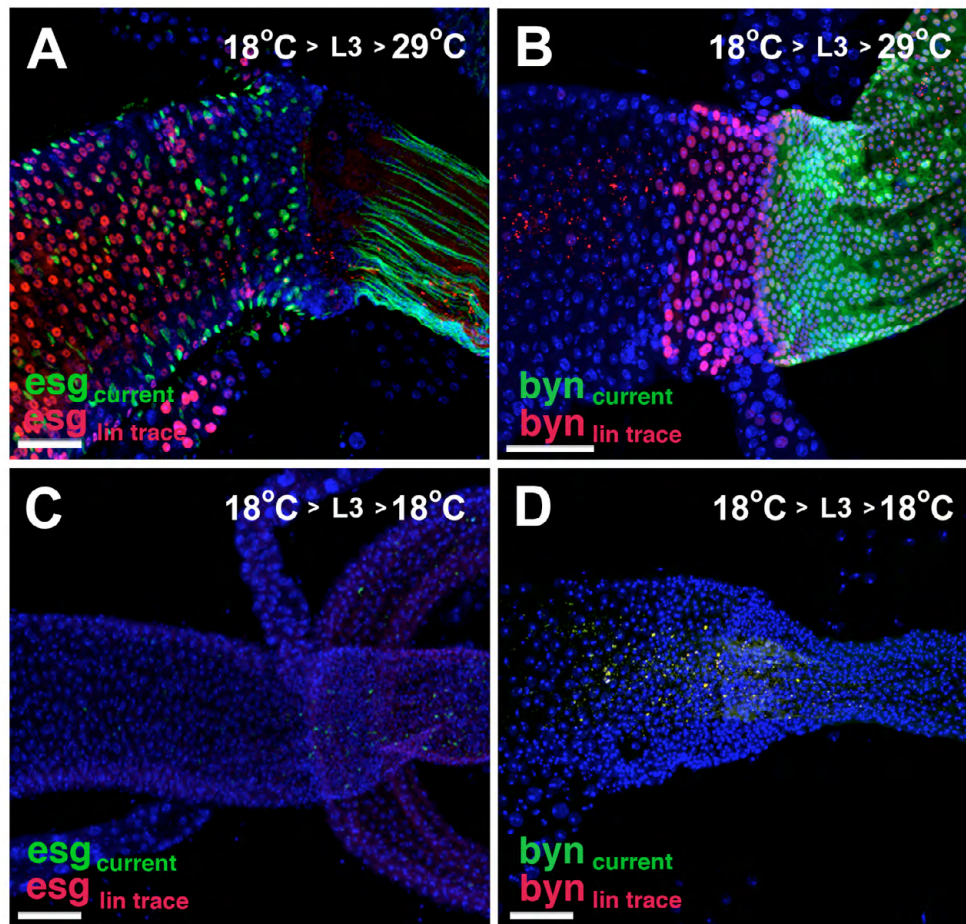


**Fig. S1. Expression of Gal4 drivers.** (A) Expression of *esg-Gal4>UAS-myr-mRFP* and (B) *byn-Gal4>UAS-GFP* in the hindgut-midgut boundary region at consecutive stages (both are shown in green). *esg-Gal4* lineage is also shown in the bottom row in A, where the lineage marker was briefly activated for 6 hours in first instar larvae and the animals were kept at 18°C until they reached L3 or the stage equivalent to P24 at 25°C (48 hours APF at 18°C) (current expression of *esg-Gal4* is not visible because of the inhibition of Gal4-UAS system by Gal80<sup>ts</sup> at this temperature). Abbreviations: ahg, adult hindgut; amg, adult midgut; amg<sub>ps</sub>, posterior terminal midgut; amp, adult midgut progenitor; aure, adult ureter; ahpz, adult hindgut proliferation zone; hpz, hindgut proliferation zone; L3, late third instar larva; lhg, larval hindgut; lhpz, larval hindgut proliferation zone; limg, larval midgut; lure, larval ureter; P6, P16, P24, P28, P48, P72, pupa aged 6, 16, 24, 28, 48, 72 hours after puparium formation; pISC, presumptive intestinal stem cell; pRSC, presumptive renal stem cell. Scale bars: 50 μm.



**Fig. S2. Negative controls for lineage analysis.** (A-D) Expression of a lineage marker (*lacZ*) driven by (A,C) *esg-Gal4* and (B,D) *byn-Gal4* in hindgut-midgut boundary region. Current expression of the *Gal4* drivers is shown in green and the lineage is shown in red. The lineage-tracing construct was activated by raising the temperature to 29°C at late third instar stage (A,B). Negative controls are shown in C,D, where the animals were reared at 18°C, which prevents activation of the construct. In the negative controls, current expression of *Gal4* driver is also not visible because of the inhibition of Gal4-UAS system by Gal80<sup>ts</sup> at this temperature. Scale bars: 50 μm.