

Supplementary information for

KIF3A binds to β -arrestin for suppressing Wnt/ β -catenin signalling independently of primary cilia in lung cancer

Minsuh Kim^{1,3}, Young-Ah Suh³, Ju-Hee Oh³, Bo Ra Lee³, Joon Kim^{1,2*} and Se Jin Jang^{3,4*}

¹ Biomedical science and engineering interdisciplinary program, KAIST, Daejeon, South Korea

² Graduate School of Medical Science and Engineering, KAIST, Daejeon, South Korea

³ Asan Institute for Life Sciences, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea

⁴ Asan Center for Cancer Genome Discovery, Department of Pathology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea

* Corresponding Authors:

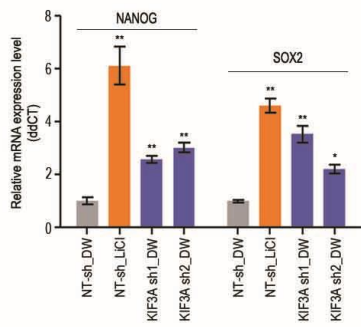
Se Jin Jang, MD, PhD & Joon Kim, PhD.

Supplementary materials and methods

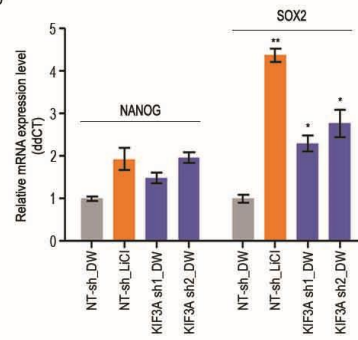
Annexin-V assay

For apoptosis analyses, we used an annexin V assay (Invitrogen). Cells treated with HPI4 were washed in ice-cold PBS without Ca^{2+} or Mg^{2+} . We then resuspended 1×10^6 cells in 1 ml of binding buffer and 200 μl of this cell suspension was incubated with 2 μl of 100 $\mu\text{g}/\text{ml}$ propidium iodide and 10 μl of Alexa Fluor® 488 annexin V for 15 min in the dark at room temperature. Flow cytometric analysis was immediately performed using a FACS Calibur Instrument (Becton Dickinson).

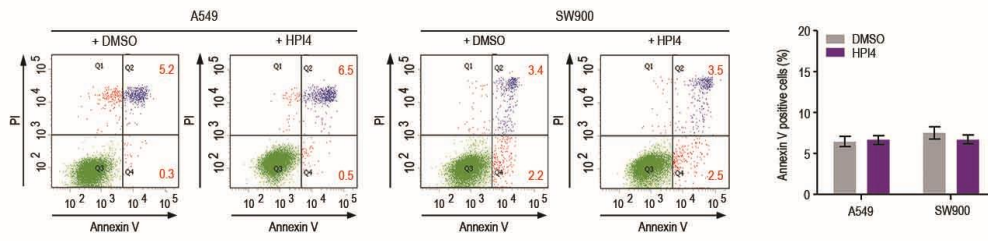
A549



SW900

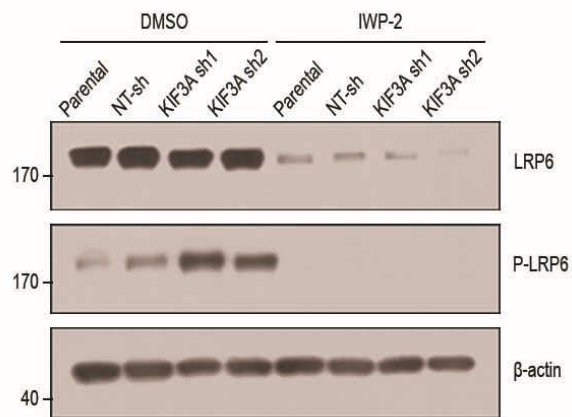


Supplementary Figure 1. LiCl increases stemness marker expression in NT-sh cells. Quantitative RT-PCR analysis confirming stemness marker expression in NT-sh cells treated to LiCl (20 mM) for 24 h. β -actin was used as an internal control for quantitative RT-PCR (n = 3; error bars indicate SEM; p-value was determined by one-way ANOVA). *, P < 0.05; **, P < 0.01.

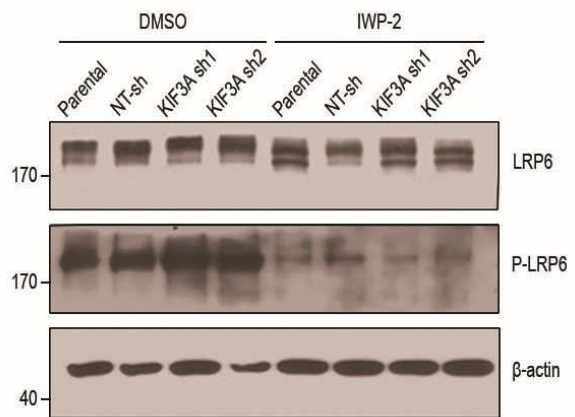


Supplementary Figure 2. HPI4 does not induce apoptosis. Annexin-V assay to examine apoptosis in cells treated with HPI4 (20 μ M). *, $P < 0.05$; **, $P < 0.01$.

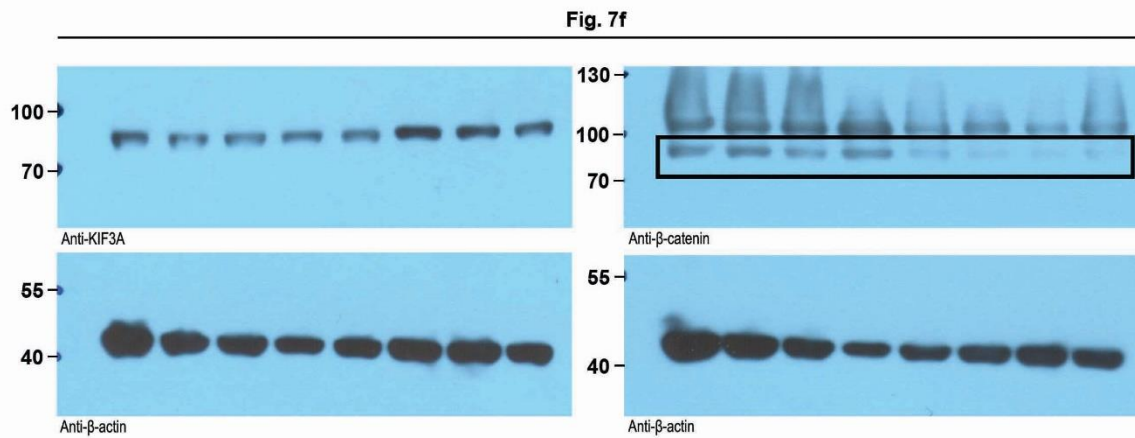
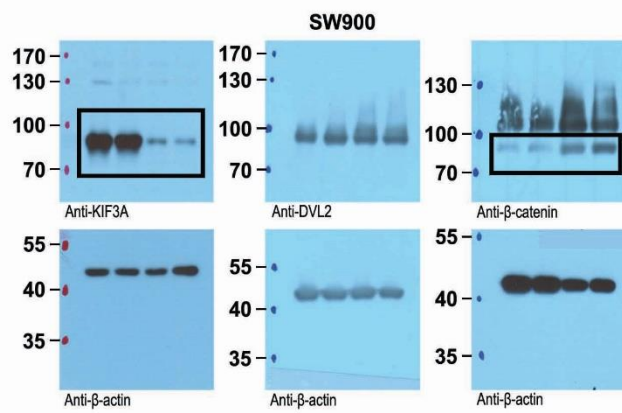
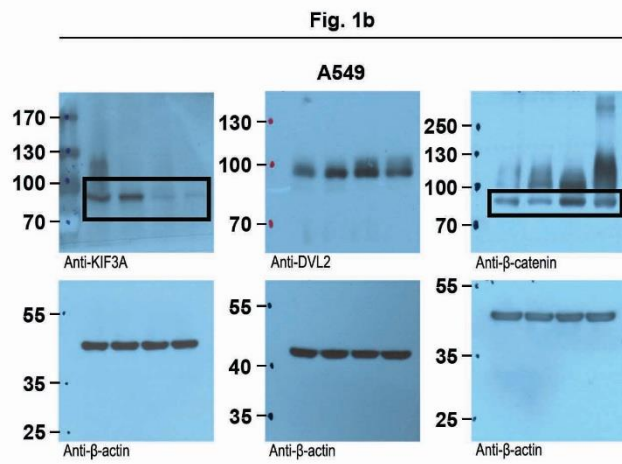
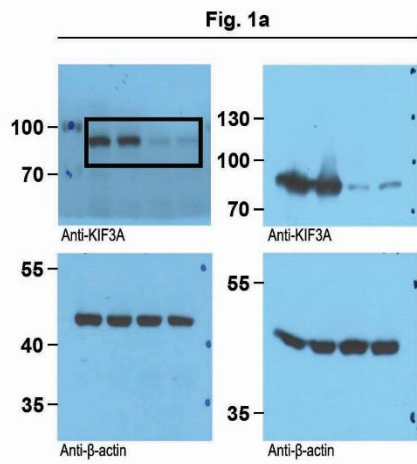
A549



SW900



Supplementary Figure 3. IWP-2 suppresses LRP6 and P-LRP6 expression in KIF3A knock down cells. Immunoblot analysis showing protein levels of LRP6 and P-LRP6 after treating IWP-2 (30 μ M) for 72 h. β -actin was used as a loading control. Full-length blot is shown in the supplementary Figure 5, and the gels have been run under the same experimental conditions.



Supplementary Figure 4. Full-length blots in this study.

Fig. 6a

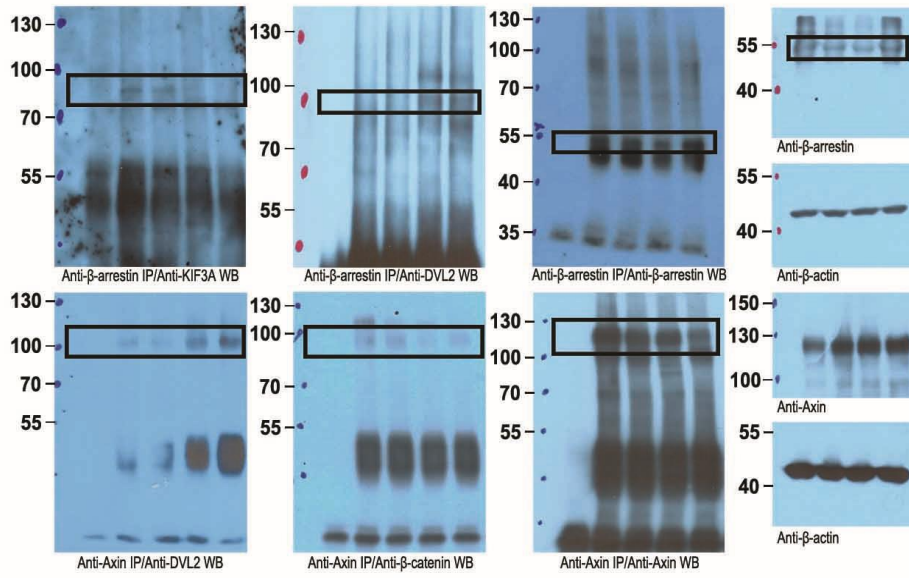
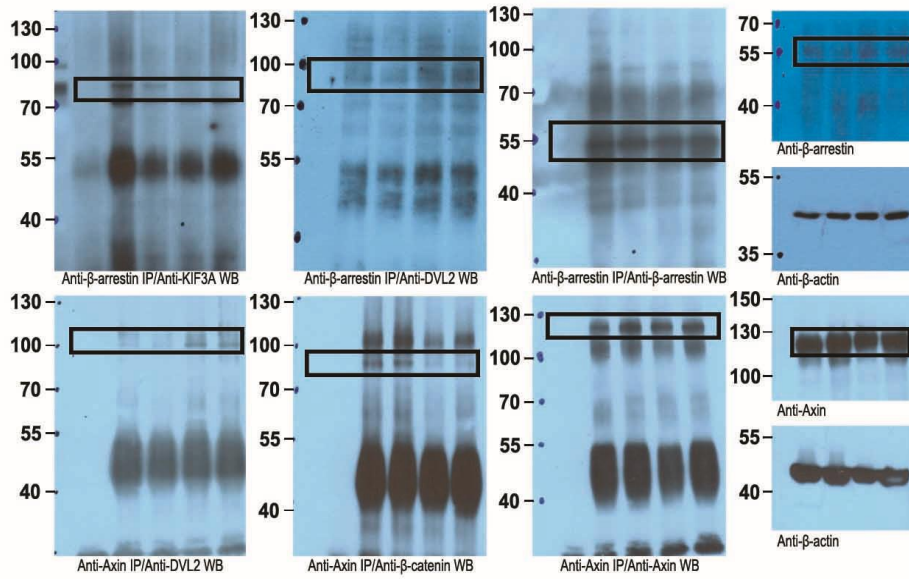
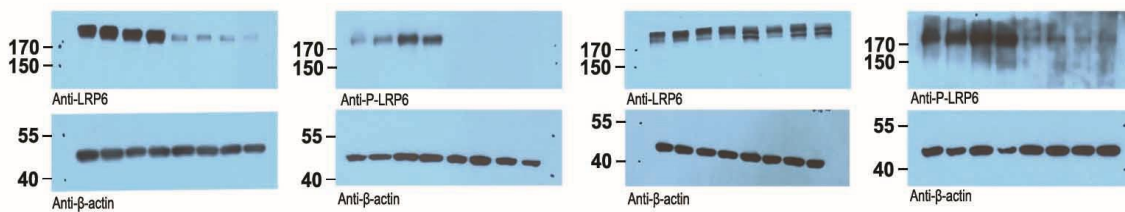


Fig. 6b



Supplementary Figure 3



Supplementary Figure 5. Full-length blots in this study.

Supplementary Table 1. KIF3A, β -catenin and Cyclin D1 expression scores according to IHC analysis.

Case #	Sex	Age	S.M.K.	KIF3A			β -catenin			CycD1			1: Adenocarcinoma, 2: Squamous cell carcinoma, 3: Adenosquamous cell carcinoma, 4: Sarcomatoid carcinoma, 5: Large cell carcinoma
				P	R1	R2	P	R1	R2	P	R1	R2	
1	F	48		1	1		4	4		45	45		1
2	M	32		0	0	1	2	3	1	30	25	10	2
3	M	79		0	1		2	2		30	1		1
4	F	54		0	2		3	3		25	65		1
5	M	70		1	2		1	1		70	55		1
6	M	61		1	2		2	2		1	1		1
7	M	54		1	1		1	1		5	5		1
8	M	52		1	2		2	1		5	10		2
9	M	56		0	0		2	2		85	75		1
10	M	40		1	1	2	1	1	4	70	5	25	1
11	F	55		2	1		3	2		10	40		1
12	M	55		1	0		1	4		80	5		5
13	F	53		2	0		1	3		5	55		1
14	M	36		0	2		3	1		35	1		2
15	M	68		0	1		4	3		1	1		5*
16	F	45		1	1		4	3		60	1		1
17	M	57		0	0		3	3		35	95		2
18	M	68		1	0		1	3		10	65		1
19	F	42		0	2	0	1	4	4	15	40	20	1
20	M	53		1	1		2	2		80	75		1

21	M	71		0	1		3	2		40	5		2
22	F	68		1	0		3	3		25	10		1*
23	M	37		1	0		2	2		85	65		1
24	M	46		2	1	0	2	3	3	1	1	1	2
25	F	58		2	n.d.		4	n.d.		75	n.d.		3
26	M	65		0	2		2	1		20	30		2
27	M	56		1	1		2	1		10	5		1
28	M	55		0	1		3	3		15	15		2
29	M	64		0	1		3	3		25	15		2
30	M	51		1	0		2	4		60	40		1
31	M	43		0	1	1	3	2	3	35	50	35	2
32	M	61		0	0	0	3	4	3	45	40	25	2
33	M	59		2	1		4	4		35	65		2
34	M	62		2	n.d.		0	n.d.		1	n.d.		1
35	F	38		0	1		3	2		25	30		1
36	M	52		1	1		1	1		40	40		2
37	M	68		1	0		1	2		80	15		1
38	M	60		0	1		4	1		70	30		2
39	M	58		2	1	2	2	2	2	15	15	25	1
40	M	61		0	0		4	3		85	75		2
41	M	67		2	2	2	2	3	3	20	60	55	1
42	M	53		0	1		2	2		40	65		2
43	M	65		0	0		2	2		35	25		4
44	F	53		1	2		4	2		80	50		1

45	M	51		1	n.d.		3	n.d.		1	n.d.		1
46	M	69		1	1		2	3		40	25		2
47	F	67		2	2		3	4		50	65		1
48	M	56		0	n.d.		4	n.d.		55	n.d.		1
49	M	61		1	2		1	2		5	40		2
50	M	65		2	2		3	3		15	25		1
51	M	60		0	1		1	1		20	5		2
52	M	71		1	0		4	4		45	75		2
53	M	71		0	n.d.		3	n.d.		55	n.d.		2
54	M	73		2	2		2	1		15	0		2
55	M	60		2	2		2	4		35	25		1
56	M	72		1	1		2	2		35	5		1
57	M	70		2	2		1	1		5	5		1
58	F	38		2	1		1	1		1	30		1
59	M	45		1	2		4	3		45	50		1
60	M	68		2	1	1	1	2	2	45	5	50	1
61	F	46		0	2		3	2		55	40		1
62	M	53		0	2		2	2		1	1		2
63	F	70		1	1		1	1		10	10		1
64	M	54		1	1		4	4		60	85		1
65	M	70		1	0		2	3		45	5		2
66	M	50		0	0		2	4		60	70		2
67	M	71		0	2		3	1		15	70		2
68	M	64		2	1		2	2		15	10		1

69	M	54		2	2		2	2		25	30		1
70	M	51		2	2	0	2	2	3	5	5	1	1
71	F	60		2	2		2	3		30	10		1
72	F	62		0	1		3	2		25	45		1
73	F	45		0	2		3	3		40	35		1
74	F	61		0	1		3	3		50	65		1
75	M	51		2	2	2	3	2	2	55	95	75	1
76	M	50		2	2		2	3		15	60		1
77	M	60		2	1		4	4		55	50		2*
78	M	57		2	1		2	3		45	85		1
79	M	60		2	1		1	2		15	50		1
80	M	56		0	2		3	3		30	35		1
81	M	59		2	0		1	1		15	5		1
82	F	37		0	1	1	4	3	2	85	25	60	1
83	F	61		1	1	1	2	2	2	1	5	15	1
84	M	75		2	2		2	4		1	25		2
85	M	66		2	2		1	1		15	30		1
86	F	63		1	1		1	1		25	50		1
87	M	68		2	2		1	2		5	35		1
88	F	58		0	0		2	3		5	1		1
89	M	74		1	2	2	2	2	3	45	55	15	2
90	M	57		1	1		2	2		70	60		1
91	M	56		1	2		3	2		80	100		2
92	M	57		2	2		1	2		25	10		1

93	M	68		0	1		1	2		10	15		2
94	M	60		1	n.d.		2	n.d.		60	n.d.		2
95	M	66		2	0		2	0		15	5		2
96	F	67		1	0		2	2		30	15		1
97	M	63		2	2		2	3		10	20		2
98	F	64		2	2		2	1		5	25		1
99	F	56		2	2		3	2		5	25		1
100	M	54		2	2		2	3		10	10		1
101	M	61		0	2		3	2		75	5		2
102	M	65		0	1		3	2		70	10		2
103	M	58		2	2	0	1	1	2	1	1	20	1
104	F	44		2	2		1	3		5	10		1
105	M	75		1	1	0	1	3	2	10	50	25	2
106	F	50		1	1		1	3		20	35		1
107	M	71		2	2		4	4		5	5		2
108	M	55		2	0	2	1	2	2	5	25	25	1
109	F	76		1	2		4	4		85	20		1
110	M	66		1	1		2	1		20	65		2

n.d; IHC data not done, M; male, F; female, S.M.K. (Smoking status): dark gray; ever smoker, empty; never smoker. P; primary, R1; 1st recurred tumors, R2; 2nd recurred tumors, score 4 in β -catenin score; nuclear expression, n*; recurred tumor showed different histologic feature with primary tumor.

Supplementary Table 2. Clinic characteristics of patient-derived primary NSCLC cells.

PCC #	Sex	Age	Histology
PCC1	58	F	Adenocarcinoma
PCC2	60	F	Adenocarcinoma
PCC3	60	F	Sarcomatoid carcinoma
PCC4	72	M	Squamous cell carcinoma
PCC5	66	M	Squamous cell carcinoma
PCC6	54	M	Squamous cell carcinoma
PCC7	61	M	Adenocarcinoma
PCC8	72	F	Adenocarcinoma

Supplementary Table 3. The list of primer sequences used for quantitative RT-PCR and RT-PCR.

Gene	Primer name	Primer sequence
NANOG	NANOG_f	5'-CTAAGAGGTGGCAGAAAAACA-3'
	NANOG_R	5'-CTGGTGGTAGGAAGAGTAAAGG-3'
OCT4	OCT4_f	5'-GACAACAATGAAAATCTTCAGGAGA-3'
	OCT4_R	5'-CTGGCGCCGGTTACAGAACCA-3
SOX2	SOX2_f	5'-ACGACGTGAGCGCCCTGCAGTACAA-3'
	SOX2_R	5'-GCTGGAGCTGGCCTCGGACTTGACC-3'
β -actin	β -actin_f	5'-AGAGCTACGAGCTGCCTGAC-3'
	β -actin_R	5'-AGCACTGTGTTGGCGTACAG-3'
GAPDH	GAPDH_f	5'-AGCCACATCGCTCAGACAC-3'
	GAPDH_R	5'-GCCCAATACGACCAAATCC-3'