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Supplemental Information

Divergent Aging of Isogenic Yeast Cells Revealed through Single-Cell Phenotypic Dynamics

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Supplemental Information

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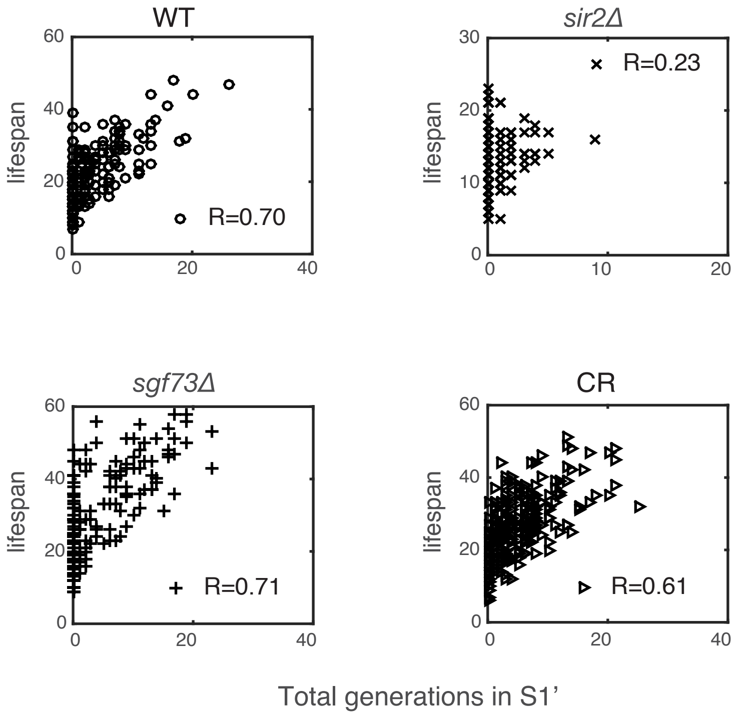

Figure. S1: Related to Figure 1. Correlation between the duration in S1' state and lifespan. Each subplot shows the correlation between the total number of generations in S1' and the replicative lifespan under indicated conditions. Each symbol represents a single aging cell. Different symbols represent various genetic or environmental conditions, as indicated. Correlation efficient (R) for each condition has been calculated.

WT cells

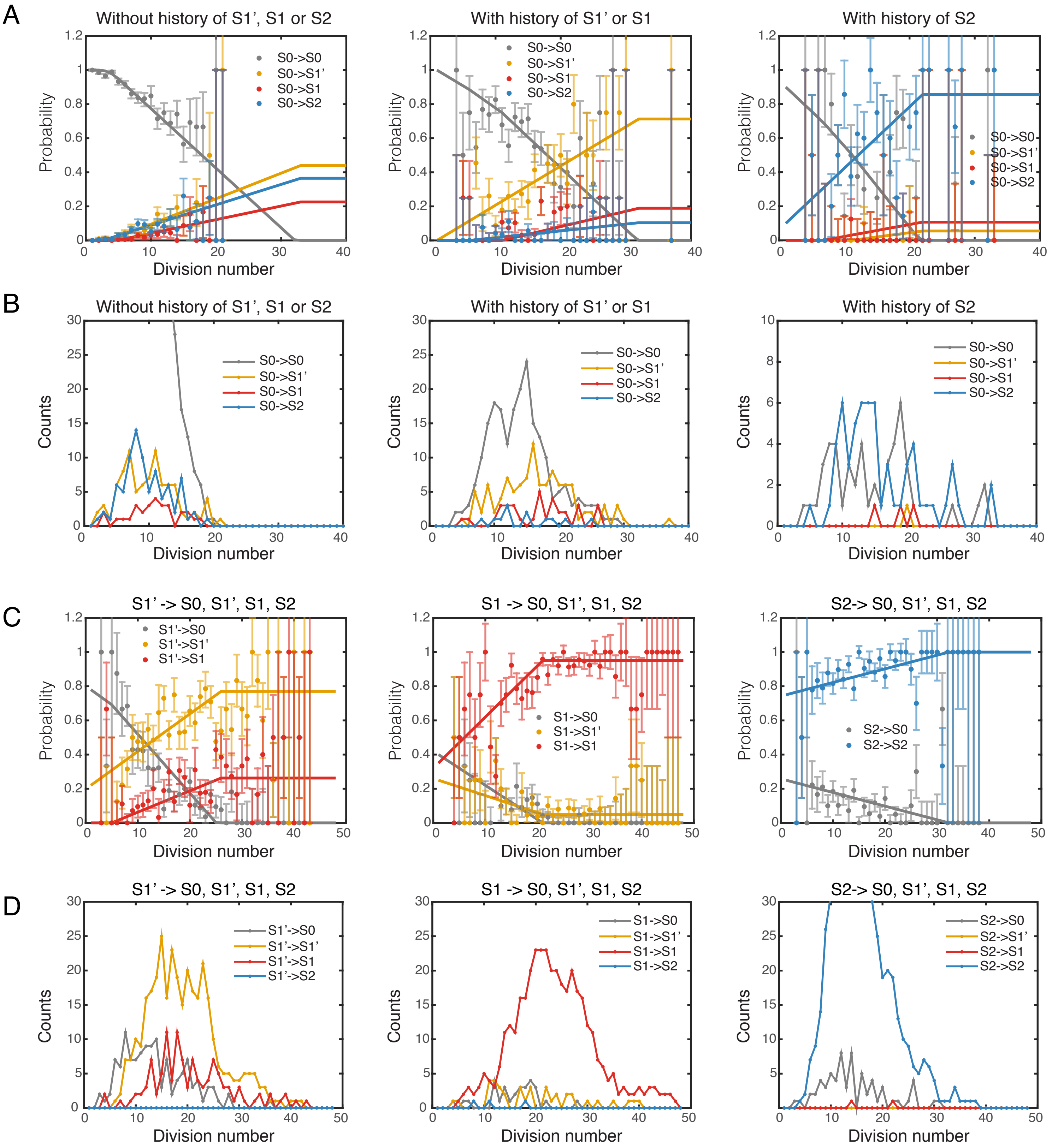

Figure. S2: Related to Figure. 1. Transition probabilities between states in WT cells. (A) Transition probabilities from S0, with three different history conditions. Dots are calculated from experimental data. Solid lines are linear fits for the state transition probabilities of the same color. Error bars indicate standard deviations for observed transitions. (B) The number of cells that underwent individual state transitions from S0, used to calculate transition probabilities in (A). (C) Transition probabilities from S1’ (left), S1 (middle) and S2 (right). Dots are calculated from experimental data. Solid lines are linear fit for the state transition of the same color. (D) The number of cells that underwent state transitions from S1’, S1 and S2. Error bars indicate expected standard deviations.

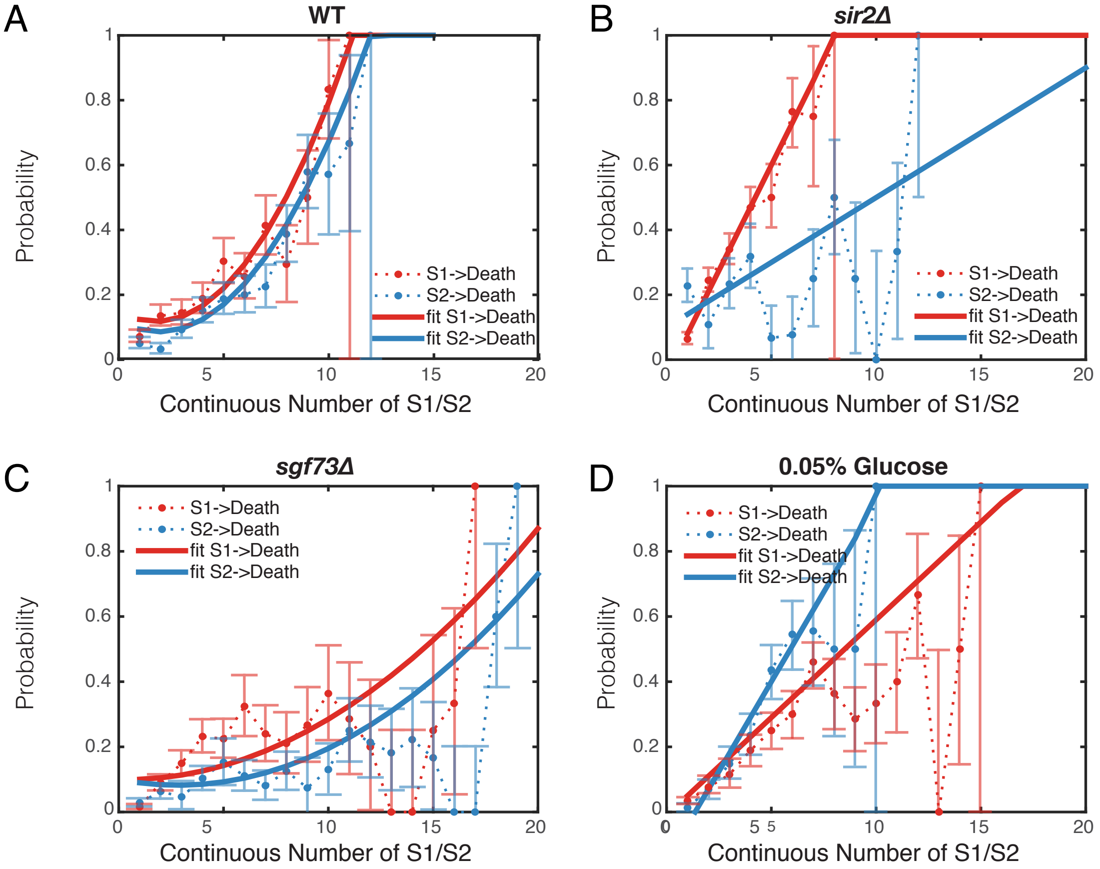

Figure. S3: Related to Figures 2-5. Transition probabilities from S1 and S2 to cell death for all experiments. (A) p_{1D} and p_{2D} for wild type cells, as in Fig 2C. Dots are calculated from experimental data. Solid lines are the corresponding fits. (B) p_{1D} and p_{2D} for *sir2Δ* cells. (C) p_{1D} and p_{2D} for *sgf73Δ* cells. (D) p_{1D} and p_{2D} for cells grown under caloric restriction (0.05% glucose). Error bars indicate expected standard deviations.

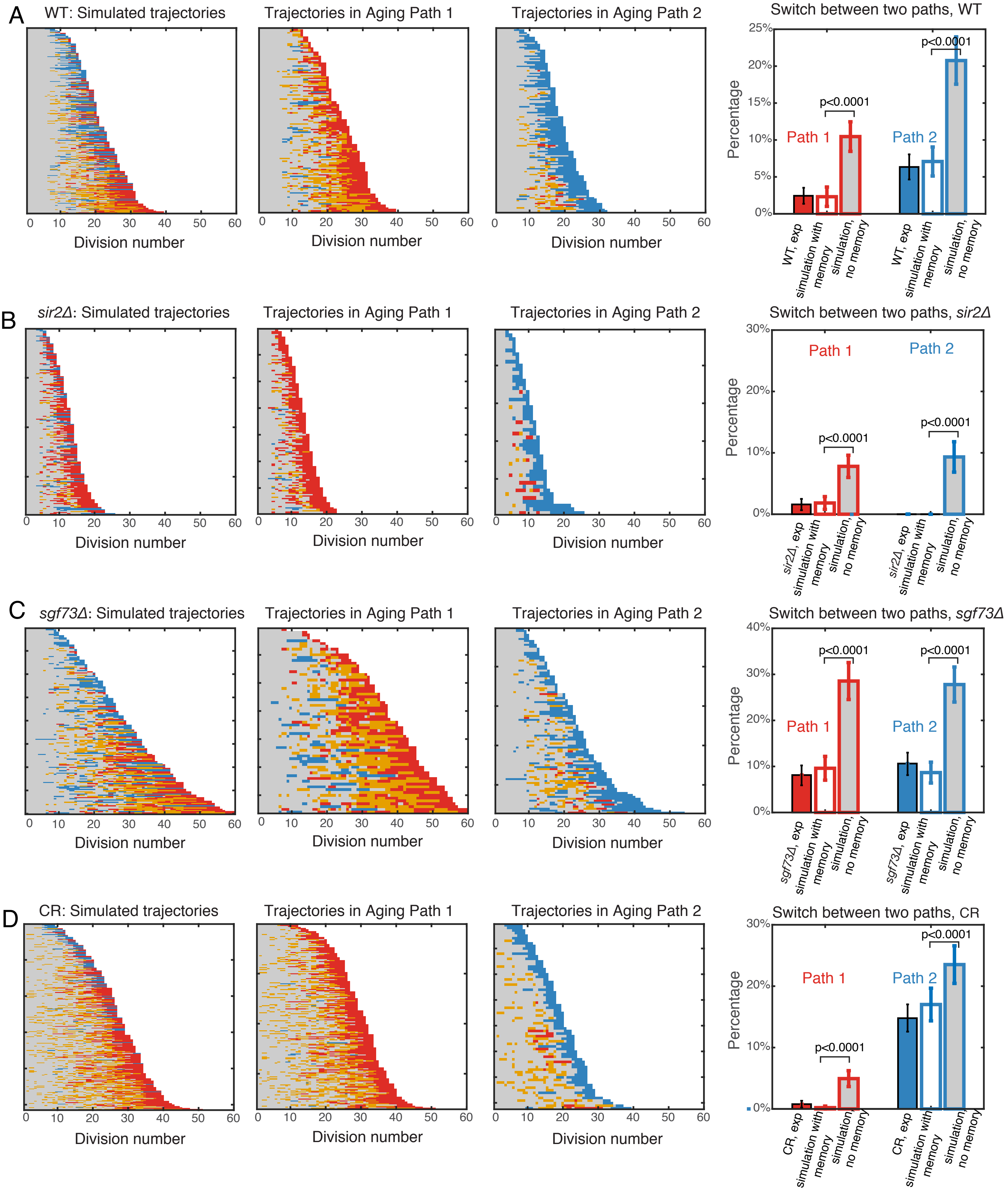

Figure. S4: Related to Figures 2-5. The effect of history dependence (memory) in the divergence of two aging paths. Single-cell state transition trajectories from one representative simulation without history dependent transition probabilities from S0 (other transition probabilities are unchanged.) for WT (A), *sir2Δ* (B), *sgf73Δ* (C), and CR treated cells (D). The switching between Aging Path 1 and Aging Path 2 became notably increased (middle columns for all cases). Bar graphs on the right column showed the percentages of cross-path switching in simulations with or without history dependence (memory) for WT, *sir2Δ*, *sgf73Δ*, and CR (top to bottom). Solid bars represent experimental data, with error bars indicating expected standard deviations as in Fig. 4D. Open bars represent simulations with history dependence; grey bars represent simulations without history dependence (memory). Error bars of simulations are standard deviations from 50 simulations. A two-sample t-test with unequal variance is used for p-value of simulations with and without memory.

sir2Δ cells

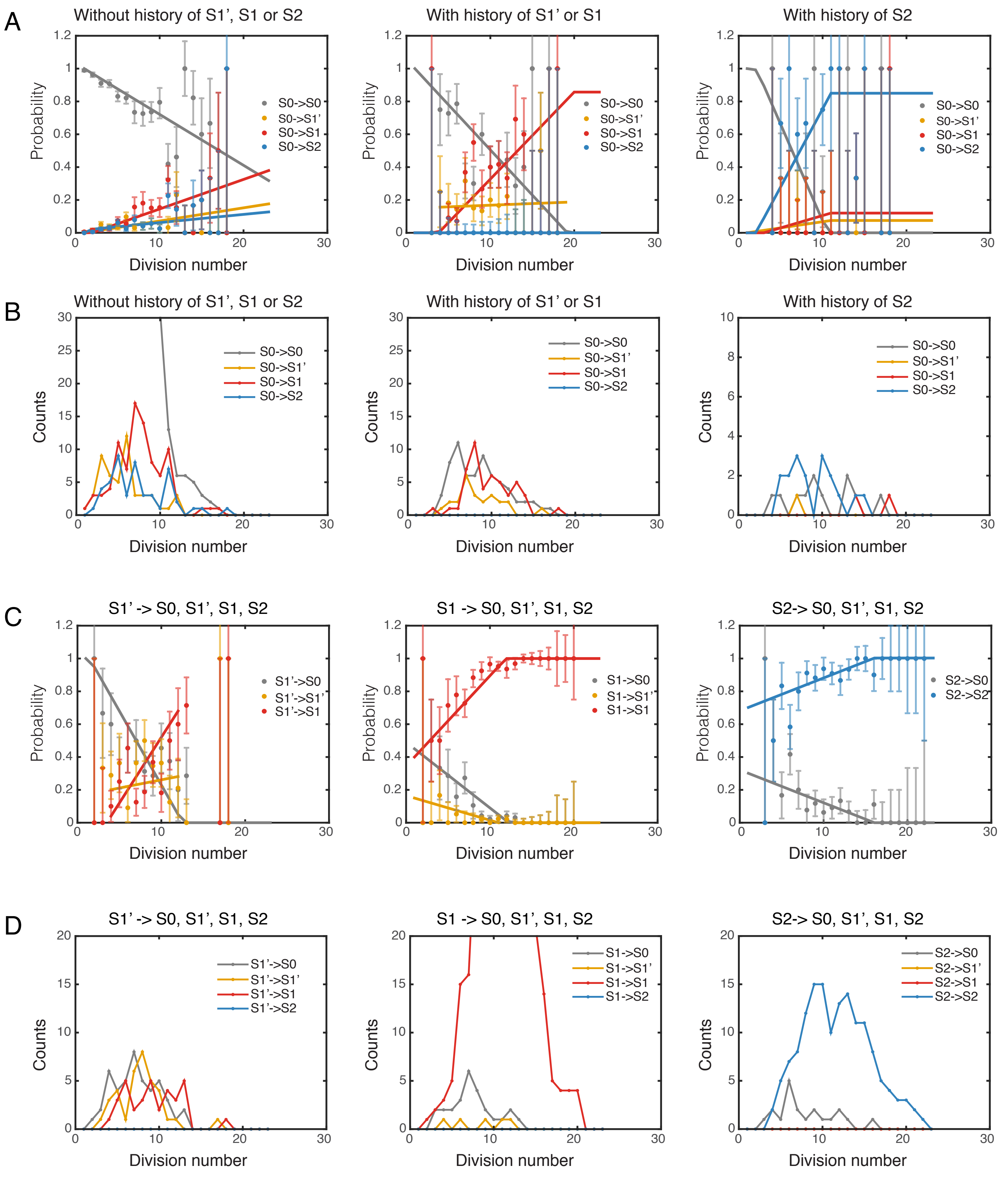

Figure. S5: Related to Figure. 3. Transition probabilities between states in *sir2Δ* cells. Dots are calculated from experimental data. Solid lines are linear fits for the state transition probabilities of the same color. (A) Transition probabilities from S0, with three different history conditions. (B) The number of cells that underwent individual state transitions from S0, used to calculate transition probabilities in (A). (C) Transition probabilities from S1' (left), S1 (middle) and S2 (right). (D) The number of cells that underwent state transitions from S1', S1 and S2, used to calculate transition probabilities in (C). Error bars indicate expected standard deviations.

sgf73Δ cells

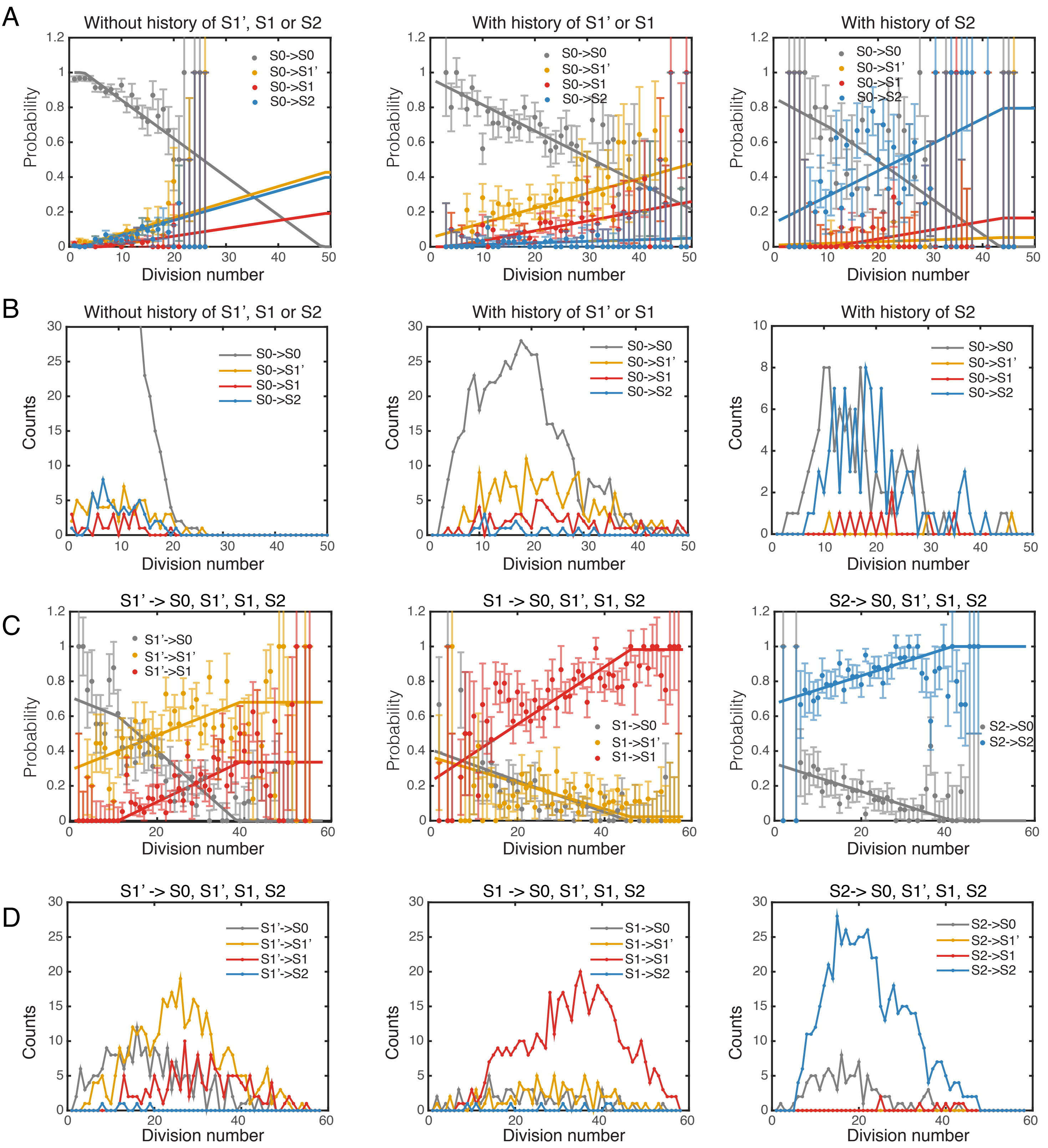

Figure. S6: Related to Figure. 4. Transition probabilities between states in *sgf73Δ* cells. Dots are calculated from experimental data. Solid lines are linear fits for the state transition probabilities of the same color. (A) Transition probabilities from S0, with three different history conditions. (B) The number of cells that underwent individual state transitions from S0, used to calculate transition probabilities in (A). (C) Transition probabilities from S1' (left), S1 (middle) and S2 (right). (D) The number of cells that underwent state transitions from S1', S1 and S2, used to calculate transition probabilities in (C). Error bars indicate expected standard deviations.

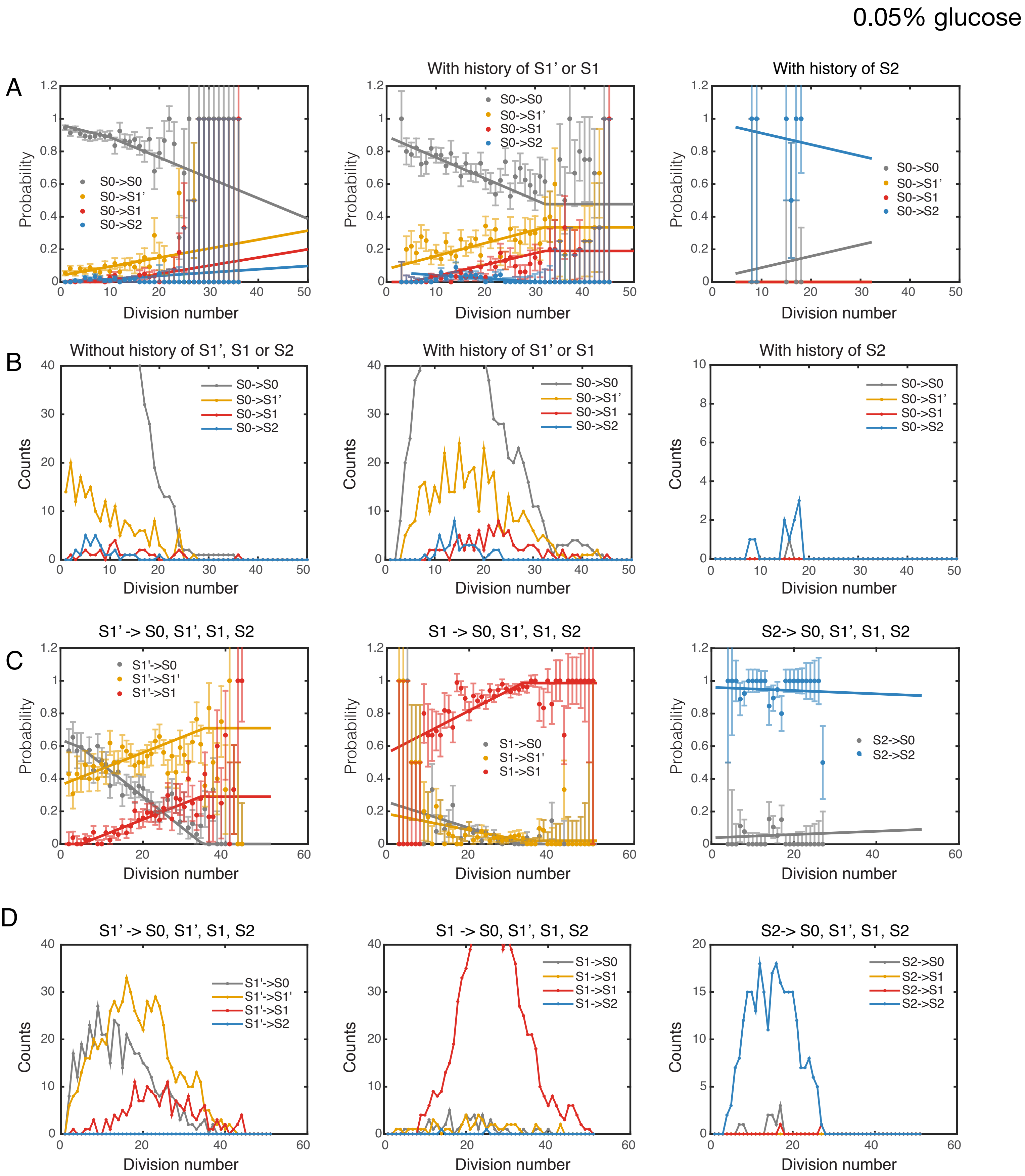

Figure. S7: Related to Figure. 5. Transition probabilities between states under CR. Dots are calculated from experimental data. Solid lines are linear fits for the state transition probabilities of the same color. (A) Transition probabilities from S0, with three different history conditions. (B) The number of cells that underwent individual state transitions from S0, used to calculate transition probabilities in (A). (C) Transition probabilities from S1' (left), S1 (middle) and S2 (right). (D) The number of cells that underwent state transitions from S1', S1 and S2, used to calculate transition probabilities in (C). Error bars indicate expected standard deviations.

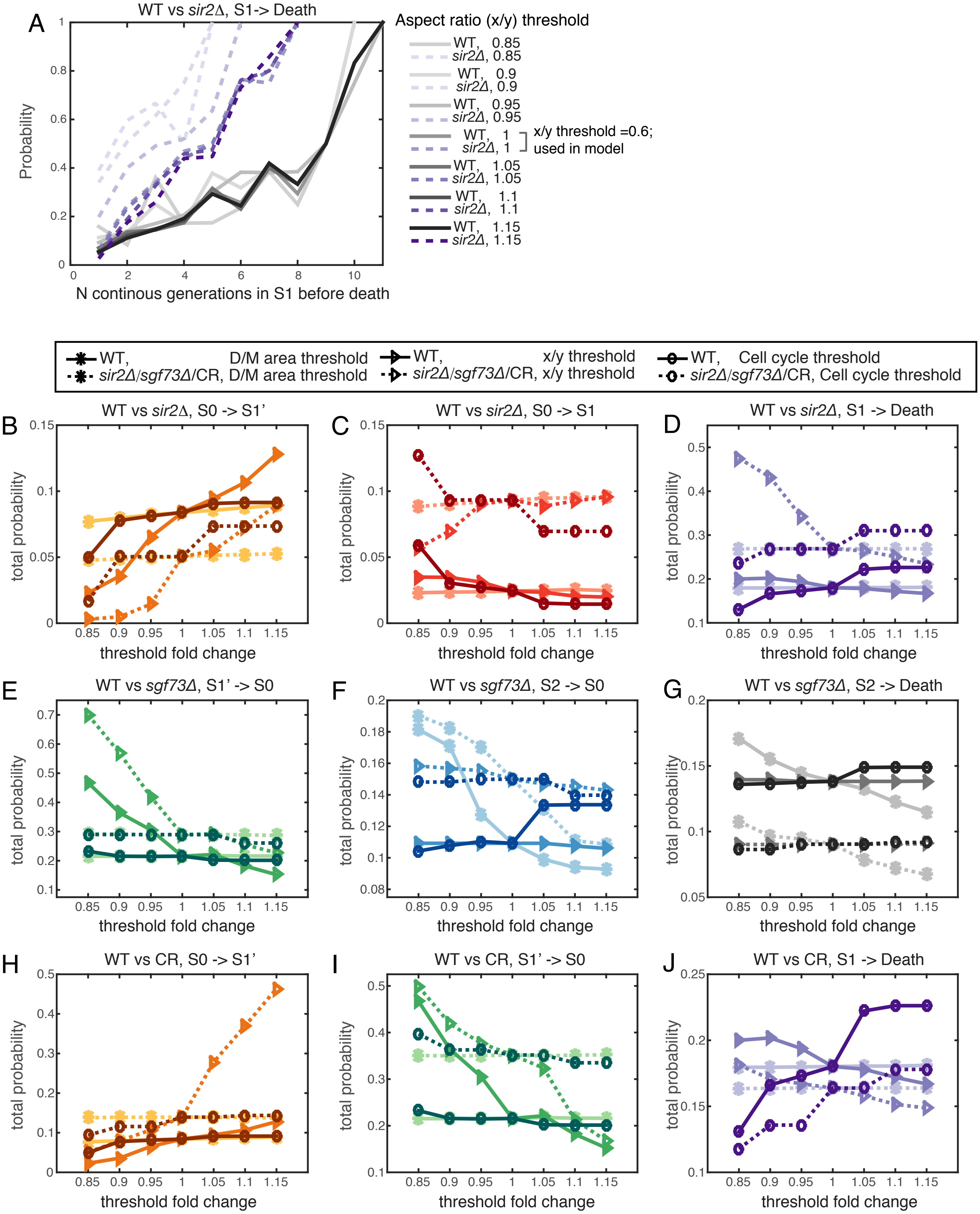

Figure. S8: Related to Figures 2-5. Sensitivity analysis for thresholds of daughter aspect ratio, Daughter/Mother area ratio and cell cycle duration. (A) An exemplary plot for how transition probability S1 to death, p_{1D}, changes by varying aspect ratio threshold from 0.85 to 1.15 folds of current value (0.6) with 0.05 intervals in WT and *sir2Δ* cells. Dashed lines for p_{1D} in *sir2Δ*, solid lines for p1D in WT. p_{1D} with the same threshold value for WT and *sir2Δ* has the same color saturation. (B-J) Effects of thresholds of Daughter/Mother area ratio, aspect ratio (x/y) and cell cycle on total probabilities, $P_{01'}$, P_{01}, and P_{1D} for WT and *sir2Δ* (B-D), $P_{1'0}$, P_{20} and P_{2D} for WT and *sgf73Δ* (E-G), $P_{01'}$, $P_{1'0}$, and P_{1D} for WT and CR treated cells (H-J). Total probability is used as it is independent of lifespan length. Solid lines are total probabilities in WT, dashed lines for those in *sir2Δ*, *sgf73Δ* and CR. Star, triangle and open circle represent thresholds for D/M area ratio, aspect ratio (x/y) and cell cycle respectively.

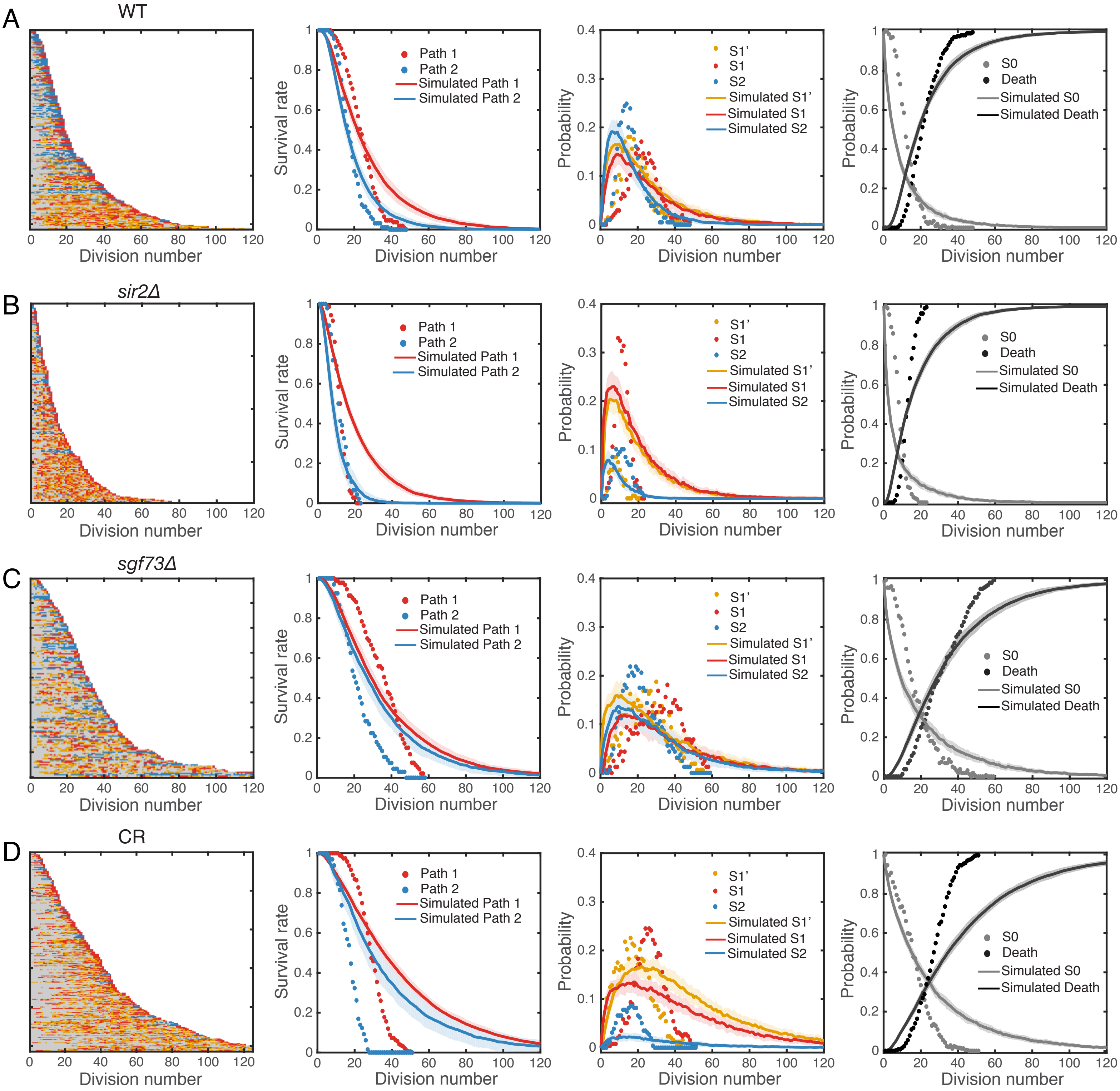

Figure. S9: Related to Figures 2-5. Justification of linear approximation of transition probabilities between states. The best simulations using constant transition rates between S0, S1', S1 and S2 for WT (A), *sir2Δ* (B), *sgf73Δ* (C), and CR treated cells (D). Note that these simulations deviated greatly from the state transition dynamics in single cells trajectories (1st column), survival curves (2nd column) and state distributions (3rd and 4th columns) for all experiments compared to Figs 2-5. Therefore, the approximation with linear transition rates describes the data better than constant transition rates.

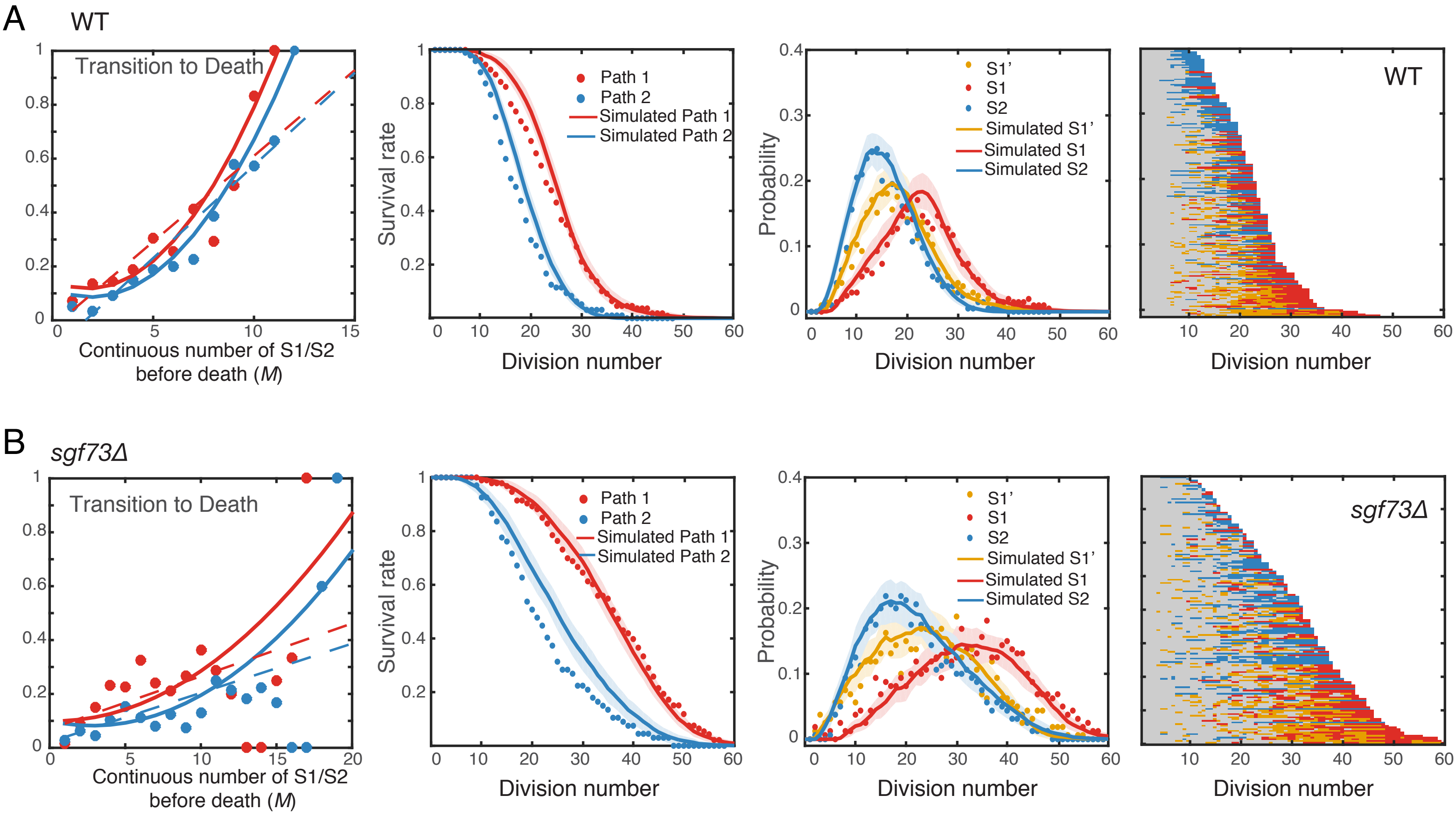

Figure. S10: Related to Figure. 2 and Figure. 4. Justification of second order polynomial approximation of transition probabilities to death in WT and *sgf73Δ* cells. (A) Simulations with the best linear fits for transition rates to death for WT cells. Left: fits for transition probabilities to death. Solid dots: experimental data. Dashed lines: linear fits. Solid lines: second order polynomial fits. Middle: survival rates and state distributions using linear death probabilities in the left panel. The mean square distances (msd) between simulation and data for survival rate are 0.0056 and 0.0073 for Path 1 and Path 2, respectively. They are 2-fold and 3-fold of that in Fig. 2E (msd for Path 1 and Path 2 are 0.0025 and 0.0014 in Fig. 2E). Right: simulated single cell state changing trajectories using linear death probabilities in (A). (B) Simulations with the best linear fits for transition rates to death for *sgf73Δ* cells. Left: fits for transition probabilities to death, same legend as WT. Middle: survival rates and state distributions using linear death probabilities in the left panel. The msd between simulation and data for survival rate are 0.0011 and 0.011 for Path 1 and Path 2, respectively. The msd for Path 1 is comparable to that in Fig. 4B, but 3-fold larger for Path 2 than that in Fig. 4B (msd for Path 1 and Path 2 are 0.0012 and 0.0037 in Fig. 4B).

Table S1, related to Figures 2-6. Transition probabilities used in simulations. Transition probabilities are deduced from data fitting using the linear function p = a x *N* + b, where *N* is the replicative age (generation). a and b obtained from fitting are listed below for each probability under each condition.

	WT		*sir2Δ*		*sgf73Δ*		CR		NAM 5mM	
	a	b	a	b	a	b	a	b	a	b
$p_{00\|\text{no history}}$	-0.035	1.089	-0.031	1	-0.022	1.04	-0.0125	1	-0.045	1
$p_{01'\|\text{no history}}$	0.015	-0.04	0.008	0	0.009	-0.005	0.0055	0.045	0.016	0.01
$p_{01\|\text{no history}}$	0.008	-0.03	0.018	-0.017	0.0045	-0.025	0.005	-0.045	0.012	0.025
$p_{02\|\text{no history}}$	0.012	-0.019	0.005	0.017	0.0085	-0.01	0.002	0	0.017	-0.035
$p_{00\|\text{nearest history of S1'S1}}$	-0.036	1.11	-0.055	1	-0.015	0.95	-0.013	0.88	-0.09	1.4
$p_{01'\|\text{nearest history of S1'S1}}$	0.023	0	0.002	0.15	0.0085	0.06	0.008	-0.087	0.018	0
$p_{01\|\text{nearest history of S1'S1}}$	0.009	-0.09	0.053	-0.15	0.0055	-0.015	0.007	-0.027	0.05	-0.3
$p_{02\|\text{nearest history of S1'S1}}$	0.004	-0.02	0	0	0.001	0.005	-0.002	0.06	0.022	-0.1
$p_{00\|\text{nearest history of S2}}$	-0.048	0.99	-0.1175	1.13	-0.021	0.89	0.007	0.025	0	0
$p_{01'\|\text{nearest history of S2}}$	0.005	-0.05	0.0075	0	0.001	0.01	0	0	0	0
$p_{01\|\text{nearest history of S2}}$	0.007	-0.04	0.015	-0.03	0.005	-0.05	0	0	0	0
$p_{02\|\text{nearest history of S2}}$	0.036	0.1	0.095	-0.1	0.015	0.15	-0.007	0.975	0	1
$p_{1'0}$	-0.0345	0.83	-0.09	1.04	-0.022	0.82	-0.02	0.67	-0.01	0.2
$p_{1'1'}$	0.022	0.22	0.01	0.16	0.01	0.3	0.01	0.37	-0.025	0.72
$p_{1'1}$	0.0125	-0.05	0.08	-0.2	0.012	-0.12	0.01	-0.04	0.035	0.08

p_{10}	-0.02	0.4	-0.04	0.45	-0.009	0.4	-0.008	0.25	-0.007	0.1
$p_{11'}$	-0.01	0.25	-0.015	0.15	-0.0075	0.36	-0.005	0.18	-0.017	0.24
p_{11}	0.03	0.35	0.055	0.4	0.0165	0.24	0.013	0.57	0.024	0.66
p_{20}	-0.008	0.25	-0.02	0.3	-0.008	0.32	0.001	0.04	-0.018	0.2
p_{22}	0.008	0.75	0.02	0.7	0.008	0.68	-0.001	0.96	0.018	0.8
p_{1D}	$0.01M^2-0.036M+0.15$		$0.13M-0.05$		$0.002M^2-0.0015M+0.1$		$0.06M-0.01$		$0.11M-0.12$	
p_{2D}	$0.009M^2-0.035M+0.12$		$0.04M+0.1$		$0.0022M^2-0.0125M+0.1$		$0.11M-0.15$		$0.08M-0.05$	