Materials and Methods for supplementary figures

Cells. Human hepatoma cell line HepG2 cells were obtained from Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). Human embryonic kidney 293T (HEK293T) cells and human neuroblastoma SH-SY5Y cells were obtained from the American Type Culture Collection (Manassas, VA, USA).

Chemicals. Notch4 (Cleaved-Val1432) antibody was obtained from Enogene (NewYork, NY, USA). Antibodies against Hes1 (D6P2U) and presenilin 2 were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA). c-Myc (9E10) antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA). The pCS2 Notch1 Δ EMV-6MT (plasmid 41737) was obtained from Addgene (Cambridge, MA, USA).

Plasmid transfection into HEK293T cells. HEK293T cells were plated at 3×10^5 cells/well in six-well collagen-coated culture plates, growing overnight, and transfected with Notch1 ΔEMV plasmid in the presence of 2.4 μg of the indicated DNA combinations with Lipofectamine2000 (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions.

siRNA transfection into HepG2 or SH-SY5Y cells. HepG2 cells were seeded at 5×10^5 cells/well in six-well culture plates and cultured for 1 day before each experiment. SH-SY5Y cells were seeded at 1×10^6 cells/well in six-well culture plates and cultured for 2 day before each experiment. Transfection of siRNA against human Notch1 into HepG2 and SH-SY5Y cells was done using Lipofectamine RNAiMAX (Invitrogen Corp.) and METAFECTENE® Pro (Biontex, Germany), respectively. After incubation for 24 h, cells were washed with medium and used for experiments.

Annexin-V and propidium iodide staining. Culture medium containing floating cells were aspirated and reserved. After trypsinization, cells were suspended in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 medium, and the culture medium was returned. The numbers of live, necrotic, and apoptotic cells were determined by the Tali® Image-Based Cytometer using a Tali® Apoptosis Kit-Annexin

V Alexa Fluor® 488 and Propidium Iodide (Life Technology Corp., Carlsbad, CA, USA) according to the manufacturer's instructions.

Nucleosome assay. After preparing the cytoplasmic fraction, histone-associated DNA fragments (mono- and oligonucleosomes) were assayed with a Cell Death Detection ELISA^{PLUS} (Roche Applied Science, Penzberg, Germany) according to the manufacturer's instructions.