C. elegans HIF-1 is broadly required for survival in hydrogen sulfide

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ABSTRACT

Hydrogen sulfide is common in the environment, and is also endogenously produced by animal cells. Although hydrogen sulfide is often toxic, exposure to low levels of hydrogen sulfide improves outcome in a variety of mammalian models of ischemia-reperfusion injury. In Caenorhabditis elegans, the initial transcriptional response to hydrogen sulfide depends on the hif-1 transcription factor, and hif-1 mutant animals die when exposed to hydrogen sulfide. In this study, we use rescue experiments to identify tissues in which hif-1 is required to survive exposure to hydrogen sulfide. We find that expression of hif-1 from the unc-14 promoter is sufficient to survive hydrogen sulfide. Although unc-14 is generally considered to be a pan-neuronal promoter, we show that it is active in many non-neuronal cells as well. Using other promoters, we show that pan-neuronal expression of hif-1 is not sufficient to survive exposure to hydrogen sulfide. Our data suggest that hif-1 is required in many different tissues to direct the essential response to hydrogen sulfide.
INTRODUCTION

Hydrogen sulfide (H\textsubscript{2}S) in the environment is produced by industrial sources and natural sources, including volcanic deposits and anaerobic bacteria (Beauchamp et al., 1984). H\textsubscript{2}S is also endogenously produced as a product of the cysteine biosynthesis through the transsulfuration pathway, and endogenous H\textsubscript{2}S has important roles in cellular signaling (Li et al., 2011; Vandiver and Snyder, 2012; Wang, 2012). Chronic exposure to relatively low concentrations of environmental H\textsubscript{2}S in humans has been associated with neurological, respiratory, and cardiovascular dysfunction (Kilburn and Warshaw, 1995; Richardson, 1995; Bates et al., 2002). However, transient exposure to low H\textsubscript{2}S has also been shown to improve outcome in many mammalian models of ischemia-reperfusion injury (Bos et al., 2015; Wu et al., 2015). It is possible that the biological effects of exogenous H\textsubscript{2}S exposure, both beneficial and detrimental, result from activation of pathways that are normally regulated by endogenous H\textsubscript{2}S.

*Caenorhabditis elegans* is an excellent system to define physiological responses to exogenous H\textsubscript{2}S. In addition to powerful genetics, all cells are directly exposed to the gaseous environment (Shen and Powell-Coffman, 2003). This feature allows for control of cellular H\textsubscript{2}S exposure without confounding factors from physiological regulation gas delivery. *C. elegans* grown in H\textsubscript{2}S are long-lived, thermotolerant, and resistant to hypoxia-induced disruptions of proteostasis (Miller and Roth, 2007; Fawcett et al., 2015). HIF-1 directs the transcriptional response to H\textsubscript{2}S in *C. elegans* (Budde and Roth, 2010; Miller et al., 2011). HIF-1 is a highly-conserved transcription factor best known for regulating the transcriptional response to low oxygen (hypoxia) in metazoans (Semenza, 2000; Semenza, 2001). *C. elegans hif-1* mutant animals are viable and fertile in room air but die if exposed to hypoxia during embryogenesis (Jiang et al., 2001; Miller
In contrast, exposure to H$_2$S is lethal for hif-1 mutant animals at all developmental stages (Budde and Roth, 2010).

Several studies have argued for neuronal-specific functions of HIF-1, though the hif-1 promoter is active in most, if not all, cells and HIF-1 protein is stabilized ubiquitously in C. elegans exposed to either hypoxia or H$_2$S (Jiang et al., 2001; Budde and Roth, 2010). Neuronal expression of hif-1 in hypoxia is reported to be sufficient to prevent hypoxia-induced diapause and to increase lifespan through induction of intestinal expression of fmo-2 (Miller and Roth, 2009; Leiser et al., 2015). Furthermore, neuronal CYSL-1 protein regulates the activity of HIF-1 to modulate behavioral responses to changes in oxygen availability (Ma et al., 2012). These data motivated us to determine if neuronal HIF-1 activity is sufficient for C. elegans to survive exposure to H$_2$S.

In this study, we used tissue-specific rescue of hif-1 to define the site of essential HIF-1 activity in low H$_2$S. We found that expression of hif-1 from the unc-14 promoter was sufficient for survival in H$_2$S. Although considered a pan-neuronal promoter (Ogura et al., 1997; Pocock and Hobert, 2008), our data indicate that the unc-14 promoter is also broadly expressed in non-neuronal cells. We show that hif-1 expressed from the pan-neuronal rab-3 promoter is not sufficient for viability in H$_2$S. We further demonstrate that expression of hif-1 in muscle, hypodermis, or intestine is not sufficient for viability in low H$_2$S. Together, our data indicate that the activity of HIF-1 may be required in multiple tissues to coordinate the organismal response to H$_2$S.
**MATERIALS AND METHODS**

**Strains**

Strains were grown at room temperature on nematode growth media plates (NGM) seeded with the OP50 strain of *E. coli* (Brenner, 1974). All strains were derived from N2 (Bristol). Full genotypes of strains used in this study are in Table 1. To sequence the *Punc-14: hif-1* junction of *otIs197*, the region was amplified with forward primer oET479 (5'-GTTGTCCACCATCAGTAATACG) and reverse primer oET480 (5'-ACGACCGCGTTCATG). The oET479 primer was used for sequencing.

**Constructs and transgenes**

All constructs were made using the multisite Gateway system (Invitrogen) where a promoter region, a gene region (*hif-1* cDNA or GFP), and a C-terminal 3'UTR were cloned into the destination vector pCFJ150 (Frøkjær-Jensen et al., 2008). The *hif-1 A* isoform was amplified from cDNA using forward primer oET467 (5'-GGGACAAGTTTGTACAAAAAAGCAGGCTCAATGGAAGACAATCGGAAAAGAAC) and reverse primer oET469 (5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAAGAGAGCATTGGAAATGGG). For the tissue-specific rescuing experiments, an operon GFP::H2B was included in the expression constructs downstream of the 3'UTR (Frøkjær-Jensen et al., 2012). This resulted in expression of untagged HIF-1 protein and histone H2B fused to GFP, which allowed for confirmation of promoter expression by monitoring GFP expression. The *unc-14* promoter (1425 bp upstream of the start codon) was amplified from genomic DNA using forward primer oET520 (5'-GGGGACAACTTTGTACAAAAAAGCAGGCTCAATGGAAGACAATCGGAAAAGAAC) and reverse primer oET507 (5'-GGGGACCACCTTTGTACAAGAAAGCTGGGTGTCAAGAGAGCATTGGAAATGGG).
GGGGACTGCTTTTTTGTACAAACTTGTTTTGGTGGAAGAATTGAGGG. All plasmids constructed were verified by sequencing. Constructs used in this study are in Table 2. Extrachromosomal arrays were made by standard injection methods (Mello et al., 1991) with 10-15 ng/µl of the expression vector. At least two independent lines were isolated for each construct.

H₂S atmospheres

Construction of atmospheric chambers was as previously described (Miller and Roth, 2007; Fawcett et al., 2012). In short, H₂S (5000 ppm with balance N₂) was diluted continuously with room air to a final concentration of 50 ppm. Final H₂S concentration was monitored using a custom-built H₂S detector containing a three-electrode electrochemical Surecell H₂S detector (Sixth Sense) as described (Miller and Roth, 2007), calibrated with 100 ppm H₂S with balance N₂. Compressed gas mixtures were obtained from Airgas (Radnor, PA) and certified standard to within 2% of the indicated concentration.

Survival assays

Twenty to forty L4 animals were picked to plates seeded with OP50. Plates were exposed to 50ppm H₂S for 20-24 hours, and then returned to room air to score viability. Death was defined as failure to move when probed with a platinum wire on the head or tail.

Imaging
For imaging expression of GFP, larval stage 1 (L1) or first-day adult animals were mounted on 2% agarose pads and anesthetized with 50 mM sodium azide for ten minutes before placing the cover slip. The images were obtained using a Nikon 80i wide-field compound microscope.

Reagent Availability

Strains are available upon request and have been deposited at the *Caenorhabditis* Genetics Center (cgc.umn.edu). Plasmid constructs are available upon request.
RESULTS AND DISCUSSION

C. elegans requires hif-1 to survive exposure to low H$_2$S (Budde and Roth, 2010).

To determine whether neuronal expression of hif-1 was sufficient for survival in H$_2$S, we used transgenic hif-1(ia04) mutant animals that expressed hif-1 from heterologous promoters. We first used the available otIs197 transgene, which expresses hif-1 from the putative pan-neuronal unc-14 promoter (Pocock and Hobert, 2008). We found that hif-1(ia04); otIs197 animals survived exposure to 50 ppm H$_2$S (Figure 1A). This result suggests that neuronal expression of hif-1, from the unc-14 promoter, is sufficient to survive exposure to H$_2$S.

To further dissect which neuronal cell type(s) HIF-1 activity was required to survive exposure to H$_2$S, we generated transgenic animals that expressed hif-1 cDNA under the control of promoters active in specific neuronal subtypes. We found that expression in neither cholinergic neurons (Punc-17) nor in GABAergic neurons (Punc-47) was sufficient to rescue the lethality of hif-1(ia04) mutant animals exposed to H$_2$S (Figure 1A). Curiously, we also observed that expression of hif-1 cDNA from the pan-neuronal rab-3 promoter did not rescue survival of the hif-1(ia04) mutant animals (Figure 1A). This was unexpected, as expression of HIF-1 from the unc-14 promoter (the otIs197 transgene) was sufficient for survival in H$_2$S. We therefore pursued the source of this discrepancy.

We first sought to verify the molecular nature of the otIs197 integrated transgene. We used PCR to amplify a region from the unc-14 promoter and the hif-1 coding region from the otIs197 transgenic animals. As expected, this reaction generated a single band of ~500 bp. However, when we sequenced the resulting PCR product we discovered an insertion of an extra G immediately following the ATG of the hif-1 cDNA. This insertion
causes a frame-shift and results in a stop codon after 13 amino acids. However, the 
*otls197* transgene must express some HIF-1 protein, as it can rescue many phenotypes of 
*hif-1* mutant animals (Pocock and Hobert, 2008; Miller and Roth, 2009; Ma et al., 2012; 
Leiser et al., 2015). The *otls197* transgene was constructed to express isoform A of *hif-1*, 
though there are six predicted isoforms (Wormbase, 2017). We noted that the ATG for 
isoform E is approximately 20 bp downstream of the original ATG in the *hif-1* cDNA. 
Thus, it could be that expression of the *hif-1e* isoform is the basis of the activity of the 
*otls197* transgene. Because our *Prab-3::hif-1* transgene expressed the *hif-1a* isoform, it 
was possible that the differences we observed from *otls197* was due to the expression of 
different *hif-1* isoforms. To test this possibility we created transgenic strains expressing 
*hif-1a* under control of the *unc-14* promoter using a *Punc-14::hif-1a(P621A)::YFP* plasmid 
(Pocock and Hobert, 2008), which we verified had had the expected *hif-1a(P621A)* 
sequence. We injected this plasmid into *hif-1(ia04)* mutant animals to generate the 
yakEx137 transgene. If the rescue we observed in *otls197* was due to expression of *hif1e* 
rather than *hif1a*, then the animals expressing *Punc-14::hif-1a(P621A)::YFP* would die in 
H2S. However, these animals survived exposure to H2S (Figure 1C), indicating that 
potential expression of different isoforms did not underlie differences in survival of 
exposure to H2S.

The HIF-1 protein expressed by the *otls197* transgene has a P621A mutation that 
prevents it from being hydroxylated and degraded by the proteasome (Pocock and 
Hobert, 2008). In contrast, the constructs we generated produced wild-type HIF-1 
protein. We did not expect this feature to be salient for our experiments, since HIF-1 
protein is stabilized in H2S due to inhibition of the hydroxylation reaction (Budde and 
Roth, 2010; Ma et al., 2012). However, it is possible that constitutive stabilization of HIF-
protein in neurons promotes survival in H$_2$S. To evaluate this possibility, we cloned wild type hif-1 cDNA under control of the unc-14 promoter, including 1.4 kb upstream of the transcription start site (Ogura et al., 1997). We found that hif-1(ia04); Punc-14::hif-1 (yakEx144) animals survived exposure to H$_2$S, similar to hif-1(ia04); otIs197 animals (Figure 1C). We conclude that the P621A mutation in otIs197 does not underlie the difference in survival in H$_2$S that we observe for animals expressing hif-1 from rab-3 and unc-14 promoters.

Given that the only other notable difference between the Prab-3::hif-1 and Punc-14::hif-1 constructs is the promoter elements, we hypothesized that differences between either the levels of expression from these promoters or the identity of the cells where these promoters are expressed should account for their different behavior. The transgenic constructs we generated all included an operon GFP::H2B downstream of the 3’UTR (Frøkjær-Jensen et al., 2012). This resulted in expression of untagged HIF-1 protein as well as GFP::H2B. We therefore visualized GFP expression to evaluate the expression levels and cellular patterns of promoter activity. As expected, GFP expression from adult hif-1(ia04); Prab-3::hif-1::operon::GFP::H2B was exclusively in neurons (Figure 2A). However, when we imaged adult hif-1(ia04); Punc-14::hif-1::operon::GFP::H2B animals that had survived exposure to H$_2$S we observed GFP expression in neurons, as expected, but also in intestinal and hypodermal cells (Figure 2B). We saw similar expression in animals that had not been exposed to H$_2$S. To corroborate this observation, we cloned the unc-14 promoter upstream of GFP and injected it into wild-type animals. We then imaged larvae (Figure 2C) and adult animals (Figure 2D) from three separate lines. We observed expression of GFP was expressed in numerous cells other than neurons including intestine, hypodermis, muscle, and the
uterus. Every animal that we imaged had expression in at least one other cell type other than neurons (n = 50).

Based on our understanding of Punc-14 expression and the fact that hif-1(ia04); Prab-3::hif-1 animals die when exposed to H$_2$S (Figure 1A), we inferred that neuronal HIF-1 activity is not sufficient for survival in H.S. We therefore explored whether expression of hif-1 exclusively in non-neuronal tissues was sufficient for survival in H$_2$S. For these experiments, we generated transgenes with hif-1 expressed under control of the unc-120 promoter, which is active in body-wall and vulval muscle; the dpy-7 promoter, which is active in hypodermis; the vha-6 promoter, which is active in intestine; and the ubiquitous efl-3 promoter. We chose these promoters because they included many of the tissues that had unc-14 driven expression of GFP (Figure 2B). As shown in Figure 3, only the ubiquitously-expressed Peft-3::hif-1 rescued the lethality of hif-1(ia04) mutants exposed to H.S. Although we did not test all possible cell and tissue types, these data suggest that HIF-1 activity in a single tissue cannot support survival in H$_2$S.

The fact that Punc-14::hif-1 was sufficient for survival in H.S (Figure 1A) suggests that activity of HIF-1 may not be required in all cells. Since we did not observe rescue when hif-1 was expressed in a single tissue, we made transgenic animals with expression of hif-1 in more than one tissue to determine if we could find a minimal expression that was sufficient for survival in H$_2$S. We found that even animals with hif-1 expression in neurons, hypodermis, and intestine (hif-1(ia04); yakEx146[Prab-3::hif-1, Pnha-6::hif-1, Pdpy-7::hif-1]) did not survive exposure to H$_2$S (Figure 3B). Together, our data suggests that that HIF-1 activity is required in many tissues to coordinate the essential response to H$_2$S. This could indicate that HIF-1 acts cell autonomously to
direct expression of many tissue-specific transcripts that are required to survive exposure to H$_2$S.

Although it was reported that *otIs197* expresses hif-1 selectively in neurons (Pocock and Hobert, 2008), our data show that the *unc-14* promoter is more broadly expressed. In fact, others have reported non-neuronal expression of transgenes expressed under the control of the *unc-14* promoter (Ogura et al., 1997; Wolkow et al., 2000; da Graca et al., 2004). However, the non-neuronal expression we have demonstrated is much more penetrant than has been previously acknowledged. This is an important consideration when interpreting the results of experiments using transgenes driven by *unc-14*, including hif-1 from *otIs197*. Our data show that non-neuronal expression from the *unc-14* promoter is significant and that rescue by *unc-14*-driven transgenes is not sufficient to infer neuronal function of HIF-1 and, presumably, other proteins.

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Figure legends

Figure 1. HIF-1 expression from the *unc-14* promoter rescues the H$_2$S lethality of *hif-1(ia04)* mutant animals.

(A) Survival of animals exposed to H$_2$S. All animals have the null *hif-1(ia04)* mutation. The *otls197* integrated array expresses a non-degradable HIF-1 variant. Other constructs were extrachromosomal arrays that express wild-type HIF-1. The *unc-14* promoter is expressed pan-neuronally (Ogura *et al.*, 1997), *rab-3* promoter is expressed in most, if not all, neurons (Nonet *et al.*, 1997), *unc-17* is expressed in cholinergic neurons (Rand *et al.*, 2000), and *unc-47* is expressed in GABAergic neurons (Eastman *et al.*, 1999). Animals were exposed to 50 ppm H$_2$S starting at L4. (B) HIF-1 gene structure and predicted A and E isoforms (Wormbase, 2017). The P621A mutation that prevents degradation of *hif-1* included in *otls197* is marked with *. (C) Survival of animals expressing HIF-1 from *unc-14* promoter exposed to H$_2$S. All animals have the null *hif-1(ia04)* mutation. Expression of HIF-1 was from extrachromosomal arrays. The *yakEx137* array expresses non-degradable HIF-1(P621A) and the *yakEx144* array expresses wild-type *hif-1*. For all panels animals were exposed to 50 ppm H$_2$S starting at L4. Average of three independent experiments is shown, each with n = 20-40 animals. Error bars are standard error of the mean (SEM).

Figure 2. The *unc-14* promoter is active in many non-neuronal cells.

(A) Visualization of GFP expressed from *Prab-3::hif-1::operon::GFP::H2B* (transgene *yak125*). Tail, head, and ventral cord neurons are shown of the ventral aspect of the same animal. VG = ventral ganglia. RVG = retrovesicular ganglia. In all images scale bar is 10 μm. (B) Representative images of adult *hif-1(ia04); Punc-14::hif-1::operon::GFP::H2B*
(transgene yakEx144) animals. GFP expression in hypodermal and intestinal cells is shown. Scale bar is 10 μm. (C,D) Representative images of (C) L1 and (D) adult transgenic animals expressing Punc-14::GFP (transgene yakEx142). Representative animals are shown with GFP expression in hypodermis, intestine, muscle, uterus, pharynx, and neurons. Scale bars are 5μm in (C) and 10μm in (D).

Figure 3. Survival in H₂S requires broad expression of hif-1.

Survival of animals exposed to H₂S. All animals have the null hif-1(ia04) mutation. (A) Lethality of animals that express hif-1 only in hypodermis (Pdpy-7::hif-1; yakEx143), intestine (Pvha-6::hif-1; yakEx136), or muscle (Punc-120::hif-1(P621A); otEx3165). As a control, hif-1 was expressed from a ubiquitous promoter (Peft-3::hif-1; yakEx131). Expression was from extrachromosomal arrays. Wild-type hif-1 was used for all constructs except the Punc-120::hif-1(P621A), which expresses the non-degradable variant. (B) Survival of hif-1(ia04); yakEx146 animals exposed to H₂S that express hif-1 simultaneously in intestine (Pvha-6::hif-1), hypodermis (Pdpy-7::hif-1), and neurons (Prab-3::hif-1). Average of three independent experiments is shown, each with n = 20-35 animals. Error bars are standard error of the mean (SEM).
Table 1: Strains used in this study

| Strain | Genotype Information |
|--------|----------------------|
| ZG31:  | hif-1(ia4) V         |
| DLM26: | hif-1(ia4) V; otEx3165[Punc-120::hif-1P621A, Pttx-3::RFP] |
| XZ2056:| hif-1(ia4) V; yakEx126[Punc-17::hif-1cDNA, Pmyo-2::mCherry] |
| DLM25: | hif-1(ia4) V; otIs197[Punc-14::hif-1P621A, Pttx-3::RFP] |
| XZ2065:| hif-1(ia4) V; yakEx131[Peft-3::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2074:| hif-1(ia4) V; yakEx136[Pvha-6::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2073:| hif-1(ia4) V; yakEx137[Punc-14::hif-1P621A::YFP, Pmyo-2::mCherry] |
| XZ2081:| hif-1(ia4) V; yakEx143[Pdpy-7::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2080:| yakEx142[Punc-14::GFP, Pmyo-2::mCherry] |
| XZ2082:| hif-1(ia4) V; yakEx144[Punc-14::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2083:| hif-1(ia4) V; yakEx145[Punc-47::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2084:| hif-1(ia4) V; yakEx125[Prab-3::hif-1cDNA, Pmyo-2::mCherry] |
| XZ2085:| hif-1(ia4) V; yakEx146[Pvha-6::hif-1cDNA, Pdpy-7::hif-1cDNA, Prab-3::hif-1cDNA, Pmyo-2::mCherry] |
Table 2: Plasmids and constructs used in this study

Gateway entry clones

| Plasmid   | Description                                      |
|-----------|--------------------------------------------------|
| pCFJ326   | tbb-2 3’UTR::OPERON::GFP [2-3]                  |
| pCFJ386   | Peft-3[4-1] 625 bp upstream and including ATG   |
| pCR110    | GFP[1-2]                                         |
| pEGB05    | Prab-3 [4-1] 1232 bp upstream of ATG            |
| pET168    | hif-1 cDNA A isoform [1-2]                      |
| pET210    | Punc-14[4-1] 1425 bp upstream of ATG            |
| pGH1      | Punc-17 [4-1] 3229 bp upstream and including the ATG |
| pMH522    | Punc-47 [4-1] 1254 bp upstream and including the ATG |
| pET187    | Pdpy-7[4-1] 351 bp upstream and including the ATG |
| pET188    | Pvha-6[4-1] 881 bp upstream and including the ATG |

Gateway expression constructs

| Gateway expression construct | Description                                      |
|------------------------------|--------------------------------------------------|
| pET171                       | Punc-47::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET172                       | Punc-17::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET182                       | Prab-3::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET187                       | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET188                       | Pvha-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET212                       | Punc-14::GFP::let-858 3’UTR_pCFJ150               |
| pET213                       | Punc-14::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
| pET216                       | Peft-3::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP_pCFJ150 |
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Figure 1

A

Percent dead after 24h H$_2$S

- wild type
- hif-1
- ols197
- yakEx125
- yakEx126
- yakEx145
- [Punc-14::hif-1P621A]
- [Prab-3::hif-1]
- [Punc-17::hif-1]
- [Punc-47::hif-1]


B

hif-1 isoforms

- hif-1a
- hif-1e


C

Percent dead after 24h H$_2$S

- hif-1
- hif-1, yakEx137
- [Punc-14::hif-1P621A]
- hif-1, yakEx144
- [Punc-14::hif-1]
Figure 2

A. Prab-3::hif-1::operon::GFP::H2B

- Head neurons
- Tail neurons
- Ventral cord neurons

B. Punc-14::hif-1::operon::GFP::H2B

- Hypodermal cells
- Intestinal cells

C. L1 Punc-14::GFP

- Head
- Mid body
- Body hypodermal cells
- Intestine
- Tail

D. Adult Punc-14::GFP

- Ventral cord neurons
- Head neurons
- Hypodermal cells
- Intestine
- Body wall muscle cells
- Uterus
- Pharynx
Figure 3

A

B

Percent dead after 24h H₂S

hif-1

yakEx136

(Pvha-6::hif-1)

dEx136

[Punc-120::hif-1P621A]

yakEx131

[Pafr-3::hif-1]

Percent dead after 24h H₂S

hif-1

yakEx146

(Pvha-6::hif-1)

yakEx143

(Pdpy-7::hif-1)

hif-1; yakEx146

(Pvha-6::hif-1)

yakEx136

(Pdpy-7::hif-1)

hif-1; yakEx143

(Pvha-6::hif-1)