

# Supporting Information

## Table of Content

### Supporting Texts

**Text S1:** Assessing the statistical significance in different types of amino acid changes by rate ratio tests.

### Supporting Tables

**Table S1.** Statistics for different types of amino acid changes for pathogenic versus benign mutations.

**Table S2.** Statistics for forward versus reverse amino acid changes among pathogenic mutations.

**Table S3.** Statistics for forward versus reverse amino acid changes among benign mutations.

### Supporting Figures

**Figure S1.** Histograms of FoldX free energy change predictions for pathogenic and benign mutations

## Supporting Texts

### Text S1. Assessing the statistical significance in different types of amino acid changes by rate ratio tests

In this section, we explain how to apply rate ratio test (Fay, 2010) to quantify the statistical significance (p-value) of the difference between pathogenic versus benign mutations for the same type of amino acid change. As an example, we will consider the mutation from amino acid type L to P.  $n_{path}(L \rightarrow P) = 1171$  and  $n_{ben}(L \rightarrow P) = 466$  mutations are for amino acid change from L to P among  $N_{path} = 31132$  pathogenic mutations and  $N_{ben} = 39465$  benign mutations, respectively. Therefore, the observed rates of L to P mutations among pathogenic and benign mutations are 0.0376 and 0.0118, respectively:

$$\begin{cases} P_{path}(L \rightarrow P) = n_{path}(L \rightarrow P)/N_{path} = 0.0376 \\ P_{ben}(L \rightarrow P) = n_{ben}(L \rightarrow P)/N_{ben} = 0.0118 \end{cases} \quad (S1)$$

Without loss of generality, we suppose  $P_{path}(L \rightarrow P) \geq P_{ben}(L \rightarrow P)$ . Note that  $P_{path}(L \rightarrow P)$  and  $P_{ben}(L \rightarrow P)$  are observed rates, while the (unknown) real rates of  $L \rightarrow P$  among pathogenic and benign mutations are  $\lambda_{path}(L \rightarrow P)$  and  $\lambda_{ben}(L \rightarrow P)$ , respective. The null and alternative of rate ratio test for  $L \rightarrow P$  are:

$$\begin{cases} H_0: RR(L \rightarrow P) = \frac{\lambda_{path}(L \rightarrow P)}{\lambda_{ben}(L \rightarrow P)} = 1 \\ H_1: RR(L \rightarrow P) = \frac{\lambda_{path}(L \rightarrow P)}{\lambda_{ben}(L \rightarrow P)} > 1 \end{cases} \quad (S2)$$

For ease of discussion, we denote:

$$N(L \rightarrow P) = n_{path}(L \rightarrow P) + n_{ben}(L \rightarrow P) \quad (S3)$$

$$p(L \rightarrow P) = \frac{N_{path} \cdot \lambda_{path}(L \rightarrow P)}{N_{path} \cdot \lambda_{path}(L \rightarrow P) + N_{ben} \cdot \lambda_{ben}(L \rightarrow P)} \quad (S4)$$

Since any  $L \rightarrow P$  mutations we discuss here are either pathogenic or benign, with probability  $p(L \rightarrow P)$  and  $1 - p(L \rightarrow P)$ , respectively, the number of  $L \rightarrow P$  mutations that are pathogenic should follow a binomial distribution:

$$n_{path}(L \rightarrow P) \sim \text{binom}(N(L \rightarrow P), p(L \rightarrow P)) \quad (S5)$$

The p-value can thus be calculated as:

$$\begin{aligned} p - \text{value}(RR(L \rightarrow P) > 1) &= P(n_{path}(L \rightarrow P) \leq \text{binom}(N(L \rightarrow P), p(L \rightarrow P))) \\ &= 1 - P(n_{path}(L \rightarrow P) > \text{binom}(N(L \rightarrow P), p(L \rightarrow P))) \end{aligned} \quad (S6)$$

Since  $n_{path}(L \rightarrow P)$  is a discrete variable (i.e. an integer), equation (S6) can be rewritten into:

$$\begin{aligned} p - \text{value}(RR(L \rightarrow P) > 1) &= 1 - P(n_{path}(L \rightarrow P) + 1 \geq \text{binom}(N(L \rightarrow P), p(L \rightarrow P))) \\ &= 1 - p\text{binom}(n_{path}(L \rightarrow P) + 1, N(L \rightarrow P), p(L \rightarrow P)) \end{aligned} \quad (S7)$$

The term  $p\text{binom}(n_{path}(L \rightarrow P) + 1, N(L \rightarrow P), p(L \rightarrow P))$  is the value of Cumulative Distribution Function (CDF) for binomial distribution  $\text{binom}(N(L \rightarrow P), p(L \rightarrow P))$  at quantile  $n_{path}(L \rightarrow P) + 1$ . Under any null hypothesis regarding the rate ratio parameter, equation (S4) can be re-written as:

$$p(L \rightarrow P) = \frac{N_{path} \cdot \lambda_{path}(L \rightarrow P)}{N_{path} \cdot \lambda_{path}(L \rightarrow P) + N_{ben} \cdot \lambda_{ben}(L \rightarrow P)} = \frac{N_{path}}{N_{path} + N_{ben}} \quad (S8)$$

Therefore, equation (S7) can be rewritten into:

$$p - value(RR(L \rightarrow P) > 1) = 1 - pbinom\left(n_{path}(L \rightarrow P) + 1, N(L \rightarrow P), \frac{N_{path}}{N_{path} + N_{ben}}\right) \quad (S9)$$

Our program for rate ratio test is available at <https://zhanglab.ccmb.med.umich.edu/RAR>, as part of our previous study (Wei, et al., 2020). Similar to the above procedure, we can also calculate the rate ratio test  $p$ -value to assess the statistical significance in difference between forward and reverse L to P mutations (i.e. mutation  $L \rightarrow P$  versus mutation  $P \rightarrow L$ ) among all pathogenic mutations (Table S2) or among all benign mutations (Table S3).

## Reference

- Fay, M.P. Two-sided Exact Tests and Matching Confidence Intervals for Discrete Data. *R J* 2010;2(1):53-58.  
Wei, X., *et al.* Detecting Gene Ontology misannotations using taxon-specific rate ratio comparisons. *Bioinformatics* 2020;36(16):4383-4388.

## Supporting Tables

**Table S1.** Statistics for different types of amino acid changes for pathogenic versus benign mutations. This table is ranked in descending order of rate differences, and only includes the 251 pairs of amino acid type change that occurs among any mutations.

| Mutation | Rate among pathogenic mutation | Rate among benign mutation | P-value of rate ratio test |
|----------|--------------------------------|----------------------------|----------------------------|
| L→P      | 3.761e-02                      | 1.181e-02                  | 2.220e-16                  |
| G→R      | 3.864e-02                      | 1.490e-02                  | 2.220e-16                  |
| R→C      | 3.649e-02                      | 1.974e-02                  | 2.220e-16                  |
| R→W      | 3.296e-02                      | 1.629e-02                  | 2.220e-16                  |
| C→Y      | 1.747e-02                      | 4.561e-03                  | 2.220e-16                  |
| Y→C      | 1.902e-02                      | 7.196e-03                  | 2.220e-16                  |
| G→D      | 1.975e-02                      | 8.235e-03                  | 2.220e-16                  |
| C→R      | 1.667e-02                      | 5.245e-03                  | 2.220e-16                  |
| G→V      | 1.551e-02                      | 5.245e-03                  | 2.220e-16                  |
| R→P      | 1.307e-02                      | 4.029e-03                  | 2.220e-16                  |
| G→E      | 1.481e-02                      | 6.689e-03                  | 2.220e-16                  |
| L→R      | 1.086e-02                      | 3.345e-03                  | 2.220e-16                  |
| C→F      | 6.874e-03                      | 1.216e-03                  | 2.220e-16                  |
| E→K      | 2.592e-02                      | 2.030e-02                  | 1.086e-06                  |
| W→R      | 8.673e-03                      | 3.953e-03                  | 1.998e-15                  |
| G→C      | 6.489e-03                      | 1.900e-03                  | 2.220e-16                  |
| R→L      | 9.026e-03                      | 4.840e-03                  | 2.389e-11                  |
| D→Y      | 6.938e-03                      | 2.838e-03                  | 3.553e-15                  |
| W→C      | 5.268e-03                      | 1.318e-03                  | 2.220e-16                  |
| F→S      | 7.709e-03                      | 3.953e-03                  | 6.138e-11                  |
| C→G      | 4.658e-03                      | 1.140e-03                  | 2.220e-16                  |
| C→W      | 4.433e-03                      | 1.090e-03                  | 2.220e-16                  |
| A→P      | 9.669e-03                      | 6.411e-03                  | 1.687e-06                  |
| C→S      | 6.456e-03                      | 3.243e-03                  | 8.749e-10                  |
| I→N      | 4.882e-03                      | 1.774e-03                  | 4.148e-13                  |
| D→V      | 5.685e-03                      | 2.661e-03                  | 4.525e-10                  |
| A→D      | 6.521e-03                      | 3.674e-03                  | 1.353e-07                  |
| G→S      | 1.844e-02                      | 1.568e-02                  | 5.812e-03                  |
| M→R      | 3.533e-03                      | 8.362e-04                  | 2.665e-15                  |
| D→G      | 9.412e-03                      | 6.943e-03                  | 3.459e-04                  |
| Y→D      | 3.180e-03                      | 8.869e-04                  | 5.151e-12                  |
| V→D      | 3.405e-03                      | 1.115e-03                  | 8.643e-11                  |
| V→F      | 4.401e-03                      | 2.331e-03                  | 2.503e-06                  |
| M→K      | 2.859e-03                      | 8.108e-04                  | 9.611e-11                  |
| V→G      | 5.461e-03                      | 3.471e-03                  | 9.428e-05                  |
| I→S      | 2.955e-03                      | 1.014e-03                  | 5.048e-09                  |
| V→E      | 3.501e-03                      | 1.571e-03                  | 3.704e-07                  |
| P→R      | 7.741e-03                      | 5.828e-03                  | 2.420e-03                  |
| F→C      | 3.276e-03                      | 1.419e-03                  | 3.615e-07                  |
| R→G      | 1.131e-02                      | 9.451e-03                  | 1.768e-02                  |
| S→F      | 8.673e-03                      | 6.867e-03                  | 7.579e-03                  |
| W→S      | 2.377e-03                      | 5.828e-04                  | 2.228e-10                  |
| Q→P      | 4.722e-03                      | 2.965e-03                  | 2.051e-04                  |
| D→H      | 5.782e-03                      | 4.029e-03                  | 1.078e-03                  |
| H→P      | 3.598e-03                      | 1.850e-03                  | 1.018e-05                  |
| W→G      | 2.345e-03                      | 6.335e-04                  | 1.970e-09                  |
| N→I      | 3.052e-03                      | 1.343e-03                  | 1.376e-06                  |
| T→R      | 3.501e-03                      | 1.951e-03                  | 9.867e-05                  |
| L→Q      | 3.148e-03                      | 1.672e-03                  | 8.005e-05                  |
| F→V      | 2.923e-03                      | 1.470e-03                  | 4.581e-05                  |
| R→H      | 2.865e-02                      | 2.724e-02                  | 2.741e-01                  |
| G→W      | 2.313e-03                      | 9.629e-04                  | 1.045e-05                  |

|     |           |           |           |
|-----|-----------|-----------|-----------|
| Y→N | 2.120e-03 | 7.855e-04 | 3.361e-06 |
| I→F | 3.373e-03 | 2.052e-03 | 9.485e-04 |
| N→K | 7.067e-03 | 5.803e-03 | 4.147e-02 |
| A→E | 3.822e-03 | 2.711e-03 | 1.191e-02 |
| Y→S | 2.538e-03 | 1.470e-03 | 1.940e-03 |
| R→S | 6.489e-03 | 5.499e-03 | 1.003e-01 |
| W→L | 1.510e-03 | 6.588e-04 | 7.656e-04 |
| L→H | 2.088e-03 | 1.292e-03 | 1.292e-02 |
| S→L | 9.508e-03 | 8.919e-03 | 4.404e-01 |
| S→W | 1.221e-03 | 6.335e-04 | 1.400e-02 |
| I→K | 8.673e-04 | 3.041e-04 | 2.692e-03 |
| N→Y | 1.735e-03 | 1.216e-03 | 9.020e-02 |
| F→I | 1.702e-03 | 1.216e-03 | 1.115e-01 |
| I→R | 8.673e-04 | 4.054e-04 | 2.098e-02 |
| H→L | 1.670e-03 | 1.267e-03 | 1.942e-01 |
| T→P | 5.075e-03 | 4.713e-03 | 5.279e-01 |
| Y→H | 5.750e-03 | 5.397e-03 | 5.656e-01 |
| S→P | 9.026e-03 | 8.691e-03 | 6.666e-01 |
| R→T | 2.248e-03 | 1.976e-03 | 4.823e-01 |
| H→D | 1.735e-03 | 1.470e-03 | 4.334e-01 |
| K→I | 7.067e-04 | 4.561e-04 | 2.196e-01 |
| L→W | 1.028e-03 | 8.362e-04 | 4.774e-01 |
| L→S | 4.015e-03 | 3.902e-03 | 8.579e-01 |
| L→G | 9.636e-05 | 0         | 0         |
| A→F | 9.636e-05 | 0         | 0         |
| G→L | 9.636e-05 | 2.534e-05 | 4.591e-01 |
| V→Q | 6.424e-05 | 0         | 0         |
| G→K | 6.424e-05 | 0         | 0         |
| A→C | 6.424e-05 | 0         | 0         |
| T→K | 2.313e-03 | 2.255e-03 | 9.341e-01 |
| E→V | 2.281e-03 | 2.230e-03 | 9.486e-01 |
| S→I | 2.505e-03 | 2.458e-03 | 9.578e-01 |
| R→E | 9.636e-05 | 5.068e-05 | 7.807e-01 |
| S→H | 6.424e-05 | 2.534e-05 | 8.238e-01 |
| F→T | 6.424e-05 | 2.534e-05 | 8.238e-01 |
| D→F | 6.424e-05 | 2.534e-05 | 8.238e-01 |
| A→I | 6.424e-05 | 2.534e-05 | 8.238e-01 |
| W→F | 3.212e-05 | 0         | 0         |
| V→P | 3.212e-05 | 0         | 0         |
| T→H | 3.212e-05 | 0         | 0         |
| R→N | 3.212e-05 | 0         | 0         |
| P→F | 3.212e-05 | 0         | 0         |
| N→V | 3.212e-05 | 0         | 0         |
| N→G | 3.212e-05 | 0         | 0         |
| M→N | 3.212e-05 | 0         | 0         |
| I→Y | 3.212e-05 | 0         | 0         |
| I→P | 3.212e-05 | 0         | 0         |
| H→T | 3.212e-05 | 0         | 0         |
| H→A | 3.212e-05 | 0         | 0         |
| G→T | 3.212e-05 | 0         | 0         |
| F→A | 3.212e-05 | 0         | 0         |
| D→I | 3.212e-05 | 0         | 0         |
| D→C | 3.212e-05 | 0         | 0         |
| C→V | 3.212e-05 | 0         | 0         |
| A→Y | 3.212e-05 | 0         | 0         |
| A→N | 3.212e-05 | 0         | 0         |
| A→K | 6.424e-05 | 5.068e-05 | 1         |
| S→R | 6.039e-03 | 6.031e-03 | 1         |
| W→K | 3.212e-05 | 2.534e-05 | 1         |
| S→V | 3.212e-05 | 2.534e-05 | 1         |

|     |           |           |   |
|-----|-----------|-----------|---|
| R→D | 3.212e-05 | 2.534e-05 | 1 |
| R→A | 3.212e-05 | 2.534e-05 | 1 |
| Q→W | 3.212e-05 | 2.534e-05 | 1 |
| Q→G | 3.212e-05 | 2.534e-05 | 1 |
| P→V | 3.212e-05 | 2.534e-05 | 1 |
| I→A | 3.212e-05 | 2.534e-05 | 1 |
| F→N | 3.212e-05 | 2.534e-05 | 1 |
| F→H | 3.212e-05 | 2.534e-05 | 1 |
| F→G | 3.212e-05 | 2.534e-05 | 1 |
| E→M | 3.212e-05 | 2.534e-05 | 1 |
| D→Q | 3.212e-05 | 2.534e-05 | 1 |
| C→H | 3.212e-05 | 2.534e-05 | 1 |
| A→L | 6.424e-05 | 7.602e-05 | 1 |
| L→Y | 3.212e-05 | 5.068e-05 | 1 |
| R→Q | 3.145e-02 | 3.147e-02 | 1 |
| Y→T | 0         | 2.534e-05 | 0 |
| Y→R | 0         | 2.534e-05 | 0 |
| Y→M | 0         | 2.534e-05 | 0 |
| Y→I | 0         | 2.534e-05 | 0 |
| W→P | 0         | 2.534e-05 | 0 |
| W→E | 0         | 2.534e-05 | 0 |
| V→T | 0         | 2.534e-05 | 0 |
| V→S | 0         | 2.534e-05 | 0 |
| T→L | 0         | 2.534e-05 | 0 |
| T→G | 0         | 2.534e-05 | 0 |
| T→D | 0         | 2.534e-05 | 0 |
| R→Y | 0         | 2.534e-05 | 0 |
| R→F | 0         | 2.534e-05 | 0 |
| Q→S | 0         | 2.534e-05 | 0 |
| Q→N | 0         | 2.534e-05 | 0 |
| Q→D | 0         | 2.534e-05 | 0 |
| P→I | 0         | 2.534e-05 | 0 |
| P→G | 0         | 2.534e-05 | 0 |
| P→D | 0         | 2.534e-05 | 0 |
| M→S | 0         | 2.534e-05 | 0 |
| M→Q | 0         | 2.534e-05 | 0 |
| L→T | 0         | 2.534e-05 | 0 |
| L→D | 0         | 2.534e-05 | 0 |
| K→V | 0         | 2.534e-05 | 0 |
| K→L | 0         | 2.534e-05 | 0 |
| K→D | 0         | 2.534e-05 | 0 |
| K→A | 0         | 2.534e-05 | 0 |
| G→Q | 0         | 2.534e-05 | 0 |
| G→N | 0         | 2.534e-05 | 0 |
| F→D | 0         | 2.534e-05 | 0 |
| E→W | 0         | 2.534e-05 | 0 |
| E→S | 0         | 2.534e-05 | 0 |
| E→R | 0         | 2.534e-05 | 0 |
| D→R | 0         | 2.534e-05 | 0 |
| C→Q | 0         | 2.534e-05 | 0 |
| C→K | 0         | 2.534e-05 | 0 |
| A→Q | 0         | 2.534e-05 | 0 |
| Y→V | 0         | 5.068e-05 | 0 |
| Y→G | 0         | 5.068e-05 | 0 |
| W→Q | 0         | 5.068e-05 | 0 |
| T→E | 0         | 5.068e-05 | 0 |
| S→D | 0         | 5.068e-05 | 0 |
| Q→Y | 0         | 5.068e-05 | 0 |
| N→E | 0         | 5.068e-05 | 0 |
| L→C | 0         | 5.068e-05 | 0 |

|     |           |           |           |
|-----|-----------|-----------|-----------|
| H→C | 0         | 5.068e-05 | 0         |
| E→T | 0         | 5.068e-05 | 0         |
| E→N | 0         | 5.068e-05 | 0         |
| E→L | 0         | 5.068e-05 | 0         |
| A→M | 0         | 5.068e-05 | 0         |
| L→A | 3.212e-05 | 1.014e-04 | 5.398e-01 |
| A→R | 0         | 7.602e-05 | 0         |
| D→N | 1.490e-02 | 1.500e-02 | 9.428e-01 |
| D→S | 0         | 1.014e-04 | 0         |
| Y→L | 0         | 1.267e-04 | 0         |
| R→M | 5.461e-04 | 7.348e-04 | 4.096e-01 |
| S→Y | 2.441e-03 | 2.661e-03 | 6.211e-01 |
| D→A | 1.959e-03 | 2.204e-03 | 5.345e-01 |
| T→N | 2.602e-03 | 2.939e-03 | 4.414e-01 |
| K→M | 7.709e-04 | 1.115e-03 | 1.785e-01 |
| R→I | 4.818e-04 | 9.375e-04 | 3.516e-02 |
| F→L | 9.444e-03 | 1.001e-02 | 4.742e-01 |
| I→T | 1.253e-02 | 1.310e-02 | 5.270e-01 |
| P→H | 2.281e-03 | 2.863e-03 | 1.512e-01 |
| H→N | 1.221e-03 | 1.824e-03 | 5.281e-02 |
| H→Y | 5.589e-03 | 6.233e-03 | 2.923e-01 |
| N→H | 1.799e-03 | 2.509e-03 | 5.374e-02 |
| P→Q | 1.895e-03 | 2.711e-03 | 3.097e-02 |
| Q→L | 1.124e-03 | 1.951e-03 | 7.376e-03 |
| N→T | 1.702e-03 | 2.534e-03 | 2.180e-02 |
| K→T | 1.927e-03 | 2.813e-03 | 2.065e-02 |
| F→Y | 5.782e-04 | 1.520e-03 | 1.852e-04 |
| Y→F | 8.994e-04 | 1.976e-03 | 2.584e-04 |
| G→A | 5.172e-03 | 6.259e-03 | 6.542e-02 |
| Q→K | 2.248e-03 | 3.446e-03 | 3.932e-03 |
| E→A | 2.184e-03 | 3.522e-03 | 1.247e-03 |
| P→L | 2.358e-02 | 2.516e-02 | 1.892e-01 |
| P→T | 4.240e-03 | 5.904e-03 | 2.505e-03 |
| I→L | 1.221e-03 | 3.041e-03 | 2.622e-07 |
| H→Q | 3.341e-03 | 5.245e-03 | 1.620e-04 |
| M→T | 6.071e-03 | 8.083e-03 | 1.907e-03 |
| S→C | 3.405e-03 | 5.448e-03 | 6.617e-05 |
| N→D | 4.208e-03 | 6.385e-03 | 1.005e-04 |
| K→Q | 1.510e-03 | 3.725e-03 | 1.421e-08 |
| M→L | 1.124e-03 | 3.345e-03 | 6.352e-10 |
| I→M | 3.533e-03 | 5.777e-03 | 1.873e-05 |
| K→E | 7.452e-03 | 9.730e-03 | 1.397e-03 |
| K→N | 4.625e-03 | 6.918e-03 | 8.690e-05 |
| M→I | 3.822e-03 | 6.132e-03 | 2.036e-05 |
| L→I | 8.994e-04 | 3.573e-03 | 5.281e-14 |
| L→M | 1.253e-03 | 4.029e-03 | 8.915e-13 |
| L→F | 8.448e-03 | 1.130e-02 | 1.815e-04 |
| E→G | 5.461e-03 | 8.337e-03 | 6.272e-06 |
| T→I | 1.038e-02 | 1.335e-02 | 3.619e-04 |
| Q→E | 1.927e-03 | 4.916e-03 | 1.790e-11 |
| E→Q | 3.244e-03 | 6.994e-03 | 5.778e-12 |
| H→R | 7.452e-03 | 1.135e-02 | 1.429e-07 |
| P→A | 2.634e-03 | 6.664e-03 | 6.310e-15 |
| S→A | 6.103e-04 | 4.916e-03 | 2.496e-29 |
| A→G | 2.345e-03 | 6.740e-03 | 5.752e-18 |
| V→L | 5.172e-03 | 9.958e-03 | 4.841e-13 |
| Q→H | 3.694e-03 | 8.615e-03 | 1.316e-16 |
| T→M | 1.086e-02 | 1.586e-02 | 1.399e-08 |
| M→V | 6.424e-03 | 1.163e-02 | 6.213e-13 |
| D→E | 3.662e-03 | 9.046e-03 | 4.506e-19 |

|     |           |           |            |
|-----|-----------|-----------|------------|
| S→T | 1.702e-03 | 7.374e-03 | 1.096e-29  |
| A→S | 2.602e-03 | 8.286e-03 | 1.170e-24  |
| R→K | 2.184e-03 | 8.159e-03 | 1.065e-28  |
| L→V | 5.172e-03 | 1.117e-02 | 2.087e-18  |
| V→M | 1.230e-02 | 1.845e-02 | 7.989e-11  |
| T→S | 1.510e-03 | 8.083e-03 | 6.867e-38  |
| S→G | 2.505e-03 | 9.122e-03 | 3.869e-31  |
| E→D | 3.790e-03 | 1.052e-02 | 5.117e-26  |
| S→N | 4.208e-03 | 1.178e-02 | 2.386e-29  |
| N→S | 9.508e-03 | 1.713e-02 | 3.686e-18  |
| K→R | 3.501e-03 | 1.133e-02 | 8.420e-34  |
| Q→R | 6.328e-03 | 1.432e-02 | 2.814e-25  |
| P→S | 1.018e-02 | 1.832e-02 | 2.959e-19  |
| V→A | 5.075e-03 | 1.460e-02 | 2.539e-37  |
| A→V | 1.683e-02 | 2.742e-02 | 7.812e-21  |
| T→A | 5.139e-03 | 1.926e-02 | 5.415e-66  |
| A→T | 1.738e-02 | 3.264e-02 | 4.106e-37  |
| I→V | 3.084e-03 | 2.374e-02 | 8.747e-134 |
| V→I | 4.658e-03 | 2.792e-02 | 4.945e-137 |

---



**Table S2.** Statistics for forward versus reverse amino acid changes among pathogenic mutations. This table is ranked in descending order of rate differences, and only includes the 117 pairs of amino acid type change that occurs among pathogenic mutations. The first column lists the forward mutation. For example, the first row means that the rates of pathogenic mutations R→Q and Q→R are 3.145e-2 and 6.328e-3, respectively.

| Mutation | Probability of forward mutation | Probability of reverse mutation | P-value of rate ratio test |
|----------|---------------------------------|---------------------------------|----------------------------|
| R→Q      | 3.145e-02                       | 6.328e-03                       | 2.220e-16                  |
| R→W      | 3.296e-02                       | 8.673e-03                       | 2.220e-16                  |
| R→H      | 2.865e-02                       | 7.452e-03                       | 2.220e-16                  |
| R→C      | 3.649e-02                       | 1.667e-02                       | 2.220e-16                  |
| A→V      | 1.683e-02                       | 5.075e-03                       | 2.220e-16                  |
| D→N      | 1.490e-02                       | 4.208e-03                       | 2.220e-16                  |
| G→V      | 1.551e-02                       | 5.461e-03                       | 2.220e-16                  |
| V→M      | 1.230e-02                       | 6.424e-03                       | 2.953e-14                  |
| S→L      | 9.508e-03                       | 4.015e-03                       | 2.220e-16                  |
| R→P      | 1.307e-02                       | 7.741e-03                       | 7.181e-11                  |
| T→M      | 1.086e-02                       | 6.071e-03                       | 8.609e-11                  |
| D→Y      | 6.938e-03                       | 3.180e-03                       | 3.797e-11                  |
| C→F      | 6.874e-03                       | 3.276e-03                       | 2.794e-10                  |
| G→A      | 5.172e-03                       | 2.345e-03                       | 8.702e-09                  |
| Q→P      | 4.722e-03                       | 1.895e-03                       | 7.487e-10                  |
| D→V      | 5.685e-03                       | 3.405e-03                       | 2.896e-05                  |
| I→F      | 3.373e-03                       | 1.702e-03                       | 4.281e-05                  |
| V→I      | 4.658e-03                       | 3.084e-03                       | 1.927e-03                  |
| V→F      | 4.401e-03                       | 2.923e-03                       | 2.800e-03                  |
| H→P      | 3.598e-03                       | 2.281e-03                       | 3.002e-03                  |
| E→Q      | 3.244e-03                       | 1.927e-03                       | 1.538e-03                  |
| S→F      | 8.673e-03                       | 7.709e-03                       | 1.990e-01                  |
| T→N      | 2.602e-03                       | 1.702e-03                       | 1.934e-02                  |
| T→P      | 5.075e-03                       | 4.240e-03                       | 1.420e-01                  |
| R→S      | 6.489e-03                       | 6.039e-03                       | 5.104e-01                  |
| I→L      | 1.221e-03                       | 8.994e-04                       | 2.678e-01                  |
| S→T      | 1.702e-03                       | 1.510e-03                       | 6.173e-01                  |
| L→M      | 1.253e-03                       | 1.124e-03                       | 7.275e-01                  |
| R→E      | 9.636e-05                       | 0                               | 0                          |
| D→F      | 6.424e-05                       | 0                               | 0                          |
| A→F      | 9.636e-05                       | 3.212e-05                       | 6.250e-01                  |
| A→L      | 6.424e-05                       | 3.212e-05                       | 1                          |
| A→I      | 6.424e-05                       | 3.212e-05                       | 1                          |
| S→V      | 3.212e-05                       | 0                               | 0                          |
| R→N      | 3.212e-05                       | 0                               | 0                          |
| R→D      | 3.212e-05                       | 0                               | 0                          |
| R→A      | 3.212e-05                       | 0                               | 0                          |
| Q→W      | 3.212e-05                       | 0                               | 0                          |
| Q→G      | 3.212e-05                       | 0                               | 0                          |
| P→F      | 3.212e-05                       | 0                               | 0                          |
| N→V      | 3.212e-05                       | 0                               | 0                          |
| N→G      | 3.212e-05                       | 0                               | 0                          |
| L→Y      | 3.212e-05                       | 0                               | 0                          |
| I→Y      | 3.212e-05                       | 0                               | 0                          |
| H→A      | 3.212e-05                       | 0                               | 0                          |
| E→M      | 3.212e-05                       | 0                               | 0                          |
| D→Q      | 3.212e-05                       | 0                               | 0                          |
| D→I      | 3.212e-05                       | 0                               | 0                          |
| D→C      | 3.212e-05                       | 0                               | 0                          |
| C→V      | 3.212e-05                       | 0                               | 0                          |
| A→Y      | 3.212e-05                       | 0                               | 0                          |
| V→L      | 5.172e-03                       | 5.172e-03                       | 1                          |
| G→L      | 9.636e-05                       | 9.636e-05                       | 1                          |

|     |           |           |           |
|-----|-----------|-----------|-----------|
| P→V | 3.212e-05 | 3.212e-05 | 1         |
| H→T | 3.212e-05 | 3.212e-05 | 1         |
| G→W | 2.313e-03 | 2.345e-03 | 1         |
| T→G | 0         | 3.212e-05 | 0         |
| P→I | 0         | 3.212e-05 | 0         |
| N→M | 0         | 3.212e-05 | 0         |
| N→F | 0         | 3.212e-05 | 0         |
| N→A | 0         | 3.212e-05 | 0         |
| K→W | 0         | 3.212e-05 | 0         |
| H→F | 0         | 3.212e-05 | 0         |
| H→C | 0         | 3.212e-05 | 0         |
| G→F | 0         | 3.212e-05 | 0         |
| F→W | 0         | 3.212e-05 | 0         |
| T→F | 0         | 6.424e-05 | 0         |
| Q→V | 0         | 6.424e-05 | 0         |
| K→G | 0         | 6.424e-05 | 0         |
| K→A | 0         | 6.424e-05 | 0         |
| H→S | 0         | 6.424e-05 | 0         |
| C→A | 0         | 6.424e-05 | 0         |
| S→Y | 2.441e-03 | 2.538e-03 | 8.724e-01 |
| D→E | 3.662e-03 | 3.790e-03 | 8.439e-01 |
| K→I | 7.067e-04 | 8.673e-04 | 5.682e-01 |
| H→Y | 5.589e-03 | 5.750e-03 | 8.314e-01 |
| I→M | 3.533e-03 | 3.822e-03 | 5.971e-01 |
| F→Y | 5.782e-04 | 8.994e-04 | 1.839e-01 |
| H→Q | 3.341e-03 | 3.694e-03 | 4.993e-01 |
| K→T | 1.927e-03 | 2.313e-03 | 3.384e-01 |
| R→I | 4.818e-04 | 8.673e-04 | 8.843e-02 |
| N→Y | 1.735e-03 | 2.120e-03 | 3.153e-01 |
| H→L | 1.670e-03 | 2.088e-03 | 2.672e-01 |
| S→I | 2.505e-03 | 2.955e-03 | 3.187e-01 |
| L→W | 1.028e-03 | 1.510e-03 | 1.147e-01 |
| H→N | 1.221e-03 | 1.799e-03 | 7.898e-02 |
| K→Q | 1.510e-03 | 2.248e-03 | 4.150e-02 |
| C→W | 4.433e-03 | 5.268e-03 | 1.501e-01 |
| L→F | 8.448e-03 | 9.444e-03 | 2.036e-01 |
| S→W | 1.221e-03 | 2.377e-03 | 8.606e-04 |
| S→P | 9.026e-03 | 1.018e-02 | 1.523e-01 |
| E→V | 2.281e-03 | 3.501e-03 | 5.668e-03 |
| R→T | 2.248e-03 | 3.501e-03 | 4.375e-03 |
| R→K | 2.184e-03 | 3.501e-03 | 2.543e-03 |
| C→Y | 1.747e-02 | 1.902e-02 | 1.631e-01 |
| E→A | 2.184e-03 | 3.822e-03 | 2.359e-04 |
| C→G | 4.658e-03 | 6.489e-03 | 2.595e-03 |
| R→L | 9.026e-03 | 1.086e-02 | 2.432e-02 |
| N→I | 3.052e-03 | 4.882e-03 | 3.469e-04 |
| S→A | 6.103e-04 | 2.602e-03 | 2.703e-10 |
| Q→L | 1.124e-03 | 3.148e-03 | 4.292e-08 |
| K→M | 7.709e-04 | 2.859e-03 | 5.684e-10 |
| T→I | 1.038e-02 | 1.253e-02 | 1.339e-02 |
| K→N | 4.625e-03 | 7.067e-03 | 8.004e-05 |
| R→M | 5.461e-04 | 3.533e-03 | 7.387e-18 |
| S→C | 3.405e-03 | 6.456e-03 | 6.417e-08 |
| H→D | 1.735e-03 | 5.782e-03 | 5.026e-17 |
| D→A | 1.959e-03 | 6.521e-03 | 5.285e-19 |
| S→N | 4.208e-03 | 9.508e-03 | 9.128e-16 |
| P→A | 2.634e-03 | 9.669e-03 | 1.671e-30 |
| E→G | 5.461e-03 | 1.481e-02 | 6.010e-32 |
| D→G | 9.412e-03 | 1.975e-02 | 4.874e-27 |
| T→A | 5.139e-03 | 1.738e-02 | 3.270e-49 |

|     |           |           |            |
|-----|-----------|-----------|------------|
| P→L | 2.358e-02 | 3.761e-02 | 1.087e-23  |
| S→G | 2.505e-03 | 1.844e-02 | 2.920e-94  |
| K→E | 7.452e-03 | 2.592e-02 | 5.939e-75  |
| R→G | 1.131e-02 | 3.864e-02 | 8.526e-109 |

---

**Table S3.** Statistics for forward versus reverse amino acid changes among benign mutations. This table is ranked in descending order of rate differences, and only includes the 133 pairs of amino acid type change that occurs among benign mutations. The first column lists the forward mutation. For example, the first row means that the rates of pathogenic mutations R→Q and Q→R are 3.147e-2 and 1.432e-2, respectively.

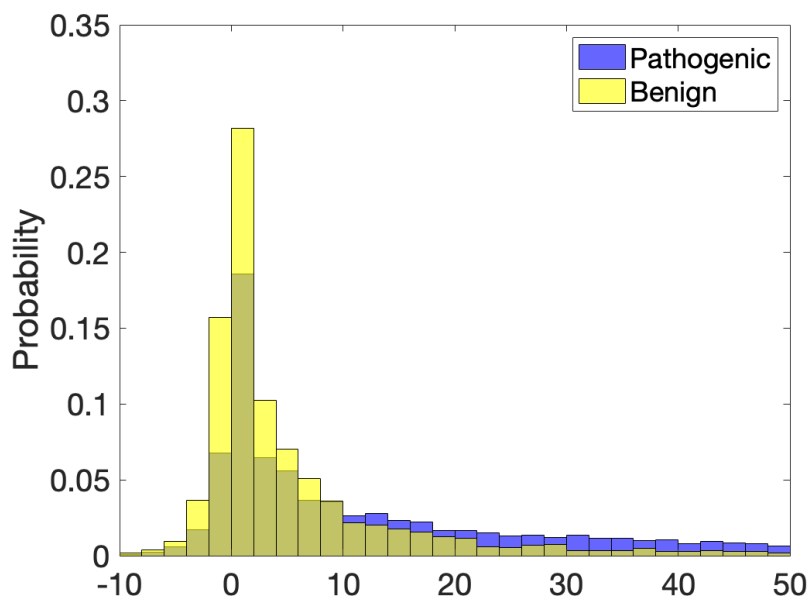
| Mutation | Probability of forward mutation | Probability of reverse mutation | P-value of rate ratio test |
|----------|---------------------------------|---------------------------------|----------------------------|
| R→Q      | 3.147e-02                       | 1.432e-02                       | 2.220e-16                  |
| R→H      | 2.724e-02                       | 1.135e-02                       | 2.220e-16                  |
| R→C      | 1.974e-02                       | 5.245e-03                       | 2.220e-16                  |
| P→L      | 2.516e-02                       | 1.181e-02                       | 2.220e-16                  |
| A→V      | 2.742e-02                       | 1.460e-02                       | 2.220e-16                  |
| R→W      | 1.629e-02                       | 3.953e-03                       | 2.220e-16                  |
| D→N      | 1.500e-02                       | 6.385e-03                       | 2.220e-16                  |
| T→M      | 1.586e-02                       | 8.083e-03                       | 2.220e-16                  |
| V→M      | 1.845e-02                       | 1.163e-02                       | 5.551e-15                  |
| S→L      | 8.919e-03                       | 3.902e-03                       | 2.220e-16                  |
| V→I      | 2.792e-02                       | 2.374e-02                       | 2.794e-04                  |
| S→F      | 6.867e-03                       | 3.953e-03                       | 2.867e-08                  |
| S→C      | 5.448e-03                       | 3.243e-03                       | 3.052e-06                  |
| E→Q      | 6.994e-03                       | 4.916e-03                       | 1.804e-04                  |
| D→Y      | 2.838e-03                       | 8.869e-04                       | 1.365e-10                  |
| G→V      | 5.245e-03                       | 3.471e-03                       | 1.900e-04                  |
| E→G      | 8.337e-03                       | 6.689e-03                       | 8.530e-03                  |
| D→V      | 2.661e-03                       | 1.115e-03                       | 6.256e-07                  |
| R→L      | 4.840e-03                       | 3.345e-03                       | 1.216e-03                  |
| S→I      | 2.458e-03                       | 1.014e-03                       | 1.220e-06                  |
| L→F      | 1.130e-02                       | 1.001e-02                       | 8.462e-02                  |
| S→Y      | 2.661e-03                       | 1.470e-03                       | 2.881e-04                  |
| K→N      | 6.918e-03                       | 5.803e-03                       | 5.485e-02                  |
| V→F      | 2.331e-03                       | 1.470e-03                       | 6.852e-03                  |
| I→F      | 2.052e-03                       | 1.216e-03                       | 4.650e-03                  |
| H→Y      | 6.233e-03                       | 5.397e-03                       | 1.352e-01                  |
| E→A      | 3.522e-03                       | 2.711e-03                       | 4.788e-02                  |
| L→M      | 4.029e-03                       | 3.345e-03                       | 1.273e-01                  |
| E→V      | 2.230e-03                       | 1.571e-03                       | 4.087e-02                  |
| K→T      | 2.813e-03                       | 2.255e-03                       | 1.374e-01                  |
| R→I      | 9.375e-04                       | 4.054e-04                       | 5.486e-03                  |
| N→Y      | 1.216e-03                       | 7.855e-04                       | 7.116e-02                  |
| T→N      | 2.939e-03                       | 2.534e-03                       | 3.074e-01                  |
| G→W      | 9.629e-04                       | 6.335e-04                       | 1.299e-01                  |
| K→M      | 1.115e-03                       | 8.108e-04                       | 2.067e-01                  |
| K→Q      | 3.725e-03                       | 3.446e-03                       | 5.523e-01                  |
| Q→L      | 1.951e-03                       | 1.672e-03                       | 4.031e-01                  |
| Q→P      | 2.965e-03                       | 2.711e-03                       | 5.477e-01                  |
| P→A      | 6.664e-03                       | 6.411e-03                       | 6.920e-01                  |
| T→I      | 1.335e-02                       | 1.310e-02                       | 7.806e-01                  |
| L→W      | 8.362e-04                       | 6.588e-04                       | 4.350e-01                  |
| K→I      | 4.561e-04                       | 3.041e-04                       | 3.616e-01                  |
| S→W      | 6.335e-04                       | 5.828e-04                       | 8.854e-01                  |
| D→S      | 1.014e-04                       | 5.068e-05                       | 6.875e-01                  |
| Q→Y      | 5.068e-05                       | 0                               | 0                          |
| E→L      | 5.068e-05                       | 0                               | 0                          |
| A→M      | 5.068e-05                       | 0                               | 0                          |
| R→E      | 5.068e-05                       | 2.534e-05                       | 1                          |
| H→C      | 5.068e-05                       | 2.534e-05                       | 1                          |
| T→G      | 2.534e-05                       | 0                               | 0                          |
| R→F      | 2.534e-05                       | 0                               | 0                          |
| P→V      | 2.534e-05                       | 0                               | 0                          |
| P→I      | 2.534e-05                       | 0                               | 0                          |

|     |           |           |           |
|-----|-----------|-----------|-----------|
| K→V | 2.534e-05 | 0         | 0         |
| K→L | 2.534e-05 | 0         | 0         |
| K→D | 2.534e-05 | 0         | 0         |
| G→L | 2.534e-05 | 0         | 0         |
| E→S | 2.534e-05 | 0         | 0         |
| E→M | 2.534e-05 | 0         | 0         |
| R→T | 1.976e-03 | 1.951e-03 | 1         |
| E→T | 5.068e-05 | 5.068e-05 | 1         |
| E→N | 5.068e-05 | 5.068e-05 | 1         |
| T→L | 2.534e-05 | 2.534e-05 | 1         |
| S→V | 2.534e-05 | 2.534e-05 | 1         |
| R→Y | 2.534e-05 | 2.534e-05 | 1         |
| R→D | 2.534e-05 | 2.534e-05 | 1         |
| Q→G | 2.534e-05 | 2.534e-05 | 1         |
| E→W | 2.534e-05 | 2.534e-05 | 1         |
| D→Q | 2.534e-05 | 2.534e-05 | 1         |
| D→F | 2.534e-05 | 2.534e-05 | 1         |
| A→I | 2.534e-05 | 2.534e-05 | 1         |
| H→L | 1.267e-03 | 1.292e-03 | 1         |
| Q→W | 2.534e-05 | 5.068e-05 | 1         |
| K→A | 2.534e-05 | 5.068e-05 | 1         |
| T→Y | 0         | 2.534e-05 | 0         |
| T→V | 0         | 2.534e-05 | 0         |
| T→F | 0         | 2.534e-05 | 0         |
| S→Q | 0         | 2.534e-05 | 0         |
| S→M | 0         | 2.534e-05 | 0         |
| Q→M | 0         | 2.534e-05 | 0         |
| Q→C | 0         | 2.534e-05 | 0         |
| Q→A | 0         | 2.534e-05 | 0         |
| P→W | 0         | 2.534e-05 | 0         |
| N→Q | 0         | 2.534e-05 | 0         |
| N→G | 0         | 2.534e-05 | 0         |
| N→F | 0         | 2.534e-05 | 0         |
| M→Y | 0         | 2.534e-05 | 0         |
| K→W | 0         | 2.534e-05 | 0         |
| K→C | 0         | 2.534e-05 | 0         |
| I→Y | 0         | 2.534e-05 | 0         |
| H→S | 0         | 2.534e-05 | 0         |
| H→F | 0         | 2.534e-05 | 0         |
| G→P | 0         | 2.534e-05 | 0         |
| G→F | 0         | 2.534e-05 | 0         |
| D→T | 0         | 2.534e-05 | 0         |
| D→P | 0         | 2.534e-05 | 0         |
| D→L | 0         | 2.534e-05 | 0         |
| A→L | 7.602e-05 | 1.014e-04 | 1         |
| R→A | 2.534e-05 | 7.602e-05 | 6.250e-01 |
| V→Y | 0         | 5.068e-05 | 0         |
| G→Y | 0         | 5.068e-05 | 0         |
| C→L | 0         | 5.068e-05 | 0         |
| L→Y | 5.068e-05 | 1.267e-04 | 4.531e-01 |
| R→M | 7.348e-04 | 8.362e-04 | 7.035e-01 |
| C→F | 1.216e-03 | 1.419e-03 | 4.926e-01 |
| C→W | 1.090e-03 | 1.318e-03 | 4.119e-01 |
| I→M | 5.777e-03 | 6.132e-03 | 5.488e-01 |
| N→I | 1.343e-03 | 1.774e-03 | 1.488e-01 |
| F→Y | 1.520e-03 | 1.976e-03 | 1.476e-01 |
| G→A | 6.259e-03 | 6.740e-03 | 4.268e-01 |
| R→S | 5.499e-03 | 6.031e-03 | 3.485e-01 |
| I→L | 3.041e-03 | 3.573e-03 | 2.157e-01 |
| H→N | 1.824e-03 | 2.509e-03 | 4.646e-02 |

|     |           |           |           |
|-----|-----------|-----------|-----------|
| S→T | 7.374e-03 | 8.083e-03 | 2.743e-01 |
| C→G | 1.140e-03 | 1.900e-03 | 7.847e-03 |
| H→P | 1.850e-03 | 2.863e-03 | 4.118e-03 |
| T→P | 4.713e-03 | 5.904e-03 | 2.451e-02 |
| V→L | 9.958e-03 | 1.117e-02 | 1.036e-01 |
| D→G | 6.943e-03 | 8.235e-03 | 4.097e-02 |
| D→A | 2.204e-03 | 3.674e-03 | 1.698e-04 |
| D→E | 9.046e-03 | 1.052e-02 | 4.015e-02 |
| R→P | 4.029e-03 | 5.828e-03 | 3.736e-04 |
| H→D | 1.470e-03 | 4.029e-03 | 4.643e-12 |
| C→Y | 4.561e-03 | 7.196e-03 | 1.581e-06 |
| R→K | 8.159e-03 | 1.133e-02 | 7.435e-06 |
| H→Q | 5.245e-03 | 8.615e-03 | 1.420e-08 |
| S→A | 4.916e-03 | 8.286e-03 | 6.109e-09 |
| S→N | 1.178e-02 | 1.713e-02 | 4.540e-10 |
| R→G | 9.451e-03 | 1.490e-02 | 4.159e-12 |
| S→G | 9.122e-03 | 1.568e-02 | 1.095e-16 |
| S→P | 8.691e-03 | 1.832e-02 | 8.397e-32 |
| K→E | 9.730e-03 | 2.030e-02 | 2.625e-34 |
| T→A | 1.926e-02 | 3.264e-02 | 1.126e-31 |

---

## Supporting Figures



**Figure S1.** Histograms of FoldX free energy change predictions for pathogenic and benign mutations. T-test  $p$ -value =  $4.7 \times 10^{-186}$ . Overall correlation of 0.96 with EvoEF values.