

**Figure S5** Identifying cryptic regulatory sites uncovered by mutations. (A) All motifs that strongly match either a wildtype or mutant 25-mer (P<0.001). Motifs are plotted by the change to match significance of each mutation (x-axis) and the fitness of each single mutant (y-axis). Points are colored based on their annotated function. (B) Inset showing motif matches that are weakened by mutations that confer fitness increases. Unannotated mutations that match multiple motifs are grouped the transcription factor motif matched is noted. Transcription factors that we hypothesize to be functionally important at that site are in bold.