TIP30 is a pulmonary tumor suppressor that negatively regulates EGFR cytoplasmic and nuclear signaling

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Running Title: TIP30, lung adenocarcinoma, EGFR signaling, tumor suppressor

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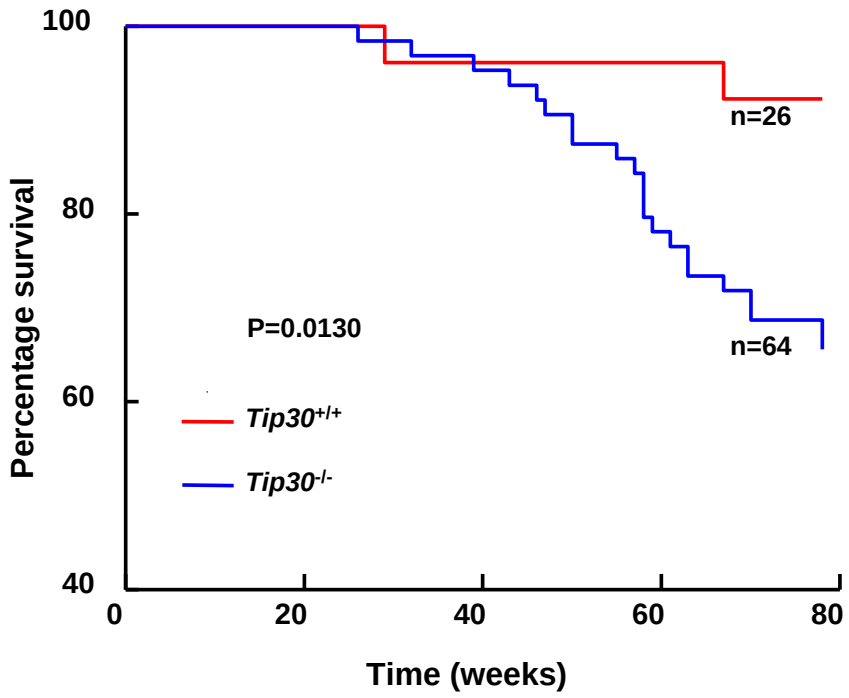
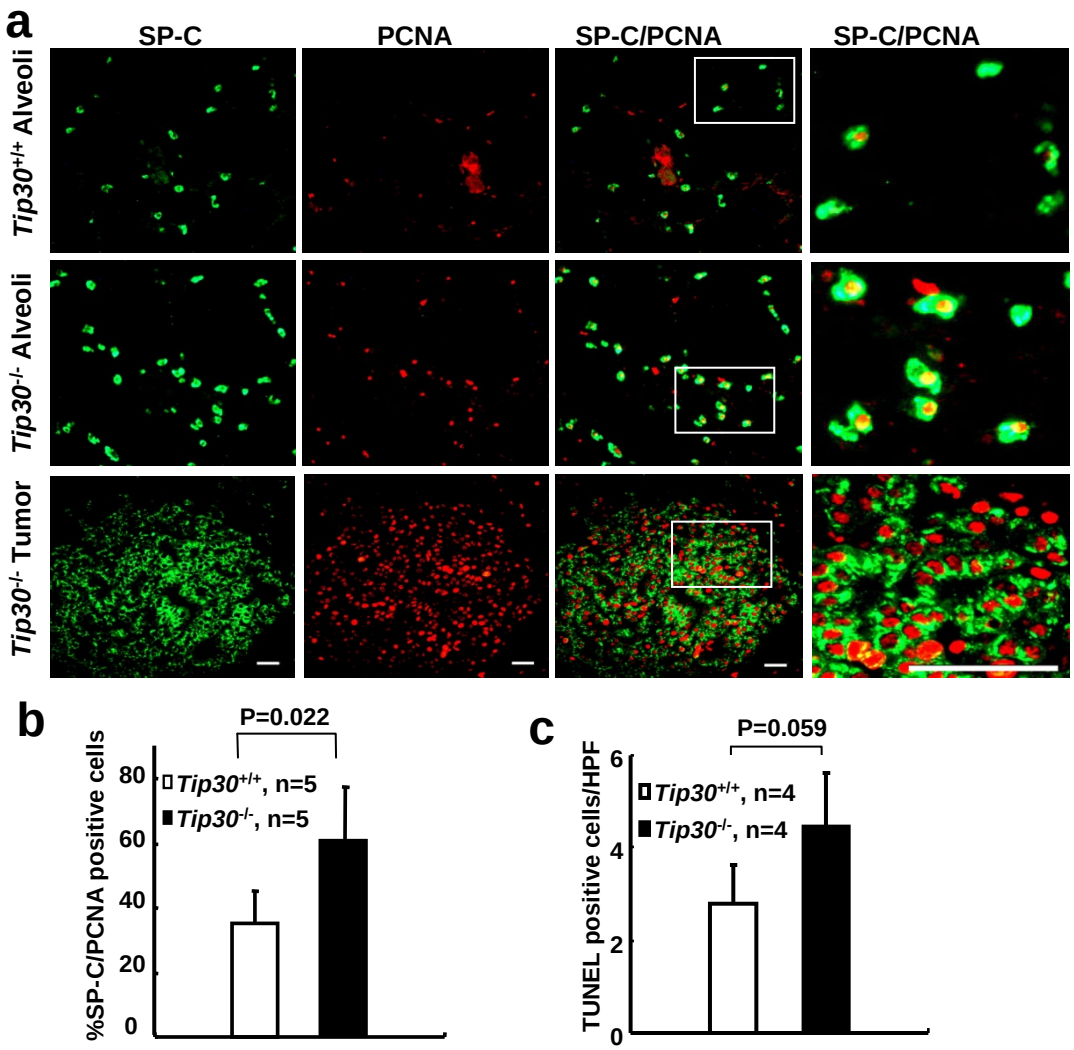
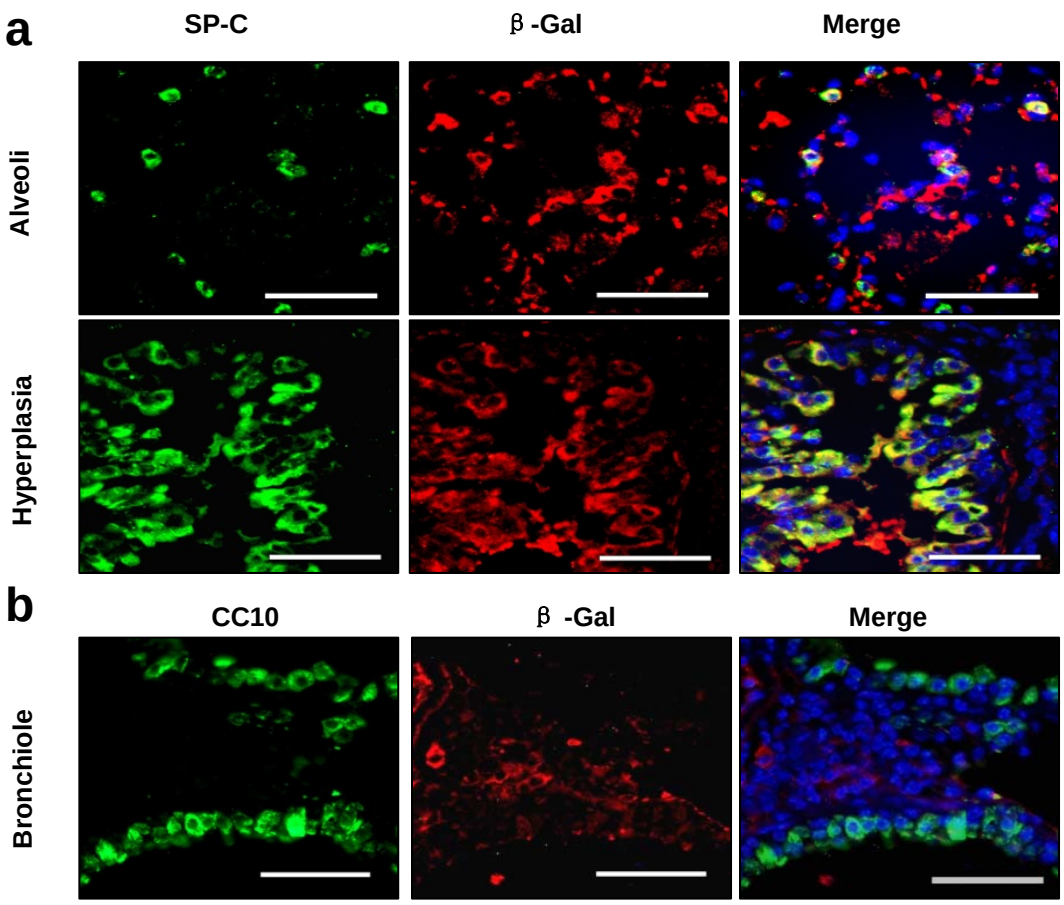


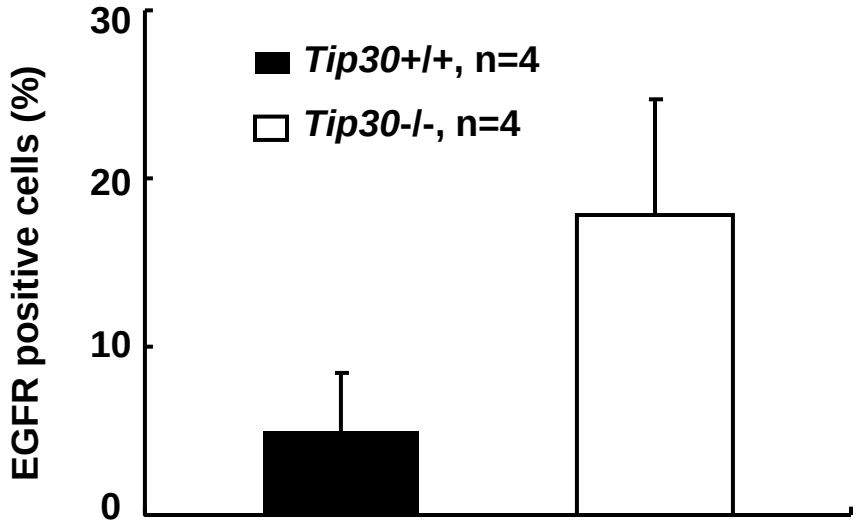
Supplemental Figure 1 Tip30 deletion resulted in shorter lifespan. Kaplan-Meier survival curves of *Tip30*$^{+/+}$ and *Tip30*$^{-/-}$ Balb/c mice. Spontaneous deaths were recorded as events. Data were compared using a Log-Rank test.



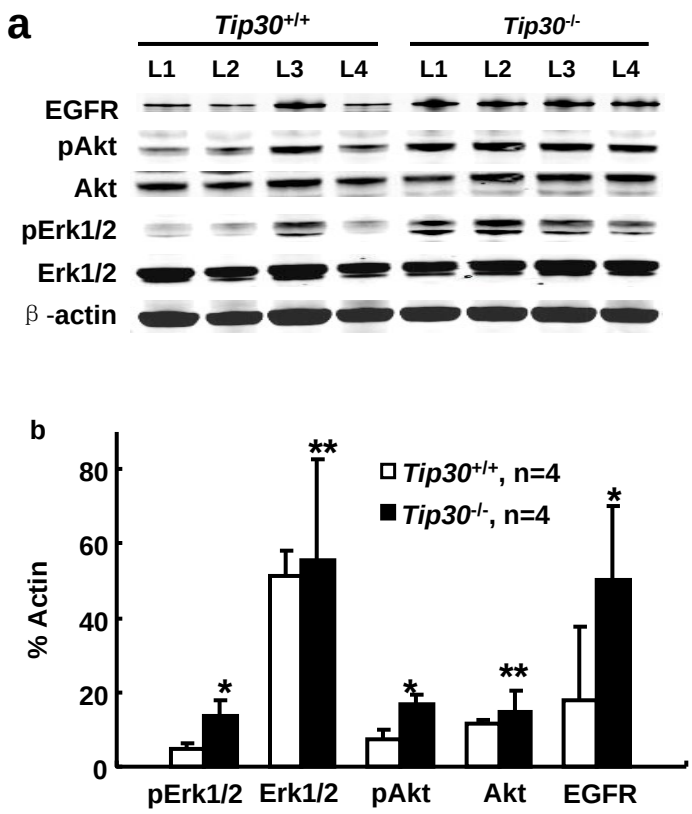
Supplementary Figure 2. Cell proliferation and apoptosis in lungs of age-matched *Tip30*$^{+/+}$ and *Tip30*$^{-/-}$ mice. (**a**) Representative images of double immunofluorescent staining for SP-C and PCNA on indicated lung tissue and tumor sections. Scale bar, 20 μm. (**b**) Quantification of dual PCNA/SP-C positive cells shown in (**a**). Values are expressed as percentage of dual positive cells relative to the total number of SP-C positive cells per field. Cells were counted in 5 fields per slide. Bars Indicate mean ±SEM. (**c**) Graph shows the numbers of the apoptotic cells in alveoli that were counted in 5 high power fields (HPF) per slide of mouse lung tissue samples using TUNEL staining. A slightly increase, but no statistical significance, in apoptosis was observed in *Tip30*$^{-/-}$ lungs. Bars Indicate mean ±SEM.



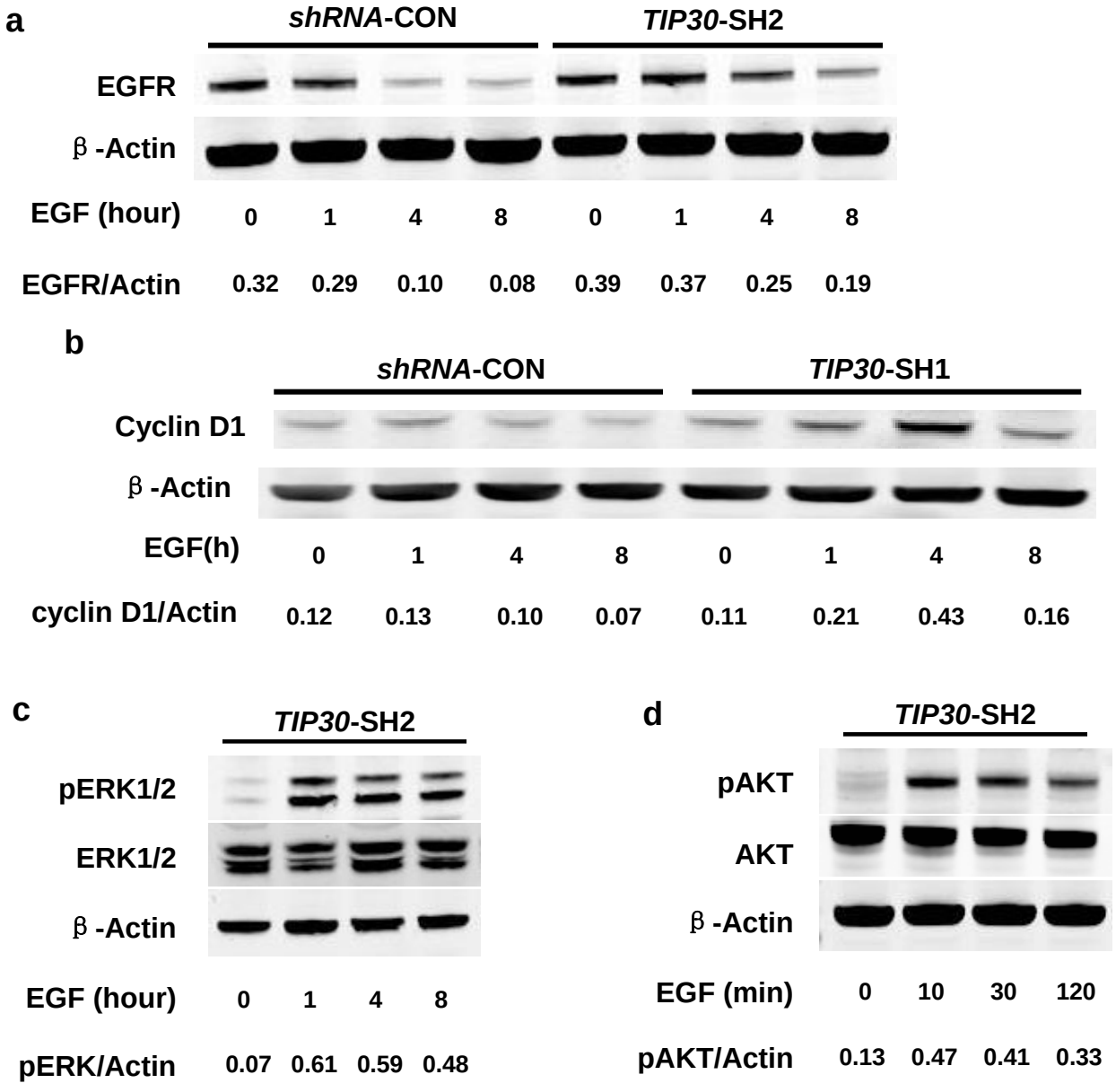
Supplemental Figure 3 Immunostaining for SP-C, CC10 and β -Gal in *Tip30*$^{-/-}$ mouse lung tissues and hyperplasia. Representative Images show that β -Gal coexpresses with SP-C (**a**), but not with CC10 (**b**) in lung alveoli and hyperplasia. Scales bar, 20μm.



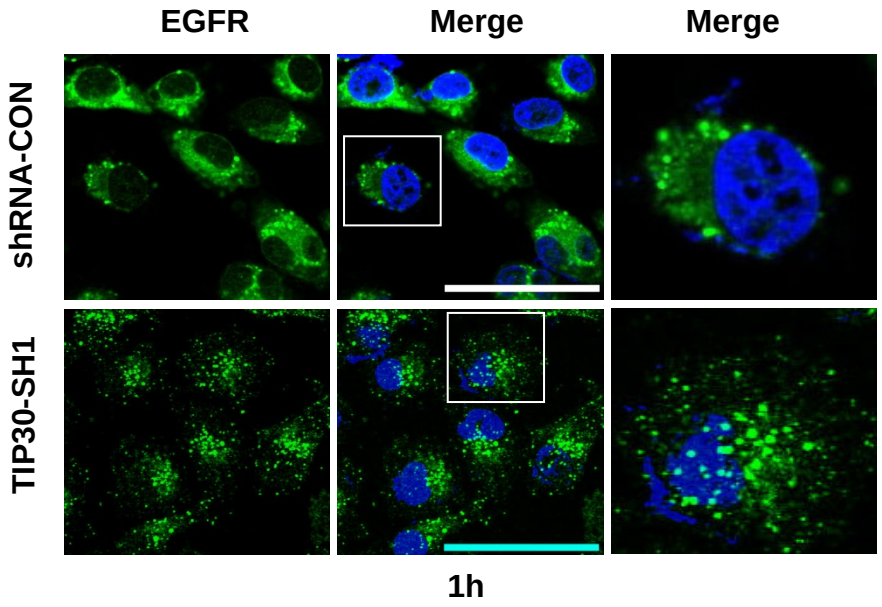
Supplemental Figure 4 Graph shows quantification of EGFR positive cells per field on *Tip30*$^{+/+}$ and*Tip30*$^{-/-}$ lung alveoli.



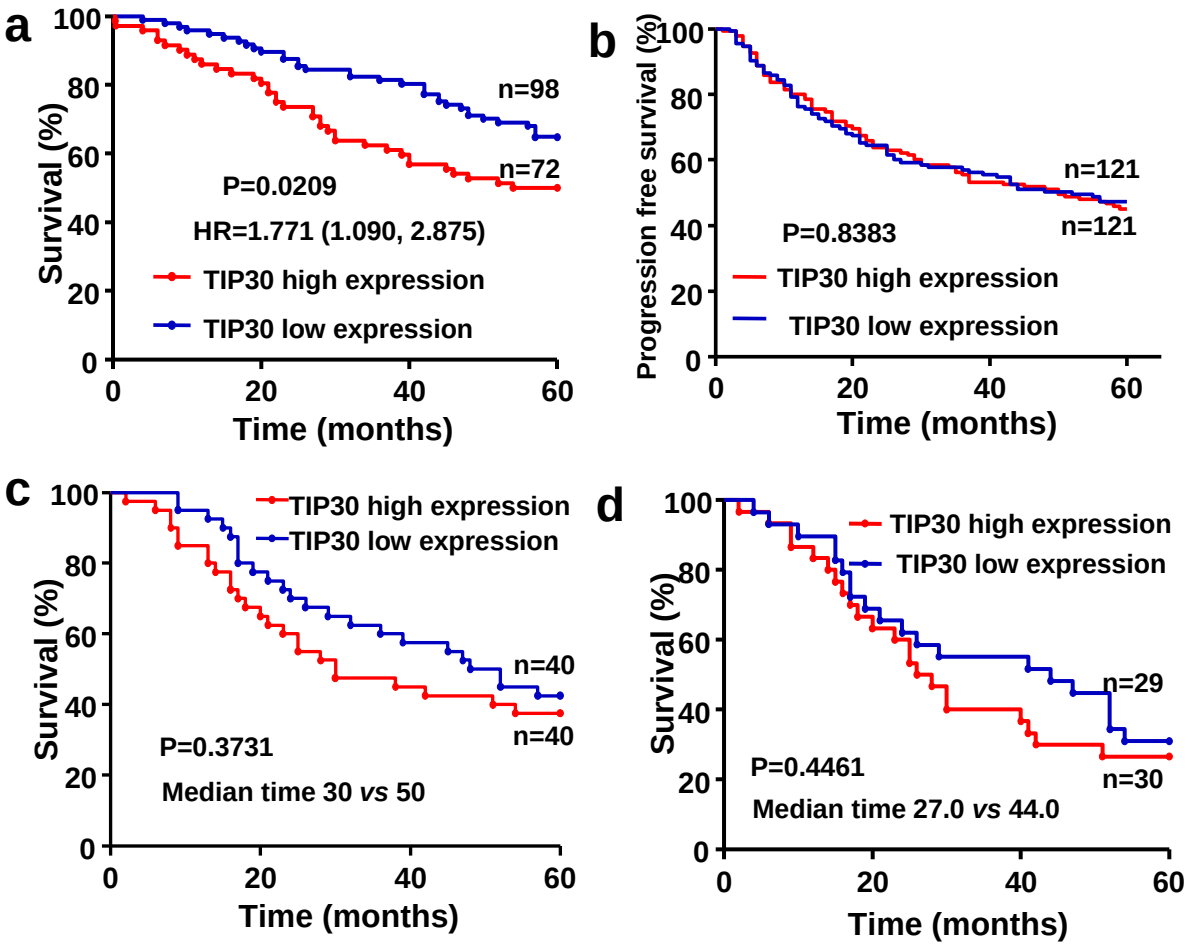
Supplemental Figure 5 Effects of TIP30 deficiency on EGFR signaling in mouse lung tissues. (**a**) Western blot analysis of EGFR, Akt, pAkt, Erk1/2, and pErk1/2 in the indicated lung tissue extracts of four mice of each genotype at 10 months of age. Each lane represents a different mouse lung. (**b**) The histogram represents the relative protein levels as percent of β-actin. *P < 0.05 versus*Tip30*$^{+/+}$, **P > 0.05 versus*Tip30*$^{+/+}$.



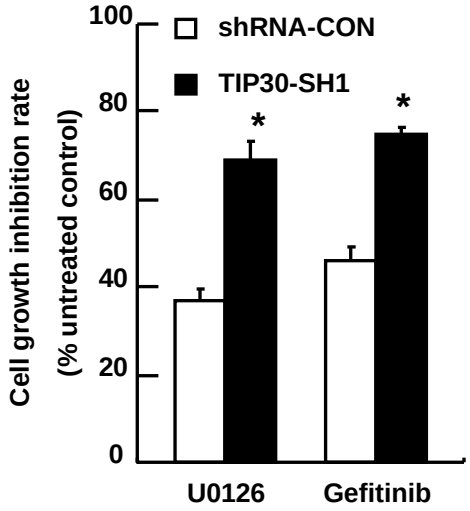
Supplemental Figure 6 Effects of TIP30 deficiency on EGFR degradation and signaling in human A549 cells. Western blot analysis of EGFR (a), Cyclin D1(b), pERK1/2 (c) and pAKT (d) in extracts of TIP30 knockdown A549 cells with TIP30–SH2 or SH1 as indicated. Cells were treated with EGF, then incubated in DMEM including cycloheximide and collected at various time points after EGF treatment and subjected to Western blot analysis with indicated antibodies. EGFR, Cyclin D1, pAKT or pERK1/2 levels were expressed as the ratio of the protein to β -actin.



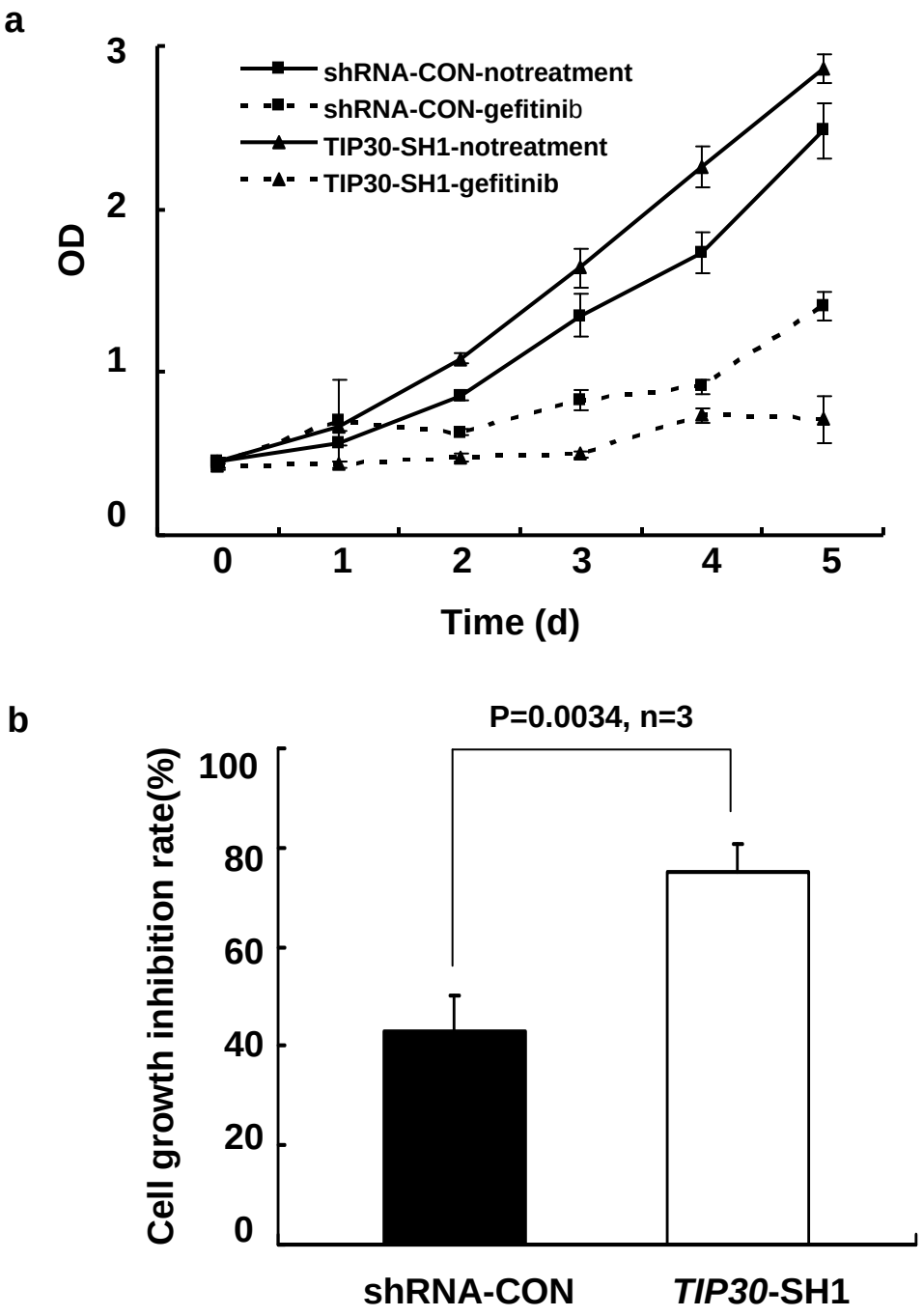
Supplemental Figure 7 Effect of TIP30 knockdown on EGFR trafficking and nuclear localization inducing by EGF in A549 cells. Representative images of EGFR (green) in human A549 cells. Cells were treated with 5 ng/ml EGF and 10 nM leptomycin B in DMEM for 1 hours were then stained for EGFR. Nuclei were stained by DAPI (blue). Scale bars, 10 μm .



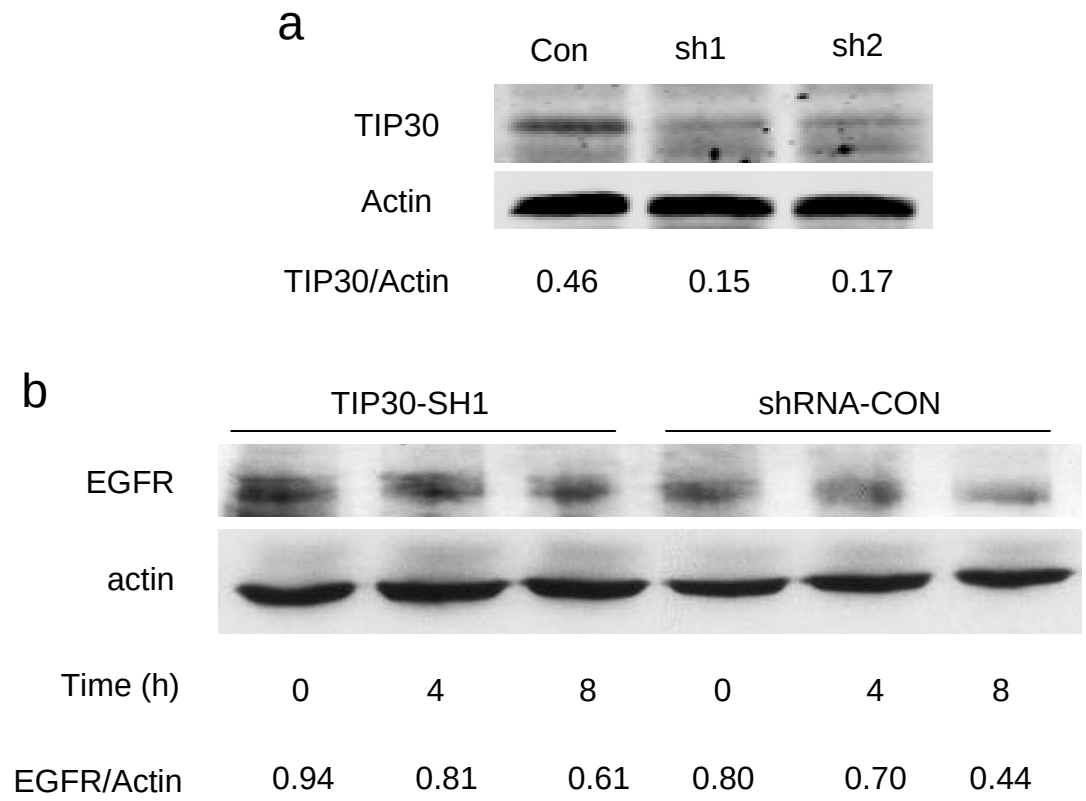
Supplemental Figure 8 Correlation of *TIP30* mRNA expression with PFS and OS in lung adenocarcinoma patients. Kaplan-Meier analysis was used to assess survival of stage I/II lung cancer patients from the NCI's caArray database. (**a**) Raw data were treated by quantile-quantile normalization. Patients with TIP30 lower expression (Z-score >0.5) have long survival time after resection. (**b**) Progression free survival of stage I/II lung cancer patients from the NCI's caArray database. (**c**) Correlation of *TIP30* mRNA expression with OS of lung adenocarcinoma patients with adjuvant chemotherapies. (**d**) Correlation of *TIP30* mRNA expression with OS of lung adenocarcinoma patients with adjuvant radiotherapies.



Supplemental Figure 9 Inhibition of A549 cell growth by gefitinib or U0126. Cell growth inhibition rate was represented as a percentage of untreated cells at day 5, *$P<0.01$, *vs.* shRNA-CON. Data were mean±SEM of three independent experiments



Supplemental Figure 10 Inhibition of H322 cell growth by gefitinib. (a) Growth curves of shRNA-CON and *TIP30*-SH1 cells treated with or without gefitinib. Cells were exposed to 20nM gefitinib for indicating times. Data were mean±SEM of three independent experiments. Cell growth inhibition rate (b) was represented as a percentage of untreated cells at day 5, **P*=0.0034*, vs.* shRNA-CON. Data were mean±SEM of three independent experiments.



Supplemental Figure 11 Effects of TIP30 knockdown on EGFR degradation in immortalized human lung BEAS-2B cells. (a) Western blot analysis of BEAS-2B cells with shRNA-control or TIP30 knockdown. Whole cell lysates were made from cells expressing a scramble shRNA-CON, TIP30-SH1 or TIP30-SH2 that specifically target TIP30. (b) Western blot analysis of EGFR in extracts of TIP30 knockdown BEAS-2B cells with TIP30–SH1 as indicated. Cells were treated with EGF, then incubated in DMEM including cycloheximide and collected at various time points after EGF treatment and subjected to Western blot analysis with indicated antibodies. EGFR level is expressed as the ratio of EGFR to β-actin.