An N-terminal motif unique to primate tau enables differential protein–protein interactions

Kristie Stefanoska, Alexander Volkerling, Josefine Bertz, Anne Poljak, Yazi Diana Ke, Lars Matthias Ittner, Arne Ittner

Supporting Information

Supporting tables

Table S1. Protein summary results for ProteinPilot v4.0 analysis. Numbers for proteins detected refer to all proteins identified at global 1% FDR or 95% confidence. Hence, this includes contaminating proteins (e.g. trypsin), proteins with no quantitation data or only one distinct peptide for identification.

iTRAQ	Proteins detected[a]	Proteins detected[b]	Proteins before grouping[b]	Distinct peptides[b]	Spectra identified[b]	%total spectra
tauFL/Δ18 8-plex	101	133	947	1239	3930	24.7

[a]1% global FDR   [b]95% confidence, equal to ProteinPilot unused score <= 1.3

Unused score	%Cov(95)	Accession	Name	Peptides (95%)
	76.9	gi|150207Add2	gi|150207Add2|xxx|xxx| AddedProtein2: htau40[c]	158
33.34	38.6	gi|755529785	PREDICTED: pyruvate kinase PKM isoform X2 [Mus musculus]	25
30.49	48.03	gi|10946574	creatine kinase B-type [Mus musculus]	21
29.23	54.9	gi|6754086	glutathione S-transferase Mu 5 [Mus musculus]	23
28.59	38.1	gi|40254595	dihydropyrimidinase-related protein 2 [Mus musculus]	26
24.33	33.3	gi|31981690	heat shock cognate 71 kDa protein [Mus musculus]	24
23.84	39.8	gi|755516109	PREDICTED: glyceraldehyde-3-phosphate dehydrogenase isoform X3 [Mus musculus]	18
23.30	43.8	gi|755567900	PREDICTED: alpha-enolase isoform X1 [Mus musculus]	14
23.27	10.8	gi|568975337	PREDICTED: clathrin heavy chain 1 isoform X3 [Mus musculus]	14
22.55	13.2	gi|755519440	PREDICTED: sodium/potassium-transporting ATPase subunit alpha-3 isoform X3 [Mus musculus]	12
20.89	54.5	gi|6755901	tubulin alpha-1A chain [Mus musculus]	28
20.04	35.4	gi|6671539	fructose-bisphosphate aldolase A isoform 2 [Mus musculus]	14
19.67	23.6	gi|110625979	elongation factor 1-gamma [Mus musculus]	11
18.71	28.4	gi|254540027	malate dehydrogenase, cytoplasmic [Mus musculus]	9
17.95	17.3	gi|40556608	heat shock protein HSP 90-beta [Mus musculus]	12
16.56	32.4	gi|6681273	elongation factor 1-alpha 2 [Mus musculus]	13
16.56	32.4	gi|6681273	elongation factor 1-alpha 2 [Mus musculus]	13
16.05	49.5	gi|6754084	glutathione S-transferase Mu 1 [Mus musculus]	24
14.97	40.0	gi|755550334	PREDICTED: 14-3-3 protein zeta/delta isoform X1 [Mus musculus]	12
14.86	23.4	gi|6678674	L-lactate dehydrogenase B chain isoform 1 [Mus musculus]	8
14.40	40.3	gi|6752954	actin, cytoplasmic 2 [Mus musculus]	16
14.39	36.1	gi|226958349	triosephosphate isomerase [Mus musculus]	9
14.21	26.3	gi|31982332	glutamine synthetase [Mus musculus]	12

13.48	28.5	gi|113680352	carbonyl reductase [NADPH] 1 [Mus musculus]	9
13.32	23.0	gi|226423909	2',3'-cyclic-nucleotide 3'-phosphodiesterase isoform 1 [Mus musculus]	9
13.15	34.3	gi|114326546	phosphoglycerate mutase 1 [Mus musculus]	7
12.39	49.5	gi|10092608	glutathione S-transferase P 1 [Mus musculus]	33
12.04	51.7	gi|6753244	calmodulin [Mus musculus]	11
11.87	26.7	gi|742670581	fructose-bisphosphate aldolase C [Mus musculus]	9
11.15	29.3	gi|6679439	peptidyl-prolyl cis-trans isomerase A [Mus musculus]	6
10.73	41.6	gi|45597447	superoxide dismutase [Cu-Zn] [Mus musculus]	7
10.67	21.3	gi|70778976	phosphoglycerate kinase 1 [Mus musculus]	9
10.00	27.7	gi|568920559	PREDICTED: carbonic anhydrase 2 isoform X1 [Mus musculus]	6
9.84	24.9	gi|31982186	malate dehydrogenase, mitochondrial precursor [Mus musculus]	8
8.26	15.5	gi|569018370	PREDICTED: lanC-like protein 1 isoform X1 [Mus musculus]	6
8.00	46.9	gi|6678467	tubulin alpha-4A chain [Mus musculus]	25
8.00	49.6	gi|568982871	PREDICTED: beta-synuclein isoform X1 [Mus musculus]	5
7.92	39.9	gi|113679874	glutathione S-transferase Mu 7 [Mus musculus]	17
7.89	8.4	gi|254553458	glucose-6-phosphate isomerase [Mus musculus]	4
7.89	29.0	gi|742068536	peroxiredoxin-6 isoform 2 [Mus musculus]	7
7.85	10.3	gi|18079339	aconitate hydratase, mitochondrial precursor [Mus musculus]	6
7.49	7.6	gi|160707903	synapsin-1 isoform b [Mus musculus]	4
7.00	34.5	gi|188219614	ubiquitin carboxyl-terminal hydrolase isozyme L1 [Mus musculus]	5
6.61	28.3	gi|6680924	cofilin-1 [Mus musculus]	4
6.59	29.2	gi|160298217	glutathione S-transferase A4 [Mus musculus]	9
6.54	27.6	gi|31980922	elongation factor 1-beta [Mus musculus]	5
6.51	6.6	gi|39204499	neurofilament light polypeptide [Mus musculus]	4
6.48	9.8	gi|6680027	glutamate dehydrogenase 1, mitochondrial precursor [Mus musculus]	4
6.39	14.5	gi|6679935	neuromodulin [Mus musculus]	3
6.37	6.4	gi|568922642	PREDICTED: putative adenosylhomocysteinase 2 isoform X1 [Mus musculus]	3
6.26	31.3	gi|69885073	myelin basic protein isoform 6 [Mus musculus]	4
6.20	12.4	gi|37202121	4-aminobutyrate aminotransferase, mitochondrial isoform 1 precursor [Mus musculus]	4
5.97	25.1	gi|703491589	gamma-enolase isoform 2 [Mus musculus]	6
5.89	7.7	gi|160298209	aspartate aminotransferase, cytoplasmic [Mus musculus]	4
5.65	32.6	gi|755506225	PREDICTED: CDK5 regulatory subunit-associated protein 2 isoform X10 [Mus musculus]	11
5.57	6.1	gi|165972307	syntaxin-binding protein 1 isoform a [Mus musculus]	3
5.54	206	gi|6754976	peroxiredoxin-1 [Mus musculus]	5
4.61	7.0	gi|6754036	aspartate aminotransferase, mitochondrial [Mus musculus]	3
4.51	2.8	gi|568912897	PREDICTED: dynamin-1 isoform X10 [Mus musculus]	3
4.47	0.8	gi|568915692	PREDICTED: microtubule-associated protein 1A isoform X1 [Mus musculus]	2
4.31	22.3	gi|226874906	14-3-3 protein epsilon [Mus musculus]	6
4.25	19.1	gi|6679078	nucleoside diphosphate kinase B [Mus musculus]	3
4.15	34.4	gi|31543976	14-3-3 protein gamma [Mus musculus]	8
4.03	22.8	gi|62460366	parathymosin [Mus musculus]	2

4.00	16.4	gi|227330544	complexin-1 [Mus musculus]	2
3.89	16.7	gi|568956465	PREDICTED: peroxiredoxin-2 isoform X1 [Mus musculus]	3
3.84	14.5	gi|6754254	heat shock protein HSP 90-alpha [Mus musculus]	10
3.83	17.8	gi|6755983	visinin-like protein 1 [Mus musculus]	3
3.46	48.4	gi|31981939	tubulin beta-4A chain [Mus musculus]	31
3.42	40.9	gi|569011811	PREDICTED: thymosin beta-4 isoform X1 [Mus musculus]	2
3.12	40.9	gi|569011811	PREDICTED: thymosin beta-4 isoform X1 [Mus musculus]	2
3.12	6.4	gi|26006861	pyridoxal kinase [Mus musculus]	2
3.03	3.7	gi|568983700	PREDICTED: tubulin polymerization-promoting protein isoform X1 [Mus musculus]	1
2.67	13.3	gi|83921595	acyl-CoA-binding protein isoform 1 [Mus musculus]	1
2.60	1.9	gi|755553090	PREDICTED: V-type proton ATPase catalytic subunit A isoform X1 [Mus musculus]	1
2.58	9.9	gi|755550928	PREDICTED: ADP-ribosylation factor 3 isoform X1 [Mus musculus]	2
2.44	1.2	gi|568991693	PREDICTED: ATP-dependent 6-phosphofructokinase, muscle type isoform X1 [Mus musculus]	1
2.31	2.9	gi|6680045	guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 [Mus musculus]	1
2.21	8.6	gi|6755114	peroxiredoxin-5, mitochondrial precursor [Mus musculus]	1
2.17	22.1	gi|126032329	elongation factor 1-alpha 1 [Mus musculus]	9
2.17	22.1	gi|126032329	elongation factor 1-alpha 1 [Mus musculus]	9
2.15	4.1	gi|13385942	citrate synthase, mitochondrial precursor [Mus musculus]	2
2.15	4.1	gi|13385942	citrate synthase, mitochondrial precursor [Mus musculus]	2
2.13	2.1	gi|124286811	neurofilament heavy polypeptide [Mus musculus]	3
2.08	3.4	gi|6753060	annexin A5 [Mus musculus]	1
2.06	5.6	gi|568904952	PREDICTED: ES1 protein homolog, mitochondrial [Mus musculus]	1
2.02	0.4	RRRRRgi|568945980	REVERSED PREDICTED: cation channel sperm-associated protein subunit gamma 1 isoform X12 [Mus musculus]	1
2.02	2.2	gi|9845253	heterogeneous nuclear ribonucleoprotein H2 [Mus musculus]	1
2.02	6.8	gi|6755588	synaptosomal-associated protein 25 isoform a [Mus musculus]	1
2.01	8.1	gi|16716569	protease, serine, 1 precursor [Mus musculus]	6
2.01	1.6	gi|755508249	PREDICTED: neurochondrin isoform X1 [Mus musculus]	1
2.00	1.1	RRRRRgi|51491856	REVERSED sodium-coupled monocarboxylate transporter 2 isoform 1 [Mus musculus]	1
2.00	43.2	gi|7106439	tubulin beta-5 chain [Mus musculus]	27
2.00	27.6	gi|568917832	PREDICTED: 14-3-3 protein beta/alpha isoform X1 [Mus musculus]	10
2.00	24.4	gi|6756037	14-3-3 protein eta [Mus musculus]	6
2.00	26.9	gi|11528516	neurogranin [Mus musculus]	2
2.00	2.2	gi|112363107	neurofilament medium polypeptide [Mus musculus]	2
2.00	4.2	gi|6753242	calbindin [Mus musculus]	1

2.00	5.2	gi|755556595	PREDICTED: protein kinase C and casein kinase substrate in neurons protein 1 isoform X1 [Mus musculus]	1
2.00	0.3	gi|568940864	PREDICTED: inositol 1,4,5-trisphosphate receptor type 1 isoform X15 [Mus musculus]	1
2.00	1.1	gi|189409138	cullin-associated NEDD8-dissociated protein 1 [Mus musculus]	2
2.00	2.0	gi|113680348	fascin [Mus musculus]	1
2.00	1.2	gi|225735584	hexokinase-1 isoform HK1 [Mus musculus]	1
2.00	4.2	gi|148539957	alpha-internexin [Mus musculus]	2
2.00	6.6	gi|755476257	PREDICTED: LOW QUALITY PROTEIN: dihydropteridine reductase-like [Mus musculus]	1
2.00	1.1	gi|568978506	PREDICTED: reticulon-1 isoform X1 [Mus musculus]	1
2.00	6.0	gi|7710086	ras-related protein Rab-10 [Mus musculus]	1
2.00	2.2	gi|755517291	PREDICTED: secernin-1 isoform X1 [Mus musculus]	1
2.00	4.2	gi|755509924	PREDICTED: protein DJ-1 isoform X1 [Mus musculus]	1
2.00	7.9	gi|47564127	dynein light chain LC8-type 1-like [Mus musculus]	1
2.00	1.6	gi|157951598	calpain-2 catalytic subunit [Mus musculus]	1
2.00	7.1	gi|9506971	profilin-2 [Mus musculus]	1
2.00	7.1	gi|84794597	calcineurin subunit B type 1 [Mus musculus]	1
2.00	4.7	gi|7304963	chloride intracellular channel protein 4 [Mus musculus]	1
2.00	12.6	gi|7110705	prothymosin alpha [Mus musculus]	1
2.00	12.4	gi|6755911	thioredoxin [Mus musculus]	1
2.00	4.5	gi|6679108	nucleophosmin isoform 1 [Mus musculus]	1
2.00	2.9	gi|6678197	synaptotagmin-1 isoform 1 [Mus musculus]	1
2.00	4.5	gi|594542556	myelin proteolipid protein isoform 3 [Mus musculus]	1
2.00	7.8	gi|568972328	PREDICTED: rho GDP-dissociation inhibitor 1 isoform X1 [Mus musculus]	1
2.00	1.0	gi|160333923	heterogeneous nuclear ribonucleoprotein U [Mus musculus]	1
1.77	2.2	gi|54607171	keratin, type II cytoskeletal 6A [Mus musculus]	1
1.77	2.1	gi|8567410	synapsin-2 isoform IIb [Mus musculus]	1
1.77	0.8	gi|755567432	PREDICTED: microtubule-associated protein 2 isoform X9 [Mus musculus]	1
1.77	22.2	gi|755524407	PREDICTED: Purkinje cell protein 2 isoform X1 [Mus musculus]	1
1.72	8.1	gi|6754524	L-lactate dehydrogenase A chain isoform 1 [Mus musculus]	2
1.70	6.6	gi|19527388	ubiquitin thioesterase OTUB1 [Mus musculus]	1
1.66	5.2	gi|7949055	hippocalcin-like protein 1 [Mus musculus]	1
1.64	5.0	gi|568946688	PREDICTED: 40S ribosomal protein S9 isoform X1 [Mus musculus]	1
1.62	1.3	gi|9790049	nitric oxide-associated protein 1 [Mus musculus]	3
1.48	16.1	gi|7305287	metallothionein-3 [Mus musculus]	2
1.46	4.1	gi|568912818	PREDICTED: adenylate kinase isoenzyme 1 isoform X1 [Mus musculus]	1
1.43	1.5	gi|568913591	PREDICTED: gelsolin isoform X1 [Mus musculus]	2
1.30	2.3	gi|85362742	calcium/calmodulin-dependent protein kinase type II subunit gamma isoform 2 [Mus musculus]	1

[c]human tau was added to the ProteinPilot search to facilitate identification of tau bait protein

Table S2. Protein functional annotation clustering with DAVID v6.8. Proteins identified in iTRAQ interaction screen and confirmed differential binding partners from gel excision/mass spectrometry approach were used for gene ontology analysis using DAVID Bioinformatics Resources 6.8. Only enrichment categories that were statistically significant after correction for multiple testing (Bonferroni-adjusted p-value <0.05) are listed.

Category	Term	Count	%	p-value	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
Enrichment Score: 3.62											
GOTERM_CC_DIRECT	GO:0030424~axon	5	62.5	4.13E-06	8	370	19662	33	2.73E-04	2.74E-04	0.0042
GOTERM_CC_DIRECT	GO:0014069~postsynaptic density	4	50	5.99E-05	8	239	19662	41	0.0039	0.0020	0.0612
UP_KEYWORDS	Synapse	4	50	1.29E-04	8	357	22680	32	0.0053	0.0053	0.1188
GOTERM_CC_DIRECT	GO:0045202~synapse	4	50	5.46E-04	8	505	19662	19	0.0354	0.0090	0.5566
UP_KEYWORDS	Cell junction	4	50	7.90E-04	8	661	22680	17	0.0319	0.0107	0.7248
Enrichment Score: 2.78											
GOTERM_CC_DIRECT	GO:0030424~axon	5	62.5	4.13E-06	8	370	19662	33	2.73E-04	2.74E-04	0.0042
GOTERM_CC_DIRECT	GO:0014069~postsynaptic density	4	50	5.99E-05	8	239	19662	41	0.0039	0.0020	0.0612
GOTERM_CC_DIRECT	GO:0030425~dendrite	4	44	5.00E-04	8	490	19662	20	0.0324	0.0109	0.5097

Table S3. Protein network summary for STRING v10.5 analysis. Medium confidence with a minimum interaction score of 0.400.

Network stats			
Number of nodes	8	Avg. local clustering coefficient	0.542
Number of edges	7	Expected number of edges	0
Average node degree	1.75	PPI enrichment p-value	$7.1*10^{-7}$
Functional enrichment			
	Molecular Function (GO)		
pathway ID	pathway description	count	false discovery rate
GO:0005543	phospholipid binding	4	0.0071
GO:1901981	phosphatidylinositol phosphate binding	3	0.0090
GO:0043168	anion binding	6	0.0229
GO:0005515	protein binding	7	0.0468
	Cellular Component (GO)		
pathway ID	pathway description	count	false discovery rate
GO:0030054	cell junction	6	0.0004
GO:0016023	cytoplasmic membrane-bound vesicle	5	0.0012
GO:0030424	axon	4	0.0012
GO:0030659	cytoplasmic vesicle membrane	4	0.0012
GO:0044456	synapse part	4	0.0027
GO:0014069	postsynaptic density	3	0.0046
GO:0045202	synapse	4	0.0051
	PFAM Protein Domains		
pathway ID	pathway description	count	false discovery rate
PF00244	14-3-3 protein	2	0.0030
	INTERPRO Protein Domains and Features		
pathway ID	pathway description	count	false discovery rate
IPR000308	14-3-3 protein	2	0.0033
IPR023409	14-3-3 protein, conserved site	2	0.0033
IPR023410	14-3-3 domain	2	0.0033

Table S4. Oligonucleotide primers used for genotyping of *Mapt*^{-/-} mice.

Strain	Forward primer (5'-3')	Reverse primer (5'-3')
tau[WT]	CTCAGCATCCCACCTGTAAC	CCAGTTGTGTATGTCCACCC
tau[KO]	AAGTTCATCTGCACCACCG	TGCTCAGGTAGTGGTTGTCG

Table S5. Oligonucleotide primers used for generation of plasmid constructs.

Strain	Forward primer (5'-3')	Reverse primer (5'-3')
tauΔ18	GGGGGCTACACCATGCAC	AGCGTGATCTTCCATCACTTCG
tauFL	AAGTTCATCTGCACCACCG	TGCTCAGGTAGTGGTTGTCG
Anxa5	GATAGAATTCATGGCTACGAGAGGCACTGTGAC	GTAACTCGAGTCAGTCATCCTCGCCCCCGCAG
Anxa5-HA	GATAGAATTCACCATGGCTACGAGAGGCACTGTGAC	GTAACTCGAGTCAGGCGTAATCTGGAACATCGTATGGGTA GTCATCCTCGCCCCCGCAG
GST-tau	GACGAATTCGCTGAGCCCCGCCAGGAG	GACCTCGAGTCACAAACCCTGCTTGGCCA