SUPPLEMENTARY FIGURE LEGENDS

Tamamouna, Rahman et al., Remodelling of oxygen-transporting tracheoles drives intestinal regeneration and tumorigenesis in *Drosophila*

Extended Data Figure 1. Infection and oxidative damage increase *esg>GFP*+ cells in the midgut and associate with increased TTC branching. a. Adult midgut intestinal progenitors labeled with *esg^{NP5130}-Gal4>UAS-srcGFP* were imaged in unchallenged conditions (4% sucrose) and upon oral *P.a.* infection (48hrs), and feeding with H_2O_2 (48hrs) and PQ (24hrs). DAPI (blue) in the upper panels stains all midgut nuclei. The bottom panels show the GFP-labeled progenitors separately. *P.a.* and PQ expanded the intestinal progenitors with a posterior midgut bias, whereas H_2O_2 exhibited an anterior midgut bias. **b-c.** Quantification of midgut mitosis (b, *n=10 each*) and TTC branching (c, *n=7,6*) in PQ-treated flies. **d-e.** Posterior midgut (R4) images of *btl-Gal4>UAS-srcGFP* flies in baseline conditions (sucrose) and upon PQ feeding. DAPI (blue) staining all the nuclei. Single channel images of the GFP are shown in d'-e'. **f-g.** Posterior midgut images of *QF6>QUAS-mtdTomato* flies in baseline conditions exhibit tracheal expression of the reporter. Midgut epithelial ECs with low expression of the reporter are visible is zoomed image (g). Single channel images of the Tomato are shown in f'-g'. Scale bars: 300 μm in a, 75 μm in d-g. Data are presented as mean values ± SD. Statistical significance (t-tested, two-sided for b, and U-tested for c): ns, not significant, * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.

Extended Data Figure 2. The FGFR/Btl is necessary and sufficient for midgut TTC branching and ISC mitosis. a-b. Brightfield images of the tracheae of *P.a.* infected R5

regions of the midgut in *trh-Gal4* control and *trh-Gal4>UAS-btl^{DN}*-expressing flies. **c-d.** Brightfield images of the tracheae of uninfected R5 regions of the midgut in *trh-Gal4* control and *trh-Gal4>UAS-λbtl*-expressing flies. **e-g.** Quantification of TTCs (e, *n=10,11,10,9,10*), TTC branching (f, *n=10,11,10,8,10*), and midgut mitosis (g, *n=8,6,10,9,6*) upon *trh-Gal4*-driven *btl* manipulation with or without *P.a*. infection. **h-k.** Brightfield images of the tracheae of *P.a.*-infected R5 regions of the midgut in *dSRF-Gal4* (h) control and *dSRF-Gal4>UAS-btl^{DN}* (i) as well as control (j) and *dSRF-Gal4>UAS-λbtl* (k) -expressing flies. **l-n.** Quantification of TTCs (l, *n=10,10,8,8,11*), TTC branching (m, *n=10,10,8,8,11*), and midgut mitosis (n, *n=11,9,12,12,9*) upon *dSRF-Gal4*-driven *btl* manipulation with or without *P.a*. infection**.** Scale bars: 75 μm. Data are presented as mean values ± SD. Statistical significance (t-tested, two-sided): ns, not significant, * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.

Extended Data Figure 3. Infection and oxidative damage induce *FGF/bnl* in the midgut epithelium. Adult midgut *bnl*-expressing cells labeled with the reporter *bnl-Gal4>UAS-srcGFP* were imaged in unchallenged conditions (4% sucrose) and upon oral *P.a.* infection (48hrs), feeding with H_2O_2 (48hrs) and PQ (24hrs). DAPI (blue) in the upper panels stained all midgut nuclei. The bottom panels show the GFP-labeled *bnl* expressing cells separately. *P.a.* and PQ induced the reporter throughout the midgut, whereas H_2O_2 exhibited an anterior midgut bias. Scale bars: 300 μm.

Extended Data Figure 4. Btl/Bnl signaling in the epithelial cells is necessary for efficient tracheal remodeling and mitosis in response to infection. a-b.

Quantification of TTC branching upon progenitor- (a) and EC-specific (b) silencing of *bnl* (*bnl*^{RNAi3}) and *btl* (*btl*^{RNAi}) (a, *n=10,8,5,10,7,7* and b, *n=10,7,9,10,9,8*). **c-d.** Quantification of midgut mitosis upon progenitor- (c) and EC-specific (d) silencing of *bnl* (*bnl*^{RNAi3}) (c, *n=6,8,12,12* and d, *n=9,9,11,13*). **e.** Quantification of *esg*+ progenitors as a percent of total number of cells in the posterior regions of the midgut upon progenitor-specific knockdown of *btl* (*btl*^{RNAi}) and *bnl* (*bnl*^{RNAi3}) (*n=12,15,15)*. **f-g.** Quantification of midgut mitosis upon progenitor- (f) and EC-specific (g) silencing of *btl* (*btl*^{RNAi}) (f, *n=8,13,11,13* and g, *n=12,11,11,16*). **h.** Quantification of *esg*+ progenitor cells/total number of cells in the posterior midgut upon progenitor-specific knockdown of *btl* (*btl*^{DN}*, n=9,9*). Data are presented as mean values ± SD. Statistical significance (t-tested, two-sided): ns, not significant, * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$ and *** $p\leq0.001$.

Extended Data Figure 5. Infection and oxidative damage activate Hif-1α/Sima in the midgut epithelium and the visceral TTCs. Hif-1α/Sima activation was monitored via the *Idh-Gal4>UAS-nlsGFP* reporter expression in the adult midgut epithelium and the intestinal trachea of the R5 region in unchallenged flies (sucrose) and upon *P.a.* and PQ treatment (a, c, e), and of the R2 region in unchallenged flies (sucrose) and upon H_2O_2 feeding (b, d). Epithelial sections (a-d) and trachea surface sections (a'-d') of the same midguts were imaged. DAPI (blue) in a-d and a'-d' stains all the nuclei. a''-d'' and a'''-d''' correspond to separated channels for reporter expression in the epithelium and the intestinal trachea, respectively. The *Idh-Gal4>UAS-nlsGFP* reporter was expressed in cells of the midgut epithelium and in the midgut TTCs in baseline conditions in the anterior (R2 in b, b') and posterior (R5 in a, a') midgut. *P.a.* (c, c'), H_2O_2 (d, d') and PQ (e, e')

induced the reporter in the epithelium and the trachea at varying degrees. All images were acquired at the same confocal settings as their respective controls. Scale bars: 75 μm.

Extended Data Figure 6. Hif-1a/Sima is necessary in the intestinal epithelium and the trachea for TTC branching. a-b. Brightfield images of the midgut TTCs (R5 region) upon trachea-specific (via *btl-Gal4*) *sima* knockdown in the background of heterozygous *simaKG* in baseline conditions. **c-d.** Bright-field images of the midgut TTCs (R5 region) upon EC-specific (via *Myo1A-Gal4*) *sima* knockdown in the background of heterozygous *simaKG* in baseline conditions. **e-f.** Bright-field images of the midgut TTCs (R5 region) upon trachea-specific (via *btl-Gal4*) *sima* knockdown in the background of heterozygous $sima^{KG}$ in *P.a.*-infected conditions. g-h. Bright-field images of the midgut TTCs (R5 region) upon EC-specific (via *Myo1A-Gal4*) *sima* knockdown in the background of heterozygous $sima^{KG}$ in *P.a.*-infected conditions. The images correspond to examples of those quantified for Fig. 4e,i. Scale bars: 75 μm.

Extended Data Figure 7. Time-course analysis of $Notch^{DN}$ progenitor-derived midgut tumors. a-d. The R4a region of control (reared for 4 days at 18^0C) (a) and tumorous midguts (reared for 4, 7 and 10 days at 29^0C) (b-d) of the *esg-Gal4 UAS-eGFP tub-*$Gal80^{ts}$*>UAS-*$Notch^{DN}$ genotype with concomitant expression of *QF6>QUAS-mtdTomato* (red) to label the trachea. DAPI (blue in a-d) is used to label all midgut nuclei and Prospero (a''-d'') labels the EEs. a'-d', a''-d'' and a'''-d''' correspond to the individual

channels for eGFP, Prospero and Tomato-labeled trachea, respectively. Scale bars: 75 μm. **e-g**. Quantification of TTC branching in the R4a of control (*Notch^{DN}* uninduced) and *Notch^{DN}*-expressing midguts (e, *n=4,8,4,6,4,7*), in the *Notch^{DN}* tumor-region vs. neighboring non-tumor area on the same image (f, *n=6,6,4,4,6,6*), and midgut mitosis of control (*Notch^{DN}* uninduced) and *Notch^{DN}*-expressing midguts (g, *n=20 each*) during a time-course analysis at 4, 7, and 10 days post-tumor induction. Scale bars: 75 μm. Data are presented as mean values ± SD. Statistical significance (t-tested, two-sided): ** $0.001<p\leq0.01$ and *** $p\leq0.001$.

Extended Data Figure 8. Time-course analysis of *Ras^{V12}* progenitor-derived midgut tumors. a-d. The R5 region of control *esg-Gal4 UAS-eGFP tub-Gal80^{ts}* (reared for 1 day at 29^{0C}) and *esg-Gal4 UAS-eGFP tub-Gal80^{ts}>UAS-Ras^{V12}*-tumor bearing midguts (reared for 1, 3 and 5 days at 29^{0}C) with concomitant expression of *QF6>QUAS-mtdTomato* (red) to label the trachea. DAPI (blue in a-d) was used to label all midgut nuclei. a'-d' and a''-d'' correspond to the individual channels for the eGFP and the Tomato-labeled trachea, respectively. Scale bars: 75 μm.