G3 · Genes | Genomes | Genetics

The *Drosophila* pro-secretory transcription factor *dimmed* is dynamically regulated in adult enteroendocrine cells and protects against Gram-negative infection

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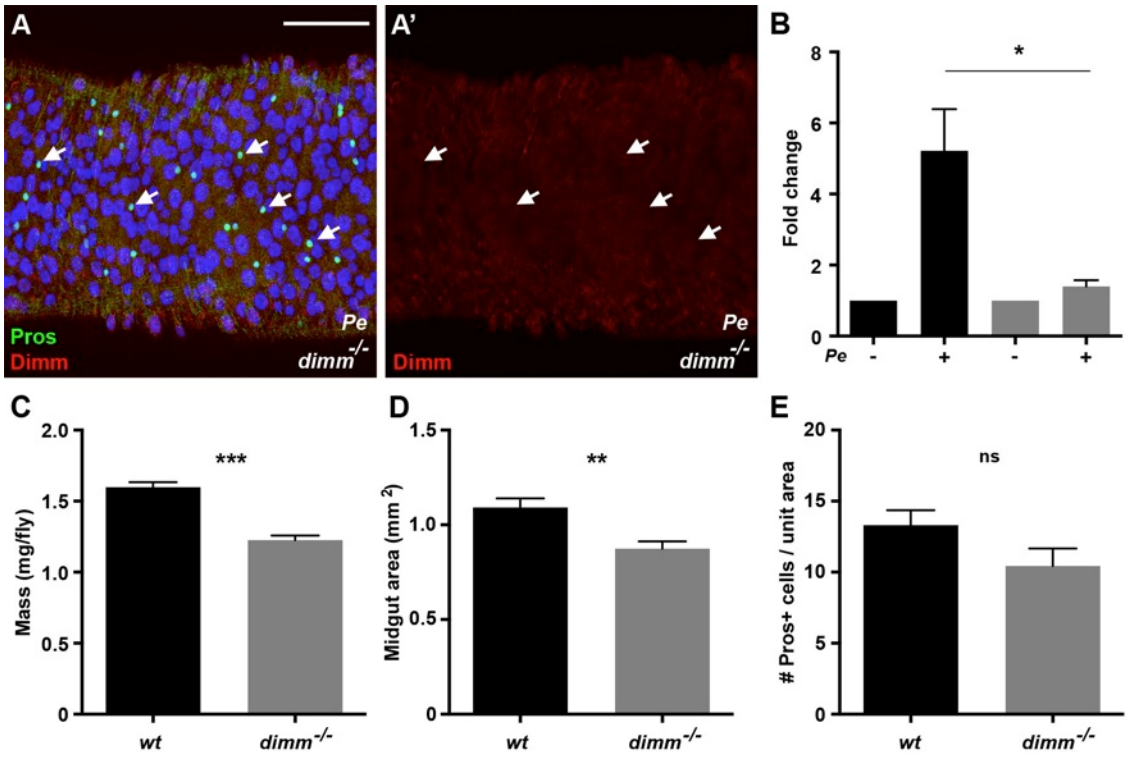

Figure S1 Analysis of *dimm* mutants.
(A-A') Confocal micrograph of the adult midgut epithelium of a *dimm* mutant midgut exposed to *Pe* (DAPI, blue; anti-Dimm, red; anti-Pros, green). Arrows reference example enteroendocrine nuclear locations to compare A to A'. Scale bar: 50 µm. (B) qPCR analysis of *dimm* mRNA in whole body tissue of wild type and *dimm* mutants (wild type, black bars; *dimm* mutant, grey bars). Fold change represents *Pe* compared to mock using the $2^{-\Delta\Delta C_T}$ method (n=3 trials, 30 flies). *Pe* dose was OD5 and time of collection 24h in A-B. (C-E) Analysis of *dimm* mutants 3 days following eclosion under RF conditions (wild type, black bars; *dimm* mutant, grey bars). (C) Body mass of wild type and *dimm* mutants (n=3 trials, 70-90 females). (D) Midgut area of wild type and *dimm* mutants (n=2 trials, 14-19 midguts). (E) Density of $Pros^{+}$ cells per unit area in wild type and *dimm* mutant midguts (n=2 trials, 16 midguts). Bars indicate mean values ± SEM.

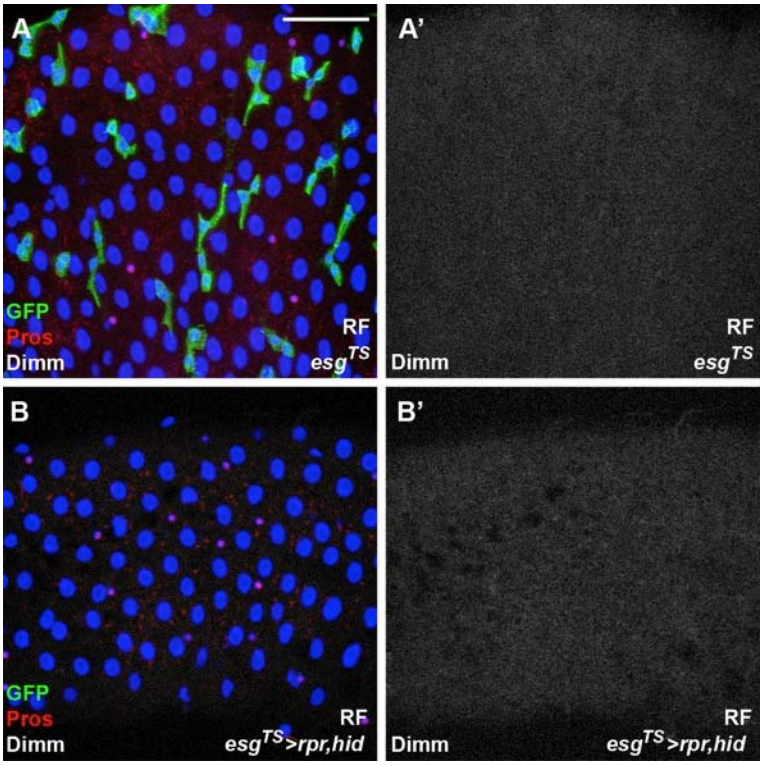

Figure S2 Ablation of *esg* cells does not induce Dimm under baseline conditions.
Confocal micrographs of adult midguts expressing either GFP (A-A') or the pro-apoptotic genes *rpr* and *hid* (B-B') using the esg^{TS} conditional system (DAPI, blue; anti-Pros, red; anti-GFP, green; anti-Dimm, white). Flies were temperature shifted to initiate transgene expression 4 days prior to dissection and were maintained on regular food (RF). Scale bar: 50 µm.

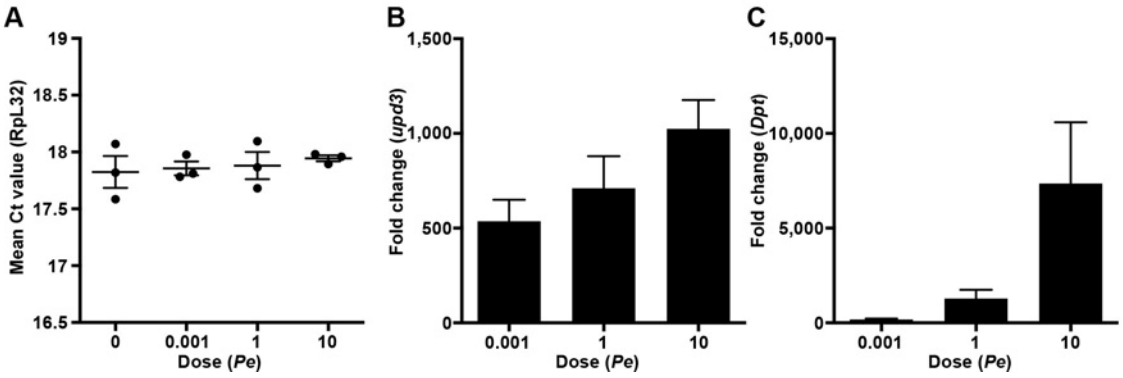

Figure S3 *upd3* and *Dpt* mRNA induction increase with *Pe* dose.
(A) Raw C_T values for *RpL32* transcript from whole body tissue. Tissue was collected in 3 separate trials, each point represents cDNA pooled from 10 whole bodies. (B) qPCR analysis of *upd3* mRNA from midgut tissue of wild type flies exposed to increasing dose of *Pe* (n=3 trials, 60 midguts). (B) qPCR analysis of *Dpt* mRNA from whole body tissue of wild type flies exposed to increasing dose of *Pe* (n=3 trials, 30 flies). Fold change represents *Pe* compared to mock using the $2^{-\Delta\Delta C_T}$ method. Tissue was collected following 24h of *Pe* exposure. Bars indicate mean values ± SEM.

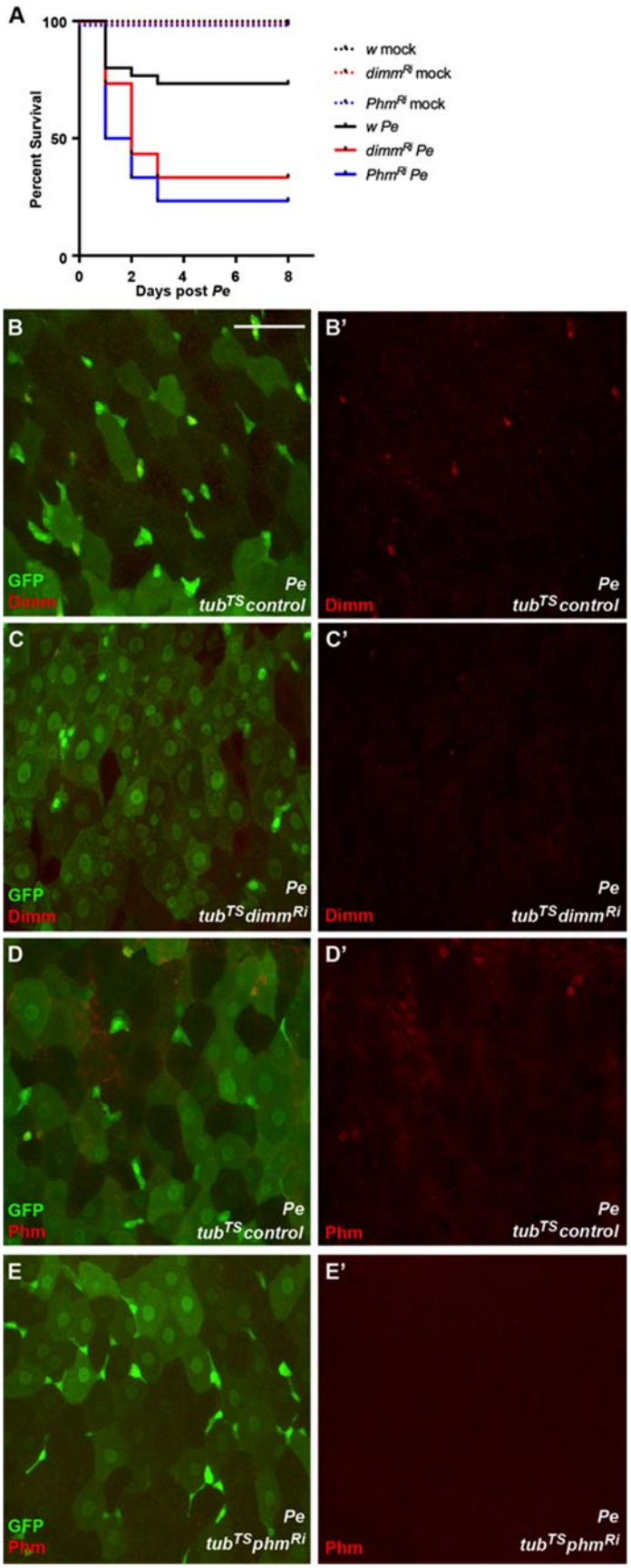
A
100
50
0
Percent Survival
0
2
4
6
8
Days post Pe
w mock
dimmRi mock
PhmRi mock
w Pe
dimmRi Pe
PhmRi Pe
B
GFP
Dimm
Pe
tubTScontrol
B'
Dimm
Pe
tubTScontrol
C
GFP
Dimm
Pe
tubTSdimmRi
C'
Dimm
Pe
tubTSdimmRi
D
GFP
Phm
Pe
tubTScontrol
D'
Phm
Pe
tubTScontrol
E
GFP
Phm
Pe
tubTSphmRi
E'
Phm
Pe
tubTSphmRi

Figure S4 Expression of RNAi targeting *dimm* or *Phm* reduces survival following *Pe*.
(A) Survival following exposure to *Pe* of *tub*^{TS} flies expressing GFP (control, *w*) or an RNAi targeting *dimm* or *Phm* (n=3 trials, 30 flies). The conditional *tub*Gal4, *tub*Gal80^{TS} genotype was used to initiate RNAi expression 3 days prior to *Pe* exposure. Flies were exposed to *Pe* at OD 10 for 24h. (B-E') Validation of RNAi knockdown by antibody staining following *Pe*. (B-B') Anti-Dimm staining in *tub*^{TS} control flies. (C-C') Anti-Dimm staining in *tub*^{TS} flies driving expression of RNAi targeting *dimm*. (D-D') Anti-Phm staining in *tub*^{TS} control flies. (E-E') Anti-Phm staining in *tub*^{TS} flies driving expression of RNAi targeting *Phm*. Flies were exposed to *Pe* at OD 5 for 24h.

Table S1 Primers used in this study.

Flybase ID	Gene name	Symbol	Forward Primer	Reverse Primer	Reference
CG7939	*Ribosomal Protein L32*	*RpL32*	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG	Neyen et al., 2014
CG8667	*dimmed*	*dimm*	AGACGAACTTCACAGCTAAGCA	GTCATCGCTTTGCGAACTGG	This study
CG8667	*dimmed* [a]	*dimm*	GATGCACAGCCTAAACGA	TTTGGCCAGTGTGAGTGT	Gauthier and Hewes, 2006
CG12763	*Diptericin*	*Dpt*	GCTGCGCAATCGCTTCTACT	TGGTGGAGTGGGCTTCATG	Neyen et al., 2014
CG10816	*drosocin*	*dro*	CCATCGTTTTCCTGCT	CTTGAGTCAGGTGATCC	Neyen et al., 2014
CG10146	*Attacin A*	*AttA*	CCCGGAGTGAAGGATG	GTTGCTGTGCGTCAAG	Neyen et al., 2014

[a] The *dimm* primers from Gauthier and Hewes, 2006 were used to verify results.
Fold change results examining *dimm* were consistent across *dimm* primer sets.

Table S2 Statistical analysis of survival of wild type adult females exposed to different doses of *Pe* (n=4 trials, 80 females). Table accompanies Figure 1G.

Dose (OD_{600})	p value for Mantel-Cox test (compared to mock)
0.001	ns, 0.3205
1	*, 0.0159
5	***, <0.0001
10	***, <0.0001
20	***, <0.0001

Table S3 Statistical analysis of survival of wild type and *dimm* mutant adult females exposed to mock or *Pe* treatment (n=3 trials, 60 females). Table accompanies Figure 5A.

Statistical Comparison	p value for Mantel-Cox test
wt, Pe compared to mock	***, <0.0001
dimm -/- , Pe compared to mock	***, <0.0001
dimm-/- Pe compared to *wt Pe*	*, 0.0462

Table S4 Estimated number of *Pe* CFUs per 0.5mL applied to each experimental vial.

Dose (OD_{600})	CFUs/0.5mL
0.001	4.53E+05
1	4.53E+08
5	2.27E+09
10	4.53E+09
20	9.06E+09