

## Supplemental Information

## Kibra Is a Regulator

## of the Salvador/Warts/Hippo Signaling Network

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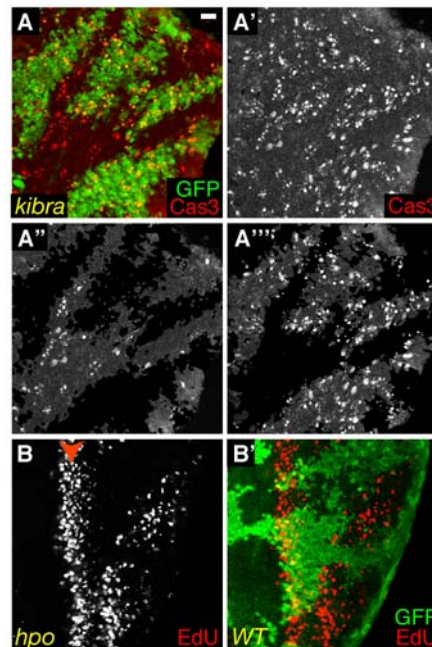
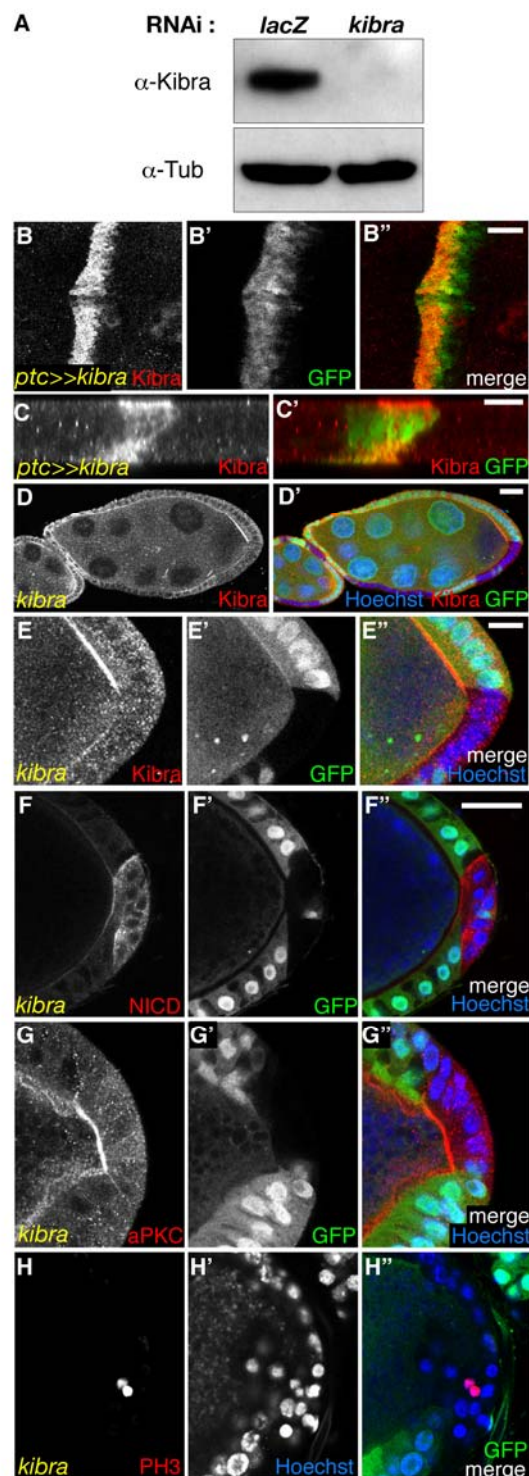


Figure S1, Genevet et al.

**Figure S1, related to Figure 1. Reduced apoptosis in *kibra* mutant tissue in the eye**  
 (A-A''') Apoptosis in 28-hour-APF retinas containing *kibra* mutant clones (absence of GFP). (A) shows a retina stained for activated Caspase 3 (Cas3) merged with GFP. (A') shows the Cas3 staining for the whole retina, (A'') for the *kibra* mutant tissue only (no GFP) and (A''') for the complementary WT tissue (presence of GFP). These images were used as a basis for the apoptotic index quantification in Figure 1P.  
 (B and B') EdU labelling (shown in grey or red), marking cells in S phase on discs containing *hpo*-mutant clones. Posterior is to the right, the second mitotic wave is indicated by a red arrowhead, scale bar = 20  $\mu$ m.



**Figure S2, Genevet et al.**

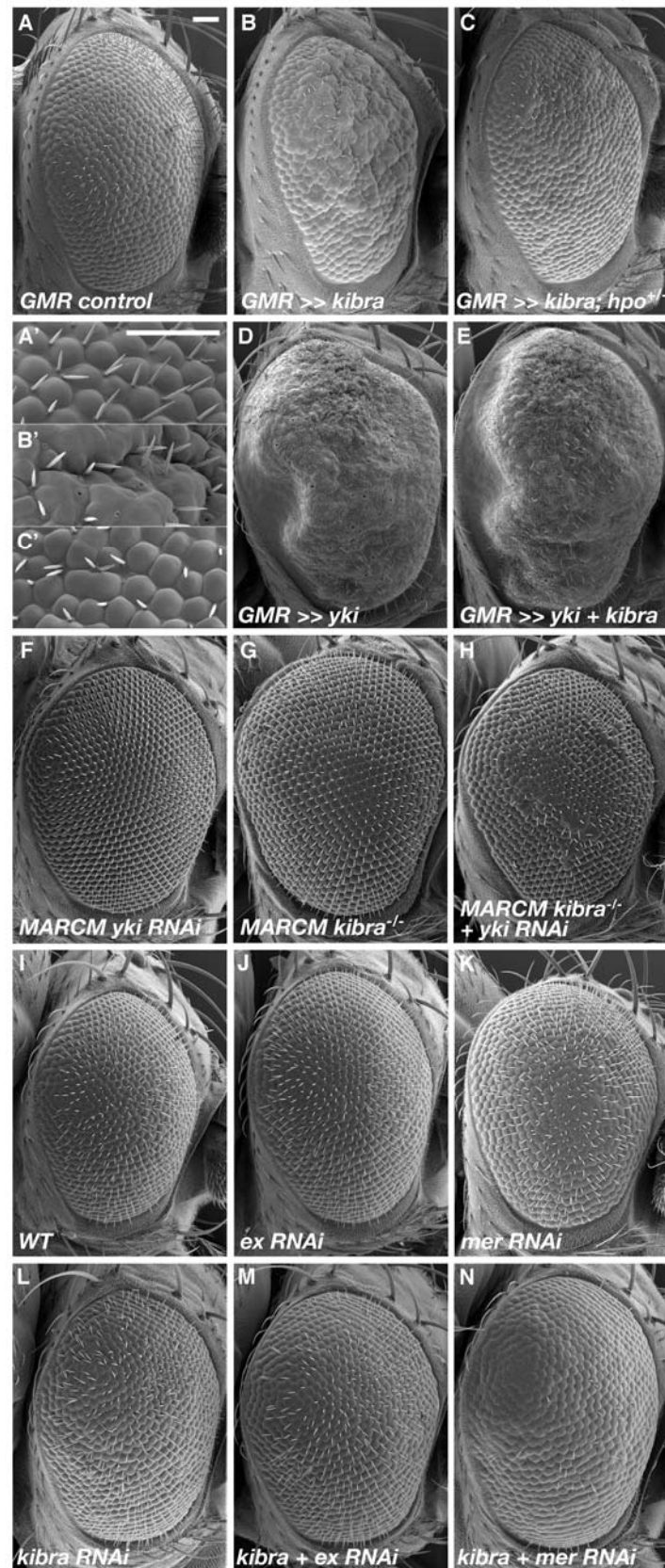
**Figure S2, related to Figure 2. Characterisation of a Kibra antibody and of the *kibra*<sup>432</sup> phenotype in the Posterior Follicle Cells**

(A) Western blot showing the disappearance of Kibra antibody signal in S2 cells treated with a *kibra* RNAi, indicating that the antibody is specific.

(B and C') Third instar imaginal wing disc overexpressing *kibra* under the control of the *patched* promoter (GFP-marked cells) and stained with the Kibra antibody.

(B and B'') is a XY image, while (C and C') is a transverse XZ section (apical is to the top).

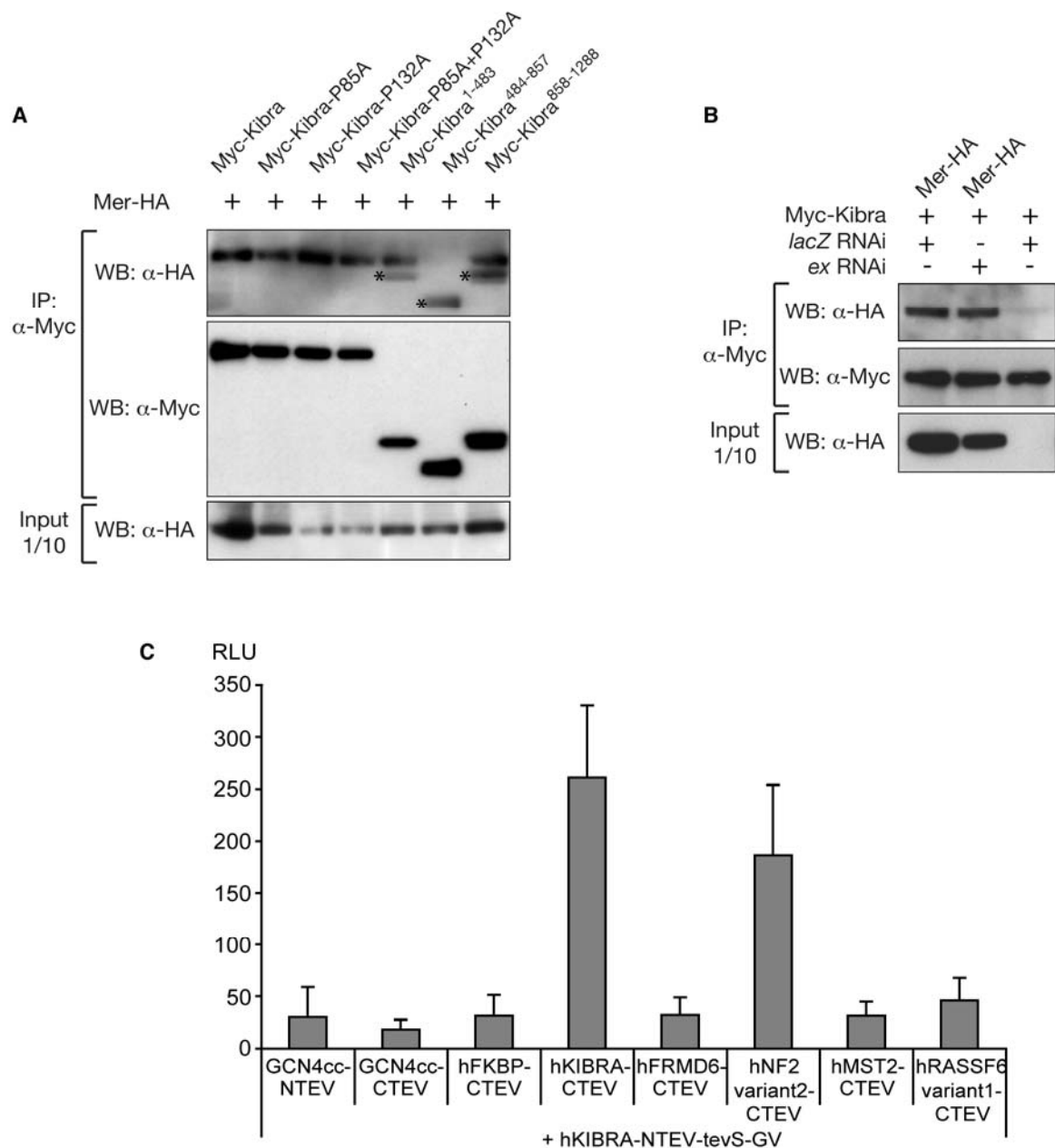
(D-H'') *kibra*<sup>432</sup> clones (marked by absence of GFP) in stage 8 (D and D'), 9 (D-E'') and 10B (F-H'') egg chambers. (E-H'') are images of the PFC region. (E-E'') is a close-up of the stage 9 egg chamber shown in (D and D'). The various egg chambers are stained for Kibra (D-E''), Notch (NICD; F and F''), aPKC (G and G'') or Phospho-Histone H3 (PH3; H and H''). Scale bars = 20  $\mu$ m (B-B'', D, D', F-H''), 10  $\mu$ m (C, C', E-E'').



**Figure S3, Genevet et al.**

**Figure S3, related to Figure 3. Genetic interaction and epistatic relationships between *kibra* and members of the SWH network**

(A-N) Scanning Electron Microscopy images of fly eyes of different genotypes as indicated. Scale bars = 50  $\mu$ m. (A',B',C' are close-ups of (A, B, C) The GMR driver was used in (A-E, I-N), while the MARCM clones in (F-H) were induced with the *eyFLP* driver.



**Figure S4, Genevet et al.**

**Figure S4, related to Figure 4. Characterisation of the Kibra/Mer/Ex interaction in *Drosophila* and in human cells**

(A) Western blots of co-IP assays between Mer-HA and different versions of Myc-tagged Kibra. P85A and P132A respectively affect Kibra's first and second WW domains. Asterisks mark residual Myc bands after stripping.

(B) Western blots of co-IP assays between Mer-HA and Myc-Kibra in presence or absence of endogenous Ex.

(C) Split TEV assay in human cells. KIBRA was fused to NTEV-tevS-GV and CTEV; all other potential interactors were fused to CTEV. KIBRA interacts with MER but not with FRMD6, MST2 and RASSF6. GCN4cc and FKBP served as negative controls while the dimerisation of KIBRA was used as positive control (Johannsen et al., 2008). HEK293 cells were transfected with combinations of indicated plasmids along with UAS-Firefly luciferase and a mix of Renilla luciferases as control (see Experimental Procedures). Cells were harvested and analysed after 24h, n=6, error bars represent standard deviation.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Drosophila stocks

*w; UAS::yki/ TM3 Sb* was a gift from D. Pan (Huang et al., 2005). *yw mer<sup>4</sup> FR19A; P[Ubi Mer] P[Ubi GFP] FRT40A/Cyo P[act::GFP]* and *w; ex<sup>e1</sup> FRT40A/ Cyo P[act::GFP]; hsFLP MKRS/TM6b* are gifts from R. Fehon (Maitra et al., 2006). *w; ex<sup>697</sup>/Cyo* (called *ex-LacZ* in the text) and *UAS::mer* are gifts from G. Halder. *UAS::Ex-GFP* is from H. McNeill. *w; FRT42D hpo<sup>5.1</sup>/ Cyo* is from (Genevet et al., 2009). The MARCM makers *yw tubGAL4 hsFLP 122 UAS-nucGFPmyc;; FRT82B CD21 y+ tubG80.LL3/TM6* and *yw tubGAL4 hsFLP 122 UAS-nucGFPmyc; FRT40A CD21 tubG80.LL3/CyO* were gifts from G. Struhl. The MARCM maker *eyFLP; G454 Act>y+>GAL4 srcGFP/+; FRT82B tub GAL80* was a gift from T. Igaki. *yw hsFLP; FRT42D GFP*, *yw hsFLP; FRT82B GFP*, *yw eyFLP; FRT42D GFP*, *yw eyFLP; FRT82B GFP*, *FRT82B, GMR>gal4/Cyo*, *hh>gal4*, *ey GMR>gal4*, *MS1096>gla4* were from Bloomington stock center. *UAS ex RNAi*, *UAS mer RNAi*, *UAS kibra RNAi* (on the X) are from the VDRC stock center.

### kibra mutant and transgenics

*w; FRT82B kibra<sup>Δ32</sup>/ TM6B*, which is homozygous lethal, was generated by mobilization of *EP747* (Bloomington stock center), which is inserted in the 5'UTR of the *kibra* gene. Excisions of *EP747* were screened by PCR with the following primers:

- 5'b : CAATTACACCTATGCTTGGGGACTACA
- 3'b : GGTCATTCGTTATGTATGCCTCATTTTC
- 3'c : GCACACAGCACCAAACGAAGTGA

Sequencing of the PCR products revealed a deletion of 1677 bp that removes part of the 5'UTR, the translation start site, and a small part of the first intron of the *kibra* gene, with 22 bp of the *EP747* sequence being left behind.

*kibra* cDNA was cloned into the pUASP vector (see Plasmid section). To create a RNAi second hairpin, a fragment of *kibra* was amplified using the following primers : 5'-

TACAACGTGCTGAGCTCCAA, 3'-ATTAGGCCAACATCGCTGAC, and inserted into the pMF3 vector ([http://www.vdrc.at/fileadmin/conferences/VDRC/protocols/pMF3\\_map.jpg](http://www.vdrc.at/fileadmin/conferences/VDRC/protocols/pMF3_map.jpg)).

The constructs were introduced into the germ line by injection in the presence of transposase as previously described (Brand and Perrimon, 1993; Rubin and Spradling, 1982).

### Quantification of inter-ommatidial cell number

40-hour-APF retinas were stained with an anti-Arm antibody to label the cell outlines. The IOCs for a single ommatidium were quantified by drawing a hexagon connecting the centres of the six surrounding neighbouring ommatidia. All IOCs within that hexagon were then counted. Cells straddling the boundary of the hexagon were counted as half a cell. For each genotype, 46 non-overlapping hexagons from the retinas of 5 different animals were counted.

### RNAi production

dsRNAs were synthesized with a Megascript T7 kit (Ambion). DNA templates for dsRNA synthesis were PCR-amplified from plasmids using primers that contained the 5' T7 RNA polymerase-binding site sequence. The following primers were used:

- For *kibra* :     - 5'- GTGGGCGAAAAGATCAATGCTCGGGAA,  
                  - 3'- GGTGGACTTCAGGGCTTCTTGCAAGTTT.
- For *lacZ* :     - 5'- TTGCCGGGAAGCTAGAGTAA,  
                  - 3'- GCCTTCCTGTTTTTGCTCAC.
- For *ex* :        - 5'- GTCTTCCTTGCTGGACTTGC,  
                  - 3'- CAAACTTTTTCCGCTCGAAG.
- For *hpo* :       - 5'- TTCTGATGGCTTATCCCCTG,  
                  - 3'- AAATTTGCACAATGCACGAA.
- For *wt* :         - 5'- GTTCTTCATGGAGCAGCACA,  
                  - 3'- CACCCAGTTATTGTCTGGCTT.
- For *mats* :      - 5'- GAGTTCTGCACCGAGGAGAC,  
                  - 3'- TCATCCTTAGCCGTCAGCTT.

## Plasmids

Expression plasmids were generated using the Gateway technology (Invitrogen). Open reading frames (ORF) were PCR-amplified from cDNA (DGRC, <https://dgrc.cgb.indiana.edu/vectors/>) and cloned into Entry vectors (pDONR207, pDONR/Zeo, pENTR-D-TOPO). For Destination vectors, the pAMW, pAWH and pAWF plasmids from the Drosophila Gateway Vector Collection (<http://www.ciwemb.edu/labs/murphy/Gateway%20vectors.html>) were used, as well as the pUASP plasmid (for transgenics). The *expanded* and *merlin* ORFs were gifts from G. Halder, the *warts* ORF was a gift from D. Pan. All Entry vectors were sequence-verified. The point mutations were generated using the Quickchange® Multi Site-Directed Mutagenesis Kit (Stratagene). For Ex-P845A, a CCG was mutated to a GCG. For Kibra-P85A, a CCC was mutated to a GCC and for Kibra-P132A, a CCG to a GCG.

## Co-immunoprecipitation of proteins

**Co-IP after transfection.** S2R+ cells, grown in Shields and Sang M3 insect medium (Sigma) supplemented with 10% FCS and 1% Penicillin/Streptomycin, were transfected with the indicated plasmids using Effectene according to the manufacturer's instructions. The cells were allowed to express the plasmids for 72 hrs, washed once in PBS and lysed in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% NP40, 1 mM EGTA, "Complete" protease inhibitor tablet (Roche), Phosphatase Inhibitor II (Sigma), 20 mM NaF). 300 µg lysate per sample was incubated with either anti-Myc (1:300, anti-rabbit, Santa Cruz) or anti-HA (1:300, anti-rabbit, Abcam) antibodies for 1 hour at 4°C, and then incubated with Protein-A sepharose beads for 1 hour at 4°C. The beads were washed 3x, and the bound proteins were denatured using NuPAGE reducing and NuPAGE loading buffer (Invitrogen). IP samples and 10% input (30 µg lysate) were run on NuPAGE Protein gels (Invitrogen) and Western blots were probed with anti-Myc (1:1000, anti-mouse, Santa Cruz) and anti-HA (1:1000, anti-rat, Roche) antibodies.

**Endogenous co-IPs.** S2 cells were cultured in 15 cm Petri dishes in Schneider's medium (Invitrogen) supplemented with 10% FCS and 1% Penicillin/Streptomycin. Cells were treated with 250 µg of RNAi for 4 days, and lysed in 2 mL of the lysis buffer described above. 5 mg lysate per sample was incubated with either anti-Yki (10 µL per IP, anti-rat, generated by Eurogentec SA against the last 15 amino acids of the protein) or with the pre-immune serum from this anti-Yki (5 µL per IP) for 1,5 hour at 4°C, and then incubated with Protein-G sepharose beads for 1,5 hour at 4°C. The beads were washed 4x, and the bound proteins were denatured using NuPAGE reducing and NuPAGE loading buffer (Invitrogen). Western blot as above, except with 1% input (50 µg lysate). Samples were probed with preblocked rabbit anti-Wts (1/10 000, gift from K. Irvine), rat anti-Yki or rabbit anti-Yki (1/2000, gift from D. Pan), rabbit anti-Kibra (see Immunostainings) and rabbit anti P-Yki (1/1000, made by Eurogentec SA against peptide HHSRAR(pS)SPA).

## Quantitative RT-PCR

*MS1096*>> (control) and *MS1096*>>*yki* wing discs were dissected in PBS and snap-frozen in liquid nitrogen. RNA isolation and subsequent qRT-PCR reactions were performed as described in (Genevet et al., 2009). All reactions were performed in four replicates. The relative amount of specific mRNAs under each condition was calculated after normalization to the *histone 3* (*his3*) transcript.

For *his3* mRNA :     - 5'- GTGAAGTAGTGAACGTGAAC,  
                              - 3'- CCGCCGAGCTCTGGAATCGC.  
For *ex* mRNA :        - 5'- CAGCAGCAGCCGAAAACCT,  
                              - 3'- GGCGGGACGTCTCATCTTTC.  
For *kibra* mRNA :     - 5'- CAGCATCTATCGACGCGGTG,  
                              - 3'- CCGCTCATCGCTTCGTAGGG

## Split-TEV luciferase assays

For split-TEV experiments, human KIBRA was recombined into a pcDNA3-DEST\_Cter-NTEV-tevS-GV vector. Also, human KIBRA, FRMD6, NF2 variant2, MST2 and RASSF6 were cloned into a pTag4C-DEST\_Cter-CTEV vector. The GCN4cc-N/CTEV and human FKBP-CTEV constructs were previously described (Wehr et al., 2006).

HEK293 cells were cultured in DMEM/GlutaMAX™ low glucose (Invitrogen) supplemented with 10% FCS and 1% Penicillin/Streptomycin.

Luciferase reporter assays were performed a 96-well format with 6 wells per condition, and 20 ng of DNA per plasmid per well was used. Indicated combinations of plasmids plus the UAS-Firefly luciferase (pFR-Luc, Stratagene) were transfected into HEK293 cells using LipofectamineLTX according to the manufacturer's instructions. 1 ng of a mix of Renilla Luciferases, driven by SV40, TK and CMV promoters at a molar ratio of 10:2:1, was co-transfected as internal control. After transfection, plasmids were allowed to express 24h, and the cells were lysed in Passive Lysis buffer (Promega). Coupled Firefly and Renilla luciferase assays were run in a BMG PHERAstar reader (IMGEN Technologies) using the Dual Luciferase Kit (Promega).

## Genotypes

Figure 1A: *w; hh>Gal4 /+ (3rd)*

Figure 1B: *UAS kibra RNAi / w; hh>Gal4 /+*

Figure 1C: *UAS wts RNAi / hh>Gal4*

Figure 1D,G: *ey>Gal4 GMR>Gal4 /+ (2nd)*

Figure 1E,H: *UAS kibra RNAi / w; ey>Gal4 GMR>Gal4 /+*

Figure 1F,I: *ey>Gal4 GMR>Gal4 /+ ; UAS wts RNAi /+*

Figure 1K,M,T: *yw eyFLP/ w;; FRT82B GFP/ FRT82B*

Figure 1L-P,U,V,S1A-A''': *yw eyFLP/ w;; FRT82B GFP/ FRT82B kibra<sup>Δ32</sup>*

Figures 1Q-S, S2D-H'': *yw hsFLP/ w;; FRT82B GFP/ FRT82B kibra<sup>Δ32</sup>*

Figure 1S: *yw hsFLP/ w;; FRT82B GFP/ FRT82B*

Figure 2A-C': *yw hsFLP/ w; ex-LacZ/ +; FRT82B GFP/ FRT82B kibra<sup>Δ32</sup>*

Figs. 2D-K'': *yw hsFLP/ w; FRT82B GFP/ FRT82B kibra<sup>Δ32</sup>*

Figure 3A-A'': *yw hsFLP/ w;; FRT42D GFP/ FRT42D hpo<sup>5.1</sup>; GMR>Gal4 UAS::kibra/+*

Figure 3B,C: *yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; FRT82B kibra<sup>Δ32</sup>/FRT82B CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; UAS::ex-GFP/+; FRT82B/FRT82B CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; UAS::ex-GFP/+; FRT82B kibra<sup>Δ32</sup>/FRT82B CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; UAS::mer/+; FRT82B/FRT82B CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; UAS::mer/+; FRT82B kibra<sup>Δ32</sup>/FRT82B CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; FRT40A ex<sup>el</sup>/FRT40A CD21 y+ tubG80.LL3, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; FRT40A/FRT40A CD21 y+ tubG80.LL3; kibra<sup>EP747</sup>/+, yw tubGAL4 hsFLP 122 UAS-nucGFPmyc/w; FRT40A ex<sup>el</sup>/FRT40A CD21 y+ tubG80.LL3; kibra<sup>EP747</sup>/+*

Figure 3D,D',F,F': *yw mer4; FRT40A p[w<sup>+</sup>mer<sup>+</sup>] UbiGFP/ FRT40A ex<sup>el</sup>; MKRS hsFLP/+*

Figure 3E,E',G-I'': *yw hsFLP; FRT42D GFP/ FRT42D hpo<sup>5.1</sup>*

Figure 3K: *MS1096>Gal4/w, MS1096>Gal4/w;; UAS::yki/+*

Figure 3L-O'': *yw hsFLP/w;; FRT42D lacZ/ FRT42D hpo<sup>5.1</sup>*

Figure S1B,B': *yw eyFLP/ w;; FRT42D GFP/ FRT42D hpo<sup>5.1</sup>*

Figure S2B-C': *w; ptc>Gal4, UAS::GFP/+; kibra<sup>EP747</sup>/+*

Figure S3A,A',I: *GMR>Gal4 /+*

Figure S3B,B': *GMR>Gal4 /+; kibra<sup>EP747</sup>/+*

Figure S3C,C': *GMR>Gal4 / FRT42D hpo<sup>5.1</sup>; kibra<sup>EP747</sup>/+*

Figure S3D: *GMR>Gal4 /+; UAS::yki/+*

Figure S3E: *GMR>Gal4 /+; kibra<sup>EP747</sup>/ UAS::yki*

Figure S3F: *eyFLP; G454 Act>y+>GAL4 srcGFP/UAS yki RNAi; FRT82B tub GAL80/ FRT82B*

Figure S3G: *eyFLP; G454 Act>y+>GAL4 srcGFP/+; FRT82B tub GAL80/ FRT82B kibra<sup>Δ32</sup>*



Figure S3H: *eyFLP; G454 Act>y+>GAL4 srcGFP/UAS yki RNAi; FRT82B tub GAL80/FRT82B kibra*<sup>A32</sup>

Figure S3J: *GMR>Gal4 /+; UAS ex RNAi/+*

Figure S3K: *GMR>Gal4 /+; UAS mer RNAi/+*

Figure S3L: *GMR>Gal4 /UAS kibra RNAi*

Figure S3M: *GMR>Gal4 /+; UAS ex RNAi/+*

Figure S3N: *GMR>Gal4 /UAS kibra RNAi; UAS mer RNAi/+*

## SUPPLEMENTAL REFERENCES

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