Expanded View Figures

Figure EV1. Continuous cultivation after contact inhibition results in progressive cell death in NMR fibroblasts through *INK4a* upregulation.

A Scheme for continuous cultivation after induction of contact inhibition (CI).
B, C Cell morphology (B) and SA-β-Gal staining (C) in mouse or NMR fibroblasts at 14 or 28 days after induction of CI. Scale bar, 100 μm. The number in the upper left corner indicates Hoechst-positive nuclei.
D, E Quantification of SA-β-Gal-positive cells (%) (D), and BrdU-positive cells (%) (E) at 14 or 28 days after induction of CI.
F qRT-PCR analysis of the expression of *INK4a* in mouse or NMR fibroblasts at 14 or 28 days after induction of CI, normalized to *ACTB* mRNA levels.
G Quantification of Annexin V-positive (early apoptotic; Annexin V^+/PI^- and late apoptotic; Annexin V^+/PI^+ double-positive) cells (%) at 14 or 28 days after induction of CI.
H, I qRT-PCR analysis of the expression of *INK4a* normalized to *ACTB* mRNA levels (H), and quantification of Annexin V-positive (early apoptotic; Annexin V^+/PI^- and late apoptotic; Annexin V^+/PI^+ double-positive) cells (%) (I) in sh*INK4a*-transduced NMR-fibroblasts at 21 days after induction of CI.
J Mouse fibroblasts were passaged 28 days after induction of CI and subjected to cell proliferation analysis.
K NMR fibroblasts were passaged 28 days after induction of CI and subjected to cell proliferation analysis.

Data information: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns, not significant. One-way ANOVA followed by Dunnett's multiple comparison test for (D–I).
Data are expressed as the mean ± SD from $n = 3$ biological replicates except for (G) ($n = 5$).

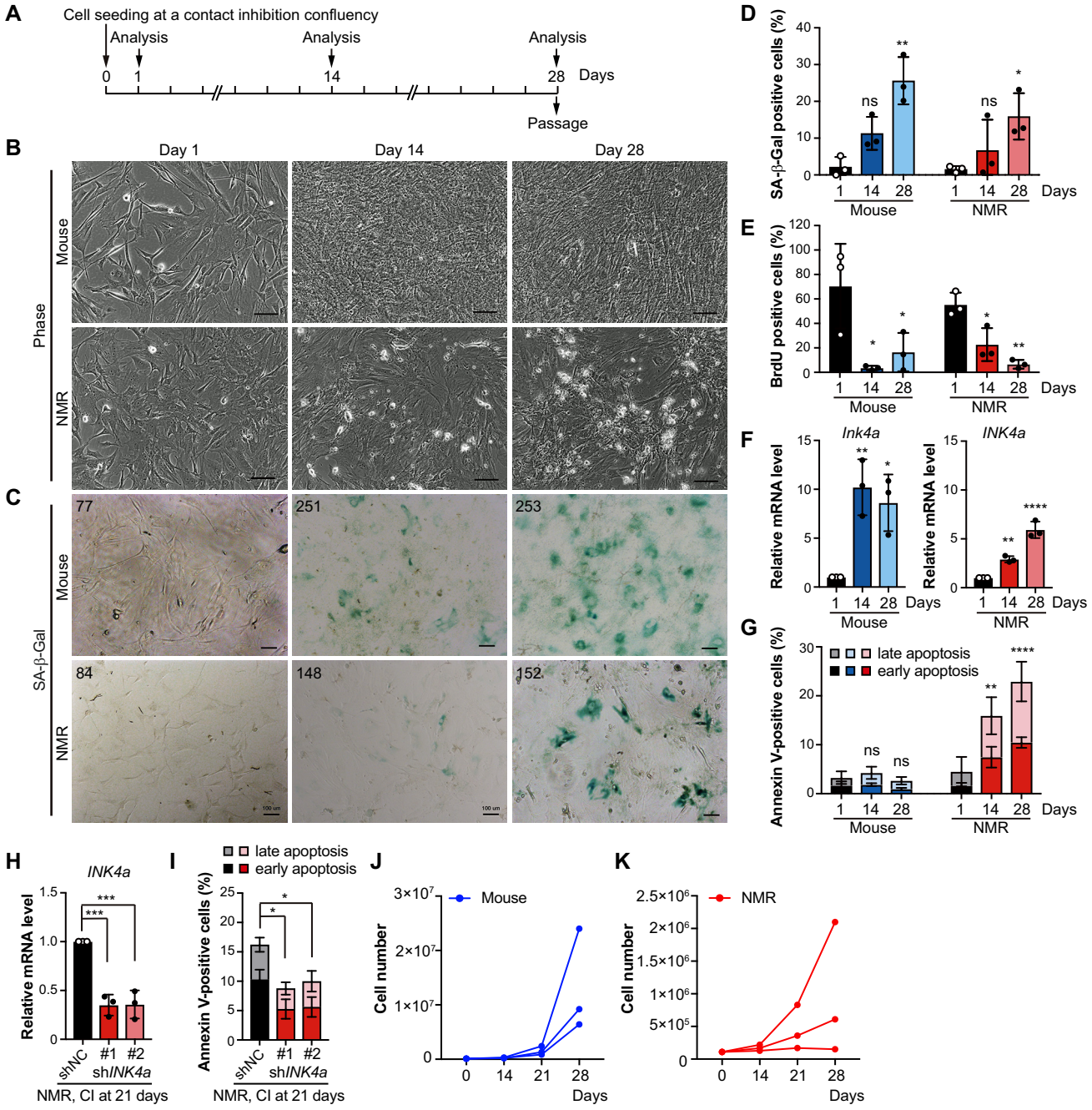
A
Cell seeding at a contact inhibition confluency
Analysis
Analysis
Analysis
0
1
14
28
Days
Passage
B
Day 1
Day 14
Day 28
Phase
Mouse
NMR
C
SA-β-Gal
Mouse
NMR
77
251
253
84
148
152
D
SA-β-Gal positive cells (%)
ns
ns
Mouse
NMR
Days
E
BrdU positive cells (%)
F
Ink4a
INK4a
Relative mRNA level
G
late apoptosis
early apoptosis
Annexin V-positive cells (%)
H
INK4a
Relative mRNA level
shNC
#1
#2
shINK4a
NMR, CI at 21 days
I
late apoptosis
early apoptosis
Annexin V-positive cells (%)
NMR, CI at 21 days
J
Mouse
Cell number
Days
K
NMR
Cell number
Days

Figure EV1.