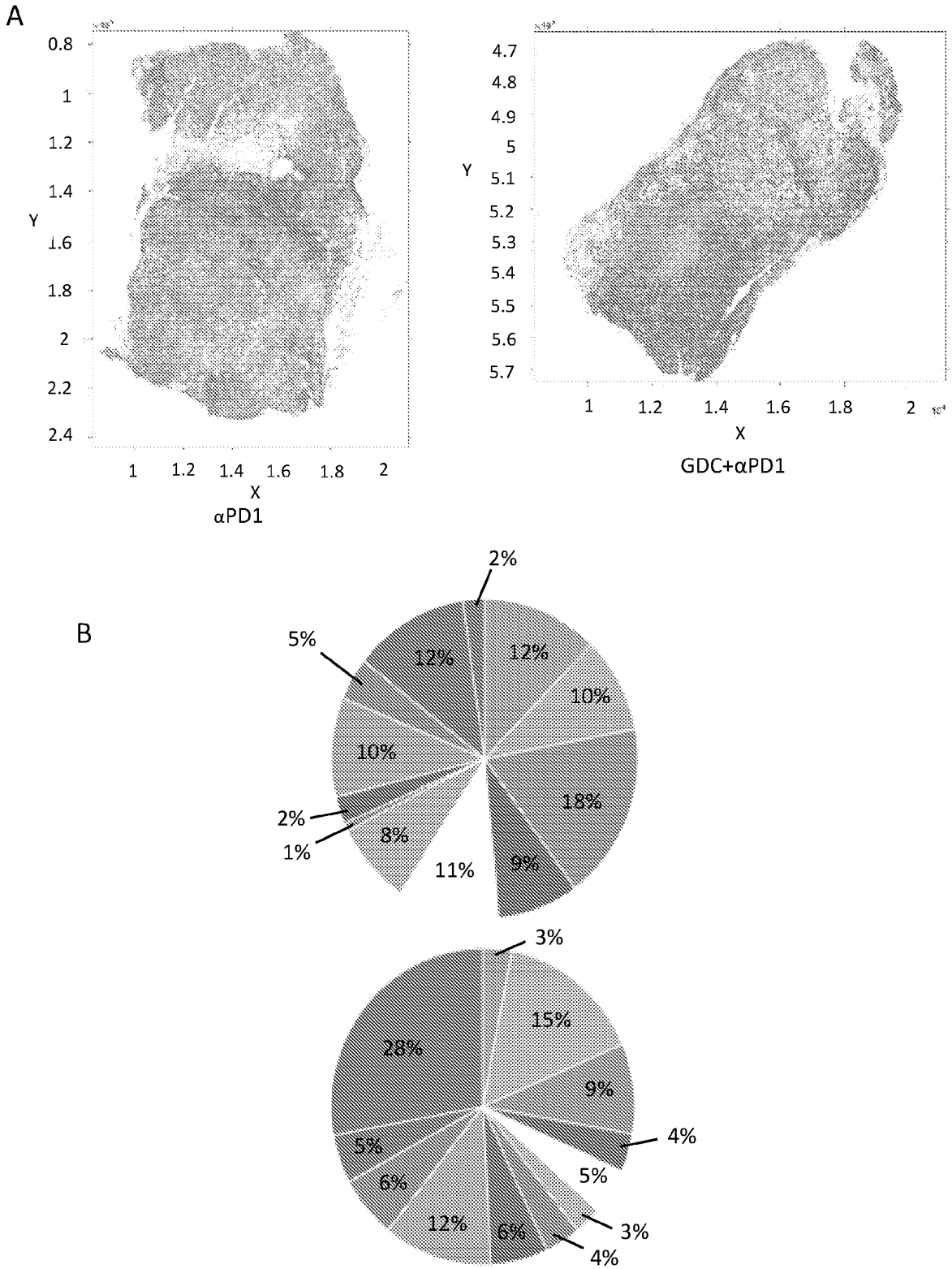

A
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
Y
1
1.2
1.4
1.6
1.8
2
X
αPD1
4.7
4.8
4.9
5
5.1
5.2
5.3
5.4
5.5
5.6
5.7
Y
1
1.2
1.4
1.6
1.8
2
X
GDC+αPD1
B
2%
5%
12%
12%
10%
10%
18%
2%
8%
1%
11%
9%
3%
15%
28%
9%
4%
5%
6%
5%
3%
12%
6%
4%

Figure 1

C

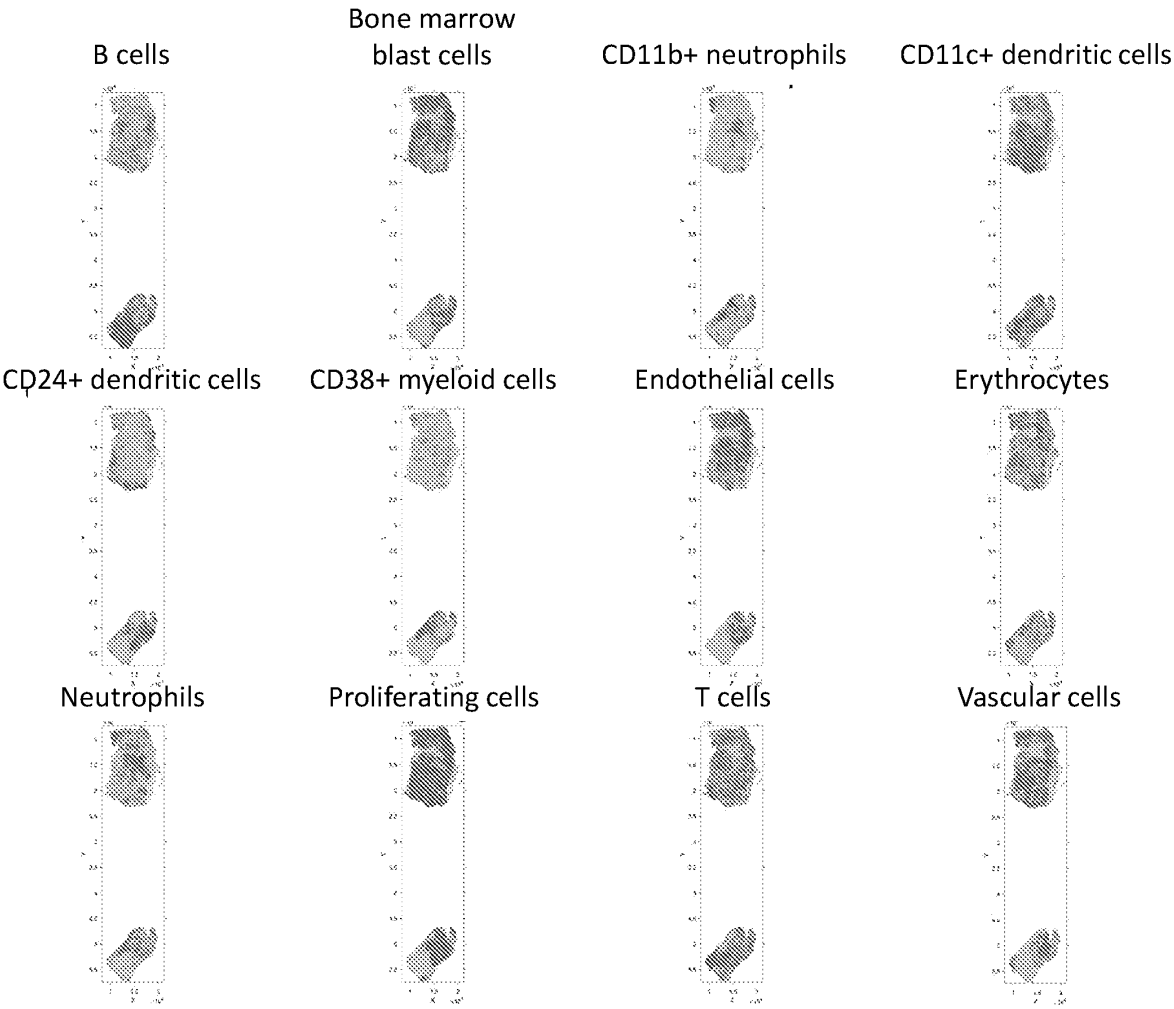


Figure 1 cont

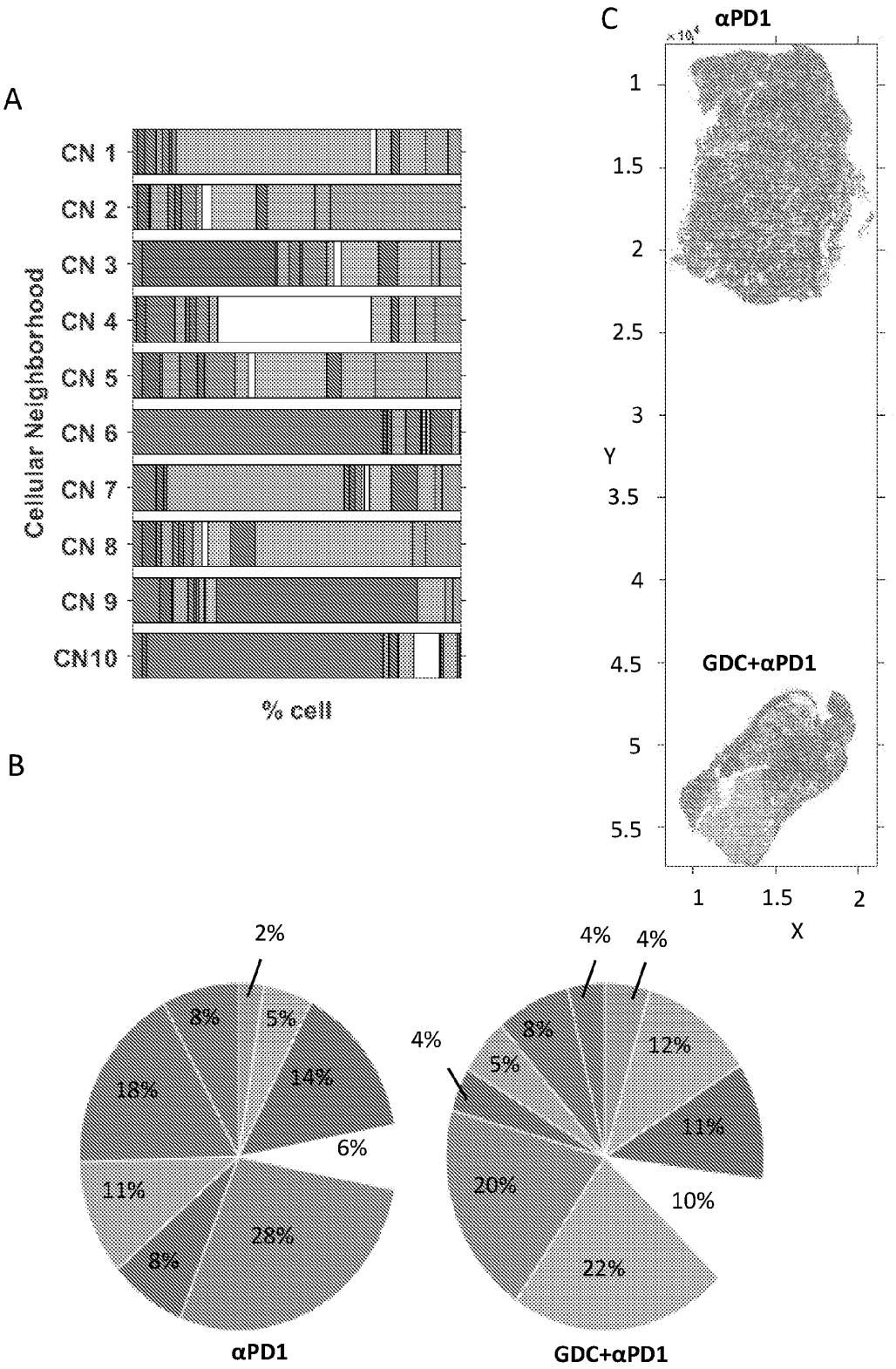

A
CN 1
CN 2
CN 3
CN 4
CN 5
CN 6
CN 7
CN 8
CN 9
CN10
Cellular Neighborhood
% cell
B
2%
8%
5%
14%
18%
6%
11%
28%
8%
αPD1
4%
4%
4%
8%
12%
5%
11%
20%
10%
22%
GDC+αPD1
C
αPD1
GDC+αPD1
Y
X
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5

Figure 2

A

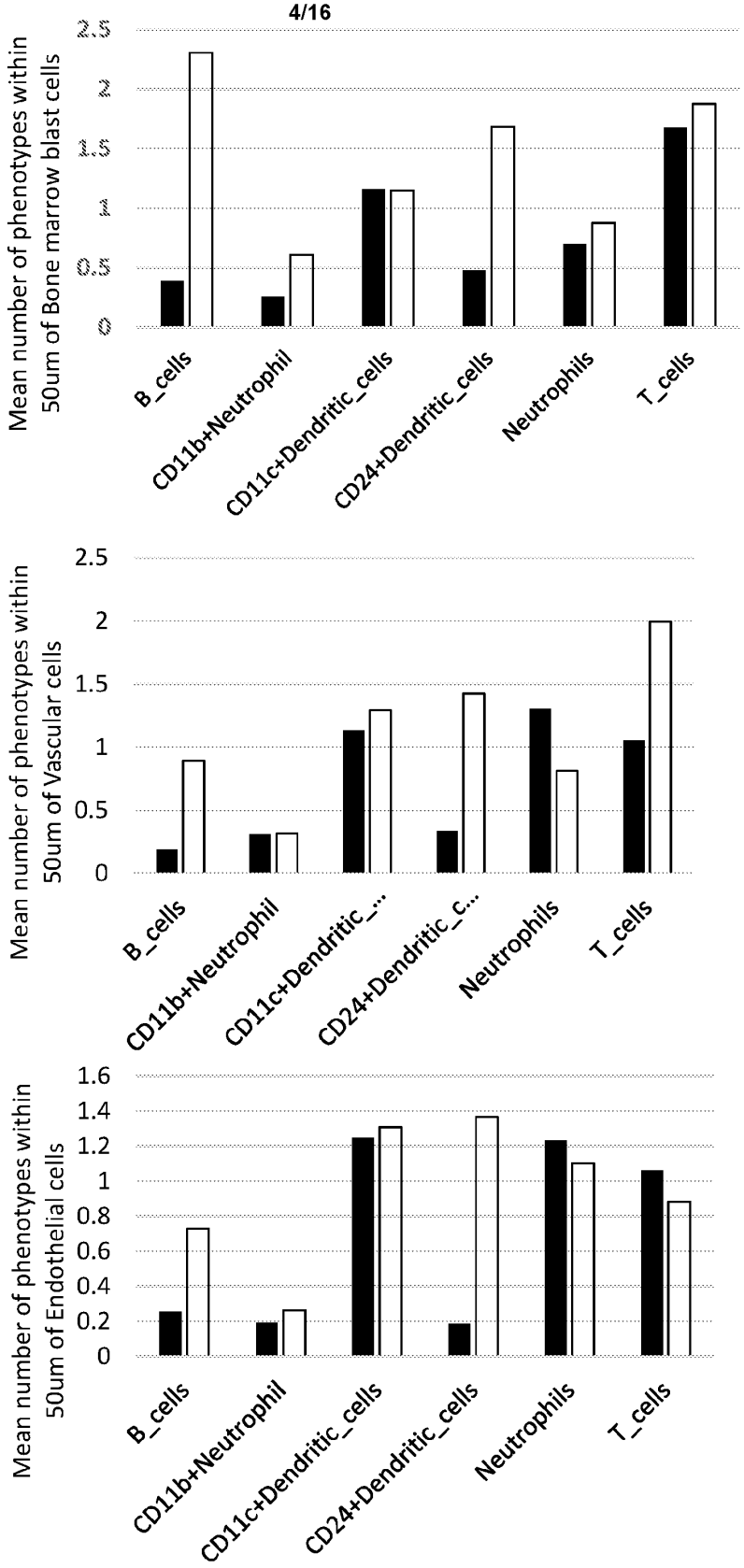

Mean number of phenotypes within 50um of Bone marrow blast cells
2.5
2
1.5
1
0.5
0
B_cells
CD11b+Neutrophil
CD11c+Dendritic_cells
CD24+Dendritic_cells
Neutrophils
T_cells
Mean number of phenotypes within 50um of Vascular cells
2.5
2
1.5
1
0.5
0
B_cells
CD11b+Neutrophil
CD11c+Dendritic_...
CD24+Dendritic_c...
Neutrophils
T_cells
Mean number of phenotypes within 50um of Endothelial cells
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
B_cells
CD11b+Neutrophil
CD11c+Dendritic_cells
CD24+Dendritic_cells
Neutrophils
T_cells

Figure 3

B

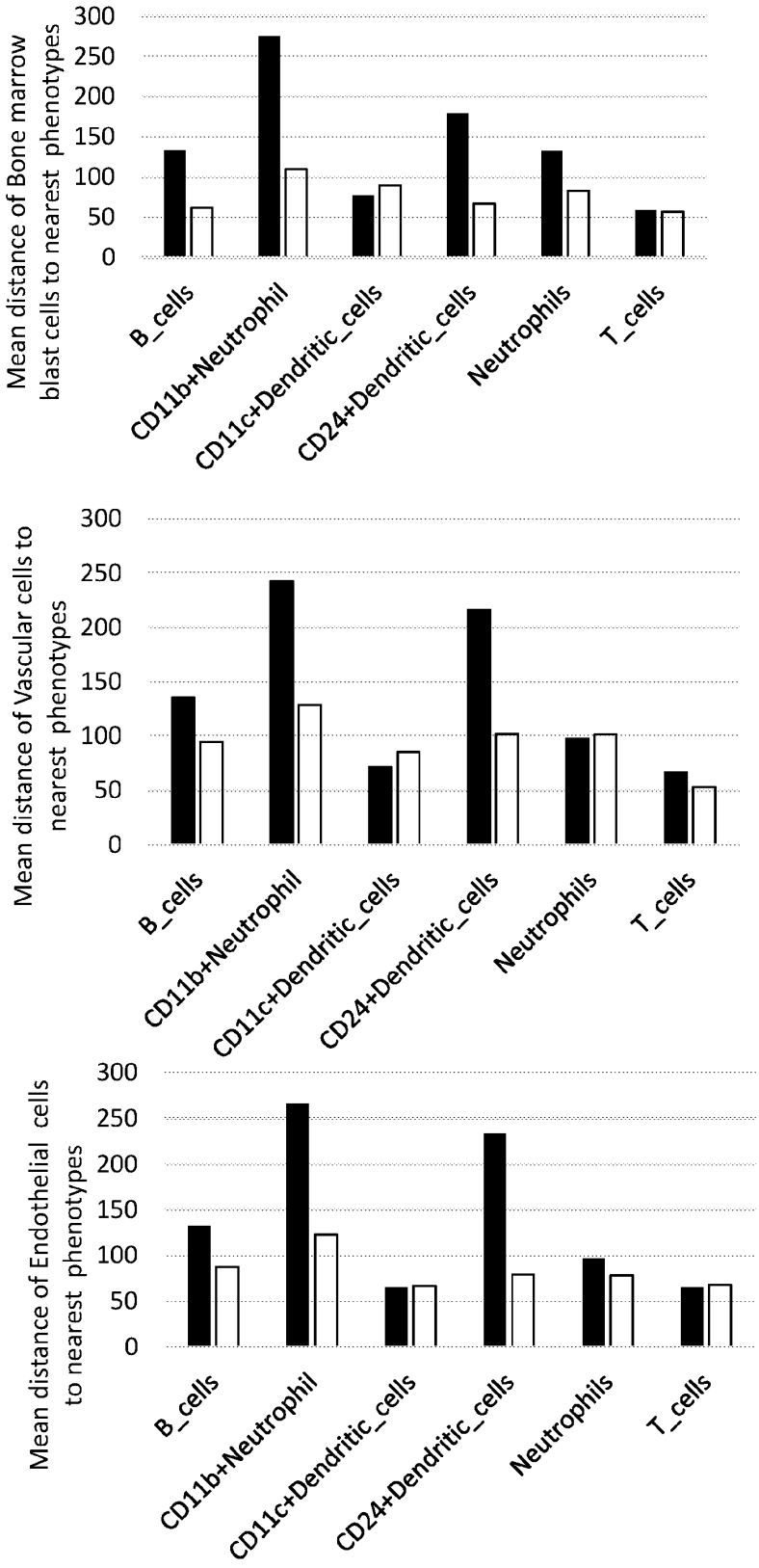


Figure 3 con't

A

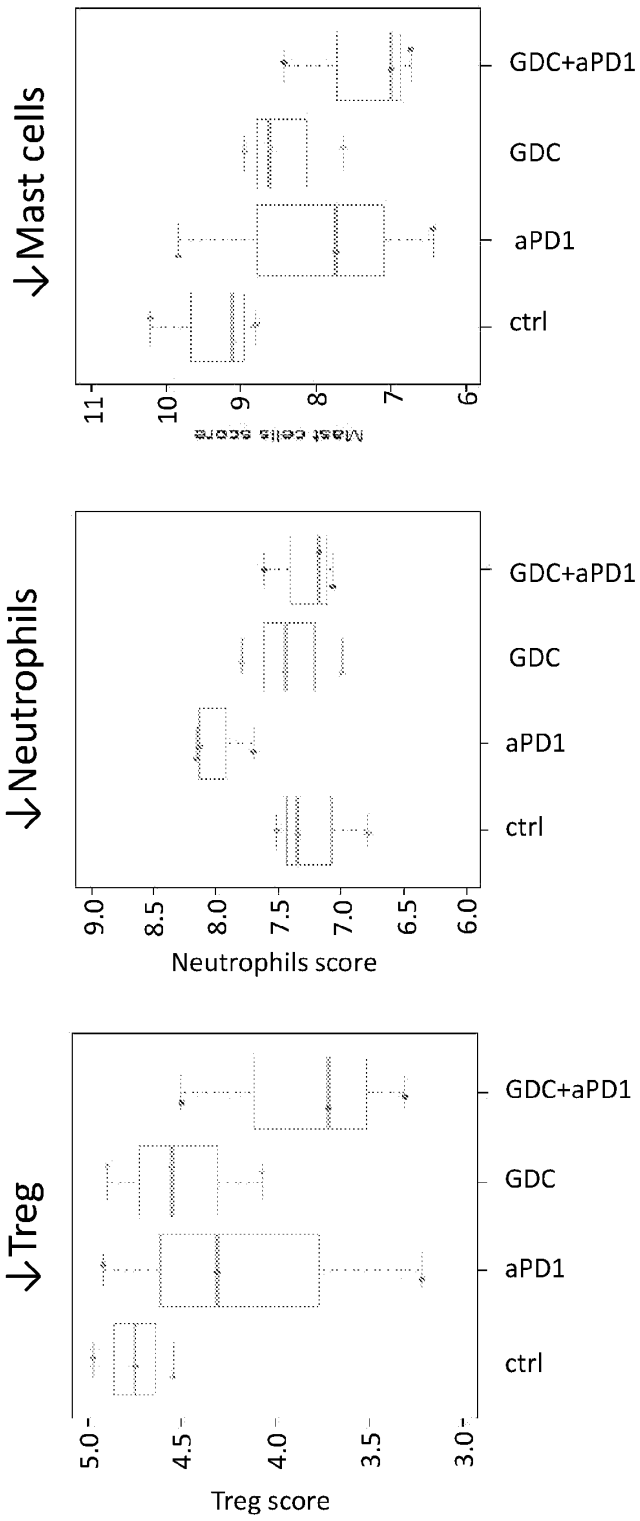

↓Mast cells
GDC+aPD1
GDC
aPD1
ctrl
11
10
9
8
7
6
↓Neutrophils
GDC+aPD1
GDC
aPD1
ctrl
9.0
8.5
8.0
7.5
7.0
6.5
6.0
Neutrophils score
↓Treg
GDC+aPD1
GDC
aPD1
ctrl
5.0
4.5
4.0
3.5
3.0
Treg score

Figure 4

B

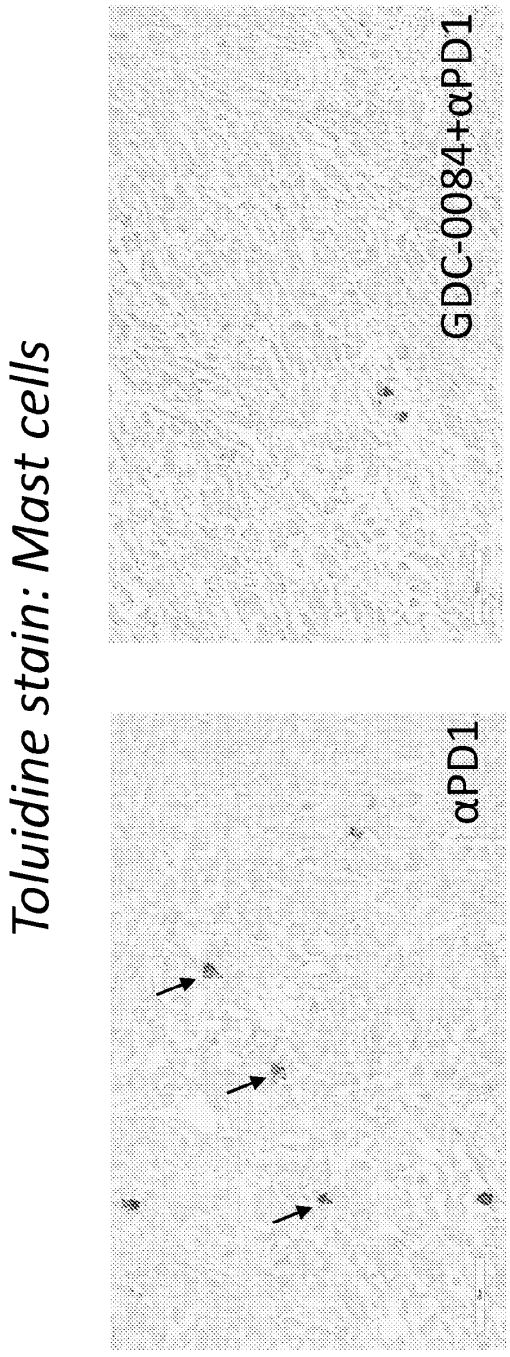


Figure 4 cont.

A

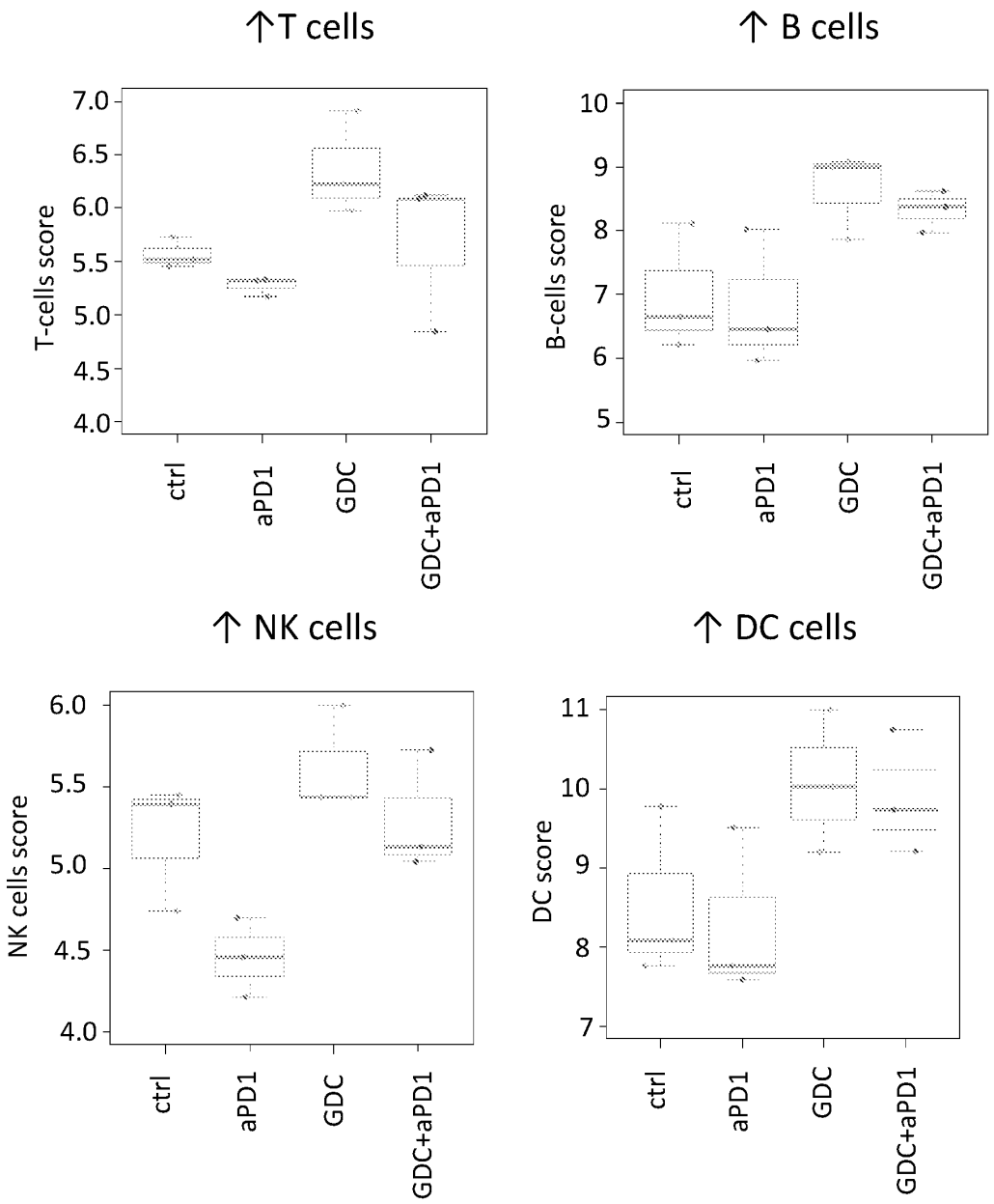
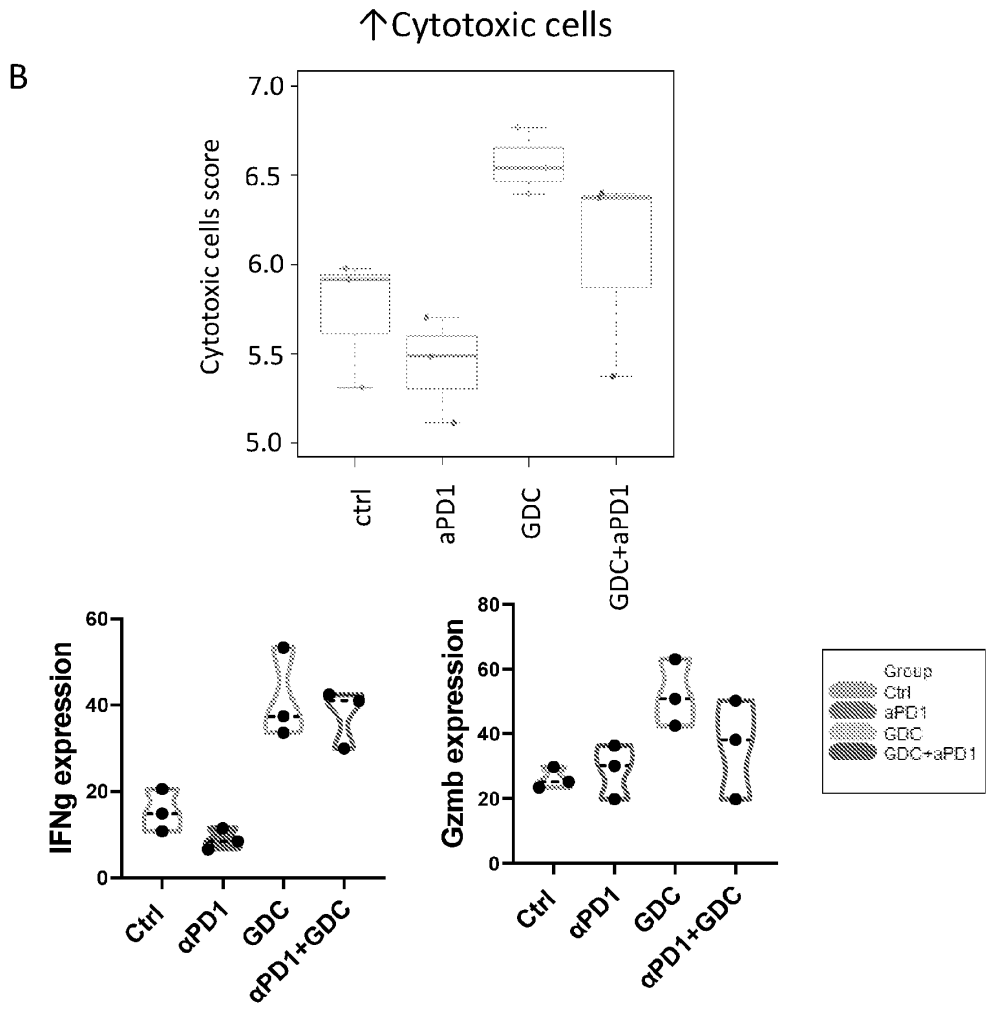

↑T cells
T-cells score
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ctrl
aPD1
GDC
GDC+aPD1
↑ B cells
B-cells score
10
9
8
7
6
5
ctrl
aPD1
GDC
GDC+aPD1
↑ NK cells
NK cells score
6.0
5.5
5.0
4.5
4.0
ctrl
aPD1
GDC
GDC+aPD1
↑ DC cells
DC score
11
10
9
8
7
ctrl
aPD1
GDC
GDC+aPD1

Figure 5



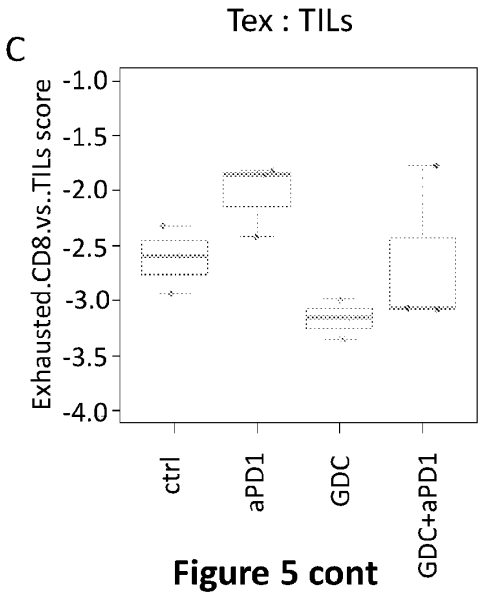


Figure 5 cont

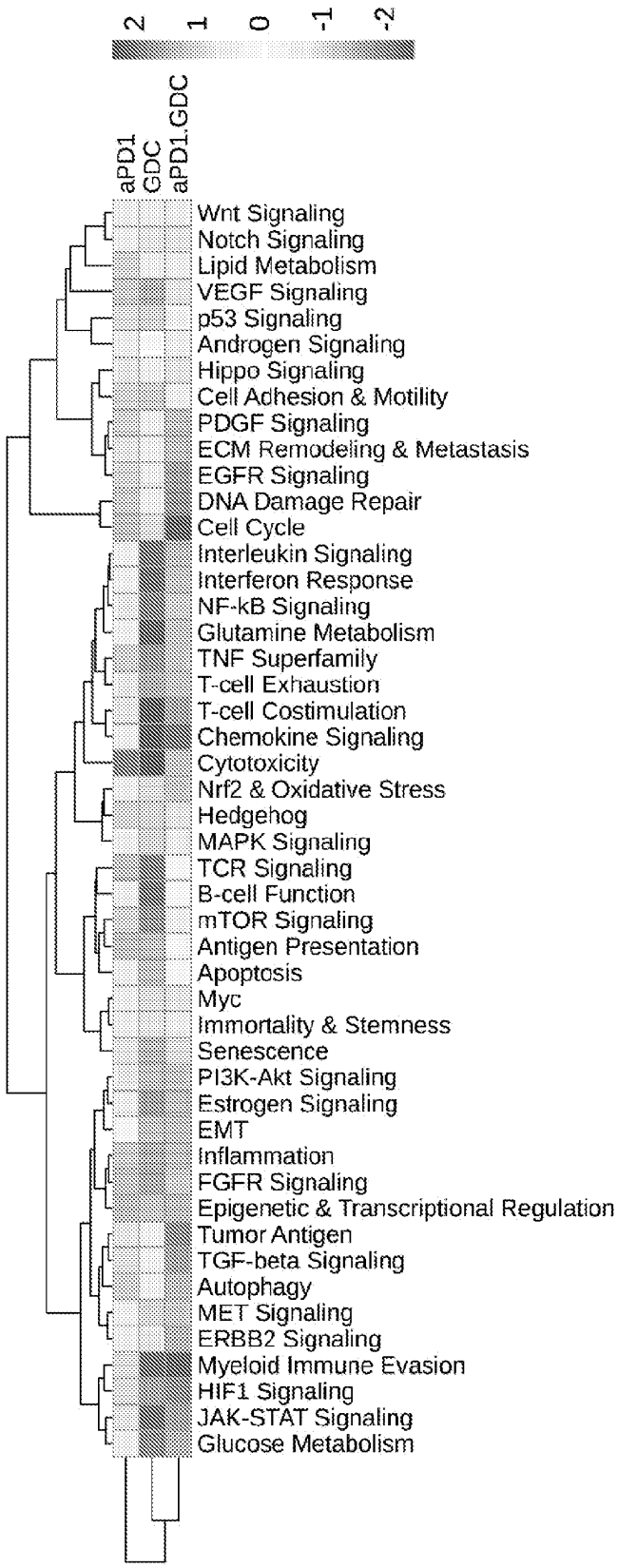

2
1
0
-1
-2
aPD1
GDC
aPD1.GDC
Wnt Signaling
Notch Signaling
Lipid Metabolism
VEGF Signaling
p53 Signaling
Androgen Signaling
Hippo Signaling
Cell Adhesion & Motility
PDGF Signaling
ECM Remodeling & Metastasis
EGFR Signaling
DNA Damage Repair
Cell Cycle
Interleukin Signaling
Interferon Response
NF-kB Signaling
Glutamine Metabolism
TNF Superfamily
T-cell Exhaustion
T-cell Costimulation
Chemokine Signaling
Cytotoxicity
Nrf2 & Oxidative Stress
Hedgehog
MAPK Signaling
TCR Signaling
B-cell Function
mTOR Signaling
Antigen Presentation
Apoptosis
Myc
Immortality & Stemness
Senescence
PI3K-Akt Signaling
Estrogen Signaling
EMT
Inflammation
FGFR Signaling
Epigenetic & Transcriptional Regulation
Tumor Antigen
TGF-beta Signaling
Autophagy
MET Signaling
ERBB2 Signaling
Myeloid Immune Evasion
HIF1 Signaling
JAK-STAT Signaling
Glucose Metabolism

Figure 6

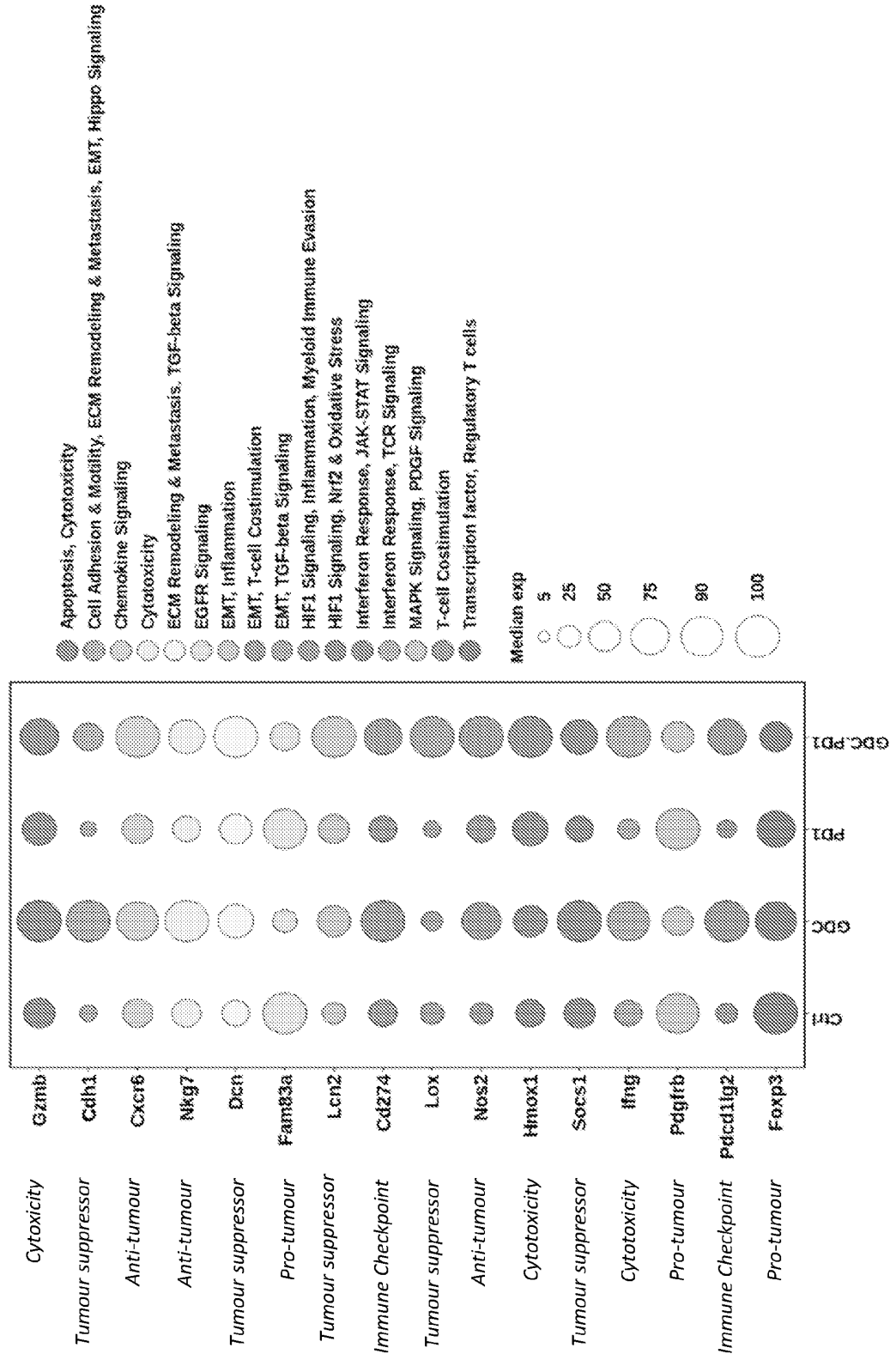
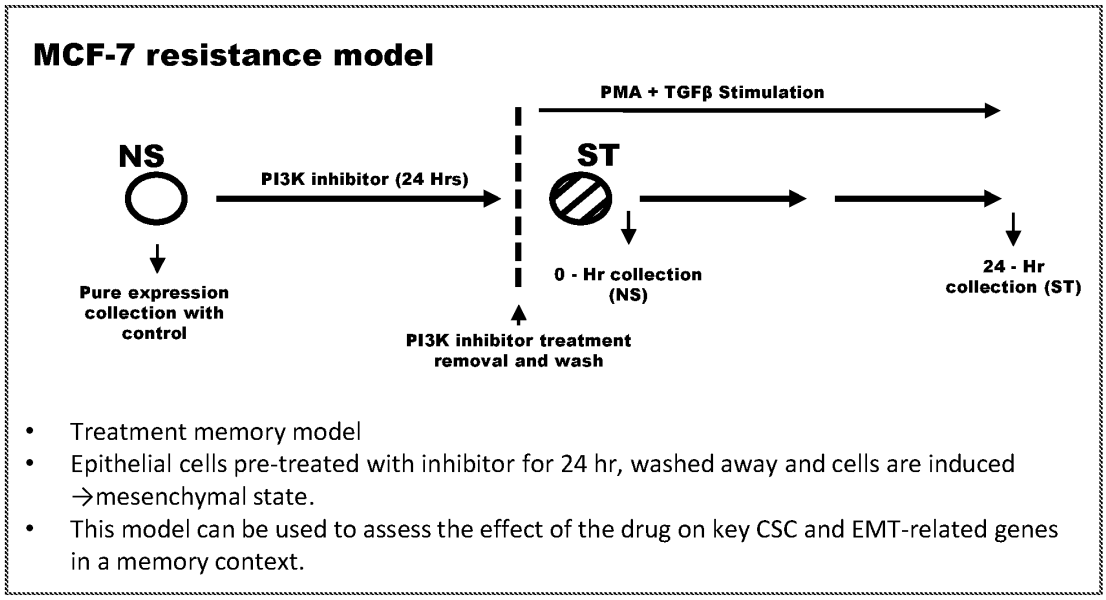

Apoptosis, Cytotoxicity
Cell Adhesion & Motility, ECM Remodeling & Metastasis, EMT, Hippo Signaling
Chemokine Signaling
Cytotoxicity
ECM Remodeling & Metastasis, TGF-beta Signaling
EGFR Signaling
EMT, Inflammation
EMT, T-cell Costimulation
EMT, TGF-beta Signaling
HIF1 Signaling, Inflammation, Myeloid Immune Evasion
HIF1 Signaling, Nrf2 & Oxidative Stress
Interferon Response, JAK-STAT Signaling
Interferon Response, TCR Signaling
MAPK Signaling, PDGF Signaling
T-cell Costimulation
Transcription factor, Regulatory T cells
Median exp
5
25
50
75
90
100
Ctl
GDC
PD1
GDC_PD1
Cytoxicity
Tumour suppressor
Anti-tumour
Anti-tumour
Tumour suppressor
Pro-tumour
Tumour suppressor
Immune Checkpoint
Tumour suppressor
Anti-tumour
Cytotoxicity
Tumour suppressor
Cytotoxicity
Pro-tumour
Immune Checkpoint
Pro-tumour
Gzmb
Cdh1
Cxcr6
Nkg7
Dcn
Fam83a
Lcn2
Cd274
Lox
Nos2
Hmox1
Socs1
Ifng
Pdgfrb
Pdcd1lg2
Foxp3

Figure 7

A



- Treatment memory model
- Epithelial cells pre-treated with inhibitor for 24 hr, washed away and cells are induced →mesenchymal state.
- This model can be used to assess the effect of the drug on key CSC and EMT-related genes in a memory context.

B

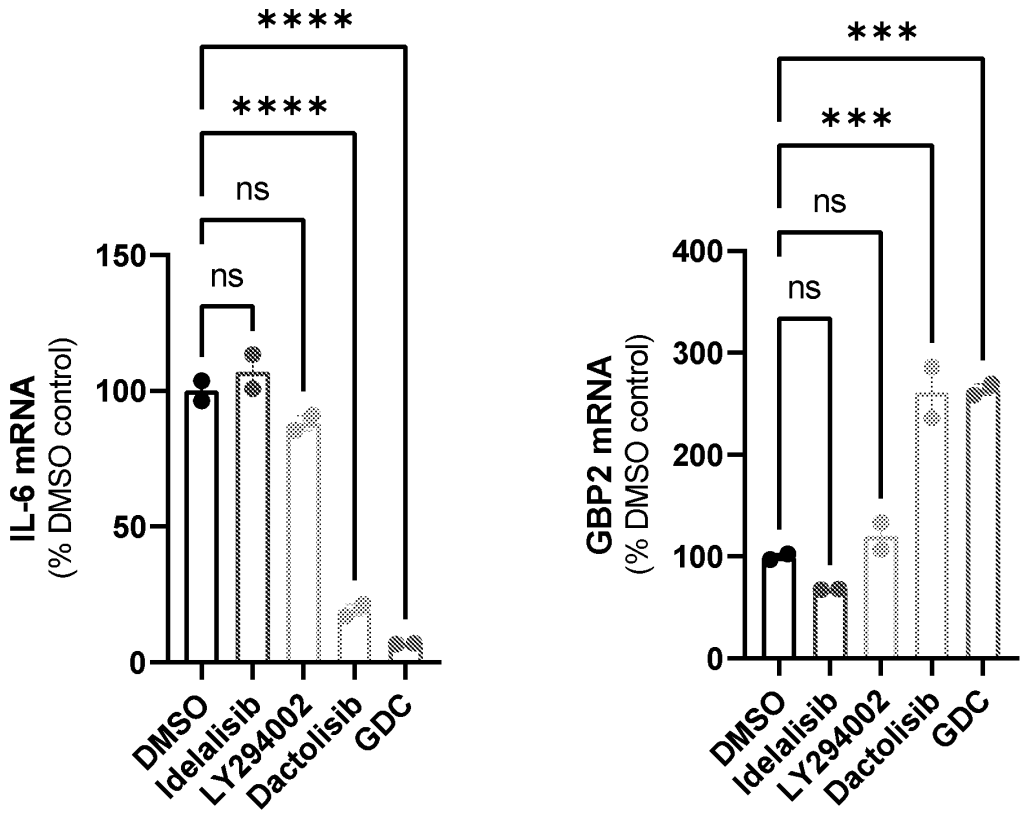
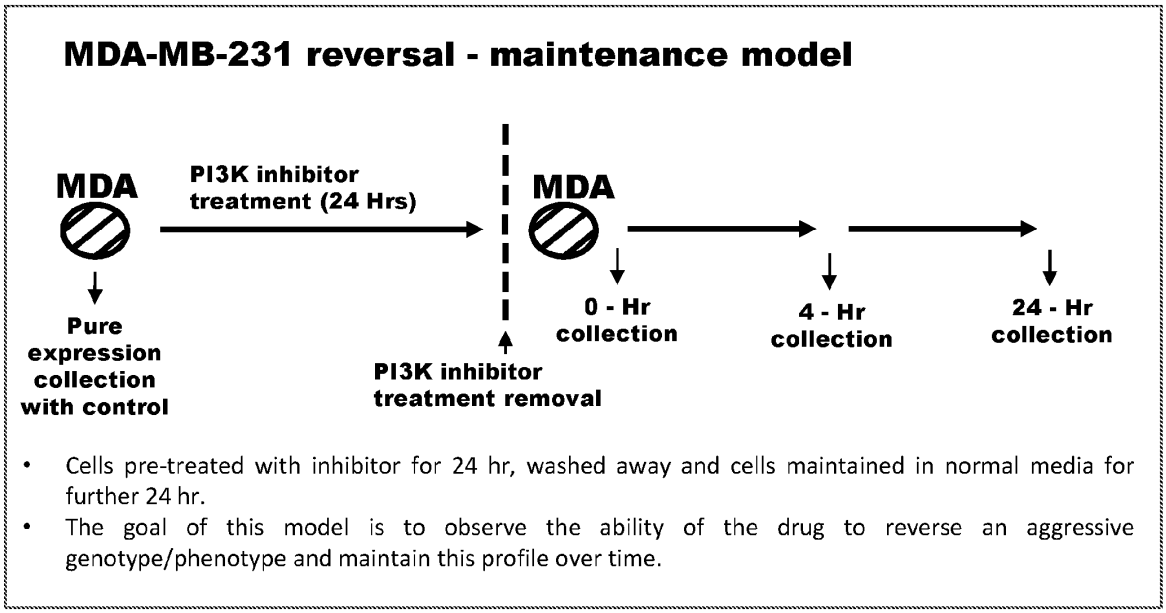


Figure 8

A


MDA-MB-231 reversal - maintenance model
MDA
PI3K inhibitor treatment (24 Hrs)
MDA
Pure expression collection with control
PI3K inhibitor treatment removal
0 - Hr collection
4 - Hr collection
24 - Hr collection
• Cells pre-treated with inhibitor for 24 hr, washed away and cells maintained in normal media for further 24 hr.
• The goal of this model is to observe the ability of the drug to reverse an aggressive genotype/phenotype and maintain this profile over time.

B

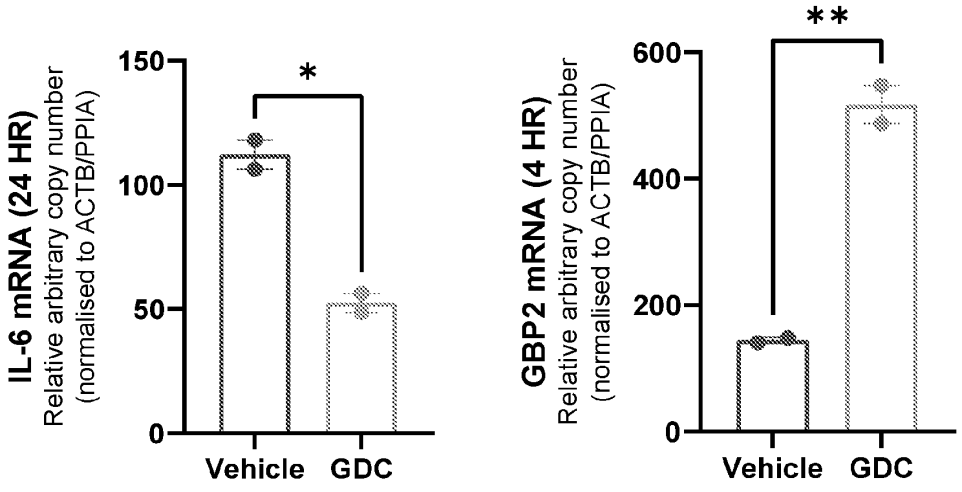

IL-6 mRNA (24 HR)
Relative arbitrary copy number (normalised to ACTB/PPIA)
150
100
50
0
*
Vehicle
GDC
GBP2 mRNA (4 HR)
Relative arbitrary copy number (normalised to ACTB/PPIA)
600
400
200
0
**
Vehicle
GDC

Figure 9

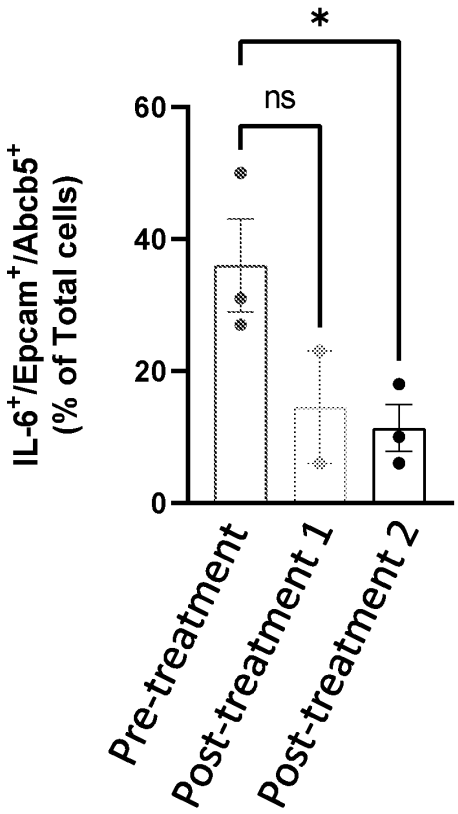
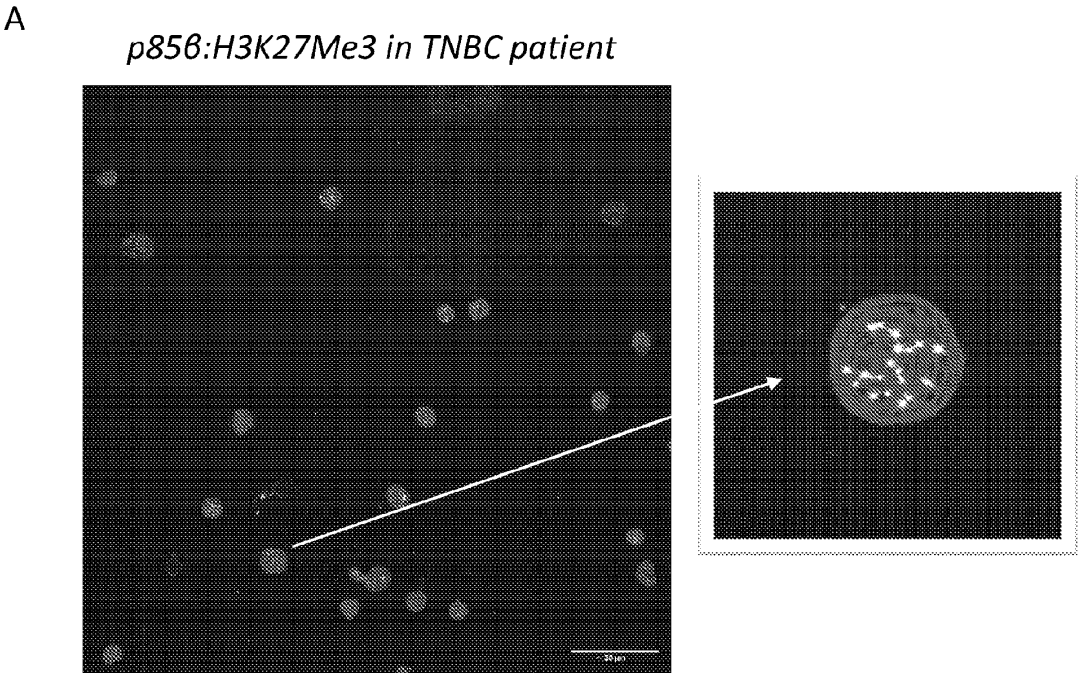

*
ns
60
40
20
0
IL-6+/Epcam+/Abcb5+
(% of Total cells)
Pre-treatment
Post-treatment 1
Post-treatment 2

Figure 10

A


p85β:H3K27Me3 in TNBC patient

B

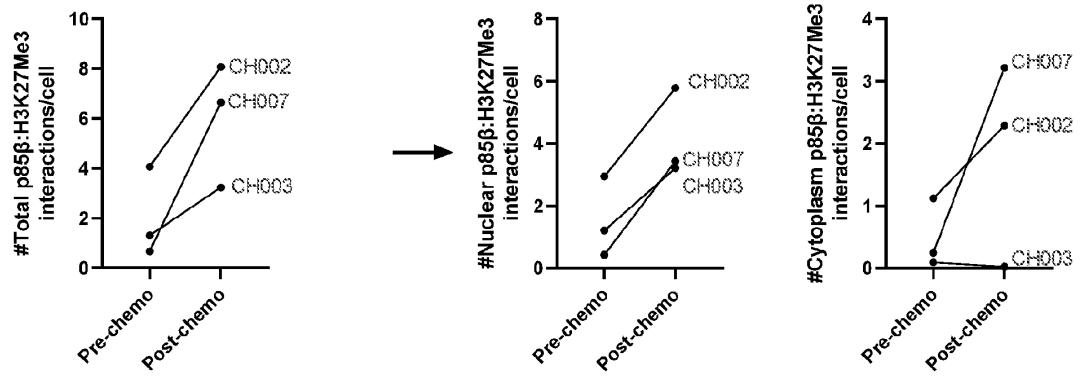

#Total p85β:H3K27Me3 interactions/cell
10
8
6
4
2
0
CH002
CH007
CH003
Pre-chemo
Post-chemo
#Nuclear p85β:H3K27Me3 interactions/cell
8
6
4
2
0
CH002
CH007
CH003
Pre-chemo
Post-chemo
#Cytoplasm p85β:H3K27Me3 interactions/cell
4
3
2
1
0
CH007
CH002
CH003
Pre-chemo
Post-chemo

Figure 11

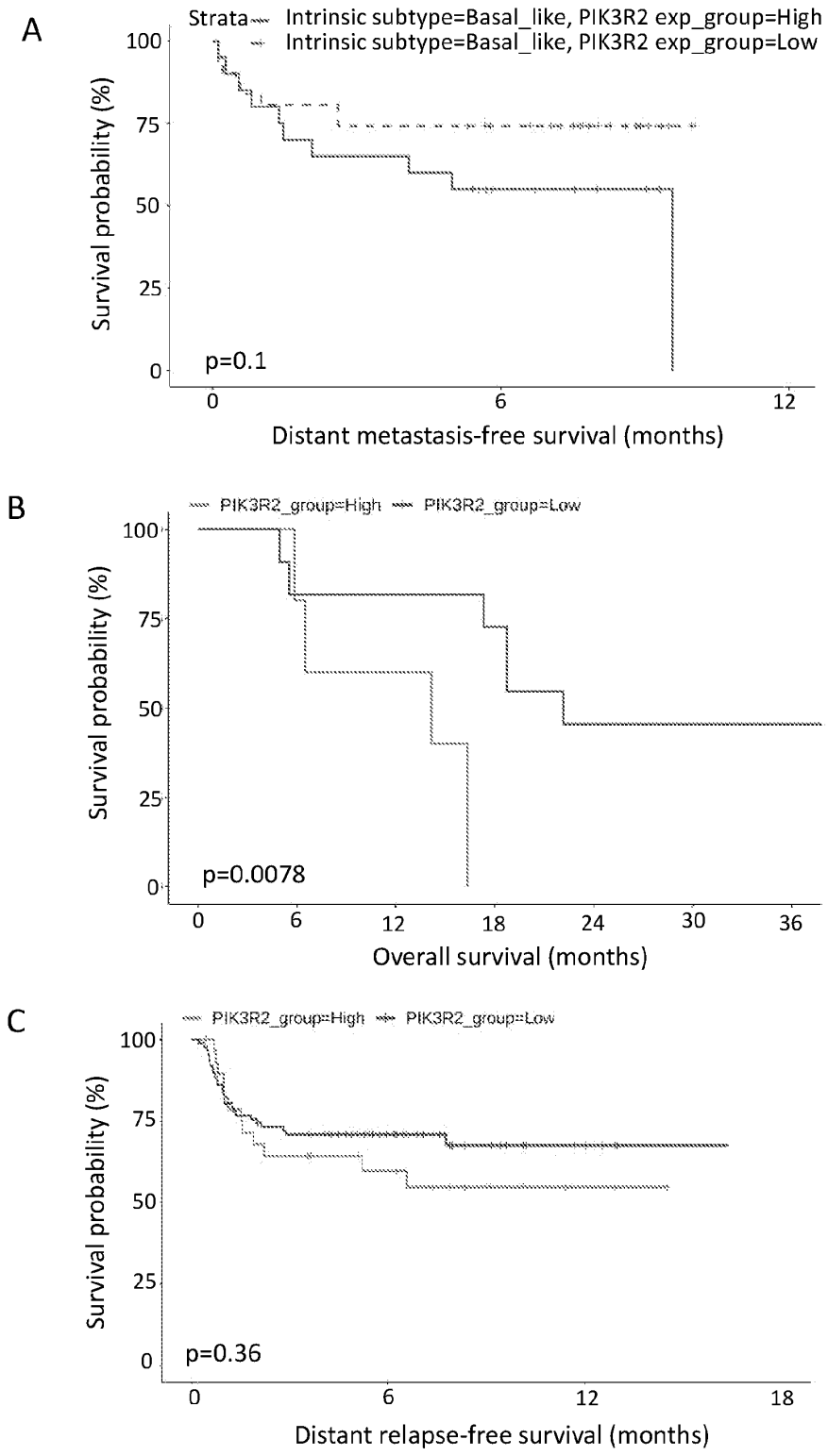

A
Strata
Intrinsic subtype=Basal_like, PIK3R2 exp_group=High
Intrinsic subtype=Basal_like, PIK3R2 exp_group=Low
Survival probability (%)
100
75
50
25
0
p=0.1
0
6
12
Distant metastasis-free survival (months)
B
PIK3R2_group=High
PIK3R2_group=Low
Survival probability (%)
100
75
50
25
0
p=0.0078
0
6
12
18
24
30
36
Overall survival (months)
C
PIK3R2_group=High
PIK3R2_group=Low
Survival probability (%)
100
75
50
25
0
p=0.36
0
6
12
18
Distant relapse-free survival (months)

Figure 12