



Figure S3: Construction of *C. albicans* *Ssa1*⁻ mutant by CRISPR/Cas9 method. (A) Scheme of *SSA1* gene in *C. albicans* NBRC 1385 wild type (*Ssa1*⁺) and *Ssa1*⁻ mutant. Based on the repair template sequence (Supplementary Table 2), nucleotides "G" and "A" were inserted into *SSA1* gene to create a stop codon and *EcoRI* recognition site. To screen *Ssa1*⁻ mutant, a 776-bp PCR product was amplified using checking primers (Supplementary Table 2), and the PCR product from the *Ssa1*⁻ mutant was digested with *EcoRI* to generate 202 bp and 574 bp DNA fragments. (B) Presence of *EcoRI* recognition site in the *SSA1* gene of *C. albicans* *Ssa1*⁻ was demonstrated by electrophoresis on a 1% agarose gel. (C) The presence of stop codon and *EcoRI* recognition site in the *SSA1* gene of *C. albicans* *Ssa1*⁻ was confirmed by sequencing. Insertion of nucleotides "G" and "A" (orange square) into the *SSA1* gene of *C. albicans* *Ssa1*⁻ indicates that a stop codon was generated, and only N-terminal 12 residues from the total 656 amino acids of the *Ssa1* protein were translated. (D) Confirmation of *C. albicans* *Ssa1*⁻ mutant by Western blot analysis. The *Ssa1* positive band from the recombinant *Ssa1* and *C. albicans* *Ssa1*⁺ lysate (red arrow) but not *C. albicans* *Ssa1*⁻ lysate was detected using anti-*Ssa1* polyclonal antibodies in mouse serum (prepared in our laboratory) and HRP-conjugated anti-mouse IgG (Abcam, Tokyo, Japan). To prepare anti-*Ssa1* polyclonal antibodies, BALB/c mice (with 7 weeks of age) were subcutaneously immunized with 10 μ g recombinant *Ssa1* mixed with Imject[®] Alum (Thermo Scientific) on Day 0 and 14. On Day 28, mice were anesthetized, and whole blood was taken. Whole blood was incubated at 37°C for 30 min and then centrifuged at 800 \times g for 15 min to obtain serum. (Left) Equal volume of whole cell lysate from *C. albicans* wild type (*Ssa1*⁺) and *Ssa1*⁻ mutant were loaded on 8% SDS-polyacrylamide gel with CBB-staining.