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851 **Extended Data:**

852 **1. Supplemental Figures S1-S5**

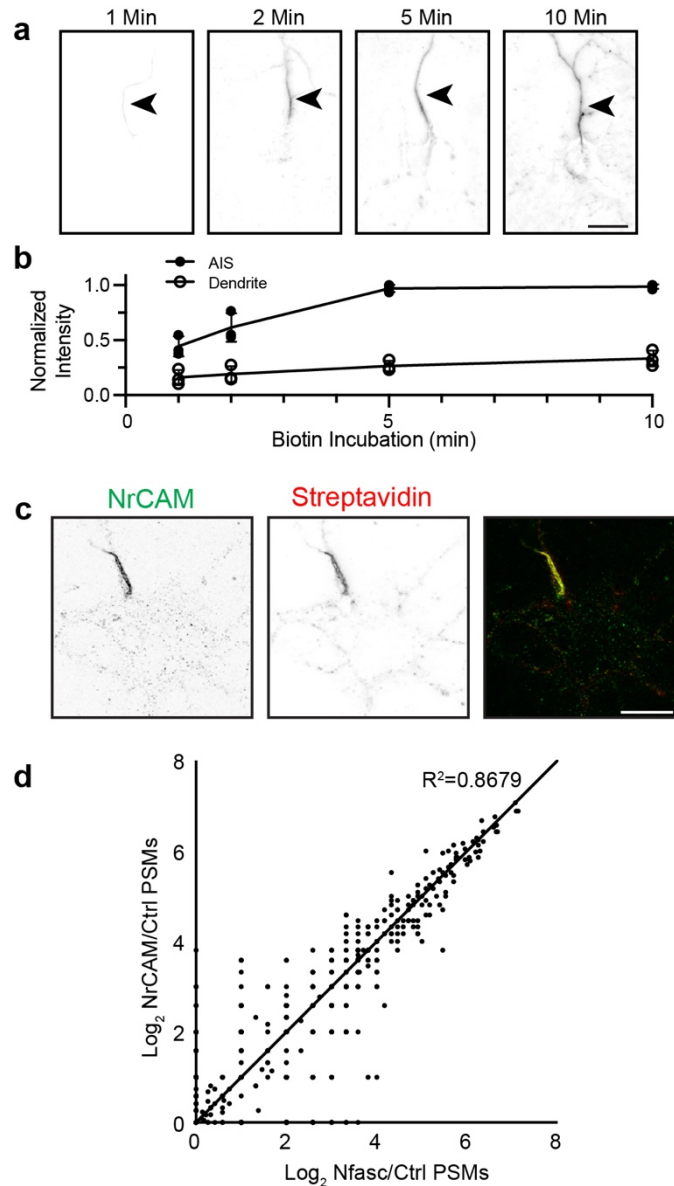
853 **2. Supplemental extended materials file**, detailed listing of all reagents, plasmids, gRNA
854 sequences, antibodies, sources, etc.

855 **3. Supplemental Table 1**, Nfasc-BAR and NrCAM-BAR proximity proteomes comparison
856 (included as excel file)

857 **4. Supplemental Table 2**, Nfasc-BAR proximity proteomes at DIV 4, 7, 14, 21, and 28
858 (included as excel file).

859 **5. Supplemental Table 3**, Nfasc-BAR fold change and p-values for all identified proteins
860 (included as excel file).

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862 **Figure S1. BAR-Nfasc and BAR-NrCAM are highly concordant. a, b,** Biotinylation of the AIS

863 (arrowhead) as a function of biotin-tyramide incubation time. N = 3 independent experiments.

864 Error bars, \pm SEM. Scale bar, 20 μ m. **c,** Fluorescence imaging of DIV14 rat hippocampal neurons

865 labeled by NrCAM-BAR. NrCAM immunofluorescence (green) enrichment defines the AIS.

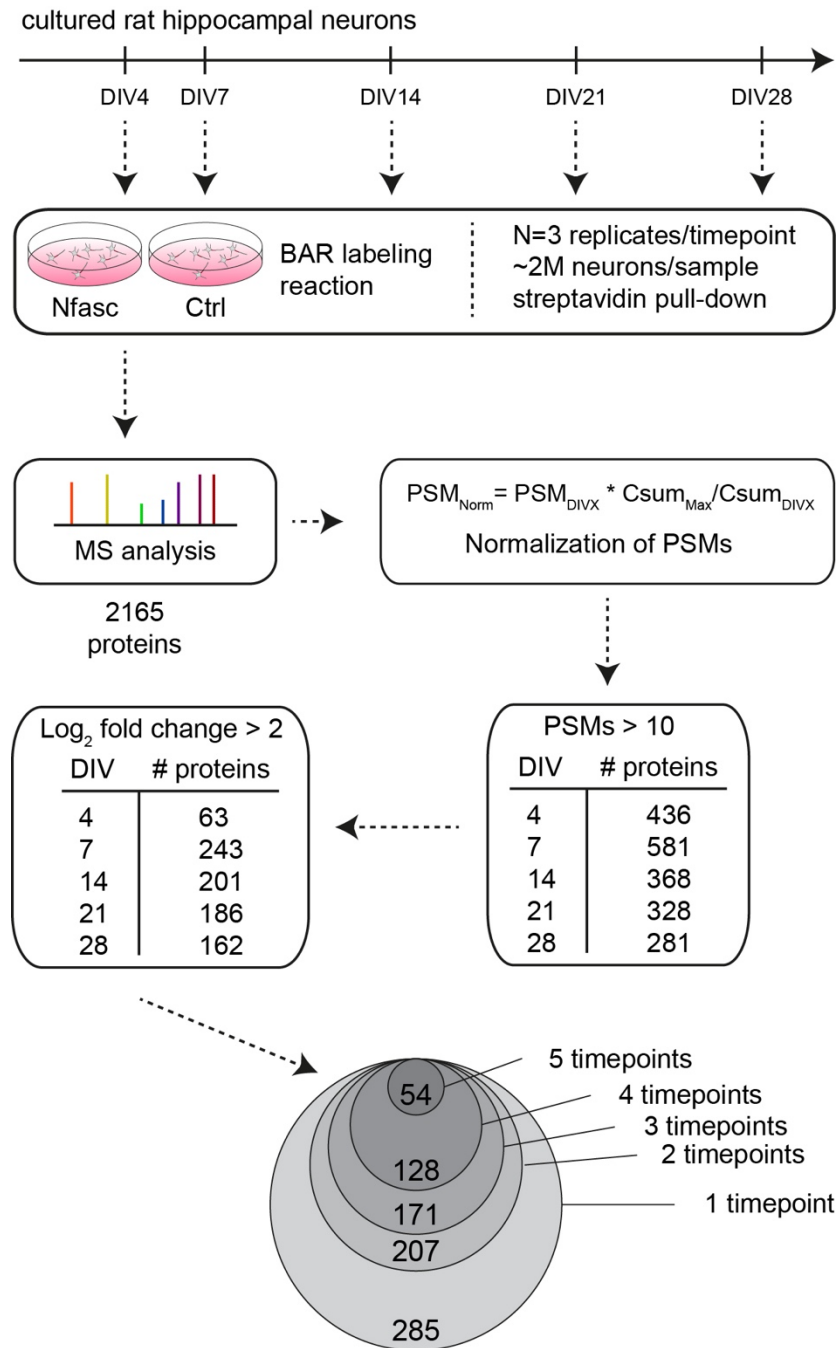
866 Biotinylated proteins were detected using Alexa594-conjugated streptavidin. Scale bar, 20 μ m.

867 **d,** Scatter plot of the number of peptide spectral matches (PSMs) for each biotinylated protein

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868 identified by mass spectrometry using either Nfasc-BAR or NrCAM-BAR. Data were highly
869 concordant since most proteins identified fell on or close to the solid line representing equal
870 enrichment in both BAR conditions.
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873 **Figure S2. Workflow of Nfasc-BAR labeling and normalization of PSMs.** Nfasc-BAR labeling was

874 performed in triplicate for each of five timepoints. Mass spectrometry analysis resulted in a

875 dataset of 2165 proteins. To compare across timepoints, PSMs were normalized using the

876 formula $\text{PSM}_{i, \text{Norm}, \text{DivX}} = \text{PSM}_{i, \text{DivX}} * \text{SPSM}_{\text{Carboxylases}}^{\text{DivY}} / \text{SPSM}_{\text{Carboxylases}}^{\text{DivX}}$, where *i* is a particular

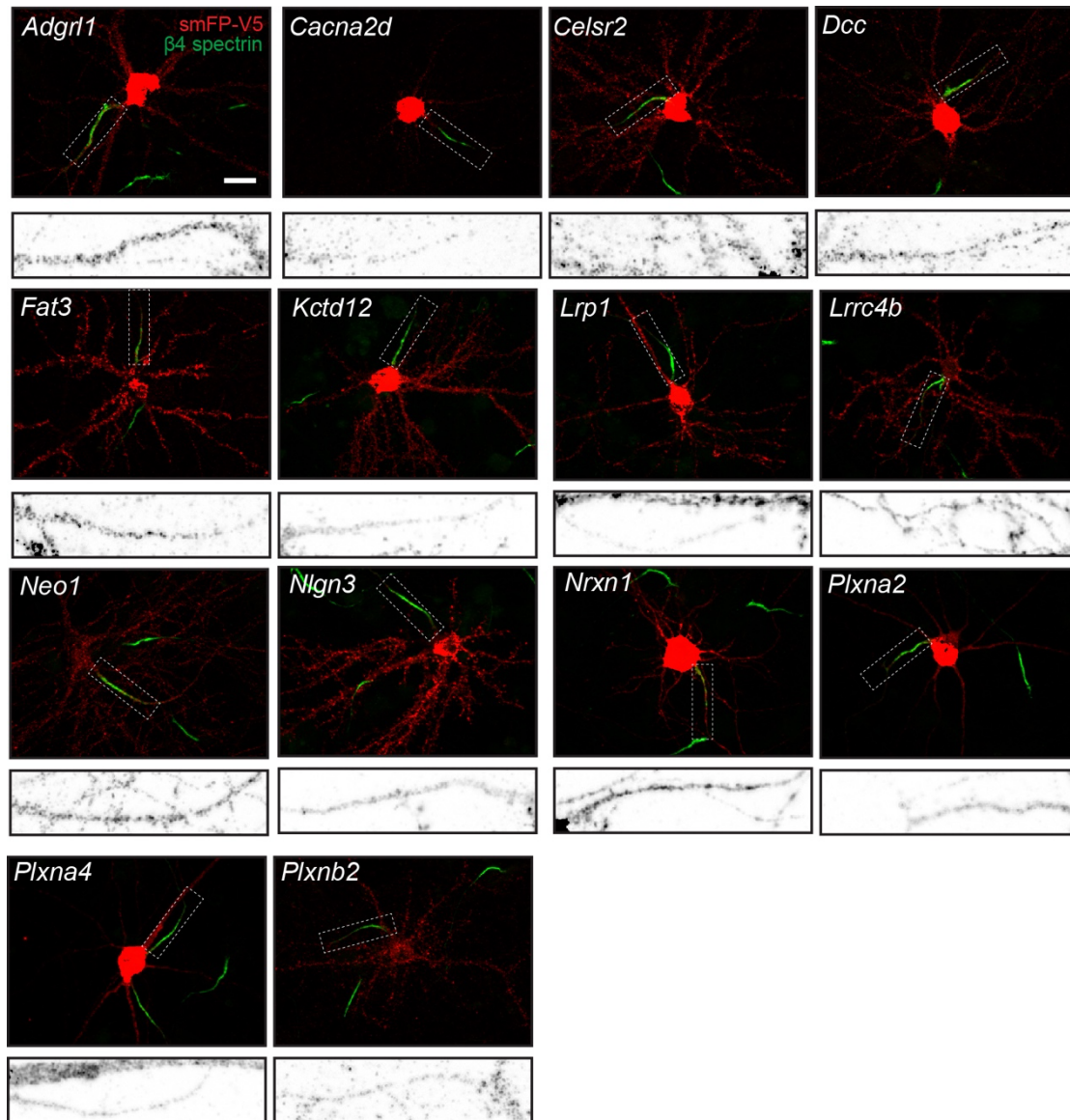
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877 protein on the set, X is a Div time point, Y is the Div time point with the maximum value for
878 summed carboxylase PSMs. Candidates were distinguished from background by filtering based
879 on the number of PSMs identified and the fold change. Tables show the number of proteins
880 that satisfied one or both criteria at a given timepoint. Circles show the number of proteins that
881 satisfied both criteria for 1-5 timepoints.
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884 **Figure S3. Fold-enrichment for the top 100 most enriched proteins identified using Nfasc-BAR**
885 **during *in vitro* neuron development. a-e, Log₂ fold-change (log₂(FC)) for the top 100 most**
886 **enriched proteins identified at DIV 4 (a), 7 (b), 14 (c), 21 (d) and 28 (e). Known AIS proteins are**
887 **indicated in red. Error bars, ±SEM.**
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892 **Figure S4. Tagging of endogenous membrane proteins.** Examples of smFP-V5 tagged

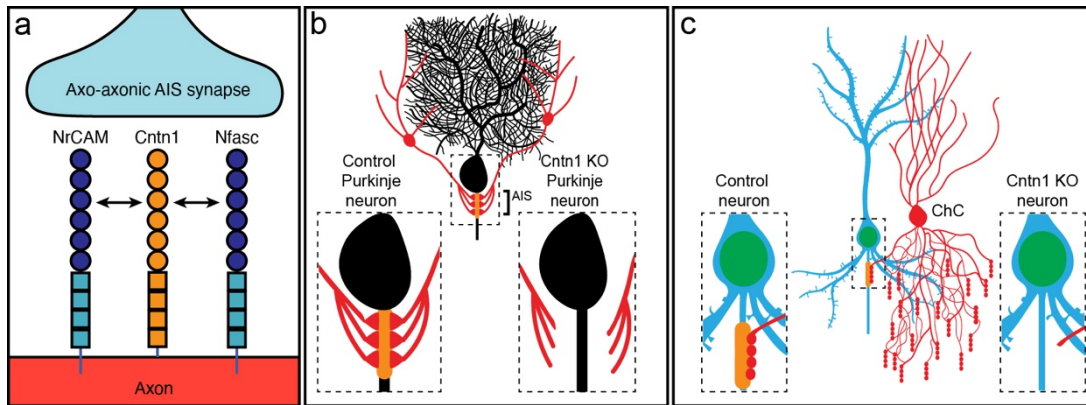
893 proteins (red). AIS are labeled for $\beta 4$ spectrin (green). The inset boxed regions

894 correspond to the tagged protein at the AIS. Scale bar, 20 μm .

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899 **Figure S5. Summary of results.** a, Cntn1 interacts with and is redundantly recruited to the AIS
900 through interactions with both Nrcam and Nfasc. b, Loss of AIS Cntn1 (orange at AIS) from
901 cerebellar Purkinje neurons disrupts basket cell innervation of the AIS and formation of pinneau
902 synapses. c, Loss of AIS Cntn1 (orange) from Pyramidal neurons results in reduced innervation
903 of AIS by Chandelier cells (ChC) and reduced numbers of AIS inhibitory synapses.

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