

# Appendix

## ***Alu* element-containing RNAs maintain nucleolar structure and function**

Maiwen Caudron-Herger, Teresa Pankert, Jeanette Seiler, Attila Németh, Renate Voit, Ingrid

Grummt and Karsten Rippe

### **Table of contents**

Appendix Tables S1 to S5

Appendix Figures S1 to S9

## Appendix Tables

**Appendix Table S1. Distribution of transcripts in total, nucleoplasmic and nucleolar RNA.**

	genomic region	total RNA	nucleoplasmic RNA	nucleolar RNA
transcripts distribution (% , $\pm$ SD)	primary transcripts (exon + intron)	70 $\pm$ 14	89 $\pm$ 1	42 $\pm$ 1
	spliced transcripts (exon only)	26 $\pm$ 15	8 $\pm$ 3	32 $\pm$ 2
	intron only	4 $\pm$ 1	4 $\pm$ 4	26 $\pm$ 3
	intron only overlapping <i>Alu</i> repeats	1 $\pm$ 2	2 $\pm$ 1	10 $\pm$ 2

**Appendix Table S2. List of read clusters overlapping intronic *Alu* elements found in the nucleolar RNA and the corresponding clusters found in nucleoplasmic and total RNA.**

NE indicates the normalized expression value for each cluster. Clusters are listed from the most enriched in the nucleolar RNA (top) to the most enriched in the nucleoplasmic and/or total RNA (bottom). The *Alu* repeat elements overlapping with those clusters are also listed.

**Appendix Table S2.** List of read clusters overlapping intronic *Alu* elements found in the nucleolar RNA and the corresponding clusters found in nucleoplasmic and total RNA.

<i>Alu</i> repeats					nucleolar RNA clusters					nucleoplasmic RNA clusters					total RNA clusters				
chr	start	end	strand	name	chr	start	end	NE	strand	chr	start	end	NE	strand	chr	start	end	NE	strand
chr8	124446700	124446848	+	AluSz	chr8	124446700	124446848	30371.1486	+	chr8	124446236	124446872	12.0699659	+	chr8	124446662	124446913	165.534542	+
chr3	53342369	53342502	-	AluIr4	chr3	53342369	53342502	6076.66833	-	chr3	53342361	53342923	6.97024694	-	chr3	53342364	53342564	52.0018171	-
chr6	125312049	125312178	+	AluIr	chr6	125312049	125312178	1843.40026	+	chr6	125311832	125312372	3.71821059	+	chr6	125311997	125312166	43.1710428	+
chr14	31577361	31577475	-	AluSq2	chr14	31577361	31577475	2339.76996	-	chr14	31577192	31577659	4.9328637	-	chr14	31576655	31578806	5.01481325	-
chr17	30525565	30525679	+	AluJb	chr17	30525565	30525679	2658.8295	+	chr17	30525456	30525739	7.57336616	+	chr17	30525565	30525673	687.797236	+
chr17	74041054	74041121	-	AluSp	chr17	74041054	74041185	1371.99295	-	chr17	74040407	74041466	4.42029299	-	chr17	74041061	74041179	613.047077	-
chr17	45739648	45739667	+	AluSx	chr17	45739648	45739760	2767.31373	+	chr17	45739454	45740543	9.52716477	+	chr17	45738595	45743615	3.05513171	+
chr17	45739668	45739760	+	AluJb	chr17	45739648	45739760	2767.31373	+	chr17	45739454	45740543	9.52716477	+	chr17	45738595	45743615	3.05513171	+
chr1	10220812	10220975	+	AluJo	chr1	10220812	10220975	826.195769	+	chr1	10220661	10221056	3.21559057	+	chr1	10220759	10220961	14.6043365	+
chrX	19363525	19363662	+	AluSq2	chrX	19363525	19363662	3003.776229	+	chrX	19363313	19363668	12.5501909	+	chrX	19363522	19363635	1405.526663	+
chr9	95054566	95054620	-	AluJb	chr9	95054566	95054871	4302.0278	-	chr9	95053493	95055546	18.3783117	-	chr9	95045146	95056014	10.5233473	-
chr1	206519071	206519141	+	AluIr	chr1	206519071	206519221	8074.64389	+	chr1	206519073	206519363	40.3330431	+	chr1	206518883	206519453	13.3539394	+
chr1	206519143	206519211	+	AluJo	chr1	206519071	206519221	8074.64389	+	chr1	206519073	206519363	40.3330431	+	chr1	206518883	206519453	13.3539394	+
chr1	206519214	206519221	+	AluJo	chr1	206519071	206519221	8074.64389	+	chr1	206519073	206519363	40.3330431	+	chr1	206518883	206519453	13.3539394	+
chr1	235291256	235291283	+	AluSx	chr1	235291122	235291283	4560.86313	-	chr1	235291026	235292198	23.8956889	-	chr1	235288776	235292283	15.7908724	-
chr5	133989397	133989526	+	AluJo	chr5	133989397	133989526	721.330535	+	chr5	133989278	133989827	4.20292883	+	chr5	133989374	133989575	29.815225	+
chr11	73560978	73561107	+	AluJb	chr11	73560978	73561107	1683.10458	+	chr11	73560850	73561186	12.186187	+	chr11	73560555	73561296	3.05066449	+
chr9	135255524	135255664	-	AluSx1	chr9	135255524	135255664	331.901586	-	chr9	135255397	135256091	3.59459818	-	chr9	135255304	135255667	6.51252014	-
chr18	12469997	12470134	-	AluIr	chr18	12469997	12470134	811.83143	-	chr18	12469988	12470250	9.67562653	-	chr18	12469992	12470347	3.72650053	-
chr6	99993898	99994040	-	AluSx	chr6	99993898	99994040	435.766709	-	chr6	99993871	99994155	5.49434601	+	chr6	99989571	99995350	19.4639994	-
chr6	151253115	151253256	+	AluSx1	chr6	151253115	151253256	249.912596	+	chr6	151252721	151253914	3.49564391	+	chr6	151253114	151253281	37.1646216	+
chr19	10672854	10673025	-	AluSx1	chr19	10672854	10673124	163.046098	-	chr19	10672529	10673697	3.21590142	-	chr19	10672789	10673144	6.47234302	-
chr19	10673025	10673124	-	AluIr	chr19	10672854	10673124	163.046098	-	chr19	10672529	10673697	3.21590142	-	chr19	10672789	10673144	6.47234302	-
chr6	31746278	31746374	-	AluJb	chr6	31746136	31746374	293.887068	-	chr6	31744878	31753727	6.24842475	-	chr6	31745288	31749385	3.98516212	-
chr3	57887592	57887736	+	AluSz	chr3	57887592	57887736	381.535465	+	chr3	57887422	57887825	8.8359191	+	chr3	57887558	57887897	3.73372567	+
chr20	37057711	37057917	-	AluJb	chr20	37055925	37058529	2251.72386	-	chr20	37054752	37063360	55.5099121	-	chr20	37052040	37064010	59.2007137	-
chrX	21862407	21862612	+	AluJb	chrX	21862407	21862612	535.917687	+	chrX	21862359	21862651	13.3258319	+	chrX	21862406	21862631	24.6321558	+
chr6	99993898	99994040	-	AluSx	chr6	99993898	99994040	435.766709	-	chr6	99993836	99994111	13.796319	-	chr6	99989571	99995350	19.4639994	-
chr5	130498578	130498802	-	AluSx	chr5	130498578	130498802	489.408408	-	chr5	130498493	130498883	18.066035	-	chr5	130494307	130500361	32.9309319	-
chr3	186504715	186504845	-	AluJb	chr3	186503918	186505686	2937.86714	+	chr3	186503889	186506010	109.607775	+	chr3	186501259	186509060	183.0339305	-
chr18	43705063	43705234	+	AluSx	chr18	43705063	43705281	103.454281	+	chr18	43704958	43705360	3.9245948	+	chr18	43705007	43705271	7.68845848	+
chr18	43705234	43705281	+	AluSg	chr18	43705063	43705281	103.454281	+	chr18	43704958	43705360	3.9245948	+	chr18	43705007	43705271	7.68845848	+
chr2	10931045	10931175	-	AluIr	chr2	10931045	10931175	722.309314	-	chr2	10930844	10931212	28.8376923	-	chr2	10930251	10931254	10.5651881	-
chr10	126762919	126763029	-	AluSq2	chr10	126762859	126763029	176.481011	-	chr10	126762832	126763509	8.8909877	-	chr10	126762842	126763030	31.39869	-
chr2	135894382	135894574	+	AluIr	chr2	135894207	135894574	69.1722163	+	chr2	135894168	135894652	4.62562652	+	chr2	135894207	135894574	25.5775053	+
chr14	20791540	20791561	+	AluJb	chr14	20791347	20791561	1161.66928	-	chr14	20790806	20792059	85.2744003	-	chr14	20790854	20791883	41.4722224	-
chr6	151345556	151345682	+	AluIr	chr6	151345556	151345765	108.617892	+	chr6	151345342	151346084	9.3630278	+	chr6	151345559	151345764	73.3127568	+
chr6	151345682	151345765	+	AluS26	chr6	151345556	151345765	108.617892	+	chr6	151345342	151346084	9.3630278	+	chr6	151345559	151345764	73.3127568	+
chr2	17928824	17929014	-	AluSz	chr2	17928824	17929014	85.9551467	-	chr2	17928767	17929326	8.50444944	-	chr2	17928874	17931084	3.06480583	-
chr19	13037137	13037386	-	AluY	chr19	13037137	13037464	58.6741203	-	chr19	13037344	13037698	7.96572214	-	chr19	13037277	13037517	24.444158	-
chr19	13037390	13037464	-	AluJo	chr19	13037137	13037464	58.6741203	-	chr19	13037344	13037698	7.96572214	-	chr19	13037277	13037517	24.444158	-
chr15	44112904	44113105	-	AluJb	chr15	44112904	44113186	18.8116221	-	chr15	44112860	44113289	3.80156412	-	chr15	44112909	44113209	6.10557455	-
chr15	44113105	44113186	-	AluJo	chr15	44112904	44113186	18.8116221	-	chr15	44112860	44113289	3.80156412	-	chr15	44112909	44113209	6.10557455	-
chr15	60675435	60675627	-	AluJb	chr15	60675435	60675834	19.3349974	-	chr15	60675073	60676639	4.83371957	-	chr15	60674404	60689870	5.23008853	-
chr5	1805384	1805466	+	AluJb	chr5	1805384	1805768	20.9982067	+	chr5	1804850	1808882	8.22967057	+	chr5	1801479	1812864	4.15774001	+
chr6	111022137	111022414	+	AluSg4	chr6	111022137	111022430	23.0532944	+	chr6	111022135	111022765	9.32338475	+	chr6	111022135	111022944	15.9378002	+
chr14	59668192	59668333	+	AluSx1	chr14	59668192	59668491	12.8399817	+	chr14	59668110	59668453	5.34036816	+	chr14	59668088	59668476	3.04296451	+
chr14	59668360	59668491	+	AluSx8	chr14	59668192	59668491	12.8399817	+	chr14	59668110	59668453	5.34036816	+	chr14	59668088	59668476	3.04296451	+
chr1	24842361	24842657	+	AluSg	chr1	24842361	24842659	31.9138192	+	chr1	24842344	24842725	18.3173541	+	chr1	24842222	24842789	15.3788911	+
chr9	130211111	130211393	-	AluSz	chr9	130210699	130211408	58.2244486	-	chr9	130210058	130213921	42.7176502	-	chr9	130206453	130214342	29.5451618	-
chr1	62491210	62491452	+	AluS26	chr1	62490955	62491452	10.8066937	+	chr1	62490897	62491500	8.75769038	+	chr1	62490937	62491426	8.16360381	+
chr17	75085816	75085828	+	AluSq	chr17	75085383	75085828	275.82981	+	chr17	75085235	75086545	238.353636	+	chr17	75084748	75091820	32.6334626	+
chr17	2233260	2233514	-	AluSp	chr17	2233260	2233620	6.07962429	-	chr17	2232824	2233611	3.42518679	-	chr17	2231984	2235871	11.3267954	-
chr19	18200164	18200391	+	AluS26	chr19	18200164	18200391	27.0901525	+	chr19	18200159	18200435	40.3742189	+	chr19	18200160	18200611	16.9034467	+
chr1	173836374	173836666	+	AluSx	chr1	173835670	173837115	29.7564661	-	chr1	173832907	173837111	18.2996409	-	chr1	173828783	173837252	44.0832754	-
chr17	47647071	47647365	-	AluSx	chr17	47647048	47647365	15.0956045	-	chr17	47647026	47647372	10.7435887	-	chr17	47647033	47647365	18.70860601	-
chr9	126147440																		

chr11	62433771	62433795	+	AluJo	chr11	62433771	62434338	4.3407252	+	chr11	62431811	62434742	3.4580893	-	chr11	62432796	62435895	16.3918911	+
chr16	27357009	27357103	+	AluSx1	chr16	27356652	27357103	4.16655936	+	chr16	27356445	27357313	3.60344133	+	chr16	27356432	27357521	4.74897886	+
chr5	138613642	138613869	+	AluJo	chr5	138613642	138613869	24.6274113	+	chr5	138611090	138613909	22.8894546	+	chr5	138609805	138622580	58.2316993	+
chr19	46191251	46191340	-	AluSx6	chr19	46191070	46191340	16.1698609	-	chr19	46190298	46192748	15.9125318	-	chr19	46190298	46193108	39.342161	+
chr19	1414038	1414312	-	AluSx	chr19	1414038	1414704	3.1963712	-	chr19	1413890	1414743	16.7173066	+	chr19	1407417	1418063	3.06597703	+
chr19	1414463	1414704	+	AluSc	chr19	1414038	1414704	3.1963712	+	chr19	1413890	1414743	16.7173066	+	chr19	1407417	1418063	3.06597703	+
chr1	145398761	145399016	+	AluSq	chr1	145398761	145399031	16.1698609	+	chr1	145398751	145399010	19.4343966	+	chr1	145398716	145399103	24.3197452	+
chr9	139621615	139621632	+	AluSx1	chr9	139620993	139621632	27.8214329	-	chr9	139618877	139621590	36.4747919	-	chr9	139618038	139622624	41.8738295	-
chr1	28906528	28906810	+	AluSx1	chr1	28906185	28906810	13.6127298	-	chr1	28905071	28908377	20.4350412	-	chr1	28902210	28908384	18.7501953	-
chr1	173833125	173833236	+	AluJo	chr1	173833078	173834668	11.6660032	-	chr1	173832907	173837111	18.2996409	-	chr1	173828783	173837252	44.0832754	-
chr1	173834074	173834348	+	AluSz	chr1	173833078	173834668	11.6660032	-	chr1	173832907	173837111	18.2996409	-	chr1	173828783	173837252	44.0832754	-
chr8	144536421	144536560	-	AluSq	chr8	144536421	144536871	9.50908345	-	chr8	144536252	144537469	15.5315944	-	chr8	144536154	144536872	49.4586014	-
chr8	144536572	144536871	-	AluY	chr8	144536421	144536871	9.50908345	-	chr8	144536252	144537469	15.5315944	-	chr8	144536154	144536872	49.4586014	-
chr18	3453402	3453709	+	AluSp	chr18	3453353	3453794	6.71926218	+	chr18	3448755	3454438	11.9474627	+	chr18	3450129	3459945	7.58020499	+
chr5	72378741	72378999	-	AluSc5	chr5	72378741	72378999	20.3296008	-	chr5	72378731	72379004	51.3895256	-	chr5	72378567	72379012	32.4916665	+
chr1	76252925	76253040	-	AluJb	chr1	76252644	76253577	4.90971658	+	chr1	76252702	76254348	12.7303621	+	chr1	76251902	76263275	25.1130162	+
chr1	28832737	28832996	-	AluSx	chr1	28832737	28833166	19.5993481	+	chr1	28832510	28833501	55.0376747	+	chr1	28832507	28840775	873.875538	+
chr19	51305758	51306042	+	AluSg	chr19	51305565	51306372	5.94777354	-	chr19	51304362	51306342	20.898747	-	chr19	51305073	51308245	13.8224813	+
chr17	16344915	16345132	-	AluJb	chr17	16344915	16345217	13.4368718	+	chr17	16344248	16349056	51.831124	+	chr17	16342193	16350716	35.825746	+
chr20	47895879	47895971	+	AluSx	chr20	47895204	47895971	3.92351611	+	chr20	47895185	47902641	18.5537597	+	chr20	47895155	47907586	24.577384	+
chr11	62433771	62433795	+	AluJo	chr11	62433771	62434338	4.3407252	+	chr11	62432800	62434810	21.0898146	+	chr11	62432796	62435895	16.3918911	+
chr19	3546836	3546892	-	AluY	chr19	3546569	3546892	12.7599962	-	chr19	3530712	3554077	90.3273933	-	chr19	3541215	3549575	13.7273115	-
chr12	49523584	49523778	-	AluJr	chr12	49523574	49524482	4.43380256	-	chr12	49522869	49525165	34.364989	-	chr12	49522475	49525201	62.9184338	-
chr7	1574535	1574596	-	AluJo	chr7	1574113	1574596	5.01310333	+	chr7	1570366	1589639	42.2611055	+	chr7	1570930	1582868	9.67684138	+
chr7	1574788	1574847	-	AluJo	chr7	1574788	1576242	4.82600551	+	chr7	1570366	1589639	42.2611055	+	chr7	1570930	1582868	9.67684138	+
chr7	1575008	1575309	+	AluJb	chr7	1574788	1576242	4.82600551	+	chr7	1570366	1589639	42.2611055	+	chr7	1570930	1582868	9.67684138	+
chr1	1413277	1413383	-	AluSp	chr1	1413277	1413393	12.8539669	+	chr1	1413119	1414036	124.352448	+	chr1	1412069	1414036	29.0583085	+
chr1	1413385	1413676	-	AluSx1	chr1	1413277	1413393	12.8539669	+	chr1	1413119	1414036	124.352448	+	chr1	1412069	1414036	29.0583085	+
chr19	58903563	58903707	+	AluSz	chr19	58903563	58904179	6.28431835	+	chr19	58902343	58905039	70.924147	+	chr19	58898134	58906458	25.6466567	+
chr19	58903710	58903998	+	AluSx3	chr19	58903563	58904179	6.28431835	+	chr19	58902343	58905039	70.924147	+	chr19	58898134	58906458	25.6466567	+
chr6	90431340	90431412	-	AluJo	chr6	90431143	90431412	17.7272116	+	chr6	90430889	90431433	214.487333	+	chr6	90430974	90431430	130.846099	+
chr19	3542491	3542779	-	AluSx1	chr19	3542491	3544730	7.21548318	-	chr19	3530712	3554077	90.3273933	+	chr19	3541215	3549575	13.7273115	-
chr19	18498921	18498941	-	AluSx1	chr19	18498921	18499695	7.91090439	+	chr19	18496769	18500210	103.181609	+	chr19	18496365	18500426	19.2428169	+
chr7	44839700	44839790	-	AluSx	chr7	44839172	44839790	3.12671648	+	chr7	44839024	44840494	42.8720631	+	chr7	44836234	44843083	59.6829872	+
chr16	87869206	87869379	-	AluY	chr16	87869206	87870208	3.67705048	-	chr16	87863675	87870971	68.8242894	-	chr16	87869344	87870370	3.93278655	-
chr9	128001877	128002024	+	AluSx	chr9	128000877	128002024	12.5266584	-	chr9	128000048	128003619	355.048018	-	chr9	127997077	128003770	75.8741305	-
chr9	128000306	128000389	+	AluSx	chr9	128000306	128000761	7.07877742	-	chr9	128000048	128003619	355.048018	-	chr9	127997077	128003770	75.8741305	-
chr11	61733577	61733745	+	AluSc8	chr11	61733577	61735144	7.97628495	-	chr11	61731564	61736302	516.86961	-	chr11	61729329	61735380	358.414701	-

**Appendix Table S3. Nucleolar-enriched *al*uRNA-containing clusters**

chr	start	end	NE nucleolar RNA	NE total RNA	strand	length	region*
chr1	10220812	10220975	826.20	14.60	+	163	Region_1
chr1	24842361	24842659	31.91	15.38	+	298	Region_2
chr1	62490955	62491452	10.81	8.16	+	497	Region_3
chr1	1.44E+08	1.44E+08	7334.69	47.93	+	149	
chr1	1.56E+08	1.56E+08	2284.79	69.33	-	122	
chr1	2.07E+08	2.07E+08	8074.64	13.35	+	150	
chr2	10931045	10931175	722.31	10.57	-	130	Region_4
chr2	17928824	17929014	85.96	3.06	-	190	Region_5
chr3	53342369	53342502	6076.67	52.00	-	133	Region_6
chr3	57887592	57887736	381.54	3.73	+	144	Region_7
chr5	1805384	1805768	21.00	4.16	+	384	
chr5	1.3E+08	1.3E+08	489.41	32.93	-	224	Region_8
chr5	1.34E+08	1.34E+08	721.33	29.82	+	129	Region_9
chr6	31746136	31746374	293.89	3.99	-	238	
chr6	99993898	99994040	435.77	19.46	-	142	Region_10
chr6	1.25E+08	1.25E+08	1843.40	43.17	+	129	Region_11
chr6	1.51E+08	1.51E+08	249.91	37.16	+	141	Region_12
chr6	1.51E+08	1.51E+08	108.62	73.31	+	209	
chr7	1068849	1069071	120.57	4.68	-	222	Region_13
chr8	1.24E+08	1.24E+08	30371.15	165.53	+	148	Region_14
chr9	95054566	95054871	4302.03	10.52	-	305	
chr9	1.3E+08	1.3E+08	58.22	29.55	-	709	Region_15
chr9	1.35E+08	1.35E+08	331.90	6.51	-	140	Region_16
chr10	1.04E+08	1.04E+08	661.39	35.40	-	122	Region_17
chr10	1.27E+08	1.27E+08	176.48	31.40	-	170	
chr11	73560978	73561107	1683.10	3.05	+	129	Region_18
chr11	93466262	93466929	374.35	40.96	-	667	
chr11	93467946	93468425	542.37	40.96	-	479	Region_19
chr14	31577361	31577475	2339.77	5.01	-	114	Region_20
chr14	59668192	59668491	12.84	3.04	+	299	
chr15	44112904	44113186	18.81	6.11	-	282	Region_21
chr15	60675435	60675834	19.33	5.23	-	399	Region_22
chr17	30525565	30525679	2658.83	687.80	+	114	Region_23
chr17	45739648	45739760	2767.31	3.06	+	112	
chr17	74041054	74041185	1371.99	613.05	-	131	
chr17	75085383	75085828	275.83	32.63	+	445	
chr18	12469997	12470134	811.83	3.73	-	137	Region_24
chr18	43705063	43705281	103.45	7.69	+	218	
chr19	1414038	1414704	3.20	3.07	+	666	Region_25
chr19	1636211	1636342	722.10	4.76	-	131	Region_26
chr19	1956418	1956567	513.26	30.19	+	149	
chr19	5140383	5140493	1658.66	3.07	+	110	
chr19	10672854	10673124	163.05	6.47	-	270	Region_27
chr19	13037137	13037464	58.67	24.44	-	327	Region_28
chr20	37055925	37058529	2251.72	59.20	-	2604	Region_29
chrX	19363525	19363662	3003.78	1405.53	+	137	Region_30
chrX	21862407	21862612	535.92	24.63	+	205	Region_31

\* Regions that were used for the alignment in Figure S2. NE indicates the normalized expression value for each cluster.

**Appendix Table S4. Sequences of DNA and RNA probes used in this study**

Sample	Sequence
<i>alu</i> RNA ASO 1	AGCGATTCTCCTGCCTCAGC
<i>alu</i> RNA ASO 2	GGCTCACTGCAACCGCCACC
<i>alu</i> RNA ASO 3	AGTAGCTGGGATTACAGGCA
control ASO	CCGTCAGCCAGTACTGCCTC
L1-repeat ASO	GGTTAGTTACATATGTATAC
7SL-RNA ASO	GGCATAGCGCACTACAGCCC
<i>alu</i> RNA LNA	AGCAATTCTCCTGCCTCAGC
B1 ASO - 1	TTTGTAGACCAGGCTGGCCT
B1 ASO - 2	ATCCGCCTGCCTCTGCCTCC
L1-repeat LNA	GCAGGTTAGTTACATATGTA
Northern blot <i>alu</i> RNA probe NB1	GGCTCACTGCAACCGCCACC
<i>alu</i> RNA FISH 1	GGGTTCAAGCGATTCTCCTGCCTCAGCCTCCCGAGTAG CTGGGACTACAG
<i>alu</i> RNA FISH 2	CGCCCGCCTCGGCCTCCCAAAGTGCTGGGATGACAGG CGTGAGCCACTGA
<i>alu</i> RNA FISH 1c	CTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGA ATCGCTTGAACCC
<i>alu</i> RNA FISH 2c	TCAGTGGCTCACGCCTGTCATCCCAGCACTTTGGGAGG CCGAGGCGGGCG
L1-repeat FISH	CATGGCACATGTATACATATGTAACCTGCACAATG TGCACATGTAC
Met tRNA FISH	GGTTTAGATCCATCGACCTCTGGGTTATGGGCCAGCA CGCTTCCGCTGC
Ala tRNA FISH	GGATCGAACCGAGGCCTCATAATGCAAAGCATGCGC TCTACCACTGAGC
RNA FISH, MS2 loops	GTCGACCTGCAGACATGGGTGATCCTCATGTTTTCTAG GCAATTA
Primer actin FWD	TATCCTGACCCTGAAGTACC
Primer actin REV	CTCGGTGAGCAGCACAGGG
Primer B1-1 FWD	AGGCCAGCCTGGTCTACAAA
Primer B1 REV	CGAGACAGGGTTTCTCTG
Primer B1-2 FWD	GGAGGCAGAGGCAGGCGGAT
forward <i>alu</i> RNA with MS2 loops	CTGGACTGACTTCATAGTTTAAACGTCAAGAGAAGGCC GGGCTCAGTGGCTCACGCCTGTCATCCCAGCACTTTG GGAGGCCGAGGCGGGCGGGTCACGAGGTCAAGAGAT CGAGACCATCCGGGCCGACACGGTGAACCCCGTCTC TATTAAGTATAAAGATTAGCTGGGCGTGGTGGCGGG CACCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGG AGAATCGCTTGAACCCAGGGAGGTGGCGGTTGCAGTG AGCCGAGATCACACCATCGCACTCCAGCCTGGGCGAC

	AGAGCGAGAC
forward <i>aluRNA<sub>L</sub></i> with MS2 loops	CTGGACTGACTTCATAGTTTAAACGTCAAGAGAAGGCC GGGCTCAGTGGCTCACGCCTGTCATCCCAGCACTTTG GGAGGCCGAGGCCGGCGGGTCACGAGGTCAAGAGAT CGAGACCATCCGGGCCGACACGGTGAAACCCCGTCTC T
forward <i>aluRNA<sub>R</sub></i> with MS2 loops	ATTAAAAGTATAAAGATTAGCTGGGCGTGGTGGCGGGC ACCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGA GAATCGCTTGAACCCAGGGAGGTGGCGGTTGCAGTGA GCCGAGATCACACCATCGCACTCCAGCCTGGGCGACA GAGCGAGAC
CDV3 RNA with MS2 loops	GATGAGGTTTTCAAAAACCAGGCCCTTAACTTCAGCT AGACAACCAATATGCTGTGCTTGAAAATCAGAAAAGCA GCCACTCACAATACAATTAAGGAATGGGCTTTGCTAAC CCTTCTGAGGTAAGTACTGACTGCAGCTAACCACCACAA CAGCCATTCATCATCTGATCTCTGCTGGATCTACAGACA CCGATGCAGACCACTCGATTTTCATGACCGGCCCTATTG CACTATGGAAGTTAAAGTGTACGACTGCTCTATGCAT ATTGGATTTAGGGGAATTTTCATTGTTACATAAATGTGT GAACTAGTTTCAACAGTGTCTTTTCATATTTACTCTGCA AATACAAAAAACCAAAACCTGCAGCCAGTGGTCATTTT AAAATCTTTTTATGTTTCAGATACTGAGCCTTCATAAGGG TTGACTACCTCAGATTTGCTGCACTCATTGTGGACTTCA TGTGGATCACAACCTTCTGGATAAGAAGATTACAACCTATT AAGTGTGATGTGAACCTTGCAACCAGCTCTACTGGAT TCTTATCAGAAATCCTGCATAAAAAGTCAGCCATCTGG GTTCTGATCTGCTGTAAAAGATGAAGATTTAAGTGACCT TAATTAACCTGTCCTGTGCCCTACCCTTAAGGAATACTC TCTGTAGTAGGCTGTTGTTATATTAGACTTCCTGGAACA CACTGCTGAAAAGAAGTGTGTTTCAGATCATCTGTGT AGGGCTGTGATTTGTAATTTAACTAATTGTATTCTGAG GTA
RepA RNA with MS2 loops	CACTCTTTTTCTATATTTTGCCCATCGGGGCTGCGGAT ACCTGGTTTTATTATTTTTCTTTGCCCAACGGGGCCGT GGATACCTGCCTTTTAATCTTTTTTATTGCCCCATCGG GGCCGCGGATACCTGCTTTTTATTTTTTTTTCTTAGCC CATCGGGGTATCGGATACCTGCTGATTCCCTTCCCCTC TGAACCCCAACACTCTGGCCCATCGGGGTGACGGAT ATCTGCTTTTTAAAAATTTCTTTTTTTGGCCCATCGGG GCTTCGGATACCTGCTTTTTTTTTTTTTATTTTCCTTGCC CATCGGGGCTCGGATACCTGCTTTAATTTTTGTTTTTC TGCCCATCGGGGCCGCGGATACCTGCTTTGATTTTTTT TTTTCATCGCCATCGGTGCTTTTTATGGATGAA
STARD7 RNA with MS2 loops	TACCAATCTTTATGTATTTATTACACATTTGATAAAAAT GTCACAGTTAGGAGTGAAATCATTACAATGACATGAGT AACTGTACAGACAGACCCCAAGTGCAGAATCAAATTGC CCTAAGTCAGAACATGGAGCAACCGCAACTCCTTCGCA CTTGTGCATGTGTGTGCGCTCGCAGACGCACACACACA CACACATATTCTCTCTCTCTTTATGCACACATCCATCC ACATCCCAACAATTGCAGGTGCTAAGTTTGGACATAAC CCAGGGACTCTTCCCTGACTTCTGTCAGGTCTTGAAA GAAGAAGTAATAAATGAAAAGCAGCTGGGACTGCTCGA TGCATCTCTCTCTTCCAACAATGACGCGGAGAAGGCA AGACATACACTGGGGCAGCTACTTCTTGGCACAAAAA TGAACAGGCAACAAGAAGGTCAAGGAAGTGTTAAGTT

	<p>AGTCTCAGGTTTAAACCACTTTTCAACACCACAAAACAG  TAGCAAGCAGGAAAACAAAAACAAAAACAAAAACAAA  ACTCTGGCCCTCAAGACTCCAGAAAAAAGGGAAGGAG  GAGGATTTAAAACCTTGATCCCTATTATTCTAACAAATTG  CAGCATGACCATAAGCAAACCAGCTCGGTCAAACCTGA  CAGCATCAGATTGTGACCTTATCTGAACAGTGTAGTTCA  CTTTTATTTTGGCTCTGAAGAGAGGAAGAAAACTTTTT  AGAGGAACTTAATGGTAACATAAACCAAATCTCCACTGT  ATTAGTATTTGAGACAAGATTACATCTATGCATTACAC  AGCTTGTCTGTAGATCTGAGAGCTCCAAGGGAGTGGCC  CAGCCCCCATTCTCTGACTTTAGCCTTCTGAAAAGAA  CAAGTCAAACCTGAAATATGAAAAATAATACCTGAATCA  AAATGGTGTCTTCTATACAATCGGACTAGGGTAGAATCC  TGCTCAATTCCTCAACTCCTATTTGACACAAAAGTAAA  AAATTTTTAACTAAATTTAAATGTGATGTTTTGAGCATC  AAAAAGCTACTATCTAAAAGGATTAGTCTCCCAGTGTTT  TTGGTAAATGGGGAAGGTTAGGAAGGAGGCAATGATC  CAATGAATATAGAAGAACTGGCCGATTCACAGGAAACT  TGCTTTGGATAAGGTGAGTCAATGGGTGATATTGTGCA  GGCAGGGAGGGAAATTTCTTTGTACAAATTCATGTCCC  TGGCCAGGCATGGCCAGGAGTTCAAGACCAGCCTGG  GAAACAGAGTGAAACTCCATCTCTACTTAAAAAAAAAAAA  AAATTAATAATAACTTCATGTCTCTAGAGATAAAAGCAA  GGTGCGGACAAGGCACTTAACATAGCCAAGTATCGTTC  ACACCCATTCACATCATGATTGTTACCAAGAGCACTCC  CAGTAGCCCCCTCATTGAGAGACTCAACTGGCCAACATA  TACTTTCCAGGTGGACCTGGAGAACTGAAGGAAATCCA  AAGCGCTGCACAATCGATGGTGGGATTTGGAATGTCAG  CAGAGGAACTACTCAGAGAAGCAAATGGAGGTCATCC  CCTTCTGGCCTGAGGTGAGAGGTTTGTCCAGATTAGC  TCAGTGAGATACCGAATTTCAAACAGTTGGCCTGAGA  ATATGACAACACTCCCACAAATGTAGCCTTCTTCTGTTT  TGATAAGAGCAATAAGGGCTCTGAATGAAATGGGACAT  CAGTTATTGAATTATCTTGCACTGGAAGTTACAGCAGAT  GCCTTCTAATACATGTGGCATGTCCCCCTTCTACAGCA  GAGTGATAACGGACTGAGACAGGGCTAGAAGCACCTT  GTCCCTTCTTATCCCAAAGCCTG</p>
<p>CORO1C RNA  with MS2 loops</p>	<p>TAATCAATGATTTTTATCTCTGAAAATGGAAGCAAGTGT  TTTGACAGAACACTATGGCCACTCTATAAGAGCCGACC  TAGGAGTAATTCAGTGTCTCTTCTGGGATGTCATGGC  TTAAAAAAAAAAAAAAAAAAGACAAAACAGGAAAATAA  TCCACAATGCTTTGGGCGCCTACTCTGAGCTTGGCTGG  GCATCCATTCATTAATAAGTCATAAGCTACAACTAAG  ATCAGGGTAACTTTCCCTTGGCCTCCTGCTTCTTTTGA  ACCACATGCCCTACGGTGTTCACCACCATCAACAACCT  TACTTCATTAATTCGATATCAGAACCCTTAAAACACTACT  GAATCTTAGACAAAAGGTCCTCAGCAGATTACATTGATA  AAGAAACAAATACAGATTTGAATATAAGTCATTGCCATT  ATTGTTTTCTTTACATCTCATGATCCACTTCAATATTA  CACATACATACACACACAAAACCACATCAAACATTCA  GATGCCCTGAAATTGTGGAGACAGCACTCCCAGTATTG  GCTGGATAAGAACATGGTCCTTTTTCTACACGGACATTA  CTCCAAGTTCGGCTGTCACCACACTCTGCCCTCACCTT  GGCTCCCTGCATACAGACGTTTCCAGCCAGTCCCATGC  CCACACCAGCACGAGCATGTGCAAGAATTCCCTTCGTG</p>



	<p>GTCCCAATGGCACTACACATTTACGTTCAATCACAGA  CAGCCCTTCCAAAACAGAGGCTCCTTAAATCTTGTTTC  CAAGCATGCACTATAAGAACTGGAAACGGGGCAAAGT  TCCAGCTAACCGAGGAATGAACAGGTGACCAGAGCTC  AGTCTCTGGCCCCCTCCGAGTCCGGGGAGCCTCTGTG  CTTTGACGCAGCCTTGGTGAGAGATGGAGAGGCAGGG  CAGGTTAGTTGTGCGGAGGGACATGTTTGTATGCATTA  AAGCTACAGCAGAGGTCTGTGCTCCTGTTTCTGCTTCA  CTCTCTATCATTCTCCTCAGTCTCAATACTCATTCAATC  GCACCCTCTGGCCTTGTCTTAGCTTAATGTGGTTAGAG  CTAAAAGGCAGGAAGGAAAGAAGGAATGGCTGGGGGA  GCCCTGAGAGAGGTGCGCAGCACATGTGAGGAAGAGGG  TAGAGCAGGAGCTTTGTTGGTGGGAAGGAGAGCCACTT  CCTCCTCCAACACAGCATGGGGACAGCACAGGGAAAC  AGGATCACCCAGGGCAAATACCCCTCTCCACATGCCCA  CATTTTTAGTCCCCACCACTGTAAGAGCTGGGCA  AGCTGCTGAAAAGGGCAAGAAGGAACACTCAGCAGTA  CACGTCTTCTGTCTGGGCACCCTTGTCAAGTATTGA  CCAAAACCTGAAACATGATGTTTAAAGTGATGAATGCAA  TATGATCCTAGGTGTGTAACAACTGCAGAAACACATG  CTAGTTTGGGTTAGATTATAATCATCTGAAGCACAGGAT  AACCGAGAAGCAAATTCATTCTGGTACAAACACCCA  ATTTCTAGAAAAGAGAAAAGGAAAAGAAGAAATACGACG  TGAGCTTTTTTGTATCAGAAGACTCCATGAAATGAGAGC  GGTGGTAATATGAATCCACGTGATTTTTCAAGTCTTCT  GTTGTACAGTCATCAAATGACCAGGTTTGTGCTGCA  AAGGAGCCAGCACCATGTGGCTACTGCTTTGATTGTTT  TCAGATGAATGTTTATACAAAATAATATCTTATCTTCATT  TAGTTTATAAACATACACAGTGCTGTCCCTTTCAAATTA  AGGAAAAAAAAACCACACACACAAATACTGCAAAGTAGC  AAAATACAAAGGAAAACAAAGCTACTTTTGGTTTTGGCA  ACATTAAGAAAGAAAGAAATATAAAAAGCAATGTGGCAT  TGGTCCCTATTCATTAAGAAAGGGTACTTGGGCA  CGACACAATCAGAATTAGTTTGTCTTAAATTCAGAG  TATCTGGGATTTTAAAAGTAGCACTTTTTAAAAGTTCA  ACAAGTCACATAACACTTAAAACATCAAAAAGCTTTCT  GATAAAAAGCTCAGCTTTTAAATCACGTTTTGTTTCTGC  AAATTTGGGAGACAAATTGAGTTCTTACTGGAATGTGG  CCTATCGCTGGTTGACAAATCTGAAATGGAATGTCTCC  AAATGGCAGTGCCTCCCTTCCGCCCTCCCTAGGACCA  CACCAATAACCAGCTCCAAGCACAGTTCTTGCTCCC  ATTTTTCTGTAGGGGTGGGGGTGGGACCT</p>
forward <i>alu</i> RNA template for T7 <i>in vitro</i> transcription	<p>TAATACGACTCACTATAGGGCTGGACTGACTTCATAGTT  TAAACGTCAAGAGAAGGCCGGGCTCAGTGGCTCACGC  CTGTATCCAGCACTTTGGGAGGCCGAGGCGGGCGG  GTCACGAGGTCAAGAGATCGAGACCATCCGGGCGGAC  ACGGTCAAACCCCGTCTCTATTAAGTATAAGATTAG  CTGGGCGTGGTGGCGGGCACCTGTAGTCCAGCTACT  CGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCAGGG  AGGTGGCGGTTGCAGTGAGCCGAGATCACACCATCGC  ACTCCAGCCTGGGCGACAGAGCGAGAC</p>
reverse <i>alu</i> RNA template for T7 <i>in vitro</i> transcription	<p>TAATACGACTCACTATAGGGGTCTCGCTCTGTCGCCCA  GGCTGGAGTGCGATGGTGTGATCTCGGCTCACTGCAA  CCGCCACCTCCCTGGGTTCAAGCGATTCTCCTGCCTCA  GCCTCCCGAGTAGCTGGGACTACAGGTGCCCGCCACC</p>

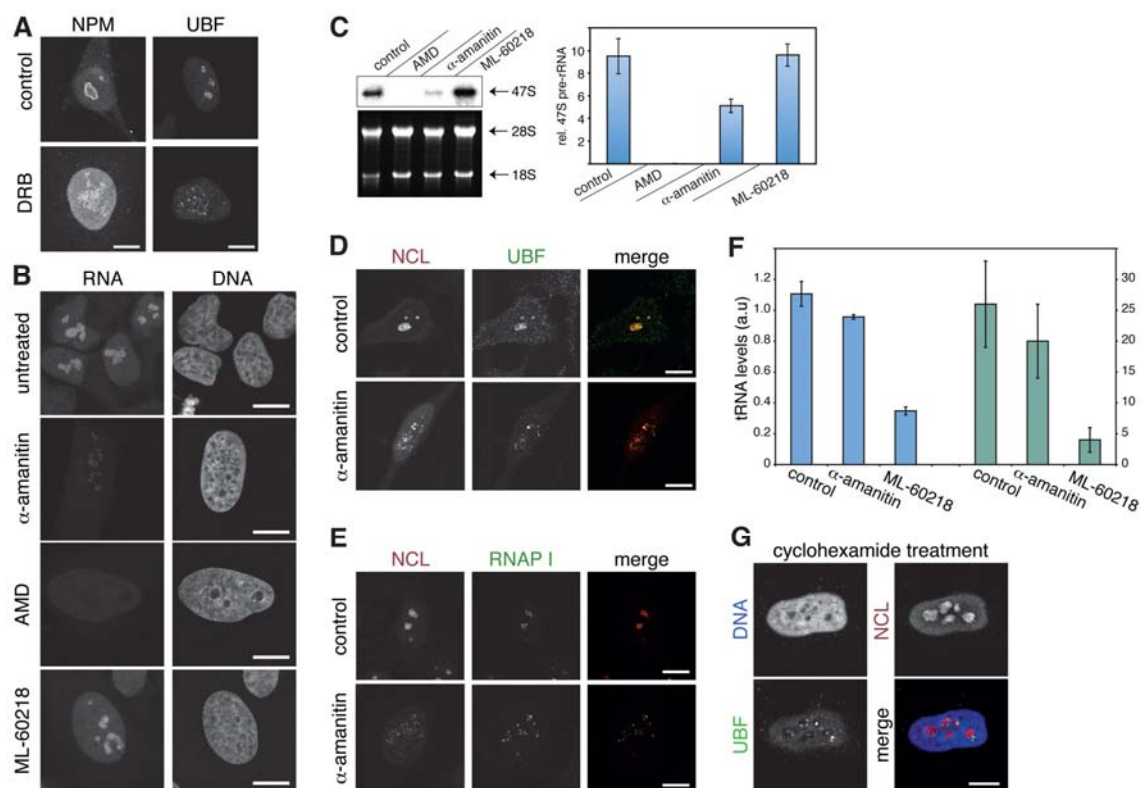
	ACGCCCAGCTAATCTTTATACTTTTAATAGAGACGGGGT TTCACCGTGTCTGGCCCCGGATGGTCTCGATCTCTTGACC TCGTGACCCGCCCGCCTCGGCCTCCCAAAGTGCTGGG ATGACAGGCGTGAGCCACTGAGCCCGGCCTTCTCTTG ACGTTTAAACTATGAAGTCAGTCCAG
RNA <sub>L</sub> template for T7 <i>in vitro</i> transcription	TAATACGACTCACTATAGGGCTGGACTGACTTCATAGTT TAAACGTCAAGAGAAGGCCGGGCTCAGTGGCTCACGC CTGTCATCCCAGCACTTTGGGAGGCCGAGGCGGGCGG GTCACGAGGTCAAGAGATCGAGACCATCCGGGGCCGAC ACGGTGAAACCCCGTCTCT
RNA <sub>R</sub> template for T7 <i>in vitro</i> transcription	TAATACGACTCACTATAGGGATTAAGTATAAAGATTA GCTGGGCGTGGTGGCGGGCACCTGTAGTCCCAGCTAC TCGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCAGG GAGGTGGCGGTTGCAGTGAGCCGAGATCACACCATCG CACTCCAGCCTGGGCGACAGAGCGGAGAC
CDV3a template for T7 <i>in vitro</i> transcription	TAATACGACTCACTATAGGGAAATTCAAGGACGAATATT TTCAAAAACCCTAGTGAAGAAGAAATATTTACTGATTAC ATTTCTTTTCCCTTAG
CDV3b template for T7 <i>in vitro</i> transcription	TAATACGACTCACTATAGGGGACCACCAGAAATCTACA GTGATACACAGTTCCCATCCCTGCAGTCAACTGCCAAG CATGTAGAAAGCCGGAA
L1-repeat template for T7 <i>in vitro</i> transcription	TAATACGACTCACTATAGGGGACAGAGGCATTATTCAC AATAGCAAAGACTTCGAACCAACCCAAATGTCCAACAA TGATAGACTGGATTAAGAAAATGTGGCACATATACACC ATGGAATACTATGCAGCCATAAAAAATGATGAGTTCATG TCCTTTGTAGGGACATGGATGAAATTGGAAATCATCATT CTCAGTAACTATCGCAAGAACAACAAAAACCAAACACCG CATATTCTCATAGGTGGGAACTGAACAATGAGATCACA TGGTCACAGGAAGGGGAATATCACACTCTGGGAACTGT GGTGGGGTGGGGGGAGGGGGGAGGGGATAGCATTGGG AGATATACCTAATGGTAGATGACGAGTTAGTGGGTGCA GCACACCAGCATGGCACATGTATACATATGTAACCTAAC CTGCACAATGTGCACATGTACGTAAAACCTTAAAG

**Appendix Table S5. Summary of reads obtained from RNA-seq**

sample	read length	# reads	# uniquely mapped	% mapped*
total RNA	36	44748728	17509543	82
nucleolar RNA	36	39049758	15077407	91
total RNA	100	64020509	35257382	73
nucleoplasmic RNA	100	72257683	26527408	68
nucleolar RNA	100	60301513	11002830	64
total RNA, control	100	45478612	21587838	65
total RNA, $\alpha$ -amanitin	100	42072870	19739852	65
total RNA, ML-60218	100	45270772	21404418	77
total RNA, control	100	62902322	34570403	87
total RNA, $\alpha$ -amanitin	100	64700649	33350538	82
total RNA, ML-60218	100	41871486	21033049	67
total RNA, mock	100	53274853	26363971	73
total RNA, <i>alu</i> RNA ASO	100	55449460	27732908	77
total RNA, control ASO	100	51555714	28641577	77

\* including multiple mapped reads and rRNA reads.

## Appendix Figures



### Appendix Figure S1. Effects of drug treatment on nucleolar structure and function.

A. CLSM images of HeLa cells treated with DRB and of untreated control. The nucleolar marker proteins NPM and UBF were visualized by immunofluorescence with antibodies specific to NPM or UBF.

B. CLSM images of HeLa cells after treatment with the indicated drugs. Nascent RNA was pulse labeled with EU, and DNA was stained with DAPI.

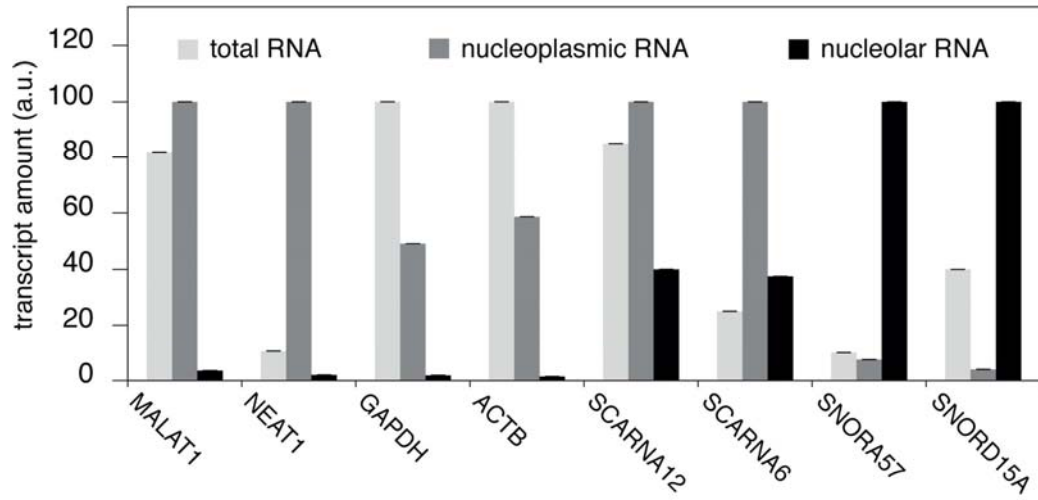
C. Northern blot showing rRNA levels in untreated-, AMD-,  $\alpha$ -amanitin- and ML-60218-treated HeLa cells (left). The 47S pre-rRNA has a very short half-life time in the range of minutes (Popov et al., 2013) as compared to 18S and 28S that are stable for days (Defoiche et al., 2009). Accordingly, 47S steady-state levels reflect the rRNA synthesis rate. For quantification, samples were normalized to 18S rRNA as determined by RT-qPCR. Error bars represent the standard deviation ( $n = 6$ ).

D. CLSM images of HeLa cells treated with  $\alpha$ -amanitin. The localization of NCL (red) and UBF (green) was detected by immunofluorescence.

E. Same as in panel D but for NCL (red) and Pol I (green).

F. Tyrosine tRNA levels normalized to 18S rRNA as measured by RT-qPCR (left y-axis, blue) or RNA-seq (right y-axis, green) in untreated (control),  $\alpha$ -amanitin- or ML-60218-treated total RNA samples. Error bars represent the standard deviation (blue bars,  $n = 2$ ) or the 95% CI (green bars).

G. Distribution of DNA (blue), UBF (green) and NCL (red) in HeLa cells treated with cycloheximide for 5 hours and visualized by CLSM. Scale bars, 10  $\mu$ m.



**Appendix Figure S2. Distribution of selected transcripts in total, nucleoplasmic and nucleolar RNA fractions.**

The relative amount of the indicated transcripts within the total, nucleoplasmic and nucleolar RNA fractions is plotted. Scale bars, 95% confidence interval (95% CI).



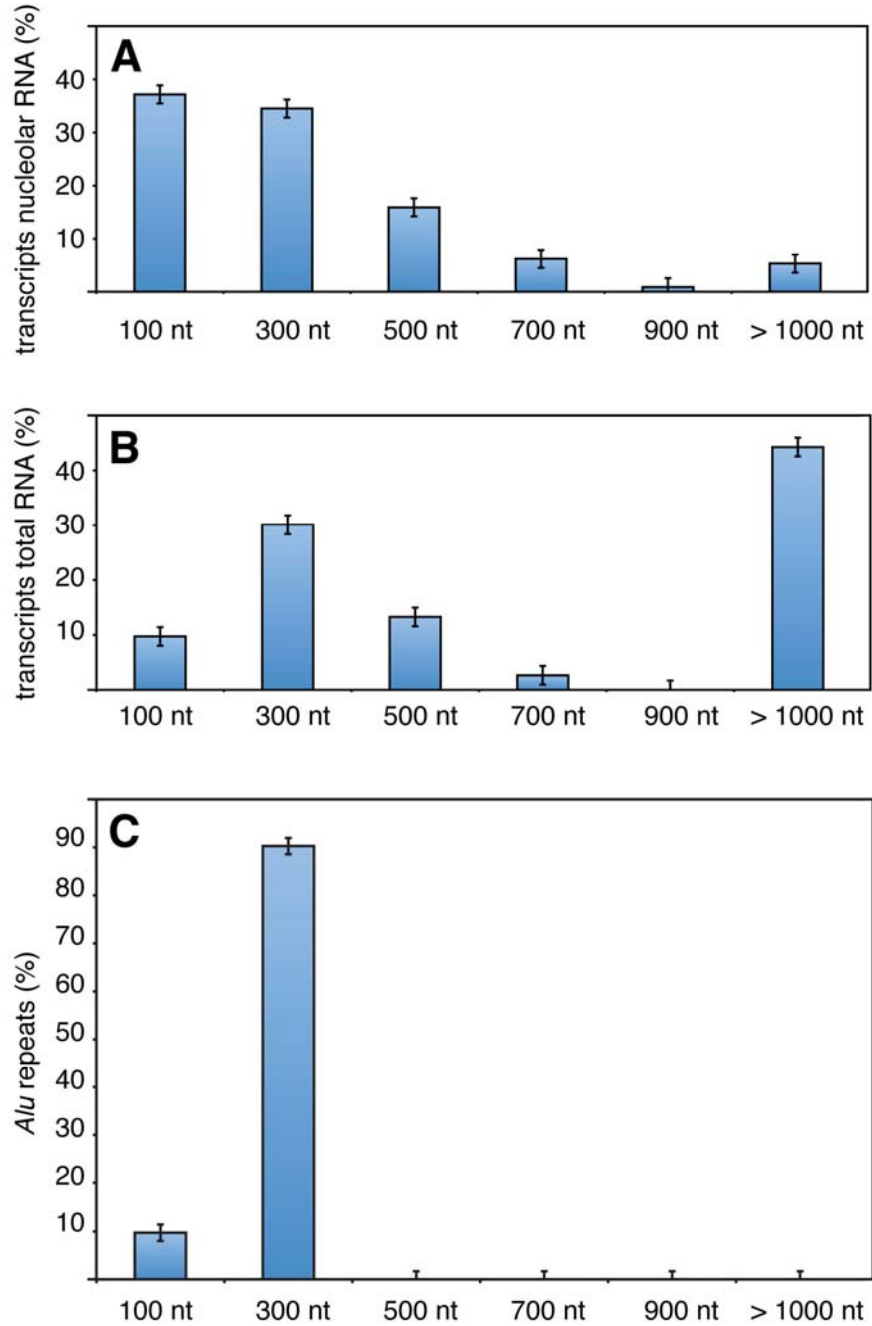
```

Region_7 -----AGAT CATGCCACCA CACTCCAGCC TG-G
Region_14 -----GGAT TGTGCCACTG TGCTCCAGCC TG-GGTGACA G
Region_30 -----AGAT CGTGCCATG CACTACGGCC T
Region_26 -----AGAT CGCACCCTG C
Region_23 AAAAAAAGGC TGGGCATGAT GGCTTATGCC TGTAATCCCA G-----CA CTTTGGGAGG C
Region_11 -----AGAT CACACCCTG CA
Region_24 -----TGAT TGCACCA
Region_2 -----AGAT TGCGCCAGCG TACTCCAGCC TG-GGCAACA GAGCGAGACT CAGTCTCAAG AAAAAAAAAA G--AAAGAAA GAAACA
Region_3 -----AGGT AGTG
Region_25 -----AGAT CGCGCCACTA CACTCC
Region_29 -----TGAC CATGCCACTG CACTC-AGCC TG-GGCAACA GAGTGAGACC CTGTCTCAAG AAAGAAAAAC GAGAAAGGGA GAGTCCCTCC ACTGTAAGGA GATCGGGTTC ATTACATTTT GGGGTGTTGG
Region_15 -----AGAT TGTGCCACTG CACTCCAGCC TG-GGTAACA G--TGAGACT CCGTCTCAA AAAAAAAAAA ---AAAGTGC CATGTAGTCC -CTGTGTGGG G--CAGGCC CAATCAGT-- -GCATGTGGG
Region_19 -----TGAT GGCACCCTG TACTCCAGCC TG-AGTAACA AAGACAGACC CTGTCTCTTA AGAAAAAAG
Region_22
Region_21
Region_28 -----AGAT CATGCCACTG CA
Region_27
Region_13
Region_31 -----TGAC CGTGCCACTG CACTCCAAC TG-GGTGA
Region_5 -----AGAT TGTGCCACTG CA
Region_8 -----AGAT CATGCCATG CACTAC
Region_4
Region_20 -----AGAT
Region_9 -----CAAT TGCACAAC TG CACTACAACC TG
Region_6 -----TTAT TCTGCCACTG CACTCCAGC
Region_17 -----TGGT GGCACCCT
Consensus agat .g.gccactg .....

```

**Appendix Figure S3. Sequence alignment of nucleolar-enriched *alu*RNAs.**

*Alu* element-containing RNAs (regions 1 to 31 from Table S2) enriched in the nucleolar RNA fraction over total RNA were aligned to derive a consensus sequence for nucleolar *alu*RNA (bottom line in the alignment). The degree of similarity to the consensus sequence is indicated by different colors: high, red; moderate, blue; neutral, black.



**Appendix Figure S4. Length distribution of *Alu* repeat-containing RNA transcripts from RNA-seq and corresponding genomic *Alu* elements.**

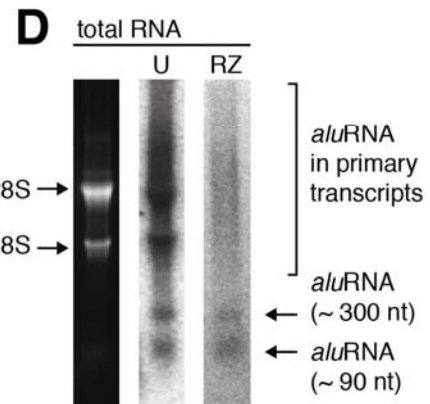
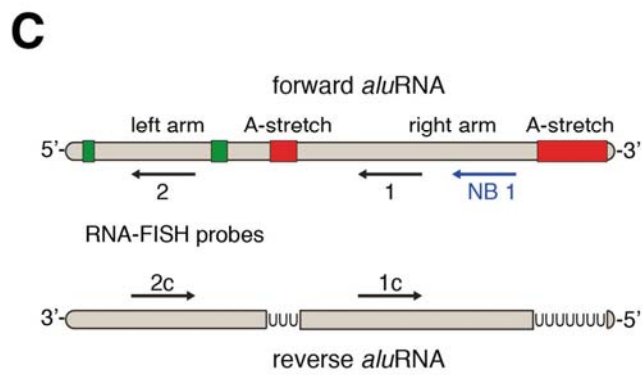
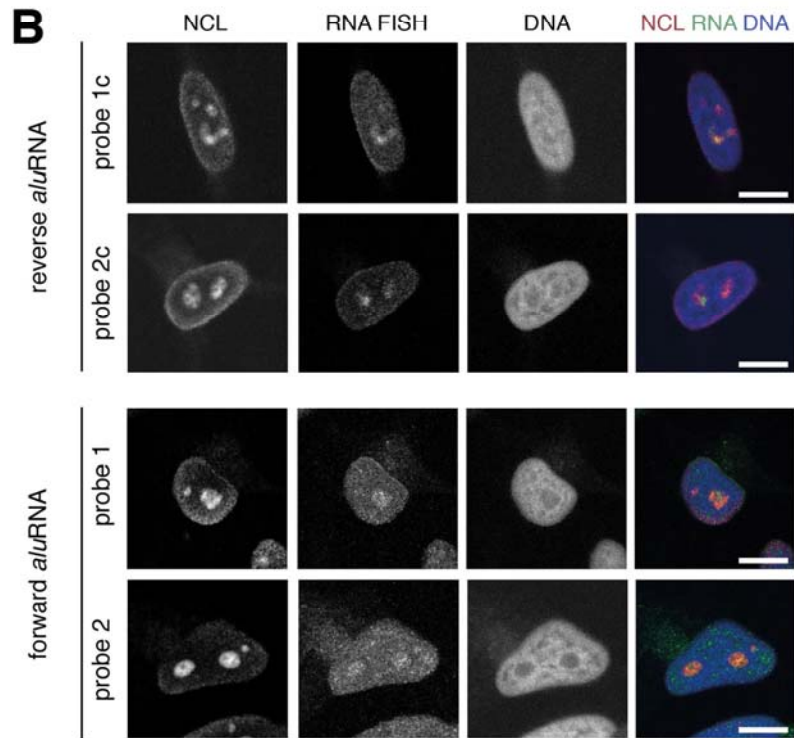
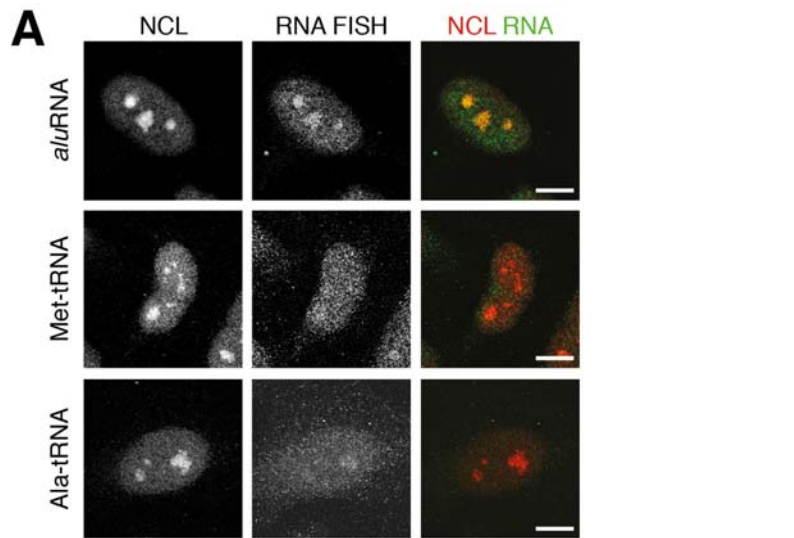
A. Length distribution of *Alu* repeat-containing nucleolar RNA sequences.

B. Length distribution of *Alu* repeat-containing total RNA sequences.

C. Length distribution of *Alu* element in the genome, which overlap with the RNA sequences from panel A and B.

Error bars, 95% CI.





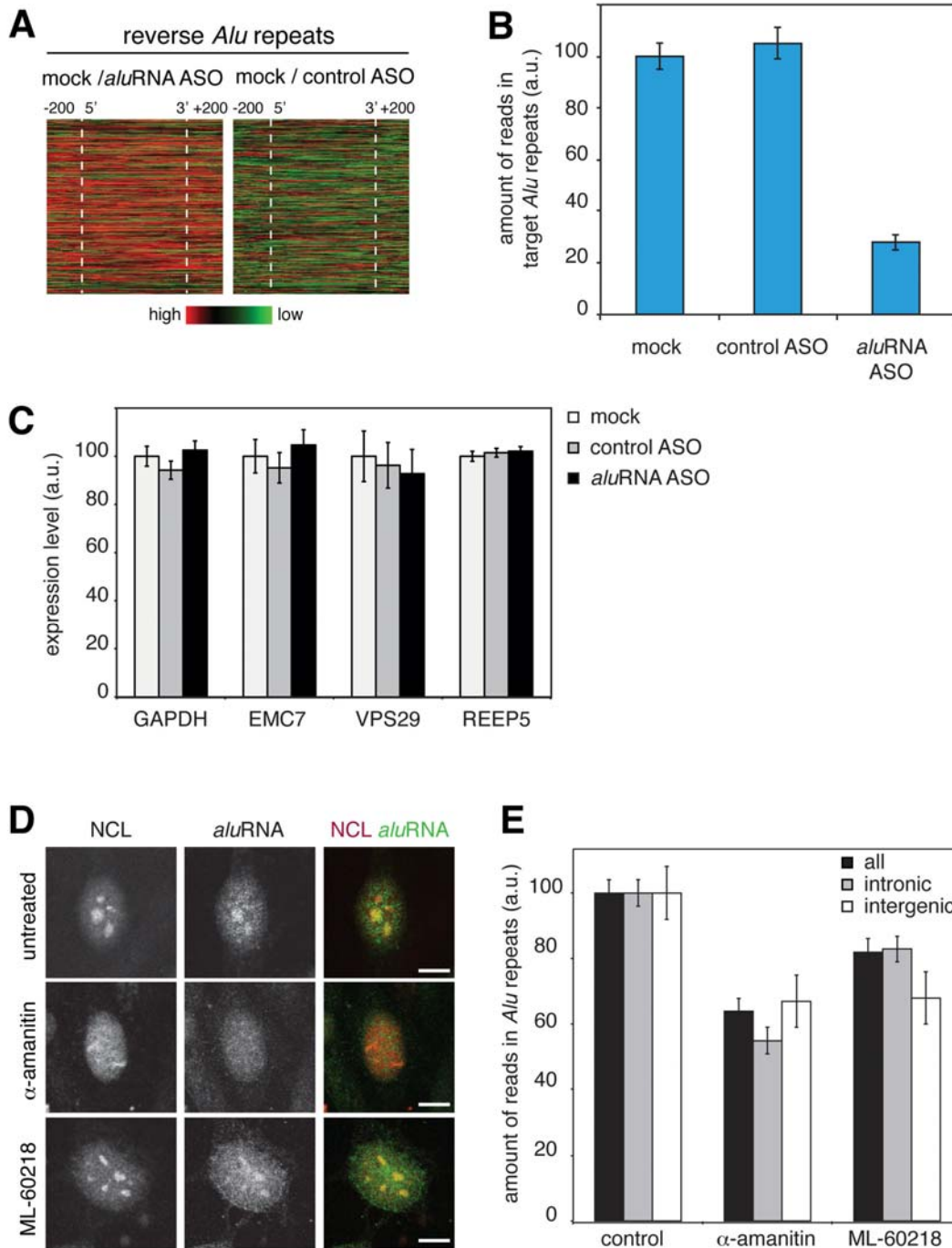
**Appendix Figure S5. Detection of *alu*RNAs in nucleoli and in total RNA.**

A. CLSM images of RNA-FISH analysis in HeLa cells using probes specific to forward *alu*RNA, methionine (Met-tRNA) and alanine (Ala-tRNA) tRNA sequences in HeLa cells. There is a weak signal in the nucleoli for the two tRNAs, which is in agreement with a small number of sequencing reads matching tRNA sequences found in the nucleolar fraction. The image intensity for the three RNA FISH images was normalized to the same nuclear intensity.

B. CLSM images of RNA-FISH against forward or reverse *alu*RNA sequences in HeLa cells. Color coding of merged images: RNA-FISH, green; NCL immunofluorescence, red; DNA (DAPI staining), blue. Scale bars, 10  $\mu$ m.

C. Scheme showing target sites of different RNA-FISH probes (1, 2, 1c and 2c, black arrows) and northern blot probe (NB 1, blue arrow) within a forward (top) or reverse (bottom) *Alu* repeat RNA sequence.

D. Northern blot analysis of purified total RNA probed with a sequence (NB1, see Appendix Table S4 and panel C) that targets the right arm of an *Alu* element. The bracket marks long primary transcripts with embedded *alu*RNA sequences. The position of the 300 nt length RNA was calculated according to co-migration of 7SL RNA and the position of the ~90 nt length RNA according to co-migration of tyrosine tRNA. In the lane labeled with RZ, total RNA after RiboZero treatment was loaded, in the lane U, an untreated total RNA sample. The position of 28S and 18S rRNA was detected by ethidium bromide staining of the untreated RNA sample.



**Appendix Figure S6.** *aluRNA* levels are reduced after *aluRNA* ASO-mediated depletion or  $\alpha$ -amanitin treatment.

A. Heatmaps of read ratios obtained from total RNA sequencing of HeLa cells. Mock (transfection reagent only) ratio to *alu*RNA-ASO or control-ASO transfected cells. Colors indicate high (red) to low (green) read ratios.

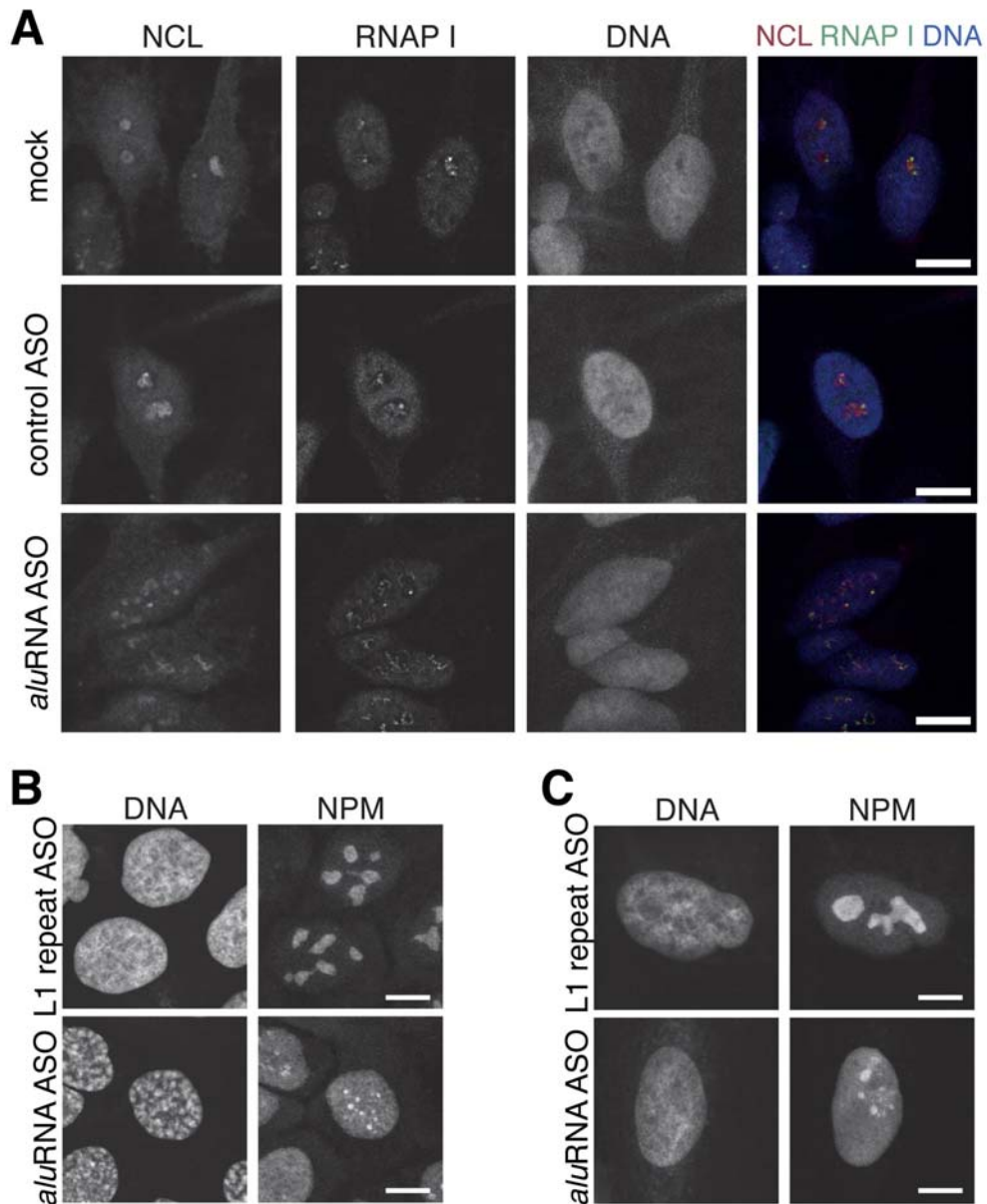
B. Graph showing the amount of reads corresponding to *Alu* repeats that were targeted by the *alu*RNA ASO (n = 3737). Cells were treated with ASO as indicated.

C. Graph showing the expression levels of house keeping genes in ASO-treated cells as in panel B.

D. CLSM images of HeLa cells treated as indicated. The distribution of NCL (immunofluorescence) and *alu*RNA (RNA FISH) are shown. Color-coding in the merge images: red, NCL and green, *alu*RNA. Scale bars, 10  $\mu$ m.

E. Graph showing the amount of reads corresponding to all (n = 330 000), intronic (n = 250 000) or intergenic (n = 70 000) *Alu* repeats position. Cells were treated as indicated.

Error bars, 95% CI.



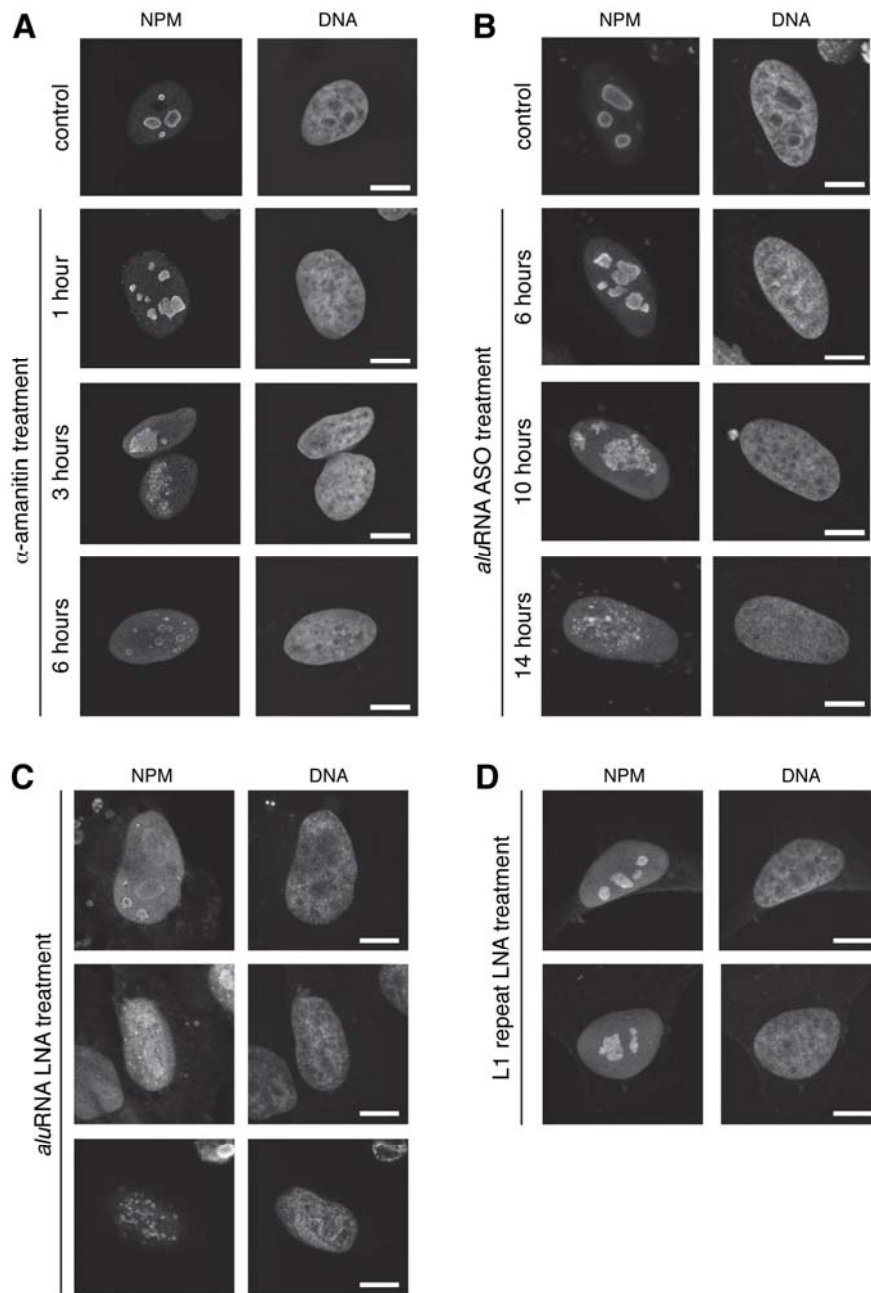
**Appendix Figure S7. Antisense oligonucleotides against *alu*RNA disrupt the nucleolar structure in different human cell types.**

A. CLSM images of HeLa cells treated with the indicated ASO. Nucleoli were visualized by immunofluorescence of NCL (red), Pol I (green), DNA was stained with DAPI.

B. CLSM images of human keratinocytes treated with the indicated ASO. Nucleoli were visualized by immunofluorescence of NPM (red), DNA was stained with DAPI.

C. CLSM images of human fibroblasts treated with the indicated ASO. Nucleoli were visualized by immunofluorescence of NPM (red), DNA was stained with DAPI.

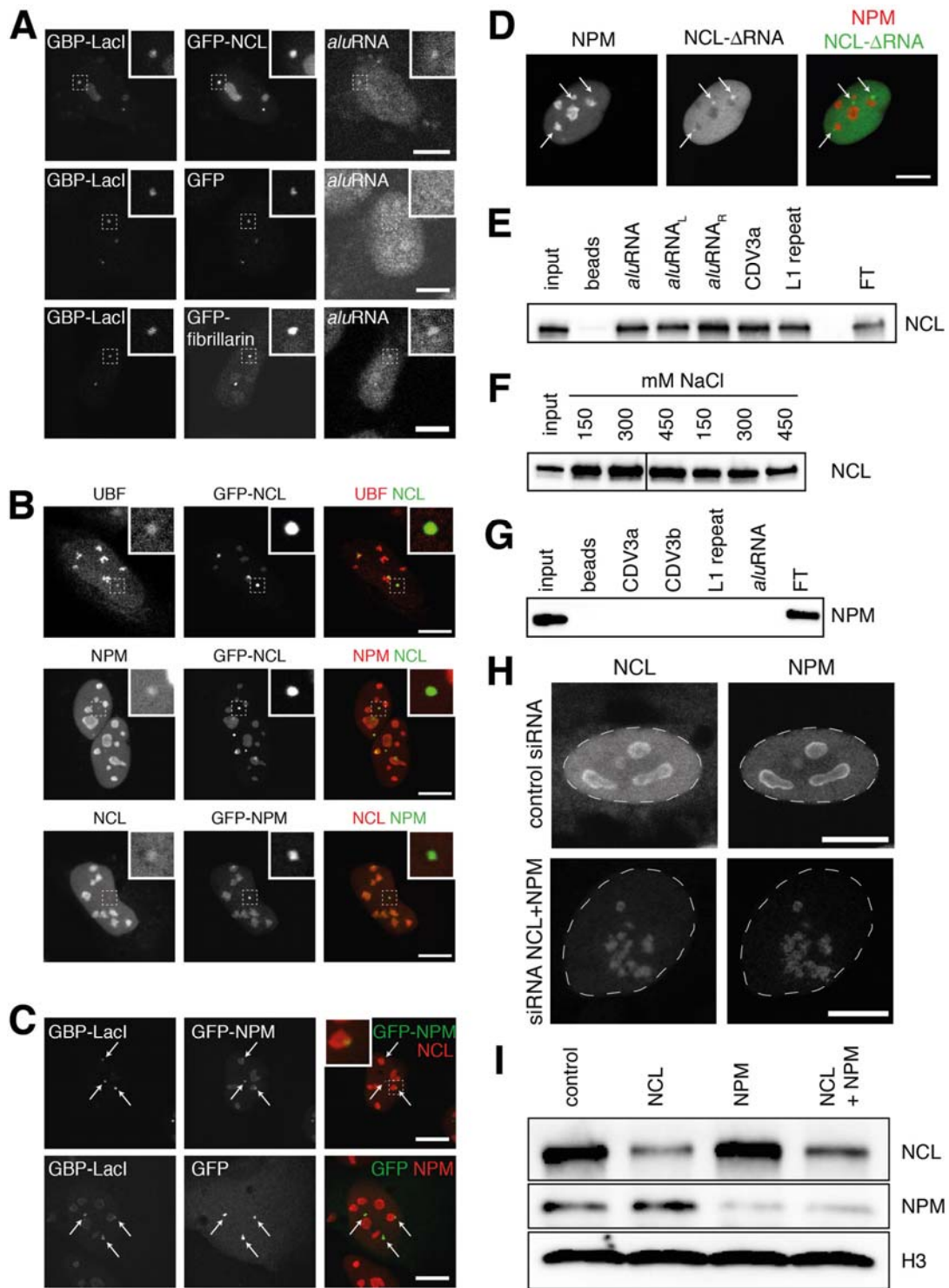
Scale bars, 10  $\mu$ m.



**Appendix Figure S8. Kinetics of nucleoli disruption upon inhibition of Pol II activity by  $\alpha$ -amanitin or depletion of *alu*RNAs are traced by time-lapse imaging.**

CLSM images of HeLa cells under different treatment conditions. Nucleoli were visualized by immunofluorescence of NPM, DNA by staining with DAPI. Scale bars in all images, 10  $\mu$ m.

A.  $\alpha$ -amanitin. B. ASO directed against the forward *alu*RNA. C. Blocking LNA probe directed against the forward *alu*RNA sequence. D. Blocking LNA probes directed against L1-repeat RNA.



**Appendix Figure S9. NCL and NPM are required to preserve the nucleolar structure and interact differently with *alu*RNA.**

A-D, H. CLSM images showing the localization of the indicated proteins or RNAs. For recruitment of proteins to *lacO* arrays (panel A-D) the U2OS F6B2 cell line was used. Scale bars, 10  $\mu$ m.

A. Co-localization of GFP-NCL (upper panel), GFP (middle panel), or GFP-fibrillarin (lower panel) with GBP-LacI-RFP and *Alu*RNA. *Alu*RNA was visualized by RNA-FISH. The insets show enlarged images of one of the *lacO* loci.

B. Upper panel: immunostaining of UBF (red) and GFP-NCL (green); middle panel: immunostaining of NPM (red) and GFP-NCL (green); lower panel: immunostaining of NCL (red) and GFP-NPM (green). Insets indicate examples of *lacO* arrays to which the GFP-tagged protein was recruited.

C. GBP-LacI-RFP, GFP-NPM or GFP (green), and NCL or NPM visualized by immunostaining (red). Arrows indicate the stable inserted *lacO* arrays. The inset shows an example of a *lacO* array that is localized in the nucleolus.

D. NPM (immunofluorescence, red) and GFP-NCL- $\Delta$ RNA (green). Arrows indicate the position of the *lacO* array.

E-G. Western blot analysis of NCL or NPM binding to RNA in pull-down experiments using nuclear extract and the indicated biotinylated RNAs immobilized on streptavidin beads (see also Appendix Table S4). The lane labeled with "beads" indicates a sample that contained only the magnetic beads without biotinylated RNA. FT corresponds to the flow through fraction.

E. Binding of NCL to different RNAs in binding buffer containing 200 mM NaCl. 0.7  $\mu$ g/ $\mu$ l nuclear proteins were used. The FT sample is separated from the others by an empty lane.

F. Salt-dependent binding of NCL to forward *Alu*RNA in the presence of 70  $\mu$ g (lanes 2-4) or 35  $\mu$ g (lanes 5-7) of nuclear extract in a final volume of 100  $\mu$ l. Due to a broken well, 2 empty lanes, were removed from the blot image between the 3<sup>rd</sup> and 4<sup>th</sup> lane as marked by the line.

G. Binding of NPM to indicated RNAs under the same conditions as in panel E.

H. Nuclear distribution of NCL and NPM in HeLa cells transfected with control siRNA or siRNA specific to NPM and NCL. NCL and NPM were visualized by immunofluorescence. Images were taken with the same settings to allow for the comparison of fluorescence signals.

I. Western blot analysis of siRNA-mediated depletion of NCL and NPM in HeLa cells. Histone H3 was used as loading control. siRNA treatments reduced the amount of the corresponding protein by more than 50% (NCL) and 80% (NPM).