Supplementary Material
Figure S1

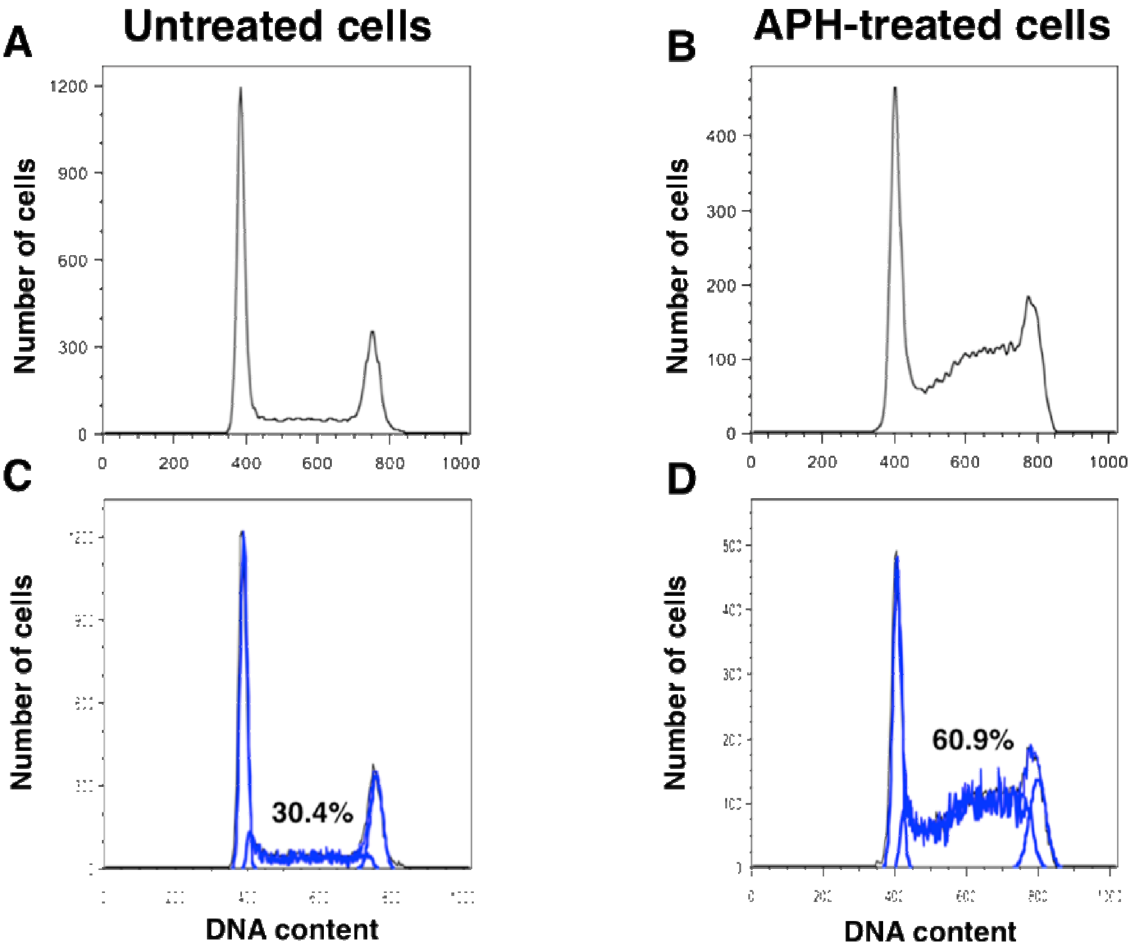
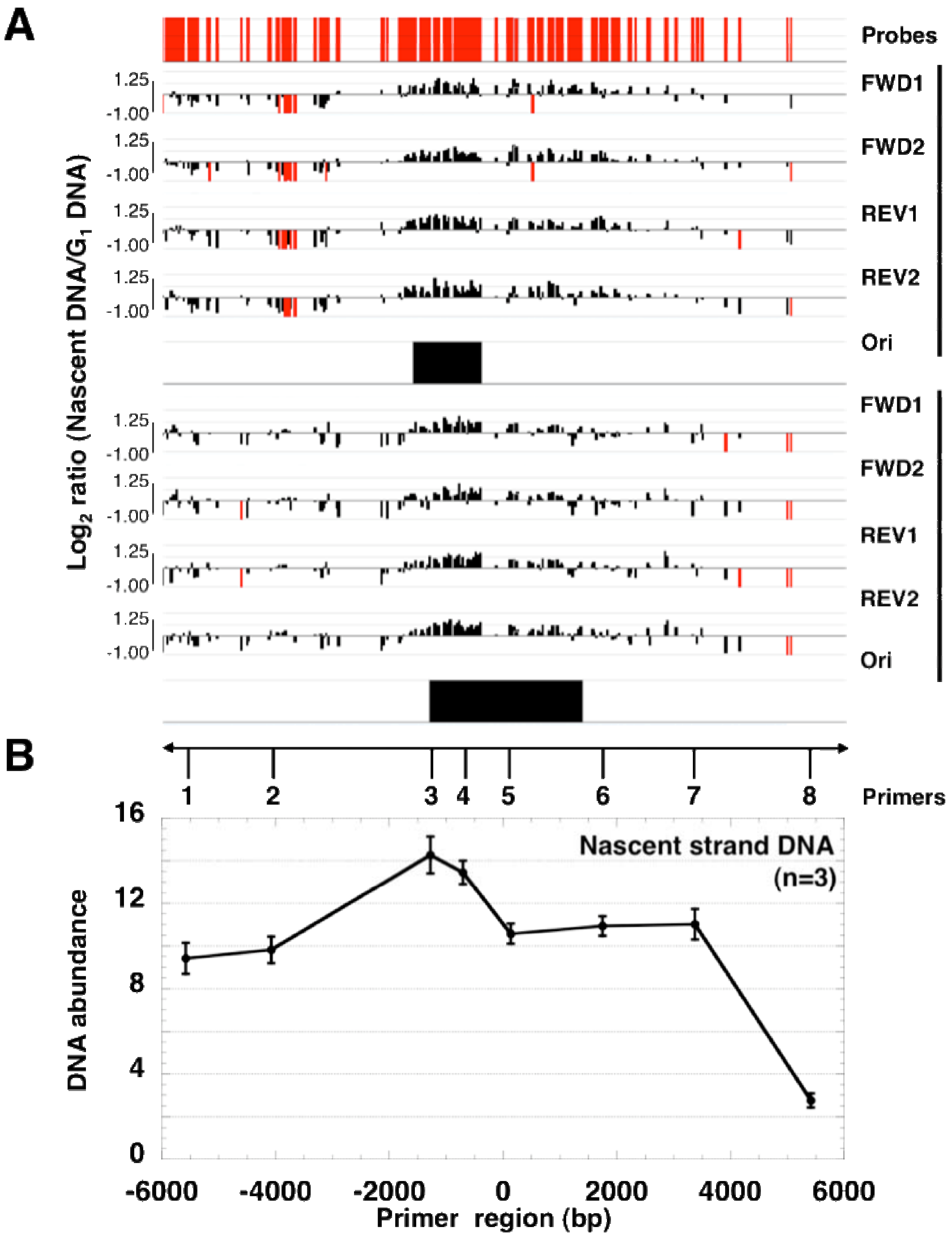


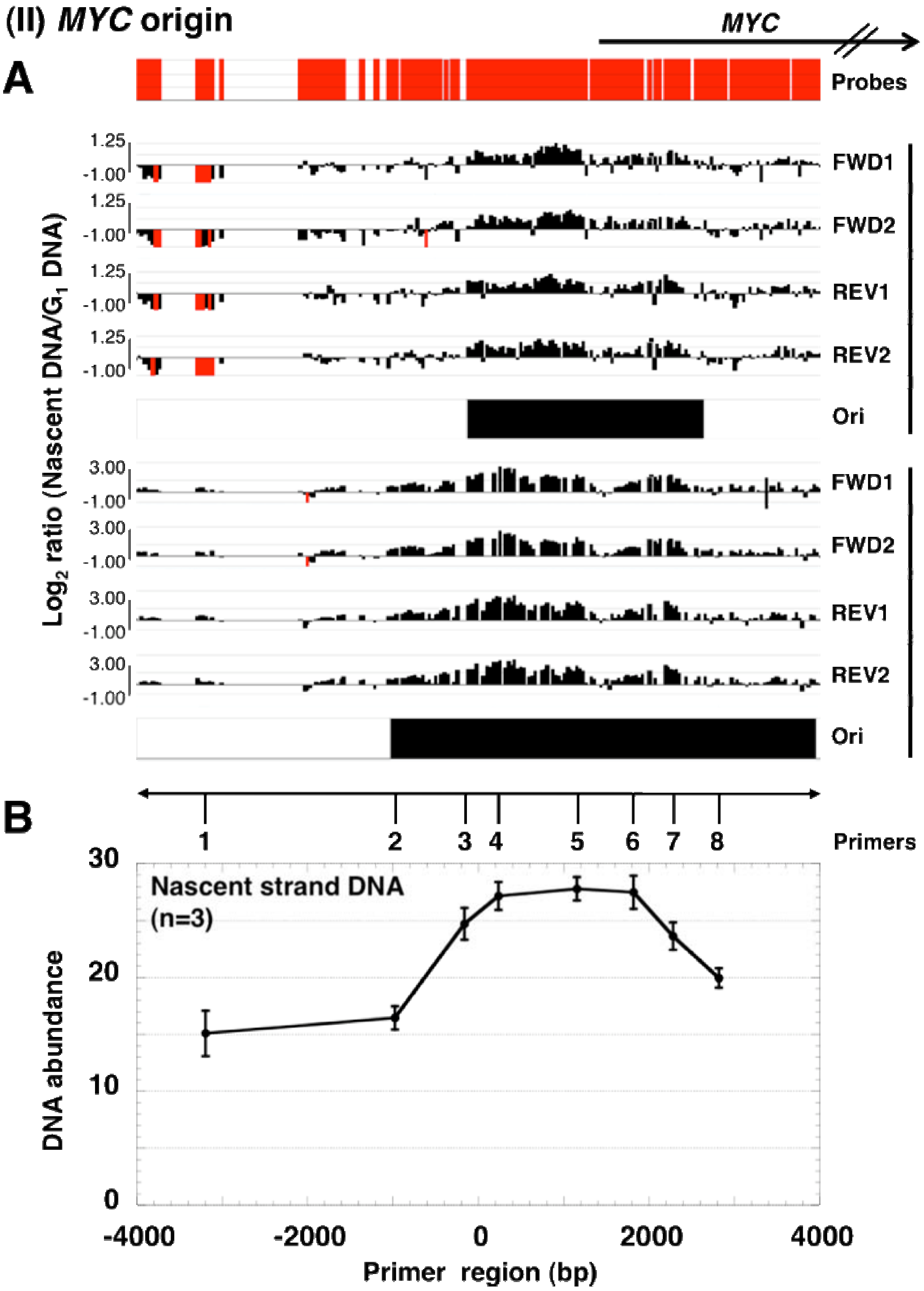
Figure S1. Estimation of the fraction of S phase cells in an asynchronous population of untreated or APH-treated cells

Representative cell cycle profile of (A) untreated and (B) APH-treated (0.4 µM for 24 h) asynchronous cells in log phase.
The percentage of cells in S phase obtained by applying a Watson Pragmatic model to the DNA content profiles is indicated for both
(C) untreated and (D) APH-treated cells (the outline of the peaks used to calculate G1, S and G2 (blue) are superimposed on the cell
cycle profile (black), FlowJo software).

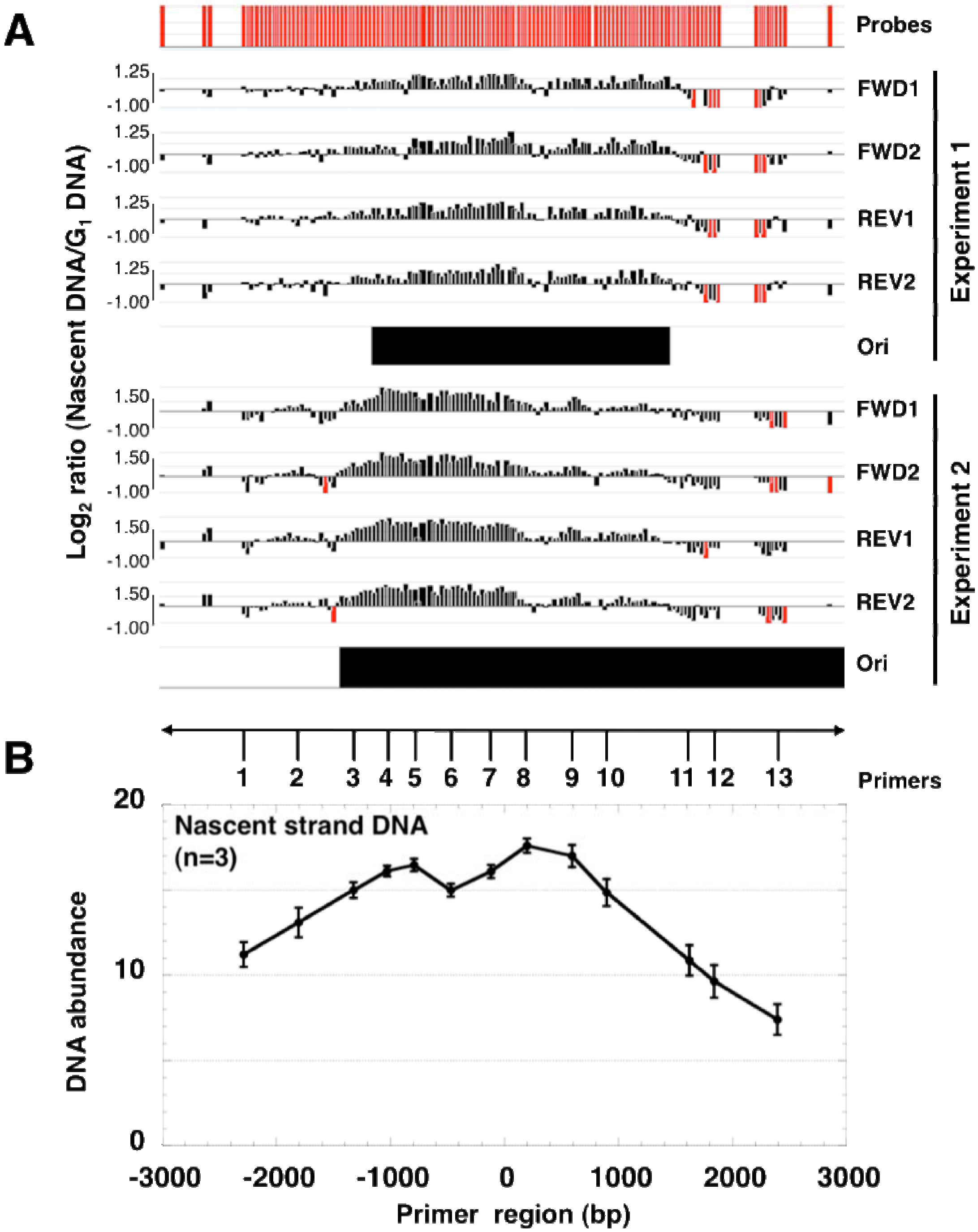
Figure S2

(I) FRA3B ori 1




(II) MYC origin
A
MYC
Probes
1.25
-1.00
FWD1
FWD2
REV1
REV2
Ori
Experiment 1
3.00
-1.00
FWD1
FWD2
REV1
REV2
Ori
Experiment 2
Log2 ratio (Nascent DNA/G1 DNA)
B
1
2
3
4
5
6
7
8
Primers
Nascent strand DNA
(n=3)
DNA abundance
30
20
10
0
-4000
-2000
0
2000
4000
Primer region (bp)

(III) *TBC1D10A* origin



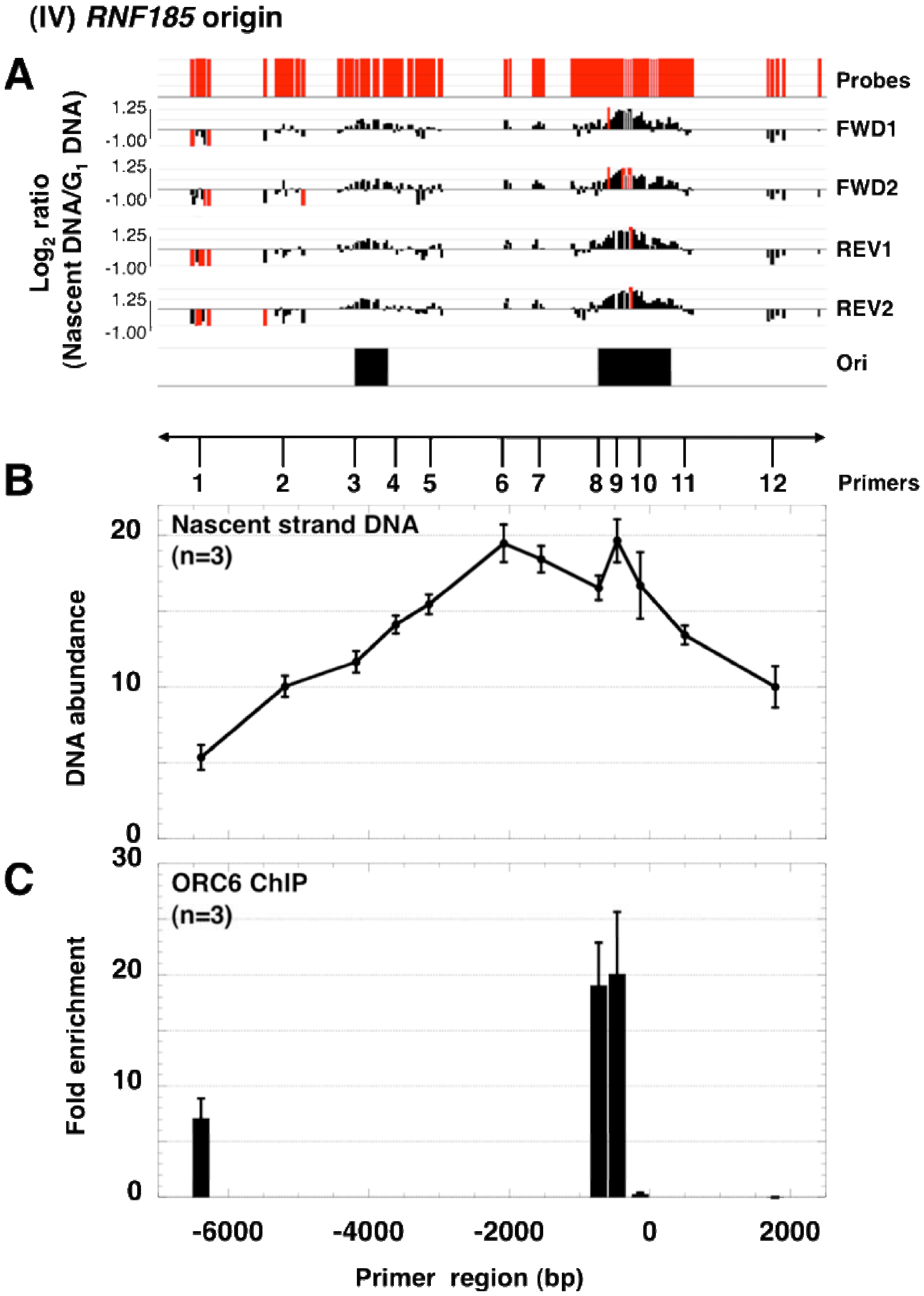

(IV) *RNF185* origin
A
Probes
Log$_2$ ratio (Nascent DNA/G$_1$ DNA)
1.25
-1.00
FWD1
1.25
-1.00
FWD2
1.25
-1.00
REV1
1.25
-1.00
REV2
Ori
B
1
2
3
4
5
6
7
8
9
10
11
12
Primers
Nascent strand DNA
(n=3)
DNA abundance
20
10
0
C
30
ORC6 ChIP
(n=3)
Fold enrichment
20
10
0
-6000
-4000
-2000
0
2000
Primer region (bp)

Figure S2. Detailed origin mapping for (I) FRA3B ori 1 and the three control origins within (II) *MYC*, (III) *TBC1D10A*, and (IV) *RNF185*.

(A) Microarray analysis of nascent strand DNA in untreated cells. The nascent strand DNA abundance data (Log$_2$ ratio of nascent strand DNA signal over G$_1$ DNA signal) obtained for each of the four sets of probes represented on the microarray (probes representing the forward and reverse strands are present in duplicate: FWD1, FWD2, REV1, REV2) for one or two independent experiments within a 6 to 12 kb region spanning (I) FRA3B ori 1, (II) *MYC*, (III) *TBC1D10A*, and (IV) *RNF185* origins. Real-time PCR quantification using closely-spaced primers (Supplementary Table 1) of (B) nascent strand DNA and (C, for the *RNF185* origin only) DNA immunoprecipitated with ORC6 antibody. The ChIP results are presented as the fold-enrichment over the value obtained using the primer 1 for FRA3B ori 2 . Of note, the Real-time PCR results of both the nascent strand DNA and ChIP experiments plotted here correspond to the average and the standard error obtained for three independent experiments (n=3). The *TBC1D10A* origin as defined by the peak finder in Experiment 2 extends farther than in Experiment 1 due to the presence of another origin centromeric of *TBC1D10A*. The two origins were merged in Experiment 2.

Figure S3

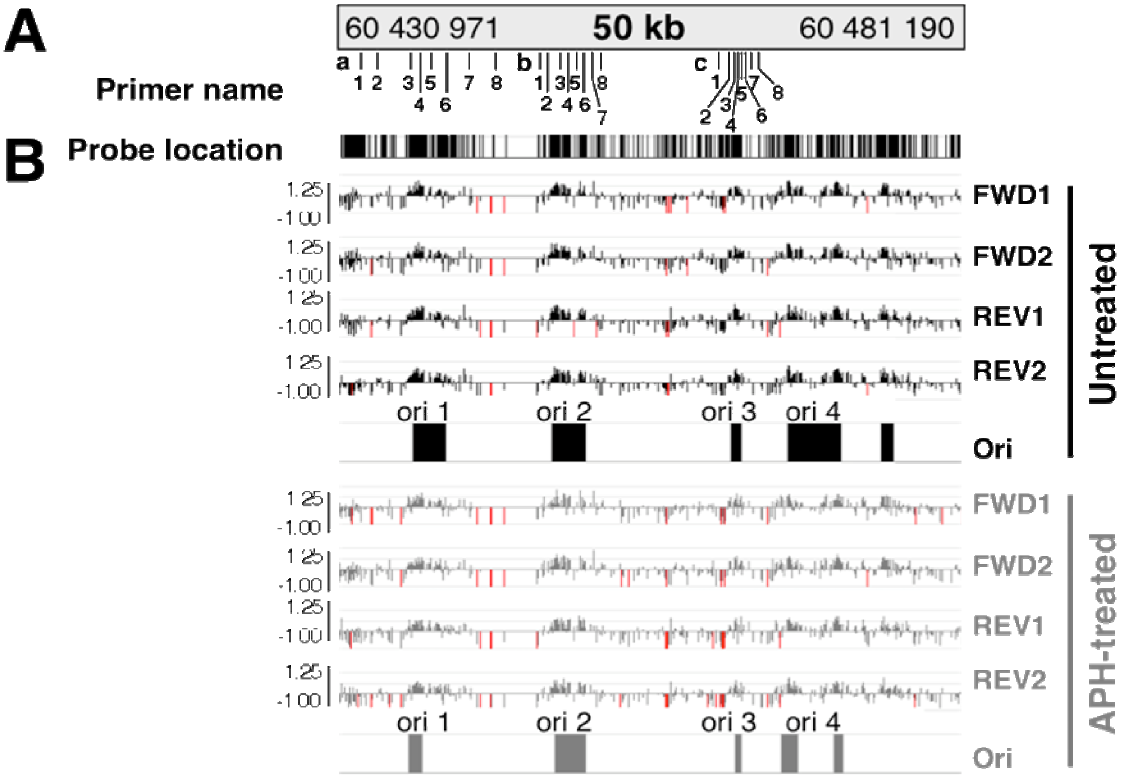


Figure S3. Mapping origins of replication within the FRA3B in untreated and APH-treated cells.

Microarray analysis of nascent strand DNA in untreated, and APH-treated cells. The nascent strand DNA abundance data (Log$_2$ ratio of nascent strand DNA signal over G$_1$ DNA signal) obtained for each of the four sets of probes represented on the microarray (probes representing the forward and reverse strands are present in duplicate: FWD1, FWD2, REV1, REV2), and the location of the mapped origins are shown across a 50 kb region within FRA3B for one experiment in untreated (black lines) and APH-treated cells (gray lines).

Table S1. Origins mapped by microarray within the 50kb region of FRA3B in untreated and APH-treated cells

Origins	Chromosome Coordinates (NCBI Build 35)		
	Untreated		APH-treated
	Exp 1	Exp 2	Exp 2
Ori 1	60 436 458 to 60 437 683 bp	60 436 741 to 60 439 454 bp	60 436 420 to 60 437 623 bp
Ori 2	60 447 814 to 60 449 397 bp	60 447 920 to 60 450 770 bp	60 448 159 to 60 450 770 bp
Ori 3	60 462 203 to 60 463 083 bp	60 462 343 to 60 463 289 bp	60 462 704 to 60 463 264 bp
Ori 4	60 470 650 to 60 471 452 bp	60 466 935 to 60 471 294 bp	60 466 440 to 60 467 821 bp 60 470 650 to 60 471 487 bp

Table S2. Primers used for determining the nascent strand DNA abundance by Real-time PCR.

Origin	**Primer name**	**Primer sequence**	**Start (bp) NCBI Build 35**	**End (bp) NCBI Build 35**	**Product size (bp)**	**Distance to origin center (bp)**
MYC	1F	CCCCATCAGACTGTTGACCT	128812849	128812868	156	-3193.5
	1R	TCTGACTAATTGCCCCTTGC	128812985	128813004		
	2F	TCCCCTTTCCCCAATAAATC	128815089	128815108	109	-977
	2R	AAACCCTAAAACGGCCAAAC	128815178	128815197		
	3F	GTGAGGGACCAAGGATGAGA	128815893	128815912	134	-160.5
	3R	CCCACACATGATTTGTTTGC	128816007	128816026		
	4F	GGGAAAGAGGACCTGGAAAG	128816285	128816304	143	236
	4R	GGGACCGGACTTCCTAAAAG	128816408	128816427		
	5F	GAGCAGCAGAGAAAGGGAGA	128817218	128817237	117	1156
	5R	CAGCCGAGCACTCTAGCTCT	128817315	128817334		
	6F	GGCACTTTGCACTGGAACTT	128817878	128817897	120	1817.5
	6R	CTTTCAGAGAAGCGGGTCCT	128817978	128817997		
	7F	GGTCGGACATTCCTGCTTTA	128818341	128818360	121	2281
	7R	GATATGCGGTCCCTACTCCA	128818442	128818461		
	8F	AACAGCTGCTACCCTTGGTG	128818886	128818905	104	2817.5
	8R	TAATAGGGGCACCTCCCTTC	128818970	128818989		
TBC1D10A	1F	GCTCAGCCTCAGCCTCTGTA	29038253	29038272	153	-2287
	1R	ACCTGATCTTGCCCTCACAG	29038386	29038405		
	2F	GAGGTCAAACAAGCCTCCAG	29038731	29038750	160	-1806
	2R	GAAGGATGCAAGGAGAACCA	29038871	29038890		
	3F	ACATGGCAGTCATGAACCAA	29039216	29039235	154	-1324
	3R	GACGGCATAAAGAGGAGCTG	29039350	29039369		
	4F	CCAACCCATTTCCCTAACCT	29039523	29039542	130	-1029
	4R	GCAGTCGTTTGAGCATGTGT	29039633	29039652		
	5F	CTGGCTGCTTTTTCCTCTGT	29039765	29039784	117	-793
	5R	GGCACTCACAGACTTGCTGA	29039862	29039881		
	6F	AGCCTCTGAAGACCTGGACA	29040096	29040115	101	-470
	6R	TGCCAACTTTGGCTTTCTCT	29040177	29040196		
	7F	CAATGCATCCTGAGCCTGTA	29040448	29040467	101	-118
	7R	ACTGTCACCCAGTGCTCCTC	29040529	29040548		
	8F	GGTCAAGACCAATGCAATCC	29040730	29040749	169	198
	8R	CTTCCAGTATGGAGGGCAGA	29040879	29040898		
	9F	CCCTCTCAGGGGAAGTAAGG	29041142	29041161	135	593
	9R	GGAGCAGCTCGTGGTTCTAT	29041257	29041276		
	10F	GTGAGGAGGGAGCACACAGT	29041459	29041478	106	896
	10R	CTGAAACAGGACCTCCCAAG	29041545	29041564		
	11F	CAGATCCCTTGCTCCCATAA	29042149	29042168	176	1621
	11R	AGGTGGTCCCTGACCTTCTT	29042305	29042324		

	12F	CCCAACCACTTCCTCTATGC	29042387	29042406	134	1838
	12R	CCAGGACCAGTTGAGGAAAA	29042501	29042520		
	13F	GGCAAGCTGCTTTCTTGTCT	29042926	29042945	168	2394
	13R	AAACTGGAGGCCTGTGAGAA	29043074	29043093		
RNF185	1F	TGCCATTGGAAAGAACACAG	29910458	29910477	193	-6388
	1R	ACGCTGTGGCTGAATACCTT	29910631	29910650		
	2F	GGGAGGAATGGTCGTTGTTA	29911697	29911716	103	-5194
	2R	GATCTCCACTGGCAAGTTCC	29911780	29911799		
	3F	AAGGATGAGGACAGCCTGAA	29912689	29912708	147	-4180
	3R	ATGGGGGCATTCACATACAC	29912816	29912835		
	4F	TTCATCAGCTCTGGTTGCAC	29913271	29913290	110	-3616.5
	4R	AGAAGGCTGTGGGGGTAAGT	29913361	29913380		
	5F	GAAAGTCTCCCTGGTCGTCA	29913737	29913756	119	-3146
	5R	CACCTTCTTTCTCCCATCCA	29913836	29913855		
	6F	GAGAAACCCAAAACGGGATT	29914775	29914794	175	-2080
	6R	ATCCCAGGCATCAGAATCAG	29914930	29914949		
	7F	CTACTGGGAAGGTGGGGATT	29915330	29915349	129	-1548
	7R	TCTAGGGCCTCCTAGCAACA	29915439	29915458		
	8F	CTTCTCCCCTTGGATGTGAA	29916151	29916170	125	-729
	8R	CCTCCCCTCCTAGCTGACTC	29916256	29916275		
	9F	ATGTAGCAGGAGAGGCTCCA	29916389	29916408	172	-467.5
	9R	AGACCATGAAGGGCTCTCAA	29916541	29916560		
	10F	CCCAGTCCTGCTCAAGCTAC	29916714	29916733	180	-138.5
	10R	TCATGGTTTCAAGGGGGATA	29916874	29916893		
	11F	CCCTGACCCTGTTGGTAAAG	29917384	29917403	110	496.5
	11R	CGAGGAGGGGTCTTCTCTCT	29917474	29917493		
	12F	ATTTACCCACCATTGCCAAG	29918591	29918610	146	1721.5
	12R	ATCATGGAAAACCTGCCAAA	29918717	29918736		
FRA3B ori 1	1F	CACCCACCTGGTCTTTTGTT	60432453	60432472	123	-5578
	1R	GGGGAAGACAGAGCAAGATG	60432556	60432575		
	2F	CTGCTCTCAAGGTGTTGCTG	60433949	60433968	139	-4074
	2R	TCTTGGAACAGTGCTCATGC	60434068	60434087		
	3F	TTGGCACAAGAACTGGCATA	60436753	60436772	127	-1276
	3R	TGCACAGCCATCAGTTTTTC	60436860	60436879		
	4F	ATCAGCTGGGAGCACTGACT	60437325	60437344	128	-704
	4R	TCCCTAGGCCCTTGAACTTT	60437433	60437452		
	5F	TATGCCTGCCTTTGGATTTC	60438175	60438194	104	135
	5R	GTGATGCCTGGCAAGAGAGT	60438259	60438278		
	6F	TCTAAGGTTGCCCAGGACAG	60439773	60439792	137	1749
	6R	GAAAGGCACGTCTGAACCAT	60439890	60439909		
	7F	GCCAAAACTAAGGGGACACA	60441377	60441396	175	3372
	7R	CCCTGCAGAGTCAGAGAGGT	60441532	60441551		
	8F	AATCTCCGGGACACAGAGTG	60443427	60443446	153	5411
	8R	TCTGCTGGCTTTTGGATTTT	60443560	60443579		

FRA3B ori 2	1F	TTGGCTATACAGAGGTCTTCATAGATT	60445108	60445134	119	-3568.5
	1R	GAAATAAGAGAAAACAAATTGGACAAC	60444940	60444966		
	2F	TTCCTATTAGAGTGGACAGCATAGATT	60446876	60446902	-100	-1780
	2R	AACAGTTTCCCTATCAATAGTCATTTG	60446749	60446775		
	3F	TGAACAATTCCCCAAAGCTC	60447209	60447228	115	-1339.5
	3R	TGCAATGCTTTCTTTGCCTA	60447304	60447323		
	4F	CTAAGCAACCCTGCCACAAG	60447745	60447764	117	-802.5
	4R	CCCAAAGAGATCAGCCAAGA	60447842	60447861		
	5F	TTTCTCCAGCCAAAGCAAAT	60448678	60448697	111	127.5
	5R	TGGCTTCACAGATGGTCTTG	60448769	60448788		
	6F	GCAGGGTAGGGGATATTCTCA	60449364	60449384	147	831.5
	6R	TGCCTGAATATAGGGCTTGG	60449491	60449510		
	7F	CAATTCACAGAGGTGGCAAA	60450032	60450051	112	1482
	7R	GCCAGGATTTTGTTGCTTTC	60450124	60450143		
	8F	CTGAACAGGAGGCTCCAAAC	60450646	60450665	108	2094
	8R	TTCCTGCCTGTCATGTCGTA	60450734	60450753		
	9F	AAGGCCAACCAGAACAAAAA	60452201	60452220	107	3648.5
	9R	TCTGGCTGTGGCTGAAGATA	60452288	60452307		
FRA3B ori 3	1F	GGTCAGCTGGCTGAAATCAT	60461594	60461613	132	-984
	1R	GAATATGTGCCAAGCCCTGT	60461706	60461725		
	2F	ATTTACGCCAGCTCTTCCAA	60462208	60462227	155	-358
	2R	AGCCAGGAAGTGGAACTCAA	60462343	60462362		
	3F	GGCGGTTCTTTGATAAGCAG	60462704	60462723	134	128
	3R	TGACAACCTAGACCCCCTTG	60462818	60462837		
	4F	CAGCAGCCTGGGAAAGATTA	60463016	60463035	130	438
	4R	GACAGCTCCTTGGAGACAGG	60463126	60463145		
	5F	TTTTCTTGCGGGTTTAGGTC	60463261	60463280	105	670
	5R	TGATGCCTGACATTATTGTGCT	60463344	60463365		
	6F	TGTAGTCATGAGGGCTGACG	60463492	60463511	141	919
	6R	CCATCTTCCTTTCTGCCATT	60463613	60463632		
	7F	GCCTATGATAGCAAAGATGC	60464197	60464216	151	1629
	7R	AGAAGTCATGCCACAACCAA	60464327	60464346		
	8F	CCAGATTATATCACAAAGTGACG	60464768	60464790	145	2196
	8R	TGTGACTGACATTGGTGGCTA	60464890	60464910		
Chr22	R1_1_F	ATGGGGTAATGGGACAACCT	29185290	29185309	141	NA
	R1_1_R	CCTGTGGGCTACAACCTCAT	29185411	29185430		
	R1_2_F	TTCCAGTGTCTGTCCTGCAC	29185554	29185573	128	NA
	R1_2_R	GGTTTGTTGGCTGAATGGTT	29185662	29185681		
FRAXA	FRAXA_R1_1_F	TAAATTTGGGGATCGTCAGC	146825195	146825214	128	NA
	FRAXA_R1_1_R	TGCTGGCTTTCCTCTCTTTC	146825303	146825322		

The primers were designed to meet universal conditions (60^0C, no extra $MgCl_2$ added to the ABI Fast SYBR Real-time PCR mix).

The shaded rows indicate the non-origin region primers used for normalizing the nascent strand DNA abundance between untreated and APH-treated samples at the *MYC*, *TBC1D10A*, *RNF185*, and FRA3B origins in Figures 5 and 6.

Table S3. Primers used for the timing of origin firing at the *MYC*, FRA3B, FRAXA, and *RNF185* loci by Real-time PCR.

Region	**Primer name**	**Primer sequence**	**Start (bp) NCBI Build 35**	**End (bp) NCBI Build 35**	**Mg (mM)**	**Temp**
***MYC* (ori)**	**5.5F**	TACAGACTGGCAGAGAGCAG	128816612	128816631	2	60
	5.5R	ATGTATGCACAGCTATCTGG	128816805	128816824		
***MYC* (non-ori)**	**0F**	GAGTTGGCAACCCTTGATGT	128808885	128808904	2	58
	0R	GTTAGGATTTCCCGCCTTTC	128809139	128809158		
FRA3B ori 1 (ori)	**3.5F**	TTTAGGATACCTCCAAAGGG	60447273	60447292	4	60
	3.5R	GGAGAAGTGTTTTGGCTTTG	60447477	60447496		
FRA3B ori 1 (non-ori)	**0F**	TCACCACTGACACCACAGAA	60443771	60443790	2.5	61
	0R	GACACCAGTTCCTCTTTGTA	60444022	60444041		
FRAXA (ori)	**FRAXA-ori-f**	AGGTCTCCTTTGGCTTCTCT	146699760	146699779	2	63
	FRAXA-ori-r	ATGGTTTTAGACGCTGAAGC	146700045	146700064		
FRAXA (non-ori)	**FRAXA-neg-f**	GAGCAGTGGTTCCTGTTGGT	146704269	146704288	4	68
	FRAXA-neg-r	CTAGCAACTGGGCCAAAGAG	146704450	146704469		
***RNF185* (ori)**	**9.5F**	CAGGATCTGGGTGACTTTGT	29916460	29916479	3	63
	9.5R	AGAAGTTAGAGGAGCAGGTG	29916745	29916764		
***RNF185* (non-ori)**	**0F**	TCACAGCACTCTGGATGAGG	29908640	29908659	2	62
	0R	TAGGGCAAGAGCCTCAAGAA	29908876	29908895		

Table S4. Real-time quantification using the primer pairs representing the origin region and a non-origin region

Time after release (h)		**0**	**2**	**4**	**6**	**8**
MYC	**ori**	0.2 ± 0.01	1.2 ± 0.04	6.9 ± 0.00	1.7 ± 0.00	0.1 ± 0.01
	non-ori	0.1 ± 0.02	0.3 ± 0.02	8.5 ± 0.19	1.9 ± 0.06	0.1 ± 0.00
RNF185	**ori**	1.2 ± 0.08	7.5 ± 0.09	323.4 ± 13.18	75.9 ± 5.75	0.3 ± 0.10
	non-ori	0.1 ± 0.01	0.5 ± 0.01	14.6 ± 1.31	3.0 ± 0.27	0.0 ± 0.00
FRAXA	**ori**	2.4 ± 0.74	9.0 ± 4.63	464.5 ± 97.37	134.5 ± 37.74	0.1 ± 0.01
	non-ori	0.2 ± 0.03	0.8 ± 0.05	19.3 ± 2.43	5.4 ± 0.15	0.0 ± 0.01
FRA3B ori 2	**ori**	0.1 ± 0.00	0.7 ± 0.28	68.8 ± 5.61	12.7 ± 0.61	0.0 ± 0.00
	non-ori	0.1 ± 0.02	0.3 ± 0.08	6.3 ± 0.08	2.3 ± 0.21	0.0 ± 0.00

The average and the standard deviation of the nascent strand DNA quantification performed in triplicate are indicated for each primer set.