Supplementary Materials

Table 1. Antibodies for flow cytometry

| Target          | Fluorophore | Company   | Catalog number | Clone |
|-----------------|-------------|-----------|----------------|-------|
|                 | FACS Aria III panel for sorting of cord blood DCs |
| CD141           | BV711       | BD        | 563155         | 1A4   |
| CD1c            | PE-Cy7      | Biolegend | 331516         | L161  |
| CD14            | PerCP-Cy5.5 | Biolegend | 325622         | HCD14 |
| CLEC9A          | APC         | Biolegend | 353806         | 8F9   |
| CD45            | AF700       | Biolegend | 304024         | Hi30  |
| Dead Cell Stain | Near-IR     | Life Technologies | L34976 |      |
|                 | FACS Aria III panel for enrichment of DCs from tumor digests |
| CD45            | BV510       | Biolegend | 304036         | Hi30  |
| CD3             | AF488       | Biolegend | 317310         | OKT3  |
| Dead Cell Stain | Near-IR     | Life Technologies | L34976 |      |
|                 | LSR Fortessa panel for cytokine expression in blood or tumor-derived DCs |
| CD11c           | BV421       | Biolegend | 301628         | 3.9   |
| CD45            | BV510       | Biolegend | 304036         | Hi30  |
| TNFα            | BV605       | Biolegend | 502936         | MAb11 |
| CD56            | BV650       | Biolegend | 318344         | HCD56 |
| CD16            | BV711       | Biolegend | 302044         | 3G8   |
| IFNα            | AF488*      | R&D Systems | MAB15981-500  | 247801|
| CD14            | PerCP-Cy5.5 | Biolegend | 325622         | HCD14 |
| CXCL9           | PE          | Biolegend | 519504         | J034D6|
| CXCL10          | PE          | Biolegend | 357904         | J1015E10 |
| CD3             | PE-Cy5      | BD        | 555341         | HIT3a |
| CD1c            | PE-Cy7      | Biolegend | 331516         | L161  |
| CLEC9A          | APC         | Biolegend | 353806         | 8F9   |
| CD19            | AF700       | Biolegend | 302226         | HiB19 |
| HLA-DR          | BUV395      | BD        | 564040         | G46-6 |
| Dead Cell Stain | Near-IR     | Life Technologies | L34976 |      |
| Antibody  | Catalog Number | Provider | Code    | Cat. No. |
|-----------|----------------|----------|---------|---------|
| CD3       | BUV395         | BD       | 740283  | HIt3a   |
| CD19      | BUV395         | BD       | 563551  | SJ25C1  |
| HLA-DR    | BUV496         | BD       | 741157  | Tu39    |
| CD16      | BUV615         | BD       | 750572  | 3G8     |
| CD56      | BUV661         | BD       | 750478  | NCAM16.2|
| CD45      | BUV805         | BD       | 612891  | HI30    |
| TNFα      | BV605          | Biolegend| 502936  | MAb11   |
| CD14      | BV650          | BD       | 740633  | MfP9    |
| IFNα      | AF488*         | R&D Systems | MAB15981-500 | 247801 |
| CXCL10    | PE             | Biolegend| 519504  | J034D6  |
| CD68      | PE-eFluor 610  | invitrogen| 61-0689-42 | Y1/82A |
| CD1c      | PE-Cy7         | Biolegend| 331516  | L161    |
| CLEC9A    | APC            | Biolegend| 353806  | 8F9     |
| CD11c     | AF700          | Biolegend| 337220  | Bu15    |
| Dead Cell Stain | Near-IR | Life Technologies | L34976 |

* anti-IFNα antibodies in AF488: in-house labeling (Invitrogen, #A30005)
Table 2. Overview of scRNA-seq studies

| Study_name     | Tissue_type                  | Status                          | Study               |
|----------------|------------------------------|---------------------------------|---------------------|
| Ovarian_Qian   | Ovarian                      | Solid tumor - Cancer            | Qian et al.         |
| OV-FTC_Cheng   | Ovarian                      | Solid tumor - Cancer            | Cheng et al.        |
| Lung_Qian      | Lung                          | Solid tumor - Cancer            | Qian et al.         |
| Breast_Qian    | Breast                        | Solid tumor - Cancer            | Qian et al.         |
| THCA_Cheng     | Thyroid Carcinoma (THCA)     | Solid tumor - Cancer            | Cheng et al.        |
| ESCA_Cheng     | Esophageal Carcinoma (ESCA)  | Solid tumor - Cancer            | Cheng et al.        |
| Kidney_Cheng   | Kidney cancer                | Solid tumor - Cancer            | Cheng et al.        |
| PAAD_Cheng     | Pancreatic adenocarcinoma (PAAD) | Solid tumor - Cancer          | Cheng et al.        |
| UCEC_Cheng     | Uterine Corpus Endometrial Carcinoma (UCEC) | Solid tumor - Cancer          | Cheng et al.        |
| HN_Cillo       | Head and Neck cancer         | Solid tumor - Cancer            | Cillo et al.        |
| Intestine_Qian | Intestine/Colorectal         | Solid tumor - Cancer            | Qian et al.         |
| Intestine_Lee  | Intestine/Colorectal         | Solid tumor - Cancer            | Lee et al.          |
| Liver_Ma       | Liver                        | Solid tumor - Cancer            | Ma et al.           |
| Lung_Maier     | Lung                         | Solid tumor - Cancer            | Maier et al.        |
| Pancreatic_Peng| Pancreatic cancer            | Solid tumor - Cancer            | Peng et al.         |
| PBMC_Cillo     | Head and Neck PBMC - cancer  | Blood sample from cancer patients | Cillo et al.       |
| PBMC_Cillo_nc  | Head and Neck PBMC - healthy | Blood sample from healthy donors | Cillo et al.       |
| Intestine_Smillie | Intestine                   | Non-cancer                     | Smillie et al.      |
| Pancreatic_Peng_nc | Pancreas                    | Non-cancer                     | Peng et al.         |
| Lung_Raredon   | Lung                         | Non-cancer                     | Raredon et al.      |
| Lung_Madissoon | Lung                         | Non-cancer                     | Madissoon et al.    |
| Tonsils_Cillo  | Tonsils                      | Non-cancer                     | Cillo et al.        |
| PBMC_Kotliarov | PBMC                         | Non-cancer                     | Kotliarov et al.    |
| Liver_Ramachandran | Liver                     | Non-cancer                     | Ramachandran et al.|
| Intestine_Martin | Intestine                  | Non-cancer                     | Martin et al.       |
| CBDCs_He       | CBDCs                        | Cord blood sample              | He et al.           |
| PBMC_He        | PBMC                         | Blood sample                   | He et al.           |
| Lung_He        | Lung                         | Solid tumor - Cancer, IFNy+TL8-506 and Poly(IC)+TL8-506 treated | He et al.           |
| Colon_He       | Colon                        | Solid tumor - Cancer, IFNy+TL8-506 and Poly(IC)+TL8-506 treated | He et al.           |
| Melanoma_He    | Melanoma                     | Solid tumor - Cancer, IFNy+TL8-506 treated | He et al.           |
### Table 3. Parameters for the filtering of high-quality cells in scRNA-seq studies

| Study_name         | standard_min_genes | standard_min_cells | standard_min_counts | standard_n_genes | standard_percent_mito | standard_max_counts |
|--------------------|---------------------|--------------------|---------------------|------------------|-----------------------|---------------------|
| Ovarian_Qian       | 800                 | 10                 | 600                 | 6000             | 0.1                   | 55000               |
| OV-FTC_Cheng       | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| Lung_Qian          | 600                 | 10                 | 1000                | 6000             | 0.15                  | 70000               |
| Breast_Qian        | 400                 | 10                 | 800                 | 5900             | 0.03                  | 70000               |
| THCA_Cheng         | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| ESCA_Cheng         | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| Kidney_Cheng       | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| PAAD_Cheng         | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| UCEC_Cheng         | 500                 | 10                 | 1000                | 6000             | 0.15                  | 50000               |
| HN_Cillo           | 400                 | 10                 | 1200                | 7000             | 0.3                   | 70000               |
| Intestine_Qian     | 800                 | 10                 | 1200                | 6000             | 0.1                   | 55000               |
| Intestine_Lee      | 500                 | 20                 | 1000                | 6000             | 0.1                   | 70000               |
| Liver_Ma           | 500                 | 30                 | 1500                | 7000             | 0.15                  | 60000               |
| Lung_Maier         | 800                 | 20                 | 1500                | 5000             | 0.1                   | 30000               |
| Pancreatic_Peng    | 500                 | 20                 | 1000                | 8000             | 0.1                   | 80000               |
| PBMC_Cillo         | 600                 | 10                 | 1200                | 5000             | 0.15                  | 40000               |
| PBMC_Cillo_nc      | 600                 | 10                 | 1200                | 5000             | 0.15                  | 40000               |
| Intestine_Smillie  | 800                 | 30                 | 2000                | 8000             | 0.2                   | 100000              |
| Pancreatic_Peng_nc | 800                 | 30                 | 2000                | 8000             | 0.2                   | 100000              |
| Lung_Raredon       | 700                 | 20                 | 1000                | 8000             | 0.15                  | 60000               |
| Lung_Madissoon     | 700                 | 20                 | 1000                | 8000             | 0.1                   | 80000               |
| Tonsils_Cillo      | 400                 | 10                 | 1700                | 7000             | 0.1                   | 70000               |
| PBMC_Kotliarov     | 500                 | 30                 | 1000                | 3000             | 0.05                  | 20000               |
| Liver_Ramachandran | 600                 | 20                 | 1000                | 7000             | 0.15                  | 60000               |
| Intestine_Martin   | 500                 | 30                 | 1000                | 6000             | 0.2                   | 50000               |
| CBDCs_He           | 800                 | 10                 | 1000                | 6500             | 0.15                  | 50000               |
| PBMC_He            | 1500                | 20                 | 1000                | 7000             | 0.18                  | 60000               |
| Lung_He            | 600                 | 10                 | 800                 | 7000             | 0.15                  | 75000               |
| Colon_He           | 600                 | 10                 | 800                 | 7000             | 0.15                  | 75000               |
| Melanoma_He        | 600                 | 10                 | 800                 | 6500             | 0.15                  | 50000               |
| Parameters for integrated DC dataset | 500 | 10 | 1000 | 6000 | 0.15 | 50000 |
| cDC1 medium vs. IFNγ + TL8-506 | cDC1 medium vs. Poly(I:C) + TL8-506 | cDC1 medium vs. Poly(I:C) + TL8-506 | cDC2 medium vs. IFNγ + TL8-506 | cDC2 medium vs. Poly(I:C) + TL8-506 | cDC2 medium vs. Poly(I:C) + TL8-506 |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| gene | log2FC | gene | log2FC | gene | log2FC | gene | log2FC | gene | log2FC | gene | log2FC |
|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------|
| B2M | 8.9  | B2M | 9.0  | B2M | 8.9  | B2M | 9.0  | B2M | 8.9  | B2M | 9.0  |
| UBD | 6.9  | UBD | 6.9  | UBD | 6.9  | UBD | 6.9  | UBD | 6.9  | UBD | 6.9  |
| CCL3L1 | 6.2 | CCL3L1 | 6.2 | CCL3L1 | 6.2 | CCL3L1 | 6.2 | CCL3L1 | 6.2 | CCL3L1 | 6.2 |
| ITGB8 | 30.9 | ITGB8 | 30.9 | ITGB8 | 30.9 | ITGB8 | 30.9 | ITGB8 | 30.9 | ITGB8 | 30.9 |
| ISG15 | 3.3 | ISG15 | 3.3 | ISG15 | 3.3 | ISG15 | 3.3 | ISG15 | 3.3 | ISG15 | 3.3 |
| IL12B | 6.5 | IL12B | 6.5 | IL12B | 6.5 | IL12B | 6.5 | IL12B | 6.5 | IL12B | 6.5 |
| IL6 | 8.1 | IL6 | 8.1 | IL6 | 8.1 | IL6 | 8.1 | IL6 | 8.1 | IL6 | 8.1 |
| SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 |
| UBD | 6.9 | UBD | 6.9 | UBD | 6.9 | UBD | 6.9 | UBD | 6.9 | UBD | 6.9 |
| NCF2 | 30.2 | NCF2 | 30.2 | NCF2 | 30.2 | NCF2 | 30.2 | NCF2 | 30.2 | NCF2 | 30.2 |
| OASL | 3.0 | OASL | 3.0 | OASL | 3.0 | OASL | 3.0 | OASL | 3.0 | OASL | 3.0 |
| OAS1 | 3.0 | OAS1 | 3.0 | OAS1 | 3.0 | OAS1 | 3.0 | OAS1 | 3.0 | OAS1 | 3.0 |
| CCL3L1 | 8.8 | CCL3L1 | 8.8 | CCL3L1 | 8.8 | CCL3L1 | 8.8 | CCL3L1 | 8.8 | CCL3L1 | 8.8 |
| CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 |
| CCL10 | 5.5 | CCL10 | 5.5 | CCL10 | 5.5 | CCL10 | 5.5 | CCL10 | 5.5 | CCL10 | 5.5 |
| CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 | CLCF1 | 2.2 |
| CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 |
| FAM9C | 25.1 | FAM9C | 25.1 | FAM9C | 25.1 | FAM9C | 25.1 | FAM9C | 25.1 | FAM9C | 25.1 |
| IL7 | 4.7 | IL7 | 4.7 | IL7 | 4.7 | IL7 | 4.7 | IL7 | 4.7 | IL7 | 4.7 |
| CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 | CCL4 | 5.0 |
| IFNL1 | 25.1 | IFNL1 | 25.1 | IFNL1 | 25.1 | IFNL1 | 25.1 | IFNL1 | 25.1 | IFNL1 | 25.1 |
| SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 |
| SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 | SIGLEC6 | 25.9 |
| TCF7L2 | 5.2 | TCF7L2 | 5.2 | TCF7L2 | 5.2 | TCF7L2 | 5.2 | TCF7L2 | 5.2 | TCF7L2 | 5.2 |
| TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 |
| IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 |
| TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 | TNF | 5.0 |
| ST6GALNAC2 | 24.1 | ST6GALNAC2 | 24.1 | ST6GALNAC2 | 24.1 | ST6GALNAC2 | 24.1 | ST6GALNAC2 | 24.1 | ST6GALNAC2 | 24.1 |
| IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 | IFNΛ | 25.1 |

Table 4. List of top 50 DE genes in treated tumor cDCs
| gene          | log2FC | gene          | log2FC | gene          | log2FC | gene          | log2FC | gene          | log2FC |
|--------------|--------|--------------|--------|--------------|--------|--------------|--------|--------------|--------|
| LTA          | 29.4   | WNT4         | 6.7    | LTA          | 29.5   | LTA          | 29.2   |             |        |
| TBKBP1       | 6.1    | IL1B         | 4.7    | IL1B         | 5.8    | CCL3         | 6.9    |             |        |
| CXCL10       | 5.6    | ZBTB32       | 6.3    | CCL4         | 5.7    | WNT4         | 6.7    |             |        |
| TNF          | 5.4    | CCL3         | 5.2    | IL1B         | 5.9    | LTA          | 7.2    |             |        |
| WNT4         | 5.4    | IL1B         | 4.7    | IL12A        | 5.8    | CCL3         | 6.9    |             |        |
| GBP5         | 5.3    | IL6          | 4.7    | CCL4         | 5.7    | WNT4         | 6.7    |             |        |
| CXCL11       | 5.2    | CXCL4        | 4.7    | CXCL11       | 5.7    | LTA          | 6.6    |             |        |
| TCF7L2       | 5.0    | LAMC1        | 4.7    | CSF2         | 5.6    | TNF          | 6.4    |             |        |
| L27          | 4.8    | TCF7L2       | 4.6    | GBP5         | 5.3    | LTA          | 6.2    |             |        |
| CXCL9        | 4.8    | RASSF8       | 4.5    | CXCL11       | 5.3    | IL6          | 6.0    |             |        |
| IL1B         | 4.7    | EXT1         | 4.3    | KCNJ2        | 5.2    | STEAP1B      | 5.9    |             |        |
| CCL3         | 4.5    | NPAI         | 4.3    | IL27         | 5.1    | IL6          | 5.8    |             |        |
| AM2          | 4.5    | ABAT         | 4.3    | WNT4         | 5.1    | EPR41L5      | 5.6    |             |        |
| SNTB1        | 4.4    | GALNT12      | 4.3    | SERPNB2      | 5.0    | ZBT32        | 5.4    |             |        |
| IL6          | 4.3    | TCF45        | 4.3    | IL6          | 4.9    | CCL5         | 4.9    |             |        |
| P2RY6        | 4.2    | IL1R1        | 4.3    | TBKBP1       | 4.9    | RASSF8       | 4.9    |             |        |
| SLAMF8       | 4.2    | B3GNT7       | 4.2    | IL1          | 4.7    | TCF45        | 4.9    |             |        |
| TCF45        | 4.2    | AP08R        | 4.1    | STEAP1B      | 4.7    | B3GNT7       | 4.8    |             |        |
| IL1R1        | 4.1    | TCF45        | 4.0    | IL1          | 4.7    | TCF45        | 4.9    |             |        |
| GBP7         | 4.1    | M2ST1        | 4.0    | MEFV         | 4.6    | EXT1         | 4.7    |             |        |
| IL1B         | 4.0    | HERC5        | 3.9    | PLXCR1       | 4.5    | IL1          | 4.6    |             |        |
| PRSS5L       | 3.9    | KISF3        | 3.8    | CSF3         | 4.4    | BAALC        | 4.6    |             |        |
| CDH1         | 3.9    | TACSTD2      | 3.7    | CCL5         | 4.4    | NPAI         | 4.6    |             |        |
| GBP4         | 3.8    | CCL5         | 3.6    | GBP1         | 4.4    | ITGA1        | 4.5    |             |        |
| GBP1         | 3.8    | IL2RA        | 3.6    | TCF7L2       | 4.3    | ITGA3        | 4.5    |             |        |
| TBX21        | 3.8    | SGMS2        | 3.6    | CACNA1A      | 4.3    | LAMC1        | 4.4    |             |        |
| APOB        | 3.8    | NFMS3        | 3.5    | BAALC        | 4.2    | ITGA5        | 4.4    |             |        |
| GBP3         | 3.8    | PDGFβ        | 3.4    | RAB33A       | 4.2    | ITGB1        | 4.4    |             |        |
| CMK2         | 3.7    | IL12B        | 3.4    | AQP9         | 4.2    | IL2A         | 4.3    |             |        |
| RAB23A       | 3.7    | PLAUR        | 3.4    | DMAF1        | 4.2    | AMOS2        | 4.2    |             |        |
| POL21F1      | 3.6    | MBP          | 3.4    | P2RX7        | 4.2    | GALNT12      | 4.2    |             |        |
| TCF45        | 3.6    | GALNT3       | 3.3    | EPB41L5      | 4.1    | HERC5        | 4.2    |             |        |
| DLX6         | 3.6    | GPRAV1       | 3.3    | CEPH2        | 4.1    | TCF7L2       | 4.2    |             |        |
| CCL5         | 3.6    | NEDD4L       | 3.3    | CDX00E       | 4.1    | SHROOM3      | 4.2    |             |        |
| SMN1         | 3.6    | TCF7         | 3.2    | TLR4         | 4.1    | IL2RA        | 4.2    |             |        |
| G0S2         | 3.5    | G0S2         | 3.2    | GBP1         | 4.1    | NEDD4L       | 4.1    |             |        |
| DMI1         | 3.4    | ZMYND11      | 3.1    | ATP10A       | 4.1    | CLCF1        | 4.0    |             |        |
| PAWR         | 3.4    | MAP4K5       | 3.1    | INHBA        | 4.0    | CDH1         | 3.9    |             |        |
| CASP9       | 3.4    | CLCFL1       | 3.1    | PPARG        | 3.9    | KAI1222      | 3.9    |             |        |
| NFIXB        | 3.4    | CD40         | 3.1    | KCCN4        | 3.9    | IL1RN        | 3.8    |             |        |
| SCARF1       | 3.3    | PMP14        | 3.1    | EXT1         | 3.8    | ITG2         | 3.8    |             |        |
| CASP3       | 3.3    | ISG2R        | 3.1    | DVRK3        | 3.8    | TRAPAP2      | 3.8    |             |        |
| ZPYVE28      | 3.3    | HAUS6        | 3.1    | TBX21        | 3.8    | TCF45        | 3.8    |             |        |
| ETV7         | 3.3    | RFL          | 3.1    | NEU4         | 3.7    | IL12B        | 3.8    |             |        |
| H3F1H4       | 3.2    | FOSS1        | 3.1    | CA8L         | 3.7    | LAMC1        | 3.8    |             |        |
| EP2C         | 3.2    | MFHAS1       | 3.0    | ITGA1        | 3.7    | MACC1        | 3.7    |             |        |
| L3RA         | 3.2    | PLQUL2       | 3.0    | AMO2        | 3.6    | SNTB1        | 3.7    |             |        |
| GAP3TCH4     | 3.2    | FAM26A       | 3.0    | ZPYVE28      | 3.6    | GALNT3       | 3.7    |             |        |
| APO1         | 3.2    | PRKCI        | 3.0    | ISG3F3       | 3.6    | DDX60        | 3.7    |             |        |
| DYSK         | 3.1    | CDC42EP3     | 3.0    | GBP4         | 3.6    | G0S2         | 3.7    |             |        |
# Table 5. Overview of patient tumor samples

| sample_ID | Tumor Indication | Subtype         | Patient Treatment | Assays                  | % CD45+ cells | Panel for flow cytometry |
|-----------|------------------|-----------------|-------------------|-------------------------|---------------|--------------------------|
| colon_1    | Colon cancer     | liver metastasis| untreated         | scRNA-seq, flow cytometry | 30            | FACS Aria III, FACSymphony A5 |
| lung_1     | Lung cancer      | Adeno carcinoma | untreated         | scRNA-seq              | 75            | FACS Aria III             |
| lung_2     | Lung cancer      | NSCLC           | untreated         | flow cytometry, ELISA   | 44            | LSR Fortessa              |
| lung_3     | Lung cancer      | NSCLC           | untreated         | flow cytometry          | 72            | LSR Fortessa              |
| lung_4     | Lung cancer      | NSCLC           | untreated         | flow cytometry          | 74            | LSR Fortessa              |
| melanoma_1 | Melanoma         | lymph node metastasis | untreated | scRNA-seq              | 96            | FACS Aria III             |
| melanoma_2 | Melanoma         | lymph node metastasis | untreated | scRNA-seq, flow cytometry | 15           | FACS Aria III, FACSymphony A5 |
| melanoma_3 | Melanoma         | lymph node metastasis | untreated | flow cytometry          | 98            | FACSymphony A5            |
| melanoma_4 | Melanoma         | lymph node metastasis | untreated | flow cytometry          | 15            | LSR Fortessa              |
| melanoma_5 | Melanoma         | brain metastasis | LGX818+MEK1 62, Ipilimumab, Nivo | flow cytometry | 21            | LSR Fortessa              |
| melanoma_6 | Melanoma         | lymph node metastasis | untreated | flow cytometry          | 18            | LSR Fortessa              |
| ovarian_1  | Ovarian cancer   | serous carcinoma | untreated         | ELISA                   | 61            | -                        |
| ovarian_2  | Ovarian cancer   | serous carcinoma | untreated         | ELISA                   | 59            | -                        |
Materials and Methods

ScRNA-seq data quality control and pre-processing

Publicly available scRNA-seq datasets of different tissues (online supplemental table 2) were collected either as raw count matrices or fastq files. Internal and external fastq files were aligned and quantified using the Cell Ranger Single-Cell Software [1] with default parameters against the GRCh38 human reference genome. The data was further pre-processed by following the standard workflow in Besca [2]. For individual datasets, the quality of cells was assured by filtering all low-quality cells and removing uninformative genes by using the parameters in online supplemental table 3. The filtering was applied to cells based on the metrics, including minimum and maximum number of genes expressed, minimum and maximum total UMI count, and maximum proportion of mitochondrial gene count. Also, genes that were expressed in less than 10 cells were filtered out. After performing quality control, the raw count data were normalized, logarithmized and used for several downstream studies.

Dimension reduction and unsupervised clustering

For each dataset, the genes showing highest variability using besca.st.highly_variable_genes function were selected (minimal mean = 0.0125, maximal mean = 3 and minimal normalized dispersion = 0.5 cutoffs). Next, the effects of total count per cell and mitochondrial gene percentage effects were regressed out and the data were standardized. Subsequently, a principal component analysis with 50 components was performed and the first 50 components retained to build a nearest neighbor graph (local neighborhood size 15) and to derive clusters using the Leiden community detection
algorithm [3]. To integrate the cells from public datasets into a shared space, the raw datasets were concatenated and a second round of preprocessing, quality control was performed. The calculated PCA matrix was subjected to the Harmony algorithm [4] as an input, and individual studies were kept as a technical covariate for the correction. The batch-corrected PCA coordinates were then used to build the integrated nearest neighbor graph and to find clusters within. For the visualization of identified clusters, the Harmony corrected PCA matrix as an input was used for the UMAP. Further, Gaussian kernel density estimation function from scanpy was used to calculate the density of tumor-derived treated and non-treated immune cells, as well as tumor-derived cells from public datasets in the UMAP space [5].

**Cell type annotation**

To identify the cell type of the clusters returned by the Leiden algorithm, curated signatures and the sig-annot workflow available in Besca were used [2] based on the expression of signature markers. After identifying major cell types in the pooled dataset, myeloid dendritic cells were separated and the third round of pre-processing and cell-type annotation was performed to explore subpopulations with higher resolution. The third round is similar to the second round of pre-processing, where the raw and unfiltered gene expression concatenated matrix of only the identified myeloid dendritic cells was used and filtering (parameters in online supplemental table 3), HVG identification, PCA computation, and batch correction by Harmony algorithm was performed.

**Differential expression analysis**

Differential expression (DE) analysis between different cell groups was performed using a Wilcoxon Rank Sum test and multiple hypothesis testing correction using the Benjamini-
Hochberg procedure (function scanpy.tl.rank_genes_groups). To avoid the possible bias resulting from the comparison of imbalanced cell groups, when needed, each cell group was downsampled to the minimum number of all cell groups. The adjusted p-value threshold was kept at 0.05 and genes with less than this value were considered as significantly differentially expressed. Top DE genes were selected based on the highest log2 fold change (log2FC). Top 50 DE genes are listed in online supplemental table 4.

**Velocity analysis**

The Velocyto 0.17.17 package [6] was used to obtain spliced and unspliced read counts from the previously aligned scRNA-seq files from melanoma, lung and colon cancer samples and RNA velocity was calculated using the scvelo 0.2.3 package [7]. To keep the embedding consistent with the integrated dataset, the batch corrected PCA space was used to calculate the nearest neighbor graph. For each cell, the RNA velocity of genes was used to generate the RNA velocity vector embedding. For this, the first and second-order moments among nearest neighbor cells in reduced PCA space were computed. Further, the data and calculated moments were subjected to the RNA velocity estimation by modeling the full transcriptional dynamics of splicing kinetics (dynamical model).

**CD8+ T cell migration assay**

Observation windows were filled with 50 µL cold PBS. Collagen mixture (4 mg/mL rat collagen, R&D, #3440-100-01, in 0.1 M HEPES, Life Technologies, #15630-122, 3.7 g/L NaHCO₃, Lonza, #BE17-613E) was prepared on ice and 2 µL was pipetted into the middle channel of a cold 3-lane OrganoPlate (MIMETAS, #4004-400-B) to build the extracellular matrix barrier. After 30 min of polymerization at 37°C, 30 µL PBS were added to the inlet
of the collagen channel to prevent collagen drying. OrganoPlate was left overnight in the cell culture incubator. The next day, PBS was removed from the collagen inlets, $2 \times 10^4$ human umbilical vein endothelial cells (HUVECs, Lonza, #C2517AS) were seeded in 2 µL HUVEC medium (EGM-2, Lonza, #CC-3162/6) into the inlet of the top channel. 50 µL HUVEC medium was added to the inlet of the top channel. HUVECs were allowed to attach to the collagen interface for 2 hours in the cell culture incubator. 50 µL HUVEC medium was then added to the outlet of the top channel. After 6 days of HUVEC vessel formation at 37°C in a CO$_2$ incubator, HUVEC medium was removed from the inlets and outlets of the top channel. 2 $\times$ 10$^5$ activated CD8$^+$ T cells in 100 µL T cell medium (RPMI, Gibco, #42401-018, 10% FBS, 1% Pen/Strep, 1% Sodium Pyruvate, 1% Non-Essential Amino Acids Solution, Gibco, #11140-050, 1% Glutamax, Gibco, #35050-061, 50 µM 2-Mercaptoethanol, Gibco, #31350-010) were plated into the top channel. CD8$^+$ T cells were isolated from PBMCs using the Miltenyi CD8$^+$ T Cell Isolation Kit (#130-096-495) and activated for 4 days using CD3/CD28 activator (STEMCELL, #10971) according to the manufacturer's protocol. CD8$^+$ T cells were labeled with 1 mM CMFDA (Life Technologies, #C7025) for 15 min at 37°C right before plating into the OrganoPlate. Supernatants of stimulated cord blood cDCs, 1:1 diluted in T cell medium were added to the bottom channel. T cell migration was measured in the collagen layer after 48 hours at 37°C in a CO$_2$ incubator using the PerkinElmer Operetta High Content Imaging System. After 72 hours of migration, cells in the collagen layer were fixed using 0.4% formaldehyde (Sigma, #47608) in PBS for 15 min at room temperature. Cells were washed 2x with PBS and permeabilized using 0.3% Triton X-100 (Sigma, #T8787) for 10 min at room temperature. The plate was washed 1x with 4% FBS in PBS and blocked with 2% FBS, 2% BSA, 0.1% Tween20 (Sigma, #P2287) in PBS for 30 min at room temperature. Cells were stained
with anti-CD8 antibodies (BD, #555635) in 2% FBS, 2% BSA, 0.1% Tween20 in PBS for 4 hours at room temperature. The plate was washed 2x with 4% FBS in PBS, 1x with PBS. Stained cells were detected using the PerkinElmer Operetta High Content Imaging System. Quantification of the migrated T cells was done using ImageJ.
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Supp. Figure 1 TL8-506 is selective for human TLR8 and does not activate human TLR7. HEK-Blue cells that were engineered to express the human TLR7 or TLR8 and the NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene were treated with the indicated agonists and concentrations for 18 hours. Real-time detection of SEAP activity by performing the colorimetric enzyme assay in HEK-Blue Detection medium, from three independent experiments, mean+SEM is shown.
**Supp. Figure 2** Human cord blood, blood and tumor cDCs show comparable gene expression. 10x Genomics scRNA-seq was performed on FACS sorted blood cDCs (PBMC), n=2 donors, from one experiment, and in vitro differentiated cord blood cDCs (CBDC) harvested on day 12, n=3 batches of cord blood from mixed donors, 6 donors in total, from two independent experiments. scRNA-seq data of tissue cDCs (non-cancer and cancer) were integrated from different public studies listed in online supplemental table 2. (A) UMAP of 59'025 cDCs profiled across different scRNA-seq studies with each cell color-coded for study or cDC subset. (B) Spearman correlation values are shown comparing cord blood cDCs with blood and tissue cDCs. Correlation values were calculated based on the mean expression of genes per cell subset and donor. (C) Fraction positive and mean expression of different cDC subset-specific markers in cord blood, blood and tissue cDC1s or cDC2s. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range. (D) Fraction positive and mean expression of different IFNRs and PRRs in cord blood, blood and tissue cDC1s or cDC2s. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range.
Figure S3

A

cord blood DCs

- Fold change to medium
- Synergy score
- Combination (single+single)

B

cDC1

- Fold change Poly(I:C) + TL8-506 to medium

- Fold change IFNγ + TL8-506 to medium

- Synergy score > 2

- Fold change > 5


cDC2

- Fold change Poly(I:C) + TL8-506 to medium

- Fold change IFNγ + TL8-506 to medium

- Synergy score > 2

- Fold change > 5
**Supp. Figure 3** IFNγ + TL8-506 or Poly(I:C) + TL8-506 synergize to upregulate different genes in cord blood cDCs. Cord blood cDCs were sorted by FACS and stimulated with the indicated stimuli for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel, n=2 batches of cord blood from mixed donors, in total 4 donors, from two independent experiments, representative data are shown for 1 batch of cord blood from mixed donors (2 donors). (A) For each gene, synergy scores for combinatorial stimulation is plotted against fold change in expression in stimulated cDCs, calculation of synergy score for each gene is depicted on the right, labeled genes have a synergy score >2 and fold change to medium >5. (B) For each gene, fold change in expression in IFNγ + TL8-506 treated cDCs is plotted against fold change in expression in Poly(I:C) + TL8-506 treated cDCs, comparing the genes highly induced by IFNγ + TL8-506 vs Poly(I:C) + TL8-506 treatment. Labeled genes have a synergy score >2 and fold change to medium >5. The following concentrations were used for DC stimulation: 50'000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.
Supp. Figure 4 cDCs with an activated phenotype are rare across different human tumor indications. (A) Percentage of cDC1s, cDC2s and activated DCs (aDCs) of all cells (CD45+ and CD45− cells) from the processed tissue samples was calculated and depicted. Only studies in which the entire tissue was proved by scRNA-seq without prior cell type enrichment were included. (B) DC subset-specific markers were analyzed in the activated DC (aDC) population from all scRNA-seq studies. Fraction positive and mean expression of DC subset-specific markers in aDCs from tissue (non-cancer, cancer), blood (PBMC) and cord blood (CBDC) is shown. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range.
Supp. Figure 5 Tumor cDCs activated by TL8-506 combinations up-regulate genes involved in co-stimulation and antigen presentation. (A) Digested tumor samples from melanoma, CRC and lung cancer patients were treated with 1 μM TL8-506 + 50'000 U/mL IFNγ or 1 μM TL8-506 + 10 μg/mL Poly(I:C) for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted CD45^+ CD3^− cells, n=4 donors, from three independent experiments. Fraction positive and mean expression of co-stimulatory molecules and genes involved in antigen presentation in tumor-derived cDC1s and cDC2s upon treatment with TL8-506 combinations is shown. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range. - = medium, IT = IFNγ + TL8-506, PT = Poly(I:C) + TL8-506. (B) Cord blood cDCs were sorted by FACS and stimulated with 1 μM TL8-506 + 50'000 U/mL IFNγ or 1 μM TL8-506 + 10 μg/mL Poly(I:C) for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel. n=2 batches of cord blood from mixed donors, 4 donors in total, from two independent experiments, colors displaying the maximum (100%) to minimum (0%) mean gene expression per column.
Figure S6

A

Supplemental material

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B

0%
50%
100%

medium

IFNγ + TL8-506
Poly(I:C) + TL8-506
cDC1

medium

IFNγ + TL8-506
Poly(I:C) + TL8-506
cDC2
**Supp. Figure 6** TL8-506 combinations induce TLR and IFN signaling in treated tumor cDCs. (A) Digested tumor samples from melanoma, CRC and lung cancer patients were treated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted CD45⁺, CD3⁻ cells, n=4 donors, from three independent experiments. Mean gene expression of components of the TLR and IFN pathways in tumor cDC1s and cDC2s is displayed, IT = IFNγ + TL8-506, PT = Poly(I:C) + TL8-506. (B) Cord blood cDCs were sorted by FACS and stimulated with 1 µM TL8-506 + 50'000 U/mL IFNγ or 1 µM TL8-506 + 10 µg/mL Poly(I:C) for 15 hours. Gene expression was analyzed in cell lysates using the NanoString human Myeloid Innate Immunity panel, n=2 batches of cord blood from mixed donors, 4 donors in total, from two independent experiments, colors displaying the maximum (100%) to minimum (0%) mean gene expression per column.
Supp. Figure 7 Tumor-derived cDCs activated by TL8-506 combinations show increased expression of activation markers compared to in situ activated cDCs from different human tumor indications. Digested patient tumor samples were treated with 1 μM TL8-506 + 50 000 U/mL IFNγ or 1 μM TL8-506 + 10 μg/mL Poly(I:C) for 4 hours. scRNA-seq was performed on FACS sorted CD45+ CD3- cells, n=4 donors, from three independent experiments. scRNA-seq data of in situ activated cDCs were integrated from different public studies listed in online supplemental table 2. (A) Fraction positive and mean expression of activation markers in activated DCs (aDCs), stratified per treatment, study and tissue of origin. Only cDCs falling into the aDC cluster were analyzed. Mean expression was calculated across all the cells in the group and then scaled to a 0-1 range. (B) Fraction positive and mean expression of activation markers in activated DCs (extended population), stratified per treatment, study and tissue of origin. Cells color-coded in orange from the aDC cluster and intermediate cluster between aDCs and cDC2s were analyzed, adc_intcdc2 = aDC cluster + intermediate cDC2 cluster. Mean expression was calculated across all the cells in group and then scaled to a 0-1 range.
Supp. Figure 8 Poly(I:C) + TL8-506 activate cord blood cDCs to induce the release of IFNγ but not IL-4 or IL-10 in DC/T cell co-cultures. Sorted cord blood cDC2s were stimulated with the indicated stimuli for 18 hours. Treated cDCs were washed and co-cultured with allogeneic naive T cells for 4 days. Cytokine concentrations in the supernatant of co-cultures were determined by ELISA, n=2 batches of cord blood from mixed donors, 3 T cell donors, from two independent experiments, one-way ANOVA was used for statistical analysis, **p≤0.002. The following concentrations were used for DC stimulation: 10 µg/mL Poly(I:C), 1 µM TL8-506.
Figure S9

A

CXCL10

pg/ml

IFNγ + TL8-506 Poly(I:C) + TL8-506

CCL4

pg/ml

IFNγ + TL8-506 Poly(I:C) + TL8-506

TNFα

pg/ml

IFNγ + TL8-506 Poly(I:C) + TL8-506

B

T cell migration
CCL4 blockade

count migrated CD8+ T cells

ns

DC supernatant

DC supernatant + anti-CCL4 antibodies

C

T cell migration
chemokines

count migrated CD8+ T cells

0.5 nM CXCL10

5 nM CCL4

1 ng/ml TNFα

10 ng/ml TNFα

0.5 nM CXCL10 + 1 ng/ml TNFα

0.5 nM CXCL10 + 10 ng/ml TNFα

5 nM CCL4 + 10 ng/ml TNFα
Supp. Figure 9 CCL4 and TNFα induce CD8⁺ T cell migration in 3D in vitro system. (A) Sorted cord blood cDC2s were stimulated for 18 hours with the indicated stimuli. Cytokine concentrations were determined in the cell culture supernatants by ELISA. CXCL10, CCL4 and TNFα concentrations in the supernatant of cDCs treated with IFNγ + TL8-506 or Poly(I:C) + TL8-506 are shown, n=3 batches of cord blood from mixed donors, 6 donors in total, from three independent experiments as described in Figure 1D. (B) Sorted cord blood cDC2s were stimulated with the indicated compounds for 18 hours. The supernatant was collected and placed into the bottom channel of a 3D tissue culture device. Activated CD8⁺ T cells were labeled with CMFDA and added to the top channel which was coated with an artificial endothelial vessel. T cell migration was measured after 48 hours by imaging of the collagen layer that separated the two channels. Cell counts of migrated CD8⁺ T cells in the absence (grey) and presence of 5 μg/mL anti-CCL4 antibodies (green) in supernatants of stimulated cDCs are shown, n=2 donors, from two independent experiments, mean±SD, Student’s t-test, ns, not significant. (C) Experimental set-up as described in B, CXCL10 + TNFα or CCL4 + TNFα dilutions in concentrations present in supernatants of IFNγ + TL8-506 or Poly(I:C) + TL8-506 treated cDC2s were placed in the bottom channel. Cell counts of migrated CD8⁺ T cells towards chemokine dilutions are depicted, n=2 donors, from two independent experiments, mean±SD, one-way ANOVA, **p≤0.002. The following concentrations were used for DC stimulation: 50'000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.
Supp. Figure 10 Poly(I:C) + TL8-506 activate cord blood cDCs in the presence of tumor-conditioned medium to produce IL-12p70. (A) Patient tumor-derived digest was cultured for 6 hours and tumor-conditioned medium was harvested. Sorted cord blood cDCs were stimulated with 1 µM TL8-506 + 10 µg/mL Poly(I:C) in the absence or presence of tumor-conditioned medium (1:1 diluted) for 16 hours. IL-12p70 concentrations were measured in cell culture supernatant by ELISA, 3 patient tumor-derived digests, 2 batches of cord blood from mixed donors (4 donors in total), from two independent experiments, mean + SD of technical replicates is shown, unpaired Student’s t-test, ***p<0.0002, PT = Poly(I:C) + TL8-506. (B) Cell culture supernatant of the human lung cancer cell line COR-L105 was collected. Sorted cord blood cDC2s were activated in the absence or presence of conditioned medium from COR-L105 cells for 16 hours. IL-12p70 concentrations were measured in cell culture supernatant by ELISA, 2 batches of cord blood from mixed donors (4 donors in total), from two independent experiments.
**Supp. Figure 11** TL8-506 combinations up-regulate cytokines in human tumor-derived B cells, plasmacytoid DCs and macrophages in a cell type-specific manner. (A) UMAP of 35'169 cells profiled from IFNγ + TL8-506 or Poly(I:C) + TL8-506 treated and control treated tumor digests, with each cell color-coded for cell type, treatment and tissue. (B) Fraction positive and mean expression of activation markers in tumor-derived B cells, plasmacytoid DCs (pDCs) and macrophages upon treatment with TL8-506 combinations. Mean expression is calculated across all the cells in the group and then scaled to a 0-1 range. (C) Volcano plots showing differentially expressed (DE) genes (blue: up-regulated, red: down-regulated) between stimulated and control treated B cells, pDCs and macrophages from human tumor digests. Top 20 DE genes scored by p-values and fold changes are labeled, Wilcoxon Rank Sum test. The following concentrations were used for stimulation: 50'000 U/mL huIFNγ, 10 µg/mL Poly(I:C), 1 µM TL8-506.
Supp. Figure 12 Human cDCs are low in percentage in tumor tissue and blood. (A) Patient tumor-derived tissues were treated with the indicated compounds for 4 hours. 10x Genomics scRNA-seq was performed on FACS sorted CD45+CD3- cells with the exception of colon_1 that was sorted for CD45+ cells, n=4 donors, from three independent experiments. Percentages of DCs from all sequenced cells are plotted in a bar graph (left) or are listed in a table (right). - = medium, IT = IFNγ + IL-12 + TNFα, PT = Poly(I:C) + TNFα. (B) DCS were differentiated in vitro from cord blood stem cells, cells were harvested on day 12 and sorted for cDC1s, cDC2s or live cells (bulk) by FACS. 10x Genomics scRNA-seq was performed on sorted cells. n=3 batches of cord blood from mixed donors, 6 donors in total, from two independent experiments. Percentages of DCs from all sequenced cells are plotted in a bar graph (left) or are listed in a table (right). (C) PBMCs were isolated fromuffy coats of healthy donors (bulk). DCS were enriched from PBMCs 1x or 2x using the Miltenyi Pan-DC Enrichment Kit, n=2 donors, from one experiment. Percentages of DCs from all CD45+ cells were quantified by flow cytometry and are plotted in a bar graph (left) or are listed in a table (right), mean+SD of technical replicates is shown.
Graphical Abstract

The graphical abstract was created with BioRender.com.
Combinations of Toll-like receptor 8 agonist TL8-506 activate human tumor-derived dendritic cells

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In Brief
- Human tumor-derived conventional dendritic cells (cDCs) from cancer patients are activated by Toll-like receptor 8 agonist combinations
- Human tumor-derived cDC1s and cDC2s show an immunostimulatory phenotype associated with Th1 responses upon treatment
- Combination-specific induction of activation markers are consistent in human cord blood, blood and tumor-derived cDCs