Figure 1

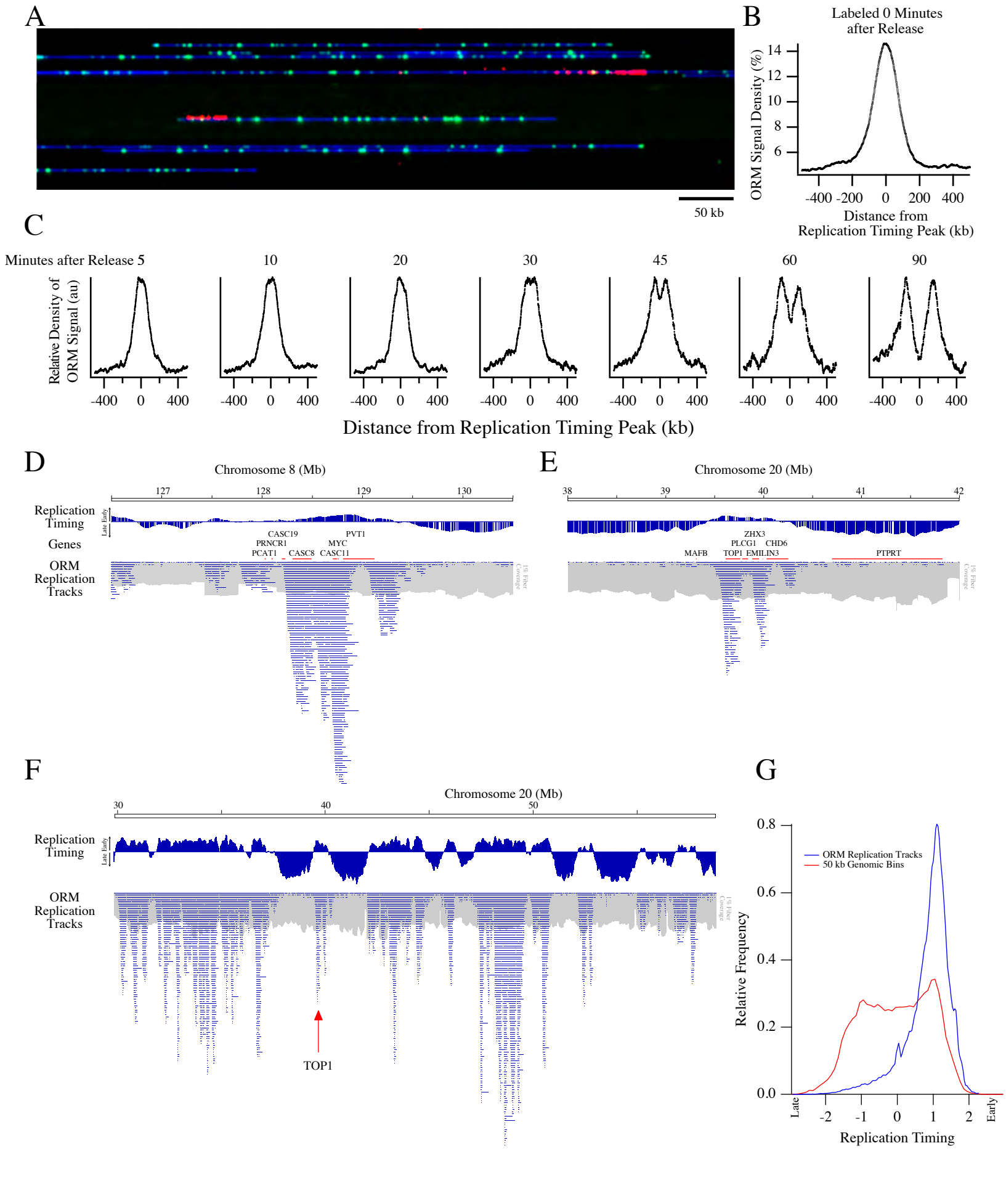

A
50 kb
B
Labeled 0 Minutes after Release
ORM Signal Density (%)
14
12
10
8
6
-400 -200 0 200 400
Distance from Replication Timing Peak (kb)
C
Minutes after Release 5
10
20
30
45
60
90
Relative Density of ORM Signal (au)
-400 0 400
Distance from Replication Timing Peak (kb)
D
Chromosome 8 (Mb)
127
128
129
130
Replication Timing
Late Early
Genes
CASC19
PVT1
PRNCR1
MYC
PCAT1
CASC8
CASC11
ORM Replication Tracks
1% Fiber Coverage
E
Chromosome 20 (Mb)
38
39
40
41
42
ZHX3
PLCG1
CHD6
MAFB
TOP1
EMILIN3
PTPRT
F
Chromosome 20 (Mb)
30
40
50
Replication Timing
ORM Replication Tracks
TOP1
G
ORM Replication Tracks
50 kb Genomic Bins
Relative Frequency
0.8
0.6
0.4
0.2
0.0
Late
-2
-1
0
1
2
Early
Replication Timing

Figure 2

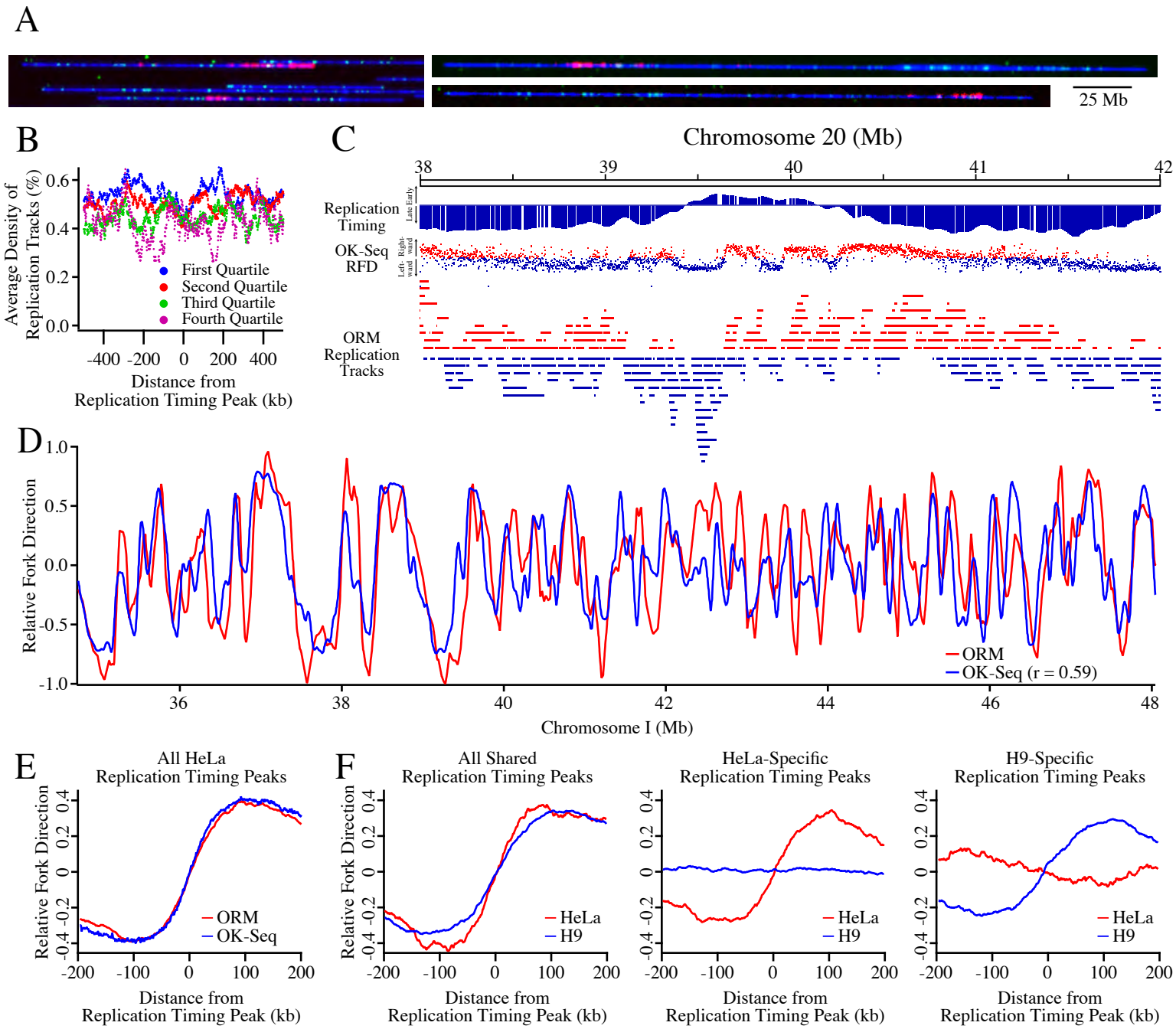

A
25 Mb
B
Average Density of Replication Tracks (%)
0.6
0.4
0.2
0.0
First Quartile
Second Quartile
Third Quartile
Fourth Quartile
-400 -200 0 200 400
Distance from Replication Timing Peak (kb)
C
Chromosome 20 (Mb)
38 39 40 41 42
Replication Timing
Late Early
OK-Seq RFD
Left-ward Right-ward
ORM Replication Tracks
D
Relative Fork Direction
1.0
0.5
0.0
-0.5
-1.0
ORM
OK-Seq (r = 0.59)
36 38 40 42 44 46 48
Chromosome I (Mb)
E
All HeLa Replication Timing Peaks
Relative Fork Direction
ORM
OK-Seq
-200 -100 0 100 200
Distance from Replication Timing Peak (kb)
F
All Shared Replication Timing Peaks
HeLa-Specific Replication Timing Peaks
H9-Specific Replication Timing Peaks
HeLa
H9
-0.4 -0.2 0 0.2 0.4
-200 -100 0 100 200
Distance from Replication Timing Peak (kb)

Figure 3

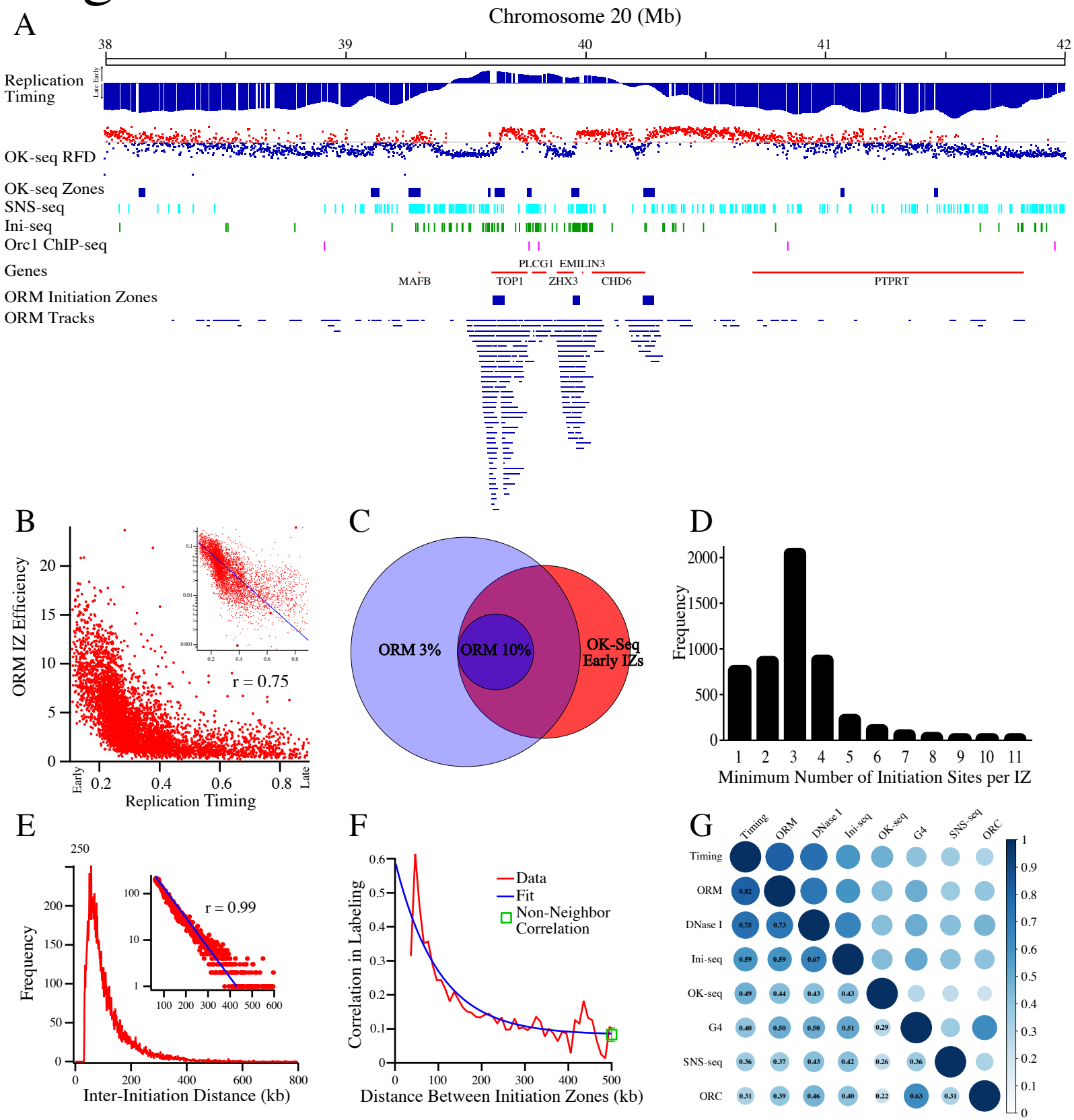

A
Chromosome 20 (Mb)
38
39
40
41
42
Replication Timing
Late Early
OK-seq RFD
OK-seq Zones
SNS-seq
Ini-seq
Orc1 ChIP-seq
Genes
PLCG1 EMILIN3
MAFB
TOP1
ZHX3
CHD6
PTPRT
ORM Initiation Zones
ORM Tracks
B
ORM IZ Efficiency
r = 0.75
Early
Late
Replication Timing
C
ORM 3%
ORM 10%
OK-Seq Early IZs
D
Frequency
Minimum Number of Initiation Sites per IZ
E
Frequency
r = 0.99
Inter-Initiation Distance (kb)
F
Correlation in Labeling
Data
Fit
Non-Neighbor Correlation
Distance Between Initiation Zones (kb)
G
Timing
ORM
DNase I
Ini-seq
OK-seq
G4
SNS-seq
ORC

Figure 4

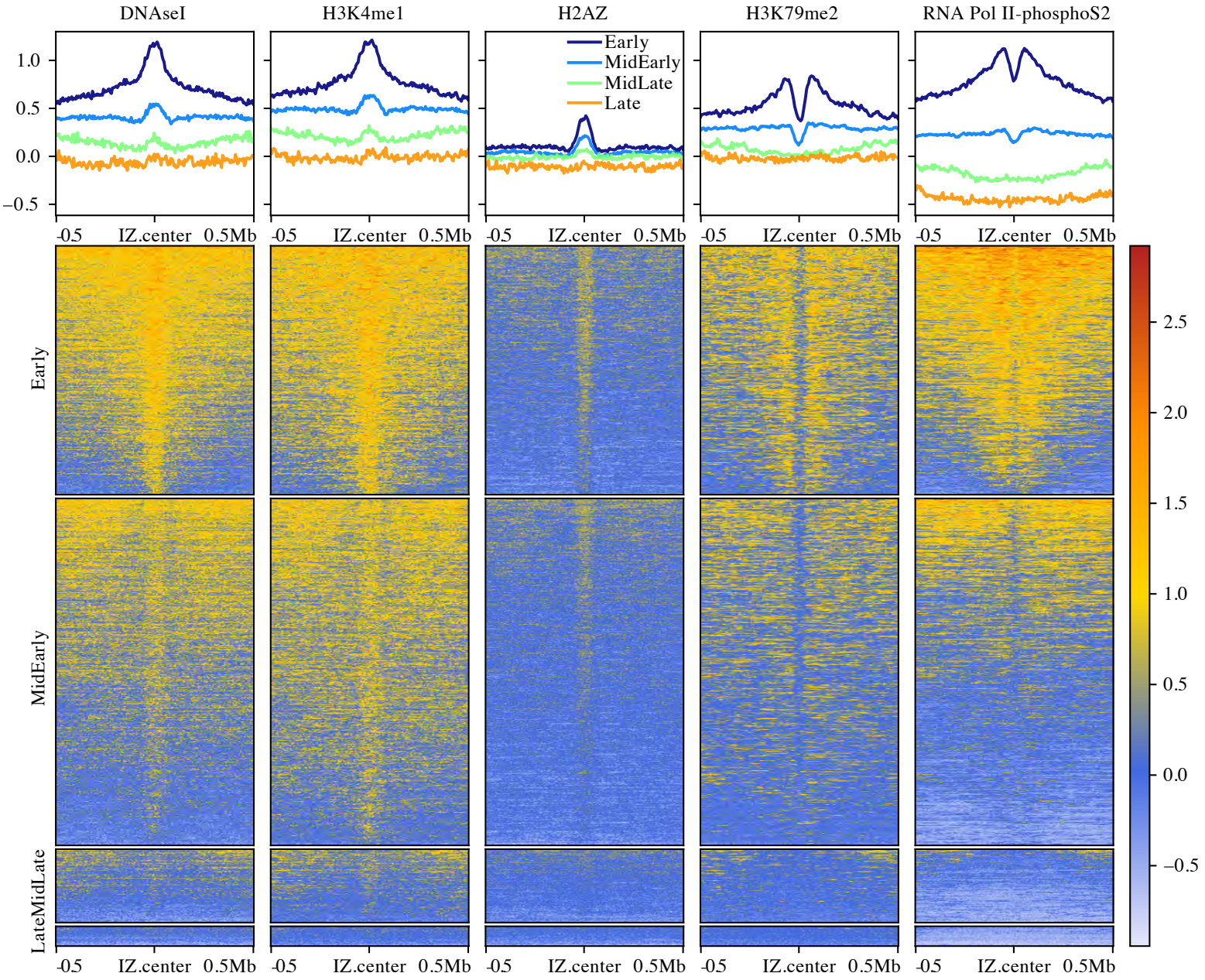

DNAseI
H3K4me1
H2AZ
H3K79me2
RNA Pol II-phosphoS2
Early
MidEarly
MidLate
Late
1.0
0.5
0.0
−0.5
-0.5
IZ.center
0.5Mb
Early
MidEarly
LateMidLate
2.5
2.0
1.5
1.0
0.5
0.0
−0.5

Figure 5

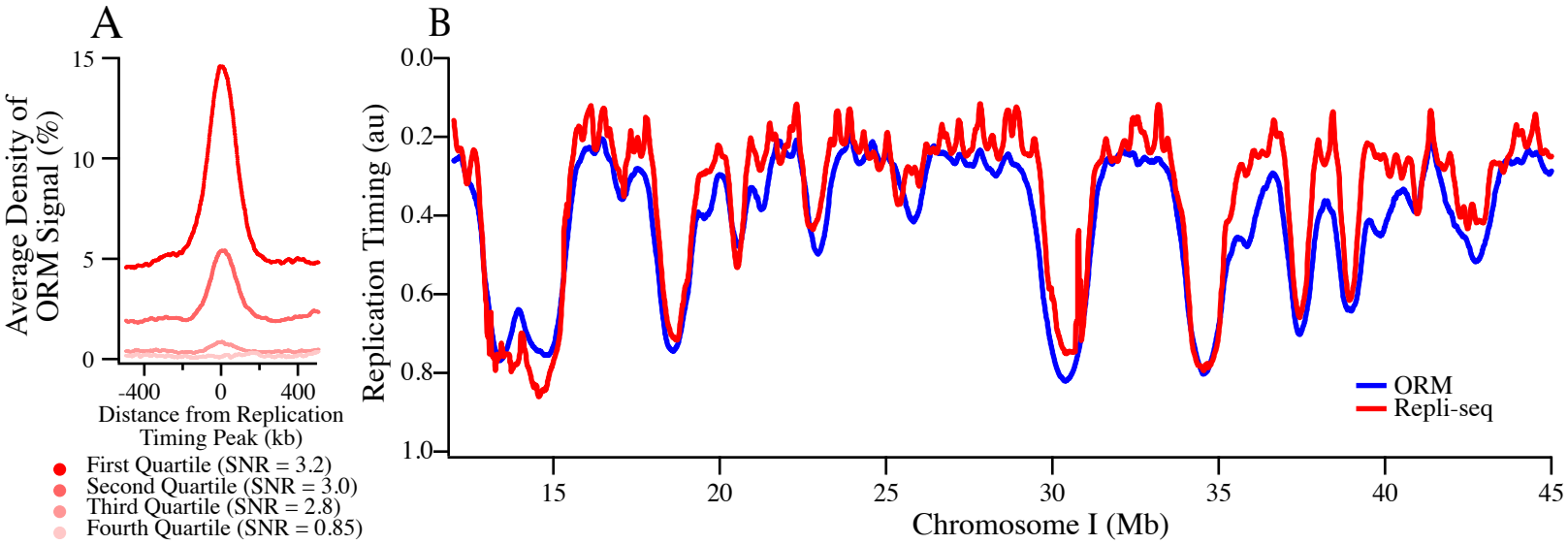
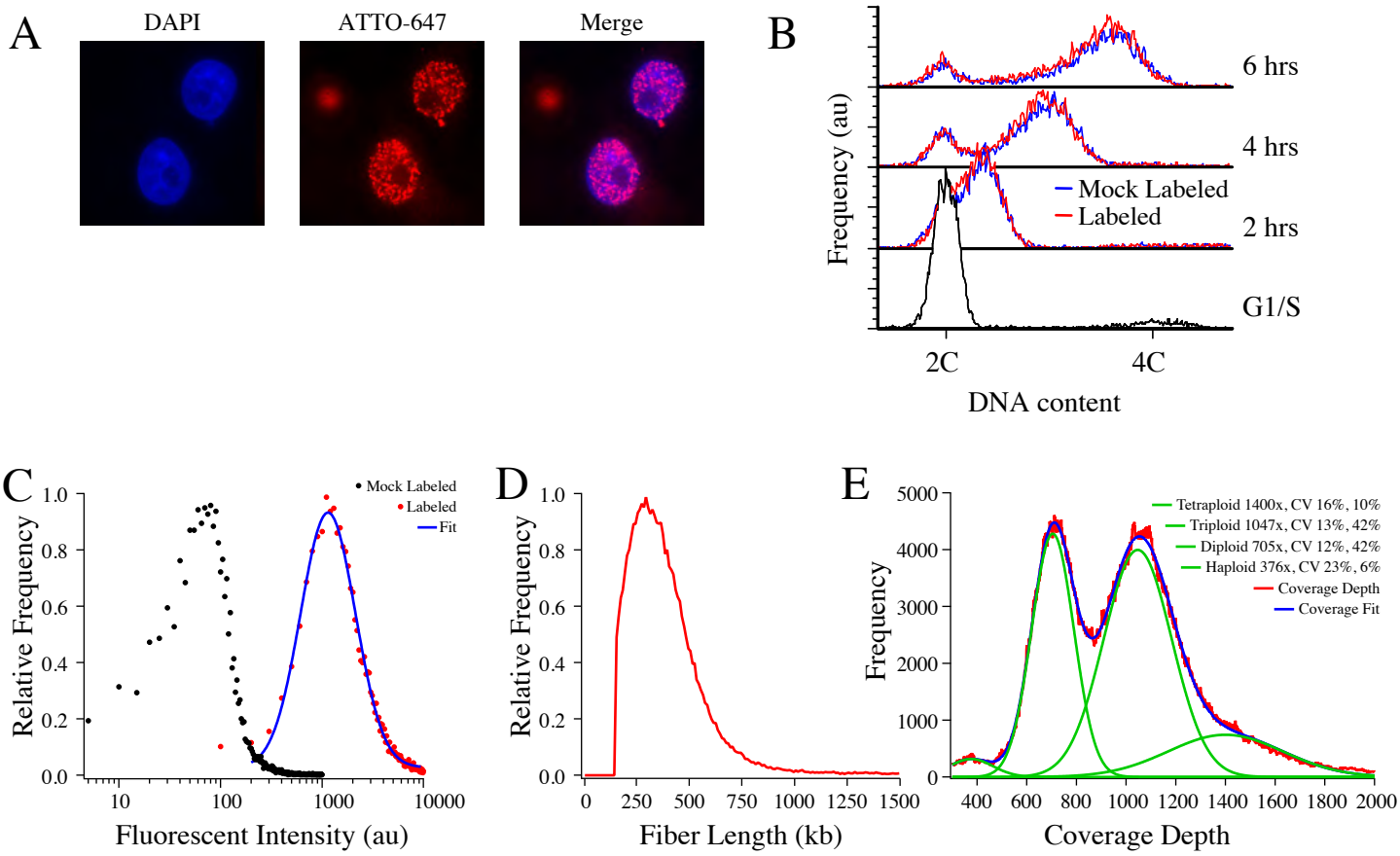
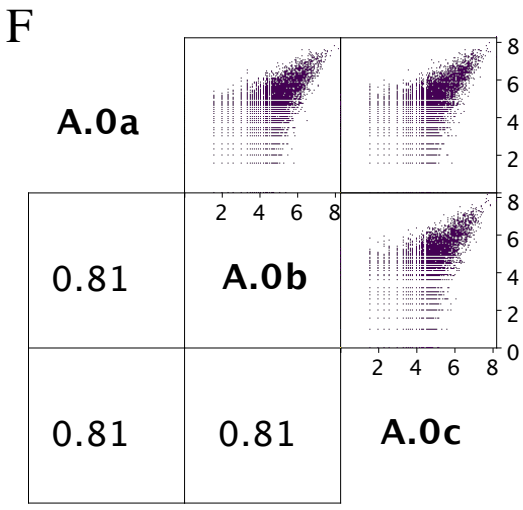

A
B
Average Density of ORM Signal (%)
15
10
5
0
-400
0
400
Distance from Replication Timing Peak (kb)
First Quartile (SNR = 3.2)
Second Quartile (SNR = 3.0)
Third Quartile (SNR = 2.8)
Fourth Quartile (SNR = 0.85)
Replication Timing (au)
0.0
0.2
0.4
0.6
0.8
1.0
15
20
25
30
35
40
45
Chromosome I (Mb)
ORM
Repli-seq

Figure S1


A
DAPI
ATTO-647
Merge
B
6 hrs
4 hrs
2 hrs
G1/S
Mock Labeled
Labeled
Frequency (au)
2C
4C
DNA content
C
Mock Labeled
Labeled
Fit
Relative Frequency
Fluorescent Intensity (au)
D
Relative Frequency
Fiber Length (kb)
E
Tetraploid 1400x, CV 16%, 10%
Triploid 1047x, CV 13%, 42%
Diploid 705x, CV 12%, 42%
Haploid 376x, CV 23%, 6%
Coverage Depth
Coverage Fit
Frequency
Coverage Depth


F
A.0a
0.81
A.0b
0.81
0.81
A.0c

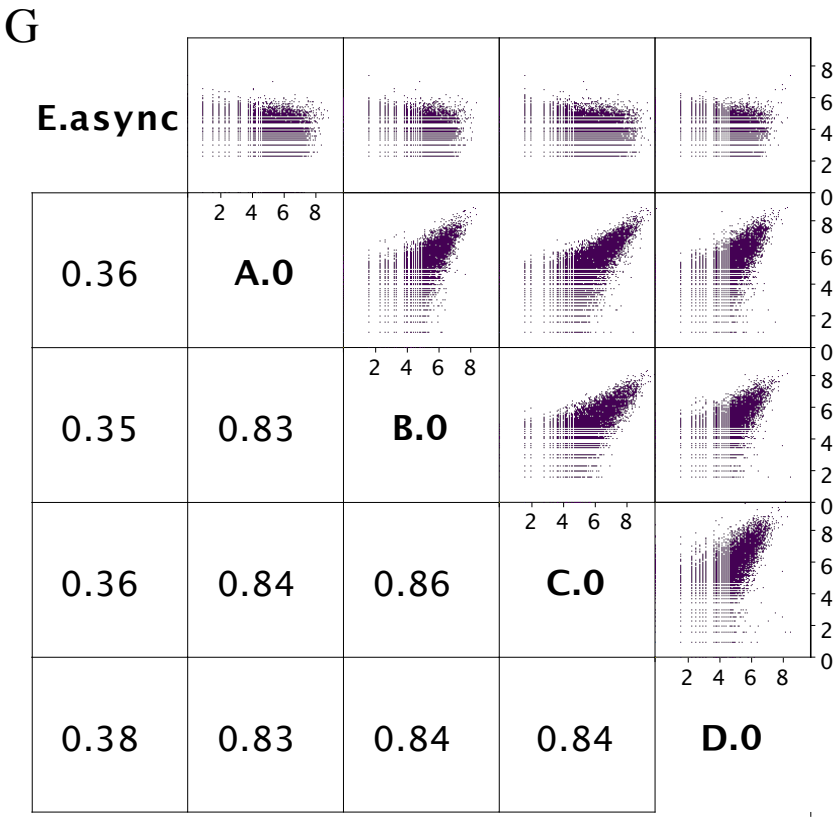

G
E.async
0.36
A.0
0.35
0.83
B.0
0.36
0.84
0.86
C.0
0.38
0.83
0.84
0.84
D.0

Figure S2

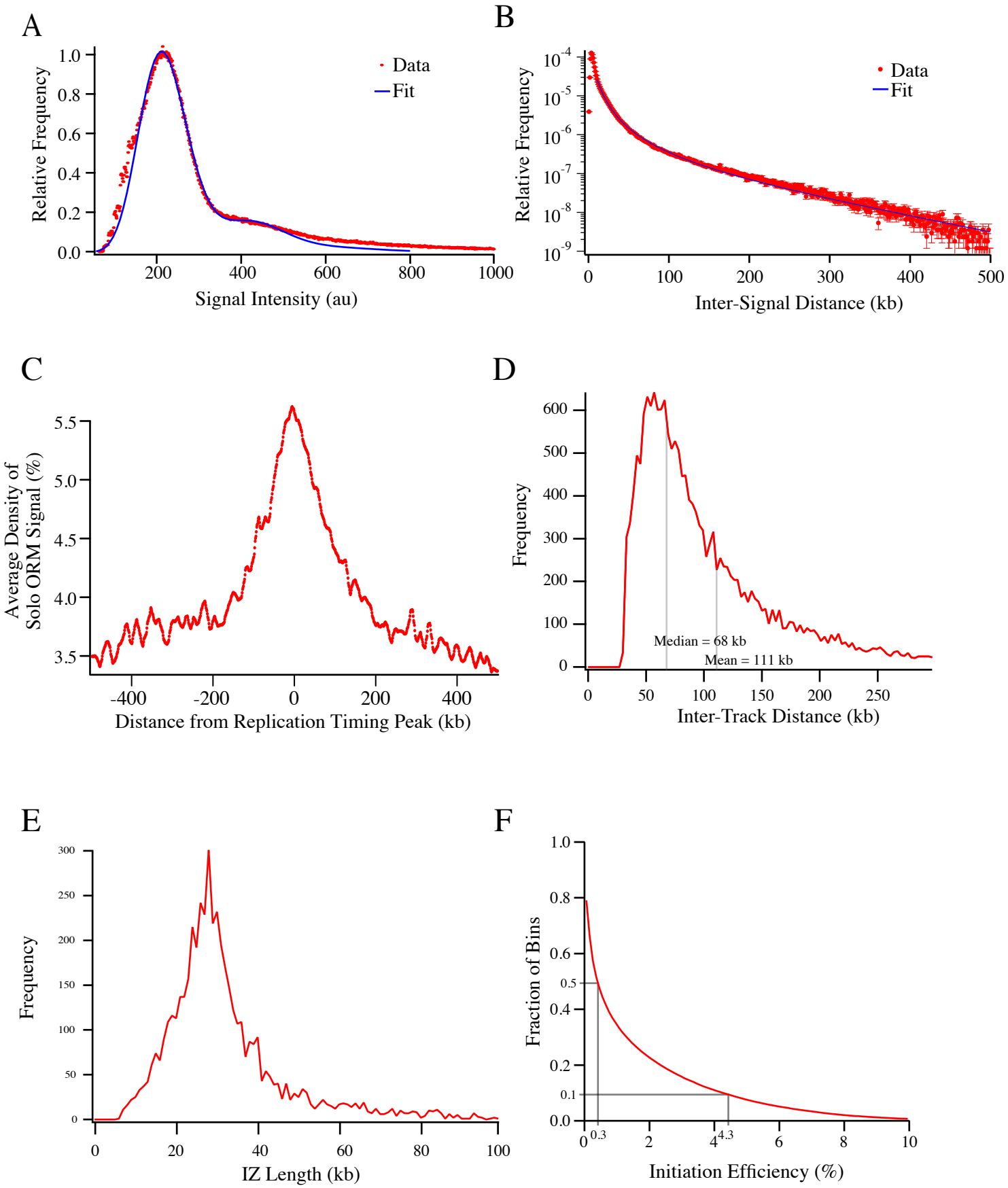

A
Relative Frequency
Signal Intensity (au)
Data
Fit
B
Relative Frequency
Inter-Signal Distance (kb)
Data
Fit
C
Average Density of Solo ORM Signal (%)
Distance from Replication Timing Peak (kb)
D
Frequency
Inter-Track Distance (kb)
Median = 68 kb
Mean = 111 kb
E
Frequency
IZ Length (kb)
F
Fraction of Bins
Initiation Efficiency (%)

Figure S3

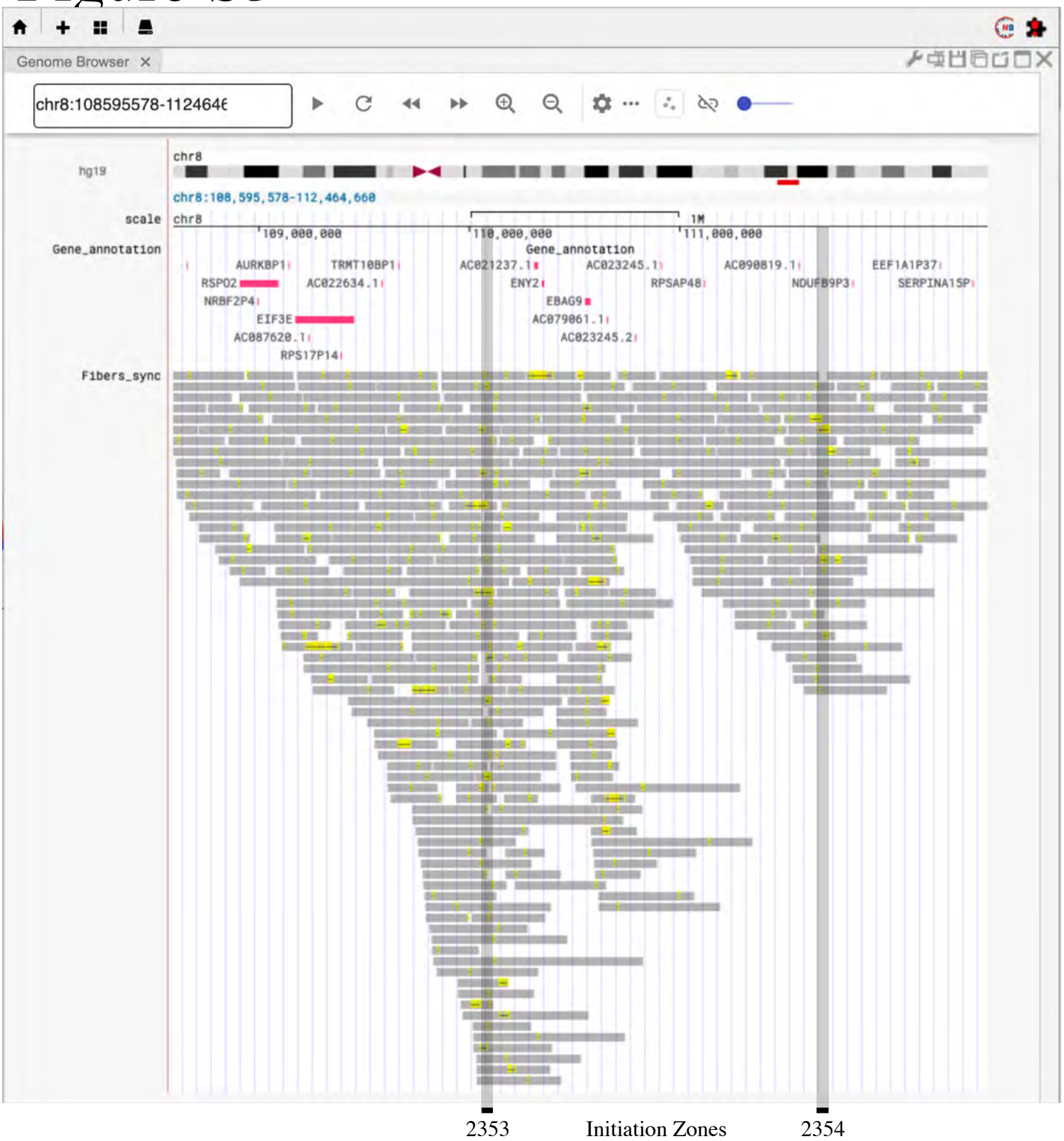


Figure S3. The ORM Genome Browser

The ORM Genome Browser allows interactive visualization the HeLa ORM data. Shown is a screen shot of the Fibers track of synchronous data. It shows fibers as gray bars, ORM signals as yellow hash marks, and inferred replication tracks as black lines. Only fibers labeled with ORM signals are displayed because only~5% of fibers are labeled; displaying all fibers is impractical. The browser can also display only the replication tracks, to provide a more easily-visualized view of replication initiation. It can also show the fibers and the replication tracks from the asynchronous data. Two low-efficiency initiation zones are shown, 2353 (3%) and 2354 (2%), because higher-efficiency initiation zones contain too many labeled fibers. Note that, although the signals and replication tracks are concentrated around the initiation zones, many lay outside of them and none are concentrated in discrete areas.

Figure S4

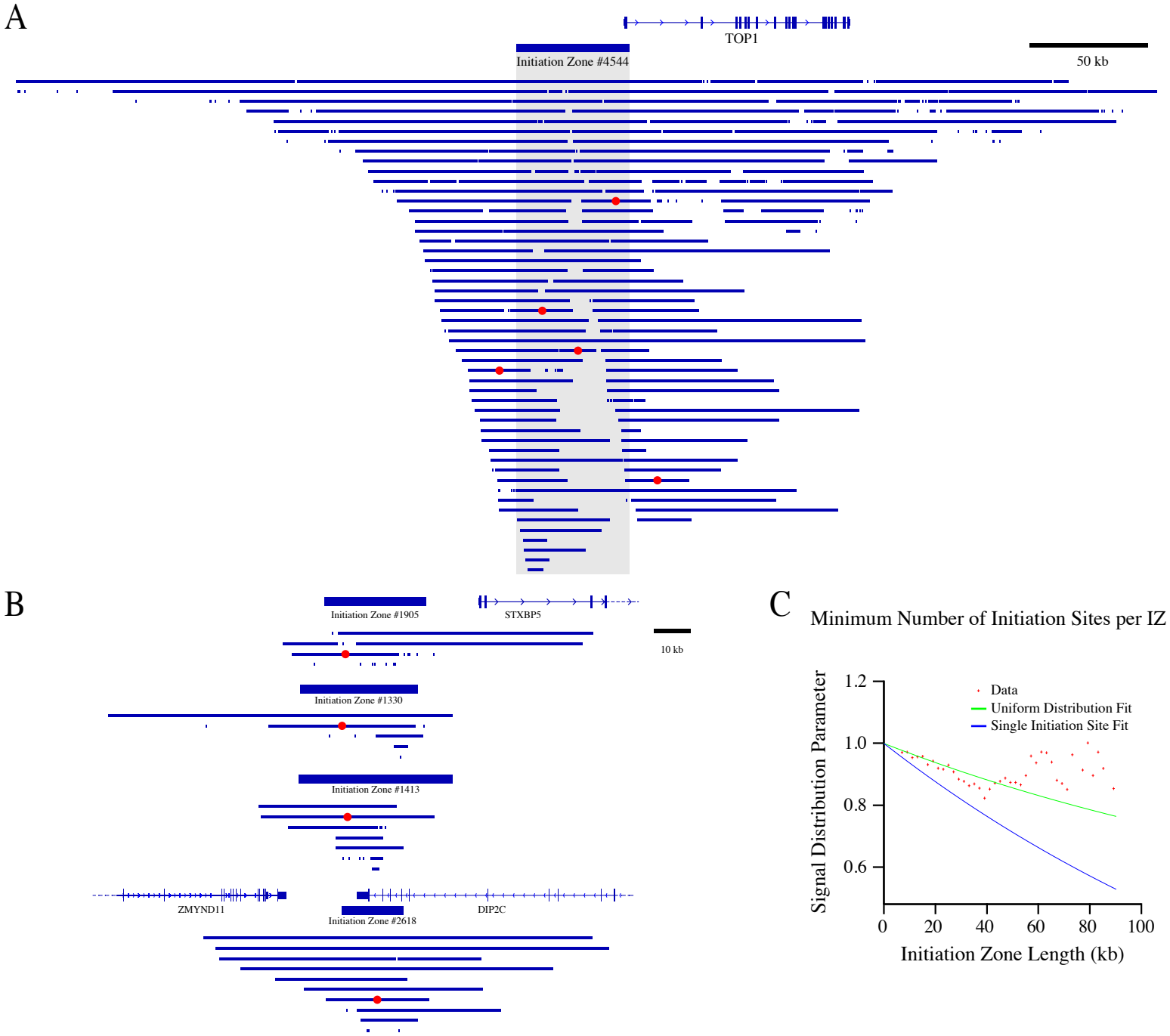


Figure S4. Distribution of Replication Tracks within Initiation Zones

A) The distribution of replication tracks in the merged 0 minute dataset at the Top1 locus. The Top1 IZ has an estimated minimum of five initiation sites because the five replication track centers indicated in red are all 15 kb away from each other.

B) The distribution of replication tracks at four examples of IZs for which our estimate of the minimum number of initiation sites is 1.

C) The distribution of signal across IZs. The ratio of signal frequency at the IZ center to the IZ boundary is plotted versus IZ length. This value is expected to decrease more quickly in IZs that predominantly have a single initiation site (Eq. 25) than if initiation is distributed across the IZ (Eq. 28, Supplemental Mathematical Methods). The distribution across IZs shorter than 55 kb is consistent with a uniform distribution of initiation sites. At longer lengths, we actually see more signal at the edges of the IZs than it the center. One possible explanation for this phenomenon is that larger IZs may actually be two smaller IZs fused together such that there is more initiation sites towards the edges of the fused IZ and less initiation in the center, which is actually between the two constituent IZs. See Supplemental Mathematical Methods for details.

Figure S5

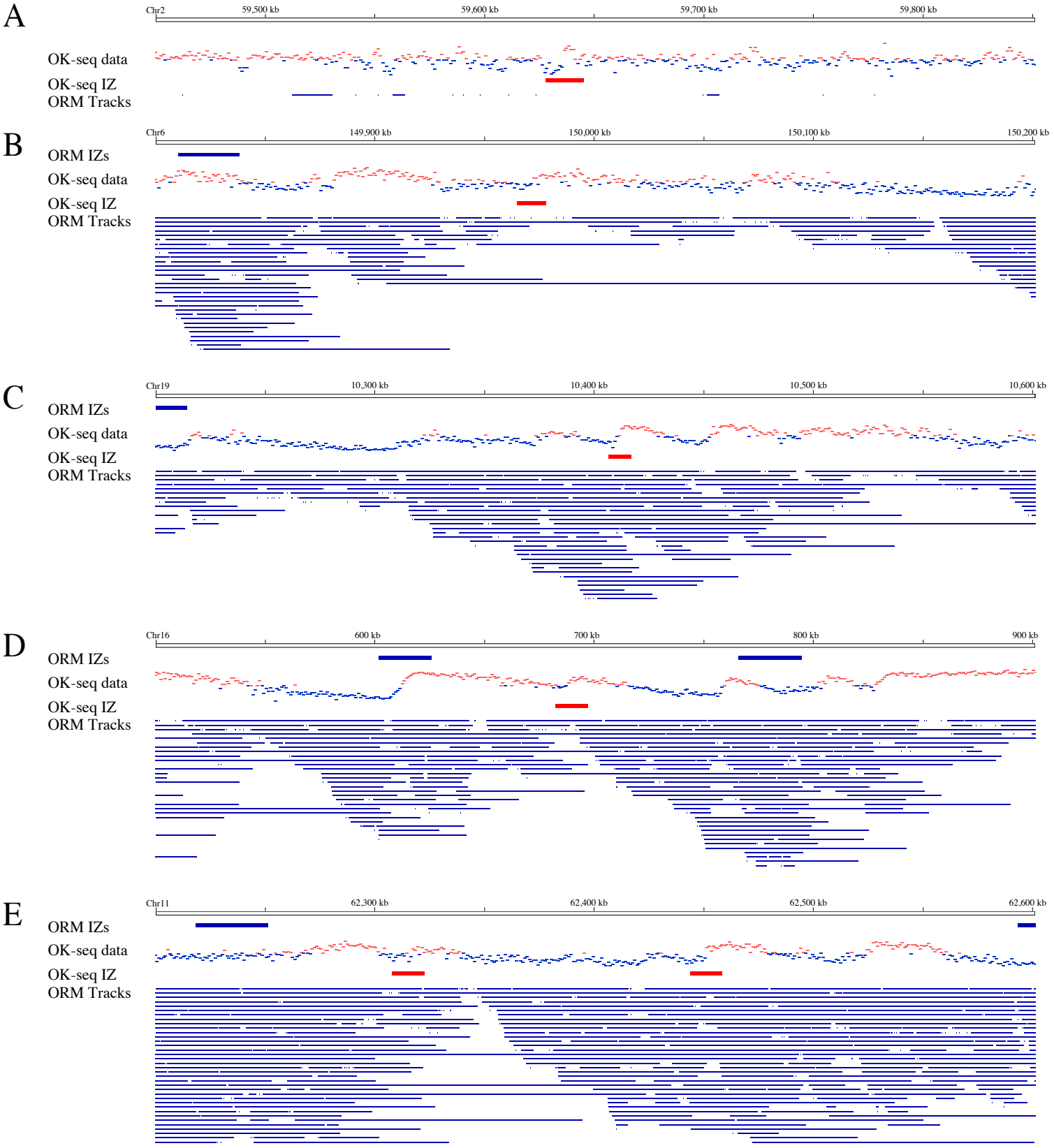

A
Chr2
59,500 kb
59,600 kb
59,700 kb
59,800 kb
OK-seq data
OK-seq IZ
ORM Tracks
B
Chr6
149,900 kb
150,000 kb
150,100 kb
150,200 kb
ORM IZs
OK-seq data
OK-seq IZ
ORM Tracks
C
Chr19
10,300 kb
10,400 kb
10,500 kb
10,600 kb
ORM IZs
OK-seq data
OK-seq IZ
ORM Tracks
D
Chr16
600 kb
700 kb
800 kb
900 kb
ORM IZs
OK-seq data
OK-seq IZ
ORM Tracks
E
Chr11
62,300 kb
62,400 kb
62,500 kb
62,600 kb
ORM IZs
OK-seq data
OK-seq IZ
ORM Tracks

Figure S6

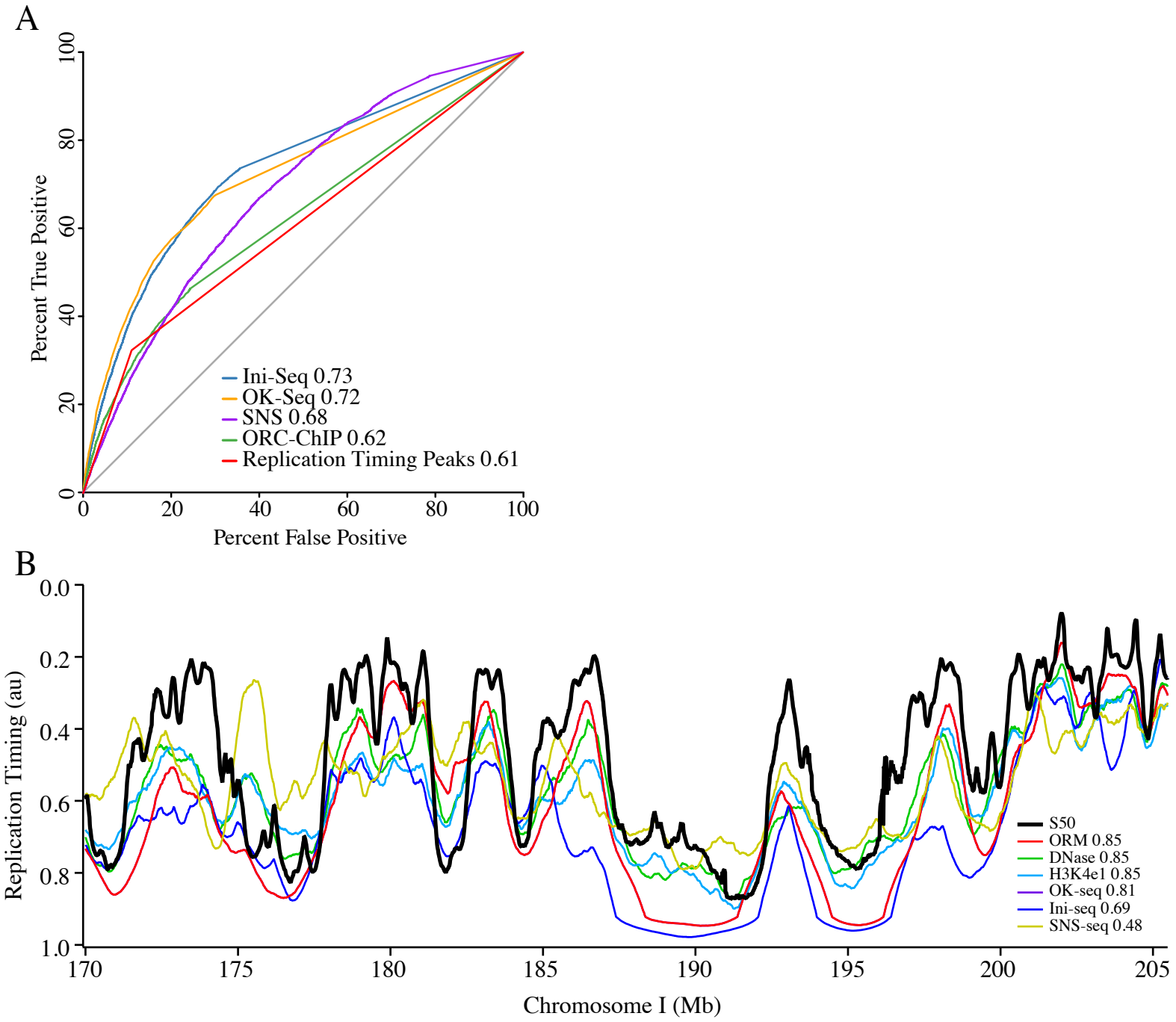


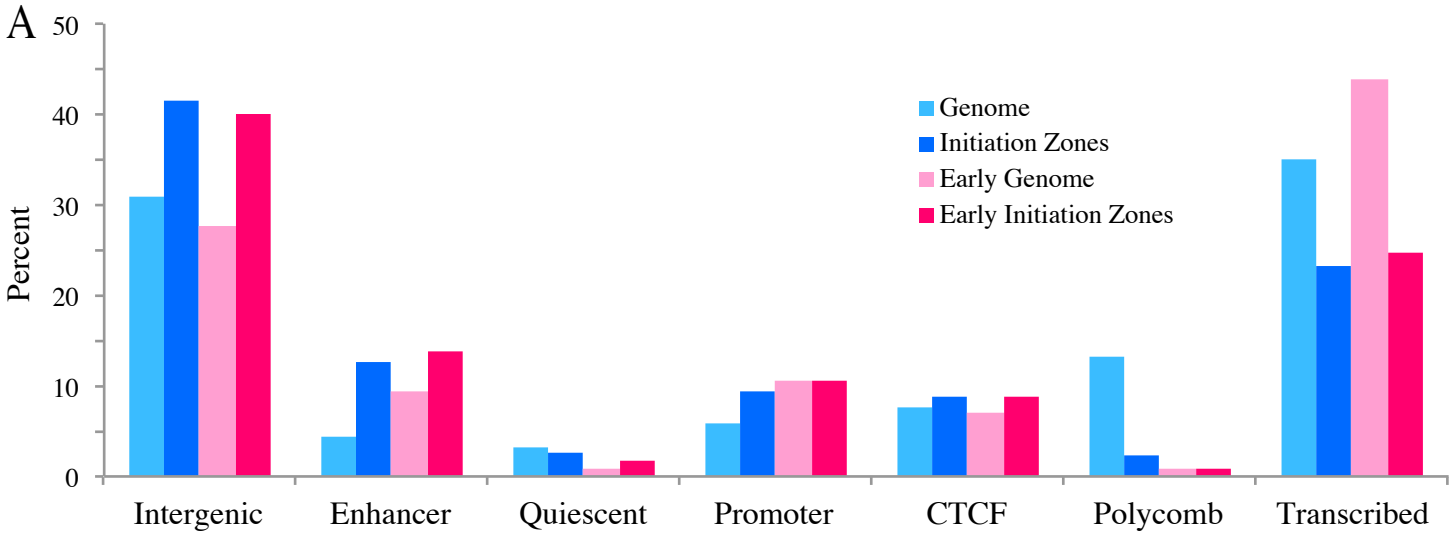
Figure S6. Simulated Replication Timing Profiles

A) ROC analysis of the association between the ORM IZs and other origin mapping data shown in Figure 3G. As shown in the ROC curves, the ORM IZs are better correlated with OK-seq IZs (AUC=0.72) and Ini-seq IZs (AUC=0.73). The bias of the SNS ROC curve towards high true positive rates only at high false negative values is consistent with that dataset having more false positive signal, whereas the bias of the replication timing and ORC datasets relatively high true positive rates only at low false negative values is consistent with those datasets having fewer, but more accurate true positives. Areas under the ROC curves (AUC) are shown in the legend.

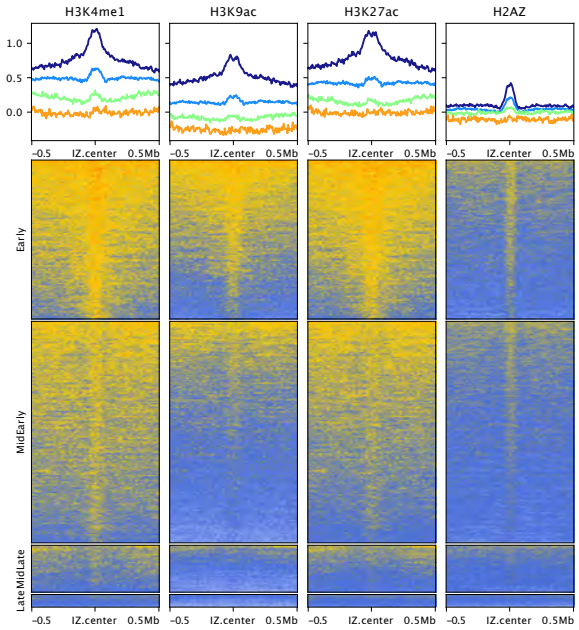
B) Comparison between experimentally determined HeLa replication timing (S50) and replication timing predicted from ORM, DNase I hypersensitivity (Bernstein et al., 2012), OK-Seq (Petryk et al., 2016), Ini-seq (Langley et al., 2016) and SNS-seq (Picard et al., 2014) data using a stochastic model (Gindin et al., 2014a). The Spearman correlation coefficients with replication timing are shown in the legend.

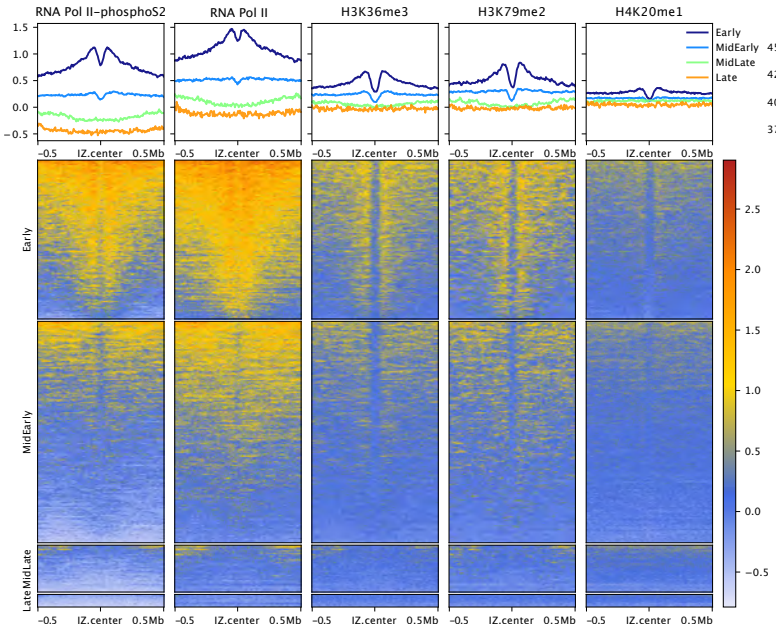
Figure S7

A

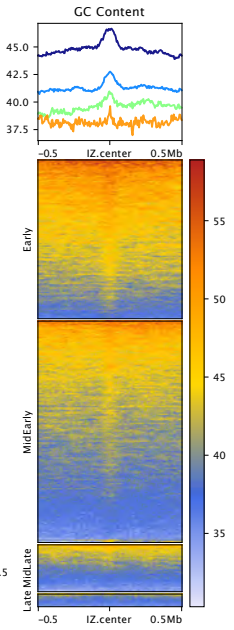


B

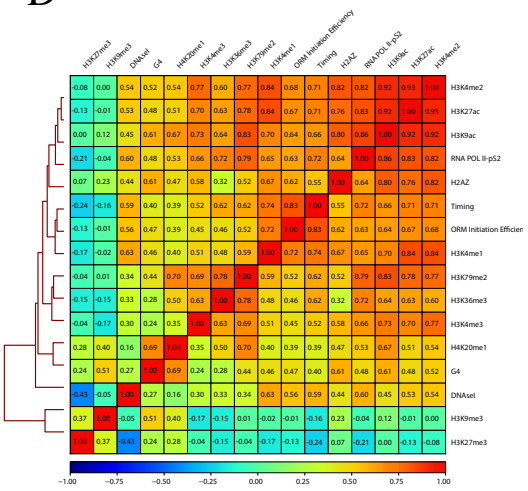




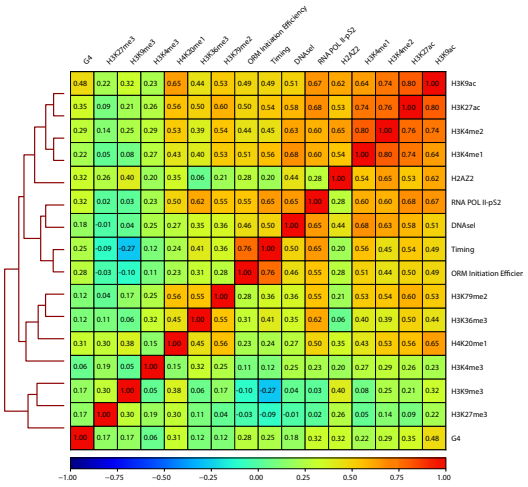
C



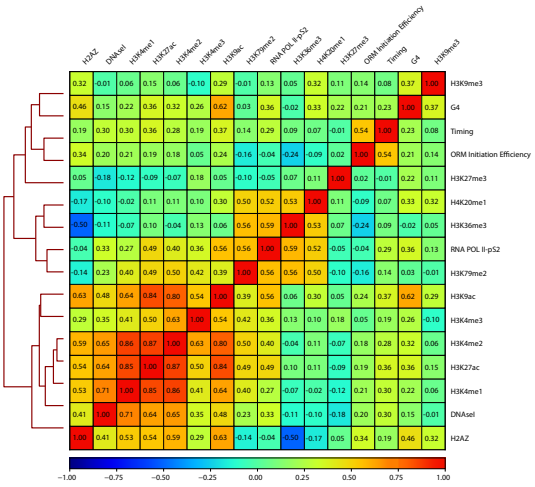
D



100 kb bins



1 kb bins



1 kb, early replicating bins