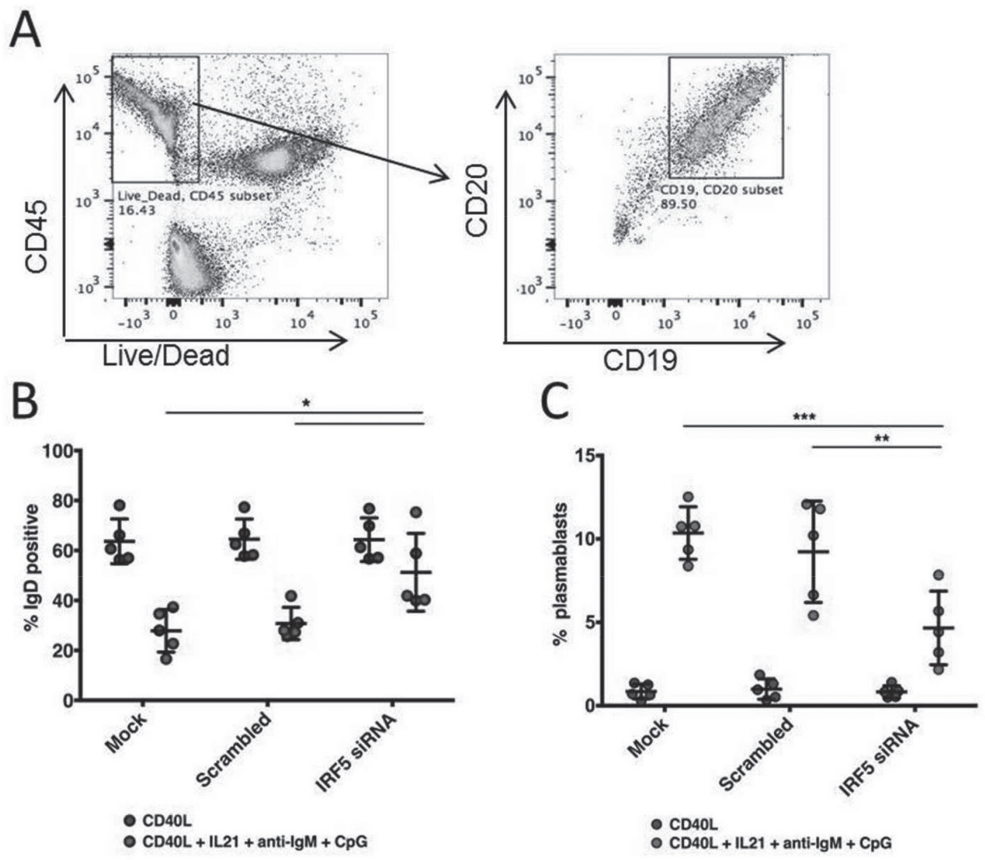

Abstract AI-02 Figure 1 Loss of IRF5 expression leads to IgD retention and reduced short-lived PCs. After nucleofection of siRNAs, cells were cultured with CD40L alone or with IL21, anti-IgM antibody, and CpG-B for 7 days. **A**, Representativegating strategy with LIVE/DEAD® Viability/ Cytotoxity discrimination dye. **B**, PercentIgD$^+$ cells is shown after gating on CD19$^+$ CD20$^+$ IgD$^+$ CD38- cells. **C**, Same as in B except IgD$^-$ CD38$^+$ B cells were gated on. Percent IgD$^-$ CD38$^+$ plasmablasts is shown. Circles represent individual healthy donors; n = 5.

AI-03 REGULATION OF AGE-ASSOCIATED B CELLS IN LUPUS

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Background A novel subset of B cells termed Age-associated B cells (ABCs) has been recently reported to preferentially accumulate with age in female mice. ABCs are characterised by high expression of CD11c and CD11b and low levels of CD21 and CD23. ABC differentiation depends on the transcription factor T-bet and is promoted by TLR7 stimulation. While ABCs have been proposed to play a key role in the development of autoimmune diseases and in the gender bias observed in these disorders the molecular mechanisms driving ABC differentiation are poorly understood. Our laboratory has previously identified a novel protein termed DEF6, which shows significant homology to SWAP-70. DEF6 and SWAP-70 are the only two members of a unique family of multifunctional proteins that regulate both Rac activation as well as IRF4 function. Mice deficient in both DEF6 and SWAP-70 (DKO mice) spontaneously develop a lupus-like disease on a C57BL/6 background. Notably, the lupus-like disorder in DKO mice shares several features with human SLE, including exhibiting a sex-bias, and the DEF6 locus has recently been identified as a new SLE risk variant.

Materials and methods CD11c+ CD11b+ B220+ CD19+ B cells (ABCs) were analysed *in vivo* in WT and DKO mice by FACS. We investigated the role of IL-21 and SAP *in vivo* by crossing DKO mice with mice lacking these molecules. ABCs were generated *in vitro* by culturing CD23+ follicular B cells with αIgM, αCD40, and IL-21 in the presence/absence of TLR7 ligand. ABC markers and gene expression were analysed by FACS and qPCR.

Results We have found that female DKO mice exhibit a marked accumulation of ABCs. The expansion of ABCs observed *in vivo* in DKO mice is dependent on both IL-21 and T-B cell interactions as demonstrated by a significant reduction of ABCs in DKO mice that also lack either IL-21 or SAP. We have also generated male Yaa-DKO mice, which carry a duplication of the TLR7 gene on the Y chromosome. Yaa-DKO males exhibit an even greater expansion of ABCs, which correlates with high titers of anti-dsDNA autoantibodies. *In vitro* stimulation of CD23$^+$B cells with αBCR, αCD40, and IL-21, furthermore demonstrates that B cells from DKO mice are hyper-responsive to IL-21 and strongly upregulate CD11c and CD11b.

Conclusions Our study demonstrates that the absence of DEF6, a genetic risk factor for lupus, leads to the aberrant expansion of ABCs, a novel B cell subset previously associated with the development of autoimmunity. We furthermore show that accumulation of ABCs in lupus is dependent on T-B cell interactions and IL-21.

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