SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Plasmid Constructs

Previously described plasmids used included, pAc-Yki:V5 and its isomers, pAc-Gal4DBD, pAc-Gal4DBD:Yki:V5 and its isomer, pAc-GFP:V5, pAc-Tkv^{Q235D}:3XFlag, pAc-3XFlag:Mad, pAc5.1-Med:HA, 5XDRE-luc (Oh and Irvine, 2011), UAS-luc , Ex-luc , pGEX-MscI-GFP:V5, pGEX-NI-Yki:V5 and its isomers (Oh et al., 2013), 3XSd2-luc (Zhang et al, 2008), p2xFlag CMV2-YAP2, p2xFlag CMV2-YAP2-1st&2nd WW mutant (Oka et al., 2008), 8XGTIIC-luciferase (Dupont et al., 2011), and pAC-hRluc (Potter et al 2010). For pQUAST-attB was created by inserting blunted SpeI fragment, which contains attB sequence, from pUAST-attB into StuI site of pQUAST. For pQUAST-Gal4DB:Yki:3XFL and pQUAST-Gal4DB:Yki-WW:3XFL, KpnI fragment of Gal4DB:Yki:3XFL and Gal4DB:Yki-WW:3XFL from their pAc versions were inserted into KpnI site of pQUAST-attB. For pAc-3XFL:dNcoA6, genomic DNA was amplified by PCR and then inserted into KpnI/XbaI sites of pAc-3XFL. For pAc-Yki:3XFL:dNcoA6 and its isomers KpnI frament of Yki from pAc-Yki:3XFL (Oh et al., 2013) was inserted into KpnI site of pAc-3XFL:dNcoA6. For pAc-Sd:3XFL, cDNA was amplified by PCR and then inserted into KpnI/XbaI sites of pAc-3XFL. For pAc-3XFL:GalDB:NcoA6 and Sd:3XFL:NcoA6, Gal4DB and Sd:3XFL were amplified by PCR and then inserted into KpnI sites of pAc-3XFL:dNcoA6. For pGST-YkiC:V5, C-terminal region of Yki (241-418 a.a.) was amplified by PCR and then inserted into SmaI site of pGEX-3X. For pCMV2-RLuc, EcoRI/XbaI fragment from copia-RLuc was inserted into EcoRI/XbaI site of p2XFL-CMV2-YAP2. For pCMV2-GFP:V5, KpnI/StuI fragment of GFP:V5 from pAc-GFP:V5 was inserted into KpnI/SmaI site of p2xFlag

CMV2-YAP2 and then KpnI site was blunted with Klenow fragment to create a stop codon before GFP sequence. For pCMV2-hNcoA6:V5, cDNA (2063 a.a.) was amplified by PCR and inserted into NotI/XbaI site of pCDNA3.1V5HisB. Mutations in the three PPxY motifs of dNcoA6 (Y262A, Y632A, and Y1464A) and two PPxY motifs of hNcoA6 (Y376A and Y973A) were introduced by primer-mediated site-directed mutagenesis using QuikChange Multi Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions.

RNA extraction, RT-PCR, and RNAi

RNA was extracted from the anterior part of ten 3rd instar larvae for each genotype using Trizol (Invitrogen), DNaseI treated and then purified with RNeasy kit (Qiagen). 1 μg of RNA was used for reverse transcription using High Capacity RNA-to-cDNA kit (Applied Biosystems) and same amount of cDNA was used for PCR. Primers for PCR were as follows: UAS-GFP (5'-tgccaagaagtaattattgaata / 5'-gcatcaccttcaccctctcc), pka (5'-agccgcactcgcgcttctac/ 5'-caatcagcagattctccggct) and GAPDH (5'-gatcggaattaacggatttgg/5'-ccacatactcggctccagca).

For Quantitative RT-PCR in cell culture, total RNA was extracted from cells in 12-well plates using the TriZol Plus RNA Kit (Invitrogen). 2 μg of RNA was used for reverse transcription using High Capacity RNA-to-cDNA kit (Applied Biosystems) and same amount of cDNA was used for quantitative PCR using the SYBR Select Master Mix (Applied Biosystems) on StepOnePlus Real time PCR Systems (Applied Biosystems). GAPDH was used as an internal control. Primers for RT-PCR were: hGAPDH (5'- ggagcgagatccctccaaaat / 5'- ggctgttgtcatacttctcatgg), BIRC3 (5'-

tttccgtggctcttattcaaact/ 5’- gcacagtggtaggaacttctcat) and dGAPDH (5’-gatcggaattaacggatttgg/5’-ccacatactcggctccagca) and trx (5’- gcagctgcaaaatggagtgg/5’-gtacgtaaagccatcttcgct)).

Primers for generation of dsRNA for S2 cells:

GFP (5’- ggtgagcaagggcgaggagct/5’- tcttgaagttcaccttgatgccg)

Sd (5’- gcatggtggatagcaaaaac/5’- gccaagttgaacgaatagatgccg)

NcoA6-GD (5’-ccacagcatccacaaacacaa/5’- tctcggcgtctcaccgtcc)

NcoA6-KK (5’- ccgtctttaaatcccccaata/5’-cgatatggcaatgttgttgctg)

trr-KK (5’- cgctgatgaacttccagacag/5’- ggcagtaactgacccacgtt)

trr-open (from Openbiosystems, sequences not available)

dSET1-c (5’- ccagcaacagccatgattcg/5’- tgcccaagtgtttgtttgtgatg)

dSET1-g (5’- cgaagctcgctcaaaccaga/5’- tccacatttagttgcgtcaattat)

trx-KK (5’- aagcagctgcaaaatggagtgg/5’- ggaggccaaccaactaaacat)

trx-open (from Openbiosystems, sequences not available).

Drosophila **NcoA6 antibody preparation**

N-terminal half (1-1504 a.a.) and C-terminal half (1499- 2437 a.a.) of dNcoA6 were cloned separately into pGEX-3X (Amersham Biosciences) at a *Sma*I site. GST:dNcoA6-N and -C were expreseed in BL21(DE3) *E. coli* (Invitrogen) by induction with 0.5 mM IPTG and then purified with B-PER GST Fusion Protein Purification Kit (Thermo Scientific) according to manufacturer’s instruction. Purified GST:dNcoA6-N and –C were mixed and were used to immunize guinea pigs (Cocalico Biologicals). The

specificity of the resulting anti-sera was confirmed by immunostaining *Drosophila* imaginal discs (Fig. S3F) and by Western blotting (Fig. S2B).

ChIP-Seq

For S2 chromatin collection, approximately 10^7 cells were fixed for 20 min. in Schneider's media with 1.8% formaldehyde. Glycine was added to the fixation solution (5 min., final glycine concentration of 225mM) to quench the formaldehyde. Fixed cells were then washed two times with lysis buffer (140 mM NaCl, 15 mM HEPES pH 7.6, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 1% Triton X-100, 0.5mM DTT, and Roche Complete EDTA-free protease inhibitors). After washing, cells were resuspended in 500uL lysis buffer plus 0.1% SDS and rotated for 20 minutes at 4°C. Chromatin was then sheared in a Bioruptor sonicator (7.5 minutes total sonication time), insoluble material was removed by centrifugation, and soluble chromatin extract was used for anti-Yki IP of protein-DNA complexes as described previously (Oh et al., 2013). Immunoprecipitated DNA was prepared for Illumina sequencing using the Epicentre Nextera DNA Sample Preparation Kit, sequenced on an Illumina HiSeq 2000, and processed as described previously. All experiments were performed in duplicate and peak calling was based on merged reads for duplicate ChIPs (and input control DNA). Briefly, for data processing alignment of sequences to the *Drosophila* genome was performed using BWA and peaks were called using MACSv2 (bandwidth of 100 and p-value cutoff of $1x1x10^{-5}$) (Zhang et al., 2008). ChIP datasets for S2 H3K4me3, S2H3K4me1, and S2 Trr were previously published (Herz et al., 2012) and are deposited in GEO under

accession number GSE41440. Datasets generated for the current study are also available at GEO accession numbers GSE38594 and GSE46305.

Supplemental References

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S.*, et al.* (2011). Role of YAP/TAZ in mechanotransduction. Nature *474*, 179-183.

Herz, H.M., Mohan, M., Garruss, A.S., Liang, K., Takahashi, Y.H., Mickey, K., Voets, O., Verrijzer, C.P., and Shilatifard, A. (2012). Enhancer-associated H3K4 monomethylation by Trithorax-related, the Drosophila homolog of mammalian Mll3/Mll4. Genes and Development *26*, 2604-2620.

Mahajan, M.A., and Samuels, H.H. (2008). Nuclear receptor coactivator/coregulator NCoA6(NRC) is a pleiotropic coregulator involved in transcription, cell survival, growth and development. Lucler Receptor Signaling *6*, 1-19.

Oh, H., and Irvine, K.D. (2011). Cooperative regulation of growth by Yorkie and Mad thgrough bantam. Developmental Cell *20*, 109-122.

Oh, H., Slattery, M., Ma, L., Crofts, A., White, K.P., Mann, R.S., and Irvine, K.D. (2013). Genome-wide Association of Yorkie with Chromatin and Chromatin-Remodeling Complexes. Cell Reports *3*, 309-318.

Oka, T., Mazack, V., and Sudol, M. (2008). Mst2 and Lats Kinases Regulate Apoptotic Function of Yes Kinase-associated Protein (YAP). J Biol Chem *283*, 27534-27546.

Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nusbaum, C., Myers, R.M., Brown, M., Li, W.*, et al.* (2008). Model-based analysis of ChIP-Seq (MACS). Genome biology *9*, R137.