

Supplementary Information

Kalium channelrhodopsins effectively inhibit neurons

Stanislav Ott¹, Sangyu Xu², Nicole Lee¹, Ivan Hong Hee Kean¹, Jonathan Anns^{2,3}, Danesha Devini Suresh¹, Zhiyi Zhang¹, Xianyuan Zhang¹, Raihanah Harion⁴, Weiying Ye⁵, Vaishnavi Chandramouli⁴, Suresh Jesuthasan⁴, Yasunori Saheki⁴, Adam Claridge-Chang^{1,2,4,6}

1. Program in Neuroscience and Behavioral Disorders, Duke-NUS Medical School, Singapore
2. Institute for Molecular and Cell Biology, A*STAR Agency for Science, Technology and Research, Singapore
3. School of Biological Sciences and Institute for Life Sciences, University of Southampton, UK
4. Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
5. Department of Pharmacy, National University of Singapore, Singapore
6. Correspondence. claridge-chang.adam@duke-nus.edu.sg

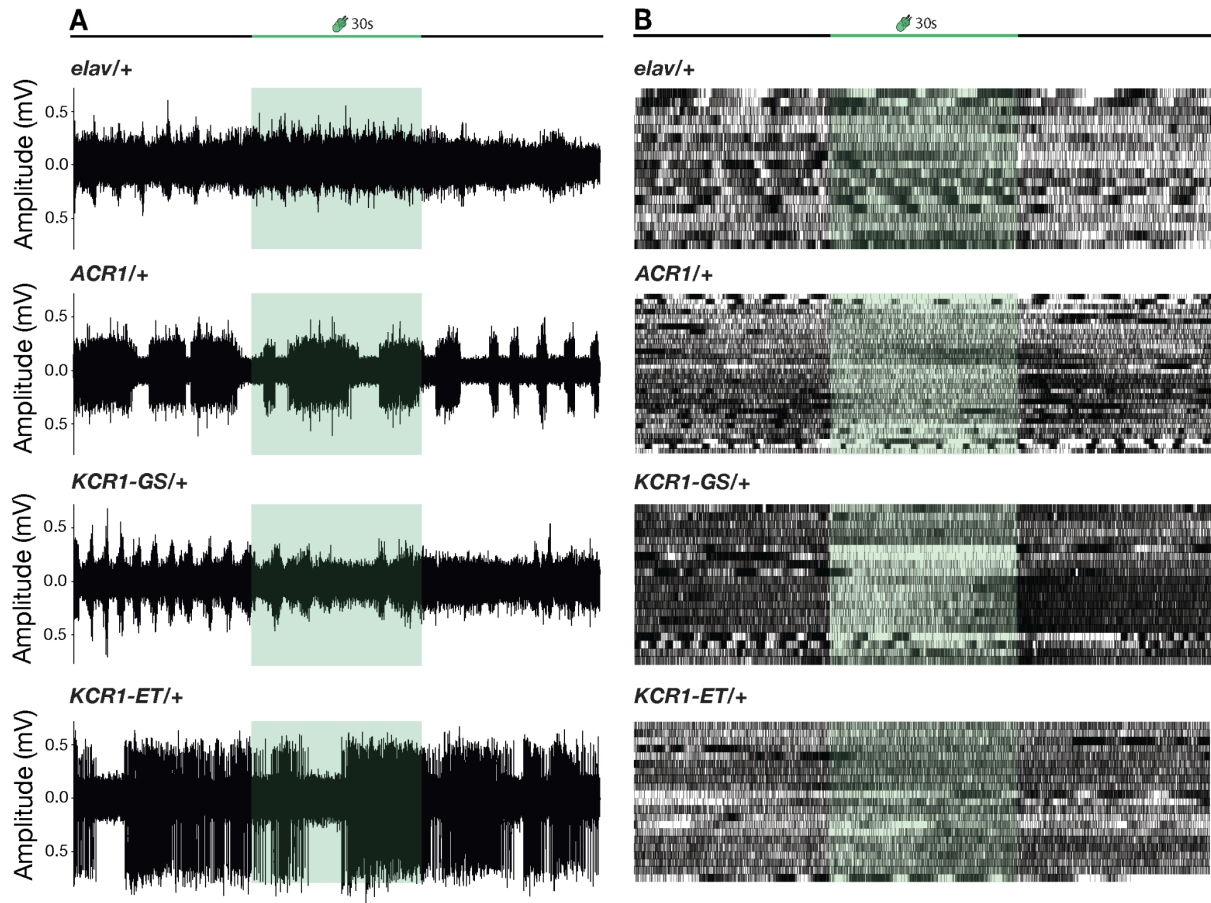


Figure S1: Effects of illumination on larval abdominal nerves in control genotypes.

Representative recordings (A) and raster summaries (B) of action potential occurrences for sibling control genotypes. Action potentials were recorded from the fly larval abdominal nerve 3. For all representative traces $n = 1$ biologically independent sample over 1 independent experiment. For all raster summaries $n = 3$ biologically independent samples over 3 independent experiments. Source data are provided as a Source Data file.

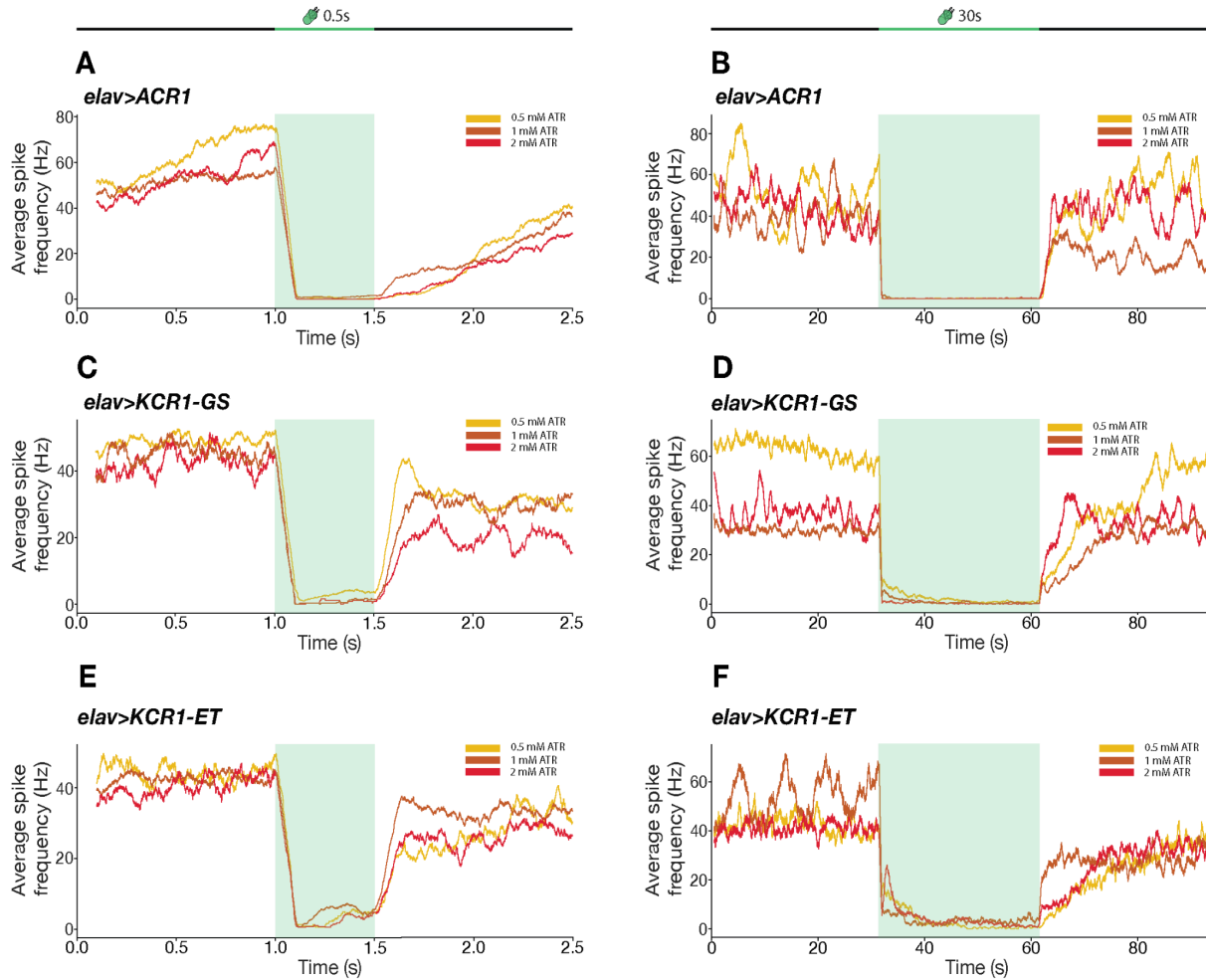


Figure S2: Inhibition of spontaneous spikes at different ATR concentrations

Average spike frequency comparisons for *Drosophila* larvae reared on food containing different ATR concentrations. Experiments with 0.5 s optogenetic actuation ($40 \mu\text{W}/\text{mm}^2$) (left: **A, C, E**) and 30s actuation ($40 \mu\text{W}/\text{mm}^2$) periods (right: **B, D, F**) are shown. ACR1-induced silencing was slightly more potent than KCR-induced silencing at all ATR concentrations. Action potential recovery after 0.5 s actuation was slower in ACR-expressing larvae as compared to KCR-expressing larvae. For all genotypes and conditions $n = 3$ biologically independent samples over 3 independent experiments. Additional statistical information for all panels is presented in Supplementary Dataset 1. Source data are provided as a Source Data file.

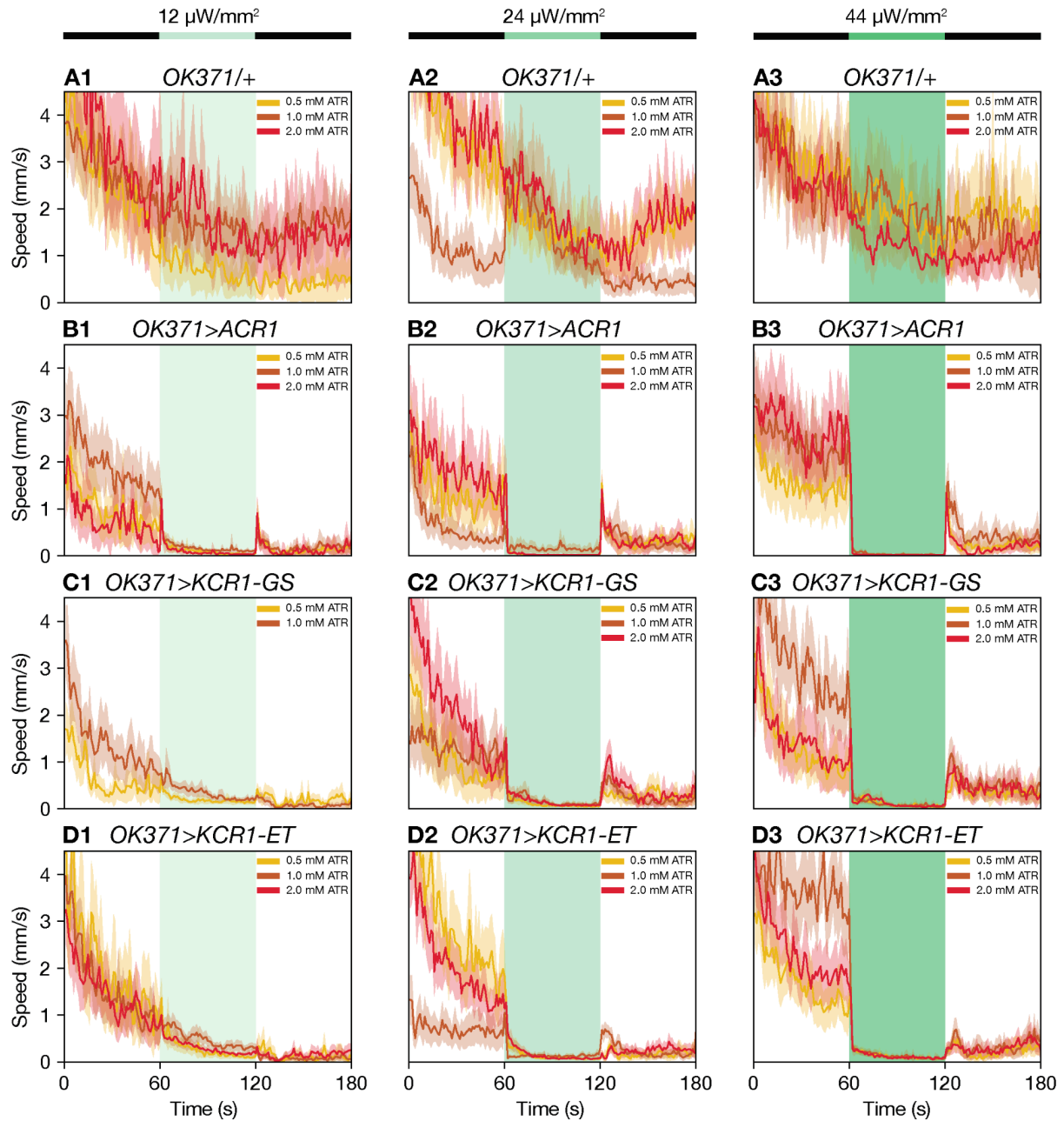


Figure S3: Walking inhibition at different ATR concentrations and light intensities

Locomotor impairment comparisons for different ATR and light dosages. Each line plot shows the average performance with a 95% CI ribbon. The schematic (top) and the green rectangle in each panel indicates the time of illumination.

A: Locomotor activity of the driver control before, during and after light actuation.

A1. *OK371/+* 0.5 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ $n = 39$ biologically independent animals over 2 independent experiments. *OK371/+* 1 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ $n = 66$ biologically independent animals over 3 independent experiments. *OK371/+* 2 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ $n = 33$ biologically independent animals over 2 independent experiments.

OK371/+ 0.5 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 59 biologically independent animals over 3 independent experiments. *OK371/+* 1 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 76 biologically independent animals over 3 independent experiments.

A2. *OK371/+* 2 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 45 biologically independent animals over 2 independent experiments. *OK371/+* 0.5 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 39 biologically independent animals over 2 independent experiments.

A3. *OK371/+* 1 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 36 biologically independent animals over 2 independent experiments. *OK371/+* 2 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 68 biologically independent animals over 3 independent experiments.

B. Locomotor activity of *OK371>ACR1* flies before, during and after light actuation.

B1. *OK371>ACR1* 0.5 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 41 biologically independent animals over 2 independent experiments. *OK371>ACR1* 1 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 65 biologically independent animals over 3 independent experiments. *OK371>ACR1* 2 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 20 biologically independent animals over 1 independent experiment.

B2. *OK371>ACR1* 0.5 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 44 biologically independent animals over 2 independent experiments. *OK371>ACR1* 1 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 72 biologically independent animals over 2 independent experiments. *OK371>ACR1* 2 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 44 biologically independent animals over 2 independent experiments.

B3. *OK371>ACR1* 0.5 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 66 biologically independent animals over 3 independent experiments. *OK371>ACR1* 1 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 70 biologically independent animals over 3 independent experiments. *OK371>ACR1* 2 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 62 biologically independent animals over 3 independent experiments.

C. Locomotor activity of *OK371>KCR1-GS* flies before, during and after light actuation.

C1. *OK371/KCR1-GS* 0.5 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 47 biologically independent animals over 2 independent experiments. *OK371/KCR1-GS* 1 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 60 biologically independent animals over 3 independent experiments.

C2. *OK371/KCR1-GS* 0.5 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 46 biologically independent animals over 2 independent experiments. *OK371/KCR1-GS* 1 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 50 biologically independent animals over 2 independent experiments. *OK371/KCR1-GS* 2 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 44 biologically independent animals over 2 independent experiments.

C3. *OK371/KCR1-GS* 0.5 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 53 biologically independent animals over 3 independent experiments. *OK371/KCR1-GS* 1 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 44 biologically independent animals over 2 independent experiments. *OK371/-KCR1-GS* 2 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 41 biologically independent animals over 3 independent experiments.

D. Locomotor activity of *OK371>KCR1-ET* flies before, during and after light actuation.

D1. *OK371>KCR1-ET* 0.5 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 22 biologically independent animals over 1 independent experiment. *OK371>KCR1-ET* 1 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 48

biologically independent animals over 3 independent experiments. *OK371>KCR1-ET* 2 mM ATR, 12 $\mu\text{W}/\text{mm}^2$ n = 54 biologically independent animals over 3 independent experiments.

D2. *OK371>ACR1* 0.5 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 44 biologically independent animals over 2 independent experiments. *OK371>KCR1-ET* 1 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 58 biologically independent animals over 3 independent experiments. *OK371>KCR1-ET* 2 mM ATR, 24 $\mu\text{W}/\text{mm}^2$ n = 66 biologically independent animals over 3 independent experiments. *OK371>KCR1-ET* 0.5 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 63 biologically independent animals over 3 independent experiments.

D3. *OK371>KCR1-ET* 1 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 52 biologically independent animals over 3 independent experiments. *OK371>KCR1-ET* 2 mM ATR, 44 $\mu\text{W}/\text{mm}^2$ n = 66 biologically independent animals over 3 independent experiments. Additional statistical information for all panels is presented in Supplementary Dataset 1. Source data are provided as a Source Data file.

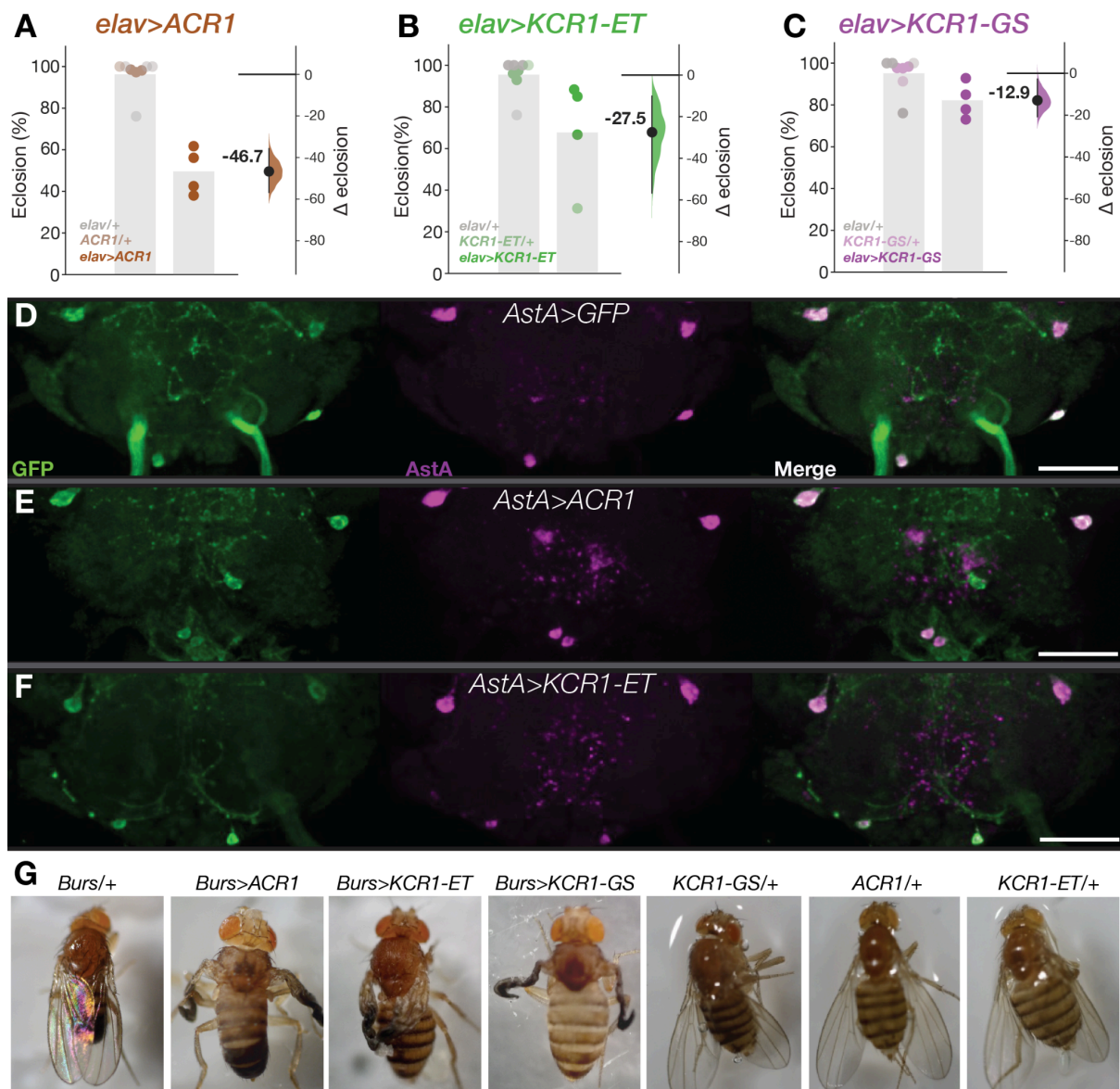


Figure S4: Toxicity is lower with KCR1 transgenes

A-C. Developmental lethality of dark-raised pan-neuronal *elav>ACR1* (**A**), *elav>KCR1-ET* (**B**) and *elav>KCR1-GS* (**C**) flies compared to genotypic controls. In each panel the left-hand axes display the percent of eclosed flies relative to the number of eggs laid. Each dot represents one experimental iteration. The right-hand axes show the $\Delta\%$ between control and test conditions. The same *elav-Gal4/+* control flies were reused in each panel. Error bars represent the 95% CI. For ACR1 genotypic controls $n = 514$ biologically independent animals over 4 independent experiments. *Elav>ACR1* $n = 299$ biologically independent animals over 4 independent experiments. For KCR1-ET genotypic controls $n = 462$ biologically independent animals over 4 independent experiments. *Elav>KCR1-ET* $n = 286$ biologically independent animals over 4 independent experiments. For KCR1-GS genotypic controls $n = 497$ biologically independent animals over 4 independent experiments. *Elav>KCR1-GS* $n = 259$ biologically independent animals over 4 independent experiments.

D-F. Representative confocal images of fly brains expressing GFP or the respective opsin with *AstA-Gal4* in four cells in the subesophageal zone after six days of illumination. Anti-GFP is shown in green and anti-AstA in magenta. Scale bar = 50 μm . For each genotype $n = 1$ biologically independent sample over 3 independent experiments.

G. Representative images of flies expressing the respective opsin with *Burs-Gal4* and control genotypes. Flies were exposed to green light ($31 \mu\text{W}/\text{mm}^2$) for all four days of metamorphosis. All of the *Burs>ACR1*, *Burs>KCR1-GS* and *Burs>KCR1-ET* flies displayed wing-expansion failure; the wings developed normally in all controls. For each genotype $n = 100$ biologically independent animals over 2 independent experiments. Additional statistical information is presented in Supplementary Dataset 1. Source data are provided as a Source Data file.

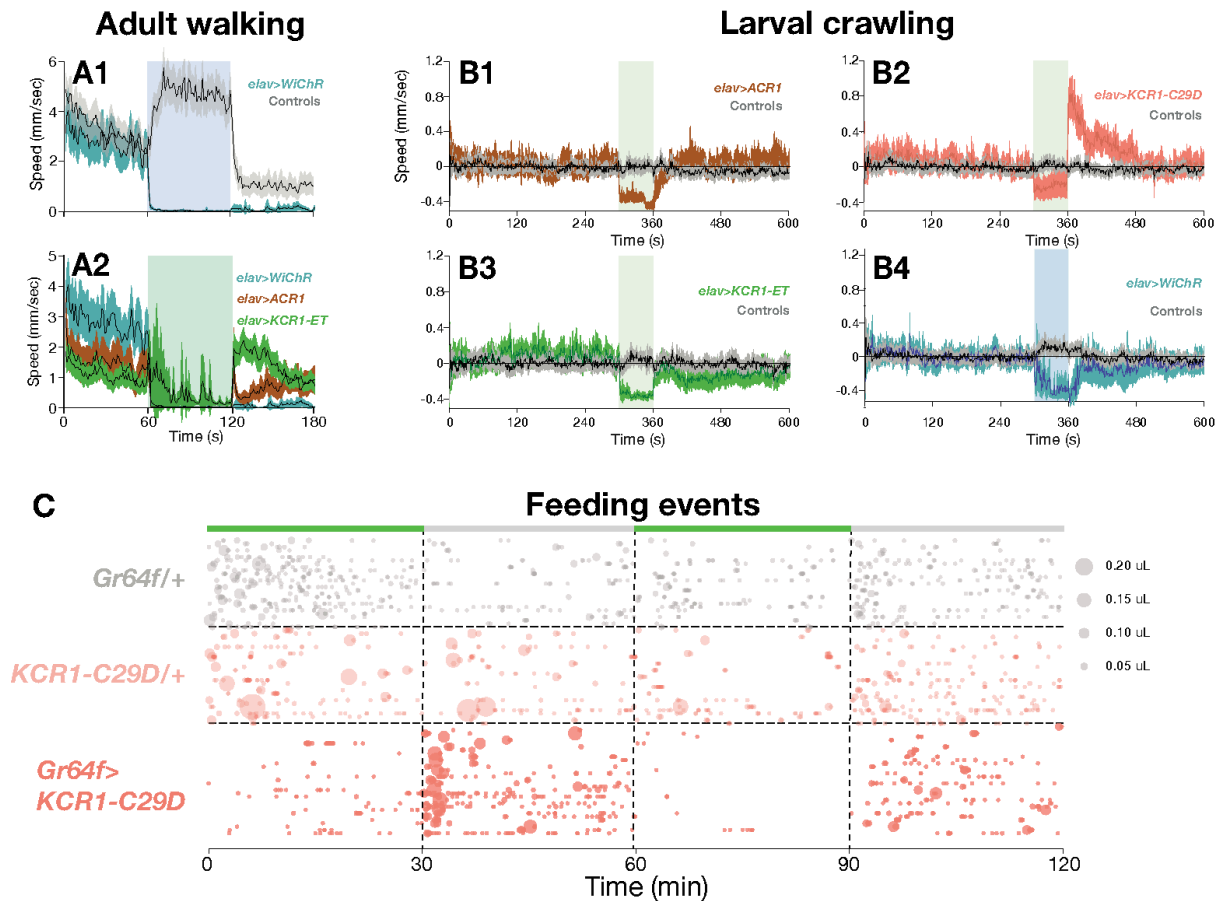


Figure S5: Inhibition of locomotion and feeding by KCR1-C29 and WiChR

A. Horizontal speed of *elav>WiChR* flies and genotypic controls before, during and after light actuation (top). Speed of the same *elav>WiChR* flies and those that express *elav>ACR1* and *elav>KCR1-ET* (bottom). Error bars represent the 95% CI. Green illumination intensity was $24 \mu\text{W}/\text{mm}^2$. Blue illumination was $24 \mu\text{W}/\text{mm}^2$. For *WiChR* genotypic controls $n = 126$ biologically independent animals over 6 independent experiments. *Elav>WiChR* $n = 55$ biologically independent animals over 3 independent experiments. *Elav>KCR1-ET* $n = 66$ biologically independent animals over 4 independent experiments. *Elav>ACR1* $n = 64$ biologically independent animals over 4 independent experiments.

B. Locomotor assessment of *Drosophila* larvae before, during and after light illumination. Each respective opsin was expressed pan-neuronally with *elav-Gal4*. All crawling activity stopped upon light exposure in larvae expressing *ACR1* (**B1**), *KCR1* variants (**B2-3**) or *WiChR* (**B4**). In addition, larvae expressing *elav>KCR1-C29D* displayed curling behaviour and increased activity immediately after light exposure (**B2**). The *elav>WiChR* larvae displayed an approximately 60 s-long activity recovery delay following light exposure (**B4**). Respective genotypic controls are shown in grey. Error bands show 95% CI. Green light illumination intensity was $92 \mu\text{W}/\text{mm}^2$. Blue light illumination was $27 \mu\text{W}/\text{mm}^2$. *ACR1* genotypic controls $n = 58$ biologically independent animals over 2 independent experiments. *Elav>ACR1* $n = 39$ biologically independent animals over 3 independent experiments. *KCR1-C29D* genotypic controls $n = 73$ biologically independent animals over 5 independent

experiments. *Elav>KCR1-C29D* n = 35 biologically independent animals over 2 independent experiments. KCR1-ET genotypic controls n = 60 biologically independent animals over 3 independent experiments. *Elav>KCR1-ET* n = 31 biologically independent animals over 2 independent experiments. WiChR genotypic controls n = 57 biologically independent animals over 2 independent experiments. *Elav>WiChR* n = 26 biologically independent animals over 3 independent experiments.

C. Feeding events of *Gr64f>KCR1-C29D* flies and genotypic controls in the presence and absence of light (24 $\mu\text{W}/\text{mm}^2$). Green bars indicate illumination epochs; the area of each bubble indicates volume of that feed event. KCR1-C29D genotypic controls n = 56 biologically independent animals over 3 independent experiments. *Gr64f>KCR1-C29D* n = 33 biologically independent animals over 3 independent experiments. Additional statistical information is presented in Supplementary Dataset 1. Source data are provided as a Source Data file.

Supplementary methods

C. elegans constructs

gBlocks (IDT) containing codon optimized cDNAs encoding either one of the opsins (ACR1, KCR1-ET, KCR1(GS) or KCR2-ET) that was tagged with YFP at its C-terminus (with three synthetic introns to enhance expression) was PCR amplified and ligated in the KpnI and EcoRI sites of *sdf-9P::mCherry* vector using the following primer sets:

ACR1-f and ACR-1-YFP-r for *sdf-9p::ACR1::YFP*; KCR-1-f and KCR-1-YFP-r for *sdf-9p::KCR1::YFP*; KCR-1-GS-f and KCR-1-GS-YFP-r for *sdf-9p::KCR1(GS)::YFP*; KCR-1-f and KCR-1-YFP-r for *sdf-9p::KCR2::YFP*.

Genomic DNA corresponding to pan-neuronal promoter (*snt-1p*) was PCR amplified from *C. elegans* genome using the following primers, *snt-1P-FseI-F* and *snt-1P-AscI-R*, and then ligated in the FseI and AscI sites, to generate *snt-1p::ACR1::YFP*, *snt-1p::KCR1::YFP*, *snt-1p::KCR1(GS)::YFP*, and *snt-1p::KCR2::YFP*. The plasmid was co-injected with *elt-2::mCherry* at 10ng/μl each into the gonads of adult N2 hermaphrodites by microinjector (InjectMan 4) to establish transgenic strains.

Live worm imaging by confocal fluorescence microscopy

For imaging experiments, L4 hermaphrodite worms were transferred to a glass slide and immobilized on 3% agarose pads using 2-3μl 1mg/μl levamisole diluted in M9 buffer. Images were then captured under a 100x objective. Multiple transgenic lines of each transgene were examined for fluorescent expression and localization patterns. Spinning disc confocal (SDC) microscopy was performed on a setup built around a Nikon Ti2 inverted microscope equipped with a Yokogawa CSU-W1 confocal spinning head, a Plan-Apo objective (100 × 1.45 NA), and a back-illuminated sCMOS camera (Prime 95B; Photometrics). Excitation light was provided by 488 nm/150 mW (Coherent) (for YFP) (power measured at optical fiber end) through DPSS laser combiner (iLAS system; Gataca systems). All image acquisition and processing were controlled by MetaMorph (Molecular Device) software. Images were acquired with exposure times in the 400–500 ms range.

Table S2: C. elegans genotypes and DNA constructs

Strain genotype	ID
<i>C. elegans: yasEX257 [snt-1p::ACR1::YFP (10ng/ul); elt-2P::mCherry (10ng/ul)]</i>	SAH743
<i>C. elegans: yasEX259 [snt-1p::KCR1::YFP (10ng/ul); elt-2P::mCherry (10ng/ul)]</i>	SAH745
<i>C. elegans: yasEX260 [snt-1p::KCR2::YFP (10ng/ul); elt-2P::mCherry (10ng/ul)]</i>	SAH746
<i>C. elegans: yasEX261 [snt-1p::KCR1(GS)::YFP (10ng/ul); elt-2P::mCherry (10ng/ul)]</i>	SAH747

Recombinant DNA constructs	ID
<i>snt-1p::ACR1::YFP</i>	RAI77
<i>snt-1p::KCR1::YFP</i>	RAI78
<i>snt-1p::KCR1(GS)::YFP</i>	RAI79
<i>snt-1p::KCR2::YFP</i>	RAI80
<i>sdf-9p::mCherry</i>	JB164

Recombinant DNA sequences	ID
acccttgGCTAGCgtcgacGGTACCggtagaaaaaATGAGTTCCATCACGTGC GACCCTGCCATCTACGGCGAGTGGTCCCGAGAGAACCAGTTCTGCGTAGAGAA GTCCTTGATAACCCTGGATGGAATTAAGTACGTCCAGCTGGTGATGGCAGTCG TGTCAGCATGTCAAGTCTTTgtaagtttaaacagttcgggtactaactaaccat acataatttaaatcttcagTTCATGGTGACAAGAGCACCGAAGGTTCTTGGA AGCGATTTATTTGCCGACCACCGAAATGATTACCTATTCATTGGCCTTTACGG GAAATGGTTACATTCGAGTCGCTAATGGCAAGTATCTTCCGTGGGCTCGAATG GCATCTTGCTTTGCACCTGCCCTATAATGCTTGGACTTGATCCAACATGGC CTTAGTAAAGTACAAATCAATCCCGCTTAACCCTATGATGATTGCCGCGTCTT CTATATGTACCGTATTCGGAATAACGGCTTCCGTAGTATTGGATCCTCTTCAT GTCTGGCTGTACTGCTTTATTTTCGAGTATATTCTTTATATTTGAAATGGTCGT AGCGTTTGCTATATTTGCTATCACGATCCACGATTTTCAGACGATAGGATCAC CAATGTCGCTTAAAGTGGTTGAAAGACTCAAACCTTATGCGAAgtaagtttaaa catgattttactaactaactaatctgattttaaattttcagTTGTATTCTACGT TTCCTGGATGGCCTATCCTATTCTCTGGTCATTCTCTCCACGGGTGCTTGTA TTATGTGCGGAGAACACTTCTTCGGTTTTATATCTTTTGGGCGATGCTCTCTGT AAAAACACGTATGGTATTCTGCTTTGGGCTACTACTTGGGGCCTCCTTAACGG CAAATGGGACCGAGACTATGTTAAGGGCCGAAACGTGGATGGAGCCGCCGCCG TGAGCAAGGGAGAGGAGCTGTTACCGGAGTGGTGCCCATCCTGGTGGAGCTG GATGGCGACGTGAACGGCCACAAGTTCTCGGTGAGCGGAGAGGGAGAGGGCGA CGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGC CCGTGCCGTGGCCAACCCTGGTGACCACCTTCGGCTACGGCCTGCAGTGCTTC GCCCGCTACCCAGATCACATGAAGCAGCAGACTTCTTCAAGTCGGCCATGCC GGAGGGATACGTGCAGGAGCGCACCATCTTCTTCAAGGATGACGGCAACTACA AGACCCGCGCCGAGGTGAAGTTCGAGGGCGATACCCTGGTGAACCGCATCGAG CTGAAGGGCATCGATTTCAAGGAGGACGGCAATATCCTGGGCCACAAGCTGGA GTACAACCTACAATAGCCACAACGTGTACATCATGGCCGACAAGCAGAAGAACG	RAI77_gBlock_A CR1

GCATCAAGGTTAATTTCAAGATCCGCCACAATATCGAGGATGGCTCCGTGCAG CTGGCCGACCACTACCAGCAGAACACCCCGATTGGCGATGGACCCGTGCTGCT gtaagtttaaacatatataactaaccctgattattttaaatTTTcagGC CAGACAATCACTACCTGAGCTACCAGTCCGCCCTGTCTGAAGGACCCCAACGAG AAGCGCGACCACATGGTGCTGCTGGAGTTTGTGACCGCCGCCGGAATTACCTT GGGAATGGACGAGCTGTATAAGttctgctacgagaacgaggtgTAAGAATTc aactgagcgccggtcgcta	
acccttgGCTAGCgtcgacGGTACCggtagaaaaaATGCCATTCTACGACAGT AGACCGCCGGAAGGTTGGCCAAAGGTTCCATCAATGATATGGACTACCCGCT CCTCGGTTCCATCTGCGCCGTCTGTTGCGTTTTCTGTTGGCGGGAAGTGGTATAT GGATGCTGTACCGTTTAGATCTGGGTATGGGATACTCTTGTAACCGTACAAA TCGGGCCGAGCGCCAGAGGTCAATTCTCTGTCCGGAATTATATGTCTGCTTTG CGGCACGATGTATGCGGCGAAATCATTGATTTCTTTGACGGCGGAGGAACTC CTTTCTCCCTGAACTGGTATTGGTATCTGGATTATGTGTTCACTTGTCCGCTG CTGATCTTAGATTTTTCGTTTACATTGGACCTCCACATAAGATTAGATACTT CTTCGCTGTTTTTTTACCCCTCTGGTGCGGCGTTGCGGCGTTTGTACACCGA GTGCATACCGTTTCGCGTACTACGCATTGGGATGCTGCTGGTTCACCCATTC GCCCTCTCCCTTATGCGACACGTGAAAGAGCGATATTTAGTATACCCGCCGAA GTGTCAGAGATGGCTCTTCTGGGCATGTGTGATATTCTTCGGTTTTTTGGCCGA TGTTTTCCGATTTTATTCATATTCAGTTGGTTGGGTACTGGCCATATATCTCAA CAGGCTTTCTACATAATCCACGCATTCTTGGACTTAACGTGTAAATCGATTTT TGGCATATTGATGACTGTATTTTCGTCTCGAGTTAGAGGAGCACACGGAAGTGC AAGGACTGCCTCTTAATGAACCAGAAACCTTATCGGCTGCTGCGAAGTCTCGT ATCACATCAGAGGGTGAATACATACCTCTGGATCAAATTGACATAAACGTTgc cgccgccaagagcaggatcaccagcgagggcgagtacatccccctggaccaga tcgacatcaacgtgGTGAGCAAGGGAGAGGAGCTGTTACCGGAGTGGTGCCC ATCCTGGTGGAGCTGGATGGCGACGTGAACGGCCACAAGTTCTCGGTGAGCGG AGAGGGAGAGGGCGACGCCACCTACGGCAAGCTgtaagtttaaacagttcggt actaactaaccatacatatTTTaaatTTTcagGACCCTGAAGTTCATCTGCACC ACCGGCAAGCTGCCCCTGCGGTGGCCAACCCTGGTGACCACCTTCGGCTACGG CCTGCAGTGCTTCGCCCGCTACCCAGATCACATGAAGCAGCACGACTTCTTCA AGTCGGCCATGCCGAGGGATACGTGCAGGAGCGCACCATCTTCTTCAAGGAT GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGATACCCTGGT GAACCGCATCGAGCTGAAGGGCgtaagtttaaacatgattttactaactaact aatctgattttaaatTTTcagATCGATTTCAAGGAGGACGGCAATATCCTGGGC CACAAGCTGGAGTACAATACAATAGCCACAACGTGTACATCATGGCCGACAA GCAGAAGAACGGCATCAAGGTTAATTTCAAGATCCGCCACAATATCGAGGATG GCTCCGTGCAGCTGGCCGACCACTACCAGCAGAACACCCCGATTGGCGATGGA CCCGTGCTGCTGCCAGACAATCACTACCTGAGCTACCAGTCCGCCCTGTCTGAA GGACCCCAACGAGAAGCGCGACCACATGGTGcgttaagtttaaacatatata ctaactaaccctgattatTTTaaatTTTcagTGCTGGAGTTTGTGACCGCCGCC	RAI321_gBlock_ KCR1

GGAATTACCCTGGGAATGGACGAGCTGTATAAGttctgctacgagaacgaggtgTGAGAATTCcaactgagcgccggtcgcta	
acccttgGCTAGCgtcgacGGTACCggtagaaaaaATGCCTTTTTACGATTCA CGTCCTCCGGAAGGCTGGCCTAAGGGCTCCATTAACGATATGGACTATCCGCT GTTGGGCTCAATTTGCGCTGTATGTTGCGTCTTCGTCGCTGGATCGGGTATCT GGATGCTGTATCGACTTGATTTAGGTATGGGCTATTCCTGTAAGCCGTATAAA TCAGGACGAGCACCGGAAGTGAAGTCTCTTTTCAGGCATAATATGTCTCCTTTG TGGTACTATGTACGCCGCAAGTCATTGATTTTTTCGACGGAGGCGGCACTC CGTTTTTCGCTCAATgtaagtttaaacagttcggtactaactaaccatacatat ttaaatcttcagTGGTACTGGTATTTAGACTATGTATTCACTTGTCCACTGTT AATCCTCGATTTTCGATTACATTGGACCTTCCACACAAGATTCGATACTTTT TTGCTGTTTTCTTAACACTCTGGTGTGGCGTGGCGGCCTTTGTAAGTCCATCT GCGTATCGATTTCGCTTATTACGCACTCGGATGTTGCTGGTTCACACCTTTTGC GCTCAGTCTGATGCGTCACGTTAAAGAGCGATATTTGGTGTATCCTCCTAAGT GCCAACGATGGTTATTCTGGGCCTGTGTGATCTTTTTTCGGCTTTTGGCCTATG TTCCCGATACTCTTTATTTTTTCGTGGCTTGAACCGGCCACATTTTCGAGCA GGCATTTTACATTATTCACGATTCTCGACCTCACTTGTAATCGATCTTTG GAATACTTATGACTGTGTTTAGATTGGAAGTGGAGGAGCATAACCGAAGTACAG GGCTTGCCATTGAATGAACCGGAGACGTTATCCACGGGAGGAGGTGGAGGATC GGGTGGTGGAGGTTACAGGAGGCGGAGGAGTGGCTCTACAGGAGGAGGAGGAG GATCAGGAGgtaagtttaaacatgattttactaactaactaatctgatttaaa ttttcagGAGGAGGATCAGGAGGAGGAGGATCaggaTCAGTGAGCAAGGGAGA GGAGCTGTTACCGGAGTGGTGCCCATCCTGGTGGAGCTGGATGGCGACGTGA ACGGCCACAAGTTCTCGGTGAGCGGAGAGGGAGAGGGCGACGCCACCTACGGC AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCGTGGCC AACCCTGGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCCTACCCAG ATCACATGAAGCAGCAGCACTTCTTCAAGTCGGCCATGCCGGAGGGATACGTG CAGGAGCGCACCATCTTCTTCAAGGATGACGGCAACTACAAGACCCGCGCCGA GGTGAAGTTCGAGGGCGATACCCTGGTGAACCGCATCGAGCTGAAGGGCATCG ATTTCAAGGAGGACGGCAATATCCTGGGCCACAAGCTGGAGTACAACATAAAT AGCCACAACGTGTACATCATGGCCGACAAGCAGAAGAACGGCATCAAGTTAA TTTCAAGATCCGCCACAATATCGAGGATGGCTCCGTGCAGCTGGCCGACCACT ACCAGCAGAACACCCCGATTGGCGATGGACCCGTGCTGCTgtaagtttaaca tatataactaactaaccctgattatttaaatcttcagGCCAGACAATCACTA CCTGAGCTACCAGTCCGCCCTGTCAAGGACCCCAACGAGAAGCGCGACCACA TGGTGCTGCTGGAGTTTGTGACCGCCGCCGAATTACCCTGGGAATGGACGAG CTGTATAAGttctgctacgagaacgaggtgGAATTCcaactgagcgccggtcg cta	RAI322_gBlock_ KCR1(GS)
acccttgGCTAGCgtcgacGGTACCggtagaaaaaATGCCATTCTACGACAGT AGACCGCCGGAAGGTTGGCCACGAGGCTCCGTGAACGACATGGACTATCCGCT TCTGGGCTCAATCTGTGCGATTTCTGCGATAGCTATTGCGGGATCGGGCATAT GGATGTTATATCGATTAGACCTGGGAATGGGCTATTCTTGCAAACCGTATAAA	RAI323_gBlock_ KCR2

TCAGGTCGTGCTCCTGAAGTAAACTCTATTTCCGGAATAGTGTGCCTGCTCTG CGGCACAATGTACGCAGCCAAATCGTTTCGATTTTTTCGACGGTGGTGGAACCTC CATTTTCACTTAACCTGGTACTGGTACTTAGATTATGTGTTTCACCTGTCCTCTG TTGATAGTTGATTTTCGCGTTCCACCCTGGACATTCCACAAAAGCTGAGATACTC CATTGCCGTATTCGTGCTCTGTGGTGTGCTGTAGCGGCGTTTCGCTACACCAT CGGCTTTTCGATTTCGCGTATTATGCGCTCGGTTGCTGTTGGTTCATCCCTCTG TCTCTGTTCTTATACGAGACGTAAAAAAGCGTTACCAGGTTTATCCGCCAAA GTGCCAGCGTCTGCTGTTTTGGGCCTGTGTTGTCTTTTGGATTCTGGCCTT TGTTTTCCATTGCTCTTTATCTTTTCGTGGCAGGGTTCTGGACACATCTCGCGT CAAGCGTATTACATCATCCATGCTTTCCTTGACTTAGTATGCAAGTCTATATT TGGATTTTTGATGACTTTCTTCCGATTAGAATTAGAGGAGCACACAGAAGTAC AGGGTCTCCCACTGAAGGAACCGAAAGTCATGGACGCTGCGGCTAAATCCCGA ATAACGTCTGAAGGAGAGTATATCCATTGGATCAAATTGACATCAACGTAgc cgccgccaagagcaggatcaccagcgaggcgagtacatccccctggaccaga tcgacatcaacgtgGTGAGCAAGGGAGAGGAGCTGTTACCGGAGTGGTGCCC ATCCTGGTGGAGCTGGATGGCGACGTGAACGGCCACAAGTTCTCGGTGAGCGG AGAGGGAGAGGGCGACGCCACCTACGGCAAGCTgtaagtttaaacagttcggt actaactaaccatacatatttaaattttcagGACCCTGAAGTTCATCTGCACC ACCGGCAAGCTGCCCCTGCGGTGGCCAACCCTGGTGACCACCTTCGGCTACGG CCTGCAGTGCTTCGCCCGCTACCCAGATCACATGAAGCAGCACGACTTCTTCA AGTCGGCCATGCCGGAGGGATACGTGCAGGAGCGCACCATCTTCTTCAAGGAT GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGATACCCTGGT GAACCGCATCGAGCTGAAGGGCgtaagtttaaacatgattttactaactaact aatctgattttaaattttcagATCGATTTCAAGGAGGACGGCAATATCCTGGGC CACAAGCTGGAGTACAATACAATAGCCACAACGTGTACATCATGGCCGACAA GCAGAAGAACGGCATCAAGGTTAATTTCAAGATCCGCCACAATATCGAGGATG GCTCCGTGCAGCTGGCCGACCACTACCAGCAGAACACCCCGATTGGCGATGGA CCCGTGCTGCTGCCAGACAATCACTACCTGAGCTACCAGTCCGCCCTGTGCAA GGACCCCAACGAGAAGCGCGACCACATGGTGCgtaagtttaaacatatatata ctaactaaccctgattatttaaattttcagTGCTGGAGTTTGTGACCGCCGCC GGAATTACCCTGGGAATGGACGAGCTGTATAAGttctgctacgagaacgaggt gTGAGAATTCaactgagcgccggtcgcta	
ttttcaggaggacccttgGCTAGCgtcgacGGTACCggtagaaaaa	RAI215_ACR1-f
ctcagttgGAATTCTTAcacctcgt	RAI216_ACR-1-Y FP-r
ttttcaggaggacccttgGCTAGCgtcgacGGTACCggtagaaaaaATGCCAT TCTACGA	RAI217_KCR-1-f
ctcagttgGAATTCTCacacctcgttctcgtagcagaaCTTATAC	RAI218_KCR-1-Y FP-r

GCTAGCgtcgcacGGTACCggtagaaaaaATGCCTTTTTACGATTC	RAI219_KCR-1-GS-f
ctcagttgGAATTCcacctcgttct	RAI220_KCR-1-GS-YFP-r
actgactgGGCCGGCCTTCCTTCAGAAGACGTGCTTTCCTTTTC	RAI229_snt-1p-FseI-F
cctctagaGGCGCGCCGGTGACTGAAAGTTTGATTGATAAATGAA	RAI230_snt-1p-AscI-R