

Increased expression of LRP5 in metastatic tumor cells

We detected the background expression of LRP5 protein in different cell lines by Western blot (*Figure S1*). LRP5 had little expression in colorectal cancer cell line SW480, but highly expressed in its high metastatic cell line SW620. Similarly, LRP5 barely expressed in ovarian cancer cell line HO8910, but highly expressed in its high metastatic cell line HO8910PM, all of which suggest that the expression of LRP5 may be related to cell migration.

Construction and verification of pLVX-IRES-ZsGreen1-DN-LRP5 and pLVX-puro-DN-LRP5 and verification of HO8910PM/DN-LRP5

To study the functions of LRP5 receptor protein and other related proteins in ovarian cancer cells both *in vitro* and *in vivo*, we shall knock down the expression of LRP5 by using dominant negative vector of LRP5. Accordingly, pLVX-puro-DN-LRP5 was constructed to obtain the LRP5 low expression cell line, while pLVX-IRES-ZsGreen1-DN-LRP5 was constructed to detect lentivirals infection efficiency.

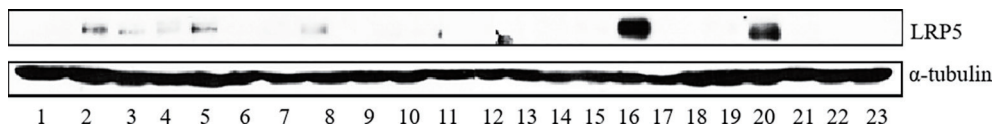


Figure S1 Increased expression of LRP5 in metastatic tumor cells. Human normal liver cells: 1: L02, 2: Chang's liver. Hepatocarcinoma cells: 3: HepG2, 4: HepG2.2.15, 5: Hep3B, 6: 7721, 7: PLC, 8: Huh7, 9: 97-H, 10: LM3, 11: FOCUS, 12: SNU398, 13: Sk-Hep-1. Colon carcinoma cells: 14: LoVo, 15: HCT116, 16: SW480, 17: SW620. OCa cells: 18: A2780, 19: HO8910, 20: HO8910PM, 21: SKOV3. Lung cancer cells: 22: H460, 23: H446.

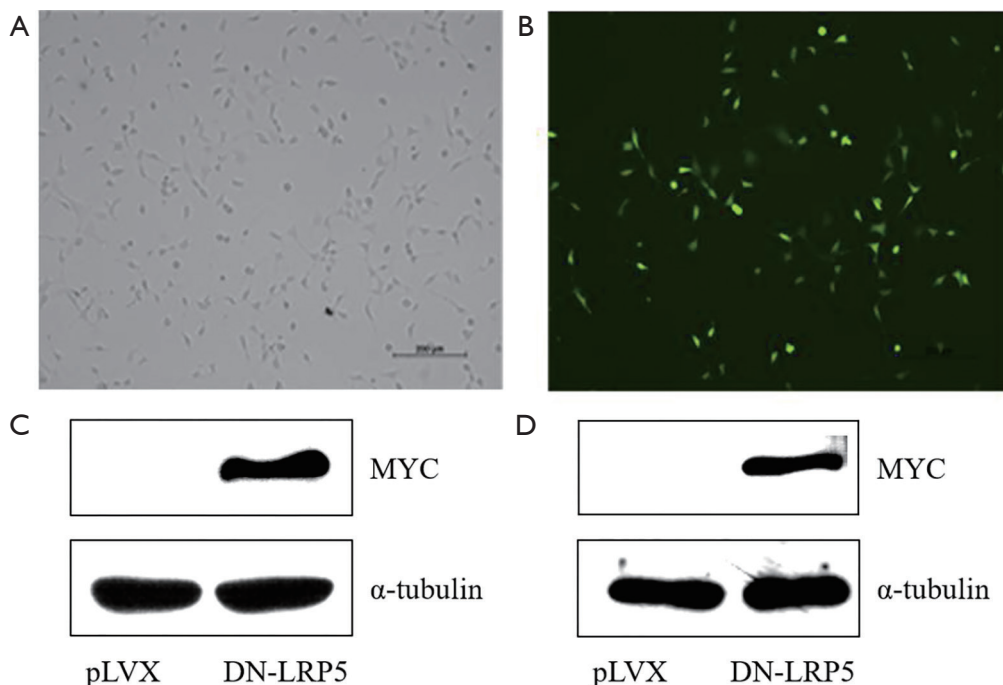


Figure S2 Verification of HO8910PM/DN-LRP5. (A) Light microscopy observation of HEK293T infected with pLVX-IRES-ZsGreen1-DN-LRP5 virals ($\times 100$); (B) fluorescence microscopy observation of HEK293T infected with pLVX-IRES-ZsGreen1-DN-LRP5 virals ($\times 100$); (C) Western blot analysis for expression of DN-LRP5 (myc) in pLVX-puro-DN-LRP5 virals in HEK293T; (D) Western blot analysis for expression of DN-LRP5(myc) in pLVX-puro-DN-LRP5 virals in HO8910PM. DN-LRP5, dominant negative plasmid of LRP5.

To obtain packaged recombinant lentivirus, HEK 293T cells were co-transfected with recombinant lentivirus expression plasmids and lentivirus packaging plasmids psPAX2 and pMD2.G. After infection, expression of a large number of green fluorescent proteins in HEK 293T cells was observed by fluorescence microscopy (Figure S2A,B), while expression of DN-LRP5 protein was detected by Western blot (Figure S2C).

To obtain the cell line stably expressing DN-LRP5, HO8910PM cells were infected with the recombinant lentivirus and resistant cell clones were selected with puromycin. Consistently, the expression of DN-LRP5 in positive clones was detected by Western blot (Figure S2D).

DN-LRP5 regulates EMT and multiple cancer-associated pathways

To see if DN-LRP5 are associated with EMT and some cancer-related pathways, multiple EMT or cancer-associated proteins are checked by Western blotting (Figure 3), and the quantitative densitograms of Figure 3 are shown in Figure S3.

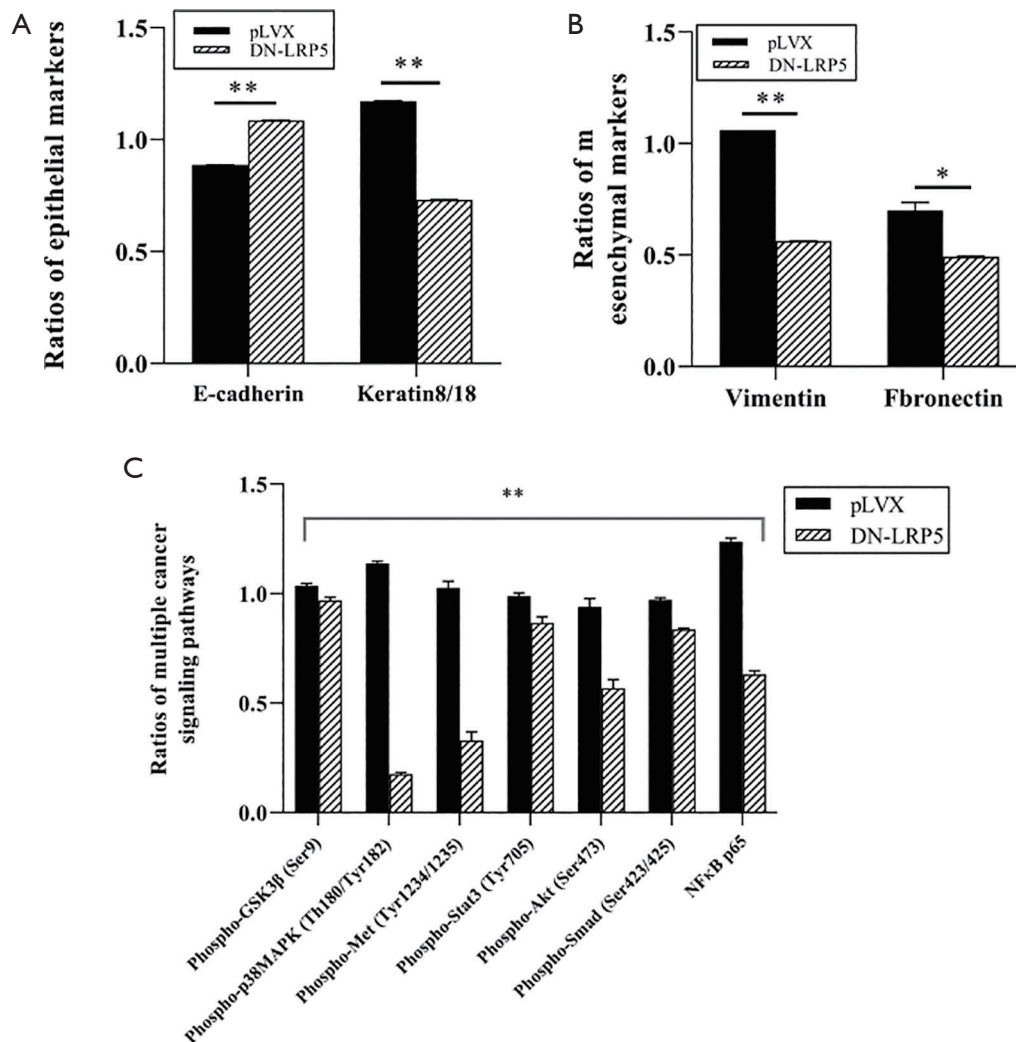


Figure S3 Western blotting densitograms of DN-LRP5 associated EMT and multiple cancer-associated pathways. (A) Ratios of epithelial markers verse tubulin; (B) ratios of mesenchymal markers verse tubulin; (C) ratios of multiple cancer signaling pathway proteins verse tubulin. DN-LRP5, dominant negative plasmid of LRP5; EMT, epithelial-mesenchymal transition.