Supporting Information

Onda et al. 10.1073/pnas.1102746108

SI Materials and Methods

Amino acid sequence of toxin present in HA22-LR-PE38:
AKASGGRHRQPRGWEQLPTG AEFLGDGGDVSFSTR GTQNW TVERLLQAHRQLEERGYVFVGYHGTFLEAAQ SIVFGGVRA RSQDLDAIWRGFYIAGDPAL AYGYAQD QEPDARGRIRNGA LLRVYVPRSSLPGFYRTSLT LAA PEAAGEVERLIGHPLPL RLDAITGPEEEGGRLETILGWP LAERTVVIPSAIPTDPRN VGGDLDPSSIPDKEQAISAL PD YASQPGKPPREDLK.

First Ala is the final residue of the HA22 VH.

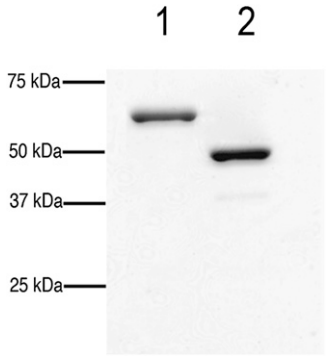

Fig. S1. Nonreducing SDS/PAGE of HA22 (1) and HA22-LR-8M (2) stained with Coomassie blue. HA22-LR-8M migrates as a single protein of the expected molecular weight of 50.5 kDa.

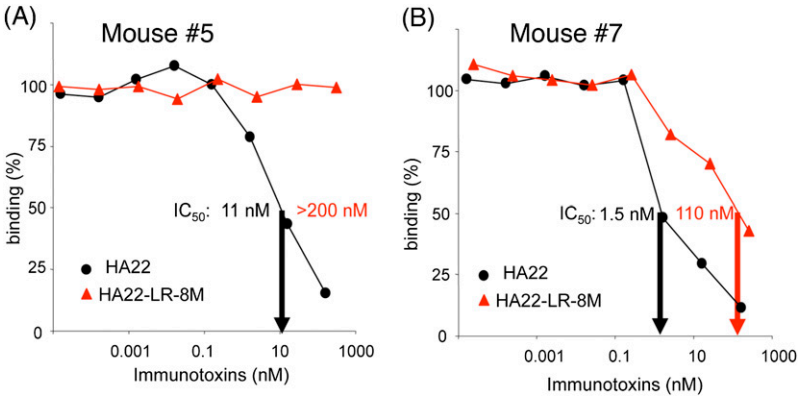

Fig. S2. Mouse antigenicity. Antidrug antibodies were produced by mice immunized with HA22. Typical results of competition assays using mouse serum #5 (*A*) and #7 (*B*) are shown. The concentration of HA22 and HA22-LR-8M at which binding to PE38 was inhibited by 50% (IC_{50}) was 11 nM and >200 nM, respectively, for serum #5 (*A*) and 1.5 nM and 110 nM, respectively, for serum #7 (*B*). The binding (IC_{50}) ratios of HA22 to HA22-LR-8M are <5.5% for mouse #5 and 1.4% for mouse #7. We analyzed serum from a total of seven mice, and their binding ratios are shown in Table 2.

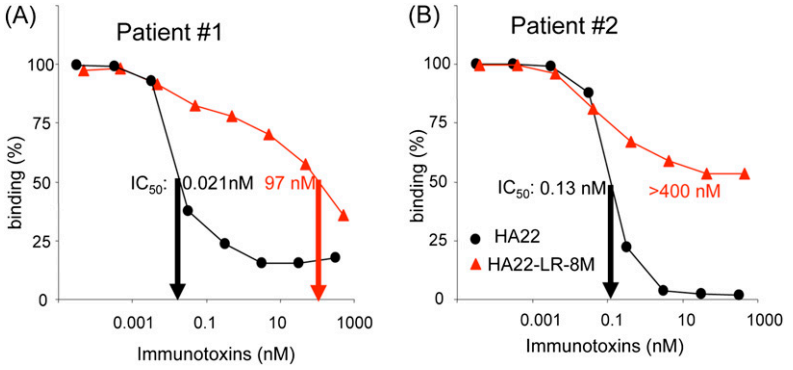

Fig. S3. Human antigenicity. Anti-drug antibodies were produced in patients undergoing clinical trials with HA22. Typical results of competition assays using human serum #1 (*A*) and #2 (*B*) are shown. The concentration of HA22 and HA22-LR-8M at which binding to PE38 was inhibited by 50% (IC_{50}) was 0.021 nM and 97 nM, respectively, for patient #1 (*A*) and 0.13 nM and >400 nM, respectively, for patient #2 (*B*). The binding (IC_{50}) ratios of HA22 to HA22-LR-8M are 0.022% for patient #1 and <0.033% for patient #2. We analyzed serum from a total of nine patients, and their binding ratios are shown in Table 3.