

Supplementary Materials for
**Single-cell chemoproteomics identifies metastatic activity signatures in
breast cancer**

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Sci. Adv. **10**, eadp2622 (2024)
DOI: 10.1126/sciadv.adp2622

The PDF file includes:

Figs. S1 to S7
Table S1
Legend for data S1

Other Supplementary Material for this manuscript includes the following:

Data S1

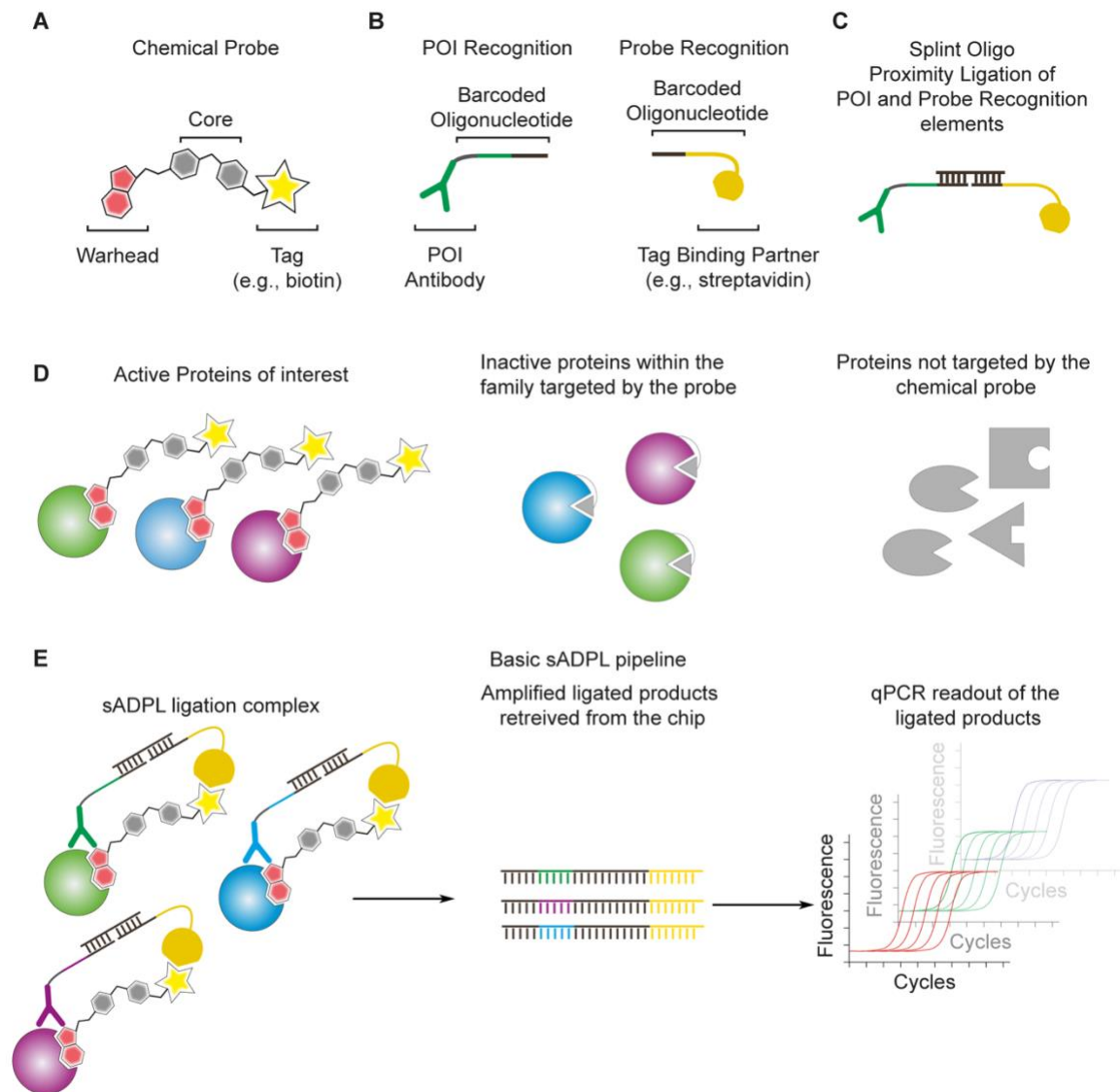


Fig. S1. Description of scADPL platform elements, targets and general workflow: A) Schematic of an activity probe showing the reactive warhead and the recognition tag B) Schematic of barcoding elements 1) antibody against the protein whose activity is measured conjugated to a barcoded oligonucleotide 2) tag binding protein conjugated to oligonucleotides C) Splint binding to the proximal DNA conjugated to antibody or tag binding protein mediating ligation. D) Whole proteome depiction after treatment with the probe showing 1) Active proteins within a protein family labelled by the activity probe 2) The inactive protein within the same family which are not labelled by the probe and 3) all the proteins that are not targeted by the activity probe. E) Schematic showing the formation of the proximity complex where the antibody oligonucleotide conjugate and the tag binding oligonucleotide conjugate are in proximity which leads to a ligation event followed by amplification of the ligated products which are then readout by qPCR.

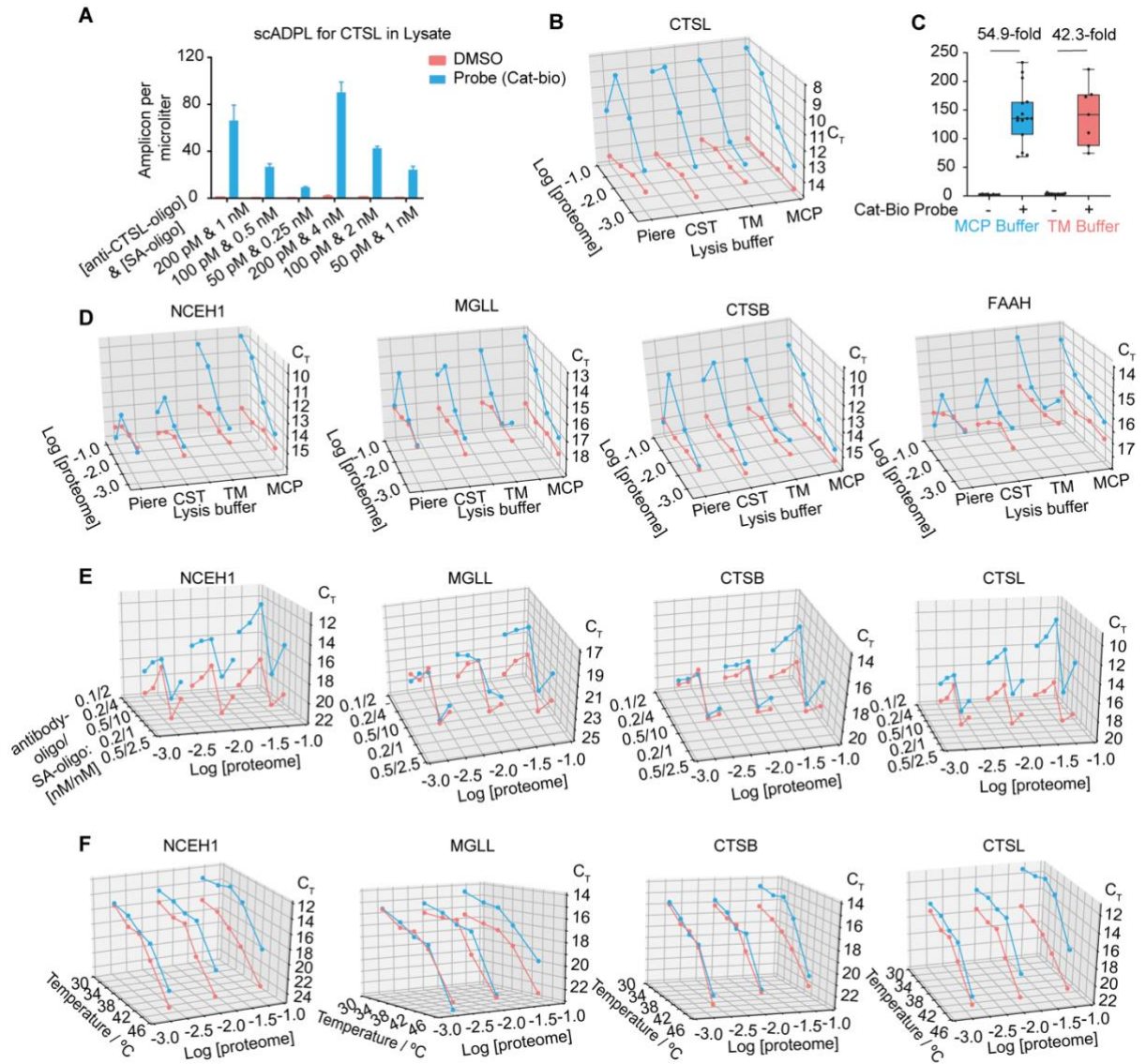


Fig. S2. Optimization of scADPL conditions: A) On-chip profiling of active CTSL in SKOV3 cell lysate on microfluidic device with varying concentrations of antibody and streptavidin oligonucleotide conjugates. The ligated amplicons were detected by ddPCR. Data shows the mean of three technical replicates from an individual bio replicate and the error bars show standard deviations. B) On-chip profiling of active CTSL in SKOV3 lysate using different lysis conditions. 3D plot depicts the activity at four different lysate concentrations with blue dots representing the cat-bio treated sample and the red dots depicts the DMSO treated sample. Data plotted are Raw C_T values from three technical replicates from an individual biological replicate. C) scADPL measurement of active CTSL in single cells using the two best lysis buffers from previous optimization in B). From the data MCP had better Signal to noise ratio than TM. Box plot depicts the median and the quartiles, the whiskers go down from the smallest to the highest values. Individual data points represent single cells. D) On-chip multiplexed profiling of active NCEH1, MGLL, CTSB and FAAH in probe (FP-bio and Cat-bio) treated SKOV3 lysate on microfluidic device with different lysis conditions. 3D plot shows the raw C_T values obtained from probe treated (blue) samples and DMSO treated (red) samples at four different lysate concentrations across four different lysis buffers. Data represents the mean from three technical replicates of one biological replicate. Data shows that MCP retained the best signal to noise ratio even in multiplexing conditions.

E) On-chip multiplexed profiling of active NCEH1, MGLL, CTSB and CTSL in probe (FP-bio and Cat-bio) treated SKOV3 lysate on microfluidic device with varying concentrations of antibody and tag binding protein oligonucleotide conjugate. 3D plot shows the raw Ct values obtained from probe treated (blue) samples and DMSO treated (red) samples at three different lysate concentrations across five different antibody and tag binding protein oligonucleotide conjugate concentrations. Data represents the mean from three technical replicates of one biological replicate. F) On-chip multiplexed profiling of active NCEH1, MGLL, CTSB and CTSL in probe (FP-bio and Cat-bio) treated SKOV3 lysate on microfluidic device with varying ligation temperatures. 3D plot shows the raw Ct values obtained from probe treated (blue) samples and DMSO treated (red) samples at three different lysate concentrations across five different ligation temperatures. Data represents the mean from three technical replicates of one biological replicate.

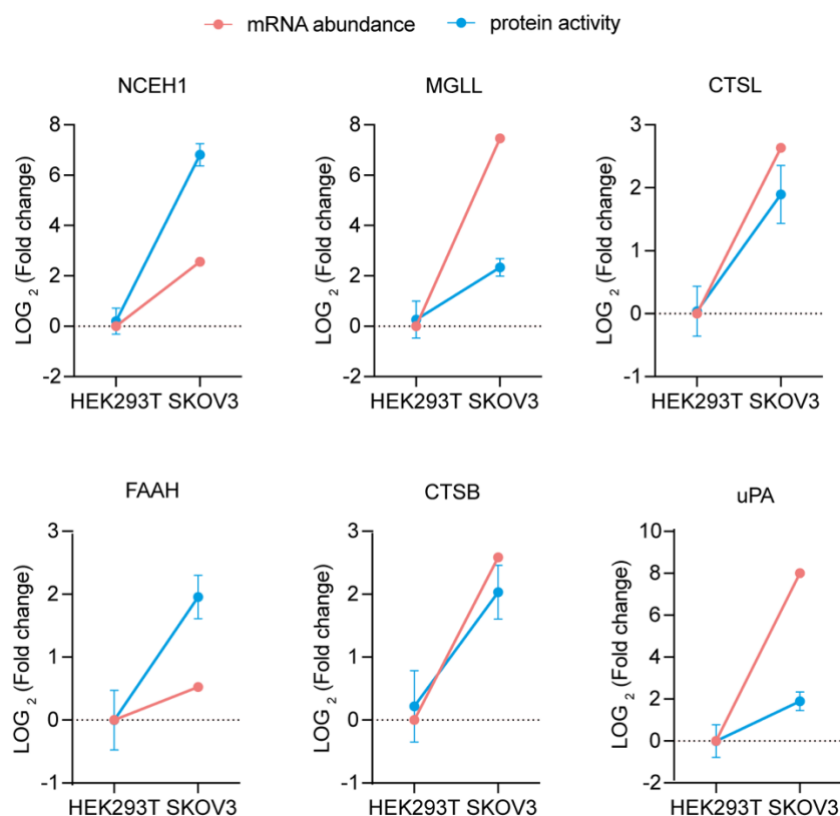


Fig. S3. mRNA abundance compared to protein activity in HEK293T and SKOV3 cells: Plots of relative mRNA abundance and active protein levels of the Ag6 panel between the cell lines HEK293T and SKOV3. mRNA measurements were obtained from the online repository, The Human Protein Atlas (<https://www.proteinatlas.org>) (51) and protein activity (in blue) of each Ag6 panel member measured using scADPL.

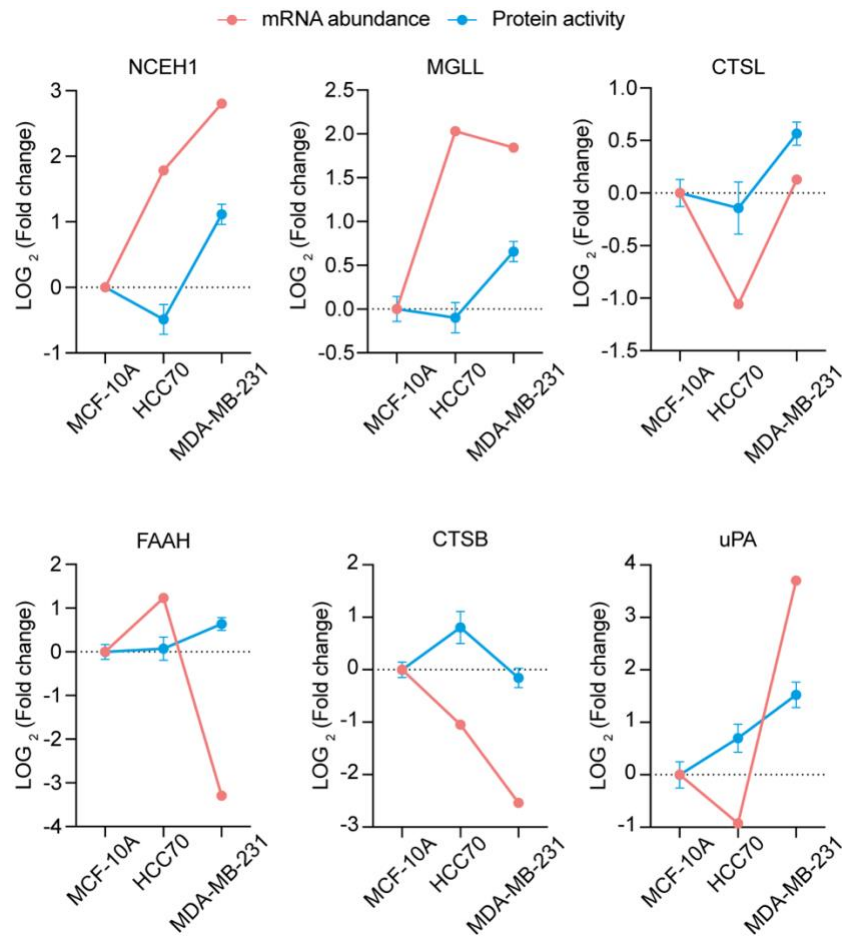


Fig. S4. mRNA abundance compared to protein activity in MCF-10A, HCC70 and MDA-MB231 cell lines: Plots of relative mRNA abundance and active protein levels of the Ag6 panel between the cell lines MCF-10A, HCC70 and MDA-MB-231. mRNA measurements were obtained from the online repository, The Human Protein Atlas (<https://www.proteinatlas.org>) (51) and protein activity (in blue) of each Ag6 panel member measured using sADPL.

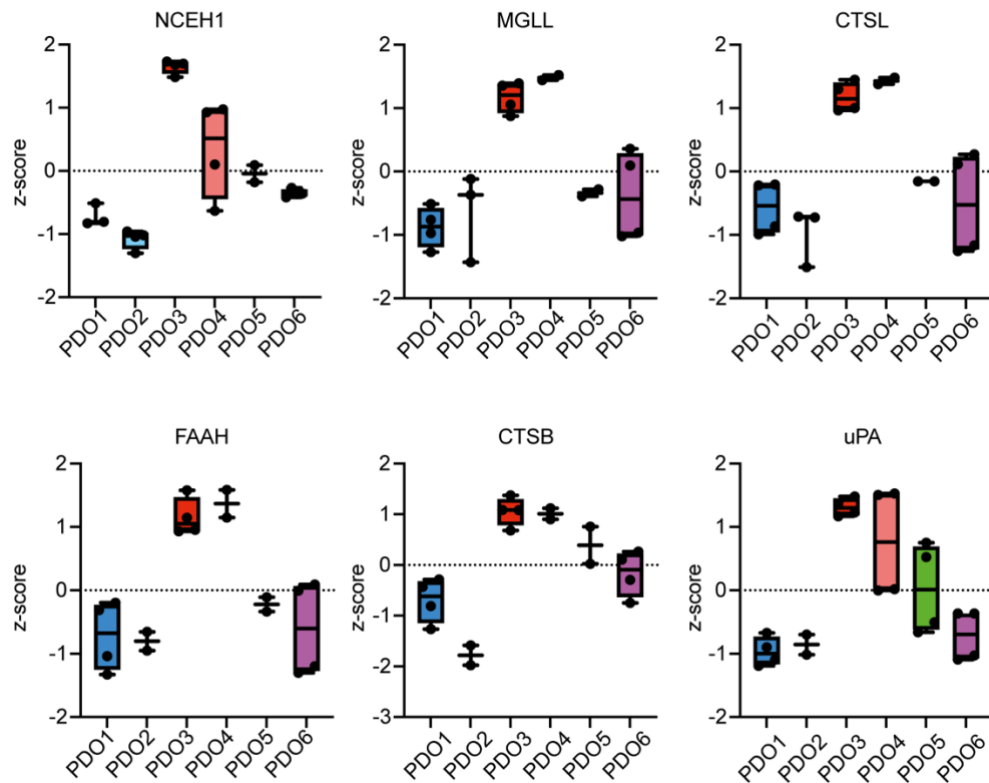


Fig. S5. sADPL comparison of tumor organoids from six different TNBC patients.

Activity measurement of six enzyme targets from two families, from PDOs derived from patient primary and metastatic tumor sites of six different patients, as listed in Table S1. Box plots represent the median and quartiles, the whiskers go down to the smallest values and the highest values. Data is a combination of four different technical replicates from one biological replicate, the whiskers go from the highest to the lowest point. All data points have been normalized to the internal control RPE. Relative activity Z-scores were calculated by normalizing further to Primary tumor – PDO1.

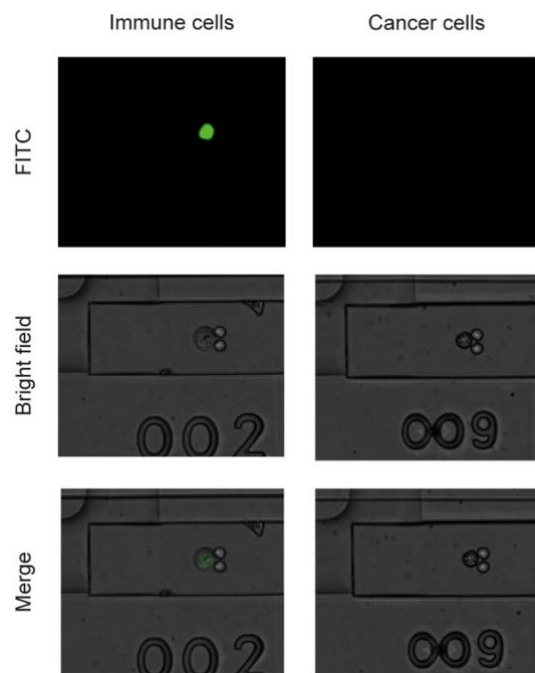


Fig. S6. On-Chip imaging can distinguish isolated immune and tumor cells. Dissociated single cells from fresh patient-derived organoids consist of heterogeneous mixture of immune cells and tumor cells. Cell staining with FITC-labeled anti-CD45 antibody prior to on-chip isolation and imaging enables per-cell identification, binning for restricted activity measurements from tumor cells.

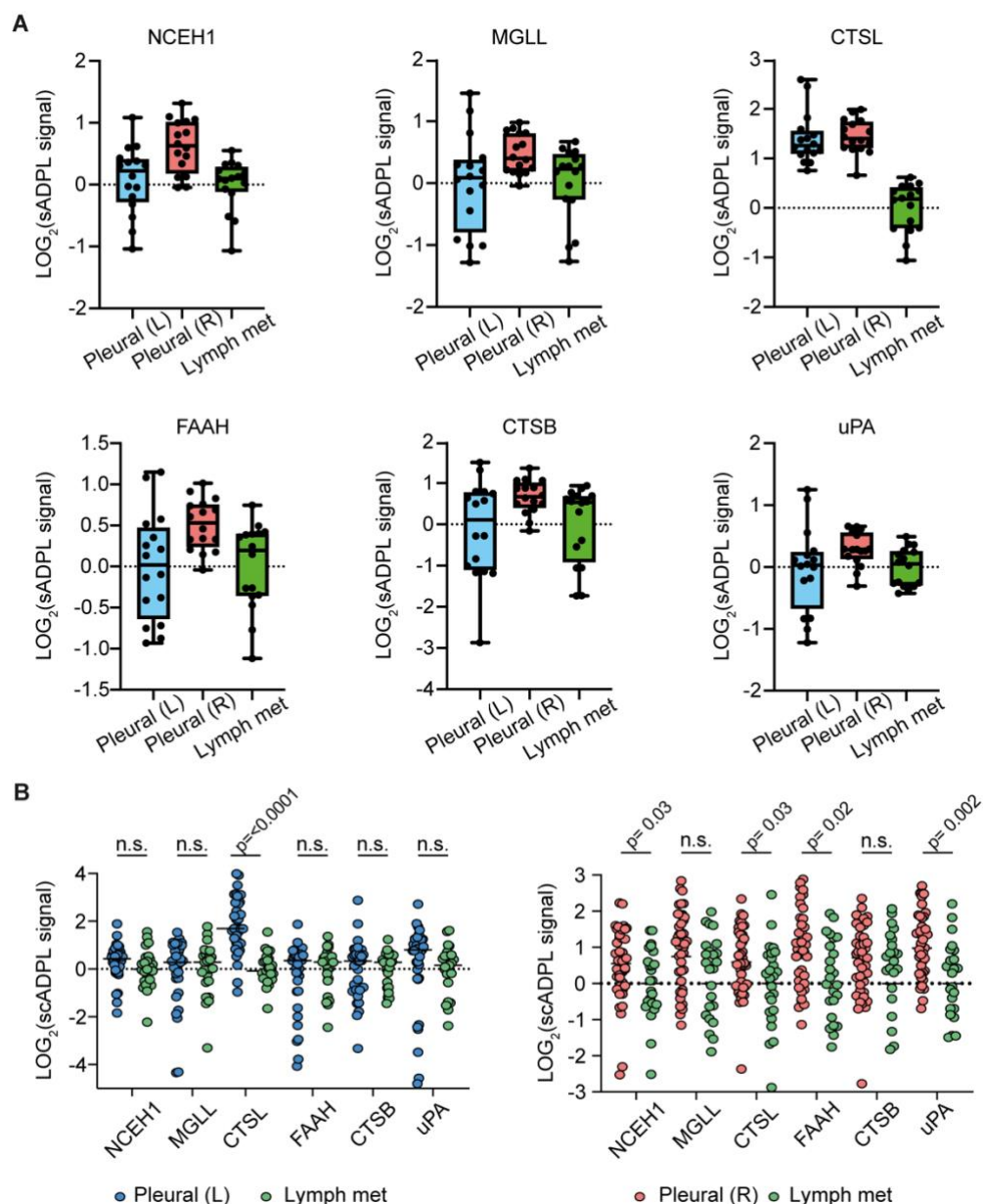


Fig. S7. Activity comparison of cancerous cells from different sites within an ER+ patient. **A)** Bulk sADPL activity measurements of Ag-6 enzyme targets compared using PDOs from multiple sites (pleural effusions during unique sample collections and lymph metastasis) on a single patient. Box plots represent the median and quartiles, the whiskers go down to the smallest values and up to the highest values. Data is representative from two biological replicates. **B)** scADPL activity comparison between dual-probe treated PDOs originating from different sites within an ER+ patient. Each point represents an individual cell and the line at the middle represents the mean of that group. Data is a representative from two biological replicates respectively. Data in **(B)** compared with Student's two-way t-test; n.s., not significant. All data points have been normalized to internal control RPE. Relative activity was calculated by further normalizing to Lymph met samples.

Table S1. Clinical features of patient-derived samples: Table below summarizes the a) specimen name of the sample, b) patient code, c) the procedure that was used to procure the specimen from the patients, d) information about stage and type of markers present in the sample. Note that pleural effusions for P0 were collected approximately one month apart and are thus unique biological specimens.

Specimen	Patient	Procedure Type	Race	Specimen Type	SUBTYPE
Pleural (L)	P0	Pleural Effusion/L	Not Spec	Pleural Effusion/ Left	ER+PR+HER2-
Pleural (R)	P0	Pleural Effusion/R	Not Spec	Pleural Effusion/ Right	ER+PR+HER2-
Lymph met	P0	LN Biopsy	Not Spec	Lymph Node Metastasis	ER+PR+HER2-
PDO1	P1	Breast Biopsy	Cauc	Breast Tumor	TNBC
PDO2	P2	Mastectomy	AA	Breast Tumor	TNBC
PDO3	P3	Liver Biopsy	White, Not	Liver Metastasis	TNBC
PDO4	P4	Liver Biopsy	Cauc	Liver Metastasis	TNBC
PDO5	P5	Lung Biopsy	White, Not	Lung Metastasis	TNBC
PDO6	P6	Skin Biopsy	Cauc	Breast Skin Metastasis	TNBC

Data S1. (Combined source data.xlsx)

Excel file containing source data for each figure in the manuscript. Tabs delineate source data for specific figures.