# nature portfolio

Corresponding author(s):	Mohr, Perrimon, Vidal, Celniker
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# **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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For	all st	atistical analyses, 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 statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	$\boxtimes$	The statistical test(s) used AND whether they are one- or two-sided  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
$\boxtimes$		A description of all covariates tested
	$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	$\boxtimes$	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	$\boxtimes$	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\times$		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on statistics for biologists contains articles on many of the points above.

# Software and code

Policy information about availability of computer code

Data collection

Code relevant to Y2H data was as published in Luck et al. 2020 and available at <a href="https://github.com/CCSB-DFCI/HuRI\_paper">https://github.com/CCSB-DFCI/HuRI\_paper</a>

Data analysis

For ChIP-seq analysis, low-quality reads and adaptor primer sequences were trimmed using Trim Galore 0.6.4 (https://github.com/FelixKrueger/TrimGalore). Trimmed reads were mapped using bowtie2 2.3.5.1 with the additional argument "-q --local"84. Samtools 1.6 was used to sort, filter unique reads, and convert file format to bam files. Peak calling was performed with MACS2 2.2.6 with additional parameter "-B --SPMR -f BAMPE -g dm". Peaks were annotated with HOMER 4.1187. DeepTools 3.4.0 were used for normalizing read counts to CPM and convert bam files to bigWig format. The code for node shuffling used to generate random networks based on the FlyBi network is available at <a href="https://github.com/moontreegy/flybi-network-analysis">https://github.com/moontreegy/flybi-network-analysis</a>. All other code is as previously published. L3 prediction code files was published in Kovacs et al. (2019) and is available at <a href="https://doi.org/10.5281/zenodo.2008592">https://doi.org/10.5281/zenodo.2008592</a>. Code relevant to Y2H and PPI analyses was published in Luck et al. 2020 and is available at <a href="https://github.com/CCSB-DFCI/HuRI\_paper">https://github.com/CCSB-DFCI/HuRI\_paper</a>. SAFE analysis software version 1.5 was used in this study; SAFE was published by Baryshnikova et al. (2016) and is available at https://github.com/baryshnikova-lab/safepy. FBgn IDs were updated and validated using <a href="https://flybase.org/convert/id">https://flybase.org/convert/id</a>. Band sizes were calculated using BioRad Quantity One software (version 4.6.9). PCR primers for ORF amplification were designed using Primer3 release 0.9 69 (the resulting primer sequences are reported in Suppl. File 9).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

FlyBi binary interaction data are as provided in Supplementary file 5 and are also available as a table and as a downloadable data file at the FlyBi project webpage ([http://flybi.hms.harvard.edu/]). These data were also integrated with other datasets at IntAct ([https://www.ebi.ac.uk/intact/]) and in the Molecular Interaction Search Tool (MIST; [https://fgrtools.hms.harvard.edu/MIST/]). MAPPIT data is provided as Suppl. File 6. RNAi data for the autophagy-related network is provided as as Suppl. File 8. Plasmid clones and information are available from at the Drosophila Genomics Resource Center (University of Indiana, Bloomington, IN) and DNASU plasmid repository (Arizona State University, Phoenix, AZ). ORFs in the Gateway donor vector were end-read sequenced and this sequence data is available at GenBank and at the FlyBi project website (see "Genbank Accession" columns on the table at [https://flybi.hms.harvard.edu/results.php]). For a subset of 954 ORFs, the end-reads sequence spanned the full ORF; this sequence data is available at the NCBI (Project Accession ID PRJNA349744) and a list of these ORFs, along with NCBI IDs, is available at the FlyBi project website (see [https://flybi.hms.harvard.edu/clones.php]). Interaction data was deposited at EBI IntAct (<https:// www.ebi.ac.uk/intact/home>) and our integrated Drosophila reference interaction (DroRI) PPIs are available at MIST (<a href="https://fgrtools.hms.harvard.edu/MIST/">https://fgrtools.hms.harvard.edu/MIST/</a>). ChIPseq data is available at NCBI GEO (Accession ID GSE220887).

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Н	ıuman.	research	partici	nants
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Human resea	h participants	
Policy information ab	it studies involving human research participants and Sex and Gender in Research.	
Reporting on sex a	gender There were no human research participants in this study	
Population charact	stics Not applicable to this study	
Recruitment	Not applicable to this study	
Ethics oversight	Not applicable to this study	
Note that full informati	on the approval of the study protocol must also be provided in the manuscript.	
1 1		
-ield-sped	fic reporting	
Please select the one	elow that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
X Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences	
	cument with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
lite scien	es study design	
All studies must discl	e on these points even when the disclosure is negative.	
	Sample size was determined by the number of unique available Drosophila open reading frame (ORF) clones (yeast two-hybrid screen) or by the results of the screen, with filters applied (e.g., Y2H-detected PPIs with specific candidates) as described in the manuscript	
Data exclusions	No data were excluded.	
Replication	The large-scale screen was performed two times in each of two different formats (four screens in total). Follow-up studies followed standard	

guidelines for study design for Drosophila or other studies, e.g., three replicates for in vivo studies, and all replicates of these studies gave comparable results.

Randomization

For the yeast two-hybrid screens, all available ORFs were included (no non-random sub-sets). Subsets of results of the large-scale screen were chosen at random for MAPPIT analysis. For the in vivo autophagy study, we used known autophagy components as the start-point to build a putative autophagy network comprised of known autophagy components (list 1), interactors with those proteins (list 2) and interactors with list 2 proteins (list 3)(see Supplemental data file 7), then tested all components in the Atg1 autophagy-related assay. Positives in that assay were included in the fat body autophagy-related assay.

Blinding

Y2H screening used pooled approaches and lab automation; researchers were blind to identify of specific ORF clones in yeast until the endpoint of the assay (sequencing) revealed identity. For the genetic screens, fly stocks were labeled and data collected based on RNAi stock IDs; researchers blind to the gene targets during the assay and data collection; identities of gene targets were only revealed when look-up tables were used to associate a stock ID with a gene target.

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed 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 & experime	ental systems	Methods
n/a Involved in the study		n/a   Involved in the study
Antibodies		ChIP-seq
Eukaryotic cell lines		
		— I —
Palaeontology and a	archaeology	MRI-based neuroimaging
Animals and other o	organisms	
Clinical data		
Dual use research o	f concern	
A #1111		
Antibodies		
Antibodies used	(A-6455, Molecular Probes) (WB) or 1:100 (IF); mouse r HA (901514, Biolegend), dil (WB); goat anti-Mouse IgG Mouse IgG (H+L) Secondary Chain Specific) (D3V2A) mA Specific) (D4W3E) mAb (HR Nanobody/VHH coupled to	mmunofluorescence (IF) or immunoblotting (WB) at the following dilutions. Rabbit polyclonal anti-GFP ), dilution factor 1:5,000 (WB); rabbit monoclonal anti-Atg8 (ab109364, Abcam), dilution factor 1:2,000 monoclonal anti-Flag (F3165, Sigma), dilution factor 1:5,000 (WB) or 1:1,000 (IF); mouse monoclonal anti-lution factor 1:1,000 (IF); rabbit polyclonal anti-GAPDH (GTX100118, GeneTax), dilution factor 1:10,000 (H+L) secondary antibody, Alexa Fluor 633 (A-21052, Invitrogen), dilution factor 1:1,000 (IF); donkey anti-y Antibody, Alexa Fluor 555 (A-31570, Invitrogen), dilution factor 1:1,000 (IF); rabbit Anti-Mouse IgG (Light the Chain the Conjugate) (58802, Cell Signaling), dilution factor 1:1000 (WB); mouse Anti-Rabbit IgG (Light-Chain the Conjugate) (93702, Cell Signaling), dilution factor 1:1000 (WB). For immunoprecipitation, we used a GFP agarose beads (ChromoTek GFP-Trap Agarose, AB_2631357). For ChIP-seq, we used anti-Flag (Sigma, by beads as included in SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology, 9005).
Validation	manufacturer and reported 1. Rabbit polyclonal anti-GF gclid=CjwKCAiArY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAY2fBhB9EivAFAIAFABOUSE IgG (HHChttps://www.thermofisher7. Donkey anti-Mouse IgG (HHChttps://www.thermofisher7. Donkey anti-Mouse IgG (Iantibody/product/Donkey-8. Rabbit Anti-Mouse IgG (Iwww.cellsignal.com/product9. Mouse Anti-Rabbit IgG (L	ercial antibodies, against well-characterized conserved protein targets or epitopes, validated by the d in publications, as noted at manufacturer websites. Please see below.  EP, validated by manufacturer (https://www.thermofisher.com/antibody/product/A-6455.html? wAWqHK6ow9ESZoZny1Nlhy2JBYrc7TiagTpf3xOzOWdGYagFy4I3Taii3xoRoCIEoQAvD_BwE&ef_id=CjwKCAi DESZoZny1Nlhy2JBYrc7TiagTpf3xOzOWdGYagFy4I3Taii3xoRoCIEoQAvD_BwE:G:s&s_kwcid=AL!3652!3! D825775!106531320406&cid=bid_pca_aup_r01_co_cp1359_pjt0000_bid00000_0se_gaw_dy_pur_con) Atg8, validated by manufacturer (https://www.abcam.com/nav/primary-antibodies/rabbit-monoclonal-bl1gabarapl2-antibody-epr4805-ab109364.html) (note, includes reports for Drosophila Atg8) Flag, validated by manufacturer (https://www.sigmaaldrich.com/SG/en/product/sigma/f3165) HA, validated by manufacturer (https://www.biolegend.com/en-us/products/anti-ha-11-epitope-tag-NPDH, validated by manufacturer (https://www.genetex.com/Product/Detail/GAPDH-antibody/GTX100118) +L) Secondary Antibody, Alexa Fluor® 633, validated by manufacturer (https://www.thermofisher.com/antibody/product/A-21052.html?CID=AFLLO-A-21052) (H+L) Secondary Antibody, Alexa Fluor™ 555, validated by manufacturer (https://www.thermofisher.com/anti-Mouse-lgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31570) Light Chain Specific) (D3V2A) mAb (HRP Conjugate), validated by manufacturer (https://cts/secondary-antibodies/rabbit-anti-mouse-igg-light-chain-specific-d3v2a-mab-hrp-conjugate/58802) Light-Chain Specific) (D4W3E) mAb (HRP Conjugate), validated by manufacturer (https://cts/secondary-antibodies/mouse-anti-rabbit-igg-light-chain-specific-d4w3e-mab-hrp-conjugate/93702)

# Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>

The S. cerevisiae (yeast) used in this study were lab strains derivative of S288C and the genotypes are described in detail in Cell line source(s)

the manuscript. The mammalian cell line used in this study was a Tavernier lab copy of HEK293T.

Authentication Authentication of HEK293T was performed by genome sequencing as described in Lin et al. (2014) PMCID: PMC4166678

Standard methods for monitoring for mycoplasma were applied; no contamination was detected. Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

"HEK," but not the "HEK293T" cell line we used, appears on the list of commonly misidentified lines. We authenticated the cell line as indicated above and further note that the identity of the cell line should have little or no impact on the findings reported, given our use of the cells, i.e., for MAPPIT assays with Drosophila proteins

# Animals and other research organisms

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in Research</u>

Laboratory animals Drosophila melanogaster lab strains

Wild animals This study did not involve wild animals

Reporting on sex

Only male Drosophila were scored for the Atg1 eye phenotype to avoid differences in eye size due to sex. For the larval fat body

assay, Drosophila larvae of both sexes were included.

Field-collected samples This study did not involve field-collected samples

Ethics oversight No ethical approval or guidance was required because the lab animal component of this study was limited to Drosophila

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

# ChIP-sea

# Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

#### Data access links

May remain private before publication.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE220887

Files in database submission

LIB045499 CHS00173889 S1 L001 R1.fastq.bz2 , LIB045499 CHS00173889 S1 L001 R2.fastq.bz2 , LIB045499\_CHS00173889\_S1\_L002\_R1.fastq.bz2, LIB045499\_CHS00173889\_S1\_L002\_R2.fastq.bz2, LIBO45499\_CHS00173889\_S1\_L003\_R1.fastq.bz2, LIBO45499\_CHS00173889\_S1\_L003\_R2.fastq.bz2, LIB045499\_CHS00173889\_S1\_L004\_R1.fastq.bz2, LIB045499\_CHS00173889\_S1\_L004\_R2.fastq.bz2, LIBO45499\_CHS00173890\_S2\_L001\_R1.fastq.bz2, LIBO45499\_CHS00173890\_S2\_L001\_R2.fastq.bz2, LIBO45499 CHS00173890 S2 L002 R1.fastq.bz2, LIBO45499 CHS00173890 S2 L002 R2.fastq.bz2, LIBO45499 CHS00173890 S2 L003 R1.fastq.bz2, LIBO45499 CHS00173890 S2 L003 R2.fastq.bz2, LIBO45499 CHS00173890 S2 L004 R1.fastq.bz2, LIBO45499 CHS00173890 S2 L004 R2.fastq.bz2,  $LIBO45499\_CHS00173892\_S4\_L001\_R1.fastq.bz2, \ LIBO45499\_CHS00173892\_S4\_L001\_R2.fastq.bz2, \ LIBO45499\_CHS0017389$ LIBO45499\_CHS00173892\_S4\_L002\_R1.fastq.bz2, LIBO45499\_CHS00173892\_S4\_L002\_R2.fastq.bz2, LIB045499\_CHS00173892\_S4\_L003\_R1.fastq.bz2, LIB045499\_CHS00173892\_S4\_L003\_R2.fastq.bz2, LIBO45499 CHS00173892 S4 L004 R1.fastq.bz2, LIBO45499 CHS00173892 S4 L004 R2.fastq.bz2, LIB045499\_CHS00173893\_S5\_L001\_R1.fastq.bz2, LIB045499\_CHS00173893\_S5\_L001\_R2.fastq.bz2,  $LIBO45499\_CHS00173893\_S5\_L002\_R1.fastq.bz2, \ LIBO45499\_CHS00173893\_S5\_L002\_R2.fastq.bz2, \ LIBO45499\_CHS0017389$ LIBO45499 CHS00173893 S5 L003 R1.fastq.bz2, LIBO45499 CHS00173893 S5 L003 R2.fastq.bz2, LIB045499\_CHS00173893\_S5\_L004\_R1.fastq.bz2, LIB045499\_CHS00173893\_S5\_L004\_R2.fastq.bz2, LIBO45499\_CHS00173895\_S7\_L001\_R1.fastq.bz2, LIBO45499\_CHS00173895\_S7\_L001\_R2.fastq.bz2, LIBO45499 CHS00173895 S7 L002 R1.fastq.bz2, LIBO45499 CHS00173895 S7 L002 R2.fastq.bz2, LIBO45499\_CHS00173895\_S7\_L003\_R1.fastq.bz2, LIBO45499\_CHS00173895\_S7\_L003\_R2.fastq.bz2, LIB045499\_CHS00173895\_S7\_L004\_R1.fastq.bz2, LIB045499\_CHS00173895\_S7\_L004\_R2.fastq.bz2, LIBO45499\_CHS00173896\_S8\_L001\_R1.fastq.bz2, LIBO45499\_CHS00173896\_S8\_L001\_R2.fastq.bz2, LIB045499\_CHS00173896\_S8\_L002\_R1.fastq.bz2, LIB045499\_CHS00173896\_S8\_L002\_R2.fastq.bz2, LIB045499 CHS00173896 S8 L003 R1.fastq.bz2, LIB045499 CHS00173896 S8 L003 R2.fastq.bz2, LIBO45499\_CHS00173896\_S8\_L004\_R1.fastq.bz2, LIBO45499\_CHS00173896\_S8\_L004\_R2.fastq.bz2, dwg\_vs\_control\_summits.bed, dwg\_vs\_input\_summits.bed

Genome browser session (e.g. <u>UCSC</u>)

No longer applicable.

# Methodology

Replicates

Two biological replicates for each condition (2 input, 2 IgG control, 2 IP-Flag-Dwg).

Sequencing depth

Sequencing Depth: LIB045499\_CHS00173889\_S1\_L001\_R1, 1.8; LIB045499\_CHS00173889\_S1\_L001\_R2, 1.8; LIB045499\_CHS00173889\_S1\_L002\_R1, 1.8; LIB045499\_CHS00173889\_S1\_L002\_R2, 1.8; LIB045499\_CHS00173889\_S1\_L003\_R1, 1.9; LIB045499\_CHS00173889\_S1\_L004\_R1, 1.8; LIB045499\_CHS00173889\_S1\_L004\_R1, 1.8; LIB045499\_CHS00173889\_S1\_L004\_R2, 1.8; LIB045499\_CHS00173890\_S2\_L001\_R1, 3; LIB045499\_CHS00173890\_S2\_L002\_R1, 2.9; LIB045499\_CHS00173890\_S2\_L002\_R2, 2.9; LIB045499\_CHS00173890\_S2\_L003\_R1, 3; LIB045499\_CHS00173890\_S2\_L003\_R2, 3; LIB045499\_CHS00173890\_S2\_L004\_R1, 2.9; LIB045499\_CHS00173891\_S3\_L001\_R1, 2.4; LIB045499\_CHS00173891\_S3\_L001\_R1, 2.4; LIB045499\_CHS00173891\_S3\_L002\_R2, 2.3; LIB045499\_CHS00173891\_S3\_L003\_R1, 2.4; LIB045499\_CHS00173891\_S3\_L004\_R2, 2.4; LIB045

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2.3; LIB045499 CHS00173892 S4 L001 R1, 3.8; LIB045499 CHS00173892 S4 L001 R2, 3.8;
LIB045499\_CHS00173892\_S4\_L002\_R1,\ 3.8;\ LIB045499\_CHS00173892\_S4\_L002\_R2,\ 3.8;\ LIB045499\_CHS00173892\_S4\_L003\_R1,\ LIB045490\_CHS00173892\_S4\_L003\_R1,\ LIB045490\_CHS00173892\_CHS00173892\_CHS00173892\_CHS00173892\_CHS00173892\_CHS00173892\_CHS00173892\_CHS00173892\_C
3.9; LIB045499 CHS00173892 S4 L003 R2, 3.9; LIB045499 CHS00173892 S4 L004 R1, 3.8;
LIBO45499 CHS00173892 S4 L004 R2, 3.8; LIBO45499 CHS00173893 S5 L001 R1, 3; LIBO45499 CHS00173893 S5 L001 R2, 3;
LIBO45499 CHS00173893 S5 L002 R1, 2.9; LIB045499 CHS00173893 S5 L002 R2, 2.9; LIB045499 CHS00173893 S5 L003 R1, 3;
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3.3; LIB045499 CHS00173894 S6 L002 R2, 3.3; LIB045499 CHS00173894 S6 L003 R1, 3.4;
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5.7; LIBO45499 CHS00173896 S8 L004 R2, 5.7; Number of uniquely mapped reads: LIBO45499 CHS00173889 S1 L001, 1236478;
LIB045499_CHS00173889_S1_L002, 1226998; LIB045499_CHS00173889_S1_L003, 1270909; LIB045499_CHS00173889_S1_L004,
1230250; LIB045499_CHS00173890_S2_L001, 2086760; LIB045499_CHS00173890_S2_L002, 2061698;
LIBO45499 CHS00173890 S2 L003, 2136062; LIBO45499 CHS00173890 S2 L004, 2067419; LIB045499 CHS00173891 S3 L001,
1492088; LIB045499 CHS00173891 S3 L002, 1456747; LIB045499 CHS00173891 S3 L003, 1532881;
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2480676; LIB045499 CHS00173892 S4 L003, 2588428; LIB045499 CHS00173892 S4 L004, 2491652;
LIB045499_CHS00173893_S5_L001, 1988063; LIB045499_CHS00173893_S5_L002, 1943597; LIB045499_CHS00173893_S5_L003,
2034599; LIB045499 CHS00173893 S5 L004, 1950723; LIB045499 CHS00173894 S6 L001, 2170419;
LIB045499 CHS00173894 S6 L002, 2109665; LIB045499 CHS00173894 S6 L003, 2223329; LIB045499 CHS00173894 S6 L004,
2119886; LIB045499_CHS00173895_S7_L001, 3105918; LIB045499_CHS00173895_S7_L002, 3047315;
LIB045499_CHS00173895_S7_L003, 3181092; LIB045499_CHS00173895_S7_L004, 3058192; LIB045499_CHS00173896_S8_L001,
3799882; LIB045499 CHS00173896 S8 L002, 3746522; LIB045499 CHS00173896 S8 L003, 3891180;
LIB045499_CHS00173896_S8_L004, 3765473; Length of reads: 150 bp; paired-end
```

### **Antibodies**

IgG antibody as included in SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology; #9005), anti-Flag (Sigma, F3165)

#### Peak calling parameters

# Specify the command line program and parameters used for read mapping and peak calling, # including the ChIP, control, and index files used

# the command line program and parameters are the same for each experiment

#### # read mapping

bowtie2 -p 16 -q --local \

- -x /n/groups/flyrnai/yifang/ref/Drosophila\_melanogaster/UCSC/dm6/Sequence/Bowtie2Index/genome \
- -1/n/groups/flyrnai/yifang/Projects/With/Hongwen/ChIP-seq/2020-03-16 ChIP-seq/trim output/ LIBO45499\_CHS00173889\_S1\_L001\_R1\_val\_1.fq \
- -2 /n/groups/flyrnai/yifang/Projects/With/Hongwen/ChIP-seq/2020-03-16\_ChIP-seq/trim\_output/ LIBO45499 CHS00173889 S1 L001 R2 val 2.fq \
- -S /n/groups/flyrnai/yifang/Projects/With/Hongwen/ChIP-seq/2020-03-16\_ChIP-seq/bowtie2\_output/ LIB045499 CHS00173889 S1 L001.sam

#### # peak calling

macs2 callpeak \

- -t bowtie2\_output/LIB045499\_CHS00173894\_S6\_unique.bam \
- -c bowtie2\_output/LIB045499\_CHS00173891\_S3\_unique.bam \
- -B --SPMR \
- -f BAMPE \
- -g dm∖
- -n odj\_vs\_control \
- --outdir macs2\_output 2> macs2\_output/odj\_vs\_control.log &

### Data quality

Peaks at 5% FDR and above 5-fold enrichment: dwg\_vs\_control, 1943; dwg\_vs\_input, 1831; odj\_vs\_control, 1202

# Software

TrimGalore 0.6.4, bowtie2 2.3.5.1, SamTools 1.6, MACS2 2.2.6, Homer 4.11, DeepTools 3.4.0