SUPPLEMENTARY DATA

Manuscript Number: MOLPHARM-AR-2022-000568R1

Journal Name: Molecular Pharmacology

Valproic acid-induced upregulation of multidrug efflux transporter ABCG2/BCRP via PPARα-dependent mechanism in human brain endothelial cells

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Supplemental Table 1

Therapeutic plasma concentrations (reference range) of antiepileptic drugs in patients with epilepsy (Johannessen, 2004)

Antiepileptic drug	Therapeutic plasma concentration range (µM)	Doses used in the present study
Phenytoin	40-80	40µM, 80µM
Carbamazepine	15-45	21µM, 42µM
Valproic acid	300-600	300µM, 600µM
Lamotrigine	10-60	15µM, 60µM
Topiramate	15-60	15µM, 60µM
Levetiracetam	35-120	40µM, 120µM

Supplemental Table 2

Sequence of primers used for qPCR

A. Primer sequences for MDTs and B2M		
Gene	**Forward Primer**	**Reverse Primer**
ABCC1	5'-TGTGTGGGCAACTGCATCG-3'	5'-GTTGGTTTCCATTTCAGATGACA-3'
ABCC2	5'-ATATAAGAAGGCATTGACCC-3'	5'-ATCTGTAGAACACTTGACCA-3'
ABCC4	5'-GAGCTGAGAATGACGCACAG-3'	5'-TACGCTGTGTTCAAAGCCAC-3'
ABCC5	5'-GGGAGCTCTCAATGGAAGAC-3'	5'-CAGCTCTTCTTGCCACAGTC-3'
ABCG2	5'- GAAGAGTGGCTTTCTACCTT -3'	5'- GTCCCAGGATGGCGTTGA-3'
B2M	5'-GGCATTCCTGAAGCTGACAG-3'	5'-TGGATGACGTGAGTAAACCTG-3'
B. Primer sequences for molecular factor genes		
Gene	**Forward Primer**	**Reverse Primer**
PXR	5'-TGCGAGATCACCCGGAAGAC-3'	5'-ATGGGAGAAGGTAGTGTCAAAGG-3'
CAR	5'- GTGCTTAGATGCTGGCATGAGGAA-3'	5'-GGCTGGTGATGGATGAACAGATGAG-3'
AhR	5'-ACATCACCTACGCCAGTCGC-3'	5'-TCTATGCCGCTTGGAAGGAT-3'
PPARA	5'-CTATCATTTGCTGTGGAGATCG-3'	5'-AAGATATCGTCCGGGTGGTT-3'
PPARG	5'-AAGGAGAAGCTGTTGGCGGAGA-3'	5'-CAGCCCTGAAAGATGCGGATGG-3'
NRF2	5'-GAGAGCCCAGTCTTCATTGC-3'	5'-TGCTCAATGTCCTGTTGCAT-3'
NFKB1	5'-GTGAAGGCCCATCCCATGGT-3'	5'-TGTGACCAACTGAACAATAACC-3'
RELA	5'-GCAGAAAGAGGACATTGAGGTG-3'	5'-CTGCATGGAGACACGCACAGGAG-3'
CREB1	5'-ACTGTAACGGTGCCAACTCC-3'	5'-GAATGGTAGTACCCGGCTGA-3'
COX-2	5'- CCTGTGCCTGATGATTGC -3'	5'-CTGATGCGTGAAGTGCTG-3'
TP53	5'-TAACAGTTCCTGCATGGGCGGC-3'	5'-AGGACAGGCACAAACACGCACC-3'
GSK3B	5'-GGTCTATCTTAATCTGGTGCTGG-3'	5'-TGGATATAGGCTAAACTTCGGAAC-3'
JNK1	5'-TGGACTTGGAGGAGAGAACC-3'	5'-CATTGACAGACGACGATGATG-3'
cJUN	5'-TTCTATGACGATGCCCTCAACGC-3'	5'-GCTCTGTTTCAGGATCTTGGGGTTAC-3'
MAPK1	5'-CGTGTTGCAGATCCAGACCATGAT-3'	5'-TGGACTTGGTGTAGCCCTTGGAA-3'
MAPK3	5'-ACCTGCGACCTTAAGATTTGTGA-3'	5'-AGCCACATACTCCGTCAGGAA-3'

PIK3CA	5'-TGGATGCTCTACAGGGCTTT-3'	5'-GTCTGGGTTCTCCCAATTCA-3'
C. Primer sequences for PPARα target genes		
Gene	**Forward Primer**	**Reverse Primer**
THBD	AGCAAGCCCCACTTATTCCC	GGGTGACTCAGGTGAGTTGG
PDK4	GAGGTGGTGTTCCCCTGAGAATT	CAAAACCAGCCAAAGGAGCATT

PXR, pregnane X receptor; CAR, constitutive androstane receptor; AhR, aryl hydrocarbon receptor; PPARA, peroxisome proliferator activated receptor alpha; PPARG, peroxisome proliferator activated receptor gamma; NRF2, nuclear factor, erythroid 2 like 2; NFKB1, nuclear factor kappa B subunit 1; RELA; RELA proto-oncogene, NF-KB Subunit; CREB1, cAMP responsive element binding protein 1; COX-2, cyclooxygenase-2; TP53, tumor protein P53; GSK3B, glycogen synthase kinase 3 beta; JNK1, c-Jun N-terminal kinase-1; cJUN, Jun proto-oncogene, AP-1 transcription factor subunit; MAPK1, mitogen-activated protein kinase 1; MAPK3, mitogen-activated protein kinase 3; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; THBD, thrombomodulin; PDK4, pyruvate dehydrogenase lipoamide kinase isozyme 4

Supplemental Table 3

Company source and assay IDs of the siRNAs used in this study.

Gene Symbol	Gene Name	Catalog/Assay ID	Company
AhR	Aryl hydrocarbon receptor	4427038/ s1198	Thermofisher
PPARA	Peroxisome proliferator activated receptor alpha	4427037/ s10881	Thermofisher
PPARG	Peroxisome proliferator activated receptor gamma	4427038/ s10886	Thermofisher
NRF2	Nuclear factor, erythroid 2 like 2	4427037/ s9491	Thermofisher
NFKB1	Nuclear factor kappa B subunit 1	4427037/ s9505	Thermofisher
RELA	RELA proto-oncogene, NF-KB Subunit	4427038/ s11914	Thermofisher
CREB1	cAMP responsive element binding protein 1	4427037/ s3489	Thermofisher
COX-2	Cyclooxygenase-2	4427037/ s11472	Thermofisher
p53/TP53	Tumor protein P53	4427038/ s607	Thermofisher
GSK3B	Glycogen synthase kinase 3 beta	4427038/ s6240	Thermofisher
MAPK1	Mitogen-activated protein kinase 1	4427038/ s11137	Thermofisher
MAPK3	Mitogen-activated protein kinase 3	4427038/ s11140	Thermofisher
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	4427038/ s10520	Thermofisher
Negative Control No. 1		4390843	Thermofisher
Gene Symbol	**Gene Name**	**Product Number/siRNA ID**	**Company**
JNK1	c-Jun N-terminal kinase-1	NM_002750/ SASI_Hs01_00010441	Sigma-Aldrich
cJUN	Jun proto-oncogene, AP-1 transcription factor subunit	NM_002228/ SASI_Hs02_00333461	Sigma-Aldrich

Supplemental Table 4

Statistical analysis of data

Figure	Comparative groups	Mean difference (95% Confidence Interval of difference)	P value	P value (Statistical test)
Fig. 1 (ABCC1/ B2M)	VC vs 300µM	0.00 (-0.05, 0.05)	0.97	0.74 (One-way ANOVA with Tukey test)
	VC vs 600µM	-0.01 (-0.06, 0.04)	0.84	
	300µM vs 600µM	0.01 (-0.04, 0.06)	0.73	
Fig. 1 (ABCC2/ B2M)	VC vs 300µM	-0.03 (-0.12, 0.07)	0.64	0.36 (One-way ANOVA with Tukey test)
	VC vs 600µM	-0.05 (-0.14, 0.05)	0.33	
	300µM vs 600µM	-0.02 (-0.11, 0.08)	0.82	
Fig. 1 (ABCC4/ B2M)	VC vs 300µM	0.05 (-0.08, 0.18)	0.50	0.15 (One-way ANOVA with Tukey test)
	VC vs 600µM	0.09 (-0.03, 0.22)	0.13	
	300µM vs 600µM	0.05 (-0.08, 0.17)	0.54	
Fig. 1 (ABCC5/ B2M)	VC vs 300µM	-0.02 (-0.09, 0.06)	0.79	0.46 (One-way ANOVA with Tukey test)
	VC vs 600µM	0.02 (-0.06, 0.09)	0.79	
	300µM vs 600µM	0.03 (-0.04, 0.11)	0.43	
Fig. 1 (ABCG2/ B2M)	VC vs 300µM	-0.23 (-0.34, -0.13)	0.001	<0.001 (One-way ANOVA with Tukey test)
	VC vs 600µM	-0.38 (-0.49, -0.28)	<0.001	
	300µM vs 600µM	-0.15 (-0.25, -0.04)	0.01	
Fig. 2 (ABCG2/ B2M)	VC vs 300 µM (6h)	-0.14 (-0.21, -0.07)	<0.001	<0.001 (By time points) <0.001 (By treatments)
	VC vs 600 µM (6h)	-0.18 (-0.25, -0.11)	<0.001	

	300 μM vs 600 μM (6h)	0.04 (-0.03, 0.11)	0.65	(Two-way ANOVA with Tukey test)
	VC vs 300 μM (12h)	-0.20 (-0.27, -0.13)	<0.001	
	VC vs 600 μM (12h)	-0.29 (-0.36, -0.22)	<0.001	
	300 μM vs 600 μM (12h)	0.09 (0.02, 0.16)	0.005	
	VC vs 300 μM (24h)	-0.23 (-0.30, -0.16)	<0.001	
	VC vs 600 μM (24h)	-0.38 (-0.45, -0.31)	<0.001	
	300 μM vs 600 μM (24h)	0.15 (0.08, 0.22)	<0.001	
	VC vs 300 μM (48h)	-0.16 (-0.23, -0.09)	<0.001	
	VC vs 600 μM (48h)	-0.24 (-0.31, -0.17)	<0.001	
	300 μM vs 600 μM (48h)	0.08 (0.01, 0.15)	0.015	
Fig. 3A (BCRP/HSC)	VC vs 300 μM (6h)	-0.20 (-0.42, 0.03)	0.08	<0.001 (By time points) <0.001 (By treatments) (Two-way ANOVA with Tukey test)
	VC vs 600 μM (6h)	-0.02 (-0.08, 0.05)	0.99	
	300 μM vs 600 μM (6h)	-0.06 (-0.12, 0.01)	0.10	
	VC vs 300 μM (12h)	-0.16 (-0.23, -0.10)	<0.001	
	VC vs 600 μM (12h)	-0.19 (-0.26, -0.13)	<0.001	
	300 μM vs 600 μM (12h)	0.03 (-0.03, 0.09)	0.89	
	VC vs 300 μM (24h)	-0.18 (-0.24, -0.12)	<0.001	
	VC vs 600 μM (24h)	-0.21 (-0.28, -0.15)	<0.001	
	300 μM vs 600 μM (24h)	0.03 (-0.03, 0.10)	0.80	
	VC vs 300 μM (48h)	-0.24 (-0.30, -0.18)	<0.001	
	VC vs 600 μM (48h)	-0.32 (-0.38, -0.26)	<0.001	
	300 μM vs 600 μM (48h)	0.08 (0.02, 0.14)	0.004	
	VC vs 300 μM (72h)	-0.18 (-0.25, -0.12)	<0.001	
	VC vs 600 μM (72h)	-0.27 (-0.33, -0.21)	<0.001	
	300 μM vs 600 μM (72h)	0.09 (0.03, 0.15)	0.001	

Fig. 3C (BCRP activity)	VC vs 300 µM (12h)	-0.03 (-0.11, 0.05)	0.98	<0.001 (By time points) <0.001 (By treatments) (Two-way ANOVA with Tukey test)
	VC vs 600 µM (12h)	-0.08 (-0.15, 0.002)	0.06	
	300 µM vs 600 µM (12h)	0.05 (-0.03, 0.13)	0.59	
	VC vs 300 µM (24h)	-0.08 (-0.16, 0.00)	0.048	
	VC vs 600 µM (24h)	-0.09 (-0.17, -0.01)	0.02	
	300 µM vs 600 µM (24h)	0.01 (-0.07, 0.09)	1.00	
	VC vs 300 µM (48h)	-0.10 (-0.17, -0.02)	0.01	
	VC vs 600 µM (48h)	-0.18 (-0.26, -0.11)	<0.001	
	300 µM vs 600 µM (48h)	0.09 (0.01, 0.17)	0.02	
	VC vs 300 µM (72h)	-0.11 (-0.19, -0.03)	0.001	
	VC vs 600 µM (72h)	-0.22 (-0.29, -0.14)	<0.001	
	300 µM vs 600 µM (72h)	0.11 (0.03, 0.18)	0.002	
Fig. 4A (PHT)	Baseline vs 20µM	0.02 (0.004, 0.04)	0.02	0.02 (One-way ANOVA with Dunnett test)
	Baseline vs 40µM	0.01 (-0.01, 0.03)	0.44	
	Baseline vs 80µM	0.02 (0.0003, 0.04)	0.047	
	Baseline vs 160µM	0.02 (0.006, 0.04)	0.015	
	Activated vs 20µM	0.01 (-0.04, 0.05)	0.99	0.71 (One-way ANOVA with Dunnett test)
	Activated vs 40µM	0.02 (-0.03, 0.07)	0.50	
	Activated vs 80µM	0.01 (-0.04, 0.06)	0.89	
	Activated vs 160µM	0.006 (-0.04, 0.05)	0.99	
Fig. 4B (CBZ)	Baseline vs 10µM	-0.01 (-0.06, 0.04)	0.93	0.31 (One-way ANOVA with Dunnett test)
	Baseline vs 20µM	0.02 (-0.04, 0.07)	0.69	
	Baseline vs 40µM	-0.02 (-0.07, 0.03)	0.54	
	Baseline vs 80µM	-0.008 (-0.06, 0.04)	0.94	
	Activated vs 10µM	-0.05 (-0.11, 0.002)	0.06	0.03

	Activated vs 20μM	-0.05 (-0.10, 0.006)	0.08	(One-way ANOVA with Dunnett test)
	Activated vs 40μM	-0.05 (-0.10, 0.01)	0.11	
	Activated vs 80μM	-0.07 (-0.13, -0.02)	0.014	
Fig. 4C (VPA)	Baseline vs. 75μM	0.07 (0.03, 0.10)	0.004	0.002 (One-way ANOVA with Dunnett test)
	Baseline vs. 150μM	0.06 (0.02, 0.09)	0.008	
	Baseline vs. 300μM	0.09 (0.05, 0.13)	0.001	
	Baseline vs. 600μM	0.07 (0.03, 0.10)	0.004	
	Activated vs. 75μM	-0.03 (-0.08, 0.02)	0.32	0.12 (One-way ANOVA with Dunnett test)
	Activated vs. 150μM	-0.02 (-0.07, 0.03)	0.47	
	Activated vs. 300μM	-0.03 (-0.08, 0.02)	0.32	
	Activated vs. 600μM	0.02 (-0.03, 0.07)	0.70	
Fig. 4D (LTG)	Baseline vs. 15μM	0.03 (0.01, 0.05)	0.005	0.001 (One-way ANOVA with Dunnett test)
	Baseline vs. 30μM	0.05 (0.03, 0.06)	0.001	
	Baseline vs. 60μM	0.05 (0.03, 0.07)	<0.001	
	Baseline vs. 120μM	0.04 (0.02, 0.05)	0.002	
	Activated vs. 15μM	-0.05 (-0.11, 0.002)	0.06	0.03 (One-way ANOVA with Dunnett test)
	Activated vs. 30μM	-0.05 (-0.10, 0.01)	0.08	
	Activated vs. 60μM	-0.05 (-0.10, 0.01)	0.10	
	Activated vs. 120μM	-0.07 (-0.13, -0.02)	0.014	
Fig. 4E (TPM)	Baseline vs. 30μM	-0.007 (-0.05, 0.03)	0.89	0.90 (One-way ANOVA with Dunnett test)
	Baseline vs. 60μM	-0.004 (-0.04, 0.04)	0.98	
	Baseline vs. 120μM	-0.008 (-0.05, 0.03)	0.86	
	Activated vs. 15μM	0.006 (-0.02, 0.03)	0.85	0.32 (One-way ANOVA with Dunnett test)
	Activated vs. 30μM	0.01 (-0.02, 0.04)	0.52	

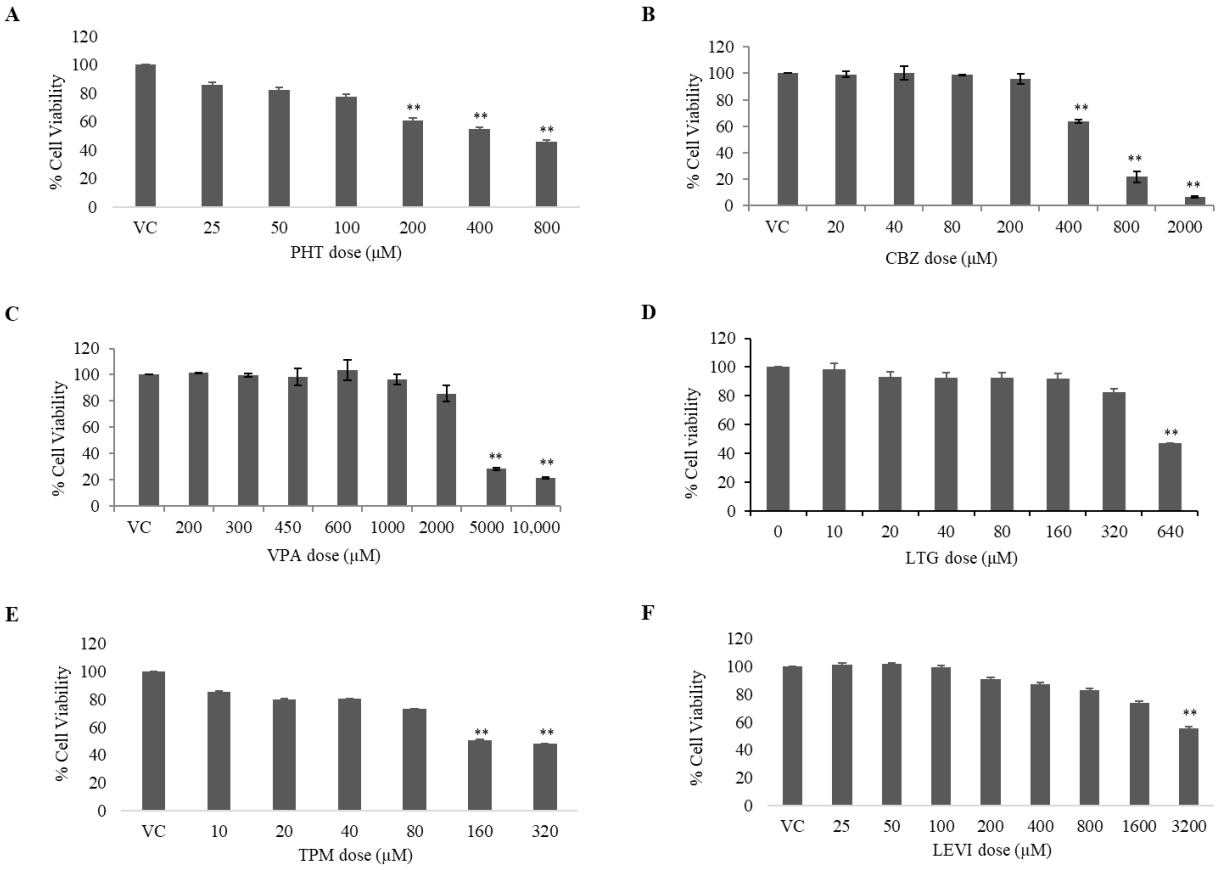
	Activated vs. 60μM	0.02 (-0.01, 0.05)	0.17	
	Activated vs. 120μM	0.007 (-0.02, 0.03)	0.82	
Fig. 4F (LEVI)	Baseline vs. 25μM	0.004 (-0.06, 0.06)	0.10	0.49 (One-way ANOVA with Dunnett test)
	Baseline vs. 50μM	-0.002 (-0.06, 0.06)	1.00	
	Baseline vs. 100μM	0.02 (-0.04, 0.08)	0.62	
	Baseline vs. 200μM	0.02 (-0.04, 0.08)	0.52	
	Activated vs. 25μM	0.003 (-0.03, 0.03)	0.98	0.38 (One-way ANOVA with Dunnett test)
	Activated vs. 50μM	0.005 (-0.02, 0.03)	0.90	
	Activated vs. 100μM	0.01 (-0.02, 0.04)	0.52	
	Activated vs. 200μM	0.02 (-0.01, 0.05)	0.25	
Fig. 5A (PHT)	VC vs 40μM	0.06 (0.03, 0.09)	<0.001	<0.001 (One-way ANOVA with Dunnett test)
	VC vs 80μM	0.10 (0.07, 0.12)	<0.001	
	VC vs KO143	0.20 (0.18, 0.23)	<0.001	
Fig. 5B (VPA)	VC vs 300μM	0.15 (0.10, 0.20)	<0.001	<0.001 (One-way ANOVA with Dunnett test)
	VC vs 600μM	0.20 (0.15, 0.25)	<0.001	
	VC vs KO143	0.20 (0.15, 0.25)	<0.001	
Fig. 5C (LTG)	VC vs 15μM	0.20 (0.15, 0.26)	<0.001	<0.001 (One-way ANOVA with Dunnett test)
	VC vs 60μM	0.19 (0.13, 0.24	<0.001	
	VC vs KO143	0.20 (0.15, 0.26)	<0.001	
Fig. 6B (ABCG2/ B2M)	SCR, VC vs SCR, VPA	1.27 (0.77, 1.77)	<0.001	<0.001 (By siRNA) <0.001 (By drug treatment) (Two-way ANOVA with Tukey test)
	SCR, VC vs siPPARα, VC	-0.19 (-0.69, 0.32)	0.66	
	SCR, VC vs siPPARα, VPA	0.19 (-0.32, 0.69)	0.65	

	SCR, VPA vs siPPARα, VC	1.46 (0.95, 1.96)	<0.001	
	SCR, VPA vs siPPARα, VPA	-1.08 (-1.59, -0.58)	0.001	
	siPPARα, VC vs siPPARα, VPA	0.37 (-0.13, 0.88)	0.16	
Fig. 6D (BCRP/ HSC)	SCR, VC vs SCR, VPA	1.16 (0.79, 1.52)	<0.001	<0.001 (By siRNA) <0.001 (By drug treatment) (Two-way ANOVA with Tukey test)
	SCR, VC vs siPPARα, VC	-0.06 (-0.43, 0.30)	0.94	
	SCR, VC vs siPPARα, VPA	0.22 (-0.14, 0.59)	0.28	
	SCR, VPA vs siPPARα, VC	1.22 (0.85, 1.59)	<0.001	
	SCR, VPA vs siPPARα, VPA	-0.93 (-1.30, -0.57)	<0.001	
	siPPARα, VC vs siPPARα, VPA	0.29 (-0.08, 0.65)	0.13	
Fig. 6E (BCRP activity)	SCR, VC vs SCR, VPA	0.57 (0.47, 0.68)	<0.001	<0.001 (By siRNA) <0.001 (By drug treatment) (Two-way ANOVA with Tukey test)
	SCR, VC vs siPPARα, VC	-0.11 (-0.21, -0.00)	0.04	
	SCR, VC vs siPPARα, VPA	-0.03 (-0.13, 0.08)	0.84	
	SCR, VPA vs siPPARα, VC	0.68 (0.58, 0.78)	<0.001	
	SCR, VPA vs siPPARα, VPA	-0.60 (-0.70, -0.50)	<0.001	
	siPPARα, VC vs siPPARα, VPA	0.08 (-0.02, 0.18)	0.14	
Fig. 7B (BCRP/ HSC)	VC vs VPA	0.36 (0.20, 0.52)	<0.001	0.003 (By -/+MK886) <0.001 (By drug treatment) (Two-way ANOVA with Tukey test)
	VC vs VC+8μMMK886	-0.11 (-0.27, 0.05)	0.21	
	VC vs VPA+8μMMK886	-0.05 (-0.21, 0.11)	0.77	
	VPA vs VC+8μMMK886	0.46 (0.30, 0.62)	<0.001	
	VPA vs VPA+8μMMK886	-0.40 (-0.56, -0.24)	<0.001	
	VC+8μMMK886 vs VPA+8μMMK886	0.06 (-0.10, 0.22)	0.65	
Fig. 7C (BCRP activity)	VC vs VPA	-0.22 (-0.31, -0.12)	<0.001	<0.001 (One-way ANOVA with Tukey test)
	VC vs VC+8μMMK886	0.08 (-0.02, 0.17)	0.11	

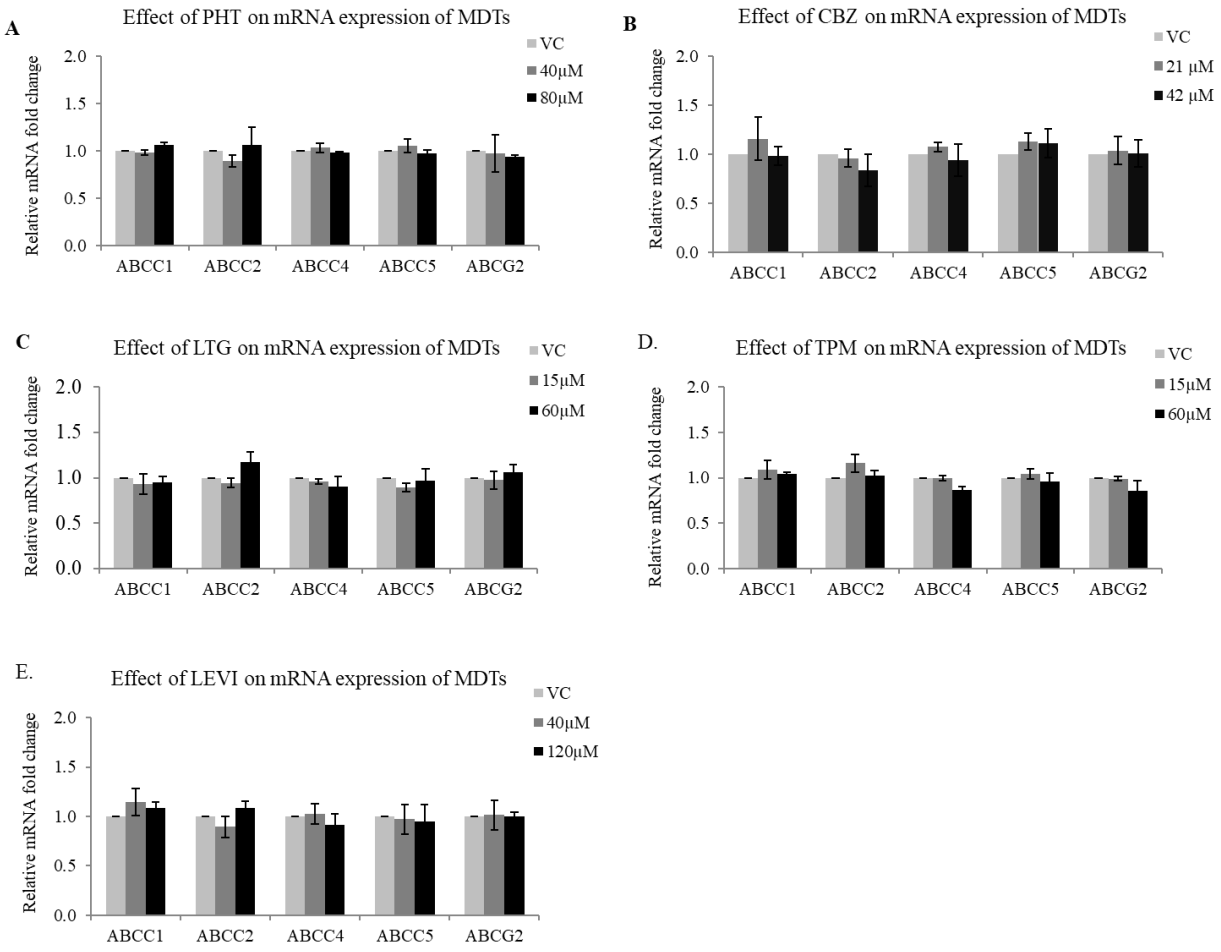
	VC vs VPA+8μMMK886	0.02 (-0.08, 0.11)	0.94	
	VPA vs VC+8μMMK886	0.29 (0.20, 0.39)	<0.001	
	VPA vs VPA+8μMMK886	0.23 (0.14, 0.33)	<0.001	
	VC+8μMMK886 vs VPA+8μMMK886	-0.06 (-0.15, 0.03)	0.24	
Fig. 8A (PPARα/ B2M)	VC vs 600 μM (1h)	-0.03 (-0.10, 0.03)	0.80	<0.001 (By time points) <0.001 (By treatments) (Two-way ANOVA with Tukey test)
	VC vs 600 μM (3h)	-0.16 (-0.22, -0.09)	<0.001	
	VC vs 600 μM (6h)	-0.30 (-0.37, -0.24)	<0.001	
	VC vs 600 μM (12h)	-0.24 (-0.31, -0.18)	<0.001	
	VC vs 600 μM (24h)	-0.23 (-0.29, -0.16)	<0.001	
	VC vs 600 μM (48h)	-0.07 (-0.14, -0.004)	0.03	
Fig. 8B (PPARα/ HSC)	VC vs 600 μM (1h)	-0.01 (-0.10, 0.07)	1.00	<0.001 (By time points) <0.001 (By treatments) (Two-way ANOVA with Tukey test)
	VC vs 600 μM (3h)	-0.21 (-0.29, -0.13)	<0.001	
	VC vs 600 μM (6h)	-0.14 (-0.22, -0.05)	<0.001	
	VC vs 600 μM (12h)	-0.05 (-0.13, 0.03)	0.57	
	VC vs 600 μM (24h)	0.01 (-0.07, 0.09)	1.00	
Fig. 8D	VC vs 600 μM (24h) – PDK4	-0.22 (-0.25, -0.19)	<0.001	(Unpaired t-test)
	VC vs 600 μM (24h) – THBD	-0.27 (-0.32, -0.22)	<0.001	
Fig. 9B	0h cytosolic vs 3h cytosolic	0.10 (0.05, 0.15)	<0.001	<0.001 (One-way ANOVA with Tukey test)
	0h cytosolic vs 6h cytosolic	0.06 (0.002, 0.11)	0.04	
	3h cytosolic vs 6h cytosolic	-0.05 (-0.10, 0.01)	0.12	
	0h nuclear vs 3h nuclear	-0.14 (-0.19, -0.08)	<0.001	
	0h nuclear vs 6h nuclear	-0.11 (-0.16, 0.06)	<0.001	
	3h nuclear vs 6h nuclear	0.03 (-0.03, 0.08)	0.67	

	3h cytosolic vs 3h nuclear	-0.24 (-0.29, -0.18)	<0.001	
	6h cytosolic vs 6h nuclear	-0.17 (-0.22, -0.11)	<0.001	
Fig. 9D	0h vs 3h	-0.13 (-0.19, -0.06)	0.001	0.001 (One-way ANOVA with Tukey test)
	0h vs 6h	-0.12 (-0.18, -0.05)	0.002	
	3h vs 6h	0.01 (-0.06, 0.07)	0.95	
Fig. 10A	VC vs VPA (IgG)	-0.20 (-0.67, 0.27)	0.30	(Unpaired t-test)
	VC vs VPA (PPARα) – 3h	-0.82 (-1.06, -0.58)	<0.001	
	VC vs VPA (PPARα) – 6h	-0.62 (-0.84, -0.40)	<0.001	
Fig. 10C	VC vs VPA (pGL2-control)	-0.01 (-0.05, 0.02)	0.44	(Unpaired t-test)
	VC vs VPA (pGL2-prom-ABCG2)	-0.17 (-0.20, -0.13)	<0.001	(Unpaired t-test)

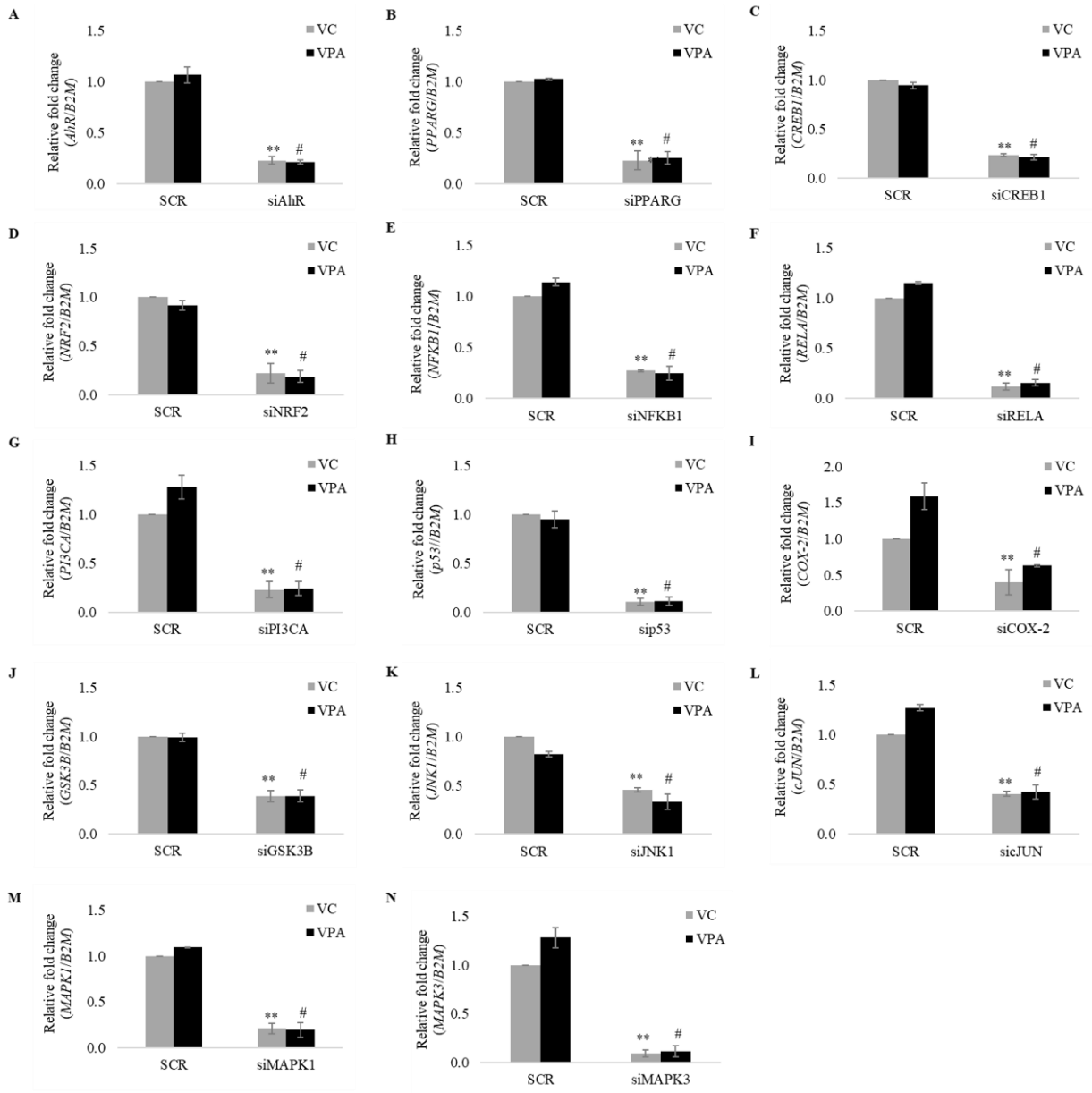
Supplemental Data



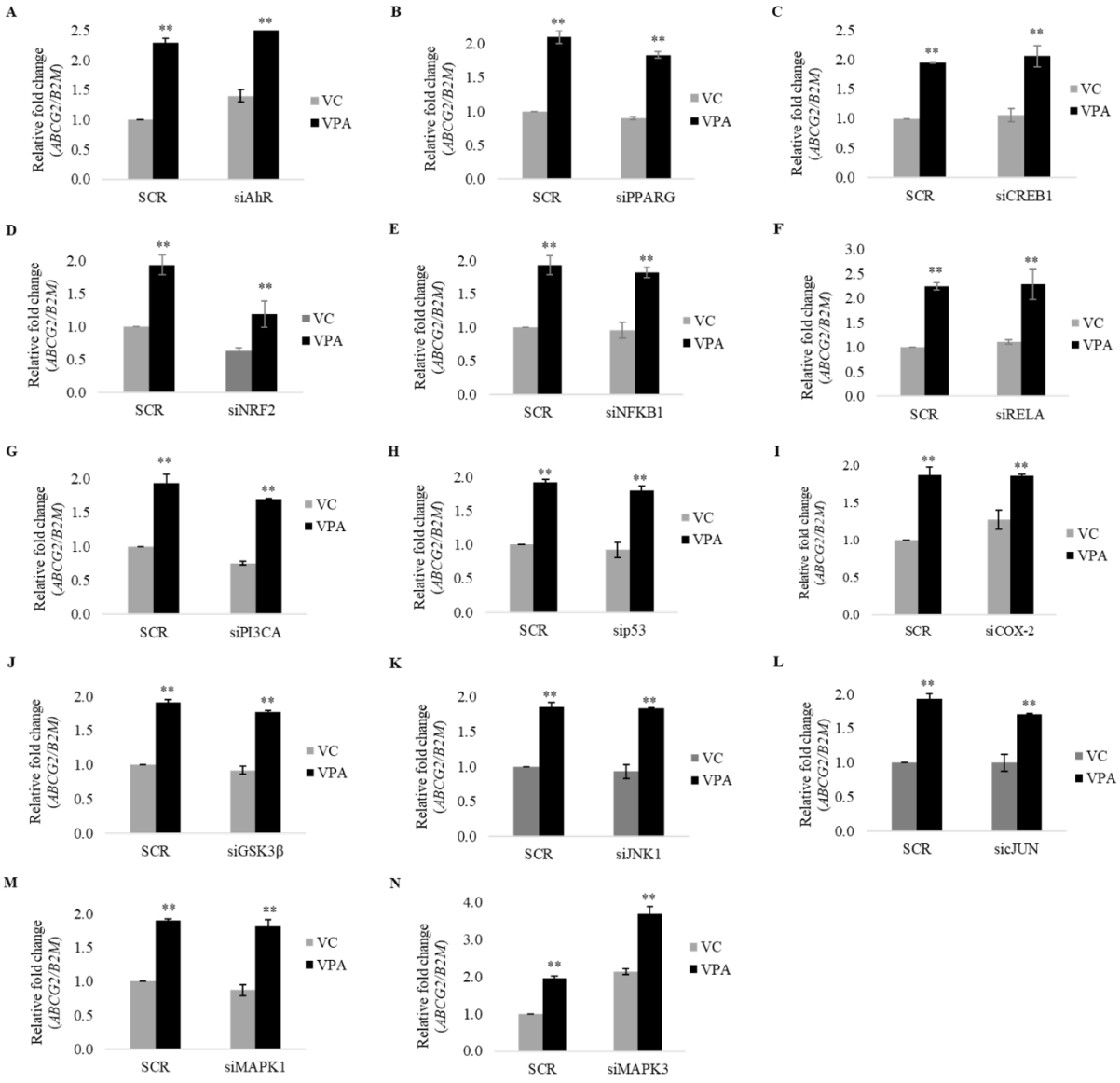
Supplemental Fig. 1. Effect of AEDs on hCMEC/D3 cell viability. Cells were treated with different doses of (A) phenytoin (PHT), (B) carbamazepine (CBZ), (C) valproic acid (VPA), (D) lamotrigine (LTG), (E) topiramate (TPM) and (F) levetiracetam (LEVI) for 72h. After treatment, cells were assessed for % viability using MTT assay. Data is the mean ± S.D. of 4 independent experiments. **p<0.01, compared to VC (One-way ANOVA with Dunnett's post hoc test).



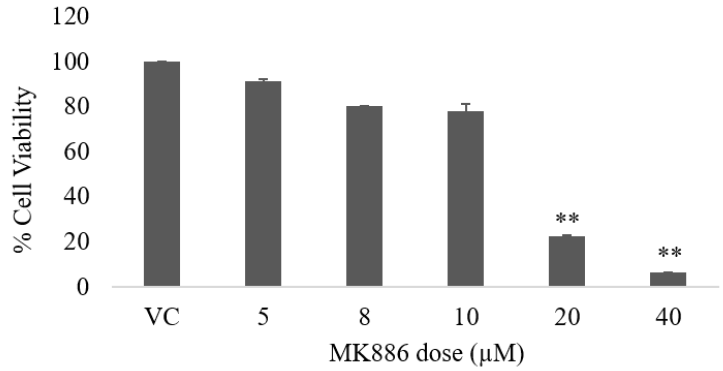
Supplemental Fig. 2. Effect of PHT, CBZ, LTG, TPM and LEVI on mRNA expression of MDTs in hCMEC/D3 cells. RT-qPCR analysis of ABCC1, ABCC2, ABCC4, ABCC5 and ABCG2 mRNA expression in hCMEC/D3 cells treated with (A) PHT (40µM, 80µM), (B) CBZ (21µM, 42µM), (C) LTG (15µM, 60µM), (D) TPM (15µM, 60µM) and (E) LEVI (40µM, 120µM) for 24h. The changes in mRNA levels of target genes were normalized with *B2M* and expressed as normalized fold change over VC (0.1% DMSO for PHT, CBZ, LTG and TPM; water for LEVI). The data is the mean ± S.D. of 3 independent experiments.



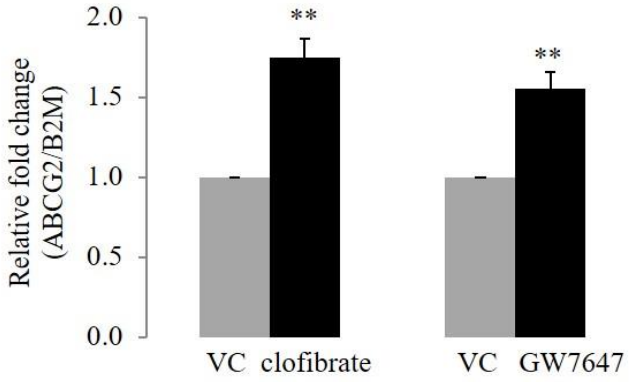
Supplemental Fig. 3. siRNA validation data for 14 molecular factors at mRNA level. hCMEC/D3 cells were transiently transfected with siRNA specific to (A) AhR, (B) PPARG, (C) CREB1, (D) NRF2, (E) NFKB1, (F) RELA, (G) PIK3CA, (H) p53, (I) COX-2, (J) GSK3B, (K) JNK1, (L) cJUN, (M) MAPK1, (N) MAPK3, or the non-targeting control (scramble, SCR). Subsequently, cells were treated with VC (0.1% DMSO) or VPA (600μM) for 24h. RT-qPCR analysis was done to check the knockdown of each factor in VC-treated as well as VPA-treated group. The changes in mRNA level of each gene were normalized with *B2M*. The data is the mean ± S.D. of 3 independent experiments. **P < 0.01, VC (SCR vs. siRNA); #P<0.01 VPA (SCR vs. siRNA) (unpaired t-test).



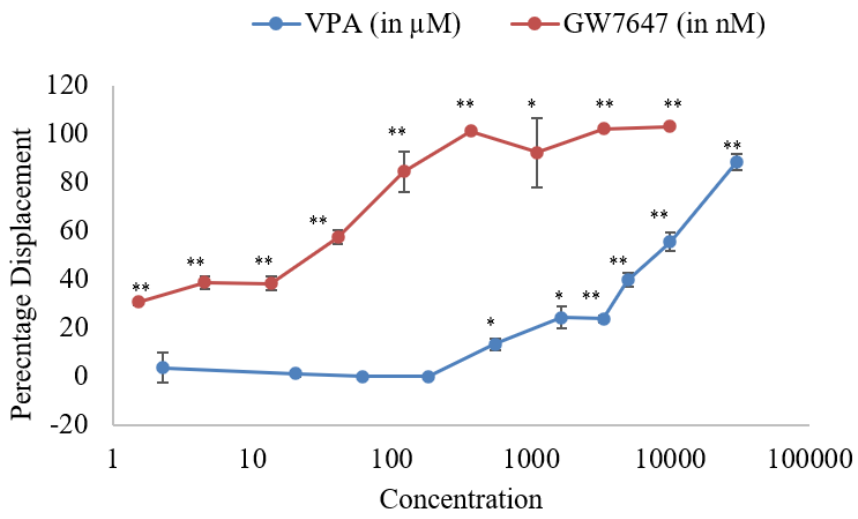
Supplemental Fig. 4. Effect of silencing of molecular factors on VPA-induced ABCG2 mRNA. Expression of the respective factors (A-N) was silenced by transient transfection of gene-specific siRNA in hCMEC/D3 cells. Then, the cells were treated with VC (0.1% DMSO) or VPA (600μM) for 24h and RT-qPCR analysis was done to check mRNA expression levels of ABCG2. Scramble (SCR) was used as non-targeting control. The changes in mRNA level of ABCG2 was normalized with *B2M*. The data is the mean ± S.D. of 3 independent experiments. **P < 0.01, SCR (VC vs. VPA) and siRNA (VC vs. VPA) (Two-way ANOVA with Tukey's post hoc test).



Supplemental Fig. 5. Effect of MK886 on hCMEC/D3 cell viability. Cells were treated with different doses of MK886 for 48h. After treatment, cells were assessed for % viability using MTT assay. Data is the mean ± S.D. of 4 independent experiments. **$p<0.01$, compared to VC (0.1% DMSO) (One-way ANOVA with Dunnett's post hoc test).



Supplemental Fig. 6. Effect of known PPARα agonist on ABCG2 mRNA in hCMEC/D3 cells. RT-qPCR analysis of ABCG2 mRNA expression in hCMEC/D3 cells treated with 100µM clofibrate and 100nM GW7647 for 24h. The changes in the mRNA level were normalized with *B2M* and expressed as normalized fold change over VC (0.1% DMSO). The data is the mean ± S.D. of 3 independent experiments. **p<0.01, compared to VC (unpaired t-test).



Supplemental Fig. 7. Binding of VPA to PPARα LBD in a TR-FRET competitive binding assay. GW7647 was used as a known PPARα agonist. Data is the mean ± S.D. of 2 independent experiments. *p < 0.05, **p < 0.01 compared to solvent control (1% DMSO, 0% displacement); One-way ANOVA with Dunnett's post hoc test.

References

Johannessen SI (2004) Therapeutic drug monitoring of antiepileptic drugs, in *Drug Monitoring and Clinical Chemistry* (Hempel G ed) pp 221-253, Elsevier: Amsterdam, The Netherlands.