Supplemental Data

Figure S1. Germination by *C. difficile* spores in defined medium at 25°C. Purified spores from (A) wildtype *C. difficile* UK1 or (B) *C. difficile* RS07 (*alr2::ermB*) or (C) *C. difficile* RS07 pRS89 (p*alr2*) were suspended germination buffer supplemented with TA alone (●), or supplemented with glycine (■) or L-alanine (▲) or D-alanine (▼). CaDPA release from the germinating spores was monitored at 25°C as an increase in Tb^{3+} fluorescence over time. Data points represent the average from three independent experiments and error bars represent the standard deviation of the mean.

Figure S2. Purity of recombinantly expressed Alr2. Ni-affinity-purified Alr2 was concentrated and separated on a Sephedex G200 size exclusion column. The peak at ~15 minutes was collected and is Alr2.

Figure S3. Purity of L / D alanine and serine used for germination, conversion and ITC. (A) L-alanine, (B) D-alanine, (C) L-serine, or (D) D-serine were labeled with FDAA and separated by HPLC. The amino acids used were >98% pure and there is minimal detection of the opposite enantiomer in the labeling and HPLC analysis.

Figure S4. L / D conversion of alanine and serine during ITC. After completion of the ITC binding for (A) L-alanine or (B) D-alanine or (C) L-serine or (D) D-serine, the samples in the ITC cell were labeled with FDAA as described in the material and methods. The reacted samples were separated by HPLC. The HPLC retention times of each amino acid were determined using labeled amino acid standards.

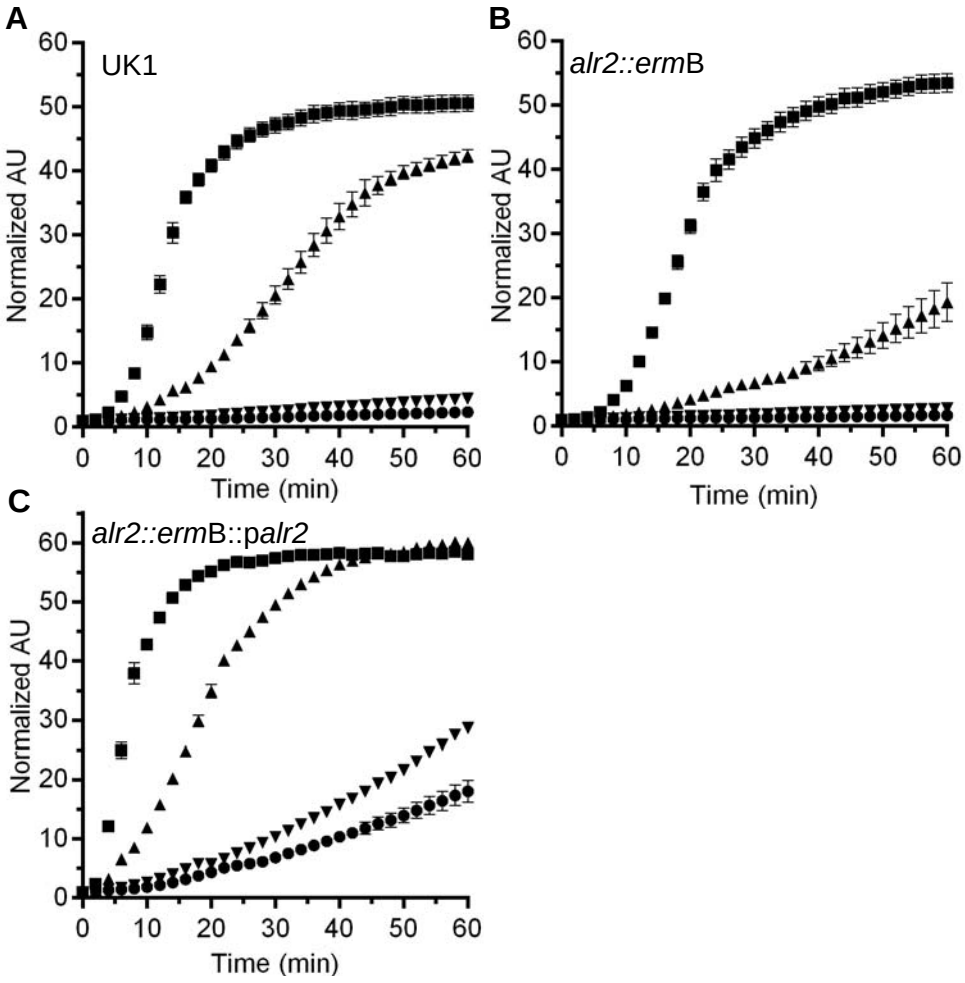

Fig S1. Germination by *C. difficile* spores in defined medium at 25 °C

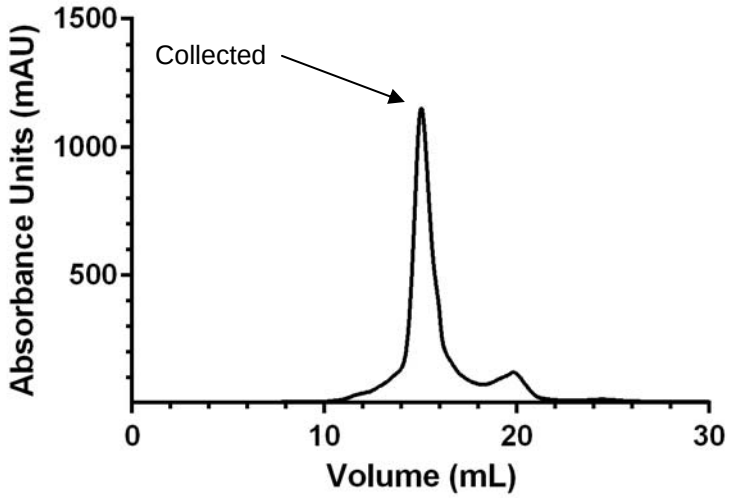

Figure S2. Purity of recombinantly expressed Alr2

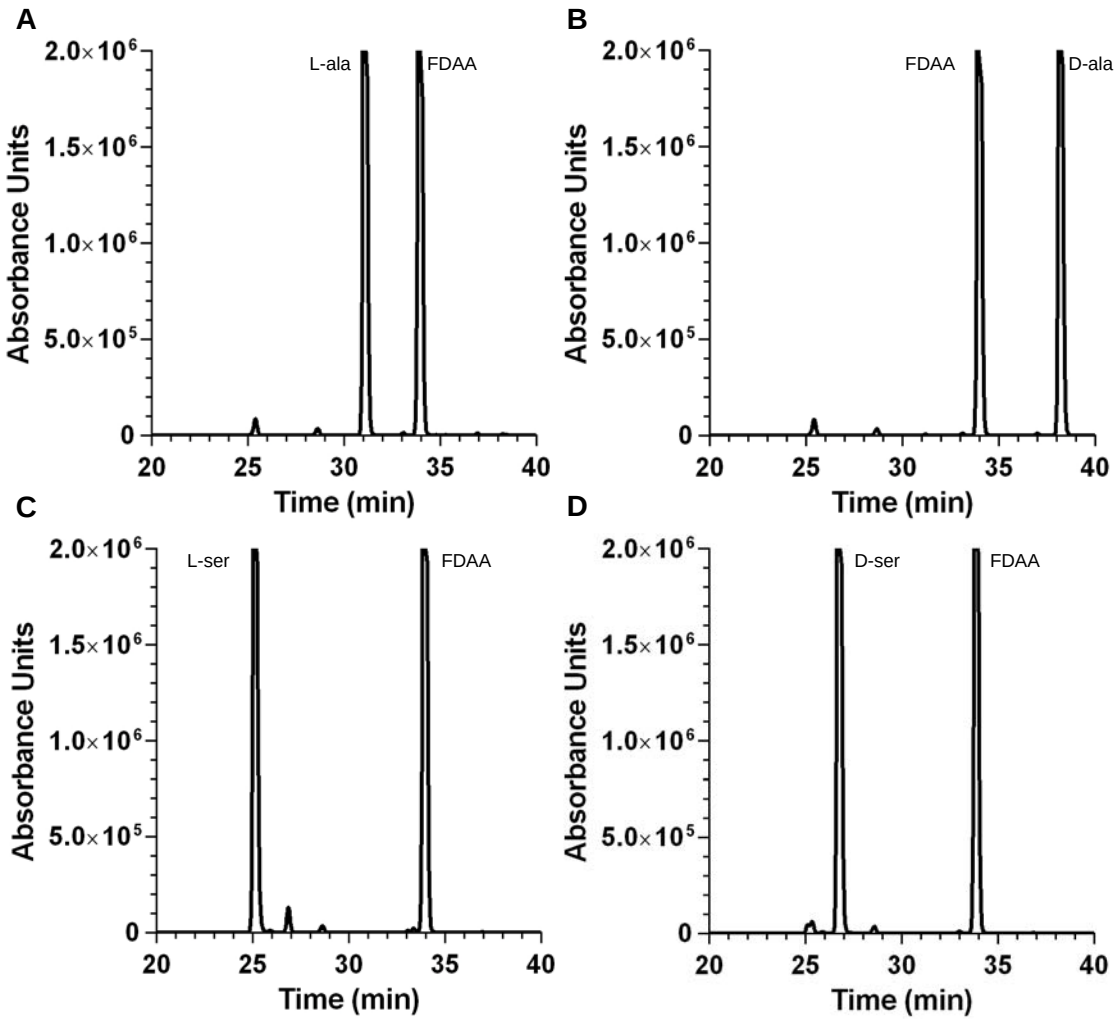

Figure S3. Purity of L / D alanine and serine used for germination, conversion and ITC

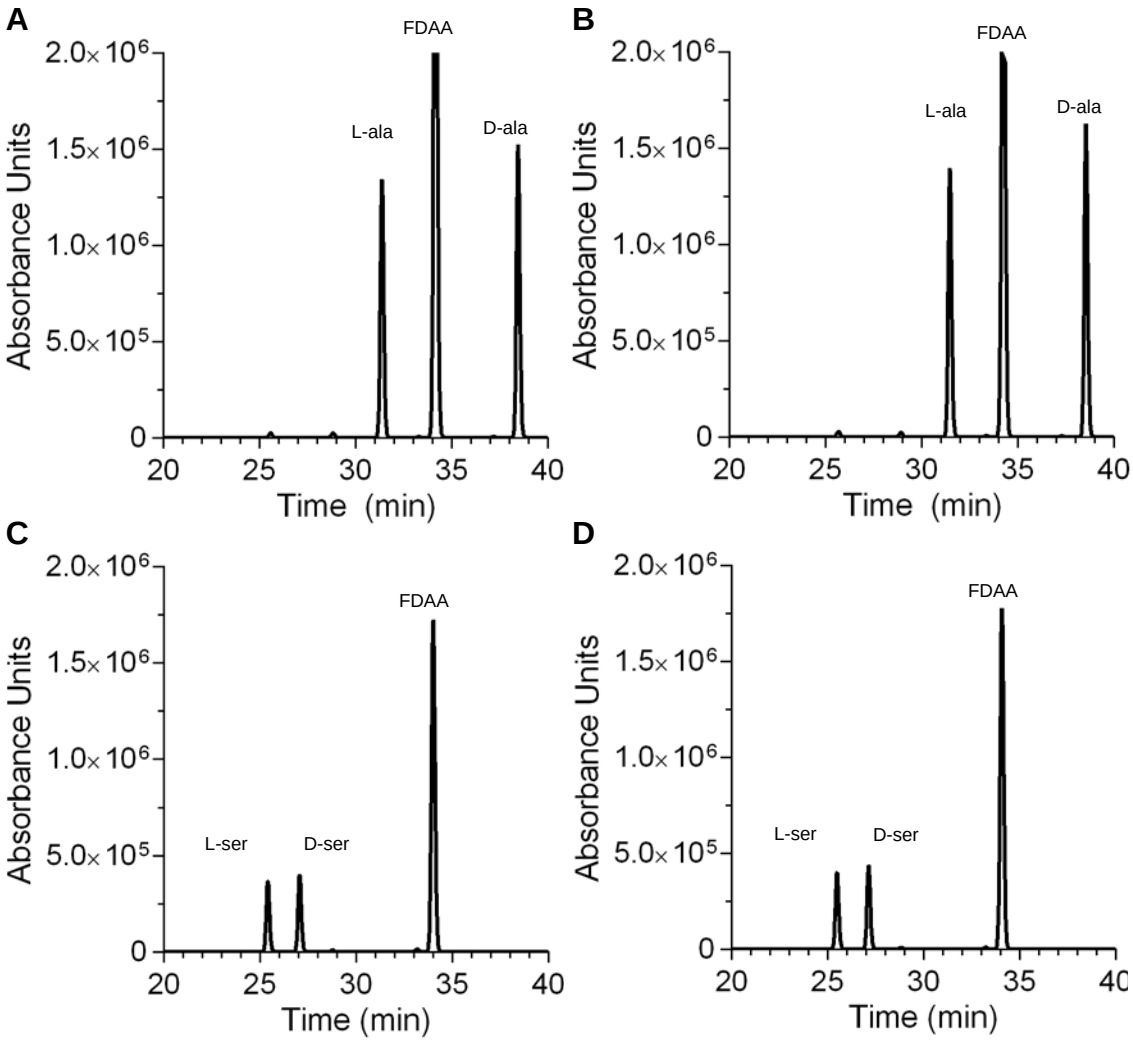

Figure S4. L / D conversion of alanine and serine during ITC