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Supplementary Materials for

Clonal dominance defines metastatic dissemination in pancreatic cancer

I-Lin Ho *et al.*

Corresponding author: Andrea Viale, aviale@mdanderson.org; I-Lin Ho, iho@mdanderson.org; Chieh-Yuan Li, alexcyli2015@gmail.com

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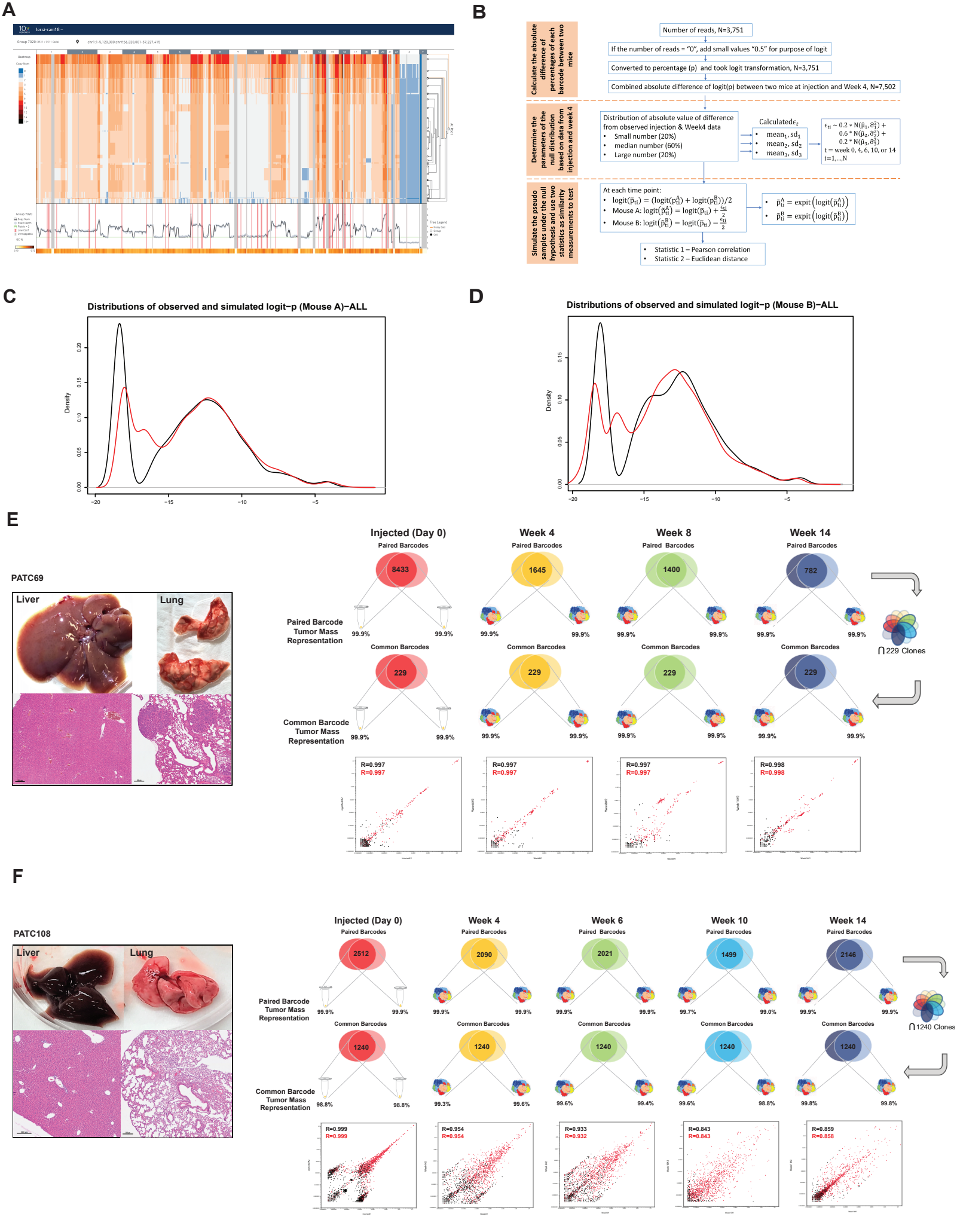
Figs. S1 to S6

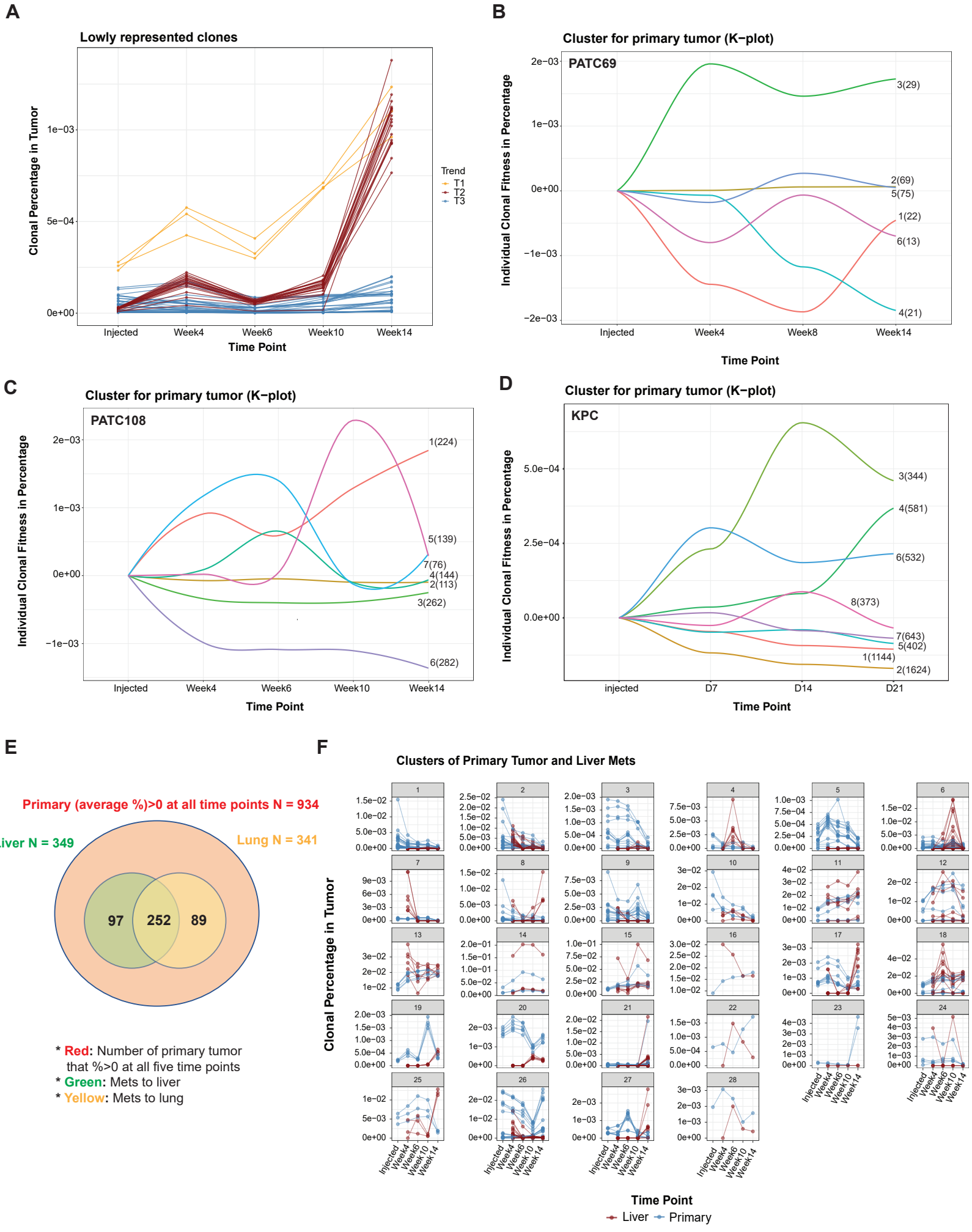
Fig. S1


A
B
Calculate the absolute difference of percentages of each barcode between two mice
Number of reads, N=3,751
If the number of reads = "0", add small values "0.5" for purpose of logit
Converted to percentage (p) and took logit transformation, N=3,751
Combined absolute difference of logit(p) between two mice at injection and Week 4, N=7,502
Determine the parameters of the null distribution based on data from injection and week 4
Distribution of absolute value of difference from observed injection & Week4 data
Small number (20%)
median number (60%)
Large number (20%)
Calculated
Simulate the pseudo samples under the null hypothesis and use two statistics as similarity measurements to test
At each time point:
Mouse A:
Mouse B:
Statistic 1 – Pearson correlation
Statistic 2 – Euclidean distance
C
Distributions of observed and simulated logit−p (Mouse A)−ALL
Density
D
Distributions of observed and simulated logit−p (Mouse B)−ALL
Density
E
PATC69
Liver
Lung
Injected (Day 0)
Week 4
Week 8
Week 14
Paired Barcodes
8433
1645
1400
782
99.9%
Paired Barcode Tumor Mass Representation
Common Barcodes
229
Common Barcode Tumor Mass Representation
∩229 Clones
R=0.997
R=0.997
R=0.998
R=0.998
F
PATC108
Liver
Lung
Injected (Day 0)
Week 4
Week 6
Week 10
Week 14
Paired Barcodes
2512
2090
2021
1499
2146
99.9%
99.7%
99.0%
Paired Barcode Tumor Mass Representation
Common Barcodes
1240
98.8%
99.3%
99.6%
99.4%
99.8%
Common Barcode Tumor Mass Representation
∩1240 Clones
R=0.999
R=0.999
R=0.954
R=0.954
R=0.933
R=0.932
R=0.843
R=0.843
R=0.859
R=0.858

Supplementary Figure 1. oCRTs capture unperturbed clonal dynamics in different PDX-derived cells.

a, Single-cell CNV analysis of PATC124 cells through 10x Genomics platform (n=3,511 cells). Columns indicate chromosome number. Red, copy number gain; blue, copy number loss; bottom, average CNV profile of all cells; right, clustering of detected subclones. **b**, Flow of barcode data processing, simulation and resampling bootstrap test for PATC124 oCRTs. **c**,**d,** Observed distribution of logit-transformed percentages of two PATC124 oCRT samples (mouse A (**c**) and B (**d**)) for all timepoints were combined (black line). At each timepoint, assuming one randomly picked mouse as A and another one as B. The simulated distributions for all timepoints were combined and shown in red. Assuming $logit(\bar{p}_{ti}) = (logit(p_{ti}^{A}) + logit(p_{ti}^{B}))/2$; $logit(\tilde{p}_{ti}^{A}) = logit(\bar{p}_{ti}) + \frac{\epsilon_{ti}}{2}, logit(\tilde{p}_{ti}^{B}) = logit(\bar{p}_{ti}) - \frac{\epsilon_{ti}}{2}$ and $\epsilon_{ti} \sim 0.2 \times N(\hat{\mu}_1, \hat{\sigma}_1^2) + 0.6 \times N(\hat{\mu}_2, \hat{\sigma}_2^2) + 0.2 \times N(\hat{\mu}_3, \hat{\sigma}_3^2)$, timepoint t = day 0 or 4, 6, 10 or 14 weeks; barcode i = 1,…,*N*; **e**, Left: metastatic organotropism of PATC69 transplanted mice. After three months, livers and lungs were collected for gross anatomical analysis (upper quadrants) and histological analysis (lower quadrants) (Scale bar = 200 μm). Right: clonal correlation among PATC69 orthotopic clonal replica tumors (oCRTs) at different time points. Top, paired barcoded lineages shared by primary tumors at each time point and their relative representation (%) of tumor mass; middle, set of 229 barcodes common to all tumors and their relative representation (%) of tumor mass; bottom, scatter plots displaying representation of individual lineages derived from n = 2 tumors collected at each time point (red, set of common clones). **f,** Left: metastatic organotropism of PATC108 transplanted mice. After three months, livers and lungs were collected for gross anatomical analysis (upper quadrants) and histological analysis (lower quadrants) (Scale bar = 200 μm). Right: clonal correlation among PATC108 oCRTs at different time points. Top, paired barcoded lineages shared by primary tumors at each time point and their relative representation (%) of tumor mass; middle, set of 1240 barcodes common to all tumors and their relative representation (%) of tumor mass; bottom, scatter plots displaying representation of individual lineages derived from n = 2 tumors collected at each time point (red, set of common clones).

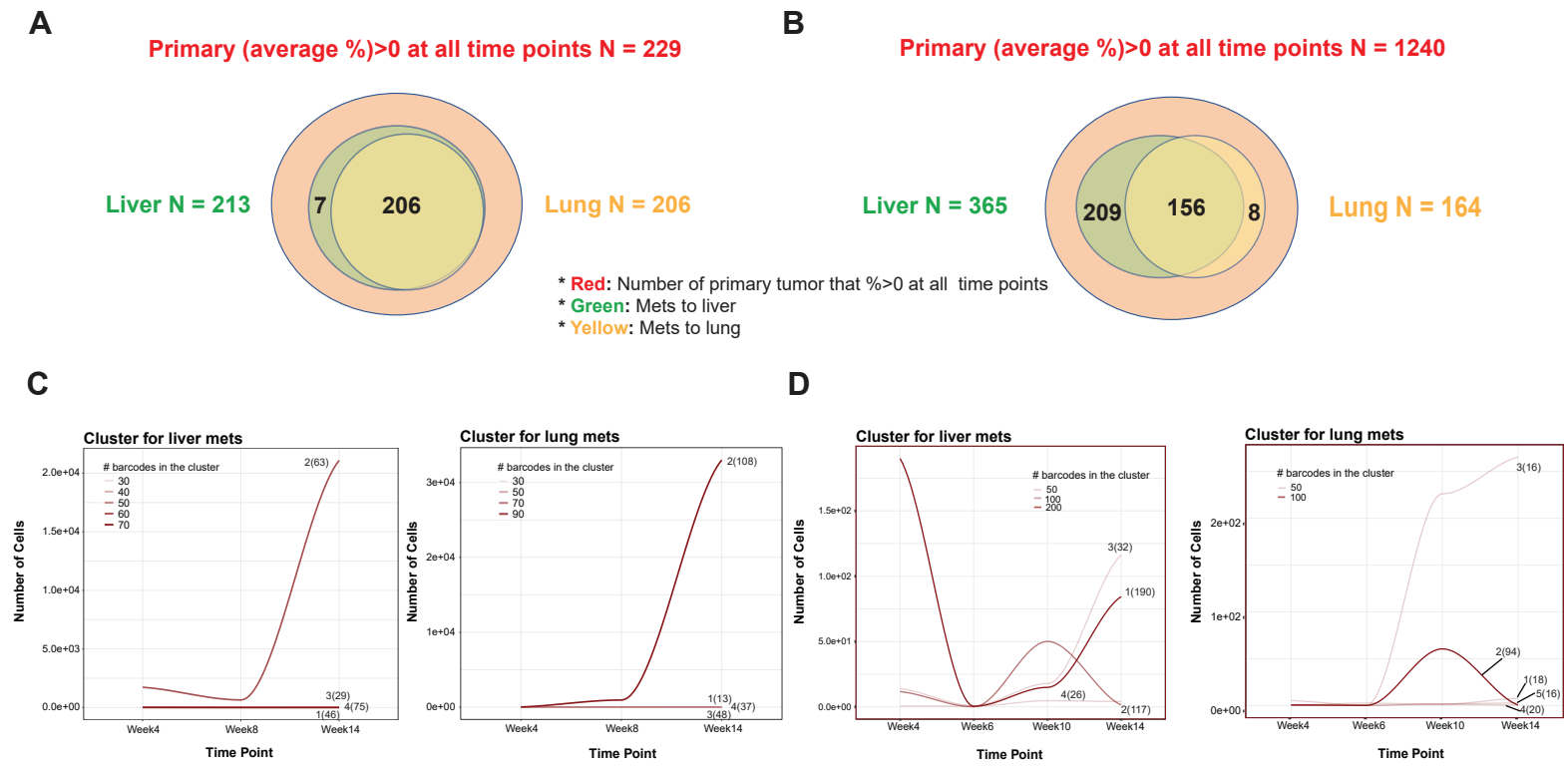
Fig. S2


A
Lowly represented clones
Clonal Percentage in Tumor
1e−03
5e−04
0e+00
Injected
Week4
Week6
Week10
Week14
Time Point
Trend
T1
T2
T3
B
Cluster for primary tumor (K−plot)
PATC69
Individual Clonal Fitness in Percentage
2e−03
1e−03
0e+00
−1e−03
−2e−03
3(29)
2(69)
5(75)
1(22)
6(13)
4(21)
Injected
Week4
Week8
Week14
Time Point
C
Cluster for primary tumor (K−plot)
PATC108
Individual Clonal Fitness in Percentage
2e−03
1e−03
0e+00
−1e−03
1(224)
5(139)
7(76)
4(144)
2(113)
3(262)
6(282)
Injected
Week4
Week6
Week10
Week14
Time Point
D
Cluster for primary tumor (K−plot)
KPC
Individual Clonal Fitness in Percentage
5.0e−04
2.5e−04
0.0e+00
3(344)
4(581)
6(532)
8(373)
7(643)
5(402)
1(1144)
2(1624)
injected
D7
D14
D21
Time Point
E
Primary (average %)>0 at all time points N = 934
Liver N = 349
Lung N = 341
97
252
89
* Red: Number of primary tumor that %>0 at all five time points
* Green: Mets to liver
* Yellow: Mets to lung
F
Clusters of Primary Tumor and Liver Mets
Clonal Percentage in Tumor
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
Injected
Week4
Week6
Week10
Week14
Time Point
Liver
Primary

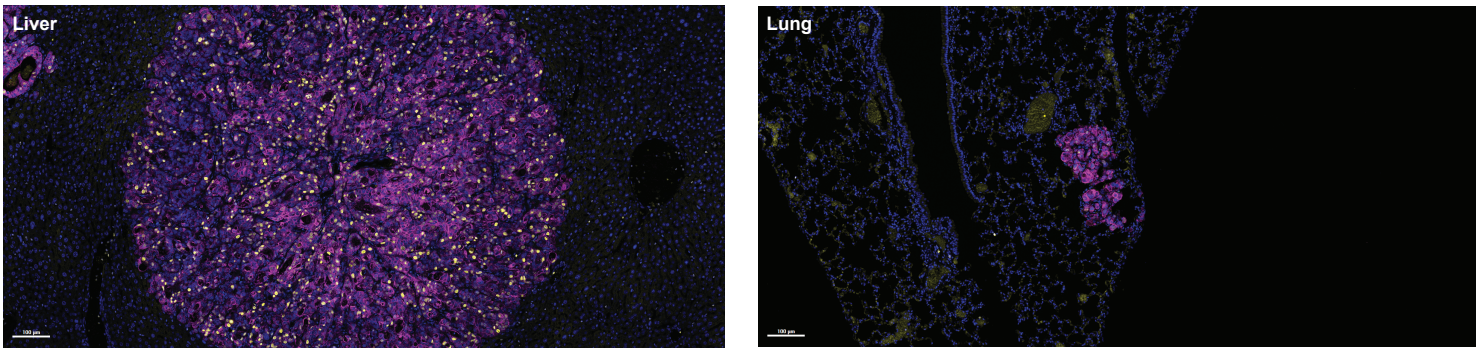
Supplementary Figure 2. Different pancreatic cancer models show ACD during tumor expansion

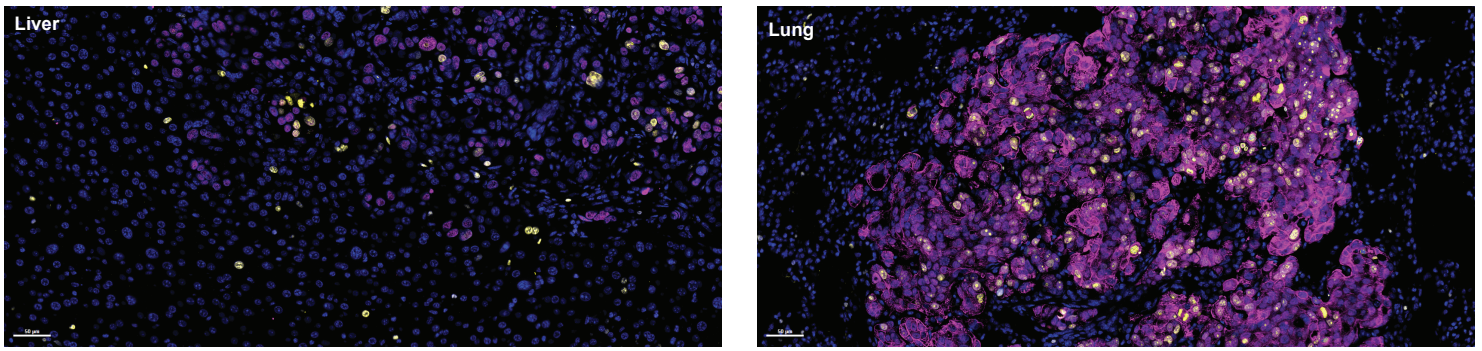
a, Clonal dynamics of lowly represented PATC124 clones. Each line represents growth dynamics of an individual barcode. **b-d,** Barcode analysis of n = 2 PATC69 (**b**), PATC108 (**c**) and KPC (**d**) tumors per timepoint was weighted smoothed and normalized to injected samples. Barcodes were grouped into discrete dynamic growth patterns (clusters) and plotted as percent of total tumor over time (colored lines). On the right of each line, the numbers outside brackets indicate the cluster name, and numbers within brackets indciate the number of barcodes within the cluster. **e,** Venn diagram of the number of clones detected in the primary tumor, liver, and lungs of PATC124 at least at one time point. **f,** Dynamic growth of PATC124 subclones in the primary tumor (blue) overlaid with their behavior in the liver (red).

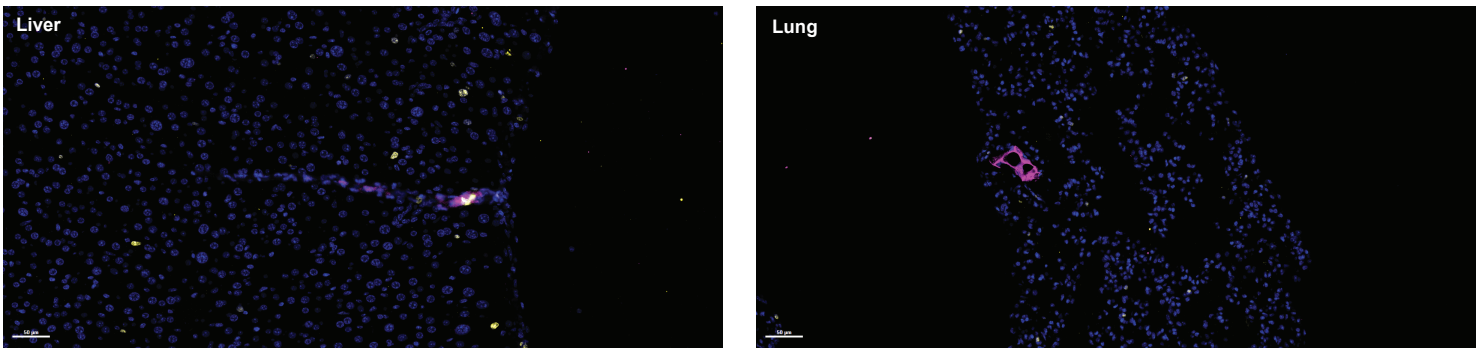
Fig. S3


A
Primary (average %)>0 at all time points N = 229
Liver N = 213
7
206
Lung N = 206
B
Primary (average %)>0 at all time points N = 1240
Liver N = 365
209
156
8
Lung N = 164
* Red: Number of primary tumor that %>0 at all time points
* Green: Mets to liver
* Yellow: Mets to lung

C
Cluster for liver mets
barcodes in the cluster
30
40
50
60
70
2(63)
3(29)
4(75)
1(46)
Number of Cells
Time Point
Week4
Week8
Week14
Cluster for lung mets
barcodes in the cluster
30
50
70
90
2(108)
1(13)
4(37)
3(48)
D
Cluster for liver mets
barcodes in the cluster
50
100
200
3(32)
1(190)
4(26)
2(117)
Cluster for lung mets
barcodes in the cluster
50
100
3(16)
2(94)
1(18)
5(16)
4(20)
Week6
Week10


E
Liver
Lung

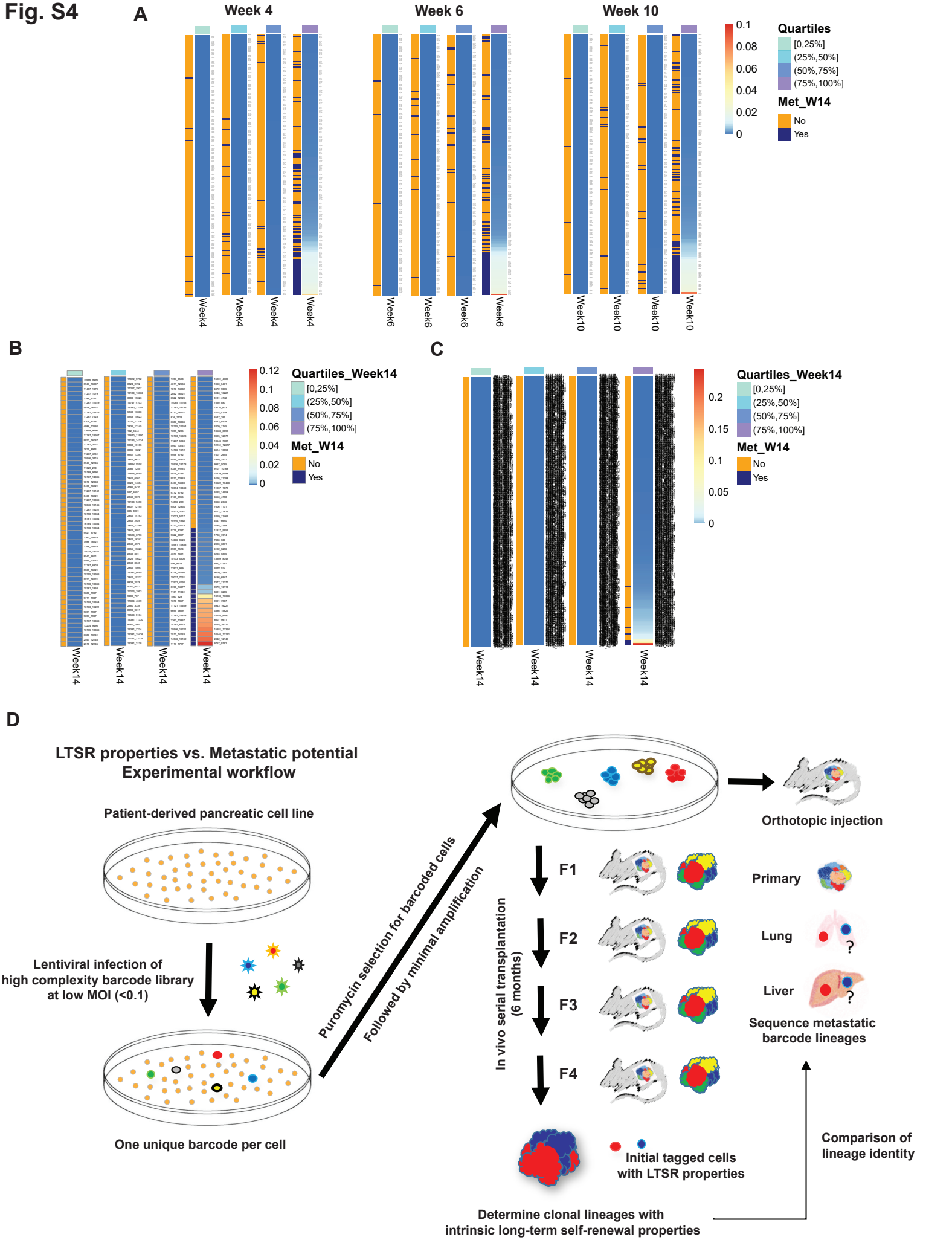

F
Liver
Lung


G
Liver
Lung

Supplementary Figure 3. PDX-derived models exhibit distinct organotropisms

a,b, Venn diagram of the number of clones detected in the primary tumor, liver, and lungs of PATC69 (**a**) and PATC108 (**b**) at least at one time point. **c**,**d**, Dynamic subclonal growth of PATC69 (**c**) and PATC108 (**d**) in liver and lung. Cell number at each time point is converted based on spike-in scales. Lineage behavior was grouped into discrete dynamic growth patterns (clusters) over time. The numbers outside the brackets indicate the cluster name, and the number within the brackets indicate the exact number of barcodes within the cluster (darker colors are associated with larger number of barcodes within the cluster). **e-g,** Liver and lungs from PATC124 (**e**), PATC69 (**f**) and PATC108 (**g**) mice at 14 weeks were harvested and stained with anti-HLA (magenta) and anti-Ki67 (yellow). Cell nuclei were stained with DAPI (blue). Scale bar=100 μm for PATC124; scale bar=50 μm for PATC69 and PATC108.

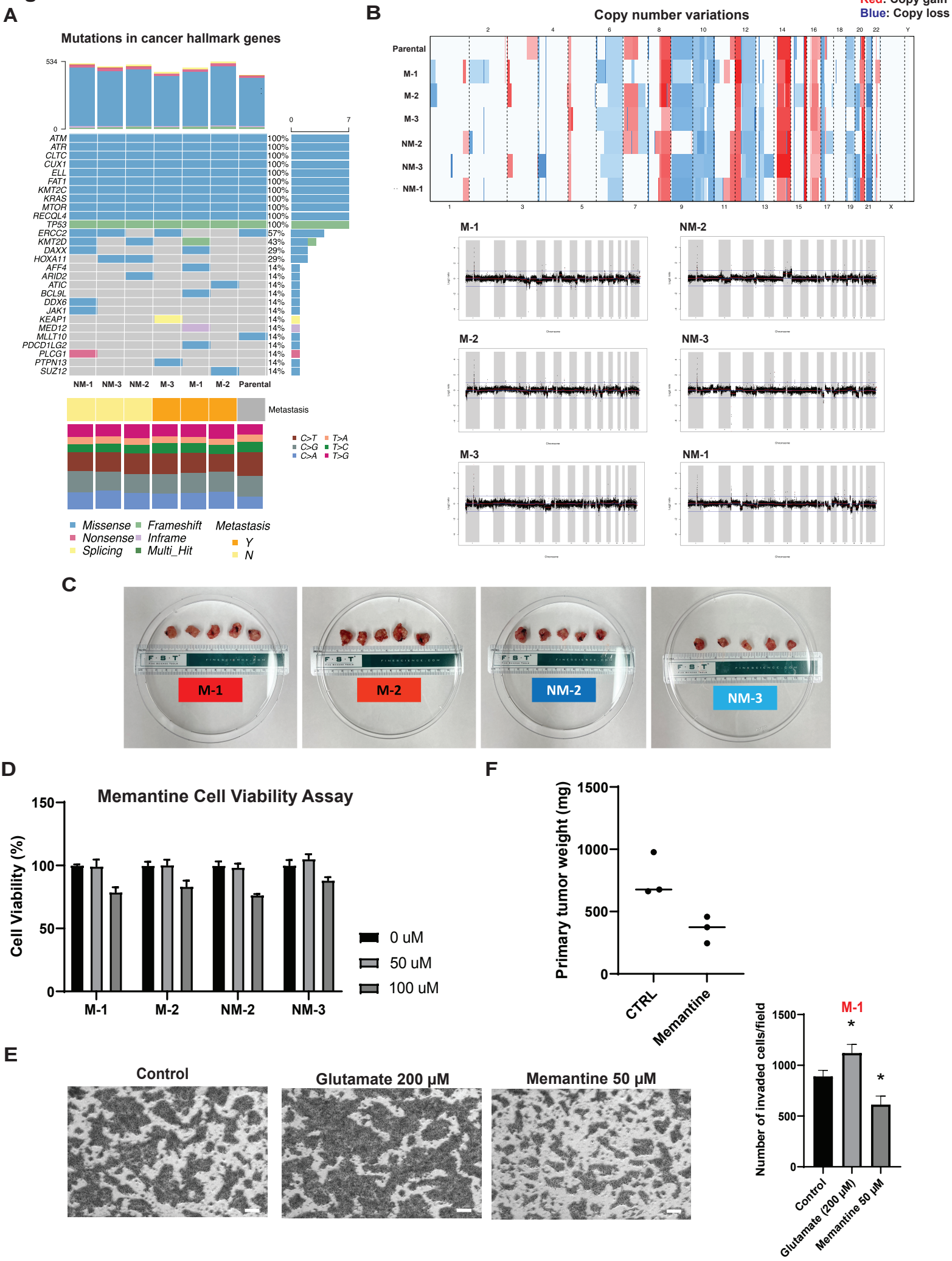
Fig. S4


A
Week 4
Week 6
Week 10
Week4
Week6
Week10
0.1
0.08
0.06
0.04
0.02
0
Quartiles
[0,25%]
(25%,50%]
(50%,75%]
(75%,100%]
Met_W14
No
Yes
B
Week14
0.12
0.1
0.08
0.06
0.04
0.02
0
Quartiles_Week14
[0,25%]
(25%,50%]
(50%,75%]
(75%,100%]
Met_W14
No
Yes
C
Week14
0.2
0.15
0.1
0.05
0
Quartiles_Week14
[0,25%]
(25%,50%]
(50%,75%]
(75%,100%]
Met_W14
No
Yes
D
LTSR properties vs. Metastatic potential
Experimental workflow
Patient-derived pancreatic cell line
Lentiviral infection of
high complexity barcode library
at low MOI (<0.1)
One unique barcode per cell
Puromycin selection for barcoded cells
Followed by minimal amplification
Orthotopic injection
In vivo serial transplantation
(6 months)
F1
F2
F3
F4
Primary
Lung
Liver
Sequence metastatic
barcode lineages
Initial tagged cells
with LTSR properties
Comparison of
lineage identity
Determine clonal lineages with
intrinsic long-term self-renewal properties

Supplementary Figure 4. Clonal abundance is highly correlated with metastatic potential

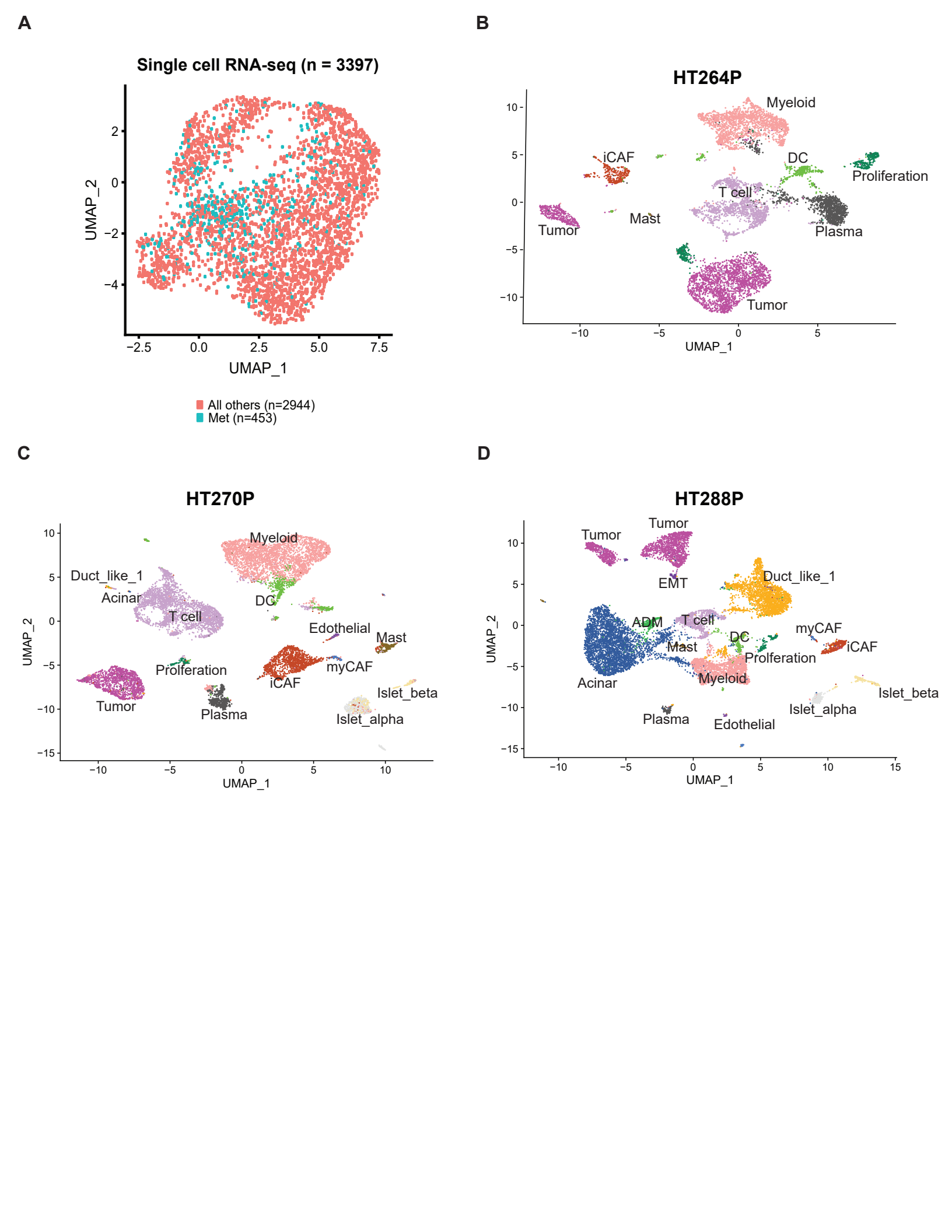
a, Heatmap of subclonal abundance (percentage) in the PATC124 primary tumor at weeks 4, 6 and 10 with metastasis status (yes >200 tumor cells / no <200 tumor cells) at week 14. Quartiles are as defined in Fig. 2. Metastasis of each subclone is indicated on the left of each heat map in quartiles (yellow, no dissemination; blue, dissemination). **b**,**c**, Heatmap of average subclonal abundance (percentage) in the PATC69 (**b**) and PATC108 (**c**) primary tumors with metastasis status (yes >200 tumor cells / no <200 tumor cells) at week 14. Barcodes were divided into quartiles (0%–25%, 25%–50%, 50%–75%, and 75%–100%) on the basis of their percent of total tumor mass. Metastasis of each subclone is indicated on the left of each heat map in quartiles (yellow, no dissemination; blue, dissemination). **d**, Experimental workflow of parallel metastatic potential and LTSR studies. PATC124 cells were barcoded with a high-complexity barcode library at low MOI and selected with puromycin. The barcoded cells were minimally amplified to achieve uniformity across barcoded clones as well as cell number. After taking an aliquot for baseline barcode analysis, the barcoded cells were split in half for the two studies. For the metastatic potential study, n = 3 animals received orthotopic injection in the pancreas, and animals were euthanized after 3 months to collect primary tumor, liver, and lungs for NGS barcode analysis. For the LTSR study, n = 3 animals were injected in the F1 generation followed by serial transplantation into recipient animals every 1.5 months through F4 animals (total duration of study was 6 months). Primary tumors were collected for NGS barcode analysis from all animals in generations F1-F4.

Fig. S5


A
Mutations in cancer hallmark genes
534
0
0
7
ATM 100%
ATR 100%
CLTC 100%
CUX1 100%
ELL 100%
FAT1 100%
KMT2C 100%
KRAS 100%
MTOR 100%
RECQL4 100%
TP53 100%
ERCC2 57%
KMT2D 43%
DAXX 29%
HOXA11 29%
AFF4 14%
ARID2 14%
ATIC 14%
BCL9L 14%
DDX6 14%
JAK1 14%
KEAP1 14%
MED12 14%
MLLT10 14%
PDCD1LG2 14%
PLCG1 14%
PTPN13 14%
SUZ12 14%
NM-1 NM-3 NM-2 M-3 M-1 M-2 Parental
Metastasis
C>T T>A
C>G T>C
C>A T>G
Missense Frameshift
Nonsense Inframe
Splicing Multi_Hit
Metastasis
Y
N
B
Copy number variations
Red: Copy gain
Blue: Copy loss
Parental
M-1
M-2
M-3
NM-2
NM-3
NM-1
M-1
NM-2
M-2
NM-3
M-3
NM-1
C
M-1
M-2
NM-2
NM-3
D
Memantine Cell Viability Assay
Cell Viability (%)
150
100
50
0
M-1 M-2 NM-2 NM-3
0 uM
50 uM
100 uM
F
Primary tumor weight (mg)
1500
1000
500
0
CTRL
Memantine
M-1
Number of invaded cells/field
1500
1000
500
0
*
*
Control
Glutamate (200 μM)
Memantine 50 μM
E
Control
Glutamate 200 μM
Memantine 50 μM

Supplementary Figure 5. Genomic and functional characterization of isolated clones

a, 6 PATC124 isogenic clones with differential metastasis potential and their parental population were submitted for WES to identify mutations in hallmark cancer genes. **b**, CNV profiling results from the 6 PATC124 isogenic clones and parental cells compared to a normal diploid genome. Data are shown in aberration plot (top, copy number gain in red and copy number loss in blue) and CNV plot (bottom; Log2 ratio; red lines indicate copy segments). **c**, Monoclonal primary tumor images of 2 metastatic (M-1 and M-2) and two non-metastatic (NM-2 and NM-3) PATC124 clones. **d**, MTT assay to evaluate effects of a range of memantine concentrations on viability of PATC124 clones. Data are presented as mean ± SD of two independent experiments**. e**, Representative images of invasion assay of PATC124 M-1 metastatic clone grown with glutamate (NMDAR agonist) or memantine (NMDAR antagonist). Quantification of invaded cells per field is shown as mean ± SD of two independent experiments. Statistical significance was assessed using one-way ANOVA. Scale bar = 200 μm. **f**, PATC124 bi-clonal (M-1 and M-2) primary tumor weight of untreated control and memantine treated group as in figure 5h.


A
Single cell RNA-seq (n = 3397)
UMAP_2
UMAP_1
All others (n=2944)
Met (n=453)
B
HT264P
Myeloid
iCAF
DC
Proliferation
T cell
Mast
Tumor
Plasma
Tumor
UMAP_1
C
HT270P
Myeloid
Duct_like_1
Acinar
DC
T cell
Edothelial
Mast
Proliferation
myCAF
iCAF
Tumor
Plasma
Islet_beta
Islet_alpha
UMAP_2
UMAP_1
D
HT288P
Tumor
Tumor
EMT
Duct_like_1
ADM
T cell
myCAF
DC
Mast
iCAF
Prolferation
Acinar
Myeloid
Islet_beta
Islet_alpha
Plasma
Edothelial
UMAP_2
UMAP_1

Supplementary Figure 6. scRNA-seq of different human pancreatic cancer cells

a, The 100 most enriched and 100 most downregulated genes from transcriptomic analysis as in figure 5a were used to derive a pro-met signature. ScRNA-seq analysis of PATC124 cells (n = 3,397) was used to identify clones with metastatic potential. **b-d,** Overview of all cell types profiled in the scRNA-seq cohorts as in Figure 6a-c.