Table 1. Sequences of primers and probes used

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Sequence</th>
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<tr>
<td>bGlobulin For</td>
<td>ATTCTGAGTCCAAGCTAGGC</td>
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<td>hGH pA Rev</td>
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<td>S25-XhoI-Rev</td>
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<td>CMV_TFor</td>
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<td>CMV_TRev</td>
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Materials

The 150kDa BoNT/A was purified, free of haemagglutinin and other accessory proteins, to a specific neurotoxicity of 1 mouse LD$_{50}$ = 5 pgs (Shone and Tranter, 1995). The following reagents were purchased: AAV vector Helper-free system (Stratagene), mouse anti-NF200 antibody and basic laboratory chemicals (Sigma-Aldrich), anti-S25 SMI-81 antibody (Sternberger Monoclonals), mouse anti-His$_6$ antibody (Amersham), α-bungarotoxin (α-BuTx) conjugated to Alexa-488 or rhodamine (Invitrogen), West-Dura ECL developer (Pierce), DNA purification kits (Qiagen), enzymes (NEB) and female Wistar rats (Trinity Bioresources Unit or Harlan).

Additional comments:

1) Female Wistar rats (8 weeks old, ~200g), acquired under license from Trinity Bioresources Unit or Harlan, were given ad libitum access to water and commercial rodent food. Animals were housed in certified facilities and handled by trained animal technicians. All techniques
were approved by animal welfare authorities in Ireland and conformed to relevant EU regulations.

2) Protein expression in virally-infected chromaffin cells was quantified by separation on 12% pre-cast SDS-PAGE gels and Western blotting. The blots were probed with different dilutions of the primary (1:5000 SMI-81, 1:1000 anti-His₆, or 1:2000 anti-hrGFP) and relevant secondary antibodies (1:4000 rabbit anti-mouse or 1:2000 goat anti-rabbit HRP-conjugated), developed with West-Dura reagents and chemiluminescence was captured using a cooled-CCD camera.

3) Images were captured on an Olympus IX51 inverted fluorescent microscope with 10x objective and a fast digital camera (Evolution QEi monochrome, MediaCybernetics). A calibrated motorized stage and ‘Stage-Pro’ software allowed the serial acquisition and stacking of image-frames to obtain a picture of the whole muscle area. Scans of 20-40 sections of the muscle were acquired per treatment regime and analyzed with ‘Image-Pro Plus 5.1’ software (MediaCybernetics). The automated ‘count’ function, after adjusting the ranges, permitted quantitation of the total endplate area, mean endplate area and size of the largest endplate in each section, along with the total muscle area. Data from more than 2000 endplates, as determined by the program, were quantified per treatment, normalized to the total muscle area, averaged over ~20 sections per treatment and compared to untreated and toxin-only treated muscles; T-test was used to determine statistical significance.

4) The controversy in the literature about the exact coordinates of the lumbar spinal levels supplying the lower limb between (Greene, 1968) and (Nicolopoulos-Stournaras and Iles, 1983) necessitated retrograde tracing using fluorogold injected into the soleus; the results obtained, T13-L2, agree with the latter.

5) There was no evidence of any adverse effect on the synapse at the microscopic or functional levels; binding of fluorescently-labeled α-Butx showed normal distribution of post-synaptic Ach receptors; accordingly, only a small, non-significant, difference could be detected in
ENT values between muscles innervated by virally-infected lumbar motor neurons and vehicle-injected control neurons.


**Additional figures**

**Fig 5.**

A, Overlay of phase contrast and fluorescent microscopic views of HEK-293 cells infected with AAV-hrGFP at a multiplicity of infection of 1. B, Expression and subcellular localization of the introduced His6-tagged S25 in HEK-293 cells, infected with AAV-His6-S25 for 5 days, were tested by SDS-PAGE of cytoplasmic (lane 1) and membrane (lane 2) fractions of lysed cells and blotted
with mouse anti-His$_6$/anti-mouse-HRP antibodies. C, Representative confocal micrograph showing membrane-association of His$_6$-S25 in HEK-293 cells upon probing with anti-S25/anti-mouse-A488 and counter-stained with DAPI. D, Fluorescence images detecting His$_6$-S25R198T (red - i), acquired as before but with the substitution of an anti-mouse-phycoerythrin secondary antibody and hrGFP (green - ii), were obtained and overlaid (iii); yellow indicates co-expression of His$_6$-S25R198T with hrGFP in HEK-293 cells infected with AAV-His$_6$-S25R198T-IRES-hrGFP (scale bars = 20 µm).

**Fig 6.**

AAV-His$_6$-S25-R198T viruses mediate partial rescue of the catecholamine release after BoNT/A blockade. The cells were treated with 10 nM BoNT/A for 24 hr before application of AAV-His$_6$-S25 (WT S25 control), AAV-His$_6$-S25R198T (resistant S25) or PBS (-ve control). Catecholamine release was assayed as before. Each bar represents the mean +/- SD of three measurements.