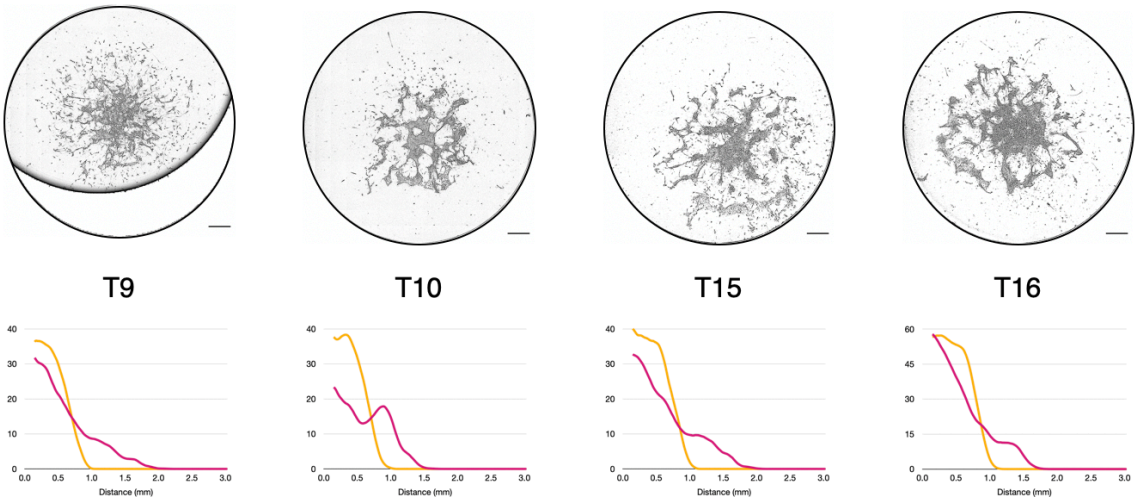
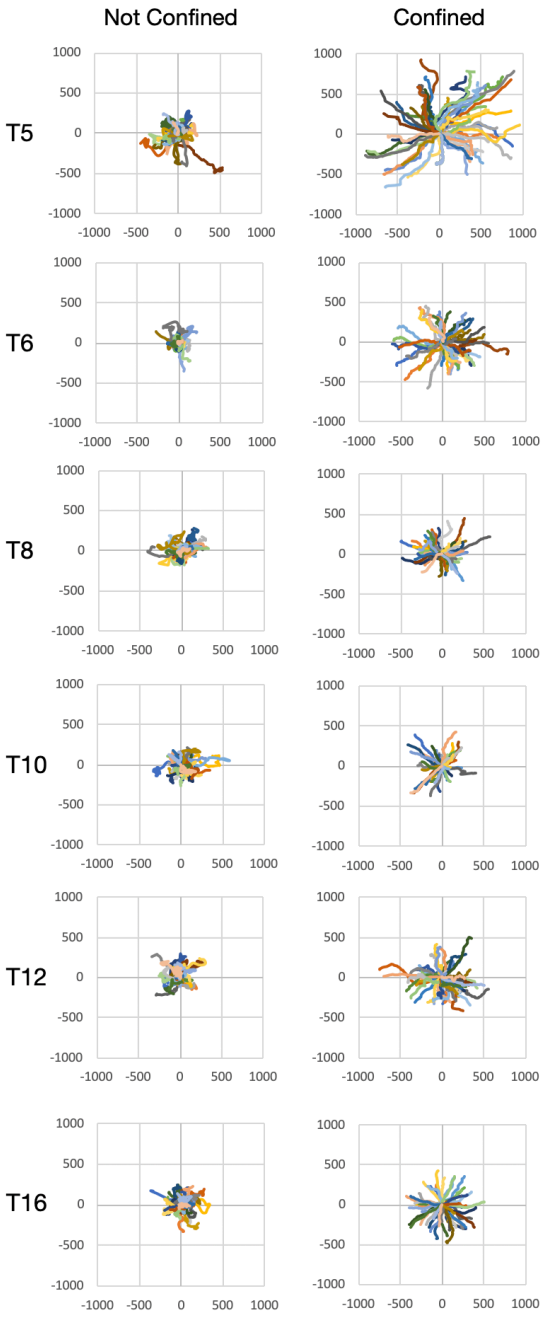
Supplementary Figures and legends

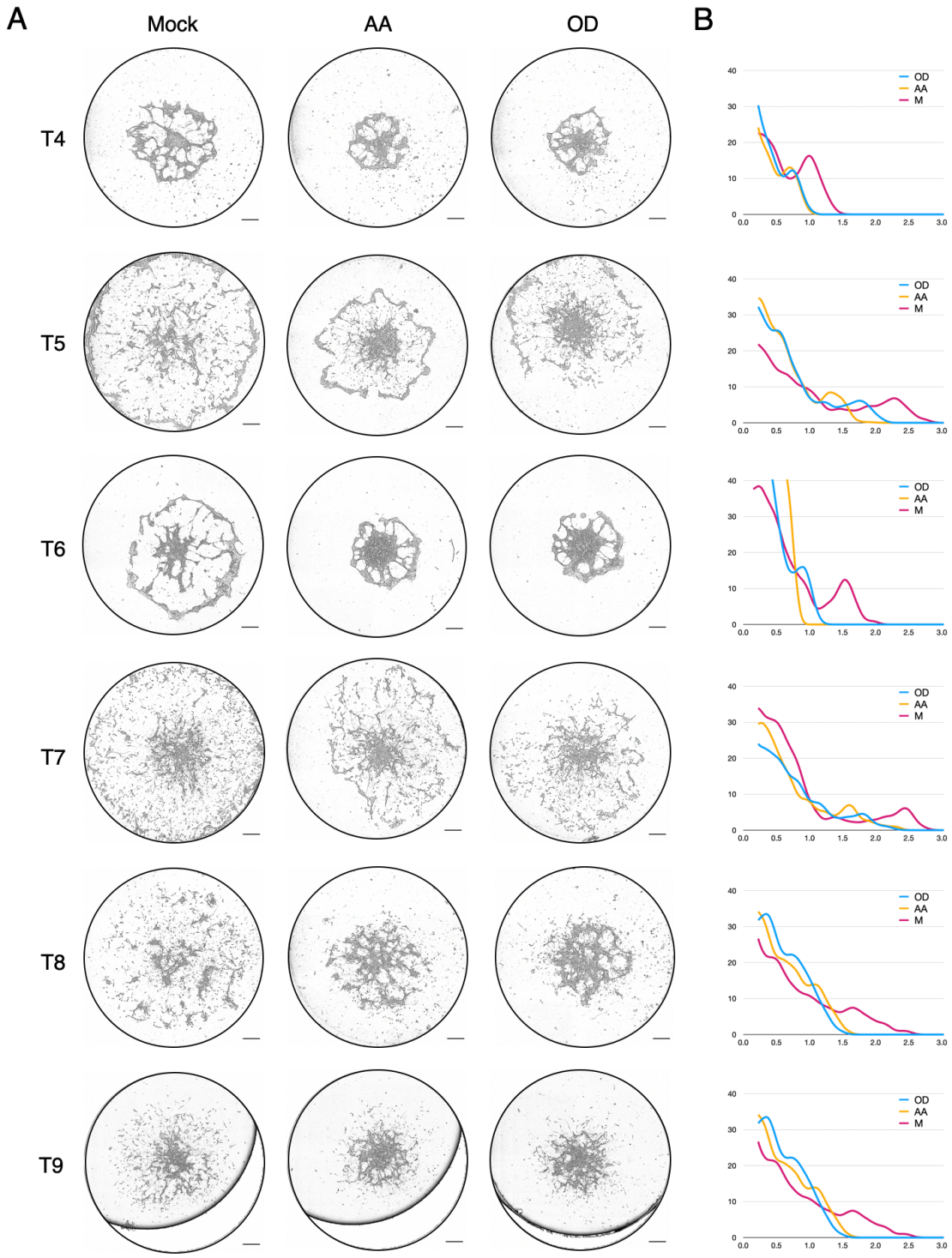


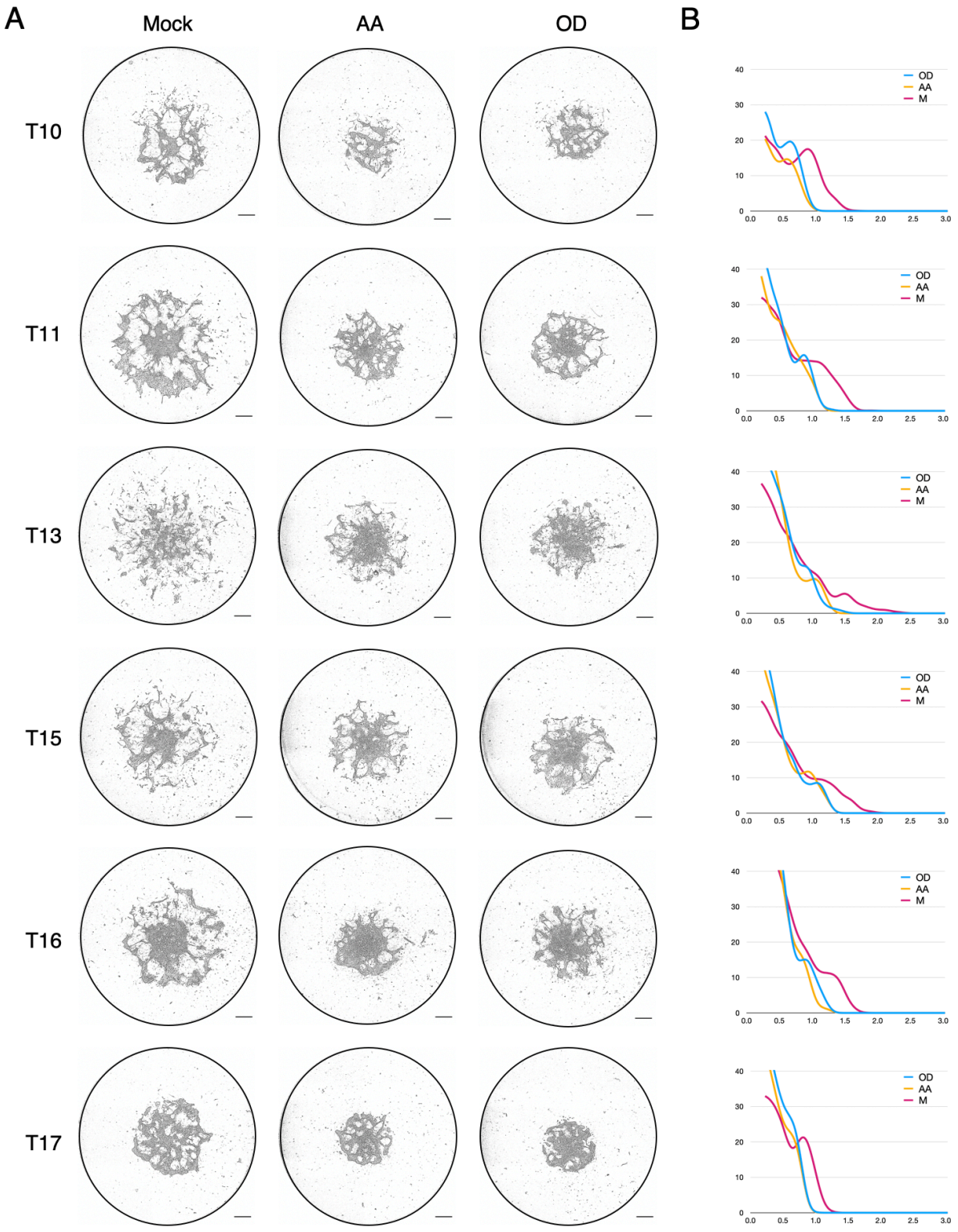
Sup Figure 1: Migration of T9, T10, T15 and T16 breast tumour cells in the aerotactic assay. Representative images taken at 48 h corresponding to the aerotactic migration of T9, T10, T15 and T16 tumour cells subjected to the aerotactic assay. Cell distribution from the centre of the well at 0 h (yellow) and 48 h (carmine) are indicated as graphs (mean of three experiments). Scale bars, 0.5 mm.



Sup Figure 2: Comparison of aerotaxis versus intrinsic migration of T5, T6, T8 T10, T12 and T16 breast cancer cells.

24 h trajectories of 50 representative cells per condition followed as XY plots (scales in µm) of the indicated cells either plated at 5% confluency (not confined) or subjected to the aerotactic assay (confined).

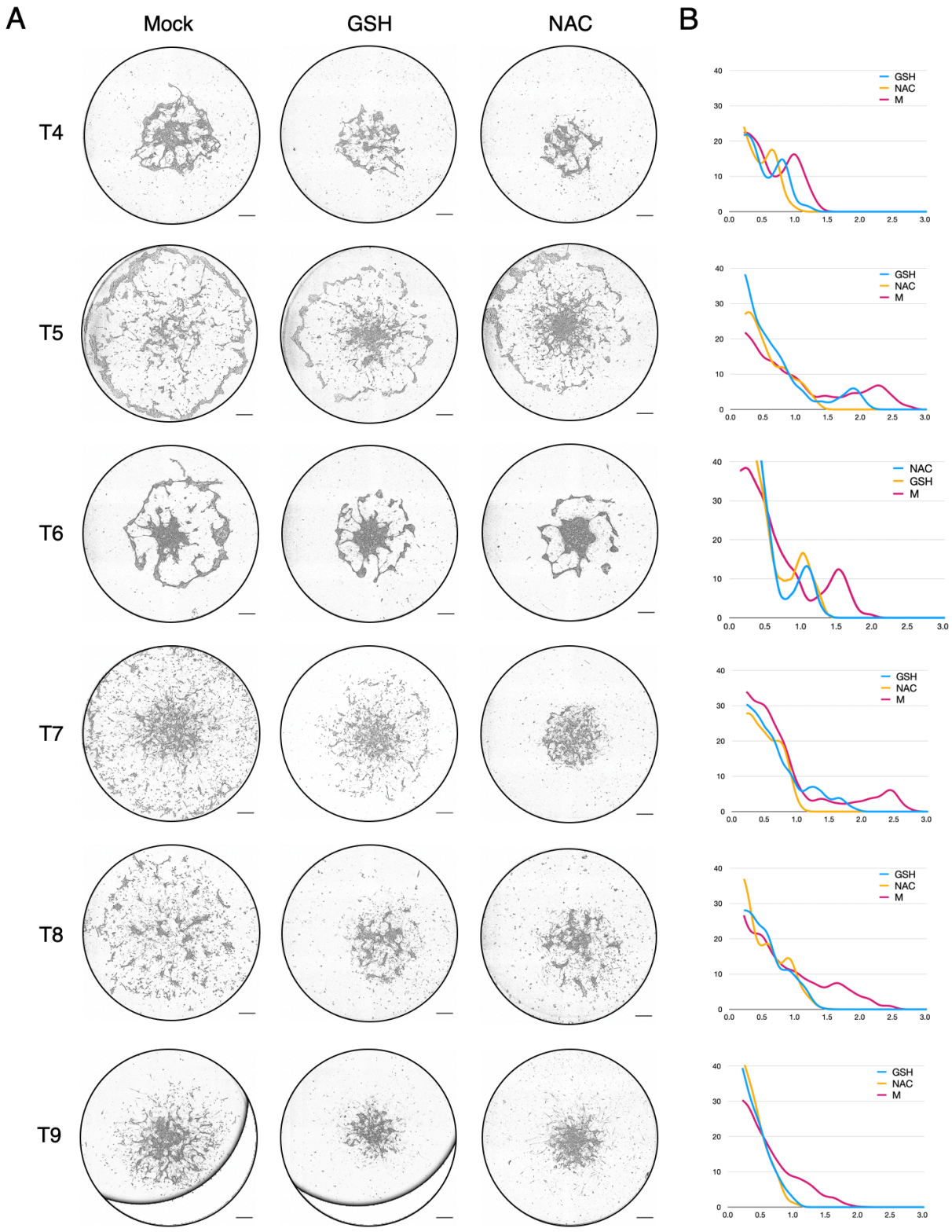

A
Mock
AA
OD
B
T4
T5
T6
T7
T8
T9
OD
AA
M
0
10
20
30
40
0.0
0.5
1.0
1.5
2.0
2.5
3.0

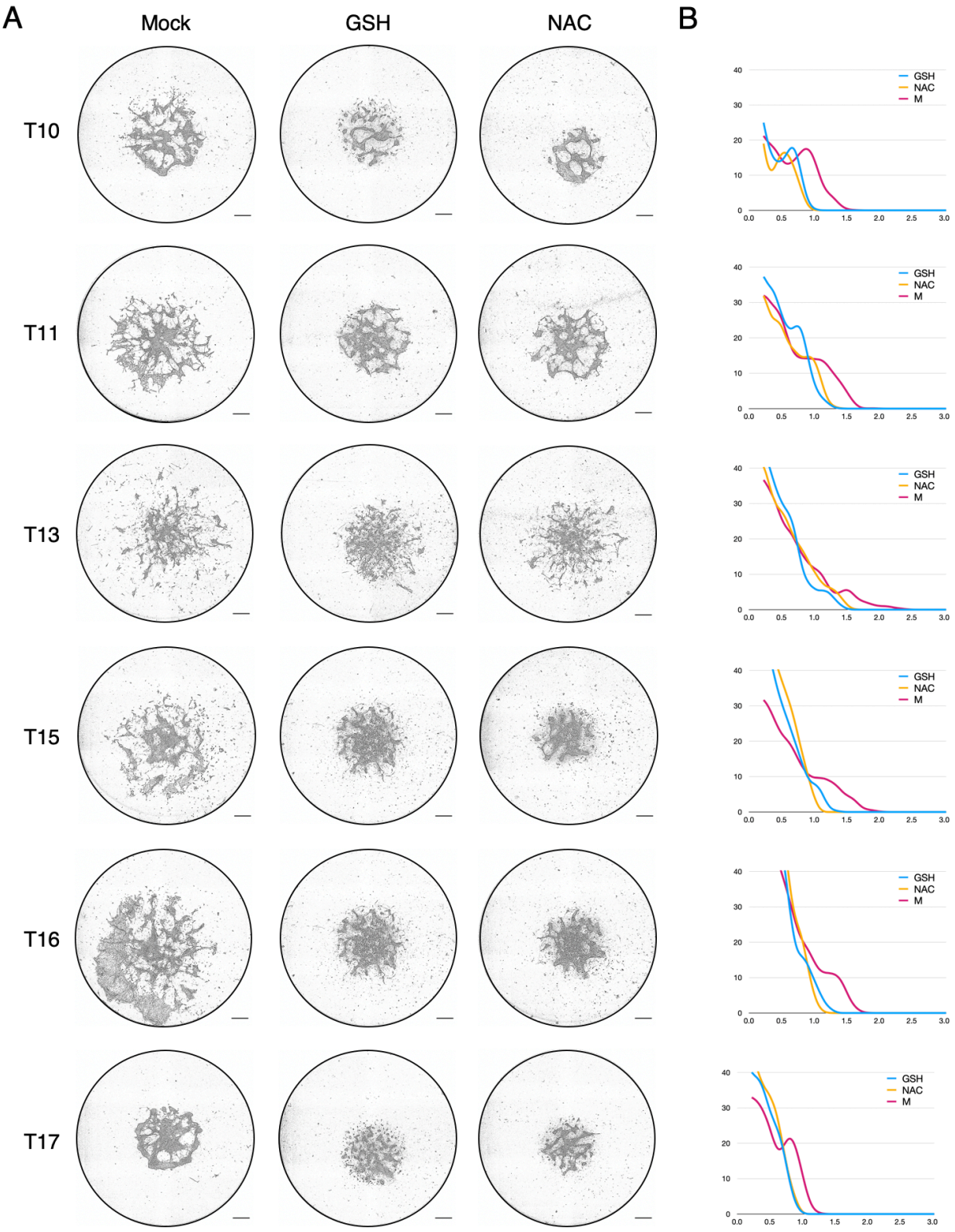


Sup Figure 3: Migration of breast cancer cells in the aerotactic assay relies on a functional oxidative phosphorylation (OXPHOS) system.

(A) Aerotactic migration at 48 h of the indicated tumour cells either untreated (Mock) or treated with Antimycin A (AA, 1 µM) or Oligomycin D (OD, 0.5 µM). Scale bars 0.5 mm. **(B)** Cell distribution from

the centre of the well at 48 h in the Mock condition (carmine, mean of three independent experiments) in cells treated with 1 μM AA (yellow) or 0.5 μM OD (blue).

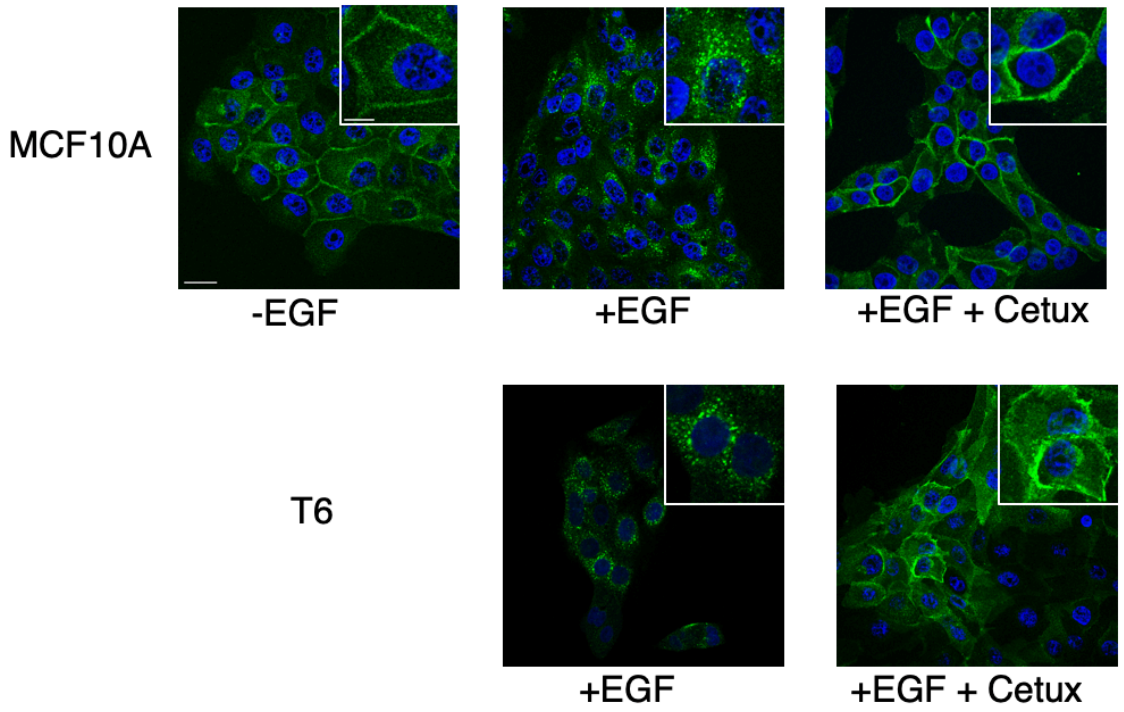

A
Mock
GSH
NAC
T4
T5
T6
T7
T8
T9
B
GSH
NAC
M
NAC
GSH
M
0
10
20
30
40
0.0
0.5
1.0
1.5
2.0
2.5
3.0



Sup Figure 4 : Aerotactic migration is impaired by antioxidants.

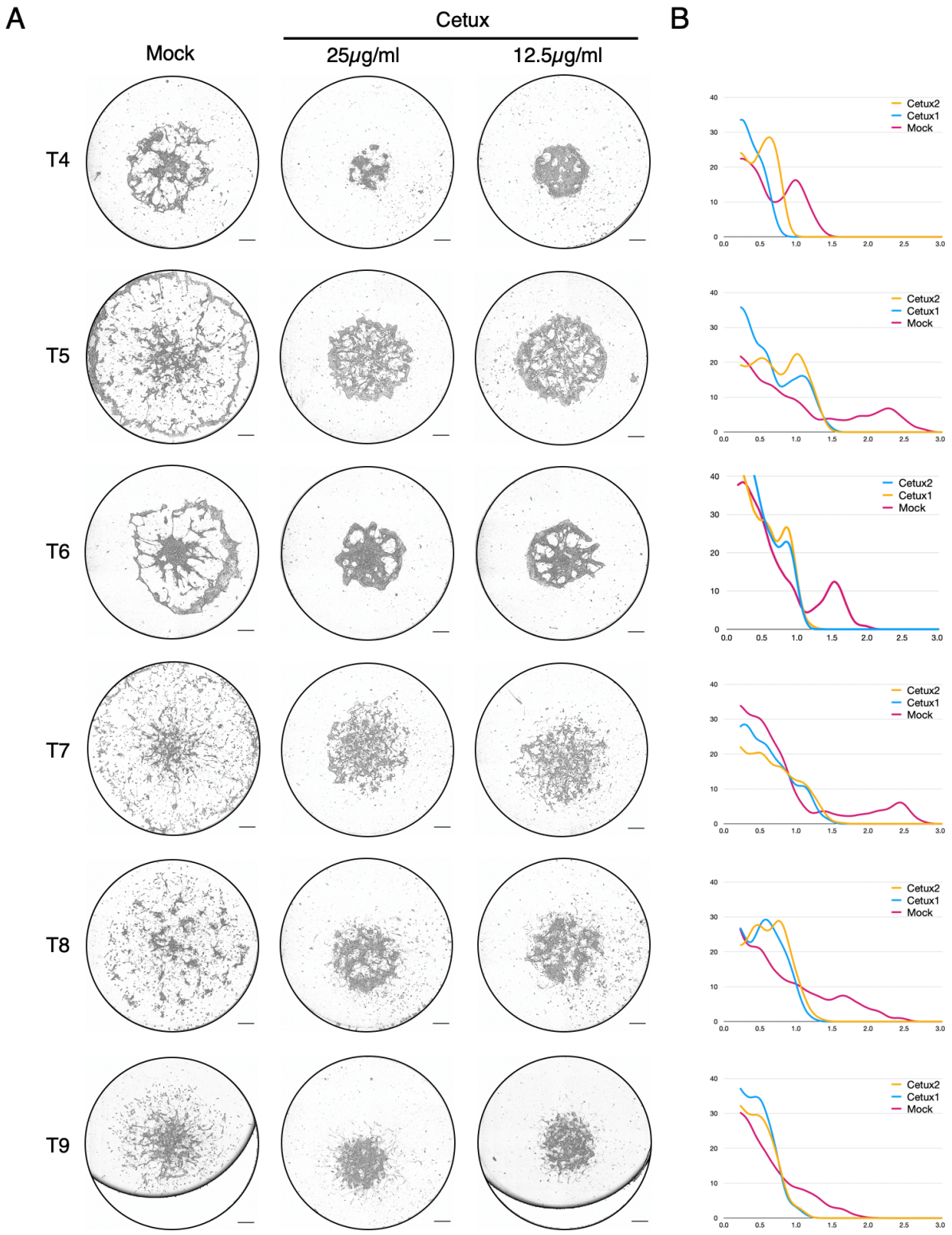
(A) Aerotactic migration at 48 h of the indicated tumour cells either untreated (Mock) or treated with 10 mM reduced glutathione (GSH, except for T7 treated with 5 mM GSH) or 10 mM N-Acetyl Cysteine (NAC). Scale bars 0.5 mm. **(B)** Cell distribution from the centre of the cell cluster (in mm) at 48 h in the

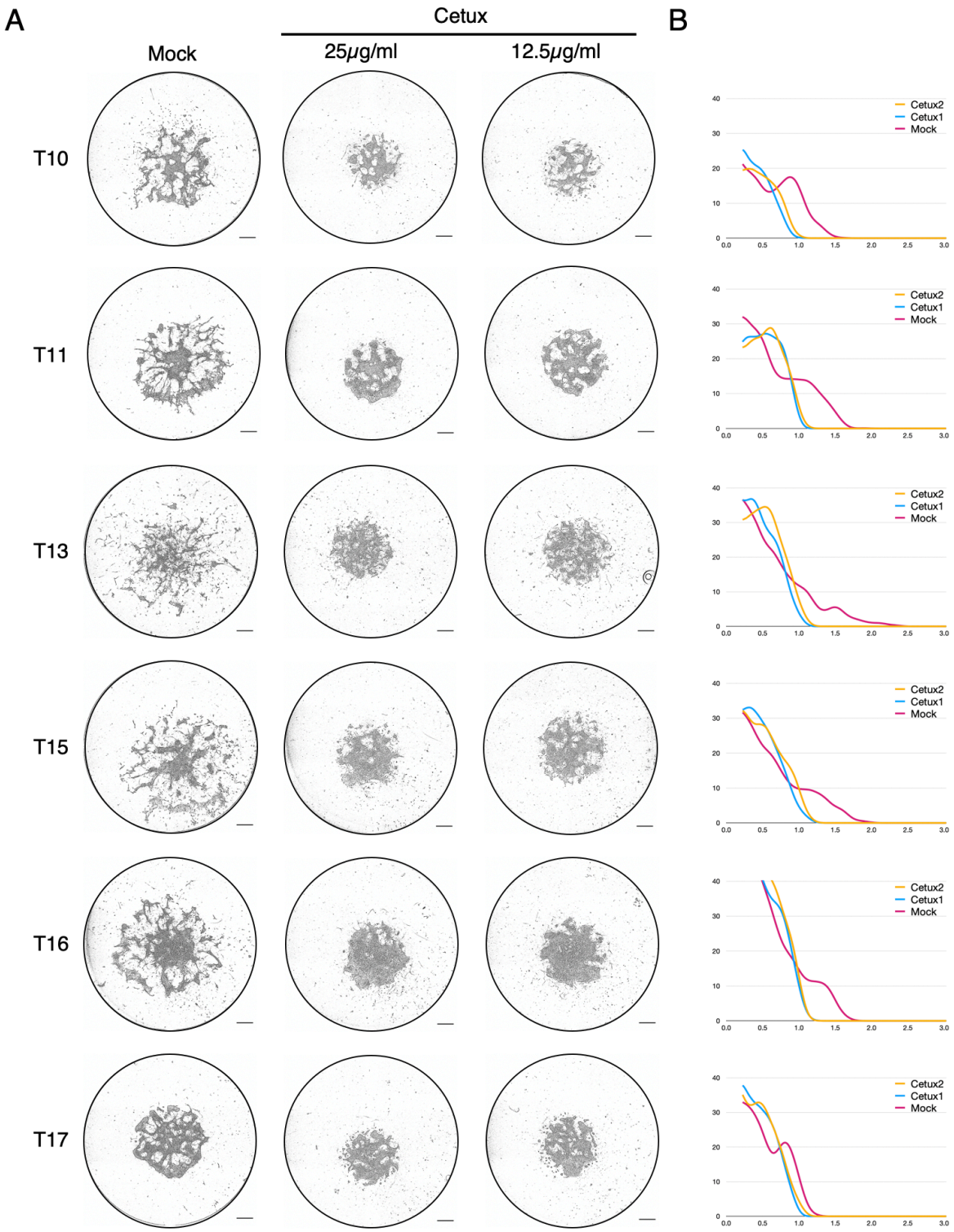
Mock condition (carmine, mean of three experiments) in cells treated with 10 mM GSH (blue) or 10 mM NAC (yellow).



Sup Figure 5: Re-localization of EGFR following Cetuximab treatment.

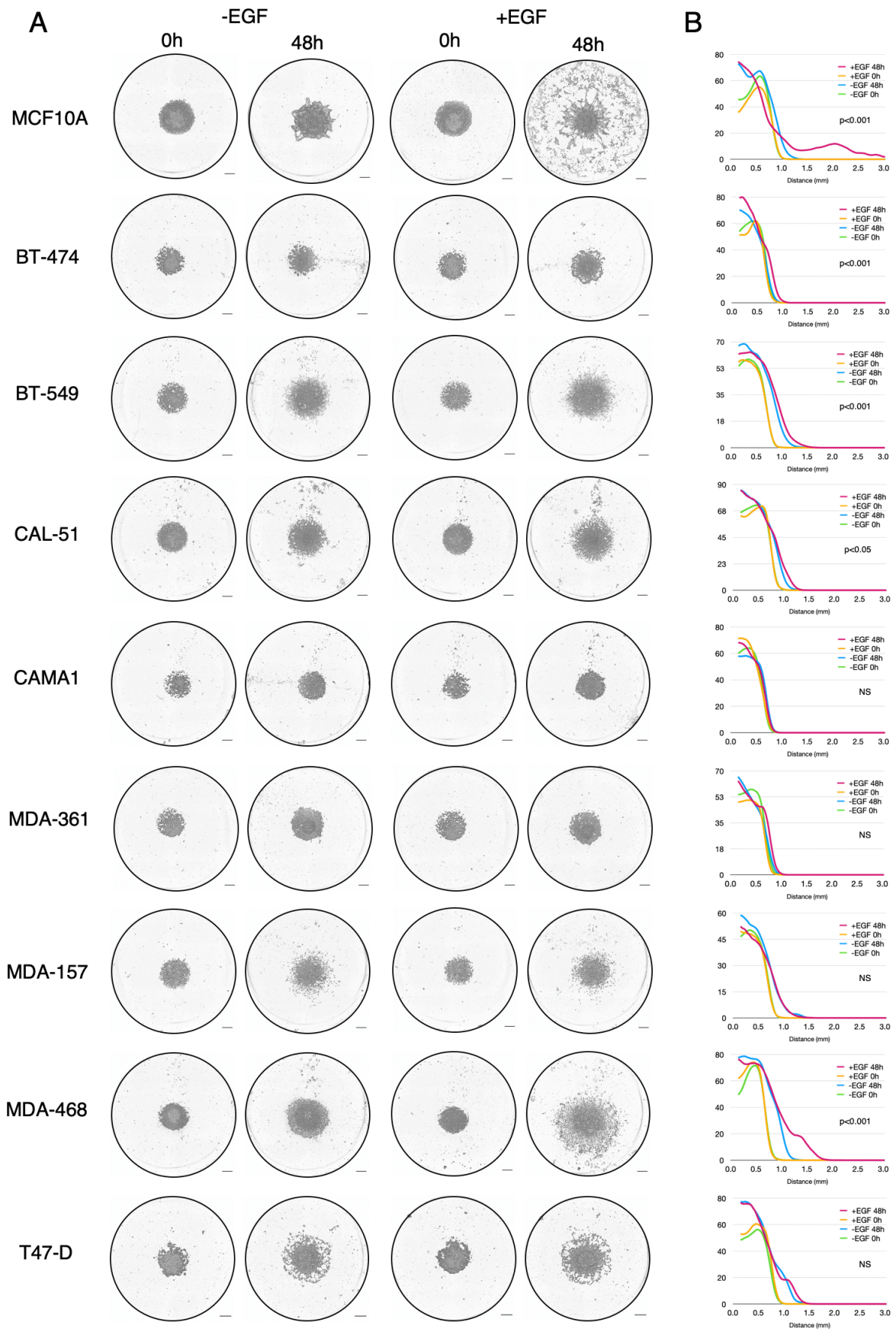
(A) Immunofluorescence imaging of EGFR in the absence of EGF (-EGF), upon activation by EGF (+EGF), or incubated with EGF and Cetuximab, a monoclonal EGFR neutralising antibody (+EGF +Cetux) in MCF10A cells and T6 cells. Scale bars 25 μm and 10 μm for higher resolution images.


A
Cetux
Mock
25μg/ml
12.5μg/ml
T4
T5
T6
T7
T8
T9
B
Cetux2
Cetux1
Mock
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40



Sup Figure 6: Aerotaxis of breast cancer cells is dependent on EGFR activation.

(A) Aerotactic migration at 48 h of the indicated tumour cells either untreated (Mock), or treated with 25 µM or 12.5 µM of Cetuximab (Cetux). Scale bars 0.5 mm. **(B)** Cell distribution from the centre of the cell cluster (in mm) at 48 h in the Mock condition (carmine, mean of three experiments) in cells treated with 10 mM GSH (blue) or 10 mM NAC (yellow).


A
-EGF
+EGF
0h
48h
0h
48h
MCF10A
BT-474
BT-549
CAL-51
CAMA1
MDA-361
MDA-157
MDA-468
T47-D
B
+EGF 48h
+EGF 0h
-EGF 48h
-EGF 0h
p<0.001
p<0.001
p<0.001
p<0.05
NS
NS
NS
p<0.001
NS
Distance (mm)

Sup Figure 7: Migration of breast cancer cell lines in the aerotactic assay.

(**A**) Representative images taken at 48 h of breast cancer cell lines subjected to the aerotactic assay in medium minus EGF (-EGF) or supplemented with 10 µg/mL of EGF (+EGF). Scale bars, 0.5 mm. (**B**) Cell distribution from the centre of the cell cluster (in mm) at 0 h (green) and 48 h (carmine) in the -EGF condition and at 0 h (yellow) and 48 h (blue) in the +EGF condition are indicated as graphs (mean of three experiments). To assess the difference between the plus and minus EGF conditions at 48 h, a Student t-test statistical was performed on D5% values. P-values are indicated within graphs. NS not significant.