Supporting Information

Liu et al. 10.1073/pnas.0906328106

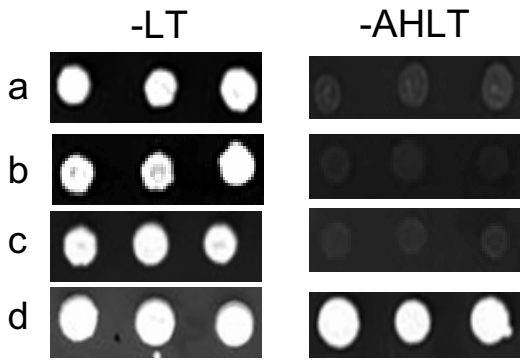

Fig. S1. Miz1 also interacts with JNK2 in yeast. JNK2 was fused to GAL4-DNA binding domain (pGBKT7), and Miz1 was fused with GAL4-transactivating domain (pACT2). The interaction between pGBKT7/JNK2 and pACT2/Miz1 was analyzed by cotransformation assay in yeast. a, pGBKT7 + pACT2; b, pGBKT7/JNK2+ pACT2; c, pGBKT7 + pACT2/Miz1; d, pGBKT7/JNK2 +pACT2/Miz1. A, adenine; L, leucine; H, histidine; T, tryptophan.

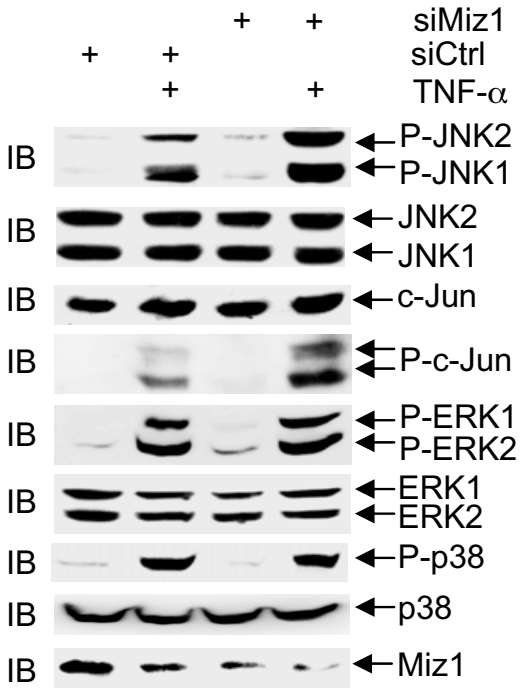

Fig. S2. Knockdown of Miz1 by a different siRNA of Miz1 (siMiz1) also augments TNF-α- induced activation of JNK1 but not p38 and ERK. WT fibroblasts were transfected with the control scramble siRNA or the second siRNA of Miz1 (CGAGACGGAAGUACUCAAA) (100 nm each). After 36 h, cells were stimulated without or with TNF-α (5 ng/mL, 15 min). Phosphorylation and expression of JNK, ERK, p38, c-Jun, and Miz1 were analyzed by immunoblotting with corresponding antibodies, respectively.

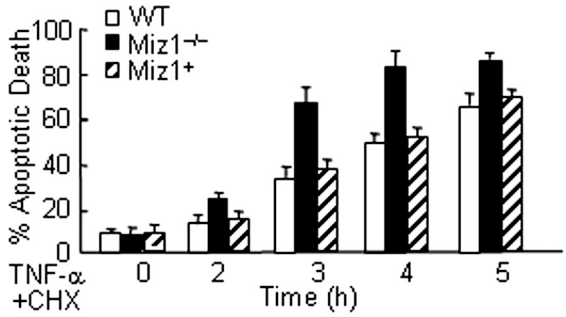

Fig. S3. The loss of Miz1 also accelerated TNF-α-induced cell death in the presence of CHX. Miz1-null MEFs were transfected with EGFP (0.8 μg) plus Express-Miz1 or empty vector (3.2 μg each) for 36 h and then treated with TNF-α (5 ng/mL) plus CHX (1 μg/mL) for various periods of time as indicated. Apoptotic death of EGFP-positive cells was determined.

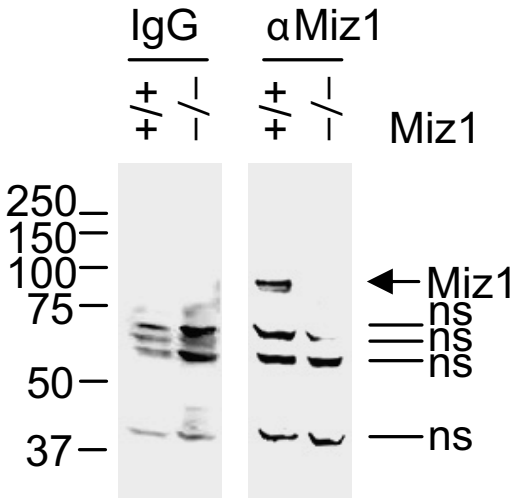

Fig. S4. Characterization of anti-Miz1 polyclonal antibody. Polyclonal anti-Miz1 antibody was generated by immunization of rabbits with a KLH-conjugated peptide corresponding to the residues (EPPEENEESAGTDSG) of the murine Miz1 sequence (Synbiosci). Cell extracts of WT and Miz1 null mouse embryonic fibroblasts were analyzed by immunoblotting with preimmune sera (IgG) or anti-Miz1 sera (1:500 dilution). ns, nonspecific proteins.