Supplemental Figures

High-throughput imaging assay for drug screening of 3D prostate cancer organoids

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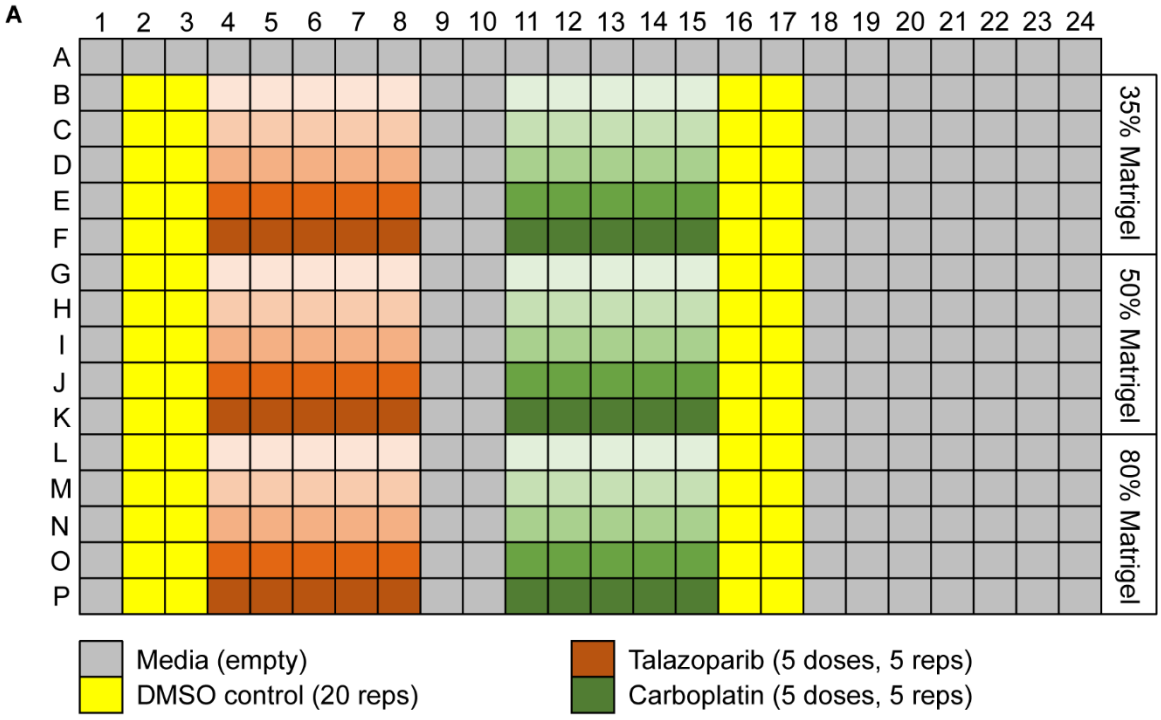
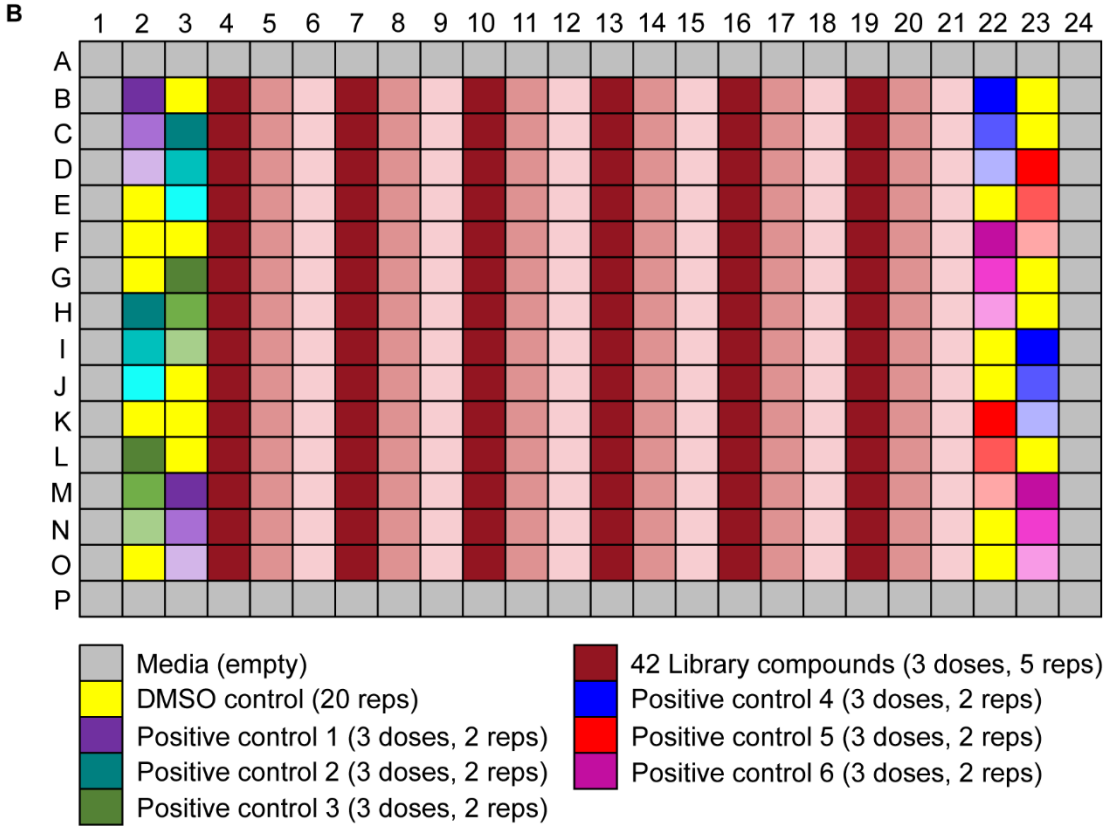
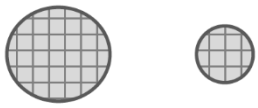
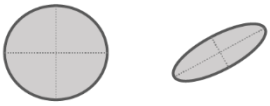
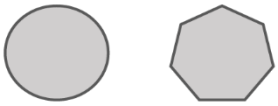
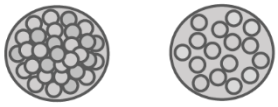
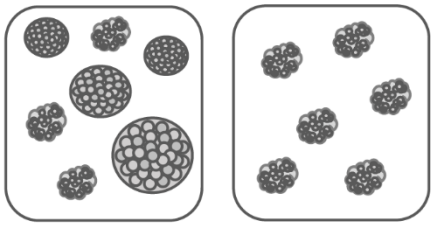
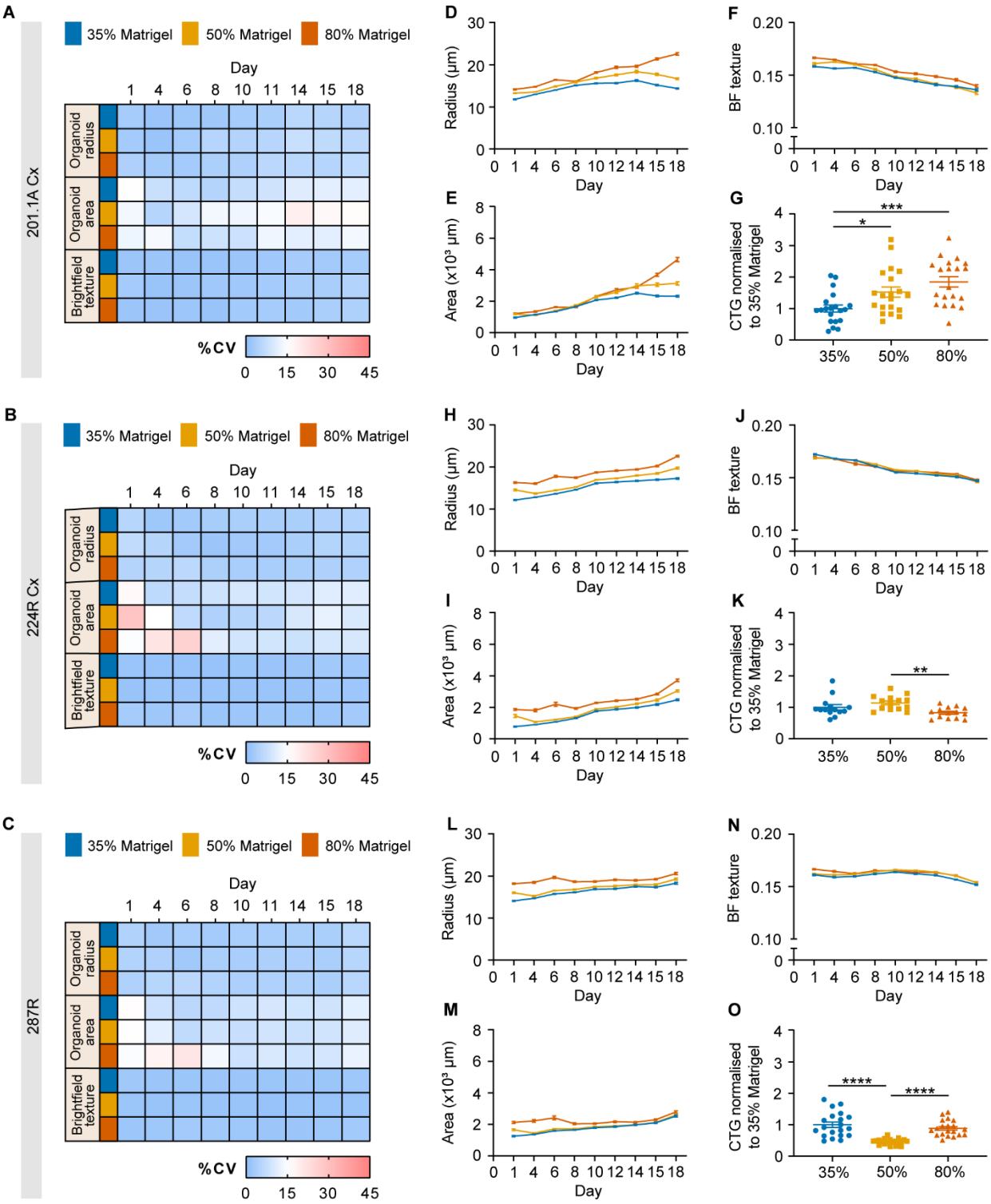

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
35% Matrigel
50% Matrigel
80% Matrigel
Media (empty)
DMSO control (20 reps)
Talazoparib (5 doses, 5 reps)
Carboplatin (5 doses, 5 reps)


B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Media (empty)
DMSO control (20 reps)
Positive control 1 (3 doses, 2 reps)
Positive control 2 (3 doses, 2 reps)
Positive control 3 (3 doses, 2 reps)
42 Library compounds (3 doses, 5 reps)
Positive control 4 (3 doses, 2 reps)
Positive control 5 (3 doses, 2 reps)
Positive control 6 (3 doses, 2 reps)

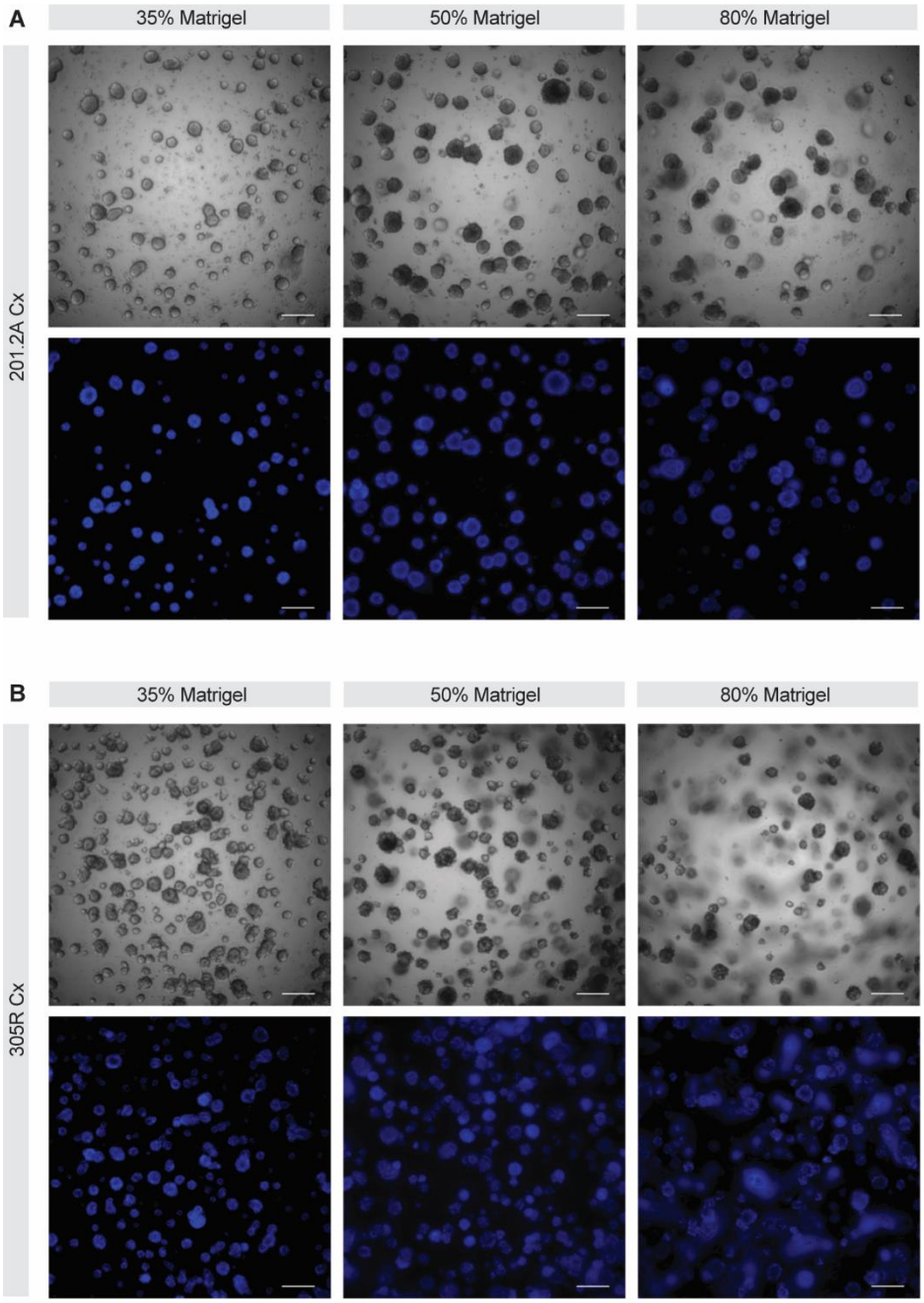
Supplementary Figure 1: Representative plate layout of a high throughput screening assay. (**A**) Plate layout of organoids grown in different concentrations of Matrigel treated with DMSO and dose responses of talazoparib or carboplatin. (**B**) Plate layout of a 3D compound screen including DMSO, six positive controls and 42 library compounds.

Imaging feature	Schematic representation
Area	
MeanRadius	
Eccentricity	
FormFactor	
MeanIntensity	
Texture	
Texture_Variance	

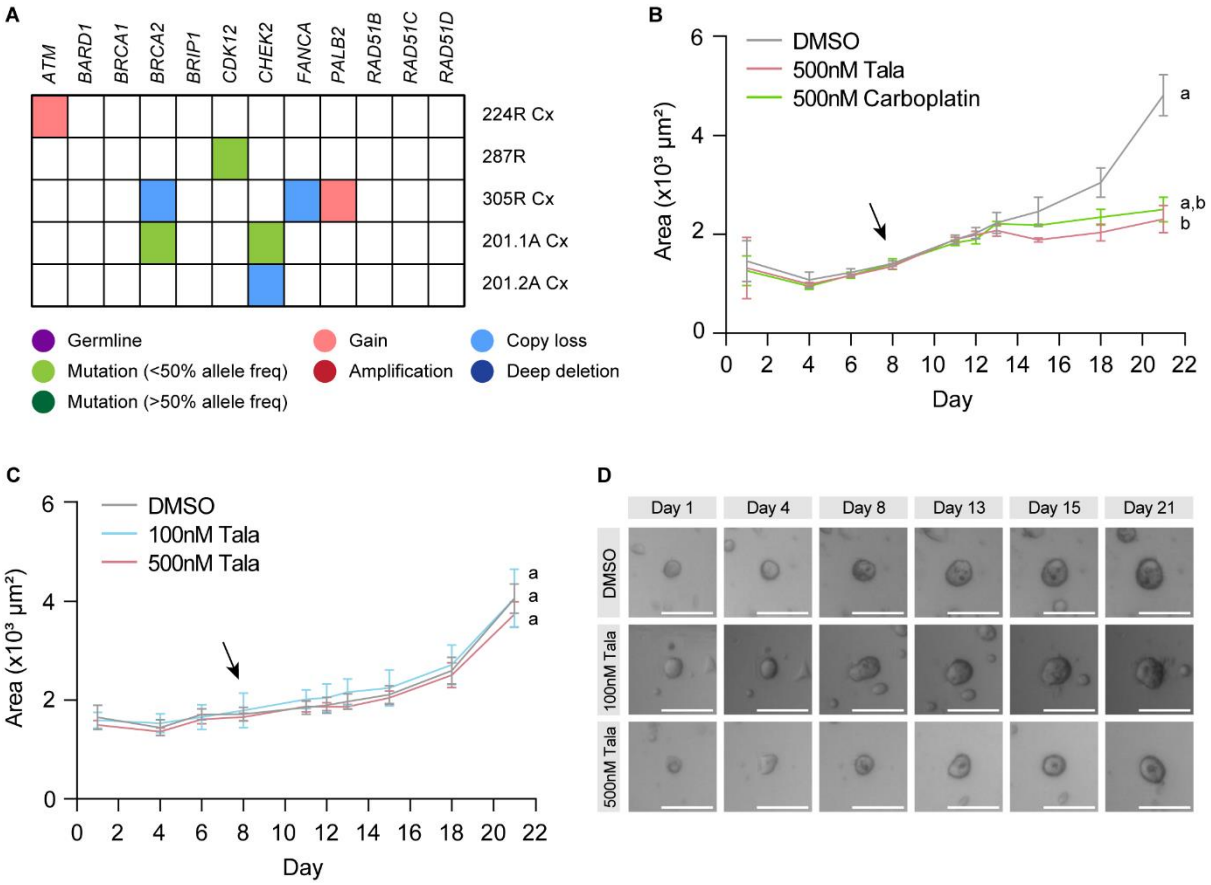
Supplementary Figure 2: Schematic representation of imaging features used for organoid analyses. Imaging features include area, mean radius, eccentricity, form factor, mean intensity, texture and texture variance. A detailed description is also provided in Table 1.


A
201.1A Cx
35% Matrigel
50% Matrigel
80% Matrigel
Day
1 4 6 8 10 11 14 15 18
Organoid radius
Organoid area
Brightfield texture
%CV
0 15 30 45
B
224R Cx
C
287R
D
Radius (µm)
0 10 20 30
0 1 4 6 8 10 12 14 15 18
Day
E
Area (x10³ µm)
0 2 4 6 8
F
BF texture
0.10 0.15 0.20
G
CTG normalised to 35% Matrigel
0 1 2 3 4
*
***
35% 50% 80%
H
I
J
**
K
L
M
N
****
O

Supplementary Figure 3: Comparison of organoid growth from diverse phenotypes of prostate cancer in different concentrations of Matrigel. (**A**-**C**) Heatmaps of percent coefficient of variation for (**A**) 201.1A-Cx, (**B**) 224R-Cx, and (**C**) 287R organoids across 18 days for organoid radius, area, and brightfield texture in 35% (blue), 50% (yellow) and 80% Matrigel (orange). The growth of organoids from 201.1A-Cx (**D**-**G**), 224R-Cx (**H-K**), and 287R (**L-O**) in different concentrations of Matrigel was measured over 18 days (n = 15 wells per tumor). Graphs show changes in organoid radius (**D, H, L**), area (**E, I, M**), and brightfield texture (**F, J, N**). Metabolic activity (**G, K, O**) was measured with CTG and normalized to 35% Matrigel. All data are mean ± SEM; *P < 0.05; **P < 0.01, ***P < 0.001, ***P < 0.0001; one-way ANOVA with post hoc Tukey's test. Abbreviations: CV – coefficient of variation, CTG – CellTiter-Glo.

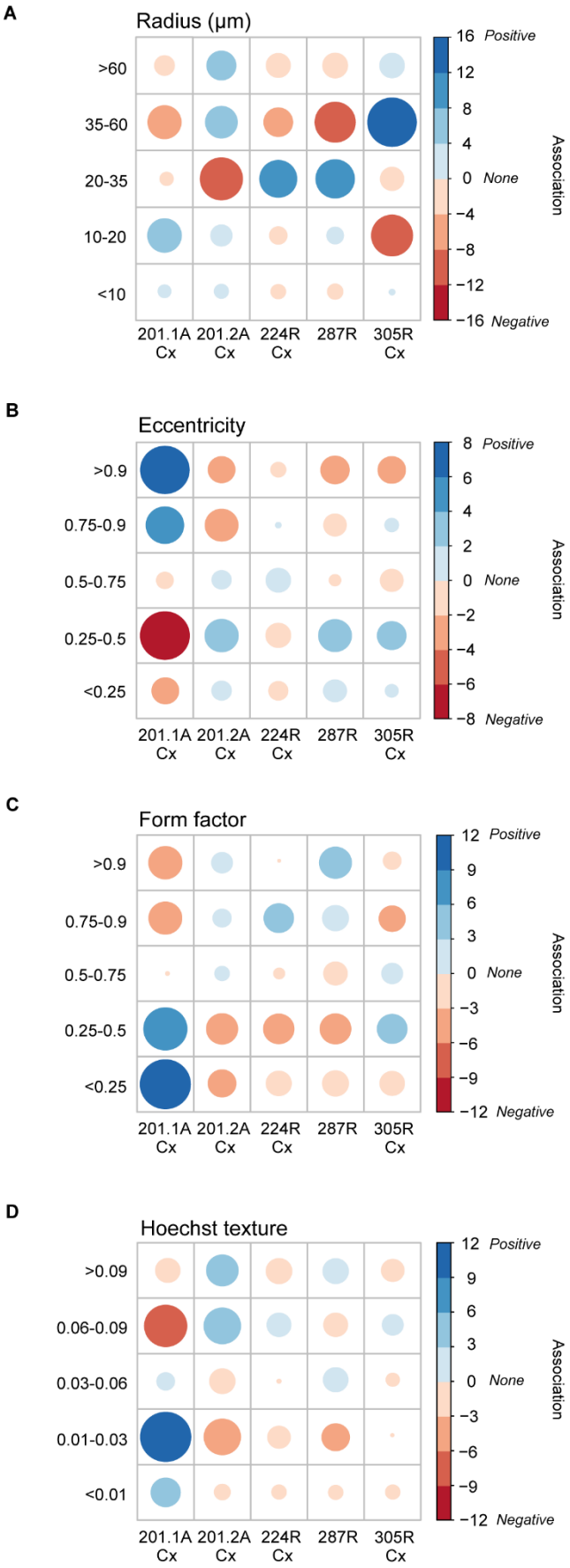


Supplementary Figure 4: Maximum projections of brightfield and fluorescence microscopy of organoids grown in different concentrations of Matrigel. Representative images of (**A**) 201.2-Cx and (**B**) 305R-Cx organoids grown for 21 days in 35%, 50% or 80% Matrigel. Images are maximum projections from three Z heights for brightfield microscopy and fluorescence microscopy of Hoechst staining. Scale bars = 500 µm.



Supplementary Figure 5: Alterations in DNA damage repair genes and change in area over time in organoids after treatment. (**A**) Plot summarising genomic alterations in DNA damage repair genes based on targeted DNA sequencing of PDX tissue. There are no homozygous mutations or deep deletions. (**B**) Graph showing the mean area of 224R-Cx organoids grown for 21 days and treated with DMSO, 500 nM talazoparib, or 500 nM carboplatin from day 8. Arrow indicates the first day of treatment. (**C**) Graph showing the mean area of 287R organoids grown for 21 days and treated with DMSO, 100 or 500nM talazoparib from day 8. Data are mean ± SEM. $^{a}P < 0.001$, statistical analysis of change in organoid area across time within each

treatment group; [b]$P < 0.001$, statistical analysis of organoid area within each treatment group compared to vehicle control; two-way ANOVA with Dunnett's post hoc test. (**D**) Representative images of 287R single organoids over time that were treated with DMSO, 100 or 500 nM talazoparib from day 8. Scale bars = 250 µm. Abbreviations: CTG – CellTiter-Glo, Tala – talazoparib.


A
Radius (µm)
>60
35-60
20-35
10-20
<10
201.1A Cx
201.2A Cx
224R Cx
287R
305R Cx
16 Positive
12
8
4
0 None
−4
−8
−12
−16 Negative
Association
B
Eccentricity
>0.9
0.75-0.9
0.5-0.75
0.25-0.5
<0.25
8 Positive
6
4
2
0 None
−2
−4
−6
−8 Negative
C
Form factor
12 Positive
9
6
3
0 None
−3
−6
−9
−12 Negative
D
Hoechst texture
>0.09
0.06-0.09
0.03-0.06
0.01-0.03
<0.01

Supplementary Figure 6: Schematic of Chi-squared analysis results for association between organoid models per bin. The graphical matrices (**A, B, C, D**) represent Chi-squared tests of the association between organoid models (column) in each bin (row). Positive (blue) and negative (red) residuals specify the association between each organoid per bin. A positive association indicates a higher proportion of organoids in a bin, whereas a negative association indicates a lower proportion of organoids in a bin. The size of residuals (circles) is proportional to the magnitude of the association, positive or negative.