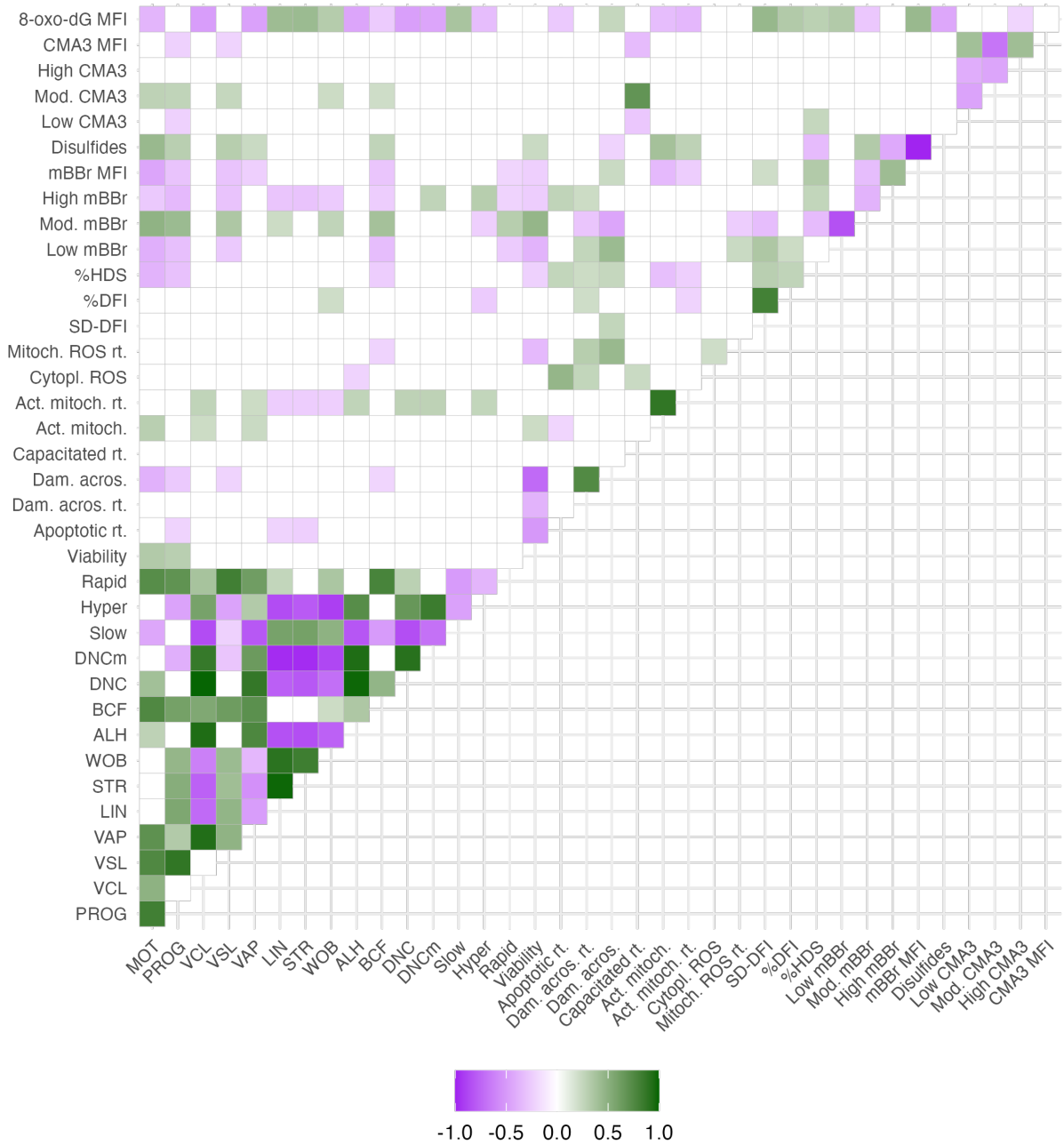

Figure S1. Correlation analysis for all the variables at day 0 of the experiment. The color gradient shows the r value. Correlations with P>0.05 (unadjusted) were removed (blank spaces). Variable names and standard abbreviations are explained in the text (rt.: ratio).

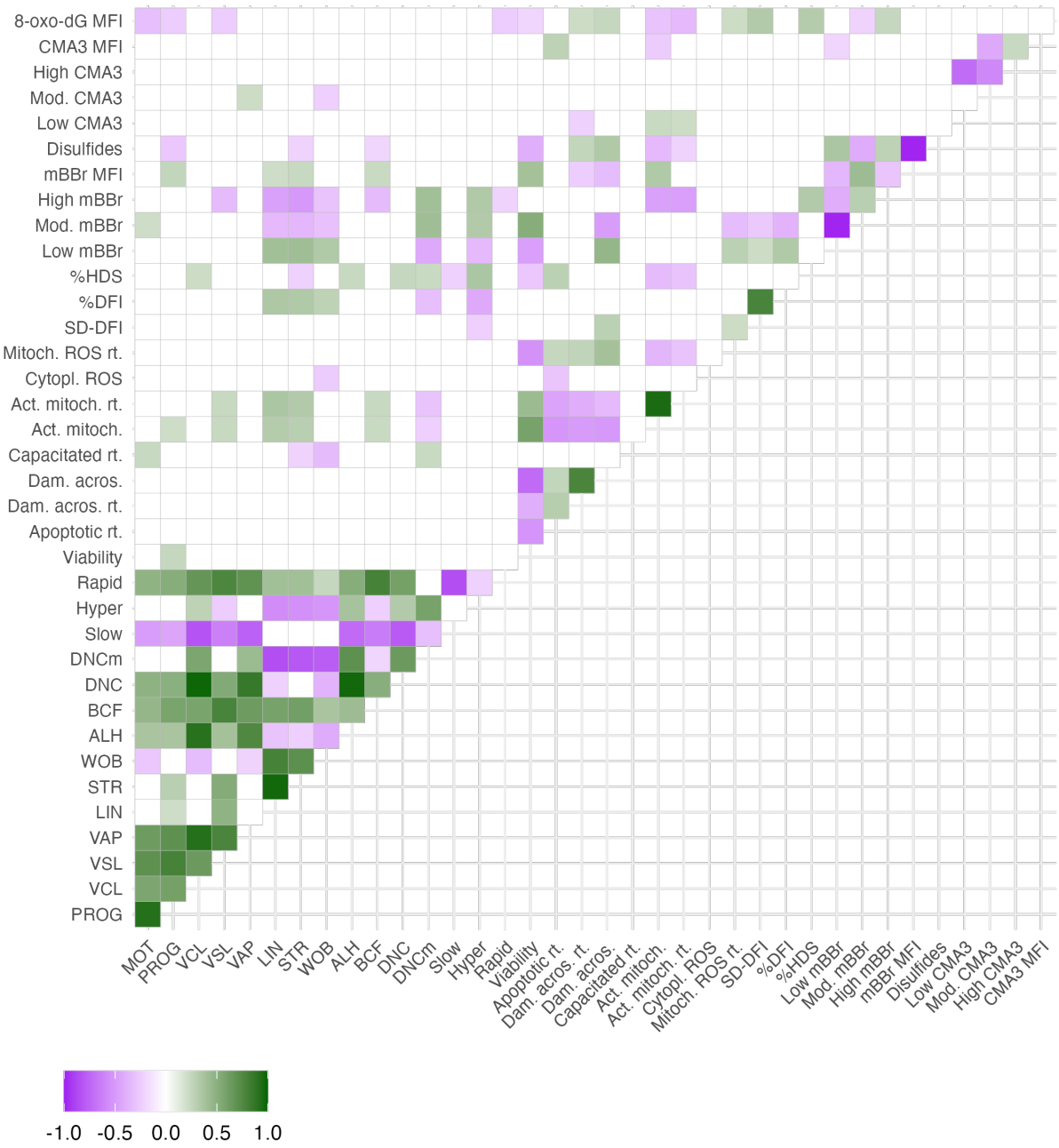

Figure S2. Correlation analysis for all the variables on day 11 of the experiment. The color gradient shows the r value. Correlations with P>0.05 (unadjusted) were removed (blank spaces). Variable names and standard abbreviations are explained in the text (rt.: ratio).

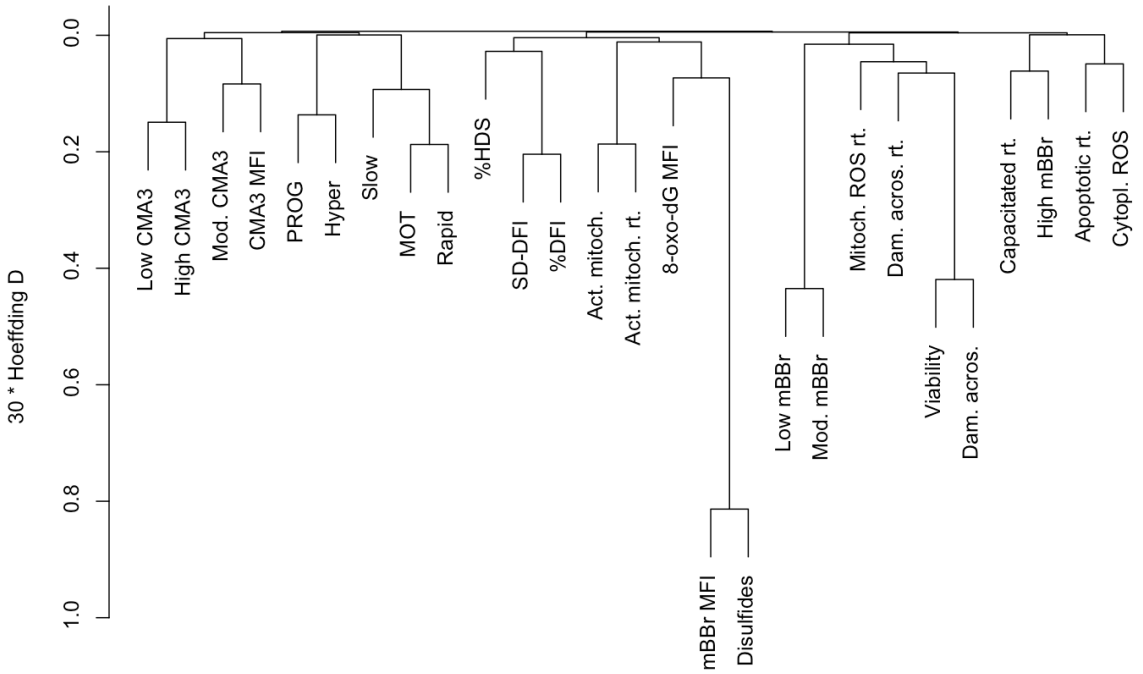

Figure S3. Hierarchical clustering of all the variables at day 0 of the experiment using the Hoeffding D statistic (times 30). Variables with shorter distances are more related and clustered together.

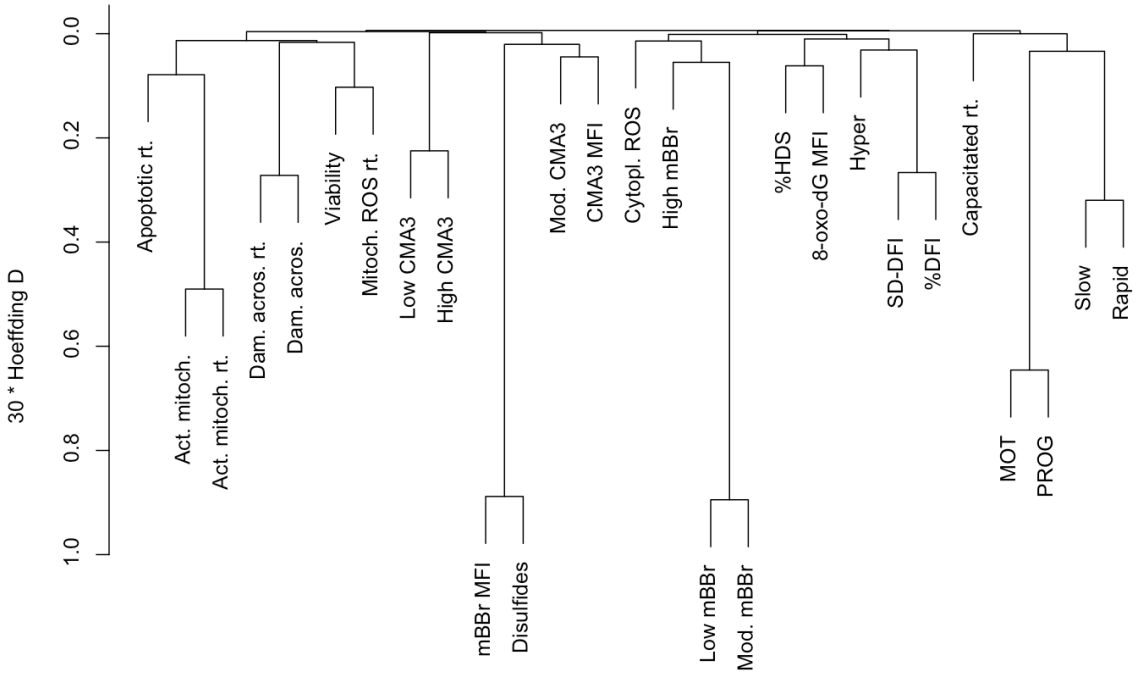

Figure S4. Hierarchical clustering of all the variables on day 11 of the experiment using the Hoeffding D statistic (times 30). Variables with shorter distances are more related and clustered together.

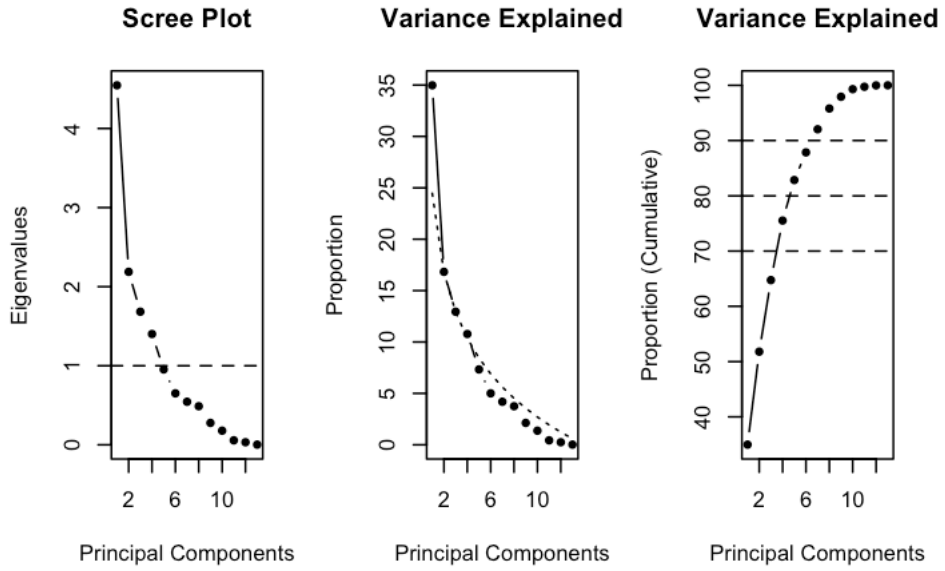

Figure S5. Scree plot for the PCA using the chromatin status variables (see Fig. 3 and Table 3) and proportion of the variance explained by the principal components (individual and cumulative). The Kaiser-Guttman criterion (eigenvalue >1, horizontal line in the scree plot) and the Explained Variance criterion (70% to 80% cumulative) suggest retaining four principal components. In the middle plot (variance explained for the individual variables), the dotted line indicates the expected variance explained; principal components are retained until the variance explained falls below this line.

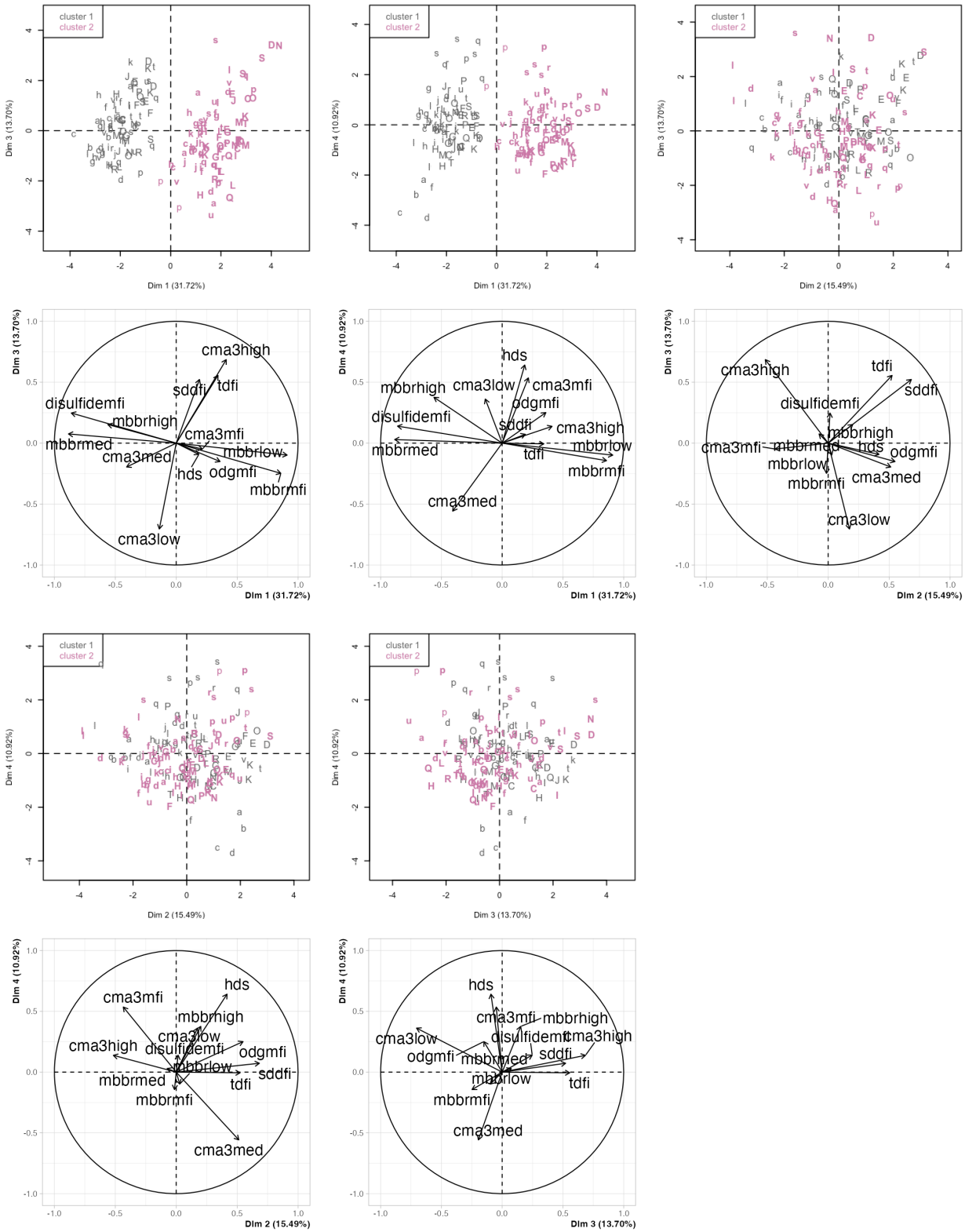

Figure S6. Principal component analysis (PCA) using the chromatin variables (PC1 vs. PC2 displayed in Fig. 3). In each row, the top panels display the observations in the space defined by the respective two principal components (PC, labeled as Dim), identified by boar (letters), day of analysis (regular font for D0 and bold for D11), and cluster (dark grey for cluster 1 and pink for cluster 2; hierarchical clustering on the PCA results). The bottom panel shows the variable loadings for the two first PCs (influence of the original

variables on the PC). Abbreviations are defined as: sddfi: SD-DFI; tdfi: %DFI; hds: %HDS; mbbrlow: Low mBBr; mbbrmed: Moderate mBBr; mbbrhigh: High mBBr; mbbrmfi: mBBr MFI; disulfidemfi: Disulfide levels; cma3low: Low CMA3; cma3med: Moderate CMA3; cma3high: High CMA3; cma3mfi: CMA3 MFI; odgmfi: 8-oxo-dG MFI.

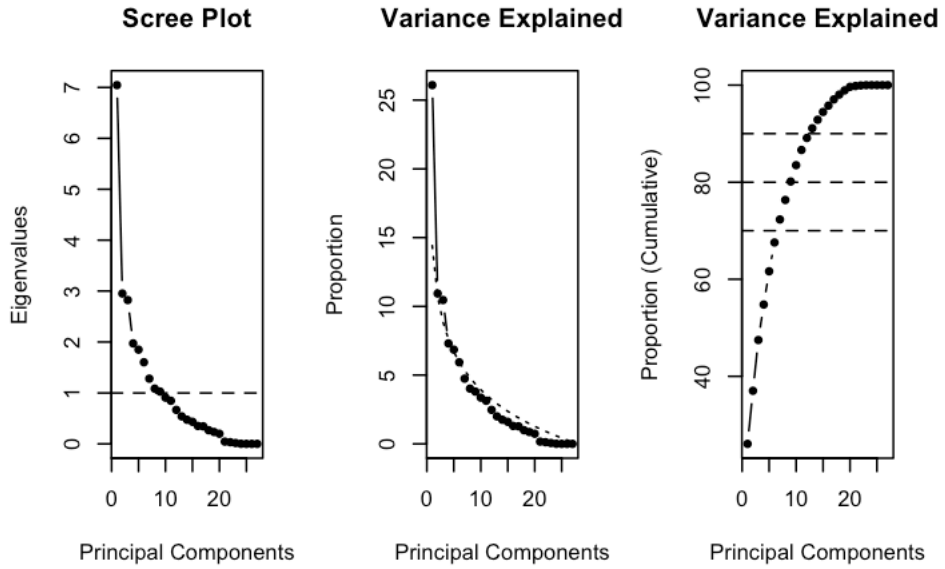

Figure S7. Scree plot for the PCA using the chromatin status variables (see Fig. 3 and Table 3) and proportion of the variance explained by the principal components (individual and cumulative). The Kaiser-Guttman criterion (eigenvalue >1, horizontal line in the scree plot) and the Explained Variance criterion (70% to 80% cumulative) suggest retaining eight principal components. In the middle plot (variance explained for the individual variables), the dotted line indicates the expected variance explained; principal components are retained until the variance explained falls below this line.

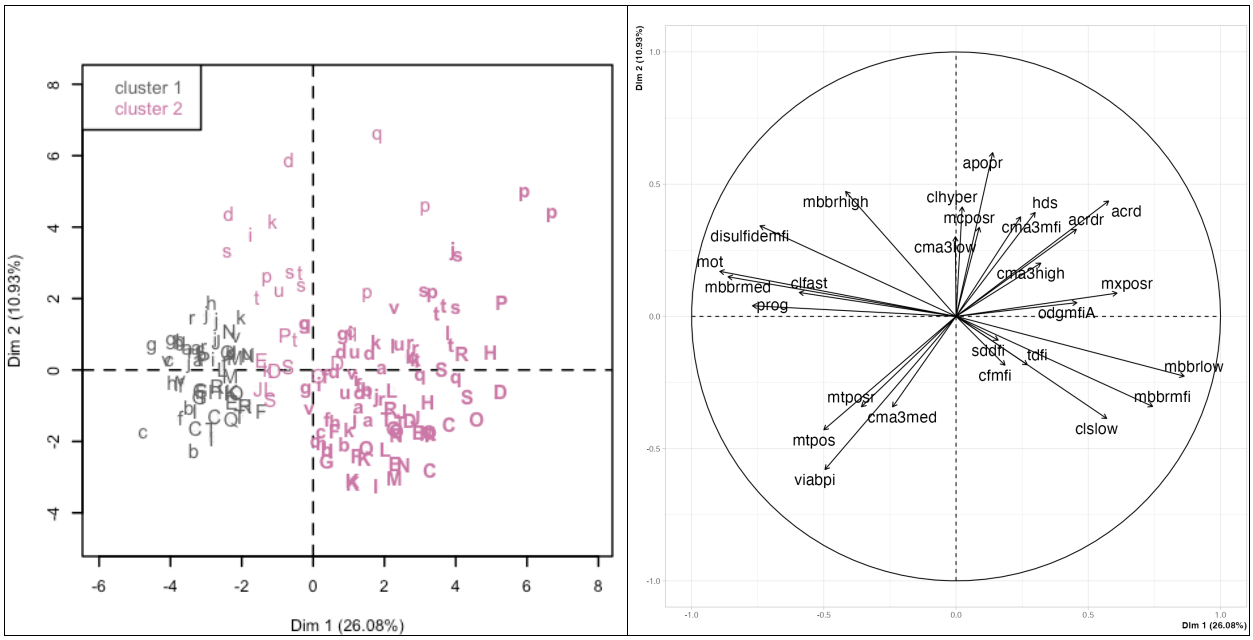

Figure S8. Principal component analysis (PCA) using all the variables in the study. The left panel shows the observations in the space defined by the first two principal components (PC, labeled as Dim 1 and Dim 2), identified by boar (letters), day of analysis (plain for day 0 and bold for day 11), and cluster (dark grey for cluster 1 and pink for cluster 2). The right panel shows the variable loadings for the two first PCs (influence of the original variables on the PC).

mot: Total motility; prog: Progressive motility; clslow: Slow subpopulation; clhyper: Hyperactivated-like subpopulation; clfast: Fast subpopulation; viabpi: Viable (PI^-); apopr: Apoptotic (ratio); acrdr: Acrosomal damage (ratio); acrd: Acrosomal damage (total); mcposr: Capacitated ($M540^+$, ratio); mtpos: Active mitochondria; mtposr: Active mitochondria (ratio); cfmfi: Cytoplasmic ROS (MFI); mxposr: Mitochondrial ROS (ratio); sddfi: SD-DFI; tdfi: %DFI; hds: %HDS; mbbrlow: Low mBBr; mbbrmed: Moderate mBBr; mbbrhigh: High mBBr; mbbrmfi: mBBr MFI; disulfidemfi: Disulfide levels; cma3low: Low CMA3; cma3med: Moderate CMA3; cma3high: High CMA3; cma3mfi: CMA3 MFI; odgmfiA: 8oxodG MFI.

Table S1. Mean ± SEM for the sperm motility and physiology (flow cytometry) parameters for each analysis day and P values for the factor effects (LME analysis with day, boar, and their interaction).

Variable	Day 0	Day 11	Day	Boar	Day×Boar
Total motility (%)	75.51±1.63	25.73±1.62	<0.001	<0.001	<0.001
Progressive motility (%)	38.23±1.52	13.68±1.48	<0.001	<0.001	<0.001
VCL (μm/s)	96.14±2.25	73.37±2.23	<0.001	<0.001	0.118
VSL (μm/s)	23.78±0.60	19.04±0.58	<0.001	<0.001	0.005
VAP (μm/s)	53.73±1.04	37.93±1.02	<0.001	<0.001	0.136
LIN (%)	30.73±1.00	30.47±0.99	0.466	<0.001	0.055
STR (%)	53.21±1.18	57.18±1.16	0.061	<0.001	0.090
WOB (%)	58.02±0.68	54.90±0.67	<0.001	<0.001	0.092
ALH (μm)	2.29±0.05	2.05±0.05	0.091	<0.001	0.055
BCF (Hz)	15.68±0.38	10.76±0.37	<0.001	<0.001	0.023
DNC (μm²/s)	241.42±10.63	157.97±10.53	<0.001	<0.001	0.068
DNCm (μm)	10.45±0.53	7.60±0.52	0.561	<0.001	0.129
Slow (%)	30.66±1.49	48.20±1.46	<0.001	<0.001	0.024
Fast (%)	49.94±1.59	34.43±1.55	<0.001	<0.001	0.009
Hyperactivated (%)	19.40±1.22	17.37±1.22	0.098	<0.001	0.060
Viability (%)	84.02±0.66	80.36±0.65	<0.001	<0.001	0.002
Apoptotic, ratio (%)	1.82±0.16	1.84±0.16	0.003	<0.001	0.217
Damaged acrosomes, ratio (%)	3.17±0.50	6.36±0.49	0.003	0.193	0.655
Damaged acrosomes (%)	14.36±0.67	19.35±0.66	<0.001	<0.001	0.869
Capacitated, ratio (%)	0.76±0.10	1.07±0.09	0.009	0.704	0.946
Active mitochondria (%)	78.28±1.71	67.46±1.69	<0.001	<0.001	<0.001
Active mitochondria, ratio (%)	89.37±1.98	81.55±1.96	<0.001	<0.001	<0.001
Cytoplasmic ROS (MFI)	5.94±0.74	8.16±0.70	0.102	0.482	0.999
Mitochondrial ROS, ratio (%)	3.49±0.39	7.91±0.39	<0.001	<0.001	0.006

VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; LIN, linearity of the curvilinear trajectory (ratio of VSL/VCL); STR, straightness; ALH, amplitude of lateral head displacement; WOB, wobble coefficient; BCF, beat cross-frequency; DNC, sperm dance; DNCm, sperm mean dance.

Table S2. Type III Analysis of Variance Table with Satterthwaite's method from the analysis of the motility and sperm physiology variables.

Total motility

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	21724.66268	21724.66268	1	107.20	771.11	<0.001
Boar	6865.57649	196.15933	35	107.10	6.96	<0.001
Day×Boar	3105.62874	88.73225	35	107.20	3.15	<0.001

Progressive motility

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	95.07067	95.07067	1	107.24	242.90	<0.001
Boar	69.99155	1.99976	35	107.12	5.11	<0.001
Day×Boar	38.65270	1.10436	35	107.24	2.82	<0.001

VCL

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.15294	0.15294	1	107.44	36.13	<0.001
Boar	0.71265	0.02036	35	107.19	4.81	<0.001
Day×Boar	0.20151	0.00576	35	107.44	1.36	0.118

VSL

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	58.57535	58.57535	1	107.31	46.72	<0.001
Boar	247.65126	7.07575	35	107.09	5.64	<0.001
Day×Boar	84.67760	2.41936	35	107.31	1.93	0.005

VAP

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.30957	0.30957	1	107.26	109.70	<0.001
Boar	0.56329	0.01609	35	107.20	5.70	<0.001
Day×Boar	0.13118	0.00375	35	107.26	1.33	0.136

LIN

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.05939	0.05939	1	107.61	0.54	0.466
Boar	27.56539	0.78758	35	107.34	7.11	<0.001
Day×Boar	5.87445	0.16784	35	107.61	1.51	0.055

STR

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	21228.09813	21228.09813	1	109.00	3.57	0.061
Boar	1471018.31710	42029.09477	35	109.00	7.07	<0.001
Day×Boar	294275.66274	8407.87608	35	109.00	1.42	0.090

WOB

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.00003	0.00003	1	107.43	13.73	<0.001
Boar	0.00055	0.00002	35	107.23	6.21	<0.001
Day×Boar	0.00013	0.00000	35	107.43	1.41	0.092

ALH

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.05884	0.05884	1	109.00	2.92	0.091
Boar	2.99391	0.08554	35	109.00	4.24	<0.001
Day×Boar	1.06928	0.03055	35	109.00	1.51	0.055

BCF

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	69.81130	69.81130	1	109.00	86.96	<0.001
Boar	119.40348	3.41153	35	109.00	4.25	<0.001
Day×Boar	47.18470	1.34813	35	109.00	1.68	0.023

DNC

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.12467	0.12467	1	107.59	15.05	<0.001
Boar	1.31753	0.03764	35	107.32	4.54	<0.001
Day×Boar	0.42677	0.01219	35	107.59	1.47	0.068

DNCm

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.02026	0.02026	1	107.62	0.34	0.561
Boar	12.73486	0.36385	35	107.35	6.11	<0.001
Day×Boar	2.79202	0.07977	35	107.62	1.34	0.129

Slow

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	268.40612	268.40612	1	109.00	77.52	<0.001
Boar	559.22535	15.97787	35	109.00	4.61	<0.001
Day×Boar	201.90437	5.76870	35	109.00	1.67	0.024

Fast

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	1417.25341	1417.25341	1	109.00	62.43	<0.001
Boar	3693.94122	105.54118	35	109.00	4.65	<0.001
Day×Boar	1462.76011	41.79315	35	109.00	1.84	0.009

Hyperactivated

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	9.85061	9.85061	1	107.34	2.78	0.098
Boar	411.72853	11.76367	35	107.24	3.32	<0.001
Day×Boar	185.61660	5.30333	35	107.34	1.50	0.060

Viability

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	5453470.89571	5453470.89571	1	107.23	31.16	<0.001
Boar	50758072.03956	1450230.62970	35	107.12	8.29	<0.001
Day×Boar	12626053.92236	360744.39778	35	107.23	2.06	0.002

Apoptotic (ratio)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	3.07432	3.07432	1	109.00	9.40	0.003
Boar	39.06730	1.11621	35	109.00	3.41	<0.001
Day×Boar	13.97482	0.39928	35	109.00	1.22	0.217

Damaged acrosomes (ratio)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	5.10383	5.10383	1	106.12	9.36	0.003
Boar	23.85832	0.68167	35	105.75	1.25	0.193
Day×Boar	16.84606	0.48132	35	106.12	0.88	0.655

Damaged acrosomes

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	5.53396	5.53396	1	106.79	24.66	<0.001
Boar	28.56622	0.81618	35	106.79	3.64	<0.001
Day×Boar	5.62702	0.16077	35	106.79	0.72	0.869

Capacitated (ratio)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	687.59179	687.59179	1	108.00	6.98	0.009
Boar	2928.57239	83.67350	35	108.00	0.85	0.704
Day×Boar	2141.70697	61.19163	35	108.00	0.62	0.946

Active mitochondria

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	21079516.13664	21079516.13664	1	106.54	48.18	<0.001
Boar	114757354.57737	3278781.55935	35	106.08	7.49	<0.001
Day×Boar	49435213.33291	1412434.66665	35	106.54	3.23	<0.001

Active mitochondria (ratio)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	11518785.46115	11518785.46115	1	109.00	16.07	<0.001
Boar	133070655.50010	3802018.72857	35	109.00	5.30	<0.001
Day×Boar	96457218.10832	2755920.51738	35	109.00	3.84	<0.001

Median of CM-H_2DCFDA fluorescence (cytoplasmic ROS)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.00901	0.00901	1	106.92	2.72	0.102
Boar	0.11587	0.00331	35	106.55	1.00	0.482
Day×Boar	0.04553	0.00130	35	106.92	0.39	0.999

Mitochondrial ROS (ratio)

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	14.33921	14.33921	1	107.20	129.68	<0.001
Boar	11.73570	0.33531	35	107.10	3.03	<0.001
Day×Boar	7.35844	0.21024	35	107.20	1.90	0.006

Table S3. Type III Analysis of Variance Table with Satterthwaite's method from the analysis of the sperm chromatin status variables.

SD-DFI

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.00000	0.00000	1	109.00	0.00	0.997
Boar	0.02764	0.00079	35	109.00	4.15	<0.001
Day×Boar	0.00662	0.00019	35	109.00	0.99	0.490

%DFI

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	2.77196	2.77196	1	106.80	9.33	0.003
Boar	41.36496	1.18186	35	106.73	3.98	<0.001
Day×Boar	10.49102	0.29974	35	106.80	1.01	0.468

%HDS

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.15137	0.15137	1	109.00	2.30	0.132
Boar	12.73631	0.36389	35	109.00	5.53	<0.001
Day×Boar	1.65134	0.04718	35	109.00	0.72	0.869

Low mBBr fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	321.06583	321.06583	1	109.00	1940.57	<0.001
Boar	49.88082	1.42517	35	109.00	8.61	<0.001
Day×Boar	27.81707	0.79477	35	109.00	4.80	<0.001

Moderate mBBr fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	148946833.94327	148946833.94327	1	109.00	1185.85	<0.001
Boar	23805819.55299	680166.27294	35	109.00	5.42	<0.001
Day×Boar	14144236.32929	404121.03798	35	109.00	3.22	<0.001

High mBBr fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	66.30380	66.30380	1	107.17	673.85	<0.001
Boar	22.42663	0.64076	35	106.80	6.51	<0.001
Day×Boar	6.68840	0.19110	35	107.17	1.94	0.005

Median mBBr fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	1.83180	1.83180	1	107.42	1210.43	<0.001
Boar	0.39361	0.01125	35	107.23	7.43	<0.001
Day×Boar	0.15850	0.00453	35	107.42	2.99	<0.001

Disulfide levels, fluor. median

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	4595001.72693	4595001.72693	1	107.39	1273.00	<0.001
Boar	938206.51839	26805.90053	35	107.23	7.43	<0.001
Day×Boar	393602.37077	11245.78202	35	107.39	3.12	<0.001

Low CMA3 fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.00209	0.00209	1	109.00	0.62	0.431
Boar	0.10437	0.00298	35	109.00	0.89	0.643
Day×Boar	0.12533	0.00358	35	109.00	1.07	0.385

Moderate CMA3 fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	224973.46298	224973.46298	1	106.69	4.33	0.040
Boar	1584304.36958	45265.83913	35	106.43	0.87	0.674
Day×Boar	1990994.15434	56885.54727	35	106.69	1.09	0.355

High CMA3 fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.02515	0.02515	1	106.95	19.23	<0.001
Boar	0.05213	0.00149	35	106.85	1.14	0.301
Day×Boar	0.04803	0.00137	35	106.95	1.05	0.412

Median CMA3 fluor.

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.01125	0.01125	1	106.94	10.43	0.002
Boar	0.03740	0.00107	35	106.99	0.99	0.495
Day×Boar	0.02613	0.00075	35	106.94	0.69	0.893

8-oxo-dG MFI A

	Sum Sq.	Mean Sq.	Num. d.f.	Den. d.f.	F value	Pr(<F)
Day	0.01914	0.01914	1	107.63	15.41	<0.001
Boar	0.90970	0.02599	35	107.36	20.93	<0.001
Day×Boar	0.13873	0.00396	35	107.63	3.19	<0.001

Table S4. Loadings and contributions of each starting variable on the two principal components computed from the PCA. The loadings correspond to the correlations of variables and components. The contributions represent the importance of each variable for each component.

Variable	Loadings		Contributions	
	PC1	PC2	PC1	PC2
Total motility	-0.893	0.170	11.33	0.98
Progressive motility	-0.770	0.040	8.42	0.05
Slow	0.570	-0.386	4.61	5.05
Fast	0.023	0.414	0.01	5.80
Hyperactivated	-0.593	0.090	4.99	0.28
Viability	-0.496	-0.579	3.49	11.36
Apoptotic (ratio)	0.139	0.619	0.27	12.99
Damaged acrosomes (ratio)	0.455	0.329	2.94	3.67
Damaged acrosomes	0.577	0.436	4.73	6.45
Capacitated (ratio)	0.088	0.337	0.11	3.84
Active mitochondria	-0.500	-0.429	3.55	6.23
Active mitochondria (ratio)	-0.358	-0.342	1.82	3.96
Cytoplasmic ROS	0.185	-0.185	0.49	1.15
Mitochondrial ROS (ratio)	0.609	0.088	5.26	0.26
SD-DFI	0.159	-0.090	0.36	0.27
%DFI	0.270	-0.183	1.03	1.13
%HDS	0.299	0.393	1.27	5.23
Low mBBr fluor.	0.863	-0.226	10.57	1.73
Moderate mBBr fluor.	-0.862	0.150	10.55	0.76
High mBBr fluor.	-0.418	0.472	2.48	7.56
mBBr MFI	0.743	-0.341	7.85	3.93
Disulfide levels	-0.741	0.342	7.80	3.97
Low CMA3 fluor.	-0.003	0.301	0.00	3.07
Moderate CMA3 fluor.	-0.240	-0.342	0.82	3.97
High CMA3 fluor.	0.321	0.202	1.46	1.38
CMA3 MFI	0.244	0.376	0.85	4.80
8-oxo-dG MFI	0.457	0.052	2.96	0.09

VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; LIN, linearity of the curvilinear trajectory (ratio of VSL/VCL); STR, straightness; ALH, amplitude of lateral head displacement; WOB, wobble coefficient; BCF, beat cross-frequency; DNC, sperm dance; DNCm, sperm mean dance; SD-DFI, the standard deviation of the DFI (DNA fragmentation index); %DFI, DNA fragmentation; HDS, chromatin immaturity; mBBr, monobromobimane; CMA3, chromomycin A; MFI: Median fluorescent intensity for the fluorescent label.