Electronic Supplementary Material (ESI) for Molecular BioSystems

Identification of a high-affinity network of secretagogin-binding proteins involved in vesicle secretion - Supplementary information

Mikael C. Bauer[1*], David J. O'Connell[2*], Magdalena Maj[3], Ludwig Wagner[3], Dolores J. Cahill[2], Sara Linse[1]†

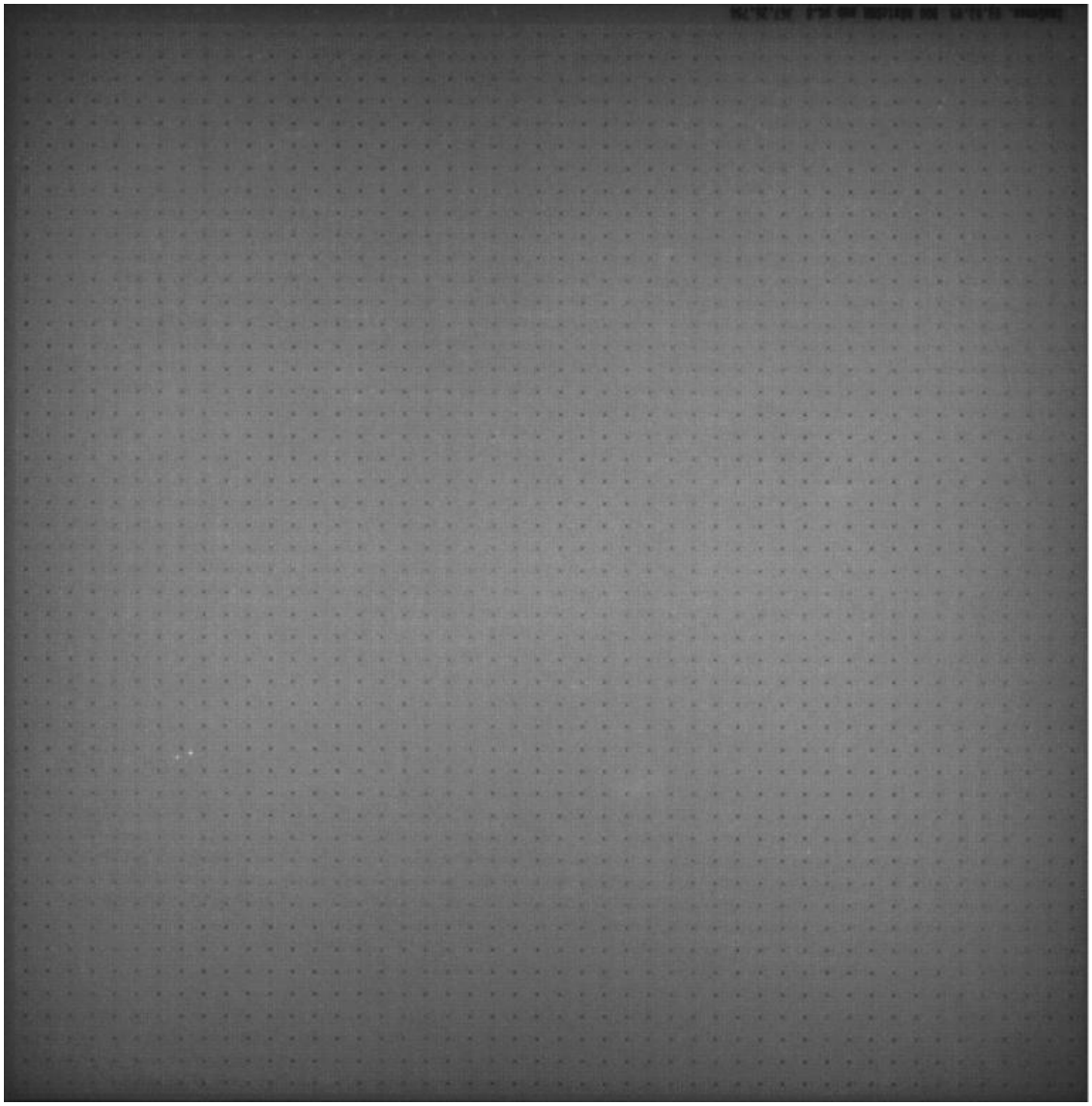

Fig. S1 The hEx1 macroarray imaged at 480 nm after overnight incubation with 1 µM Alexafluor488 labeled secretagogin in TBS with 1 mM Ca^{2+} and then washed six times in TBS-T with 1 mM Ca^{2+}.

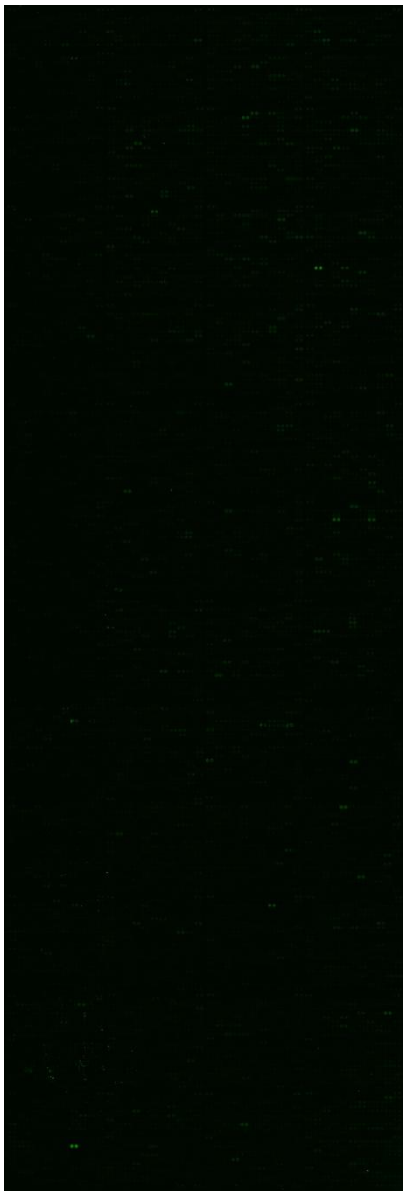

Fig S2. The ProtoArray imaged at 532 nm after 90 min incubation with 1 µM Alexafluor546 labeled secretagogin in TBS with 1 mM Ca^{2+} and then washed five times in TBS with 1 mM Ca^{2+}.

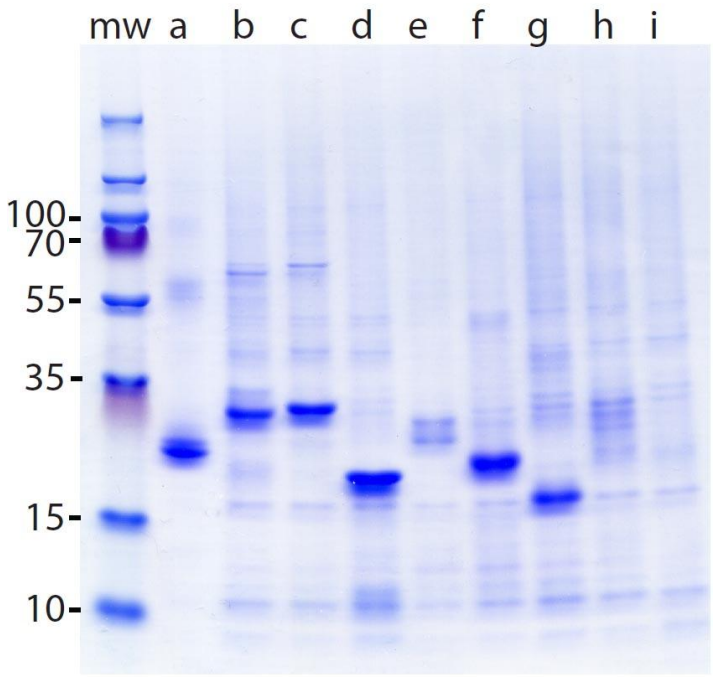

Fig S3. SDS-PAGE gel of the protein solutions used in SPR validation experiments. (a) DDAH-2, (b) myeloid leukaemia factor 2, (c) kif5b (d) ARFGAP2, (e) ATP synthease, (f) doc2-alpha, (g) SNAP-23, (h) rootletin, (i) beta tubulin.

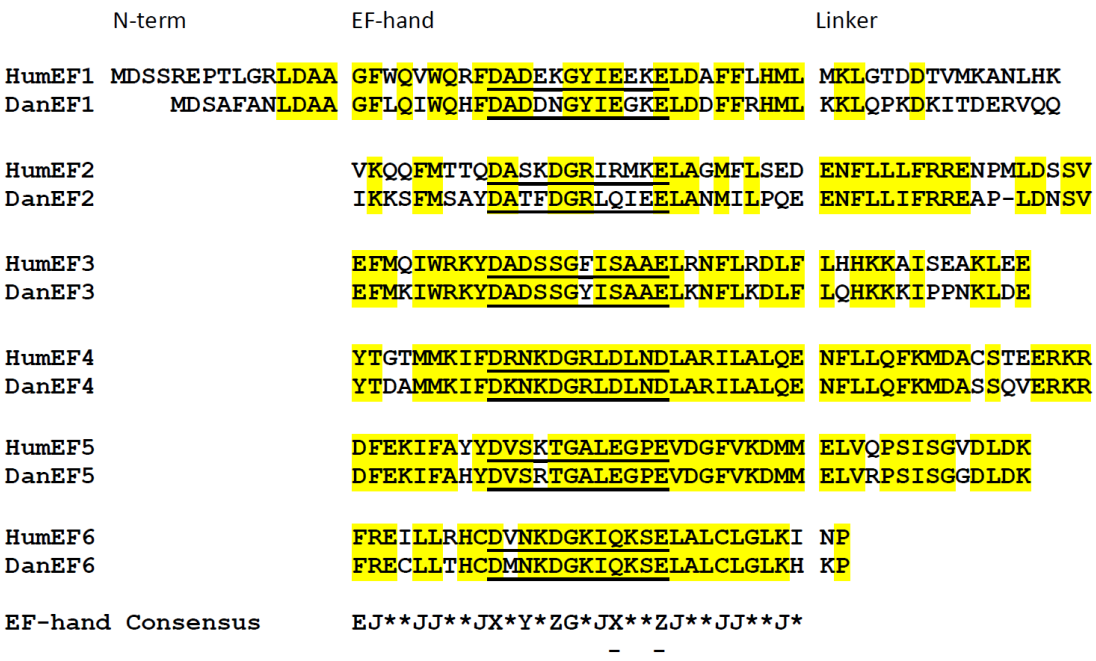

Figure S4. Amino acid sequences of human (Hum) and zebra fish (Dan) secretagogin with identities in yellow. The EF-hand loops are underlined. The EF-hand consensus sequence is shown at the bottom with the calcium coordinating positions labeled X, Y, Z, -X and –Z, J=hydrophobic residue, E = glutamate and G = glycine.

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Table S1. Proteins names, uniprot IDs and sizes of the clones for the putative targets found in the screen of the hEx1 library. *This clone has a very high homology to the proteins rootletin and rootletin-like 1, the sequence expressed by the clone shows a identity >90% with the given region of rootletin

Name	UniProt ID	Size of clone
Validated		
N(G),N(G)-dimethylarginine dimethylaminohydrolase 2 (DDAH-2)	O95865	76-285
synaptosomal-associated protein 23 (SNAP-23)	O00161	79-211
myeloid leukemia factor 2	Q15773	43-248
ADP-ribosylation factor GTPase-activating protein 2 (ARFGAP2)	Q8N6H7	362-521
kinesin heavy chain (kif5b)	P33176	755-963
double C2-like domain-containing protein alpha (DOC2alpha)	Q14183	
ATP synthase O subunit	P48047	
rootletin	Q5TZA2	483-653*
tubulin, beta	P07437	299-430
Non-Validated		
hemoglobin alpha subunit	P69905	1-142
ribosomal protein S2	P15880	1-293
proteasome subunit beta type 5	P28074	25-160

Table S2. Protein name, Gene ID and signal intensity of top ranking target from ProtoArray screen. Proteins in italic was also found in a parallel screen for calmodulin targets.

Piccolo	BC001304.1	614.5
SNAP23	NM_003825.2	556
kinesin light chain 2	NM_022822.1	299.5
cortactin	NM_138565.1	274.5
chromosome 11 open reading frame 52	NM_080659.1	228
sciellin	BC047536.1	217.5
chromosome 19 open reading frame 43	NM_024038.2	217

Table S3. A summary of the validated and putative secretagogin targets grouped according to known functions related to vesicles. Proteins in brackets are non-validated putative targets.

Vesiclular fusion	
SNAP-23	Part of SNARE-complexes in non-neuronal cells which control fusion of vesicles with membranes.
ADP-ribosylation factor GTPase-activating protein 2, ARFGAP2 (ARFGAP 3)	Essential proteins for vesicle transport from ER to Golgi.
double C2-like domain-containing protein alpha, DOC2alpha	Regulates Ca^{2+}-dependent lysosome exocytosis.
(cortactin)	Constituent of clathrin-coated pits, involved in endocytosis.
Vesicular transport	
kinesin heavy chain 1, KIF5B (kinesin light chain 2, KLC2) (kinesin light chain 4, KLC4)	Parts of the kinesin assembly. Moves along microtubili and bind vesicles. Binds SNAP proteins.
β-tubulin	Together with α-tubilin the constituent of microtubulin filaments used for transport of vesicles and organelles.
Vesicular scaffolds	
rootletin	Monomer of the rootlet a large structural unit in rod-cells. Binds vesicles and interacts with kinesins.
(piccolo)	Acts as a scaffold for vesicles and for protein assemblies in the presynaptic active zone.

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Supplementary discussion

Four of the top seven putative targets from the ProtoArray screen are involved in vesicle trafficking thus have a functional link to the validated ones from the hEX1 array. Piccolo is a huge protein, over 5000 amino acids, which is found in the presynaptic active zone and is thought to act as a scaffold for vesicles and protein assemblies (*2*, *3*). Kinesin light chain 2 is part of the kinesin assembly that transports vesicles (*1*) and cortactin is part of the clathrin-coated pits, where endocytosis is initiated (*4*). SNAP-23 is among the validated proteins found in the human protein (hEx1) screen. Among the proteins with lower intensities in the ProtoArray screen is ARFGAP3, a functional homologue of the validated protein ARFGAP2. A brief summary of both validated and non-validated target proteins involved in vesicle trafficking grouped after functions is found in Table S3.

References

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4. Cao, H., Orth, J.D., Chen, J., Weller, S.G., Heuser, J.E. and McNiven, M.A. Cortactin is a component of clathrin-coated pits and participates in receptor-mediated endocytosis. *Mol. Cell. Biol.* 2003, 23, 2162-2170.