Figure S1. RACK1 is highly expressed in cervical cancer and regulates SiHa cell migration and invasion *in vitro*. (A) Expression of RACK1 in CC and normal cervical epithelial tissues from the GEO database. (B) Expression of RACK1 in CC with and without LNM from the GEO database. (C) Transwell assays were performed to investigate the effects of RACK1 on the invasion and migration of SiHa cells (left panel), with the quantified bands assessed (right panel). Data were analyzed using the Student's t-test and are presented as the mean ± SD. Data were compared with the shNON group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001). RACK1, receptor for activated C kinase 1; GEO, Gene Expression Omnibus.

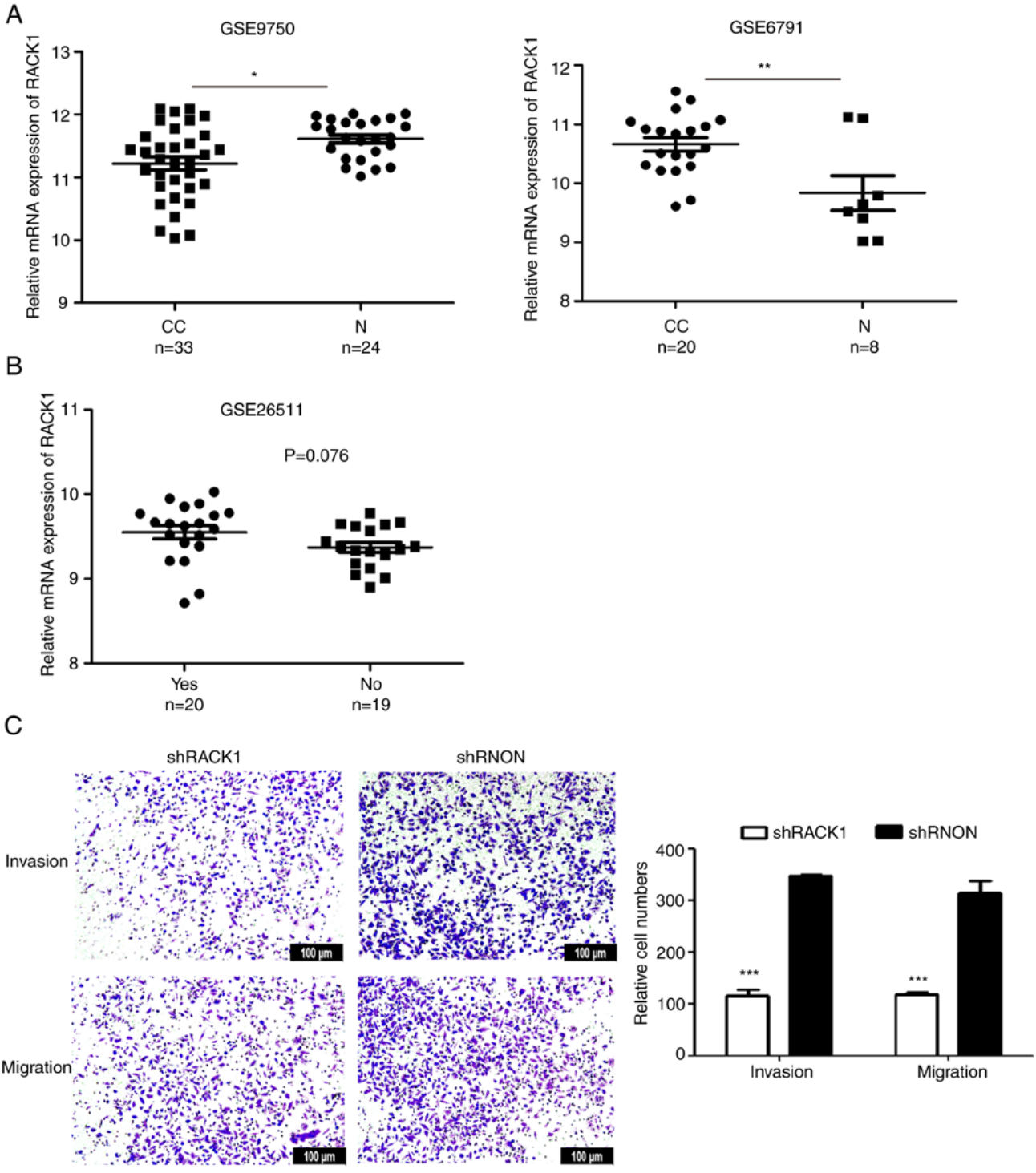

Figure S2. RACK1 interacts with IGF1R and promotes glycolysis and aggressiveness by activating IGF1R/AKT/mTOR signaling in SiHa cells. (A) Co-IP assays were used to detect the interaction between RACK1 and IGF1R in SiHa cells (left panel). Co-IP assay was used to detect the interaction between RACK1 and IGF1R in shRACK1/SiHa and shNON/SiHa cells (right panel). (B) shRACK1/SiHa cells was stimulated for different time periods (6, 12 and 24 h) with 200 ng/ml IGF1. The RACK1, p-AKT (ser472 + ser474 + ser473), p-mTOR (ser2448), AKT, mTOR, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression was examined using western blot analysis. (C) Effect of IGF1 and IGF1 combined with Rapa on glucose uptake and lactate production in SiHa cells. (D) shRACK1/SiHa cells were stimulated for 24 h with 200 ng/ml IGF1 and various concentrations (10 and 20 nM) of Rapa. The expression of RACK1, p-mTOR(ser2448), mTOR, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin was examined using western blot analysis. (E) Transwell assays were performed to investigate the effects of IGF1 and IGF1 combined with Rapa on the invasion and migration of SiHa cells (right panel), with the quantified bands assessed (left panel). Data were analyzed using one-way ANOVA and are presented as the mean ± SD. Data were compared with the shRACK1 group ($^{***}$P<0.001) or the shRACK1 + IGF1 group ($^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001). RACK1, receptor for activated C kinase 1; mTOR, mammalian target of rapamycin; GLUT1, glucose transporter 1; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; PKM2, pyruvate kinase M2; Rapa, rapamycin; IGF1, insulin-like growth factor 1.

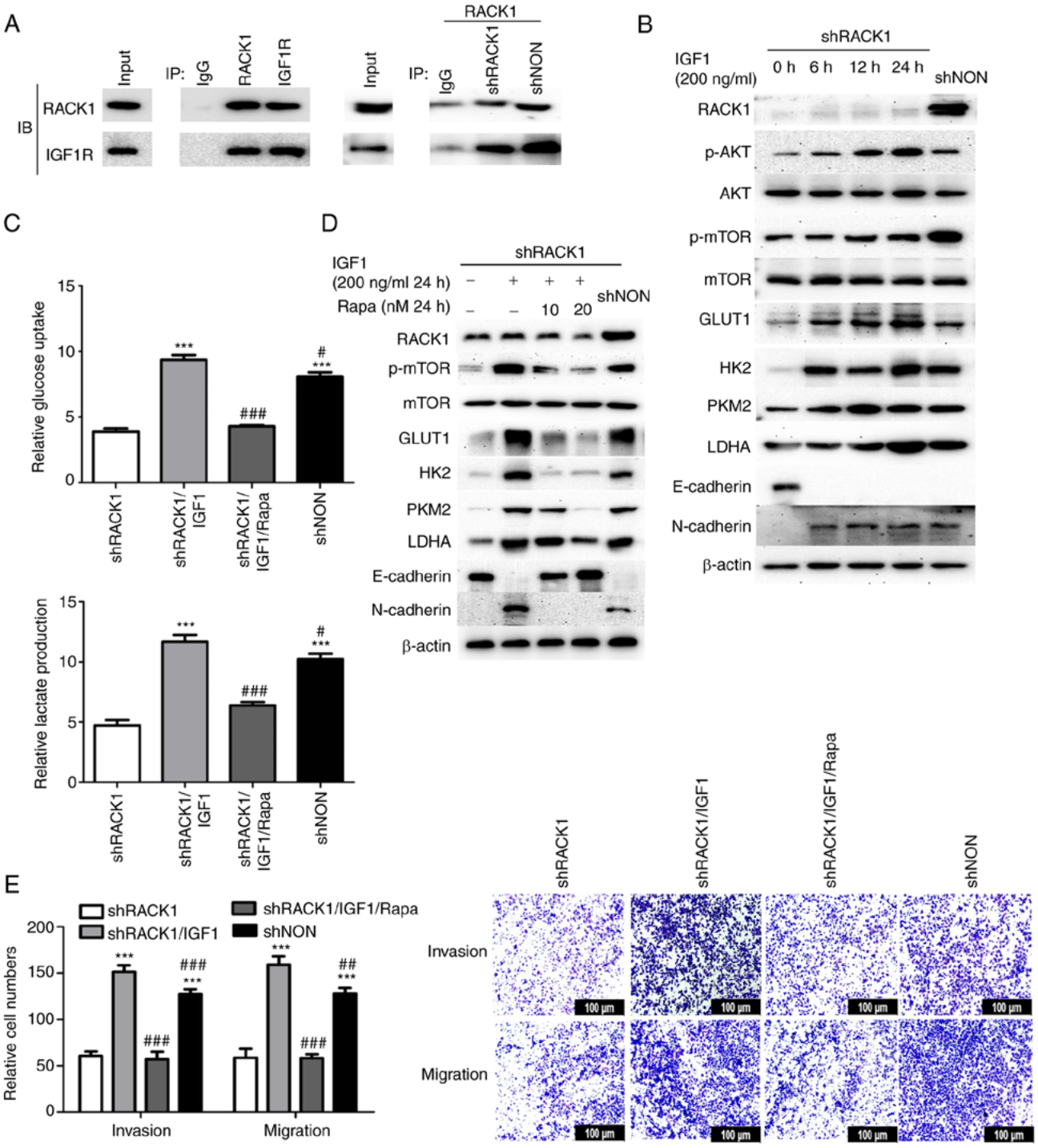

Figure S3. (A) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression of MS751 cells following treatment for different time periods (6, 12 and 24 h) with 200 ng/ml IGF1. (B) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression of MS751 cells following stimulation for 24 h with 200 ng/ml IGF1 and various concentrations (100 and 200 nM) of Rapa. (C) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression following transfection with POU2F2 overexpression lentivirus into shRACK1/MS751 cells. Data were analyzed using one-way ANOVA and are presented as the mean ± SD. Data were compared with the shRACK1 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001) or shRACK1 + IGF1 group ($^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001) or shRACK1/NC POU2F2 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001). RACK1, receptor for activated C kinase 1; mTOR, mammalian target of rapamycin; GLUT1, glucose transporter 1; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; PKM2, pyruvate kinase M2; Rapa, rapamycin; IGF1, insulin-like growth factor 1.

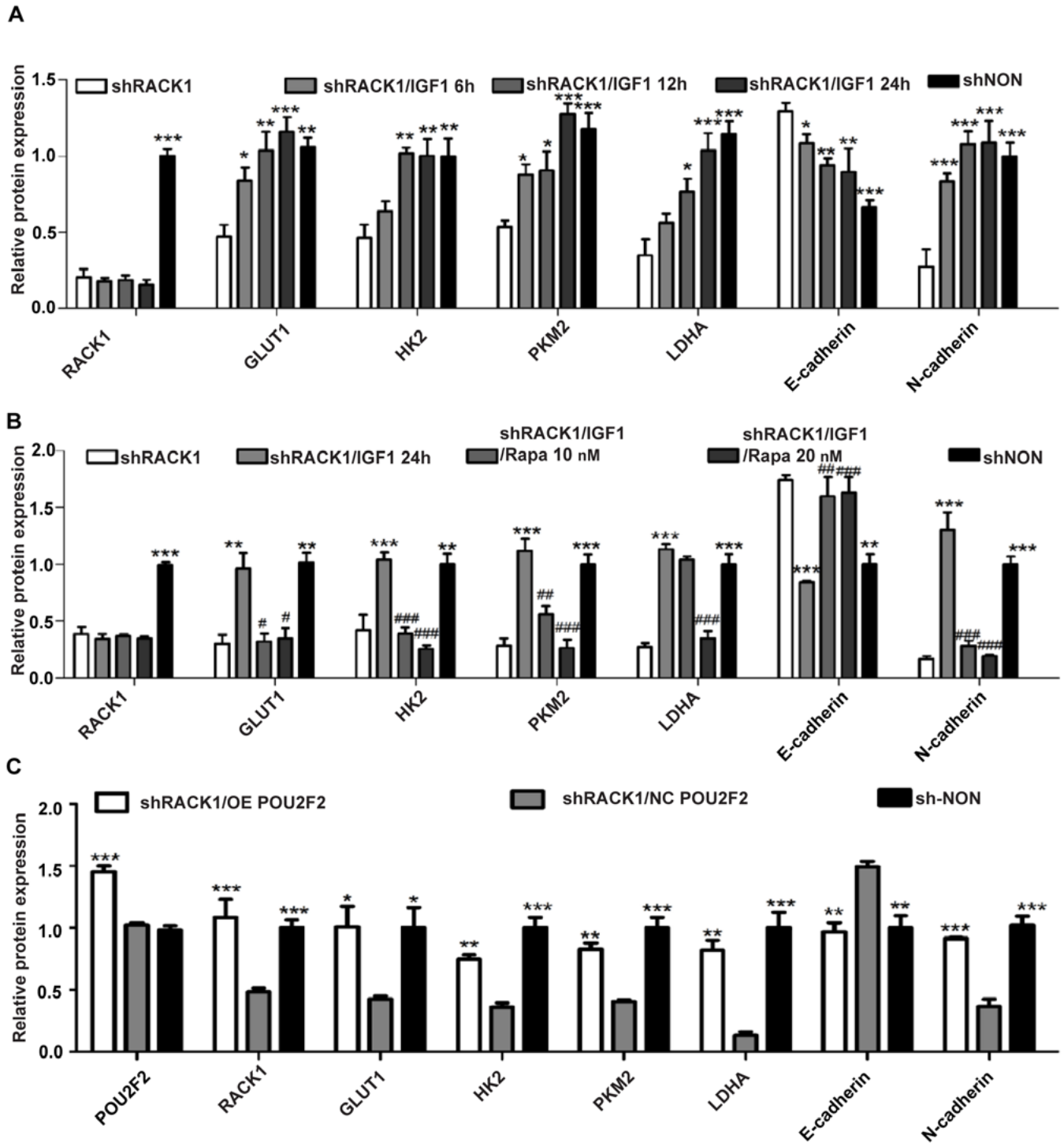

Figure S4. (A) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression of SiHa cells following treatment for different time periods (6, 12 and 24 h) with 200 ng/ml IGF1. (B) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression of SiHa cells following stimulation for 24 h with 200 ng/ml IGF1 and various concentrations (10 and 20 nM) of Rapa. (C) Graph with the semi-quantification of RACK1, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin expression after transfected POU2F2 overexpression lentivirus into shRACK1/SiHa cells. Data were analyzed using one-way ANOVA and are presented as the mean ± SD. Data were compared with the shRACK1 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001) or shRACK1 + IGF1 group ($^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001) or shRACK1/NC POU2F2 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001). RACK1, receptor for activated C kinase 1; mTOR, mammalian target of rapamycin; GLUT1, glucose transporter 1; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; PKM2, pyruvate kinase M2; Rapa, rapamycin; IGF1, insulin-like growth factor 1.

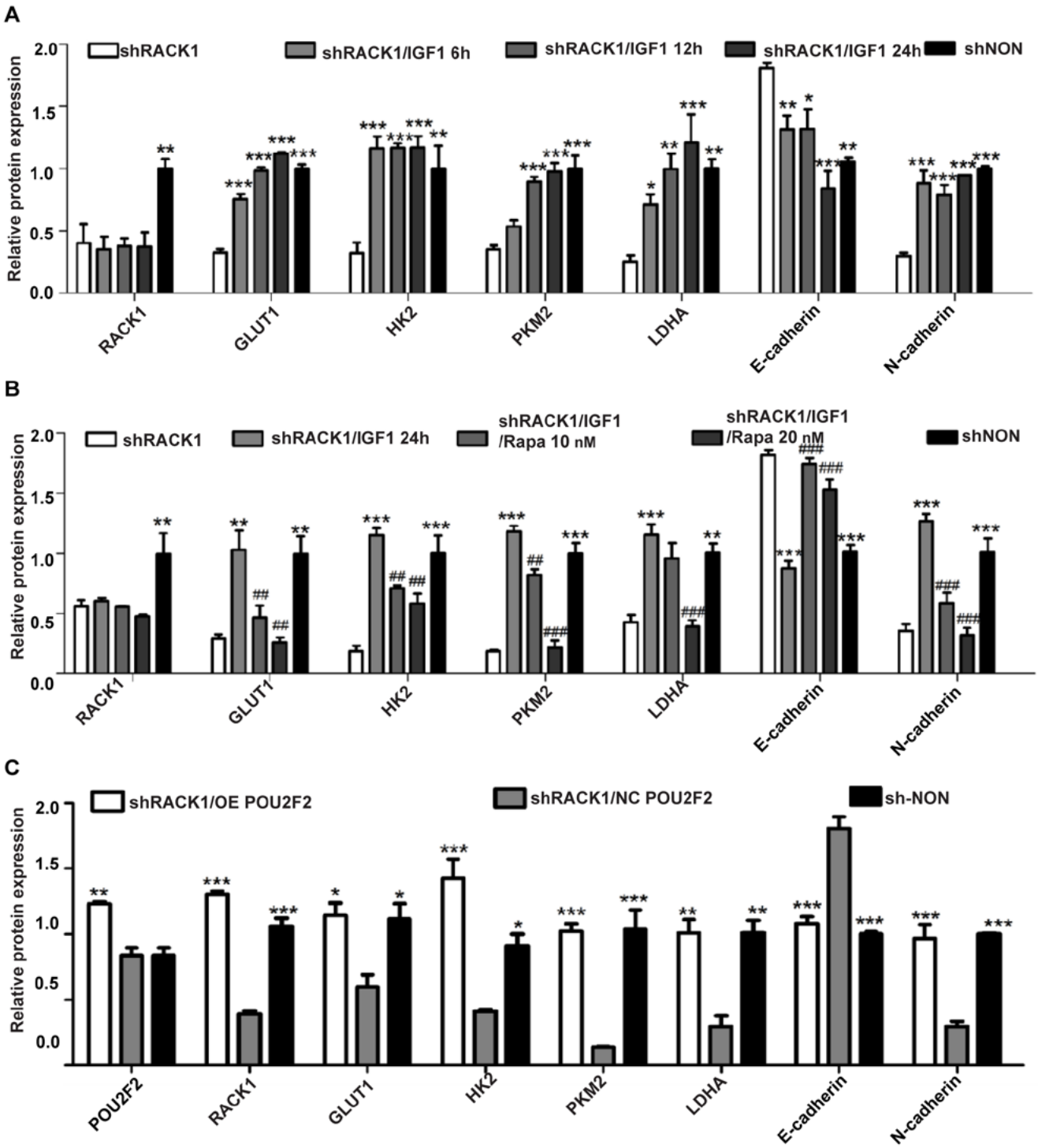

Figure S5. (A) Graphs with the ratios of p-AKT (ser472 + ser474 + ser473)/AKT and p-mTOR (ser2448)/mTOR in MS751 and SiHa cells transfected with specific shRACK1 lentiviral vectors (shRACK1-1 and shRACK1-2). (B) Graphs with the ratios of p-AKT (ser472 + ser474 + ser473)/AKT and p-mTOR (ser2448)/mTOR in MS751 and SiHa cells following treatment for different periods (6, 12 and 24 h) with 200 ng/ml IGF1. (C) Graph with the ratios of p-AKT (ser472 + ser474 + ser473)/AKT and p-mTOR (ser2448)/mTOR in MS751 and SiHa cells following stimulation for 24 h with 200 ng/ml IGF1 and various concentrations (10 and 20 nM) of Rapa. (D) Graphs with the ratios of p-AKT (ser472 + ser474 + ser473)/AKT and p-mTOR (ser2448)/mTOR following transfection with POU2F2 overexpression lentivirus into shRACK1/MS751 and shRACK1/SiHa cells. Data were analyzed using one-way ANOVA and are presented as the mean ± SD. Data were compared with the shRACK1 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001). or shRACK1 + IGF1 group ($^{\#}$P<0.05 and $^{\#\#}$P<0.01) or NC POU2F2/shRACK1 group ($^{**}$P<0.01 and $^{***}$P<0.001). RACK1, receptor for activated C kinase 1; IGF1R, insulin-like growth factor 1 receptor; POU2F2, POU class 2 homeobox 2.

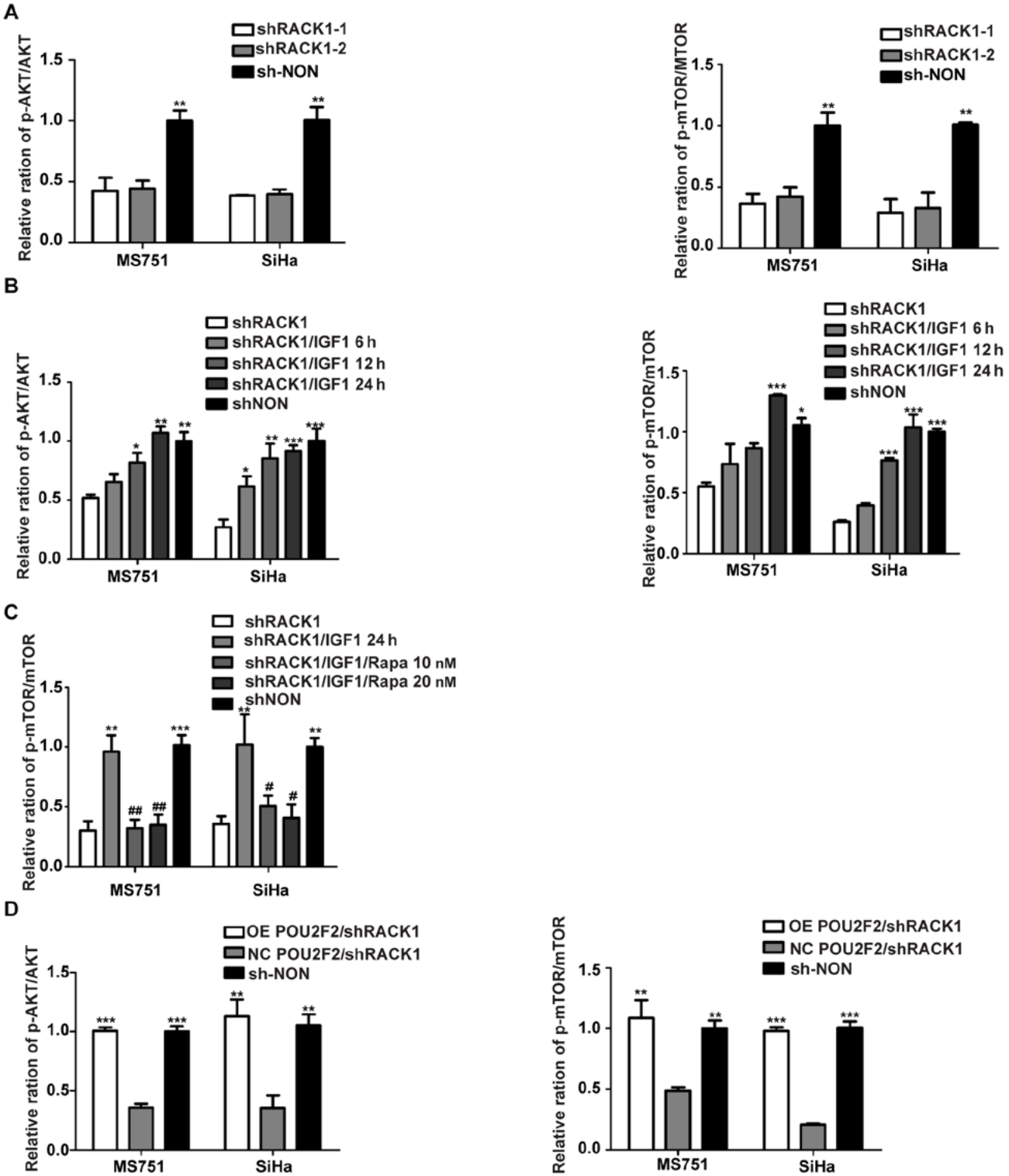

Figure S6. RACK1 is a direct target of POU2F2. (A) Luciferase reporter assays of WT and MUT RACK1 luciferase reporters transfected with POU2F2 in SiHa cells. (B) ChIP-PCR assay to detect POU2F2-binding sites in the sequence of the RACK1 promoter. (C) POU2F2 expression in MS751 and SiHa cells transfected with POU2F2 overexpression plasmid (OE POU2F2) and control plasmid (NC POU2F2) was examined using western blot analysis. (D) Graph with the semi-quantification of POU2F2 expression of MS751 and SiHa cells transfected with POU2F2 overexpression plasmid (OE POU2F2) and control plasmid (NC POU2F2). (E) POU2F2 expression in MS751 and SiHa cells transfected with POU2F2 overexpression plasmid (OE POU2F2) and control plasmid (NC POU2F2) was analyzed using reverse transcription-quantitative PCR. (F) Effect of POU2F2 on the expression of RACK1, p-AKT (ser472 + ser474 + ser473), p-mTOR (ser2448), AKT, mTOR, GLUT1, HK2, PKM2, LDHA, E-cadherin and N-cadherin in shRACK1/SiHa cells, as evaluated using western blot analysis. (G) Effect of POU2F2 on glucose uptake and lactate production in shRACK1/SiHa cells. (H) POU2F2 OE/shRACK1 cells were stimulated for 24 h with 10 mM 2-DG. Effect of POU2F2 and 2-DG combined with POU2F2 on the invasion and migration of shRACK1/SiHa cells (left panel), with the quantified bands assessed (right panel). Data were analyzed using one-way ANOVA and are presented as the mean ± SD. Data were compared with the shRACK1 group ($^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001) or with the NC POU2F2/shRACK1 group ($^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001). RACK1, receptor for activated C kinase 1; POU2F2, POU class 2 homeobox 2; WT, wild-type; MUT, mutant type; 2-DG, 2-deoxy-D-glucose; mTOR, mammalian target of rapamycin; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; PKM2, pyruvate kinase M2; OE, overexpression.

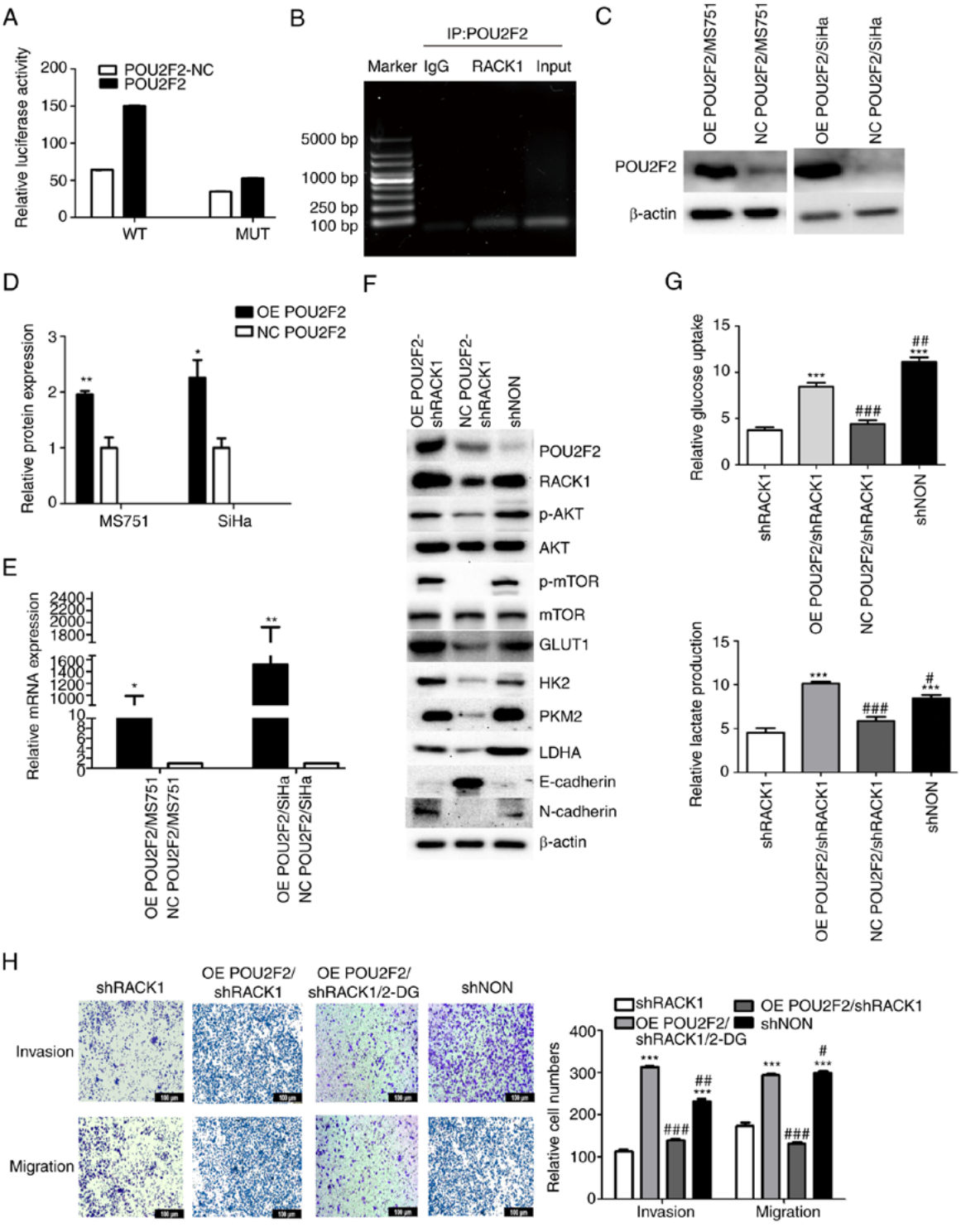

Figure S7. RACK1 enhances the LNM of SiHa cells *in vivo*. (A) Representative images of inguinal LNs in different groups of nude mice (n=8, left panel). Representative images of inguinal lymph nodes of H&E staining in different groups of nude mice (middle panel). Representative images of anti-GFP IHC analysis for inguinal lymph nodes in different groups of nude mice (right panel). (B) Representative images of footpads primary tumor in different groups of nude mice (left panel). Representative images of footpads primary tumor tissues of H&E staining (middle panel) and percentages of PDPN-indicated lymphatic vessels density in different groups of nude mice (right panel). (C) Representative images of RACK1, GLUT1, PKM2, HK2, LDHA, E-cadherin and N-cadherin expressions of footpad primary tumor tissues in IHC analysis. (D) Image of all lymph nodes with a ruler. (E) The bar graph summarizes the tumor volume assessed (left panel), and lymph node volume assessed (right panel). Data are presented as the mean ± SD and were analyzed using the t-test. Data were compared with the shNON group ($^{**}P<0.01$ and $^{***}P<0.001$). IHC, immunohistochemistry; RACK1, receptor for activated C kinase 1; LNM, lymph node metastasis; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; PKM2, pyruvate kinase M2; PDPN, podoplanin.

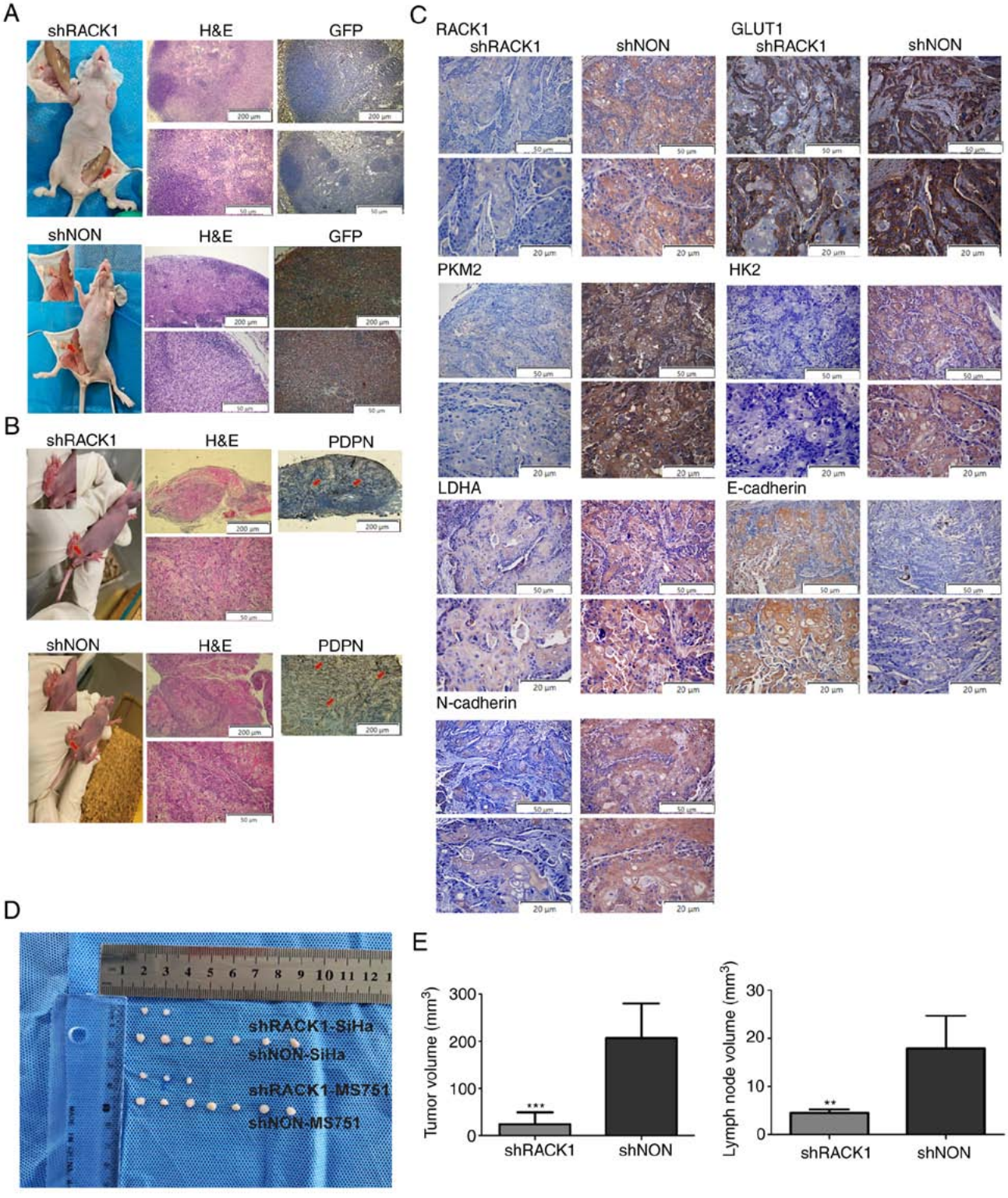

Figure S8. RACK1 expression correlates with IGF1R, POU2F2 and HK2 expression in tissues from patients with CC. (A) Location of RACK1 in cervical cancer and normal cervical epithelial tissues. (B) Spearman's correlation analysis of RACK1 and IGF1R expression in CC tissues. (C) Spearman's correlation analysis of RACK1 and HK2 expression in CC tissues. (D) Spearman's correlation analysis of RACK1 and POU2F2 expression in CC tissues. CC, cervical cancer; NC, normal control; RACK1, receptor for activated C kinase 1; HK2, hexokinase 2; IGF1R, insulin-like growth factor 1 receptor; POU2F2, POU class 2 homeobox 2.