##### **Supplementary File 1a. Absolute values and statistics.** All comparisons, Ns (animals, NMJs, AZs (AZs), and statistical tests used in this study. All values are mean ± SEM.

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| **Figure. Panel** | **Comparison** | **Ns** | **Values/Statistical tests** |
| Fig. 1C | Average Pr in type Ib and type Is | 6 animals, 6 NMJs | Ib Average Pr = 12.47 ± 0.88%Is Average Pr = 25.69 ± 1.20%Paired t test, p < 0.0001 |
| Fig. 1E | CacTd-Tomato-N to Pr correlation in type Is | 6 animals, 6 NMJs | Average type Is Pearson’s r = 0.617 ± 0.028 |
| Fig. 1F | CacTd-Tomato-N to Pr correlation in type Ib | 6 animals, 6 NMJs | Average type Ib Pearson’s r = 0.576 ± 0.035 |
| Fig. 1H | CacsfGFP-N levels at type Ib vs type Is | n = 12 animals, 36 NMJs | Type Ib mean: 1.000 ± 0.017Type Is mean: 0.991 ± 0.028t test, p = 0.78 |
| Fig. 1J | Slopes of CacTd-Tomato-N to Pr correlation at type Ib vs type Is | 6 animals, 6 NMJsType Ib: n = 531 AZsType Is: n = 365 AZs | Ib Slope = 0.085 ± 0.006Is Slope = 0.154 ± 0.011ANCOVA test, p < 0.0001 |
| Fig. 2F | CacHaloTag-N cluster area in type Ib and type Is | 5 animals, 14 NMJsType Ib: n = 80 boutons5 animals, 19 NMJsType Is: n = 96 boutons | Type Ib = 0.0093 ± 0.0004 µm2Type Is = 0.0098 ± 0.0004 µm2Mann-Whitney test, p = 0.5906 |
| Fig. 2G | CacHaloTag-N localizations per cluster in type Ib and type Is | 5 animals, 14 NMJsType Ib: n = 80 boutons5 animals, 19 NMJsType Is: n = 96 boutons | Type Ib = 69.39 ± 4.76 Type Is = 83.52 ± 5.67 Mann-Whitney test, p = 0.0928 |
| Fig. 2H | CacHaloTag-N cluster density in type Ib and type Is | 5 animals, 14 NMJsType Ib: n = 80 boutons5 animals, 19 NMJsType Is: n = 96 boutons | Type Ib = 7185 ± 234.7 µm-2Type Is = 8277 ± 342.6 µm-2Mann-Whitney test, p = 0.0278 |
| Fig. 2I | CacHaloTag-N cluster density per NMJ in type Ib and type Is | Type Ib: n = 5 animals, 14 NMJsType Is: n = 5 animals 19 NMJs | Type Ib = 7300 ± 362.2 µm-2Type Is = 8569 ± 584.0 µm-2Wilcoxon test, p = 0.0076 |
| Fig. 3B | Brp levels at type Ib and type Is | n = 12 animals,36 NMJs | Type Ib mean: 1.00 ± 0.02Type Is mean: 0.79 ± 0.03Unpaired T-test, p < 0.0001 |
| Fig. 3C | CacsfGFP-N :Brp ratio at type Ib vs type Is | n = 12 animals,36 NMJs | Type Ib mean: 1.00 ± 0.01Type Is mean: 1.30 ± 0.05Paired T-test, p < 0.0001  |
| Fig. 3D | CacsfGFP-N :Brp correlation | 12 animals, 36 NMJsIb: n = 5349 AZsIs: n = 2625 AZs | Type Ib slope: 0.874 ± 0.005Type Is slope: 0.787 ± 0.009ANCOVA, p < 0.0001 |
| Fig. 3G | CacsfGFP-N levels in type Ib vs Is in Control and *brp-/-*  | 9 animals Control Ib: n = 29 NMJs*brp-/-* Ib: n = 31 NMJsControl Is: n = 29 NMJs*brp-/-* Is: n = 31 NMJs | Control Ib mean = 1.00 ± 0.03*brp-/-* Ib mean = 0.33 ± 0.01; p < 0.0001 Control Is mean = 1.00 ± 0.03*brp-/-* Is mean = 0.45 ± 0.02; p < 0.0001Kruskal-Wallis test adjusted p values vs Control |
| Fig. 3H | *brp-/-*:Control ratio of CacsfGFP-N levels in type Ib vs Is | n = 9 animals,31 NMJs  | Ib ratio = 0.334 ± 0.010Is ratio = 0.451 ± 0.018Mann-Whitney test, p < 0.0001 |
| Fig. 4C | Brp levels at *cacsfGFP-N* type Ib and type Is terminals in control vs PhTx | Control: n = 12 animals, 40 NMJsPhTx: n = 12 animals, 40 NMJs | Control Ib = 1.000 ± 0.047PhTx Ib = 1.488 ± 0.068; p < 0.0001 Control Is = 1.000 ± 0.050PhTx Is = 1.493 ± 0.076; p < 0.0001Kruskal-Wallis test adjusted p values vs Control |
| Fig. 4D | CacsfGFP-N levels at *cacsfGFP-N* type Ib and type Is terminals in control vs PhTx | Control: n = 12 animals, 40 NMJsPhTx: n = 12 animals, 40 NMJs | Control Ib = 1.000 ± 0.044PhTx Ib = 1.325 ± 0.059; p < 0.0001 Control Is = 1.000 ± 0.048PhTx Is = 1.300 ± 0.058; p = 0.0002ANOVA test adjusted p values vs Control |
| Fig. 4G | CacsfGFP-N levels at *cacsfGFP-N*; *brp-/-* type Ib and type Is terminals in control vs PhTx | Control: n = 12 animals, 43 NMJsPhTx: n = 12 animals, 43 NMJs  | Control Ib = 1.000 ± 0.041PhTx Ib = 1.029 ± 0.038; p = 0.8475 Control Is = 1.000 ± 0.046PhTx Is = 0.8288 ± 0.035; p = 0.0058ANOVA test adjusted p values vs Control |
| Fig. 5C | EJPs of endogenously tagged subunits | WT: n = 9 NMJsStj: n = 8 NMJsStolid: n = 11 NMJsCa-β: n = 9 NMJs | WT mean = 30.21 ± 4.09 mVStj mean = 41.17 ± 4.32 mV; p = 0.14Stolid mean = 26.44 ± 2.82 mV; p = 0.80Ca-β mean = 36.49 ± 4.09 mV; p = 0.52ANOVA, adjusted p values vs WT |
| Fig. 5D | mEJPs of endogenously tagged subunits | WT: n = 9 NMJsStj: n = 8 NMJsStolid: n = 11 NMJsCa-β: n = 9 NMJs | WT mean = 1.02 ± 0.08 mVStj mean = 1.12 ± 0.07 mV; p = 0.92Stolid mean = 1.11 ± 0.09 mV; p = 0.99Ca-β mean = 1.16 ± 0.09 mV; p = 0.57Kruskal-Wallis test adjusted p values vs WT |
| Fig. 5E | QC of endogenously tagged subunits | WT: n = 9 NMJsStj: n = 8 NMJsStolid: n = 11 NMJsCa-β: n = 9 NMJs | WT mean = 29.63 ± 2.96Stj mean = 37.02 ± 3.93; p = 0.34Stolid mean = 24.73 ± 2.63; p = 0.60Ca-β mean = 32.79 ± 4.34; p = 0.86ANOVA, adjusted p values vs WT |
| Fig. 6E | Ca-βV5-C levels at type Ib vs type Is | n = 11 animals, 27 NMJs  | Type Ib mean: 1.00 ± 0.04Type Is mean: 0.90 ± 0.04Mann-Whitney Test, p = 0.06 |
| Fig. 6F | StjV5-N levels at type Ib vs type Is | n = 9 animals, 28 NMJs | Type Ib mean: 1.00 ± 0.05Type Is mean: 0.76 ± 0.05Unpaired t test, p = 0.0008 |
| Fig. 6G, H | CacsfGFP-N :StjV5-N correlation | 5 animals, 22 NMJsIb: n = 4012 AZsIs: n = 2211 AZs | Type Ib slope: 0.912 ± 0.009Type Is slope: 0.914 ± 0.012ANCOVA, p = 0.90 |
| Fig. 7C | StjV5-N levels at type Ib and type Is terminals in control vs PhTx | Control: n = 6 animals, 34 NMJsPhTx: n = 6 animals, 26 NMJs | Control Ib = 1.00 ± 0.01PhTx Ib = 1.27 ± 0.03; p < 0.0001 Control Is = 1.00 ± 0.02PhTx Is = 1.26 ± 0.02; p < 0.0001Kruskal-Wallis test adjusted p values vs Control |
| Fig. 7F | StjV5-N levels at type Ib and type Is terminals in control vs *GluRIIA-/-* | Control: n = 7 animals, 37 NMJs*GluRIIA-/-*: n = 7 animals, 26 NMJs | Control Ib = 1.00 ± 0.02*GluRIIA-/-* Ib = 1.18 ± 0.03; p < 0.0001 Control Is = 1.00 ± 0.02*GluRIIA-/-* Is = 1.17 ± 0.03; p < 0.0001ANOVA test adjusted p values vs Control |
| Fig. S1D | EJPs of Cac endogenous tags | Control: n = 11 NMJs*cacHaloTag-N*: n = 8 NMJs*cacTd-Tomato-N*: n = 8 NMJs | Control mean = 42.51 ± 2.08 mV*cacHaloTag-N* mean = 43.02 ± 1.92 mV; p = >0.99*cacTd-Tomato-N* mean = 40.07 ± 0.99 mV; p = 0.25Kruskal-Wallis test adjusted p values vs Control |
| Fig. S1E | mEJPs of Cac endogenous tags | Control: n = 11 NMJs*cacHaloTag-N*: n = 8 NMJs*cacTd-Tomato-N*: n = 8 NMJs | Control mean = 0.88 ± 0.07 mV*cacHaloTag-N* mean = 0.77 ± 0.07 mV; p = 0.47*cacTd-Tomato-N* mean = 0.93 ± 0.06 mV; p = 0.86ANOVA, adjusted p values vs Control |
| Fig. S1F | QC of Cac endogenous tags | Control: n = 11 NMJs*cacHaloTag-N*: n = 8 NMJs*cacTd-Tomato-N*: n = 8 NMJs | Control mean = 49.97 ± 2.56 mV*cacHaloTag-N* mean = 59.83 ± 6.76 mV; p = 0.23*cacTd-Tomato-N* mean = 44.34 ± 2.95 mV; p = 0.60ANOVA, adjusted p values vs Control |

**Supplementary File 1b. Imaging details.** This table contains detailed information on how each protein was labeled and visualized using live and fixed confocal microscopy and STORM imaging. All secondary antibodies were incubated at RT for 2 hours at a concentration of 1:500.

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| **Imaging Method** | **Protein**  | **Genetic line**  | **Imaging Reagents** | **Sample processing** |
| Confocal | Cac | *cacsfGFP-N* (Gratz et al., 2019) | anti-GFP AF488 conjugate (ThermoFisher- RRID:AB\_221477) | Fix: Bouins or 4% PFAStaining: 1:500, 2 hours at RT |
| Confocal | Stj | *stjV5-N* ( this study) | *Primary:* anti-V5 monoclonal (ThermoFisher- RRID:AB\_2556564)*Secondary:* Goat anti-Mouse IgG Highly Cross-Adsorbed AF488 (ThermoFisher- RRID:AB\_2534088) | Fix: MethanolPrimary staining: 1:500, overnight at 4°C\*Note, best in 488 channel |
| Confocal | Stolid | *stolidV5-N* ( this study) | *Primary:* anti-V5 monoclonal (ThermoFisher- RRID:AB\_2556564)*Secondary:* Goat anti-Mouse IgG Highly Cross-Adsorbed AF488 (ThermoFisher- RRID:AB\_2534088) | Fix: BouinsPrimary staining: 1:500, overnight at 4°C |
| Confocal | Ca-β | *Ca-βV5-C* (this study) | *Primary:* anti-V5 monoclonal (ThermoFisher- RRID:AB\_2556564)*Secondary:* Goat anti-Mouse IgG Highly Cross-Adsorbed AF488 (ThermoFisher- RRID:AB\_2534088) | Fix: BouinsPrimary staining: 1:500, overnight at 4°C |
| Confocal | Brp |  | *Primary:* anti-Brp *(*DSHB- RRID:AB\_2314866)*Secondary:* Goat anti-Mouse IgG Highly Cross-Adsorbed AF568 (ThermoFisher- RRID:AB\_144696 ) | Fix: Bouins or MethanolPrimary staining: 1:100 overnight at 4°C |
| Confocal | HRP |  | anti-HRP AF488 conjugate (Jackson ImmunoResearch- RRID:AB\_2338965),anti-HRP AF647 conjugate (Jackson ImmunoResearch- RRID:AB\_2338967) | Fix: Bouins or MethanolPrimary staining: 1:500, 2 hours at RT |
| LiveImaging | Cac | *cacTd-Tomato-N*(this study) |  |  |
| STORM | Cac | *cacHaloTag-N*(this study) | JaneliaFluor 646 HaloTag Ligand (Promega #GA1120) | Live label: 500nM for 20 min in dark box at RTFix: 4% PFA for 30 min at RT in dark box |