ING5 inhibits aerobic glycolysis of Lung cancer cells by promoting TIE1-mediated phosphorylation of pyruvate dehydrogenase kinase 1 (PDK1) at Y163

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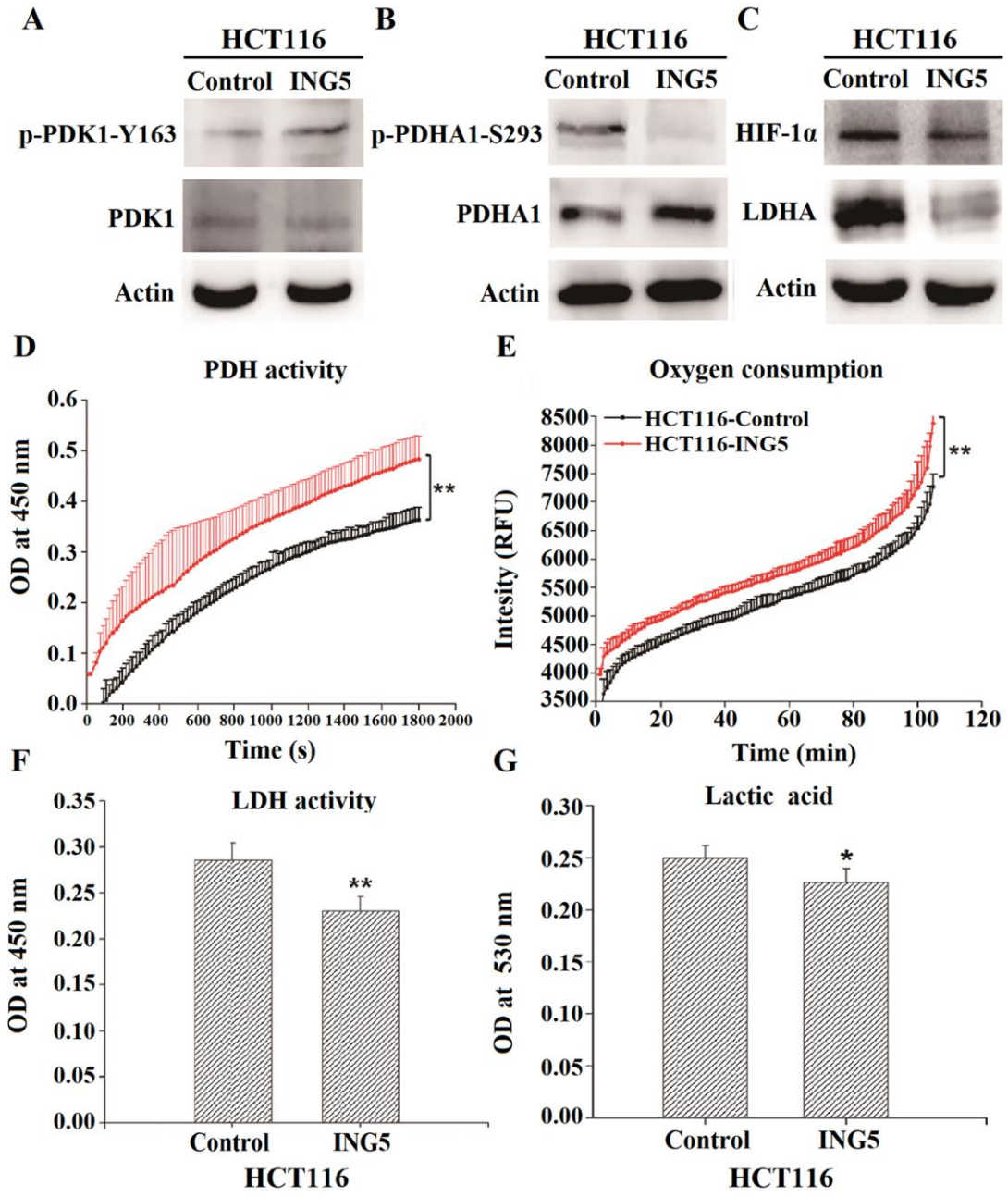

Fig. S1 ING5 promoted PDK1 (Y163) phosphorylation negatively regulates its kinase activity which leads to dephosphorylation and activation of PDHA1 enzyme and inhibition of Warburg effect in colorectal HCT116 cells. A Phosphorylation of PDK1 Y163 is up-regulated in ING5 overexpressing HCT116 (ING5) cells by western blot. Actin was used as an internal loading control. B ING5 overexpression

decreased phosphorylation of PDHA1 S293 by western blot. Actin was used as an internal loading control. C ING5 overexpression downregulated HIF-1α and LDHA1 by western blot. Actin was used as an internal loading control. D ING5 overexpression increased the enzyme activity of PDH. Data are shown as the mean plus standard error of three independent experiments. $^{**}P < 0.01$ compared to Control group ($P = 0.0062$). E ING5 overexpression enhanced oxygen consumption. Data are shown as the mean plus standard error of three independent experiments. $^{**}P < 0.01$ compared to Control group ($P = 0.0076$). F ING5 overexpression inhibited LDH activity. Data are shown as the mean plus standard error of three independent experiments. $^{**}P<0.01$ compared to Control group ($P = 0.0081$). G ING5 overexpression decreased lactate production. Data are shown as the mean plus standard error of three independent experiments. $^{*}P < 0.05$ compared to Control group ($P = 0.0116$). (n = 3 samples).

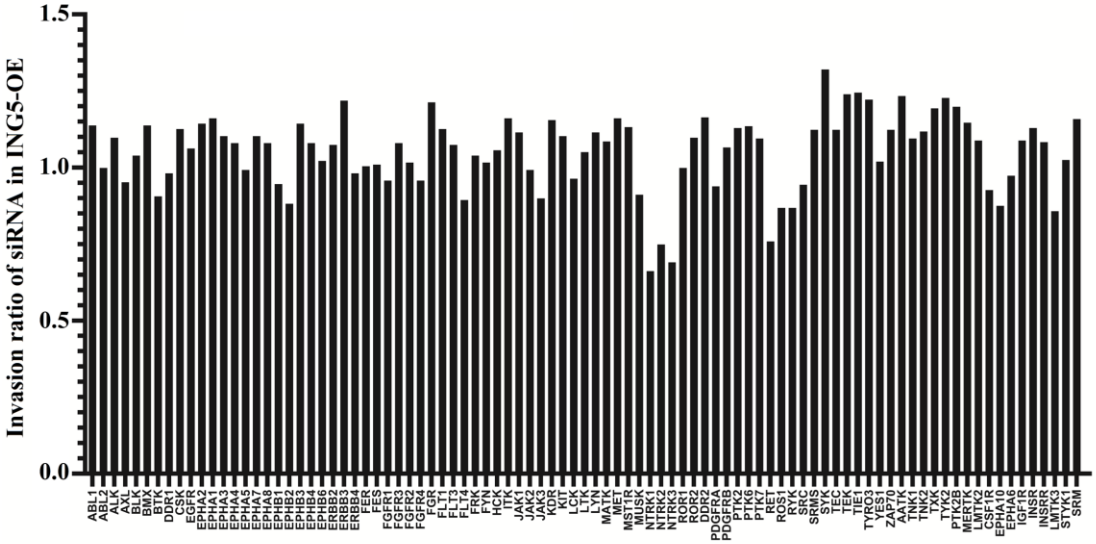

Fig. S2 High-throughput tyrosine kinase siRNA screening combined with high-content functional analysis in A549 cells with ING5 overexpression. The results of invasion assay with siRNA transfected A549 cells overexpressing ING5.

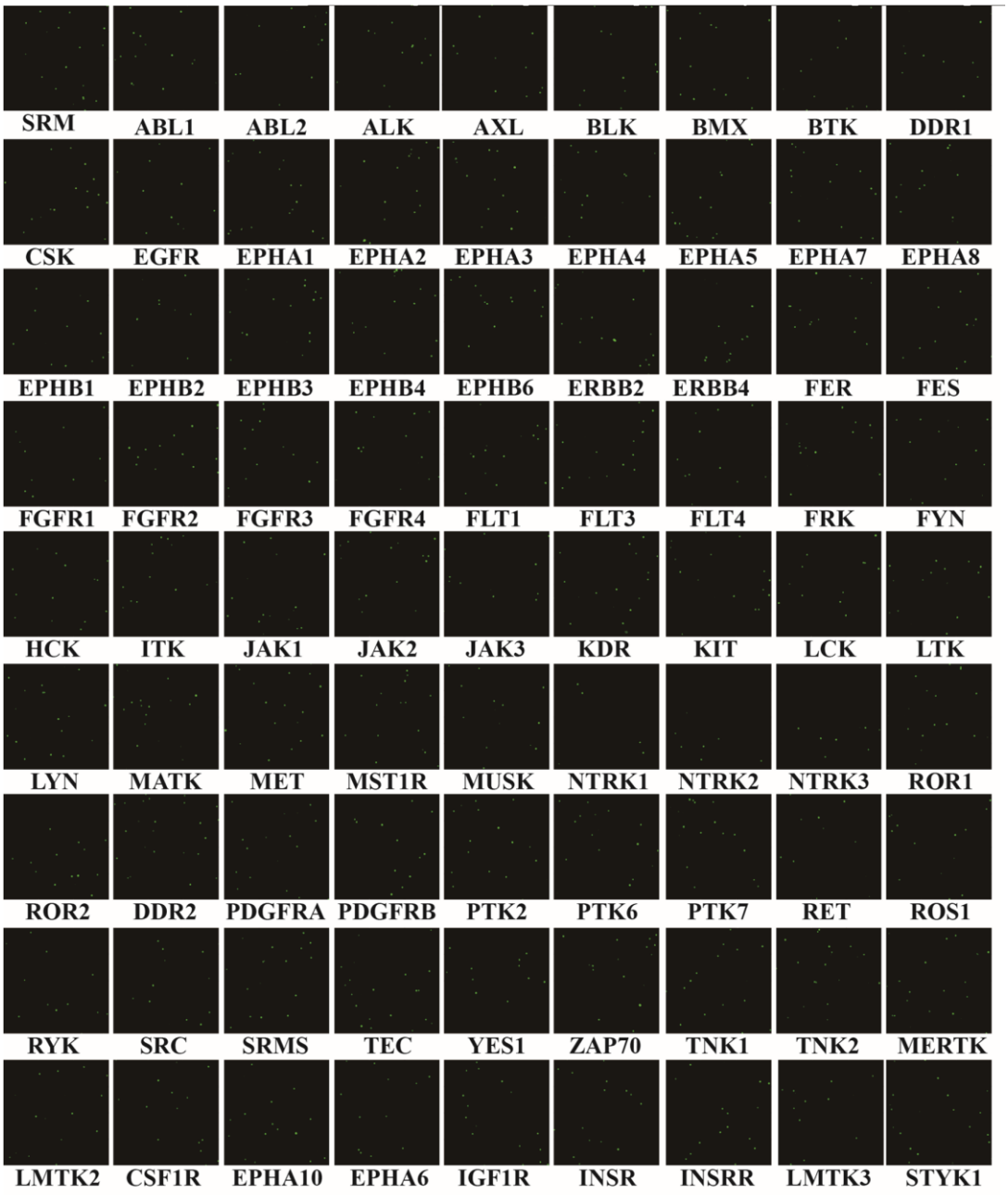

Fig. S3 Tyrosine protein kinase siRNA library-based high content screening of invasion assay. Representative images of invasion results of ING5-overexpressing A549 cells with knockdown of targeted genes.

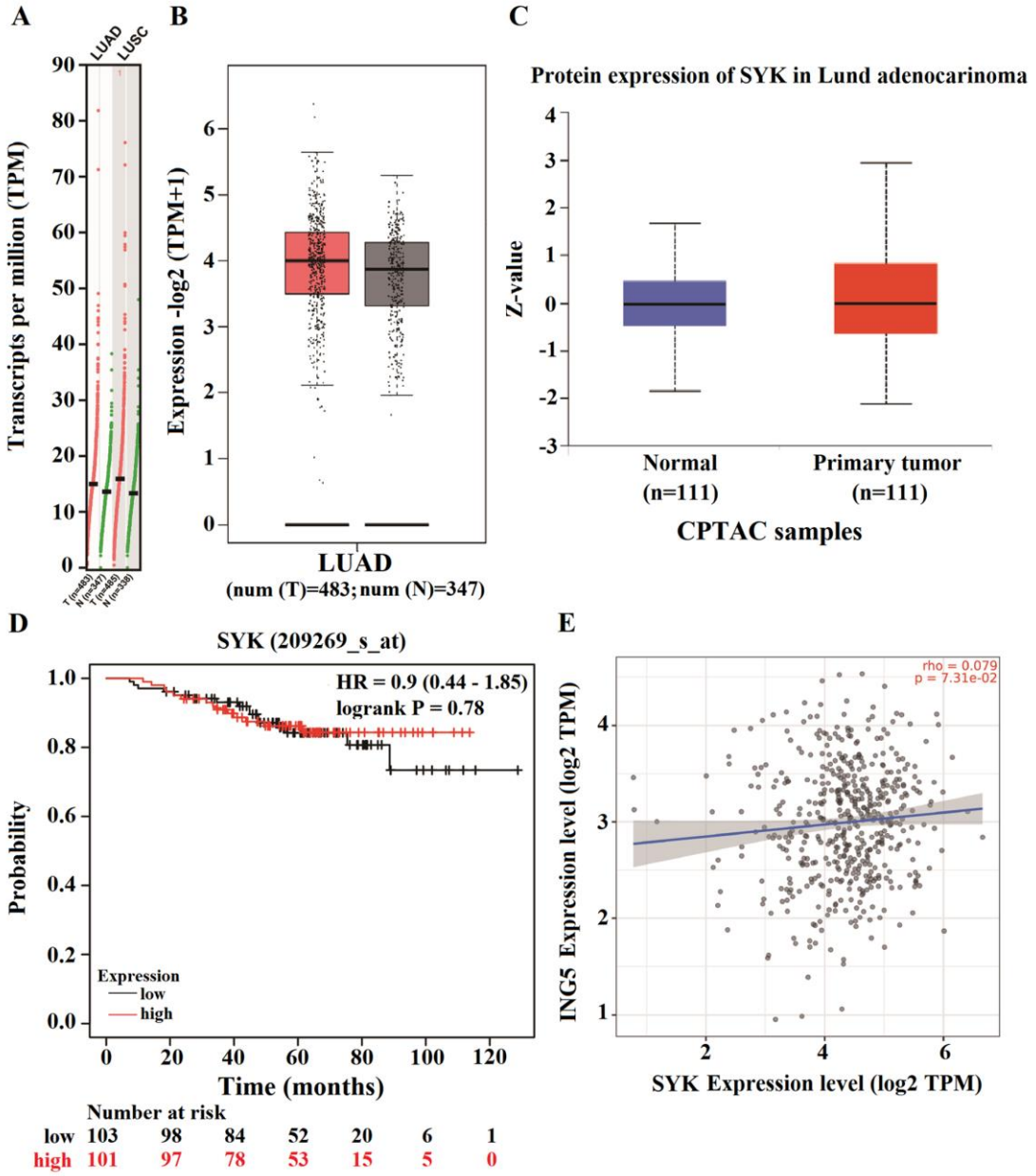

Fig. S4 The expression of SYK had no significant difference between normal and LUAD samples. A GEPIA2 database showed that SYK level had no significant difference compared with the normal sample in LUAD and LUSC. B SYK mRNA level had no significant difference between normal control and LUAD samples. C With the CPTAC database, no significant difference in SYK protein expression was shown between normal and LUAD tissues (P = 0.22). D K-M plotter database showed that the overall survival of the patients with high expression of SYK

(Affymetrix ID: 209269_s_at) has no difference with those with low SYK expression (HR = 0.9, 95% CI = 0.44-1.85, *P* = 0.78). E The TIMER database showed that the expression of SYK was not correlated with ING5 expression (*r* = 0.079, *P* = 0.07) by Spearman's rho value. (n = 3 samples). Statistical analysis was performed by one-way ANOVA. ns vs. NC group.

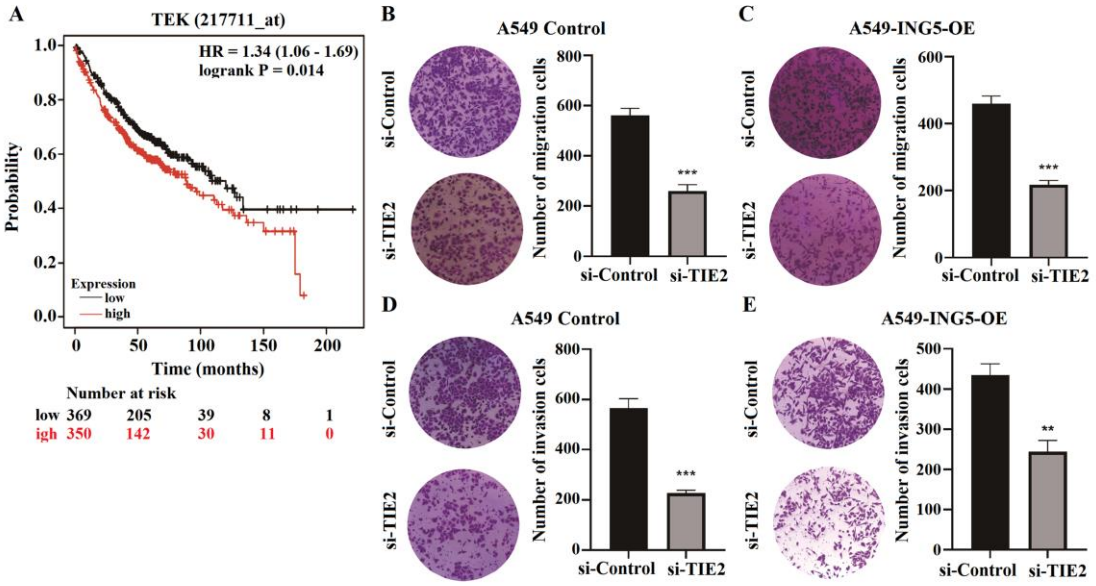

Fig. S5 High expression of TIE2 correlates with poor survival in lung cancer patients and TIE2 promotes migration and invasion of A549 control and ING5 overexpression cells. A Kaplan-Meier database showed the overall survival analysis of TIE2 (TEK, Affy ID: 217711_at) expression in patients with LUAD samples (n = 719, *P* = 0.014, HR = 1.34, 95% CI = 1.06-1.69, log-rank test). B and C si-TIE2 A549 cells and si-TIE2 A549 overexpression cells were subjected to migration assays. D and E Representative images and results of invasion assays using si-TIE2 A549 cells and si-TIE2 A549 ING5 overexpression cells. (n = 3 samples).

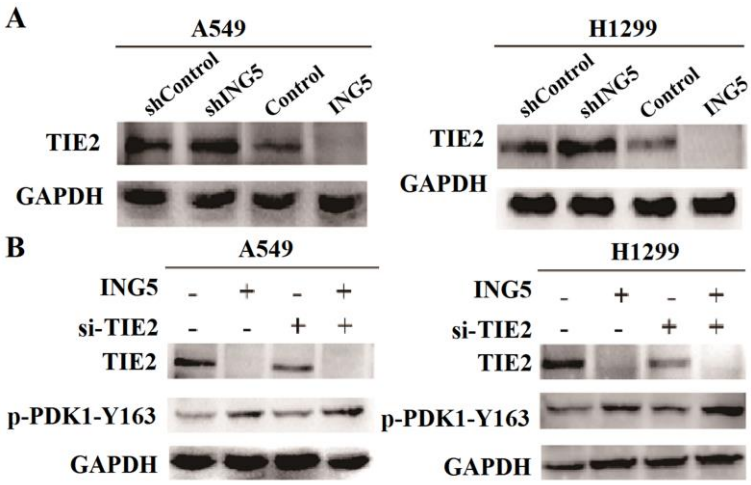

Fig. S6 ING5 inhibits TIE2 expression, and Knockdown of TIE2 by siRNA led to increased expression of p-PDK1-Y163 in lung cancer cells. A Western blot showed that TIE2 was up-regulated in ING5 knockdown lung cancer cells, while down-regulated in ING5 overexpression lung cancer cells. B si-TIE2 led to decreased expression of TIE2, while increased p-PDK1-Y163 in lung cancer cells. (n = 3 samples).