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| **Key Resources Table** |
| **Reagent type (species) or resource** | **Designation** | **Source or reference** | **Identifiers** | **Additional information** |
| genetic reagent (*D. melanogaster*) | *ftz-f1ex7* | (Yamada et al., 2000)*.* | FLYB:FBal0120497 |  |
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|
| genetic reagent (*D. melanogaster*) | *ftz-f1::GFP.FLAG* | BDSC | BDSC:38645 ; |  |
| FLYB: FBst0038645 |
| RRID:BDSC\_38645 |
| genetic reagent (*D. melanogaster*) | *ftz-f1fs(3)*2877 | (Karpen and Spradling, 1992) |  |  |
| genetic reagent (*D. melanogaster*) | *Mmp2::GFP/Cyo* | (Deady et al., 2015) |  |  |
| genetic reagent (*D. melanogaster*) | *Vm26Aa-Gal4* | (Peters et al., 2013) |  |  |
| genetic reagent (*D. melanogaster*) | *Oamb-RFP* | (Knapp et al., 2019) |  |  |
| genetic reagent (*D. melanogaster*) | *47A04-LexA* | BDSC | BDSC:54873 ;RRID:BDSC\_54873 |  |
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|
| genetic reagent (*D. melanogaster*) | *lexAop2-6XGFP* | BDSC | BDSC:52265; RRID:BDSC\_52265 |  |
|
| genetic reagent (*D. melanogaster*) | *sim3.7-Gal4* | BDSC | BDSC: 26784 | (Xiao et al., 1996) |
| FLYB: FBst0026784 |
| RRID:BDSC\_26784 |
|  |
| genetic reagent (*D. melanogaster*) | *UAS-EcRDN* | BDSC | BDSC: 6872 |  |
| FLYB: FBst0006872 |
| RRID:BDSC\_6872 |
| genetic reagent (*D. melanogaster*) | *UAS-ttkRNAi* | VDRC | VDRC: 101980 FLBY:FBst0473852 RRID:FlyBase\_FBst0473852 |  |
| genetic reagent (*D. melanogaster*) | *UAS-Cyp18a1* | (Rewitz et al., 2010), |  |  |
| genetic reagent (*D. melanogaster*) | *UAS-Cyp18a1RNAi* | VDRC | VDRC: 5602 |  |
| FLYB: FBst0470059 |
| RRID:FlyBase\_FBst0470059 |
| genetic reagent (*D. melanogaster*) | *UAS*-*ftz-f1RNAi1* | BDSC | BDSC: 33625 |  |
| FLYB: FBst0033625 |
| RRID:BDSC\_33625 |
| genetic reagent (*D. melanogaster*) | *UAS- ftz-f1RNAi2* | VDRC | VDRC: 104463 |  |
| FLYB: FBst0476321 |
| RRID:FlyBase\_FBst0476321 |
| genetic reagent (*D. melanogaster*) | *UAS-ftz-f1* | (Yussa et al., 2001) |  |  |
| genetic reagent (*D. melanogaster*) | *UAS-simRNAi* | VDRC | VDRC: 26888 |  |
| FLYB: FBst0456642 |
| RRID:FlyBase\_FBst0456642 |
| genetic reagent (*D. melanogaster*) | *UAS-sim-3xHA* | FlyORF | FlyORF: 000719 |  |
| FLYB: FBst0501520 |
| RRID:FlyBase\_FBst0501520 |
| genetic reagent (*D. melanogaster*) | *UAS-mSF1* | Yussa et al., 2001 |  |  |
| genetic reagent (*D. melanogaster*) | *hsGal4DBD-EcRLBD, UAS-nlacZ* | (Kozlova and Thummel, 2002) |  | gift by Wu-Min Deng |
| chemical compound, drug | 20E (20-Hydroxyecdsone) | Cayman Chemical | Item No. 16145 |  |
|  |
| CAS No. 5289-74-7 |
|  |
| chemical compound, drug | Octopamine (OA) | Sigma-Aldrich | CAS No. 770-05-8 |  |
| chemical compound, drug | Ionomycin (Iono) | Cayman Chemical | Item No. 10004974 CAS No. 56092-81-0  |  |
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| chemical compound, drug | L-012 | Wako Chemical | Cas No. 143556-24-5 |  |
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|
| antibody | anti-Hnt | DSHB | Cat#1G9 | IF (1:75) |
| (Mouse monoclonal) | AB\_528278 RRID:AB\_528278 |
| antibody | anti-Cut | DSHB | Cat#2B10 | IF (1:15) |
| (Mouse monoclonal) | AB\_528186 RRID:AB\_528186 |
| antibody | anti-Br-C (Mouse monoclonal) | DSHB | Cat#25E9.D7 | IF (1:15) |
| AB\_528104 RRID:AB\_528104 |
| antibody | anti-EcR.A (Mouse monoclonal) | DSHB | Cat#15G1a | IF (1:30) |
| AB\_10659792 |
| RRID:AB\_10659792 |
| antibody | anti-EcR.B1 (Mouse monoclonal) | DSHB | Cat# AD4.4 | IF (1:30) |
| AB\_2154902 RRID:AB\_2154902 |
| antibody | anti-Ftz-f1 (Rabbit polyclonal) | gift from Dr. Hitoshi Ueda, Okayama University, Japan | RRID:AB\_2569929 | IF (1:50000) |
| antibody | anti-Ttk69 (Rabbit polyclonal) | a gift from Dr. Wanzhong Ge, Zhejiang University, China |  | IF (1:100) |
| antibody | anti-GFP (Rabbit polyclonal) | Invitrogen | AB\_221569 ; RRID:AB\_221569 | IF (1:4000) |
| antibody | anti-GFP | Invitrogen | AB\_221568; RRID:AB\_221568 | IF (1:500) |
| (Mouse monoclonal) |
| antibody | anti-RFP | MBL international | AB\_591279 RRID:AB\_591279 | IF (1:2000) |
| (Rabbit polyclonal ) |
| antibody | anti-β-Gal | Abcam | Cat# ab9361 AB\_307210 RRID:AB\_307210 | IF (1:500) |
| (Chicken polyclonal) |
| antibody | anti-Sim (Guinea-pig polyclonal) | from Dr. Stephen Crews, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, USA |  | IF (1:1000) |
| antibody | Alexa Fluor 488 anti-Guinea pig (Goat polyclonal) | Invitrogen | AB\_2534117 RRID:AB\_2534117 | IF (1:1000) |
| antibody | Alexa Fluor 488 anti-Rabbit (Goat polyclonal) | Invitrogen | AB\_143165 RRID:AB\_143165 | IF (1:1000) |
| antibody | Alexa Fluor 488 anti-Mouse (Goat polyclonal) | Invitrogen | AB\_2534069 RRID:AB\_2534069 | IF (1:1000) |
| antibody | Alexa Flour 568 anti-chicken (Goat polyclonal) | Invitrogen | AB\_2534098 RRID:AB\_2534098 | IF (1:1000) |
| antibody | Alexa Flour 568 anti-guinea pig (Goat polyclonal) | Invitrogen | AB\_2534119 RRID:AB\_2534119 | IF (1:1000) |
| antibody | Alexa Flour 568 anti-Mouse (Goat polyclonal) | Invitrogen | AB\_144696 RRID:AB\_144696 | IF (1:1000) |
| antibody | Alexa Flour 568 anti-Rabbit (Goat polyclonal) | Invitrogen | AB\_10563566 RRID:AB\_10563566 | IF (1:1000) |
| commercial assay or kit | Click-iT™ Plus EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 555 dye | Invitrogen | Cat# C10638 |  |
| Chemical compound, drug | TRIzol | Life Technologies | Cat #15596018 |  |
| commercial assay or kit | Direct-zol™ RNA MicroPrep Kit | Zymo Research |  |  |
| commercial assay or kit | using Illumina TruSeq Stranded mRNA Sample Preparation kit | Illumina |  |  |
| Software, algorithm | Sickle | Sickle | OMICS\_01077 RRID:SCR\_006800 |  |
|
| Software, algorithm | HISAT2 | HISAT2 | RRID:SCR\_015530 | (Kim et al., 2015) |
| Software, algorithm | HTSeq-count | HTSeq-count | RRID:SCR\_011867 | (Anders et al., 2015) |
| Software, algorithm | DESeq2 |  | RRID:SCR\_015687 | (Love et al., 2014). |
| Software, algorithm | Prism 7 | Graphpad | RRID:SCR\_002798 |  |
| Recombinant DNA reagent | pAG/MNase | Addgene | ID# 123461 RRID:Addgene\_123461 |  |
| (Plasmid ) |
| commercial assay or kit | NEBNext Ultra II DNA Library Prep Kit (NEB) |  |  | ( Liu et al., 2018) |
| antibody | anti-FLAG M2 (Mouse monoclonal) | Sigma-Aldrich | Cat# F1804 AB\_262044 RRID:AB\_262044 | C&R: (1:250) |
| antibody | IgG1 Negative contronl clone Ci4 (Mouse monoclonal) | Sigma-Aldrich | AB\_97846 RRID:AB\_97846 | C&R: (1:125) |
| Software, algorithm | CUT&RUNTools workflow | This paper |  | modified from (Zhu et al., 2019) |