

Supplementary Table S1

JGI protein IDs of cy-aaRS from *N. vectensis*, mitochondrial targeting sequences and their MitoProt scores

	JGI Nemvel Protein ID	MitoProt score	Signal sequence
A	189992	0.9361	MVLRQLFSKNLSLSFYLVLPQATRIHFSRGLSNMDSSL
C	159064	0.9249	MQFLRVSVLWVKRTKNFLSKGRL
D	205625	0.999	MLSSTRIAARFLRTLVKPAFPPTRFTHPTTRVFHARGYRL
EP	197245	0.931	MWTRCLLLIPVRPVKLPFSVVQRARSLCSVNSFSS
Fa	215283		
Fb	211098		
G	162025	0.9959	MLRLCFRRLFRINSPLRYLSIAQSCHRSHRGHFKSTFVIGTLWARQNNFQDSIALVGTKRQFVMASAN
H	165986	0.8213	MKSLRVVSQVVKGFRVTP
I	168885	0.9937	MIRNPCGRSWPIFRNCLRTARALKTPQIQVVIPRV
K	240113	0.9668	MFLRALPFRTCARALTGLSRVS
L	207871	0.9673	MRRLGARVVRVSGRIFGVNSDLV
M	215489	0.9859	MVGYSAGLFRRVWPVIRISLRYLHNRSLLSSLCQD
N	184858	0.8759	MSVDNYIFLCFLFSKLHCRSLRSSKVQL
Q	167786	0.9923	MPRAYIRSFTVSVSRL
R	243135	0.9968	MKFLIWRFSRFLGFQRELRIVLGSPWSPTRCSRRLHVSRTLCLTRG
S	239510	0.9794	MKFFSPSLHQLRTVRISFVFRRILSSSSSKM
T	248119	0.9979	MVRIPKSLFGSALQTRVFIAKRCFARNVLKT
V	20110	0.9164	MQYWKAISPQLRHLVKNNLFL
W	217049	0.9806	MHVLYRSNLAVNRLFITNFLWPIARRMASPKNEESTGE
Y	247577	0.8815	MLRSASRACHLAKNLISLDRSSSSSLRF

Supplementary Table S2

Refseq accession numbers of cy-aaRS from *H. sapiens*, mitochondrial targeting sequences and their MitoProt scores

	NCBI Refseq Accession #	MitoProt score	Signal sequence
A	NP_001596	0.9191	MAGRQPF AVRVLGGCRLHRGRYPATYRWSLVQIGPSPGLLLLTAASPF / KPIFLNTIDPSHPMAKLSRAANTQKCIRA
C	NP_001014437		
D	NP_001340	0.8908	MRFLLSLPLYLHLYTYTGTGNPCCLGKPTALLCPNKQGNGLRRGLLRPQAATSPRGRE
EP	NP_004437		
Fa	NP_004452		
Fb	NP_005678	0.8922	MAIFKTTRTRRG
G	NP_002038	0.9884	MPSRPVLLRGARAALLLLPPRLLARPSLLLRSLSAASCPP
H	NP_002100		
I	NP_038203	0.8854	MRLAKHLKRHFKTSLIRDFTFYDGPP
K	NP_001123561	0.9733	MLTQAAVRLVRGSLRK
L	NP_064502		
M	NP_004981	0.9242	MAISFSTTREAAAASVTALQQRKRIKVRLALKPKRLFWISRKKVLLCVVPFLTRP
N	NP_004530	0.9988	MNSTSLHHLGIGATSLVWFARGPARLQLGRNWKIQKGKLGRGRKGAK / VTLCQGLQGRGAPGQAAPSELHKRVGRGVSGSGSCLCPHQRWPRRRS
Q	NP_005042		
R	NP_002878		
S	NP_006504	0.9419	MSLWKSFLSSLSKRQPHFPKAVLLRVGKVTIWFIQRF
T	NP_689508	0.9792	MVSRSPKTSRKFRPRPTYYLRC
V	NP_006286	0.9428	MDLPFGPHQAAPCWAPRYRVGNALHRQGRPTLLGQFPSCPLGAALATRPR / TRTRCPWRRLTGASCSGVSSRPASAPSAPRPALFLVRAVLGALGRALSPLEEWL
W	NP_998810		
Y	NP_003671	0.6498	MQPGWTRVTVPGTRRRR

Supplementary Tables S3-S10. Reciprocal Best Hits Data. Results are listed according to their aaRS specificities. Query = NCBI Accession of sequence used to query the target genome. Top Hit = NCBI accession of the highest scoring hit in the target genome. RBH = NCBI Accession of the highest scoring reciprocal hit in the query genome. RBH Score = The normalized BLAST score of the reciprocal best hit. Tables are titled as follows: "<compartment> <query> / <target>", where <compartment> is the subcellular compartment of the query aaRS sequences, <query> is the query organism and <target> is the target organism. Queries for which the RBH accession is not the same as the Query accession (i.e putative paralogs) are in bold. Sequences that represent bacterial contamination are highlighted in green.

S3. Mitochondrial Yeast / Nematostella

	Query	Top Hit	RBH	RBH Score
D	NP_015221	XP_001638035	NP_010322	641
E	NP_014609	XP_001632077	NP_014811	559
F	NP_015372	XP_001637777	NP_015372	68.2
I	NP_015285	XP_001630713	NP_009477	1168
K	NP_014326	XP_001638035	NP_010322	641
L	NP_013486	XP_001621348	NP_013486	237
M	NP_011687	XP_001626448	NP_011780	622
N	NP_009953	XP_001634172	NP_011883	455
P	NP_011010			
R	NP_011959	XP_001632819	NP_010628	190
S	NP_011875	XP_001639063	NP_010306	408
T	NP_012727	XP_001623293	NP_116578	681
W	NP_010554	XP_001633179	NP_010554	243
Y	NP_015228	XP_001617599	NP_015228	165

S4. Mitochondrial Yeast / Human

	Query	Top Hit	RBH	RBH Score
D	NP_015221	NP_060592	NP_015221	302
E	NP_014609	NP_001077083	NP_014609	285
F	NP_015372	NP_006558	NP_015372	303
I	NP_015285	NP_060530	NP_015285	592
K	NP_014326	NP_001123561	NP_010322	649
L	NP_013486	NP_056155	NP_013486	528
M	NP_011687	NP_612404	NP_011687	310
N	NP_009953	NP_078954	NP_009953	287
P	NP_011010	NP_689481	NP_011010	169
R	NP_011959	NP_064716	NP_010628	410
S	NP_011875	NP_060297	NP_011875	221
T	NP_012727	NP_689508	NP_116578	795
W	NP_010554	NP_056651	NP_010554	248
Y	NP_015228	NP_001035526	NP_015228	254

S5. Cytosolic Yeast / Nematostella

	Query	Top Hit	RBH	RBH Score
A	NP_014980	XP_001628752	NP_014980	871
C	NP_014152	XP_001640747	NP_014152	535
D	NP_013083	XP_001634294	NP_013083	556
E	NP_011269	XP_001632077	NP_014811	559
Fa	NP_116631	XP_001626580	NP_116631	469
Fb	NP_013161	XP_001629909	NP_013161	507
G	NP_009679	XP_001637887	NP_009679	619
H	NP_015358	XP_001633827	NP_015358	455
I	NP_009477	XP_001630713	NP_009477	1168
K	NP_010322	XP_001638035	NP_010322	641
L	NP_015165	XP_001632504	NP_015165	500
M	NP_011780	XP_001626448	NP_011780	622
N	NP_011883	XP_001634172	NP_011883	455
P	NP_011884	XP_001626358	NP_011884	90.5
Q	NP_014811	XP_001632077	NP_014811	559
R	NP_010628	XP_001632819	NP_010628	190
S	NP_010306	XP_001639063	NP_010306	408
T	NP_116578	XP_001623293	NP_116578	681
V	NP_011608	XP_001620731	NP_011608	283
W	NP_014544	XP_001625239	NP_014544	430
Y	NP_011701	XP_001624530	NP_011701	400

S6. Cytosolic Yeast / Human

	Query	Top Hit	RBH	RBH Score
A	NP_014980	NP_001596	NP_014980	919
C	NP_014152	NP_001014437	NP_014152	607
D	NP_013083	NP_001340	NP_013083	540
E	NP_011269	NP_004437	NP_011884	600
Fa	NP_116631	NP_004452	NP_116631	456
Fb	NP_013161	NP_005678	NP_013161	503
G	NP_009679	NP_002038	NP_009679	598
H	NP_015358	NP_002100	NP_015358	461
I	NP_009477	NP_038203	NP_009477	1199
K	NP_010322	NP_005539	NP_010322	651
L	NP_015165	NP_064502	NP_015165	907
M	NP_011780	NP_004981	NP_011780	615
N	NP_011883	NP_004530	NP_011883	554
P	NP_011884	NP_004437	NP_011884	600
Q	NP_014811	NP_005042	NP_014811	515
R	NP_010628	NP_064716	NP_010628	410
S	NP_010306	NP_006504	NP_010306	406
T	NP_116578	NP_689508	NP_116578	795
V	NP_011608	NP_006286	NP_011608	1001
W	NP_014544	NP_998810	NP_014544	452
Y	NP_011701	NP_003671	NP_011701	404

S7. Mitochondrial Human / Nematostella

	Query	Top Hit	RBH	RBH Score
A	NP_065796	XP_001628752	NP_001596	1115
C	NP_078813	XP_001640747	NP_001014437	720
D	NP_060592	XP_001638035	NP_001123561	91.3
E	NP_001077083	XP_001632077	NP_005042	1012
F	NP_612387	XP_001619520	NP_612387	131
H	NP_036340	XP_001633827	NP_002100	689
I	NP_060530	XP_001630713	NP_038203	1473
L	NP_056155	XP_001621348	NP_056155	226
M	NP_612404	XP_001626448	NP_004981	848
N	NP_078954	XP_001634172	NP_004530	663
P	NP_689481			
R	NP_064716	XP_001632819	NP_002878	788
S	NP_060297	XP_001639063	NP_006504	672
T	NP_079426	XP_001623293	NP_689508	904
V	NP_065175	XP_001618894	NP_006286	284
W	NP_056651	XP_001633179	NP_056651	327
Y	NP_001035526	XP_001617599	NP_001035526	145

S8. Mitochondrial Human / Yeast

	Query	Top Hit	RBH	RBH Score
A	NP_065796	NP_014980	NP_001596	919
C	NP_078813	NP_014152	NP_078813	607
D	NP_060592	NP_015221	NP_060592	302
E	NP_001077083	NP_014609	NP_001077083	285
F	NP_006558	NP_015372	NP_006558	303
H	NP_036340	NP_015358	NP_002100	461
I	NP_060530	NP_015285	NP_060530	592
L	NP_056155	NP_013486	NP_056155	528
M	NP_612404	NP_011687	NP_612404	310
N	NP_078954	NP_009953	NP_078954	287
P	NP_689481	NP_011010	NP_689481	169
R	NP_064716	NP_010628	NP_064716	410
S	NP_060297	NP_011875	NP_060297	221
T	NP_079426	NP_116578	NP_689508	795
V	NP_065175	NP_011608	NP_006286	1001
W	NP_056651	NP_010554	NP_056651	248
Y	NP_001035526	NP_015228	NP_001035526	254

S9. Cytosolic Human / Nematostella

	Query	Top Hit	RBH	RBH Score
A	NP_001596	XP_001628752	NP_001596	1115
C	NP_001014437	XP_001640747	NP_001014437	720
D	NP_001340	XP_001634294	NP_001340	669
EP	NP_004437	XP_001632077	NP_005042	1012
Fa	NP_004452	XP_001626580	NP_004452	644
Fb	NP_005678	XP_001629909	NP_005678	742
G	NP_002038	XP_001637887	NP_002038	875
H	NP_002100	XP_001633827	NP_002100	689
I	NP_038203	XP_001630713	NP_038203	1473
K	NP_001123561	XP_001638035	NP_001123561	813
L	NP_064502	XP_001632504	NP_064502	744
M	NP_004981	XP_001626448	NP_004981	848
N	NP_004530	XP_001634172	NP_004530	663
Q	NP_005042	XP_001632077	NP_005042	1012
R	NP_002878	XP_001632819	NP_002878	788
S	NP_006504	XP_001639063	NP_006504	672
T	NP_689508	XP_001623293	NP_689508	904
V	NP_006286	XP_001618894	NP_006286	284
W	NP_998810	XP_001625239	NP_998810	543
Y	NP_003671	XP_001624530	NP_003671	680

S10. Cytosolic Human / Yeast

	Query	Top Hit	RBH	RBH Score
A	NP_001596	NP_014980	NP_001596	919
C	NP_001014437	NP_014152	NP_001014437	607
D	NP_001340	NP_013083	NP_001340	540
EP	NP_004437	NP_011884	NP_004437	600
Fa	NP_004452	NP_116631	NP_004452	456
Fb	NP_005678	NP_013161	NP_005678	503
G	NP_002038	NP_009679	NP_002038	598
H	NP_002100	NP_015358	NP_002100	461
I	NP_038203	NP_009477	NP_038203	1199
K	NP_005539	NP_010322	NP_005539	651
L	NP_064502	NP_015165	NP_064502	907
M	NP_004981	NP_011780	NP_004981	615
N	NP_004530	NP_011883	NP_004530	554
Q	NP_005042	NP_014811	NP_005042	515
R	NP_002878	NP_010628	NP_064716	199
S	NP_006504	NP_010306	NP_006504	406
T	NP_689508	NP_116578	NP_689508	795
V	NP_006286	NP_011608	NP_006286	1001
W	NP_998810	NP_014544	NP_998810	452
Y	NP_003671	NP_011701	NP_003671	404