

Supplementary Material

corresponding to:

Expression of *Hey* marks a subset of enteroendocrine cells in the *Drosophila* embryonic and larval midgut

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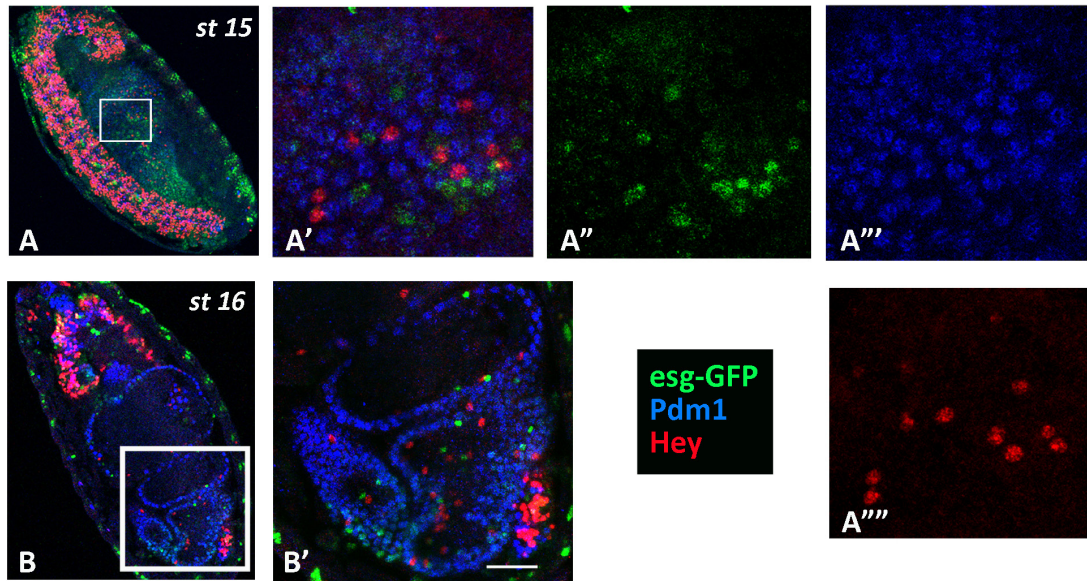


Fig. S1. Hey expression in the midgut primordia during late embryonic stages. Late stage embryos stained for Esg-GFP (green), Pdm1 (blue) and Hey (red). ECs, AMPs and EEs comprise three separate populations at these stages. **(A'-A''''')** High magnification images of the boxed area in the middle region of a stage 15 **(A)** midgut primordium. Anterior is at the top and ventral to the left. Images focus on the epithelium of the closed midgut sac, which is formed by this stage, showing Esg-GFP⁺ AMPs (green) and Hey⁺ EEs (red) intermingled with Pdm1⁺ ECs (blue). **(B-B'')** Horizontal view of a stage 16 embryo displaying the midgut lumen enclosed by the epithelium. Higher magnification image **(B')** at the middle-posterior area showing three of the four midgut domains formed at this stage. Esg-GFP⁺ AMPs (green) and Hey⁺ EEs (red) are located apically to Pdm1⁺ ECs (blue) and towards the lumen. Scale bar, 40 μ m (A, B), 20 μ m (B'), 13 μ m (A'-A''').

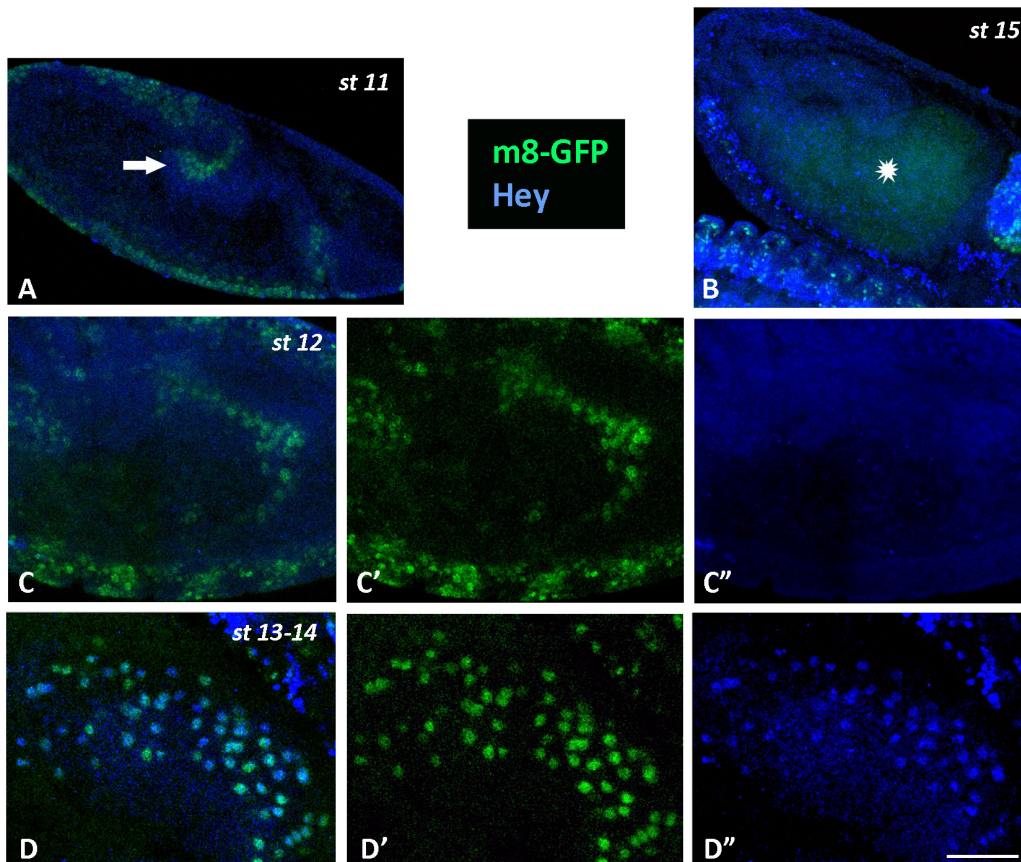


Fig. S2. E(spl)m8 is co-localized with Hey in embryo midgut primordia. Different stage embryos of the *E(spl)-m8GFP* line were stained for m8GFP (green) and Hey (blue) expression. E(spl)-m8GFP appears in amg/pmg primordia at earlier stages than Hey; later it is co-expressed in the same cells, but in subsequent stages it disappears while Hey remains. **(A)** Sagittal view of stage 11 embryo displaying E(spl)-m8GFP expression (green) in the pmg (arrow). Hey is not expressed at this stage. **(B)** Sagittal view of a stage 15 embryo showing absence of E(spl)-m8GFP staining (green) from the midgut sac (star). **(C-D'')** High magnification images of the midgut primordium region in stage 12 and stage 13/14 embryos. **(C-C'')** E(spl)-m8GFP (green) is expressed in many cells of the stage 12 primordium (C,C') while Hey expression (blue) is still not on (C''). **(D-D'')** In stage 14, both proteins are expressed and co-localize in the same EE subpopulation. Scale bar, 40 μ m (A, B), 35 μ m (C-C''), 20 μ m (D-D'').

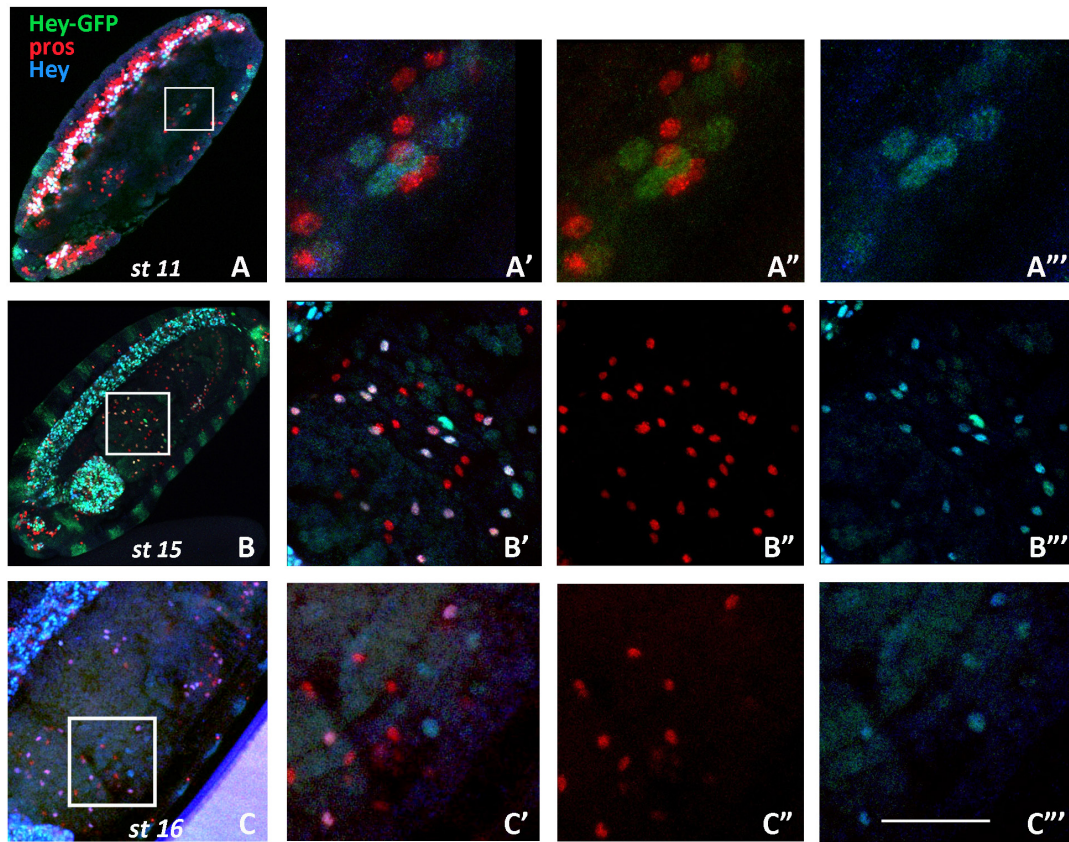


Fig. S3. Hey-GFP expression pattern in embryonic midgut primordia. Different stage embryos from the *Hey-GFP* line stained for Hey (blue), Prospero (red) and GFP (green) indicating Hey-GFP fusion expression. **(A''', B''', C''')** Hey-GFP expression pattern (green) is completely matched with Hey (blue) staining. **(B-B'', C-C'')** Co-localization is in a subpopulation of Pros⁺ (red) cells in stage 15-16 embryos. **(A-A''')** In stage 11, Hey-GFP (green) and Hey (blue) co-localize only in a few large nuclei that are Pros-negative (presumptive ICPs). Scale bar, 17 μ m (A'-A'''), 30 μ m (B'-B'''), 22 μ m (C'-C''').