



Figure 3. Increasing doses of UO126 inhibit ERK phosphorylation after anti-CD3 crosslinking.

Thymocytes (1×10^7 /ml) were stimulated with $10 \mu\text{g/ml}$ anti-CD3 (2C11) and washed in medium (RPMI medium plus 10% FCS and β ME). Cells were treated with UO126 at the concentrations shown for 15 min at 37C, and bound antibody was then crosslinked by the addition of $75 \mu\text{g/ml}$ of goat anti-hamster Ig for 2min. Stimulation was terminated by placing cells on ice in RIPA buffer. Total cell extracts were analysed for phospho-ERK (anti-phosphoMAPK; Cell Signaling) and anti-ERK2 (D2) (SC-1647; Santa-Cruz Biotech, Inc) by immunoblot.