Figure S1. FXYD5 is upregulated and predicts poor survival in patients with various types of cancer. (A) In the cohort of patients from TCGA, a significant association was identified between mRNA expression level and DNA copy number alteration of FXYD5. (B) In three dependent datasets from Oncomine (http://www.oncomine.org), respectively, FXYD5 mRNA was upregulated in the tumor vs. normal tissue, and lymphatic and distant metastasis positive vs. negative, in patients with EOC. (C) Cross-cancer alterations summary for FXYD5 in 25 studies. Data were analyzed using the cBio Cancer Genomics Portal (http://cbioportal.org). (D) FXYD5 mRNA expression in di□erent tumor types from Oncomine. The graphic compares the number of datasets that had significant FXYD5 mRNA overexpression (left column, red) and underexpression (right column, blue) in tumor tissues vs. normal tissues. The datasets were filtered with the following parameters: P<0.05, >1.5 fold change and top 10% of the gene rank. (E) Oncomine box plots of FXYD5 expression in multiple advanced human malignant tumors. Expression and survival analysis of FXYD5 mRNA expression in (F) subtypes of ovarian cancer and (G) another three types of malignant tumors using Kaplan-Meier plotter (http://kmplot.com/analysis). Student's t-test was used to compare quantitative data between two groups, and one-way ANOVA with Least-Significant Difference post hoc tests were used to compare the means among multiple groups (n>3). The error bars represent the SD. **P<0.01; ***P<0.001. TCGA, The Cancer Genome Atlas; FXYD5, FXYD domain-containing ion transport regulator 5; EOC, epithelial ovarian cancer; HR, hazard ratio.

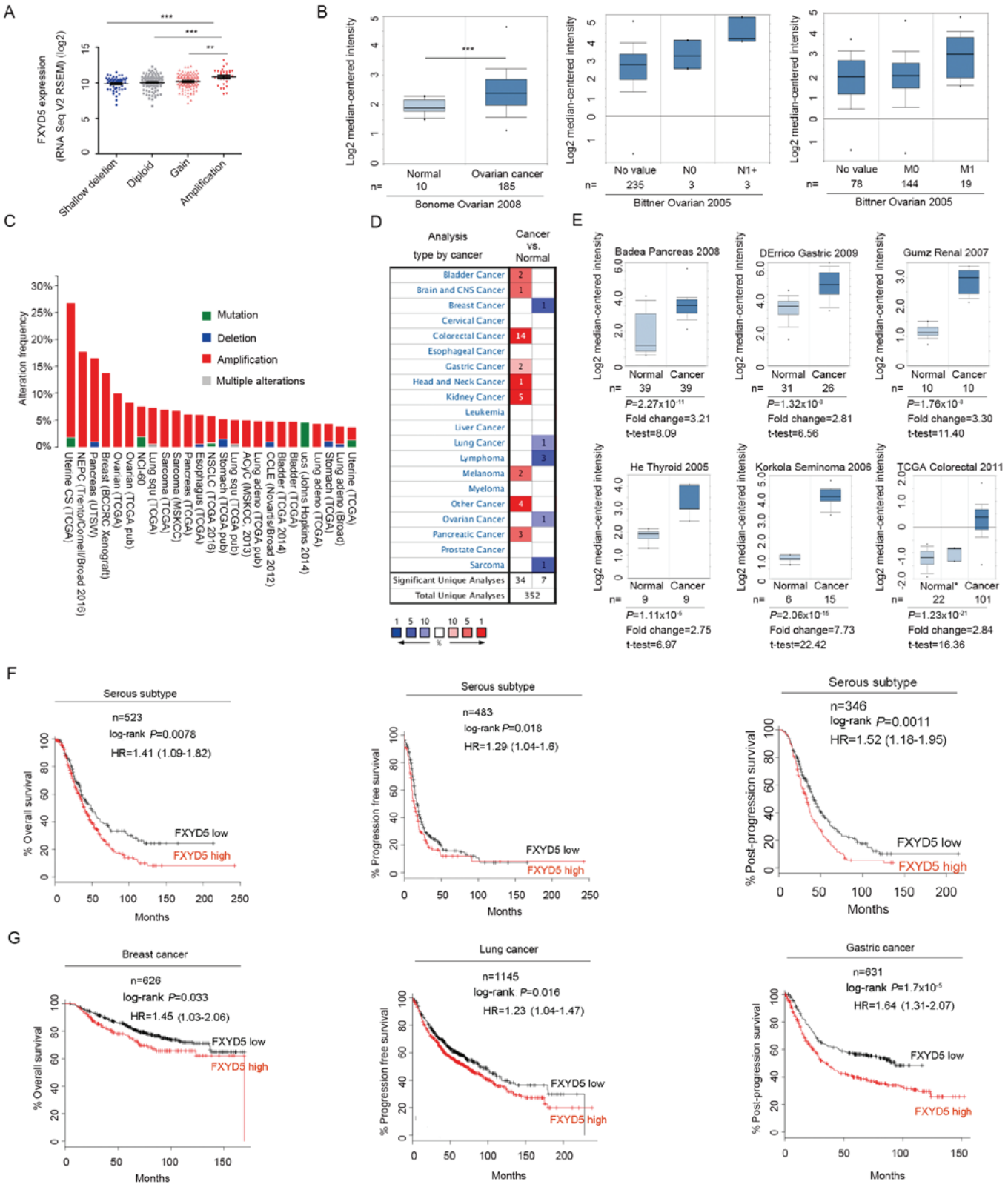

Figure S2. Top 30 GO terms enriched for the differential genes in the RNA sequencing datasets. Global canonical GO analysis. Differential genes of the RNA-sequencing datasets from SKOV3-control and SKOV3-FXYD domain-containing ion transport regulator 5 cell lines were mapped to the GO terms. GO, Gene Ontology; TGF-β, transforming growth factor-β.

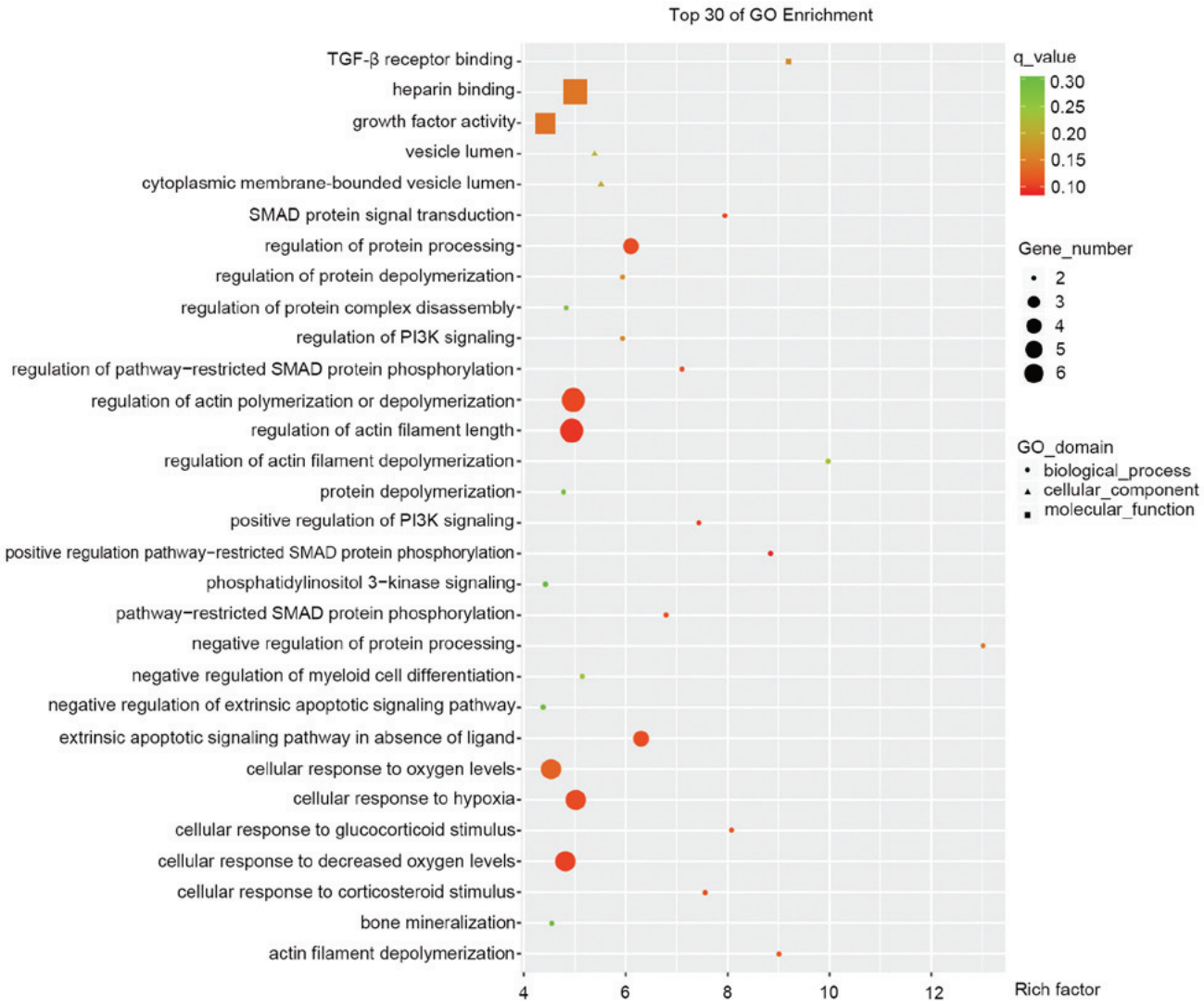

Figure S3. Biological pathway and molecular function enrichment analysis for differential genes. Enrichment analysis of biological pathways for the differentially expressed genes following (A) FXYD5 overexpression and (B) FXYD5 deletion and for (C) co-expression genes with FXYD5 from the TCGA dataset. Enrichment analysis of molecular functions for the differential genes caused by (D) FXYD5 overexpression and (E) FXYD5 deletion and for (F) co-expression genes with FXYD5 from the TCGA dataset. (B) #1, Stabilization and expansion of the E-cadherin adherens junction; #2, E-cadherin signaling in the nascent adherens junction; #3, Posttranslational regulation of adherens junction stability and disassembly. Data analysis was performed utilizing FunRich, an open access standalone functional enrichment and interaction network analysis tool. *P<0.05; **P<0.01; ***P<0.001; FXYD5, FXYD domain-containing ion transport regulator 5; TCGA, The Cancer Genome Atlas; EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; CSI, cellular surface interactions.

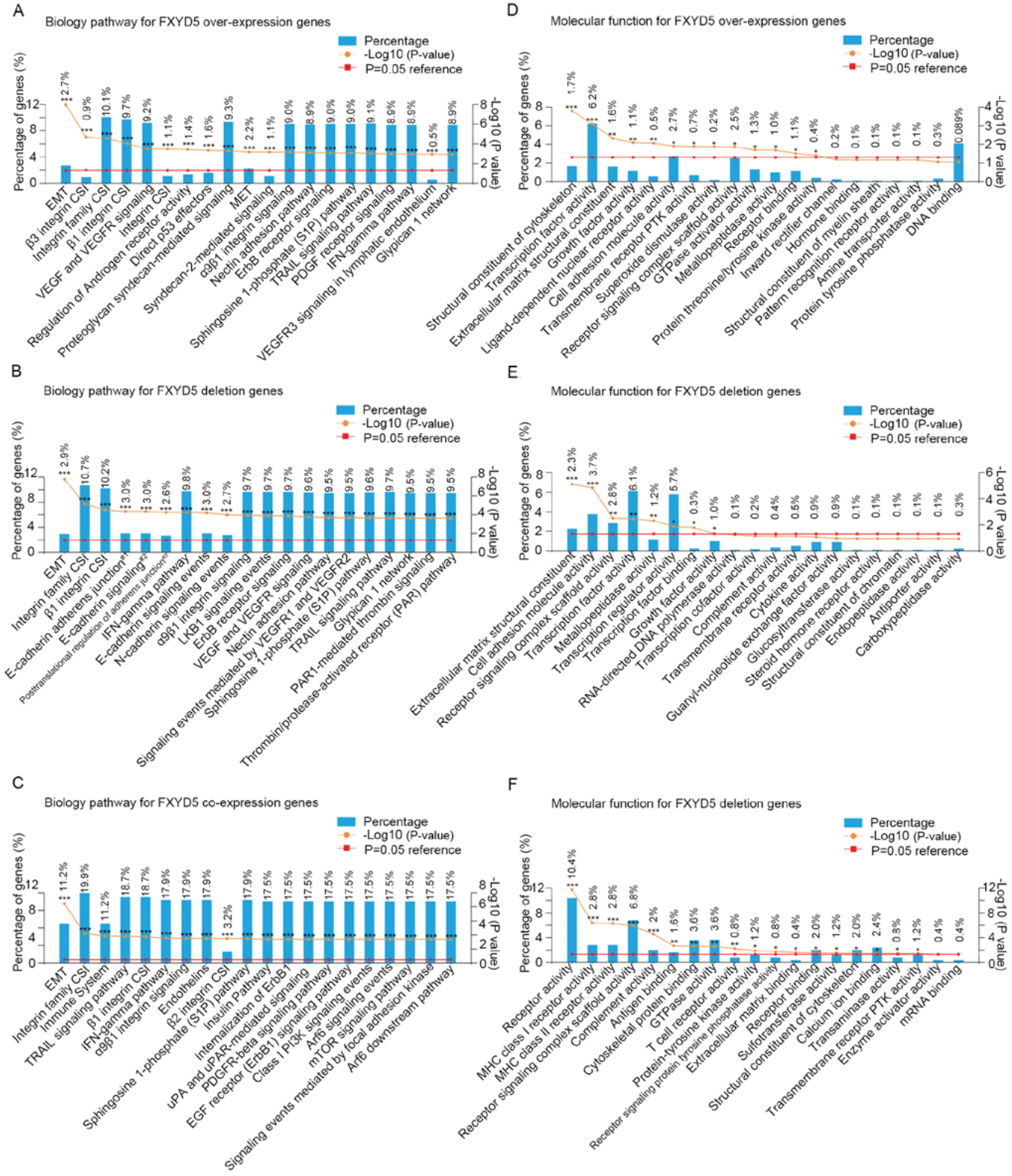

Figure S4. Cellular component and protein domain enrichment analysis for differential genes. Enrichment analysis of cellular component for the differential genes caused by (A) FXYD5 overexpression and (B) FXYD5 deletion, and for (C) co-expression genes with FXYD5 from the TCGA dataset. Enrichment analysis of protein domains for the differential genes caused by (D) FXYD5 overexpression and (E) FXYD5 deletion and for (F) co-expression genes with FXYD5 from TCGA dataset. Data analysis was performed utilizing FunRich, an open access standalone functional enrichment and interaction network analysis tool. *P<0.05; **P<0.01; ***P<0.001; FXYD5, FXYD domain-containing ion transport regulator 5; TCGA, The Cancer Genome Atlas.

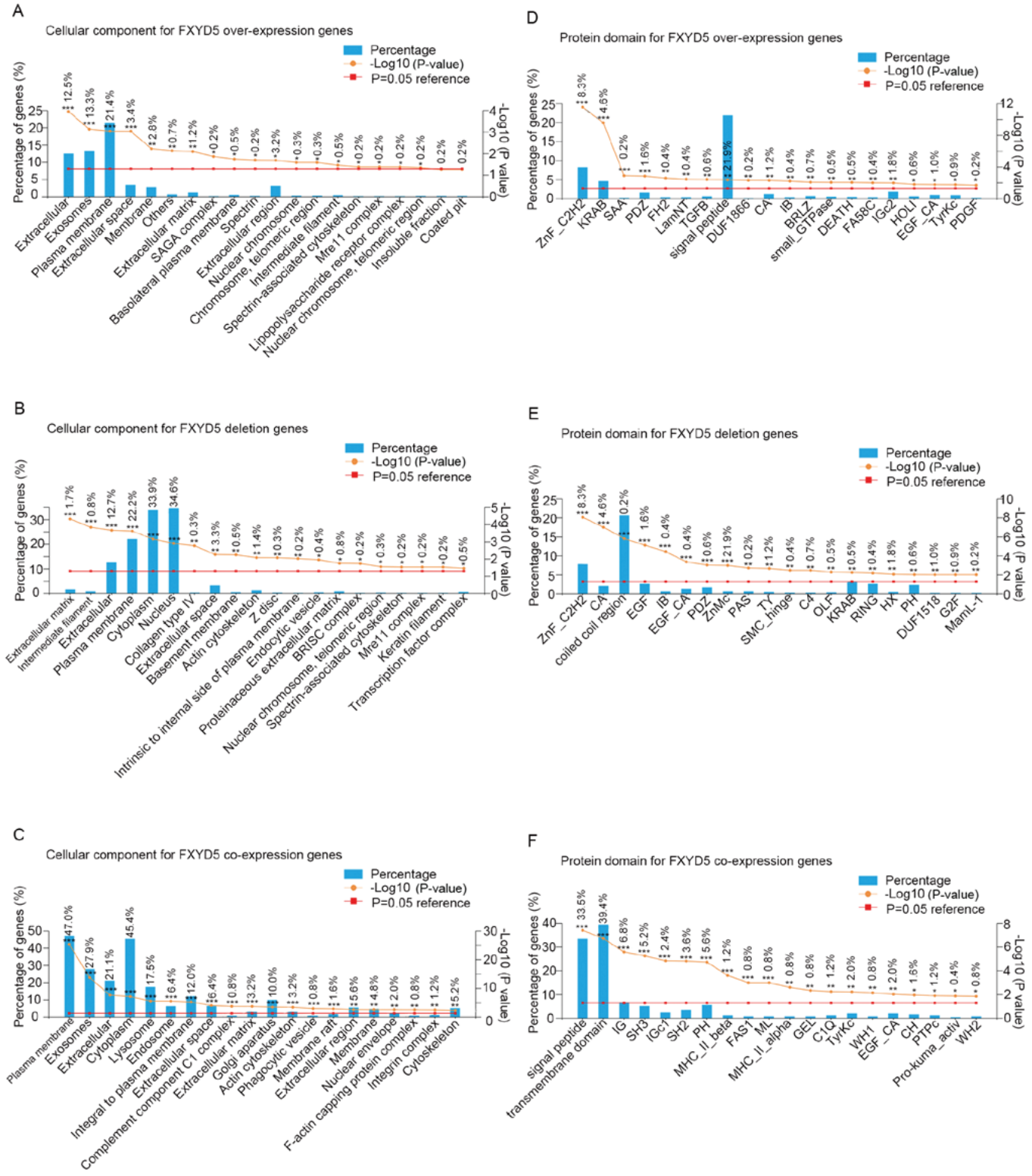

Figure S5. FXYD5 activates the TGF-β/SMADs signaling pathway. (A and B) Migration and invasion abilities of SKOV3 cells following ectopic FXYD5 expression and/or TGF-β treatment were evaluated by Transwell assays *in vitro*. (A) Images of representative fields of migratory and invasive cells. Scale bar, 10 μm. (B) Histograms of the results. (C) WB analysis of the AMPK/ERK and AKT signaling status in the SKOV3 cells following FXYD5 overexpression and in SKOV3-IP cells following FXYD5 deletion. (D) TGFB1 and TGFB1I1, and TGFB1I1 and FXYD5 were co-expressed in two TCGA datasets. Correlation values and P-values were determined using Spearman and Pearson's correlation. (E) FXYD5 mRNA level in SKOV3 cells treated with TGF-β at the indicated concentrations for 48 h. (F) WB analysis of p-SMAD2/3 and total SMAD2/3 expression in SKOV3 cells treated with TGF-β (10 ng/ml) for 60 min to confirm that the signaling pathway could be activated in response to TGF-β. (G) WB analysis of p-SMAD2/3 and total SMAD2/3 expression in SKOV3 cells treated with GW788388 and DMSO for 30 min to confirm that TGF-β signaling could be blocked by GW788388. (H) WB analysis of FXYD5 expression in SKOV3 cells incubated with GW788388 and DMSO for 48 h. (I) WB analysis of FXYD5 expression in SKOV3 cells treated with si-SMAD2 in the absence or presence of TGF-β for 48 h. Student's t-test was used to compare quantitative data between two groups, and one-way ANOVA with (E) Dunnett's or (B) Least-Significant Difference post hoc tests were used to compare the means among multiple groups (n>3). The error bars represent the SD. *P<0.05; ***P<0.001; ns, no significant difference. DMSO, dimethyl sulfoxide; TGF-β, transforming growth factor-β; FXYD5, FXYD domain-containing ion transport regulator 5; WB, western blotting; AMPK, protein kinase AMP-activated catalytic subunit alpha 2; TCGA, The Cancer Genome Atlas; p-, phosphorylated-; si, small interfering RNA; TGFB1I1, TGF-β-induced transcript 1.