SPARC and SPARC2 Flies: Inserted with CRISPR-HDR into a region near the *attP40* locus (CR-P40; see supplemental methods). **Bolded stocks** are available from the Bloomington Drosophila Stock Center (BDSC; https://bdsc.indiana.edu/stocks/misc/sparc.html).

1. ***+; Tl{20XUAS-SPARC-D-GCaMP6f}CR-P40; +***
2. ***+; Tl{20XUAS-SPARC-I-GCaMP6f}CR-P40; +***
3. ***+; Tl{20XUAS-SPARC-S-GCaMP6f}CR-P40; +***
4. ***+; Tl{20XUAS-SPARC-D-jGCaMP7f}CR-P40; +***
5. ***+; Tl{20XUAS-SPARC-I-jGCaMP7f}CR-P40; +***
6. ***+; Tl{20XUAS-SPARC-S-jGCaMP7f}CR-P40; +***
7. ***+; Tl{20XUAS-SPARC-I-ASAP2f}CR-P40; +***
8. *+; Tl{20XUAS-SPARC-D-mCD8::GFP}CR-P40; +*
9. *+; Tl{20XUAS-SPARC-I-mCD8::GFP}CR-P40; +*
10. *+; Tl{20XUAS-SPARC-S-mCD8::GFP}CR-P40; +*
11. *+; Tl{20XUAS-SPARC-D-LexA::p65}CR-P40; +*
12. *+; Tl{20XUAS-SPARC-I-LexA::p65}CR-P40; +*
13. *+; Tl{20XUAS-SPARC-S-LexA::p65}CR-P40; +*
14. ***+; Tl{20XUAS-SPARC2-D-LexA::p65}CR-P40; +***
15. ***+; Tl{20XUAS-SPARC2-I-LexA::p65}CR-P40; +***
16. ***+; Tl{20XUAS-SPARC2-S-LexA::p65}CR-P40; +***
17. ***+; Tl{20XUAS-SPARC2-D-Syn21-CsChrimson::tdTomato-3.1}CR-P40; +***
18. ***+; Tl{20XUAS-SPARC2-I-Syn21-CsChrimson::tdTomato-3.1}CR-P40; +***
19. ***+; Tl{20XUAS-SPARC2-S-Syn21-CsChrimson::tdTomato-3.1}CR-P40; +***
20. ***+; Tl{20XUAS-SPARC2-D-mCD8::GFP}CR-P40; +***
21. ***+; Tl{20XUAS-SPARC2-I-mCD8::GFP}CR-P40; +***
22. ***+; Tl{20XUAS-SPARC2-S-mCD8::GFP}CR-P40; +***

PhiC31 transgenic strains:

1. ***y w; P{nSyb-IVS-PhiC31}su(Hw)attP5; +***
2. ***y w; P{20XUAS-IVS-PhiC31}su(Hw)attP5; +***
3. ***y w; P{αTub84-IVS-PhiC31}su(Hw)attP5; +***
4. ***y w, P{nSyb-IVS-PhiC31}attP18; +***
5. ***y w, P{20XUAS-IVS-PhiC31}attP18; +***
6. ***+, P{nSyb-IVS-PhiC31}attP18; S/Cyo; Pr/TM6B***
7. ***+, P{20XUAS-IVS-PhiC31}attP18; S/Cyo; Pr/TM6B***

Supplementary Table 5: Transgenic flies generated in this study.

R2 neuron transgene expression	Resting membrane potential (mean ± SEM, mV)*	Input resistance (mean ± SEM, MΩ)*
CsChrimson::tdTomato+, GFP+	-38 ± 5	220 ± 14
GFP+	-48 ± 2	628 ± 131

Supplementary Table 7: Resting membrane potential and input resistance of R2 neurons from Figure 6.

Supplementary Note - Fly genotypes (by figure):

Figure 2b: +*; TI{20XUAS-SPARC-D-GCaMP6f}CR-P40/+; P{VT025965-Gal4}attP2/ P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 2c: +*; TI{20XUAS-SPARC-D-GCaMP6f}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 2d: +*; TI{20XUAS-SPARC-I-GCaMP6f}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 2e: +*; TI{20XUAS-SPARC-S-GCaMP6f}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 2g-g”:+*; TI{20XUAS-SPARC-I-LexA::p65}CR-P40/+; P{GMR19F01-Gal4}attP2/ P{UAS-mCD8::GFP}attP1 P{13XlexAop-IVS-myr::tdTomato}attP2*

Figure 2h-h”: +*; TI{20XUAS-SPARC2-I-LexA::p65}CR-P40/+; P{GMR19F01-Gal4}attP2/ P{10XUAS-mCD8::GFP}attP1 P{13XlexAop-IVS-myr::tdTomato}attP2*

Figure 2i-i”: +*; TI{20XUAS-SPARC2-D-LexA::p65}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-mCD8::GFP}attP1, P{13XlexAop-IVS-myr::tdTomato}attP2*

Figure 2j-j”: +*; TI{20XUAS-SPARC2-I-LexA::p65}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-mCD8::GFP}su(Hw)attP1, P{13XlexAop-IVS-myr::tdTomato}attP2*

Figure 2k-k”: +*; TI{20XUAS-SPARC2-S-LexA::p65}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-mCD8::GFP}su(Hw)attP1, P{13XlexAop-IVS-myr::tdTomato}attP2*

Figure 3a: +*; TI{20XUAS-SPARC2-D-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3b: +*; TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3c: +*; TI{20XUAS-SPARC2-S-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3d: +*; TI{20XUAS-SPARC2-D-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3e: +*; TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3f: +*; TI{20XUAS-SPARC2-S-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3g: +*; TI{20XUAS-SPARC2-D-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GH146-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3h: +*; TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GH146-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3i: +*; TI{20XUAS-SPARC2-S-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GH146-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3j: *P{nSyb-IVS-PhiC31}attP18; PBac{R17A04-p65.ADZp}VK00027/TI{20XUAS-SPARC2-D-mCD8::GFP}CR-P40; P{R35B06-ZpGAL4.DBD}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3k: *P{nSyb-IVS-PhiC31}attP18; PBac{R17A04-p65.ADZp}VK00027/TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40; P{R35B06-ZpGAL4.DBD}attP2 / P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3l: *P{nSyb-IVS-PhiC31}attP18; PBac{R17A04-p65.ADZp}VK00027/TI{20XUAS-SPARCS-I-mCD8::GFP}CR-P40; P{R35B06-ZpGAL4.DBD}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3m: +*; TI{20XUAS-SPARC2-D-mCD8::GFP}CR-P40 P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR27B03-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3n: +*; TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR27B03-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 3o: +*; TI{20XUAS-SPARC2-S-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR27B03-Gal4}attP2/ P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 4a-c': +*; TI{20XUAS-SPARC2-I-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GH146-Gal4}/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 4d-g': +*; TI{20XUAS-SPARC2-S-mCD8::GFP}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GH146-Gal4}/P{10XUAS-IVS-myr::tdTomato}attP2*

Figure 5a-e:

SPARC: +*; TI{20XUAS-SPARC-S-GCaMP6f}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{VT025965-Gal4}attP2/* +

FlpOut: +*/y w, P{hsFLP}; P{20XUAS-IVS-GCaMP6f}attP40/P{αTub84b{FRT.Gal80}}2; P{VT025965-Gal4}attP2/*+

Figure 6b,c: *P{nSyb-IVS-PhiC31}attP18/+; TI{20XUAS-SPARC2-D-Syn21-CsChrimson::tdTomato-3.1}CR-P40/{20XUAS-IVS-mCD8::GFP}attP40; P{GMR19C08-Gal4}attP2/+*

Figure 6d,e: *P{nSyb-IVS-PhiC31}attP18/y w; TI{3XP3-DsRed=20XUAS-SPARC2-D-Syn21-CsChrimson::tdTomato-3.1}CR-P40/ P{20XUAS-IVS-mCD8::GFP}attP40; P{GMR19C08-Gal4}attP2/+*

Extended Data Figure 1c-d": +*; TI{20XUAS-34bp_attP-inversion-GCaMP6f}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Extended Data Figure 1f-f": +*; TI{20XUAS-Lex-OR-Flp}CR-P40/P{nSyb-IVS-PhiC31}su(Hw)attP5; P{GMR19F01-Gal4}attP2/P{UAS-FRT-stop-FRT-mCD8::GFP}, P{13XlexAop-IVS-myr::tdTomato}attP2*

Extended Data Figure 3a, e-e": +*/w; TI{20XUAS-SPARC-D-GCaMP6f}CR-P40/P{R19B03-p65.ADZp}attP40; P{R26E07-ZpGAL4.DBD}attP2/P{10X UAS-myr::tdTomato}attP2*

Extended Data Figure 3b, f-f": +*; TI{20XUAS-SPARC-D-GCaMP6f}CR-P40/P{nSyb-PhiC31}su(Hw)attP5 P{R19B03-p65.ADZp}attP40; P{R26E07-ZpGAL4.DBD}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Extended Data Figure 3c, g-g": +*; TI{20XUAS-SPARC-I-SPARC-GCaMP6f}CR-P40/P{nSyb-PhiC31}su(Hw)attP5 P{R19B03-p65.ADZp}attP40; P{R26E07-ZpGAL4.DBD}attP2/ P{10XUAS-IVS-myr::tdTomato}attP2*

Extended Data Figure 3d, h-h": +*; TI{20XUAS-SPARC-S-GCaMP6f}CR-P40/P{nSyb-PhiC31}su(Hw)attP5, P{R19B03-p65.ADZp}attP40; P{R26E07-ZpGAL4.DBD}attP2/P{10XUAS-IVS-myr::tdTomato}attP2*

Supplementary Note - Origin of transgenes

1. *P{GMR19F01-Gal4}attP2* from BDSC, stock number 48852[1].
2. *P{VT025965-Gal4}attP2* from VDRC[2].
3. *P{GH146-Gal4}*[3] *and P{UAS-FRT-stop-FRT-mCD8::GFP}*[4] *were generous gifts from ChiChi Xie and Liqun Luo.*
4. *P{R19B03-p65.ADZp}attP40; P{R026E07-ZpGAL4.DBD}attP2* and *PBac{R17A04-p65.ADZp}VK00027; P{R35B06-ZpGAL4.DBD}attP2* from Janelia FlyLight collection.
5. *P{GMR27B03-Gal4}attP2*[5] from BDSC, stock number 49211.
6. *P{GMR19C08-Gal4}attP2* from BDSC, stock number 48845, and is described in[6].
7. *P{10XUAS-myr::tdTomato}attP2* from BDSC, stock number 32221.
8. *P{13XlexAop-myr::tdTomato}attP2* was a generous gift from Heather Dionne and Gerald Rubin.
9. *P{10XUAS-IVS-mCD8::GFP}su(Hw)attP1* from BDSC, stock number 32187.

10. *P{20XUAS-IVS-mCD8::GFP}attP40* was a gift from Barret Pfeiffer and Gerald Rubin and is described in[7].
11. *CyO, P{w[+mC]=Crew}DH1* from BDSC, stock number 1092, and is described in[8].
12. *P{20XUAS-IVS-GCaMP6f}attP40* from BDSC, stock number 42747, and is described in[9].
13. *P{hsFLP}1* from BDSC stock number 6, and is described in[10].
14. *P{αTub84b(FRT.Gal80)}2* from BDSC, stock number 38880, and is described in[11].

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