Supplementary Materials and Methods for
Frequent copy number gain of *MCL1* is a therapeutic target for osteosarcoma

Cell culture

MG-63, NY, and HOS cells were cultured in MEM (Wako) containing 10% FBS (CORNING), 1% non-essential amino acids solution (Wako), and 100 μg/mL kanamycin (MEIJI). HuO9, HuO-3N1, SJSA-1, NOS-1, and NOS-10 cells were cultured in an RPMI-1640 medium (Wako) containing 10% FBS and 100 μg/mL kanamycin. G-292 cells were cultured in a McCoy's 5a medium (Sigma–Aldrich) containing 10% FBS and penicillin-streptomycin solution (Wako). Sa-xeno-147 and Sa-xeno-184 cells were cultured in a collagen-coated dish with an RPMI1640/Ham's F12 (1:1) medium containing 15% FBS, 20 mM HEPES, and antibiotic antimycotic solution (Wako). The cells were cultured at 37°C and 5% CO_2. Cell lines were authenticated, tested Mycoplasma free by vendors, and used for experiments within a short period of culture from thawing. If cell stocks were over about five times of passage, cells were authenticated by short tandem repeat (STR) analysis.

RNA interference for gene knockdown

An Opti-MEM I Reduced Serum Medium (Thermo Fisher Scientific) with a final concentration of 2.4 nM siRNAs and 1.0 μL of lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) was allowed to stand at 24°C for 10 min; then, it was mixed into a cell suspension diluted to 2000 to 4000 cells/100 μL. After 3 min of incubation at room temperature, cells were seeded in a 96-well plate at 2000 cells/100 μL/well for cell viability assay or in a 6-well plate at 4×10^5 cells/2 mL for immunoblotting and qPCR. Cell lysate was collected after 24 or 48 h of incubation. The following siRNA were purchased from Dharmacon (Lafayette): SMARTpool siRNA for human *BCL2* (L-003307-00-0005), human *BCL2L1* (L-003458-00-0005), and human *MCL1* (L-004501-00-0005). Silencer Negative Control No. 1 siRNA (Thermo) was used as a control.

Cell viability assay

Cells were seeded in triplicates at a density of 1000-2000 cells/well in 96-well plates or collagen-coated plates. After indicated time points, the cells were incubated with a CellTiter-Glo reagent (Promega) for 2 min on a Titramax 100 plate shaker (Heidolph). After equivalation for 10 min, luminescence was measured using a Tristar LB941 Multimode Microplate Reader (Berthold Technologies). The relative cell viability was

calculated as a ratio of the DMSO control or siCTRL-treated cells. GraphPad Prism was used to analyze and graphically display the data. The experiments were independently repeated thrice.

Caspase 3/7 assay

Cells were incubated with a Caspase-Glo 3/7 Assay reagent (Promega) for 30 sec on a Titramax 100 plate shaker (Heidolph). After equivalation for 30-60 min, luminescence was measured using a Tristar LB941 Multimode Microplate Reader (Berthold Technologies). The relative cell viability was calculated as a ratio of the siCTRL-treated cells.

Tissue microarray with OS specimens

The tumors in patients with OS were surgically dissected at the Cancer Institute Hospital of JFCR between January, 2004 and December, 2015. Malignancy of the specimens was confirmed via histologic examination. Small biopsies were extracted from FFPE specimens using a tissue microarrayer (KIN-2, Azumaya, Tokyo, Japan). The 2-mm cores were placed in a standard-sized recipient array block. After the construction of the array block, multiple 4-μm sections were cut, and FISH and IHC analyses were performed as described below. Electronic medical records were reviewed retrospectively to obtain clinical information in accordance with a protocol approved by the IRB.

IHC staining

Antigen retrieval was performed by heating the slides for 30 min at 95°C in an antigen retrieval solution with a pH of 9.0 (Nichirei Biosciences, Inc., Tokyo, Japan). Antigen–antibody complexes were visualized using Histofine Simple Stain MAX PO detection reagent (Nichirei Biosciences, Inc.). A 1:200 dilution of an anti-Mcl-1 monoclonal antibody (CST Cat#39224) was used. The staining procedure was performed using the Histostainer 36A automated staining system (Nichirei Biosciences, Inc.).