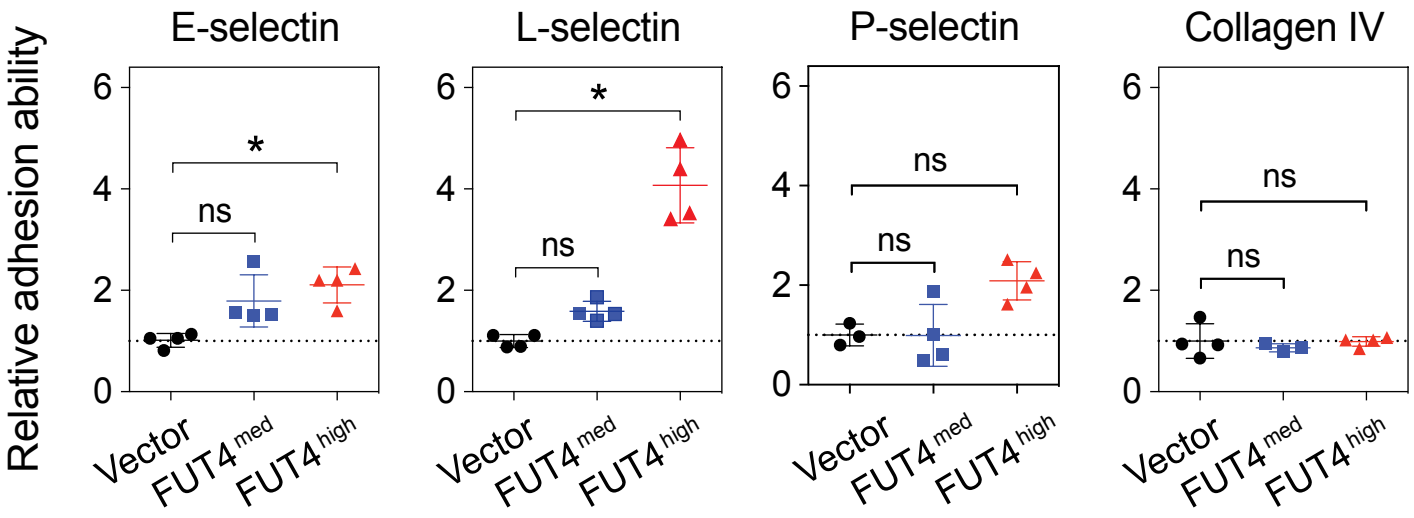
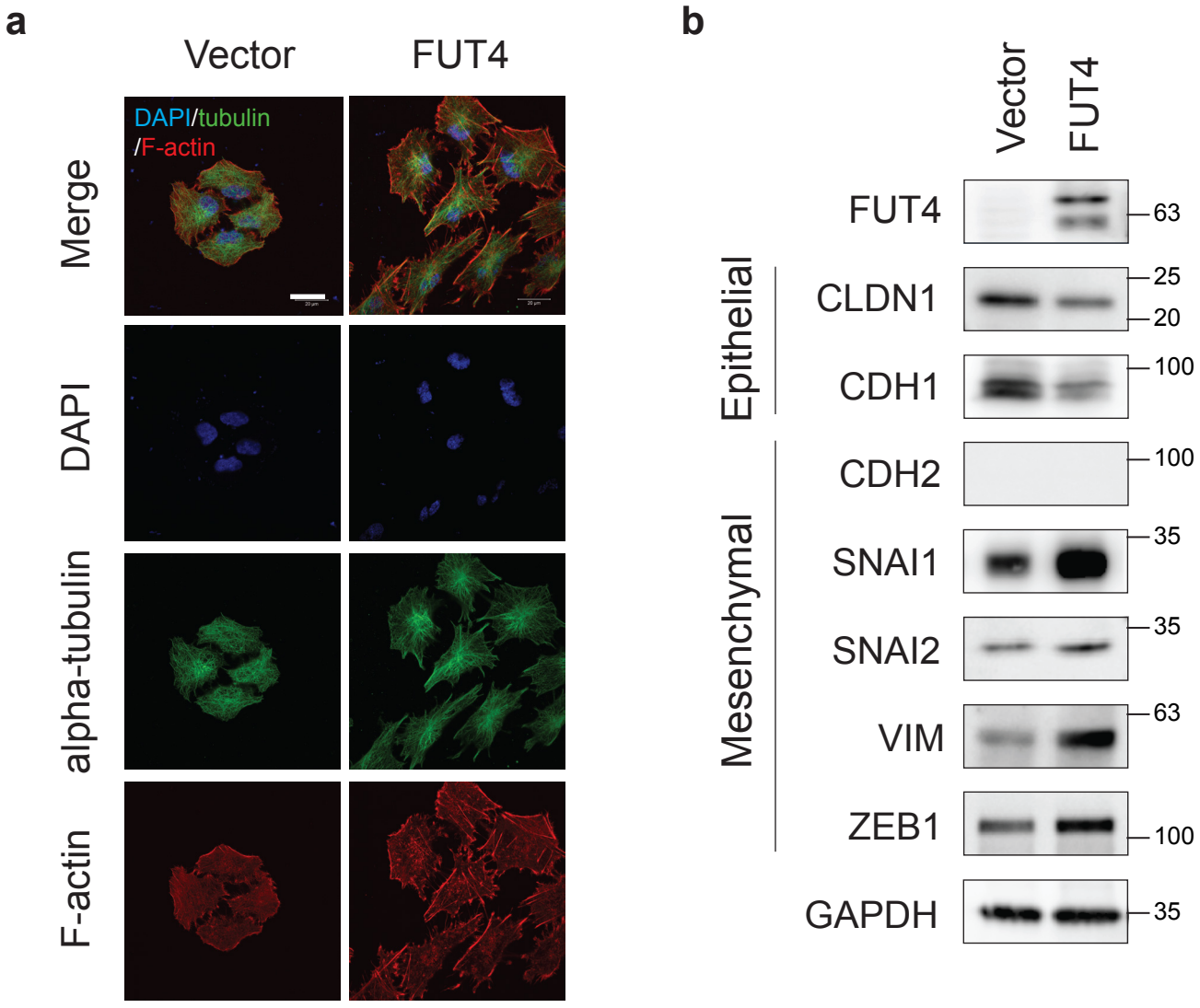
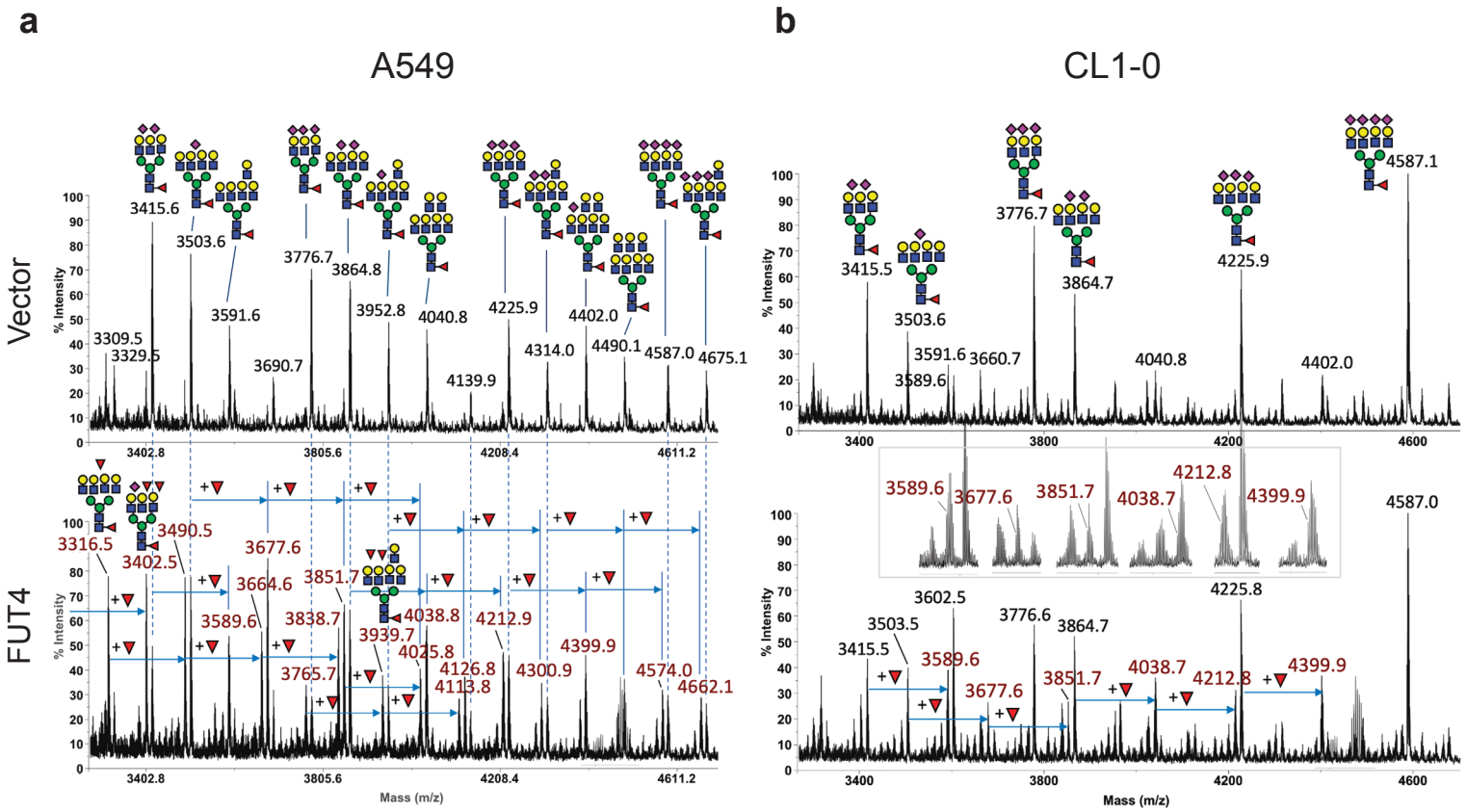
Supplemental Figures



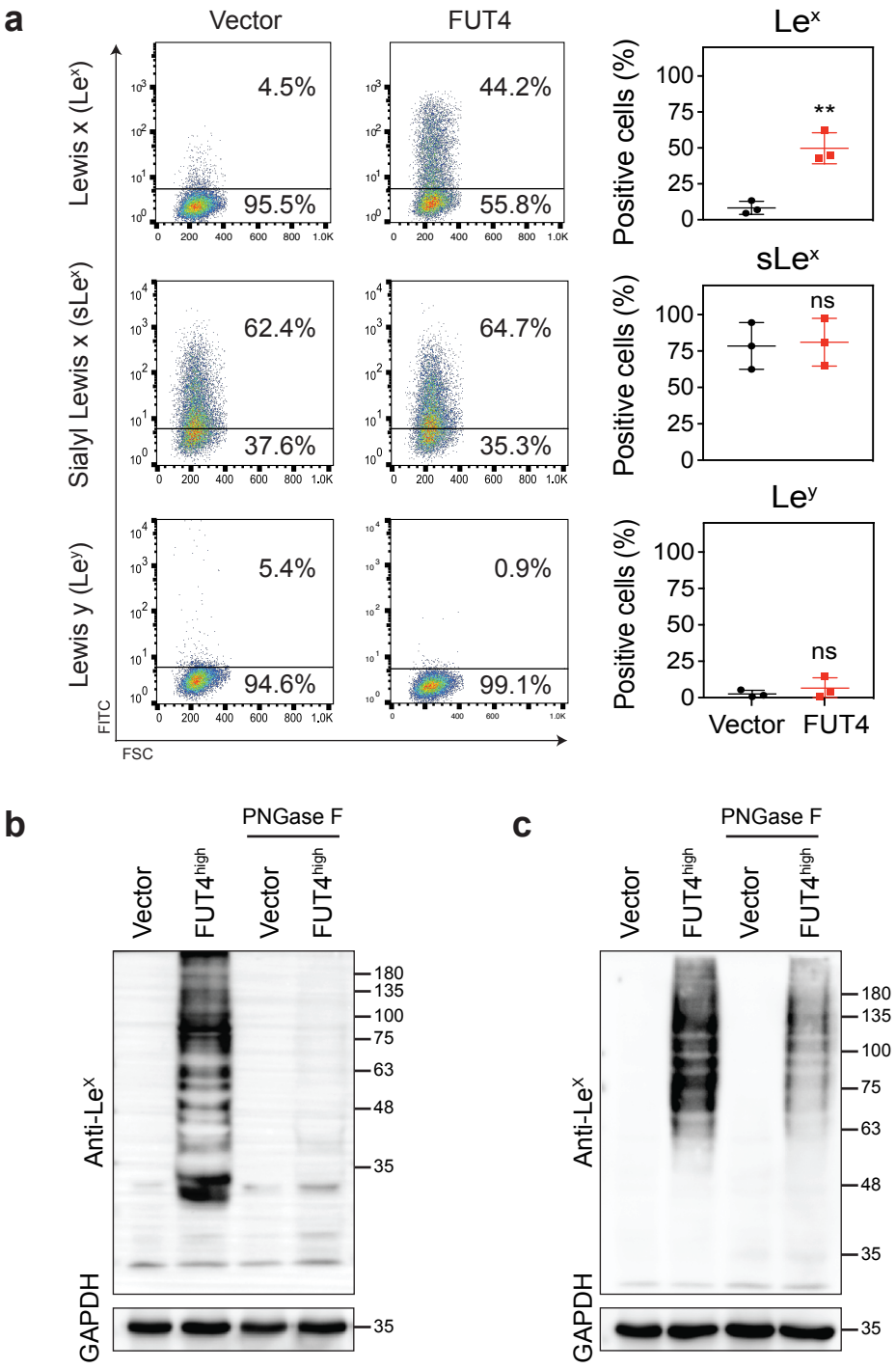
Supplemental Fig. 1. Dot plots showing relative adhesion abilities of FUT4-overexpressing A549 lung cancer cells to collagen IV, E-selectin, L-selectin and P-selectin. Cells bound to the adhesion molecules precoated on 96-well plates were stained by DAPI and counted using Metamorph® software. Relative adhesion ability refers to the numbers of adhering cells divided by those of the vector control.



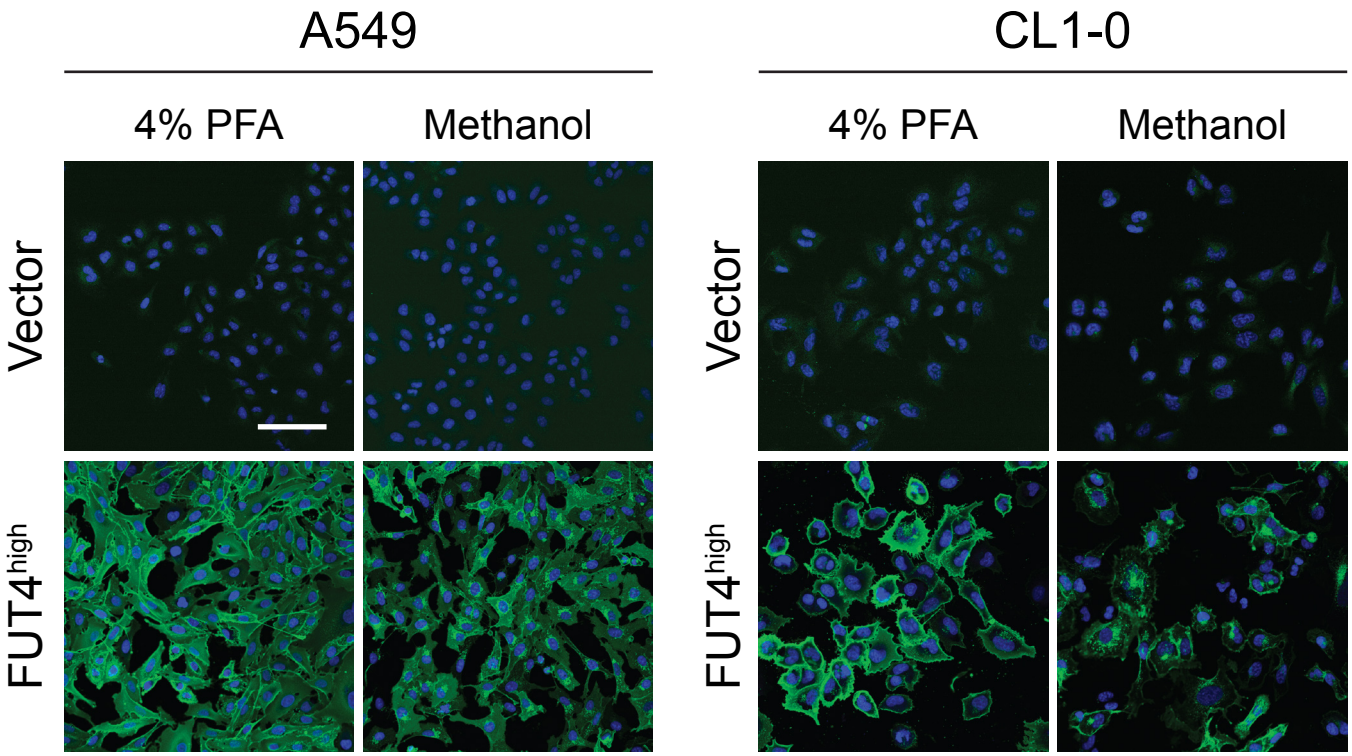
Supplemental Fig. 2. (a) Immunofluorescent imaging analysis of CL1-0 lung cancer cells with FUT4 over-expression (CL1-0_FUT4) compared to vector control (CL1-0_vector). Blue fluorescence: DAPI (4',6-diamidino-2-phenylindole). Green fluorescence: alpha-tubulin. Red fluorescence: F-actin. Scar bar: 20 μm. (b) Western blot analyses of EMT marker proteins in CL1-0 lung cancer cell lines with FUT4 overexpression. Epithelial markers: CLDN1 (claudin-1) and CDH1 (E-cadherin). Mesenchymal markers: CDH2 (N-cadherin), SNAI1 (snail), SNAI2 (slug), VIM (vimentin) and ZEB1 (Zinc Finger E-Box Binding Homeobox 1).



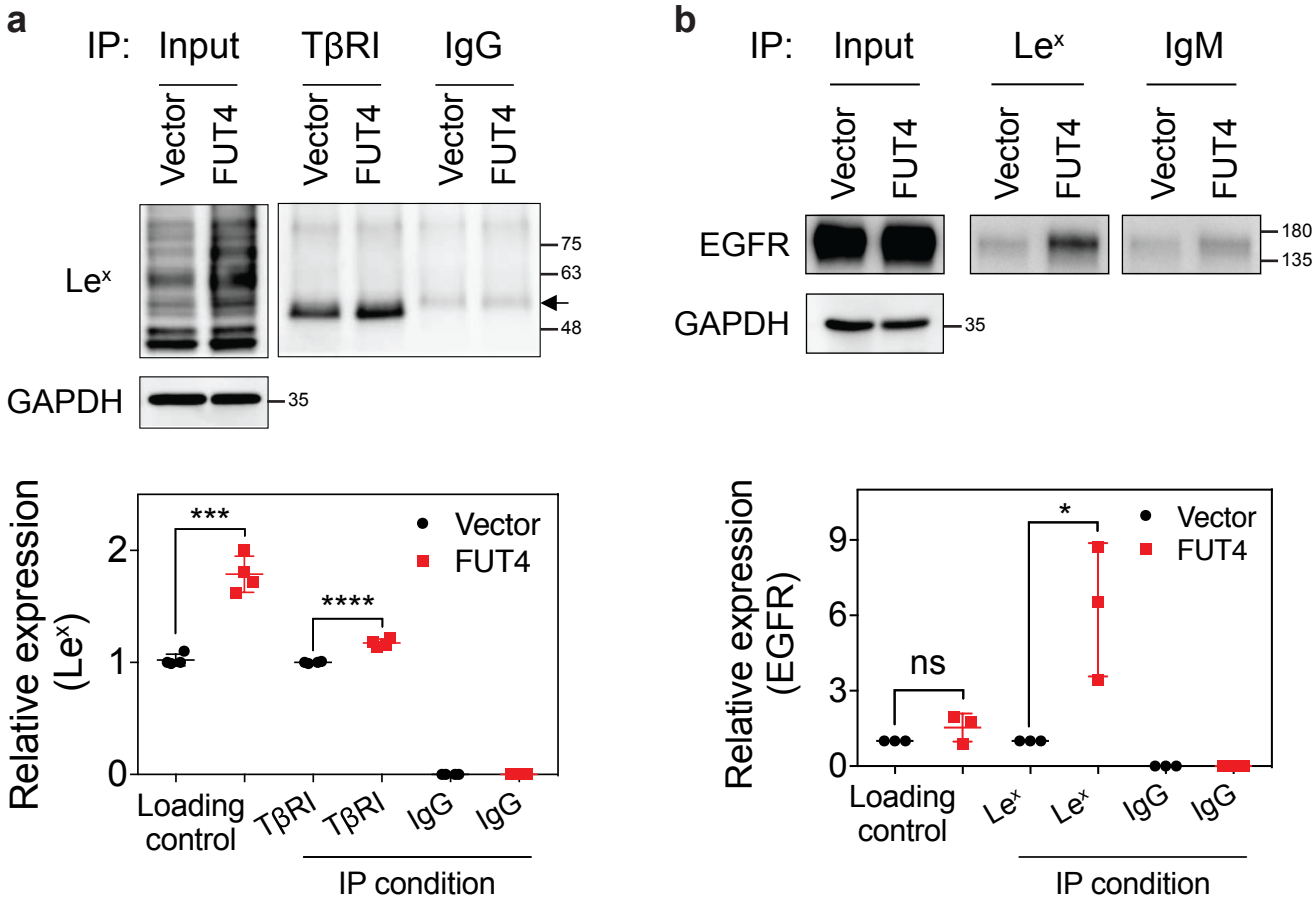
Supplemental Fig. 3. MALDI-MS mapping of the N-glycomic changes in A549 (a) and CL1-0 (b) upon overexpressing FUT4. Major signals were assigned based solely on glycosyl composition but annotated as the most probable N-glycan structures, using the symbol nomenclature for glycans system (Varki et al. 2015). Structures carrying more than 4 LacNAc units were annotated as having polyLacNAc. In cells without overexpression of FUT4, the majority of N-glycans contained only a single fucose, most likely as the core fucose, as annotated (upper panels). The increase in fucose content is depicted as arrows from the major peaks present in the controls. The changes in CL1-0 were less obvious by MALDI-MS mapping. The inset in the lower panel in (b) are zoom-ins on the precursors to identify the resolved monoisotopic peaks. All peaks were annotated by the m/z of monoisotopic peaks, which are a few mass units lower than the most abundant peak of that isotopic cluster at the mass range around 4000. Substituting a NeuAc with 2 fucose results in 13 u lower. 2 NeuAc + 1 fucose is 2 u lower than 2x LacNAc. These mass differences make assignment less definitive at high mass range due to the isotopic clusters and possible undermethylation (-14 u). Only a portion of the entire mass range is shown here to show the changes in fucosylation for the complex type N-glycans. No attempt was made to comprehensively analyze the glycome.



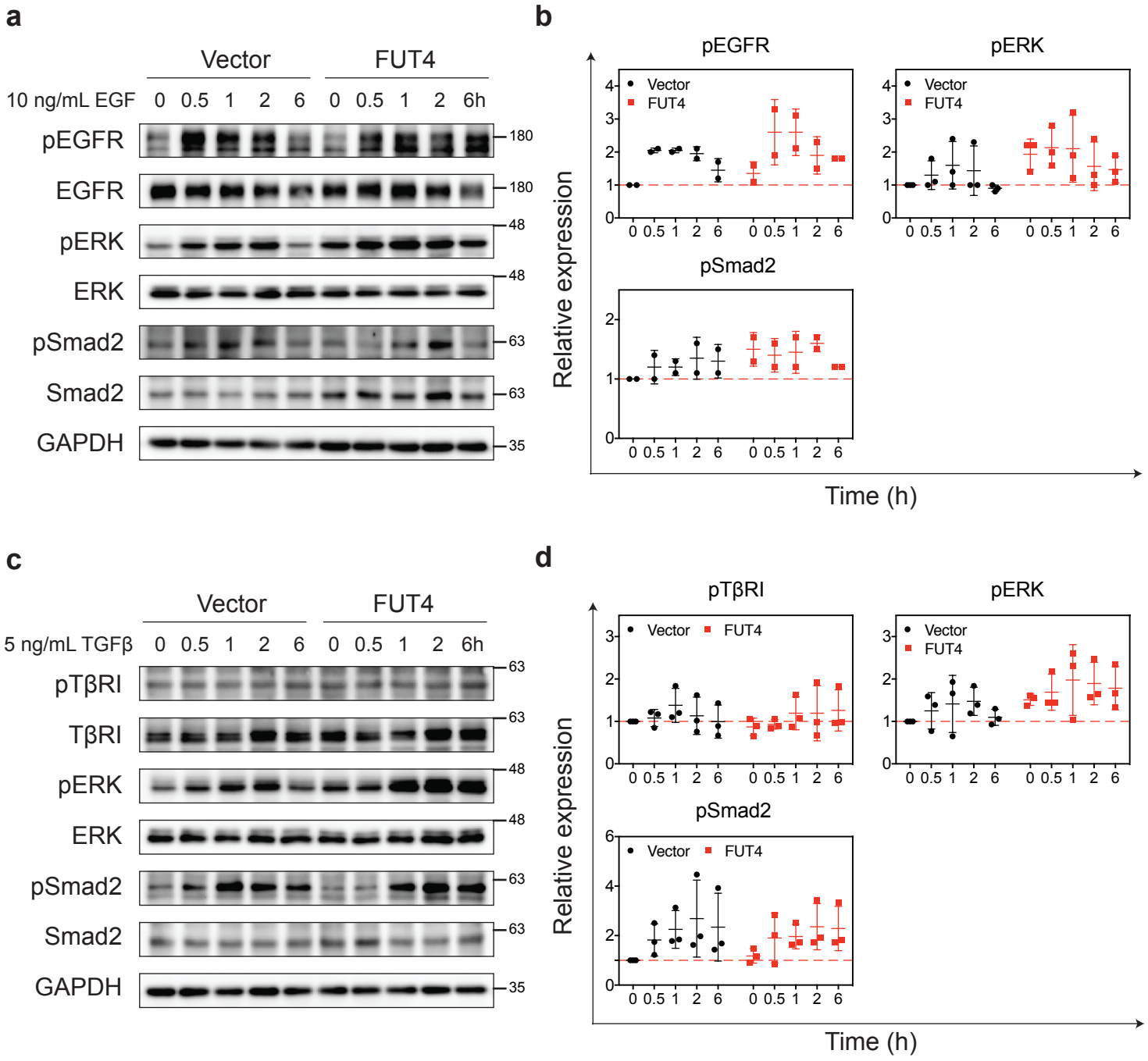
Supplemental Fig. 4. (a) Flow cytometric analysis of cell surface glycans, Lewis x (Le^x), sialyl Lewis x (sLe^x) and Lewis y (Le^y) in FUT4-overexpressing CL1-0 lung cancer cells. Left panels, representative flow cytometric dot plots of Le^x, sLe^x and Le^y expression are shown. Right panels, dot plots showing percentages of cells expressing individual surface glycans in three biological replicates. (b-c) Western blot analysis of proteins that carry Le^x antigen in A549 (b) and CL1-0 (c) cells with FUT4 overexpression as compared to those of the vector control.



Supplemental Fig. 5. Immunofluorescence staining of Le^x in FUT4-overexpressed A549 and CL1-0 lung cancer cells with 4% paraformaldehyde (PFA) or Methanol fixation. There is a decrease in Le^x expression in methanol-fixed cells due to removal of glycolipids by methanol. Scale bar: 100 μm.



Supplemental Fig. 6. Immunoprecipitation-western blot analysis of Lewis X (Le^x), TβRI and EGFR in A549_FUT4high cells as compared to A549_vector cells. (a) Quantifications of Le^x in anti-TβRI pull-down lysates from both cell lines are shown in dot plots. (b) Quantifications of EGFR in anti-Le^x pull-down lysates from both cell lines are shown in dot plots. p value was calculated by Mann-Whitney test. All experiments were performed in three biological replicates and presented as mean ± SEM. * p <0.05, ** p < 0.01, *** p <0.0005, **** p <0.0001. ns: not significant.



Supplemental Fig. 7. (a) Western blot analyses of signaling cascade proteins in the EGF/EGFR pathway in CL1-0_FUT4 versus CL1-0_vector cells at 0, 0.5, 1, 2, and 6 hrs following the addition of 10 ng/mL EGF. Quantifications of signal intensities on western blots for phospho-EGFR, phospho-ERK and phospho-Smad2 from three biological replicates are graphed on the right panel. (b) Western blot analyses of signaling cascade proteins in the TGFβ signaling pathway in CL1-0_FUT4 versus CL1-0_vector cells at 0, 0.5, 1, 2, and 6 hrs following the addition of 5 ng/mL TGFβ. Quantifications of signal intensities on western blots for phospho-TGFβ receptor I, phospho-ERK and phospho-Smad2 from three biological replicates are graphed on the right panel. All experiments were performed in three biological replicates and presented as mean ± SEM.