Predictions were made using SNAP (Screening for Non-Acceptable Polymorphisms), a neural network based method for prediction of functional effects of single amino acid substitutions. By default, for each submitted substitution, SNAP reports a single binary prediction (Neutral/Non-neutral), which is associated with an RI (reliability index, range 0-9) and a value of “expected accuracy” (0-100%; in testing, the accuracy of SNAP predictions at the given RI). The SNAP RI scores are computed by taking a mean of ten neural nets trained on different data sets – the raw score. Unfortunately, none of these values map directly to percentage change in proliferation rate. We have previously observed that increased scores correlate fairly well with the severities of protein function change. Thus, we used the raw score as reference and followed the steps below in making predictions. Here, our “standard deviation” is computed from the variation in predictions on different networks:

1. For neutral predictions (negative raw score) we report no change (0.5 proliferation rate)
2. For non-neutral predictions we assumed a score of over 60 to mean 1.0 proliferation. All scores between 0 and 60 were normalized to represent the range from 0.5 wild-type proliferation to 1.0 positive-control proliferation

Overall, it is important to note that while we can attempt to adapt SNAP output to the various prediction challenges, accounting for all possible variations on the theme is nearly impossible. Thus, it is more realistic to leave the interpretation of mutation neutrality to the expert experimentalists working with the protein at hand.