Supplemental Materials and Methods for *Using a robust and sensitive GFP-based cGMP sensor for real time imaging in intact Caenorhabditis elegans*

Molecular Biology: FlincG3 was codon optimized by Genscript for use in *C. elegans*. The *C. elegans* codon-optimized FlincG3 was inserted into a worm specific expression (Fire) vector, pPD95.75, which facilitates transcription and translation of the sensor by providing worm-specific introns (Evans 2006).

The *gcy-5* promoter (2003 bp upstream of start site) was inserted using *Xba*I and *Eag*I into pPD95.75 to make *gcy-5p::FlincG3*. *myo-3p*::CyclOp::SL2::mCherry was made as described (Gao *et al.* 2015). The plasmid *gcy-5p::FlincG3* was digested with *Xba*I and *Pci*I and the *gcy-5* promoter fragment was replaced with the *myo-3* promoter fragment to yield the *myo-3p::FlincG3* plasmid construct.

pCFJ104 [*myo-3p*::mCherry::unc-54] was a gift from Erik Jorgensen (Addgene Plasmid # 19328) (Frøkjær-Jensen *et al.* 2008). To generate *myo-3p*::bPAC::SL2::mCherry, a 1193 bp long bPAC fragment was amplified using primers 5'-TCCATCTAGAGGATCCTTCCGCATCTCTTGTTCAAGGG-3' and 5'-CAGCGGTACCGTCGACTTACTTGTCGTTTTCCAGGGTCTG-3'. This was ligated into a *myo-3p*::SL2::mCherry backbone, obtained by digestion with *Bam*HI and *Sal*I, using 'in-fusion cloning' (Clontech). To generate *myo-3p*::bPGC::SL2::mCherry, a 3486 bp long *myo-3p*::bPGC fragment was amplified using primers 5'-ATTACGCCAAGCTTGCGGCTATAATAAGTTCTTGAA-3' and 5'-TACCGTCGACGCTAGTTACTTGTCGTTTTCCAGGG-3'. This was ligated into a SL2::mCherry backbone, obtained by digestion with *Nhe*I and *Sph*I, using 'in-fusion cloning' (Clontech).

pJN55 [*myo-3p*::tax-2::GFP] and pJN58 [*myo-3p*::tax-4::GFP] plasmid construction have been previously reported (Gao *et al.* 2015). pWSC13 [*myo-3p*::bPGC::eYFP]: pPD96.52 was cut with *Nhe*I and *Bam*HI to yield a 6010 bp fragment, and bPGC synthetic construct, a gift from Peter Hegemann, was cut with *Bam*HI and *Nhe*I to yield a 2106 bp fragment that was then inserted into cut pPD96.52. To generate pJN59 [*myo-3p*::bPGC(K265D)::eYFP], K265D was introduced into the parent plasmid pWSC13 (*myo-3p*::bPGC::eYFP) using the Q5 site-directed mutagenesis kit (New England Biolabs Inc.) and primers oJN146 (5'-CCTGAAGATGGACCACGGCCTGC-3') and oJN147 (5'-CTGCTGCCCATGTTGCCC-3').

To generate pFG248 (the *gcy-8p::FlincG3* construct), ~ 2.2 kb of the *gcy-8* promoter was isolated from pFG148 using *Sph*I and *Bam*HI, which was then inserted into these sites of pFG216, replacing the o*sm-10* promoter. pFG216 (*osm-10p::FlincG3)* was made by isolating ~ 900 bp of the *osm-10* promoter from pFG1 (Krzyzanowski *et al.* 2013) using *Sph*I and *Bam*HI, which was then inserted into these sites of *gcy-5p::FlincG3*, replacing the *gcy-5* promoter. pFG148 was made by isolating ~ 2.2 kb of the *gcy-8* promoter from *gcy-8 in MC10* (from Piali Sengupta) using *Sph*I and *Bam*HI, which was then inserted into these sites of Fire vector pPD49.26 (Fire Lab *C. elegans* Vector Kit, Addgene).

To generate pSRW1JZ (the *flp-6p::FlincG3* construct, Addgene Plasmid # 129528), the *flp-6* promoter fragment was amplified from *flp-6p::GCaMP6*, a gift from Shawn Lockery, using the primers 5'-ATTACGCCAAGCTTGCATGGCAGCGCTTGACTTCTGATG-3' and 5'-

CCGGGGATCCTCTAGTGCAGGCATGCAAGCTTGTC-3'. The plasmid *nlp-1p::FlincG3* was digested with *Xba*I and *Sph*I-HF and the *nlp-1* promoter fragment was replaced with the *flp-6* promoter fragment to yield the *flp-6p::FlincG3* plasmid construct using 'in-fusion' cloning (Clontech). The plasmid *str-2p::jRCaMP1b*, a gift from Cornelia Bargmann, was digested with *Hin*dIII-HF and *Xma*I and the *str-2* promoter fragment was replaced with the *flp-6* promoter fragment to yield the *flp-6p::jRCaMP1b* plasmid construct using 'in-fusion' cloning (Clontech). The plasmid *str-2p::jRGECO1a*, a gift from the Bargmann lab, was digested with *Hin*dIII-HF and *Asc*I and the *str-2* promoter fragment was replaced with the *flp-6* promoter fragment to yield the *flp-6p::jRGECO1a* plasmid construct using 'in-fusion' cloning (Clontech).

To generate the *nlp-1p::FlincG3* construct, FlincG3 from *gcy-5p::FlincG3* was inserted into the vector *nlp-1p*::pSMΔ (Barsi-Rhyne *et al.* 2013) using the NEBuilder High-Fidelity DNA Assembly Cloning Kit (NEB) and the following primers: MVP598 WincG FWD (5'-ggattggccaaaggacATGGCACACCACCACCAC-3'), MPV599 WincG REV (5'-ggtcctcctgaaaatgttcTTATCGTCCGAATCCTCCG-3'), MVP597 *pSMΔ::nlp-1p* FWD (5'-GAACATTTTCAGGAGGACCC-3'), and MVP596 *nlp-1p::pSMΔ* REV (5'-GTCCTTTGGCCAATCCCG-3'). The construct was then sequence-verified.