Figure S1. Visualisation of RNAi screening -kMAD scatter plots of U87MG and LN18 cells. (A and B) A total of 2 sets of 96-well plates were used for screening 113 kinase genes, and different siRNA pools were coated inside each well. Each plate had its respective cut-off point. (A) Plate 2, with a cut-off point of 2.525, had 13 potential hits (black). PLK1 (dark grey) had the lowest value, suggesting a robust positive control for the assay. (B) Plate 1 had a cut-off point of 2.741, with 10 hits (black) with PLK1 (dark grey) also in the list, suggesting the robustness of this assay. (C and D) Visualisation of RNAi screening -kMAD scatter plots of LN18 cells. A total of 2 sets of 96-well plates were used for screening 113 kinase genes, and different siRNA pools were coated inside each well. Each plate had its respective cut-off point. Plate 1, with a cut-off point of 2.826, had 3 potential hits: *CDKN1A*, *CDC2*, and *AURKA* (black). PLK1 (dark grey) had the lowest value, suggesting a robust positive control for the assay. (D) Plate 2, with a cut-off point of 2.805, had 17 hits (black); PLK1 (dark grey) was also in the list, suggesting a robust assay. kMAD, k-median absolute deviation; siRNA, small interfering RNA.

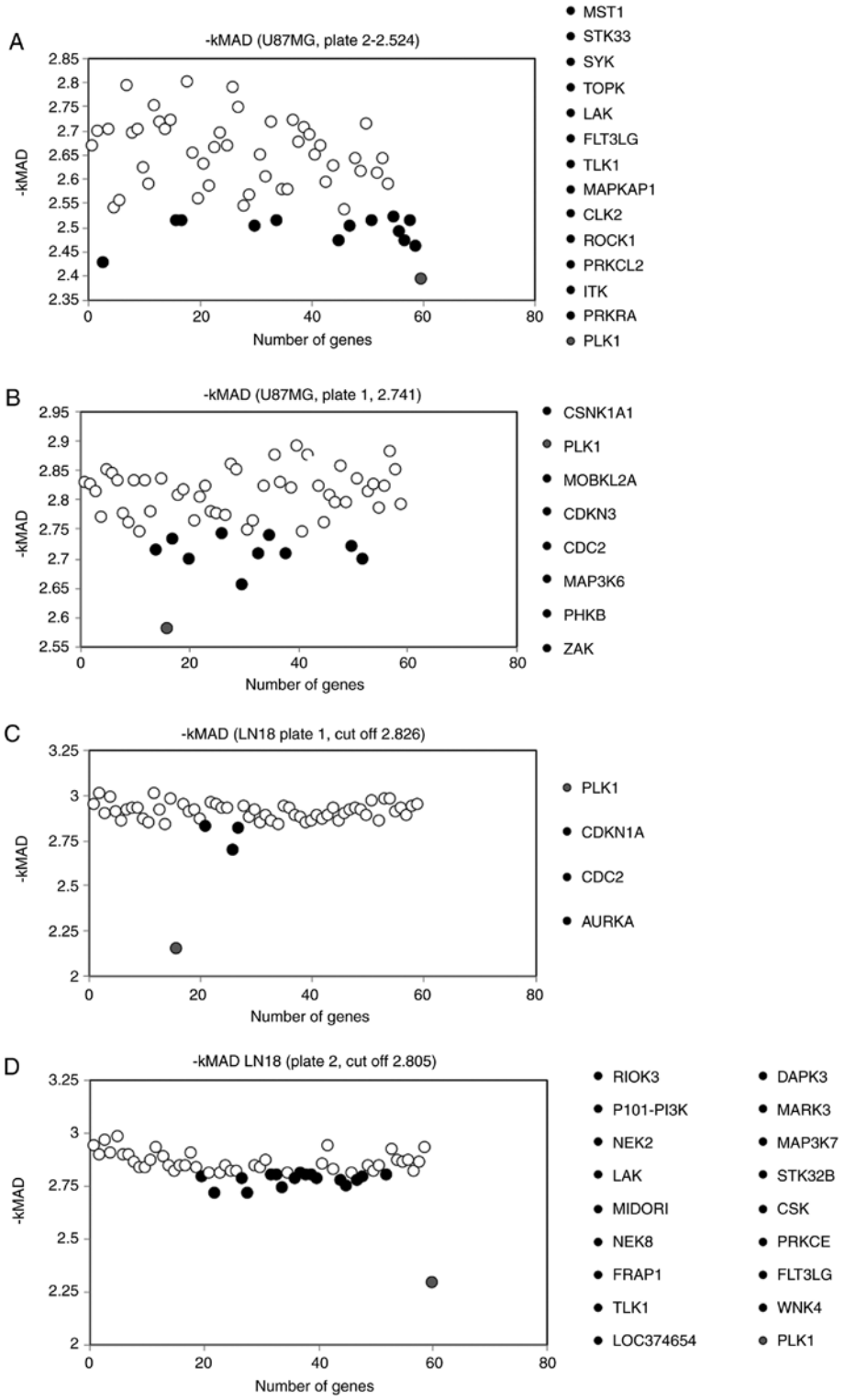

Figure S2. Decrease in the expression of mRNA of TLK1, FLT3, CDC2 and LAK demonstrates efficient knockdown in both U87MG and LN18 cells. mRNA was harvested 48 h post-transfection. si, small interfering RNA; TLK1, tousled-like kinase 1; FLT3, Fms related tyrosine kinase 3; CDC2; LAK.

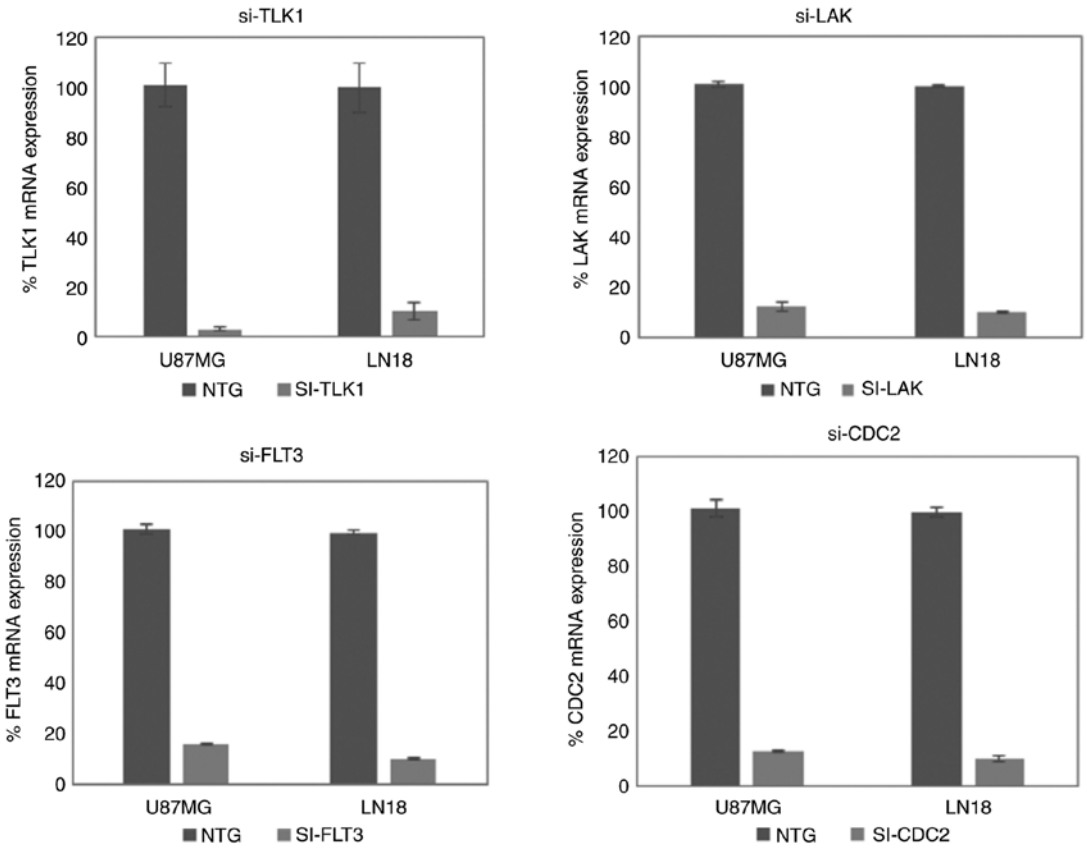

Figure S3. Analysis was performed 76 h post-transfection. A decrease in cell viability only started to significantly occur at 96 h post si-TLK1 transfection. Analysis was performed using Kruskal-Wallis with Dunn's post hoc test. $^{**}P<0.05$. *TLK1*, tousled-like kinase 1; si, small interfering RNA; NTG, non-targeting siRNA.

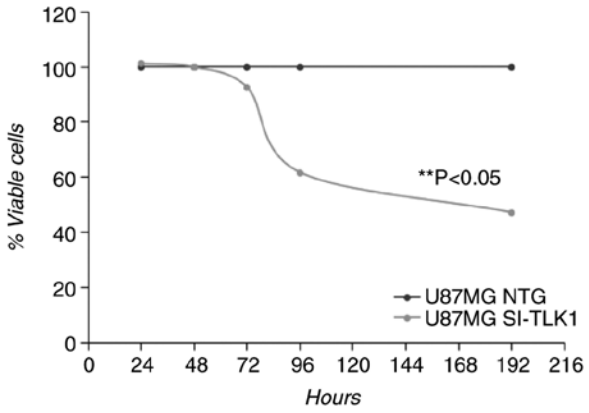

Figure S4. Confirmation of caspase-3 and caspase-7 apoptotic pathway involvement due to TLK1 knockdown following qualitative analysis of LN18 and U87MG cells. Confocal microscopy images are presented. Fluorescence dots, DEVD complex formation due to caspase-3/caspase-7 activation. Analysis was performed at 76 h post-transfection.

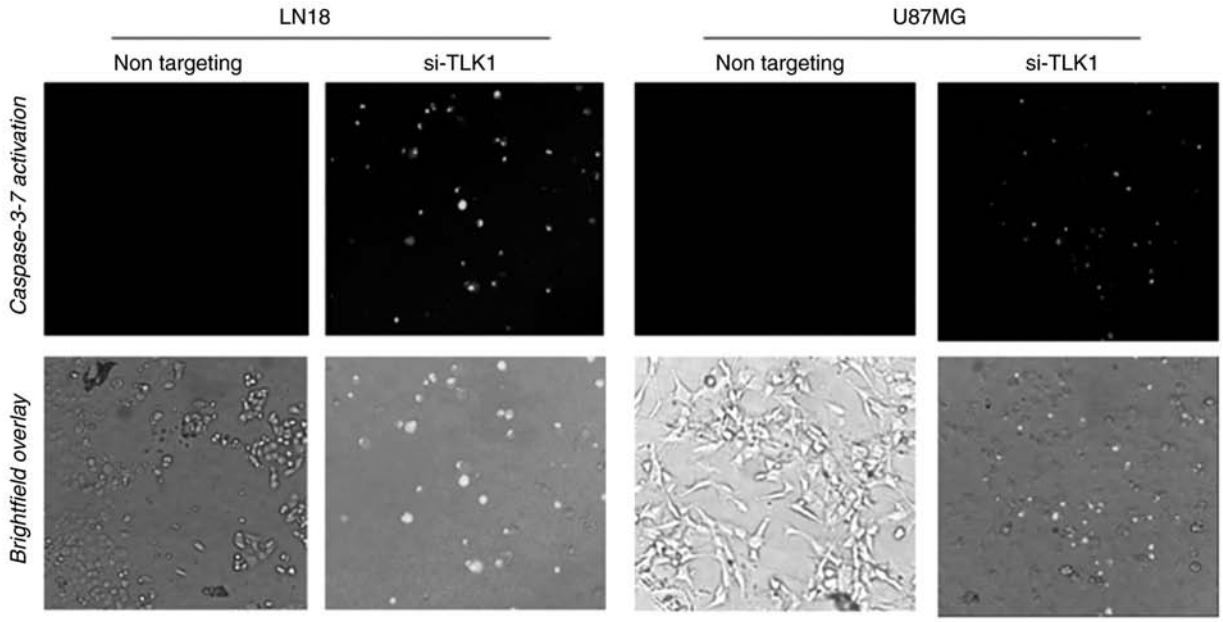

Figure S5. Brdu and cell cycle analysis. (A) Decreased signal of brdU compared with non-targeting in U87MG cells. **P<0.05. (B) Increased cellular percentage at S-phase of cell cycle in U87MG overexpressed-TLK1 cells. **P<0.05. All experiments were performed in triplicate and the results were compared with the si-NTG control. Analysis was performed using Kruskal-Wallis test with Dunn's post hoc test. *TLK1*, tousled-like kinase 1; si, small interfering RNA; NTG, non-targeting siRNA.

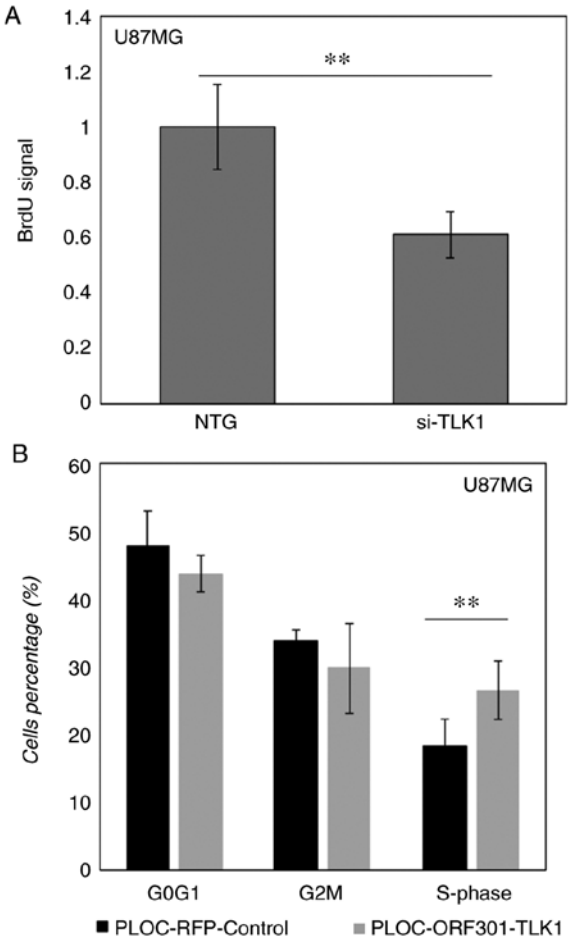

Figure S6. Knockdown efficiency of sh-455 and sh-461 in U87MG. (A) Relative TLK1 mRNA expression following sh-TLK1 transfection. (B) Transfection efficiency observation using fluorescence microscopy. Successful transfection was based on fluorescence signals within the cells tagged with RFP. (C) Monitoring successful transfection by flow cytometry. TLK1, tousled-like kinase 1; sh, short hairpin RNA; NTG, non-targeting small interfering RNA.

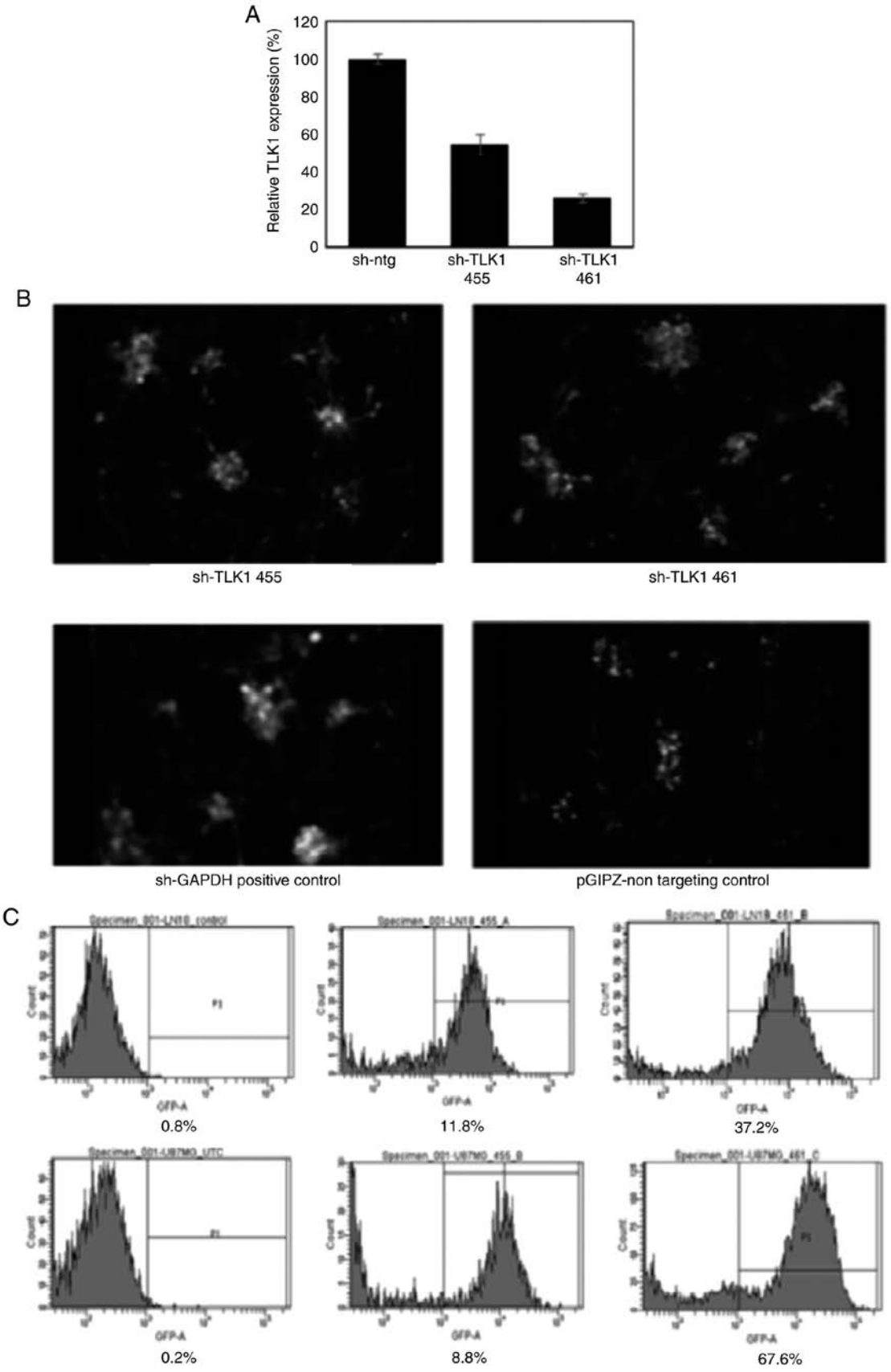

Figure S7. Understanding the potential role of TLK1 downstream kinome signalling using an ELISA-based assay in U87MG and LN18 cells. Both cell lines were transduced with GIPZ shRNA and lentiorf clones. Cell lysates were later extracted after 72 h and respective kinase levels were measured. (A) The ratio of phosphorylated TP53 over total TP53 increased significantly in TLK1-knockdown U87MG cells. Alternately, a decreased phosphorylated TP53 over total TP53 ratio was observed in over-expressed TLK1 cells. **P<0.05. (B) A significant increase of AKT 1/2/3 activation signals in sh-TLK1-455 U87MG only was observed. **P<0.05. (C) A significant decrease in p70s6k signals was observed in both TLK1-knockdown U87MG cells. **P<0.05. (D) A significant increase in relative ERK 1/2/3 protein signals of both sh-TLK1 sequences in U87MG cells. **P<0.05. (E) The ratio of phosphorylated TP53 over total TP53 protein signals was increased in the TLK1 knockdown sh-TLK1-455 LN18 cells. **P<0.05. (F) Significant activation of AKT 1/2/3 in sh-TLK1-455 LN18 only. (G) No significant p70s6k activation was observed in the LN18 cells. (H) A significant increase in ERK activation was observed in both sh-TLK1 and TLK1-overexpressing LN18 cells. **P<0.05. All analysis was performed using Kruskal Wallis with Dunn's multiple correction test. TLK1, tousled-like kinase 1; GIPZ, ; TP53, tumor protein 53; sh, short hairpin.

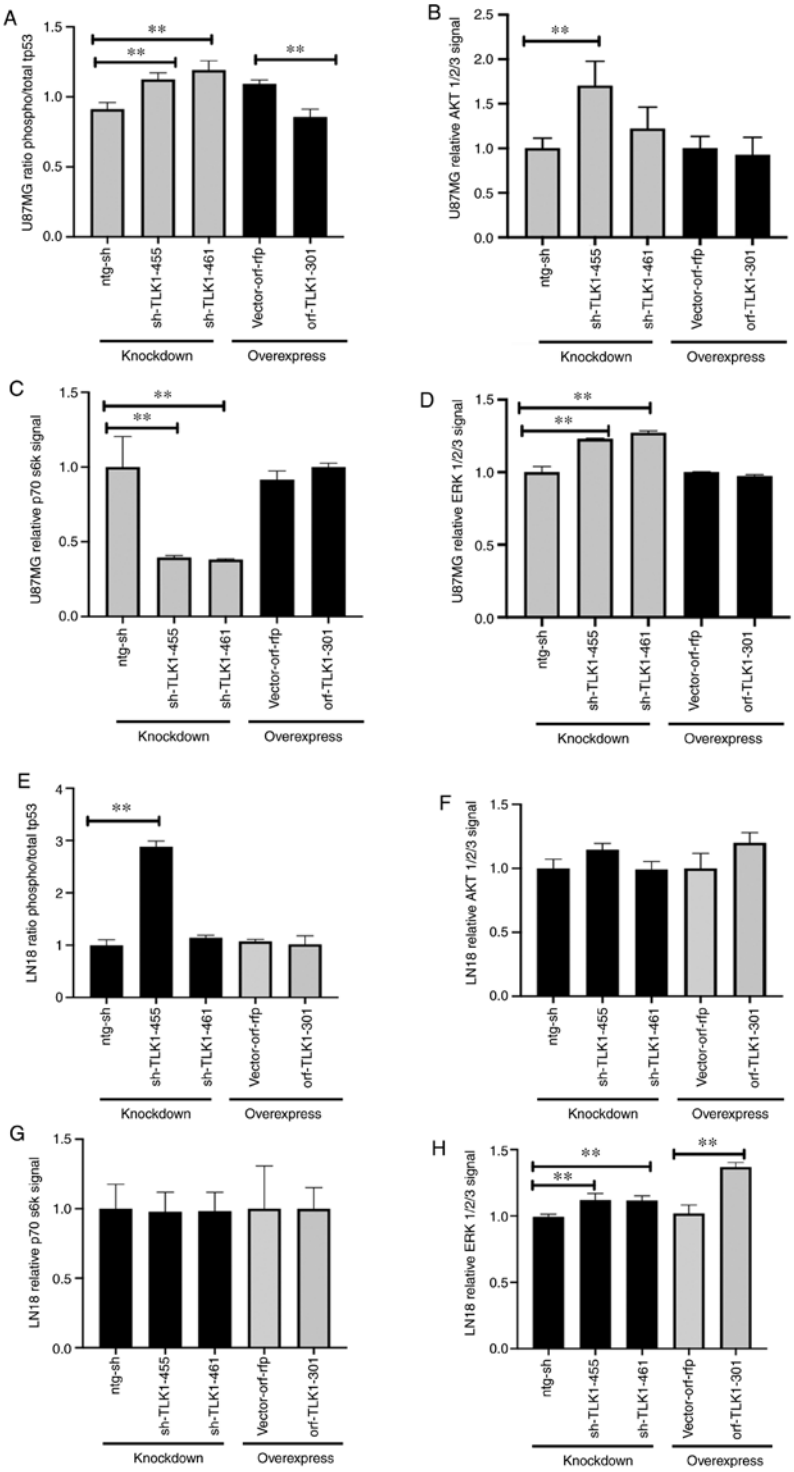

Figure S8. Functional analysis of NHA transiently transfected with si-*TLK1*. (A) Transfection of si-TLK1 in NHA with >90% efficiency. (B) Morphological observations indicated that si-TLK1 did not affect cellular changes and viability. Magnification, x10. (C) No significant difference in cell viability at 48 h post-transfection was observed. (D) No significant difference in apoptosis following Annexin V staining was observed, suggesting that NHA were not affected by TLK1 knockdown. NHA; si, small interfering RNA; TLK1, tousled-like kinase 1.