Tonic signaling from O_2 sensors sets neural circuit activity and behavioral state

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Supplementary Information

Supplementary Figure 1. The behavioral assay setup.

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Supplementary Film 1
Halorhodopsin activation causes slowing in *npr-1 lite1; pgcy-32::NpHR-mCherry* animals kept at 21% O_2. Movie is speeded up 18x.

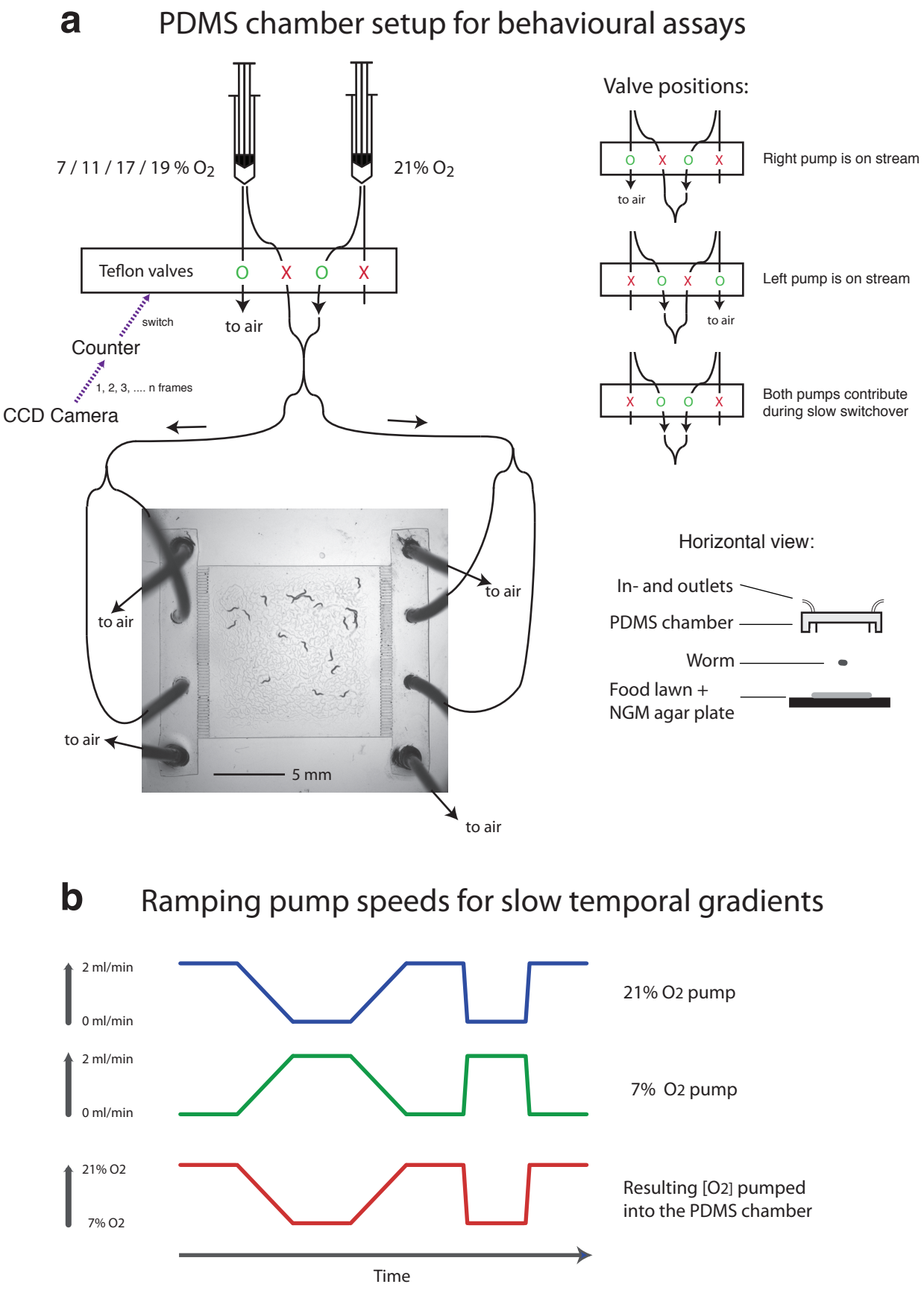
Supplementary Film 2
Channelrhodopsin activation causes speeding in *npr-1 lite1; pgcy-32::ChR2-mCitrine* animals kept at 11% O_2. Movie is speeded up 18x.

Supplementary Film 3
Selective channelrhodopsin activation of URX using the programmable array microscope elicits reversal behavior in *npr-1 lite1; pgcy-32::ChR2-EYFP* animals kept at 7% O_2. Movie is in real time.

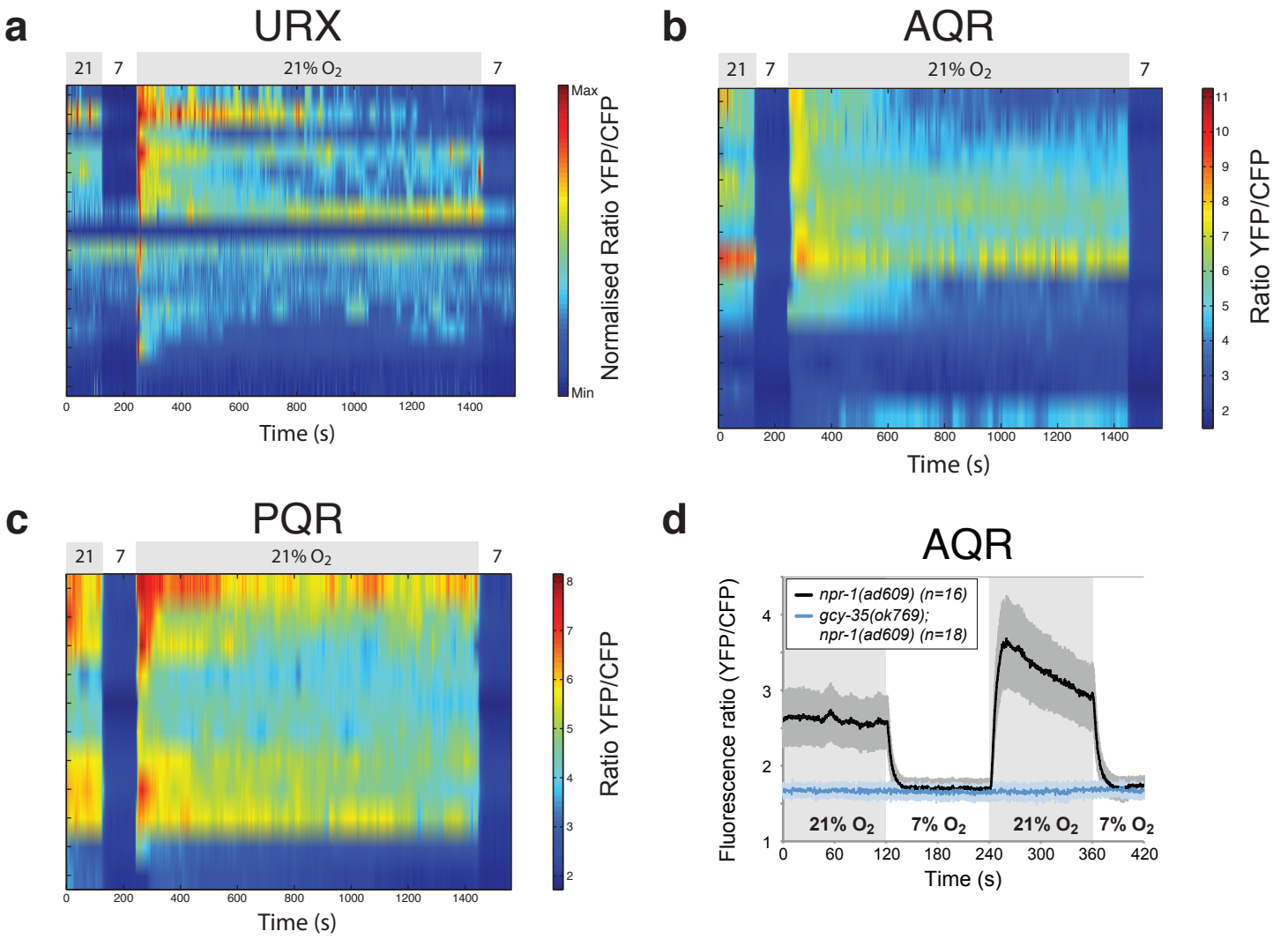
Supplementary Film 4
Selective channelrhodopsin activation of PQR using the programmable array microscope elicits accelerated forward movement in *npr-1 lite1; pgcy-32::ChR2-EYFP* animals kept at 7% O_2. Movie is in real time.

Supplementary Film 5
A puff of 21% O_2 directed at the head elicits reversal behavior in *npr-1* animals kept at 7% O_2. Movie is in real time.

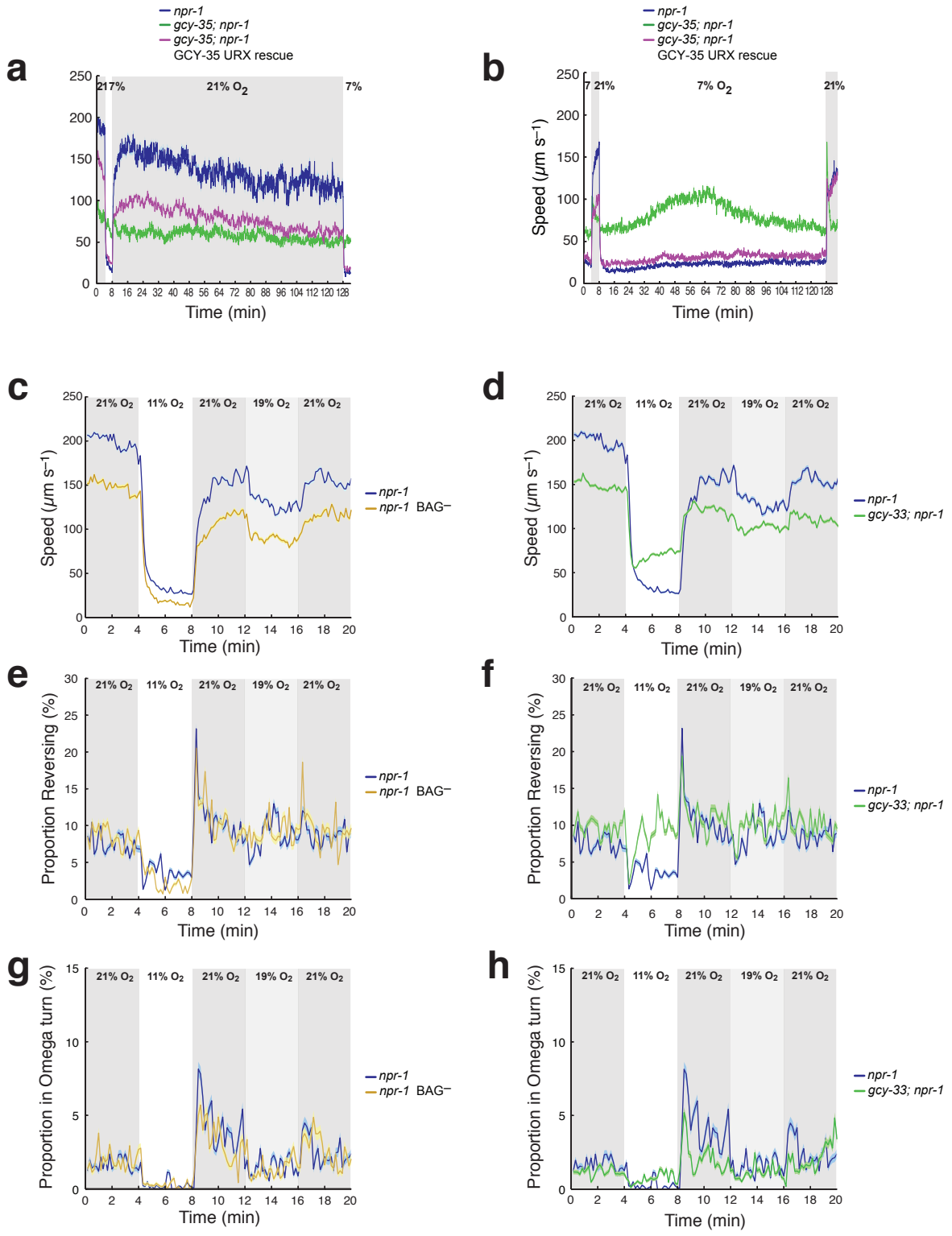
Supplementary Film 6
A puff of 21% O_2 directed at the tail elicits forward acceleration in *npr-1* animals kept at 7% O_2. Movie is in real time.



Supplementary Figure 1. The behavioral assay setup.
(**a**) The microfluidics device used to analyse behavioral responses to temporal changes in [O_2]. Animals can move freely in a square arena of 1.4 cm^2 seeded with a bacterial food lawn, while their behavior is recorded with a CCD camera. Two outer chambers are located on opposite sides of the arena, separated from it by pillars with 70 μm gaps in between, each containing two gas inlets connected to syringe pumps, and outlets that exhaust to air. Teflon valves were used to switch between different gas supplies at specified time points. (**b**) Gas supply regime used for slow temporal changes of ambient [O_2]. The pumping speed of two syringe pumps was changed simultaneously, one ramped up over a period of time (e.g. 2 min), the other ramped down. This resulted in a linear change of the gas flow contribution from one pump to the other, while overall gas flow into the chamber remained constant.



Supplementary Figure 2. O_2–evoked Ca^{2+} responses of O_2 sensors.
(**a – c**) Individual traces of O_2-evoked Ca^{2+} responses measured in the cell body of URX (**a**), AQR (**b**) and PQR (**c**) neurons using YC3.60. Traces are presented as heatmaps and ordered by similarity. For AQR and PQR the heat maps show the actual YFP/CFP ratios; because of the range of responses we see in URX, traces for this neuron are internally normalized for each trace. The highest ratios, representing high Ca^{2+}, are shown in red; blue are lowest ratios representing low Ca^{2+}. Light blue boxes above the heatmaps indicate exposure to 21% O_2. The averages of the data presented here correspond to the mean ratios presented in Figure 2b – d.
(**d**) O_2-evoked Ca^{2+} responses in AQR in *npr-1* and *gcy-35; npr-1* animals. Mutations in *gcy-35* abrogate AQR O_2 responses.

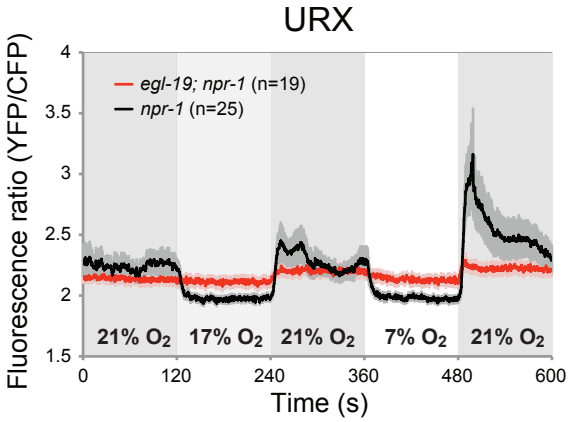


Supplementary Figure 3. Contributions of URX and BAG neurons to O_2-evoked locomotory responses of feeding *npr-1* animals.

(**a, b**) A transgene that expresses *gcy-35* cDNA in URX but not AQR and PQR neurons can partially restore coupling between [O_2] and locomotory activity in *gcy-35; npr-1* animals. n> 80 animals for each genotype.

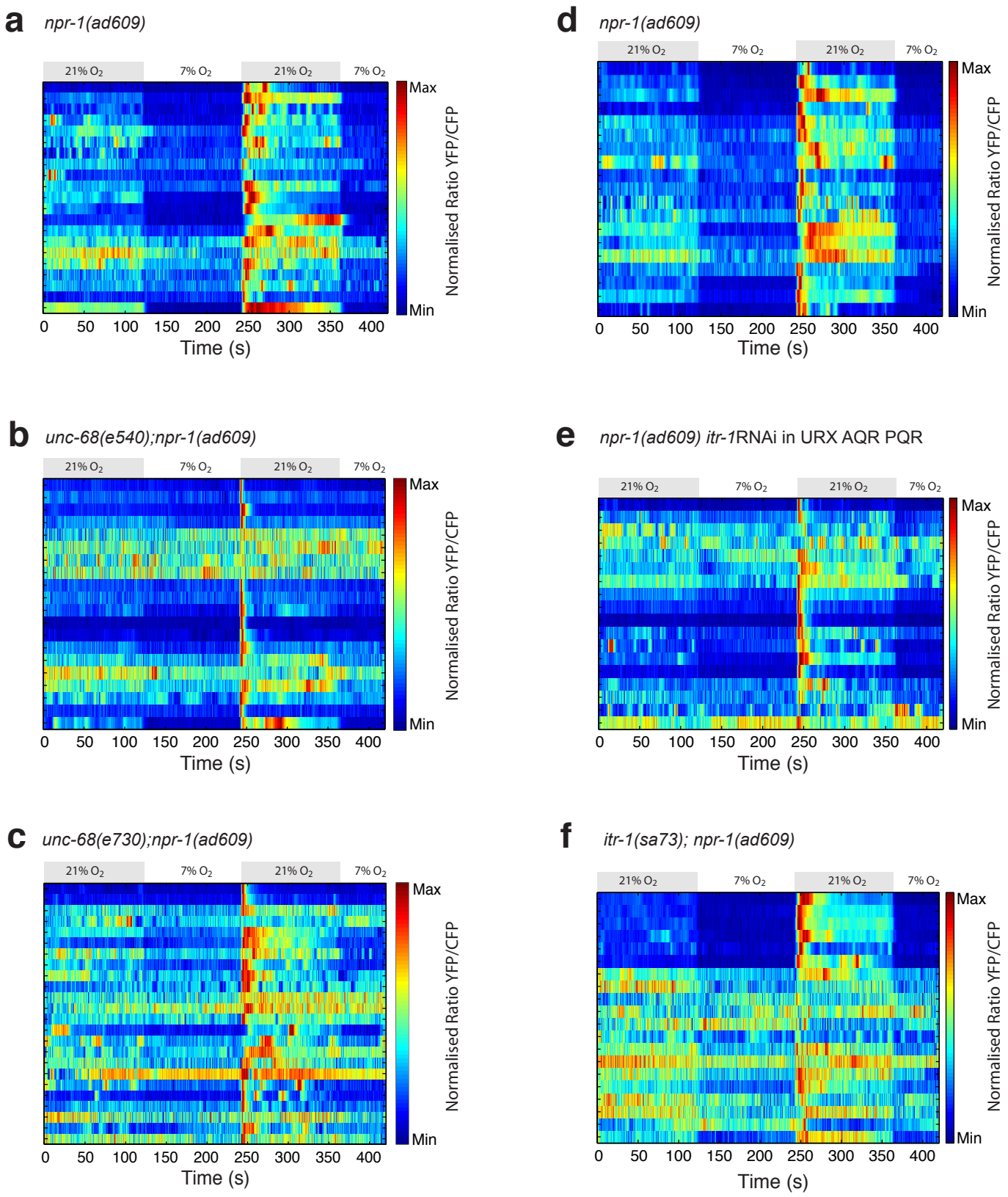
(**c, e, g**) Behavior of animals in which the BAG neurons have been genetically ablated by expressing the cell death gene *egl-1* from the *gcy-33* promoter. Speed at 21% O_2 is reduced in the ablated animals, but speed at 11% O_2, and the transient increases of reversals and omega turns in response to a sharp increase of [O_2] from 11% to 21%, are similar to those of *npr-1* control animals. n> 70 animals for both genotypes.

(**d, f, h**) The same behavioral assay in (c) carried out with a *gcy-33* guanylate cyclase mutant (*gcy-33(ok232); npr-1(ad609*). GCY-33 is expressed in AQR, PQR, URX and BAG, and is required for BAG neural responses to O_2. The speed of *gcy-33; npr-1* animals is reduced at 21% O_2, but increased at 11% O_2, compared to *npr-1* animals, while the transient increases of reversals and omega turns in response to a sharp rise of [O_2] are similar. n>120 animals for both genotypes.

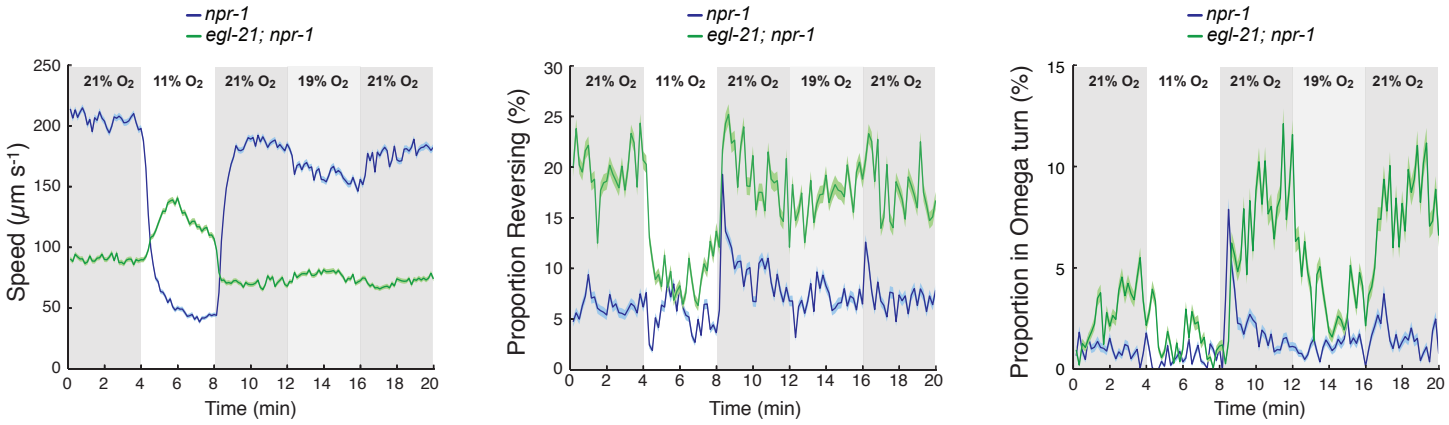


Supplementary Figure 4. A partial loss-of-function mutation in the *egl-19* L-type voltage-gated Ca^{2+} channel disrupts O_2-evoked Ca^{2+} responses in URX.
O_2-evoked Ca^{2+} responses in URX neurons measured using YC3.60.

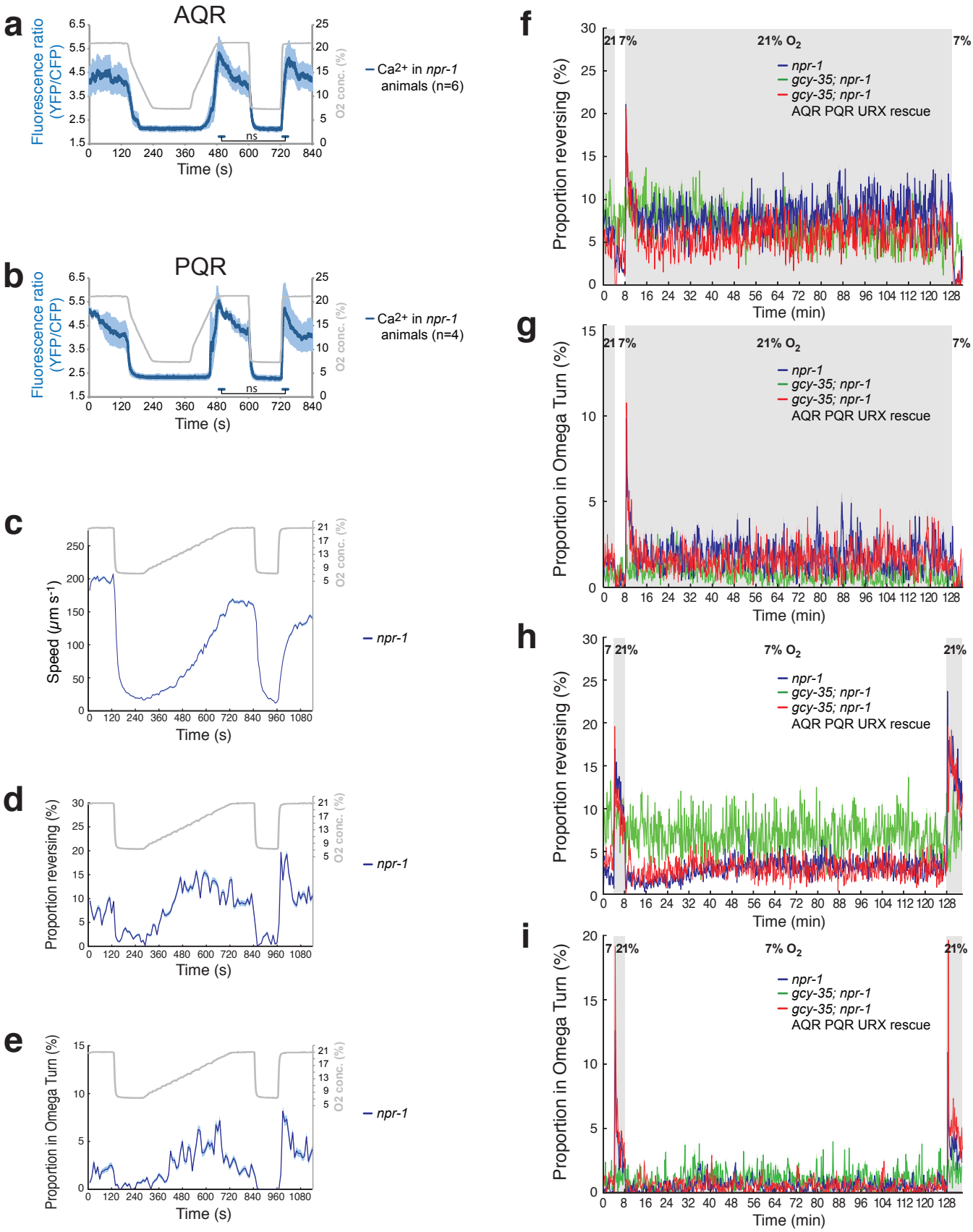
URX calcium imaging *pgcy32::YC3.60*



Supplementary Figure 5. O_2–evoked Ca^{2+} responses of URX O_2 sensors defective in the *unc-68* ryanodine receptor or the *itr-1* IP_3 receptor.
Shown are individual traces of O_2-evoked Ca^{2+} responses measured in the cell body of URX. Traces are presented as heatmaps and ordered by similarity. Traces are internally normalized for each trace. The highest ratios, representing high Ca^{2+}, are shown in red; blue are lowest ratios representing low Ca^{2+}. Light blue boxes above the heatmaps indicate exposure to 21% O_2. The averages of the data presented here correspond to the mean ratios presented in Figure 2.



Supplementary Figure 6. Mutations in *egl-21* carboxypeptidase disrupt normal behavioral responses to O_2.
Responses of *egl-21(n611); npr-1(ad609)* worms (green line) and *npr-1(ad609)* animals (blue line) to a 21% - 11% - 21% - 19% - 21% ambient O_2 stimulus train in the presence of bacterial food. Differently shaded areas of blue indicate periods of 19 and 21% O_2 stimuli. n> 100 animals for both genotypes.



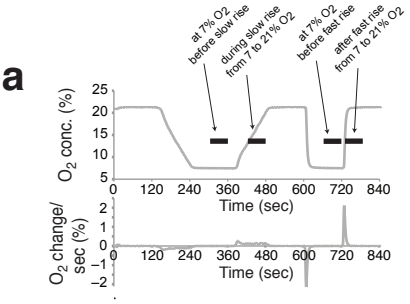
Supplementary Figure 7. Encoding dO_2/dt.

(**a, b**) Ca^{2+} responses evoked in AQR (**a**) and PQR (**b**) neurons by different rates of dO_2/dt. The blue line indicates the fluorescence ratio (Y-axis on the left), whereas the changing O_2 levels are indicated by the gray line (Y-axis on the right).

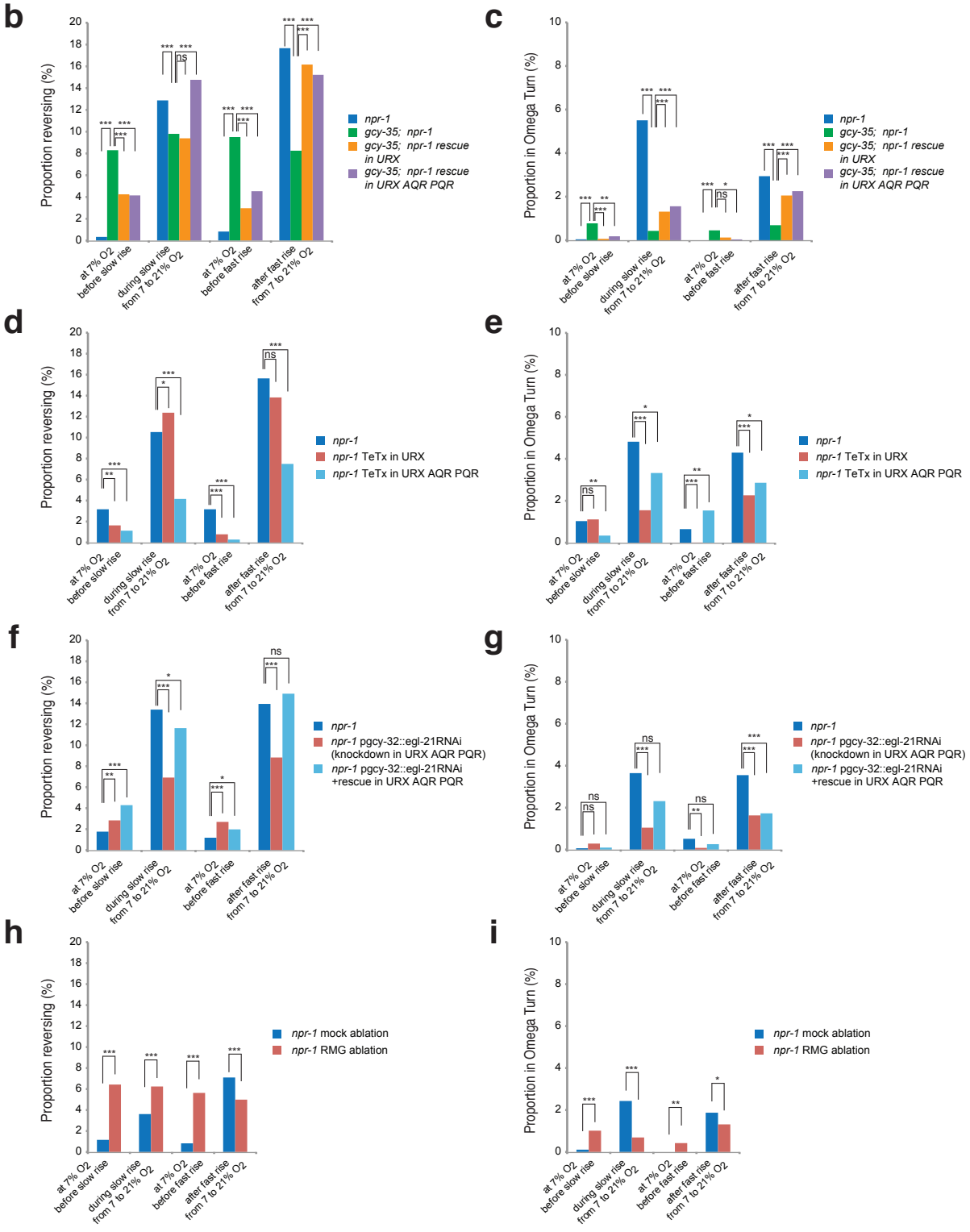
(**c – e**) Behavioral responses evoked in *npr-1* animals by very slow (0.03% sec^{-1}) rates of dO_2/dt compared to very fast (2% sec^{-1}) rates of dO_2/dt. Data plotted are speed (**c**), proportion reversing (**d**), and proportion turning (**e**). Changing in O_2 levels are indicated by the gray line (Y-axis on the right).

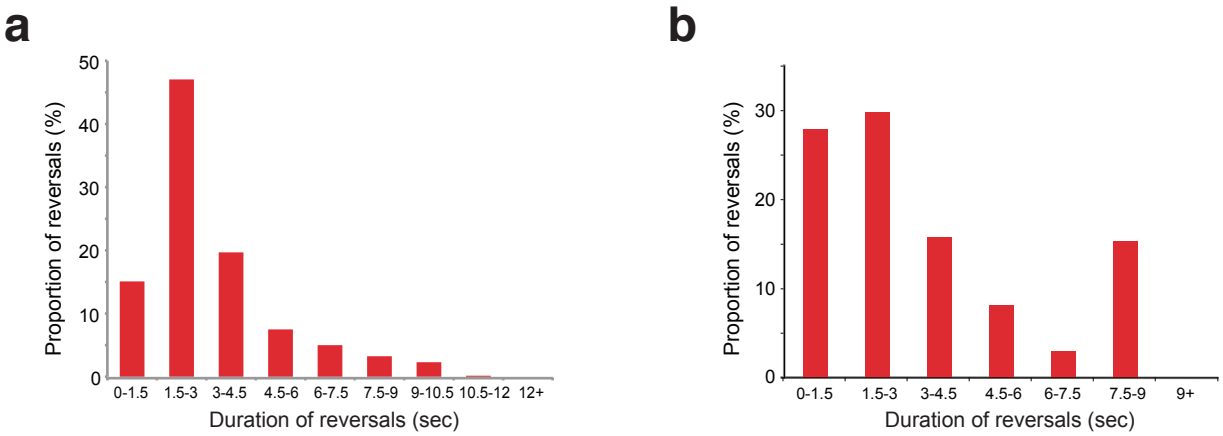
(**f – i**) Proportion of animals reversing (**f, h**) and turning (**g, i**) for indicated genotypes when animals are subjected to long-lasting [O_2] changes (data are from same videos analysed for Figure 1b, c).

Supplementary Figure 8

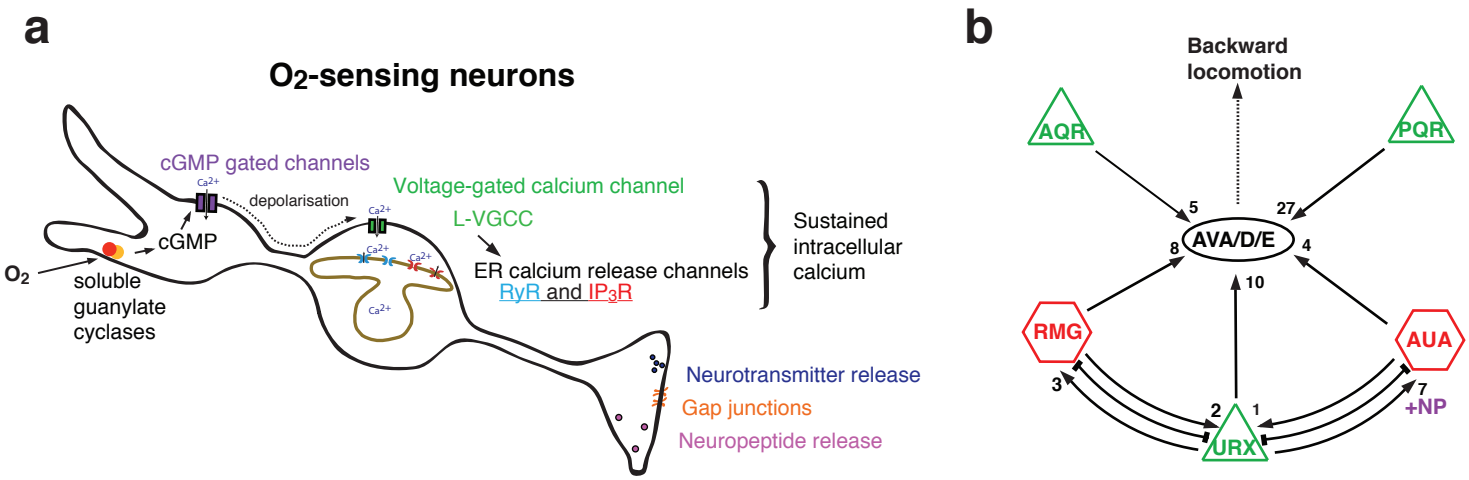


Supplementary Figure 8. Behavioral responses to different d[O_2]/dt.
(**a**) Animals were exposed to slow (0.2% s^{-1}) and fast (2% s^{-1}) switches between 7% and 21% O_2. Plotted in grey are the changes in [O_2] measured with an optode.
(**b–i**) Mean proportion of animals reversing or executing omega turns in the 1 min periods before and during slow and before and after fast rises of [O_2]. The averaged periods are indicated by horizontal black bars in (**a**). Statistical significance of genotype differences was assessed with two-tailed chi-square tests (*** $P < 0.001$). Data correspond to those shown in Figure 7(c–l).
(**b,c**) The proportion of *npr-1* animals reversing (**b**) or turning (**c**) increases during both slow and rapid rises in [O_2]. *gcy-35;npr-1* mutants change little in their reversal or turning patterns in response to O_2 changes. Expressing a *gcy-35::gfp* operon in AQR, PQR, URX (from the *gcy-32* promoter) or in URX alone (from the *flp-8* promoter) in *gcy-35; npr-1* mutants restores reversal and turning responses to both slow and fast O_2 changes. n> 80 animals for each genotype.
(**d,e**) Animals expressing tetanus toxin in AQR, PQR and URX show a partial reduction in O_2-evoked reversal (**d**) and turning (**e**) behaviors. Animals showing Tetx expression only in URX neurons show weak reversal, but strong omega turn phenotypes. n> 70 animals.
(**f,g**) RNAi knockdown of *egl-21* carboxypeptidase E in AQR, PQR and URX neurons significantly reduces O_2-evoked reversals and turning. n> 80 animals.
(**h,i**) Ablation of the RMG interneurons suppresses O_2-evoked reversal and turning. n> 40 animals.





Supplementary Figure 9. Distribution of reversal duration in animals kept at 21% O_2. Spontaneous reversals of freely moving *npr-1* animals kept at 21% O_2 usually last less than 12 seconds. Data for panel (**a**) were obtained from the same videos analyzed in Figures 1b and Supplementary Figure 7f and g (n=28676 reversal events); data for panel (**b**) were obtained from the freely moving imaging experiments show in Figure 8b.



Supplementary Figure 10. Model for the generation of tonic responses in O_2-sensing neurons, and their neural connectivity.

(a) Model showing how the tonic response of O_2-sensing neurons to persistent high O_2 is mediated by a Ca^{2+} relay involving the soluble guanylate cyclases GCY-35/36, cGMP-gated channels, the L-type voltage-gated Ca^{2+} channel EGL-19, and the ryanodine/IP_3 receptor Ca^{2+} release channels UNC-68/ITR-1. Tonic signaling evokes tonic release of neuropeptides that promote a sustained behavioral state switch.

(b) Simplified circuitry diagram showing signaling between the AQR, PQR and URX O_2-sensing neurons and downstream interneurons.