

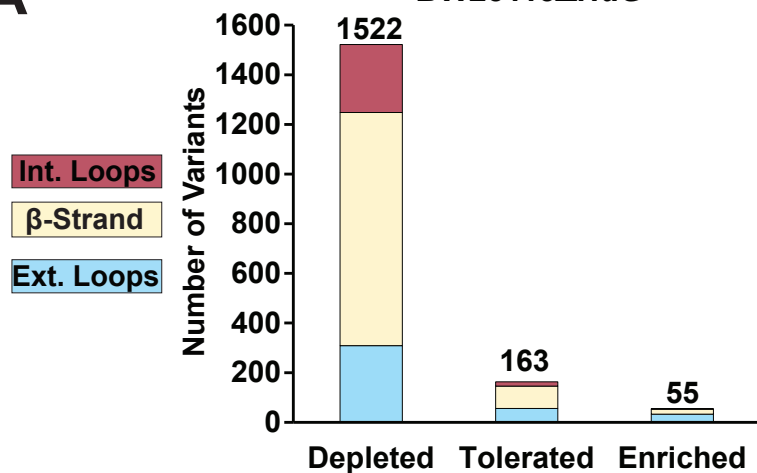
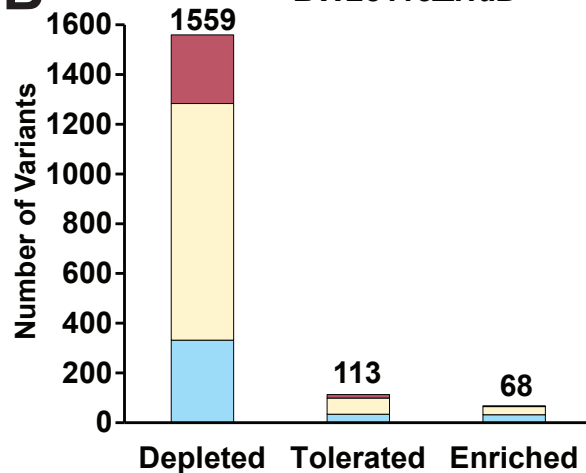
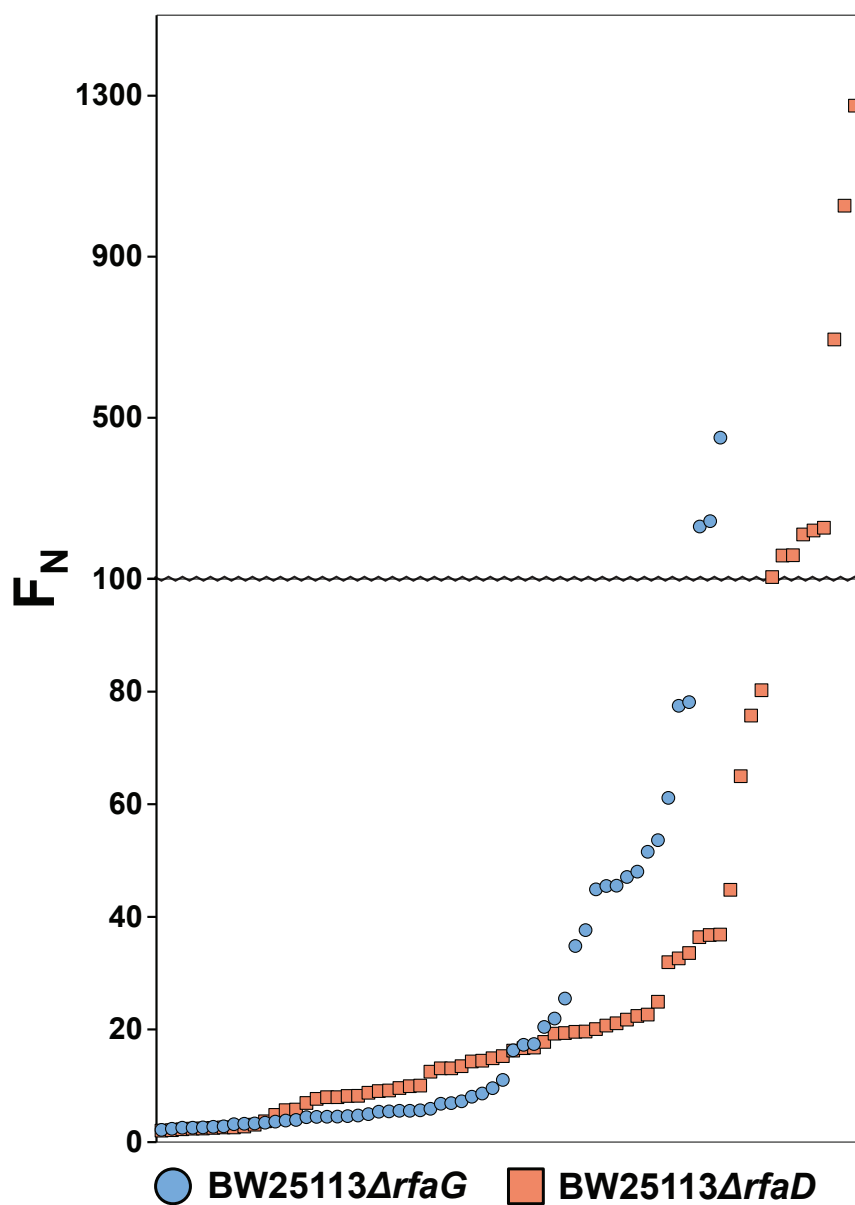
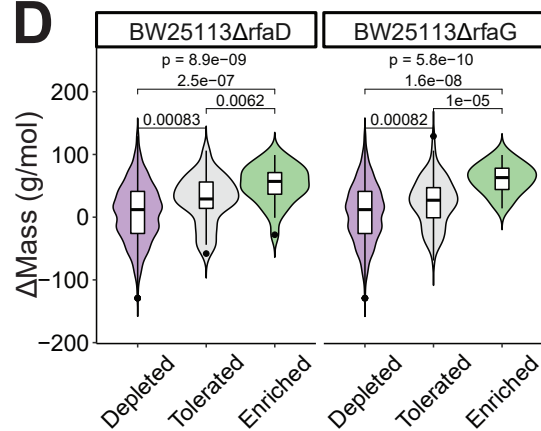
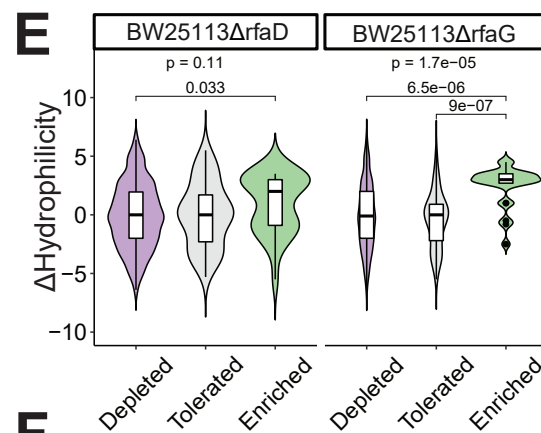
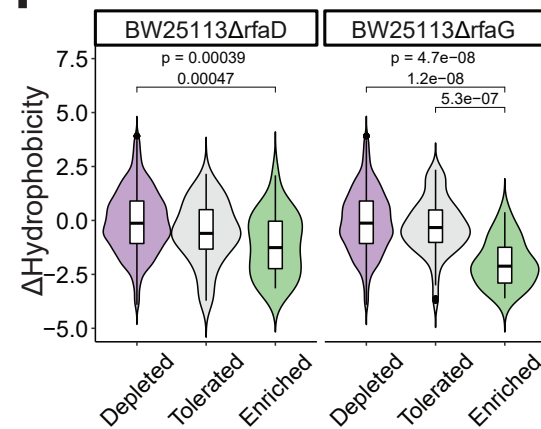
A**BW25113 Δ rfaG****B****BW25113 Δ rfaD****C****D****E****F**

Figure 4 – Figure Supplement 2. Distribution, enrichment profile and physicochemical properties of variants after selection on BW25113 Δ *rfaG* and BW25113 Δ *rfaD*.

Number of variants that were depleted ($F_N \leq 0.1$) tolerated ($F_N > 0.1$ and < 2) or enriched ($F_N \geq 2$) after selection on **(A)** BW25113 Δ *rfaG* or **(B)** BW25113 Δ *rfaD*, separated by topology of the tip domain color coded as interior loops (red), β -sheets (beige) and exterior loops (blue). **(C)** Average F_N of enriched variants ($F_N \geq 2$) for BW25113 Δ *rfaD* (orange squares) and BW25113 Δ *rfaG* (blue circles) ordered left to right from lowest to highest F_N . Violin plots comparing **(D)** change in mass, **(E)** change in hydrophilicity, and **(F)** change in hydrophobicity for grouped depleted ($F_N \leq 0.1$) generally tolerated ($F_N > 0.1$ and < 10) or well enriched ($F_N \geq 10$) substitutions on *E. coli* BW25113 Δ *rfaD* and BW25113 Δ *rfaG*. The upper p-value is the result of a Kruskal-Wallis test among all three groups while pairwise p-values from a Wilcoxon test are shown linking each group; p-values are shown if only if < 0.05 .