

Fig. S1: AT mutant lytic activity on rabbit RBC (A) and human A549 cell line (B). (A) Washed RBCs were incubated with serial dilutions of AT WT or AT mutants (2.5 to 0.0024 $\mu\text{g/ml}$). Hemolysis was measured by the amount of hemoglobin released in the supernatant, and calculated as followed: $100 \cdot [(\text{OD}_{\text{AT}}) / (\text{OD}_{\text{SDS}})]$. (B) A549 were incubated with serial dilutions of AT WT or AT mutants (20 to 0.01 $\mu\text{g/ml}$). LDH was measured in supernatants after two hour incubation at 37°C. % cytolysis was calculated as follows: $100 \cdot [100 - (\text{OD}_{\text{AT}}) / (\text{OD}_{\text{SDS}})]$. Results are a mean of triplicates and representative of two separate experiments.

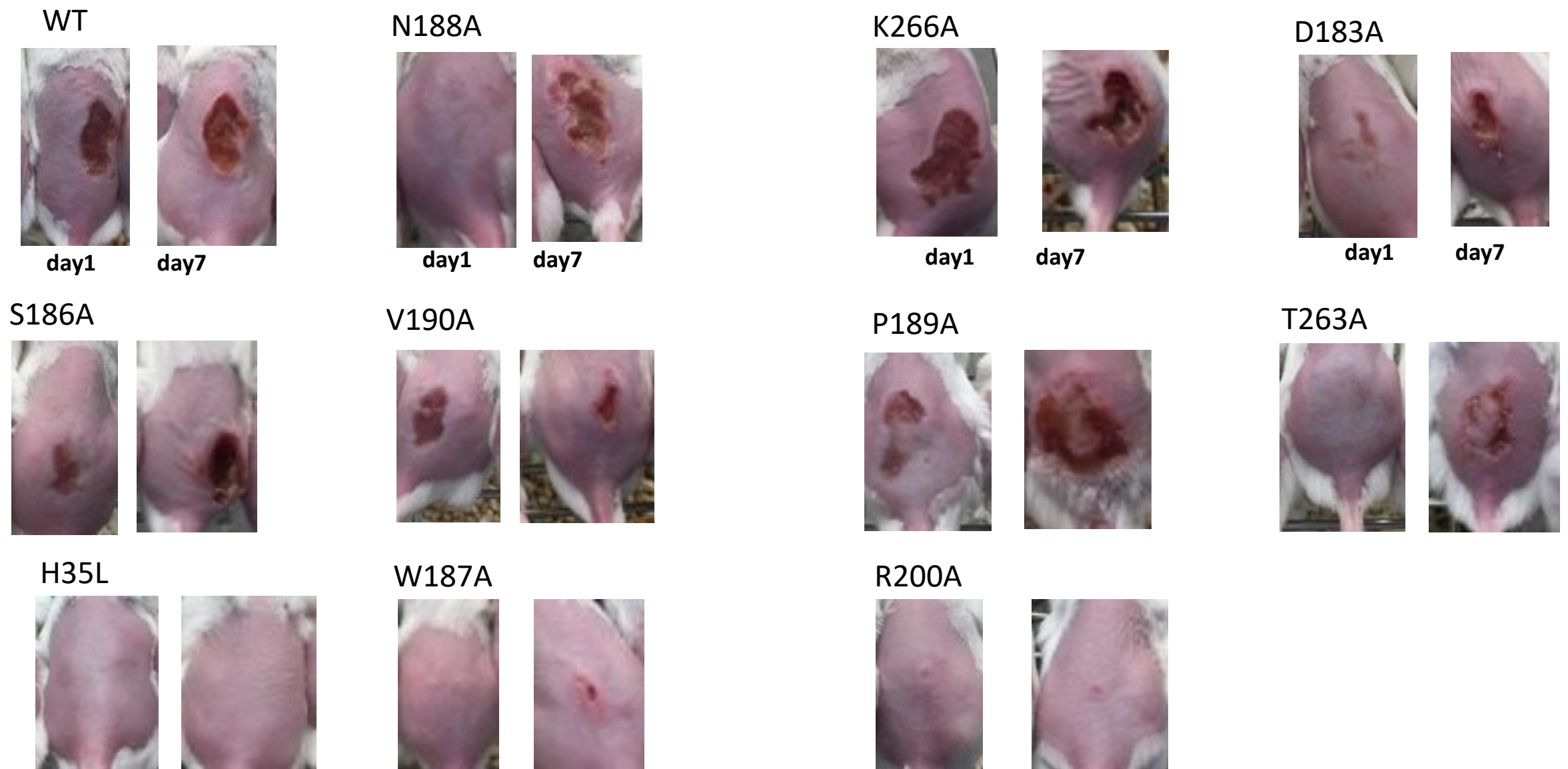


Fig. S2: Purified toxin-induced dermonecrosis. Balb/c mice (n=5) were injected intra-dermal with AT molecules (1 μ g). Wild type AT (WT) and AT_{H35L} (H35L) were used as positive and negative controls, respectively. Lesions were recorded at days 1 and 7 post injection. The data are representative of 3 independent experiments.

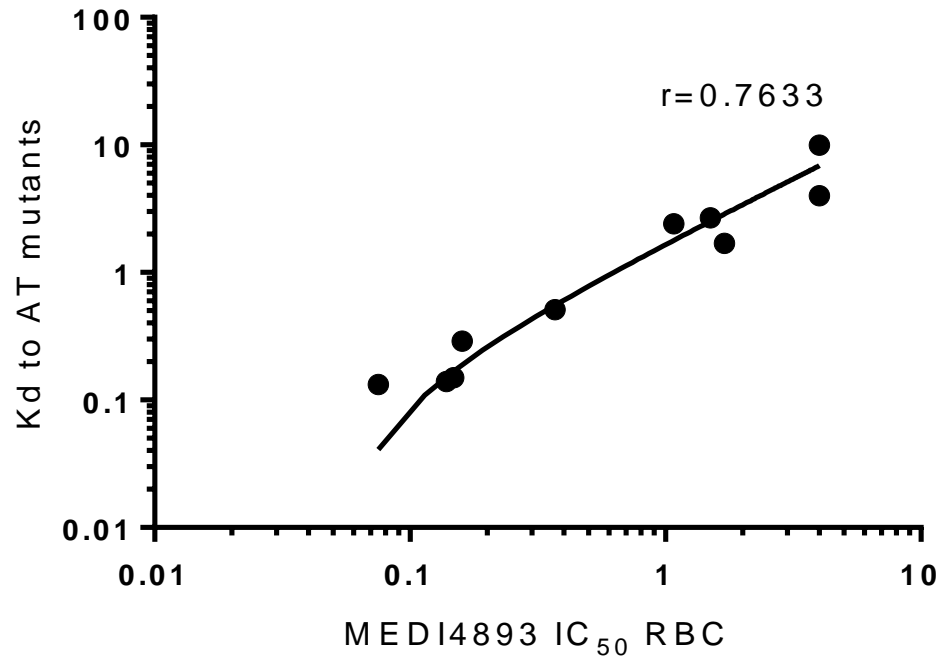


Fig. S3: Correlation between MEDI4893 neutralization in hemolysis and KD binding to mutants. Linear regression constant was measured between Constant affinity binding (Kd) and in vitro activity (IC₅₀ in the hemolytic assay) for each AT mutant IC₅₀ were graphed and linear regression constant calculated.

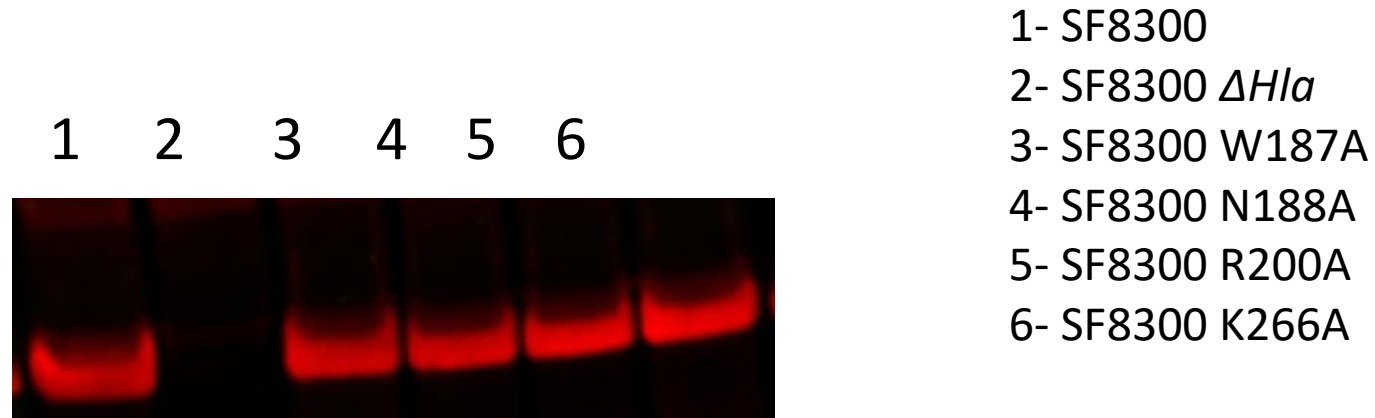


Fig. S4: AT expression in SF8300 knock-in mutant

Strains were cultured overnight in TSB, and AT level measured by western blot



Fig. S5: MEDI4893* inhibits USA300 knock-in mutant induced-dermonecrosis. Balb/c mice (n=10) were IP immunized with c-IgG or MEDI4893* (10mpk), and ID injected with SF8300 WT or SF8300 knocking mutants (5e7cfu). Lesion sizes were photographically recorded 24h post infection. This experiment is representative of 3 independent experiments.

	H35L	WT	D183A	S186A	W187A	N188A
no mAb	307	415	216	62	24	28
c-IgG	312	470	159	40	32	21
MEDI4893*	3	2	1	1	5	2

	P189A	V190A	R200A	T263A	K266A
no mAb	68	58	5	160	79
c-IgG	61	61	8	185	55
MEDI4893*	2	0	4	2	0

Table S1: Median of fluorescence intensity of AT mutants and nAT on A549 human cells in presence of anti-AT mAb (MEDI4893*) or irrelevant mAb (c-IgG). Mean for staining control has been subtracted.