Supplemental Figure 1. CD5 stimulation increases the phosphorylation detected by antibodies reacting with inhibitory tyrosine residues of Src-family kinases. JKHM cells were activated with Y-2/178 for the times indicated or incubated with isotype-control antibody (NS) at 37°C. (A) Subsequently, cells were collected, fixed and permeabilized for 30 min at 4°C. Cells were stained with anti-pSrc(+) or anti-Src(-) polyclonal antibodies followed by a FITC-labeled goat anti-rabbit (GAR) polyclonal antibody, or FITC-GAR alone. Histograms are from one of three independent experiments. (B) Graphs show the mean values and standard deviation of the variation of pSrc(+) and pSrc(-) phosphorylation relative to time 0 from the three experiments. (C) Following activation with Y-2/178 for the indicated times, cells were collected and lysed, and lysates were run on SDS-PAGE. Detection of phosphorylation of Src activatory and inhibitory tyrosine residues was done by Western blotting using anti-pSrc(+) or anti-pSrc(-) polyclonal antibodies and peroxidase-conjugated GAR followed by enhanced chemiluminescence detection.