Since DREAM and KChIP-2 proteins display different functions in different cellular compartments, we examined the early and late signaling pathways that activate cytokine gene expression by analyzing whether expression of EFmDREAM can modify the early Ca\(^{2+}\) response. Flow cytometry analysis of T cells loaded with fluo-3AM showed that the pattern of the Ca\(^{2+}\) response, in terms of the percentage of responsive cells and maximum intensity in the [Ca\(^{2+}\)],, was not different in wild-type and transgenic thymocytes or splenocytes following αCD3 stimulation (Supplementary figure S2A and B). This suggests that the expression of the Ca\(^{2+}\)-insensitive EFmDREAM does not interfere with the early Ca\(^{2+}\) signal. Supporting this conclusion, we found that T cell exposure to PMA and ionomycin, a stimulation that bypasses TCR proximal signaling, resulted in a lower IL-2 and IFN\(\gamma\) production in transgenic as compared to wild-type T lymphocytes (Supplementary figure S2C). To examine the downstream signaling events prior to cytokine induction, we analyzed ERK activation in transgenic T cells following PMA stimulation. Notably, in these experiments the levels and the kinetics of ERK phosphorylation were not different in wild-type and transgenic thymocytes (Supplementary figure S2D).
Figure S2: Signaling pathways downstream of TCR engagement are not modified in transgenic mice. (A-B) The intracellular Ca\(^{2+}\) concentration in wild-type or transgenic thymocytes loaded with Fluo-3AM was recorded before and after stimulation with biotinylated αCD3 and streptavidin. (A) The percentage of cells that display a fluorescence greater than the average fluorescence of unstimulated cells plus 2 times the standard deviation. (B) The mean fluorescence intensity of T cells. (C) Wild-type or transgenic thymocytes were stimulated with ionomycin (1 µM) and PMA (10 ng/ml) for 24 h and cytokine productions were quantified by ELISA. Results are expressed as mean ± sd of 4 mice per group and are representative of 4 experiments. Asterisks represent statistical significance versus the appropriate control in each case *p<0.05 and **p<0.01. (D) Lysates from wild-type or transgenic thymocytes, non-stimulated or stimulated for the indicated times (min) with PMA (10 ng/ml), were electrophoresed and immunoblotted with the αphospho ERK antibody. Membranes were stripped and reprobed with the αERK2 antibody.