Ayyaz et al. Supplementary Information

METHODS

Fly lines and husbandry

Flies were cultured on yeast/molasses-based food at 25°C with a 12 hours light/dark cycle and only female animals were used in all experiments.

The following fly lines were used: W^{1118} (WT), y^1w^1, FRT40, FRTG13, FRT82, upd1^{RNAi} (BL28722), upd2^{RNAi} (BL33949), upd3^{RNAi} (BL28575), Dpp$^{RNAi\ \#1}$ (BL33618) [18], Dpp$^{RNAi\ \#2}$ (BL25782), Sax^{RNAi} (BL36131) [21], Smox^{RNAi} (BL26756), Mad^{RNAi} (BL31315), Gbb^{RNAi} (BL34898) [21,22], UAS::Dpp (BL1486), UAS::Tkv (BL51653), UAS::Tkv^{QD} (BL36536) [18], UAS::CD8-GFP (BL5137), UAS::rpr (BL5823), Smox::FLAG (BL43958), EGFR-GOF (BL9536: p{Egfr.1.A887T.UAS}c12-7), Mad1-2,FRT40/cyo (BL7323), Med13,FRT82/TM3 (BL7340), Sax4,FRTG13/cyo (BL5404), SmoxMB388,FRT19A/FM7c (BL44384), tubG80^{ts} (BL7017 and BL7108), hmlΔ::Gal4,UAS::GFP (BL30140, BL30142), hmlΔ::Gal4 (BL30141), p[He-Gal4] (BL8699), Dpp::Gal4 (BL1553), Dpp::Gal4^{Gut} (BL45111) [20], How::Gal4 (BL1767) were provided by Bloomington Drosophila Stock Center. Tkv^{RNAi} (vdrc3059), Spi^{RNAi} (vdrc103817), Vein^{RNAi} (vdrc109437), STAT^{RNAi} (vdrc106980), pvf1^{RNAi} (vdrc46875), pvf2^{RNAi} (vdrc7629), Bsk^{RNAi} (vdrc34138), and Keren^{RNAi} (vdrc104299) were obtained from the Vienna Drosophila RNAi Center. Cardia-Gal4 (103522), dve::Gal4 (113273) and UAS::Sax (F001576) were obtained from Drosophila Genomics Resource Center (DGRC) and FlyORF (Zurich ORFeome Project), respectively. The line UAS::hid was a gift from J.K. Billeter, dad::nlsGFP from G. Pyrowolakis, UAS::DppGFP from A. Lander, Vkg-GFP from B. Biteau [11], Mad12,FRT40/cyo, Tkv8,FRT40/cyo [18], 5966::GeneSwitch [49], MARCM40A and MARCM82A from B. Ohlstein, tkva12,FRT40/cyo[18], Btl::Gal4^{ts} from D. Bohmann, byn-Gal4 from V. Hartenstien, Eater::DsRed from T. Tokusumi [30], NP1G80^{ts} from D. Ferrandon [16], esg::Gal4 [49], UAS::GFP from S. Hayashi, esg::Gal4,Su(H)GBE::G80,UAS::2XEYFP,tub::G80^{ts} from S. Hou [66], hmlΔ::RFP from K. Bruckner [33], tkv8,FRT40/cyo, tkv04415,FRT40/cyo [20], Notch^{RNAi}, hsFlp, yw; X.15.29 and yw; X.15.33 [38] from N. Perrimon, 2XSTAT::GFP from E.A. Bach, UAS::HopTumL from D. Bilder, and UAS::upd2 from M. Zeidler. hmlΔ::GeneSwitch and hmlΔ::Dpp^{RNAi} were made in this study. Hemoless and HDD flies were made by crossing hml::Gal4,UAS::GFP line with UAS::hid and Dpp^{RNAi}, respectively, and animals were allowed to develop at 25°C. Also see Supplementary Table 1 for specific genotypes used for individual experiments in each figure.

Generation of transgenic animals:

To construct the hmlΔ::GeneSwitch line, a ~839 bp fragment was amplified from crude DNA of *w1118* flies using primer sequences: *Fw*: ACG CGT CAA AAG TTA TTT CTG TAG GC, *Rev*: ACG CGT TTT GTT AGG CTA ATC GGA AAT TG (also see [33]). The resulting hmlΔ promoter fragment was cloned into pP[UAS-GeneSwitch] vector [67] at a single MluI restriction site. To generate hmlΔ::Dpp^{RNAi} transgenic animals, a ~1.5 Kbp DNA fragment containing Dpp hairpin was amplified from DNA extract of a UAS::Dpp^{RNAi} fly line generated at Transgenic RNAi Project (TRiP): TRiP # HMS00011 (Hairpin ID: SH.00007.N); Bloomington Stock # 33618 (also see [68] for construction of the original TRiP fly line and the specificity of the hairpin construct against Dpp), using primer sequences: *Fw*: agg cct tct agc agt TCG TTC AGT GAT AGT GAT AAA tag tta tat tca agc ata, and *Rev*: aac cgg ttg ttg ttg gtt ggc aca cca caa ata tac tg; while actual Dpp short hairpin sequence is shown in capital letters. The resulting Dpp^{RNAi} fragment was then cloned into StuI/AgeI sites of *hmlΔ::pRed H-Pelican* vector, a kind gift from Dr. Katja Brueckner [33], to generate *hmlΔ::Dpp^{RNAi}-pRed H-Pelican* vector. Transgenic flies were generated by standard procedures (Genetic Services, MA).

Selection of Dpp^{RNAi} lines

A recent study [21] has reported contradictory effects of Dpp on ISC proliferation and self-renewal when Dpp was knocked down in ECs using two different RNAi lines: Bloomington Stock Number 33628, termed DppRNAi^{S} by the authors, and Bloomington Stock Number 25782 (called DppRNAi^{W}). A recent comment on flybase.com explains, however, that, based on flybase release 5.43, DppRNAi^{S} (Bloomington Number 33628) does not match Dpp transcripts accurately (http://flystocks.bio.indiana.edu/Reports/33628.html), while DppRNAi^{W} line (Bloomington Number BL25782) as well as a third DppRNAi line (Bloomington Number BL33618) specifically target Dpp transcripts and have no reported off targets. Both of the later lines efficiently knock down Dpp transcripts (Supplementary Fig.3E) yet do not inhibit ISC proliferation during stress when expressed by an EC-specific driver (Supplementary Fig.3D and [21]). Due to their target specificity, we have used these two lines in our analyses.

Immunostaining and Microscopy

Adult female *Drosophila* guts were dissected in 1x phosphate-buffered saline (PBS), fixed for 45 minutes at room temperature in fixative (100 mM glutamic acid, 25mM KCl, 20 mM MgSO4, 4 mM sodium phosphate, 1 mM MgCl2, and 4% formaldehyde), washed for 1 hour at 4°C in

washing buffer (1X PBS, 0.5% bovine serum albumin and 0.1% Triton X-100), and then incubated in primary antibodies (4°C overnight) and secondary antibodies (4°C for 4 hours or overnight) in washing buffer, between which was 1 hour washing at 4°C. Staining with pSMad3 antibody was performed using a phosphatase inhibitor (Roche, 4906837001) during fixation, 1 hour wash, and primary antibody incubation following the same protocol above. Staining with Delta and FLAG antibodies was performed following the methanol-heptane fixation method described in [18].

In order to monitor dynamics of hemocyte attachment to the intestine, flies were held with thin entomological pins in Sylgard coated small petri dish and dissected directly into fixation solution (100 mM glutamic acid, 25mM KCl, 20 mM MgSO4, 4 mM sodium phosphate, 1 mM MgCl2, and 4% formaldehyde). Forceps were used to gently remove dorsal cuticle in order to expose the body cavity to fixative for at least one hour. Observations were then made either directly under fluorescent microscope or opened animals were incubated in a described staining solution. Animals were finally washed 3X and guts were carefully removed, preserving all tissues attached to the gut, and mounted to perform confocal microscopy. To examine the morphology of circulating hemocytes (Supplementary Fig.2D), hemolymph was collected in PBS (as described below) from 20 flies and transferred to glass slides. Hemocytes were then allowed to settle for 30 minutes at room temperature followed by fixation for 15 minutes and subsequent incubation in Phalloidin solution overnight at 4°C.

Primary antibodies and dilution: rabbit anti-pSMad3 (Epitomics, 1:300); rabbit anti-β-galactosidase (Cappel, 1:5000); rabbit anti-GFP (Invitrogen, 1:500); rabbit anti-phospho-Histone H3 Ser10 (Upstate, 1:1000), mouse anti-Prospero (Developmental Studies Hybridoma Bank, DSHB, 1:250), mouse monoclonal anti-Cut (DSHB, 1:100), mouse anti-Armadillo (DSHB, 1:100), rabbit anti-Sax (abcam, ab42105, 1:200), rabbit anti-FLAG (Sigma, 1:300), rat anti-Delta (gift from M. Rand, 1:1000), mouse anti-NimC1 (gift from István Andó, 1:30)[31] and rabbit anti-Tkv (gift from M. Gonzalez-Gaitan, 1:100). Fluorescent secondary antibodies were from Jackson Immunoresearch. DAPI was used to stain DNA and Phalloidin (Invitrogen) to stain actin. The images of whole fly were taken on Zeiss dissecting fluorescent microscope, and all other images were taken on a Zeiss LSM 700 confocal microscope and processed using Adobe Photoshop, Illustrator and Image J.

Conditional expression of UAS-linked transgenes

The TARGET system was used in combination with indicated Gal4 driver to conditionally express UAS-linked transgenes [69]. Flies were developed at 18ºC, and then shifted to 29°C to induce transgene expression. For GeneSwitch drivers, flies were developed on normal food at 25°C while 2-5 days old adults were transferred to Mifepristone (RU486) containing food for 2 days before performing experiments.

Bacterial infection, Paraquat treatment and feeding assays

Previously described procedures [10,13,15] were followed for oral bacterial challenge. Briefly, the bacterial strains, Ecc15 or *P. entomophila*, were cultured in LB medium overnight at 30°C. Flies were fed on 500ul of concentrated bacteria in 5% sucrose (OD100) for the time indicated and the same volume of 5% sucrose as control. To test feeding efficiency, 5% Bromophenol blue was mixed in infection solution before flies were transferred to feed for 90 minutes. CAFÉ assay was performed as described previously [18]. For paraquat exposure, flies were fed on either 5 mM Paraquat in 5% sucrose solution or only on 5% sucrose as control, for an indicated time. In case of each of the above treatments, flies were starved in empty vials for 2 hours. For survival experiments, flies were either orally infected with *P. entomophila* for 2 days and shifted for another 2 days to a cocktail of antibiotics as described previously [10], before start monitoring rate of fly survival (Fig. 7A), or a tungsten needle was used to directly inject *P. entomophila* into fly hemolymph as described in [28], followed by monitoring their survival (Supplementary Fig.1F).

Hemolymph extraction and transfer

Hemolymph was extracted or re-injected into flies using NanojectII (Drummond). Transgene expression in donor flies was induced through the TARGET system by incubating approximately 9 days old adult animals for 3 days at 29ºC. Maximum amount of hemolymph was then collected from both sides of thorax of donor flies. Thin sterile glass capillaries were used with NanojectII apparatus which extracts the fly hemolymph primarily by capillary action thus making it sure that only sterile and clean hemolymph containing circulating hemocytes was collected. Quantification (see below) shows that hemolymph collected from a single fly in this manner should contain about 85 hemocytes. The collected hemolymph was then quickly transferred into the recipients without exposing it to any external media. 2-3 donor flies were used to transfer hemolymph into a single recipient fly of the same age (about 12 days old). Thus, in this way each recipient fly should receive at least 171-257 hemocytes (see below for calculations).

Finally, the injected animals were allowed to recover overnight before Paraquat treatment or bacterial challenge for the described time period.

Hemocyte Quantification

In order to quantify the number of hemocytes transferred from donor flies into the recipients, maximum amount of hemolymph was collected from 5 wild-type hmlΔ::Gal4,UAS::GFP adult flies of a given age by the method described above, and dissolved into 100uL of PBS. Total number of retrievable circulating GFP-positive hemocytes was then manually counted using a fluorescent microscope after platting the solution on glass slides. Since number of hemocytes extracted from the hemolymph of 12 days old flies was significantly higher, animals of this age were always used in hemolymph transfer experiments. A slightly separate method was used to compare number of hemocytes in 3 days old wild-type and HDD flies, where hemolymph collected from only one side of the thorax cuticle of 20 flies was collected in 10uL PBS and hemocyte quantification was performed using hemocytometer (LifeTechnologies) following manufacturer's recommendations.

LacZ clone and MARCM clone induction

LacZ marked clones were generated in hsFlp; X.15.29/X.15.33 flies [38] combined with indicated genotypes. 2-3 days old flies were heat-shocked for 45 minutes at 37°C and kept at room temperature (RT) for 7 days before dissected. For MARCM clone induction [34], 2-3 days old flies were heat-shocked for 45 minutes at 37°C. The flies were either kept for 7 days at RT before dissected, or after 1 day subjected to bacterial infection for 24 hours, and then kept for 3 days at RT before dissected.

Lifespan experiments

Lifespan experiments were performed following previously described procedure [49]. Briefly, hmlΔ::Gal4 driver was backcrossed 7 times with the wild-type flies ($y^1.w^1$). Resulting hmlΔ::Gal4/+ flies were then crossed either with wild-type (+/+) or UAS::Hid or UAS::DppRNAi (Bloomington Stock # 33618; back crossed four times with the wild-type) in separate bottles, each with at least 4 replicates. Progenies with different genotypes (as described in Supplementary Fig.8F) from each bottle were then split into two and transferred to separate rearing cages, with a maximum population density of 100 animals per cage. Thus, survival of

each cohort of about 50-100 flies in a certain cage could directly be compared with the sister fly group that had developed together as larvae in the same bottle but had different genotype.

qRT-PCR analysis

Total RNA from intestines of 20 flies (Supplementary Figs.4A and 3E), or hemolymph of 50 flies (Supplementary Fig. 3J), or carcass of 10 flies (Supplementary Fig. 3E) was extracted using Trizol (Invitrogen). cDNA was synthesized using an oligo-dT primer. Real-time PCR was performed on a Bio-Rad CFX96 detection system. Relative expression was normalized to Actin5C or Rp49 (RpL32). Primer sequences:

Actin5C (F): 5'- CTCGCCACTTGCGTTTACAGT -3'

Actin5C (R): 5'- TCCATATCGTCCCAGTTGGTC-3'

Rp49 (F): 5'-TCCTACCAGCTTCAAGATGAC-3'

Rp49 (R): 5'-CACGTTGTGCACCAGGAACT-3'

Dpp (F): 5'-CAGCACGCCTTCGGCACCTT-3'

Dpp (R): 5'-GGCACTCGCTGTACGTGGACTTCTC-3'

Upd3 (F): 5'- ACAAGGCCAGGATCACCACCAAT-3'

Upd3 (R): 5'- TGTACAGCAGGTTGGTCAGGTTGA-3'

Statistical Analyses

Animals of same age and genotype that received diverse treatments in different experiments were indiscriminately selected in all experiments. No randomization was performed among differentially treated animals. In order to blind the investigators for allocation of treatments and the outcomes from experiments shown in Figs.1A-C,3A-C,3E,5C,7A,7F and Supplementary Figs.3A,4F,6F,8F genetically different cohorts were numerically or alphabetically marked at the time of parental crosses. Once a particular experiment was complete and required data had been collected blindly, the arbitrary marks were matched with parental genotypes to designate final results to actual genotypes of tested animals. For all other experiments, investigators were not blinded to allocation during experiment and outcome assessment. Sample size, mean, median, range, variation and number of replicates for individual experiments are described in corresponding figure legends. No statistical method was used to predetermine sample size. Prism and MS Excel software were used for all statistical analyses.

SUPPLEMENTARY REFERENCES

66. Wang, L., Zeng, X., Ryoo, H.D. & Jasper, H. Integration of UPRER and oxidative stress signaling in the control of intestinal stem cell proliferation. *PLoS Genet* **10**, e1004568 (2014).

67. Osterwalder, T., Yoon, K.S., White, B.H. & Keshishian, H. A conditional tissue-specific transgene expression system using inducible GAL4. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 12596-601 (2001).

68. Ni, J.Q. et al. A genome-scale shRNA resource for transgenic RNAi in Drosophila. *Nat Methods* **8**, 405-7 (2011).

69. McGuire, S.E., Mao, Z. & Davis, R.L. Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. *Sci STKE* **2004**, pl6 (2004).