Drug Metabolism and Disposition

Supplementary Materials for:

Synthesis and characterization of BODIPY-FL-cyclosporine A as a substrate for multidrug resistance-linked P-glycoprotein (ABCB1)

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Table S1: Docking scores of the 9 lowest energy poses of CsA, NBD-CsA and BD-CsA docked into human P-gp structure (6QEX.pdb).

	Docking energy (kcal/mol)		
Docking pose number	CsA	NBD-CsA	BD-CsA
1	-14.5	-14.5	-14.5
2	-14.5	-13.8	-13.8
3	-14.2	-13.8	-13.2
4	-14.1	-13.8	-12.2
5	-13.5	-13.8	-12.1
6	-13.3	-13.8	-12.1
7	-13.2	-13.7	-12.1
8	-13.2	-13.7	-12
9	-13.2	-13.6	-11.9

Autodock Vina program was used for docking ligands in the drug-binding pocket of human P-gp as described in the Materials and Methods Section.

Table S2: List of amino acid residues present within a 5Å distance of CsA, NBD-CsA and BD-CsA in pose 1 obtained by docking in the drug-binding pocket of human P-gp structure (6QEX.pdb).

Residues within 5Å of pose 1 for CsA	Residues within 5Å of pose 1 for NBD-CsA	Residues within 5Å of pose 1 for BD-CsA
LEU 65	LEU 65	LEU 65
MET 68	MET 68	MET 68
MET 69	MET 69	MET 69
PHE 72	GLN 195	MET 192
TRP 232	TRP 232	GLN 195
ALA 302	PHE 239	SER 196
PHE 303	ASN 296	THR 199
ILE 306	PHE 303	SER 228
TYR 307	ILE 306	ALA 229
TYR 310	TYR 307	TRP 232
PHE 336	TYR 310	ALA 233
LEU 339	PHE 336	LEU 236
ILE 340	LEU 339	ILE 299
PHE 343	ILE 340	PHE 303
GLN 347	PHE 343	ILE 306
GLN 725	SER 344	TYR 307
PHE 728	GLN 347	TYR 310
PHE 732	ASN 721	PHE 336
GLU 875	GLY 722	LEU 339
MET 876	LEU 724	ILE 340
LEU 879	GLN 725	PHE 343
GLN 946	PHE 728	SER 344

MET 949	PHE 770	GLN 347
TYR 950	GLN 773	ASN 721
TYR 953	SER 831	GLY 722
PHE 957	ALA 834	LEU 724
LEU 975	VAL 835	GLN 725
PHE 978	GLN 838	PHE 728
SER 979	ASN 842	SER 766
VAL 982	GLU 875	PHE 770
PHE 983	MET 876	GLN 773
MET 986	MET 949	GLN 838
ALA 987	TYR 953	ASN 842
GLN 990	SER 979	GLU 875
	PHE 983	MET 876
	MET 986	LEU 879
	ALA 987	GLN 946
	GLN 990	MET 949
	VAL 991	TYR 953
	PHE 994	VAL 982
	ALA 995	PHE 983
	PRO 996	MET 986
		ALA 987
		GLN 990
		VAL 991
		PHE 994

HPLC trace of **6**

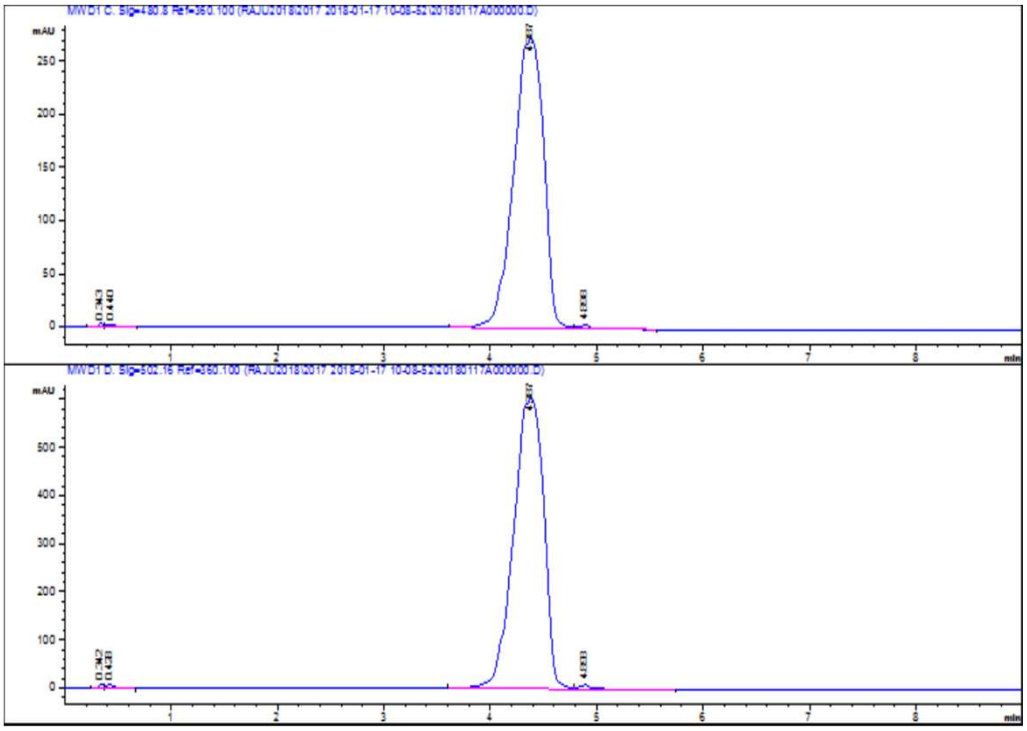

Figure S1. HPLC trace of purified BD-CsA: Histogram showing the HPLC trace of compound 6,referring to BD-CsA purity.

Mass spectrum of **6**

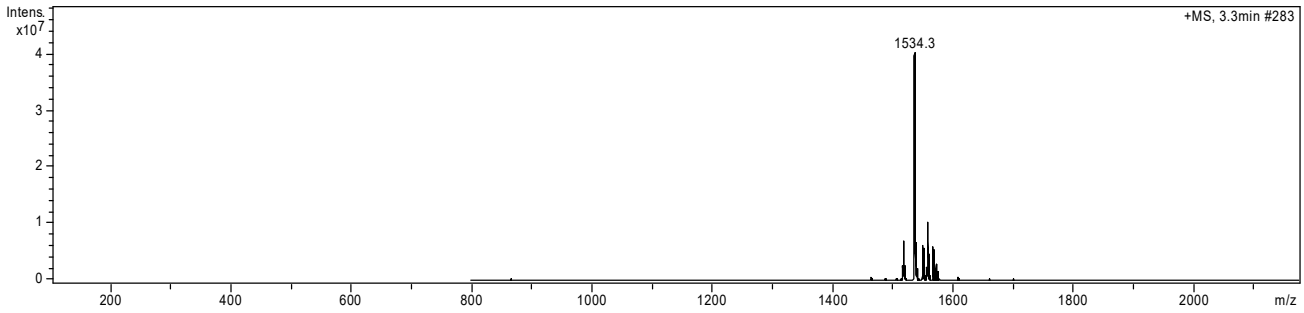

Figure S2. Mass spectrum of BD-CsA: Histogram showing MS spectra of compound 6, purified as BD-CsA. The single peak at 1534.3 Da indicates compound purity.

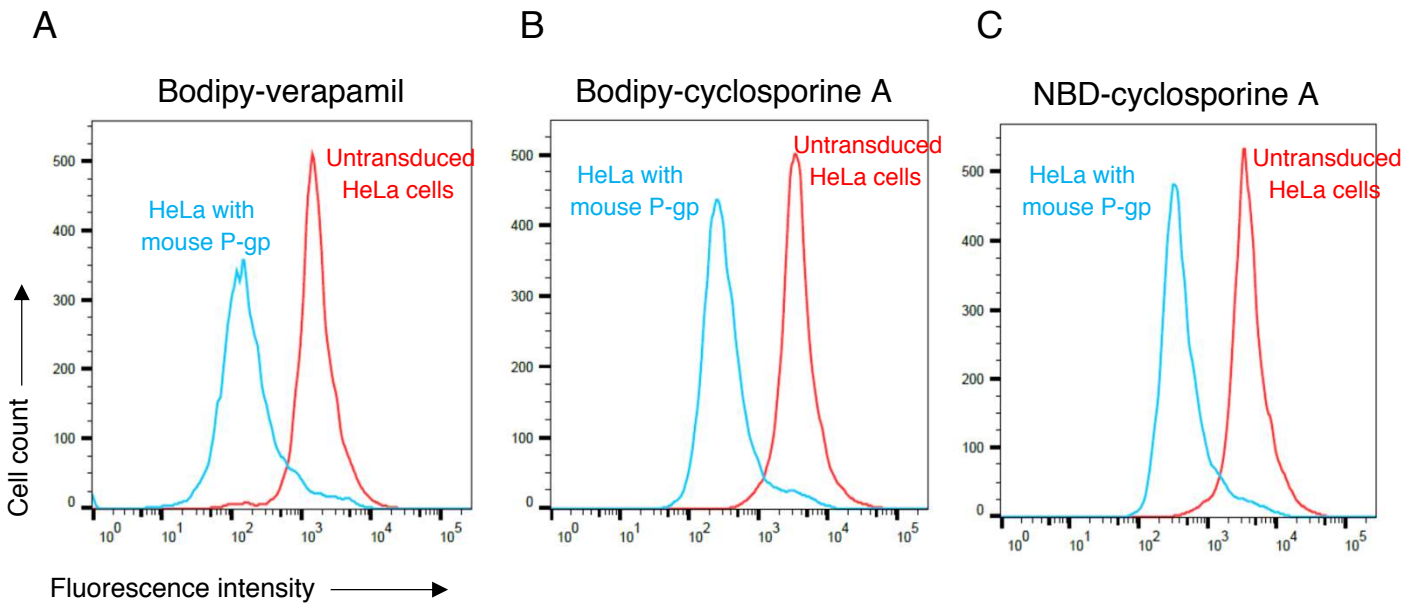

Figure S3. BD-CsA is a substrate for mouse P-gp: HeLa cells were transduced with BacMam baculovirus to express mouse P-gp and untransduced cells were used as a control. Cells were incubated with BD-CsA or NBD-CsA (0.5 μM, each) for 45 minutes at 37°C and fluorescence was measured using flow cytometry. Histogram traces show the transport of substrates by mouse P-gp, (A) BD-verapamil, (B) BD-CsA and (C) NBD-CsA. The efflux by P-gp was assayed by comparing the fluorescence intensity of cells expressing P-gp (blue traces) with those that do not express P-gp (untransduced cells, red traces).

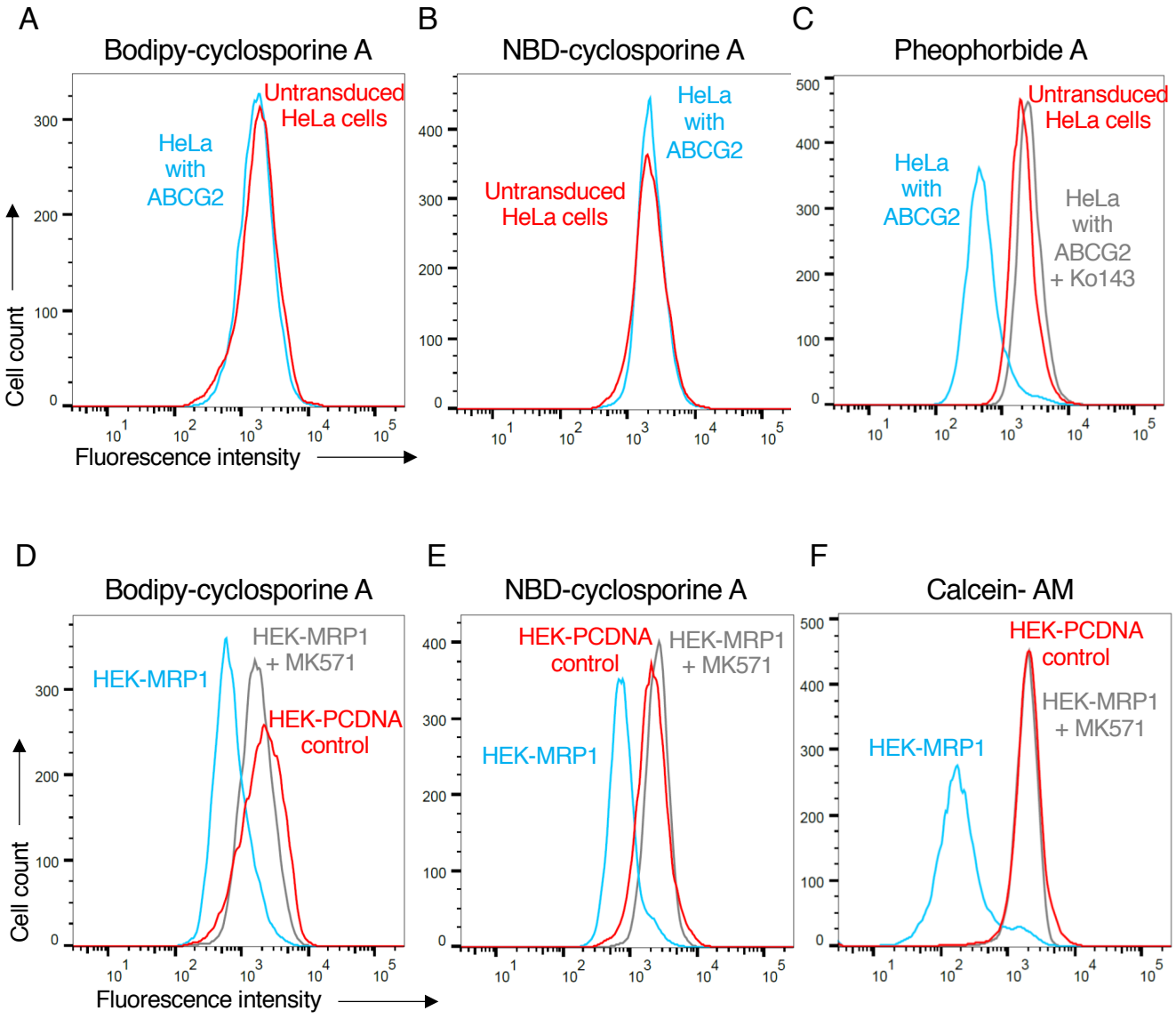

Figure S4. Efflux of BD-CsA and NBD-CsA by human ABCG2 and MRP1: (A-C) HeLa cells were transduced with BacMam baculovirus to express human ABCG2 and untransduced cells were used as a control. Cells were incubated with BD-CsA, NBD-CsA (0.5 μM, each) or pheophorbide A (2 μM) for 45 minutes at 37°C and fluorescence was measured using flow cytometry. Histogram traces show the transport of substrates by ABCG2, (A) BD-CsA, (B) NBD-CsA and (C) Pheophorbide A (known ABCG2 substrate). The ABCG2 inhibitor Ko143 (2.5 μM) was used to indicate the specificity of ABCG2. (D-F) HEK-cells transfected with MRP1 or PCDNA3.1 (vector control) were used. Histogram traces show the transport activity of MRP1 with (D) BD-CsA, (E) NBD-CsA and (F) calcein-AM (known MRP1 substrate). The MRP1 inhibitor MK571 was used at 25 μM. The efflux was assayed by comparing the fluorescence intensity of cells expressing the transporter (blue traces) with control (untransduced or parental) cells (red traces). The traces of cells expressing the transporters in the presence of inhibitors are shown in grey.

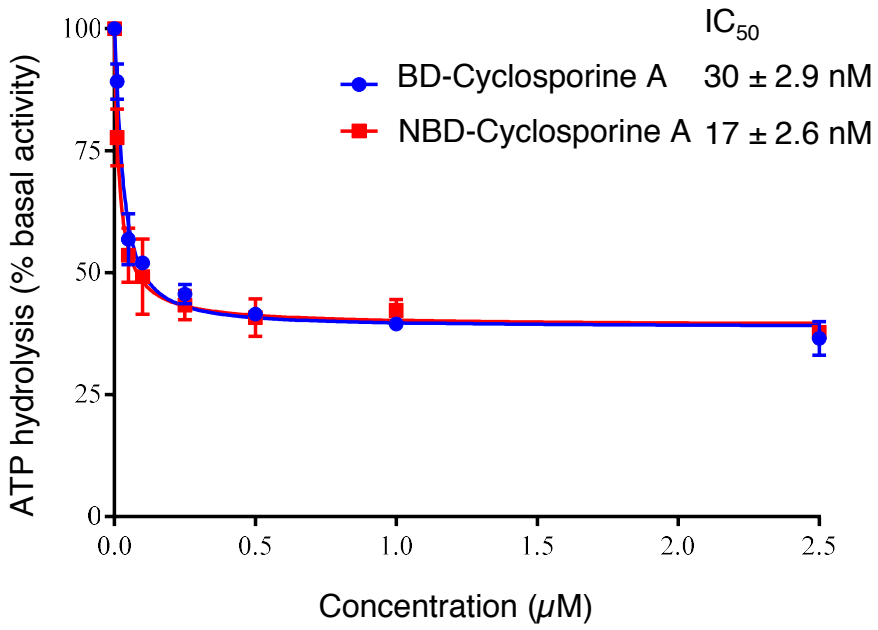

Figure S5. Effect of BD-CsA and NBD-CsA on ATPase activity of P-gp: ATPase activity of human P-gp was assayed in insect cell membrane vesicles and the effect of BD-CsA or NBD-CsA at indicated concentrations was measured as described in the Materials and Methods. Both compounds partially inhibited the ATPase activity with IC50 of 30±2.9 nM for BD-CsA and 17±2.6 nM for NBD-CsA.

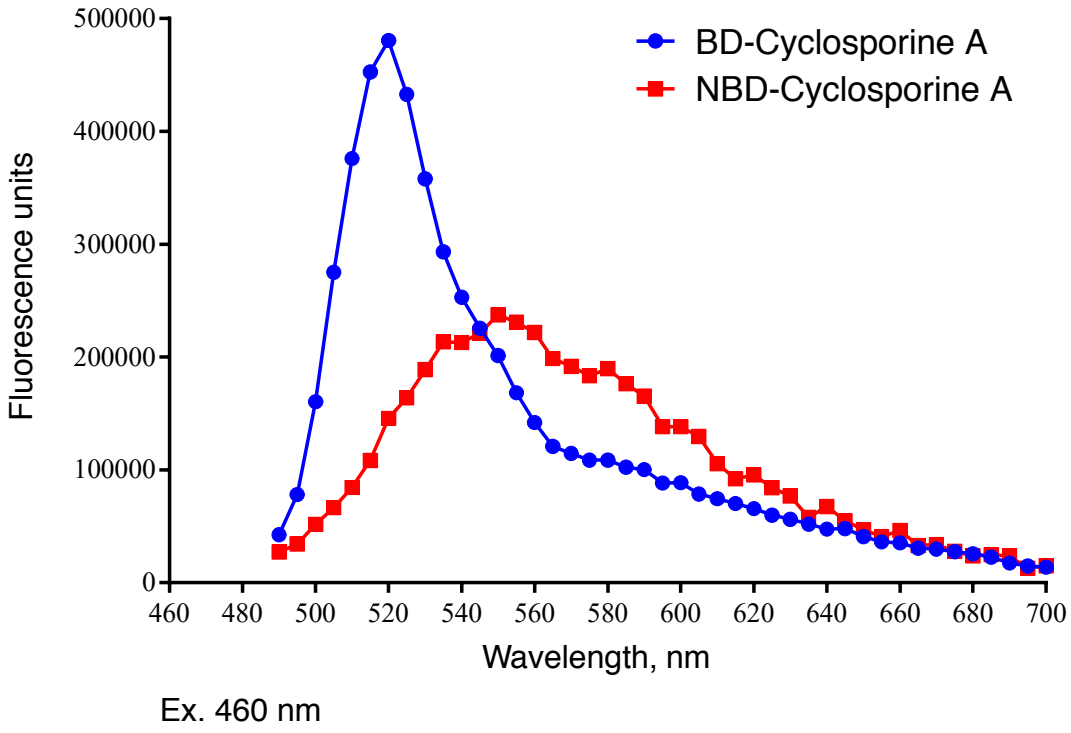

Figure S6. Fluorescence emission spectra of BD-CsA and NBD-CsA: Histogram showing the fluorescence emission spectra of BD-CsA (blue circles) and NBD-CsA (red squares), with excitation at 460 nm.