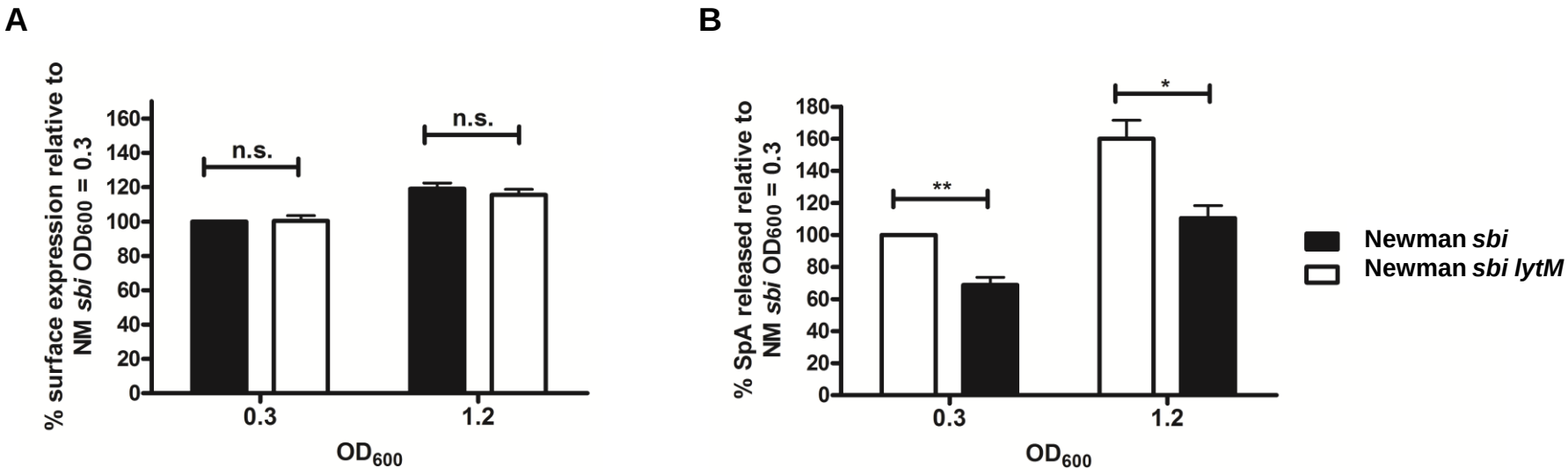

Figure S1. LytM-mediated release of protein A by *S. aureus* Newman.

A) Protein A on the surface of Newman *sbi* and Newman *sbi lytM* was detected using FITC-labelled rabbit IgG and the fluorescence intensity was measured by flow cytometry. Values are plotted as a percentage of the mean fluorescence intensity measured for NM *sbi* grown to an OD_{600} of 0.3. Bars represent the mean values and error bars indicate the SEM of three independent experiments.

B) Protein was captured from culture supernatants using chicken anti-SpA polyclonal IgY and detected using biotin-conjugated mouse monoclonal anti-SpA IgG followed by streptavidin-HRP in an ELISA. Values are expressed as a percentage of total released SpA measured for Newman *sbi* grown to an OD_{600} = 0.3. Bars represent the mean percentage release from four independent experiments. Error bars represent the SEM. ** $p = 0.003$, * $p = 0.014$, n.s. = not significant, $p > 0.05$.

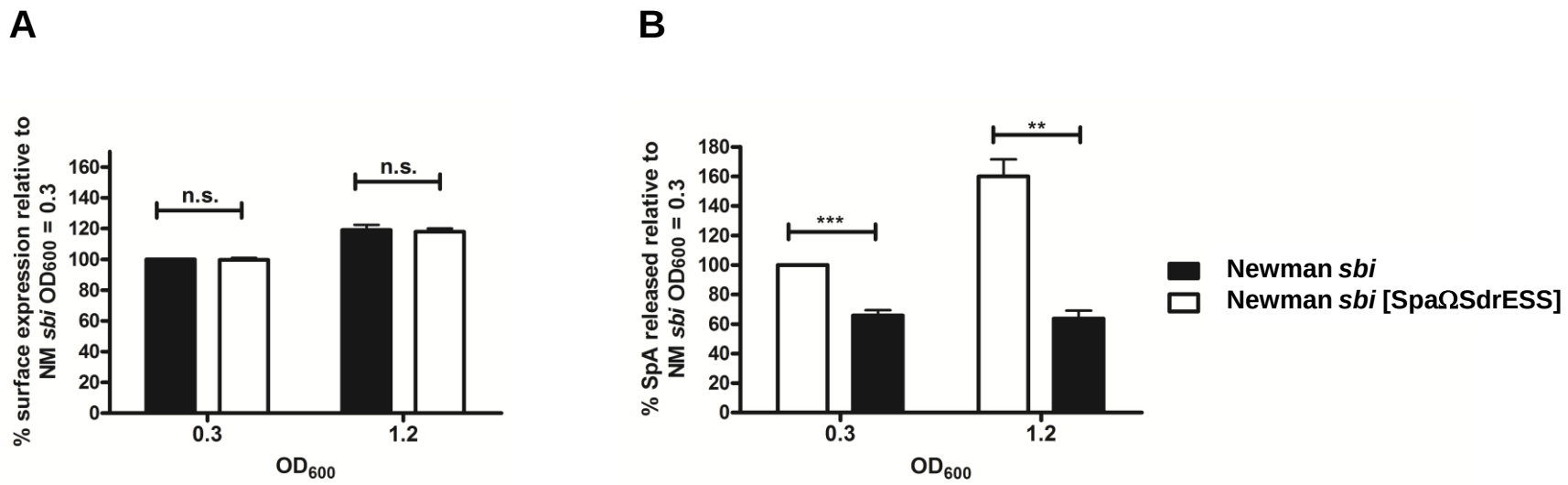

Figure S2. **Replacing the SpA sorting signal with the sorting signal from SdrE reduces release of SpA by *S. aureus* Newman**

A) Protein A on the surface of Newman *sbi* and Newman *sbi* [SpAΩSdrESS] was detected using FITC-labelled rabbit IgG and the fluorescence intensity was measured by flow cytometry. Values are plotted as a percentage of the mean fluorescence intensity measured for Newman *sbi* grown to an OD_{600} of 0.3. Bars represent the mean values and error bars indicate the SEM of three independent experiments.

B) Protein was captured from culture supernatants using chicken anti-SpA polyclonal IgY and detected using biotin-conjugated mouse monoclonal anti-SpA IgG followed by streptavidin-HRP in an ELISA. Values are expressed as a percentage of total released SpA measured for Newman *sbi* grown to an OD_{600} = 0.3. Bars represent the mean percentage release from four independent experiments. Error bars represent the SEM. *** p = <0.001, ** p = 0.02, n.s. = not significant, $p > 0.05$.

TABLE S1. Oligonucleotides used in this study.

Oligonucleotide name	5' - 3' sequence[a, b]
pSLF	GAATTCACTGGCCGTCGTTTTACAACGTCG
pSLR	GAGCTCAGATCTGTTAACGGTACCATCATA
SpAF	TATGATGGTACCGTTAACAGATCTGAGCTCTACAGGGGGTATTAATTTG
SpAR	CGACGTTGTAAAACGACGGCCAGTGAATTCTTATAGTTCGCGACGACG
RESF	CAGATGCTAACAAAGCTCAAGCATTACCAGAAACAGGTAGTG
RESR	GTAAAACGACGGCCAGTGAATTCTTATTTGTTTTGTTTTTTGCGAC
DWrR	TGGCGCCACTTTCTTTTCAGCATC
DSpF	GATGCTGAAAAGAAAGTGGCGCCATTACCAGAAACTGGTGAAG
DSdF	GATGCTGAAAAGAAAGTGGCGCCATTACCAGAAACAGGTAGTG
SEQF	ATTCAGGCTGCGCAAC
SEQR	TTGTTGACATTATATCATTG
lytm-A	GGAGGTACCCAATCACTATACGATGTATCAGAC
lytM-B	CATGTATAAAACATCCTCCATTAAAG
lytM-C	TTAATGGAGGATGTTTTATACATGTAATACAGAAAATCCCAAGTTG
lytM-D	GGAGAGCTCAAAGACAAATACAAAATCACATC
pSRER	TTATTTGTTTTGTTTTTTGCGAC
SpADSF	CGTCGCAAAAAACAAAACAAATAAAAACAAACAATACACAACGATAG
SpADSR	CGACGTTGTAAAACGACGGCCAGTGAATTCATTTCAACAAGCGGTTCATTAC
pIREF	CCTCACTAAAGGGAACAAAAGCTGGGTACCAAAGCCTTAAAGACGATC
pIRER	CGACTCACTATAGGGCGAATTGGAGCTCTCAACAAGCGGTTCATTAC
pIMAYF	GGTACCCAGCTTTTGTTCCCTTTAGTGAGG
pIMAYR	GAGCTCCAATTCGCCCTATAGTGAGTCG

[a] * indicates a 5' phosphorylation modification

[b] 5' extensions with homology to vector sequences were added to facilitate sequence and ligase-independent cloning (underlined).