

## Supplementary Information

### Supplementary Figure Legends

**Figure S1. Nucleotide sequence of the 1.7-kb genomic DNA around the *Hck* transcription start site.**

Positions are given relative to the transcription start site (TSS, +1) of the *Hck* gene. The boxed regions indicate ChIP-qPCR products. The potential Gli binding motif is indicated.

**Figure S2. Comparison of human and mouse Gli1 protein sequences.** The conserved Tyr residues are marked with upper case “Y” below the sequence. The lower case “y” indicates the tyrosine residues present only in mouse or human Gli1. The zinc-finger domain is indicated.

**Figure S3. Phosphorylation of Gli1 mutants by Hck.** (A) Map of Gli1 constructs relative to the full-length Gli1 protein. Several conserved Tyr residues are marked. (B) NIH3T3 cells were co-transfected with constructs expressing Hck and different HA-tagged Gli1 proteins. Cell lysates were precipitated with 4G10 antibody and probed with anti-HA. Phosphorylation of all Gli1 constructs was detected.

**Figure S4. Sufu interacts with non-phosphorylated Gli1 proteins.** NIH3T3 cells were transfected with constructs expressing HA-Gli1 and Flag-Sufu proteins in the presence or absence of exogenous HA-Hck. Cell lysates were precipitated with anti-Flag antibodies and antibodies against HA or Flag were used for western blot. Phosphorylated Gli1 bands (which migrate more slowly) were detected in the input protein lysate (marked by a \*) but not in the Flag-precipitated compartment in the presence of exogenous Hck.

**Figure S5. Hck is highly expressed in Shh-type medulloblastoma** (A) Microarray analyses (NCBI Geo Datasets GSE9299) indicate that both *Gli1* and *Hck* but not *Src* mRNA levels are higher in SmoM2 induced medulloblastoma than in normal cerebellum controls. (B) Microarray analyses (GSE34126) indicate that *Hck* mRNA levels in mouse *Ptch1*-null Shh-type medulloblastomas are higher than those in normal stem cells and those in Myc-driven Group 3 like medulloblastomas.

## Supplementary Tables

**Table S1 Sequences of PCR-Primers**

Gli1F	GGTCTCGGGGTCTCAAACCTGC
Gli1R	CGGCTGACTGTGTAAGCAGAG
Gli15UTRF	GCTGGAGGTCTGCGTGGTAGAG
Gli15UTRR	GAGGAGGAGGAGGAAAGAGAGATCC
HckhmF	TCACTGGTGTGTAAGATTGCTG
HckhmR	GGGTTTGACATACCTGGGTAAG
HckmF	CAAGTCTTCGTCGCTTGCTC
HckmR	CGGAGGAACCTGGACTTCAC
Ptch1F	GAAGCCACAGAAAACCCTGTC
Ptch1R	GCCGCAAGCCTTCTCTAGG
SufuF	ATGGCCGGCACTTCACCTACAAG
SufuR	GGCCAGCTGTA CTCTTTGGGAAG
GapdhF	GTGGTGAAGCAGGCATCTGA
GapdhR	GCCATGTAGGCCATGAGGTC

Figure S1

-1575	GCTGCCTTTA	TTGGCTTCTT	TCCCATCTCA	ATCTCCTTCA	CTCAGTAGAT	AACAAGCATT	GTTTACTGCT	ACACAGACTA
	CGACGAAAT	AACCGAAGAA	AGGGTAGAGT	TAGAGGAAGT	GAGTCATCTA	TTGTTTCGTAA	CAAAATGACGA	TGTGTCTGAT
-1495	ATTTCACTTC	TGTGTCCAAG	TCAATTAAAC	CTGTTCAAGA	CTCGGTCTAT	TTTTAGAAAT	AGGGCATGAA	TATATTAATA
	TAAAGTGAAG	ACACAGGTTT	AGTTAATTTG	GACAAGTTCT	GAGCCAGATA	AAAATCTTTA	TCCCGTACTT	ATATAATTTT
-1415	TACATAGCAA	ACACATTAGT	GTTGGGTGAC	TGGGATTTCT	ACGTGGGCTG	GGGACCAGGC	TGTGTAGGAG	GCAGCTCTGG
	ATGTATCGTT	TGTGTAATCA	CAACCCACTG	ACCCTAAAGA	TGCACCCGAC	CCCTGGTCCG	ACACATCCTC	CGTCGAGACC
							<i>EcoRI</i>	
-1335	TTGTCAGAAC	TGCTACTGGG	CCTAGCGGGT	GGAActCTAG	AGCTGCTAAC	ACCGCTCGCG	CTCA <b>GAATTC</b>	ATACTTAAAA
	AACAGTCTTG	ACGATGACCC	GGATCGCCCA	CCTTGAGATC	TCGACGATTG	TGGCGAGCGC	GAGTCTTAAG	TATGAATTTT
-1255	CATAGTACTG	CTAAACGCAT	GCCTACATGC	ACGCGTAAGC	GAATGAATGA	GAGATACTGG	TGGTCTGGCC	TGGCTAGAGG
	GTATCATGAC	GATTTGCGTA	CGGATGTACG	TGCGCATTCG	CTTACTTACT	CTCTATGACC	ACCAGACCCG	ACCGATCTCC
			<i>PstI</i>					
-1175	AGGGAGGACT	CCTGTACGTT	<b>CTGCGAG</b> GTG	TGTGTTGCAG	TTTAGAGAGT	GTTGGTCCCT	GTCGTTTTGA	ATGATGTCTC
	TCCCTCCTGA	GGACATGCAA	AGACGTCCAC	ACACAACGTC	AAATCTCTCA	CAACCAGGGA	CAGCAAAACT	TACTACAGAG
							<b>Gli binding site</b>	
-1095	AGGCTGAGTG	GAATATGCCA	TGCAAACAGG	ACCTCAGTGG	TCTGCCAGTA	AGACTTCCTC	TCTTCAACTT	<b>GGGAGGTCCT</b>
	TCCGACTCAC	CTTATACGGT	ACGTTTGTCC	TGGAGTCACC	AGACGGTCAT	TCTGAAGGAG	AGAAGTTGAA	CCCTCCAGGA
-1015	CTGAGCAGTC	CCATATTCAG	CCCAGAATAA	GGCAAAGAAC	TCCCGGGGGC	GCCAGACTTT	GCATGGAGTT	TGAATAAGCG
	<b>GACTCGTCAG</b>	<b>GGTATAAGTC</b>	<b>GGGTCTTATT</b>	<b>CCGTTTCTTG</b>	<b>AGGGCCCGCC</b>	<b>CGGTCTGAAA</b>	<b>CGTACCTCAA</b>	<b>ACTTATTCGC</b>
-935	GTGGGTGGGC	GGGTGGAGGG	AAAACCGCTG	CATGTGGGAG	GGACCGAAGT	GGAAGGGCGG	TGGAGACCCA	CCAGAATCCT
	CACCCACCCG	CCCACCTCCC	TTTTGGCGAC	GTACACCCTC	CCTGGCTTCA	CCTTCCCGCC	ACCTCTGGGT	GGTCTTAGGA
-855	AGAGGGAGAG	GTTAGACTTA	TCAGGGCGTG	GTTAGACTTA	AAGGGAGCTA	CATTCCTTCT	GCGGCTGGTT	TAGGATCTTT
	TCTCCCTCTC	CAATCTGAAT	AGTCCGCAC	CAATCTGAAT	TTCCCTCGAT	GTAAGGAAGA	CGCCGACCAA	ATCCTAGAAA
-775	GAAAGGAAGG	ATAGGATCGG	TTGATTGCTC	CAGTGCAGGA	TTTAGGACCT	ATGGGATTAA	GGTCTCGTAG	GGGGCTGGTG
	CTTTCCTTCC	TATCCTAGCC	AACTAACGAG	GTCACGTCTC	AAATCCTGGA	TACCCTAATT	CCAGAGCATC	CCCCGACCAC
							<i>XhoI</i>	
-695	GCTGTAGTAC	AAACTACGGA	GAGAGAGGCC	CTGGATTCCA	TTCTCTAGAA	CACACAAGCG	CGCGCGCGCG	CGCT <b>CTCGAG</b>
	CGACATCATG	TTTGATGCCT	CTCTCTCCGG	GACCTAAGGT	AAGAGATCTT	GTGTGTTTCG	GCGCGCGCGC	GCGAGAGCTC
-615	ATTAAAACCC	TTTCAGAAAA	TTTGATTACA	AATCTAGAGA	ATTTGTAGGG	GTTAGGACTG	GGAACAGAAT	AGAAAGTGAG
	TAATTTTGGG	AAAGTCTTTT	AAACTAATGT	TTAGATCTCT	TAAACATCCC	CAATCCTGAC	CCTTGCTCTA	TCTTTCACTC
-535	ATTGAAAATC	TTTCCCCAGG	AGGAGGCAGA	TGGTTTTGCA	GGTAAAGACG	CCTGGTGCCA	AGCGTGGCTG	CTTGAGTTCA
	TAACTTTTAG	AAAGGGGTCC	TCTCCGTCT	ACCAAAACGT	CCATTTCTGC	GGACCACGGT	TCGCACCAGC	GAACTCAAGT
-455	GTCCCCGGAA	CTCGGAAGGT	GGAAGGTAAG	ATCCTAGTCC	TCCAAATTGT	TCTCTAACCT	CTACACCAGC	CTGAGGCTCC
	CAGGGGCCCT	GAGCCTTCCA	CCTTCCATTC	TAGGATCAGG	AGGTTTAAACA	AGAGATTGGA	GATGTGGCGA	GACTCCGAGG
-375	AGGGTACTTA	CACACATAA	CGGAAACAAT	CAACCAGTCA	AACAATAAAC	GTAATAATAT	ACAATTTCTT	AGGGAATCCA
	TCCCATGAAT	GTGTGTATGT	GCCTTTGTTA	GTTGGTCAAG	TTGTTATTTG	CATTTTATAA	TGTTAAGAAA	TCCCTTAGGT
-295	AGGGTATTTG	GTCTCTTAGG	TAGGGGTCCA	AAATCTCTGG	GGATAGGGTT	TAGAATCCTT	TGGACAGGCT	CATTACATCA
	TCCCATAAAC	CAGAGAATCC	ATCCCCAGGT	TTTAGAGACC	CCTATCCCAA	ATCTTAGGAA	ACCTGTCCGA	GTAATGTAGT
			<i>SpeI</i>					
-215	TGGGGGGCTT	AAACGC <b>ACTA</b>	<b>GT</b> GGGGGAGG	GGGACTGGGA	ATCTTGGGAG	GGCGGGTCAA	ACCTTGGTGT	CTGAGTAAGT
	ACCCCCGAA	TTTGCGTGAT	CACCCCTCC	CCCTGACCCT	TAGAACCCTC	CCGCCAGTT	TGGAACCACA	GACTCATTCA
-135	CCTTCAAGGA	CTGGTTAGCG	CCCTTGAGGA	CAGGGTTAGC	AAAGAAATGC	TGAAATTTCT	TGGAGTGAA	TTTAGAGCAG
	GGAAGTTCCT	GACCAATCGC	GGGAACTCCT	GTCCCAATCG	TTTCTTTACG	ACTTTAAGAA	ACCTCCACTT	AAATCTCGTC
							<b>TSS</b> →	
-55	GAGACAAGAG	AGCCAGAGTG	GGAGTTAGGC	TCTTAGAGGG	CGGAGTCGGG	GTATTTGGGG	GCGGGGTTTG	CATCACCCAG
	CTCTGTTCTC	TCGGTCTCAC	CCTCAATCCG	AGAATCTCCC	GCCTCAGCCC	CATAAACCCC	CGCCCCAAAC	GTAGTGGGTC
+26	<b>GGTGC</b> GGCTA	<b>AGGC</b> GGCCAG	<b>ATGG</b> CCAGTG	<b>AGCG</b> CCACTG	<b>GGTT</b> GCGGCT	<b>CCC</b> AGCTTGC	<b>ACT</b> CCCCGCG	<b>GCG</b> GCTCTCA
	CCACGCCGAT	TCCGCGGGTC	TACCGGTCAC	TCGCGGTGAC	CCAACGCCGA	GGGTCGAACG	TGAGGGGCGC	CGCCGAGAGT
+106	GACGGGTGGG	AGGGGACCAA	AGTCGCCCGT	GAAG				
	<b>CTGCC</b> ACCC	<b>TCCC</b> TGGTT	<b>TCAG</b> CGGGCA	<b>CTTC</b>				

Figure S2

Gli1_m	1	MFNPMTPPQVNSYSEPCCLRPLHSQGVPSMGTEGLSGLPFCHQANFMSGSQGYGAARETS	60
Gli1_h	1	MFNSMTPPPISYGEPCCLRPLPSQGAPSVGTEGLSGPPFCHQANLMSGPHSYGPARETN Y Y	60
Gli1_m	61	SCTEGSLFPPPPPRSSVKLTKKRALSISPLSDASLDLQTVIRTSPSSLVAFINSRCTSP	120
Gli1_h	61	SCTEGPLFSSP---RSAVKLTKKRALSISPLSDASLDLQTVIRTSPSSLVAFINSRCTSP	117
Gli1_m	121	GGSYGHLSIGTMSPSLGFPPQMSHQKGTSPPYGVQPCVPHDSTRGSMMLHPQARGPRATC	180
Gli1_h	118	GGSYGHLSIGTMSPSLGFPAQMNHQKGPSFVQPCGPHDSARGGMIPHPQSRGPFPTC Y y	177
Gli1_m	181	QLKSELDMMVGKCPEDPLEGDMSSPNSTGIQDHLGMLDGREDLEREEKREPE	240
Gli1_h	178	QLKSELDMLVGKCREEPLGDMSSPNSTGIQDPLLGMMLDGREDLEREEKREPE Y	237
Gli1_m	241	RWDGCSQEFDSQEQLVHHINSEIHGERKEFVCHWGGCSRELRPFKAQYMLVVMRRHTG	300
Gli1_h	238	RWDGCSQEFDSQEQLVHHINSEIHGERKEFVCHWGGCSRELRPFKAQYMLVVMRRHTG Y	297
Gli1_m	301	EKPHKCTFEGCRKSYSRLENLKTHLRSHTEGKPYMCEQEGCSKAFSNASDRAKHQNRTHS	360
Gli1_h	298	EKPHKCTFEGCRKSYSRLENLKTHLRSHTEGKPYMCEHEGCSKAFSNASDRAKHQNRTHS Y Y	357
Gli1_m	361	NEKPYVCKLPGCTKRYTDPSSLRKHVKTVHGPDHAVTKRHRGDGPLPRAQPLSTVEPKRE	420
Gli1_h	358	NEKPYVCKLPGCTKRYTDPSSLRKHVKTVHGPDHAVTKRHRGDGPLPRAPSISTVEPKRE Y Y	417
Gli1_m	421	REGGSGREESRLTVPEASAM-PQQSPGAQSSCSSDHPAGSAANTDSGVEMAGNAGGSTED	479
Gli1_h	418	REGGPIREESRLTVPEGAMKQPSPGAQSSCSSDHPAGSAANTDSGVEMTGNAGGSTED	477
Gli1_m	480	LSSLDEGPCVSATGLSTLRLENLRDLQLHQLRPIGSRGLKPLSLTHAGAPVSRRLGPPV	539
Gli1_h	478	LSSLDEGPCIAGTGLSTLRLENLRDLQLHQLRPIGTRGLKPLSLSHTGTTVSRRVGPV	537
Gli1_m	540	SLDRSSSSSSMSSAYTVSRRSSLASFPFPGTPPENASSLPLTPAQHYMLRARYASAR	599
Gli1_h	538	SLERRSSSSSSISSAYTVSRRSSLASFPFPGSPPENASSLPLMPAQHYLLRARYASAR Y Y Y	597
Gli1_m	600	GSQTPTAAHSLDRMGGLSVPPWRSRTEYPGYNPNAVTRRASDPARAADHPAPARVQRF	659
Gli1_h	598	GGTSPTAASSLDRIGGLMPPWRSRAEYPGYNPNAVTRRASDPAAADRPAPARVQRF Y Y	657
Gli1_m	660	KSLGCVHTPPSVA-TGRNFDPHHPTSVYSPQPPSITENVAMDTRGLQEEPEVGTSMVGN	718
Gli1_h	658	KSLGCVHTPPTVAGGGQNFDPYLPSTSVYSPQPPSITENAAMDARGLQEEPEVGTSMVGS y Y	717
Gli1_m	719	LNPYMDFSSTDTLGYGGPEGTAAPYEARPGSLPLGPGPPTNYGPGHCAQQVSYDPDTP	778
Gli1_h	718	LNPYMDFPPTDTLGYGGPEGAAAPYARGPGSLPLGPGPPTNYGPNPCQQASYDPDTP Y Y Y Y Y	777
Gli1_m	779	ENWGEFSPHAGVYPSNKAPGAAYSQCPRLEHYGQVQVKPEQGCPVGSSTGLAPCLNAHP	838
Gli1_h	778	ETWGEFSPHSGLYPGPKALGGTYSQCPRLEHYGQVQVKPEQGCPVGSSTGLAPCLNAHP Y Y Y	837
Gli1_m	839	SEGSPGPQLFSSHHPQLPQPYPQSGYPQPPHYLSTEPRLGLNFPSSSHSTGQLKAQ	898
Gli1_h	838	SEGPPHPQLFSHYPQSPPPQYLSGYPYQPPDYLPSEPRCLDFD-SPTHSTGQLKAQ y Y Y Y	896
Gli1_m	899	LVCNYVQSQQELLWEGRNRRGLPNQELPYQSPKFLGGSQVSPAKTPAAAAAYGSGFA	958
Gli1_h	897	LVCNYVQSQQELLWEGGGREDAPAQEPSYQSPKFLGDSQVSPSRAKAP---VNTYGPFG Y Y Y	953
Gli1_m	959	PASANKSGSYAPSPCHETFTVGNRPSHRPAAPPRLPLSPCYGPKLVGDTNPSCGH	1018
Gli1_h	954	PNLPNHKSGSYPTSPCHENFVVGANRASHRAAAPRLPLPTCYGPKLVGDTNPSCGH Y Y	1013
Gli1_m	1019	PEVGRLGAGPALYPPPEGQVCNALDSLDLNNTQLDFVAILDEAQGLSPLSHEQGDSSKN	1078
Gli1_h	1014	PEVGRLGAGPALYPPPEGQVCNPLDSLDLNNTQLDFVAILDEPQGLSPPPSHDQRGSSGH Y	1073
Gli1_m	1079	TPSPSGPPNMAVGNMSVLLGSLPGETQFLNSSA	1111
Gli1_h	1074	TPPPSGPPNMAVGNMSVLLRSLPGETQFLNSSA	1106

Figure S3

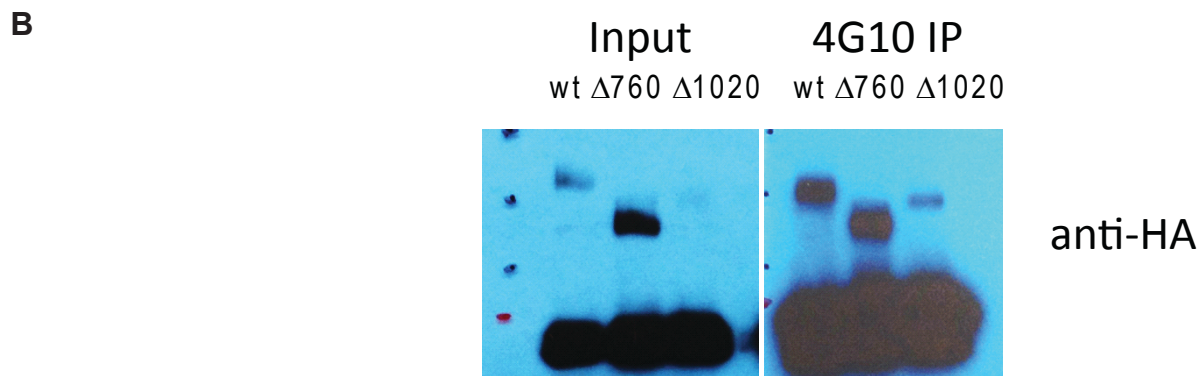
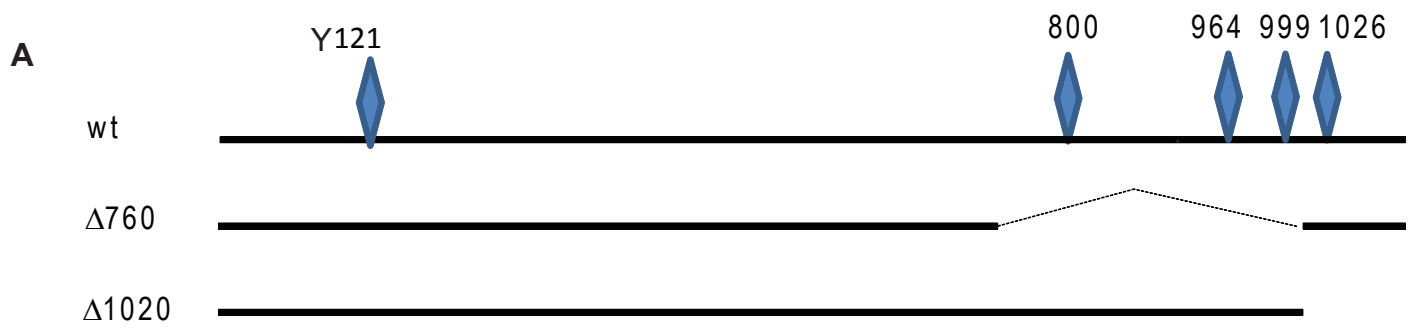


Figure S4

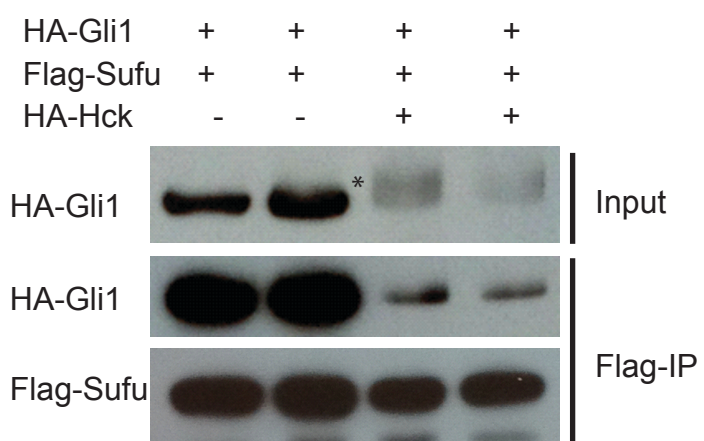
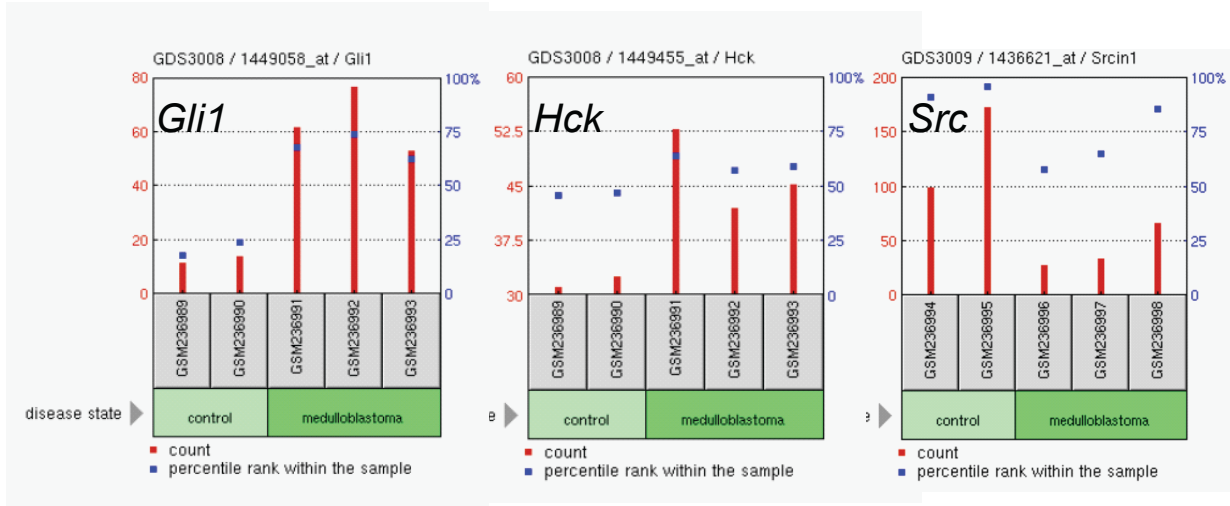


Figure S5 Hck level in human medulloblastoma and mouse medulloblastoma model

**A**



**B**

