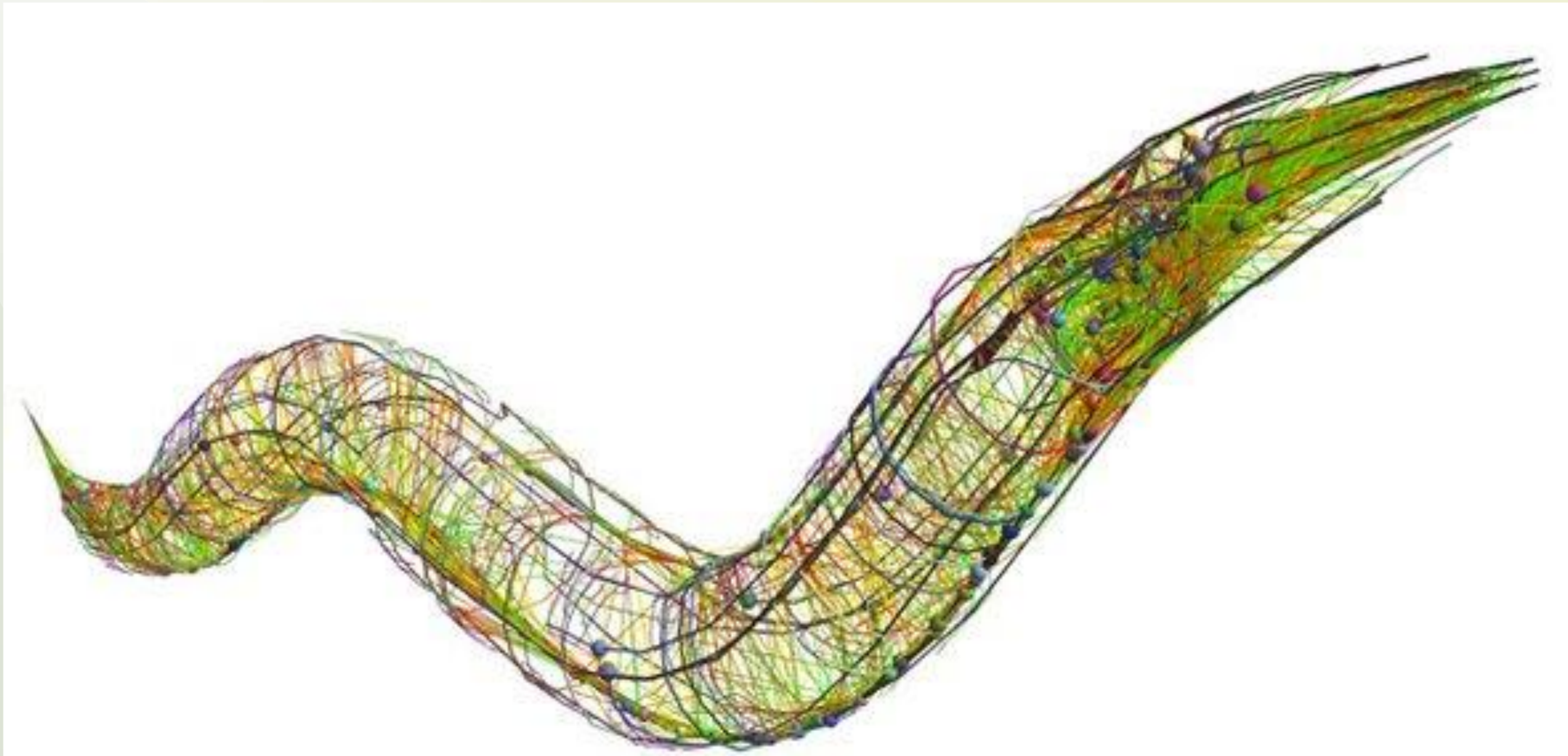
A druggable in vivo model of paraganglioma



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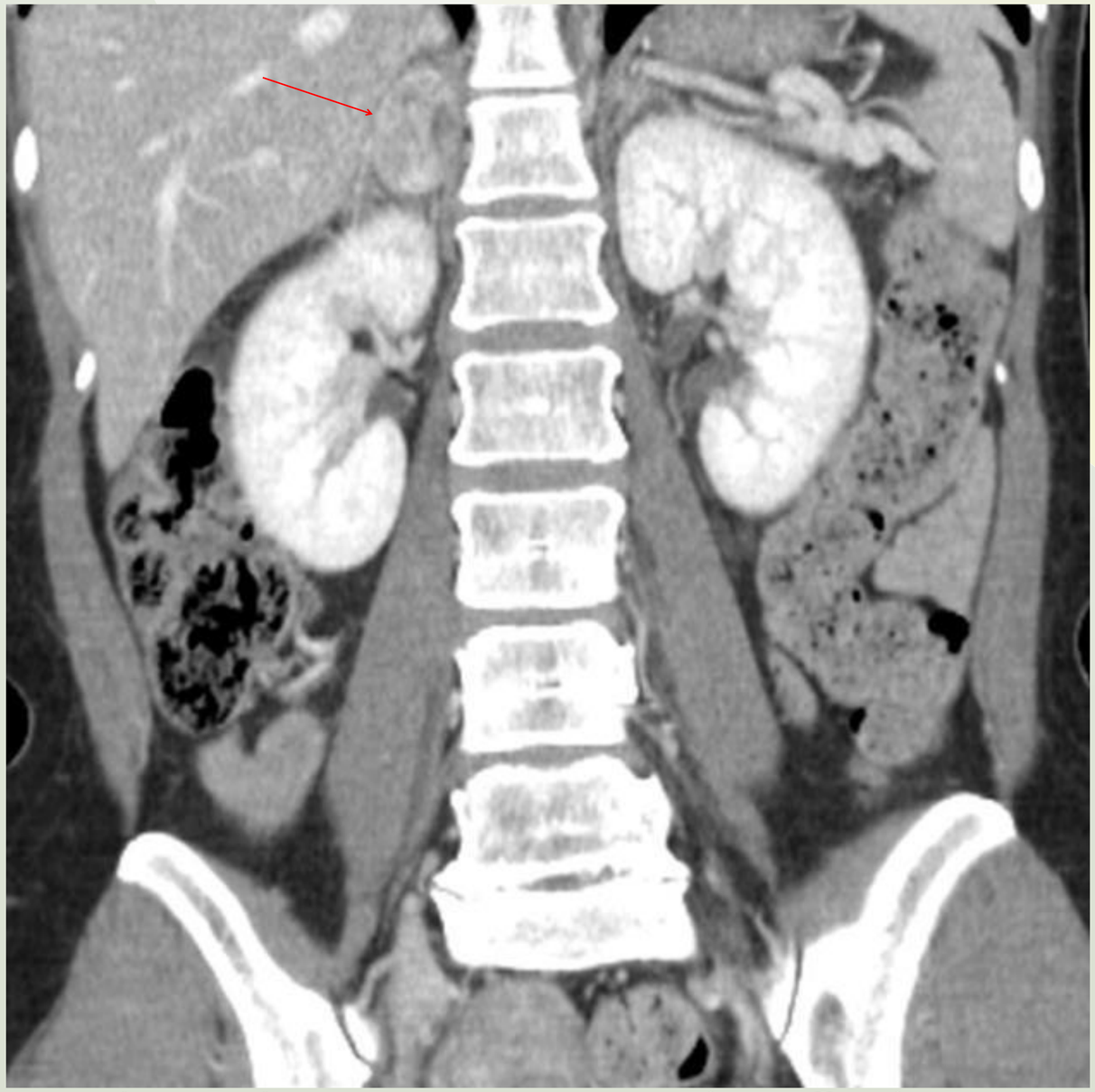
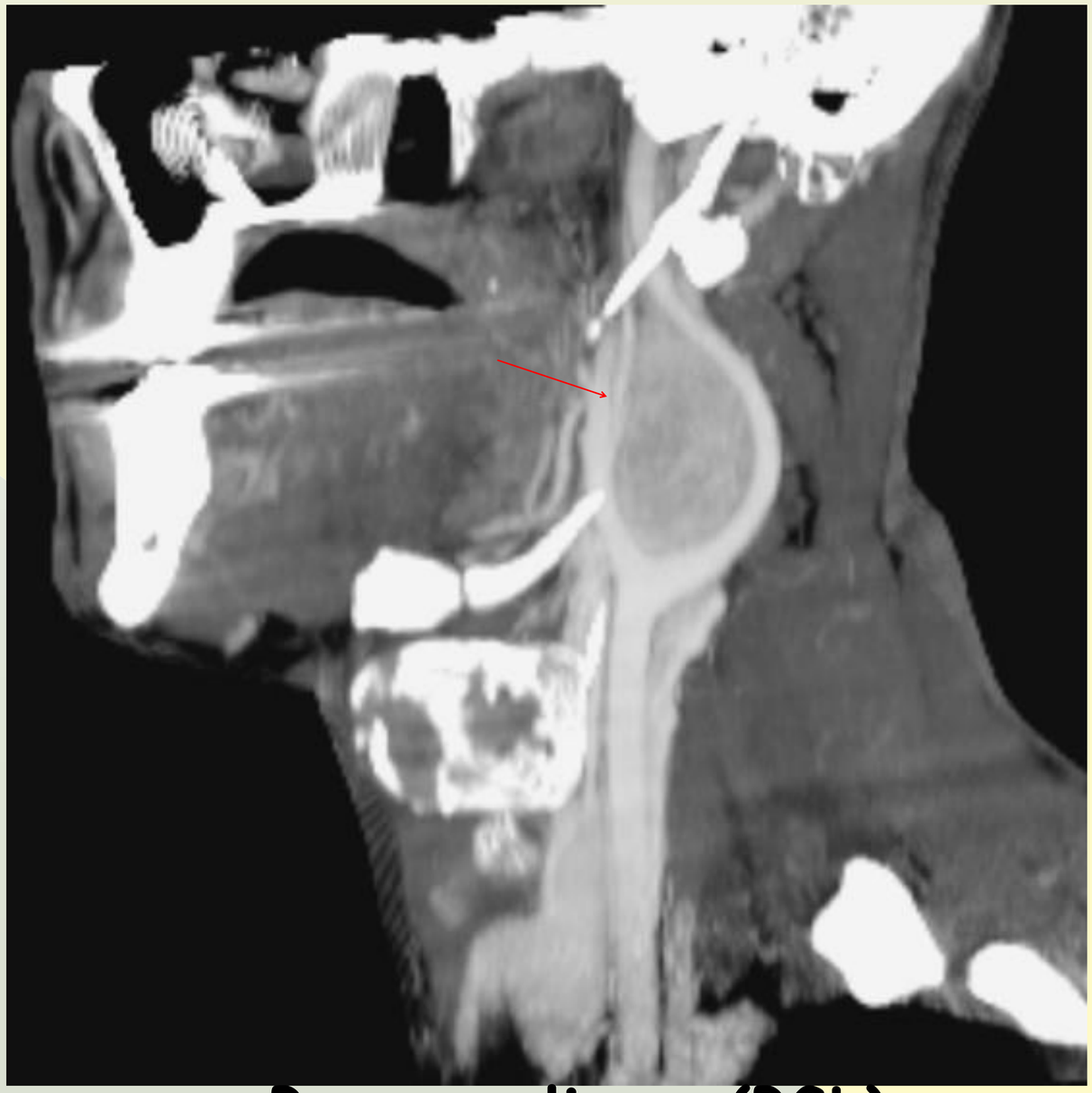
Pheochromocytoma (PHEO) and paraganglioma (PGL) - PPGL

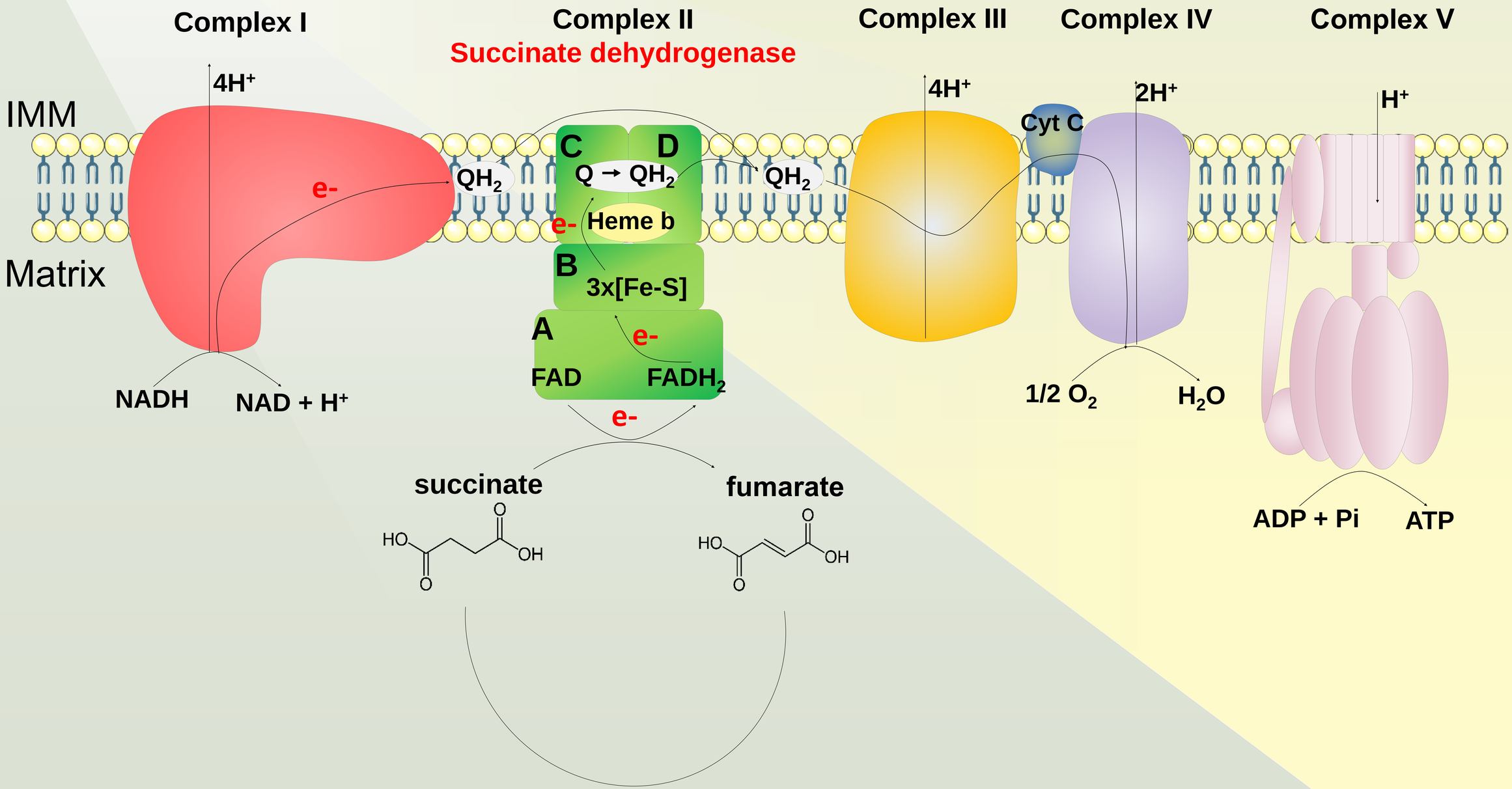


Pheochromocytoma (PHEO)



Paraganglioma (PGL)

The SDH (succinate dehydrogenase) complex



TCA cycle

SDHB mutations predispose to malignant PPGLs

Caenorhabditis elegans encodes a highly conserved *SDHB* orthologue *sdhb-1*


```
SDHB_HUMAN    --------------MAAVVALSLRRRLPATTLGGACLQASRGAQTAAATAPRIKKFAIYRW
SDHB_CAEL     MLARSARLLHSAELAANAIRAASGAPATAAAAEASFPSTDDVAAKTKKTGNRIKTFEIYRF

SDHB_HUMAN    DPDKAGDKPHMQTYEVDLNKCGPMVLDALIKIKNEVDSTLTFRRSCREGICGSCAMNINGG
SDHB_CAEL     NPEAPGAKPTVQKFDVDLDQCGTMILDALIKIKNEVDPTLTFRRSCREGICGSCAMNIGGQ

SDHB_HUMAN    NTLACTRRIDTNLNKVSKIYPLPHMYVIKDLVPDLSNFYAQYKSIEPYLKKKDESQEGKQQ
SDHB_CAEL     NTLACICKIDSDTSKSTKIYPLPHMFVVKDLVPDMNLFYAQYASIQPWIQKKTPLTLGEKQ

SDHB_HUMAN    YLQSIEEREKLDGLYECILCACCSTSCPSYWWNGDKYLGPAVLMQAYRWMIDSRDDFTEER230
SDHB_CAEL     MHQSVAERDRLDGLYECILCACCSTSCPSYWWNADKYLGPAVLMQAYRWVIDSRDDYATER244

SDHB_HUMAN    LAKLQDPFSLYRCHTIMNCTRTCPKGLNPGKAIAEIKKMMATYKEKKASV----   280
SDHB_CAEL     LHRMHDSFSAFKCHTIMNCTKTCPKHLNPAKAIGEIKSLLTGFTSKPAAEPSAF   298
```

Ubiquinone bindig site
2Fe-2S cluster
4Fe-4S cluster
3Fe-4S cluster

Human:	Arg230His	(G689A)
C. elegans:	Arg244His	(G731A)

Identity in amino acid sequences: 60%
Similarity in amino acid sequences: 84%

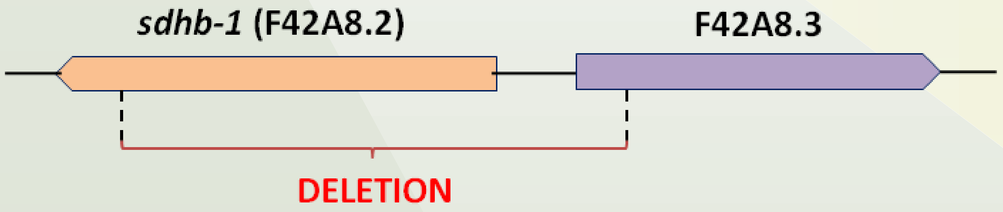
A wild-type *sdhb-1* transgene rescued the *gk165* null mutation efficiently

sdhb-1(gk165)/mIn1 [mIs14 dpy-10(e128)] II.

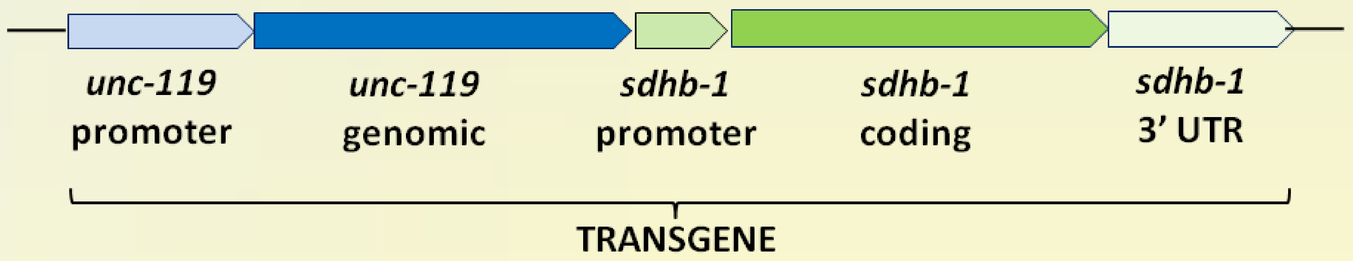
X

EG6705. 3[pNU637 (Psdhb-1_sdhb-1(genomic wt)_UTRsdhb-1;unc-119(+))]X;unc-119(ed3)III

Chromosome: II



Strain: EG6705.3
Chromosome: X



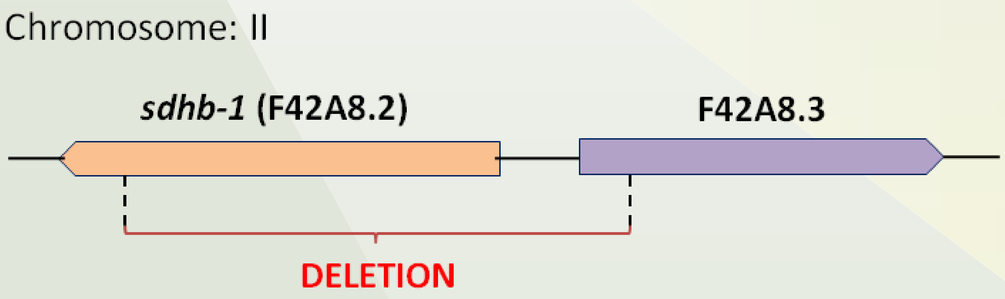
With help of Knudra Transgenics, Murray, Utah

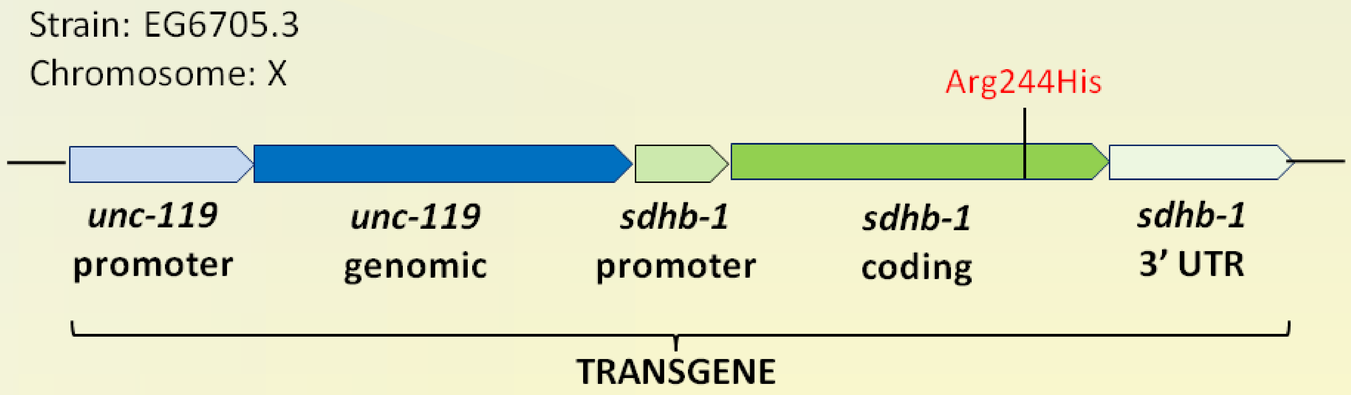
The Arg244His point mutant version did not rescue the *gk165* null mutation

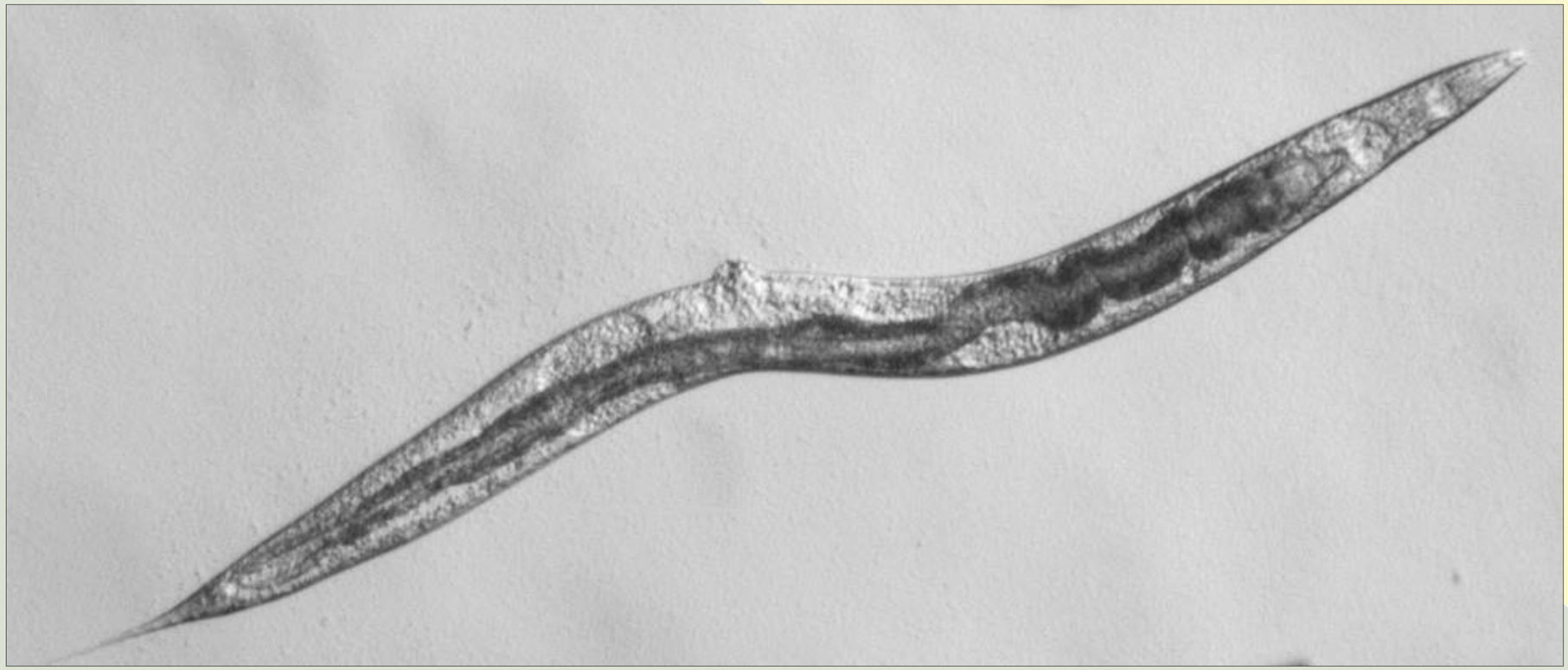
sdhb-1(gk165)/mIn1 [mIs14 dpy-10(e128)] II.

X

EG6705. 3[pNU637 (Psdhb-1_sdhb-1(G731A) _UTRsdhb-1;unc-119(+))]X;unc-119(ed3)III

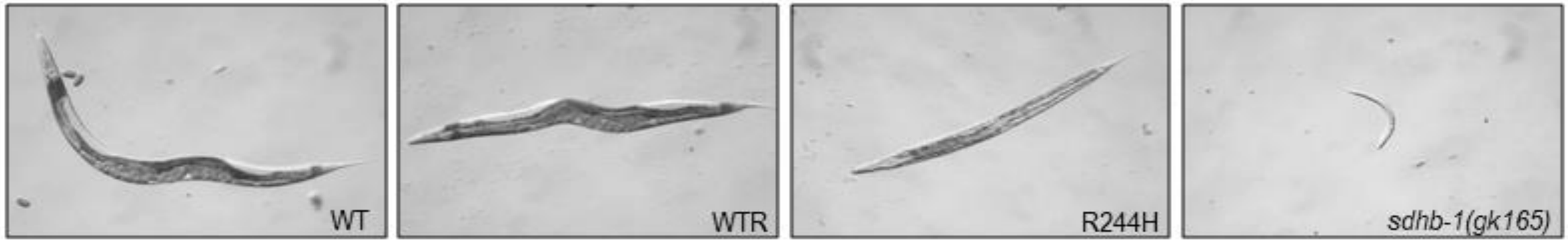


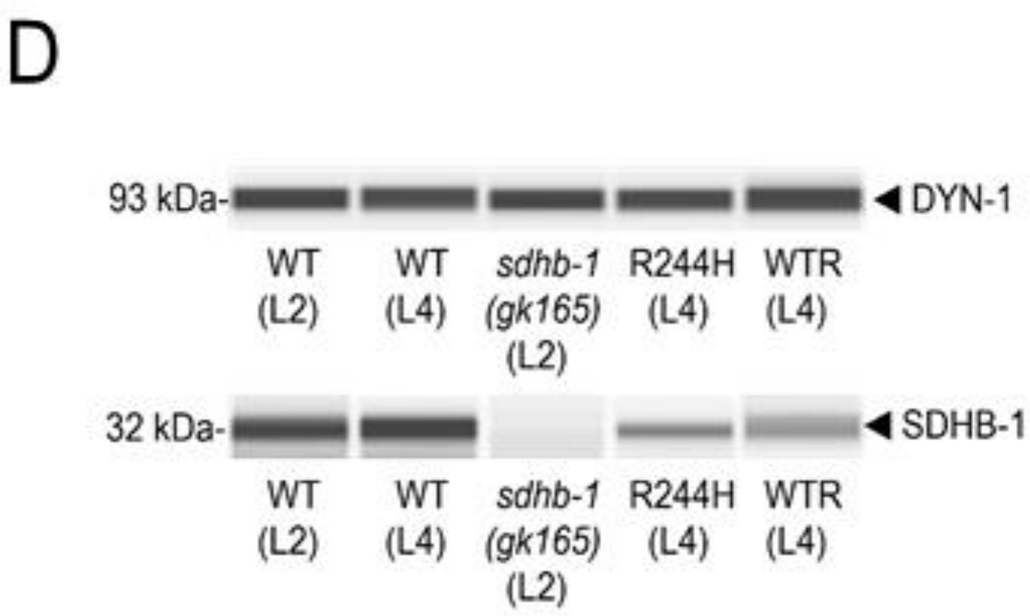


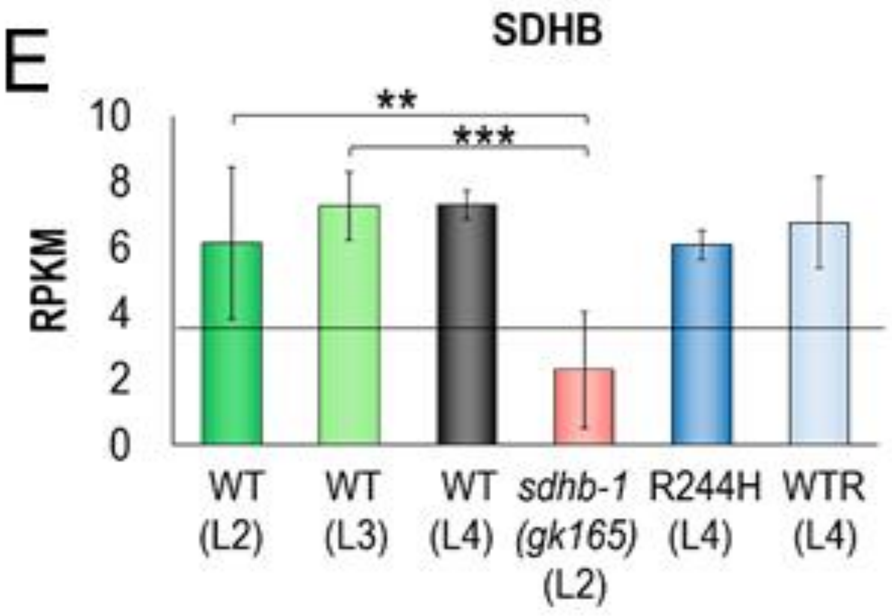


Our model: the Arg244His point mutant, which shows a protruding vulva (Pvl) and sterility

To characterize our model, we compared R244H worms with null mutants and wild-type worms



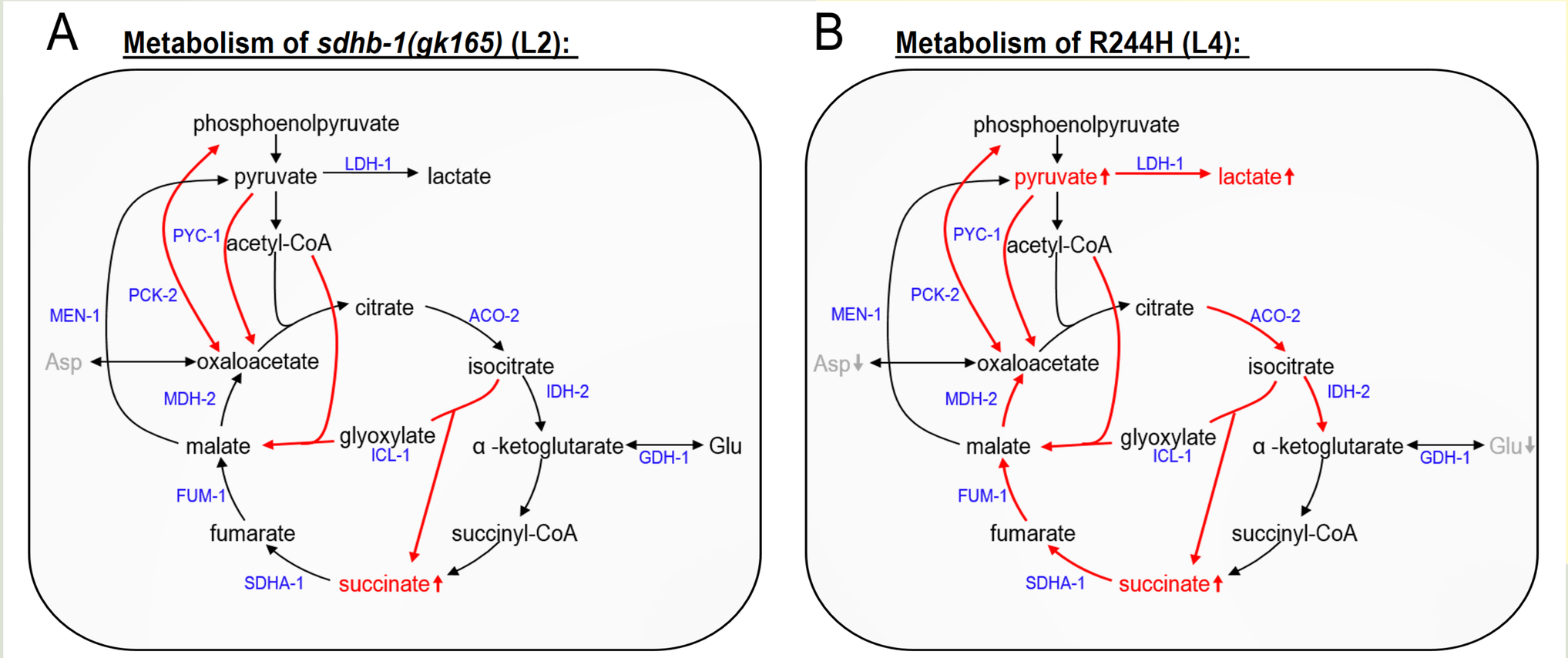




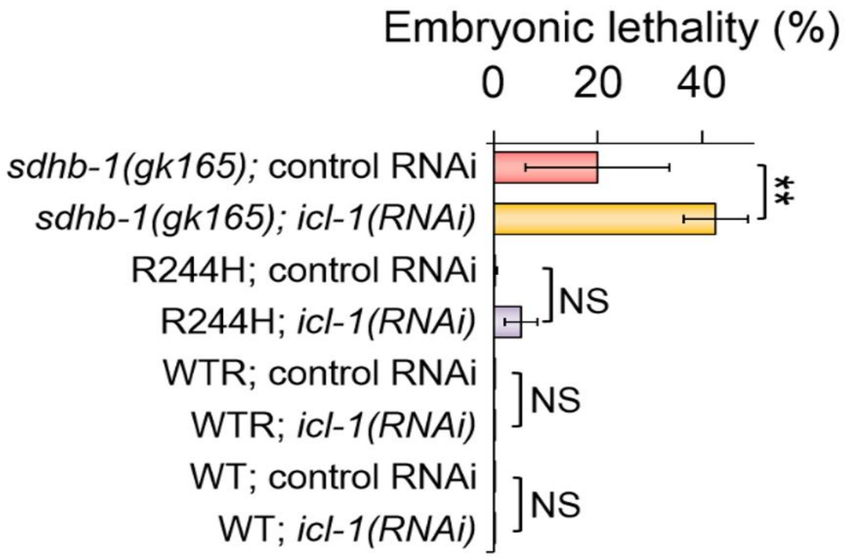
Tools used: Wes, LC-MS to measure TCA cycle metabolites, Seahorse to measure oxygene consumption,measuring mitochondrial and ATP content, bioinformatics, transcriptomics, lifespan measurements.

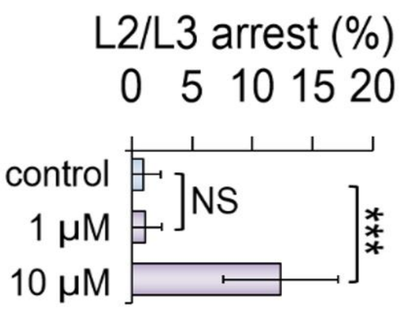
R244H worms develop further than *sdhb-1(-)* null mutants and show high glycolytic activity

- R244H worms and *gk165* null mutants have a similar succinate/fumarate ratio and oxygene comsumption
- Based on the above data in R244H mutants the SDH enzyme complex is inactive (this is supported by bioinformatics data)
- The glyoxylate cycle is of key importance in the metabolism of null mutants
- **R244H animals show elevated pyruvate and lactate levels, increased lactate dehydrogenase (LDH-1) activity: rewired metabolism reminiscent of tumor cells**



Our nematode model is drug responsive





RNAi treatment specific for *icl-1*, the key enzyme of the glyoxylate cycle caused embryonic lethality of *sdhb-1* null mutant worms

LDHA inhibitor treatment resulted in arrested development of R244H point mutant worms

Future experiments:

-We intend to examine whether the increased glycolitic activity is linked to HIF1 activation.

-Analysis of the neuroendocrine uv1 cells in *sdhb-1* R244H mutant background

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RESEARCH ARTICLE

The SDHB Arg230His mutation causing familial paraganglioma alters glycolysis in a new *Caenorhabditis elegans* model

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Anil Mehta, Jo Williamson, Gordon Stewart

The Phaeo and Para Cancer Charity

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