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Supplementary Materials for

Restoring tumor immunogenicity with dendritic cell reprogramming

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Methods

Molecular cloning

To express OVA using a lentiviral-based expression system, the coding sequence for a truncated cytoplasmatic form of the protein (cOVA) was subcloned into the pHAGE (*67*) lentiviral vector modified to express the puromycin resistance gene after an IRES element. To induce reprogramming in mouse cancer cell lines, we modified the lentiviral vector SFFV-eGFP (*29*) to have an empty cassette (SFFV-MCS) or express the polycistronic cassette containing mouse PU.1, IRF8 and BATF3 transcription factors (*28*) with or without eGFP (SFFV-PIB-eGFP, SFFV-PIB) or mOrange (SFFV-mOrange). Alternatively, doxycycline inducible lentiviral system composed of FUW-TetO-PIB and pFUW-UbC-M2rtTA (*29*) was used to induce reprogramming. Sequences were verified by Sanger sequencing. Plasmids and primers used in this study are listed in Data File S8.

Bone marrow (BM) and spleen isolation

Total BM cells were harvested from long bones of the leg (tibias and femurs) by crushing. Cells were harvested in PBS supplemented with 2% FBS (fluorescence activated cell sorting (FACS) buffer) and filtered with a 40 μm cell strainer. Freshly isolated spleens were homogenized with syringe pestles against 40 μm cell strainers. Red blood cells were lysed with Pharm Lyse (BD Biosciences) for 8 min protected from light at room temperature.

Generation of BM derived CD103+ DC cultures

Total BM cells recovered after red blood cell lysis were plated in Petri dishes ($15x10^6$ cells per plate) in RPMI complete media supplemented with $5x10^{-6}$ mg mL^{-1} GM-CSF and $2x10^{-4}$ mg mL^{-1} Fltl3L (Peprotech) as previously described (*69*).

Flow cytometry and FACS

Analysis of surface marker expression was performed in dissociated cells. Briefly, cells were washed, incubated with 1% mouse or rat serum and a mixture of anti-human or anti-mouse fluorescently labeled antibodies for 20-30 min, and washed again with FACS buffer. To exclude dead cells, 7-Aminoactinomycin D (7AAD, Thermo Fisher Scientific) or 4′,6-diamidino-2-phenylindole (DAPI) were added shortly before analysis or sorting. Alternatively, fixable viability dye eFluor520 or LIVE/DEAD Near IR Fixable Stain was used to determine live cells. Viability assays were done using markers for apoptosis (annexin V) and necrosis (propidium iodide, PI). Flow cytometry analysis was performed with LSR Fortessa, LSR Fortessa X20, LSRII, CantoII flow cytometers (BD Biosciences) or NovoCyte Quanteon (Agilent). FACS-sorting was performed on a BD FACS Aria III sorter, using a 100 μm nozzle. FACS data were analyzed using FlowJo v.10.0.7 (FlowJo LLC) and NovoExpress software v.1.5.0. (Agilent). Gates were determined according to fluorescence minus one (FMO) controls. All antibodies and materials are listed in Table S1.

Magnetic-activated cell sorting (MACS)

Purification of reprogrammed cancer cells for functional assays was performed either at day 5 or day 9 post-transduction. Cells were dissociated and resuspended in staining FACS buffer supplemented with 2% penicillin-streptomycin. Briefly, 10^7 cells were incubated with 2 μL rat serum for 15 min, followed by 5 min incubation with 60 ng rat anti-mouse MHC-II coupled with biotin. Cells were washed twice before incubation with anti-biotin magnetic beads (Miltenyi Biotec) for 15 min. All incubations were performed

on ice. Labeled cells were purified in LS MACS columns (Miltenyi) according to manufacturer's recommendations. Purity of enriched populations was assessed by washing and consequently staining with fluorescent-conjugated antibodies for CD45, MHC-II and MHC-I.

Immunofluorescence

Reprogrammed B16 cells were MACS-enriched and fixed with 4% paraformaldehyde for 20 min and permeabilized with 0.4% Triton X-100. Blocking for 30 min with 2.5% bovine serum albumin and 10% goat serum (Abcam), was followed by overnight incubation at 4 ºC with anti-mouse MHC-II and anti-mouse B2M. Cells were then washed and incubated with secondary antibodies coupled with fluorochromes (Invitrogen) for 1 h 30 min. Cells were washed and imaged on CellDiscoverer 7 microscope (Zeiss), at 20x magnification. Data acquisition, image analysis and export were performed with the ZEN 2.5 blue software (Zeiss).

Scanning electron microscopy

T98G-derived CD45+HLA-DR+ and control eGFP+ cells were FACS-purified 9 days after transduction with PIB or eGFP vectors respectively, plated in 0.1% gelatin-coated coverslips, cultured overnight and prepared as described previously (*28*). Analysis was performed with Jeol JSM-7800F FEG-SEM.

Western Blotting

LLC untransduced or transduced with eGFP or PIB and CD103+ BM-DCs were sorted by FACS and lysed in radio- immunoprecipitation assay buffer (Thermo Fisher) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher), 5 mM sodium fluoride (Sigma-Aldrich) and 1 mM phenylmethylsulfonylfluoride (Sigma-Aldrich) for 20 min. After centrifugation (3500 g, 10 min) protein containing supernatants were diluted 1:2 in Laemmli buffer (Bio-Rad) with 5% 2-mercaptoethanol (Sigma) and boiled at 98 °C for 8 min. The samples were run on NuPAGE 4 to 12% bis-tris (Invitrogen) SDS-PAGE gels using the Mini Gel Tank (Invitrogen) and Bolt MES SDS running buffer (Invitrogen). Thereafter, transfer was performed on nitrocellulose membranes (Invitrogen) using the iBlot dry system (Thermo Fisher) for 8 min. Membranes were blocked with 2% ECL blocking agent (Cytiva) and incubated with unconjugated primary antibodies against PSMB10, PSMB9 or calnexin and donkey anti-rabbit horseradish peroxidase-conjugated secondary antibody (Cytiva) diluted $1{:}10^4$. For detection, membranes were incubated with ECL Western Blotting Detection Reagent (Cytiva) and the chemiluminescence signal was acquired by the ChemiDoc (Bio-Rad).

T cell killing assays

CD8+ T cells from spleen of OT-I mice or pmel-1 mice were enriched using a mouse CD8+ T cell isolation kit (Miltenyi Biotec) according to manufacturer's protocol. 6-well untreated plates were coated with anti-CD3 and anti-CD28 at $2x10^{-3}$ mg mL^{-1} for 2 h at 37 ºC and washed 3x before seeding $1x10^6$ T cells per mL in complete growth media (RPMI) supplemented with murine IL-2 (Peprotech, 100 U mL^{-1}) and IL-12p70 (Peprotech, $2.5x10^{-3}$ mg mL^{-1}). After 24 h of activation, T cells were re-seeded at $1x10^6$ cells per mL in complete RPMI supplemented with murine IL-2 for 48 h on new untreated plates to allow T cell expansion. MACS-sorted reprogrammed mOrange+ B16-OVA cells or IFN-γ treated cells were seeded with non-fluorescent B16-OVA (mOrange-) in equal

numbers, 24 h before co-culture with T cells. Expanded T cells were added in ratios of 0:1, 1:1, 5:1, 10:1 T cell to target cell. B16 cells not expressing OVA were used to assess assay specificity. For flow cytometry analysis, cells were resuspended and stained for viability (DAPI) and anti-CD3 and measured at indicated time points post co-culture with T cells**.** For evaluating cell killing towards natural endogenous antigens, mOrange+ B16 and non-fluorescent B16 were used in co-cultures with pmel-specific T cells at 1:1 and 10:1 T cell to target cell ratio.

RNA-seq analysis

Paired-end reads were mapped to the human or murine genomes (Ensembl, release 93) using STAR v2.5.3a (*70*) with default settings except sjdbOverhang 74 --quantMode GeneCounts. Resulting gene counts were further processed with R package DESeq2 (*71*) and normalized using RLE method. DESeq2 package and used for performing differential expression analysis based on Wald test. We defined upregulated genes by a fold change (FC) > 0 and Benjamini Hochberg (BH) corrected p-value < 0.05 and downregulated genes by FC < 0 and BH-corrected p-value < 0.05. Principal Component Analysis (PCA) was performed using plotPCA function from DESeq2 package. Normalized counts were $\log_2$ transformed and visualized using ggplot2 R package (https://ggplot2.tidyverse.org/) or GraphPad Prism 9 software (Data file S1 to S3, and S6). Previously published dataset (GSE103618) was re-analyzed together with newly generated data.

Gene set enrichment analysis

For the human dataset, functional enrichment analysis was performed for differentially expressed genes for each cancer cell line and tumor-APC signature using clusterProfiler (BH-adjusted P < 0.1) (*72*) and top 6 categories were selected. For the mouse dataset, gene list enrichment analyses with KEGG pathways, gene ontologies (GO) biological processes and molecular functions were obtained through EnrichR (*73*). Input gene set consisted of commonly upregulated genes in all murine reprogramming processes (fold-change >9, 27 genes). Results for each database were ordered based on $\log_{10}$(p-value) and the top 6 pathways, processes and functions plotted accordingly (Data file S1, S2).

Tumor-APC signature

The tumor-APC signature was defined as commonly upregulated genes between CD45+HLA-DR+ cells at day 9 and eGFP transduced cells (day 0), in at least 75% of human cancer lines. To order cell lines by reprogramming efficiency, we calculated for each gene the average difference between day 9 and day 0 and normalized it to the difference between cDC1 and day 0 for individual cancer cell lines. The median for each cancer cell line was used as reprogramming efficiency (Data File S1, S3, S4).

Antigen presentation signature

Gene lists for antigen presentation gene, IFN-γ and STING pathways were curated based on human or murine KEGG pathways list for antigen presentation, cytosolic-DNA sensing pathway, and literature review (Data file S1, S2). The expression value of those genes was z-transformed and subjected to MinMax function (values higher than defined Max value were set to Max value, and lower than Min value were set to Min value). Gene lists were clustered using complete linkage method and visualized using pheatmap R package (*https://cran.r-project.org/web/packages/pheatmap/index.html*). To estimate activation of antigen presentation signature, we used a similar procedure as described for tumor-APC signature gene list (Data file S1, S3, S4).

Cell proliferation signature

A cell proliferation gene list was curated based on the 25 most periodic genes expressed during cell cycle (cyclebase.org). For each gene we calculated the average difference between day 9 and day 0, normalized it to the expression levels in day 0, and used the median of cell proliferation gene list for each cancer cell line (Data file S1, S3).

IRF8 and BATF3 endogenous gene expression

To estimate endogenous expression, we calculated the number of reads in the 5' untranslated region (UTR) and 3' UTR of corresponding transcription factors using multicov from bedtools v2.27.0 (https://bedtools.readthedocs.io). For total expression, multicov software was also used considering the window (start of the gene, end of the gene) (Data file S1 to S3).

Assay for transposase accessible chromatin with sequencing (ATAC-seq) data analysis

In total, we obtained 1,384,592,926 ATAC-seq reads, with a median sample coverage of approximately 46.7 million reads. To remove Illumina universal adapters, we used NGmerge (https://github.com/jsh58/NGmerge) by setting adapter-removal mode. Reads were mapped to the GRCh38 reference genome using HISAT2 v2.0.4 (*74*) with the following parameters: --very-sensitive -k 20. Peak calling was performed with Genrich (v0.6.1, available at https://github.com/jsh58/Genrich, parameters: -m 30 -j -y -r -e chrM) separately for each sample. A combined peak list for all samples was obtained by using PEPATACr R library (*https://pepatac.databio.org*). Finally, read counts on a combined peak list were calculated with bedtools multicov (https://bedtools.readthedocs.io). The resulting read counts were processed with R package DESeq2 (*72*) and normalized using the RLE method. PCA was performed using plotPCA function from DESeq2 package. For peak annotation we used ChIPseeker R library (*75*). To map common chromatin changes, we used a modified procedure for ATAC-seq data as described for tumor-APC gene expression signature. Briefly, for each peak associated with individual genes from the tumor-APC signature we calculated an average difference between day 9 and day 0 and normalized it to the difference between cDC1 and day 0 for individual phenotype or time point of reprogramming. After that, we took the median of normalized peaks value separately for each phenotype or time point of reprogramming and plotted. For genome tracks, bigwig files were created from bam files with deeptools (*https://deeptools.readthedocs.io*). Genome tracks were explored using the UCSC Genome Browser. For motif discovery, findMotifsGenome.pl procedure from HOMER (*76*) was used on differential ATAC-seq peaks with default parameters. Functional enrichment analysis for differential ATAC-seq peaks was performed by Great software (*77*) using GO biological process ontology (Data file S4).

scRNA-seq data analysis

Paired-end sequencing reads of scRNA-seq were processed using publicly available 10x Genomics software Cell Ranger v6.1.2. Firstly, we used cellranger mkfastq to convert binary base call files to FASTQ files and to decode multiplexed samples simultaneously. Next, we applied cellranger count to FASTQ files and performed alignment to human (hg38) genome assemblies using STAR v2.7.6a. Then, we combined output files from each run to produce one single matrix using cellranger aggr. The sparse expression matrix generated by cellranger analysis pipeline was used as input to Scater library, and cells and genes passing quality control thresholds were included according to the following criteria:

1) total number of unique molecular identifiers detected per sample greater than 3 lower median absolute deviations (MADs); 2) number of genes detected in each single cell greater than 3 lower MADs; 3) percentage of counts in mitochondrial genes less than 7.5%. The resulting expression matrix was filtered by Scater analysis pipeline and used as input to the Seurat library v4 (*78*). To account for technical variation, we performed batch integration. Firstly, we normalized each batch separately using "LogNormalize" with the scale factor of 10,000 and identified 5,000 variable features. Next, we performed batch integration by finding corresponding anchors between the batch using 30 dimensions. We then computed 50 principal components and tested their significances by JackStraw. We selected the first 30 principal components for subsequent tSNE, UMAP and clustering analyses. Previously published dataset (GSE162650) was integrated with newly generated data as a separate batch.

Reprogramming trajectory analysis

To order cells on a reprogramming pseudotime, we used Monocle 3 library (*79*). It was run on t-SNE with default settings except for the following parameter: use_partition=FALSE that assumes all cells in the dataset descending from a common transcriptional ancestor. The root of the trajectory was selected automatically among day 0 cells. We identified genes varying over a trajectory using graph_test function and grouped them into 6 distinct modules using find_gene_modules function (Data file S6).

Single-cell DC classification

We used scPred (*80*) and publicly available DC single-cell expression data for DC subset affiliation (*34*). Training of classifiers with available DC data was performed using 7,000 variable genes. Next, to adjust for cancer line expression background, we normalized cells by gene expression levels in non-reprogrammed cells. We then classified normalized expression levels from the reprogramming dataset. We used a probability threshold of 0.99 to classify cells into classes (Data file S6). Previously available data (GSE94820) were re-normalized according to the parameters used in our newly generated data sets.

Aneuploidy prediction

We performed copy number variation (CNV) inference using CopyKAT (*43*) to separate malignant and non-malignant cells based on aneuploidy and diploidy. CopyKAT identified structural genomic heterogeneity within control and reprogrammed cancer samples using default parameters and a window size of 50. The cells that had a low confidence classification were assigned to unaffiliated.

Purification MHC-I-eluted peptides

Anti–MHC-I monoclonal antibody (28-8-6S) was purified from the supernatant of HB-51 hybridoma cells (ATCC) using Protein A–Sepharose 4B beads (Invitrogen). Antibodies were cross-linked to Protein A–Sepharose 4B beads at a concentration of 5 mg of antibodies per 1 mL volume of beads. For this purpose, the antibodies were incubated with the Protein A–Sepharose 4B beads for 1 hour at room temperature. Chemical cross-linking was performed by the addition of dimethyl pimelimidate dihydrochloride (Sigma-Aldrich) in 0.2 M sodium borate buffer pH 9 (Sigma-Aldrich) at a final concentration of 20 mM for 30 min. The reaction was quenched by incubation with 0.2 M ethanolamine pH 8 (Sigma-Aldrich) for 2h. Cross-linked antibodies were kept at 4°C until use. To improve sensitivity, two groups of samples were processed: 3 biological replicates of $2x10^8$/each B16 cells intended for data dependent acquisition (DDA) measurement to generate a spectral library generation, and 3 biological replicates of

samples of 10^6 sorted B16 cells that were either GFP transduced, reprogrammed, or IFN-γ treated, and control cells, for data independent acquisition (DIA) measurement. B16 cells were lysed in phosphate buffered saline containing 0.50% sodium deoxycholate (Sigma-Aldrich), 0.2 mM iodoacetamide (Sigma-Aldrich), 1 mM EDTA, 1:200 Protease Inhibitor Cocktail (Sigma-Aldrich), 1 mM phenylmethylsulfonylfluoride (Roche), and 1% octyl-β-D glucopyranoside (Sigma-Aldrich) at 4°C for 1 hour. Samples of $2x10^8$ cells were lysed in 4 mL, samples of $1x10^6$ cells in 1 mL lysis buffer. Lysates were cleared by centrifugation with a table-top centrifuge (Eppendorf Centrifuge) at 4°C at 20,000 g for 50 min. MHC-I molecules were purified by incubating the cleared lysates with HIB antibodies cross-linked to Protein A–Sepharose 4B beads in affinity columns for 3h at 4°C. 300 µL of beads were used for samples of $2x10^8$ and 200 µL for samples of 10^6 cells. The affinity columns were then washed as follows: 2 column volumes of 150 mM sodium chloride (NaCl) in 20 mM Tris-HCl pH 8, 2 column volumes of 400 mM NaCl in 20 mM Tris-HCl pH 8, and again 2 column volumes of 150 mM sodium chloride in 20 mM Tris-HCl pH 8. Finally, the beads were washed in 1 column volume of 20 mM Tris-HCl pH 8. MHC complexes and the bound peptides were eluted at room temperature by adding twice 500 µL of 1% trifluoroacetic acid (TFA). Sep-Pak tC18 96-well plates (Waters), preconditioned with 1 mL of 80% acetonitrile (ACN) in 0.1% TFA and then with 2 mL of 0.1% TFA, were used for the purification and concentration of MHC-I peptides. Elutions containing MHC-I molecules and peptides were loaded in the Sep-Pak tC18 96-well plates and the C18 wells were then washed with 2 mL of 0.1% TFA. The MHC-I peptides were eluted twice with 250 µL of 28% ACN in 0.1% TFA. MHC-I peptides containing elutions were transferred into Eppendorf tubes. Recovered peptides were dried using vacuum centrifugation (Thermo Fisher Scientific) and stored at −20°C.

Mass spectrometry (MS) acquisition of immunopeptidome data

Prior to MS analysis, MHC-I peptide samples were resuspended in 8µL of 2% ACN and 0.1% formic acid (FA), and 10% iRT suspension (Biognosys AG). Then, two technical replicates of 3 µL per biological samples were loaded on the column for each measurement by LC-MS/MS. The LC-MS system consists of an Easy-nLC 1200 (Thermo Fisher Scientific) coupled on-line to Q Exactive HF and or HF-X mass spectrometer (Thermo Fisher Scientific). Peptides were separated on a 450-mm homemade column of 75-µm inner diameter packed with ReproSil Pur C18–AQ 1.9-µm resin (Dr. Maisch GmbH). For DDA the analytical separation was performed for a period of 130 min using a gradient of H_2O/FA 99.9%/0.1% (solvent A) and CH_3CN/FA 80%/0.1% (solvent B). The gradient was run in sequential, linear steps: 0 to 110 min (2%-25% B), 110 to 114 min (25%-35% B), 114 to 115 min (35%-100% B), and 115 to 130 min (constant at 100% B) at a flow rate of 250 nL/minute. The mass spectrometer was operated as follows: full-scan MS spectra were acquired from m/z = 300–1,650 at a resolution of 60,000 (m/z = 200) with a maximum injection time of 80 ms. The auto gain control (AGC) target value was set to 3×10^6 ions. MS/MS spectra were acquired at a resolution of 30 000 (m/z = 200) using a top 20 method with an isolation window of 1.2 m/z, and a collision energy of 27 (HCD). For all MS/MS scans ions were accumulated to an AGC target value of 2*105 with a maximum injection time of 120 ms. Precursors with a charge of 4 or more were excluded from fragmentation. Dynamic exclusion was set to 20s. For DIA the analytical separation was performed for a period of 65 min using a gradient of H_2O/FA 99.9%/0.1% (solvent A) and CH_3CN/FA 80%/0.1% (solvent B). The gradient was run in sequential, linear steps: 0 to 52 min (2%-25% B), 52 to 54 min (25%-35% B), 54 to 55 min (35%-100% B), and 55 to 65 min (constant at 100% B) at a flow rate of 250 nL/minute. The DIA method consisted of one full-scan MS spectra, acquired from m/z =

300–1,650 (Resolution = 60 000, ion accumulation time = 60 ms), and 22 MS/MS scans (Data file S5). For each MS/MS scan the automatic gain control (AGC) target value was set to 3×10^6 ion, resolution was set at 30 000 and a stepped normalized collision energy (25.5, 27, 30) approach was used. Maximum ion accumulation was set to auto, fixed first mass to 200 m/z. The overlap between consecutive MS/MS scans was 1 m/z.

MS data processing

All DDA files were used to generate a library with a database search using fragpipe v17.1, including MSFragger v3.5. The FASTA file contained all reviewed mouse uniprot entries, as well as the manually added uniprot sequences P11269, P03386, Q3UFS3, and Q2TA50. The search space comprised unspecifically digested peptides with a length of 8 to 14 amino acids. Variable modifications included methionine oxidation and N-terminal acetylation and no fixed modifications were applied. Maximal precursor and fragment ion deviation was set to 20 ppm. Peptide, ion, and peptide spectrum match FDRs were set to 0.01, no protein FDR was applied. DIA files were searched with the library described above using SpectroNaut 16 with a precursor q-value cut-off of 0.01.

MS data analysis

Peptide binding affinities were predicted with NetMHCpan (*81*) and MixMHC (*82*). Peptides were considered as binders when they received a rank percentage of 2% or less by either of the two tools for at least one allele. The intensity of each peptide and the number of identifications per biological replicate were averaged for each replicate. MHC-I peptides predicted as binders were ranked by intensity (highest intensity = rank 1). The ranks were then normalized by dividing each rank by the total amount of binders per sample and subsequently transformed ($f(x) = x - 1$), yielding in an intensity score that ranged from 0 to 1, where 0 indicates the lowest intensity in a sample and 1 indicates the highest intensity in a sample. Data analyses were done with Julia and R, visualizations with R only (Data file S5).

DC populations from peripheral blood

Human cDC1 were obtained from peripheral blood of healthy donors provided by the Department of Transfusion Medicine, Skåne University Hospital. Erythrocytes were removed using BD Pharm Lyse. Total DCs were enriched via negative selection using human pan-DC Enrichment Kit (Miltenyi Biotec) and stained with anti-CD11c, anti-CD123, anti-HLA-DR, anti-CD141, anti-CD1c. Dead cells were excluded using 7-AAD. Peripheral blood cDC1 (HLA-DR+CD11c+CD141+), cDC2 (HLA-DR+CD11c+CD141-CD1c+), and pDC (HLA-DR+CD11c-CD123+) cells (*34*) were purified via FACS on a FACSAriaIII.

Live Cell Imaging

$5x10^3$ MACS-purified PIB-transduced LLC cells expressing either CD45 or MHC-II were seeded per well on a black 96-well µ-Plate (Ibidi) and incubated in the presence of $10x10^{-3}$ mg mL^{-1} AlexaFluor-647-labeled OVA (Thermo Fisher) or 0.1 mg mL^{-1} DQ-OVA (Thermo Fisher), followed by continuous recording for up to 4 h. $5x10^3$ B16 PIB-transduced cells expressing either CD45 or MHC-II were incubated for 12 h in the presence or absence of OVA peptides (SIINFEKL) and $2x10^4$ CellTrace Violet (CTV, Invitrogen)-labeled OT-I $CD8^+$ T cells were added to the media and co-cultures imaged during 72 h. All live cell imaging was performed using the CellDiscoverer 7 microscope

(Zeiss). Acquisition, image analysis and export were performed with ZEN 2.5 blue edition software (Zeiss) (Movies S1-S5).

Cytokine secretion

Day 9 reprogrammed cells (T98G, FACS-purified 10 000/well eGFP+CD45+HLA-DR+ or eGFP+CD45-HLA-DR+ or bulk primary melanoma cells, 20 000/well) were seeded into flat bottom 96-well plates and stimulated overnight with LPS (100 mg/ml), Poly(I:C) (25 µg/ml), and R848 (5 µg/ml) (Invivogen). Subsequently, 50 µl of culture supernatants were harvested and processed with LEGENDplex Human Inflammation Panel 1 (Biolegend) according to manufacturer's instructions. eGFP transduced cells were used as controls. Human DCs (separated by negative selection using pan-DC enrichment kit (Miltenyi Biotec) from healthy peripheral blood) and monocyte-derived DCs served as reference. Alternatively, mouse CD103+ BM-DCs, non-transduced, eGFP transduced, and reprogrammed CD45+MHC-II+ MACS-enriched B16 and LLC cells were seeded at a density of $6.5x10^4$ cells/well in 96-well plates and incubated overnight in the presence or absence of Poly(I:C). Supernatants were collected 10 h post-seeding and analyzed with LegendPlex Mouse Anti-Virus Response Panel (13-plex) according to manufacturer's protocol. Triplicates were performed per condition. Data were acquired with BD Fortessa flow cytometer and analyzed in LEGENDPlex software.

Antigen uptake and processing analysis

Alexa-Fluor 647-labeled chicken ovalbumin (Ova-AF647, Thermo Fisher Scientific) was used as a model antigen. Reprogrammed cells were resuspended at $1x10^6$ cells/ml (LLC, MACS-purified) or at $0.5x10^6$ cells/ml (T98G) of growth medium, followed by addition of $10x10^3$ mg mL^{-1} OVA-AF647 or 0.1 mg mL^{-1} of DQ-OVA and incubated at 37 ºC for 30-60 min. Fluorescence microscopy required up to 2 h of incubation for imaging OVA uptake and up to 4 h of incubation for visualization of OVA processing. Alternatively, the experiment was conducted on ice to inhibit active antigen uptake and processing. Changes in fluorescence referring to the internalization of labeled ovalbumin were analyzed by flow cytometry. Additionally, cells were pre-incubated with endocytosis receptor inhibitor mannan (Sigma-Aldrich, 3 mg mL^{-1}), protease and proteasome inhibitors leupeptin (Sigma-Aldrich, 0.1 mg mL^{-1}) or lactacystin (Sigma-Aldrich, 2.02 mM), 30 min before addition of OVA-AF647 or DQ-OVA and flow cytometry data acquisition.

Dead cell phagocytosis

B16 or LLC cells were harvested, washed, and exposed to UV light (45 min/3x10 min with 24h intervals. Dead cells were fluorescently labeled with CellVue Claret Far Red Fluorescent Cell Linker Kit (Sigma). Reprogrammed human eGFP+CD45+HLA-DR+ or mouse eGFP+CD45+MHC-II+ and control eGFP+ T98G/LLC cells were FACS-sorted on day 9 of reprogramming and seeded into a glass-bottom 96-well plate (Ibidi). Cell death and Far-Red staining was confirmed by flow cytometry and fluorescently labeled dead cells were added into individual wells containing adhered reprogrammed cancer cells. Images were acquired every 20 min over 16-hour timeframe using CD7 inverted microscope (Zeiss). Images were processed using Zeiss Blue and Adobe Illustrator software (Movies S3, S4). Alternatively, dead cell phagocytosis was evaluated by flow cytometry after 4-8h co-culture and quantified as an increase in CellVue Claret Far Red fluorescence.

***In vitro* stimulation of CMV-specific CD8+ T cells**

eGFP-transduced T98G glioblastoma cells (HLA-A2-positive) and T98G-derived tumor-APCs (day 9) were treated with LPS, Poly(I:C), and R848 overnight. Cells were then pulsed with 1 μg/peptide/ml CMV peptide mix (ProMix CMV Peptide Pool, ProImmune) for 3 h and washed thoroughly. 10,000 cells were incubated with 100,000 CD8+ T cells from a CMV-seropositive HLA-A2-positive donor in RPMI with 10% FBS, 1% Penicillin-Streptomycin. CMV-positivity was validated using flow cytometry detection of CMV-specific CD8+ T cells with CMV Dextramer (Immunodex) staining. 24 h later, supernatants were collected, and T cell activation was measured by quantification of IFN-γ levels with ELISA (BD OptEIA Human IFN-γ ELISA Kit, BD) using GloMax Discover Microplate Reader (Promega).

MHC-tetramer staining

MART-1 and CMV-specific T cells were assessed with PE- and allophycocyanin-linked specific tetramers (Immunitrack). CD8+ T cells were harvested on day 8 of *in vitro* stimulation (IVS) and transferred to a round-bottom 96-well plate, washed twice with FACS buffer, and incubated for 30 min at 37°C in the presence of the protein kinase inhibitor (PKI) dasatinib (100 nM). Afterwards, 1 μl of each tetramer was added to the corresponding wells and cells were incubated at 37°C. After 15 min, cells were washed twice with FACS buffer and stained with surface markers as described before. Then, cells were washed twice, resuspended in 100 μl FACS buffer and acquired on the flow cytometer.

TNF-α and IFN-γ intracellular staining

Activation of MART-1- and CMV- specific CD8+ T cells was assessed using the production of IFN-γ and TNF-α by intracellular staining. CD8+ T cells were harvested on day 8 of IVS and transferred to a round-bottom 96-well plate. 1 μM of the MART-1 or CMV peptides were added to the corresponding wells with a total volume of 150 μl D10 media per well. After 30 min of incubation at 37°C, the protein transport inhibitors brefeldin A (GolgiPlug) and Monensin (GolgiStop) were added to every well, and the cells were incubated at 37 °C for 4 h. Afterwards, cells were washed twice with FACS buffer and stained with LIVE/DEAD Near IR Fixable Stain and adequate antibodies for 20-30 min at 4 °C. Subsequently, cells were washed twice and fixated in 200 μl/well of Fixation Buffer for 1 h at room temperature. Then cells were washed twice in 150 μl/well of Permeabilization Buffer and stained for intracellular cytokines for 20-30 min at 4°C. Lastly, cells were washed twice with Permeabilization Buffer and resuspended in FACS buffer before acquisition on the flow cytometer.

Isolation of monocyte-derived DCs (moDCs)

Human monocytes were obtained from peripheral blood of healthy donors provided by the Department of Transfusion Medicine, Skåne University Hospital. Erythrocytes were removed using BD Pharm Lyse. Monocytes were enriched via positive selection using CD14 microbeads (Miltenyi Biotec) by MACS according to the manufacturer's protocol. CD14+ monocytes were cultured in RPMI supplemented with 10% FBS, 1% Pen-Strep, IL-4 (350 ng/mL), and GM-CSF (850 ng/ml). Medium was replaced every 2-3 days. At day 6, IL-6 (15ng/mL), PGE2 (10 μg/mL), TNFα (10 ng/mL), and IL-1β (5 ng/mL) were added to the culture medium for 24 h to generate mature moDCs.

Isolation and expansion of tumor infiltrating lymphocytes (TILs)

Young TIL cultures were generated from tumor fragments in RPMI 1640 supplemented with 10% human serum (HS) and 6000 U/mL IL-2. Briefly, on day 0, tumor fragments were placed separately in the wells of a 24-well/plate with 2 ml of media. Plates were

incubated at 37°C, with 5% CO_2. Half of the medium was replaced in all wells no later than 1 week after culture initiation, and this was subsequently repeated three times per week. Young TILs were propagated by splitting an individual confluent well into two additional wells and maintaining cells that originated from each initial fragment as an independent culture. Young TIL cultures were further expanded using a standard rapid expansion protocol (REP) for the generation of REP-TILs. Briefly, on day 0, irradiated (40 Gy) allogeneic feeder cells (2×10^7), OKT3 antibody (30 ng/mL), 10 ml KM media (RPMI-1640 + 10% HS + 6000 IU/mL IL-2), 10 ml RM media (AIM-V + 10% HS and 6000 U/mL IL-2) and TILs (1×10^5) were mixed and aliquoted to a T25 tissue culture flask. Flasks were incubated upright at 37 °C in 5% CO_2. On day 5, 10 mL of culture supernatant was removed by aspiration (cells are retained on the bottom of the flask) and media were replaced with a 1:1 mixture of KM/RM supplemented with 10% HS. On day 7 and every day thereafter, cell concentration was determined, and cells were split into further flasks with additional RM without HS as needed to maintain cell densities around $1–2\times10^6$ cells/mL. On day 14, cells were harvested and cryopreserved at −150°C in HS + 10% DMSO.

TIL activation and human cytotoxicity assay

The ability of REP-TILs to kill autologous melanoma cell lines before or after reprogramming was assessed by xCELLigence Real-Time Cell Analysis Assay. For that, day 8 reprogrammed primary melanoma cells were seeded (30 000 cells/well/100 µL D10 medium). After 24h, autologous 90 000 autologous REP-TILs were added to each well (3:1 ratio) and the co-culture was measured in 15 minute intervals for 72h. Non-transduced and eGFP transduced cells were used as controls. To determine cytolysis, the cell index of co-cultures has been normalized by cultures in the absence of REP-TILs. All conditions were measured in triplicates, and data shown is the average of all measurements. Furthermore, REP-TIL activation profile was evaluated with a long intracellular staining. $0.3x10^6$ REP-TILs and $0.1x10^6$ tumor cells (3:1 ratio) were co-cultured for 8h in the presence of Brefeldin A, Monensin and anti-CD107a antibodies. Afterwards, cells were washed, and stained with antibodies as previously described. Cells were then washed twice and fixed in 200 µL/well of Fixation Buffer overnight at 4°C. The next day cells were permeabilized and stained for intracellular markers as described above before analysis by flow cytometry. For each patient, a negative control with only REP-TILs and no stimuli, and a positive control with REP-TILs + PMA/Ionomycin were included. When mentioned, combination of anti-PD1 and anti-CTLA4 (10 µg mL^{-1}) were added to the cultures.

***In vitro* proliferation and soft agar colony formation assays**

To evaluate proliferation, transduced cancer cells were FACS-sorted at reprogramming day 9. Purified cell populations were stained with CellTrace Violet Cell Proliferation Kit (Thermo Fisher Scientific). Every second day, cells were harvested, washed, and stained with anti-CD45 and anti-HLA-DR antibodies. Proliferation was quantified as decreasing fluorescence up to 10 days after seeding. For soft agar assay, eGFP+CD45+HLA-DR+ cells or eGFP+ transduced cells were FACS-purified on day 9. Soft agar was prepared as described previously (*83*). Briefly, 20,000 cells were resuspended in 2x concentrated DMEM, mixed with 0.6% sterile Noble agarose (Sigma-Aldrich) and plated on top of 0.5% agarose-DMEM layer in a 6-well plate. Alternatively, cancer cells were reprogrammed with inducible PIB in the presence of doxycycline (DOX). After FACS purification, DOX was removed and tumor-APCs allowed to grow in soft agar as described before. After 4-6 weeks, colonies were counted using an inverted microscope.

Colonies were then stained with 0.1% Crystal Violet solution (Sigma-Aldrich) and imaged using Chemidoc (Bio-Rad). Images were processed with Adobe Illustrator Software. Experiments were performed in triplicates.

T cell isolation from peripheral blood and tetramer staining

14 days after B16-OVA tumor establishment and 7 days after the first intra-tumoral tumor-APC injection, peripheral blood was collected from the tail vein into heparin tubes to prevent clotting. Erythrocytes were lysed using BD Pharm Lyse according to the manufacturer's protocol and cells labeled with H-2Kb OVA (SIINFEKL) or H-2Kb MuLV p15E (KSPWFTTL) tetramers and anti-CD45, anti-CD8 or anti-TCR antibodies.

***IVS* of CD8+ T cells**

On day 18, tumor draining lymph nodes (TdLN) and non-draining lymph nodes (NdLN) were excised from animals and mechanically digested. TdLNs and NdLNs were passed through a 50µm filter to generate a cell suspension. To recall melanoma-associated antigen Pmel-reactive CD8+ T cells from TdLN and NdLN, cell suspensions were plated in U-bottom 96-well plates and re-stimulated with 10 µg/ml mouse Pmel peptide (EGSRNQDWL) and 10 µg/ml Poly(I:C) for 20h. For re-stimulation of CD8+ T cells isolated from peripheral blood at day 14, $1x10^5$ CD103+ BM-DCs were plated in U-bottom 96-well plates and stimulated with Pmel peptide or TRP2 peptide (SVYDFFVWL) and 10 µg/ml Poly(I:C) 24 h before addition of T cells. Co-culture was performed for 20 h. Thereafter, 5 µg/ml Brefeldin A (Biolegend) was added and kept for an additional 4 h. Re-stimulated T cells were stained according to the Fixation/Permeabilization Kit (BD) protocol extracellularly for CD8, TCR and CD44 and intracellularly for IFN-γ.

Immunophenotyping of tumors, TdLN and NdLN

On day 18, tumors were excised and chopped into pieces of 2 mm diameter. Tumor tissue was further mechanically and enzymatically processed for 1 h at 37°C using a digestion mixture of 1 mg/ml Collagenase D (Sigma-Aldrich), 10 µg/ml DNase I (Sigma-Aldrich) and 5 mM $MgCl_2$ in PBS under rotation. The resulting cell suspension was passed through a 70µm filter and divided equally for flow cytometry analysis using separate antibody staining panels. The lymphoid panel included antibodies for CD45, TCRβ, CD8α, CD4, CD44, PD-1 and NK1.1. The myeloid panel included antibodies for CD45, CD11c, MHC-II, CD64, Ly6C, CD103, CD11b, Ly6G. Populations were defined according to: CD8+ T cells (CD45+TCRb+CD8+), CD4+ T cells (CD45+TCRb+CD4+), NK cells (CD45+NK1.1+), neutrophils (CD45+MHC-II-CD11b+Ly6G+), monocytes (CD45+MHC-II-CD11b+Ly6c+), macrophages (CD45+MHC-II+CD11c+Ly6c-CD64+) and dendritic cells (CD45+MHC-II+CD11c+CD64-Ly6c-CD11b+).

Supplementary Figures

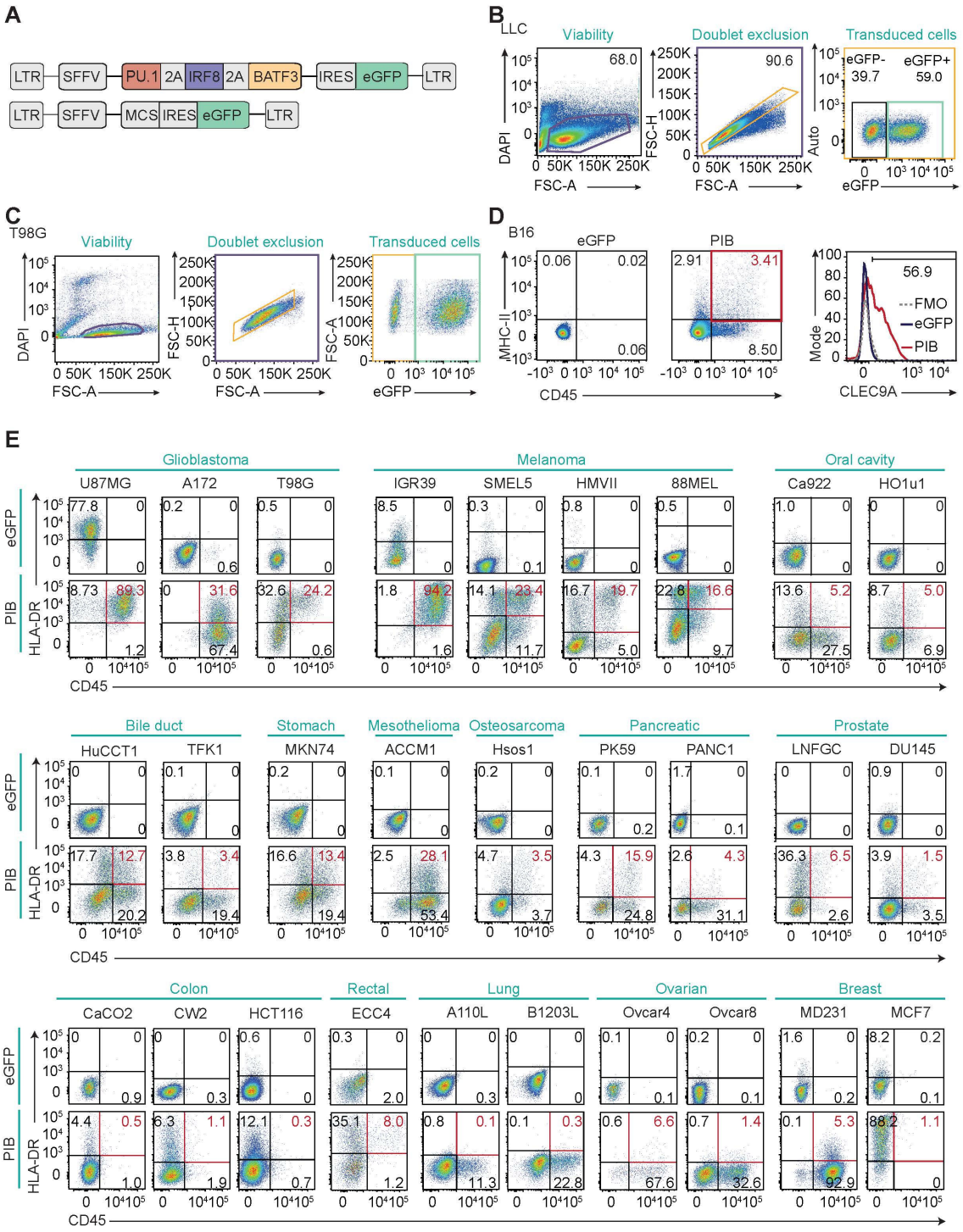

fig. S1. PU.1, IRF8 and BATF3 induce cDC1 reprogramming in human and mouse cancer cell lines derived from solid tumors. (A) Schematic representation of lentiviral vectors used in cDC1 reprogramming experiments. The polycistronic vector (upper panel) encoding the three mouse or human reprogramming factors (PU.1, IRF8, BATF3 in the order depicted separated by self-cleaving 2A peptides), followed by an IRES-eGFP

was used to elicit reprogramming and identify transduced cells. An empty vector containing a multiple cloning site (MCS) followed by an IRES-eGFP (lower panel) was used as control. The splenic focus-forming virus (SFFV) promoter was used to drive expression in both vectors. **(B)** Flow cytometry gating strategy applied in reprogramming experiments of murine and **(C)** human cancer cells. Reprogramming efficiency was evaluated in live, single, eGFP+ cells. Percentages of cells in each gate are indicated. **(D)** Reprogramming efficiency of murine melanoma cancer cells (B16) and representative flow cytometry plot for CLEC9A expression gated in CD45+MHC-II+ cells (PIB, red). **(E)** Solid tumor cell lines were transduced with PIB-IRES-eGFP or eGFP and analyzed by flow cytometry after 9 days. Reprogramming efficiency was determined as a percentage of cells co-expressing CD45 and HLA-DR gated in transduced eGFP+ cells. Representative flow cytometry plots indicating reprogramming efficiencies (red) and the percentages of intermediate populations (CD45+HLA-DR- and CD45-HLA-DR+ cells, black) induced by PIB in 28 human cancer cell lines derived from solid tumors.

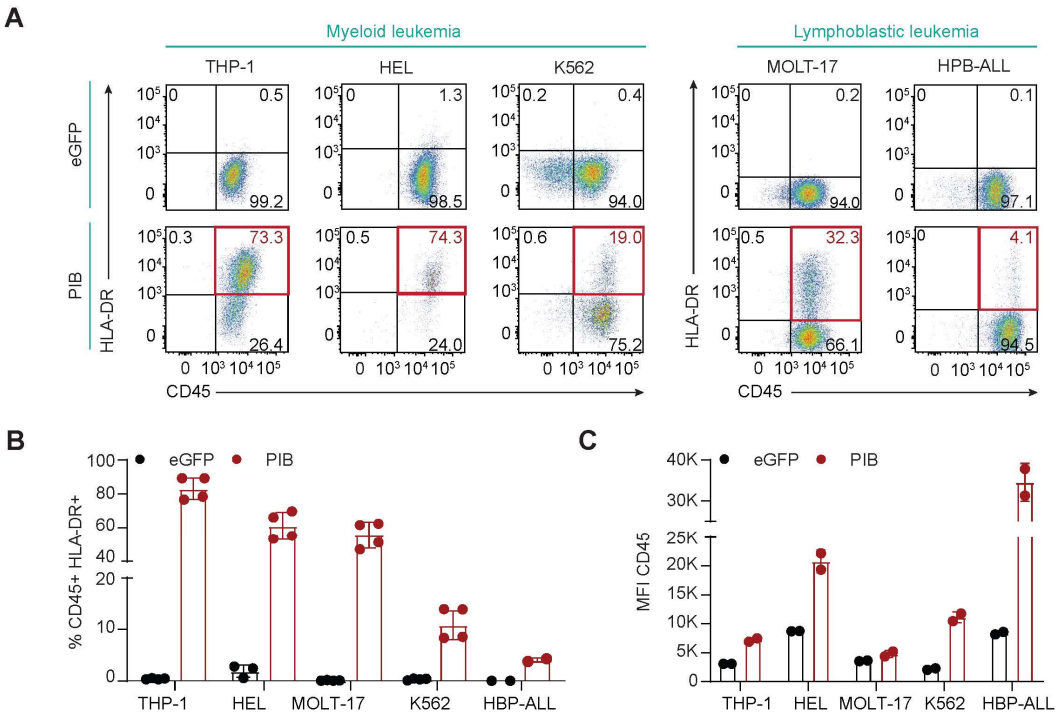

fig. S2. Reprogramming of human leukemic cell lines to cDC1-like cells. (**A**) Myeloid leukemia (THP-1, HEL, and K562), and lymphoblastic leukemia (MOLT-17 and HPB-ALL) cell lines were transduced with PIB or eGFP empty vector as control and analyzed by flow cytometry after 9 days. Reprogramming efficiency was determined as the percentage of cells co-expressing CD45 and HLA-DR gated in transduced eGFP+ cells (red). (**B**) cDC1 reprogramming efficiency in 5 leukemia-derived cell lines at reprogramming day 9. (n=2-4) (**C**) Quantification of median fluorescence intensity (MFI) of CD45 in leukemic cell lines in PIB-transduced or eGFP transduced cells (n=2). Mean±SD is represented. n= biological replicates.

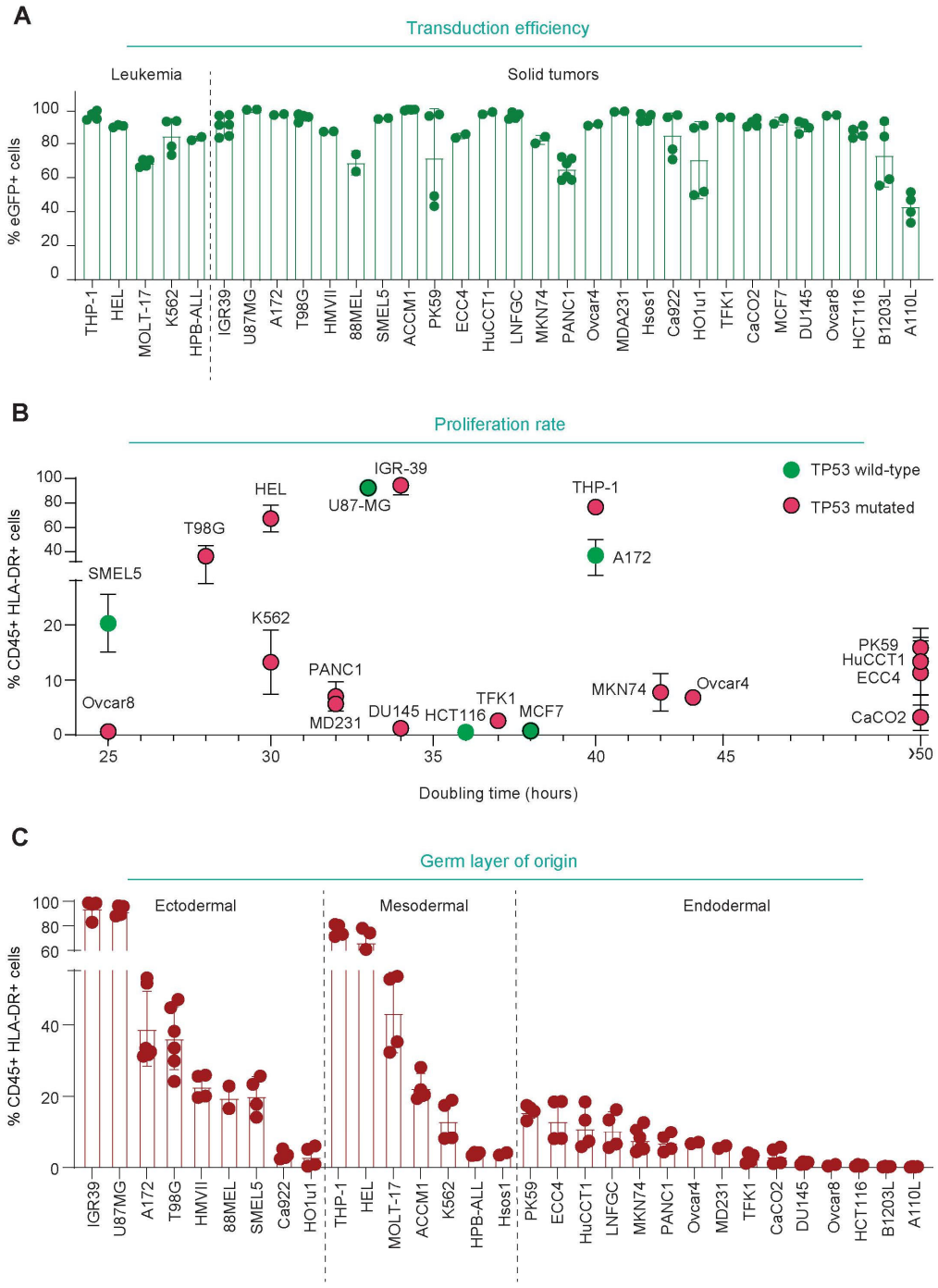

fig. S3. cDC1 reprogramming across germ layers and mutational profiles. (**A**) Leukemic and solid tumor cell lines were transduced with eGFP empty vector and analyzed by flow cytometry. Transduction efficiency was determined as the percentage of eGFP+ cells at day 9 by flow cytometry. Cell lines are ordered according to reprogramming efficiency (% CD45+HLA-DR+ cells) when transduced with PIB.(n=2-4) (**B**) Reprogramming efficiency plotted against doubling time of the parental cell line. Color code refers to the reported *TP53* status of each cell line. (n=2-6). (**C**) Reprogramming efficiency according to the cell line germ layer of origin (n=2-6). Mean±SD is represented. n= biological replicates.

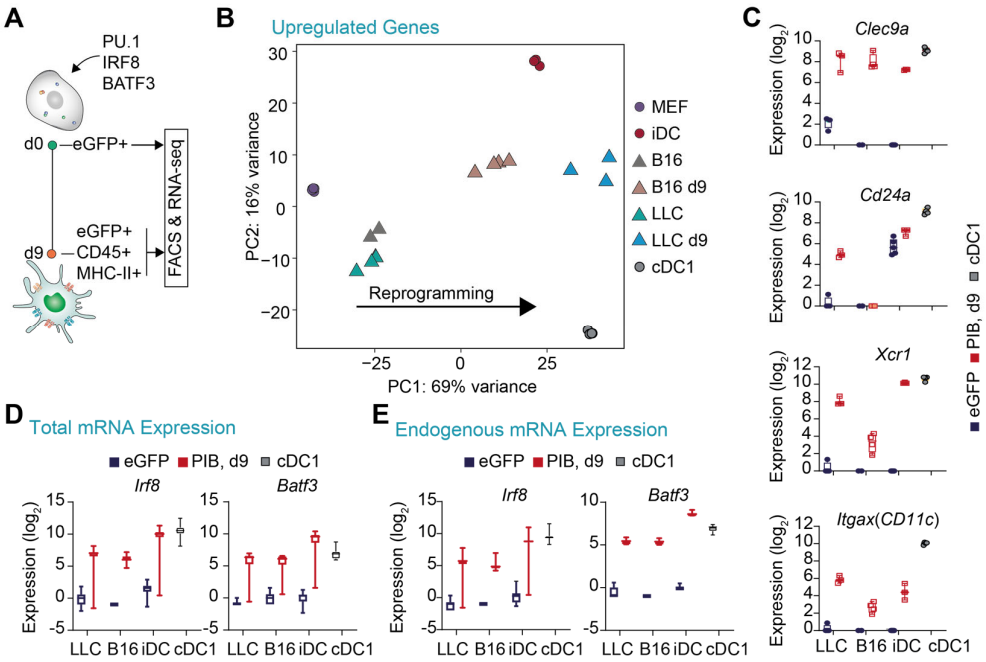

fig. S4. Induction of cDC1 gene signature in murine cancer cells. (A) Lewis Lung Carcinoma (LLC) and melanoma B16 cancer cells were transduced with PU.1, IRF8 and BATF3 (PIB) and purified by FACS at day 9 (d9) based on the co-expression of eGFP, CD45 and MHC-II and profiled by RNA-sequencing (RNA-seq). Cancer cells transduced with an eGFP vector were included as day 0 (d0) controls.(n=2-4; biological replicates) **(B)** Principal component (PC) analysis for transduced murine cancer cells, along with mouse embryonic fibroblasts (MEF), induced dendritic cells (iDC) and splenic cDC1 (GSE103618). Arrow highlights reprogramming trajectory. **(C)** mRNA expression levels of the cDC1 markers *Clec9a*, *Xcr1*, *Cd24* and *Itgax.* **(D)** Whisker box plots showing total (endogenous + exogenous) gene expression and **(E)** endogenous expression of *Irf8* and *Batf3*.

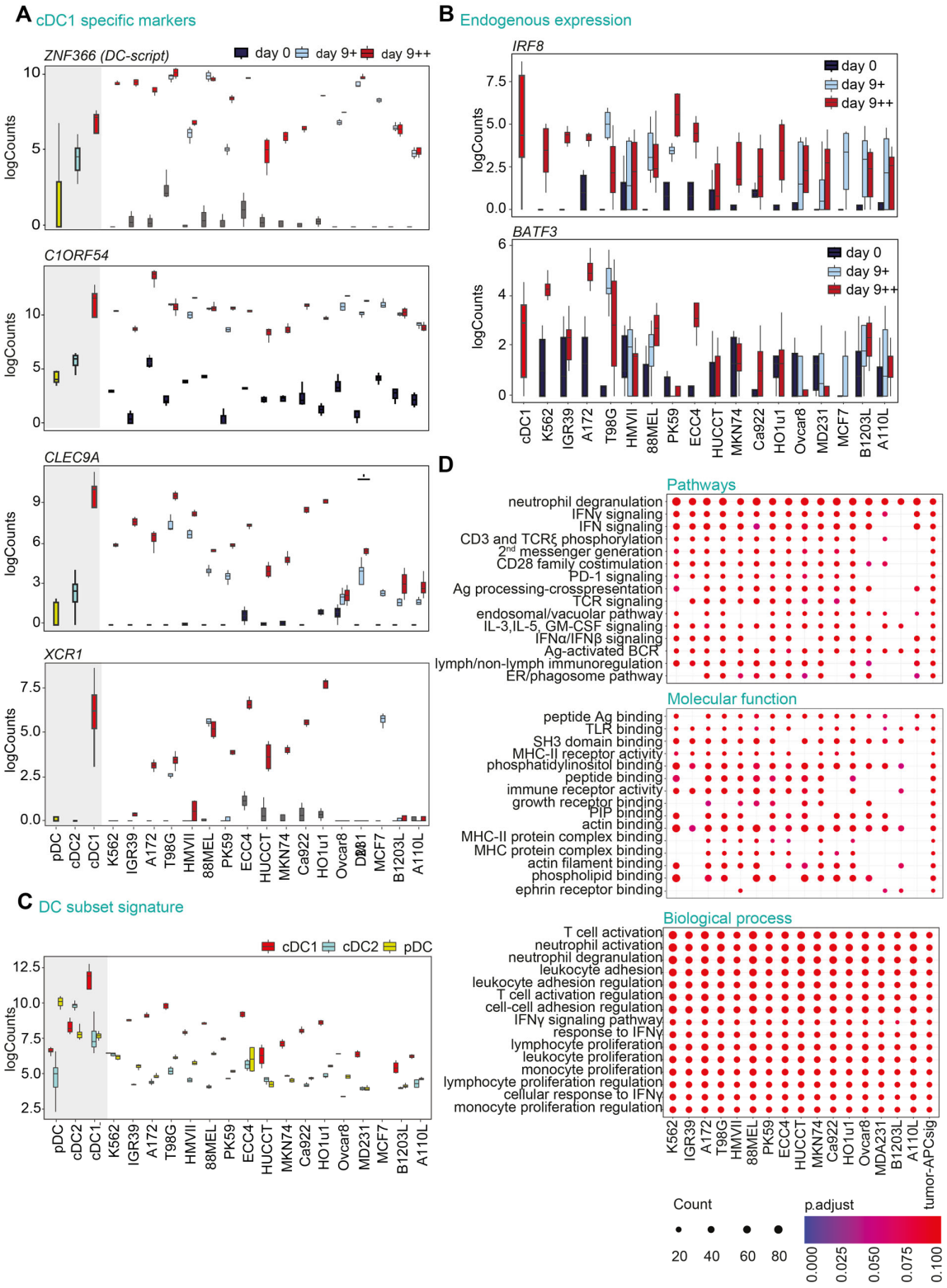

fig. S5. Induction of cDC1 gene signature in human cancer cells. (**A**) 17 cell lines were transduced with PIB and reprogrammed (CD45+HLA-DR+, day 9++) or partially reprogrammed cells (expressing either CD45 or HLA-DR, day 9+) were FACS-purified

after 9 days for RNA-seq profiling. Cancer cells transduced with an eGFP-empty vector were profiled as controls (day 0). Sorted cDC1 (HLA-DR+CD11c+CD141+), cDC2 (HLA-DR+CD11c+CD1c+CD141-) and pDC (HLA-DR+CD11c-CD123+) populations from donor peripheral blood (grey) served as reference. Cell lines are ordered according to the reprogramming efficiency (%CD45+HLA-DR+ cells). The panels show mRNA expression for cDC1 specific genes *ZNF366*, *C1ORF54*, *CLEC9A*, and *XCR1* (n=3-8). (**B**) Endogenous mRNA expression of *IRF8* and *BATF3*. (**C**) DC-subset gene signatures (cDC1, cDC2 and pDC) evaluated in reprogrammed CD45+HLA-DR+ cells. (**D**) Top 15 Reactome pathways, GO molecular function and biological process for genes differentially expressed between CD45+HLA-DR+ cells and eGFP transduced cells. Tumor-APC signature (tumor-APCsig) of commonly upregulated genes is shown (right). Circle size refers to the number of genes within each group intersected with enrichment analysis categories. Color gradient depicts adjusted p-values based on hypergeometric test. Ag, antigen; PIP, phosphatidylinositol phosphate.

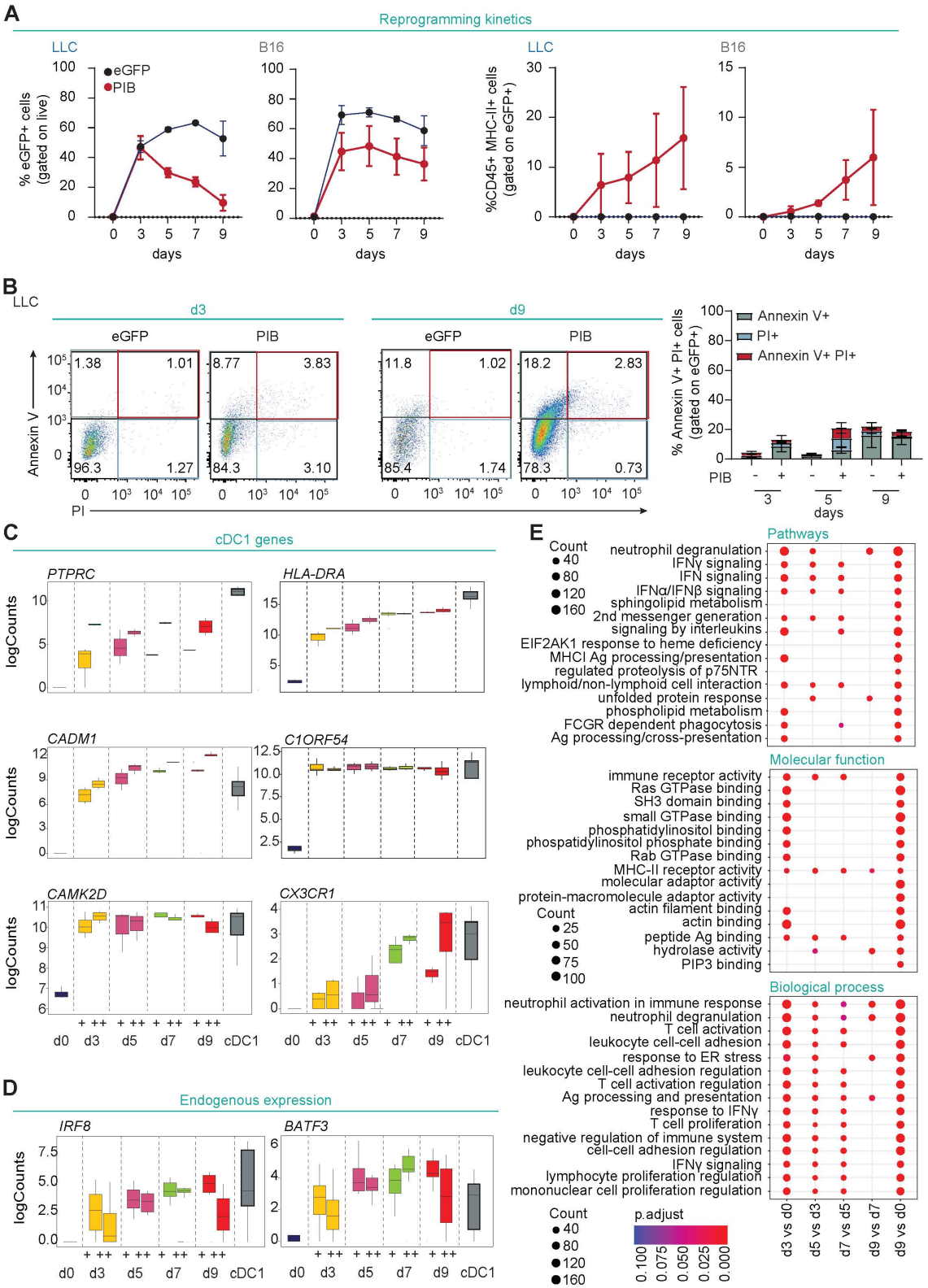

fig. S6. cDC1 reprogramming dynamics in mouse and human cancer cells. (A) Flow cytometry analysis of LLC and B16 murine cancer cell lines at reprogramming day 3, 5, 7 and 9. The percentage of transduced cells is shown over time when cells were transduced with PIB or eGFP control vector (left; LLC n=4-10; B16 n=2-15). Reprogramming kinetic was evaluated by assessing the percentage of CD45+MHC-II+

cells gated in eGFP+ cells (right, LLC n=2-16; B16 n=3-20). **(B)** Flow cytometry analysis of CD45+MHC-II+ and eGFP+ LLC-derived cells assessing apoptosis (Annexin V) and necrosis (propidium iodide, PI) at day 3, 5 and 9. Representative flow cytometry plots and quantification of Annexin V+, PI+ and double positive populations are shown (n=2-5; biological replicates). Mean±SD is represented. **(C)** T98G cells were transduced with PIB and reprogrammed (CD45+HLA-DR+, ++) or partially reprogrammed (CD45-HLA-DR+, +) cells were FACS-sorted and profiled by RNA-sequencing at day 3, 5, 7 and 9. Plots show mRNA expression of the pan-hematopoietic marker *PTPRC* (CD45), antigen presentation marker *HLA-DRA* and cDC1-specific genes *(C1ORF54*, *CADM1*, *CAMK2D*, *CX3CR1*). eGFP transduced T98G cells served as day 0 control. cDC1 cells from peripheral blood were used as reference. (**D**) Endogenous mRNA expression of *IRF8* and *BATF3* is visualized using box plots (n=2-8). (**E**) Top 15 Reactome Pathways, GO molecular function and biological process identified by stepwise enrichment analysis of upregulated genes. Circle size refers to the number of genes intersecting with the respective database category. Color gradient depicts adjusted p-values based on hypergeometric test.

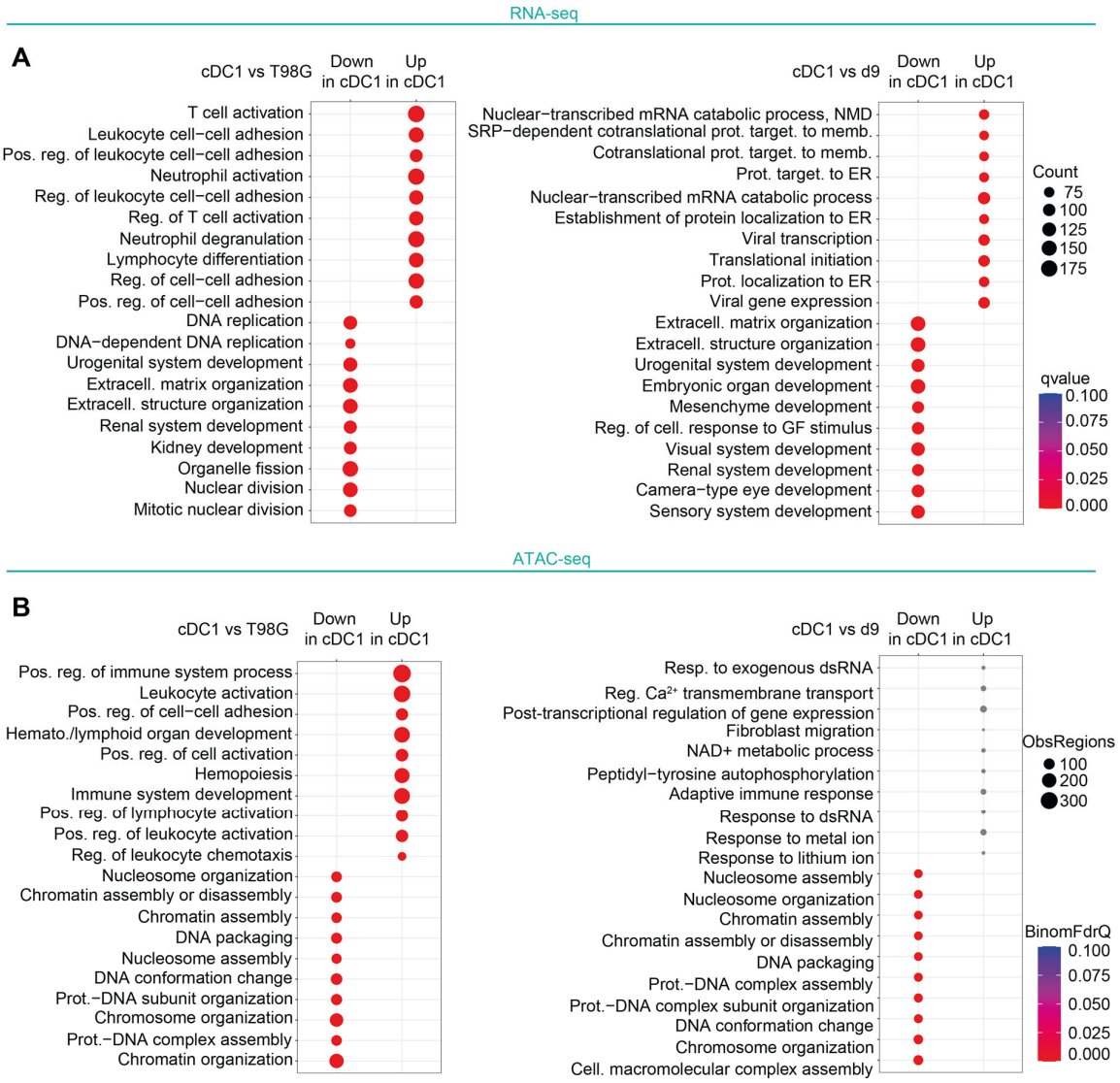

fig. S7. Comparison of tumor-APCs and cDC1 highlights transcriptional and epigenetic remodeling. (A) T98G cells were transduced with PIB and reprogrammed CD45+HLA-DR+ cells were FACS-sorted and profiled by bulk RNA-sequencing (RNA-seq) or ATAC-sequencing (ATAC-seq) at day 9 (d9) and compared to eGFP transduced cells (T98G) and peripheral blood cDC1. Gene Ontology (GO) biological process enrichment analysis of differentially expressed genes or (**B**) differential ATAC-seq peaks between cDC1 and T98G cells (left) or tumor-APCs at day 9 (right). Pos. – positive; reg. – regulation; extracell. – extracellular; cell. – cellular; NMD – non-sense-mediated mRNA decay; SRP – signal recognition particle; prot. – protein; target. – targeting; memb. – membrane; ER – endoplasmic reticulum; GF – growth factor; hemato. – hematopoiesis; resp. – response; NAD+ - nicotinamide adenine dinucleotide. Circle size refers to the number of genes (A) or peaks (B) intersecting with the respective database category. Color gradient depicts adjusted p-values based on hypergeometric test (A) or binomial test.

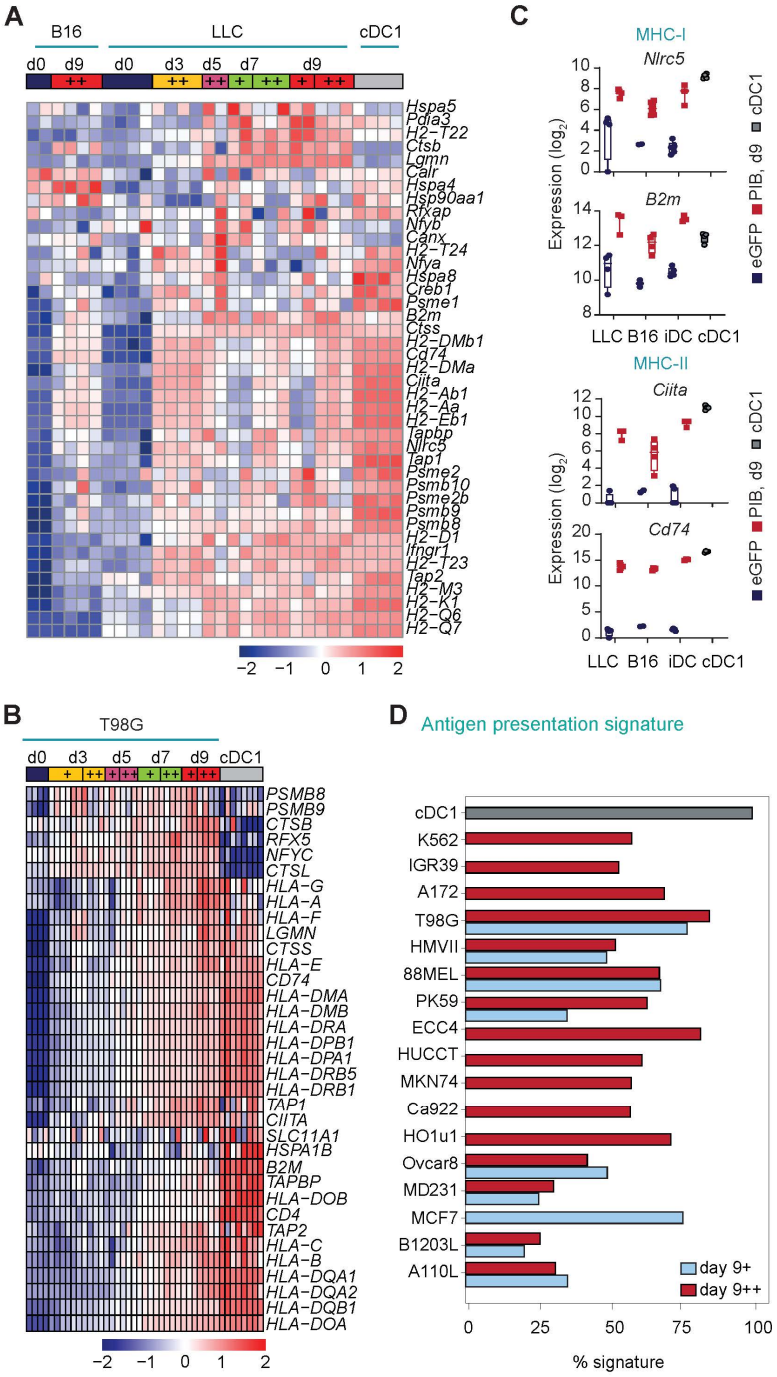

fig. S8. Induction of an antigen presentation gene signature in mouse and human cancer cells. (A) Murine B16 and LLC cell lines were transduced with PIB and at reprogramming day 3 (d3), 5 (d5), 7 (d7), and 9 (d9), partially (CD45+MHC-II-, +) and fully reprogrammed cells (CD45+MHC-II+, ++) were FACS-sorted and profiled by bulk RNA-sequencing. Heatmap shows expression of antigen processing and presentation genes during reprogramming time course (day 0, 3, 5, 7 and 9) and comparison with splenic cDC1. **(B)** T98G-derived partially (CD45-HLA-DR+, +) and fully reprogrammed cells (CD45+HLA-DR+, ++) were FACS-sorted and profiled by RNA-sequencing. Heatmap shows gene expression changes in antigen processing and presentation genes during reprogramming. Peripheral blood cDC1 were used as a reference and eGFP-

transduced cells were included as day 0 controls. **(C)** Whisker box plots show gene expression of the MHC-I and MHC-II regulators *Nlrc5* and *Ciita*, and surface molecules *B2m* and *Cd74*. Minimum, maximum, mean, and individual values are represented (n=2-5). (**D**) Acquisition of an antigen processing and presentation gene signature imposed by cDC1 reprogramming in 17 cancer lines is shown at reprogramming day 9. Cell lines are ordered according to reprogramming efficiency (% CD45+HLA-DR+ cells) (n=3-8). n= biological replicates.

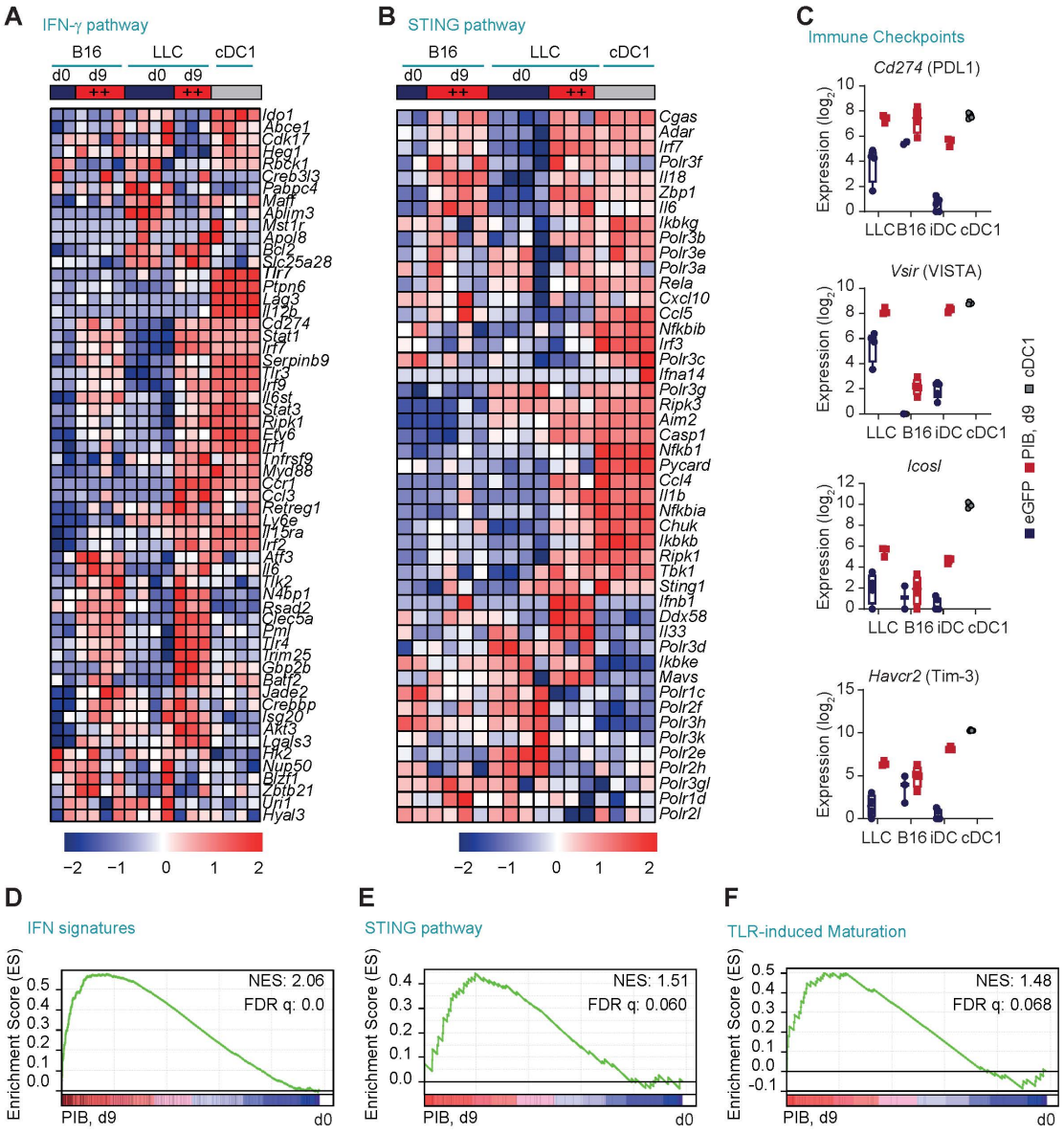

fig. S9. cDC1 reprogramming induces an inflammatory profile in mouse and human cancer cells. (A) Murine LLC and B16-derived reprogrammed cells (eGFP+CD45+MHC-II+, ++) were transduced with PIB and purified by FACS on day 9 (d9). Cancer cells transduced with eGFP were included as controls (d0). Heatmaps show expression genes related to IFN-γ and (**B**) STING pathways. Splenic dendritic cells type 1 (cDC1) were included as reference (GSE103618). (**C**) Whisker box plots showing gene expression levels of the immune checkpoint molecules *Cd274*, *Vsir*, *Icosl* and *Hvacr2*. Minimum, maximum, mean, and individual data points are represented (n=3-5; biological replicates). **(D)** Gene set enrichment analysis for IFN-related genes (**E**), STING pathway related genes and (**F**) TLR-induced maturation signature in tumor-APCs derived from all human cancer cell lines (PIB, d9) compared to parental lines (d0). Normalized enrichment score (NES) and false discovery rate q-values (FDR q) are shown.

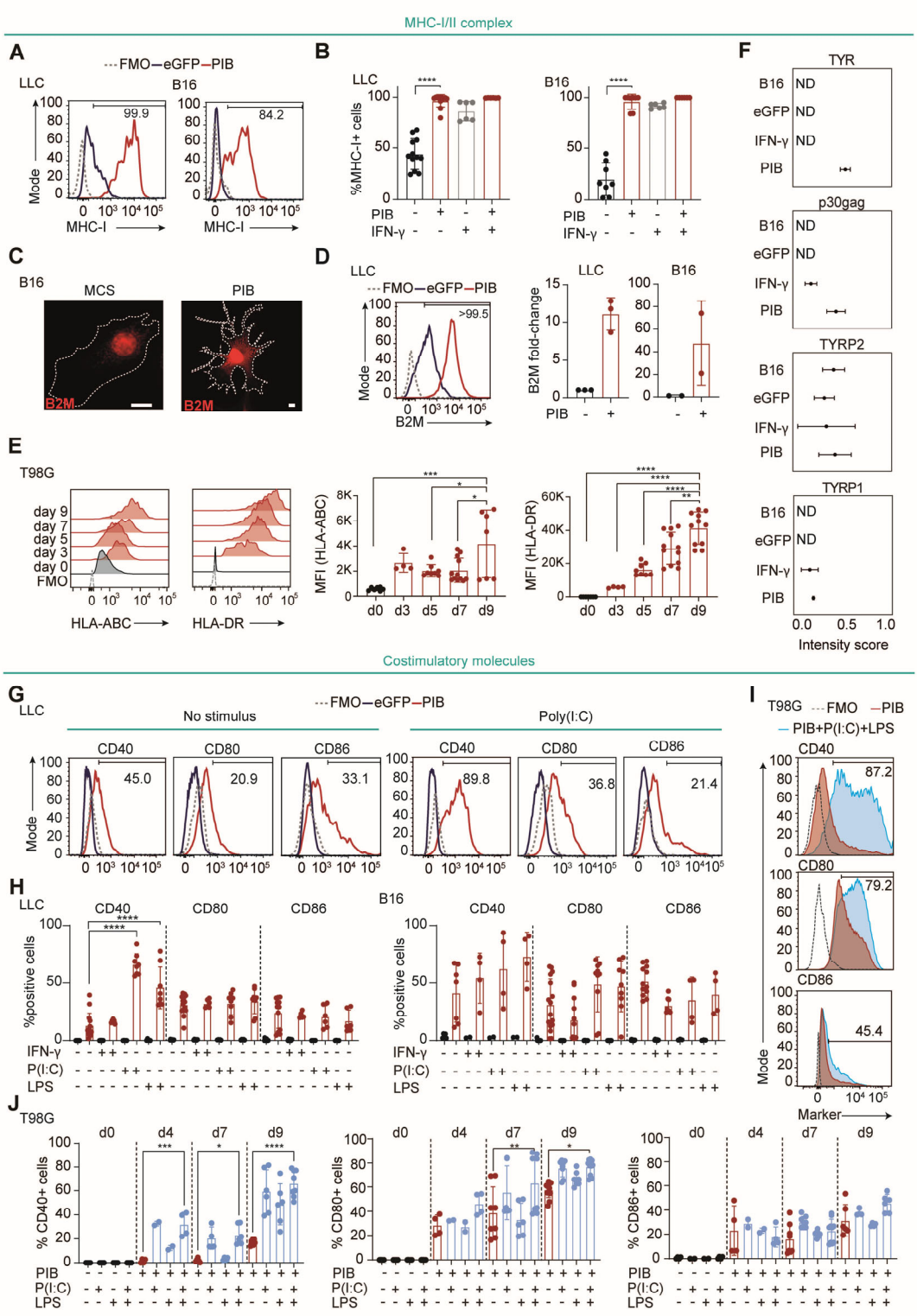

fig. S10. Reprogramming towards cDC1 induces antigen presentation machinery in cancer cells. (A) Murine B16 and LLC cell lines were transduced with PIB or eGFP and analyzed by flow cytometry after 9 days for surface MHC-I expression. Representative plots of MHC-I expression (left) and quantification (right) in transduced cancer cells. **(B)** Comparison with IFN-γ treatment overnight is shown (n=6-12). Fluorescence minus one (FMO) was included for PIB transduced cells to define the negative population. **(C)**

Micrographs depicting expression of beta-2-microglobulin (B2M) in fixed and permeabilized B16 cells at reprogramming day 9. Scale bars = 20 μm. Mean±SD is represented. **(D)** Flow cytometry analysis of B2M in tumor-APCs at day 9 showing a representative plot (left) and B2M mean-intensity fluorescent intensity (MFI) fold-change between eGFP and PIB transduced (right, LLC: n=3; B16: n=2). **(E)** T98G cells were transduced with PIB and analyzed for surface expression of HLA-ABC (left) and HLA-DR (right) upon cDC1 reprogramming. Representative flow cytometry plots are depicted (left) and quantification of MFI CD45+HLA-DR+ cells (red) and eGFP-transduced cells (day 0, black) (n=4-12). **(F)** Normalized intensity score of peptides originated from Tyrosinase (TYR), Gag polyprotein (GAG), Tyrosinase-related protein 1 and 2 (TYRP1, TYRP2) calculated for untransduced, eGFP transduced, reprogrammed (PIB) and IFN-γ stimulated B16 melanoma cells. Mean±SD is represented. ND – not detected. **(G)** Surface expression of costimulatory molecules CD40, CD80 and CD86 in reprogrammed LLC, gated within CD45+MHC-II+ cells (red) on day 9 of reprogramming. 24 h prior analysis, cells were stimulated with IFN-γ or TLR agonists Poly(I:C) (P(I:C)) or LPS. **(H)** Quantification of the percentage of CD40, CD80 or CD86-positive cells assessed by flow cytometry with or without individual TLR stimulation in LLC (n=4-12, left panel) and B16 cells (n=2-17, right panel). (**I)** Flow cytometry plots and **(J)** quantification of surface expression of CD40, CD80, and CD86 costimulatory molecules in CD45+HLA-DR+ T98G-derived cells before (red) or after P(I:C) and LPS stimulation (blue) at day 9 (n=2-12). Mean±SD is represented. n= biological replicates. p-value was calculated using One-way ANOVA followed by Tukey´s multiple comparison test. *p<0.1, **p<0.01, ***p<0.001, ****p<0.0001.

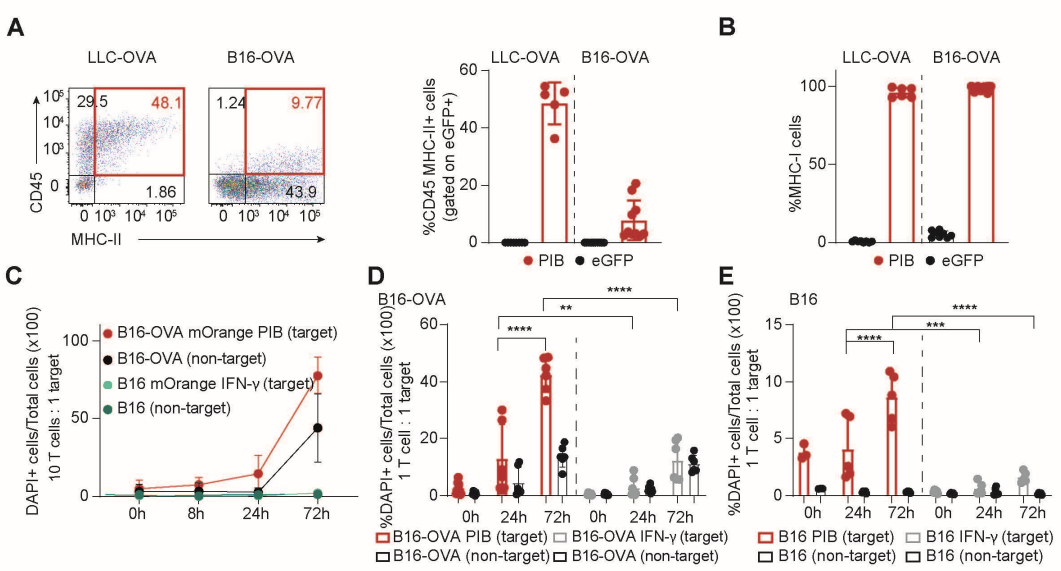

fig. S11. **Restored immunogenicity is fast and antigen specific. (A)** Murine B16 and LLC cell lines, expressing a cytoplasmatic truncated version of ovalbumin (OVA), were transduced with PIB and analyzed by flow cytometry (left). Reprogramming efficiency was quantified (right) by the percentage of CD45+MHC-II+ cells at reprogramming day 9 (n=5-10). Cells transduced with eGFP were used as control (-). **(B)** Quantification of the percentage of MHC-I+ cells gated in eGFP+ transduced LLC-OVA or B16-OVA cells (n=6-8). **(C)** Quantification of T cell killing of either target or non-target tumor cells when co-cultured with activated OT-I T cells (T cell cancer cell ratio of 10:1) after 8, 24 and 72 h. Target cells were B16-OVA-mOrange+ cells reprogrammed with PIB (red), while B16-OVA (mOrange-) in the same well were defined as non-target. Assessment of T cell killing assay specificity was performed with B16 mOrange cells treated with IFN-γ that did not express OVA (grey) (n=6-9). **(D)** Quantification of T cell killing of either target or non-target tumor cells when co-cultured with activated OT-I (n=6-8) or (**E**) Pmel T cells (1:1 ratio) after 0, 24 and 72h. Target cells were reprogrammed B16-OVA-mOrange+ or B16-mOrange+ cells transduced with PIB (red) or treated with IFN-γ (gray). Parental B16-OVA or B16 cells (mOrange-) served as non-target cells in co-culture with target cells (black) (n=3-5). Mean±SD is represented. n=biological replicates. p-value was calculated using One-way ANOVA followed by Tukey´s multiple comparison test. **p<0.01, ***p<0.001, ****p<0.0001.

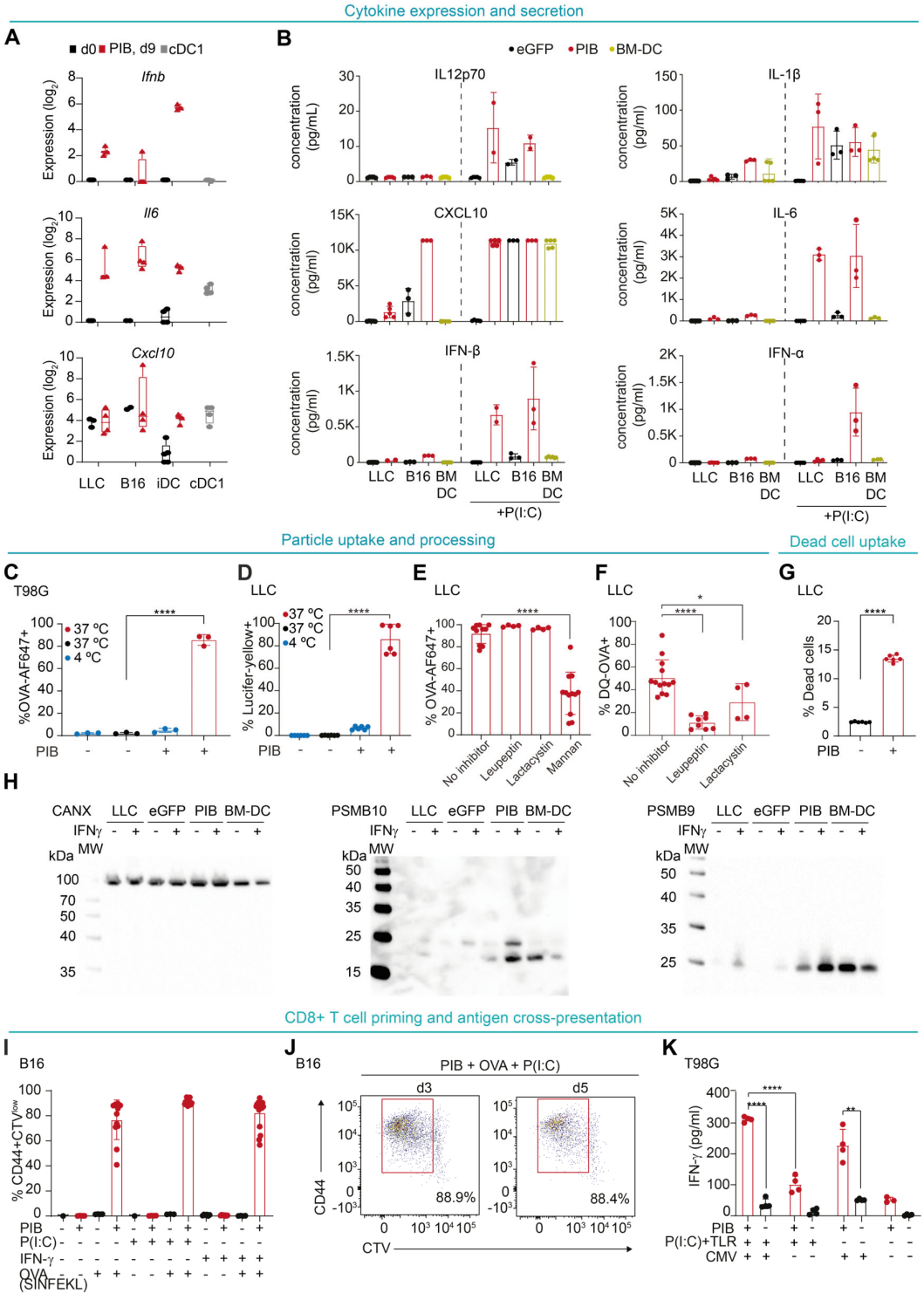

fig. S12. Induction of cDC1 function in murine and human cancer cells with PU.1, IRF8 and BATF3. (A) Whisker box plots showing mRNA expression levels of *Ifnb*, *Il6* and *Cxcl10*. B16 and LLC cell lines were transduced with PIB and purified by FACS on day 9 based on co-expression of eGFP, CD45 and MHC-II (red). Cancer cells transduced with eGFP were included as control (d0, black). Splenic conventional dendritic cells type 1 (cDC1, CD45+MHC-II+CD8+, grey), mouse embryonic fibroblasts (MEFs) and

induced dendritic cells (iDCs, Clec9a-tdTomato+CD45+MHC-II+) were used as reference (GSE103618) (n=3-5). **(B)** Quantification of IL12p70, IL-1β, CXCL10, IL-6, IFN-β and IFN-α, in supernatants of eGFP (black) and MACS-purified PIB-transduced B16 and LLC cells at reprogramming day 9 (red), in the presence or absence of Poly(I:C) (P(I:C)) stimulation. CD103+ bone marrow (BM)-DCs were used as reference (green, n=3-5). **(C)** Quantification of OVA-AlexaFluor647 (OVA-AF647) uptake by reprogrammed (red) and eGFP transduced T98G (black) at 4ºC and 37ºC. (n=3). **(D)** Quantification of lucifer-yellow+ cells, gated in CD45+MHC-II+ cells (red, +) or LLC cells transduced with control vector (MCS, black, -) (n=6). **(E)** Quantification of OVA-AF647 cells gated in CD45+MHC-II+ cells at reprogramming day 9 in the absence or presence of Leupeptin, Lactacystin and Mannan (n=4-12). **(F)** Quantification of DQ-OVA+ cells gated in CD45+MHC-II+ cells in the presence of Leupeptin and Lactacystein (n=4-13). (**G**) Quantification of dead cell uptake by eGFP (-) or PIB (+) transduced LLC cells (n=6). (**H**) Uncut western blot membranes showing the expression of PSMB10 and PSMB9 and CANX (loading control). **(I)** Quantification of proliferative CD44+CTV^{low} CD8+ OT-I T cells after a 3-day co-culture period with reprogrammed B16 cells pre-incubated with OVA peptide (SINFEKL; n=3-16). **(J)** Representative flow cytometry plots for CD8^{+} T cell proliferation (CTV dilution) and activation (CD44+) after co-culture with reprogrammed B16 at day 3 and 5. **(K)** Quantification of IFN-γ in supernatants collected after co-culture of reprogrammed T98G (red) with CMV-specific CD8+ T cells, after CMV peptide pulse. T98G cells transduced with eGFP were used as controls (black, n=6). Mean±SD is represented. n= biological replicates, p-value was calculated with One-way ANOVA followed by Tukey´s multiple comparison test. *p<0.1, **p<0.01, ***p<0.001, ****p<0.0001.

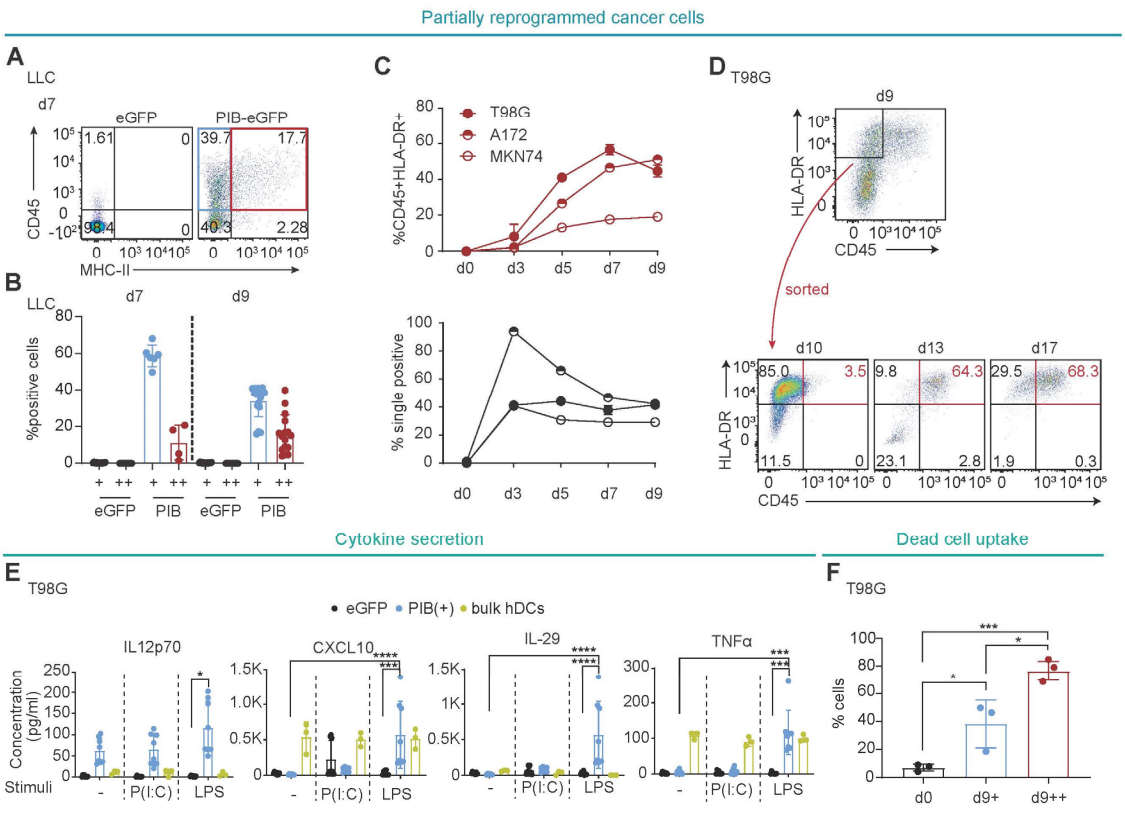

fig. S13. Partially reprogrammed cells acquire cDC1 features. (A) The LLC cell line was transduced with PIB and reprogrammed cells (eGFP+CD45+MHC-II+, red) or partially reprogrammed cells (eGFP+CD45+MHC-II-, blue) were analyzed by flow cytometry. **(B)** Quantification of reprogrammed (++) and partially reprogrammed (+) populations at day 7 (d7, n=4) and 9 (d9, n=12-15). **(C)** Human glioblastoma (T98G and A172) and human gastric carcinoma (MKN74) cell lines were transduced with PIB to induce cDC1 reprogramming. The percentage of reprogrammed CD45+HLA-DR+ cells (red, upper panel) and partially reprogrammed cells expressing either CD45 or HLA-DR (black, lower panel) was quantified by flow cytometry at day 3 (d3), 5 (d5), 7 (d7) and 9 (d9) (n=2). (**D**) T98G-derived partially reprogrammed CD45-HLA-DR+ cells were FACS-purified at day 9 and cultured. Flow cytometry analysis for CD45 and HLA-DR after 1 (d10), 4 (d13) and 8 days (d17). (**E**) Secretion of IL-12, CXCL10, IL-29, and TNFα by eGFP-transduced (day 0, black) and partially reprogrammed T98G cells (CD45-HLA-DR+, day 9+, blue), unstimulated or after Poly(I:C) (P(I:C)) or LPS stimulation. Enriched pan-DCs (green) served as reference (n=3-8) and eGFP transduced T98G cells were used as control. **(F)** Purified reprogrammed (CD45+ HLA-DR+, day 9++), partially reprogrammed (CD45- HLA-DR+, day 9+) T98G-derived cells were co-cultured with fluorescently labeled dead cells. The percentage of cells that incorporated dead cells was quantified by flow cytometry after 8h (n=3). Mean±SD is represented. n= biological replicates. p-value was calculated using Two-way ANOVA (E) or One-way ANOVA (F) followed by Tukey´s multiple comparison test. *p<0.05, **p<0.01, ***p<0.001****p<0.0001.

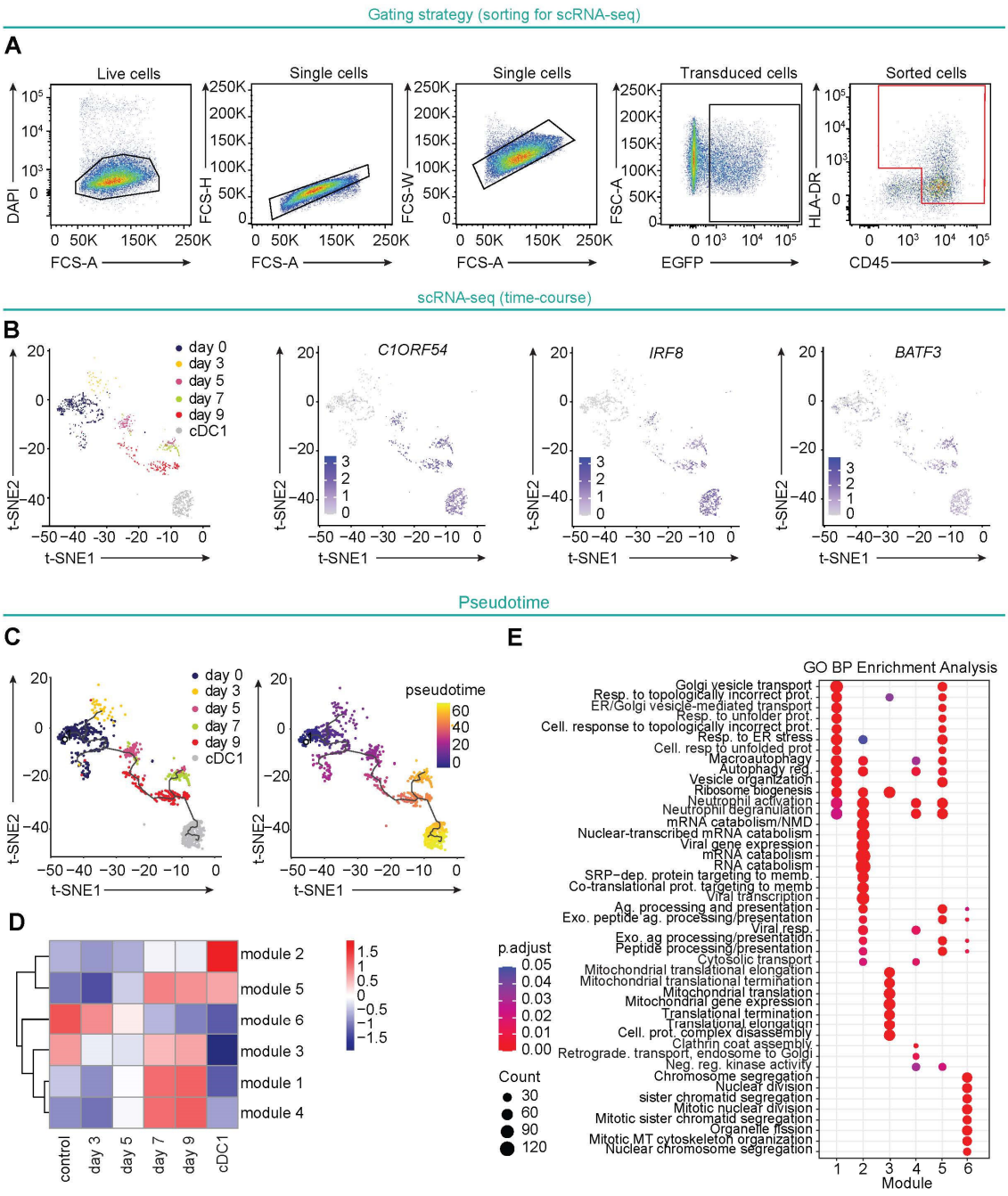

fig. S14. Pseudo-temporal ordering of single cancer cells to tumor-APCs. (**A**) Gating strategy to purify tumor-APCs for scRNA-seq. Transduced eGFP+ cells expressing at least one of the reprogramming markers (CD45 or HLA-DR, red) were FACS-sorted. Representative data from U3P2E2 bladder carcinoma is shown. (**B**) scRNA-seq analysis of reprogrammed T98G cell line at day 3 (d3), 5 (d5), 7 (d7), and 9 (d9) including complete and partially reprogrammed cells. t-SNE plots show cells according to the reprogramming timepoints, highlighting the expression of *C1ORF54*, and endogenous expression of *IRF8* and *BATF3*. Color intensity indicates expression levels. eGFP transduced cells were used as day 0 control and peripheral blood cDC1 as reference. (**C**) Monocle3 reconstruction of single-cell trajectories. UMAP plots depict pseudo time of cDC1 reprogramming trajectory (black line) colored according to the timepoint (left) and

relative position in pseudotime (right). (**D**) Heatmap showing mean gene expression of identified gene modules during cDC1 reprogramming time course. (**E**) GO biological process (BP) enrichment analysis of gene modules. Circle size refers to the number of genes within each module. Color gradient depicts adjusted p-values based on hypergeometric test. Resp. – response; prot. – protein; cell. – cellular; ER – endoplasmic reticulum; reg. – regulation; NMD – nonsense-mediated mRNA decay; SRP – signal recognition particle; memb. – membrane; ag. – antigen; exo. – exogenous; transport. – transportation; neg. – negative; MT – microtubule.

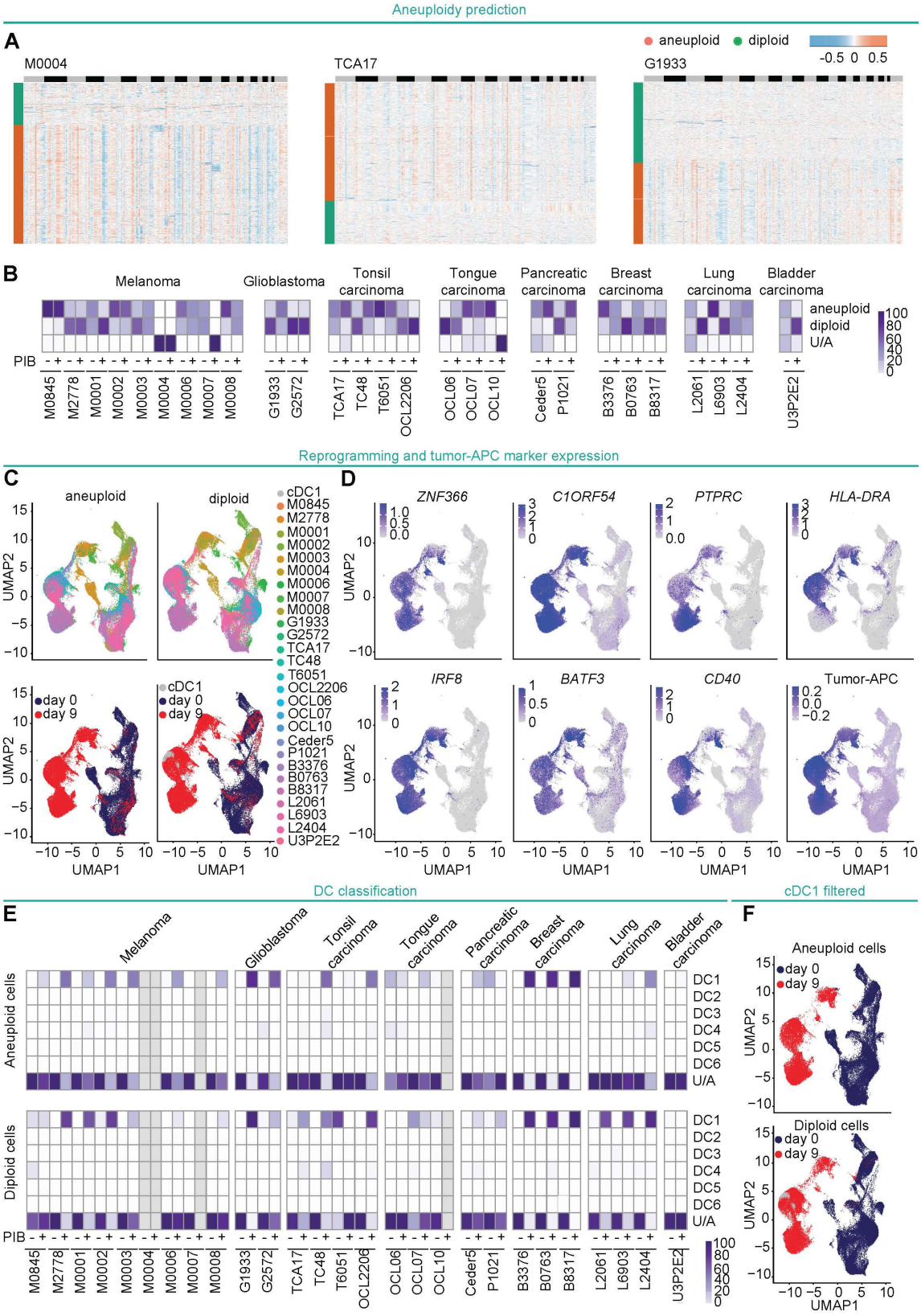

fig. S15. cDC1 reprogramming of aneuploid patient-derived cancer cells. **(A)** Example of copy number variation profiles estimated from scRNA-seq in primary cancer

cells to identify aneuploid (malignant) and diploid cells. Clustered heatmap of scRNA-seq copy number profiles in melanoma (M0004), tonsil carcinoma (TCA17), and glioblastoma (G1933). Color gradient depicts copy number status and clusters show aneuploid or diploid cells. (**B**) Heatmap showing percentages of aneuploid and diploid cells from 27 patient-derived cancer cell samples or unaffiliated (U/A). Cells were transduced with PIB and FACS purified on day 9. eGFP transduced cells were used as controls. (**C**) UMAP analysis of single-cell transcriptomes showing aneuploid and diploid cells from 27 primary cancer cells according to their origin (upper panels) or induced reprogramming (day 9 PIB or day 0 eGFP, bottom panels). Peripheral blood cDC1 were used as reference. (**D**) UMAP plots showing expression of cDC1 genes *ZNF366* and *C1ORF54*, reprogramming markers *PTPRC* and *HLA-DRA*, endogenous expression of *IRF8* and *BATF3*, the costimulatory molecule *CD40*, and the tumor-APC signature in aneuploid cells. (**E**) Integration of aneuploid (top) and diploid (bottom) single cells from reprogrammed primary tumor samples with published DC subset data (GSE94820).# Heatmap shows the percentage of single cells affiliated to individual cDC subsets or unaffiliated. (**F**) UMAP showing single-cell transcriptomes of cDC1-affiliated aneuploid and diploid cells.

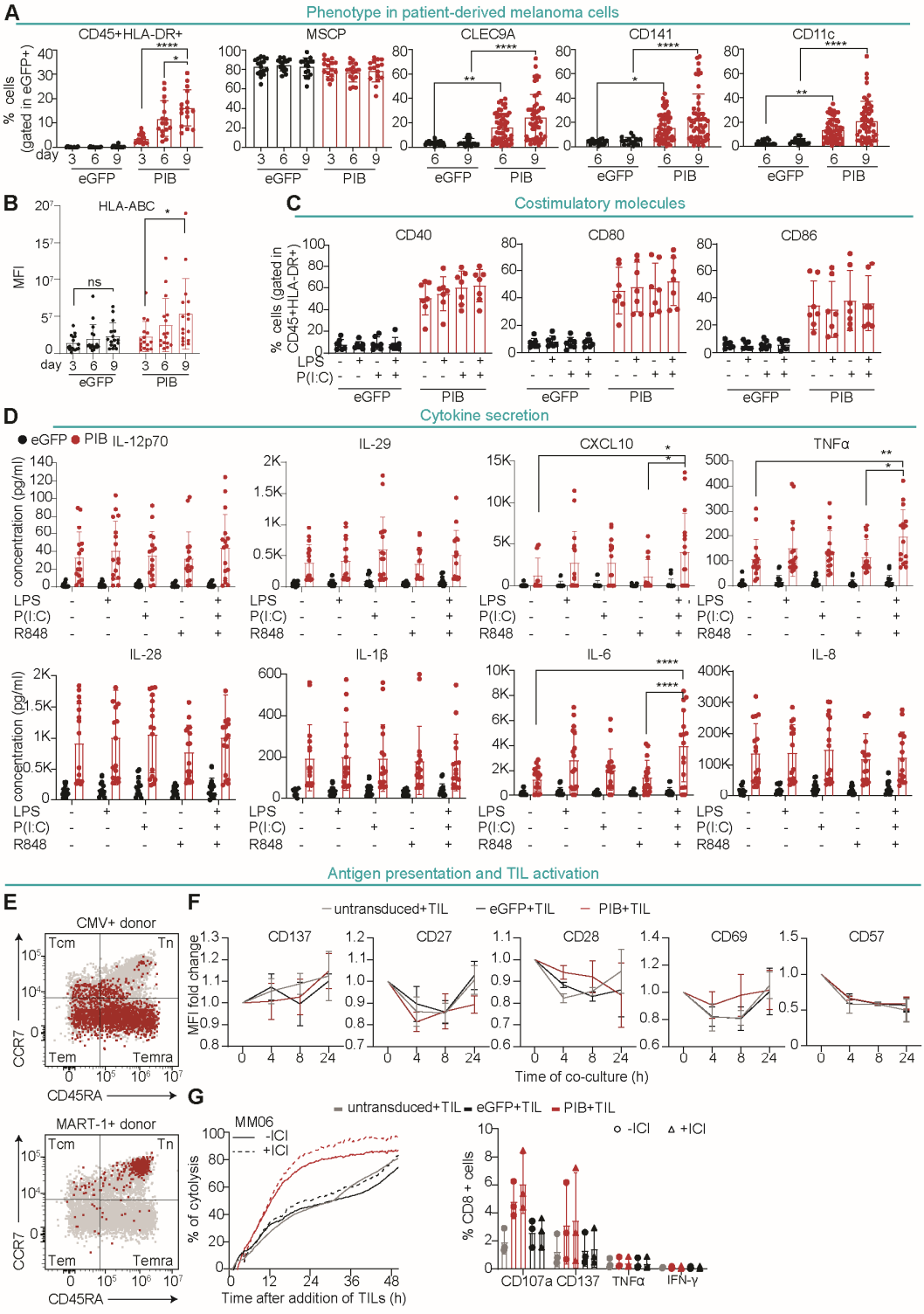

fig. S16. cDC1 reprogramming elicits cDC1 phenotype and function in primary melanoma cells. (A) Quantification of expression of CD45 and HLA-DR, the tumor antigen MSCP and the DC markers CLEC9A, CD141, and CD11c by primary melanoma cells at day 3, 6, and 9 after transduction with PIB. Cells transduced with eGFP were used as controls. (n= 16-55) (**B**) Quantification of mean intensity fluorescence (MFI) of induced HLA-ABC during reprogramming kinetics. (n=16) (**C**) Quantification of expression of costimulatory molecules CD40, CD80, and CD86 by reprogrammed

CD45+HLA-DR+ cells with and without stimulation with LPS and PolyI:C (P(I:C)). (n=8) **(D)** Secretion of pro-inflammatory cytokines (IL12p70, Il-29, CXCL10, TNFα, IL-28, IL-1β, IL-6 and IL-8) by eGFP-transduced (black) and day 9 reprogrammed CD45+HLA-DR+ cells (red) with or without P(I:C), LPS or R848 stimulation. (n=16). (**E**) Phenotype of donor peripheral blood CMV+ and MART-1+ CD8+ T cells (highlighted in red). CD45RA+ expressing effector memory T cells (CD45RA+CCR7-, Temra), central memory T cells (CD45RA-CCR7+, Tcm), naïve T cells (CD45RA+CCR7+, Tn) and effector memory T cells (CD45RA-CCR7-, Tem). (**F**) Quantification of the MFI of the reactivity markers CD137, CD27, CD28, CD69, and CD57 in CD3+ T cells after co-culture of reprogrammed melanoma cells with autologous tumor infiltrating lymphocytes (TIL) for 24h. (n=4). **(G)**) TIL mediated cytolysis of reprogrammed melanoma cells in the presence or absence of immune checkpoint inhibitors (ICI, anti-PD1, anti-CTLA-4). Untransduced and eGFP-transduced melanoma cells were used as controls (right, n=4). Quantification of expression of CD107a, CD137, TNFα and IFN-γ in TIL mediating cytolysis, in the presence or absence of ICI. Mean±SD is represented. n= biological replicates. p-value was calculated using One-way ANOVA (A-B) or Two-way ANOVA (D) followed by Tukey´s multiple comparison test. *$p<0.05$, **$p<0.01$, ***$p<0.001$****$p<0.0001$.

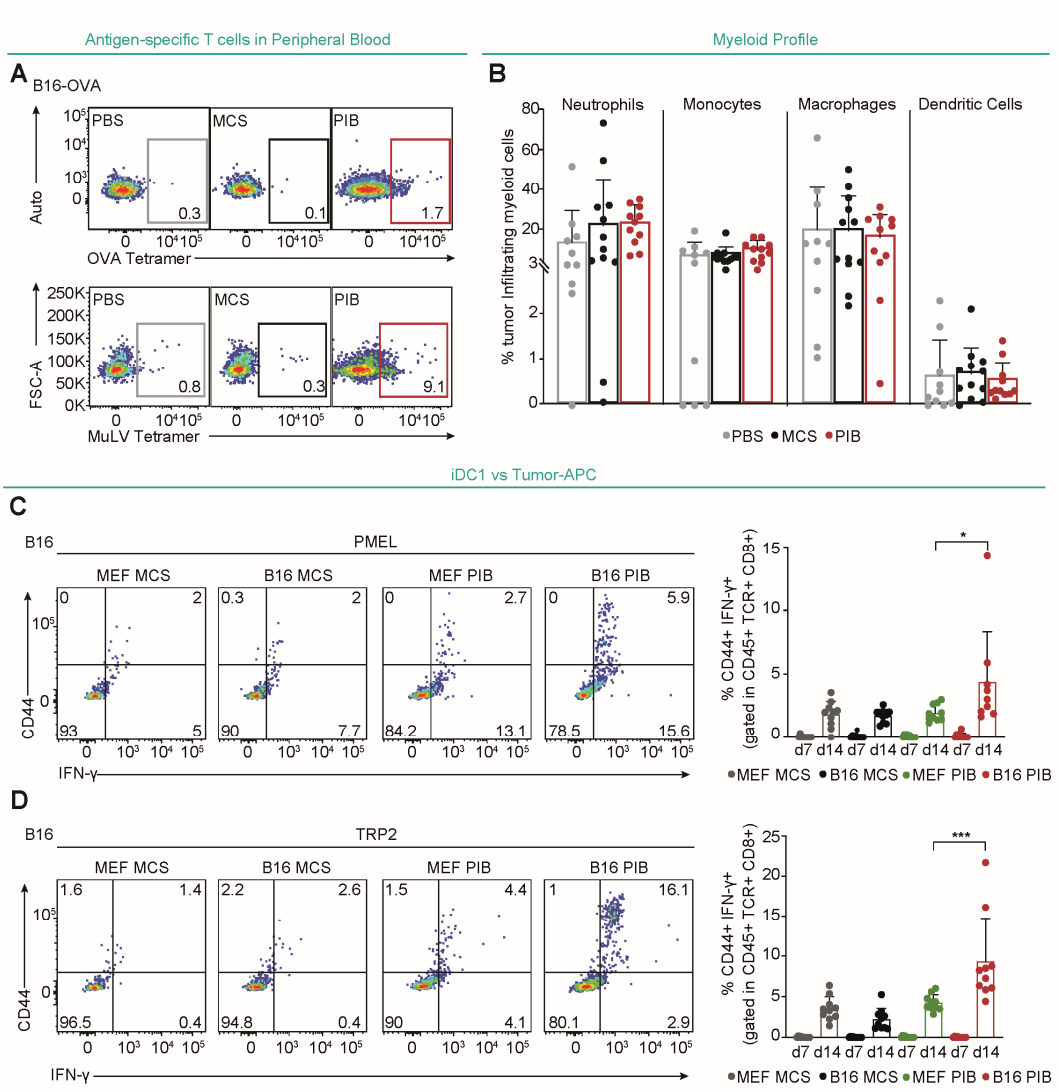

figure S17. Tumor-APCs expand tumor-specific T cells *in vivo*. (A) Tumors were established subcutaneously in mice using B16-OVA cancer cells. On day 7, 10 and 13 B16-derived tumor-APCs were injected intratumorally after overnight incubation with Ovalbumin (OVA) protein and P(I:C). Flow cytometry plots of peripheral blood T cells stained with OVA tetramer (top) or Murine Leukemia Virus (MuLV) tetramer (bottom) 14 days after B16-OVA tumor establishment. Mice injected with B16-derived tumor-APCs (PIB) were compared with mice injected with PBS or B16 cells transduced with control virus (MCS). **(B)** Flow cytometry quantification of tumor infiltrating myeloid cells isolated from tumors 18 days after tumor establishment. Neutrophils (CD45+ MHC-II- CD11b+ Ly6G+), monocytes (CD45+ MHC-II- CD11b+ Ly6C+), macrophages (CD45+ MHC-II+ CD11c+ Ly6C- CD64+) and dendritic cells (CD45+ MHC-II+ CD11c+ CD64- Ly6C- CD11b+) were assessed. (n=10) **(C)** Flow cytometry plots (left) and quantification (right) of CD44+ IFN-γ+ peripheral blood CD8+ T cells after *in vitro* re-stimulation with Pmel peptide (n=10) or **(D)** TRP2 peptide 14 days post B16 tumor establishment (n=10). Tumors were injected with B16-derived tumor-APCs (B16 PIB), mouse embryonic fibroblast (MEF)-derived iDC1 (MEF PIB) and B16 cells or MEFs

transduced with control virus (MCS) after overnight stimulation with Poly(I:C) (P(I:C)). Mean±SD is represented. n= biological replicates. p-value was calculated using Mann-Whitney test. *$p<0.05$, ***$p<0.001$.

Data file S1. RNA-seq data analysis of human tumor-APCs and peripheral blood DCs.
Bulk RNA-seq data of 17 human cancer cell lines 9 days after transduction with PIB or eGFP and peripheral blood DC subsets. The table contains normalized gene counts, tumor-APC, antigen presentation, and proliferation signatures and associated gene counts, pathway and gene ontology analysis, and endogenous gene counts for reprogramming factors. Related to bulk RNA-seq analysis presented in Figure 1, 7, S5 and S9.

Data file S2. RNA-seq data analysis of murine tumor-APCs.
Bulk RNA-seq data analysis of melanoma (B16) and lung (LLC)-derived tumor-APCs, including normalized gene counts, differentially expressed genes, pathway, and gene ontology analysis and intronic and exonic gene counts of reprogramming factors. Splenic cDC1 data from GSE103618. Related to bulk RNA-seq analysis presented in Figure 1, 3, S4, S8, S9 and S12.

Data file S3. RNA-seq data analysis of reprogramming time-course of human cancer cells.
Bulk RNA-seq data at 5 timepoints during reprogramming of the glioblastoma cell line T98G and human embryonic fibroblasts (HEFs) compared to peripheral blood cDC1s. The table contains gene counts, tumor-APC, antigen presentation, and proliferation signatures and associated gene counts, pathway and gene ontology analysis, and endogenous gene counts for reprogramming factors. Related to bulk RNA-seq analysis presented in Figure 2, 7, S6, S7 and S8.

Data file S4. ATAC-seq data analysis of reprogramming time-course of human cancer cells and human peripheral blood cDC1.
Bulk ATAC-seq data acquired at 5 timepoints during the reprogramming of the glioblastoma cell line T98G as well as peripheral blood cDC1. The table contains peak counts, peaks in genes affiliated to tumor-APC signature, gene ontology peak enrichment analysis, and transcription factor binding motif enrichment analysis. Related to bulk ATAC-seq analysis presented in Figure 2 and S7.

Data file S5. Immunopeptidomics data analysis.

List of peptides bound to MHC-I identified in untransduced B16, eGFP-transduced, IFN-γ treated and reprogrammed CD45+ tumor-APCs . Related to immunopeptidomics data presented in Figure 3 and S10.

Data file S6. Human single-cell RNA-seq data analysis.
scRNA-seq data acquired at 5 timepoints during reprogramming of the T98G cell line and 27 patient-derived cancer cells after 9 days of reprogramming. eGFP transduced cells were included as controls. The table contains a list of identified gene modules along reprogramming trajectory, gene ontology analysis, and DC subset classification. Related to Figure 5, S14, and S15.

Data file S7. Raw data
Flow cytometry data and mouse survival curves for figures 1 to 8 and S1 to S17.

Data file S8. Cells, antibodies and materials used in this study.
Murine cancer cell lines, human cell lines and primary cancer samples as well as antibodies, plasmids, cloning and sequencing primers used in the study.

Supplementary movies

Supplementary movie S1. Protein uptake by reprogrammed murine cancer cells. LLC cancer cells were transduced with PIB and cells expressing either CD45 or MHC-II were MACS-purified after 9 days. Reprogrammed LLC cells were incubated with Ovalbumin-AlexaFluor647 (OVA-AF647) and immediately imaged by live microscopy. The video shows cellular morphology (brightfield) overlayed with the fluorescence signal resultant of uptake of OVA-AF647 (red). After approximately 37 min, OVA-AF647 starts to be detected in the cytoplasm and increases in intensity with time. Pictures were taken every 12 min over a period of 148 min.

Supplementary movie S2. Protein processing by reprogrammed murine cancer cells. LLC cancer cells were transduced with PIB and cells expressing either CD45 or MHC-II were MACS-purified after 9 days. Reprogrammed LLC cells were incubated with DQ-Ovalbumin (DQ-OVA) and immediately imaged by live microscopy. The video shows cell morphology (brightfield) overlayed with the fluorescence signal resultant of DQ-OVA processing within the cytoplasm (green). After 97 min, DQ-OVA starts to be detected (top right corner) and increases in intensity over time. Pictures were taken every 24 min over a period of 395 min.

Supplementary movie S3. Dead cell uptake by human glioblastoma-derived tumor-APCs. T98G cancer cells were transduced with PIB and eGFP+CD45+HLA-DR+ cells FACS-purified on day 9. Tumor-APCs were incubated with dead cells labelled with Cell Vue Claret Far Red dye and immediately imaged by live cell microscopy. The video shows reprogrammed cells (brightfield, green) actively searching and engulfing dead cells (red). Pictures were taken every 26 min over a period of 16 h.

Supplementary movie S4. Dead cell uptake by control eGFP transduced human glioblastoma cells. T98G cancer cells were transduced with control eGFP lentiviral vectors and eGFP+ cells FACS-purified on day 9. Sorted cells were incubated with dead cells fluorescently labelled with CellVue Claret Far Red dye and immediately imaged by live cell microscopy. The video shows eGFP+ cancer cells (brightfield, green) unable to capture dead cells (red). Pictures were taken every 26 min over a period of 16 h.

Supplementary movie S5. T cell priming and cytotoxicity elicited by murine tumor-APCs. A, B16 cells were transduced with PIB. After 9 days cells expressing either CD45 or MHC-II were MACS-purified and incubated with SIINFEKL peptide and co-cultured with CTV labeled naïve OT-I CD8+ T cells. Video shows cancer cell morphology (brightfield) overlayed with T cell-derived CTV fluorescence (blue). Priming and activation of CD8+ T-cells by reprogrammed cancer cells are highlighted by T cell cluster formation leading to cancer cell cytotoxicity**. B**, Co-culture in the absence of SIINFEKL does not lead to cancer cell killing. Pictures were taken every hour over a period of 72 h.