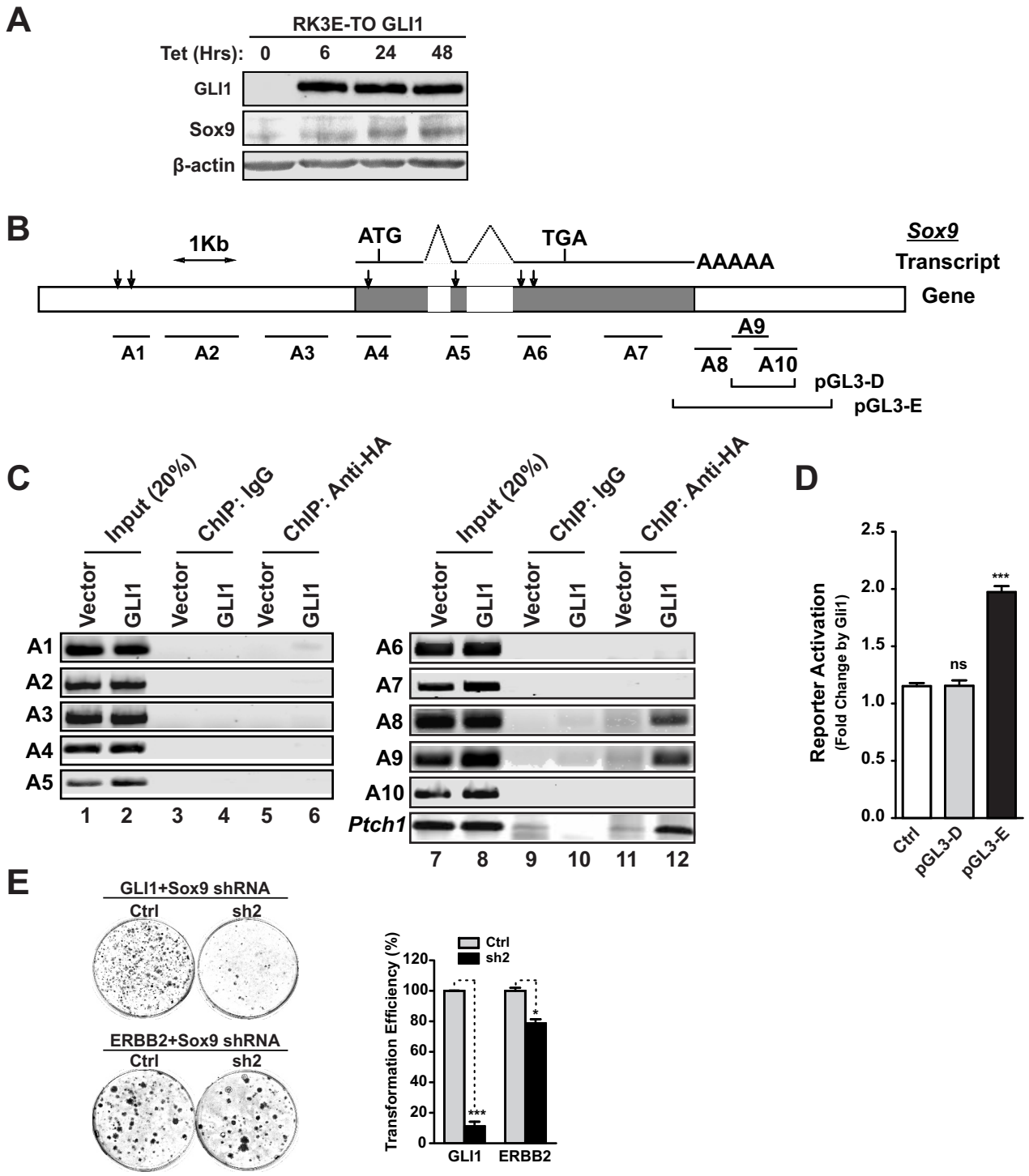

Figure S1. Sox9 is activated by GLI1 in the rat RK3E epithelial model and is required for GLI1-mediated malignant transformation. (A) RK3E-derived, tet-on (TO) GLI1 cells were induced with tet and cell extracts were analyzed by immunoblot. **(B)** Schematic of the RnSox9 locus. Potential GLI-binding sites (arrows) each contain one mismatch to the consensus GACCACCCA. ChIP-analyzed fragments (A1-A10) and fragments inserted into a luciferase reporter (pGL3-D, pGL3-E) are indicated below. Primer sequences are listed in Table S3 in the supplemental material. **(C)** ChIP analysis of Sox9 gene in RK3E cells. Chromatin was prepared from RK3E cells stably-transduced with HA-GLI1 retrovirus (GLI1) or empty vector (Vector). Ptch1 served as a positive control. **(D)** Regulation of luciferase reporter activity by GLI1 relative to empty vector in HEK293 cells. **(E)** In vitro transformation by GLI1 or ERBB2 was assessed in RK3E cells. ***, P<0.001; ns, not significant.

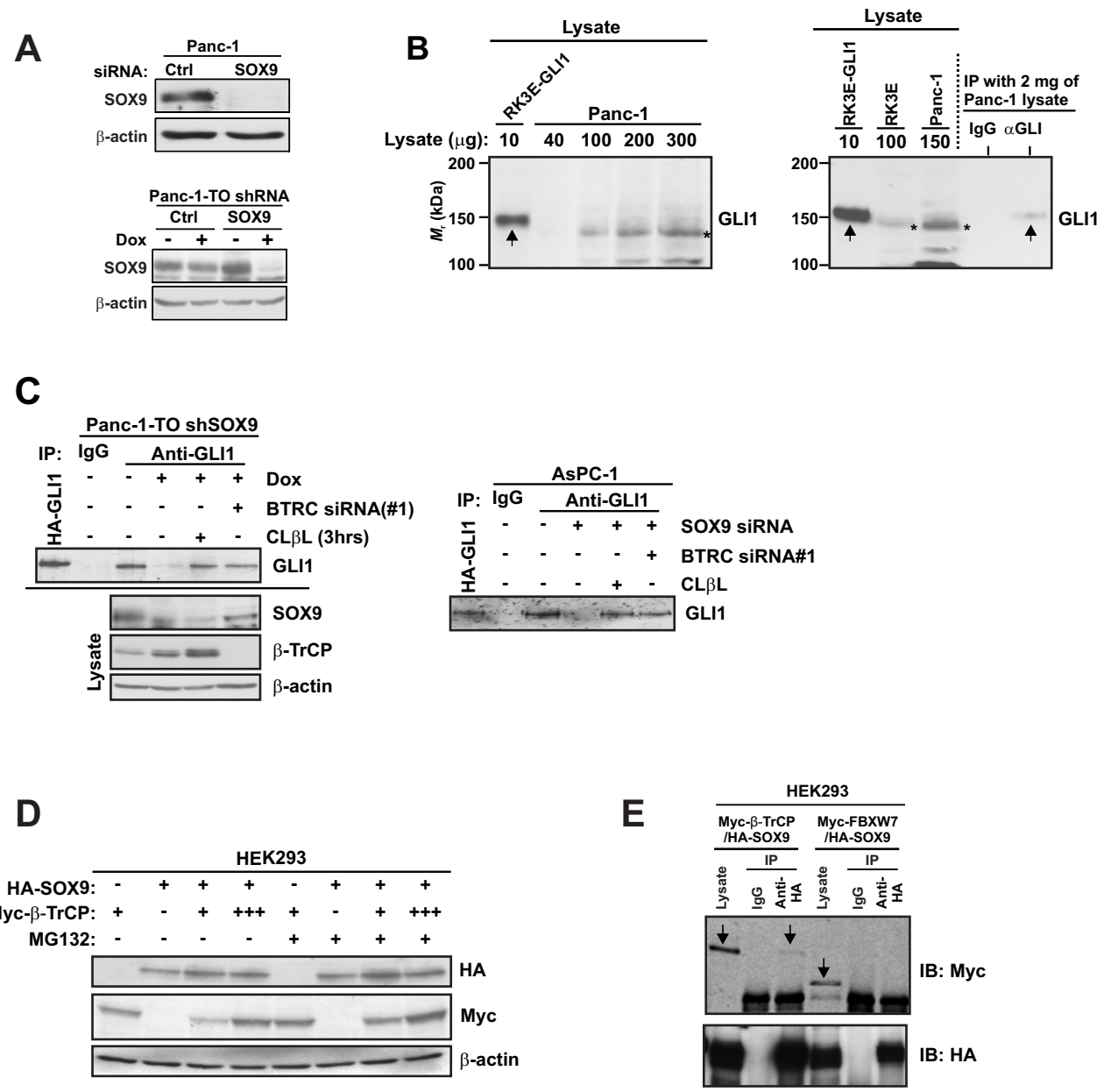

Figure S2. SOX9 interrupts β-TrCP-mediated GLI1 degradation. (A) SOX9 suppression mediated by transient siRNA or stable, conditional vector (TO-shSOX9) in Panc-1 cells. These two strategies target distinct sequences within SOX9. **(B)** Analysis of GLI1 protein in Panc-1 cells by immunoblot or IP-immunoblot analysis. Left panel: RK3E cells expressing exogenous GLI1 served as a control. By immunoblot a background species (asterisk) is detected in Panc-1 cells. In contrast, IP-immunoblot detects endogenous GLI1 (right panel). **(C)** SOX9 stabilizes the GLI1 protein in PDA cells. Left panel: Cells were treated with dox or vehicle control for 5 days and then analyzed by IP-immunoblot analysis. Right panel: Cells were treated as indicated and then analyzed by IP-immunoblot analysis. **(D)** Despite interacting with β-TrCP, SOX9 expression is not suppressed by the exogenous F-box protein. Instead, SOX9 suppresses β-TrCP expression in a proteasome-dependent fashion. Cells were transfected with the indicated vectors and treated with proteasome inhibitor (MG132) or vehicle (DMSO) prior to immunoblot analysis. The Myc-β-TrCP plasmid quantity in the transfection mixture was three-fold higher in the indicated lanes (+++). **(E)** SOX9 selectively interacts with β-TrCP as compared to another F-box protein, FBXW7. Co-IP analysis of HA-SOX9 with Myc-tagged F box proteins in HEK293 cells after transient expression. Lysate lanes represent 5% of the input extract.

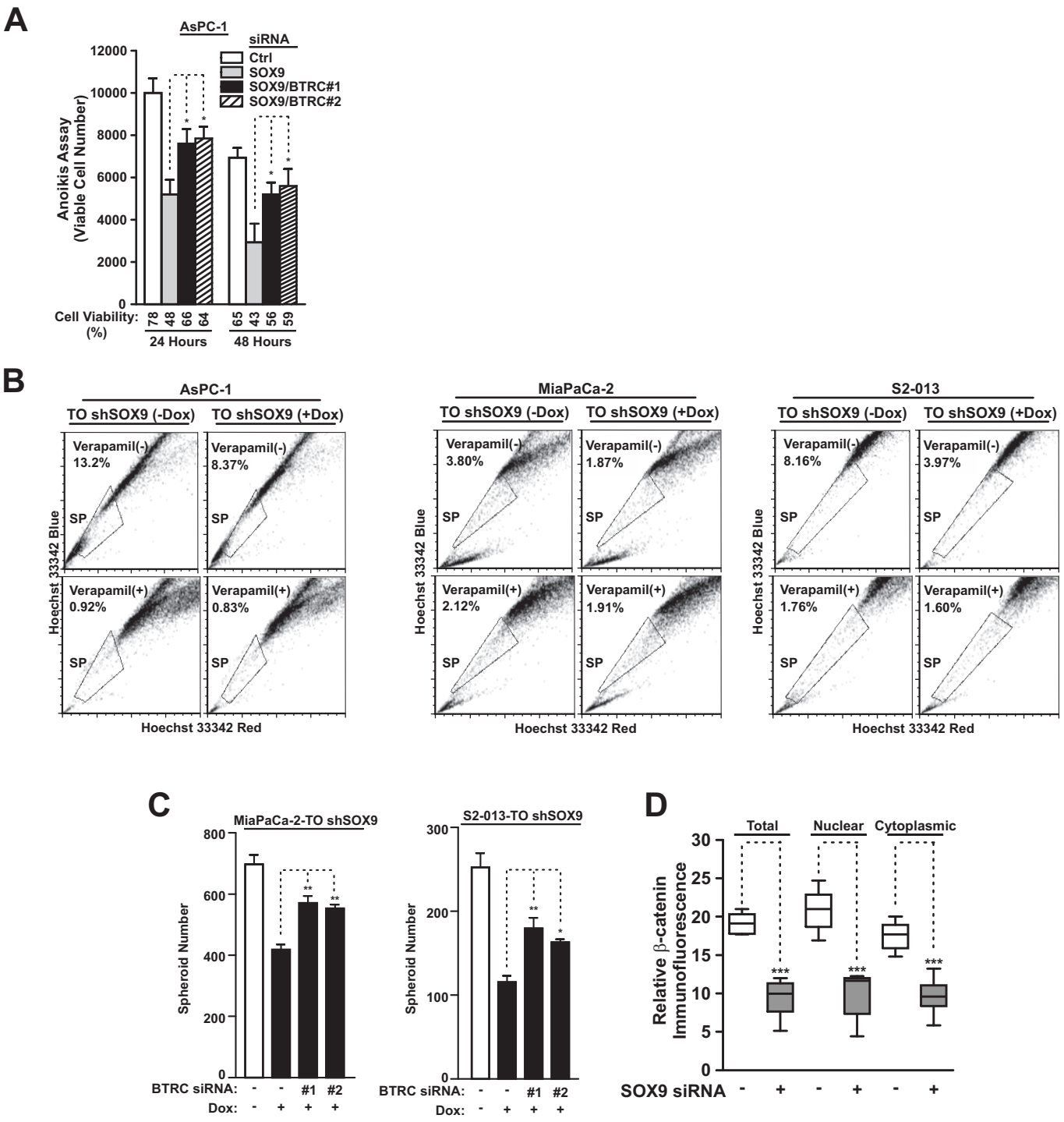

Figure S3. SOX9 is critical for cancer cell malignant properties, cancer stem cell properties and expression of β-catenin in PDA cells. (A) Anoikis assays were performed in AsPC-1 using two β-TrCP siRNAs. **(B)** PDA cells were treated with dox or vehicle for 5 days and then analyzed for Side population (SP) cells. **(C)** Roles of SOX9 and β-TrCP in tumor spheroid growth. **(D)** Regulation of β-catenin by SOX9. Panc-1 cells were treated with the indicated siRNA and the expression of β-cateninin was quantitated in the nucleus and cytoplasm. For each sample, confocal images were captured for ten randomly selected fields and immunofluorescence was quantitated using Image J. Boxes show the 25th-75th percentiles and the median is indicated. The whiskers show the range.

Figure S4. Gene expression profile analysis of human PDA. Human pancreatic carcinoma samples and normal pancreatic tissue were analyzed for expression of *SOX9* and *BTRC*. Published studies that showed upregulation of established PDA markers such as *SFN* (14-3-3σ) were selected for analysis of *SOX9* and *BTRC*. Box plots indicate median and inter-quartile range (box), 10th and 90th percentiles (whiskers), and minimum and maximum values (asterisk).

Table S1: Microarray expression analyses of Sox9 and β-TRCP in primary pancreatic cancer.

Data Source (Reference)	Sox9 (Cancer vs Normal)			β-TrCP (Cancer vs Normal)			Stratifin (Cancer vs Normal)		
	Probe	P value	Fold	Probe	P value	Fold	Probe	P value	Fold
Badea et al., 2008	202936_s_at	0.0020	1.87	204901_at	6.5E-07	-1.22	33323_r_at	1.8E-11	6.66
				222374_at	1.2E-05	-1.21			
Iacobuzio-Donahue et al., 2003	IMAGE:753184	0.19	1.35	IMAGE:461628	0.061	-1.25	IMAGE:2027515	1.1E-06	20.2
Pei et al., 2009	202936_s_at	0.014	1.79	222374_at	0.034	-1.12	33323_r_at	2.3E-08	19.0
Segara et al., 2005	202936_s_at	0.50	1.00	204901_at	0.026	-1.23	33323_r_at	3.3E-06	9.74

Table S2: Oligonucleotide sequences for siRNA and shRNA

siRNA/shRNA	Target sequence (5'-3')	Source
Ctrl	Firefly luciferase	siGENOME Non-Targeting siRNA #2, Thermo Scientific (Dharmacon), D-001210-02
Ctrl2	GATGGGGAGTTTCCAGGTTCA	Thermo Scientific (Dharmacon), custom
BTRC #1	GCGACATAGTTTACAGAGA	siGENOME siRNA, Thermo Scientific (Dharmacon), D-044048-04
BTRC #2	Smart Pool	siGENOME SMARTpool siRNA, Thermo Scientific (Dharmacon), M-003463-01
GLI1	Smart Pool	ON-TARGETplus SMARTpool siRNA, Thermo Scientific (Dharmacon), L-003896-00
SOX9	GAACGCACATCAAGACGGA	siGENOME siRNA, Thermo Scientific (Dharmacon), D-059108-01
TO shCtrl	Scrambled sequence	Non-silencing TRIPZ lentiviral inducible shRNAmir control (Open Biosystems, RHS4743)
TO shSOX9	ACCCGCTCACAGTACGACT	The hairpin sequence from pGIPZ-shRNAmir (Open Biosystems, V3LHS_396212) was inserted into pTRIPZ.
Rat Sox9 #1	AGAACAAGCCACACGTCAA	Thermo Scientific (Dharmacon), custom
Rat Sox9 #2	CTGCTGAACGAGAGCGAGA	Thermo Scientific (Dharmacon), custom
Rat shCtrl	Scrambled sequence	Hairpin sequence was inserted into the pSilencer 2.1-U6 neo vector (Life Technologies, AM5764).
Rat Sox9 sh1	GAGAGCGAGGAAGATAAAT	Hairpin sequence was inserted into the pSilencer 2.1-U6 neo vector (Life Technologies, AM5764).
Rat Sox9 sh2	GGAAGATAAATTCCCAGTGTG	Hairpin sequence was inserted into the pSilencer 2.1-U6 neo vector (Life Technologies, AM5764).

Table S3: Oligonucleotides for real-time quantitative PCR analysis

Gene	Sense (5'-3')	Antisense (5'-3')
BTRC	CTGCAGGGACACTCTGTCTAC	GAAGTCCCAGATGAGGATTGTG
CTNNB1	CCCACTGGCCTCTGATAAAGG	ACGCAAAGGTGCATGATTTG
c-Myc	CGACGAGACCTTCATCAAAA	TGCTGTCGTTGAGAGGGTAG
CD24	AAACAACAACTGGAACTTCAAGTAACT	GGTGGTGGCATTAGTTGGATTT
CD44	TGCCGCTTTGCAGGTGTAT	GGCCTCCGTCCGAGAGA
CD133	TGGATGCAGAACTTGACAACGT	ATACCTGCTACGACAGTCGTGGT
CXCR4	GCCTTATCCTGCCTGGTATTGTC	GCGAAGAAAGCCAGGATGAGGAT
ESA	GCAGCTCAGGAAGAATGTG	CAGCCAGCTTTGAGCAAATGAC
GAPDH	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA
GLI1	ACAGAAGGACTGTCTGGCCCGC	GGTGAGATGGACAGTGCCCGC
GLI2	GCCCTTCCTGAAAAGAAGAC	CATTGGAGAAACAGGATTGG
KLF4	AGAGTTCCCATCTCAAGGCA	GTCAGTTCATCTGAGCGGG
OCT4	GAAGCAGAAGAGGATCACCTTG	TTCTTAAGGCTGAGCTGCAAG
SNAI1	TCTGAGTGGGTCTGGAGGTG	CTCTAGGCCCTGGCTGCTAC
SOX2	AACCCCAAGATGCACAACTC	GCTTAGCCTCGTCGATGAAC
SOX9	CCCTTCGTGGAGGAGGCGGA	CCGGAGGAGGAGTGTGGCGA
Rat *Bcl2*	CGGAGGCTGGGATGCCTTTGT	TGTGGCCCAGGTATGCACCCA
Rat *Gapdh*	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA
Rat *Ptch1*	CCAGGCTGCTGTGGTGGTGG	CCGGCTGACACAGGGGCTTG
Rat *Sox9*	GCGAGGAAGATAAATTCCCAGTG	GTGGTCTTTCTTGTGCTGCACGC