

## Supplementary Table S5

### Steady-state kinetics parameters for the reduction of CB1954 by NADH catalysed by NfsB mutants.

Initial rates were monitored at 420 nm at a series of concentrations of both substrates and all the data fitted to the equation for a bi-bi substituted enzyme reaction (equation4), by nonlinear regression with equal weighting all points, using the programme Sigmaplot 14 (Systat software, San Jose, CA). The data for N71S/T41Q/F124T/M127V is from this work. The data for the other mutants is from [1]

Protein	$k_{cat}$ $s^{-1}$	$K_{mCB1954}$ $\mu M$	$k_{cat}/K_{mCB1954}$ $\mu M^{-1} s^{-1}$	$K_{mNADH}$ $\mu M$	$k_{cat}/K_{mNADH}$ $\mu M^{-1} s^{-1}$
N71S/T41Q/F124T/M127V	$270 \pm 17$	$480 \pm 60$	$0.57 \pm 0.04$	$100 \pm 13$	$2.7 \pm 0.2$
Wild type	$140 \pm 32$	$17200 \pm 4800$	$0.007 \pm 0.0006$	$40 \pm 12$	$3.46 \pm 0.6$
T41L/N71S	$153 \pm 8$	$216 \pm 33$	$0.71 \pm 0.08$	$253 \pm 41$	$0.62 \pm 0.08$
T41Q/N71S/F124T	$181 \pm 7$	$569 \pm 45$	$0.32 \pm 0.02$	$136 \pm 12$	$1.33 \pm 0.077$

1 Jarrom, D., Jaberipour, M., Guise, C. P., Daff, S., White, S. A., Searle, P. F. and Hyde, E. I. (2009) Steady-state and stopped-flow kinetic studies of three Escherichia coli NfsB mutants with enhanced activity for the prodrug CB1954. *Biochemistry*. **48**, 7665-7672