Supplementary Material April-Monn et al. (2020), *Neuroendocrinology*

Supplementary Table 1. Table summarizing clinical information of the primary PanNET patient cohort.

Supplementary Fig. 1.

(A) Association table showing estimated p-values from χ^2-test of independence using Monte Carlo simulation. Association was estimated from all variables of interest from all PanNET patients used in this study (n=16). Iso-Success = Isolation success; Age_gr = Age grouped; Grade_WHO= WHO tumor grading system; TNM_AJCC= American Joint Committee on Cancer TNM-staging system; T_stage= Tumor stage; N_stage= Lymph node stage; M_stage= Metastasis stage; Cell_yield= Cell yield at isolation

(B-D) Stainings of formalin fixed paraffin embedded (FFPE) original tumor tissue (hematoxylin-eosin (HE), left) and Micro-Cell-Blocks (HE or synaptophysin (SYN), right) of samples derived from necrotic or acellular fibrotic tissue (B), samples with fibroblast overgrowth (C), and samples with few target cells (D). All stainings were assessed by two pathologists (M.T., A.P.). Scale bar = 250 µm and 50 µm.

Supplementary Fig. 2.

(A) Mean expression values of growth factor receptors in 26 PanNET patients. RNAseq data form Scarpa *et al.* [2] was downloaded from the ICGC Data Portal (PAEN-AU project). FPKM normalization method was applied to raw gene counts. A list of all available growth factor receptors was acquired from the UniProt Knowledgebase [39]. Complete expression data of growth factor receptor is available in Supplementary Data Sheet 1. Data represent mean ± SD (n=26).

(B) Representative light micrographs of PanNET islet-like tumoroids from primary tumor (B992, left) and liver metastasis (B563m, right). Isolated cells were cultured in 24-well ultra-low attachment plates (ULA) for 14 days. Scale bar = 200 µm.

(C) Representative time points from 12 days live-cell imaging of primary human PanNET (B992). A clear formation of islet-like tumoroids can be observed after 72 hours. Snapshots were taken from Supplementary Video. Specific time points (hh:mm) are indicated in every image.

Supplementary Fig. 3

(A) IHC staining and quantification of NET specific biomarker synaptophysin (SYN) in formalin fixed paraffin embedded (FFPE) in original tumor tissues and respective Micro-Cell-Blocks (MCB) of all screened patients (n=7). Tumor content (%) in original tumor tissue (left) was assessed by a

cytopathologist (M.T.) followed by building a classifier in QuPath software [35] to automate quantification (right). MCBs were individually analyzed by a cytopathologist (M.T.) comparing hematoxylin-eosin (HE) and SYN staining to estimate tumor content (%). Data (left) represent mean+SD (n=1-2 tissue punches per patient). Scale bar = 100bμm (left), 50bμm (right). Tissue= Original tumor tissue.

(B) Bar graph quantifying percentage of synaptophysin positive (SYN+) cells in original tumor tissue and 3-D human primary PanNET culture at day of isolation (MCB D0) and at 15 days (MCB D15), respectively. Data represent mean ± SD (n=7 individual patients). Tissue= Original tumor tissue.

Supplementary Fig. 4

(A) Line graphs of all screened PanNET patients (n=7) displaying IC50 for sunitinib (SUN), everolimus (EVE), temozolomide (TEM) 7 days after treatment. Treatment responses were fitted into a 4-parameter logistic regression model in GraphPad software to calculate absolute IC50. Data represent mean ± SEM (n=1 per patient, 3 technical replicates). Dotted line = Absolute IC50.

(B) Heat map comparing absolute IC50 for SUN, EVE, and TEM in 3D human primary PanNET culture at short-term (3 days, _d3) and long-term (7 days, _d7) drug treatment. Heat map was derived using WardD2 clustering method with displaying Pearson's clustering distance using ComplexHeatmap R-package. Color code represents scaled IC50 (z-score) for each drug. Vertical dashed line displays k-value from consensus clustering analysis. Each row represents patient response at d3 or d7 of the treatment.

Supplementary Video

Upon single cell isolation of sample B992, cells were seeded in a 96-well ULA plate (5000 cells/well). After two days of recovery, the plate was transferred to the Cell-IQ® (CM Technologies Oy, Tampere, Finland), a fully integrated incubator (37°C, 21% O_2, 5% CO2) including an image acquisition system. Phase contrast images (20x) were captured with an integrated CDD camera every two hours for a duration of 235 hours with pre-defined positions. Images were processed using the Cell-IQ Analyser™ Cell Activation (Yokogawa) software.

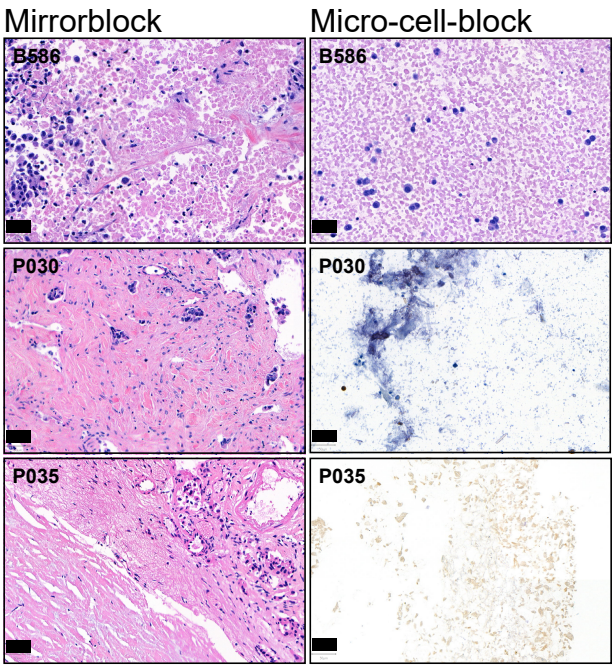
	Age	Grade [WHO]	Sex	Ki67 [%]	Size [cm]	T	N	M	TNM-staging [ENETS]	Site	Hormone secretion	Cohort
P005	65	G2	female	3	3	T3	N1	M0	3B	pancreas	Insulin	Method
B563m	65	G2	male	15	18.1	T4	NA	M1	4	liver	NF	Screen/Method
B586m	48	G2	female	5	2.5	NA	NA	M1	4	liver	NF	Method
B719	63	G2	female	5	5.5	T4	NA	M1	4	liver	NF	Method
B849	64	G1	female	1	2.4	T2	N0	M0	2A	pancreas	NF	Method
B931	46	G2	female	5	2.8	T1	N0	M0	1	pancreas	NF	Method
B992	81	G1	female	<2	2.3	T1	N0	M0	1	pancreas	NF	Method
P030	79	G2	male	7	4	T2	N1	M0	3A	pancreas	NF	Method
P032x	49	G3	male	25	3.2	T2	N1	M0	3A	pancreas	NF	Method
P033	67	G2	male	7	3	T2	N1	M0	3A	pancreas	NF	Method
P035	42	G2	male	4	2.2	T2	N0	M0	2A	pancreas	NF	Method
P040	55	G2	female	10	2.5	T2	N0	M0	2A	pancreas	NF	Screen
P044	19	G2	female	18	3,5	T3	N1	M0	3A	pancreas	NF	Screen
P049	66	G1	female	1	3.5	T2	N0	M0	2A	pancreas	NF	Screen
P050	58	G1	male	<1	2.5	T2	N1	M0	3A	pancreas	NF	Screen
P051	25	G1	female	<1	7.5	T3	N0	M0	2B	pancreas	NF	Screen/Method

Fig. S1

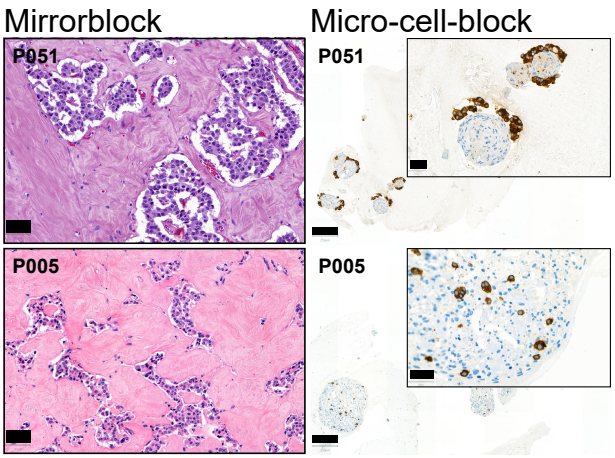
A

Iso_Success									
0.4	Age_gr								
0.1	0.8	Sex							
0.09	0.8	0.3	Grade_WHO						
0.4	1	0.4	0.5	TNM_AJCC					
0.6	0.8	0.2	0.9	2e−04	T_stage				
0.1	1	0.1	0.3	7e−04	0.6	N_stage			
1	0.8	1	1	0.3	0.6	0.6	M_stage		
0.007	0.05	0.6	0.3	0.2	0.07	0.1	1	Cell_Yield	
0.04	1	0.1	0.7	0.6	0.8	1	1	0.2	Tissue

B



C



D

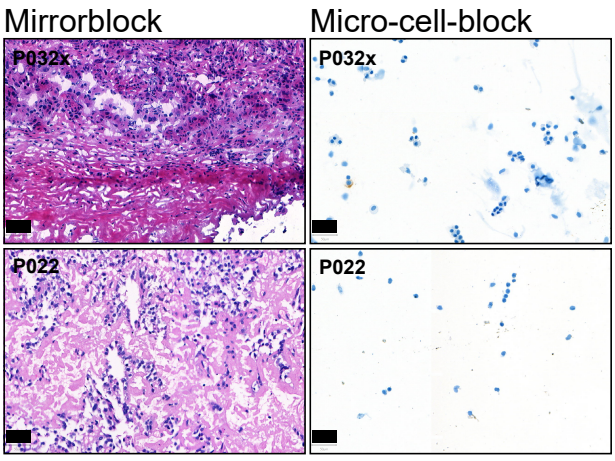
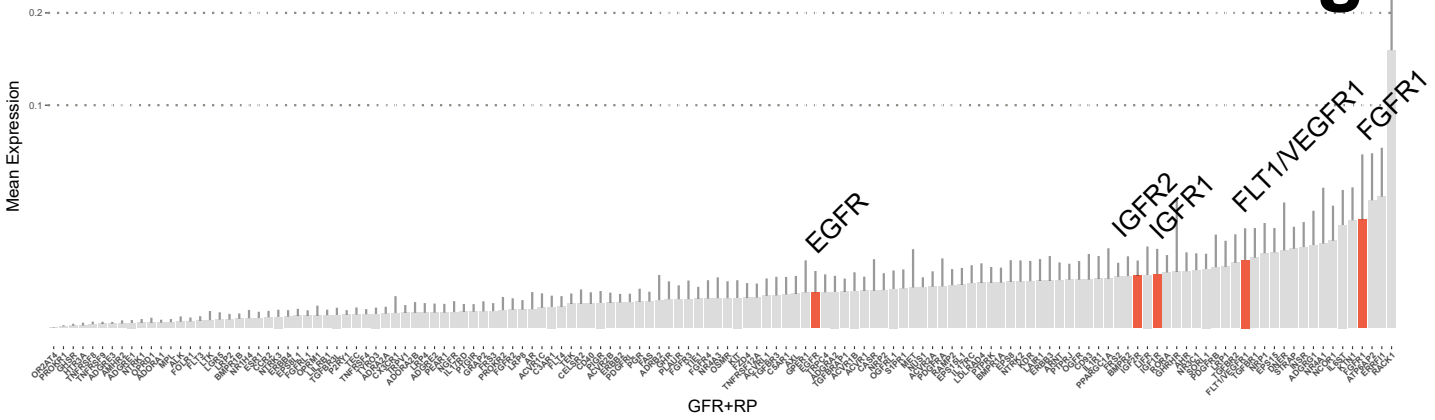
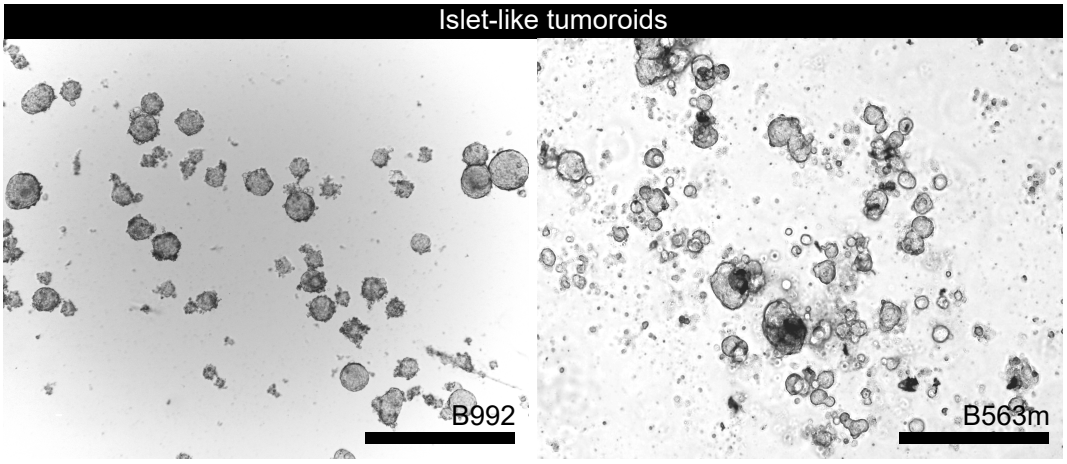


Fig. S2

A


Mean Expression
0.2
0.1
EGFR
IGFR2
IGFR1
FLT1/VEGFR1
FGFR1
GFR+RP

B


Islet-like tumoroids
B992
B563m

C

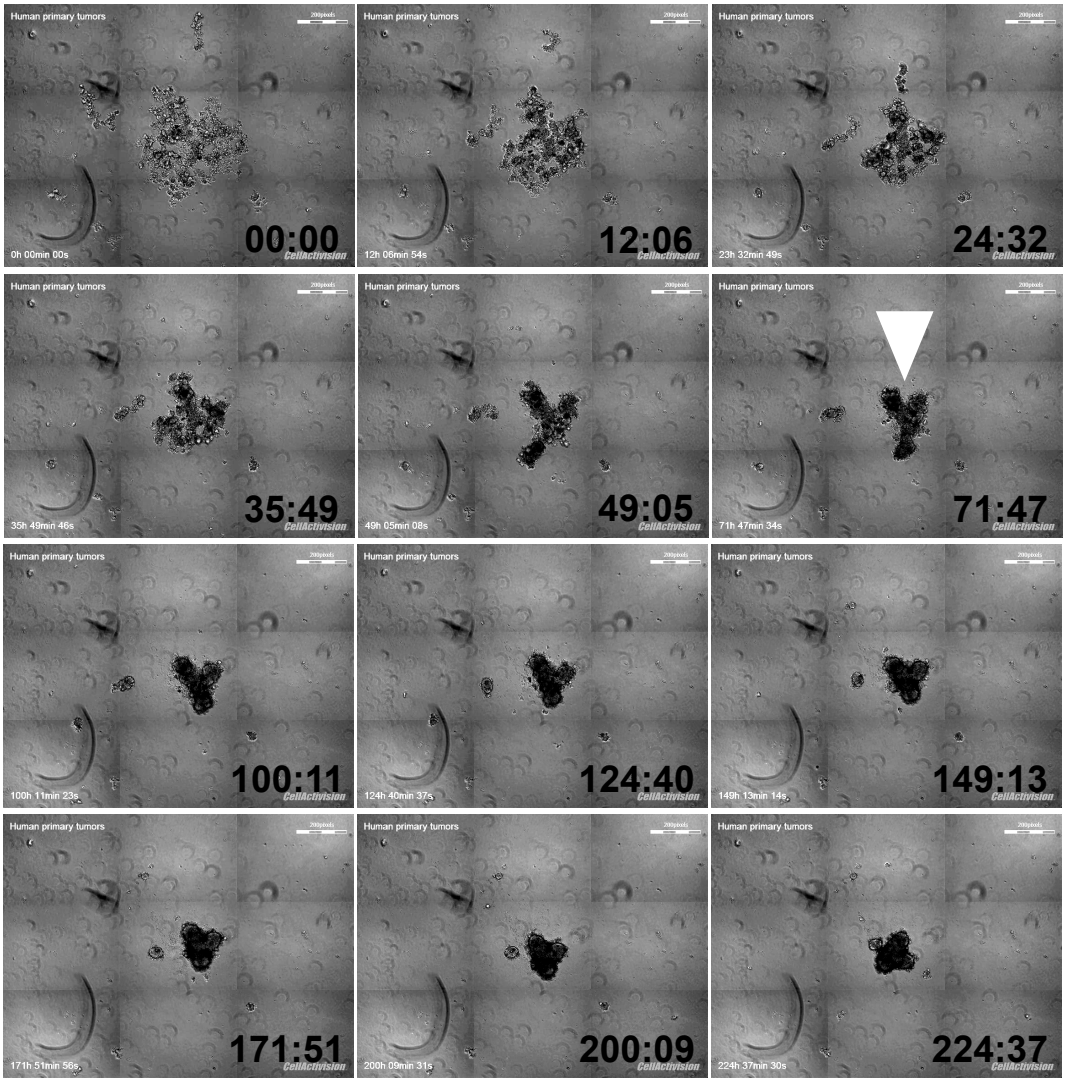
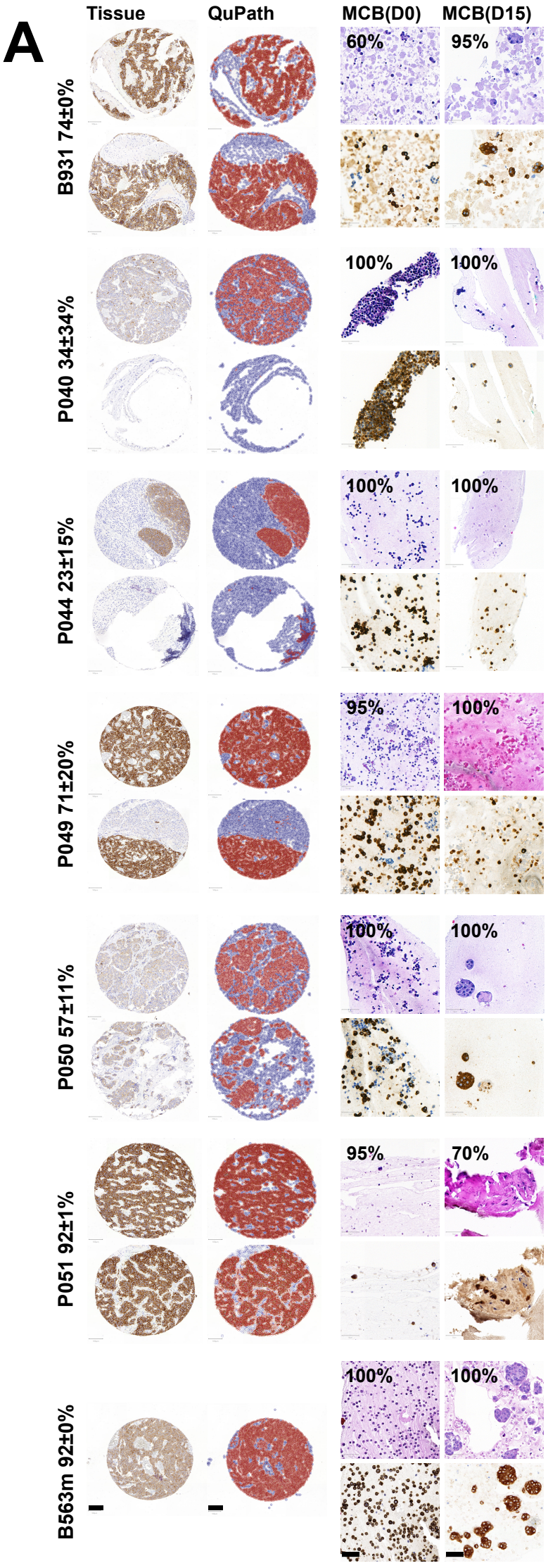

Human primary tumors
00:00
12:06
24:32
35:49
49:05
71:47
100:11
124:40
149:13
171:51
200:09
224:37

Fig. S3


A
Tissue
QuPath
MCB(D0)
MCB(D15)
B931 74±0%
P040 34±34%
P044 23±15%
P049 71±20%
P050 57±11%
P051 92±1%
B563m 92±0%
60%
95%
100%
100%
100%
100%
95%
100%
100%
100%
95%
70%
100%
100%

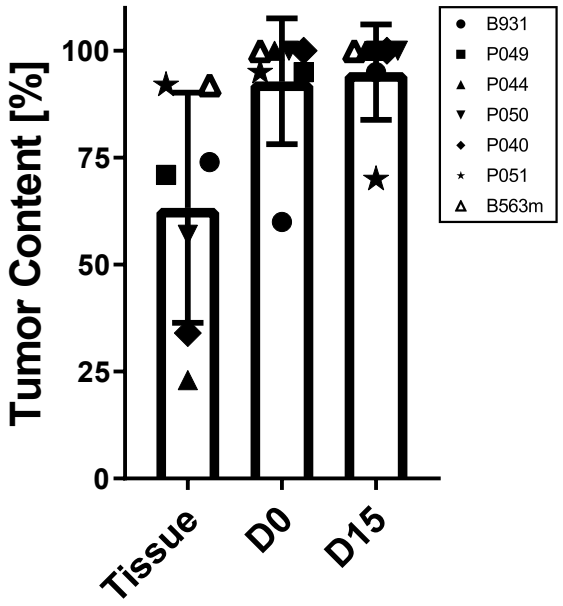
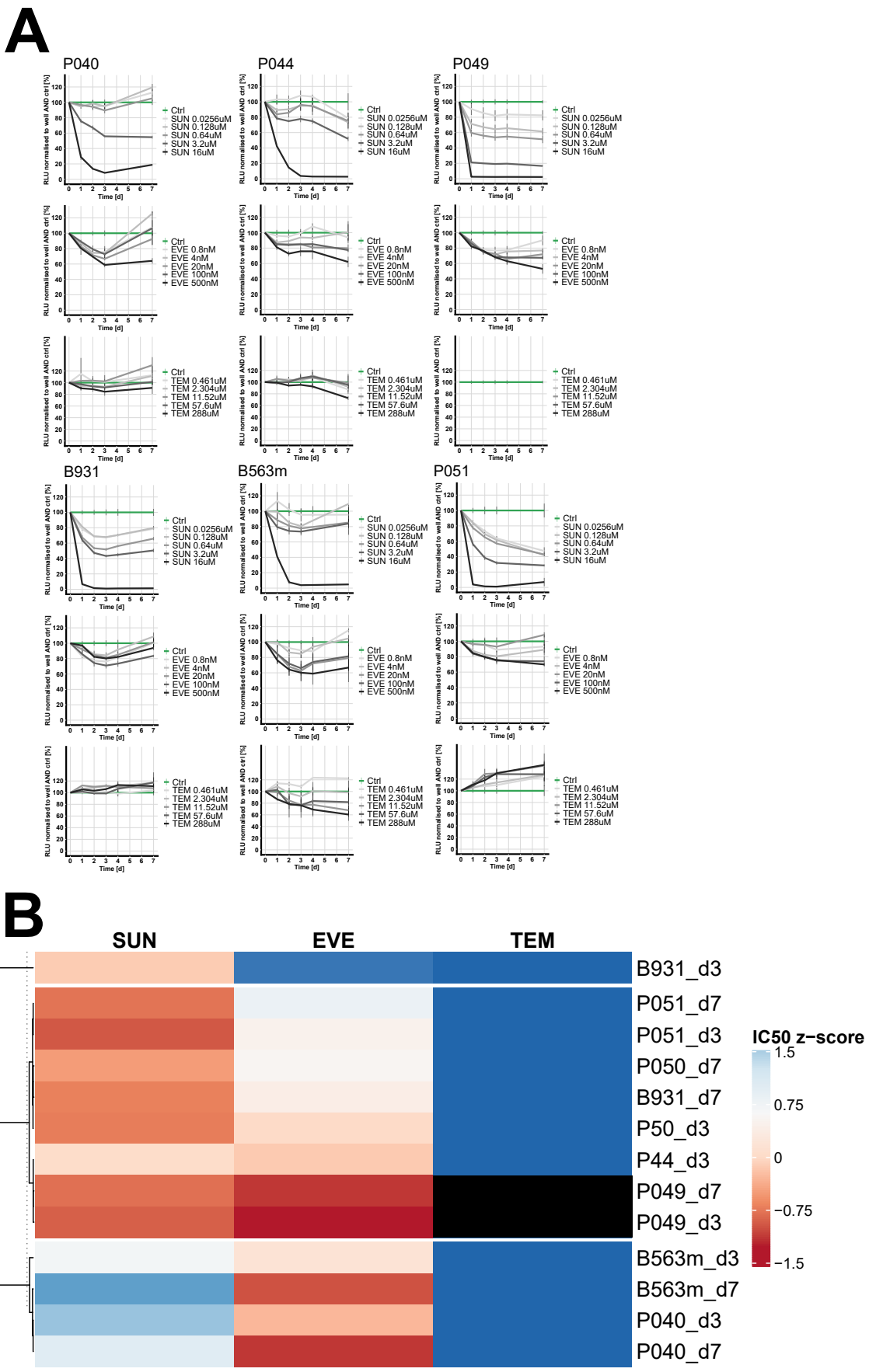

B
Tumor Content [%]
100
75
50
25
0
Tissue
D0
D15
B931
P049
P044
P050
P040
P051
B563m

Fig. S4


A
P040
P044
P049
B931
B563m
P051
RLU normalised to well AND ctrl [%]
Time [d]
Ctrl
SUN 0.0256uM
SUN 0.128uM
SUN 0.64uM
SUN 3.2uM
SUN 16uM
EVE 0.8nM
EVE 4nM
EVE 20nM
EVE 100nM
EVE 500nM
TEM 0.461uM
TEM 2.304uM
TEM 11.52uM
TEM 57.6uM
TEM 288uM
B
SUN
EVE
TEM
B931_d3
P051_d7
P051_d3
P050_d7
B931_d7
P50_d3
P44_d3
P049_d7
P049_d3
B563m_d3
B563m_d7
P040_d3
P040_d7
IC50 z−score
1.5
0.75
0
−0.75
−1.5