SUPPLEMENTARY FIGURES

Figure S1: Distribution of various codon usage metrics for *M. extorquens* AM1 protein-coding genes, obtained from the codon usage bias database (http://cub-db.cs.umt.edu/index.shtml). Data for *fae* are indicated with a dashed blue line. Numbers in parentheses show the percentile values for *fae*, for each metric.

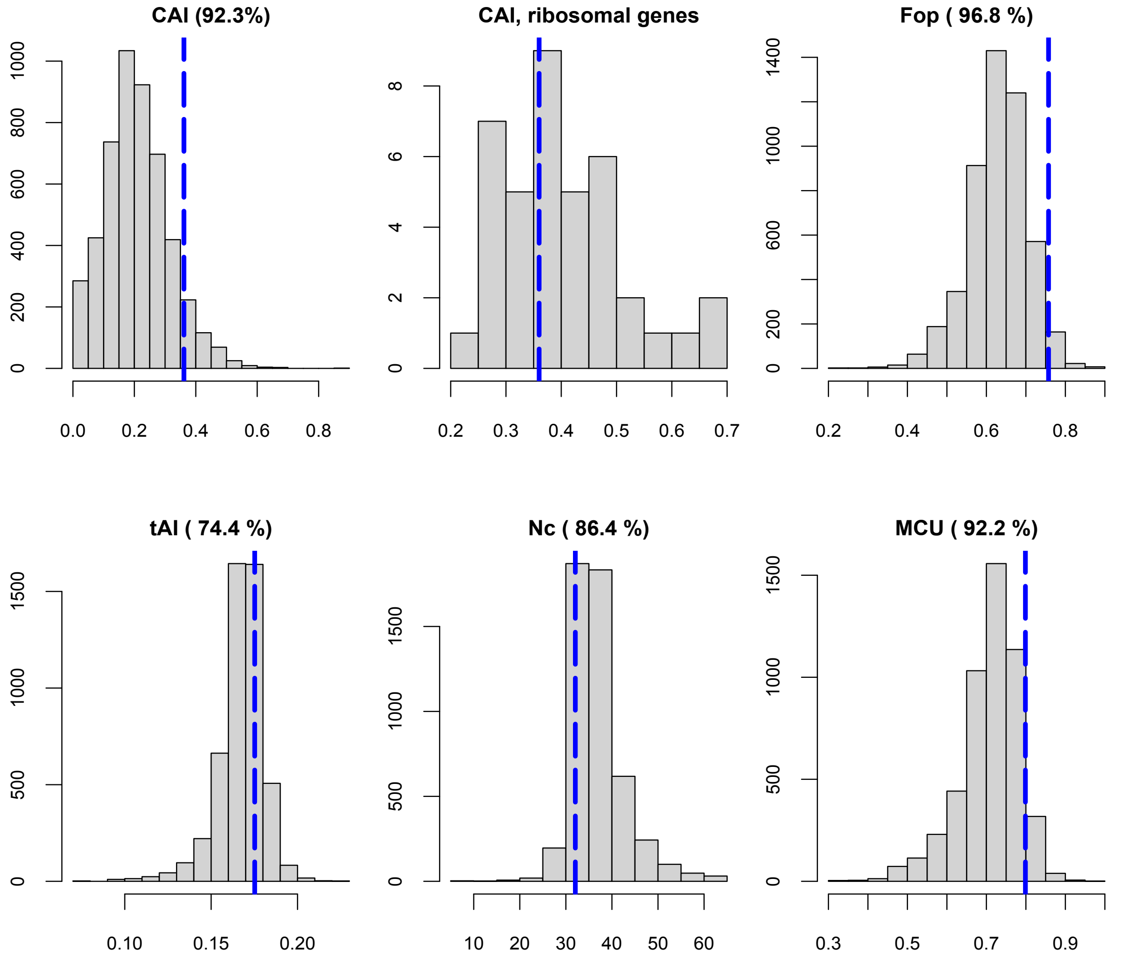

Figure S2: Growth rate (mean ± 2 se; n = 4) on methanol is strongly correlated with fitness during competition with fluorescently labeled wild type strain. The open triangle shows data for the wild type strain without FLAG tags (“WT*), and the diamond indicates the *fae* knockout (Del).

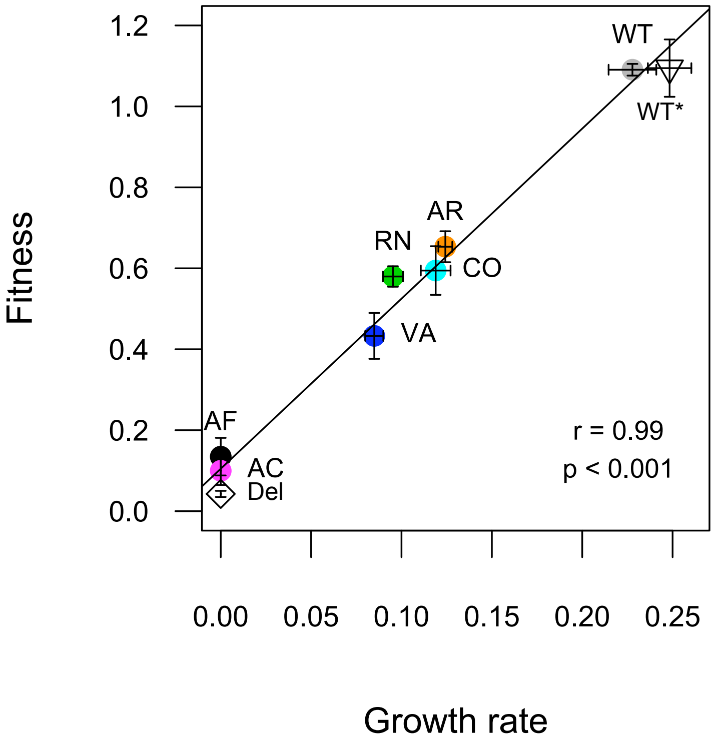

Figure S3: (a) Minimum folding energy for each *fae* allele calculated for 50 nt windows, starting 100 nt upstream of the start codon. Dashed lines indicate windows that overlap the start codon. (b) P values for the correlation between protein levels of each mutant and folding energy for each window. The dashed line shows the threshold p value after correcting for multiple tests using the Bonferroni correction.

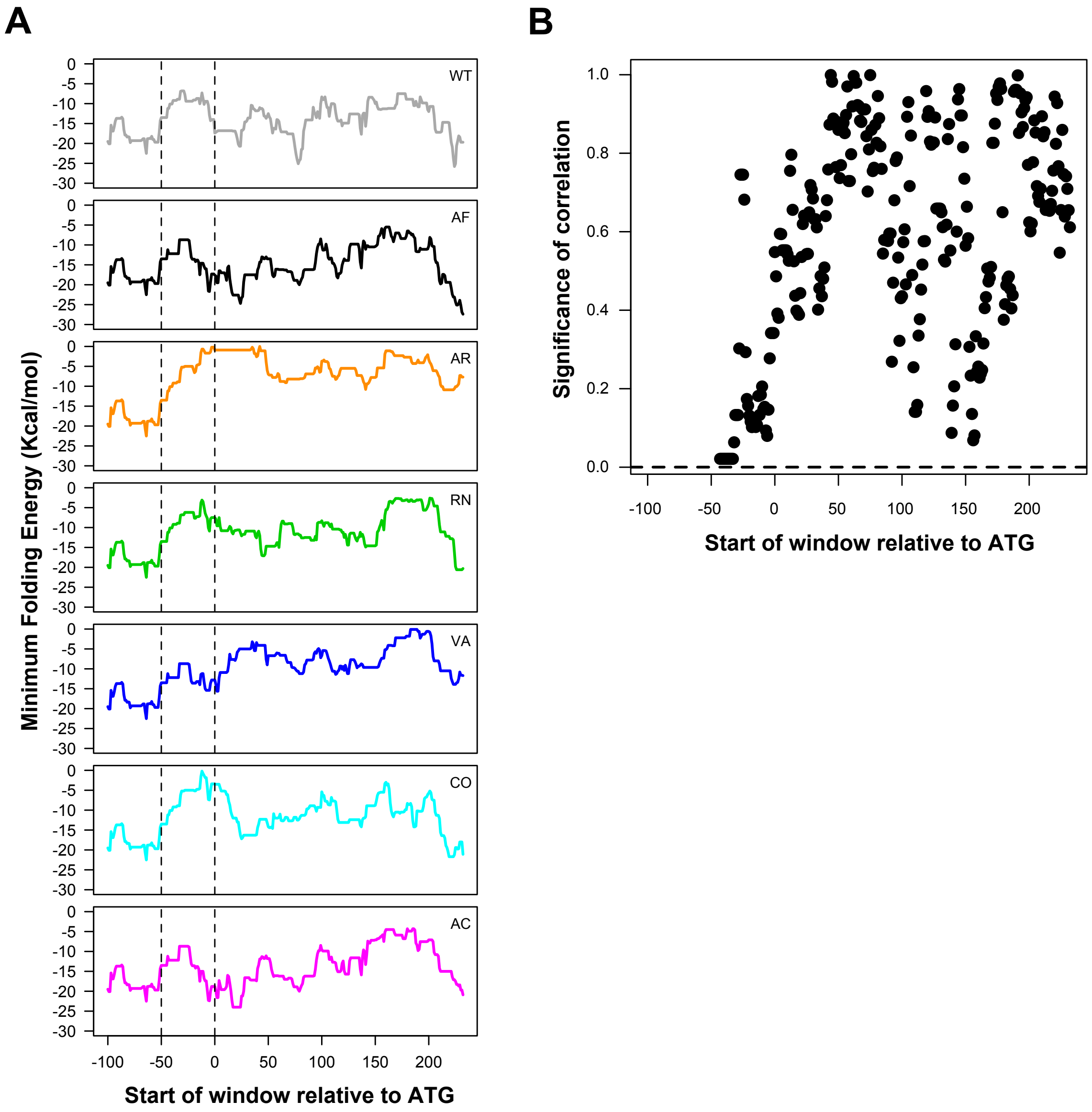

Figure S4: Local tAI for each codon as a function of distance from the start codon, for each *fae* allele. No 5′ “ramp” of low tAI codons is observed for any allele.

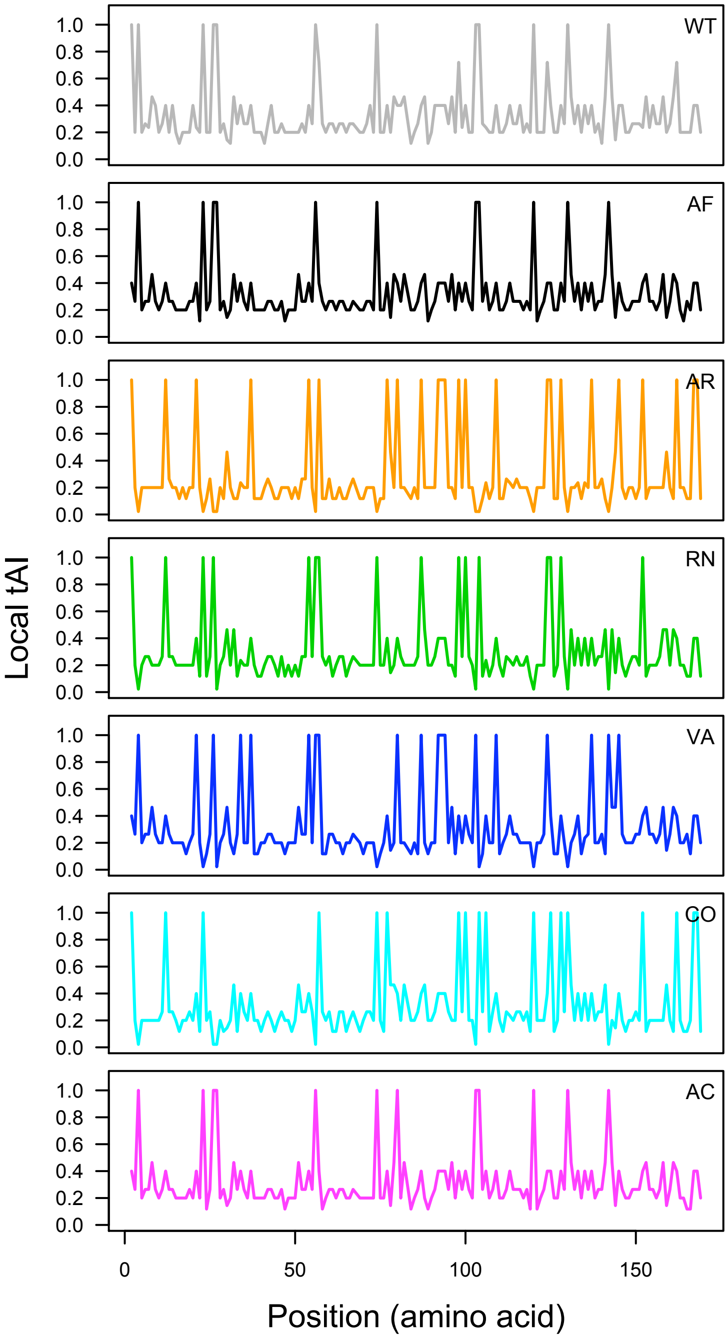

Figure S5: FAE protein production in each *fae* mutant, as a function of the number of hexamers GAAGAA and TGGCCA.

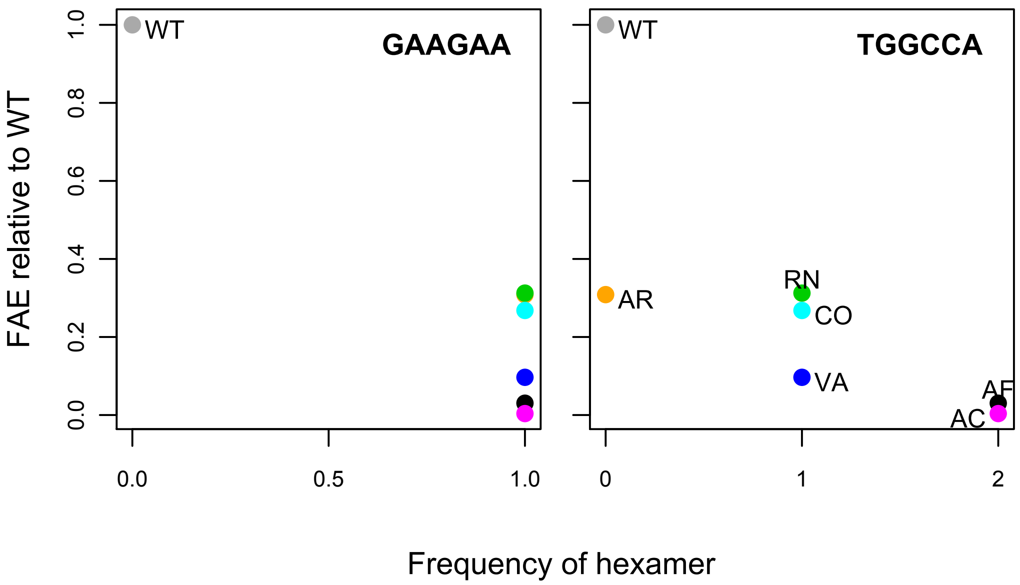

Figure S6: Soluble and insoluble FAE fractions in mutant strains as seen in Western Blots.

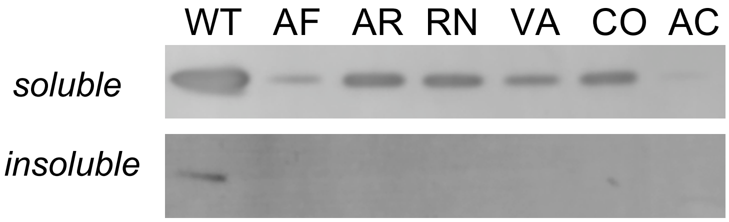

Figure S7: The relationship between isoaccepting tRNA fractions and codon fractions for 12 amino acids with two or more isoaccepting tRNAs, calculated as in Qian et al 2012 [18]. For amino acids with at least three codons, best fit regression lines are shown; for amino acids with only two codons, lines simply connect the two points. A positive correlation indicates that relative codon usage is proportional to tRNA availability. Grey points and lines show wild type *fae* (WT); orange points and lines indicate the mutant with only rare codons (AR), and black points and dotted black lines indicate the mutant with only frequent codons (AF).