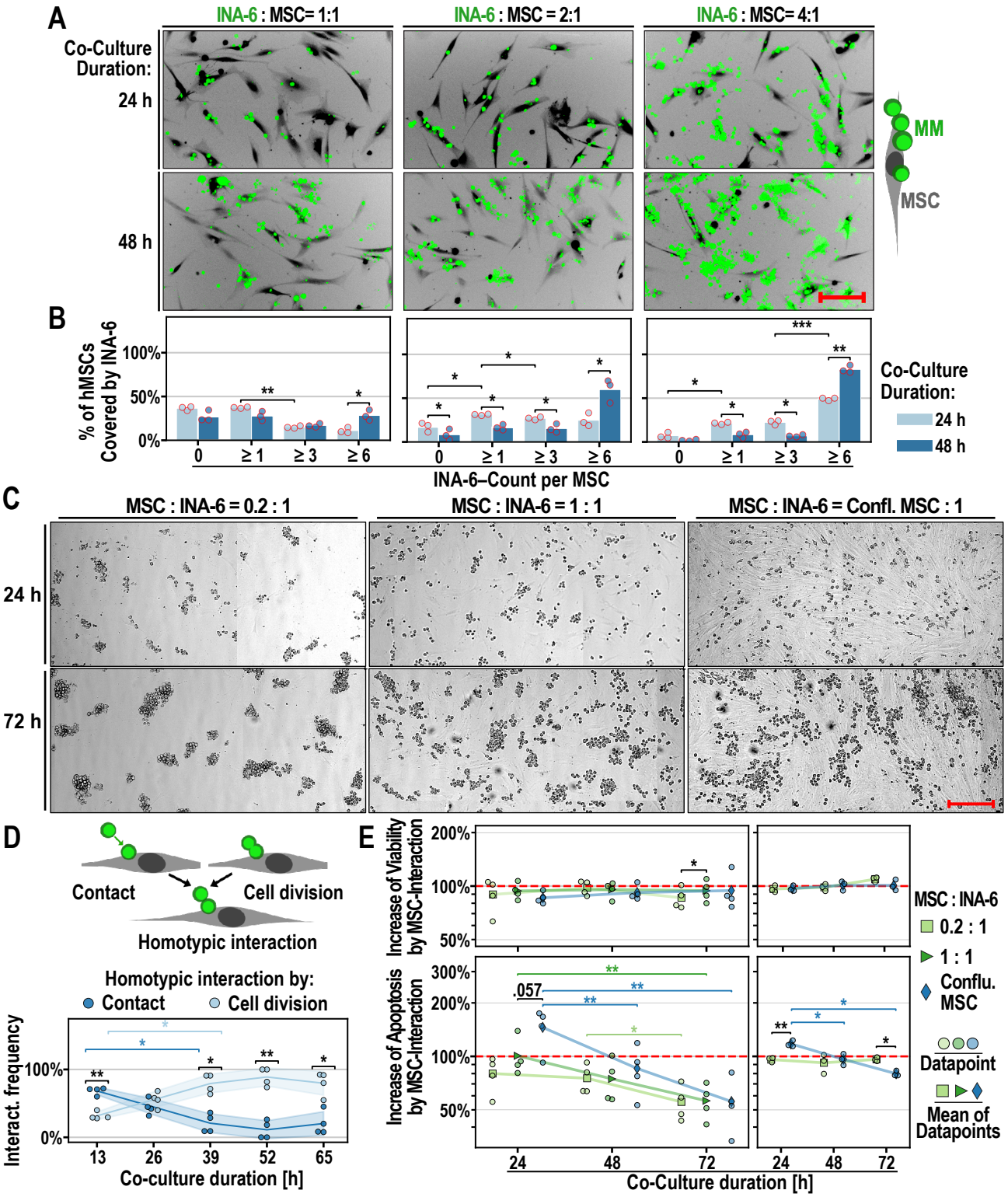
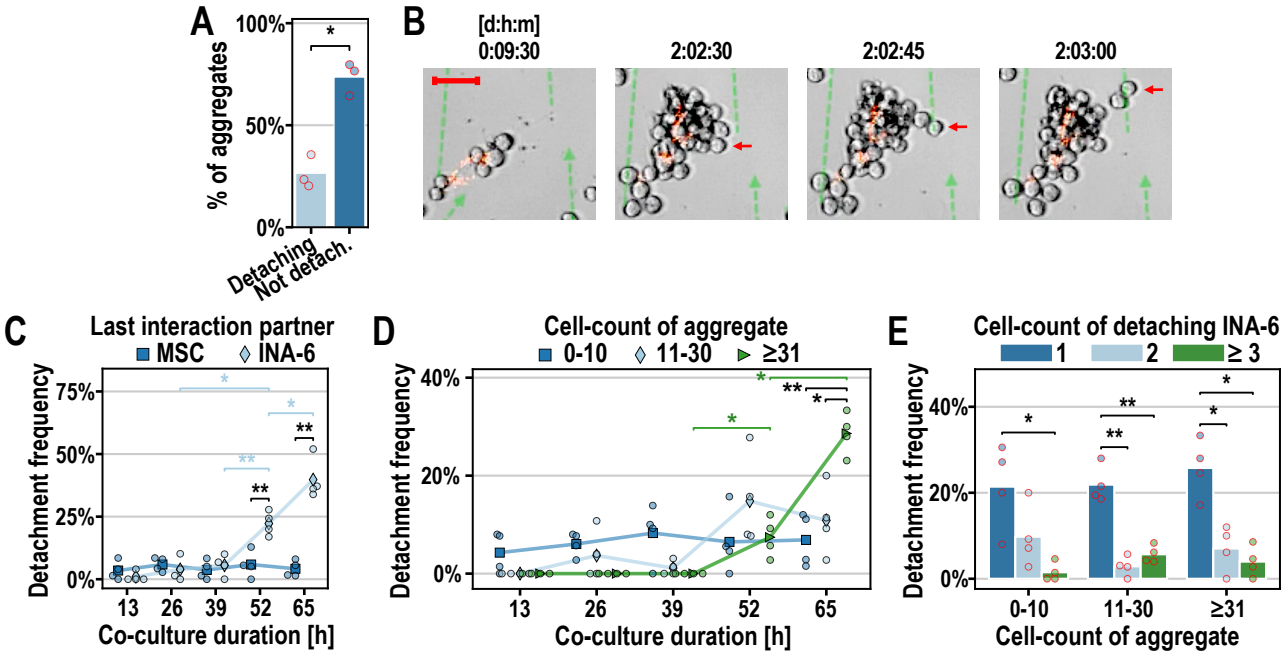
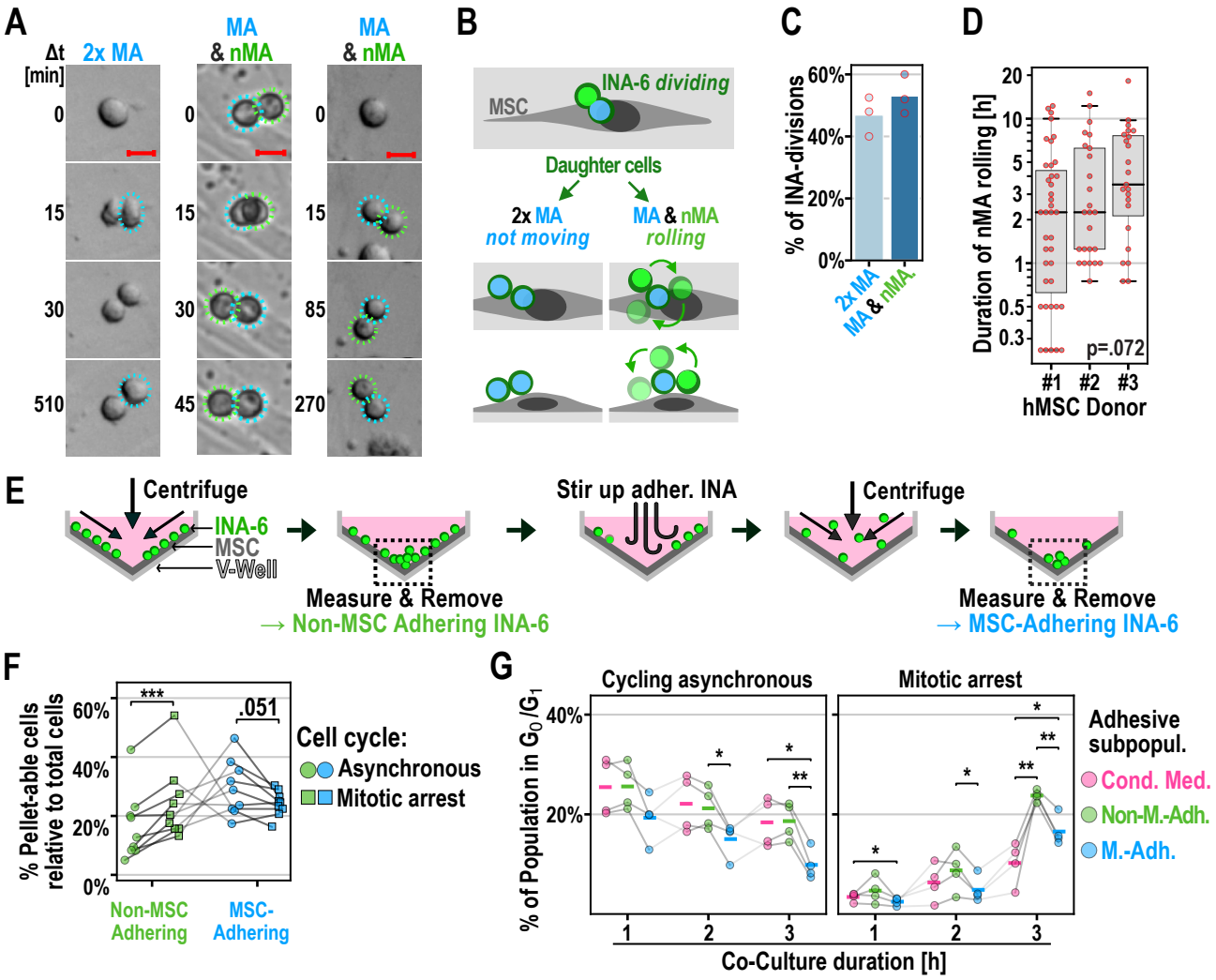
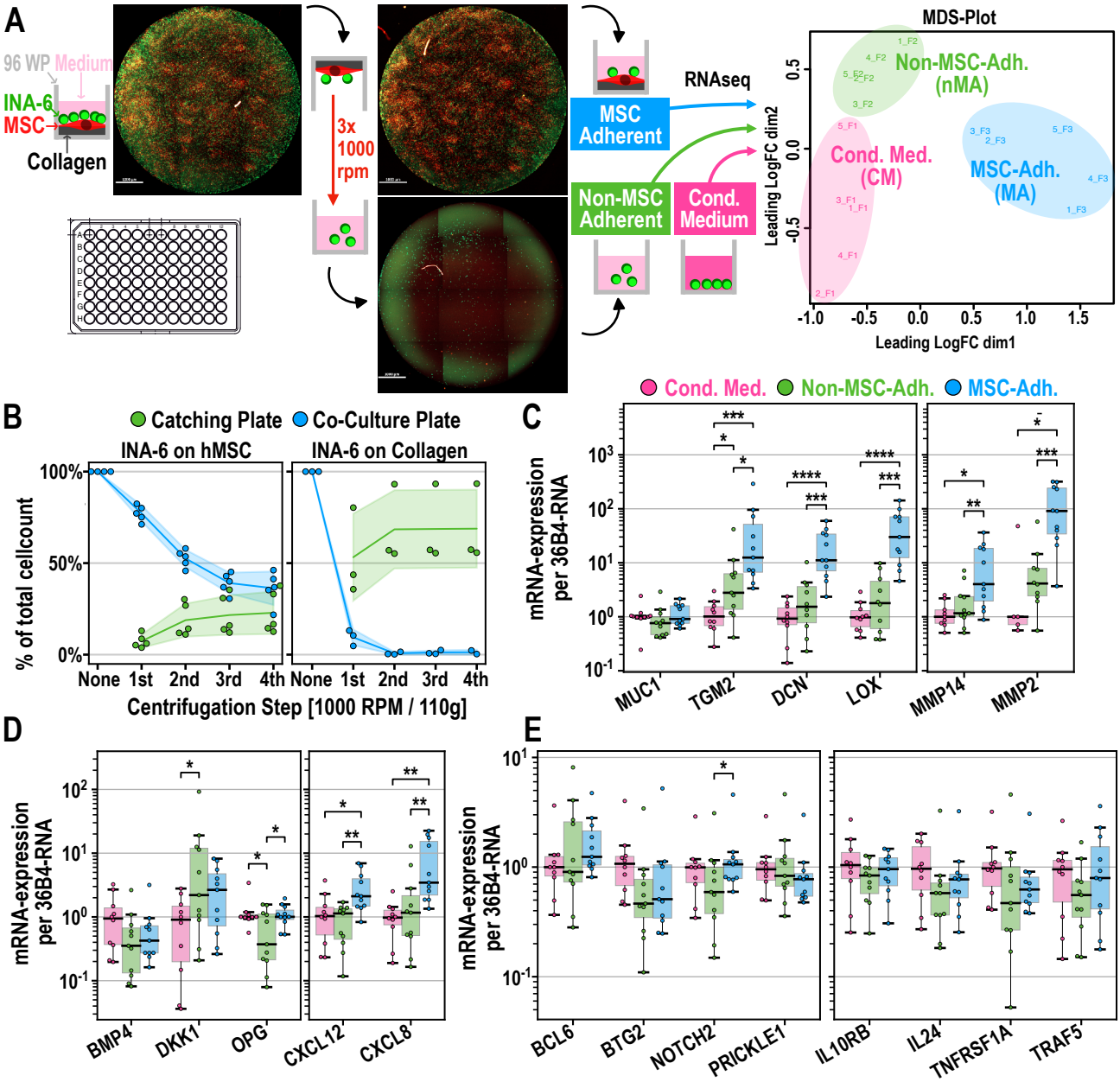
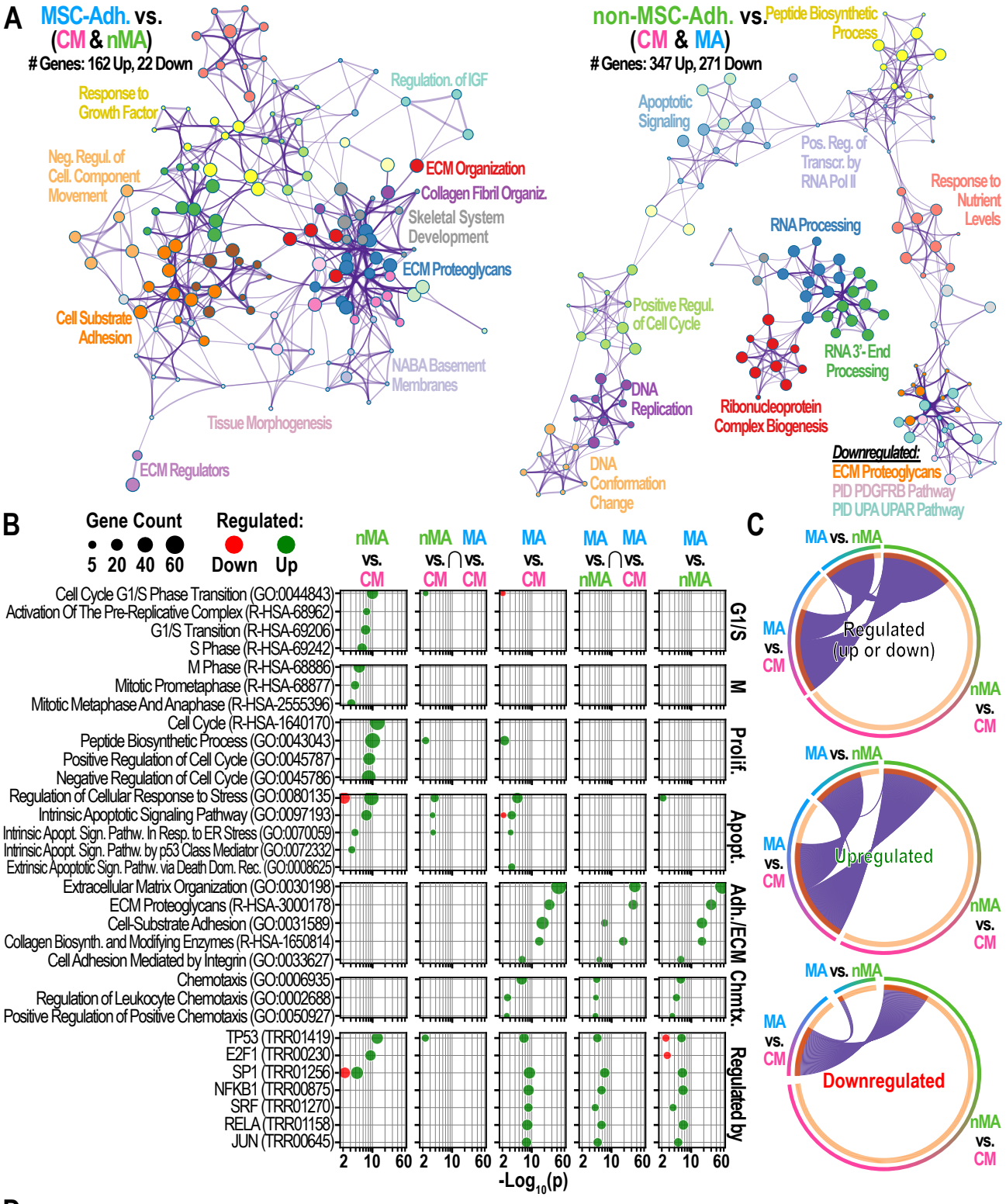
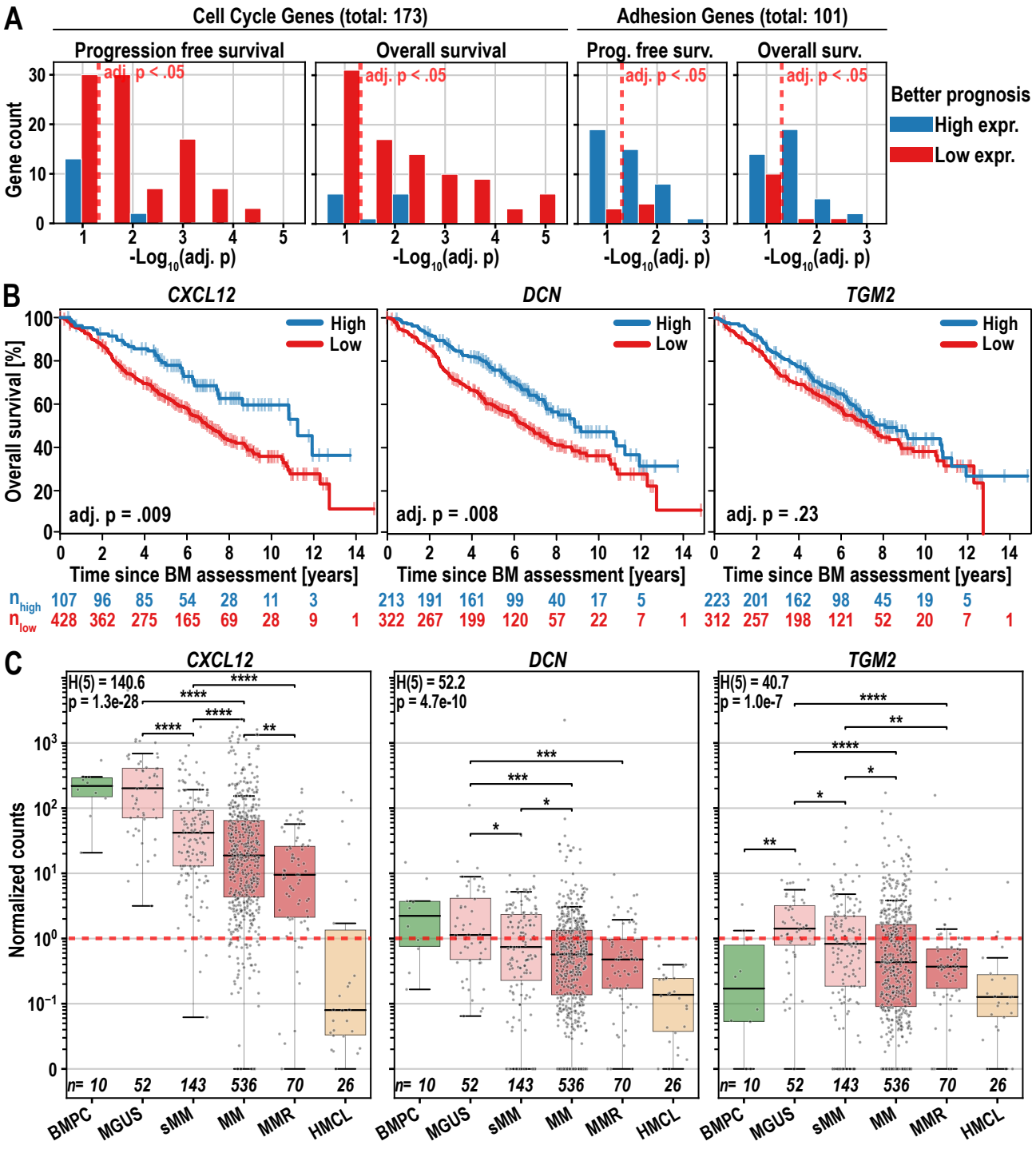

A
INA-6 : MSC= 1:1
INA-6 : MSC = 2:1
INA-6 : MSC= 4:1
Co-Culture Duration:
24 h
48 h
MM
MSC
B
% of hMSCs Covered by INA-6
100%
50%
0%
0
≥ 1
≥ 3
≥ 6
INA-6–Count per MSC
Co-Culture Duration:
24 h
48 h
C
MSC : INA-6 = 0.2 : 1
MSC : INA-6 = 1 : 1
MSC : INA-6 = Confl. MSC : 1
24 h
72 h
D
Contact
Cell division
Homotypic interaction
Homotypic interaction by:
Contact
Cell division
Interact. frequency
100%
0%
13
26
39
52
65
Co-culture duration [h]
E
Increase of Viability by MSC-Interaction
Increase of Apoptosis by MSC-Interaction
200%
100%
50%
300%
200%
100%
50%
.057
24
48
72
Co-Culture duration [h]
MSC : INA-6
0.2 : 1
1 : 1
Confl. MSC
Datapoint
Mean of Datapoints

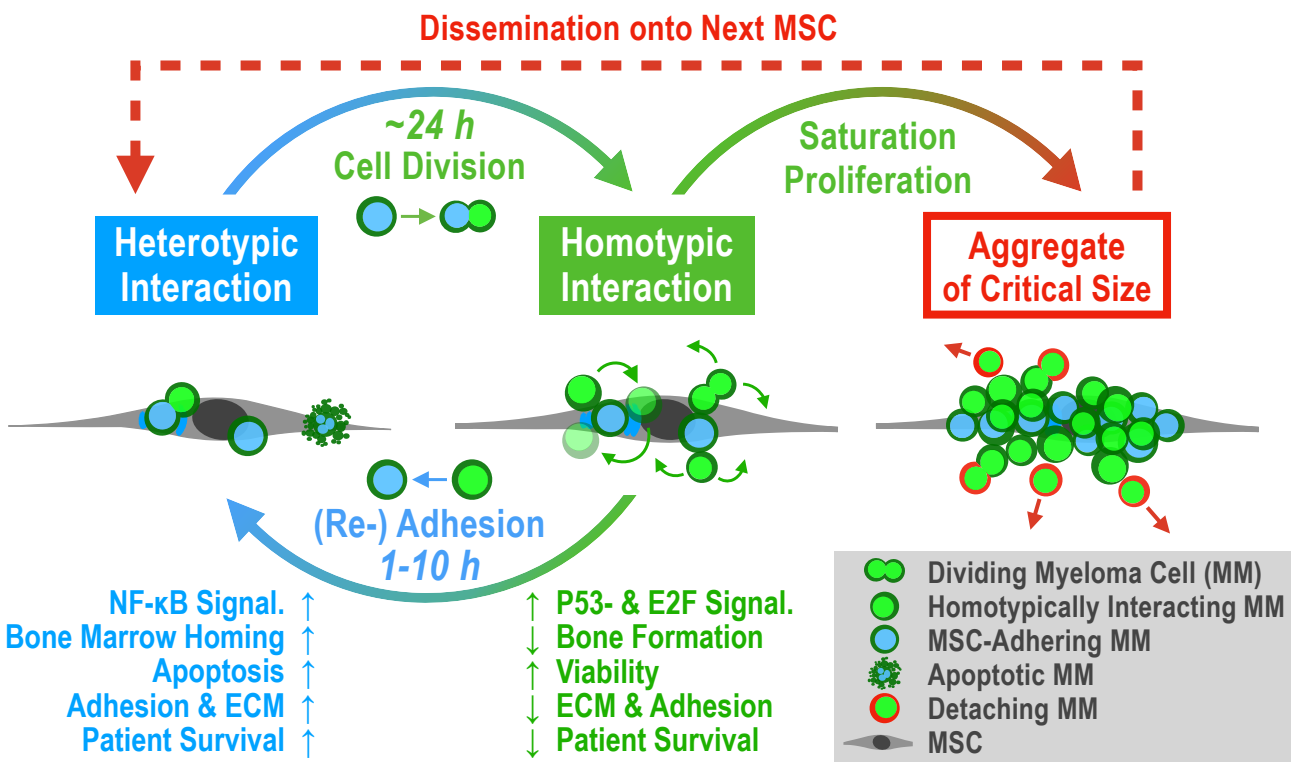

A
100%
50%
0%
% of aggregates
*
Detaching
Not detach.
B
[d:h:m]
0:09:30
2:02:30
2:02:45
2:03:00
C
Last interaction partner
MSC
INA-6
Detachment frequency
75%
50%
25%
0%
13
26
39
52
65
Co-culture duration [h]
D
Cell-count of aggregate
0-10
11-30
≥31
Detachment frequency
40%
20%
0%
13
26
39
52
65
Co-culture duration [h]
E
Cell-count of detaching INA-6
1
2
≥ 3
Detachment frequency
40%
20%
0%
0-10
11-30
≥31
Cell-count of aggregate


A
Δt [min]
2x MA
MA & nMA
MA & nMA
0
15
30
510
0
15
30
45
0
15
85
270
B
MSC
INA-6 dividing
Daughter cells
2x MA not moving
MA & nMA rolling
C
% of INA-divisions
60%
40%
20%
0%
2x MA
MA & nMA
D
Duration of nMA rolling [h]
20
10
5
3
2
1
0.5
0.3
p=.072
#1 #2 #3
hMSC Donor
E
Centrifuge
INA-6
MSC
V-Well
Measure & Remove
→ Non-MSC Adhering INA-6
Stir up adher. INA
Centrifuge
Measure & Remove
→ MSC-Adhering INA-6
F
% Pellet-able cells relative to total cells
60%
40%
20%
0%
***
.051
Non-MSC Adhering
MSC-Adhering
Cell cycle:
Asynchronous
Mitotic arrest
G
Cycling asynchronous
Mitotic arrest
% of Population in G0/G1
40%
20%
Co-Culture duration [h]
Adhesive subpopul.
Cond. Med.
Non-M.-Adh.
M.-Adh.


A
96 WP
Medium
INA-6
MSC
Collagen
3x
1000
rpm
MSC
Adherent
Non-MSC
Adherent
Cond.
Medium
RNAseq
MDS-Plot
Non-MSC-Adh.
(nMA)
Cond. Med.
(CM)
MSC-Adh.
(MA)
Leading LogFC dim1
Leading LogFC dim2
B
Catching Plate
Co-Culture Plate
INA-6 on hMSC
INA-6 on Collagen
% of total cellcount
100%
50%
0%
None
1st
2nd
3rd
4th
Centrifugation Step [1000 RPM / 110g]
C
Cond. Med.
Non-MSC-Adh.
MSC-Adh.
mRNA-expression
per 36B4-RNA
MUC1
TGM2
DCN
LOX
MMP14
MMP2
D
BMP4
DKK1
OPG
CXCL12
CXCL8
E
BCL6
BTG2
NOTCH2
PRICKLE1
IL10RB
IL24
TNFRSF1A
TRAF5


A
MSC-Adh. vs. (CM & nMA)
Genes: 162 Up, 22 Down
Regulation. of IGF
Response to Growth Factor
Neg. Regul. of Cell. Component Movement
ECM Organization
Collagen Fibril Organiz.
Skeletal System Development
ECM Proteoglycans
Cell Substrate Adhesion
NABA Basement Membranes
Tissue Morphogenesis
ECM Regulators
non-MSC-Adh. vs. (CM & MA)
Genes: 347 Up, 271 Down
Peptide Biosynthetic Process
Apoptotic Signaling
Pos. Reg. of Transcr. by RNA Pol II
Response to Nutrient Levels
RNA Processing
Positive Regul. of Cell Cycle
RNA 3'- End Processing
DNA Replication
Ribonucleoprotein Complex Biogenesis
DNA Conformation Change
Downregulated:
ECM Proteoglycans
PID PDGFRB Pathway
PID UPA UPAR Pathway
B
Gene Count
5 20 40 60
Regulated:
Down Up
nMA vs. CM
nMA vs. ∩ vs. MA CM
MA vs. CM
MA vs. ∩ vs. MA nMA
MA vs. nMA
Cell Cycle G1/S Phase Transition (GO:0044843)
Activation Of The Pre-Replicative Complex (R-HSA-68962)
G1/S Transition (R-HSA-69206)
S Phase (R-HSA-69242)
M Phase (R-HSA-68886)
Mitotic Prometaphase (R-HSA-68877)
Mitotic Metaphase And Anaphase (R-HSA-2555396)
Cell Cycle (R-HSA-1640170)
Peptide Biosynthetic Process (GO:0043043)
Positive Regulation of Cell Cycle (GO:0045787)
Negative Regulation of Cell Cycle (GO:0045786)
Regulation of Cellular Response to Stress (GO:0080135)
Intrinsic Apoptotic Signaling Pathway (GO:0097193)
Intrinsic Apopt. Sign. Pathw. In Resp. to ER Stress (GO:0070059)
Intrinsic Apopt. Sign. Pathw. by p53 Class Mediator (GO:0072332)
Extrinsic Apoptotic Sign. Pathw. via Death Dom. Rec. (GO:0008625)
Extracellular Matrix Organization (GO:0030198)
ECM Proteoglycans (R-HSA-3000178)
Cell-Substrate Adhesion (GO:0031589)
Collagen Biosynth. and Modifying Enzymes (R-HSA-1650814)
Cell Adhesion Mediated by Integrin (GO:0033627)
Chemotaxis (GO:0006935)
Regulation of Leukocyte Chemotaxis (GO:0002688)
Positive Regulation of Positive Chemotaxis (GO:0050927)
TP53 (TRR01419)
E2F1 (TRR00230)
SP1 (TRR01256)
NFKB1 (TRR00875)
SRF (TRR01270)
RELA (TRR01158)
JUN (TRR00645)
G1/S
M
Prolif.
Apopt.
Adh./ECM
Chmtx.
Regulated by
2 10 60
-Log10(p)
C
MA vs. nMA
Regulated (up or down)
Upregulated
Downregulated
D
Co-Culture duration (before sub-culturing for 48 h)
24 h
48 h
Fold change Viability (ATP)
Foldchange Apoptosis (Cas3/7 Activity)
10000%
1000%
100%
10%
INA-6
IL-6 + INA-6
CM-INA6
nMA-INA6
MA-INA6


A
Cell Cycle Genes (total: 173)
Adhesion Genes (total: 101)
Progression free survival
Overall survival
Prog. free surv.
Overall surv.
adj. p < .05
Gene count
-Log10(adj. p)
Better prognosis
High expr.
Low expr.
B
CXCL12
DCN
TGM2
High
Low
Overall survival [%]
adj. p = .009
adj. p = .008
adj. p = .23
Time since BM assessment [years]
n high 107 96 85 54 28 11 3 213 191 161 99 40 17 5 223 201 162 98 45 19 5
n low 428 362 275 165 69 28 9 1 322 267 199 120 57 22 7 1 312 257 198 121 52 20 7 1
C
H(5) = 140.6
p = 1.3e-28
H(5) = 52.2
p = 4.7e-10
H(5) = 40.7
p = 1.0e-7
Normalized counts
n= 10 52 143 536 70 26
BMPC MGUS sMM MM MMR HMCL


Dissemination onto Next MSC
~24 h
Cell Division
Saturation
Proliferation
Heterotypic Interaction
Homotypic Interaction
Aggregate of Critical Size
(Re-) Adhesion
1-10 h
NF-κB Signal. ↑
Bone Marrow Homing ↑
Apoptosis ↑
Adhesion & ECM ↑
Patient Survival ↑
↑ P53- & E2F Signal.
↓ Bone Formation
↑ Viability
↓ ECM & Adhesion
↓ Patient Survival
Dividing Myeloma Cell (MM)
Homotypically Interacting MM
MSC-Adhering MM
Apoptotic MM
Detaching MM
MSC