Figure S1. Generation of cells in which LINC00460 was knocked down by CRISPR/Cas9. (A) Schematic illustration of the process to generate LINC00460 knockout (KO) cells. Dual gRNAs and Cas9 eliminated the region of the human genome encoding LINC00460. (B) Structure of the vector system expressing dual gRNAs and Cas9 from a single plasmid. The two independent sgRNAs for LINC00460 KO are driven by the human H1 and U6 promoter sequences. (C) Screening of positive LINC00460 KO clones by RT-qPCR. Results are presented as the means of 3 independent experiments. GAPDH mRNA was used as an internal control. (D) Validation of the KO clone by genomic PCR. The PCR products were detected by agarose gel electrophoresis. The positions of the primers are illustrated in (A). (E) DNA sequence of the KO clone involving deletion. (F) Relative expression of the LINC00460 transcript in LINC00460 KO cells and LINC00460-overexpressing lung cancer cells (H1299). The LINC00460 overexpression vector or control vector was introduced into the KO cells. WT, wild type; KO, knockout; EV, empty vector (control); OE, overexpression. Results are presented as the means ± SD of 3 independent experiments. GAPDH mRNA was used as an internal control.