Supporting Information: Methods

RNA and DNA isolation

All specimens were obtained from the cooperative Human Tissue Network or the University of Washington Fetal Tissue Bank. Cell lines were obtained from American Type Culture Collection and were cultured according to supplied protocols. RNeasy and DNeasy kits (Invitrogen) were used for RNA and DNA isolation, respectively.

Identification, cloning, northern blotting, polysome and antibody studies

We used 17 strand specific reverse transcriptase primers spaced between 0.1-1 kb, covering a 12 kb region extending up and downstream of the *H19* gene region together with 16 PCR primers to map the *HOTS* transcript (Fig. 1a and Supplementary Table 4). To determine the transcript boundaries, we performed rapid amplification of cDNA ends (5' /3' RACE). The 5' and 3' RACE of cDNA ends (5' /3' RACE) was performed using Roche's 5'/3' RACE Kit, 2nd generation, following supplied protocols. Gene specific primer 1144 was used to reverse transcribe *HOTS* cDNA from human fetal kidney RNA as described (1). The resulting cDNA obtained using the 5' end and 3' end primers HOT-NAT-F2 and HOT-NAT-R spanning *HOTS* 453 bp open reading frame was cloned into pEGFP.N3 (Clontech) and p3XFLAG-Myc-CMV-24 (Sigma-Aldrich) plasmid vectors. RNA probes were prepared using the Riboprobe Combination System-SP6/T7 kit following supplied protocols (Promega). The template for the strand specific RNA probes was either a 397 bp DNA fragment (PCR primers 63F and 10R) encompassing introns 2, 3 and 4 of *H19* cloned in the dual promoter plasmid vector pCRII TOPO T7/Sp6 (Invitrogen) or H19 3' untranslated region (PCR primers 1F and 62R) cloned into the pcDNA 3.1 T7 promoter vector (Fig. 1a). *GAPDH* loading control probe was generated

by random priming using the Prime It-II kit (Stratagene) using a 133 bp *GAPDH* PCR template obtained using *GAPDH* forward and reverse primers and an oligo-dT cDNA synthesized from fetal kidney RNA**.** Northern blots were purchased from Ambion, hybridizations and detection was performed as described (2). Multiple sequence alignment, gene feature searches and protein localization predictions were done as described previously (1). Membrane bound polysomal RNA and free RNA were purified by sucrose gradient sedimentation equilibrium centrifugation as described (3). Briefly, 5 x 10^8 HeLa or HEK293 cells was treated with cycloheximide (10 μg/ml) for 10 min, pelleted (5min, 4 ^oC, 1000g), washed twice in 125 ml ice cold PBS with cycloheximide (10μg/ml), cells were resuspended at 2.5 x 10^8/ml in hypotonic-lysis buffer (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-Cl, pH 7.4, 10 μg/ml cycloheximide), homogenized in a Dounce homogenizer. The homogenate was centrifuged (2 min, 4 ^oC, 2000g) and 2 ml of supernatant was then loaded onto a successively layered 2.5 M, 1.95 M, 1.3 M sucrose gradient in gradient buffer (150 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-Cl, pH 7.4, 10 μg/ml cycloheximide) and ultracentrifuged (5 hr, 4 ^oC, 90000g). Gradients were harvested by collecting 1.5 ml fractions after puncturing the bottom of the tube. Protein concentrations were determined by reading OD_{280}. RNA was extracted using Trizol following supplied protocols (Invitrogen). Quantitation of gene expression levels was performed by real-time PCR as following manufactures protocols. Strand specific cDNA templates were generated for *HOTS* and *H19* using gene specific primers 1144R and 1F, respectively (Fig. 1a, Supplementary Table 4). Real-time PCR data were normalized against oligo-dT synthesized *GAPDH* cDNA amplified using the *GAPDH* primers mentioned above (Supplementary Table 4). To purify HOTS protein for antibody

generation, we cloned *HOTS* tagged with $Hist_6$ into the pDEST17 vector (Invitrogen), transformed BL21 *E. coli* and purified HOTS-$Hist_6$ protein using the ProBand purification system kit (Invitrogen). Purified protein was sent to Covance (Denver, PA) for antibody production.

Pre-immune serum was drawn prior to immunization of the selected rabbits, and serum was drawn after immunization every month for a total of three months. Antibody purification was carried out using Protein-G Dynabeads (Invitrogen) following manufacture's protocols. Specificity of the antibodies was assayed by western blot using purified HOTS-$Hist_6$, mouse 11p15 uniparental disomy (UPD) BWS and WT samples.

Sub-cellular and immunohistochemical localization of HOTS

HOTS cDNA was cloned N-terminus in-frame to green fluorescent protein (GFP) into the *Bam*HI and *Eco*RI sites of the pEGFP-N3 plasmid vector (Clontech). SiHa and Cos7 cells were transfected with empty or HOTS-GFP vector, and fluorescent microscopy and digital image capture were done as described (1). For antibody staining, both SiHa and Cos7 cells were cultured on cover slips, fixed, blocked with 3% BSA, hybridized with HOTS antibodies, washed, hybridized to FITC-conjugated goat anti-rabbit antibodies, washed and visualized under fluorescent microscopy as described (1). To identify the nucleolus we used antibodies against nucleophosmin (4).

Tumor cell growth assays

SiHa, Cos7, RD, JEG-3, SK-NEP-1 and G401 were transfected with two types of constructs cloned in pEGFP-N3 and unmodified pEGFP-N3. The first construct was *HOTS* fused to GFP, and generated using the primers: HOT-NAT-F2 and HOT-NAT-R, and the second was mutated *HOTS* with nine stop codons amplified with primers: HOT-MUT-F and HOT-MUT-R. Transfection efficiency was determined by flow cytometry, by evaluating the percentage of GFP positive cells of the co-transfected unmodified pEGFP-N3. H19 cDNA used for transfection was generated as outlined under the methods section: Identification, cloning, northern blotting, polysome and antibody studies. Growth media was supplemented with G418 (neomycin) antibiotic to select for transformants and was changed every third day. The concentration of G418 used was as follows: SiHa (1.2mg/ml), Cos7 (0.6 mg/ml), RD (0.6mg/ml), SK-NEP-1 (0.8mg/ml) and G401 (0.4mg/ml). Cells were harvested and counted after 12-14 days.

***HOTS* RNAi assays for tumor cell proliferation, anchorage dependent growth and *in vivo* growth in nude mice**

We used the BLOCK-iT Inducible H1 RNAi Entry vector kit to clone double stranded oligonucleotides targeting nucleotides 257 to 279 of HOTS in the provided pENTRH1/TO entry vector following the manufacturer's recommendation (Invitrogen). A sequence-verified HOTS shRNA clone was transfected into HeLa-TREx cells, transfected clones using 500 μg/ml zeocin, and shRNA expression was induced using 2 μg/ml tetracyline. Colonies were counted under light microscopy three weeks post transfection. For siRNA experiments siRNA probes were designed by RNAi Design (IDT) and purchased from IDT. Transfections of siRNA were performed using HiPerFect

(Qiagen) following manufacture's protocols. *In vivo* tumor cell growth assays were performed as previously described (5). Quantitative real-time PCR was performed as previously described (6).

Protein interactions

We cloned *HOTS* into the p3XFLAG-Myc-CMV-24 (Sigma-Aldrich), and tagged at the N-terminus with FLAG and at the C-terminus with Myc epitope. Candidate HOTS-interacting proteins were cloned into the pEF6/V5-His TOPO TA vector (Invitrogen), dual tagged with His_6-tag and V5 tag. Myc-tagged antibodies were purchased from Roche, His_6-tagged purification nickel beads and V5 antibodies were obtained from Invitrogen and ERH antibodies were purchased from Abcam. Lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.8, 1% NP40, 1 mM EDTA) was added to cells prior to sonication, and lysates were pre-cleared by centrifugation, at 10,000g, 4 ^{o}C. For immunoprecipitation, 10 μg of antibody or 50 μl of nickel slurry was used per 10^7 cells (0.5 ml lysate). SDS-PAGE was performed using precast Nu-PAGE gels (Invitrogen). Western blotting was performed according to the ECL protocol (Amersham) and the Chemidoc XRS imaging system (Bio-Rad) was used for detection and digital imaging.

Bisulfite sequence analysis of HOTS CpG Island

DNA was extracted from Wilms tumor samples as outlined under DNA and RNA isolation above. We used the EZ DNA methylation-Gold Kit for bisulfate DNA treatment and purification following the included protocol (Zymo Research, Orange, CA). For analysis of the HOTS CpG Island (chr11:2013334-2013617, UCSC browser, hg19), we used primers: HOTCPG-1F, 5-GAATTTATGTTGAGATATAG-3' and HOTCPG-395R,

5’- ATAAAAACCAAATCATCC-3’ for first round amplification, then used HOTCPG-23F, 5’-TTATATATAGTTAGGTTTAGG-3’ and HOTCPG-350R, 5’- TACTACTACT-ATAACCAC-3’ for nested amplification. For HOTS sequence conserved putative promoter we used primers: HOTSP-BS-2F, 5’-GTGTTTGTGAGTAGAGG-3’ and HOTSP-BS-387R, 5’-AACCTACTCCTAATAACACC-3’ for first round amplification and nested primers: HOTSP-BS-21F, 5’-TTGGGTAGGAGGAGGAGGAG-3’ and HOTSP-BS-387R, 5’-AACCTACTCCTAATAACACC-3’ for second round amplification. PCR amplified products were either sequenced following purification or cloned into ZeroBlunt TOPO Cloning kit for Sequencing (Invitrogen) and sequenced using either the forward or reverse nested primer. Sequencing was performed on a 3130xl Genetic Analyzer-Hitachi (Applied Biosystems) and analyzed using two softwares, Sequencing Analysis v5.3.1 (Applied Biosystems) and Sequencher (Gene Codes Corporation, Ann Arbor, MI).

References for Methods

1.Onyango P, et al (2002) SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc Natl Acad Sci U S A* 99:13653-8

2.Onyango P, et al (2000) Sequence and comparative analysis of the mouse 1-megabase region orthologous to the human 11p15 imprinted domain. *Genome Res*. 10:1697-710.

3. Mechler BM. (1987) Isolation of messenger RNA from membrane-bound polysomes. *Methods Enzymol*. 152:241-8

4. Michalik J, Yeoman LC, Busch H.(1981) Nucleolar localization of protein B23 (37/5.1) by immunocytochemical techniques. *Life Sci.* 28:1371-9

5. Farmer H, et al (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 43:917-21.

6. Kaneda A et al, (2007) Enhanced sensitivity to IGF-II signaling links loss of imprinting of IGF2 to increased cell proliferation and tumor risk. *Proc Natl Acad Sci U S A*. 104:20926–20931.

Supporting Information: Figures

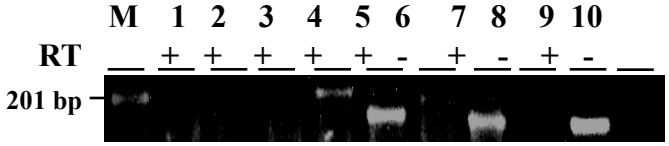

Fig. S1. Mapping *HOTS* transcript by RT PCR analysis. RT-PCR products separated on an agarose gel from cDNA generated using either strand specific RT primers (Lanes 1-8) or oligo-dT (Lanes 9 and10), using human placenta poly A RNA as template. M, Marker. Lane 1, RT primer 1065R (located downstream of *HOT* transcript) and PCR primer pair 619F/629R . Lane 2, RT primer 2061R (located upstream of *HOT* transcript), PCR primer pair 1847F/1948R. Lane 3, RT primer 3240R (located upstream of *HOT* transcript), PCR primer pair 3033F/3152R. Lane 4, RT primer 6140R, PCR primer pair 57F/59R. Lane 5, RT primer 5010R, PCR primer pair 47F/49R. Lane 6, No RT enzyme control, RT primer 5010R, PCR primer pair 47F/49R . Lane 7, RT primer 7045R, PCR primer pair 67F/69R. Lane 8, No RT enzyme control, RT primer 7045R, PCR primer pair 67F/69R. Lane 9, 3' RACE product using oligo-dT as RT primer and PCR primer pairs 10F/ PCR anchor(from 5' /3' RACE kit from Roche). Lane 10, No RT enzyme included, control for 3' RACE.

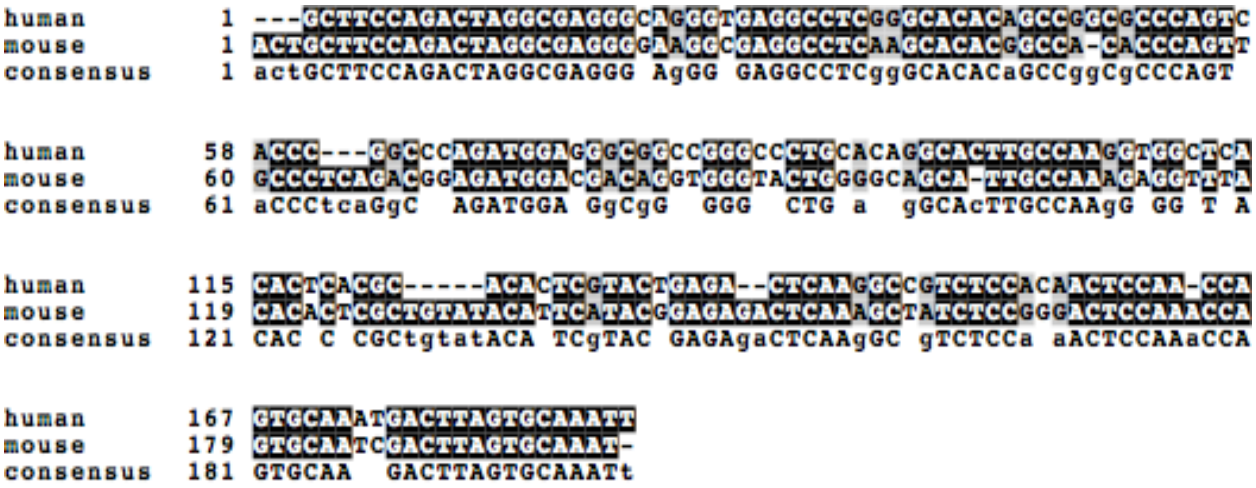

Fig. S2. *HOTS* conservation analysis. Alignment of human and predicted mouse *HOTS* sequences. Black shading represents conserved nucleotides. Gray shading depicts G/A purine alignment. The consensus sequence is shown at the bottom and depicts identical nucleotides in uppercase. Alignment was done using the Clustal W multiple sequence alignment tool (http://searchlauncher.bcm.tmc.edu/multi-align /Option/clustalw.html). The overall nucleotide sequence identity between the human and the predicted mouse *HOTS* is 43%.

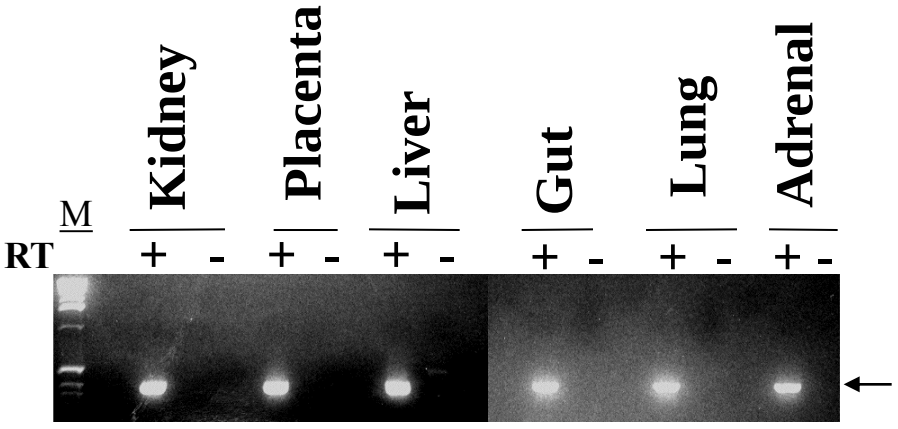

Fig. S3. *HOTS* is ubiquitously expressed. Reverse transcriptase PCR (RT-PCR) of *HOTS* cDNA fragment generated using strand specific RT primer 1144R and PCR primers 24F and 62R. Samples were separated on a 1 % agarose gel and stained with ethidium bromide. The expected 404 bp cDNA fragment is shown by an arrow. Lane 1 (M), 1 kb DNA ladder (Invitrogen). Amplifications of cDNA were performed in the presence (+), or absence (-) of the RT enzyme. The expression of *HOTS* appears global as indicated here by its presence in the following tissues; kidney, placenta, live, gut, lung and adrenal.

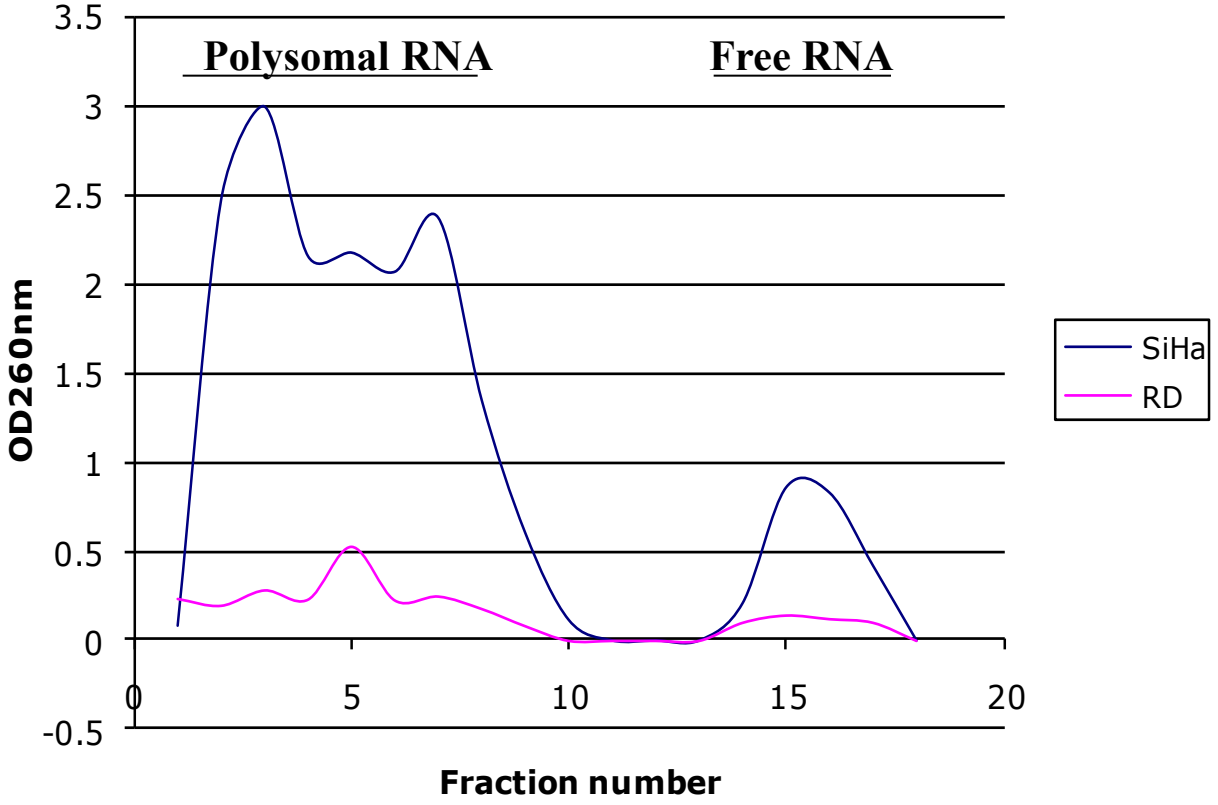

Fig. S4. Fractionation of polysome associated and free RNA by sucrose gradient centrifugation. Polysomal and free RNA were isolated following protocols outlined in the Methods section. The amount of RNA in each fraction was determined using a Nanodrop, ND-1000 spectrophotometer (NanoDrop Technologies, US). Samples were obtained from the cervical carcinoma cell line SiHa (blue) and the rhabdoid tumor cell line RD (pink). Because RD cells grew much slower the input amount of cells was about a quarter of SiHa cells used, which explains the lower RNA yields for RD. The polysomal RNA fractions and the free RNA fractions were separated by a number of fractions (11-14), that showed no RNA yield.

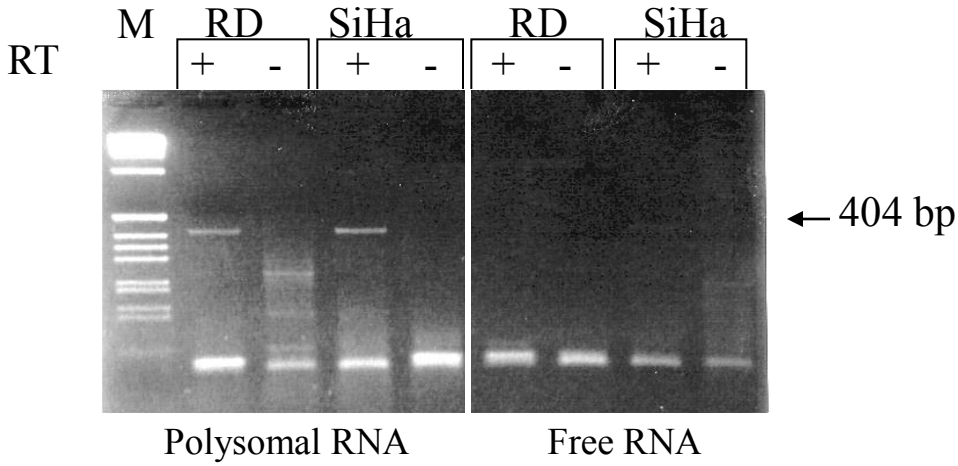

Fig. S5. *HOTS* is associated with polysomes. Polysomal and free RNA were isolated from RD and SiHa cell lines by sucrose gradient centrifugation as outlined in the methods section. RT-PCR of *HOTS* from polysomal and free RNA using strand specific RT primer 1144R followed by PCR amplification with primers 24F and 62R. PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide. The expected 404 bp PCR cDNA product was present in cases where RT enzyme was included (+), but not in cases where RT enzyme was absent (-). Lane1 (M), 1 kb DNA ladder (Invitrogen). *HOTS* was readily amplified from polysome derived RNA fractions of both SiHa and RD cell lines, compared to Free RNA fractions, suggesting a significant association of *HOTS* with polysomes.

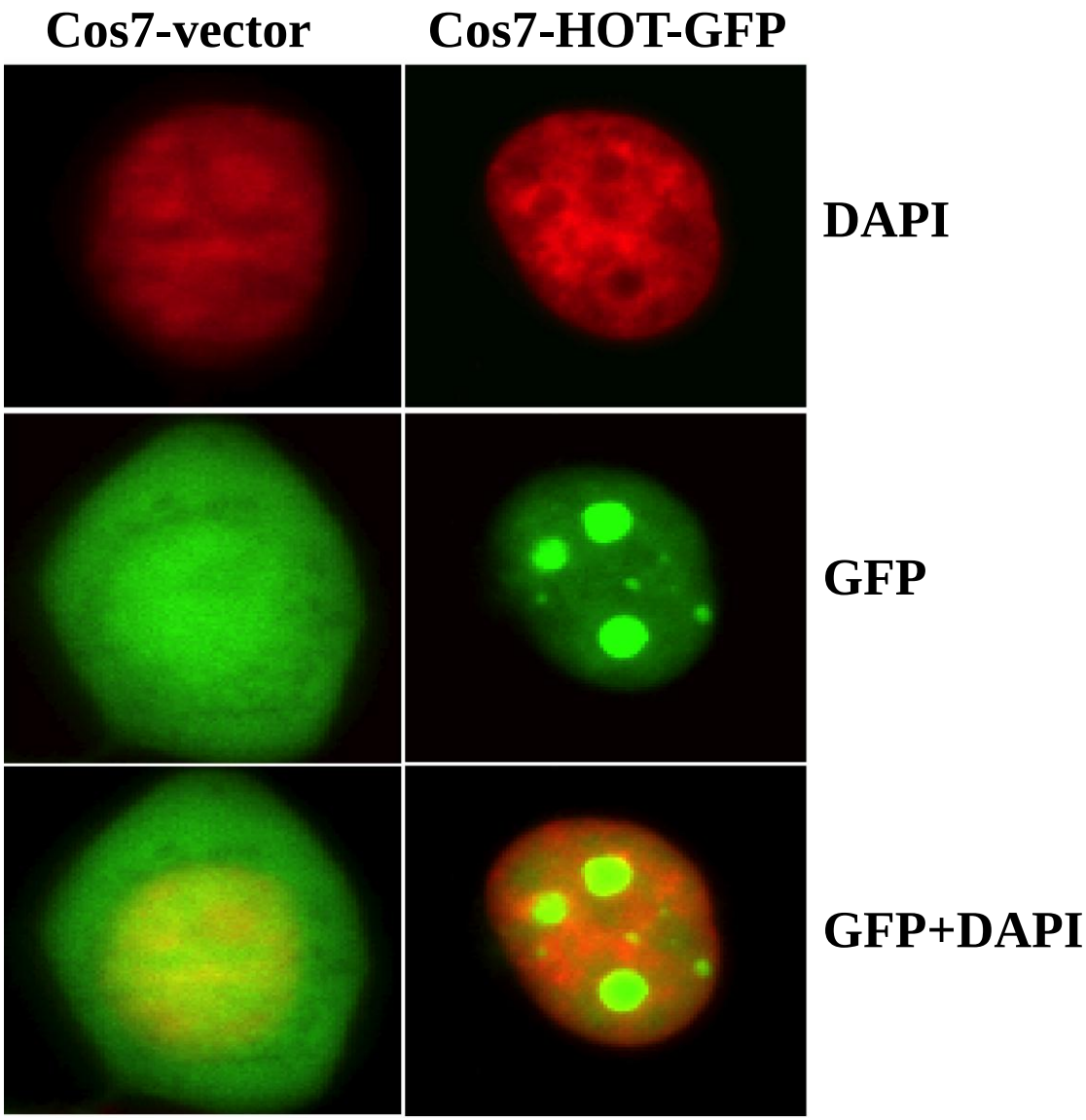

Fig. S6. *HOTS* is both a nuclear and subnuclear protein. Digitally acquired images from a fluorescent microscope showing COS7 cells transfected with pEGFP.N3 vector (left panels) or HOTS-GFP cloned into the pEGFP.N3 vector (right panels). Cells were counterstained with DAPI (red, first row) to reveal the nucleus, middle panels (green) show green fluorescent protein(GFP) expressed from the vector (left) and HOTS fused to GFP (right). The bottom panels are overlays of the DAPI images and the corresponding GFP images from the top and the middle panels. In contrast to the apparent diffused GFP staining throughout the vector transfected cells (left panels), HOTS-GFP signals is restricted to the nucleus with pronounced sequestration into a nuclear substructure (right panels).

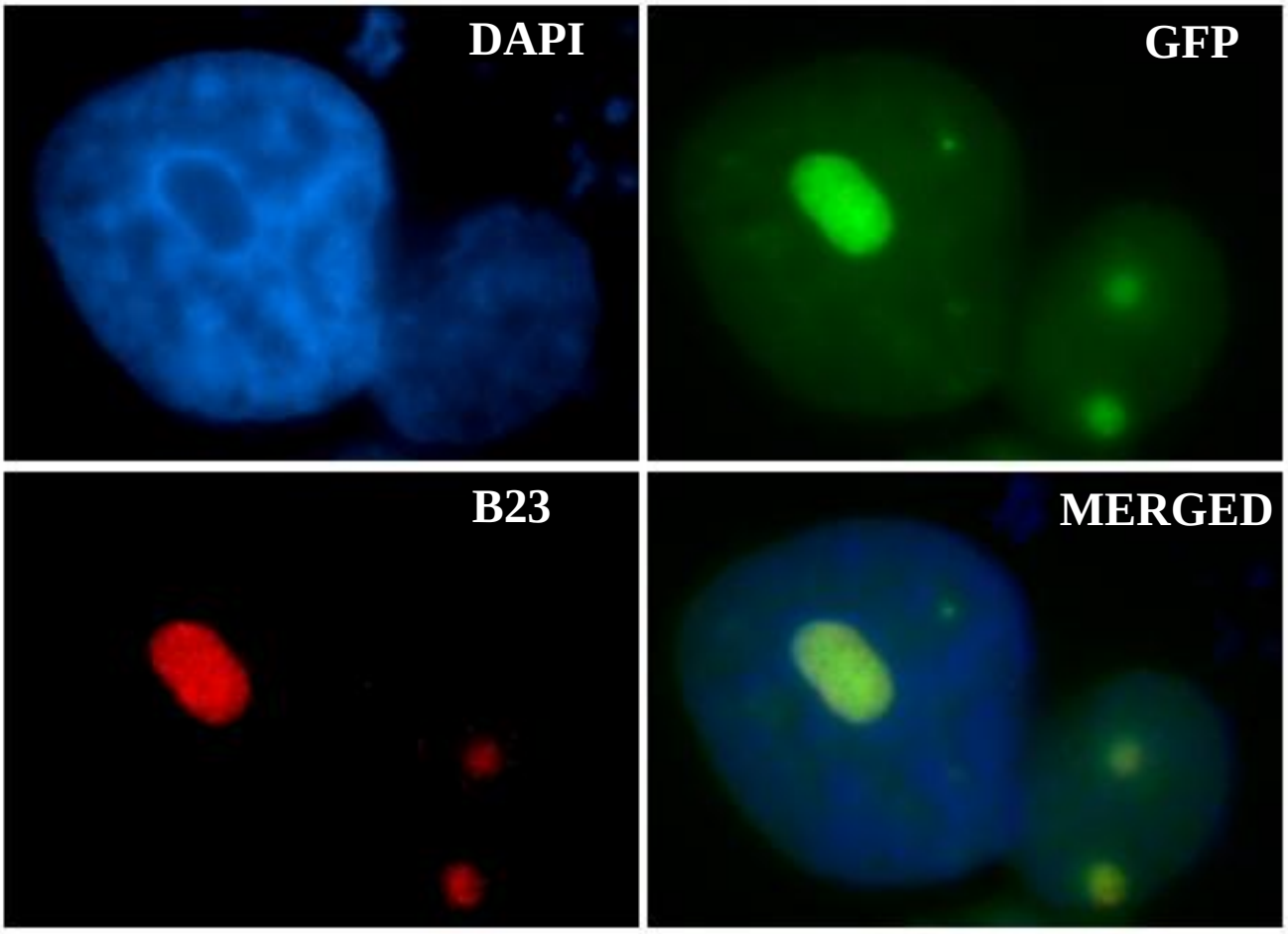

Fig. S7. *HOTS* is sequestered to the nucleolus. Fluorescent microscopy of COS7 cells transfected with HOTS protein fused to GFP in pEGFP.N3 vector and hybridized with antibodies against B23 (nucleophosmin), an authentic nucleolus protein. Top left panel depict cells counterstained with DAPI to reveal the nucleus. Top right, HOTS-GFP expression pattern (green). Bottom left, B23 staining pattern(red). Bottom right, merged image of DAPI, GFP and B23 stains. The homogeneity of the staining for HOTS-GFP and B23 in the subnuclear structure suggest that HOTS is sequestered to the nucleolus.

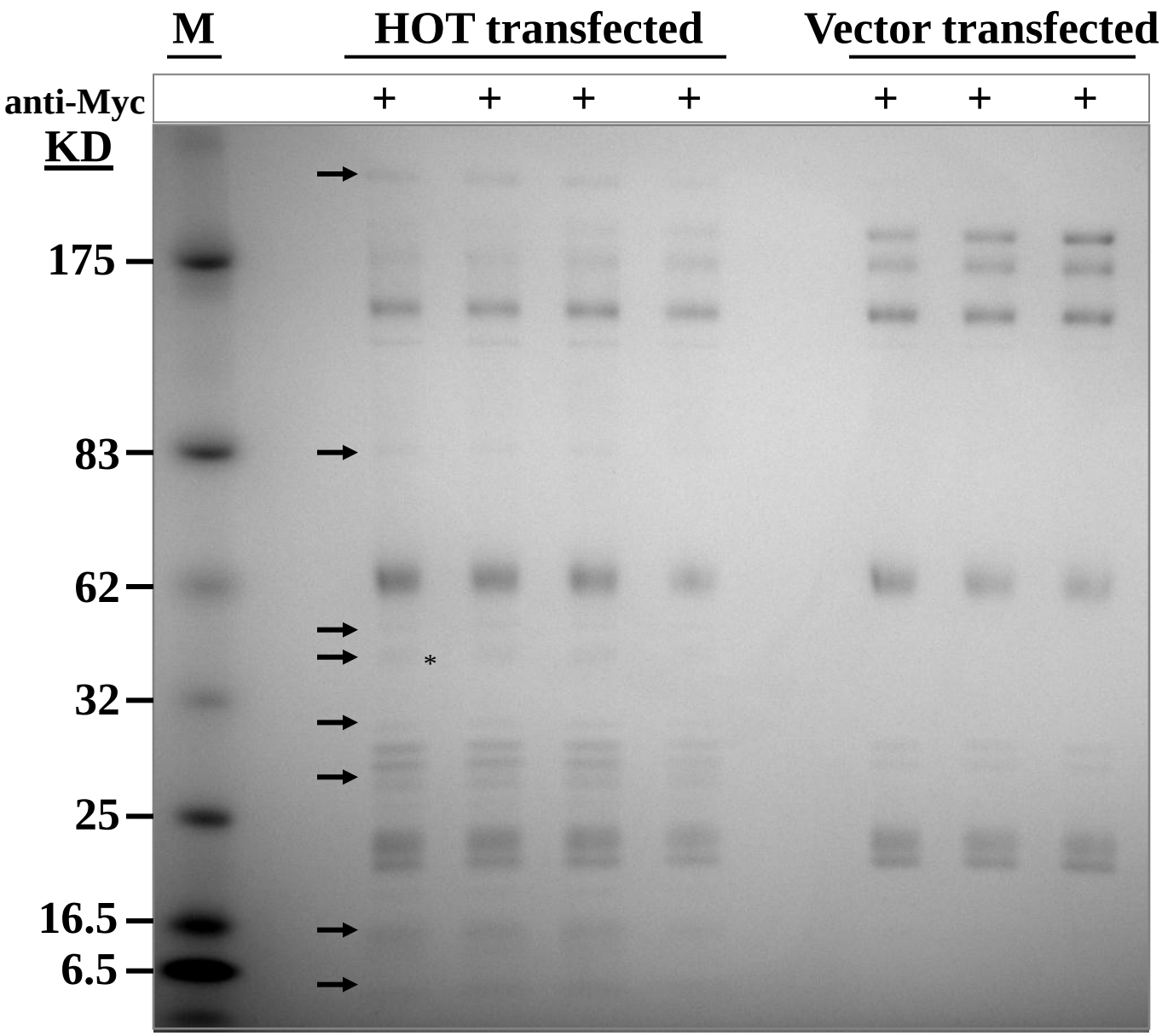

Fig. S8. *HOTS* interacting proteins. Coomassie stained SDS-PAGE of candidate HOTS interacting proteins discovered by anti-myc immunoprecipitation (IP) of HOTS protein from human HEK293 cells transfected with the myc tagged HOTS plasmid and the empty vector as control. IP for HOTS were done in quadruplet and in triplicate for the vector. Protein bands (pointed by arrows) visible in the HOTS IP lanes but not in the vector IP lanes were cut from the gel and subjected to mass spectrometry for identification. The asterisk mark is placed to the right most edge of the candidate HOTS interacting protein subsequently identified to be enhancer of rudimentary (ERH).

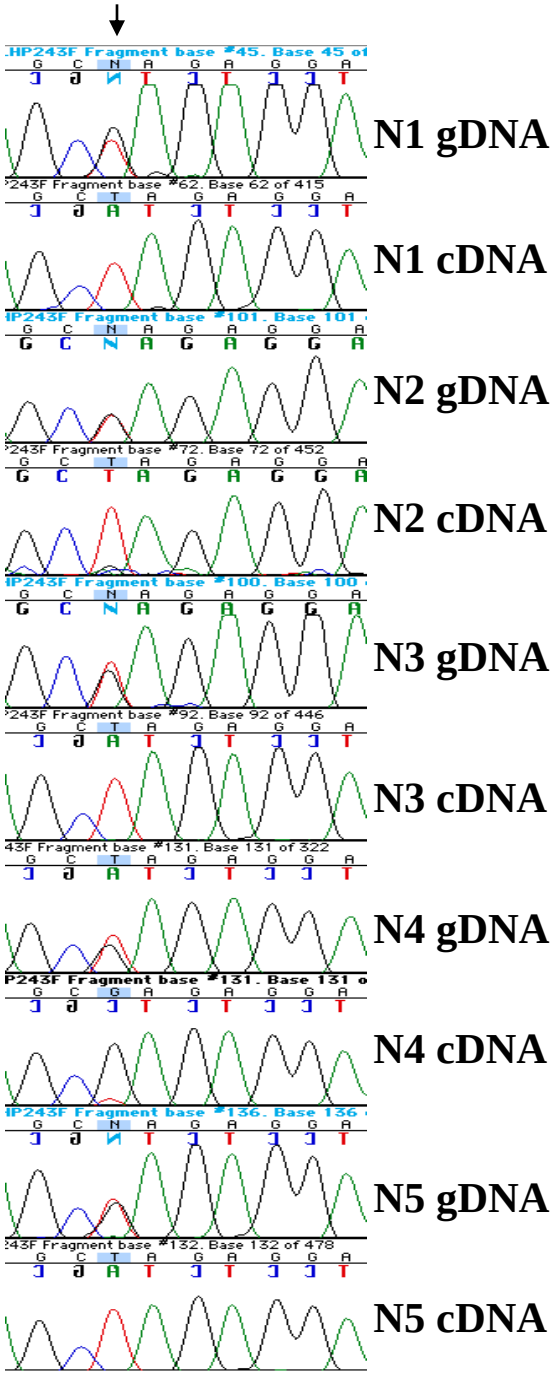

Fig. S9. *HOTS* is monoallelically expressed**.** Sequence chromatograms showing the +588 (G/T) polymorphism (marked by an arrow) at the 3' UTR of *HOT*, the genomic DNA (gDNA) and kidney cDNA of five individuals (N1-N5) are shown.

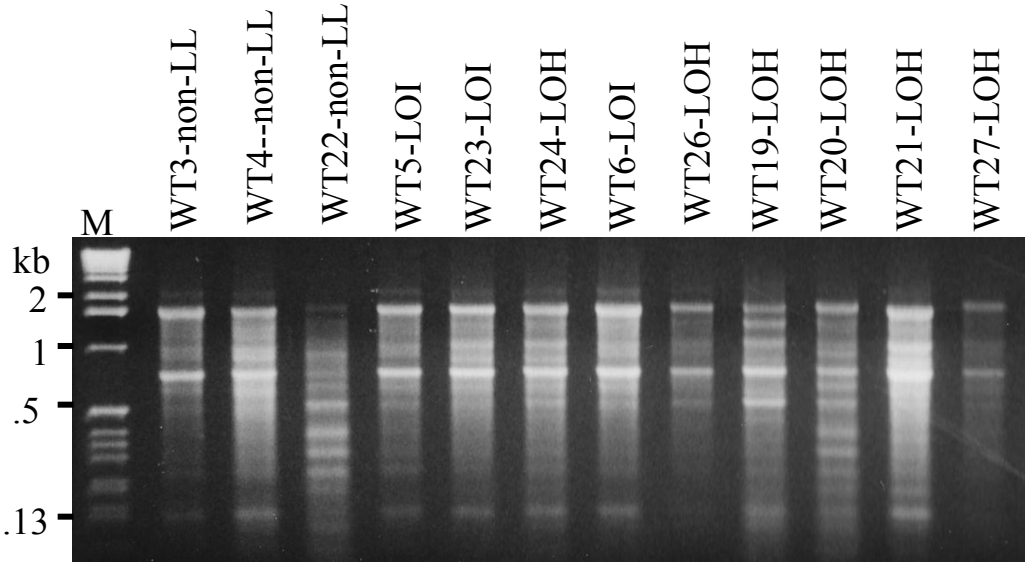

Fig. S10. Agarose gel fractionation of DNase treated total RNA from Wilms tumor samples. Lanes are labeled with the sample name followed by the abbreviations non-LL for Non-LOH and non-LOI, and LOI or LOH as indicated. Samples were treated with amplification grade DNase I (Invitrogen).

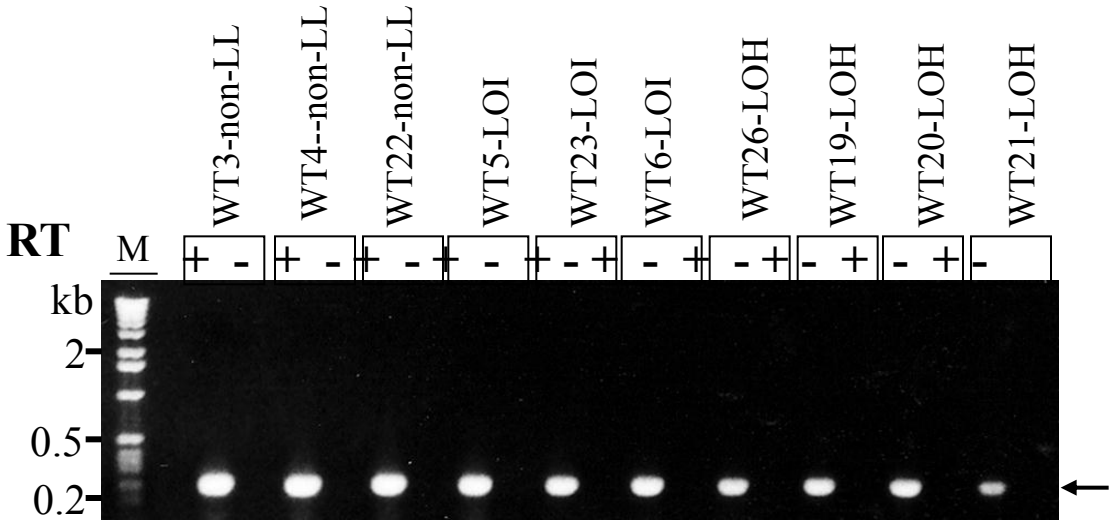

Fig. S11. *GAPDH* RT-PCR from DNase I treated Wilms tumor RNA. Samples shown are the same as most shown in figure S13. cDNA synthesis was performed in the presence (+) and absence of (-) of the RT enzyme. Oligo-dT was used to prime RNA for cDNA synthesis and PCR amplification was performed with GAPDH-ReTP-483F and GAPDH-ReTP613R primers. The 133 bp expected PCR fragment is pointed by an arrow.

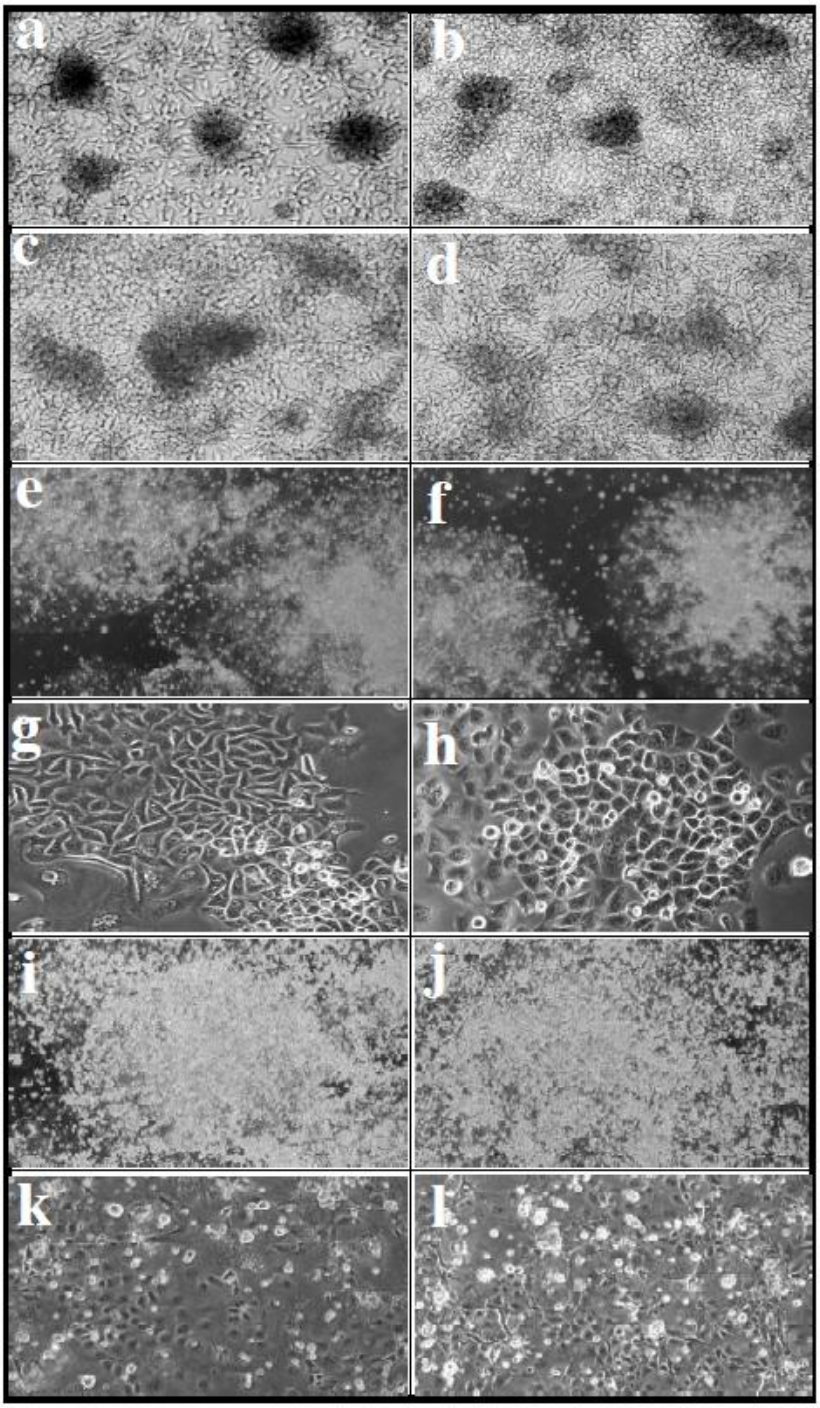

Fig. S12. *H19* does not suppress tumor cell growth. Cells were transfected either with *H19* cDNA in pcDNA3.1D/V5-His-TOPO vector or with the control vector and cultured in the presence of neomycin for 2 weeks. Images were acquired digitally under light microscopy. **a.** RD cells transfected with control vector, **b.** RD cells transfected with *H19* cDNA, **c.** WT49 transfected with control vector, **d.** WT49 cells transfected with *H19* cDNA, **e.** JEG3 cells transfected with control vector, **f.** JEG3cells transfected with *H19* cDNA **g.** G401 cells transfected with control vector, **h.** G401 cells transfected with *H19* cDNA, **i.** SiHa cells transfected with control vector, **j.** SiHa cells transfected with *H19* cDNA, **k.** Cos7 cells transfected with control vector, **l.** Cos7 cells transfected with *H19* cDNA.

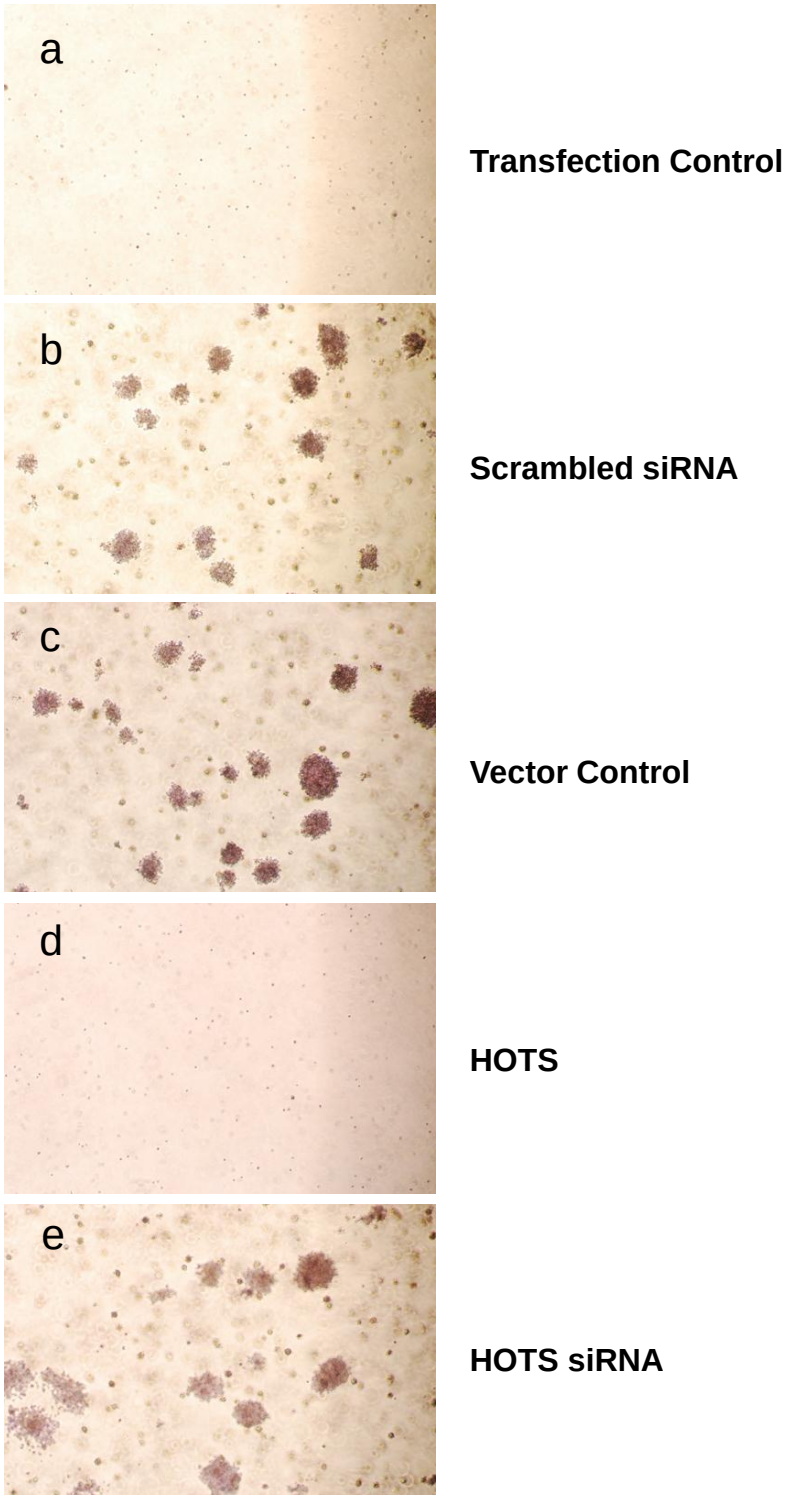

Fig. S13. HOTS suppresses HeLa cell growth in soft agar assays. **a.** Cells untransfected and selected with 0.5 ug/ml zeocin. **b**. Cells transfected with scrambled siRNA. **c**. Cells transfected with empty vector. **d**. Cells transfected with HOTS cDNA. **e**. Cells transfected with HOTS siRNA.

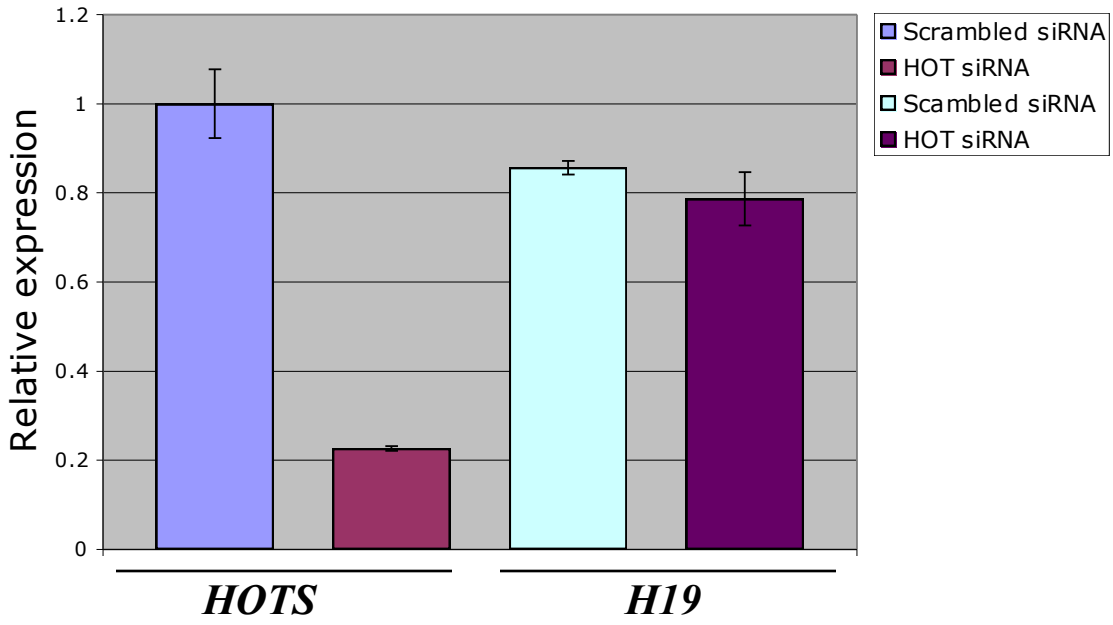

Fig. S14. *HOTS* siRNA specifically targets *HOTS* expression. Realtime expression analysis of *HOTS* expression and *H19* expression following siRNA targeting of both genes with a HOTS siRNA (HOT siRNA) and a scrambled *HOTS* siRNA in HeLa cells. cDNA synthesis was carried out using strand-specific primers 1144R for *HOTS* and 1F for *H19;* real time amplification primers were 139R and 1F. *GAPDH* was used as an internal control to normalize for variations in RNA amounts. Scrambled HOT siRNA did not have an effect on either *HOTS* (blue rectangle) or *H19* (cyan rectangle) gene expression, whereas, HOT siRNA had a dramatic and significant decrease in *HOTS* expression with a 5 fold decrease (maroon rectangle) for *HOTS* (p-Value = 0.01). In contrast, HOTS siRNA showed an insignificant decrease (purple rectangle) in *H19* expression (p-Value = 0.13). n=3, Error bars; s.d.

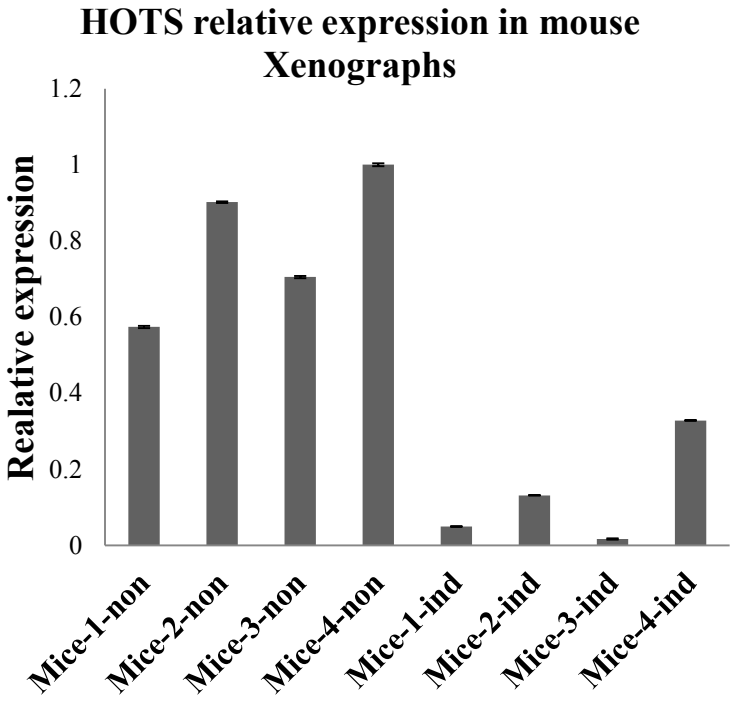

Fig. S15. *HOTS* expression in tumors from athymic nude mice. Quantitative realtime PCR expression analysis of *HOTS* from tumors dissected from athymic nude mice 4 weeks after injection with either *HOTS* expressing construct, first four (-non) or *HOTS* knockdown construct, *HOTS* shRNA knockdown (tetracycline inducible (-ind), last four. *GAPDH* was used as an internal control to normalize for variations in RNA amounts. The difference in expression of *HOTS* between the *HOTS* knockdown and *HOTS* expressing tumors was statistically significant, p-Value = 0.0005 (n = 4). Error bars: s.d.

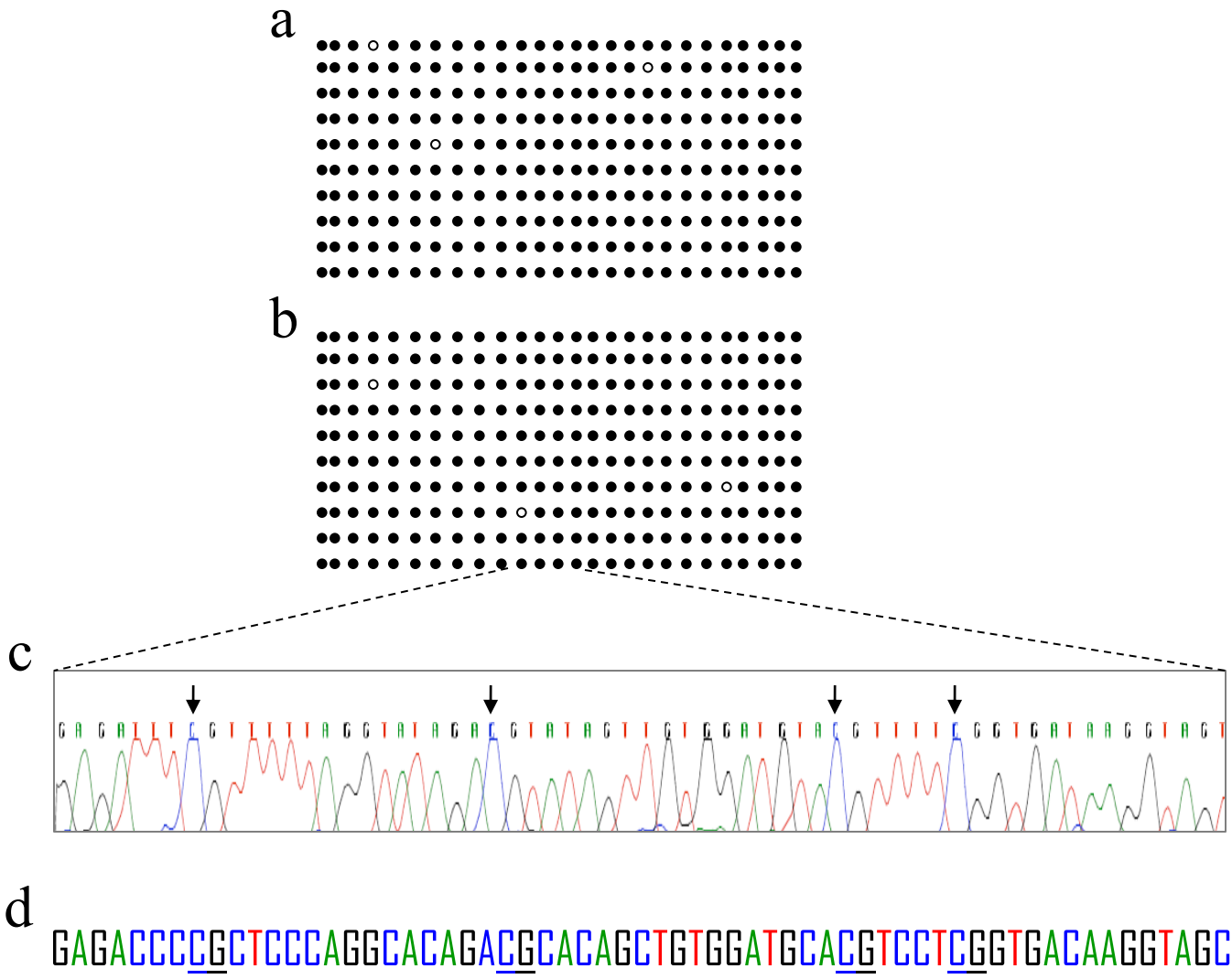

Fig. S16. *HOTS* CpG island is methylated in non-LOI and LOI Wilms tumors. Bisulfite sequence analysis of the 26 CpG dinucleotides in the HOTS CpG island (chr11:2013334-2013617, UCSC browser, hg19). (**a**) Representative methylation analysis results of 10 non-LOI Wilms tumors. Each line represents an individua'sl sample. Analysis was performed in triplicate (**b)** Methylation results for 10 LOI Wilms tumors. Bisulfite sequences analysis was carried out in triplicate for each WT sample. (**c**) Representative bisulfite sequence chromatogram of a portion of the island (magnification of the dotted lines section), methylated CpG dinucleotides are shown by arrows. (**d**) Genomic sequence of the chromatogram shown in c, corresponding CpG dinucleotides are underlined.

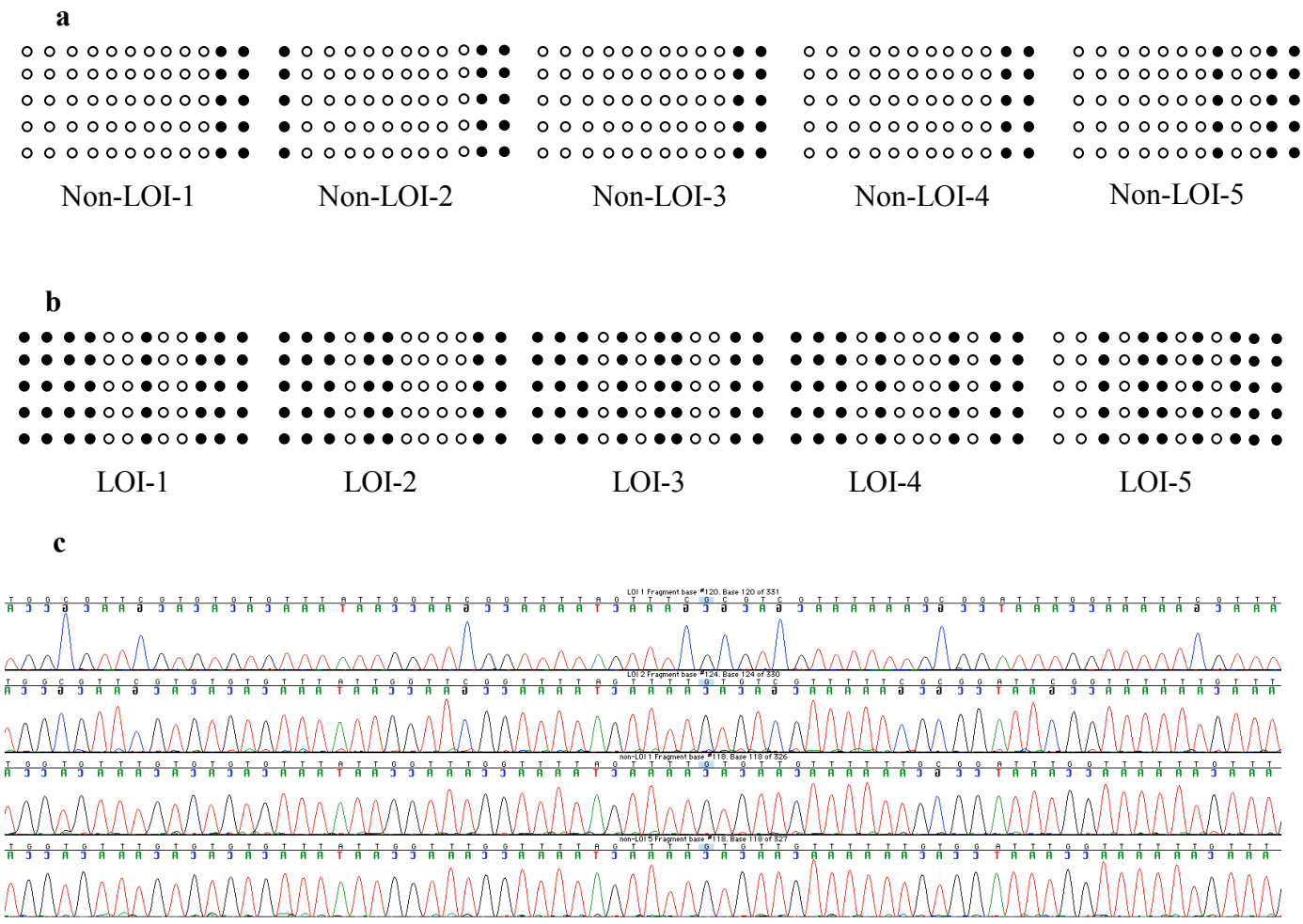

Fig. S17. Bisulfite sequencing of the putative *HOTS* promoter located at chr11:2012237-2012566, UCSC genome browser, hg19. **(a)** 5 non-LOI and **(b)** 5 LOI samples are shown each with 5 cloned sequences. **(c)** Representative chromatograms of the sequenced region is shown for 2 LOI (Top 2 sequences) and 2 non-LOI samples (Bottom 2 sequences).

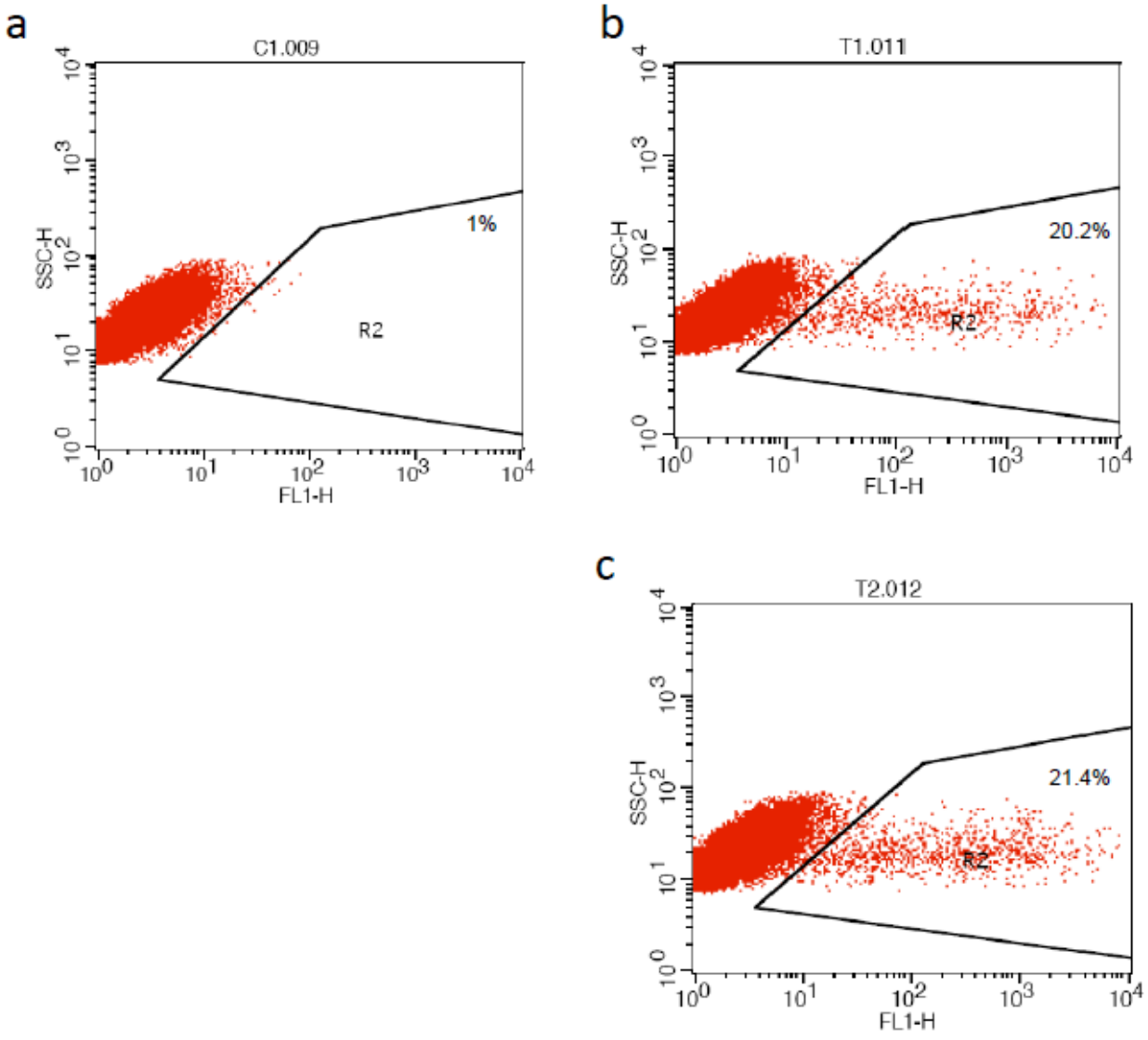

Fig. *S18*. Flow cytometry of GFP transfected cells. a. Untransfected control. b. Transfected with native HOT-GFP. c.Transfected with mutated HOTS –GFP.

Supporting Information: Tables

Table S1. Candidate HOT Interacting Proteins

Gene	Gene Symbol	Gene ID Number	Chromosomal Location	Biological Process
Heat shock 70kDa protein 1-like	HSPA1L	386785	6p21.3	Protein stabilization and folding
Heterogeneous nuclear ribonucleoprotein G	HNRPG	542850	Xq26	mRNA processing
LIM domain only 7	LMO7	57162283	13q22.2	Organ development, growth
Biogenesis of ribosomes in Xenopus 1	BRX1	19070357	5p13.2	Nucleolar protein, constituent of 66S pre-ribosomal particles
Enhancer of Rudimentary	ERH	76827483	14p24.1	Pyrimidine metabolic process, cell cycle
Human splicing factor SRp55-3	SRp55-3	1049086	20q13.11	Pre-mRNA-splicing factor
Ribosomal protein L 27	RP L27	4506623	17q21.31	Ribosomal assembly
Histone 2 B	H2B	1568551	6p22.1	Structure of chromatin

Table S2. Growth suppression activity of HOTS on tumor cell lines

Cell line	GFP vector	Mutant-HOT[a]	HOT[b]	pValue (n=3)
SKNEP1	$1X10^2$	$1.71X10^2$	6	0.03
RD	$2X10^5$	$3X10^5$	$6X10^4$	0.05
JEG3	$3.1X10^2$	$1.25 X 10^2$	1	0.04
G401	$5X10^5$	$1X10^6$	2	0.05
Cos7	$8X10^5$	$6X10^5$	27	0.004

a-Transfected with mutant HOT construct, b-Transfected with native HOT fused to GFP

Table S3. ***H19*** **cDNA fails to suppress growth of tumor cells**

Cell line	Vector [a]	H19[b]	pValue (n=3)
RD	$5.53X10^5$	$7.4X10^5$	0.11
WT49	$4.6X10^5$	$6.7X10^5$	0.71
JEG3	$3.9 X 10^4$	$4.4X10^4$	0.19
G401	$3.7X10^4$	$6.8X10^4$	0.12
SiHa	$3.2X10^6$	$3.16X10^6$	0.45
Cos7	$8.1X10^5$	$11.3X10^5$	0.95

a-Transfected with vector without insert , b-Transfected with *H19* cDNA

Table S4. Primer Sequences

Primer name	Primer Sequence (5'>3')
1065R	AGTACGTGGAGGAAGCTCTAG
619F	CAAGCTCCTTATCTCGAATGCT
829R	CCAATGCAGTGGTCTCCAGC
2061R	CCTGTGACACAGGTGCATGG
1847F	CCTGTCCACCCTCTCACCAGTGG
1948R	CCAGACCTCTGTGAAGCCAG
3240R	GGTGCAGAGCAAGCTCCTGG
3033F	GGTGCATGCTGCAGTGCAGC
3152R	CCCACGTTGCAGAAAACTGG
4381R	GCTGTGTCTCAGCATGAATTC
4066F	GGCTCTGAGTGCTCAGATCC
4267R	CCAACTCTACGAAGGAAGTG
4534R	TCTGCATGCTGACCAGTTAACC
44R	GGGTGGTGTTCCCTGGAGG
5010R	CCTGCACTGACTTCAGTGC
47F	CTGGATGACTTGGTCTTCACTC
49R	GAGGAACTTCCACCGTTCAGG
6140R	CCATCTCATGGAGGAGGTCA
57F	GCAGAGTCTTCCATGTTCTCC
59R	CATCCAACTCTGCAGGAGGT
7045R	CCACAAGGTGCTCTGG
67F	CTTGGAGCTCCAGCAGCAAG
69R	GGCAGAGGTCCTAAGGAGG
11115R	CCTTCCACATCCCAGAGG
10F	GCCTCACAGACTCTCCCAGG

11R	GACACTCGAGACTTGAGGTGA
1119R	CCTTCCTGGTGAGCGTGTCTGC
87F	GGAAGGAGTGAGGTCCCACTCAGC
63F	CCTCTAGCTTCACCTAGAGATAGC
10R	GTCTTCTACAGAACACCTTAGG
689R	GCTCCTTCCGGAAGCCTCCAG
110R	CTGAGGATCCCCTGCAGCCTCCTTGCTGCGCAATGTCC
24F	GCACACTCGTACTGAGACTC
62R	CACTGCACTACCTGACTCA
115R	CCTTCAAGCATTCCATTACG
139R	CTCGCCTAGTCTGGAAGCTCC
1R	CACCGGGGCAACCAGGGGAAGATGGGG
1F	GCTGTAACAGTGTTTATTGA
1144R	GAGTGAATGAGCTCTCAG
1272R	CCACAGTGGACTTGGTGACG
1214R	GCAGACAGTACAGCATCCA
HOTNAT-F2	TGTAAGAATTCGTGTGCCACCATGATGAGTCCAGGGCTCC
HOTNAT-R	TTACAGGATCCCAGAGCTGAGCTCCTCCAGC
HOTMUT-F	TAGTTGAATTCAGTGTTTATTGTGAATGAGTCCAGG
HOTMUT-R	TACTAGGATCCTCAATCCCAGAGCTGAGCTCCTCCAGC
GAPDH-F	CCATGAGAAGTATGACAACAGC
GAPDH-R	CATGGACTGTGGTCATGAGTCC