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Supplementary material

Fibroblast growth factor–14 acts as tumor suppressor in lung adenocarcinomas

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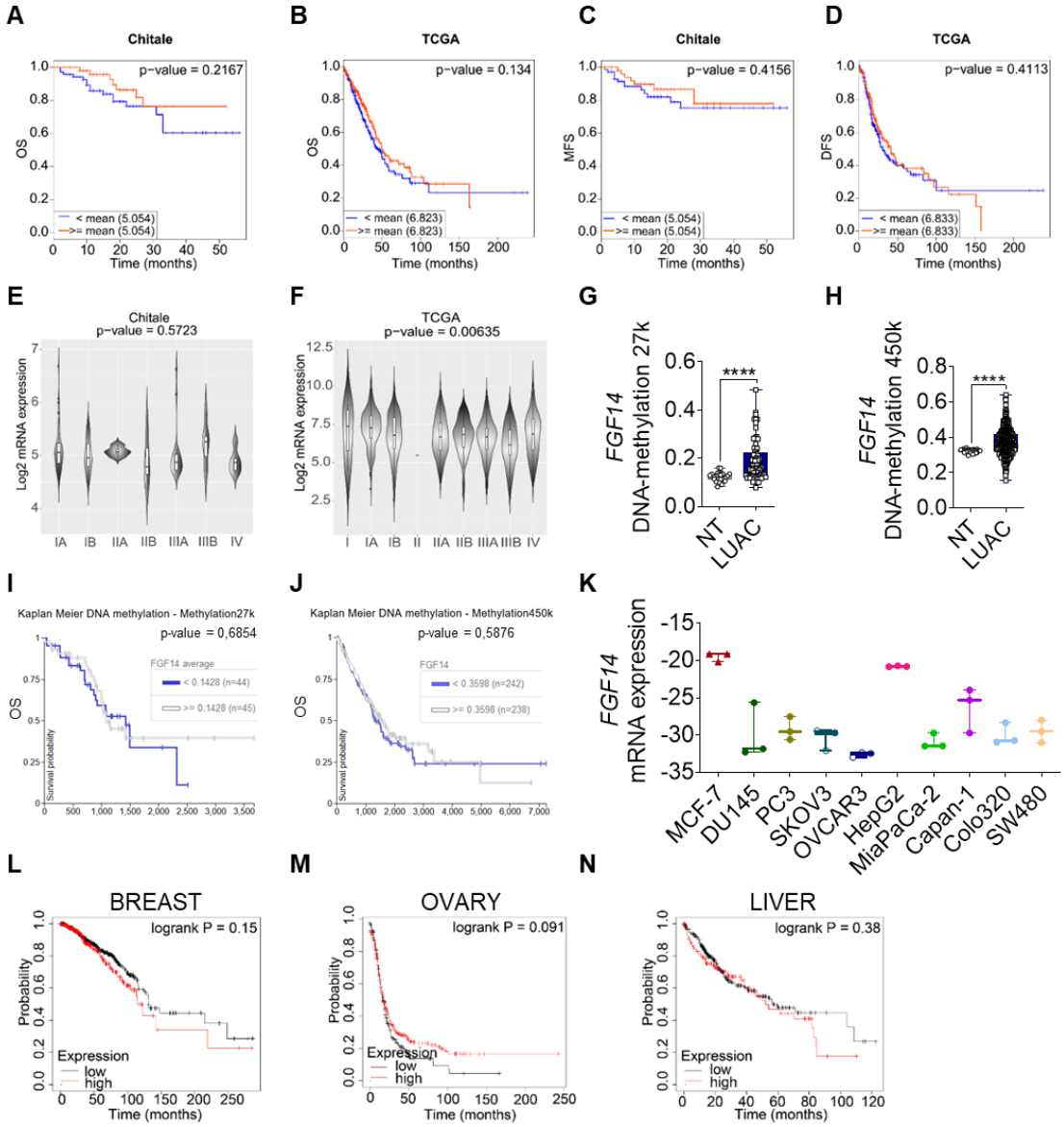

Figure S1. *In silico* comparative analyses of FGF14 mRNA expression in lung, breast, ovary and liver cancers. (**A-F**) Graphics include Kaplan–Meier curves representing overall survival (OS) (**A, B**), metastasis-free survival (MFS) (**C**), and disease-free survival (DFS) (**D**) of patient groups selected according to the expression of *FGF14* in the datasets designated as indicated above each graphic. (**E–F**) Violin plots depict the expression of FGF14 among cancer specimens of the indicated group of tumors in the different datasets. The pathological stage is indicated as IA, IB, II, IIA, IIB, IIIA, IIIB, or IV. An analysis of variance test was performed to compare the mean among groups [32]. FGF14-DNA methylation (**G**) 27k and (**H**) 450k in LUAC compared with non-tumor tissue. (**I-J**) OS of LUAC patients according to FGF14-DNA methylation. (**K**) *FGF14* mRNA expression in different cancer cell lines, such as breast (MCF-7), prostate (DU145 and PC3) ovary (SKOV3 and OVCAR3), liver (HEP2G), pancreatic (MiaPaCa-2 and Capan-1), and colon (Colo320 and SW480 (**L–N**). Adjusted *p*-values were calculated using the Benjamini–Hochberg (BH) method. Methylation data (**G–J**) was obtained from The Cancer Genome Atlas LUAC cohort using Xena browser [33].

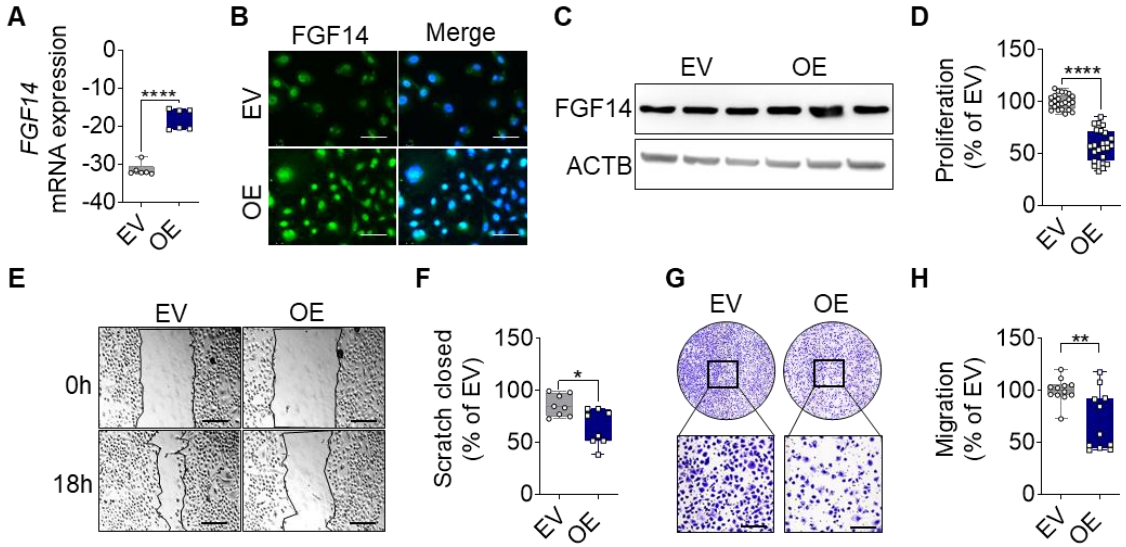

Figure S2. FGF14 overexpression in H838 cells. Validation of FGF14 overexpression by H838 cells via (**A**) real-time polymerase chain reaction, (**B**) immunocytochemistry staining, and (**C**) western blot. (**D**) Cellular proliferation was quantified by bromodeoxyuridine incorporation in H838-FGF14-expression vector (OE) cells compared with H838-empty vector (EV) control cells. The migratory ability of FGF14-OE cells was assessed via scratch assay (**E, F**) and Boyden chamber assay (**G, H**) compared with the EV control cells. Representative photographs were taken at 0 and 18 h after scratching (5× magnification). The cells in B were labeled using FGF14 antibody and revealed using an AlexaFlour 488 secondary antibody (green). DNA was stained with 4′,6-diamidino-2-phenylindole (blue), scale bars, 100 μm. Data are shown as mean± standard error of the mean using Student's t-test. *P*-values ≤ 0.05 were considered statistically significant for all analyses. *$p \leq 0.05$, **$p \leq 0.01$, and ****$p \leq 0.0001$.

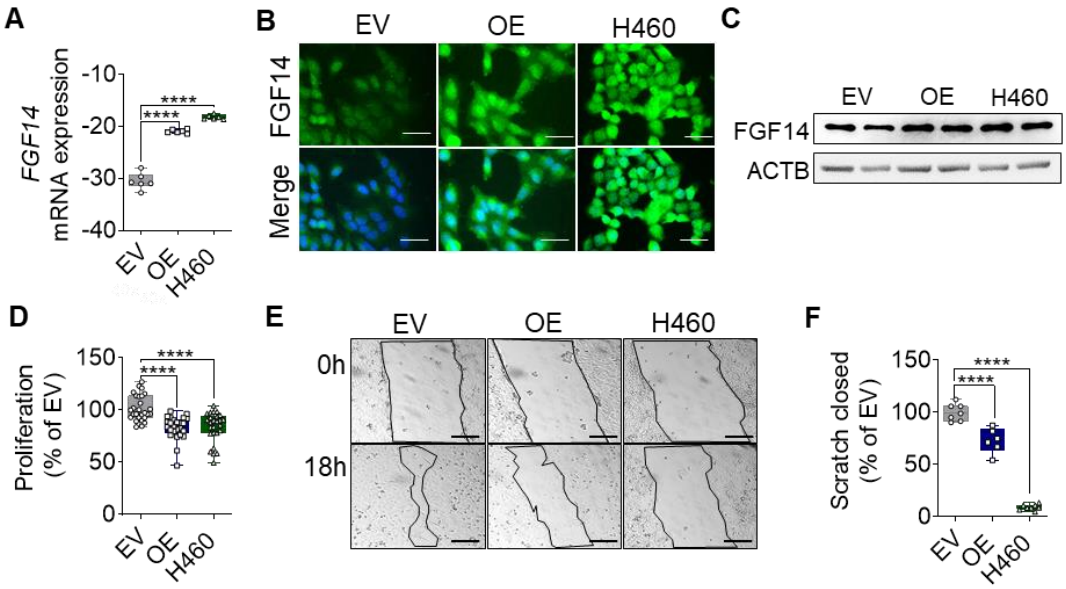

Figure S3. FGF14 expression in H460 cells. Validation of FGF14 expression by H460 parental cells compared with A549-empty vector (EV) and A549-FGF14-expression vector (OE)-transfected cells via (**A**) real-time polymerase chain reaction, (**B**) immunocytochemistry staining, and (**C**) western blot. (**D**) Quantification of protein expression by H460 and H838-FGF14-OE cells compared with A549-EV control cells by relative density normalized to β-actin. (**E**) Cellular proliferation was quantified by bromodeoxyuridine incorporation in H460 and A549-FGF14-OE cells compared with H838-EV control cells. (**F**) The migratory ability of H460 and A549-FGF14-OE cells was evaluated by scratch assay compared to the A549-EV control cells. Representative photographs were taken at 0 and 18 h after scratching (5× magnification). Cells in B were labeled using FGF14 antibody and revealed by an AlexaFlour 488 secondary antibody (green). DNA was stained with 4′,6-diamidino-2-phenylindole (blue), scale bars, 100 μm. Data are shown as mean ± standard error of the mean using a one-way analysis of variance. *P*-values ≤ 0.05 were considered statistically significant for all analyses; ****$p \leq$ 0.0001.

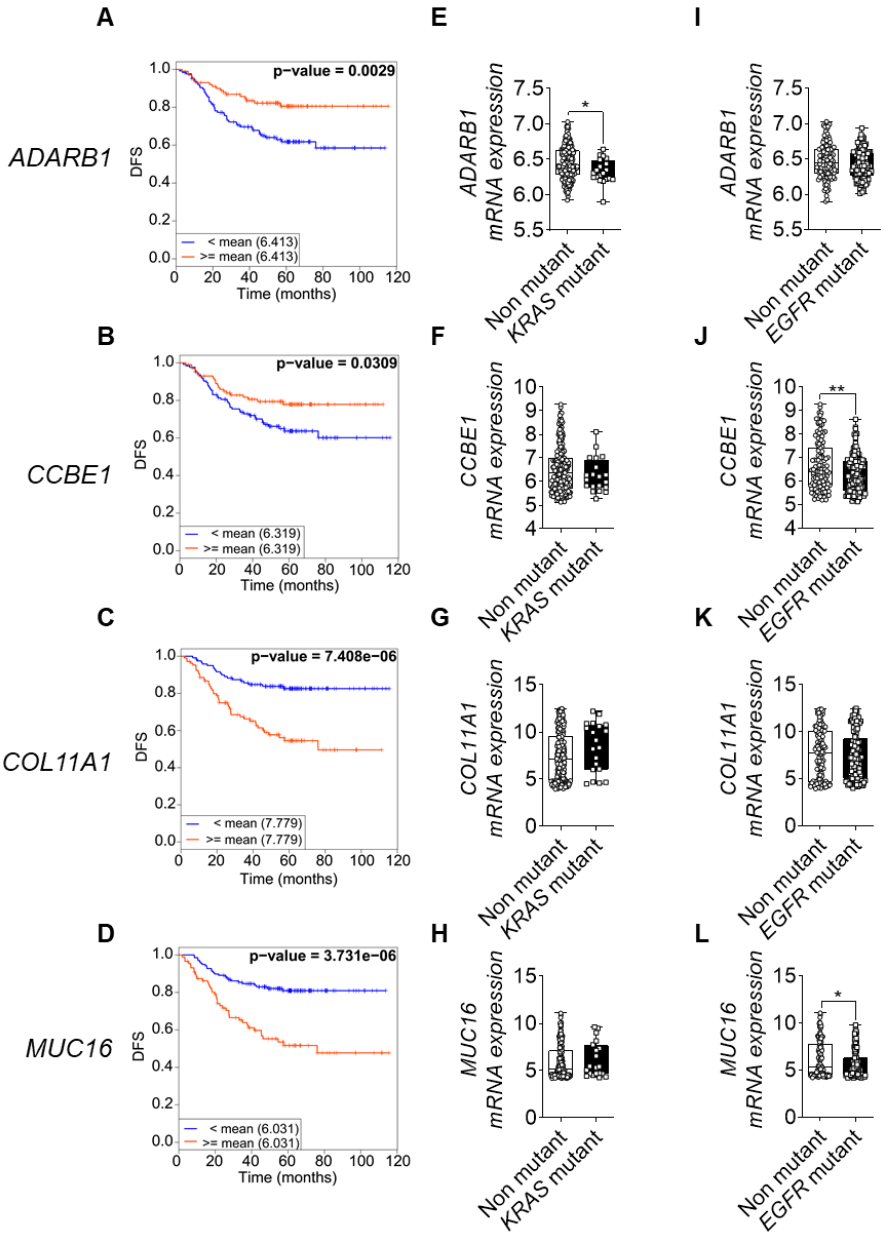

Figure S4. Kaplan–Meier estimate of overall survival in lung adenocarcinoma patients. (**A-D**) Kaplan–Meier estimate of overall survival among Okayama patient dataset with LUAC that are classified according to the m-RNA expression levels of *CCBE1, ADARB1, COL11A1* and *MUC16*: high (above the mean value of mRNA levels) and low (below the mean value of mRNA levels). (**E-H**) Analysis of expression in *KRAS* mutant vs non-mutant patient samples and (**I-L**) *EGFR* mutant vs non-mutant samples. Data obtained from CANCERTOOL. Data are shown as mean ± standard error of the mean using Student's t-test. *P*-values ≤0.05 were considered statistically significant for all analyses. **p* ≤ 0.05 and ***p* ≤ 0.01.

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