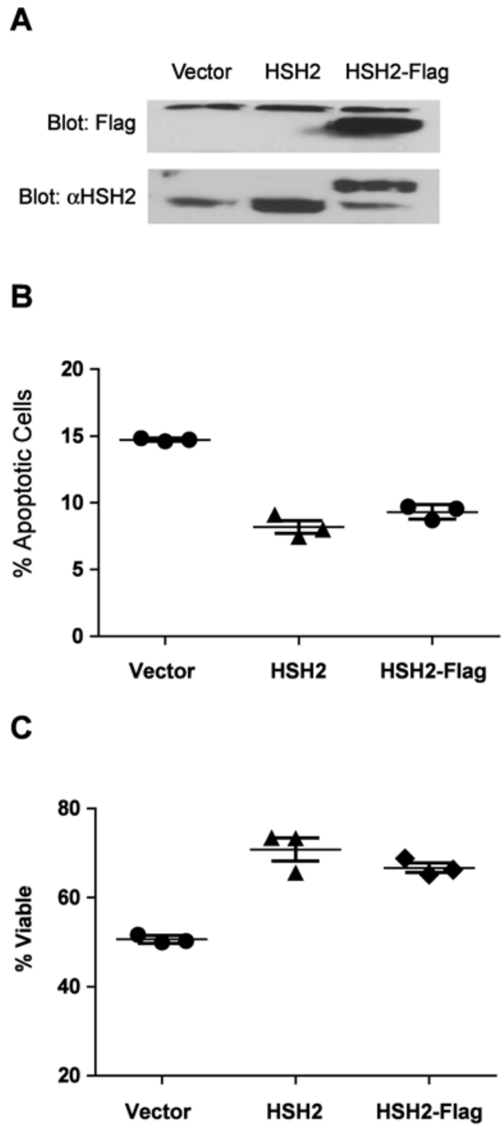
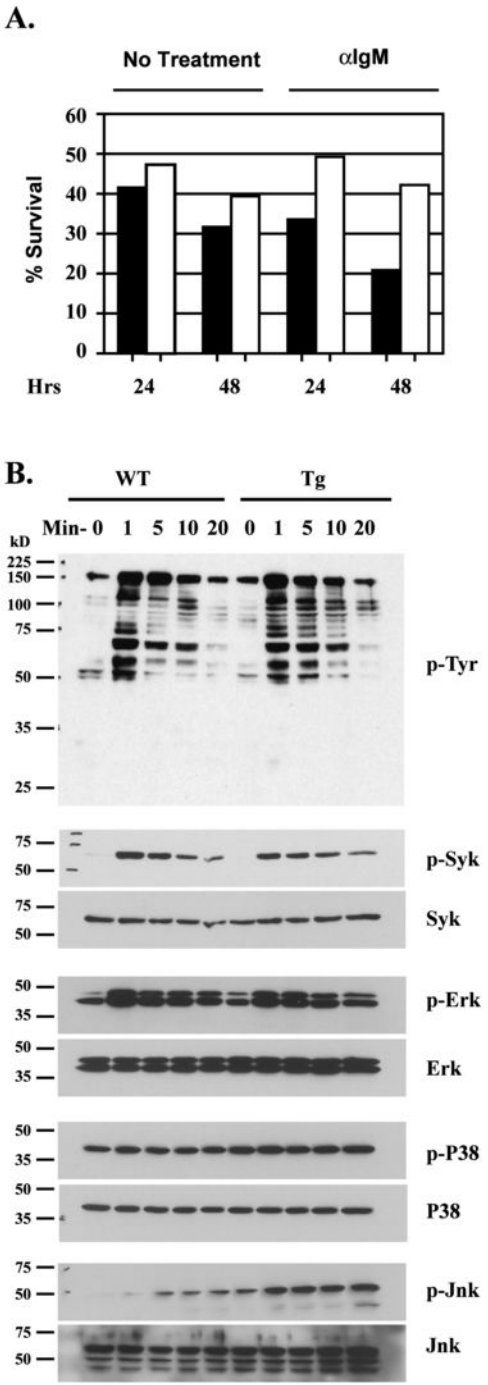


Supplemental Figure 1. Epitope tagging of HSH2 with Flag does not affect its biological function. (A) WEHI-231 cells were retrovirally transduced with the MSCV-Puro construct alone (Vector) or in which the cDNA encoding HSH2 in either an untagged (HSH2) or a C-terminally Flag-tagged form (HSH2-Flag) was cloned. The transduced cells were selected in medium containing puromycin for 24 h prior to analysis of HSH2 expression by Western blotting. WEHI-231 cells ($2x10^7$/sample) were lysed in buffer containing 1% NP-40 and the proteins in the cleared cell lysates were separated by SDS-PAGE on a 10% polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane and the membrane was probed with anti-Flag mAb to detect the expression of Flag-tagged HSH2. The membrane was stripped and reprobed with the anti-HSH2 mAb 3-e1 to detect total HSH2 expression. **(B and C)** WEHI-231 cells were cultured in the presence of 3 μg/ml polyclonal $F(ab')_2$ fragments of anti-mouse IgM for 16 h. The cells were then harvested and stained with 7AAD and DiOC6 to monitor the percent of cells that were induced to undergo apoptosis, as well as the percent viable by flow cytometry.



Supplemental Figure 2. Expression of the HSH2 transgene protects B cells from BCR-induced apoptosis in vitro but does not globally affect BCR signal transduction. A. Constitutive HSH2 expression protects B cells from BCR-induced apoptosis. B cells isolated from either HSH2 Tg (White) or littermate control (Black) mice were cultured for the indicated times in the presence or absence of 10 μg/mL polyclonal anti-IgM F(ab')2 fragments. The viability of cultured B cells was measured by the exclusion of 7AAD using flow cytometry. The graph depicted is representative of three independent experiments. **B.** Analysis of B cell activation following membrane IgM cross-linking. B cells isolated from HSH2 Tg or littermate control mice were stimulated for the indicated times with 20 μg/ml of polyclonal anti-IgM F(ab')2 fragments. B cell lysates were subjected to Western blot analysis to detect total protein tyrosine phosphorylation using the anti-PTyr mAb 4G10 coupled to HRP. Syk activation was measured based on Western blotting with a phospho-specific anti-Syk Ab after which the blot was stripped and reprobed with anti-Syk Ab as a loading control. Similar procedures were used to measure activation of the MAP kinases Erk, p-38 and Jnk. The data depicted are representative of 3 independent experiments.