Supplementary data

Figure 1. Cellular accumulation of ICG and Bodipy-verapamil in MDCK-MDR1 (MDR1) and MDCK-CT (CT) cells. Shown is the cell protein concentration-normalized NIR fluorescence of 1 μM ICG (A) or 1 μM Bodipy-verepamil (B) in the presence and the absence of the MRPs and MDR1 inhibitors MK-571 (50 μM) and cyclosporine A (CsA; 10 μM), respectively, following 1 h incubation. Results in A and B are presented as means ± SD. au, arbitrary units. A. [a] Significantly different from DMSO-treated cells, $P < 0.01$; [b] significantly different from DMSO-treated cells, $P < 0.05$. B. [a] Significantly different from DMSO -treated and from MK-571-treated cells, $P < 0.01$.

Fig. 2. Scatter chart of bidirectional ICG transfer (present as percent of initial ICG concentration in the donor side) in DMSO-treated MDCK-MDR1 cells (A), verapamil-treated MDCK-MDR1 cells (B), and MDCK-CT cells (C). Results are presented as means ± SEM.

Figure 3. Membrane ATPase studies with ICG. ATPase activity was evaluated in P-gp, BCRP and MRP2 membranes. Values represent the mean ± SD of individual experiments, in duplicates, as suggested by the ATPase activity kit manufacturer.

Figure 4. Cellular accumulation of ICG and Bodipy-prazosin in MDCK-BCRP (BCRP) and MDCK control cells (CT). A. Cell protein concentration-normalized NIR fluorescence of 1 μM ICG in MDCK-BCRP and MDCK-CT cells in the presence and the absence of the BCRP inhibitor fumitremorgin C (FTC; 10 μM) following 1 h incubation. B. Bodipy-prazosin accumulation under the conditions described for ICG. C. BCRP protein concentrations in MDCK-BCRP cells and in MDCK control cells. Also shown is the concentration of the reference protein β-actin. Results in A and B are presented as means ± SD. au, arbitrary units. [a]

Significantly different from MDCK control cells, $P < 0.01$; [b] Significantly different from DMSO-treated cells, $P < 0.01$.

Fig. 1

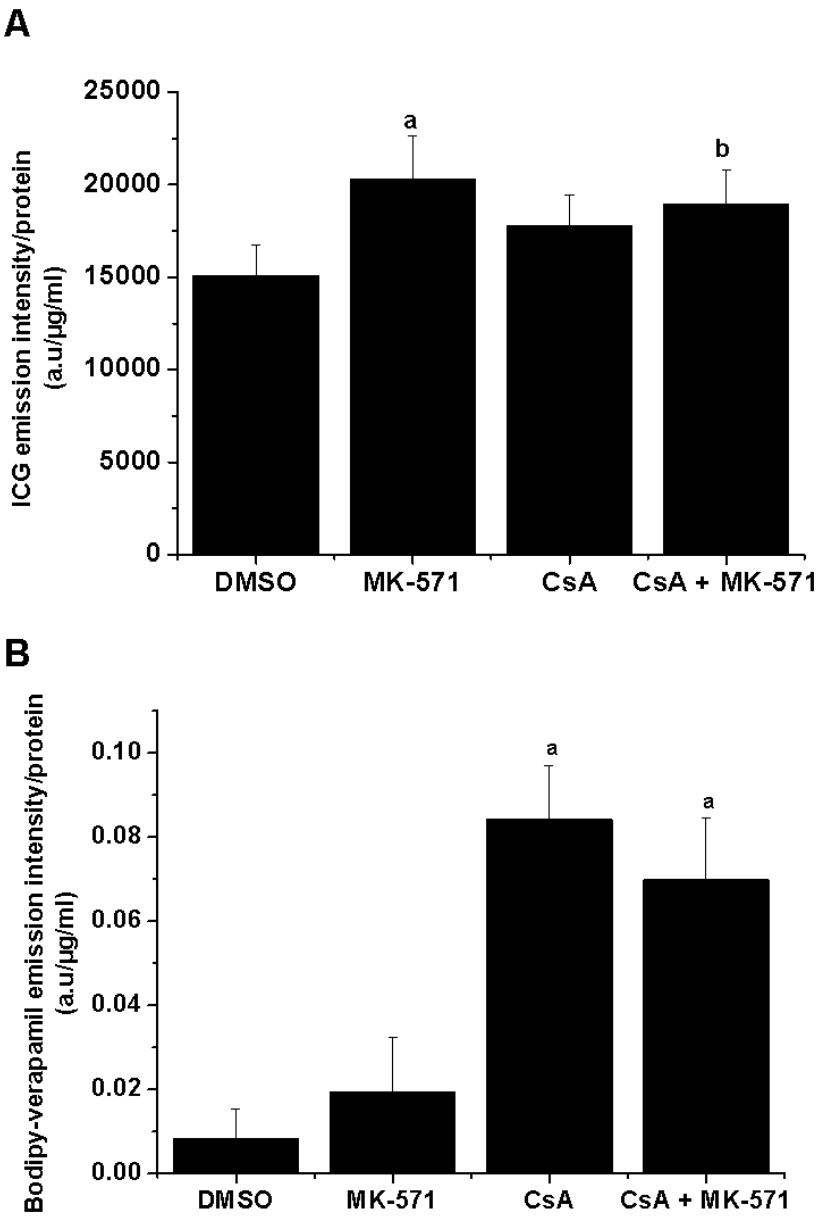

A
ICG emission intensity/protein (a.u/µg/ml)
25000
20000
15000
10000
5000
0
a
b
DMSO
MK-571
CsA
CsA + MK-571
B
Bodipy-verapamil emission intensity/protein (a.u/µg/ml)
0.10
0.08
0.06
0.04
0.02
0.00
a
a
DMSO
MK-571
CsA
CsA + MK-571

Fig. 2

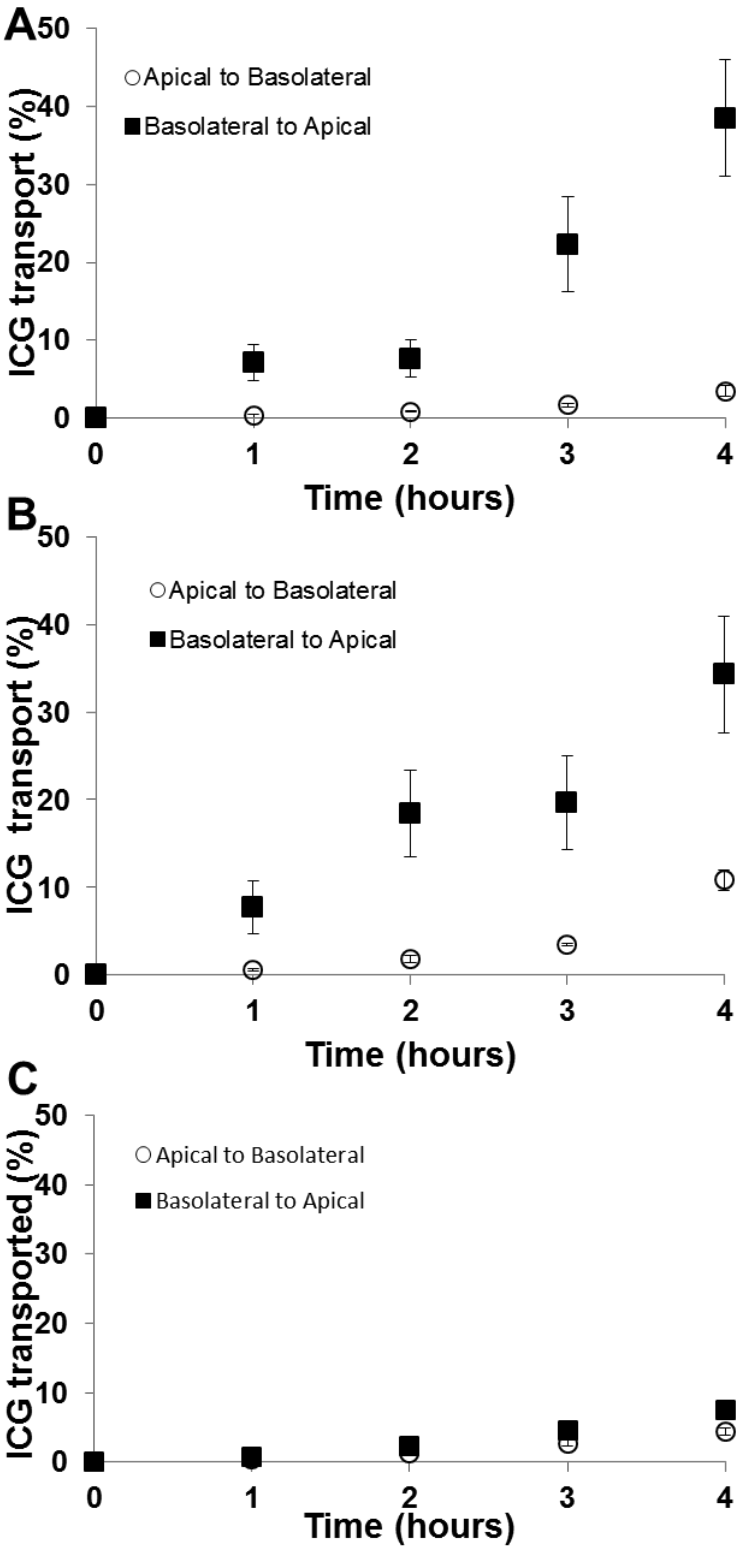

A
Apical to Basolateral
Basolateral to Apical
ICG transport (%)
0
10
20
30
40
50
0
1
2
3
4
Time (hours)
B
Apical to Basolateral
Basolateral to Apical
ICG transport (%)
0
10
20
30
40
50
0
1
2
3
4
Time (hours)
C
Apical to Basolateral
Basolateral to Apical
ICG transported (%)
0
10
20
30
40
50
0
1
2
3
4
Time (hours)

Fig. 3

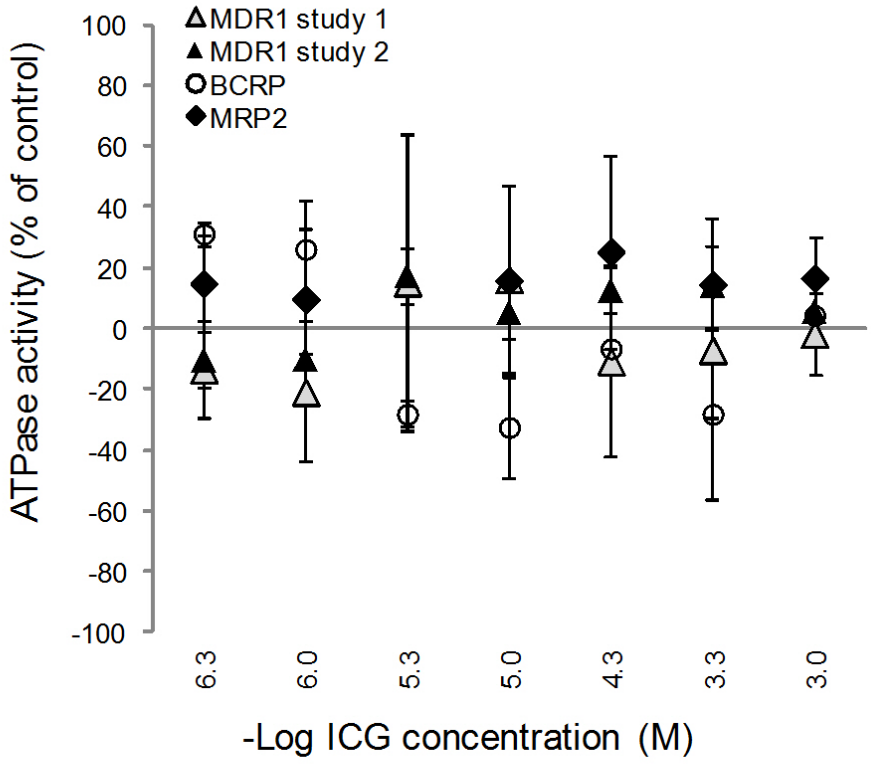
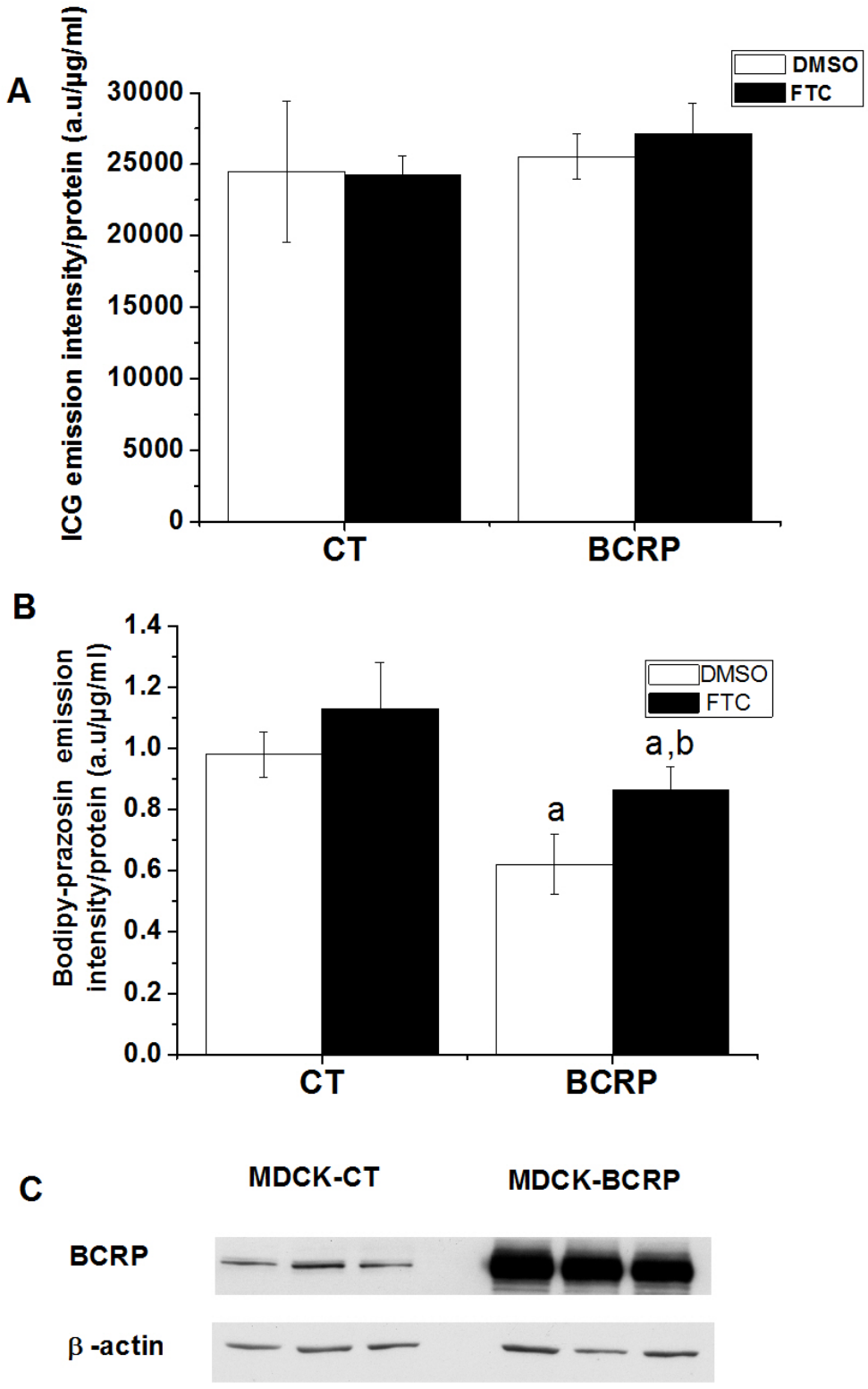

MDR1 study 1
MDR1 study 2
BCRP
MRP2
ATPase activity (% of control)
100
80
60
40
20
0
-20
-40
-60
-80
-100
6.3
6.0
5.3
5.0
4.3
3.3
3.0
-Log ICG concentration (M)

Fig. 4


A
ICG emission intensity/protein (a.u/µg/ml)
30000
25000
20000
15000
10000
5000
0
DMSO
FTC
CT
BCRP
B
Bodipy-prazosin emission intensity/protein (a.u/µg/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
DMSO
FTC
a
a,b
CT
BCRP
C
MDCK-CT
MDCK-BCRP
BCRP
β -actin