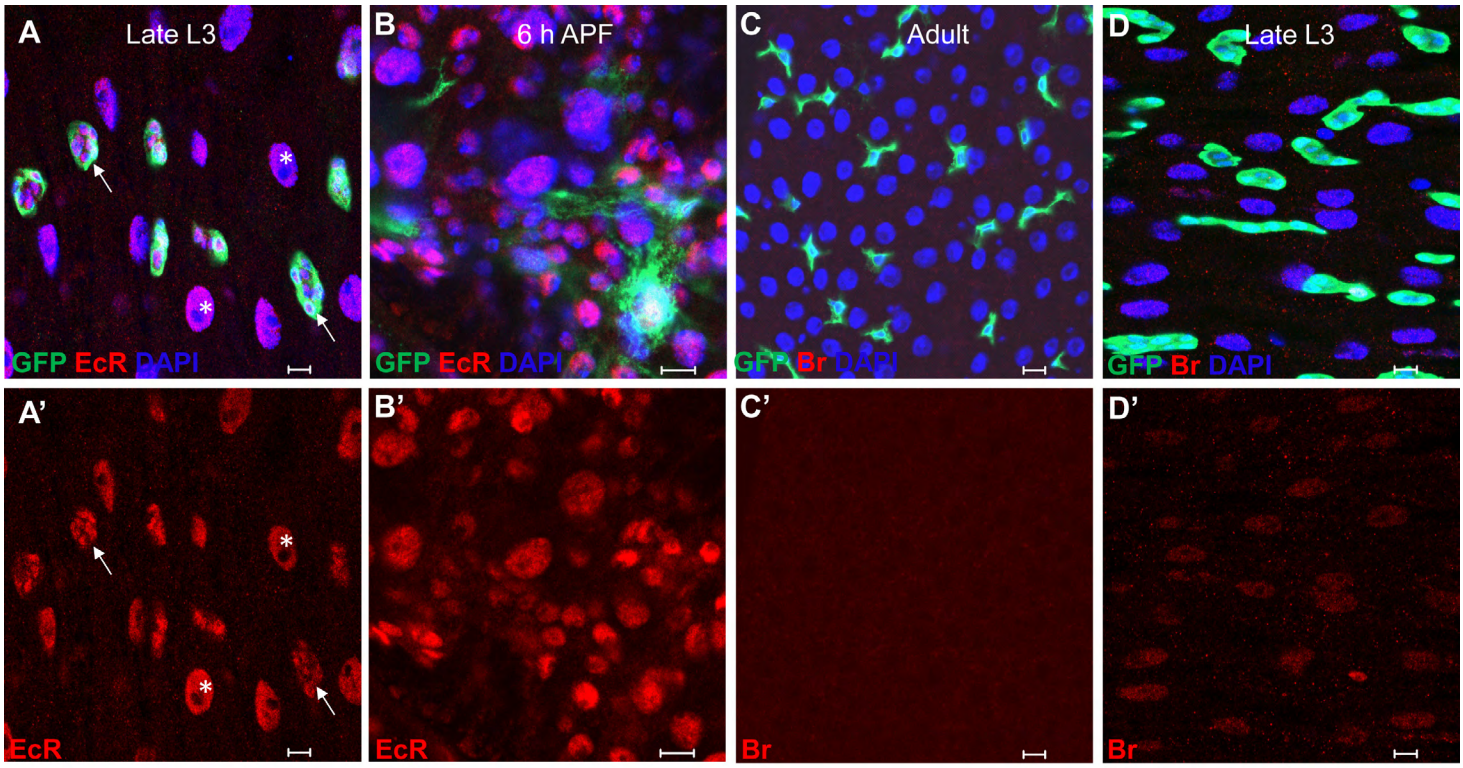

Fig. S1. EcR is expressed in all cell types in the midgut. (**A-B′**) *Su(H)GBE-Gal4, UAS-mCD8-GFP* flies were stained at late L3 and 6 hours APF using the EcR (common) antibody. EcR was expressed evenly in all cell types, including AMPs (arrow) and ECs (star). GFP, green; EcR (common), nuclear red; DAPI, nuclear blue. (**C**,**C′**) Adult *Su(H)GBE-Gal4, UAS-mCD8-GFP* flies were stained at 7 days after eclosion using the Br core antibody. The expression of Br was undetectable in the adult midgut. (**D**,**D′**) Br was efficiently depleted in GFP-labeled cells, in which *UAS-br*^{IR} was driven by *esg*^{ts}. GFP, green; Br core, nuclear red; DAPI, nuclear blue. Scale bars: 10 μm.

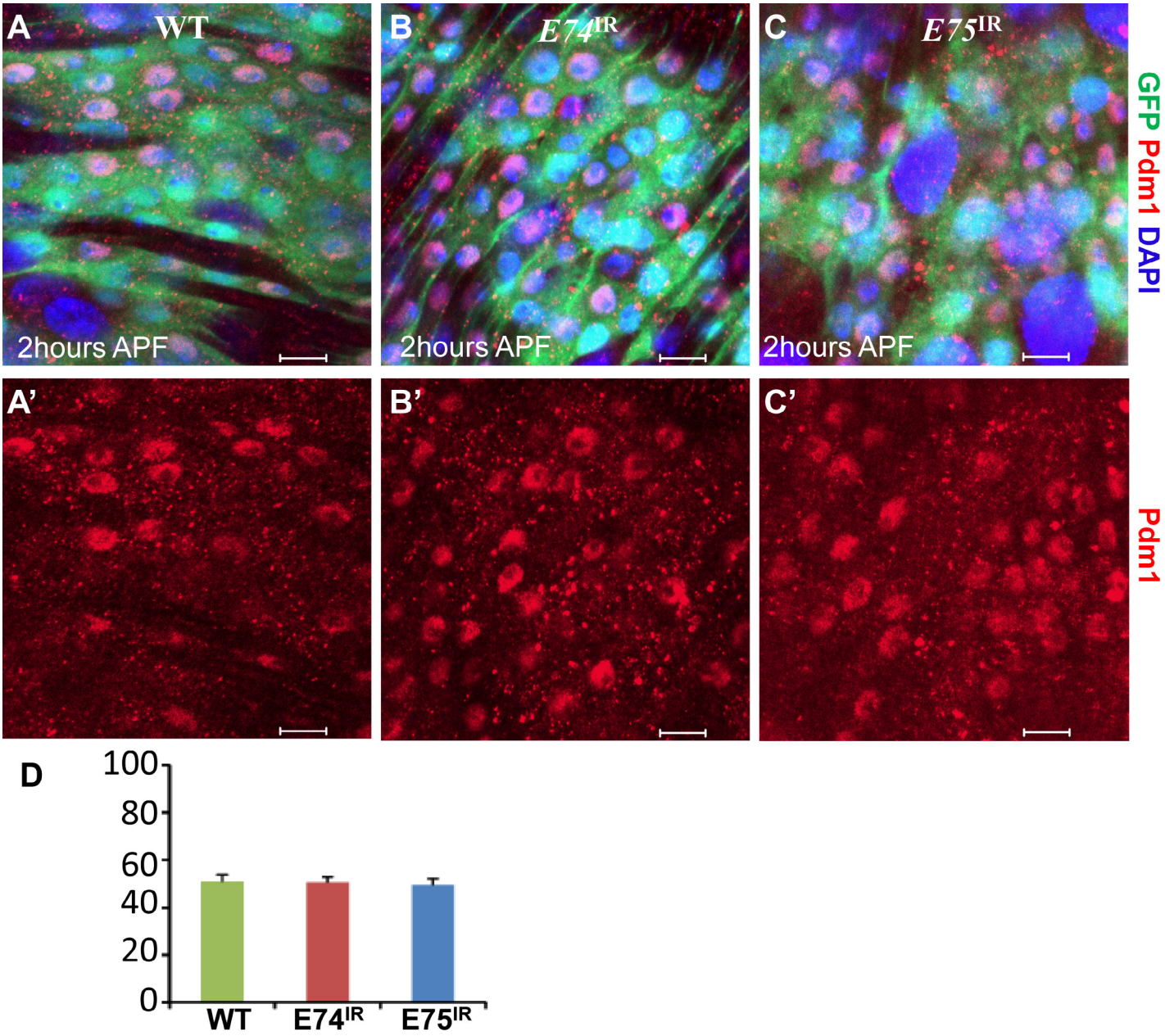

Fig. S2. Depletion of *E74* or *E75* does not block AMP differentiation. (**A-C′**) Wild-type (A,A′), *UAS-E74*^{IR}, (B,B′) or *UAS-E75*^{IR} (C,C′) was driven by *esg*^{ts} at 29°C and stained with indicated antibodies at 2 hours APF. *E74* or *E75* depletion did not block AMP release from islands and differentiation into Pdm1-positive ECs. (**D**,**D′**) The percentage of Pdm1-positive ECs in GFP-labeled cells in wild-type control and *E74* and *E75*-RNAi flies at 2 hours APF. Data are represented as mean±s.e.m., no significant difference was found between the wild-type control and *E74*- and *E75*-RNAi flies. GFP, green; Pdm1, nuclear red; DAPI, nuclear blue. Scale bars: 10 μm.

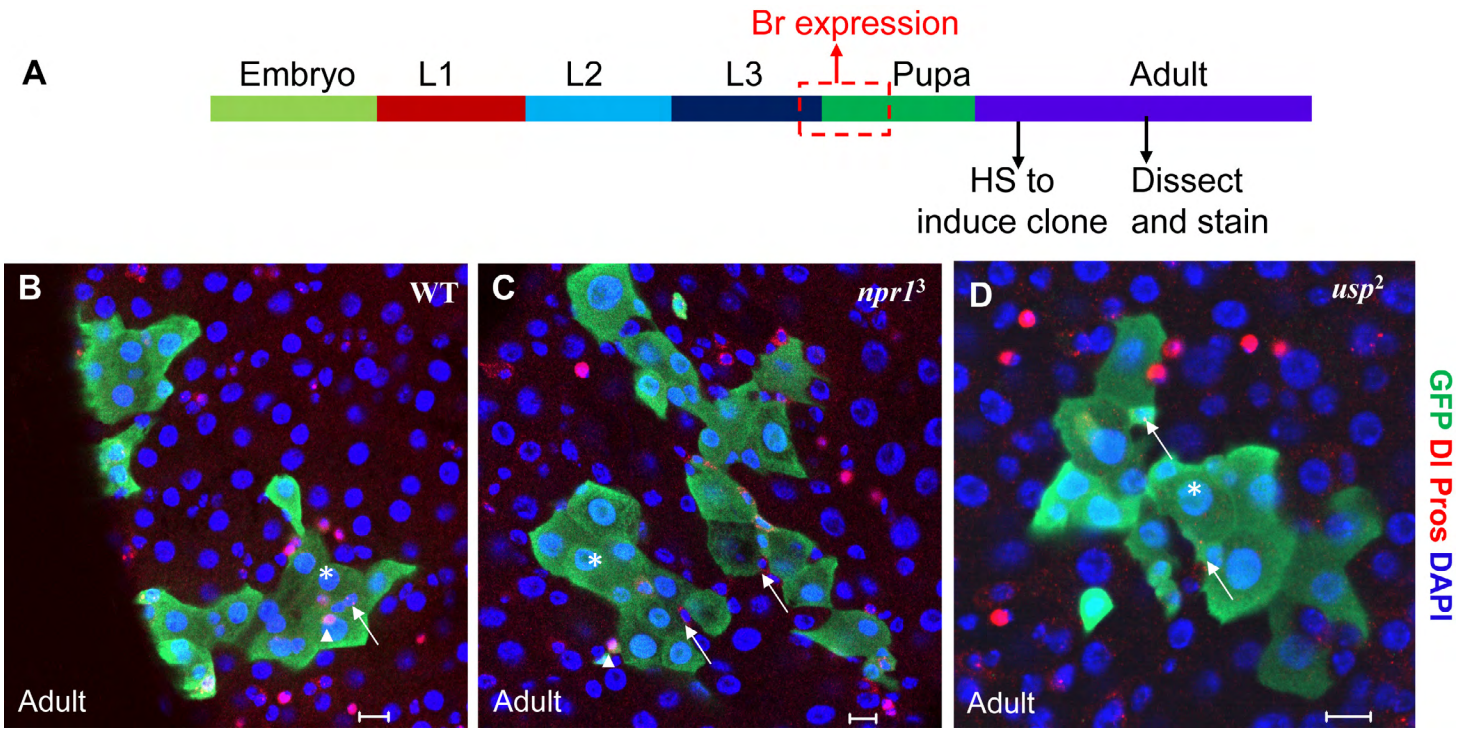

Fig. S3. Broad and EcR signals are not required for adult ISC differentiation. (**A**) The experimental design for generating ISC MARCM clones by heat shock (HS) in the adult midgut. One-week-old flies were heat shocked twice for 45 minutes and were dissected for staining in 5 days. (**B-D**) *br* (C) and *usp* (D) are not required for adult ISC differentiation and their mutant ISC MARCM clones were similar to wild-type MARCM clones (B). Arrow, ISC; arrowhead, EE; Star, EC. GFP, green; Dl, cytoplasmic red, Pros, nuclear red; DAPI, nuclear blue. Scale bars: 10 μm.

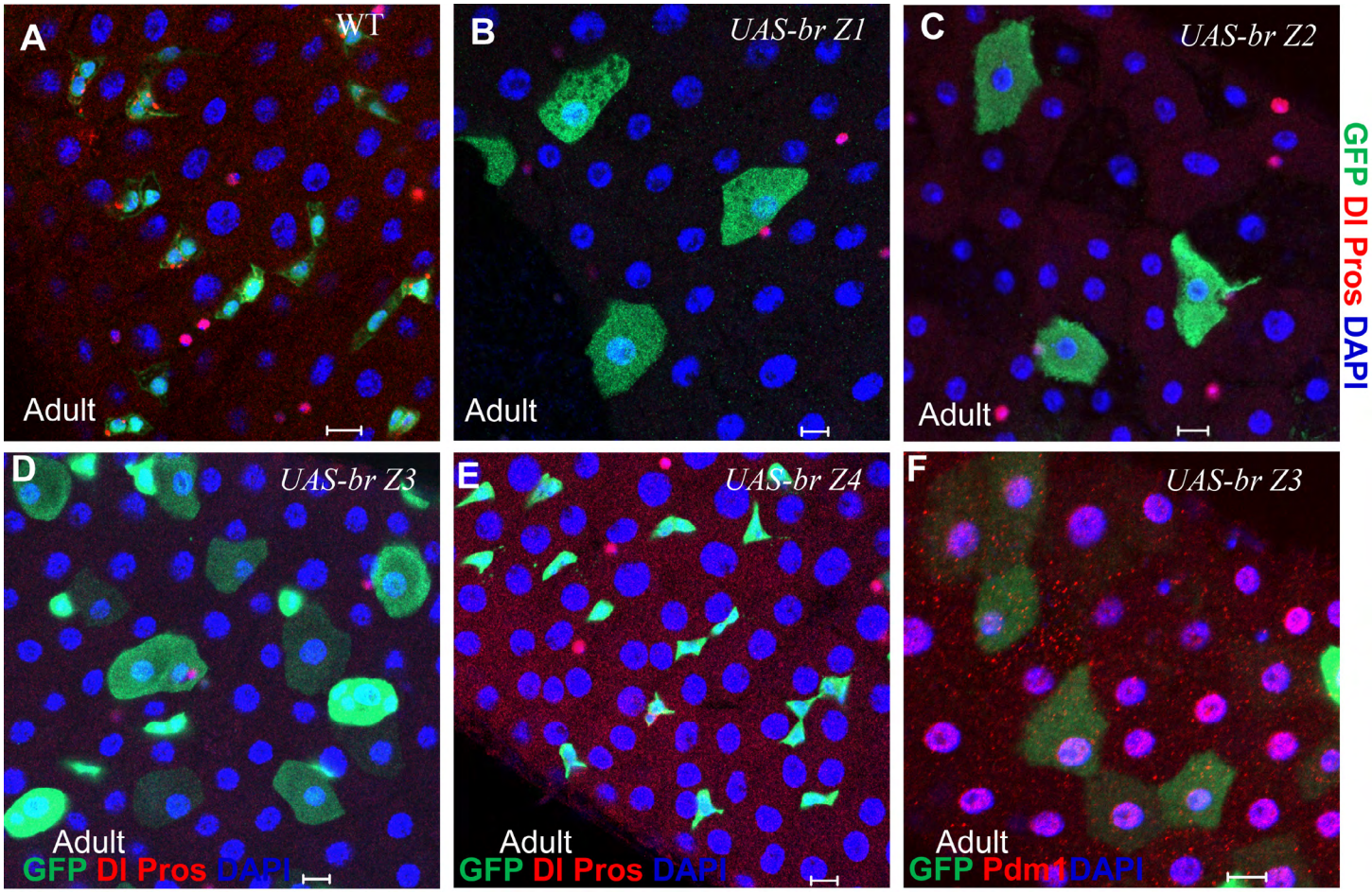

Fig. S4. Ectopic expression of Br drives ISC differentiation into EC-like cells in the adult midgut. (**A-F**) A wild-type control (A), *UAS-br Z1* (B), *UAS-br Z2* (C), *UAS-br Z3* (D,F) or *UAS-br Z4* (E) was driven by *esg*^{ts} in adult ISC at 29°C for 7 days. Ectopic overexpression of *br* in adult ISCs drove ISCs to differentiate into EC-like cells, which can be labeled by EC-specific marker Pdm1 (F). GFP, green; Dl, cytoplasmic red; Pros, nuclear red (in F: Pdm1, nuclear red); DAPI, nuclear blue. Scale bars: 10 μm.

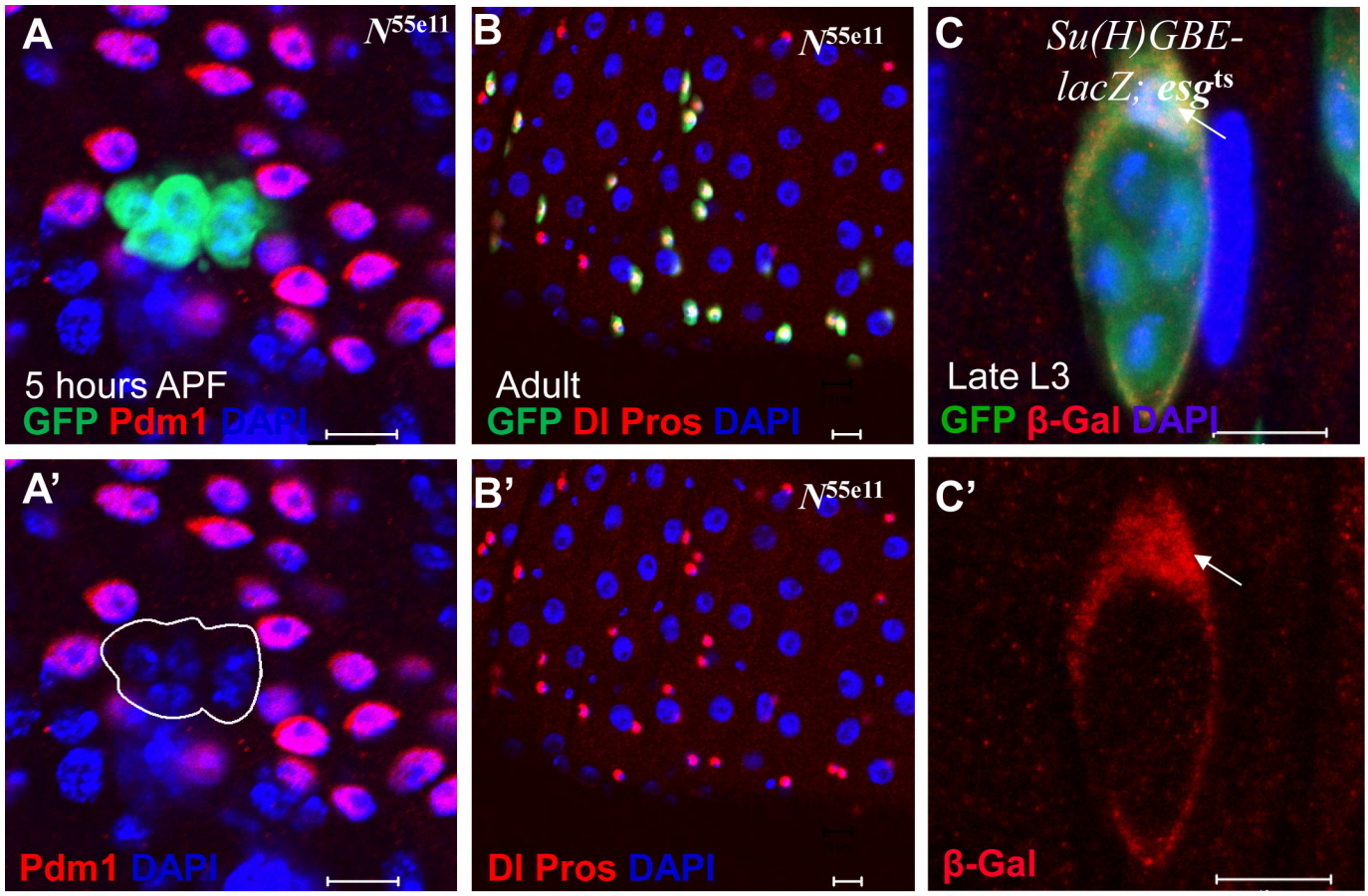

Fig. S5. Notch signaling is required for AMPs to develop into ECs. (**A**,**A**′) *N*-mutant MARCM is generated using the method shown in Fig. 3A. Compared with the wild-type control in Fig. 3B,B′, GFP-labeled *N*-mutant AMPs were still Pdm1-negative at 5 hours APF. GFP, green; Pdm1, nuclear red; DAPI, nuclear blue. (**B**,**B**′) GFP-labeled *N* mutant AMPs generated using the methods shown in Fig. 4A were traced to the adult stage. All GFP-labeled *N* mutant AMPs developed into EEs labeled with nuclear staining of Pros in the adult midgut. GFP, green; Dl cytoplasmic red; Pros, nuclear red; DAPI, nuclear blue. (**C**,**C**′) A wild-type control was driven by *Su(H) GBE-lacZ; esg*^{ts}. The expression of *Su(H)GBE-lacZ*, an N activity reporter, was detected in PC (arrow). GFP, green; β-galactosidase, red; DAPI, nuclear blue. Scale bars: 10 μm.

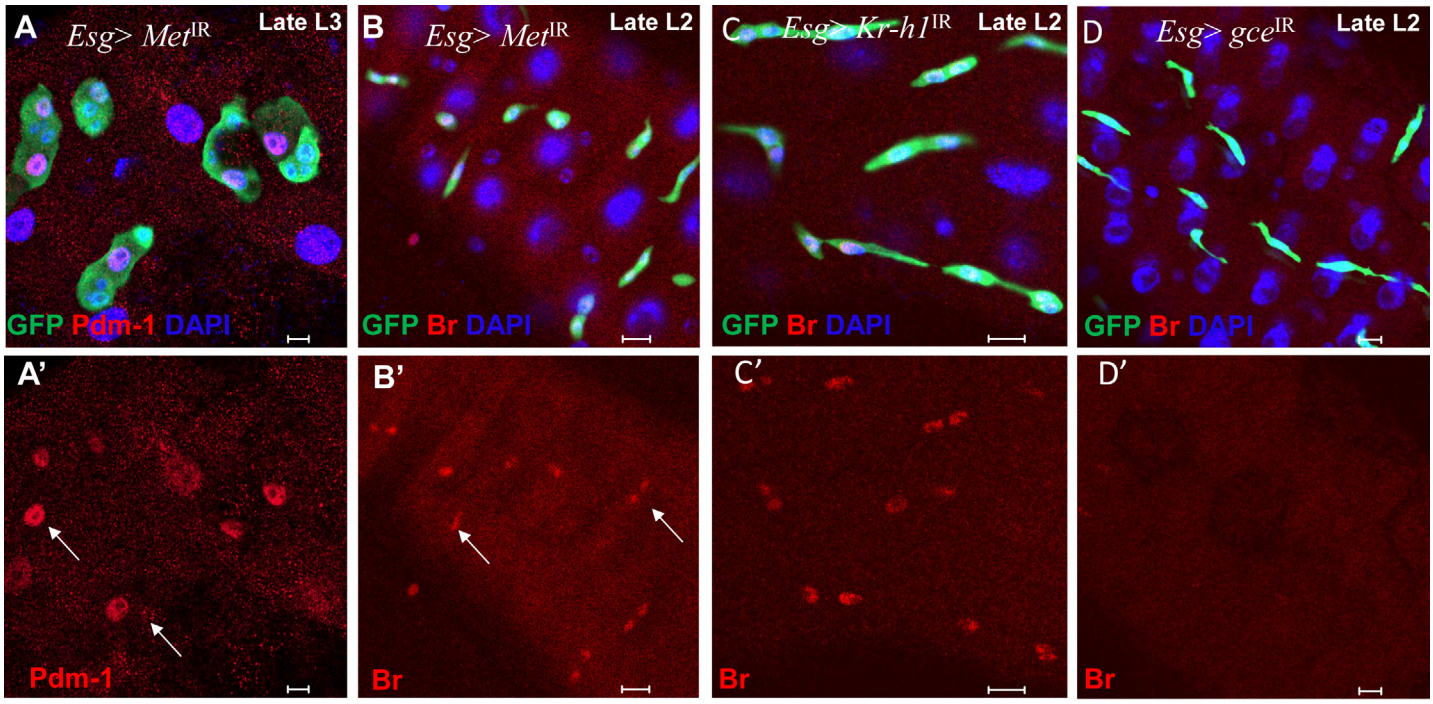

Fig. S6. *Met* knockdown induced AMP premature differentiation. (**A-D′**) *UAS-Met*^{IR} (A,A′,B,B′), *UAS-Kr-h1*^{IR} (C,C′) or *UAS-gce*^{IR} (D,D′) was driven by *esg*^{ts}. *Met* knockdown resulted in AMP premature differentiation and precocious Br expression. *Kr-h1* knockdown induced precocious Br expression too, whereas *gce* knockdown did not. GFP, green; Pdm1 or Br, nuclear red; DAPI, nuclear blue. Scale bars: 10 μm.

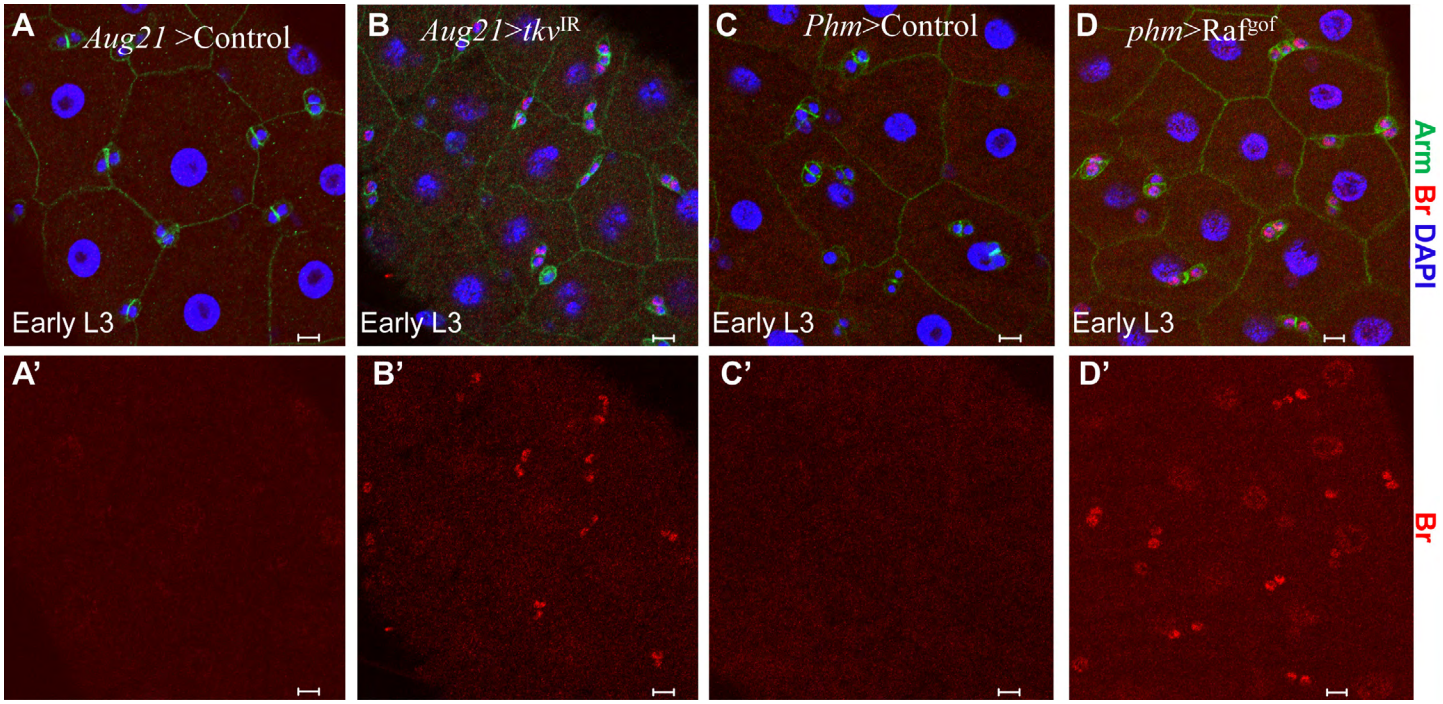

Fig. S7. The Dpp signal transduction pathway in CA and the Ras-Raf signal transduction pathway in PG regulate Br expression in AMPs. (**A-D′**) When compared with wild-type controls driven by CA-specific *Aug21-Gal4* (A,A′) or PG-specific *phm-Gal4* (C,C′), CA-specific knockdown of *tkv* (*Aug21>tkv*^{IR}; B,B′) or PG-specific expression of a constitutively activated form of Raf (*phm>Raf*^{gof}; D,D′) induced precocious Br expression. All flies were fixed and stained at early L3 with indicated antibodies. Arm, green; Br, nuclear red; DAPI, nuclear blue. Scale bars: 10 μm.