Figure 1

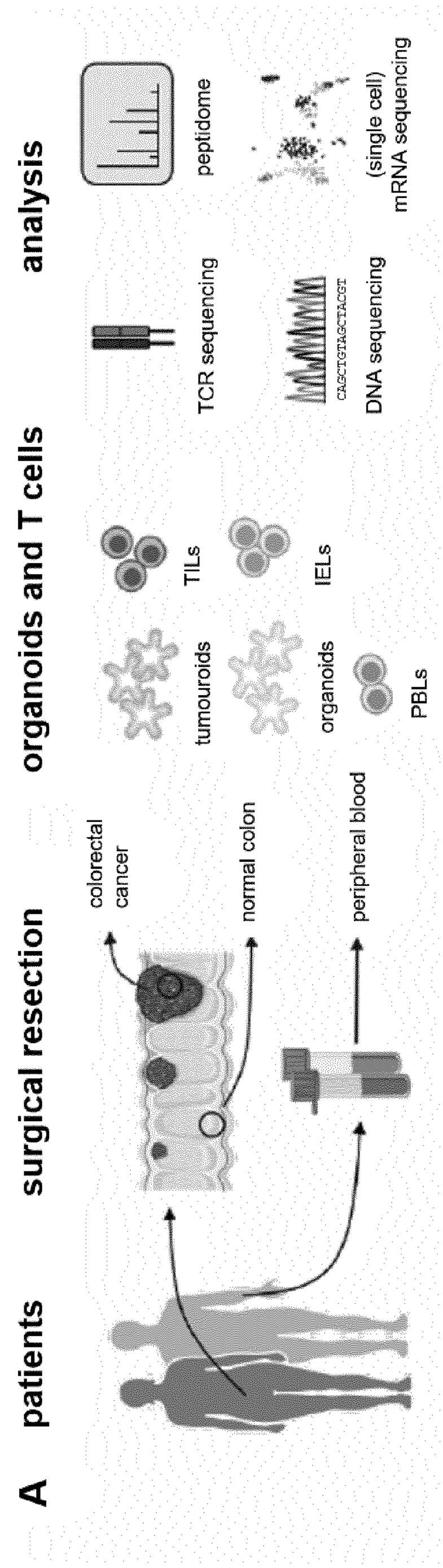

A patients
surgical resection
colorectal cancer
normal colon
peripheral blood
organoids and T cells
tumouroids
TILs
TCR sequencing
organoids
IELs
CAGCTGTAGCTACGT
DNA sequencing
PBLs
analysis
peptidome
(single cell) mRNA sequencing

Figure 1

B organoid cultures **C T-cell enriched cultures** **Flow analysis**

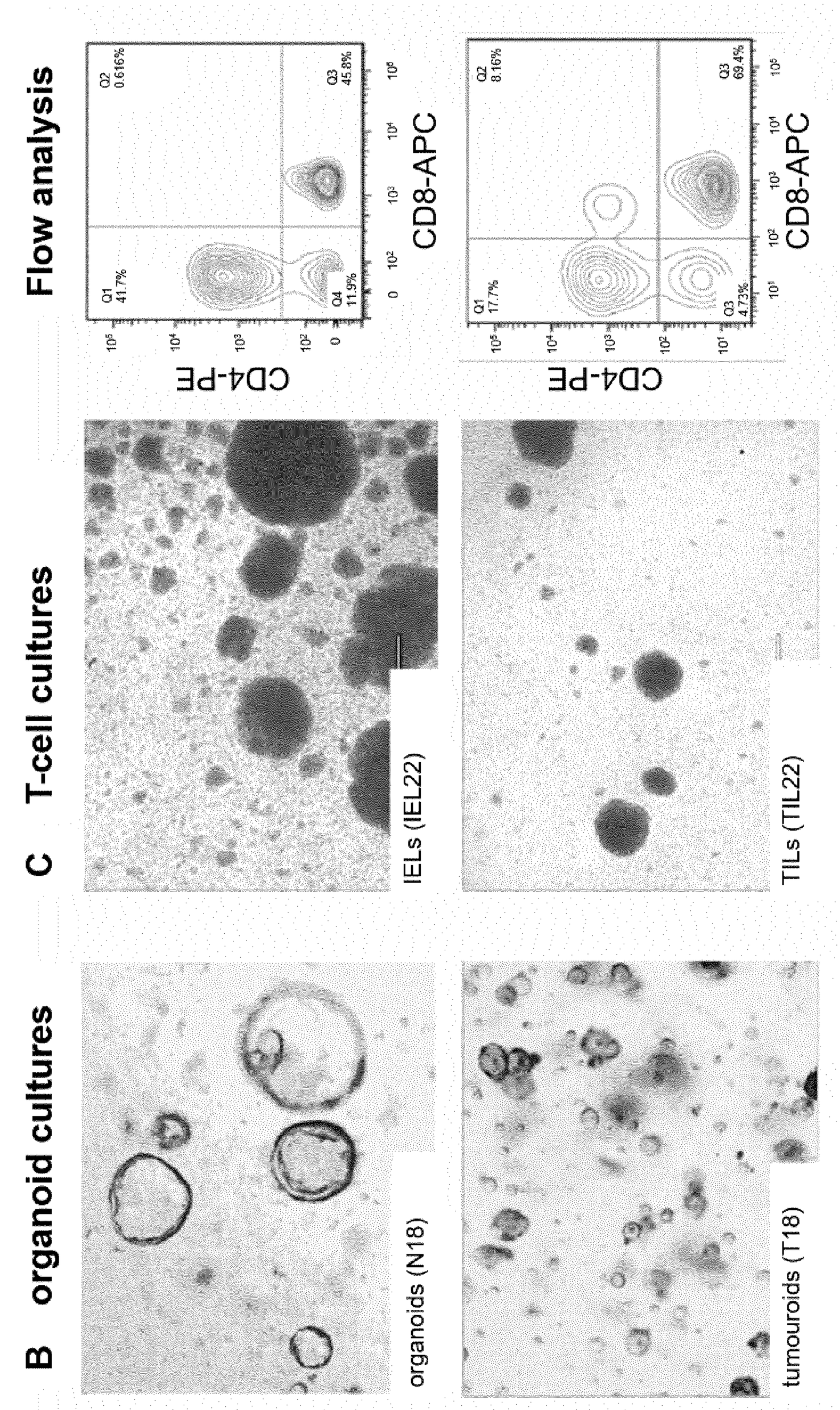


Figure 2

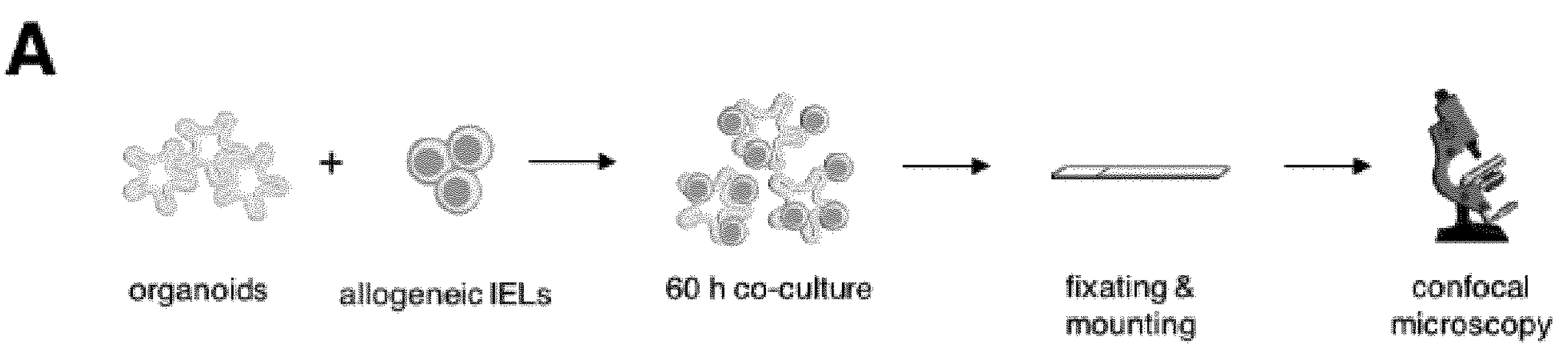

A
organoids
allogeneic IELs
60 h co-culture
fixating & mounting
confocal microscopy
B
C

Figure 3

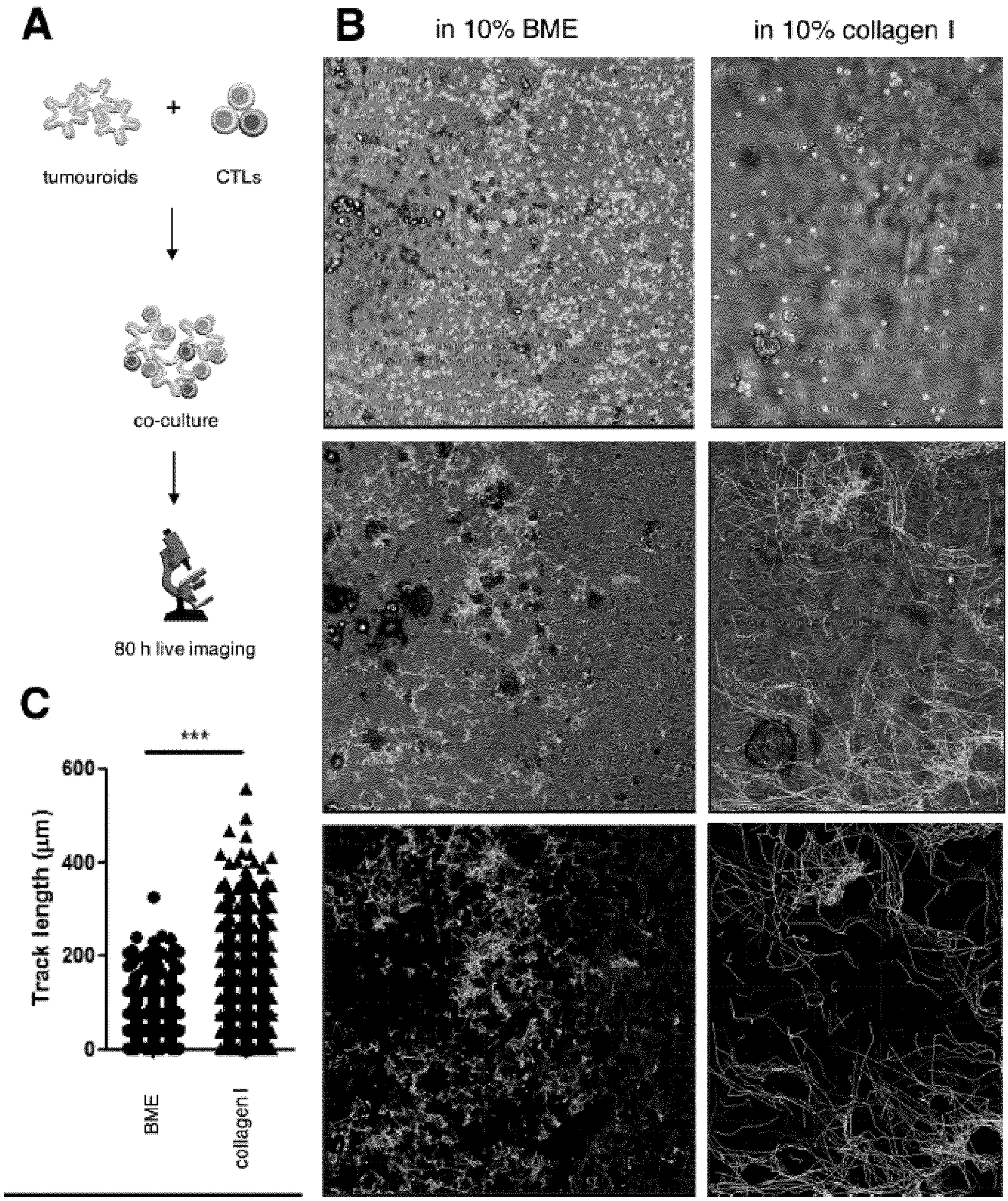

A
tumouroids
+
CTLs
co-culture
80 h live imaging
B
in 10% BME
in 10% collagen I
C
***
Track length (µm)
600
400
200
0
BME
collagen I

Figure 4

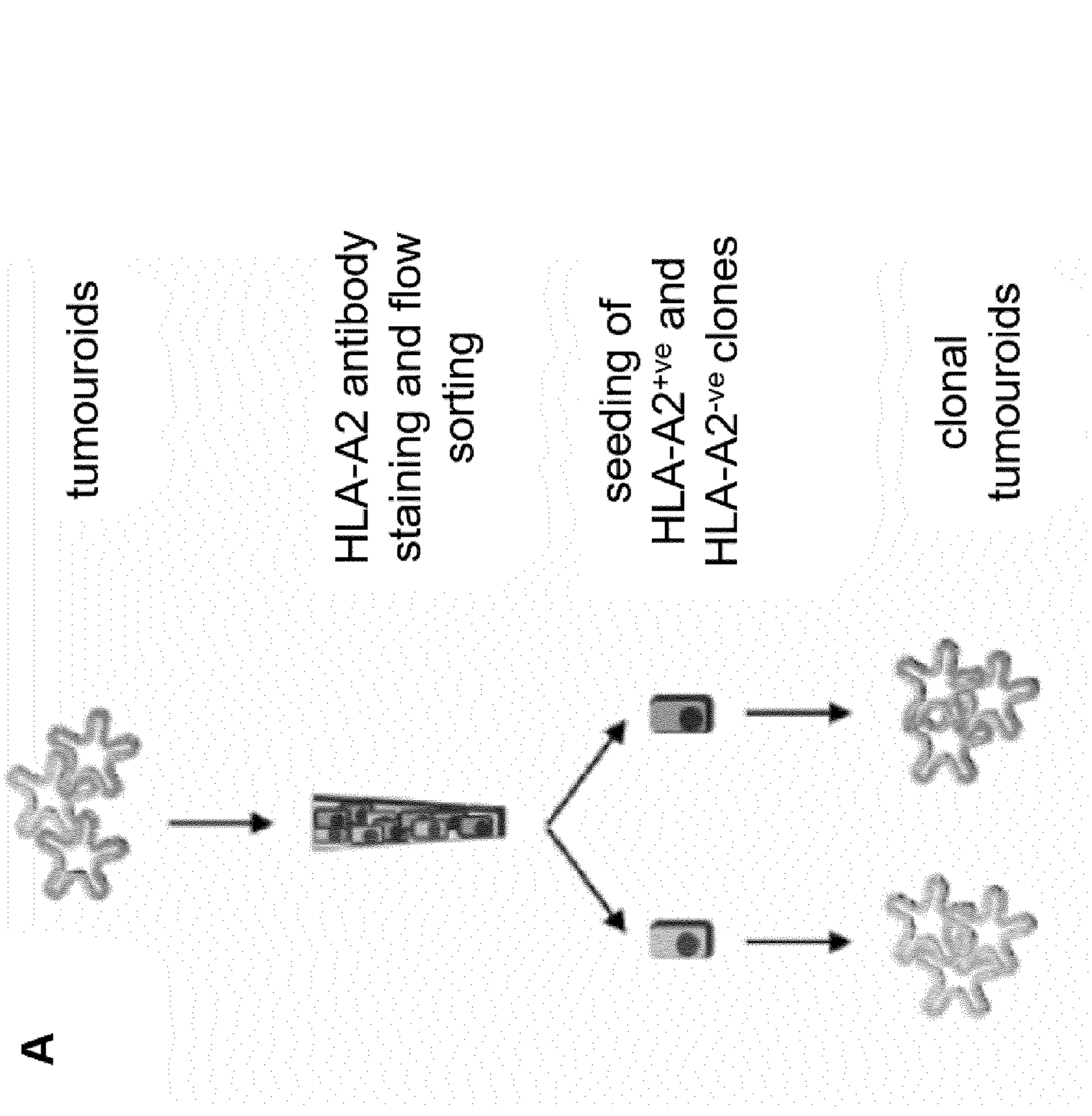

tumouroids
HLA-A2 antibody staining and flow sorting
seeding of HLA-A2+ve and HLA-A2-ve clones
clonal tumouroids
A

Figure 4

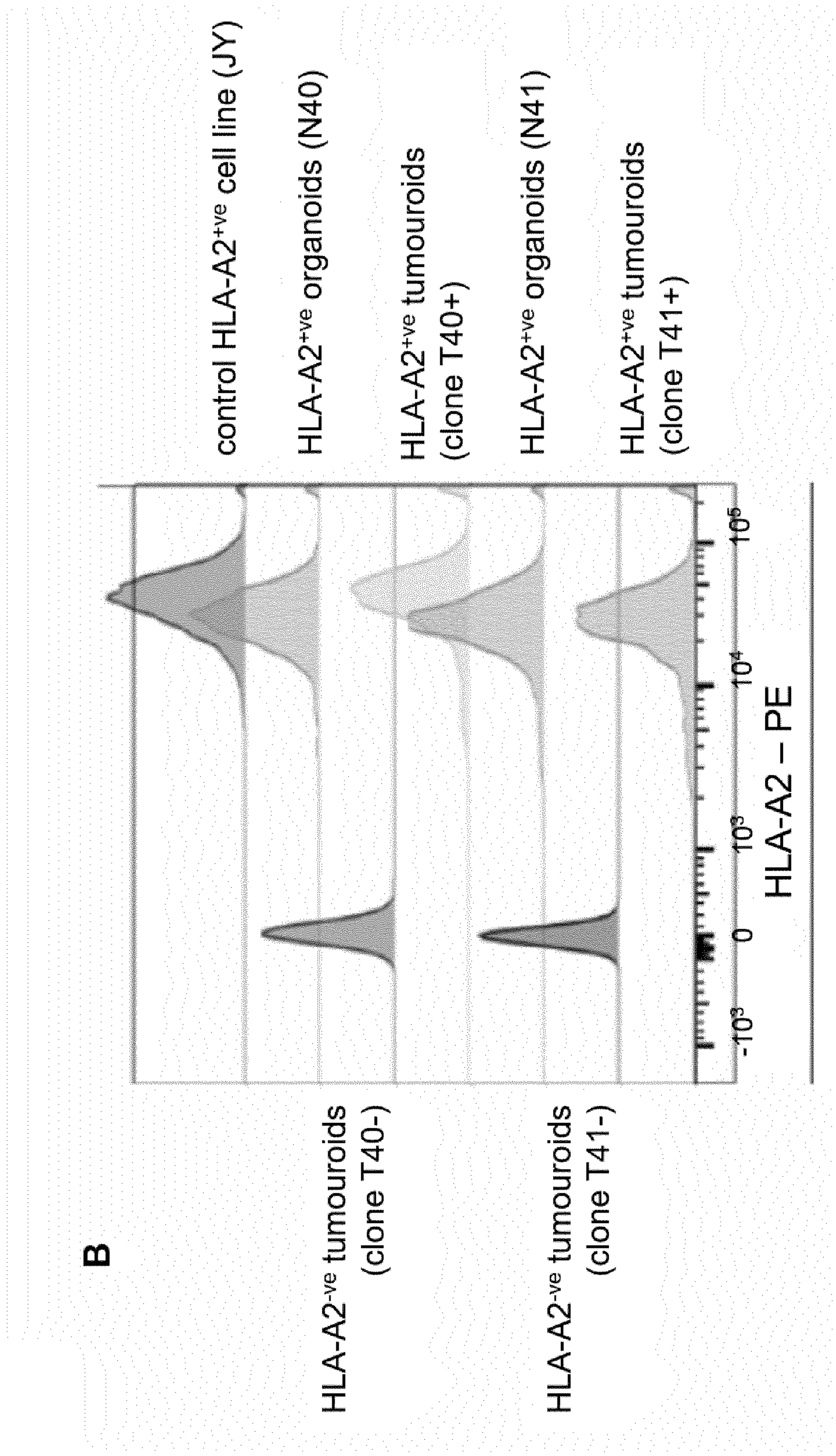
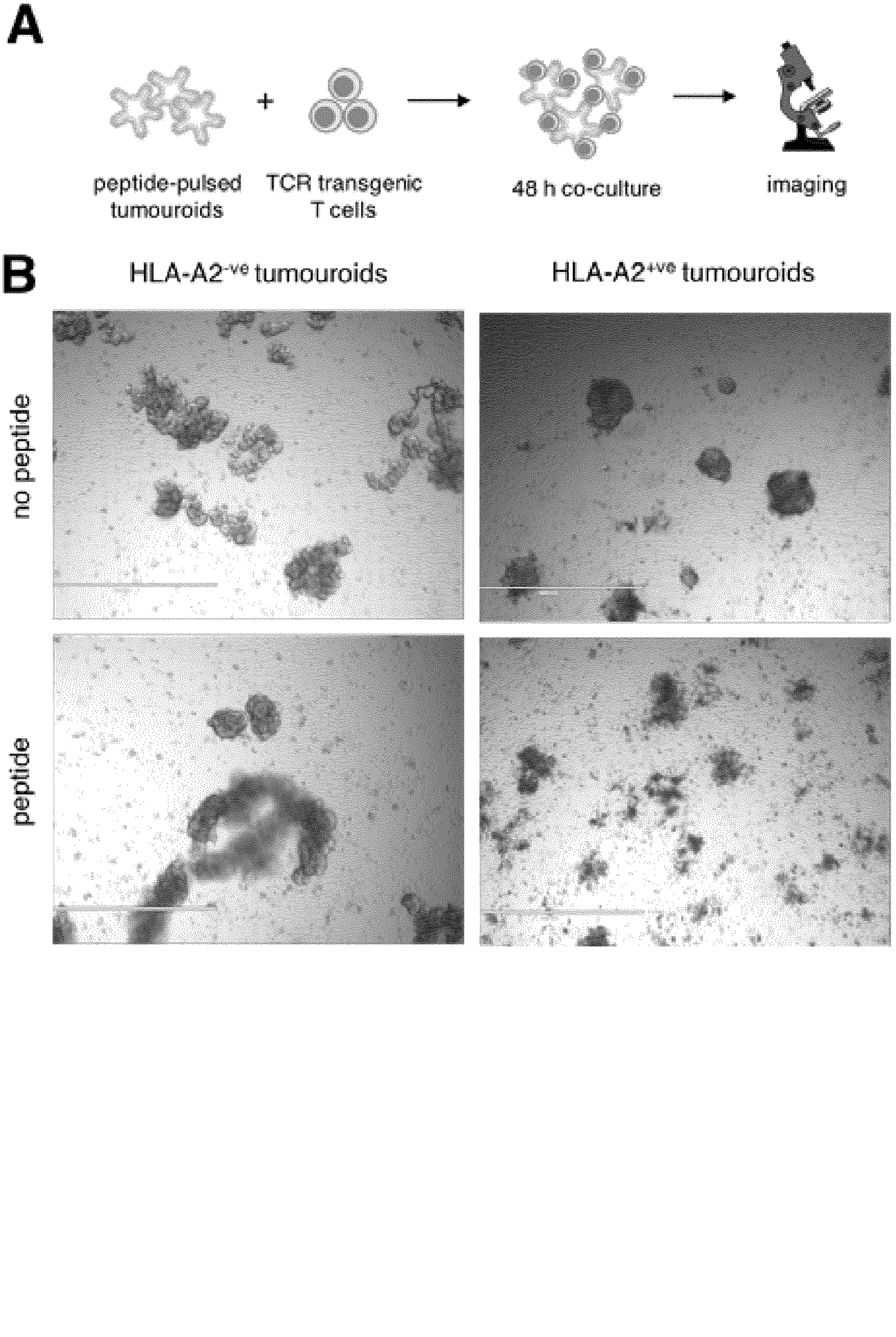

control HLA-A2+ve cell line (JY)
HLA-A2+ve organoids (N40)
HLA-A2+ve tumouroids (clone T40+)
HLA-A2+ve organoids (N41)
HLA-A2+ve tumouroids (clone T41+)
HLA-A2 – PE
-10^3
0
10^3
10^4
10^5
B
HLA-A2-ve tumouroids (clone T40-)
HLA-A2-ve tumouroids (clone T41-)

Figure 5


A
peptide-pulsed tumouroids
TCR transgenic T cells
48 h co-culture
imaging
B
HLA-A2-ve tumouroids
HLA-A2+ve tumouroids
no peptide
peptide

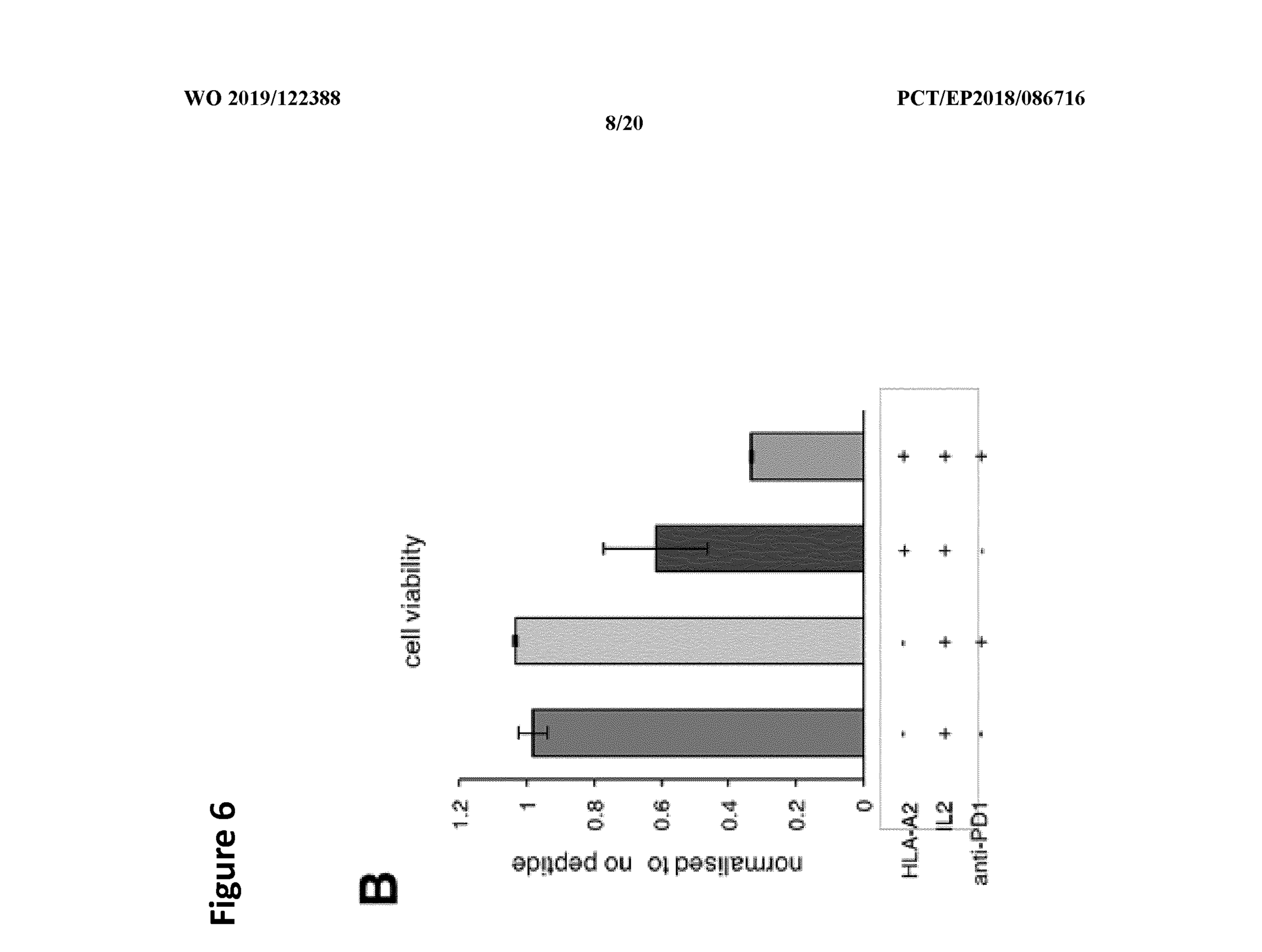

Figure 6
A
peptide-pulsed tumouroids
+
TCR transgenic T cells
12 h co-culture
cell viability assay
B
cell viability
normalised to no peptide
1.2
1
0.8
0.6
0.4
0.2
0
HLA-A2
IL2
anti-PD1
-
+
+
+
+
+
-
+
+
+

Figure 7

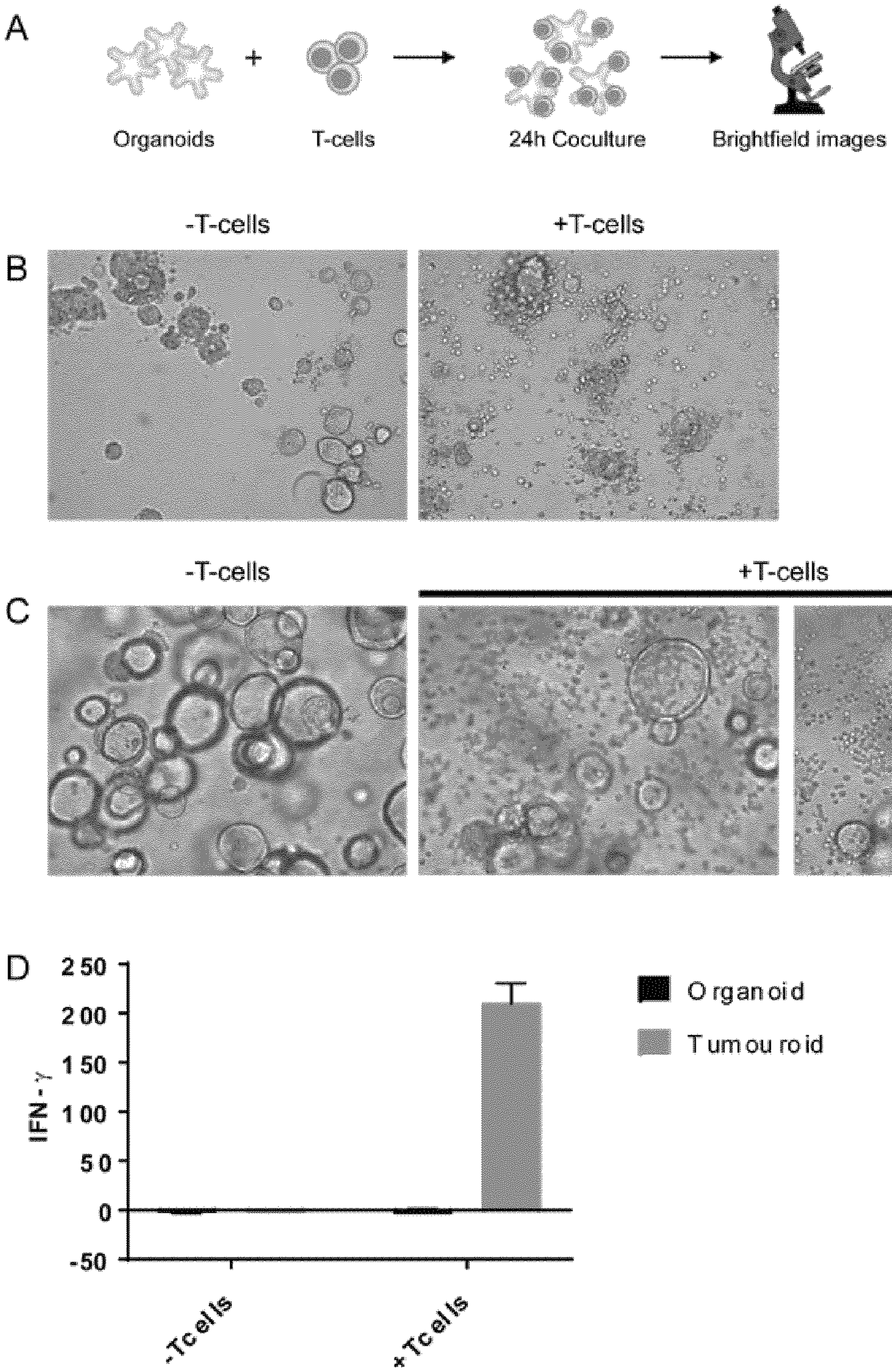

A
Organoids
T-cells
24h Coculture
Brightfield images
-T-cells
+T-cells
B
-T-cells
+T-cells
C
D
IFN-γ
250
200
150
100
50
0
-50
Organoid
Tumouroid
-Tcells
+Tcells

Figure 8

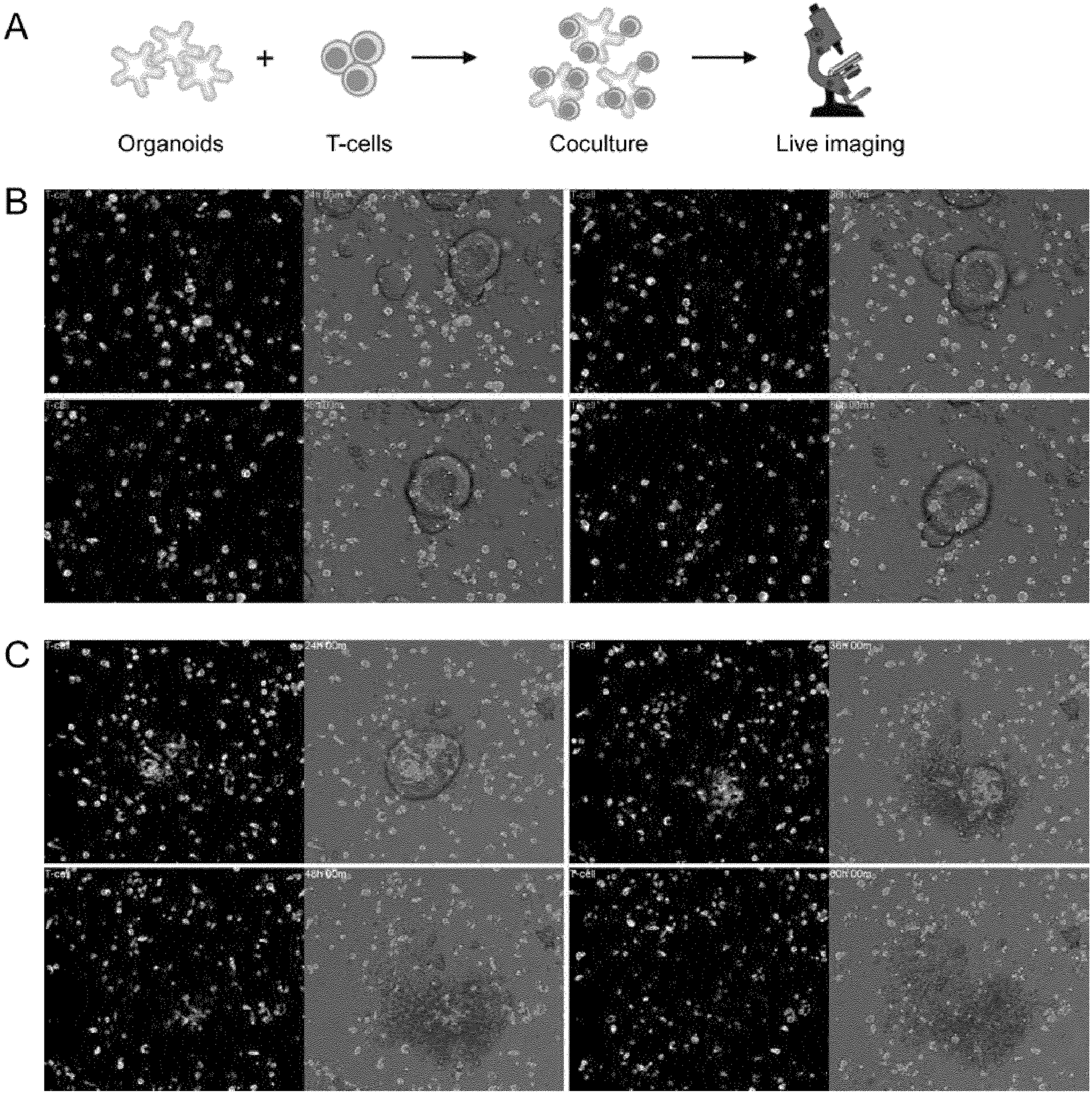


Figure 9

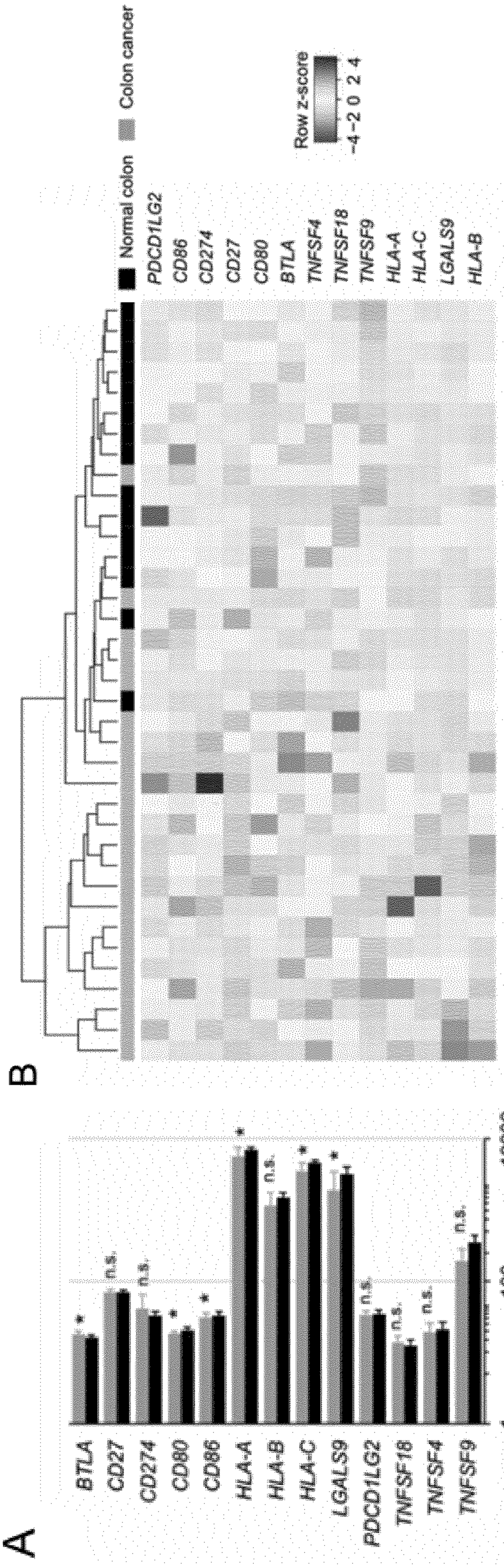
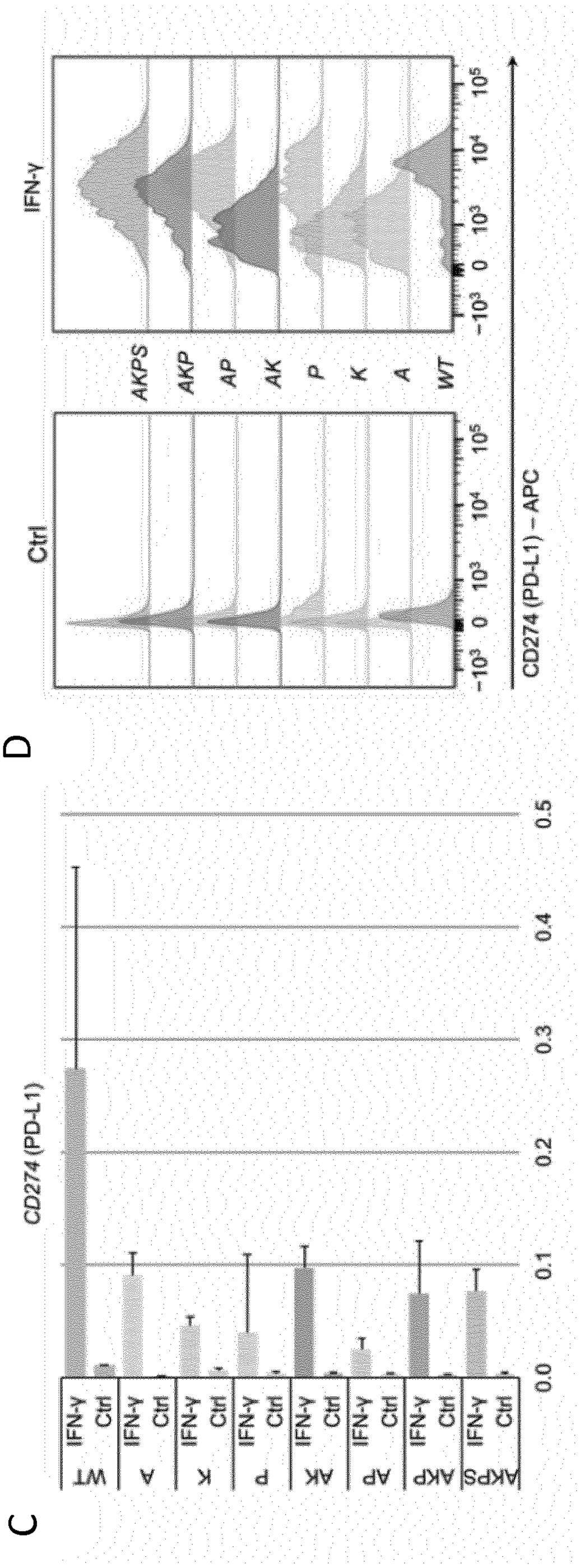

Normal colon
Colon cancer
Row z-score
−4 −2 0 2 4
PDCD1LG2
CD86
CD274
CD27
CD80
BTLA
TNFSF4
TNFSF18
TNFSF9
HLA-A
HLA-C
LGALS9
HLA-B
B
A
10000
100
1
n.s.
Colon cancer
Normal colon

Figure 9


D
Ctrl
AKPS
AKP
AP
AK
P
K
A
WT
IFN-γ
-10^3 0 10^3 10^4 10^5
CD274 (PD-L1) – APC
C
CD274 (PD-L1)
0.0
0.1
0.2
0.3
0.4
IFN-γ
Ctrl
WT
A
K
P
AK
AP
AKP
AKPS

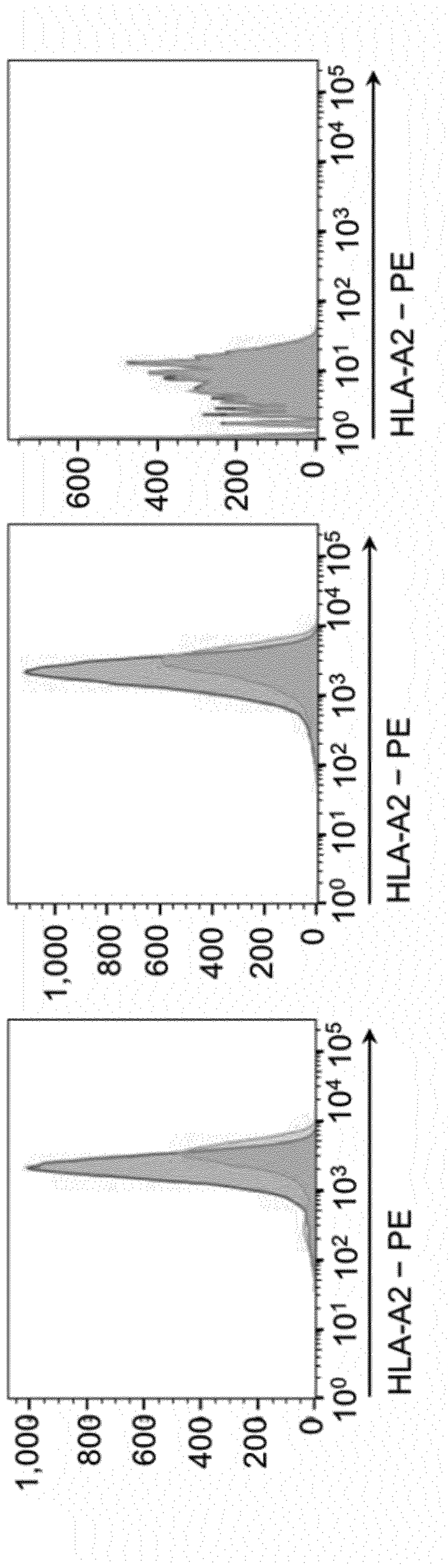

600
400
200
0
10^0 10^1 10^2 10^3 10^4 10^5
HLA-A2 – PE
1,000
800
600
400
200
0
10^0 10^1 10^2 10^3 10^4 10^5
HLA-A2 – PE
1,000
800
600
400
200
0
10^0 10^1 10^2 10^3 10^4 10^5
HLA-A2 – PE
Ctrl (no stimulation)
IFN-γ stimulation

Figure 10

Figure 11

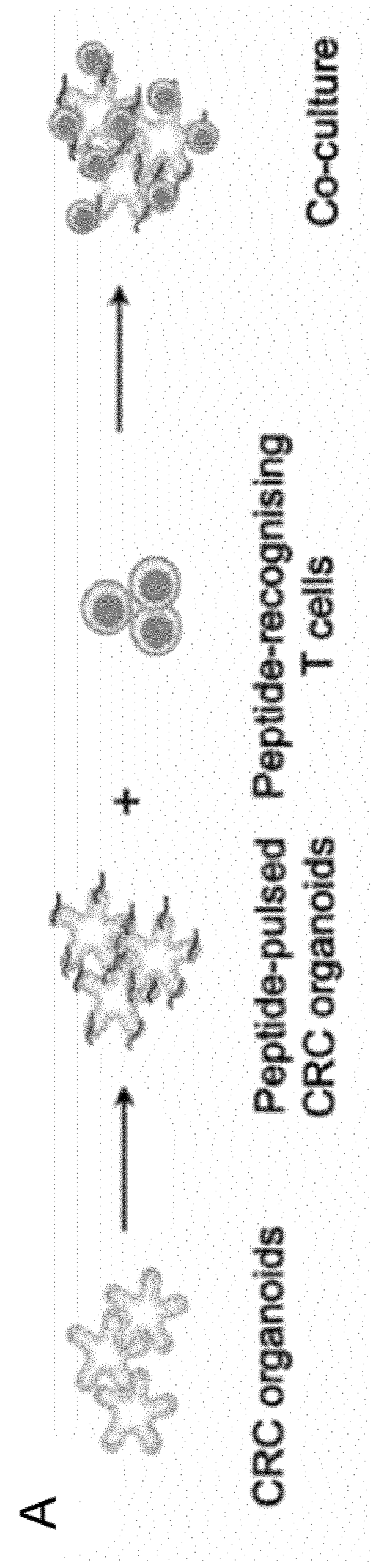

Co-culture
Peptide-recognising
T cells
+
Peptide-pulsed
CRC organoids
CRC organoids
A

Figure 11

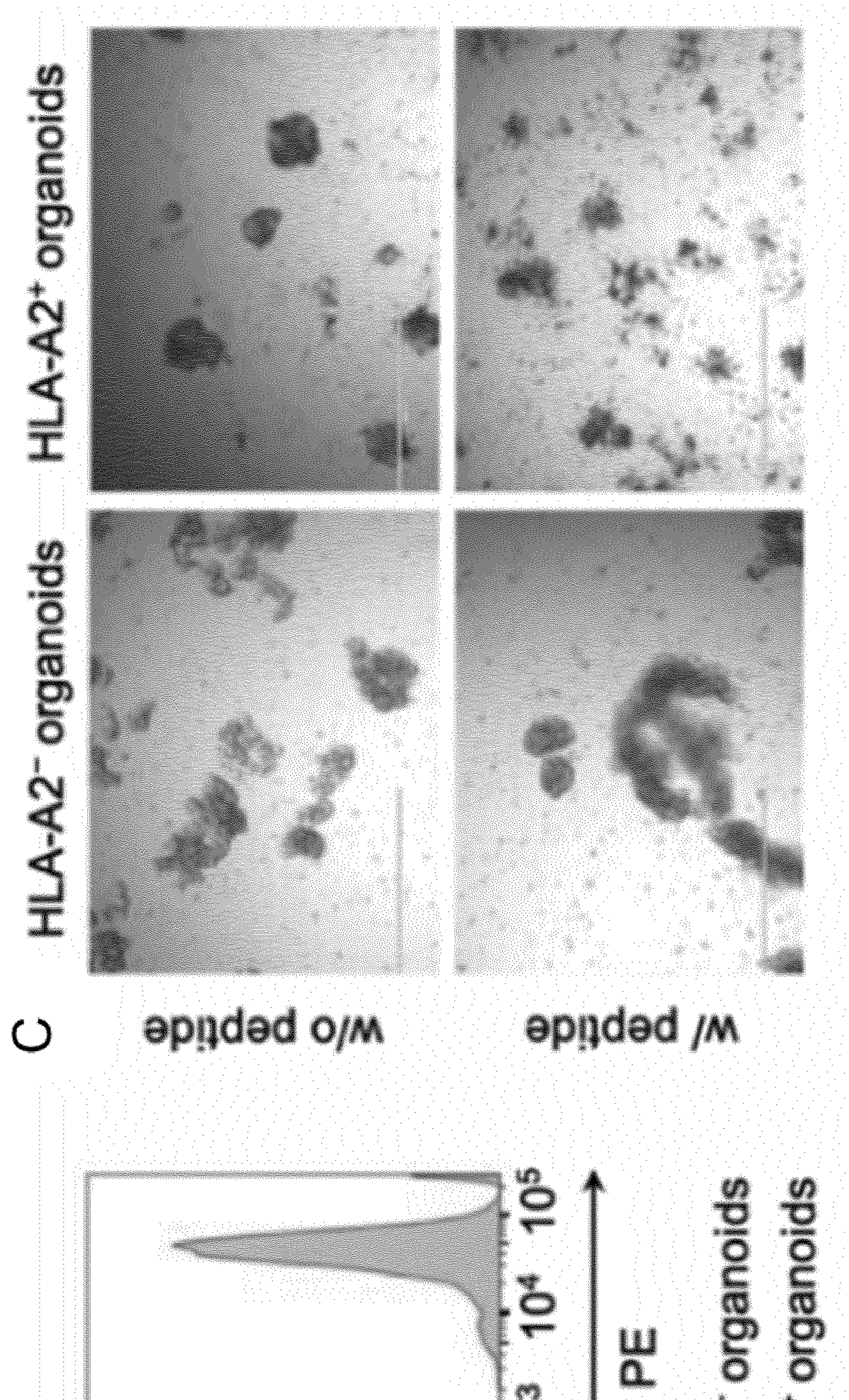

HLA-A2⁻ organoids
HLA-A2⁺ organoids
w/o peptide
w/ peptide
C
B
100
80
60
40
20
0
−10³ 0 10³ 10⁴ 10⁵
HLA-A2 – PE
HLA-A2⁻ organoids
HLA-A2⁺ organoids

Figure 11

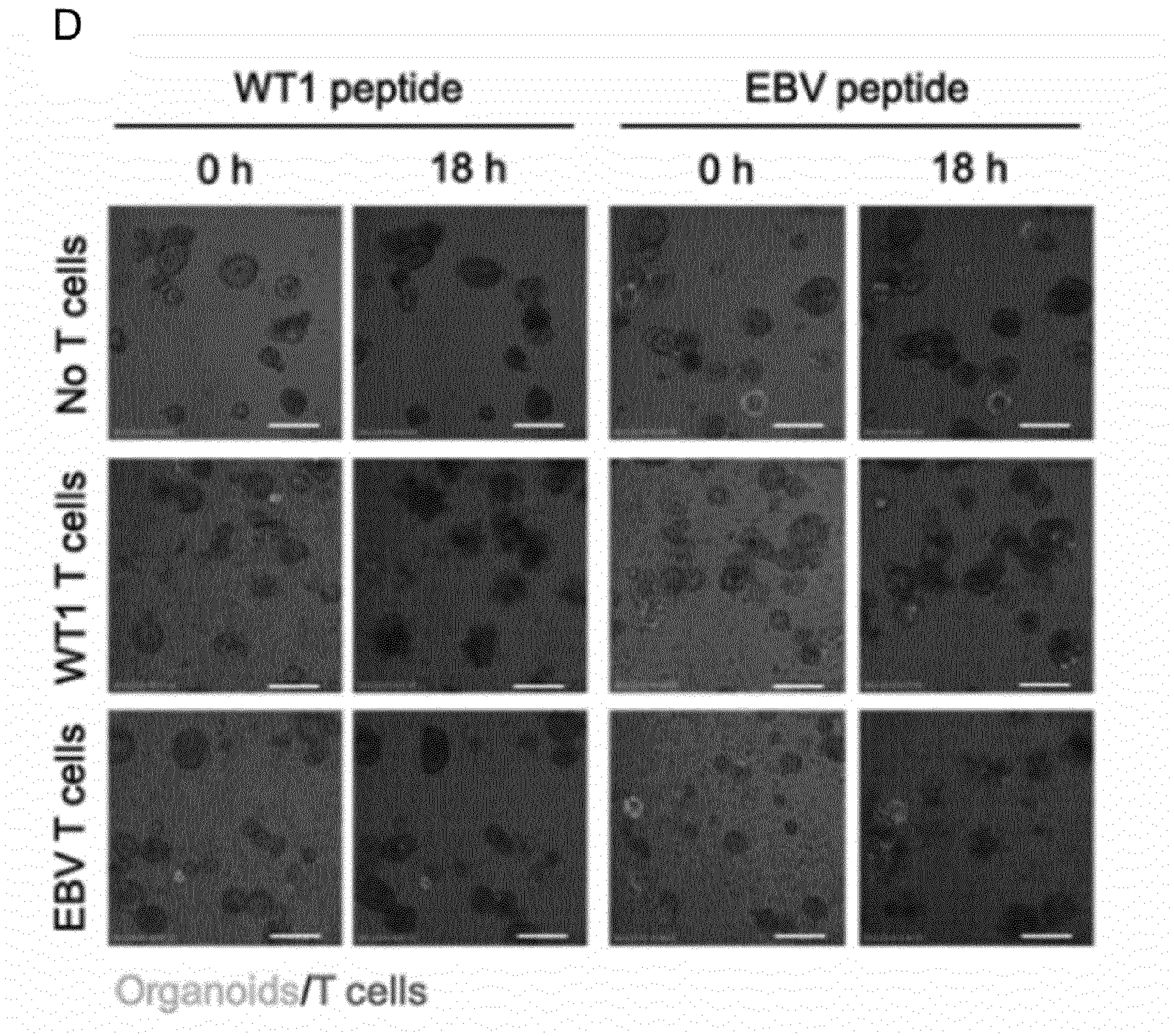

D
WT1 peptide
EBV peptide
0 h
18 h
0 h
18 h
No T cells
WT1 T cells
EBV T cells
Organoids/T cells

Figure 11

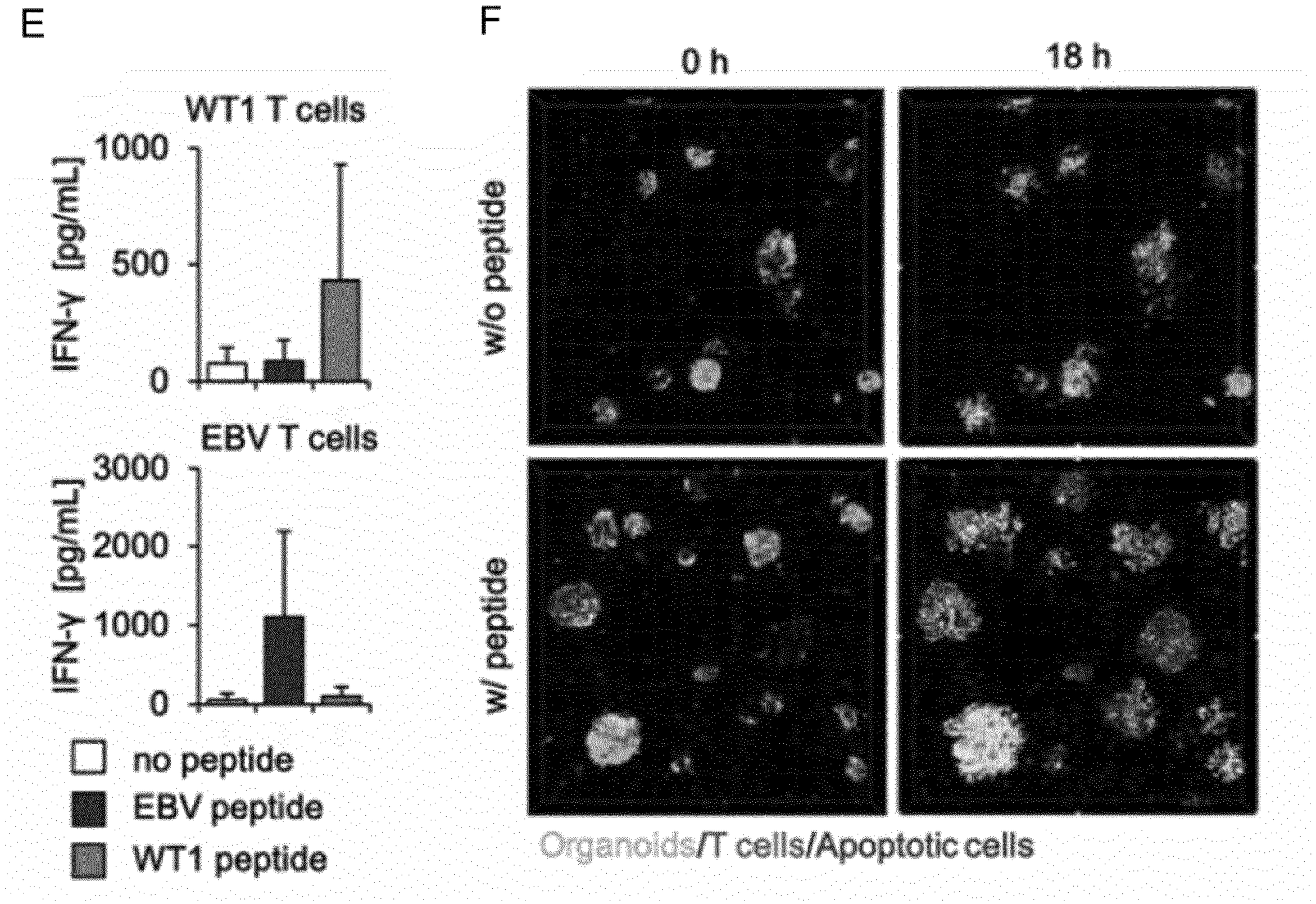

E
WT1 T cells
IFN-γ [pg/mL]
1000
500
0
EBV T cells
3000
2000
1000
0
no peptide
EBV peptide
WT1 peptide
F
0 h
18 h
w/o peptide
w/ peptide
Organoids/T cells/Apoptotic cells

Figure 11

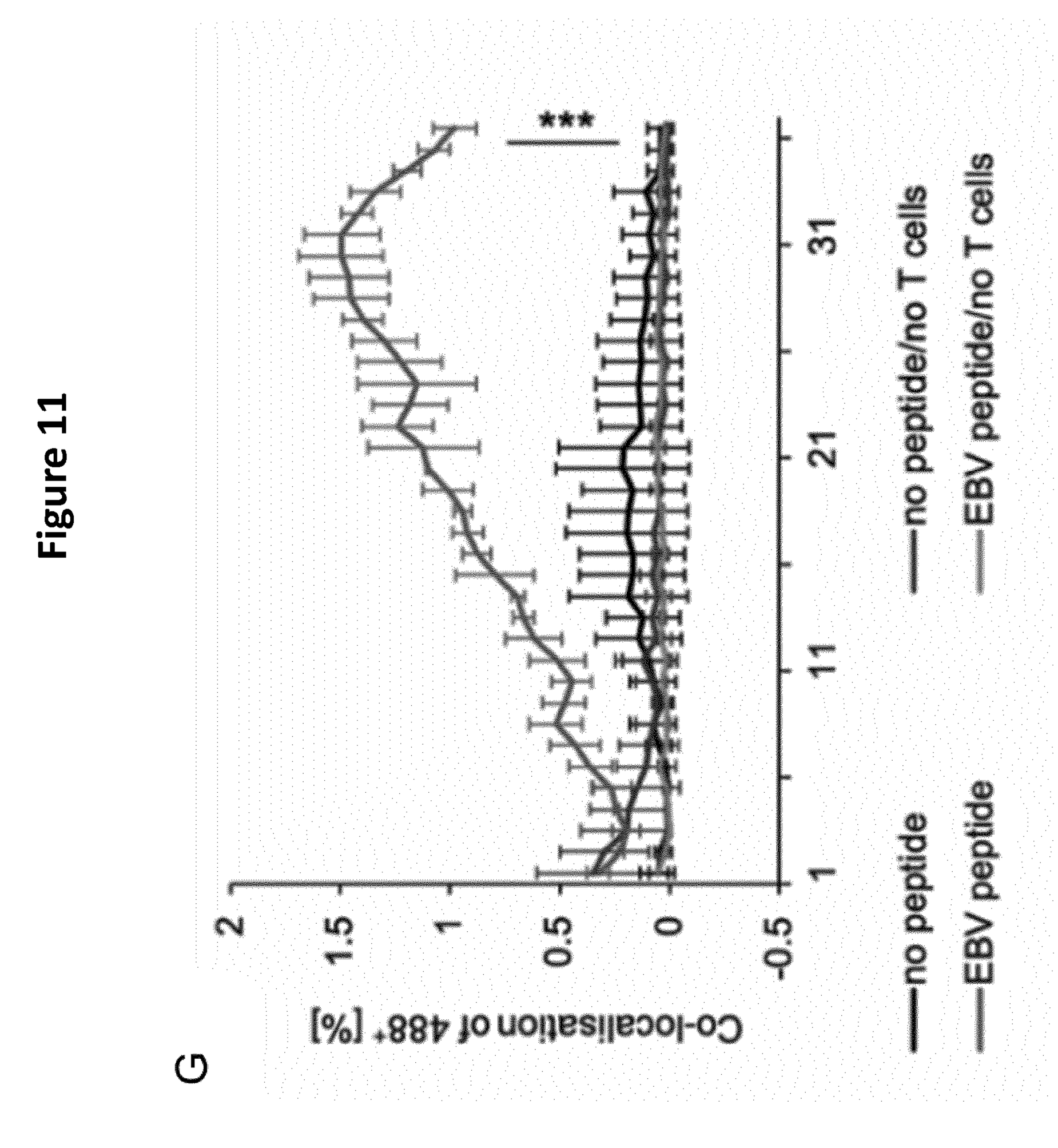

G
Co-localisation of 488+ [%]
2
1.5
1
0.5
0
-0.5
1
11
21
31
***
no peptide
EBV peptide
no peptide/no T cells
EBV peptide/no T cells

Figure 11

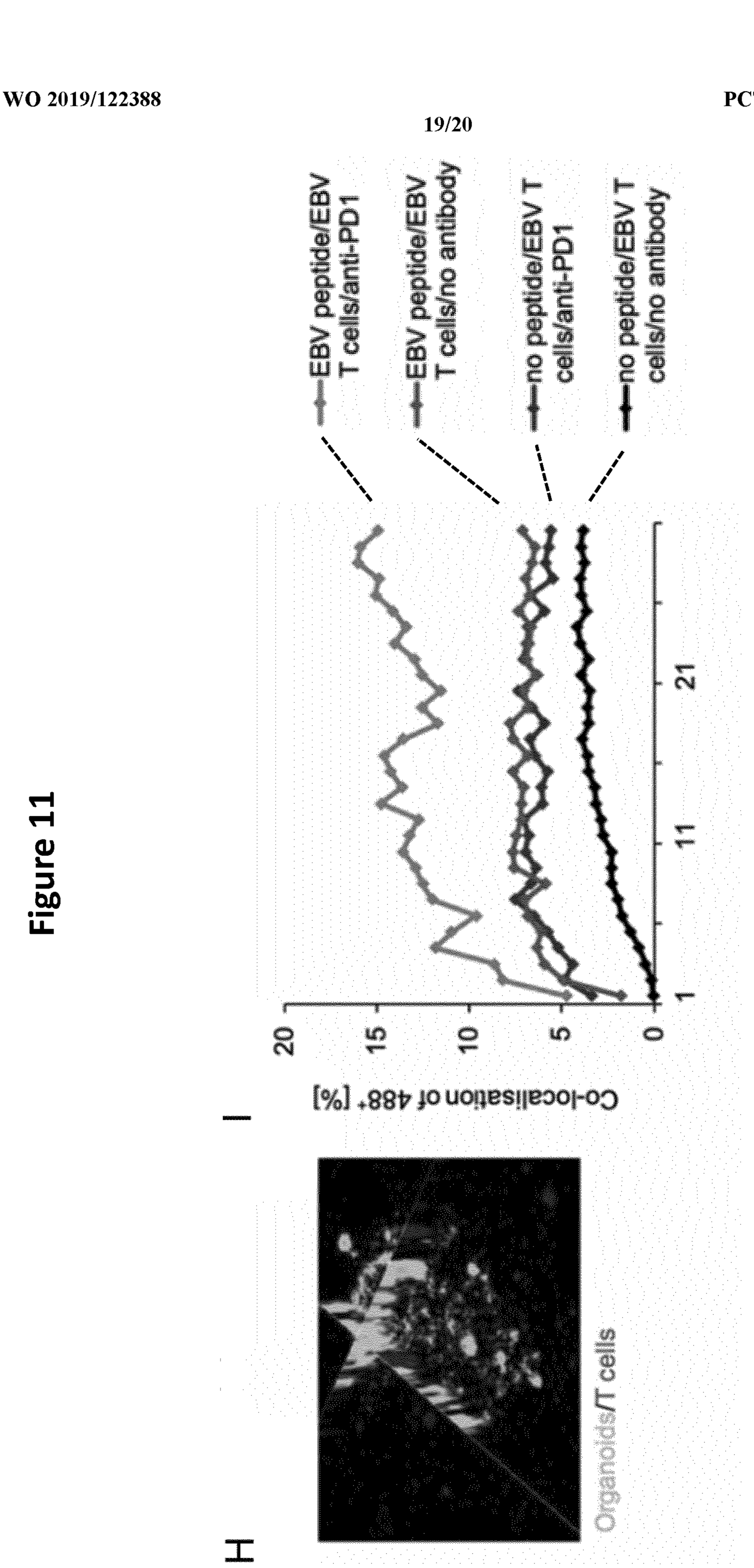

EBV peptide/EBV T cells/anti-PD1
EBV peptide/EBV T cells/no antibody
no peptide/EBV T cells/anti-PD1
no peptide/EBV T cells/no antibody
Co-localisation of 488+ [%]
20
15
10
5
0
1
11
21
Organoids/T cells

Figure 11

