**Table S3**. Relative fitness measured in different conditions and different genetic backgrounds.

| Genetic background | Fitness assay at | Mutant | Codon Change | Relative fitness (±95%CI)[1] | p-value[2] | n | Phenotypic effect |
|---|---|---|---|---|---|---|---|
| REL 1206 (Ancestral background) | 42.2°C in DM25 | $A^{Rif}1$ | I572F | 1.246 ± 0.012 | **<0.0001∗∗∗** | 6 | **24.6% Advantage** |
| | | $A^{Rif}2$ | I572L | 1.211 ± 0.018 | **<0.0001∗∗∗** | 6 | **21.1% Advantage** |
| | | $A^{Rif}3$ | I572N | 1.182 ± 0.020 | **0.0001∗∗∗** | 6 | **18.2% Advantage** |
| | 37°C in DM25 | $A^{Rif}1$ | I572F | 0.981 ± 0.013 | 0.0893 | 12 | Neutral |
| | | $A^{Rif}2$ | I572L | 0.944 ± 0.017 | **0.011∗** | 6 | **5.6% Cost** |
| | | $A^{Rif}3$ | I572N | 0.942 ± 0.016 | **0.0064∗∗** | 6 | **5.8% Cost** |
| | 42.2°C in DM1000 | $A^{Rif}1$ | I572F | 0.969 ± 0.055 | 0.2944 | 12 | Neutral |
| | | $A^{Rif}2$ | I572L | 0.981 ± 0.015 | 0.1155 | 12 | Neutral |
| | | $A^{Rif}3$ | I572N | 1.005 ± 0.017 | 0.6065 | 12 | Neutral |
| | 37°C in DM1000 | $A^{Rif}1$ | I572F | 0.956 ± 0.017 | **0.0135∗** | 12 | **4.4% Cost** |
| | | $A^{Rif}2$ | I572L | 0.963 ± 0.015 | **0.0147∗** | 12 | **3.7% Cost** |
| | | $A^{Rif}3$ | I572N | 0.947 ± 0.017 | **0.0051∗∗** | 12 | **5.3% Cost** |
| | 42.2°C in LB | $A^{Rif}1$ | I572F | 0.868 ± 0.014 | **0.0001∗∗∗** | 6 | **13% Cost** |
| | | $A^{Rif}2$ | I572L | 0.836 ± 0.033 | **0.002∗∗** | 6 | **13% Cost** |
| | | $A^{Rif}3$ | I572N | 0.928 ± 0.030 | **0.03∗** | 6 | **9.8% Cost** |
| REL 606 | 42.2°C in DM25 | $R^{Rif}1$ | I572F | 1.286 ± 0.030 | **<0.0001∗∗∗** | 6 | **28.6% Advantage** |
| | | $R^{Rif}2$ | I572L | 1.302 ± 0.036 | **0.0002∗∗∗** | 6 | **30.2% Advantage** |
| | | $R^{Rif}3$ | I572N | 1.297 ± 0.038 | **0.0003∗∗∗** | 6 | **29.7% Advantage** |
| K12 (MG1655) | 42.2°C in DM25 | $K^{Rif}1$ | I572F | 0.908 ± 0.018 | **0.0004∗∗∗** | 6 | **9.2% Cost** |
| | | $K^{Rif}2$ | I572L | 0.975 ± 0.010 | **0.0218∗** | 6 | **2.5% Cost** |

[1]Mean relative fitness and 95% confidence interval based on n replicates.

[2]Significance values based on one-tailed t-distribution with n-1 degrees of freedom; the null hypothesis is that the mean fitness equals 1.