Supplemental Materials and Methods

Yeast strains and growth conditions

All strains were derived from W303 and Anchor-Away genetic backgrounds (see Supplemental Table S2) (Haruki et al. 2008). Cells were grown in YEPD medium (1% yeast extract, 1% peptone) supplemented with 2% glucose as carbon source**.** The BrdU incorporation cassette was introduced according to (Viggiani and Aparicio 2006). Anchor-away of Nrd1-AA*,* Nrd1-AA *set2Δ,* Rrp6-AA or Ysh1-AA strains was induced by adding 1µg/ml of rapamycin to the medium.

G1-phase synchronization and BrdU labeling

Cells grown at 30°C to an OD_{600}= 0.4 were synchronized in G1-phase with α-factor (20 ng/ml, Sigma) for 3h in total. After two washes with distilled water, cells were released into S-phase at 18°C. Depending on the experiment, cells were released in YEPD medium containing BrdU (100 µg/ml, Sigma) and treated or not with rapamycin. Cells were then collected at different times after G1-release and treated with 0.1% Sodium Azide (Sigma). Flow cytometry was performed on ethanol-fixed cells using propidium iodide (Sigma). Flow cytometry profiles were obtained using Gallios flow cytometer (Beckman-Coulter) before data analyses using FlowJo software (LLC).

RNA extraction and Reverse Transcription-qPCR

RNAs were extracted using TRIzol (Invitrogen). Purified nucleic acids were first treated with DNase (Ambion) before reverse transcription using SuperScript II (Invitrogen). cDNA was then amplified using the SYBR select master mix for CFX (Applied Biosciences) on a CFX96 Real-time detection system (Bio-Rad). Primers are available upon request.

BrdU immunoprecipitation

Genomic DNA was sonicated into 300-400 bp fragments and denatured. 5µg BrdU antibody (BD PharMingen, 555627) coupled to 75µl of Dynabeads Protein G (Invitrogen) were added to 5µg of denatured BrdU-containing genomic DNA in BrdU IP buffer (PBS +

0.0625% Triton X-100). After 1 hour incubation at room temperature on a wheel, beads were washed twice with BrdU IP buffer and eluted in Tris-HCl 10mM (pH 8.0), EDTA 1mM, 1% SDS. Eluates were cleared of SDS using the NucleoSpin Gel and PCR Clean-up (Macherey-Nagel).

Chromatin Immunoprecipitation (ChIP)

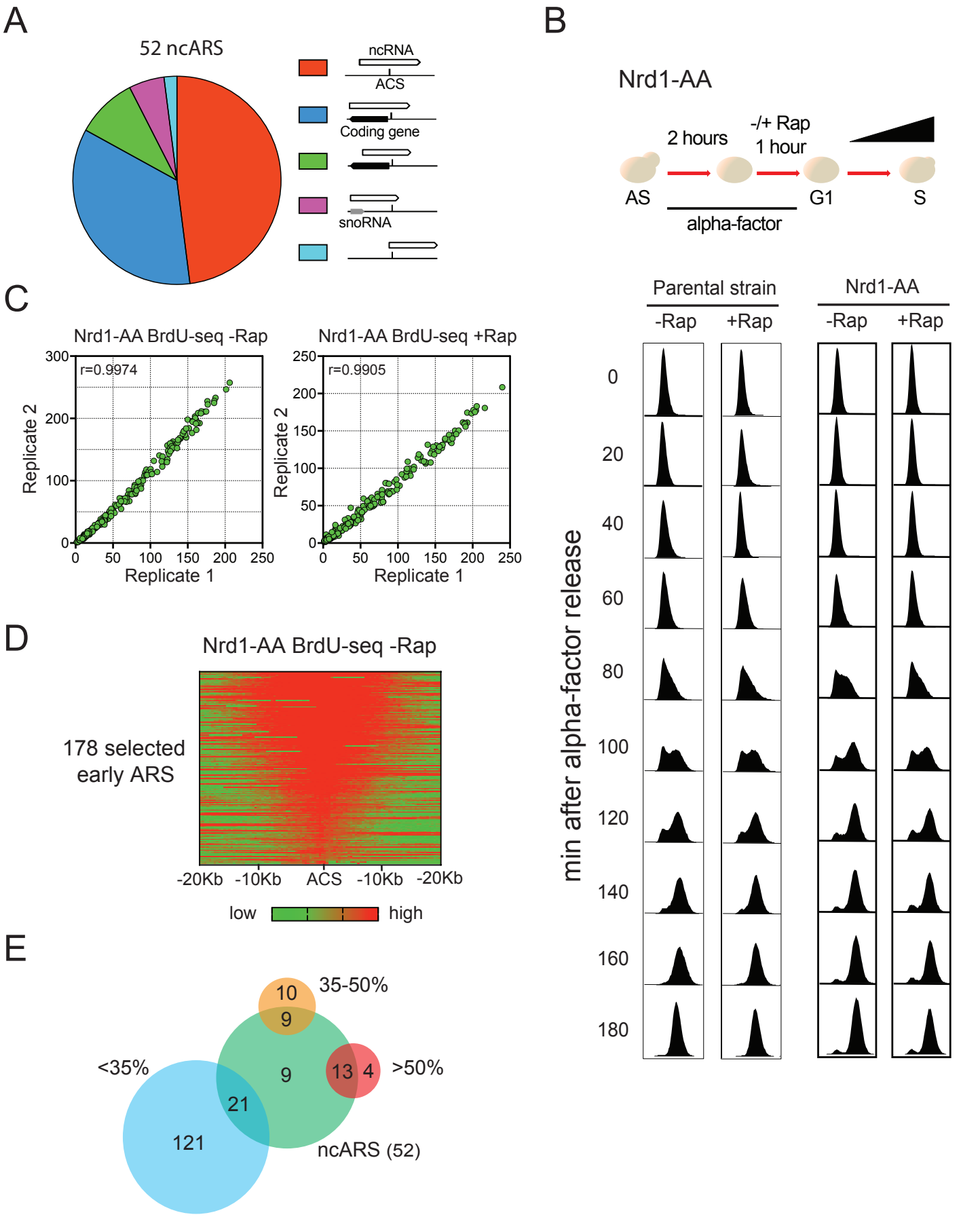
Antibodies against H3 (Abcam ab1791), H3K36me3 (Abcam ab9050) and H3K18ac (Abcam ab1191) were incubated with Protein G dynabeads (Invitrogen) before being mixed with sonicated chromatin and incubated on a wheel at 4°C for 2 hours. After washes, immunoprecipitated chromatin was eluted before being purified on columns (Macherey-Nagel).

Transcriptional readthrough calculation for RNA Pol II PAR-CLIP

Induced transcriptional readthrough was calculated as follows. First, mean densities of Top and Bottom strands in each condition were calculated on oriented ARS between the ACS to +100bp considering this region as 1bin using HTS bioscript. A pseudo-count of 1 was added to each value to correct for low values, which could lead to overestimated ratios defined hereafter. The total readthrough was then calculated by adding the values obtained for Top and Bottom in +Rap divided by the sum of Top and Bottom in –Rap. For Fig. 3, Top and Bottom strand values were added to get the total amount of natural readthrough. Three classes of ARS with different levels of natural readthrough (High, Mid and Low) were then defined through the use of *k*-means clustering (http://scistatcalc.blogspot.ch/2014/01/k-means-clustering-calculator.html).

Viggiani CJ, Aparicio OM. 2006. New vectors for simplified construction of BrdU-Incorporating strains of Saccharomyces cerevisiae. *Yeast* **23**: 1045-1051.

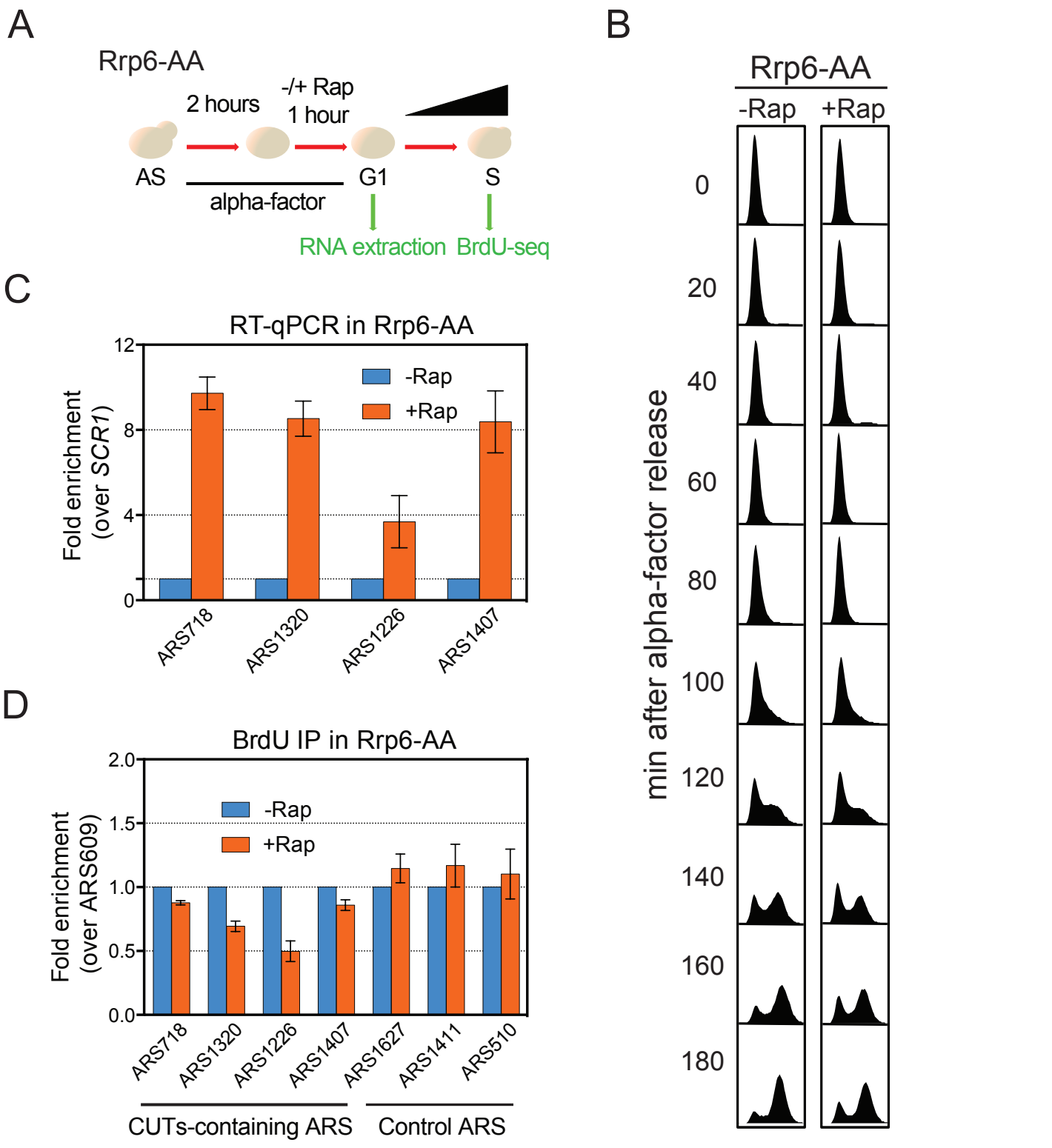
Supplemental Fig. S1



<u>Supplemental Fig. S1</u>: related to Fig. 1.

(A) Configuration distributions of the 52 ncRNAs-containing ARS (ncARS) with respect to the ACS. **(B)** FACS profiles of S-phase progression for the Nrd1-AA strain. **(C)** The mean coverage of BrdU nascent DNA in a 5Kb window centered around the ACS was measured for the 178 ARS taken into account in Fig. 1E. The plots represent this mean coverage for the 2 replicates in either –Rap or +Rap. **(D)** Heatmap representing the BrdU-seq densities (log_2) 20Kb around the ACS of the 178 replication origins considered as active in the experiment. **(E)** Overlap between the 52 ncRNA-containing ARS and the different classes of BrdU incorporation-defective replication origins in +Rap versus -Rap. The ncARS strongly overlap with the most affected ARS.

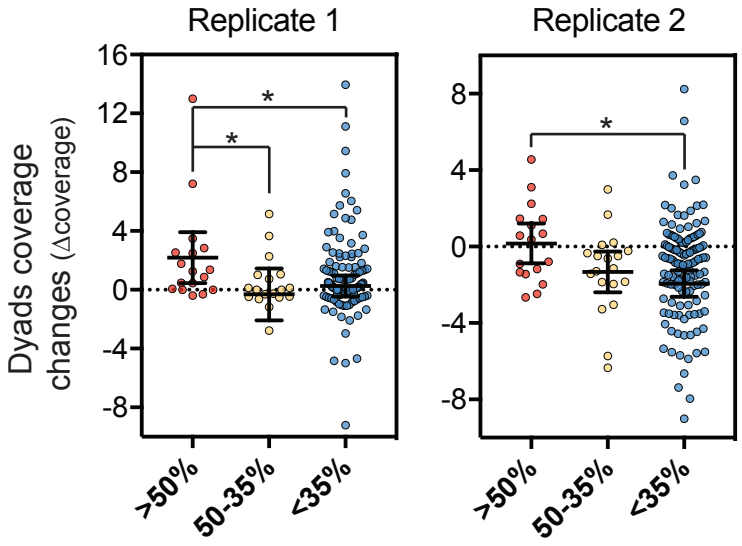
Supplemental Fig. S2



<u>Supplemental Fig. S2</u>: related to Fig. 1.

(A) Rrp6-AA cells were treated as in Fig. 1C. RNA was extracted in G1-phase while DNA extraction and BrdU immunoprecipitation were performed at the 80 min time point following release into S-phase. **(B)** FACS profiles of S-phase progression for the Rrp6-AA strain. **(C)** RT-qPCR analysis of CUTs-containing ARS in G1-phase synchronized cells with or without 1h rapamycin treatment. Measured ncRNAs were normalized to *SCR1* RNA. **(D)** Analysis of BrdU incorporation after 80 min of S-phase release at 18°C. Three ARS which do not contain CUTs were used as controls. Fold enrichment represents the ratio of immunoprecipitated BrdU for a given ARS over the value of the very late replicated origin ARS609. For **(C)** and **(D)**, the fold enrichment was artificially set to 1 for the –Rap condition (n=3). Error bars represent SEM.

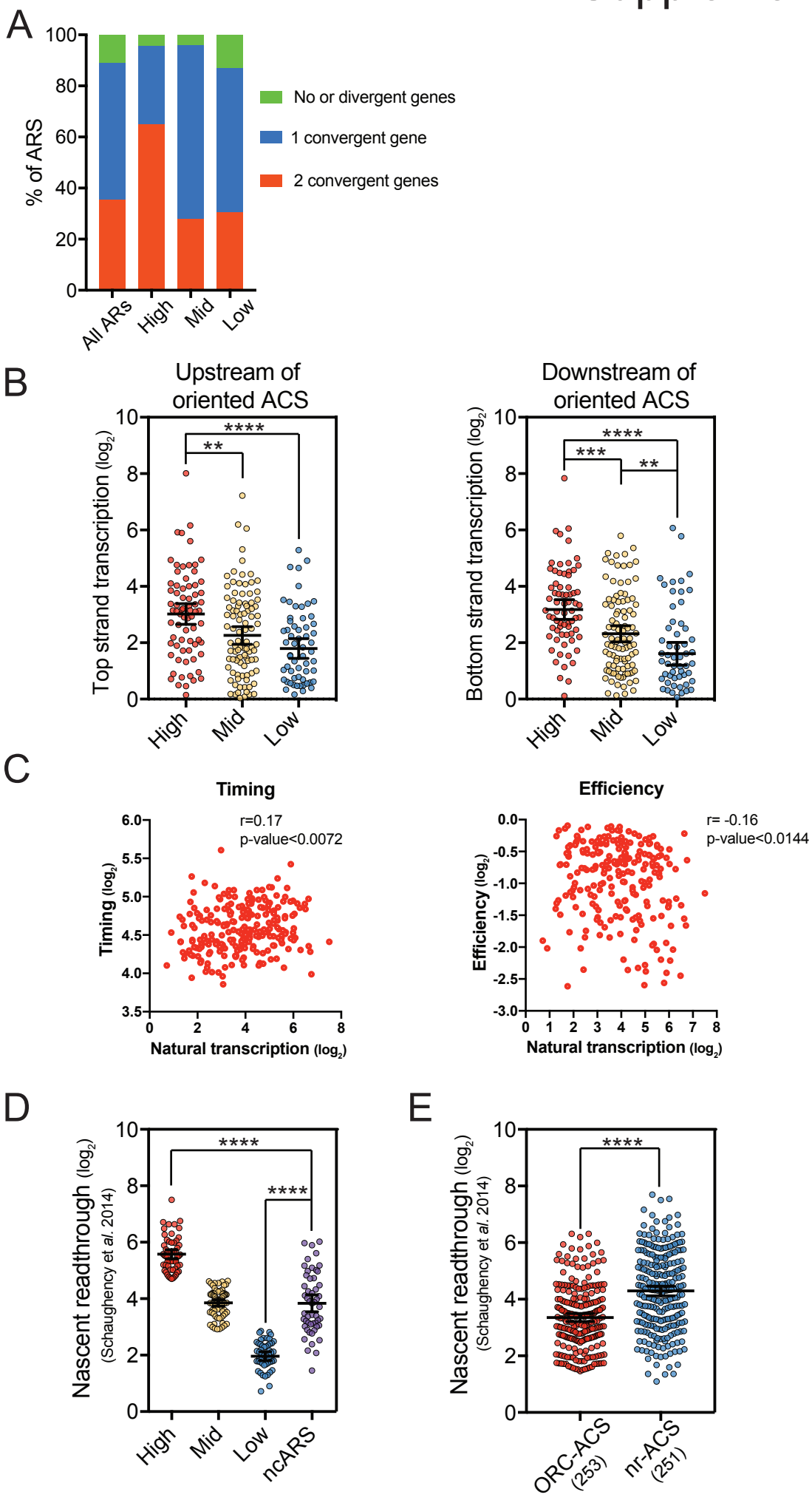
Supplemental Fig. S3



<u>Supplemental Fig. S3:</u> related to Fig. 2.

Scatter dot plot representing the difference of dyads coverage (Δcoverage= coverage (+Rap) - coverage (-Rap)) between the ACS to +100 of oriented ARS when comparing +Rap and –Rap conditions separately for Replicate 1 and 2. For each replicate, the class of ARS affected >50% in replication is significantly different from the class affected <35% (p-values<0.015 for replicate 1 and <0.024 for replicate 2).

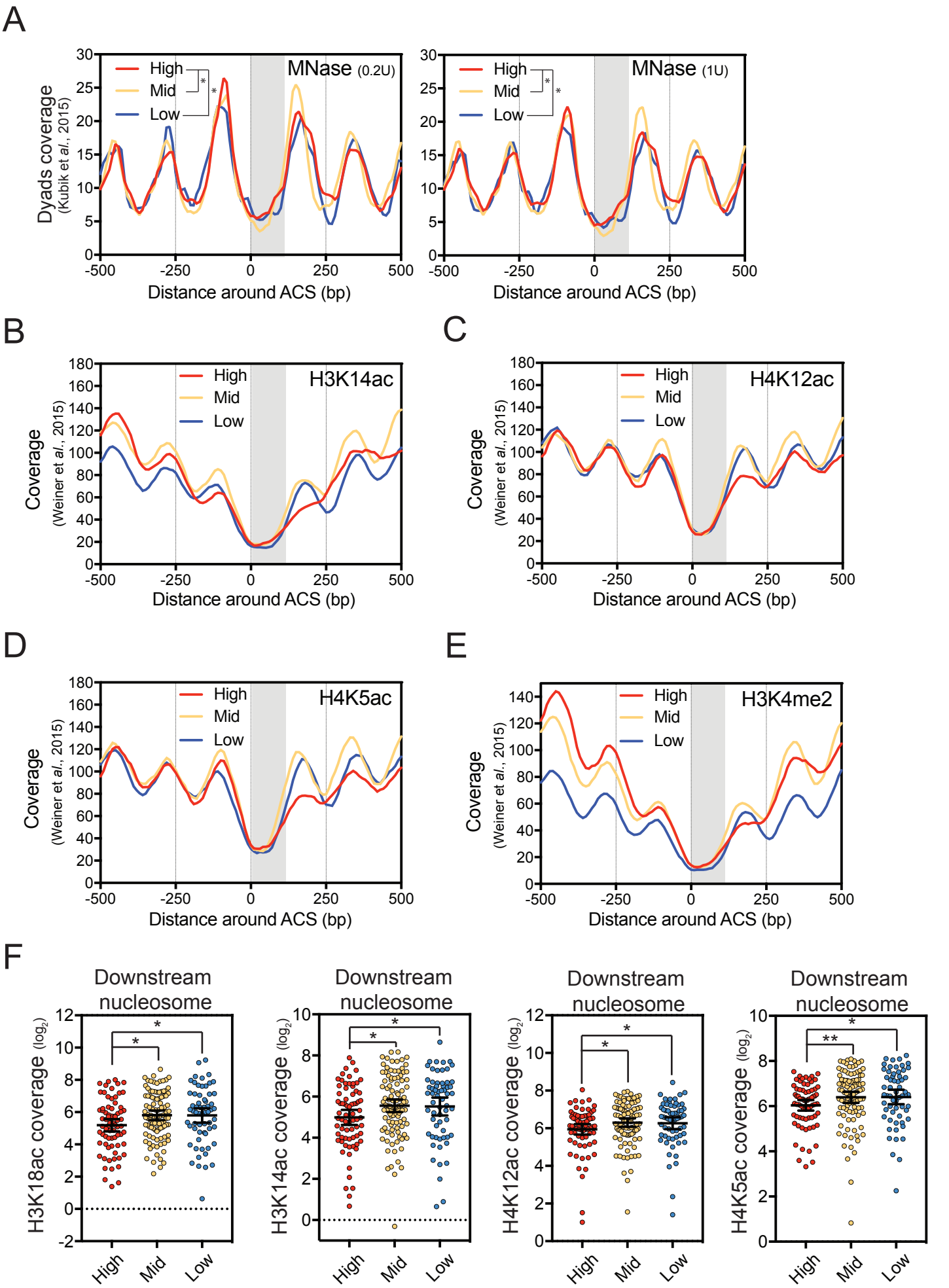
Supplemental Fig. S4



Supplemental Fig. S4: related to Fig. 3.

(A) Gene configuration around All ARS, High, Mid and Low classes of transcribed ARS. A gene was considered as convergent if pointing to the ARS within a distance <500bp to the ACS. **(B)** Scatter dot plot of nascent transcription pointing toward the ARS either Upstream (-400 to -300bp) or Downstream (+400 to +500bp) relative to the oriented ACS. **(C)** Scatter dot plot showing the global correlation between nascent transcription in the ACS to +100bp and Timing or Efficiency, respectively. r corresponds to the Pearson correlation coefficient. **(D)** Scatter dot plot representing natural readthrough of CUTs/NUTs-containing ARS (ncARS) versus High, Mid and Low classes. **(E)** Scatter dot plot presenting nascent transcription in the ACS to +100bp region for ORC-bound ACS (ORC-ACS) and non-replicated ACS (nrACS) as defined in Eaton et al., 2010.

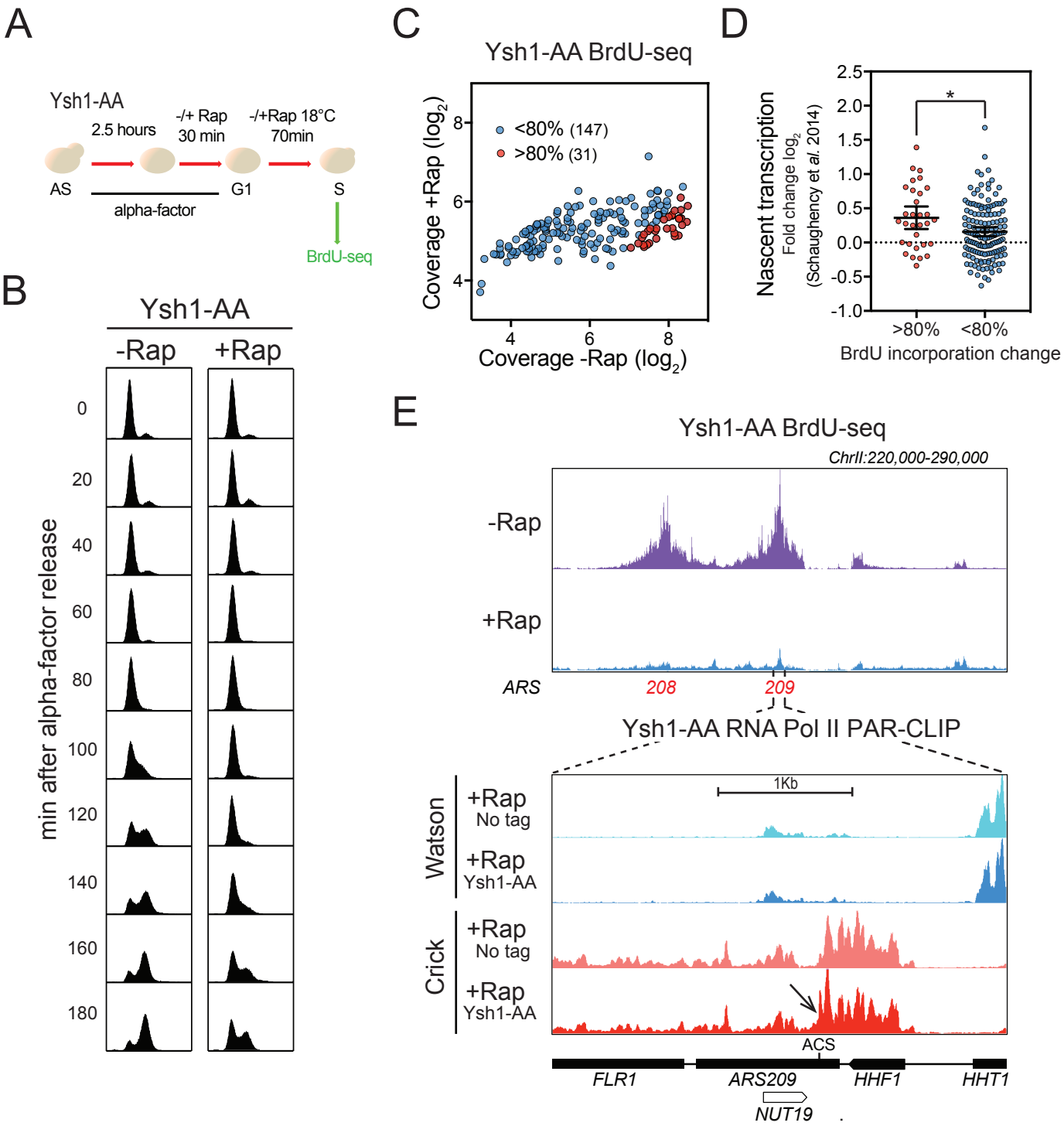
Supplemental Fig. S5



<u>Supplemental Fig. S5</u>: related to Fig. 3.

(A)(B)(C)(D)(E) Metagene analysis of MNase-seq performed with two different concentrations of MNase, H3K14ac, H4K12ac, H4K5ac and H3K4me2 profiles around the ACS of the High, Mid and Low classes of ARS. Data were retrieved from (Belsky et al. 2015; Kubik et al. 2015; Weiner et al. 2015). Results were smoothed over a 10bp-moving window. No significant differences were detected over the ACS to +100bp region. **(F)** H3K18ac, H3K14ac, H4K12ac, and H4K5ac coverages were measured over the downstream nucleosome (between 160bp to 200bp from the ACS) of the 3 classes considering this region as 1 bin.

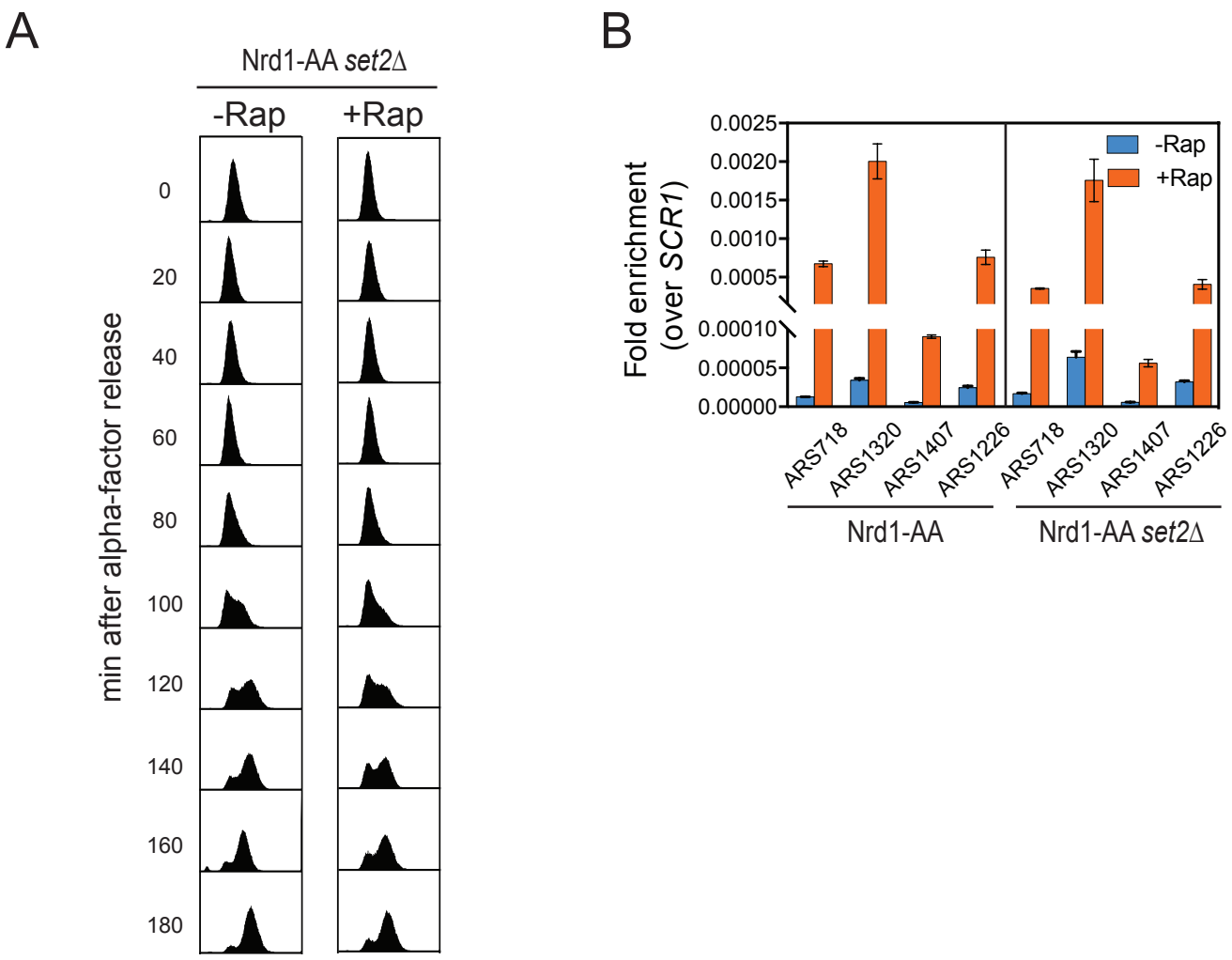
Supplemental Fig. S6



Supplemental Fig. S6: related to Fig. 4.

(A) Ysh1-AA cells were synchronized in G1-phase with alpha-factor during 3 hours at 30°C. During the last 30min, rapamycin (Rap) was added or not in the medium. Cells were then washed and released into the cell cycle at 18°C in the presence of BrdU and -/+Rap. After 70min, cells were collected for DNA extraction and BrdU-seq. **(B)** FACS analysis of the Ysh1-AA strain. Cells were treated as indicated in A. **(C)** Scatter plot depicting the mean coverage of BrdU nascent DNA over the same 178 ARS as in Fig. 1E. The 31 red dots and 147 blue dots represent the ARS showing >80% and <80% decrease in BrdU incorporation in +Rap versus -Rap respectively. **(D)** Scatter dot-plots representing the ratio $\log_2$ +Rap/-Rap of the RNA PolII PAR-CLIP signal from both strands between the ACS and +100 of oriented ARS in the Ysh1-AA strain at the 2 classes of ARS defined above. **(E)** Top: Snapshot illustrating the BrdU incorporation defect in the Ysh1-AA strain. Bottom: zoom over the ARS209 showing the *HHF1* gene readthrough transcription over the ACS by RNA PolII PAR-CLIP. Transcriptional readthrough is indicated by an arrow.

Supplemental Fig. S7



Supplemental Fig. S7: related to Fig. 5.

(A) FACS analysis of the Nrd1-AA *set2Δ* strain. Cells were treated as shown in Fig. 5A. **(B)** RT-qPCR analysis of NUTs-containing ARS in the Nrd1-AA and Nrd1-AA *set2Δ* strains with or without 1h rapamycin treatment. Measured ncRNAs were normalized to *SCR1* RNA.

Supplemental Table 1. List of the 234 ARS features used in this study.

Chr	ACS	ARS	Orientation	Timing (min)	Efficiency (%)
chrI	147536	ARS108	-	23.735	0.668
chrI	124521	ARS107	-	27.404	0.741
chrI	70432	ARS106	-	31.291	0.218
chrI	42059	ARS105	+	28.660	0.326
chrII	237875	ARS208	-	15.588	0.695
chrII	63373	ARS202	-	19.066	0.616
chrII	486897	ARS216	-	19.982	0.760
chrII	255081	ARS209	-	24.969	0.501
chrII	418010	ARS215	-	25.267	0.488
chrII	774020	ARS227	-	27.550	0.431
chrII	802270	ARS229	-	28.119	0.588
chrII	170263	ARS207	-	31.714	0.377
chrII	408041	ARS214	-	34.844	0.391
chrII	622754	ARS220	+	21.272	0.700
chrII	326193	ARS211	+	27.165	0.766
chrII	632018	ARS221	+	29.128	0.301
chrII	611771	ARS219.5	+	32.332	0.255
chrIII	39591	ARS305	-	16.238	0.591
chrIII	108972	ARS307	-	17.568	0.345
chrIII	74523	ARS306	+	14.501	0.777
chrIII	224858	ARS315	+	17.452	0.837
chrIII	166657	ARS310	+	18.158	0.409
chrIII	132042	ARS309	+	21.817	0.543
chrIII	315816	ARS319	+	23.144	0.247
chrIV	484036	ARS417	-	16.834	0.536
chrIV	555399	ARS418	-	17.234	0.924
chrIV	1159443	ARS432	-	18.662	0.478
chrIV	1166175	ARS432.5	-	19.201	0.644
chrIV	1302759	ARS435	-	20.016	0.704
chrIV	505519	ARS417.5	-	20.838	0.562
chrIV	329742	ARS413	-	21.937	0.660
chrIV	212593	ARS409	-	29.550	0.558
chrIV	46222	ARS404	-	30.060	0.668
chrIV	629309	ARS420	-	31.775	0.587
chrIV	640065	ARS421	-	34.626	0.415
chrIV	1461904	ARS442	+	19.074	0.278
chrIV	408132	ARS414	+	20.703	0.374
chrIV	913862	ARS428	+	21.341	0.401
chrIV	921742	ARS429	+	21.572	0.243
chrIV	316878	ARS412	+	24.365	0.289

chrIV	123677	ARS406	+	27.245	0.512
chrIV	1057893	ARS431	+	29.587	0.506
chrIX	73953	ARS907	-	19.888	0.350
chrIX	105966	ARS909	-	21.972	0.790
chrIX	412000	ARS922	-	22.149	0.660
chrIX	342028	ARS919	-	27.051	0.579
chrIX	310739	ARS916	-	30.660	0.557
chrIX	214733	ARS913	+	15.871	0.643
chrIX	175171	ARS912	+	26.345	0.628
chrIX	357223	ARS920	+	26.473	0.604
chrIX	80373	ARS908	+	28.215	0.413
chrV	173807	ARS511	-	18.779	0.890
chrV	59469	ARS507	-	19.620	0.798
chrV	145713	ARS510	-	19.651	0.583
chrV	406902	ARS517	-	19.977	0.881
chrV	94056	ARS508	+	17.251	0.914
chrV	353583	ARS516	+	20.327	0.833
chrV	569630	ARS523	+	21.305	0.403
chrV	287566	ARS514	+	26.321	0.502
chrV	212455	ARS512	+	26.816	0.727
chrV	498900	ARS520	+	27.741	0.758
chrV	549586	ARS522	+	30.072	0.372
chrVI	167731	ARS606	-	20.287	0.756
chrVI	118678	ARS603.5	-	26.961	0.556
chrVI	136037	ARS605	-	36.277	0.247
chrVI	199402	ARS607	+	15.378	0.936
chrVI	127869	ARS604	+	19.365	0.299
chrVI	68832	ARS603	+	25.803	0.659
chrVII	485115	ARS719	-	16.890	0.771
chrVII	834669	ARS731	-	17.174	0.860
chrVII	508911	ARS720	-	19.346	0.464
chrVII	653836	ARS726	-	19.528	0.603
chrVII	388849	ARS717	-	20.561	0.773
chrVII	977910	ARS733	-	21.676	0.608
chrVII	421285	ARS718	-	21.747	0.825
chrVII	352866	ARS716	-	25.985	0.717
chrVII	64458	ARS702	-	30.001	0.633
chrVII	660004	ARS727	-	31.979	0.335
chrVII	17906	ARS700.5	-	33.251	0.294
chrVII	1083677	ARS131a	+	17.176	0.268
chrVII	888418	ARS731.5	+	17.603	0.786
chrVII	715319	ARS728	+	21.386	0.803
chrVII	163242	ARS707	+	23.729	0.666
chrVII	203978	ARS710	+	32.839	0.466
chrVIII	392250	ARS818	-	20.133	0.462

chrVIII	501949	ARS822	-	20.436	0.657
chrVIII	447794	ARS820	-	20.891	0.891
chrVIII	359698	ARS816	-	21.387	0.699
chrVIII	45778	ARS804	-	27.409	0.216
chrVIII	297102	ARS815	+	19.650	0.908
chrVIII	64301	ARS805	+	21.111	0.637
chrVIII	168599	ARS809	+	23.838	0.563
chrVIII	245791	ARS813	+	25.611	0.834
chrVIII	556152	ARS824	+	38.395	0.163
chrX	417311	ARS1014	-	18.061	0.769
chrX	654465	ARS1020	-	20.441	0.483
chrX	442416	ARS1015	-	22.269	0.271
chrX	113736	ARS1007	-	22.705	0.662
chrX	67713	ARS1005	-	24.249	0.834
chrX	228567	ARS1009	-	26.895	0.449
chrX	374861	ARS1012	+	16.843	0.790
chrX	683928	ARS1021	+	21.242	0.836
chrX	161448	ARS1007.5	+	22.882	0.679
chrXI	55867	ARS1103	-	20.460	0.859
chrXI	329502	ARS1109	-	23.609	0.669
chrXI	213310	ARS1106.7	-	24.563	0.611
chrXI	153125	ARS1106	-	24.774	0.742
chrXI	457168	ARS1114.5	-	26.925	0.386
chrXI	612053	ARS1120	-	27.419	0.660
chrXI	388668	ARS1112	+	23.775	0.708
chrXI	98390	ARS1104.5	+	23.943	0.806
chrXI	257590	ARS1107	+	24.564	0.823
chrXI	642422	ARS1123	+	24.832	0.285
chrXI	416884	ARS1113	+	27.822	0.508
chrXII	373328	ARS1213	-	16.165	0.924
chrXII	513085	ARS1217	-	18.729	0.929
chrXII	412853	ARS1215	-	20.842	0.791
chrXII	794192	ARS1226	-	21.585	0.724
chrXII	231251	ARS1211	+	18.443	0.886
chrXII	1007238	ARS1232	+	22.050	0.653
chrXII	659895	ARS1220	+	23.299	0.616
chrXII	730540	ARS1222	+	24.034	0.617
chrXII	243743	ARS1211.5	+	31.538	0.195
chrXII	622861	ARS1219	+	31.913	0.218
chrXII	1013787	ARS1233	+	34.939	0.206
chrXIII	503627	ARS1319	-	18.955	0.693
chrXIII	535769	ARS1320	-	20.864	0.788
chrXIII	94390	ARS1305	-	22.511	0.759
chrXIII	554598	ARS1322	-	23.191	0.351
chrXIII	897975	ARS1332	-	25.787	0.812

chrXIII	40132	ARS1304	-	30.096	0.243
chrXIII	758416	ARS1327	-	31.011	0.569
chrXIII	815391	ARS1330	+	18.728	0.702
chrXIII	31767	ARS1303	+	20.124	0.346
chrXIII	263127	ARS1309	+	20.780	0.589
chrXIII	634521	ARS1324	+	21.295	0.449
chrXIII	433030	ARS1315	+	21.650	0.590
chrXIII	137322	ARS1307	+	21.664	0.675
chrXIII	805162	ARS1329	+	23.111	0.410
chrXIII	371020	ARS1312	+	23.242	0.832
chrXIII	649362	ARS1325	+	23.833	0.570
chrXIII	159062	ARS1307.5	+	27.358	0.438
chrXIII	611318	ARS1323	+	33.987	0.479
chrXIV	89756	ARS1407	-	22.498	0.673
chrXIV	280067	ARS1414	-	23.015	0.690
chrXIV	691682	ARS1427	-	23.675	0.672
chrXIV	635835	ARS1426	-	26.400	0.509
chrXIV	412443	ARS1417	-	27.879	0.727
chrXIV	546150	ARS1421	-	28.955	0.352
chrXIV	449538	ARS1419	-	30.269	0.616
chrXIV	322006	ARS1415	+	20.947	0.780
chrXIV	609538	ARS1424	+	23.739	0.779
chrXIV	499042	ARS1420	+	25.434	0.725
chrXIV	61696	ARS1406	+	28.945	0.584
chrXV	277733	ARS1511	-	19.353	0.889
chrXV	309249	ARS1512	-	19.762	0.424
chrXV	874369	ARS1526	-	22.955	0.616
chrXV	155258	ARS1509.5	-	28.308	0.165
chrXV	337484	ARS1513	-	31.274	0.389
chrXV	85360	ARS1508	-	31.364	0.341
chrXV	167004	ARS1510	+	21.679	0.788
chrXV	113910	ARS1509	+	22.066	0.746
chrXV	766691	ARS1523	+	23.198	0.767
chrXV	436793	ARS1513.5	+	24.732	0.610
chrXV	72689	ARS1507	+	25.645	0.581
chrXV	908311	ARS1528	+	33.991	0.278
chrXV	35714	ARS1506.5	+	34.024	0.199
chrXVI	777096	ARS1626.5	-	17.945	0.838
chrXVI	43150	ARS1604	-	19.931	0.170
chrXVI	695620	ARS1625	-	25.280	0.689
chrXVI	842852	ARS1628	-	33.042	0.415
chrXVI	289531	ARS1614	+	18.272	0.623
chrXVI	73105	ARS1605	+	20.350	0.745
chrXVI	384595	ARS1618	+	20.991	0.560
chrXVI	633924	ARS1623	+	21.153	0.895

chrXVI	511707	ARS1621	+	22.719	0.693
chrXVI	116594	ARS1607	+	23.358	0.636
chrXVI	684408	ARS1624	+	31.888	0.333
chrI	215011	ARS111	-	18.780	0.670
chrI	6572	ARS201	-	26.714	0.379
chrII	539400	ARS218	-	18.339	0.356
chrII	517285	ARS217	-	22.380	0.192
chrII	143692	ARS206	-	26.351	0.586
chrII	741781	ARS224	-	28.218	0.521
chrII	591434	ARS219	+	18.575	0.447
chrII	93553	ARS203	+	22.068	0.315
chrII	379119	ARS212	+	26.964	0.386
chrII	757485	ARS225	+	30.997	0.319
chrIII	273027	ARS316	-	22.416	0.465
chrIV	1033263	ARS430.5	-	16.136	0.434
chrIV	236050	ARS409.5	-	20.600	0.681
chrIV	101241	ARS405.5	-	21.706	0.471
chrIV	1487095	ARS446	-	24.132	0.454
chrIV	852	ARS400	-	25.988	0.566
chrIV	1110136	ARS431.5	-	27.711	0.629
chrIV	86124	ARS405	-	42.903	0.189
chrIV	702929	ARS422	+	21.481	0.701
chrIV	1404327	ARS440	+	22.368	0.489
chrIV	1276272	ARS434	+	27.188	0.651
chrIV	1240924	ARS433	+	27.513	0.820
chrIV	845128	ARS426.5	+	28.403	0.359
chrIV	253841	ARS410	+	29.972	0.183
chrIX	136287	ARS911	-	22.478	0.561
chrV	520952	ARS521	+	30.851	0.392
chrVII	187407	ARS709	+	28.594	0.394
chrVII	1063520	ARS735.5	+	33.917	0.580
chrVIII	535621	ARS823.5	-	28.884	0.540
chrX	730035	ARS1023	+	17.486	0.196
chrX	337270	ARS1011	+	20.057	0.494
chrX	298838	ARS1010	+	25.888	0.678
chrX	23928	ARS1004	+	48.747	0.337
chrXII	52108	ARS1203	-	24.886	0.337
chrXII	888740	ARS1227.5	-	25.101	0.768
chrXII	289421	ARS1212	-	28.695	0.684
chrXII	1024153	ARS1234	-	29.394	0.409
chrXII	822105	ARS1227	+	28.461	0.668
chrXIII	468237	ARS1316	+	23.344	0.441
chrXIII	688918	ARS1326	+	28.514	0.392
chrXIII	878735	ARS1331.7	+	30.640	0.262
chrXIII	772677	ARS1328	+	32.178	0.310

chrXIV	250465	ARS1413	-	25.503	0.530
chrXIV	764337	ARS1429	-	26.026	0.416
chrXIV	169749	ARS1411	-	27.602	0.622
chrXIV	196226	ARS1412	-	31.148	0.626
chrXIV	126679	ARS1410	-	37.763	0.342
chrXIV	738730	ARS1428.5	+	35.238	0.442
chrXV	566598	ARS1516	-	21.689	0.780
chrXV	681350	ARS1520	-	29.163	0.622
chrXV	729797	ARS1521	+	19.448	0.316
chrXV	783388	ARS1524	+	22.563	0.346
chrXV	656703	ARS1519	+	26.471	0.632
chrXVI	584397	ARS1622.7	-	29.081	0.259
chrXVI	331771	ARS1617	+	23.235	0.477
chrXVI	880921	ARS1630	+	30.530	0.613

The 178 ARS analyzed for BrdU incorporation (Fig. 1, 4 and 5) are indicated in grey.

Supplemental Table 2. List of strains used in this study

Strain	Lab name	Genotype
Nrd1-AA	FSY5348	*MAT a, tor1-1, fpr1::NAT, RPL13A-2×FKBP12::TRP1, NRD1-FRB::KanMX6, bar1::LEU2, BrdU-inc::HIS3, ura3-1, ade2-1*
Nrd1-AA *set2Δ*	FSY7284	*MAT a, tor1-1, fpr1::NAT, RPL13A-2×FKBP12::TRP1, NRD1-FRB::KanMX6, bar1::LEU2, BrdU-inc::HIS3, set2 ::URA3, ade2-1*
Rrp6-AA	FSY5725	*MAT a, tor1-1, fpr1::loxP-LEU2-loxP, RPL13A-2×FKBP12::loxP, RRP6-FRB::KanMX6, bar1::URA3, BrdU-inc::HIS3, ade 2-1 trp1-1, leu2-3,112*
Ysh1-AA	FSY7715	*MAT a, tor1-1, fpr1::loxP-LEU2-loxP, RPL13A-2×FKBP12::loxP, YSH1-FRB::KanMX6, bar1::URA3, BrdU-inc::HIS3, ade 2-1 trp1-1, leu2-3,112*