**Supplementary File 1**

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| **Key Resources Table** | | | | |
| **Reagent type (species) or resource** | **Designation** | **Source or reference** | **Identifiers** | **Additional information** |
| gene (*Drosophila melanogaster*) | *fru* | FlyBase | FLYB:FBgn0004652 |  |
| strain, strain background (*Drosophila melanogaster*) | Canton-S | Hoyer et al., 2008 (DOI:10.1016/j.cub.2007.12.052) |  | Gift from David Anderson (California Institute of Technology) |
| strain, strain background (*Drosophila melanogaster*) | *nos-phiC31*, *y1*, *sc1*, *v1*, *sev21*; *P{CaryP}attP2* | Bloomington *Drosophila* Resource Center | BDSC:25710; FLYB: FBst0025710; RRID:BDSC\_25710 | Used to create the *R71G01-LexA* (in attP2) transgenic reagent (see below) |
| genetic reagent (*Drosophila melanogaster*) | *NP2631* | Yu et al., 2010 (DOI:10.1016/j.cub.2010.08.025) | DGGR:104266; FLYB:FBti0034959; RRID:DGGR\_104266 | Gift from Daisuke Yamamoto (Tohoku Univ.) |
| genetic reagent (*Drosophila melanogaster*) | *R15A01-p65AD:Zp* (in attP40) | Hoopfer et al., 2015 (DOI:10.7554/eLife.11346) | BDSC:68837; FLYB: FBti0187848; RRID:BDSC\_68837 | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *R71G01-Zp:GAL4DBD* (in attP2) | Hoopfer et al., 2015 (DOI:10.7554/eLife.11346) | BDSC:69507; FLYB: FBti0191802; RRID:BDSC\_69507 | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *R15A01-LexA* (in attP2) | Hoopfer et al., 2015 (DOI:10.7554/eLife.11346) | FLYB:FBtp0087961 | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *20XUAS-IVS-Syn21-CsChrimson:tdTomato* (in VK00022) | Rubin lab (HHMI Janelia Research Campus) |  | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *20XUAS>myr:TopHAT2>CsChrimson:tdTomato* (in VK00022) | Rubin lab (HHMI Janelia Research Campus) |  | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *20XUAS>myr:TopHAT2>CsChrimson:tdTomato* (in VK00005) | Duistermars et al., 2018 (DOI:10.1016/j.neuron.2018.10.027) |  | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *13XlexAop2-IVS-Syn21-GCaMP6f* (codon-optimized)*-p10* (in su(Hw)attP5) | Rubin lab (HHMI Janelia Research Campus) |  | Gift from David Anderson (California Institute of Technology) |
| genetic reagent (*Drosophila melanogaster*) | *fruM* | Demir & Dickson, 2005 (DOI:10.1016/j.cell.2005.04.027) | BDSC:66874; FLYB:FBal0179807; RRID:BDSC\_66874 | Gift from Barry Dickson (HHMI Janelia Research Campus) |
| genetic reagent (*Drosophila melanogaster*) | *fruF* | Demir & Dickson, 2005 (DOI:10.1016/j.cell.2005.04.027) | BDSC:66873; FLYB:FBal0191136; RRID:BDSC\_66873 | Gift from Barry Dickson (HHMI Janelia Research Campus) |
| genetic reagent (*Drosophila melanogaster*) | *fruFLP* | Yu et al., 2010 (DOI:10.1016/j.cub.2010.08.025) | BDSC:66870; FLYB:FBal0248671; RRID:BDSC\_66870 | Gift from Barry Dickson (HHMI Janelia Research Campus) |
| genetic reagent (*Drosophila melanogaster*) | *dsxGAL4* | Rideout et al., 2010 (DOI:10.1038/nn.2515) | FLYB:FBal0248671 | Gift from Stephen Goodwin (Univ. Oxford) |
| genetic reagent (*Drosophila melanogaster*) | *dsxFLP* | Rezaval et al., 2014 (DOI:10.1016/j.cub.2013.12.051) | FLYB:FBal0296301 | Gift from Stephen Goodwin (Univ. Oxford) |
| genetic reagent (*Drosophila melanogaster*) | *fruP1.LexA* | Mellert et al., 2010 (DOI: 10.1242/dev.045047) | BDSC:66698; FLYB:FBal0244777; RRID:BDSC\_66698 | Gift from Bruce Baker (HHMI Janelia Research Campus) |
| genetic reagent (*Drosophila melanogaster*) | *fru4-40* | Bloomington *Drosophila* Resource Center | BDRC:66692; FLYB:FBal0126397; RRID:BDSC\_66692 |  |
| genetic reagent (*Drosophila melanogaster*) | *8XlexAop2-GAL80* (in attP40) | Bloomington *Drosophila* Resource Center | BDSC:32214; FLYB: FBti0131966; RRID:BDSC\_32214 |  |
| genetic reagent (*Drosophila melanogaster*) | *R71G01-LexA* (in attP2) | This paper |  | Created by targeting the GMR71G01-LexA plasmid (gift from Rubin lab; see below) into the attP2 landing site. See also Materials and Methods for details. |
| antibody | anti-DsRed (Rabbit polyclonal) | Clontech | Cat # 632496; RRID: AB\_10013483 | IHC (1:1,000) |
| antibody | anti-BRP (Mouse monoclonal nc82) | Developmental Studies Hybridoma Bank | nc82 (concentrate); RRID: AB\_2314866 | IHC (1:100) |
| antibody | anti-GFP (Chicken polyclonal) | Abcam | Cat # ab13970; RRID:AB\_300798 | IHC (1:1,000) |
| antibody | anti-FruM (Rabbit polyclonal) | Stockinger et al., 2005 (DOI:10.1016/j.cell.2005.04.026) |  | Gift from Barry Dickson (HHMI Janelia Research Campus). IHC (1:10,000) |
| antibody | anti-FruM (Guinea pig polyclonal) | This paper |  | Generated by Michael Perry (UC San Diego) and provided to the authors. IHC (1:100) |
| antibody | anti-DsxM (Rat polyclonal) | This paper |  | Generated by Michael Perry (UC San Diego) and provided to the authors. IHC (1:100) |
| antibody | anti-chicken IgY Alexa 488 (Goat polyclonal) | ThermoFisher Scientific | Cat # A11039; RRID:AB\_2534096 | IHC (1:100) |
| antibody | anti-rat IgG Alexa 488 (Goat polyclonal) | ThermoFisher Scientific | Cat # A11006; RRID:AB\_2534074 | IHC (1:100) |
| antibody | anti-rabbit IgG Alexa 568 (Goat polyclonal) | ThermoFisher Scientific | Cat # A11036; RRID:AB\_10563566 | IHC (1:100) |
| antibody | anti-mouse IgG Alexa 633 (Goat polyclonal) | ThermoFisher Scientific | Cat # A21052; RRID:AB\_2535719 | IHC (1:100) |
| antibody | anti-guinea pig IgG Alexa 633 (Goat polyclonal) | ThermoFisher Scientific | Cat # A21105; RRID:AB\_2535757 | IHC (1:100) |
| recombinant DNA reagent | GMR71G01-LexA plasmid | HHMI Janelia Research Campus | FLYB:FBtp0079698 | Details of plasmid design are described in Pfeiffer et al., 2010 (10.1534/genetics.110.119917) and Jennet et al., 2012 (10.1016/j.celrep.2012.09.011). |
| chemical compound, drug | Vectashield | Vector Laboratories | Cat # H-1000; RRID:AB\_2336789 |  |
| chemical compound, drug | FocusClear | CelExplorer Labs, Taiwan | Cat # FC-101 |  |
| chemical compound, drug | MountClear | CelExplorer Labs, Taiwan | Cat # MC-301 |  |
| chemical compound, drug | all-*trans* retinal | MilliporeSigma | Cat # R2500 | Used at 0.2mM |
| software, algorithm | Fiji | Schindelin et al., 2012 (DOI:10.1038/nmeth.2019) | RRID:SCR\_002285 | https://fiji.sc/ |
| software, algorithm | Computational Morphometry Tookit (CMTK) | Rohlfing & Maurer, 2003 (DOI:10.1109/titb.2003.808506) | RRID:SCR\_002234 | https://www.nitrc.org/projects/cmtk |
| software, algorithm | Fiji plugin for CMTK | Jefferis et al., 2007 (DOI:10.1016/j.cell.2007.01.040) |  | https://github.com/jefferis/fiji-cmtk-gui |
| software, algorithm | FluoRender | Wan, Otsuna, Chien, & Hansen, 2009 (DOI:10.1109/TVCG.2009.118) | RRID:SCR\_014303 | https://github.com/SCIInstitute/fluorender |
| software, algorithm | MATLAB | The Mathworks, Inc. | RRID:SCR\_001622 |  |
| software, algorithm | BIAS | IORodeo |  | https://bitbucket.org/iorodeo/bias |
| software, algorithm | FlyTracker | Eyjolfsdottir et al., 2014 (DOI:https://doi.org/10.1007/978-3-319-10605-2\_50) |  | http://www.vision.caltech.edu/Tools/FlyTracker/ |
| software, algorithm | JAABA | Kabra et al., 2013 (DOI: 10.1038/nmeth.2281) |  | https://sourceforge.net/projects/jaaba/files/ |