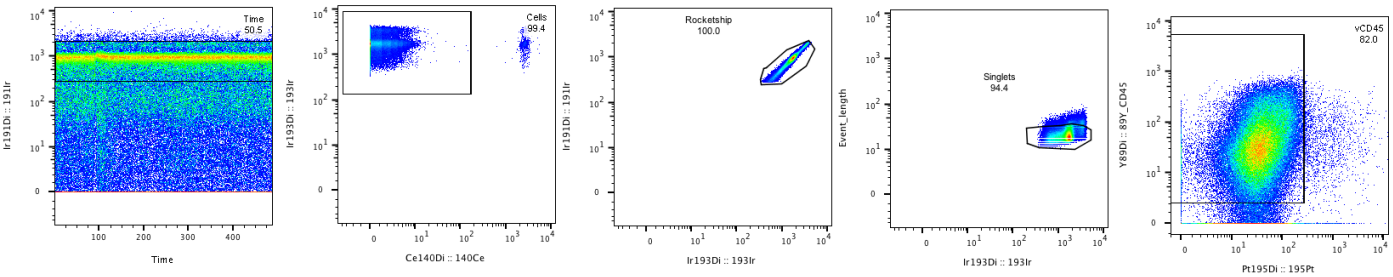
Supplementary Figure 1: Biopsy sample exclusion criteria. a, Scatterplot between percentage of CD8 T cells by immunohistochemistry and *CD8A* gene expression by RNA-seq. PT0285_tx and PT0325_base show high T cell infiltration by immunohistochemistry and was not captured by RNA-seq and therefore they were excluded from the analysis. **b, c,** Immunohistochemistry images for PT0285_tx (**b**) and PT0325_base (**c**). Slides were stained with CD8 (top) and S100 (bottom). **d**, Principal component analysis revealed four outlier samples (n = 64)**. e**, Heatmap showing that these samples are outliers with respect to overexpression of keratinocyte biomarkers *KRT15* and *KRT5*, flagging them for exclusion from further analysis (n= 64).

Supplementary Figure 2

a



b



Supplementary Figure 2: **Gating strategy for CD8 depletion validation**. Splenocytes were first gated based on physical parameters (FSC-H vs SSC-H). Then, we excluded doublets using FSC-H vs FSC-A. Singlets were selected for viable cells (LiveDead vs SSC-H) and then gated for CD45 positive cells (CD45-Brilliant Violet vs SSC-H). We then gate CD4 vs CD8 to quantify the number of CD8 positive cells. b, Gating strategy for CD45+ cells. First, we checked the stability over time (data not shown) and excluded the beads (140Ce vs 193Ir). Singlets were gated based on 193Ir and CD45 cells were selected and used as an input for CyTOF analysis using Cytofkit.