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| **Key Resources Table** |
| **Reagent type (species) or resource** | **Designation** | **Source or reference** | **Identifiers** | **Additional information** |
| antibody | Rat anti-RFP (monoclonal) | Bulldog Bio | ITEM# RMA5F8 | (1:500) |
| antibody | Mouse Ig1 anti-24B10 (monoclonal) | Developmental Studies Hybridoma Bank (DSHB) | ID# AB\_528161  | (1:20) |
| antibody | Mouse Ig1 anti-GFP-G1 (monoclonal) | DSHB | ID# AB\_ 2619561 | (1:500) |
| antibody | Mouse Ig2b anti-Highwire (monoclonal) | DSHB | ID# AB\_528277  | (1:20) |
| antibody | Mouse Ig2a anti-eyeless (monoclonal) | DSHB | ID# AB\_2253542 | (1:20) |
| antibody | Mouse Ig2a anti-dachshund (monoclonal) | DSHB | ID# AB\_579773  | (1:100) |
| antibody | Rat anti-Elav (monoclonal) | DSHB | ID# [AB\_528218](http://antibodyregistry.org/AB_528218)  | (1:100) |
| antibody | Guinea pig anti-Dichaete  | Gift from Claude Desplan |  | (1:50) |
| antibody | Guinea pig anti-Bsh  | Gift from Makoto Sato |  | (1:600) |
| antibody | Rat anti-dilp2  | Gift from Pierre Leopold |  | (1:500) |
| antibody | Rat anti-drifter  | Gift from Sarah J Certel  |  | (1:1000) |
| antibody | Guinea pig anti-Toy  | Gift from Uwe Walldorf |  | (1:100) |
| antibody | Rat anti-HA (monoclonal) | Sigma-Aldrich | Cat# 11867423001 | (1:500) |
| antibody | Rabbit anti-GFP (monoclonal) | Thermo Fisher Scientific | Cat# G-10362 | (1:500) |
| antibody | Rabbit anti-GFP (polyclonal) | Thermo Fisher Scientific | Cat# A-11122 | (1:2000) |
| antibody | Mouse Ig2a anti-V5 (monoclonal) | Thermo Fisher Scientific | Cat# R960-25 | (1:500) |
| antibody | Mouse Ig2a anti-GFP (monoclonal) | Thermo Fisher Scientific | Cat# A-11120 | (1:500) |
| antibody | Rat anti-CD8 (monoclonal) | Thermo Fisher Scientific | Cat# MCD0800 | (1:500) |
| antibody | Goat anti-rabbit Alexa 488 | Thermo Fisher Scientific | Cat# A-11034 | (1:500) |
| antibody | Goat anti-rabbit Alexa 568 | Thermo Fisher Scientific | Cat# A-11036 | (1:500) |
| antibody | Goat anti-rabbit Alexa 647 | Thermo Fisher Scientific | Cat# A-21245 | (1:500) |
| antibody | Goat anti-mouse Ig1 Alexa 488  | Thermo Fisher Scientific | Cat# A-21121 | (1:500) |
| antibody | Goat anti-mouse Ig1 Alexa 568 | Thermo Fisher Scientific | Cat# A-21124 | (1:500) |
| antibody | Goat anti-mouse Ig1 Alexa 647 | Thermo Fisher Scientific | Cat# A-21240 | (1:500) |
| antibody | Goat anti-mouse Ig2a Alexa 488  | Thermo Fisher Scientific | Cat# A-21131 | (1:500) |
| antibody | Goat anti-mouse Ig2a Alexa 568 | Thermo Fisher Scientific | Cat# A-21134 | (1:500) |
| antibody | Goat anti-mouse Ig2a Alexa 647 | Thermo Fisher Scientific | Cat# A-21241 | (1:500) |
| antibody | Goat anti-mouse Ig2b Alexa 488  | Thermo Fisher Scientific | Cat# A-21141 | (1:500) |
| antibody | Goat anti-mouse Ig2b Alexa 568 | Thermo Fisher Scientific | Cat# A-21144 | (1:500) |
| antibody | Goat anti-mouse Ig2b Alexa 647 | Thermo Fisher Scientific | Cat# A-21242 | (1:500) |
| antibody | Goat anti-guinea pig Alexa 488  | Thermo Fisher Scientific | Cat# A-11073 | (1:500) |
| antibody | Goat anti-guinea pig Alexa 568 | Thermo Fisher Scientific | Cat# A-11075 | (1:500) |
| antibody | Goat anti-guinea pig Alexa 647 | Thermo Fisher Scientific | Cat# A-21450 | (1:500) |
| antibody | Goat anti-rat Alexa 568 | Thermo Fisher Scientific | Cat# A-11077 | (1:500) |
| antibody | Goat anti-rat Alexa 647 | Thermo Fisher Scientific | Cat# A-21247 | (1:500) |
| chemical compound, drug | PCR Master Mix | Thermo Fisher Scientific | Cat# K0171 |  |
| chemical compound, drug | Low-melting point agarose | Thermo Fisher Scientific | Cat# 16520050 |  |
| chemical compound, drug | Protease K | Thermo Fisher Scientific | Cat#EO0491 |  |
| chemical compound, drug | G418 | Sigma Aldrich | Cat# G5013 |  |
| chemical compound, drug | GreenGlo DNA dye | Denville Scientific | Cat# CA3600  |  |
| chemical compound, drug | VECTASHIELD | Vector Laboratories | Cat# H1000; RRID: AB\_2336789 |  |
| chemical compound, drug | Paraformaldehyde 20% Solution | Electron Microscopy Sciences | Cat# 15713-S |  |
| chemical compound, drug | Restriction enzymes | New England Biolabs  |  |  |
| chemical compound, drug | DNA Gel Loading Solution (5x) | Quality Biological | Cat# 351-028-661 |  |
| chemical compound, drug | TAE (10x) | Crystalgen | Cat# 221-197-01 |  |
| commercial assay or kit | Nucleobond Midiprep Kit | Promega | Cat# 740420.10  |  |
| Biological sample | Xenopus oocyte  | EcoCyte |  |  |
| genetic reagent (*D. melanogaster*) | hsFLP1 | Bloomington *Drosophila* Stock Center (BDSC) | BDSC\_6 |  |
| genetic reagent (*D. melanogaster*) | hsFLP122 | BDSC | BDSC\_23649 |  |
| genetic reagent (*D. melanogaster*) | GMR-Gal4 | BDSC | BDSC\_1104 |  |
| genetic reagent (*D. melanogaster*) | longGMR-Gal4 | BDSC | BDSC\_8121 |  |
| genetic reagent (*D. melanogaster*) | Actin5c-Gal4 | BDSC | BDSC\_4414 |  |
| genetic reagent (*D. melanogaster*) | GMR24F06-Gal4 | BDSC | BDSC\_49087 |  |
| genetic reagent (*D. melanogaster*) | GMR27G05-Gal4 | BDSC | BDSC\_48073 |  |
| genetic reagent (*D. melanogaster*) | GMR9B08-Gal4 | BDSC | BDSC\_41369 |  |
| genetic reagent (*D. melanogaster*) | GMR24F06-LexA | BDSC | BDSC\_52695 |  |
| genetic reagent (*D. melanogaster*) | UAS-mCD8::GFP | BDSC | BDSC\_32184 |  |
| genetic reagent (*D. melanogaster*) | UAS-mCD8::mCherry | BDSC | BDSC\_27392 |  |
| genetic reagent (*D. melanogaster*) | UAS-Torwt | BDSC | BDSC\_7012 |  |
| genetic reagent (*D. melanogaster*) | UAS-InRwt | BDSC | BDSC\_8262 |  |
| genetic reagent (*D. melanogaster*) | UAS-InRK1409A | BDSC | BDSC\_8253 |  |
| genetic reagent (*D. melanogaster*) | InR deletion Df(3R)ED6058 | BDSC | BDSC\_24140 |  |
| genetic reagent (*D. melanogaster*) | In(3R)GC25, InR93Dj-4 | BDSC | BDSC\_9554 |  |
| genetic reagent (*D. melanogaster*) | FRT2A, SREBP189  | BDSC | BDSC\_38392 (Kunte et al., 2006) |  |
| genetic reagent (*D. melanogaster*) | FRT40,Thork07736 | Kyoto Stock Center | 114605 (Spradling et al., 1999) |  |
| genetic reagent (*D. melanogaster*) | FRT40, Atg7d06996 | Kyoto Stock Center | 114560 |  |
| genetic reagent (*D. melanogaster*) | FRT40,DrefKG09294  | Kyoto Stock Center | 114408 (Bellen et al., 2004) |  |
| genetic reagent (*D. melanogaster*) | FRT82, S6kl-1 | BDSC | BDSC\_32552(Montagne et al., 1999) |  |
| genetic reagent (*D. melanogaster*) | FRT82, Df(3R)PI3K92EA | BDSC | BDSC\_25900 (Weinkove et al.,1999) |  |
| genetic reagent (*D. melanogaster*) | Imp-L2-RA-Gal4 | Gift from Ernst Hafen | (Bader et al., 2013) |  |
| genetic reagent (*D. melanogaster*) | UAS-Dilp2 | Gift from Ernst Hafen | (Ikeya et al., 2002)  |  |
| genetic reagent (*D. melanogaster*) | UAS-Dilp6 | Gift from Ernst Hafen | (Ikeya et al., 2002) |  |
| genetic reagent (*D. melanogaster*) | FRT40,Chico1 | Gift from Ernst Hafen  | (Bohni et al., 1999) |  |
| genetic reagent (*D. melanogaster*) | FRT40,Chicofs(2)4 | Gift from Ernst Hafen  | (Bohni et al., 1999) |  |
| genetic reagent (*D. melanogaster*) | FRT40,Pten2L117 | Gift from Ernst Hafen  | (Oldham et al.,2002) |  |
| genetic reagent (*D. melanogaster*) | FRT82,Dp1101C1  | Gift from Ernst Hafen  | (Willeckeet al., 2011)  |  |
| genetic reagent (*D. melanogaster*) | FRT82,Rheb3M2 | Gift from Ernst Hafen  | (Stocker et al.,2003) |  |
| genetic reagent (*D. melanogaster*) | FRT82,Tsc11A2 | Gift from Ernst Hafen  | (Stocker et al.,2003) |  |
| genetic reagent (*D. melanogaster*) | Dilp2-Gal4 | Gift from Edwin Levitan | (Rulifson et al.,2002) |  |
| genetic reagent (*D. melanogaster*) | UAS-Dilp2::GFP | Gift from Edwin Levitan | (Wong et al., 2012)  |  |
| genetic reagent (*D. melanogaster*) | UAS-IVS-R::PEST  | Gift from Gerrald Rubin | (Nern et al, 2011)  |  |
| genetic reagent (*D. melanogaster*) | FRT19A, rictorΔ2 | Gift from Ville Hietakangas  | (Hietakangas et al, 2007) |  |
| genetic reagent (*D. melanogaster*) | FRT19A, RaptorDel | Gift from Jianzhong Yu | (Li et al., 2019) |  |
| genetic reagent (*D. melanogaster*) | DIP-γ-Gal4 | Gift from Kai Zinn  | (Carrillo et al, 2015) |  |
| genetic reagent (*D. melanogaster*) | 6-60-Gal4 | Gift from Larry Zipursky  | (Nern et al., 2008) |  |
| genetic reagent (*D. melanogaster*) | FRT40,TorΔP | Gift from Mary Lily  | (Zhang et al., 2000) |  |
| genetic reagent (*D. melanogaster*) | FRT82,Tsc1Q87X | Gift from Mary Lily  | (Tapon 2001 Cell) |  |
| genetic reagent (*D. melanogaster*) | FRT82, InR273 | Gift from Leslie Pick | (Song et al., 2003) |  |
| genetic reagent (*D. melanogaster*) | FRT82,InR353 | Gift from Leslie Pick  | (Songet al., 2003) |  |
| genetic reagent (*D. melanogaster*) | InRE19 | Gift from Hwei-Jan Hsu | (Kao et al., 2015) |  |
| genetic reagent (*D. melanogaster*) | InR339 | Gift from Hwei-Jan Hsu | (Kao et al., 2015) |  |
| genetic reagent (*D. melanogaster*) | arm-lacZ, FRT19A | Gift from Hwei-Jan Hsu |  |  |
| genetic reagent (*D. melanogaster*) | FRT82,FoxoΔ94 | Gift from Linda Partridge | (Slack et al., 2011) |  |
| genetic reagent (*D. melanogaster*) | Rh4-Brp::shortmCherry | Gift from Takashi Suzuki | (Berger-Muller et al., 2013) |  |
| genetic reagent (*D. melanogaster*) | UAS-CD4::spGFP1-10 | Gift from Kristin Scott | (Gordon et al., 2009) |  |
| genetic reagent (*D. melanogaster*) | GMR-CD4::spGFP11 | Lee Lab | (Ting et al., 2014) |  |
| genetic reagent (*D. melanogaster*) | OrtC2b-Gal4 | Lee Lab | (Gao et al., 2008) |  |
| genetic reagent (*D. melanogaster*) | OrtC1a-Gal4 | Lee Lab | (Karuppudurai et al., 2014) |  |
| genetic reagent (*D. melanogaster*) | Dm8-LexA | Lee Lab | (Karuppudurai et al., 2014) |  |
| genetic reagent (*D. melanogaster*) | Rh3-Syb::spGFP1-10 | This study |  | Materials & Methods Section |
| genetic reagent (*D. melanogaster*) | Rh4-Syb::spGFP1-10 | This study |  | Materials & Methods Section |
| genetic reagent (*D. melanogaster*) | UAS-Ort::HA::spGFP11 | This study |  | Materials & Methods Section |
| genetic reagent (*D. melanogaster*) | UAS-FSF-tdTom::T2A::spGFP1-10 | This study |  | Materials & Methods Section |
| genetic reagent (*D. melanogaster*) | InR::V5::spGFP11 | This study |  | Materials & Methods Section |
| genetic reagent (*D. melanogaster*) | UAS-Dilp2-RNAi | Vienna *Drosophila* Research Center (VDRC) | VDRC\_102158 |  |
| genetic reagent (*D. melanogaster*) | UAS-Dilp6-RNAi | VDRC | VDRC\_ 102465 |  |
| genetic reagent (*D. melanogaster*) | UAS-BaboDN | Gift from Tzu-Min Lee |  |  |
| genetic reagent (*D. melanogaster*) | UAS-BaboDA | BDSC | BDSC\_64293 |  |
| genetic reagent (*D. melanogaster*) | UAS-SREBPWT | BDSC | BDSC\_8236 |  |
| genetic reagent (*D. melanogaster*) | UAS-SREBPCA | BDSC | BDSC\_8242 |  |
| genetic reagent (*D. melanogaster*) | LexAop-mCD8::GFP | Gift from Gerrald Rubin | (Pfeiffer et al., 2010) |  |
| genetic reagent (*D. melanogaster*) | LexAop-FSF-mCD8::GFP | Lee lab | (Ting et al., 2014) |  |
| genetic reagent (*D. melanogaster*) | LexAop-FSF-rCD2::mCherry | Lee lab | (Ting et al., 2014) |  |
| genetic reagent (*D. melanogaster*) | *SevE2* | Lee lab | (Ting et al., 2014) |  |
| sequence-based reagent | InR tagging  | This paper (custom made by Operon) | PCR primers | For 5’ homologous fragment of InR:Fwd: 5’-CGAATGCATCTAGATATCGACTTACGTTGTGCCTGATGC-3’Rev: 5’-GGGATAGGAGCCGATATCCGCCTCCCTTCCGATGAATC-3’For 3’ homologous fragment of InR:Fwd1: 5’-TTCTAGGGTTAATACGTATCGTTACGAACTGTAGTTCTGTAG-3’Rev1: 5’-GTGTCATGGATCTACGGAAAGGTGTGTTATTTTACGAAG-3’Fwd2: 5’-CGTAGATCCATGACACCTTTTCTTCTTGGTGTCGAACCA-3’Rev2: 5’-CCGGGATCCGATTACGTAGAAAGTGGTATTCCTAGAGCAGAGC-3’ |
| sequence-based reagent | For pCaST-Rh3::Syb::spGFP1-10: | This paper (custom made by Operon) |  | Fwd: 5’-AGGGCGAATTCGTTTAAACATGGCGGACGCTGCAC-3’Rev: 5’-ACAAAGATCCTCTAGACTATGTTCCTTTTTCATTTGGATCTTTGCT-3’For pCaST-Rh4::Syb::spGFP1-10:Fwd: 5’-CGGGTTGACCGGTTTAAACATGGCGGACGCTGCAC-3’Rev: 5’-ACAAAGATCCTCTAGACTATGTTCCTTTTTCATTTGGATCTTTGCT-3’ |
| sequence-based reagent | for UAS-spGFP11::HA::Ort  | This paper (custom made by Operon) |  | 5’-TTCAACAAAGTCTGGCCATACGCGACCACATGGTGCTGCACGAGTACGTGAACGCCGCCGGCATCACCGGCTCCTACCCCTACGACGTGCCCGACTACGCCGGCTATCCCTATGACGTCCCGGACTATGCAGGCTCCTTGGCCATAACCGACATCC-3’ |
| sequence-based reagent | For UAS-FSF-myr::TdTom::dT2A::R::PEST  | This paper (custom made by Operon) |  | Fwd: 5’-GGCCCTGGTAGCAGGCCTATGCAGCTTACCAAGGACAC-3’Rev: 5’-AATTCGTTTAAACGGCCGGCCAGATCGATCCAGACATGATAAGATAC-3’ |
| sequence-based reagent | Probe synthesis for *in situ* hybridization  | This paper (custom made by Operon) |  | T3-dilp2-sense Forward:AATTAACCCTCACTAAAGGGCGGCTCGACCCAACTTAATCdilp2-sense Reverse:CGCGCTTGTGTGGAATCACdilp2-a-sense Forward:CGGCTCGACCCAACTTAATCT7-dilp2-antisense Reverse: TAATACGACTCACTATAGGGCGCGCTTGTGTGGAATCACT3-dilp2-sense-2 Forward: AATTAACCCTCACTAAAGGGGATCTGGACGCCCTCAATCCdilp2-sense-2 Reverse: CAGAGATAATCGCGTCGACCdilp2-antisense-2 Forward: GATCTGGACGCCCTCAATCCT7-dilp2-antisense-2 Reverse: TAATACGACTCACTATAGGGCAGAGATAATCGCGTCGACC |
| recombinant DNA reagent | pUC57-InR::V5::spGFP11  | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pCaST-Rh3(Rh4)::Syb::spGFP1-10 | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pCaST-Rh3(Rh4)::Syb::spGFP1-10 | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pUAST-spGFP11::HA::Ort | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pUAST-myr::TdTom::dT2A::spGFP1-10 | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pUAST-FSF-myr::TdTom::dT2A::spGFP1-10 | This paper |  | Maintained in Lee lab |
| recombinant DNA reagent | pUAST-FSF-myr::TdTom::dT2A::R::PEST | This paper |  | Maintained in Lee lab |
| software | Fiji/Image J | NIH | https://imagej.net/Fiji;RRID:SCR\_002285 |  |
| software | Imaris 8.0 | Bitplane | http://www.bitplane.com/ |  |
| software | GraphPad Prism 7.0 | GraphPad Software | https://www.graphpad.com/; RRID: SCR\_002798 |  |
| software | HuygensDeconvolution | Scientific Volume Imaging | https://svi.nl/ |  |