

Impairment of TGF- β directed class switching to IgA in Runx3 KO splenic B cells

In vitro studies have previously shown that Runx3 participates in TGF- β mediated immunoglobulin class switching to IgA by mouse splenic B cells (Shi and Stavnezer, 1998). We therefore examined the TGF- β mediated class switching to IgA in WT and Runx3 KO splenic B cells and also measured the *in vivo* production of IgA. Splenocytes from KO and WT mice were incubated in the presence of TGF- β and LPS and after 4 days analyzed for the presence of germline (GL) Ig α and rearranged IgA transcripts by RT-PCR (Figure S4A). Significantly, GL and post-switch IgA (ps IgA) transcripts were observed in RNA isolated from WT splenocytes, but not in RNA from Runx3 KO splenocytes, whereas IgM mRNA was readily detected in both. Consistent with these results, supernatants of cultured WT splenocytes contained higher levels of IgA than supernatants of the KO, whereas levels of IgM and IgG were similar (Figure S4B). These results further support the findings that indicate a role for Runx3 as mediator of TGF- β signaling. Intriguingly, however, the level of IgA in BAL of Runx3 KO mice was elevated (~6 fold) as compared to WT (Figure S4C) as were the levels of IgA in the serum and fecal pellets of the KO mice (data not shown). It thus appears that Runx3 is required for class switching to IgA in cultured splenocytes *in vitro*, but not for the production of IgA *in vivo*, indicating that more than one pathway may play a role in the switch to IgA.

Figure S4

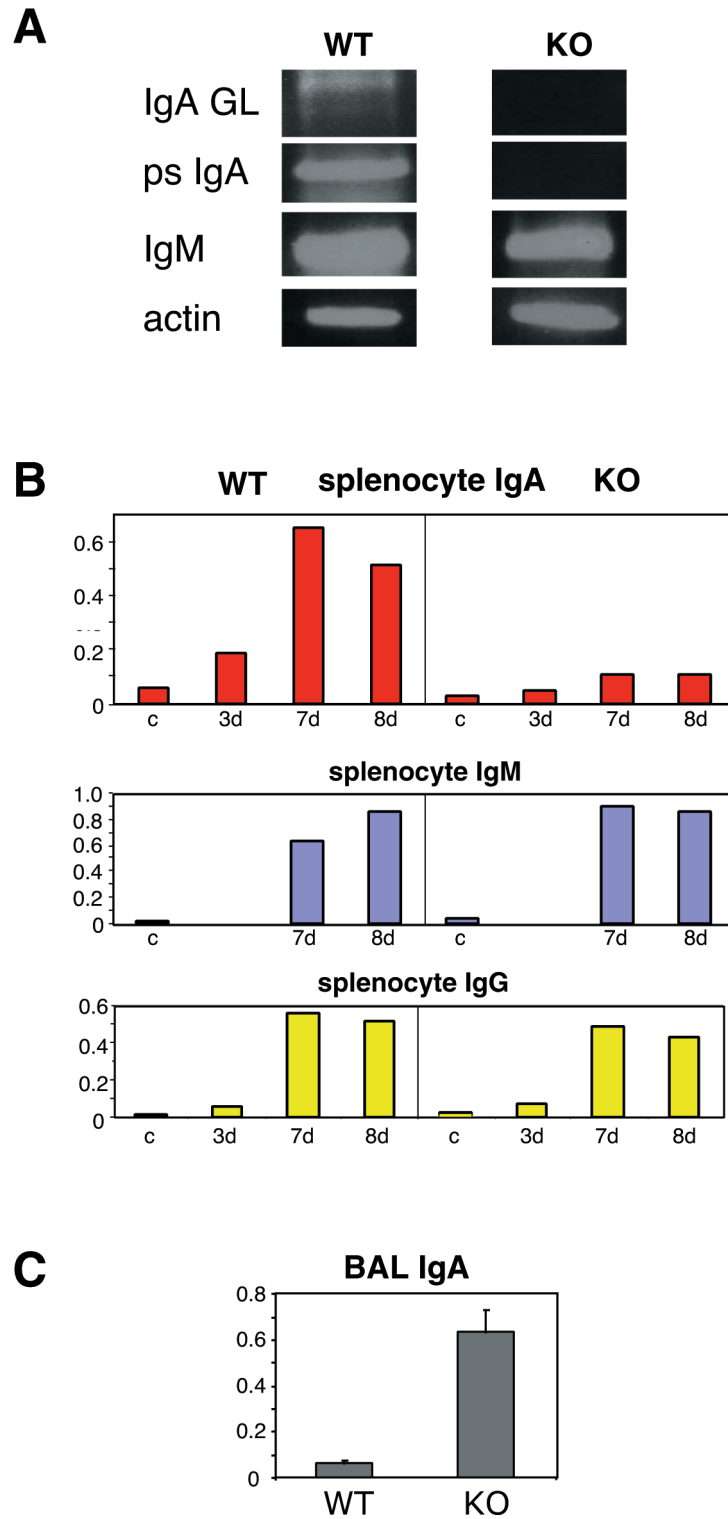


Figure. S4. TGF- β dependent IgA class switching in cultured Runx3 KO splenocytes is abrogated. **(A)** Splenocytes were cultured in the presence of TGF- β and LPS to induce IgA class switching. At day 4 RNA was prepared and analyzed by RT-PCR using the primers: IgA germline (IgA GL) Forward 5'-CCTGGCTGTTCCCCTATGAA-3' Reverse 5'-GAGCTGGTGGGAGTGTCAGTG-3';

IgA post switch (ps IgA) Forward 5'-CTCTGGCCCTGCTTATTGTTG-3' Reverse 5'-GAGCTGGTGGGAGTGTCAGTG-3';

IgM Forward 5'-CTCTGGCCCTGCTTATTGTTG-3' Reverse 5'-GAAGACATTTGGGAAGGACTGACT-3';

Actin Forward 5'-GATGACGATATCGCTGCGCTG-3' Reverse 5'-GTACGACCAGAGGCATACAGG-3'.

IgA germline (IgA GL) and IgA post switch (ps IgA) transcripts were detected only in WT splenocytes, but not in the KO. Levels of IgM mRNA in WT and KO were similar. **(B)** Aliquots of supernatant from cultured WT and KO splenocytes were removed on culture days 0,3,7, and 8 and levels of IgA, IgM and IgG were determined by ELISA. IgA production was detected in days 7 and 8, but only in WT splenocytes. Production of IgM and IgG was similar. **(C)** BAL from KO and WT mice were analyzed for IgA levels by ELISA (n=4, p=0.001). ELISA results are presented as the optical density readouts of the machine.