

## Supplementary Information

### Supplementary Material and Methods

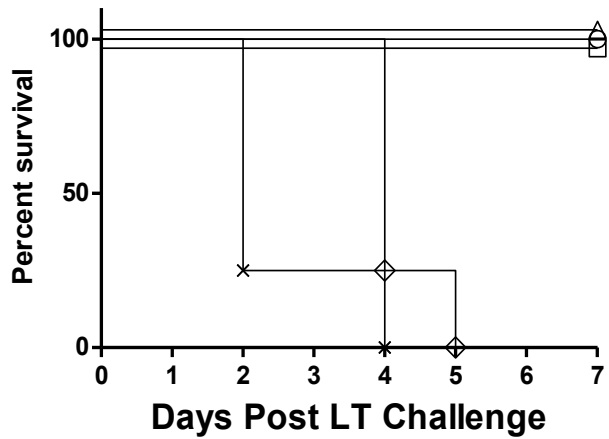
**Ricin and LT cytotoxicity assays.** Vero cell assays utilized 10 ng/mL of ricin toxin (Vector Labs), whereas J774 assays employed 300 ng/mL LT, with a 1:1 mass ratio of PA and LF. In both assays, 5,000 cells per well were seeded in a 96 well opaque cell culture treated plate in DMEM plus 10% fetal bovine serum. Serum samples were serially diluted and then mixed with toxin before being applied to 96 well plates. In the Vero cell assays, the toxin-antisera mixtures was allowed to incubate at 37°C for 2 hours, at which point the media was changed. CellTiter-Glo® (Promega, Madison, WI) was then used to determine cell viability 48 hours later. In the J774 assays, the media was not changed after the toxin-antisera mixtures were added to the cells. Cell viability was determined using CellTiter-Glo® 24 hours later.

**Table S1. Serum analysis of mice immunized with RiVax, DNI or the combination.**

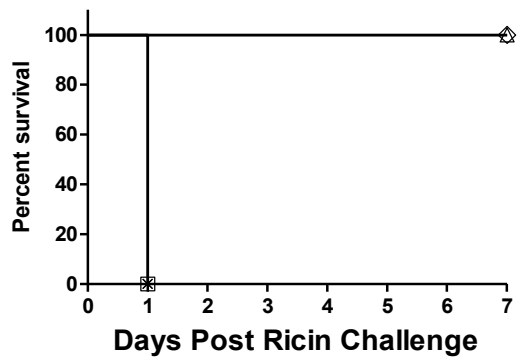
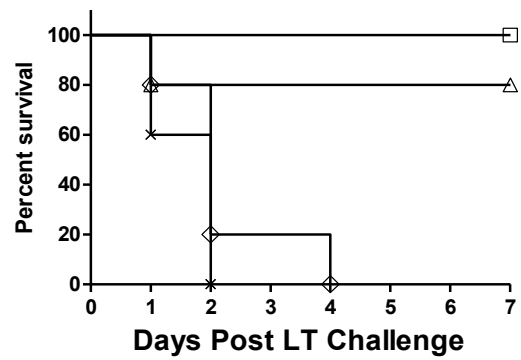
Grp	Mouse <sup>a</sup>	Ricin Endpoint		PA Endpoint		Ricin TNA		LT TNA		Survival <sup>b</sup>	
		d 20	d 200	d 20	d 200	d 20	d 200	d 20	d 200	Ricin	LT
RiVax	A1	204800	102400	1 <sup>c</sup>	1	1	800	1	1		No
	A2	204800	204800	1	1	1	1600	1	1		No
	A3	204800	102400	1	1	1	800	1	1		No
	A4	204800	51200	1	1	100	100	1	1		No
	A5	204800	102400	1	1	1	400	1	1		No
	B1	204800	51200	1	1	1	100	1	1	Yes	
	B2	204800	102400	1	1	1	800	1	1	Yes	
	B3	204800	102400	1	1	1	200	1	1	Yes	
	B4	204800	204800	1	1	1	1600	1	1	Yes	
	B5	204800	204800	1	1	1	400	1	1	Yes	
DNI	A1	1	1	204800	409600	1	1	1600	3200	(No)	Yes
	A2	1	1	204800	204800	1	1	800	800	(No)	Yes
	A3	1	1	204800	204800	1	1	800	1600	(No)	Yes
	A4	1	1	204800	102400	1	1	1600	1600	(No)	Yes
	A5	1	1	204800	102400	1	1	1600	1600	(No)	Yes
	B1	1	1	102400	204800	1	1	200	1600	No	
	B2	1	1	204800	204800	1	1	800	3200	No	
	B3	1	1	204800	102400	1	1	1600	1600	No	
B4	1	1	204800	204800	1	1	3200	1600	No		
Dual	A1	51200	102400	102400	51200	1	800	400	800	(Yes)	Yes
	A2	204800	102400	204800	51200	1	800	800	800	(Yes)	Yes
	A3	204800	204800	102400	51200	1	800	100	200		No
	A4	102400	204800	6400	51200	1	800	200	1600	(Yes)	Yes
	A5	102400	51200	51200	51200	1	400	200	400	(Yes)	Yes
	B1	102400	102400	25600	51200	1	400	1	400	Yes	
	B2	102400	51200	12800	102400	1	100	400	1600	Yes	
	B3	204800	102400	204800	102400	1	400	3200	800	Yes	
	B4	102400	51200	12800	51200	1	200	100	1600	Yes	
	B5	204800	102400	51200	25600	1	400	800	1600	Yes	
Ctrl	A1	1	1	1	1	1	1	1	1		No
	A2	1	1	1	1	1	1	1	1		No
	A3	1	1	1	1	1	1	1	1		No
	A4	1	1	1	1	1	1	1	1		No
	A5	1	1	1	1	1	1	1	1		No
	B1	1	1	1	1	1	1	1	1	No	
	B2	1	1	1	1	1	1	1	1	No	
	B3	1	1	1	1	1	1	1	1	No	
	B4	1	1	1	1	1	1	1	1	No	
	B5	1	1	1	1	1	1	1	1	No	

<sup>a</sup>, Each group of immunized mice was arbitrarily divided into two subsets (A, B) following the second immunization. The A subset received a primary challenge with LT, while the B subset (shaded) received a primary challenge with ricin. Survival (far right columns) following challenge is indicated as “yes” or “no” for each animal. <sup>b</sup>, The DNI-immunized and the dual-immunized A subsets of animals that survived LT challenge received a secondary challenge with ricin. The outcome of the secondary challenge is indicated in parentheses.

## SUPPLEMENTAL INFORMATION



**Figure S1. LT LD<sub>100</sub> Determination.** A dose response experiment was performed to determine the LD<sub>100</sub> of a stock of LF and PA. Using a 1:1 mass ratio of PA and LF, groups of four naïve mice received vehicle (circle), or 25 µg (square), 50 µg (triangle), 100 µg (diamond) or 200 µg (cross) µg of LT. Based on the observation that all mice given  $\geq 100$  µg or more died within 5 days of challenge, the LD<sub>100</sub> was set at 100 µg, which equates to 50 µg each of PA and LF. For the studies shown in Table 3, mice were challenged with 250 µg LT, equal to 2.5xLD<sub>100</sub>.

**A****B**

**Figure S2. Survival plots following primary ricin and LT challenges.** Groups of immunized mice, as described in the text that had been immunized with RiVax (diamonds), DNI (squares), the combination of RiVax and DNI (triangles) or vehicle (cross) were challenged with (A)  $10 \times LD_{50}$  of ricin or (B)  $2.5 \times LD_{100}$  of LT. Survival was monitored for 7 days.