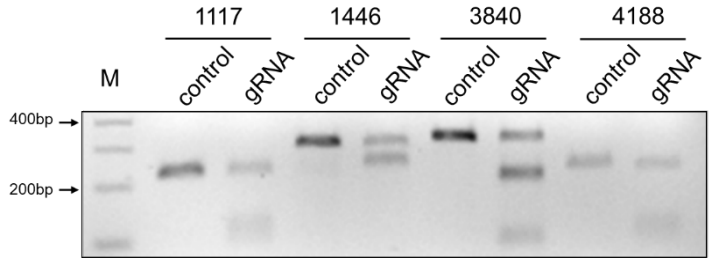
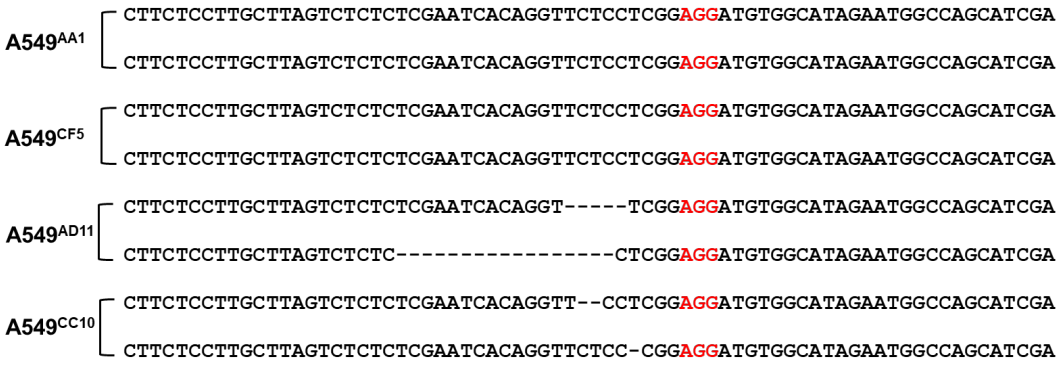
Supplementary figures

Supplementary Fig. 1



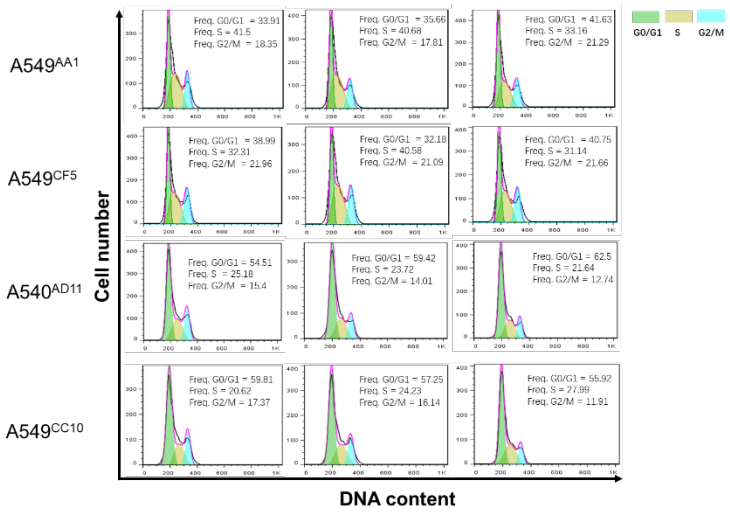
Supplementary Fig. 1 The T7 endonuclease I assay was performed to assess indels in the HUWE1 locus. The gRNA-3840 was considered to be the most-active gRNA and used in the following experiment. The empty vector was used as a control.

Supplementary Fig. 2



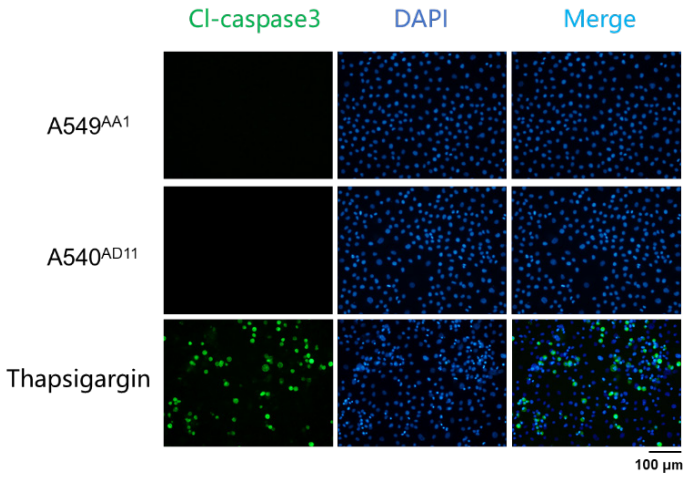
Supplementary Fig. 2 The schematic showing *HUWE1* mutations in A549^{AD11} and A549^{CC10} clones. The HUWE1 gRNA-3840 plasmid was transfected into A549 cells and single-cell culture was established using the limiting dilution method. Sanger sequencing was performed to detect *HUWE1* mutations in A549^{AA1}, A549^{CF5}, A549^{AD11}, and A549^{CC10} clones. The PAM sequence was marked in red.

Supplementary Fig. 3



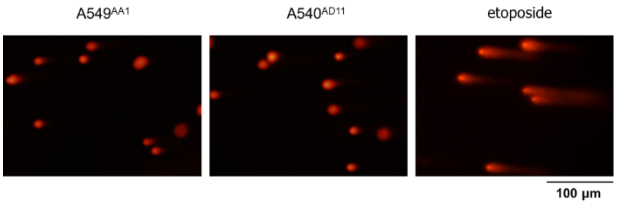
Supplementary Fig. 3 Flow cytometry analysis for the cell cycle of the indicated cells. The cells were collected when they were in a confluency between 50-60%, and cell cycle analysis was performed as described in materials and methods.

Supplementary Fig. 4



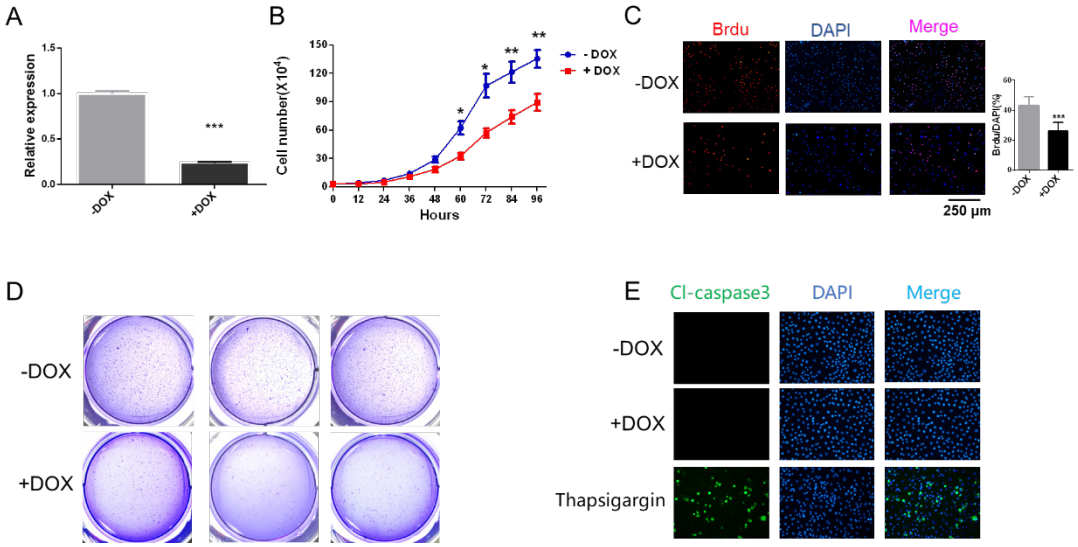
Supplementary Fig. 4 Deletion of HUWE1 didn't induce apoptosis in A549 cell. Cleaved-caspase 3 (green) and DAPI (blue) stained in indicated cells. Thapsigargin was used as the positive control to induce A549 cells apoptosis.

Supplementary Fig. 5



Supplementary Fig. 5 The comet assay for DNA damage in the indicated cells. Etoposide (1 μg/ml) was used as the positive control to induce DNA damage in A549 cells.

Supplementary Fig. 6



Supplementary Fig. 6 HUWE1 knockdown impaired cell proliferation and colony formation capacity

(A) Realtime quantitative RT-PCR was used to determine HUWE1 expression level in A549/teton-shHUWE1 cells with or without doxycycline (DOX). The results are presented as the mean ± SD (***, $P < 0.001$, unpaired t-test).

(B) Growth curves for A549/teton-shHUWE1 cells with or without DOX. A549/teton-

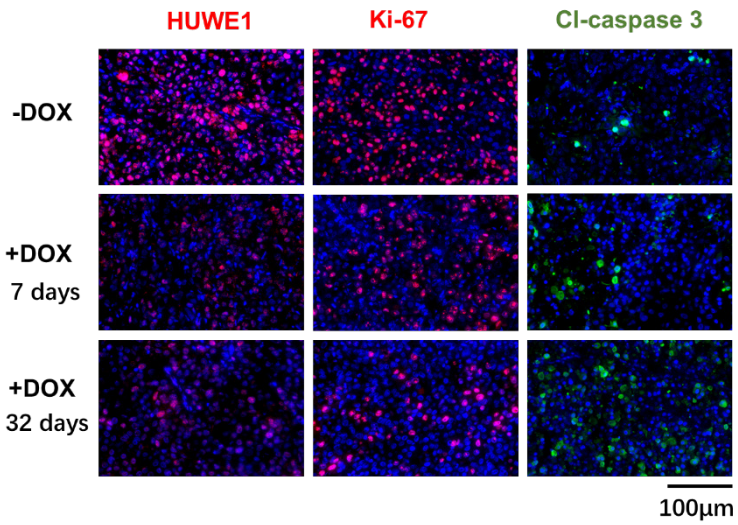
shHUWE1 cells were treated with Dox for 2 days, then planted in 12-well plates. The number of cells was counted every 12 hours. The data were presented as the means ± SD (**, $P < 0.01$, unpaired t-test).

(C) BrdU incorporation assay was used to evaluate the DNA synthesis and proliferation rates of A549/teton-shHUWE1 cells with or without DOX. The data are presented as the means ± SD (***, $P < 0.01$, unpaired t-test).

(D) Soft agar colony formation assays for A549/teton-shHUWE1 cells with or without DOX.

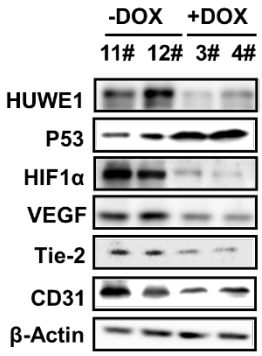
(E) A549/teton-shHUWE1 cells were treated with Dox for 5 days, then cleaved-caspase 3 antibody was used to detect cell apoptosis. Thapsigargin was used as the positive control to induce A549 cells apoptosis.

Supplementary Fig. 7



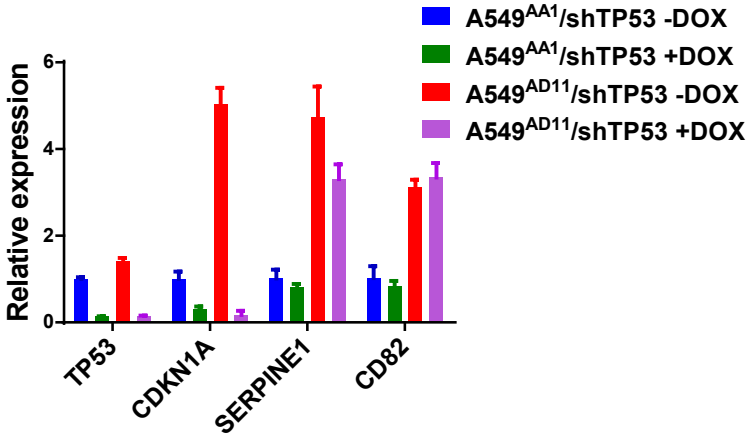
Supplementary Fig. 7 HUWE1 knockdown impaired the tumorigenicity of lung cancer cells. HUWE1, Ki67, and cleaved (Cl)-caspase 3 staining in cancer tissues described in Fig.3E at day 7 and day 32 after doxycycline (DOX) treatment.

Supplementary Fig. 8



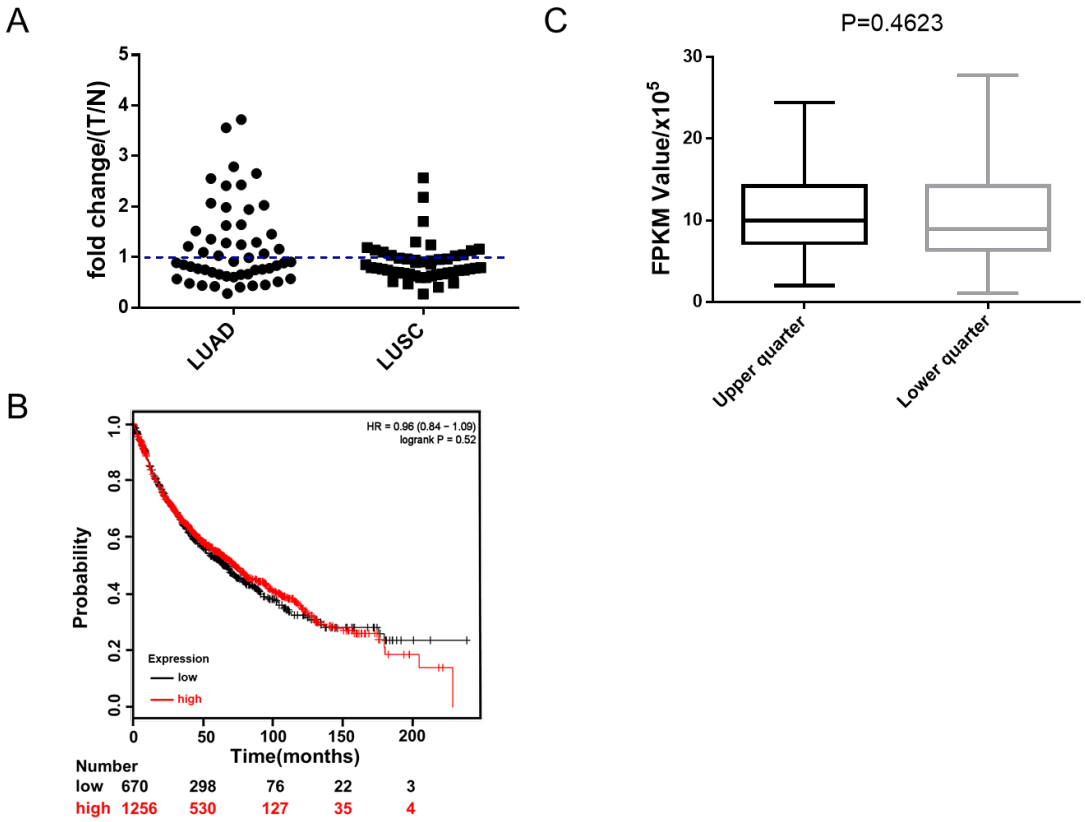
Supplementary Fig. 8 HUWE1 knockdown inhibited angiogenesis in xenograft tumors. Proteins were extracted from the tumors formed by A549/teton-shHUWE1 cells in BALB/c nude mice described in Fig. 3E. Markers of angiogenesis in the lysates were detected by western blotting; 3#, 4#, 11# and 12# indicated the serial number of mice. β-actin was used as a loading control.

Supplementary Fig. 9



Supplementary Fig. 9 Realtime quantitative RT-PCR showed the expression of the indicated genes. HUWE1 wild type cells and HUWE1-null cells were infected with teton-shTP53 lentivirus. *TP53* knockdown was induced by doxycycline (DOX), and quantitative RT-PCR was performed as described in materials and methods. The data are presented as the means ± SD.

Supplementary Fig. 10



Supplementary Fig. 10 The association between MDM2 expression and prognosis

(A) The fold change of MDM2 mRNA expression in lung cancer(T) and adjacent normal tissue(N). The RNAseq data was downloaded from the TCGA website. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma. 49% of LUAD and 27% of LUSC have elevated MDM2 expression compared with paired normal tissue (fold change>1).

(B) Kaplan-Meier plot showing overall survival of non-small-cell lung cancer patients stratified by high or low *MDM2* mRNA expression (lower tertile).

(C) The box plot displaying the mRNA expression of *CDKN1A* in the *MDM2* low (upper quarter) and *MDM2* high (lower quarter) expression group (P =0.4623, unpaired t-test).