

Supplemental Material for Manuscript:

HEY1 functions are regulated by its phosphorylation at Serine 68

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A

ANTIGEN:

Sequence: DRINN(pS68)LSELRRL-cys

MW: 1769,89g/mol (MS)

Purity: 85,56% (HPLC)

Peptide ID: P120814-XP308028

ANTIGEN

PREPARATION:

Conjugation to carrier (KLH)

PRODUCT ID:

A003481

LOT NO:

A120730-ZJ1097

HOST:

Rabbit

ELISA RESULTS - DRINN(PS68)LSELRRL-CYS:

Dilution	Blank	Negative	1:1000	1:4000	1:16000	1:32000	1:64000
Rabbit1	0,063	0,057	1,942	1,762	1,578	1,312	1,057

B

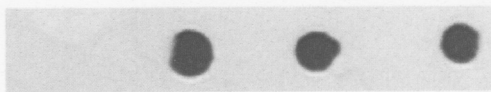


Fig1: Phosphor-antibody rabbit1

Antigen: 1h , RT

Line1: Negative control Non-Phosphor-peptide 100ng PO# 308029

Line2-4: Phosphor-peptide 100ng PO# 308028

5% Milk blocking 2h,RT

Primary Antibody: 1h , RT

Line1: Phosphor-antibody Rabbit1 1:1000 dilution

Line2: Phosphor-antibody Rabbit1 1:1000 dilution

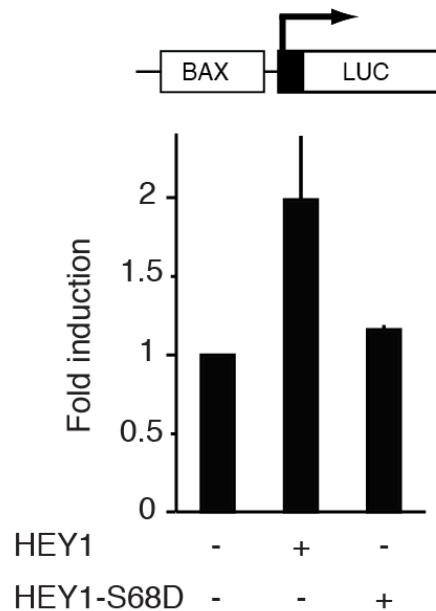
Line3: Phosphor-antibody Rabbit1 1:4000 dilution

Line4: Phosphor-antibody Rabbit1 1:16000 dilution

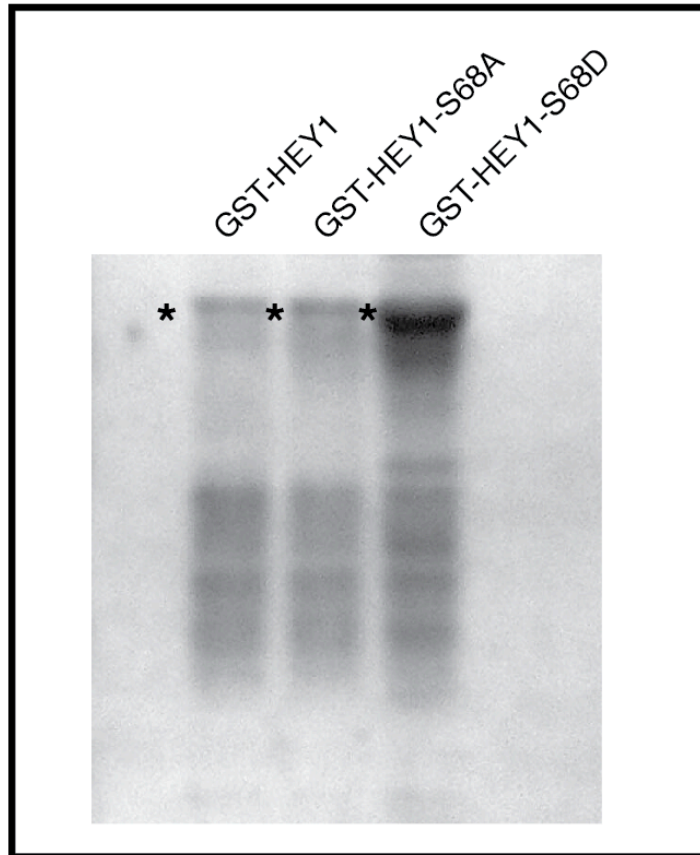
Secondary Antibody: 1h, RT

ECL

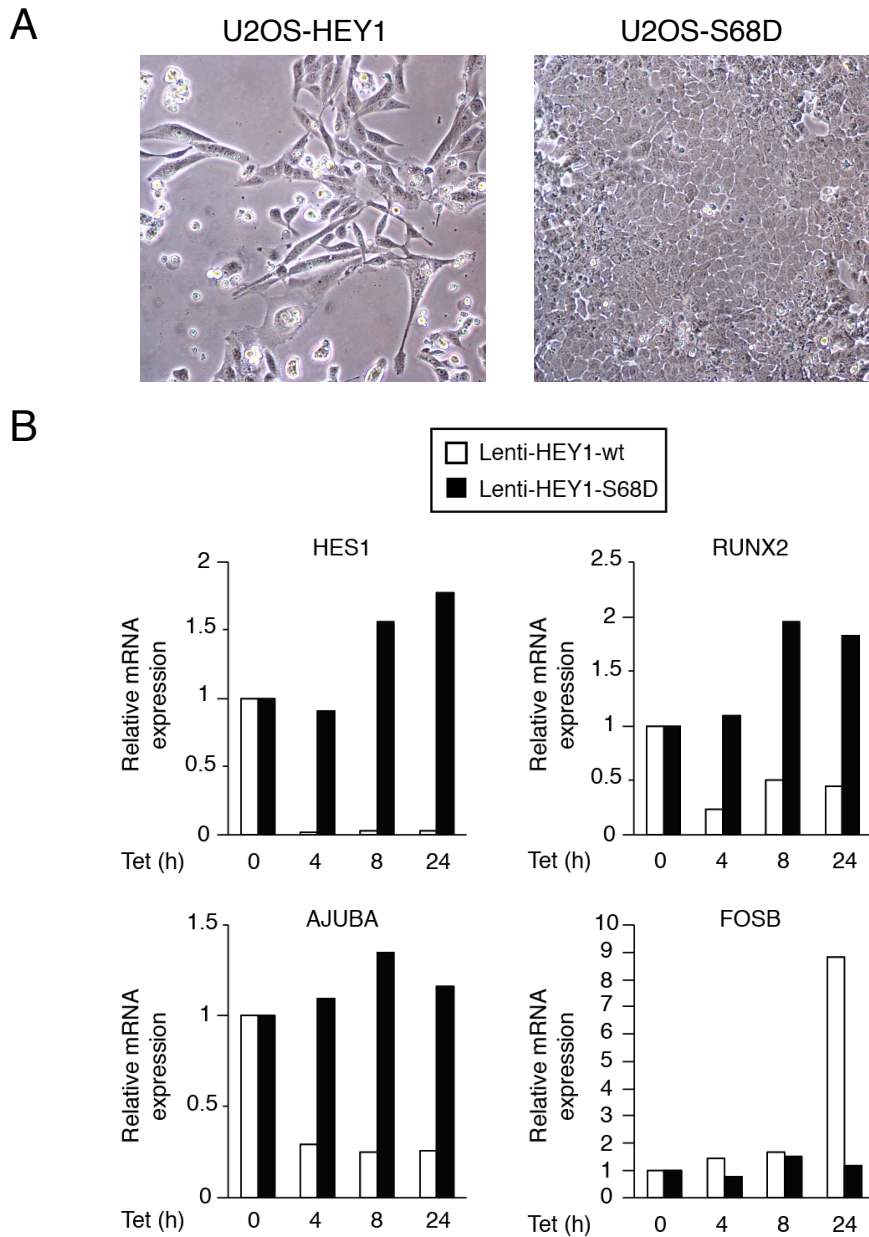
Supplemental Figure S1. Antibody specificity validation using ELISA (A) and DOT BLOT (B)



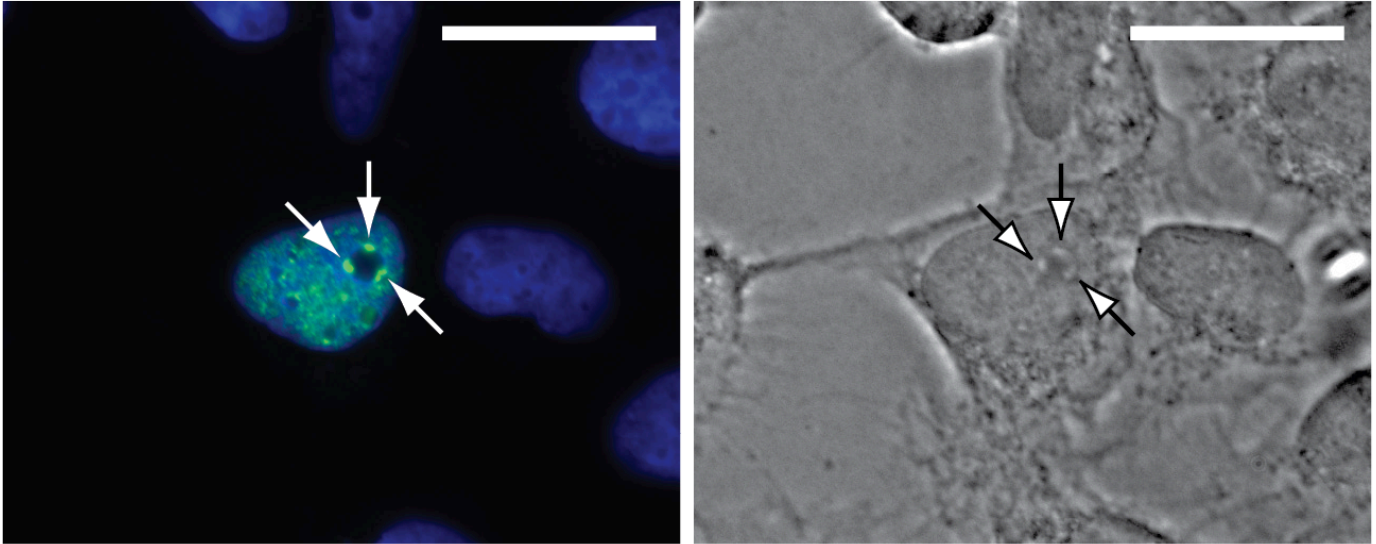
Supplemental Figure S2. Simulation of HEY1 phosphorylation at residue Ser-68 inhibits its ability to enhance p53 transcriptional activity. (A) U2OS cells were transfected with 100 ng of BAX-LUC and 200 ng of expression vectors for HEY1 or HEY1-S68D. After transfection, cells were incubated 24 hours. Subsequently cell lysates were assayed using a dual luciferase reporter system. Normalized values are expressed relative to the activity of the reporter in the absence of HEY1. The results shown represent the averages of results of three independent experiments assayed in duplicate + s.d.



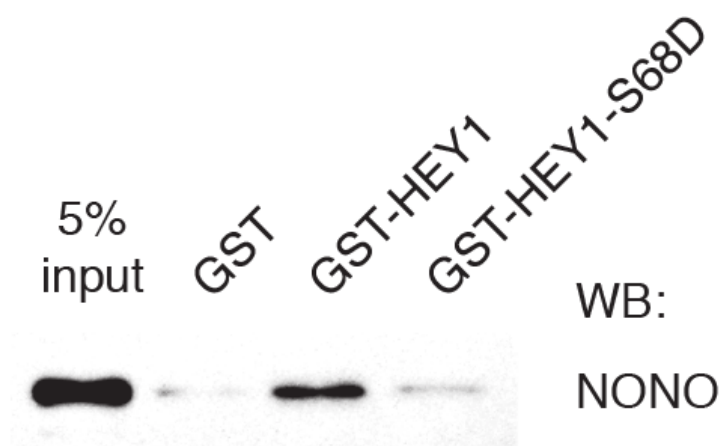
Supplemental Figure S3. Coomassie-stained gel show the typical expression levels of the bacterially expressed GST fusion proteins used in the pull-down assays marked with an asterisk. To perform the assays all proteins were diluted at similar concentrations.



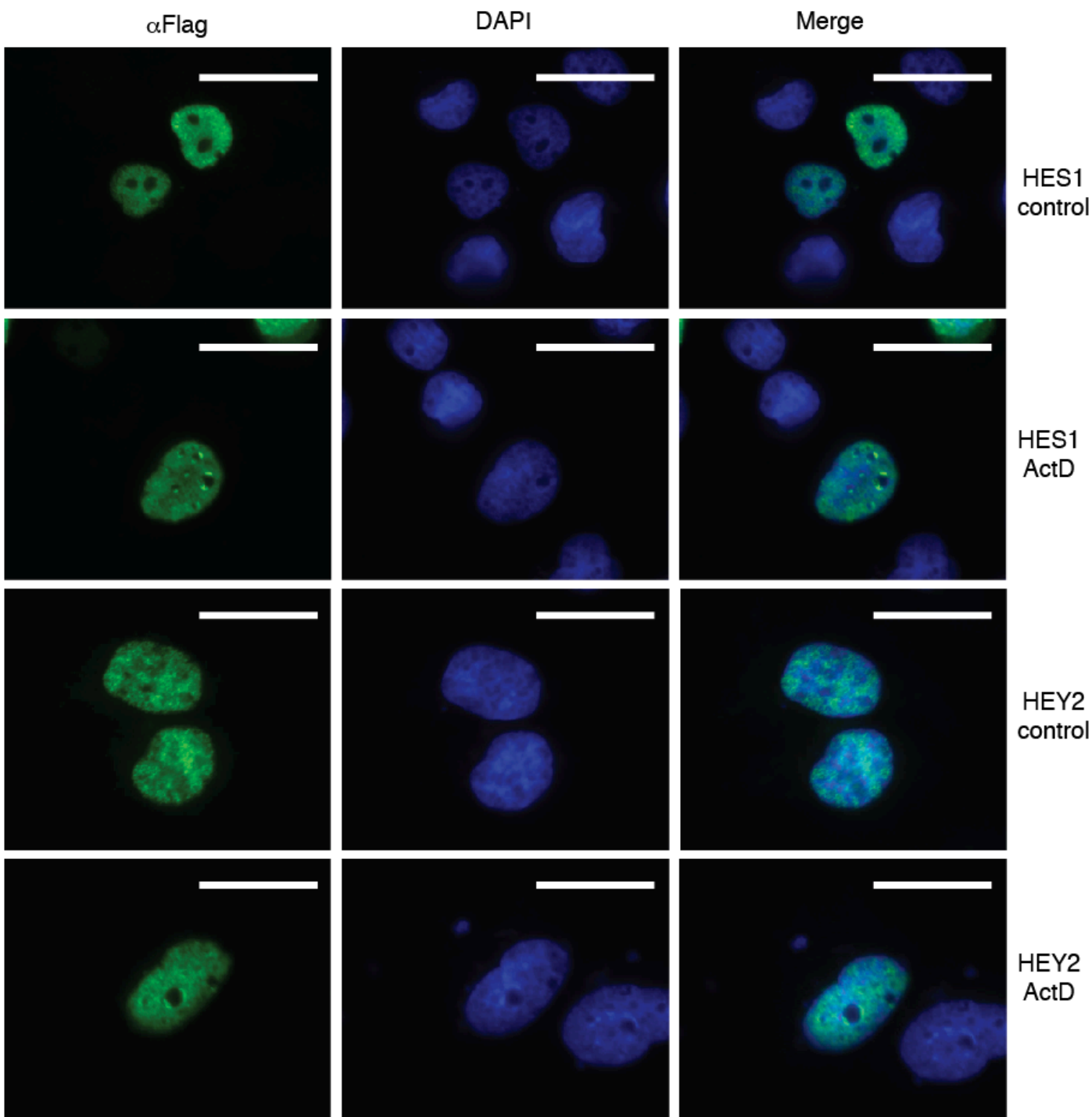
Supplemental Figure S4. Characterization of U2OS-HEY1 and U2OS-S68D cell lines. **(A)** Phase-contrast micrograph of monolayers of U2OS-HEY1 and U2OS-S68D cells cultured for 7 days in the presence of 1 $\mu\text{g/ml}$ tetracycline. **(B)** Quantitative RT-PCR analysis expression for the expression of HES1, RUNX2, AJUBA and FOSB transcripts in U2OS-HEY1 (white bars) and U2OS-S68D (black bars) cells treated with 1 $\mu\text{g/ml}$ tetracycline (Tet) for 4 h, 8 h or 24 h. Results from a representative experiment are shown. qRT-PCR analysis was performed as previously described (PMID: 19802006). The human-specific primers used were: HES1-forward; CGGACATTCTGGAAATGACA, HES1-reverse; CATTGATCTGGGTCATGCAG, RUNX2-forward; CAGACCAGCAGCACTCCATA, RUNX2-reverse; CAGCGTCAACACCATCATTC; AJUBA-forward; CTGGAAATGATGACTCCTGACA, AJUBA-reverse; ACAAGGTGCATTTCCCTC. FOSB-forward; GAAGAGGAGAAGCGAAGGGT, FOSB-reverse; CACCAGCACAACTCCAGAC.



Supplemental Figure S5. Ribosomal stress induced by Actinomycin D cause HEY1 localization in phase light nucleolar caps. U2OS cells previously transfected with expression vector for Flag-Tagged HEY1 were treated with Actinomycin D (5 nM) for 6h and assayed by indirect immunofluorescence with anti-Flag antibody. The left picture shows the indirect immunofluorescence with anti-Flag antibody (green) and DAPI staining of DNA (blue) and the right picture shows the phase contrast. Arrows indicate nucleolar caps. Bars, 20 μ m.



Supplemental Figure S6. Figure 8. In vitro interaction of NONO with HEY1. Whole cell extracts from U2OS cells previously transfected with expression vector for Flag-tagged NONO were incubated with glutathione S-transferase (GST) fusion proteins of HEY1 or HEY1-S68D coupled with Sepharose beads. Bound proteins were detected by western blotting using anti-Flag antibody.



Supplemental Figure S7. Ribosomal stress induced by Actinomycin D causes HES1 and HEY2 perinucleolar localization. (A) U2OS cells previously transfected with expression vector for Flag-Tagged HES1 or Flag-Tagged HEY2 were treated with Actinomycin D (5nM) for 6h and assayed by indirect immunofluorescence with anti-Flag antibody. The first column shows the indirect immunofluorescence with anti-Flag antibody, the second column shows DAPI staining of DNA and the third column shows the merge image indicating the degree of colocalization. Bars, 20 μ M.

Supplementary Table S1: List of serine/threonine-protein kinases that co-immunoprecipitate with HEY1 in the proteomic study					
47 centrosome-related proteins (out of 278 total proteins)		Accession Number	Molecular Weight	Unique peptides detected	Protein coverage(%)
1	Gene_Symbol=STK38 Serine/threonine-protein kinase 38	MSIPI:IPI00027251.1 SWISS-PROT:Q15208	60 kDa	39	30
2	Gene_Symbol=STK38L Serine/threonine-protein kinase 38-like	MSIPI:IPI00237011.5 SWISS-PROT:Q9Y2H1	56 kDa	10	8,5
3	Gene_Symbol=CSNK2B Casein kinase II subunit beta	MSIPI:IPI00010865.1 SWISS-PROT:P67870	34 kDa	3	21
4	Gene_Symbol=MARK3 MAP/microtubule affinity-regulating kinase 3	MSIPI:IPI00917102.2 TREMBL:A2SY06	49 kDa	2	5,6

Supplementary Table S2: List of centrosome associated proteins that co-immunoprecipitate with HEY1 in the proteomic study

	47 centrosome-related proteins (out of 278 total proteins)	Accession Number	Molecular Weight	Unique peptides detected	Protein coverage(%)
1	Gene_Symbol=TUBA1B Tubulin alpha-1B chain	MSIPI:IPI00930688.1 SWISS-PROT:P68363 (+1)	130 kDa	60	20
2	Gene_Symbol=TUBB2C Tubulin beta-2C chain	MSIPI:IPI00007752.1 SWISS-PROT:P68371	78 kDa	57	32
3	Gene_Symbol=TUBA1A Tubulin alpha-1A chain	MSIPI:IPI00180675.4 SWISS-PROT:Q71U36	119 kDa	6	21
4	Gene_Symbol=TUBB2A Tubulin beta-2A chain	MSIPI:IPI00013475.1 SWISS-PROT:Q13885	87 kDa	7	25
5	Gene_Symbol=TUBB Tubulin, beta	MSIPI:IPI00645452.1 TREMBL:B4DQN9	48 kDa	10	50
6	Gene_Symbol=TUBB3 Tubulin beta-3 chain	MSIPI:IPI00013683.2 SWISS-PROT:Q13509 (+2)	57 kDa	8	29
7	Gene_Symbol=TUBA3D Isoform 1 of Tubulin alpha-3C/D chain	MSIPI:IPI00179709.4 SWISS-PROT:Q13748-1	83 kDa	7	27
8	Gene_Symbol=TUBA4A Tubulin alpha-4A chain	MSIPI:IPI00007750.1 SWISS-PROT:P68366 (+1)	50 kDa	2	38
9	Gene_Symbol=TUBB4 Tubulin beta-4 chain	MSIPI:IPI00023598.2 SWISS-PROT:P04350	73 kDa	2	30
10	Gene_Symbol=FLNA Isoform 2 of Filamin-A	MSIPI:IPI00302592.2 SWISS-PROT:P21333-2 (+2)	475 kDa	50	4,2
11	Gene_Symbol=ACTB Actin, cytoplasmic 1	MSIPI:IPI00021439.1 SWISS-PROT:P60709 (+3)	143 kDa	14	5,9
12	Gene_Symbol=VIM Vimentin	MSIPI:IPI00418471.6 SWISS-PROT:P08670	103 kDa	13	6,4
13	Gene_Symbol=HSPA8 Isoform 1 of Heat shock cognate 71 kDa protein	MSIPI:IPI00003865.1 SWISS-PROT:P11142-1	102 kDa	77	29
14	Gene_Symbol=HSPA5 HSPA5 protein	MSIPI:IPI00003362.2 SWISS-PROT:P11021	73 kDa	54	31
15	Gene_Symbol=HSPA1A Heat shock 70 kDa protein 1	MSIPI:IPI00304925.5 SWISS-PROT:P08107 (+1)	95 kDa	12	11
16	Gene_Symbol=HSP90AB1 Heat shock protein HSP 90-beta	MSIPI:IPI00414676.6 SWISS-PROT:P08238	118 kDa	8	3,6
17	Gene_Symbol=CCT5 T-complex protein 1 subunit epsilon	MSIPI:IPI00010720.1 SWISS-PROT:P48643	83 kDa	20	8,9
18	Gene_Symbol=CCT6A T-complex protein 1 subunit zeta	MSIPI:IPI00027626.3 SWISS-PROT:P40227 (+1)	78 kDa	10	6,9
19	Gene_Symbol=CCT8 59 kDa protein	MSIPI:IPI00302925.4 (+1)	59 kDa	2	4
20	Gene_Symbol=CCDC78 Isoform 1 of Coiled-coil domain-containing protein 78	MSIPI:IPI00216820.2 SWISS-PROT:A2IDD5-1	75 kDa	2	4,5
21	Gene_Symbol=CCDC88A Isoform 1 of Girdin	MSIPI:IPI00171134.5 SWISS-PROT:Q3V6T2-1 (+2)	234 kDa	2	1,8
22	Gene_Symbol=CCDC124 Coiled-coil domain-containing protein 124	MSIPI:IPI00060627.2 SWISS-PROT:Q96CT7	31 kDa	7	13
23	Gene_Symbol=CCDC72 Coiled-coil domain-containing protein 72	MSIPI:IPI00006378.3 SWISS-PROT:Q9Y2S6	8 kDa	9	27
24	Gene_Symbol=TTN titin isoform N2-A	MSIPI:IPI00759613.2 TREMBL:A2TKE6	5162 kDa	85	0,54
25	Gene_Symbol=TTN Isoform 1 of Titin	MSIPI:IPI00759754.1 SWISS-PROT:Q8WZ42-1	4875 kDa	10	0,59
26	Gene_Symbol=SYNE1 1011 kDa protein lng=8797	MSIPI:IPI00745471.3	1011 kDa	21	1
27	Gene_Symbol=SYNE2 Isoform 1 of Nesprin-2	MSIPI:IPI00239405.4 SWISS-PROT:Q8WXH0-1 (+1)	1056 kDa	10	0,86
28	Gene_Symbol=TNKS Isoform 1 of Tankyrase-1	MSIPI:IPI00021493.2 SWISS-PROT:O95271-1 (+1)	169 kDa	2	1,3
29	Gene_Symbol=TNKS1BP1 Isoform 1 of 182 kDa tankyrase-1-binding protein	MSIPI:IPI00304589.4 SWISS-PROT:Q9C0C2-1	243 kDa	2	1,7
30	Gene_Symbol=AKAP9 Isoform 3 of A-kinase anchor protein 9	MSIPI:IPI00220625.1 SWISS-PROT:Q99996-3	546 kDa	4	1,3
31	Gene_Symbol=CEP192 213 kDa protein	MSIPI:IPI00455675.9 (+1)	213 kDa	7	4,9
32	Gene_Symbol=VCP Transitional endoplasmic reticulum ATPase	MSIPI:IPI00022774.3 SWISS-PROT:P55072	114 kDa	19	7,6
33	Gene_Symbol=PLEC1 Isoform 1 of Plectin-1	MSIPI:IPI00014898.3 SWISS-PROT:Q15149-1	731 kDa	14	0,93
34	Gene_Symbol=EWSR1 Ewing sarcoma breakpoint region 1 isoform 1	MSIPI:IPI00009841.6 TREMBL:Q96MX4 (+4)	69 kDa	17	11
35	Gene_Symbol=STK38 Serine/threonine-protein kinase 38	MSIPI:IPI00027251.1 SWISS-PROT:Q15208	60 kDa	39	30
36	Gene_Symbol=STK38L Serine/threonine-protein kinase 38-like	MSIPI:IPI00237011.5 SWISS-PROT:Q9Y2H1	56 kDa	10	8,5
37	Gene_Symbol=CSNK2B Casein kinase II subunit beta	MSIPI:IPI00010865.1 SWISS-PROT:P67870	34 kDa	3	21
38	Gene_Symbol=CSNK2A1 CSNK2A1 protein	MSIPI:IPI00016613.2 TREMBL:B2R6D7 (+2)	46 kDa	3	14
39	Gene_Symbol=C14orf106 Isoform 1 of Kinetochores-associated protein KNL-2 homolog	MSIPI:IPI00104907.3 SWISS-PROT:Q6P0N0-1	153 kDa	2	1,5
40	Gene_Symbol=PTPN13 Isoform 1 of Tyrosine-protein phosphatase non-receptor type 13	MSIPI:IPI00030246.1 SWISS-PROT:Q12923-1	377 kDa	7	1,4
41	Gene_Symbol=RAB1A Isoform 1 of Ras-related protein Rab-1A	MSIPI:IPI00005719.1 SWISS-PROT:P62820-1 (+4)	25 kDa	2	12
42	Gene_Symbol=APC Isoform Long of Adenomatous polyposis coli protein	MSIPI:IPI00012391.3 SWISS-PROT:P25054-1 (+1)	525 kDa	7	0,41
43	Gene_Symbol=DCTN1 Putative uncharacterized protein DCTN1 lng=1283	MSIPI:IPI00872359.1 TREMBL:A8MY36	142 kDa	4	1,8
44	Gene_Symbol=FMNL1 Isoform 2 of Formin-like protein 1	MSIPI:IPI00025202.6 SWISS-PROT:O95466-2 (+2)	125 kDa	2	3,1
45	Gene_Symbol=CDC6 Cell division control protein 6 homolog	MSIPI:IPI00014575.1 SWISS-PROT:Q99741	85 kDa	2	4,7
46	Gene_Symbol=BIRC6 baculoviral IAP repeat-containing 6	MSIPI:IPI00299635.7 (+1)	686 kDa	2	0,58
47	Gene_Symbol=KIAA0101 PCNA-associated factor	MSIPI:IPI00014147.3 SWISS-PROT:Q15004 (+2)	18 kDa	2	14

Supplemental Table S3:GO Analysis; Cellular components						
	GO_id	Term	NumberOfGenes	p-value	p-value_fdr	p-value_bonferroni
1	GO:0022626	cytosolic ribosome	20	2.3E-18	3.11E-15	3.32E-15
2	GO:0070062	extracellular vesicular exosome	69	8.6E-18	3.11E-15	1.25E-14
3	GO:0022627	cytosolic small ribosomal subunit	14	2.06E-17	5.95E-15	2.97E-14
4	GO:0043232	intracellular non-membrane-bounded organelle	94	1.81E-16	3.74E-14	2.62E-13
5	GO:0005634	nucleus	134	2.28E-15	4.12E-13	3.3E-12
6	GO:0030529	ribonucleoprotein complex	35	5.02E-15	8.07E-13	7.27E-12
7	GO:0044445	cytosolic part	22	1.39E-14	2.01E-12	2.01E-11
8	GO:0032991	macromolecular complex	104	4.26E-14	5.6E-12	6.17E-11
9	GO:0031974	membrane-enclosed lumen	85	8.29E-14	9.99E-12	1.2E-10
10	GO:0043233	organelle lumen	83	2.23E-13	2.49E-11	3.23E-10
11	GO:0005829	cytosol	81	3.25E-13	3.36E-11	4.71E-10
12	GO:0044428	nuclear part	75	7.07E-13	6.82E-11	1.02E-9
13	GO:0031981	nuclear lumen	68	1.48E-12	1.34E-10	2.14E-9
14	GO:0070013	intracellular organelle lumen	80	1.62E-12	1.38E-10	2.34E-9
15	GO:0044391	ribosomal subunit	17	1.93E-12	1.55E-10	2.8E-9
Supplemental Table S3:GO Analysis; Molecular Function						
	GO_id	Term	NumberOfGenes	p-value	p-value_fdr	p-value_bonferroni
1	GO:0044822	poly(A) RNA binding	92	3.62E-44	1.4E-40	1.4E-40
2	GO:0003723	RNA binding	91	2.16E-37	4.19E-34	8.39E-34
3	GO:0000166	nucleotide binding	76	3.28E-13	3.26E-10	1.27E-9
4	GO:1901265	nucleoside phosphate binding	76	3.36E-13	3.26E-10	1.3E-9
5	GO:0036094	small molecule binding	78	1.15E-11	8.95E-9	4.48E-8
6	GO:0003676	nucleic acid binding	91	7.5E-11	4.85E-8	2.91E-7
7	GO:0017111	nucleoside-triphosphatase activity	33	3.39E-10	1.88E-7	1.32E-6
8	GO:0016462	pyrophosphatase activity	33	1.2E-9	5.17E-7	4.65E-6
9	GO:0005515	protein binding	153	1.14E-8	4.02E-6	4.43E-5
10	GO:0005200	structural constituent of cytoskeleton	12	2.21E-8	7.14E-6	8.57E-5
11	GO:0032550	purine ribonucleoside binding	54	4.44E-8	1.25E-5	1.72E-4
12	GO:0001883	purine nucleoside binding	54	4.73E-8	1.25E-5	1.83E-4
13	GO:0032549	ribonucleoside binding	54	4.83E-8	1.25E-5	1.87E-4
14	GO:0035639	purine ribonucleoside triphosphate binding	54	5.24E-8	1.27E-5	2.03E-4
15	GO:0001882	nucleoside binding	54	5.92E-8	1.35E-5	2.3E-4
Supplemental Table S3:GO Analysis; Biological Processes						
	GO_id	Term	NumberOfGenes	p-value	p-value_fdr	p-value_bonferroni
1	GO:0016071	mRNA metabolic process	52	7.91E-26	9.79E-22	9.79E-22
2	GO:0006413	translational initiation	23	1.38E-19	8.54E-16	1.71E-15
3	GO:0006414	translational elongation	21	1.8E-18	7.43E-15	2.23E-14
4	GO:0006396	RNA processing	43	3.48E-18	1.08E-14	4.31E-14
5	GO:0019083	viral transcription	19	2.71E-17	6.72E-14	3.36E-13
6	GO:0006415	translational termination	19	4.36E-17	8.73E-14	5.4E-13
7	GO:0043933	macromolecular complex subunit organization	61	4.94E-17	8.73E-14	6.11E-13
8	GO:0045047	protein targeting to ER	20	9.47E-17	1.42E-13	1.17E-12
9	GO:0070972	protein localization to endoplasmic reticulum	21	1.03E-16	1.42E-13	1.27E-12
10	GO:0072599	protein localization to endoplasmic reticulum	20	1.16E-16	1.43E-13	1.43E-12
11	GO:0019080	viral gene expression	19	2.58E-16	2.9E-13	3.19E-12
12	GO:0044033	multi-organism metabolic process	19	3.91E-16	3.84E-13	4.84E-12
13	GO:0008380	RNA splicing	30	4.03E-16	3.84E-13	4.99E-12
14	GO:0006397	mRNA processing	33	8.5E-16	7.52E-13	1.05E-11
15	GO:0006614	SRP-dependent cotranslational protein targeting	19	1.29E-15	1.06E-12	1.59E-11

Supplemental Table S4: List of ribosomal proteins that co-immunoprecipitate with HEY1 in the proteomic study

	Gene Symbol / Protein name	Accession Number	Molecular Weight(kDa)	Unique peptides detected	Protein coverage (%)
1	RPL38 / 60S ribosomal protein L38	MSIPI:IPI00215790.6 SWISS-PROT:P63173	23	8	18
2	RPS10 / 40S ribosomal protein S10	MSIPI:IPI00008438.1 SWISS-PROT:P46783	23	9	43
3	RPS25 /40S ribosomal protein S25	MSIPI:IPI00012750.3 SWISS-PROT:P62851 (+1)	17	5	23
4	HNRNPK / Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	MSIPI:IPI00216049.1 SWISS-PROT:P61978-1 (+4)	63	9	23
5	RPS19 / 40S ribosomal protein	MSIPI:IPI00215780.5 SWISS-PROT:P39019	73	8	8
6	RPS7 / 40S ribosomal protein S7	MSIPI:IPI00013415.1 SWISS-PROT:P62081	36	8	32
7	RPL15 / Ribosomal protein L15	MSIPI:IPI00382749.1 TREMBL:Q96S84	24	2	9
8	RPS16 / 40S ribosomal protein S16	MSIPI:IPI00221092.8 SWISS-PROT:P62249	22	5	26
9	RPS18 / 40S ribosomal protein S18	MSIPI:IPI00013296.3 SWISS-PROT:P62269	24	7	28
10	RPL12 / Isoform 1 of 60S ribosomal protein L12	MSIPI:IPI00024933.3 SWISS-PROT:P30050-1	19	3	22
11	RPS14 / 40S ribosomal protein S14	MSIPI:IPI00026271.5 SWISS-PROT:P62263	28	7	22
12	RPL23/ 60S ribosomal protein L23	MSIPI:IPI00010153.5 SWISS-PROT:P62829 (+1)	27	5	17
13	RPS3 / 40S ribosomal protein S3	MSIPI:IPI00011253.3 SWISS-PROT:P23396	67	8	13
14	RPS29 / 40S ribosomal protein S29	MSIPI:IPI00182289.6 SWISS-PROT:P62273	7	2	34
15	RPS20 / 40S ribosomal protein S20	MSIPI:IPI00012493.1 SWISS-PROT:P60866 (+1)	20	4	15
16	RPL9 / 60S ribosomal protein L9	MSIPI:IPI00031691.1 SWISS-PROT:P32969	24	4	32
17	HNRNPA1 / Isoform 2 of Heterogeneous nuclear ribonucleoprotein A1	MSIPI:IPI00797148.1 SWISS-PROT:P09651-3	35	5	23
18	SNRPD1 / Small nuclear ribonucleoprotein Sm D1	MSIPI:IPI00302850.4 SWISS-PROT:P62314	13	2	28
19	SNRPD3 / Small nuclear ribonucleoprotein Sm D3	MSIPI:IPI00017964.1 SWISS-PROT:P62318 (+2)	14	2	15
20	RPL22L1 / 60S ribosomal protein L22-like 1	MSIPI:IPI00856049.1 SWISS-PROT:Q6P5R6 (+2)	17	3	33
21	SNRPB / Isoform SM-B' of Small nuclear ribonucleoprotein-associated proteins	MSIPI:IPI00027285.1 SWISS-PROT:P14678-1 (+7)	46	1	2
22	RPS17 / 40S ribosomal protein S17	MSIPI:IPI00221093.7 SWISS-PROT:P08708	27	2	13
23	SNRNP27 / U4/U6.U5 small nuclear ribonucleoprotein 27 kDa protein	MSIPI:IPI00017289.3 SWISS-PROT:Q8WVK2	22	3	19
24	RPS5 / 40S ribosomal protein S5	MSIPI:IPI00008433.4 SWISS-PROT:P46782	31	2	17
25	FAU / ubiquitin-like protein fubi and ribosomal protein S30 precursor	MSIPI:IPI00019770.3 SWISS-PROT:P62861	43	1	2
26	HNRNPH1 / Heterogeneous nuclear ribonucleoprotein H	MSIPI:IPI00013881.6 SWISS-PROT:P31943 (+1)	60	2	6
27	RPS15A / 40S ribosomal protein S15a	MSIPI:IPI00221091.9 SWISS-PROT:P62244	24	3	18
28	RPL11 / Isoform 1 of 60S ribosomal protein L11	MSIPI:IPI00376798.3 SWISS-PROT:P62913-1	76	2	3
29	HNRNPD / Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	MSIPI:IPI00028888.1 SWISS-PROT:Q14103-1 (+5)	50	2	5
30	HNRNPA2B1/ Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	MSIPI:IPI00396378.3 SWISS-PROT:P22626-1 (+2)	42	2	6
31	RPS26P11 / Putative 40S ribosomal protein S26-like 1	MSIPI:IPI00187140.1 SWISS-PROT:Q5JNZ5 (+2)	13	1	8
32	HNRPLL / Isoform 2 of Heterogeneous nuclear ribonucleoprotein L-like	MSIPI:IPI00554613.1 SWISS-PROT:Q8WV9-2 (+1)	31	1	2

Supplemental Table S5: List of nucleolar-related proteins that co-immunoprecipitate with HEY1 in the proteomic study

	Gene Symbol / Protein name	Accession Number	Molecular Weight(kDa)	Unique peptides detected	Protein coverage (%)
1	SYNE1 / Nesprin-1	MSIPI:IPI00745471.3 SWISS-PROT:Q8NF91	1011	21	1
2	EWSR1/ Ewing sarcoma breakpoint region 1 isoform 1	MSIPI:IPI00009841.6 TREMBL:Q96MX4 (+4)	69	17	11
3	VCP / VCP Transitional endoplasmic reticulum ATPase	MSIPI:IPI00022774.3 SWISS-PROT:P55072	114	19	7,6
4	FRG1 / FSHD Region Gene 1	MSIPI:IPI00004655.1 SWISS-PROT:Q14331	60	16	11
5	FEN1 / Flap endonuclease 1	MSIPI:IPI00026215.1 SWISS-PROT:P39748	61	11	10
6	FBL / Fibrillarin	MSIPI:IPI00025039.1 SWISS-PROT:P22087	43	5	11
7	SYNE2 / Nesprin-2	MSIPI:IPI00239405.4 SWISS-PROT:Q8WXH0-1 (+1)	1056	10	0,86
8	DDX5 / DEAD Box Helicase 5	MSIPI:IPI00017617.1 SWISS-PROT:P17844	84	4	5,5
9	NOLC1 / Nucleolar phosphoprotein p130	MSIPI:IPI00292387.6 SWISS-PROT:Q14978-1	89	2	6
10	YLPM1 / YLP motif containing 1	MSIPI:IPI00165434.4 TREMBL:B4DMQ9 (+1)	290	3	1,5
11	NONO / Non-POU domain-containing octamer-binding protein	MSIPI:IPI00304596.3 SWISS-PROT:Q15233	75	2	4,3
12	YBX1 / Y box binding protein 1	MSIPI:IPI00479509.2 TREMBL:Q2VIK8	35	2	7,2
13	EZR / Ezrin	MSIPI:IPI00872684.1 TREMBL:Q9UJZ8	69	2	4,1
14	HSPA8/ Heat shock cognate 70 kDa protein 8	MSIPI:IPI00003865.1 SWISS-PROT:P11142-1	102	77	29
15	DDX46 / DEAD box polypeptide 46	MSIPI:IPI00329791.11 SWISS-PROT:Q7L014 (+1)	138	4	3,4
16	IQSEC1 / IQ Motif And Sec7 Domain 1	MSIPI:IPI00457114.2 SWISS-PROT:Q6DN90-1 (+3)	114	3	4