

Cell Reports, Volume 41

Supplemental information

**MicroRNA mediated regulation
of the onset of enteroblast differentiation
in the *Drosophila* adult intestine**

Sromana Mukherjee, Brian R. Calvi, Heather A. Hundley, and Nicholas S. Sokol

Supplemental Information

Supplemental Table

Table S1: Oligo List, Related to STAR Methods	
Oligo sequence	Oligo Identifier
5'-acgaagacgctaactgtacaagtaTCTAGAATGTATTAGTAGAAATTTGTTAGCACTTA-3'	Oligo 4854
5'-CGAGGCCCTTTCGTCTTCAAGAATTCGTTTaaaaagtctggcagcgctgcatattggt-3'	Oligo 4855
5'-ACCCGTGGCCAGGGCCGCAAGCTTGCATGGCCCCGCACAGAAGTCCTTTCCCAGGGCCTT-3'	Oligo 3989
5'-ATCACGAGGCCCTTTCGTCTTCAAGAATTCGAGATGGCATCCAAAGCAAACCTCTGACTG-3'	Oligo 4024
5'-AGATTACCCAGGTCCTGCTG-3'	m3 qPCR forward
5'-AGTCGCGTCGATAGGAATTT-3'	m3 qPCR reverse
5'-GCAAGACAGTGATGACCCAT-3'	m5 qPCR forward
5'-TCCTCGTTATCGCTGTGGTA-3'	m5 qPCR reverse
5'-AATCGTCGTGCAGTCAACAT-3'	m7 qPCR forward
5'-GTGGCCATTGTGTGTTTGT-3'	m7 qPCR reverse
5'-GGGACGCGTCTACAAGAACT-3'	m8 qPCR forward
5'-TTGTCCATTGAGGAGCAGTC-3'	m8 qPCR reverse
5'-CAACTGGACTCCGGATGATA-3'	mbeta qPCR forward
5'-TCAAGTCCAGCACCTCTTG-3'	mbeta qPCR reverse
5'-AATGAGGTCTCCCGTCACT-3'	mgamma qPCR forward
5'-GGGTCATTAGCTGAGTGCCT-3'	mgamma qPCR reverse
5'-CGCAACATCGCAACTTTATT-3'	mdelta qPCR forward
5'-TTGCGGTAATGCTGAGTCTT-3'	mdelta qPCR reverse

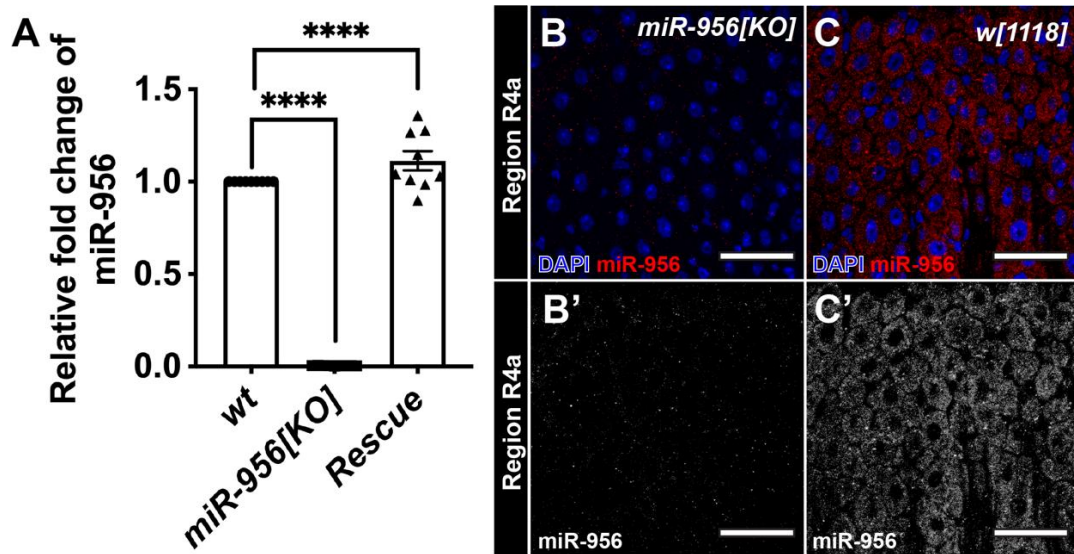


Figure S1 Mature *miR-956* is absent in *miR-956*[KO], restored by a Rescue transgene, and expressed in all intestinal cell types in region R4a. Related to Figure 1 and 2.

(A) qPCR analysis of *miR-956* levels in *miR-956*[KO] and Rescue; *miR-956*[KO] relative to *w*[1118] control animals. Statistical significance of the difference in miRNA levels in intestinal and carcass samples relative to whole tissue is indicated. Data shown as Mean \pm SEM. Significance values are **** $p < 0.0001$. (B-C) Midgut expression in region R4a of *miR-956* (red) using RNA *in situ* probes in (B) *miR-956*[KO] mutants used as a control and (C) wildtype (*w*[1118]) animals with counterstaining of all cell nuclei (DAPI in blue). (B'-C') Grayscale images of *miR-956* RNA *in situ* probes. Scale bar, 25 μ m.

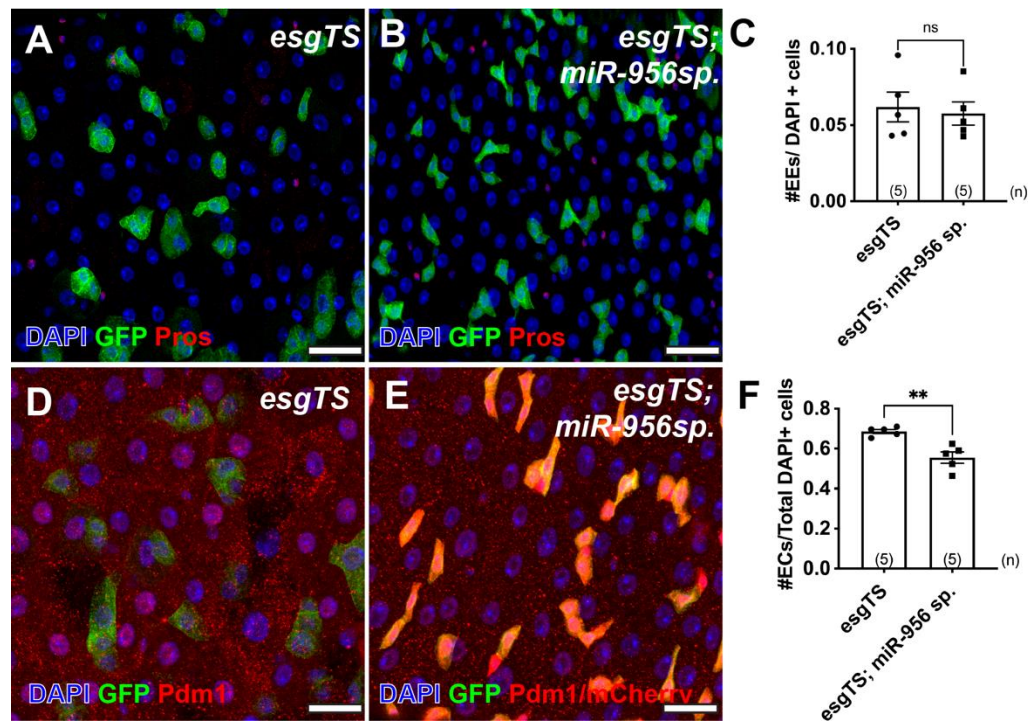


Figure S2 *miR-956* activity in progenitors does not affect EE numbers but regulates EC numbers. Related to Figure 2.

(A-B,D-E) *esgTS* labelled progenitor cells (green) in (A,D) *esgTS*, (B,E) *esgTS; miR-956sp.* midguts counterstained for all cell nuclei (DAPI in blue), EEs marked using Prospero (red) in (A-B), ECs marked using nuclear Pdm1 in (D,E), *miR-956sp.* transgene marked using mCherry (red) in (E). Quantification of (C) EEs (F) ECs in *esgTS*, *esgTS; miR-956sp.* animals. ECs were quantified based on the presence of nuclear Pdm1 signal and absence of GFP signal. Data shown as Mean ± SEM. Significance values are n.s., not significant; ** p < 0.01. Scale bar, 25µm. n values in the graphs indicate the number of intestines.

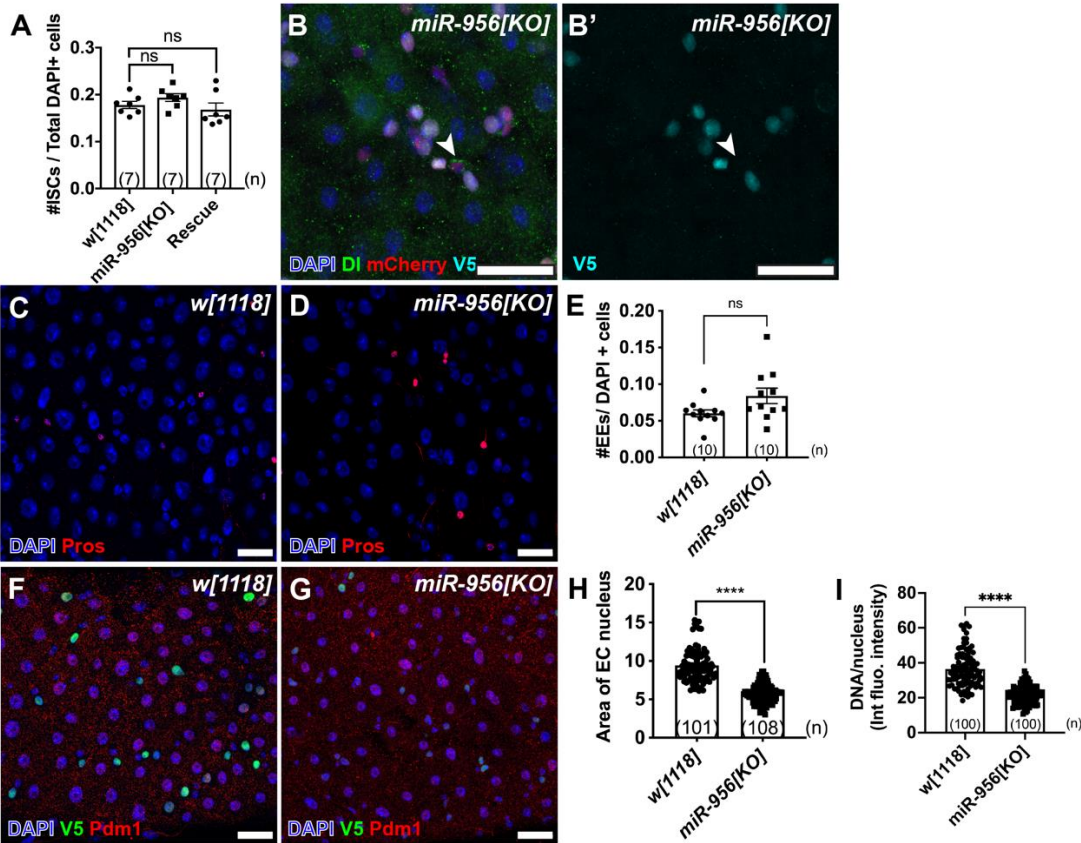


Figure S3 *miR-956* does not affect ISC and EE numbers but regulates EC nuclear size and ploidy. Related to Figure. Related to Figure 2.

(A) Quantification of ISC numbers in *miR-956[KO]* mutants in comparison to wildtype and rescued controls. (B) *miR-956[KO]* mutants counterstained for ISCs marked using DI (green), progenitors (anti-mCherry in red), EBs marked with Su(H)-GBE-V5 (anti V5 in cyan) and all cell nuclei (DAPI in blue). (B') V5 reporter channel from B. (C-D) Midguts in (C) wildtype (*w[1118]*) and (D) *miR-956[KO]* mutants counterstained for EE cell marker(anti Prospero in red) and all cell nuclei(DAPI in blue). (E) Quantification of EEs in wildtype vs *miR-956[KO]* mutants. (F-G) Midguts in (F) wildtype (*w[1118]*) and (G) *miR-956[KO]* mutants showing EBs marked with Su(H)-GBE-V5 (anti V5 in green), ECs (anti Pdm1 in red) and all cell nuclei (DAPI in blue). (H) Quantification of area of EC nucleus in wildtype and *miR-956[KO]* mutant animals. (I) Quantification of DNA content per EC nucleus in wildtype and *miR-956[KO]* mutant animals. Data shown as Mean \pm SEM. Significance values are n.s., not significant; **** $p < 0.0001$. Scale bar, 25 μ m. n values in graphs A and E indicate the number of intestines. n values in graphs H and I indicate the number of cells quantified from at least five intestines.

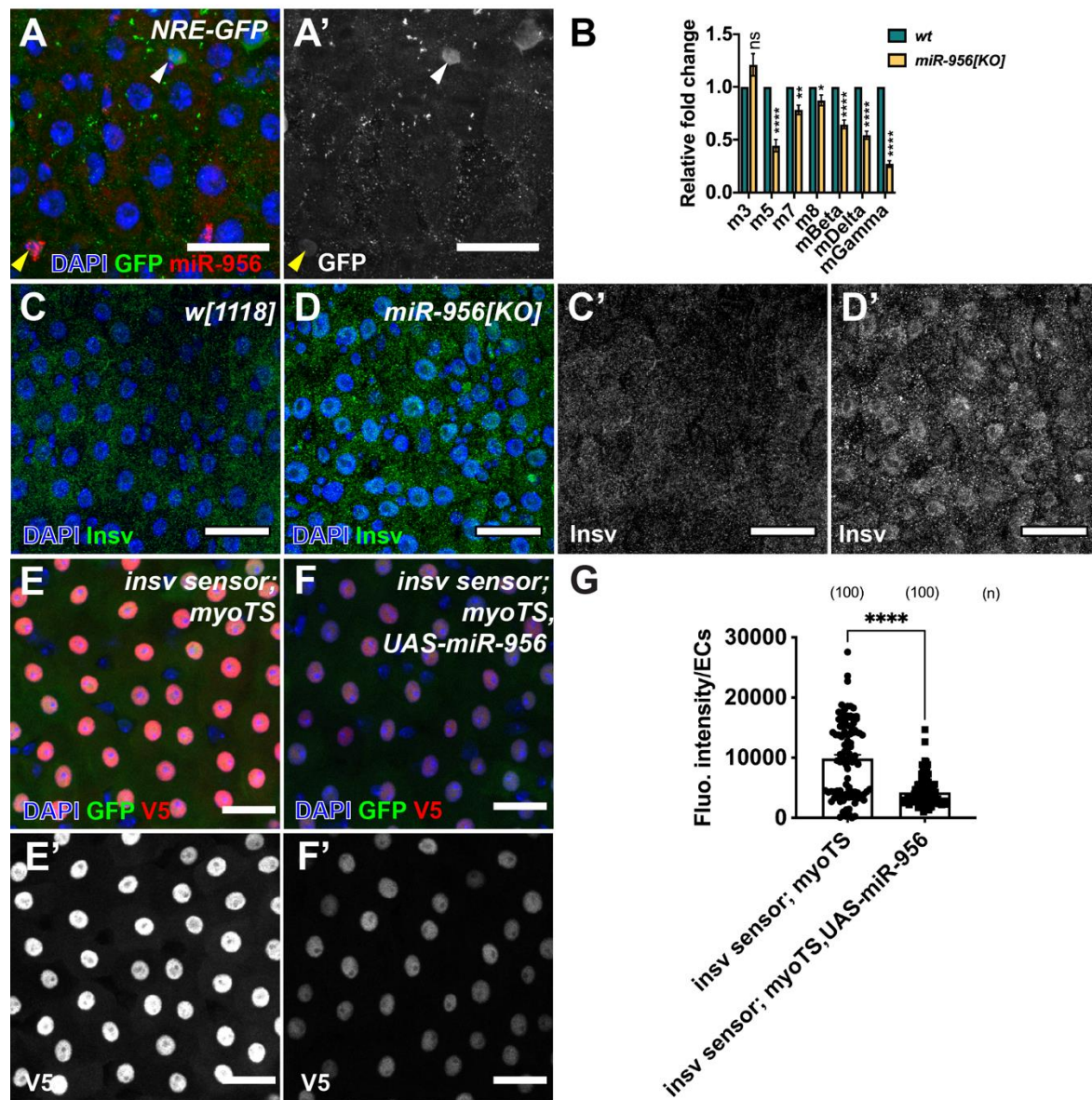


Figure S4 *miR-956* promotes Notch activity by suppressing *insv*. Related to Figure 4 and 5.

(A) Midgut showing Notch signaling reporter expression (GFP in green), *miR-956* using RNA *in situ* probes (red) and all cell nuclei (DAPI in blue). (A') Grayscale image of GFP reporter channel shown in A. Yellow arrowheads indicate high *miR-956* and low Notch activity in A and A' respectively. White arrowheads indicate low *miR-956* and high Notch activity in A and A' respectively. (B) qPCR analysis of E(spl)-C genes in *miR-956*[KO] mutants relative to wildtype (*w*[1118]) animals. (C-D) Midgut expression of Insv protein expression (green) in (C) wildtype (*w*[1118]) animals and (D) *miR-956*[KO] mutants with counterstaining of all cell nuclei in (DAPI in blue). (C'-D') Grayscale images of Insv protein expression in indicated channels from C-D. (E-F) Midguts from (E) *myoTS* or (F)

myoTS; *UAS-miR-956* animals stained for *smGFP.V5.insv 3'UTR* (red), ECs marked with GFP (green) and cell nuclei (blue). (E'-F') Grayscale images of indicated channels from F-G. (G) Fluorescence intensity of V5 reporter expression in ECs of *myoTS* and *myoTS*; *UAS-miR-956* animals stained for *smGFP.V5.insv 3'UTR* (red). Data shown as Mean \pm SEM. Significance values are n.s., not significant; * $p < 0.1$; ** $p < 0.01$; **** $p < 0.0001$. Scale bar, 25 μ m. n values in the graph indicate the number of cells quantified from at least five intestines.

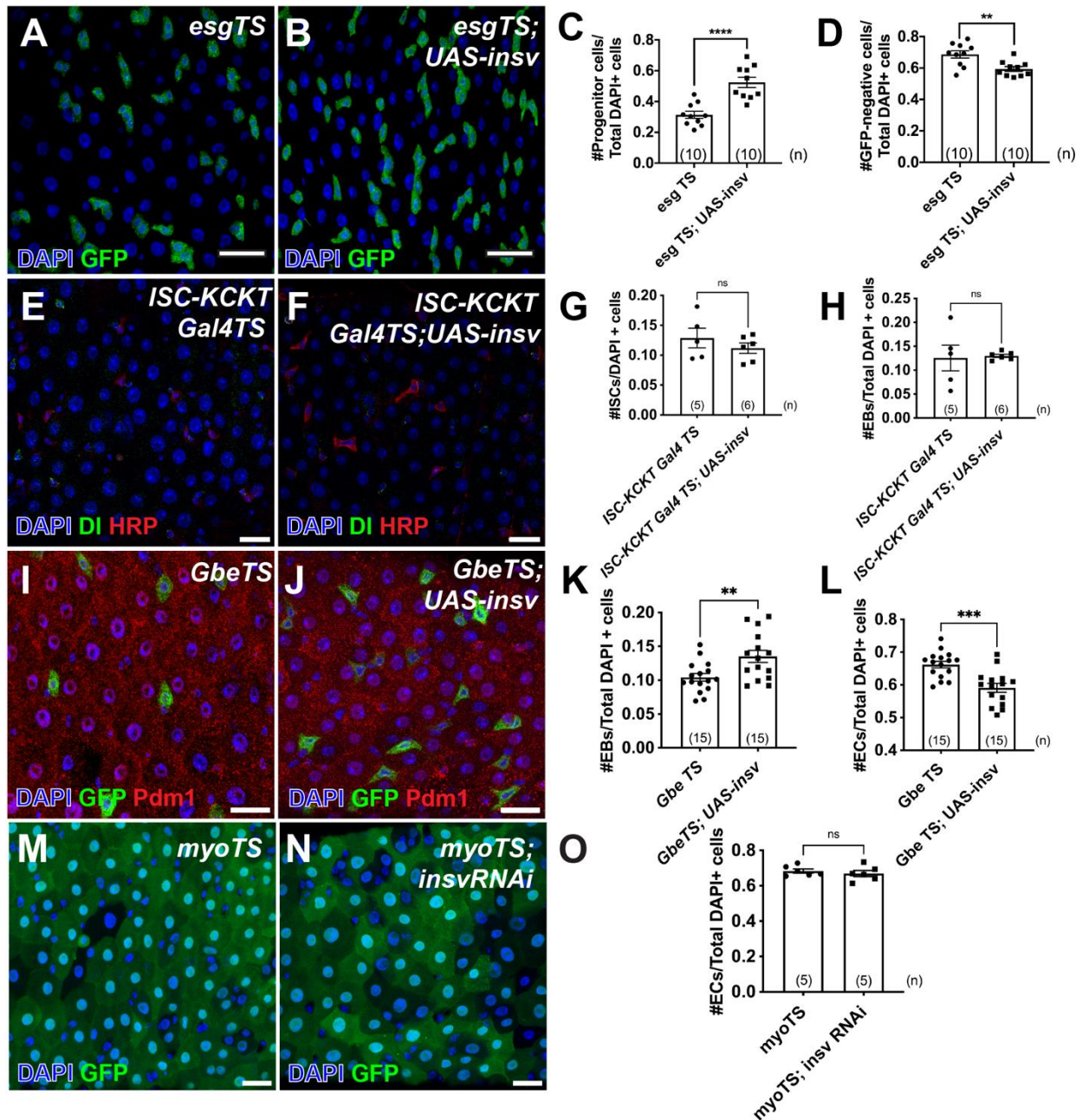


Figure S5 *insv* acts in EBs to regulate EB and EC numbers. Related to Figure 5.

(A-B) *esgTS* labelled progenitor cells (green) in (A) *esgTS*, (B) *esgTS, UAS-insv* midguts counterstained for all cell nuclei (DAPI in blue). (C-D) Quantification of (C) progenitor cell numbers and (D) GFP-negative differentiated cell numbers in *esgTS, esgTS, UAS-insv* animals. (E-F) Progenitor cells stained for HRP (red), ISCs stained for D1 (green) in (A) *ISC-KCKT Gal4 TS*, (B) *ISC-KCKT Gal4 TS, UAS-insv* midguts counterstained for all cell nuclei (DAPI in blue). (G-H) Quantification of (G) ISCs (H) HRP positive, D1 negative EBs in *ISC-KCKT Gal4 TS, ISC-KCKT Gal4 TS, UAS-insv* animals. (I-J) *Gbe TS* labelled EBs (green) in (I) *GbeTS*, (J) *GbeTS, UAS-insv* midguts counterstained for EC cell marker Pdm1 (red) and all cell nuclei (DAPI in blue). (K-L) Quantification of (K) EBs (L) ECs in *GbeTS, GbeTS, UAS-insv* animals. (M-N) *myoTS* labelled EC cells (green) in (M) *myoTS*, (N) *myoTS, insv RNAi* midguts counterstained for all cell nuclei (DAPI in blue). (O) Quantification of EC numbers in *myo TS* and *myoTS, insv RNAi* animals. Data shown as Mean \pm SEM. Significance values are ** $p < 0.01$; **** $p < 0.0001$. Scale bar, 25 μ m. n values in the graphs indicate the number of intestines.

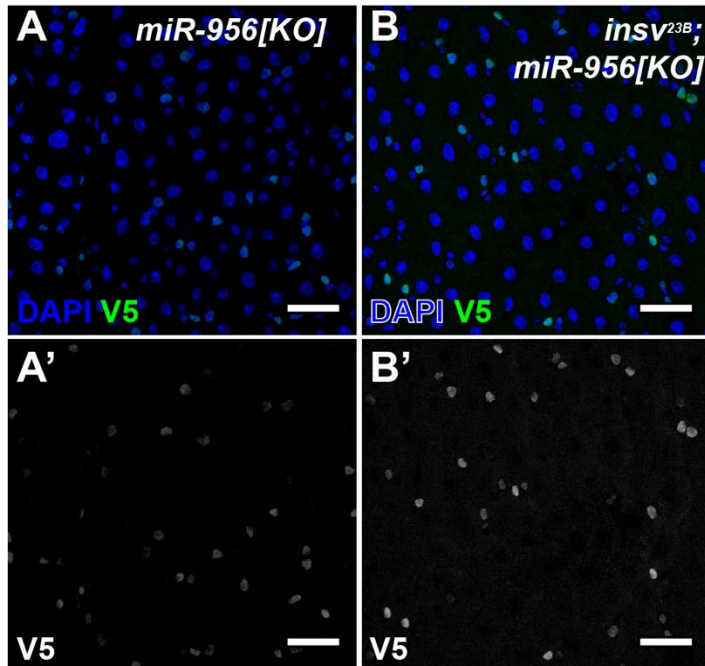


Figure S6 *miR-956* regulates Notch signaling activity in EBs via *insv*. Related to Figure 5.

(A-B) Notch signaling reporter expression using Su(H)-GBE-V5 (anti-V5 in green) in *miR-956[KO]* mutants that harbor (A) two versus (B) one wild-type alleles of *insv* with counterstaining of all cell nuclei (DAPI in blue). (A'-B') Grayscale images of reporter expression in indicated channels from A-B. Scale bar, 25 μ m.