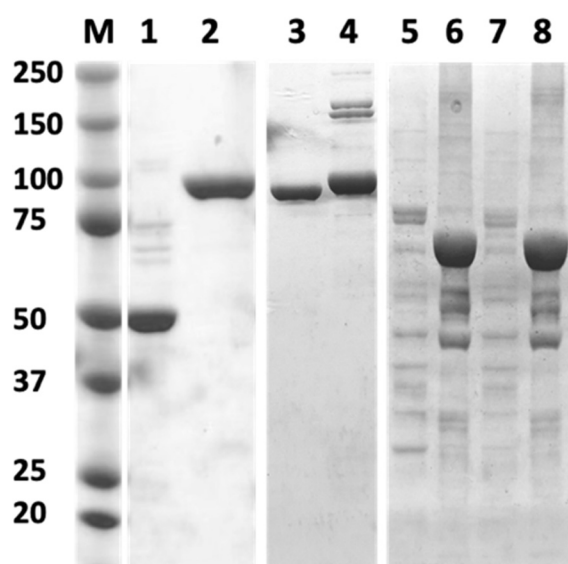


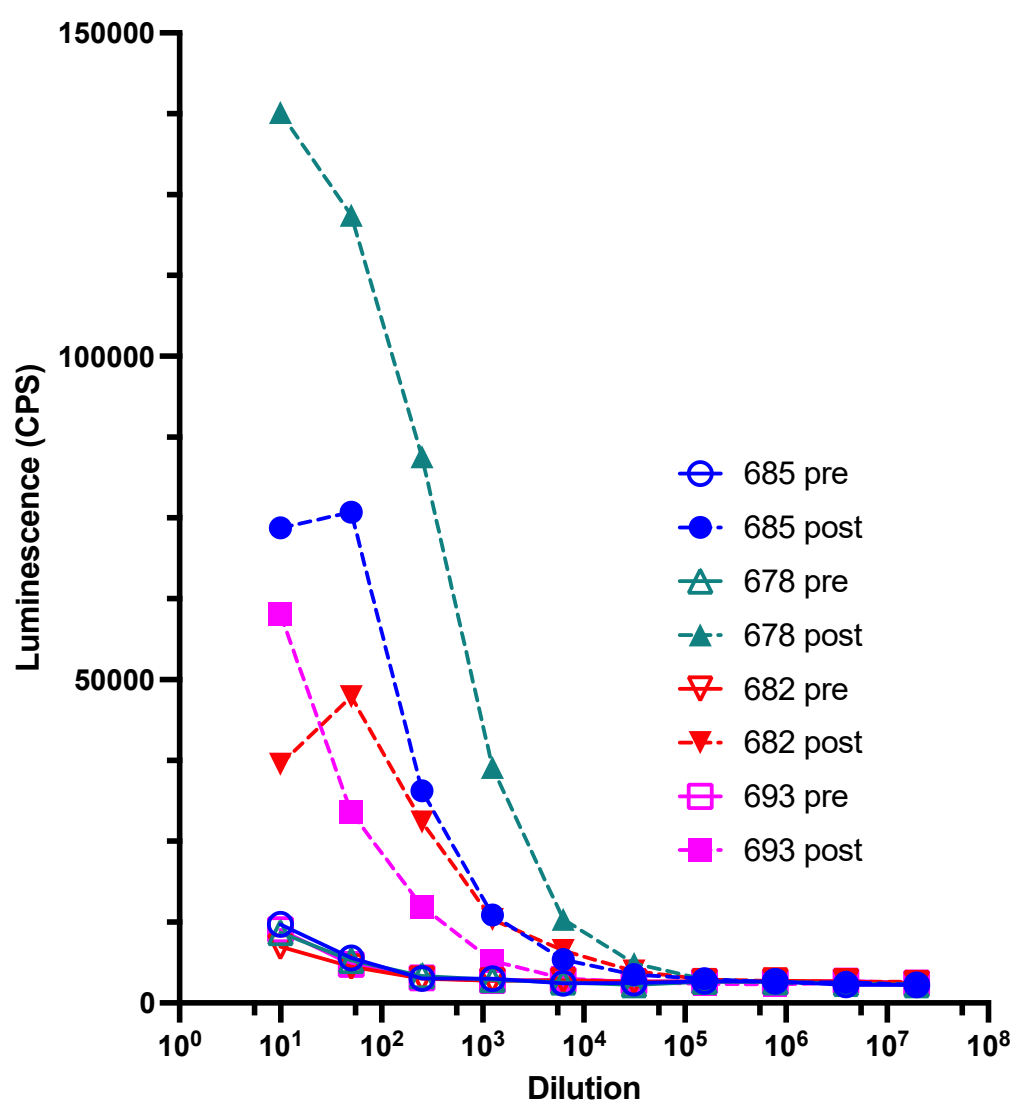
# A three-monoclonal antibody combination potently neutralizes BoNT/G toxin in mice

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**Figure S1.** BoNT/G fragment preparation, SDS PAGE analysis.

SDS-PAGE analysis of the preparation of BoNT/G fragments LC, LCH<sub>N</sub> and Hc. BoNT/G LC (50kDa) showed high expression yield and good purity (lane 1, LC in reduced buffer showing a monomer with 50kDa; lane 2, LC in non-reduced buffer showing a dimer with 100kDa). G LCH<sub>N</sub> (100kDa) also had good expression yield (lane 3, LCH<sub>N</sub> and lane 4, LCH<sub>N</sub>-myc fusion protein). Hc expression was low. Hc variants with various tags. Lane 5: His-tagged GHc 873-1297. Lane 6: MBP-fused Hc 873-1297. Lane 7: His-tagged Hc 877-1297. Lane 8: His tagged Hc 877-1297.

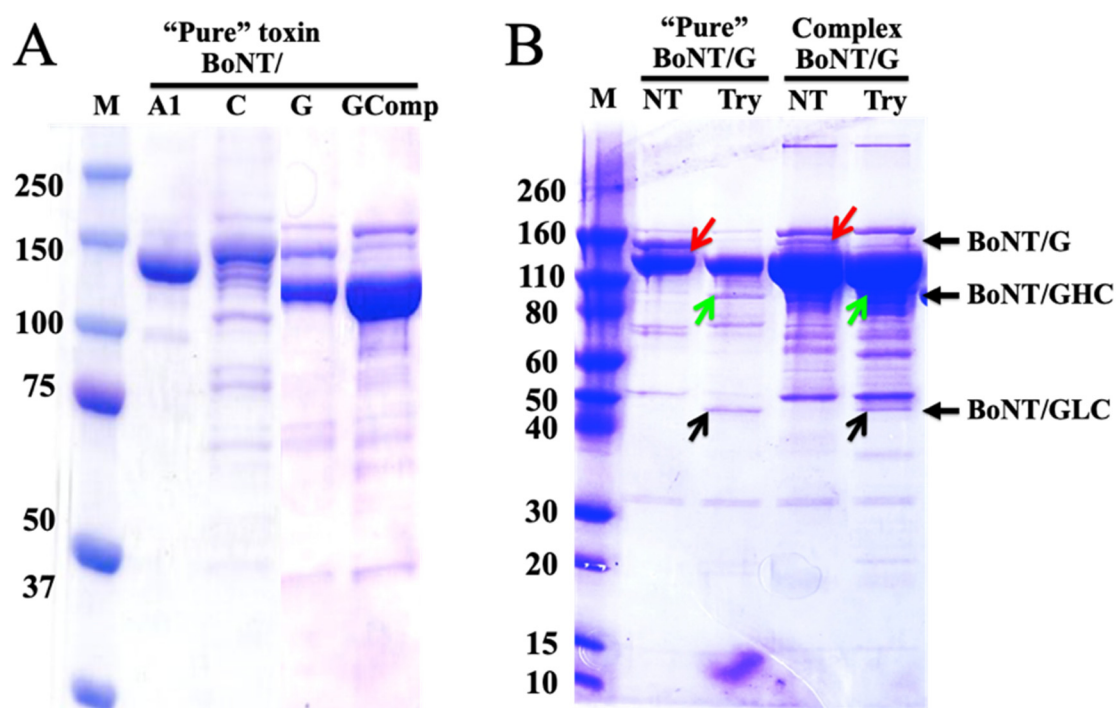


**Figure S2.** ELISA of mouse serum following mouse immunization with BoNT/Gi.

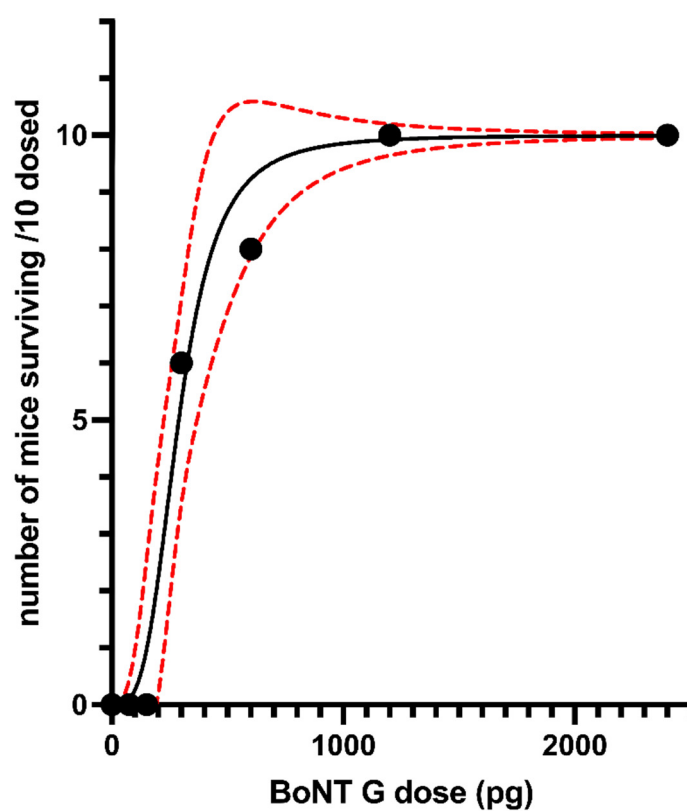
ELISA plate coated with Toxogen BoNT/Gi. Numbers are mouse identifiers. Open symbols indicate pre-immunization solid symbols, final bleed. The number is the mouse identifier.

**Table S1.** Steps in humanization and affinity maturation for the lead mAbs.

| Starting mAb | Step 1             | Resulting mAb | Step 2             | Resulting mAbs                     | Step 3             | Resulting mAbs |
|--------------|--------------------|---------------|--------------------|------------------------------------|--------------------|----------------|
| 6G6          | Humanization       | Hu6G6         | Random mutagenesis | Hu6G6.1                            |                    |                |
| 6G7          | Random mutagenesis | 6G7.1         | humanization       | Hu6G7.1                            | Random mutagenesis | Hu6G7.2        |
| 6G9          | Humanization       | Hu6G9         | Random mutagenesis | Hu6G9.1                            |                    |                |
| 6G10         | Humanization       | Hu6G10        | Random mutagenesis |                                    |                    |                |
| 6G11         | Humanization       | Hu6G11        | Random mutagenesis | Hu6G11.1,<br>hu6G11.2,<br>Hu6G11.3 |                    |                |

**Figure S3.** Metabionics Inc. BoNT/G holotoxin characterization.

**Panel A** SDS-PAGE of the “pure” BoNT/G and the Complex BoNT/G from Metabionics Inc. side by side with BoNT/A and BoNT/C. The molecular weight of the major band in BoNT/G is smaller than BoNT/A1 and BoNT/C and similar to BoNT/G complex. **Panel B** shows SDS-PAGE of trypsinized BoNT/G and BoNT/G complex. Compared with non-trypsinized samples, both BoNT/G and BoNT/G complex lost the band at 150 kDa (red arrows) and gained a band at 100kDa (green arrows) and a band at 50 kDa (black arrows). 4 µg of toxin was used for trypsinization at 37°C.



**Figure S4.** MLD<sub>50</sub> determination for recombinant BoNT/G (Toxogen GmbH) using least squares regression.

Toxin was delivered intraperitoneally to female mice in cohorts of 10. The dotted lines indicate the 95% confidence interval.  $R^2 = 0.978$ .

| BoNT /G Dose (pg)   | Number of mice dead /10 mice dosed |
|---------------------|------------------------------------|
| 2400                | 10/10                              |
| 1200                | 10/10                              |
| 600                 | 8/10                               |
| 300                 | 6/10                               |
| 150                 | 0/10                               |
| 75                  | 0/10                               |
| 0 (Gelatin control) | 0/10                               |