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Supplementary Materials for

Neuronal delivery of antibodies has therapeutic effects in animal models of botulism

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Other Supplementary Material for this manuscript includes the following:

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Supplementary Material

Materials and Methods

***Characterization of sdAb B8 binding to LC/A1 and ciBoNT/A1*:** ELISA was used to compare the selectivity of sdAb B8 for isolated LC/A1 *vs* the heterodimer. sdAbs were tested for binding to LC/A1 or a catalytically inactive BoNT/A1 mutant (*ci*BoNT/A1; used as a substitute for active toxin to minimize risk to laboratory personnel, (*52*) in a 1:5 dilution series ELISA using *Nunc Maxisorb* plates coated overnight at 4°C with 100 µL of 1 µg/mL protein (LC/A1 or *ci* BoNT/A1) (*52*). ELISA plates were blocked with 4% skim milk in DPBS/0.1% Tween-20. sdAbs were diluted to 0.004, 0.02, 0.11, 0.53, 2.6, 13, 66, and 330 nM in the blocking agent, added to wells, incubated 1 h at room temperature, washed with DPBS/0.1% Tween-20, and incubated with HRP-anti-E-tag mAb. sdAb binding was detected using chromogenic HRP substrate 3,3',5,5'-tetramethylbenzidine (sdAbs B8 and JDQ-H7 were expressed with E-tags at the C-terminus).

Expression and processing of B8C1ad: The gene for B8C1*ad* was engineered with tRNA bias typical for the *Sf9* cell translational machinery and synthesized *de novo*. The full-length B8C1*ad* DNA was incorporated into recombinant baculovirus as previously described (*17*), and the protein was expressed as a secreted proprotein, in accordance with CDC regulations. Protein was expressed and purified using the same baculovirus methodology described for BoNT/C1*ad* (*17*).

Proteomic characterization of B8C1ad heterodimer: Mass-spectrometry characterization of processed B8C1*ad* heterodimer was performed at Proteomics laboratory, part of core facilities at NYU Grossman School of Medicine, as previously described (*17*).

Western blot characterization of B8C1ad heterodimer: B8C1*ad* protein was loaded on SDS PAGE gels under non-reducing or reducing conditions (β-mercaptoethanol) and separated by SDS PAGE on a 4-12% Criterion gel (*Bio-Rad*), followed by transfer to 0.2 μm nitrocellulose membranes. Membranes were blocked and incubated with primary antibodies recognizing OLLAS tag; *c-myc* tag; E-tag; BoNT/C1 light chain; BoNT/C1 heavy chain; or HA tag, followed by incubation with HRP-conjugated secondary antibodies. SuperSignal West Pico chemiluminescent substrate was used for visualization. Western blot images were acquired using LiCor Odyssey Fc imaging system.

***Immunocytochemistry, biochemical properties of B8C1ad, and pattern of protein internalization into primary neuronal cultures*:** E18 rat cortical tissue (BrainBits LLC, Springfield, IL) was dissociated and plated at a density of 78,000 cells/cm^2 on poly-D-lysine coated glass coverslips as previously described (*47*). Neuronal cultures were maintained at 5% CO_2, 37°C, and 95% humidity in NbActiv4 medium. Experiments were performed no earlier than 14 days after cell plating to allow for emergence of synaptic activity. B8C1*ad* was diluted from 10 mg/mL stock in NbActiv4 50% fresh / 50% conditioned medium to a final concentration of 20 nM. Neuronal cultures were exposed to B8C1*ad* for 24 h, washed 2 times with 37°C CO_2-equilibrated fresh NbActiv4 medium, and chased with 25% fresh / 75% conditioned medium for another 24 h to complete the process of compound internalization. Specimens were briefly

washed with room temperature DPBS, fixed with ice-cold 4% formaldehyde for 15 minutes at room temperature, permeabilized, and blocked with 3% IgG-free BSA and 0.1% saponin in DPBS for 45 minutes (*54*). Fixed, permeabilized, and blocked specimens were probed with mouse anti-**STX1** mAb (dilution 1:1,000/Alexa 555 donkey anti-mouse IgG); mouse anti-**SNAP-25** mAb (dilution 1:1,000/Alexa 555 donkey anti-mouse IgG); for synaptic markers, and with human anti-LC/C1 mAb (4C10.2, Dr. J. Marks, UCSF, dilution 1:1,500) for B8C1*ad* in DPBS overnight at 4°C, followed by incubation for 1 h at room temperature with Alexa 555-labeled secondary antibodies (dilution 1:3,000) for synaptic markers, and with secondary Alexa 488-labeled donkey anti-human IgG (dilution 1:3,000) for B8C1*ad*. Coverslips were mounted onto slides with Prolong Gold DAPI mounting medium, and images were collected by confocal microscopy Z-stack – for B8LC and synaptic markers by epifluorescence – for DAPI nuclear staining, with standard excitation and emission filters using a Zeiss LSM 710 microscope (*Carl Zeiss Inc*). Images were processed using ImageJ software.

***B8LC co-localization with Endosomal/Lysosomal/Autophagosomal (ELA) markers in neurons*:** E18 rat cortical tissue (BrainBits, LLC) was dissociated and plated at 78,000 cells/cm^2 on glass coverslips and maintained in Neurobasal medium with NeuroCult SM1 Supplement. At 14 d after plating, neurons were exposed to vehicle (50% conditioned Neurobasal medium/SM1) or 50 nM B8C1*ad*. After 24 h, medium was aspirated, cells were washed with DPBS and chased with 50% conditioned Neurobasal medium/SM1 for 24 h. Cells were washed, fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100 in DPBS, and blocked with 10% goat serum in DPBS for 45 min at room temperature. Cells were probed with antibodies against mouse anti-Early Endosome Antigen 1 (EEA1) and Alexa 555 donkey anti-mouse IgG1; rabbit

anti-Lysosomal Associated Membrane Protein-1 (LAMP-1) and Alexa 555 donkey anti-rabbit IgG1; or mouse anti-Rab-7 and with Alexa 555 donkey anti-mouse IgG1. Cells were also probed with anti-LC/C1 human mAb 4C10.2 (Dr. J. Marks, UCSF) and Alexa 555 donkey anti-human or Alexa 488 donkey anti-human IgG. Images were collected on a Zeiss LSM 880 confocal microscope and analyzed using Zeiss LSM confocal microscopy software.

***B8C1ad effects on SNAP-25 cleavage after exposure of primary neurons to BoNT/A1*:** E18 rat cortical tissue (BrainBits LLC) was plated at a density of 125,000 cells/cm^2 in polyethylenimine-coated 6-well dishes and maintained at 5% CO_2, 37°C, and 95% humidity in NbActiv4 medium. At 14 d after plating, neurons were exposed to 5 pM BoNT/A1 for 1.5 h, washed with 50% conditioned medium, chased with fresh medium for 2 h, incubated with 50 nM B8C1*ad* or saline vehicle for 24 h, washed and incubated for an additional 1, 4, or 7 days. Neurons were lysed on ice with Triton X-100 lysis buffer with protease inhibitors after 1, 4 or 7 d and immunoblots were probed with rabbit anti-E-tag antibody (to detect B8LC), mouse anti-β-actin, and mouse anti-SNAP-25, followed by incubation with HRP-conjugated secondary antibodies. SuperSignal West Pico chemiluminescent substrate was used for visualization. Western blot images were acquired using a LiCor Odyssey Fc imaging system and analyzed by densitometry using instrument software. This experiment was replicated four times with similar results.

Fig. S1.

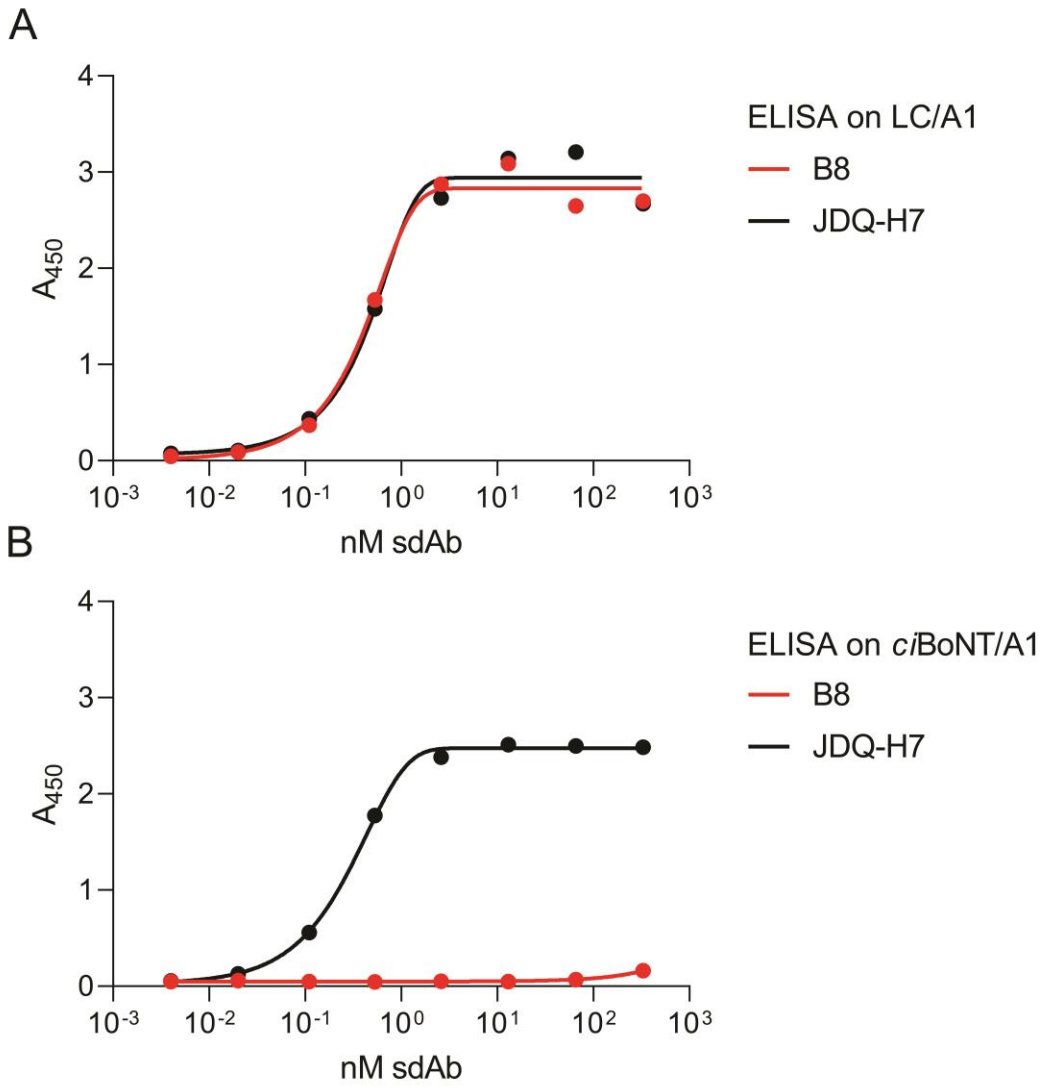

Fig. S1. sdAb B8 binds LC/A1 but not the intact BoNT/A1 heterodimer. ELISA assay evaluating binding of sdAbs B8 and positive control JDQ-H7 to (**A**) purified LC/A1 and (**B**) catalytically inactive BoNT/A1 holotoxin (*ci*BoNT/A1) (*52*).

Fig. S2.

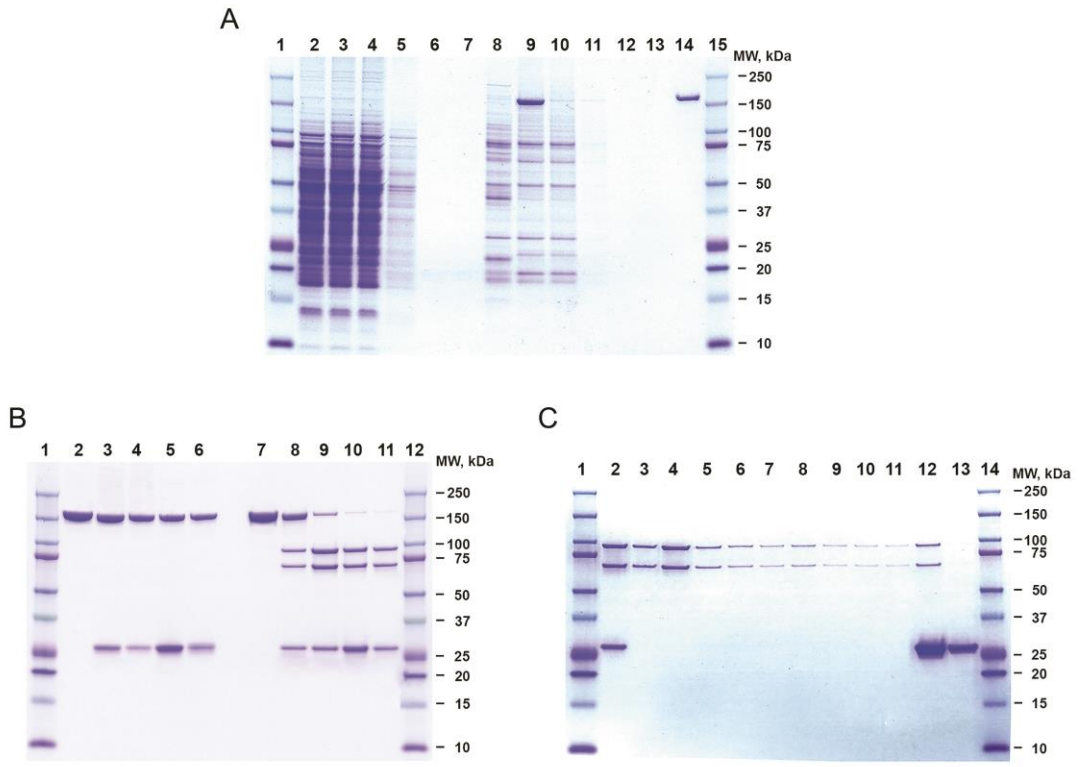

Fig. S2. B8C1*ad* expression, purification, and processing. Coomassie blue-stained, SDS-PAGE gel demonstrating each step of B8C1*ad* proprotein purification. (**A**) Tandem affinity chromatography to capture protoxin from SF9 cell supernatant: Lanes 1, 15: protein MW ladder; lanes 2–8: products of Ni^{2+}-NTA affinity chromatography: lanes 2-3: concentrated and dialyzed culture supernatant containing B8C1*ad* proprotein; lane 4: flow through; lane 5: wash with 15 mM imidazole buffer; lanes 6-7: washes with 15 mM imidazole buffer; lane 8: wash with 45 mM imidazole buffer; lane 9: eluate obtained with 250 mM imidazole buffer; lanes 10-14: StrepTactin affinity chromatography of eluate from 9; lane 10: flow through; lanes 11-12: 1 M NaCl washes; lane 13: low salt wash; lane 14: eluate obtained with 5 mM *D*-desthiobiotin. (**B**) Processing of B8C1*ad* proprotein to heterodimer by proteolytic cleavage with TEV protease: lanes 1, 12: protein MW ladder; lanes 2-6: non-reduced samples; lanes 7-11: samples reduced by incubation with β-mercaptoethanol; lanes 2, 7: no TEV controls; lanes 3, 8: B8C1*ad* proprotein incubated with TEV for 1 h at 25°C; lanes 4, 9: incubated with TEV for 6 h; lanes 5, 10: incubated with TEV for 24 h; lanes 6, 11: incubated with TEV for 48 h. (**C**) Removal of TEV protease (MW ≈ 27 kDa) from the reaction mixture by Ni^{2+}-NTA affinity chromatography: lanes 1, 14: protein MW ladder; lane 2: loading material; lanes 3-4; flow through; lanes 5–8: washes with 15 mM imidazole buffer; lanes 9-11: washes with 45 mM imidazole buffer; lanes 12-13: elution with 250 mM imidazole buffer. Pull-down fractions from lanes 3-11 were pooled for subsequent use.

Fig. S3.

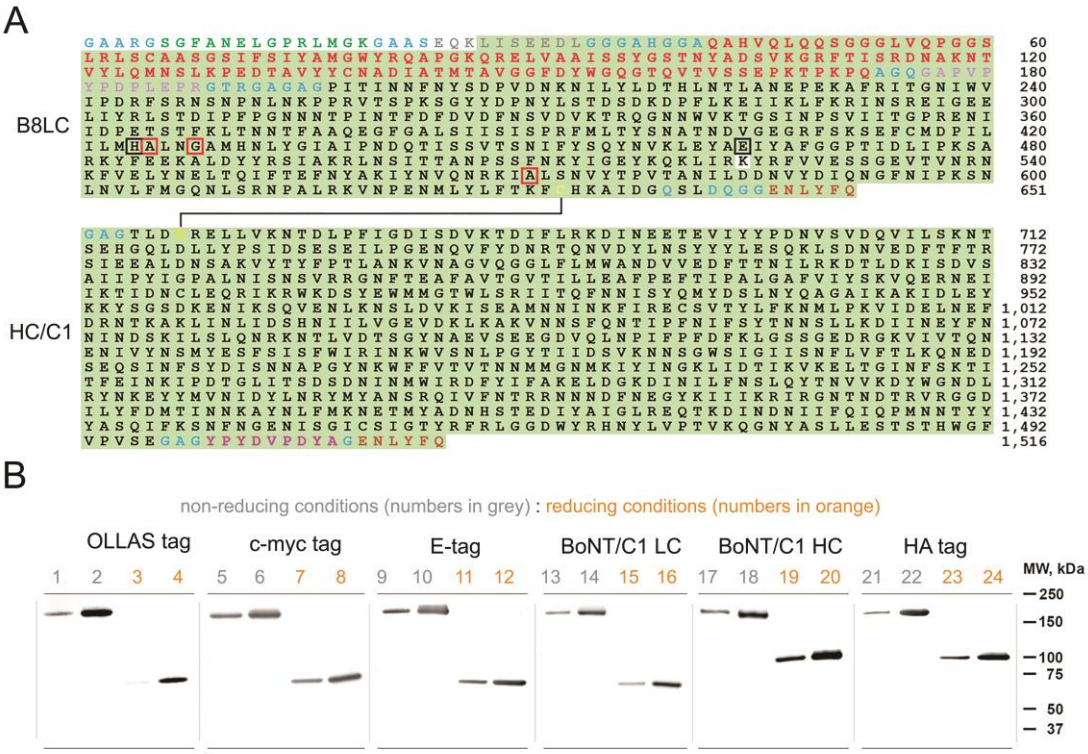

Fig. S3. Proteomic and immunological characterization of B8C1*ad* heterodimer. (**A**) Sequence of B8LC and HC/C1. Letters on light green background represent confirmed sequences identified among one or more corresponding peptides from tryptic and chymotryptic digests of the recombinant protein. Letters on white background represent known sequences not recovered from any of the digests. Light blue letters represent exogenous linkers/spacers introduced into the recombinant protein. Red letters represent the sequence of sdAb B8. Black letters represent conserved sequences of the C1*ad* heterodimer. Boxed letters mark positions of five non-adjacent amino acids – *H, E, H, E, Y* – that form the active site of Zn^{2+}-metalloprotease; letters boxed with red outline among this group represent mutations E_{425}>A; H_{428}>G; Y_{570}>A introduced into the metalloprotease active site, rendering it inactive. Light brown letters represent the TEV protease recognition sequence. Yellow letters represent the two cysteine residues that form the disulfide bridge between light and heavy chains of the heterodimer. Green, gray, lavender, and magenta letters represent OLLAS, *c-myc*, E, and HA tags, respectively, introduced for detection purposes at the N-terminus of the construct, at the C-terminus of sdAb B8, or at the C-terminus of the heavy chain. (**B**) Western blot characterization of B8C1*ad* heterodimer using primary antibodies recognizing OLLAS, *c-myc*, and E tags, BoNT/C1 light/ heavy chains and HA tag, under reducing and non-reducing conditions. Dual lanes shown on each Western blot probe represent 50 ng (odd) and 200 ng (even) B8C1*ad* loaded.

Fig S4.

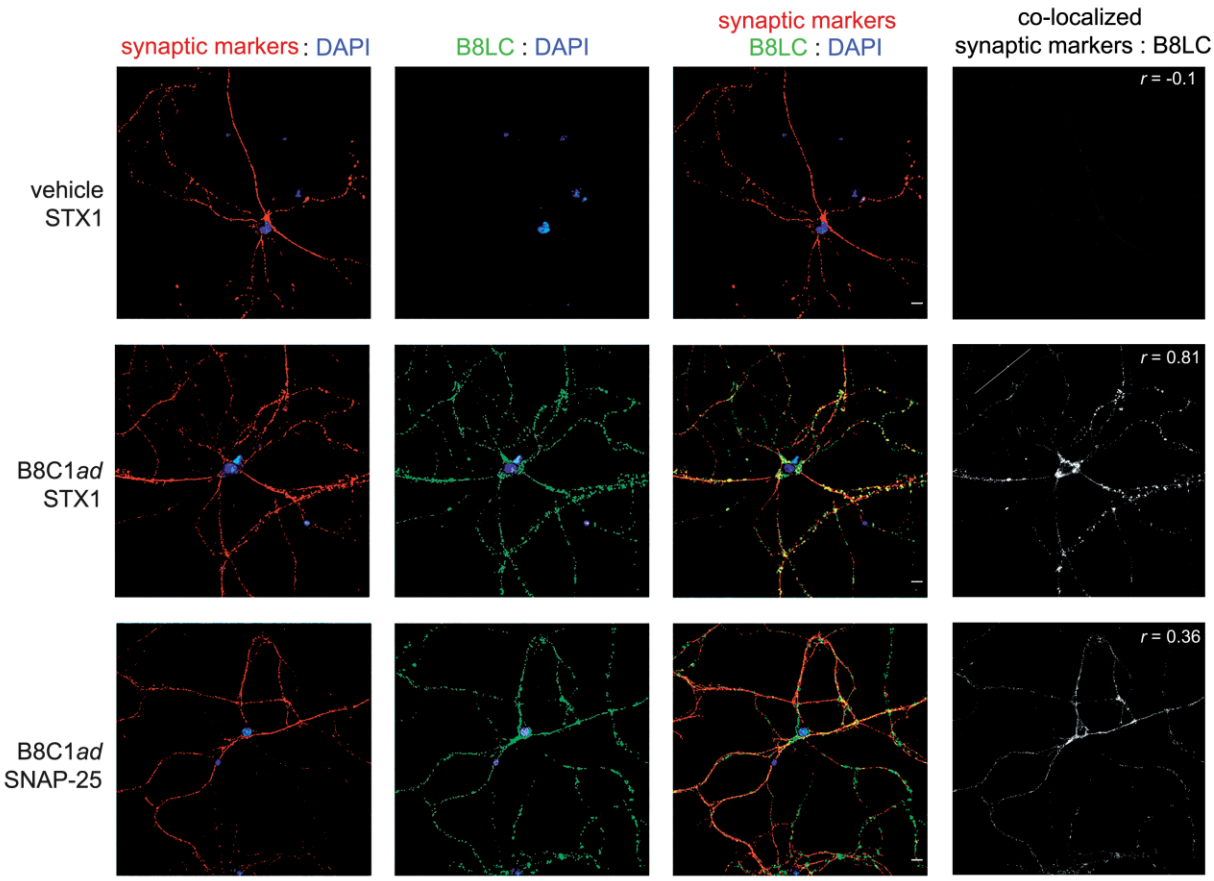

Fig. S4. Internalized B8LC colocalizes with presynaptic markers in primary neurons. Rat E18 primary cortical neurons (14 d) were treated for 24 h with vehicle (A) or B8C1*ad* (B-C) and analyzed for co-localization of B8LC (red) with presynaptic markers (green) by staining for: (**A, B**) STX1; or (**C**) SNAP-25. Pearson's above-threshold *r* value is presented on each image. Panels on the right identify merged pixels from red and green channels. Scale bar=20 µm.

Fig. S5.

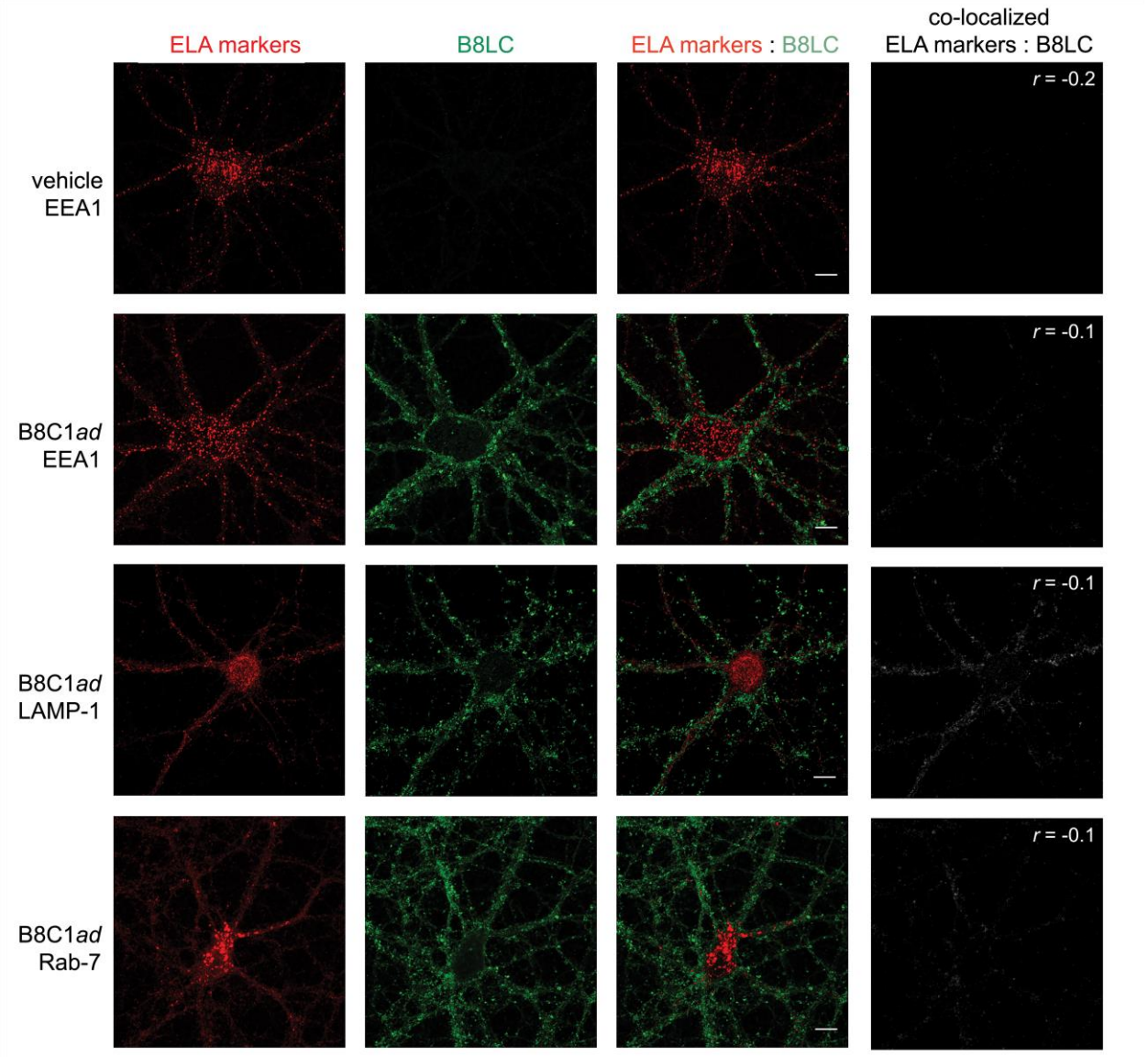

Fig. S5. Internalized B8LC does not colocalize with endosomal, lysosomal, or autophagosomal markers in primary neurons. Rat E18 primary cortical neurons (14 d) were treated for 24 h with B8C1*ad* and analyzed for co-localization of B8LC (green) with endosomal, lysosomal and autophagosomal markers (red): (**A**) EEA1, (**B**) LAMP-1 or (**C**) Rab-7. Pearson's above-threshold R value is presented in white letters on each image. Panels on the right identify merged pixels from red and green channels. Details of statistical comparisons are presented in Table S2. Scale bar=10 µm.

Fig. S6.

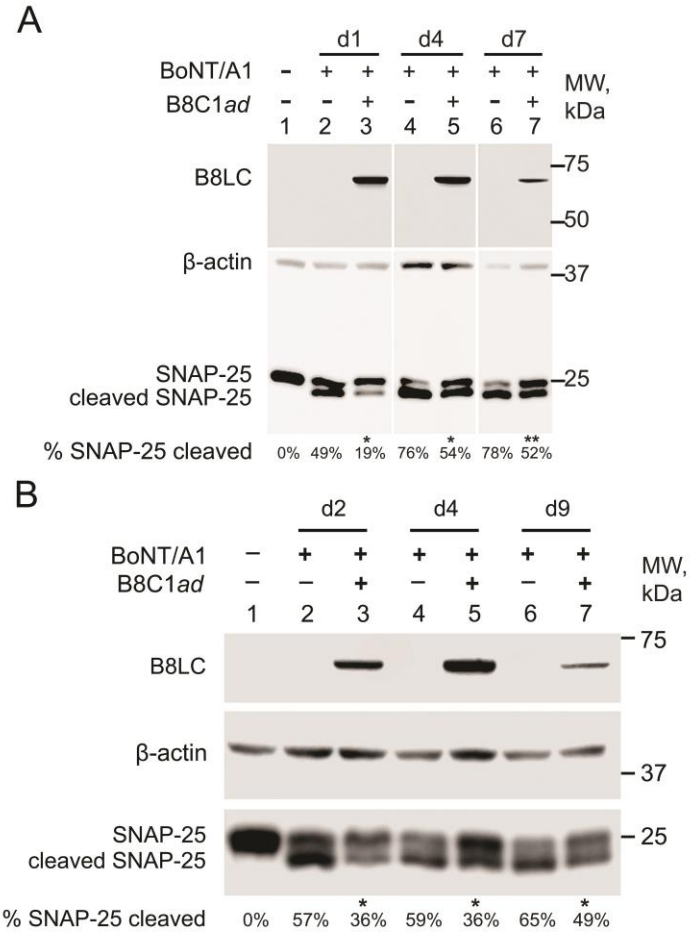

Fig. S6. B8C1*ad* protects against BoNT/A1 challenge in primary neurons. (**A**) Representative Western blots showing SNAP-25 cleavage in neurons incubated with 5 pM BoNT/A1 for 1.5 h, chased with fresh medium for 2 h, incubated with 50 nM B8C1*ad* or vehicle for 24 h and lysed after 1, 4, or 7 d. Treatment conditions are marked above each lane and the average percentage of cleaved SNAP-25 is shown below each lane. Bolded percentages indicate significant reduction in SNAP-25 cleavage *vs* vehicle (N=5, n=3-5 for each condition, unpaired Welch's t-test). Lane 1 is naïve neurons harvested at 1 d. (**B**) Representative Western blot of primary neurons exposed to B8C1*ad* or vehicle for 24 h, challenged with BoNT/A1 after 1, 3 or 8 d, and harvested 24 h later. β-actin is used as a loading control in panels A and B. The average percentage of cleaved SNAP-25 is shown below each lane (N=3, n=3 for each condition, unpaired Welch's t-test). *p<0.05; ** p<0.01. Additional details of statistical comparisons are presented in Table S2.

Table S1. Dose-dependent toxicities of B8C1*ad* in mice, guinea pigs, and rhesus macaques.

Species (route) and number per dose	B8C1*ad* dose (mg/kg)	Max CSS score within cohort: adverse events observed
Mouse (i.p.) n=5-10 per dose	0.20	0: none observed
	0.40	**0: none observed; identified as NOAEL dose**
	0.60	1: mild paradoxical abdominal breathing; resolved by 48 h
	0.80	2: mild paradoxical abdominal breathing, lethargy; resolved by 48 h
	1.00	9: severe paradoxical abdominal breathing, limb weakness; resolved by 72 h
	1.20	16: 2/5 mice euthanized or found dead at 24 h
	1.60	16: 3/5 mice euthanized or found dead at 24 h
	2.00	16: 4/5 mice euthanized or found dead at 24 h
	2.40	16: 5/5 mice euthanized or found dead at 24 h
Guinea pig (i.p.) n=3 per dose	0.01	0: none observed
	0.02	0: none observed
	0.03	**0: none observed; identified as NOAEL dose**
	0.04	3: paradoxical abdominal breathing, lethargy; resolved by 48 h
	0.08	7: forced abdominal breathing, lethargy, lacrimation; resolved by 72 h
	0.40	20: agonal breathing, moribund; euthanized by 48 h
Rhesus macaque (i.v.) n=1 per dose	0.02	0: none observed
	0.06	**0: none observed; identified as NOAEL dose**
	0.08	1: possible mild abdominal breathing; resolved by 24 h
	0.12	2: abdominal breathing and muscle weakness; resolved by 60 h
	0.20	16: agonal breathing, prostrate unable to rise; euthanized by 24 h
	0.40	16: agonal breathing, prostrate unable to rise; euthanized by 12 h

B8C1*ad* dose-dependent acute toxicities were determined by monitoring animals for adverse physiological effects after treatment. (**Top row**) Mice were administered 0.2-2.4 mg/kg B8C1*ad* (i.p.) and physiological status was evaluated at 24 h intervals over 10 d. (**Second row**) Guinea pigs were administered 0.01-0.40 mg/kg B8C1*ad* (i.p.) and physiological status was evaluated at 24 h intervals over 10 d; (**Bottom row**) Rhesus macaque monkeys were administered 0.02–0.40 mg/kg B8C1*ad* (i.v.), followed by quarter-dose supplemental administration of B8C1*ad* (i.v.) at 3 d intervals. Physiological status was assessed at 6 h intervals for up to 10 d.

Table S2. Emergence of respiratory signs of botulism in mice challenged with 2 mipLD_{50} BoNT/A1.

Respiratory sign*	Median time of onset (range), h	Incidence
Mild abdominal paradox	8 (6-8)	100% (10/10)
Severe abdominal paradox	16 (12-16)	100% (10/10)
Agonal breathing	20 (18-24)	100% (10/10)
Death	27 (22-34)	100% (10/10)

* Mice were observed for respiratory signs of botulism at 2 h intervals

Table S3. Sample sizes, statistical analyses, and significance thresholds for all comparisons.

Figure #	group: number of replicates	Comparison	primary test	primary test result (significance at $P < 0.05$)	secondary test	secondary test result (significance at $P < 0.05$, unless Bonferroni correction is listed in next column)	Bonferroni-corrected significance threshold
1C FRET-based cleavage assay	C1*ad*: 2 replicates per concentration B8C1*ad*: 2 replicates per concentration	FRET ratio (normalized to vehicle)	Welch's t-test with Bonferroni-Dunn's correction	1:1: B8C1*ad* (18.8%) *vs* C1*ad* (99.9%): **$P < 0.0001$** 9:1: B8C1*ad* (3.0%) *vs* C1*ad* (99.9%): **$P < 0.0001$** 81:1: B8C1*ad* (2.2%) *vs* C1*ad* (99.9%): **$P < 0.0001$**			
2A Co-localization studies in primary neurons	*wt*LC/A1 *vs* B8LC*ad*, 3 technical replicates	co-localization	Pearson's r (above threshold)	$r = 0.59$ (PSF = 3.0)			
2C SNAP-25 cleavage at 1 d in primary neurons	vehicle: 5 biological replicates B8C1*ad*: 5 biological replicates sdAb B8: 3 biological replicates C1*ad*: 4 biological replicates	percentage of cleaved SNAP-25	Mixed-effects, one-way ANOVA	$F(1.48, 4.45) = 36.58$, **$P = 0.0021$**	Dunnett's multiple comparisons test	vehicle *vs* B8C1*ad*: **$P = 0.0006$** vehicle *vs* B8 sdAb: $P = 0.4879$ vehicle *vs* C1*ad*: $P = 0.3277$	
3A LD_{50} determinations in mice	BoNT/A: 12 mice per dose, 7 doses	non-linear regression	R^2 95% CI	$R^2 = 0.99$ 95% CI = 0.15-0.19 ng/kg			
	B8C1*ad*: 5 mice per dose, 8 doses	non-linear regression	R^2 95% CI	$R^2 = 0.97$ 95% CI = 1.18-1.72 mg/kg			
3B Efficacy in mice 2 mipLD$_{50}$, i.p.	vehicle: 15 B8 alone: 10 C1ad alone: 10 B8C1*ad*: 15	median survival time	Mantel-cox log-rank test	$\chi^2(3,50) = 42.3$, **$P < 0.0001$**	pairwise Mantel-Cox log-rank test with adjusted significance threshold	vehicle *vs* B8C1*ad*: $\chi^2(1,30) = 30.3$, **$P < 0.0001$** vehicle *vs* B8 sdAb: $\chi^2(1,25) = 0.25$, $P = 0.62$ vehicle *vs* C1*ad*: $\chi^2(1,25) = 0.25$, $P = 0.62$	$P < 0.0167$
		survival rate 10 d	Chi-square contingency test	$\chi^2(3,50) = 45.4$, **$P < 0.0001$**	pairwise two-sided Fisher's exact test with adjusted significance threshold	vehicle (0/15) *vs* B8 sdAb (0/10): $P < 0.99$ vehicle (0/15) *vs* C1*ad* (0/10): $P < 0.99$ vehicle (0/15) *vs* B8C1*ad* (14/15): **$P < 0.0001$**	$P < 0.0167$
3C Efficacy in mice 4, 6 mipLD$_{50}$; i.p.	4 mipLD$_{50}$: vehicle *vs* B8C1*ad*: 5 each	median survival time	Mantel-cox log-rank test	$\chi^2(1,10) = 6.0$, **$P = 0.0143$**			
		survival rate 10 d	two-sided Fisher's exact test	vehicle (0/5) *vs* B8C1*ad* (3/5): $P = 0.17$			
	6 mipLD$_{50}$: vehicle *vs* B8C1*ad*: 5 each	median survival time	Mantel-cox log-rank test	$\chi^2(1,10) = 6.0$, **$P = 0.014$**			
		survival rate 10 d	two-sided Fisher's exact test	vehicle (0/5) *vs* B8C1*ad* (1/5): $P > 0.99$			
3D Prophylaxis in mice 2 mipLD$_{50}$, i.p.	-2 d vehicle: 10 -2 d B8C1*ad*: 12 -3 d B8C1*ad*: 10 -4 d B8C1*ad*: 10	median survival time	Mantel-cox log-rank test	$\chi^2(3,42) = 32.1$**, $P < 0.0001$**	pairwise Mantel-Cox log-rank test with adjusted significance threshold	vehicle *vs* -2 d B8C1*ad*: $\chi^2(1,22) = 17.69$, **$P < 0.0001$** vehicle *vs* -3 d B8C1*ad*: $\chi^2(1,20) = 12.91$, **$P = 0.003$** vehicle *vs* -4 d B8C1*ad*: $\chi^2(1,20) = 12.91$, **$P = 0.003$**	$P < 0.0167$
		survival rate 10 d	Chi-square contingency test	$\chi^2(3,42) = 18.6$, **$P = 0.0003$**	pairwise two-sided Fisher's exact test with adjusted significance threshold	vehicle (0/10) *vs* -2 d B8C1*ad* (10/12): **$P = 0.0001$** vehicle (0/10) *vs* -3 d B8C1*ad* (6/10): **$P = 0.011$** vehicle (0/10) *vs* -4 d B8C1*ad* (2/10): $P = 0.47$	$P < 0.0167$
4A B8C1*ad* *vs* antitoxin in mice 2 mipLD$_{50}$; i.p.	3 h: antitoxin *vs* B8C1*ad*: 20 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (14/20) *vs* B8C1*ad* (20/20): **$P = 0.020$**			
	6 h: antitoxin *vs* B8C1*ad*: 20 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (13/20) *vs* B8C1*ad* (20/20): **$P = 0.008$**			
	8 h: antitoxin *vs* B8C1*ad*: 20 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (6/20) *vs* B8C1*ad* (20/20): **$P < 0.0001$**			
	12 h: antitoxin *vs* B8C1*ad*: 30 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (7/20) *vs* B8C1*ad* (30/30): **$P < 0.0001$**			
	16 h: antitoxin *vs* B8C1*ad*: 20 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (0/20) *vs* B8C1*ad* (16/20): **$P < 0.0001$**			
	20 h: antitoxin *vs* B8C1*ad*: 30 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (0/20) *vs* B8C1*ad* (17/30): **$P < 0.0001$**			
4B B8C1*ad* *vs* antitoxin in mice 2 mipLD$_{50}$; i.v.	12 h: antitoxin *vs* B8C1*ad*: 5 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (1/5) *vs* B8C1*ad* (5/5): **$P = 0.048$**			
	16 h: antitoxin *vs* B8C1*ad*: 5 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (0/5) *vs* B8C1*ad* (5/5): **$P = 0.008$**			
	20 h: antitoxin *vs* B8C1*ad*: 5 each	survival rate 10 d	two-sided Fisher's exact test	antitoxin (0/5) *vs* B8C1*ad* (5/5): **$P = 0.008$**			

4C, D B8C1ad ED_{50} in mice 2 mipLD_{50}; i.v. B8C1*ad* at 10 h	vehicle: 23 0.003 mg/kg: 10 0.01 mg/kg: 8 0.02 mg/kg: 17 0.03 mg/kg: 8 0.10 mg/kg: 25 0.20 mg/kg: 10 0.30 mg/kg: 25 0.40 mg/kg: 10	non-linear regression	R^2 95% CI	R^2 = 0.98 95% CI = 0.023-0.028 mg/kg			
		survival rate 10 d	Chi-square contingency test	$\chi^2(136,8) = 101.3$, ***P* < 0.0001**	Dunnett's multiple comparisons test	vehicle (0/23) *vs* 0.003 (0/10): $P > 0.9999$ vehicle (0/23) *vs* 0.01 (1/8): $P = 0.9445$ vehicle (0/23) *vs* 0.02 (3/17): $P = 0.1458$ vehicle (0/23) *vs* 0.03 (6/8): ***P* < 0.0001** vehicle (0/23) *vs* 0.10 (23/25): ***P* < 0.0001** vehicle (0/23) *vs* 0.20 (9/10): ***P* < 0.0001** vehicle (0/23) *vs* 0.30 (24/25): ***P* < 0.0001** vehicle (0/23) *vs* 0.40 (10/10): ***P* < 0.0001**	
		CSS at 10 d	Kruskal-Wallis one-way ANOVA	$H(8) = 96.0$, ***P* < 0.0001**	Dunn's multiple comparisons test	vehicle *vs* 0.003: $P > 0.9999$ vehicle *vs* 0.01: $P > 0.9999$ vehicle *vs* 0.02: $P > 0.9999$ vehicle *vs* 0.03: $P < 0.0757$ vehicle *vs* 0.10: ***P* < 0.0001** vehicle *vs* 0.20: ***P* < 0.0001** vehicle *vs* 0.30: ***P* < 0.0001** vehicle *vs* 0.40: ***P* < 0.0001**	
5A, B Efficacy in guinea pigs 19 mipLD_{50}/kg; *i.m.*	vehicle: 14 single dose (SD) B8C1*ad*: 9 multi-dose (MD) B8C1*ad*: 18	median survival time	Mantel-cox log-rank test	$\chi^2(2,41) = 35.06$, ***P* < 0.0001**	pairwise Mantel-Cox log-rank test with adjusted significance threshold	vehicle *vs* single-dose B8C1*ad*: $\chi^2(1,23) = 15.73$, ***P* < 0.0001** vehicle *vs* multiple-dose B8C1*ad*: $\chi^2(1,32) = 33.59$, ***P* < 0.0001**	$P < 0.025$
		survival rate 10 d	Chi-square contingency test	$\chi^2(2,41) = 23.76$, ***P* < 0.0001**	pairwise two-sided Fisher's exact test with adjusted significance threshold	vehicle (0/14) *vs* single-dose B8C1*ad* (1/9): $P = 0.39$ vehicle (0/14) *vs* multiple-dose B8C1*ad* (14/18)**: *P* < 0.0001**	$P < 0.025$
		CSS comparison	two-way ANOVA with Dunnett's	time x treatment: $F(20,380) = 23.51$, ***P* < 0.0001**			
6A, B Efficacy in monkeys 40 mipLD_{50}/kg; i.v.	vehicle: 7 B8C1*ad*: 6	median survival time	Mantel-cox log-rank test	$\chi^2(1,13) = 12.51$, ***P* = 0.0004**			
		survival rate 10 d	two-sided Fisher's exact test	vehicle (0/7) *vs* B8C1*ad* (6/6): ***P* = 0.0006**			
		CSS comparison	two-way ANOVA with Sidak's	time x treatment: $F(41,451) = 96.93$, ***P* < 0.0001**			
S4 Co-localization with presynaptic markers	STX1 *vs* vehicle, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = -0.1 (PSF = 3.0)			
	STX1 *vs* B8C1*ad*, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = 0.81 (PSF = 3.0)			
	SNAP-25 *vs* B8C1*ad*, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = 0.36 (PSF = 3.0)			
S5 Co-localization with ELA markers	EEA1 *vs* vehicle, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = -0.2 (PSF = 3.0)			
	EEA1 *vs* B8C1*ad*, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = -0.1 (PSF = 3.0)			
	LAMP-1 vs B8C1*ad*, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = -0.1 (PSF = 3.0)			
	Rab-7 vs B8C1*ad*, 3 technical replicates	co-localization	Pearson's R value (above threshold)	r = -0.1 (PSF = 3.0)			
S6A SNAP-25 cleavage after 1, 4 and 7 d of treatment	vehicle: 3 biological replicates/time point B8C1*ad*: 3 biological replicates/time point	SNAP-25 cleavage	unpaired Welch's t-test	1 d: t = 3.8, ***P*= 0.0191** 4 d: t = 8.2, ***P*= 0.0144** 7 d: t = 12.2, ***P*= 0.0066**			
S6B SNAP-25 cleavage at 2, 4 and 9 d latencies	vehicle: 3 biological replicates/time point B8C1*ad*: 3 biological replicates/time point	SNAP-25 cleavage	unpaired Welch's t-test	2 d: t = 4.2, ***P*= 0.0133** 4 d: t = 3.8, ***P*= 0.0195** 9 d: t = 3.3, ***P*= 0.0327**			

Summary of all experimental conditions, number of biological replicates per condition, the type of statistical analysis, statistical outcomes and significance thresholds for all comparisons.

Data file S1. Individual-level details for each experiment.

Provided as a separate Word file.

Data file S2. Individual CSS scores at all time points for guinea pig and rhesus macaque survival studies.

Provided as a separate Excel file.