Supplemental Data

Fibrinogen Alpha Chain Knockout Promotes Tumor Growth and Metastasis through Integrin-AKT Signaling Pathway in Lung Cancer

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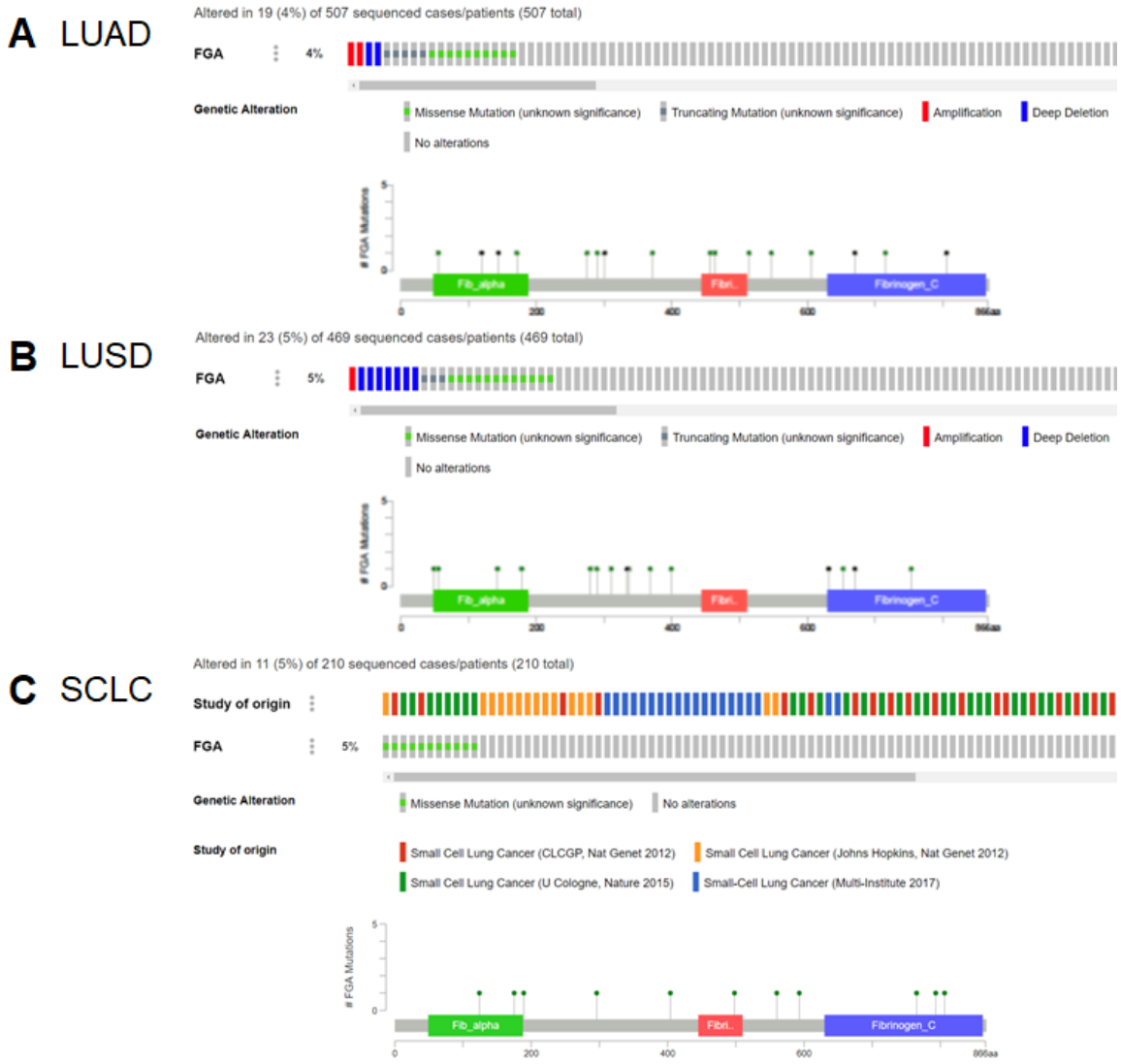
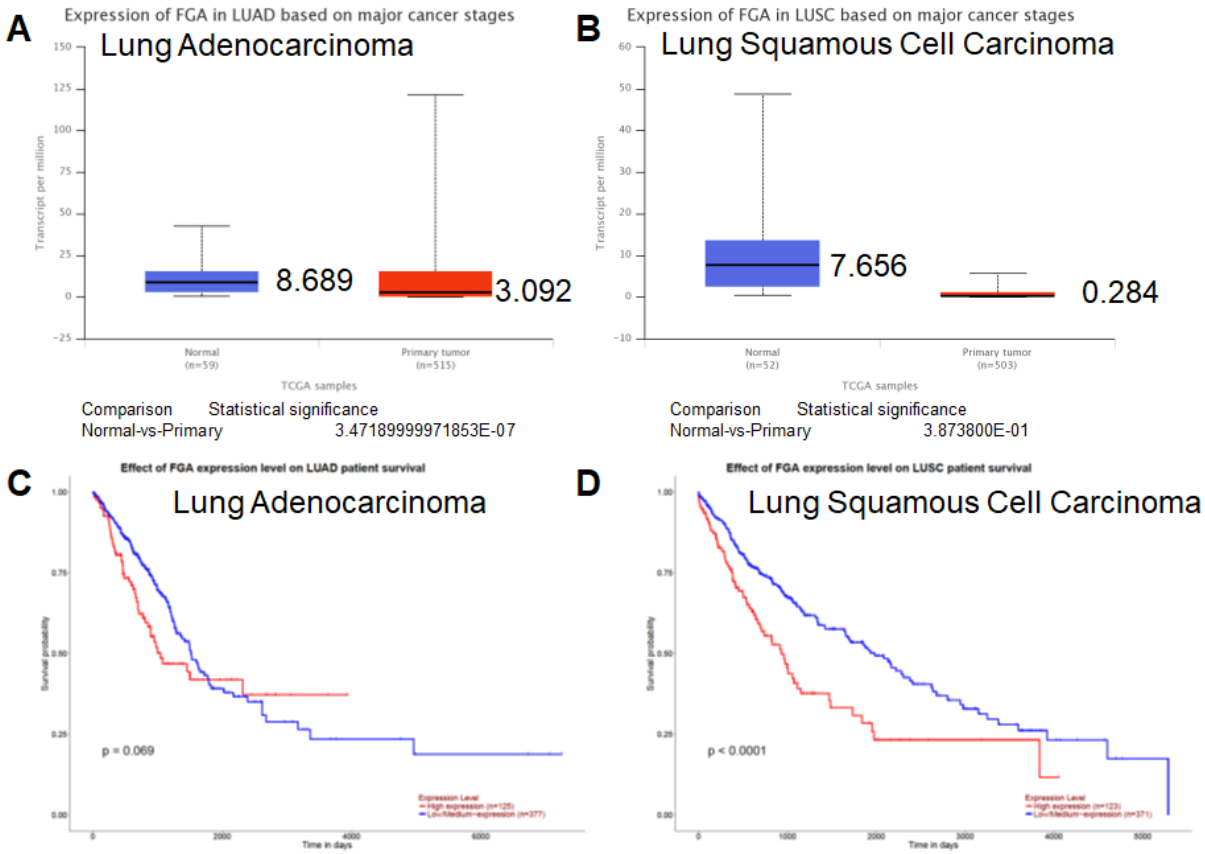
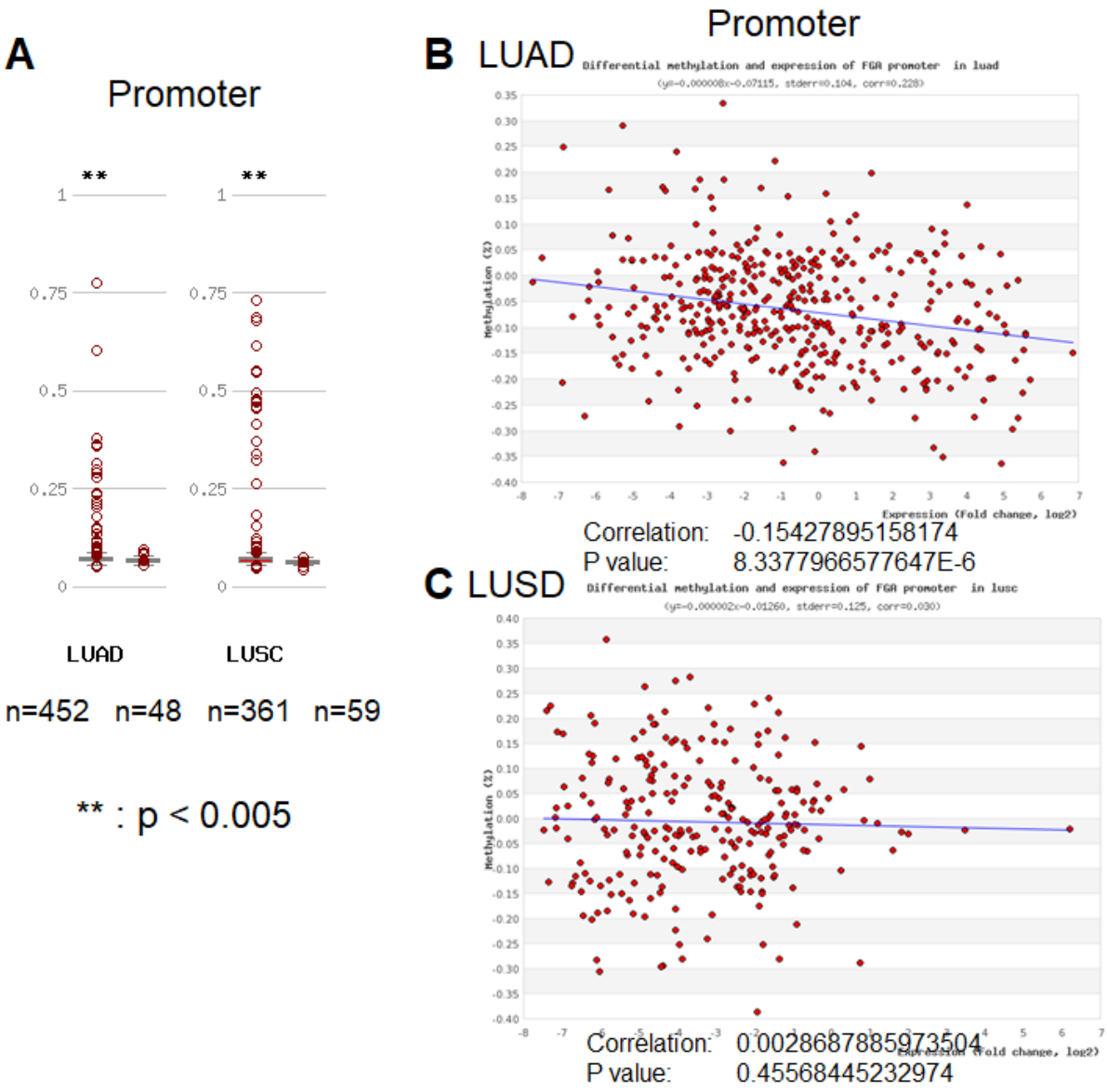


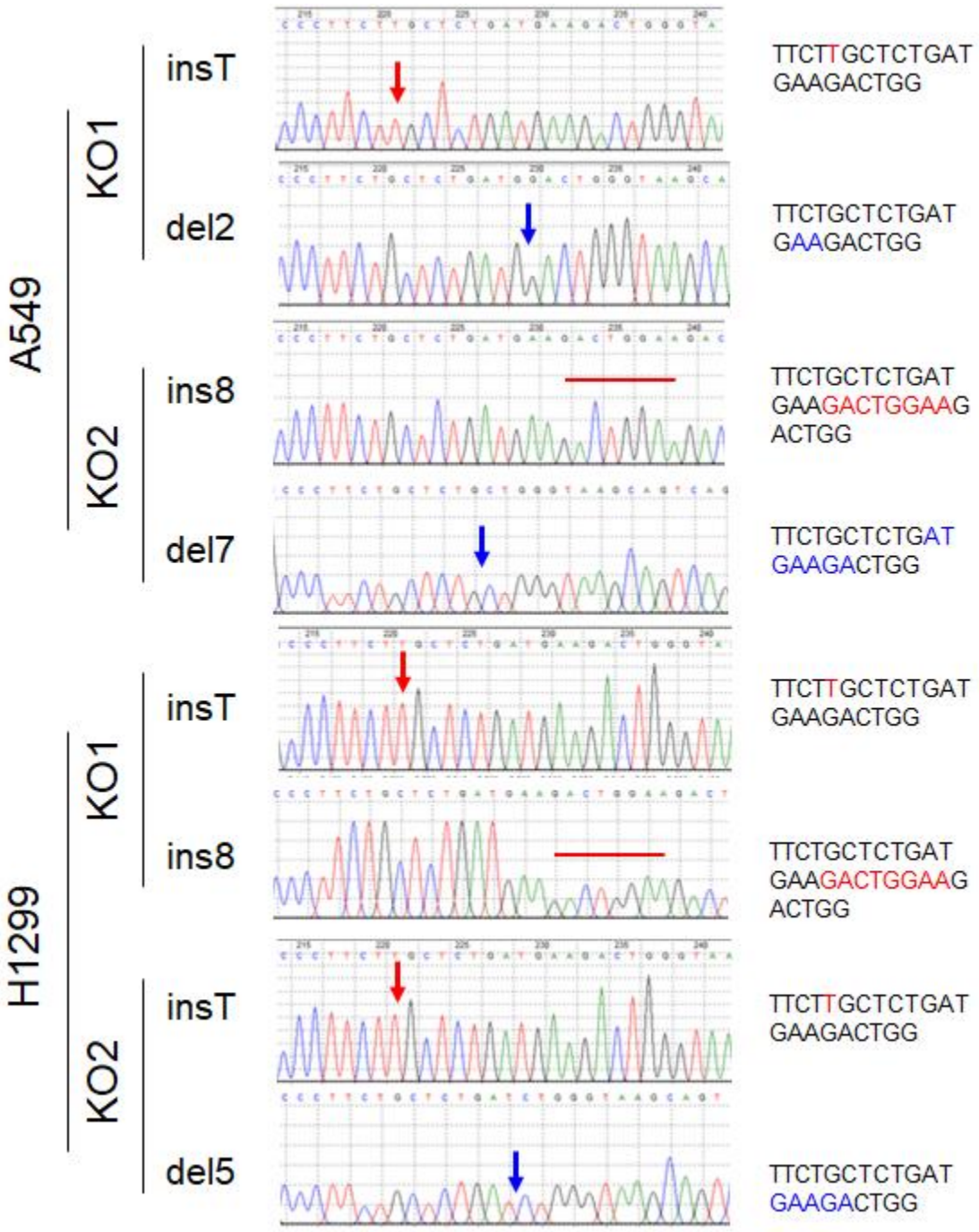
Supplementary Figure S1. Public dataset-based analysis of *FGA* genetic alterations in human lung cancer samples. The data mining and summary of *FGA* genetic alterations for lung adenocarcinoma (LUAD) samples **(a)**, lung squamous cell carcinoma (LUSD) samples **(b)**, and small cell lung cancer (SCLC) samples **(c)** were performed using the cBioPortal with multiple public datasets, which are available at http://www.cbioportal.org. The database query was based on deregulation (amplifications, deletions, mutants) of the FGA gene.



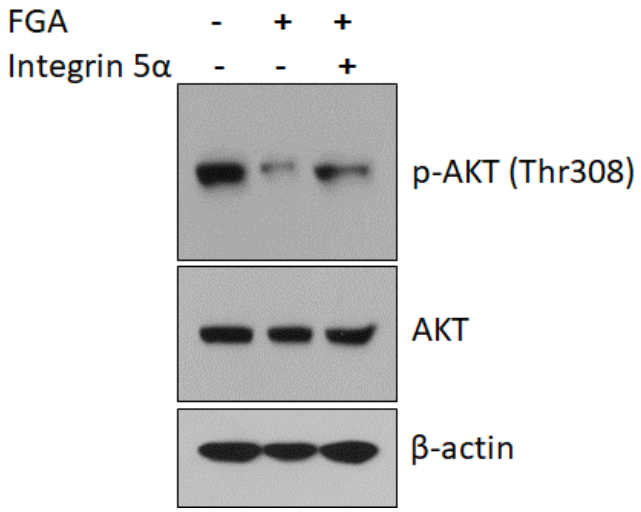
Supplementary Figure S2. Bioinformatics and survival analyses of mRNA expression of *FGA* in human lung cancer samples using the dataset from TCGA. Comparison of *FGA* expression in **(a)** primary lung adenocarcinoma tissues vs. normal lung tissues and **(b)** primary lung squamous cell carcinoma tissues vs. normal lung tissues using the nonparametric Mann–Whitney *U* test. Box plots of samples are displayed by maximum, upper quartile, median, lower quartile, and minimum. Overall survival analysis for patients with lung adenocarcinoma **(c)** and lung squamous cell carcinoma **(d)** was performed between high and low or medium mRNA expressions of the *FGA*. The gene expression and survival analysis were performed using the UALCAN 23 (http://ualcan.path.uab.edu/index.html).



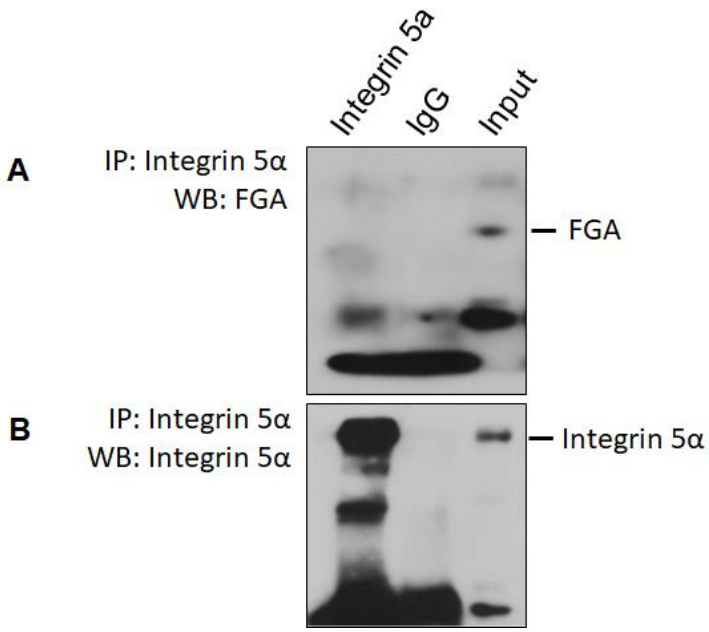
Supplementary Figure S3. DNA methylation status in the promoter region of *FGA* in human lung cancer samples using the dataset from TCGA. (a) The DNA methylation status in the promoter region of FGA in lung adenocarcinoma (LUAD) samples and lung squamous cell carcinoma (LUSD) samples vs. normal lung tissue samples, respectively. Correlation of DNA methylation levels with mRNA expression levels of *FGA* in (**b**) primary lung adenocarcinoma (LUAD) samples and in **(c)** primary lung squamous cell carcinoma (LUSD) samples using the Pearson correlation method of analysis. The gene expression and survival analysis were performed using the MethHC 24 (http://methhc.mbc.nctu.edu.tw).



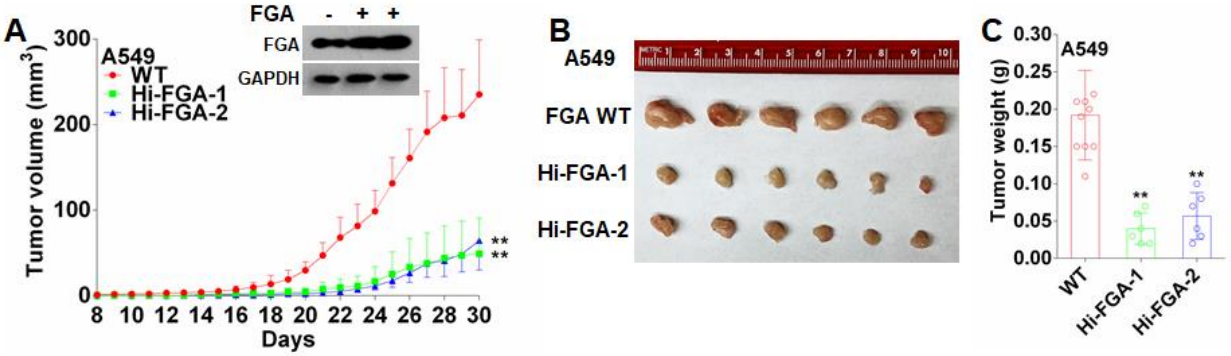
Supplementary Figure S4. Sanger sequencing of CRISPR/Cas9 genome editing in *FGA* KO A549 and H1299 cells. Down-arrows and DNA sequences (right panel) indicate insert (red) and knockout (blue) editing. KO, knockout; ins, inserts; del, deletion.



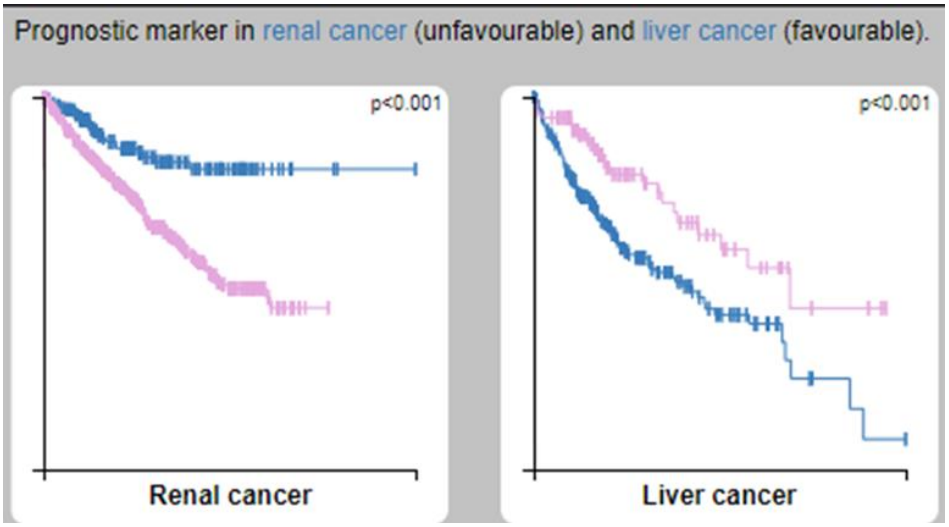
Supplementary Figure S5. Effect of FGA mutation on interaction of FGA and integrin 5α in FGA KO A549 cells. In *FGA* KO A549 cells after treatment with recombinant FGA or integrin α5 for 6 hours, phosphorylation and expression of AKT was determined by Western blot analysis with specific antibodies.



Supplementary Figure S6. Effect of FGA mutation on interaction of FGA and integrin 5α in FGA KO A549 cells. In *FGA* KO A549 cells after treatment with mutant FGA for 6 hours, the immunoprecipitation (IP) of integrin α5 and 10-fold diluted input were determined by Western blot analysis with (**A**) anti-FGA antibody and (**B**) anti-integrin α5 antibody. The mutant recombinant human FGA does not include the two Arg-Gly-Asp (RGD) sequences, which are required for a specific binding of integrin 5α/3β to FGA.



Supplementary Figure S7. Effect of FGA over-expression on xenograft tumor growth of A549 cells *in vivo*. (**A**) Tumor growth in nude mice subcutaneously injected with FGA WT and FGA over-expressed A549 cells (n=6 mice including 3 male and 3 female mice each group). *Inserted image*, expression of FGA was determined by Western blot analysis with specific antibodies. Data are presented as means ± SD of the tumor volumes. Representative images (**B**) and weights (**C**) of xenograft tumors at day 30 after injection. ** $p < 0.01$ by two-way ANOVA test or two-tailed t-test *vs.* the WT control group. WT, wild-type. This experiment was repeated two times.



Supplementary Figure S8. Survival analyses of mRNA expression of *FGA* in human renal and liver cancer samples using the dataset from TCGA. Overall survival analysis for patients with renal cancer (left panel) and liver cancer (right panel) was performed between high and low mRNA expressions of the *FGA*. The survival analysis was performed using the Human Protein Atlas (https://www.proteinatlas.org).