

# Supporting Information

## Genomic Deoxyxylulose Phosphate Reductoisomerase (DXR) Mutations Conferring Resistance to the Antimalarial Drug Fosmidomycin in *E. coli*

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Content:

**Fig. S1** - A detailed example for a CREATE cassette.

**Fig. S2** - Sequence alignment of FSM-resistant colonies.

**Fig. S3** - Correlations between repeats.

**Fig. S4** - CREATE plasmid deep sequencing enrichment data.

**Fig. S5** - Extended genomic sequencing enrichment results.

**Fig. S6** - Correlation plots between plasmid and genomic sequencing.

**Fig. S7** - Proline 274 in its structural context.

**Fig. S8** - Growth curves of the P274K mutant relative to controls.

**Table S1** - Mutant enrichment values.

**Table S2** - EC<sub>50</sub> values of selected mutants

# Figure S1

ccgcgagggtttgcagggTTCACCTCAATGGTGCCTATCAGGACGGCAGTGTTTC  
                  (GGG) (CCG)  
TGGCGCAGCTGggcGAAaaaGATATGCGTACGCCAATTGCCACACCATGGCAT  
                  (G→G) (P→K)  
GGCCGAATCgatcTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTcagtgt  
tctggcgcagctggGTTTTAGAGCTAGAAATAGCAAGT

Supplementary Figure 1. A detailed example for a CREATE cassette. The P274K mutation is shown. Colors correspond to the color scheme shown in Figure 1A. Wild-type sequences are shown in parentheses, above target sites.

# Figure S2

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1 (P274R) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAACGTGATATGCGTACGCCAATTGCCAC
2 (P274R) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGGTTTTAGAGCTAGAAAATAGCAAGTAAAAATAA
3 (S186Q) ...GGGTTGGGATACGCTGACCTTGAG-CAAAATGGCGTGGTGTCCATTTTACTTACGGGGCAGGGT---GGCCCT-TTCGTGAGACG
4 (WT) ...GGGTTGGACCCGTAAAGCAGAGCAAAGCGCAAT-TGTTACCCGT--CGATTGCGAACATAACG-CGATTTTTCAGAGTTTACCG
5 (P274K) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAAAAGATATGCGTACGCCAATTGCCAC
6 (P274K) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAAAAGATATGCGTACGCCAATTGCCAC
7 (P274R) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAACGTGATATGCGTACGCCAATTGCCAC
8 (WT)
9 (WT) ...GGGTTCAACTCTGGATGTTTCATAAGCAACTCAC-CATTTCTGGGC-TCGACGGGCTCGATTACC---TGCAGCACGCTGGACGTG
10 (S186Q) ...GGGTTGGGATACGCTGACCTTGAG-CAAAATGGCGTGGTGTCCATTTTACTTACGGGGCAGGGT---GGCCCTTTCGGTGAGACG
11 (WT) ...GGGTTGGGATACGCTGACCTTGAGCAAAATGGCG-TGTTCCATT-TTACTACGGGGCCGGG---GGCCCTTTCGGTGAGACG
12 (P274H) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAACATGATATGCGTACGCCAATTGCCAC
13 (P274K) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAAAAGATATGCGTACGCCAATTGCCAC
14 (WT) ...GGGTATCCGAACTGGTCGATGGGGCGTAAATTT---CTGTCGAT-TCGGCTACGATGATGAAC-CCGGTCTGGAATACATTGA
15 (WT) ...GGGTTGTACCTACGCTTGCTGCATCCCGCGGGTAAACCATT-TTGTGGCGAATATGGAA---TCACTGGTTACCTCGGGA
16 (P274H) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAACATGATATGCGTACGCCAATTGCCAC
17 (S186A) ...GGGTTGGGATACGCTGACCTTGAGGAAAATGGCGTGGTGTCCATTTTACTTACGGGGGGGGG---GGCCCTTTCGGTGAGACG
18 (WT) ...GGGTTCAACTCTGGATGTTTCATGAAGCAACTCACCATTCTGGGC-TCGACGGGCTCGATTGTG---TGCAGCACGCTGGACGTG
19 (D275W) ...GGGTCACTCAATGGTGCCTATCAGGACGGCATGTTCTGGCGCA---GCTGGCGAACCGTGG-ATGCGTACGCCAATTGCCA
20 (WT)
21 (P274K) ...GGGTTTGAAGCGGTTGGCTGTTTAAACGCCAGCGCAGCCA-GA--TGGAAGTCTGATTCACC-CGAGTCAGTGATTATATCA
22 (P274M) ...GGGTTTCACTCAATGGTGCCTATCAGACCGGCAGTGTCTGGCG-CAGCTGGCGAAATGATATGCGTACGCCAATTGCCAC
23 (M276I) ...--AGGCTACTCAATGGTGCCTATCAGGACCGGCAGTGTCTGGCG-CAGCTGGCGAACCGGATATTCGTACGCCAATTGCCAC
24 (WT) ...GGGTATCCGAACTGGTCGATGGGGCGTAAATTT---CTGTCGAT-TCGGCTACGATGATGAAC-CCGGTCTGGAATACATTGA

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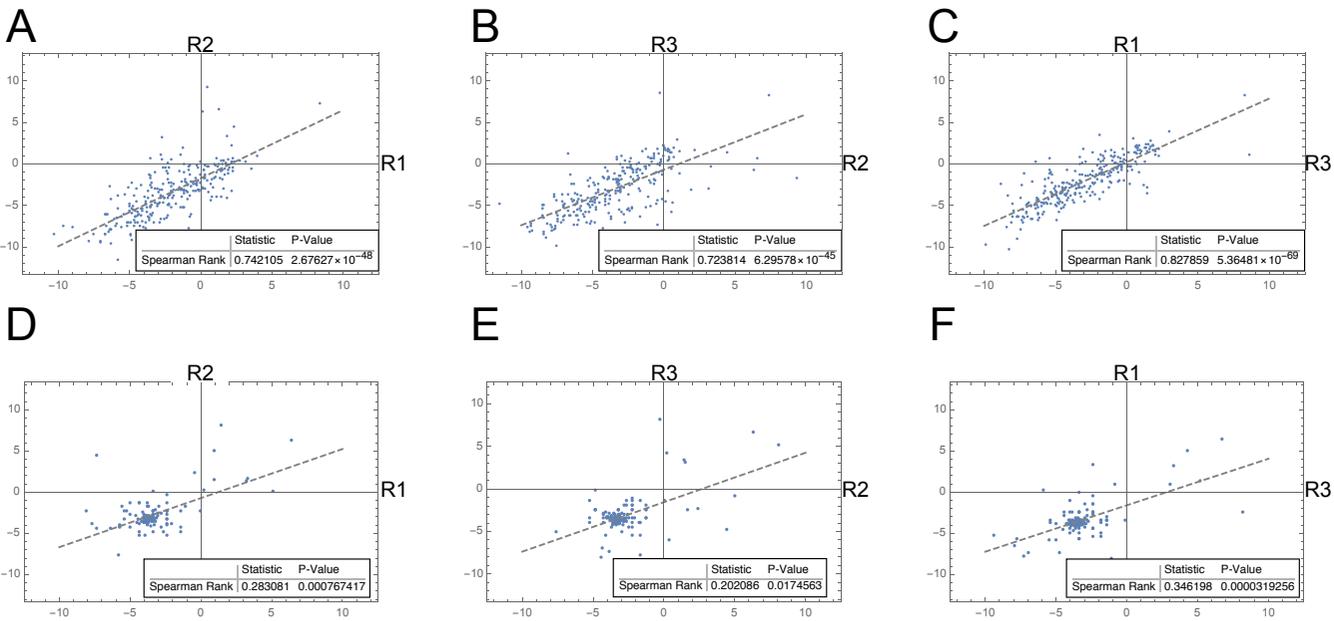
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1 (P274R) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
2 (P274R) -----
3 (S186Q) CCATTGCGGATTTGGCAACAAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTACGGAAA-GGGCCACCAGACCSTTT...
4 (WT) CAACCTATCCAGCATAATCTGGGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTATCECTGC-TTTCAAGGGCGAASTTT...
5 (P274K) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
6 (P274K) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
7 (P274R) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTTAAGTGTT-CTGGCGCAGCTGGGTTT...
8 (WT) -----
9 (WT) GTGCGCCATAATCCGAACACTGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTTATTTTT-TTCAAGGGCGAASTTT...
10 (S186Q) CCATTGCGGATTTGGCAACAAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTACGGAAA-GGGCCACCAGACCSTTT...
11 (WT) CCATTGCGGATTTGGCAACAAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTTGGTG--ATAAGCGTGCTCTSTTT...
12 (P274H) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
13 (P274K) ACCATGGCATGGCCGAATCGA---TCTTGACAGCAGC---CAGTCTAGGTATAATACTAGTCAGTGT-TGGCGCAGC-GGSTTT...
14 (WT) AGCGCGTTGGCTGTTAAACGCCAGATCTTGACAGTAGCTCAGTCTAGGTATAATACTAGTCTGGGA--ATAAGCGTGCTCTSTTT...
15 (WT) CGTCTGTTATGGACCGCGTAAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTGGCGAA-ATTTGATAATTGCTSTTT...
16 (P274H) ACCATGGCATGGCCGAATCGA---TCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
17 (S186A) CCATTGCGGATTTGGCAACAAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTACGGAAA-GGGCCACCAGACCSTTT...
18 (WT) GTGCGCCATAATCCGAACACTGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAAGTGGTATAAAATGGGGGGCAAASTTT...
19 (D275W) CACCATGGCATGGCCGAATCGCGATCTTGACAGCTAGCTCAGTCTCGTATAATACTANTCAGTGT-CTGGCGCAGCTGGGTTT...
20 (WT) -----
21 (P274K) ATGGTGCCTATCAGGACGGCAGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTGCGTAA--AATTTCTGTGATTSTTT...
22 (P274M) ACCATGGCATGGCCGAATCGATC---TTGACAGCTAGCTCAGT---TAGGTATAATACTAGTCAGTGT-TGGCGCAGCTGGGTTT...
23 (M276I) ACCATGGCATGGCCGAATCGCGGATCTTGACAGCTAGCTCAGTCTAGGTATAATACTAGTCAGTGTT-CTGGCGCAGCTGGGTTT...
24 (WT) AGCGCGTTGGCTGTTAAACGCCAGATCTTGACAGTAGCTCAGTCTAGGTATAATACTAGTCTGGGA--ATAAGCGTGCTCTSTTT...

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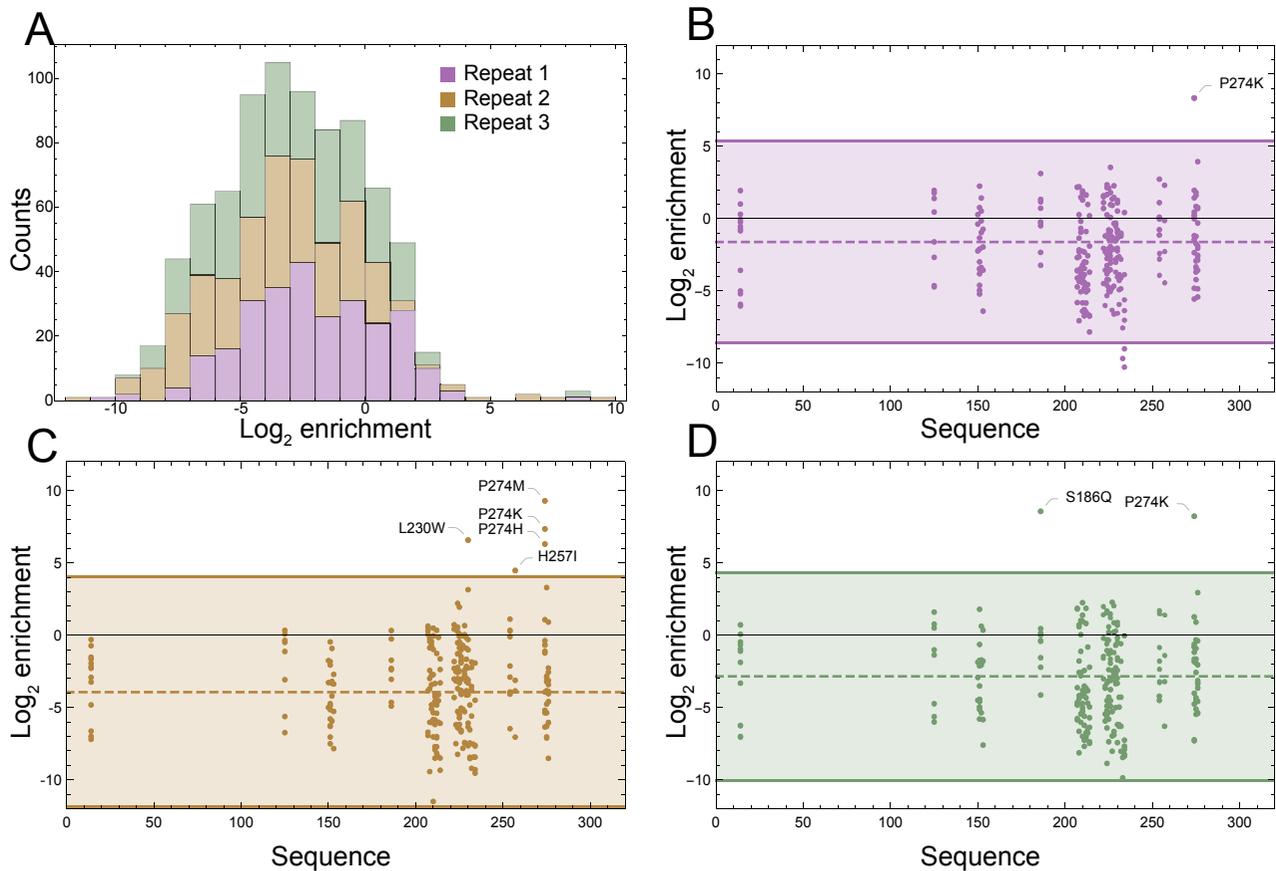
Supplementary Figure 2. Sequence alignment of all 24 FSM-resistant colonies. Numbers correspond to colony numbers, and genomic mutations are indicated. WT, no genomic editing had occurred. Colors correspond to the cassette elements, as shown in Fig. 1A and S1. Magenta circles indicate mismatches with cassette design. Black rectangles highlight cross-overed gRNA spacers. Sequences were aligned using Clustal Omega, and some corrections were made manually.

# Figure S3



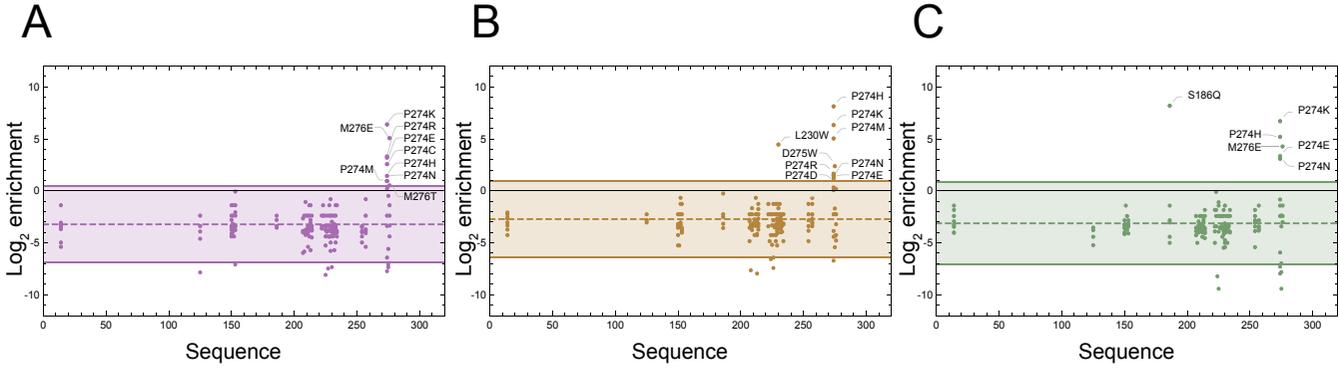
Supplementary Figure 3. Correlations between repeats. Correlation was calculated using the Spearman Rank test for the CREATE plasmids deep sequencing (A-C), and for the genomic deep sequencing (D-F). 'R' indicates the repeat number and dashed lines indicate the linear model fit for each comparison.

# Figure S4



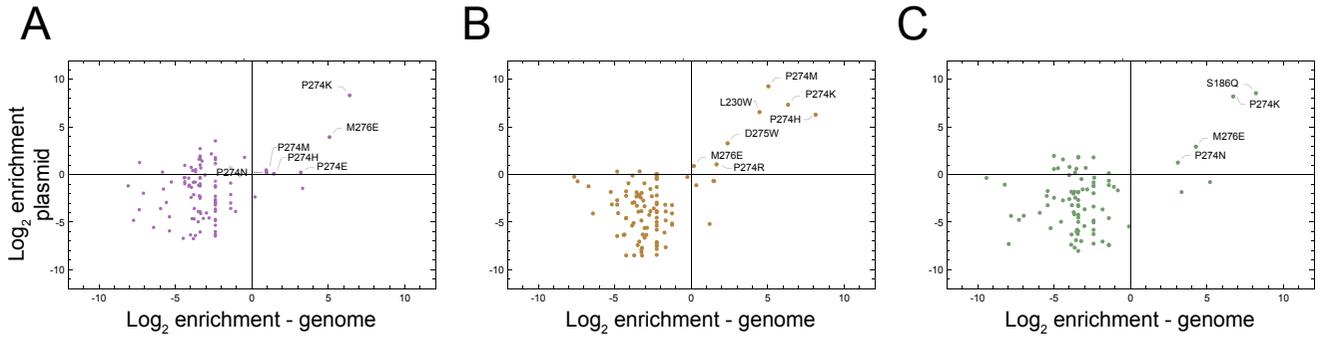
Supplementary Figure 4. CREATE plasmid deep sequencing enrichment data. Mutant enrichment was calculated between the overnight samples that were either treated or not treated with FSM (G and H in Fig. 1). A, Enrichment stacked histogram. B-C plots representing the enriched mutations according to their position in DXR. Colors correspond to the repeat identity. Dashed lines indicate the mean enrichment of silent mutations. Solid lines mark two standard deviations from the mean, with the area shaded between them. Significantly enriched mutations are labeled.

# Figure S5



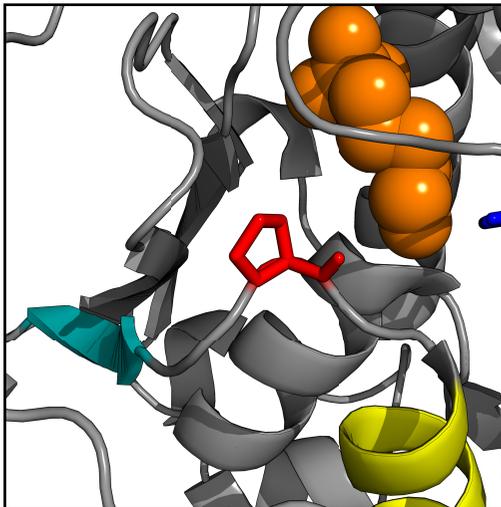
Supplementary Figure 5. Extended genomic sequencing enrichment results. This figure corresponds to Fig. 3 and includes the G14 saturation control which was omitted for clarity.

# Figure S6



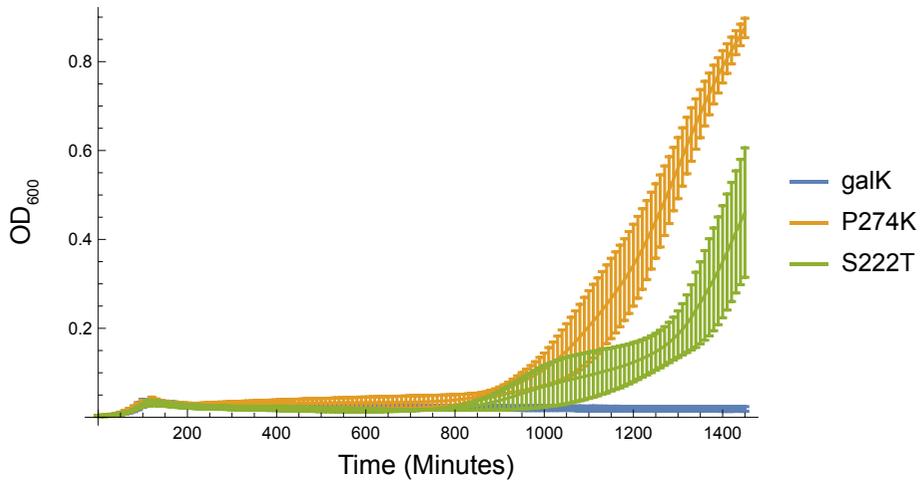
Supplementary Figure 6. Correlation plots between plasmid and genomic sequencing. A plot is shown for every repeat (A-C). Positively correlated mutations are labeled.

# Figure S7



Supplementary Figure 7. Proline 274 in its structural context. P274 is in a cis conformation and induces turn between two structural elements. P274 is shown in red, beta sheet is colored in cyan, and the alpha helix is shown in yellow. FSM is colored in orange. Structure PDB ID: 1q0l.

# Figure S8



Supplementary Figure 8. Growth curves of the P274K, the control galk edit and the previously reported S222T mutations (Armstrong et al., 2015). The P274K mutants was recreated from an FSM-resistant colony (Fig. 2b). The S222T mutation was made by synthesizing a single CREATE cassette, with the same design used for the library. Both strains were sequence-validated, while the galk mutation was isolated using a pink/white colony screen (see Materials and Methods, Library preparation). Bacteria growth was measured by BioTek Synergy 2 microplate reader in the presence of FSM (100uM). Optical density was measured in 10 minutes intervals up to 24 hours. The mean and SD from three independent experiments are shown.

# Table S1

The statistically significantly enriched mutations following FSM treatment, both at the genomic and the plasmid levels, as shown in Fig. 3 and Fig. S4, respectively.

Repeat 1		Repeat 2		Repeat 3	
Mutation	Log2 enrichment	Mutation	Log2 enrichment	Mutation	Log2 enrichment
Genomic sequencing					
P274K	6.391	P274H	8.130	S186Q	8.190
M276E	5.075	P274K	6.332	P274K	6.710
P274R	3.316	P274M	5.043	P274H	5.195
P274E	3.200	L230W	4.461	M276E	4.268
P274C	2.569	D275W	2.375	P274E	3.336
P274H	1.437	P274R	1.653	P274N	3.089
P274N	0.959	P274N	1.501		
P274M	0.937	P274E	1.447		
M276T	0.522	P274D	1.206		
Plasmid sequencing					
P274K	8.340	P274M	9.293	S186Q	8.567
		P274K	7.347	P274K	8.227
		L230W	6.576		
		P274H	6.305		
		H257I	4.475		

# Table S2

EC<sub>50</sub> values for selected mutants describes in this study, along with their 95% confidence intervals (CI). Data shown represents three independent replicates.

<b>DXR</b>	<b>EC<sub>50</sub> (<math>\mu</math>M)</b>	<b>95% CI (<math>\mu</math>M)</b>
WT	1.245	1.073 to 1.475
P274K	6.697	5.912 to 7.637
P274M	3.360	2.798 to 3.950
P274R	5.532	3.998 to 7.943
S222T	6.330	5.531 to 7.232