Supplementary Methods

SiRNA Design and Synthesis:

siRNA targeting human cyclophyilin B (peptidyl prolyl isomerase, PPIB, BC001125), cyclo 1, and MEK1 (MAP2K1, NM_002745),MEK1-2 and MEK1-4 were designed at Dharmacon, Inc. using the SMARTselection algorithm [1]. Following sequences (sense strand) were used:

cyclo 1 5'GAAAGAGCAUCUACGGUGA

MEK1-2 5'GCAGAGAGAGCAGAUUUGA

MEK1-4 5'GAGCAGAUUUGAAGCAACU

dTdT overhang was used for all 3 siRNA.

Sense and antisense strands of siRNA were synthesized using 2'-ACE chemistry [2], deprotected, and annealed to generate stock solutions.

Lipofectamine 2000 Transfection: For lipid-mediated transfection using Lipofectamine 2000 (L2K, Invitrogen), HeLa cells (ATCC, Manassas, VA) were plated in 96 well plates (10^3 cells/well) and cultured over night before being transfected with siRNA (100nM) using LP2000 (0.3 l/well). After 24 hours, cells were lysed to retrieve mRNA for quantitation of human cyclophilin B mRNA levels and gene profiling studies. For quantifaction, Cyclophilin B and MEK1 mRNA levels were assessed using a branched DNA assay (Genospectra, Fremont, CA). Tissue processing and transcript quantitation

was performed according the manufacturers instructions, using GAPDH as a reference control.

For the study of gene expression in cells treated with lipid transfection reagent alone Hela cells were incubated with Lipofectamine2000 in concentrations indicated . Cells were harvested for mRNA 24h after lipid addition.

Electroporation: For electroporation, cells were harvested in mid-log phase and electroporated with cyclo 1, MEK1-2 or MEK1-4 siRNA (100nM, 3 x 10^6 cells/ml) or without siRNA (mock) using a Cyto Pulse PA-4000. The cuvette gap was 2 mm and a total of six 100 sec pulses were applied with an interval time of 0.125 seconds (2200 volts /cm). Branched DNA assays were similarly performed to assess cyclophilin B and MEK1 mRNA levels relative to the level of GAPDH mRNA.

Microarray experiments: For each sample, 1ug of total RNA isolated from cyclo 48-treated cells was amplified and Cy5- or Cy3-labeled (Cy-5 and Cy-3 CTP, Perkin Elmer) using Agilent's Low Input RNA Fluorescent Linear Amplification Kit. Hybridizations were performed using Agilent's Human 1A (V2) Oligo Microarrays (22,000 sequences) according to the published protocol, with 750 ng each of Cy-3 and Cy-5 labeled material loaded onto each array. Slides were washed using 6X and 0.06X SSPE (each with 0.025% N-lauroylsarcosine) and dried using Agilent's nonaqueous drying and stabilization solution. Biological replicates of each sample array were scanned on an Agilent Microarray Scanner (model G2505B) and the raw image was processed using Feature Extraction software (v6.1.1 or v7.5.1). Further analysis was performed using

Spotfire Decision Site 7.2 software and the Spotfire Functional Genomics Module. Low signal genes (those with a Log(total intensity)<2.8 in a self-self hybridization, calculated from the log base 10 of the green and red processed signal sum) were removed from the analysis. A 2-fold cutoff (Log Ratio of >0.3 or <–0.3) was applied to genes used in comparative analysis. Outlier flagging was not used. To generate scatter plots, genes modulated by two-fold or more in two independent replicates were downloaded into an Excel file and plotted using fold change (Y-axis) vs relative expression (X-axis).

Dye swap hybridizations were performed to identify targets that demonstrated dye-biased labeling and/or hybridization artifacts and such targets were excluded from analysis.

References

1. Reynolds, A. et al. *Nature Biotechnology* **22**, 326-330 (2004).
2. Scaringe, S. A. *Methods in Enzymology* **317**, 3-18 (2000).