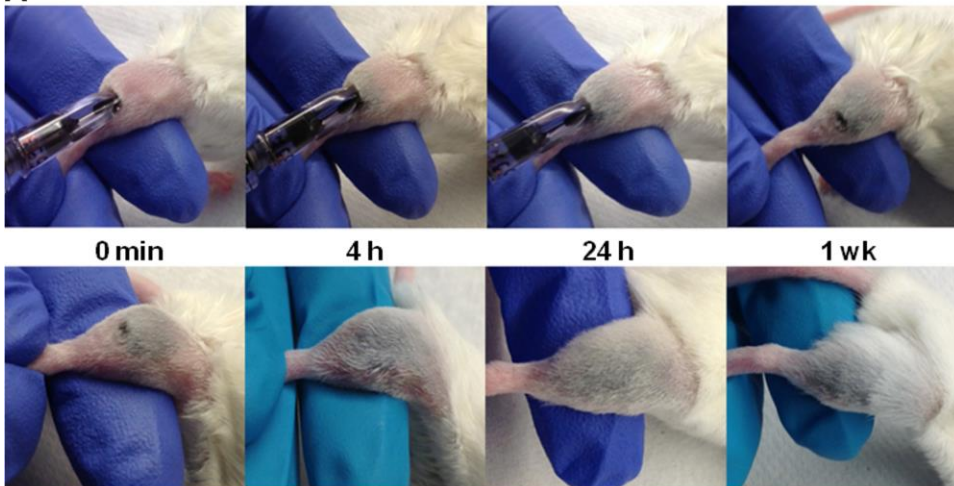
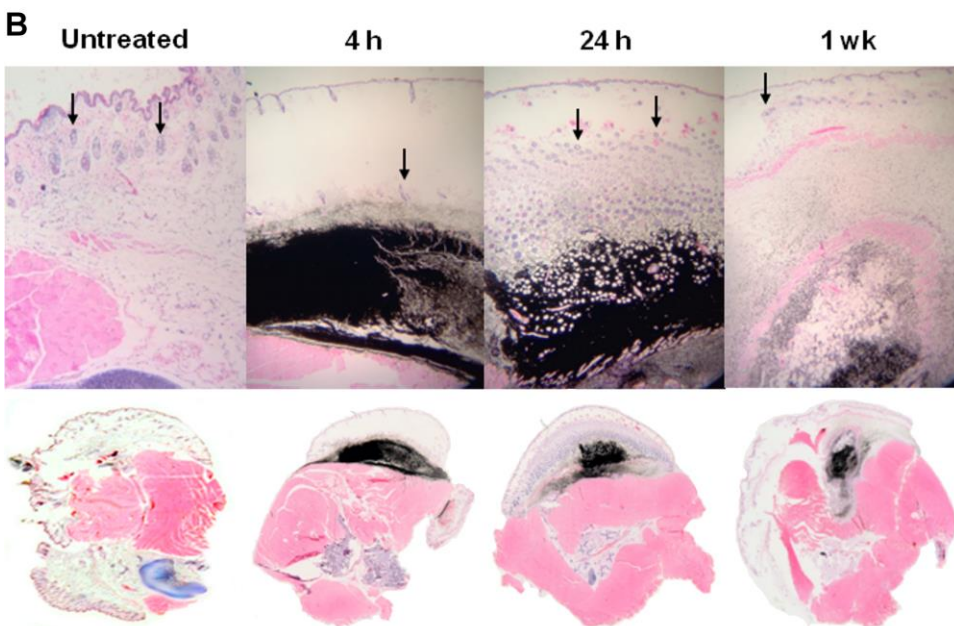


Supplemental Figure 1.

A



B



Supplemental Figure 1. Intradermal injection and uptake of injected dye. 25 μ l of India Ink dye was administered i.d. into the shaved thigh of Balb/c mice using the NanoPass MicronJet 600 needle. (A) Macroscopic picture of the injection site during the procedure (top panel), immediately after injection, at 4 and 24 h and 1 wk after injection (bottom panel). (B) Histological images by light microscopy of H&E stained thigh tissue sections following i.d. India ink injection obtained with higher magnification (40x) to focus on the cellular infiltrate surrounding the injected fluid (top panel) and lower magnification (2x) to display the entire tissue section (bottom panel). Arrows indicate the location of hair follicles in the epidermal layer. Tissue from untreated mice served as controls. The low magnification images of the entire section (bottom row) show: 1) the inoculum delivered into the dermis and above the muscle (4 h after injection); 2) its progressive diffusion to nearby areas and influx of phagocytic cells with dense black cytoplasm (24 h); and 3) its dispersion into the subcutaneous layer (1 wk) (Supplemental Figure 1B, bottom). These observations were consistent from histological analysis of five individual mice.

Supplemental Table I. Comparison of serum IgG antibody responses against vaccine antigens

Experiment	Route	Vaccine	Serum IgG titer		
			IpaB ^a	IpaD ^a	dmLT ^a
1	i.d.	IpaB/IpgC+IpaD+dmLT (High)	239,452	131,476	3,053,612
2	i.d.	IpaB+IpaD+dmLT (High)	388,638	130,203	3,278,591
1	i.d.	IpaB/IpgC+IpaD+dmLT (Medium)	225,112	89,558	3,392,942
2	i.d.	IpaB+IpaD+dmLT (Medium)	696,761*	105,378	3,826,246
1	i.n.	IpaB/IpgC+IpaD+dmLT	846,368	718,362*	12,773,227*
2	i.n.	IpaB+IpaD+dmLT	1,259,924	394,740	7,636,112
1	i.d.	PBS	28.15	12.5	519.39
2	i.d.	PBS	20.33	17.13	98.87

^aMean EU/ml values in serum samples from day 55 (n=10 for experiment 1 and n=20 for experiment 2). Asterisks denote significant differences ($p < 0.05$) between Ab titers against individual antigens from all samples in Experiment 1 compared to the same group in Experiment 2, determined by one-Way ANOVA with Tukey's multiple comparisons test.