

Appendix

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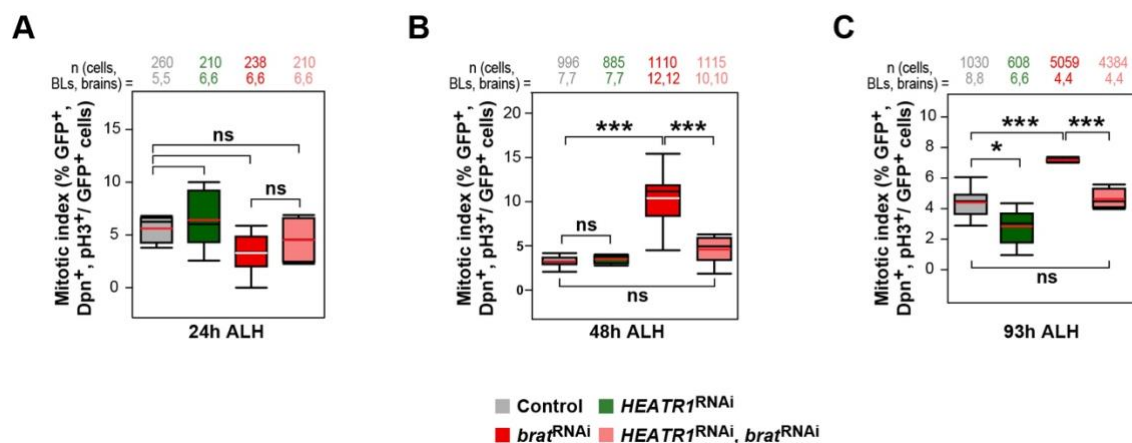
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Appendix Figure S1

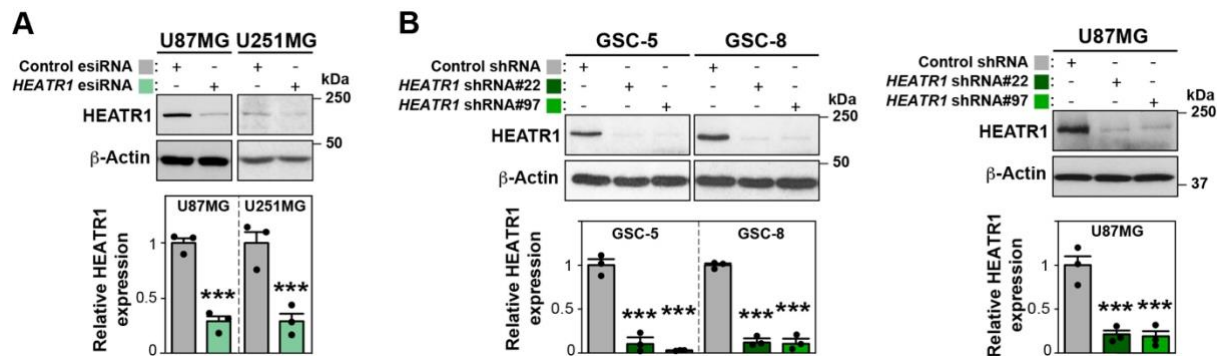


Appendix Figure S1. Mitotic cell index of *brat*-deficient tumour and control brains following *HEATR1* knockdown

A-C Mitotic cell index quantifications (% GFP⁺Dpn⁺pH3⁺/ GFP⁺ cells) of type II NSC lineages expressing *CD8-GFP* (control) and *HEATR1*^{RNAi}, *brat*^{RNAi} (tumour) or both *HEATR1*^{RNAi}, *brat*^{RNAi} (*HEATR1*-deficient tumour) immunostained with GFP, Dpn and pH3 at 24 h (A; biological replicates: 5-6), 48 h (B; biological replicates: 7-12) and 93 h (C; biological replicates: 4-8) ALH. Unpaired two-tailed t-tests, except in B (Mann-Whitney test, *HEATR1*^{RNAi} versus control). Representative images in Figures 2A-D, L-O and Q-T for 24 h, 48 h and 93 h ALH, respectively.

Data Information: Box plots represent 25th and 75th percentiles, central black bands indicate medians, central red or white bands specify means, whiskers indicate 10th and 90th percentiles. ***p ≤ 0.001; *p ≤ 0.05; p > 0.05, ns (non-significant).

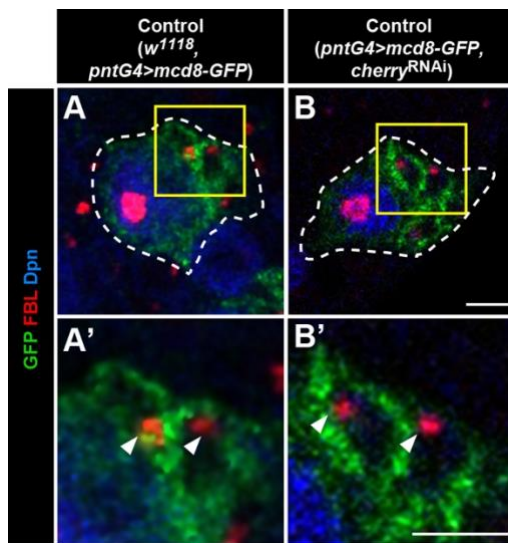
Appendix Figure S2



Appendix Figure S2. Efficient *HEATR1* knockdown in GBM cell lines and GSCs

A, B. Immunoblots of *HEATR1* in GBM cell lines (U87MG; U251MG) 48 hpt with *HEATR1*-esiRNA or control *GFP*-esiRNA (A) and of GSCs (GSC-5; GSC-8) and U87MG cells 168 hpi with *HEATR1*-shRNAs or control shRNAs (B). β-Actin: loading control. *HEATR1* signal quantifications, error bars: s.e.m, biological replicates: 3. Unpaired two-tailed t-tests (***) $p \leq 0.001$).

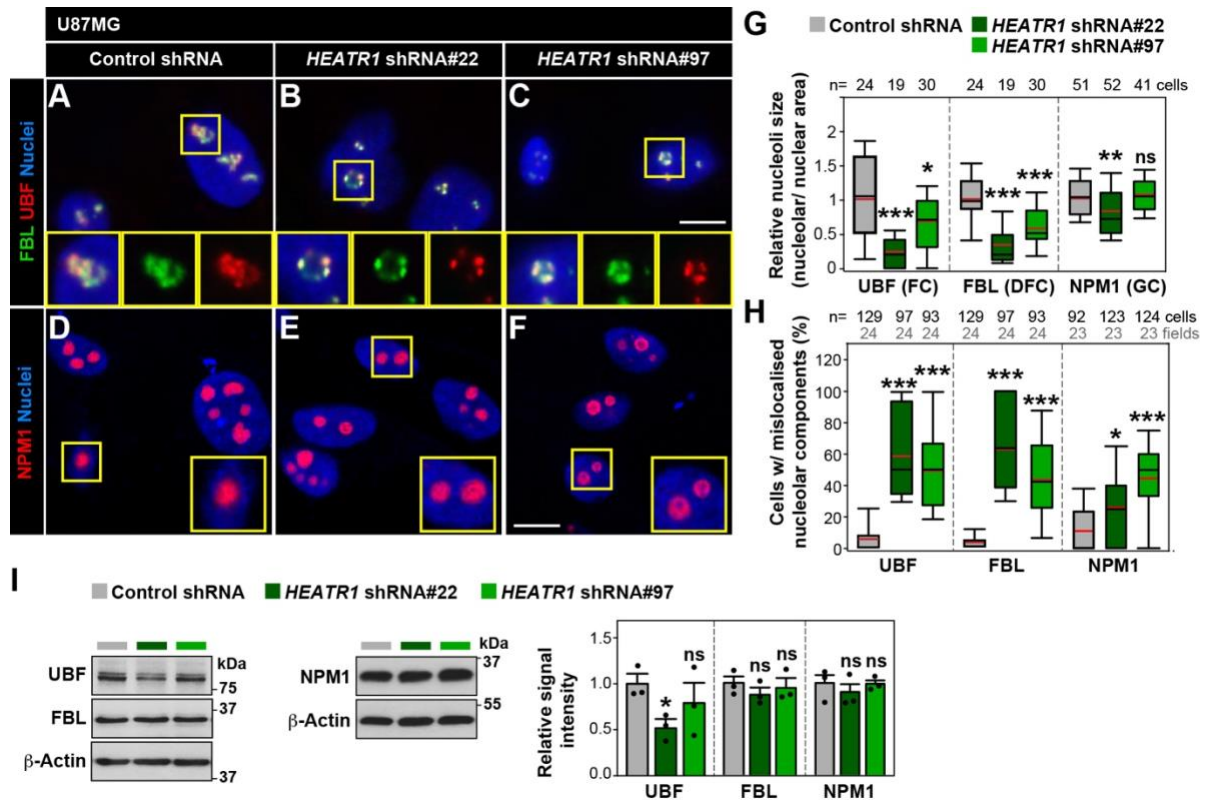
Appendix Figure S3



Appendix Figure S3. Representative images of control *Drosophila* RNAi expression assays

A-B' Immunostainings of GFP, FBL and Dpn in control type II NSC lineages expressing *CD8-GFP* in the GD RNAi library host isogenic background (A) or simultaneously expressing *cherry^{RNAi}* (B) at 24 h ALH. Insets: higher magnification in A', B'. Arrowheads: nucleoli. Scale bars: 5 μ m. Quantification of nucleoli sizes and sample numbers in Figure 4E.

Appendix Figure S4



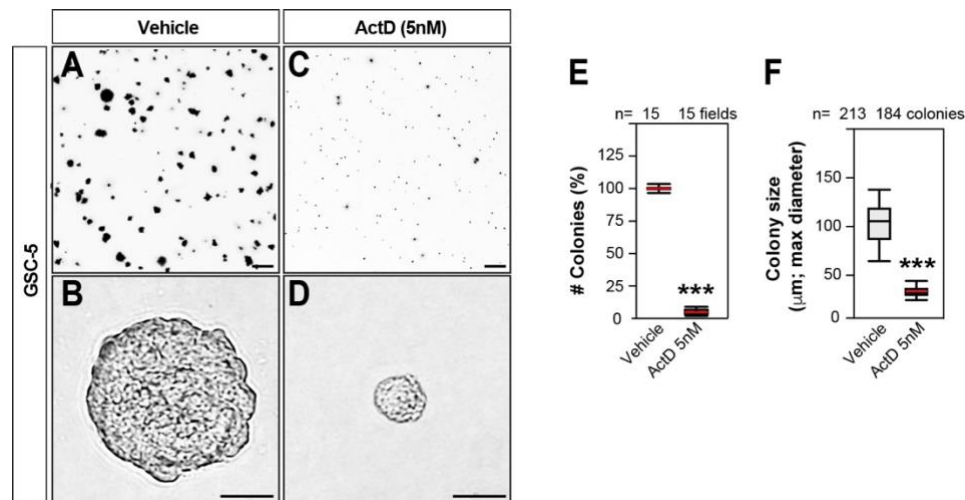
Appendix Figure S4. *HEATR1* inhibition impairs nucleoli size and architecture in GBM cells

A-H. Immunostainings of FBL, UBF (A-C) and NPM1 (D-F) in U87MG GBM cells 168 hpi with control shRNAs (A, D) or *HEATR1*-shRNAs (B-C, E-F). FBL, UBF and NPM1 mark dense fibrillar centres (DFCs), fibrillar centres (FCs) and granular components (GCs) of nucleoli functional domains, respectively. Nuclei (DAPI). Insets: higher magnification. Scale bars: 10 μ m. Nucleoli size quantification relative to nucleoli domain areas (maximum areas; G): 19-30 (UBF), 19-30 (FBL) and 41-52 (NPM1) cells from 3 biological replicates. Number of cells (%) with mislocalised nucleolar components (H): 24 (UBF), 24 (FBL) and 23 (NPM1) cell images (fields) from 3 biological replicates. Unpaired two-tailed t-tests except in G (Mann-Whitney tests: UBF, FBL and NPM1 analysis upon *HEATR1*-shRNA #22 versus control) and in H (Mann-Whitney tests: UBF and FBL analysis upon *HEATR1*-shRNA #22 or #97 versus controls).

I. Immunoblots of U87MG cells 168 hpi with *HEATR1*-shRNAs or control shRNAs with indicated antibodies. β -Actin: loading control. Quantifications of relative signals. Error bars: s.e.m., biological replicates: 3, unpaired two-tailed t-tests. UBF and FBL blots share the same loading β -Actin controls as in Appendix Figure S6A since immunoblot membrane was cut and probed with the different indicated antibodies (see data source files).

Data Information: Box plots represent 25th and 75th percentiles, central black bands indicate medians, central red bands specify means, whiskers indicate 10th and 90th percentiles. ***p $\leq$ 0.001; **p $\leq$ 0.01; *p $\leq$ 0.05; p > 0.05, ns (non-significant).

Appendix Figure S5



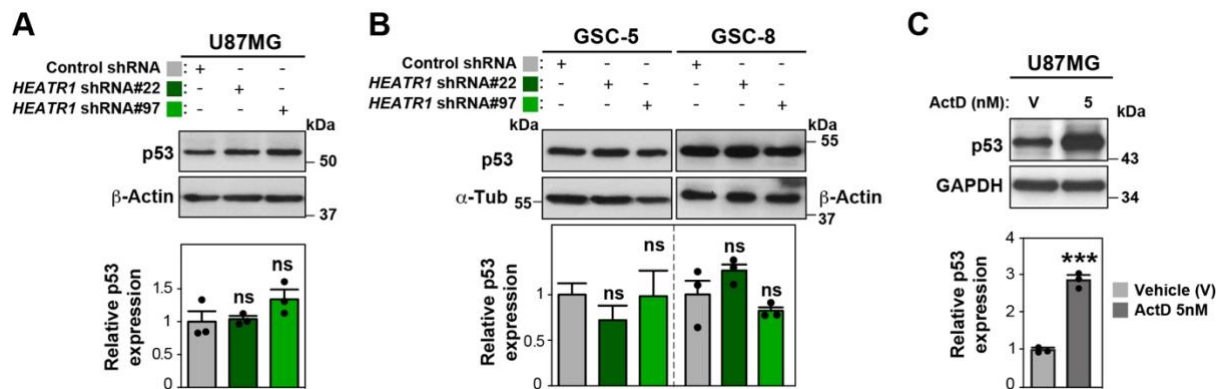
Appendix Figure S5. RNA Pol I inhibition by ActD abolishes GSC tumourigenic potential

A-F Soft agar colony formation assay of GSC-5 treated with vehicle (A, B) or ActD (C, D). Colony number (%) quantification (E): 15 fields per condition from 3 biological replicates. Colony size quantification (F): 184-213 colonies from 3 biological replicates. Mann-Whitney tests.

Scale bars: 400 μm (A, C); 50 μm (B, D).

Data Information: Box plots represent 25th and 75th percentiles, central black bands indicate medians, central red bands specify means, whiskers indicate 10th and 90th percentiles. ***p ≤ 0.001.

Appendix Figure S6



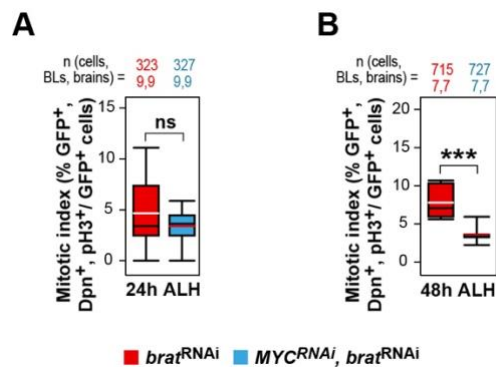
Appendix Figure S6. p53 total levels are not affected by *HEATR1* inhibition in GBM cells or GSCs

A, B Immunoblots of p53 in U87MG cells (A) and GSCs (GSC-5; GSC-8; B) 168 hpi with *HEATR1* or control shRNAs. β -Actin, α -Tubulin: loading controls. p53 signal quantifications. Biological replicates: 3 (U87MG; GSC-8), 6 (GSC-5). Error bars: s.e.m. Unpaired two-tailed t-tests except in B (Mann-Whitney test: GSC-8 upon *HEATR1* shRNA #97 versus control).

C Immunoblots of p53 in U87MG cells treated with ActD (5 nM) or vehicle for 24 h. GAPDH: loading control. p53 signal quantifications, error bars: s.e.m, biological replicates: 3, unpaired two-tailed t-test.

Data Information: ***p $\leq$ 0.001; p > 0.05, ns (non-significant).

Appendix Figure S7



Appendix Figure S7. Mitotic cell index of *brat*-deficient tumour brains following *MYC* knockdown

A, B Mitotic cell index quantifications (% GFP⁺Dpn⁺pH3⁺/ GFP⁺ cells) of type II NSC lineages expressing *CD8-GFP* and *brat*^{RNAi} (tumour) or both *MYC*^{RNAi}, *brat*^{RNAi} (*MYC*-deficient tumour) immunostained with GFP, Dpn and pH3 at 24 h (A; biological replicates: 9) and 48 h (B; biological replicates: 7) ALH. Unpaired two-tailed t-tests. Representative images in Figure 6A, B and E, F for 24 h and 48 h ALH, respectively.

Data Information: Box plots represent 25th and 75th percentiles, central black bands indicate medians, central red or white bands specify means, whiskers indicate 10th and 90th percentiles. ****p* ≤ 0.001; *p* > 0.05, ns (non-significant).

Appendix Table S1

Sample	Age (years)	Gender	Clinical Diagnosis (WHO grade)	Brain region	IDH status	RT-qPCR	WB	IHC
CB9	33	M	Normal*	Fr	N/A	+	+	-
CB10	32	M	Normal	Fr	N/A	+	+	-
CB18	61	M	Normal*	Fr	N/A	+	-	-
CB19	70	M	Normal	Te	N/A	+	-	-
CB20	57	F	Normal	Fr	N/A	+	+	-
CB21	57	M	Normal	Fr	N/A	+	-	-
CB77	77	M	Normal	Fr	N/A	-	+	-
CB78	61	M	Normal	Ce	N/A	-	+	-
CB79	65	M	Normal	Ce	N/A	-	+	-
CB84	36	M	Normal	Te	N/A	-	-	+
CB85	52	F	Normal	Pa	N/A	-	-	+
CB86	62	M	Normal	Oc	N/A	-	-	+
CB87	72	M	Normal	Fr	N/A	-	-	+
CB88	37	M	Normal	Fr	N/A	-	-	+
CB89	34	M	Normal	Fr	N/A	-	-	+
CB90	34	M	Normal	Fr	N/A	-	-	+
CB91	70	M	Normal	Te	N/A	-	-	+
CB92	73	M	Normal	Te	N/A	-	-	+
CB93	52	F	Normal	Te	N/A	-	-	+
CB55	38	F	DA (II)	Fr	mut	+	+	+
CB56	38	M	DA (II)	Fr	mut	+	+	+
CB57	44	M	DA (II)	Iv/Pv	mut	+	+	+
CB58	53	F	DA (II)	Oc	mut	+	-	+
CB60	34	F	DA (II)	Te	mut	+	-	+
CB61	31	M	DA (II)	Fr	mut	+	+	+
CB62	63	M	DA (II)	Te	mut	+	-	+
CB63	37	F	DA (II)	N/A	mut	+	+	+
CB76	25	M	DA (II)	Fr	mut	+	-	+
CB13	40	F	GBM (IV)	Fr	wt	+	-	-
CB17	69	F	GBM (IV)	Fr	wt	+	-	-
CB22	73	F	GBM (IV)	Te	wt	-	+	-
CB23	53	M	GBM (IV)	Fr	wt	+	-	-
CB24	53	F	GBM (IV)	Fr	wt	+	-	-
CB29	68	M	GBM (IV)	Pa	wt	-	+	-
CB30	73	M	GBM (IV)	Fr	wt	+	-	-
CB31	64	M	GBM (IV)	Fr	wt	+	-	-
CB32	63	M	GBM (IV)	Fr	wt	+	-	-
CB33	58	M	GBM (IV)	Fr	wt	+	-	-
CB34	70	F	GBM (IV)	Fr	wt	+	-	-
CB35	65	F	GBM (IV)	Fr	wt	+	+	-
CB42	73	F	GBM (IV)	Fr	wt	-	+	-
CB43	69	F	GBM (IV)	Fr	wt	-	+	-
CB44	41	F	GBM (IV)	Fr	wt	-	+	-
CB45	54	M	GBM (IV)	Fr	wt	-	+	-
CB65	23	F	GBM (IV)	Fr	wt	+	+	+
CB66	62	F	GBM (IV)	Te	wt	+	-	+
CB67	70	M	GBM (IV)	Oc	wt	+	-	+
CB68	77	M	GBM (IV)	Fr	wt	+	+	+
CB69	66	M	GBM (IV)	Te	wt	+	-	+
CB70	61	M	GBM (IV)	Te	wt	+	+	+
CB72	47	M	GBM (IV)	Te	wt	+	-	+
CB73	51	F	GBM (IV)	Tpa	wt	+	+	+
CB80	84	M	GBM (IV)	Pa	wt	+	-	-
CB82	40	M	GBM (IV)	Fr	wt	-	-	+

Appendix Table S1. Human tissue samples used for gene and protein expression analysis

M: male. F: female. DA: Diffuse astrocytoma (grade II). GBM: glioblastoma (grade IV).
*Normal appearing brain from adjacent to glioma. Fr: frontal. Ce: cerebellum. Te: temporal.
Pa: parietal. Oc: occipital. Iv: intraventricular. Pv: periventricular. Co: cortex. Ft:
frontotemporal. Tpa: temporoparietal. N/A: not available. WB: Western blotting. IHC:
immunohistochemistry.

Appendix Table S2

Gene (symbol)	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Source
<i>Drosophila</i>			
<i>ase</i>	CACCTACCAACTGCTGACG	GCTGCTGCTGCTAATGTTG	This paper
<i>CG6724</i>	ACACGGAGACACATTCCACC	GGTGGACTGGATATCGCTGG	This paper
<i>Ilk</i>	TTCTCGCCGTCAGATCATCG	TGTTGCGAGAGGAATGGCTT	This paper
<i>CG2126</i>	GGCAGGATCCGTGAAACGTA	AGCTGTGCACTGGATGTTGT	This paper
<i>Hsf</i>	ATGCCGATACCAATCGCTTGA	CTTGCGGAATTGCGCTTGA	This paper
<i>nop5</i>	GCCTCTGGTTGGTAGGTGAA	GCGGCTCACAAGGTTAAACT	This paper
<i>NS1</i>	AAGCACAAAGATCGAAAAGAAGGT	TGTCATCCTTGAAGGGACAGA	This paper
<i>Prp3</i>	AATAGCTTCTTCTGGGCCG	AACATACGCGCCAAGAAACG	This paper
<i>en</i>	AGCGATTCTCGTTGAACTCC	CAGCCAAAGGACAAGACCA	This paper
<i>SC35</i>	CCGTCTAGCATGCGACCAT	CGGATTCGCATTTGTTGCT	This paper
<i>Kap-α1</i>	AGAGGCTCTAGAATGCCGGA	GCTCTCCAGTCCCATGATG	This paper
<i>link</i>	TTTCAAGCCAGCAGGAGGAG	CTGTGGTCTGGTCAGGTCAC	This paper
<i>ATPsynγ</i>	CCCACGCAGAACACCTTAGT	CTCATCGCAGTGACTTCGGA	This paper
<i>GC3499</i>	TCTTCGTCGGCTCTTCTCACA	CGCTTTTGTCCGCCTCAA	This paper
<i>gus</i>	GGCAAGGGTTCGGGATCTAA	TTGCGTTTAGAGGACTGCGA	This paper
<i>Mob3</i>	GGGCAAGACCTTTAGGCCAAA	CGATTGAAGAAGTCCACAACGTG	This paper
<i>Nop60B</i>	CGCAGCATAGACTCGTCTT	AGTCTGAGCGCGATGGTATG	This paper
<i>mRpL12</i>	CGAACTTGACCAGCTTGACCT	AAACTGAACCTGCCCGAGAC	This paper
<i>Obp44a</i>	GTTCTTGTCGGCGCACTT	GGGCTTCAAGGTGGAGAAC	This paper
<i>Fis1</i>	TAGTCACGCCTTCCATCTGG	TACGCATTCTGTCTGGTCCG	This paper
<i>Kr-h2</i>	GCTACGTGCTGCCATCTTTA	GGCAATCTCTGGTGCAAACG	This paper
<i>Prosβ7</i>	ATTTGGCTTCGGTTTGGCTG	GTAACAAGTGGGAGGGCGA	This paper
<i>eIF3-S9</i>	CTTTCACCTTCCATGACGAGAC	GGACTCCACCAACGATTACATC	This paper
<i>Smox</i>	GATCGCCGCAATTGTAATCGG	TCCATCCTGGTGCCCAAATC	This paper
<i>U2af50</i>	AATTTCCACGCTCCGCAC	CCGACTGAGGTTCTTTGCTTG	This paper
<i>blw</i>	TACCGAAGACCACAACACCG	GCCGATGAGATGGTGGAGTT	This paper
<i>ana1</i>	AGGCAGTGGAGTTGGAATCA	TCACGGAAAGCGACCATTCT	This paper
<i>SPARC</i>	TGCAGCACAATCTACTCAATCC	GTAGAATCGAGACCGAGGAGAG	(Gaddam <i>et al</i> , 2013)
<i>mtrm</i>	ATGCCAGTTCACCAGATCG	CGTCGTCTTCCATCAAAGC	This paper
<i>l(2)k09022</i>	GCAGTTGCCCTTCTGTGTTT	CAGCGCAAGAGTGAGTTGAG	This paper
<i>Nopp140</i>	CTTGCCGTTTGTAGTGCA	AGATCCCAGCAATCAAGGC	This paper
<i>RpL5</i>	GAGCTGCAATCTTAGTCTCCG	CACCAGGCAATTCGTAACG	This paper
<i>l(2)35Di</i>	TTGATGGGGTTGTTCAGCTC	TGAAGCCGATGGTAATTGCG	This paper
<i>Dredd</i>	CGCCTCCACATTGTATCCCA	CCAGCAGAAGTTTCACCGGA	This paper
<i>Hakai</i>	GAAGGTGCTGAACCCGATGAT	GAGGCAAAATACATGCTTGCA	This paper
<i>CG12262</i>	CTCCACAATGAACCCGGTGA	AGTGGGTAAATCAACGGCCAG	This paper
<i>CG2862</i>	GCAATGAGATGTCCAGCA	AAATGCGTTGCCTTCCAC	This paper
<i>Spn42Da</i>	GAACCTGGGCAATTTGAGCG	GATCGTACTGCCAACACCA	This paper
<i>rp49 (calibrator)</i>	GCTAAGCTGTCGCACAAATG	GTTGATCCGTAACCGATGT	(Kohyama-Koganeya <i>et al</i> , 2008)
Human			
<i>MOB3A</i>	ATCCAGTCCATCAGCAGGTC	ACCTCATCTACGGCACCATC	This paper
<i>JKAMP</i>	AACCAGAGGAAGCATTGCCA	GGACAGAGAACGAATGCACAG	This paper
<i>SPARC</i>	TCCAGGCGCTTCTCATTCTC	GCTCAAGAACGTCTGGTCA	This paper
<i>FIS1</i>	CGTACTCAAACGCGTGCTC	CTGAACGAGCTGGTGTCTGT	This paper
<i>HEATR1</i>	GGTCTGAACACTTCGCTCCA	TACTGGCCTGGAAGAGTTGC	This paper
<i>YME1L</i>	ATTCTCCAACCTGCTCCG	GCAAGTTACAGTTCCAAGGC	This paper
<i>SMAD3</i>	TGGTGGTCACTGGTTTCTCC	CACTGGACGACTACAGCCAT	This paper
<i>KPNA6</i>	CTTGCCCTCTTGCTCTCAA	TCTACTGGGCTGCATCAA	This paper

<i>WDR12</i>	CCATGTGTTTGTCCAAGGGC	CTGCTGCCTCTGAAATTGCC	This paper
<i>ILK</i>	ATGAGGAGCAGGTGGAGACT	TGCTGAAGGTTTCGAGACTGG	This paper
<i>ATP5A1</i>	TGAAGAGGACAGGAGCCATTG	ACCAAGGGCATCAACTACAC	This paper
<i>SPSB1</i>	AGAACCAGCCAAGCAAAACA	AAGCCACTCCCATGTACTGT	This paper
<i>TMEM33</i>	TGCTCTGAGGCTGCATCAA	AACAGGTAGTGGCAGCTGTC	This paper
<i>HINT1</i>	TTTGGCTGGTATTTCTTGC	GCGGGTTCCTCCCTTCTT	(Hua <i>et al</i> , 2011)
<i>SERPINI1</i>	GAGCTGGGCACTCAGAATGT	GGACACCCTCAGCATGTTCA	This paper
<i>PRPF3</i>	ACAGATGATGGAAGCAGCAA	GGTTGGGAGGATGAAGGAGT	This paper
<i>ATP5C1</i>	GAGCTGCAGCACCAGAGATA	GAGCAGAGTGCCAGGATGA	This paper
<i>ACADM</i>	AAATCATCCCAGTGGCTGCA	ACCAAGTTCCCAGGCTCTTC	This paper
<i>CBLL1</i>	CGCAGACGAATTCCTATAAAGC	CCTTCTTCATCACCAGGTGG	This paper
<i>*TRIM3</i>	GGCTGACTGGGGCAACAGCCGCATC	ATCTGCCAGAACCACTGTATGGTCCA	(Chen <i>et al</i> , 2014)
<i>47S pre-rRNA (5'ETS)</i>	CCTGCTGTTCTCTCGCGCTCCGAG	AACGCCTGACACGCACGGCACGGAG	(Grandori <i>et al</i> , 2005)
<i>18S</i>	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA	(Karahan <i>et al</i> , 2015)
<i>5.8S</i>	CTCTTAGCGGTGGATCACTC	GACGCTCAGACAGGCGTAG	(Karahan <i>et al</i> , 2015)
<i>28S</i>	CAGGGGAATCCGACTGTTTA	ATGACGAGGCATTTGGCTAC	(Karahan <i>et al</i> , 2015)
<i>MYC</i>	CCTCTCAACGACAGCAGCT	CAGAAGGTGATCCAGACTCTG	(Rossetti <i>et al</i> , 2018)
<i>RRN3</i>	CGGAAACCTGAAAGAAGGTTTGC	CTGGCGATTGTTCTCTCAATG	(Rossetti <i>et al</i> , 2018)
<i>POLR1B</i>	TATGGAAGATGCCATGATTGTGA	TGTAATACGGATCTCCGTACTG	(Rossetti <i>et al</i> , 2018)
<i>RPL32 (calibrator)</i>	CATCTCCTTCTCGGCATCA	AACCCTGTTGTCAATGCCTC	(Reber <i>et al</i> , 2006)

Appendix Table S2. Primers used for PCR and RT-qPCR gene expression analysis.

*Reverse primer contains an extra cytosine.