Supplementary Information

Materials and Methods.

Immune complex kinase assays. V-H69 and -H69 cells in SFM were washed three times in RPMI 1640, and 2 x 106 cell aliquots were incubated in this medium for 30 min at 37oC. Cells were treated in the presence or absence of 40 μM (TI-TITAT, (TI-TITAT or TITAT peptide for 4 h as indicated, prior to performing an immune complex kinase assay for S6K1 or S6K2 as described (Pardo et al., 2001). In separate experiments, HEK293Tet cells expressing tetracycline-inducible kinase active cytoplasmic forms of S6K1 or S6K2 were incubated with or without tetracycline prior to lysis and immune complex kinase assay.

In vitro kinase assay. 0.5 μg recombinant His-S6K and 4 μg recombinant PKCε were incubated on ice in 50mM TRIS (pH 7.5), 100 mM NaCl, 0.1 mM EDTA and 0.1% TritonX-100, 0.3 (v/v)% β-mercatptoethanol and 1 mM Na3VO4 in the presence or absence of recombinant active V600EB-Raf. The reaction was started by adding an ATP-mix resulting in a final concentration of 100 μM ATP, 10mM MgCl2 with or without 33 nCi/μl [γ-^{32}P]ATP and incubated for 30 min at 30ºC. As a positive control recombinant GST-MEK was used as a substrate for V600EB-Raf. Reactions were terminated in SDS-sample buffer and analysed by SDS-PAGE and autoradiography.

RNAi sequences. RNAi-mediated downregulation of PKCα, PKCε, S6K1, S6K2, B-Raf and Raf-1 was achieved using short-hairpin sequences cloned into pSUPER Retro constructs or oligonucleotide siRNA and are listed in the supplementary data. For pSUPER Retro-mediated downregulation, each protein was simultaneously targeted using three different short-hairpin sequences. Sequences were as follow: PKCα, ..CAAGGCTTCCAGTGCCAAG, GGAACACATGATGGATGGA, CATGGAACTCAGGCAGAAA; PKCε, TCTGCGAGGCCGTGAGCTT, CTACAAGGTCCCTACCTTC, GGAAGGGATTCTGAATGGT; S6K1, GGTTCTGGGCCAGGGATCC, GCTCTATCTCATTCTGGAC, CATCATCACTCTGAAAGAT; S6K2, GGGGGGCTATGGCAAGGTG, CGGAATCCCAGCCAGCGGA, GATACGGCCTGCTTCTACC; B-Raf, CAACAGTTATTGGAATCTC, CCTATCGTTAGAGTCTTCC, GAATTGGATCTGGATCATT; Raf-1, CAGTGGTCAATGTGCGAAA, GAACTTCAAGTAGATTTCC, CATCAGACAACTCTTATTG. Oligonucleotide siRNA against S6K2 and S6K1 were purchased from Dharmacon as SMARTpools.

Sequences were as follow: S6K2, GCAAGGAGUCUAUCCAUGAUU, GACGUGAGCCAGUUUGAUAUU, GGAAGAAAACCAUGGAUAAUU, GGAACAUUCUAGAGUCAGUUU; AS, 5'-PACUGACUCUAGAAUGUUCCUU; S6K1, GCAGGAGUGUUUGACAUAG, GACAAAAUCCUCAAAUGUA, CAUGGAACAUUGUGAGAAA, CCAAGGUCAUGUGAAACUA. Oligonucleotide targeting of B-Raf was achieved using a single sequence: AAAGAAUUGGAUCUGGAUCAU.

Reagents. Etoposide was purchased from Calbiochem. PKCα, PKCβ, PKCδ, PKCλ, PKCγ, PKCζ, PKCα, PKCε, Bcl-X_L and XIAP antibodies were purchased from Becton Dickinson. The phospho-PKCα and phosphor-S6 protein antibody was from Cell Signalling. The phospho-PKCε antibody against Ser729 and an additional PKCε antibody (for Western-blotting only) were obtained from Upstate. The phospho-PKCε antibody against Thr566 was as described previously (Parekh et al., 1999). S6K1, B-Raf, Raf-1, Lamin B and Actin antibodies were purchased from Santa Cruz. The S6K2 antibody was as described previously (Gout et al., 1998). Protein A and G were obtained from Amersham. Lipofectin, Lipofectamin Plus, G418, zeocin and puromycin were obtained from Invitrogen. The activated ERK antibody, etoposide, polybrene and crystal violet were obtained from Sigma. FGF-2, PD098059, Gö6976, Hispidin, BAPTA, Rottlerin and GF109203X were purchased from Calbiochem.