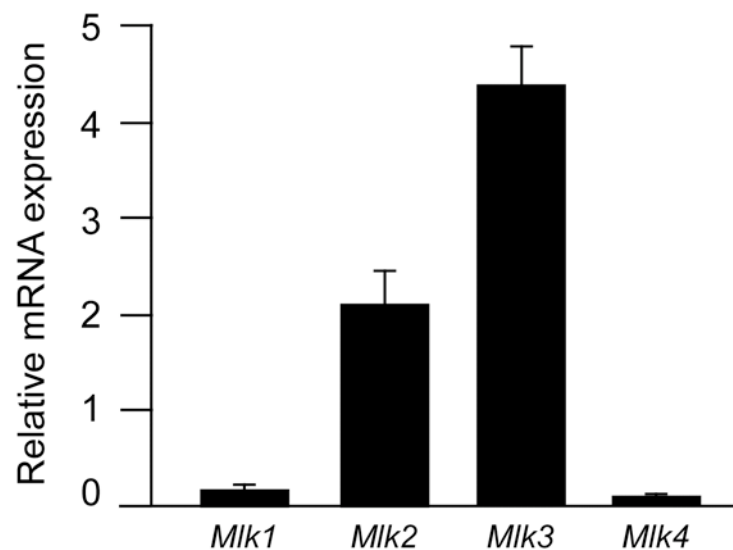


***Supplemental Information***

**TNF-stimulated MAP kinase activation mediated by a Rho family GTPase signaling pathway.**

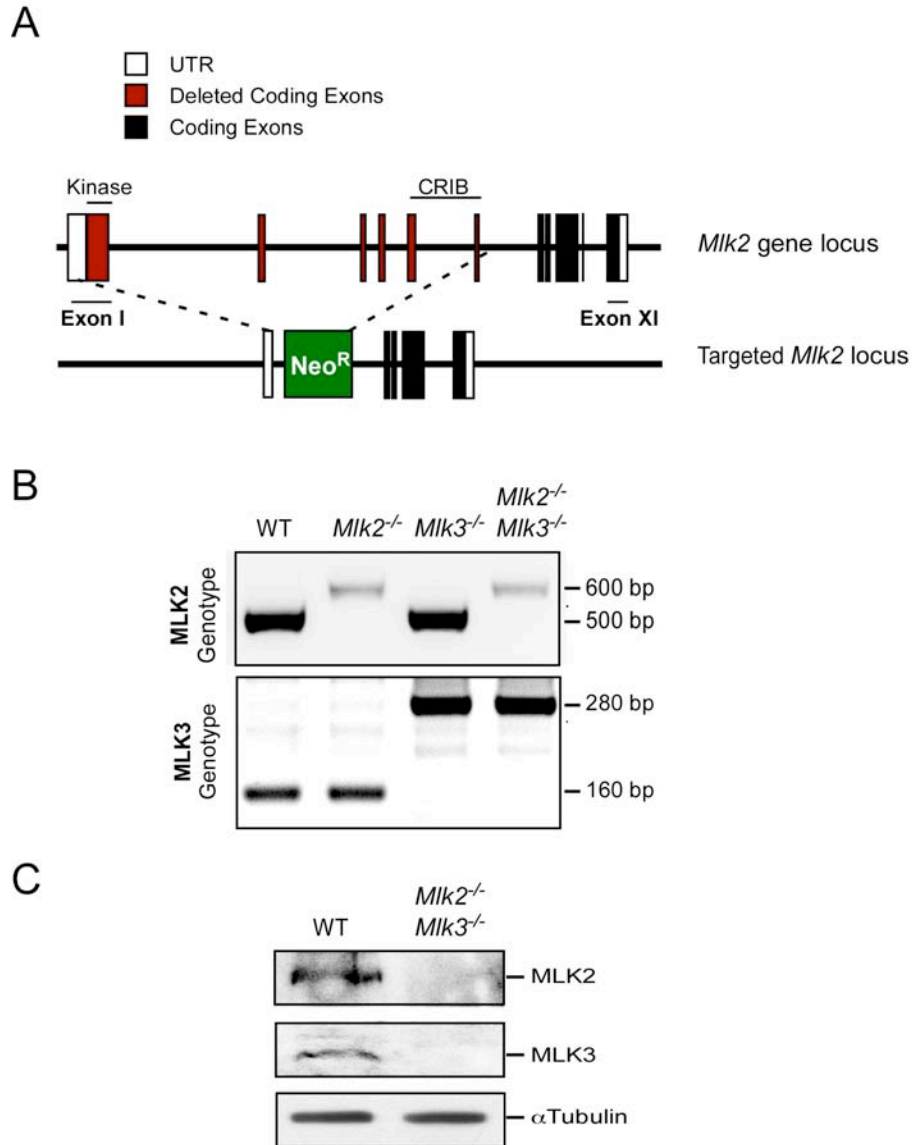
Shashi Kant, Wojciech Swat, Sheng Zhang, Zhong-Yin Zhang, Benjamin G. Neel, Richard A. Flavell, and Roger J. Davis

Supplemental Figures S1 – S4



**Supplementary Figure S1. Expression of MLK isoforms.**

Total RNA isolated from wild-type MEF was employed to measure the expression of *Mik1*, *Mik2*, *Mik3*, and *Mik4* mRNA by quantitative Taqman<sup>®</sup> RT-PCR analysis. The data are normalized for the amount of *Gapdh* mRNA detected in each sample (mean ± SD; n = 4).



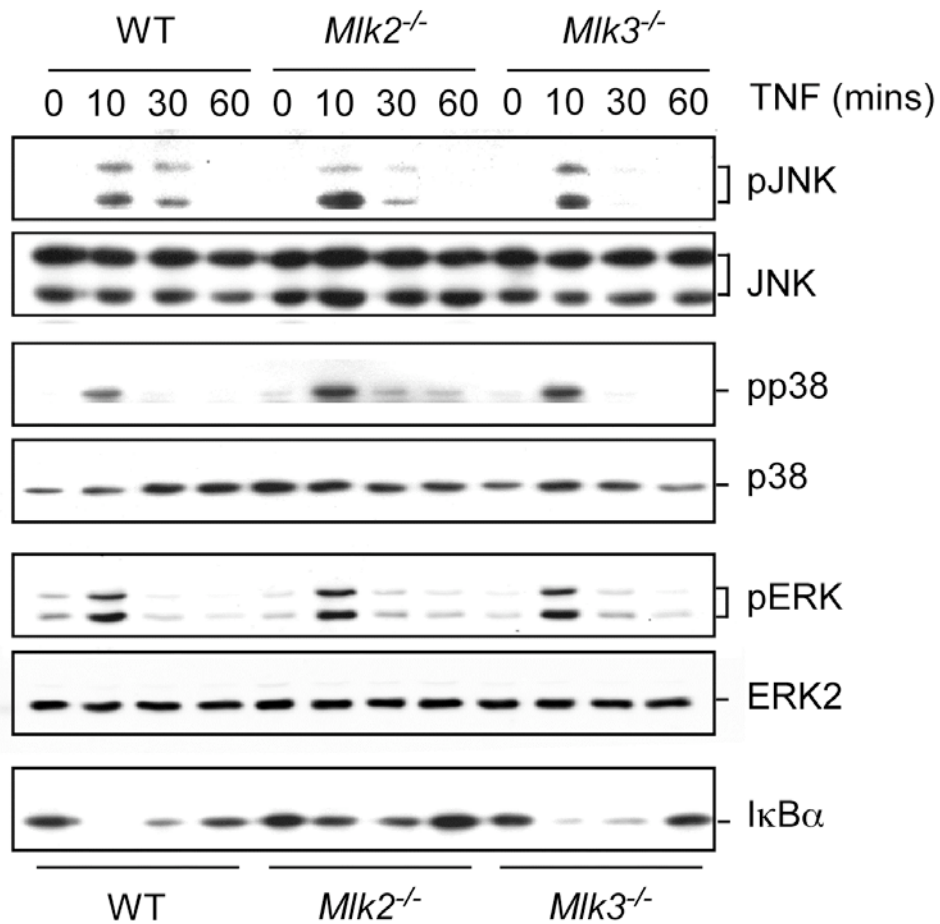
### Supplementary Figure S2. Construction of MLK2-deficient mice.

**(A)** Strategy for the creation of *Mik2*<sup>-/-</sup> mice. The structure of the *Mlk2* genomic locus and the targeted locus are illustrated. The kinase domain is encoded by exon I and the CRIB domain is encoded by exons V and VI. Homologous recombination was employed to replace *Mlk2* exons I - VI with a *Neo*<sup>R</sup> cassette. The deletion starts within exon I (260 bp 5' of the translation initiation site) and extends to intron VI.

**(B)** Genomic DNA isolated from mouse tails was examined by PCR to detect wild-type and disrupted alleles of *Mik2* and *Mik3*.

**(C)** Extracts prepared from WT and *Mik2*<sup>-/-</sup> *Mik3*<sup>-/-</sup> MEF were examined by immunoblot analysis using antibodies to MLK2, MLK3, and α-Tubulin.

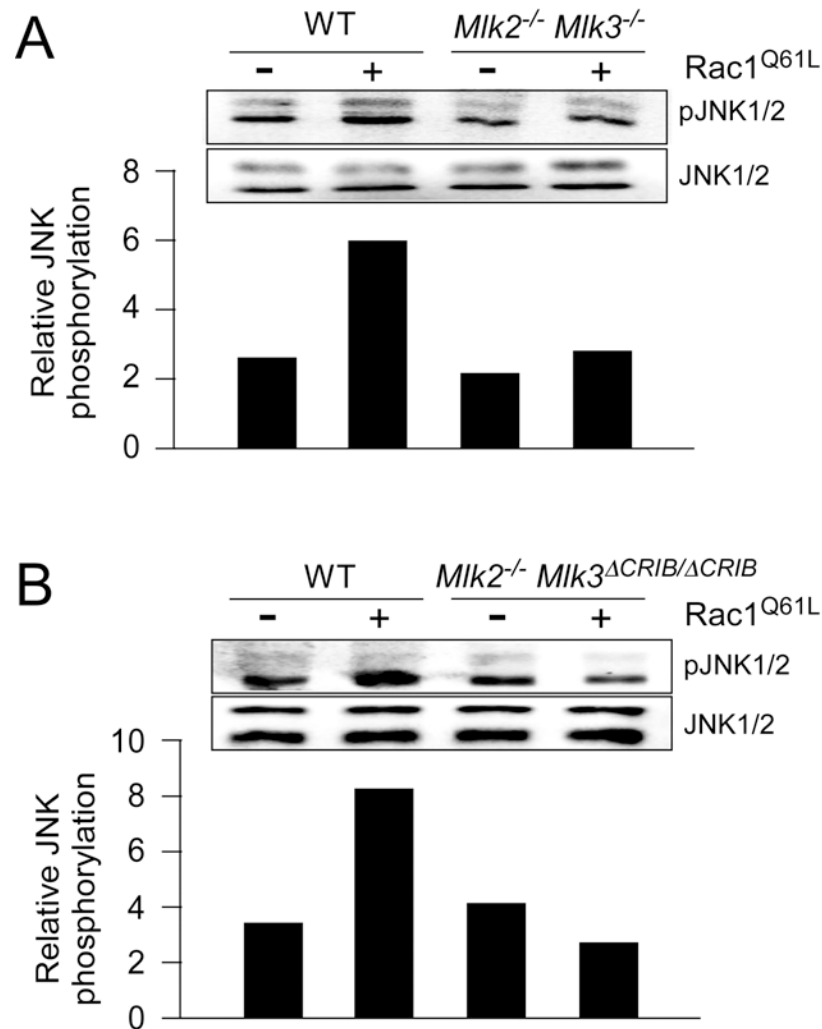
Quantitative RT-PCR assays of WT and *Mik2*<sup>-/-</sup> *Mik3*<sup>-/-</sup> MEF demonstrated no significant differences in the expression of *Tak1*, *Tnfr1*, and *Tnfr2* mRNA (*data not shown*).



**Supplementary Figure S3. Effect of MLK2 or MLK3 -deficiency on the response to TNF.**

WT, *Mik2*<sup>-/-</sup>, and *Mik3*<sup>-/-</sup> MEF were treated without or with 10 ng/ml TNF $\alpha$ . Protein extracts were examined by immunoblot analysis by probing with antibodies to MAP kinases, phospho-MAP kinases, and I $\kappa$ B $\alpha$ .

MLK2 or MLK3 -deficiency did not alter the activation of the NF- $\kappa$ B pathway, as monitored by measurement of I $\kappa$ B $\alpha$  degradation. Similarly, MLK2 and MLK3 -deficiency did not cause major defects in MAP kinase activation.



**Supplementary Figure S4. Effect of MLK-deficiency on Rac-stimulated JNK activation.**

(A) WT and *Mlk2*<sup>-/-</sup> *Mlk3*<sup>-/-</sup> MEF were transduced with a retroviral vector that expresses activated Rac (pBABE-Rac1<sup>Q61L</sup>-Puro) or with a control vector (pBABE-Puro). Transduced MEF were selected by incubation with medium supplemented with 2 μg/ml puromycin (72 hrs) at 48 hrs post-infection. Cell lysates prepared from the MEF were examined by immunoblot using antibodies to JNK and phospho-JNK. The immunoblots were quantitated using an ImageQuant LAS4000 machine and ImageQuant TL software (General Electric).

(B) Rac-stimulated JNK activation was compared using cultures of WT and *Mlk2*<sup>-/-</sup> *Mlk3*<sup>ΔCRIB/ΔCRIB</sup> MEF.