Supplemental Figure Legends

Supplemental Figure 1. The EEC subtypes in zebrafish larvae. (A) UMAP plots of the zebrafish intestine single-cell RNA sequencing showing the zebrafish EECs and the five EEC subtypes in zebrafish larvae. The zebrafish scRNA dataset is from Wen J. et al., 2021. (B) The hormone profiles in the five zebrafish EEC subtypes. (C-E) Immunofluorescence staining of the PYY+EEC subtype. Note that the PYY+EECs are distributed in the proximal zebrafish intestine (C-C'). It overlaps with the secretory cell marker 2F11 (D) but does not overlap with the marker for other EEC subtypes, such as trpa1b (E). (F-K) Immunofluorescence staining of the Trpa1+EEC subtype. The single-cell RNA seq data above demonstrate that the Trpa1+EECs (EEC5) express the peptide enkephalin (ENK) and the enzyme that synthesizes serotonin (tph1b). (F-G) Immunofluorescence staining of ENK confirms that only Trpa1+EECs express ENK (G). Interestingly, ENK is only expressed in the Trpa1+EECs in the proximal intestine (F-F'). (H) Trpa1+EECs do not express sst2, a marker for the EEC subtype 1. (I-J) Tph1b is expressed in the EECs. (K) Immunofluorescence staining showing part of the Trpa1+EECs express 5-HT.

Supplemental Figure 2. Commensal microbiota colonization promotes the formation of "neuropod"-like structure in EECs. (A-D') Confocal projections of the GF and CV *Tg(neurod1:lifeActin-EGFP)* zebrafish. The yellow stars in A indicate EECs with thin actin filaments at the basal lateral membrane. The White arrows in C and D indicate the "neuropod" like elongated basal lateral membrane protrusions in CV EECs. (E) Quantification of the EEC percentage that has "neuropod" like structure in GF and CV conditions. Student T-test was used in E. Each dot represents an individual zebrafish. * $P<0.05$.

Supplemental Figure 3. Gut microbiota did not alter the proximal intestine mitochondria abundance. (A) Quantification of the intracellular mitochondria abundance in the 3dpf to 6dpf zebrafish EECs. The mitochondrial abundance is represented by the *neurod1:mitoEOS* and *neurod1:RFP* fluorescence ratio in individual EECs. Each dot represents an EEC. 4 zebrafish were analyzed. (B-C) Quantification of the intracellular mitochondria abundance in the proximal and distal intestines of the GF and CV zebrafish. The mitochondrial abundance is represented by the *neurod1:mitoEOS* and *neurod1:RFP* fluorescence ratio in individual EECs. Each dot represents an EEC. More than 7 zebrafish in GF and CV groups were quantified. One-Way Anova followed by Tukey post-test was used in A. Student T-test was used in B and C. *** $P<0.001$, ** $P<0.01$.

Supplemental Figure 4. Nutrient stimulation prominently promotes mitochondrial Ca2+ to arise near the basal membrane. (A-B) Zoom in view shows two linoleic acid activated EECs in *Tg(neurod1:Gcamp6f); Tg(neurod1:mitoRGECO)* zebrafish. The cytoplasmic calcium was indicated with Gcamp6f (green fluorescence), and the mitochondrial calcium was indicated with mitoRGECO (magenta fluorescence). White arrows show activated mitochondria near the basal membrane.

Supplemental Figure 5. Model figure showing gut microbiota modulate EEC maturation and mitochondrial function. (A) During early development, the immature EECs exhibit low cytoplasmic Ca^{2+} and low mitochondrial Ca^{2+} levels. These immature EECs have active filapodial filaments at the basal lateral membrane. After the zebrafish hatched out and commensal microbiota started to colonize the zebrafish intestine, the EECs continued to develop and mature. Shortly after commensal microbiota colonization, the EECs increase both cytoplasmic and mitochondrial Ca^{2+} significantly ("EEC awakening"). After the EEC awakening, the EECs continue to mature and lose the basal lateral filapodial filaments. Some EECs form a neuropod. The mature EECs have low cytoplasmic Ca^{2+} but high mitochondria-to-cytoplasm Ca^{2+} ratio. (B) Commensal microbiota promotes EEC mitochondrial respiration function and increases mitochondrial inner membrane electronic potential ($\Delta\Psi m$). When nutrient stimulants, like fatty acids, stimulate the EECs, the EEC cytoplasmic Ca^{2+} rises. The high $\Delta\Psi m$ permits the cytoplasmic Ca^{2+} to flux into the mitochondrial matrix and power mitochondrial ATP production, which then promotes EEC vesicle release.

Supplemental Tables

Supplemental Table 1. The change of transcriptomics in GF and CV EECs.

Supplemental Table 2. The zebrafish lines and primary antibodies used in this manuscript.

Supplemental Videos

Supplemental Video 1. The 3dpf enterocytes do not have active filopodia filaments. The enterocyte actin was visualized via the *Tg(gata5:lifeActin-EGFP)* zebrafish line. The actin filaments were enriched in the brush border at the apical site.

Supplemental Video 2. The active EEC filopodia filaments in 3dpf EECs. The EEC filopodia at the EEC base is visualized via the *Tg(neurod1:lifeActin-EGFP)* zebrafish line.

Supplemental Video 3. The 6dpf EECs do not display active filopodia structure at the base. The majority of the 6dpf EECs displayed enriched actin filaments at the apical brush border.

Supplemental Video 4. Use the *Tg(neurod1:Gcamp6f); Tg(neurod1:mitoRGECO)* to simultaneously image the EECs' cytoplasmic and mitochondrial calcium.

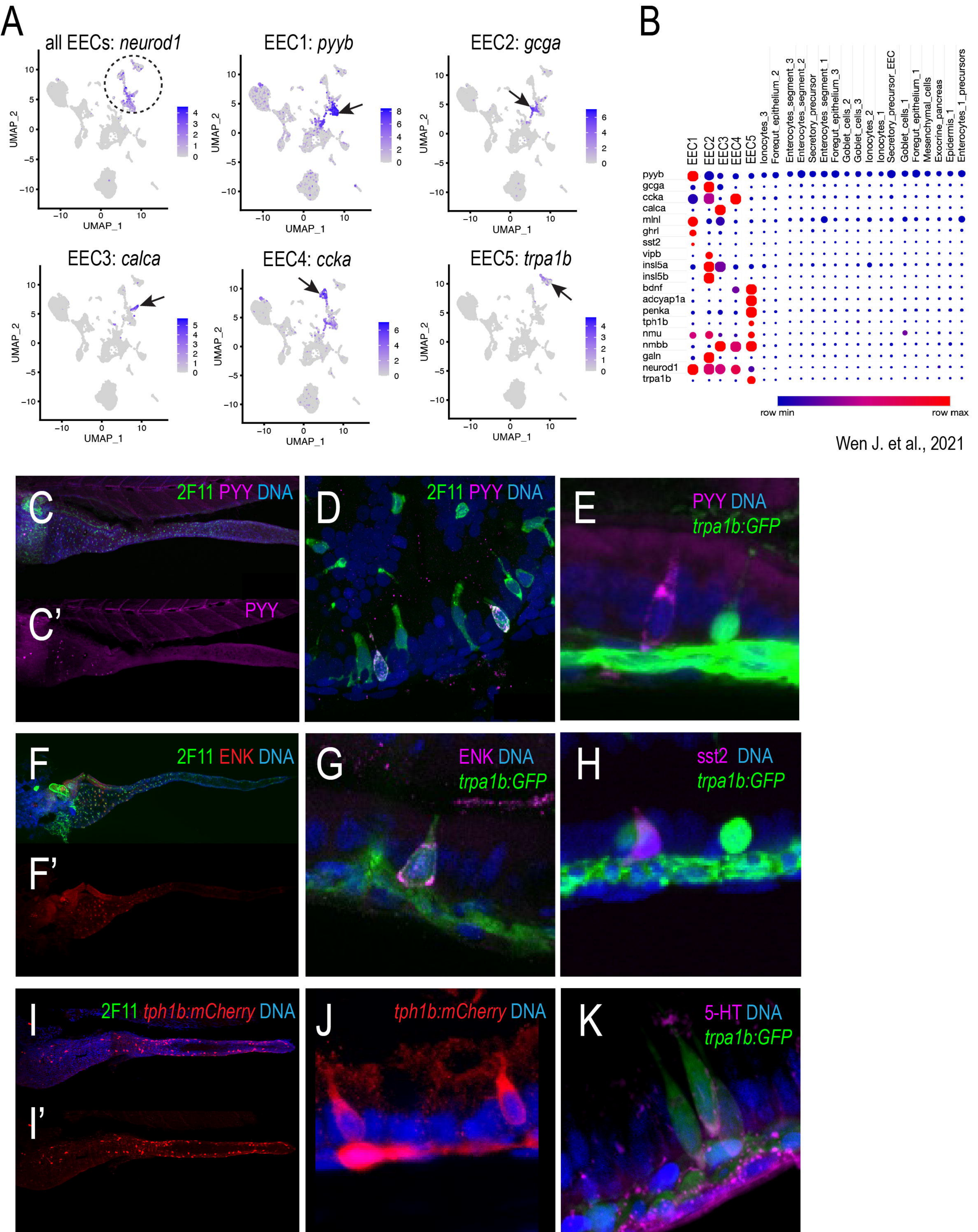
Supplemental Video 5. The dynamic of the EEC mitochondrial calcium at the resting conditions in conventionally raised zebrafish.

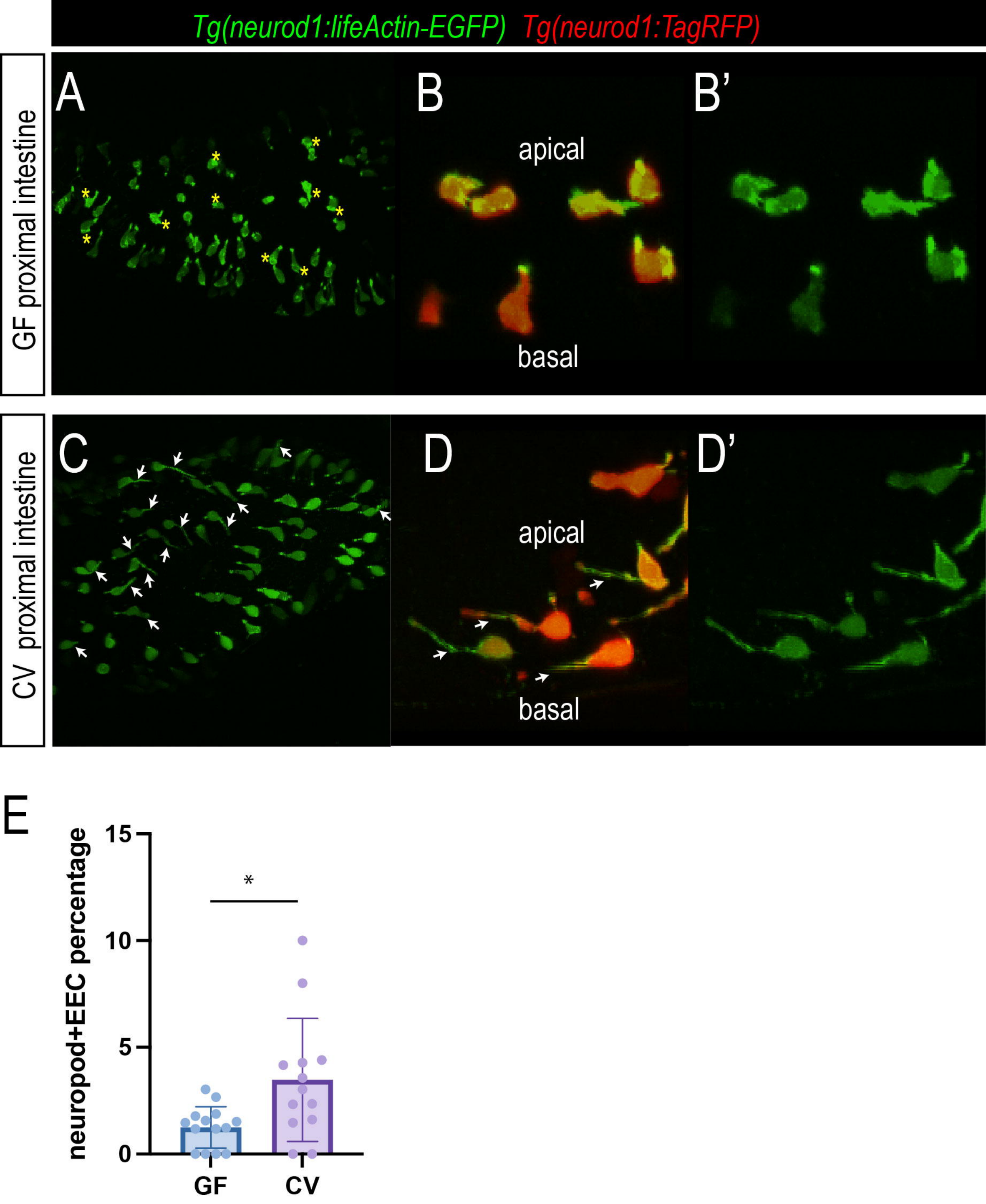
Supplemental Video 6. The spontaneous calcium fluctuations in conventionalized (CV) zebrafish EECs. The EEC calcium dynamics were visualized using the *Tg(neurod1:Gcamp6f)* zebrafish.

Supplemental Video 7. The reduced calcium fluctuations in germ-free zebrafish EECs. The EEC calcium dynamics were visualized using the *Tg(neurod1:Gcamp6f)* zebrafish.

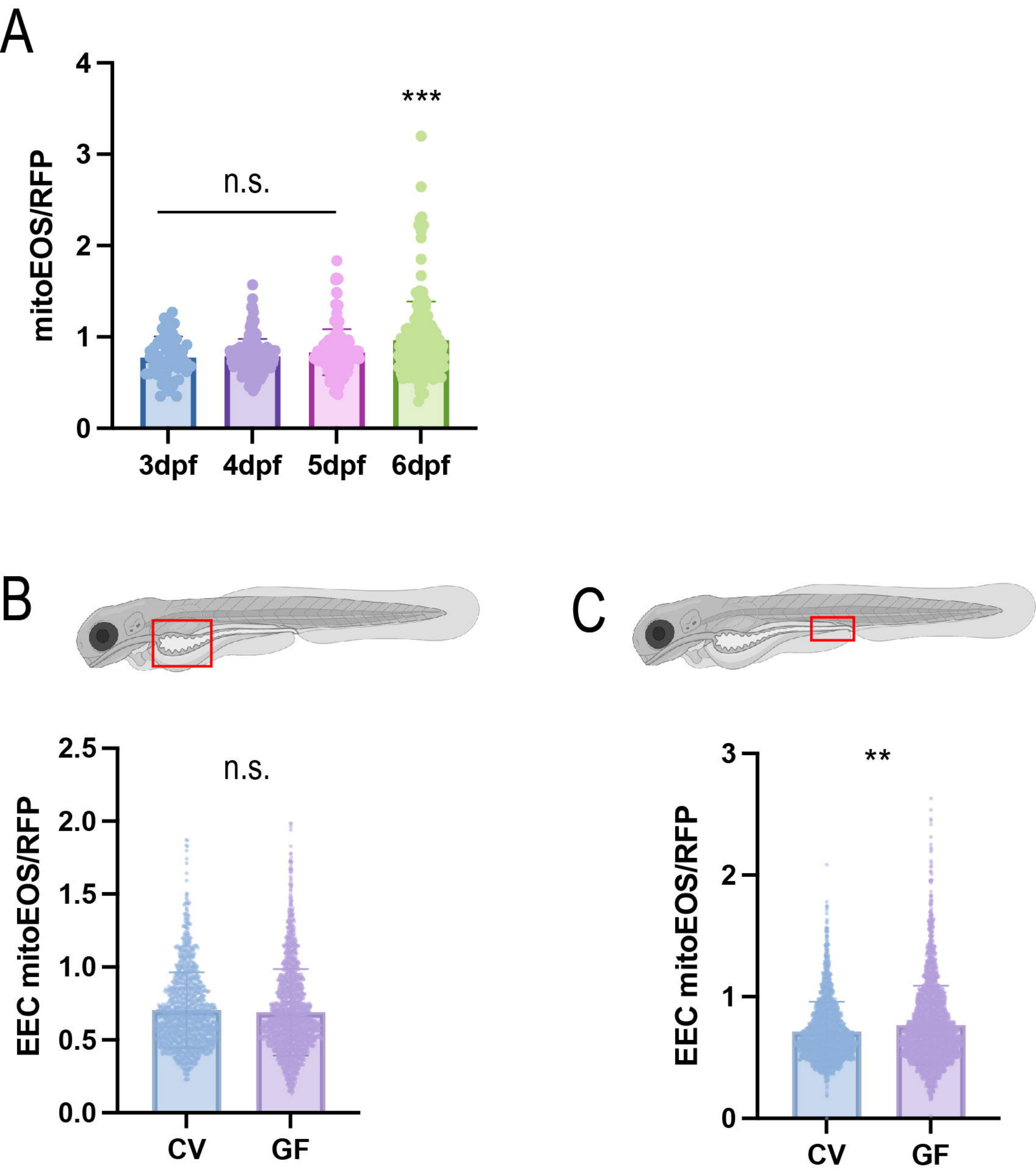
Supplemental Video 8. The Linoleic acid stimulation increases EEC cytoplasmic and mitochondrial calcium in conventionally raised zebrafish. The cytoplasmic calcium was visualized via the *Tg(neurod1:Gcamp6f)* (green) and the mitochondrial calcium was visualized via the *Tg(neurod1:mitoRGECO)* (magenta).

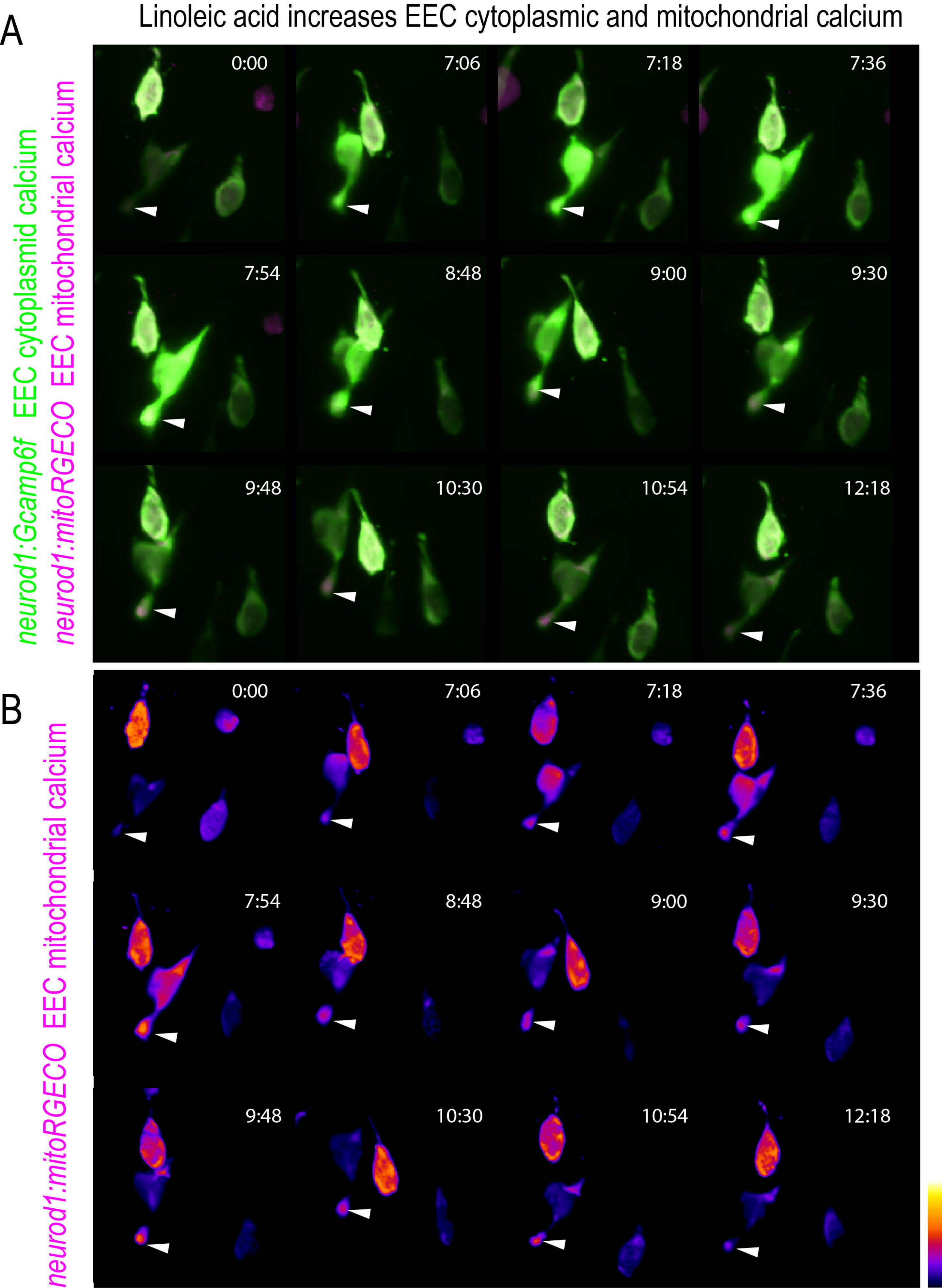
Supplemental Video 9. The Linoleic acid stimulation increases EEC mitochondrial calcium in conventionally raised zebrafish.


A
all EECs: neurod1
EEC1: pyyb
EEC2: gcga
EEC3: calca
EEC4: ccka
EEC5: trpa1b
UMAP_1
UMAP_2
B
EEC1
EEC2
EEC3
EEC4
EEC5
Ionocytes_3
Foregut_epithelium_2
Enterocytes_segment_3
Enterocytes_segment_2
Secretory_precursor
Enterocytes_segment_1
Foregut_epithelium_3
Goblet_cells_2
Goblet_cells_3
Ionocytes_2
Ionocytes_1
Secretory_precursor_EEC
Goblet_cells_1
Foregut_epithelium_1
Mesenchymal_cells
Exocrine_pancreas
Epidermis_1
Enterocytes_1_precursors
pyyb
gcga
ccka
calca
mlnl
ghrl
sst2
vipb
insl5a
insl5b
bdnf
adcyap1a
penka
tph1b
nmu
nmbb
galn
neurod1
trpa1b
row min
row max
Wen J. et al., 2021
C
C'
2F11 PYY DNA
PYY
D
2F11 PYY DNA
E
PYY DNA
trpa1b:GFP
F
F'
2F11 ENK DNA
G
ENK DNA
trpa1b:GFP
H
sst2 DNA
trpa1b:GFP
I
I'
2F11 tph1b:mCherry DNA
J
tph1b:mCherry DNA
K
5-HT DNA
trpa1b:GFP

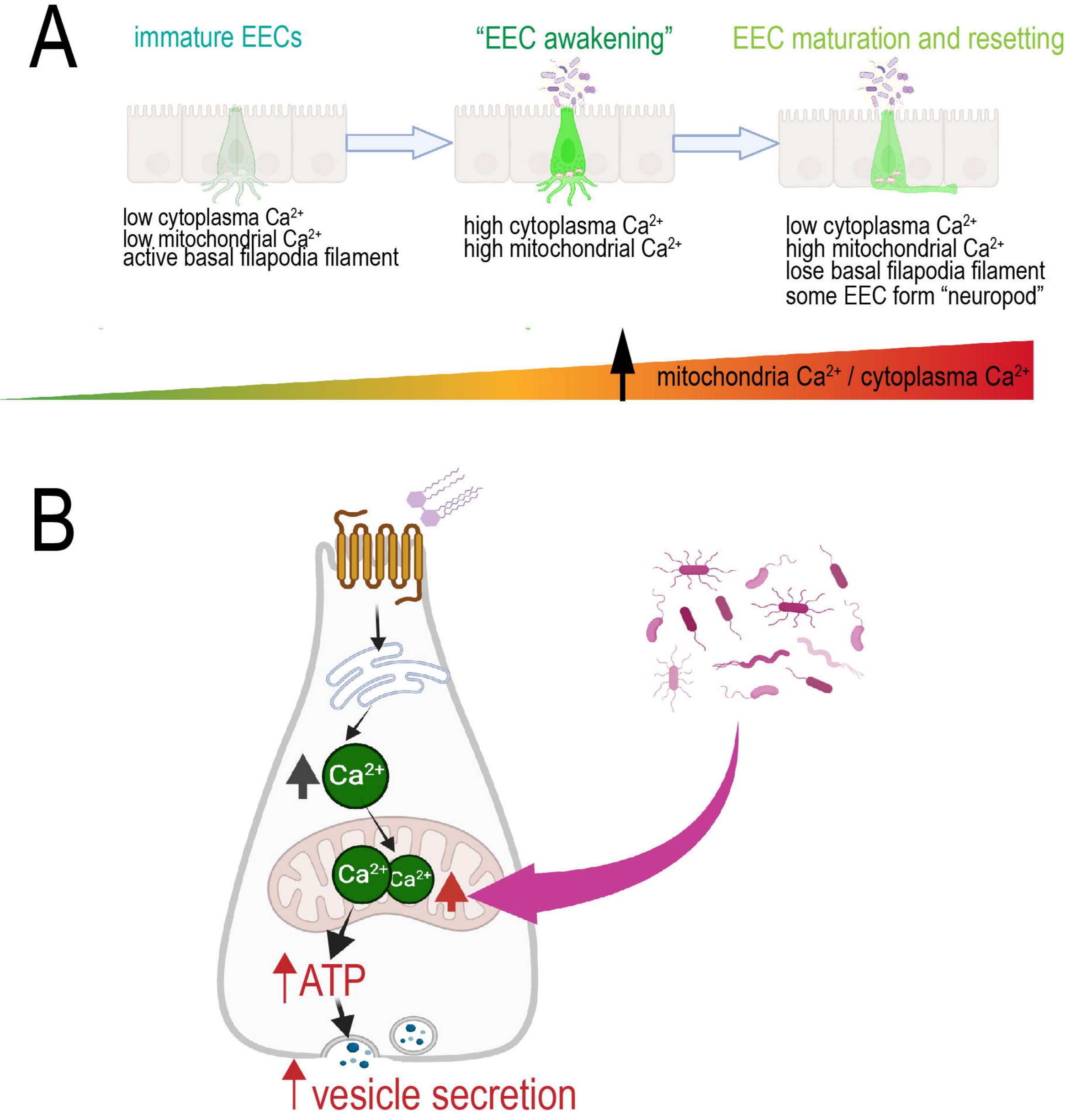


Tg(neurod1:lifeActin-EGFP) Tg(neurod1:TagRFP)
GF proximal intestine
A
B
B'
apical
basal
CV proximal intestine
C
D
D'
apical
basal
E
neuropod+EEC percentage
15
10
5
0
*
GF
CV


A
mitoEOS/RFP
n.s.
***
3dpf
4dpf
5dpf
6dpf
B
C
EEC mitoEOS/RFP
n.s.
CV
GF
EEC mitoEOS/RFP
**
CV
GF



A
Linoleic acid increases EEC cytoplasmic and mitochondrial calcium
neurod1:Gcamp6f EEC cytoplasmid calcium
neurod1:mitoRGECO EEC mitochondrial calcium
0:00
7:06
7:18
7:36
7:54
8:48
9:00
9:30
9:48
10:30
10:54
12:18
B
neurod1:mitoRGECO EEC mitochondrial calcium
0:00
7:06
7:18
7:36
7:54
8:48
9:00
9:30
9:48
10:30
10:54
12:18


A
immature EECs
"EEC awakening"
EEC maturation and resetting
low cytoplasma Ca^{2+}
low mitochondrial Ca^{2+}
active basal filapodia filament
high cytoplasma Ca^{2+}
high mitochondrial Ca^{2+}
low cytoplasma Ca^{2+}
high mitochondrial Ca^{2+}
lose basal filapodia filament
some EEC form "neuropod"
mitochondria Ca^{2+} / cytoplasma Ca^{2+}
B
Ca^{2+}
Ca^{2+}
Ca^{2+}
ATP
vesicle secretion