SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Secondary structure predictions for human NMNAT2. The annotated secondary structures of NMNAT1 (PDB:1kku_A) and NMNAT3 (PDB:1nur_A) are collectively represented in line 9 (1kku/1nur). Common elements are written in upper case. X designates non-resolved regions. Porter, Sable, Jpred3, PROFking, SSpro, and PSIPRED are secondary structure prediction tools based on comparative prediction algorithms. The corresponding predictions for NMNAT2 are displayed. The amino acid stretch Lys107 to Thr148 of ISTID2 seems to be rather unstructured, while the second part, Ala149 to Leu192, might harbor several structural elements. Fold recognition was performed using mGenTHREADER, which detected NADPH dependent prostaglandin E(2) 9 reductase (rabbit; PDB:1q5m) as a structural template for prediction of the ISTID fold (line 8). ISTID2 of NMNAT2 is highlighted in red. Structural elements are designated as follows: C, coiled (unstructured); H, α-helical; E, β-strand.

Figure S2. Schematic representation of the NMNAT constructs generated and used in this study. Human NMNAT cDNA sequences were introduced into eukaryotic expression vectors. The various constructs were generated as described in Experimental Procedures. The specification of tags, mutations and other alterations is indicated in the box. The designation of the constructs has been used throughout this study.

Figure S3. Fine localization of NMNAT2. NMNAT2 does not co-localize with TGN and other post-Golgi compartments. N-terminally Flag-tagged NMNAT2 wild-type was over-expressed in HeLa S3 cells and subjected to immunofluorescence analyses using mouse anti-Flag antibody as well as IgGs specific for endogenous markers of different Golgi compartments: *cis*-Golgi (GM130), ER-Golgi intermediate compartment (ERGIC-53), and trans-Golgi network/late endosomes (CI-MPR).

Figure S4. Multiple sequence alignment of vertebrate NMNAT orthologs. From the ENSEMBL database (release 53) (45), 15 sequences for NMNAT1s (length of the gap free alignment is 275 codons), 13 sequences for NMNAT2 (300 codons), and 10 sequences for NMNAT3 (232 codons) from various vertebrate species, including humans, were extracted. For NMNAT2 an additional sequence was obtained from the lamprey (*Petromyzon marinus*) genome assembly (http://genome.wustl.edu/pub/organism/Other_Vertebrates/Petromyzon_marinus/assembly/Petromyzon_marinus-3.0/output/) using GeneWise gene prediction (46). Corresponding protein sequences were aligned using MUSCLE (67) and prepared with PAL2NAL (68). Manual inspection revealed a low alignment quality for the C-terminus of NMNAT3, thus the alignment was trimmed in order to reduce the probability of overaligning. Residues belonging to the ISTIDs of vertebrate NMNATs are collectively surrounded by the red box.
(A) The multiple sequence alignment of vertebrate NMNAT1 orthologs is shown.
(B) The multiple sequence alignment of vertebrate NMNAT2 orthologs is shown.
(C) The multiple sequence alignment of vertebrate NMNAT3 orthologs is shown.

SUPPLEMENTAL REFERENCES

67. Edgar, R. C. (2004) *Nucleic Acids Res.* **32,** 1792-1797
68. Suyama, M., Torrents, D., and Bork, P. (2006) *Nucleic Acids Res.* **34,** W609-W612

Figure S1

```
                      10        20        30        40        50        60
                      |         |         |         |         |         |
      NMNAT2 MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMC
      Porter CCCCCCCEEEEEEEECCCCCCHHHHHHHHHHHHHHHHCCCEEEEEEEEECCCCCCCCCCCCCHHHHHHHH
       Sable CCCCCCCEEEEEECCCCCCCHHHHHHHHHHHHHHHHHCCCEEEEEEEECCCCCCCCCCCCCCCHHHHHHH
      Jpred3 -------EEEEEE--------HHHHHHHHHHHHHHHH---EEEEEEEEE-------------HHHHHHHH
    PROFKing CCCCCCEEEEEEECCCCCCCHHHHHHHHHHHHHHHCCCCEEEEEEEEECCCCCCCCCCCCCCCCHHHHHH
       Sspro CCCCCCCEEEEEEECCCCCCCHHHHHHHHHHHHHHHHCCCEEEEEEEECCCCCCCCCCCCCHHHHHHHHH
     PSIPRED CCCCCCCCEEEEECCCCCCCCHHHHHHHHHHHHHHCCCCCCEEEEEEEEECCCCCCCCCCCCHHHHHHHH
MGenTHREADER CCCCCCCEEEEEEECCCCCCCHHHHHHHHHHHHHHHHCCCEEEEEEEECCCCCCCCCCCCCCHHHHHHHH
   1kku/1nur ------EEEEEEEE------HHHHHHHHHHHHHHHHHH-eEEEEEEEEEE----------hHHHHHHHHH
      NMNAT1 MENSEKTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGRYTVVKGIISPVGDAYKKKGLIPAYHRVIMA
      NMNAT3 --MKSRIPVVLLACGSFNPITNMHLRMFEVARDHLHQTGMYQVIQGIISPVNDTYGKKDLAASHHRVAMA

                      80        90        100       110       120       130
                      |         |         |         |         |         |
      NMNAT2 QLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHRDLMKRVTGCILSNVNTPSMTPVIGQPQNETPQPIYQN
      Porter HHHHCCCCCEEECCHHHHCCCHHHHHHHHHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
       Sable HHHHCCCCCEEECCCCCCCCCCCCHHHHHHHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
      Jpred3 HHHH-----EEEEEEEE-----EEEHHHHHHHHHHHHHHH------------------------------
    PROFKing HHHHCCCCEEEECCCCCHHHHHHHHHHHHHCCCHHHHHCCCEEEECCCCCCCCCCCCCCCCCCCCCCCCC
       Sspro HHHHCCCCEEEECCHHHHHHHHHHHHHHHHHHHHHHHHHCCCEECCCCCCCCCCCCCCCCCCCCCCCCCC
     PSIPRED HHHHCCCCCEEEEEHHHHCCCCEECHHHHHHHHHHCCCCCEEEEECCCCCCCCHHHCCCCCCCHHHHHHH
MGenTHREADER HHHHCCCCCEEEEHHHCCCCCCCCHHHHHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCHHHHC
   1kku/1nur HHHHh----EEEHHHHHHh---HHHHHHHHHHHHHH--xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
      NMNAT1 ELATKNSKWVEVDTWESLQKEWKETLKVLRHHQEKL--EASDCDHQQNSPTLERPGRKRKWTETQDSSQK
      NMNAT3 RLALQTSDWIRVDPWESEQAQWMETVKVLRHHHSKL---------LRSPPQMEGP------------DHG

                      150       160       170       180       190       200
                      |         |         |         |         |         |
      NMNAT2 SNVATKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLLESFCIP
      Porter CCCCCCCCCCHHCCCCCCCCCCEEEEECCCCEEEEECCCCCCCCHCHHHHHHHHHHHHHHHHHHHHHCCC
       Sable CCCCCCCCHHHHHCHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCEEEEEECCHHHHHHCCC
      Jpred3 -----HHHHHHHHHHH-----------------------HHHHHHHH-------EEEEEHHHHHH-----
    PROFKing CCCCCCCCCCCCCCCCCCCCCCEEEECCCCCEEEEECCCCCCCHHHHHHHHHHHHHEECCCCHHHHCCCC
       Sspro CCCCCCCCHHHHHHHHHHHHHHHCCCCCCEEEEEECCCCCCCCCCCCCHHHHHHHHHHCCCHHHHHCCCC
     PSIPRED CCEEEECCCCCCHHCCHHHHHHHHHHHHHHHHHHCCCCCCCCCCCCCCHHHEEEHEEEECHHHHHHCCCC
MGenTHREADER CCCCCCCCCHHHHHHCCCCEEEEECCCCCCCCCCEEEECCCHHHHHHHHCCCCCEEEEECHHHHHHCCCC
   1kku/1nur xxxxxxxxxxx------------------------------------------EEEEEEHHHHHHHHhh-
      NMNAT1 KSLEPKTKAVP------------------------------------------KVKLLCGADLLESFAVP
      NMNAT3 KALFSTPAAVP------------------------------------------ELKLLCGADVLKTFQTP

                      220       230       240       250       260       270
                      |         |         |         |         |         |
      NMNAT2 GLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHG
      Porter CCCCHHHHHHHHHHCCEEEECCCCCCHHHHHHHHHHHHHHCCCEEEECCCCCCCCCCCCHHHHHHHHHCC
       Sable CCCCHHHHHHHHHCCCEEEEECCCCCHHHHHHHCHHHHHHCCCEEEEECCCCCCCCEECHHHHHHHHHCC
      Jpred3 -----HHHHHHHHH--EEEEE------HHH--------------EEEE-------HHHHHHHHHHHHHH-
    PROFKing CCCCCCCHHHHHHCCCEEEEECCCCCHCCEEECCHHHHHHCCCCEEEECCHCCCCEEEECHHHHHHHHCC
       Sspro CCCCHHHHHHHHHHCCEEEEECCHCCHHHHHCHHHHHHHHCCCEEEEECCCCCCCCCCCHHHHHHHHHCC
     PSIPRED CCCCHHHHHHHHHHCCEEEEECCCCCHHHHHHHHHHHHHCCCCCEEEEEECCCCHHHCCHHHHHHHHHCC
MGenTHREADER CCCCHHHHHHHHHHCCEEEEECCCCCHHHHHHHHHHHHHCCCCEEEECCCCCCCHHHHHHHHHHHHHHCC
   1kku/1nur ---HHHHHHHHHHHh-EEEEe-hhhHHHHHHHHHHHHHHH---EEEEe------xxx-HHHHHHHHHxxh
      NMNAT1 NLWKSEDITQIVANYGLICVTRAGNDAQKFIYESDVLWKHRSNIHVVNEWIAND---ISSTKIRRAL--R
      NMNAT3 NLWKDAHIQEIVEKFGLVCVGRVGHDPKGYIAESPILRMHQHNIHLAKEPVQNE---ISATYIRRAL--G

                      290       300       310       320
                      |         |         |         |
      NMNAT2 DGHVVDYLSQPVIDYILKSQLYINASG----------------------
      Porter CCCEEECCCHHHHHHHHHCCCCCCCCC----------------------
       Sable CCCCEEECCHHHHHHHHHCCCCCCCCC----------------------
      Jpred3 ---------HHHHHHHHH-------------------------------
    PROFKing CCCEEEECCCHHHHHHHHHCCCCCCCC----------------------
       Sspro CCCEEEECCHHHHHHHHHCCCEECCCC----------------------
     PSIPRED CCCCCCCCCHHHHHHHHHHCCCCCCCC----------------------
MGenTHREADER CCCCCCCCCHHHHHHHHHCCCCCCCCC----------------------
   1kku/1nur h-----hhHHHHHHHHHhh------------------------------
      NMNAT1 RGQSIRYLVPDLVQEYIEKHNLYSSESEDRNAGVILAPLQRNTAEAKT-
      NMNAT3 QGQSVKYLIPDAVITYIKDHGLYTKGSTWK------GKSTQSTEGKTS-
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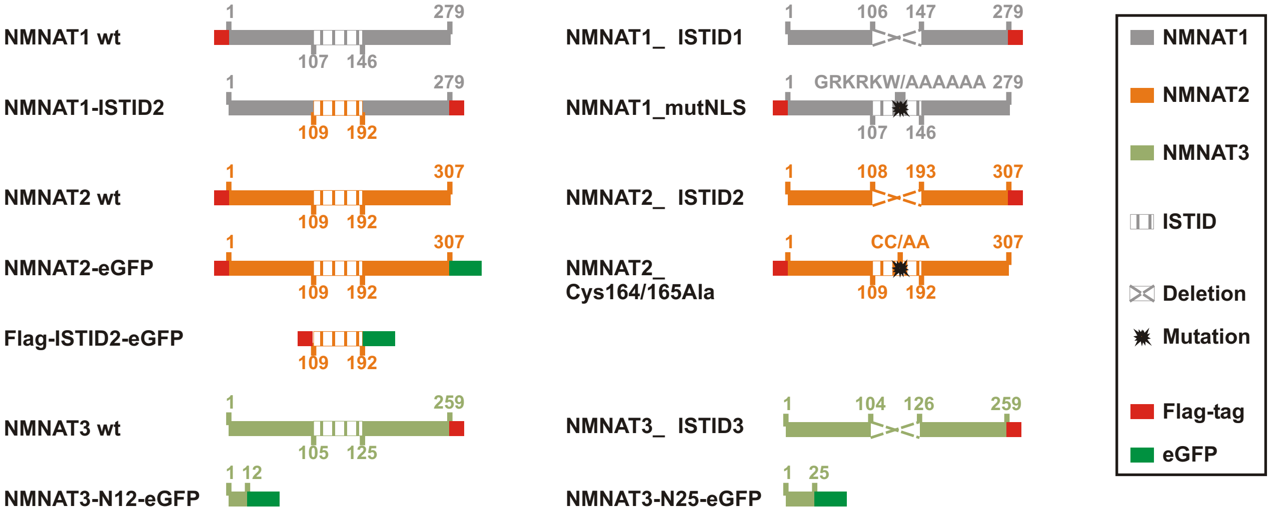
Figure S2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107
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NMNAT1-ISTID2
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NMNAT2 wt
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NMNAT2-eGFP
1
307
109
192
Flag-ISTID2-eGFP
109
192
NMNAT3 wt
1
259
105
125
NMNAT3-N12-eGFP
1 12
NMNAT1_ ISTID1
1
106
147
279
NMNAT1_mutNLS
1
GRKRKW/AAAAAA
279
107
146
NMNAT2_ ISTID2
1
108
193
307
NMNAT2_
Cys164/165Ala
1
CC/AA
307
109
192
NMNAT3_ ISTID3
1
104
126
259
NMNAT3-N25-eGFP
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NMNAT2
NMNAT3
ISTID
Deletion
Mutation
Flag-tag
eGFP

Figure S3

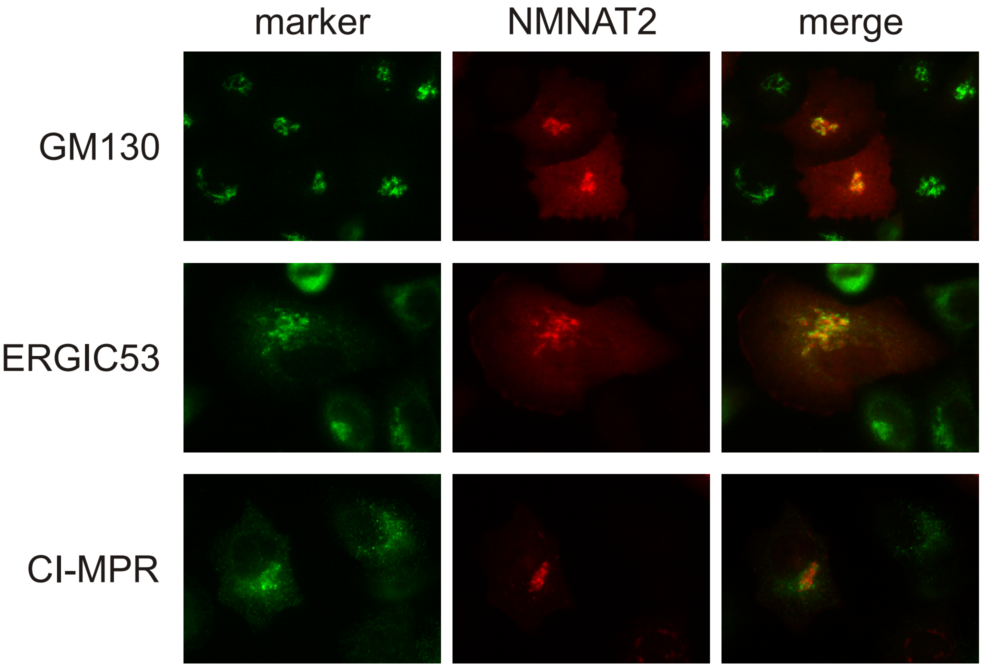
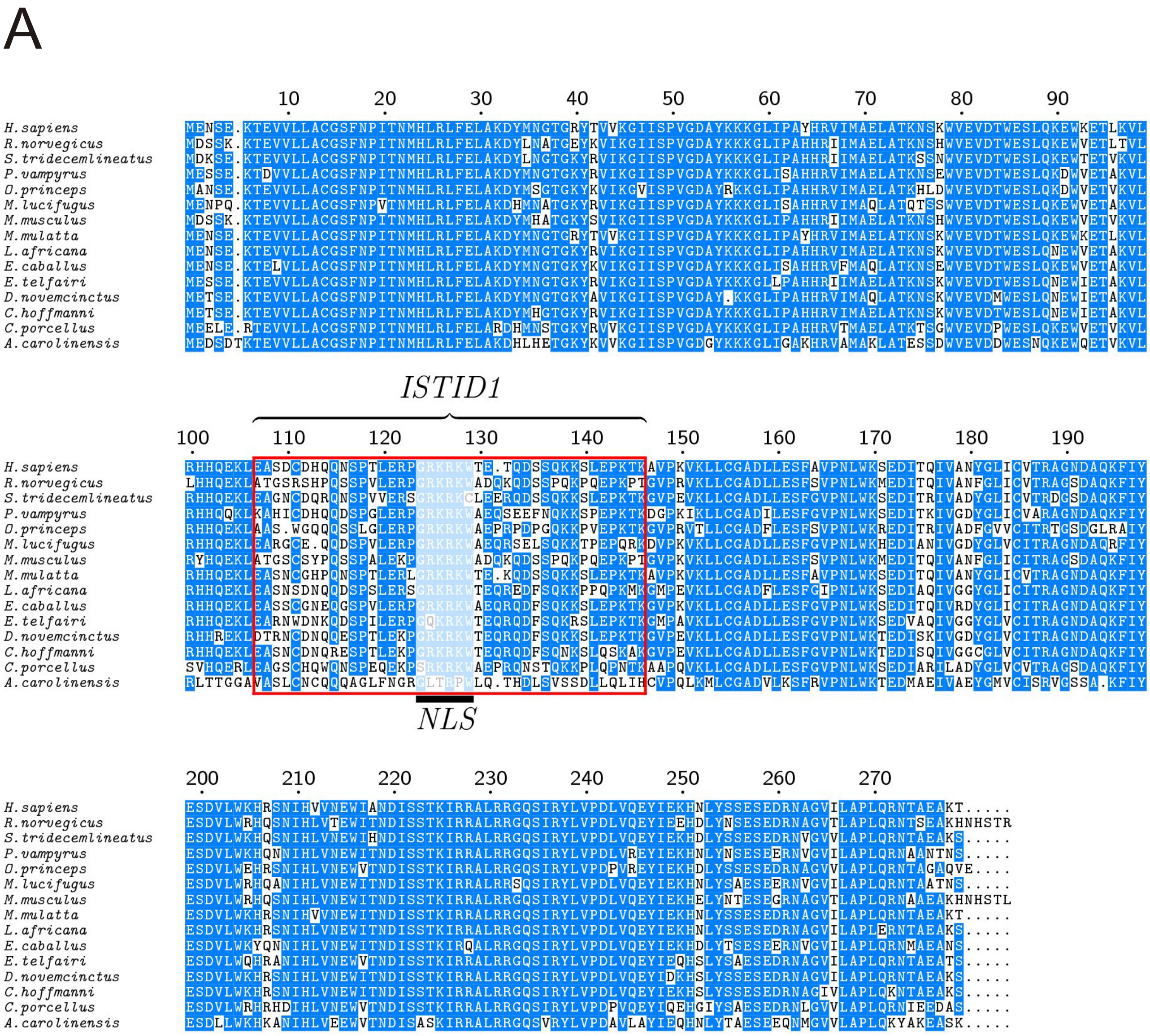


Figure S4



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R.norvegicus        MDSSK.KTEVVLLACGSFNPITNMHLRLFELAKDYLNATGEYKVIKGIISPVGDAYKKKGLIPAHHRIIMAELATKNSHWVEVDTWESLQKEWVETLTVL
S.tridecemlineatus  MDKSE.KTEVVLLACGSFNPITNMHLRLFELAKDYLNGTGKYRVIKGIISPVGDAYKKKGLIPAHHRIIMAELATKSSNWVEVDTWESLQKEWTETVKVL
P.vampyrus          MESSE.KTDVVLLACGSFNPITNMHLRLFELAKDYMNGTGKYRVIKGIISPVGDAYKKKGLISAHHRVIMAELATKNSEWVEVDTWESLQKDWVETAKVL
O.princeps          MANSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMSGTGKYKVIKGVISPVGDAYRKKGLIPAHHRVIMAELATKHLDWVEVDTWESLQKDWVETVKVL
M.lucifugus         MENPQ.KTEVVLLACGSFNPVTNMHLRLFELAKDHMNATGKYRVIKGIISPVGDAYKKKGLISAHHRVIMAQLATQTSSWVEVDTWESLQKEWVETAKVL
M.musculus          MDSSK.KTEVVLLACGSFNPITNMHLRLFELAKDYMHATGKYSVIKGIISPVGDAYKKKGLIPAHHRIIMAELATKNSHWVEVDTWESLQKEWVETVKVL
M.mulatta           MENSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGRYTVVKGIISPVGDAYKKKGLIPAYHRVIMAELATKNSKWVEVDTWESLQKEWKETLKVL
L.africana          MENSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGKYRVIKGIISPVGDAYKKKGLIPAHHRVIMAELATKNSKWVEVDTWESLQNEWVETAKVL
E.caballus          MENSE.KTELVLLACGSFNPITNMHLRLFELAKDYMNGTGKYKVIKGIISPVGDAYKKKGLISAHHRVFMAQLATKNSEWVEVDTWESLQKEWVETAKVL
E.telfairi          MESSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGKYRVIKGIISPVGDAYKKKGLLPAHHRIIMAELATKNSKWVEVDTWESLQNEWIETAKVL
D.novemcinctus      METSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGKYAVIKGIISPVGDAY.KKGLIPAHHRVIMAQLATKNSKWVEVDMWESLQNEWIETAKVL
C.hoffmanni         METSE.KTEVVLLACGSFNPITNMHLRLFELAKDYMHGTGKYRVIKGIISPVGDAYKKKGLIPAHHRVIMAELATKNSKWVEVDTWESLQNEWIETAKVL
C.porcellus         MEELE.RTEVVLLACGSFNPITNMHLRLFELARDHMNSTGKYRVVKGIISPVGDAYKKKGLIPAHHRVTMAELATKTSGWVEVDPWESLQKEWVETVKVL
A.carolinensis      MEDSDTKTEVVLLACGSFNPITNMHLRLFELAKDHLHETGKYKVVKGIISPVGDGYKKKGLIGAKHRVAMAKLATESSDWVEVDDWESNQKEWQETVKVL

                   100       110       120       130        140       150       160       170       180       190
H.sapiens           RHHQEKLEASDCDHQQNSPTLERPGRKRKWTE.TQDSSQKKSLEPKTKAVPKVKLLCGADLLESFAVPNLWKSEDITQIVANYGLICVTRAGNDAQKFIY
R.norvegicus        LHHQEKLATGSRSHPQSSPVLERPGRKRKWADQKQDSSPQKPQEPKPTGVPRVKLLCGADLLESFSVPNLWKMEDITQIVANFGLICVTRAGSDAQKFIY
S.tridecemlineatus  RHHQEKLEAGNCDQRQNSPVVERSGRKRKCLEERQDSSQKKSLEPKTKGVPEVKLLCGADLLESFGVPNLWKSEDITRIVADYGLICVTRDGSDAQKFIY
P.vampyrus          RHHQQKLKAHICDHQQDSPGLERPGRKRKWAEQSEEFNQKKSPEPKTKDGPKIKLLCGADILESFGVPNLWKSEDITKIVGDYGLICVARAGNDAQKFIY
O.princeps          RHHQEKLAAS.WGQQQSSLGLERPGRKRKWAEPRPDPGQKKPVEPKTKGVPRVTLLCGADFLESFSVPNLWKREDITRIVADFGVVCITRTGSDGLRAIY
M.lucifugus         RHHQEKLEARGCE.QQDSPVLERPGRKRKWAEQRSELSQKKTPEPQRKDVPKVKLLCGADLLESFGVPNLWKHEDIANIVGDYGLVCITRAGNDAQRFIY
M.musculus          RYHQEKLATGSCSYPQSSPALEKPGRKRKWADQKQDSSPQKPQEPKPTGVPKVKLLCGADLLESFSVPNLWKMEDITQIVANFGLICITRAGSDAQKFIY
M.mulatta           RHHQEKLEASNCGHPQNSPTLERLGRKRKWTE.KQDSSQKKSLEPKTKAVPKVKLLCGADLLESFAVPNLWKSEDITQIVANYGLICVTRAGNDAQKFIY
L.africana          RHHQEKLEASNSDNQQDSPSLERSGRKRKWTEQREDFSQKKPPQPKMKGMPEVKLLCGADFLESFGIPNLWKSEDIAQIVGGYGLICITRAGNDAQKFIY
E.caballus          RHHQEKLEASSCGNEQGSPVLERPGRKRKWAEQRQDFSQKKSLEPKTKGVPKVKLLCGADLLESFGVPNLWKSEDITQIVRDYGLICITRAGNDAQKFIY
E.telfairi          RHHQEKLEARNWDNKQDSPILERPGQKRKWTEQRQDFSQKRSLEPKTKGMPAVKLLCGADLLESFGVPNLWKSEDVAQIVGGYGLVCITRAGNDAQKFIY
D.novemcinctus      RHHREKLDTRNCDNQQESPTLEKPGRKRKWTEQRQDFSQKKSLEPKTKGVPEVKLLCGADLLESFGVPNLWKTEDISKIVGDYGLVCITRAGNDAQKFIY
C.hoffmanni         RHHQEKLEASNCDNQRESPTLEKPGRKRKWTEQRQDFSQNKSLQSKAKGVPEVKLLCGADLLESFGVPNLWKTEDISQIVGGCGLVCITRAGNDAQKFIY
C.porcellus         SVHQERLEAGSCHQWQNSPEQEKPSRKRKWAEPRQNSTQKKPLQPNTKAAPQVKLLCGADLLESFGVPNLWKSEDIARILADYGLVCVTRAGSDAQKFIY
A.carolinensis      RLTTGGAVASLCNCQQQAGLFNGRGLTRPWLQ.THDLSVSSDLLQLIHCVPQLKMLCGADVLKSFRVPNLWKTEDMAEIVAEYGMVCISRVGSSA.KFIY

                   200       210       220       230       240       250       260       270
H.sapiens           ESDVLWKHRSNIHVVNEWIANDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHNLYSSESEDRNAGVILAPLQRNTAEAKT.....
R.norvegicus        ESDVLWRHQSNIHLVTEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIEEHDLYNSESEDRNAGVTLAPLQRNTSEAKHNHSTR
S.tridecemlineatus  ESDVLWKHQSNIHLVNEWIHNDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHDLYSSESEDRNVGVVLAPLQRNTAEAKS.....
P.vampyrus          ESDVLWKHQNNIHLVNEWITNDISSTKIRRALRRGQSIRYLVPDLVREYIEKHNLYNSESEERNVGVILAPLQRNAANTNS.....
O.princeps          ESDVLWEHRSNIHLVNEWVTNDISSTKIRRALRRGQSIRYLVPDPVREYIEKHDLYSSESEDRNAGVVLAPLQRNTAGAQVE....
M.lucifugus         ESDVLWRHQANIHLVNEWITNDISSTKIRRALRRSQSIRYLVPDLVQEYIEKHNLYSAESEERNVGVILAPLQRNTAATNS.....
M.musculus          ESDVLWRHQSNIHLVNEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHELYNTESEGRNAGVTLAPLQRNAAEAKHNHSTL
M.mulatta           ESDVLWKHRSNIHVVNEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHNLYSSESEDRNAGVILAPLQRNTAEAKT.....
L.africana          ESDVLWKHRSNIHLVNEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHNLYSSESEDRNAGVILAPLERNTAEAKS.....
E.caballus          ESDVLWKYQNNIHLVNEWITNDISSTKIRQALRRGQSIRYLVPDLVQEYIEKHDLYTSESEERNVGVILAPLQRNMAEANS.....
E.telfairi          ESDVLWQHRANIHLVNEWVTNDISSTKIRRALRRGQSIRYLVPDLVQEYIEQHSLYSAESEDRNAGVILAPLQRNTAEATS.....
D.novemcinctus      ESDVLWKHRSNIHLVNEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIDKHSLYSSESEDRNAGVILAPLQRNTAEAKS.....
C.hoffmanni         ESDVLWKHRSNIHLVNEWITNDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHSLYSSESEDRNAGIVLAPLQKNTAEAKS.....
C.porcellus         ESDVLWRHRHDIHLVNEWVTNDISSTKIRRALRRGQSIRYLVPDPVQEYIQEHGIYSAESEDRNLGVVLAPLQRNIEEDAS.....
A.carolinensis      ESDLLWKHKANIHLVEEWVTNDISASKIRRALRRGQSVRYLVPDAVLAYIEQHNLYTAESEEQNMGVVLAPLQKYAKEASK.....
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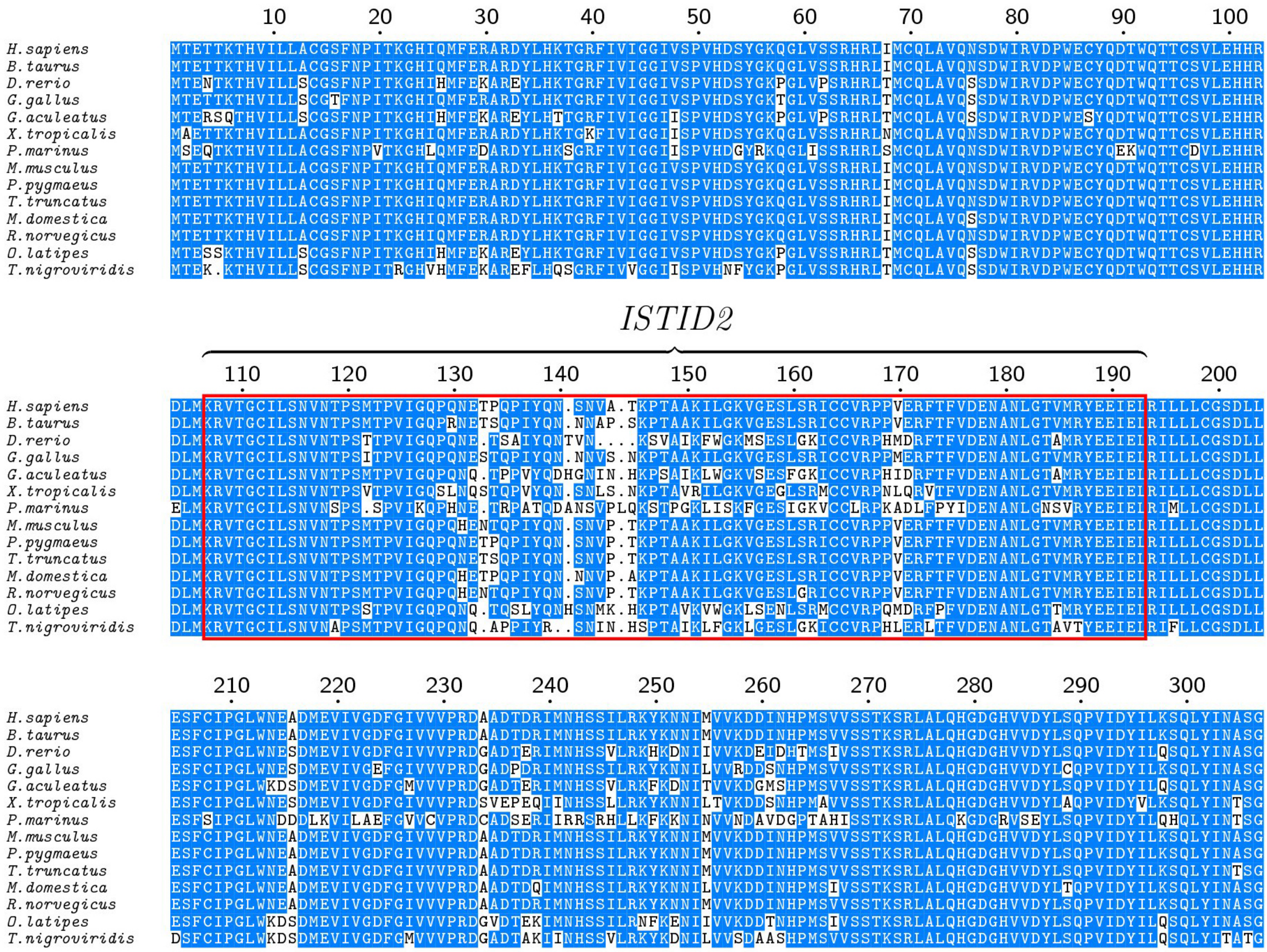
Figure S4

B



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B.taurus        MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
D.rerio         MTENTKTHVILLSCGSFNPITKGHIHMFEKAREYLHKTGRFIVIGGIVSPVHDSYGKPGLVPSRHRLTMCQLAVQSSDWIRVDPWECYQDTWQTTCSVLEHHR
G.gallus        MTETTKTHVILLSCGTFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKTGLVSSRHRLTMCQLAVQSSDWIRVDPWECYQDTWQTTCSVLEHHR
G.aculeatus     MTERSQTHVILLSCGSFNPITKGHIHMFEKAREYLHTTGRFIVIGGIISPVHDSYGKPGLVPSRHRLTMCQLAVQSSDWIRVDPWESYQDTWQTTCSVLEHHR
X.tropicalis    MAETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGKFIVIGGIISPVHDSYGKQGLVSSRHRLNMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
P.marinus       MSEQTKTHVILLACGSFNPVTKGHLQMFEDARDYLHKSGRFIVIGGIISPVHDGYRKQGLISSRHRLSMCQLAVQNSDWIRVDPWECYQEKWQTTCDVLEHHR
M.musculus      MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
P.pygmaeus      MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
T.truncatus     MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
M.domestica     MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQSSDWIRVDPWECYQDTWQTTCSVLEHHR
R.norvegicus    MTETTKTHVILLACGSFNPITKGHIQMFERARDYLHKTGRFIVIGGIVSPVHDSYGKQGLVSSRHRLIMCQLAVQNSDWIRVDPWECYQDTWQTTCSVLEHHR
O.latipes       MTESSKTHVILLSCGSFNPITKGHIHMFEKAREYLHKTGRFIVIGGIVSPVHDSYGKPGLVSSRHRLTMCQLAVQSSDWIRVDPWECYQDTWQTTCSVLEHHR
T.nigroviridis  MTEK.KTHVILLSCGSFNPITRGHVHMFEKAREFLHQSGRFIVVGGIISPVHNFYGKPGLVSSRHRLTMCQLAVQSSDWIRVDPWECYQDTWQTTCSVLEHHR

                                                       ISTID2
                      110       120       130       140          150       160       170       180       190        200
H.sapiens       DLMKRVTGCILSNVNTPSMTPVIGQPQNETPQPIYQN.SNVA.TKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
B.taurus        DLMKRVTGCILSNVNTPSMTPVIGQPRNETSQPIYQN.NNAP.SKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
D.rerio         DLMKRVTGCILSNVNTPSTTPVIGQPQNE.TSAIYQNTVN....KSVAIKFWGKMSESLGKICCVRPHMDRFTFVDENANLGTAMRYEEIELRILLLCGSDLL
G.gallus        DLMKRVTGCILSNVNTPSITPVIGQPQNESTQPIYQN.NNVS.NKPTAAKILGKVGESLSRICCVRPPMERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
G.aculeatus     DLMKRVTGCILSNVNTPSMTPVIGQPQNQ.TPPVYQDHGNIN.HKPSAIKLWGKVSESFGKICCVRPHIDRFTFVDENANLGTAMRYEEIELRILLLCGSDLL
X.tropicalis    DLMKRVTGCILSNVNTPSVTPVIGQSLNQSTQPVYQN.SNLS.NKPTAVRILGKVGEGLSRMCCVRPNLQRVTFVDENANLGTVMRYEEIELRILLLCGSDLL
P.marinus       ELMKRVTGCILSNVNSPS.SPVIKQPHNE.TRPATQDANSVPLQKSTPGKLISKFGESIGKVCCLRPKADLFPYIDENANLGNSVRYEEIELRIMLLCGSDLL
M.musculus      DLMKRVTGCILSNVNTPSMTPVIGQPQHENTQPIYQN.SNVP.TKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
P.pygmaeus      DLMKRVTGCILSNVNTPSMTPVIGQPQNETPQPIYQN.SNVP.TKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
T.truncatus     DLMKRVTGCILSNVNTPSMTPVIGQPQNETSQPIYQN.SNVP.TKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
M.domestica     DLMKRVTGCILSNVNTPSMTPVIGQPQHETPQPIYQN.NNVP.AKPTAAKILGKVGESLSRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
R.norvegicus    DLMKRVTGCILSNVNTPSMTPVIGQPQHENTQPIYQN.SNVP.TKPTAAKILGKVGESLGRICCVRPPVERFTFVDENANLGTVMRYEEIELRILLLCGSDLL
O.latipes       DLMKRVTGCILSNVNTPSSTPVIGQPQNQ.TQSLYQNHSNMK.HKPTAVKVWGKLSENLSRMCCVRPQMDRFPFVDENANLGTTMRYEEIELRILLLCGSDLL
T.nigroviridis  DLMKRVTGCILSNVNAPSMTPVIGQPQNQ.APPIYR..SNIN.HSPTAIKLFGKLGESLGKICCVRPHLERLTFVDENANLGTAVTYEEIELRIFLLCGSDLL

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H.sapiens       ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINASG
B.taurus        ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINASG
D.rerio         ESFCIPGLWNESDMEVIVGDFGIVVVPRDGADTERIMNHSSVLRKHKDNIIVVKDEIDHTMSIVSSTKSRLALQHGDGHVVDYLSQPVIDYILQSQLYINASG
G.gallus        ESFCIPGLWNESDMEVIVGEFGIVVVPRDGADPDRIMNHSSILRKYKNNILVVRDDSNHPMSVVSSTKSRLALQHGDGHVVDYLCQPVIDYILKSQLYINASG
G.aculeatus     ESFCIPGLWKDSDMEVIVGDFGMVVVPRDGADTERIMNHSSVLRKFKDNITVVKDGMSHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILQSQLYINASG
X.tropicalis    ESFCIPGLWNESDMEVIVGDFGIVVVPRDSVEPEQIINHSSLLRKYKNNILTVKDDSNHPMAVVSSTKSRLALQHGDGHVVDYLAQPVIDYVLKSQLYINTSG
P.marinus       ESFSIPGLWNDDDLKVILAEFGVVCVPRDCADSERIIRRSRHLLKFKKNINVVNDAVDGPTAHISSTKSRLALQKGDGRVSEYLSQPVIDYILQHQLYINTSG
M.musculus      ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINASG
P.pygmaeus      ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINASG
T.truncatus     ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINTSG
M.domestica     ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDQIMNHSSILRKYKNNILVVKDDINHPMSIVSSTKSRLALQHGDGHVVDYLTQPVIDYILKSQLYINASG
R.norvegicus    ESFCIPGLWNEADMEVIVGDFGIVVVPRDAADTDRIMNHSSILRKYKNNIMVVKDDINHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILKSQLYINASG
O.latipes       ESFCIPGLWKDSDMEVIVGDFGIVVVPRDGVDTEKIMNHSSILRNFKENIIVVKDDTNHPMSIVSSTKSRLALQHGDGHVVDYLSQPVIDYILQSQLYINASG
T.nigroviridis  DSFCIPGLWKDSDMEVIVGDFGMVVVPRDGADTAKIINHSSVLRKYKDNILVVSDAASHPMSVVSSTKSRLALQHGDGHVVDYLSQPVIDYILQSQLYITATG
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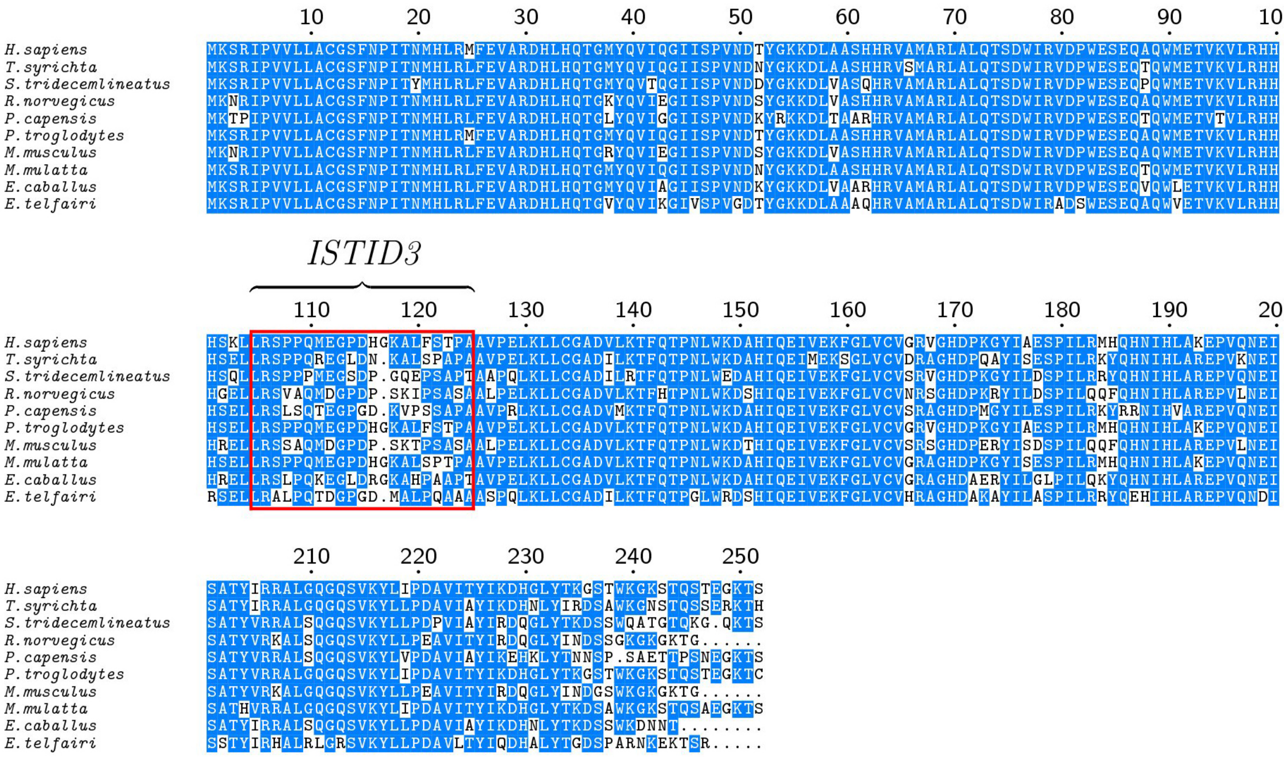
Figure S4

C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T.syrichta
S.tridecemlineatus
R.norvegicus
P.capensis
P.troglodytes
M.musculus
M.mulatta
E.caballus
E.telfairi