SUPPLEMENTAL METHODS and MATERIALS

An in vitro compartmentalization based method for the selection of bond-forming enzymes from large libraries

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Primers and sequences used.

Primers used to amplify BirA with RTS extensions:

BirA.RTS.F	CGCTTAATTAAACATATGACCATGAAGGATAACACCGT
BirA.RTS.R	TAGTTAGTTACCGGATCCCTTATTATTATTTTTCTGCACTACGCAG

Oligonucleotides used to generate sortase A gene:

ml.stra.1	ATGCAGGCGAAACCGCAGATCCCGAAAG
ml.stra.2	CGCGTCCGGGATTTCGATGTAACCCGCAACTTTAGATTTGTCTTTCGGGATCTGCGGTTT
ml.stra.3	CGAAATCCCGGACGCGGACATCAAAGAGCCGGTGTACCCTGGTCCGGCGACCCCGGAACA
ml.stra.4	TCCAGAGATTCGTTTTCTTCCGCGAAAGAAACACCACGGTTCAGTTGTTCCGGGGTCGCC
ml.stra.5	CGGAAGAAAACGAATCTCTGGACGATCAGAACATTTCTATCGCGGGTCACACCTTCATCG
ml.stra.6	TTTTCGCCGCTTTCAGGTTGGTGAACTGGTAGTTCGGACGGTCGATGAAGGTGTGACCCG
ml.stra.7	ACCTGAAAGCGGCGAAAAAAGGTTCTATGGTTTACTTCAAAGTTGGTAACGAAACCCGTA
ml.stra.8	CGGTCGGTTTAACGTCACGGATAGAGGTCATTTTGTATTTACGGGTTTCGTTACCAACTT
ml.stra.9	CGTGACGTTAAACCGACCGACGTTGGTGTTCTGGATGAGCAGAAAGGTAAAGATAAACAG
ml.stra.10	TTTTCGTTGTAGTCGTCGCAGGTGATCAGGGTCAGCTGTTTATCTTTACCTTTCTGCTCA
ml.stra.11	TGCGACGACTACAACGAAAAGACCGGTGTTTGGGAAAAACGTAAAATCTTCGTTGCGACC
ml.stra.12	TTTAACTTCGGTCGCAACGAAGATTTTACG

Sortase-Roche adaptor primers:

pg.strA.nHis.OLE.F	CGCTTAATTAAACATATGACCGGGCAGGCGAAACCGCAGATCCCG
pg.strA.nHis.OLE.R	TTAGTTAGTTACCGGATCCCTTATTATTTAACTTCGGTCGCAACGAAGAT

RTS Extension primers:

RTS.ext.F	CGCTTAATTAAACATATGACC
RTS.ext.R	TTAGTTAGTTACCGGATCCCTTATTA

The sequence of the upstream regulator region (RocheUp) was:

GATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAG
ACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGTCTGGTTCTCATCATCA
TCATCATCATAGCAG CGGCATCGAAGGCCGCGGCCGCTTAATTAAACATATGACC

The sequence of the downstream regulator region (RocheDown) was:

GGGATCCGGTAACTAACTAAGATCCGGTAAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGC
CACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAA
CTATATCCGGATA TCCACAGGACGGGTGTGGTCGCC

The primer used to append the RocheUp and RocheDown regulator regions to the assembled gene sequences were:

5.RTS.F	TACGATGCCGGCCACGATGCGT
RTS.hisR	GGCGACCACACCCGTCCTGTGGATATCC

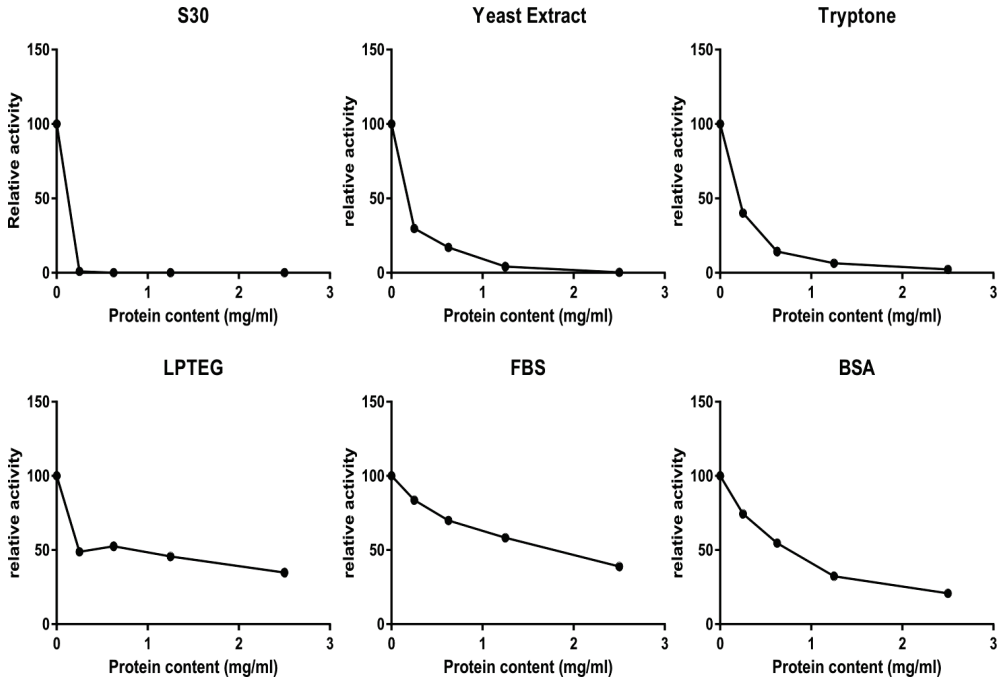

Figure S1: Sortase activity is inhibited by the presence of cell lysates and other additives. Sortase activity was measured using a bead-based FACS assay. Poisoning assays were performed using a bead based activity assay. Neutravidin coated 1 um polystyrene microbeads were saturated with biotinylated LPETG peptide. These beads were then incubated with 1.5 uM purified recombinant sortase A in the presence of varying concentrations of each additive. These reactions were allowed to proceed for 12 hrs at 37°C. The beads were then collected by centrifugation, washed in TBS + 0.1% tween 20 and analyzed using an Eclypse iCyt flow cytometer. The values reported are the median value from the histogram obtained.

In cell lysate based additives (S30, Yeast Extract, Tryptone) sortase activity was completely inhibited at concentrations far below physiological levels. In addition to the calcium dependence of sortase, this is likely an additional obstacle to observing intracellular sortase activity.

Because the mechanism of inhibition is not known for any of these inhibitors, we have simply connected the points as a visual aid.

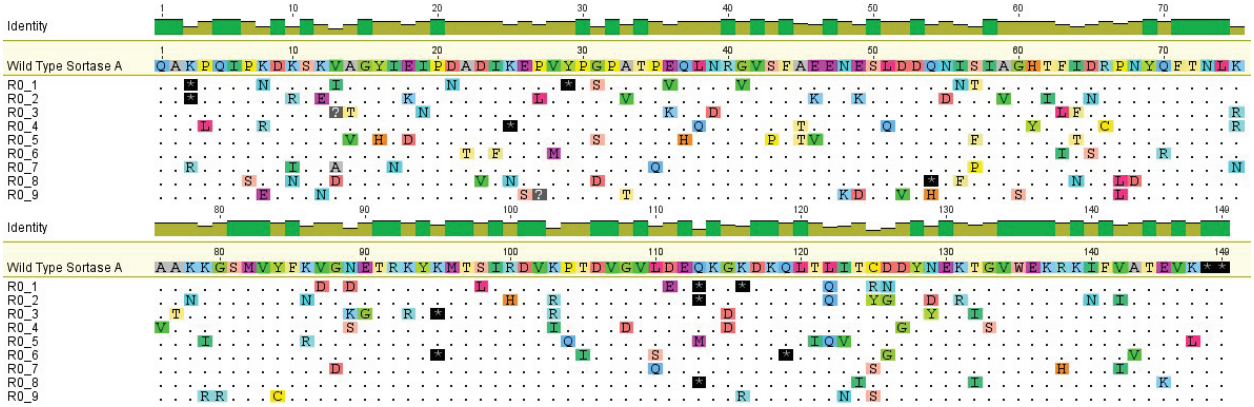

Figure S2: Round 0 sequencing data. The sequences of 9 clones isolated from Round 0 were used to determine the mutagenesis level of the starting library. Base on this analysis the starting library contained an average mutagenesis level of ~10%.

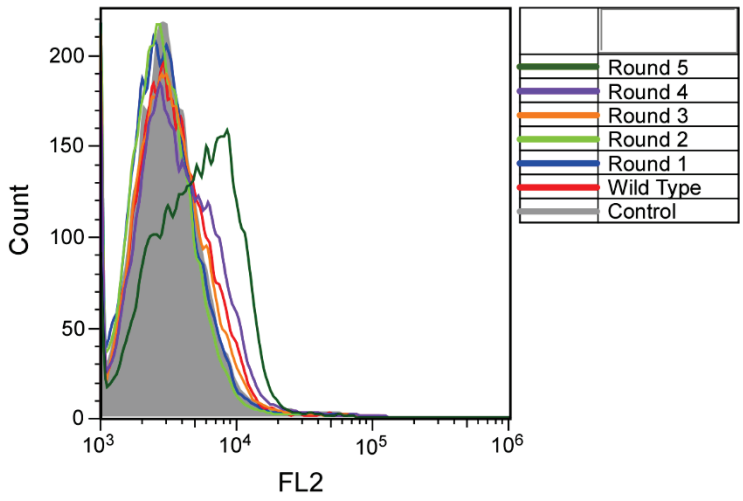

Figure S3: Sortase R1-5 selection progress. Reactions were carried out in a manner similar to the selection. Briefly, reactions for each library were set up with approximately 100 genes per bead and 100,000 peptides per bead. The reactions were set up, emulsified and recovered as described previously and analyzed on an Eclypse iCyt measuring fluorescence in the FITC channel. For the first 5 rounds of selection, the activity of the library showed steady improvement compared to wild type sortase A.

Figure S4: Round 15 sequencing data. Sequences were grouped by similarity using ClustalW for a first pass and then further refined manually.

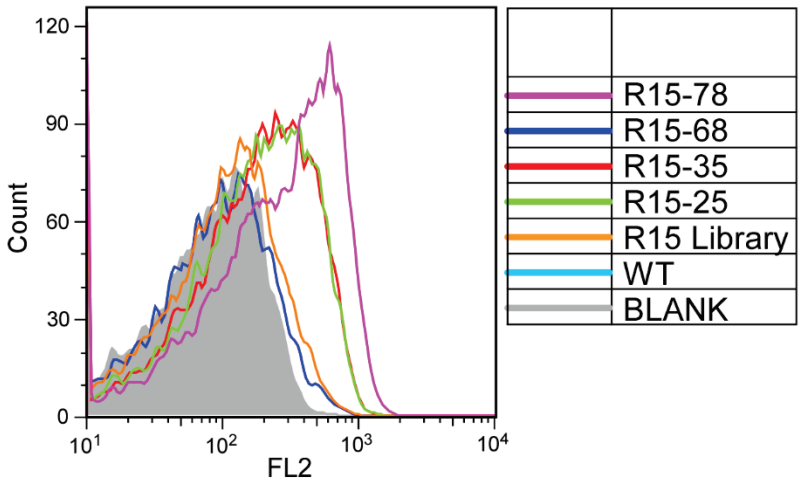

Figure S5: Despite being from the most abundant family in the selection (making up approximately 2/3 of sequences) R15-68 showed relatively poor activity compared to other clones tested. Reactions were performed in emulsion as described previously. On average 100 DNA molecules were displayed on each bead.

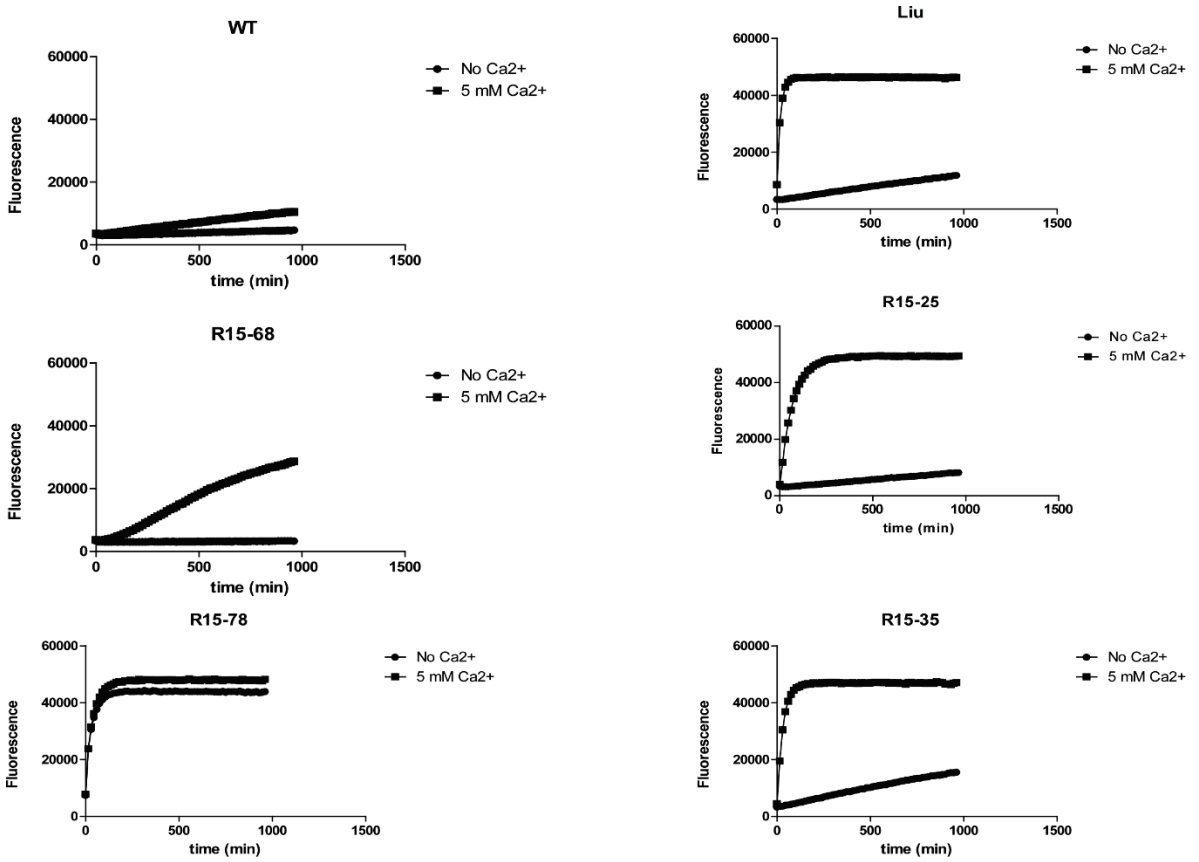

Figure S6: Progress curves of sortase variants with and without calcium. Reactions were performed in 50 mM Tris pH 7.5 and 150 mM NaCl with or without 5 mM $CaCl_2$. 1 uM Sortase Substrate II from Anaspec was incubated with 1 uM enzyme and 5 mM triglycine at 37°C for 16 hrs. Fluorescence was measured every 15 min using an excitation of 335 nm and emission of 493 nm. While all performed better than wild type, R15-78 showed nearly identical reaction rates both in the presence and absence of calcium ions.

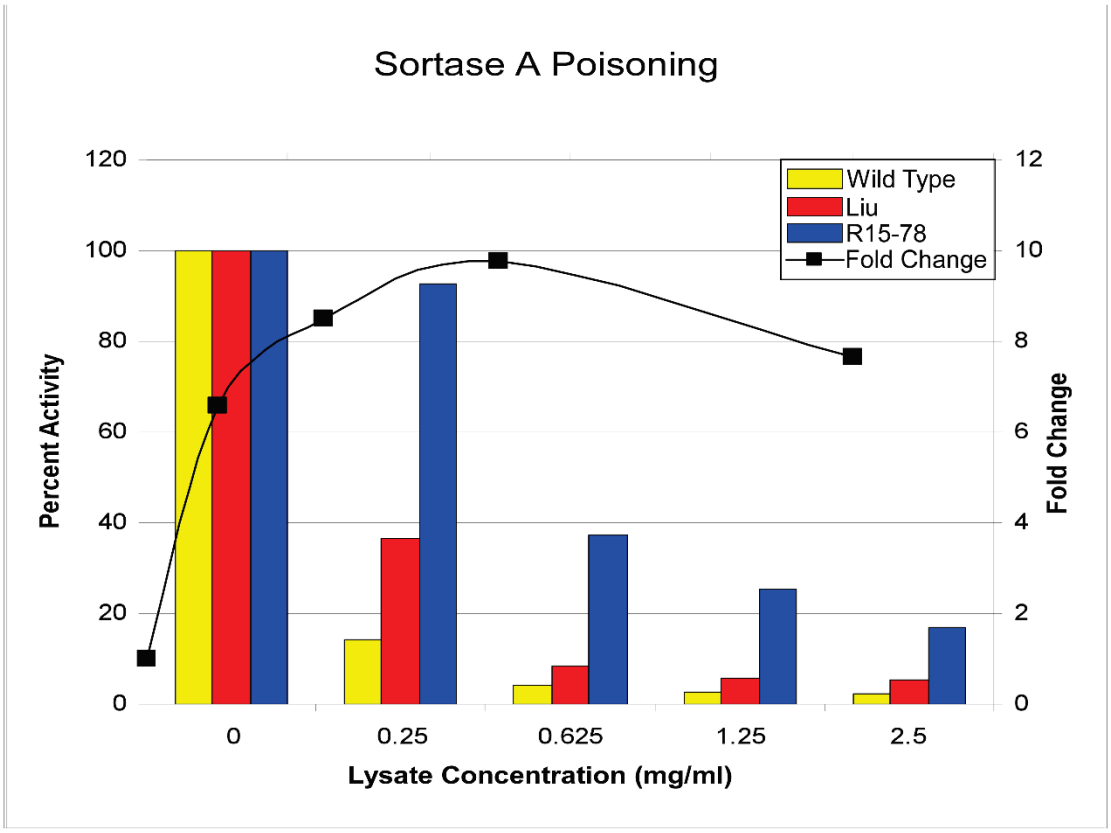

Figure S7: R15-78 shows increased resistance to inhibition by *E.coli* lysate. Using the bead based FACS assay described previously to measure sortase activity, purified wild type sortase A, Liu-tase and R15-78 were tested in the presence of increasing amounts of lysate. Compared to wild type sortase, R15-78 shows up to a 10-fold increase in resistance to lysate inhibition.