Supplementary Information

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Multiscale modeling allows to study the different modes of cancer cell invasion

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Outline

Connecting the agent-based model to the Boolean Model

We have described here the links of the variables of the agent-based model (ABM) model and of the Boolean model (BM).

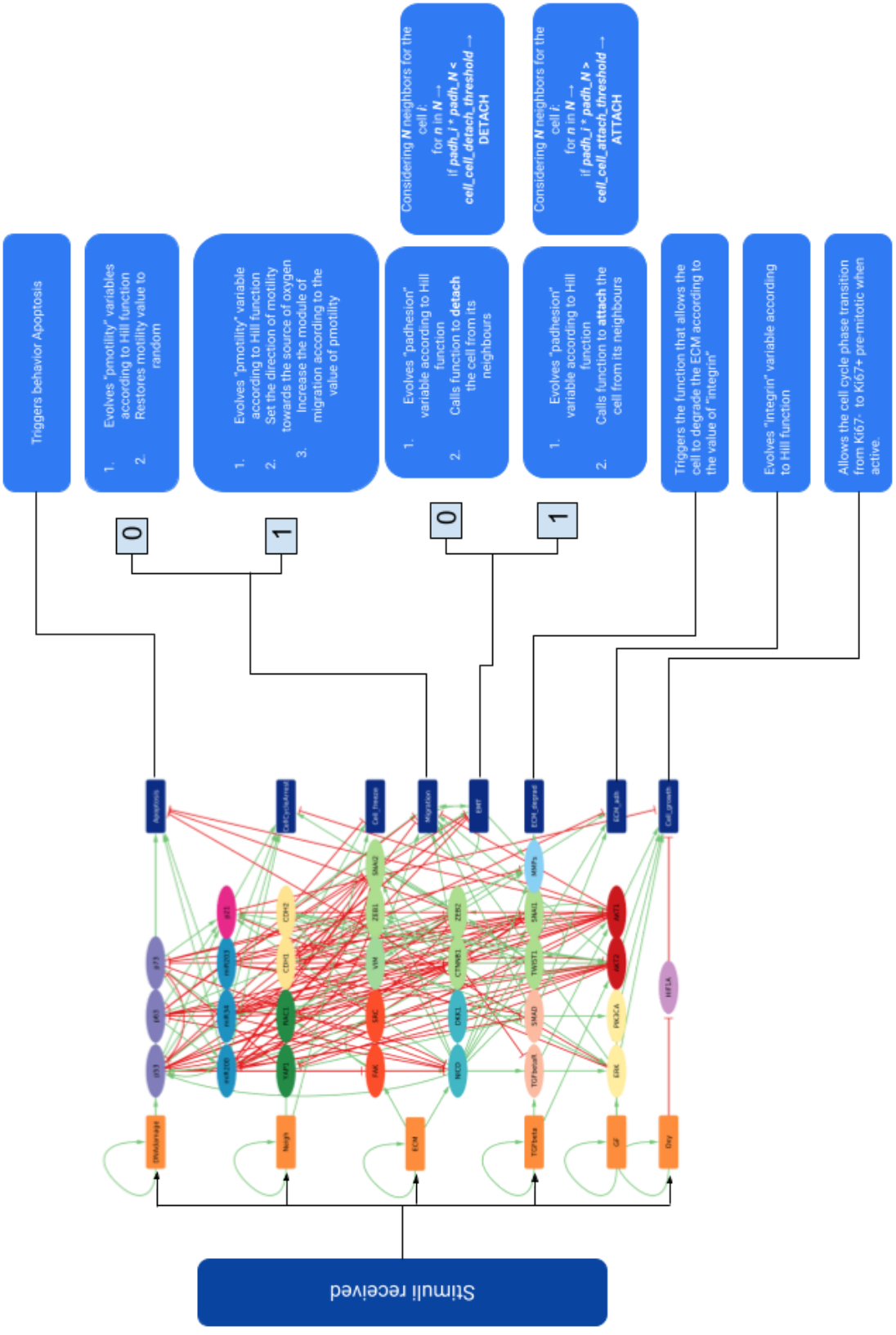


Figure S1: *Scheme of the links between the intracellular model and the behaviors of the agents.*

Cell Cycle model in PhysiBoSS

PhysiBoSS already provides the user with different options for cell cycle description.
For this study, we chose the model of the cell cycle based on Ki67 (Advanced Ki67 CellCycle model).

This cell cycle is composed of 3 phases and 3 transitions. Each cell starts in interphase Ki67-. Once the *Cell_growth* output node is activated, it triggers the transition to the next phase, Ki67+ pre-mitotic.

The cell then starts growing and once it reaches a certain threshold, it divides into two daughter cells that inherit the phenotype of the mother cell. Both daughter cells stay in Ki67+ post-mitotic phase and switch again to an early cell cycle phase Ki67- at a default transition rate provided by PhysiCell.

Logical formulae of the intracellular model

The notation for the logical connectors is:
& for AND
| for OR
! for NOT

Variable	**Logical rule**
HIF1A	!Oxy
FAK	(ECM | (SRC)) & !p53
YAP1	((!AKT1 | ! AKT2) & SRC)
RAC1	(SRC | FAK) & !(AKT1 | AKT2)
PIK3CA	(GF | RAC1)
MMPs	(MMPs & (((NICD & SMAD)| RAC1) & !p73)) | p63
SRC	FAK
NICD	(!p73 & !p53 & !p63 & !miR34 & !miR200 & (ECM | FAK))
CTNNB1	(!DKK1 & !p53 & !AKT1 & !p63 & !miR34 & !miR200 & !CDH1 & CDH2 & !SRC)
DKK1	(!NICD & CTNNB1) | (NICD)
AKT2	TWIST1 & (TGFbeta | GF | CDH2) & !(miR203 | miR34 | p53)

ZEB1	((TWIST1 & SNAI1) | CTNNB1 | SNAI2 | NICD) & ! miR200
SNAI1	(NICD | TWIST1) & ! miR203 & ! miR34 & ! p53 & ! CTNNB1
ZEB2	(SNAI1 | (SNAI2 & TWIST1) | NICD) & ! miR200 & ! miR203
p73	(!AKT2 & !ZEB1 & !p53 & !AKT1 & DNAdamage & !YAP1)
p53	(DNAdamage | CTNNB1 | NICD | miR34) & ! SNAI2 & ! p73 & ! AKT1 & ! AKT2
AKT1	(CTNNB1 & (NICD | TGFbetaR | GF | CDH2) & ! p53 & ! miR34 & ! CDH1)
p63	(!NICD & !AKT2 & !p53 & !AKT1 & DNAdamage & !miR203)
miR34	!(SNAI1 | ZEB1 | ZEB2) & (p53 | p73) & AKT2 & ! p63 & ! AKT1
SNAI2	(TWIST1 | CTNNB1 | NICD) & ! miR200 & ! p53 & ! miR203
miR200	(p63 | p53 | p73) & !(AKT2 | SNAI1 | SNAI2 | ZEB1 | ZEB2)
TWIST1	CTNNB1 | NICD | SNAI1
CDH1	(!AKT2 & !ZEB1 & !ZEB2 & !SNAI1 & !SNAI2 & !TWIST1 & !SRC & Neigh)
CDH2	(TWIST1 | SRC)
TGFbetaR	(NICD & !CTNNB1 & TGFbeta)
miR203	(!ZEB1 & !ZEB2 & !SNAI1 & p53)
ERK	((SMAD | CDH2 | GF | NICD) & !AKT1)
SMAD	(!miR200 | !miR203) & (TGFbetaR | YAP1)
p21	((SMAD & NICD) | p63 | p53 | p73 | AKT2) & !(AKT1 | ERK)
VIM	CTNNB1 | ZEB2 | SRC
EMT	(!CDH1 & CDH2) | EMT & (!CDH1 & CDH2)
Migration	(AKT2 & !AKT1 & !miR200 & ERK & VIM & EMT & ((CDH2 & SMAD) | (CTNNB1)) & !p63)
Apoptosis	(p53 | p63 | p73 | miR200 | miR34) & ! ZEB2 & ! AKT1 & ! ERK
ECM_adh	(NICD & !CDH1 & SMAD) | RAC1
ECM_degrad	MMPs
CellCycleArrest	(miR203 | miR200 | miR34 | ZEB2 | p21) & !AKT1
Cell_freeze	(Neigh & !CDH2 & CDH1)
Cell_growth	((ERK & !p21) | (AKT1 & AKT2 & PIK3CA)) & !HIF1A

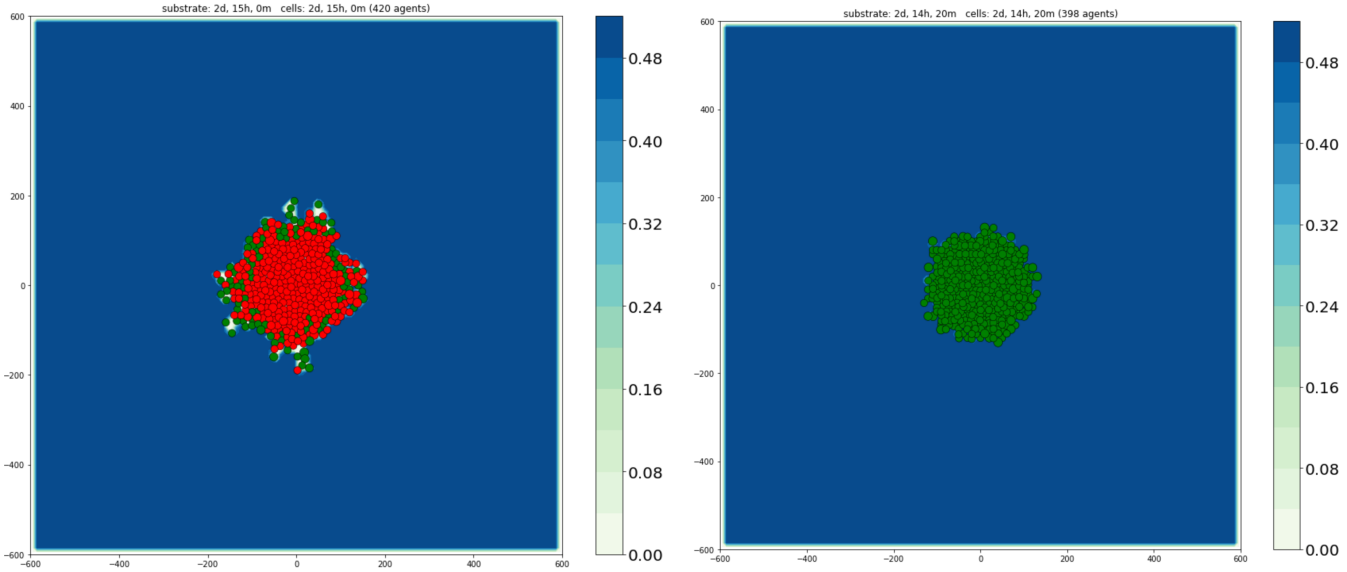
List of parameters of the model

Here we report a brief list of the main parameters of the simulation with a short description.

Parameter	Description	Value
Domain	3D / 2D space domain	600x600(x600)μm
∆space	voxel's unit measure	10 μm
Cell-substrates interaction parameters		
ecm_adhesion_min	set the min adhesion between cells and ECM	1
ecm_adhesion_max	set the max adhesion between cells and ECM	2
cell_ecm_repulsion	set the value of ECM repulsion	15μm/min
Cell parameters		
max_interaction_factor	set the max distance of interaction	1.3μm
homotypic_adhesion_min	set the min adhesion between cells of the same type	0.4
homotypic_adhesion_max	set the max adhesion between cells of the same type	0.8
Threshold parameters		
contact_ECM_threshold	change the threshold needed to trigger ECM interaction	0.05
contact_TGFβ_threshold	change the threshold needed to trigger TGFβ interaction	0.02
contact_cell_cell_threshold	change the threshold needed to trigger Neigh node	0.3
epith_cell_junctions_attach_threshold	change the threshold needed to attach cells in cluster with cell junction	0.05
mes_cell_junctions_detach_threshold	change the threshold needed to detach cells in cluster with cell junction	0.03
Motility parameters		
migration_bias	change value of migration bias for cells with migration node active	0.85
migration_speed	change value of migration speed for cells with migration node active	0.5μm/min
motility_amplitude_min	change the min value of motility amplitude	0.1
motility_amplitude_max	change the max value of motility amplitude	0.8
Substrates parameters		
config_radius	change the initial radius of the tumor	100μm
TGFβ_radius	change radius of the TGFβ substrate	90μm
densityβ	change initial density of the TGFβ substrate	0.4
density_ECM	change initial density of the ECM substrate	0.5
ECM_degradation	change the amount of ECM degraded by the cells	0.05
ECM_TGFβ_ratio	change the amount of TGFβ degraded by the cells	0.002
TGFβ_degradation	change the threshold needed to start sensing TGFbeta inside a voxel with ECM	0.75

CTNNB1 overexpressing mutation

Following the analysis of the intracellular model (see Supplementary_file2-Intracellular_model_analysis) we tested a possible overexpressing



mutation of CTNNB1. As shown in the figure, compared to the standard condition, this mutation is not preventing the tumor from growing, but greatly affects the invasive capacity.

Figure S2: On the left, simulation of the model in standard initial condition with no mutation. Green cells represent mesenchymal cells, red cells epithelial. On the right, knock-out mutation of CTNNB1.

Sensitivity analysis on model parameters

We run a sensitivity analysis on the parameters of the ABM model that are linked to the BM. The goal is to measure how each parameter affects the amount of cells that migrate as single cells or as clusters. We select 7 parameters among the ones shown in the previous section. For each parameter, we choose a range of values to test based on our previous experience. For each value we did 50 runs and took the mean value and the squared mean error to see how the stochasticity of the simulation affected the results.

Each parameter has been tested independently. To limit the computational cost, we performed the sensitivity analysis on one parameter at a time.
The simulations took almost 90 hours on the cluster abacus at the Curie Institute (28 nodes for a total of 1120 cores, 5.25Tb of RAM).

To measure the amount of single and collective migration, we printed on a csv file the amount of interactive neighbors for each cell at each time step of the simulation. To separate the clusters and the single cells, we took advantage of a representation of the simulation as a network, using NetworkX to read the csv. Finally we counted the

disconnected components of the resulting network, excluding the strongly connected component, which represents the core of the tumor.

The parameters are the following:

Parameters	Range	Potential range	Number of values selected
cell_ecm_repulsion regulates the amount of repulsion between cell and ECM	5 < **15** < 50	[0-infinite]	10
epith_cell_attach_threshold changes the activation threshold needed to attach cells in cluster with cell junction	0.001 < **0.05** < 1	[0-1]	25
mes_cell_detach_threshold change the activation threshold needed to detach cells in cluster with cell junction	0.001 < **0.03** < 1	[0-1]	25
cell_cell_contact_threshold changes the activation threshold of the value *cell_contact* needed to trigger Neigh node	0.01 < **0.3** < 3.5	[0-infinite]	18
cell_ecm_contact_threshold changes the activation threshold of the value *ecm_contact* needed to trigger ECM node	0.001 < **0.05** < 1	[0-1]	27
migration_bias changes the value of migration bias for cells with *Migration* node active	0.5 < **0.85** < 1	[0-1]	9
migration_speed changes the value of migration speed for cells with *Migration* node active	0.3 < **0.7** < 1	[0-1]	7

The graphs below show the proportions of cells that are found as single cells (blue) or in clusters (orange) for various values of the parameters. Some results and interpretations are discussed in the main text.

- *epith_cell_attach_threshold*

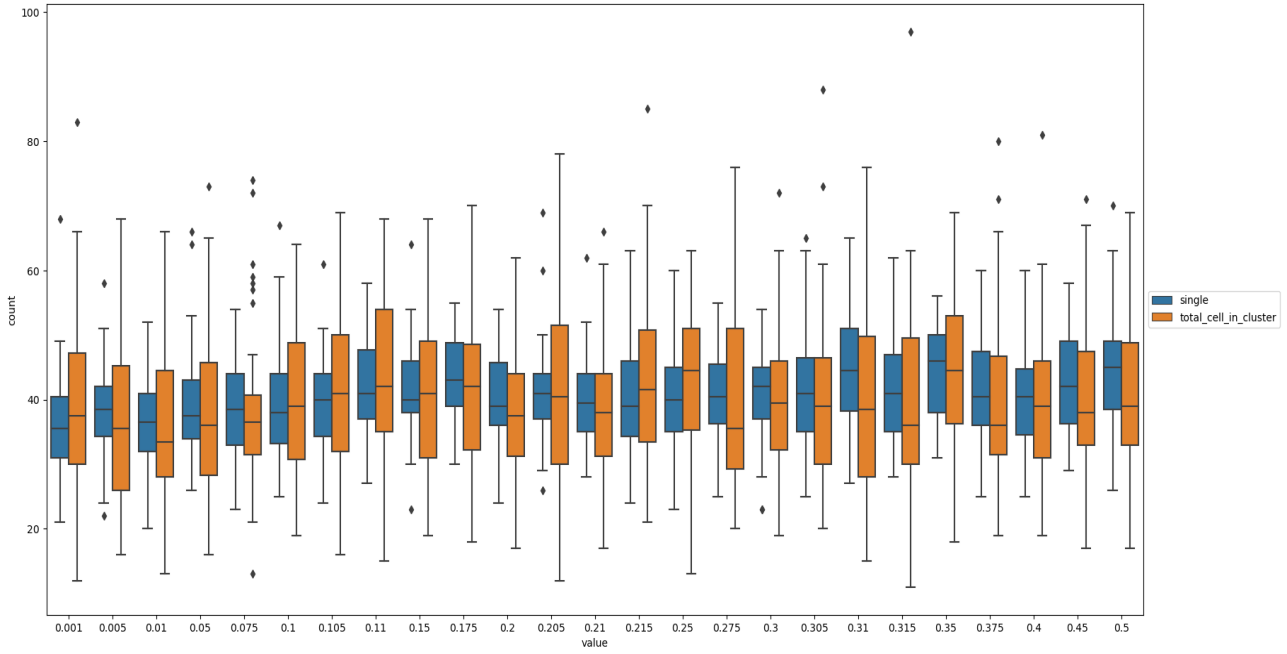


Figure S2: *Amount of single cell vs cell in cluster for different values of epith_cell_attach_threshold*

From the analysis, this parameter seems robust and shows a moderate change in the amount of single vs. collective migrating cells.

- *mes_cell_detach_threshold*

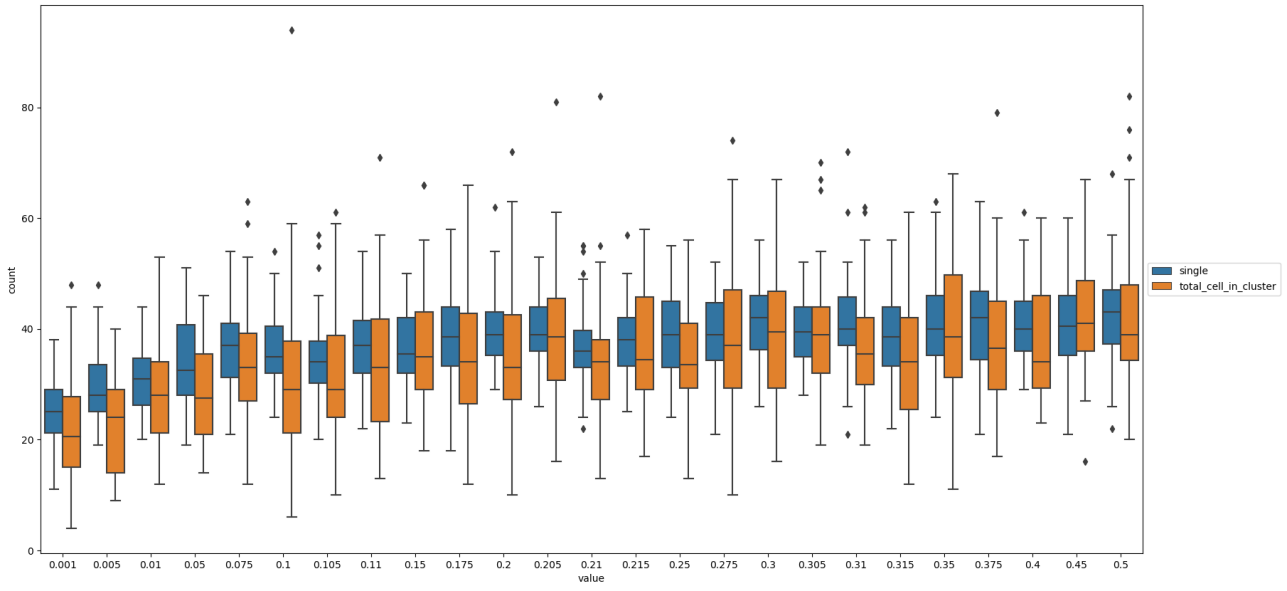


Figure S3: *Amount of single cell vs cell in cluster for different values of mes_cell_detach_threshold*

From the analysis, this parameter seems robust for values higher than 0.05 and it shows no changes in the rate between single and collective migrating cells. For values lower than 0.05, the amount of single vs. collective cells tends to diminish.

- *migration_bias*

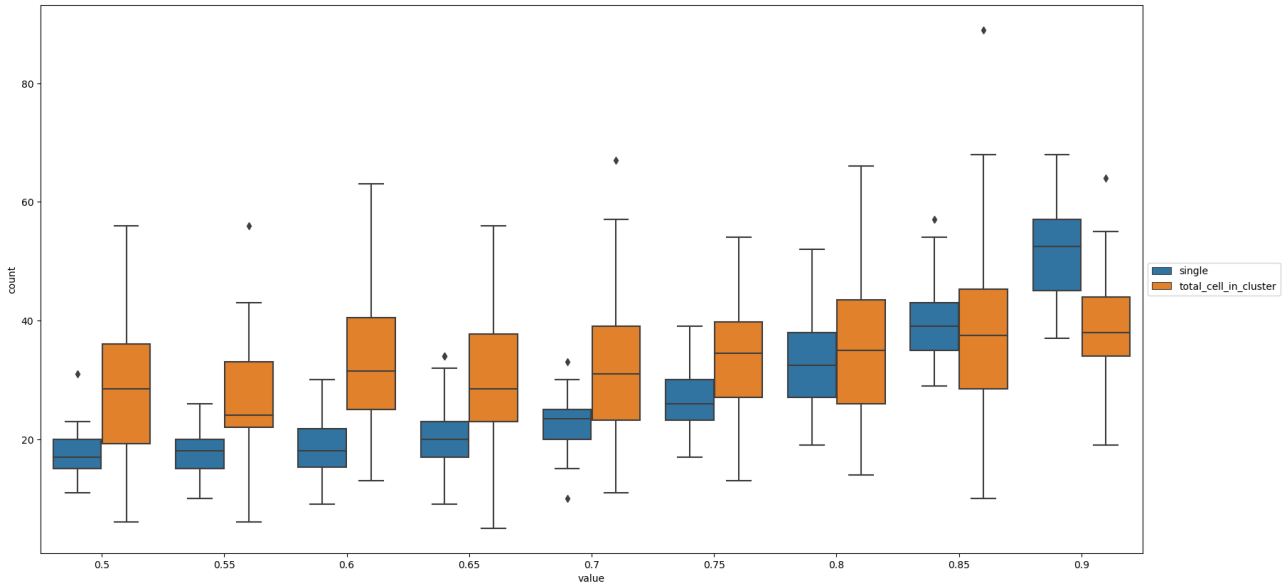


Figure S4: *Amount of single cell vs cell in cluster for different values of migration_bias*

From the analysis, this parameter heavily influences the amount of single and collective migration: for values minor than 0.85 the amount of cells migrating in clusters is higher than the single cells. For higher values, the number of single cells exponentially increases.

- *migration_speed*

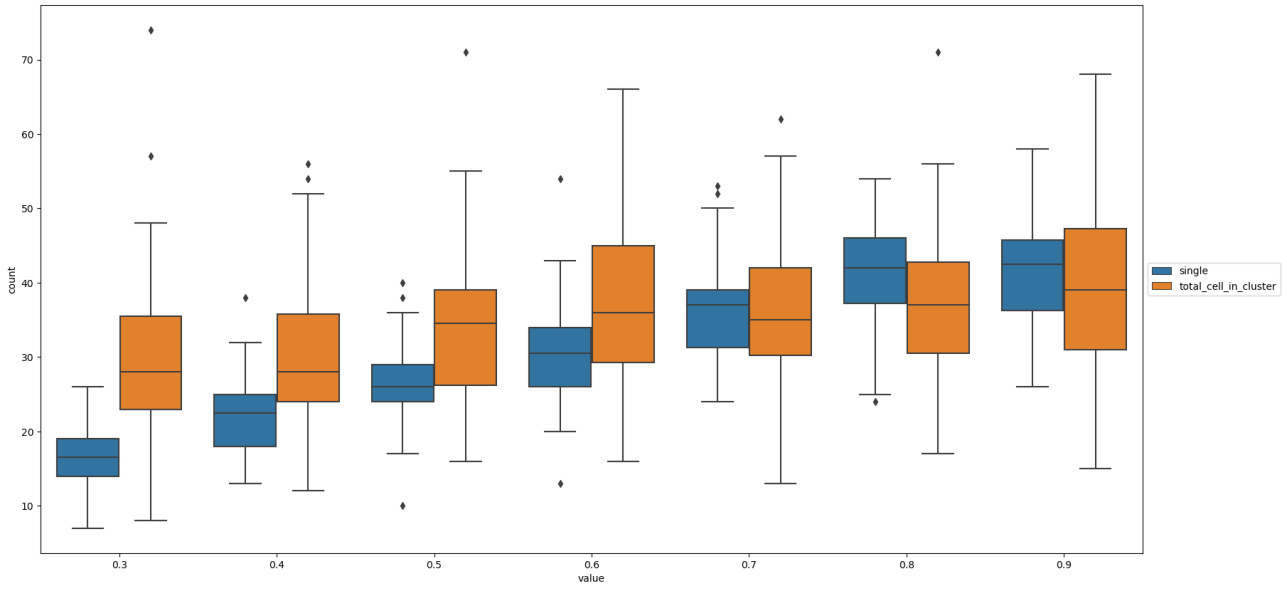


Figure S5: *Amount of single cell vs cell in cluster for different values of migration_speed*

From the analysis, this parameter heavily influences the amount of single and collective migration: for values minor than 0.7 the amount of cells migrating in clusters is higher than the single cells. For higher values, the number of single cells linearly increases.

- *cell_ecm_contact_threshold*

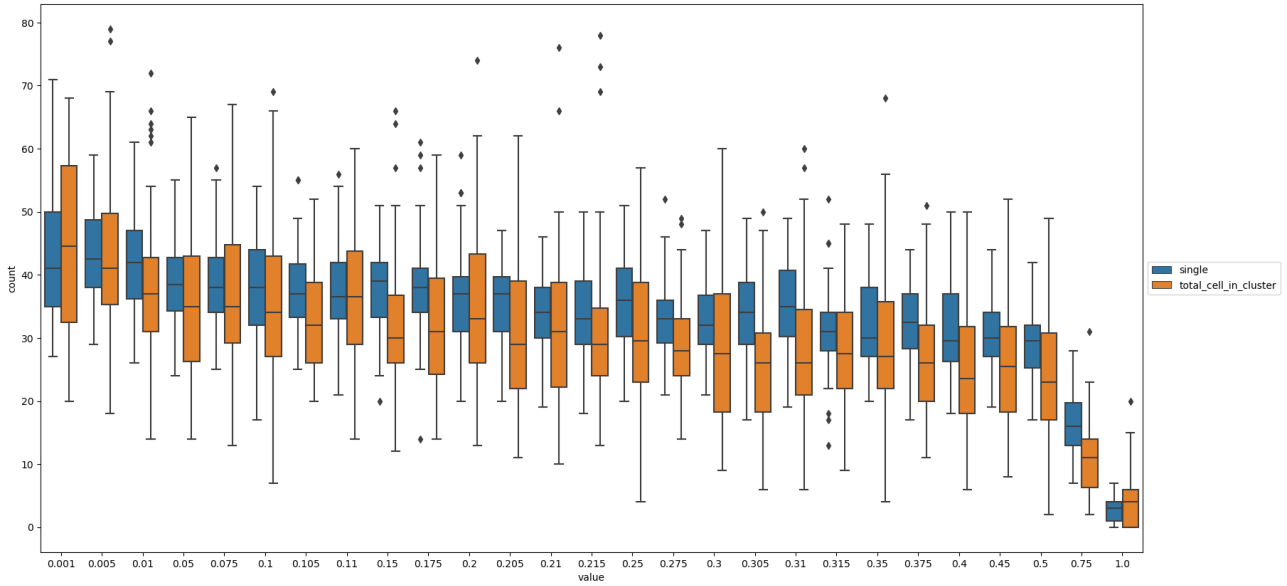


Figure S6: *Amount of single cell vs cell in cluster for different values of cell_ecm_contact_threshold*

From the analysis, this parameter seems to influence the amount of single and collective migration: the rate between single and collective migrating cells seems to be constant, decreasing slightly up to 0.5, after that it rapidly decreases.

- *cell_cell_contact_threshold*

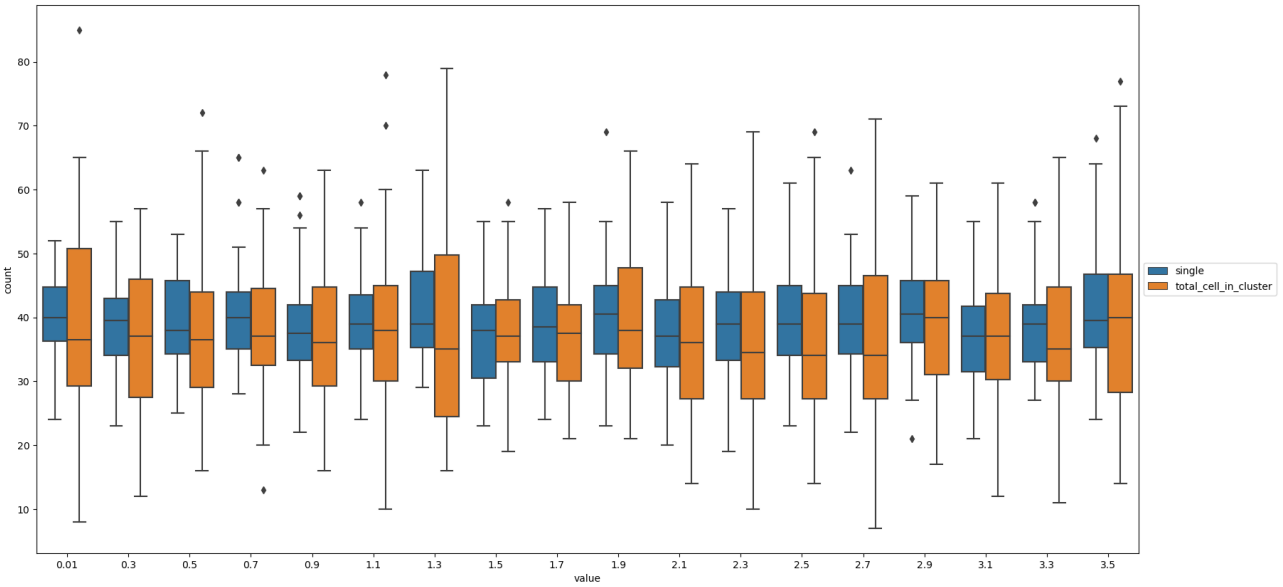


Figure S7: *Amount of single cell vs cell in cluster for different values of cell_cell_contact_threshold*

The analysis shows that the parameter has little impact on the separation of clusters and single cells.

- *cell_ecm_repulsion*

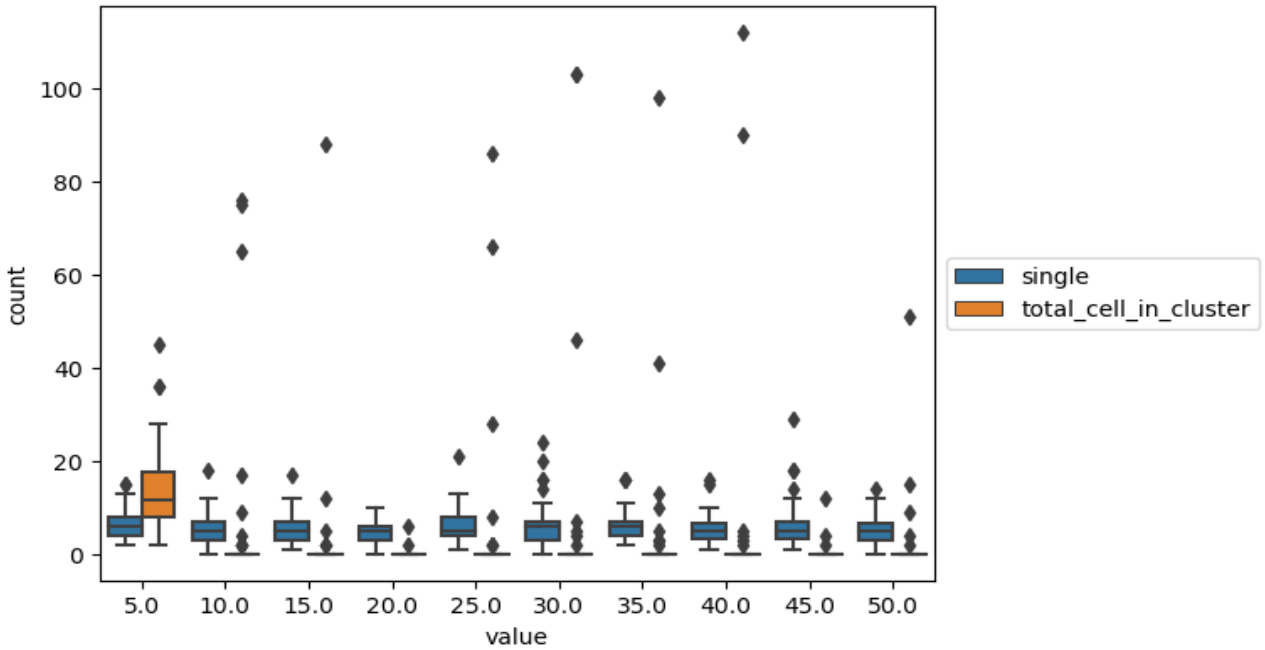


Figure S8: *Amount of single cell vs cell in cluster for different values of cell_ecm_repulsion*

With values higher than 5, it is difficult to see cells in clusters. This is due to the fact that, in these conditions, there are very few cells that touch the ECM and thus, that can become mesenchymal.