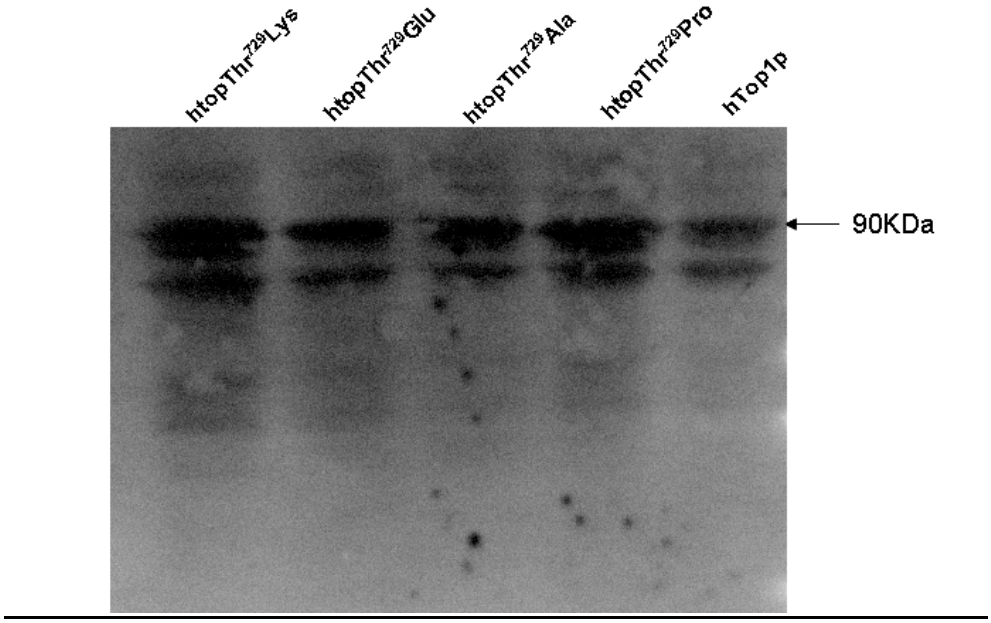
<u>FIGURE 1S</u>: Western Blot analysis of the hTopIp variants expression in EKY3 cells.



In order to confirm that the different CPT sensitivities of yeast expressing different TopIp variants could not be ascribed to different expression levels, the amount of human TopIp expressed in the different cells has been compared by western blotting. Equal amounts of total protein yeast extract were boiled for 3 min at 90° C in sample buffer and subjected to SDS–PAGE electrophoresis on 12% gel. For Western blot analysis proteins were electrophoretically transferred to a nitrocellulose membrane at 35 V overnight. The nitrocellulose was blocked with 3% milk powder in Tris-buffered saline (TBS; 150 mM NaCl, 10 mM Tris–HCl buffer pH 7.4) for 2 h and incubated for 1 h with the AntiFlagM2 peroxidase-conjugated (SIGMA) monoclonal antibody, diluted 1:25 in TBS containing 3% bovine serum albumin. After one wash for 20 min in 3% milk powder T-PBS and several washes in T-PBS without milk, the filters were incubated with the detection solution of the luminol-based ECL system (Pierce). Fluorographies were carried out at room temperature using the ChemiDoc XRS system (BioRad). The arrow indicate the TopIp molecular weight.