# nature research

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# **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all st	atistical an	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed	
	$\boxtimes$	The exact	sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	$\boxtimes$	A stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	$\boxtimes$		tical test(s) used AND whether they are one- or two-sided on tests should be described solely by name; describe more complex techniques in the Methods section.
$\boxtimes$		A descript	ion of all covariates tested
$\boxtimes$		A descript	ion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	$\boxtimes$	A full desc AND varia	cription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) tion (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	$\boxtimes$	For null hy Give P value	pothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted as as exact values whenever suitable.
$\boxtimes$		For Bayesi	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$		For hierar	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$		Estimates	of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated
			Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
So	ftw	are an	d code
Poli	cy inf	formation a	about <u>availability of computer code</u>
Da	ata co	ollection	Zeiss LSM880 was used for microscopy image acquisition.
Da	ata ai	nalysis	Statistical analysis was performed using GraphPad Prism (v9.0.0). fastp (v0.19.4) HISAT2 (v2.0.4) exomePeak (v1.9.1) HOMER (v4.1) ChIPseeker (v1.18.0) StringTie (v1.3.4) edgeR (v4.1) rMATS (v4.0.1)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

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High-throughput m6 files, and bigWig files	5A-seq data were deposited into the GEO database with accession number:GSE155662. It includes all raw files, peak text files, FPKM value text is for visualization.
Field-spe	ecific reporting
Please select the o	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
\times Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>
Life scier	nces study design
	sclose on these points even when the disclosure is negative.
Sample size	No statistical methods were used to predetermine sample size. Sample sizes were chosen based on previous experience and similar experiments in the literature. Sample sizes were indicated in the figure legends and Methods.
Data exclusions	No data were excluded from the analysis.
Replication	Repeat numbers of the experiments were indicated in the figure legends. All attempts at replication were successful.
Randomization	Samples were randomly assigned.
Blinding	The experiments were not blinded as the obvious mutant phenotypes make blinding surplus.
Reportin	g for specific materials, systems and methods
We require informati	ion from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each materia ited is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
Materials & ex	perimental systems Methods
n/a Involved in th	· · · · · · · · · · · · · · · · · · ·
Antibodies	S ChIP-seq
Eukaryotic	c cell lines Flow cytometry

Materials & experimental systems	Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
Animals and other organisms	·
Human research participants	
Clinical data	
Dual use research of concern	

#### **Antibodies**

Antibodies used

mouse anti-Fl(2)d (9G2, DSHB), rat anti-Ecad (DCAD2, DSHB), mouse anti-Sxl (M18, DSHB), rabbit anti-Flacc (self-made), rabbit anti-Hakai (self-made), rabbit anti-Vir (self-made), rabbit anti-Mettl3 (self-made), rabbit anti-Mettl14 (self-made), rabbit anti-GFP (A6455, Molecular Probes), rat anti-HA (3F10, Roche), rabbit anti-m6A (202003, Synaptic Systems), Donkey anti-Mouse IgG (H+L) (Alexa Fluor 488) (Thermo Fisher Scientific, A21202), Donkey anti-Rabbit IgG (H+L) (Alexa Fluor 488) (Thermo Fisher Scientific, A21206), Cy3conjugated Donkey anti-Mouse IgG (H+L) (Jackson ImmunoResearch, 715-165-150), Cy3-conjugated Donkey anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 711-165-152). The concentrations used were described in the methods section.

Validation

Hakai antibody was validated by immunostaining in RNAi, CRISPR and mutant wing discs (Fig. 2d-e, 4g, 4f'). Vir antibody was validated by immunostaining in RNAi wing discs (Fig. 4m). Mettl3 and Mettl14 antibodies were validated by immunostaining in mutant wing discs (Fig. S5a-d). rabbit anti-Flacc (self-made) was validated previously in: Guo, J et al. PNAS, 115(14):3674-3679 (2018). mouse anti-FI(2)d (9G2, DSHB), rat anti-Ecad (DCAD2, DSHB), and mouse anti-SxI (M18, DSHB) were obtained from Developmental Studies Hybridoma Bank and their information are described in https://dshb.biology.uiowa.edu. rabbit anti-GFP (A6455, Molecular Probes), rat anti-HA (3F10, Roche), and rabbit anti-m6A (202003, Synaptic Systems) are widely-used commercial antibodies and their information are described in the companies' websites.

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Drosophila S2 cells were kindly provided by Prof Yongzhen Xu at Wuhan University as described in: Li, L et al. Nat. Commun. 11:5608 (2020). HeLa cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Authentication

None

Mycoplasma contamination

Not tested for mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

None

### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Drosophila Melanogaster of the following genotypes were used:

yw Mettl3SK2 Mettl14SK1 Df(3R)Exel6197

ap-Gal4

w1118

Hakai shRNA (VDRC 330548)

vir shRNA (HMC03908, Bloomington 55694) fl(2)d shRNA (HMC03833, Bloomington 55674)

flacc shRNA (VDRC 35212GD) actin-Cas9 (Bloomington 54590) nanos-Cas9 (Bloomington 78782)

U6-Hakai-sgRNA HakaiSH2 HakaiSH4

Hakai shRNA (TH14422.S) Hakai shRNA (TH14423.S)

Immunostaining was performed on 3rd instar larval wing discs. Adult flies used in the paper were about 1-2 days old; the sex of these flies were indicated in Fig. 3b, Fig. 6d-g, Fig. 54, Fig. 2 legend, Fig. 3 legend, Fig. 6 legend, and Fig. 7 legend.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight

Not applicable.

Note that full information on the approval of the study protocol must also be provided in the manuscript.