Supporting Information

Proteome alterations of hippocampal cells caused by *Clostridium botulinum* C3 exoenzyme

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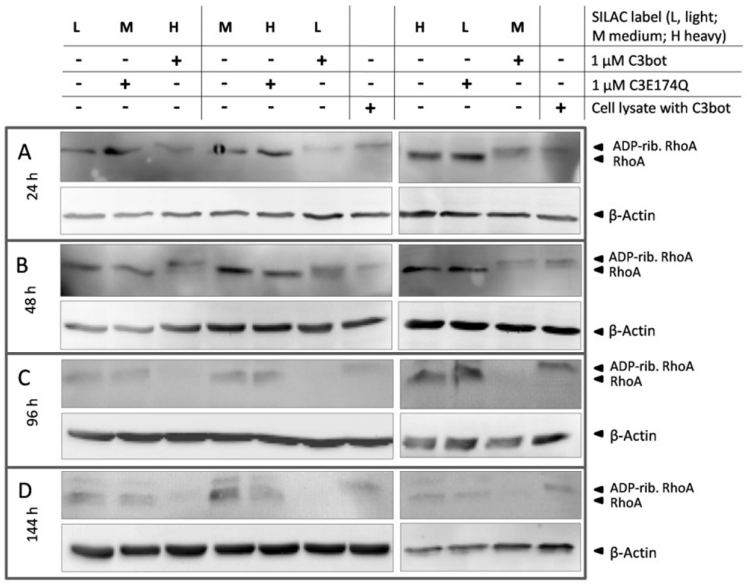


Figure S1. Western blot analysis of RhoA

Cells were exposed to 1000nM C3bot or C3E174Q for 24-144 h. Cell lysates were submitted to western blot analysis for RhoA including β-actin for protein load. In cell lysates which originated from C3bot treatment exhibit a shift of RhoA after 24-48 h to a higher molecular mass in SDS-PAGE which indicates ADP-ribosylation of GTPase. Lack of RhoA after 96-144 h C3bot treatment indicates a degradation or downregulation of RhoA.

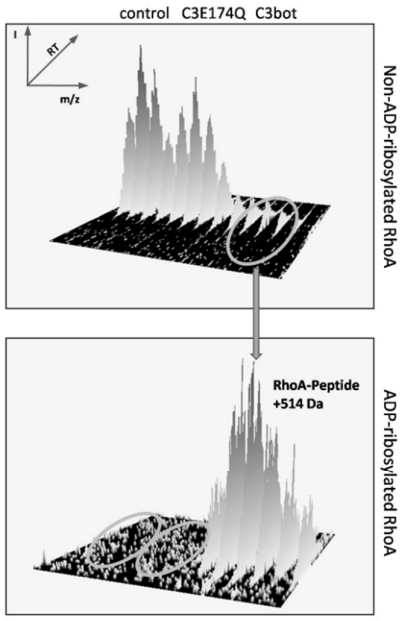
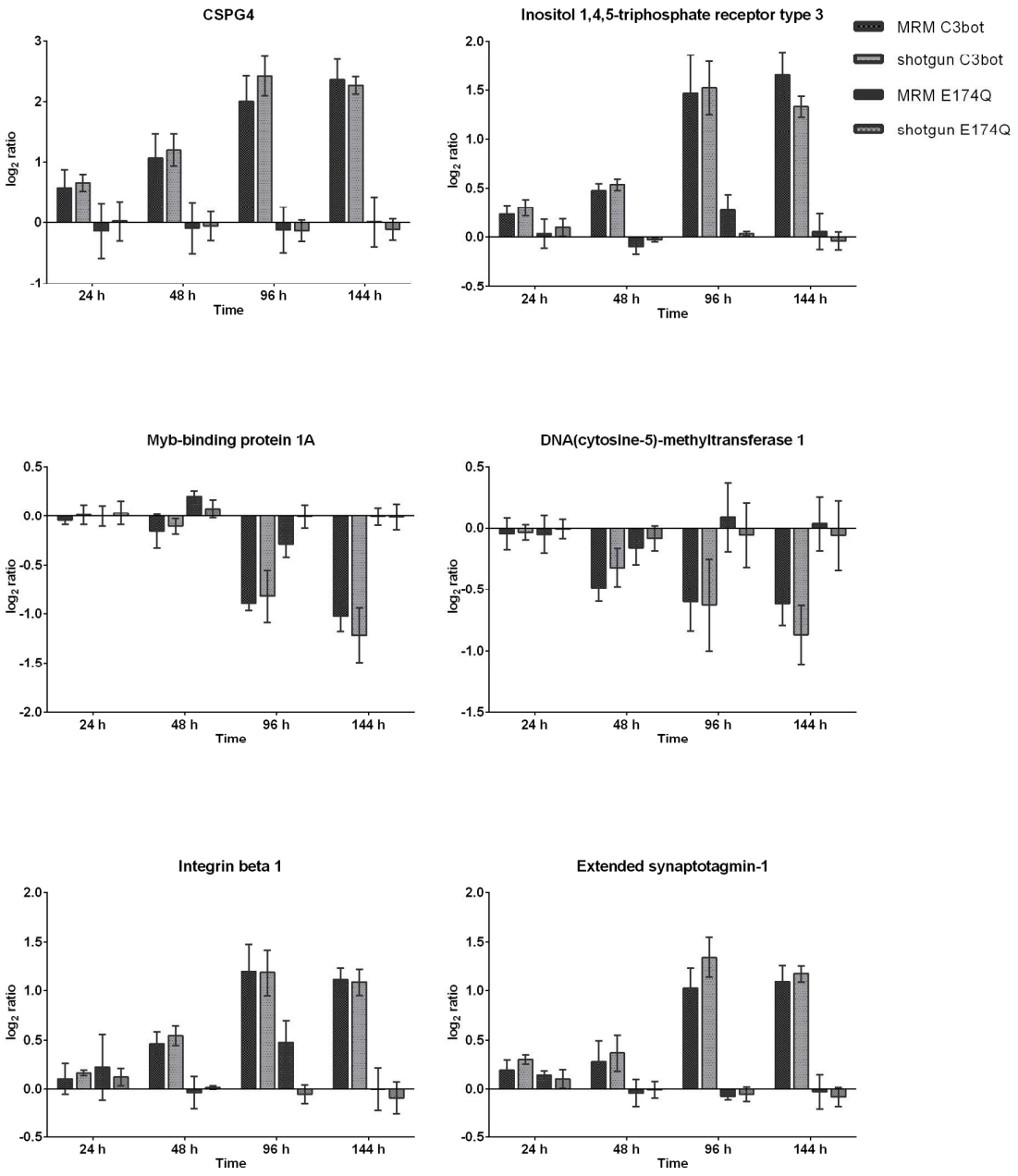


Figure S2. Mass shift of ADP-ribosylated RhoA

ADP-ribosylated Rho proteins exhibit a mass shift of 514 Da. The tryptic peptide of RhoA which contains the Asparagine-41 could be identified in the modified and unmodified state. The upper panel shows a SILAC triplet of the unmodified RhoA peptide. The heavy state which was treated with C3bot is decreased in intensity compared to the light (control) and medium (C3E174Q) state. Compatible to this finding shows the lower image only the heavy state of the SILAC triplet due to the absent modified light and heavy state peptides.


CSPG4
Inositol 1,4,5-triphosphate receptor type 3
MRM C3bot
shotgun C3bot
MRM E174Q
shotgun E174Q
Myb-binding protein 1A
DNA(cytosine-5)-methyltransferase 1
Integrin beta 1
Extended synaptotagmin-1
log2 ratio
Time
24 h
48 h
96 h
144 h

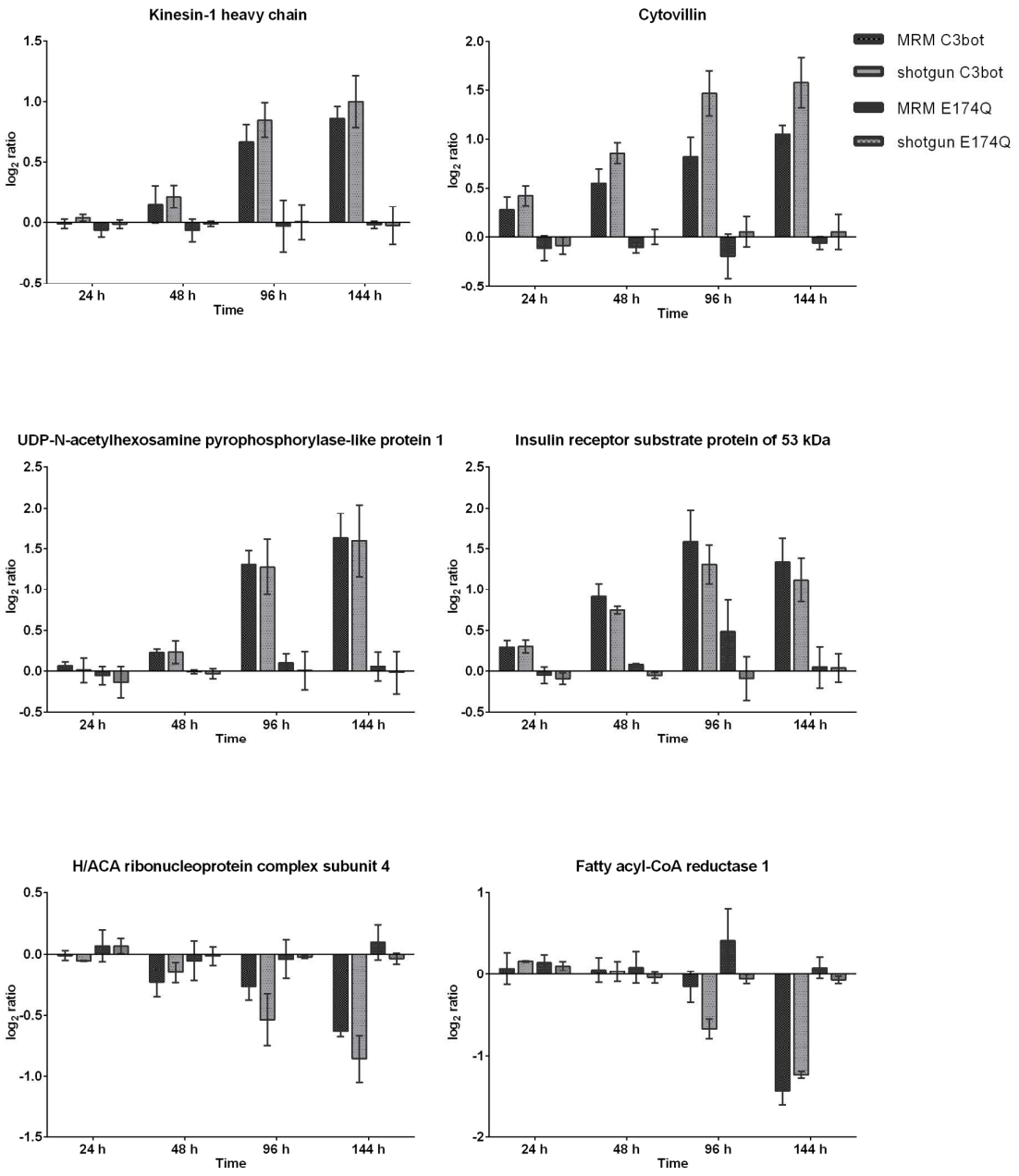


Figure S3. Verified protein ratios of regulated proteins

Protein ratios of 12 regulated proteins were logarithmized and compared to ratios obtained by MRM analysis. Overall protein ratios of both techniques correlate with each other for C3bot and C3E174Q treatment.