Figure S1. Immunohistochemistry intensity scoring of nucleolar and spindle-associated protein 1. The staining intensity was determined in 10-20 areas at x400 magnification by visual assessment, regardless of the percentage of staining, and the score for staining intensity was classified as follows: 0, Negative (no positive cells); 1, weak; 2, moderate; and 3, strong.

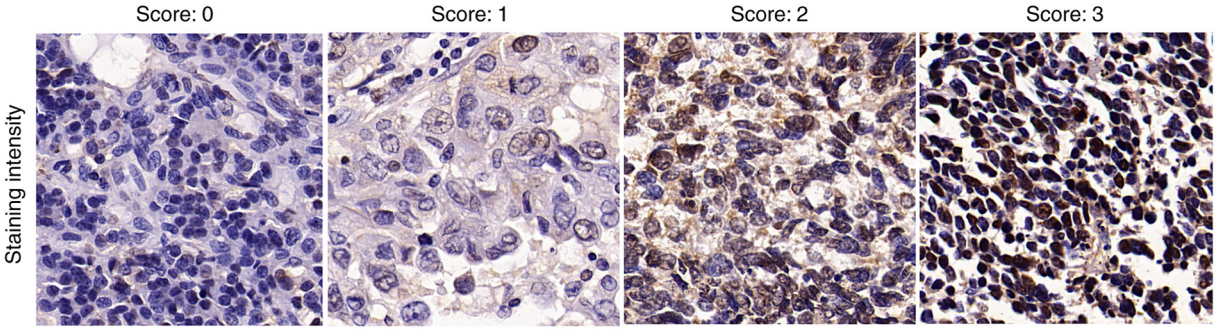

Figure S2. Transfection of non-small cell lung cancer cell lines with U6-shRNA-CMV-puromycin+RFP vectors or pcDNA3.1 CMV-gene-neomycin+EGFP vectors. (A) H1299 and A549 cells were transfected with U6-shRNA-CMV-puromycin+RFP vectors. After 72 h of transfection, H1299 and A549 cells were selected with 2 or 1 μg/ml puromycin, respectively, for 14 days. After screening using a fluorescence microscope, cell transfection efficiency was >95% (magnification, x100). (B) H1299, A549 and HCC827 cells were transfected with pcDNA3.1 CMV-gene-neomycin+EGFP vectors. After 72 h of transfection, H1299, A549 and HCC827 cells were selected with 200, 500 or 400 μg/ml G418, respectively for 14 days. After screening using a fluorescence microscope, cell transfection efficiency was >95% (magnification, x100). shRNA, short hairpin RNA.

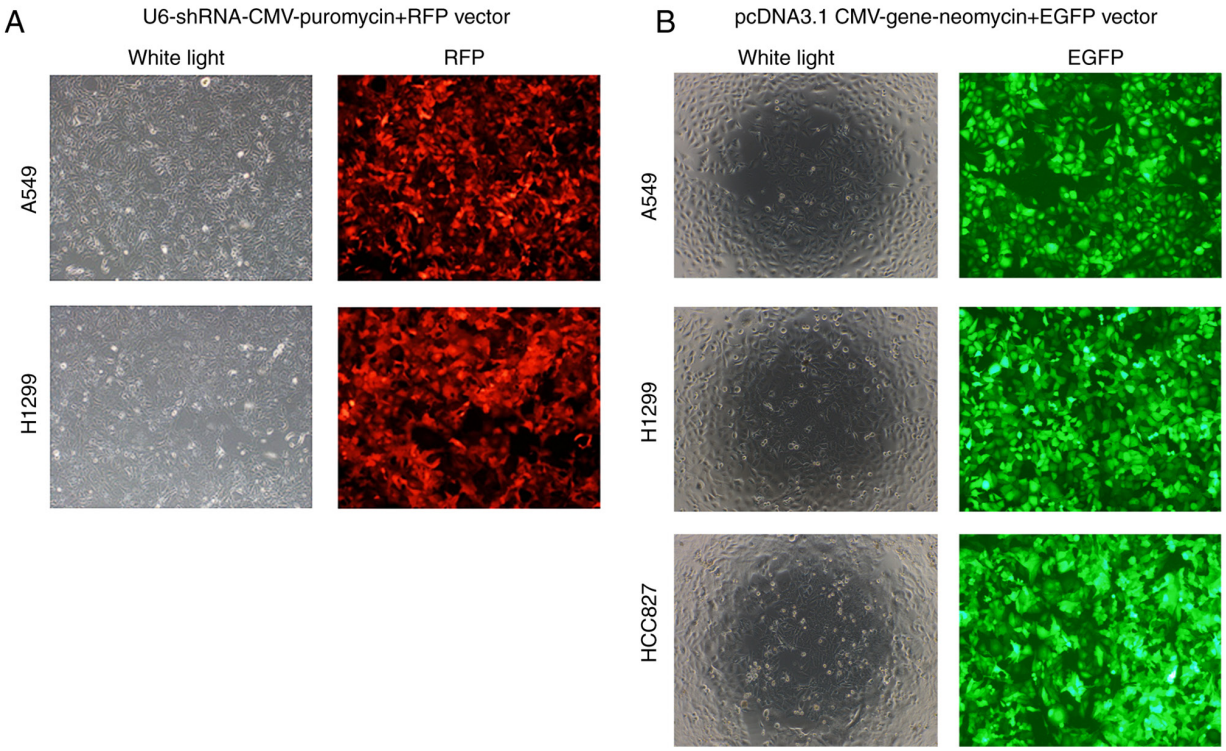

Figure S3. MEF2D-knockdown inhibits NUSAP1 mRNA expression in A549. (A) A549 cells were transfected with shRNA plasmids against MEF2D (MEF2D RNAi), ZEB1 (ZEB1 RNAi) or E2F1 (E2F1 RNAi), or a universal NC plasmid. The protein expression levels of MEF2D, ZEB1 and E2F1 were determined by western blotting. (B) A549 cells were transfected with MEF2D RNAi, ZEB1 RNAi, E2F1 RNAi or NC. The expression levels of NUSAP1 were determined by reverse transcription-quantitative PCR. The experiments were independently repeated thrice. $^{**}P<0.01$. MEF2D, myocyte enhancer factor 2D; NC, negative control; NUSAP1, nucleolar and spindle-associated protein 1; shRNA, short hairpin RNA; RNAi, RNA interference; ZEB1, zinc finger E-box binding homeobox 1; E2F1, E2F transcription factor 1.