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SUPPLEMENTARY DATA

Directed evolution of P-glycoprotein cysteines reveals site-specific, non-conservative substitutions that preserve multidrug resistance

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Table S1 Oligonucleotide sequences used for site-saturation mutagenesis and site-directed mutagenesis of single TM Cys mutants

Only the forward mutagenic primer sequences are shown. Reverse primer sequences are identical but complimentary to the forward primer.

Primer name	Sequence (5′ to 3′)
Mutagenic primers	
C427-NNN	CGGAAATTCTGGTNNNGGCAAGTCCAC
C638-NNN	GGAAATGAGGCTNNNAAGTCTAAGGACGAG
C669-NNN	CACTAGAAAGTCCATTNNNGGTCCACACGATC
C1070-NNN	GGTTCTTCCGGTNNNGGCAAGTCCACTG
C1121-NNN	CTATTCTTTTCGACNNNAGCATTGCAGAG
C1223-NNN	CCAGAGAAGGAAGAACTNNNATCGTCATTGCTC
C133A	TCCTTCTGGGCCCTGGCCGCTGGAAGACAAATCCACAAGATTAGACAGAAG
C713A	GGTATCTTCGCTGCCATCATTAACGGTGGTTTGCAGCCAGCTTTTTCTGTG
C952A	TACGCTGCCGCATTCAGATTCGGTGCTTATTTGGTGACTCAGCAATTGATG
Flanking primers	
NBD1/linker	CCTTGTTTCTTTTTCTGCACAA (Forward)
	CTGTCTTTGGGTTTCTGGTGGACCACC (Reverse)
NBD2	CCATACCGTAACGCTATGAAAAAGGCTC (Forward)
	GAAGTGTCAACAACGTATCTACCAACG (Reverse)

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Table S2 Relative frequency of each amino acid found at the nine native Cys positions in closely related Pgp orthologs

Position	Cys^{133}*	Cys^{427}	Cys^{638}	Cys^{669}	Cys^{713}	Cys^{952}	Cys^{1070}	Cys^{1121}	Cys^{1223}
A									
C	84	100	11	5	98	98	100	96	98
D			34						
E			5						
F								4	
G	2		23						
H				23					
I									
K				16					
L	2								
M									
N			7						
P									
Q				2					
R				49					2
S			14	2	2	2			
T	11			2					
V									
W									
Y			7						
Total%	100	100	100	100	100	100	100	100	100

*Human and mouse Pgp were aligned with 46 closest related orthologs (⩾70% sequence identity) from the Ensembl database. The relative frequency (%) of amino acids found at each Cys position is shown.

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