Supporting Methods

Construction of egl-9 and hif-1 plasmids: To create Pegl-9::egl-9::tag, 1.6 kb of sequence 5’ to the egl-9 translational start was amplified and cloned into the pPD95.75 vector (from Andrew Fire) (primers: CGCCTGAGCGTCTATGTGTGTGAAAGAG and GGCGTGACGGActTTTTTCTGTCAATTCAG). The remaining coding sequence for egl-9 (exons 3 to end) was amplified from cDNA, using primers 5’GCCGTCGACCACATGACATGAGCAGTGCCCATAATG and 5’GGCGGATCCGGATGTAATCTCGGGTGGTTGTTGGAAGG. The resulting fragment was digested with SalI and BamHI and inserted into the construct containing the egl-9 promoter. Finally, the genomic sequence including the first three egl-9 exons was amplified (primers: 5’GCCGGTCGACCACATGACATGAGCAGTGCCCATAATG and 5’GGATTGGAATCGATGGCTCTGG) and added to the construct using SalI and ClaI restriction sites. Pegl-9::egl-9(H487A)::tag was made by mutating codon His487 to encode alanine.

The Phif-1::hif-1::tag construct contains 5.2 kb of hif-1 5’ regulatory sequence, the genomic sequence for the first exon and first intron of hif-1, and cDNA sequence for exons 2 - 9. The cDNA sequence is from the predominant hif-1 mRNA isoform (hif-1a). This construct also includes an epitope tag that consists of one copy of HA and five copies of c-myc (from clone CD3-128, Arabidopsis Biology Resource Center), inserted in frame after the hif-1 coding sequences. The coding sequences are followed by a stop codon and 400 bp of genomic sequence 3’ to the hif-1 coding region. To create the Phif-1::hif-1 (P621G)::tag construct, the codon for proline 621 was modified to encode glycine, and the HA sequence was removed from the epitope tag.

Hypoxia treatment and protein blots: To achieve 0.5% oxygen, nitrogen was mixed with room air. To assess the levels of HIF-1 or Phnhr-57::GFP, 40 – 100 synchronized L4-stage animals were boiled in 10ul M9 and 10ul 2x SDS buffer [50mM Tris-HCl PH6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 100mM DTT, 0.1% (w/v) bromphenol blue] for 10 minutes before loading to 10% or 12% polyacrylamide gels. Phnhr-57::GFP is expressed at much higher levels in animals carrying strong loss-of-function mutations in egl-9, and a smaller fraction of the lysate was loaded in each lane of the gel (the equivalent of 4 animals). After separation, proteins were transferred to nitrocellulose membranes. The blots were probed with monoclonal antibodies recognizing the following epitopes: GFP (antibody from Roche at 1:1000 dilution); HA (antibody from Cell Signaling Technology clone 6E2 at 1:1000 dilution); myc (mouse ascites, clone 9E10, from the Developmental Studies Hybridoma Bank at 1:1000 dilution), or AHA-1 [Jiang et al. 2001] (1:100 dilution). The secondary antibody (goat anti-mouse IgG+IgM from Biorad) was diluted 1:2000. The relative intensity of protein bands were quantified by Image J software.