Supplementary Materials for Manuscript Entitled

α-Catulin promotes cancer stemness by antagonizing WWP1-mediated KLF5 degradation in lung cancer

Chia-Hao Tung[1], Meng-Fan Huang[1], Chen-Hsien Liang[2], Yi-Ying Wu[3], Jia-En Wu[1], Cheng-Lung Hsu[4], Yuh-Ling Chen[2,5,#] and Tse-Ming Hong [1,3,#]

[1]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

[2]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

[3]Clinical Medicine Research Center, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

[4]Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan.

[5]Institute of Oral Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

Running title: α-Catulin promotes lung cancer stemness

Supplementary figure legends

Figure S1. α-Catulin promotes EMT in NSCLC cells. (A) Representive images of CL1-0-pLKO and pLKO-α-Catulin cells. Scale bar, 50 μm. **(B)** Protein expression of α-Catulin and EMT markers in CL1-0 cells with or without α-Catulin overexpression. **(C)** Representive images of CL1-5-shLuc and shCTNNAL1#2 cells. Scale bar, 100 μm. **(D)** Protein expression of α-Catulin and EMT markers in CL1-5 cells with or without *CTNNAL1* silencing.

Figure S2. α-Catulin expression is positively associated with stemness-associated signatures and genes. (A) The expression levels of genes related to cancer stemness in our previous microarray analysis (GSE40141). The data were presented as $\log_2$ ratios of the normalized expression in A549-pLKO-α-Catulin to the A549-pLKO-control cells. **(B)** GSEA-enrichment plots of the stemness-associated gene sets in TCGA lung adenocarcinomas ($n = 509$) ranked by Pearson correlation to *CTNNAL1* expression. NES, normalized enrichment score; Pval, nominal *P*-value; FDR, false discovery rate.

Figure S3. Overexpression of α-Catulin enhances the proportion of CD133 positive cells in NSCLC cells. (A) Protein expression of α-Catulin in CL1-0 cells with or without α-Catulin overexpression. β-acitn was used as a loading control. Relative protein intensities were calculated by Image J software and normalized to control cells. **(B)** Flow cytometry analysis of CD133 positive cells in CL1-0 cells with or without α-Catulin overexpression. ***$P < 0.001$ by two-tailed Student *t* test.

Figure S4. Silencing of *CTNNAL1* suppresses the characteristics of lung CSCs. (A) Protein

expression of α-Catulin in CL1-5 and HOP-62 cells with or without α-Catulin depletion. β-actin was used as loading control. **(B)** Sphere formation assay of CL1-5 and HOP-62 cells with or without α-Catulin depletion. Formed spheres were photographed and quantified. Scale bar, 100 μm. **(C)** Protein expression of α-Catulin in CL1-5 and HOP-62 cells expressing tet-on-shCTNNAL1#2 with or without treatment of DOX (1 μg/ml). β-acitn was used as a loading control. Relative protein intensities were calculated by ImageJ software and normalized to control cells. DOX, doxycycline; DMSO: Dimethyl sulfoxide. **(D)** Aldehyde dehydrogenase (ALDH) activity of CL1-5 and HOP-62 cells with or without α-Catulin depletion. **(E)** Flow cytometry analysis of CD133 positive cells in CL1-5 cells expressing tet-on-shCTNNAL1#2 with or without treatment of DOX (1 μg/ml). $^{**}P < 0.01$ and $^{***}P < 0.001$ by two-tailed Student's *t* test.

Figure S5. α-Catulin and KLF5 co-expression increases the binding of KLF5 to the *POU5F1* promoter. (A) The scheme showed the predicted KLF5 DNA binding sites and the regions which amplified by ChIP primers 1 to 4 in the promoters of *POU5F1* and *NANOG*. **(B)** CL1-0 cells were overexpressed α-Catulin and KLF5. ChIP assay was performed with anti-KLF5 antibody, and the precipitated DNA was amplified with primers 1 to 4, respectively. NTC, non-template control.

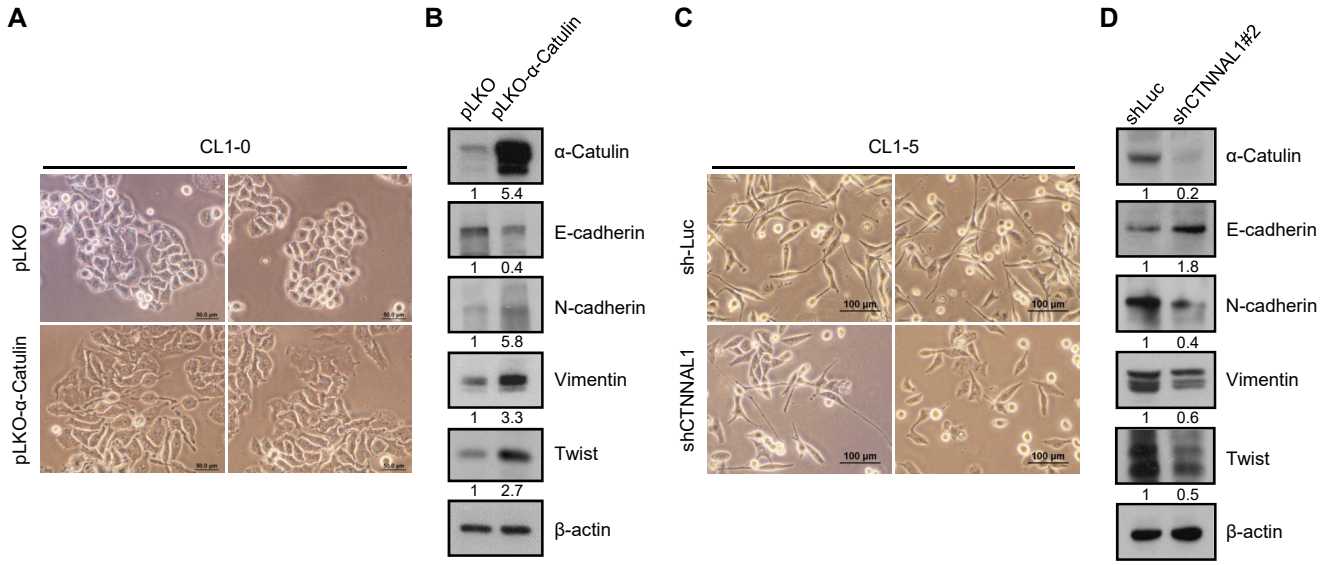
Figure S6. The reduction of KLF5 by α-Catulin knockdown could be blocked by the proteasome inhibitor MG132. (A) Western blot analysis of α-Catulin, KLF5, Lamin B1 (nuclear marker) and β-actin in cytoplasmic (C) and nuclear (N) fractions of CL1-5 and HOP-62 cells with or without α-Catulin depleted. **(B)** α-Catulin-depleted and control CL1-5 and

HOP-62 cells were treated with or without MG132 (10 μM, 24 h). Western blot analysis was performed with the indicated antibodies.

Figure S7. Overexpression of KLF5 partially rescues the OSU-T315-suppressed cancer stemness. (A) The qRT-PCR analysis of *CTNNAL1* and *KLF5* shows that OSU-T315 does not reduce the mRNA levels of these two genes. **(B)** OSU-T315-reduced protein stability of KLF5 could be blocked by the proteasome inhibitor MG132. **(C)** Overexpression of kinase-dead ILK mutant (ILK-A262V) suppressed the expression of KLF5 in CL1-0 cells. **(D)** Overexpression of KLF5 partially rescues the OSU-T315-suppressed cancer sphere formation.

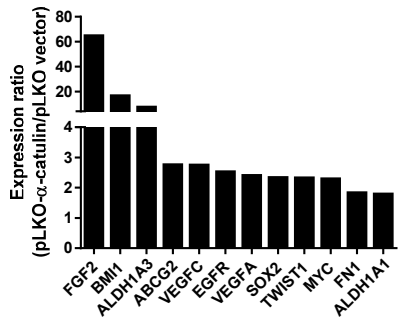
Figure S8. Time-dependent receiver operating characteristic (ROC) curve analysis for the *CTNNAL1*/*ILK*/*KLF5* 3-gene signature in lung adenocarcinoma. AUC, area under the curve.

Supplementary Figure S1

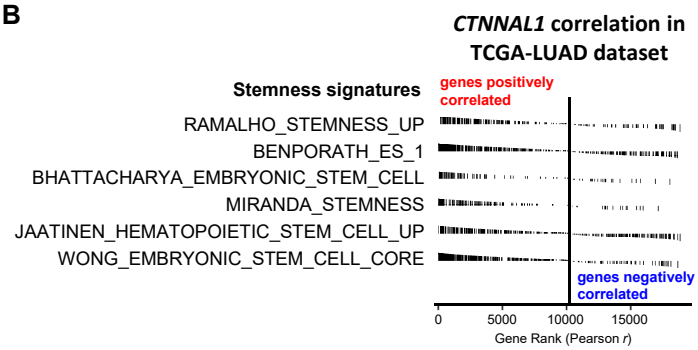


Supplementary Figure S2

A

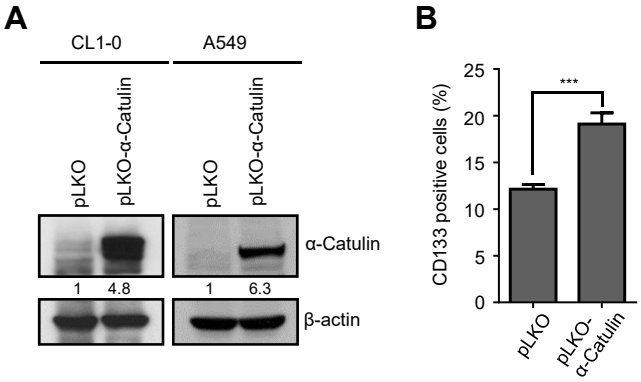


B

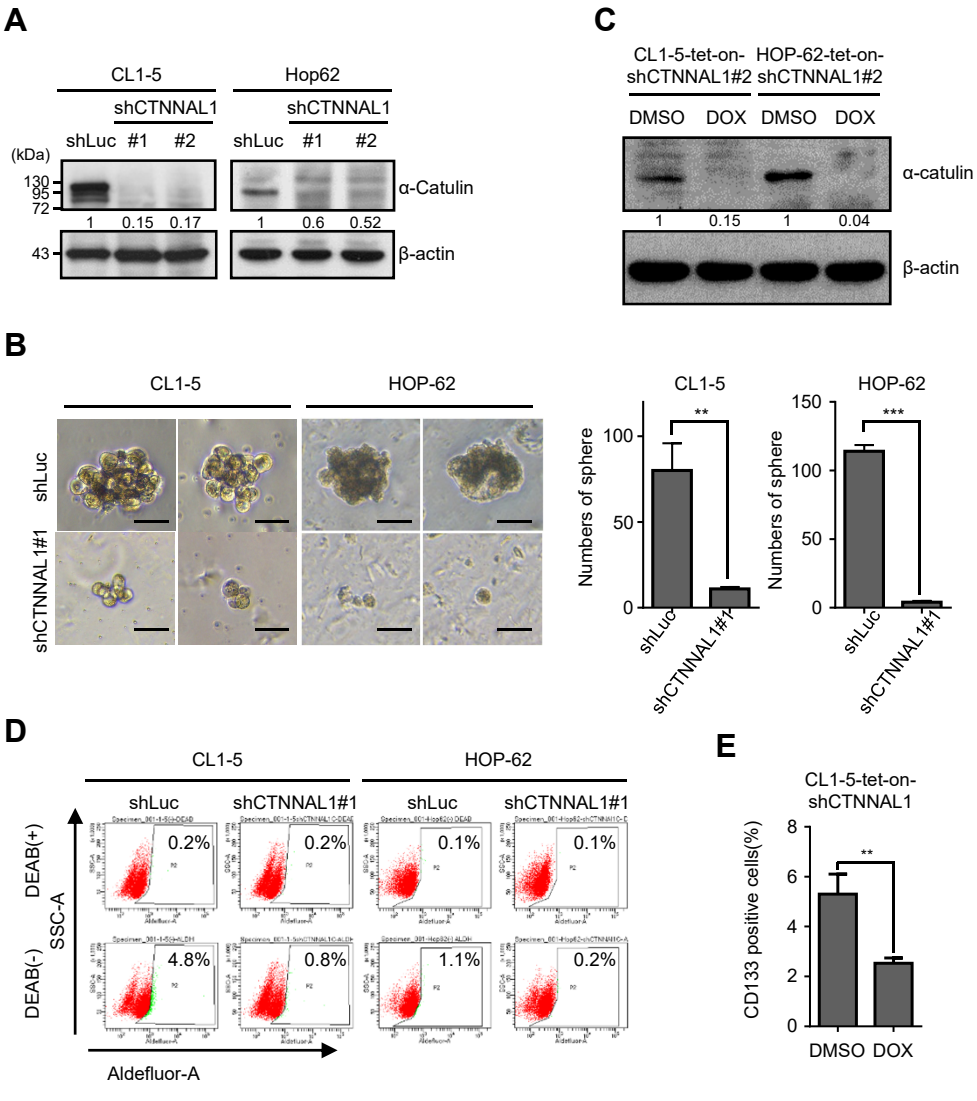


Stemness signatures	NES	Pval	FDR
RAMALHO_STEMNESS_UP	1.93	1.2e-4	1.1e-3
BENPORATH_ES_1	2.41	1.1e-4	1.1e-3
BHATTACHARYA_EMBRYONIC_STEM_CELL	2.50	1.3e-4	1.1e-3
MIRANDA_STEMNESS	2.51	1.3e-4	1.1e-3
JAATINEN_HEMATOPOIETIC_STEM_CELL_UP	1.42	2.7e-3	1.0e-2
WONG_EMBRYONIC_STEM_CELL_CORE	2.72	1.1e-4	1.1e-3

Supplementary Figure S3

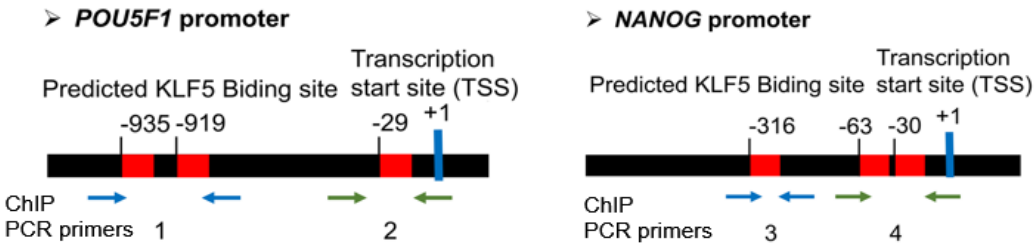


Supplementary Figure S4

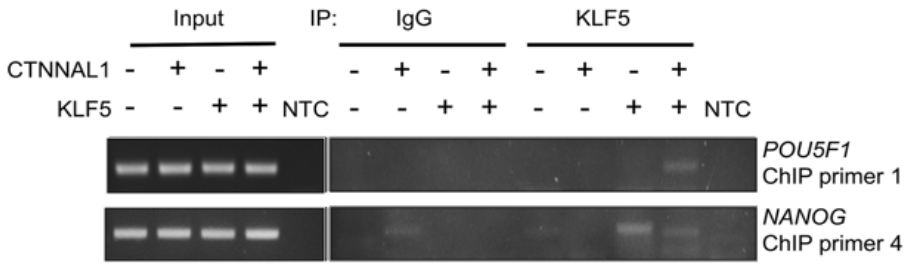


Supplementary Figure S5

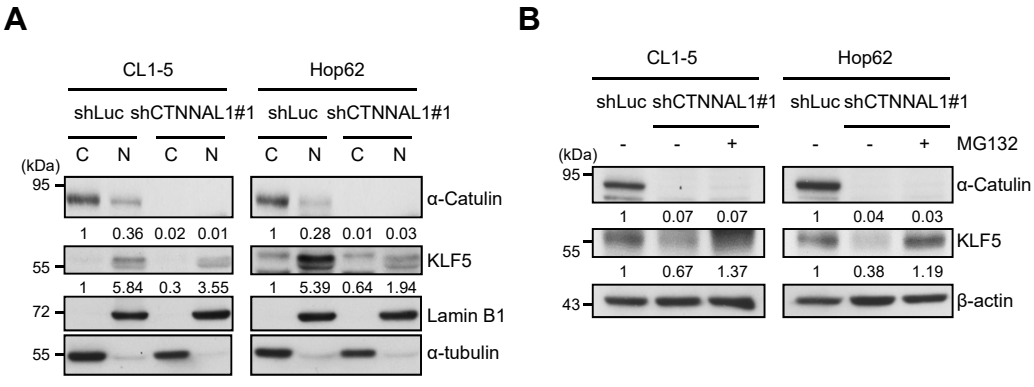
A



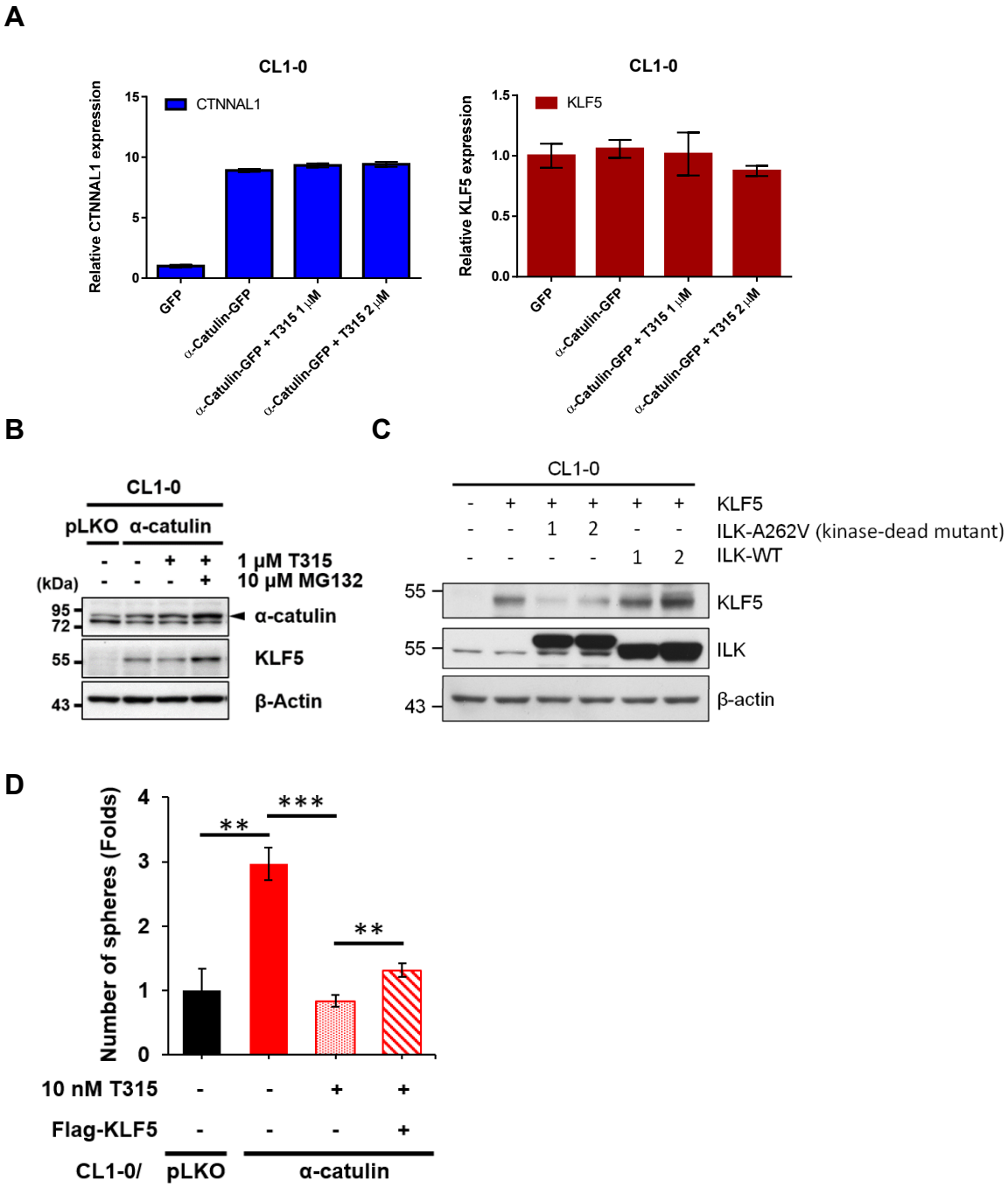
B



Supplementary Figure S6



Supplementary Figure S7



Supplementary Figure S8