Supplementary Data

Supplemental Figures

Fig. S1. Up-regulation of Notch pathway components in *spen* clones. Confocal micrograph of *spen* eye discs clones stained (in red) for (A,B) Scabrous, (C,D) extracellular Notch ($Notch^{EC}$, 1:200 C458.2H, DSHB) and (E,F) E(spl)-bHLH (mAb323). s*pen* mutant tissue lacks GFP (green). Sca expression is broadened in *spen* clones (A,B, brackets) as compared to its restricted expression in IG cells. Notch expression is elevated in clones (C,D, arrows). E(spl)-bHLH expression is broadened in *spen* clones (E,F, brackets) and elevated posterior to the MF. E(spl)-bHLH is normally restricted to alternating groups of cells (E, arrowheads). Discs are oriented anterior to the left. Scale bars = 20 μm.

Fig. S2. *E(spl)-bHLH* transcripts are elevated in *spen* mutant discs. qRT-PCR was performed to measure relative (A) *E(spl)-m3* and (B) *E(spl)-mβ* mRNA levels in wild-type and *spen*/cell lethal eye discs (three samples, mean + st. dev.). Elevated levels are significant (*), $p = 0.0026$ and 0.018, respectively.

Fig. S3. s*pen* loss affects EGFR signaling pathway components. (A,B) Confocal micrographs of third instar eye imaginal discs oriented with anterior to the left in *spen* clones stained (in red) for (A,B) dpERK or (C,D) Yan. s*pen* mutant tissue lacks GFP (green). dpERK is lost at the MF in *spen* clones (A,B, asterisks *) but appears unchanged posterior to the MF. Yan is up-regulated in *spen* clones both in and posterior to the MF (B,D, arrows). Scale bars = 20 μm.

Fig. S4. Yan is up-regulated in *spen* wing clones that express ectopic Yan from a cDNA transgene. (A,B) confocal migraphs of third instar wing imaginal discs that co-expression NLS-tagged GFP with (A,B) Yan^{WT} or (B) Yan^{ACT} under the control of the *dpp*-GAL4 driver. *dpp*>Yan^{WT} (A,B, arrows) is expressed in a subset of cells where *dpp*>GFP^{NLS} is driven (A,B, arrowheads). However, *dpp*>Yan^{ACT} co-localizes well (C,D, arrows) with *dpp*>GFP^{NLS}. (E,F) *dpp*>Yan^{WT} is expressed in a *spen* clones background. *spen* clones lack GFP (green). More red Yan-positive cells are seen in *spen* mutant tissue (E,F, arrowheads) than in control tissue (E,F, arrow). Discs are oriented dorsal up. Scale bars = 20 μm.

Fig. S5. Loss of organization and cell types in *spen* mutant pupal tissue. Confocal micrographs of eye imaginal discs 42 hr APF (after puparium formation) in wild-type (A,C,E) and *spen* clones (B,D,F). Discs were stained for (A,B) DE-cadherin (DE-cad, 1:50 DCAD2, DSHB), (C,D) Elav, and (E,F) Cut. *spen* mutant tissue is marked by lack of GFP (green). C,E and D,F correspond to the same sample, respectively. DE-cad (A,B) is a cell boundary marker that especially marks cone, pigment, and bristle cells. *spen* mutant tissue (B) shows loss of these cell types and loss of the regular ommatidial organization found in wild-type (A). In *spen* clones, there are fewer Elav-positive R cells (D) and Cut-positive cone cells (F). Scale bars = 10 μm.

Fig. S1

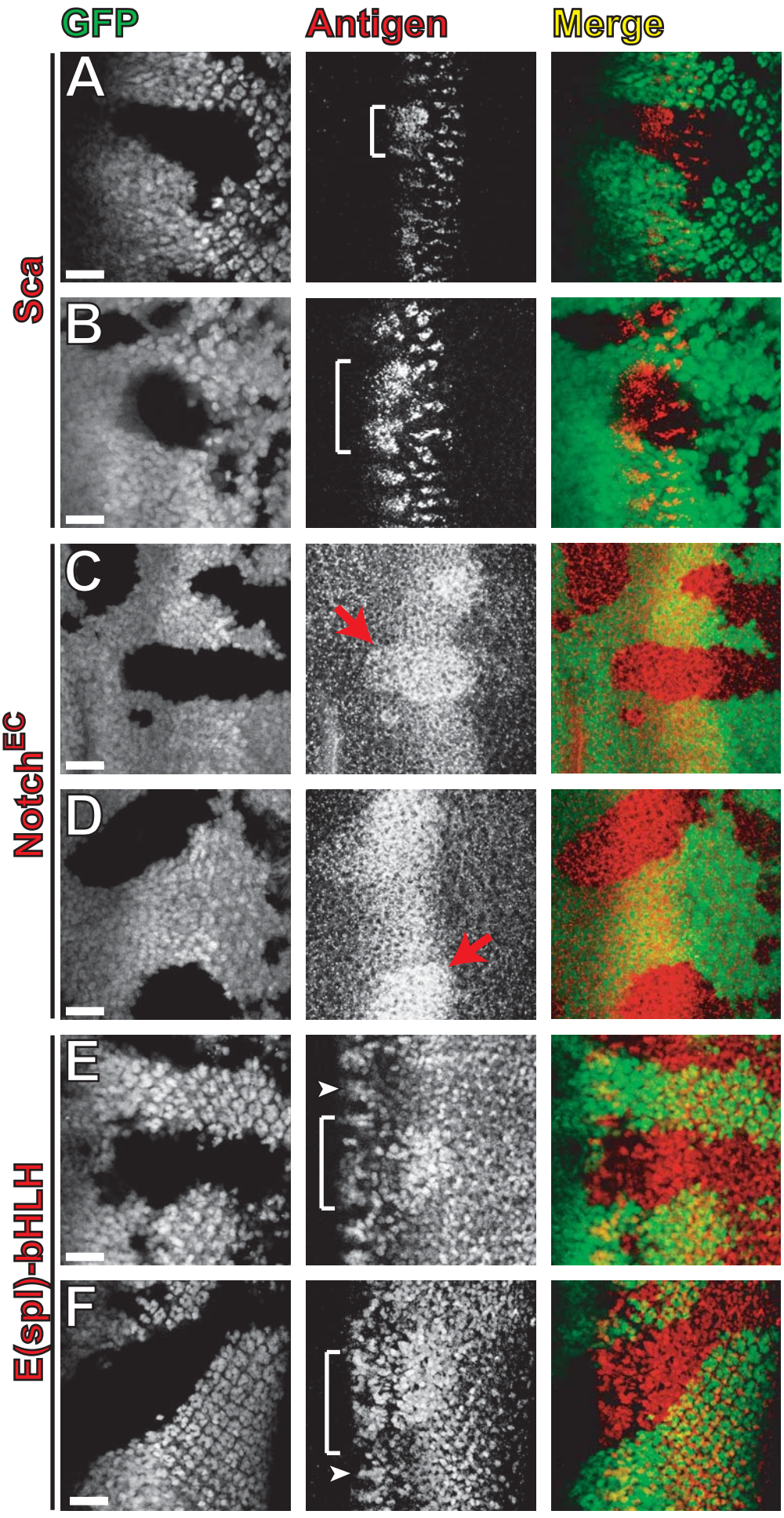


Fig. S2

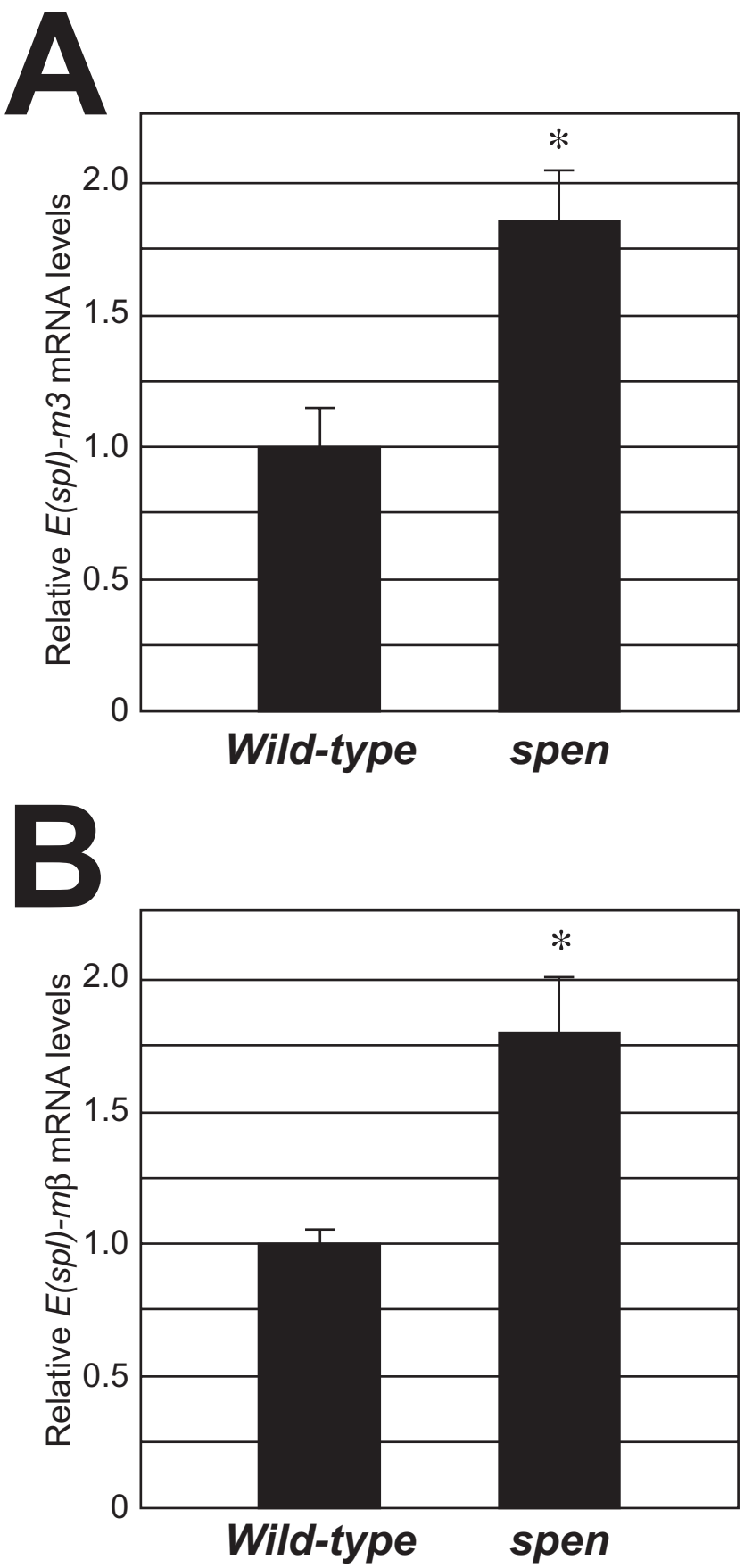

A
Relative E(spl)-m3 mRNA levels
2.0
1.5
1.0
0.5
0
*
Wild-type
spen
B
Relative E(spl)-mβ mRNA levels
2.0
1.5
1.0
0.5
0
*
Wild-type
spen

Fig. S3

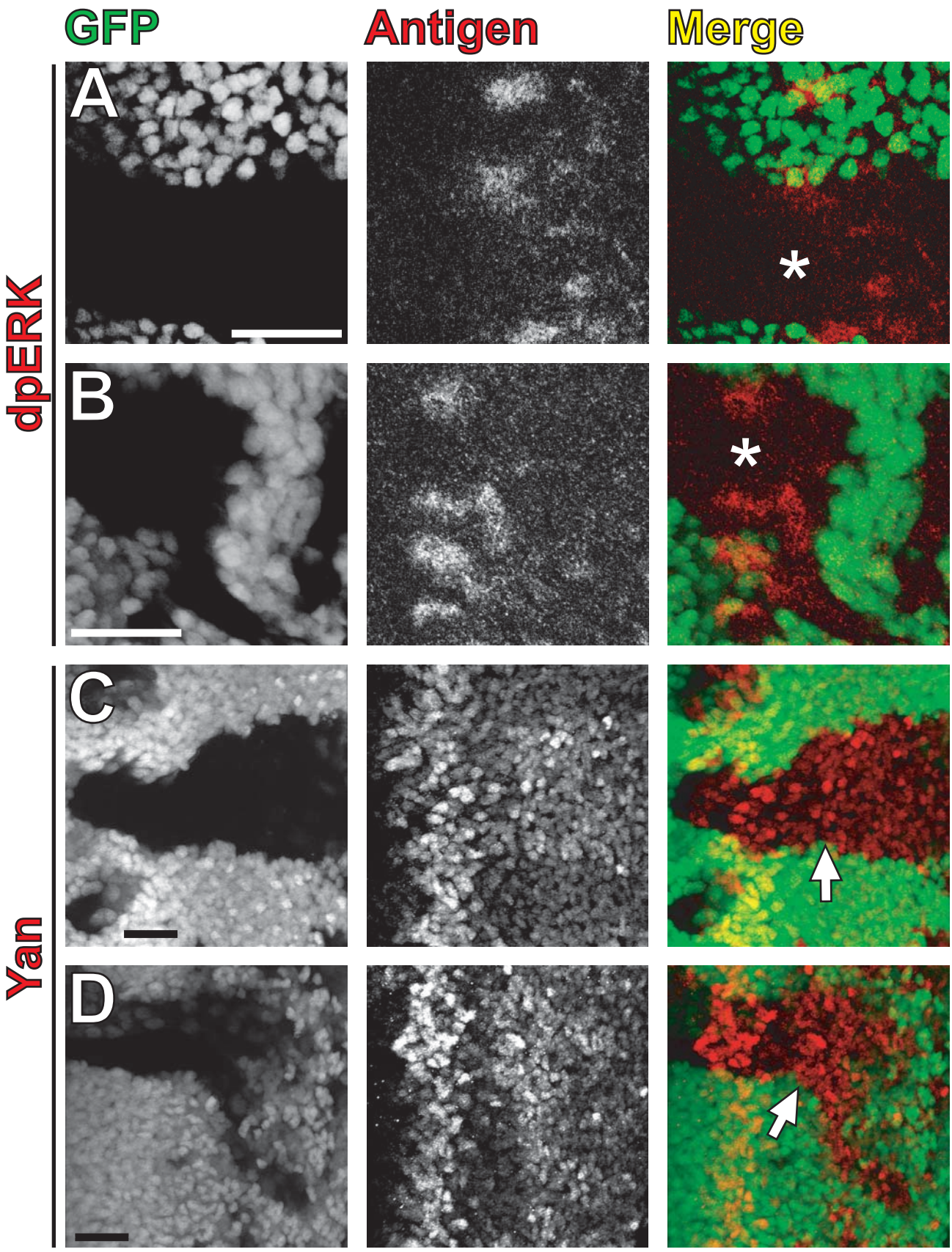


Fig. S4

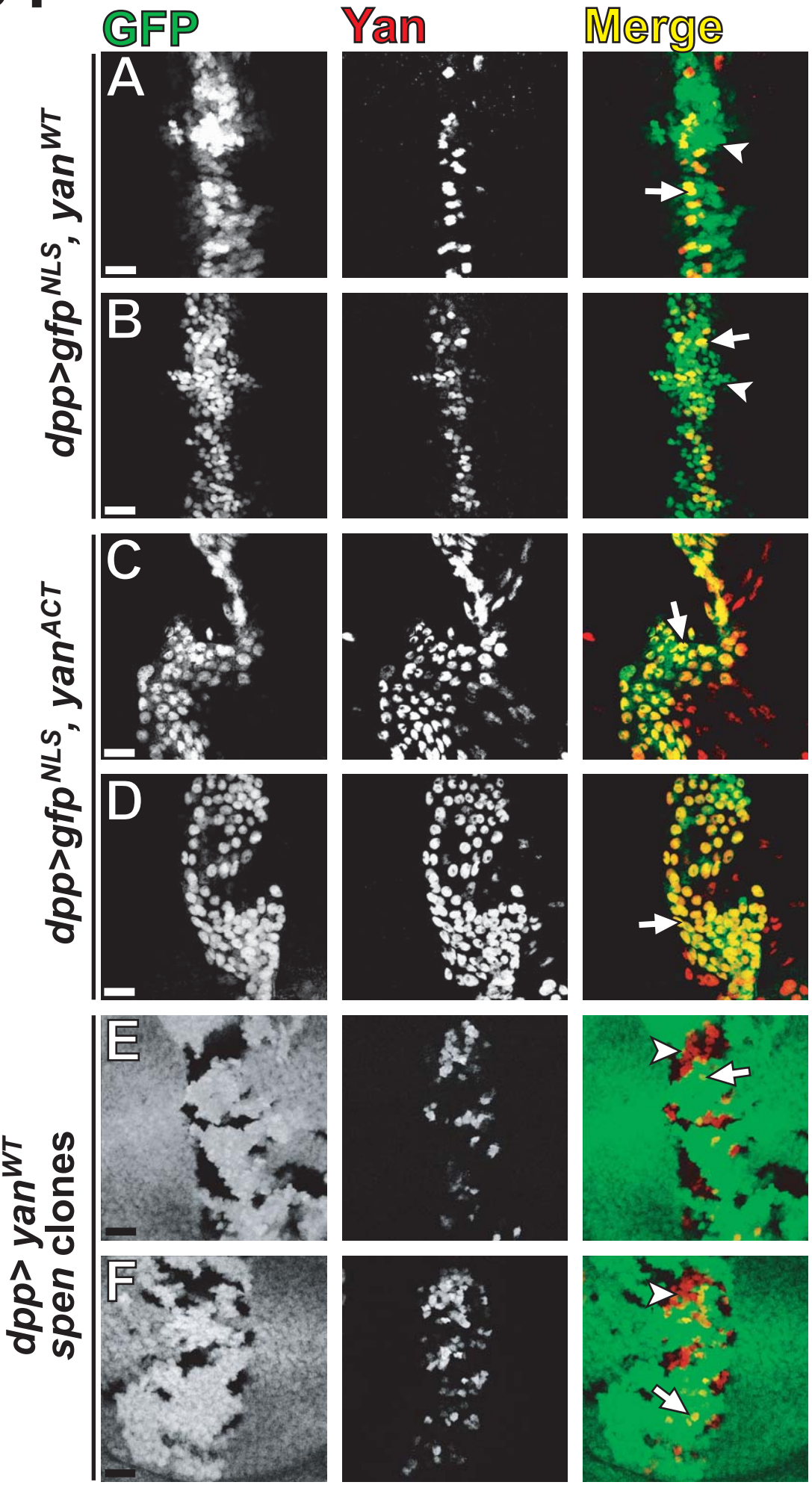
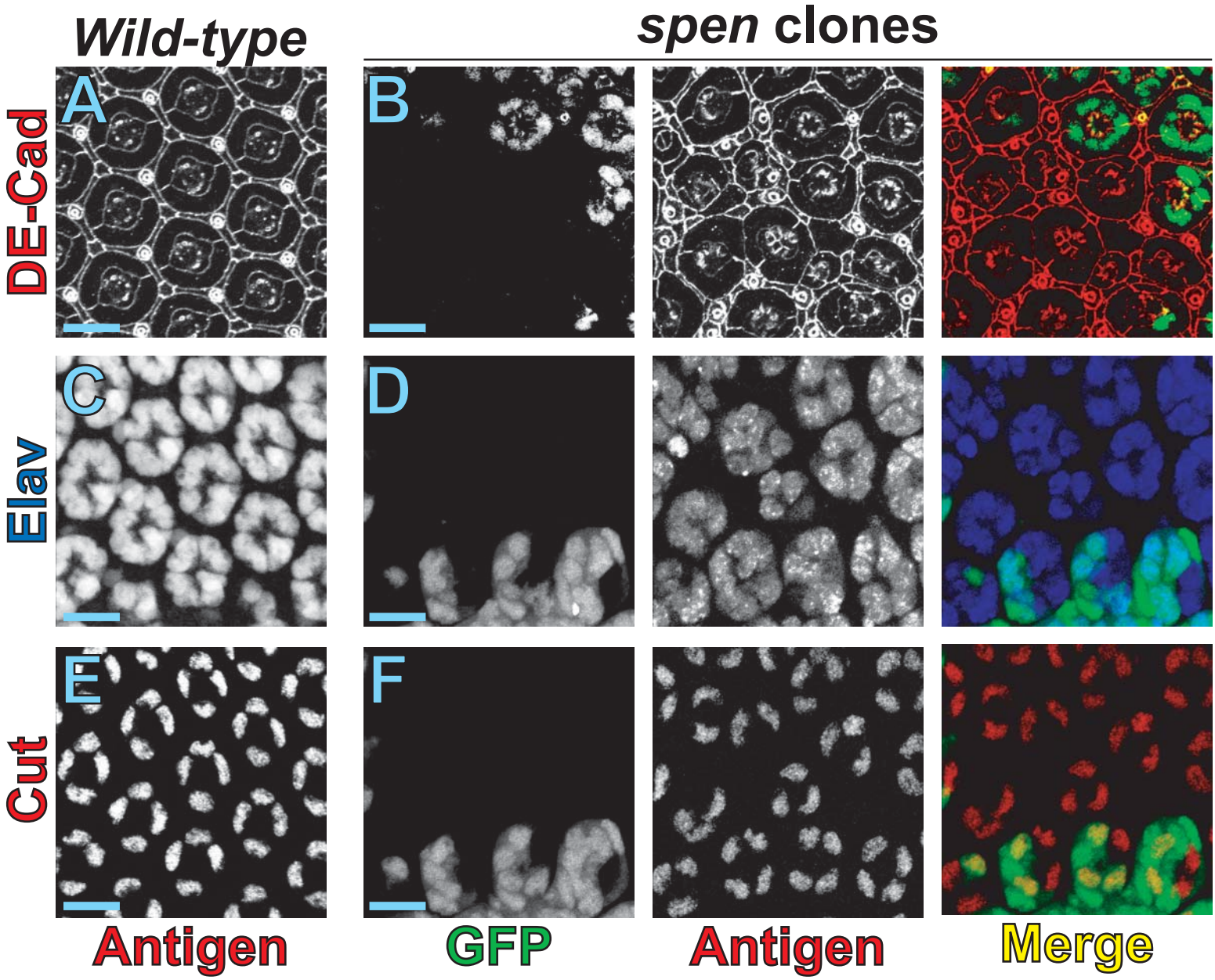

GFP
Yan
Merge
A
B
C
D
E
F
dpp>gfp^NLS, yan^WT
dpp>gfp^NLS, yan^ACT
dpp> yan^WT
spen clones

Fig. S5


Wild-type
spen clones
DE-Cad
Elav
Cut
A
B
C
D
E
F
Antigen
GFP
Antigen
Merge