Bacillus subtilis RecA and its accessory factors, RecF, RecO, RecR and RecX, are required for spore resistance to DNA double strand break

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Supplementary Table S1 *Bacillus subtilis* strains used in this study

Strain	Relevant genotype	Source or reference[a]
168	*trpC*2	Laboratory stock
WN463[a]	+ *recA*	(1)
LAS600[b]	Δ*upp*	(2)
LAS523[b]	+ *dinR3* [*lexA*(Ind$^-$)]	(2)
BG214[c]	*trpCE metA*5 *amyE1 ytsJ*1 *rsbV*37 *xre*1 *xkd*A1 *att*$^{SP\beta}$ *att*^{ICEBs1}	Laboratory stock
BG809[c]	+ Δ*ykoV*	(3)
BG849[c]	+ Δ*ykoV* Δ*recA*	(3)
BG190[c]	+ Δ*recA*	(4)
BG277[c]	+ Δ*recN*	(5)
BG125[c]	+ *addA*5	(6)
BG189[c]	+ *addA*5 *addB*72	(5)
BG705[c]	+ Δ*recQ*	(7)
BG425[c]	+ Δ*recS*	(8)
BG713[c]	+ *addA*5 Δ*recJ*	(7)
BG675[c]	+ Δ*recJ*	(7)
BG129[c]	+ *recF15*	(6)
BG439[c]	+ Δ*recO*	(9)
BG128[c]	+ Δ*recR*	(10)
BG1065[c]	+ Δ*recX*	(11)
BG1053[c]	+ Δ*recX recF15*	(11)

[a]Strains isogenic with 168 (DSMZ, DMS402). [b]Strains derived from prototrophic 168 bearing a null *upp* mutation. [c]Strains isogenic with BG214 (a 168 derivative).

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Supplementary Figures

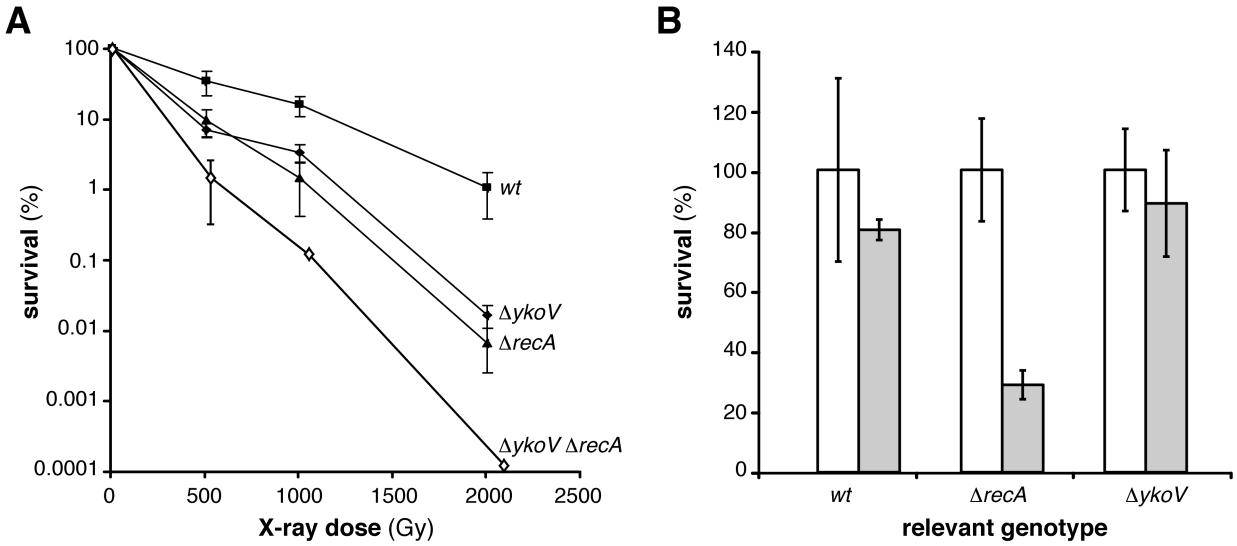


Figure S1. Survival of *B. subtilis wt* and mutant spores deficient in HR or NHEJ after X-ray radiation (A) and UHV (B). (A) X-ray dose dependent survival of *wt* (■), Δ*recA* (▲), Δ*ykoV* mutant (♦) or Δ*ykoV* Δ*recA* (◊) mutant spores. (B) Survival of untreated (white column) and treated spores (gray column) after 7 days UHV exposure.

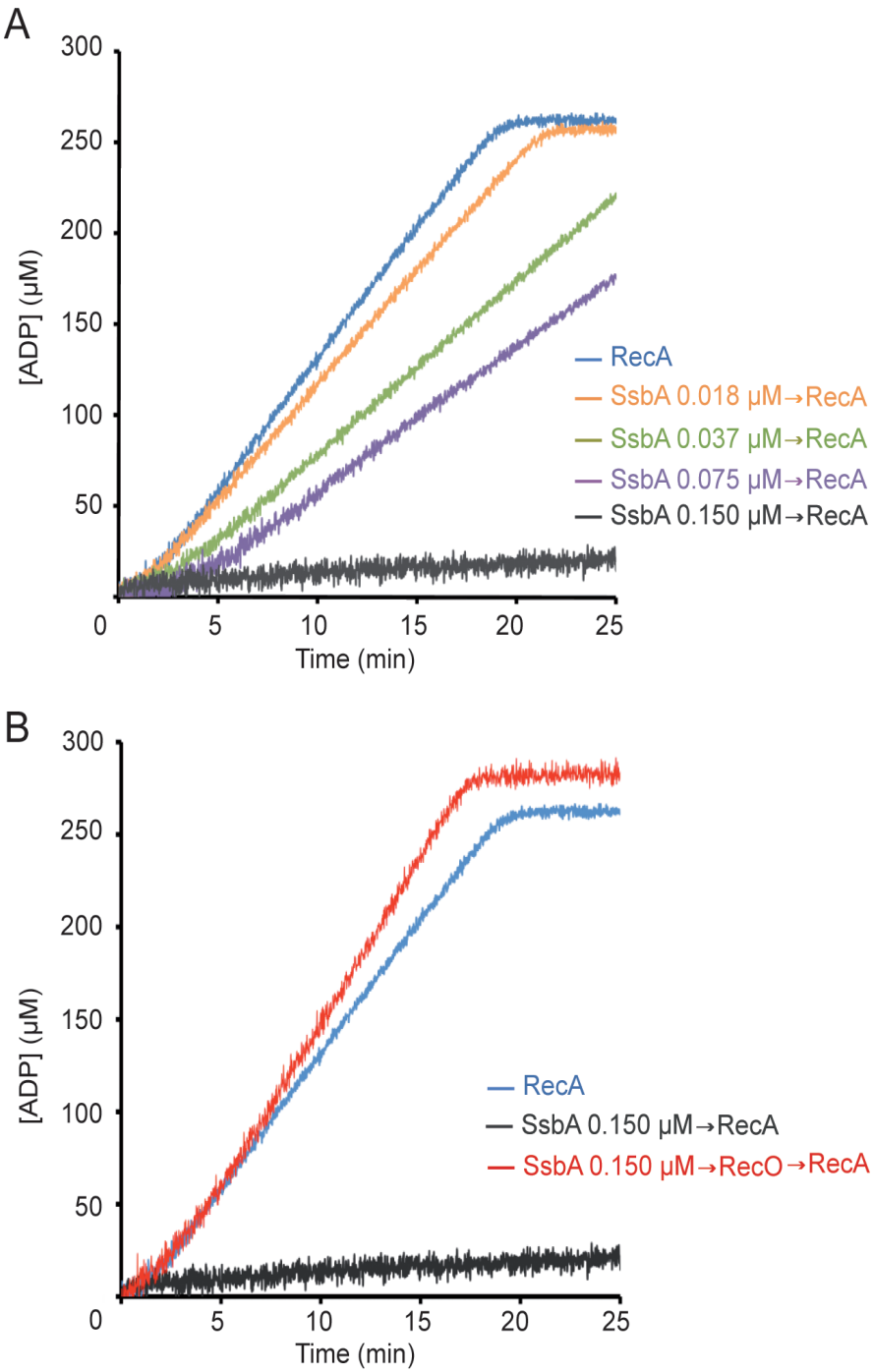
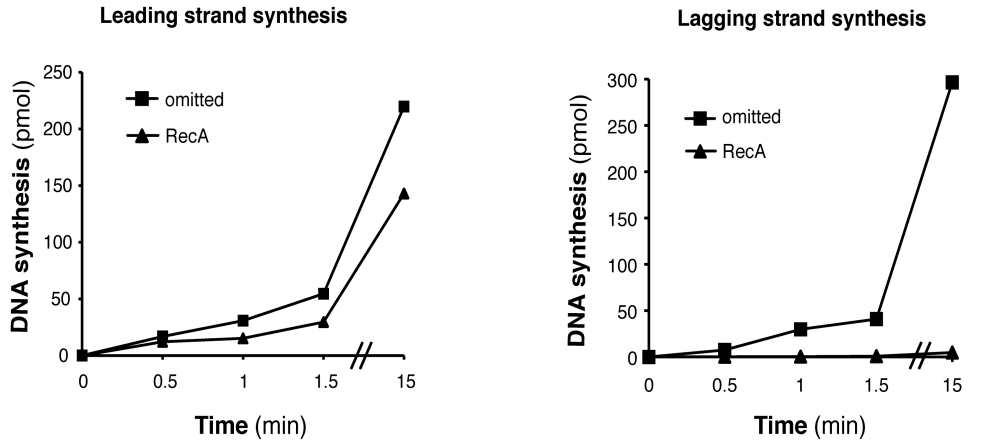


Figure S2. SsbA blocks RecA assembly and RecO overcomes this kinetic barrier. (A) Circular ssDNA (10 μM in nt) was pre-incubated with increasing SsbA concentrations (0, 0.018, 0.037, 0.075 and 0.150 μM) for 5 min at 37° C in buffer A containing 5 mM ATP. Then, RecA (1 μM) was added, and the ATPase activity measured for 25 min. (B), circular ssDNA was pre-incubated with SsbA for 5 min at 37° C, and then RecO (0.2 μM) was added to the preformed SsbA·ssDNA complexes and incubation continued for 5 min at 37° C. Finally RecA (1 μM) was added, and the ATPase activity was measured for 25 min. All reactions were repeated three or more times with similar results. The amount of ATP hydrolysed was calculated as described in Materials and methods.

A. In absence of SsbA



B. In presence of SsbA

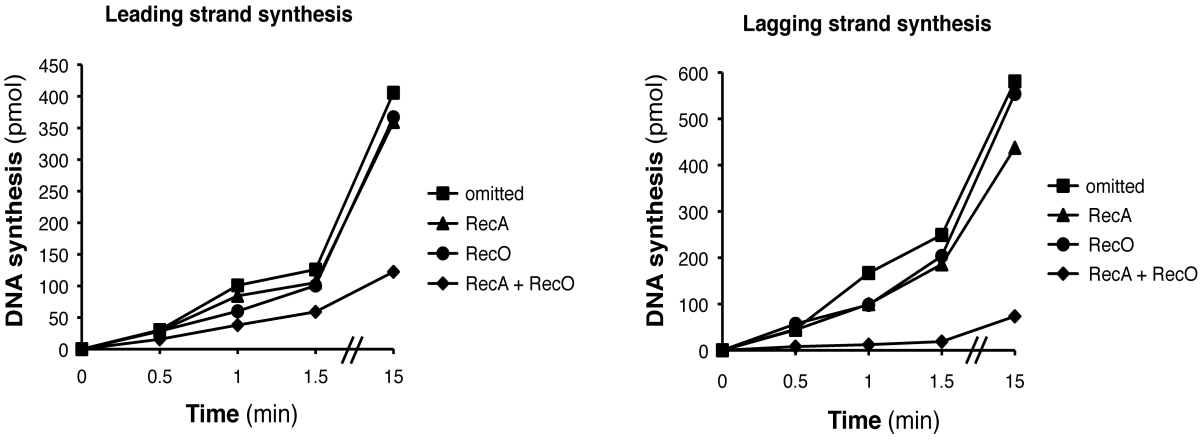


Figure S3. RecA inhibits *in vitro* the *B. subtilis* replisome in concert with RecO. Quantification of leading and lagging strand synthesis obtained measured by radioactivity incorporation as a function of time in the absence of SsbA (A) or in the presence of SsbA (B). The reactions and the measurement of DNA synthesis on both leading and lagging strands were performed as described in Materials and methods.