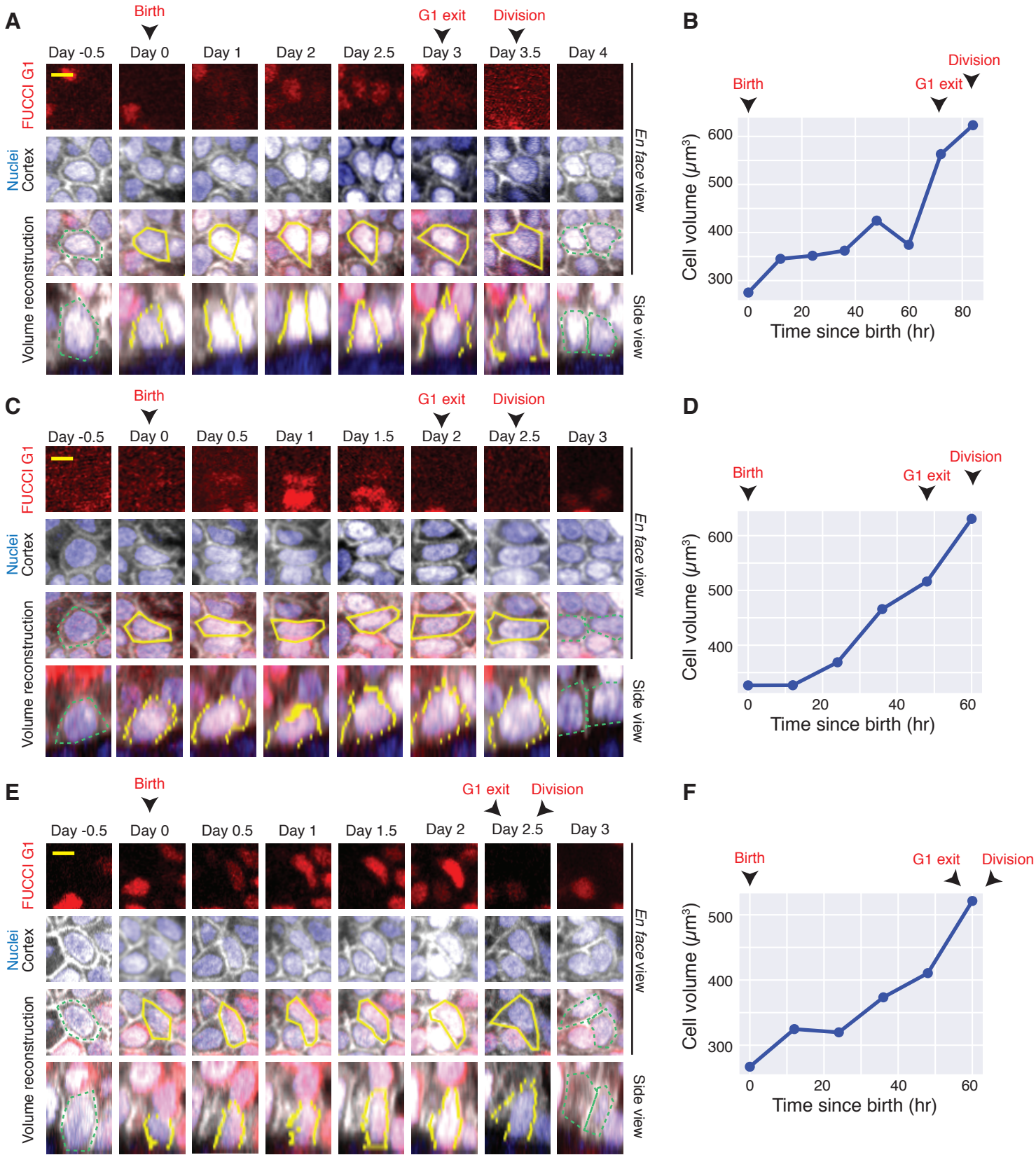
A
Birth
G1 exit
Division
Day -0.5
Day 0
Day 1
Day 2
Day 2.5
Day 3
Day 3.5
Day 4
FUCCI G1
Nuclei
Cortex
Volume reconstruction
En face view
Side view
B
Division
Birth
G1 exit
Cell volume (μm³)
600
500
400
300
0
20
40
60
80
Time since birth (hr)
C
Birth
G1 exit
Division
Day -0.5
Day 0
Day 0.5
Day 1
Day 1.5
Day 2
Day 2.5
Day 3
FUCCI G1
Nuclei
Cortex
Volume reconstruction
En face view
Side view
D
Division
Birth
G1 exit
Cell volume (μm³)
600
500
400
0
20
40
60
Time since birth (hr)
E
Birth
G1 exit
Division
Day -0.5
Day 0
Day 0.5
Day 1
Day 1.5
Day 2
Day 2.5
Day 3
FUCCI G1
Nuclei
Cortex
Volume reconstruction
En face view
Side view
F
Birth
G1 exit
Division
Cell volume (μm³)
500
400
300
0
20
40
60
Time since birth (hr)

Figure S1. Examples of 3D reconstruction of epidermal stem cells. Related to Figure 1.

A-F. Three examples of the volume reconstruction of single epidermal stem cells. The FUCCI-G1 reporter is shown in red, the nucleus in blue, and the actin cortex in gray. The manually segmented cell outlines are shown in yellow on top of the merged images showing *en face* and side views. The cell cycle landmarks birth, G1 exit, and division are annotated. The parent cell and daughter cells are outlined in dotted green. The volume growth curve is quantified for the cells shown in their respective neighboring panels.

Scale bars are 5 μm.

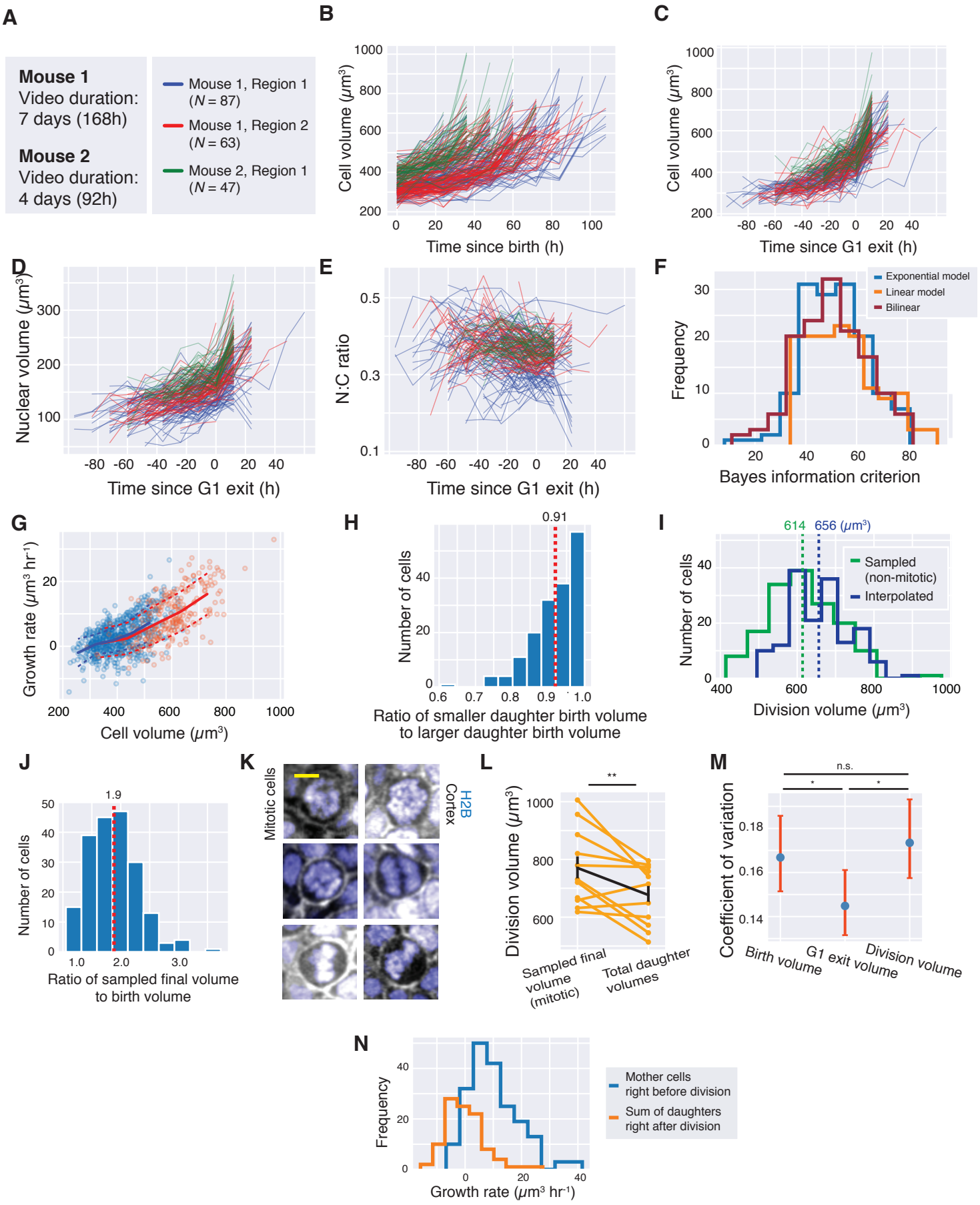
A
Mouse 1
Video duration:
7 days (168h)
Mouse 2
Video duration:
4 days (92h)
Mouse 1, Region 1 (N = 87)
Mouse 1, Region 2 (N = 63)
Mouse 2, Region 1 (N = 47)
B
Cell volume (μm³)
Time since birth (h)
C
Cell volume (μm³)
Time since G1 exit (h)
D
Nuclear volume (μm³)
Time since G1 exit (h)
E
N:C ratio
Time since G1 exit (h)
F
Exponential model
Linear model
Bilinear
Frequency
Bayes information criterion
G
Growth rate (μm³ hr⁻¹)
Cell volume (μm³)
H
0.91
Number of cells
Ratio of smaller daughter birth volume to larger daughter birth volume
I
614
656 (μm³)
Sampled (non-mitotic)
Interpolated
Number of cells
Division volume (μm³)
J
1.9
Number of cells
Ratio of sampled final volume to birth volume
K
Mitotic cells
H2B
Cortex
L
**
Division volume (μm³)
Sampled final volume (mitotic)
Total daughter volumes
M
n.s.
*
*
Coefficient of variation
Birth volume
G1 exit volume
Division volume
N
Frequency
Growth rate (μm³ hr⁻¹)
Mother cells right before division
Sum of daughters right after division

Figure S2. Epidermal stem cell growth dynamics *in vivo*. Related to Figure 2.

A. Summary of the multiple skin regions used in this study. Two regions were analyzed from mouse 1, which was imaged for 7 days. One region was analyzed from mouse 2, which was imaged for 4 days.

B. Volume growth curves aligned by birth ($N = 197$). Each independent region is as indicated in (A).

C. Volume growth curves aligned by G1 exit.

D. The nuclear volumes aligned by G1 exit ($N = 197$).

E. The N:C volume ratios are plotted with respect to time and aligned by G1 exit.

F. The volume growth rate (backwards differences) is plotted against cell volume, both estimated from unsmoothed data ($N = 946$). Blue dots denote G1 cells, orange dotes denote S/G2 cells. Binned means are shown in blue (G1) and orange (S/G2). Dotted lines show standard deviations.

G. The ratio of the birth volume of the smaller daughter cell to its larger sister cell ($N = 167$). Dotted line denotes the median.

H. The distribution of the sampled final volume (green) and the interpolated division volume (blue) are shown for cells whose last frame is not during mitosis. Dotted lines denote the means for the distribution of corresponding color.

I. The distribution of the ratio between the sampled final volume to birth volume. Dotted line denotes the mean.

J. Examples of mitotic cells. The histone reporter is shown in blue. The actin cortex is shown in gray. Scalebar is 5μm.

K. The differences between the sampled final volume and the interpolated division volume are shown for cells that are in mitosis in their last frame ($N = 11$). Black line denotes the mean. Cells in mitosis are larger than the sum of the two daughter cell volumes. (**: one-tailed paired T-test, $P < 0.01$).

L. The CV of cell volume at cell birth, G1 exit, and cell division. $N = 197$. Error bars show the 95% confidence interval estimated using bootstrapping. (*: $P < 0.05$, n.s.: $P > 0.5$; one-tailed bootstrap test).

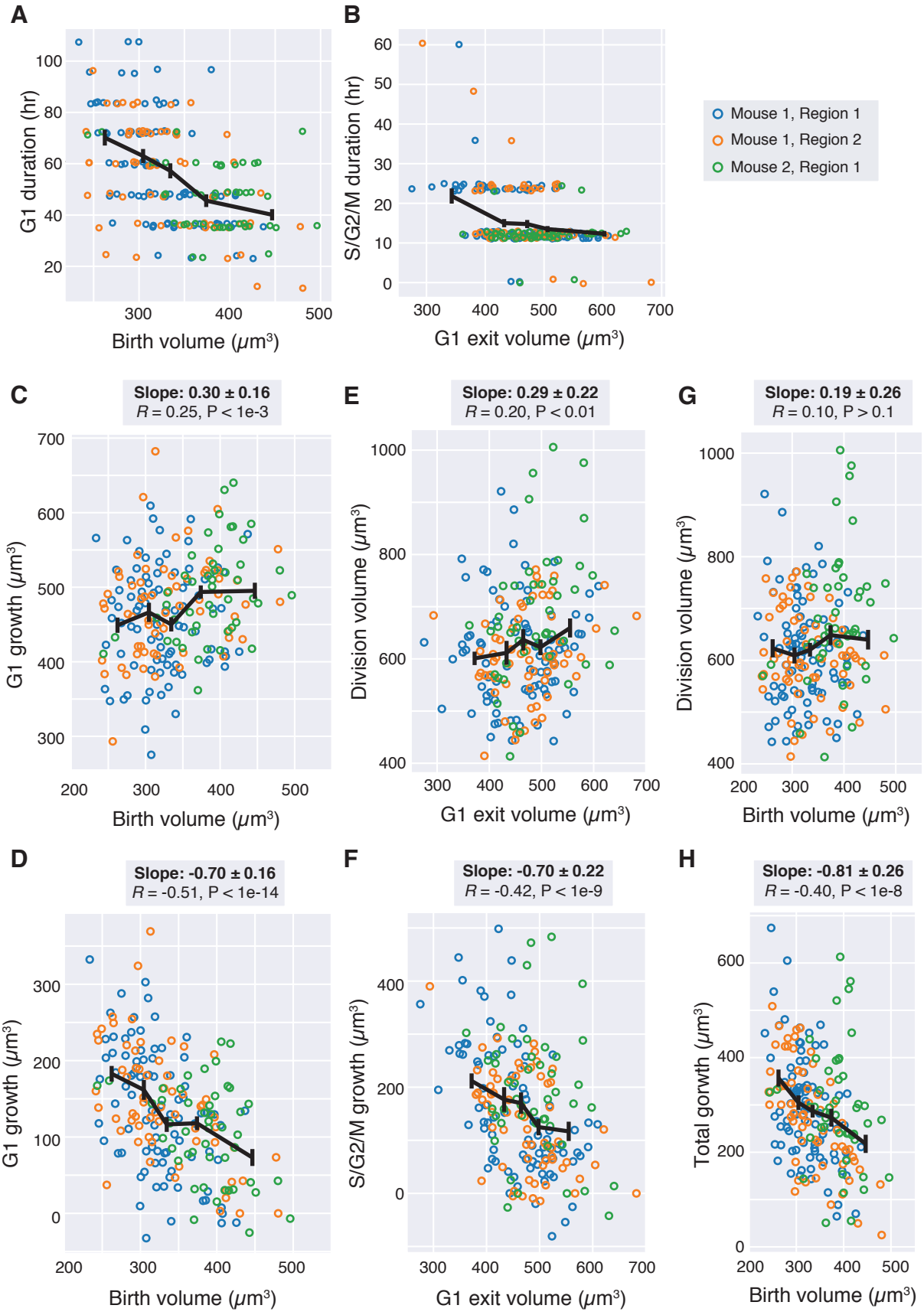
A
G1 duration (hr)
Birth volume (μm³)
B
S/G2/M duration (hr)
G1 exit volume (μm³)
Mouse 1, Region 1
Mouse 1, Region 2
Mouse 2, Region 1
C
Slope: 0.30 ± 0.16
R = 0.25, P < 1e-3
G1 growth (μm³)
Birth volume (μm³)
E
Slope: 0.29 ± 0.22
R = 0.20, P < 0.01
Division volume (μm³)
G1 exit volume (μm³)
G
Slope: 0.19 ± 0.26
R = 0.10, P > 0.1
Division volume (μm³)
Birth volume (μm³)
D
Slope: -0.70 ± 0.16
R = -0.51, P < 1e-14
G1 growth (μm³)
Birth volume (μm³)
F
Slope: -0.70 ± 0.22
R = -0.42, P < 1e-9
S/G2/M growth (μm³)
G1 exit volume (μm³)
H
Slope: -0.81 ± 0.26
R = -0.40, P < 1e-8
Total gorwth (μm³)
Birth volume (μm³)

Figure S3. Cell size control correlations showing unsmoothed data from individual regions. Related to Figure 3.

A. Cell birth volume is plotted against the duration of the G1 phase. Cells from each individual region are shown in different colors.

B. The volume at which cells exit G1 is plotted against the duration of S/G2/M phases.

C-D. Birth volume is plotted against the volume at G1 exit (C) or the amount of growth during G1 (D).

E-F. The G1 exit volume is plotted against the volume at division (E) or the amount of growth during S/G2/M (F).

G-H. The birth volume is plotted against the division volume (G) or the total amount of growth (H).

All data are unsmoothed and contain no daughter volume interpolation. Black lines show binned means ± SEM. Slopes reported correspond to the slope of the linear regression and 95% confidence intervals. R values are Pearson's correlations, with P-values reported against the null-hypothesis of $R = 0$. We note that not using smoothed data leads to the same qualitative conclusions as using smoothed data.

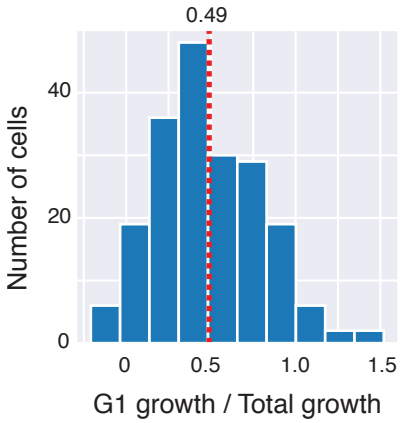
0.49
40
20
0
Number of cells
0
0.5
1.0
1.5
G1 growth / Total growth

Figure S4. The distribution of the ratio between growth during G1 over total growth. Related to Figure 4. *N* = 167, dotted line denotes the mean.