

Intolerant Tolerant Functional

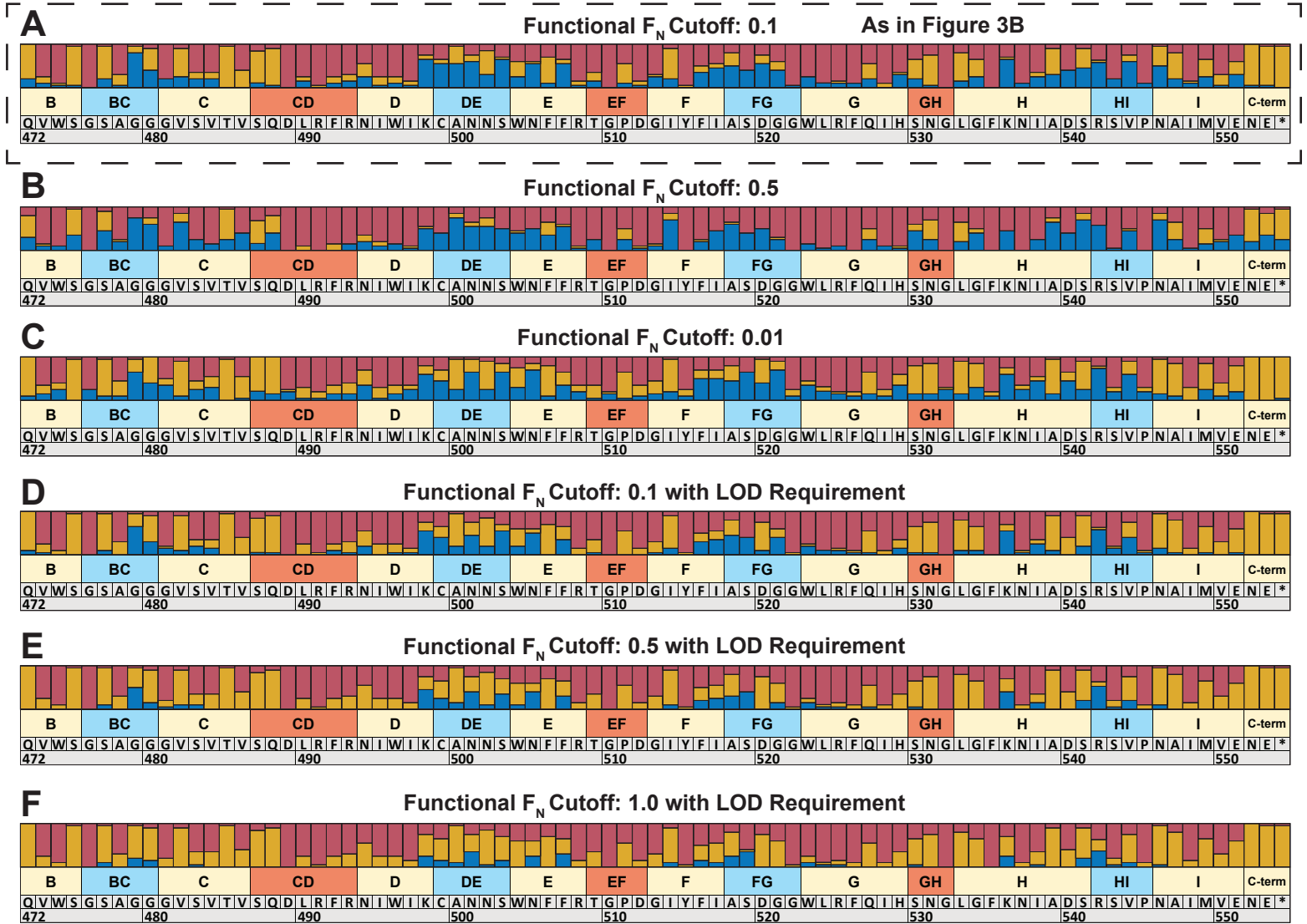


Figure 3 - Figure Supplement 2. Classification of tolerant, intolerant and functional regions based on different cutoff conditions.

Phenotypic classification of each position is determined by aggregating scores of all substitutions in all hosts at that position. **(A)** Substitutions are classified using an F_N cutoff of 0.1 as intolerant ($F_N < 0.1$ in all hosts), tolerant ($F_N \geq 0.1$ in all hosts), or functional ($F_N < 0.1$ in one host, $F_N \geq 0.1$ in another host) as seen in Figure 3B. Variations of this cutoff **(B)** using an F_N cutoff of 0.5 or **(C)** using an F_N cutoff of 0.01. An alternative, more conservative approach to classifying a functional substitution requires an F_N below the limit of detection (LOD) in one host and a higher F_N of **(D)** 0.1, **(E)** 0.5, or **(F)** 1.0 in another host. All cutoff conditions broadly result in similar overall trends.