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Supplemental information

HES1 protein oscillations are necessary for neural stem cells to exit from quiescence

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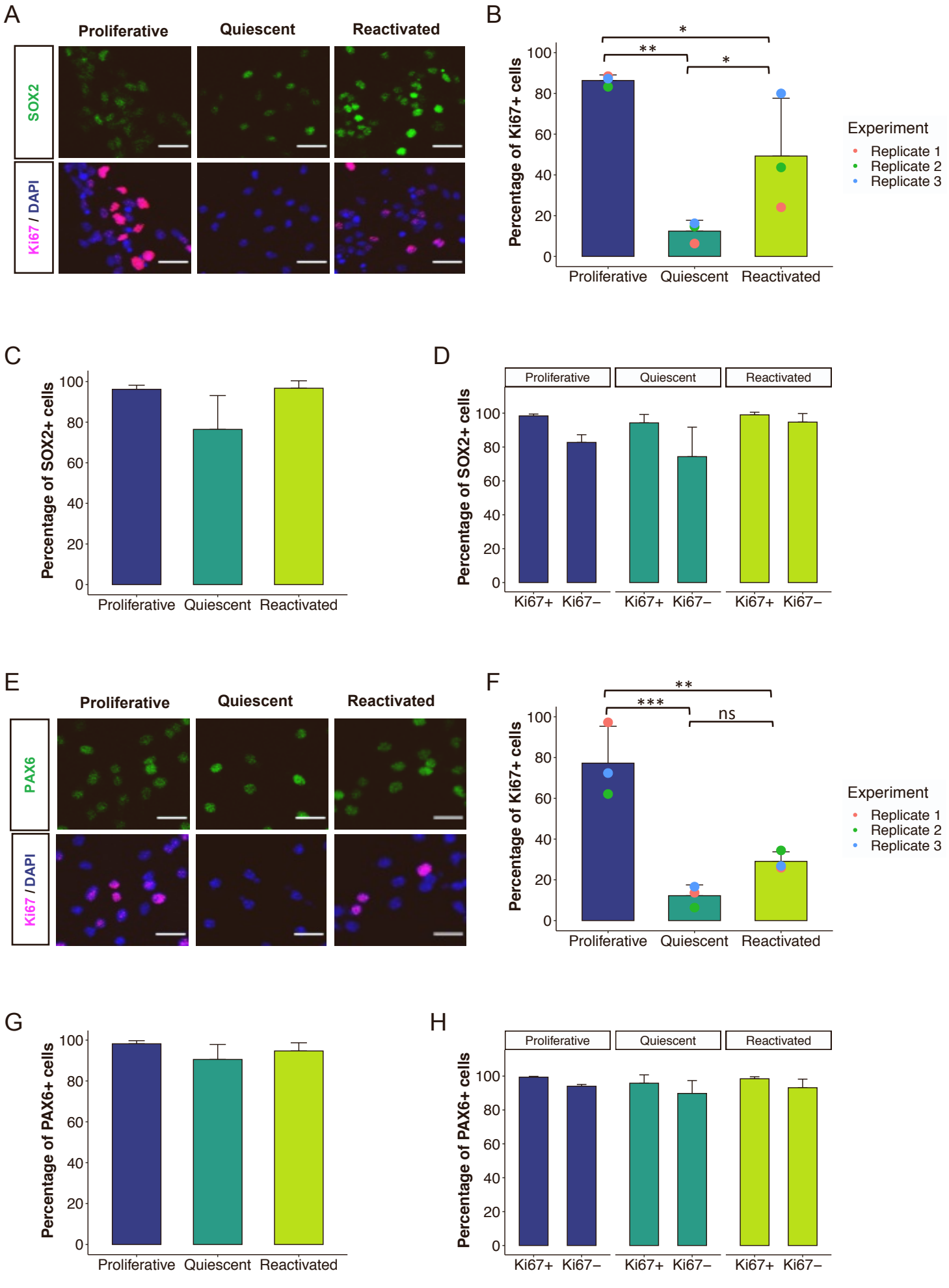
A
Proliferative
Quiescent
Reactivated
SOX2
Ki67 / DAPI
B
Percentage of Ki67+ cells
100
80
60
40
20
0
*
**
*
Proliferative
Quiescent
Reactivated
Experiment
Replicate 1
Replicate 2
Replicate 3
C
Percentage of SOX2+ cells
100
80
60
40
20
0
Proliferative
Quiescent
Reactivated
D
Proliferative
Quiescent
Reactivated
Percentage of SOX2+ cells
100
80
60
40
20
0
Ki67+
Ki67–
Ki67+
Ki67–
Ki67+
Ki67–
E
Proliferative
Quiescent
Reactivated
PAX6
Ki67 / DAPI
F
Percentage of Ki67+ cells
100
80
60
40
20
0
**
***
ns
Proliferative
Quiescent
Reactivated
Experiment
Replicate 1
Replicate 2
Replicate 3
G
Percentage of PAX6+ cells
100
80
60
40
20
0
Proliferative
Quiescent
Reactivated
H
Proliferative
Quiescent
Reactivated
Percentage of PAX6+ cells
100
80
60
40
20
0
Ki67+
Ki67–
Ki67+
Ki67–
Ki67+
Ki67–

Figure S1. SOX2 and PAX6 expression in NSCs in proliferative, quiescent and reactivated conditions, related to Figure 1

A) Example pictures of immunofluorescence staining for SOX2 expression in E13.5 LUC2:HES1 NSCs in proliferative, quiescent and reactivated conditions. Top panels show expression of SOX2, bottom panels show expression of DAPI and Ki67 (scale bars =30μm). B) Percentage of Ki67 positive cells in proliferative, quiescent and reactivated conditions of E13.5 LUC2:HES1 NSCs immunostained for SOX2 expression (error bars represent standard deviation, dots represent biological replicates, n=3 biological experiments, One-way ANOVA with Uncorrected Fisher's LSD multiple comparison test, proliferative vs quiescent **p=0.004, quiescent vs reactivated *p=0.04, proliferative vs reactivated *p=0.04). C) Percentage of SOX2 positive cells in proliferative, quiescent and reactivated conditions (error bars represent standard deviation, n=3 biological experiments). D) Percentage of SOX2 positive cells within the Ki67 positive and Ki67 negative population of cells in proliferative, quiescent and reactivated conditions (error bars represent standard deviation, n=3 biological experiments). E) Example pictures of immunofluorescence staining for PAX6 expression in E13.5 LUC2:HES1 NSCs in proliferative, quiescent and reactivated conditions. Top panels show expression of PAX6, bottom panels show expression of DAPI and Ki67 (scale bars =30μm). F) Percentage of Ki67 positive cells in proliferative, quiescent and reactivated conditions of E13.5 LUC2:HES1 NSCs immunostained for PAX6 expression (error bars represent standard deviation, dots represent biological replicates, n=3 biological experiments, One-way ANOVA with Uncorrected Fisher's LSD multiple comparison test, proliferative vs quiescent ***p=0.0007, proliferative vs reactivated **p=0.001, ns=not significant). G) Percentage of PAX6 positive cells in proliferative, quiescent and reactivated conditions (error bars represent standard deviation, n=3 biological experiments). H) Percentage of PAX6 positive cells within the Ki67 positive and Ki67 negative population of cells in proliferative, quiescent and reactivated conditions (error bars represent standard deviation, n=3 biological experiments).

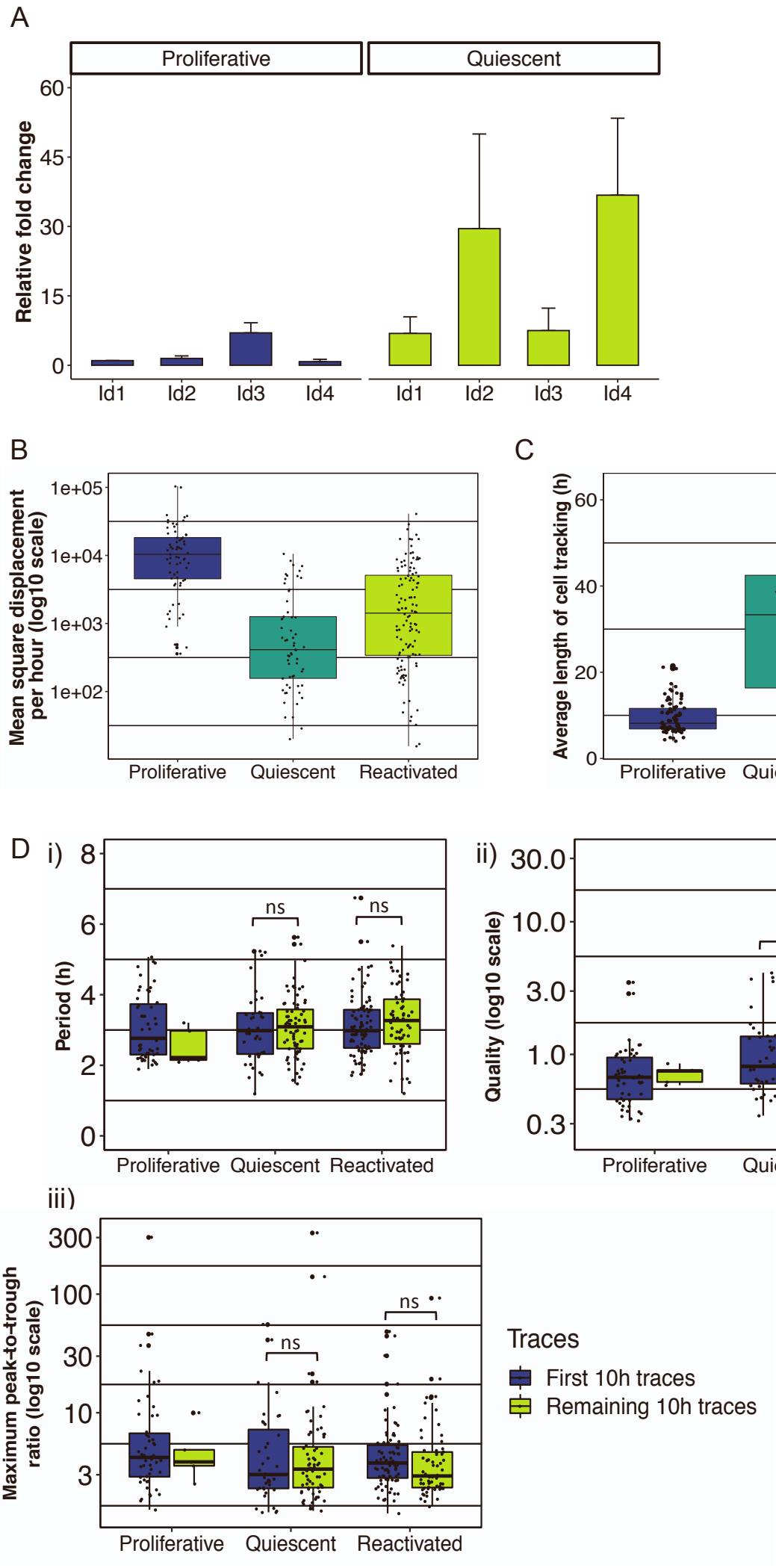
A
Proliferative
Quiescent
Relative fold change
60
45
30
15
0
Id1
Id2
Id3
Id4
Id1
Id2
Id3
Id4
B
Mean square displacement per hour (log10 scale)
1e+05
1e+04
1e+03
1e+02
Proliferative
Quiescent
Reactivated
C
Average length of cell tracking (h)
60
40
20
0
Proliferative
Quiescent
Reactivated
D
i)
Period (h)
8
6
4
2
0
ns
ns
Proliferative
Quiescent
Reactivated
ii)
Quality (log10 scale)
30.0
10.0
3.0
1.0
0.3
ns
ns
Proliferative
Quiescent
Reactivated
iii)
Maximum peak-to-trough ratio (log10 scale)
300
100
30
10
3
ns
ns
Proliferative
Quiescent
Reactivated
Traces
First 10h traces
Remaining 10h traces

Figure S2. *Id* gene expression and HES1 dynamic expression profile in proliferative, quiescent and reactivated conditions, related to Figure 1

A) qPCR analysis for *Id1*, *Id2*, *Id3* and *Id4* mRNA expression in E13.5 LUC2:HES1 NSCs in proliferative, quiescent and reactivated conditions. Fold change expression is relative to *Id1* mRNA levels in proliferative conditions (error bars represent standard deviation, n=3 biological experiments). B) Box plots representing the mean square displacement (MSD) for each cell tracked in proliferative, quiescent and reactivated conditions. This is defined as the square of the distance of the cell from the starting point of tracking plotted against the relative time since the beginning of tracking (dots represent full-length individual cell traces, black horizontal lines represent median, number of cell traces analysed: 67 proliferative from n=6, 61 quiescent from n=4, 120 reactivated from n=5 biological experiments). C) Average length of tracking for each cell in proliferative, quiescent and reactivated conditions (dots represent full-length individual cell traces, black horizontal lines represent median, number of cell traces analysed: 90 proliferative from n=8, 61 quiescent from n=4, 120 reactivated from n=5 biological experiments). D) Compare (i) period, (ii) quality and (iii) maximum peak-to-trough ratio between the first 10h traces and the remaining 10h traces in proliferative, quiescent and reactivated conditions (dots represent individual 10h oscillatory cell traces, black horizontal lines represent median, number of 10h oscillatory cell traces analysed: 56 proliferative (51 first, 5 remaining) from n=7, 110 quiescent (39 first, 71 remaining) from n=4, 149 reactivated (87 first, 62 remaining) from n=5 biological experiments, no statistical analysis was performed for the proliferative cells between the first and remaining 10h traces due to very low number of samples in remaining traces. Two-tailed unpaired t-test was performed for the quiescent cells in (i), Mann-Whitney test was performed for all other statistical analysis, ns=not significant).

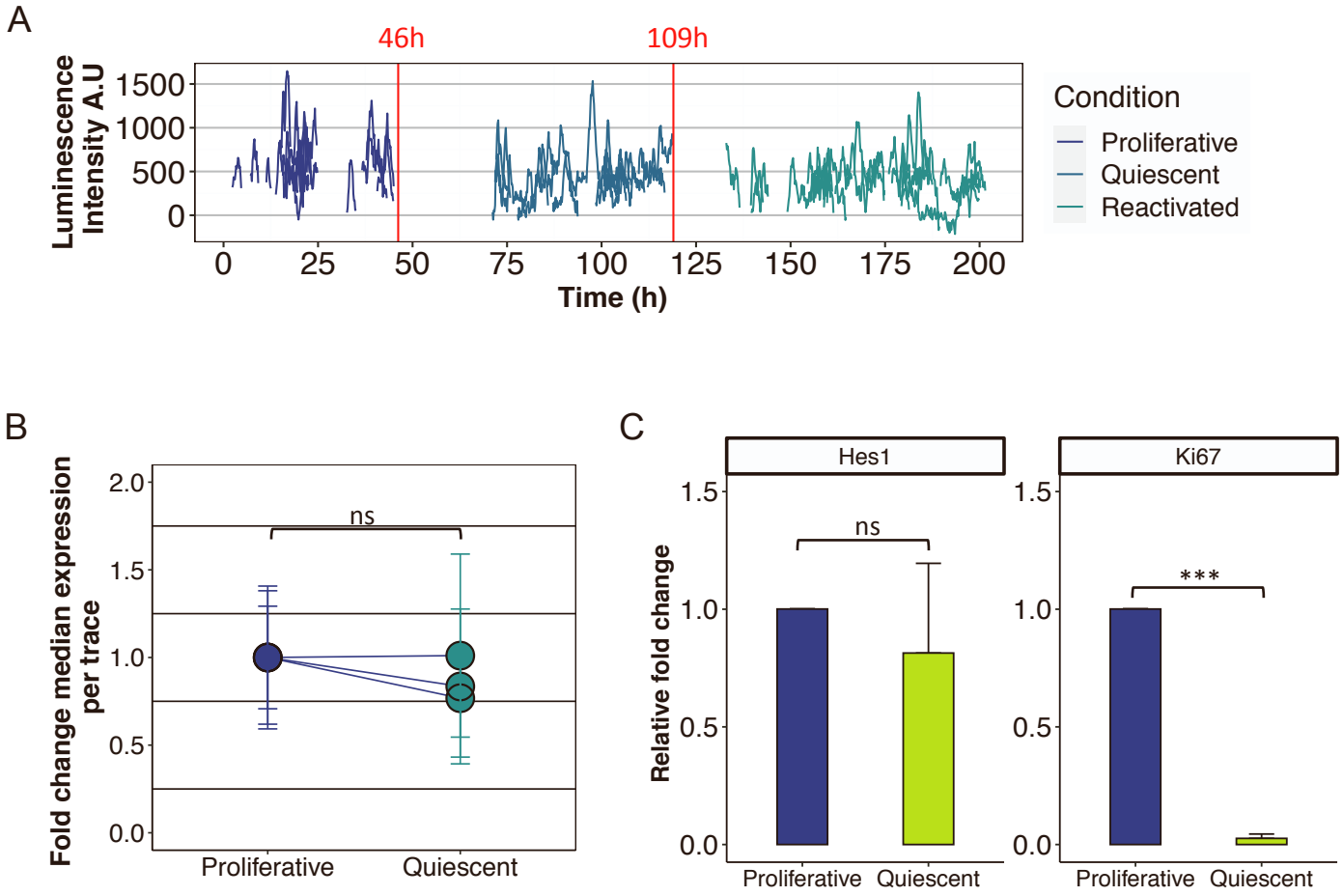

Figure S3. HES1 level does not increase as NSCs transition from proliferation into quiescence, related to Figure 2

A) Graph showing luminescence expression of LUC2:HES1 NSCs as they transition from proliferation into quiescence and then into proliferation again upon reactivation. Red vertical lines indicate the time at which proliferation media was replaced by quiescence media (46h) and then by proliferation media again (109h). Luminescence expression is absent immediately after the change of media. B) Graph showing the relative fold change of median luminescence expression between proliferative and quiescent conditions per experiment. Luminescence expression was estimated by the median luminescence expression per cell trace and per condition (error bars represent standard deviation, two-tailed paired t-test, ns=not significant). C) qPCR analysis for *Hes1* and *Ki67* mRNA expression in E13.5 LUC2:HES1 NSCs in proliferative and quiescent conditions. Fold change expression is relative to proliferative conditions for each gene (error bars represent standard deviation, n=3 biological experiments, Wilcoxon test for *Hes1*, ns=not significant, two-tailed Paired t-test for *Ki67*, ***p=0.0001).

Figure S4. DNA sequence of the *Hes1* locus in the HOM HES1:mSCARLET-I transgenic mice, related to Figure 3

DNA sequence encoding for a linker protein, a 3xFlag epitope and the mSACRLET-I protein has been inserted downstream of the last *Hes1* exon and before the 3'UTR.

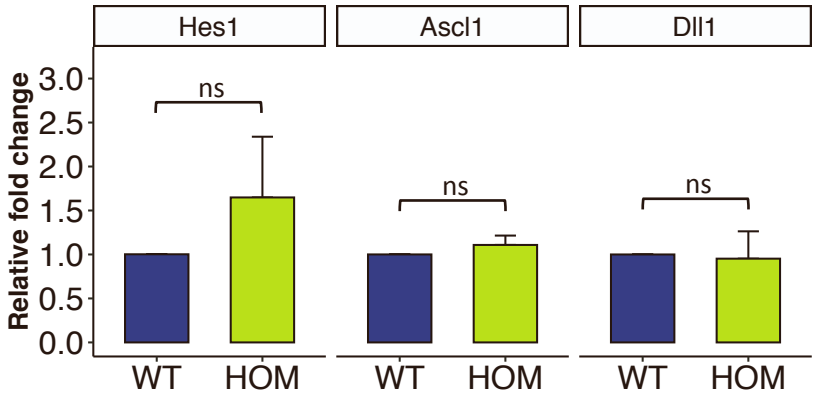

Figure S5. *Hes1*, *Ascl1* and *Dll1* mRNA expression in WT vs HOM HES1:mSCARLET-I NSCs, related to Figure 3

qPCR analysis comparing expression of *Hes1*, *Ascl1* and *Dll1* mRNA in E13.5 LGEs isolated either from WT mice or from HOM HES1:mSCARLET-I mice and kept in proliferative conditions. Fold change expression is relative to WT sample for each gene (error bars represent standard deviation, n=3 biological experiments, two-tailed Paired t-test, ns=not significant).

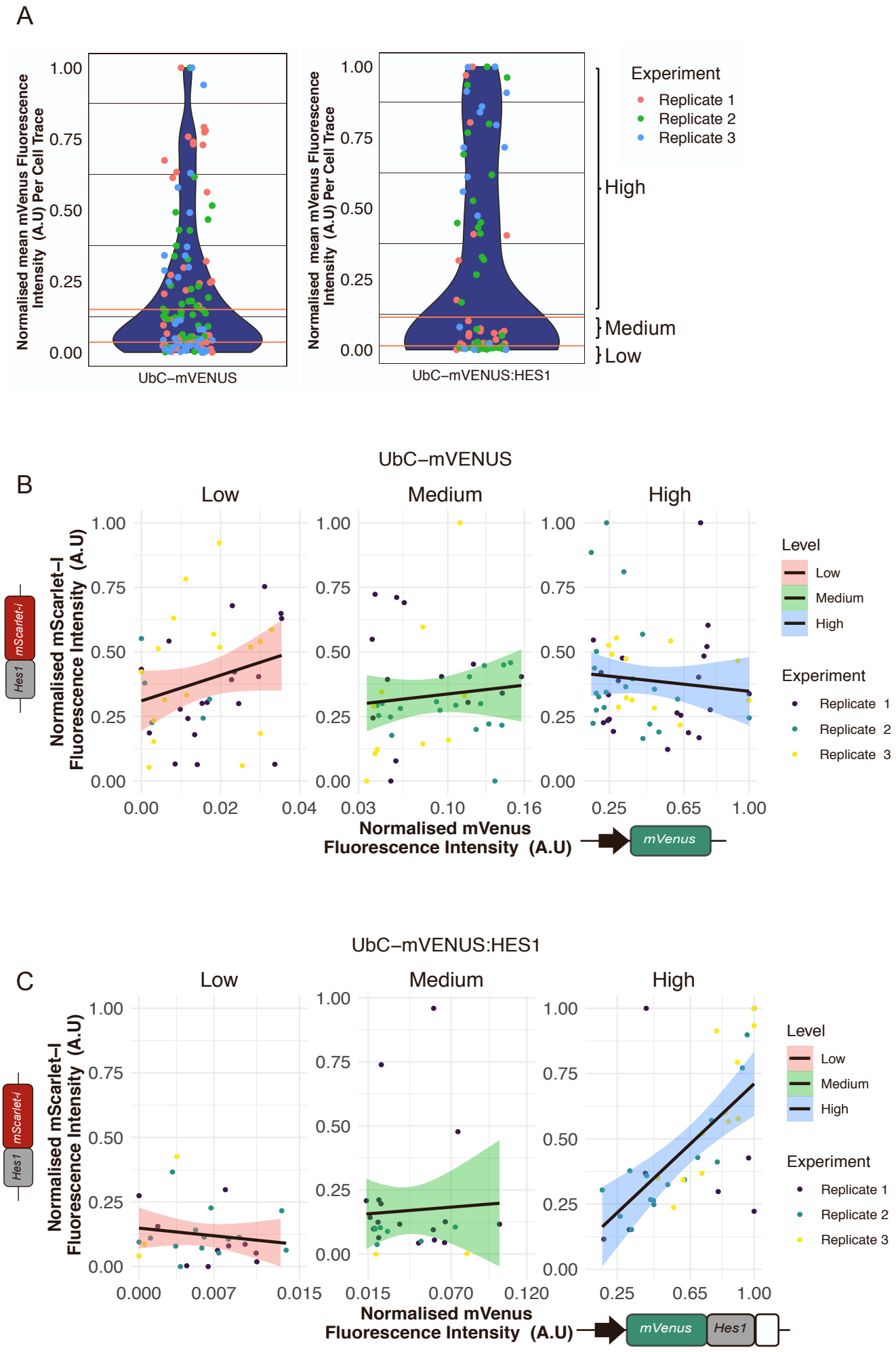
A
Normalised mean mVenus Fluorescence Intensity (A.U) Per Cell Trace
1.00
0.75
0.50
0.25
0.00
UbC–mVENUS
UbC–mVENUS:HES1
Experiment
Replicate 1
Replicate 2
Replicate 3
High
Medium
Low
B
UbC–mVENUS
Low
Medium
High
Normalised mScarlet–I Fluorescence Intensity (A.U)
mScarlet-I
Hes1
0.00
0.02
0.04
0.03
0.10
0.16
0.25
0.65
1.00
Normalised mVenus Fluorescence Intensity (A.U)
mVenus
Level
Low
Medium
High
Experiment
Replicate 1
Replicate 2
Replicate 3
C
UbC–mVENUS:HES1
Low
Medium
High
Normalised mScarlet–I Fluorescence Intensity (A.U)
mScarlet-I
Hes1
0.000
0.007
0.015
0.015
0.070
0.120
0.25
0.65
1.00
Normalised mVenus Fluorescence Intensity (A.U)
mVenus
Hes1
Level
Low
Medium
High
Experiment
Replicate 1
Replicate 2
Replicate 3

Figure S6. Correlation of HES1 endogenous expression with ectopic UbC-mVENUS and UbC-HES1:mVENUS expression, related to Figure 3

A) Violin plots showing the range of mVENUS expression in UbC-mVENUS and UbC-mVENUS:HES1 transfected HOM HES1:mSCARLET-I NSCs. For each replicate experiment the absolute mVENUS expression intensity values were rescaled from 0 to 1 and plotted together per condition. The mVENUS distribution was then divided in 3 quartiles (separated by horizontal orange lines) and was characterised as low, medium and high expression (A.U. = arbitrary units, n=3 biological experiments per condition, total number of cells analysed for UbC-mVENUS=135 and for UbC-mVENUS:HES1 = 88). B) Plots showing correlation of transfected ectopic UbC-mVENUS expression level vs endogenous HES1-mSCARLET level in the same cell for low, medium and high mVENUS expressing cells. The absolute mSCARLET-I expression intensity values were also rescaled from 0 to 1 and plotted together from all replicate experiments (A.U. = arbitrary units, n= 3 biological experiments, Pearson correlation, the shaded coloured area represents 95% confidence interval, correlation coefficient values are: Low= 0.26, Medium= 0.11, High= -0.1). C) Plots showing correlation of transfected ectopic UbC-mVENUS:HES1 expression level vs endogenous HES1-mSCARLET level in the same cell for low, medium and high mVENUS expressing cells. The absolute mSCARLET-I expression intensity values were also rescaled from 0 to 1 and plotted together from all replicate experiments (A.U. = arbitrary units, n= 3 biological experiments, Pearson correlation, the shaded coloured area represents 95% confidence interval, correlation coefficient values are: Low= -0.16, Medium= 0.05, High= 0.64).

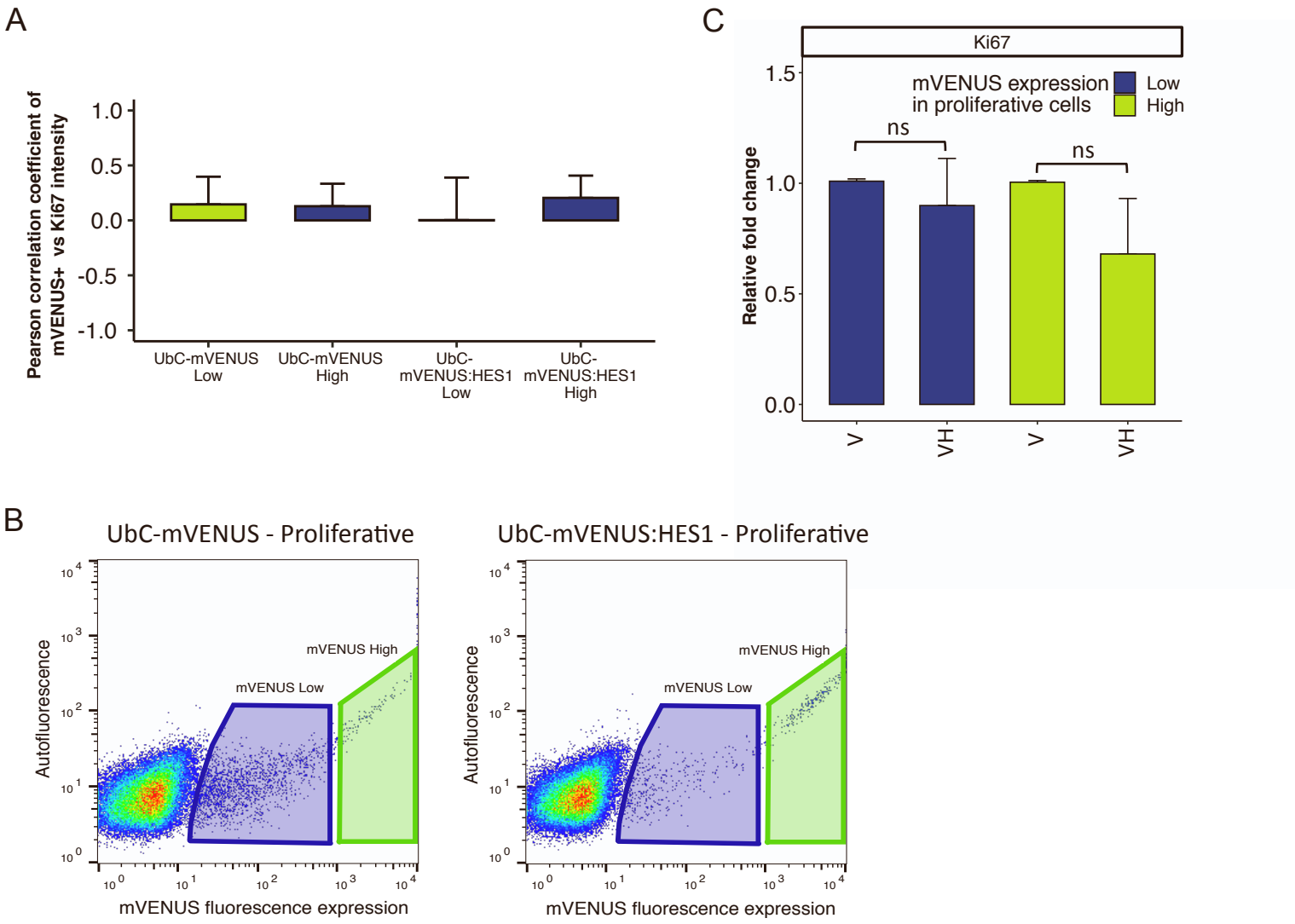

Figure S7. Correlation of Ki67 expression with ectopic UbC-mVENUS and UbC-HES1:mVENUS expression, related to Figure 4

A) Graph showing the Pearson correlation coefficient of mVENUS protein expression intensity (within mVENUS positive cells) vs Ki67 protein expression intensity in proliferative WT NSCs transfected with either UbC-mVENUS or UbC-mVENUS:HES1 and immunostained with anti-GFP (against mVENUS) and anti-Ki67 (1 or 2 days post transfection) (error bars represent standard deviation, n=2 biological experiments, correlation values: UbC-mVENUS Low = 0.15, UbC-mVENUS High = 0.13, UbC-mVENUS:HES1 Low = 0.002, UbC-mVENUS:HES1 High = 0.2). B) Flow cytometry plots showing the gating strategy for sorting mVENUS low and mVENUS high expressing cells. WT proliferative NSCs were transfected with either UbC-mVENUS or UbC-mVENUS:HES1 and were sorted 2d post transfection for low or high mVENUS expression. C) qPCR analysis for *Ki67* mRNA expression in proliferative WT NSCs sorted for UbC-mVENUS low or high and UbC-mVENUS:HES1 low or high expression (V=UbC-mVENUS, VH=UbC-mVENUS:HES1). Fold change is relative to the UbC-mVENUS sample within each condition (low or high mVENUS expression) (error bars represent standard deviation, n=3 biological experiments, two-tailed Paired t-test, ns=not significant).

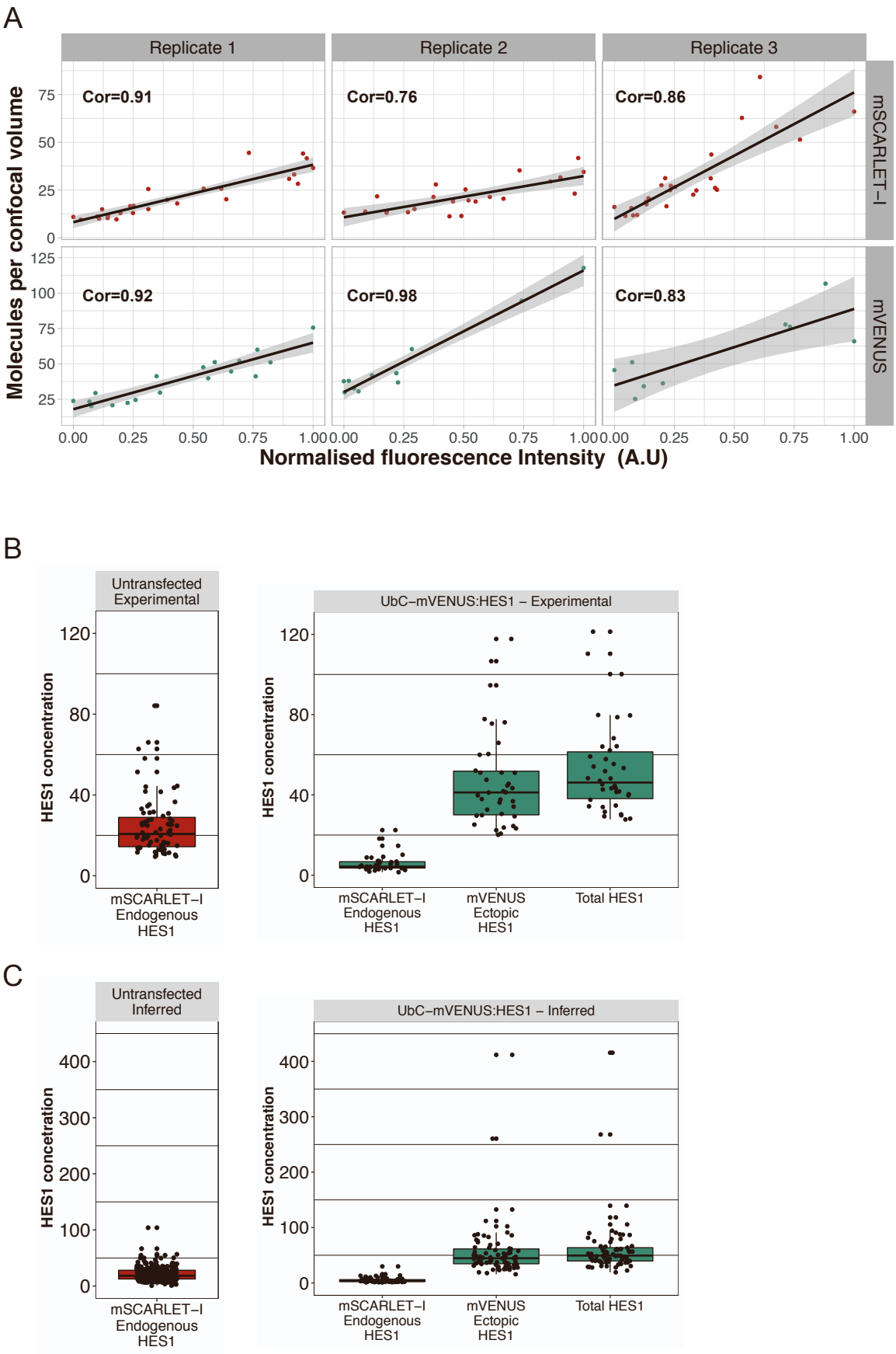
A
Replicate 1
Replicate 2
Replicate 3
mSCARLET–I
mVENUS
Cor=0.91
Cor=0.76
Cor=0.86
Cor=0.92
Cor=0.98
Cor=0.83
Molecules per confocal volume
Normalised fluorescence Intensity (A.U)
B
Untransfected Experimental
UbC–mVENUS:HES1 – Experimental
HES1 concentration
mSCARLET–I Endogenous HES1
mVENUS Ectopic HES1
Total HES1
C
Untransfected Inferred
UbC–mVENUS:HES1 – Inferred
HES1 concetration
HES1 concentration

Figure S8. Ectopic persistent HES1 expression does not increase total HES1 level above physiological range, related to Figure 5

A) Correlation of HES1:mSCARLET-I fluorescence intensity vs HES1:mSCARLET-I concentration and UbC-mVENUS:HES1 fluorescence intensity vs UbC-mVENUS:HES1 concentration per cell from 3 replicate experiments. E13.5 HES1:mSCARLET-I NSCs were transfected with UbC-mVENUS:HES1. HES1:mSCARLET-I fluorescence intensity and HES1:mSCARLET-I concentration was estimated from untransfected cells while UbC-mVENUS:HES1 fluorescence intensity and UbC-mVENUS:HES1 concentration was estimated from transfected cells in the same culture (Pearson correlation, the shaded gray area represents 95% confidence interval) B-C) Box plots showing total HES1 concentration in untransfected (left panels) and UbC-mVENUS:HES1 transfected (right panels) E13.5 HES1:mSCARLET-I NSCs. In the reporter transfected cells, the total HES1 concentration is the sum of UbC-mVENUS:HES1 concentration (ectopic HES1) and HES1:mSCARLET-I concentration (endogenous HES1) depicted on separate box plots. In (B) all concentrations have been estimated experimentally by FCS (apart from the HES1:mSCARLET-I concentration in transfected cells which was inferred). In (C) all concentrations have been inferred (black horizontal lines represent median, dots represent individual cells, number of cell analysed in (B) untransfected= 75, transfected=38, n=3 biological replicates, in (C) untransfected=316, transfected=77, n=3 biological experiments).

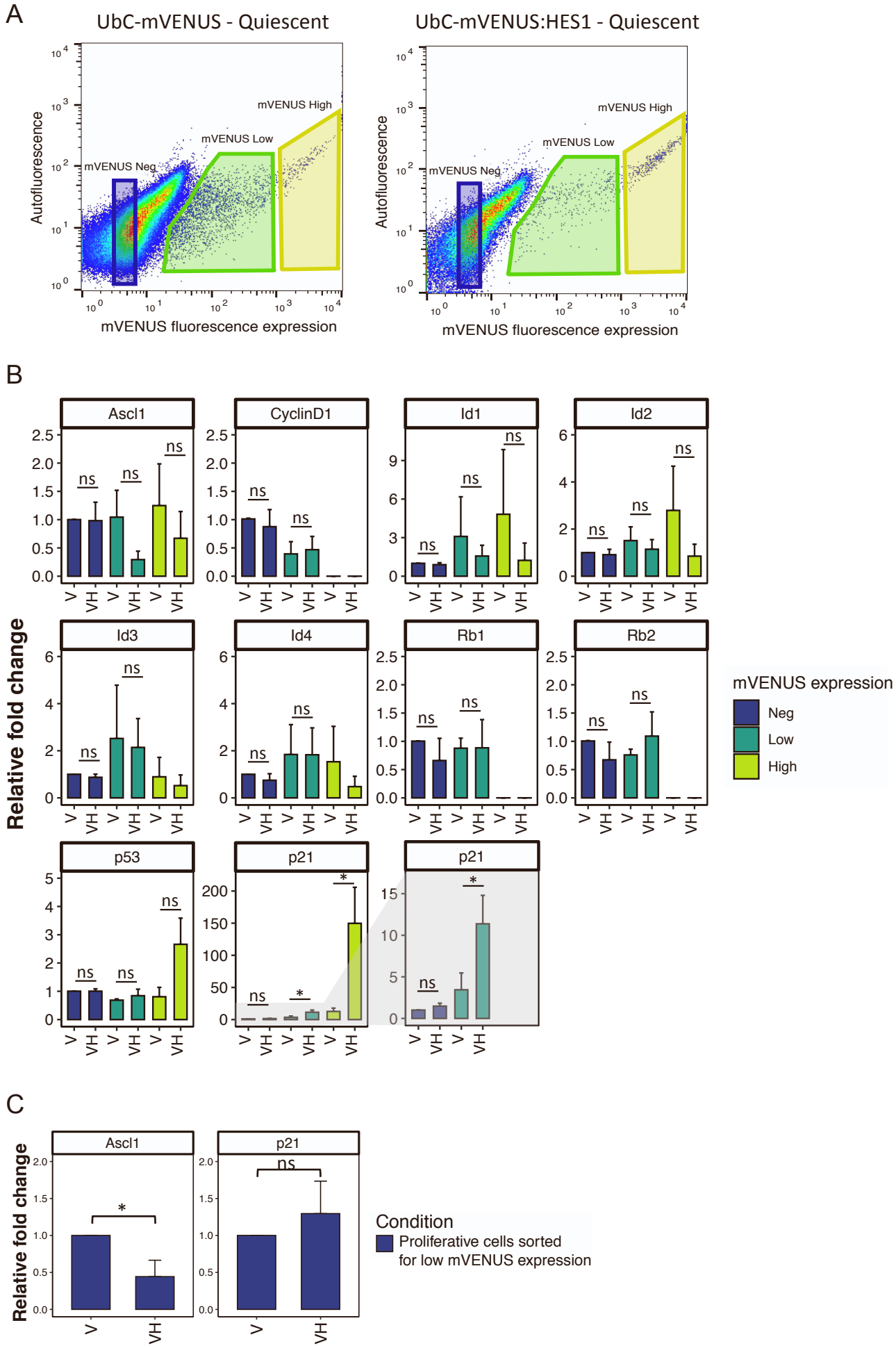
A
UbC-mVENUS - Quiescent
UbC-mVENUS:HES1 - Quiescent
mVENUS Neg
mVENUS Low
mVENUS High
Autofluorescence
mVENUS fluorescence expression
B
Ascl1
CyclinD1
Id1
Id2
Id3
Id4
Rb1
Rb2
p53
p21
p21
Relative fold change
ns
*
V
VH
mVENUS expression
Neg
Low
High
C
Ascl1
p21
Relative fold change
Condition
Proliferative cells sorted
for low mVENUS expression

Figure S9. Effect of UbC-mVENUS:HES1 persistent expression on other genes' expression, related to Figure 5

A) Flow cytometry plots showing the gating strategy for sorting mVENUS negative, mVENUS low and mVENUS high expressing cells in quiescent NSCs previously transfected with either UbC-mVENUS or UbC-mVENUS:HES1. B) qPCR analysis for a panel of genes in quiescent NSCs sorted for UbC-mVENUS negative, low or high and UbC-mVENUS:HES1 negative, low or high (V=UbC-mVENUS, VH=UbC-mVENUS:HES1). Fold change is relative to the UbC-mVENUS negative sample. For the p21 gene a zoomed in region of lower fold change values is shown in the grey shaded panel (error bars represent standard deviation, n=3 biological experiments apart from the samples which are missing statistics where reliable mRNA values could be obtained only from 2 out of 3 experiments, two-tailed Paired t-test (apart from stats in Id1 in high mVENUS expression where a Wilcoxon test was performed) , ns=not significant. For p21 gene in low mVENUS expression *p=0.027, in high mVENUS expression *p=0.043). C) qPCR analysis of *Ascl1* and *p21* mRNA expression in proliferative WT NSCs sorted for UbC-mVENUS or UbC-mVENUS:HES1 low expression (V=UbC-mVENUS, VH=UbC-mVENUS:HES1). Fold change is relative to the UbC-mVENUS sample (error bars represent standard deviation, n=3 biological experiments, two-tailed Paired t-test, *p=0.048, ns=not significant).