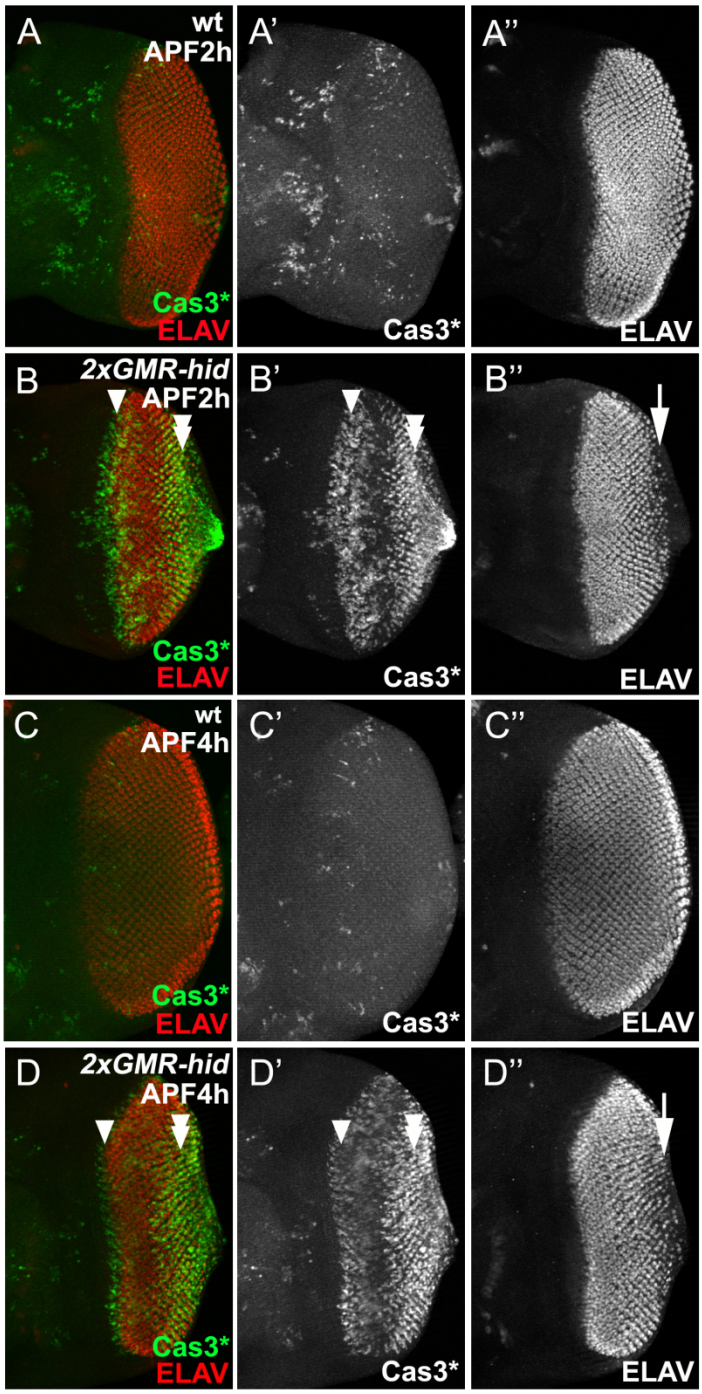

Figure S1 (related to Figure 1). *GMR-hid*-induced cell death in pupal eye discs.

(A, B) Early pupal eye discs (2 hours after puparium formation, APF2h) labeled with Cas3* and ELAV. Compared to wild type **(A)** and larval *GMR-hid* eye discs (Figure 1), the second apoptotic wave gets wider in *GMR-hid* pupal discs **(B).** Accordingly, the density of photoreceptor neurons, especially at the posterior end, is reduced (arrow, B'').

(C, D) APF4h pupal eye discs labeled with Cas3* and ELAV. Compared to wild type **(A)**, the second apoptotic wave covers half of the *GMR-hid* eye disc **(D).** Density of photoreceptor neurons is further declining and the disc is now clearly reduced in size.

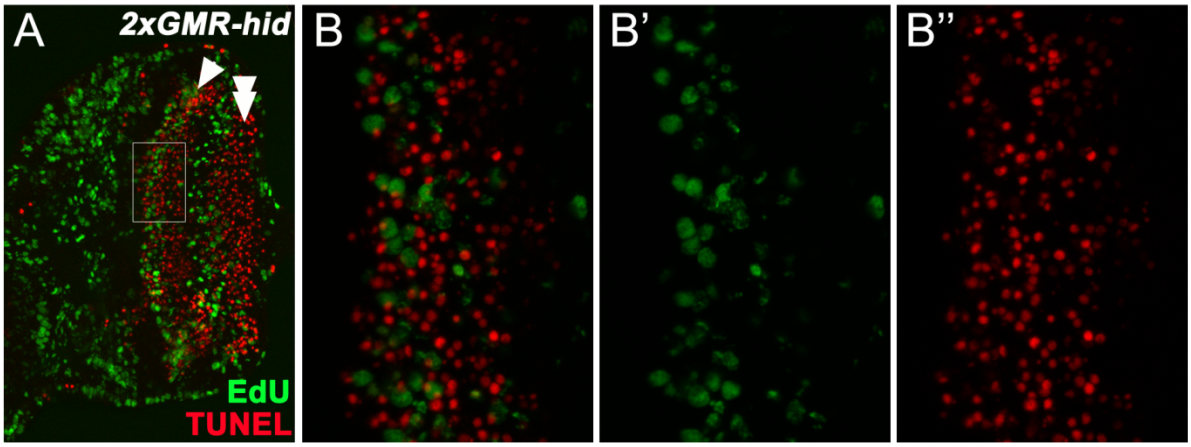

Figure S2 (related to Figure 2). Proliferation and apoptosis markers are spatially very close in the first apoptotic wave in *GMR-hid* eye discs.

(A) EdU (green) and TUNEL (red) double labeling of *2xGMR-hid* eye discs. Arrowhead and double arrowheads highlight the two apoptotic waves in *GMR-hid*. The wave of compensatory proliferation is visible in (A) between the two TUNEL-positive waves.

(B,B',B'') An image stack of 3µm shows an increased magnification view of the white box in (A). These markers do not overlap because apoptotic cells do not enter S phase.

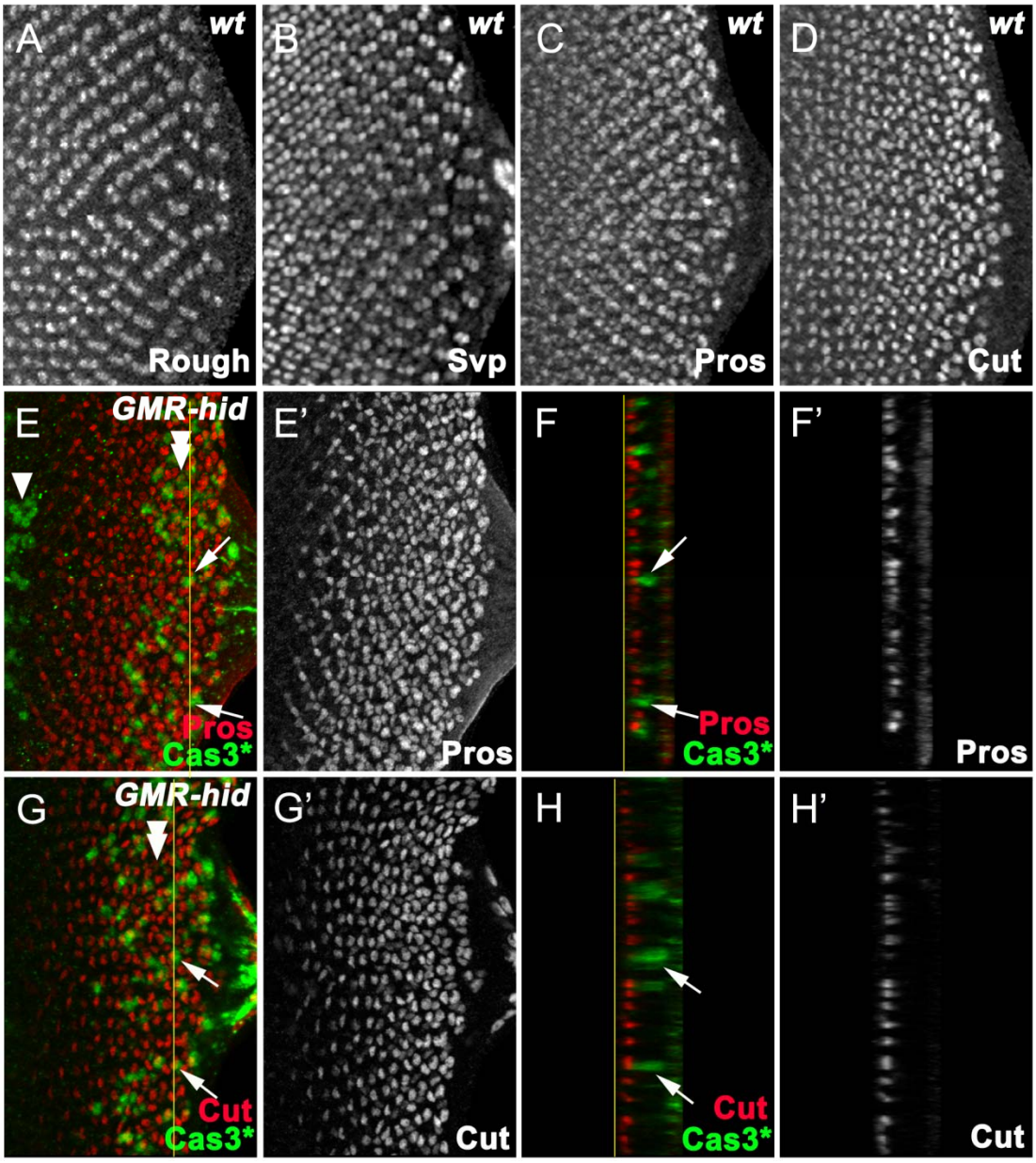

Figure S3 (related to Figure 3). R7 and cone cells do not die in the second apoptotic wave.

(A-D) Wild type late 3rd instar eye discs labeled with Rough **(A)**, Svp **(B)**, Prospero (Pros; labels R7 and its precursor cells) **(C)** or Cut (labels cone cells and theirs precursors) (**D**).

(E, F) Late 3rd instar *GMR-hid* eye discs labeled with Cas3* and Pros. **(F)** shows a cross section of **(E)**. The yellow line indicates location of the cross section in (E) and marks its apical surface in (F). Pros-positive cells are not labeled with Cas3* (arrows) and they do not die in the second apoptotic wave.

(G, H) Late 3rd instar *GMR-hid* eye discs labeled with Cas3* and Cut. **(H)** shows a cross section of **(G)**. The yellow line indicates location of the cross section in (G) and its apical surface in (H). Cut-positive cells are not labeled with Cas3* (arrows) and they do not die in the second apoptotic wave.

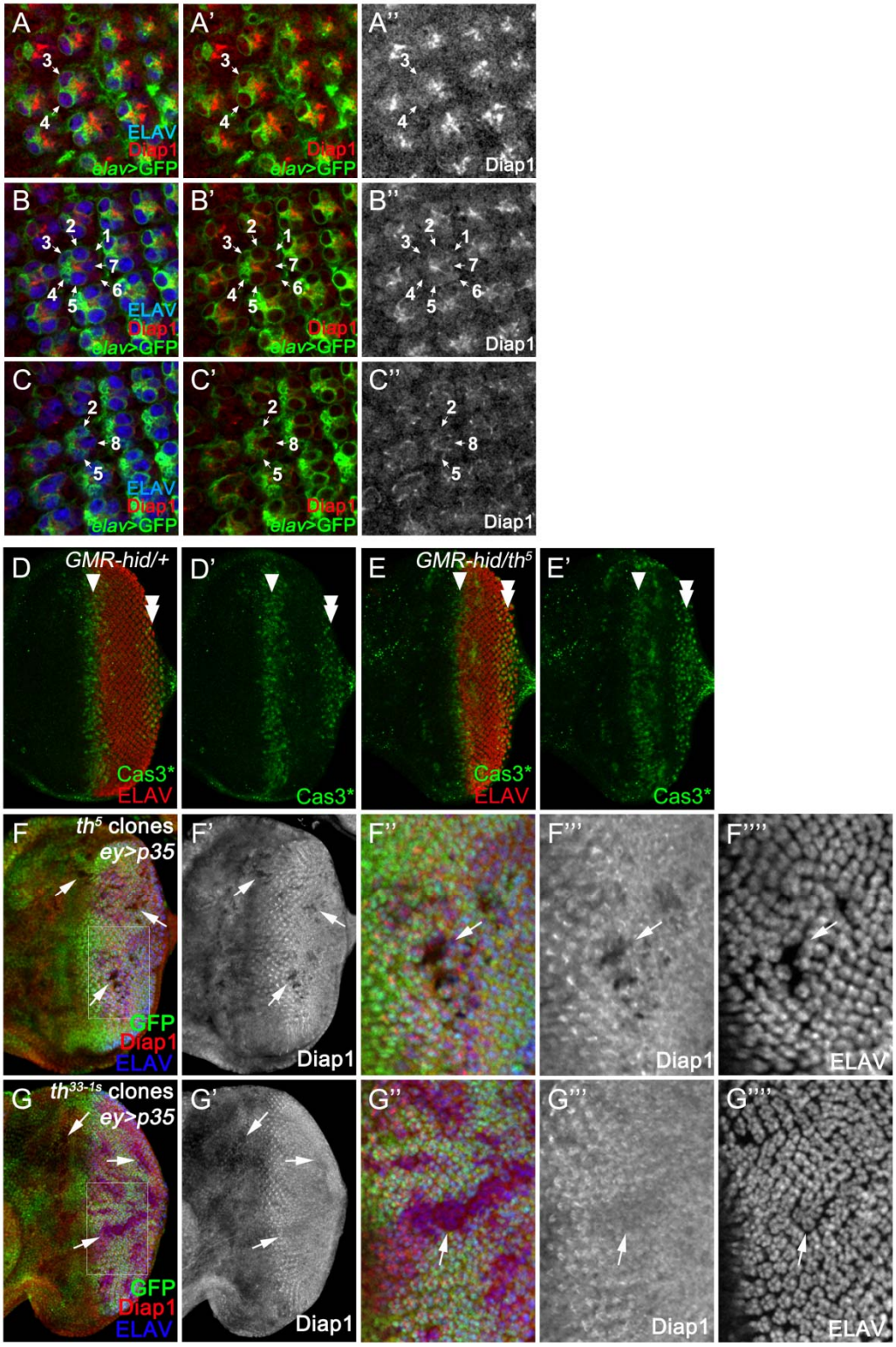

Figure S4. Histochemical and mutant analysis of Diap1 in the developing larval retina (related to Figure 5).

(**A-C’’**) **Diap1 accumulates in all photoreceptor neurons**.
Shown are different high-resolution confocal sections of the same wild-type eye imaginal discs expressing *elav-Gal4*-driven membrane-bound GFP (*elav-Gal4 UAS-CD8GFP*). Photoreceptor neurons are indicated by numbers.

(D-E). Loss one copy of *diap1* enhances *GMR-hid*-induced apoptosis.
Late 3rd instar eye discs labeled by Cas3* and ELAV. th^{5} is a null allele of *diap1*. Compared to *GMR-hid* alone **(D,D’)**, both first and second waves of apoptosis are broader in *GMR-hid/+;*th^{5} */+* eye discs **(E,E’)**. Accordingly, the apoptosis free zone is reduced in (E,E’).

(F-G) Verification of Diap1 antibodies.
Late 3rd instar eye discs labeled by Diap1 and ELAV. *diap1* null (th^{5}) **(F)** or hypomorphic ($th^{33\text{-}1s}$) **(G)** mutant clones are negatively labeled for GFP. $th^{33\text{-}1s}$ lacks the RING domain of Diap1, but leaves the BIR domains intact. *p35* was expressed in the eye (*ey>p35*) to block apoptosis in *diap1* mutant clones. (F’’, G’’) show enlarged views of the outlined regions in (F, G), respectively. Photoreceptor neurons do not form (ELAV-negative) in th^{5} clones. Diap1 labeling is missing in th^{5} clones (arrows in F-F’’’). In $th^{33\text{-}1s}$ clones, photoreceptor neurons do form. Although the RING domain is deleted in $th^{33\text{-}1s}$, $Diap1^{33\text{-}1s}$ protein levels are not increased, but rather reduced compared to neighboring wild-type tissue (arrows in G-G’’’).

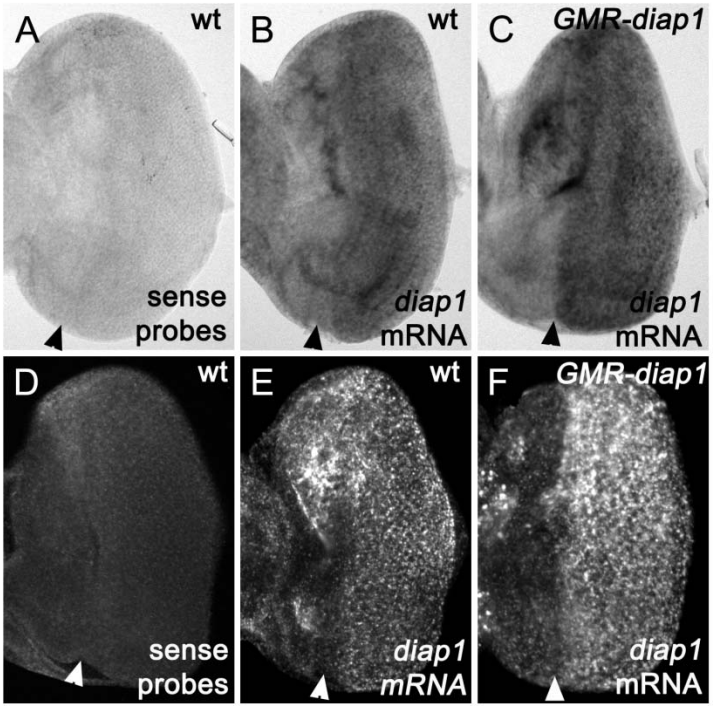

Figure S5. *diap1* transcription is not increased in photoreceptor neurons (related to Figure 6).

In situ hybridization of late 3rd instar eye discs with DIG-labeled probes detected with either NBT/BCIP **(A-C)** or Tyramide Signal Amplification **(D-F)**. Compared to the control discs using sense probes (A, D), *diap1* is ubiquitously expressed in wild type eye discs (B, E). The *diap1* probe worked as it detects high levels of *diap1* in *GMR-diap1* discs posterior to the MF (C, F). Levels of *diap1* transcripts are not specifically increased in photoreceptor neurons.

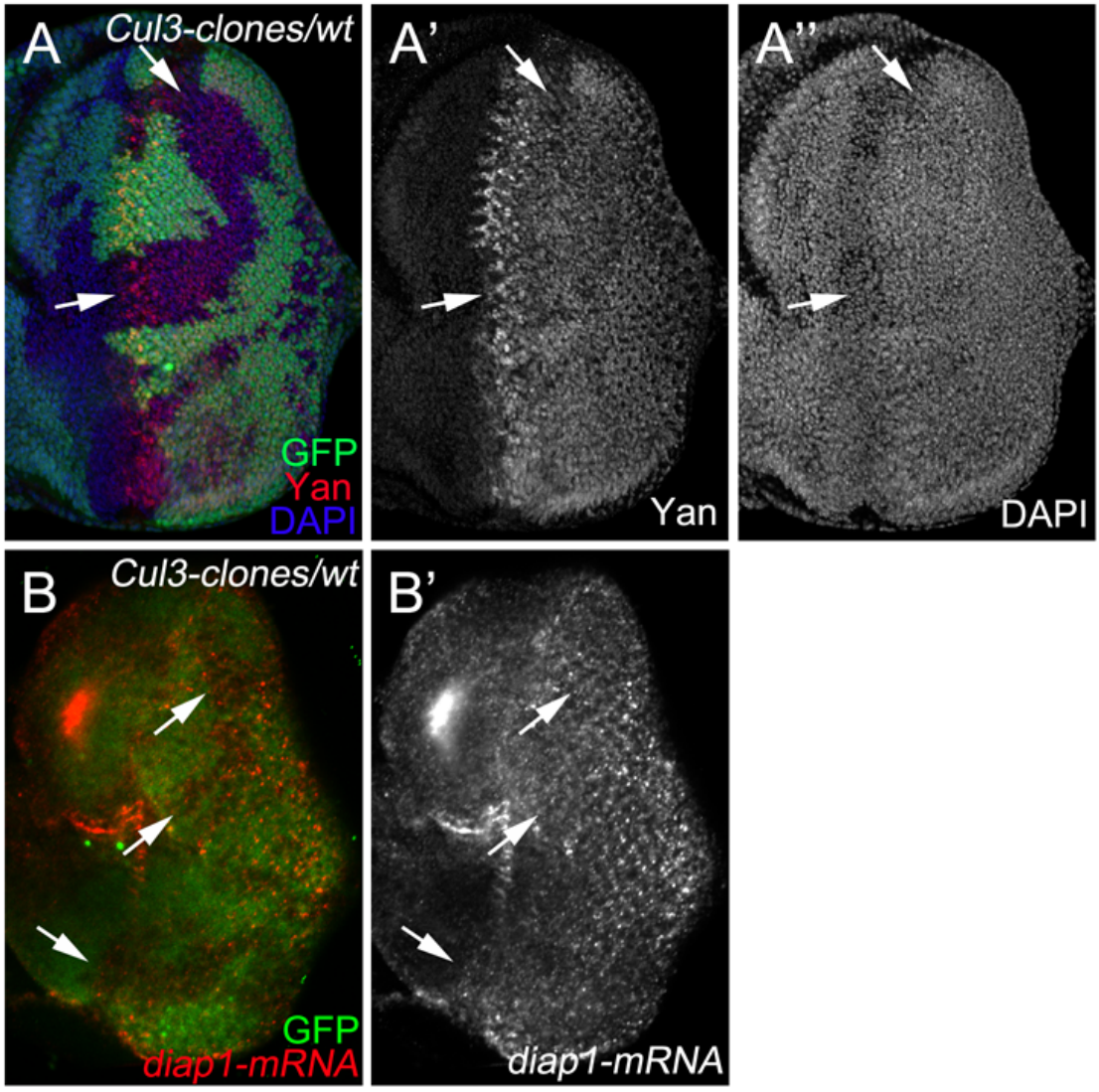

Figure S6. Cell number does not increase in *Cul3* mutant clones (related to Figure 7).

(**A,B**) Late 3rd instar eye disc labeled by (**A**) Yan (marker of unspecified cells) and (B) *diap1* in situ probe. DAPI as nuclear marker was added in (A). *Cul3* clones (arrows) are labeled by the lack of GFP. Numbers of unspecified cells (A') and total cells (A'') do not increase in *Cul3* clones. *diap1* mRNA is not increased in *Cul3* clones (B, B').