Venom protection by antibody from a snakebite hyperimmune subject

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Abstract:
Snake envenomation is a neglected tropical disease, causing over 100,000 deaths and 300,000 permanent disabilities in humans annually. Here, we recover broadly neutralizing antivenom antibody lineages from the B-cell memory of a human subject with extensive history of snake venom exposure. Centi-3FTX-D09, an antibody from these lineages, recognized a conserved neutralizing epitope on 3-finger toxins (3FTXs), a dominant snake neurotoxin. Crystal structures of Centi-3FTX-D09 in complex with 3FTXs from mamba, taipan, krait, and cobra revealed epitope mimicry of the interface between these neurotoxins and their host target, the nicotinic acetylcholine receptor. Centi-3FTX-D09 provided *in-vivo* protection against diverse 3FTXs, whole venom challenge from cobras, black mamba, and king cobra, and, when combined with the phospholipase inhibitor varespladib, protection against tiger snake, krait, eastern brown, and taipans.

Introduction:

In 2018, snakebite envenoming became ranked in the World Health Organization's list of neglected tropical diseases [1], every year causing 81,000-138,000 deaths and leaving another 300,000-400,000 permanently disabled [2,3]. For over a century, the standard of care for snakebite envenoming has been antivenom: a polyclonal serum preparation derived from animals immunized with venom from one or more snakes [4]. While effective, antivenom serotherapies present several challenges. Treatment with non-human antibodies results in early-onset adverse reactions in 59% of subjects, which can lead to more severe reactions if the antivenom were to be ever used again in the same patient [5]. Envenomed patients typically require 5 or more doses of antivenom, as polyclonal sera derived Fab formulations have short half-lives, are contaminated with 5-22% non-antibody proteins, and have been reported to contain only 9-15% of venom-specific antibodies to any given snake in the case of polyvalent polyclonal antivenoms [6–8]. Moreover, antivenom developed for a single species requires the correct identification of the specific snake that bit the victim, which is often not possible for victims or healthcare workers not well-versed in snake phenotypes [6,9]. Although a global public health problem, research into improved envenoming treatments is hampered by the heterogeneity of venom toxins across species, the relatively low market value of a disease primarily afflicting the developing world, and the great fracturing of that market by differing requirements of the many different species-restricted antivenoms [10].

Although desirable, a single broad-spectrum, fully-human, antivenom has never been developed, predominantly due to complexity of snake venom and diversity of snake species. There are over 500 genetically diverse venomous snake species globally that span 167 million years of Toxicofera evolution [11]. Each snake produces 5-70 fractions, or unique proteins, in their venom, and there is abundant polymorphism of venom proteins even between individuals of a single snake species [12]. Thus, even if an antibody were discovered for every venom fraction of every venomous snake, it would not be possible to combine all of these antibodies into a single antivenom, as each antibody would be at far too low of a dose to be therapeutically relevant [13–15].

In this study, we sought to determine whether broadly neutralizing antibodies could protect mice against entire classes of snake toxins as well as whole venom challenge. We chose to begin with the dominant neurotoxin of the elapid family of snakes [16–18]. The elapid family of snakes account for ~60% of venomous snake species and includes over 50 genera, 300 species, and over 170 subspecies [19]. The α-neurotoxin three-finger toxins (3FTXs) are amongst the deadliest toxins in elapid venom [20]. 3FTX α-neurotoxin orthologs are found in nearly all elapid neurotoxic snakes, where they bind with high affinity to both muscular (alpha-1/CHRNA1) and neuronal (alpha-7/CHRNA7) nicotinic acetylcholine receptors (nAChR) and impair neuromuscular and neuronal transmission [21]. The extreme conservation of the nAChR/acetylcholine interface across all vertebrates enables a single toxin to productively paralyze neurons from mammals, birds, reptiles, amphibians, and fish. This evolutionary constraint also requires that 3FTXs from all snake species maintain a conserved interface with the nAChR [22], thereby presenting a plausible epitope target for broadly neutralizing anti-3FTX antibodies. Here we report the isolation of such a broadly neutralizing antibody, and characterization of *in-vivo* protection by this antibody alone and in combination with varespladib [23], a broad-spectrum small-molecule PLA2 inhibitor.

Results:

***In-vivo* and *in-vitro* selection for broadly neutralizing 3FTx antibodies.**
Broadly neutralizing antibodies (bnAbs) against snake neurotoxins were isolated from an adult male subject with an extensive history of diverse snake venom exposure (**Fig. 1a**).

A hyper-immune human subject was identified with a documented 18-year history of 756 immunizations to venoms spanning 2001 to 2018 (**Fig. 1b**). Immunizations included mambas (*D. polylepis*, *D. viridis*, *D. angusticeps*, *D. jamesoni*), cobras (*N. kaouthia*, *N. haje*, *N. melanoleuca*, *N. nivea*), rattlesnakes (*C. atrox*, *C. scutulatus*), water cobras (*N. annulata*, *N. cristyi*), taipans (*O. scutellatu*s, *O. scutellatus canni*), as well as *M. fulvius* (coral snake), *Bungarus caeruleus* (common krait), *Bungarus multicinctus* (banded krait), *Notechis scutatus* (tiger snake), and *Pseudonaja textilis* (eastern brown snake) (**Fig. 1b**). Hypothesizing that this repeated cyclical pattern of diverse venom exposure may have selected for broadly-reactive antivenom antibodies that recognized conserved epitopes shared across the venom toxins of multiple snake species, we sought to isolate such bnAbs from this subject.

In a non-interventional study design, two 20ml samples of blood were collected during the subject's standard self-immunization schedule: one 20ml collection following a three-month venom immunization holiday (Day 0), and the second 20ml collection 7 days following the end of the subject's 21-day routine self-immunization schedule with black mamba, western diamondback, and coastal taipan (Day 28). Blood samples were separated into plasma and PBMCs (see **Methods:Plasma & PBMCs**). Relative to snake venom-naive healthy control plasma, significantly elevated antibodies were detected in hyperimmune subject plasma against a panel of α-neurotoxin from black mamba, cape cobra, coastal taipan, and common krait to a level of 1:10,000 dilution (**Fig. 1c, see Methods:Serum ELISA**). From the PBMCs, B-cell receptor repertoire DNA was isolated from each blood sample, and the isolated repertoires underwent high-throughput sequencing (**see Methods:Amplicons**). The total VH repertoire somatic hypermutation (SHM) rate was elevated in the hyperimmune subject at Day 0 (7.32 +/- 2.69) compared with a panel of healthy adult controls (5.66 +/- 2.52) (**Fig. 1d; see Methods:Informatics**). The SHM rate increased at Day 28 following the immunization schedule (7.65 +/- 2.61). The SHM rate was further significantly elevated in the venom-specific repertoire of the subject (8.61 +/- 2.23), obtained by enrichment panning against biotinylated whole venom from 4 snake species. The SHM rate was highest in the 64 broadly cross-reactive antivenom antibodies isolated against homologs of α-neurotoxin (12.06 +/- 1.62)**.** Thus, the hyperimmune subject appeared to have an elevated total SHM profile compared with healthy controls, this elevated SHM profile was enriched in the antivenom-specific repertoire, and particularly elevated in isolated broadly neutralizing antibodies.

From the hyperimmune subject's PBMCs, broadly neutralizing anti-3FTX monoclonal antibodies were isolated by phage display **(Fig. 1f)**. From 40ml of blood, containing approximately 16 million B cells, antibody Fv VH and VL domains were amplified by multiplex phage adaptor PCR. Sequencing of 4.3 million amplicons identified at least 45,174 unique CDR-H3 VH sequences and 36,817 unique CDR-L3 sequences, excluding singletons. These domains were assembled combinatorially into an m13 VH-VL scFv-pIII fusion display vector and transformed to a final library size of 2.21e9, such that every observed VH domain was associated

with every VL domain in the library, including every original native VH/VL pair (**see Methods:Library**).

To isolate broadly reactive antibodies from the hyperimmune subject antibody library, the library was sequentially panned against four recombinant α-neurotoxin ortholog representatives from four diverse genera of elapidae. The four orthologs chosen were alpha elapitotoxin-Dpp2a from *Dendroaspis polylepis* (black mamba) of Sub-Sarahan Africa, alpha-cobratoxin/long neurotoxin 1 from *Naja nivea* (Cape cobra) of Southern Africa, Long neurotoxin 1 from *Oxyuranus microlepidotus* (inland taipan) from Australia, and Alpha-delta bungarotoxin from *Bungarus caeruleus* (common krait) from Asia. Evolutionarily, these four distinct genera of elapids span 40 million years since most recent common ancestor, with highly diverse 3FTX orthologs that share only 48-64% identity at the amino acid level (**Fig. S1**). Clinically, they represent some of the deadliest elapids to humans. The four recombinant α-neurotoxin orthologs were expressed with C-terminal site-specific biotinylation avitags in HEK293 cell culture, tag-purified, and lethal neurotoxic functional activity was confirmed *in vivo* in C57BL/6 mouse Maximum Tolerated Dose (MTD) studies (**Methods:Recombinants**).

Four sequential rounds of soluble-phase automated panning were performed, using a different ortholog of α-neurotoxin in each subsequent round to select for breadth (**Fig. 1a, Methods:Panning**). Following the third round, Illumina MiSeq repertoire sequencing was performed, observing approximately one thousand unique enriched VH clonal variants (**Fig. 1f**), as well as a significant enrichment of clones with more somatic hypermutation than observed in the hyperimmune subject's total repertoire (**Fig. 1e**). Following the fourth round of selection, 376 clones were isolated and screened for reactivity to the cobra, taipan, krait, and mamba α-neurotoxin by ELISA (**Fig. 1f, see Methods:PPE Screening**). Sixty-four broadly reactive neurotoxin-specific clones were identified, and upon sequencing 61 of the 64 (95%) were found to be from a single dominant lineage D09 that utilized the same VDJ rearrangement and contained the clone Centi-3FTX-D09 as a member (**Fig 1g,h; see Methods:Informatics**). The D09 lineage utilized IGHV3-13 V-gene segment, a long (19 amino acids) CDR-H3 loop with a 7 amino acid non-templated V-D junctional region, and bore evidence of extensive somatic hypermutation, with 22 amino acid mutations relative to germline (77.4% ID) in the heavy chain. The mutations were concentrated in the CDRs, with 9 mutations in CDR-H2, 2 mutations in CDR-H1, and 1 mutation and 8 non-templated amino acids in CDR-H3 (**Fig. 1g**). Six definitive heavy chain SHM variants were identified, all showing evidence of extensive SHM and a common origin from a single parental B-cell clone. The light chain had a significant restriction to IGKV1-39 (85%), with SHM mutations including 3 in CDR-L1, 1 in CDR-L2, 4 putative non-templated base mediated amino acids in CDR-L3, and 4 mutations in the frameworks. Neither germline reversion of all 8 framework SHM mutations in the VH domain nor all 4 framework SHM mutations in the VK domain impacted binding, and both Centi-3FTX-D09 and germlined D09 lineage variants exhibited unusually high thermostability and aggregation resistance (75 °C Tm1, 75 °C Tagg 266) (**Fig. S2**).

Affinity and breadth of Centi-3FTX-D09

Initial ELISA screening indicated broad reactivity of lineage D09 to the representative recombinant long neurotoxins from *Naja nivea* (cape cobra), *Bungarus caeruleus* (common krait), *Oxyuranus scutellatus* (coastal taipan), and *Dendroaspis polylepis* (black mamba) (**Table S1**).

Kinetics assays using Surface Plasmon Resonance (SPR) performed on Biacore 8K demonstrated picomolar affinity monovalent interactions between lineage member Centi-3FTX-D09 and long neurotoxins from diverse species: <74pM to black mamba, <37pM to Cape cobra, and 490pM to coastal taipan, as well as high affinity interactions to these toxins by Centi-3FTX-B11, another member of the lineage (**Fig. 2a-c; Table S2; Methods:Kinetics**).

To further characterize the breadth of Centi-3FTX-D09 reactivity, we used biolayer interferometry to assess the reactivity of Centi-3FTX-D09 to whole venom from 20 elapid species. In 18 species, we observed clear reactivity (**Fig. 2d**) and, in 2, *Naja mossambica* and *Pseudechis papuanus*, we observed no or marginal reactivity (**Fig. 2e**). Consistent with this observation, *Pseudechis papuanus* venom has been reported to contain no α-neurotoxin [24], and for both species, no long neurotoxin 3FTX family members were found in Uniprot. As negative controls, we also tested two viperid venoms not expected to contain α-neurotoxin (from *Daboia russelii* and *Crotalus atrox)* and observed no/marginal reactivity (**Fig. 2f**).

To confirm the reactivity in venom corresponding to recognition by Centi-3FTX-D09 of a 3FTX, we performed immunoprecipitation pull-down and mass-spectrometry peptide sequencing experiments with Centi-3FTX-D09 and whole venom and observed clear evidence for 3FTX binding with *D. polylepsis*, *N. naja*, *N. annulifera,* and *H. haje*, but not with *D. russelii*, *C. atrox* (**Fig. 2g).** Mass-spectrometry confirmed the specific identity of the pulled-down peptides to be α-neurotoxin in every instance where a band was observed.

Overall, these binding experiments demonstrate high affinity of Centi-3FTX-D09, with breadth extending to most 3FTXs.

Crystal structures of Centi-3FTX-D09 with 3FTXs reveal similarity in recognition between antibody and acetylcholine receptor

To provide an atomic-level explanation for the broad reactivity of Centi-3FTX-D09 with 3FTXs, we determined crystal structures of the antigen-binding fragment (Fab) of Centi-3FTX-D09 in complex with 3FTXs from *Naja nivea* (cape cobra), *Bungarus caeruleus* (common krait), *Oxyuranus scutellatus* (coastal taipan), and *Dendroaspis polylepis* (black mamba) (**Fig. 3a** and **Table S3**). Analysis of the interfaces between Centi-3FTX-D09 Fab and 3FTXs from these diverse 3FTXs revealed buried surfaces areas of ~1600 $Å^2$, shared half between Fab and toxin (**Table S3**). The interactive surface area was contributed primarily by the heavy chain 3rd complementarity determining region (CDR H3), although CDR H1 and L1 and L2 contributed a mixture of hydrophobic interactions and H-bonds.

Comparison with the previously determined structure of the Krait 3FTX with the nicotinic acetylcholine receptor [25] revealed the antibody CDR H3 to approximate the position of "loop C" of the acetylcholine receptor, as part of an interface that was in total ~15% larger (**Fig. 3b** and **Table S4**). On the toxin, the key $Arg36_{Toxin}$ extends over $Phe32_{Toxin}$, to form critical hydrogen bonds with the phenol of $Tyr95_{Receptor}$ and the backbone carbonyl of $W151_{Receptor}$; on antibody Centi-3FTX-D09, the aliphatic portion of the $Arg36_{Toxin}$ sidechain extends over $Phe32_{Toxin}$, is further sandwiched by $Tyr106_{HC}$, and forms a bridge with $Asp111_{HC}$ (**Fig. 3c**). Meanwhile, $Tyr32_{LC}$ on the light chain was positioned similarly to $Trp151_{Receptor}$ helping to cradle $Phe32_{Toxin}$ and $Arg36_{Toxin}$.

Analysis of the conservation in acetylcholine receptor and toxin revealed the interface to be highly conserved (**Fig. 3d; Fig. S1; see Methods:Conservation**). In general, antibody and receptor interacted with similar toxin residues (**Fig. 3e**).

Collectively, these results reveal antibody mimicry of acetylcholine receptor to enable broad recognition of toxin by antibody.

In-vivo protection by Centi-3FTX-D09 with live challenge by recombinant α-neurotoxin and whole venom

To determine to what degree broad reactivity *in vitro* would translate to protective neutralization *in vivo*, we performed recombinant neurotoxin and whole venom live challenge experiments in C57BL/6 mice. (**see Methods:In Vivo**).

We began by evaluating whether Centi-3FTX-D09 could provide *in-vivo* protection versus live challenge of recombinant 3FTXs of *Naja nivea* (Cape cobra), *Bungarus caeruleus* (common krait), *Oxyuranus scutellatus* (coastal taipan), and *Dendroaspis polylepis* (black mamba). The recombinant 3FTX from these four snakes, when injected at the pre-determined LD_{100} of 0.5 mg/kg for common krait and 1.0 mg/kg for the other three species, caused 100 percent lethality starting at 60min to 2 hours, with the exception of one mouse surviving the *Naja nivea* injection. Pre-mixed injections of recombinant 3FTX with 30mg/kg Centi-3FTX-D09 provided durable protection beyond 24 hours in all cases (**Fig. 4a**). Thus, Centi-3FTX-D09 provided broad neutralizing protection from 3FTXs from diverse elapids.

Whole venom contains multiple toxic fractions, each of which could contribute to lethality. In order to deconstruct the relative contribution of the 3FTX, we first performed whole venom challenge protection studies with Centi-3FTX-D09 as a single agent, on a panel of snakes where the 3FTX was the majority constituent, representing 63-88% of whole venom [12]. As a single agent, Centi-3FTX-D09 provided robust protection from lethal whole-venom challenge for multiple species of cobra (**Fig. 4b**). Complete protection by Centi-3FTX-D09 was observed for *Naja nivea* (Cape cobra), *Naja kaouthia* (Monocled cobra), and *Naja haje* (Egyptian cobra), and for *Naja naja* (Indian cobra), 9/10 mice were protected by Centi-3FTX-D09 (**Fig. 4b**). Centi-3FTX-D09 additionally provided complete protection from lethal whole-venom challenge for the non-cobra species *Dendroaspis polylepis* (black mamba) and *Ophiophagus hannah* (King cobra) (**Fig. 4c**).

After 3FTX, Phospholipase A2 (PLA2) is the next most abundant toxin in most elapid venoms. To further deconstruct the contribution of other toxins, we performed whole venom challenge studies with varespladib, a broad-spectrum PLA2 inhibitor, alone and in combination with Centi-3FTX-D09, in a panel of five additional elapid genera.

Notechis scutatus (tiger snake) venom, consisting of 74.5% PLA2 and 5.6% 3FTX [12], was 100% lethal after 2 hours post-injection (**Fig. 4d**). In the mice receiving Centi-3FTX-D09 alone, 1 out of 5 mice survived a total of 10 hours and 4 out of 5 mice survived an additional 30 minutes relative to venom alone. Varespladib alone or in combination with Centi-3FTX-D09 was protective against Tiger Snake lethality for 24 hours, and complete protection was obtained with when varespladib was redosed every 8 hours to account for short PK.

A similar pattern was observed for taipans. *Oxyuranus microlepidotus* (inland taipan) venom, consisting of 38% PLA2 and 12.2% 3FTX [12], was 100% lethal in under two hours after injection (**Fig. 4e**). *Oxyuranus scutellatus* (coastal taipan) was 100% lethal at 24 hours. The use of Centi-3FTX-D09 marginally extended time to death by a few minutes for venom from both species. Varespladib alone or in combination with Centi-3FTX-D09 was completely protective against a lethal dose of inland and coastal taipan venom.

Bungarus caeruleus (common krait) venom, consisting of 64.5% PLA2 and 19% 3FTX [12], caused 100% lethality in mice after 3 hours post-injection (**Fig. 4f**). Both Centi-3FTX-D09 and varespladib doubled survival time to 6 and 7 hours, respectively. The combination of Centi-3FTX-D09 and varespladib demonstrated synergistic protective effects, with 4 out of 5 mice surviving 12 hours, and one mouse recovering completely. Redosing with varespladib every 8 hours did not further protect from death.

Pseudonaja textilis (eastern brown) venom was 100% lethal after 2 hours after injection (**Fig. 4g**). When Centi-3FTX-D09 was administered with venom, 2 out of 5 mice had extended survival to 6 hours. Varespladib alone or in combination with Centi-3FTX-D09 extended survival of all mice dosed to 6 hours. With repeated redosing of varespladib every 8 hours, two mice survived to 24 hours but then died.

Laticauda colubrina (Banded sea krait) venom, consisting of 33% PLA2 and 66% 3FTX [12], was lethal in 4 out of 5 mice injected 40 minutes after injection. Varespladib alone did not provide protection. Two of 5 mice receiving venom and Centi-3FTX-D09 survived indefinitely (**Fig 4h**). Varespladib and Centi-3FTX-D09 extended survival of mice to 60-120 minutes, although all mice had died by 120 minutes.

In a phylogeny of α-neurotoxin homologs from elapidae, Centi-3FTX-D09 provided complete *in-vivo* protection from venom from 6 species spanning 3 genera in clades A, C, and D, and partial protection to members of clades B, E, and F (**Fig 4i**). When combined with varespladib, complete protection was extended to members of clades A-E, spanning Africa, Asia, and Oceana, and partial protection for clade F (oceans). Protection for three species (banded sea krait, eastern brown, and common krait) remained partial for the cocktail at the doses of venom and cocktail evaluated here. Group G, the only clade exhibiting some venoms non-reactive to Centi-3FTX-D09, was not evaluated *in vivo*.

Discussion

Here we report Centi-3FTX-D09, a broadly neutralizing anti-3FTX antibody capable of binding and neutralizing 3FTXs from diverse genera of snake venom. A broadly neutralizing antibody against the 3FTX neurotoxins is useful, as no small molecule inhibitors for neurotoxins have been identified, unlike the small molecule inhibitors reported for PLA2, SVMP, and SVSP toxins [26].

As a single agent, Centi-3FTX-D09 provided complete protection from lethal challenge by four cobra species, black mamba, and king cobra venom, as well as partial protection against tiger snake, eastern brown, and common krait. Marginal partial protection was also observed for Javan spitting cobra, green mamba, and banded sea krait. Given the diversity of genera recognized, it is

possible that Centi-3FTX-D09 could provide protection against venom neurotoxicity for additional members of the 300 venomous species of elapidae.

When combined with a broad-spectrum inhibitor of PLA2 (varespladib), we observe broad protection against a representative panel of 11 snake species, including 8 genera of elapidae. We observed synergistic protection in some venoms, as well as some venoms that were unilaterally protected by Centi-3FTX-D09 or varespladib alone. Given diversity of genera recognized, it is possible that this cocktail could provide complete protection against whole venom for additional members of the 300 venomous species of elapidae.

In some live challenges, we observed delayed death of all animals at 6, 12, or 24 hours following the use of Centi-3FTX-D09 and varespladib as a cocktail. When providing the cocktail followed by redosing of varespladib at 8-hour intervals, full protection was achieved in some additional venoms, confirming that the short (5.5 hour) pK_a of a single injection of *varespladib* was the source of delayed death [27]. In future studies, replacing varespladib with a broadly neutralizing anti-PLA2 antibody may be a path to extend protection indefinitely with a single injection.

These antibodies were isolated from a hyper-immune individual with a history of heterosubtypic venom immunization exposures that resulted in the generation of potent and broadly cross-neutralizing antibodies against snake venom homologous toxins. From structural and homology analysis of the resulting antibodies, it is evident that the broadly neutralizing antibodies function by exploiting the conserved active sites in this toxin family that have been unable to vary due to a requirement over evolutionary history of being able to target conserved receptor sites in a wide array of vertebrates. As active sites and binding sites are more conserved as a general property of functional proteins, we can anticipate that similar broadly neutralizing antibodies may be able to be recovered for other snake toxin families.

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List of Supplementary Material

Methods and Materials

Figure S1. 3FTX conservation.

Figure S2. Nicotinic acetylcholine receptor (nAChR) conservation.

Figure S3. Centi-3FTX-D09 – recombinant monovalent SPR kinetics.

Table S1. ELISA screening.

Table S2. X-ray data collection and refinement statistics.

Table S3. Antibody D09-3FTX interface analysis.

Table S4. Acetylcholine receptor- Krait 3FTX interface details.

Table S5. Identification of immunoprecipitated proteins with mass spectrometry.

Figure Legends

Figure 1. In-vivo and in-vitro selection for broadly neutralizing 3FTx antibodies. a, Immune library from hyperimmune subject was sequentially panned against recombinant a-neurotoxins from cobra, taipan, mamba and krait. **b,** Documented immunization history of hyperimmune subject, grouped by genus. **c,** Serum reactivity to recombinant a-neurotoxins, hyperimmune subject versus venom-naive donors. **d**, Antibody repertoire somatic hypermutation. **e,** Down-selection and screening of 16 million B cells to identify final lead D09 antibody lineage. **f**, D09 lineage mutational variants for confirmed binders of **(top**) heavy and (**bottom)** light chain Fv domains (mutations from germline in purple; VDJ non-templated base positions in salmon.

Figure 2. Affinity and breadth of Centi-3FTX-D09. a-c, Monovalent affinity by Biacore 8K surface plasmon resonance kinetics of Centi-3FTX-D09 versus monovalent recombinant for **a**, black mamba, **b**, cape cobra, and **c**, coastal taipan. **d-f**, Whole venom Biolayer interferometry reactivity of Centi-3FTX-D09 versus **d**, eighteen elapid species with confirmed reactivity; **e,** two elapid species with no/marginal reactivity, and **f,** two negative control viperid venoms with no known 3FTX (black: offtarget mAb vs venom at 500nM, Red: Centi-3FTX-D09 vs venom 500nM, orange: Centi-3FTX-D09 vs venom 250nM) **g,** Immunoprecipitation by Centi-3FTX-D09 of 3FTXs from whole venom (3FTX identity confirmed by mass-spectrometry as detailed in Table S5).

Figure 3. Crystal structures of D09 with 3FTXs reveal similarity in recognition between antibody and acetylcholine receptor (nAChR). a, Crystal structures of Fab Centi-3FTX-D09 in complex with Taipan, Cobra, Mamba and Krait toxins. **b**, Toxin recognition of acetylcholine receptor (left) and Centi-3FTX-D09 (right) **c**, Details, **d**, Binding surfaces on nAChR and toxin (left); Centi-3FTX-D09 recognized a conserved surface on toxins (right) which closely overlaps with the surface used by toxin to bind acetylcholine receptor (middle). Molecular surfaces colored by sequence conservation, with white indicating 100% conservation. **e,** Toxin residues involved in binding of Centi-3FTX-D09 and nAChR receptor. Both Centi-3FTX-D09 and nAChR bound the same toxin residues.

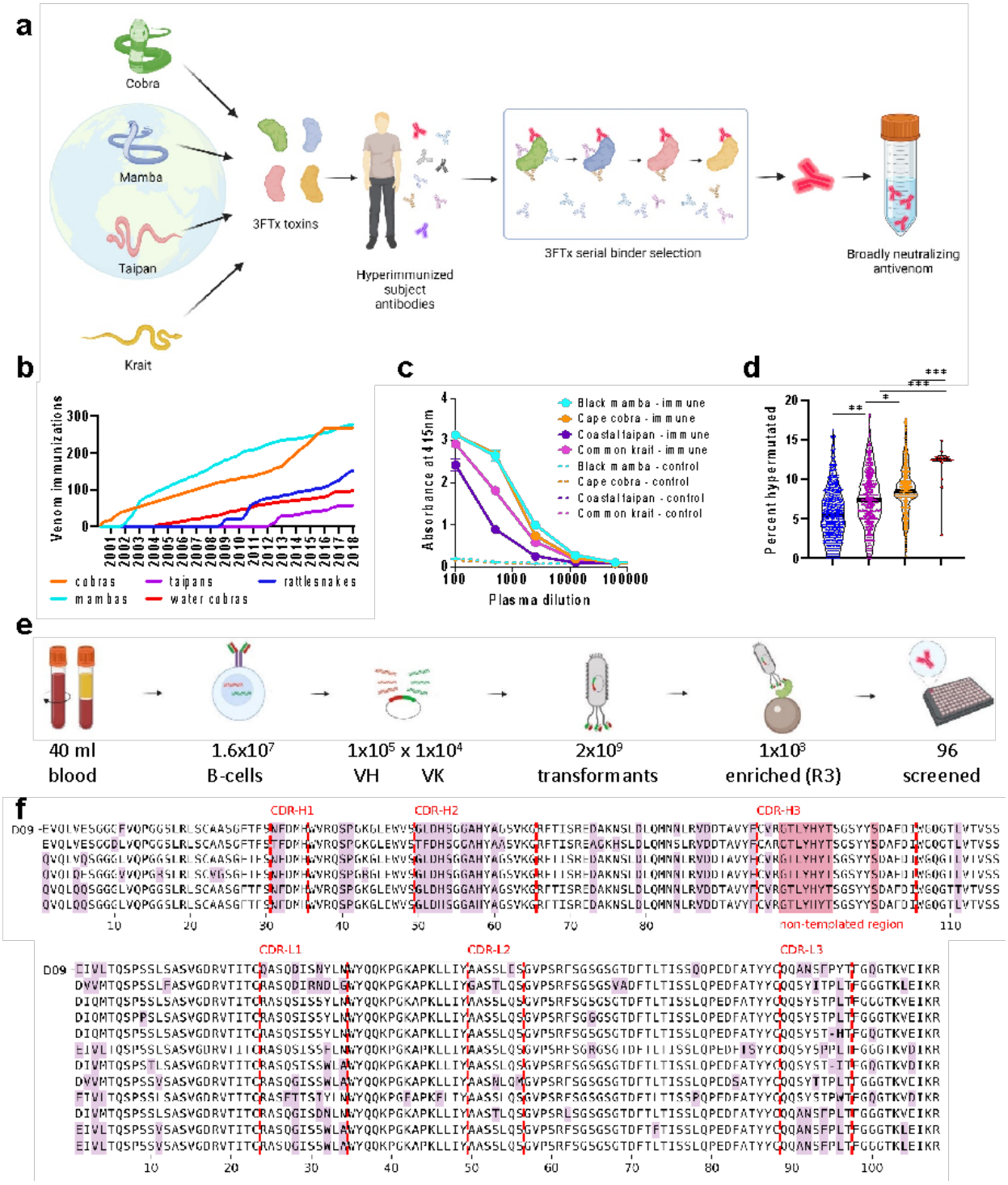
Figure 4. In-vivo protection by D09 with live challenge by recombinant a-neurotoxin and whole venom. a-c, Kaplan Meier survival curves for C57BL/6 mice injected intraperitoneally. Centi-3FTX-D09 full protection *in vivo* challenge with **a**, recombinant 3FTX from four elapid species (n=10) and whole venom live challenge (n=5), including **b,** four species of cobra, and **c**, whole venom from two non-cobra elapids. **d-h**, Full protection for cocktail of Centi-3FTX-D09 and PLA2 inhibitor (varespladib) for **d**, whole venom from tiger snake, and **e**, whole venom from two species of taipan. Partial protection for cocktail of Centi-3FTX-D09 and varespladib for **f**, common krait, **g** eastern brown snake, and **h**, yellow lipped sea krait. **i**, Phylogenetic and geographic breadth of Centi-3FTX-D09 and varespladib *in-vivo* protection to snake venom (lower case letters on dendrogram related to panel of this figure where venoms were assessed).

Supplementary Figure S1. 3FTX conservation. a, Sequence alignment of 3FTXs from diverse snake venoms. **b**, Pairwise identity (%) between 3FTXs.

Supplementary Figure S2. Nicotinic acetylcholine receptor (nAChR) conservation. a, Sequence alignment of diverse nAChRs **b**, Neighbor-joining tree of diverse nAChRs.

Supplementary Figure S3. Centi-3FTX-D09 binding kinetics. a, Sensorgrams for mamba, taipan, and cobra 3FTX interaction with Centi-3FTX-D09. **b**, Binding kinetic parameters.

Figures



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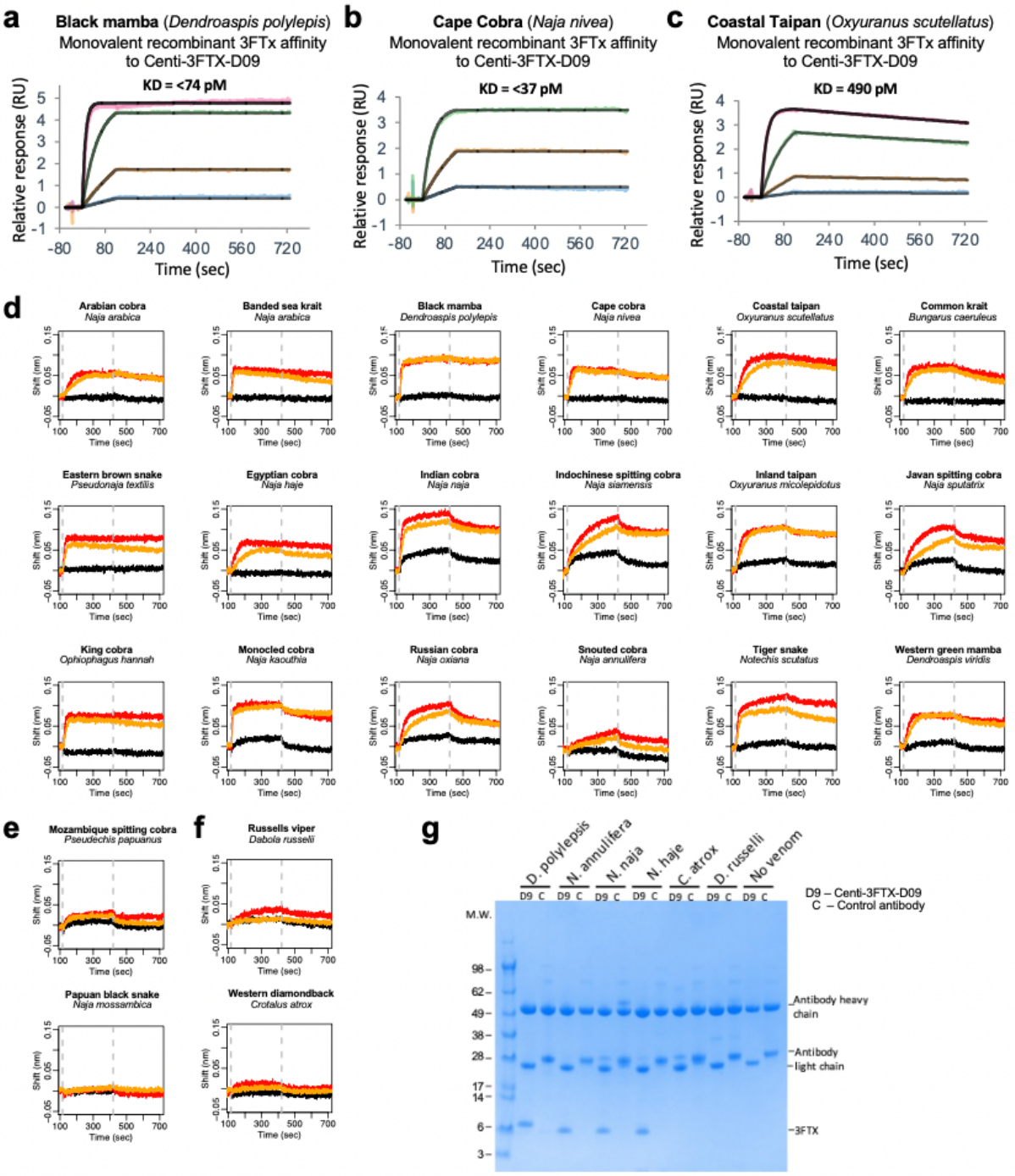

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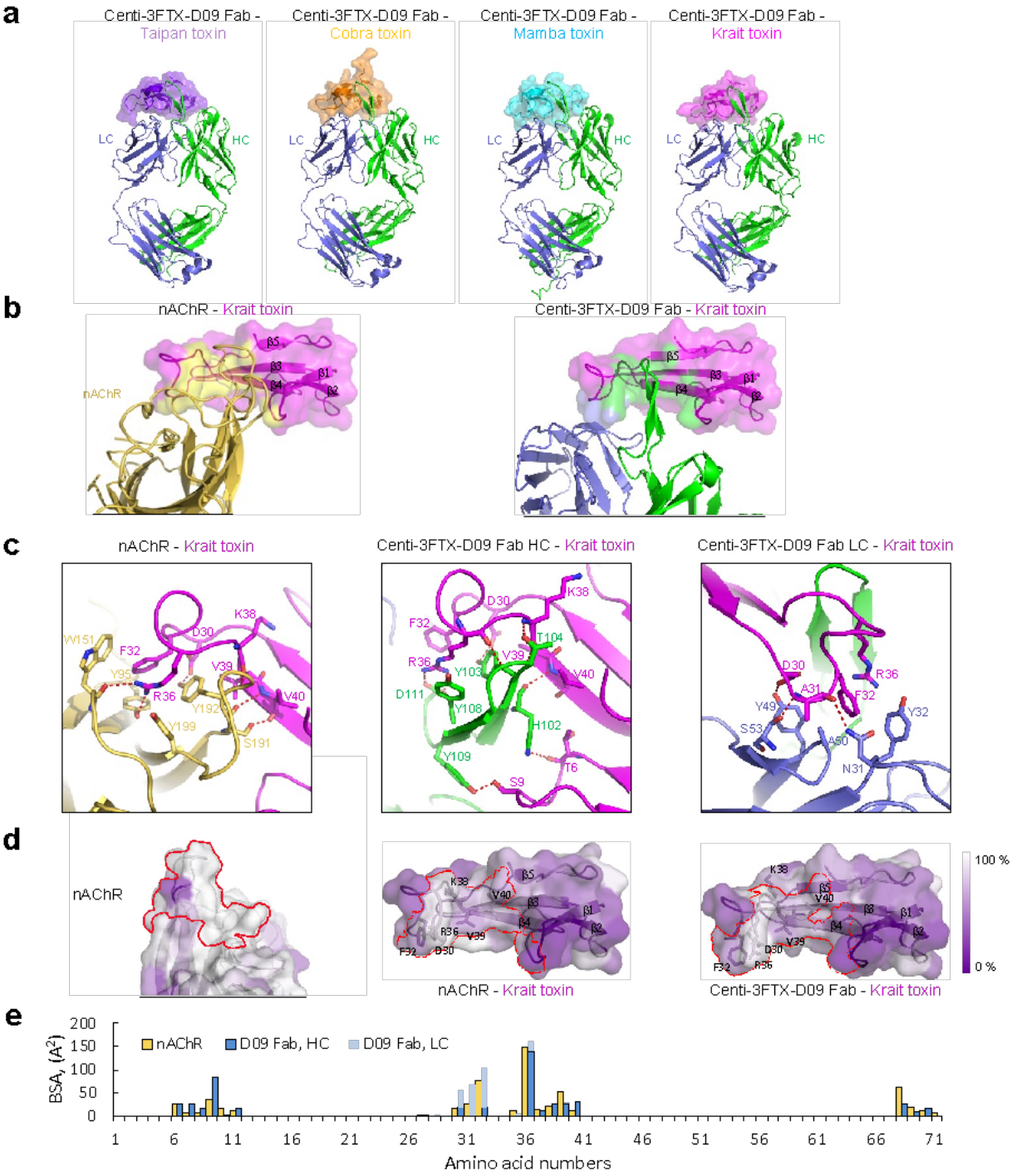

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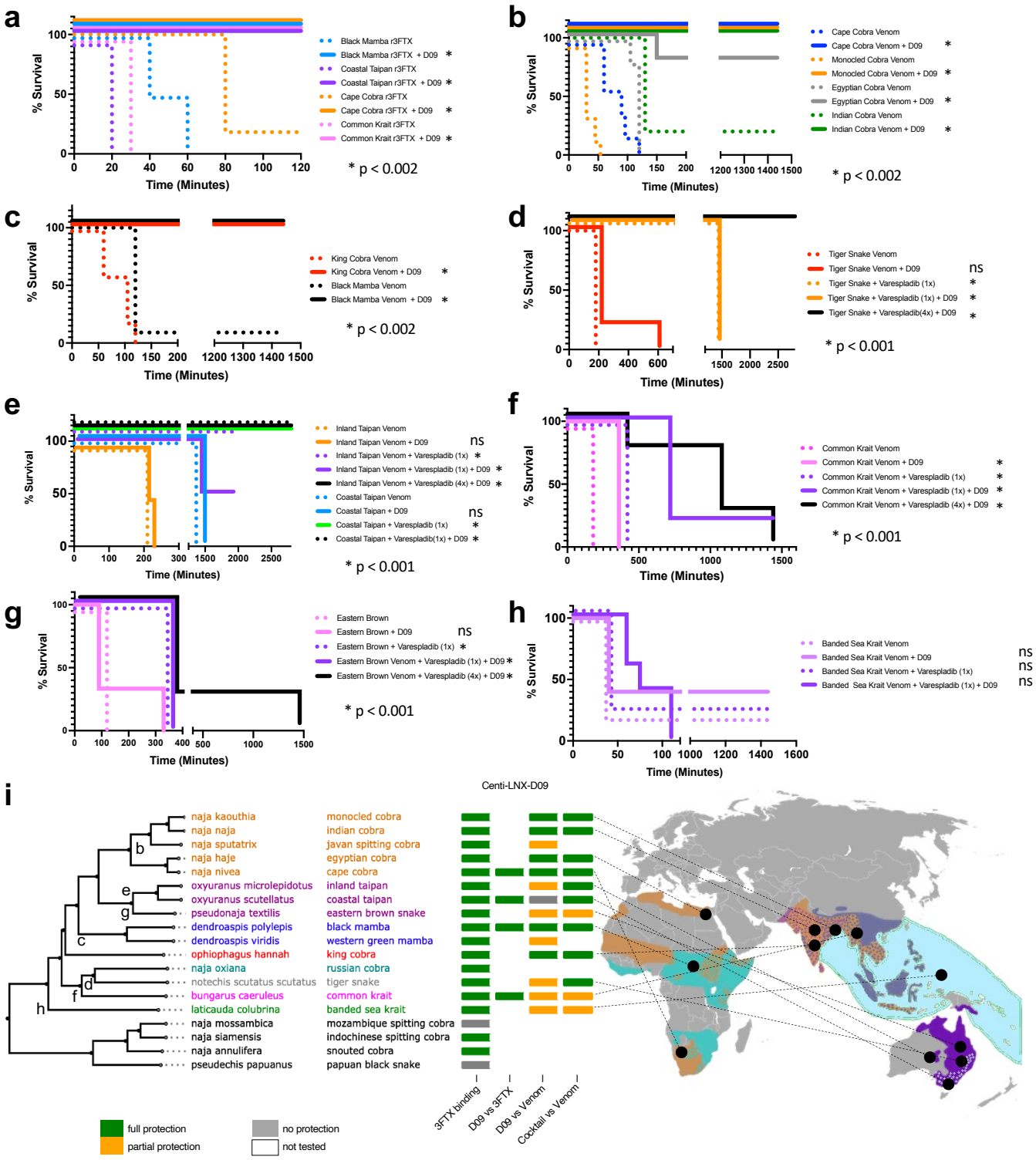

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