**mStayGold**

* **UAS-mStayGold in su(Hw)attP5:**
  + w; P{y[+t7.7] w[+mC]=20X-UAS-IVS-mStayGold}su(Hw)attP5/CyO; +
  + Comment: Expresses mStayGold under the control of UAS for cell labeling or live-cell imaging.
* **UAS-mStayGold in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-mStayGold}VK00005/TM3, Sb
  + Comment: Expresses mStayGold under the control of UAS for cell labeling or live-cell imaging.
* Associated Genes:
  + mStayGold (no flybase entry)
    - mStayGold is a bright and photostable green fluorescent protein for live-cell imaging.
    - [https://www.fpbase.org/protein/mstaygold/](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.fpbase.org%2Fprotein%2Fmstaygold%2F&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886684195%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=qhHZdoyEVlniBRH1MOUbFPGS2T20fx%2BWTD5ZV%2FaRyt0%3D&reserved=0)
* Generation:
  + The mStayGold sequence was taken directly from Ando 2023 using the sequence from the addgene Plasmid #212017. This sequence includes the “N1”’ adapter or for N-terminus tagging or cytosolic expression (Ando, R., Shimozono, S., Ago, H. et al. StayGold variants for molecular fusion and membrane-targeting applications. Nat Methods 21, 648–656 (2023). [https://doi.org/10.1038/s41592-023-02085-6](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoi.org%2F10.1038%2Fs41592-023-02085-6&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886696700%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=NVeRG9aL9UT9uBTx%2BZlKYidX%2Fj9Ac269HTvvssqufVc%3D&reserved=0)).
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + These constructs were crossed to several Gal4 drivers to test fluorescence using a conventional upright brightfield fluorescence microscope with an eGFP excitation/collection cube. 2-photon fluorescence was verified using a standard laser-scanning 2-photon microscope with typical GCaMP collection optics (525/50nm emission filter). No obvious trafficking issues were present.

**mCD8::mStayGold**

* **UAS-mCD8::mStayGold in su(Hw)attP5:**
  + w; P{y[+t7.7] w[+mC]=20X-UAS-IVS-mCD8::mStayGold}su(Hw)attP5/CyO; +
  + Comment: Expresses membrane-targeted mStayGold under the control of UAS for cell labeling or live-cell imaging.
* **UAS-mCD8::mStayGold in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-mCD8::mStayGold}VK00005/TM3, sb
  + Comment: Expresses membrane-targeted mStayGold under the control of UAS for cell labeling or live-cell imaging.
* Associated Genes:
  + mStayGold (no flybase entry)
    - mStayGold is a bright and photostable green fluorescent protein for live-cell imaging.
    - [https://www.fpbase.org/protein/mstaygold/](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.fpbase.org%2Fprotein%2Fmstaygold%2F&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886709933%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=PR7%2BU00uymSRAYsehHcZC1sNjOxfLWVT0jSUlUiF1lw%3D&reserved=0)
* Generation:
  + The mCD8 membrane targeting sequence was taken from the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + The mStayGold sequence was taken directly from Ando 2023 using the sequence from the addgene Plasmid #212017. This sequence includes the “N1”’ adapter or for N-terminus tagging or cytosolic expression (Ando, R., Shimozono, S., Ago, H. et al. StayGold variants for molecular fusion and membrane-targeting applications. Nat Methods 21, 648–656 (2023). [https://doi.org/10.1038/s41592-023-02085-6](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoi.org%2F10.1038%2Fs41592-023-02085-6&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886724342%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=2AVU8Lc5nVIsfoxd%2Bs8efcjHnLL250%2FMf43QvmZdED8%3D&reserved=0)).
  + The mCD8 was fused to the mStayGold sequence at its N-terminus with a short, flexible linker sequence (GGGSGGGS), to create mCD8::mStayGold.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + These constructs were crossed to known ‘weak’ Gal4 drivers difficult to target for patch-clamp electrophysiology. To qualitatively test relative fluorescence we used a conventional upright brightfield fluorescence microscope with an eGFP excitation/collection cube. We also verified 2-photon fluorescence was verified using a standard laser-scanning 2-photon microscope with typical green collection optics (525/50nm emission filter). No obvious trafficking issues were present.

**mNeonGreen**

* **UAS-mNeonGreen in su(Hw)attP5:**
  + w; P{y[+t7.7] w[+mC]=20X-UAS-IVS-mNeonGreen}su(Hw)attP5/CyO; +
  + Comment: Expresses mNeonGreen under the control of UAS for cell labeling or live-cell imaging.
* **UAS-mNeonGreen in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-mNeonGreen}VK00005/TM3, sb
  + Comment: Expresses mNeonGreen under the control of UAS for cell labeling or live-cell imaging.
* Associated Genes:
  + mNeonGreen [http://flybase.org/reports/FBto0000126.htm](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fflybase.org%2Freports%2FFBto0000126.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886736209%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=IGjf1o9%2BcklyvG6mGu7aC140dPN%2FRDq%2B93UD1hZicHM%3D&reserved=0)
    - mNeonGreen is a bright super-resolution compatible green fluorescent protein (Shaner, N.C. et al. (2013) ‘A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum’, Nature methods, 10(5), pp. 407–409.)
* Generation:
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. et al. (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, Genetics, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + These constructs were crossed to several Gal4 drivers to test fluorescence using a conventional upright brightfield fluorescence microscope with an eGFP excitation/collection cube. 2-photon fluorescence was verified using a standard laser-scanning 2-photon microscope with typical GCaMP collection optics (525/50nm emission filter). Live STED fluorescence was verified using a commercial STED microscope with a 592nm de-excitation laser line. No obvious trafficking issues were present.

**mCyRFP3::Kir2.1**

* **UAS-mCyRFP3::Kir2.1 in su(Hw)attP8:**
  + w, P{y[+t7.7] w[+mC]=20X-UAS-IVS-mCyRFP3::Kir2.1}su(Hw)attP8/FM7i; +; +
  + Comment: Expresses human Kir1.2 (KCNJ2) tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3  under the control of UAS for inhibiting neuronal activity.
* **UAS-mCyRFP3::Kir2.1 in VK00002:**
  + w; PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-mCyRFP3::Kir2.1}VK00002/CyO; +
  + Comment: Expresses human Kir1.2 (KCNJ2) tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3  under the control of UAS for inhibiting neuronal activity.
* **UAS-mCyRFP::Kir2.1 in VK00033:**
  + w; +; PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-mCyRFP3::Kir2.1}VK00033/TM6B
  + Comment: Expresses human Kir1.2 (KCNJ2) tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3  under the control of UAS for inhibiting neuronal activity.
* **LexAop-mCyRFP::Kir2.1 in VK00002:**
  + w; PBac{y[+mDint2] w[+mC]=13XLexAop-IVS-mCyRFP3::Kir2.1}VK00002/CyO; +
  + Comment: Expresses human Kir1.2 (KCNJ2) tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3  under the control of LexAop for inhibiting neuronal activity.
* **LexAop-mCyRFP::Kir2.1 in VK00033:**
  + w; +; PBac{y[+mDint2] w[+mC]=13XLexAop-IVS-mCyRFP3::Kir2.1}VK00033/TM6B
  + Comment: Expresses human Kir1.2 (KCNJ2) tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 under the control of LexAop for inhibiting neuronal activity.
* Associated Genes:
  + Kir2.1 [https://flybase.org/reports/FBto0000566.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000566.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886749342%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=mVCUdcrOKTnU6QBux3zNWSJhdS4FU6lsoSotvL%2Bqfz8%3D&reserved=0)
    - Kir2.1 (human KCNJ2) is an inwardly rectifying potassium channel
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The Kir2.1 sequence was taken from genbank.
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from addgene Plasmid #193323. (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The mCyRFP3 was fused to the Kir2.1 sequence at its N-terminus with a short, flexible linker sequence (GGGSGGGS), to create mCyRFP3::Kir2.1.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + These constructs were tested for test live fluorescence by crossing to several Gal4 drivers and using a conventional upright brightfield fluorescence microscope with a GFP long pass excitation/collection cube. Expression filled cells, however red puncta were often observed, in particular near the cell body, similar to that observed for other red fluorescent proteins. Antigenicity of mCyRFP3 confirmed by antibody staining for RFP and confocal imaging. 2-photon red fluorescence was confirmed using 2-photon microscopy by imaging with a standard laser-scanning 2-photon microscope with typical red/green collection optics. Some bleedthrough into the green channel is present using a 525/50nm emission filter, as expected with the published spectra.
  + Cells were targeted for whole-cell patch-clamp to verify inhibition: expression effectively clamps expected spiking activity during fly walking in both “PFL” and “EPG” cells.

**CsChrimson::mCyRFP3**

* **UAS-CsChrimson::mCyRFP3 in VK00002:**
  + w; PBac{y[+mDint2] w[+mC]=20X-UAS-IVS-CsChrimson::mCyRFP3}VK00002/CyO; +
  + Comment: Expresses the light-gated ion channel CsChrimson tagged with the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 under the control of UAS for light-activating neurons.
* **LexAop-CsChrimson::mCyRFP3 in VK00002:**
  + w; PBac{y[+mDint2] w[+mC]=13XLexAop-IVS-CsChrimson::mCyRFP3}VK00002/CyO; +
  + Comment: Expresses the light-gated ion channel CsChrimson tagged with the cyan-excitable red-fluorescent protein fluorophore construct mCyRFP3 under the control of LexAop for light-activating neurons.
* Associated Genes:
  + CsChrimson [https://flybase.org/reports/FBto0000558.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000558.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886761415%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=eyTIDvpHn%2BkMiVSb%2Fan%2B%2BPf%2BPmSoZpYWQxt5JcjF7j8%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The coding sequence for CsChrimson was taken from Klapoetke 2014 using the sequence from addgene Plasmid #111544. (Klapoetke NC, Murata Y, Kim SS, Pulver SR, Birdsey-Benson A, Cho YK, Morimoto TK, Chuong AS, Carpenter EJ, Tian Z, Wang J, Xie Y, Yan Z, Zhang Y, Chow BY, Surek B, Melkonian M, Jayaraman V, Constantine-Paton M, Wong GK, Boyden ES Nat Methods. 2014 Mar;11(3):338-46. doi: 10.1038/nmeth.2836. Epub 2014 Feb 9.).
  + The coding sequence for mCyRFP3 was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The CsChrimson sequence was fused to the mCyRFP3 sequence at its C-terminus with a short, flexible linker sequence (GGGSGGGS), to create CsChrimson::mCyRFP3.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + These constructs were tested for test live fluorescence by crossing to several Gal4 drivers and using a conventional upright brightfield fluorescence microscope with a GFP long pass excitation/collection cube. Expression filled cells, however red puncta were often observed, in particular near the cell body, similar to that observed for other red fluorescent proteins. 2-photon red fluorescence was confirmed using 2-photon microscopy by imaging with a standard laser-scanning 2-photon microscope with typical red/green collection optics. Some bleedthrough into the green channel is present using a 525/50nm emission filter, as expected with the published spectra.
  + Cells were targeted for whole-cell patch-clamp to verify light-driven excitation: expression effectively drives spiking activity in “PFL” cells. 2-photon activation was tested by co-expressing GCaMP8m and simultaneously imaging with a 920nm laser while stimulating with a 1064nm laser line. Cells were effectively driven by typical amounts of laser activation.

**jGCaMP7f::mCyRFP3**

* **UAS-jGCaMP7f::mCyRFP3 in VK00033:**
  + w; +; PBac{y[+mDint2] w[+mC]=20XUAS-IVS-jGCaMP7f::mCyRFP3}VK00033/TM6B
  + Comment: Expresses the calcium indicator jGCaMP7f fused to the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 for ratiometric imaging with a common excitation source.
* Associated Genes:
  + jGCaMP7f [https://flybase.org/reports/FBto0000470.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000470.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886772954%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=R88QhOwA%2B4bTWCJyk4eiUOjR%2BX8ZoIojlqWebepO5as%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP7f coding sequence was taken from Dana et al 2019 using the sequence from addgene plasmid #104483.
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The jGCaMP7f sequence was fused to the mCyRFP3 sequence at its C-terminus with a short, flexible linker sequence (GGGSSGGGSS), to create jGCaMP7f::mCyRFP3.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + This construct was tested using 2-photon calcium by imaging movements of the EPG calcium “bump” during fly menotaxis behavior, and imaging stimulus-driven responses axons of primary mechanosensory neurons. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. No obvious changes in calcium kinetics were observed when using this fusion construct in these cell-types. Some accumulation of the fusion was observed in the bipolar sensory neurons, however there were not noticeable instances of clumping typical of red fluorophores. Bleedthrough into the green channel by mCyRFP3 was observed, as expected based on the published emission spectrum. This could be removed by using a longer shifted emission filter.

**jGCaMP7f-T2A-mCyRFP3**

* **UAS-jGCaMP7f-T2A-mCyRFP3 in su(Hw)attP5:**
  + w; P{y[+t7.7] w[+mC]=20XUAS-IVS-jGCaMP7f-T2A-mCyRFP3}su(Hw)attP5/CyO; +
  + Comment: Expresses both the calcium indicator jGCaMP7f and the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 using the T2A self-cleaving peptide sequence under the control of UAS.
* **UAS-jGCaMP7f-T2A-mCyRFP3 in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-GCaMP7f-T2A-mCyRFP3}VK00005/TM3, sb
  + Comment: Expresses both the calcium indicator jGCaMP7f and the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 using the T2A self-cleaving peptide sequence under the control of UAS.
* Associated Genes:
  + jGCaMP7f [https://flybase.org/reports/FBto0000470.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000470.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886785130%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=uRUlKxOjda%2FWnUrVjXNHd0IVWdWO6CAdhDn0T5bldpQ%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP7f coding sequence was taken from Dana et al 2019 using the sequence from addgene plasmid #104483.
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + Coding sequences were linked by a “T2A” sequence to allow for bicistronic expression of two separate proteins.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + Constructs were tested for expression both mCyRFP3 and jGCaMP7f using a standard laser-scanning 2-photon microscope with typical red/green collection optics. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. Some bleedthrough into the green channel was present using a 525/50nm emission filter, as expected with the published spectra. Red puncta were visible in the somata of some cell types at levels typical for red fluorophores.

**jGCaMP8s::mCyRFP**

* **UAS-jGCaMP8s::mCyRFP in VK00033:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-jGCaMP8s::mCyRFP3}VK00033/TM6
  + Comment: Expresses the calcium indicator jGCaMP8s fused to the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 for ratiometric imaging with a common excitation source.
* Associated Genes:
  + jGCaMP8s [https://flybase.org/captcha/reports/FBto0000736](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Fcaptcha%2Freports%2FFBto0000736&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886796821%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=nmYAtfQAkh4%2BIh8NhJ%2BrOxtzmL00jo1stsobh2GaeY0%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP8s coding sequence was taken from addgene Plasmid #162386. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The jGCaMP8s sequence was fused to the mCyRFP3 sequence at its C-terminus with a short, flexible linker sequence (GGGSSGGGSS), to create jGCaMP8s::mCyRFP3.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + This construct was tested using 2-photon calcium by imaging movements of the EPG calcium “bump” during fly menotaxis behavior. No obvious changes in calcium kinetics were observed when using this fusion construct in these cell-types. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. Some accumulation of the fusion was observed in the bipolar sensory neurons, however there were not noticeable instances of clumping typical of red fluorophores. Bleedthrough into the green channel by mCyRFP3 was observed, as expected based on the published emission spectrum. This could be removed by using a longer shifted emission filter.

**jGCaMP8s-T2A-mCyRFP3**

* **UAS-jGCaMP8s-T2A-mCyRFP3 in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-jGCaMP8s-T2A-mCyRFP3}VK00005/TM3, sb
  + Comment: Expresses both the calcium indicator jGCaMP8s and the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 using the T2A self-cleaving peptide sequence under the control of UAS.
* Associated Genes:
  + jGCaMP8s [https://flybase.org/captcha/reports/FBto0000736](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Fcaptcha%2Freports%2FFBto0000736&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886809977%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=GfOa2%2FVSBefG57PZrfncqVKW2Bh%2BJsGF3etw7vfPT78%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP8s coding sequence was taken from addgene Plasmid #162386. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + Coding sequences were linked by a “T2A” sequence to allow for bicistronic expression of two separate proteins.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + Constructs were tested for expression both mCyRFP3 and jGCaMP8s using a standard laser-scanning 2-photon microscope with typical red/green collection optics. jGCaMP8s behavior was tested using 2-photon calcium by imaging movements of the EPG calcium “bump” during fly menotaxis behavior. No obvious changes in calcium kinetics were observed when using this fusion construct in these cell-types. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. Some bleedthrough into the green channel was present using a 525/50nm emission filter, as expected with the published spectra. We did not note red puncta, however we expect them to be present in this construct based on prior experience.

**jGCaMP8m::mCyRFP**

* **UAS-jGCaMP8m::mCyRFP in VK00033:**
  + w; +; PBac{y[+mDint2] w[+mC]=20XUAS-IVS-jGCaMP8m::mCyRFP3}VK00033/TM6B
  + Comment: Expresses the calcium indicator jGCaMP8m fused to the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 under the control of UAS for ratiometric imaging with a common excitation source.
* Associated Genes:
  + jGCaMP8m [https://flybase.org/reports/FBto0000735.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000735.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886821594%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=%2FofuTS9qC%2BlRxitifMgOc1RlcGTMteFtIlrJI4ie9Yg%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP8m coding sequence was taken from addgene Plasmid #162387. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The mCyRFP3 coding sequence was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The jGCaMP8m sequence was fused to the mCyRFP3 sequence at its C-terminus with a short, flexible linker sequence (GGGSSGGGSS), to create jGCaMP8s::mCyRFP3.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + This construct was tested using 2-photon calcium by imaging movements of the EPG calcium “bump” during fly menotaxis behavior. No obvious changes in calcium kinetics were observed when using this fusion construct in these cell-types. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. Bleedthrough into the green channel by mCyRFP3 was observed, as expected based on the published emission spectrum. This could be removed by using a longer shifted emission filter. We did not note any accumulation of this fusion construct, but expect it based on experience with other sensors.

**jGCaMP8m-T2A-mCyRFP3**

* **UAS-jGCaMP8m-T2A-mCyRFP3 in su(Hw)attP5:**
  + w; P{y[+t7.7] w[+mC]=20XUAS-IVS-jGCaMP8m-T2A-mCyRFP3}su(Hw)attP5/CyO; +
  + Comment: Expresses both the calcium indicator jGCaMP8m and the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 using the T2A self-cleaving peptide sequence under the control of UAS.
* **UAS-jGCaMP8m-T2A-mCyRFP3 in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-jGCaMP8m-T2A-mCyRFP3}VK00005/TM3, sb
  + Comment: Expresses both the calcium indicator jGCaMP8m and the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 using the T2A self-cleaving peptide sequence under the control of UAS.
* Associated Genes:
  + jGCaMP8m [https://flybase.org/reports/FBto0000735.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000735.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886833038%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=zTdHbVR13VOMyZLwAzK%2FXYLyyONTCkZBfh4iWcgtc6M%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The jGCaMP8m coding sequence was taken from addgene Plasmid #162387. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The coding sequence for mCyRFP3 was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + Coding sequences were linked by a “T2A” sequence to allow for bicistronic expression of two separate proteins.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + Constructs were tested for expression both mCyRFP3 and jGCaMP8m using a standard laser-scanning 2-photon microscope with typical red/green collection optics. jGCaMP8m behavior was tested using 2-photon calcium by imaging movements of the EPG calcium “bump” during fly menotaxis behavior. No obvious changes in calcium kinetics were observed when using this fusion construct in these cell-types. Strong red channel signal was present throughout the neurons, which showed bleaching at higher laser intensities, and proceeded faster than bleaching in the green channel. Some bleedthrough into the green channel was present using a 525/50nm emission filter, as expected with the published spectra. We did not note red puncta, however we expect them to be present in this construct based on prior experience.

**syt1::jGCaMP8m::mCyRFP3**

* **UAS-syt1::jGCaMP8m::mCyRFP3 in VK00033:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-syt1::jGCaMP8m::mCyRFP3}VK00033/TM6B
  + Comment: Expresses synapse-targeted calcium indicator jGCaMP8m fused to the cyan-excitable red-fluorescent protein fluorophore mCyRFP3 under the control of UAS for ratiometric calcium imaging of synapses with a common excitation source.
* Associated Genes:
  + syt1 [https://flybase.org/reports/FBgn0004242.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBgn0004242.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886844520%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=OqOe8oy9RyAMC3%2FLJ6gLD8l0kj%2FPLm8O%2FFu%2BGeJAfvs%3D&reserved=0)
  + jGCaMP8m [https://flybase.org/reports/FBto0000735.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000735.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886856084%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=6rJuZODbOlFUYfn%2B9rNCezGTXtx0o4u5%2BE4oiFQymaQ%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The syt1 coding sequence was taken from addgene Plasmid #190896.
  + The jGCaMP8m coding sequence was taken from addgene Plasmid #162387. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The coding sequence for mCyRFP3 was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The syt1 coding sequence was fused to the  jGCaMP8m coding sequence N-terminus using a flexible linker (GGGGSGGGGS), the mCyRFP3 coding sequence was fused to the C-terminus of the same jGCaMP8m sequence using a different flexible linker (GGSSSGGSSS).
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + Construct was tested by crossing to driver lines with known syt1-fused jGCaMP8m expression and activity. We looked for similar expression patterns and GCaMP responses using a conventional laser-scanning 2-photon microscope. Strong signal was present in both red and green channels, some bleedthrough into the green channel was present using a 525/50nm emission filter, as expected with the published spectra (removable by using a longer shifted emission filter).

**syt1::jGCaMP8m-T2A-syt1::mCyRFP3**

* **UAS-syt1::jGCaMP8m-T2A-syt1::mCyRFP3 in VK00005:**
  + w; +;  PBac{y[+mDint2] w[+mC]=20XUAS-IVS-syt1::jGCaMP8m-T2A-syt1::mCyRFP3}VK00005/TM3, sb
  + Comment: Expresses synapse-targeted calcium indicator jGCaMP8m and synapse-targeted cyan-excitable red-fluorescent protein fluorophore mCyRFP3 under the control of UAS.
* Associated Genes:
  + syt1 [https://flybase.org/reports/FBgn0004242.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBgn0004242.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886867646%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=T1YFzwHF3Zfqgi75i7Pk9U7LendfktQN0HkXLSTNetY%3D&reserved=0)
  + jGCaMP8m [https://flybase.org/reports/FBto0000735.htm](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflybase.org%2Freports%2FFBto0000735.htm&data=05%7C02%7Cgm119%40universityofcambridgecloud.onmicrosoft.com%7C88d6c9a3a7074cd0e3c808dd574d8193%7C49a50445bdfa4b79ade3547b4f3986e9%7C1%7C0%7C638762712886881986%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=US3%2FilZVeM6LKshHOx1qFguioYOA1Kvvfrraq75J2lE%3D&reserved=0)
  + mCyRPF3 (no flybase entry)
    - From: Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.
* Generation:
  + The syt1 coding sequence was taken from addgene Plasmid #190896.
  + The jGCaMP8m coding sequence was taken from addgene Plasmid #162387. (Zhang Y, Rózsa M, Bushey D, Zheng J, Reep D, Broussard GJ, Tsang A, Tsegaye G, Patel R, Narayan S, Li JX, Zhang R, Ahrens MB, Turner GC, Wang SS-H, Svoboda K, Korff W, Schreiter ER, Hasseman JP, Kolb I, Looger LL Janelia Research Campus, Online Resource (2020) doi:10.25378/janelia.13148243)
  + The coding sequence for mCyRFP3 was taken from Kim et al 2022 using the sequence from the addgene Plasmid #193323 (Kim BB, Wu H, Hao YA, Pan M, Chavarha M, Zhao Y, Westberg M, St-Pierre F, Wu JC, Lin MZ Sci Rep. 2022 Mar 7;12(1):3678. doi: 10.1038/s41598-022-07313-1.).
  + The syt1 coding sequence was fused to the  jGCaMP8m coding sequence N-terminus using a flexible linker (GGGGSGGGGS), syt1 was also fused to the mCyRFP3 coding sequence N-terminus using the same flexible linker. These two fused coding sequences were linked by a “T2A” sequence to allow for bicistronic expression of two separate polypeptides.
  + This sequence was codon optimized for Drosophila, synthesized, and inserted into the pJFRC7-20XUAS-IVS-mCD8::GFP plasmid backbone (Pfeiffer, B.D. *et al.* (2010) ‘Refinement of tools for targeted gene expression in Drosophila’, *Genetics*, 186(2), pp. 735–755.), addgene Plasmid #26220.
  + Plasmids were purified and injected into embryos for PhiC31 integrase-mediated transgenesis. Transformants were kept for several generations and the healthiest were then selected.
* Testing:
  + Construct was tested by crossing to driver lines with known syt1-fused jGCaMP8m expression and activity. We looked for similar expression patterns and GCaMP responses using a conventional laser-scanning 2-photon microscope. Strong signal was present in both red and green channels, some bleedthrough into the green channel was present using a 525/50nm emission filter, as expected with the published spectra (removable by using a longer shifted emission filter).