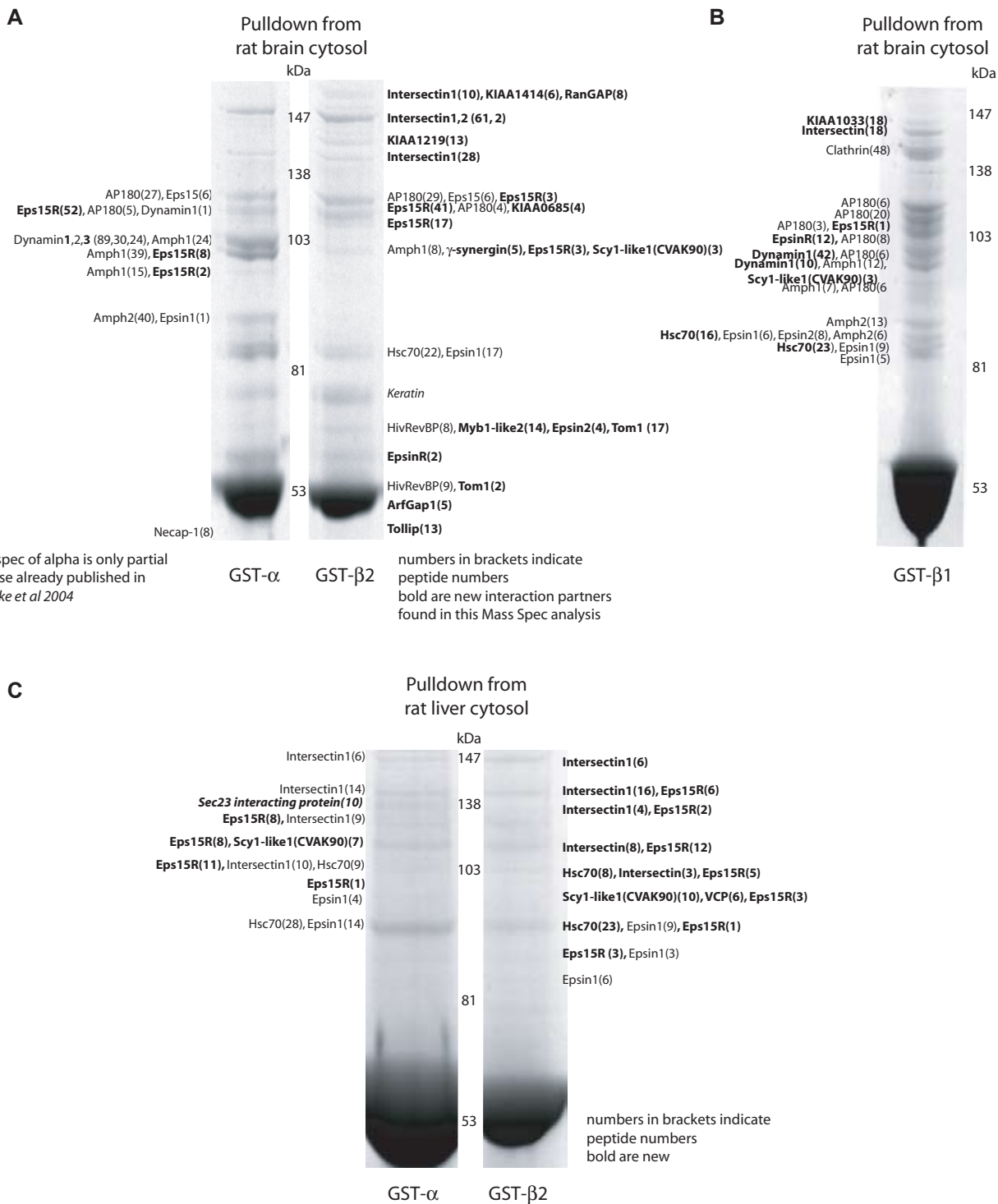


Figure S1

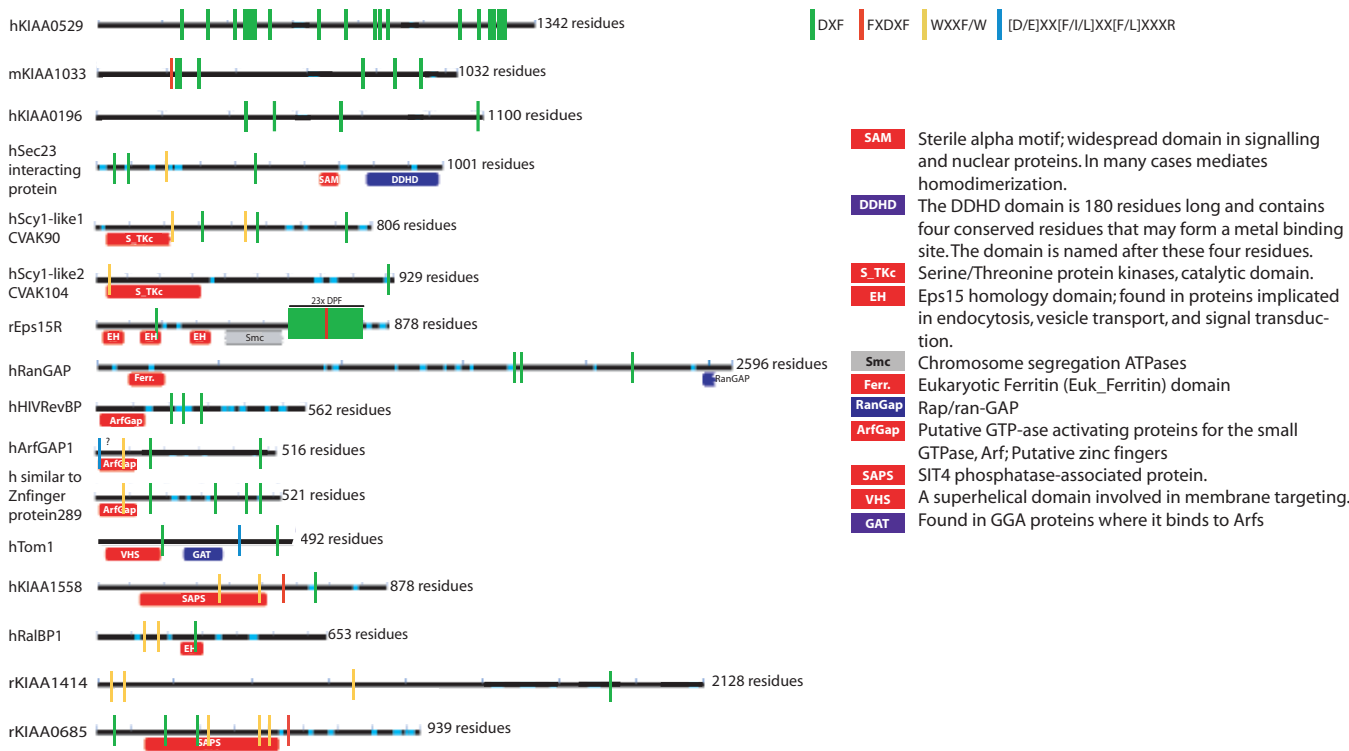


Fishing in rat brain/liver cytosol with GST-appendages

One rat brain was homogenised in 4 mls of 150 mM NaCl, 20 mM HEPES pH 7.4 with 2 mM DTT and protease inhibitors and 0.1% Tx-100 and spun for 20 min at 40 000 rpm in a Beckman TLA100 ultracentrifuge rotor. For interaction experiments 500 μ l extract was added 30-100 μ g of GST fusion-protein + glutathione-sepharose beads, incubated for 1 hour at 4 deg. C. Then the bead bound proteins were washed 3-4 times with the same buffer with Tx-100.

For preparation of liver extract an initial spinning step was included to deplete the lipid content. However, our analysis of liver extract was not so exhaustive as for brain of HeLa cells. All proteins used were purified by Q Sepharose and gel filtration.

Schematics of short peptide motifs and domains in new proteins obtained in the current study



Accession numbers for proteins found in mass spec analysis:

HeLa Extract (Figure 1) – human

α :	
KIAA0592	AAH82258
Intersectin1	NP_001001132 (EH, RhoGEF and SH3 domain containing protein)
Intersectin2	AAF63600 (EH, RhoGEF and SH3 domain containing protein)
γ-synergin	AAD49732 (gamma adaptin binding)
GAK	NP_005246 (G-associated kinase, Auxilin2)
KIAA1033	BAA82985
AAK1	NP_055726 (Adaptor associated kinase1)
KIAA0196	NM_014846
Sec23IP	Q9Y6Y8 (putative phospholipase, SAM (sterile alpha motif) and DDHD domain)
Stonin2	NP_149095 (μ-like domain, AP2 binding)
Scy1-like1	NP_065731 (CVAK90)
Scy1-like2	NP_060458 (CVAK104)
Eps15	CAI13031 (EH domain containing, adaptor and clathrin binding)
Eps15R	NP_067058 (Eps15 related, EH domain containing, α, β and γ adaptor/clathrin binding)
Dynamin2	P50570 (ubiquitous scission molecule/GTPase)
Dab2	AAF05540 (disabled2, binds PI(4,5)P2 clathrin, adaptors and cargo)
RanGAP1	NP_055805 (Ferritin domain, truncated RanGAP domain)
Hsc70	NP_006588 (involved in uncoating)
Sortingnexin9	NP_057308 (SH3 and BAR protein, binds dynamin/clathrin/adaptors)
Epsin1	NP_037465 (membrane curvature driving, clathrin/adaptor binding)
Epsin2	Q95208 (membrane curvature driving, clathrin/adaptor binding)
EpsinR	Q14677 (Epsin4, Enthoprotein, CLINT, membrane curvature molecule binds PI(4)P)
CALM	AAB07762 (non-neuronal form of AP180)
HivRevBP	NP_004495 (hRIP-Rev interacting protein, ArfGAP domain, nucleoporin-like, Znfinger-like)
Znfinger	NP_115765 (similar to Znfinger protein289, ArfGAP domain)
pascin2	AAH08037 (Syndapin, FCH and SH3 domain)
ArfGAP1	CAG30268 (ADP-ribosylation factor GTPase activating protein 1)
Necap-1	AAH67367 (adaptin-ear-binding coat-associated protein 1)

β2 :

Intersectin1	NP_001001132
Intersectin2	AAF63600
Clathrin HC	NP_004850 (coat protein)
KIAA1219	BAA86533 (does not have good adaptor interaction motifs)
γ-synergin	AAD49732
AAK1	NP_055726
Eps15	CAI13030
Eps15R	NP_067058
KIAA1558	BAB13384 (SAPS domain, sporulation induced transcript4 associated protein)
Aftiphilin	Q6ULP2 (binds γ adaptin)
KIAA0196	BAA12109
Rho/RacGEF2	CAH72629
Scy1-like1	NP_065731
RalBP1	AAK34942 (EH domain containing)
Dab2	AAF05540
Epsin1	NP_037465
EpsinR	Q14677
Hsc70	NP_006588
Sortingnexin9	NP_057308
HivRevBP	NP_004495
Tom1	NP_005479 (target of myb1) Has also a clathrin interaction motif.

Rat brain (Supplemental Figure1) – rat

α :	
AP180	NP_113916 (ANTH domain, PI(4,5)P2 and clathrin/adaptor binding)
Eps15	AAP12671
Eps15R	AAH98004
Dynamin1	NP_542420
Dynamin2	NP_037331
Dynamin3	Q08877
Amphipysin1	NP_071553 (BAR and SH3 binds clathrin/adaptors)
Amphipysin2	CAA73807 (BAR and SH3 binds clathrin/adaptors)
Epsin1	NP_476477
Necap-1	P69682 (Adaptin ear-binding coat-associated protein 1)

Only partially analysed because already published in Praefcke et al

β2 :

Intersectin1	XP_573259
Intersectin2	XP_233945
KIAA1414	XP_343001 (no predicted domains and not many interaction motifs)
RanGAP	XP_578542
KIAA1219	AAH47482
AP180	NP_113916
Eps15	AAP12671
Eps15R	AAH98004
KIAA0685	XP_217014 (SAPS1, contains SAPS domain) Rik protein
Amphipysin1	NP_071553
γ-synergin	AAD49733
Scy1-like1	AAH87141
Hsc70	CAA49670
Epsin1	NP_476477
HivRevBP	XP_343609
Tom1	AAH83873
ArfGAP1	AAH70895
Tollip	XP_341962

β1 :

KIAA1033	XP_235003
Intersectin1	XP_573259
Clathrin	NP_062172
AP180	NP_113916
Eps15R	AAH98004
Dynamin1	NP_542420
Scy1-like1	AAH87141
Amph1	NP_071553
Amph2	CAA73807
Hsc70	CAA49670
Epsin1	NP_476477
Epsin2	Q9Z123
EpsinR	AAH76397

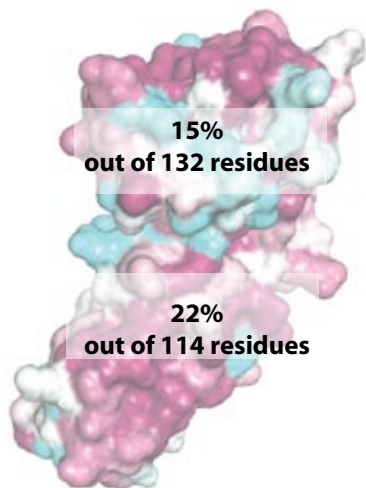
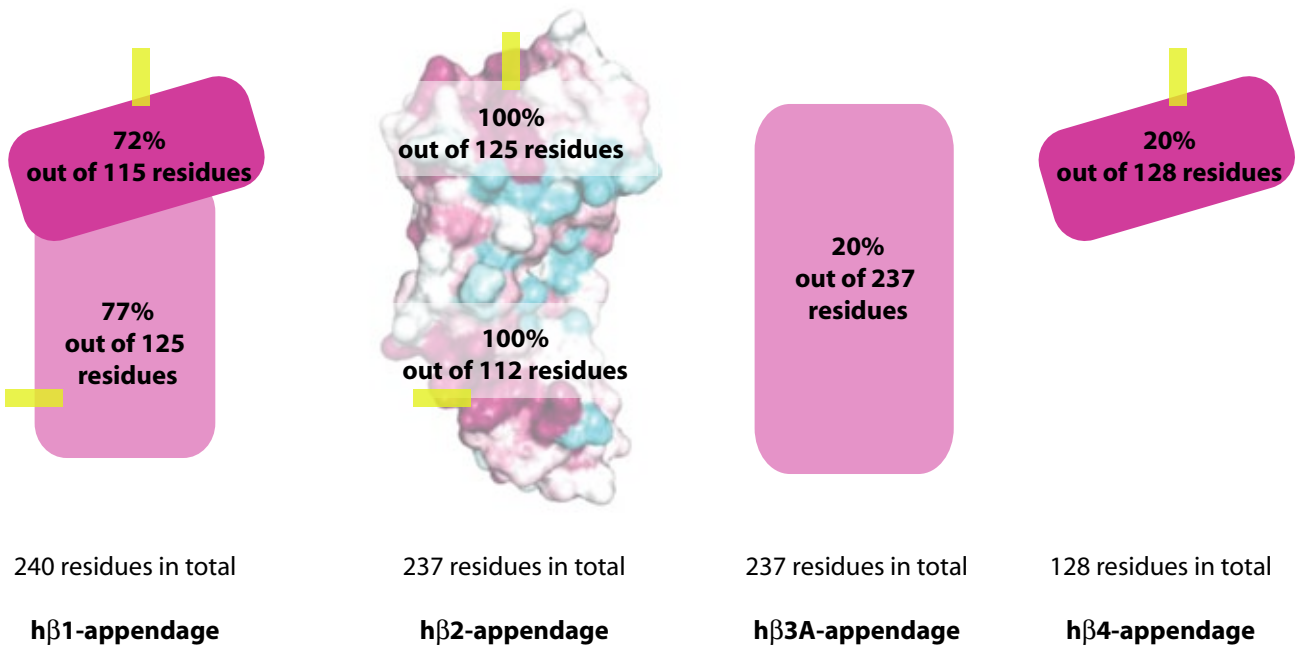
Rat liver cytosol (Supplemental Figure 1) – rat

α :	
Intersectin1	XP_573259
Sec23IP	AAH98824 (Sec23 interacting protein)
Eps15R	AAH98004
Scy1-like1	AAH87141
Epsin1	NP_476477
Hsc70	CAA49670

b2 :

Intersectin1	XP_573259
Eps15R	AAH98004
Hsc70	CAA49670
Scy1-like1	AAH87141
VCP	NP_446316 (valosing containing protein, clathrin binding, NSF homologue)
Epsin1	NP_476477

Figure S3



246 residues in total

m α 2-appendage

Homologies between α and β 1-4 appendage domains

We have divided the β 2 appendage into platform and β sandwich subdomains between the residues VL:FV. The figure shows that this division is not apparent for β 3 and that the top binding site is conserved in β 1,2 and 4 while the side site is conserved in β 1 and β 2.