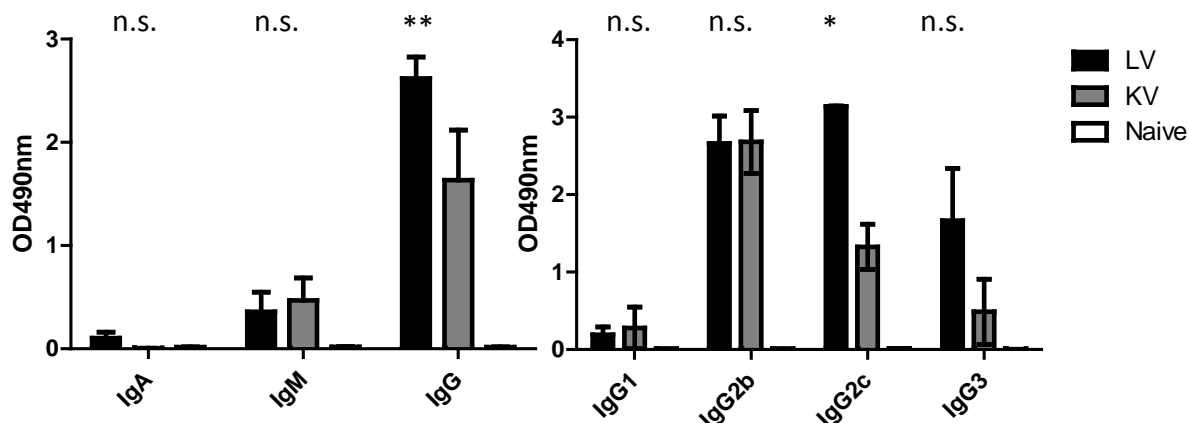


Figure S4: Anti-*Salmonella* antibody isotype and IgG subclass response following immunisation.

To monitor the antibody response following immunisation, anti-*Salmonella* LPS IgG subclass immunoglobulin was measured by ELISA in serum from mice in the following groups:

LV: 3 months after immunisation with live attenuated STm SL3261 ($n=3$)

KV: 2 weeks post-boost immunisation with acetone-killed virulent STm SL1344 ($n=4$).



ELISA Method: *Salmonella enterica* serovar Typhimurium LPS (Sigma) was dissolved in water to 1 mg/ml, mixed with an equal volume of sodium deoxycholate (0.5% w/v), incubated at 37 °C for 15 min, aliquoted and stored at -20 °C. Half-area microtitre plates (Greiner Bio-One) were coated overnight at 37 °C with 50 µl LPS at 5 µg/ml in carbonate buffer (0.15% w/v Na₂CO₃; 0.1% w/v MgCl₂·6H₂O; 0.3% w/v NaHCO₃), washed three times with PBS-Tween (0.05%) and blocked with 150 µl PBS+2% BSA for 1 hr at 37 °C. Following three further washes, 50 µl serum dilutions (1/800 in PBS-Tween+1% BSA) were applied in duplicate and incubated for 2 hr at 37 °C. Plates were washed and total antibody detected with horseradish peroxidase-conjugated goat anti-mouse Ig antibody (Southern Biotech), detection was with SigmaFast OPD substrate (Sigma). Bars show mean ± SEM. Tests of significance were by 2-way ANOVA with Bonferroni post-test to compare the LV vs KV response for each subclass or isotype (*: $P<0.05$).