Supplementary Methods

Identification of germline mutations in HRR genes

Confirmation of a participant's germline mutation status was performed using a variety of sequencing platforms at the Peter MacCallum Molecular Pathology NATA accredited clinical laboratory. Variants were assigned a class C4–C5 (pathogenic) mutation status according to a 5-tier clinical classification introduced by ENIGMA http://www.enigmaconsortium.org/. Variants are listed in Table S2.

Differential expression and normalization

We used the RUV R package (Removal of Unwanted Variation)[1,2] for normalization and differential gene expression analysis. For differential expression analysis we used RUV-4, with genes annotated as housekeeping, positive control and negative control genes by Nanostring as control genes. We chose a *k* that resulted in a uniform distribution of empirical p-values. Resulting nominal p-values were adjusted using the Benjamini & Hochberg method. Genes with an adjusted p-value less than 0.05 and were considered to be significant. For normalization, we used RUVIII. We extended our set of control genes used for differential expression analysis with RUV-4 to also include genes that showed no signal of differential expression (adjusted p-value > 0.5 and absolute log2 fold change less than 0.3).

Tumor inflammation signature (TIS)

We used the following 16 genes to perform TIS[3] analyses: *CXCR6*, *TIGIT*, *CD27*, *CD274*, *PDCD1LG2*, *LAG3*, *PSMB10*, *CMKLR1*, *CD8A*, *IDO1*, *CCL5*, *CXCL9*, *HLA-DQA1*, *CD276*, *STAT1*, *HLA-E*. Two additional genes of the original signature, *NKG7* and *HLA-DRB1*, were not present in the Nanostring PanCancer Immune Profiling Panel and were excluded. This reduced TIS signature has been published previously[4]. TIS scores were calculated for individual samples by averaging the normalized gene expression of these genes.

Tissue-resident memory T-cell (TRM)

We used 37 genes from previously published TRM signature of 179 genes[5] that were present in the Nanostring PanCancer Immune Profiling Panel platform. A similar procedure has been previously carried out by other authors[6]. TRM scores were calculated for individual samples by averaging the normalized gene expression of these genes.

Multiplex immunohistochemistry

Sections from formalin-fixed paraffin embedded (FFPE) tumor blocks were stained with H&E and marked by two pathologists for tumor-rich regions. We used a T cell panel with primary antibodies for CD3 (clone SP7 1:500, Abcam), CD4 (clone SP35, 1:100 Abcam), CD8 (clone4B11, 1:500, Leica Biosystems), FOXP3 (1: 200, Bio SB), PD-L1 (clone SP142, 1:500 Abcam), AMACR (13H4 1:1000, Cell Marque) and DAPI for cell visualization and identification, as we have done previously[4,7,8]. Our combination of markers allowed us to distinguish $CD3^+CD4^+$ (helper T cells), $CD3^+CD8^+$ (cytotoxic T cells), $CD3^+CD4^+FOXP3^+$ (regulatory T cells), tumor cells ($AMACR^+$) and $PD\text{-}L1^+$ cells.

Slides (4uM sections) were baked at 60^{o}C, dewaxed prior to antigen retrieval followed by primary antibody for 30min and 0.3% H_2O_2 block for 10min at room temperature (RT). Anti-rabbit or Anti-mouse HRP-conjugated secondary antibody from Perkin Elmer (1:500 dilution) was applied for 10min at RT. Signal amplification was carried out using TSA Plus (1:100 in TSA amplification diluent, PerkinElmer) for 10min. Three (2min each) washes were performed in between each step using TBST (0.05% Tween-20). Slides were microwaved as per PerkinElmer instructions to strip the primary-secondary-HRP complex allowing introduction of the next antibody. After the final antibody, the slides were incubated with DAPI for 1 min and coverslips were placed with mounting medium. Single antibody

controls were included with each antibody. Visualization of the seven-color OPAL slides was performed using Perkin Elmer's Vectra 3.0 automated imaging system. Tissue segmentation, cell segmentation and phenotyping of images was performed using the inForm Advanced Image Analysis Software (versions 2.3 and 2.4). We took 15 representative multispectral images of 1.34 mm^2 of the tumor area identified by a pathologist. Cell types were quantified in each image, and then averaged per tumor sample. The density of T cells was calculated as the ratio of the number of T cells in an image and the image size (1.34 mm^2). The field resolution was 20x (0.5μm) with an image size of 1338 μm x 1004 μm.

Deriving the FIS signature

We derived gene expression signatures for the FIS profile by calculating the Spearman correlation between the ratio of free and clustered CD8+ cells and the genes available in the Nanostring platform and selecting the top 5 genes, which had a positive Spearman correlation greater than 0.25 and p-values < 0.05. Signature levels were measured using *singscore*[9].

References

1. Molania R, Gagnon-Bartsch JA, Dobrovic A, Speed TP. A new normalization for Nanostring nCounter gene expression data. *Nucleic Acids Res.* 2019;47(12):6073-6083.
2. Gagnon-Bartsch JA, Speed TP. Using control genes to correct for unwanted variation in microarray data. *Biostatistics.* 2012;13(3):539-552.
3. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest.* 2017;127(8):2930-2940.
4. Keam SP, Halse H, Nguyen T, et al. High dose-rate brachytherapy of localized prostate cancer converts tumors from cold to hot. *J Immunother Cancer.* 2020;8(1).
5. Savas P, Virassamy B, Ye C, et al. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nature Medicine.* 2018;24(7):986-993.
6. Solomon B, Young RJ, Bressel M, et al. Identification of an excellent prognosis subset of human papillomavirus-associated oropharyngeal cancer patients by quantification of intratumoral CD103+ immune cell abundance. *Ann Oncol.* 2019;30(10):1638-1646.
7. Wang M, Huang Y-K, Kong JC, et al. High-dimensional analyses reveal a distinct role of T-cell subsets in the immune microenvironment of gastric cancer. *Clinical & translational immunology.* 2020;9(5):e1127-e1127.
8. Halse H, Colebatch AJ, Petrone P, et al. Multiplex immunohistochemistry accurately defines the immune context of metastatic melanoma. *Sci Rep.* 2018;8(1):11158.
9. Foroutan M, Bhuva DD, Lyu R, Horan K, Cursons J, Davis MJ. Single sample scoring of molecular phenotypes. *BMC Bioinformatics.* 2018;19(1):404.