Expression and purification of MAPKs. BL21(DE3)pLysS *E. coli* (Invitrogen) were freshly co-transformed with expression constructs for the MAPK and its activating kinase(s) and selected on LB plates with 100 μg/ml ampicillin, 50 μg/mL kanamycin and 34 μg/ml chloramphenicol. Kinase expression was induced at 30°C under full antibiotic selection in 250 ml log phase cultures (OD_{600} 0.5-0.8) with 0.4 mM IPTG. After 6 hr the cultures were pelleted, washed once with TBS (10 mM Tris, 140 mM NaCl, pH 8.0) and snap frozen. The frozen pellet was resuspended in 5 ml ice-cold resuspension buffer (20 mM Tris, 140 mM NaCl, 10 mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 μg/ml pepstatin A and 400 μM PMSF at pH 7.5). The cell suspension was digested with 100 μg/ml lysozyme for 10 minutes on ice and lysed by adding sodium deoxycholate to 0.05% and incubating 15 min at room temperature. Genomic DNA was digested with 30 U/ml DNAse I and 50 mM $MgCl_2$ at room temperature for 15 min. Lysed cultures were cleared by centrifugation at 20,000 × g for 15 min at 4 °C. GST-ERK2 was recovered by incubating the cleared lystate with 500 μl of prewashed glutathione sepharose 4B slurry (GE Healthcare) at 4 °C for 2 hr. The bound resin was washed twice for 5 min with ice-cold TBS + 0.05 % Ipegal CA-630 (Sigma) and washed once for 5 min with ice-cold GST wash buffer (20 mM Tris, 50 mM NaCl and 1 mM DTT at pH 7.5). GST-ERK2 was eluted from the resin by incubation for 10 min at 4 °C with 500 μl GST wash buffer containing 10 mg/ml reduced glutathione. The eluate was dialyzed at 4 °C overnight in 10,000 MWCO dialysis tubing against 1 l GST wash buffer. Dialyzed protein was aliquoted, snap frozen and stored at -80 °C. Purified MAP kinases were quantified in triplicate by absorbance at 280 nm (in 6 M guanidinium HCl, 40 mM NaOAc, pH 6.5) and assayed for purity by resolving 0.2 μg and 2 μg aliquots on 12 % SDS-PAGE and staining with Coomassie Brilliant Blue.

Consensus peptide kinase assays. Peptides were diluted rapidly from DMSO stocks to 10X aqueous stocks in 20 mM HEPES, pH 7.4. Kinase reactions were initiated at 20 sec intervals in 25 μl at 30 °C (20 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 0.1 % Tween-20, 100 μM ATP, 15 μCi γ-[^{33}P]-ATP). After 10 min, 20 μl of the reaction was spotted, in 20 sec intervals, onto 2.5 cm circles of P81 phosphocellulose paper and immediately quenched in 75 mM H_3PO_4. Three additional 5 min washes in 75 mM H_3PO_4 were performed to remove unincorporated radiolabel. After a brief wash in acetone to facilitate drying, the P81 circles were immersed in 5 ml of OptiFluor scintillation fluid (Perkin Elmer) and ^{33}P incorporation was measured on a Beckman Coulter LS6500 scintillation counter. The specific activity of radiolabel for each set of experiments was measured by pooling the excess reaction volumes, spotting them in 20 μl aliquots on P81 circles, and measuring the radioactivity of the unwashed circles, in triplicate. All kinase assays were performed in three independent reactions and are reported as the rate of phosphate incorporation in pmol phosphate/sec/nmol kinase.