Supplemental information

Experimental procedures

Cell culture and reagents

Human fibrosarcoma cell line HT1080, human embryonic kidney (HEK) 293A cells, and mouse embryonic fibroblasts (MEF) were grown in Dulbecco's Modified Eagle Medium (DMEM), 10% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin solution, at 37 °C under a 5% CO_2 atmosphere. All reagents were obtained from commercial sources; PMB, NAC (Wako, Tokyo, Japan), FasL (Enzo Life Science), Z-VAD-fmk (Peptide Institute, Osaka, Japan), ML385 (Merck Millipore, Burlington, USA). The anti- bodies used were against caspase-8 (Enzo Life Science), caspase-3, caspase-9 (Cell Signaling, Danvers, USA), and β-actin (Wako, Tokyo, Japan).

Colorimetric cell viability assay

Cells were seeded on 96-well plates. After indicated stimulation, cell viability was measured by phenazine methosulfate (PMS)/3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay using Cell Titer 96 Cell Proliferation Assay kit (Promega, Madison, USA), according to the manufacturer's protocol. The absorbance was read at 492 nm using the iMark microplate reader (Bio-Rad, Hercules CA, USA). Data were normalized to control (100%) without stimulus.

Cell death assay

Cell death was monitored using an LDH-Cytotoxic Test Kit (Wako, Tokyo, Japan) according to the manufacturer's protocol. The activity level of the LDH released into the culture media was quantified as a percentage of the total activity level of LDH.

Immunoblot

Cells were lysed with the 1 % Triton X-100 buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 % Triton-X100, 10 % Glycerol, and 1 % protease inhibitor cocktails (Nacalai Tesque, Kyoto, Japan)]. After centrifugation, the cell extracts were resolved by SDS-PAGE, and analyzed by immunoblotting. The blots enhanced by chemiluminescence ECL (Merck Millipore, Burlington, USA) were developed using ChemiDoc Touch (Bio-Rad, Hercules CA, USA).

Generation of knockout cell lines

Knockout cells were generated using Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated protein-9 nuclease (CRISPR/Cas9) system. Guide RNAs (gRNAs) were designed to target a region in the exon 5 of caspase-3 gene (5'-CATACATGGAAGCGAATCAATGG-3') using CRISPRdirect. gRNA-encoding oligonucleotide was cloned into lentiCRISPRv2 plasmid (addgene, Watertown, USA), and knockout cells were established.

Bioimaging and quantification of ROS

HT1080 cells were seeded on glass plates. After stimulation, cells were treated with 10 μM DCFH-DA for 30 min at 37 °C. After washing with PBS, the intracellular ROS generation was observed using a Zeiss LSM800 laser confocal microscope (Carl Zeiss, Oberkochen, Germany), and the images were processed with Zen software (Carl Zeiss,

Oberkochen, Germany). The fluorescence images were obtained from three deferent fields of view.