Supplementary Information

Table S1: Zfh1 DamID peaks and associated genes from S2 cells

Click here to Download Table S1

Table S2: Transcripts with altered expression levels in S2 cells overexpressing Zfh1-CIDm vs. Zfh1

Click here to Download Table S2

Table S3: Zfh1 DamID peaks and associated genes from CySCs

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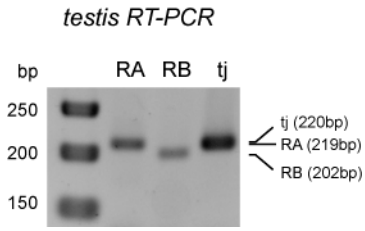

Figure S1 - Expression of Zfh1 isoforms

RT-PCR on adult testis mRNA extracts reveals expression of both Zfh1-RA and -RB isoforms. tj, expression control.

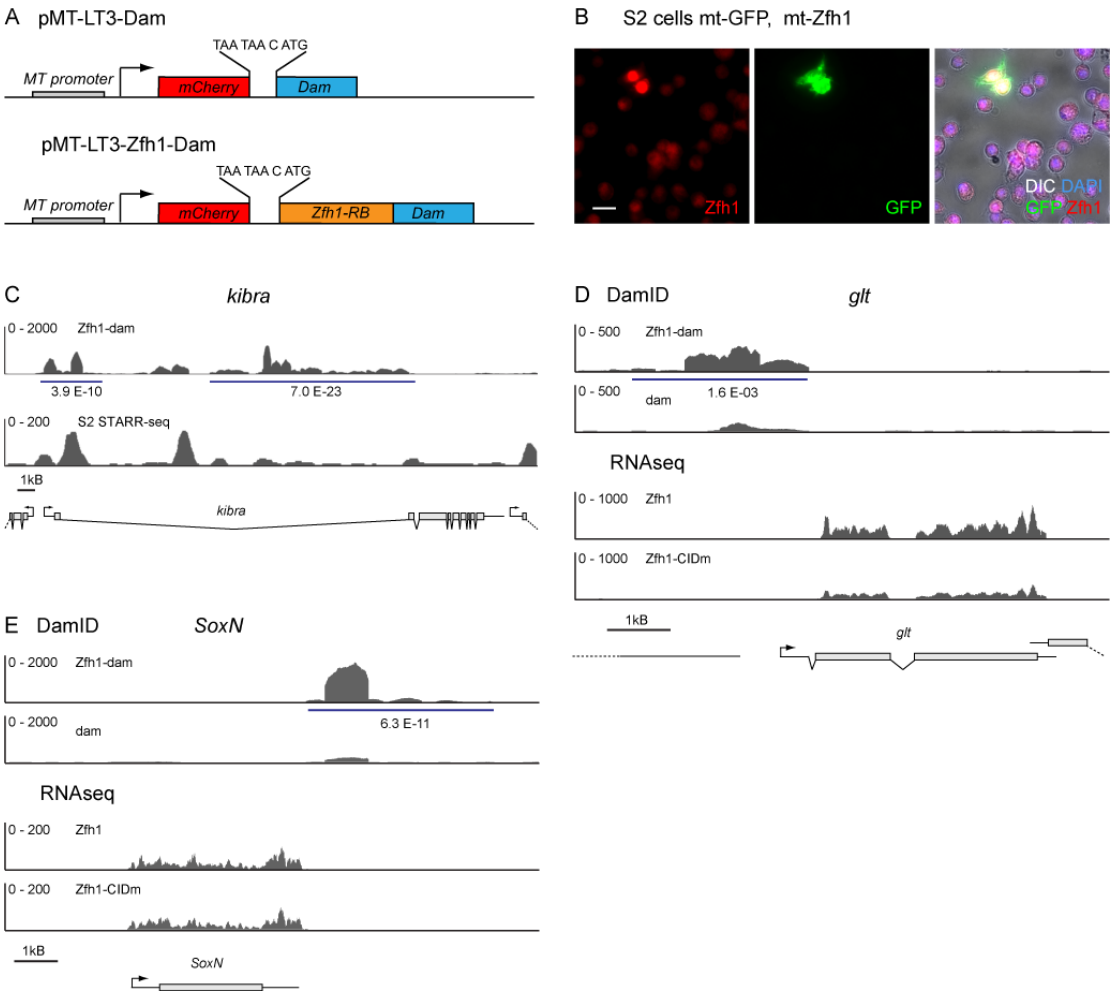

Figure S2 - Zfh1 in S2 cells

A Schematic representation of the DamID vectors used for stable transfection. Levels of Dam and the Zfh1-Dam fusion protein are minimized by the presence of an mCherry leader ORF termed LT3 followed by two stop codons and a frame shift that limits expression to rare transcriptional reinitiation events.

B S2 cells transiently cotransfected with plasmids expressing either Zfh1 or GFP under control of the metallothionein (mt) promoter. Following a 24h induction, strong Zfh1 immunostaining (red) is visible in GFP positive (green) nuclei, while a weaker, endogenous Zfh1 signal can also be seen in the nontransfected cells. Nuclei marked by DAPI (blue), cell outlines by DIC (grayscale).

C One of the two DamID peaks identified at the *kibra* locus that is localized near the transcriptional start site overlaps with a transcriptional activator region identified by STARR-seq.

D The *glt* gene is associated with a single, 5´ localized Zfh1 DamID peak and exhibits reduced transcription following overexpression of the Zfh1-CIDm construct unable to bind the CtBP transcriptional corepressor.

E The *SoxN* gene is associated with a single, 3´ localized Zfh1 DamID peak but does not exhibit any change in transcription following Zfh1-CIDm overexpression.

Peaks called by damid_seq marked by blue line; FDR indicated.

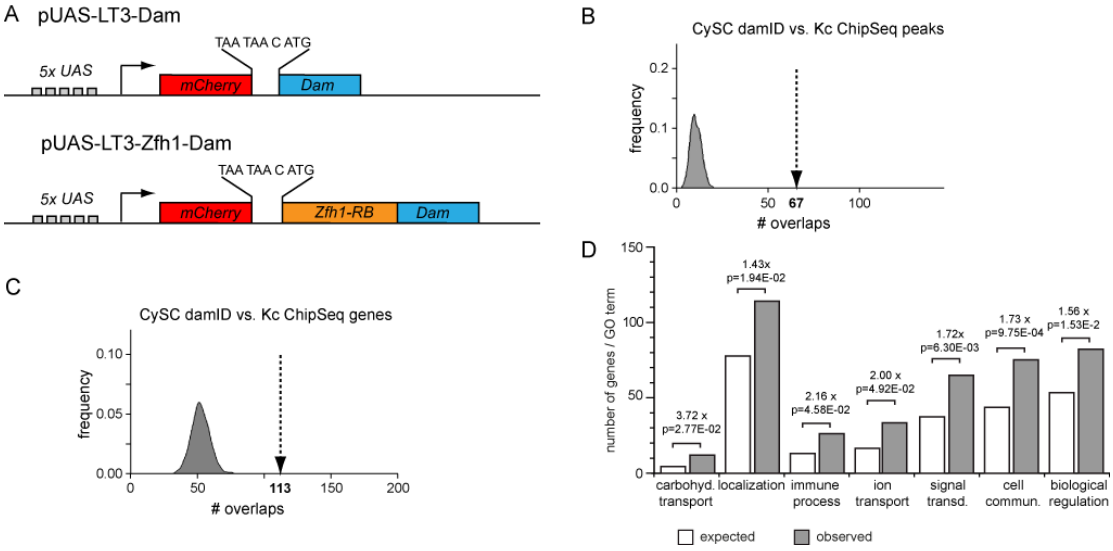

Figure S3 - Zfh1 DamID in S2 cells

A Schematic representation of the DamID vectors used for UAS/Gal4 driven expression in the CySCs. Levels of Dam and the Zfh1-Dam fusion protein are again minimized by the presence of an mCherry leader ORF termed LT3 followed by two stop codons and a frame shift.

B Zfh1 DamID peaks in CySCs significantly colocalize with Zfh1 ChIP peaks in Kc167 cells ($p<0.001$, permutation test). Distribution of overlaps from 1000 resamplings plotted against frequency; dashed arrow indicates observed overlap.

C Same as **B** for the respective associated genes (DamID peak falling within transcript region ± 1kB, $p<0.001$, χ^2-test).

D GO-slim terms overrepresented for genes associated with at least one Zfh1 DamID peak in CySCs. Expected and observed numbers of genes, enrichment factor, and p-value given for terms exhibiting significant overrepresentation.

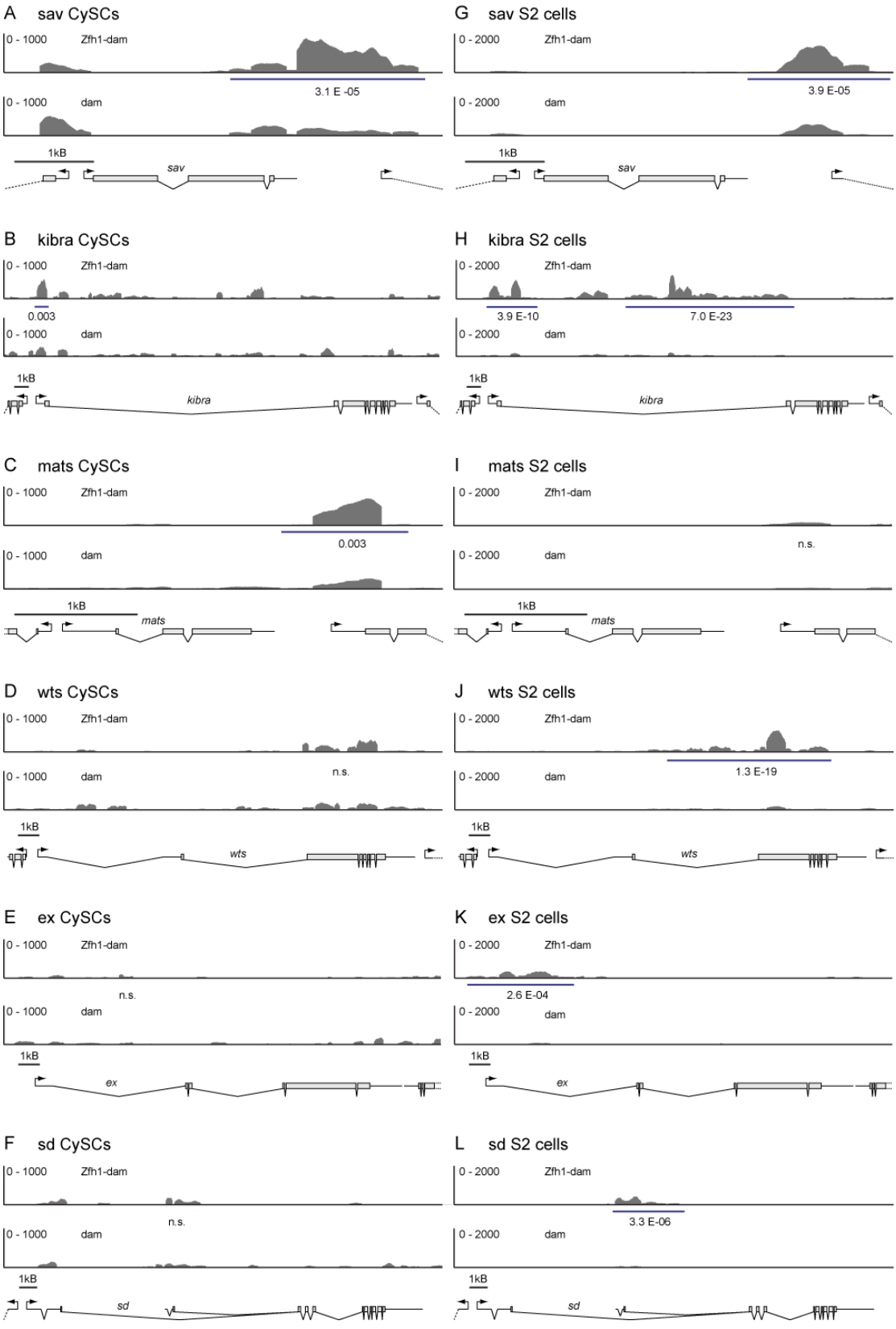

Figure S4 - Zfh1 DamID peaks near Hippo pathway components

A-E NGS reads from Zfh1-Dam (top panel) and Dam only control samples (bottom) panels for selected components of the Hippo pathway reveals Zfh1 binding peaks in CySCs.

G-K Same for S2 cells.

DamID peaks as called by damid_seq, blue line; FDR indicated.

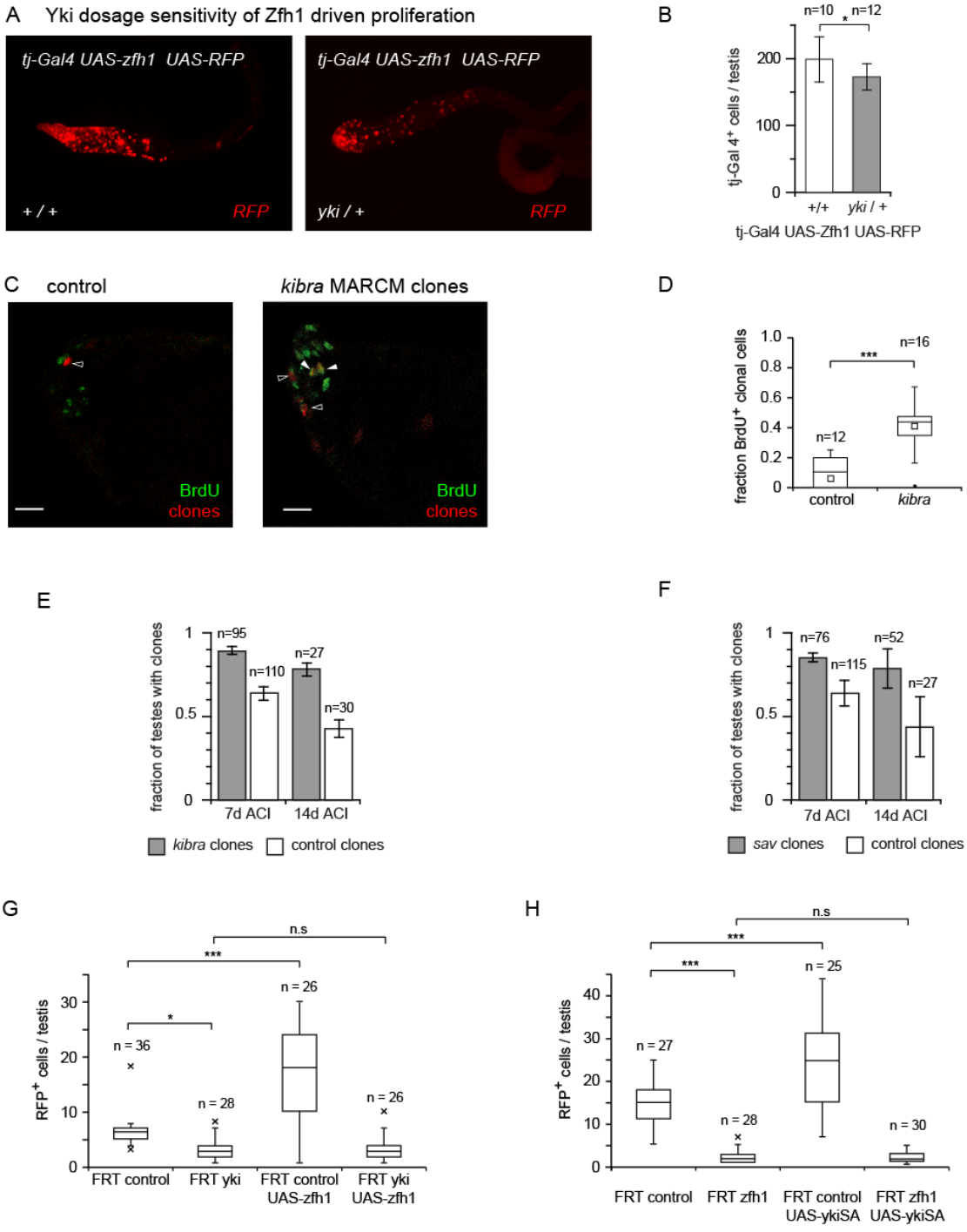

Figure S5 - Hippo signalling and CySC maintenance and proliferation

A Overexpression of UAS-Zfh1 under Tj-Gal4 control for five days causes expansion of somatic cells as visualized by co-overexpression of UAS-nlsRFP.

B Quantification of the RFP positive nuclei reveals a 14% reduction in number when one copy of *yki* is removed.

C Following an 8h pulse of BrdU feeding, *kibra* MARCM clones (RFP, red) contained a larger fraction of BrdU (green) positive cells than corresponding, neutral control clones.

D Quantification of the fraction of BrdU positive cells relative to the total number of cells per clone.

E Compared with control clones, which are gradually lost over time, homozygous *kibra* clones are retained in a larger fraction of testes one or two weeks ACI.

F Same as **E** for homozygous *sav* and control clones.

G,H Epistasis analysis for Yki and Zfh1. **G,** Loss of Yki reduces clone size (as reflected by the number of RFP positive cells per testis). This cannot be rescued by clonal overexpression of Zfh1, even though this is in control clones sufficient to increase the number of marked cells. **H**, Overexpression of activated, nonphosphorylatable Yki can conversely not rescue the loss of RFP positive cells homozygous mutant for *zfh1* even though it is sufficient to expand control clones.

Scale bars, 10μm. **B,E,F**: Columns indicate mean, error bars standard deviation **D,G,H**: Box indicates first and third quartile and median. Whiskers indicate data range up to 1.5x interquartile distance. Outliers marked individually. *, $p<0.05$; ***, $p<0.001$ (**B**,**D**: t-test, **G,H**: Anova); n, number of testes.

Plasmid sequences

Annotated sequences for the following plasmids are provided in genbank flat (.gb) format:

pRK2-zfh1_GFP

pRK2-zfh1_T2A_Gal4

pBSKS-attB kibra exon 5-9

pattB-sav_T2A_GFPnls

pattB -sav-ATG_GFPnls full

pattB -sav-ATG_GFPnls ΔNsiPst

pattB -sav-ATG_GFPnls ΔRCSI

pUAST-LT3-Zfh1::Dam

pMT-LT3-Dam

pMT-LT3-zfh1::Dam

pMT-zfh1-eGFP

pMT-zfh1-CIDm-eGFP

Click here to Download the plasmid sequences