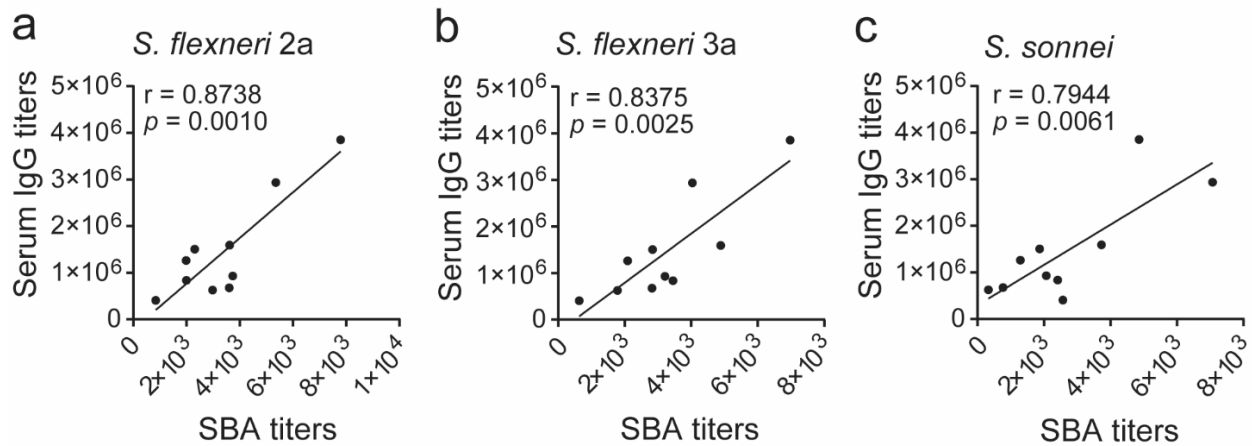


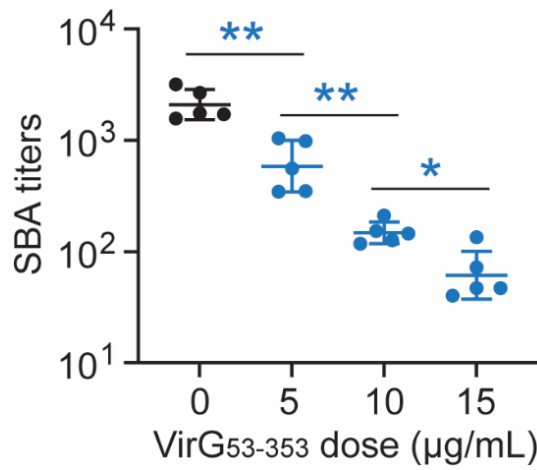
Supplementary Information

VirG-specific antibodies mediate antimicrobial functions and confer cross-protection against *Shigella*

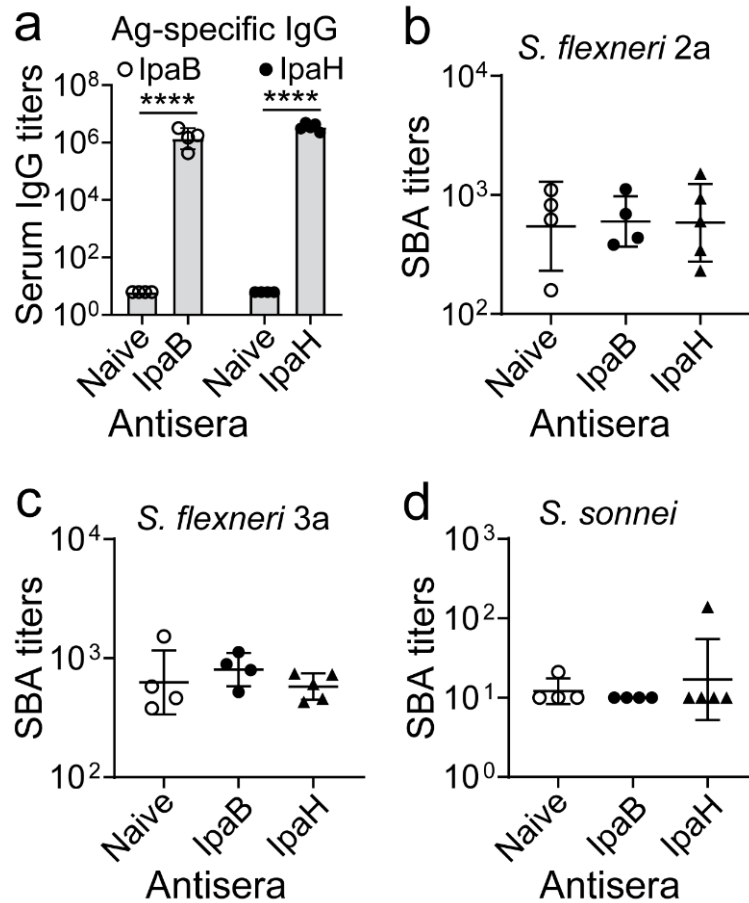
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Supplementary Fig. 1. Correlation between anti-VirG IgG and SBA titers. Datapoints represent VirG-specific IgG and SBA titers against *S. flexneri* 2a (a), *S. flexneri* 3a (b) or *S. sonnei* (c) measured in sera from 10 rabbits vaccinated with VirG₅₃₋₃₅₃ and VirG α (combined) as described in Figure 1a. The correlation coefficient (r) was calculated by Pearson's correlation test.



Supplementary Fig. 2. Absorption of rabbit immune sera with VirG inhibits bactericidal activity in a dose-dependent manner. Anti-VirG₅₃₋₃₅₃ rabbit sera collected at day 70 were preincubated for 15 min with increasing doses (0, 5, 10, or 15 μg/mL) of VirG₅₃₋₃₅₃ and tested for SBA against *S. flexneri* 2a. VirG₅₃₋₃₅₃ adsorption inhibited bactericidal activity in a dose-dependent manner. Data are presented as GMT ± GSD; * $p < 0.05$, ** $p < 0.01$ by Mann-Whitney test. GMT, geometric mean titer; GSD, geometric standard deviation.



Supplementary Fig. 3. Serum IgG and bactericidal activity of IpaB and IpaH rabbit immune sera. Adult NZW rabbits (n=5/group) were immunized IM with 100 µg of IpaB or IpaH on days 0, 7, 21 and 49 using complete Freund's adjuvant for primary immunization and incomplete Freund's adjuvant for boosters (as described in Fig. 1a). Data correspond to rabbit sera collected on day 70 post-vaccination. **a**) IpaB- and IpaH-specific serum IgG titers (EU/mL) were determined by ELISA as described in the Methods. Sera from unvaccinated rabbits were used as a negative control. Data are presented as GMT ± GSD; $p < 0.0001$ by Mann–Whitney test. **(b–d)** SBA titers against *S. flexneri* 2a (**b**), *S. flexneri* 3a (**c**), and *S. sonnei* (**d**). SBA titers from IpaB and IpaH rabbit immune sera were compared with those of non-vaccinated rabbits. Data are presented as GMT ± GSD; no significant differences were observed in SBA titers between groups ($p > 0.4$, Mann–Whitney test). GMT, geometric mean titer; GSD, geometric standard deviation.