Figure S1. (A) Analysis of FXYD5 mRNA expression in 23 normal samples, 26 astrocytoma, 50 oligodendroglioma and 81 glioblastoma patient samples in glioma using GSE4290 dataset. (B) Analysis of FXYD5 mRNA expression in 23 normal samples, 45 grade 2, 31 grade 3 and 77 grade 3 patient samples in glioma using GSE4290 dataset. (C) mRNA expression of FXYD5 in LN229 cells (left) and U251 (right) cells after FXYD5 knockdown, showing the knockdown efficiency of shFXYD5 virus. (D and E) Flow cytometry examined the apoptotic rate (%) of (D) LN229 and (E) U251 cells after FXYD5 knockdown. (F) Correlation analysis of positively correlation between FXYD5 and the key genes of lipid metabolism, ACSL4, GPAT3 and SOAT1. (G) Level of free fatty acid concentration of U251 cells after FXYD5 knockdown. (H) Nile Red indicator assay evaluating the effect of FXYD5 knockdown on intracellular lipid titer in U251 cells. Scale bars, 500 μm. (I) Level of total cholesterol content and triglyceride content of U251 cells after FXYD5 knockdown. (J) mRNA expression of lipid metabolism, ACSL4, GPAT3 and SOAT1 in U251 cells after overexpression of FXYD5 and knockdown of ACSL4. (K) Western blot analysis of ACSL4, GPAT3 and SOAT1 protein expression in U251 cells after overexpression of FXYD5 and knockdown of ACSL4. Right is the bar graph obtained after quantification of protein bands. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. SOAT1, sterol O-acyltransferase 1; ACSL4, long-chain acyl-coenzyme A (CoA) synthase 4; GPAT3, glycerol-3-phosphate acyltransferase 3; LV, lentivirus; sh-, short hairpin; NC, negative control.

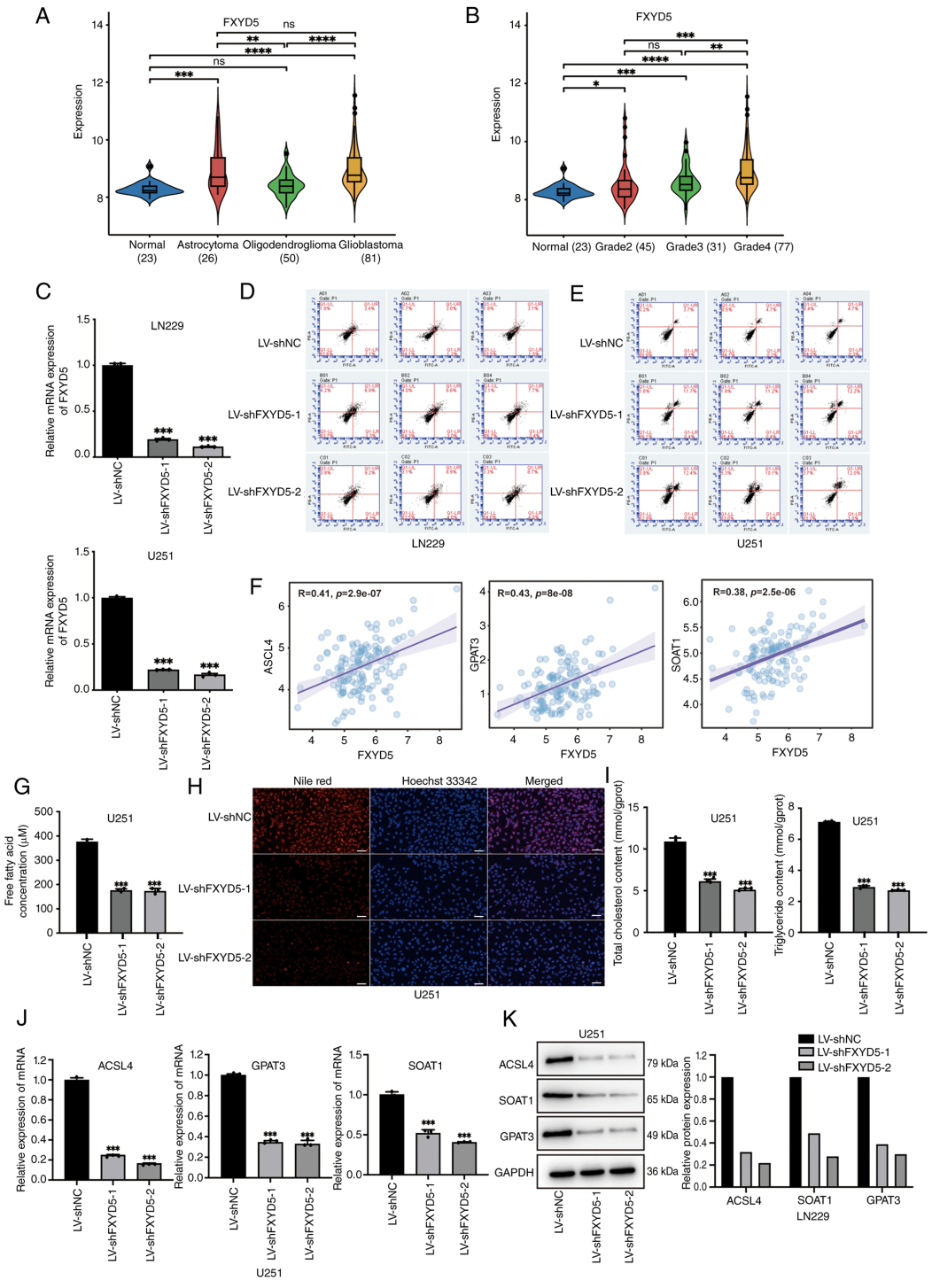

Figure S2. (A) Western blot analysis of FXYD5 protein expression in LN229 (Left) and U251 (Right) cells after overexpression of FXYD5. (B) Western blot analysis of ACSL4 protein expression in LN229 (Left) and U251 (Right) cells after knockdown of ACSL4. (C) The bar graph obtained after quantification of Fig. 3B protein bands. (D) mRNA expression of FXYD5 and ACSL4 in U251 cells after overexpression of FXYD5 and knockdown of ACSL4. (E) Western blot analysis of FXYD5 and ACSL4 protein expression in U251 cells after overexpression of FXYD5 and knockdown of ACSL4. The bar graph obtained after quantification of protein bands is in below. (F) Level of free fatty acid concentration of U251 cells after overexpression of FXYD5 and knockdown of ACSL4. (G) Nile Red indicator assay evaluating the effect of overexpression of FXYD5 and knockdown of ACSL4 on intracellular lipid titer in U251 cells. Scale bars, 500 μm. (H) Level of total cholesterol content and triglyceride content of U251 cells after overexpression of FXYD5 and knockdown of ACSL4. (I) Western blot analysis of ACSL4 protein expression in LN229 (Left) and U251 (Right) cells after overexpression of ACSL4. (J) The bar graph obtained after quantification of Fig. 3G protein bands. ***P<0.001. ACSL4, long-chain acyl-coenzyme A (CoA) synthase 4; LV, lentivirus; sh-, short hairpin; NC, negative control; OE, overexpression; ns, not significant.

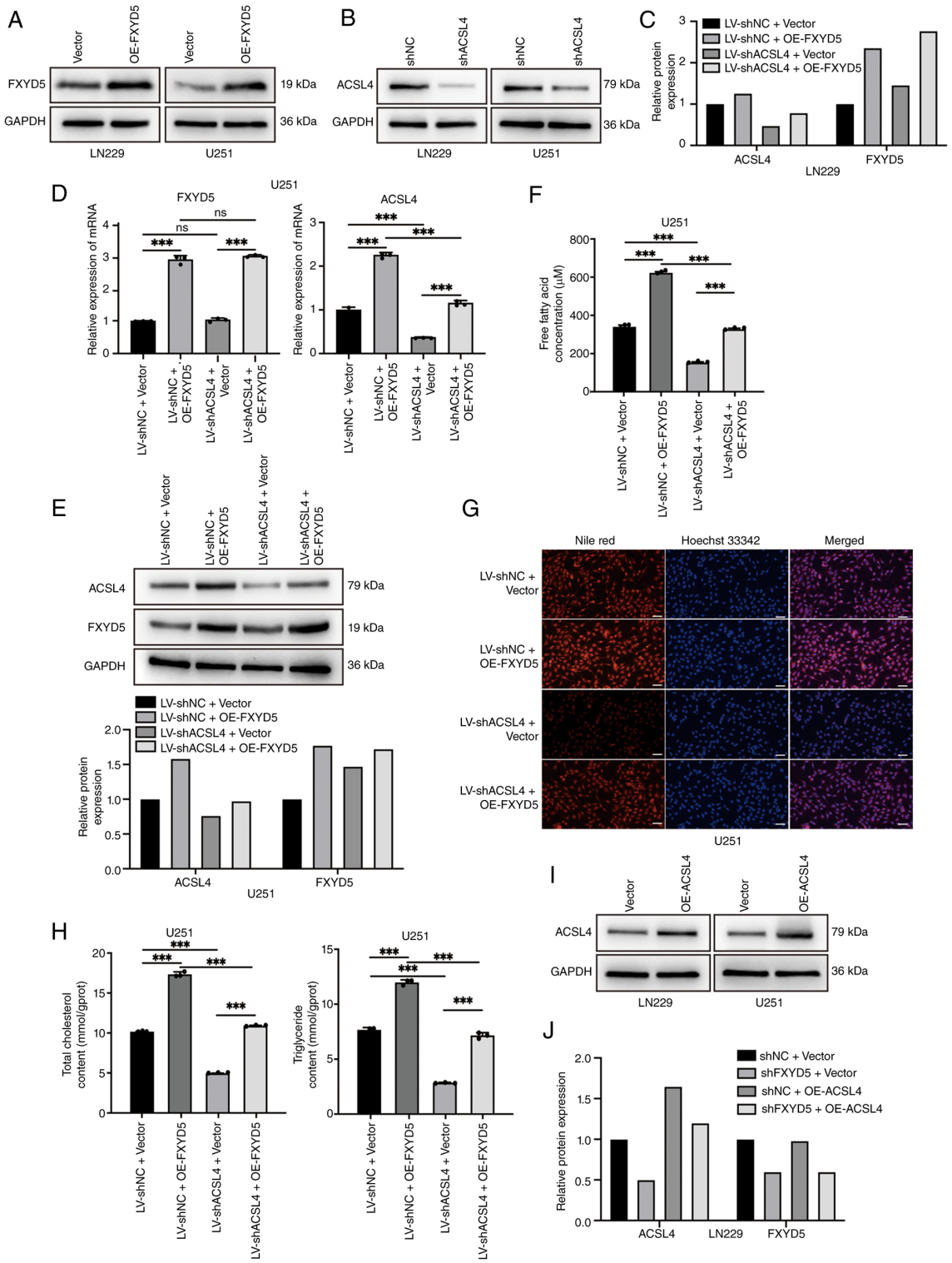

Figure S3. (A) ssGSEA Score of PI3K/AKT pathway between 38 FXYD5-low or 106 FXYD5-high clusters. (B) The bar graph obtained after quantification of Fig. 4C protein bands. (C) Western blot analysis of PI3K, AKT, p-PI3K and p-AKT protein expression in U251 cells after FXYD5 knockdown. The bar graph obtained after quantification of protein bands is in right. (D) Co-immunoprecipitation was detected the contents of FXYD5, PI3K-p85 and PI3K-p110α after overexpression of FXYD5 in U251 cells and LN229 cells. (E) Cell Counting Kit-8 assay examined the relative cell proliferation of U251 cells after overexpression of FXYD5 and using AKT inhibitor. (F) Transwell assays demonstrated the inhibition of migratory and invasive capabilities in U251 cells after overexpression of FXYD5 and using AKT inhibitor. Scale bars, 50 μm. (G) The bar graph obtained after quantification of Fig. 4F protein bands. (H) Western blot analysis of AKT, p-AKT and ACSL4 protein expression in U251 cells after overexpression of FXYD5 and using AKT inhibitor (E)-Akt inhibitor-IV. The bar graph obtained after quantification of protein bands is in right. ***P<0.001. p-, phosphorylated; ACSL4, long-chain acyl-coenzyme A (CoA) synthase 4; LV, lentivirus; sh-, short hairpin; NC, negative control; OE, overexpression; IP, immunoprecipitation; ns, not significant.

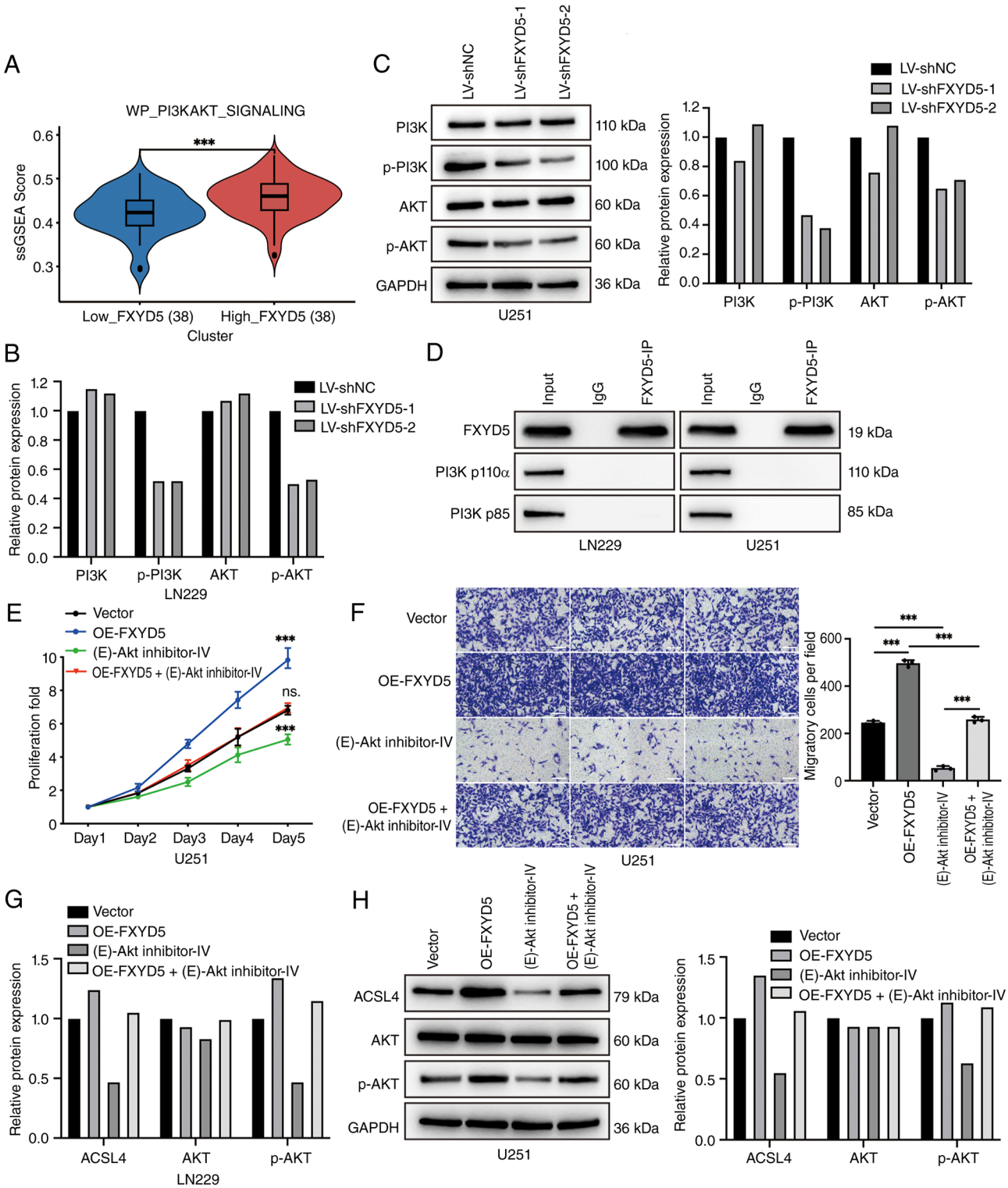

Figure S4. (A) Bar graph obtained after quantification of Fig. 4I protein bands. (B) Cell Counting Kit-8 assay was used to examine the relative cell proliferation of U251 cells after FXYD5 knockdown and overexpression of PIK3CA-H1047R. (C) Transwell assays demonstrated the inhibition of migratory and invasive capabilities in U251 cells after FXYD5 knockdown and overexpression of PIK3CA-H1047R. Scale bars, 50 μm. (D) Immunohistochemical analysis of the negative controls for all three antibodies: isotype-matched non-immune IgG controls for FXYD5, p-AKT and ACSL4. Montage scale bar, 100 μm. **P<0.01 and ***P<0.001. p-, phosphorylated; ACSL4, long-chain acyl-coenzyme A (CoA) synthase 4; sh-, short hairpin; NC, negative control; OE, overexpression.