

Supporting information

Comparative hepatic and intestinal efflux transport of statins

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Supplementary table S1: The characteristic multiple reaction monitoring (MRM) transitions of the analytes and internal standards.

| Analyte | Analyte mass-to-charge ratios (m/z) | Internal standard (concentration in stop solution) | Internal standard mass-to-charge ratios (m/z) | Ionization (+/-) | Limit of quantification (analyte) |
|-------------------------------|-------------------------------------|--|---|-------------------|-----------------------------------|
| Atorvastatin | 559.1-440.1 | Atorvastatin-d5 (25 ng/ml) | 564.1-445.1 | Positive | 0.077 nM |
| 2-hydroxyatorvastatin | 575.1-440.1 | 2-hydroxyatorvastatin-d5 (25 ng/ml) | 580.1-445.1 | Positive | 0.185 nM |
| 2-hydroxyatorvastatin lactone | 557.1-448.1 | 2-hydroxyatorvastatin lactone-d5 (25 ng/ml) | 562.1-453.1 | Positive | 0.741 nM |
| 4-hydroxyatorvastatin | 575.1-440.1 | 4-hydroxyatorvastatin-d5 (25 ng/ml) | 580.1-445.1 | Positive | 0.167 nM |
| 4-hydroxyatorvastatin lactone | 557.1-448.1 | 4-hydroxyatorvastatin lactone-d5 (25 ng/ml) | 562.1-453.1 | Positive | 0.741 nM |
| Fluvastatin (racemic) | 410.1-348.1 | Fluvastatin-d8 (25 ng/ml) | 418.1-356.1 | Negative | 0.226 nM |
| 3R,5S-fluvastatin | 410.1-348.1 | Fluvastatin-d8 (25 ng/ml) | 418.1-356.1 | Negative | 0.226 nM |
| 3S,5R-fluvastatin | 410.1-348.1 | Fluvastatin-d8 (25 ng/ml) | 418.1-356.1 | Negative | 0.226 nM |
| Pitavastatin | 422.1-290.1 | Pitavastatin-d5 (25 ng/ml) | 427.1-295.1 | Positive | 0.3 nM |
| Pravastatin | 442.1-269.1 | Pravastatin-d9 (25 ng/ml) | 451.1-269.1 | Positive | 0.29 nM |
| Rosuvastatin | 482.1-258.1 | Rosuvastatin-d6 (25 ng/ml) | 488.1-264.1 | Positive | 0.29 nM |
| Simvastatin acid | 437.1-303.1 | Simvastatin acid-d6 (25 ng/ml) | 443.1-303.1 | Positive | 0.293 nM |
| Estradiol-17-glucuronide | 471-199 | Tauroursodeoxycholic acid -d5 (20 ng/ml) | 503-80 | Positive/Negative | N.D. |
| N-methyl-quinidine | 339-58 | Quinine-d3 (25 ng/ml) | 328-79 | Positive | N.D. |

Supplementary table S2: Summary of screening results. The mean and SD of uptake in the presence (+ATP) and absence of ATP (-ATP), ATP-dependent transport, and ratio of statin uptake between +ATP and -ATP from each transport-statin combination were obtained from a single experiment, performed with triplicate samples. One-way ANOVA was performed to evaluate the transport rates and the uptake ratio compared to control. * p < 0.05, ** p < 0.01.

| Statin | Transporter | +ATP | | -ATP | | ATP-dependent transport | | Uptake ratio of +ATP / -ATP | | |
|-------------------------|-------------|----------------|------|----------------|------|-------------------------|------|-----------------------------|------|------|
| | | (pmol/m in/mg) | SD | (pmol/m in/mg) | SD | (pmol/min/mg) | SD | * | SD | * |
| 10 µM atorvastatin | BCRP | 10.32 | 0.66 | 6.42 | 1.24 | 3.90 | 1.41 | * | 1.61 | 0.33 |
| | MRP2 | 7.61 | 0.45 | 5.04 | 0.46 | 2.57 | 0.64 | | 1.51 | 0.16 |
| | MRP3 | 12.28 | 1.35 | 5.53 | 1.30 | 6.75 | 1.87 | ** | 2.22 | 0.58 |
| | MRP4 | 6.45 | 1.23 | 4.64 | 0.53 | 1.81 | 1.34 | | 1.39 | 0.31 |
| | MRP8 | 7.56 | 0.39 | 6.30 | 0.41 | 1.26 | 0.57 | | 1.20 | 0.10 |
| | P-gp | 12.48 | 1.75 | 4.07 | 0.57 | 8.41 | 1.84 | ** | 3.06 | 0.60 |
| | Control | 5.22 | 1.04 | 4.03 | 0.24 | 1.19 | 1.07 | | 1.30 | 0.27 |
| 10 µM 3R,5S-fluvastatin | BCRP | 21.84 | 2.41 | 5.75 | 1.21 | 16.09 | 2.70 | ** | 3.80 | 0.91 |
| | MRP2 | 8.21 | 0.27 | 6.46 | 2.58 | 1.75 | 2.59 | | 1.27 | 0.51 |
| | MRP3 | 11.08 | 0.44 | 5.24 | 1.43 | 5.84 | 1.49 | * | 2.12 | 0.58 |
| | MRP4 | 8.58 | 0.75 | 3.68 | 0.25 | 4.90 | 0.79 | | 2.33 | 0.26 |
| | MRP8 | 10.83 | 0.50 | 8.01 | 1.05 | 2.82 | 1.16 | | 1.35 | 0.19 |
| | P-gp | 12.16 | 0.13 | 4.28 | 0.50 | 7.89 | 0.52 | ** | 2.84 | 0.34 |
| | Control | 7.16 | 0.26 | 5.47 | 1.34 | 1.70 | 1.36 | | 1.31 | 0.32 |
| 10 µM 3S,5R-fluvastatin | BCRP | 16.68 | 5.21 | 5.40 | 2.13 | 11.28 | 5.63 | ** | 3.09 | 1.56 |
| | MRP2 | 3.99 | 0.69 | 2.81 | 2.08 | 1.18 | 2.19 | | 1.42 | 1.08 |
| | MRP3 | 8.91 | 2.31 | 2.81 | 0.51 | 6.10 | 2.37 | * | 3.17 | 1.00 |
| | MRP4 | 6.51 | 1.05 | 2.08 | 0.43 | 4.43 | 1.14 | | 3.13 | 0.82 |
| | MRP8 | 6.43 | 1.44 | 3.34 | 0.75 | 3.09 | 1.63 | | 1.92 | 0.61 |
| | P-gp | 8.45 | 1.52 | 3.58 | 0.99 | 4.87 | 1.82 | | 2.36 | 0.78 |
| | Control | 4.67 | 2.06 | 3.89 | 0.80 | 0.78 | 2.21 | | 1.20 | 0.58 |
| 10 µM pitavastatin | BCRP | 9.93 | 1.24 | 2.17 | 0.14 | 7.76 | 1.25 | ** | 4.57 | 0.64 |
| | MRP2 | 2.61 | 0.34 | 1.86 | 0.06 | 0.75 | 0.35 | | 1.40 | 0.19 |
| | MRP3 | 3.44 | 0.24 | 1.42 | 0.31 | 2.02 | 0.40 | | 2.43 | 0.56 |
| | MRP4 | 3.06 | 0.39 | 1.76 | 0.72 | 1.30 | 0.82 | | 1.74 | 0.75 |
| | MRP8 | 4.54 | 1.17 | 2.54 | 0.31 | 2.00 | 1.21 | | 1.79 | 0.51 |
| | P-gp | 5.79 | 0.61 | 1.78 | 0.52 | 4.01 | 0.80 | * | 3.25 | 1.01 |
| | Control | 3.67 | 1.10 | 2.11 | 0.22 | 1.56 | 1.12 | | 1.74 | 0.55 |
| 10 µM pravastatin | BCRP | 2.25 | 0.03 | 1.10 | 0.22 | 1.15 | 0.23 | | 2.04 | 0.42 |
| | MRP2 | 1.77 | 0.22 | 1.02 | 0.57 | 0.75 | 0.61 | | 1.73 | 0.99 |
| | MRP3 | 4.19 | 0.02 | 2.29 | 1.73 | 1.90 | 1.73 | | 1.83 | 1.38 |
| | MRP4 | 2.81 | 0.98 | 1.21 | 0.18 | 1.60 | 1.00 | | 2.33 | 0.89 |
| | MRP8 | 1.80 | 0.59 | 1.36 | 0.69 | 0.44 | 0.91 | | 1.32 | 0.80 |
| | P-gp | 1.64 | 0.07 | 0.98 | 0.39 | 0.66 | 0.40 | | 1.67 | 0.68 |
| | Control | 1.31 | 0.29 | 0.84 | 0.30 | 0.47 | 0.42 | | 1.55 | 0.65 |
| 10 µM rosuvastatin | BCRP | 23.19 | 2.93 | 2.78 | 0.95 | 20.42 | 3.08 | ** | 8.35 | 3.05 |
| | MRP2 | 1.80 | 0.23 | 1.84 | 0.49 | -0.05 | 0.55 | | 0.97 | 0.29 |
| | MRP3 | 2.88 | 0.66 | 2.29 | 0.24 | 0.59 | 0.71 | | 1.26 | 0.32 |
| | MRP4 | 5.53 | 3.72 | 2.14 | 0.49 | 3.39 | 3.75 | | 2.59 | 1.84 |
| | MRP8 | 5.73 | 3.68 | 2.15 | 0.42 | 3.59 | 3.71 | | 2.67 | 1.79 |
| | P-gp | 3.65 | 0.78 | 1.79 | 0.29 | 1.87 | 0.83 | | 2.04 | 0.55 |
| | Control | 3.07 | 0.24 | 2.09 | 0.41 | 0.98 | 0.48 | | 1.47 | 0.31 |
| 1 µM simvastatin | BCRP | 0.22 | 0.04 | 0.15 | 0.01 | 0.06 | 0.04 | | 1.40 | 0.27 |
| | MRP2 | 0.16 | 0.02 | 0.08 | 0.08 | 0.09 | 0.08 | | 2.08 | 2.09 |
| | MRP3 | 0.17 | 0.05 | 0.21 | 0.04 | -0.04 | 0.07 | | 0.79 | 0.29 |
| | MRP4 | 0.16 | 0.03 | 0.16 | 0.02 | 0.00 | 0.04 | | 0.99 | 0.22 |
| | MRP8 | 0.31 | 0.11 | 0.26 | 0.09 | 0.06 | 0.14 | | 1.23 | 0.60 |
| | P-gp | 0.17 | 0.05 | 0.20 | 0.05 | -0.04 | 0.07 | | 0.82 | 0.31 |
| | Control | 0.20 | 0.04 | 0.13 | 0.03 | 0.07 | 0.05 | | 1.53 | 0.46 |

Supplementary table S3: Summary of atorvastatin metabolite transport. The mean and SD of uptake in the presence (+ATP) and absence of ATP (-ATP), ATP-dependent transport and ratio between +ATP and -ATP from each transport-metabolite combination were obtained from a single experiment, performed with triplicate samples. One-way ANOVA was performed to evaluate the transport rates and the uptake ratio compared to control. * p < 0.05, ** p < 0.01

| Metabolite | Transporter | +ATP | | -ATP | | ATP-dependent transport | | Uptake ratio of +ATP / -ATP | |
|-------------------|-------------|----------------|------|----------------|------|-------------------------|------|-----------------------------|---------|
| | | (pmol/m in/mg) | SD | (pmol/m in/mg) | SD | (pmol/min/mg) | SD | | SD |
| 10 µM 2OH-ATV | BCRP | 16.52 | 4.14 | 4.72 | 0.22 | 11.80 | 4.15 | ** 3.50 | 0.89 * |
| | MRP2 | 8.68 | 1.98 | 3.74 | 0.67 | 4.94 | 2.09 | 2.32 | 0.67 |
| | MRP3 | 11.47 | 1.47 | 3.47 | 0.51 | 8.00 | 1.55 | * 3.30 | 0.64 * |
| | MRP4 | 4.69 | 2.36 | 3.08 | 0.08 | 1.61 | 2.36 | 1.52 | 0.77 |
| | P-gp | 8.42 | 2.64 | 2.63 | 0.11 | 5.79 | 2.65 | 3.20 | 1.01 |
| | Control | 4.13 | 1.39 | 2.18 | 0.14 | 1.95 | 1.39 | 1.90 | 0.65 |
| 10 µM 4OH-ATV | BCRP | 17.58 | 1.87 | 5.96 | 2.65 | 11.61 | 3.24 | ** 2.95 | 1.34 |
| | MRP2 | 6.18 | 1.71 | 3.05 | 0.50 | 3.13 | 1.78 | 2.02 | 0.65 |
| | MRP3 | 11.13 | 0.96 | 2.53 | 0.82 | 8.61 | 1.26 | ** 4.41 | 1.48 ** |
| | MRP4 | 5.15 | 0.27 | 3.62 | 1.59 | 1.54 | 1.61 | 1.42 | 0.63 |
| | P-gp | 10.64 | 1.41 | 2.70 | 0.62 | 7.95 | 1.54 | ** 3.95 | 1.05 ** |
| | Control | 2.16 | 0.45 | 1.73 | 0.92 | 0.44 | 1.02 | 1.25 | 0.71 |
| 10 µM 2OH-ATV lac | BCRP | 6.99 | 1.60 | 8.76 | 0.80 | -1.77 | 1.79 | 0.80 | 0.20 |
| | MRP2 | 9.93 | 2.18 | 10.93 | 0.81 | -1.00 | 2.32 | 0.91 | 0.21 |
| | MRP3 | 9.38 | 2.21 | 10.78 | 0.19 | -1.40 | 2.21 | 0.87 | 0.21 |
| | MRP4 | 7.62 | 0.60 | 10.51 | 2.25 | -2.89 | 2.33 | 0.73 | 0.17 |
| | P-gp | 8.96 | 2.67 | 9.62 | 1.13 | -0.66 | 2.90 | 0.93 | 0.30 |
| | Control | 5.53 | 1.73 | 6.57 | 1.29 | -1.04 | 2.16 | 0.84 | 0.31 |
| 10 µM 4OH-ATV lac | BCRP | 3.94 | 0.56 | 5.19 | 0.91 | -1.24 | 1.07 | 0.76 | 0.17 |
| | MRP2 | 5.58 | 1.77 | 5.02 | 0.42 | 0.56 | 1.82 | 1.11 | 0.36 |
| | MRP3 | 4.90 | 0.65 | 6.03 | 1.69 | -1.13 | 1.81 | 0.81 | 0.25 |
| | MRP4 | 4.48 | 0.57 | 5.20 | 0.97 | -0.72 | 1.13 | 0.86 | 0.20 |
| | P-gp | 4.38 | 0.63 | 4.96 | 0.42 | -0.58 | 0.76 | 0.88 | 0.15 |
| | Control | 4.03 | 0.57 | 4.29 | 0.88 | -0.26 | 1.05 | 0.94 | 0.23 |

Supplementary table S4: Summary of screening results. The mean and SD of uptake in the presence (+ATP) and absence of ATP (-ATP), ATP-dependent transport and ratio between +ATP and -ATP from each transport-statin combination studied were obtained from a single experiment, performed with triplicate samples. Two-way ANOVA was performed to evaluate the transport rate and uptake ratio compared to control. * p < 0.05, ** p < 0.01

| Statin | Transporter | ATP-dependent transport at 5 min (pmol/min/mg) | | Ratio of +ATP / -ATP at 5min | | ATP-dep. transport at 10 min | | Ratio at 10 min | | ATP-dep. transport at 15 min | | Ratio at 15 min | |
|-------------------|-------------|--|------|------------------------------|------|------------------------------|----|-----------------|------|------------------------------|------|-----------------|----|
| | | SD | | SD | | SD | | SD | | SD | | SD | |
| Atorvastatin | BCRP | 196.6 | 54.5 | ** | 2.53 | 0.43 | ** | 121.1 | 31.7 | ** | 2.70 | 0.58 | * |
| | MRP2 | 66.7 | 19.1 | | 1.61 | 0.21 | | 90.7 | 13.3 | * | 2.09 | 0.27 | |
| | MRP3 | 143.2 | 15.7 | * | 2.16 | 0.23 | * | 131.8 | 16.1 | ** | 2.57 | 0.21 | * |
| | MRP4 | 43.8 | 5.8 | | 1.49 | 0.09 | | 53.4 | 3.8 | | 1.75 | 0.08 | |
| | P-gp | 186.7 | 34.0 | ** | 2.46 | 0.36 | ** | 108.7 | 52.0 | ** | 3.12 | 1.03 | ** |
| | Control | 65.5 | 59.5 | | 1.45 | 0.41 | | 27.0 | 12.2 | | 1.27 | 0.13 | |
| 3R,5S-fluvastatin | BCRP | 344.9 | 92.8 | ** | 4.05 | 0.85 | ** | 408.3 | 39.9 | ** | 4.10 | 0.55 | ** |
| | MRP2 | 84.8 | 9.5 | * | 2.33 | 0.17 | ** | 118.9 | 10.8 | ** | 2.60 | 0.25 | ** |
| | MRP3 | 177.6 | 39.7 | ** | 3.06 | 0.63 | ** | 320.3 | 52.4 | ** | 3.81 | 0.65 | ** |
| | MRP4 | 91.2 | 22.1 | * | 2.50 | 0.47 | ** | 195.2 | 27.3 | ** | 2.76 | 0.30 | ** |
| | MRP8 | 136.7 | 11.3 | ** | 2.03 | 0.12 | * | 10.8 | 8.6 | | 1.36 | 0.30 | |
| | P-gp | 195.1 | 23.7 | ** | 2.96 | 0.32 | ** | 204.3 | 10.5 | ** | 2.78 | 0.12 | ** |
| | Control | 4.3 | 4.8 | | 1.14 | 0.17 | | -1.8 | 5.4 | | 0.97 | 0.10 | |
| 3S,5R-fluvastatin | BCRP | 326.3 | 29.1 | ** | 5.38 | 2.05 | ** | 409.6 | 41.5 | ** | 4.49 | 0.48 | ** |
| | MRP2 | 0.1 | 13.2 | | 1.00 | 0.25 | | 79.8 | 10.2 | ** | 2.79 | 0.31 | ** |
| | MRP3 | 128.5 | 25.6 | ** | 3.07 | 0.75 | * | 157.3 | 26.8 | ** | 3.47 | 0.50 | ** |
| | MRP4 | 49.6 | 9.5 | | 2.16 | 0.45 | | 99.1 | 13.1 | ** | 3.56 | 0.82 | ** |
| | MRP8 | 48.3 | 14.6 | | 1.71 | 0.25 | | 86.0 | 10.4 | ** | 2.04 | 0.13 | |
| | P-gp | 124.9 | 18.6 | ** | 2.74 | 0.33 | | 182.2 | 12.8 | ** | 3.08 | 0.32 | ** |
| | Control | 22.4 | 19.6 | | 1.27 | 0.27 | | 11.1 | 6.4 | | 1.42 | 0.33 | |

Supplementary table S5: Summary of screening results. The mean and SD of uptake in the presence (+ATP) and absence of ATP (-ATP), ATP-dependent transport and ratio between +ATP and -ATP from each transport-statin combination studied were obtained from a single experiment, performed with triplicate samples. Two-way ANOVA was performed to evaluate the transport rate and uptake ratio compared to control. * p < 0.05, ** p < 0.01

| Statin | Transporter | ATP-dependent transport at 5 min (pmol/min/mg) | SD | Ratio of +ATP / -ATP at 5min | | ATP-dep. transport at 10 min | SD | Ratio at 10 min | SD | ATP-dep. transport at 15 min | SD | Ratio at 15 min | SD |
|--------------|-------------|---|------|------------------------------|-------|------------------------------|----|-----------------|------|------------------------------|-------|-----------------|----|
| | | | | | | | | | | | | | |
| Pitavastatin | BCRP | 357.6 | 18.0 | ** | 5.24 | 0.58 | ** | 348.8 | 58.5 | ** | 5.71 | 0.80 | ** |
| | MRP3 | 68.2 | 26.8 | * | 1.92 | 0.46 | | 78.9 | 40.5 | | 1.91 | 0.50 | |
| | MRP8 | 16.7 | 27.0 | | 1.16 | 0.28 | | 51.8 | 14.9 | | 1.68 | 0.23 | |
| | P-gp | 100.1 | 15.4 | ** | 3.63 | 1.12 | ** | 74.2 | 7.5 | | 2.82 | 0.46 | |
| | Control – A | 31.6 | 7.6 | | 1.72 | 0.20 | | 38.1 | 12.7 | | 1.82 | 0.28 | |
| | MRP2 | 21.9 | 11.5 | | 1.8 | 0.5 | | 40.5 | 21.5 | | 2.4 | 0.9 | |
| | Control – B | 6.2 | 4.8 | | 1.3 | 0.2 | | 19.5 | 7.2 | | 1.8 | 0.4 | |
| Pravastatin | BCRP | 14.1 | 15.4 | | 1.30 | 0.33 | | 17.2 | 8.0 | | 1.54 | 0.32 | |
| | MRP2 | -5.7 | 17.2 | | 0.89 | 0.32 | | 8.6 | 27.0 | | 1.18 | 0.58 | |
| | MRP3 | 39.5 | 15.5 | ** | 2.24 | 0.53 | ** | 32.4 | 19.9 | | 1.91 | 0.60 | |
| | MRP4 | 0.6 | 11.0 | | 1.02 | 0.30 | | 13.9 | 14.9 | | 1.48 | 0.63 | |
| | Control | -1.6 | 7.9 | | 0.95 | 0.25 | | 5.0 | 12.4 | | 1.14 | 0.39 | |
| Rosuvastatin | BCRP | 343.1 | 41.4 | ** | 18.49 | 2.76 | ** | 238.9 | 17.3 | ** | 15.46 | 4.08 | ** |
| | MRP2 | -0.6 | 4.4 | | 0.98 | 0.14 | | 5.6 | 5.7 | | 1.33 | 0.36 | |
| | MRP4 | 20.7 | 10.3 | | 1.98 | 0.66 | | 25.7 | 4.2 | * | 2.84 | 0.55 | |
| | MRP8 | 2.9 | 16.4 | | 1.08 | 0.50 | | 4.2 | 4.7 | | 1.28 | 0.35 | |
| | P-gp | 3.9 | 5.0 | | 1.14 | 0.18 | | 21.7 | 16.6 | | 1.85 | 1.14 | |
| | Control | -2.4 | 7.9 | | 0.86 | 0.41 | | 5.6 | 7.0 | | 1.46 | 0.61 | |

Supplementary table S6: The uptake ratios (the ratio between +ATP and -ATP) and their standard deviation of studied statins in concentration-dependent transport studies at 12 µM statin concentration. N indicates the number of independent experiments carried out with triplicate samples.

| Statin | Transporter | Uptake ratio | SD | N |
|--------------|-------------|--------------|------|-------|
| Atorvastatin | BCRP | 2.34 | 0.21 | n = 3 |
| Atorvastatin | MRP3 | 2.44 | 0.36 | n = 3 |
| Atorvastatin | P-gp | 3.36 | 0.24 | n = 3 |
| Fluvastatin | BCRP | 4.62 | 0.88 | n = 3 |
| Fluvastatin | MRP2 | 1.81 | 0.44 | n = 3 |
| Fluvastatin | MRP3 | 2.37 | 0.38 | n = 3 |
| Fluvastatin | MRP4 | 2.11 | 0.34 | n = 3 |
| Fluvastatin | MRP8 | 2.02 | 0.09 | n = 3 |
| Fluvastatin | P-gp | 2.39 | 1.09 | n = 3 |
| Fluvastatin | Control | 1.26 | 0.15 | n = 3 |
| Pitavastatin | BCRP | 6.68 | 1.67 | n = 3 |
| Pitavastatin | MRP3 | 1.75 | 0.23 | n = 3 |
| Pitavastatin | P-gp | 2.18 | 0.28 | n = 3 |
| Pitavastatin | Control | 0.91 | 0.17 | n = 2 |
| Pravastatin | MRP3 | 2.19 | 0.38 | n = 4 |
| Rosuvastatin | MRP4 | 1.95 | 0.37 | n = 3 |
| Rosuvastatin | P-gp | 3.01 | 1.52 | n = 3 |

Supplementary table S7: The transporter abundance in vesicle preparations and protein-specific peptides used in proteomic measurements.

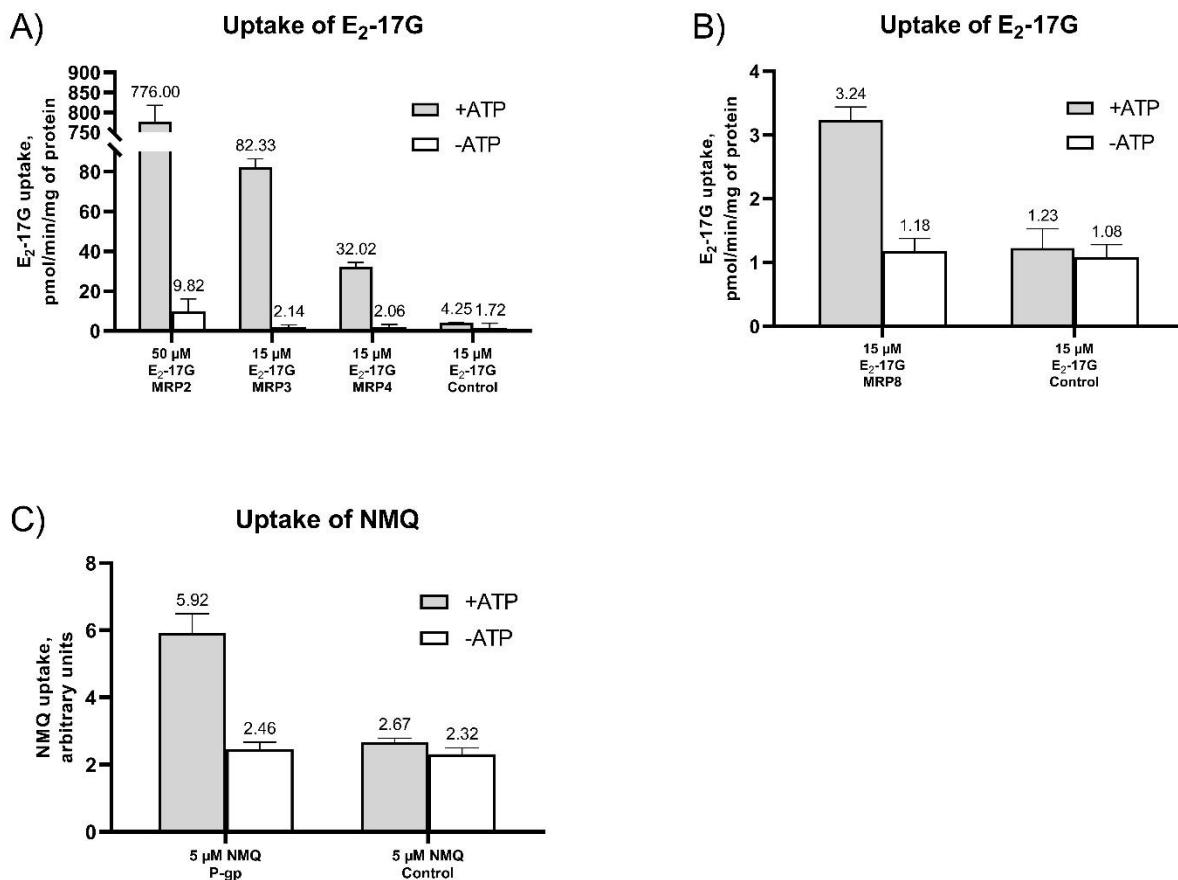
| Peptide | Protein-specific peptides | Protein expression _{in vitro} , (pmol/mg of protein) |
|---------|---------------------------|--|
| BCRP | SSLLDVLAAR | 146.08 |
| MRP2 | LTIIPQDPILFSGSLR | 85.33 |
| MRP3 | IDGLNVADIGLHDLR | 54.24 |
| MRP4 | SSLISALFR | 43.85 |
| P-gp | AGAVAEVLAIR | 59.12 |

Supplementary table S8: The transporter abundance in small intestine and liver according to previous literature and the calculated tissue-specific active efflux clearance values and fractions. The abundance data of Burt et al. 2016 was converted from pmol/10⁶ hepatocytes to fmol/mg of tissue according to the hepatocellularity measurements of Sohlenius-Sternbeck 2006^a, where in human 1 g of liver contained 139 x 10⁶ hepatocytes.

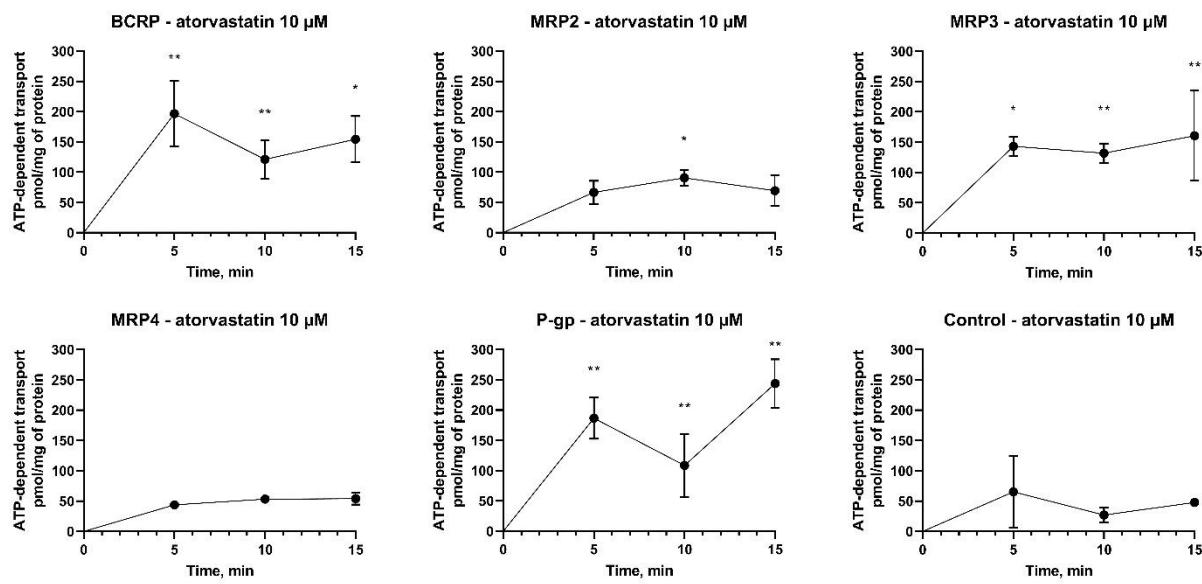
| | Drozdizk et al. 2018 Mean abundance (fmol/mg tissue) | | | Burt et al. 2016 Mean abundance (fmol/mg of tissue) | Burt et al. 2016 Mean abundance pmol/10 ⁶ hepatocytes |
|-------------|---|---------|-------|---|--|
| Transporter | Duodenum | Jejunum | Ileum | Liver | Liver |
| BCRP | 5.51 | 23.27 | 30.47 | 8.095 | 0.058 |
| MRP2 | 11.88 | 22.44 | 19.84 | 62.865 | 0.452 |
| MRP3 | 17.28 | 30.86 | 22.58 | 23.783 | 0.171 |
| MRP4 | 13.37 | 13.87 | 15.18 | 7.594 | 0.055 |
| P-gp | 7.67 | 40.21 | 70.78 | 27.816 | 0.200 |

| Statin | Adj. CL (nL/min/pmol) | CL, duodenum (nL/min/mg tissue) | CL, jejunum (nL/min/mg tissue) | CL, ileum (nL/min/mg tissue) | CL, liver (nL/min/mg tissue) |
|---------------------|--------------------------|--|---|------------------------------------|------------------------------------|
| Atorvastatin | | | | | |
| BCRP | 34.9 | 0.2 | 7.1 % | 0.7 | 8.8 % |
| MRP3 | 140.7 | 1.2 | 50.0 % | 2.1 | 26.0 % |
| P-gp | 162.9 | 1.0 | 42.8 % | 5.3 | 65.3 % |
| Total | | 2.4 | 100.0 % | 8.2 | 100.0 % |
| Fluvastatin | | | | | |
| BCRP | 338.5 | 1.6 | 32.0 % | 7.0 | 52.7 % |
| MRP2 | 71.3 | 0.6 | 11.5 % | 1.1 | 8.5 % |
| MRP3 | 199.7 | 1.7 | 32.8 % | 3.0 | 22.9 % |
| MRP4 | 122.4 | 1.0 | 19.8 % | 1.1 | 8.0 % |
| P-gp | 31.7 | 0.2 | 3.9 % | 1.0 | 7.9 % |
| Total | | 5.1 | 100.0 % | 13.2 | 100.0 % |
| Pitavastatin | | | | | |
| BCRP | 156.1 | 0.8 | 38.3 % | 3.2 | 50.2 % |
| MRP3 | 110.8 | 0.9 | 47.2 % | 1.7 | 26.2 % |
| P-gp | 45.9 | 0.3 | 14.5 % | 1.5 | 23.6 % |
| Total | | 2.0 | 100.0 % | 6.4 | 100.0 % |
| Rosuvastatin | | | | | |
| BCRP | 435.9 | 2.1 | 86.8 % | 9.0 | 90.8 % |
| MRP4 | 22.3 | 0.2 | 7.6 % | 0.2 | 2.0 % |
| P-gp | 21.8 | 0.1 | 5.6 % | 0.7 | 7.2 % |
| Total | | 2.4 | 100.0 % | 9.9 | 100.0 % |

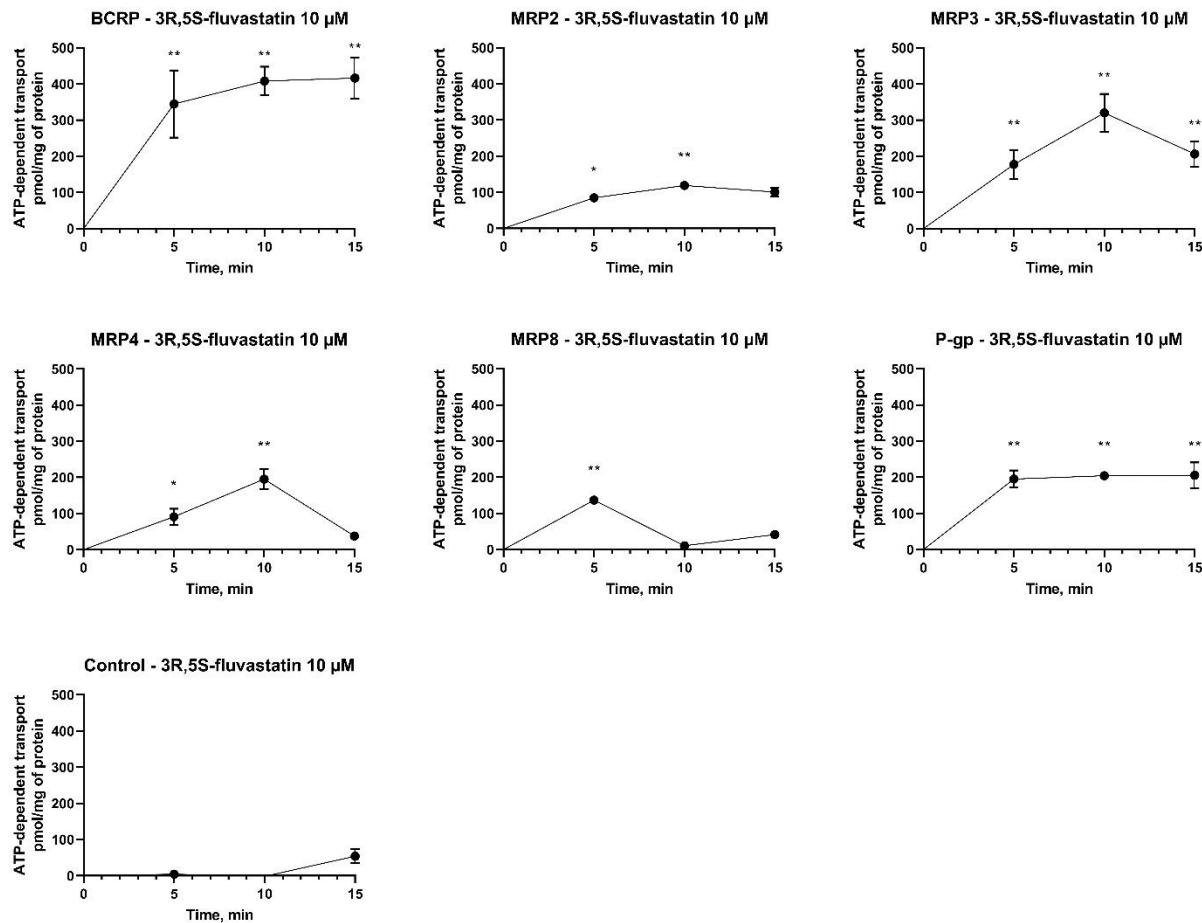
^a: Sohlenius-Sternbeck A. K. (2006). Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements. Toxicology in vitro: an international journal published in association with BIBRA, 20(8), 1582–1586.



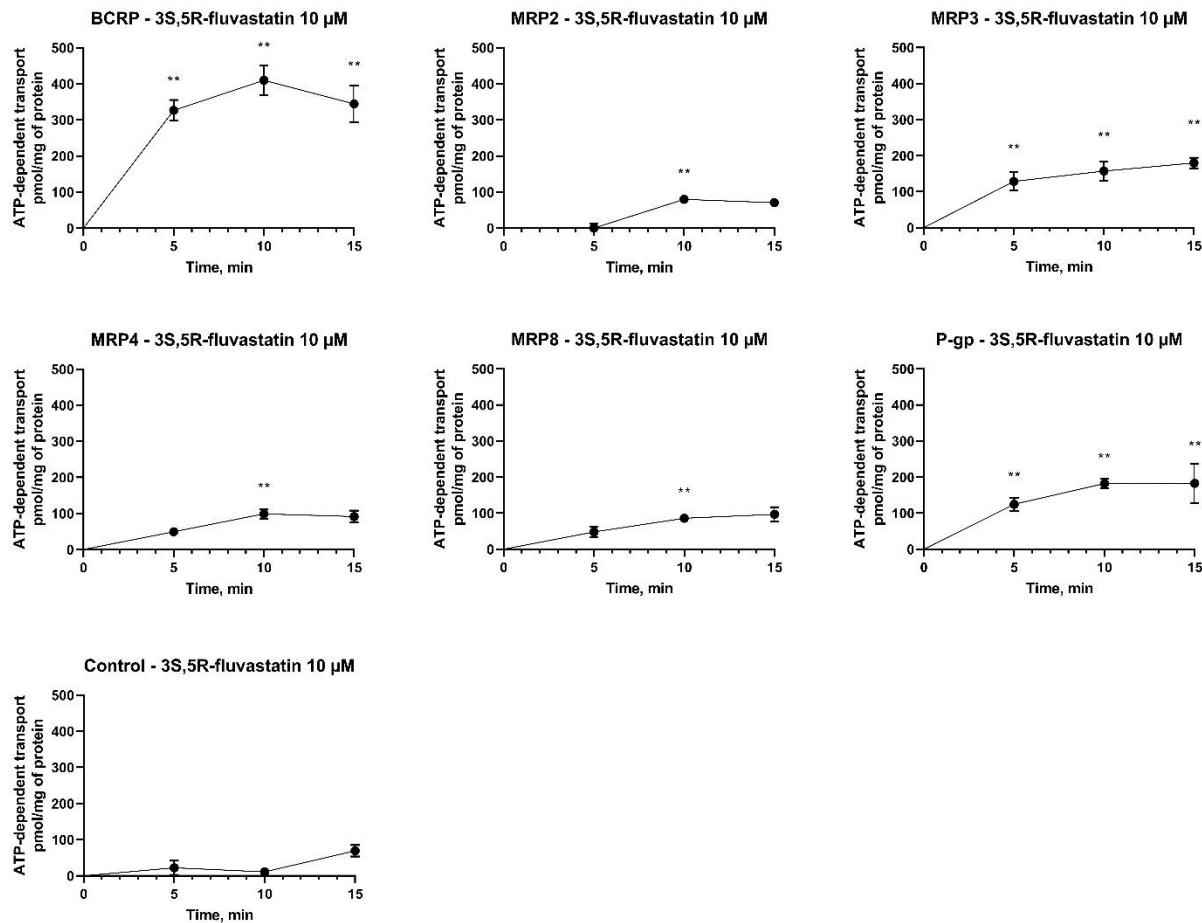
Supplementary figure S1: The transport of estradiol-17-glucuronide (E₂-17G) and N-methyl-quinidine (NMQ) in MRP2, MRP3, MRP4, MRP8, P-gp, and control vesicles. A single transport experiment performed with triplicate samples was conducted to verify the function of each membrane vesicle preparation. The amount of vesicles was 7.5 μ g in every experiment. The concentration and incubation time of E₂-17G was 50 μ M and 5 min, respectively for MRP2 vesicles, and 10 μ M and 10 minutes, respectively, for MRP3, MRP4, MRP8, and control vesicles. The concentration and incubation time of NMQ was 5 μ M and 5 min, respectively. A) The uptake of E₂-17G into the MRP2, MRP3, MRP4 and control vesicles in presence (+ATP) and absence (-ATP) of ATP is presented as mean \pm SD. B) The uptake of E₂-17G in a separate experiment into the MRP8 and control vesicles in presence (+ATP) and absence (-ATP) of ATP is presented as mean \pm SD. C) The uptake of NMQ in into the P-gp and control vesicles in presence (+ATP) and absence (-ATP) of ATP is presented as mean \pm SD. Arbitrary units of NMQ uptake stands for the ratio between the peak area of NMQ and internal standard.



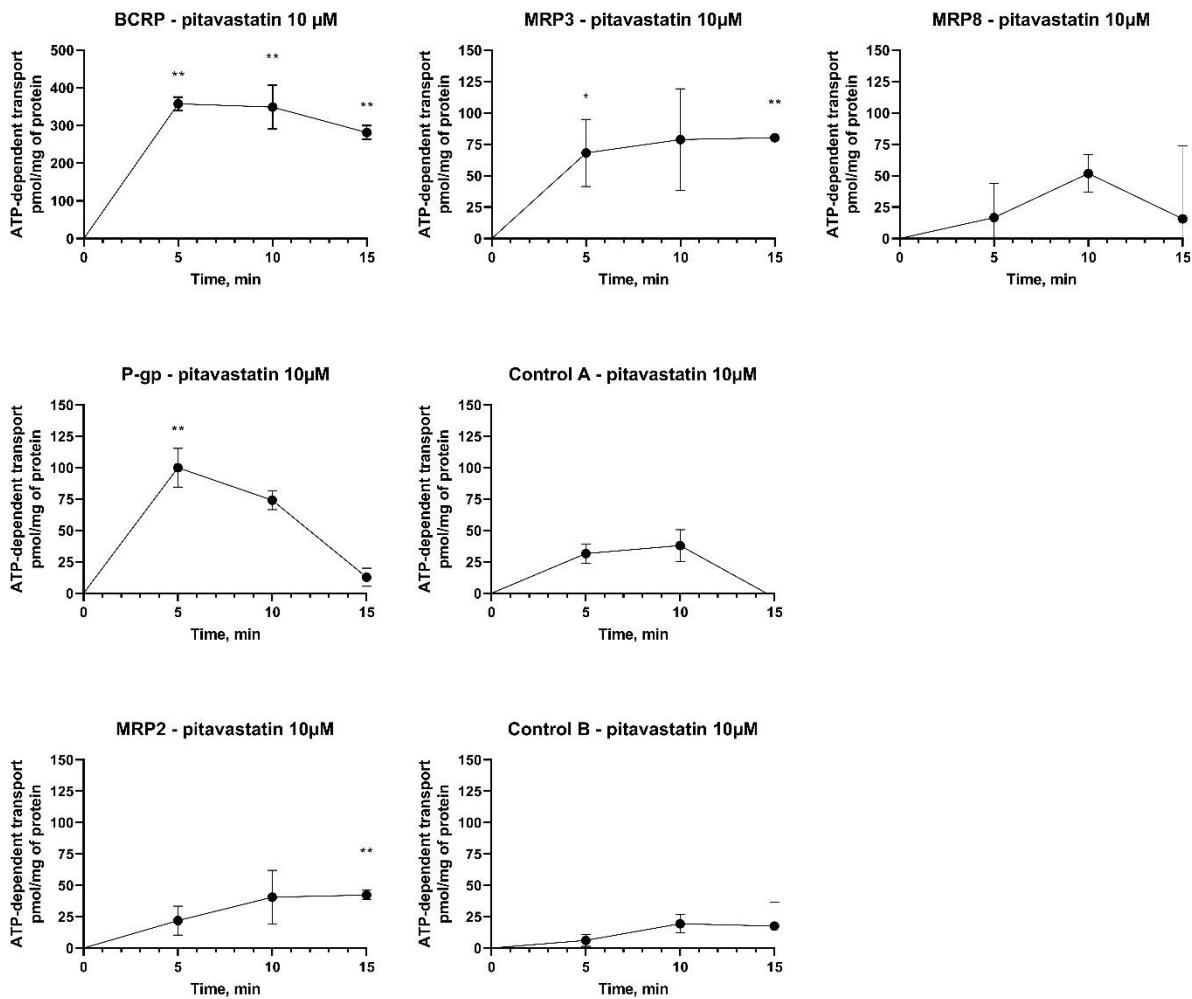
Supplementary figure S2: The time-dependent transport of atorvastatin in BCRP, MRP2, MRP3, MRP4, P-gp, and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 µM, 15 min, and 7.5 µg, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. * p < 0.05, ** p < 0.01.



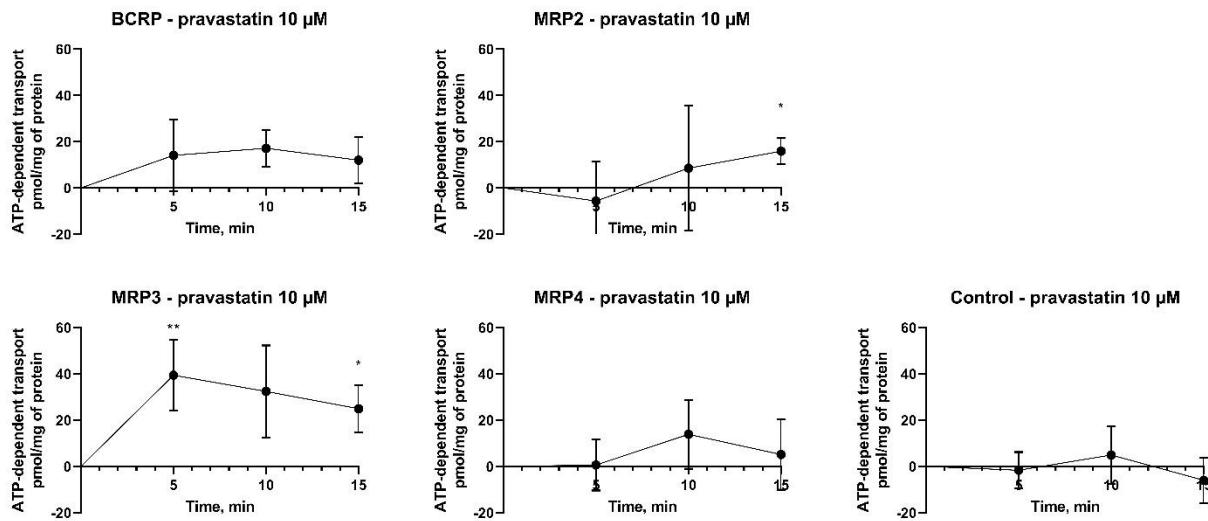
Supplementary figure S3: The time-dependent transport of 3R,5S-fluvastatin in BCRP, MRP2, MRP3, MRP4, MRP8, P-gp, and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 μ M, 15 min, and 7.5 μ g, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. * $p < 0.05$, ** $p < 0.01$.



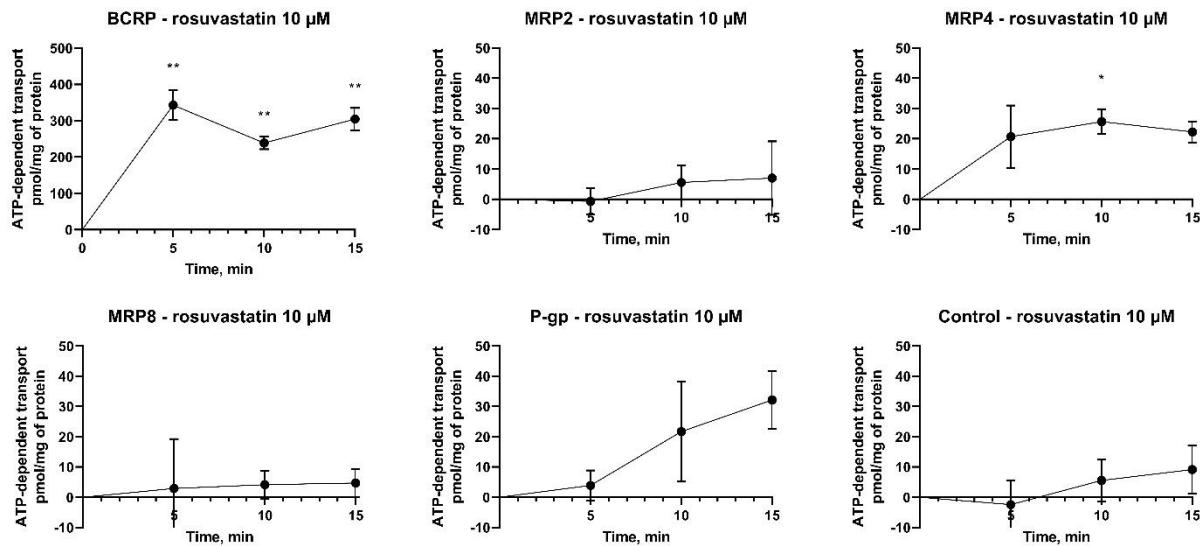
Supplementary figure S4: The time-dependent transport of 3S,5R-fluvastatin in BCRP, MRP2, MRP3, MRP4, MRP8, P-gp, and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 μ M, 15 min, and 7.5 μ g, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. * $p < 0.05$, ** $p < 0.01$.



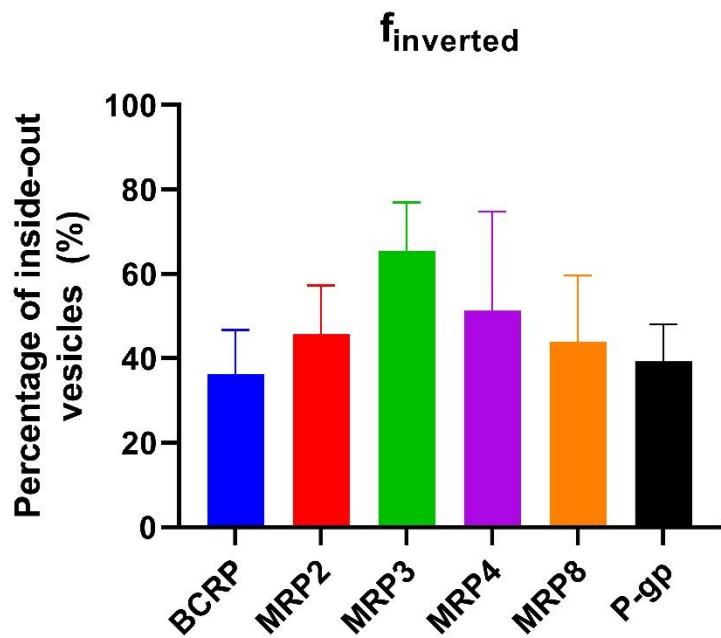
Supplementary figure S5: The time-dependent transport of pitavastatin in BCRP, MRP2, MRP3, MRP8, P-gp, and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 μ M, 15 min, and 7.5 μ g, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. Note the different y-axis of the BCRP subfigure. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. The transport in BCRP, MRP3, MRP8, and P-gp vesicles was compared to control A, and MRP2 experiment, which was performed on a separate occasion, was compared to control B. * $p < 0.05$, ** $p < 0.01$.



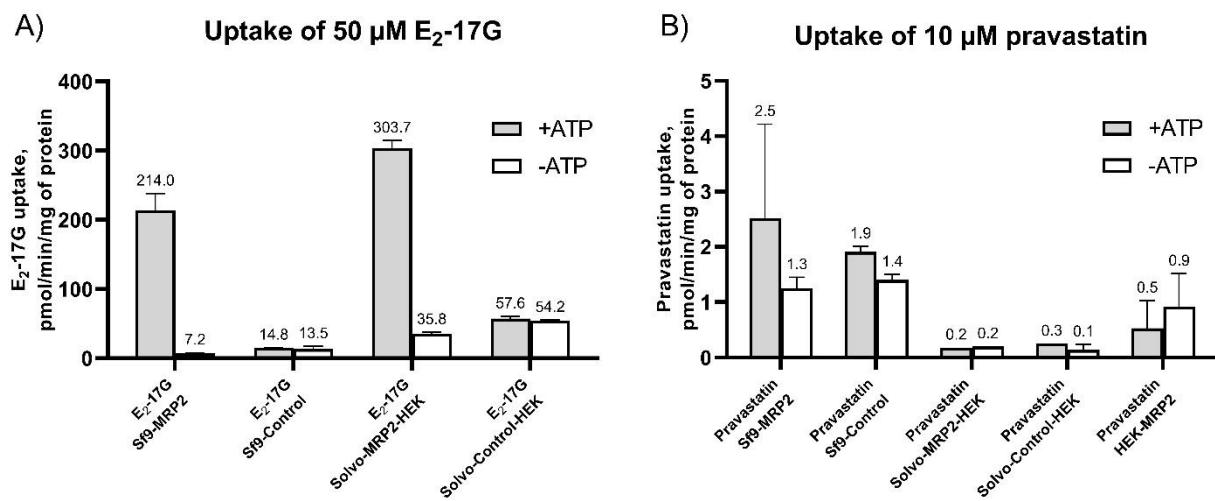
Supplementary figure S6: The time-dependent transport of pravastatin in BCRP, MRP2, MRP3, MRP4, and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 µM, 15 min, and 7.5 µg, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. * $p < 0.05$, ** $p < 0.01$



Supplementary figure S7: The time-dependent transport of rosuvastatin in BCRP, MRP2, MRP4, MRP8, P-gp and control vesicles. Substrate concentration, maximum time of incubation, and vesicle amount were 10 μ M, 15 min, and 7.5 μ g, respectively. Results are presented as mean \pm SD ATP-dependent transport obtained from a single experiment, performed with triplicate samples. Note the different y-axis of the BCRP subfigure. One-way ANOVA and uncorrected Fisher's LSD analysis were performed to evaluate the transport rate in transporter of interest compared to control. * $p < 0.05$, ** $p < 0.01$.



Supplementary figure S8: The fraction of inverted membrane vesicles (f_{inverted}) in BCRP, MRP2, MRP4, MRP8, P-gp and control vesicle preparations. Results are presented as mean \pm SD obtained from a single experiment, performed with triplicate samples.



Supplementary figure S9: The transport of 50 µM E₂-17G (panel A) and 10 µM pravastatin (panel B). Compounds of interest were incubated with membrane vesicles supplemented with 3 mM glutathione in the presence and absence of 4 mM ATP for 10 minutes. Sf9 vesicles were generated from the Sf9 insect cells expressing the human recombinant MRP2 or defective mutant of MRP3 (control) (Järvinen et al. 2019^B). Solvo-MRP2-HEK and Solvo-Control-HEK vesicles were purchased from Solvo Biotechnology (Szeged, Hungary) and HEK-MRP2 from PharmTox at the Radboud University Medical Center (PharmTox, Radboud UMC, Nijmegen, The Netherlands). Results are presented as mean ± SD uptake in the presence (+ATP) and absence (-ATP) of ATP obtained from a single experiment, performed with triplicate samples.

^B: Järvinen, E., Kidron, H., & Finel, M. (2020). Human efflux transport of testosterone, epitestosterone and other androgen glucuronides. The Journal of steroid biochemistry and molecular biology, 197, 105518.