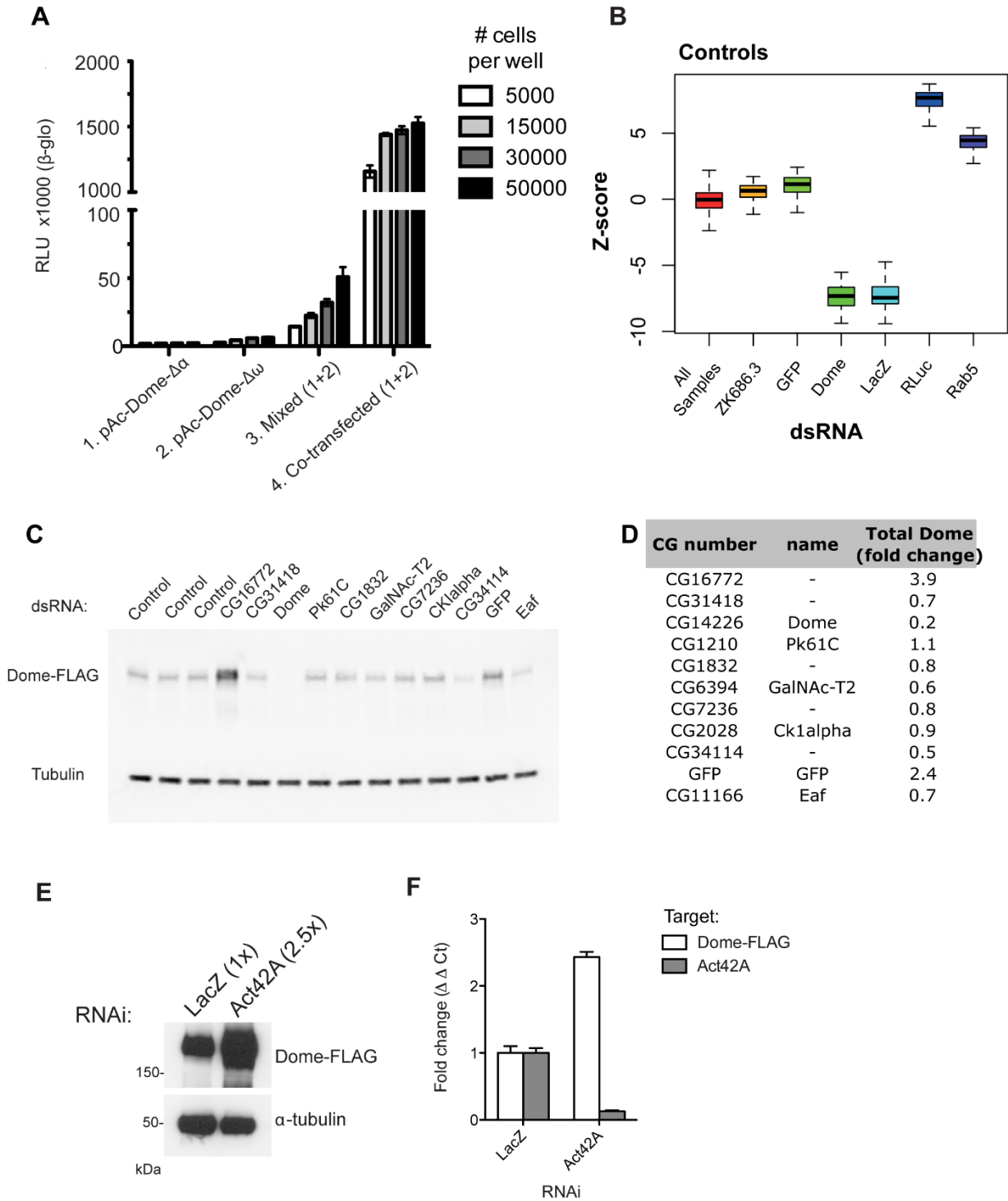
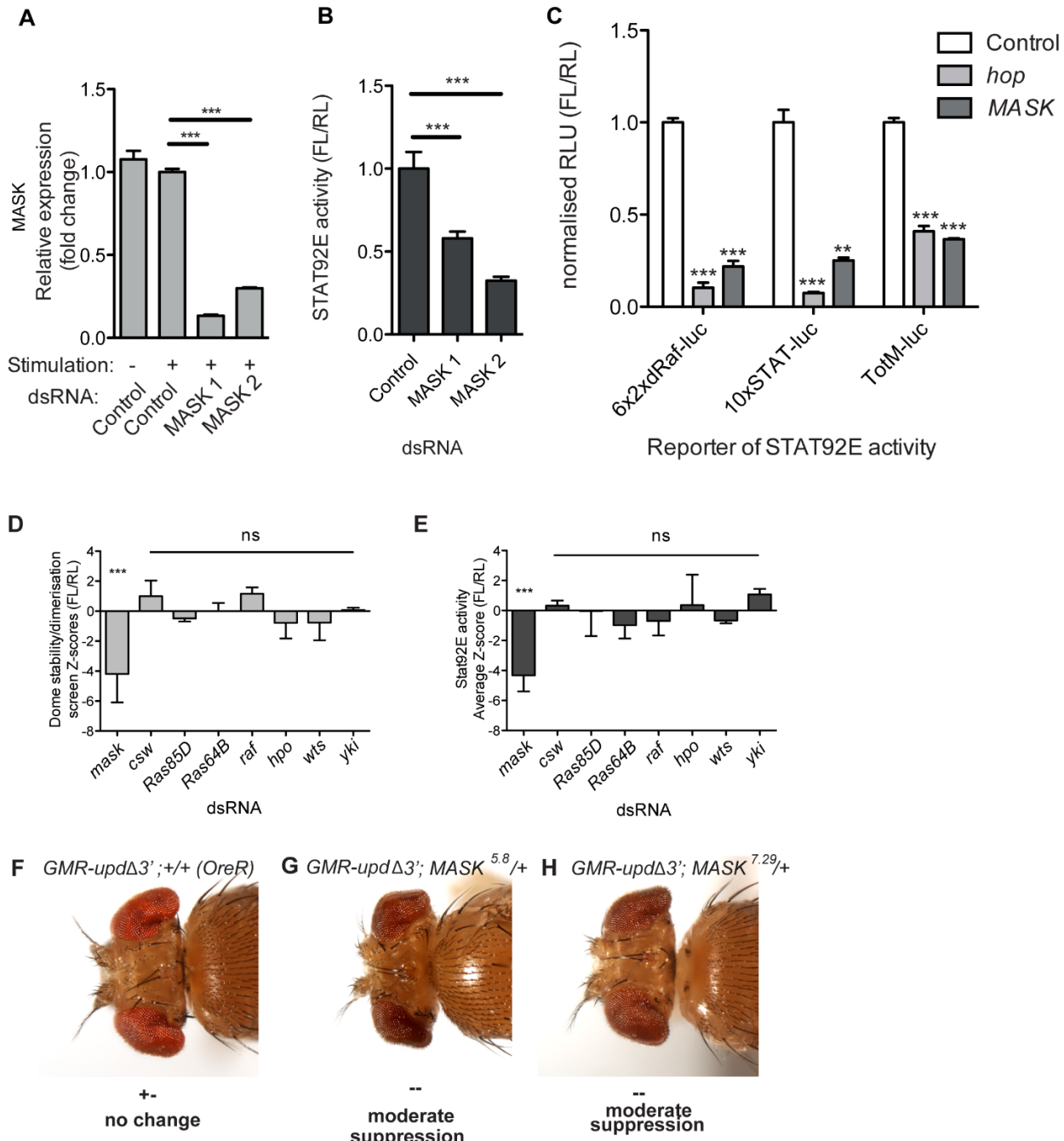


## Supplemental Figure 1



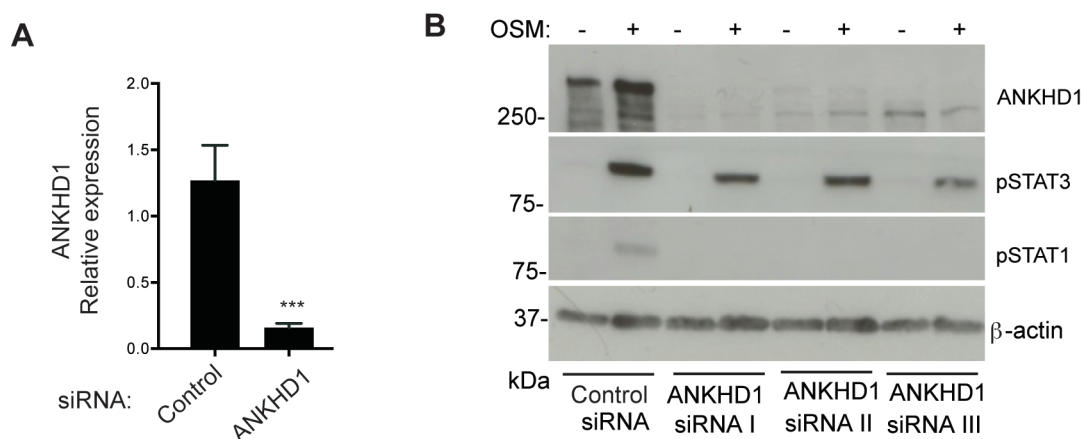
**Figure S1. A)** A significant increase in  $\beta$ -gal activity is observed in cells co-transfected with both plasmids (4), compared to cells transfected with individual plasmids and mixed together (3), demonstrating the specificity of the assay. **B)** Box-and-whisker plots showing Z-scores of negative control RNAi (*C.elegans* gene ZK686.3 or GFP) show little variation from the median, whereas technical positive RNAi controls, targeting the transfected plasmids (*Dome*, *LacZ*, *RLuc*) show significant Z-scores. Further positive control (*Rab5*), targeting the endocytic machinery and causing an increase in *Dome* stability, shows a significant increase in enzyme activity. **C-D)** Example western blot (C) and quantification (D) from secondary RNAi screen analysis, measuring *Dome* protein levels. **E)** *Dome*-FLAG protein levels increase in  $KC_{167}$  cells upon knockdown of *Act42A*, resulting in a 2.5-fold increase compared to a *LacZ* control. **F)** qPCR of the *Dome*-FLAG construct also shows an approximately 2.5-fold increase in expression upon knockdown of *Act42A*. Efficiency of RNAi is confirmed by qPCR of *Act42A*.

## Supplemental Figure 2



**Figure S2. A** mRNA expression levels of *MASK* in  $Kc_{167}$  cells assayed by qPCR after indicated RNAi treatment (*MASK1*= BKN20625; *MASK2* = HFA16018) relative to housekeeping gene *RpL32*. Knockdown of *MASK* levels are confirmed. \*\*\*  $p < 0.01$ . **B** *6x2xdRafLuc* STAT92E reporter assay is reduced after indicated RNAi treatment. **C** Three different STAT92E-dependent luciferase reporters were used to measure JAK/STAT activity after stimulation with Upd. Significant changes were observed after indicated RNAi treatment for all STAT92E-dependent reporters. **D** Z-scores derived from the Dome dimerisation genome-scale RNAi screen comparing the effect of *MASK* knockdown (column 1) to the Ras/Raf pathway components *csw*, *Ras85D*, *Ras64B*, *raf* and for the Hippo pathway genes *hpo*, *wts* and *yki*. None of the interactions were significant (ns). **E** Z-scores derived from a previous genome-scale RNAi screen for modulators of the *6x2xdRafLuc* STAT92E activity reporter (Fisher et al., 2012). The effect of *MASK* (column 1) is compared to the Ras/Raf pathway genes *csw*, *Ras85D*, *Ras64B*, *raf* and to the Hippo pathway genes *hpo*, *wts*, *yki*. None of the interactions were significant (ns). **F-H**) Dorsal view of eye overgrowth phenotypes caused by ectopic Upd ligand expression driven by *GMR-UpdΔ3<sup>+</sup>*. Panels show an alternative control (*OreR*), which was scored as normal, and two further *MASK* alleles (*MASK<sup>5,8</sup>* and *MASK<sup>7,29</sup>*), which were scored as having moderate suppression.

## Supplemental Figure 3



### Figure S3

**A**) mRNA expression of *ANKHD1* indicated from HeLa cells, after siRNA treatment against *ANKHD1* or non-targeting control. Measurements were taken relative to β-actin.

**B**) phospho-STAT1 (pSTAT1) and phospho-STAT3 (pSTAT3) protein are increased by ligand stimulation (OSM) in HeLa cells treated with control siRNA. Induction of phosphorylated STATs was suppressed when treating cells with non-overlapping siRNA reagents targeting *ANKHD1*. β-actin was used as a loading control.

### Table S1.

[Click here to Download Table S1](#)

### Table S2.

[Click here to Download Table S2](#)

**Table S3: Primers used in this study**

Gene	Purpose	Primers
<i>MASK-A1A2</i>	Gateway cloning	F: caccCGGCTGCTTTGCAAGG R: CTTAAGAGGAGCAGCCTGTTGTGTGGCAG
<i>RpL32</i>	qPCR	F: GACGCTTCAAGGGACAGTATCTG R: AAACGCGGTTCTGCATGAG
<i>MASK</i>	qPCR	F: CCGTTTCAGAGGACGATATTC R: CTTCCGACTCTTCCTCCGTTT
<i>Socs36E</i>	qPCR	F: AGTGCTTTACTGCTGCGACT R: TCGTCGAGTATTGCGAAGT
<i>SOCS3</i>	qPCR	F: AGCTGGTCTCCTTTTCCTACTCATACTA R: GGTGAAAGATGTCCCGTCTCC probe: TGGGTGGATGGAGCGGGAGGA
<i>ANKHD1</i>	qPCR	F: AGCGGTACGGGCGGAG R: AAATAAATGATTCAACCTCGGACAC probe: CGCTGGATTTCAAGTTGGCGGC
<i>β-actin</i>	qPCR	F: ATCATTGCTCCTCCTGAGCG R: GACAGCGAGGCCAGGATG probe: TACTCCGTGTGGATCGGCGGCT