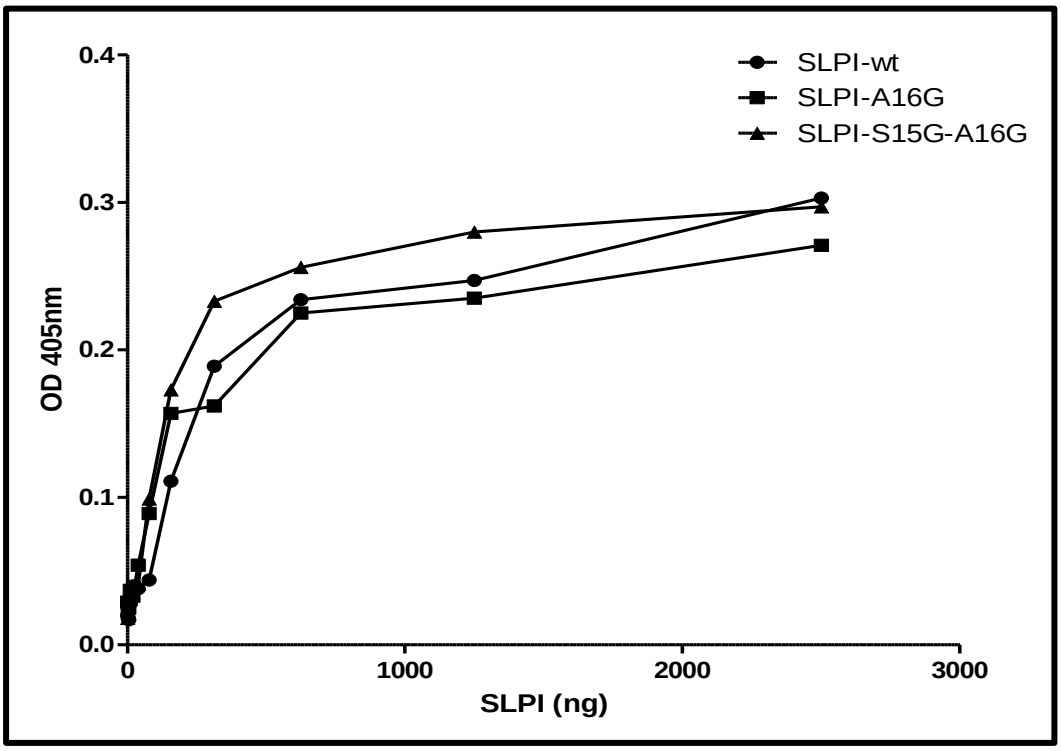
Figure 4



SLPI (ng)		2500	1250	625	312.5	156.25	78.125	39.0625	19.53125	9.765625	4.882813	2.441406	0
SLPI-wt	Abs 405nn	0.303	0.247	0.234	0.189	0.111	0.044	0.038	0.04	0.028	0.017	0.018	0.02
SLPI-A16G	Abs 405nn	0.271	0.235	0.225	0.162	0.157	0.089	0.054	0.033	0.037	0.025	0.025	0.029
SLPI-S15G-A16G	Abs 405nn	0.297	0.28	0.256	0.233	0.173	0.099	0.041	0.035	0.031	0.033	0.026	0.018

LPS binding assay

Binding of recombinant SLPI-WT and SLPI-A16G and SLPI-S15G-A16G variants to P. aeruginosa LPS was assessed by indirect ELISA as previously described with some minor modifications 10,48. Briefly, Greiner® high binding 96 well plates were coated with serial dilutions of recombinant SLPI-WT, SLPI-A16G and SLPI-S15G-A16G variants. The plate was incubated at 37°C for 2 h and then washed three times with PBS containing 0.05% (v/v) Tween 20. The plate was blocked for 1 h at room temperature

with 200 µl of 1% (w/v) BSA PBS containing 0.05% (v/v) Tween 20 per well. Biotinylated P. aeruginosa LPS (100 ng) was then added to each well and the plate was incubated at 37°C for 3 h. Control wells received serum-free medium alone. Again, the plate was washed three times with PBS containing 0.05% (v/v) Tween 20 before addition of 100 µl per well of streptavidin-conjugated HRP (1:2,500 dilution). After incubation at room temperature for 20 min, the plate was washed with PBS containing 0.05% (v/v) Tween 20. After incubation, ABTS single solution substrate (Life Technologies) was added and the plate was incubated at room temperature for 20 min. The absorbance at 405 nm of the wells was measured on a Biotek Synergy HT plate reader.