Supplementary Files

for

DDK regulates replication initiation by controlling

the multiplicity of Cdc45-GINS binding to Mcm2-7

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**Supplementary File 1a. Parameters used for fitting of Cdc45 fluorescence-intensity histograms.**

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| --- | --- |
| **Fit parametersb****[90% CI]** | **Fraction of time that DNAs with loaded Mcm2-74SNAP549 were bound by this number of Cdc45SORT649 molecules (± SE)d** |
| **Condition****(Figure)** | ***q*** | ***s*****(× 10-3)** | **λ** | ***x*****(× 10-3)** | **0** | **1** | **2** | **3** | **4** | **5** | **n** |
| Cdc45, 1.3 nM DDK, –SDPGC(Figure 2C – panel i)a | 0.69[0.66, 0.72] | 13.41[13.36, 13.49] | 0.092[0.090, 0.094] | 4.12[4.03, 4.21] | 0.682(± 0.001) | 0.222(± 0.002) | 0.079(± 0.001) | 0.0159(± 0.0004) | - | - | 78 |
| Cdc45, 1.3 nM DDK, +SDPGC(Figure 2C – panel ii)a | 0.31[0.29, 0.32] | 11.00[10.95, 11.05] | 0.101[0.010, 0.103] | 4.19[4.16, 4.23] | 0.560(± 0.001) | 0.294(± 0.001) | 0.116(± 0.001) | 0.0262(± 0.0004) | - | - | 131 |
| Cdc45, 1.3 nM DDK, +SDPGC(Figure 3C – panel i)c | 0.33[0.30,0.38] | 6.13[6.00, 6.28] | 0.123[0.116, 0.129] | 2.78[2.69, 2.86] | 0.512(± 0.004) | 0.295(± 0.009) | 0.144(± 0.004) | 0.040(± 0.003) | - | - | 48 |
| Cdc45, 6.5 nM DDK, +SDPGC(Figure 3C – panel ii)c | 0.346[0.340, 0.353] | 5.79[5.74, 5.84] | 0.310[0.306, 0.314] | 2.10[2.07, 2.12] | 0.295(± 0.001) | 0.137(± 0.004) | 0.216(± 0.004) | 0.194(± 0.002) | 0.109(± 0.002) | 0.039(± 0.001) | 87 |
| Cdc45, 6.5 nM DDK, +SDPGC, -Sld3/7 (Figure 3 – panel iii)c | 7[4, 10] | 4.54[4.39, 4.67] | 0.02[0.01, 0.03] | 0.73[0.60, 0.88] | 0.982(± 0.001) | 0.017(± 0.001) | - | - | - | - | 32 |
| 1:20 Cdc45, 1.3 nM DDK,+SDPGC(Figure 4 – supp 1A)c | -0.96[-0.98, -0.57] | 6.20[6.16, 6.25] | 0.0003[0e, 0.0033] | 3.12[3.03, 3.18] | 0.934(± 0.001) | 0.059(± 0.001) | - | - | - | - | 40 |
| 1:20 Cdc45, 6.5 nM DDK,+SDPGC(Figure 4 – supp 1B)c | 0.6[0.4, 0.8] | 6.37[6.27, 6.49] | 0.048[0.042, 0.055] | 2.05[1.94, 2.18] | 0.797(± 0.002) | 0.169(± 0.003) | 0.030(± 0.002) | - | - | - | 72 |
| Cdc45, 6.5 nM DDK, +SDPGC, Mcm2-76AD/E + ASP/Q(Figure 6C – panel iii)c | 0.27[0.24, 0.30] | 5.8[5.6, 5.9] | 0.19[0.18, 0.20] | 3.3[3.2, 3.4] | 0.356(± 0.005) | 0.272(± 0.002) | 0.226(± 0.002) | 0.107(± 0.003) | 0.032(± 0.001) | - | 43 |
| Cdc45, 6.5 nM DDK, +SDPGC, Mcm2-74AD/E + ASP/Q(Figure 6C – panel iv)c | 0.12[0.08, 0.17] | 4.8[4.6, 4.9] | 0.16[0.15, 0.17] | 2.2[2.1, 2.3] | 0.331(± 0.004) | 0.34(± 0.01) | 0.223(± 0.003) | 0.083(± 0.005) | 0.020(± 0.002) | - | 33 |
| Cdc45, 6.5 nM DDK, +SDPGC, Mcm2-7Δ6N(Figure 6 – supp 1B)c | 0.8[0.3, 0.9] | 7.85[7.56, 7.89] | 0.017[0.012, 0.019] | 4.628[4.485, 4.632] | 0.925(± 0.003) | 0.064(± 0.004) | 0.010(± 0.003) | - | - | - | 89 |

aContinuous image acquisition protocol; see Materials and Methods.

bSee Materials and Methods

cAlternating image acquisition protocol; see Materials and Methods.

dValues less than 0.01 not shown.

eConfidence interval lower limit uncertain because of minimal binding in this experiment (see Materials and Methods).

**Supplementary File 1b. Parameters used for fitting of GINS fluorescence-intensity histograms.**

|  |  |
| --- | --- |
| **Fit parametersa****[90% CI]** | **Fraction of time that DNAs with loaded Mcm2-74SNAP549 were bound by this number of GINSSORT649 molecules (± SE)c** |
| **Condition****(Figure)** | ***q*** | ***s*****(× 10-3)** | **λ** | ***x*****(× 10-3)** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **n** |
| GINS, 1.3 nM DDK, +SDPGC, On Mcm2-7(Figure 5C – panel i)b | 0.68[0.56, 0.70] | 6.11[6.06, 6.15] | 0.270[0.231, 0.273] | 2.33[2.29, 3.01] | 0.45(± 0.01) | 0.14(± 0.02) | 0.183(± 0.006) | 0.136(± 0.006) | 0.063(± 0.006) | 0.019(± 0.003) | - | 84 |
| GINS, 6.5 nM DDK, +SDPGC, On Mcm2-7(Figure 5C – panel ii)b | 0.071[0.070, 0.073] | 5.15[5.05, 5.24] | 0.41[0.40, 0.42] | 1.65[1.62, 1.67] | 0.080(± 0.002) | 0.078(± 0.004) | 0.188(± 0.005) | 0.258(± 0.002) | 0.222(± 0.003) | 0.123(± 0.004) | 0.042(± 0.002) | 57 |
| GINS, 6.5 nM DDK, +SDPGC, On Mcm2-7, –Sld3/7(Figure 5C – panel iii)b | 1.35[1.31, 1.39] | 4.94[4.88, 5.01] | 0.155[0.152, 0.159] | 2.25[2.20, 2.30] | 0.684(± 0.003) | 0.162(± 0.002) | 0.105(± 0.001) | 0.038(± 0.001) | - | - | - | 49 |
| GINS, 6.5 nM DDK, +SDPGC, On Mcm2-7, –Cdc45(Figure 5C – panel iv)b | 1.60[1.59, 1.61 | 5.67[5.62, 5.71] | 0.264[0.262, 0.265] | 3.31[3.30, 3.32] | 0.648(± 0.001) | 0.095(± 0.002) | 0.086(± 0.001) | 0.039(± 0.001) | 0.0110(± 0.0003) | - | - | 79 |
| GINS, 6.5 nM DDK,+SDPGC, Non-Mcm2-7(Figure 5 – supp 1)b | 1.55[1.53, 1.58] | 5.35[5.27, 5.42] | 0.351[0.346, 0.357] | 2.13[2.10, 2.16] | 0.621(± 0.002) | 0.053(± 0.001) | 0.101(± 0.001) | 0.109(± 0.001) | 0.074(± 0.001) | 0.032(± 0.001) | - | 84 |
| GINS, 6.5 nM DDK, +SDPGC, On Mcm2-7, –Dpb11(Figure 5 – supp 2)b | 1[-1, 3] | 3.4[3.2, 3.5] | 0.0048[0d, 0.0101] | 0.82[0d, 0.96] | 0.983(± 0.001) | 0.017(± 0.001) | - | - | - | - | - | 32 |

aSee Materials and Methods

bAlternating image acquisition protocol; see Materials and Methods.

cValues less than 0.01 not shown.

dConfidence interval lower limit uncertain because of minimal binding in this experiment (see Materials and Methods).