

Supplementary Material

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2 SUPPLEMENTARY TABLES AND FIGURES

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2.1 Figures



Figure S1. Enter the caption for your figure here. Repeat as necessary for each of your figures



Figure S2. This is a figure with sub figures, (A) is one logo, (B) is a different logo.

2.2 tables

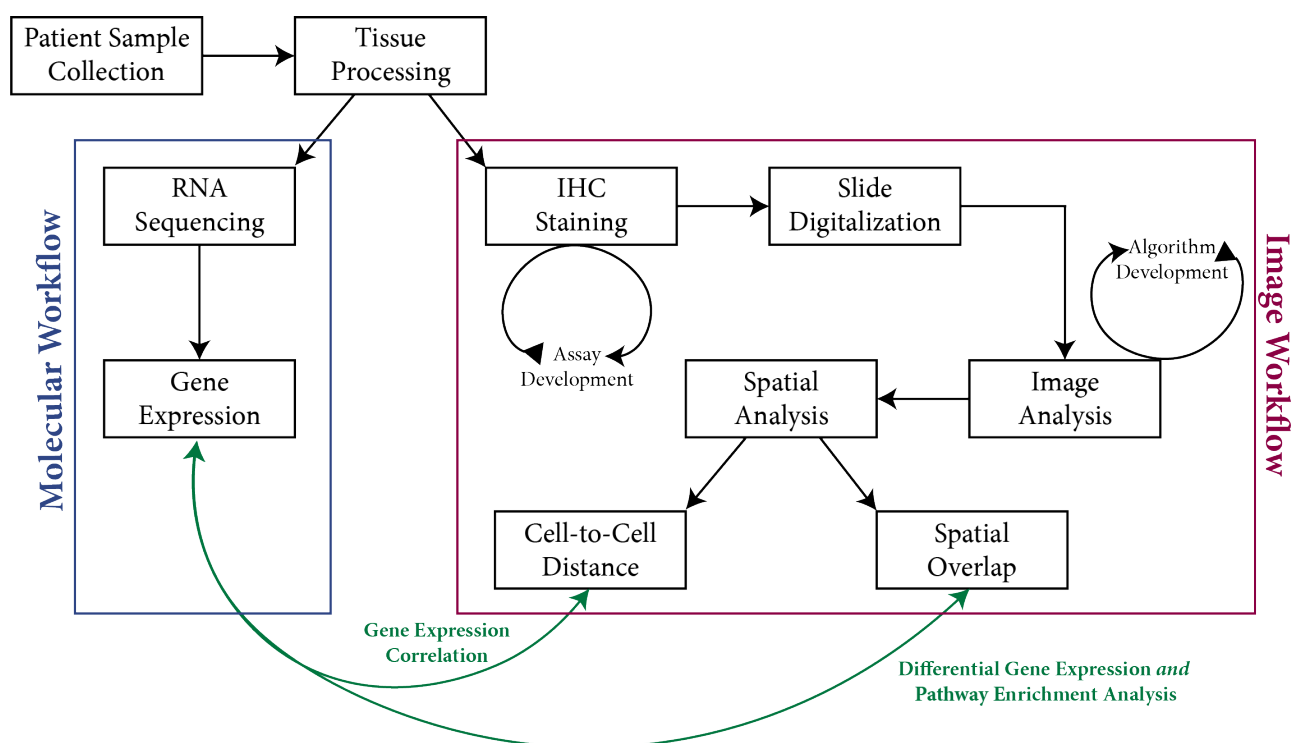


Figure S3. Proposed Study Workflow. This figure represents the schematics of the tissue analysis workflow used in the presented study. Image Workflow (red box) shows all the steps, starting from IHC assay development through slide digitalization up to spatial image analysis. This combined with the Molecular Workflow (blue box), including RNA Sequencing and Gene Expression Profiling.

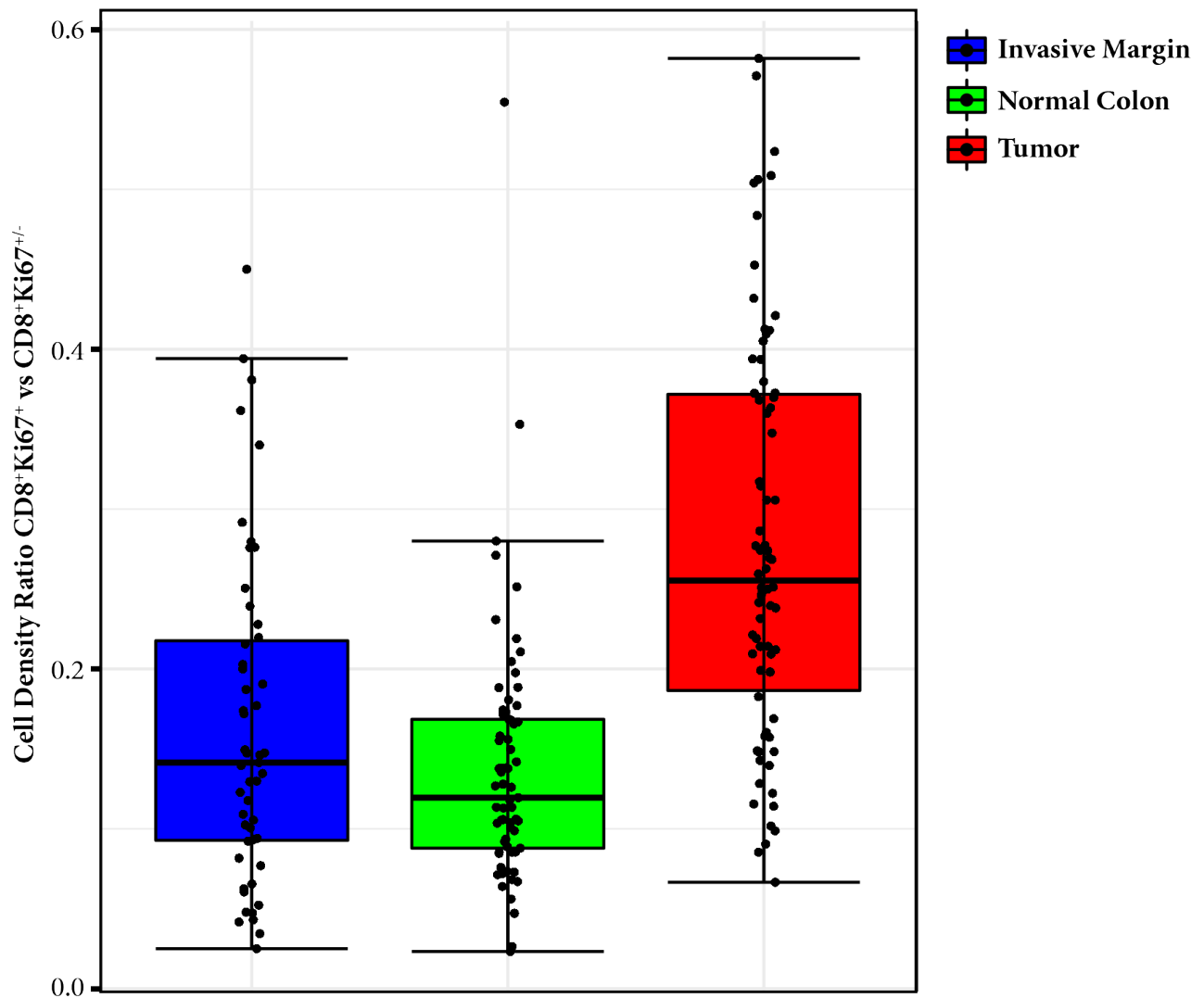


Figure S4. Ratio between CD8⁺Ki67⁺ and CD8⁺Ki67^{+/-} T cell densities. The proportion of proliferating cytotoxic T cells is presented for all three ROIs, including invasive margin, normal colon and tumor. Generally, the highest proliferation activity of CD8⁺ T cells is detected within tumor ROI.

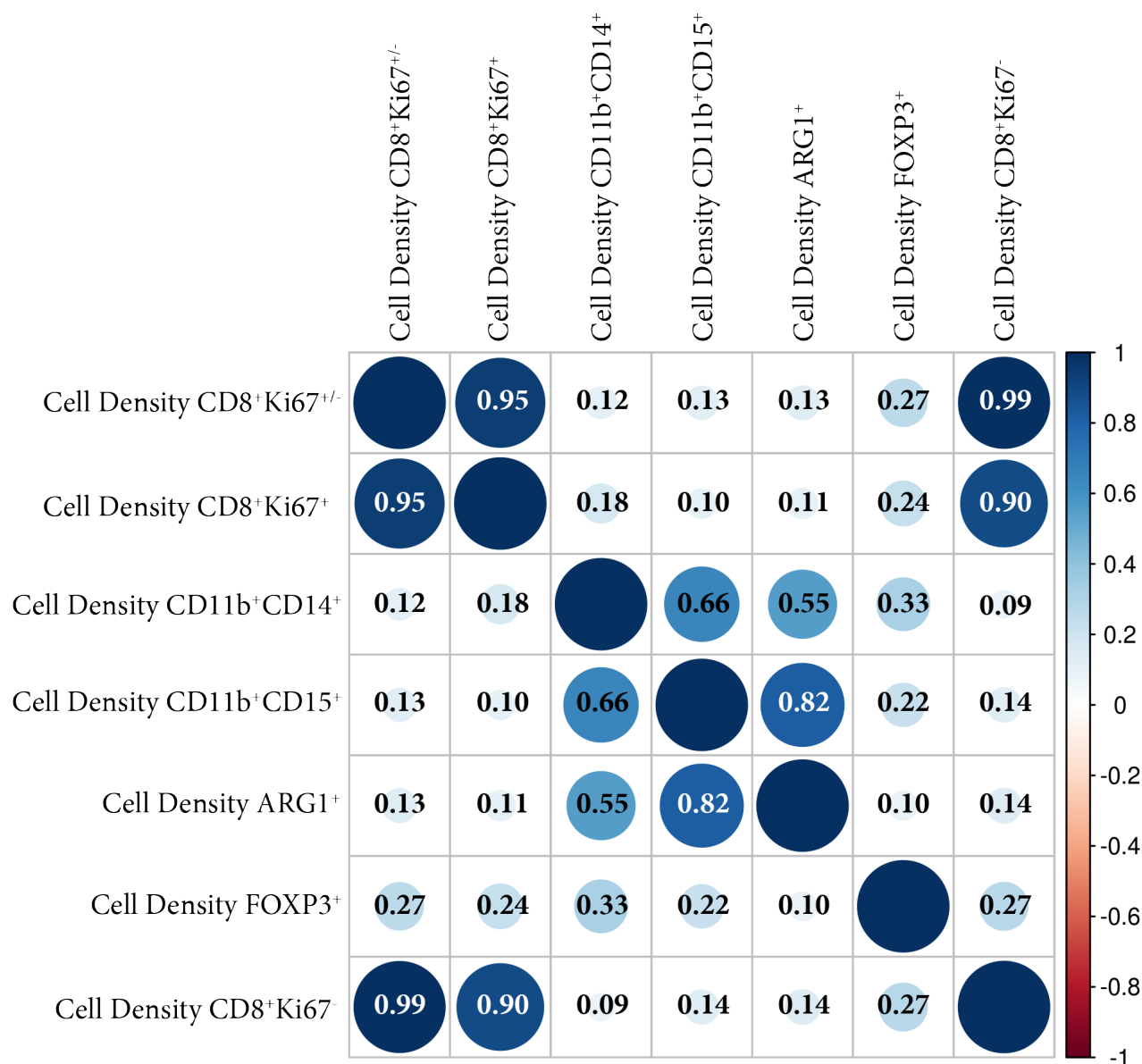


Figure S5. Cell Density Correlation Analysis in the invasive margin ROI. The circle size corresponds to the strength of the Pearson correlation coefficient and the color corresponds to either positive (blue) or negative (red) correlation. The correlation between cell densities is comparable to computed correlations in the tumor ROI.

Table S1. Technical abbreviations and short descriptions.

Abbreviation	Parameter	Description
CIT	Cancer Immunotherapy	Type of cancer treatment with immunomodulating medicines.
CMS	Consensus Molecular Subtypes	Colorectal Cancer subtyping according to gene expression profiling.
CRC	Colorectal Cancer	Tumor indication.
FFPET	Formalin fixed and paraffin embedded tissue	Processed tumor specimens in the form of a paraffin block.
GAD	Global Average Distance	Non-normalized parameter characterizing the average distance between myeloid and T cells computed in the tumor region of interest of each colorectal cancer sample.
GAD _{norm}	Normalized Global Average Distance	GAD parameter normalized against the random distribution and the density of myeloid cells in the tumor area.
GAD_CD14	Normalized GAD for CD11b ⁺ CD14 ⁺ myeloid cells	Normalized parameter characterizing the average distance between monocytic myeloid and T cells computed in the tumor region of interest of each Colorectal Cancer sample.
GAD_CD15	Normalized GAD for CD11b ⁺ CD15 ⁺ myeloid cells	Normalized parameter characterizing the average distance between granulocytic myeloid and T cells computed in the tumor region of interest of each Colorectal Cancer sample.
IHC	Immunohistochemistry	Antibody-based method of detecting protein antigens in a fixed tissue.
MDSC	Myeloid-Derived Suppressor Cells	Immature myeloid cells with immunosuppressive function.
MMR	Mismatch Repair Status	Tumor subtyping according to the mutational status of the mismatch repair proteins.
MSI	Microsatellite Instability	Deficiency in mismatch repair machinery.
MSS	Microsatellite Stability	Proficiency in mismatch repair machinery.
MTO	Myeloid-T cell Overlap	Parameter representing the tiles infiltrated by myeloid and T cells (spatial overlap), normalized against the total number of tiles infiltrated by T cells.
MTO_CD14	Myeloid-T cell Overlap for CD11b ⁺ CD14 ⁺ myeloid cells	Parameter representing the tiles infiltrated by monocytic myeloid and T cells (spatial overlap), normalized against the total number of tiles infiltrated by T cells.
MTO_CD15	Myeloid-T cell Overlap for CD11b ⁺ CD15 ⁺ myeloid cells	Parameter representing the tiles infiltrated by granulocytic myeloid and T cells (spatial overlap), normalized against the total number of tiles infiltrated by T cells.
ROI	Region of Interest	Annotated areas in the slide images used for density and spatial analysis.
TCGA	The Cancer Genome Atlas	Public repository of broad of spectrum of tumors, including clinical and molecular information.
TiME	Tumor Immune Microenvironment	The immune components of the tumor milieu.
TIL	Tumor-infiltrating lymphocytes	T lymphocytes infiltrating the tumor bed.
TLS	Tertiary Lymphoid Structure	Immune cell aggregates having a certain composition and architectural pattern.
TMB	Tumor Mutational Burden	Number of mutations per base pair.

Table S2. Genes positively and negatively correlating with distance derived GAD parameters. Listed genes represent the respective surrogate gene signatures for GAD₁₄ and GAD_CD15, used for mapping to TCGA dataset. The cutoffs after gene expression correlation with continuous values were set at $|R| > 0.4$ for GAD₁₄ and at $|R| > 0.3$ for GAD_CD15.

Distance Parameter	Genes
GAD_CD14	ATF5, TNFRSF9, CRTC3, CHST12, FCHSD2, FOXP3, PRPF31, USF2, GYPC, TTBK1, CXCL13, CD2, SCPEP1, ALKBH5, ZNF831, SENP3, LLGL1, CST7, CALM3, CASS4, PNMA3, ARSF, RTN1, C18orf25, MTMR9LP, KLHL22, RNF34, FAM26F, METRNL, ITGAE, TANGO2, ICAM5, -FEZF1, -BHMT, -MTRNR2L1, -RANBP6, -NOTUM, -ZP3, -PCLO, -SLC26A8, -CASC9, -KCTD4, -SLC35D2, -DRD1, -KRT39, -PGAP3, -KLHL9, -C5orf63, -TTC30A, -MIR374C, -ETV4, -SPATA24, -GABRB1, -WNK4, -TRIM67, -ALAS2, -SOX14, -ERV3-1, -SHANK2-AS3, -TM4SF20, -RAB3GAP2, -DSG1, -SPATA6L, -TAF7
GAD_CD15	-ITPKB-IT1, -EPN3, -PABPC4, -ABCC12, -KPNA4, -CKB, -BANF2, -PRDM14, -XKR3, -VPS28, -SLC10A5, -TOB1, -HOXC11, -ITLN1, -CEACAM22P, -SHARPIN, -ZFAND1, -OR6B1, -NMU, -CREG2, -LRP2, -MIR6837, -FAM13A, -UGT1A4, -UQCRB, -IGFBP2, -OBFC1, -FA2H, -RAB3GAP2, -LINC00622, -YOD1, -OR5AK2, -KRT1, -OCIAD2, -C16orf80, -CA12, -CANT1, -B4GALNT2, -HERC2P10, -ATAD1, -EFHB, -CCDC47, -PPP1R15B, -MIR6887, -SPATC1L, -SLC26A4-AS1, -TMEM105, -CA5A, -DRP2, -ARL14, -SMGIP5, -TMEM68, -KRT80, -MTSS1L, -GSTO2, -UGT3A1, -YWHAQ, -PRKAG3, -C4orf36, -ZDHHC11, -CCDC42B, -CXADRP3, -NT5C2, -EGR4, -GYS2, -FAM84B, -GOLT1A, -AGPAT9, -SPRED2, -PWWP2B, -LINC00521, -CSN1S2AP, -LYPLA1, -CEMIP, -FRAS1, -ANKRD30B, -ANKRD22, -KRT23, -COX6C, -PCOLCE2, -TEX26, -PHGDH, -PKHD1, -TCEA1, -CCER2, -ULBP1, -COMMD2, -PDCL2, -BEGAIN, -KCNJ16, -LGALS12, C2, ICAM5, OR2T5, FAM231A, GLCCI1, MSL3, KIAA1328, DCC, ZBTB40, KCTD20, HAND2-AS1, FAM181A, ZNF271, RHOF1, RNF216P1, TFPT, CHD5, PURA, CNTF, DNAJB6, TECTA, CXorf40A, OR11H12, PLXNA3, HTR7, MIR4644, C18orf25, ZNF175, RCN3, CBL, UBR2, UBE4A, ZNF543, CD99L2, NUBP1, RRAGB, SYT15, CYBSR4, PLEKHA8, C14orf183, EGFL8, FAXDC2, STAG3L4, LMOD2, PSMC2, POLI, MIR4744, PIK3C3, C2orf71, KMT2A, A1BG, MIR135A1, ZNF18, FAM21A, ERMARD, ZNF101, SEPT1, ZCCHC5, PPP2R2B, FKBP14, POLC3, TBX19, SNORD11B, ZNF324, HAVCR1P1, NRG4, IFNG, ANKRD34B, TRNT1, C1QTNF9, ATP11C, ZNF547, SLC10A4, CD19, SIT1, RARS, OGDH, ABCD1, SLFN1-AS1, PPP1R14A, GOLGA6L6, SORCS2, LINC00506, IRX6, DPF2, PSORS1C3, MIR3183, TRA2A, NEFH, YPEL2, PACS1, RPA3OS, GOLGA6L1, RNF216, OR2T29, CNPY3, NAPA-AS1, NUPL2, MIR3939, MIR218-1, TCP11L2, ELP2, MIR5739, RAP2C, S100B, ARSF, FOXE1, LINC00630, C10orf107, IMPG2, INCA1, METTL16, CALCOCO1, UBASH3A, TBC1D22A, PMM2, ZNF583, RPL36A-HNRNP2, MICA, ZNF354A, SNRNP70, ANKRD44-IT1, SCML4, SPACA3, IL6, XPC, C4B, PNRC1, C20orf166, HAUS1, TBRG1, SUMF2, MIR3683, OR11H2, KIAA1024, FBLN7, SLC8A2, ZNF783, LSMEM1, LIME1, VAMP2, TIRAP, METTL25, MIR381, CUL9, MIR450A1, KBTBD8, FOXO3, ANKRD49, ACSM5, EVL, TMEFF2, APBB1, MIR197, GNA14-AS1, GYPC, MAGOH2, TSPY1, CHST12, RNASEK, FAM226A, FAM226B, APBB3, PRPF31, ZNF79, RANBP17, ACTL8, ZC2HC1B, MIR5582, IRAK4, POLN, SLC6A7, MIR181A1HG, GHRL, CD160, FUNDCC2P2, SNORD109B, ZNF655, ITPR1, TBP