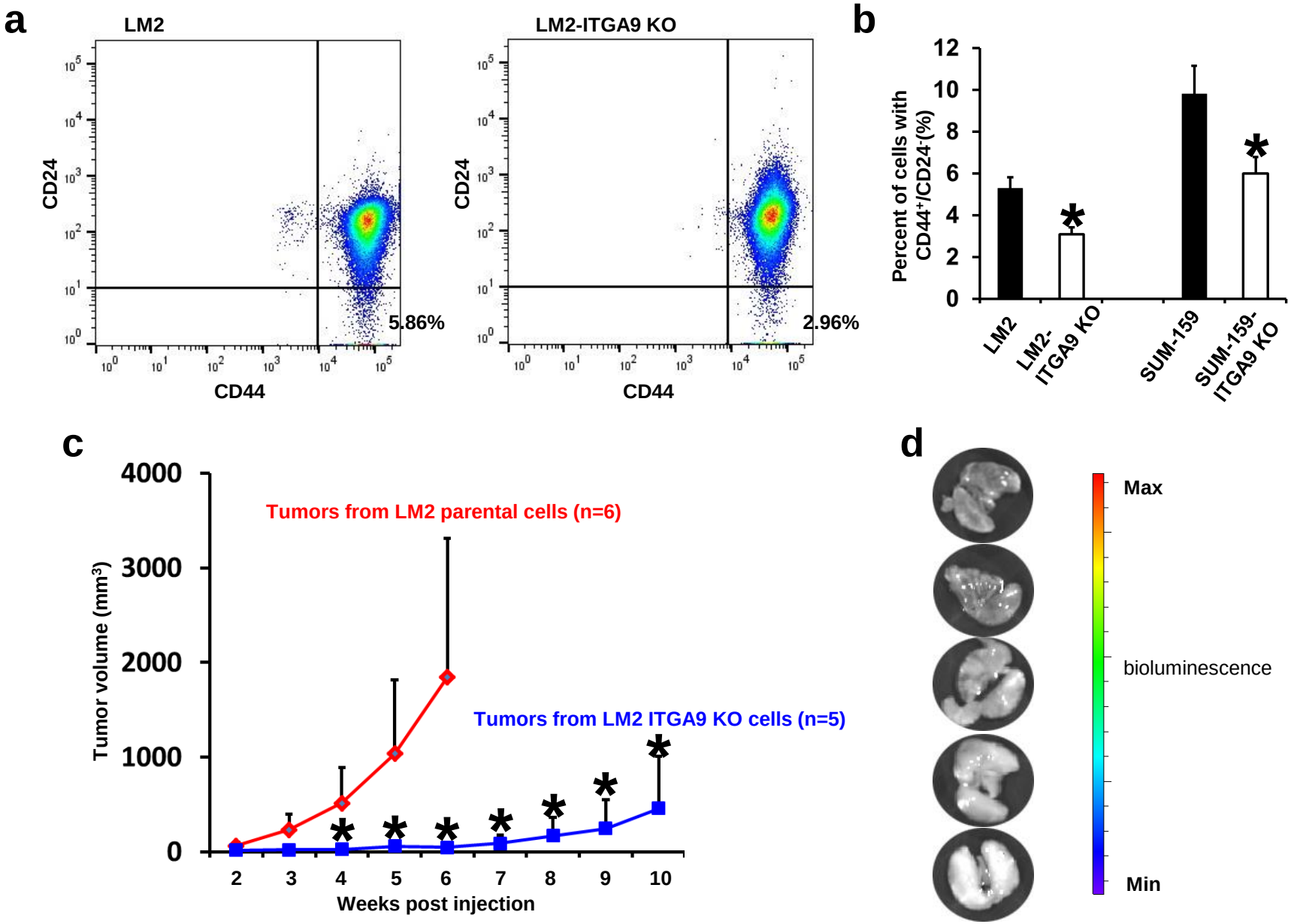
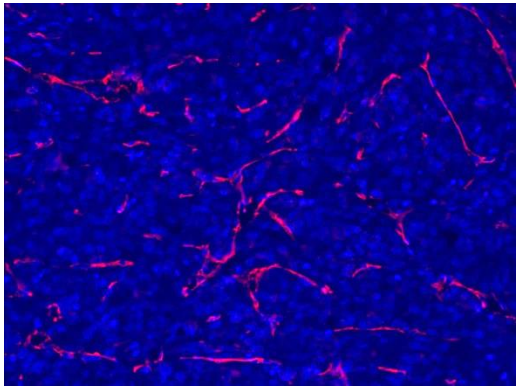


Fig. S1 ITGA9 KO in TNBC cells significantly reduces their CSC-like property and mouse orthotopic mammary xenograft tumor growth and metastasis. **a** and **b** Representative histograms of flow cytometry analysis of CD44+/CD24- cells (a) and quantitation (mean ± SD, n=3. * $p<0.05$) (b), respectively. **c** ITGA9 KO reduces tumor growth. One million of LM2 parental cells or LM2 ITGA9 KO cells were injected into nude mouse mammary fat pad and mice were euthanized 6-7 or 10 weeks post injection, respectively. Tumor volumes are presented as mean ± SD (n=5-6). * $p<0.05$. **d** Images of lung ex vivo IVIS bioluminescence imaging analysis from mice injected with LM2 ITGA9 KO cells and euthanized 10 weeks post injection.

Mouse mammary tumor section from injection of LM2 cells



Mouse mammary tumor section from injection of LM2-ITGA9 KO cells

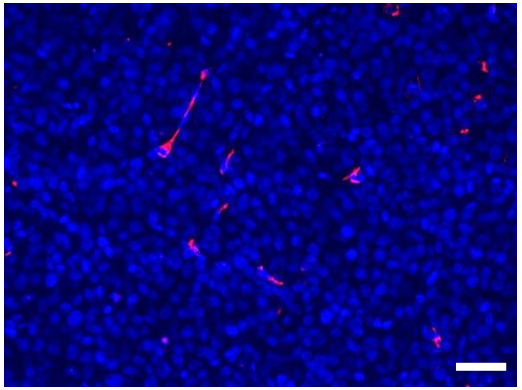


Fig. S2 ITGA9 KO in TNBC cells significantly reduces nude mouse orthotopic mammary xenograft tumor angiogenesis. Representative overlaid images of IF staining of CD31 (red) and nuclear DNA DAPI (blue) in mouse mammary xenograft tumor resulting from injection of parental LM2 cells or LM2 ITGA9 KO cells, respectively. One million of parental LM2 cells or LM2 ITGA9 KO cells were injected into nude mouse mammary fat pad. Seven weeks after injection, mice were euthanized and mammary tumors were collected and used for IF staining. Scale bar, 50 µm.

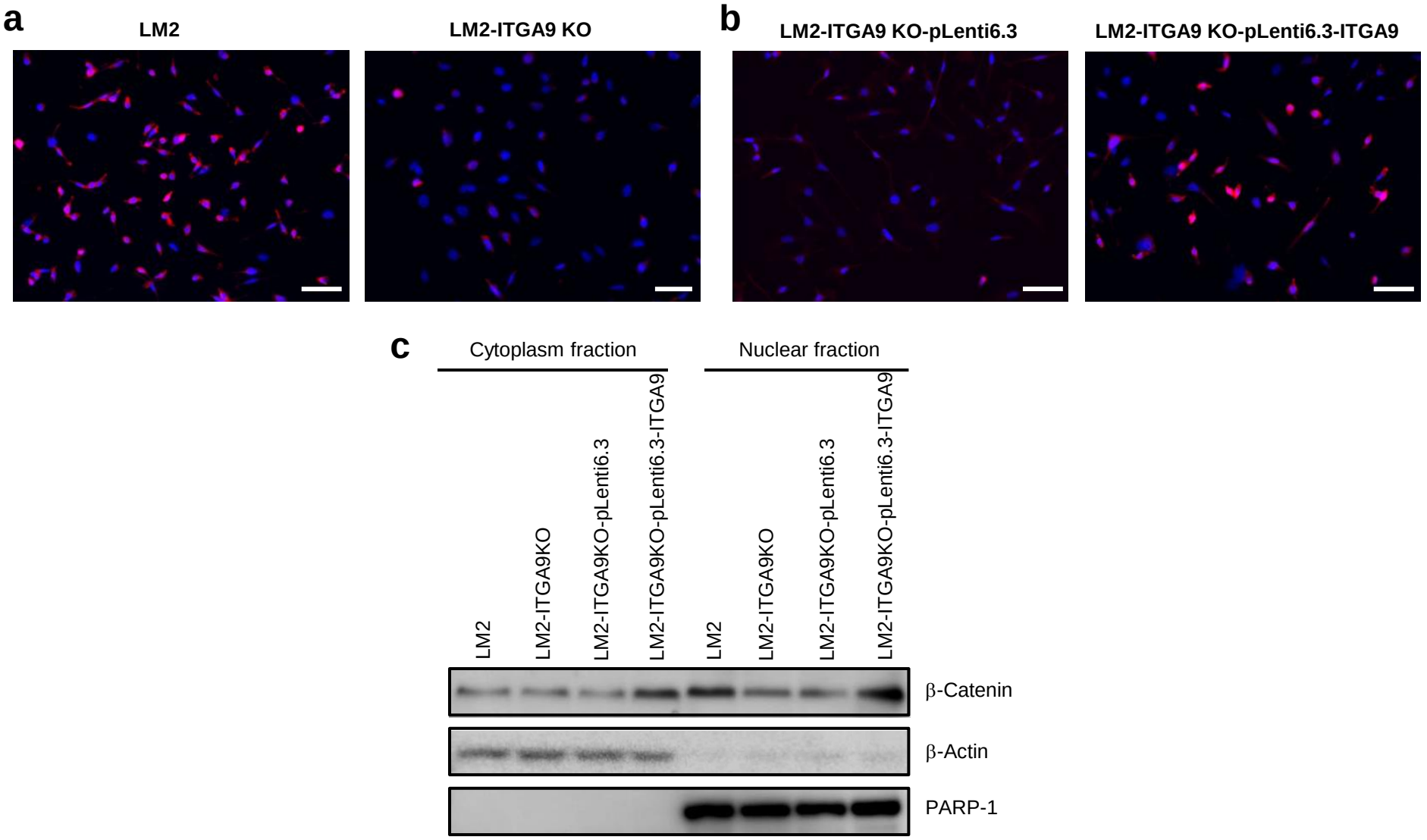


Fig. S3 ITGA9 KO reduces β-catenin nuclear localization in TNBC cells. **a** and **b** Representative overlaid images of IF staining of β-catenin (red) and nuclear DNA DAPI (blue) in LM2 parental, ITGA9 KO, ITGA9 KO vector control and ITGA9 re-expressing cells. Scale bar, 50 μm. **c** Representative Western blot analysis of β-catenin protein levels in cytoplasm and nuclear fractions. Cytoplasmic protein β-actin was used as an indicator of cytoplasm fraction and the nuclear protein poly (ADP-ribose) polymerase 1 (PARP-1) was used as an indicator of nuclear fraction.

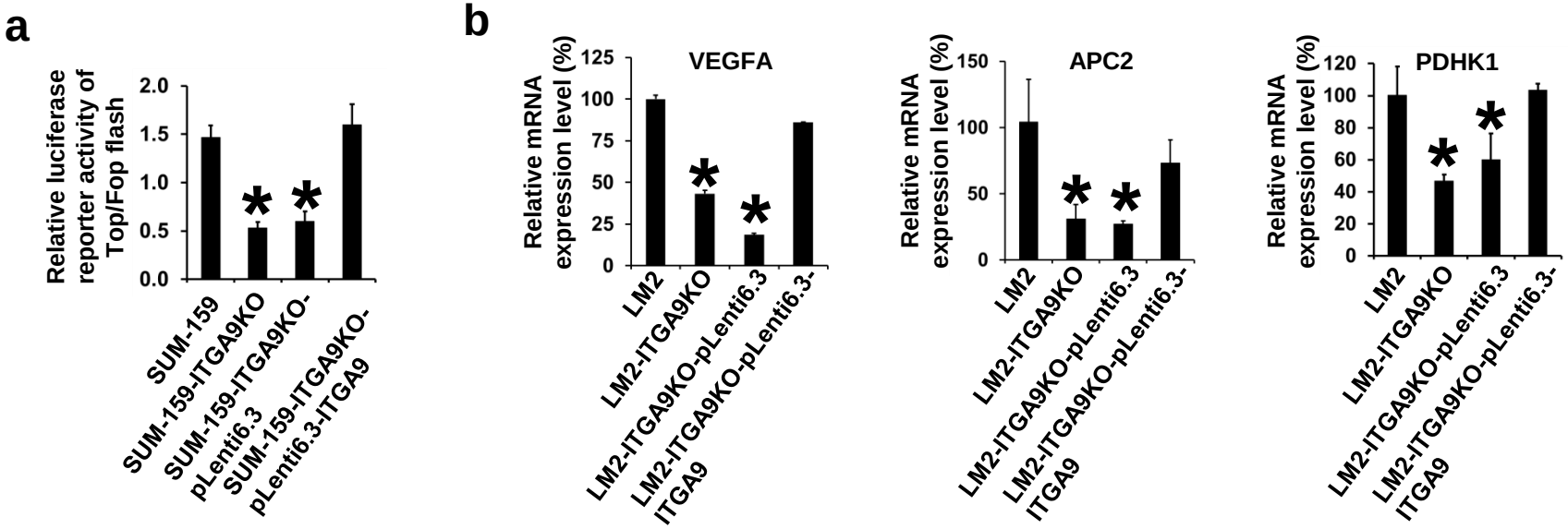
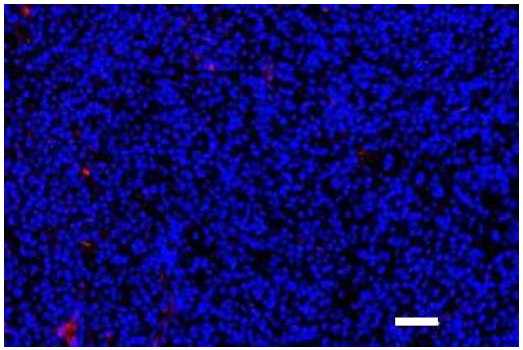


Fig. S4 ITGA9 KO reduces β-catenin transcriptional activity and its target gene expression in TNBC cells. **a** Quantifications of cellular TOPflash and FOPflash luciferase reporter assays. The results are presented as the ratio of the TOPflash luciferase activity divided by the FOPflash luciferase activity (mean ± SD, n=3). *p<0.05, compared to parental or ITGA9 re-expressing cells. **b** Q-PCR analysis of β-catenin target gene expression levels. The mRNA level of each gene was determined by ABI specific gene expression assay and normalized by β-actin (mean ± SD, n=3). *p<0.05, compared to parental or ITGA9 re-expressing cells.

Mouse mammary tumor section from injection of LM2-ITGA9 KO-pLenti6.3 cells



Mouse mammary tumor section from injection of LM2-ITGA9 KO-pLenti6.3-β-catenin cells

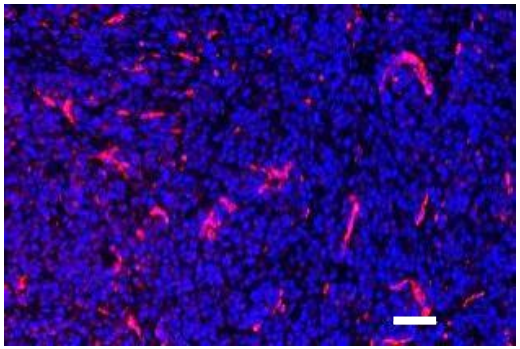


Fig. S5 β-Catenin overexpression reverses the inhibitory effect of ITGA9 KO on tumor angiogenesis. Representative overlaid images of IF staining of CD31 (red) and nuclear DNA DAPI (blue) in mouse mammary xenograft tumor resulting from injection of LM2 ITGA9 KO vector control cells or LM2 ITGA9 KO β-catenin overexpressing cells, respectively. One million of vector control cells or β-catenin overexpressing cells were injected into nude mouse mammary fat pad. Six weeks after injection, mice were euthanized and mammary tumors were collected and used for CD31 IF staining. Scale bar, 50 μm.

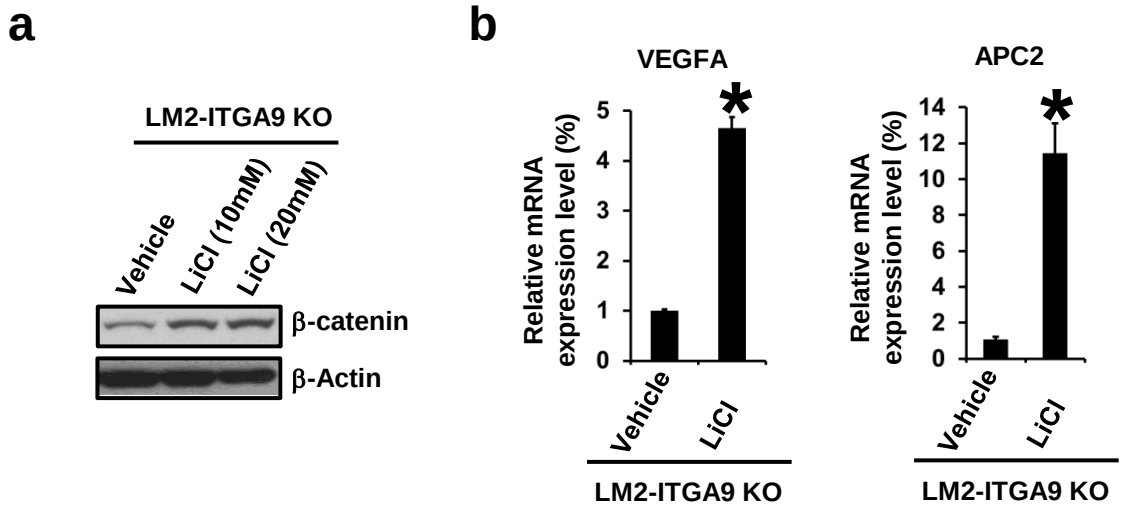


Fig. S6 Inhibition of GSK3 activity in ITGA9 KO cells significantly increases β-catenin protein level and β-catenin target gene expression. **a** Representative images of Western blot analysis of cellular β-catenin protein level. LM2 ITGA9 KO cells were treated with a vehicle control ,10 mM or 20 mM of a GSK3 inhibitor LiCl for 24 h, respectively. At the end of treatment, cells were collected for Western blot analysis of β-catenin protein level. **b** Q-PCR analysis of β-catenin target gene expression levels. LM2 ITGA9 KO cells were treated with a vehicle control or 10 mM LiCl for 24 h, respectively. At the end of treatment, cells were collected for Q-PCR analysis. The mRNA level of each gene was determined by ABI specific gene expression assay and normalized by β-actin (mean ± SD, n=3). * p <0.05.

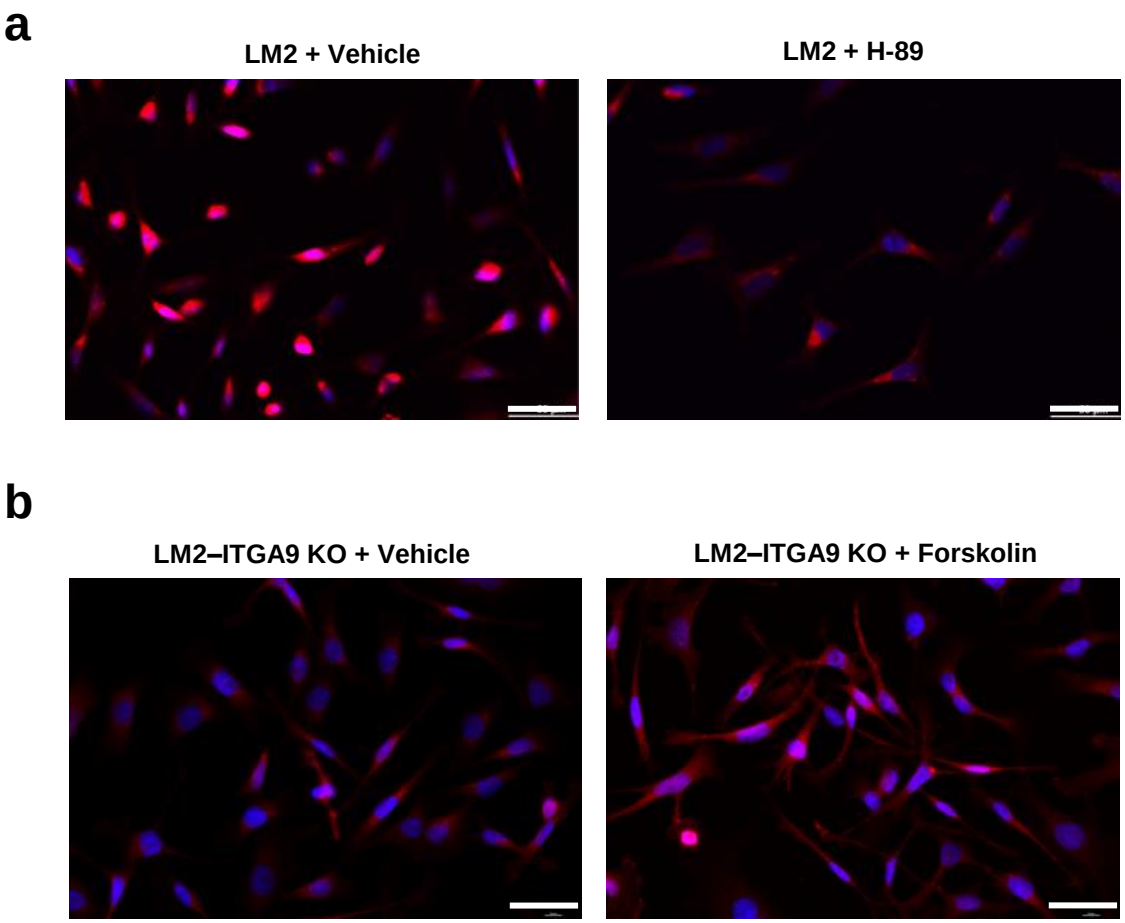


Fig. S7 Inhibiting PKA in LM2 parental cells or activating PKA in LM2 ITGA9 KO cells significantly reduces or increases β-catenin level and nuclear localization, respectively. Representative overlaid images of IF staining of β-catenin (red) and nuclear DNA DAPI (blue) in LM2 parental cells treated with a Vehicle control or a PKA inhibitor H-89 (10 μM, 24 h) (**a**); or in LM2 ITGA9 KO cells treated with a Vehicle control or a PKA agonist Forskolin (10 μM, 24 h) (**b**), respectively. Scale bar, 50 μm.

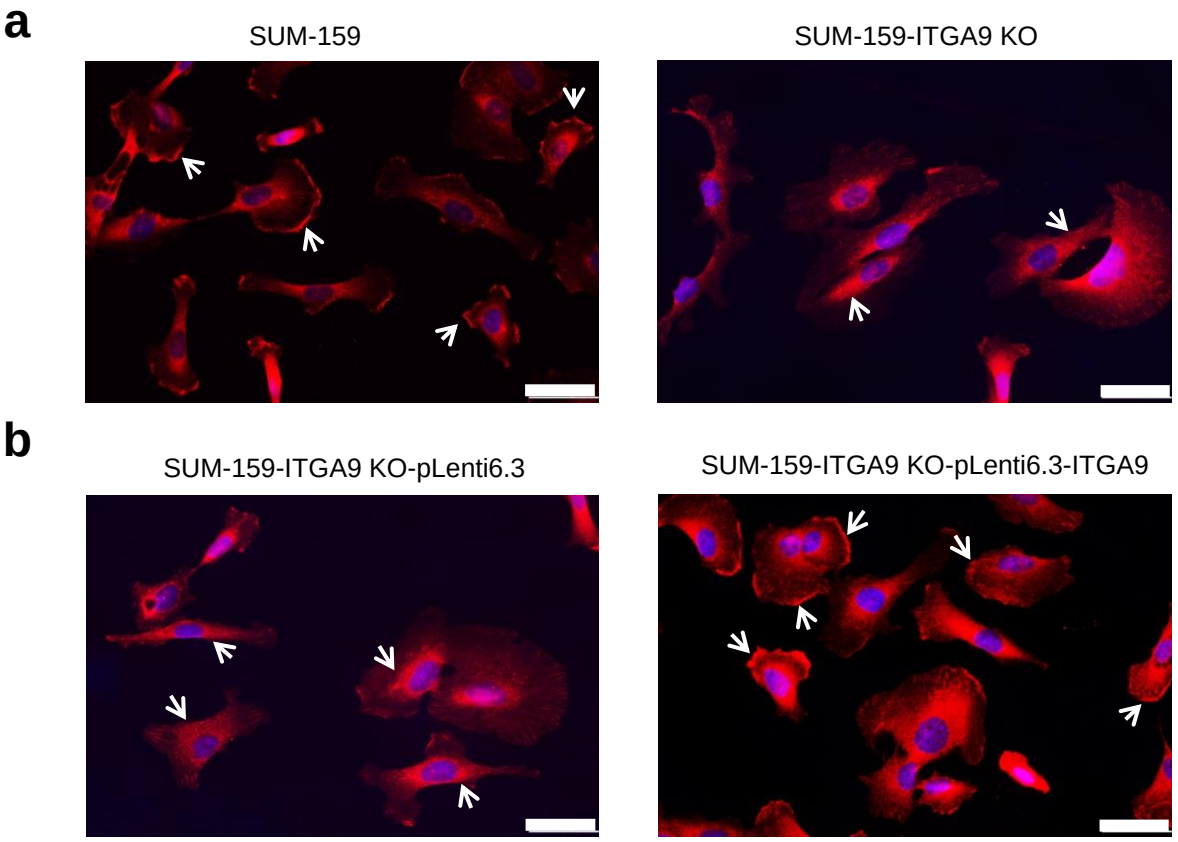


Fig. S8. ITGA9 KO leads to ILK relocate from cellular membrane region to cytoplasm and re-expressing ITGA9 in ITGA9 KO cells greatly recovers ILK cellular membrane localization. a, Representative overlaid images of IF staining of ILK (red) and nuclear DNA DAPI (blue) in parental and ITGA9 KO SUM-159 cells. While arrows point to representative ILK cellular membrane staining or cytoplasm staining in SUM-159 parental cells and SUM-159 IGTA9 KO cells, respectively. Scale bar, 50 μm. b**,** Representative overlaid images of IF staining of ILK (red) and nuclear DNA DAPI (blue) in SUM-159 ITGA9 KO cell vector control cells and ITGA9-reexpression cells. While arrows point to representative ILK cytoplasm staining or cellular membrane staining in SUM-159 ITGA9 KO cell vector control cells and ITGA9-reexpression cells, respectively. Scale bar, 50 μm.

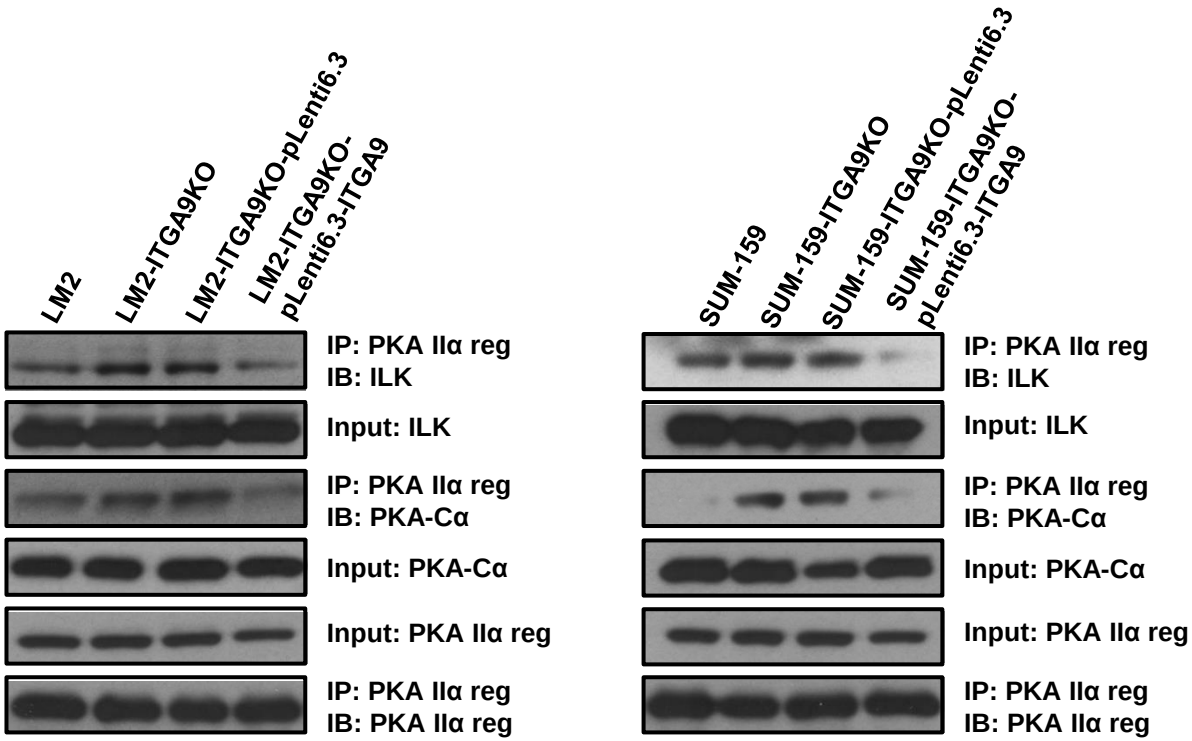


Fig. S9 ITGA9 KO leads to ILK relocate from cellular membrane region to cytoplasm and increases the interaction among ILK, PKA regulatory subunit IIα (PKA IIα reg) and PKA catalytic subunit α (PKA-Cα). Representative Western blot images of Co-IP analysis determining the interaction among ILK, PKA regulatory subunit IIα and PKA catalytic subunit α in parental, ITGA9 KO and re-expressing LM2 and SUM-159 cells. IP: immuno-precipitating; IB: immunoblotting.

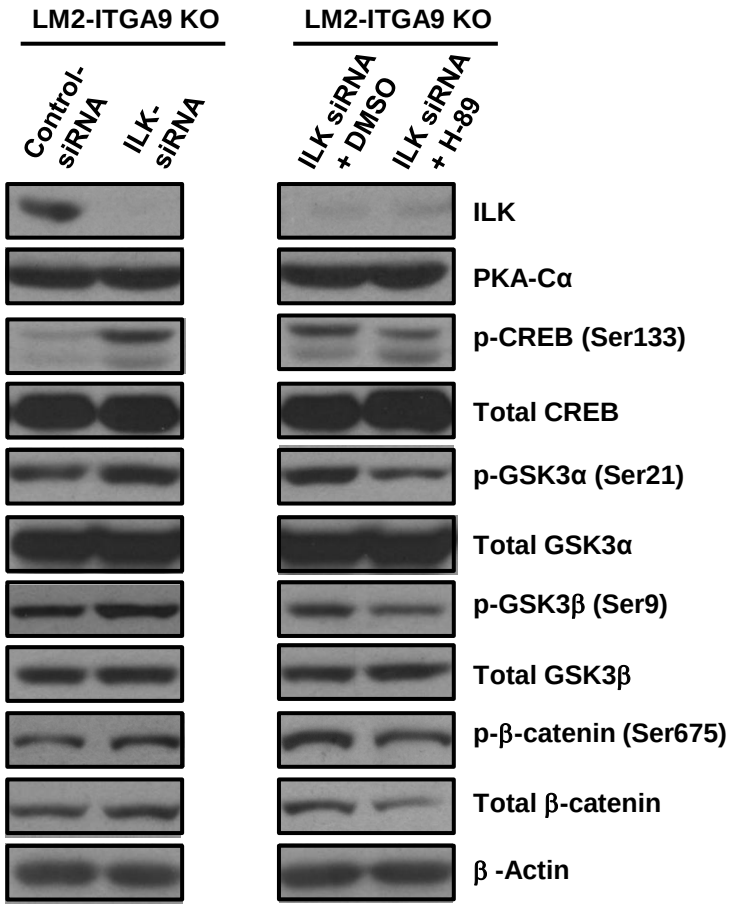


Fig. S10 SiRNA knocking down ILK expression level in LM2 ITGA9 KO cells increases GSK3 phosphorylation and β-catenin level via activating PKA. The presented images are representative Western blot images. LM2 ITGA9 KO cells were transfected with Control siRNA oligoes or a pool of 4 ILK siRNA oligoes (100 nM). Forty-eight h after transfection, cells were collected for Western blot analysis. For the H-89 treatment experiment, cells were first transfected with a pool of 4 ILK siRNA oligoes (100 nM). Twenty-24 h after transfection, cells were treated with H-89 (10 μM) for another 24 h. Cells were then collected for Western blot analysis.