Figure 3

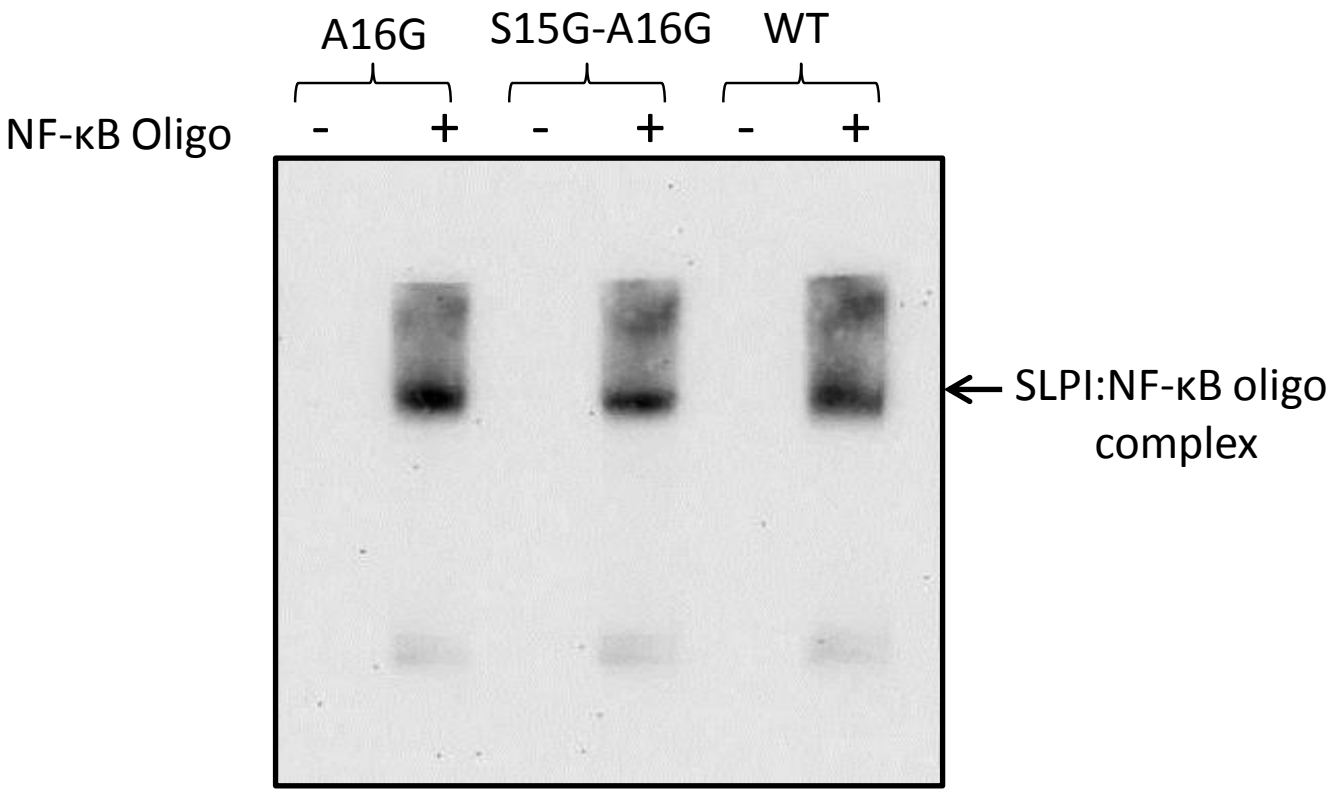

Figure 3. Binding of SLPI and the SLPI variants to DNA. SLPI-WT, SLPI-A16G and SLPI-S15G-A16G (1 µg) were incubated with a biotinylated consensus NF-κB DNA oligonucleotide, electrophoresed on a 15% polyacrylamide gel and transferred onto nitrocellulose membrane. SLPI:NF-κB oligonucleotide complexes were detected by incubating the blot with streptavidin-HRP and detected using chemiluminescence.

Methods

EMSA

The binding of SLPI-WT and the SLPI-A16G and SLPI-S15G-A16G variants to an NF-κB consensus DNA binding site was assessed by Electrophoretic Mobility Shift Assay (EMSA) as described previously [10,24]. Recombinant SLPI-WT and SLPI-A16G and SLPI-S15G-A16G variants (1 µg) were incubated with double-stranded biotinylated NF-κB consensus oligonucleotide 5'-AGTTGAGGGGACTTTCCCAGGC-3' (100 pmol; Life Technologies, Paisley, UK) and Poly(dI-dC).Poly(dI-dC) (2 µg; Sigma-Aldrich) for 30 min at r.t. in binding buffer containing 4% (v/v) glycerol, 0.1 mg/ml nuclease-free BSA, 1 mM EDTA, 5 mM DTT, 100 mM NaCl and 10 mM Tris-HCl, pH 7.5 (20 µl). After incubation, the mixtures were electrophoresed on native 15% polyacrylamide gels. The gels were transferred onto 1 µm pore size nitrocellulose membranes in 1 x TBE for 30 min at 380 mA, 100 V and then cross-linked under UV light for 10 min. A chemiluminescence nucleic acid detection kit (Pierce, Fisher Scientific UK,

Leicestershire) was used to detect the SLPI:DNA complexes which were analysed using the Syngene G:Box and GeneSnap software.