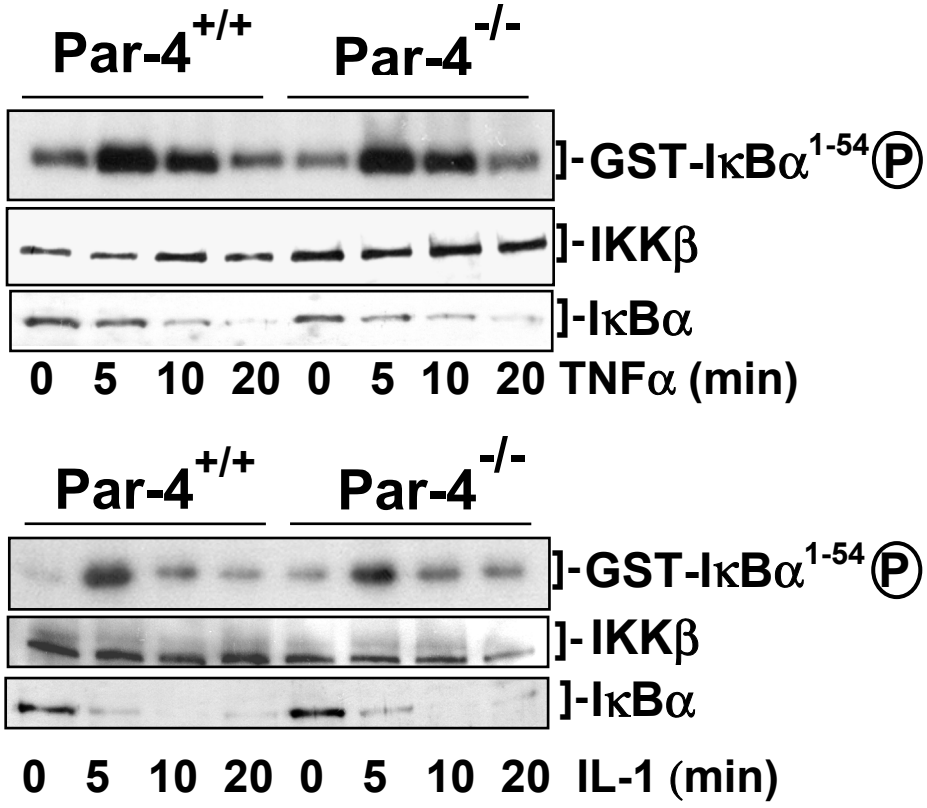
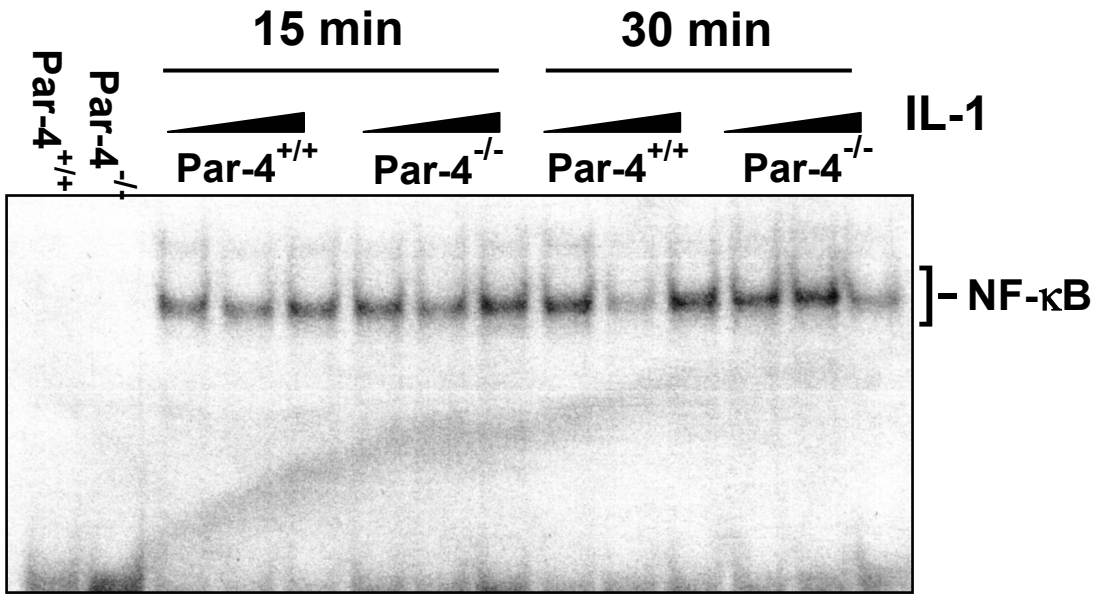
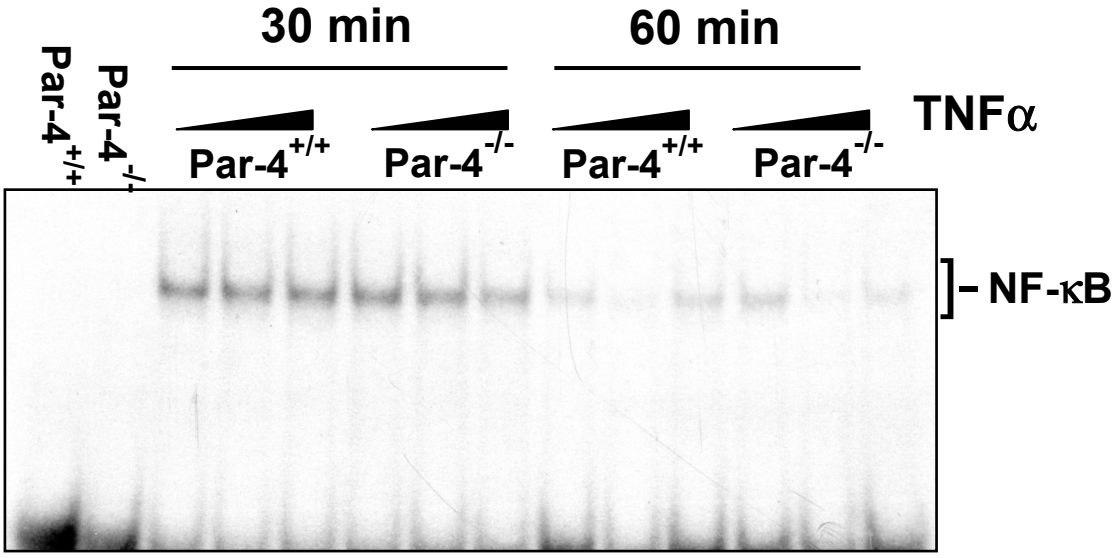
Supplementary data



Supplementary figure 1

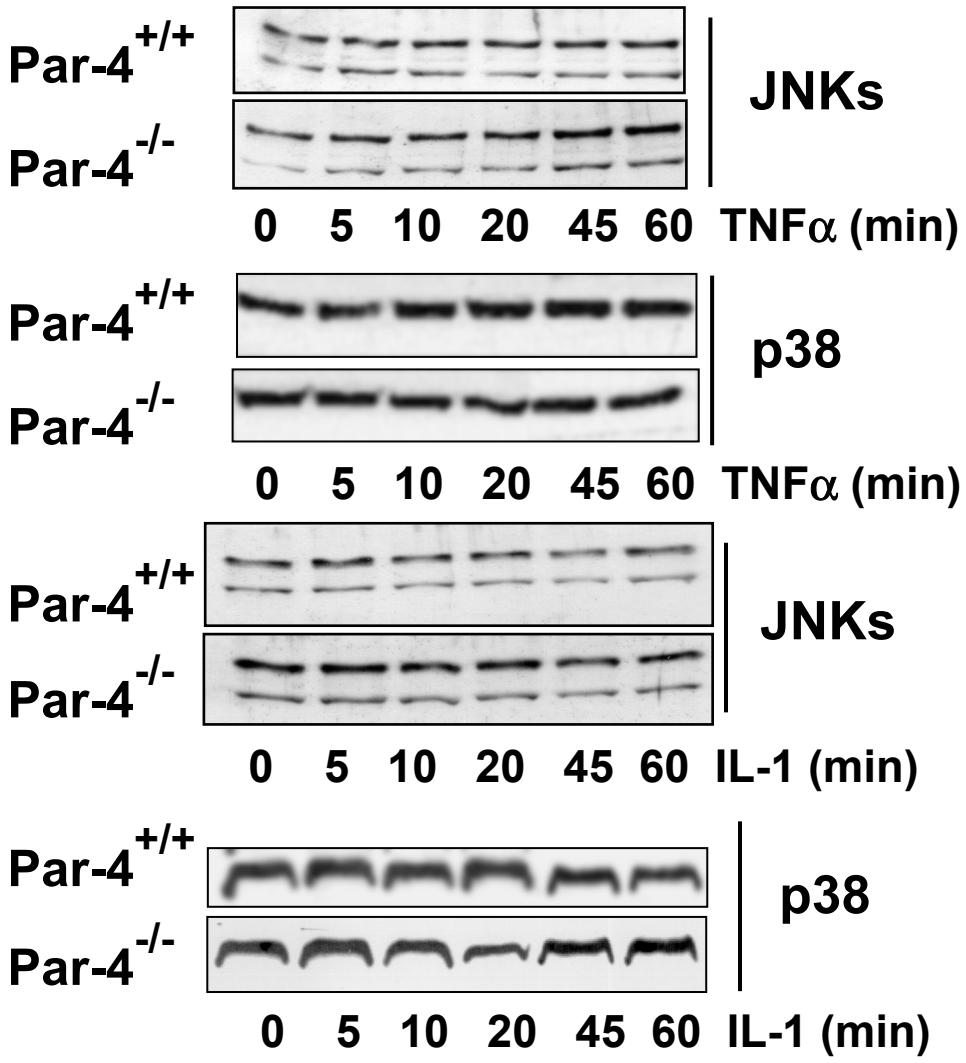
EFs were stimulated with 10 ng/ml of either TNFα (upper panel) or with 5 ng/ml of IL-1 (lower panel) for different times after which cell extracts were prepared and IKK activity was determined in immunoprecipitates of IKKγ as described previously (Leitges et al., 2001). Also, the amount of IKKβ immunoprecipitated was determined with an anti-IKKβ antibody and the degradation of IκBα was determined by immunoblotting of extracts with an anti-IκBα antibody. This is a representative experiment of another two with similar results.





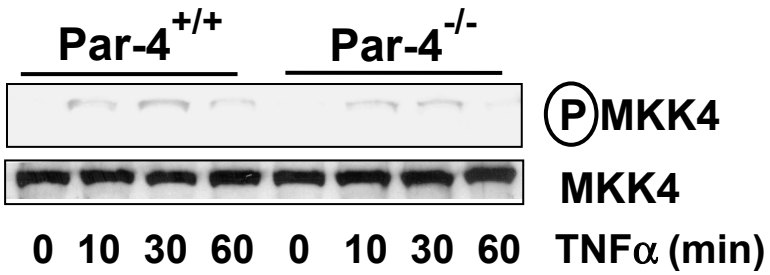
Supplementary figure 2

EFs were stimulated with different concentrations of either TNFα (5, 10 and 20 ng/ml; lower panel) or IL-1 (1, 5 and 10 ng/ml; upper panel) for different times after which nuclear cell extracts were prepared and electrophoretic mobility shift assays were determined as previously described (Leitges et al., 2001). This is a representative experiment of another two with similar results.



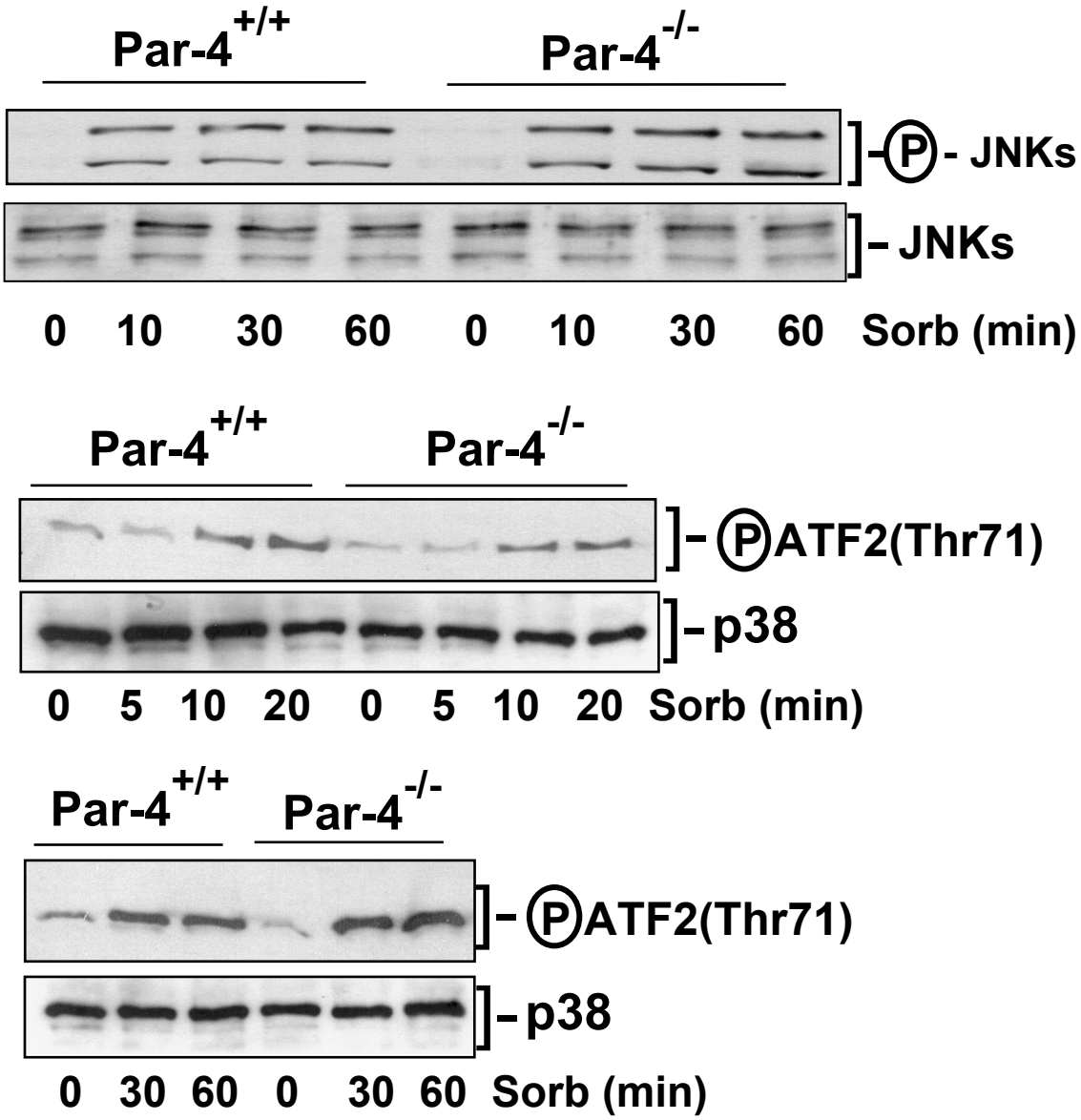
Supplementary figure 3

Extracts from the experiments of Fig. 4 were analyzed by immnublotting with anti-JNK and anti-p38 antibodies.



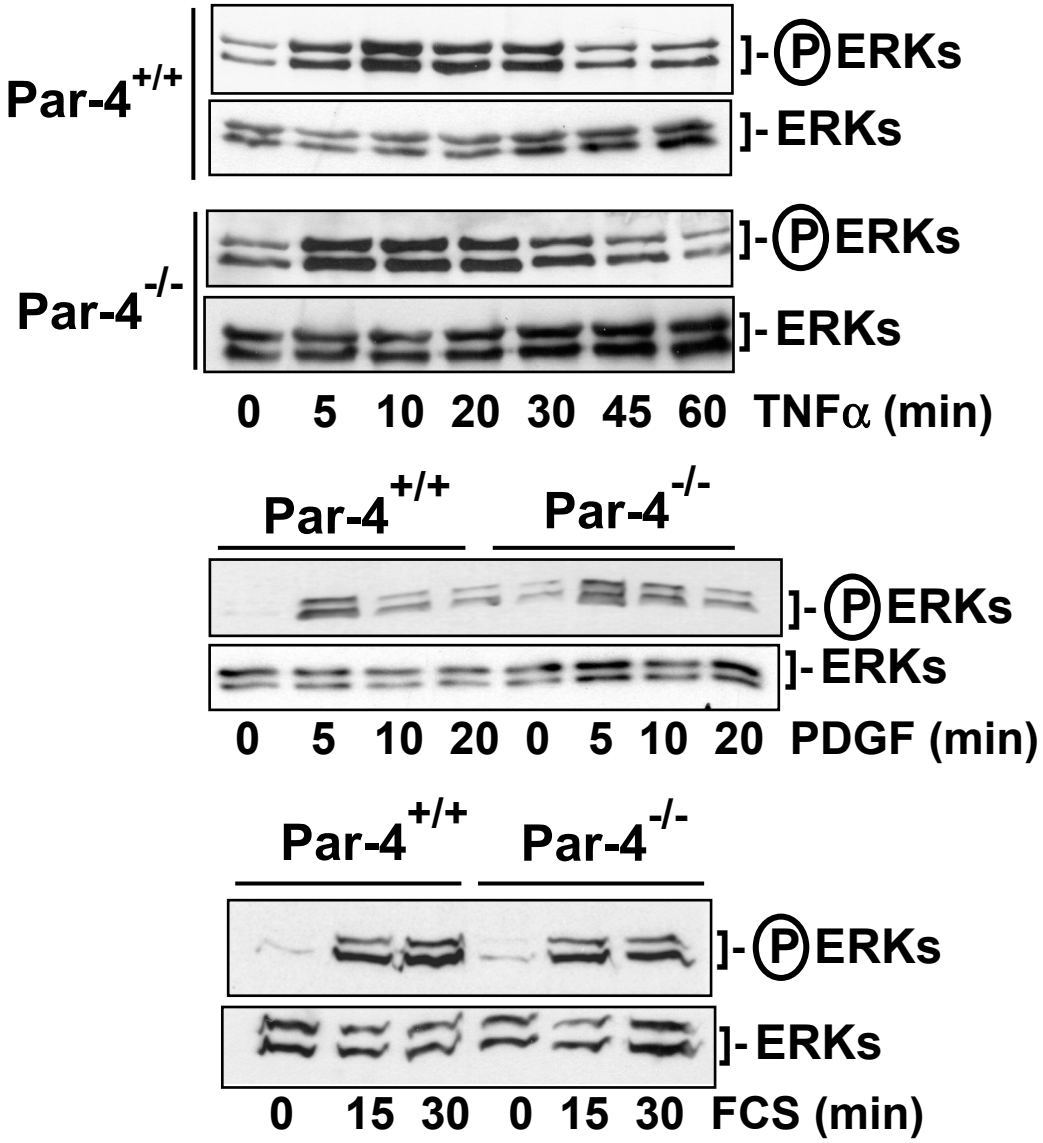
Supplementary figure 4

Extracts from EFs either wild type or Par-4-/- that had been stimulated or not with TNFα (20 ng/ml) for different times, were analyzed by immunoblotting with an anti-phospho-MKK4 antibody or an anti-MKK4 antibody. These are representative experiments of another two with similar results.



Supplementary figure 5

EFs either wild type or Par-4-/- were stimulated with sorbitol (0.6 M) for different times, after which cell extracts were analyzed by immunoblotting with an anti phospho-JNK antibody (upper panel). Extracts were also immunoprecipitated with an anti-p38 antibody and p38 activity was determined. The levels of JNKs and p38 are shown as control. These are representative experiments of another two with similar results.



Supplementary figure 6

EFs either wild type or Par-4-/- were stimulated either with 10 ng/ml TNFα (upper panel), 50 ng/ml PDGF (middle panel), or fetal calf serum (FCS; lower panel) for different times, after which cell extracts were analyzed by immunoblotting with an anti phospho-ERK antibody. The levels of ERKs are shown as control. These are representative experiments of another two with similar results.

References

Leitges, M., Sanz, L., Martin, P., Duran, A., Braun, U., Garcia, J.F., Camacho, F., Diaz-Meco, M.T., Rennert, P.D. and Moscat, J. (2001) Targeted Disruption of the zetaPKC Gene Results in the Impairment of the NF-kappaB Pathway. *Mol Cell*, **8**, 771-780.