

Supplementary information for

**Probing the killing potency of tumor-infiltrating lymphocytes
on microarrayed colorectal cancer tumoroids**

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This file includes:

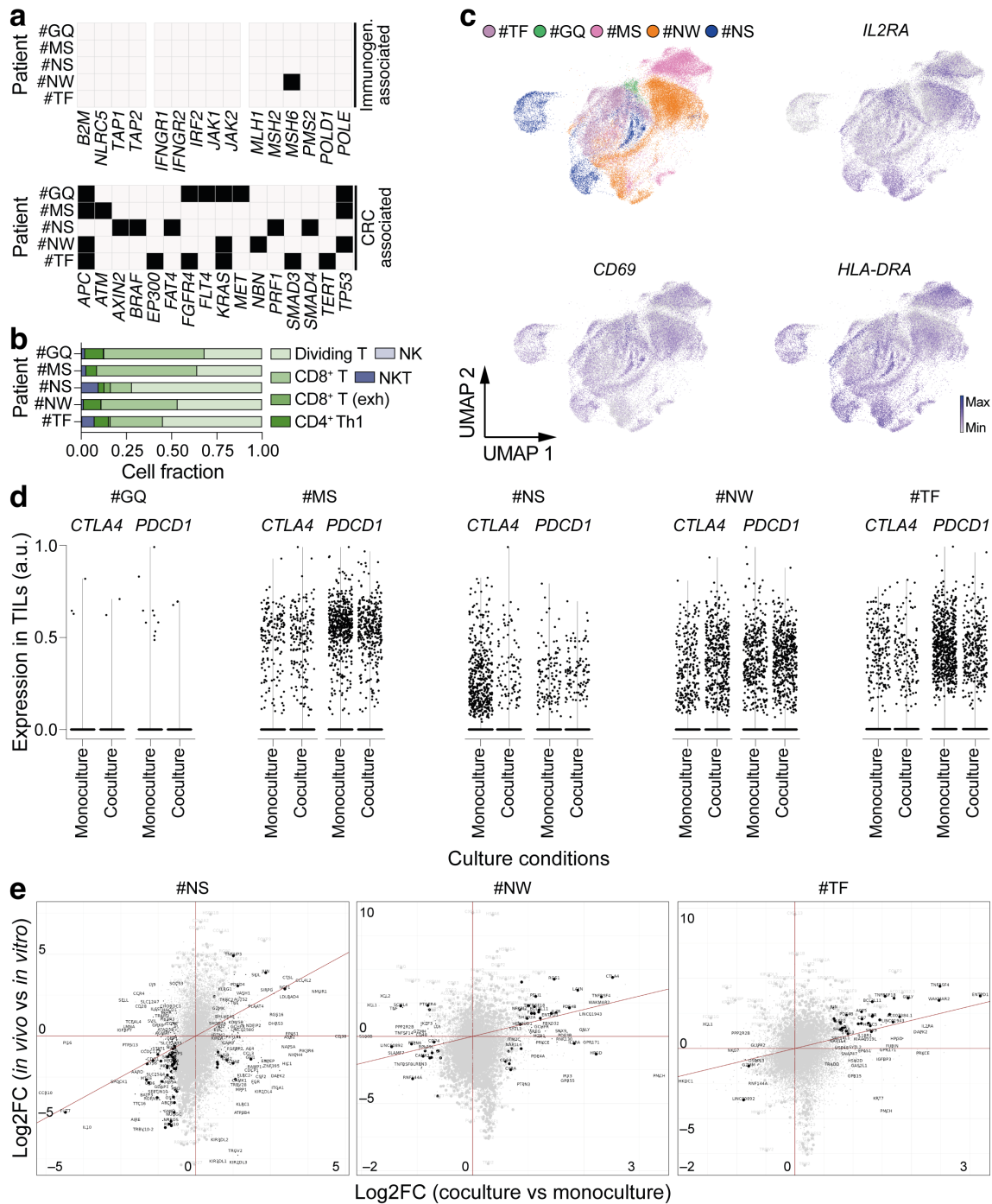
Supplementary Table 1

Supplementary Figures 1-4

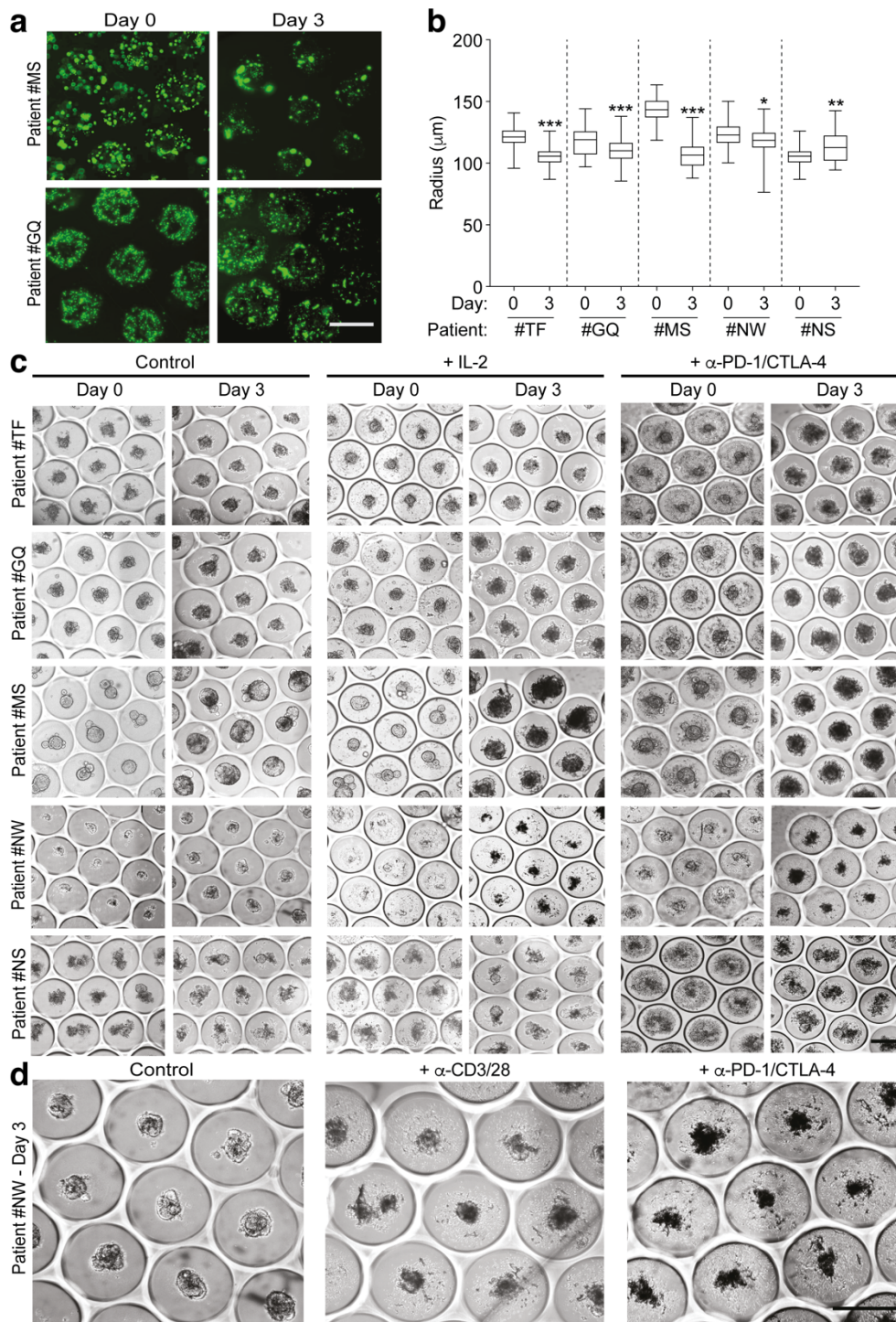
Supplementary Table 1. Main clinical parameters of the patients involved in this study

ID	Age	Sex	Disease stage (TNM)	Histological type	Sample anatomical region	Starting material	MSS status
#GQ	54	F	pT4b pN0 L0 V1 Pn0 G2 R0	Adenocarcinoma	Liver metastasis	Resection piece	MSS
#MS	54	F	pT4 pN1 cM1	Adenocarcinoma	Liver metastasis	Resection piece	MSI
#NS	59	M	pT4a pN2 pM1, L0, P0, Pn1, R0	Adenocarcinoma	Colon (primary tumor)	Resection piece	MSS
#NW	72	M	Initial: pT3 pN1b cM0	Adenocarcinoma	Liver metastasis	Resection piece	MSS
#TF	84	M	pT3 pN0 L0 V0 Pn0 pM1a G2 R0	Adenocarcinoma	Colon (primary tumor)	Resection piece	MSS

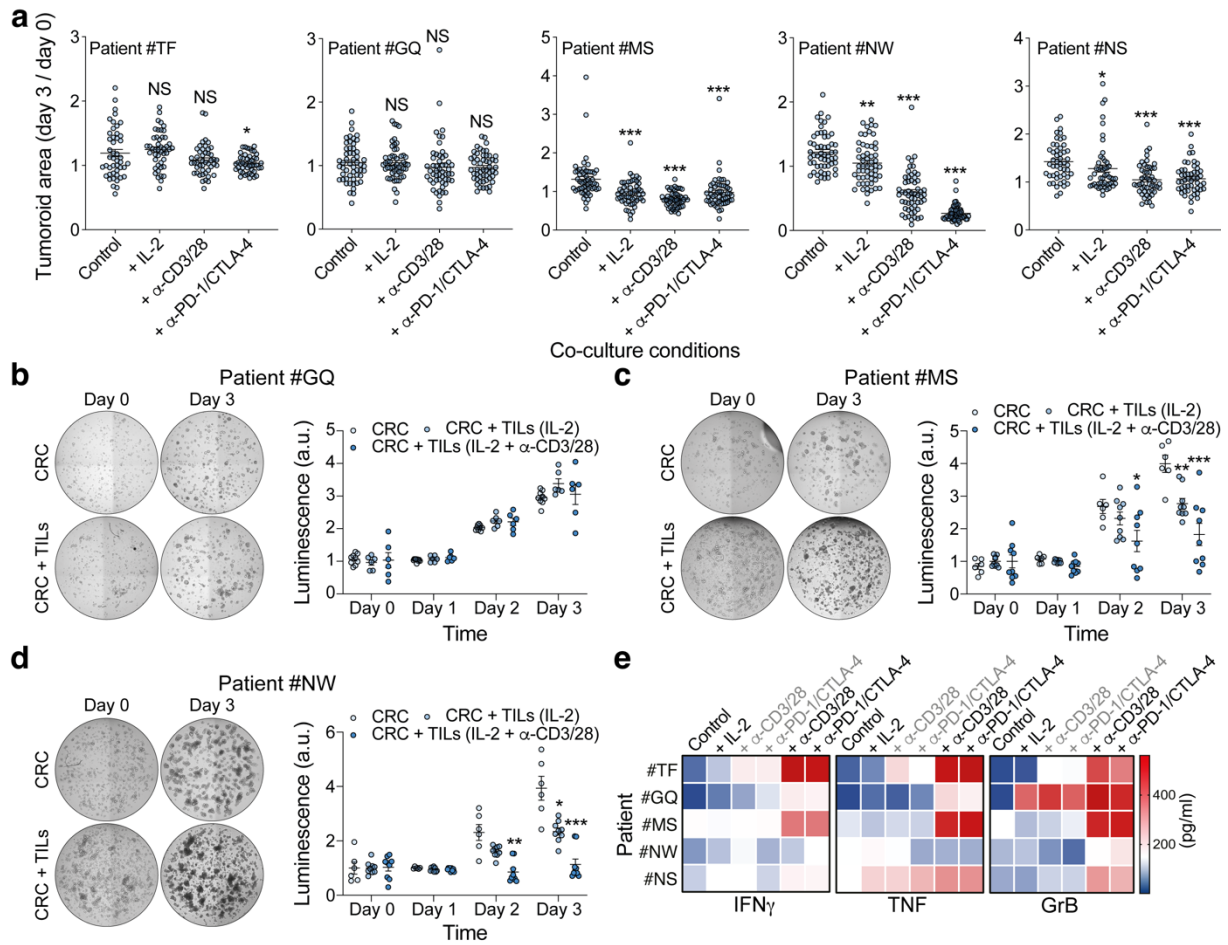
Supplementary Table 1. Main clinical parameters of the patients involved in this study. The patient ID, age, sex, colorectal cancer disease stage (TNM), histological type, anatomical region, starting material for organoid derivation, and microsatellite stability (MSS) status are indicated.



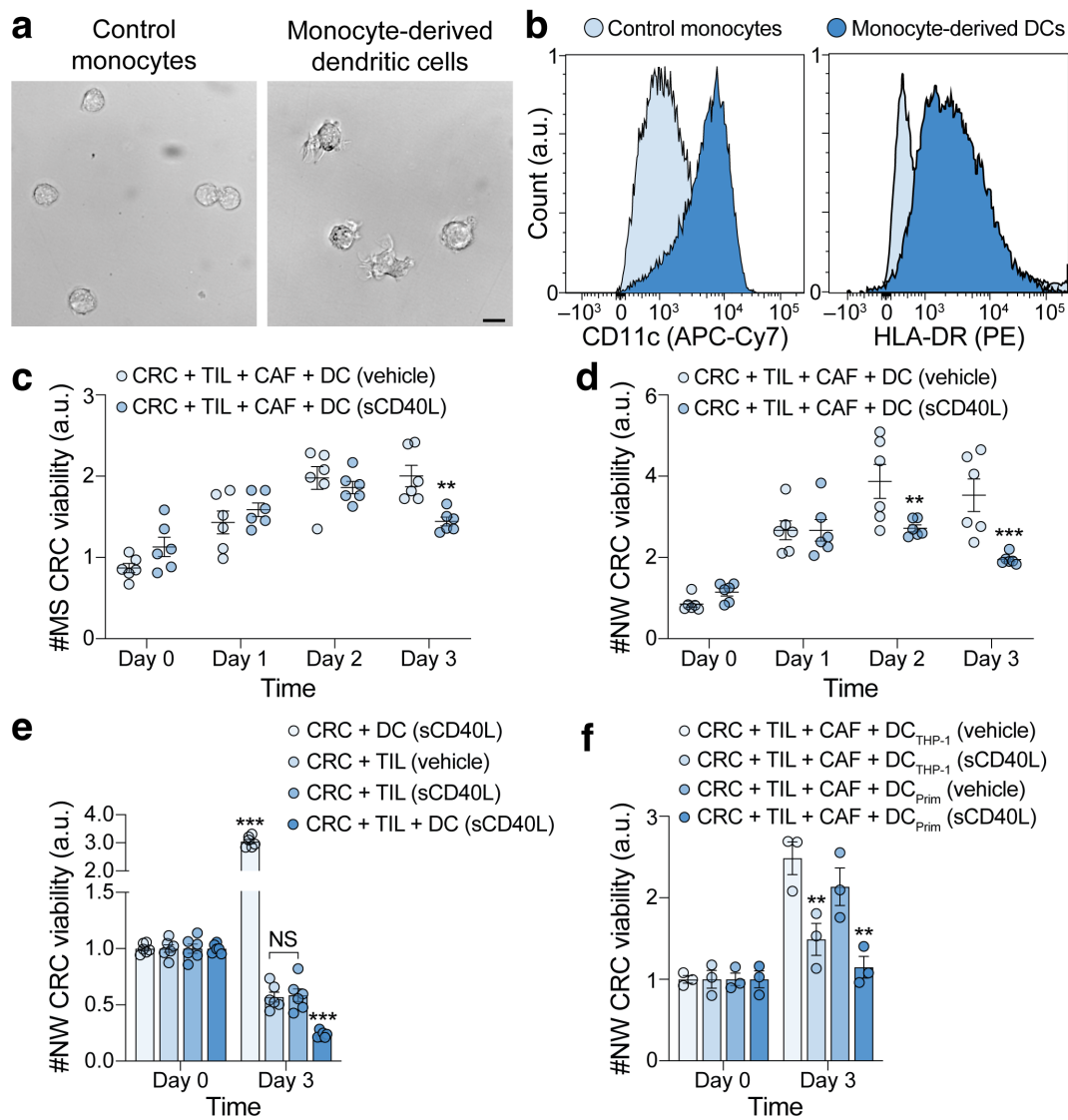
Supplementary Figure 1. The microcavity-based platform has been validated using patient-derived material with both cellular and molecular diversity. **a**, Heatmap showing the main pathogenic mutations (black cells) found in the indicated patients. Panels are arranged (left to right, top to bottom) based on genes involved in antigen presentation, IFN γ sensitivity, mismatch repair, and CRC overall pathogenicity. Immunogen., immunogenicity. **b**, Bar plot showing the cell type distribution in the TIL populations from the indicated patients. **c**, Uniform Manifold Approximations and Projections (UMAPs) of the TILs used in this study showing the expression of the indicated genes. Patient labels are shown in top left UMAP. **d**, Dot plots showing the expression of the indicated genes by the TILs of the indicated patients when cultured alone (monoculture) or with autologous CRC tumoroids (coculture). **e**, Scatter plots showing the correlation between the gene expression changes that occur in TILs *in vitro* upon co-culture with autologous CRC tumoroids, CAFs and macrophages (*x*-axis) and the ones observed in the same TILs between *in vivo* and *in vitro* conditions (*y*-axis). Patient ID is indicated on top. All the plots based on single-cell data have been generated using the CRC-TME atlas portal (<https://crc-tme.com/>).



Supplementary Figure 2. The microcavity-based T cell killing assay allows visual inspection of cytotoxic activity. **a**, Representative images illustrating TIL migration in microarrayed co-cultures from the indicated patients. Scale bar, 500 μm . **b**, Quantification of TIL migration for the indicated patients in conditions of autonomous activation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-tailed t -test, $n = 58, 60, 60, 54$, and 47 for patients #TF, #GQ, #MS, #NW, and #NS, respectively). The box plots show the median (centre lines), the first and third quartiles (box limits) and the minimum and maximum values (whiskers). **c,d**, Representative images of microarrayed tumoroids with autologous TILs in the indicated conditions, patients and days. Scale bars, 500 μm . Culture conditions: autonomous activation (IL-2), ectopic activation (IL-2 + $\alpha\text{-CD3/CD28}$), immunotherapy (IL-2 + $\alpha\text{-CD3/CD28}$ + $\alpha\text{-PD-1/CTLA-4}$).



Supplementary Figure 3. Luciferase-reporter assays and cytokine profiling support the readouts from the microarrayed system. **a**, Quantification of microarrayed tumoroid area over time for the indicated patients and conditions. NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (ANOVA and Tukey's tests, $n = 46, 53, 55, 56$, and 50 for patients #TF, #GQ, #MS, #NW, and #NS, respectively). **b-d**, Representative brightfield images and luminescence quantifications of the indicated co-cultures of luciferase-labeled CRC organoids and autologous TILs. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (ANOVA and Dunnett's multiple comparisons test, n as indicated in the graph). **e**, Heatmaps showing the amount of interferon γ (IFN γ), tumor necrosis factor (TNF), and granzyme B (GrB) released by the TILs of the indicated patients in the indicated conditions. Black and grey labels indicate 10:1 and 1:1 TIL:CRC cell ratios, respectively. Culture conditions: autonomous activation (IL-2), ectopic activation (IL-2 + α -CD3/CD28), immunotherapy (IL-2 + α -CD3/CD28 + α -PD-1/CTLA-4). In **a-d**, data represent mean $\pm$ SEM.



Supplementary Figure 4. Dendritic cells can promote T cell cytotoxicity in microarrayed co-cultures. **a**, Representative brightfield images of undifferentiated (left) and DC-differentiated (right) monocytes. Scale bar, 10 μ m. **b**, Quantitation of the expression of the dendritic cell marker CD11c and HLA-DR in undifferentiated and DC-differentiated monocytes. **c,d**, Luminescence-based measurements of the promotion of TIL killing activity induced by CD40-mediated DC activation in CRC tumoroids derived from patients #MS (**c**) and #NW (**d**). $**P < 0.01$, $***P < 0.001$ (ANOVA and Sidak's tests, $n = 6$). **e**, Luminescence-based measurements of CRC viability in patient #NW under the indicated cell and treatment combinations. $***P < 0.001$; NS, not significant (ANOVA and Sidak's tests, $n = 6$). **f**, Luminescence-based measurements of CRC viability in patient #NW under the indicated conditions when using dendritic cells derived from THP-1 (DC_{THP-1}) or primary monocytes (DC_{Prim}). $**P < 0.01$ (ANOVA and Sidak's tests, $n = 3$). In **c-f**, data represent mean $\pm$ SEM.