

SUPPLEMENTARY INFORMATION

**G protein-coupled receptor-based thermosensation determines temperature
acclimatization of *Caenorhabditis elegans***

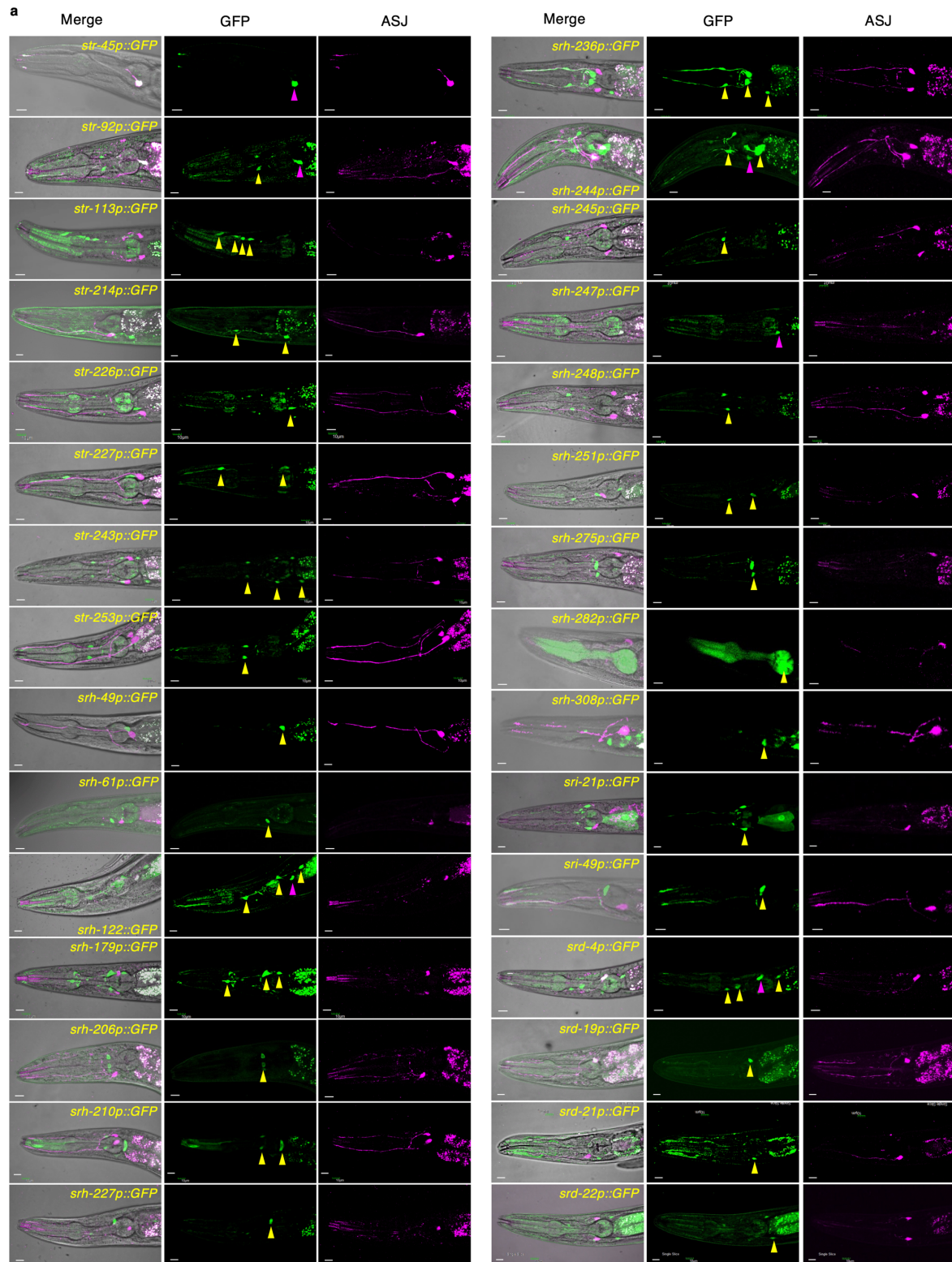
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3</sup>, Atsushi Kuhara^{*1-3,7}**

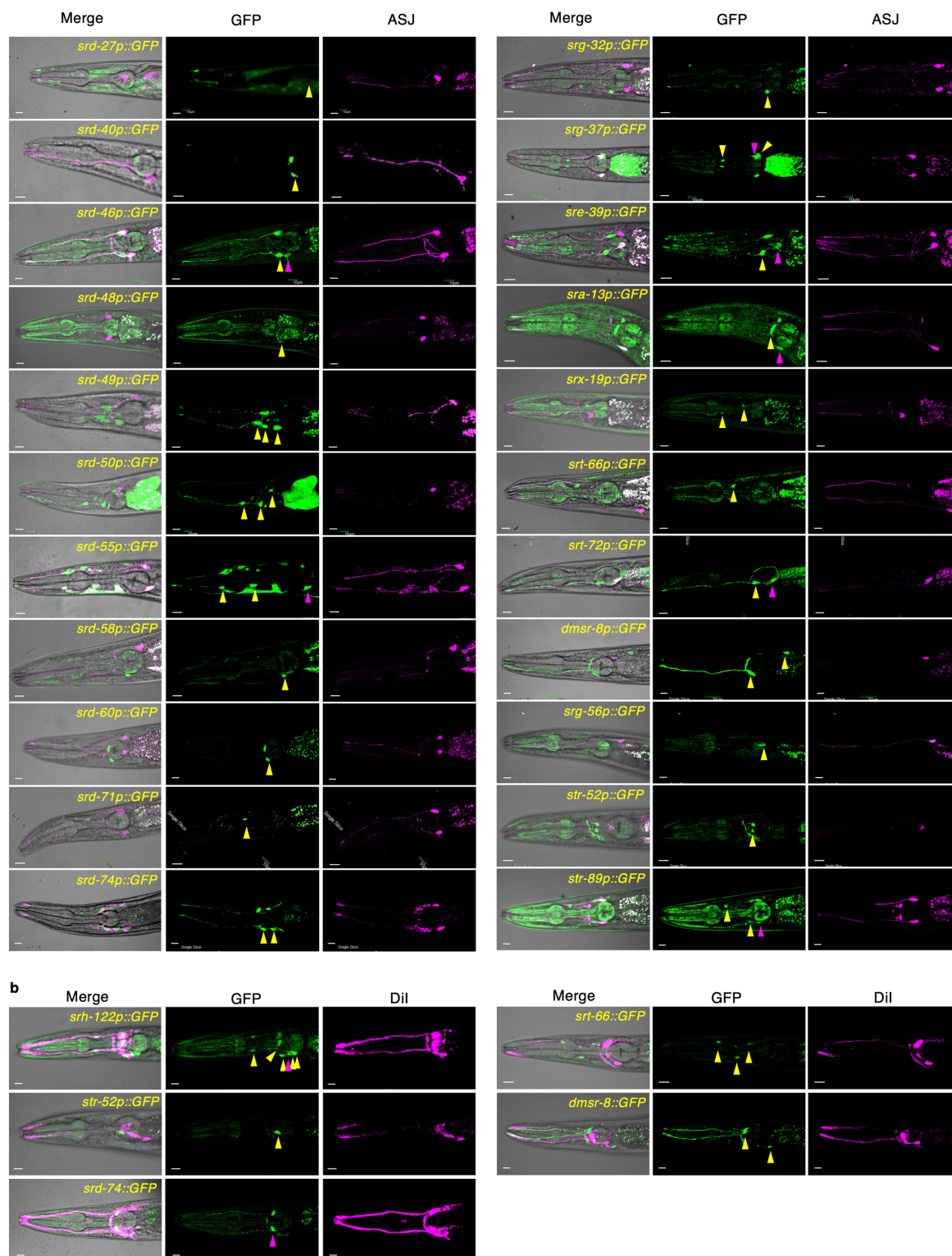
Supplementary Fig. 1 – Fig. 4

Supplementary Table 1 – Table 4

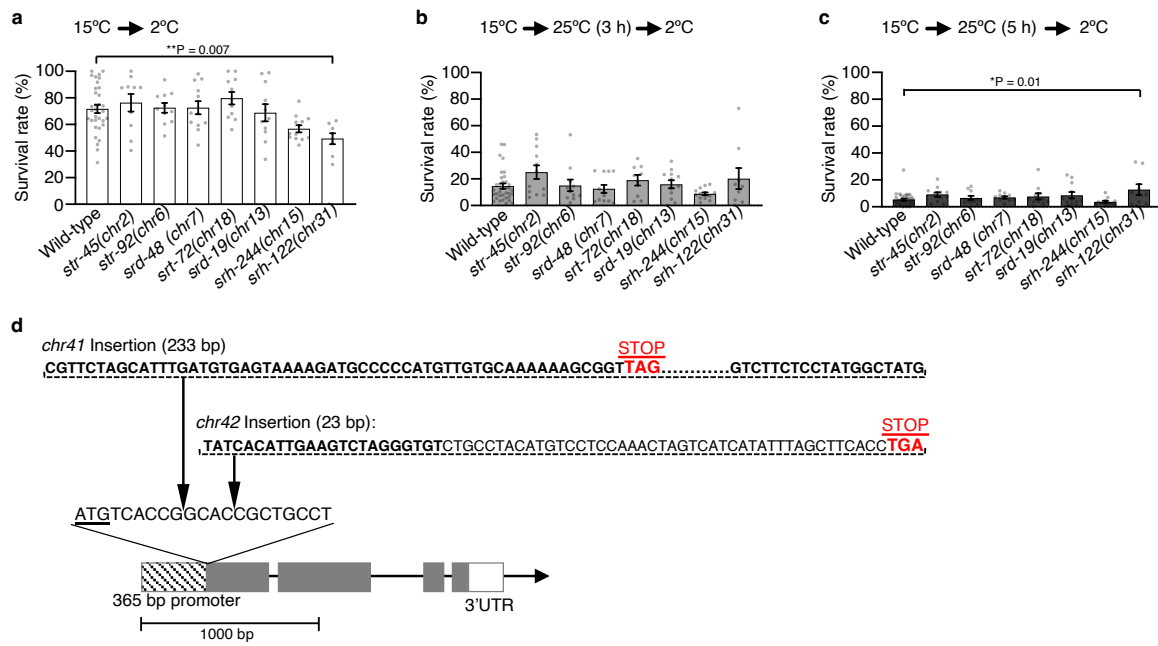
Supplementary Data 1. (separated excel file)

Supplementary Data 2. (separated excel file)

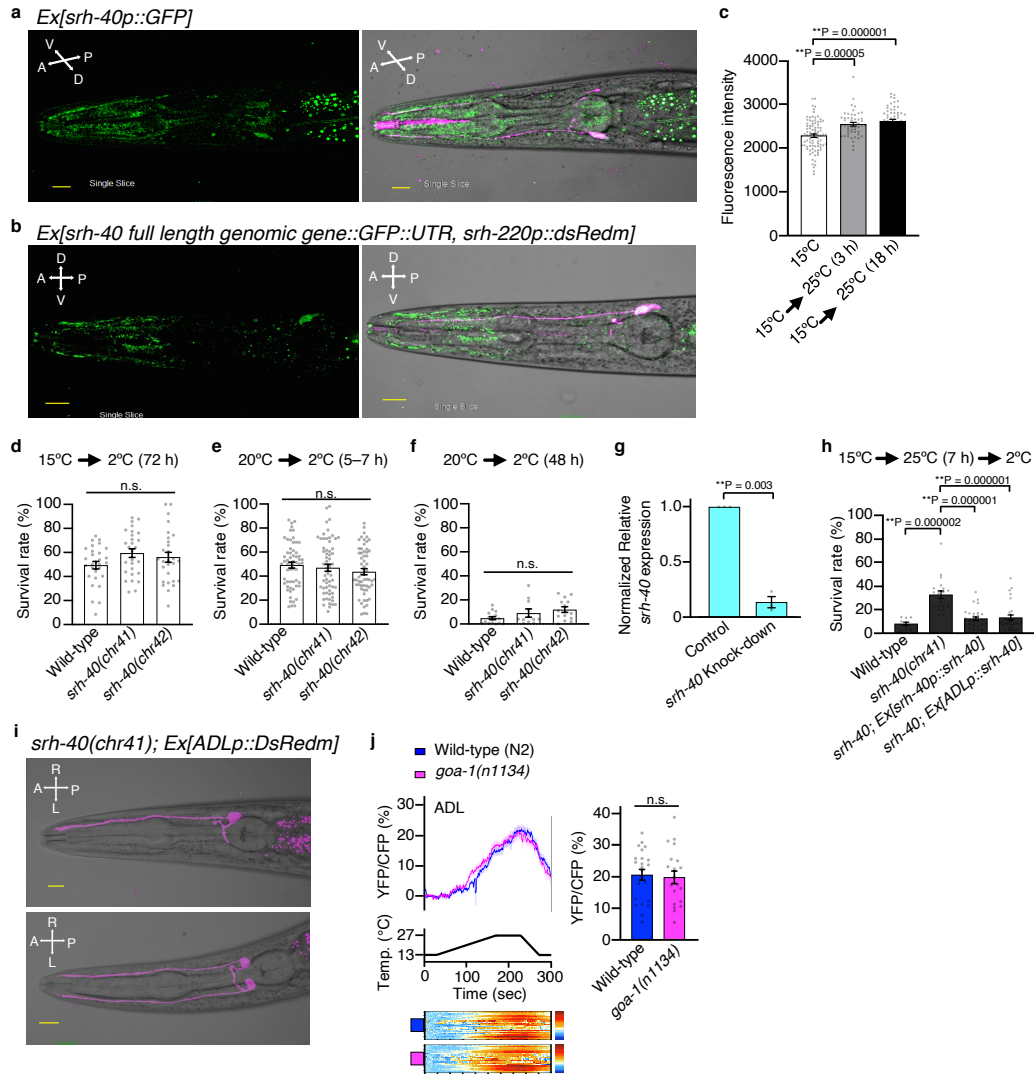




Supplementary Fig. 1. Expression patterns of 50 GPCR genes. Expression patterns visualized in transgenic animals, wherein GFP was expressed under the upstream promoter regions of each GPCR gene (Green). **a**, GFP fluorescence was merged with *trx-1p::DsRedm* that labels ASJ (Magenta). **b**, GFP fluorescence were merged with Dil that labels six pairs of amphid neurons in the head with red fluorescence (Magenta). All scale bars indicate 10 μ m. Magenta or yellow arrowheads indicate GFP fluorescence in the cell body of ASJ or ADL thermosensory neuron, respectively.



Supplementary Fig. 2. Temperature acclimatization of knockout mutants of 12 GPCRs. **a–c**, Temperature acclimatization of GPCR knockout mutants constructed by CRISPR/Cas9 and assayed under [15°C→25°C (0, 3, or 5 h)→2°C]. *n* = 33, 10, 11, 12, 11, 11, 12, and 8 (**a**), *n* = 34, 12, 11, 12, 9, 12, 13, and 9 (**b**), *n* = 35, 11, 11, 10, 11, 12, 13, and 9 (**c**). *n* indicates independent experiments (shown from left in the bar graph). Bar graphs represent mean ± SEM. *p*-values were calculated using two-sided one-way ANOVA with Dunnett's test (**a–f**). n.s. *P* ≥ 0.05; **P* < 0.05; ***P* < 0.01. Source data are provided as a Source Data file. **d**, Genomic structure of *srh-40* and the KO mutants. The shaded box and gray boxes indicate the promoter region and exons, respectively. The positions of nonsense mutations caused by each insertion in *chr41* and *chr42* are shown. In each mutant, an aberrant stop codon occurred in the first exon by frameshift.



Supplementary Fig. 3. Expression analysis, cold tolerance, and Ca²⁺ imaging. **a, b**, Wild-type expressing *srh-40p::gfp* (a) or *srh-40 full length::gfp* (b), and *srh-220p::DsRedm* that labels ADL (Magenta). **c**, GFP fluorescence intensity induced by the *srh-40* promoter in the ADL of wild-type grown at 15°C, 15°C→25°C (3 h), or 15°C→25°C (18 h) (n = 88, 49, and 57). **d–f** Cold tolerance of *srh-40* mutants ([15°C→2°C (72 h)], n = 24) or [20°C→2°C (5–7 h); n = 60, 60, and 59] to observe the mutant phenotype under conditions where the survival rate of the wild-type is approximately 50%, and ([20°C→2°C (48 h)]; n = 18, 13, and 17). **g**, Relative *srh-40* mRNA expression in *eri-1; lin-15B* mutant in which knocked-down *srh-40* gene, measured by qPCR. Bars represent fold changes relative to control with no-treated *eri-1; lin-15B* mutant. n = 3. **h**, Temperature acclimatization of the rescued *srh-40* mutant ([15°C→25°C (7 h)→2°C], n = 10, 20, 30, 30). **i**, Representative confocal image of ADL in the *srh-40* mutant. n = 39. A, anterior; P, posterior; D, dorsal; V, ventral; L, left; R, right. **j**, Ca²⁺ imaging of ADL in *goa-1* mutant (n = 23, 20). Traces indicate the averaged YFP/CFP ratio of YC3.60 in response to warming and cooling. Bar graphs indicate the averaged YFP/CFP ratio between 211–220 sec, the maximum point in each strain. Each row in the color maps represents relative changes in Ca²⁺ concentrations from one worm; excluded values >100% are shown in white. The colors of bar graphs and color maps correspond to the colors of response traces. Scale bars indicate 10 μm (a, b, i). n indicates independent experiments (shown from left in the bar graph) (c–h, j). Bar graphs represent mean ± SEM. p-values were calculated using unpaired *t*-test (g, j), one-way ANOVA with Dunnett's test (d–f) or Tukey–Kramer's test (c), and Kruskal–Wallis's test with Steel–Dwass's test (h). n.s. *P* ≥ 0.05; ***P* < 0.01. All statistical tests were two-sided. Source data are provided as a Source Data file.

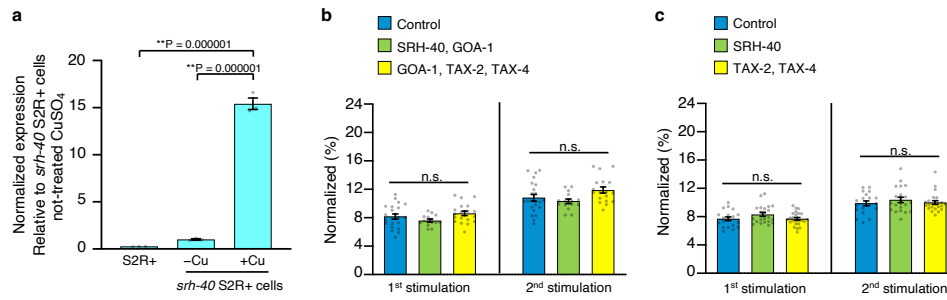


Fig 4. Ca²⁺ imaging of *Drosophila* S2R+ cells for negative control. **a**, Relative *srh-40* mRNA expression in naïve S2R+ cells, or *srh-40* stable S2R+ cells treated or not treated with CuSO₄, measured by qPCR. Bars represent fold changes relative to *srh-40* stable S2R+ cells that were not treated with CuSO₄. $n = 3$. **b**, **c**, Comparison of the maximum increase in Ca²⁺ concentrations in response to the first and second heat stimulation in the control and cells expressing various genes as indicated. $n = 828, 538$, and 688 ($951, 538$ and 652 in second stimuli) cells examined over 21, 15, and 19 (23, 15, and 18 in second stimuli) independent experiments (**b**), $n = 630, 758$, and 766 ($630, 731$, and 728 in second stimuli) cells examined over 19, 22, and 24 (19, 21, and 23 in second stimuli) independent experiments (**c**). n indicates independent experiments (shown from left in the bar graph). Bar graphs represent mean $\pm$ SEM. p -values were calculated using one-way ANOVA with Dunnett's test (**c** and first stimuli in **b**) or Tukey–Kramer's test (**a**), and Kruskal–Wallis's test with Steel's test (second stimuli in **b**). n.s. $P \geq 0.05$; $**P < 0.01$. All statistical tests were two-sided. Source data are provided as a Source Data file.

Supplementary Table 1. GPCRs expressed in thermosensory neurons.

Gene	Sequence name	ASJ	ADL
<i>str-43</i>	T23D5.9	ND (CeNGEN -)	ND (CeNGEN -)
<i>str-45</i>	T09F5.3	+	-
<i>str-52</i>	C45H4.12	-	+
<i>str-89</i>	F59A1.4	+	ND (CeNGEN -)
<i>str-92</i>	F59A1.3	+	-
<i>str-113</i>	Y39H10B.1	-	-
<i>str-214</i>	C31A11.9	-	-
<i>str-226</i>	C07G3.4	-	ND (CeNGEN NO DATA)
<i>str-227</i>	C07G3.3	-	ND (CeNGEN -)
<i>str-243</i>	K02H11.7	-	ND (CeNGEN -)
<i>str-253</i>	F41B5.8	-	-
<i>sra-13</i>	F49E12.5	+	ND (CeNGEN -)
<i>srh-40</i>	Y40D12A.3	-	+
<i>srh-49</i>	C10G11.4	- (CeNGEN +)	ND (CeNGEN -)
<i>srh-61</i>	T21B4.8	-	ND (CeNGEN -)
<i>srh-122</i>	R08H2.5	+	ND (CeNGEN NO DATA)
<i>srh-179</i>	ZK228.7	-	ND (CeNGEN -)
<i>srh-206</i>	Y102A5C.15	-	ND (CeNGEN +)
<i>srh-210</i>	D1065.4	- (CeNGEN +)	ND (CeNGEN +)
<i>srh-227</i>	C35D6.2	-	ND (CeNGEN NO DATA)
<i>srh-236</i>	Y68A4A.9	-	ND (CeNGEN NO DATA)
<i>srh-244</i>	C03G6.9	+	ND (CeNGEN -)
Gene	Sequence name	ASJ	ADL
<i>srh-282</i>	T21D12.5	- (CeNGEN +)	-
<i>srh-308</i>	F58E10.6	-	+
<i>sri-21</i>	T24A6.4	-	ND (CeNGEN -)
<i>sri-49</i>	F22E5.14	-	ND (CeNGEN -)
<i>srd-4</i>	ZK863.5	+	ND (CeNGEN -)
<i>srd-19</i>	F53F1.11	+	- (CeNGEN +)
<i>srd-21</i>	W06G6.3	-	-
<i>srd-22</i>	F26G5.3	-	-
<i>srd-27</i>	T26E4.12	-	-
<i>srd-40</i>	F17A2.12	-	ND (CeNGEN -)
<i>srd-46</i>	F17A2.10	+	ND (CeNGEN -)
<i>srd-48</i>	F17A2.9	- (CeNGEN +)	-
<i>srd-49</i>	R04B5.8	-	ND (CeNGEN -)
<i>srd-50</i>	F15A2.4	-	ND (CeNGEN -)
<i>srd-55</i>	K02A2.2	+	ND (CeNGEN -)
<i>srd-58</i>	E04F6.13	-	ND (CeNGEN NO DATA)
<i>srd-60</i>	C13B7.3	-	ND (CeNGEN NO DATA)
<i>srd-71</i>	C13B7.4	-	ND (CeNGEN NO DATA)
<i>srd-74</i>	C24H11.4	-	-
<i>sre-39</i>	F10G7.7	+	ND (CeNGEN +)
<i>srg-32</i>	T21C9.7	-	ND (CeNGEN -)
<i>srg-37</i>	K04C1.1	+	-

<i>srh-245</i>	F31F4.9	–	–
<i>srh-247</i>	C31B8.13	+	– (CeNGEN NO DATA)
<i>srh-248</i>	H05B21.2	–	ND (CeNGEN NO DATA)
<i>srh-251</i>	R08H2.4	–	ND (CeNGEN NO DATA)
<i>srh-275</i>	C03G6.7	– (CeNGEN +)	ND (CeNGEN +)

<i>srg-56</i>	C24B9.10	–	ND (CeNGEN +)
<i>srt-66</i>	B0238.14	–	–
<i>srt-72</i>	C36C5.9	+	ND (CeNGEN –)
<i>srx-19</i>	T05A12.1	–	ND (CeNGEN –)
<i>dmsr-8</i>	C35A5.7	–	ND (CeNGEN –)

“+” and “–” indicate positive and negative GFP expression driven by each gene promoter in this study, respectively. ND indicates “not determined”; “(CeNGEN +)” and “(CeNGEN –)” indicate positive and negative expression in CeNGEN, the *C. elegans* Neuronal Gene Expression database (Select threshold: 2), respectively.

Supplementary Table 2. *C. elegans* strain

Genotype	Strains	Source
<i>eri-1(mg366); lin-15B(n744)</i>	KP3948	CGC
<i>tax-4(p678)</i>	PR678	CGC
<i>osm-9(ky10)</i>	CX10	CGC
<i>ocr-2(ak47)</i>	CX4544	CGC
<i>osm-9(ky10); ocr-2(ak47)</i>	CX4652	CGC
<i>osm-9(ky10) ocr-2(ak47); ocr-1(ak46)</i>	FG125	CGC
<i>srh-40(chr41); osm-9(ky10) ocr-2(ak47); ocr-1(ak46)</i>	–	This study
<i>srh-40(chr41); osm-9(ky10) ocr-2(ak47)</i>	–	This study
<i>egl-30(ad806)</i>	DA1084	CGC
<i>egl-30(ep271)</i>	CE1047	CGC
<i>gcy-5(tm897)</i>	OH4844	CGC
<i>glr-3(tm6403)</i>	–	NBRP
<i>Ex[flp-6p::CeGCaMP8, gcy-5p::tagRFP]</i>	–	Takagaki et al., 2019
<i>glr-3(tm6403); Ex[flp-6p::CeGCaMP8, gcy-5p::tagRFP]</i>	–	Ohnishi et al. 2020
<i>Ex[ges-1p::NLS::GFP, AIYp::GFP, pBluescript II SK+]</i>	–	Ujisawa et al., 2016
<i>Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	Ujisawa et al., 2016
<i>gpa-3(pk35)</i>	NL335	CGC
<i>goa-1(n1134)</i>	MT2426	CGC
<i>goa-1(n1134); gpa-3(pk35)</i>	KHR80	Ujisawa et al., 2016
<i>ExUDA30[<i>str-45</i> full length genomic gene::gfp, <i>trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-92p::gfp, trx-1p::NLS::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-113p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-214p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-226p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-227p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-243p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>str-253p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-49p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>ExUDA32[<i>srh-61p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-122p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-179p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-206p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-210p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-227p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-236p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-245p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-247p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-248p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-251p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>Ex[<i>srh-275p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>ExUDA34[<i>srh-282p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study
<i>ExUDA28[<i>srh-308p::gfp, trx-1p::DsRedm, rol-6(gf)</i>]</i>	–	This study

<i>Ex[sri-21p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-4p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA27[sri-49p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA45[srd-19p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-21p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-22p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-27p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA42[srd-40p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA39[srd-46p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA40[srd-48p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA48[srd-49p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-50p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-55p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-58p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-60p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-71p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srd-74p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srg-32p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srg-37p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[sre-39p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>ExUDA29[srx-19p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[sra-13p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srt-66p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srt-72p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[dmsr-8p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srh-40p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srh-40 full length genomic gene::gfp::srh-40 3'UTR, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[srg-56p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[str-52p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>Ex[str-89p::gfp, trx-1p::DsRedm, rol-6(gf)]</i>	–	This study
<i>str-45(chr2)</i>	KHR44	This study
<i>str-45(chr3)</i>	KHR45	This study
<i>str-92(chr4)</i>	KHR61	This study
<i>str-92(chr6)</i>	KHR63	This study
<i>srd-48(chr7)</i>	KHR70	This study
<i>srd-48(chr8)</i>	KHR71	This study
<i>srd-19(chr13)</i>	KHR98	This study
<i>srd-19(chr14)</i>	KHR99	This study
<i>srh-244(chr15)</i>	KHR100	This study
<i>srh-244(chr16)</i>	KHR102	This study
<i>srt-72(chr18)</i>	KHR090	This study
<i>srh-122(chr31)</i>	KHR123	This study
<i>str-43(chr39)</i>	KHR151	This study
<i>srg-56(chr40)</i>	KHR152	This study
<i>srh-40(chr41)</i>	KHR153	This study
<i>srh-40(chr42)</i>	KHR154	This study
<i>str-52(chr43)</i>	KHR155	This study

<i>str-89(chr44)</i>	KHR156	This study
<i>str-89(chr45)</i>	KHR157	This study
<i>glr-3(tm6403)</i> backcross 3 times	KHR197	This study
<i>Ex[srh-220p::DsRedm, rol-6(gf)]</i>	–	This study
<i>srh-40(chr42); Ex[ges-1p::NLS::gfp, AIYp::gfp, pBluescript II SK+]</i>	–	This study
<i>srh-40(chr42); Ex[srh-40p::srh-40 cDNA, ges-1p::NLS::gfp, AIYp::gfp, pBluescript II SK+]</i>	–	This study
<i>srh-40(chr42); Ex[srh-220p::srh-40 cDNA, ges-1p::NLS::gfp, AIYp::gfp, pBluescript II SK+]</i>	–	This study
<i>srh-40(chr42); Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>srh-40(chr42); Ex[srh-220p::srh-40cDNA, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[srh-220p::egl-30sense, srh-220p::egl-30anti-sense, AIYp::GFP, ges-1p::NLS GFP, pBluescript II SK+]</i>	–	This study
<i>Ex[srh-220p::egl-30(Q205L) cDNA, AIYp::gfp, ges-1p::NLS::gfp, pBluescript II SK+]</i>	–	This study
<i>Ex[srh-220p::egl-30sense, srh-220p::egl-30anti-sense, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[srh-220p::egl-30(Q205L) cDNA, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[srh-220p::srh-40, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[srh-220p::srh-40, sre-1p::yc3.60, srh-220p::egl-30sense, srh-220p::egl-30anti-sense, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[sre-1p::yc4.12, rol-6(gf)]</i>	–	This study
<i>ocr-2(ak47); Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	Ohnishi et al. 2020
<i>srh-40(chr41); ocr-2(ak47); Ex[srh-220p::srh-40, sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>srh-40(chr41); osm-9(ky10) ocr-2(ak47); ocr-1(ak46); Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>srh-40(chr41); osm-9(ky10) ocr-2(ak47); Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>goa-1(n1134); Ex[sre-1p::yc3.60, rol-6(gf)]</i>	–	This study
<i>Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>glr-3(tm6403); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>goa-1(n1134); gpa-3(pk35); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>gpa-3(pk35); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>goa-1(n1134); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>goa-1(n1134); gpa-3(pk35); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>gpa-3(pk35); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>goa-1(n1134); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>gcy-5(tm897); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>gcy-5(tm897); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>tax-4(p678); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study
<i>tax-4(p678); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>egl-30(ad806); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP]</i>	–	This study
<i>egl-30(ad806); Ex[flp-6p::CeG-CaMP8, gcy-5p::tagRFP, gcy-5p::srh-40]</i>	–	This study

CGC: The *Caenorhabditis* Genetic Center

NBRP: The National BioResource Project (Japan)

Supplementary Table 3. The mutation of mutants constructing by CRISPR/Cas9.

Strain	Allele	Gene	Mutation
KHR44	<i>chr2</i>	<i>str-45</i>	Insertion of 5 bp containing stop codon and NheI restriction site in 1 st exon
KHR45	<i>chr3</i>	<i>str-45</i>	Deletion of 243 bp in 1 st exon.
KHR61	<i>chr4</i>	<i>str-92</i>	Deletion of 527 bp and insertion of 3 bp in 3 rd and 4 th exon.
KHR62	<i>chr5</i>	<i>str-92</i>	Deletion of 626 bp and insertion of 96 bp in 3 rd , 4 th and 5 th exon.
KHR70	<i>chr7</i>	<i>srd-48</i>	Insertion of 4 bp NheI restriction site containing stop codon in 3 rd exon.
KHR71	<i>chr8</i>	<i>srd-48</i>	Insertion 16 bp and stop codon (TGA) in 1 st exon.
KHR98	<i>chr13</i>	<i>srd-19</i>	Deletion of 502 bp in 2 nd and 3 rd exon.
KHR99	<i>chr14</i>	<i>srd-19</i>	Deletion of 299 bp in 2 nd and 3 rd exon.
KHR100	<i>chr15</i>	<i>srh-244</i>	Insertion of 5 bp containing stop codon and NheI restriction site in 2 nd exon.
KHR102	<i>chr16</i>	<i>srh-244</i>	Deletion of about 400 bp in 2 nd and 3 rd exon.
KHR090	<i>chr18</i>	<i>srt-72</i>	Insertion of 3 bp, which induce non-sense mutation.
KHR123	<i>chr31</i>	<i>srh-122</i>	Deletion of 4 bp and insertion 26 bp in 1 st exon.
KHR151	<i>chr39</i>	<i>str-43</i>	Insertion of 93 bp containing stop codon in 1 st exon.
KHR152	<i>chr40</i>	<i>srg-56</i>	Insertion of 2 bp in 1 st exon, which induce non-sense mutation.
KHR153	<i>chr41</i>	<i>srh-40</i>	Insertion of 233 bp in 1 st exon, which induce non-sense mutation.
KHR154	<i>chr42</i>	<i>srh-40</i>	Insertion of 23 bp in 1 st exon, which induce frame shift and non-sense mutation.
KHR155	<i>chr43</i>	<i>str-52</i>	Insertion 74 bp in 1 st exon, which induce non-sense mutation.
KHR156	<i>chr44</i>	<i>str-89</i>	Insertion of 113 bp in 1 st exon, which induce non-sense mutation.
KHR157	<i>chr45</i>	<i>str-89</i>	Deletion of 134 bp containing start codon in 1 st exon.

Supplementary Table 4. The plasmids in this paper

Name	Construct	Name	Construct
Expression analysis		For CRISPR/Cas9 system	
pMAI1	<i>str-45 full length genomic gene::gfp</i>	pKOH020	<i>str-45 gRNA_no.5</i>
pMAI3	<i>srh-308p::gfp</i>	pKOH010	<i>dpy-10 gRNA</i>
pMAI4	<i>sri-49p::gfp</i>	pKOH021	<i>str-92 gRNA no.2</i>
pMAI5	<i>srx-19p::gfp</i>	pKOH022	<i>str-92 gRNA no.3</i>
pUDA23	<i>str-214p::gfp</i>	pKOH019	<i>srd-48 gRNA</i>
pUDA24	<i>srh-282p::gfp</i>	pKOH034	<i>srd-19 gRNA no.1</i>
pUDA27	<i>srd-46p::gfp</i>	pKOH055	<i>srd-19 gRNA no.2</i>
pUDA28	<i>srd-48p::gfp</i>	pKOH046	<i>srh-208 gRNA</i>
pUDA30	<i>srd-40p::gfp</i>	pKOH047	<i>srh-244 gRNA</i>
pUDA31	<i>srd-50p::gfp</i>	pMIU014	<i>srt-72 gRNA</i>
pUDA32	<i>srh-61p::gfp</i>	pMIU015	<i>srh-122 gRNA no.1</i>
pUDA37	<i>srd-19p::gfp</i>	pMIU016	<i>srh-122 gRNA no.2</i>
pUDA39	<i>srd-49p::gfp</i>	pMIU017	<i>srh-40 gRNA</i>
pMIU001	<i>srd-71p::gfp</i>	pMIU018	<i>srg-56 gRNA</i>
pMIU002	<i>srd-74p::gfp</i>	pMIU019	<i>str-43 gRNA</i>
pMIU003	<i>str-113p::gfp</i>	pMIU020	<i>str-52 gRNA</i>
pMIU004	<i>srd-21p::gfp</i>	pMIU021	<i>str-89 gRNA</i>
pMIU005	<i>srd-22p::gfp</i>	For analysis of <i>srh-40</i> in <i>C. elegans</i>.	
pMIU006	<i>srh-122p_gfp</i>	pKOH141	<i>srh-220p::DsRedm</i>
pMIU007	<i>sra-13p::gfp</i>	pMIU022	<i>srh-40p::gfp</i>
pMIU008	<i>srt-66p::gfp</i>	pMIU066	<i>srh-40 full length genomic gene::gfp::srh-40 3'UTR</i>
pMIU009	<i>srt-72p::gfp</i>	pMIU028	<i>srh-220p::srh-40cDNA</i>
pMIU010	<i>dmsr-8p::gfp</i>	pMIU029	<i>srh-40p::srh-40cDNA</i>
pKOH003	<i>srd-4p::gfp</i>	pMIU030	<i>gcy-5p::srh-40cDNA</i>
pKOH004	<i>srg-37p::gfp</i>	pKOH241	<i>pPD95.75-egl30(Q205L)</i>
pKOH011	<i>str-92p::gfp</i>	pKOH244	<i>srh-220p::egl-30(Q205L)cDNA</i>
pKOH012	<i>str-227p::gfp</i>	pKOH252	<i>srh-220p::egl-30 anti-sense</i>
pKOH013	<i>str-253p::gfp</i>	pKOH253	<i>srh-220p::egl-30 sense</i>
pKOH014	<i>str-243p::gfp</i>	pMIU156	<i>sre-1p::yc4.12</i>
pKOH015	<i>srh-49p::gfp</i>	Ca²⁺ imaging in S2R+ cells.	
pKOH023	<i>srd-55p::gfp</i>	pMIU097	<i>pMT srh-40</i>
pKOH024	<i>srd-60p::gfp</i>	pMIU136	<i>pAc5.1 osm-9::T2A::ocr-2</i>
pKOH026	<i>str-226p::gfp</i>	pMIU133	<i>pMT egl-30::T2A::mCherry</i>
pKOH027	<i>srd-27p::gfp</i>	pKOH297	<i>pAc5.1 tax-2::T2A::tax-4</i>
pKOH029	<i>srh-179p::gfp</i>	pMIU146	<i>pAc5.1 goa-1::T2A::mCherry</i>

pKOH030	<i>srh-206p::gfp</i>
pKOH032	<i>srh-210p::gfp</i>
pKOH035	<i>srh-227p::gfp</i>
pKOH036	<i>srh-236p::gfp</i>
pKOH037	<i>srh-244p::gfp</i>
pKOH038	<i>srh-245p::gfp</i>
pKOH039	<i>srh-247p::gfp</i>
pKOH040	<i>srh-248p::gfp</i>
pKOH041	<i>srh-251p::gfp</i>
pKOH042	<i>srh-275p::gfp</i>
pKOH043	<i>sre-39p::gfp</i>
pKOH044	<i>sri-21p::gfp</i>
pKOH045	<i>srg-32p::gfp</i>
pMIU023	<i>srg-56p::gfp</i>
pMIU024	<i>str-52p::gfp</i>
pMIU025	<i>str-89p::gfp</i>

pKOH233	<i>pAc5.1-mCherry</i>
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