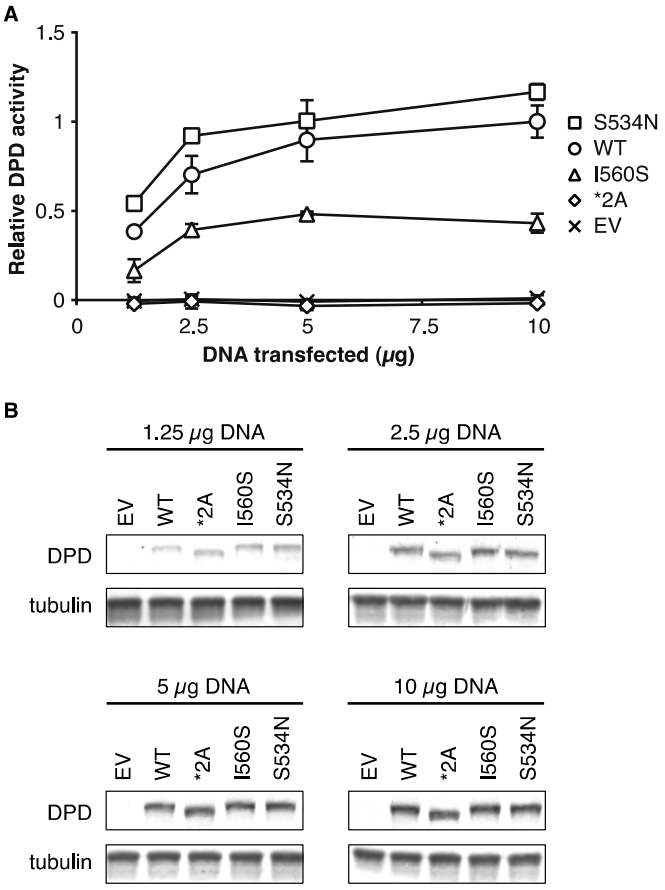
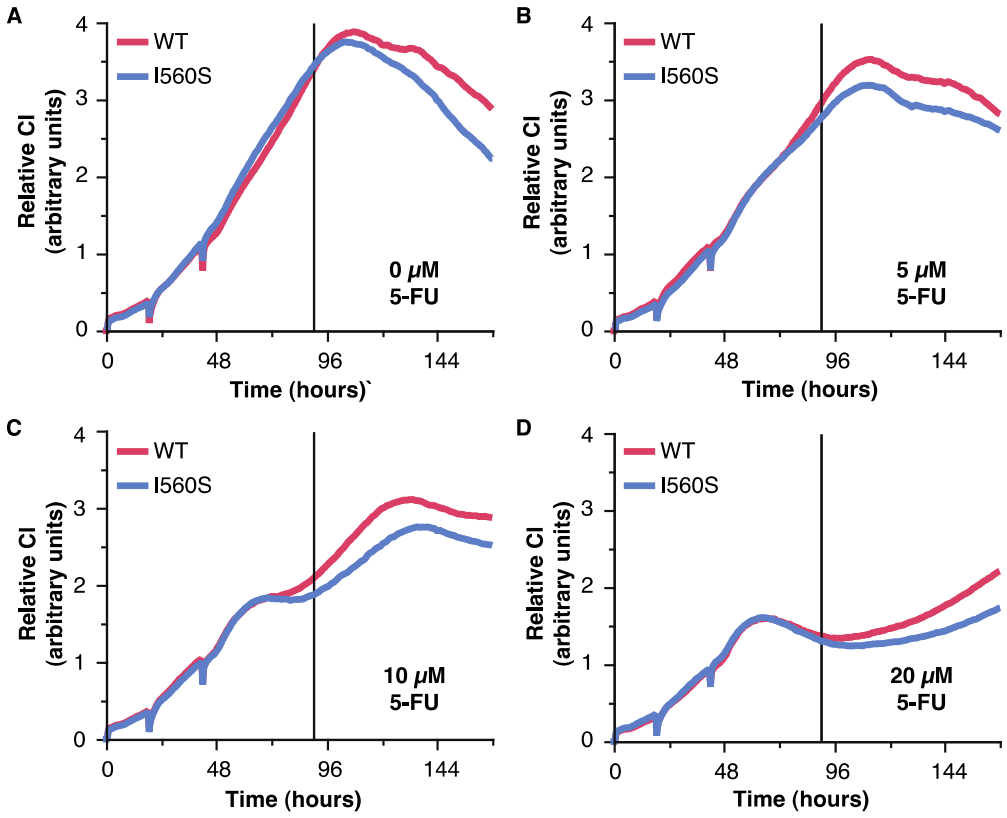
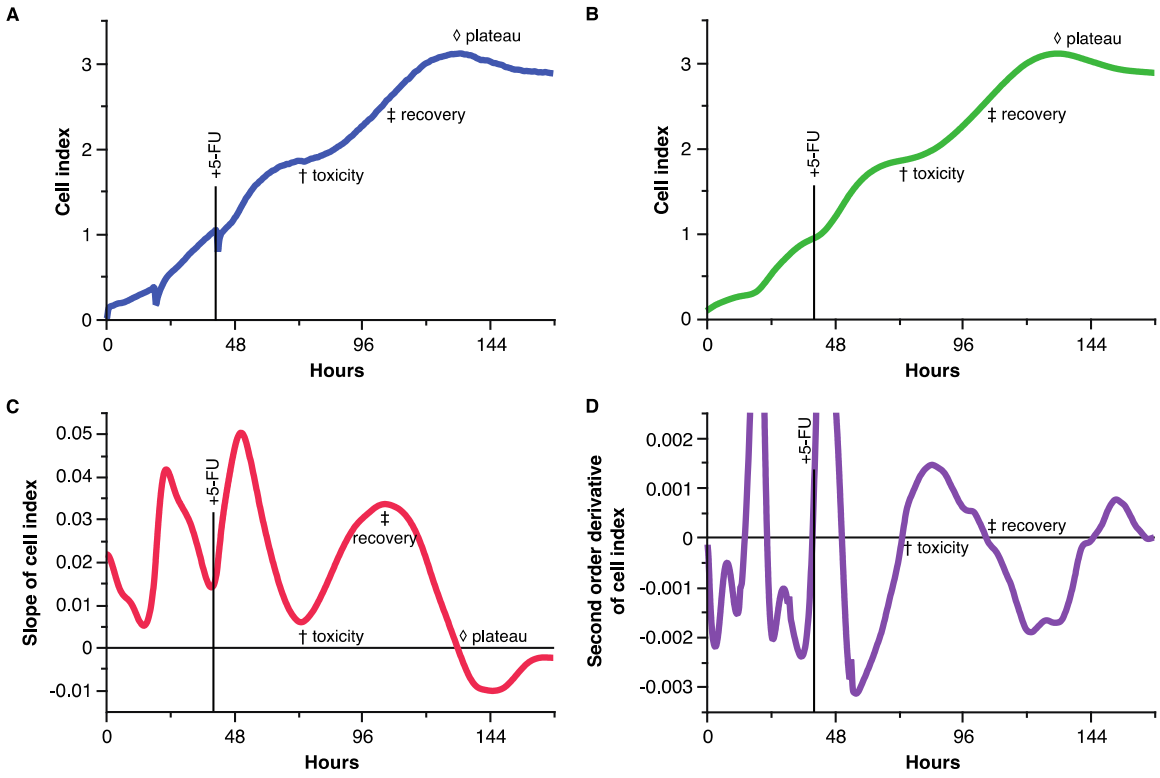
Supplementary Figures



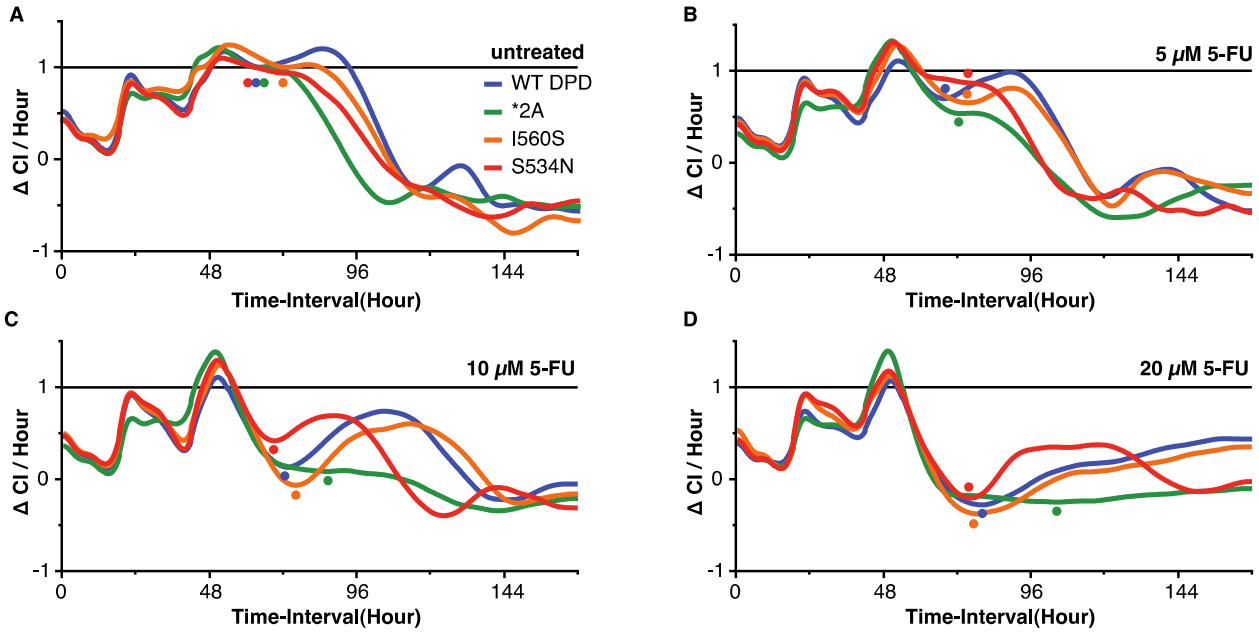
Supplementary Figure S1. Determining the range of linear response for DPD expression and enzyme activity. A, the indicated amounts of expression plasmids were transfected into HEK293T/c17 cells and lysates assayed for DPD enzyme activity. **B**, western blots demonstrating similar expression of variants at each transfection level.



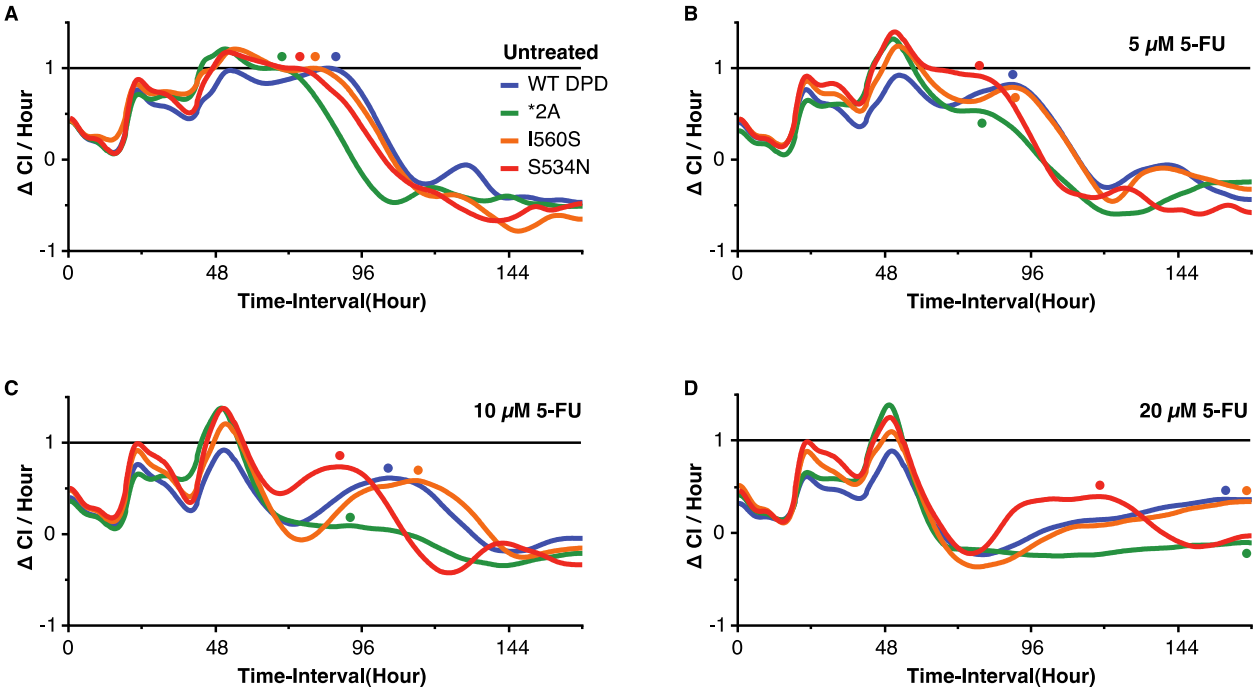
Supplementary Figure S2. Analysis of CI profiles using a single analysis time. HEK293T/c17 cells were transfected with wt *DPYD* and I560S. **A-D**, cultures were treated with varying concentrations of 5-FU and the CI was monitored by RTCA. The vertical line indicates the time at which a conventional endpoint would routinely be measured (48 hours after treatment). At the indicated analysis time, only small differences in relative CI are noted at all drug treatment concentrations except 10 μM. However, definate separation between treatments is noted at later times for those cells receiving the higher doses of 5-FU. Changing to a later fixed analysis time to measure this difference would invalidate the results at lower drug concentrations as the measurement would be taken after the CI plateau, which indicates culture confluency.



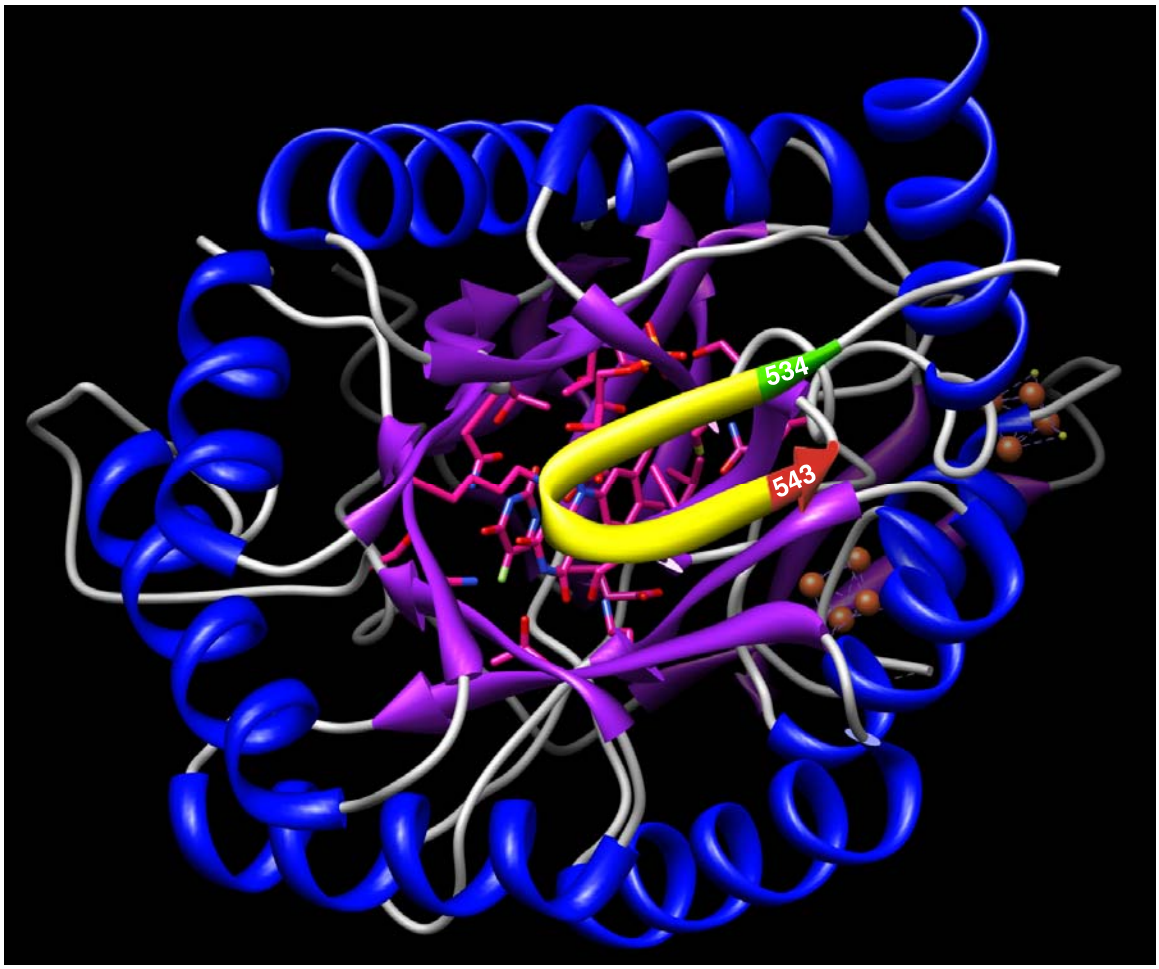
Supplementary Figure S3. Establishment of measurement times independently for each culture. A representative CI profile for cells transfected with wt *DPYD* and treated with 10 μM 5-FU is presented. Raw CI profiles (**A**) were smoothed using a spline smoothing algorithm with a smoothing coefficient (λ) equal to 1000 (**B**). The slope (derivative, **C**) and second order derivate (**D**) of the smoothed CI profile were determined for each time interval recorded. Similar trends were noted for all CI slope curves. Following a sharp increase in slope after the media change at the time of drug treatment, the slope decreased to an inflection point, which we have termed the "toxicity" time point. Following the decrease, the slope trended toward positive again where it reached a relative maximum, which we termed the "recovery" time point. Finally the slope reached zero, which corresponded to the plateau of the CI profile. The toxicity and recovery times were defined as the time at which the second order derivative of the CI equaled or approached zero (minimum of the absolute value of the second order derivative in the relevant analysis window).



Supplementary Figure S4. Comparison of slope at the toxicity time point. First order derivative (slope) CI (Δ CI hour^{-1}) profiles were generated for cells expressing *DPYD* variations treated with 0 (**A**), 5 (**B**), 10 (**C**), and 20 (**D**) μM 5-FU. Profiles were normalized to Δ CI hour^{-1} calculated at the toxicity time point for untreated cells expressing a given *DPYD* variant.



Supplementary Figure S5. Comparison of slope at the recovery time point. First order derivative (slope) CI (Δ CI hour^{-1}) profiles were generated for cells expressing *DPYD* variations treated with 0 (**A**), 5 (**B**), 10 (**C**), and 20 (**D**) μM 5-FU. Profiles were normalized to the Δ CI hour^{-1} calculated at the recovery time point for untreated cells expressing a given *DPYD* variant.



Supplementary Figure S6. Location of amino acids 534 and 543 within the 5-FU binding pocket of DPD. The chemical structure of the 5-FU binding pocket of DPD is shown as a complex with 5-Iodouracil [PDB ID: 1GTH (1)]. The coordinating side chains within the pocket are also shown. A beta-strand loop that is made up of amino acids 534-543 covers the opening to the active site. The locations for the S534N variation and the I543V variation within the structure are depicted as green and red residues, respectively. Image was prepared using UCSF Chimera (2).

References for Supplementary Figures

1. Dobritzsch D, Ricagno S, Schneider G, Schnackerz KD, Lindqvist Y. Crystal structure of the productive ternary complex of dihydropyrimidine dehydrogenase with NADPH and 5-iodouracil. Implications for mechanism of inhibition and electron transfer. J Biol Chem. 2002;277:13155-66.

2. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25:1605-12.