

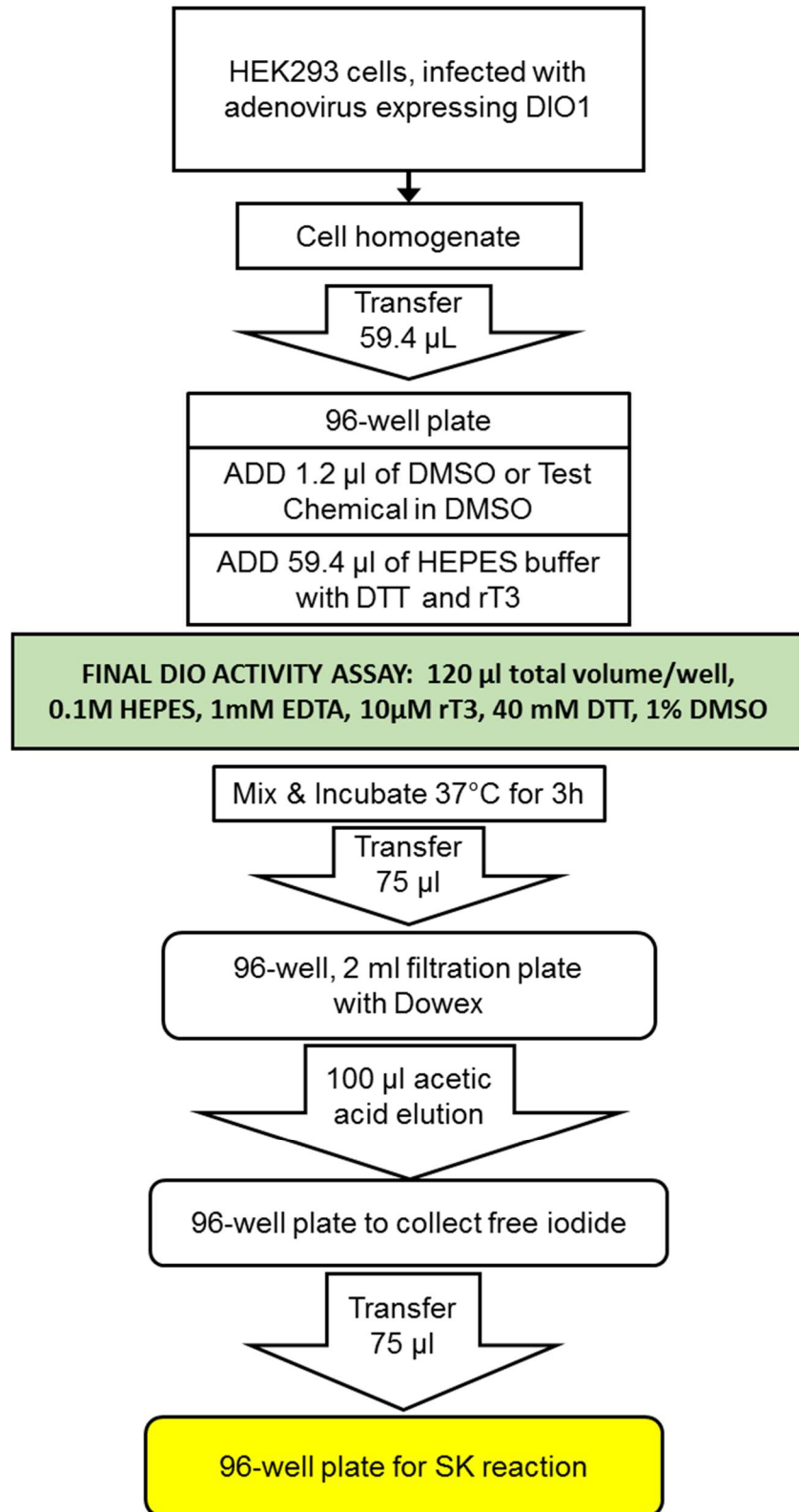
**Supplemental Figures.**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Yellow	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
B	Yellow	White	Blue	White	Blue	Blue	Blue	Blue	Red	Blue	Blue	Blue
C	Yellow	Blue	Blue	Blue	*	Blue	Red	Blue	Blue	Blue	Blue	White
D	Yellow	Blue	Blue	Blue	Blue	Blue	Blue	Blue	White	Blue	Blue	Blue
E	Yellow	Blue	Blue	Red	Blue	White	Blue	Blue	Blue	Blue	Blue	Blue
F	Yellow	Blue	Red	Blue	Blue	Blue	Blue	Blue	*	Blue	Red	Blue
G	Yellow	Blue	Blue	Blue	*	Blue	Red	Blue	Blue	Blue	White	Blue
H	White	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	Yellow	PTU Concentration Response Curve: 1000 to 0.03 $\mu$ M										
	White	DMSO										
	Red	200 $\mu$ M PTU										
	Blue	Sample										
	*	Sample that is repeated on two or more chemical plates										

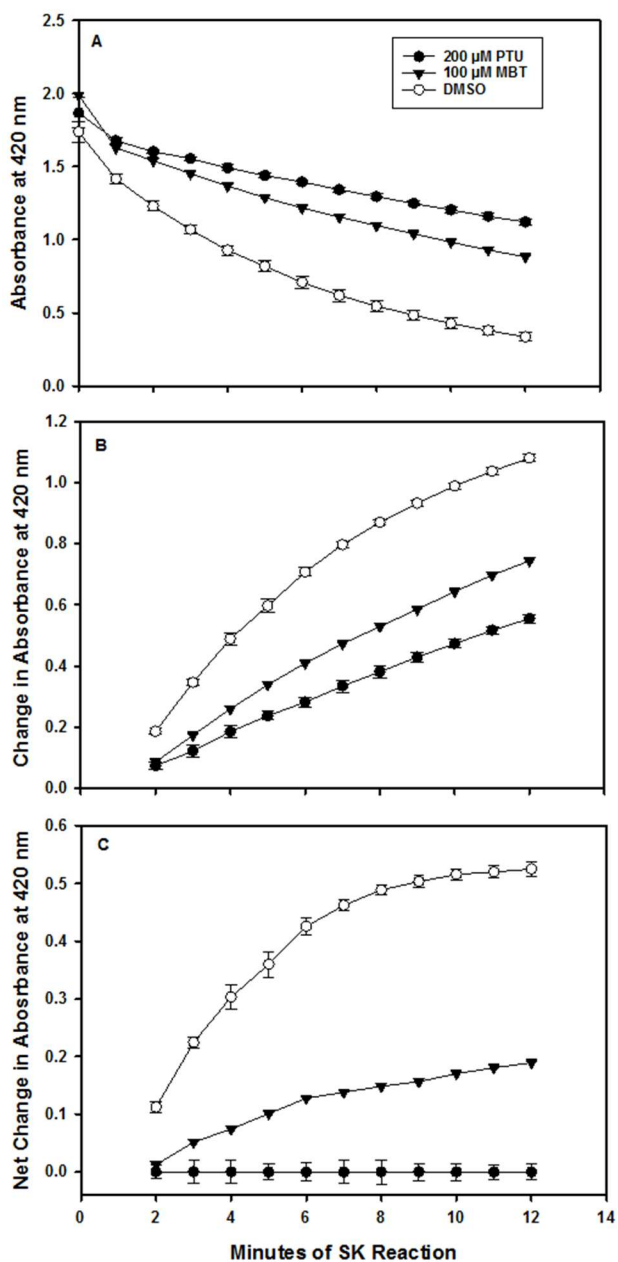
**Supplemental Figure 1.** An example plate layout for the chemical source plate for the single concentration screen. Plates were received with column 1 empty to which a 6-propylthiouracil (PTU) concentration response curve as added at our laboratory. Twelve additional wells were left empty on the source plate to which DMSO was added to 6 of these wells (white blocks) and 20 mM PTU (red blocks) was added to the remaining 6 wells to establish the fully inhibited response level. Sample chemicals repeated across multiple plates are indicated with an asterisk. These were randomly assigned to wells on each plate.

Concentration Response Plate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
B	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
C	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
D	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
E	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
F	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
G	Yellow	Chem 1	Chem 2	Chem 3	Chem 4	Chem 5	Chem 6	Chem 7	Chem 8	Chem 9	Chem 10	Chem 11
H	White	Red	White	Red	White	Red	White	Red	White	Red	White	Red
	Yellow	PTU Concentration Response Curve: 1000 to 0.03 $\mu$ M										
	White	DMSO										
	Red	200 $\mu$ M PTU										
	Blue	11 Chemicals in concentration response (200 to 0.03 $\mu$ M). One chemical per column.										

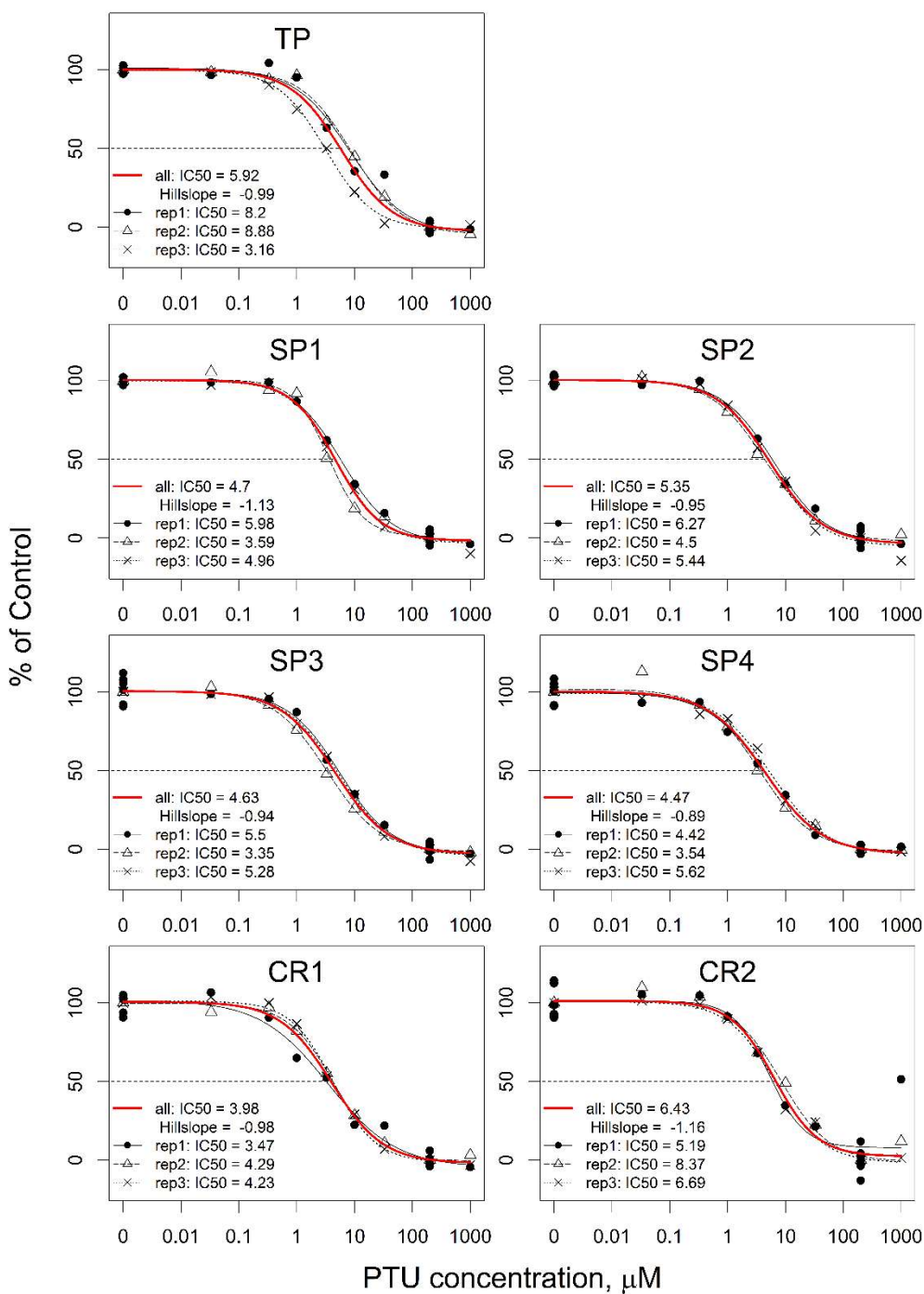
**Supplemental Figure 2.** Plate layout for concentration-response screening.



**Supplemental Figure 3.** Summary of steps in assay procedure.



**Supplemental Figure 4.** Example absorbance data for determining deiodinase type 1 activity in one well of 100  $\mu$ M 2-mercaptobenzothiazole (MBT) from one test plate. Panel A shows raw absorbance values over time for the Sandell-Kolthoff (SK) reaction. Symbols represent 1 well of MBT at 100  $\mu$ M (▼), DMSO controls giving maximal activity (○; mean & sd; n=7 wells), and fully inhibited response by PTU at 200  $\mu$ M (●; mean & sd; n=6 wells). Panel B shows raw data converted to change in absorbance at each time-point. Panel C shows data converted to net change in absorbance by subtracting the mean of the fully inhibited response from the PTU wells. The net change in absorbance at the 10 min time-point is used to calculate the % of control activity for the test chemical data point. Final results reported are the median, min, and max across three replicated test plates.



**Supplemental Figure 5.** Concentration-response curves for DIO1 inhibition by 6-propylthiouracil (PTU) across initial screening (TP), single concentration (SP1-4), and concentration-response (CR1, CR2) plates. Each triplicate test of a plate has different symbols and fit line, solid circle with solid line = rep 1, open triangle with dashed line = rep 2,  $\times$  with dotted line = rep 3.