Supplemental Data

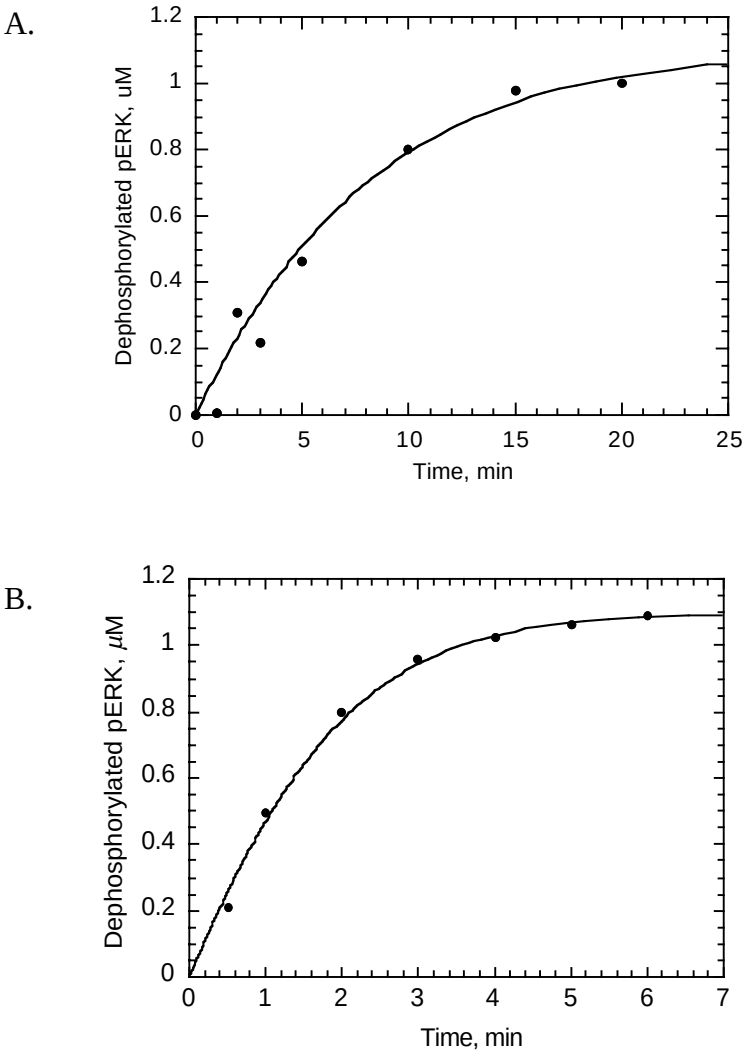
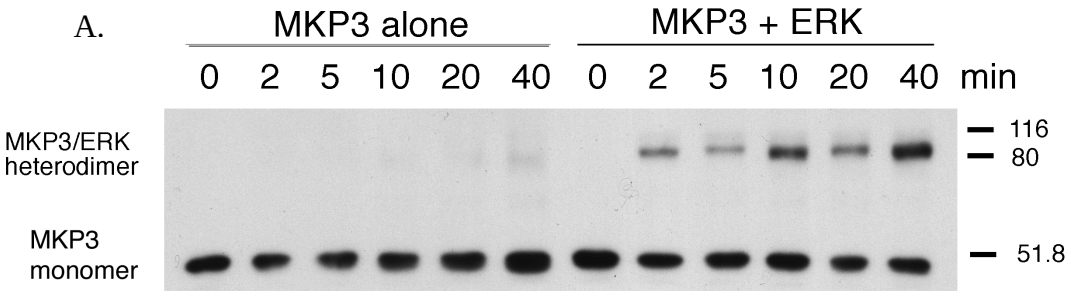
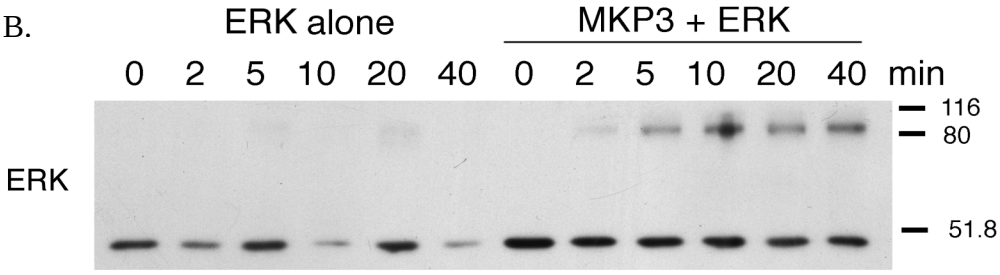


Figure 1. Time-dependent dephosphorylation of pERK by VHR and the MKP3/VHR chimera using a radioactive kinase assay. VHR (A) and MKP3/VHR chimera (B) (0.1 μM) were combined with 1 μM pERK and incubated for up to 20 min at 25 °C. Aliquots were withdrawn at the indicated times and ERK kinase assays were performed as described in Materials and Methods. The data were fitted to the integrated Michaelis-Menten equation (Eq. 2). The graph is an average of three different experiments.

A.


MKP3 alone
MKP3 + ERK
0 2 5 10 20 40 0 2 5 10 20 40 min
MKP3/ERK heterodimer
MKP3 monomer
116
80
51.8

B.


ERK alone
MKP3 + ERK
0 2 5 10 20 40 0 2 5 10 20 40 min
ERK
116
80
51.8

C.

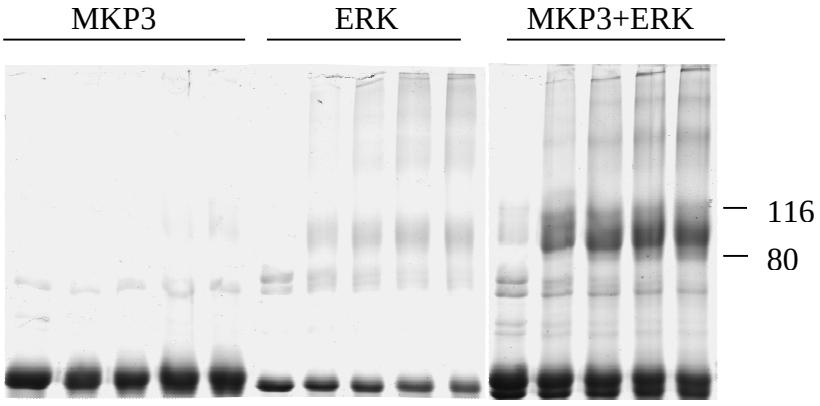

MKP3
ERK
MKP3+ERK
116
80

Figure 2. Glutaraldehyde cross-linking of MKP3, ERK and the mixture of MKP3 and ERK. (A-B) MKP3 (8 μM), ERK (8 μM), or 1:1 mixture of MKP3 and ERK (8 μM each) were cross-linked with 5 mM glutaraldehyde up to 15 min at 25 °C. The reactions were terminated by adding glycine (pH 9.0) at a final concentration of 0.2 mM. The cross-linked species were determined with SDS-PAGE and western blotting with appropriate antibodies. (C) Coomassie staining of SDS-PAGE of cross-linked species.

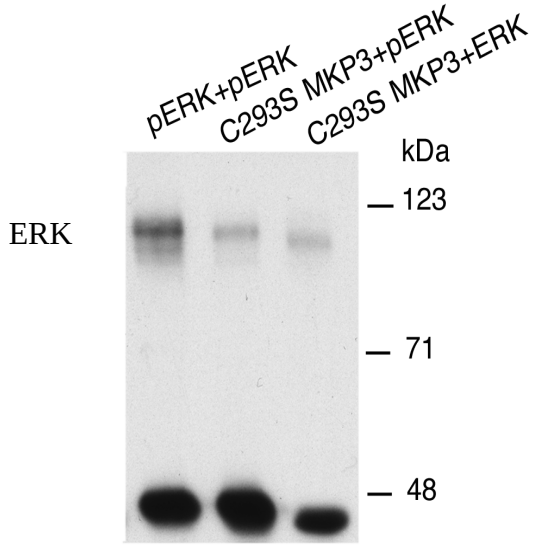


Figure 3. Mobility differences in cross-linked species. ERK-MKP3, pERK-pERK, ERK-pERK cross-linked species were separated by SDS-PAGE and probed with antibody (polyclonal) that recognizes ERK.