SUPPLEMENTAL MATERIAL

Supplemental Figure Legends

Figure S1. Diffusion of GFP through follicle cell ring canals contributes little to variegation patterns.

Clearly, diffusion through ring canals cannot generate variegation de novo if expression is equal in all cells. Hence the fundamental cause of variegation must be independent of ring canals. However, diffusion could in principle generate clusters of cells with equalized GFP levels that represent an average of the expression within the component cells. (A) Similar variegation patterns were generated using normal GFP, or modified GFP proteins thought to be non-diffusible: YPS-mRFP described in [24], membrane-tethered (membrane-GFP) and nuclear-localized (nls-mRFP). (B) Graphs below the micrographs show the calculated change probabilities from the last 4 divisions derived from the observed variegation patterns of each fluorescent protein variant. The profiles are very similar suggesting that diffusion between cells contributes little to the variegation patterns observed with normal GFP

Figure S2. Modeling the generation of variegated GFP patterns as independent color-picking events

(A) A schematic of modeling pattern generation as independent color-picking events. (B) Upper row: Simulation of variegation using normal change probabilities (shown in graph at left). Middle row: Simulation of variegation using constant high change probability. Bottom row: Simulation of variegation using rapidly stabilizing change probabilities.

Figure S3. Lsd1 acts in follicle cells to maintain plasticity

Knocking down *lsd1* expression in follicle cells but not germ cells using the *R10H05*-Gal4 driver and either of two *lsd1*-specific RNA constructs suppressed GAL4::UAS-GFP variegation. (N = 20 (Ctrl), 30 *(R10H05::lsd1$^{RNAI\#1}$)* and 21 *(R10H05::lsd1^{RNAI21})).* Scale bar: 20 μm. Error bars: standard errors. (**: p<0.01).

Figure S4. *lsd1$^{\Delta N}$*/+ does not alter average levels of GFP expression.

To determine if the *lsd1$^{\Delta N}$*/+ might suppress variegation by changing the average level of GFP expression, we measured the level of expression in variegating cell patches from control and *lsd1$^{\Delta N}$*/+ follicles expressing GAL4::GFP. The average intensity within individual patches from individual follicles was determined for large patches (A), or for patches of 4 cells or less (B) and plotted (circles). Control (Ctrl, black); *lsd1$^{\Delta N}$*/+ (green). Averages are represented as open bars. Significance: standard errors were used; NS = not significant.

Figure S5. Specificity of anti-Lsd1 immunostaining.

Immunostaining of follicle cell nuclei was observed in wild type ovarioles using mouse anti-Lsd1 antibody. No staining was apparent in *lsd1$^{\Delta N/Df(3L)ED4858}$* follicles. Scale bar: 50 μm.

Figure S6. Knocking-down dSet1 does not restore GFP variegation patterns in *lsd1$^{\Delta N}$*/+ follicles.

Unlike *Trx*, knocking-down dSET1 expression using two different dSET1 RNAi lines did not rescue lsd1-mediated suppression on variegation patterns. (N= 15 and 16 ovarioles respectively). (*: p<0.05; **:p<0.01; Student's t-test.)

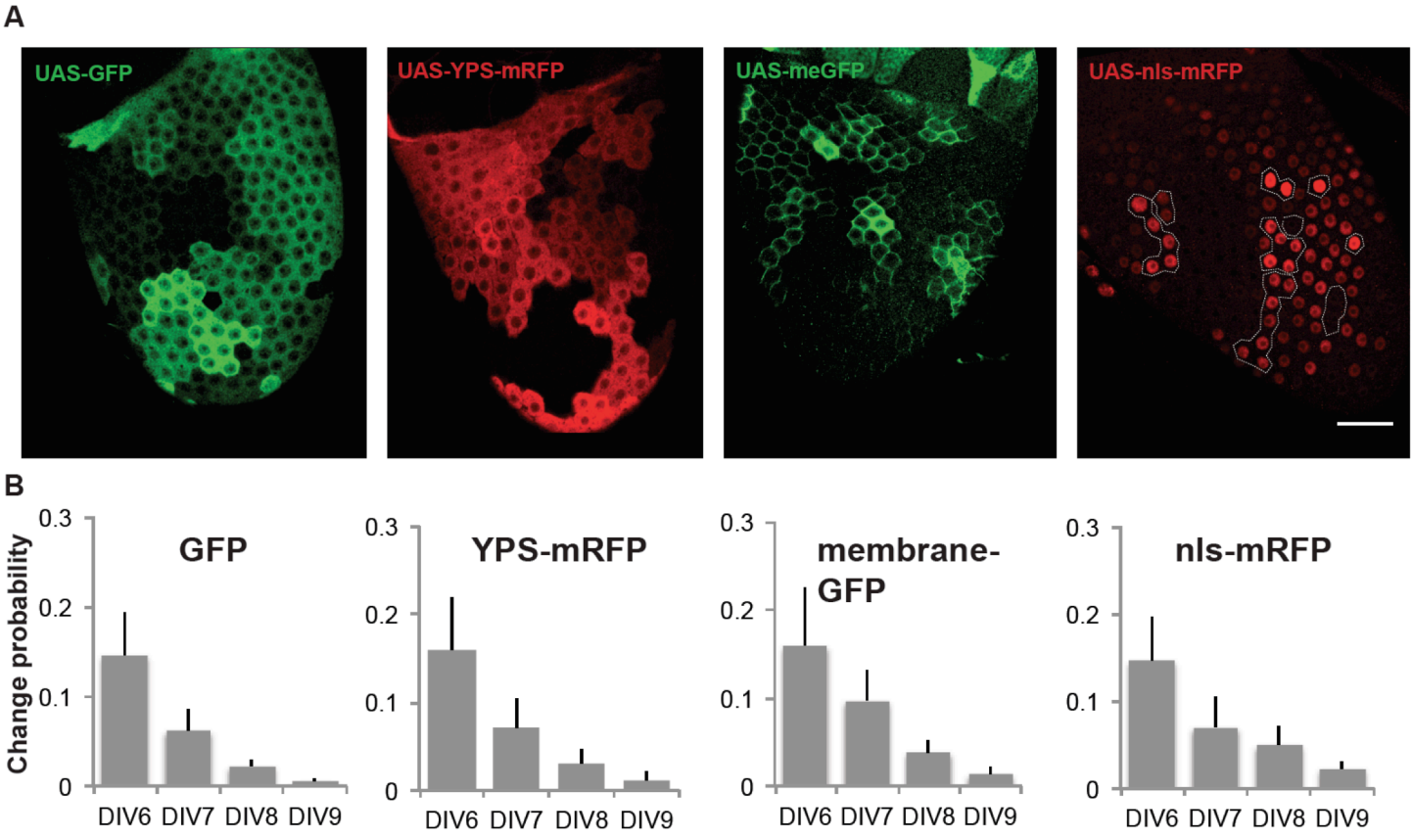
Figure S7. Reduced mitotic proliferation in *lsd1*$^{\Delta N}$/+ follicles.

Follicle cell proliferation was measured by determining the number of phosphohistone H3 positive nuclei per ovariole by immunostaining. In wild type ovarioles an average of 13 nuclei were observed, but this number was reduced in *lsd1*$^{\Delta N/+}$ follicles. (N= 21(Ctrl) and 25 (*lsd1*$^{\Delta N}$/+) ovarioles). (*: $p<0.05$; Student's t-test.)

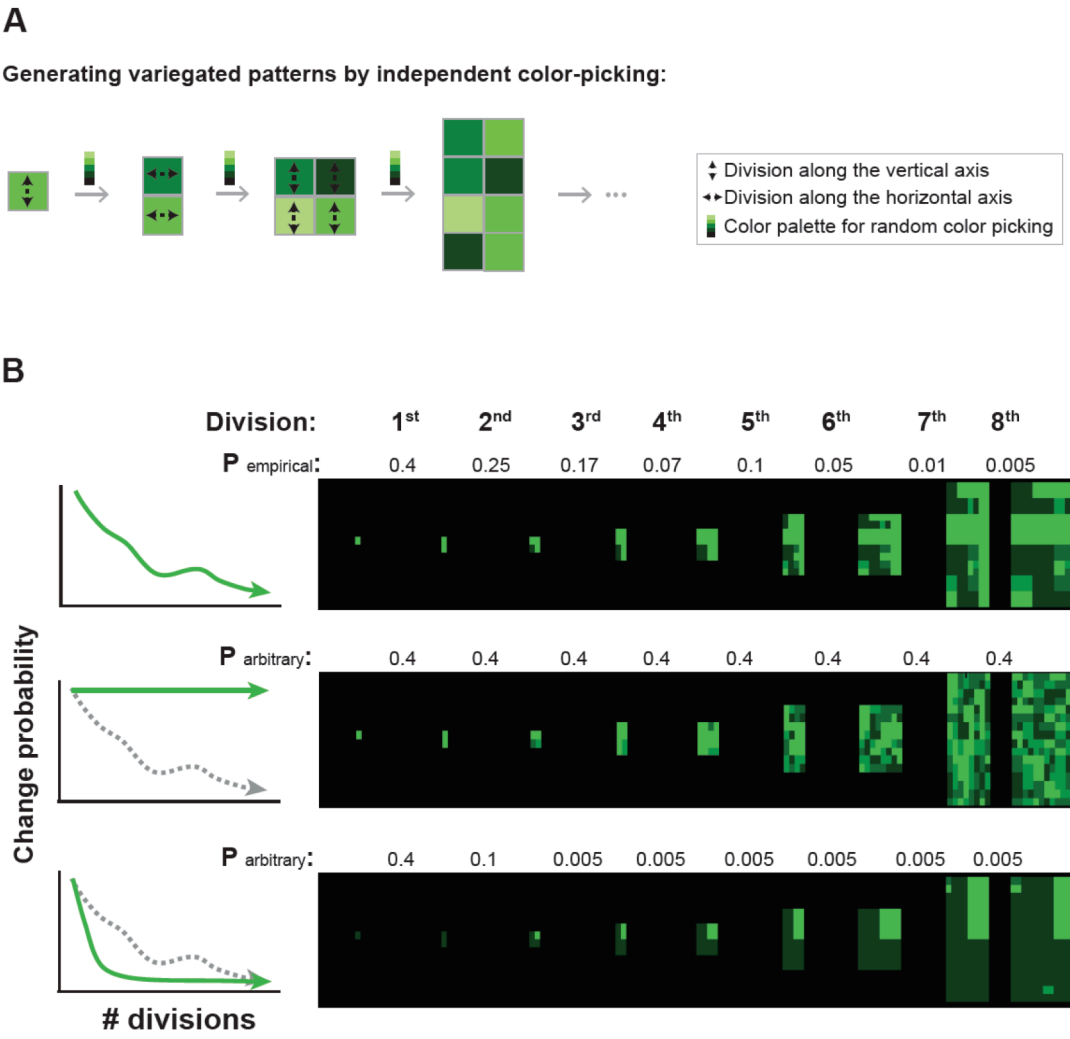
Figure S8. Premature N^{ICD} expression driven by the R10H05-Gal4 driver

Driving Notch intracellular domain in follicle cells using the *R10H05*-Gal4 driver (*R10H05::N*^{ICD}) led to premature N^{ICD} expression visualized by antibody staining. Scale bar: 50 μm.

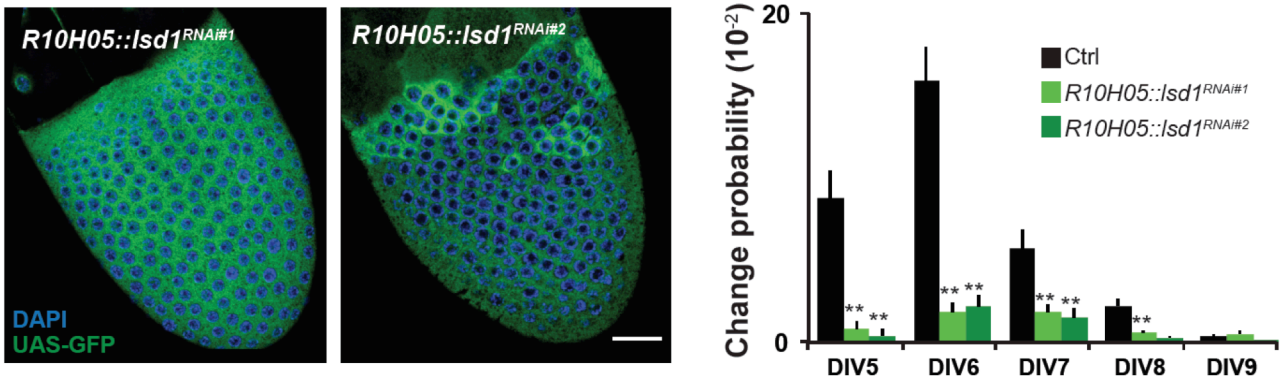
Supplemental Figure 1



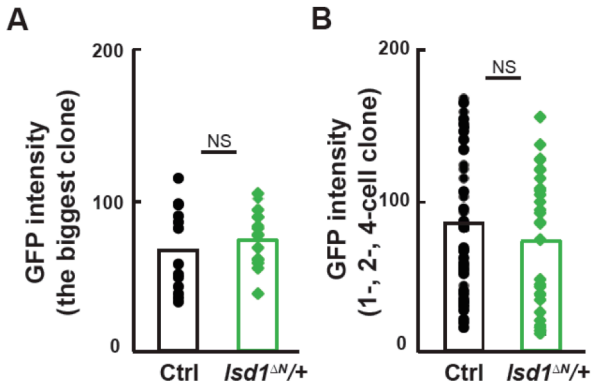
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



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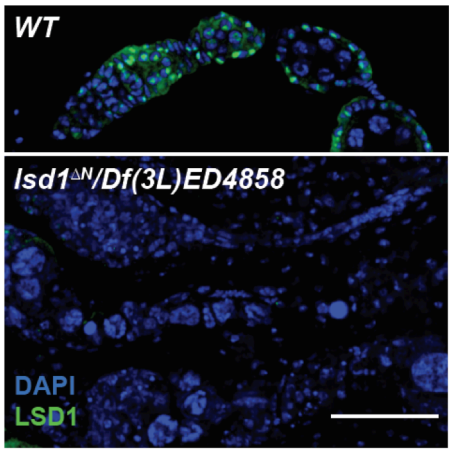

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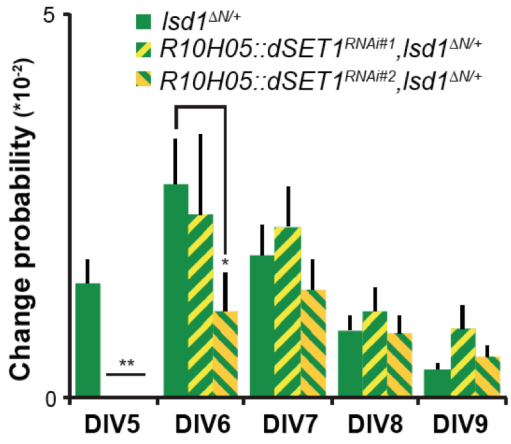
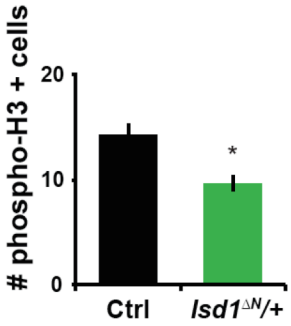


Supplemental Figure 7



Supplemental Figure 8