Figure S1. (A) The GEO DataSets (http://www.ncbi.nlm.nih.gov/geo/gds), hosted by the National Center for Biotechnology Information, was employed as the database for dataset retrieval. The search query used was '[(lung adenocarcinoma) AND (EGFR-TKI resistance) OR (gefitinib resistance) OR (osimertinib resistance)]', with additional filters set for the organism as 'Homo sapiens' and entry type as either 'DataSets' or 'Series'. The resulting datasets were then screened to include only samples derived directly from patients, excluding those from cell lines. Ultimately, dataset GSE231938 was selected, comprising samples from one EGFR-TKI-sensitive patient and two EGFR-TKI-resistant patients. The levels of TLE1 mRNA in these samples were subsequently analyzed using Geo2R, with expression levels reported in Transcripts Per Million (TPM). (B) Reanalysis of Kaplan-Meier curve of overall survival (OS) up to six years after the initial diagnosis for high and low expression levels of TLE1 in patients with lung squamous cell carcinoma. TLE1, transducin-like enhancer of split 1; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.

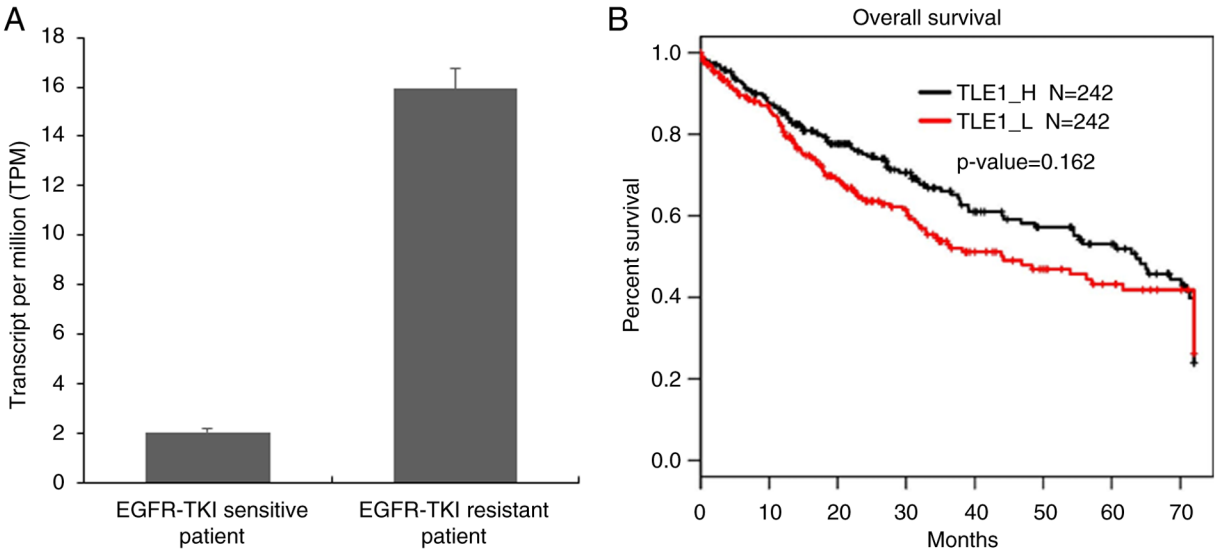

Figure S2. TLE1 expression regulates sensitivity of A549 cells to osimertinib. (A and B) Control GFP and GFP-TLE1 A549 cells were treated with the indicated concentration of osimertinib. A total of 48 h post-treatments, cells were subjected to (A) Alamar Blue cell viability and (B) apoptosis assays. (C and D) A549 were transfected with a pool of TLE1 specific or control siRNAs, and 6 h post-transfection, cells were treated with osimertinib for 48 h followed by (C) cell viability and (D) apoptosis assays. The results are representative of three independent experiments. $^{*}P<0.05$ and $^{**}P<0.01$ (Student's t-test). Error bars indicate SD. TLE1, transducin-like enhancer of split 1; siRNA, small interfering RNA.

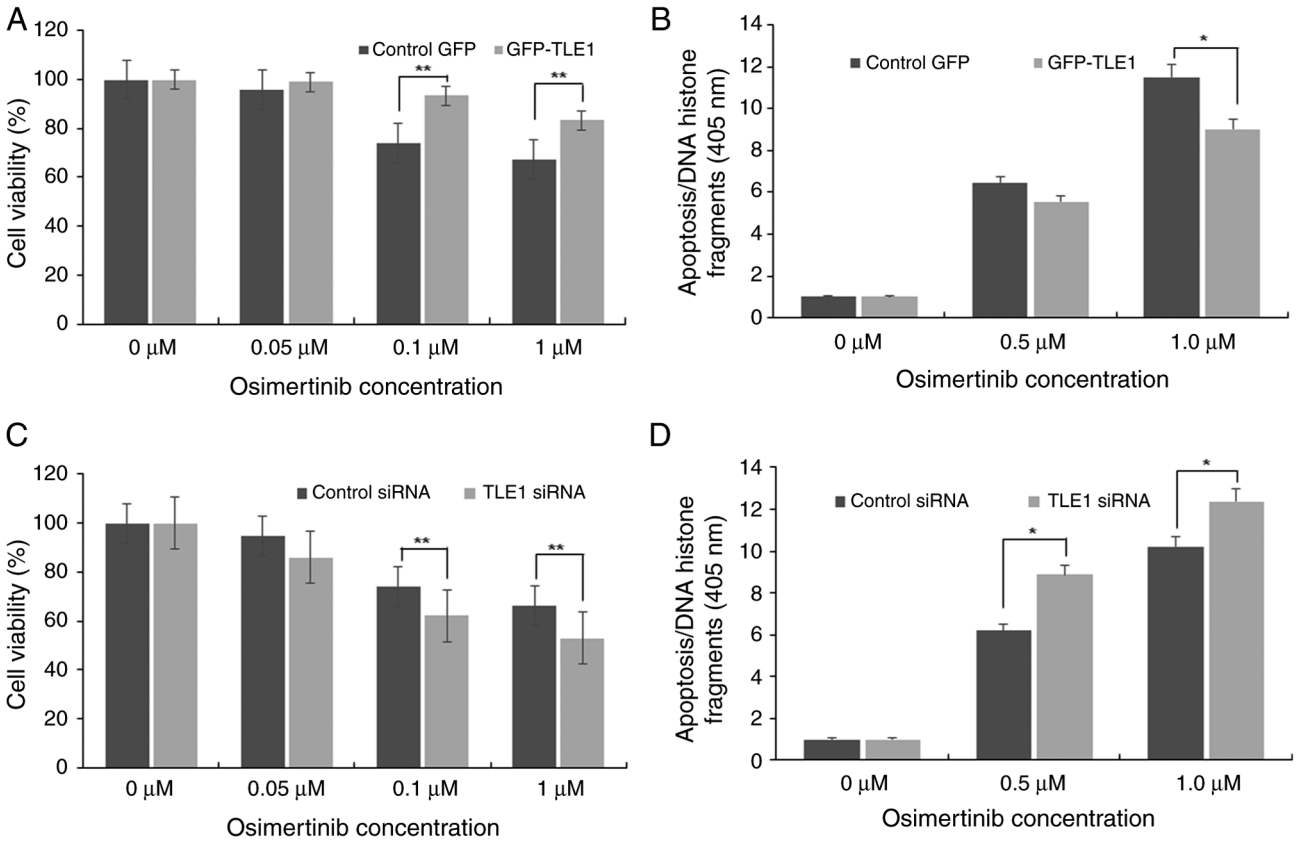

Figure S3. Exogenous TLE1 expression confers gefitinib resistance in the human lung adenocarcinoma epidermal growth factor receptor-mutant HCC827 cell line. (A-C) HCC827 expressing control GFP or GFP-TLE1 construct were subjected to (A) western blotting against specific antibodies to GFP, E-cadherin and β-actin and treated with the indicated concentration of gefitinib for 48 h followed by (B) Alamar blue assay and (C) apoptosis assay. The results are representative of three independent experiments. *P<0.05 and **P<0.01 (Student's t-test). Error bars indicate SD. TLE1, transducin-like enhancer of split 1.

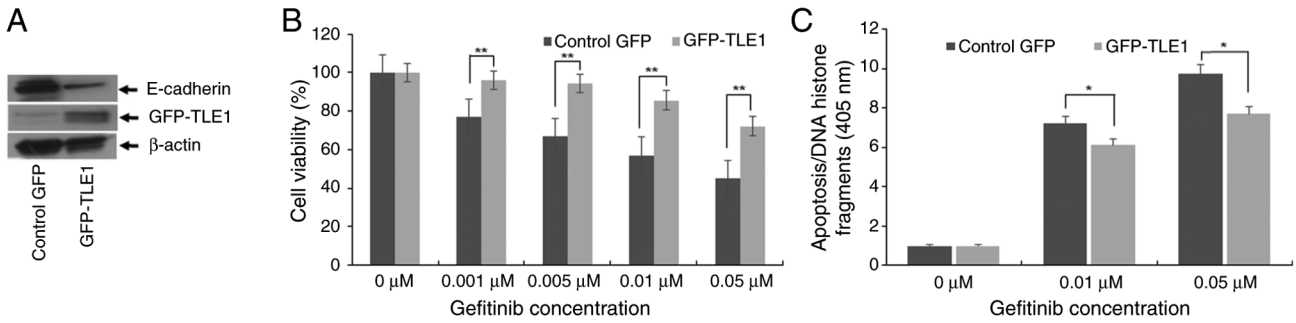

Figure S4. Exogenous expression of E-cadherin attenuates the TLE1-mediated resistance to gefitinib in HCC827 cell line. (A and B) GFP-TLE1 HCC827 cells were transfected with the vector or E-cadherin expressing construct and subjected to (A) western blotting with the indicated antibodies or further cultured in the indicated concentration of gefitinib for 48 h followed by (B) Alamar Blue viability assay. The results are representative of three independent experiments. $^{*}P<0.05$ and $^{**}P<0.01$ (One-way ANOVA with post hoc Tukey's test). Error bars indicate SD. TLE1, transducin-like enhancer of split 1.

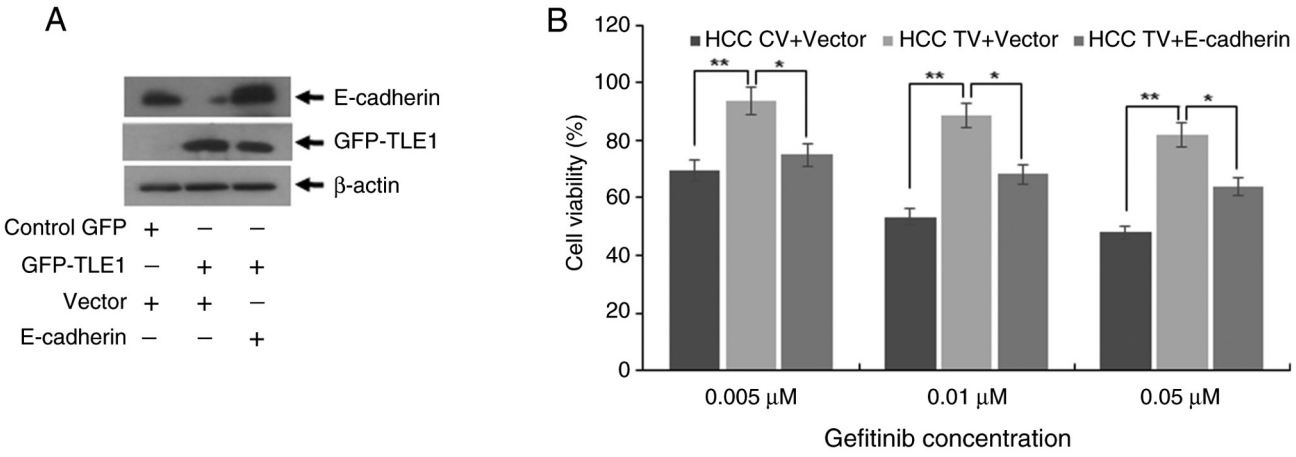

Figure S5. A549 cells are transfected with a pool of TLE1-specific, AES-specific siRNAs or a non-targeting control siRNA with the Lipofectamine RNAiMAX Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and 24 h later cells are subjected to western blotting with specific antibodies against TLE1, AES, or β-actin. TLE1, transducin-like enhancer of split 1; AES, Amino Enhancer Split; siRNA, small interfering RNA.