|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Protein** | **Endogenous localization** | **Tag location** | **Design rationale** |
| *Actb*  | β-actin | Cytoskeleton throughout cell, enriched in spines [1] | N-terminal | N-terminal tag is most commonly used, does not interfere with actin localization in neurons, F-actin polymerization or neuronal morphology. See for instance [2], [3], and ﻿[4] |
| *Arpc5* | Arp2/3 complex subunit 5 | Throughout cell, enriched in spines [5] | N-terminal | N-terminal tag does not interfere with Arpc5 localization [3] |
| *Bsn* | Bassoon | Presynaptic active zone [6, 7] | N-terminal | N-terminal GFP tag does not affect localization in neurons [6] |
| C-terminal | C-terminal GFP tag does not affect localization in neurons [6] |
| *Doc2a* | Doc2a | Potentially enriched at synapses [8] | C-terminal | C-terminal GFP tag does not affect function in neurons ([9]) |
| *Cacna1a* | CaV2.1, P/Q type | Presynaptic terminals, dendritic spines [10] | N-terminal | N-terminal tag does not affect protein localization or function [11]. C-terminus contains multiple important localization sequences [12]and [13] |
| *Cacna1e* | CaV2.3, R type | Around presynaptic terminals, inside dendritic spines and shaft [14] | N-terminal | See *CACNA1A* based on homology.  |
| *Cacnb1* | CaV β1 | Punctate pattern in axons and dendrites, including localization to presynaptic terminals and dendritic spines [15, 16] | N-terminal | Last exon is not included in all splice variants [17] and has few good PAM sites. N-terminal tag does not affect localization or function in homologous proteins [18] |
| *Cacnb2* | CaV β2 | Punctate pattern in axons and dendrites, including localization to presynaptic terminals and dendritic spines [16] | C-terminal | c-terminal tag does not interfere with localization [16] |
| *Cacnb3* | CaV β3 | Punctate pattern in axons and dendrites, including localization to presynaptic terminals and dendritic spines [15, 16] | C-terminal | c-terminal tag does not interfere with localization [16] |
| *Cacnb4* | CaV β4 | Punctate pattern in axons and dendrites, including localization to presynaptic terminals and dendritic spines [15, 16] | C-terminal | c-terminal tag does not interfere with localization [16] |
| *Cacng2* | TARP gamma-2 / Stargazin | Punctate pattern in dendrites, enriched in postsynaptic density [19] | Internal, in C-terminal tail | Internal tag in C-tail does not interfere with localization [20] |
| *Cacng8* | TARP gamma-8 | Punctate pattern in dendrites, enriched in post-synaptic density [19] | Internal, in C-terminal tail | See *Cacng2,* based on homology |
| *Capds* | CAPS1 | Axonal, punctate [21] | N-terminal | Many splice variants lack C-terminus [22] |
| *Camk2a* | CaMKIIα | Soluble throughout cell, moderate enrichment in spines [23] | N-terminal | N-terminal tag does not interfere with localization [24] |
| *Clta* | Clathrin light chain A | Punctate throughout cell [25] | N-terminal | N-terminal tag does not interfere with localization and function [26] |
| *Cplx1* | Complexin1 | Soluble throughout cell [27] | C-terminal | C-terminal GFP overexpression has similar pattern as endogenous [27, 28] |
| *Cplx2* | Complexin2 | Soluble throughout cell [27] | C-terminal | See *Cplx1,* based on homology |
| *Dlg4*  | PSD95 | Core component of the postsynaptic density [29] | C-terminal | N-terminal residues important for interaction with membranes. C-terminal tagging is commonly used, see for instance [30], [31], [32], and [33] |
| *Frrs1l* | FRRS1L / C9orf4 | Plasma membrane and ER [34] | C-terminal | N-terminal contains potentially important poly-glycine region. C-terminal tagging did not interfere with localization and co-immunoprecipitation experiments in heterologous cells ﻿[34] |
| *Gria1* | GluA1 | Enriched at the postsynaptic density [35] | C-terminal | Both N and C-terminal tags do not interfere with localization, see for instance [36] and [37] C-terminal tagging was chosen based on availability of PAM sites |
| *Gria2*  | GluA2 | Enriched at the postsynaptic density [35] | C-terminal | See *Gria1*, based on homology |
| *Gria3* | GluA3 | Enriched at the postsynaptic density [35] | C-terminal | See *Gria1*, based on homology |
| *Grin1* | GluN1 | Enriched at the postsynaptic density [38] | N-terminal, after signal peptide | C-terminal residues mediate intracellular interactions [39] and some splice variants leak parts of the C-terminal [40] |
| *Grin2a* | GluN2a | Enriched at the postsynaptic density [38] | N-terminal, after signal peptide | C-terminal residues mediate intracellular interactions [39] ﻿ |
| *Grin2b* | GluN2b | Enriched at the postsynaptic density [38] | N-terminal, after signal peptide | C-terminal residues mediate intracellular interactions [39] ﻿ |
| *Gsg1l* | GSG1-l | Punctate pattern in dendrites, enriched at the postsynaptic density [41] | N-terminal | C-terminal tag does not interfere with localization and function [42] |
| *Nlgn3* | Neuroligin-3 | Concentrated in excitatory and inhibitory synapses [43] | N-terminal, after signal peptide | C-terminus mediates intracellular interactions [44]  |
| *Pclo* | Piccolo | Enriched at presynaptic active zone [45] | N-terminal | N-terminal domains linked to GFP localize to presynaptic terminals [46]. N-terminus was chosen because PAM sites were predicted to be more specific, based on MIT score |
| *Rab11a* | Rab11 | Punctate labeling of recycling endosomes throughout cell [47, 48] | N-terminal | N-terminal fusion does not affect protein function or localization. See for instance [47, 49] |
| *Rims1* | RIM1 | Enriched at presynaptic active zone [50, 51] | C-terminal | Deletion of C-terminal domains does not interfere with localization [50]. N-terminus contains important interaction sequences [52] |
| *Rims2* | RIM2 | Presynaptic active zone [50, 51] | C-terminal | See *Rims1,* based on homology |
| *Shank1* | Shank1 | Core component of the postsynaptic density [29] | C-terminal | Shanks are commonly tagged at the N-terminus (see e.g. [53], but also [54]), but C-terminal tagging was chosen here due to unavailability of N-terminal PAM sites. We found C-terminal tagging does not interfere with synaptic localization of Shank1 and Shank2 |
| *Shank2* | Shank2 | Core component of the postsynaptic density [29] | C-terminal | See *Shank1*. |
| *Syt7* | Synaptotagmin-7 | Presynaptic terminal [55] , potentially also postsynaptic [56] | N-terminal | Based on homology with *Syt1*: N-terminal tag does not interfere with function, while a C-terminal GFP does interfere with its function in vesicle fusion [57, 58] |
| *Tubb3*  | β3-tubulin | Soluble and somatodendritic microtubules [59] | C-terminal | Previously shown to correctly tag *Tubb3* and to be incorporated in the microtubule network. See [60] and [61] |
| *Unc13a* | Munc13-1 | Presynaptic active zone [62] | C-terminal | c-terminal tag does not interfere with localization or function [63] |
| *Wasf1* | WASP1/Wave1 | Cytoplasmic, enriched in spines [64] | C-terminal | C-terminal tag does not interfere with localization [3] |

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