Figure S1. Physical maps of pLenti-CMV-MDM2-EGFP-puro and GFP fluorescence images observed under a microscope for 293T, U251, A549 and MCF7 cells. (A) Physical maps of pLenti-CMV-MDM2-EGFP-puro, which was constructed based on pLenti-CMV-Puro. MDM2-EGFP fusion protein full length cDNA was joined under the CMV promoter. (B) Core sequence of pLenti-CMV-MDM2-EGFP-puro. The clone sites region was marked. (C) pLenti-CMV-MDM2-EGFP-puro, pLenti-DVPR and pLenti-VSVG plasmids were co-transfected into 293T cells (magnification, x100) for 36 h, the lentivirus was harvested and a fluorescence microscope was used to observe GFP expression in 293T cells (magnification, x100). Vector referred to the 293T cell line, which overexpressed plenti-CMV-puro empty vector. (D) Virus was added into U251 cells after filtering, and cells were cultured in medium containing 2 µg/ml puromycin to obtain stable cells. GFP fluorescence was observed under a fluorescence microscope in stable U251 cells (magnification, x100). Vector referred to the U251 cell line, which stably overexpressed plenti-CMV-puro empty vector. (E) Virus was added into A549 cells after filtering, and cells were cultured in medium containing 2 µg/ml puromycin to obtain stable cells. EGFP fluorescence was observed under a fluorescence microscope in stable A549 cells (magnification, x100) Ctrl referred to the A549 cell line, which stably overexpressed plenti-CMV-puro empty vector. (F) Virus was added into MCF7 cells after filtering, and cells were cultured in medium containing 2 µg/ml puromycin to obtain stable cells. EGFP fluorescence was observed under a fluorescence microscope in stable MCF7 cells (magnification, x100). Ctrl referred to the MCF7 cell line, which stably overexpressed plenti-CMV-puro empty vector. Ctrl, control; GFP, green fluorescent protein; MDM2, MDM2 proto-oncogene, E3 ubiquitin protein ligase.
Figure S2. Overexpression of MDM2, p53 and 14-3-3 was confirmed by qPCR or western blotting. (A) MDM2 gene expression in the A549 MDM2-GFP stable cell line was analyzed by qPCR. Ctrl referred to the A549 cell line, which stably overexpressed plenti-CMV-puro empty vector. (B) Protein expression levels of MDM2 in A549 MDM2-GFP stable cells were analyzed by western blotting of GFP tag antibody. Ctrl referred to the A549 cell line, which stably overexpressed plenti-CMV-puro empty vector. (C) MDM2 gene expression in MCF7 MDM2-GFP stable cells was analyzed by qPCR. Ctrl referred to the MCF7 cell line, which stably overexpressed plenti-CMV-puro empty vector. (D) Protein expression levels of MDM2 in MCF7 MDM2-GFP stable cells were analyzed by western blotting of GFP tag antibody. Ctrl referred to the MCF7 cell line, which stably overexpressed plenti-CMV-puro empty vector. (E) p53 gene expression in U251 control stable cells and MDM2-GFP stable U251 cells, which were transfected with overexpression pcDNA3.1-p53 or pcDNA3.1 empty vector, was analyzed by qPCR. Ctrl referred to the U251 cell line, which stably overexpressed plenti-CMV-puro empty vector. (F) 14-3-3 gene expression in U251 control stable cells and MDM2-GFP stable U251 cells, which were transfected with overexpression pcDNA6B-Flag-14-3-3 or pcDNA6B empty vector, was analyzed by qPCR. Ctrl referred to the U251 cell line, which stably overexpressed plenti-CMV-puro empty vector. For all quantifications, data are presented as the mean ± SD derived from three independent experiments. **P<0.01; ***P<0.001. 14-3-3, tyrosine 3-monoxygenase activation protein ε; Ctrl, control; GFP, green fluorescent protein; IB, immunoblotting; MDM2, MDM2 proto-oncogene, E3 ubiquitin protein ligase; qPCR, quantitative PCR.