Supplementary Figures and Tables

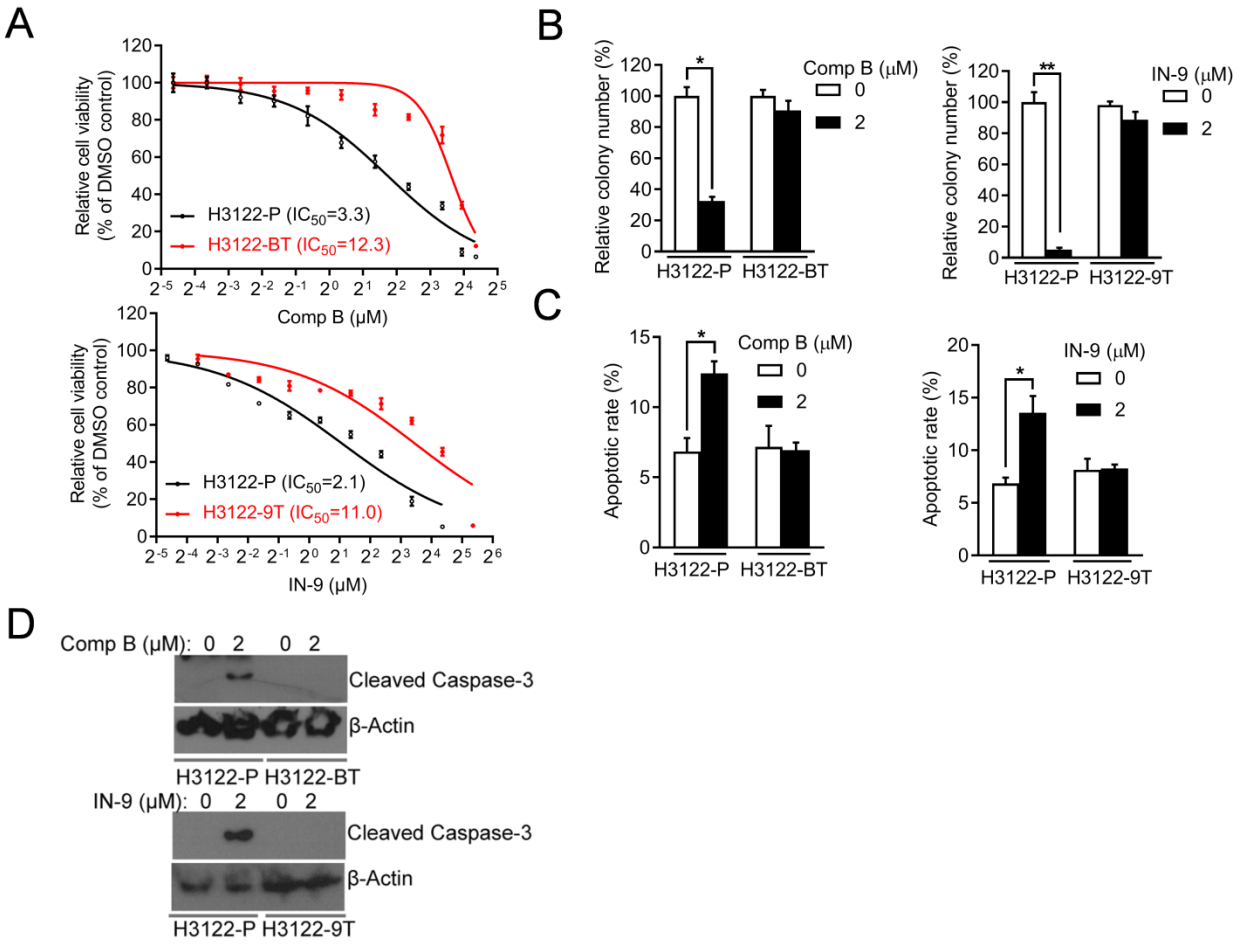

Figure S1 Characterization of drug-tolerant H3122 cells. (A) Proliferation assays on parental (P), compound B-tolerant (BT), and IN-9-tolerant (9T) H3122 cells. Cells were treated for 4 days with the indicated concentrations of compound B or IN-9. Half-maximal inhibitory (IC_{50}) concentrations were determined. (B) Colony formation assay on cell lines described in (A). Values were normalized to DMSO controls. (C) Apoptosis quantified by Annexin V/PI flow cytometry analysis after treatment with 2 μM compound B (left bar graph) or 2 μM IN-9 (right bar graph). (D) Western blot (WB) analysis of cleaved caspase-3 in H2122-P and H2122-BT cells treated for 48 hours with 2 μM Comp B (upper gels) or 2μM IN-9 (lower gels). Values were normalized to vehicle control. Data are shown as mean ± SEM of triplicate biological replicates. *P* values: two-sided t-test. *, $P<0.05$ and **, $P<0.01$.

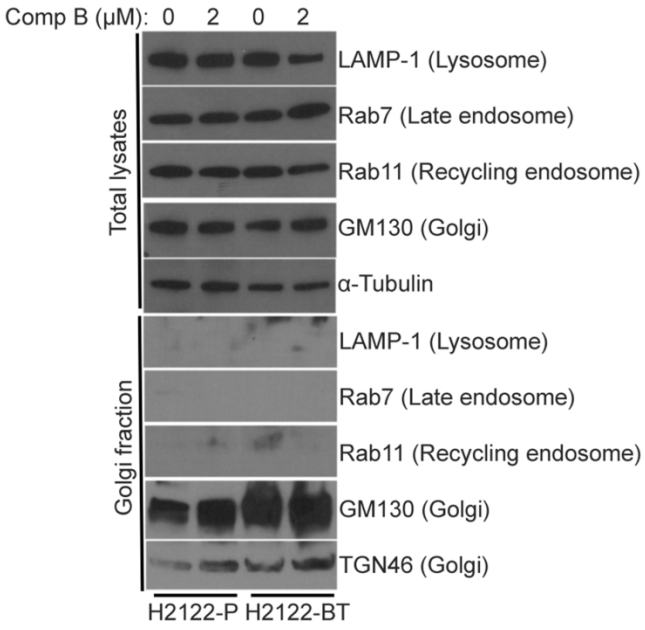

Figure S2 WB analysis of lysosomal, late endosomal, recycling endosomal, and Golgi markers in Golgi-enriched cellular fractions that were subjected to PI4P ELISA. Total cell lysates included as a control.

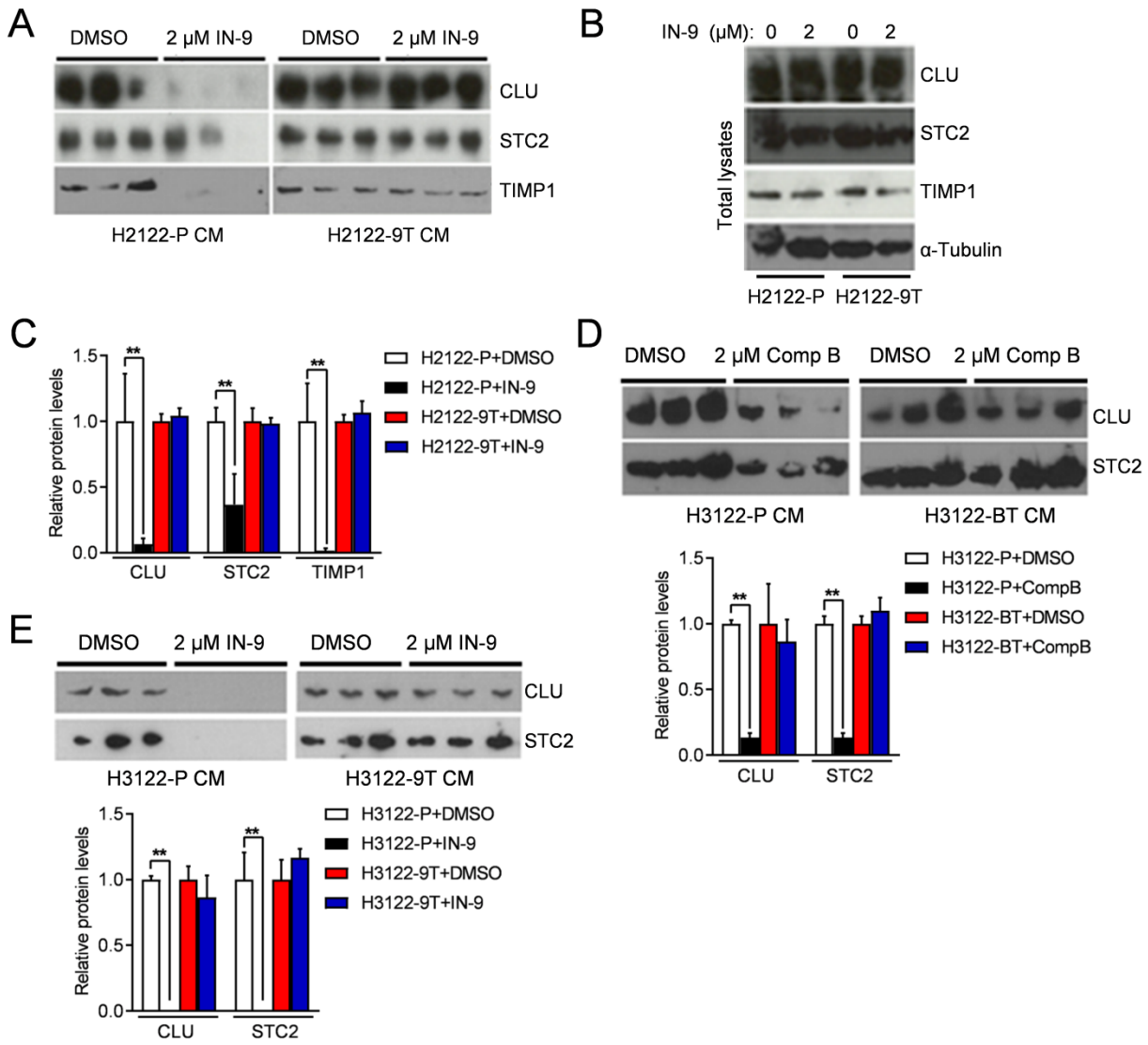

Figure S3 Drug-tolerant 1q-LUAD cells maintain PI4P-dependent secretion. (A and B) WB analysis of H2122 cells (-P or -9T) after treatment with IN-9 or vehicle (DMSO) to detect CLU, STC2 and TIMP1 in conditioned medium (CM) samples (A) and cell lysates (B). (C) Protein levels in CM were densitometrically quantified and values were normalized to DMSO control. (D and E) WB analysis of CM samples from H3122 cells (-P, -BT, or -9T) after treatment with compound B (D) or IN-9 (E). Protein levels in CM described were densitometrically quantified (bar graphs). Values were normalized to DMSO controls. Data are shown as mean ± SEM of triplicate biological replicates. *P* values: one-way ANOVA. **, $P<0.01$.

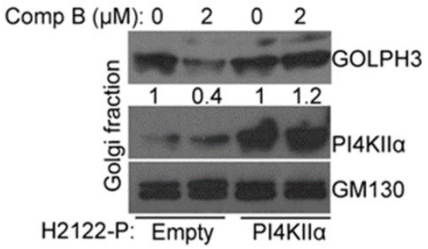

Figure S4 WB analysis of PI4KIIα and GOLPH3 in Golgi-enriched cellular fractions of H2122 cells stably transfected with PI4KIIα or empty expression vectors. GM130 served as loading control.

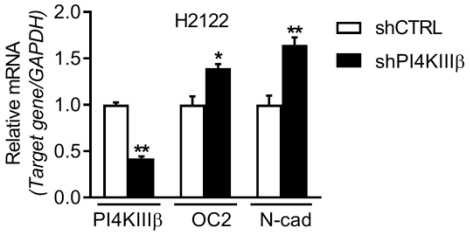

Figure S5 PI4KIIIβ depletion upregulates predicted miR-218-5p targets. qPCR analysis of PIKIIIß, OC2 and N-cad mRNA levels. Values were normalized to shRNA control (shCTRL). Data are shown as mean ± SEM of triplicate biological replicates. *P* values: two-sided t-test. *, *P*<0.05 and **, *P*<0.01.

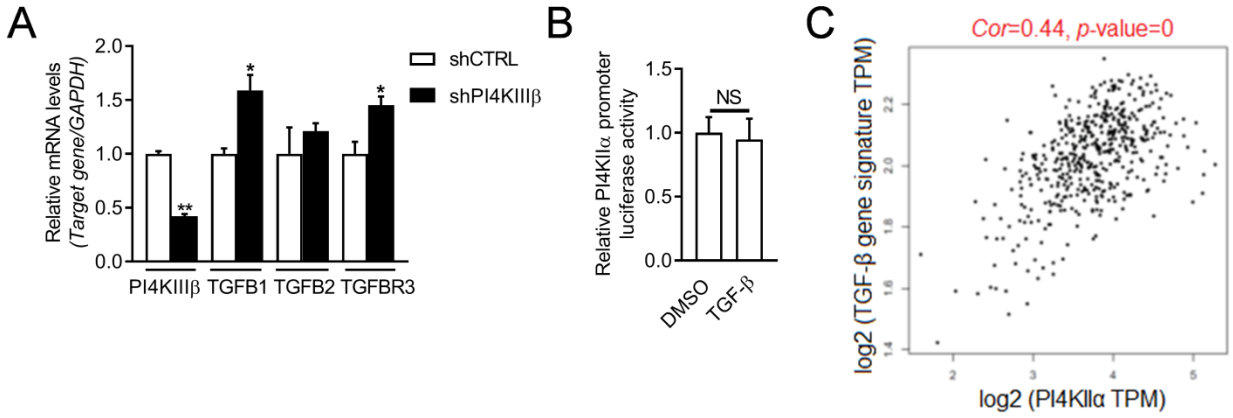

Figure S6 PI4KIIIβ deficiency activates TGFβ-dependent signaling. (A) qPCR analysis of TGF-β1 and TGFβ receptor family members in *PI4KB*-deficent and –replete H2122 cells. Values were normalized to shRNA control. (B) Luciferase assays on H2122 cells transfected with a *PI4K2A* gene promoter reporter and treated with 10 ng/ml TGF-β or vehicle (DMSO). Values were normalized to DMSO control. (C) Correlation between PI4KIIα mRNA levels and a TGF-β gene expression signature in the TCGA LUAD cohort. Data are shown as mean ± SEM of triplicate biological replicates. *P* values: two-sided t-test. NS, not significant. *, *P*<0.05 and **, *P*<0.01

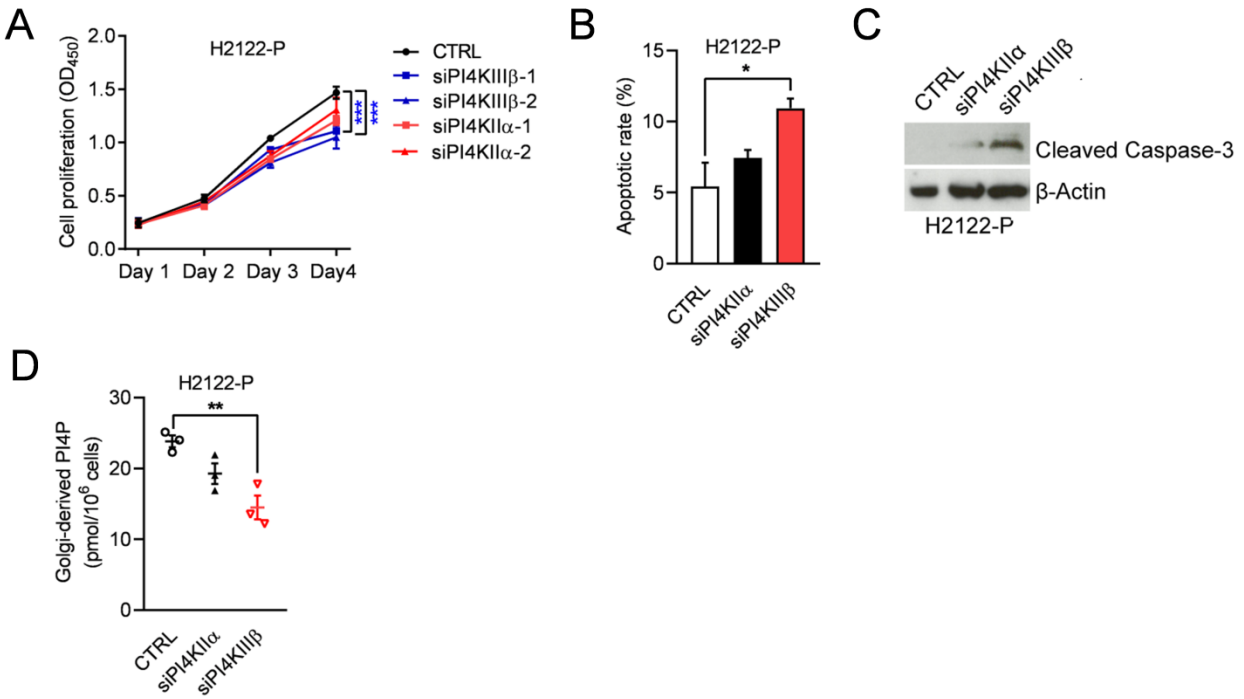

Figure S7 Parental H2212 cells rely on PI4KIIIβ for survival. (A) Relative densities of siRNA-transfected H2122-P cells in monolayer culture. (B, C) Apoptosis quantified by flow cytometric analysis of Annexin V/PI staining (B) and WB analysis of cleaved caspase-3 (C) in siRNA-transfected H2122-P cells. (D) PI4P ELISA on Golgi-enriched fractions from siRNA-transfected H2122 cells (dot plot). Data are shown as mean ± SEM of triplicate biological replicates. *P* values: two-way ANOVA (A), one-way ANOVA (B and D). *, *P*<0.05, **, *P*<0.01 and ***, *P*<0.001.

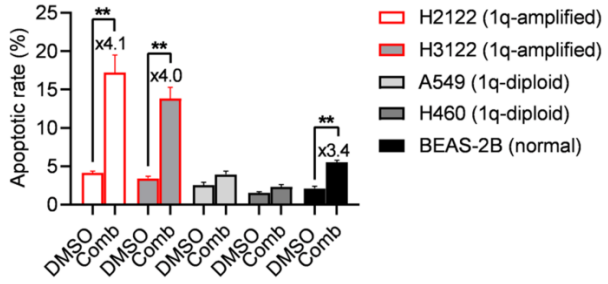

Figure S8 Apoptosis quantified by flow cytometric detection of Annexin V/PI staining of indicated cells treated for 48 h with a combination of 2 μM compound B and 5 μM PI-273 (Comb) or vehicle (DMSO) control. Data are shown as mean ± SEM of triplicate biological replicates. *P* values: two-sided t-test. **, *P*<0.01.

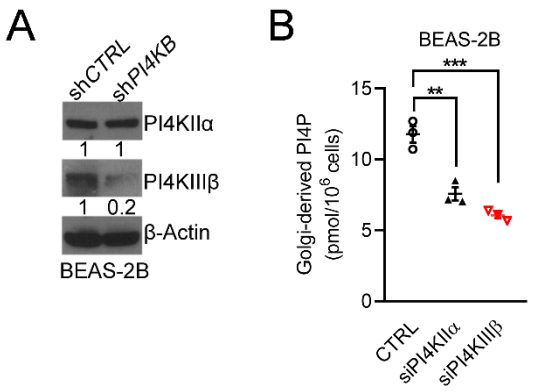

Figure S9 PI4KIIIβ depletion did not activate the feedforward pathway in BEAS-2B cells. (A) WB analysis of PI4KIIIβ and PI4KIIα in BEAS-2B cells stably infected with lentiviruses expressing control (shCTRL) or PI4KIIIβ shRNAs. β-Actin served as loading control. Values were normalized to shCTRL. (B) PI4P ELISA on Golgi-enriched fractions from siRNA-transfected BEAS-2B cells (dot plot). Data are shown as mean ± SEM of triplicate biological replicates. *P* values: one-way ANOVA. **, $P<0.01$ and ***, $P<0.001$.

Supplementary Table 1 Predicted miRNA binding sites on the 3'UTR of PI4KIIα (www.TargetScan.org).

miRNA	Position in the 3'-UTR	Seed math conserved sites
has-miR-218-5p	159-166	8mer
has-miR-9-5p	1296-1302	7mer-m8
has-miR-9-5p	1459-1466	8mer
has-miR-140-3p.1	1655-1662	8mer
has-miR-323a-3p	2260-2267	8mer

Supplementary Table 2 Gene ontology enrichment analysis on significantly up-regulated genes in PI4KIIIβ-depleted H2122 cells.

Category	Term	Count in Selected Genes	Count in Total Population	P-value
GO	SMAD protein signal transduction	5	15	0.000181
GO	Positive regulation of SMAD protein import into nucleus	3	11	0.007414
GO	Common-partner SMAD protein phosphorylation	2	6	0.019983
GO	SMAD protein import into nucleus	2	7	0.027271

Supplementary Table 3 Oligonucleotides used in this study.

Name	Sequence 5'-3'
PI4K2A 3UTR-XbaI-F	CTGTCTAGAAGCGAGTCCTACACACAGAG
PI4K2A 3UTR-NotI-R	ATAGCGGCCGCTCTCAGAAATTCTTCCTG
2A 3UTR MUT-R	ATTTGTTTCCTCCCTGTGGGTGGAAAG
2A 3UTR MUT-F	AGGGAGGAAACAAATCAGGAACAGTGAGTGC
OC2 3UTR-XbaI-F	CTGTCTAGAAGCGAGTCCTACACACAGAG
OC2 3UTR-NotI-R	ATAGCGGCCGCAATCACATGAAAACGAG
OC2 3UTR MUT-R	GACCAATCGCAAGGTGCCAGAATTGTGA
OC2 3UTR MUT-F	TCACAATTCTGGCACCTTGCGATTGGTC
OC2 3UTR M3-F	ACTGTCGGCCAGTTTTAAATATGGACGT
OC2 3UTR M3-R	AACTGGCCGACAGTAATTACCATTCTGA
OC2 3UTR M4-F	GCTTCTCGGCCATTTTGCAGTTTGCTACA
OC2 3UTR M4-R	GCAAAATGGCCGAGAAGCCATATTCTCATCA
CDH2 3UTR-NotI-R	ATAGCGGCCGCAGAGGTGTATCATTTATATTC
CDH2 3UTR-M-F	GGAGAAAAGTTCTTACGACCAATGTTTTACATAATTTGTAC
CDH2 3UTR-M-R	TGGTCGTAAGAACTTTTCTCCCTCC
CDH2 3UTR-XbaI-F	CTGTCTAGAGTATGGTGGAGGTGATGACTG
PI4K2A P-NheI-F	TATGCTAGCGGTTCCGGTTCCGGTTCCGGT
PI4K2A P-HindIII-R	TATAAGCTTCGGCGCGCTCCACACCAGCGA
PI4K2A-XhoI-F	TATCTCGAGATGTACCCATACGATGTTCCAGATTACGCTGACGAGACGAGCCCACTAGT
PI4K2A-XhaI-R	CAGTCTAGATTACCACCATGAAAAGAAGGGCTTCC
PI4K2A P-F1	TGTGTAATCTTGGCCGTCCT
PI4K2A P-R1	GGCGGATGCCTTTAAACCTT
PI4K2A P-F2	CAGCAAGTGCCTTAGCTGTT
PI4K2A P-R2	GCTTGCAGGAGTGCAATGTA
GAPDH-F	GTCTCCTCTGACTTCAACAGCG
GAPDH-R	ACCACCCTGTTGCTGTAGCCAA
PI4KA-F	GCTTTAGACCGCAGTCATCCTC
PI4KA-R	CCAGCACAGATGATGAAGGAGC
PI4K2A-F	CGAGGCAATGACAACTGGCTGA
PI4K2A-R	GCCACCTTGATAACAGGCTCCT
PI4KB-F	TGGTCGGTGGATGACATAGGCG
PI4KB-R	CTGGTGATGCTGTCCACAGAGA
SACM1L-F	GCTGAAGCTGCATATCACACC
SACM1L-R	ACGGTCAATGGTAAGTACGTCA
GOLPH3-F	CTAGAGGCTTGTGGAATGAGACG
GOLPH3-R	GACCGTTTCTGGAGGCTGAGTT
OC2-F	CAGGATGTGGAAGTGGCTTCAG
OC2-R	TGAACACCAGGCGGGACTTCTT
CDH2-F	CCTCCAGAGTTTACTGCCATGAC
CDH2-R	GTAGGATCTCCGCCACTGATTC

LEF1-F	CTACCCATCCTCACTGTCAGTC
LEF1-R	GGATGTTCCTGTTTGACCTGAGG
TGFB1-F	TACCTGAACCCGTGTTGCTCTC
TGFB1-R	GTTGCTGAGGTATCGCCAGGAA
TGFBR2-F	GTCTGTGGATGACCTGGCTAAC
TGFBR2-R	GACATCGGTCTGCTTGAAGGAC
TGFB2-F	AAGAAGCGTGCTTTGGATGCGG
TGFB2-R	ATGCTCCAGCACAGAAGTTGGC
TGFB3-F	CTAAGCGGAATGAGCAGAGGATC
TGFB3-R	TCTCAACAGCCACTCACGCACA
TGFBRAP1-F	CTGCCTGGCTAGAGAAGCACAA
TGFBRAP1-R	TCATACAGGTCTGAGCGTGTGG