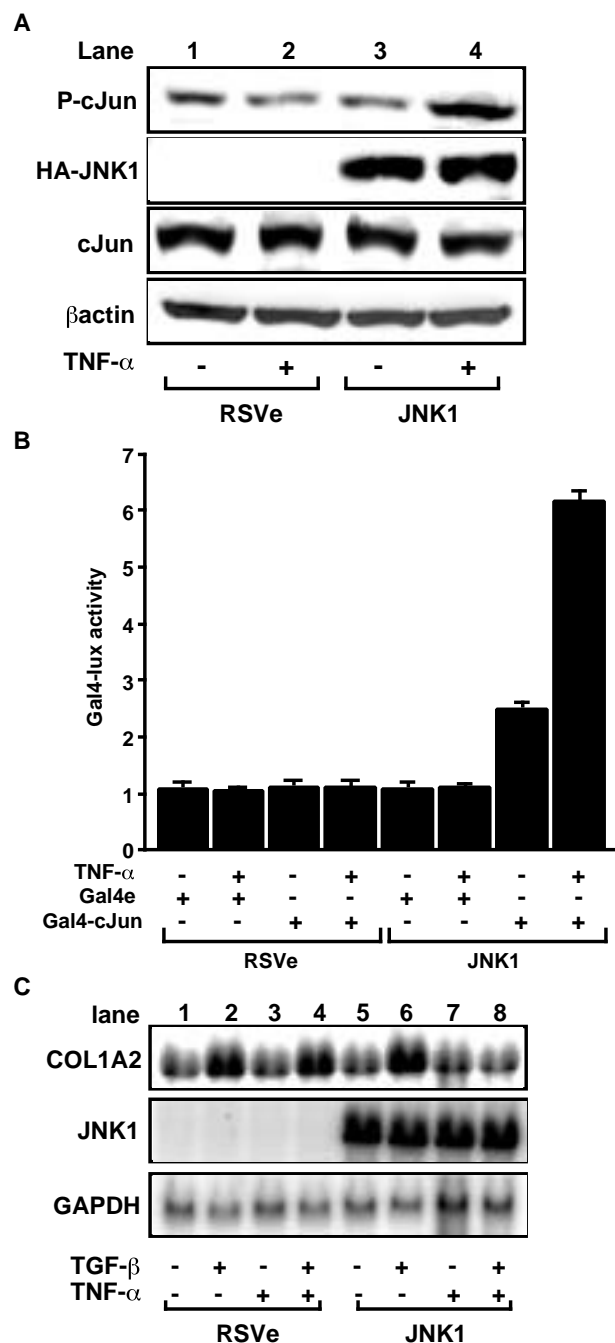


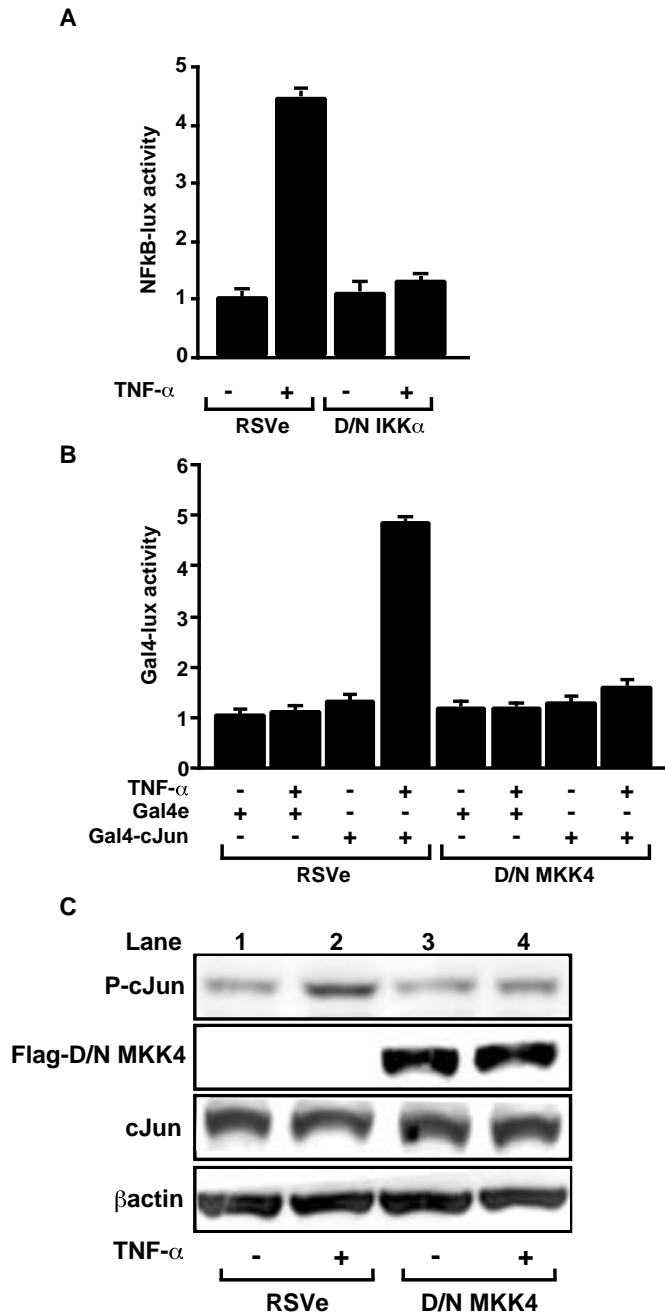
Supplementary data



Supplementary figure 1

Ectopic JNK1 expression in *JNK*^{-/-} fibroblasts restores JNK-dependent activities of TNF- .

A, *JNK*^{-/-} fibroblast cultures were electroporated with either empty pRSVe or a HA-tagged *jnk1* expression vector using a Nucleofector™ (Amaxa GmbH, Koeln, Germany). Electroporation efficiency, estimated by FACS analysis of a co-transfected GFP expression vector, was typically in the 65-75% range. One day later, TNF- was added and incubations continued for another 30 min. after which whole cell extracts were subjected to Western blot analysis of transfected Ha-tagged JNK1 and endogenous phospho-cJun, c-Jun and actin contents. Note the restoration of c-Jun phosphorylation in response to TNF- upon expression of JNK1 (lane 4 vs. lane 2). **B**, Sub-confluent *JNK*^{-/-} fibroblast cultures were co-transfected with Gal4-lux and either empty Gal4e or Gal4BD-cJun expression vectors, together with either pRSVe or *jnk1* expression vectors. Eighteen hours following glycerol shock, TNF- was added and luciferase activity was measured 24 hours later. Note the restoration of c-Jun-dependent transactivation in the presence of *jnk1*. **C**, *JNK*^{-/-} fibroblasts in which ectopic *jnk1* expression was allowed for 1 day (see panel A) were incubated for 24h in the presence or absence of TGF- and/or TNF- . *COL1A2* and *GAPDH* mRNA steady-state levels were then measured using Northern hybridization of total RNA. Note that *jnk1* expression restores the inhibitory effect of TNF- against TGF- -induced *COL1A2* expression (lane 8 vs. lane 6 compared to lane 4 vs. lane 2).



Supplementary figure 2

Dominant-negative activities of mutant MKK4 and IKK- kinases.

A, sub-confluent human dermal fibroblast cultures were transfected with pNF- κ B-lux together with either empty pRSVe or D/N IKK- expression vectors. Six hours later, TNF- was added and incubations continued for another 6 hours. Note that D/N IKK- abolishes TNF- -induced NF- B-dependent transcription. B, sub-confluent human dermal fibroblast cultures were co-transfected with Gal4-lux and either empty Gal4e or Gal4-cJun expression vectors, together with either pRSVe or D/N MKK4 expression vectors. Eighteen hours following glycerol shock, TNF- was added and luciferase activity was measured 24 hours later. Note that D/N MKK4 completely abolishes TNF- -induced Gal4-lux transactivation. C, dermal fibroblasts were transfected with either pRSVe or Flag-tagged D/N MKK4 expression vectors using the NucleofectorTM. The next day, the cultures were treated with TNF- for 1h before Western analysis of total protein extracts. Note that D/N MKK4 abolishes TNF- -induced c-Jun phosphorylation (lane 4 vs. lane 2).