

## Supporting Information for

Rpd3 regulates single-copy origins independently of the rDNA array by opposing origin stimulation by Fkh1.

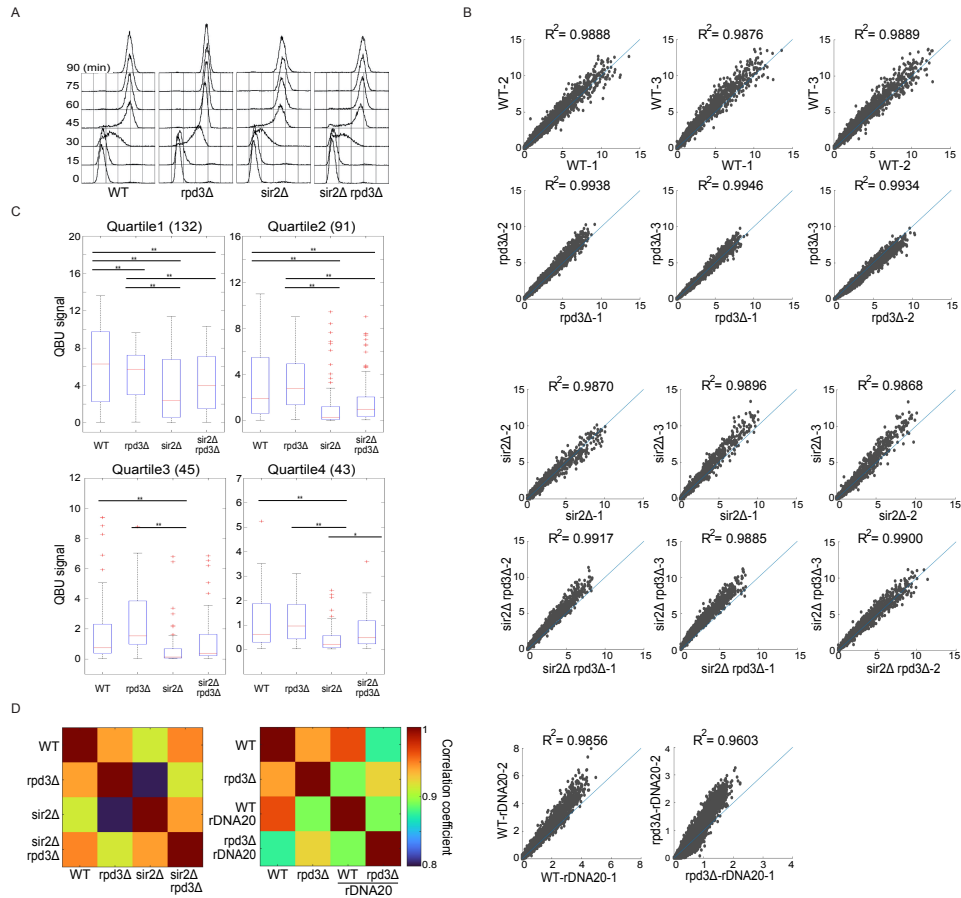
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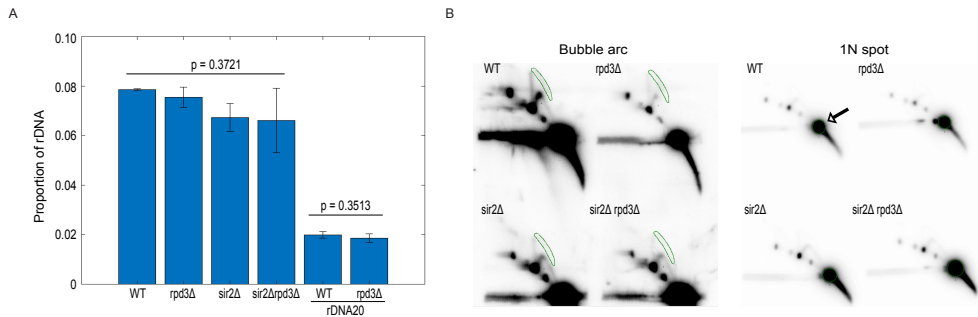
### This PDF file includes:

Figures S1 to S5  
Legends for Figures S1 to S5

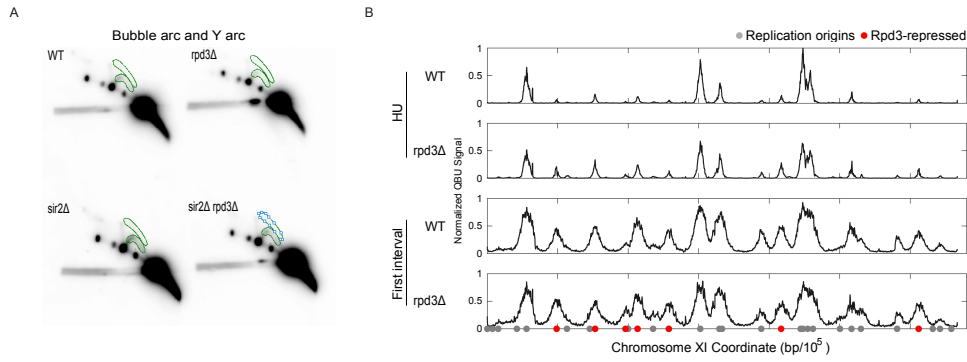
Tables S1 to S3  
Legends for Tables S1 to S3



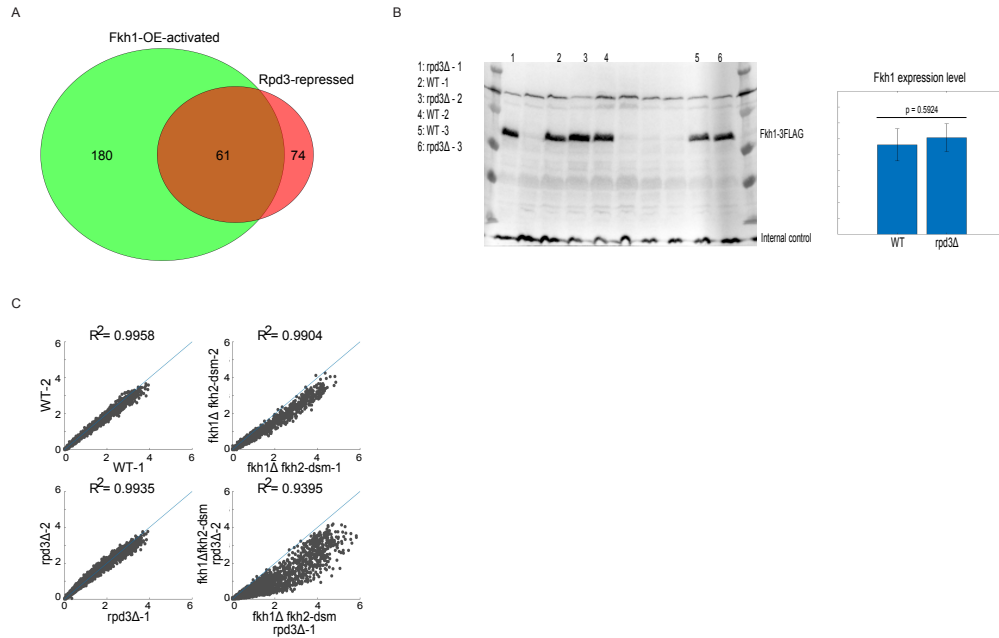
**Figure S1. DNA replication analysis of *WT*, *rpd3Δ*, *sir2Δ*, and *rpd3Δ sir2Δ* strains.** (A) Strains CVy43 (*WT*), CVy44 (*rpd3Δ*), YHy3 (*sir2Δ*), and YHy6 (*sir2Δ rpd3Δ*) were synchronized in G1 phase and released into S phase without HU for DNA content analysis. (B) QBU values (1kbp bins) for individual experimental replicates described in Figure 1 legend are plotted against each other. (C) Boxplot distributions of QBU counts across for 500bp regions aligned on origins of the indicated  $T_{Rep}$  quartiles; the number of origins in each group is indicated within parentheses; two-sided t-tests were performed on all pairs of strains and results are indicated as: \*= $p < 0.05$ , \*\*= $p < 0.01$ . (D) Heatmaps of correlation coefficients based on QBU signals for 5kbp regions centered on 625 potential origins.



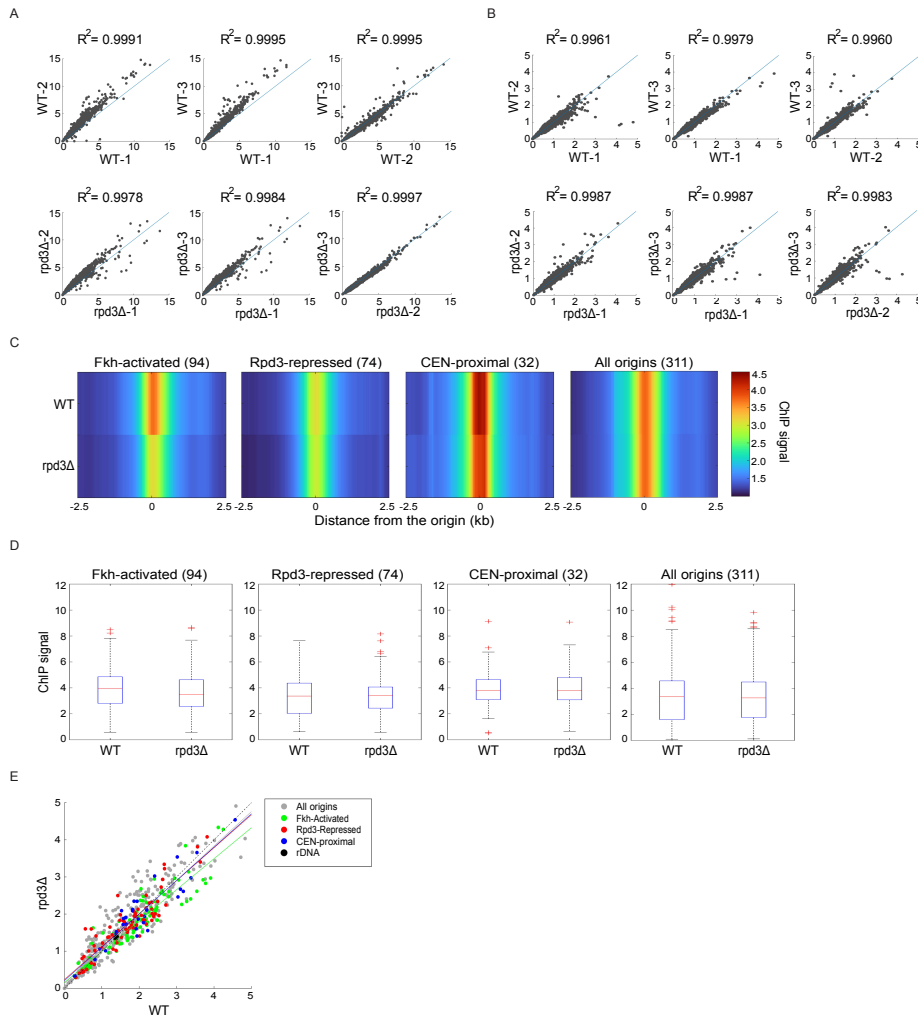
**Figure S2. Analysis of rDNA copy numbers and quantification of 2D gels. (A)** Total genomic DNA isolated from G1-arrested cells of strains described in Figure 1 were subjected to high-throughput sequencing, and read counts for the rDNA region were divided by the total read counts mapping to the nuclear genome; results of ANOVA test comparing the first four strains is given, and result of two-sided t-test for the rDNA20 strains are given. **(B)** Images of 2D gels (one set of two replicates) showing the actual areas analyzed for quantification shown in Figure 2.



**Figure S3. *RPD3* deletion de-represses similar origins with or without HU. (A)** Images of 2D gels (one set of three replicates) showing the actual areas analyzed for quantification shown in Figure 3. **(B)** Chromosome plots comparing QBU origin-firing profiles in HU versus the first interval of the time-course.



**Figure S4. Fkh1 overexpression stimulates firing of Rpd3-repressed origins. (A)** Venn diagram shows intersection of Fkh1-overexpression-activated origins (Table S3) with Rpd3-repressed origins called in this study (Table S1). **(B)** Immunoblot analysis of Fkh1-3xFLAG levels in independent transformants of *WT* and *rpd3Δ* strains (see key); blank lanes are transformants lacking Fkh1-3xFLAG, and outside lanes contain molecular weight markers. Quantification of the Fkh1-3xFLAG band was determined against the band labeled Internal control; result of t-test comparing protein levels in *WT* versus *rpd3Δ* is shown. **(C)** QBU values (1kbp bins) for individual experimental replicates described in Figure 4 legend are plotted against each other.



**Figure S5. Mcm4 origin association unaffected by *RPD3* deletion.** (A, B) ChIP enrichment values (1kbp bins) for individual experimental replicates described in Figure 5 legend and below are plotted against each other. (C-E) Strains MPy102 (*MCM4-3xHA*) and YHy37 (*rpd3Δ*, *MCM4-3xHA*) were synchronized in G1 phase and subjected to ChIP-seq. (C) Heatmaps of ChIP-seq enrichment at selected origin loci; the number in each group is indicated within parentheses. (D) Boxplots distributions of ChIP-seq enrichments for 500bp regions aligned on origins of the indicated sub-groups; the number of origins in each group is indicated within parentheses. Two-sided t-tests were performed on all pairs of strains and results are indicated as: \*= $p < 0.05$ , \*\*= $p < 0.01$ . (E) Two-dimensional scatter plots of ChIP-seq signals for 500bp regions centered on

626 origins plus the indicated origin groups; origins and sub-groups are color-coded as indicated.

A linear regression deriving the best-fit line is shown for each origin group.

**Table S1. List of RPD3-repressed origins.**

Chr	Start	End
1	124350	124599
2	773918	774348
2	389245	390368
2	801930	802617
2	378434	379194
4	46181	46237
4	316719	317111
4	1353494	1353667
4	232140	232618
4	1057828	1058076
4	1302579	1302819
4	123617	123902
5	212381	212630
5	301565	302061
5	429861	431061
6	68690	68869
6	216344	216692
7	352695	352917
7	653611	654091
7	607176	607619
7	318745	319455
7	64279	64528
7	659809	660054
7	163180	163447
8	392148	392391
8	245719	245968
8	168531	168773
8	380153	382157
8	45589	46076
8	501751	501992
8	56650	59172
9	136094	136335
9	310583	311070
9	80058	80557
10	654069	654309
10	161435	161860
10	353463	355722
10	67467	67949
11	152934	153173
11	416822	417055
11	213080	213385
11	98329	98568
11	611874	612107
11	257390	257839
11	196038	196284
12	622672	623123
12	1007180	1007470
12	76711	77163
12	947908	948358



12	794020	794269
13	554392	554750
13	836823	838167
13	94216	94463
13	420429	421629
14	279875	280108
14	449343	449588
14	61597	61894
14	498987	499232
14	89528	89802
15	514258	515458
15	766617	766862
15	759221	766221
15	72636	72872
15	85195	85444
15	489645	490129
15	854735	855228
15	154972	155462
16	584037	584486
16	427273	428757
16	456557	456805
16	89758	92975
16	384536	384784
16	687133	694133
16	684383	684632

**Table S2. List of CEN-proximal origins.**

Chr	Start	End
1	146703	147690
1	159906	160127
2	237644	237879
2	254890	255136
3	114314	114933
3	108775	109291
4	462430	462700
4	435056	435388
5	145539	145782
5	173636	173874
6	135979	136080
6	167606	168041
7	484932	485160
7	508729	508978
8	115683	117257
8	111293	111766
9	357156	357393
9	341853	342096
10	442248	442658
10	416888	417134
11	447657	447892
11	454453	459197
12	150914	151421
12	139293	140447
13	263062	263296
13	286782	287067
14	635660	635901
14	609458	609706
15	337279	337528
15	308969	309462
16	563822	564061
16	552403	554287

**Table S3. List of FKH1-OE-activated origins.**

Chr	Start	End
1	42208	42209
1	70349	70350
1	124576	124577
1	137379	137380
2	28963	28964
2	93681	93682
2	177531	177532
2	198366	198367
2	209246	209247
2	407858	407859
2	486848	486849
2	802115	802116
2	379233	379234
2	170386	170387
2	631830	631831
3	166850	166851
4	123689	123690
4	253815	253816
4	316820	316821
4	476950	476951
4	505554	505555
4	567568	567569
4	629330	629331
4	703096	703097
4	878261	878262
4	1302607	1302608
4	1486966	1486967
4	639849	639850
4	86193	86194
4	753440	753441
4	1057719	1057720
4	46066	46067
4	1462344	1462345
4	407794	407795
4	1353196	1353197
4	213203	213204
4	1017480	1017481
4	228790	228791
5	301986	301987
5	316847	316848
5	353874	353875
5	287925	287926
5	438519	438520
5	407472	407473
5	213535	213536

5	164297	164298
6	216425	216426
6	118532	118533
6	68203	68204
7	204083	204084
7	977842	977843
7	574963	574964
7	64219	64220
7	163609	163610
7	319635	319636
7	568305	568306
7	660411	660412
7	999032	999033
7	765799	765800
8	133557	133558
8	297363	297364
8	380923	380924
8	502050	502051
8	168397	168398
8	245566	245567
8	359199	359200
8	391532	391533
8	78716	78717
9	73807	73808
9	80481	80482
9	105993	105994
9	136324	136325
9	247698	247699
9	310658	310659
9	196640	196641
10	67886	67887
10	161644	161645
10	228315	228316
10	337217	337218
10	354353	354354
10	374791	374792
10	299049	299050
10	455549	455550
10	654623	654624
11	153151	153152
11	98573	98574
11	612115	612116
11	642646	642647
11	195981	195982
11	302607	302608
11	388987	388988
11	517034	517035
11	213518	213519

11	417191	417192
11	581850	581851
11	16317	16318
11	329776	329777
11	258184	258185
11	530926	530927
12	76746	76747
12	450643	450644
12	730434	730435
12	928208	928209
12	947991	947992
12	1007319	1007320
12	623203	623204
12	659711	659712
12	745314	745315
12	889006	889007
12	412469	412470
12	794525	794526
12	688195	688196
12	290047	290048
12	139853	139854
12	198446	198447
13	40138	40139
13	94377	94378
13	158926	158927
13	421301	421302
13	433282	433283
13	554466	554467
13	689069	689070
13	837710	837711
13	878637	878638
13	865281	865282
13	898047	898048
13	31678	31679
13	611499	611500
13	535533	535534
13	370902	370903
13	804926	804927
13	226807	226808
13	504481	504482
14	126850	126851
14	169687	169688
14	343561	343562
14	412385	412386
14	449430	449431
14	546038	546039
14	691523	691524
14	322229	322230

14	61919	61920
14	89827	89828
14	498922	498923
14	279620	279621
15	72714	72715
15	85214	85215
15	155155	155156
15	227853	227854
15	348277	348278
15	354524	354525
15	371940	371941
15	436914	436915
15	464305	464306
15	490091	490092
15	854790	854791
15	908318	908319
15	874443	874444
15	308932	308933
15	766504	766505
15	981900	981901
15	566938	566939
15	783043	783044
16	90544	90545
16	179437	179438
16	210534	210535
16	241741	241742
16	261434	261435
16	684383	684384
16	881098	881099
16	289715	289716
16	842632	842633
16	819129	819130
16	116468	116469
16	418083	418084
16	584547	584548
16	456925	456926
16	42825	42826
16	384955	384956
16	72806	72807