

**Supplementary Table S4:****Selection of quadruple mutations of NfsB**

NfsB was mutated at 8 positions within the active site and the gene introduced into lambda phage. E coli lysogens were made and mutants were selected using the NfsB reduction of CB1954 to activate the SOS response and release phage enriched with more active enzyme.

Amino-acid substitutions, frequency and prodrug sensitivity of mutants analysed after 15 rounds of selection:

<b>T41</b>	<b>N71</b>	<b>G120</b>	<b>F124</b>	<b>M127</b>	<b>H128</b>	<b>W138</b>	<b>NUMBER</b>	<b>APPROX IC<sub>50</sub>- REPLICA PLATE (<math>\mu</math>M CB1954)</b>	<b>IC<sub>50</sub> colony assay (<math>\mu</math>M CB1954)</b>
Q	S		T	V			3	<10	4.5
Q	S		T	T				<10	5.2
Q	S		T	I			2	<10	5.5
Q	S		T						5.8
Q	S		T	Y				<10	6.5
L	S			F			1	<20	10.8
L	S			V				<20	10.9
L	S			I			2	<20	11.3
L	S			Y				<20	11.3
L	S								14.5
L	S	S	T	L			1	<20	nd
L	S		T	T			1	<20	nd
L	S		T	Y			1	<15	nd
Q	S			Y			2	<10	nd
Q	S	S	T	L				<15	nd
Q	S		T	S				<20	nd
Q	S			T				<15	nd
	S		T					20	nd

