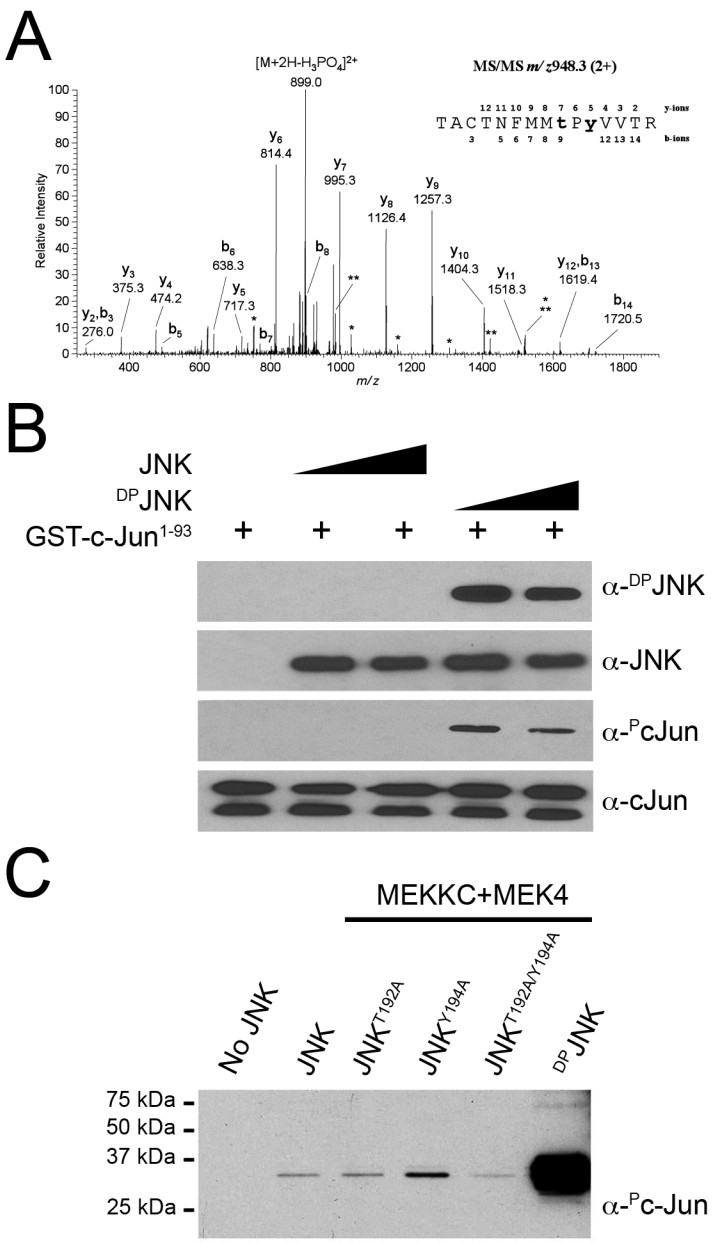
Supplemental Figure 1: Recombinant rat ^{DP}JNK phosphorylates *c*-Jun in response to diphosphorylation of the TPY motif in the activation loop. A, Spectrum of rat JNK$^{175-189}$ peptide fragment encompassing the phosphorylation sites at Thr183 and Tyr185 in the activation loop. Detected *C*-terminal (y-series) and *N*-terminal (b-series) ions are indicated on the sequence. Loss of phosphoric acid from a fragment ion is designated by * for y-ions and ** for b-ions. B, The kinase activity was assessed using a GST-*c*-jun$^{1-89}$ substrate. Note that only the ^{DP}rJNK was able to phosphorylate *c*-jun *in vitro*. C, Phosphorylation of both residues is required for maximal JNK activity. Single and double point mutants in the JNK activation loop were tested for kinase activity. Note that residual kinase activity exists with either of the single or double JNK point mutants, which is comparable to non-phosphorylated JNK.

Supplemental Figure 2: Recombinant MKP-5 is catalytically active. Recombinant MKP-5 protein was incubated with pNPP at 25° C and hydrolysis monitored at 405 nm. Increasing concentrations of MKP-5 promoted dose-dependent cleavage of pNPP, whereas MKP harboring a cysteine to serine inactivating mutation in the catalytic core at residue 408 abolished phosphatase activity.

Supp. Figure 1



Supp. Figure 2