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Supplemental information

**The TFAM-to-mtDNA ratio defines inner-cellular
nucleoid populations with distinct activity levels**

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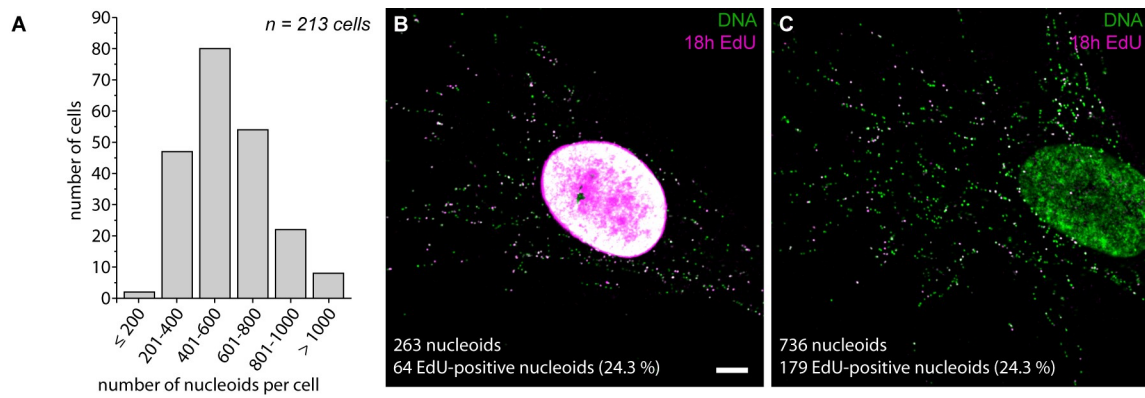


Figure S1: Variation of the nucleoid number between single cells. Related to Figure 1. **(A)** STED super-resolution microscopy reveals that the number of nucleoids in single cells have a wide range from below 200 to over 1000 nucleoids per cell. **(B, C)** HDFa cells after 18 h of EdU incubation. B shows a cell that went through the S-Phase of the cell cycle as it has incorporated EdU in the nucleus. C shows a cell without an EdU signal in the nucleus. Although both cells are in different states of the cell cycle and have a different number of total nucleoids, the proportion of replication active, EdU-positive nucleoids is identical. Scale bar, 2 μm .

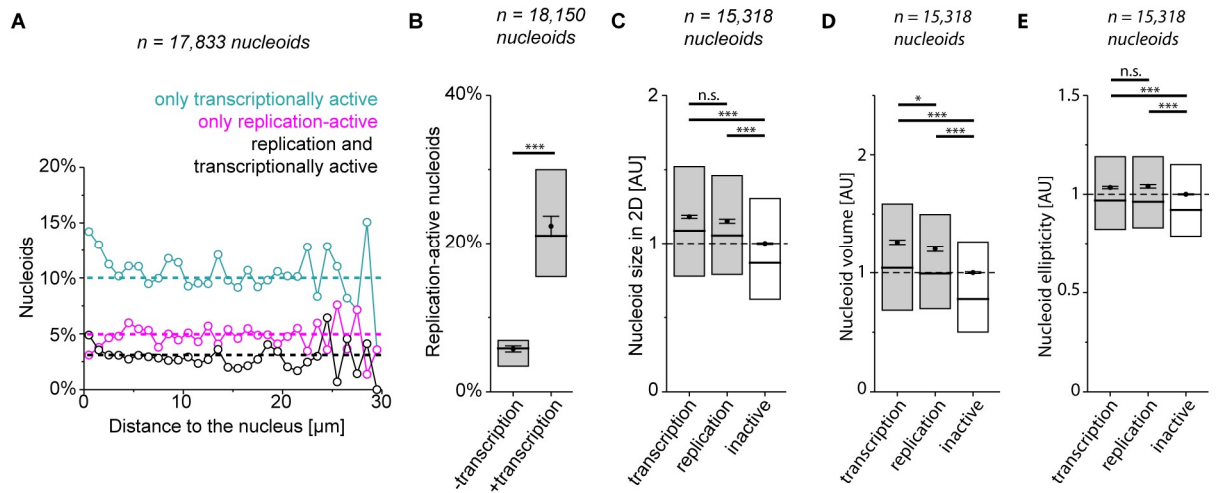


Figure S2: Properties of replication and transcriptionally active nucleoids. Related to Figure 2. **(A)** The activity of nucleoids is independent from their distance to the nucleus. Cells were incubated with EdU (70 min) and BrU (25 min). After fixation and immunodecoration, the closest distance of the nucleoids of 51 cells to the surface of the respective nucleus was determined together with the activity status of the individual nucleoid (replication, magenta; transcription, cyan; involved in both processes, black). All analysis was performed on STED images. **(B)** A transcriptionally active nucleoid has a higher probability to be also engaged in replication than a transcriptionally inactive nucleoid. **(C)** Replication and transcriptionally active nucleoids cover a larger area in 2D STED recordings than inactive nucleoids. **(D)** Transcriptionally and replication active nucleoids occupy larger volumes. The volumes were calculated based on the assumption that the nucleoids have the shape of prolate spheroids. **(E)** Nucleoids engaged in replication or transcription exhibit a more ellipsoid shape than inactive nucleoids. Boxplots show the inter-quartile range (25%-75%), the horizontal line represents the median, the dot represents the mean value and error bars indicate the standard error (B-E). Statistical significance was determined using two-tailed Student's t-test; n.s. $P > 0.05$, $*P \leq 0.05$, $***P \leq 0.001$. The exact P-values are given in Table S3 (B-E).

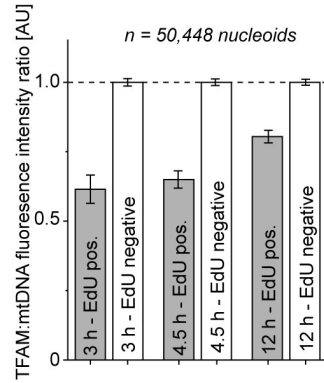


Figure S3: Replication-active (EdU-positive) nucleoids exhibit a reduced TFAM-to-mtDNA ratio. Related to Figure 3. Fibroblasts were incubated for different times with EdU, as indicated. Subsequently, the TFAM:mtDNA ratio was determined based on the fluorescence intensity in STED images. Data are represented as the mean and error bars indicate the standard error.

	Model B – Independency model		Model C – Inactivation model	
	Best fit	95 % Range	Best fit	95 % Range
Fast duplicating (active) population: <i>size</i>	21 %	15-27 %	50 %	45- 58 %
<i>duplication rate</i> (per nucleoid, per day)	0.95	0.72-1.47	0.51	0.40-0.68
<i>degradation rate</i> (per nucleoid, per day)	0.85	0.62-1.37	0.31	0.21-0.45
Slow duplicating (inactive) population: <i>size</i>	79 %	73-85 %	50 %	42-55 %
<i>duplication rate</i> (per nucleoid, per day)	0.10	0.10-0.10	0	
<i>degradation rate</i> (per nucleoid, per day)	0.00	0.00-0.00	0	
Transfer from fast to slow (per nucleoid, per day)	0		0.10	0.08-0.15
Net growth rate of entire nucleoid population per day (7 days doubling time)	0.10			
Time until 50 % of the fast duplicating (active) population is EdU-labeled	0.36 days	0.24-0.48 days	0.68 days	0.51-0.88 days
Time until 90 % of the fast duplicating (active) population is EdU-labeled	1.21 days	0.78-1.59 days	2.25 days	1.70-2.91 days
Time until 50 % of the slow duplicating (inactive) population is EdU-labeled	3.50 days	3.50 – 3.50 days	8.03 days	7.10-8.33 days
Time until 90 % of the slow duplicating (inactive) population is EdU-labeled	11.63 days	11.63 days	24.29 days	23.36-24.59 days
Time until 50 % of all nucleoids are EdU- labeled	2.33 days	2.03-2.68 days	2.19 days	1.91-2.67 days
Time until 90 % of all nucleoids are EdU- labeled	10.43 days	10.01-10.80 days	17.40 days	16.57-18.61 days

Table S1: Parameters and outcomes of modeling of the duplication activity.

Related to Figure 1. Color highlighted values were fixed in the respective model describing the nucleoid population dynamics.

	TFAM fluorescence signal intensity active nucleoids (photon counts)	TFAM fluorescence signal intensity inactive nucleoids (photon counts)	TFAM fluorescence signal intensity ratio active:inactive	DNA fluorescence signal intensity active nucleoids (photon counts)	DNA fluorescence signal intensity inactive nucleoids (photon counts)	DNA fluorescence signal intensity ratio active:inactive	TFAM:DNA ratio active:inactive
EdU 1.5 h	241.65	288.09	0.84	506.80	483.07	1.05	0.80
EdU 3 h	122.62	155.41	0.79	615.52	479.84	1.28	0.62
EdU 4.5 h	149.64	192.98	0.78	639.60	536.09	1.19	0.65
EdU 12 h	174.64	224.82	0.78	457.18	473.46	0.97	0.80
EdU 24 h	294.42	390.57	0.75	578.62	558.16	1.04	0.73
BrU 50 min	790.50	711.27	1.11	995.06	843.33	1.18	0.94

Table S2: Raw Values of the TFAM and DNA fluorescence intensity of active and inactive nucleoids.

Related to Figure 3. Given are averaged accumulated photon counts of individual nucleoids recorded by STED microscopy.

Figure	Compared datasets	p-value of the t-test
Figure 2H	Measured and calculated values of nucleoids involved in replication and transcription	3.70×10^{-8}
Figure 2I	Transcriptionally active nucleoids with and without replication activity	3.51×10^{-13}
Figure 2J	DNA fluorescence intensity of transcriptionally active and replication-active nucleoids	4.01×10^{-6}
	DNA fluorescence intensity of transcriptionally active and inactive nucleoids	3.10×10^{-91}
	DNA fluorescence intensity of replication-active and -inactive nucleoids	2.33×10^{-23}
Figure S2B	Replication-active nucleoids with and without transcription activity	4.80×10^{-17}
Figure S2C	Size in 2D of transcriptionally active and replication-active nucleoids	8.38×10^{-2}
	Size in 2D of transcriptionally active and inactive nucleoids	3.21×10^{-52}
	Size in 2D of replication-active and -inactive nucleoids	8.58×10^{-25}
Figure S2D	Calculated size in 3D of transcriptionally active and replication-active nucleoids	4.34×10^{-2}
	Calculated size in 3D of transcriptionally active and inactive nucleoids	3.41×10^{-40}
	Calculated size in 3D of replication-active and -inactive nucleoids	6.84×10^{-18}
Figure S2E	Ellipticity of transcriptionally active and replication-active nucleoids	0.58
	Ellipticity of transcriptionally active and inactive nucleoids	1.11×10^{-7}
	Ellipticity of replication-active and -inactive nucleoids	1.17×10^{-5}

Table S3: p-values of all performed t-tests.

Related to Figure 2 and Figure S2. All t-tests were two-tailed and heteroscedasticity was assumed.