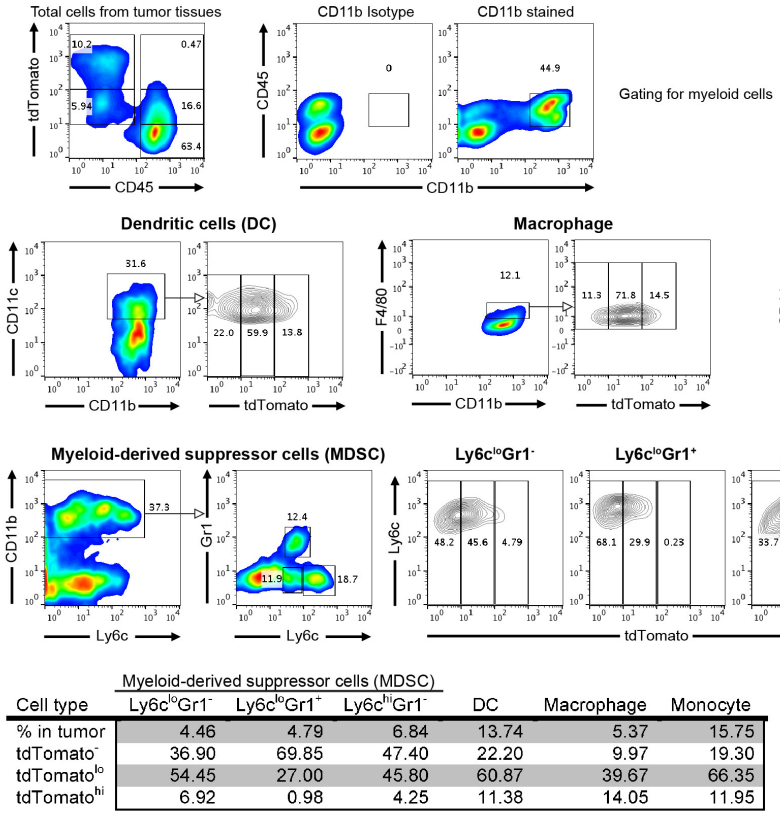
Supplemental Information

Supplemental Figures, Table and Video legends

A



Cell type	Myeloid-derived suppressor cells (MDSC) Ly6c^{lo}Gr1^{-}	Ly6c^{lo}Gr1^{+}	Ly6c^{hi}Gr1^{-}	DC	Macrophage	Monocyte
% in tumor	4.46	4.79	6.84	13.74	5.37	15.75
tdTomato^{-}	36.90	69.85	47.40	22.20	9.97	19.30
tdTomato^{lo}	54.45	27.00	45.80	60.87	39.67	66.35
tdTomato^{hi}	6.92	0.98	4.25	11.38	14.05	11.95

B

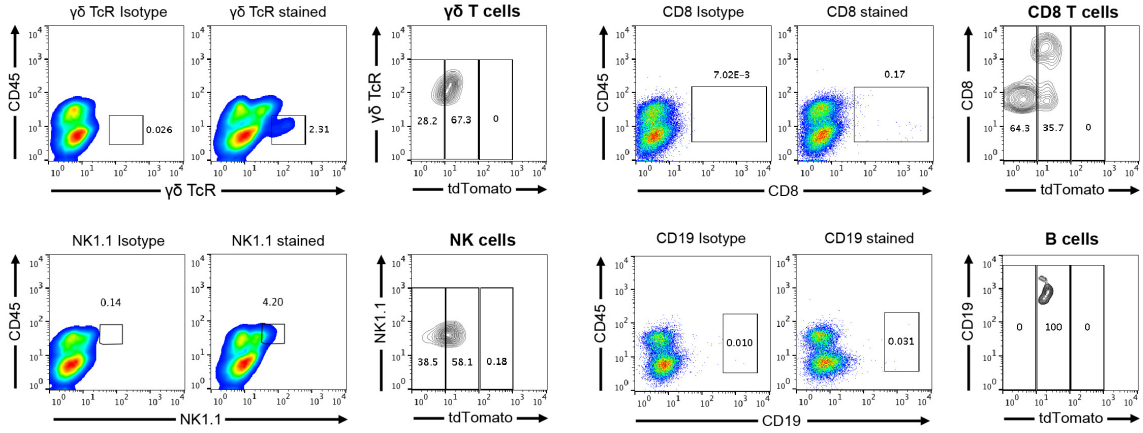


Figure S1. Analysis of hematopoietic markers and the cancer reporter tdTomato double positive cells in the murine model of colon caner liver metastasis.

Flow cytometry analysis of liver metastasized tumor samples in the murine model of colorectal cancer. Colon cancer cells carrying mutations in *Apc*, *Kras*, *Tp53* and *Smad4* genes with the

reporter gene tdTomato were injected to C57BL/6 mice via splenic vein to generate metastatic tumor formation in liver. **(A)** Analysis of myeloid cell markers and tdTomato positive cells in tumor tissues. The first panel displays the intensity of the hematopoietic marker CD45 versus cancer cell reporter tdTomato. Using anti-mouse CD11b antibody and its isotype control antibody, myeloid cells were identified and gated as CD45+CD11b+ cells. Myeloid lineage cells were further identified by CD11c, F4/80, CD14 and Ly6c/Gr1 expressions as dendritic cells, macrophages, monocytes and myeloid-derived suppressor (MDSC) cells, respectively. Percentiles of each cell type in tumor tissues and tdTomato positive cells are presented in the table. **(B)** Analysis of lymphoid cells in tumor tissues using antibodies detecting γδTcR, CD8, NK1.1 and CD19 for γδ T cells, CD8 T cells, natural killer (NK) cells and B cells, respectively. Results are representative of two independent experiments.

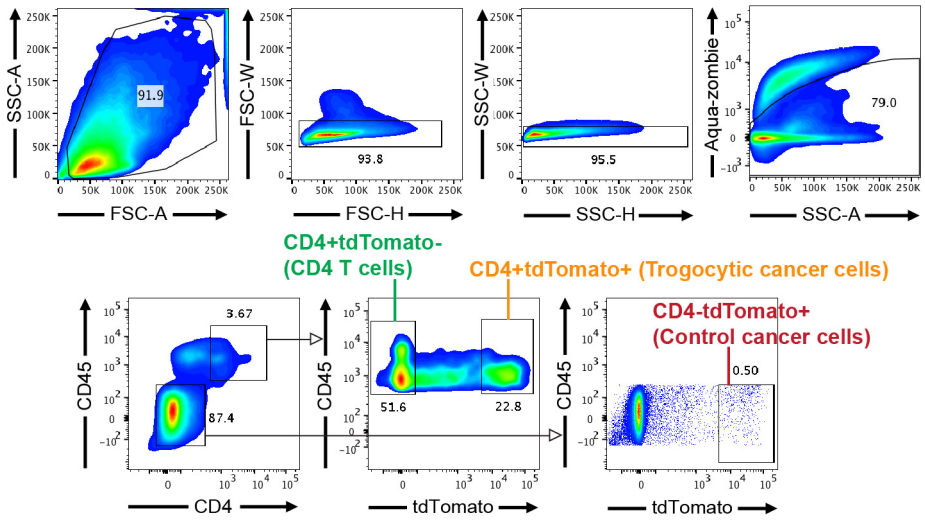


Figure S2. Sorting strategy to isolate CD4+tdTomato- T cells, CD4+tdTomato+ trogocytic cancer cells and CD4-tdTomato+ control cancer cells in the murine metastasis model of colorectal cancer.

Gating strategies to isolate tumor infiltrated T cells, trogocytic cancer cells and non-trogocytic cancer cells are shown. Single cells were gated using width of FSC and SSC (FSC-W and SSC-W). Live cells were gated as negative cells for live/dead aqua-zombie staining. CD4 and CD45 double positive cells were gated and further divided by tdTomato to sort CD4+CD45+tdTomato- T

cells and CD4+CD45+tdTomato+ trogocytic cancer cells. CD4 and CD45 double negative cells were gated then CD4-CD45-tdTomato+ cells were sorted as control cancer cells.

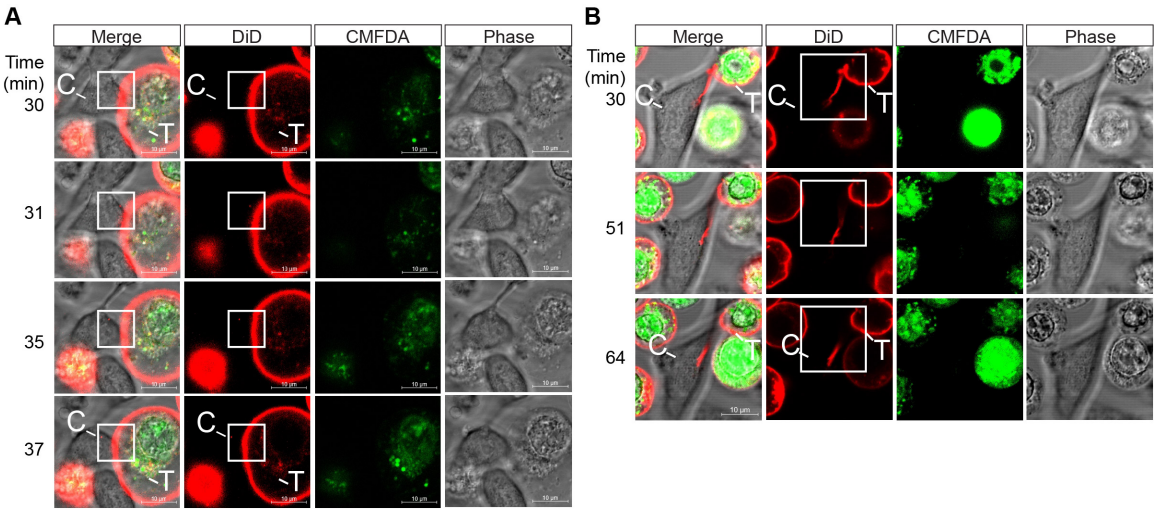


Figure S3. High-resolution live imaging analysis of *in vitro* trogocytosis

DiD and CMFDA labeled EL4 T cells were co-cultured with unlabeled MC38 colon cancer cells as described in Figure 3B. **(A-B)** Time-lapse confocal live imaging analysis of membrane protein transfer with 630x magnification. T; T cells, C; colon cancer cells, Time indicates minutes after the addition of the labeled EL4 cells to MC38 cells plated in chamber wells.

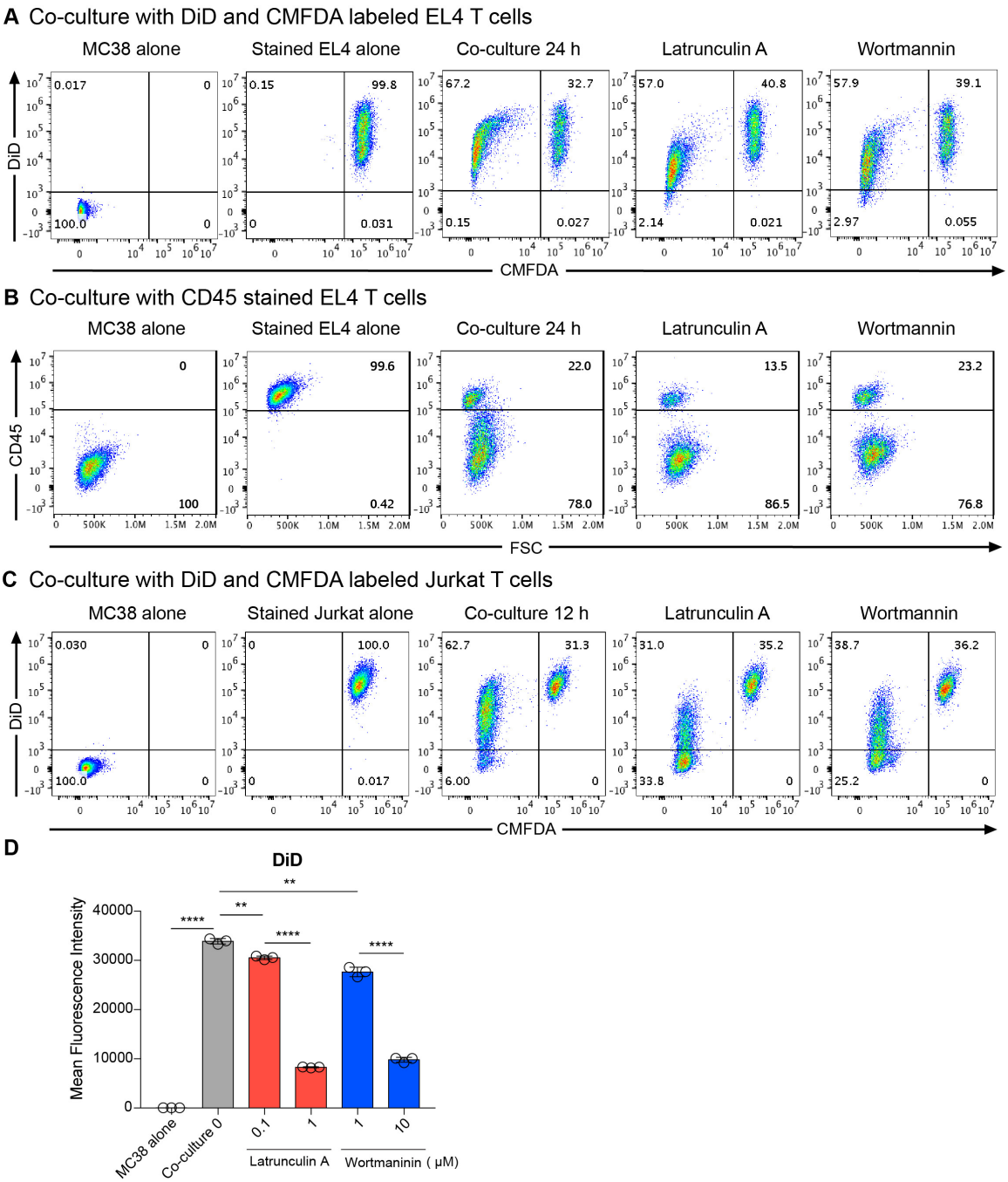


Figure S4. Transfer of membrane proteins including CD45 from mouse and human T cells to MC38 colon cancer cells *in vitro*.

(A-B) Representative flow cytometry data of DiD (A) and surface stained CD45 (B) in MC38 cells after 24 hours co-culture with the labeled EL4 T cells as described in Figure 3C and 3D. Actin-polymerization inhibitor, Latrunculin A (1 uM) and PI3-Kinase inhibitor, Wortmannin (10 uM) were used to test inhibition of trogocytosis. **(C)** DiD and CMFDA labeled Jurkat T cells were co-cultured

with unstained MC38 cells for 12 hours. Representative flow cytometry analysis of DiD transfer from Jurkat T cells to cancer cells in the presence of Latrunculin A (1 uM) or Wortmannin (10 uM). **(D)** Statistical analysis of the intensity of DiD in (C). ** = P < 0.01, **** = P < 0.0001; two-tailed Welch's t-test. Error bars indicate mean ± s.d. Results are representative of two independent experiments.

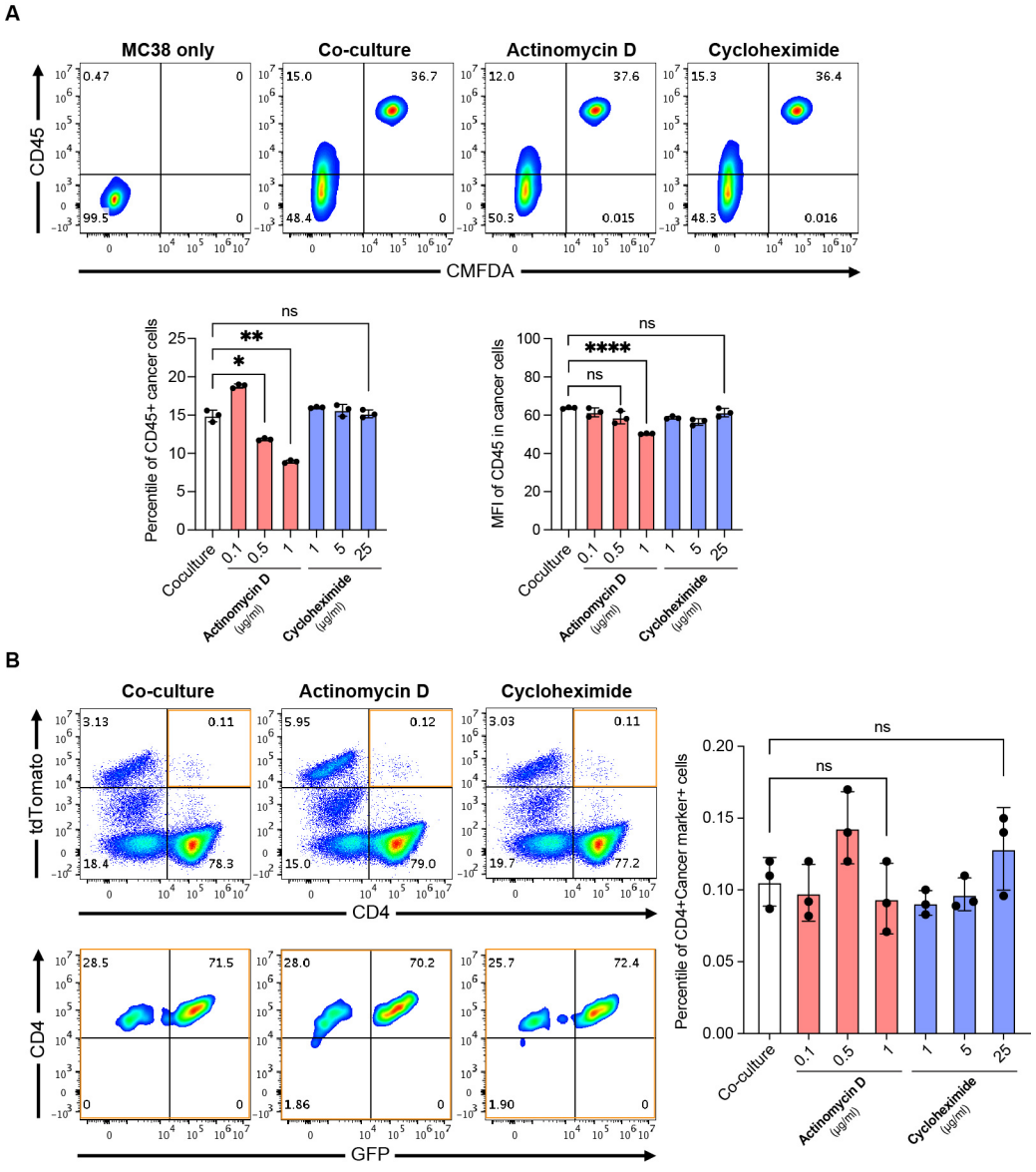


Figure S5. Flow cytometry analysis of trogocytic membrane transfer in the presence of transcription or translation inhibitors.

Colon cancer cells were pre-incubated with transcription inhibitor, Actinomycin D or translation inhibitor, Cycloheximide for 1 hour. Pre-incubated cells were washed with PBS then, co-cultured

with labeled T cells. **(A)** EL4 mouse T cells were stained with the cytosolic dye, CMFDA. The labeled EL4 cells were co-cultured with Actinomycin D or Cycloheximide treated MC38 colon cancer cells for 24 hours. Representative flow cytometry data is shown (Actinomycin D 0.5 μg/ml and Cycloheximide 25 μg/ml). Bar graphs display the percentile of CD45 acquired (CD45+) MC38 cells and mean fluorescence intensity (MFI) of CD45 in total MC38 cells. **(B)** *In vitro* organoid co-culture was performed for 2 days as described in Figure 2 using Actinomycin D or Cycloheximide pre-treated AKPS colon cancer organoids and activated primary CD4 T cells carrying the GFP reporter gene. CD4 and tdTomato double positive cells are highlighted by orange rectangle in the upper right panel of flow cytometry data. GFP levels in these double positive cells are displayed in the bottom row. Percentiles of the double positive cells in the presence or absence of Actinomycin D or Cycloheximide are shown in the graph. * = $P < 0.05$, ** = $P < 0.01$, **** = $P < 0.0001$, n.s = not significant; two-tailed Welch's t-test. Error bars indicate mean ± s.d.

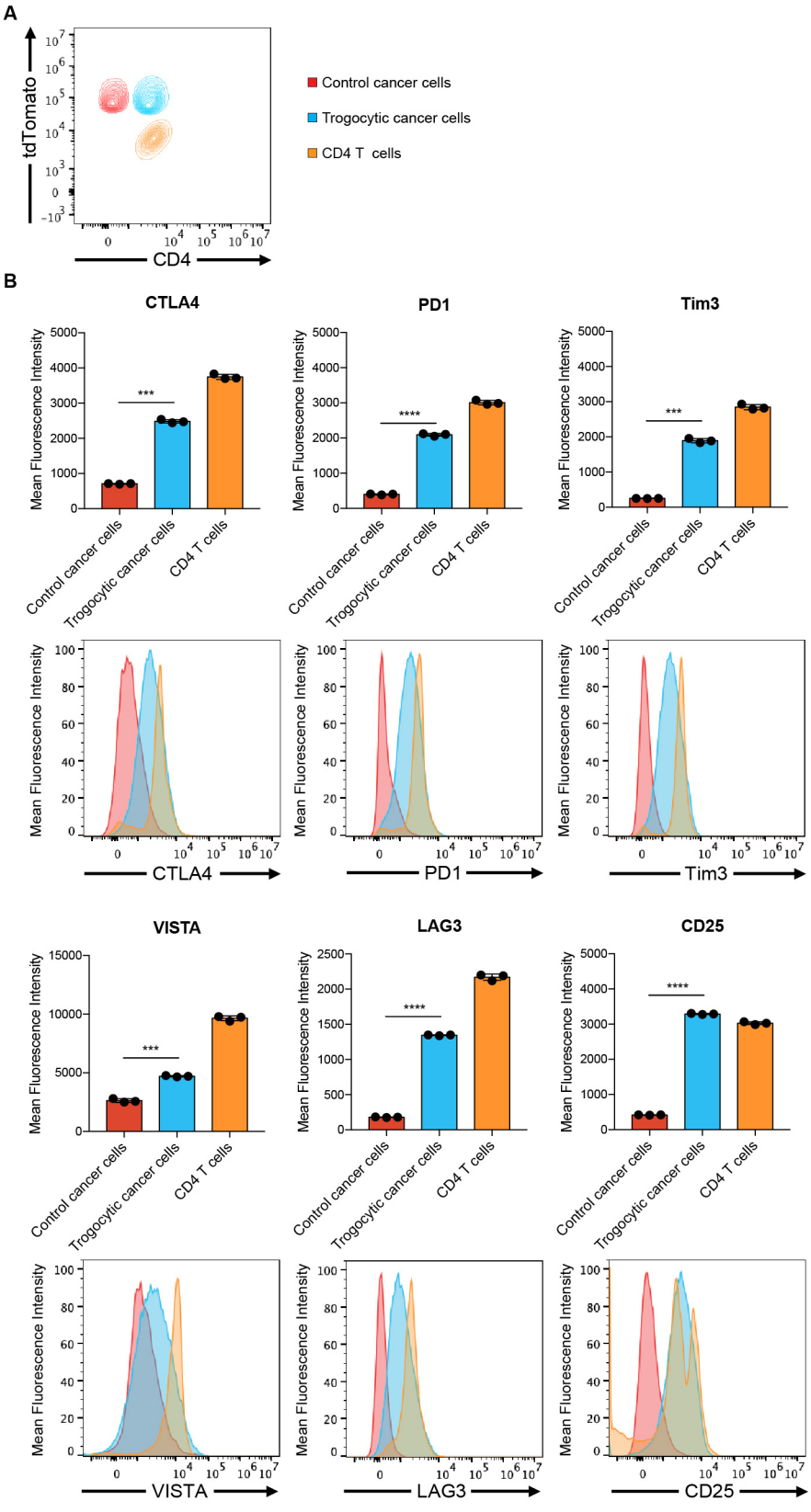

A
tdTomato
CD4
Control cancer cells
Trogocytic cancer cells
CD4 T cells
B
CTLA4
PD1
Tim3
VISTA
LAG3
CD25
Mean Fluorescence Intensity
Control cancer cells
Trogocytic cancer cells
CD4 T cells
***
****

Figure S6. Flow cytometry analyses of the transfer of immune regulatory molecules from tumor-infiltrated hematopoietic cells to metastatic colon cancer cells by cell surface staining using a single antibody.

(A) Representative clusters of non-trogocytic control cancer cells, trogocytic cancer cells and CD4 T cells in Figure 4 are displayed in the dot plot for CD4 and tdTomato. **(B)** To avoid overlaps of fluorescent signals from multiple antibodies for immune regulatory molecules, single antibody was used to detect each immune regulatory molecule. Cell surface staining of metastasized tumor tissue cells was performed with the single antibody for immune regulatory molecules and anti-CD4 antibody. Control cancer cells, trogocytic cancer cells and CD4 T cells were identified by tdTomato and CD4 as displayed in (A). Then, mean fluorescence intensity of immune regulatory molecules was analyzed. Interleukin-2 receptor alpha chain, CD25 was tested instead of CD38. *** = $P < 0.001$, **** = $P < 0.0001$; two-tailed Welch's t-test. Error bars indicate mean ± s.d. Results are representative of two independent experiments.

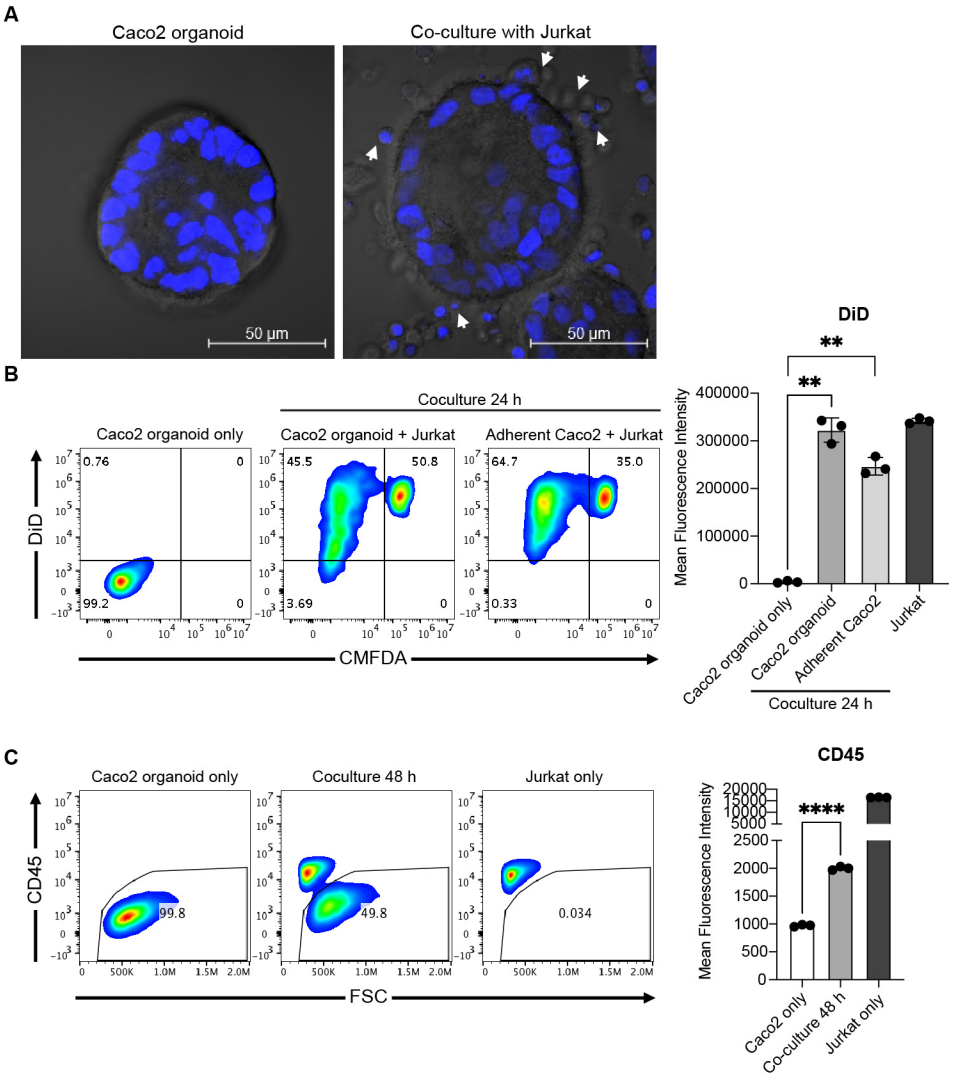


Figure S7. Membrane protein transfer from human Jurkat T cells to human colon cancer organoids derived from Caco-2 cell line.

(A) Representative microscope images of Caco-2 organoids in the presence or absence of Jurkat T cells in co-culture for 24 hours. Blue represents nuclear staining with DAPI. White arrows indicate Jurkat T cells in contact with the organoid. **(B)** Unlabeled Caco-2 organoids or adherent cells were co-cultured with CMFDA and DiD labeled Jurkat T cells for 24 hours. Labeling was performed as described in Figure 3A and organoid co-culture was performed as Figure 2A. Mean fluorescent intensity (MFI) of DiD in Caco-2 cells were analyzed. CMFDA negative cells (upper left plus lower left quadrants) were gated as Caco-2 cells and CMFDA positive cells (the upper right quadrant) were gated as Jurkat cells. **(C)** Caco-2 organoids were co-cultured with Jurkat T

cells for 48 hours then, the MFI of CD45 was analyzed. Caco-2 cells were gated as shown in dot plots. MFI of CD45 in the co-cultured Caco-2 cells (gated population) were compared to the control Caco-2 only and Jurkat only. ** = P < 0.01, **** = P < 0.0001; two-tailed Welch's t-test. Error bars indicate mean ± s.d.

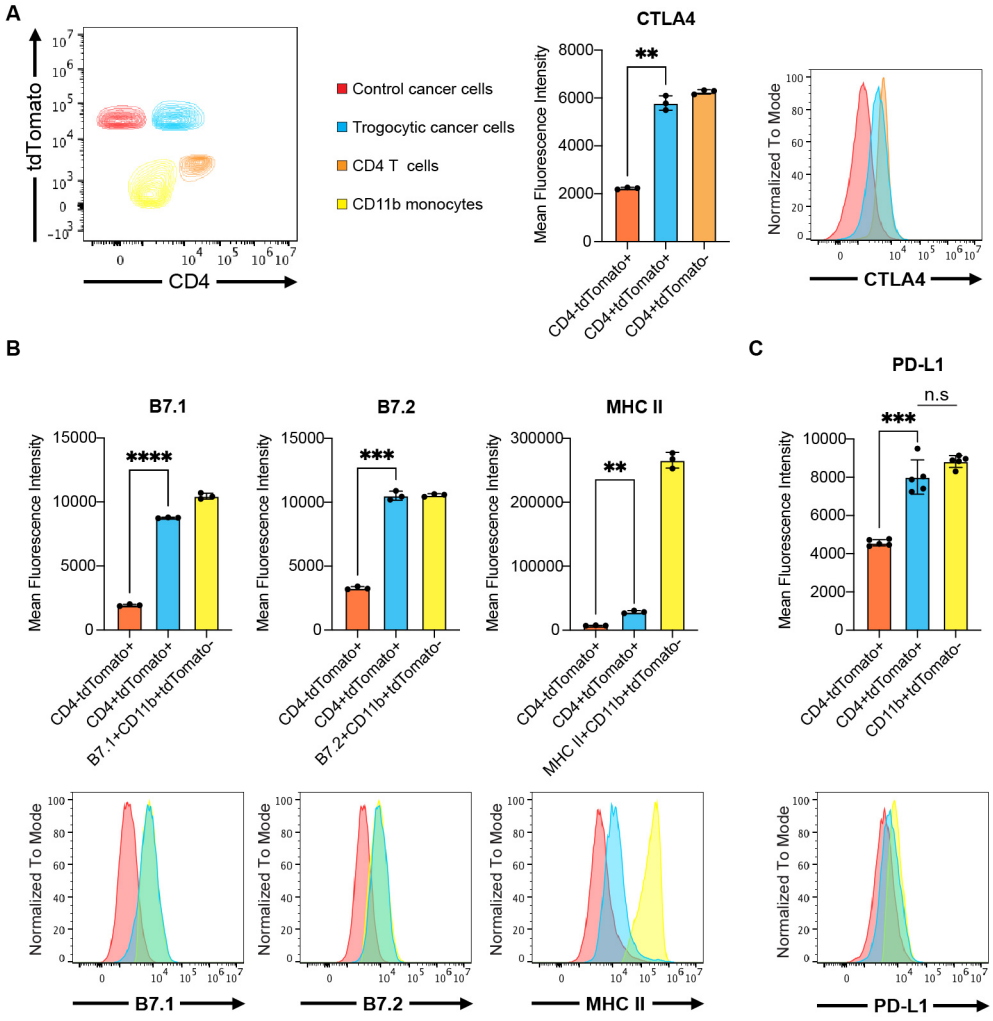


Figure S8. Flow cytometry analyses of the transfer of B7.1, B7.2, MHC class II and PD-L1 molecules from tumor-infiltrated immune cells to metastatic colon cancer cells.

Co-stimulatory ligands B7.1 and B7.2 and major histocompatibility complex (MHC) class II and PD1 ligand, PD-L1 protein levels in trogocytic cancer cells were analyzed compared to the non-trogocytic cancer cells and tumor-infiltrated immune cells using the murine metastasis model of colorectal cancer in Figure 4. **(A)** Representative clusters of non-trogocytic control cancer cells,

trogocytic cancer cells, CD4 T cells and CD11b positive monocytes are presented as shown as in Figure S6. CD4, tdTomato and CD11b were used to identify each cluster. CD11b monocytes were used as positive controls for the expression levels of B7.1, B7.2, MHC class II and PD-L1 in the tumor microenvironment. Mean fluorescence intensity (MFI) of CTLA4 was analyzed in trogocytic cancer cells compared to non-trogocytic cancer cells and CD4 T cells. **(B)** The protein levels of B7.1, B7.2 and MHC class II molecules in the same tumor samples analyzed in (A) are presented. **(C)** PD-L1 protein levels in trogocytic cells were compared to non-trogocytic cancer cells and tumor-infiltrated CD11b monocytes. Gray-filled line in the histogram indicates PD-L1 negative cells in the tumor samples. ** = P < 0.01, *** = P < 0.001, **** = P < 0.0001; two-tailed Welch's t-test. Error bars indicate mean ± s.d. Results are representative of two independent experiments.

Table S1. The list of primers used in genomic DNA PCR in Figure 5.

HLA-B	Forward (5' -3')	CTTGTTCCAGAGAGGTGGGG
	Reverse (5' -3')	AACAGACTCAGCACAGCGAA
H2-K1	Forward (5' -3')	AGCCTATGGACTCAATGTGAAGA
	Reverse (5' -3')	AAATCAGCCCTAGGTCAAGATGATA
HPRT (Hprt)	Forward (5' -3')	GTTTGTGTCATTAGTGAAACT
	Reverse (5' -3')	GCAGCAACTGACATTTCTAAA

Video S1. Live imaging analysis of membrane protein transfer from T cells to cancer cells. EL4 mouse T cells were labeled with the membrane tracker, DiD (Red) and the cytosolic tracker, CMFDA (Green). Labeled EL4 T cells (indicated as 'T') were added into the culture of MC38 colon cancer cells (indicated as 'C'). Co-culture images were captured every 5 minutes for 6 hours using confocal microscopy.

Supplementary Methods

Development of colon cancer organoids

Colonic crypts were isolated from *Rosa-LSL-tdTomato* mice to generate normal colonic organoids. Organoids were cultured in the WRN media with recombinant mouse EGF (50 ng/ml, Peprotech 315-09). Then, organoids were transfected with *pSECC-Apc* plasmid (containing *Cre*, *Cas9* and *sgApc*) to generate $Apc^{-/-}tdTomato^{+}$ organoids. $Apc^{-/-}Kras^{G12D/+}Tp53^{-/-}tdTomato^{+}$ organoids were generated using $LSL\text{-}Kras^{G12D/+}Tp53^{fl/fl}$*Rosa-LSL-tdTomato* mice. *Apc* deletion was selected by Wnt3A withdrawal, $Tp53^{-/-}$ was selected by Nutlin-3 addition (10 μM) and $Kras^{G12D}$ was selected by EGF withdrawal. After establishment of the $Apc^{-/-}Kras^{G12D/+}Tp53^{-/-}tdTomato^{+}$ organoid line, subsequent transfection with *pSECC-Smad4* plasmid (containing *Cre, Cas9* and *sgSmad4*) was performed. *Smad4* deletion was selected by addition of TGFβ (10 ng/ml) to the media to generate $Apc^{-/-}Kras^{G12D/+}Tp53^{-/-}Smad4^{-/-}tdTomato^{+}$ organoids.

Tumor dissociation

Live metastasized mouse colon cancer and patient-derived xenograft colon cancer and head and neck cancer tissues were collected and dissociated in the digestion buffer containing 200 U/ml of type IV collagenase (Worthington, LS004188), 125 μg/ml of type II dispase (Sigma, D4693), 2.5 % fetal bovine serum, 1x Penicillin/Streptomycin in advanced DMEM F-12 media at 37 °C for 30-60 min with agitation. Isolated cells were filtered through the 40 μm pore size strainer.

Flow cytometry

Surface staining of cell marker proteins or immune regulatory molecules was performed. Isolated organoid or tumor cells were treated with live/dead staining solution (LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit, Invitrogen, L34957) and the Fc blocker (TrueStain FcX™ anti-mouse CD16/32 Antibody (BioLegend, 101320, clone: 93) following the manufacturer's instructions. Antibodies were used as follow. CD45 Monoclonal Antibody, Pacific Blue (Invitrogen, MCD4528, clone: 30-F11), CD4 Monoclonal Antibody, APC (Invitrogen, 17-0041-81, clone: GK1.5), CD8a Monoclonal Antibody, APC (Invitrogen, 17-0081-82, clone: 53-6.7), TCR gamma/delta Monoclonal Antibody, APC (Invitrogen, 17-5711-82, clone: GL3), CD19 Monoclonal

Antibody, APC (Invitrogen, 17-0191-81, clone: MB19-1), CD11b Monoclonal Antibody, APC (Invitrogen, 17-0112-82, clone: M1/70), CD11b Monoclonal Antibody, APC-eFluor 780 (Invitrogen, 47-0112-80, clone: M1/70), CD11c Monoclonal Antibody, APC (Invitrogen, 17-0114-81, clone: N418), F4/80 Monoclonal Antibody, APC (Invitrogen, 17-4801-80, clone: BM8), APC Anti-Mouse Ly-6G (Tonbo, 20-1276-U025, clone: 1A8), Ly-6C Monoclonal Antibody, PerCP-Cyanine5.5 (Invitrogen, 45-5932-82, clone: HK1.4), NK1.1 Monoclonal Antibody, APC (Invitrogen, 17-5941-82, clone: PK136), CD14 Monoclonal Antibody, PerCP-Cyanine5.5 (Invitrogen, 45-0141-82, clone: Sa2-8), CD80 (B7-1) Monoclonal Antibody, eFluor 450 (Invitrogen, 48-0801-80, clone: 16-10A1), CD86 (B7-2) Monoclonal Antibody, FITC (Invitrogen, 11-0862-81, clone: GL1), MHC Class II (I-A/I-E) Monoclonal Antibody, eFluor 450 (Invitrogen, 48-5321-80, clone: M5/114.15.2), CD274 (PD-L1, B7-H1) Monoclonal Antibody, PE-Cyanine7 (Invitrogen, 25-5982-80, clone: MIH5), Rat IgG2b kappa Isotype Control, APC (Invitrogen, 17-4031-81, eB149/10H5), Rat IgG2b kappa Isotype Control, APC (Invitrogen, 17-4031-81, eB149/10H5), Rat IgG2b kappa Isotype Control, eFluor450 (Invitrogen, 48-4031-80, eB149/10H5), American Hamster IgG Isotype Control, APC (Invitrogen, 17-4888-81, eBio299Arm), Mouse IgG2a kappa Isotype Control, APC (Invitrogen, 17-4724-81, eBM2a), APC Mouse IgA, κ Isotype Control (BD, 562140, clone: M18-254), BUV737 Rat Anti-Mouse CD326 (EpCAM) (BD, 741818, clone: G8.8), CD152 (CTLA-4) Monoclonal Antibody, PE-Cyanine7 (Invitrogen, 17-0041-81, clone: UC10-4B9), Brilliant Violet 421 anti-mouse CD279 (PD-1) Antibody (BioLegend, 135217, clone: 29F.1A12), BUV395 Mouse Anti-Mouse CD366 (Tim-3) (BD, 747620, clone: 5D12), VISTA Monoclonal Antibody, Super Bright 600 (Invitrogen, 63-1083-80, clone: MIH64), CD223 (LAG-3) Monoclonal Antibody, Super Bright 702 (Invitrogen, 67-2231-80, clone: eBioC9B7W), APC/Cyanine7 anti-mouse CD38 Antibody (BioLegend, 102727, clone: 93), Anti-human CD4 Monoclonal Antibody, APC (Invitrogen, 17-0049-42, clone: RPA-T4), PE anti-human CD298 Antibody (BioLegend, 341704, clone: LNH-94), Anti-human CD45 Monoclonal Antibody, PE-Cyanine7 (BioLegend, 368531, clone: 2D1),Flow cytometry was performed using the CytoFlex LX.

In vitro co-culture of organoids with CD4 T cells

The minimal medium containing 1x B27 supplement (Thermo, 12587010), 1x Glutamax (Gibco, 35050061) and 1x Penicillin/Streptomycin (Thermo, 15140122) in advanced DMEM-F12 (Thermo, 12634010) was prepared. *Apc*$^{-/-}$*Kras*$^{G12D/+}$*Tp53*$^{-/-}$*Smad4*$^{-/-}$*tdTomato*$^{+}$ (AKPS) organoids were plated in 50 μl of 1:1 ratio mixed Matrigel and the minimal medium. Then, cultured in the 500 μl of minimal medium with 50 ng/ml of EGF. GFP positive normal colonic organoids (generated from Actin-GFP reporter mice) and *Apc*$^{-/-}$*tdTomato*$^{+}$ (A) organoids were cultured in the WERN medium, 50 ng/ml of EGF added WRN supernatants from L-WRN cell line. Culture medium was replaced every other day.

Organoids are digested using TrypLE™ (Gibco, 12605010) at 37°C for 5 min. 8-well chamber slides (ThermoScienctific, 154534PK) were coated with 100 μl of 1:1 mixed matrigel and the minimal medium (200 μl of the mixture used per well in 48-well tissue culture plates). Isolated organoids (10^5 cells) were added on top of the solidified matrigel (1:1 ratio mixed with the minimal medium). GFP positive CD4 T cells were purified and stimulated for 3 days as described above. Activated CD4 T cells (10^6 cells) were added to the organoid culture then, co-cultured for 3 days in 500 μl of the culture medium. Culture medium was refreshed at day 2.

Inhibition of transcription and translation for AKPS colon cancer organoids during the co-culture with primary CD4 T cells was performed using Actinomycin D (R&D, 1229/10) and Cycloheximide (R&D, 0970/100), respectively. Actinomycin D (0.1 to 1 μg/ml) or Cycloheximide (1 to 25 μg/ml) was added into the cultured colon cancer cells for 1 hour. Treated cells were washed with PBS three times then, co-cultured with GFP positive CD4 T cells for 2 days.

Confocal microscopy

In vitro co-cultured organoids and T cells were fixed with 4% paraformaldehyde in the PME buffer (50 mM PIPES, 2.5 mM $MgCl_2$ and 5 mM EDTA) at room temperature for 20 min. Cells were washed with PBS containing 0.05 % Tween20, then stained with CD4 Monoclonal Antibody, APC (Invitrogen, 17-0041-81, clone: GK1.5) at 4°C for 30 min. Samples were mounted with ProLong™ Gold Antifade Mountant with DAPI (ThermoScientific, P36941).

EL4 cells or Jurkat cells were labeled with 10 mM CMFDA (Invitrogen, C2925) at 37°C for 15 min. Cells were washed twice with PBS then labeled with DiD (Invitrogen, d7757) at 37°C for 5 min followed by 4°C for 25 min. Labeled cells were washed three times with PBS. 0.5 million labeled cells were co-cultured with 0.5 million unlabeled MC38 cells up to 24 hours as indicated in Figure 2A and S5A-D in the presence or absence of Latruncullin A (0.01 – 1 μM, Sigma, L5163) or Wortmannin (0.1 – 10 μM, Cell Signaling, 9951S). For live imaging analysis, cells were co-cultured in a one-well chambered cover glass (Thermo Scientific, 12-565-472). Pictures were captured using a Leica TCS SP5 or a Zeiss LSM780 confocal laser-scanning microscope.

Ex vivo co-culture

Trogocytic cancer cells and non-trogocytic cancer cells were sorted as described in Figure S2A. 200 μl of matrigel solution coated the bottom of culture wells in 48-well plates. 10^4 sorted cells were plated to the solidified matrigel. 5 x 10^5 splenocytes from Actin-GFP reporter mice were added in a total 1 ml of complete RPMI medium (10 % FBS, 1x Pen/Strep, 0.05 mM beta-mercaptoethanol) then, co-cultured for 5 days. Co-cultured cells were harvested and their total RNA was isolated using RNeasy Plus Micro Kit (Qiagen, 74034) following the manufacturer's protocol. Supernatants of co-cultured cells were analyzed for cytokine profiles using Mouse High Sensitivity T-cell discovery array 18-flex quantification assay (Eve Technologies, MDHSTC18).

RNA-seq analysis

RNA-seq libraries were constructed using NEBNext Low Input RNA kit by YCGA (Yale Center for Genome Analysis). The RNA-seq libraries were sequenced on the Illumina NovaSeq6000 instrument platform (100 bp paired-end sequencing). The sequencing reads were aligned onto Mus musculus GRCm38/mm10 reference genome using the HISAT2. The mapped reads were transformed into the count matrix with default parameters using the StringTie v1.3.3 software, then normalized using the DESeq v2 software. Differentially expressed genes (DEGs) were identified using the same software based on a negative binomial generalized linear model.

Genomic DNA real time PCR

Patient-derived tumor tissues were digested and stained with live/dead staining solution, PE anti-human CD298 Antibody (BioLegend, 341704, clone: LNH-94), Pacific Blue anti-mouse CD45 Monoclonal Antibody (Invitrogen, MCD4528, clone: 30-F11) and APC anti-mouse CD4 Monoclonal Antibody (Invitrogen, 17-0041-81, clone: GK1.5) as described above. Gated cells in Figure 4B and 4E were sorted using BD FACSAria™. Genomic DNA from the sorted cells were isolated using QIAamp DNA Mini Kit (Qiagen, 51304) following manufacturer's manual. Quantitative real time PCR was performed in 10 μl reactions using the iTag universal SYBR Green supermix on the CFX96 Touch™ real-time PCR detection system. PCR primers are listed in Table S1.