
Figures and figure supplements

Regulation of hippocampal mossy fiber-CA3 synapse function by a Bcl11b/C1ql2/Nrxn3(25b+) pathway

Artemis Koumoundourou *et al.*

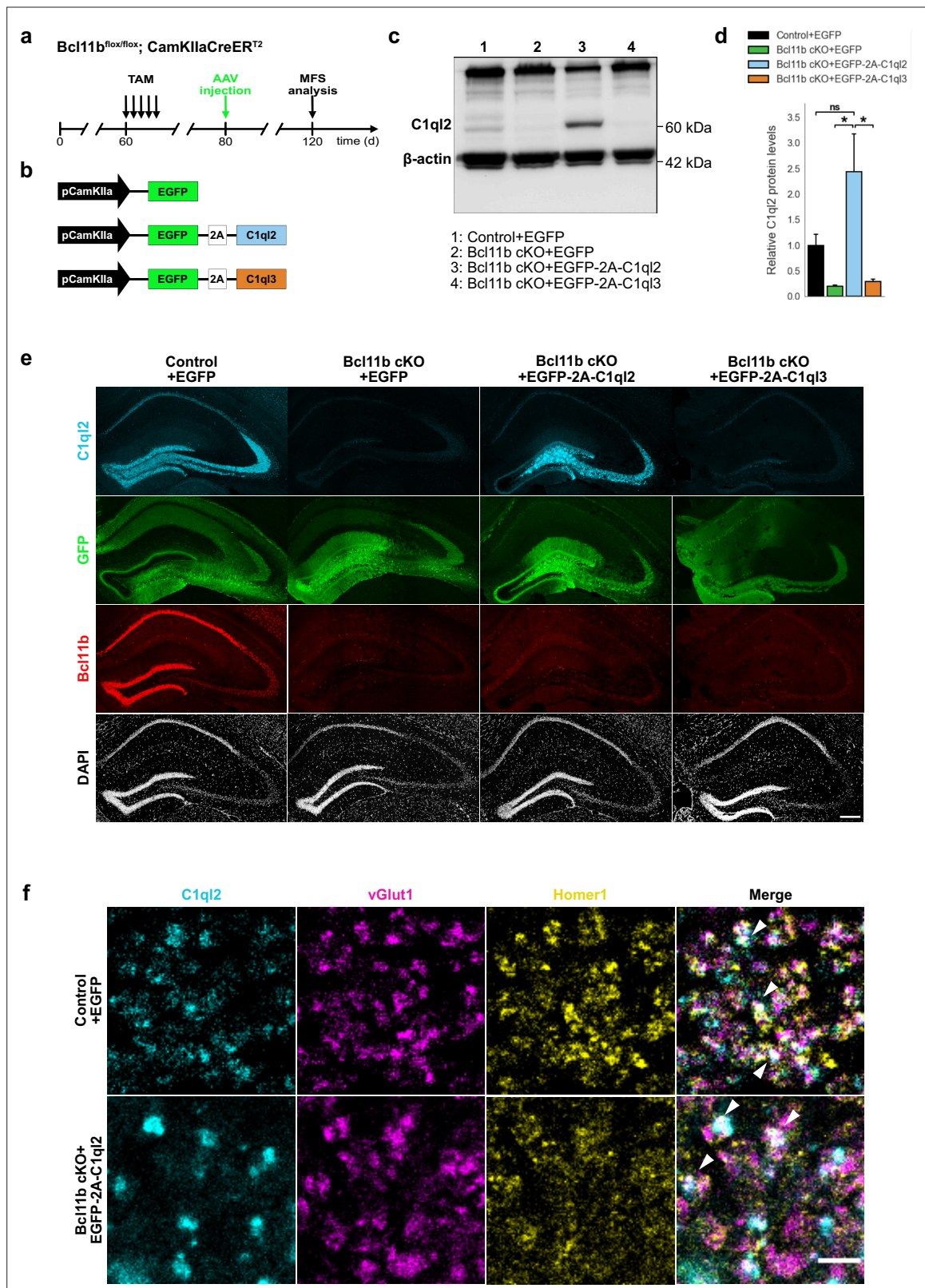


Figure 1. Stereotaxic injection of C1ql2-expressing AAV into *Bcl11b* cKO DGN restores C1ql2 levels. (a) Experimental design to analyze the functions of C1ql2 in the MFS as a downstream target of *Bcl11b*. (b) AAV constructs injected in the DG of *Bcl11b* cKO and control littermates. (c) Western blot and (d) relative C1ql2 protein levels in mouse hippocampal homogenates. N=3. All data are presented as means; error bars indicate SEM. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. *Bcl11b* cKO +EGFP-2A-C1ql2: ns, $p=0.11$; *Bcl11b* cKO +EGFP-2A-C1ql2 vs. *Bcl11b* cKO +EGFP: $*p=0.015$; *Bcl11b*

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cKO +EGFP-2A-C1ql2 vs. Bcl11b cKO +EGFP-2A-C1ql3: * $p=0.019$; ns, not significant. **(e)** Immunohistochemistry of C1ql2 (cyan), GFP (green), and Bcl11b (red) on hippocampal sections. Scale bar: 200 μm . **(f)** Immunohistochemistry of C1ql2 (cyan), vGlut1 (magenta), and Homer1 (yellow) in the SL of CA3. White arrowheads indicate co-localizing puncta of all three proteins. Scale bar: 15 μm .

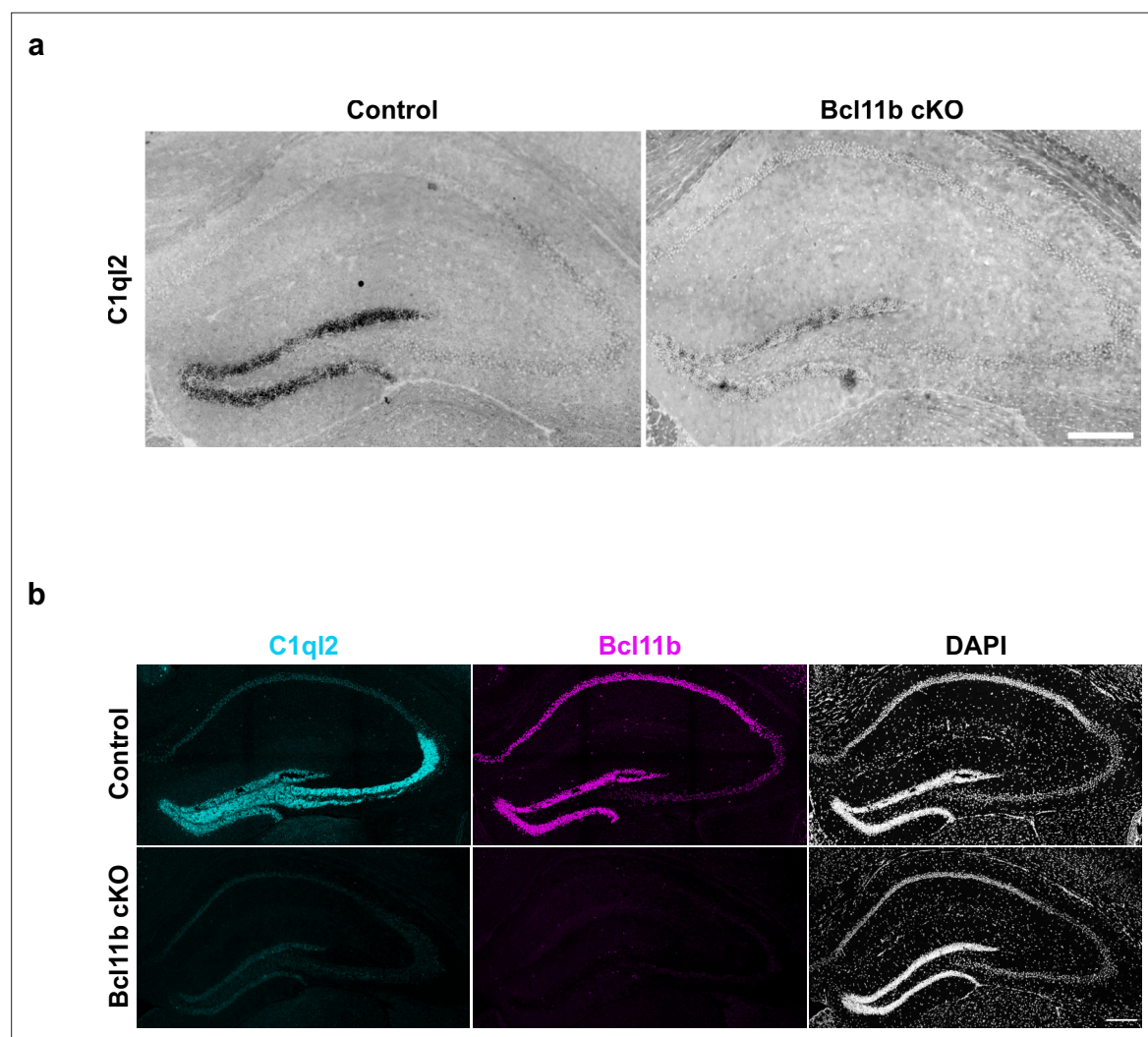


Figure 1—figure supplement 1. *C1ql2* mRNA and protein are lost upon *Bcl11b* cKO in DGN. (a) mRNA in situ hybridization of *C1ql2* on hippocampal sections. Scale bar: 200 μ m. (b) Immunohistochemistry of *C1ql2* (cyan) and *Bcl11b* (magenta) on hippocampal sections. Scale bar: 200 μ m.

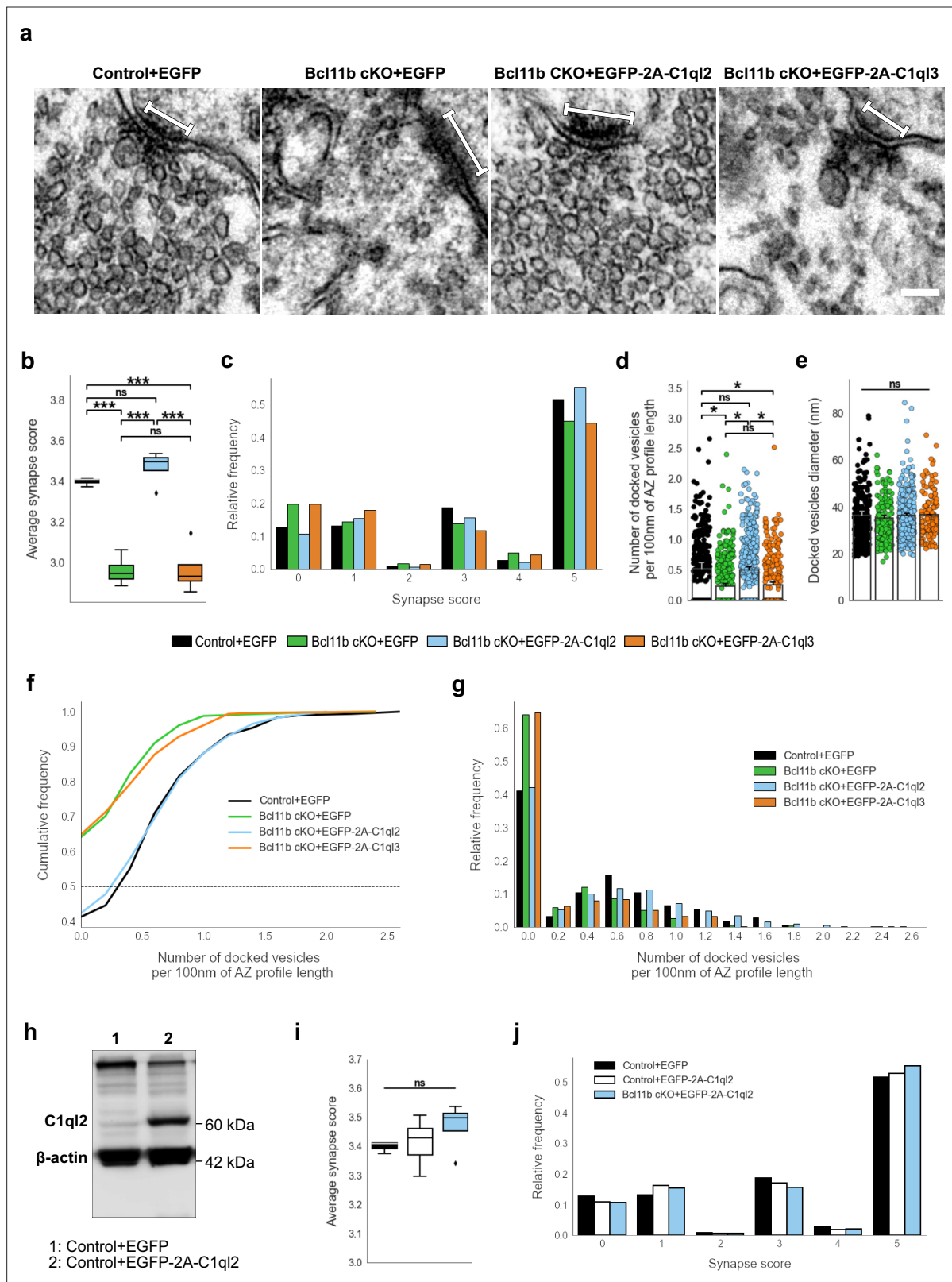


Figure 2. C1q12 reintroduction in *Bcl11b* cKO DGN rescues SV recruitment in MFS. **(a)** Electron microscope images of MFS and proximal SV. White bars mark synapse length from the postsynaptic side. Scale bar: 100 nm. **(b)** Average synapse score. Control +EGFP, N=3; Bcl11b cKO +EGFP, Bcl11b cKO +EGFP-2A-C1q12, Bcl11b cKO +EGFP-2A-C1q13, N=4. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. Bcl11b cKO +EGFP: *** $p=0.0002$, and vs. Bcl11b cKO +EGFP-2A-C1q13: *** $p=0.0003$; Bcl11b cKO +EGFP-2A-C1q12 vs. Bcl11b cKO +EGFP and vs. Bcl11b cKO +EGFP-2A-C1q13:

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*** $p < 0.0001$; ns, not significant. **(c)** Relative frequency of synapse scores. **(d)** Number of docked vesicles per 100 nm AZ profile length. Control +EGFP, Bcl11b cKO +EGFP-2A-C1ql3, N=3; Bcl11b cKO +EGFP, Bcl11b cKO +EGFP-2A-C1ql2, N=4. All data are presented as means; error bars indicate SEM. Points represent the individual examined AZ and SV, respectively. Two-way ANOVA and Tuckey's PHC. Control +EGFP vs. Bcl11b cKO +EGFP: * $p = 0.024$, and vs. Bcl11b cKO +EGFP-2A-C1ql3: * $p = 0.045$; Bcl11b cKO +EGFP-2A-C1ql2 vs. Bcl11b cKO +EGFP: * $p = 0.026$, and vs. Bcl11b cKO +EGFP-2A-C1ql3: * $p = 0.049$; ns, not significant. **(e)** Diameter of docked vesicles. Control +EGFP, Bcl11b cKO +EGFP-2A-C1ql3, N=3; Bcl11b cKO +EGFP, Bcl11b cKO +EGFP-2A-C1ql2, N=4; Two-way ANOVA. ns, not significant. **(f)** Cumulative and **(g)** relative frequency of the number of docked vesicles per 100 nm AZ profile length. **(h)** Western blot of mouse hippocampal homogenates. **(i)** Average synapse score. Control +EGFP, N=3; Control +EGFP-2A-C1ql2, N=6; Bcl11b cKO +EGFP-2A-C1ql2, N=4. Two-way ANOVA. ns, not significant. **(j)** Relative frequency of synapse scores. Data for Control +EGFP-2A-C1ql2 from i-j in this figure are compared with Control +EGFP and Bcl11b cKO +EGFP-2A-C1ql2 data from **(b-c)**.

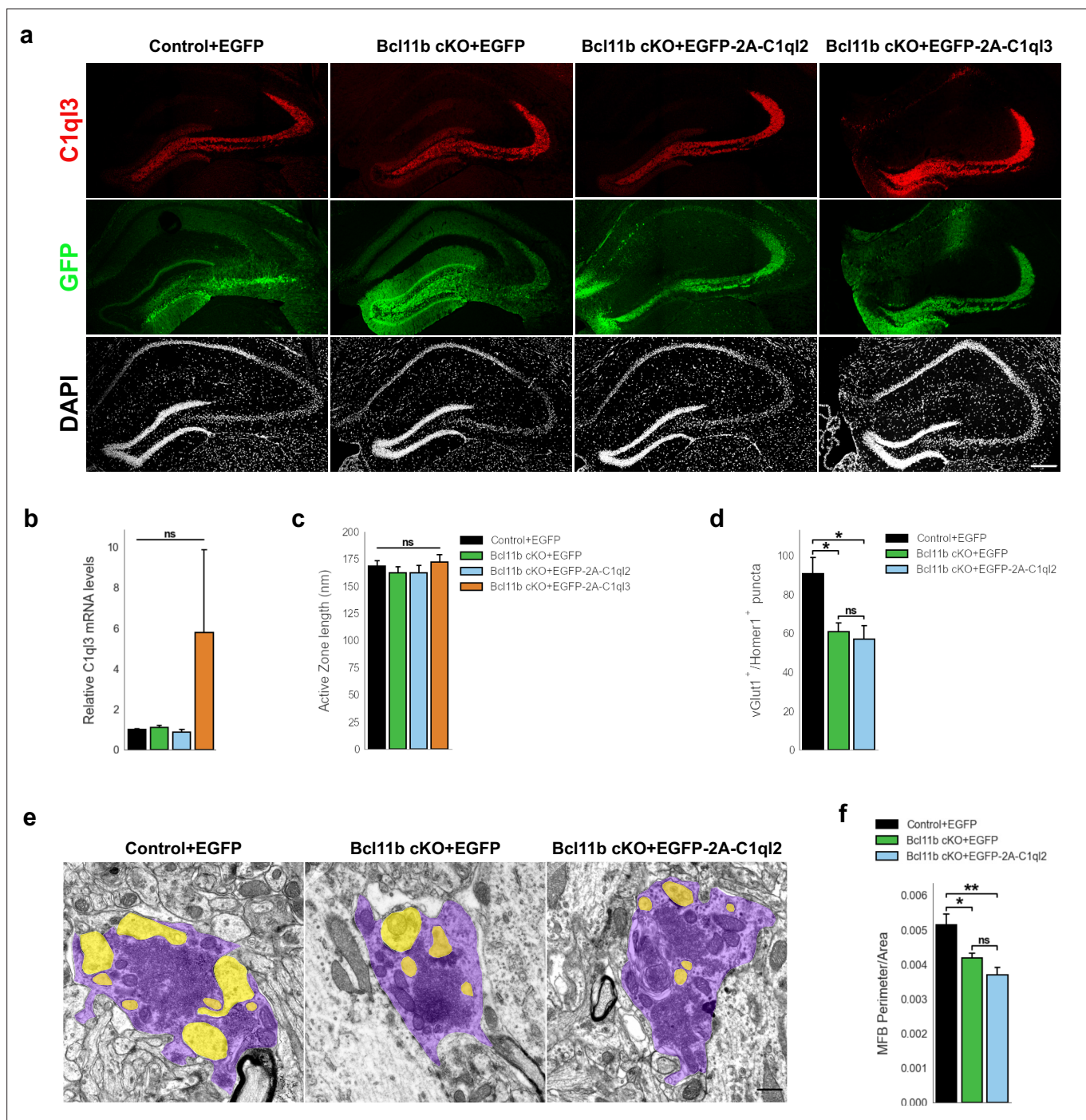


Figure 2—figure supplement 1. C1ql2 reintroduction in *Bcl11b* cKO DGN does not rescue MFS number and MFB complexity. (a)

Immunohistochemistry of C1ql3 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μ m. (b) Relative C1ql3 mRNA levels in DGN. Control +EGFP, Bcl11b cKO +EGFP, Bcl11b cKO +EGFP-2A-C1ql2, N=4; Bcl11b cKO +EGFP-2A-C1ql3, N=3. All data are presented as means; error bars indicate SEM. Two-way ANOVA. ns, not significant. (c) Active zone length. Control +EGFP, Bcl11b cKO +EGFP-2A-C1ql3, N=3; Bcl11b cKO +EGFP, Bcl11b cKO +EGFP-2A-C1ql2, N=4. All data are presented as means; error bars indicate SEM. Two-way ANOVA. ns, not significant. (d) vGlut1 and Homer1 double positive puncta in selected CA3 SL ROIs. N=3. All data are presented as means; error bars indicate SEM. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. Bcl11b cKO +EGFP: *p=0.047, and vs. Bcl11b cKO +EGFP-2A-C1ql2: *p=0.029; ns, not significant. (e) Electron microscopy images of MFBs (purple) and contacting postsynaptic spines (yellow). Scale bar: 500 nm. (f) MFB perimeter-to-area ratio. N=5. All data are presented as means; error bars indicate SEM. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. Bcl11b cKO +EGFP: *p=0.035, and vs. Bcl11b cKO +EGFP-2A-C1ql2: **p=0.0014; ns, not significant.

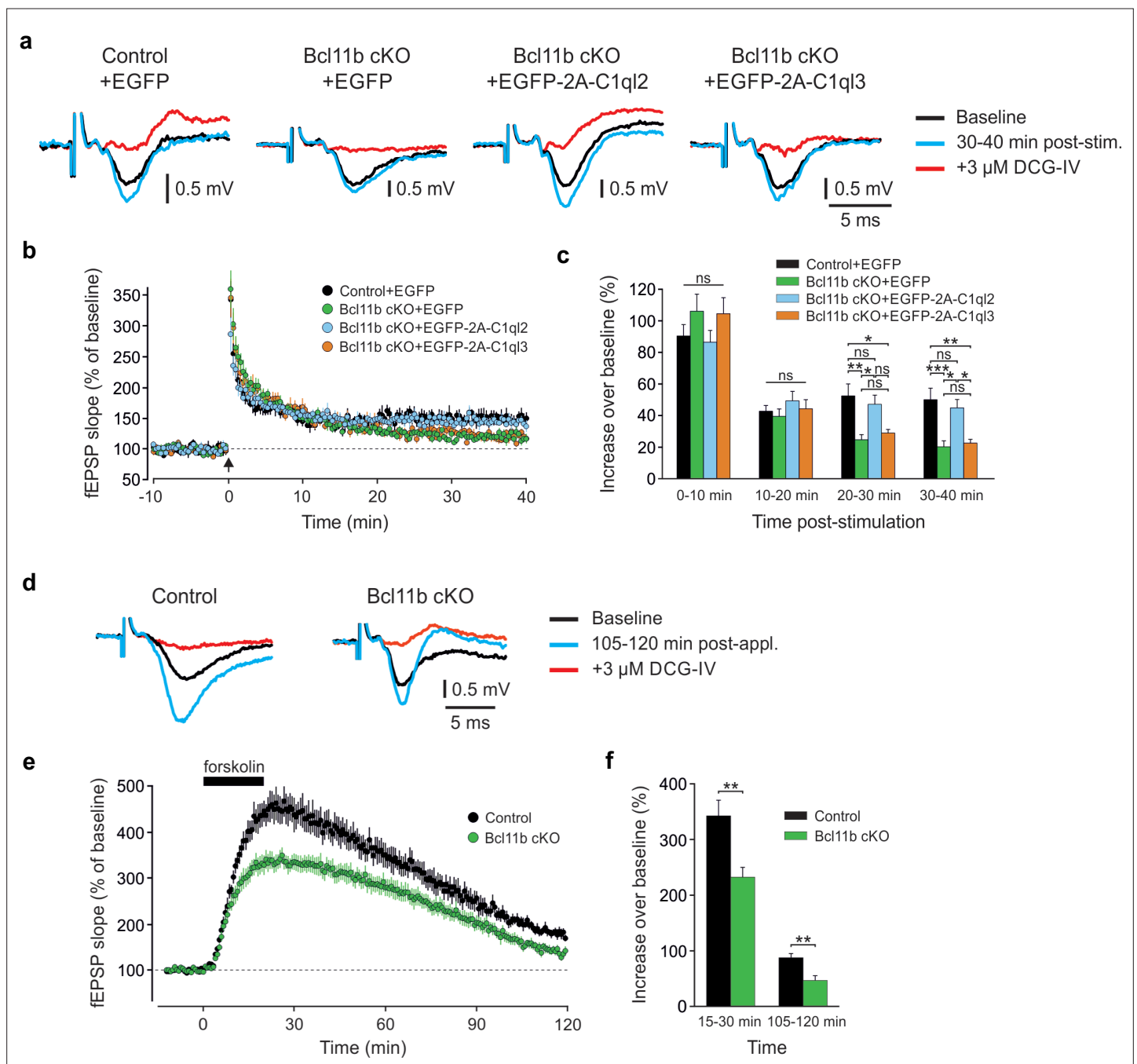


Figure 3. C1ql2 reintroduction in *Bcl11b* cKO DGN rescues mossy fiber LTP. (a) Representative fEPSP traces showing baselines before HFS (black), fEPSP changes 30–40 min after HFS (cyan) and following the application of 3 μM DCG-IV (red). (b) Time course of fEPSP slopes. The black arrow indicates HFS and the dashed line is the baseline level. (c) Quantification of fEPSP facilitation at four different time intervals after HFS. Changes in the fEPSP slope are shown as the percentage of the mean baseline fEPSP. Control +EGFP, 7 slices from 6 mice; Bcl11b cKO +EGFP, 8 slices from 5 mice; Bcl11b cKO +EGFP-2A-C1ql3, 8 slices from 6 mice; Bcl11b cKO +EGFP-2A-C1ql2, 6 slices from 4 mice; All data are presented as means; error bars indicate SEM. One-way ANOVA followed by Bonferroni's PHC for each time interval. 20–30 min: Control +EGFP vs. Bcl11b cKO +EGFP: ***p*=0.002, and vs. Bcl11b cKO +EGFP-2A-C1ql3: **p*=0.011; Bcl11b cKO +EGFP-2A-C1ql2 vs. Bcl11b cKO +EGFP: **p*=0.023; 30–40 min: Control +EGFP vs. Bcl11b cKO +EGFP: ****p*<0.001, and vs. Bcl11b cKO +EGFP-2A-C1ql3: ****p*=0.002; Bcl11b cKO +EGFP-2A-C1ql2 vs. Bcl11b cKO +EGFP: **p*=0.01 and vs. Bcl11b cKO +EGFP-2A-C1ql3: **p*=0.023; ns, not significant. (d) Representative fEPSP traces showing baselines before forskolin application (black), fEPSP changes 105–120 min after the start of application (cyan) and following the addition of 3 μM DCG-IV (red). (e) Time course of fEPSP slopes. The black solid line indicates forskolin perfusion and the dashed line is the baseline level. (f) Quantification of fEPSP facilitation at two different time intervals after the start of the forskolin application. Changes in fEPSP slope are shown as percentage of the mean baseline fEPSP. 8 slices from 5 mice. All data are presented as means; error bars indicate SEM. Unpaired t-test for both time intervals. 15–30 min: ***p*=0.005; 105–120 min: ***p*=0.0025.

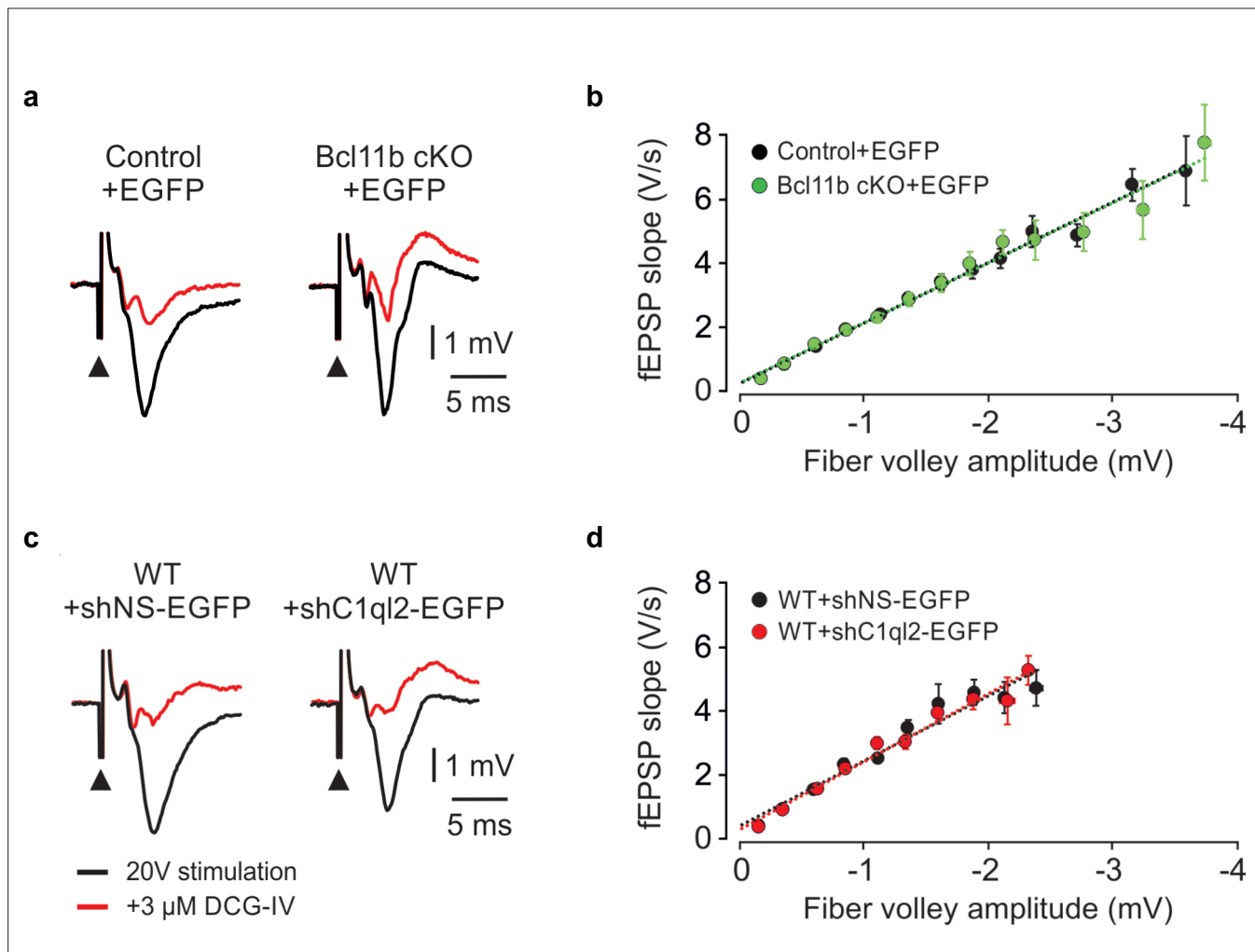


Figure 3—figure supplement 1. *Bcl11b* cKO and *C1ql2* KD in DGN do not affect basal synaptic transmission. (a) Representative fEPSP traces recorded in slices from a control and *Bcl11b* cKO animal showing responses to a 20 V electrical stimulation (black, black arrowheads indicate stimulation). The signal is almost entirely blocked by the application of 3 μ M DCG-IV (red). (b) Input-output curves generated by plotting fEPSP slope against fiber volley amplitude at increasing stimulation intensities. Control +EGFP, 35 slices from 16 mice; *Bcl11b* cKO +EGFP, 32 slices from 14 mice. The data are presented as means, error bars represent SEM. (c) Representative fEPSP traces recorded in slices from a control and *C1ql2* KD animal, showing responses to a 20 V electrical stimulation (black, black arrowheads indicate stimulation). The signal is almost entirely blocked by the application of 3 μ M DCG-IV (red). (d) Input-output curves generated by plotting fEPSP slope against fiber volley amplitude at increasing stimulation intensities. + shNS EGFP, 16 slices from 9 mice; +shC1ql2-EGFP, 12 slices from 8 mice. The data are presented as means, error bars represent SEM.

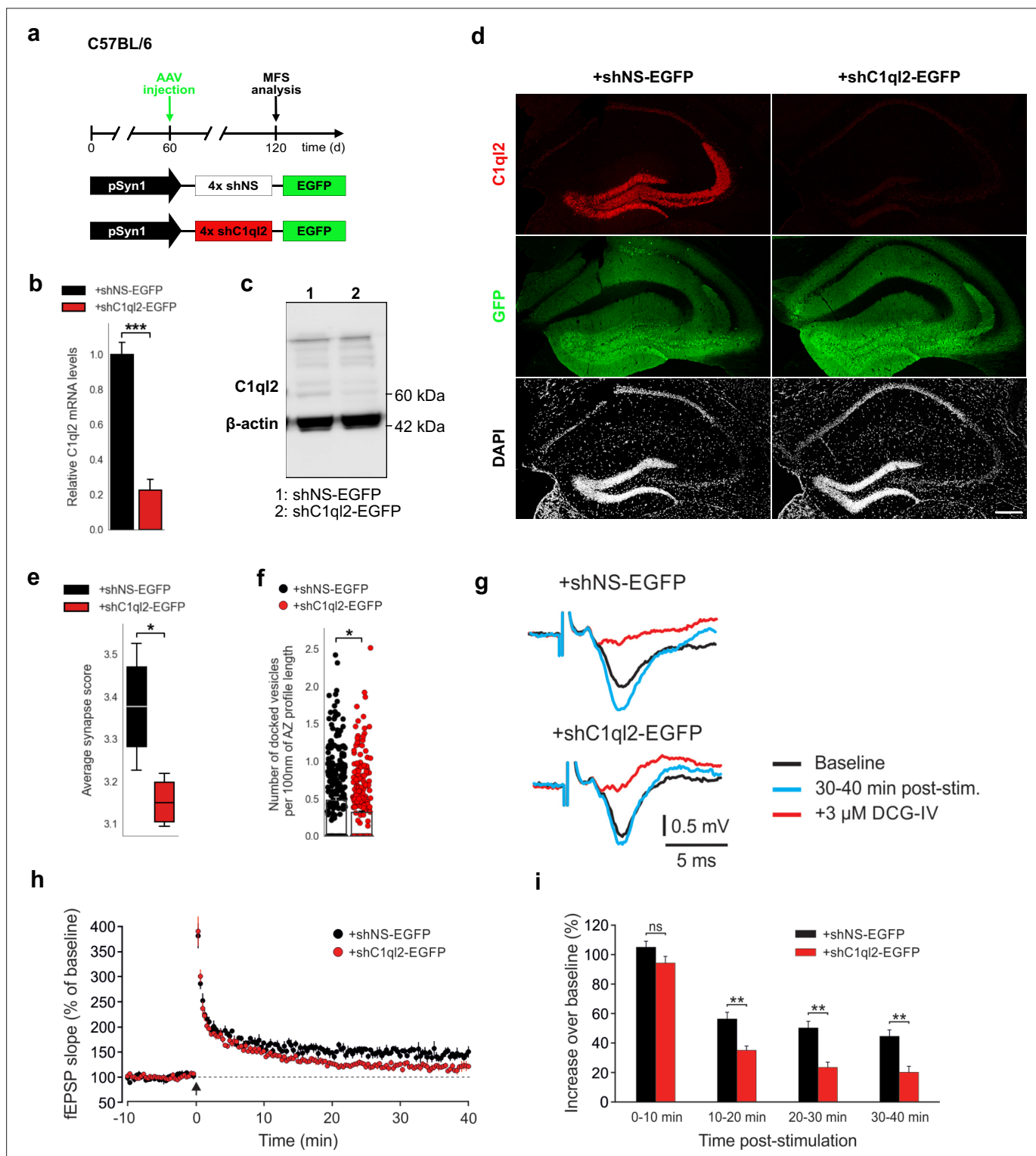


Figure 4. KD of C1q12 in DGN of WT mice impairs SV recruitment and LTP. **(a)** Experimental design to analyze the MFS after AAV-mediated KD of C1q12 in WT DGN. **(b)** Relative C1q12 mRNA levels in DGN. N=4. All data are presented as means; error bars indicate SEM. Unpaired t-test: ***p=0.0002. **(c)** Western blot of mouse hippocampal homogenates. **(d)** Immunohistochemistry of C1q12 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μm. **(e)** Average synapse score. N=4. Unpaired t-test. *p=0.025. **(f)** Number of docked vesicles per 100 nm AZ profile length. N=3. All data are presented as means; error bars indicate SEM. Points represent the individual examined AZ. Unpaired t-test. *p=0.018. **(g)** Representative fEPSP traces showing baselines before HFS (black), fEPSP changes 30–40 min after HFS (cyan) and following the application of 3 μM DCG-IV (red). **(h)** Time course of fEPSP slopes. The black arrow indicates HFS and the dashed line the baseline level. **(i)** Quantification of fEPSP facilitation at four different time intervals

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after HFS. Changes in fEPSP slope are shown as percentage of the mean baseline fEPSP. +shNS EGFP, 6 slices from 6 mice; +shC1ql2-EGFP, 7 slices from 7 mice. All data are presented as means; error bars indicate SEM. Mann-Whitney U-test for each time interval. 10–20 min: ** $p=0.0012$; 20–30 min: ** $p=0.0023$; 30–40 min: ** $p=0.0023$; ns, not significant.

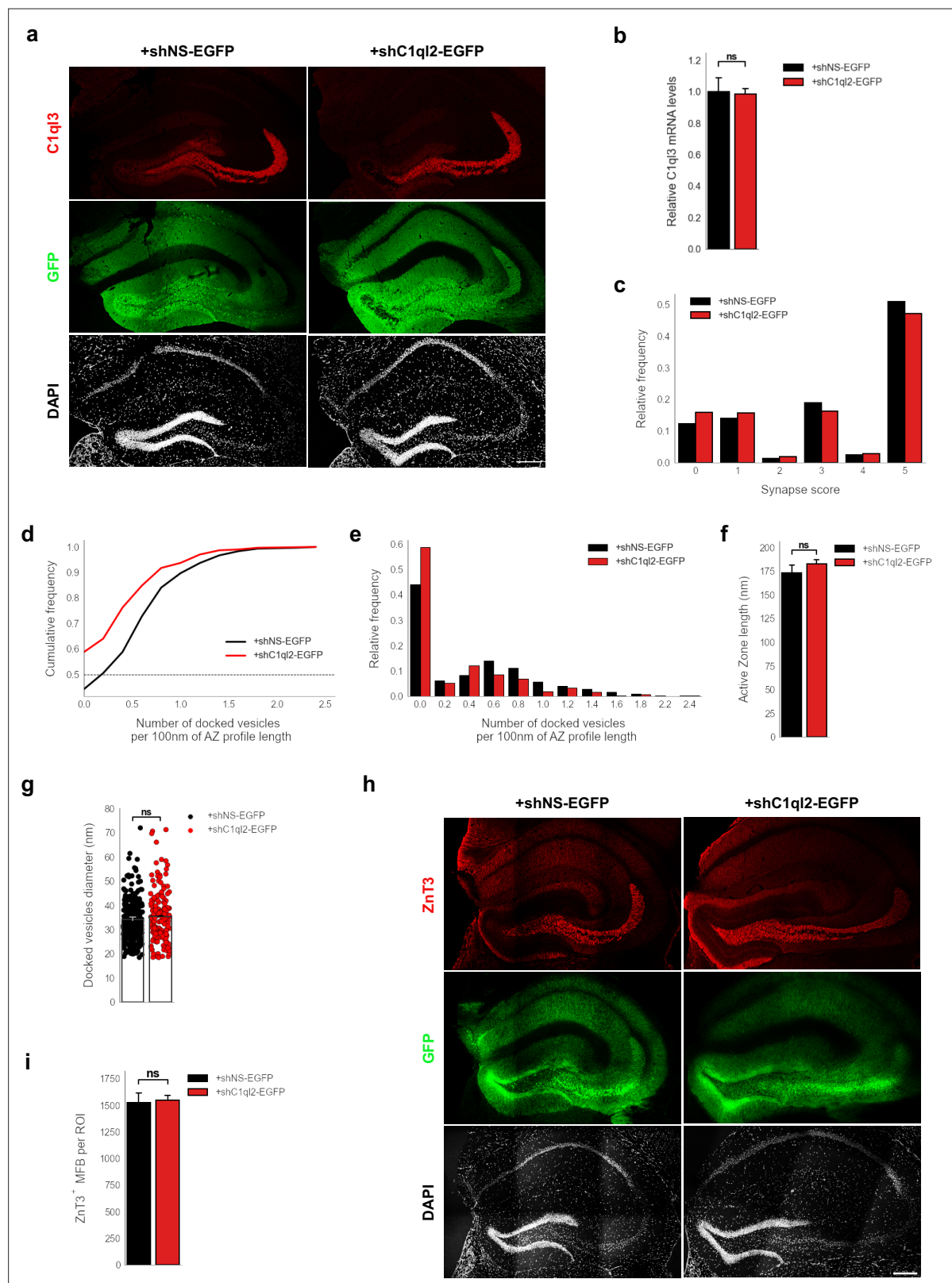


Figure 4—figure supplement 1. *C1ql2* KD in DGN of WT mice impairs SV recruitment. (a) Immunohistochemistry of C1ql3 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μ m. (b) Relative C1ql3 mRNA levels in DGN. N=4. All data are presented as means; error bars indicate SEM. Unpaired t-test. ns, not significant. (c) Relative frequency of synapse scores (refer to **Figure 4e**). (d) Cumulative and (e) relative frequency of the number of docked vesicles per 100 nm AZ profile length (refer to **Figure 4f**). (f) Active zone length. N=3. All data are presented as means; error bars indicate SEM.

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Unpaired t-test. ns, not significant. **(g)** Diameter of the docked vesicles. N=3. All data are presented as means; error bars indicate SEM. Points represent the individual examined SV. Unpaired t-test. ns, not significant. **(h)** Immunohistochemistry of ZnT3 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μ m. **(i)** Number of MFB from selected ROIs in SL of CA3.+shNS-EGFP: N=4;+shC1ql2-EGFP: N=3. All data are presented as means; error bars indicate SEM. Unpaired t-test. ns, not significant.

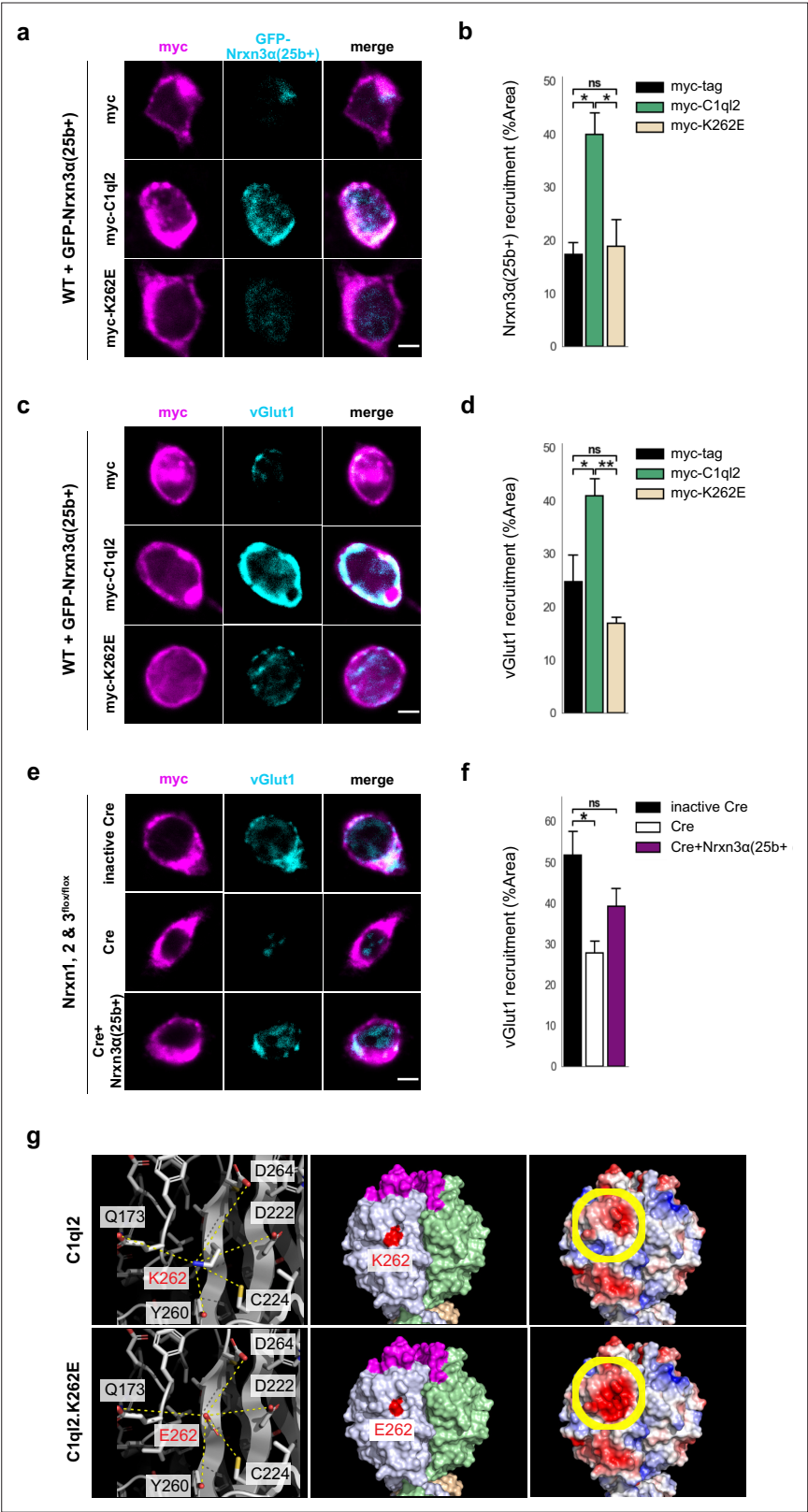


Figure 5. C1ql2-Nrxn3 interaction recruits vGlut1 in vitro. **(a)** Immunocytochemistry of myc-tagged C1ql2, C1ql2-K262E or myc-tag (magenta) expressing HEK293 cells and GFP-Nrxn3α(25b+) (cyan) from contacting hippocampal neurons. Scale bar: 5 μm. **(b)** Nrxn3α(25b+) recruitment by differentially transfected HEK293 cells. N=3. All data are presented as means; error bars indicate SEM. One-way ANOVA and Tuckey's PHC. myc-C1ql2 vs. myc-tag; *Figure 5 continued on next page*

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* $p=0.016$, and vs. myc-K262E: * $p=0.022$; ns, not significant. (c) Immunocytochemistry of myc-tagged C1ql2, C1ql2.K262E or myc-tag (magenta) expressing HEK293 cells and vGlut1 (cyan) from contacting hippocampal neurons. Scale bar: 5 μm . (d) vGlut1 recruitment by differentially transfected HEK293 cells. $N=3$. All data are presented as means; error bars indicate SEM. One-way ANOVA and Tuckey's PHC. myc-C1ql2 vs. myc-tag: * $p=0.04$, and vs. myc-K262E: ** $p=0.007$; ns, not significant. (e) Immunocytochemistry of myc-tagged C1ql2 (magenta) expressing HEK293 cells and vGlut1 (cyan) from contacting control, *Nrxn123* KO or *Nrxn123* KO with *Nrxn3 α (25+)* rescued hippocampal neurons. Scale bar: 5 μm . (f) vGlut1 recruitment by HEK293 cells in presence or absence of neuronal *Nrxns*. $N=3$. All data are presented as means; error bars indicate SEM. One-way ANOVA and Tuckey's PHC. inactive Cre vs. Cre: * $p=0.023$, and vs. Cre +*Nrxn3 α (25+)*: $p=0.21$; ns, not significant. (g) Trimeric structures of C1ql2 (PDB_ID: 4QPY, upper panels) and the variant C1ql2.K262E (lower panels). Residue 262 is the central residue (red, left and middle panels) of a larger area underneath the C1ql2-specific calcium and receptor binding loops (magenta, middle panel). The mutation K262E alters the charge of that surface area negative (yellow-circled area, right panels) and makes it potentially repulsive to bind *Nrxn3(25b+)*.

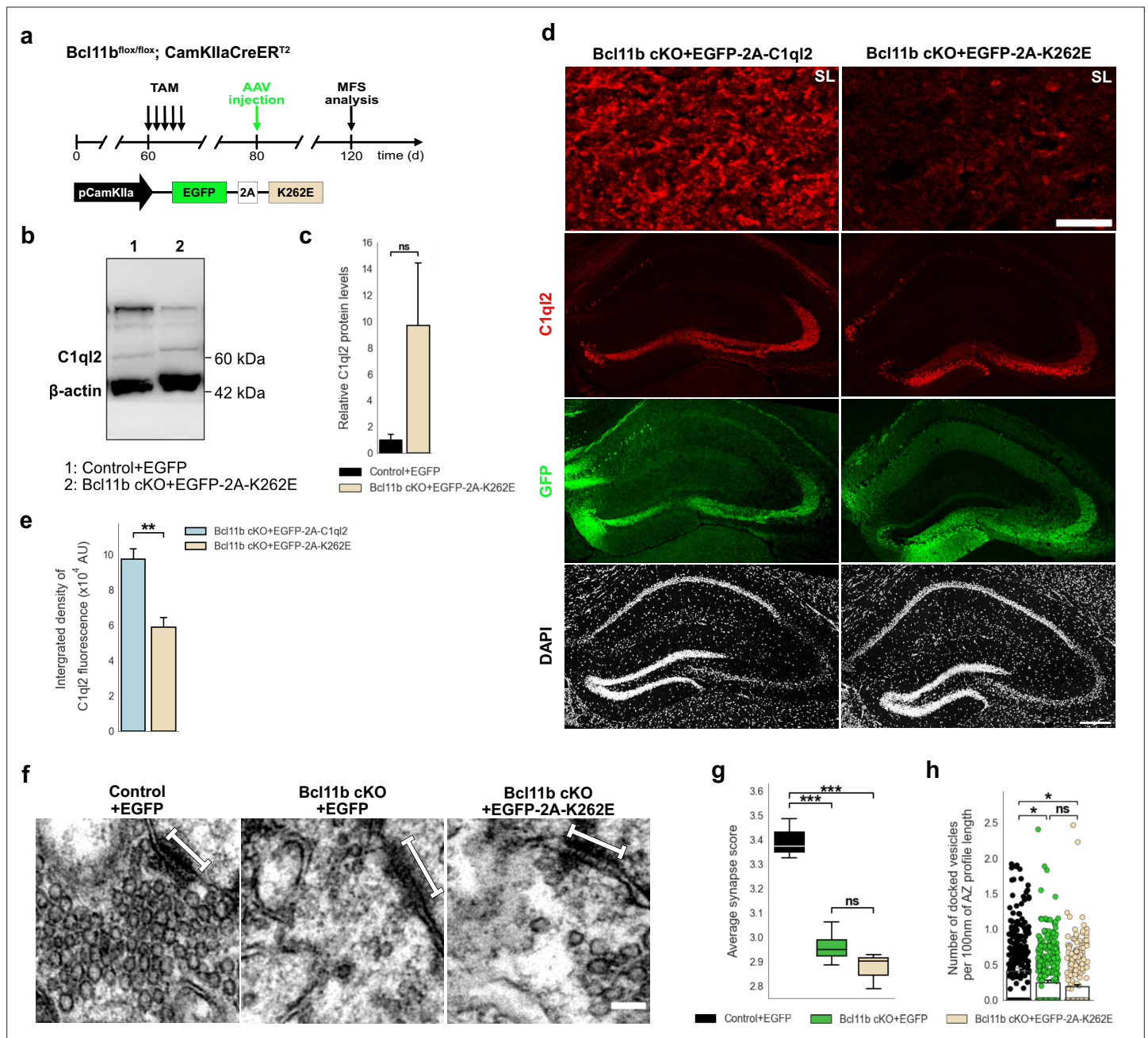


Figure 6. C1ql2-Nrxn3(25b+) interaction is important for C1ql2 localization at the MFS and SV recruitment. **(a)** Experimental design to analyze the MFS after AAV-mediated expression of C1ql2.K262E in *Bcl11b* cKO DGN. **(b)** Western blot and **(c)** relative C1ql2.K262E protein levels in mouse hippocampal homogenates. N=3. All data are presented as means; error bars indicate SEM. Mann-Whitney U-test. ns, not significant. **(d)** Immunohistochemistry of C1ql2 (red) and GFP (green) in hippocampal sections. Scale bar: 200 μ m. Upper panels depict close-ups of C1ql2 staining from the SL of CA3. Scale bar: 15 μ m. **(e)** Integrated density of C1ql2 fluorescence in the SL of CA3. N=3. All data are presented as means; error bars indicate SEM. Unpaired t-test. * $p=0.008$. **(f)** Electron microscope images of MFS and proximal SV. White bars mark synapse length from postsynaptic side. Scale bar: 100 nm. **(g)** Average synapse score. Control +EGFP, *Bcl11b* cKO +EGFP-2A-K262E: N=3; *Bcl11b* cKO +EGFP: N=4. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. *Bcl11b* cKO +EGFP: *** $p=0.0004$, and vs. *Bcl11b* cKO +EGFP-2A-K262E: *** $p=0.0002$; ns, not significant. **(h)** Number of docked vesicles per 100 nm AZ profile length. Control +EGFP, *Bcl11b* cKO +EGFP-2A-K262E: N=3; *Bcl11b* cKO +EGFP: N=4. All data are presented as means; error bars indicate SEM. Points represent the individual examined AZ. Two-way ANOVA and Tukey's PHC. Control +EGFP vs. *Bcl11b* cKO +EGFP: * $P=0.0434$, and vs. *Bcl11b* cKO +EGFP-2A-K262E: * $p=0.0196$; ns, not significant. Data for Control +EGFP and *Bcl11b* cKO +EGFP-2A-K262E from f-h in this figure are compared with *Bcl11b* cKO +EGFP data from **Figure 2**.

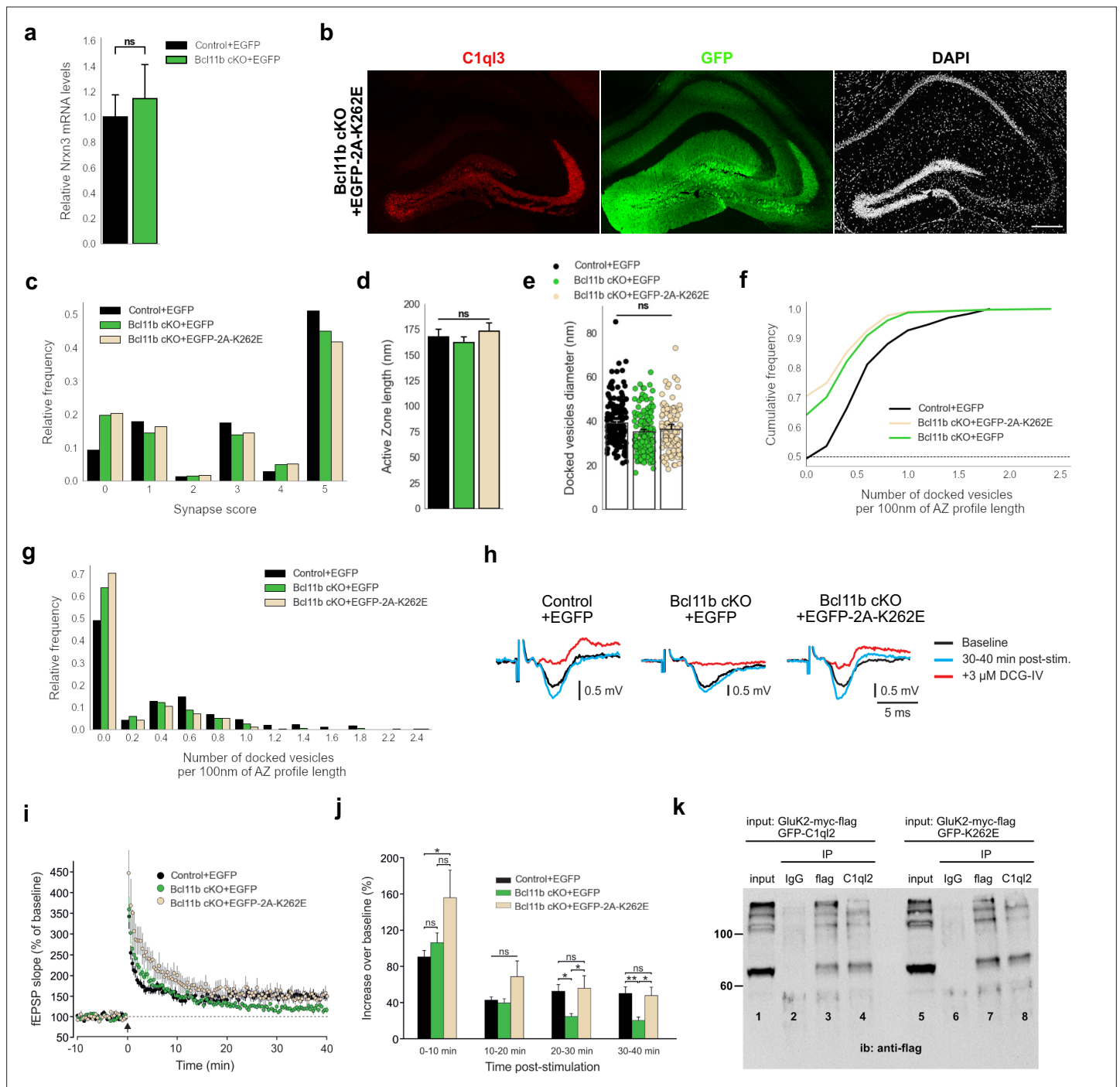


Figure 6—figure supplement 1. C1ql2-Nrxn3(25b+) interaction is important for SV recruitment at the MFS. (a) Relative *Nrxn3* mRNA levels in DGN. N=3. All data are presented as means; error bars indicate SEM. Unpaired t-test. ns, not significant. (b) Immunohistochemistry for C1ql3 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μ m. (c) Relative frequency of synapse scores (refer to **Figure 6g**). (d) Active zone length. Control +EGFP, Bcl11b cKO +EGFP-2A-K262E: N=3; Bcl11b cKO +EGFP: N=4. All data are presented as means; error bars indicate SEM. Two-way ANOVA. ns, not significant. (e) Diameter of the docked vesicles. Control +EGFP, Bcl11b cKO +EGFP-2A-K262E: N=3; Bcl11b cKO +EGFP: N=4. All data are presented as means; error bars indicate SEM. Points represent the individual examined SV. Two-way ANOVA. ns, not significant. Data for Control +EGFP and Bcl11b cKO +EGFP-2A-K262E from b-f in this figure are compared with Bcl11b cKO +EGFP data from **Figure 2**. (f) Cumulative and (g) relative frequency of the number of docked vesicles per 100 nm AZ profile length (refer to **Figure 6h**). (h) Representative fEPSP traces showing baselines before HFS (black), fEPSP changes 30–40 min after HFS (cyan) and following the application of 3 μ M DCG-IV (red). (i) Time course of fEPSP slopes. The black arrow indicates HFS and the dashed line is the baseline level. (j) Quantification of fEPSP facilitation at four different time intervals after HFS. Changes in the fEPSP slope are shown as the percentage of the mean baseline fEPSP. Data for C1ql2.K262E from f-h in this figure are compared with control and Bcl11b

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cKO data from **Figure 3a–c**. Control +EGFP, 7 slices from 6 mice; Bcl11b cKO +EGFP, 8 slices from 5 mice; Bcl11b cKO +EGFP-2A-K262E, 5 slices from 4 mice. All data are presented as means; error bars indicate SEM. One-way ANOVA followed by Bonferroni's PHC for each time interval. 0–10 min: Control +EGFP vs. Bcl11b cKO +EGFP-2A-K262E: * $p=0.037$; 20–30 min: Control +EGFP vs. Bcl11b cKO +EGFP: * $p=0.047$; Bcl11b cKO +EGFP vs. Bcl11b cKO +EGFP-2A-K262E: * $p=0.042$; 30–40 min: Control +EGFP vs. Bcl11b cKO +EGFP: ** $p=0.009$; Bcl11b cKO +EGFP vs. Bcl11b cKO +EGFP-2A-K262E: * $p=0.031$; ns, not significant. **(k)** Western blot analysis with flag-tag antibody upon co-immunoprecipitation of HEK293 cells protein extract expressing GluK2-myc-flag and GFP-C1ql2 (lanes 1,2,3, and 4) or GluK2-myc-flag and GFP-K262E (lanes 5,6,7, and 8). Protein extract was precipitated with magnetic beads coupled with anti-flag antibody (lanes 3 and 7) or anti-C1ql2 antibody (lanes 4 and 8). Anti-IgG antibody was used as negative. Expression of GluK2 was verified using protein lysate as input for the co-immunoprecipitation (lanes 1 and 5).

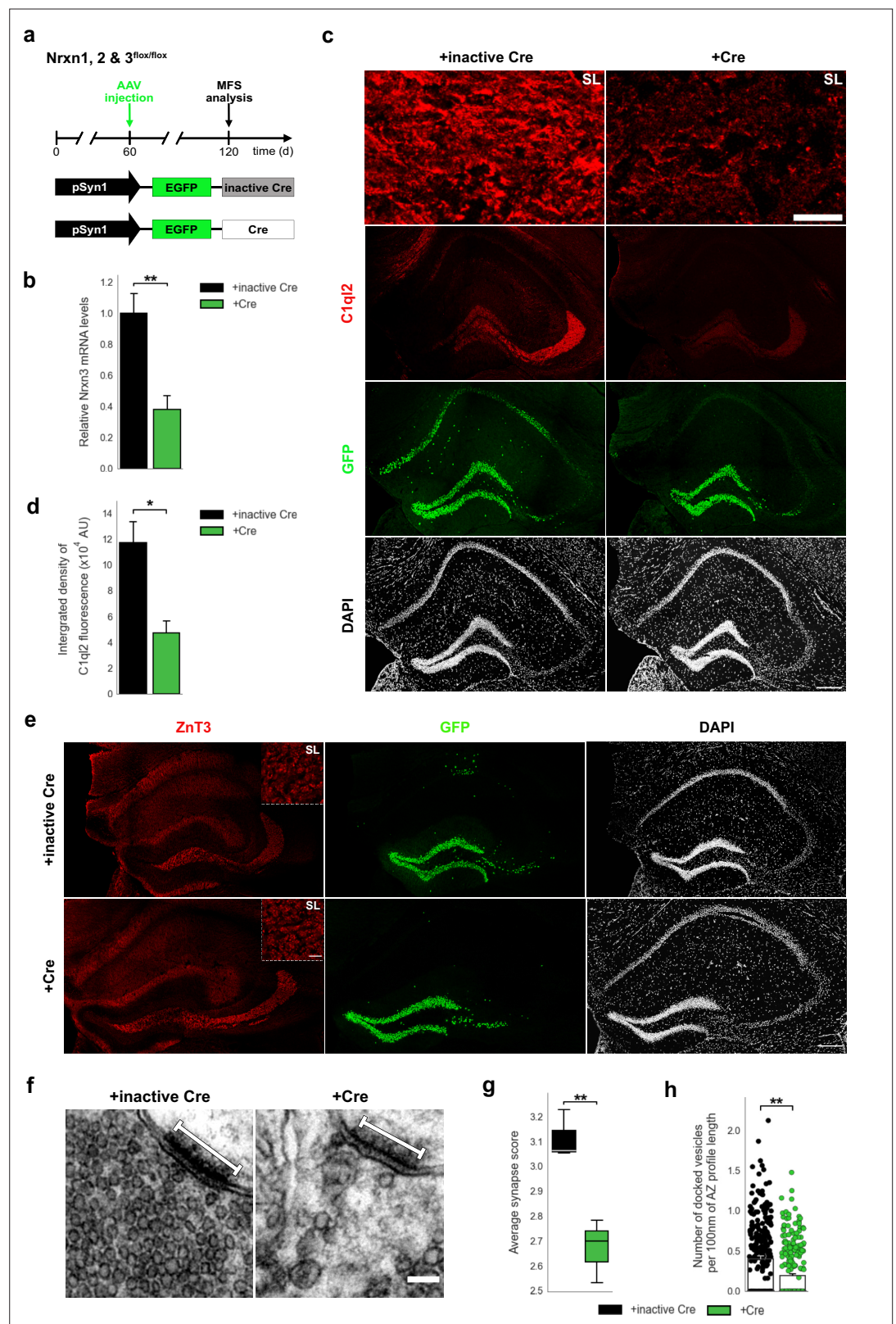


Figure 7. *Nrxn* KO perturbs C1ql2 localization at the MFS and SV recruitment. **(a)** Experimental design to analyze the MFS after AAV-mediated *Nrxn* KO. **(b)** Relative *Nrxn3* mRNA levels. $N=4$. All data are presented as means; error bars indicate SEM. Unpaired t-test. $**p=0.007$. **(c)** Immunohistochemistry of C1ql2 (red) and GFP (green) in hippocampal sections. Scale bar: 200 μ m. Upper panels depict close-ups of C1ql2 staining from the SL of CA3.

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Scale bar: 15 μm . **(d)** Integrated density of C1ql2 fluorescence in the SL of CA3. N=3. All data are presented as means; error bars indicate SEM. Unpaired t-test. * $p=0.02$. **(e)** Immunohistochemistry of ZnT3 (red) and GFP (green) in hippocampal sections. Scale bar: 200 μm . Upper right corner of ZnT3 panels depicts close-ups from the SL of CA3. Scale bar: 15 μm . **(f)** Electron microscope images of MFS and proximal SVs. White bars mark synapse length from postsynaptic side. Scale bar: 100 nm. **(g)** Average synapse score. N=3. Unpaired t-test. ** $p=0.009$. **(h)** Number of docked vesicles per 100 nm AZ profile length. N=3. All data are presented as means; error bars indicate SEM. Points represent the individual examined AZ. Unpaired t-test. ** $p=0.007$.

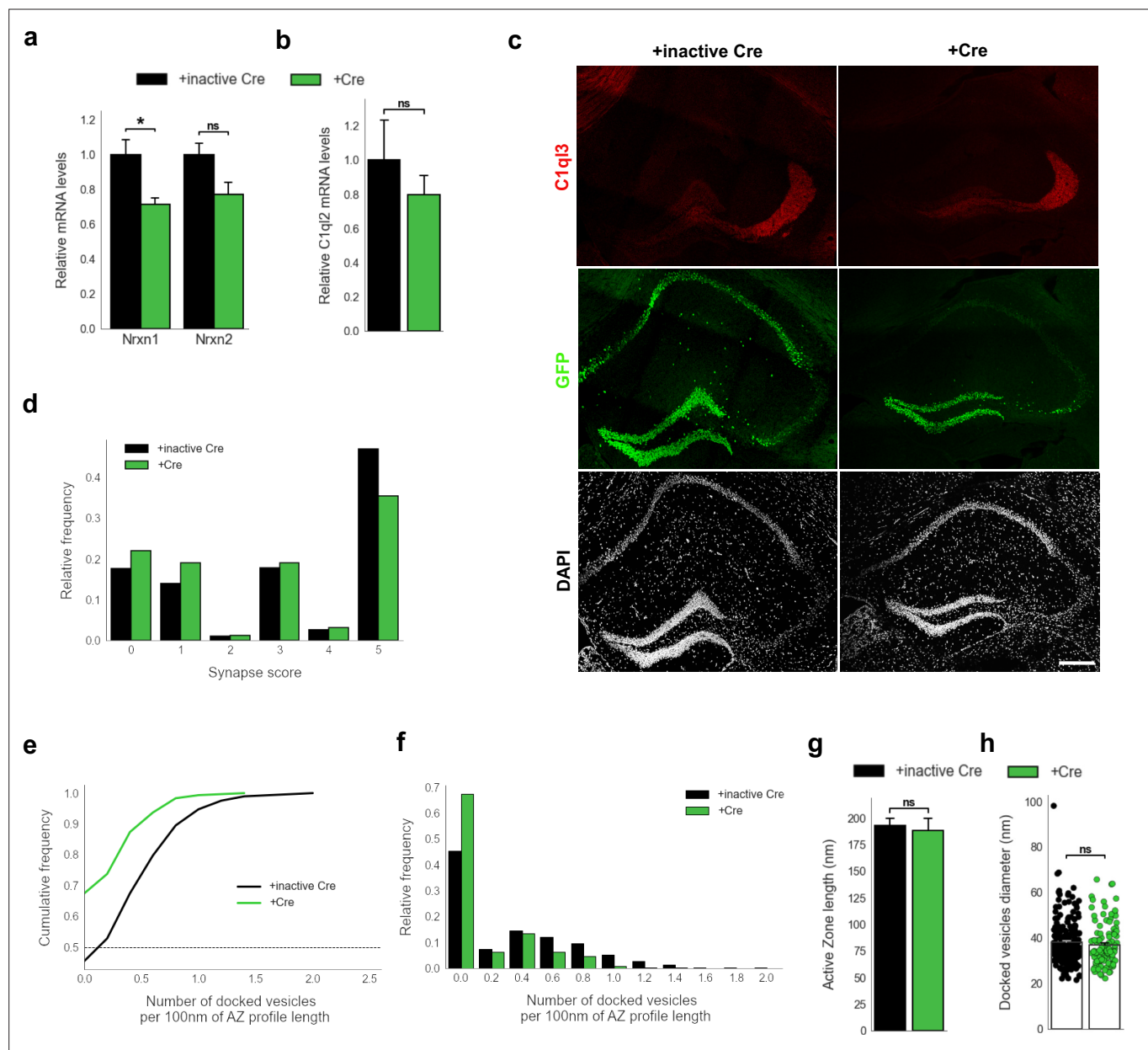


Figure 7—figure supplement 1. *Nrxn* KO perturbs SV recruitment at the MFS. (a) Relative *Nrxn1* and *Nrxn2* mRNA levels in DGN. N=4. All data are presented as means; error bars indicate SEM. Unpaired t-test. *=0.02; ns, not significant. (b) Relative *C1q2* mRNA levels in DGN. N=4. All data are presented as means; error bars indicate SEM. Unpaired t-test. ns, not significant. (c) Immunohistochemistry for C1q3 (red) and GFP (green) on hippocampal sections. Scale bar: 200 μ m. (d) Relative frequency of synapse scores (refer to **Figure 7g**). (e) Cumulative and (f) relative frequency of the number of docked vesicles per 100 nm AZ profile length (refer to **Figure 7h**). (g) Active zone length. N=3. All data are presented as means; error bars indicate SEM. Unpaired t-test. ns, not significant. (h) Diameter of the docked vesicles. N=3. All data are presented as means; error bars indicate SEM. Points represent the individual examined SV. Unpaired t-test. ns, not significant.