Supplementary Information

Genome editing retraces the evolution of toxin resistance in the monarch butterfly

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Contents

Supplementary Text

Ancestral sequence reconstruction of coevolving sites in ATPα, and associations with feeding/sequestration

Insect supertree reconstruction

To study the evolution of organismal traits and of ATPα sites associated with specialization on cardiac glycoside-containing plants, we first estimated a phylogeny of insect species adapted to feeding on plants that produce cardenolides and/or bufadienolides[6], the non-adapted close relatives of these insects, and two vertebrate outgroups, *Sus scrofa* and *Squalus acanthias*. Phylogenetic relationships between all surveyed species were obtained from previous studies (see **Suppl. Table 1** for references) using a supertree approach. These phylogenetic studies used overlapping but non-identical molecular markers, and thus, branch lengths were estimated on this fixed species topology. We used the full-length coding sequences of *ATPα*, and the *COI* gene that codes for the mitochondrial cytochrome *c* oxidase subunit 1 and is widely used in phylogenetic studies, which were available for most species (see **Suppl. Table 2** for ID numbers). The data was implemented in the IQ-TREE web server version 1.6.6[31], in which all settings were left to default, with the exception of using the FreeRate heterogeneity model[55,56]. The output tree was used for all subsequent analyses. We obtained *ATPα* sequences and information on the level of specialization on cardiac glycoside-producing host plants, including presence/absence of feeding on plants producing cardiac glycosides and presence/absence of cardiac glycoside sequestration from the literature (no new data was collected, see **Suppl. Table 3** for references)[7-11,28,57-60]. Even though the aphid *Myzus persicae* has been reported to (passively) sequester cardiac glycosides, this species has a wide host range and is not a specialist on cardiac glycoside-producing host plants like the aphid *Aphis nerii*[60]. Therefore, we decided not to categorize *M. persicae* with insects that are specialized on cardiac glycoside-producing host plants for ASR (see below).

Based on the literature cited, we assumed that amino acid substitutions in the H1-H2 extracellular loop of ATPα are fixed substitutions in the included species. Data for multiple individuals per species were only available for some of the *Danaus* and *Drosophila* species, and polymorphic non-synonymous substitutions were only detected in *D. subobscura*[27]. Because some studies did not include closely related non-cardiac glycoside-specialized species, additional sequences from close relatives were downloaded from PDB, GenBank, LepBase, and InsectBase for the Coleoptera, Diptera and Lepidoptera, when sequences of *ATPα* were available. These sequences included those from additional species of Diptera to reconstruct the evolution of resistance in this group[7,27,59]. Additional Coleoptera and Lepidoptera species were included to reconstruct the four separate instances of the evolution of cardiac-glycoside resistance in each order[9,10,57,58]. A complete list of sequences included, along with their source databases, ID numbers, and references, can be found in **Suppl. Table 2**.

Associations between sequence evolution of ATPα residues and feeding on cardiac glycoside-producing plants and toxin sequestration

We focused our analyses on the first extracellular loop (H1-H2 domain) of ATPα, and, using TraitRateProp[37], identified residues whose rate of amino acid sequence evolution was associated with changes in character states for feeding on cardiac glycoside-producing plants. The joint likelihood framework of TraitRateProp tests the hypothesis of an association between a trait and the evolutionary rate of a sequence, and where an association is found, it computes the site-specific Bayes factor between the rate of sequence evolution at each site and the trait. This analysis required an ultrametric rooted tree, a multi-sequence alignment, and trait data. The ultrametric tree was constructed using the makeChronosCalib function in the ape package in R[33], using a calibration range of 346-401 MYA for the origin of Hemiptera + Thysanoptera, given by the confidence interval estimated from Misof *et al.*[61]. Trait data can be found in **Suppl. Table 3**.

Epistasis/co-evolution of ATPα amino acid sites

To detect candidate intramolecular epistatic interactions or co-evolution between amino acid sites within the H1-H2 loop of ATPα that might constrain or enable the evolution of TSI to cardiac glycosides, we used the Spidermonkey/Bayesian graphical model (BGM) method in HyPhy version 2.3.14[35,38]. We used the default substitution models (HKY85 nucleotide and Muse-Gaut codon substitution models) and assumed a maximum of two parents per node. The MCMC chain was run for 100,000 iterations, with 10,000 burn-in, and sampled every 1,000 iterations. Sites in this analysis were filtered based on a minimum number of five substitutions across the phylogeny. Interactions between sites with a marginal posterior probability (PP) > 0.5 were considered statistically significant. For the full results of this analysis, see **Extended Data Fig. 1**.

ASR of amino acid sequence and feeding/sequestering states

ASR of amino acid sequences of the H1-H2 extracellular loop of ATPα was performed using joint maximum likelihood methods implemented in HyPhy version 2.3.14[34,35]. A custom code to execute this analysis was generously provided by Art Poon and is available[38]. ASR of cardiac glycoside feeding and sequestration was performed using the ace function of the ape package in R[33], with feeding and sequestering considered as one ordinal trait. Three models for discrete character evolution were considered (equal rates, symmetric, and all rates different). Statistical significance between likelihoods for each model was tested using a chi-square test. The amino acid sites identified as coevolving and significantly associated with feeding and sequestering were 111, 119, and 122. The number of substitutions at these three sites that occurred across each branch based on the ASR of the H1-H2 loop was then mapped onto the insect tree, along with ancestral states of feeding and sequestering, and visualized using the Interactive Tree Of Life (iTOL)[36].

Additional References for Supplementary Text

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Supplementary Tables 1 – 8

Supplementary Table 1. Phylogenetic relationships used in reconstruction of the insect supertree.

Clade	References for insect species phylogeny reconstruction
Pterygota (winged insects)	Misof, Science, 2014
	Rainford, PLoS ONE, 2014
	Wiens, Nat Commun, 2015
Hemiptera	Bramer, Proc R Soc B, 2015
	Dobler, PNAS, 2012
	Li, Sci Rep, 2015
	Rainford, PLoS ONE, 2014
	Thorpe, BMC Genomics, 2016
	Wiens, Nat Commun, 2015
	Zhen, Science, 2012
Hymenoptera	Boevé, BMC Evol Biol, 2013
	Dobler, PNAS, 2012
	Dobler, Entomol Exp et Appl, 2015
	Peters, Curr Biol, 2017
	Rainford, PLoS ONE, 2014
	Wiens, Nat Commun, 2015
Coleoptera	Dobler, PNAS, 2012
	Gómez-Zurita, PLoS ONE, 2007
	Hunt, Science, 2007
	Labeyrie and Dobler, Mol Biol Evol, 2004
	McKenna, Syst Entomol, 2015
	Wiens, Nat Commun, 2015
	Zhen, Science, 2012
Lepidoptera	Aardema, Evolution, 2016
	DaCosta, Ann ESA, 2006
	Espeland, Curr Biol, 2018
	Heikkilä, Proc R Soc B, 2012
	Kawahara, Proc R Soc B, 2014
	Kunte, Evolution, 2009
	Mitchell, Syst Entomol, 2006
	Petschenka, Evolution, 2013
	Wiens, Nat Commun, 2015
	Zahiri, Zool Scripta, 2011
	Zaspel, PLoS ONE, 2014
Diptera	Pegueroles, Sci Rep, 2016
	Petschenka, Am Nat, 2017

	San Jose, Mol Phylogenet Evol, 2017
	Seetharam, PeerJ, 2013
	Schneider, Toxicon, 2017
	Virgilio, Mol Phylogenet Evol, 2015
	Wiegmann, PNAS, 2011
	Wiens, Nat Commun, 2015

Supplementary Table 2. List of *ATPα* and *COI* sequences used for molecular evolution analyses (when multiple paralogs are present the copies used in the phylogeny are highlighted in yellow).

Clade	**Species**	***ATP α* Copy**	***ATPα* Source**	***ATPα* ID Number**	**Reference**	***COI* ID Number from GenBank**
Vertebrata	*Sus scrofa*	1A1	PDB	3N23_A	Yatime et al, J Struct Biol, 2011	KT372092.1
	Squalus acanthias	1A1	PDB	3A3Y_A	Ogawa et al, PNAS, 2009	KP192399.1
Hemiptera	*Oncopeltus fasciatus*	1C	GenBank	JQ771518.1	Zhen et al, Science, 2012	KR565226.1
	Oncopeltus fasciatus	1B	GenBank	JQ771519.1	Zhen et al, Science, 2012	KR565226.1
	Oncopeltus fasciatus	1A	GenBank	JQ771520.1	Zhen et al, Science, 2012	KR565226.1
	Lygaeus kalmii	1C	GenBank	JQ771515.1	Zhen et al, Science, 2012	KR581102.1
	Lygaeus kalmii	1B	GenBank	JQ771514.1	Zhen et al, Science, 2012	KR581102.1
	Lygaeus kalmii	1A	GenBank	JQ771513.1	Zhen et al, Science, 2012	KR581102.1
	Largus sp.	1	GenBank	HE956738.1	Dobler et al, PNAS, 2012	Not available
	Pyrrhocoris apterus	1	GenBank	HE956739.1	Dobler et al, PNAS, 2012	KM023058.1
	Halyomorpha halys	1	GenBank	XM_014416433.2	i5K Consortium, J Hered, 2013	KR070751.1
	Boisea trivittata / Leptocoris trivittatus	1	GenBank	JQ771499.1	Zhen et al, Science, 2012	KR035416.1
	Cimex lectularius	1	GenBank	XM_014394882.2	i5K Consortium, J Hered, 2013	KR044731.1
	Nilaparvata lugens	1	GenBank	XM_022328639.1	Xue, Genome Biol, 2014	JN391181.1
	Aphis nerii	1	GenBank	JQ771496.1	Zhen et al, Science, 2012	KR581182.1
	Acyrthosiphon pisum	1	GenBank	XM_016802834.1	International Aphid Genomics Consortium, PLoS Biol, 2010	AB506719.1
	Myzus persicae	1	GenBank	XM_022311823.1	Mathers, Genome Biol, 2017	KR575138.1
	Bemisia tabaci	1	GenBank	XM_019041062.1	Chen, BMC Biol, 2016	LN614545.1
Psocodea	*Pediculus humanus*	1	GenBank	XM_002427669.1	Kirkness, PNAS, 2010	AY695985.1
Hymenoptera	*Apis mellifera*	1	GenBank	XM_006564160.2	Honeybee Genome Sequencing Consortium, Nature, 2006	KX051673.1
	Camponotus floridanus	1	GenBank	XM_011260609.3	Bonasio, Science, 2010	AY334397.1
	Cephus cinctus	1	GenBank	XM_015729628.2	i5K Consortium, J Hered, 2013	KR879345.1
	Monophadnus latus	1	GenBank	LN736262.1	Dobler, Entomol Exp Appl, 2015	Not available
	Pachyprotasis variegata	1	GenBank	LN736263.1	Dobler, Entomol Exp Appl, 2015	LN736268.1
	Pachyprotasis rapae	1	GenBank	LN736264.1	Dobler, Entomol Exp Appl, 2015	LN736267.1
Coleoptera	*Rhyssomatus lineaticollis*	1A	GenBank	JQ771524.1	Zhen et al, Science, 2012	Not available
	Rhyssomatus lineaticollis	1B	GenBank	JQ771523.1	Zhen et al, Science, 2012	Not available
	Dendroctonus ponderosae	1	GenBank	XM_019913697.1	Keeling, Genome Biol, 2013	JQ308436.1
	Cyrtepomistus castaneus	1	GenBank	JQ771502.1	Zhen et al, Science, 2012	Not available, but also not in original phylogeny
	Tetraopes tetrophthalmus	1	GenBank	JQ771526.1	Zhen et al, Science, 2012	KR485899.1
	Anoplophora glabripennis	1	GenBank	XM_018706533.2	McKenna, Genome Biol, 2016	AB439154.1

	Megacyllene robiniae	1	GenBank	JQ771517.1	Zhen et al, Science, 2012	KU255678.1
	Alticinae sp.	1	GenBank	HE956742.1	Dobler et al, PNAS, 2012	Not available for this submitted individual
	Labidomera clivicollis	1	GenBank	JQ771511.1	Zhen et al, Science, 2012	KR486314.1
	Leptinotarsa decemlineata	1	GenBank	XM_023161024.1	Schoville, Sci Rep, 2018	AY165708.1
	Gastrophysa viridula	1	GenBank	HE956744.1	Dobler et al, PNAS, 2012	MH323135.1
	Plagiodera versicolora	1	GenBank	JQ771522.1	Zhen et al, Science, 2012	KM448255.1
	Chrysochus auratus	1B	GenBank	JQ771501.1	Zhen et al, Science, 2012	AY165728.1
	Chrysochus auratus	1A	GenBank	JQ771500.1	Zhen et al, Science, 2012	AY165728.1
	Chrysochus cobaltinus	1B	GenBank	AJ549438.1	Labeyrie and Dobler, Mol Biol Evol, 2004	Not available
	Chrysochus asclepiadeus	1	GenBank	HE956740.1	Dobler et al, PNAS, 2012	KP306805.1
	Chrysochus chinensis	1	GenBank	AJ617744.1	Labeyrie and Dobler, Mol Biol Evol, 2004	KU188478.1
	Platycorynus sauteri	1	GenBank	AJ617746.1	Labeyrie and Dobler, Mol Biol Evol, 2004	Not available
	Aethina tumida	1	GenBank	XM_020018205.1	i5K Consortium, J Hered, 2013	KC966652.1
	Tenebrio molitor	1	GenBank	KY921817.1	Moreira, J Insect Physiol, 2017	KR915361.1
	Agrilus planipennis	1	GenBank	XM_018472182.1	i5K Consortium, J Hered, 2013	KT250504.1
Lepidoptera	*Plutella xylostella*	1	GenBank	XM_011549930.1	You, Nat Genet, 2013	KM588487.1
	Papilio glaucus	1	GenBank	JQ771498.1	Zhen et al, Science, 2012	GU089129.1
	Papilio xuthus	1	GenBank	XM_013315255.1	Nishikawa Nat Genet, 2015	EU105383.1
	Paplio machaon	1	GenBank	XM_014516575.1	Li, Nat Commun, 2015	MH420066.1
	Papilio polytes	1	GenBank	XM_013283743.1	Nishikawa Nat Genet, 2015	KC158441.1
	Lerema accius	1	LepBase	lerema_accius_v1x1_core_32_85_1__protein__lac1010.8.mRNA	Cong, BMC Genomics, 2015	GU089979.1
	Pieris rapae	1	GenBank	XM_022259192.1	Shen, F1000Res, 2016	EU105237.1
	Pieris napi	1	LepBase	pieris_napi_pnv1x1_core_32_85_1__protein__PIENAPT00000006454	Meslin, PNAS, 2017	GU013649.1
	Phoebis sennae	1	LepBase	phoebis_sennae_v1x1_core_32_85_1__protein__pse2358.15	Cong, Genome Biol Evol, 2016	MF547337.1
	Calycopis cecrops	1	LepBase	calycopis_cecrops_v1x1_core_32_85_1__protein__cce3094.7.mRNA	Cong, Sci Rep, 2016	GU089704.1
	Mechanitis polymnia	1	GenBank	HF945460.1	Petschenka, Evolution, 2013	EU068852.1
	Lycorea halia	1	GenBank	JQ771512.1	Zhen et al, Science, 2012	GQ864788.1
	Idea leuconoe	1	GenBank	HF945457.1	Petschenka, Evolution, 2013	GQ864778.1
	Euploea phaenareta	1	GenBank	HF945456.1	Petschenka, Evolution, 2013	Not available
	Euploea mulciber	1	GenBank	HF945466.1	Petschenka, Evolution, 2013	MF804644.1
	Euploea core	1	GenBank	HF945455.1	Petschenka, Evolution, 2013	GU012616.1
	Amauris tartarea	1	GenBank	HF945450.1	Petschenka, Evolution, 2013	DQ071867.1
	Ideopsis juventa	1	GenBank	HF945458.1	Petschenka, Evolution, 2013	KP007720.1
	Parantica aglea	1	GenBank	HF945461.1	Petschenka, Evolution, 2013	GU012559.1
	Tirumala petiverana	1	GenBank	HF945463.1	Petschenka, Evolution, 2013	Not available

	Tirumala septentrionis	1	GenBank	HF945464.1	Petschenka, Evolution, 2013	KU183736.1
	Tirumala limniace	1	GenBank	HF945462.1	Petschenka, Evolution, 2013	HQ990432.1
	Danaus chrysippus	1	GenBank	LN880853.1	Pierce, Biol J Linn Soc, 2016	HQ990426.1
	Danaus gilippus	1	GenBank	JQ771506.1	Zhen et al, Science, 2012	MF547087.1
	Danaus genutia	1	GenBank	HF945453.1	Petschenka, Evolution, 2013	GU012555.1
	Danaus erippus	1	GenBank	LN880851.1	Pierce, Biol J Linn Soc, 2016	MF547376.1
	Danaus plexippus	1	GenBank	JQ771507.1	Zhen et al, Science, 2012	KT133515.1
	Limenitis archippus	1	GenBank	JQ771509.1	Zhen et al, Science, 2012	KT127045.1
	Heliconius melpomene melpomene	1	LepBase	heliconius_melpomene_melpomene_hmel2_core_32_85_1__protein__HMEL005691g1.t2	Heliconius Genome Consortium, Nature, 2012	KJ174931.1
	Heliconius erato demophoon	1	LepBase	heliconius_erato_demophoon_v1_core_32_85_1__protein__evm.model.Herato0801.124.5	Van Belleghem, Nat Ecol Evol, 2017	KP074780.1
	Junonia coenia	1	LepBase	junonia_coenia_jc10_core_32_85_1__protein__JC_0012304-RA	Zhang, PNAS, 2017	GU089963.1
	Melitaea cinxia	1	LepBase	melitaea_cinxia_core_32_85_1__protein__MCINX014373-PA	Ahola, Nat Commun, 2014	MH420044.1
	Bicyclus anynana	1	GenBank	XM_024099163.1	Nowell, GigaScience, 2017	KR139591.1
	Plodia interpunctella	1	LepBase	plodia_interpunctella_v1_core_32_85_1__protein__maker-scaffold13-augustus-gene-0.99-mRNA-1	Challis, bioRxiv, 2016	KT145708.1
	Amyelois transitella	1	GenBank	XM_013336683.1	i5K Consortium, J Hered, 2013	Not available
	Saucrobotys futilalis	1	GenBank	HE956749.1	Dobler et al, PNAS, 2012	KT148281.1
	Chilo suppressalis	1	InsectBase	CSU-MIDGUT012369	Ma, PLoS ONE, 2012	KT955025.1
	Trichoplusia ni	1	LepBase	trichoplusia_ni_hi5v02_core_32_85_1__protein__evm.model.Scaffold82.5	Fu, eLife, 2018	KX862206.1
	Spodoptera frugiperda	1	LepBase	spodoptera_frugiperda_sf21v02_core_32_85_1__protein__evm.model.Scaffold329.10	Kakumani, Genomics, 2014	MF197868.1
	Trichordestra legitima	1	GenBank	JQ771525.1	Zhen et al, Science, 2012	GU094372.1
	Helicoverpa armigera	1	GenBank	XM_021340407.1	Pearce, BMC Biol, 2017	GU686955.1
	Empyreuma pugione	1	GenBank	LN736266.1	Dobler, Entomol Exp Appl, 2015	MF924216.1
	Lophocampa caryae	1	GenBank	JQ771510.1	Zhen et al, Science, 2012	MF924301.1
	Cycnia oregonensis	1	GenBank	HE956750.1	Dobler et al, PNAS, 2012	GU091040.1
	Cycnia tenera	1	GenBank	JQ771504.1	Zhen et al, Science, 2012	GU093833.1
	Euchaetes egle	1	GenBank	JQ771508.1	Zhen et al, Science, 2012	GU092329.1
	Pygoctenucha terminalis	1	GenBank	HE956753.1	Dobler et al, PNAS, 2012	MF924291.1
	Lerina incarnata	1	GenBank	HE956754.1	Dobler et al, PNAS, 2012	MF922623.1

	Operophtera brumata	1	LepBase	operophtera_brumata_v1_core_32_85_1__protein__OBRU01_04616-PA	Derks, Genome Biol Evol, 2015	HQ649012.1
	Bombyx mori	1	GenBank	XM_004921770.3	International Silkworm Genome Consortium, Insect Biochem Mol Biol, 2008	AB649195.1
	Manduca sexta	1	GenBank	S51591.1	Holzinger, FEBS Lett, 1992	JN678236.1
	Daphnis nerii	1	GenBank	LN736265.1	Dobler, Entomol Exp Appl, 2015	MK188140.1
Siphonaptera	*Ctenocephalides felis*	1	GenBank	S66043.1	Reeves and Yamanaka, Insect Biochem Mol Biol, 1993	KY048350.1
Diptera	*Anopheles gambiae*	1	GenBank	XM_003436221.1	Holt, Science, 2002	DQ465336.1
	Aedes aegypti	1	GenBank	XM_021837787.1	Nene, Science, 2007	KX420491.1
	Mayetiola destructor	1	EnsemblMetazoa	Mdes001311-RA	i5K Consortium, J Hered, 2013	KR742285.1
	Rhagoletis zephyria	1	GenBank	XM_017619496.1	Ragland, Mol Ecol, 2015	KU511175.1
	Ceratitis capitata	1	GenBank	XM_004535986.3	i5K Consortium, J Hered, 2013	EU733510.1
	Zeugodacus cucurbitae	1	GenBank	XM_011190816.1	Sim and Geib, G3, 2017	MH751503.1
	Bactrocera oleae	1	GenBank	XM_014232491.1	i5K Consortium, J Hered, 2013	KY111512.1
	Bactrocera latifrons	1	GenBank	XM_018929031.1	Peterson, PLoS ONE, 2009	JX129505.1
	Bactrocera dorsalis	1	GenBank	XM_011214825.2	Zheng, BMC Genomics, 2016	KX051723.1
	Dacus siliqualactis	1	Publication	10.1016/j.toxicon.2017.09.011	Schneider, Toxicon, 2017	EU679328.1
	Liriomyza eupatoriae	1	GenBank	LT795109.1	Petschenka, Am Nat, 2017	LT795097.1
	Liriomyza sp. Mexico	1	GenBank	HE956748.1	Dobler et al, PNAS, 2012	HE862404.1
	Liriomyza asclepiadis	1	GenBank	HE956747.1	Dobler et al, PNAS, 2012	HE862403.1
	Napomyza lateralis	1	GenBank	LT795079.1	Petschenka, Am Nat, 2017	EF104710.1
	Napomyza scrophulariae	1	GenBank	LT795080.1	Petschenka, Am Nat, 2017	LT795091.1
	Chromatomyia horticola	1	GenBank	LT795081.1	Petschenka, Am Nat, 2017	LT795089.1
	Phytomyza crassiceta	1	GenBank	LT795078.1	Petschenka, Am Nat, 2017	KR747618.1
	Phytomyza digitalis	1	GenBank	LT795083.1	Petschenka, Am Nat, 2017	LT795093.1
	Phytomyza ilicis	1	GenBank	LT795077.1	Petschenka, Am Nat, 2017	EU367541.1
	Phytomyza fallaciosa	1	GenBank	LT795082.1	Petschenka, Am Nat, 2017	EU367595.1
	Phytomyza hellebori A	1A	GenBank	LT795110.1	Petschenka, Am Nat, 2017	LT795095.1
	Phytomyza hellebori B	1B	GenBank	LT795111.1	Petschenka, Am Nat, 2017	LT795095.1
	Drosophila grimshawi	1	GenBank	XM_001993757.1	Drosophila 12 Genomes Consortium, Nature, 2007	GU597459.1
	Drosophila virilis	1	GenBank	BK008742.1	Carvalho and Clark, Genome Res, 2013	DQ471577.1
	Drosophila mojavensis	1	GenBank	BK008743.1	Carvalho and Clark, Genome Res, 2013	DQ383730.1
	Drosophila willistoni	1	GenBank	XM_015177397.2	Drosophila 12 Genomes Consortium, Nature, 2007	JN705934.1
	Drosophila ananassae	1	GenBank	XM_001953862.2	Drosophila 12 Genomes Consortium, Nature, 2007	JQ679117.1
	Drosophila erecta	1	GenBank	XM_001979324.2	Drosophila 12 Genomes Consortium, Nature, 2007	JQ679121.1
	Drosophila yakuba	1	GenBank	XM_002096454.2	Drosophila 12 Genomes Consortium, Nature, 2007	KJ767248.1
	Drosophila melanogaster	1	GenBank	NM_169936.3	Adams, Science, 2000	KJ767244.1

	Drosophila sechellia	1	GenBank	XM_002044256.1	Drosophila 12 Genomes Consortium, Nature, 2007	KJ426007.1
	Drosophila simulans	1	GenBank	XM_002102615.2	Drosophila 12 Genomes Consortium, Nature, 2007	KT112992.1
	Drosophila persimilis	1	GenBank	XM_002013682.1	Drosophila 12 Genomes Consortium, Nature, 2007	AF451101.1
	Drosophila pseudoobscura	1	GenBank	XM_001358575.3	Richards, Genome Res, 2005	AF519412.1
	Drosophila madeirensis	1	GenBank	JN882377.1	Pegueroles, Heredity, 2013	U51613.1
	Drosophila subobscura (O3+4)	1	GenBank	JN882412.1	Pegueroles, Heredity, 2013	AF519409.1
	Drosophila subobscura (O3+4+1)	1	GenBank	KT318951.1	Pegueroles, Sci Rep, 2016	AF519409.1
	Drosophila subobscura (O3+4+7)	1	GenBank	KT318959.1	Pegueroles, Sci Rep, 2016	AF519409.1

Supplementary Table 3. References for ASR of cardiac glycoside feeding and sequestration.

Clade	References for ancestral state reconstruction of cardiac glycoside feeding and sequestration.
Hemiptera	Bramer, Proc R Soc B, 2015
	Dobler, PNAS, 2012
	Zhen, Science, 2012
	Züst and Agrawal, Func Ecol, 2016
Hymenoptera	Boevé, BMC Evol Biol, 2013
	Dobler, PNAS, 2012
	Dobler, Entomol Exp et Appl, 2015
Coleoptera	Dobler, PNAS, 2012
	Labeyrie and Dobler, Mol Biol Evol, 2004
	Zhen, Science, 2012
Lepidoptera	DaCosta, Ann ESA, 2006
	Dobler, PNAS, 2012
	Dobler, Entomol Exp et Appl, 2015
	Petschenka, J Insect Physiol, 2012
	Petschenka, Evolution, 2013
	Zaspel, PLoS ONE, 2014
	Zhen, Science, 2012
Diptera	Dobler, PNAS, 2012
	Kearney, Entomol Exp et Appl, 1983 Pegueroles, Sci Rep, 2016
	Petschenka, Am Nat, 2017
	Schneider, Toxicon, 2017

Supplementary Table 4. Sequences of gRNAs for generation of knock-in fly lines.

sgRNA1_Round 1	GCAGGGTCTAAGTCACGCCAAGG (+ strand)
sgRNA2_Round1	GTTCGCCACCGTCATCCGCGAGG (+ strand)
sgRNA1_Round 2	GAGAGGGATAATATCGTTAGTGG (- strand)
sgRNA2_Round 2	GAAACTCACGCTGCGCGCTGAGG (+ strand)

Protospacer in yellow; PAM in blue; underlined base in each of the sgRNAs of the second round were altered in the donor sequence to avoid retargeting of the newly formed allele.

Supplementary Table 5. Primer sequences for screening and validating knock-in fly lines.

Primers for Screening by GV	**Sequence (5’-3”)**	**Purpose**
For Deletion Line		
Δ_5’Arm_F	AAGGAAATATGGCCTTGTACTAATACCAAG	Validate sequence of 5’ homology arm
Δ_5' Arm_Junction GFP_R	AAGTCGCCATGTTGGATCGACT	
Δ_3' Arm_ Junction GFP_F	CCTGGGCATGGATGAGCTGT	Validate sequence of 3’ homology arm
Δ_3' Arm_R	CCTCGGTTTCGTGGATGGAC	
For Knock-in Lines		
PrimerF_GV_Screen	TGTCGGCTAATGGGTCTCGC	
PrimerR_GV_Screen	AAGTTGCGCGCCTCGATGAT	
For Validating Sequence of Knock-in Lines (Flanking Homology Arms)		
Fw1_left/right arm:	ATTCGCTATGTATAACGATGGCC (65 °C)	
Fw2_left/right arm:	CACTGGCGAAATGTTTACAGCG	
Rv1_left/right arm:	CATGTAGGCATTGTTGAACGCC (68 °C)	
Rv2_left/right arm:	CTCCTCGTCCAGAACCTTCTCC	

Supplementary Table 6. Sequences of knock-in and w^{1118} control (QAN*) fly lines.

QAN* - Exons in green	>QAN* (w^{1118} wild type) Sequenced *ATPα* region ATTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGCCACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCTGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCAGGCCAGCACCAGCGAGGAGCCGGCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAGGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGCGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAATG
QAN - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue	>QAN (engineered control) Sequenced *ATPα* region CGCTATGTATAACGATGGCCAATAATAATGTCATAAATATGGCCATTTTTGAATATCTCGGAGTTTATTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCAGGCCAGCACCAGCGAGGAGCCGGCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGTGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATACGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAATG
LAN - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue - Point mutation for Q111L in red	>LAN Sequenced *ATPα* region TTTTGATATCTCGGAGTTTATTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCTGGCCAGCACCAGCGAGGAGCCGGCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTC

	AAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGCGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAATG
LSN - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue - Point mutations for Q111L and A119S in red	>LSN Sequenced *ATPα* region TTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCTGGCCAGCACCAGCGAGGAGCCGTCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGCGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCC
VSN - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue - Point mutations for Q111V and A119S in red	>VSN Sequenced *ATPα* region TTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTAGTTCCTTTGTTTTGTATGCGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGCGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACAATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTGTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCGTGGCCAGCACCAGCGAGGAGCCGTCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGTGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAA
VSH - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue	>VSH Sequenced *ATPα* region CGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAG

- Point mutations for Q111V, A119S and N122H in red	GAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCGTGGCCAGCACCAGCGAGGAGCCGTCCGACGATCATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGTGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAATGAAGGGAG
QSN - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue - Point mutation for A119S in red	>QSN Sequenced *ATPα* region TTTTTGATATCTCGGAGTTTATTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCTGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCAGGCCAGCACCAGCGAGGAGCCGTCCGACGATAATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGCGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGANNCCCGCTACTTGCTCGTAATG
QAH - Homology arms underlined - Exons in green - Point mutations to prevent Cas9 retargeting in blue - Point mutation for N122H in red	>QAH Sequenced *ATPα* region TGTATAACGATGGCCAATAATAATGTCATAAATATGGCCATTTTTGAATATCTCGGAGTTTATTTTCGAATCTCCATTCATTAAAGAATATATTATTGTTCAAGGAAATATGGCCTTGTACTAATACCAAGCAGTTTTTTGTCTTTTGCTCGATTTTAAAAGTTTTCCAAATGAACAGGAGTTCAACTAGTTGTTGTTTTTTTCCATTTGTTTTTCCTTGATTGCATGGCAACCCCAACAATGACGTCAGAGAGCAAAAAATCACTGCCCGTGCGTCTTGAAAAAAAGAGAACACTTATAGACACTGTTAAAGATAGAATGCCAGACACTAGACAGCTGCTAAGACTATATATATAATATAACTTTTGAATCCACATATATTTTATTTAGAATTCTCTTTCTTTTCCTTCTTATTTTTTTTATTTGGCCAATTCAAAGGCTTTCGAGGTCAGCGAATGCATAGTAAATCTATATATTTTTGGTTGCACTGTAGGAAAAGTTACTTTTCATTCATTTTTTTCGTATTTAATTTTTTTTTTTAGTTCCTTTGTTTTGTATACGTAGCATATGTGTAGGAACAAATGTTTGTATAATTGCTTGATGTTTTGTTTTGTGCACCTCAATTCATTTACGTGCTCTTCTCATTTCGATCGGGAGCGTCATTTAGCGCGCATTTTGCATGCCTTAGATTGCGATTACTAGAGCTTTACGTATCACAGAGGTCGTTTTGAATATTCTCACGCTCGAACTCATTTCGGAATGGGTTCGCCCAAGTCTATAACCACATCCCCGTGCACAGGTTCGGAAAATCACTTGACAAGTAGTAAATTTAAGTCGGGTAAGTAGTTAACAACGGGTACCGGTACTTAACTTATCTCTAACTGACCCCTAACTCTTAATGGTAAACGGAACAGTTGTACTATTTTGAGGGTTTCTTCAGTGCAAACCTATCGTAAACTCTTCTCTATCCACTTCCTCTGTAACCAATCCCAAAACACACTGGTAATATTGAATCCTTTACGCCTTTTTTGAAAGGCCTAACCAAGGCATGAAGTAAATCGAATTTTGTGTCGGCTAATGGGTCTCGCTGCAGTGCATCATTAACTTCCGACACTAACGATATTATCCCTCTCAACTCAACAGTCAAGACGCAAGATGCCGGCCAAAGTTAATAAAAAGGAGAATTTAGATGATCTCAAACAGGAGTTGGATATCGATTTTCATAAAATCTCTCCCGAGGAATTGTATCAGCGCTTTCAGACACATCCCGAAAATGTAAGTGCAGCATTTGAGTAAATGGTATCCCATTCGATATGTGAACTTATACGTTTTTGATCTTCCGCAGGGTCTAAGTCACGCCAAGGCCAAGGAAAACTTGGAGCGCGATGGTCCCAATGCGCTGACGCCACCCAAGCAGACGCCCGAATGGGTGAAGTTCTGTAAGAACCTGTTCGGTGGCTTCGCCATGTTGCTGTGGATCGGTGCTATTCTCTGCTTTGTGGCCTATTCTATCCAGGCCAGCACCAGCGAGGAGCCGGCCGACGATCATTTGTATCTGGGTATTGTACTTTCCGCTGTCGTCATCGTGACGGGCATTTTCTCATACTATCAGGTATGTGTCCCTCAAATCTGAGCAGCTAAAGATCGGCTTACTAACTGTGGTATGTGTTCCGCAGGAATCGAAAAGTTCTAAGATCATGGAATCGTTCAAGAACATGGTACCCCAGTTCGCCACCGTCATCCGCGAGGGCGAGAAACTCACGCTGCGCGCTGAAGATCTCGTTCTGGGCGATGTCGTTGAGGTGAAGTTCGGCGACCGTATCCCCGCTGATATCCGCATCATCGAGGCGCGCAACTTCAAGGTGGACAACTCCTCGCTGACCGGCGAGTCGGAGCCGCAGTCCCGCGGTGCCGAGTTCACCCATGAGAATCCGCTGGAGACCAAGAATCTGGCCTTCTTCTCCACCAACGCCGTCGAGGGCACTGCCAAGGGTGTGGTCATCAGCTGTGGCGATCACACCGTCATGGGCCGCATTGCTGGCCTTGCCTCCGGTCTAGACACCGGCGAGACGCCCATTGCCAAGGAGATCCACCATTTCATCCACCTTATTACCGGCGTGGCCGTGTTCCTGGGCGTCACCTTCTTCGTGATTGCCTTCATCCTGGGCTACCACTGGCTGGACGCTGTCATCTTTTTGATCGGTATCATCGTCGCCAACGTGCCCGAGGGTCTGCTGGCCACCGTAACTGTGTGCCTGACCCTTACCGCCAAGCGTATGGCCTCAAAGAACTGTCTGGTGAAGAATCTGGAGGCCGTGGAGACCCTTGGCTCCACATCGACCATCTGCTCCGATAAGACCGGCACCCTCACCCAGAACCGAATGACGGTCGCCCACATGTGGTTCGATAATCAGATCATCGAGGCCGACACAACTGAGGATCAGTCGGGTGTTCAATACGATAGAACCAGCCCTGGATTCAAGGCGCTCTCTCGCATTGCCACTCTCTGTAACCGTGCCGAGTTCAAGGGAGGCCAAGATGGCGTCCCAATCCTCAAGAAAGAAGTCAGTGGAGATGCCTCCGAGGCTGCTCTGCTCAAGTGCATGGAACTGGCTCTGGGCGATGTGATGAACATTCGCAAGCGTAATAAGAAGATTGCCGAGGTGCCCTTCAACTCCACCAACAAATACCAAGTGTCCATCCACGAAACCGAGGATACCAACGATCCCCGCTACTTGCTCGTAATG

Supplementary Table 7. Injections and number of independent lines established.

***ATPα* genotype**	**Deletion (GFP+)**	**Engineered control (QAN)**	**LAN**	**LSN**	**VSN**	**VSH**	**QSN**	**QAH**
Nr of embryos injected	720	720	1200	720	720	720	720	720
Nr of independent lines brought to homozygous state for knock-in allele	1	1	3	3	1	1	1	2

Supplementary Table 8. Primers used for RT-qPCR.

Primer	**Sequence (5' to 3')**	**Amplification Efficiency**
Rpl32_F	ATGCTAAGCTGTCGCACAAATG	100%
Rpl32_R	GTTCGATCCGTAACCGATGT	
ATPa_F1	TGCTGTGGATCGGTGCTATTC	90%
ATPa_R1	AGCGGAAAGTACAATACCCAGA	

* *Rpl32* primers from Ponton *et al.*, 2011.

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