**A**

Inter-Origin Distances (kbp)

Frequency x 10^3

0 1 10 100 1000 10000

**B**

Replication Origins (%)

Mean number of DFSs

0 40 60 80 100 120 140 160

Computer model: IMR90
Mathematical model

Computer model: HeLa

**C**

S/G2

mitosis

G1

S

**D**

Untreated

Aphidicolin (0.2 μM)

**E**

G1 Cells

Poisson Distr. (λ = 0.95)

Frequency of cells (%)

Number of 53BP1 bodies per cell

0 1 2 3 4 5 6 7

**F**

Mean 53BP1 bodies

G1 (h after nocodazole release)

2 4 6 8

**G**

Mean 53BP1 bodies

S phase stage (EdU morphology)

I II III IV V
Figure 4

**A**

Microscopy image of cells stained with DAPI and EdU, showing a 10 µm scale bar.

**B**

Bar graph showing the mean number of mitotic EdU foci in cells treated with RNAi Control and RNAi Mcm5. The graph includes error bars for each group.

**C**

Western blot images of Mcm5 and Tubulin proteins after RNAi treatment, with quantification values 1.00 and 0.23.

**D**

Microscopy image of cells stained with DAPI and BLM, showing a 3 µm scale bar.

**E**

Bar graph showing the mean number of UFBs in cells treated with RNAi Control and RNAi Mcm5. The graph includes error bars for each group.

**F**

Histogram showing the frequency of cells with different numbers of UFBs per cell, with Poisson distributions for RNAi Control (λ=1.4) and RNAi Mcm5 (λ=2).

**G**

Table showing RNAi treatments: siControl, siMcm5, si53BP1, siMcm5 + si53BP1. Western blot images of 53BP1, Mcm5, and Tubulin proteins with corresponding quantification bands.

**H**

Graph showing the relative number of colonies (log) in cells treated with different RNAi treatments, plotted against HU (µM) concentrations.
Figure S1. Dynamics of 53BP1 Nuclear Bodies.
A) Mean number of G1-specific 53BP1 nuclear bodies in untreated and aphidicolin treated cells. Error bars = S.E.M of 3 replicates. B) Representative image of 53BP1 nuclear body identification at different stages of the cell cycle. Cells were labelled for 53BP1 (green), EdU (orange) and Cyclin A (red). C) Frequency of 53BP1 nuclear bodies at different stages of the cell cycle. Only G1-specific nuclear bodies fit a Poisson distribution. D) Frequency of G1-specific 53BP1 nuclear bodies in untreated and aphidicolin treated cells. n = 100 E) Frequency of cells at different stages of the cell cycle after release from nocodazole arrest. Cells in G1 were assessed for the frequencies of 53BP1 nuclear bodies from T2-T8 (green area) and cells from T8-T12 were used to identify the S-phase pattern (yellow area).
**Figure S2. Quantification of origin reduction**

A) Western Blot for replication origin depletion in HeLa cells using RNAi against Cdt1. Cells were transfected for the indicated time before harvesting. B) Representative FACS plot of chromatin associated Mcm2 in early S phase cells after Cdt1 depletion. C) PI/EdU FACS profiles for Mcm5 and Cdt1 depletion in HeLa cells. D) Frequency distribution of 53BP1 nuclear bodies in Mcm5 RNAi treated cells (left) and Cdt1 RNAi treated cells (right). E) Mean number of G1-specific 53BP1 nuclear bodies in response to varying degrees of licensing knockdown as measured in B) after Cdt1 RNAi. 53BP1 nuclear bodies were quantified in triplicate in at least 100 cells.
Figure S3. Cdc6 overexpression.
A) Mean number of 53BP1 nuclear bodies in un-induced and overexpression of Cdc6 in HBEC cells. Error bars = S.E.M of 3 replicates, p = 0.00662. B) FACS profiles of the distribution of chromatin associated Mcm2 and the EdU incorporation of un-induced and Cdc6 overexpressing HBEC cells.
Figure S4. G1-specific γ-H2AX foci.
A) Representative staining of γ-H2AX foci in asynchronous HeLa cells. Intense γ-H2AX foci were quantified in cells that stained negatively for EdU and CyclinA. B) Transfected cells used for microscopy were tested for successful depletion by immunoblotting for Mcm5. Quantification of Mcm5 reduction indicated below the corresponding treatments.
Moreno et al, Supplementary Figure S5

### Figure S5. Details of 53BP1 ChIP-Seq

A) Experiment names, key and number of reads. Details of the alignment can be found in Supplementary Material. B-C) ChIP-Seq quality analysis for 53BP1 (B) and IgG (C). Quality score with respect to the length of the reads (left) and quality distribution (right). D) Plot of the average 53BP1/IgG signal ratio per kilobase against replicon size including S.E.M. (error bars not reported when <3 values are present).

<table>
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<tr>
<th>Experiment</th>
<th>Key</th>
<th>Reads</th>
<th>Aligned reads</th>
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<tr>
<td>53BP1 Experiment 1 – Technical replica 1</td>
<td>1</td>
<td>15'085'705</td>
<td>13'431'878 (89.04 %)</td>
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<td>25'316'792</td>
<td>22'624'955 (89.37 %)</td>
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<tr>
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<td>35'869'224</td>
<td>31'546'518 (87.95 %)</td>
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<td>35'869'224</td>
<td>31'546'518 (87.95 %)</td>
</tr>
<tr>
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<td>16'632'221 (89.21 %)</td>
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Figure S6. 53BP1 ChIP-Seq distributions.
A) Total intensity of 53BP1 in the nucleus and in nuclear bodies obtained by quantifying microscopy images of cells expressing GFP-53BP1. B) Immunoblot of 53BP1 in CSK-extracted pellets and CSK-solubilised fractions of HeLa cells. C) Distribution of ChIP-Seq reads for 53BP1 and control IgG across the entire human genome, with DNA grouped into 1 kb bins. D) ChIP-Seq 53BP1/IgG ratio for Common Fragile Sites. E) Mean replication timing computed for each 1kb genomic region enriched in 53BP1 using timing data from Weddington et al (BMC Bioinformatics 9:530). The null distribution, using all the values reported for HeLa, is plotted for comparison (left). The distributions are significantly different (Wilcoxon signed rank test p-value <10^{-10}). F) Frequency of 53BP1+ and 53BP1- replicons of different sizes (Chi-Squared p-value = 5x10^{-4}). G) Plating efficiency of cells for the clonogenic assay. Error bars = S.E.M. of three independent experiments for untreated cells and for the four different genotypes used in the clonogenic assays.