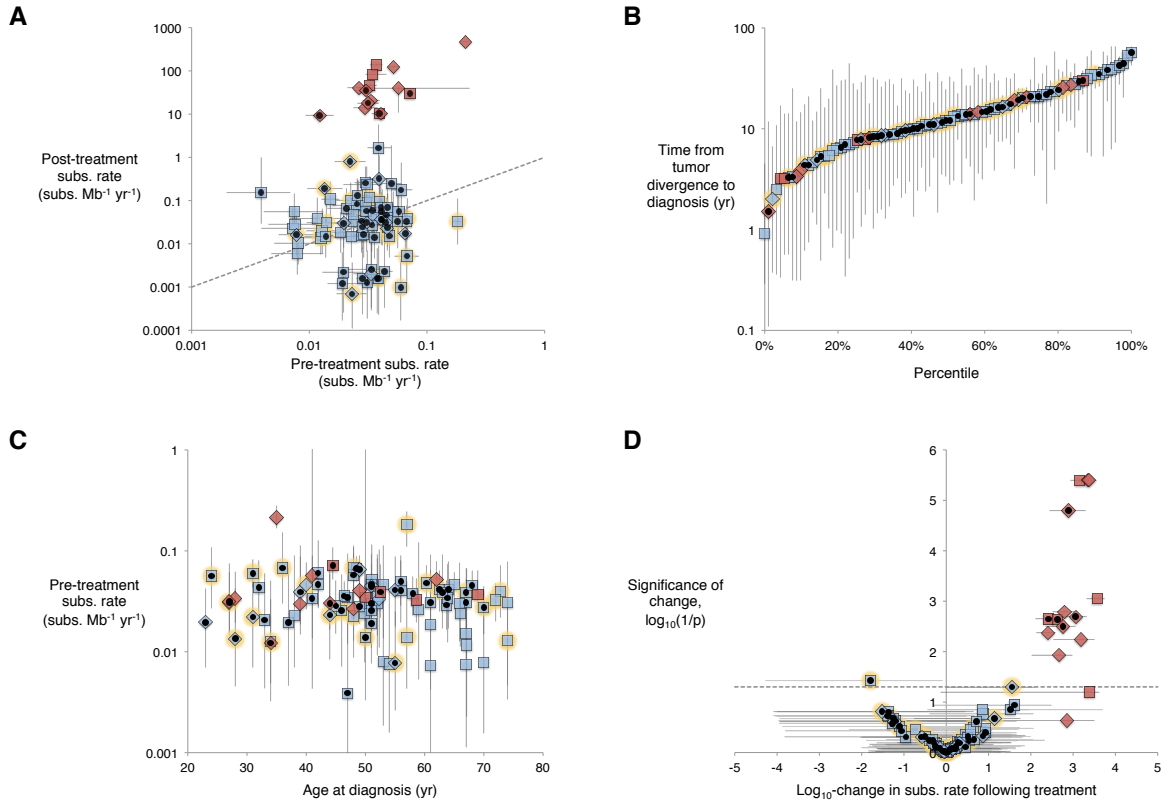
Comparison to allele frequency clonality cutoff

As cellular frequencies are themselves estimated by a model (see Main Text, Methods), we also re-ran the evolutionary model using raw allele frequencies. Since most somatic variants are heterozygous, and since tumor purity is rarely near 100%, we used allele frequency clonality cutoffs of 0.1, 0.15, and 0.2. While overall cohort fits are similar to that of the model using cellular frequencies (Additional Figures M6, M7, M8), the R^2 values comparing cellular frequency ≥0.95 and allele frequency ≥0.15 cutoffs are only moderate, ranging from 0.30 to 0.53 (Additional Figures M3D, M4D, M5D). Furthermore, using the allele frequency cutoff produces greater separation in post-treatment substitution rate between the hypermutated and non-hypermutated cases (Figure 3C and Additional Figures M1A, M2A versus Additional Figures M6A, M7A, M8A). This difference appears to stem from the fact that mutations unique to hypermutated recurrences are more likely to be classified as subclonal under the cellular frequency cutoff than by the allele frequency cutoff (median among hypermutated cases is 2,566 subclonal, 83 clonal using cellular frequency ≥0.95; versus 201 subclonal, 1497 clonal using allele frequency ≥0.15). While a large number of clonal mutations in the recurrence must be explained by an elevated post-treatment substitution rate, a large number of *subclonal* mutations may be explained either by an elevated rate or by population dynamics contributing to greater intratumor heterogeneity (ITH). The model implicitly captures ITH in the recurrent sample with the effective sample size parameter,

s_R. Accordingly, the model using cellular frequency ≥0.95 estimates this parameter to be twice as large in hypermutated than in non-hypermutated cases (median s_R=25.7 versus 12.1 cells), while the model using allele frequency ≥0.15 cutoff estimates it to be sixfold *smaller* in hypermutated cases (median s_R=2.4 versus 13.3 cells). This result suggests that, if we take the estimated cellular frequencies to be accurate determinants of clonality, ITH is roughly twice as important in hypermutated recurrent tumors as compared to non-hypermutated ones.



Additional Figure M6. Evolutionary model analysis, using mutant allele frequency ≥0.1 as clonality cutoff. All panels and legend as in Additional Figure M1.