Expanded View Figures

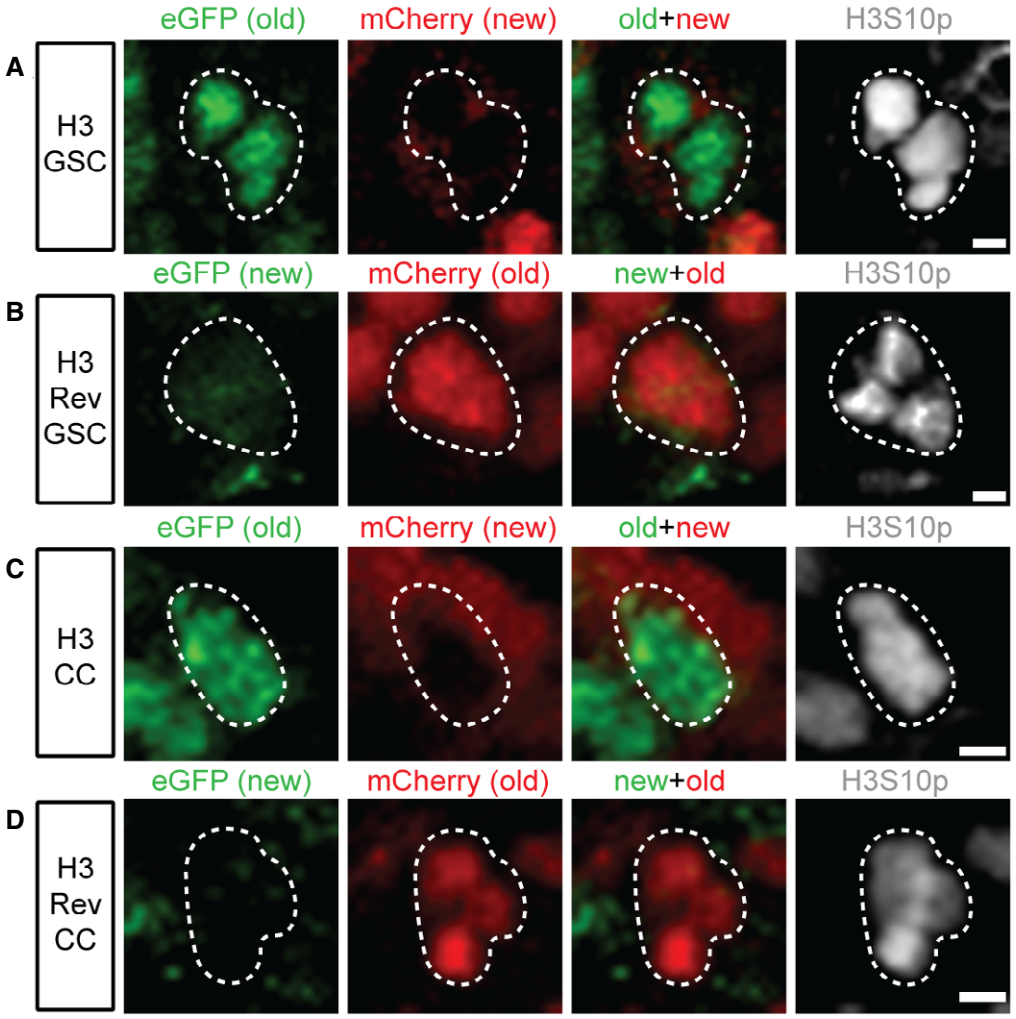

Figure EV1. Old versus new histone H3 distribution patterns in *Drosophila* female germline stem cells (GSCs) and cystocytes (CCs) during the first mitosis after heat shock-induced genetic switch.

A Old (green, eGFP) versus new (red, mCherry) histone H3 patterns in a prometaphase female GSC marked by anti-H3S10p (gray).
B Old (red, mCherry) versus new (green, eGFP) histone H3 patterns for H3Rev in a prophase female GSC marked by anti-H3S10p (gray).
C Old (green, eGFP) versus new (red, mCherry) histone H3 patterns in a prophase female CC marked by anti-H3S10p (gray).
D Old (red, mCherry) versus new (green, eGFP) histone H3 patterns for H3Rev in a prometaphase female CC marked by anti-H3S10p (gray). During the first mitosis after heat shock, the new histone has not yet been globally incorporated into the chromatin, shown as low to undetectable fluorescent signals.

Data information: Scale bars: 2 μm.

© 2021 The Authors

Figure EV2. Quantification of overlap between old and new histones, for H3, H3Rev, H4, H3.3, and H2A in different staged germ cells.

A Spearman values of single Z-slice from 15 individual female GSCs re-plotted from Fig 1L, compared to the average Spearman values obtained from the same 15 individual female GSCs by quantifying every single slice of the entire Z-stack followed by averaging them. Data collected over 9 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. n.s.: not significant.

B, C Quantification of overlap between old and new H3 in late prophase and prometaphase germ cells at distinct differentiation stages. In these analyses, a slight difference between the H3 (B) and H3Rev (C): From the 4-cell stage to 8-cell stage, there is a slight decrease of correlation coefficient in the H3 line, but not the H3Rev line. This difference could arise from subtle protein stability differences between eGFP and mCherry used as the "old" histone tag and/or lower expression levels, as *GreenEye-nanos-Gal4* expression tapers off in the later staged 4-cell and 8-cell. H3: GSC ($n = 57$), CB ($n = 51$), 2-cell ($n = 70$), 4-cell ($n = 48$), and 8-cell ($n = 35$). Data collected from 20 separate experiments. H3Rev: GSC ($n = 50$), CB ($n = 50$), 2-cell ($n = 54$), 4-cell ($n = 39$), and 8-cell ($n = 37$). Data collected from 12 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. ****: $P < 0.0001$. ***: $P < 0.001$. *: $P < 0.05$. n.s.: not significant.

D, E Quantification of overlap between old and new H4 (D) and H3.3 (E) in late prophase and prometaphase cells, which show no significant change among distinct differentiation stages. H4: GSC ($n = 60$), CB ($n = 51$), 2-cell ($n = 61$), 4-cell ($n = 39$), and 8-cell ($n = 42$). Data collected from 16 separate experiments. H3.3: GSC ($n = 55$), CB ($n = 46$), 2-cell ($n = 52$), 4-cell ($n = 51$), and 8-cell ($n = 34$). Data collected from 7 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. n.s.: not significant.

F Quantification of overlap between old and new H2A in late prophase and prometaphase cells at distinct differentiation stages. There is a slight but statistically significant decrease in overlap between GSCs and CBs for H2A, which could be due to many of the correlation values for the H2A in GSCs are compressed at or near the maximum possible correlation value at 1.0. This distribution of data points flattens the measurement variances within one sample and thus when comparing to another sample with more variance above and below the mean value, stringent statistical analysis could identify this as a significant difference between the two samples. H2A: GSC ($n = 79$), CB ($n = 80$), 2-cell ($n = 67$), 4-cell ($n = 41$), and 8-cell ($n = 38$). Data collected from 11 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. ****: $P < 0.0001$. n.s.: not significant.

G Direct comparison of overlap between old and new H3 as well as old and new H2A at both GSC and CB stages. Data collected over 12 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. ****: $P < 0.0001$. n.s.: not significant.

Data information: See Dataset EV5 for individual data points for Fig EV2A–G.

© 2021 The Authors

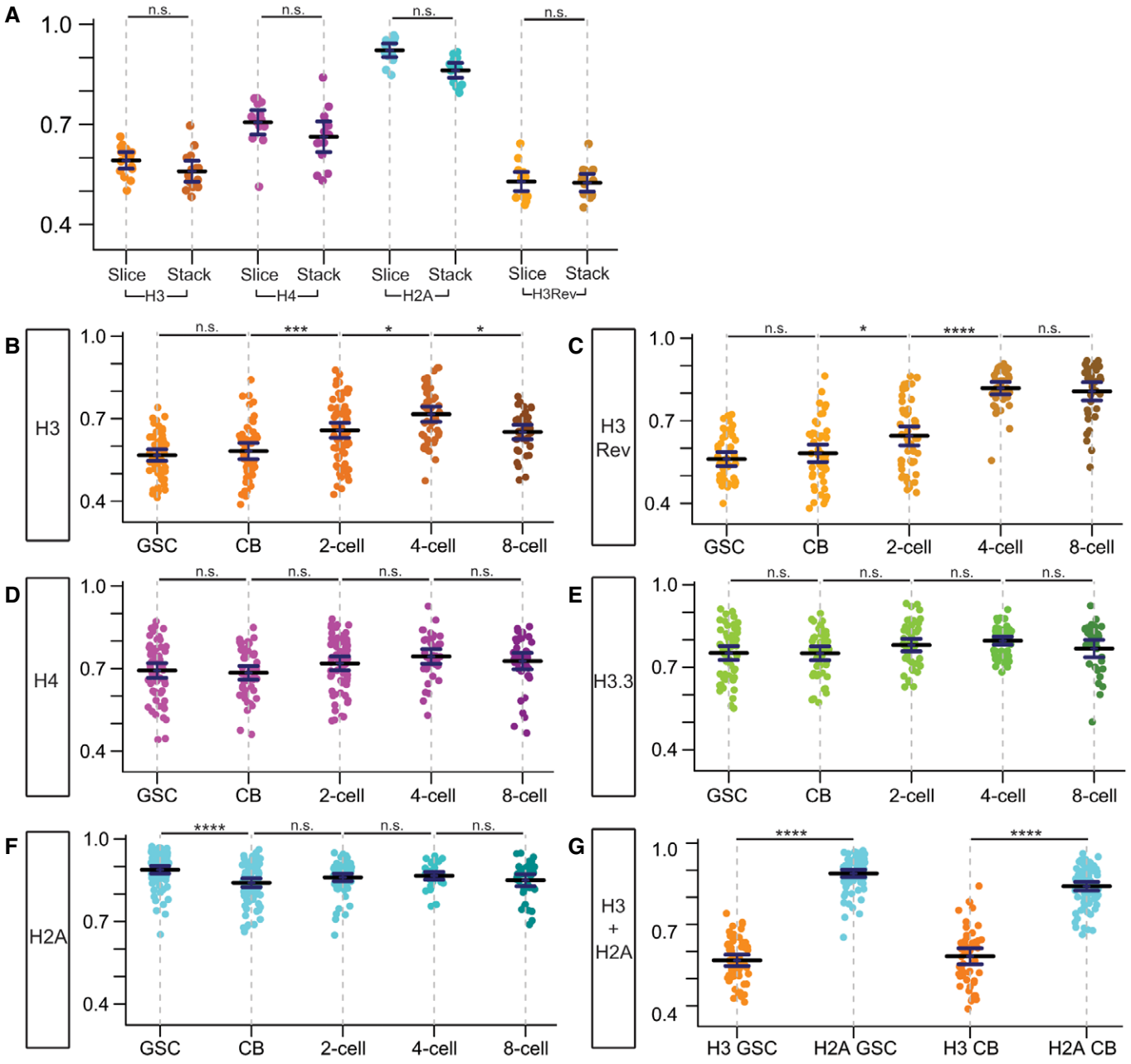

Figure EV2.

© 2021 The Authors

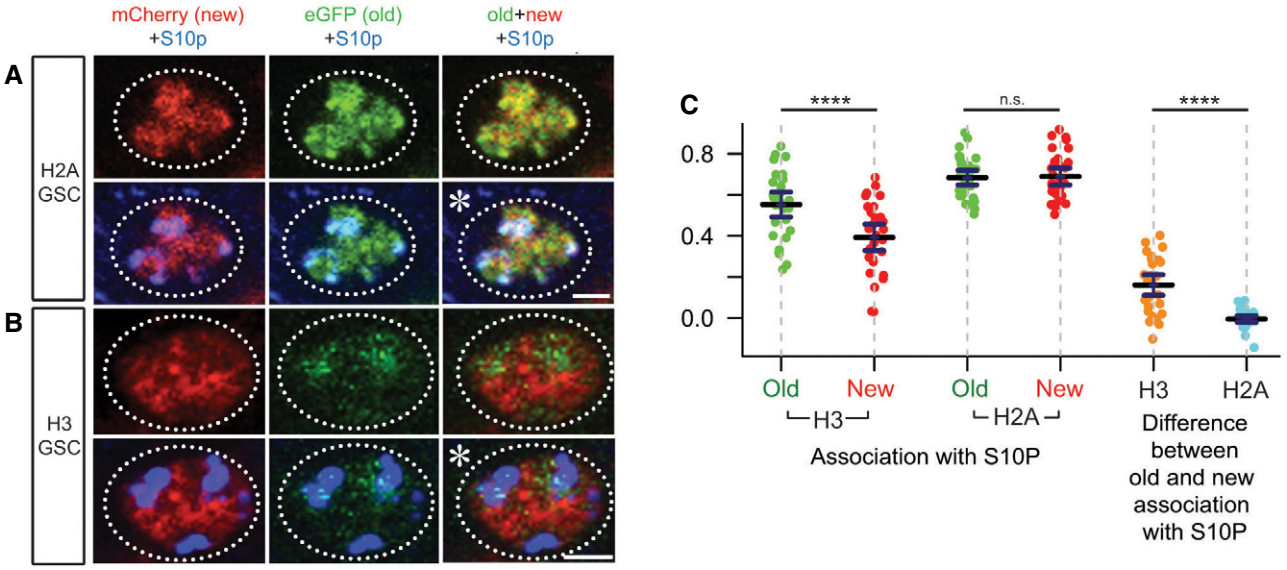

Figure EV3. H3S10p association with old versus new H3 and H2A histones in prophase GSCs.

A, B Old (green) versus new (red) histone patterns for H2A (A) and H3 (B) in early prophase female GSCs marked by anti-H3S10p (blue). Asterisk: niche. Scale bars: 2 μm.

C Spearman correlation quantification of overlap between old histone signal and H3S10p (green dots) and new histone signal and H3S10p (red dots) shows a significant difference in H3 GSCs (n = 30, mean = 0.553 for old and 0.392 for new) but not H2A GSCs (n = 31, mean = 0.684 for old and 0.689 for new). For each GSC, the Spearman value for old histone associated with H3S10p was subtracted from the Spearman value for new histone associated with H3S10p to quantify the magnitude of difference for each individual cell (right side). There was a significant difference in the amount of old versus new H3 associated with H3S10p (orange dots, mean = 0.160) compared to the amount of old versus new H2A associated with H3S10p (blue dots, mean = −0.006). Data collected over 7 separate experiments. Values are mean ± 95% CI. P-value: pairwise ANOVA test with Bonferroni correction. ****P < 0.0001. n.s.: not significant. See Dataset EV6 for individual data points.

© 2021 The Authors

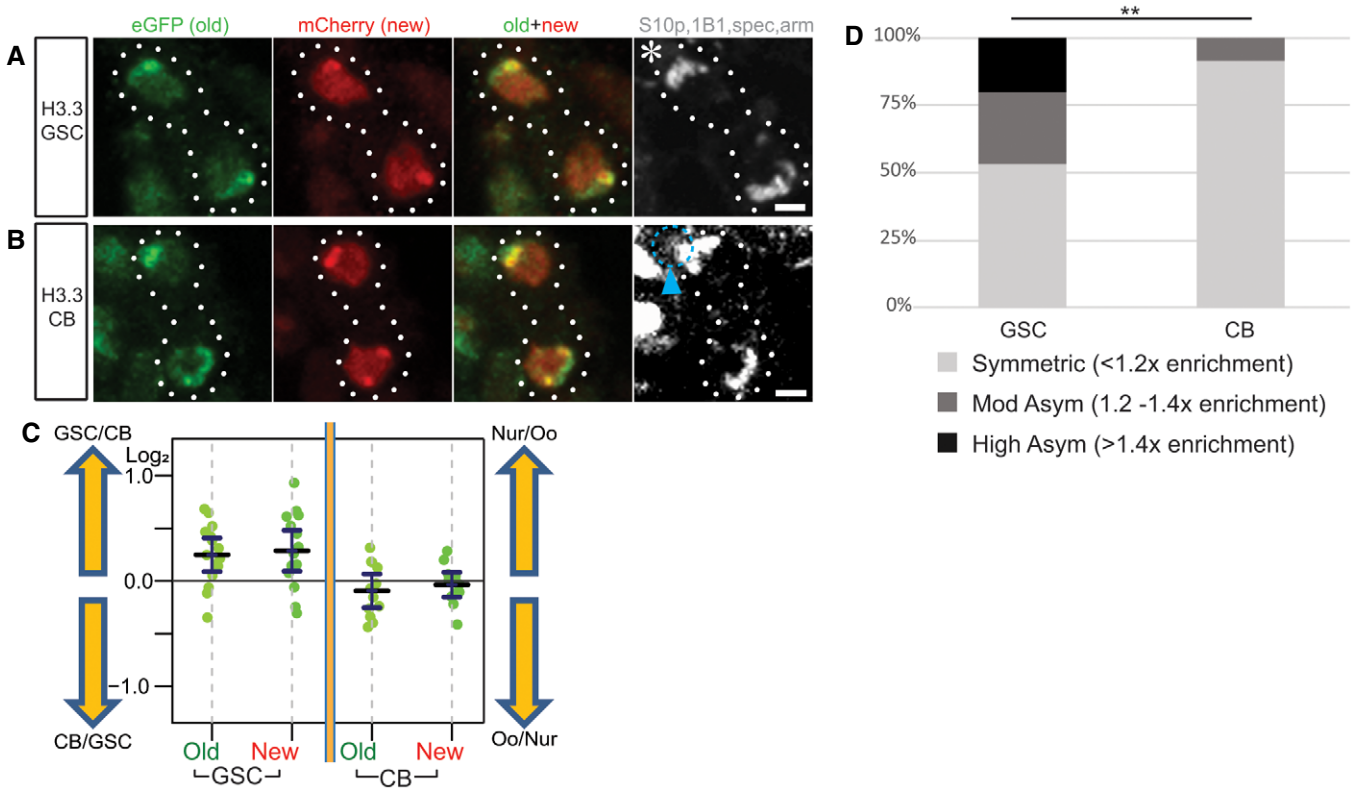

Figure EV4. The inheritance patterns of old versus new histone variant H3.3 in GSCs and CBs.

A, B Telophase cells marked by H3S10p (gray) show overlapping old (green) and new (red) histone variant H3.3 patterns during the GSC (A) and CB (B) divisions. Asterisk: niche. Arrowhead (cyan): biased spec inheritance. Scale bars: 2 μm.

C Quantification of $\log_2$ ratios of total old and new H3.3 histone inherited by each future daughter cell of the GSC and CB divisions, where a value of 0 is equal inheritance at exactly a 1:1 ratio. Data gathered over 7 separate experiments for GSCs and 9 separate experiments for CBs. Values are mean ± 95% CI. See Dataset EV7 for individual data points.

D Summary of total old histone variant H3.3 inherited in GSCs and CBs: < 1.2-fold is symmetric, 1.2-fold to 1.4-fold is moderately asymmetric, and > 1.4-fold is highly asymmetric, using previously established criteria for the degree of histone or histone variant asymmetry (Ranjan *et al*, 2019). **: $P < 0.01$, based on pairwise ANOVA test with Bonferroni correction.

 © 2021 The Authors

Figure EV5. Histone transgene expression in *bam* mutant ovaries and RNA FISH results show *bgcn* gene is actively transcribed in the early-stage female germline, including both GSCs and CBs.

A Old (eGFP) and new (mCherry) H3 or H2A expression in *bam* mutant ovaries. Scale bars: 20 μm. Asterisk: niche.

B Spearman correlation coefficients are re-plotted from Figs EV2B and 3D for direct comparison of the overlap between old and new H3 in WT GSCs, WT CBs, and *bam* mutant GSC-like germ cells. Data collected over 12 separate experiments. Values are mean ± 95% CI. *P*-value: pairwise ANOVA test with Bonferroni correction. n.s.: not significant. See Dataset EV8 for individual data points.

C RNA FISH using probes targeting nascent *bgcn* RNA (cyan) reveals active transcription in both GSCs and CBs, and nuclei are marked by DAPI (gray). Asterisk: niche. Scale bars: 5 μm.

D RNA IF-FISH using probes targeting nascent *bgcn* RNA (cyan) reveals active transcription in both GSCs and CBs, and nuclei are marked by histone H3 (gray) driven by the *GreenEye-nanos-Gal4* promoter. Asterisk: niche. Arrowheads: actively transcribed nascent *bgcn*. Scale bars: 5 μm. The *bgcn* transcript was detected in both GSCs and CBs in 100% of the germaria ($n = 17$).

E Probes recognize genes (magenta) that change their epigenetic state in a 2:2 ratio with biased old:new H3-enriched regions. In this instance, both sister chromatids for maternal or paternal chromosome have an "agreement" on old (green at the maternal chromosomes indicated by the pink outline) versus new (red at the paternal chromosomes indicated by the blue outline) H3.

F Two examples showing probe signals condensed into 2 foci (gray) amid old (green) versus new (red) H3 distribution for *dad* gene (top) and *ss* gene (bottom) in *bam* mutant GSC-like cells at prometaphase marked by anti-H3S10p (blue). Yellow arrowheads: FISH probe signal. Scale bars: 1 μm.

© 2021 The Authors

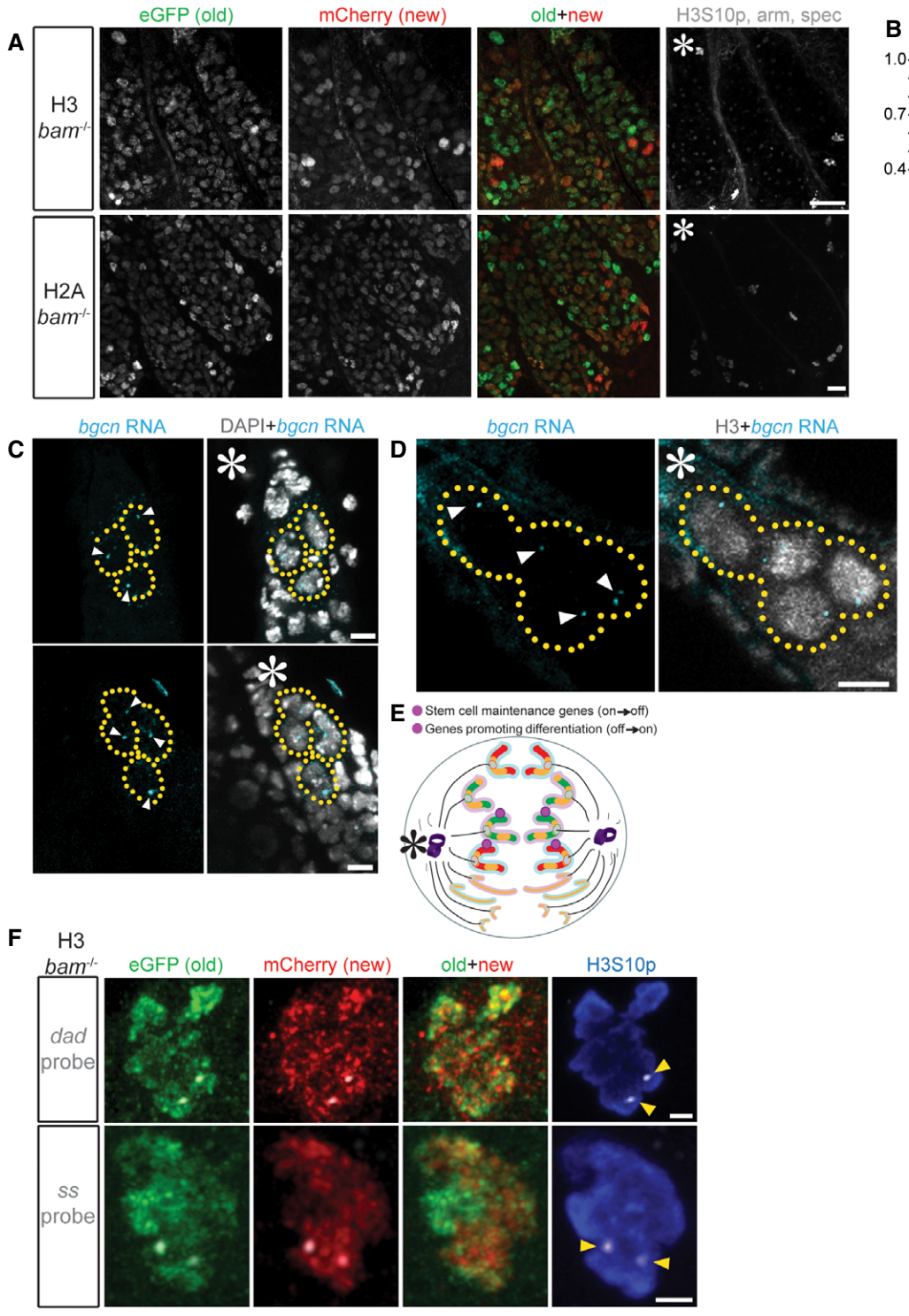

Figure EV5.

© 2021 The Authors

Figure EV6. Oligopaint IF-FISH reveals differential old versus new H3 inheritance at key genes for maintaining stem cell fate or promoting differentiation in WT female GSCs.

A, B, Top Old (red) versus new (green) histone H3Rev patterns with a single genomic locus labeled with fluorescent probes (cyan) for *dad* (A) and *bam* (B) in WT telophase female GSCs, marked by anti-H3S10ph (gray).

A, B, bottom Old (green) versus new (red) histone H3 inheritance patterns with single genes labeled with fluorescent probes (cyan) for *dad* (A) and *bam* (B) in WT telophase and metaphase female GSCs, marked by anti-H3S10ph (gray).

Data information: Zoomed-in images of each probe are displayed to the right of each figure panel and include the ratio probe association with old versus new histone, and the percentage of red versus green signal strength within each probe signal. Asterisk: niche. Arrowheads: probe signal. Scale bars: 1 μm. Zoomed-in scale bars: 0.1 μm. See Dataset EV4 telophase cells for individual data points.

© 2021 The Authors

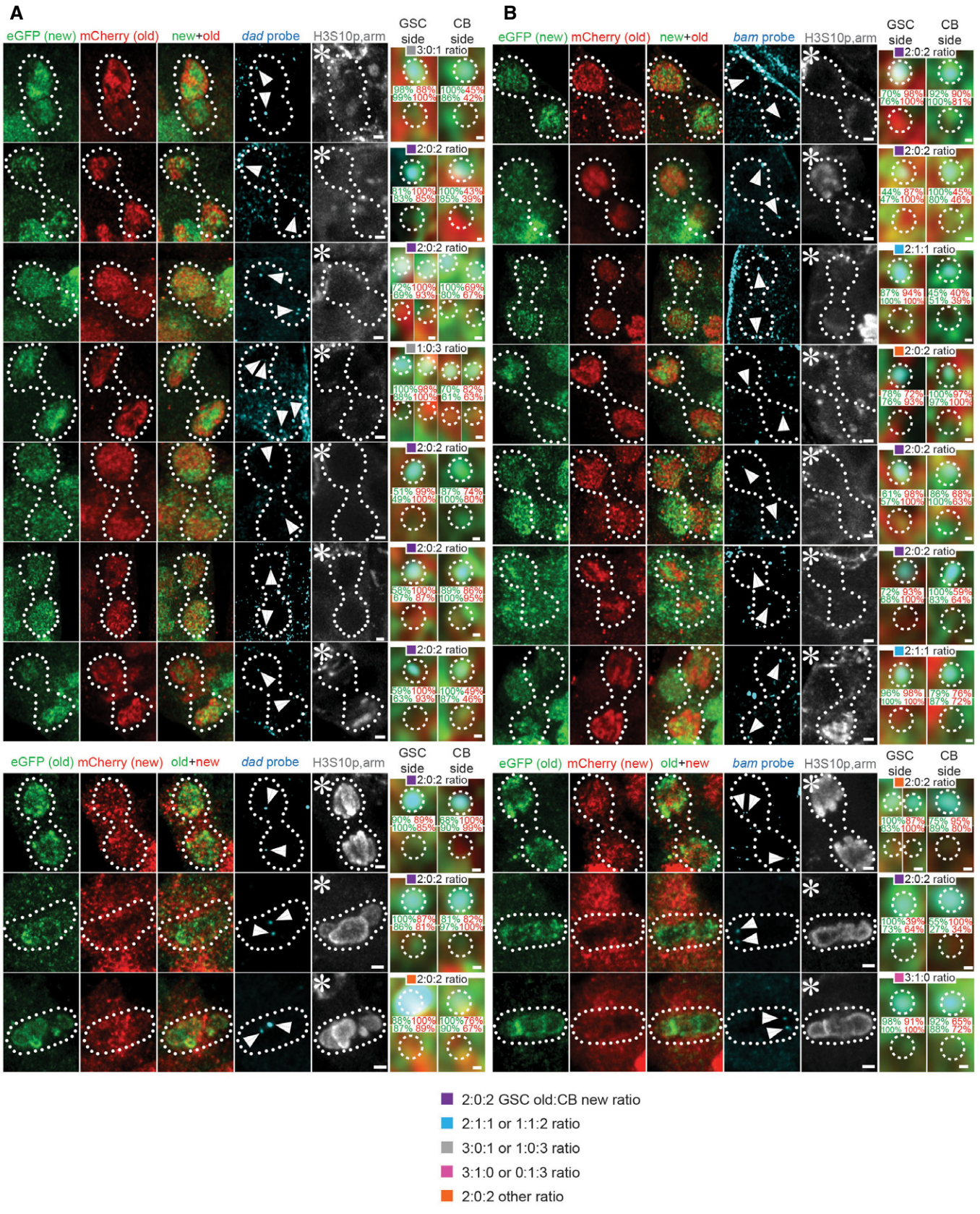

Figure EV6.

© 2021 The Authors