Supplementary Figure S1. DPD kinetics. A, DPD enzyme activity reactions were performed using lysates from cells expressing wildtype DPD and consisted of 8.2 µmol L⁻¹ [6-C¹⁴]-5-FU and the indicated final concentrations of NADPH. The K_M of NADPH was estimated to be 3.37 µmol L⁻¹ as determined by fitting a two-parameter Michaelis-Menten model (blue line) using JMP version 9.0.3. Three replicates were tested for each concentration. B, DPD enzyme activity reactions were performed using 200 µmol L⁻¹ NADPH and varying concentrations of [6-C¹⁴]-5-FU. Closed circles indicate the quantity of [6-C¹⁴]-DHFU produced in reactions (presented in arbitrary units). The K_M of [6-C¹⁴]-5-FU was estimated to be 3.35 µmol L⁻¹ by fitting a two-parameter Michaelis-Menten model (blue line). Open circles represent the sum of [6-C¹⁴]-DHFU and [6-C¹⁴]-5-FU present at the end of each reaction. As expected, the additive amounts increased linearly with increasing input amounts of [6-C¹⁴]-5-FU (red line). Three replicates were tested for each concentration. C, [6-C¹⁴]-DHFU formation increased linearly with time (blue line, R²=0.99) within the 30-minute incubation window. Reactions consisted of 8.2 µmol L⁻¹ [6-C¹⁴]-5-FU and 200 µmol L⁻¹ NADPH. Three independent reactions were performed for each time point. The sum of replicates is indicated by “×” ± the standard deviation. Note that for all three graphs, data on the y-axis are presented in arbitrary units.