Supporting Information

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SI Materials and Methods

***Drosophila* Stocks and Culture.** We used the second chromosome *esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}* cassette (1). To track *esg-Gal4* cells with luciferase, we used a *UAS-luciferase* transgene (2) integrated at attP2 to create *esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}*; UAS-luciferase stock. To create flies with *esg-Gal4*–driven tumors that could be tracked with either GFP or luciferase, we crossed *UAS-Raf^{gof}* (gain-of-function allele of the serine-threonine kinase Raf) (3) to *esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}; UAS-luciferase* flies to generate F1 *Raf^{gof}/+; esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}/+; UAS-luciferase*/+. We used these F1 flies for our chemical screens and follow-up analyses. The F1 flies were raised at room temperature (22 °C), and as adults were shifted to 29 °C to induce expression of *UAS-Raf^{gof}*, *UAS-GFP*, and *UAS-luciferase*. For wild-type (WT) controls, we crossed *esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}; UAS-luciferase* to *yw* flies to create F1 *esg-Gal4, UAS-GFP, tubulin-Gal80ts/+; UAS-luciferase*/+. To monitor Upd-3 expression, we used *upd3-Gal4;UAS-GFP* stock (4).

Tumor Transplantation. Adult tissue donors were incubated at 29 °C for 3 d before their posterior midguts were harvested for injection into host recipients. Posterior midgut donor tissue was obtained from (*i*) RAF^{gof} intestinal stem cell (ISC) tumor flies of the genotype *UAS-Raf^{gof}/+; esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}/+; UAS-luciferase*/+ and (*ii*) WT flies of the genotype *esg-Gal4, UAS-GFP, tubulin-Gal80^{ts}/+; UAS-luciferase*/+ flies. Posterior midguts were dissected in PBS, minced into small fragments, and loaded into a glass capillary needle suitable for an Eppendorf FemtoJet Injection System. Tissue fragments were transferred to anesthetized *w1118* female adults by injection into the midventral abdomen, as previously described (5).

Drug Screening. The screen was conducted in 96-well plates as follows: (*i*) fly chemical screening food was boiled, cooled to 37 °C, and aliquoted to plates at 300 μL/well; (*ii*) drugs were added at 3 μL/well and mixed by pipetting up and down five times; (*iii*) after food solidified, flies were added to the wells (three females per well for the screen of 88 FDA-approved drugs and two females per well for the screen of 6,100 drugs). Flies were reared in the plates for 3 d at 29 °C and then homogenized for luciferase assays. The 88 FDA-approved drug set was screened in duplicate: in the first round, the flies were fed drugs for 3 d and, in the second round, they were fed for a total of 6 d (with a transfer to fresh drug preparations on day 3). Drugs that reduced luciferase activity by 50% or greater in both biological replicates relative to the DMSO controls were scored as hits. The library of 6,100 compounds was screened in triplicate. Drugs that reduced luciferase activity by 50% or more in at least two of the three biological replicates relative to DMSO were scored as hits. Complete screen data are available in Dataset S1.

Luciferase Assay. Flies were anesthetized with CO_2 and transferred from 96-well drug-screening plates to standard-sized 96-well plates (Costar 3917). They were homogenized with a 96-well plate multi-homogenizer (Burkard Scientific, BAMH-96 1911101). The luciferase activity was measured in fly lysates as previously described (2). Lysates were stored at −80 °C, defrosted on ice, and aliquoted to 96-well plates for luciferase assays.

Immunofluorescence and Microscopy. We used the following primary antibodies: mouse monoclonal anti-Delta 1:50 (Developmental Studies Hybridoma Bank), rabbit monoclonal anti-dpERK 1:200 (Cell Signaling), and rabbit polyclonal anti-phosphohistone H3 1:10,000 (Millipore). The secondary antibodies were obtained from Molecular Probes (Invitrogen): Alexa 555-conjugated donkey anti-mouse, Alexa 647-conjugated goat anti-rabbit, and Alexa 647-conjugated donkey anti-mouse secondary. Adult females were anesthetized and decapitated and then dissected in PBS and fixed for 20 min as described (6). Dissected intestines were incubated in 5% normal donkey serum blocking solution (for 1 h), primary antibodies (overnight), secondary antibodies (for 1.5 h), and DAPI (for 6 min). Samples were mounted in Vectashield (Vector) mounting media and imaged with Leica TCS SP2 and Zeiss LSM700 confocal microscopes using a 40× oil immersion objective.

1. Micchelli CA, Perrimon N (2006) Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* 439(7075):475–479.
2. Markstein M, Pitsouli C, Villalta C, Celniker SE, Perrimon N (2008) Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. *Nat Genet* 40(4):476–483.
3. Brand AH, Perrimon N (1994) Raf acts downstream of the EGF receptor to determine dorsoventral polarity during *Drosophila* oogenesis. *Genes Dev* 8(5):629–639.
4. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N (2003) Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev Cell* 5(3):441–450.
5. Caussinus E, Gonzalez C (2005) Induction of tumor growth by altered stem-cell asymmetric division in *Drosophila* melanogaster. *Nat Genet* 37(10):1125–1129.
6. Ohlstein B, Spradling A (2007) Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* 315(5814):988–992.

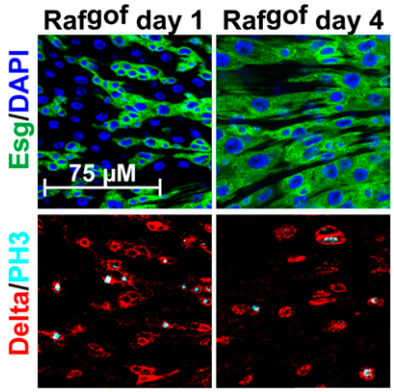

Fig. S1. RAF^{gof} tumors maintain a population of mitotic ISC-like cells. Mitotically active cells in RAF^{gof} ISC tumors are *esg+* (green) and express the mitotic marker PH3 (cyan) and the ISC marker Delta (red). Nuclei are stained with the DNA dye DAPI (blue).

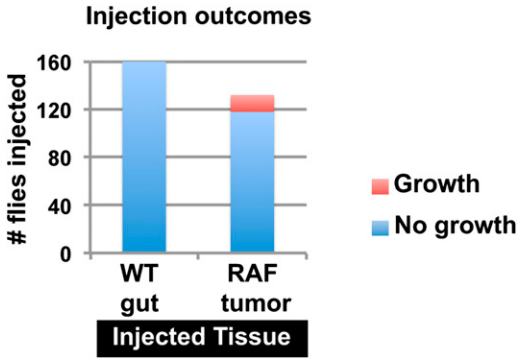

Fig. S2. The fate of WT and RAF^{gof} intestinal fragments injected into WT hosts. Over 100 injections were performed for each genotype.

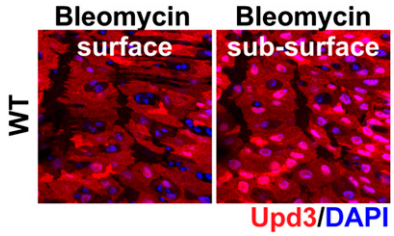

Fig. S3. The expression of Upd-3 viewed from the surface and subsurface of the intestinal epithelium, using bleomycin-induced Upd-3 expression as an example. In the surface view, the nuclei of stem and progenitor cells are in focus, and in the subsurface view, 1 μM lower, the enterocyte (EC) nuclei are in focus. Upd-3 is clearly absent from the stem and progenitor nuclei and present in the EC nuclei. Additionally, Upd-3 is present in the EC cytoplasm.

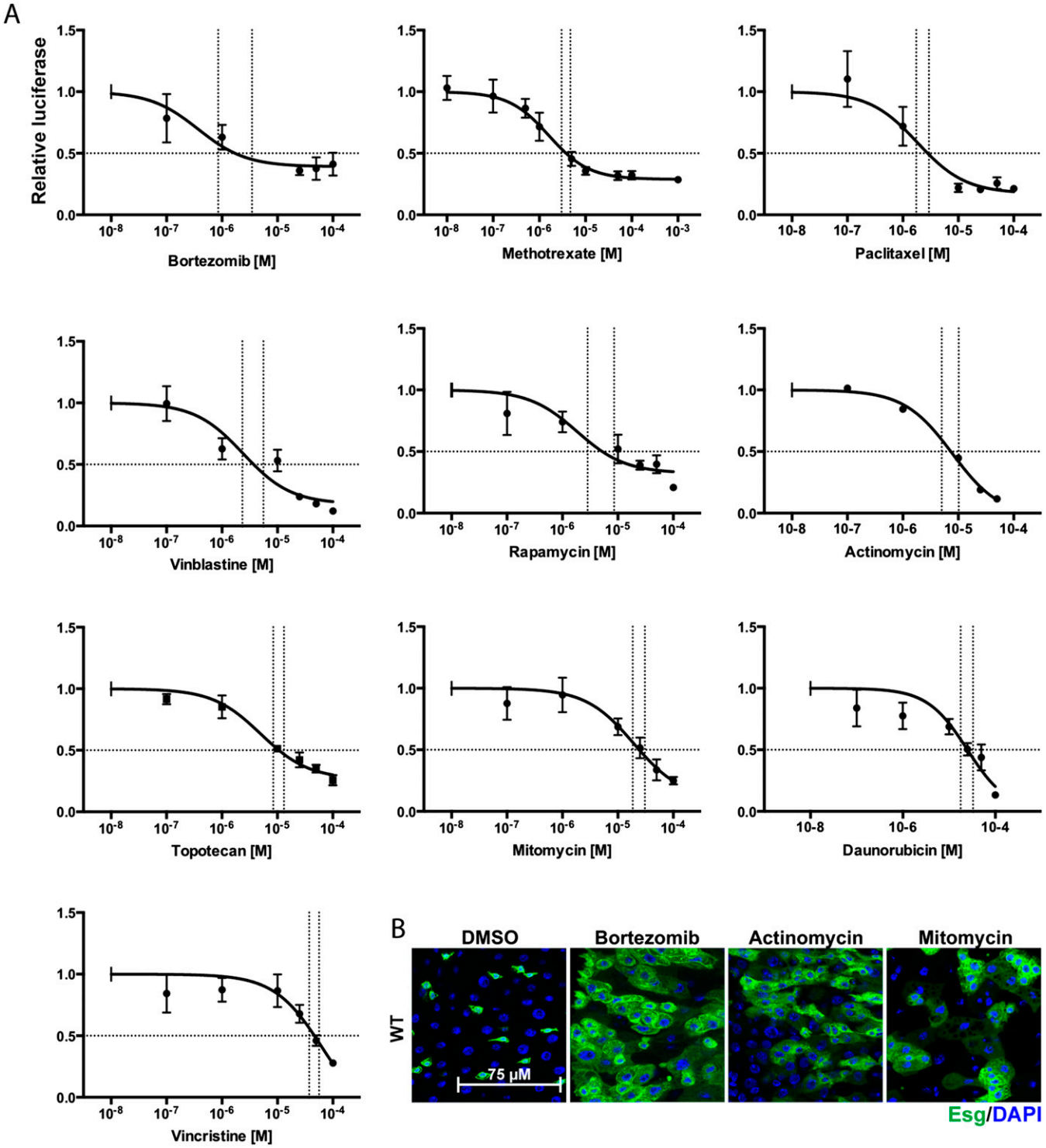

Fig. S4. The IC_{50} of class II drugs. (*A*) Dose–response curves for the class II drugs and three class I drugs displayed in order from drugs with the lowest to the highest average IC_{50} values. The IC_{50} values ranged from 2 μM for bortezomib to about 50 μM for vincristine. Each point represents the average of eight independent biological replicates; error bars = 1 SD. The vertical dotted lines delimit the 95% confidence level of the IC_{50} values. (*B*) Confocal images of posterior midguts dissected from WT flies after treatment with class II drugs at IC_{50} values within 95% confidence ranges: actinomycin (2.5 μM), bortezomib (3.5 μM), and mitomycin (4 μM). To visualize the effects of the IC_{50} doses, we fed the drugs to flies for 3 d and then dissected their intestines. Half of the class II drugs—paclitaxel, vinblastine, vincristine, and daunorubicin—when tested at IC_{50} levels, failed to be highly effective against the tumors and likewise failed to induce significant proliferation in WT ISCs. The other class II drugs—bortezomib, actinomycin, and mitomycin—when tested at IC_{50} levels, remained effective against the tumors but also still promoted the side effect on WT ISCs. These results indicate that the therapeutic windows of class II drugs may not be easily separable from their side effect on WT ISCs, suggesting that the best way to circumvent the side effect is to find drugs that avoid the side effect altogether.

Table S1. Small-molecule screening data

Category	Parameter	Description
Assay	Type of assay	Whole-animal assay in *Drosophila* for tumor reduction
	Target	Stem-cell–initiated RAF(gof) tumors expressing luciferase
	Primary measurement	Luciferase from whole-animal homogenates
	Key reagents	Promega Steady-Glo luciferase kit
	Assay protocol	See *SI Materials and Methods*
Library	Library size	6,100 compounds
	Library composition	Known bioactives, synthetic and natural products
	Source	Harvard Institute of Chemistry and Cell Biology (http://iccb.med.harvard.edu/)
Screen	Format	96-well plates
	Concentration(s) tested	100 μM (followed up with dose–response)
	Plate controls	DMSO and methotrexate
	Reagent/compound dispensing system	Pin transfer with Epson and Seiko robots
	Detection instrument and software	Analyst plate reader from molecular devices
	Assay validation	Three biological replicates; hits further validated by dissection to visualize GFP-expressing tumors
	Normalization	Normalize to number of flies per well and the median of eight DMSO wells per plate
Analysis	Hit criteria	Must score at or below luciferase cutoff of reduction of 50% luciferase activity compared with DMSO controls in two of three biological replicates
	Hit rate	1/500
	Additional assay(s)	Hits validated by dissection of flies and direct observation of GFP-expressing tumors

Other Supporting Information Files

Dataset S1 (XLSX)