Supplementary materials for Niu et al.

Supplementary figures

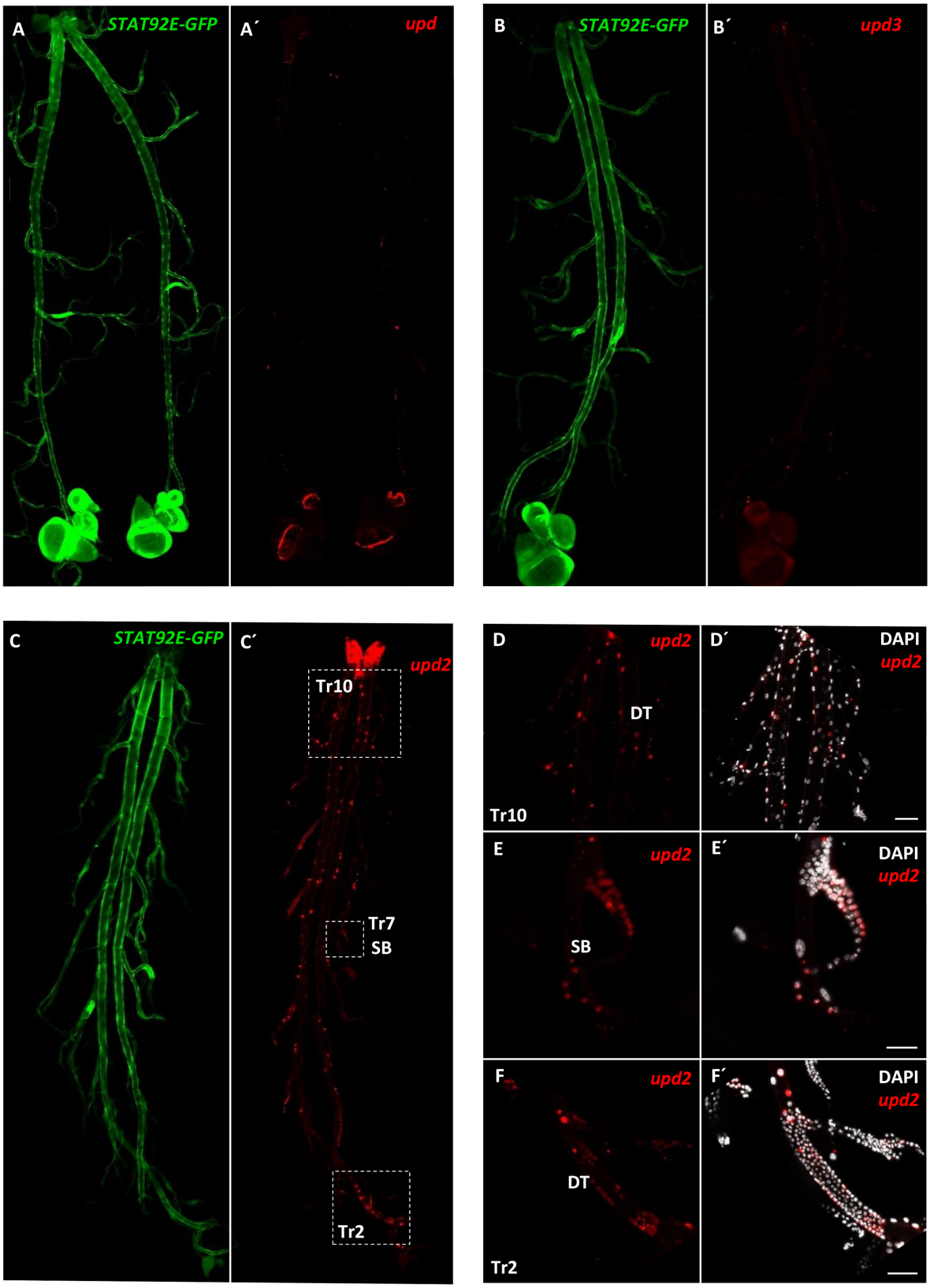
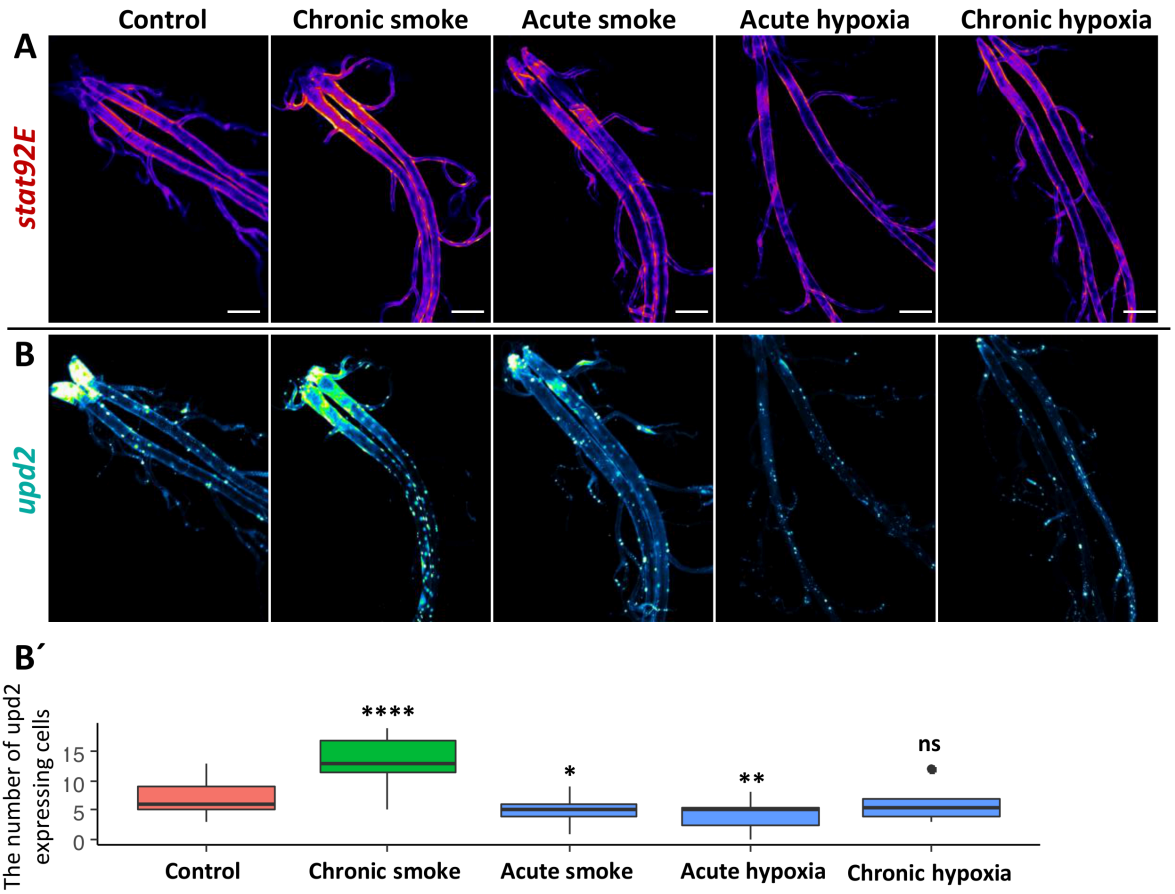


Figure S1 JAK/STAT signaling is activated in the larval trachea. (A-C) Fluorescence micrographs of the trachea of ligands (*upd*, *upd2* or *upd3*)-*Gal4*, *UAS-LacZ.nls*; *STAT92E-GFP* larvae stained to show ligand expressing cells (anti-ß-galactosidase, red) and JAK/STAT signaling activated cells (anti-GFP, green). *Upd2* displayed a higher transcript level in the trachea compared to *upd* (showed a strong expression in the other tissue, like an imaginal disc) and *upd3* (which expression in the trachea could be induced by CS (Fig. 5 and (Prange et al., 2018)). (D-F) Fluorescence micrographs of Tr2 DT, Tr7 SB and Tr10 DT of *upd2-Gal4, UAS-LacZ.nls*; *STAT92E-GFP* larvae. In the dividing active regions such as Tr2 DT and Tr7 SB there is stronger JAK/STAT activity and a high intensity of *upd2* positive cells (E-F) compared to their adjoining somatic cells (D). Nuclei are stained with DAPI. Scale bar: 50 µm.


Control
Chronic smoke
Acute smoke
Acute hypoxia
Chronic hypoxia
A
stat92E
B
upd2
B´
The number of upd2 expressing cells
15
10
5
0
****
*
**
ns
Control
Chronic smoke
Acute smoke
Acute hypoxia
Chronic hypoxia

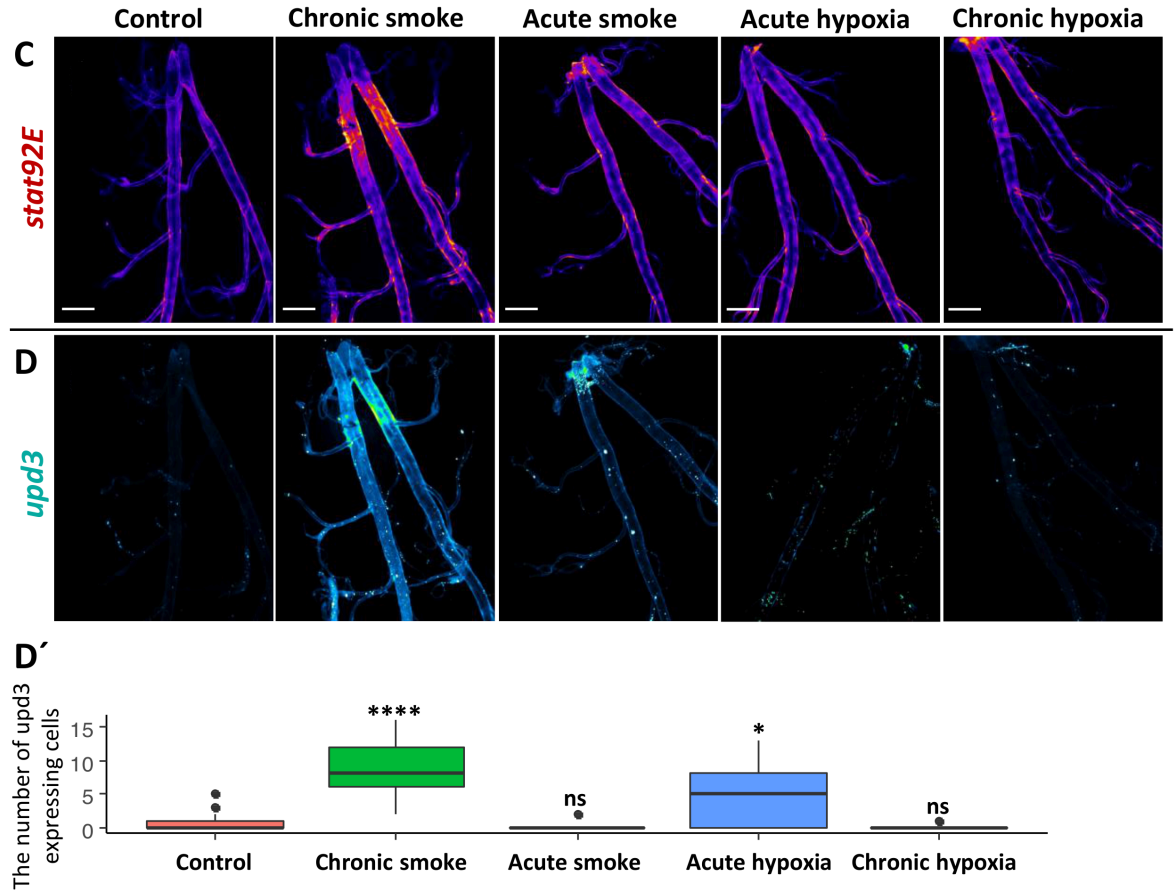

Control
Chronic smoke
Acute smoke
Acute hypoxia
Chronic hypoxia
C
stat92E
D
upd3
D´
The number of upd3 expressing cells
15
10
5
0
****
ns
*
ns
Control
Chronic smoke
Acute smoke
Acute hypoxia
Chronic hypoxia

Figure S2 The activity of JAK/STAT pathway and the expression of its ligands *upd2* or *upd3* were observed in the trachea of larvae that were exposed to smoke and hypoxia. Fluorescence micrographs of the trachea of *upd2-Gal4* (A-B) *or upd3-Gal4* (C-D); *STAT92E-GFP*; *UAS-LacZ.nls* larvae that were exposed for 2 days smoke (chronic smoke), heavy smoke (acute smoke), strong hypoxia (acute hypoxia), and 2 days hypoxia (chronic hypoxia). Trachea were stained for GFP (A and C; red, JAK/STAT pathway activated zones) and Beta-galactosidase (green, cells that expressed *upd2* or *upd3*), respectively. The numbers of cells that expressed *upd2* (B') or *upd3* (D') were counted under these different conditions. ns means not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by Student's t-test. Scale bar: 200 μm.

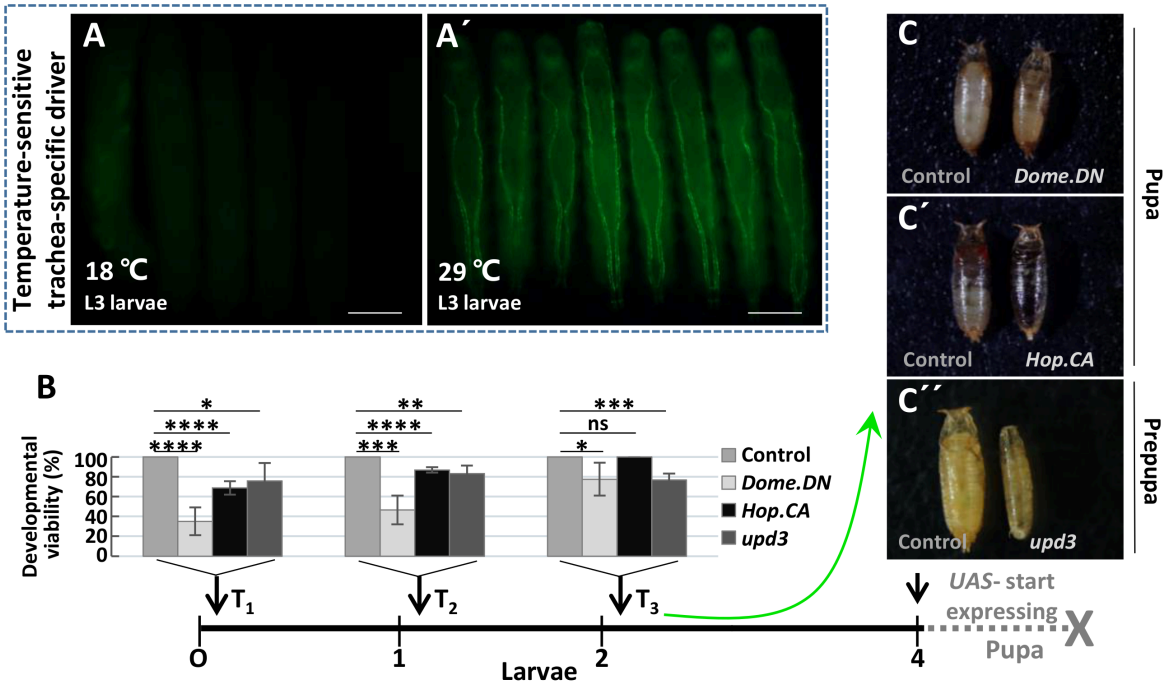


Figure S3 The temperature sensitive tracheal-specific driver line and the developmental viability analysis of larvae with abnormal JAK/STAT signaling. (A) *btl-Gal4, UAS-GFP; tub-Gal80[ts]* (*btl.ts*) larvae were raised at 18 ℃ (nonpermissive, A) and 29 ℃ (permissive, A´), respectively. Using the Gal4/UAS system, comprising the temperature-sensitive repressor Gal80[ts], were used to time ectopic gene expression. (B) Developmental viability of larvae with different genotypes (including *Dome.DN*, *Hop.CA*, and *upd3* expression in the trachea driven by *btl.ts*) and different start points of expression (indicated by black arrows). (C) None of these manipulations allowed survival up to adults shown by pictures of the non-eclosed pupae. ns means not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by Student's t-test.

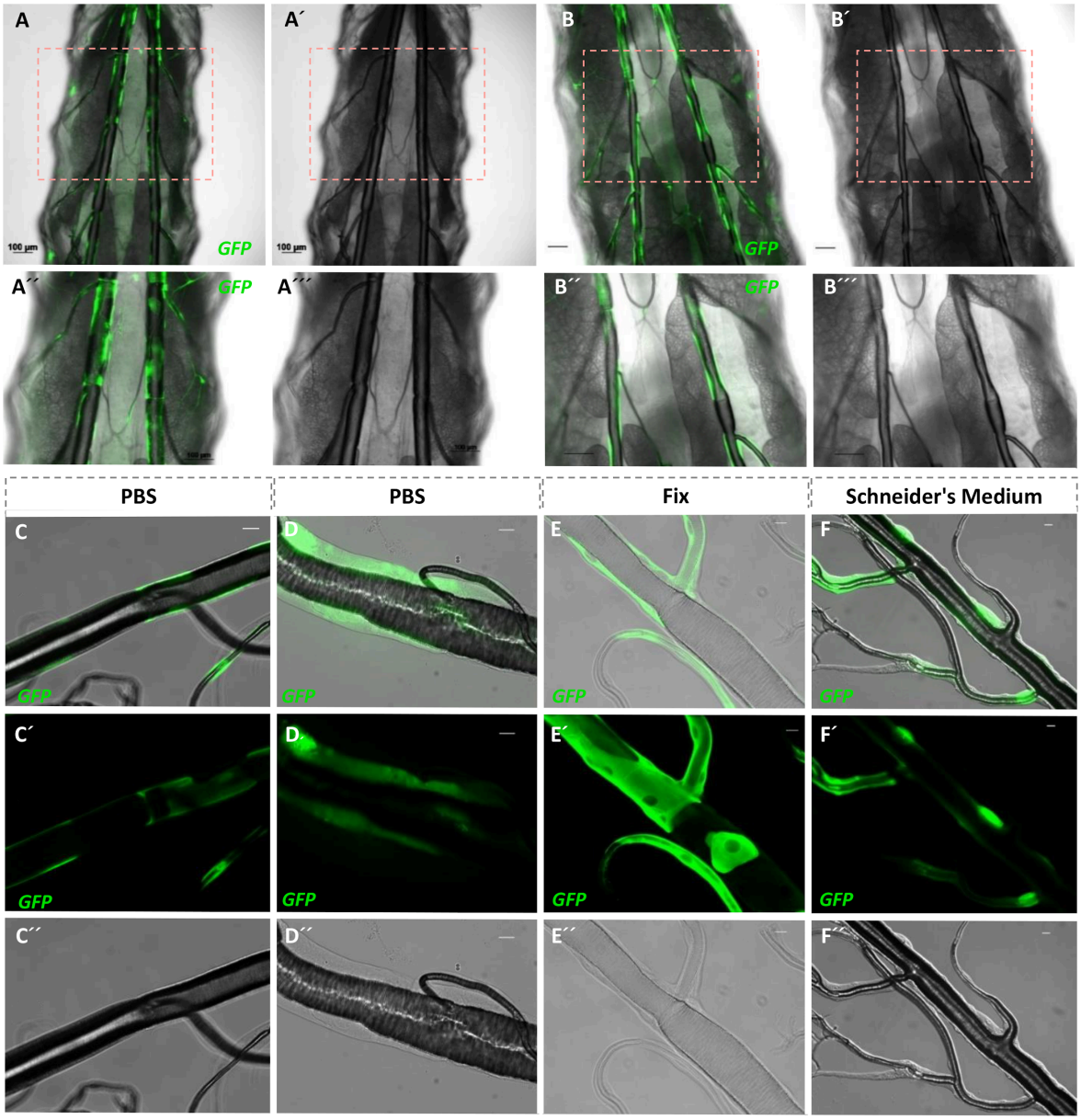


Figure S4: Validation that the increase in cell volume is not an artefact. (A-B) Micrographs of the posterior of *vvl-coin* larvae (A) and *vvl-coin>Hop.CA* larvae (B). Microscopy of the trachea in vivo displayed a thicker epithelium in affected clones (green) compared to the neighboring cells (B). The corresponding cells were not thicker in the control trachea (A). (C-F) Trachea of *vvl-coin* larvae (C) *vvl-coin>Hop.CA* larvae (D-F) were transferred to PBS (D) or Schneider's medium (F) or fixed immediately in 4% paraformaldehyde (E). All of them showed a significant increase in cell volume. Scale bar: 100 µm in A-B; 20 µm in C-F. 50 specimens are used in each experiment.

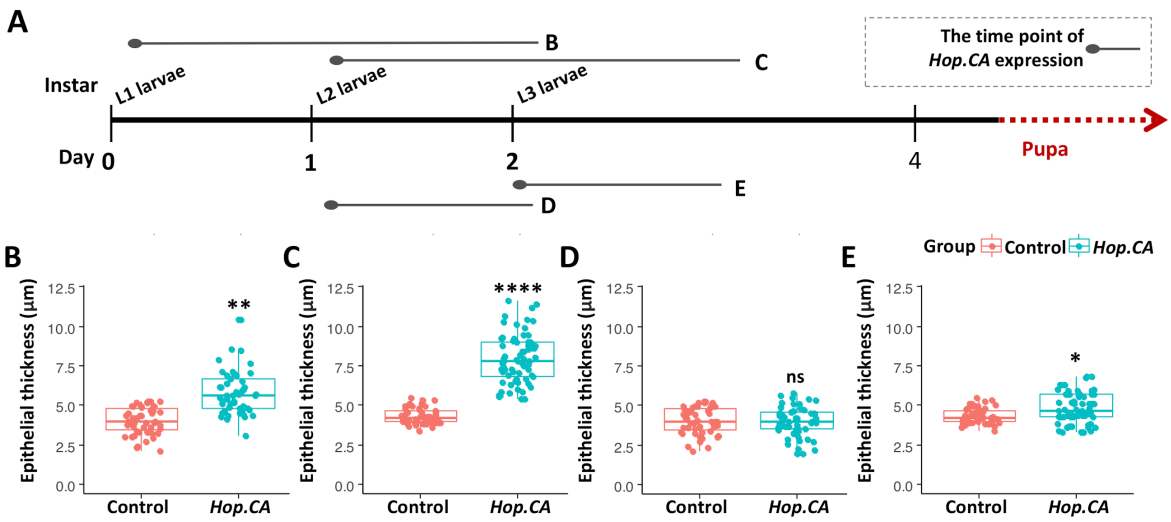


Figure S5 Time point and period of activation of *Hop.CA* expression by using the *btl.ts* driver. (A) Illustration of differing start and end points of *Hop.CA* expression by exposing them to the permissive temperature for one or two days in different larval stages. (B-E) Quantification of epithelial thickness of the DT8 region of control larvae (*btl.ts* driver line) and those experiencing ectopic manipulation (*btl.ts>Hop.CA* line). Each group contains 50 replicates in D-E. ns means not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by Student's t-test.

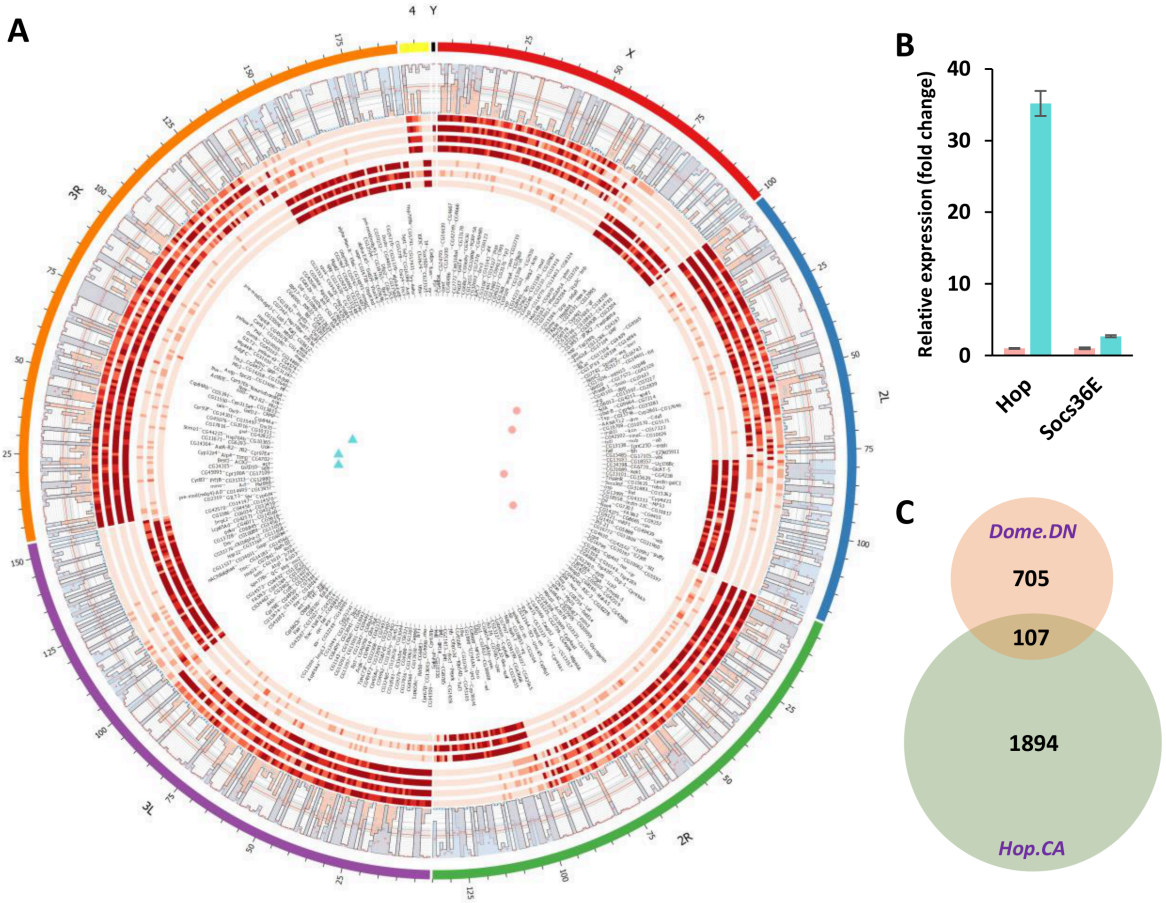


Figure S6: Changes at the transcript level in the airway epithelium of JAK/STAT mutants. Altered gene expression levels in the trachea caused by 16 hours persistent expression of *Hop.CA* driven by *btl.ts*. In total, 2004 genes were regulated significantly ($p < 0.05$), in which 1128 genes were down-regulated, 876 genes were up-regulated. (A) 707 genes (fold change > 2, $p < 0.01$) were used to visualize the differences of the transcript levels between *Hop.CA* expressed trachea and control trachea. From the outside to the inside, a histogram for expression mean values (red, control; blue, *Hop.CA*), a heatmap for controls, a heatmap for *Hop.CA* overexpression, gene names, and PCA analysis, are shown. According to transcriptome analysis of the tracheal epithelium 1128 genes ($p < 0.05$) were down-regulated, 876 genes ($p < 0.05$) were up-regulated. The changes in the gene expression total value were not significant in the ectopic expression trachea compared to the controls. (B) Relative expression levels of *Hop* and *Socs36E* in *Hop.CA* trachea compared to control trachea. (C) The inhibition of the JAK/STAT signaling by ectopic expressing *Dome.DN* for 18 hours affects the transcription of 812 genes, however, which only 102 genes were regulated in both types of airways; with ectopically activated and inhibited JAK/STAT signaling.

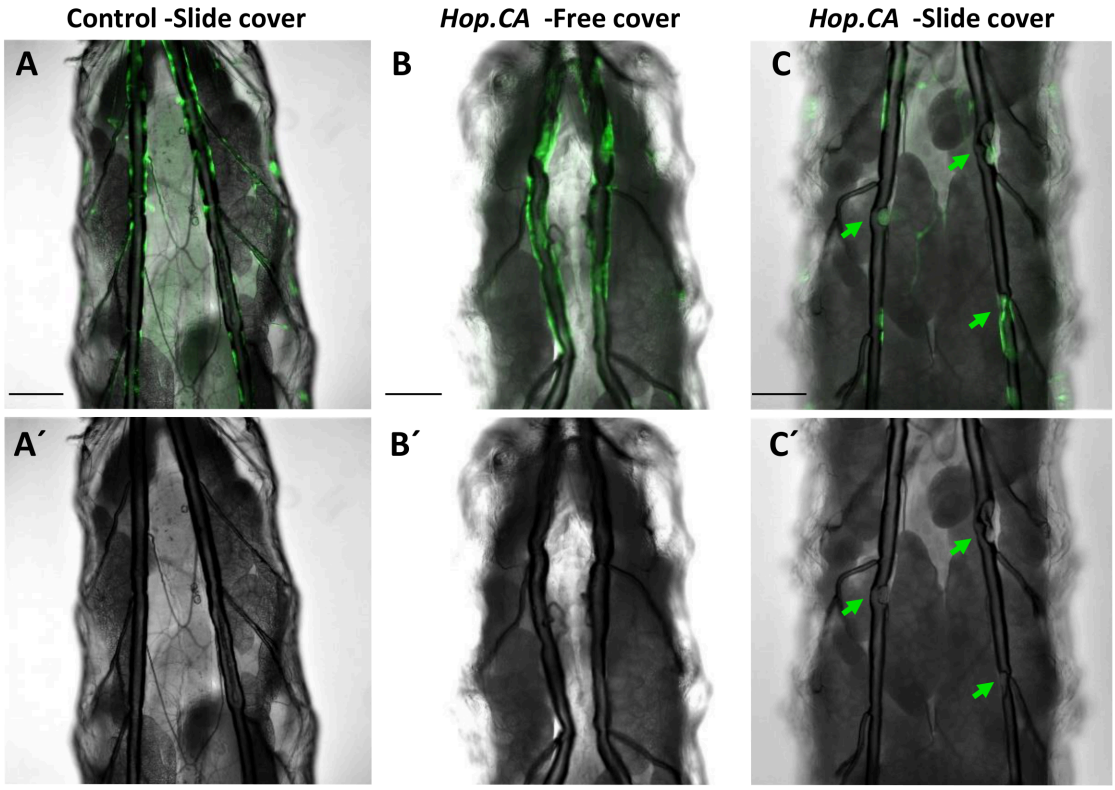


Figure S7 Micrographs of the trachea of *vvl-coin* larvae under different conditions. In A, the control is shown, in B, the undisturbed larvae of animals experiencing *Hop.CA* overexpression are displayed. (C) similar animals as in B, but the animals covered with a slide, inducing a certain degree of compression. The arrows show the collapsed sites in the tracheal tube. 30 larvae in each group were investigated in A-C. Scale bar: 200 µm.

Supplementary tables

Table S1: Kegg pathway analysis of genes, whose expression is regulated in response to *Hop.CA* overexpression.

Term	# regulated gene	#REF	corrective p-value
Protein processing in endoplasmic reticulum	37	122	0.000196415
Metabolism of xenobiotics by cytochrome P450	21	62	0.005458041
Glutathione metabolism	20	62	0.006987233
Drug metabolism - cytochrome P450	20	62	0.006987233
Metabolic pathways	143	924	0.010283682
Fatty acid metabolism	14	42	0.033601606

Table S2: 32 regulated genes in GO9 and GO10.

Name	Max group mean	Fold change	FDR p-value
CG4293	30.32889	1.492049	0.004753
Cog7	15.97701	1.44363	0.012736
deltaCOP	103.7106	1.56551	0.00068
CG9536	23.15629	1.351438	0.02827
Sec22	72.30199	1.402676	0.031492
sau	77.49881	1.431877	0.01605
epsilonCOP	76.10362	1.399189	0.011535
CG7456	23.9483	1.537314	0.001188
Syx18	28.09394	1.355287	0.021462
CG11857	79.55307	1.348644	0.047345
zetaCOP	96.3113	1.510022	0.003362
CG31729	46.75703	1.881828	7.98E-07
CG5946	43.55012	-1.48145	0.006965
CG7011	52.77113	1.986896	2.63E-08
alphaCOP	74.65062	1.44925	0.029706
GABPI	24.92601	1.662457	5.17E-05
CG4293	30.32889	1.492049	0.004753
p115	23.55302	1.500032	0.00275
bai	264.6093	1.394544	0.025263
KdelR	165.6429	1.434894	0.034105
betaCOP	95.96036	1.602758	0.001899
Bet1	36.37198	1.509118	0.005981
Sec24AB	22.08689	1.427689	0.012235
Tango1	26.83374	1.451327	0.021437
Sec13	103.0673	1.556802	0.000783
PAPLA1	18.71817	1.703538	5.78E-05
Sec16	12.41264	1.385734	0.041951
Sec23	122.68	1.544822	0.010929
Sec31	49.0174	1.658867	0.000545
loj	137.7512	1.573849	0.000975
Sec24CD	53.79686	1.555751	0.002476
eca	159.2751	1.512845	0.003406
ergic53	180.2582	1.749912	0.000928
alphaCOP	74.65062	1.44925	0.029706

Table S3: Transcription factor-binding site motifs enriched in 32 regulated genes in GO9 and GO10.

Matrix ID	Matrix Name	P-value
MA0230.1	lab	0.00226225
MA0244.1	slbo	0.00555053
MA0446.1	fkh	0.00663537
MA0186.1	Dfd	0.010245
MA0165.1	Abd-B	0.0114577
MA0170.1	C15	0.0120483
MA0013.1	Br (var.4)	0.0219955
MA0203.1	Scr	0.0261687
MA0206.1	abd-A	0.0264781
MA0448.1	H2.0	0.0340574
MA0197.2	nub	0.0356034
MA0174.1	Dbx	0.0369558
MA0458.1	slp1	0.0372846
MA0221.1	eve	0.0412541
MA0219.1	ems	0.0429901
MA0225.1	ftz	0.0474096
MA0012.1	Br (var.3)	0.0476299