

Supplementary Materials for

Recurrent phases of protein depletion rescue tumor phenotypes in a *Drosophila* intestinal cancer model

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This file includes Supplementary Figures S1 to S7 and Table S1

Fig. S1

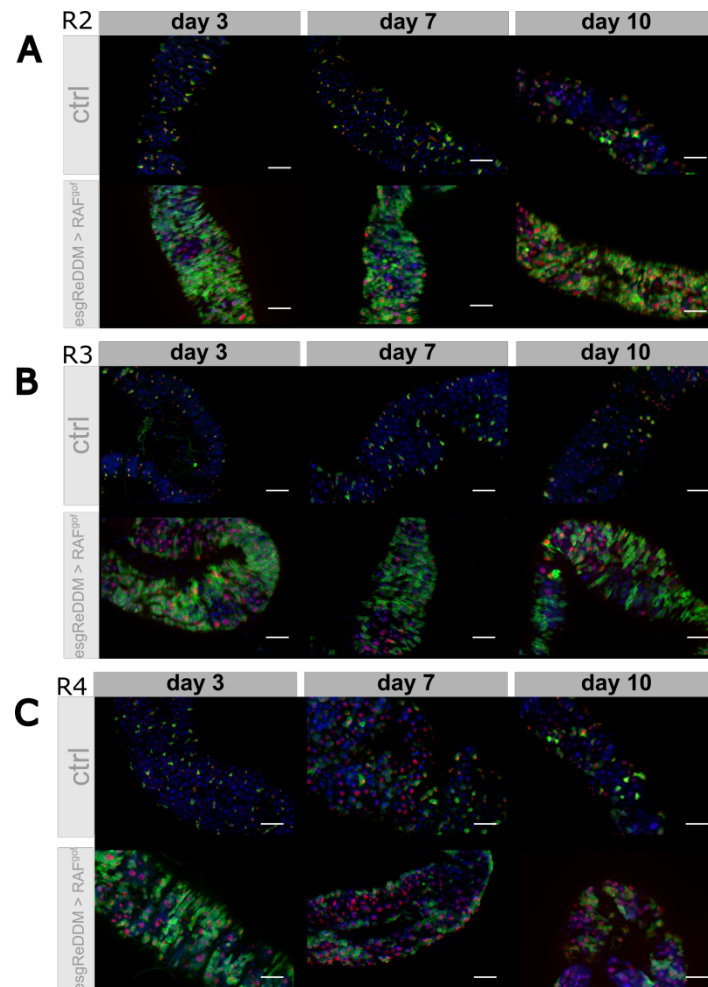


Fig. S1: Lineage tracing in midgut region R2 (A), R3 (B), and R4 (C) of control flies and flies expressing RAF^{gof} in ISCs and EBs. The ReDDM system (1) was used to visualize the development of ISCs (expressing GFP) into enteroblasts (expressing RFP). Intestines were dissected after 3, 7 or 10 days of tumor induction from flies on control diet. During differentiation the short-lived GFP fades, leaving fully differentiated enteroblasts marked with RFP only (scale bar $50\mu\text{m}$). ctrl = $esgReDDM > w1118$.

- (1) Antonello ZA, Reiff T, Ballesta-Illan E, Dominguez M. Robust intestinal homeostasis relies on cellular plasticity in enteroblasts mediated by miR-8-Escargot switch. *EMBO J.* 201534(15):2025-2041.

Fig. S2

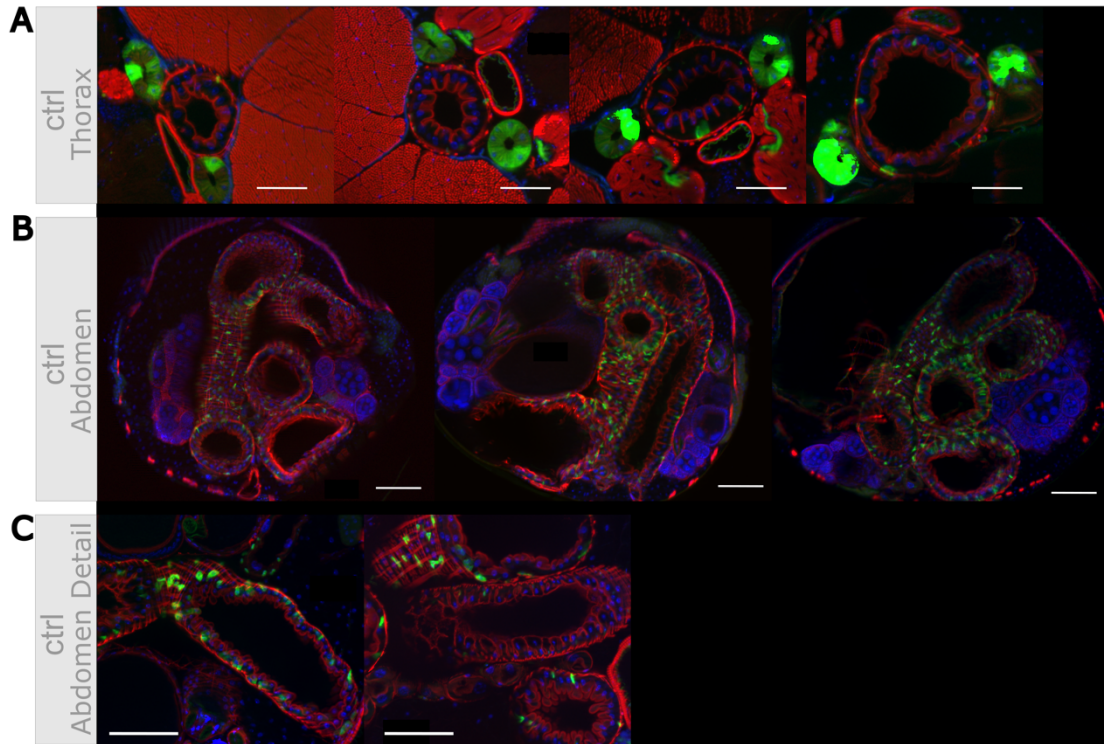


Fig. S2: Intestinal structure and phenotype variability of healthy control flies and oncogene expressing flies on a nutritious control diet. A-C, top: control flies (EGT;Luc2 > w¹¹¹⁸) and A-C, bottom: oncogene-bearing flies (EGT;Luc2 > RAF^{gof}) after 3 days of induction, showing (A) sections through the thorax (anterior midgut, R2) (scale bar 50 μm); (B) section showing multiple intestinal loops (R3-5 midgut region) (scale bar 100 μm); and (C) detail of an intestinal loop (region not specified, scale bar 100 μm).

Fig. S3

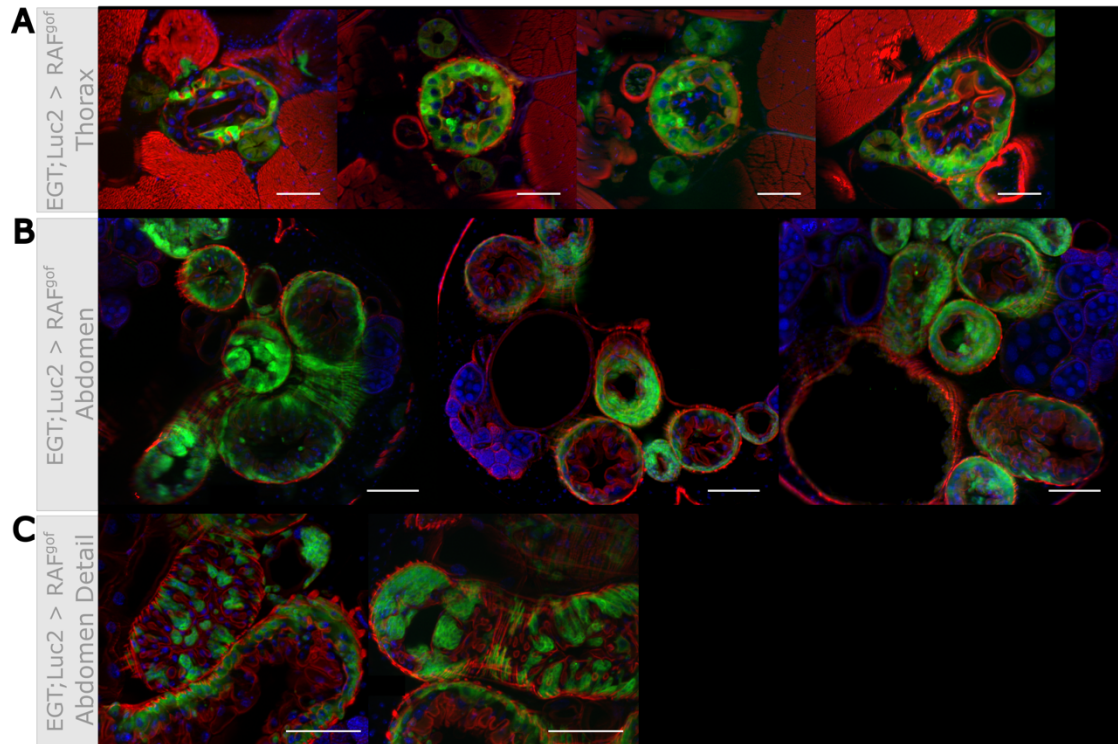


Fig. S3: Intestinal structure and phenotype variability of oncogene-expressing EGT;Luc2 > RAF^{9of} flies feeding on PDD. (A) Sections through the thorax (anterior midgut, R2) (scale bar 50 μm). (B) Section through the abdomen (R3-5 midgut region) showing multiple intestinal loops (scale bar 100 μm). (C) Detail of intestinal loop (scale bar 100 μm). The flies were fed for three days on a protein-depleted diet containing 0.1% yeast extract.

Fig. S4

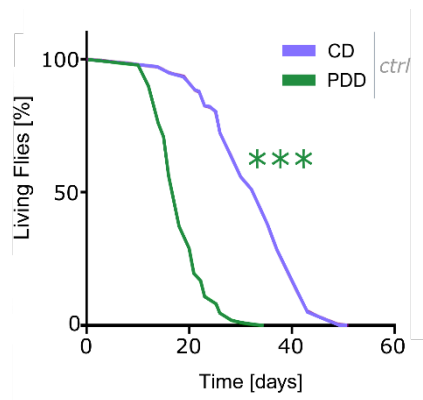


Fig. S4: Lifespan of control flies subjected to PDD or CD at 29°C (n = 200-205). ctrl = w^{1118} , CD = normal medium, PDD = protein depletion medium, *** p < 0.001.

Fig. S5

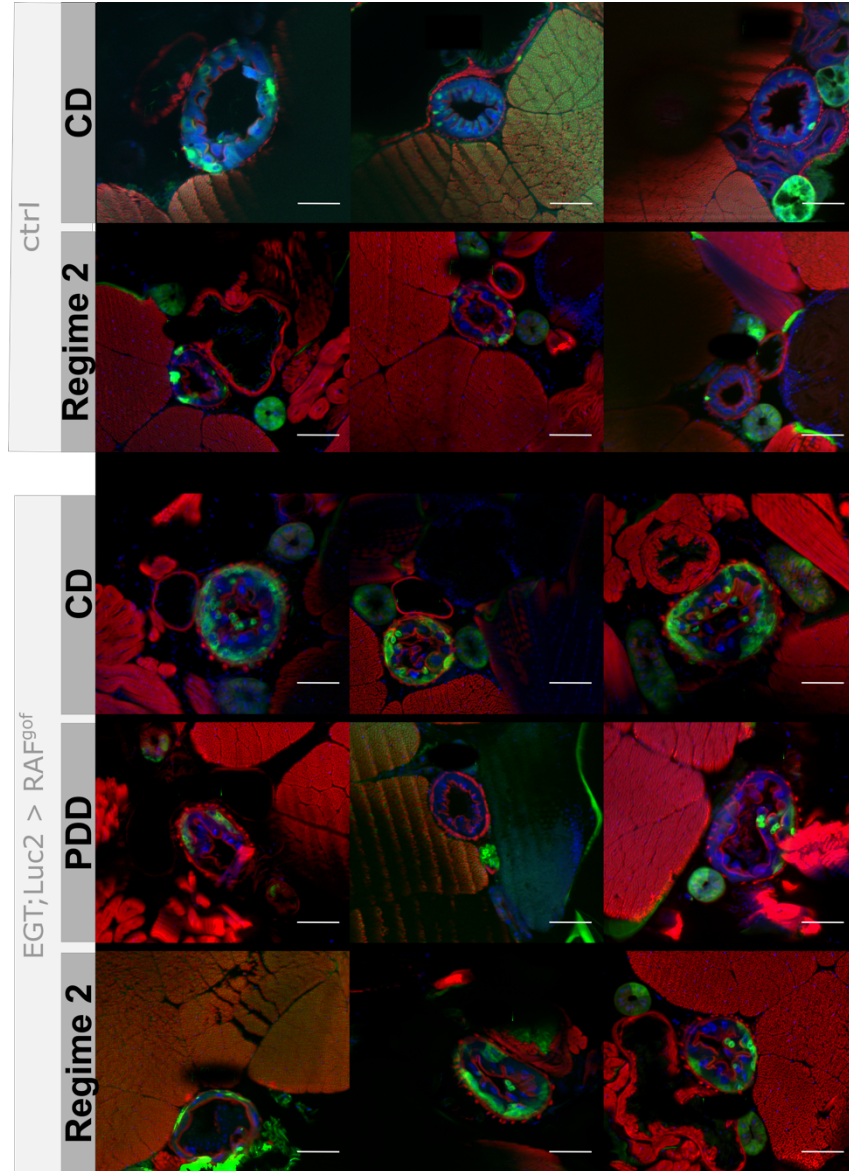


Fig. S5: Phenotypic observations in cross sections of the anterior R2 midgut region of flies feeding for 10 days on CD, feeding for 3 days on PDD followed by 3 days on CD and 4 days on PDD (PDD/CD) and 10 days on PDD (scale bar 50 μ m). ctrl = EGT;Luc2 > w¹¹¹⁸, CD = control diet with nutritious medium, PDD = protein depleted diet.

Fig. S6

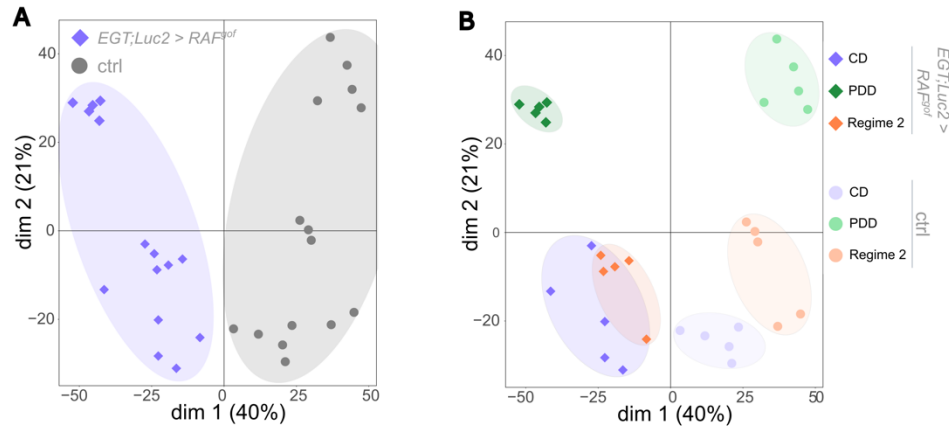


Fig. S6: PCoA analysis of transcriptomic data. Analysis of variation between and among samples using multi-dimensional scaling (MDS) was based on all transcripts (unweighted). The mRNA of oncogene-bearing and control flies was sequenced after 13 days of subjection to the respective feeding regime (n = 5). (A) Manually added ellipses represent tumor bearing (blue) and healthy controls (grey). (B) The same dataset as in A, now with ellipses representing feeding regimes as well as tumor or control phenotype (n = 5).

Fig. S7

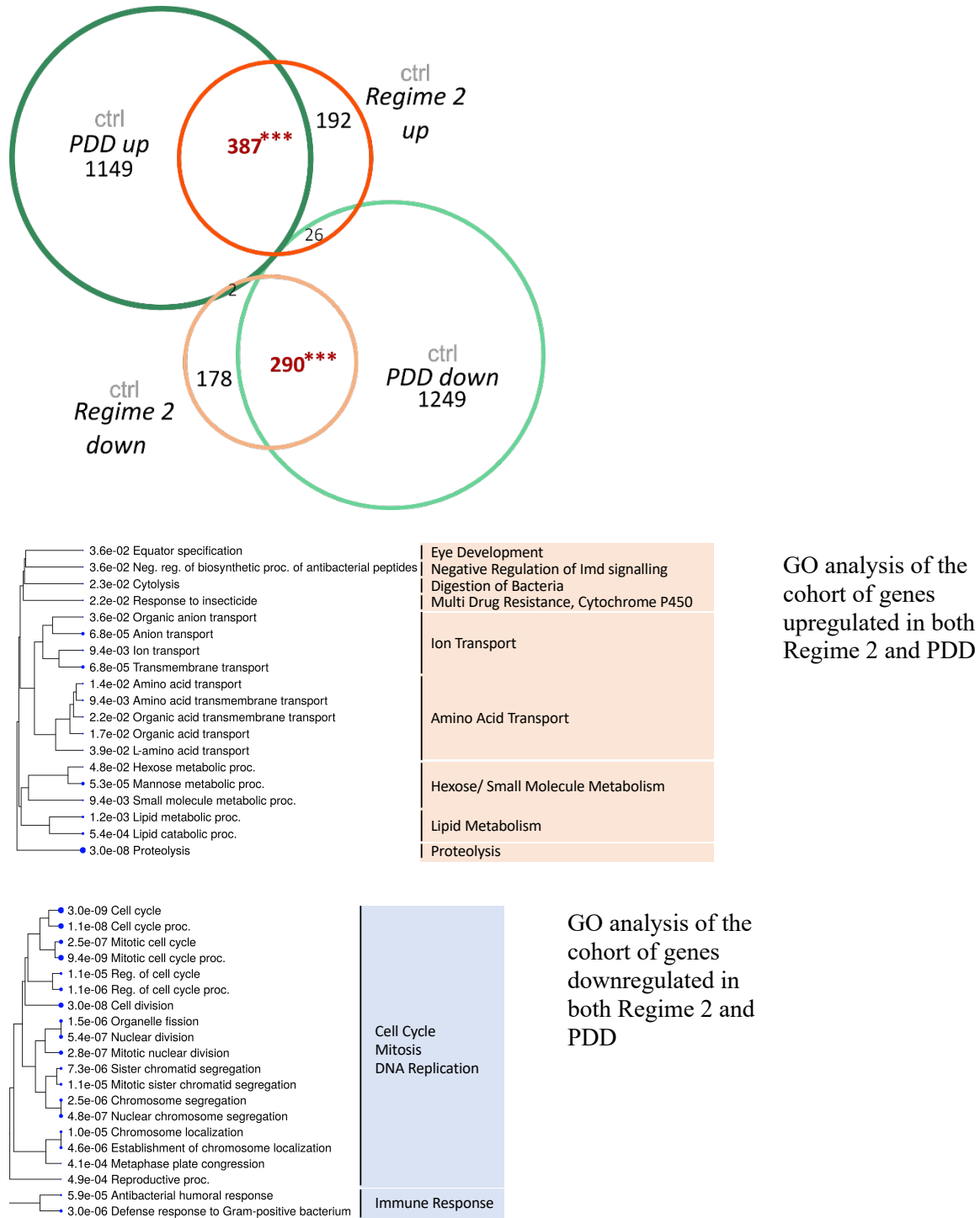


Fig. S7. Venn diagram identifying genes displaying a transcriptional memory effect (displayed in red). The GO terms of the commonly upregulated genes in Regime 2 and PDD (middle) and of the commonly downregulated genes (bottom) are shown as hierarchical clustering trees. The size of the solid circle corresponds to the enrichment FDR.

Fig. S8

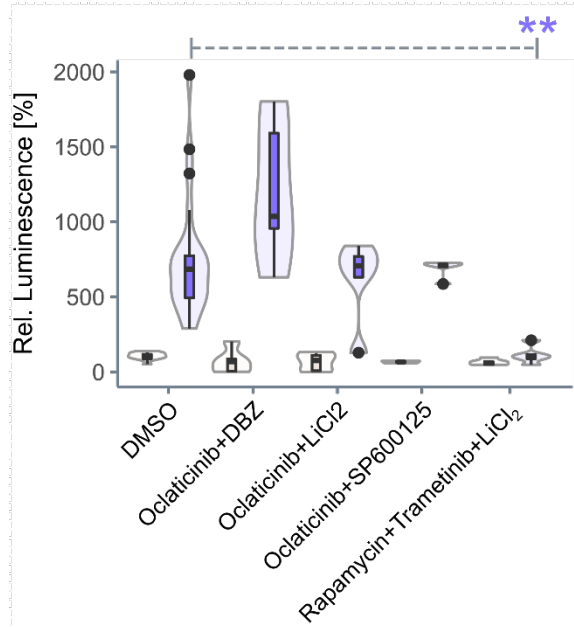


Fig. S8: Multi-drug combinations do not mimic PDD. RAF^{gof} co-expressed luciferase signal was determined in control flies and in flies expressing RAF^{gof} after 3 days of tumor induction. The flies were fed with CD containing the indicated combinations of pathway-specific inhibitors. is expressed with 100% for control flies given DMSO solvent. The combination of rapamycin, trametinib and LiCl₂ served as a positive control.

Table S1: Enrichment of GO terms for genes that were downregulated in healthy flies on PDD while upregulated in tumor-bearing flies on CD after 3 days of oncogene induction (n = 5).

Term	Identifier	log10^{-x}
cell division	GO:0051301	12.89
mitotic cell cycle	GO:0000278	9.86
spindle organization	GO:0007051	4.18
DNA replication initiation	GO:0006270	3.70
multicellular organismal reproductive process	GO:0048609	3.59
cytoskeleton organization	GO:0007010	3.51
cell differentiation	GO:0030154	1.94
defense response to Gram-positive bacterium	GO:0050830	1.76
anatomical structure development	GO:0048856	1.71
embryonic organ development	GO:0048568	1.69
actin filament -based process	GO:0030029	1.41
animal organ development	GO:0048513	1.34
DNA replication	KEGG:03030	3.42
pentose and glucuronate interconversions	KEGG:00040	3.16
ECM -receptor interaction	KEGG:04512	1.81
retinol metabolism	KEGG:00830	1.63
ascorbate and aldarate metabolism	KEGG:00053	1.49

Datasheet S1

Datasheet S2