SUPPLEMENTARY DATA

A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion

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Figure S1. The tGLI1 variant is frequently expressed in human breast cancer cells. Total RNA extracted from seven human breast cancer cell lines were subjected to RT-PCR to detect GLI1 and tGLI1 transcripts. Levels of β-actin transcripts were also determined to control for equal loading. The results showed that these cells expressed comparable levels of both GLI1 and tGLI1 transcripts. Lanes: 1, MCF-7; 2, BT-20; 3, SK-BR-3; 4, ZR75-1; 5, MDA-MB-361; 6, MDA-MB-453; 7, MDA-MB-468.

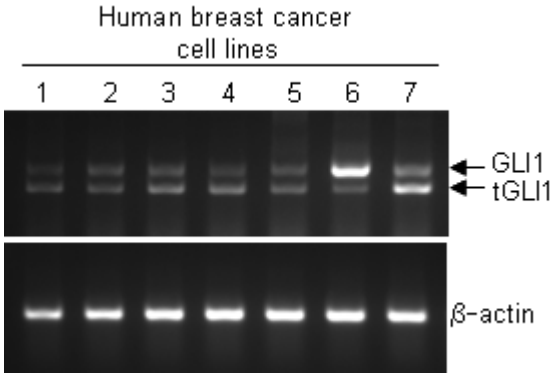


Figure S2. Cluster analysis of genes that are significantly and differentially expressed between U87MG-GLI1 and U87MG-tGLI1 cells. Based on the DNA microarray data and subsequent ANOVA analysis, genes differentially expressed between U87MG-GLI1 and U87MG-tGLI1 cells were subjected to cluster analysis. A total of 101 genes were identified to be significantly up-regulated or down-regulated (≥ 2 fold; $p<0.05$) in U87MG-tGLI1 cells relative to U87MG-GLI1 and U87MG-vector cells. In U87MG-tGLI1 cells, 75 genes were expressed at a significantly higher level and 26 genes were more suppressed compared to U87MG-vector and U87MG-GLI1 cells.

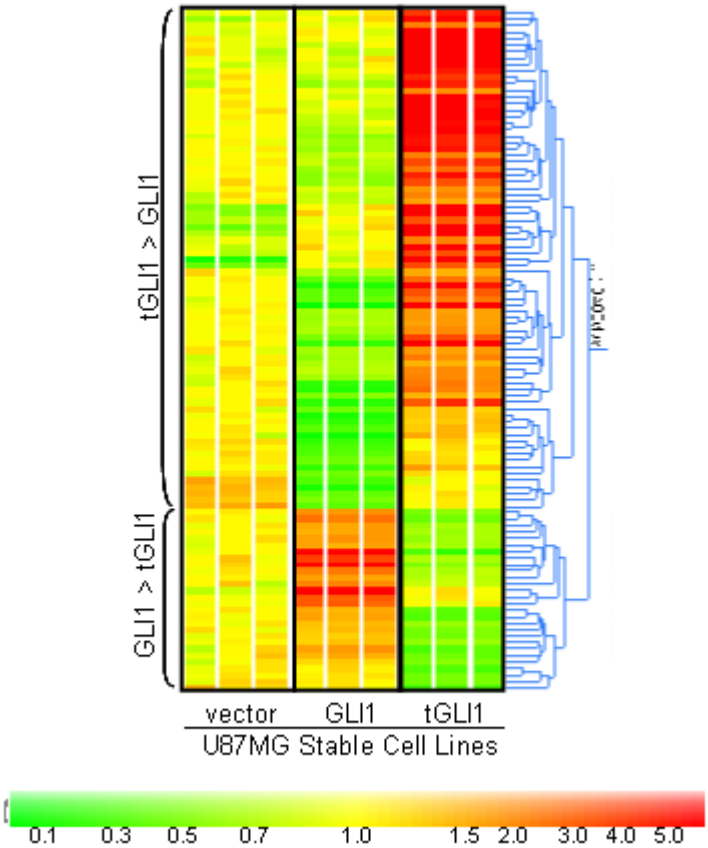


Figure S3. MEST gene expression is enhanced by tGLI1 but is not involved in tGLI1-mediated invasiveness in U87MG GBM cells.

A: MEST gene transcription is increased in U8MG-tGLI1 cells compared to U87MG-vector and U87MG-GLI1 cells. U87MG stable transfectants were subjected to RT-PCR (top) and RT-qPCR (bottom) to determine the levels of MEST and GAPDH transcripts. DNA sequences for primers that amplify MEST gene transcripts are 5'-TTTCCAACATCCAGCTACGA-3' (forward) and 5'-GGCCTGCTCAAATATG GAAT-3' (reverse).

B: **Activity of the MEST gene promoter is higher in U8MG-tGLI1 cells compared to U87MG-vector and U87MG-GLI1 cells.** The luciferase reporter construct, pMEST-Luc, contains a 1 kb human MEST gene promoter fragment, which was generated by PCR using primers, 5'-ATGCTAGCCACTTCGTGA TACTCTAC-3' (forward) and 5'-GGAGATCTCCGACTTTTAGAGCCCAC-3' (reverse). The promoter fragment was restricted by NheI and BglII and then ligated into the cohesive ends of the promoter-less pGL3-basic luciferase vector (Promega). The pMEST-Luc and pRL-TK (normalization control) reporter constructs were co-transfected into U87MG stable transfectants for 48 hrs and luciferase activity determined. Three independent experiments were performed to derive means and standard deviations.

C,D: **MEST expression down-regulation did not affect the invasiveness of U87MG-tGLI1 cells.** U87MG-tGLI1 cells were transfected with non-targeting control siRNA, CD24 siRNA and MEST siRNA for 48 hrs and subjected to RT-PCR and western blotting (C) and invasion assay (D). As indicated by results in panel C, MEST siRNA specifically down-regulated MEST but not CD24 and β-actin transcripts. In panel D (top), cell proliferative rates were also determined using Celltitier Blue Cell Viability assay kit to derive net invasiveness (invasiveness:proliferation). Three independent experiments were conducted to derive means and standard deviation. The bottom panel shows representative invaded tumor cells that have been stained by 0.5% crystal violet.

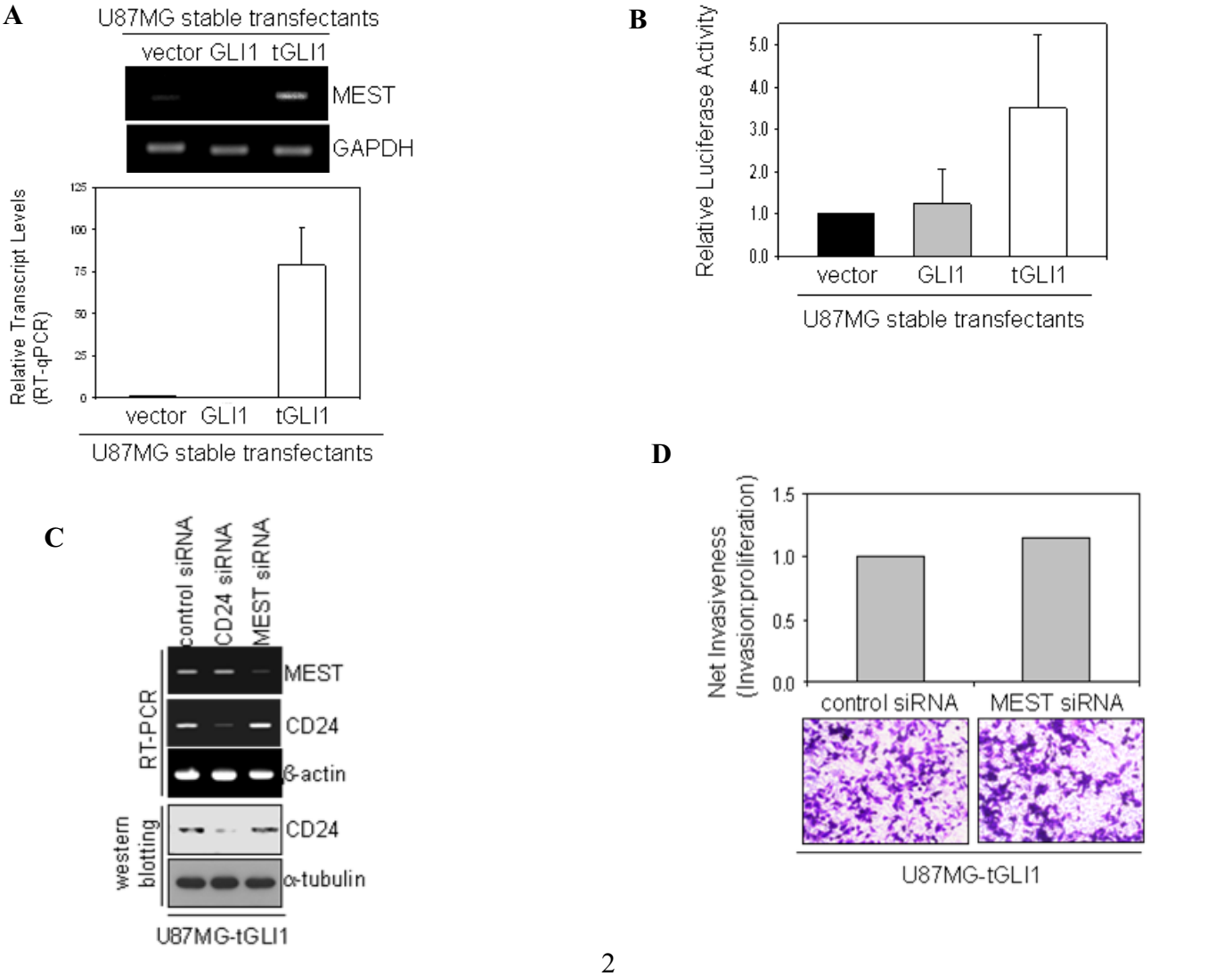
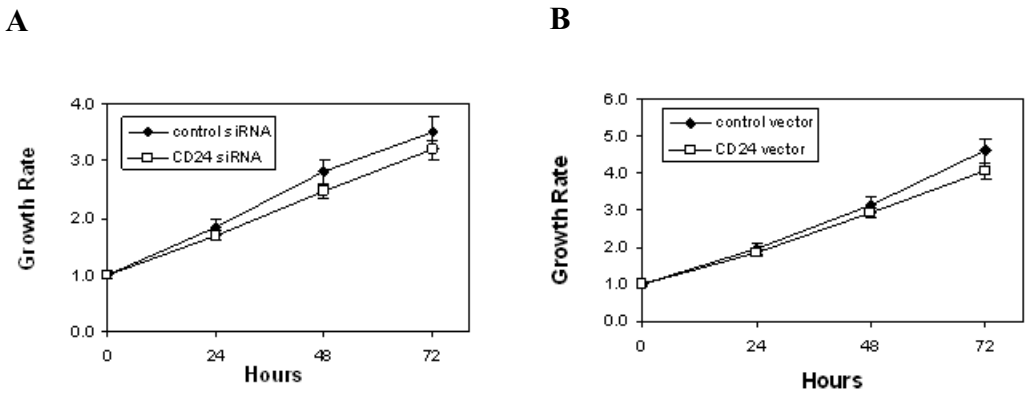


Figure S4. Transcriptional over-expression and down-regulation of CD24 did not significantly affect cell proliferation of U87MG GBM cells. To determine the effects of CD24 expression on U87MG cell proliferation, we transcriptionally down-regulated CD24 gene expression using U87MG-tGLI1 cells who express high levels of CD24 **(A)** and over-expressed CD24 using U87MG cells with low levels of CD24 **(B)**. These cells were also transfected with non-targeting siRNA and parental vector to control for CD24 siRNA and CD24 expression vector, respectively. Cell viability was then determined using Celltiter Blue Cell Viability assay kit (Promega) for 0-72 hrs. The viability at 0 hr was regarded as 1.0. Three independent experiments were performed to derive means and standard deviations. The student t-test was conducted and showed that CD24 expression did not significantly affect proliferation of U87MG GBM cells.



SUPPLEMENTARY TABLE I & II

Table I Genes significantly up-regulated in U87MG-tGLI1 cells compared to U87MG-GLI1 and U87MG-vector cells. DNA microarray data were subjected to ANOVA analysis to identify genes that are expressed in U87MG-tGLI1 cells at a significantly higher level, namely, at least 2-fold and $p<0.05$, compared to U87MG-GLI1 and U87MG-vector cells. The analysis revealed 79 hits corresponding to 75 genes in which both EPB41L4B and GREM2 genes had two hits each and for the FHL1 gene, three hits.

Probe Set ID	Gene Name	Gene Symbol	Fold change tGLI1 versus GLI1	p-value
202016_at	mesoderm specific transcript homolog (mouse)	MEST	260.2	1.01E-04
227099_s_at	hypothetical LOC387763	LOC387763	150.3	6.70E-05
209771_x_at	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	CD24	55.3	6.70E-05
227522_at	similar to mouse 2310016A09Rik gene	LOC134147	50.2	1.01E-04
223120_at	fucosidase, alpha-L- 2, plasma	FUCA2	49.0	1.02E-04
1558692_at	Hypothetical protein MGC13102	MGC13102	44.4	6.70E-05
228260_at	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	ELAVL2	25.3	6.70E-05
228280_at	similar to RIKEN cDNA 1200014N16 gene	MGC14289	24.1	1.02E-04
226702_at	hypothetical protein LOC129607	LOC129607	24.0	9.96E-05
202310_s_at	collagen, type I, alpha 1	COL1A1	20.8	1.02E-04
203060_s_at	3'-phosphoadenosine 5'-phosphosulfate synthase 2	PAPSS2	20.4	1.01E-04
1552658_a_at	neuron navigator 3	NAV3	19.0	1.40E-04
219295_s_at	procollagen C-endopeptidase enhancer 2	PCOLCE2	15.4	1.01E-04
201540_at	four and a half LIM domains 1	FHL1	14.4	1.01E-04
223710_at	chemokine (C-C motif) ligand 26	CCL26	14.2	1.32E-04
232027_at	spectrin repeat containing, nuclear envelope 1	SYNE1	11.8	9.94E-05
230085_at	Transcribed locus		11.7	1.01E-04
216095_x_at	myotubularin related protein 1	MTMR1	11.1	1.02E-04
1569290_s_at	glutamate receptor, ionotrophic, AMPA 3	GRIA3	9.7	1.02E-04
218918_at	mannosidase, alpha, class 1C, member 1	MAN1C1	9.3	1.01E-04
223502_s_at	tumor necrosis factor (ligand) superfamily, member 13b	TNFSF13B	9.2	7.66E-05
220161_s_at	erythrocyte membrane protein band 4.1 like 4B	EPB41L4B	9.1	1.01E-04
235603_at	heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	HNRPU	9.0	1.02E-04
204319_s_at	regulator of G-protein signalling 10	RGS10	8.4	1.10E-04
223427_s_at	erythrocyte membrane protein band 4.1 like 4B	EPB41L4B	7.9	1.01E-04
230560_at	syntaxin binding protein 6 (amisyn)	STXBP6	7.8	1.16E-04
225975_at	protocadherin 18	PCDH18	7.4	1.12E-04
225786_at	hypothetical protein LOC284702	LOC284702	7.3	1.02E-04
224901_at	stearoyl-CoA desaturase 5	SCD5	7.2	1.01E-04
240509_s_at	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)	GREM2	7.1	1.01E-04
210298_x_at	four and a half LIM domains 1	FHL1	6.9	1.31E-04
201539_s_at	four and a half LIM domains 1	FHL1	6.9	1.01E-04

201310_s_at	chromosome 5 open reading frame 13	C5orf13	6.6	1.45E-04
201162_at	insulin-like growth factor binding protein 7	IGFBP7	6.5	1.02E-04
219938_s_at	proline-serine-threonine phosphatase interacting protein 2	PSTPIP2	6.5	1.14E-04
230272_at	CDNA FLJ30810 fis, clone FEBRA2001440		6.5	1.01E-04
227001_at	NIPA-like domain containing 2	FLJ13955	6.4	6.70E-05
228959_at	CDNA clone IMAGE:5262734		6.3	9.94E-05
203700_s_at	deiodinase, iodothyronine, type II	DIO2	6.1	1.02E-04
1564837_at	hypothetical LOC151760	LOC151760	5.9	1.01E-04
209529_at	phosphatidic acid phosphatase type 2C	PPAP2C	5.7	1.10E-04
209121_x_at	nuclear receptor subfamily 2, group F, member 2	NR2F2	5.5	1.18E-04
238673_at	Transcribed locus		5.1	1.02E-04
223340_at	spastic paraplegia 3A (autosomal dominant)	SPG3A	4.9	1.07E-04
47550_at	leucine zipper, putative tumor suppressor 1	LZTS1	4.7	1.25E-04
219230_at	hypothetical protein FLJ10970	FLJ10970	3.9	1.01E-04
228191_at	CDNA FLJ33420 fis, clone BRACE2020028		3.7	1.01E-04
201506_at	transforming growth factor, beta-induced, 68kDa	TGFBI	3.6	1.01E-04
229285_at	ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent)	RNASEL	3.6	1.14E-04
235683_at	sestrin 3	SESN3	3.5	1.31E-04
203126_at	inositol(myo)-1(or 4)-monophosphatase 2	IMPA2	3.4	1.02E-04
209200_at	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	MEF2C	3.4	1.01E-04
235504_at	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)	GREM2	3.3	1.01E-04
236769_at	hypothetical protein LOC158402	LOC158402	3.1	1.02E-04
210222_s_at	reticulon 1	RTN1	3.1	1.01E-04
241722_x_at	Transcribed locus, moderately similar to XP_512541.1 PREDICTED: similar to hypothetical protein [Pan troglodytes]		3.1	1.24E-04
225904_at	chromosome 1 open reading frame 96	C1orf96	3.0	1.14E-04
235518_at	solute carrier family 8 (sodium/calcium exchanger), member 1	SLC8A1	3.0	1.02E-04
212944_at	Mitochondrial ribosomal protein S6	MRPS6	2.9	1.02E-04
226462_at	syntaxin binding protein 6 (amisyn)	STXBP6	2.9	9.94E-05
229256_at	phosphoglucomutase 2-like 1	PGM2L1	2.9	1.01E-04
225585_at	RAP2A, member of RAS oncogene family	RAP2A	2.7	1.01E-04
219918_s_at	asp (abnormal spindle)-like, microcephaly associated (Drosophila)	ASPM	2.7	1.01E-04
227576_at	CDNA FLJ43311 fis, clone NT2RI2009855		2.6	1.02E-04
213093_at	protein kinase C, alpha	PRKCA	2.6	1.14E-04
228306_at	Cornichon homolog 4 (Drosophila)	HSPC163	2.5	1.01E-04
209250_at	degenerative spermatocyte homolog 1, lipid desaturase (Drosophila)	DEGS1	2.5	1.12E-04
200762_at	dihydropyrimidinase-like 2	DPYSL2	2.5	1.07E-04
1555797_a_at	actin related protein 2/3 complex, subunit 5, 16kDa	ARPC5	2.4	1.16E-04
233286_at	PDZ domain containing RING finger 3	PDZRN3	2.3	1.41E-04

219481_at	tetratricopeptide repeat domain 13	TTC13	2.2	1.01E-04
218035_s_at	RNA-binding protein	FLJ20273	2.2	1.12E-04
212089_at	lamin A/C	LMNA	2.2	1.45E-04
202620_s_at	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	PLOD2	2.1	1.31E-04
203814_s_at	NAD(P)H dehydrogenase, quinone 2	NQO2	2.1	1.01E-04
236294_at	HECT, UBA and WWE domain containing 1	HUWE1	2.1	1.01E-04
217788_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	GALNT2	2.0	1.02E-04

Table II Genes significantly down-regulated in U87MG-tGLI1 cells compared to U87MG-GLI1 and U87MG-vector cells. Following DNA microarray and subsequent ANOVA analysis, we identified 26 genes that are significantly down-regulated (at 2-fold and $p<0.05$) in U87MG-tGLI1 cells compared to U87MG-GLI1 and U87MG-vector cells. The list shows 28 hits but 26 genes as both C9orf72 and ST7L genes had two hits each.

Probe Set ID	Gene Name	Gene Symbol	Fold change tGli1 versus Gli1	p-value
225331_at	chemokine (C-X-C motif) ligand 3	CXCL3	0.02	1.27E-05
1562048_at	interleukin 1, beta	IL1B	0.06	1.45E-03
220058_at	vanin 1 ; vanin 1	VNN1	0.12	9.94E-05
217990_at	serpin peptidase inhibitor, clade B (ovalbumin), member 7	SERPINB7	0.18	1.02E-04
204540_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	ABCC9	0.18	7.91E-05
210415_s_at	kynureninase (L-kynurenine hydrolase)	KYNU	0.19	1.01E-04
225919_s_at	synaptotagmin I	SYT1	0.22	1.01E-04
213889_at	dynein, axonemal, light intermediate polypeptide 1	DNALI1	0.25	1.21E-04
1552738_a_at	AN1, ubiquitin-like, homolog (Xenopus laevis)	ANUBL1	0.28	1.14E-04
217995_at	chromosome Y open reading frame 15B	CYorf15B	0.28	1.01E-04
211368_s_at	solute carrier family 22 (organic cation transporter), member 15	SLC22A15	0.28	1.02E-04
211163_s_at	ring finger protein 149	RNF149	0.29	1.01E-04
1552739_s_at	chromosome 9 open reading frame 72	C9orf72	0.30	1.02E-04
1553159_at	decay accelerating factor for complement (CD55, Cromer blood group system)	DAF	0.33	1.01E-04
201925_s_at	dynein, axonemal, heavy polypeptide 11	DNAH11	0.33	1.12E-04
1553134_s_at	suppression of tumorigenicity 7 like	ST7L	0.35	1.13E-04
225414_at	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	TNFRSF10C	0.37	1.01E-04
228497_at	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	CASP1	0.37	1.28E-04
214131_at	sulfide quinone reductase-like (yeast)	SQRDL	0.39	1.01E-04
223624_at	suppression of tumorigenicity 7 like	ST7L	0.40	1.14E-04
227081_at	phosphatidylinositol glycan, class L	PIGL	0.41	1.12E-04
203999_at	chromosome 9 open reading frame 72	C9orf72	0.41	1.29E-04
210662_at	outer dense fiber of sperm tails 2	ODF2	0.43	1.07E-04
208561_at	eukaryotic translation elongation factor 1 alpha 2	EEF1A2	0.44	1.02E-04
206421_s_at	guanosine monophosphate reductase 2	GMPR2	0.45	1.02E-04
205844_at	chromosome 17 open reading frame 39	C17orf39	0.46	1.01E-04
205067_at	hypothetical protein LOC152225	LOC152225	0.46	1.07E-04
207850_at	chromosome 3 open reading frame 6	C3orf6	0.49	1.38E-04

SUPPLEMENTARY METHODS

Plasmids. GLI1- and tGLI1-expressing plasmids were constructed using the parental pCMV-Tag2b vector (Stratagene) with a 5'-flag tag. Briefly, full-length GLI1 and tGLI1 cDNAs were obtained via RT-PCR using the primers 5'-GGGGATATCATGTTCAACTCGATGACCC (forward) and 5'-GGGGTCGACGGC ACTAGAGTTGAGGAATTC-3' (reverse), restricted by EcoRV and SalI, and ligated into the pCMV-Tag2b vector. The CD24 mammalian expression plasmid, pCMV-SPORT6-CD24, was from Open Biosystems (Huntsville, AL) and the parental pCMV-SPORT6 vector was obtained from it by restriction digestion with SmaI and ApaI to release the CD24 cDNA and ligation. pCD24-0.14kb-Luc was obtained by releasing a MluI-BmgBI fragment from the pCD24-0.3kb-Luc construct. pCD24-0.25kb-Luc and pCD24-0.2kb-Luc was obtained via mutagenesis.

Reverse transcription-PCR (RT-PCR). Total RNA isolation and RT were conducted using SV Total RNA Isolation System (Promega) and Superscript II First-Strand cDNA synthesis system (Invitrogen), retrospectively. The forward and reverse primers used for the PCR were: 5'-TGTTCAACTCGATGACCC-3' and 5'-GTCATGGGGACCACAAGG-3' (exons 1-4 of GLI1 and tGLI1reverse), 5'-GGCGGCA CCACCATGTACCC-3' and 5'-AGGGGCCGGACTCGTCATACT-3' (β-actin), 5'-ATGGGCAGAG CAATGGTGGCCA-3' and 5'-AGAGTGAGACCACGAAGAGACT-3' (CD24), 5'-TCTGGAGCAGAT TTCCAAGGGGAA-3' and 5'-AGGATTAAACATAGCCTCTTC TCC-3' (PTCH1), and 5'-TTTCCAAC ATCCAGCTACGA-3' and 5'-GGCCTGCTCAAATATGGAAT-3' (MEST). qRT-PCR was performed in the Mx3005P qPCR System (Stratagene) using the SuperScript III platinum SYBR green one-step qRT-PCR system (Invitrogen). GAPDH gene was used as normalization controls and all experiments were done in triplicates.

Transfection and luciferase assay.
All transfections were performed with cells in exponential growth using lipofectamine 2000 (Invitrogen, Carlsbad, CA) and FuGENE HD (Roche). A Renilla luciferase expression vector, pRL-CMV was used to control for transfection efficiency. Forty-eight hrs after transfection, the cells were lysed and luciferase activity measured using the Firefly and Renilla Luciferase Assay Kit (Biotium, Hayward, CA), as previously described (1-4).

Nuclear fractionation, immunofluorescence staining/confocal microscopy.
These were performed as previously described (1, 2, 5). In the immunofluorescence staining experiments, a mouse monoclonal flag-tag antibody (Sigma) was used to detect flag-tagged GLI1/tGLI1 and a fluorescein-conjegated secondary antibody was used to generate green fluorescence signals. Propidium iodide was used to label nuclei. Fluorescence signals were scanned using a Zeiss LSM 510 confocal microscope.

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