The sterol transporter Npc2c controls intestinal stem cell mitosis and host-microbiome interactions in *Drosophila*

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Table S1: Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-Npc2c	Boster	DZ41252
Rabbit anti-phospho Histone H3(Ser10)	Millipore	06-570
Chicken anti-GFP	Invitrogen	A10262
Rabbit anti-GFP	Invitrogen	A6455
Rabbit anti-Cleaved Caspase-3	Cell Signaling	9661
Mouse anti-Prospero	DSHB	MR1A
Mouse anti-Delta	DSHB	C594.9B
Alexa Fluor ™ 488 donkey anti-rabbit IgG	Invitrogen	A21206
Alexa Fluor ™ 488 goat anti-chicken IgG	Invitrogen	A32931
Alexa Fluor™ 555 goat anti-rat IgG	Invitrogen	A-21434
Alexa Fluor™ 555 donkey anti-mouse IgG	Invitrogen	A31570
Alexa Fluor™ 555 donkey anti-rabbit IgG	Invitrogen	A31572
Alexa Fluor™ 555 goat anti-rat IgG	Invitrogen	A-21434
Streptavidin, Cy3	Vector Laboratories	SA-1300
Bacterial Strains		
Pseudomonas aeruginosa, PA14	Apidianakis & Rahme, 2009	NA
Chemicals, Peptides, and Recombinant Proteins		
Drosophila Agar Type II	Apex	66-103
Cornmeal	Local market	NA
Inactivated dried yeast	Local market	NA
Sucrose	Local market	NA
Tegosept	Apex	20-258
Propionic acid	Scharlau	AC18941000
Acetic acid glacial, ACS reagent, ≥99.7%	Sigma	695092
Luria Broth	Condalab	1231.00
Luria Broth agar	Invitrogen	22700-025
Brain Heart Infusion (BHI) broth	Millipore	110493
Hemin	Sigma	51280
Vitamin K_1	Sigma	V3501
QIAzolÒ Lysis Reagent	QIAGEN	79306
Bovine Serum Albumin	Sigma	A7888
Triton™ X-100	Sigma	T8787
Filipin III from Streptomyces filipinensis	Sigma	F4767
RH5849	DrEhrenstorfer	DRE-C16813000
Cholesterol	Sigma	C8667-500MG
20 Hydroxyecdysone	Sigma	H5142
DAPI	Sigma	D9542-10mg
Vectashield Antifade Mounting Medium	Vector	H-1000
Bromophenol blue	Sigma	114391
KAPA SYBR Fast Master Mix (2x) Universal	KAPA Biosystems	KK4602
UltraPure Distilled Water DNase/RNase Free	Invitrogen	10977-035
Ethanol absolute	Scharlau	ET00072500
Methanol	Honeywell	34860
2-Propanol	Honeywell	24137
Chloroform	Millipore	1.02445.2500
DMSO	Sigma	D2650
Agarose	Cleaver Scientific	9012-36-6
Ethidium bromide solution	Sigma	E-1385
Critical Commercial Assays		
RQ1 reagent kit	Promega	M6101
PrimeScript RT Master Mix	Takara	RR036A

KAPA HiFi HotStart Ready Mix PCR Kit	Roche	07958889001
NucleoSpin Gel and PCR Clean up	Macherey-Nagel	740609.50
Experimental Models: *Drosophila melanogaster* Strains		
w^{1118}	BDSC	6326
w; esg-Gal4 UAS-GFP tub-Gal80^{ts} (*esg^{ts}-Gal4*)	Micchelli & Perrimon, 2006	NA
w; esg-Gal4 UAS-GFP tub-Gal80^{ts}; Su(H)-Gal80 (*ISC^{ts}-Gal4*)	Zheng & Hou, 2015	NA
w; Su(H)-Gal4 UAS-CD8GFP tub-Gal80^{ts20}/CyO (*SuH^{ts}-Gal4*)	Tamamouna et al, 2020	NA
w tub-Gal80^{ts}/FM7; Myo1A-Gal4 UAS-EGFP/CyO (*Myo1A^{ts}-Gal4*)	Apidianakis et al, 2009	NA
w hsFLP tub-Gal4 UAS-nls-GFP/FM7; FRT82B tub-Gal80/TM6B	Pitsouli & Delidakis, 2005	NA
w; FRT82B arm-lacZ/TM6B	BDSC	BDSC-7369
w; UAS-Npc2c^{RNAi1}/CyO; FRT82B arm-lacZ/TM6C	This study	NA
w; esg-Gal4 UAS-eGFP tub-Gal80^{ts}/CyO; UAS-Ras1^{Q13}/TM6C	Neophytou & Pitsouli, 2021	NA
w; UAS-Npc2a^{RNAi} (KK108573)	VDRC	v106771
w; UAS-Npc2b^{RNAi} (KK103974)	VDRC	v101233
w; UAS-Npc2c^{RNAi1} (KK103904)	VDRC	v101583
w; UAS-Npc2c ^{RNAi2} (GD6798)	VDRC	v31139
w; UAS-Npc2d ^{RNAi} (GD5679)	VDRC	v31095
w; UAS-Npc2e ^{RNAi} (KK104354)	VDRC	v100445
w; UAS-Npc2f ^{RNAi} (KK111008)	VDRC	v102172
Oligonucleotides		
See Table S2 for primer sequences	Integrated DNA Technologies (IDT)	NA
Deposited Data		
Metagenomic sequencing data	https://www.ncbi.nlm.nih.gov/sra/PRJNA804129	PRJNA804129
Software and Algorithms		
ImageJ	NIH	https://imagej.nih.gov/ij/
Prism 9	GraphPad	https://www.graphpad.com/scientific-software/prism/
Photoshop 2023	Adobe	https://www.adobe.com/cy_en/products/photoshop.html
BLAST	NCBI	https://blast.ncbi.nlm.nih.gov/Blast.cgi
Microsoft Office 2023	Microsoft	https://www.office.com/
Mann-Whitney U Test Calculator	Social Science Statistics	www.socscistatistics.com
Log-Rank Test	MedCalc statistical software	www.medcalc.org
FlyAtlas2	Leader et al, 2017	https://motif.mvls.gla.ac.uk/FlyAtlas2

Table S2: List of primer sequences

Primers used for 16S rRNA amplification			
Name		**Sequence**	
27F		AGAGTTTGATCCTGGCTCAG	
1492R		GGTTACCTTGTTACGACTT	
Primers used in RT-qPCR experiments			
Gene Name	**Gene symbol**	**Forward Primer**	**Reverse Primer**
Alpha tubulin	α-tub	GCTGTTCCACCCCGAGCAGCTGATC	GGCGAACTCCAGCTTGGACTTCTTGC
Attacin A	*AttA*	CACAATGTGGTGGGTCAGG	GGCACCATGACCAGCATT
Broad	*Br*	TAACCTCGGCGTTCGAGAATC	TTTGCAGGGTGTGCTCTTGA
Cyclin A	*CycA*	TCTGGAATATTTCCGGGAGAG	AGCGCATATTGTGGCTAATGT
Cyclin B	*CycB*	GATGCGGCACAGAAAGACTC	TTCTTCCAGTGGCTGTCCA
Cyclin E	*CycE*	ACAAATTTGGCCTGGGACTA	GGCCATAAGCACTTCGTCA
Death-associated inhibitor of apoptosis 1	*Diap1*	CCCCAGTATCCCGAATACGC	TCTGTTTCAGGTTCCTCGGC
decapentaplegic	*dpp*	GTATCTGTGTGCGCTGCTG	CCTAAGCATACTCCTCTTTTCCTC
Delta	*Dl*	GCGACAAGCCCAATCAAT	GGTTCCAGAACGCACTCG
Diptericin A	*Dipt*	GGCTTATCCGATGCCCGACG	TCTGTAGGTGTAGGTGCTTCCC
Drosomycin	*Drs*	GACTTGTTCGCCCTCTTCG	CTTGCACACAGACGACAG
Dual oxidase	*Duox*	CACGCGCAGCAGGATGTAAGGTTT	GCTGCACGCCAACCACAAGAGACT
Ecdysone-induced protein 75B	*Eip75B*	ATGCAACAGAGCACCCAGAAT	CATGGAGTAGGAGGGGCAATC
eiger	*egr*	AGCTGATCCCCCTGGTTTTG	GCCAGATCGTTAGTGCGAGA
glass bottom boat	*gbb*	GAGTGGCTGGTCAAGTCGAA	GAAGCCGATCATGAAGGGCT
hedgehog	*hh*	CAAGGAGCAGTTGCACAGTT	TGTAGAGCGCATTGGCATAC
Insulin-like peptide 3	*Dilp3*	CCGTTCCCTGCTGGAAAGAC	AGGCAACACTCGTCGAAGAC
Insulin-like peptide 7	*Dilp7*	CCTGGCTGCACGTGAACTAT	TGGATGGACAATACTCGGCG
Keren	*Krn*	CCGCTTTAATCGGCGCTTAC	GGTCTCGGCGTTGTGGATAT
Mitf	*Mitf*	AGTATCGGAGTAGATGTGCCAC	CGCTGAGATATTGCCTCACTTG
NADPH oxidase	*Nox*	ACCCATCAACCAGCAGTCAG	GTGCATCCAAATCGAGTACGG
Niemann-Pick type C-2c	*Npc2c*	TCCCTGTGCCTAGTGCTTTCT	GGCTGAGGATAGTTGCTGTCG
puckered	*puc*	GCCACATCAGAACATCAAGC	CCGTTTTCCGTGCATCTT
Ribosomal protein L32	*RpL32*	CGGATCGATATGCTAAGCTGT	CGACGCACTCTGTTGTCG
short gastrulation	*sog*	GAGCGAACCAATCACTCACTC	CAAGTTCTCATTGCTGCCAC
spatzle	*spz*	GTGATTCTGGAAAATGGGATTC	TCTGTGGTGGGTGAAACTTCT
spitz	*spi*	TGCGGTGAAGATAGCCGATC	TTCGCATCGCTGTCCCATAA
Suppressor of cytokine signaling at 36E	*Socs36E*	ATGGGTCATCACCTTAGCAAGT	TCCAGGCTGATCGTCTCTACT
unpaired 1	*upd1*	TGCAGTTGCCGTTCTAGTCA	GCGTGGCGAATAATACTTTCC
unpaired 2	*upd2*	CGGAACATCACGATGAGCGAAT	TCGGCAGGAACTTGTACTCG
unpaired 3	*upd3*	GCAAGAAACGCCAAAGGA	CTTGTCCGCATTGGTGGT
vein	*vn*	TCACACATTTAGTGGTGGAAGC	CGTGACCTCTGCGTTCTGT
wingless	*wg*	CAGGGACGCAAGCATAATAGA	CTGCCGCAGGTTCTTCTC

Table S3: qPCR amplification program

Step	Temperature (°C)	Time (seconds)	Repeats of Cycles
Initial Denaturation	95	30	1
Denaturation	95	10	
Annealing	60	30	40
Extension	65	30	
Final extension	65	60	1

Table S4: 16S colony PCR amplification program

Step	Temperature (°C)	Time	Repeats of Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	20 sec	
Annealing	60-75	30 sec	25
Extension	72	90 sec	
Final extension	72	5 min	1

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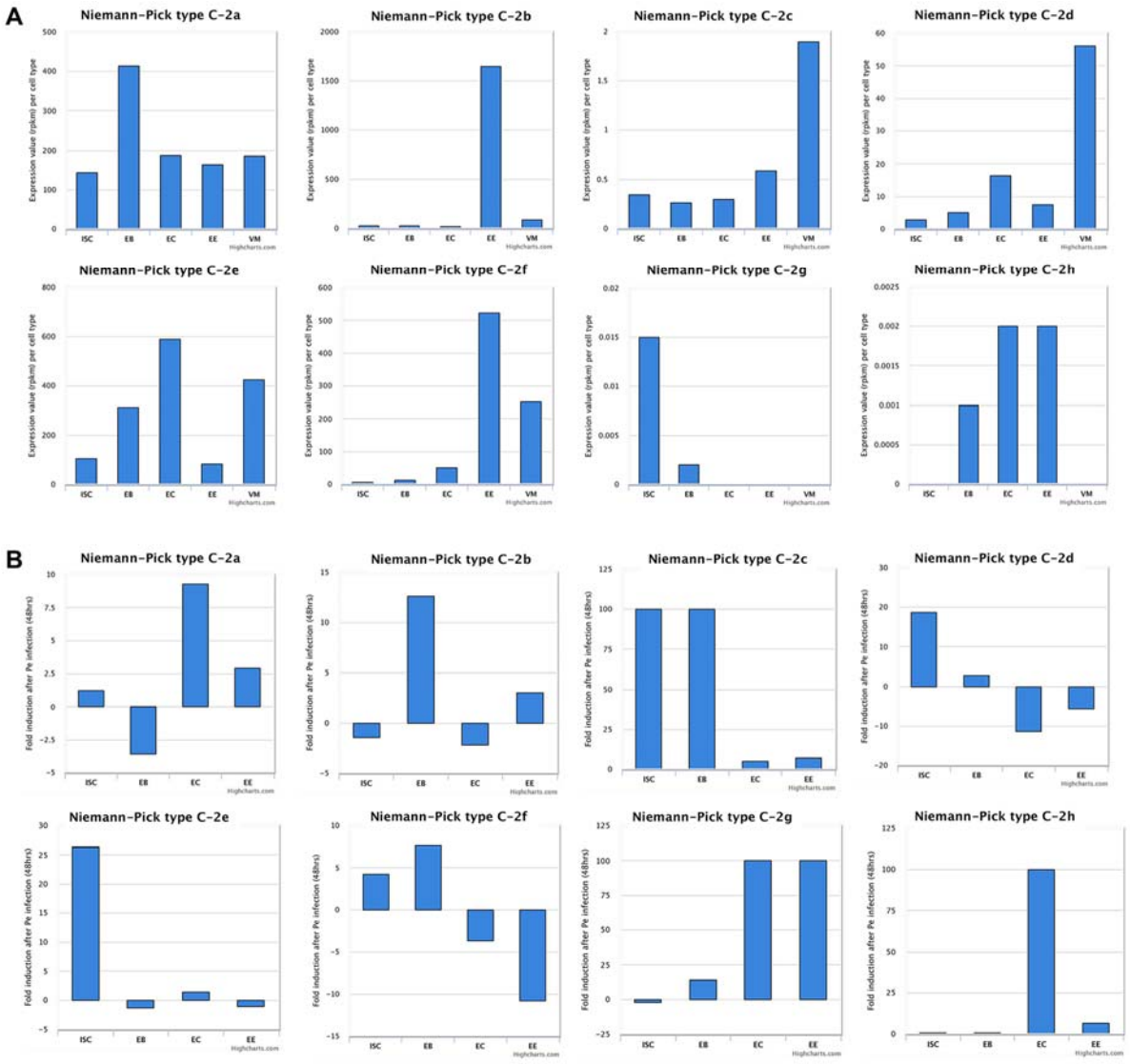

Figure S1. *Npc2* gene family expression in adult midguts. (**A**) *Npc2a-h* transcript expression in different adult midgut cell types. (**B**) *Npc2a-h* transcript expression in different adult midgut cell types upon *P. entomophila* infection. Data retrieved from FlygutSeq (http://flygutseq.buchonlab.com/).

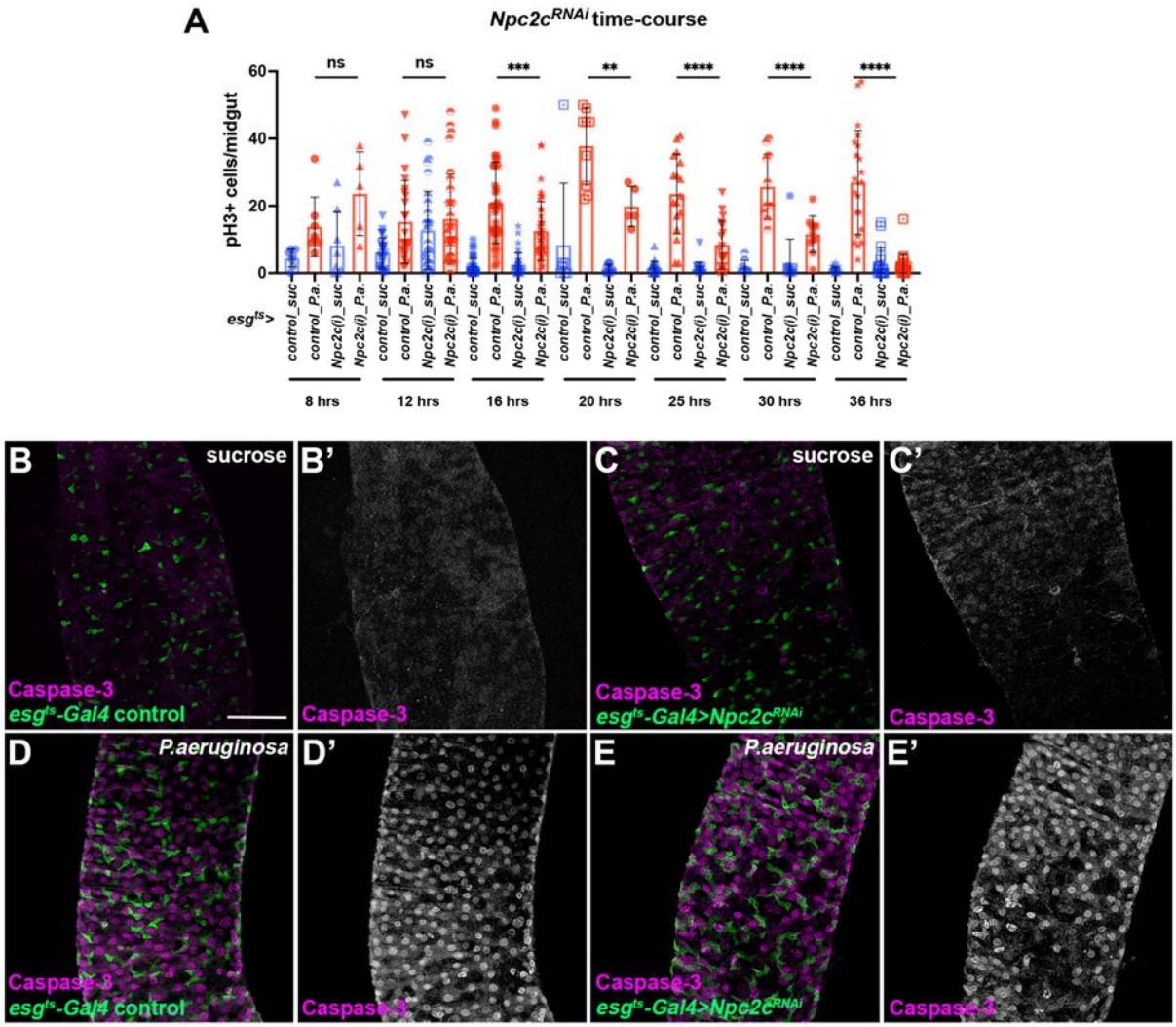

Figure S2. Progenitor-specific *Npc2c* silencing reduces mitosis effectively, but it does not cause apoptosis. (**A**) Quantification of the mitotic index (pH3-positive cells) in *esg^{ts}-Gal4* control and *esg^{ts}-Gal4>Npc2c^{RNAi1}* adult midguts during a time-course experiment of 8, 12, 18, 20, 25, 30, and 36 hrs post-transgene induction in uninfected (blue bars) and *P.a.*-infected (red bars) flies. Mitosis is gradually reduced and by 36 hrs it is eliminated in both uninfected and *P.a.*-infected midguts. (**B-E**) *esg^{ts}-Gal4* control (B,D) and *esg^{ts}-Gal4>Npc2c^{RNAi1}* (C,E) adult posterior midguts without (B-C) and with *P.a.* infection (D-E) stained for anti-cleaved Caspase-3 (magenta). GFP (green) labels the intestinal progenitors. The Caspase-3 channel is shown separately in grey in B'-E'. Although infection induces apoptosis, this is restricted to mature intestinal cells. Scale bars 75 μm. Experiments were repeated at least twice. Statistical significance in A was tested with the Student's t-test: ns, not significant, * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$, *** $0.0001<p\leq0.001$, **** $p\leq0.0001$.

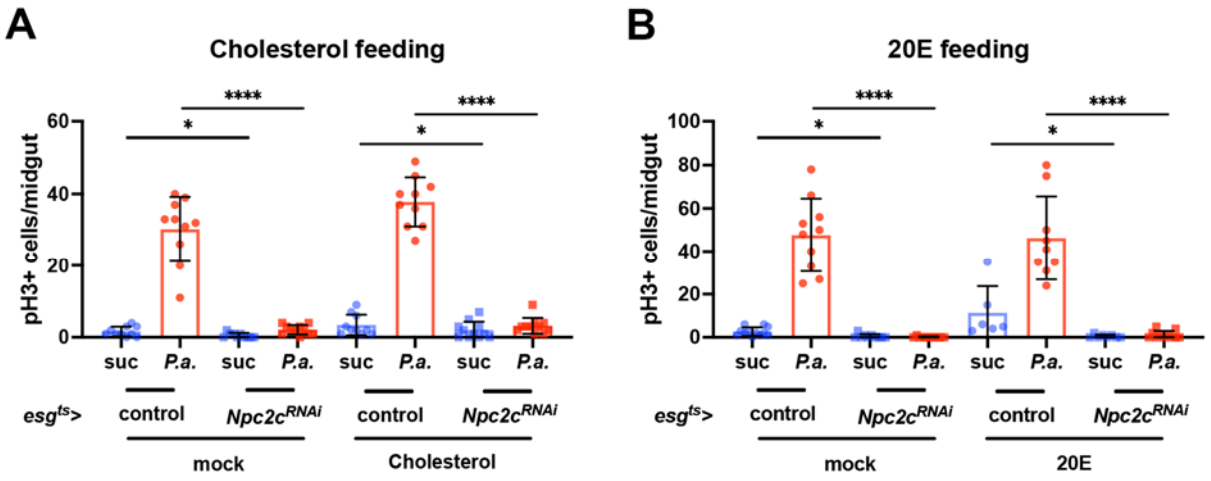

Figure S3. Supplementation of the diet with cholesterol or 20E does not rescue reduced mitosis caused by *Npc2c* silencing. (**A**) Quantification of the mitotic index (pH3-positive cells) in *esg^{ts}-Gal4* control and *esg^{ts}-Gal4>Npc2c^{RNAi1}* uninfected and *P.a.*-infected adult midguts upon cholesterol supplementation. (**B**) Quantification of the mitotic index (pH3-positive cells) in *esg^{ts}-Gal4* control and *esg^{ts}-Gal4>Npc2c^{RNAi1}* uninfected and *P.a.*-infected adult midguts upon 20E supplementation. Error bars correspond to the standard deviation. Experiments were repeated at least twice. Statistical significance in E was tested with the Student's t-test: ns, not significant, * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$, *** $0.0001<p\leq0.001$, **** $p\leq0.0001$.