

Supplementary Fig. 3 – Comparison of B cells from hSMUG1-transgenic and control littermates mice. (A) Analysis of in vitro proliferation. Splenocytes from two pairs of hSMUG1-transgenic and control littermates were purified by CD43-depletion and cultured (10⁶ cells/ml) with LPS + IL4. Viable cell numbers were determined on the days indicated, by flow cytometry after staining with propidium iodide using a constant amount of calibrite beads (BD Biosciences) as a normalisation standard. (B) Scatter analysis of purified B cells cultured with LPS + IL4 at different days of cultures. The percentages of CD45R(B220)⁺ cells within the lymphocyte scatter is indicated. The proportion of events scored within the viable lymphocyte scatter gate decreases as the cultures proceed, but with SMUG1-transgenic and control littermates exhibiting indistinguishable kinetics. (C) Flow cytometric analysis of necrotic/apoptotic cells (as judged by PI uptake and annexin V staining) in day 8 LPS + IL4 cultures from two pairs of SMUG1-transgenic and control mice. (D) Morphology of splenic germinal centres in normal (WT) and SMUG1-transgenic mice as analysed at day 11 post-immunisation with 2-phenyl-oxazolone conjugated to chicken serum albumin and 10⁹ heat inactivated *B. pertussis*. Germinal centres were identified by staining with FITC-peanut agglutinin and goat anti-IgD and Cy5rabbit anti-goat antibodies.