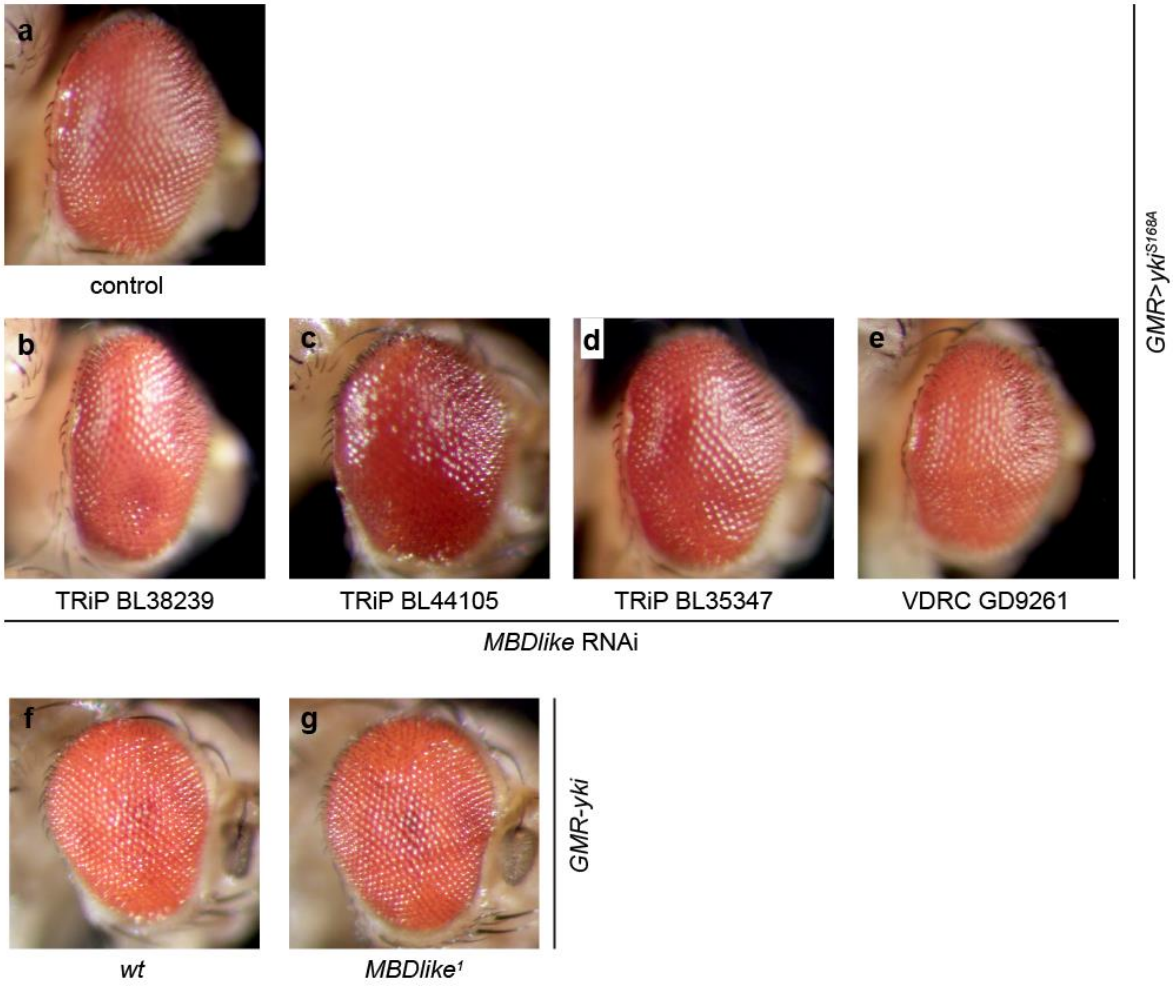
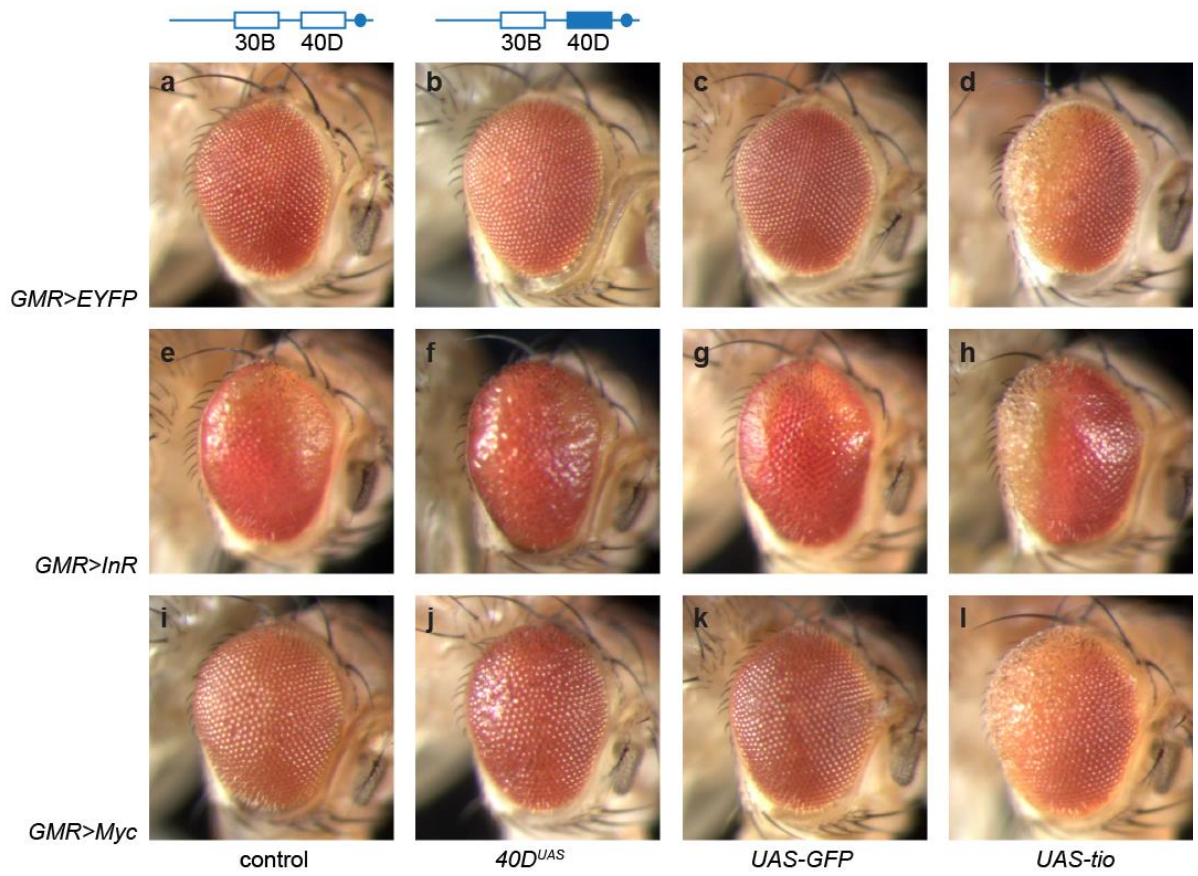
## SUPPLEMENTARY FIGURES

Supplementary Figure 1




**Supplementary Figure 1. Neither knockdown using alternative RNAi lines, nor mutation of *MBDlike* enhances the Yki eye-specific overexpression phenotype.**
**(a-e)** Adult eye phenotypes of $F_1$ flies carrying *GMR-Gal4, UAS-Yki*$^{S168A}$ transgenes crossed to a control RNAi line (*β galactosidase* RNAi VDRC GD51446) **(a)**, and *MBDlike* RNAi lines Bloomington TRiP 38239 **(b)**, 44105 **(c)**, 35347 **(d)** and VDRC GD9261 **(e)**.
**(f,g)** Adult eye phenotypes of *MBDlike* wild type **(f)** or mutant **(g)** flies carrying a *GMR-yki* transgene.

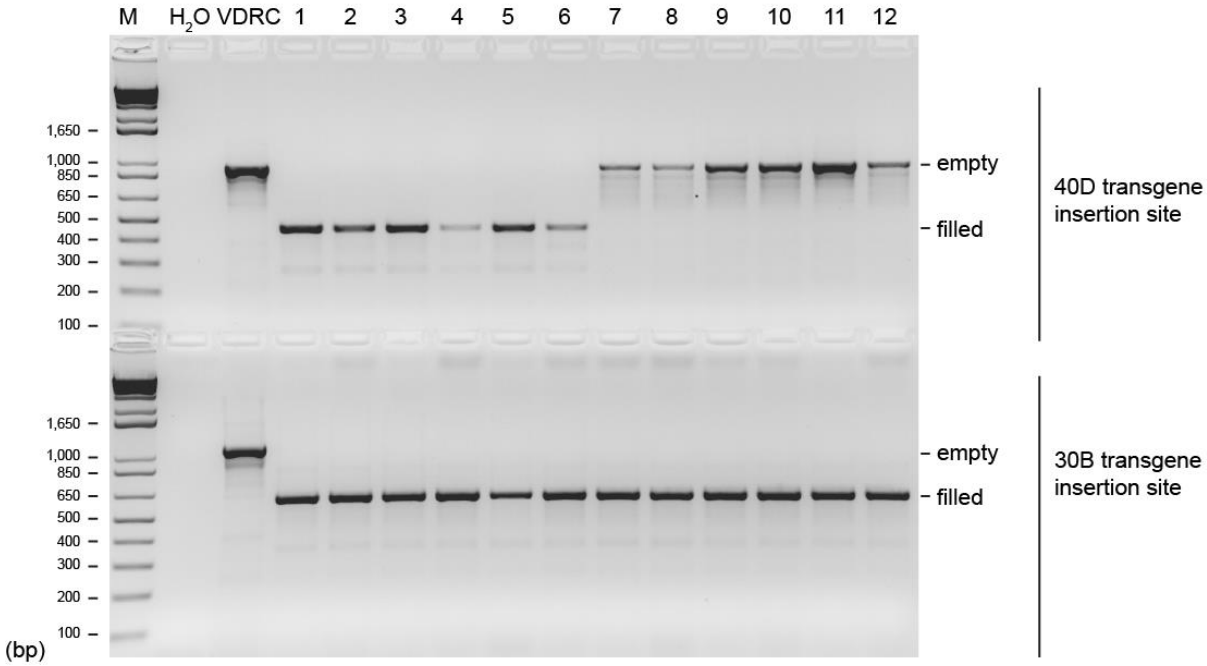
Supplementary Figure 2




**Supplementary Figure 2. Neither transgene insertion at 40D, nor *tiptop* overexpression enhances *dMyc* and *InR* eye-specific overexpression phenotypes.**

Adult eye phenotypes of $F_1$ flies carrying *GMR-Gal4* and either *UAS-EYFP* **(a-d)**, *UAS-InR* **(e-h)**, or *UAS-dMyc* **(i-l)** transgenes crossed to; VDRC genetic background **(a, e, i)**, $40D^{UAS}$ **(b, f, j)**, *UAS-GFP* **(c, g, k)**, *UAS-tio* **(d, h, l).**

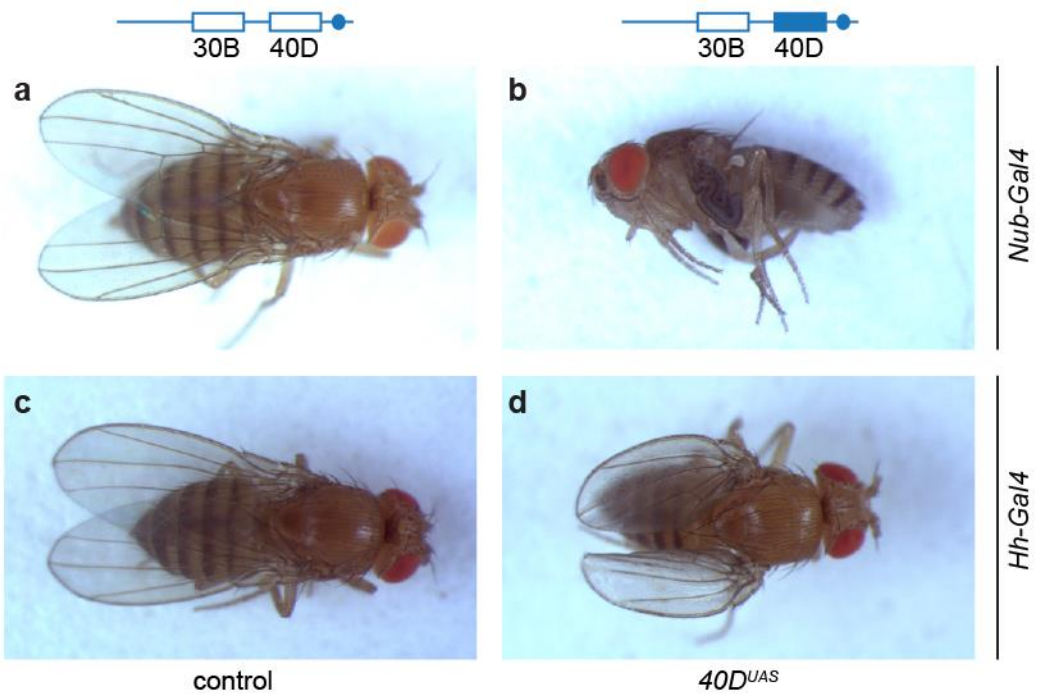
Supplementary Figure 3

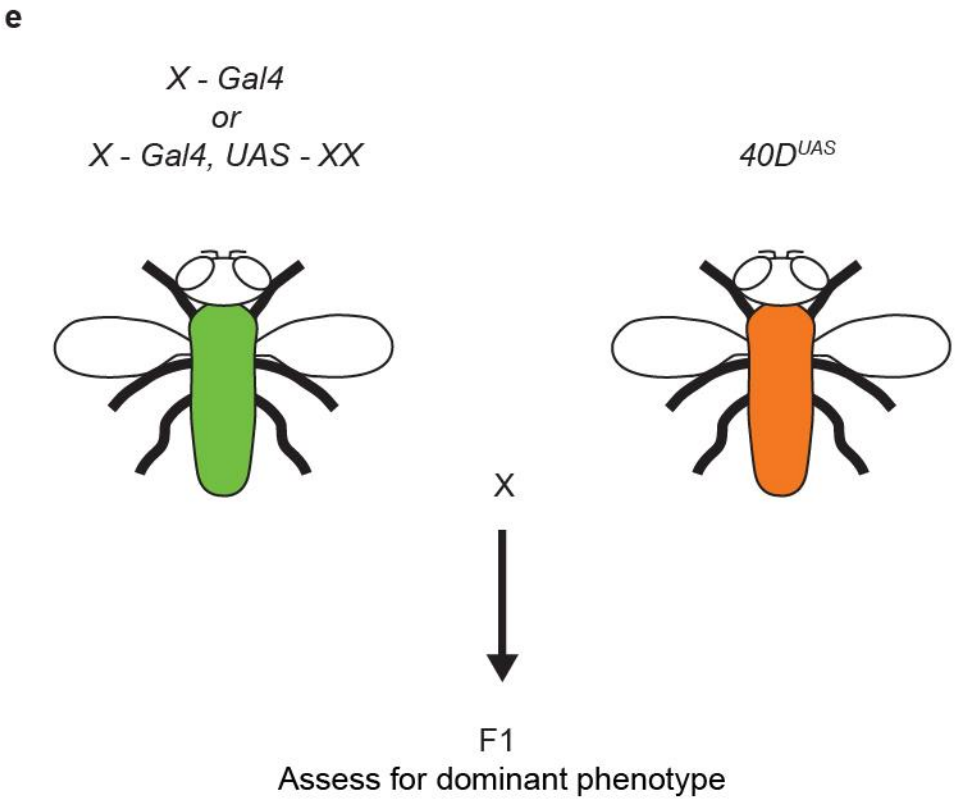



**Supplementary Figure 3. Analysis of site occupancy in KK RNAi lines targeting known Hippo pathway components.**

PCRs were performed as described in Methods[1] and run on 2% agarose gel. Numbering according to Supplementary Table 1. $H_2O$: water control. VDRC: VDRC genetic background.

Supplementary Figure 4



**Supplementary Figure 4. The *40D*$^{UAS}$ line can be used to determine whether screening system is affected by transgene integration at 40D in VDRC KK RNAi lines.**

**(a,b)** F1 flies carrying *Nubbin-Gal4* and VDRC genetic background (control) **(a)** or *40D*$^{UAS}$ **(b)**.

**(c,d)** F1 flies carrying *Hedgehog-Gal4* and VDRC genetic background (control) **(c)** or *40D*$^{UAS}$ **(d)**.

**(e)** A typical screening line will harbour a *Gal4* driver of interest, and optionally a *UAS*-driven transgene. Shown is a schematic representation of a cross to the *40D*$^{UAS}$ line described in this manuscript. Based on whether the F1 progeny of this cross shows a dominant phenotype(s), researchers can assess whether VDRC KK RNAi lines are suitable for their experiments and/or genetic screens.

**SUPPLEMENTARY TABLE 1**

| | Transformant ID | Construct ID | Gene CG number | Gene symbol | pKC26 integrated at non-annotated pKC43 (30B) | pKC26 integrated at annotated pKC43 (40D) |
|---|---|---|---|---|---|---|
| 1 | 104523 | 109756 | *CG4005* | *yki* | x | x |
| 2 | 106174 | 101055 | *CG12072* | *wts* | x | x |
| 3 | 101323 | 107562 | *CG33193* | *sav* | x | x |
| 4 | 107645 | 108458 | *CG14217* | *Tao* | x | x |
| 5 | 108254 | 107857 | *CG17090* | *HipK* | x | x |
| 6 | 105093 | 113571 | *CG5651* | *pix* | x | x |
| 7 | 104169 | 101704 | *CG11228* | *hpo* | x | |
| 8 | 106507 | 111409 | *CG33967* | *kibra* | x | |
| 9 | 109281 | 100573 | *CG4114* | *ex* | x | |
| 10 | 101497 | 108877 | *CG8544* | *sd* | x | |
| 11 | 108863 | 101190 | *CG3352* | *fat* | x | |
| 12 | 108260 | 108304 | *CG11009* | *Wbp2* | x | |

**SUPPLEMENTARY TABLE 2**

| **Gal4 driver** | **Control** | ***40D*$^{UAS}$** |
|---|---|---|
| *Actin* | NP | pupal lethal |
| *Tubulin* | NP | pupal lethal |
| *Eyeless* | NP | NP |
| *GMR* | NP | NP |
| *Dorsal* | NP | smaller, held out wing |
| *Patched* | NP | smaller wings, held up and out |
| *Fringe* | NP | small, deformed, held back wing |
| *Lozenge* | NP | NP |
| *Serrate* | NP | smaller, held out wing |
| *Hedgehog* | NP | smaller, held out wing |
| *Engrailed* | NP | held back wing |
| *Scalloped* | NP | small, held out wing |
| *Apterous* | mildly curled wing | small, deformed wing |
| *Rotund* | NP | smaller, held out wing |
| *MS1096* | NP | small, deformed, held back wing |
| *Nubbin* | NP | small, uninflated wing |
| *32B* | NP | small, held back wing |
| *71B* | NP | smaller, held up wing |
| *Spalt major* | NP | smaller, held out wing |
| *C5* | NP | NP |
| *Pannier* | NP | NP |
| *Myosin 1A* | NP | NP |

| | | |
|---|---|---|
| *Twist* | NP | NP |
| *dNab* | NP | NP |
| *Elav* | NP | NP |

(NP: no phenotype)

## SUPPLEMENTARY METHODS

### *Drosophila* genetics

Male flies from the Bloomington TRiP and VDRC 'GD' RNAi collections, the VDRC genetic background (GD60100), *40D*$^{UAS}$, *w;;UAS-GFP* and *w;; UAS-tio*[2] strains were crossed to *w; GMR-Gal4, UAS-Yki*$^{S168A}$*-YFP/TM6B* [3], *w;; GMR-Gal4, UAS-EYFP/TM6B, w;; GMR-Gal4, UAS-Myc/TM3,* or *w; GMR-Gal4, UAS-InR/CyO* virgin female flies.

For the experiment described in Supplementary Figure 1f,g, genotypes were *w; GMR-Yki/+* and *w; GMR-Yki/+; MBDlike*$^{1}$. Flies carrying the *MBDlike*$^{1}$ allele (*P{EPgy2}MBD-like*$^{EY04582}$)[4] were from Bloomington, USA. Flies carrying the *GMR-Yki* transgene have been described in[5].

To generate the genotypes in Supplementary Figure 4 and Supplementary Table 2, we crossed *40D*$^{UAS}$ or VDRC genetic background (GD60100) virgin females to males carrying the indicated Gal4 drivers.

At least 30 adult eyes of 1-2 day old females were assessed.

## SUPPLEMENTARY REFERENCES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3 Poon, C. L., Lin, J. I., Zhang, X. & Harvey, K. F. The sterile 20-like kinase Tao-1 controls tissue growth by regulating the Salvador-Warts-Hippo pathway. *Developmental cell* **21**, 896-906, doi:10.1016/j.devcel.2011.09.012 (2011).

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5 Huang, J., Wu, S., Barrera, J., Matthews, K. & Pan, D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. *Cell* **122**, 421-434, doi:10.1016/j.cell.2005.06.007 (2005).