Supplementary Information

This PDF file includes:
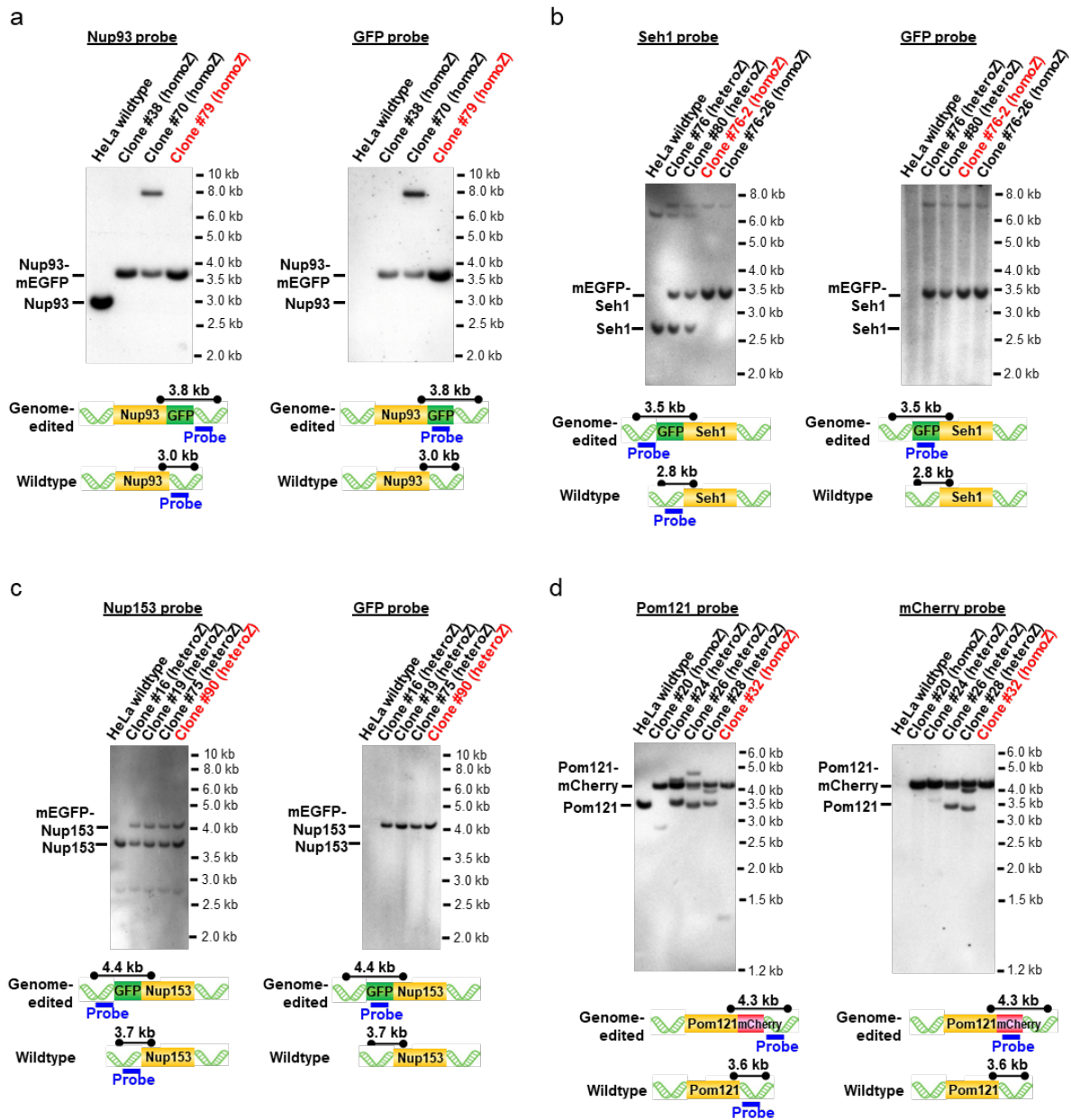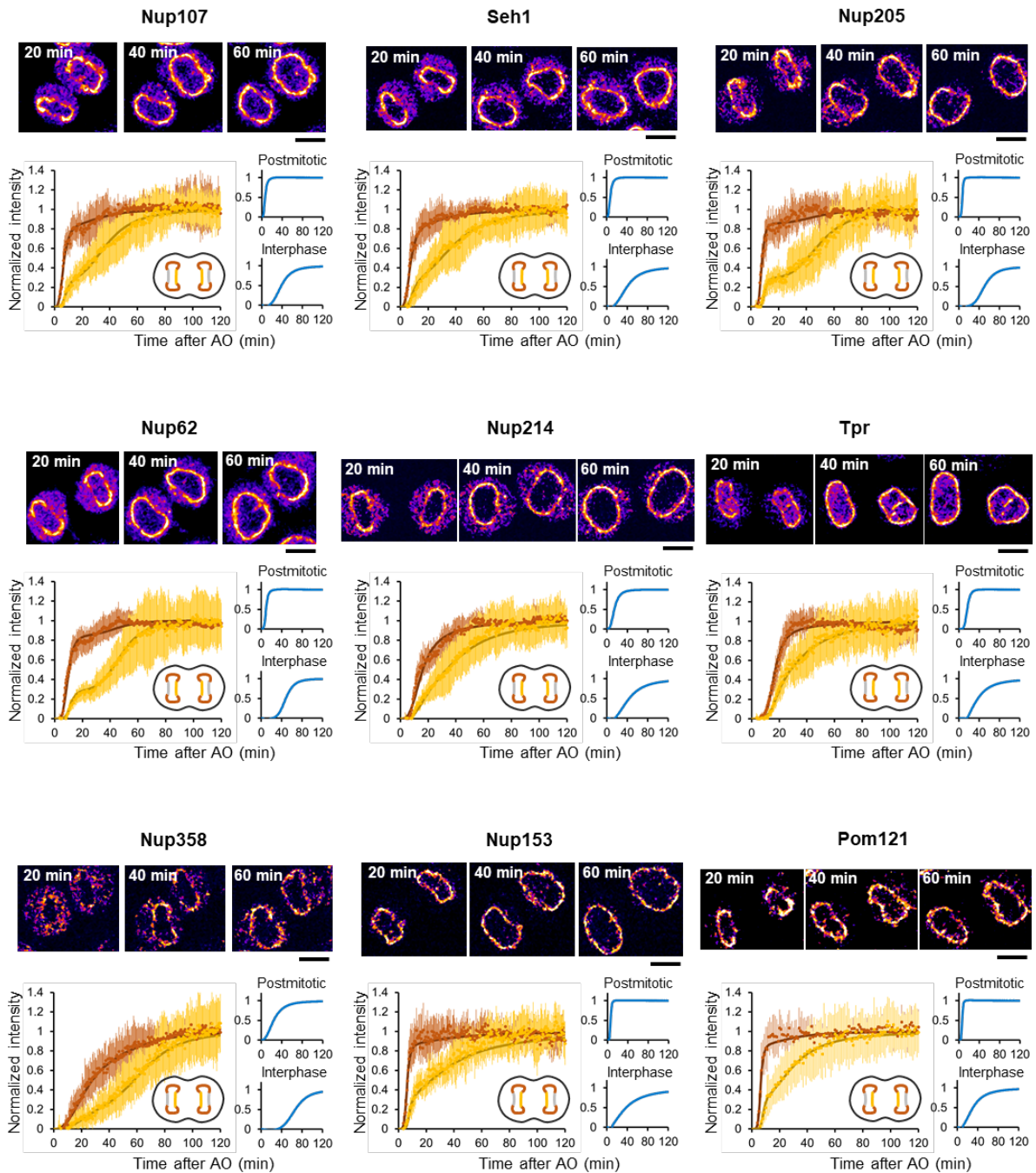
Extended Data Fig. 1 | Southern blotting of genome-edited cell clones.
Genomic DNA of each cell clone was digested with restriction enzymes and the fragments were detected by probes for Nup93 (**a**), Seh1 (**b**), Nup153 (**c**), and Pom121 (**d**), as well as GFP (**a–c**) and mCherry (**d**). The size of the fragments and the probe-binding regions are illustrated in the bottom panels. The clones indicated in red were used for this study. The following Nup cell lines were validated to be homozygously-tagged in previous reports (Tpr, Nup214 and Nup358[22]; Nup107[12]; Nup205[11]; Nup62[33]).
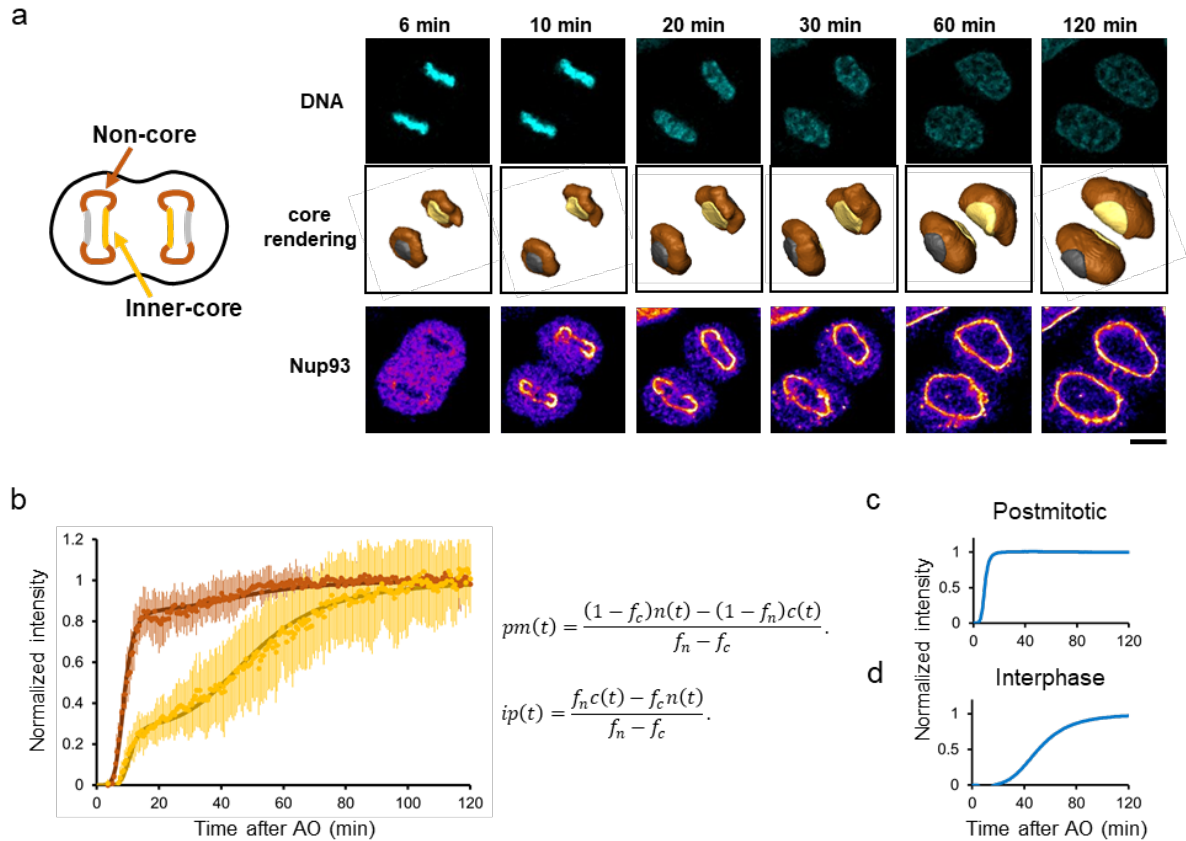
Extended Data Fig. 2 | Postmitotic and interphase assembly are spatially distinguished for the first hour after mitotic exit and thus their kinetics can be decomposed.
**a**, Time-lapse 3D imaging of Nup93-mEGFP genome-edited cell. DNA was stained with silicon–rhodamine (SiR) Hoechst. Single confocal sections of SiR and GFP channels are shown. Images were filtered with a median filter (kernel size: 0.25 × 0.25 μm). Scale bar, 10 μm. Time after AO is indicated. **b**, The fluorescence intensities at non-core (brown) and inner-core (yellow) regions were quantified. Dots represent the average and s.d. of measurements from 14 cells. The intensities were fitted with a sequential model of NPC assembly (bold lines) that allows for different population and rate constants for postmitotic and interphase assembly (described in detail in Methods and Extended Data Table 2). **c**, **d**, Decomposed kinetics of postmitotic (**c**) and interphase (**d**) assembly from (**b**).

The equations shown in panel b:

$$pm(t) = \frac{(1 - f_c)n(t) - (1 - f_n)c(t)}{f_n - f_c}.$$

$$ip(t) = \frac{f_n c(t) - f_c n(t)}{f_n - f_c}.$$

Extended Data Fig. 3 | Kinetic decomposition of the two assembly processes for each nucleoporin.
The fluorescence intensities at non-core (brown) and inner-core (yellow) regions were plotted and fitted with a sequential model of NPC assembly (bold lines) as in Extended Data Fig. 2. The plot is from 15, 20, 13, 14, 22, 22, 19, and 24 cells for Nup107, Seh1, Nup205, Nup93, Nup62, Nup214, Tpr and Nup358, respectively. Single confocal slices of cells at 20 min, 40 min, and 60 min after AO are shown. Images were filtered with a median filter (kernel size: 0.25 × 0.25 μm) for presentation purposes. Scale bars, 10 μm.
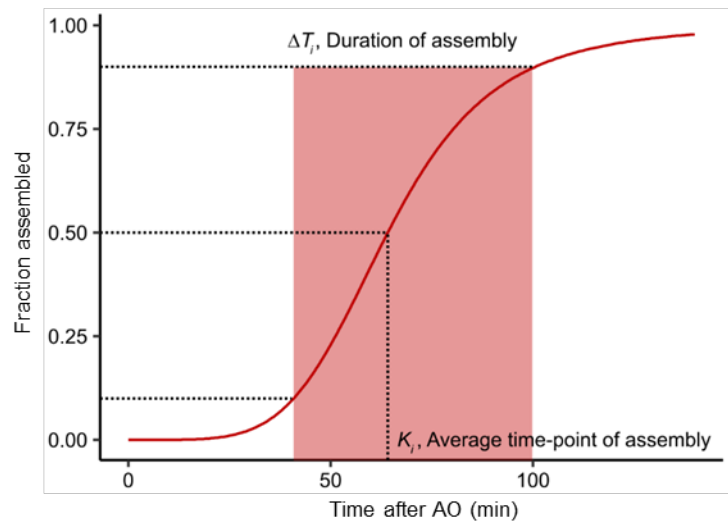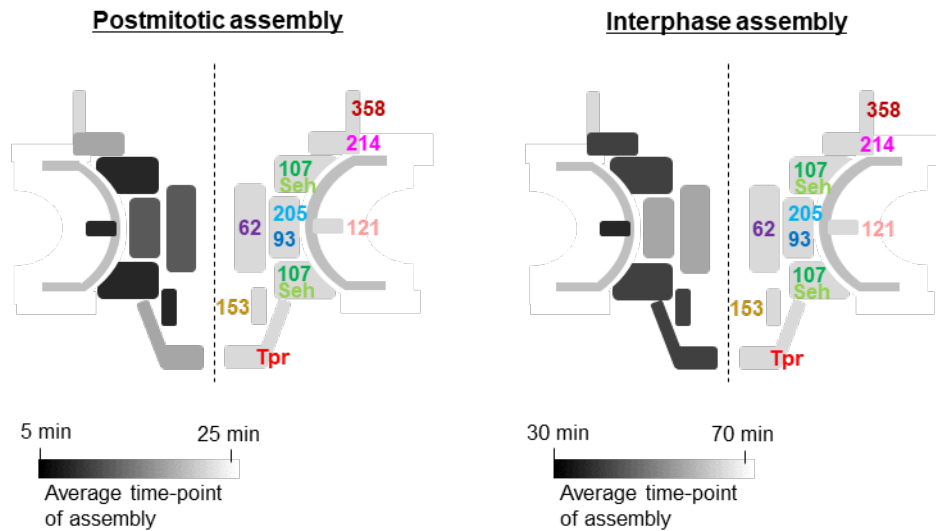
Extended Data Fig. 4 | Computed parameters for the nucleoporin assembly kinetics and illustrations of average time-point of assembly.
a, An example of computed quantities (Nup358, interphase assembly). **b**, Illustrations of average time-point of assembly of nucleoporins in postmitotic (left) and interphase (right) assembly superimposed onto representing NPC structures. The average time-points of assembly of individual nucleoporins are displayed on a pseudo-colour scale.

	Interphase			Metaphase	
	Cytoplasm	Nucleoplasm	Nuclear envelope	Cytosol	Nucleoplasm
Nup107	62 ± 17 (133)	93 ± 20 (133)	650 ± 63 (47)	200 ± 33 (49)	240 ± 36 (12)
Seh1	61 ± 16 (31)	56 ± 14 (32)	560 ± 61 (37)	180 ± 26 (20)	250 ± 28 (12)
Nup205	15 ± 7.9 (34)	23 ± 7.7 (34)	350 ± 54 (41)	90 ± 14 (21)	59 ± 5.1 (10)
Nup93	60 ± 21 (12)	65 ± 12 (12)	860 ± 110 (20)	320 ± 29 (10)	210 ± 11 (8)
Nup62	170 ± 52 (28)	84 ± 27 (28)	1000 ± 210 (41)	400 ± 54 (15)	240 ± 22 (11)
Nup214	34 ± 12 (16)	22 ± 2.8 (16)	450 ± 70 (26)	120 ± 26 (15)	68 ± 7.9 (18)
Tpr	6.2 ± 8.3 (12)	45 ± 18 (12)	420 ± 63 (55)	120 ± 20 (10)	84 ± 19 (9)
Nup358	13 ± 7.5 (6)	9.7 ± 3.4 (6)	390 ± 61 (28)	88 ± 18 (10)	65 ± 5.0 (14)

Extended Data Table 1 | Concentration of nucleoporins (nM). Concentration of mEGFP-tagged Nups in interphase. Data represent mean ± S.D. from the number of cells indicated in parentheses.

Nup	Postmitotic assembly			Interphase assembly			Fraction post-mitotic*	
	n_p	K_p (min)	ΔT_p (min)	n_i	K_i (min)	ΔT_i (min)	f_n, non-core	f_c, core
Nup107	3.38 [2.67, 4.42]	6.51 [5.95, 7.13]	9.1 [6.69, 11.99]	3.48 [2.72, 4.56]	36.17 [32.87, 39.7]	48.72 [36.73, 65.61]		
Seh1	3.3 [2.67, 4.21]	7.25 [6.61, 7.98]	10.39 [7.77, 13.54]	2.78 [2.16, 3.58]	37.36 [33.45, 41.83]	65.41 [48.96, 92.83]		
Nup205	6.73 [5.11, 9.4]	7.91 [7.41, 8.51]	5.25 [3.62, 7.3]	4.3 [3.33, 5.78]	47.73 [43.8, 52.16]	50.93 [37.11, 69.41]		
Nup93	5.71 [4.84, 6.96]	8.84 [8.4, 9.31]	6.97 [5.59, 8.46]	3.94 [3.19, 4.97]	48.66 [45.42, 52.37]	57.16 [44.15, 74.26]		
Nup62	4.71 [4.03, 5.6]	9.6 [9.11, 10.14]	9.29 [7.56, 11.27]	5.53 [4.31, 7.51]	45.8 [42.85, 49.39]	37.34 [26.53, 50.54]	0.857 [0.76, 0.95]	0.295 [0.17, 0.4]
Nup214	3.13 [2.79, 3.6]	14.83 [13.61, 16.08]	22.54 [18.24, 26.91]	2.61 [2.05, 3.66]	41.12 [36.32, 47.71]	77.68 [52.38, 115.29]		
Tpr	5.35 [4.64, 6.29]	16.37 [15.66, 17.15]	13.83 [11.58, 16.3]	2.56 [2.05, 3.22]	34.88 [30.66, 41.46]	67.46 [48.48, 102.15]		
Nup358	2.54 [2.17, 3]	24.55 [22.6, 26.78]	48.02 [38.54, 60.61]	4.88 [3.63, 6.7]	64.13 [59.28, 70.9]	59.76 [42.21, 87.08]		
Nup153	5.67 [3.97, 9.14]	6.15 [5.64, 6.74]	4.89 [2.97, 7.23]	1.83 [1.45, 2.32]	35.27 [30.47, 44.88]	106.24 [71.42, 183.97]		
Pom121	6.38 [3.89, 17.09]	6.39 [5.48, 7.46]	4.49 [1.63, 7.65]	2.41 [1.64, 3.65]	28.25 [23.19, 36.07]	58.98 [35.46, 114.84]		

Extended Data Table 2 | Parameter of the mathematical model. The number in brackets represents the 95% confidence interval as estimated using profile likelihood. *The fraction of postmitotic assembly in the noncore and core region represent the average for all Nups. See Methods. The assembly in the core region is delayed by $d = 2$ min.

Gene	Sequences
Nup153	AGGAGGCCGAGGGCTCCGGT
	CGGACGCGGCGTTGCCACCA
Nup93	TGTCAGTACATCAGGCACAT
	AGCATGGCACTTAATTCATG
Pom121	GGACAAAGGCTACTTTTTGC
	CCTTCCCTAAATCTGGACCT
Seh1	CCACAAACATGGTTTCCGTC
	CAGCATCGCGGCGGACCACA

Extended Data Table 3 | gRNA sequences used for genome-editing. The first sequence is the antisense gRNA-binding site, the second one is the sense gRNA-binding site.