Supplementary Information and Methods

BONLAC: A Combinatorial Proteomic Technique to Measure Translational Profiles in Rodent Brain Slices in Response to Acute Stimuli

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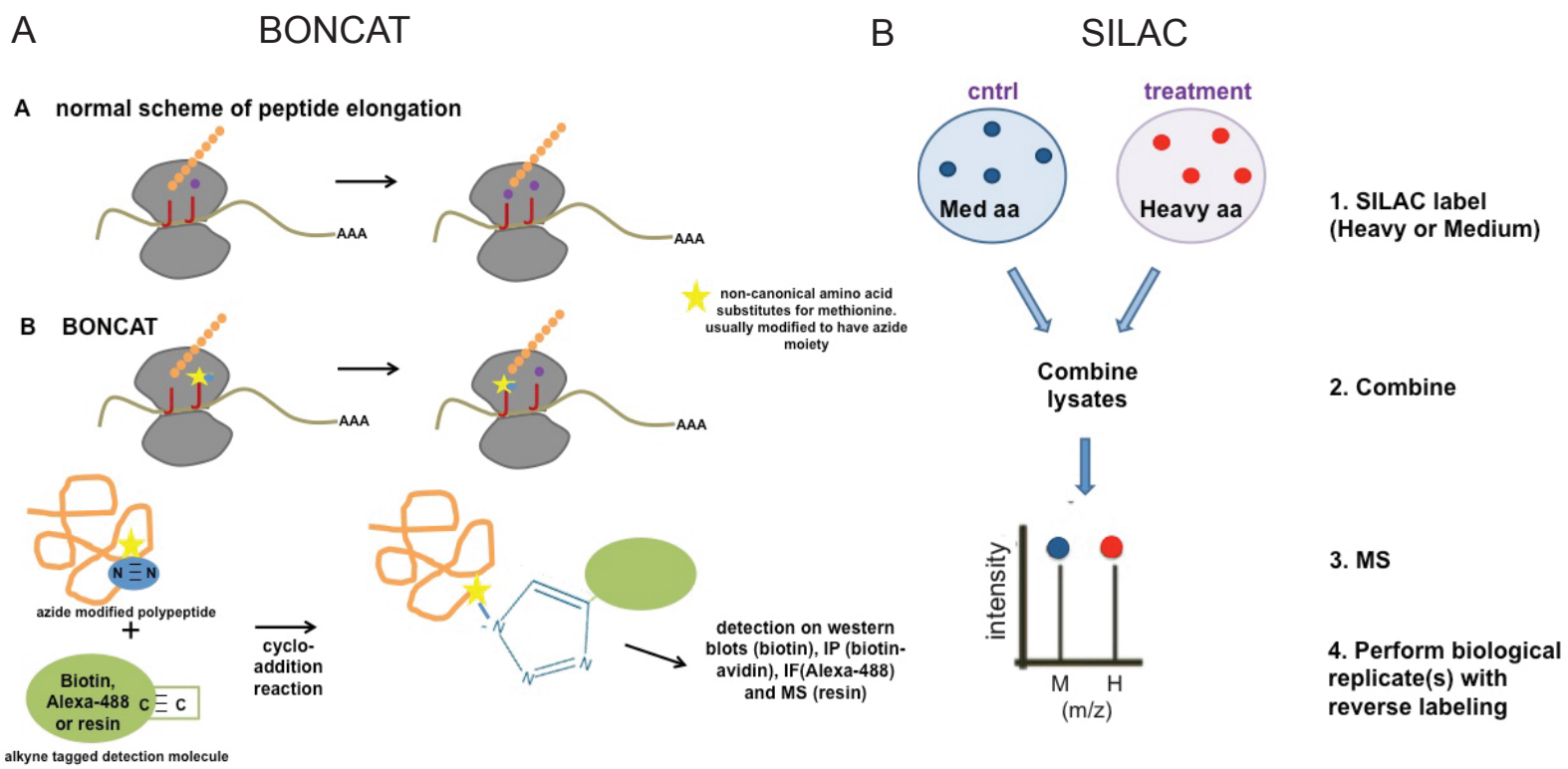
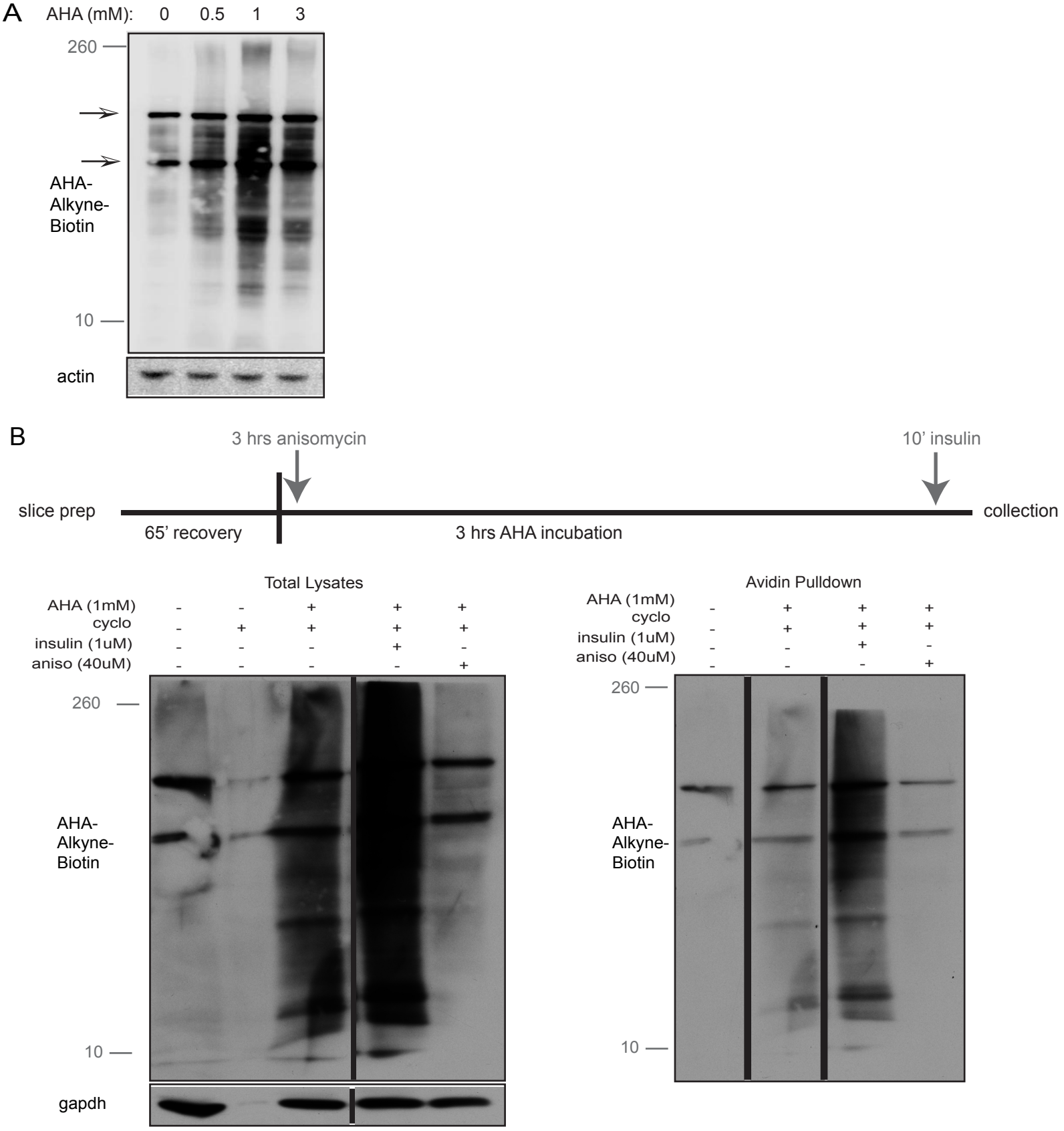
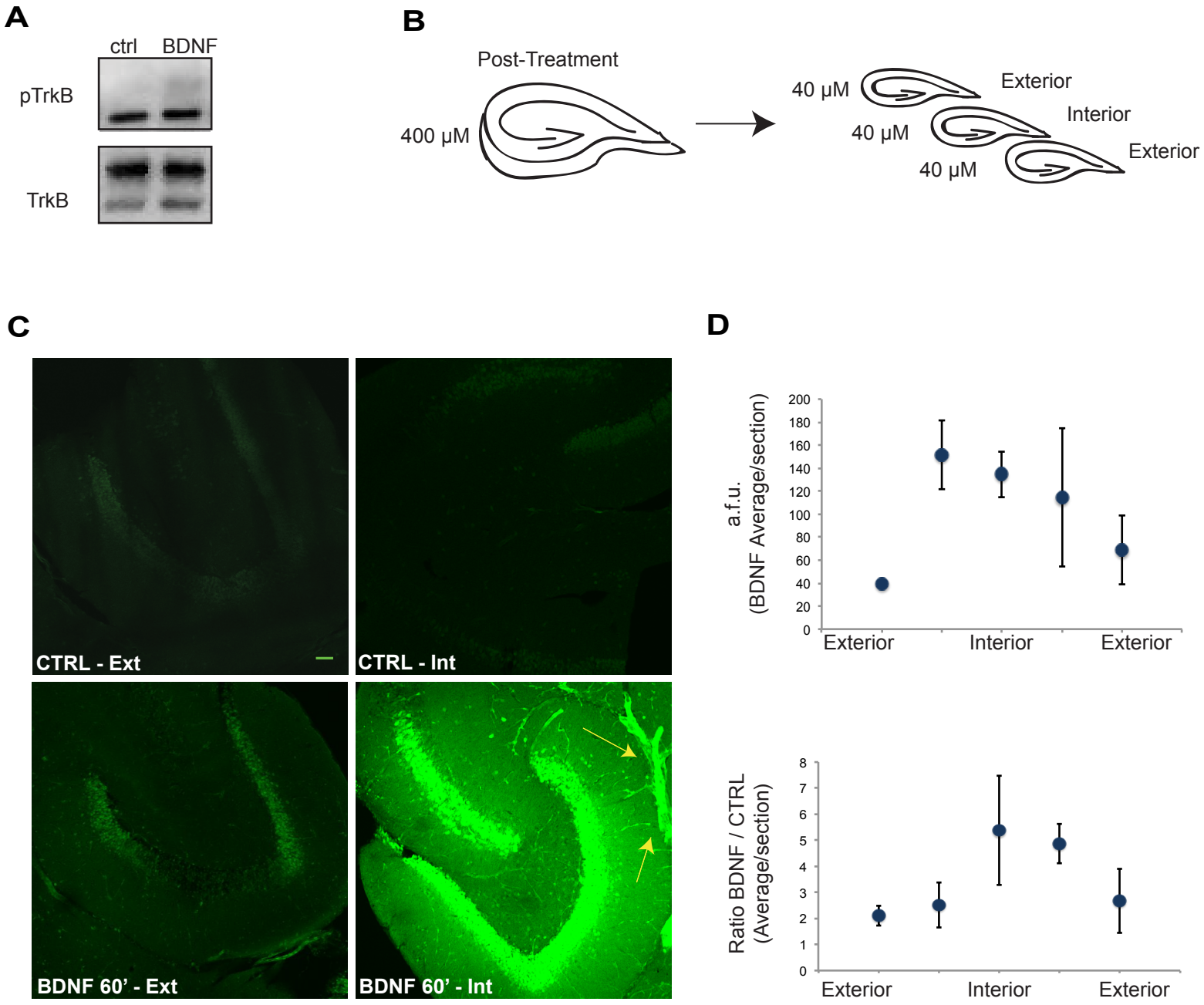
Figures (with Legends):



Supplementary Figure 1. Detailed schema of BONLAC and SILAC. A) In BONCAT, cells/neurons/slices are fed AHA (denoted by a star), a noncanonical amino acid with an azide tag that substitutes methionine during protein synthesis. After labeling for the desired period of time, cells/neurons/slices can be fixed or lysed and the azide can be covalently conjugated to a biotin, fluorophore, or alkyne resin for detection of nascent proteins by western blot, microscopy (IF), or mass spectrometry (MS). B) In SILAC, neurons are fed isotopically labeled heavy or medium amino acids which are incorporated into nascent proteins. After treatment, the lysates of the two treatment groups can be combined and jointly prepared for mass spectrometry where differences in the treatment group abundance can be quantified based on the ration of medium to heavy peptides. There are limitations on detection for SILAC in post-mitotic cells, with neurons in particular requiring enrichment of nascent proteins for incubation periods less than 48 hours[23].



Supplementary Figure S2.Dose finding and validation of BONCAT in slices using adapted method A) Dose response curve to standardize AHA labeling in wild-type hippocampal slices. n=3 slices per dose, 3 mice each.White arrows represent pre-existing proteins conjugated to biotin. They do not change in abundance with treatment or dose of AHA. B) BONCAT signal in slices is sensitive to changes in levels of protein synthesis. Top panel is a schematic showing the timeline for the experiment. Slice were obtained, left to recover, then treated were not treated, treated with 1mM AHA, AHA for 2 hours and 50 minutes, followed by a10 minutes stimulation of Insulin, or concurrently with AHA and anisomycin. Left panel shows AHA-alkyne signal in total lysates after the indicated treatments. We also performed a control for the specifity of cycloaddition "cyclo" where samples treated without AHA underwent the cycloaddtion protocol. Right panel shows the same samples after affinity precipitation using avidin beads to enrich for AHA labelled peptides. n=4-5 slices, 2 mice each.



Supplementary Figure S3. Analysis of BDNF's effect on acute adult rodent hippocampal slices A) Acute adult hippocampal slices were treated with 25 ng/ml BDNF for 1 hour, lysed, sonicated and processed by western blot. Blots were probed with phospho- and total TrkB to assess BDNF-mediated activation. n=5-6 independent experiments. B) Schematic representation of serial sectioning of 400 µm slices into 40 µm sections to test penetration of AHA and subsequent efficiency of the FUNCAT reaction. C) Representative images of interior and exterior of the DG of hippocampal slices to ascertain the penetration of AHA and the maximal translational response to BDNF. Scale bar, 50µm. Non-neuronal components noted with yellow arrows. D) Quantification of increase in AHA-alkyne signal from exterior to interior of 400 µm slices in the BDNF-treated slices (top) and comparing BDNF to control-treated slices as a ratio (bottom). Three random ROIs of 112 pixels x 112 pixels were chosen in each slice and integrated intensity measured in ImageJ. These ROI were transferred to each successive 40 µm serial section and averaged per section. For comparison of BDNF/CTRL the brightest exterior slices were matched with each other, then each subsequent slice was compared. n= 3-4 independent 400 µm slices, for CTRL and BDNF. Average ± SEM shown.

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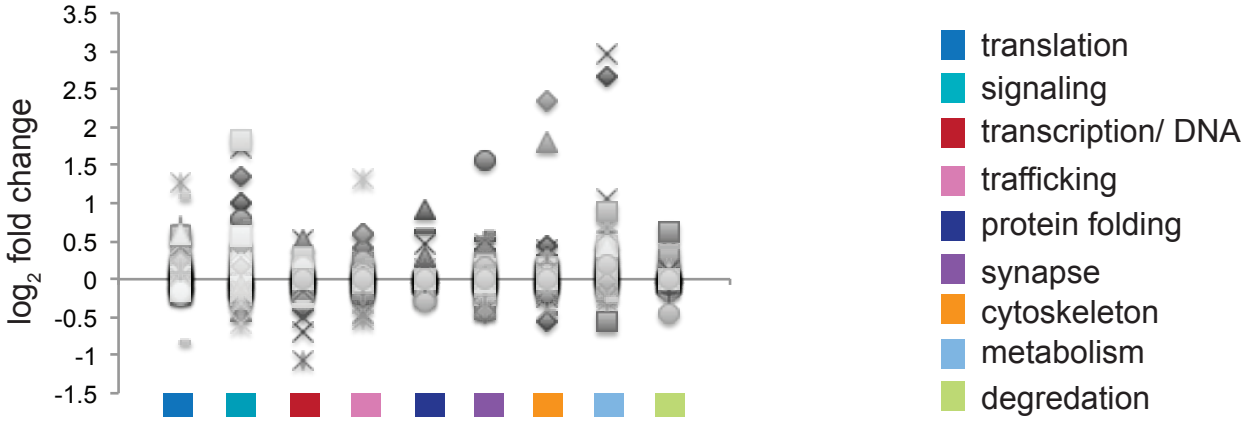
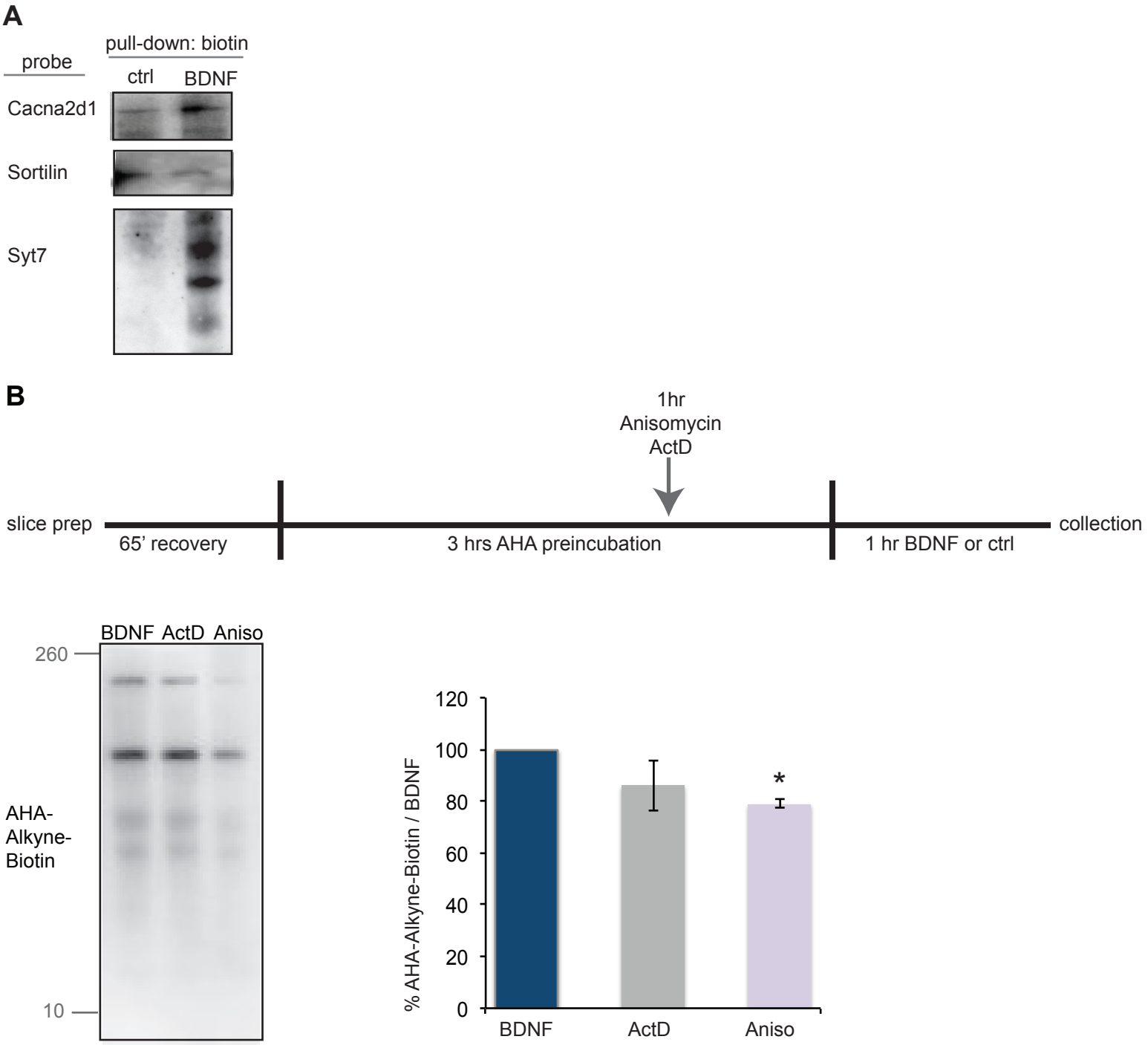


Supplementary Figure S4: Functional classification of the protein hits from all runs of the slice BONLAC screen separated into functional categories. Protein IDs were queried on the UNIPROT database and functional classification based on broad categories listed on the right. Each point represents a unique protein hit plotted for its average relative log2 fold change in all runs and each column represents one functional category. The symbols do not denote a specific classification. There is no specific enrichment for one protein class over another by BONLAC.



Supplementary Figure S5:Confirmation of BONLAC candidates by nascent protein isolation and dissecting BDNF-induced signaling changes that affect protein synthesis A) Representative western blot of biotin-avidin pulldown of BDNF-treated slices versus controls, to detect differences in levels of de novo synthesized Cacna2d1 and Syt7 increased with BDNF treatment and Sort1 decreased. n=3 slices, 3 independent experiments B) Representative BONCAT western blot showing the effects of inhibiting transcription (act D) and translation (aniso) on the elevated BONCAT signals observed in BDNF-treated slices. Slices were dissected, left to recover, then AHA was administered for 2 hours prior to the application of the inhibitor. Inhibitors were added 1 hour before BDNF application to measure their relative contributions to BDNF-induced protein synthesis. Application of both inhibitors individually negatively regulated BDNF-induced translation, with a greater effect of anisomycin. n=3-4 slices, 4 independent experiments.

Supplementary Table S1: Spreadsheet showing candidates from BONALC screen using hippocampal slices that fulfilled the consistency "C-score" criteria and the corresponding average log_2 Significance_B , average log_2 change and intensity average.

Supplementary Table S2: Raw output file from DAVID GO functional clustering analyses of the candidate protein set yielded by 'C-score' analyses of hippocampal slices and corresponding FDRs. For comparison the background dataset uploaded comprised of an in-house dataset comprising of all peptides measured by different BONLAC screens using mouse brain tissue.

Supplementary Table S3: Spreadsheet showing candidates from BONLAC screen using neuronal cultures that fulfilled the consistency "C-score" criteria and the corresponding average log_2 significance_B , average log_2 change and intensity average.

Supplementary Table S4: Raw output file from DAVID GO functional clustering analyses of the candidate protein set yielded by 'C-score' analyses of neuronal cultured cells and corresponding FDRs. For comparison the background dataset uploaded comprised of an in-house dataset comprising of all peptides measured by different BONLAC screens in neuronal culture.

Supplementary Table S5: Table of all filtered proteins that were quantified at least once using BONLAC following BDNF treatment compared to control treatment in hippocampal slices.

Supplementary Table S6: Table of all filtered proteins that were quantified at least once using BONLAC following BDNF treatment compared to control treatment in cultured neurons.