**Protocadherin PCDH10 Functions as a Tumor Suppressive Scaffold Protein Antagonizing Oncogenic WNT/β-catenin Signaling in Breast Carcinogenesis**

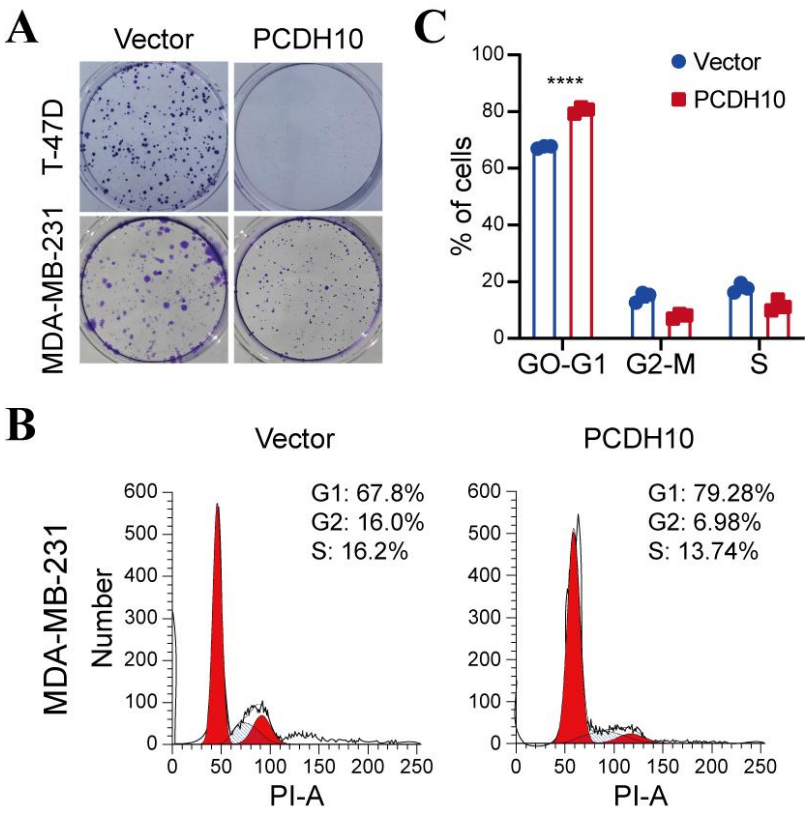


**Figure S1. PCDH10 Regulates Proliferation and Apoptosis in Breast Cancer Cells. A** Representative images of colony formation of T-47D and MDA-MB-231 cells in vector versus PCDH10-OE group (n=3, two-way ANOVA). **B-C** Representative figures of cell cycle examined by FC (B) and statistical analysis of cell cycle (C) of MDA-MB-231 cells in vector versus PCDH10-OE group (n=3, two-way ANOVA). ****$p < 0.0001$.

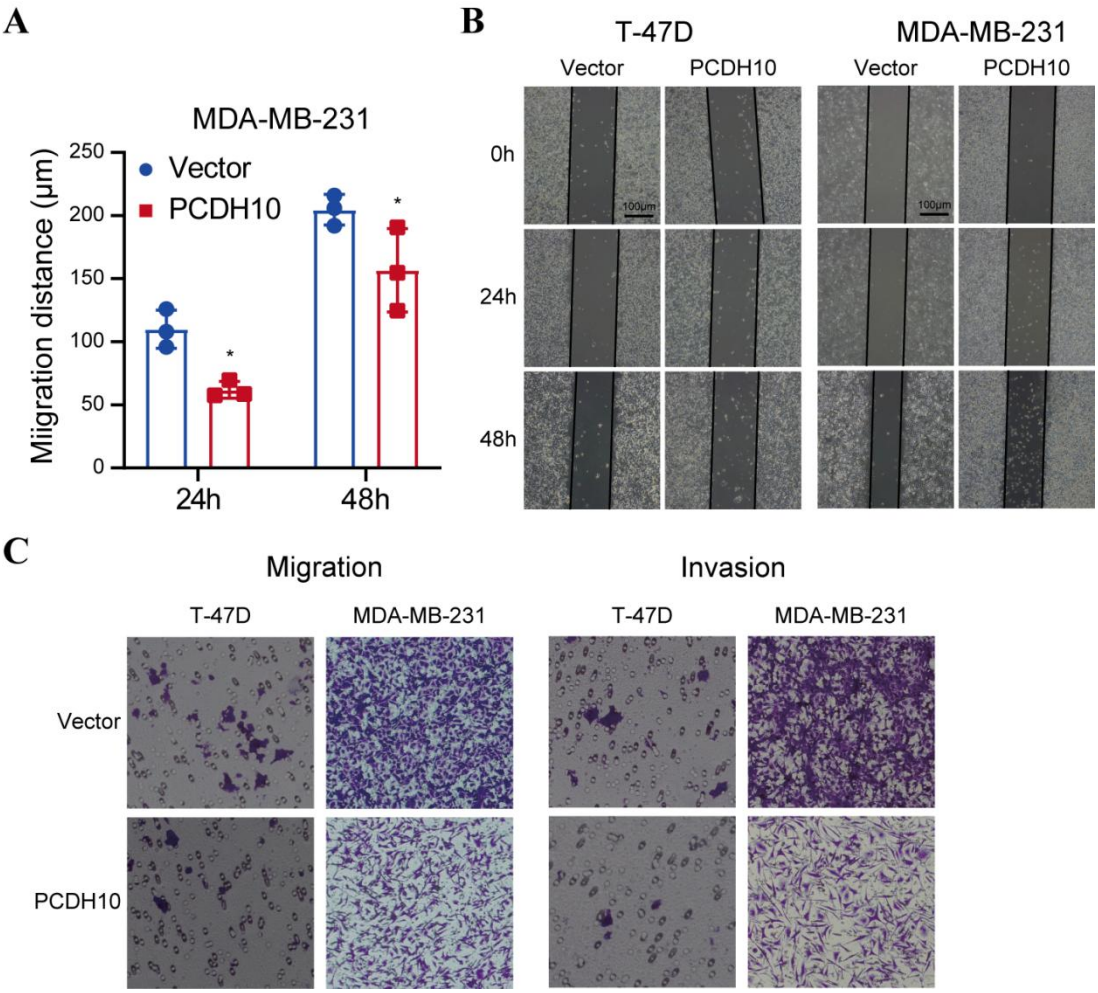


**Figure S2. PCDH10 impairs the migratory and invasive capacities of breast cancer cells. A** Statistical analysis of wound healing assay of MDA-MB-231 cells in vector versus PCDH10-OE group (n=3, two-way ANOVA). **B** Scratch assays indicating the effect of PCDH10 on motility of T-47D and MDA-MB-231 cells. **C** Transwell assays showing the effect of PCDH10 on migration and invasion of T-47D and MDA-MB-231. *$p < 0.05$.

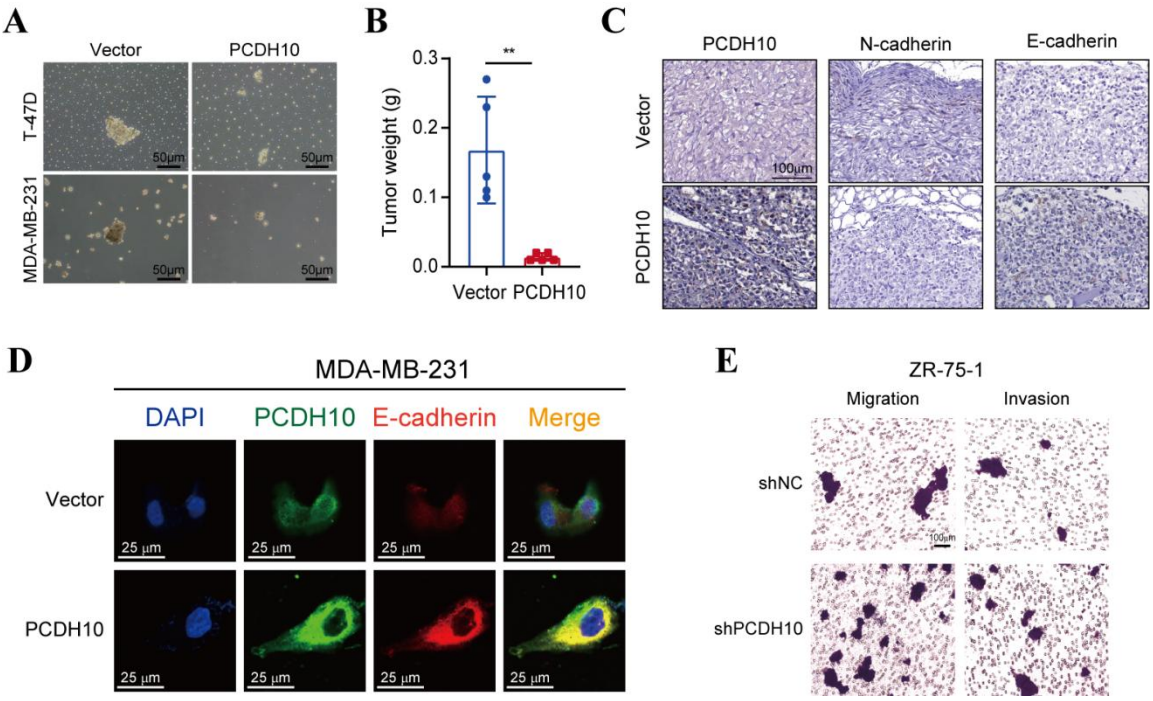


**Figure S3. PCDH10 reverses EMT and reduces stem-like properties. A** Representative images of cell spheroid-forming assay of T-47D and MDA-MB-231 cells in vector versus PCDH10-OE group (n=3, two-way ANOVA). **B** Tumor weights from nude mice xenografted with vector versus PCDH10-OE MDA-MB-231 cells (n=5, t test). **C** Immunohistochemical (IHC) staining of N-cadherin and E-cadherin in xenograft tumor sections derived from vector and PCDH10-OE MDA-MB-231 cells. **D** IF staining showed representative images of PCDH10 (green) and E-cadherin (red) in MDA-MB-231 cells. **E** Transwell assays showing the effect of PCDH10 on migration and invasion of ZR-75-1 cells. $^{**}p < 0.01$.

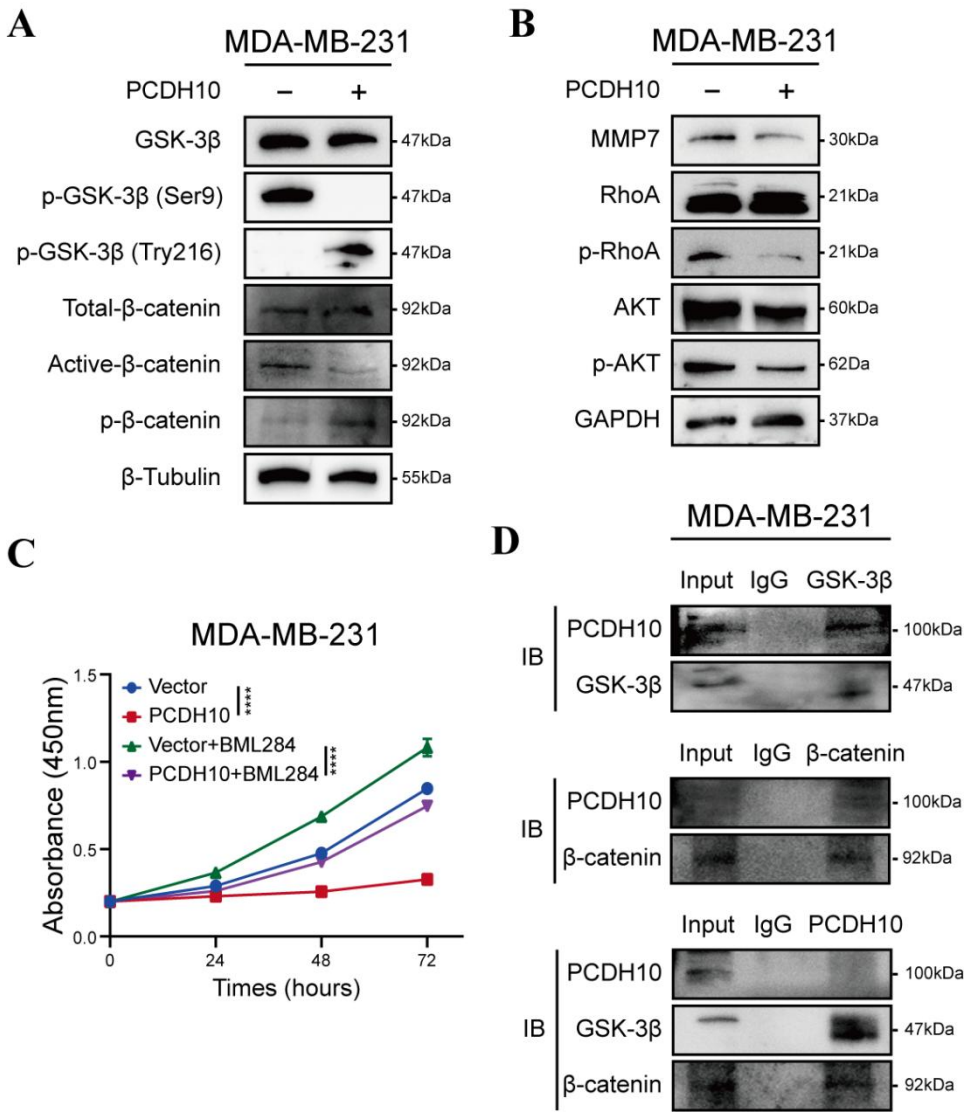


**Figure S4. PCDH10 expression confirms the inhibition of Wnt/ β -catenin signaling in MDA-MB-231 cells. A** Western blot analysis of GSK-3β and β-catenin status in vector versus PCDH10-OE MDA-MB-231. **B** Western blot analysis of MMP7, p-RhoA, and p-AKT in vector versus PCDH10-OE of MDA-MB-231. **C** CCK8 was applied to detect inhibitive effect of PCDH10 on Wnt/β-catenin pathway, and BML-284 was applied to activate β-catenin signaling in MDA-MB-231 (n=3, two-way ANOVA). **D** Co-IP isolated extracts were applied for IB to confirm binding of PCDH10, GSK-3β and β-catenin in MDA-MB-231. ****$p < 0.0001$. IB: Immunoblot.

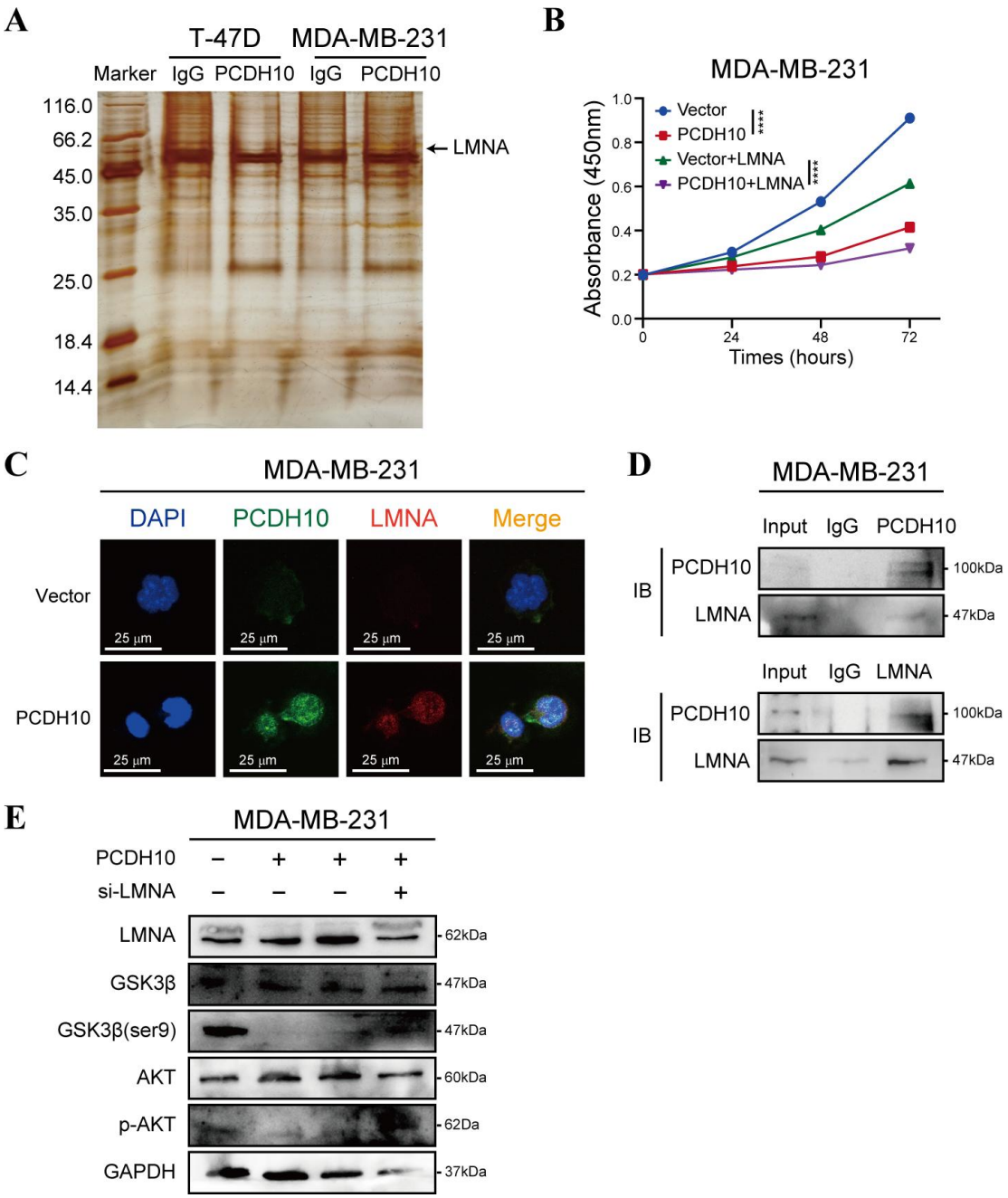


**Figure S5. PCDH10 upregulates LMNA and inhibits Akt signaling in MDA-MB-231 cells. A** Endogenous PCDH10 was immunoprecipitated with anti-PCDH10 antibody, and silver staining was performed to visualize the proteins interacting with PCDH10. **B** CCK8 determining LMNA effect on tumor cells growth in vector versus PCDH10-OE MDA-MB-231 cells (n=3, two-way ANOVA). **C** IF staining showed representative images of PCDH10 (green) and LMNA (red) in vector versus PCDH10-OE MDA-MB-231 cells. **D** IB and Co-IP performing to confirm protein-protein combination in MDA-MB-231. **E** Western

blot analysis of LMNA, GSK-3β, GSK-3β (ser9), p-AKT and AKT in vector versus PCDH10-OE MDA-MB-231 cells. IB: Immunoblot; WB: Western Blot. ****$p < 0.0001$.

**Supplementary Table 1. List of PCR primers used in this study**

| PCR | Primer | Sequence (5'-3') | |
|---|---|---|---|
| RT-PCR / qRT-PCR | *PCDH10 F* | ACTGCTATCAGGTATGCCTG | **219bp** |
| | *PCDH10 R* | GTCTGTCAACTAGATAGCTG | |
| | *Wnt5bF* | AAATGCCACGGCGTCTCG | **163bp** |
| | *Wnt5bR* | GGGTGAAGCGGCTGTTGA | |
| | *HIF1AF* | ACCATCAGCTATTTGCGTGTGA | **143bp** |
| | *HIF1AR* | ATCATGTCACCATCATCTGTGAG | |
| | *VEGFAF* | CACACAGGATGGCTTGAAGA | **136bp** |
| | *VEGFAR* | AGGGCAGAATCATCACGAAG | |
| | *VEGFCF* | CTTGCTGGGCTTCTTCTC | **180bp** |
| | *VEGFCR* | GACCGTAACTGCTCCTCC | |
| | *EGFRF* | TTCCTATGCCTTAGCAGTCTTAT | **142bp** |
| | *EGFRR* | GATGCTCTCCACGTTGCACAG | |
| | *Axin2F* | *GCAGCTCAGCAAAAAGGGAAAT* | **114bp** |
| | *Axin2R* | *TACATGGGGAGCACTGTCTCGT* | |
| MSP | *PCDH10-bM1* | TCGTTAAATAGATACGTTACGC | **153bp** |
| | *PCDH10-bM2* | TAAAAACTAAAAACTTTCCGCG | |
| | *PCDH10-bU1* | GTTGTTAAATAGATATGTTATGT | **155bp** |
| | *PCDH10-bU2* | CTAAAAACTAAAAACTTTCCACA | |

Note. qPCR: quantitative Polymerase Chain Reaction;
RT-PCR: Reverse Transcription-Polymerase Chain Reaction;
MSP: Methylation-specific PCR.