Supplementary Information for:

Identification of evolutionary and kinetic drivers of NAD-dependent signalling

 $Mathias \ Bockwoldt^1, \ Dorothée \ Houry^2, \ Marc \ Niere^3, \ Toni \ I. \ Gossmann^{4,5}, \ Ines \ Reinartz^{6,7}, \ Alexander \ Schug^8, \ Mathias \ Ziegler^3, \ and \ Ines \ Heiland^{1,\$}$

¹Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Biologibygget, Framstredet 39, 9017 Tromsø, Norway

²Department of Biological Sciences, University of Bergen, Thormøhlens gata 53 A/B, 5006 Bergen, Norway

 $^{3}\mathrm{Department}$ of Biomedicine, University of Bergen, Jonas Lies ve
i $91,\,5009$ Bergen, Norway

⁴Department of Animal and Plant Sciences, Western Bank, University of Sheffield, Sheffield, S10 2TN, United Kingdom

⁵Department of Animal Behaviour, Bielefeld University, 33501 Bielefeld, Germany

⁶Department of Physics, Karlsruhe Institute of Technology, Wolfgang-Gaede-Str. 1, 76131 Karlsruhe, Germany

⁷Steinbuch Centre for Computing, Karlsruhe Institute of Technology, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

⁸John von Neumann Institute for Computing, Jülich Supercomputing Centre, Forschungszentrum Jülich, 52425 Jülich, Germany § Corresponding author: ines.heiland@uit.no

This PDF file includes:

Figures S1 to S9 Tables S1 to S4

Other supplementary materials for this manuscript include the following:

Scripts used to run the phylogenetic analysis are available here:

https://github.com/MolecularBioinformatics/Phylogenetic-analysis

The SBML files of the mathematical models used for the pathway simulations in figures 3, 4, 6, S3 and S6 are accessible through the Biomodels database (https://www.ebi.ac.uk/biomodels/models) accession no. MODEL1905220001 and MODEL1905220002.



The phylogenetic distribution of NamPT and NNMT in birds and reptiles is scattered. The phylogenetic distribution of birds and reptiles was adopted from Prum et al. 2015 [1]. Families are marked with a green circle if they possess NamPT without NNMT or a blue circle if they possess both NamPT and NNMT.

Homo sapiens Pongo abelii Pan troglodytes Macaca mulatta Saimiri boliviensis boliviensis Callithrix jacchus Propithecus coquereli Nomascus leucogenys Otolemur garnettii Microcebus murinus Nanospalax galili Jaculus jaculus Chinchilla lanigera Fukomys damarensis Heterocephalus glaber Octodon degus Cavia porcellus Rattus norvegicus Mus musculus Peromyscus maniculatus bairdii Cricetulus griseus Dipodomys ordii Marmota marmota marmota Oryctolagus cuniculus Ochotona princeps Bubalus bubalis Ovis aries musimon Bos taurus Vicugna pacos Camelus ferus Sus scrofa Ceratotherium simum simum Mammal Equus przewalskii Loxodonta africana Balaenoptera acutorostrata scammoni Physeter catodon Orcinus orca Leptonychotes weddellii Odobenus rosmarus divergens Acinonyx jubatus Felis catus Ursus maritimus Ailuropoda melanoleuca Mvotis lucifuqus Eptesicus fuscus Pteropus alecto Echinops telfairi Condvlura cristata Sorex araneus Erinaceus europaeus Erinaceus europaeus Orycteropus afer afer Galeopterus variegatus Tupaia chinensis Chrysochloris asiatica Trichechus manatus latirostris Mondelphis domestica Dasypus novemcinctus Ornithorhynchus anatinus Struthio camelus australis Podiceps cristatus Apteryx australis mantelli Calidris pugnax Chlamydotis macqueenii Buceros rhinoceros silvestris Tauraco erythrolophus Picoldes pubescens Picoides pubescens Tinamus guttatus Leptosomus discolor Merops nubicus Colius striatus Apaloderma vittatum Cuculus canorus Cariama cristata Pterocles gutturalis Cathartes aura Gavia stellata Fulmarus glacialis lves Opisthocomus hoazin Melopsittacus undulatus Nestor notabilis Amazona aestiva Pygoscelis adeliae Aptenodytes forsteri Egretta garzetta Phaethon lepturus Phaethon lepturus Pelecanus crispus Phalacrocorax carbo Manacus vitellinus Corvus brachyrhynchos Taeniopygia guttata Acanthisitta chloris Geospiza fortis Zonotrichia albicollis Sturnus vulgaris Pseudopodoces humilis

Deuterostomia

20 30 60 70 80 40 50 VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKDVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKDVYKEHFQ VTHYKOYPPNTSKVYSYFECREKKTENSKI RKVKYFETVEYGI OYTI NKYI KGKVVTKEKTOFAKDTYKEHEO VIHTQ(YPMISAVISTECEKKTENSKLRKVKYEETVFGLQYILINKILKGKVTKEKIQEAKUYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFGLQYILINKYLKGKVVTKEKIQEAKEVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILINKYLKGKVITKEKIQEAKEVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFGLQYLLNKYLKGKVVTKEKIQGAKEVYKAHO VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFGLQYLLNKYLKGKVVTKEKIQGAKEVYKEHO VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFGLQYLLNKYLKGKVVTKEKIQGAKEVYREHO VTHYKQPPNTSKVYSYFECREKKTENSKLRKVKYEETVFGLQYLLNKYLKGKVVTKEKIQGAKEVYREHO VTHYKQYPPNT----YYFECHEKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYREHFQ V HTKQUPPNI SKVTSYFECREKKTENSKVRKWYTEET VFOLUYILINNYLKOKVVI KERLUGAREVYREHO VTHYKQVPPNTSKVYSYFECREKKTENSKVRKWYTEETVFOLUYILINNYLKGKVVTKEKIQEAKEVYREHO VTHYKQVPPNTSKVYSYFECREKKTENSKVRKWYTEETVFOLUYILINNYLKGKVVTKEKIQEAKEVYREHO VTHYKQVPPNTSKVYSYFECREKKTENSKVRKWYTEETVFOLUYILINNYLKGKVVTKEKIQEAKEVYREHO VTHYKQVPPNTSKVYSYFECREKKTENSKVRKWYTEETVFOLUYILINNYLKGKVVTKEKIQEAKEVYREHO VTHYKQVPPNTSKVYSYFECREKKTENSKVRKWYTEETVFOLUYILINNYLKGKVVTKEKIQEAKEVYREHO VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVSYFECREKKTENSKVRKWYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHFQ VTILINGUTPINTSKVJSTECREKKTEINSKURKVKTEETVEGLOVILLINGUKGKVUTKEKAGEAKEVYREIHEO VTHYKQVPPNISKVJSTECREKKTEINSKURKVYEETVEGLOVILLINKUKKKVUTKEKADEAKEVYREIHEO VTHYKQVPPNISKVJSTECREKKTEINSKURKVYEETVEGLOVILLINKUKKKVUTKEKADEAKEVYREIHEO VTHYKQVPPNISKVYSYEECREKKTEINSKURKVYEETVEGLOVILLINKUKKKVVTKEKADEAKEVYREIHEO VTHYKQVPPNISKVYSYEECREKKTEINSKURKVEETVEGLOVILLINKUKKKVVTKEKADEAKEVYREIHEO VTHYKQVPPNTSKVYSYFECREKKTENSKIRKWKYEETVFVGLQYLLNKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQVPPNTSKVYSYFECREKKTENSKIRKWKYEETVFVGLQYLLNKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQVPPNTSKVYSYFECREKKTENSKIRKWKYEETVFVGLQYLLNKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQVPPNTSKVYSYFECREKKTENSKIRKWKYEETVFVGLQYLLNKYLKGKVVTREKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENKKRKVKYEETVFYGLQYILNKYLRGKVVTEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENKKRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKWKYEETVFYGLQYLLINKYLKGKVVTKEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKWKYEETVFYGLQYLLINKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIKKWKYEETVFYGLQYLLNKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIKKWYYEETYFGLQYILINKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIKKWYYEETYFGLQYILINKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIKKWYKETYFYGLQYILINKYLKGKVVTKEKIQEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKWKYEETVFYGLQYILINKYLKGKVVTKEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKWKYEETVFYGLQYILINKYLKGKVVTKEKIQEAKEVYKEHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKIRKWKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHPQ V IHTKQUPPNISKVYSYEECREKKTEINSKIRKWYEET VFYGLQYLLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKIRKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKIRKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKWYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKVYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQVPPNISKVYSYEECREKKTEINSKURKVYEETVFYGLQYLINKYLKGKVVTKEKIQEAKEVYKEHO VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKVRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYREHFQ VTITIKQVPPNTSKVYSYEECREKKTENSKLRKVKYEETVFGLQYLLNKLKKKVVTREKIKEAKEVYREHFQ VTIHYKQVPPNTSKVYSYEECREKKTENSKLRKVKYEETVFGLQYLLNKYLKGKVVTKEKIKEAKEVYREHFQ VTIHYKQVPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYLLNKYLKGKVVTKEKIKEAKEVYREHFQ VTHYKQYPPNTSKVYSYECREKKTENSKLRKVKYEETVFGLQYILNKYLKGKVVTKEKIKEAKEVYREHFQ VTHYKQYPPNTSKVYSFECREKKTENSKLRKVKYEETVFGLQYILNKYLKGKVVTKEKIKEAKEVYREHFQ VTHYKQYPPNTSKVYSFECREKKTENSKLRKVKYEETVFGLQYILNKYLKGKVVTKEKIKEAKEVYREHFQ VTHYKQYPNTSKVYSFECREKKTENSKLRKVKYEETVFGLQYILNKYLKGKVVTKEKIKEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYLLNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYLLNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYLLNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILINKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILINKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILINKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKOYPPNTSKVYSYFECREKKTENSKI RKVKYFETVEYGI OYTI NKYI KGKVVTKEKTKEAKEVYREHEO VIHTQ(YPMISAVISTECREAKTENSKLRKVYEETVFGLQYILINKLKGKVVTKEKKEAKEVYREHQ VTHYKQYPPNTSKVYSYEECREKKTENSKLRKVKYEETVFGLQYILINKYLKGKVVTKEKKEAKEVYREHQ VTHYKQYPPNTSKVYSYECREKKTENSKLRKVKYEETVFGLQYILNKYLKGKVVTKEKKEAKEVYREHQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILINKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILINKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLKKLKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLKKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLKKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ

Continued at next page.

Figure S2 continued

VTHYKQYPPNTSKVYSYFECREKKTENSKLKKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHFQ Parus major Parus major Coturnix japonica Meleagris gallopavo Gallus gallus Aquila chrysaetos canadensis Haliaeetus albicilla Falco peregrinus Columba livia Calvote anna VTHYK0YPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGL0YILNKYLKGKVVTKEKIKEAKEVYREHF0 VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIREAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIREAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHPQ Aves Calypte anna Chaetura pelagica Anser cygnoides domesticus Alligator mississippiensis VTHYKOYPPNT5KVY5YFECBEKKTEN5KLBKVKYEETVFYGLOYILNKYLKGKVVTKEKIKEAKEVYBEHFO VITTIKYQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHO VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLKGKVVTKEKIKEAKEVYREHO VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEEIVFYGLQYILNKYLKGKVVTKEKIQEAKEVYREHO Pelodiscus sinensis Chrysemys picta bellii Chelonia mydas Python bivittatus VTHYKQYPPNTSKVYSYFECREKKTENSKLKKVKYEETVFYGLQYILNKYLKGKVITKEKIQEAKEVYREHFQ VTHYLQYPPNTSKVYSYFECREKKTENSRLKKVKYEETVFYGLQYILNKYLKGKVITKEKIQEAKEVYREHFQ VTHYLQYPPNTSKVYSYFECREKKTENSRLKKVKYEETVFYGLQYILNKYLKGKVITKEKIQEAKEVYREHFQ Reptilia VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLQGKVVTKEKIQVAKEVYKEHFQ Gekko japonicus Protobothrops mucrosquamatus Anolis carolinensis VTHYKQYPPNTSKVYSYFECREKKTDNSKLKKMKYEETVFYGLQYILHKYLKGKVVTKEKIQEAKEVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKLRKVKYEETVFYGLQYILNKYLQGKVVTKEKIQIAKAVYKEHFQ VTHYKQYPPNTSKVYSYFECREKKTENSKFRKVKYEETVFYGLQYILNKYLKGKVVTKEKIQEAKEVYKEHFQ Xenopus laevis Latimeria chalumnae Callorhinchus milii Amphibia VTHYKOYPPNTSRVYSYFECREKKTENSRTRKVKYFETVEYGI OYTI KKYI EGKVVTKEKTOFAKEVYREHEO VIHTIQITFNIJAVIJTECKENIENSLINVVIETVFOLQVILKAILUKKUVTEKLOKALEVIREHO VTHYKQPPNITKVYSYFECREKRTEDSTRIKVYEKTVFOLQVILKKYLAGVVVTEKLOZAKEVYREHO VTHYKQYPPNTTKVYSYFECREKRTEDSGKNKVKYDTVFGLQVILKKYLAGVVVTEKLOZAKEVYREHO VTHYKQYPPNTSKVYSYFECREKRTDPGKNRVKYDKTVFYGLQVILHKYLKGKVVSPEKIQEAKEVYREHO Cynoglossus semilaevis Deuterostomia Stegastes partitus Scleropages formosus Tetraodon nigroviridis VTHYKQYPPNTSKVYSYFECREKRTDPTKSRKVKYDKTVFYGLQYILHKYLKGKVVTPEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKKTDSTKDRKVKYEKTVFYGLQYILHKYLKGKVVTPEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKRTDPSKSRKVTYDKTVFYGLQYILHKYLKGKVVTPEKIQEAKDVYREHFQ Takifugu rubripes Maylandia zebra Neolamprologus brichardi VTHYK0YPPNTSKVYSYFECREKKTDPSKSHKVKYDKTVFYGL0YILHKYLKGKVVTPEKI0EAKNVYREHF0 VTHYKQYPPNTSKVYSYFECREKRTDPSKNRKVKYDKTVFYGLQYILHKYLKGKVVTPEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKRTDPSKNRKVKYDKTVFYGLQYILHKYLKGKVVTPEKIQEAKEVYREHFQ VTHYKQYPPNTSKVYSYFECREKRTDPSKNRKVKYDKTVFYGLQYILHKYLKGKVVTPEKIQEAKEVYREHFQ Oreochromis niloticus Oryzias latipes Larimichthys crocea Dicentrarchus labrax VTHYKQYPPNTSKVYSYFECREKRIDPTKTRKVKYDOTVFYGLQYILHKYLKGKVVTPEKIOEAKEVYKEHFO ITHYKQYPPNVSKIYSYFECRHK------KGSQLNEVVFFGLQYLLKKYLAGPVITEEKIOEAKEVYKEHFO ITHYKQYPPNVSKIYSYFECRHK------KGSQLNEVVFFGLQYLLKKYLAGPVITEEKIOEAKEVYKEHFO VTHYKQYPPNASKVYSYFECRETKTEPTKLRKVKYDKTVFYGLQYLLQRYLKGQVVTKGKIOEAKEVYKEHFO Carassius gibelio Cyprinus carpio Danio rerio Austrofundulus limnaeus Fish TITHYKQYPPNVSKVYSYFECRRK------KGAQFNEVVFFGLQYLLKKYLAGPVVTEEKIQAKVFYQMHFK ITHYKQYPPNINKVYSYFECRHK------KGAQFSEVVFFGLQYLLKKYLTGPVITEEKIQAKVFYQMHFK ITHRSQYPPNIDKLYSYFECRRK------KGSQFSEVVFFGLQYLLKKYLTGPVVTEEKIQEAKLFFQMHFK Xiphophorus maculatus ITHYKOYPPNVSKIYSYFECROK------KGSOFNEVVFFGLOYLLKKYLTGPVVTEDKIOEAKLFYOMHFK Xiphophorus maculatus Nothobranchius furzeri Cyprinodon variegatus Poecilia reticulata Fundulus heteroclitus Salmo salar Oncorhynchus mykiss Y THYKQYPPNTSKWYSYFECREKRTDSTKSRKVKYDQTVFYGLQYLLHKYLKGKVVTPEKIQEAKEVYREHO VTHYKQYPPNTSKVYSYFECREKRTDSTKSRKVKYDQTVFYGLQYLLHKYLKGKVVTPEKIQEAKEVYREHO VTHYKQYPPNTSKVYSYFECREKRTDPSKSRKVKYDKTVFYGLQYLLHKYLKGKVVTPEKIQEAKEVYREHO VITTIKYQYPPNTSKVYSYFECREKKRIDPGKSRKVKYDKTVFGLQYILHKYLKGKVVTPEKIQEAKUYPQHIO ITHYKQYPPNVNKVYSYFECRRK-COMPAGENEIVFFGLQYLLKKYLSGVVTEEKIQEAKUFYQMHFR ITHYKQYPPNVSKVYSYFECRRK-----RGGTQFSEIVFFGLQYLLKKYLSGRVITEEKIQEAKUFYQMHFR ITHYKQYPPNVNKVYSYFECBRK-----KGGTQLNEVVFFGLQYLLKKYLSGRVITEEKIQEAKIFYQMHFK Esox lucius Clupea harengus Lepisosteus oculatus Branchiostoma floridae TITHYKQYPPNVSKVYSYFECRRR------KOAQFHEVVFFGLQYLLKKYLGRVITEEKIQEAKLFFQMHR ITHYKQYPPDVDKVYSYFECRRK------KOAQFHEVVFFGLQYLLKKYLSGPVVTEEKIQQAKQMYQQHFK VTHHMQYPPETTTIFSYFESRG------GKFKETVFFGLQYLIKRWLVGQVVTREKIAEAKDIYKKHFG Oikopleura dioica Strongylocentrotus purpuratus VSHHLQYPPKTSYCYSYFESBG------GKEKNVIFFG------KDSGSDEEILSEHLG VSMILGUFFKISTCISTCISTCS VTHHRQYPPOTTLVSSYFESRG VTHHRQYPPNTTVIYSYFESRG VTHHRQYPPOTTTVSSYFESRG VTHHLQYPPGTTTVSSYFESRG VTHHLQYPPGTTTVSSYFESRG Lingula anatina Limulus polyphemus Octopus bimaculoides Crassostrea gigas VINHCUTFGTITUSTESAG ISHHLQYPOGITNVYSYFESRG VTHHLQYPANTTAVYSYFESRG VTHHLQYPANTTAVYSYFESRG VTHHRQYPPNTTKVYSYFESRG VTHHRQYPPNTTKVYSYFESRG Crassostrea gigas Lottia gigantea Biomphalaria glabrata Aplysia californica Capitella teleta Heloddella robusta Trichuris suis Trichinella murrelli Ancylostoma ceylanicum Nacator americanuc Mollusca VTHHL0YPPNTTTTCSYFESRG------GKEPYTVEEGL0YTLKRWLVGPVVTKEKTKEAKDTYHLHEG VTHHRQYPPNTSIVYSYFESRG------GKFPATCFFGLQYILKRWLVGQVVTREKIEEAREIFGLHFG VTHHLQYPPNTTHVYSYFESRG------GKFPSTIFFGLQYILKRWLLGQVVTDEKIEEATAVYQMHFG VTHHK0YPADTTYAYSYFESRG------GKFDKTLFFGL0YILKRWMVGEVVTEKKIIEAKEVFEKHFG Annelida Protostomia VTHYNQYPPQTTKIYSYFECRG------GKFEQVCFFGLQYVLKRWMVGRVVTHANDEAKEFYNQHFG VTHYNQYPPQTTKIYSYFECRG------GKFEDVCFFGLQYVLKRWMVGCVVTHANDEAKEFYNKHFT ITHHNQYPQGTTYVYSYFESRG------GKFPEVCFFGLQYILKRWLVGVVVTHKMIDEAKEFYKLHFN Necator americanus Strongyloides ratti Loa loa TTHHNOYPEGTTHVYSYEESRG------GKEPEVCEEGLOYTLKBWLVGVVVTHEMTDEAKEEYK-ATHHAQYPDNTTSIYSYFESRG------GKFEKTVFFGLQYFIKRYLCGRVVNK0MIEEATEFYHCHFG VTHHNQYPEGTTHVYSYFESRG------GKFSEVCFFGLQYIIKRWLVGPVVTKAMIEQAKQFYKSHFG Nematoda Wuchereria bancrofti VTHHN0YPEGTTHVYSYFESRG-----GKFSEVCFFGL0YIIKRWLVGPVVTKEMIE0AK0FYKSHFG Brugia malayi Toxocara canis Echinococcus multilocularis VITHINQYPEGTTHVYSYFESRG------GKFSEVCFGLQYIIRRWLVGPVVTRENIDEAKQYKSHF ITHISQYPKGTSHVYSYFESRG------GKFSEVCFFGLQYILKKWVGPVVNKHMIQQAKHFYKVHFG -----YPPGTTEVYSYFESRG------GKFSETIFFGLQYILKKYLVGSVITERKIREAKEVMREHFG Schistosoma haematobium VSHYS0YPAGTEETYSYFESBG------GREPNSVEEGL0YTLKKNLVG0VTTHEKTDEAKSTLLSHEG Platyhelminthes VITIYRQYPPGTTTVYSYFECRG-------GKFPEIVFFGLQYVIKWLIGQVVTKEKIEEAKEFYKLHFG VSHHKQYPPGTSILFSYFESRG------GKFPEVCFFGLQYLIKKWLVGQVVTAEKIRQAKDFYQLHFG VSHHKQYPKNTSIVFSYFESRG------GKFQSVCFFGLQYILKKWLVGQVVTKSKIEEAKQLYKLHFG Saccoglossus kowalevskii Acropora digitifera Cnidaria Exaiptasia pallida Nematostella vectensis Amphimedon queenslandica Suberites domuncula VSHIRQYPDGTSTUFSYFESRG------GKFPEVCFFGLQYIIKKWLGKVVTEEKIQEAKSLYKLHG NTHYLQYPPGTTTVSYFESRG------GKFPEVCFFGLQYIIKKWLGKVVTEKIQEAKSLYKLHG NTHYLQYPPGAEHVYSYFESRG------GKFPETVFFGLQYILKKSLVGKVVTREKIEEAAAVFDAHLG Porifera Priapulus caudatus Trichoplax adharens Rhizophagus irregularis DAOM 197198w Rasamsonia emersonii CBS 393.64 Byssochlamys spectabilis No. 5 Capronia epimyces CBS 606.96 Exophiala dermatitidis NIH/UT8656 Conidiobolus coronatus NRRL 28638 Batrachochytrium dendrobatidis JAM81 Spizellomyces punctatus DAOM BR117 Allomyces macrogynus ATCC 38327 Chlorella variabilis Gonium pectorale Chlamydomonas reinhardtii Monosiga brevicollis MX1 VSHYKOYPPGTTALWGYFESRG-----GKFPTTVFFGLOYILKRWLVGPVLNKOMIREAAEFYKLHFV Priapulus caudatus VSHYKYYPPGITALWGYFESKG------GKFPITVFFGLQYILKKWLVGPULNKQMIREAAEFYKLHN FTHFKYPPKTTKVSYFESKG------GKFENVFFGLQYIIKRLLGKVVTKEKIDEAAFSKAHFG PSHSLLFPDSVKSV-AYGEFRK----SYDNDKEDTRVLFGCIQYIIKRLLGKVVTKEKIDEAAFSKAHFG HSHWNLYPPGTRVJSSYIESKG------GEYPAHLFFGLQAFIKQHL-RPITIDDIDEAEIVTRQHOI HSHWNLYPPGTRHVSSYIESKG------GEYPAHLFFGLQAFIKQHL-RPITIDDIDEAEIVTRQHOI HSHWNLYPPGTRHVSSYIESKG------GIYPAHHFVGLQAFIKKHL-RPITIDDIDEAEIVTRQHOI LSHYXAYP-KAEKLIAYGEFRT----SYLKDPNDSRLIYYQMEYILENYLN-RPITOPDLDKLDNFCOTHW VAHNOLYP Funai ATTAAPHLLTSYLPIAYGEFRQ....GYDKOTRDTRAVFYGLRYILENFVA -RRWTLQDVELADRFFGSHMA ATTAAPHLLTSYLPIAYGEFRQ....GFNKDKDTRMVSYGMRYLVENYIA - KRWTNEDVDMAEAFYRCAAV ATHFLQYP-KAQKMVAYGEFRQ....GFNKDKTDTRLVSYGMRYLVETYIS -RQWTMEDVEMADAFYRTHMA ISHHLQYPPNTTRVSYFERSG.....GOFQETCFFGLQYILKRWLVGQVVTQAKIDEAAELYQLHFG Viridiplantae Monosiga brevicollis MX1 Monosiga Drevicollis MAI Aureococcus anophagefferens Chrysochromulina sp. CCMP291 Acanthamoeba castellanii str. Neff Capsaspora owczarzaki ATCC 30864 Guillardia theta CCMP2712 ISHHLQYPFWITKVJSTFESKG------GVPEUTLFFGLQYILKNWLVGVVTQAKIDEAAELYULHFG VSHVRQVPATTTVSYFESKG------GVPEEVVFFGLQYFIKRYLCGVVTKAKIDEAERFYGEHF VSHVRQVPATTTVSYFESKG------GVFEEVVFFGLQYFIKRYLCGVVTTEAKIDSHKG ASHFTMYP-DSKKAVGYGEFRK-----PYGGDKTDNRFVYYGIRYLVENFLC-RQWTKEDVEKADLFYSTHNA IAHTRQYPFKTTVYSYFESKG------GDFPETVFFGLQYILERYLAGPVVTQAKIDEAKEVFALHFG AGHFLMYP-ECKSMSAYGEFRE-----PFPNMEDNRFVFYGMRHYIENFVN-RKWCKADVDAAEIFYKTHKA

The structurally unresolved loop of NamPT. Sequence alignment of NamPT of different species cropped to the region around the unresolved loop structure.



Influence of enzyme expression, SAM concentration and inhibition constants on systems behaviour. We used the dynamic model of NAD biosynthesis and consumption to analyse the effect of NMNAT, NamPT (A and B) and NNMT (C and D) expression on NAD consumption flux (A and C) and free NAD concentration (B and D), NamPT/NNMT flux ratio (C) and Nam concentration (D). We furthermore varied the inhibition constants $K_i(Nam)$ and $K_i(NAD)$ for SIRT1 and NamPT, respectively, in a model without NNMT (E and F). This mimics the potential effect of inhibition relaxation due to reduced Nam or NAD concentrations. In addition, we simulated the effect of changes in the NNMT cofactor S-adenosyl methionine (SAM) on NAD consumption flux, NamPT/NNMT flux ratio (G), Nam and free NAD concentration (H).

Figure S4



Purification of wildtype NamPT and $\Delta 42-51$ NamPT, NMR spectra and NamPT substrate affinity measurements A) Elution profile of wildtype (wt) and Δ 42-51 NamPT on size-exclusion chromatography using a Superdex 200 16/60 column. B) Coomassie stained denaturating SDS-PAGE analysis of $\Delta 42$ -51 NamPT (lane 1) and wt NamPT (lane 2). $3 \mu g$ of pooled enzyme eluted from SEC was loaded onto the gel. C) The column was calibrated with apronitin 6.5 kDa, ovalbumine 42.7 kDa, coalbumine 75 kDa and blue dextran 2000 kDa. The partition coefficient (Kav) was determined for each standard (light grey squares) and plotted versus \log_{10} molecular weight. The apparent molecular weight of wt NamPT and $\Delta 42-51$ NamPT was calculated to be 135 kDa and 110 kDa, respectively. D) Exemplary 1D 1H NMR NMR spectra of NMN formation used to quantify the activity of wildtype and mutant NamPT. Inset: molecular structure of NMN with the atom detected by NMR indicated by an arrow. The range used for NMN detection in typical 1D-1H-NMR spectra of the enzymatic reactions is shown. Samples and standards were supplemented with 1mM of DSS as internal standard. NMN quantification was done with the singlet detected at 9.52 ppm. From the top to the bottom, peak detection of NMN standard (200 µM), wt NamPT (1 mM Nam and 1 mM PRPP), $\Delta 42$ -51 NamPT (1 mM Nam and 1 mM PRPP), wildtype NamPT with FK866, and $\Delta 42$ -51 NamPT with FK866. Incubation with inhibitor FK866 was done for 30 min at 30 °C. E) To compare the substrate affinity of wtNamPT and Δ 42-51 NamPT, 2 μ M enzyme were incubated for 5 min at 30 °C with 1mM ATP and PRPP and 1 μ M to 1mM Nam in 300 μ l reaction buffer (20mM Tris-HCl pH 8.0, 500mM NaCl, 6mM MgCl2, 0.03% (w/v) BSA). Reaction was stopped with 100µM of FK866 and frozen in liquid nitrogen. The protein was removed using Amicon Ultra Centrifugal Filters (Millipore - 10 kDa cut-off). NMN formation was measured by LC-MS using an LC Dionex Ultimate 3000 instrument coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific). For LC separation, an Ascentis Express C18, (10 cm x 2.1mM, particle size 2.7 µm) column was used (Sigma-Aldrich) with a stepwise gradient form 10 mM ammonium acetate pH 5 and 2 mM tetrabutylammonium bromide (TBAB) to 10 mM ammonium acetate pH 6.8, 2 mM TBAB and 90% acetonitrile at a flow rate of $0.4 \frac{ml}{min}$. Electrospray was used as ionization source, and samples were analysed in positive mode. Xcalibur software (Thermo Scientific, Waltham, MA, USA) was used for data visualization and peak integration. NMN measurement data are available at: https://doi.org/10.15490/fairdomhub.1.datafile.2944.1



Molecular dynamics simulations NamPT. Root mean square deviation (RMSD) with respect to initial structure for simulation of wildtype (wt) NamPT (red) and mutant $\Delta 42$ -51 NamPT (blue), respectively. The RMSD values for the entire simulation (in total 1000 ns) show stable structures with small fluctuations.





Potential impact of NADA on the evolution of high affinity NamPT. We simulated competition between two compartments, one containing only NADA (red lines) and one containing either NamPT, NADA and NNMT or twice the amount of NamPT to keep the sum of the amount of NADA+NamPT constant between simulations. As can be seen, the addition of NADA to NNMT and a low affinity NamPT, provides a slight advantage for A) NAD consumption flux and B) NAD concentration over NADA alone. As soon as the affinity of NamPT is high enough, double amounts of NamPT together with NNMT outcompete the combination of all three enzymes. The fact that this latter combination is actually found in some invertebrates might indicate that there is indeed an advantage over NADA alone.

	140	150	160	170	180	190 20
Ornithorhynchus_anatinus						
Erinaceus_europaeus						
Pteropus_alecto						
Pteropus_vampyrus						
Chrysochloris_asiatica						
Loxodonta_africana						
Otolemur_garnettii						
Eptesicus_fuscus						
Myotis_davidii						
Myotis_brandtii						
Myotis_lucifugus						
Tarsius_syrichta						
Tupaia_chinensis						
Propithecus_coquereli						
Microcebus murinus						
Acinonyx jubatus						
Panthera tigris altaica						
Mustela putorius furo						
Canis lupus familiaris						
Leptonychotes weddellii						
Ailuropoda melanoleuca						
Ursus maritimus						
Nomascus leucogenys						
Pongo abelii						
Pan paniscus						
Gorilla gorilla gorilla						
Homo sapiens						
Pan troglodytes						
Mandrillus leucophaeus						
Chlorocebus sabaeus						
Cercocebus atvs						
Bhinopithecus roxellana						
Panio anubis						
Macaca fascicularis						
Macaca mulatta						
Macaca_nemestrina						
Colobus angolensis palliatus						
Saimiri boliviensis boliviensis						
Callithrix jacchus						
Ceratotherium simum simum						
Orcipus orce						
Tursions truncatus						
lipotos vovillifor						
Physoton catedon						
Balaepontera acutorostrata scammoni						
Nappospalay galili						
Cricotulus gricous						
Microtuc achrogastan						
Macaaniaatus ausatus						
Mesocricetus_duratus						
mus_musculus						
Rattus_norvegicus						
Jaculus_Jaculus						
riar inota_inar inota_inarmota						
ICTIdomys_tridecemlineatus						
monodelphis_domestica						
Sarcophilus_harrisii						
PTEROCLES_gutturalis						
Acanthisitta_chloris						
Manacus_vitellinus						
	8-			Т		





Site specific positive selection in NNMT Branch specific test of positive selection conducted for NNMTs from various vertebrate species reveals a signature of positive selection specific to residue 171 occurring at the lineage leading to placentalia. Shown is a cropped fingerprint alignment using biochemical colour-coding for NNMTs of the species under consideration with the critical residue 171 indicated. Underlying statistics and tree are shown in Supplementary Figure S8.



Site specific positive selection in NNMT Branch specific test of positive selection conducted for NNMTs from various vertebrate species reveals a signature of positive selection specific to residue 171 occurring at the lineage leading to placentalia. A) Output of the codeml runs (Branch-site model A of positive selection), the likelihood between a model with no positive selection ($\omega 2a/b = 1$) is compared to a model with positive selection ($\omega 2a/b > 1$). Significance between the two models is assessed using a likelihood ratio test assuming that twice the likelihood difference is χ^2 distributed. The critical value is 3.84 at the 5% level. B) The underlying tree topology for the codeml runs including the tested branch indicated with #1. The alignment is shown in Supplementary Figure S7.



Clustering of NMNAT protein sequences of various eukaryotic species. Protein sequences were found using protein Blast with the human sequences as seeds. Protein names in red, blue, and green were found with the lowest expect value with the human NMNAT1, 2, and 3, respectively. Clustering was done with BAli-Phy version 3.0 (Suchard and Redeling 2006). The tree was visualised with Figtree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). Names were spread manually and dotted lines were added for better readability.

Table S1

#	Enzyme group	Gene name	Enzyme name	EC number	Uniprot ID	Species	Min. length
1	ADPRcyc	CD38	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1	3.2.2.6	P28907	H. sapiens	200
2	ADPRcyc	BST1	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2	3.2.2.6	Q10588	H. sapiens	200
3	ART	ART1	GPI-linked NAD(P)(+)arginine ADP-ribosyltransferase 1	2.4.2.31	P52961	H. sapiens	200
4	ART	ART3	Ecto-ADP-ribosyltransferase 3	2.4.2.31	Q13508	H. sapiens	200
5	ART	ART4	Ecto-ADP-ribosyltransferase 4	2.4.2.31	Q93070	H. sapiens	200
6	ART	ART5	Ecto-ADP-ribosyltransferase 5	2.4.2.31	Q96L15	H. sapiens	200
14	NADA	NIC1	Nicotinamidase 1	3.5.1.19	Q8S8F9	A. thaliana	170
15	NADA	pnc-1	Pyrazinamidase and NiCotinamidase	3.5.1.19	Q9N426	C. elegans	170
16	NADA	pncA	Pyrazinamidase/nicotinamidase	3.5.1.19	P21369	E. coli	170
17	NADA	PNC1	Nicotinamidase	3.5.1.19	P53184	S. cerevisiae	170
25	NamPT	NPT1	Nicotinamide phosphoribosyltransferase	2.4.2.12	D9l2J1	C. reinhardtii	400
26	NamPT	NAMPT	Nicotinamide phosphoribosyltransferase	2.4.2.12	P43490	H. sapiens	400
38	NNMT	B0303.2	Uncharacterized methyltransferase	2.1.1	P34254	C. elegans	150
39	NNMT	NNMT	Nicotinamide N-methyltransferase	2.1.1.1	P40261	H. sapiens	150
40	PARP1-3	PARP1	Poly [ADP-ribose] polymerase 1	2.4.2.30	Q9ZP54	A. thaliana	300
41	PARP1-3	PARP2	Poly [ADP-ribose] polymerase 2	2.4.2.30	Q11207	A. thaliana	300
42	PARP1-3	PARP3	Poly [ADP-ribose] polymerase 3	2.4.2.30	Q9FK91	A. thaliana	300
43	PARP1-3	pme-1	Poly(ADP-ribose) polymerase	2.4.2.30	Q9N4H4	C. elegans	300
44	PARP1-3	pme-2	Poly(ADP-ribose) polymerase	2.4.2.30	Q09525	C. elegans	300
45	PARP1-3	PARP1	Poly [ADP-ribose] polymerase 1	2.4.2.30	P09874	H. sapiens	300
46	PARP1-3	PARP2	Poly [ADP-ribose] polymerase 2	2.4.2.30	Q9UGN5	H. sapiens	300
47	PARP1-3	PARP3	Poly [ADP-ribose] polymerase 3	2.4.2.30	Q9Y6F1	H. sapiens	300
48	PARP4	PARP4	Poly [ADP-ribose] polymerase 4	2.4.2.30	Q9UKK3	H. sapiens	450
49	PARP6/8	PARP6	Poly [ADP-ribose] polymerase 6	2.4.2.30	Q2NL67	H. sapiens	175
50	PARP6/8	PARP8	Poly [ADP-ribose] polymerase 8	2.4.2.30	Q8N3A8	H. sapiens	175
51	PARP7/9-15	TIPARP	TCDD-inducible poly [ADP-ribose] polymerase	2.4.2.30	Q7Z3E1	H. sapiens	130
52	PARP7/9-15	PARP10	Poly [ADP-ribose] polymerase 10	2.4.2.30	Q53GL7	H. sapiens	130
53	PARP7/9-15	PARP11	Poly [ADP-ribose] polymerase 11	2.4.2.30	Q9NR21	H. sapiens	130
54	PARP7/9-15	PARP12	Poly [ADP-ribose] polymerase 12	2.4.2.30	Q9H0J9	H. sapiens	130
55	PARP7/9-15	PARP14	Poly [ADP-ribose] polymerase 14	2.4.2.30	Q460N5	H. sapiens	130
56	PARP7/9-15	PARP15	Poly [ADP-ribose] polymerase 15	2.4.2.30	Q460N3	H. sapiens	130
57	PARP7/9-15	PARP9	Poly [ADP-ribose] polymerase 9	2.4.2.30	Q8IXQ6	H. sapiens	130
58	PARP16	PARP16	Mono [ADP-ribose] polymerase	2.4.2.30	Q8N5Y8	H. sapiens	225
63	SIRT	SRT1	NAD-dependent protein deacetylase	3.5.1	Q9FE17	A. thaliana	180
64	SIRT	SRT2	NAD-dependent protein deacylase	3.5.1	Q94AQ6	A. thaliana	180
65	SIRT	sir-2.1	NAD-dependent protein deacetylase	3.5.1	Q21921	C. elegans	180
66	SIRT	sir-2.4	NAD-dependent protein deacetylase	3.5.1	Q95Q89	C. elegans	180
67	SIRT	sir-2.2	NAD-dependent protein deacylase	3.5.1	Q20480	C. elegans	180
68	SIRT	sir-2.3	NAD-dependent protein deacylase	3.5.1	Q20481	C. elegans	180
69		SIRII	NAD-dependent protein deacetylase sirtuin-1	3.5.1	Q96EB6	H. sapiens	180
70	SIRI		NAD-dependent protein deacetylase sirtuin-2	3.5.1	Q8IXJ6	H. sapiens	180
/1	SIRI		NAD-dependent protein deacetylase sirtuin-3	3.5.1		H. sapiens	180
12	SIRI		NAD-dependent protein deacetylase sirtuin-4	3.5.1	Q9Y6E7	H. sapiens	180
73		SIRIS	NAD-dependent protein deacetylase sirtuin-5	3.5.1	Q9NXA8	H. sapiens	180
74	SIRI	SIRI 6	NAD-dependent protein deacetylase sirtuin-6	3.5.1	Q8N617	H. sapiens	180
75	SIRI	SIRI /	NAD-dependent protein deacetylase sirtuin-7	3.5.1	Q9NRC8	H. sapiens	180
10			NAD-dependent protein deacetylase	3.5.1	PE2600	S. cerevisiae	180
	SIKI Tanlauraci	INDIZ		3.5.1	007701	S. cerevisiae	180
18	Tankyrase	pme-5	roly(ADF-ribose) polymerase	2.4.2.30	Q91XQ1	c. elegans	150
19	Tankyrase			2.4.2.30	095271	п. sapiens	150
00	тапкугазе	11NN32	DNA 21 phosphotropeforoso Tetl / KetA family	2.4.2.30		n. sapiens	170
82			TINA 2 -pilosphotransferase, Tpt1 / NptA Idilily	2.7.1.100		H sanions	170
02			uning z -phosphouansierase I	2./.1.100	Q001114	n. sapiens	1/0

Query proteins used for Blast searches.

Table S2

Name	ADPRcyc	ART	PARP1-3	PARP4	PARP6/8	PARP7/9-15	PARP16	SIRT	TRPT	Tankyrase	SUM
Mammalia	0.99	1.00	0.98	0.99	0.99	0.99	1.00	1.00	0.98	1.00	9.90
Hemichordata	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	9.00
Sauropsida	0.98	1.00	0.98	0.96	0.43	0.96	1.00	1.00	0.13	1.00	8.44
Neopterygii	0.92	0.24	0.96	0.88	0.72	0.76	0.96	0.96	0.84	0.96	8.20
Lophotrochozoa	0.80	0.00	1.00	1.00	0.80	0.80	1.00	1.00	0.80	1.00	8.20
Echinodermata	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	8.00
Branchiostoma	0.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	7.00
Cnidaria	1.00	0.00	1.00	1.00	1.00	0.00	0.50	1.00	0.50	1.00	7.00
Amphibia	0.67	0.67	0.67	0.67	0.67	0.33	0.67	0.67	0.33	0.67	6.00
Arthropoda	0.00	0.00	0.84	0.65	0.02	0.00	0.00	1.02	0.51	0.95	3.98
Platyhelminthes	0.00	0.00	1.00	0.00	0.33	0.00	0.00	1.00	0.50	1.00	3.83
Tunicata	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	3.00
Nematoda	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	3.00

Fraction of species with given NAD consumers per clade. For all clades at leaf positions in Figure 2B, the fraction of species in the respective clade possessing the respective NAD consumer is shown. For easier optical identification, table cell backgrounds are the darker, the higher the fraction is. The fraction of species with a given NAD consumer for clades that are not leaves is the sum of the values of all child nodes.

Table S3

Enzyme	EC number	Kinetic parameter	References	Rate Law
NADA	3.5.1.19	$K_M:9.6\mu M$ $K_{iP}:120\mu M$ $k_{cat}:0.65s^{-1}$	[2]	Product inhibition
NADS	6.3.5.1	$K_M:190\mu M$ $k_{cat}:21s^{-1}$	[3]	HMM
NMNAT	2.7.7.1 2.7.7.18	$\begin{array}{c} \hline K_{M_{NaMN}}:67.7\mu \mathrm{M} \\ k_{cat_{NaMN}}:42.9s^{-1} \\ K_{M_{NMN}}:22.3\mu \mathrm{M} \\ k_{cat_{NMN}}:53.8s^{-1} \\ K_{M_{NAD}}:59\mu \mathrm{M} \end{array}$	[4] ¹	Substrate Competition
		$\begin{array}{c} k_{cat_{NAD}}:129.1s^{-1} \\ K_{M_{NaAD}}:502\mu \mathrm{M} \\ k_{cat_{NaAD}}:103.8s^{-1} \end{array}$	[5] ² [5]	
NNMT	2.1.1.1	$K_{M_{Nam}}$:400 μ M $K_{M_{SAM}}$:1.8 μ M K_{iP} :60 μ M k_{cat} :8.1 s^{-1}	[6]	Bi irreversible with product inhibition
NamPT	2.4.2.12	$K_M:5nM$ $k_{cat}:0.0077s^{-1}$ $K_{i_{NAD}}: 2.1\mu M$	[8]	Competitive inhibition
NAPRT	2.4.2.11	$K_M:1.5\mu M$ $k_{cat}:3.3s^{-1}$	[8]	HMM
SIRT1	3.5.1	$K_M:29\mu M$ $K_{iP}:60\mu M$ $k_{cat}:0.67s^{-1}$	[9]	Product inhibition
NT5	3.1.3.5	$\begin{array}{c} K_{M_{NaMN}}:3.5 \mathrm{mM} \\ k_{cat_{NaMN}}:2.8 s^{-1} \\ K_{M_{NMN}}:5 \mathrm{mM} \\ k_{cat_{NMN}}:0.5 s^{-1} \end{array}$	[10]	HMM
PNP	2.4.2.1	$K_M:1.48\mathrm{mM}$ $k_{cat}:40s^{-1}$	[11]	HMM
NRK	2.7.1.173	$K_M: 3.4 \mu M$ $k_{cat}: 0.23 s^{-1}$	[12]	HMM

Overview of kinetic constants used for the construction of the model.

Additional parameter and model description

The total enzyme concentration was set to 10 times the scaling factor, for all enzymes except NamPT and NADA. For NamPT the concentration was set to 400 times the scaling factor if not stated otherwise. For NADA the enzyme concentration was set to 400 times the scaling factor of 0.1 μ M was applied to all enzymatic reactions to achieve consumption rates that are in the range of reported values [13]. Concentration of potential co-substrates except SAM were assumed to be constant and not-limiting for the reaction. If not stated otherwise, the SAM concentration was set to 80 μ M refelecting the concentration of SAM in liver tissues [14]. Thus being implicitly represented by maximal velocities consisting of total enzyme concentration times turnover rates. Nam import rates for import into the system was set to 0.1 μ M/s for all simulations, being in the range of nammalian cells [15]. In addition to the reactions listed above an additional NAD consumption was simulated using HMM-kinetics with a substrate affinity of 0.3 mM and a turnover rate of 1. Furthermore, reversible NAD binding to proteins was simulated using reversible mass actions kinetics with an equilibrium constant of 0.1, which is in a range of values reported in the literature, dissociation and association constants where set to 10 and 100s⁻¹ respectively. For the two compartment simulation, compartment size was equal for both compartments and set to 1 μ I. The actual compartment size does

¹Values for NMNAT1 used

²Equilibrium constant used for calculation of turnover rate of reverse reaction

not change the outcome of the simulations as long as both compartments have equal volumes. The Nam import rates were set to $100s^{-1}$ for both compartments. The amount of NADA present was set to 400. Thus equal to the amount of NamPT used.

To account for cell growth, we added an outflow reaction to each simulated metabolite. For this reaction we simulated a constant flux based on mass action kinetics. The reaction rate was equal for each metabolite and simulated to be in a range between $2.7 \cdot 10^{-6} s^{-1}$ and $2.8 \cdot 10^{-5} s^{-1}$, corresponding to a doubling in volume once every 0.01 to 1 hour, denoted as cell division rate in Figure 3.

Rate Laws referred to in Table S3

Product inhibition

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S + \frac{K_M \cdot P}{K_{iP}}} \tag{1}$$

Bi irreversible with product inhibition

$$v = \frac{E_T \cdot k_{cat} \cdot A \cdot B}{K_{ma}(1 + \frac{P}{K_{iP}})(B + K_{mb}) + A \cdot K_{mb}}$$
(2)

Competitive inhibition

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S + \frac{K_M \cdot I}{K_{iI}}} \tag{3}$$

Henry-Michaelis Menten for irreversible reactions (HMM)

$$v = \frac{E_T \cdot k_{cat} \cdot S}{K_M + S} \tag{4}$$

Substrate competition at NMNAT

$$v = E_T \cdot \frac{\frac{k_{cat_A} \cdot A \cdot B}{K_{M_A}} - \frac{k_{cat_P} \cdot P \cdot Q}{K_{M_P}}}{1 + \frac{A}{K_{M_A}} + \frac{B}{K_{M_B}} + \frac{P}{K_{M_P}} + \frac{Q}{K_{M_Q}}}$$
(5)

Table S4

PDB Code	2H3D	3DGR	3DHD	$3\mathrm{DHF}$	3DKJ	3DKL
2H3D	-	0.95	0.85	0.86	0.88	0.88
3DGR		-	0.61	0.61	0.55	0.57
3DHD			-	0.43	0.40	0.43
3 D H F				-	0.42	0.33
3DKJ					-	0.39
3DKL						-

Root mean square deviation (RMSD) values between different structures (in Å). The alignment and RMSD calculation was done with PyMOL[16]. The structures are 2H3D (human NAMPT) [17], 3DGR (human NAMPT·AMPcP complex) [18], 3DHD (human NAMPT·NMN·Mg₂PPi complex) [18], 3DHF (human BeF₃--NAMPT·NMN·Mg₂PPi complex) [18], 3DKJ (human NAMPT·PRPP·BzAM complex) [18], and 3DKL (human BeF₃--NAMPT·Mg₂PRPP·BzAM complex) [18]. The structural resolution of the PDB structures ranges from 1.8 Å to 2.1 Å.

References

- Prum RO, et al. (2015) A comprehensive phylogeny of birds (aves) using targeted next-generation dna sequencing. Nature 526(7574):569-73.
- [2] Smith BC, et al. (2012) Structural and kinetic isotope effect studies of nicotinamidase (Pnc1) from saccharomyces cerevisiae. Biochemistry 51(1):243-256.
- [3] Yi CK, Dietrich LS (1972) Purification and properties of yeast nicotinamide adenine dinucleotide synthetase. Journal of Biological Chemistry 247(15):4794–4802.
- [4] Sorci L, et al. (2007) Initial-rate kinetics of human NMN-adenylyltransferases: Substrate and metal ion specificity, inhibition by products and multisubstrate analogues, and isozyme contributions to NAD+ biosynthesis. *Biochemistry* 46(16):4912– 4922.
- Berger F, Lau C, Dahlmann M, Ziegler M (2005) Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. *Journal of Biological Chemistry* 280(43):36334– 36341.
- [6] Aksoy S, Szumlanski CL, Weinshilboum RM (1994) Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. Journal of Biological Chemistry 269(20):14835–14840.
- [7] Alston TA, Abeles RH (1988) Substrate specificity of nicotinamide methyltransferase isolated from porcine liver. Archives of biochemistry and biophysics 260(2):601–608.
- Burgos ES, Schramm VL (2008) Weak coupling of ATP hydrolysis to the chemical equilibrium of human nicotinamide phosphoribosyltransferase. *Biochemistry* 47(42):11086–11096.
- Borra MT, Langer MR, Slama JT, Denu JM (2004) Substrate Specificity and Kinetic Mechanism of the Sir2 Family of NAD
 + -Dependent Histone/Protein Deacetylases †. Biochemistry 43(30):9877–9887.
- [10] Kulikova V, et al. (2015) Generation, release, and uptake of the NAD precursor nicotinic acid riboside by human cells. Journal of Biological Chemistry 290(45):27124–27137.
- [11] Wielgus-Kutrowska B, Kulikowska E, Wierzchowski J, Bzowska A, Shugar D (1997) Nicotinamide riboside, an unusual, non-typical, substrate of purified purine-nucleoside phosphorylases. *European Journal of Biochemistry* 243(1-2):408–414.
- [12] Dölle C, Ziegler M (2009) Application of a coupled enzyme assay to characterize nicotinamide riboside kinases. Analytical Biochemistry 385(2):377–379.
- [13] Liu L, et al. (2018) Quantitative analysis of NAD synthesis-breakdown fluxes. Cell Metabolism 27(5):1067–1080.e5.
- [14] Reed MC, Nijhout HF, Sparks R, Ulrich CM (2004) A mathematical model of the methionine cycle. J Theor Biol 226(1):33– 43.
- [15] Billington RA, et al. (2008) Characterization of nad uptake in mammalian cells. J Biol Chem 283(10):6367-74.
- [16] Schrödinger, LLC (2010) The {PyMOL} Molecular Graphics System, Version~1.4.1.
- [17] Wang T, et al. (2006) Structure of Nampt/PBEF/visfatin, a mammalian NAD+ biosynthetic enzyme. Nature Structural & Molecular Biology 13(7):661–662.
- [18] Burgos ES, Ho MC, Almo SC, Schramm VL (2009) A phosphoenzyme mimic, overlapping catalytic sites and reaction coordinate motion for human NAMPT. Proceedings of the National Academy of Sciences 106(33):13748–13753.