

Supplemental Information for:

Arrested oocyst maturation in *Plasmodium* parasites lacking type II NADH:ubiquinone dehydrogenase

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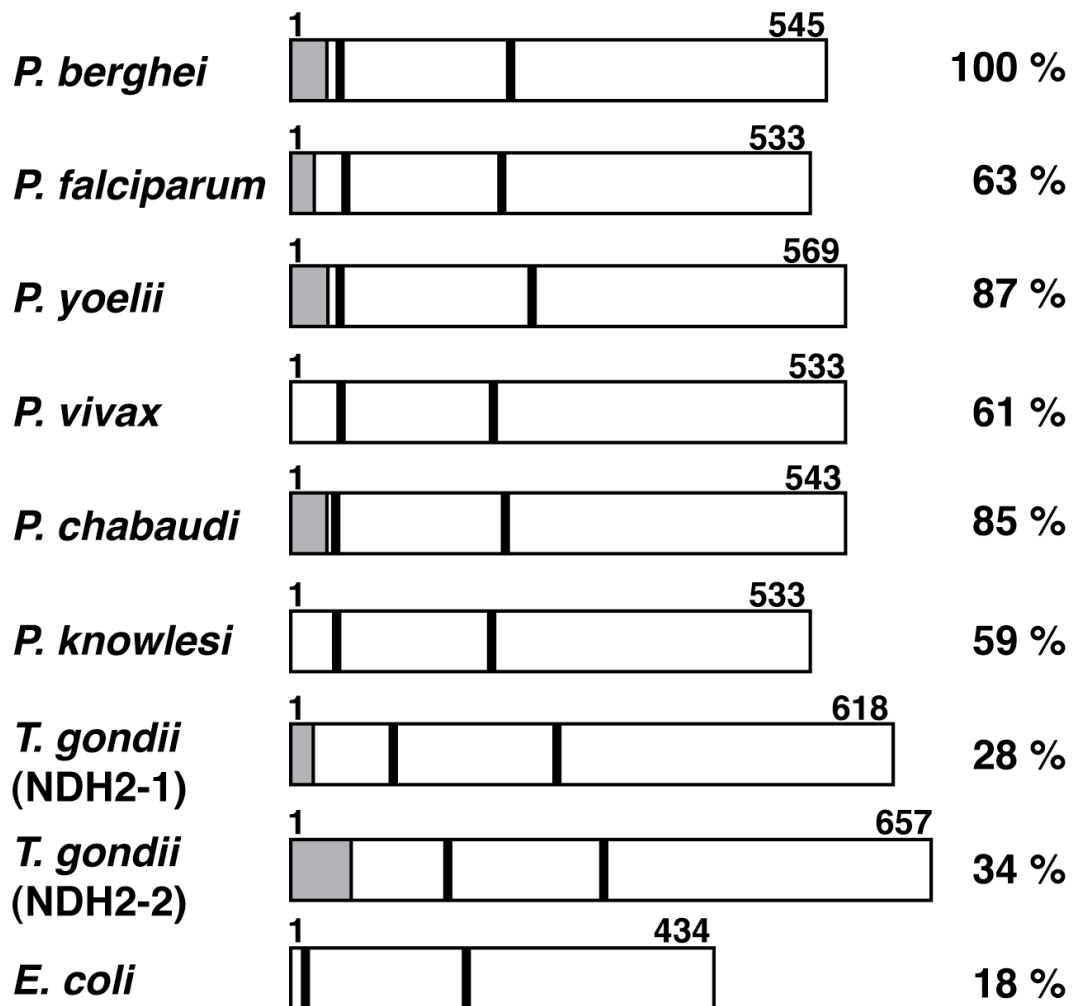
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Supplemental Movie 1: Representative gliding motility of two *ndh2(-)* ookinetes in Matrigel. Time lapse is indicated (upper right). Spherical bodies: p28-labelled magnetic beads (Dynabeads) used for purification of ookinetes.

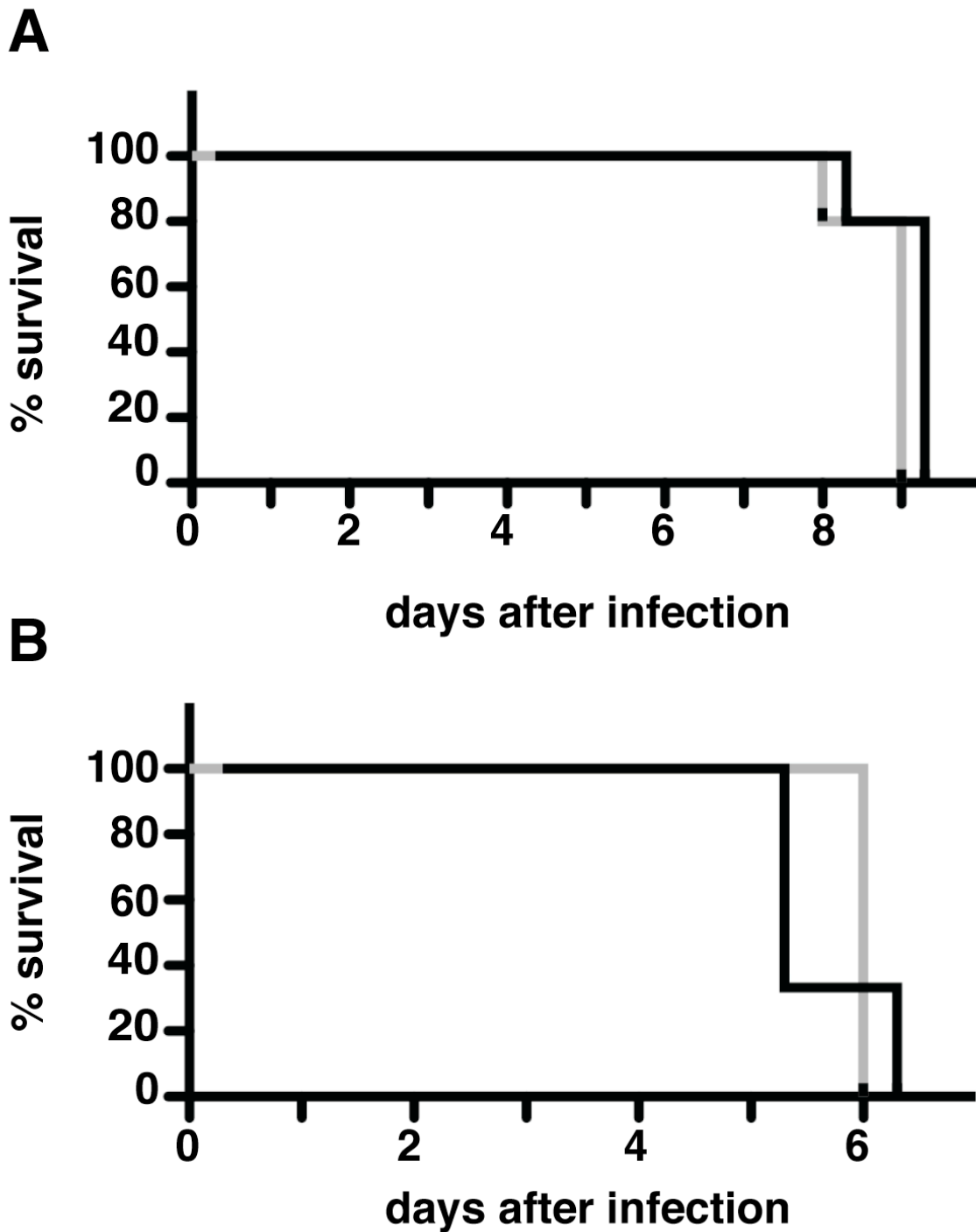
Supplemental Movie 2: Representative gliding motility of two wild type ookinetes in Matrigel. Time lapse is indicated (upper right). Spherical bodies: p28-labelled magnetic beads (Dynabeads) used for purification of ookinetes.

Boysen, Suppl. Figure 1



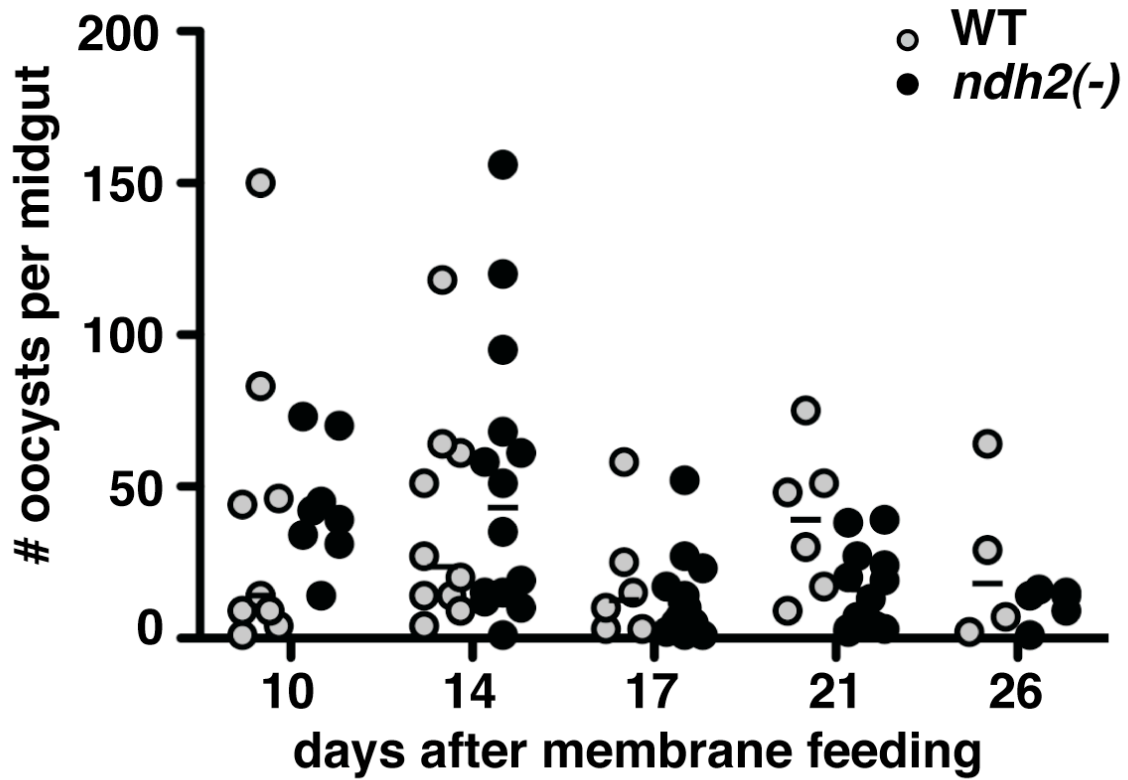
Supplemental Figure 1: Primary structure of the type II NADH:oxidoreductases (NDH2).

Shown are the overall sequence structures and amino acid sequence identities (%) of NDH2 orthologs in *Plasmodium falciparum* (gi:124506848), *P. yoelii* (gi:83318041), *P. vivax* (gi:156097305), *P. chabaudi* (gi:70935187), *P. knowlesi* (gi:221054565), *Toxoplasma gondii* (NDH2-1, gi: 237840755; NDH2-2, gi: 78057337) and *Escherichia coli* (gi: 16129072) in comparison to the predicted *P.berghei* protein (gi:68062086). Mitochondrial targeting sequences (MitoProt (1)) and signature GxGxxG motives are displayed as grey boxes and black bars, respectively. The N-terminal GxGxxG motif most likely binds flavin, while the more C-terminal GxGxxG motif is expected to bind NADH.



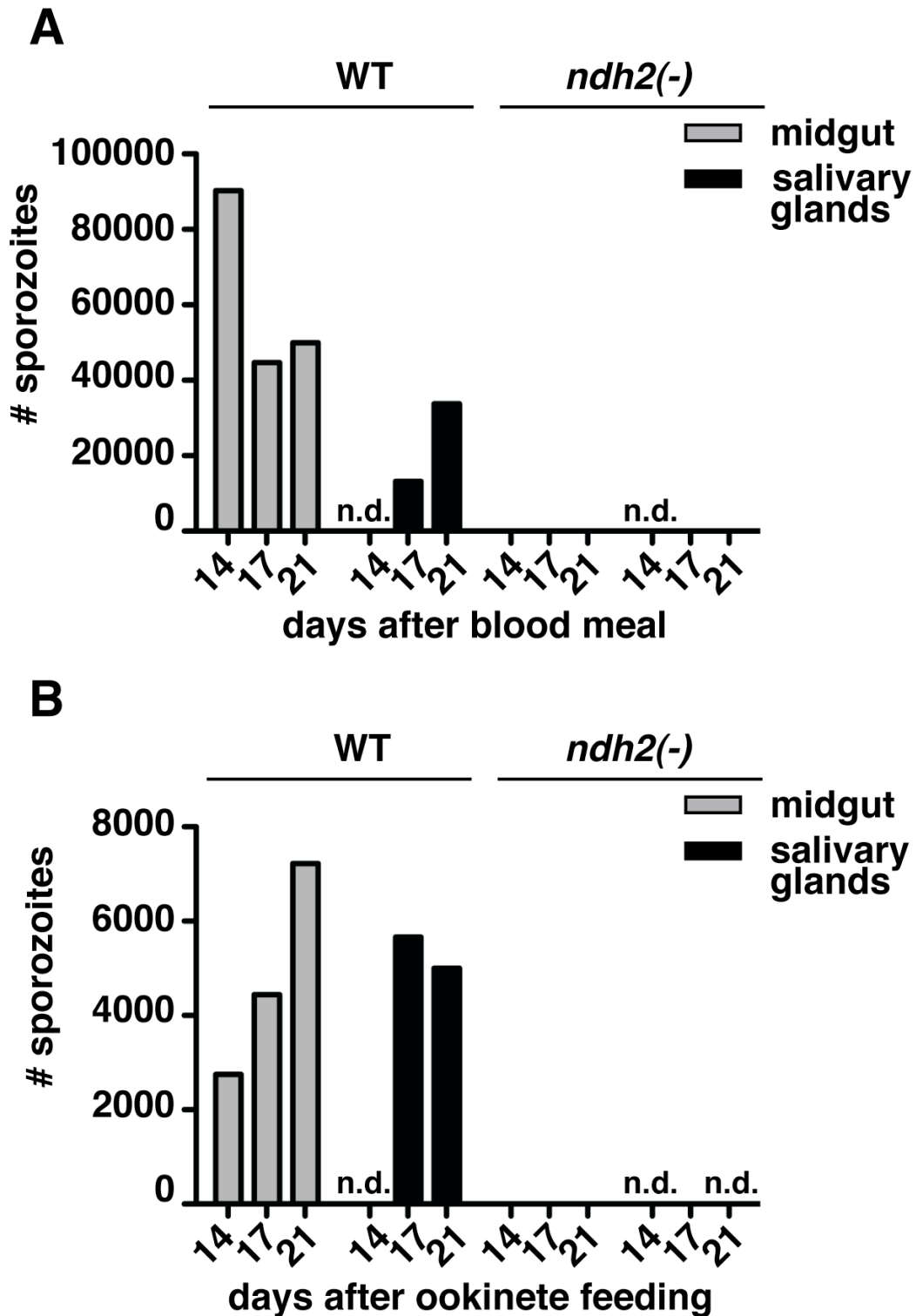
Supplemental Fig. 2: Animals infected with *ndh2*⁻ parasites develop signature symptoms of experimental cerebral malaria.

Shown is a Kaplan-Meier survival analysis of C57bl/6 mice infected with WT (ANKA-GFP, grey line) and *ndh2*⁻ (black line) parasites after injection of 1,000 (A) and 1,000,000 (B) blood stage parasites. N = 5 (A) and 3 (B), respectively.



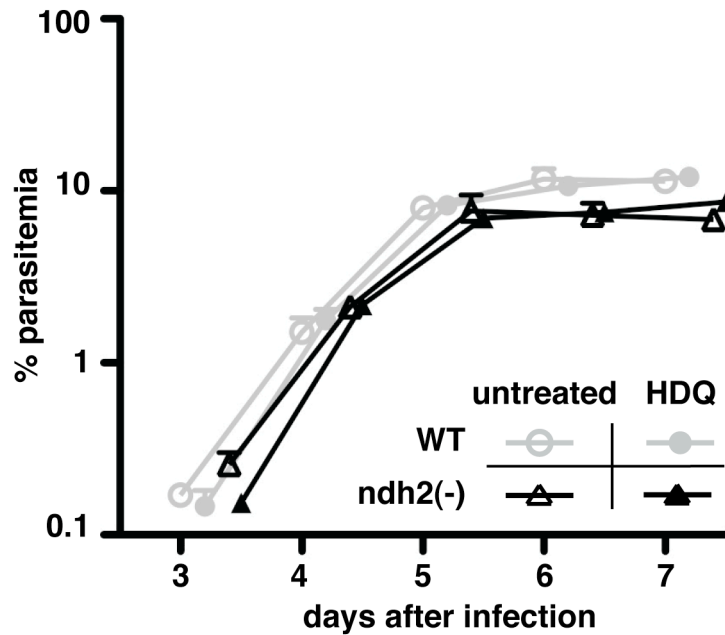
Supplemental Fig. 3: Oocyst load after membrane feeding of cultured *ndh2(-)* and WT ookinetes.

Oocysts were scored from infected midguts between day 10 and day 26 after infection with WT (grey) or *ndh2(-)* (black) parasites. *ndh2(-)* data are based on two feedings with ko1 and ko2 ookinetes. The median is indicated. The Mann-Whitney test was applied for each day shown and revealed no significant differences in oocyst numbers.



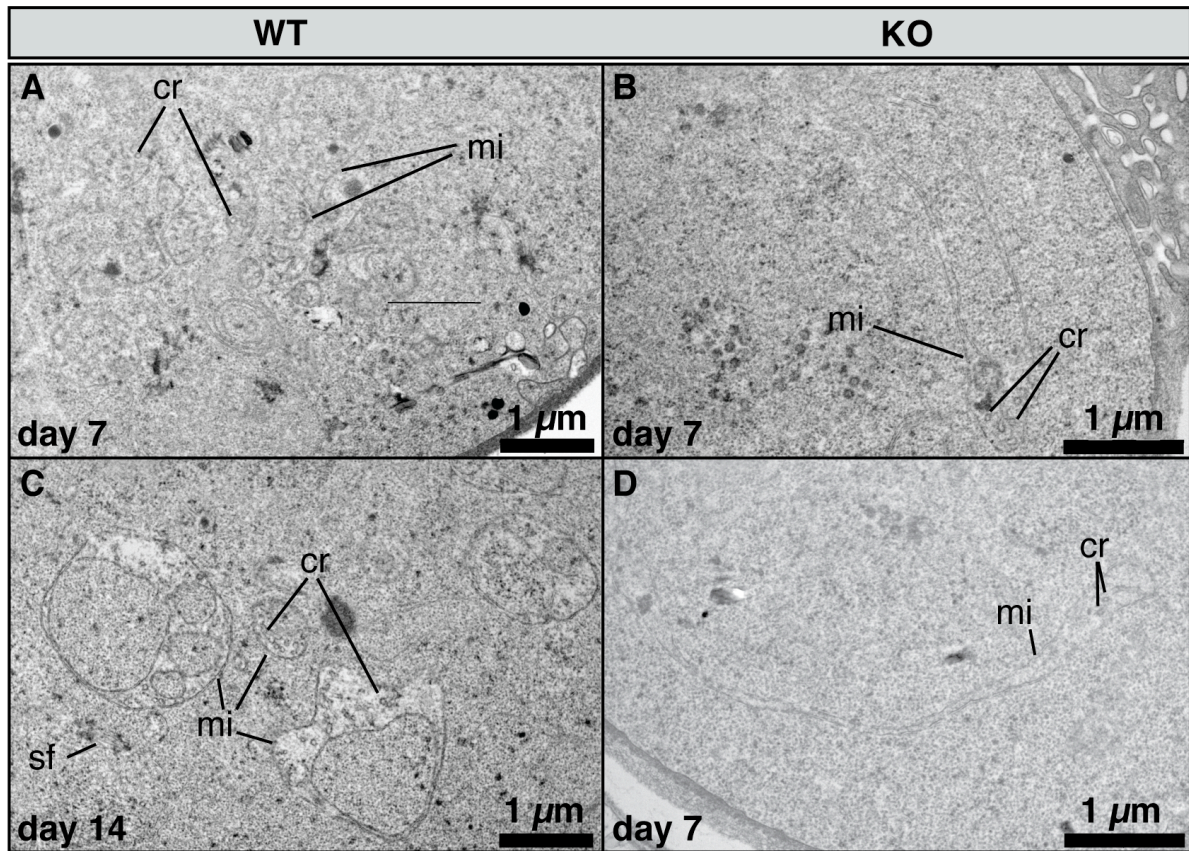
Supplemental Fig. 4: *ndh2(-)* oocysts do not develop into sporozoites.

Sporozoites isolated from oocysts or salivary glands were counted after a blood meal (A) or membrane feeding of cultured ookinetes (B). *ndh2(-)* data are based on two independent feedings with ko1 and ko2 parasites. Shown are the numbers of sporozoites isolated from 20 mosquitoes, normalized to infectivity.



Supplemental Fig. 5: *ndh2(-)* parasites in combination with HDQ treatment cause high-level parasitemia *in vivo*.

Displayed are *in vivo* growth curves of WT (grey) and *ndh2(-)* (black) parasites, either under treatment with 50 mg/kg body weight HDQ (filled symbols) or untreated (open symbols). Animals (n=3) were injected intravenously with 1,000,000 asexual parasites of the respective parasite populations. HDQ was injected daily, starting on the second day of infection. Parasitemia was determined every 24 hours by microscopic examination of Giemsa-stained blood smears.



Supplemental Fig. 6: Mitochondria in both WT and *ndh2(-)* oocysts are elongated, branched and cristate.

Transmission electron microscopy on immature oocysts. **A** and **B** are close ups of pictures shown in **Fig. 6**. cr: cristae, mi: mitochondrion, sf: spindle fibers

Supplemental Reference:

1. Claros, M. G., and Vincens, P. (1996) *Eur. J. Biochem.* **241**, 779-786

Supplemental Table 1: Nucleotide primers used in this study

Experiment	Oligonucleotide	Sequence 5' → 3'	Restriction site
RT qPCR	ndh2(-)GFP for	acacacctttgcatggtaac	
	ndh2(-)GFP rev	tgttggccatggaacaggtag	
	NDH2 for	gttatttaggatcaggatggggtg	
	NDH2 rev	cactacataaacaaggtaacaaaggag	
	GFP for	gatggaagcgttcaactagcagacc	
	GFP rev	agctgttacaactcaagaaggacc	
	Hsp70 for	aagaagctgaagctgatgctctcc	
	Hsp70 rev	agttcatacctcctggcattctcc	
	Qarts for	gagaggggaagaaatattatcagg	
	Qarts rev	gagcactctctaaacctatacc	
	G3PDH for	gttggaacaacagatgaacagcgtcc	
	G3PDH rev	ctaaaggctgaaatccacaccaagcag	
	DHOD for	gcctctagttttgtaaattggctc	
	DHOD rev	cactgactcctcctttttatcttcg	
	MQO for	gaatatagttgttacctgtggcagg	
	MQO rev	cagctgcaaattggcaatgctg	
SucDH for	gatcggattggctaggtgatcag		
SucDH rev	gcttgcctcctttaccgt		
ndh2(-) vector	5' KO flank for ^a	atcaagc actagtt ctaattgtgcgtggtatac	<i>SpeI</i>
	5' KO flank rev	caatata catatg ttaacctgcaaaggtgtg	<i>NdeI</i>
	3' KO flank for	taagctt ggccattctactgatctgacatgttag	<i>HindIII</i>
	3' KO flank rev ^b	taggtacc gtacaaatccgagctttccctc	<i>KpnI</i>
ndh2(-) test	5' integration for	aacctgaaagggtagaaagatgtccatcac	
	5' integration rev	cccgcacggacgaatccagatgg	
	3' integration for	cgcattatatgagttcattttacacaatcc	
	3' integration rev	atattcaggaaaggatatacacatgtcgtc	
	WT for ^c	ggag cgggccg caaattctttaataataaaag gag	<i>NotI</i>
	WT rev ^d	catgt cactagt gtagaatggcctacc	<i>SpeI</i>
NDH2_ mCherry vector	NDH2mCherry for ^c	ggag cgggccg caaattctttaataataaaag gag	<i>NotI</i>
	NDH2mCherry rev ^d	catgt cactagt gtagaatggcctacc	<i>SpeI</i>
NDH2_ mCherry test	5' integration for ^a	atcaagc actagtt ctaattgtgcgtggtatac	<i>SpeI</i>
	5' integration rev	cagcttcaagtagtcggggatgtcg	
	WT for ^a	atcaagc actagtt ctaattgtgcgtggtatac	<i>SpeI</i>
	WT rev ^b	taggtacc gtacaaatccgagctttccctc	<i>KpnI</i>

Oligonucleotides used for two applications are labeled ^{a-d}.