

Supplementary Figures



Figure S1. Workflow to identify candidate scaffold proteins. For comparison, the number of components of transcriptional condensates that were identified by light-induced proximity-based biotinylation [105] are shown in panel D.

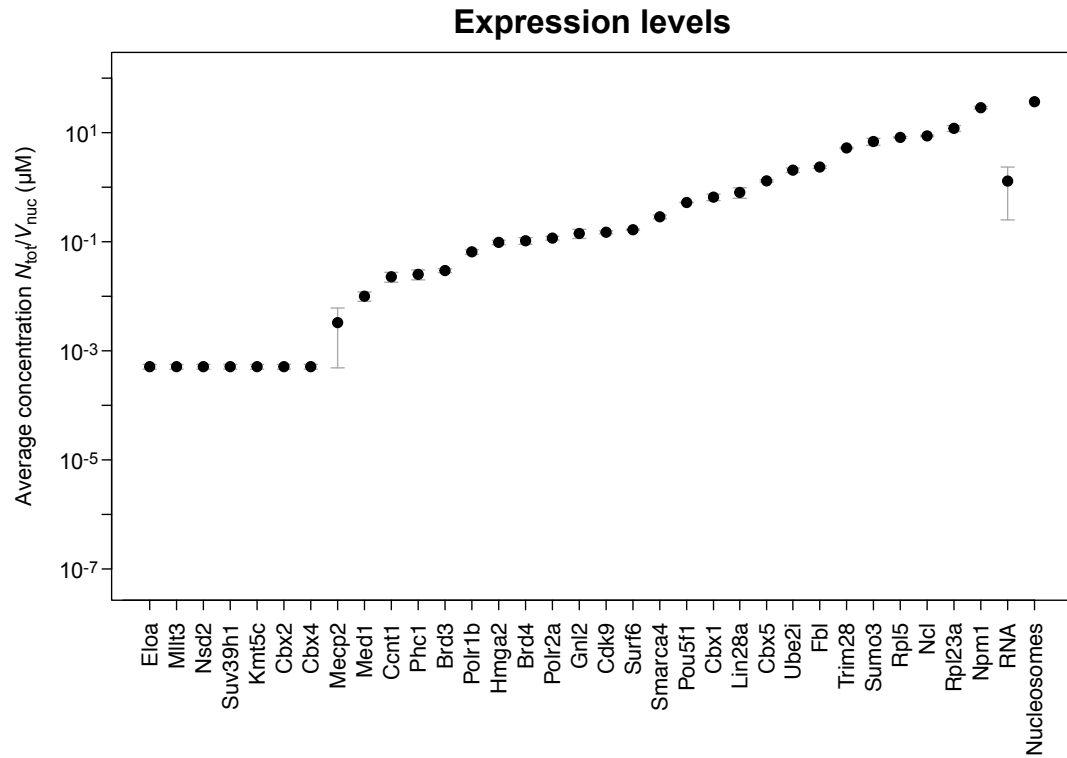


Figure S2. Expression levels of candidate scaffold proteins in the cell nucleus. Values are given as average concentrations that correspond to the copy number determined from mass spectrometry divided by the nuclear volume.

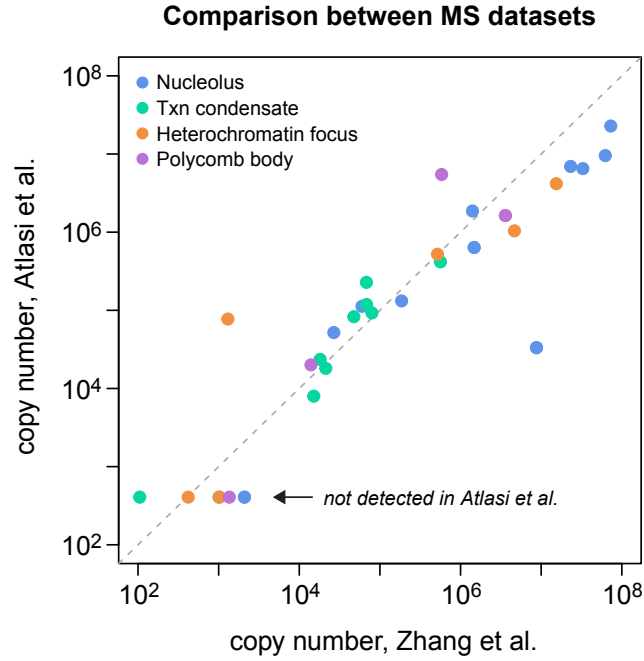


Figure S3. Copy numbers of candidate scaffold proteins in mouse embryonic stem cells based on two datasets from different studies [31, 87]. Copy numbers are color-coded according to the respective organelle as indicated.

Supplementary Tables

Organelle	Marker protein(s)	Volume (μm^3)	Reference (volume)	Number per cell	Reference (number)
Nucleus	-	1320 ± 374	[106]	1	-
Nucleolus, total	-	480 ± 134	[106]	1	[107]
Nucleolus, GC	Npm1	432 ± 121	[106, 108]	1	[37]
Nucleolus, DFC	Fbl	1.1 ± 0.3		43	[37]
Transcriptional condensate	Med1, Brd4	0.004 (small) 0.1 (large)	[42]	983 ± 102 (total)	[43]
Heterochromatin focus	Cbx5, Mecp2	4.8 ± 0.6	[94]	10 ± 1	[94]
Polycomb body	Cbx2	0.04 ± 0.01	[51]	13 ± 4	[51]

Table S1. Cellular organelles studied in this work, along with their volume, number per mouse ES cell and the marker protein(s) that were used to identify them. GC, granular component; DFC, dense fibrillar component.

Organelle	Total number of scaffold proteins per organelle	Number of RNAs per organelle ^a	Number of nucleosomes per organelle	Reference (nucleosomes)
Nucleus	62,496,930 ± 4,375,630	1,029,540 ± 829,688	29,348,434	-
Nucleolus, GC	34,181,482 ± 12,178,788	195,986 ± 121,723	2,711,831 ± 1,667,835	[109]
Nucleolus, DFC	8,926 ± 3,565	506 ± 317	7,007 ± 4,337	[109]
Transcriptional condensate	15 ± 10 (small) 400 ± 271 (large)	8 ± 6 (small) 216 ± 154 (large)	131 ± 86 (small) 3,535 ± 2,326 (large)	[43]
Heterochromatin focus	64,120 ± 19,979	6,028 ± 2,485	176,186 ± 59,805	[110]
Polycomb body	434 ± 207	35 ± 19	1,370 ± 629	[111]

^a Note that RNA copy numbers in nucleoli refer to rRNA molecules, while RNA copy numbers in other organelles refer to shorter RNA molecules (median mRNA size). RNA copy numbers in the entire nucleus refer to a mixture of both types of RNA.

Table S2. Overview of the content of membraneless organelles studied in this work, according to the class of macromolecules. The total number of candidate scaffold proteins was determined based on quantitative mass spectrometry data for each candidate protein [31]. Note that the number indicated for the entire nucleus refers only to the candidates considered for the selected membraneless organelles studied here (not to all candidates for all membraneless organelles in the nucleus). The number of RNA molecules was determined based on the known amount of total RNA in the nucleus, the median length of different species of RNA molecules, and the spatial distribution of RNA molecules taken from stainings with an RNA-specific fluorescent probe (Extended Data Fig. 4e in [35]). The number of nucleosomes in the nucleus was calculated based on the genome size and nucleosome repeat length, while the numbers of nucleosomes in the individual organelles were determined based on the chromatin distribution seen in microscopy images (references in the last column). See **Methods** section for details.

Protein name	Copy number per cell	Enrichment in organelle	Reference (enrichment)	Copy number per organelle	Concentration in organelle (μM)
Nucleolus, granular component (GC)					
Npm1	22,803,667 $\pm$ 1,195,488	4.7 $\pm$ 0.3	[110] Fig. 3c (IF)	15,824,200 $\pm$ 5,502,760	60.8 $\pm$ 21.2
Rpl23a	9,542,514 $\pm$ 1,175,100	3.8 $\pm$ 0.1	[112] Fig. 2C (Rpl23-GFP)	6,167,027 $\pm$ 2,247,853	23.7 $\pm$ 8.7
Rpl5	6,506,182 $\pm$ 224,448	17.3 $\pm$ 0.9	[113] Fig. 7B (GFP-Rpl5)	5,814,283 $\pm$ 2,147,395	22.3 $\pm$ 8.3
Ncl	6,931,051 $\pm$ 225,884	10.1 $\pm$ 0.6	[110] Fig. 3b (IF)	5,763,236 $\pm$ 2,061,265	22.2 $\pm$ 8.0
Lin28a	636,709 $\pm$ 139,637	3.6 $\pm$ 0.2	[114] Fig. 1C (IF)	404,911 $\pm$ 165,255	1.6 $\pm$ 0.6
Gnl2	112,651 $\pm$ 21,403	∞	-	112,651 $\pm$ 21,403	0.4 $\pm$ 0.1
Surf6	131,920 $\pm$ 3,574	5.3 $\pm$ 0.1	[115] Fig. 1G (IF)	94,915 $\pm$ 32,763	0.4 $\pm$ 0.1
Eloa	407 $\pm$ 41	3.7 $\pm$ 0.1	[116] Fig. 5C (mCh-Eloa)	263 $\pm$ 94	0.001 $\pm$ 0.001
Nucleolus, dense fibrillar component (DFC)					
Fbl	1,863,156 $\pm$ 103,110	4.7 $\pm$ 0.2	[110] Fig. 3d (IF)	6,499 $\pm$ 189	9.8 $\pm$ 0.3
Lin28a	636,709 $\pm$ 139,637	3.9 $\pm$ 0.1	[114] Fig. 1C (IF)	1,918 $\pm$ 852	2.9 $\pm$ 1.3
Polr1b	51,889 $\pm$ 5,305	19.2 $\pm$ 1.6	[117] Fig. 1C (Polr1b-GFP)	508 $\pm$ 20	0.8 $\pm$ 0.1
Eloa	407 $\pm$ 41	5.7 $\pm$ 0.2	[116] Fig. 5C (mCh-Eloa)	2 $\pm$ 1	0.003 $\pm$ 0.002
Transcriptional condensate (small population)					
Polr2a	92,668 $\pm$ 7,370	23.1 $\pm$ 0.9	[118] Fig. 3a (IF)	6.2 $\pm$ 4.1	2.6 $\pm$ 1.7
Brd4	82,798 $\pm$ 11,405	10.1 $\pm$ 0.2	[43] Fig. 1A (IF)	2.6 $\pm$ 1.7	1.1 $\pm$ 0.7
Pou5f1	416,624 $\pm$ 1,720	1.5 $\pm$ 0.1	[119] Fig. 2a (Pou5f1-YPet)	2.0 $\pm$ 1.3	0.8 $\pm$ 0.5
Smarca4	227,873 $\pm$ 18,084	1.7 $\pm$ 0.1	[120] Fig. 6D (IF)	1.2 $\pm$ 0.8	0.5 $\pm$ 0.3

Med1	7,971 ± 1,557	49.2 ± 2.4	[43] Fig. 1A (IF)	1.0 ± 0.7	0.4 ± 0.3
Cdk9	118,650 ± 7,659	1.5 ± 0.1	[121] Movie S1 (Cdk9-mCh)	0.6 ± 0.4	0.2 ± 0.2
Brd3	23,528 ± 1,961	4.8 ± 0.3	[122] Fig. 3C (GFP-Brd3)	0.4 ± 0.2	0.2 ± 0.1
Ccnt1	18,100 ± 3,762	5.2 ± 0.2	[123] Fig. 4A (GFP-Ccnt1)	0.3 ± 0.2	0.1 ± 0.1
Nsd2	407 ± 41	∞	-	0.3 ± 0.3	0.1 ± 0.1
Mllt3	407 ± 41	∞	-	0.3 ± 0.3	0.1 ± 0.1
Heterochromatin focus					
Trim28	4,167,789 ± 137,584	1.5 ± 0.1	[124] Fig. 5C (IF)	22,822 ± 7,114	7.9 ± 2.5
Ube2i	1,631,766 ± 151,670	2.9 ± 0.1	[125] Fig. S2e (Ube2i-YFP)	16,035 ± 4,978	5.5 ± 1.7
Cbx5	1,038,731 ± 51,797	3.5 ± 0.4	[126] Fig. 6D (IF)	12,190 ± 3,829	4.2 ± 1.3
Cbx1	522,356 ± 71,442	7.5 ± 0.8	[109] Fig. 6B (IF)	11,482 ± 3,562	4.0 ± 1.2
Hmga2	77,331 ± 7,375	6.4 ± 0.9	[127] Fig. 1A (IF)	1,513 ± 436	0.5 ± 0.2
Mecp2	2,614 ± 2,227	8.2 ± 0.2	[57] Fig. 1 (Mecp2-GFP)	61 ± 55	0.021 ± 0.019
Kmt5c	407 ± 41	12.5 ± 2.5	[128] Fig. S2C (Kmt5c-GFP)	13 ± 4	0.004 ± 0.001
Suv39h1	407 ± 41	2.6 ± 0.1	[129] Fig. 3A (GFP-Suv39h1)	4 ± 1	0.001 ± 0.001
Polycomb body					
Sumo3	5,465,502 ± 801,634	1.8 ± 0.1	[130] Fig. EV3A (IF)	265 ± 130	11.0 ± 5.4
Ube2i	1,631,766 ± 151,670	2.9 ± 0.1	[125] Fig. S2e (Ube2i-YFP)	130 ± 62	5.4 ± 2.6
Cbx4	407 ± 41	∞	-	31 ± 10	1.3 ± 0.4
Phc1	20,041 ± 4,147	14.3 ± 0.6	[51] Fig. 1d (IF)	8 ± 4	0.3 ± 0.2
Cbx2	407 ± 41	9.6 ± 0.4	[51] Fig. 1d (YFP-Cbx2)	0.1 ± 0.1	0.004 ± 0.004

Table S3. Copy numbers, enrichments and resulting concentrations of candidate LLPS scaffold proteins in nucleoli, transcriptional condensates, heterochromatin foci and Polycomb bodies of mouse embryonic stem cells. Copy numbers per cell (second column) were determined based on quantitative mass spectrometry data [31], enrichment values (third column) were estimated from immunofluorescence (IF) images or from images of cells expressing a fusion of the candidate scaffold protein to a fluorescent protein (see references and annotations in the fourth column). Proteins written in italics were not detected in the respective mass spectrometry experiments and were thus assigned a value that corresponds to the estimated detection limit (407 ± 41 copies per cell). The copy numbers in large transcriptional condensates are 27-times larger than the numbers in small transcriptional condensates given here. See **Methods** section for details.

Protein name	UniProt ID	Diameter (nm) - AlphaFold	Diameter (nm) - Relaxed	Diameter (nm) - Expanded
Nucleolus, granular component (GC)				
Npm1	Q61937	7.5	9.7	12.2
Rpl23a	P62751	8.4	8.4	8.4
Rpl5	P47962	5.5	6.3	8.5
Ncl	P09405	9.3	16.8	21.7
Lin28a	Q8K3Y3	6.4	6.8	9.3
Gnl2	Q99LH1	8.3	13.2	24.8
Surf6	P70279	8.6	12.9	18.5
Eloa	Q8CB77	10.8	19.8	28.4
Nucleolus, dense fibrillar component (DFC)				
Fbl	P35550	5.6	8.6	10.0
Lin28a	Q8K3Y3	6.4	6.8	9.3
Polr1b	P70700	7.3	7.3	7.3
Eloa	Q8CB77	10.8	19.8	28.4
Transcriptional condensate				
Brd4	Q9ESU6	11.3	26.6	39.3
Pou5f1	P20263	7.0	11.8	17.9
Polr2a	P08775	9.4	20.4	41.4
Smarca4	Q3TKT4	11.7	21.7	42.5
Med1	Q925J9	10.8	26.5	41.9
Cdk9	Q99J95	4.7	6.3	7.6
Brd3	Q8K2F0	9.0	17.2	27.4
Ccnt1	Q9QWV9	8.9	16.9	27.4

Nsd2	Q8BVE8	9.6	20.1	38.7
Mllt3	A2AM29	10.0	16.2	22.3
Heterochromatin focus				
Trim28	Q62318	10.6	18.7	29.6
Ube2i	P63280	3.2	3.2	3.2
Cbx5	Q61686	5.3	7.4	11.0
Cbx1	P83917	5.8	7.2	9.9
Hmga2	P52927	9.3	9.3	9.6
Mecp2	Q9Z2D6	9.9	16.7	21.9
Kmt5c	Q6Q783	6.5	10.8	20.7
Suv39h1	O54864	5.5	5.5	5.5
Polycomb body				
Sumo3	Q9Z172	3.7	3.7	3.7
Ube2i	P63280	3.2	3.2	3.2
Cbx4	O55187	9.7	17.2	23.5
Phc1	Q64028	11.2	23.9	32.8
Cbx2	P30658	9.9	16.6	22.8

Table S4. Sizes of candidate LLPS scaffold proteins in nucleoli, transcriptional condensates, heterochromatin foci and Polycomb bodies. The predicted sizes in the third column were taken from AlphaFold [60], the sizes for more expanded conformations in the other columns are based on the scaling relationships determined in [97]. See **Methods** section for details.