Supplementary Information

for

Integrating molecular and network biology to decode endocytosis

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Contents:

Part 1: Predicting the timescale of events in a pathway from interactomes

Having plotted a pathway protein interactome we can see the approximate time course of protein interactions within a pathway. This comes from analysing the path-lengths between the initiation point of CME (cargo binding) and the node of interest or the number of links required to get from one protein to another (protein complexes such as AP2 are treated as a single node and this type of analysis discounts the effects of protein concentration and affinities). To have a component work later in a pathway then an extra path-length/link gives a molecular-clock delay to this event. We can give the approximate time line of events in CME by concentrating on path-lengths in the pathway protein interactome (Supplementary Fig. 2a). There is one path-length between cargo and AP2, there are two path-lengths connecting cargo to accessory proteins and clathrin, and there are three path-lengths to dynamin and Hsc70. Dynamin is connected into the network through accessory proteins such as snx9, intersectin and amphiphysin (amphiphysin shown in Fig. 4a) and not via the clathrin hub, as dynamin function is spatially separated from clathrin (dynamin acts at the neck of the nascent vesicle). *In vivo* fluorescence studies of clathrin coated pit dynamics validate the time line (Supplementary Fig. 2b) where dynamin and auxilin/Hsc70 are seen to act just before clathrin spots leave the visualisation field in total internal reflection microscopy experiments. The actin machinery comes into play after this, where actin modulators in our pathway protein interactome would have a path-length of three to four from cargo binding.

While overexpression of labelled proteins can give much information about the pathway, there are limitations of these visualisation approaches. Here we can be informed by the pathway protein interactome. Firstly, the pathway protein interactome time line gives information on early events that are difficult to probe with fluorescent markers. Clathrin is generally the marker used to identify coated pit formation, and so events occurring before clathrin begins to polymerise may well be difficult to identify, since in early stages of network assembly AP2 may not be as highly concentrated and may be much more dynamic. Even after clathrin is detected as a spot the ability to detect AP2 in this spot will depend on the labelling efficiency[1]. Secondly, the pathway protein interactome tells us

that overexpression of nodes will frequently cause the system to work sub-optimally if is able to disrupt a hub and so the resulting data should be treated with care.

Part 2: Interactomes aid in experimental interpretations

Network diagrams can help biologists in interpreting or even predicting experimental outcomes. We argued in the main text that effects of depletion or overexpression of proteins depend on their status in the network (hub vs. node). Hub proteins are predicted to not have a major phenotype when overexpressed but depletion of hubs has disastrous effects and vice versa for node proteins. We see this in the overexpression of clathrin and AP2 components having no effects on transferrin endocytosis while depletions of these hubs do[2-4]. Deletion of only one AP2 appendage will lead to a clustered AP2 hub zone with fewer appendages. This will only show an effect if one looks for internalisation of specific cargo that is dependent on an alternative cargo adaptor which is specific for the now missing appendage. Precise examples for this are the combined data from the following references[5-7]. AP2 complexes without the α-appendage showed no effect in transferrin uptake in HeLa cells. Depletion of α-ear in *Drosophila* on the other hand showed a severe phenotype on notch uptake which is dependent on the alternative cargo adaptor numb that is only recruited via the α- but not the β-appendage.

Depletions of many accessory proteins have a minimal phenotype while overexpression is much more effective at producing phenotypes[7-10]. Overexpression of any individual cargo molecule would automatically lead to a reduced incorporation of other components that bind to the same cargo adaptor. This could easily have devastating consequences on vesicles that require multiple cargos to function (like synaptic vesicles). Overexpression of a protein not directly linked to the hubs in the pathway, like dynamin, will also have few consequences[11] as these do not titrate out the organising centres. Depletion of dynamin has significant effects on CME because it needs to oligomerise to function and it has significant effects on a cell[8] because the protein is positioned between pathways. The further one moves away from the hubs in a pathway the more difficult it is to predict phenotypes. Although we have only considered proteins, PtdIns(4,5)P_2 could also be considered to be a hub (see Fig. 3bii), as many of the adaptors and accessory proteins

bind to PtdIns(4,5)P_2. Like dynamin, PtdIns(4,5)P_2 is on the boundary between many different processes and manipulation will give widespread effects.

A word of caution: a negative result from siRNA could mean that the protein is a node, but one also needs to be sure that there was sufficient depletion and no functional redundancy. Also, when overexpressing proteins one needs to make sure there is sufficient overexpression to test if the network can be distorted and overexpressed proteins can also have indirect consequences (for example if one inhibits exocytosis of a receptor then there will consequently be none available for endocytosis).

In summary a pathway protein interactome gives a rational basis for predictable and testable phenotypes from RNAi versus overexpression experiments and helps them to be appropriately interpreted.

Part 3: Clustered-hubs

Clustered-hubs are a new subtype of hubs not previously described. Proteins with multiple interaction surfaces have previously been recognised as a distinct type of hub that can interact with multiple partners simultaneously[12]. However in our pathway network we find hubs that can be composed of *clustered* proteins each with multiple interaction surfaces that can interact with multiple proteins simultaneously (Supplementary Fig. 3). There is also another possible type of *clustered-hub* composed of proteins with one interaction surface that might be able to interact with multiple proteins, but only at different times or locations (Supplementary Fig. 3). This second type of protein is likely to contain an interaction domain like an SH3 domain, SH2 domain, EH domain, PTB domain or adaptor appendage domain, that can bind to short sequence motifs dispersed in the interaction partners. From a pathway-centric viewpoint we do not consider proteins with sequential interactions as functional hubs unless they are clustered. Supplementary Figure 3 illustrates the different oligomerised hub possibilities.

Part 4: Hub conservation and tissue specificity

We mentioned in the main text that a hub-centric pathway has the advantage of easily being able to add additional modules to the system. In CME, introducing alternative cargo adaptors was such an example. Moreover, addition of such alternative adaptors

could also be an evolutionary consideration, to ensure that the retrieval of some ligands is less dependent on adaptors like AP2 which could well be partially occupied by other activated receptors[13,14]. It has been noted previously by network biologists that alternative routes provide robustness and account for apparent redundancy in networks [15-17].

We investigated the conservation of the CME network across species in great detail and observed that conservation of endocytic proteins through the animal kingdom was high and the connectivity of accessory proteins is also mostly conserved (Supplementary Table 1 and 2). In lower organisms there is less duplication of proteins and thus less redundancy and so knock-out phenotypes tend to be stronger. It has to be mentioned that in *Saccharomyces cerevisiae* the core proteins and many of the accessory proteins are present but it appears that the process is not as dependent on clathrin and actin may play a more important role[18]. The network would consequently look different and thus is not included here. There is a trend for higher eukaryotes to have brain-specialised isoforms as well as ubiquitous forms of many proteins, while one form of the protein seems to suffice in other multicellular organisms with nervous systems such as *Drosophila melanogaster, Caenorhabtidis elegans*, and *Strongylocentrotus purpuratus* (sea urchin). It is tempting to suggest that these specialised forms of many node proteins may have allowed the development of the brain as we know it, and would appear to have arisen from gene duplication events in higher organisms. It is interesting that there is also a duplication of genes for exocytic components and synaptic vesicle proteins (unpublished observation). It would be interesting to know if these specialised proteins form a transcriptional cluster to make it easier to turn on all these proteins in the brain.

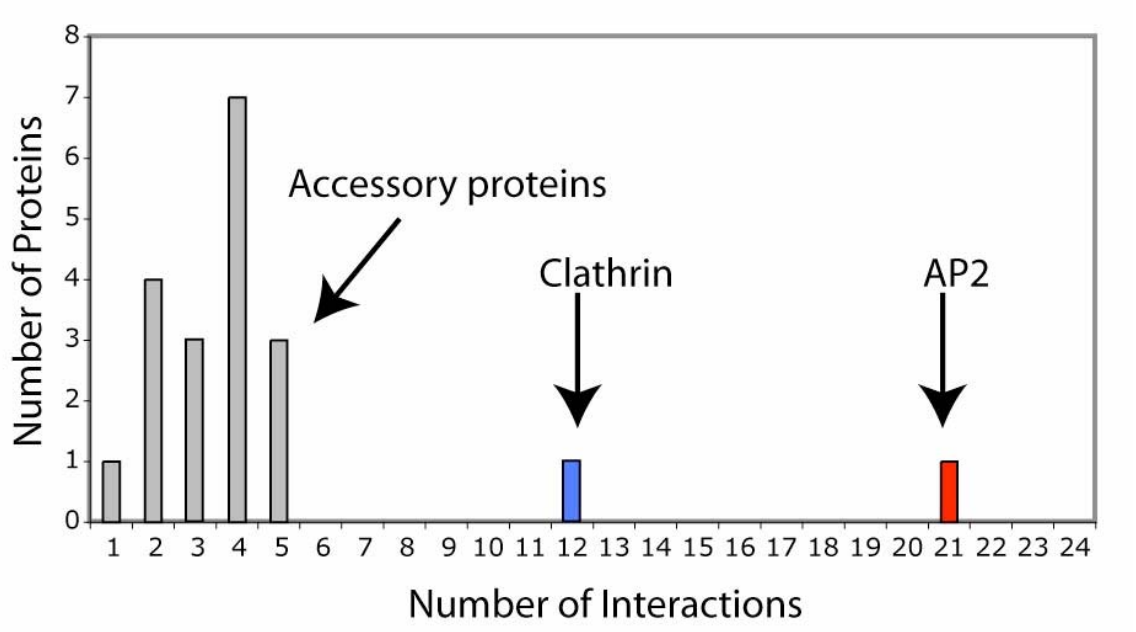
Accessory protein splice variants involved in different pathways

When organising a pathway into a network diagram and identifying hub proteins some caution needs to be applied. In higher eukaryotes many proteins have multiple splice variants and these often lead to their involvement in different interaction networks. One example in the clathrin pathway is amphiphysin which in some tissues has a splice form that has clathrin and AP2 interactions motifs and so the protein functions in CME. In other tissues like in muscle the expressed splice form does not contain clathrin and AP2 interaction motifs and the protein does not function in CME but is instead involved in T-tubule formation[19]. Thus we need to be very careful in extrapolating from interactomes

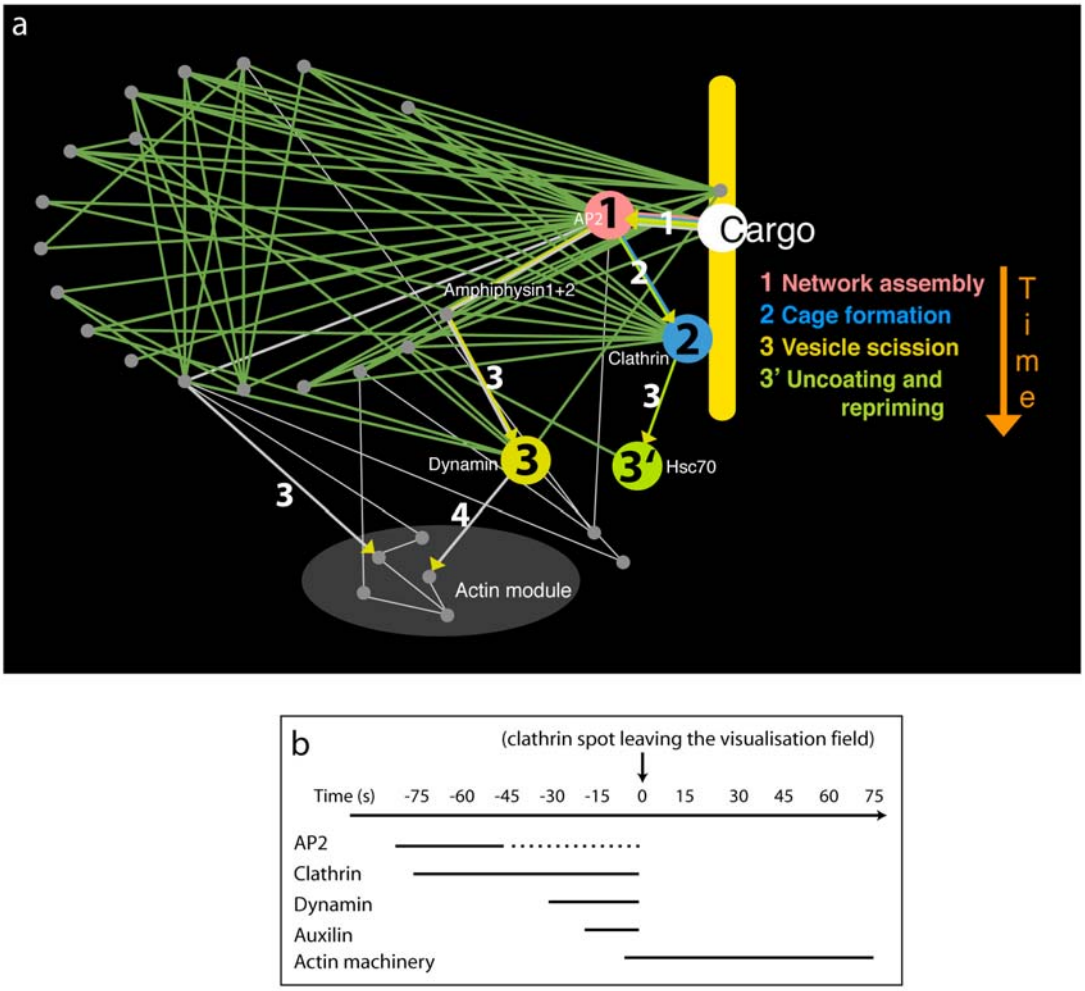
which will depict the multiple pathways that amphiphysin is involved in together. Amphiphysin should not be described as a date-hub in the sense that it is the connection between these processes (especially since these splice variants are expressed in different tissues).

CME interactome: brain versus peripheral tissues

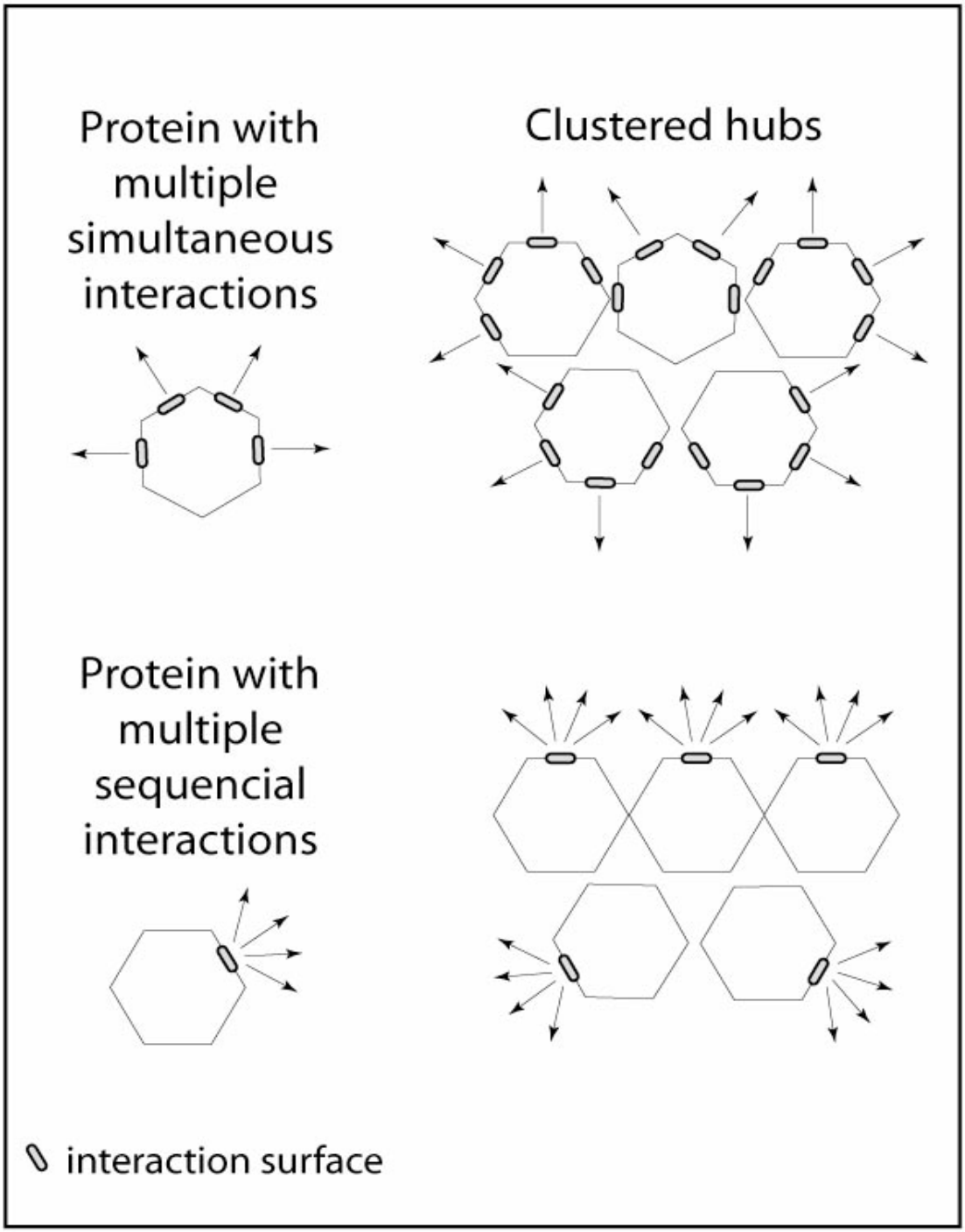
Epsin1, dynamin1, dynamin3, AP180, amphiphsin1, amphiphysin2, intersectin1 and NECAP1 are all brain enriched and other isoforms of these proteins are also found in other tissues. There are differences, in that AP180 in the brain is replaced by CALM in the periphery and brain-enriched amphiphysins are generally replaced by sorting-nexin9 in the periphery. This means that the CME interactome has a conserved architecture, but the accessory adaptors (add-ons) do vary widely with cell type.



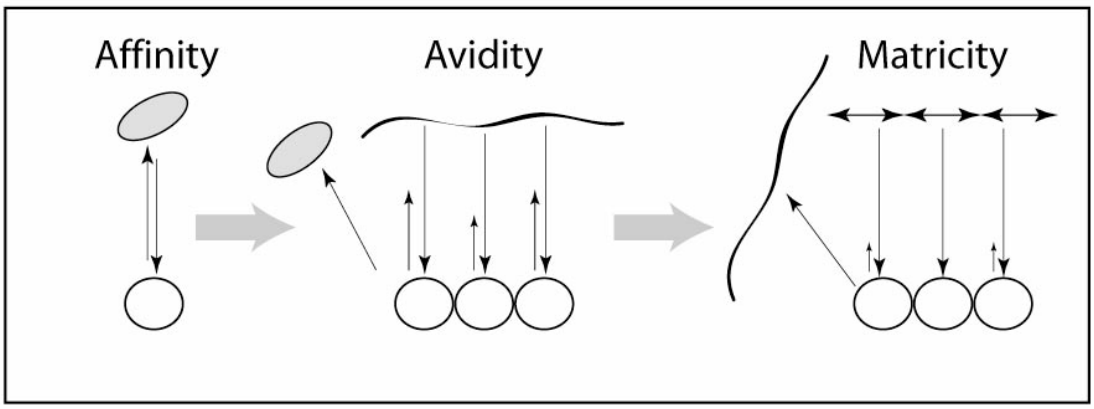
Supplementary Figure 1 'Frequency plot' of endocytic interactions. This shows that the majority of proteins involved in CME interact with up to five protein partners, whereas only two proteins have the ability to interact with significantly more proteins and are thus hubs (clathrin has 12 interactors and AP2 has 21 interactors). Interactions were taken from the network diagram in Figure 1b.



Supplementary Figure 2 Time line and hub progression for CME. a) The path-lengths between membrane cargo and downstream proteins give an approximate time course of events. Thus AP2 has a path length of 1 (cargo – AP2), while clathrin has a path length of 2 (cargo – AP2/accessory protein – clathrin) and dynamin and hsc70 (cargo – AP2 – amphiphysin/clathrin – dynamin/hsc70) have a path length of 3. Further downstream event may have longer path lengths and thus will occur with a greater molecular-clock delay. **b)** *In vivo* imaging using total internal reflection fluorescence microscopy has given us a time course for the recruitment of AP2, clathrin, dynamin and auxilin to coated pits which agrees well with the time-line derived from the interactome. These data come from the following papers: (AP2, clathrin)[1,20], (dynamin, clathrin)[21,22], (auxilin, clathrin)[23,24], (actin, clathrin)[25,26]. AP2 is seen to be present at the same time as clathrin spots leave the visualisation field in one study[1] but leaves before clathrin in another[27]. This difference is indicated by the dotted line in the AP2 time-line.



Supplementary Figure 3 Representation of 'hub'-possibilities. Clustered hubs are a new subtype of hubs that we find in CME. These can be composed of proteins with multiple interaction surfaces or a single interaction surface that can bind to different proteins.



Supplementary Figure 4 Directionality through changing interaction modes. Affinity-driven interactions have equal on- and off-rates whereas in avidity-driven interactions the off-rates are significantly reduced due to multiple interaction points. The third form of interactions, matricity-driven interactions, involve a rigid matrix which leads to a further reduction in off-rates or the absence of off-rates altogether (in our case polymerised clathrin has much less flexibility than any of the accessory proteins).

Supplementary Table 1 Domain structures and functions of CME proteins and their presence in different species. A list of proteins involved in CME was generated using published information, primarily the following papers[28-32]. The list is not exhaustive but includes the main components. The domain structure is illustrated and the descriptions of each domain and their occurrences in different proteins can be found at http://www.sanger.ac.uk/cgi-bin/Pfam/dql.pl. Clathrin interaction motifs (marked as 'x') are incomplete as they are difficult to detect given their sequence variations. Homologues were found by NCBI blast searches and http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=7668 for sea urchin sequences. We have not given the protein conservation percentage as the interaction regions of most accessory proteins are not folded domains, but weakly conserved regions containing interaction motifs. Thus we have searched for the conservation of overall domain structure combined with key interaction motifs. The details can be found in the expanded supplementary table. Mammalian brain enriched proteins were identified using http://symatlas.gnf.org/SymAtlas/ and immunoblotting[33] and are shaded in grey. Where only one form of a protein is found in a genome then it is

frequently difficult to assign it as closer to one homologue over another. In other cases we can assign this, for example AP180 versus CALM can be distinguished through the presence of NPF motifs in CALM only, showing that the brain specialised form is absent in lower organisms. When we do not find clathrin or adaptin interaction motifs in homologues then we generally assume that the protein in not involved in CME. In the case of amphiphysin we know that a *Drosophila* form of amphiphysin does not have any clathrin or adaptor interaction and does not function in CME and so is not annotated in this table (see asterisk). Abbreviations not explained in the table: AP180 **A**dapter **p**rotein **180**kDa, CALM **C**lathrin **a**ssembly **l**ymphoid **m**yeloid leukaemia protein, HIP1 /R **H**untingtin **i**nteracting **p**rotein1 /**r**elated, Eps15 /R **E**pidermal **g**rowth **f**actor **r**eceptor **p**athway **s**ubstrate **15** /**r**elated, Tom1 **T**arget **o**f **m**yb1, NECAP1 Adapti**n** **e**ar-binding **c**oat-**a**ssociated protein **1**, Hsc70 **H**eat **s**hock **c**ognate **70**kDa protein, ENTH **E**psin **N**-**t**erminal **h**omology, UIM **U**biquitin **i**nteracting **m**otif, ANTH **A**P180 **N**-**t**erminal **h**omology, BAR **B**in/**A**mphiphysin/**R**vs, SH3 **S**rc **h**omology **3**, PX **P**hox **h**omology, EH **E**ps15 **h**omology, PH **P**leckstrin **h**omology, PRD **P**roline **r**ich **d**omain, PTB **P**hospho**t**yrosine **b**inding

Supplementary Table 2 Extended Table 1 with functions, domains, motifs and accession numbers of proteins identified in Blast searches.

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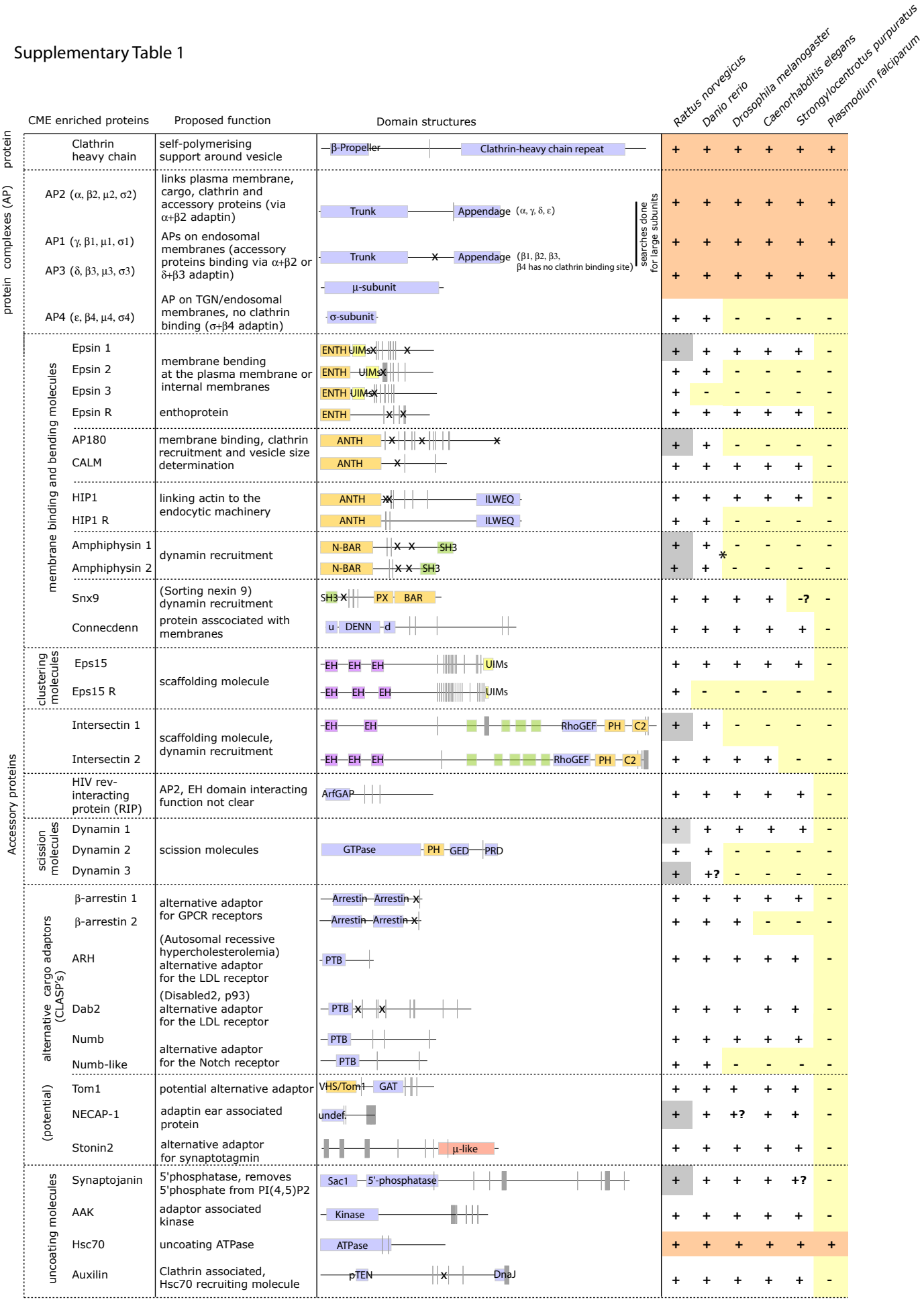
Supplementary Table 1



Supplementary Table 2

CME enriched proteins	Proposed function	Interesting domains/motifs (rat)	Rattus norvegicus	*Danio rerio*	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Strongylocentrotus purpuratus*	*Plasmodium falciparum*
Clathrin heavy chain	self-polymerising support around vesicle	N-terminal beta propeller domain (binds to DLL and WxxW motifs)	NP_062172	NP_001005391	NP_477042	NP_499260	hmm5669	XP_001350511
AP2, alpha and beta2 adaptin	heterotetrameric adapter protein, links plasma membrane, cargo, clathrin and accessory proteins	alpha ear binding sites (human): top-W840, side-F740, beta2 ear binding sites (human): top-Y888, side: Y815, beta hinge: clathrin binding site: 631-LLNLD	NP_112270 alpha adaptin C (2 alpha forms present in mammals) , NP_001273 beta2 adaptin, all sites conserved	XP_686432 alpha, top site conserved, side F-V NP_956213 beta2, both sites conserved, Clathrin LLNLD	NP_476819 alpha top and side conserved, NP_523415 beta1/2, top and side conserved, Clathrin: LLSMD	NP_509572 alpha, top and side conserved, NP_001022939 beta1/2, difficult to align, if only ear: side conserved, top uncertain, Clathrin:LLSLD	hmm91445 alpha, top and side site conserved, XP_001187613 beta 1/2, top and side NOT conserved	CAG24987 alpha, W conserved, XP_001351835, beta
AP1, gamma and beta1 adaptin	heterotetrameric adapter protein, links endosomal membrane, cargo, clathrin and accessory proteins	gamma bindig sites: ?, beta1 binding sites conserved from beta2, clathrin also LLNLD	XP_214197 gamma adaptin, NP_058973 beta1 adaptin, all sites conserved	NP_955976 gamma, XP_686642 beta1	NP_572527 gamma, see above for beta	NP_740937, gamma, see above for beta	XP_792773, gamma, see above for beta	XP_001348703, gamma
AP3, delta and beta 3 adaptin	heterotetrameric adapter protein, links endosomal membrane, cargo, clathrin and accessory proteins	delta binding sites:? beta3 binding sites?, clathrin:974-LLDLD	XP_234908 delta adaptin, XP_226666 beta3 adaptin, all sites conserved	XP_685921 delta, XP_691776 beta 3	P5436, delta, NP_525071, beta3, clathrin: LLDLD	NP_494570, delta, NP_492171, beta3, clathrin: LIDVD	XP_001192784, delta, XP_001201562 beta3?	
AP4, epsilon and beta 4 adaptin	heterotetrameric adapter protein, function unknown	no clathrin binding	XP_001078375 epsilon adaptin, XP_001065231 beta 4 adaptin	XP_691349 epsilon, NP_956632 beta4	no homologue	no homologue	XP_795821 could be epsilon, very weak homology	XP_001349197 beta4? (Only C-terminus shows homolgy)
Epsin 1	membrane bending molecule, plasma membrane	ENTH domain, 2 UIMs 10 DxF/W, 2 FxxF, 2 clathrin binding sites, 3 NPF	NP_476477	XP_698227, 4 DxF/W, 1 FxxF, 3 NPF	AAF05113, liquid fascets, 11 DxF/W, 1 FxxF, 2 NPF	NP_510459, 2-4 DxF/W, 1FxxF, 4 NPF	XP_782786, Epsin2, 7 DxF/W, 3 NPF	not present
Epsin 2	membrane bending molecule, golgi membranes	ENTH, 6 DxF/W, 3 FxxF/W, 1 FxxFxxxR, 3 NPF, 1 clathrin binding site	Q9Z1Z3	XP_686465, 7 DxF/W, 2 FxxF, 3NPF	no additional epsins	no additional epsins	no additional epsins	not present
Epsin 3	membrane bending molecule	ENTH, 8 DxF/W, 1 FxxF, 3NPFs	AAH97500	no homologue	no additional epsins	no additional epsins	no additional epsins	not present
Epsin R	enthoprotein, binds PI4P, internal trafficking	ENTH, 3 DxF/W, 4 F/WxxF/W, no NPFs, 2 clathrin binding sites	AAH76397	XP_687829, 7 DxF/W, 6 W/FxxF/W	AAL28154, 6 DxF, 7 FxxF (overlapping)	NP_509973, RNAi spreading defective, 5 DxF, 1 FxxF	XP_001191369 Epsin4, 2DxF/W, no NPF, 2 FxxF, 1 FxxFxxF	not present
AP180	PI(4,5)P2, AP2 and clathrin binding, vesicle size determination	ANTH domain, 13 DxF/W, 3 clathrin binding sites	NP_113916	XP_693753 11 DxF/W, 1 FxxF	no homologue	no homologue	no homologue	not present
CALM	ubiquit.ous AP180, contains additional NPF motif	ANTH domain, 1 DxF, WxxF, 1 DLL, 1 NPF, one clathrin binding site	AAU06231	NP_957221, 1 DxF, 1 NPF	NP_524252, 2 DxF, 1 FxDxF, 2 NPF	NP_001021015, unc11, 3 DxF, 1 FxxF, 6 NPF	XP_797001, 2 DxF, 1 NPF	not present
HIP1	linking actin to the endocytic machinery	ANTH domain, Actin binding domain, 5DxF, 2 clathrin binding sites (LLR at 485 binds to light	XP_347169	XP_689999, 4 DxF, LLR	NP_648597, 1 DxF	S44664, no adaptor binding sites	XP_785542, 2 FxxF	not present
HIP1 R	linking actin to the endocytic machinery	ANTH domain, Actin binding domain, 2 DxF	XP_001072438	XP_690629, 3 DxF	no additional HIPs	no additional HIPs	no additional HIPs	not present

Amphiphysin 1	role in dynamin recruitment to the vesicle neck region	BAR and SH3 domain, 3 DxF/W, 2 FxxF/W, 1WxxW	NP_071553	NP_957125, 4 DxF/W, 1 WxxW, 1 FxxF	NP_523717, no adaptor binding sites outside BAR and SH3 domains, 1 NPF	NP_501711, no adaptor binding sites outside BAR and SH3 domains, 2 NPFs	XP_782507, BAR, SH3 no adapter binding motifs	not present
Amphiphysin 2	role in dynamin recruitment to the vesicle neck region	BAR and SH3 domain, 2 DxF/W, 1 WxxW (overlapping),	CAA73807	XP_692019 , 1DxW,	no additional amphs	no additional amphs	no additional amphs	not present
Connecdenn	function unknown, potential membrane binder	uDENN, DENN and dDENN domains in N-terminus, 3DxFs (1FxDxF), 1WxxF and 1 FxxF in C-terminus	XP_231184	XP_683977, has DENN domains, 4DxF (1FxDxF), 1WxxF, 1FxxF	NP_665880, has DENN domains, 3DxF (1FxDxF), 1WxxF,	NP_509739, has DENN domains, 2 DxF (1FxDxF), 1WxxF,	XP_001185658 similar to myotubularin, has DENN domains, 5DxF/W (1FxDxF), 3WxxF, 4 FxxF	not present
Sorting nexin 9	Snx9, dynamin recruitment	SH3, PX, 4 DxF/W. 1 FxxF, 1 WxxW, 1 FxxFxxxR	XP_001067064	AAH91825, 1DxF, 2 FxxFxxxR	NP_648348 4 DxF/W, 1 WxxF, 1 FxxW	NP_872090, 3 DxF/W, 3 F/WxxF/W	XP_786190 , starts with PX domain, a few PX domain proteins, none aligns over full protein	not present
Eps15	scaffolding molecule	3 EH + 1 UIM domain, 16 DxF/W, 1 FxDxF. 1FxxF	AAP12671	XP_696575, 25 DxF, 5 FxxF/W	NP_611965, 25 DxF/W, 12 FxxF, 5 FxDxF	AAK13051, 6 DxF/W, 1 FxDxF, 1 FxxF	XP_001192039, 39 DxF, 2 FxxF, 1 FxxFxxF. 1 NPF	not present
Eps15R	scaffolding molecule	3 EH + 1 UIM domain, 23 DxF, 1FxDxF, 3 FxxF, 1possible Clathrin site (LxExE) binds to CLC	AAH98004	no homologue	no homologue	no homologue	XP_781924	not present
Intersectin 1	scaffolding protein dynamin recruitment	2 EH, 5 SH3, 1 RhoGEF, 1 PH and 1 C2 domain, 9 DxF/W, 2 WxxF/W	XP_573259	NP_997065, 7 DxF/W, 2 W/FxxF/W,	Dap160, AAC39139, 2 DxF/W	NP_503037, 3 DxF/W, 2 FxxF, 1 FxDxF	no homologue	not present
Intersectin 2	scaffolding protein dynamin recruitment	3 EH, 5 SH3, 1 RhoGEF, 1 PH and 1 C2 domains,9 DxF/W, 2 WxxF/W	XP_233945	CAI21104, 1EH, 4 1/2 SH3	no additional intersectin	no additional intersectin	no homologue	not present
HIV-rev interacting protein (RIP)	function in endocytosis unknown	ArfGAP domain, 2 DxF, 1FxxF, 4 NPFnucleoporin -like protein	Q4KLH5	NP_956129, 2 DxF, 4 FxxF, 4 FxxFxxF, 4 NPF	NP_477239, 2 DxF, 2 FxxF, 3 NPF	NP_499364. 6 Dxf, 7 FxxF, no NPF	XP_001194344, 1 DxF, 2 FxxF, no NPF	not present
Dynamin 1	scission molecule,	GTP domain, middle domain, GED, PH, PRD, no clathrin binding sites, maybe adaptor binding via a few motifs	NP_542420	XP_695077, looks like an incomplete sequence	NP_727910, shibire, some possible FxxFs	AAB72228, some possible FxxFs	XP_802061, no adaptor binding sites	XP_00134765 orCAD33906 (dynamin1-like, no PRD)
Dynamin 2	scission molecule		NP_037331	NP_998407	no addit. Dynamins (excluding Dynamin like and mitofusins)	no addit. Dynamins (excluding Dynamin like and mitofusins)	XP_001183998, similar to Dynamin 2 GTP domain, middle domain, PH, GED, but no PRD, 1 FxxF, 1DxF	not present
Dynamin 3	scission molecule		Q08877	NP_001025299, has PH domain but no PRD	no addit. Dynamins (excluding Dynamin like and mitofusins)	no addit. Dynamins (excluding Dynamin like and mitofusins)	no additional dynamins	not present
beta arrestin 1	alternative cargo adaptor for GPCR receptors	2 arrestin "wings", specific beta binder (FxxFxxxR), overlapping adaptor-clathrin site (LIEFD)	P29066	NP_999846 FxxFxxxR	NP_523976	T34297, FxxFxxxR	only one arrestin-like molecule	not present
beta-arrestin 2	alternative cargo adaptor for GPCR receptors	2 arrestin "wings", specific beta binder (FxxFxxxR), overlapping adaptor-clathrin site (LIEFD)	NP_037043	NP_957418, FxxFxxxR	NP_523976 (arrstin2) FxxFxxxR,	no additional beta-arrestins	XP_792277, 2 arrestin wings, second truncated, therefore no C-terminus with adaptor or clathrin binding	not present

ARH	alternative cargo adaptor for the LDL receptor	PTB, 1 DxF, 1 FxxW,	XP_001067557 (shorter PTB to human ARH)	NP_001074104, 1 DxF, 1 FxxW	ced-6 NP_610488	NP_001024439, DYstrophin-like	XP_001175617 ceg6 like, PTB domain, partial protein? no adapter binding sites, no C-terminus	not present
Dab2	alternative cargo adaptor for LDL receptor	PTB domain, 10 DxF, 1 FxxF, 5 NPF	AAF05540	XP_692633, 5 DxF , 3 NPF	AAB08527 disabled 20 DxF, 2 FxxF, 2WxxF , no NPFs (larger protein)	A88230, 9 DxF/W, 2 NPF, NP_495732 (Dab1 homologue)	hmm140144, Dab2 homology domain, only ?	not present
Numb	alternative cargo adaptor for the Notch receptor	PTB domain, 3 DxF/W, 1 FxxF, 1 NPF	BAE45130	AAT85678, 3 DxF/W, 1FxxF, 1NPF	NP_523523, 1DxF, 1FxxF, 2 NPF	NP_001024098, 3 DxF	XP_001200286, PTB domain, 1 DxW, 2 NPF	not present
Numb-like	alternative cargo adaptor for the Notch receptor	PTB domain, 1 DxF. 1 FxxF, 1 NPF	NP_001029060	BAD89560, 1 FxxF, 1 NPF	no additional numbs	no additional numbs	no additional numbs	not present
NECAP-1	adaptin ear associated protein	undefined domain, 3DxF/W, 2 WxxF	P69682	NP_957016, 3 DxF/W, 2 WxxF,	NP_996490, 2 DxF, 1WxxF	NP_494398 alignement ok, 2 DxF, no WxxFs (unlikely homolgue?)	XP_001195208, no domains, 2 DxF. 2 FxxF	not present
Stonin2	Synaptotagmin binder	mu homolgy domain in C-terminus, 5 DxF, 7 F/WxxF/W, 2 NPF	NP_149095	NP_001028915, 3 DxF/W, 6 F/WxxW/F, 2 NPF	Q24212 (stonedB), 9 DxF/W, 3 FxxF	NP_505566, 7 DxF/W, 4 WxxF	XP_795059, 5 WxxF, 2 FxxF, 3 NPF	not present
Tom1	potential alternative adaptor	VHS/Tom1 domain (similar to ENTH), GAT domain, FxxFxxxR	target of myb AAH83873	XP_688819 , 3 DxF, 2 FxxF	NP_648315, 2 DxF	NP_508777, shorter protein, 2 DxF inside domains	hmm136178, 3 DxF, 1 FxxF, 2 FxxFxxxR,	not present
Synaptojanin	5'phosphatase, removes 5'Phos from PI(4,5)P2	Sac1 homology domain, 5'phosphatase domain, PRD, 6 DxF/W, 1FxDxF, 2 WxxF, 1 NPF	Q62910	NP_001007031, 5 DxF/W, 3 FxxF/W	NP569729, 1 DxF, 2 WxxF/W	NP_001023266, unc26, 2 DxF/W	hmm103351 , Sac1 homology domain, breaks off after phosphatase domain (?)	not present
AAK	adaptor associated kinase	kinase domain, 6 DxF/W, 1WxxW, 1 NPF	P0C1X8	XP_709671, 1DxF, 1WxxF, 1NPF	NP_995725 Numb associated kinase, 3 DxF/W, 2 WxxF, 1DLL, AAF15596 longer C-terminus 6DxF, 4 F/WxxF, 1DLL	NP_001022563 (sel-5), Kinase domain, 2 DxF/W, 1 WxxF, 1 NPF	XP_001193157, predicted AAK1, truncated kinase domain (?), 13 DxF, 1NPF, 1DLL	not present
Hsc70	uncoating	almost entire protein is HSP70 domain, 2 DxF motifs therein	CAA49670	XP_692936	AAN71116, hsp, many more	NP_503068 hsp1 and many more	XP_802129	XP_001349336
Auxilin	uncoating	Kinase and DNAJ domain, 8 DxF/W, 5 F/WxxF/W	DNAj, GAK, NP_112292	CAI21335 , Gak similar, no DNAJ domain, wrong assembled?, XP_001331947 is DNAJ domain (partial protein)	NP_649438, Kinase and DNAJ domain, 8 DxF/W, 2 FxxF, 1 WxxF 1 NPF	NP_508971, no DNAJ domain (still GAK similar in blast), 3 DxF/W, 1 FxxF	XP_001201563, predicted GAK, Kinase and DNAJ domain, 11 DxF/W, 6 FxxF, 2 FxxFxxF, 2 WxxF, 1 FxxFxxxR	not present
	no homolgue present							
	brain enriched according to expression profiles	motifs indicated are not all tested to be functional	motifs indicated are from rat proteins unless otherwise stated	domain stucture is always the same as in mammalian proteins unless otherwise stated	UIMs are not detected by NCBI blast search as yet	DxF are indicated and in some cases FxDxFs are indicated		