

Supplementary material

The first characterization of multidrug and toxin extrusion (MATE/SLC47) proteins in zebrafish (*Danio rerio*)

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Table S1. Primer sequences used for relative quantification of zebrafish *mates* using qRT-PCR analysis.

Protein name	Primer sequence 5→3	Final conc.(nM)	Slope	Intercept	Efficiency[%]
DrMate3	F TCGGATGTACCAAAGATGT	300	-3.69	35.1	86.7
	R CGGTAATGCCCGCTATGG	300			
DrMate4	F GCAGCACAGGAGAACACTGA	300	-3.23	34.6	104
	R CCAACTGTGACCAATGCAAC	300			
DrMate5	F GGCTGTGATGTGAATGATGC	300	-3.04	27.4	113
	R GGCGCAGCACTAAAACACTC	300			
DrMate6	F TCACGCTGTACGTTTTCTG	300	-3.35	31.6	98.8
	R AACTGCTGCAATCTTCTGC	300			
DrMate7	F CTCAGGATCAGGACGAGAGG	300	-3.22	26.4	105
	R CTGAGTGGTGGACAGCAGAA	300			
DrMate8	F TCTGCAATGACAGCTCCTGATAGTAT	300	-3.44	38.8	95.3
	R GGCTCCACGTTCCGGATCG	300			
EF1 α	F CCTGGGAGTGAAACAGCTGATC	300	-3.43	42.5	95.5
	R GCTGACTTCCTGGTGATTCC	300			

Table S2. Primers used for cloning of zebrafish *mates*. Primer sequences, restriction enzyme sites introduced in the primer sequences (bold letters), and T_m values are shown.

Gene name	Primer sequence 5 -> 3	Restriction enzymes	T_m [°C]
Mate3	F TTAGCTAGCGAATTCATGGACAGTATTACCAGTTACAA	NheI-EcoRI	57
	R TTAGCGGCCGCGGTACCTCATCTAAGATATCTAACAAGCA	NotI-KpnI	55
Mate4	F TTAGCTAGCGAATTCATGGAAATGAACAGTATTCATAC	NheI-EcoRI	57
	R TTAGCGGCCGCGGTACCTCAAATAACAAATCTAATCAGCA	NotI-KpnI	58
Mate5	F TTAGCTAGCGAATTCATGAACGAAAGAATGAACGAAG	NheI-EcoRI	62
	R TTAGCGGCCGCGGTACCTCATCTTTAAGACTGTACTTGA	NotI-KpnI	55
Mate6	F TTAGCTAGCGAATTCATGCCTGAGGCTGGCG	NheI-EcoRI	67
	R TTAGGTACCTCTAGATTAGCTCATTGTAATATTGAGAA	KpnI-XbaI	55
Mate7	F TTAAAGCTTGAATTCATGGACGTCTCCAGTCCTGG	HindIII-EcoRI	67
	R TTAGCGGCCGCCGGGTCAAATGAAACTCGTTGAATAGA	NotI-SmaI	61
Mate8	F TTAGCTAGCGAATTCATGGACGTTTCAAGTCCCAC	NheI-EcoRI	64
	R TTAGGTACCTCTAGATTATAATGACGCAGAAGTGAAT	KpnI-XbaI	62

Table S3. Protein annotation and accession numbers of protein sequences used in the phylogenetic analysis of MATE superfamily (Multidrug Extrusion; gene name *SLC47A*) in metazoa. Species abbreviations: Hs, *Homo sapiens*; Mm, *Mus musculus*; Rn, *Rattus norvegicus*; Gg, *Gallus gallus*; Tg, *Taeniopygia guttata*; Xt, *Xenopus tropicalis*; Ac, *Anolis carolinensis*; Lc, *Latimeria chalumnae*; Dr, *Danio rerio*; Ga, *Gasterosteus aculeatus*; Gm, *Gadus morhua*; Ol, *Oryzias latipes*; Tr, *Takifugu rubripes*; Bf, *Branchiostoma floridae*; Sk, *Saccoglossus kowalevskii*; Lg, *Lottia gigantea*; Ct, *Capitella teleta*; Nv, *Nematostella vectensis*; Aq, *Amphimedon queenslandica*; Ta, *Trichoplax adhaerens*; Mb, *Monosiga brevicollis*; Sc, *Saccharomyces cerevisiae*; Ec, *Escherichia coli*; Vc, *Vibrio cholera*. Protein sequences with accession numbers starting with 'NP', 'XP', 'YP' or 'WP' were retrieved from NCBI database; the ones starting with 'ENS' or 'PAC' were retrieved from ENSEMBL database; and protein IDs in number format were retrieved from JGI database.

Protein annotation	Accession number	Length (aa)
HsMATE1	NP_060712.2	570
HsMATE2	NP_690872.2	602
HsMATE2-K	NP_001093116.1	566
HsMATE2-B	BAF37007.1	219
HsMATE2_tv3	NP_001243592.1	580
MmMate1a	AAH31436	532
MmMate1b	NP_080459.2	567
MmMate2	NP_001028714.1	573
RnMate1	NP_001014140.1	566
RnMate2	NP_001178849.1	572
GgMate1	NP_001129151.1	579
GgMate2	NP_001025891.2	475
TgMate1	XP_012424121.1	536
TgMate2	XP_012424122.1	622
AcMate1	XP_008123627.1	527
AcMate2	XP_003229800.2	373
XtMate1	NP_001096440.1	574
XtMate2	XP_012812968.1	600
XtMatelike1	XP_002934402.2	562
XtMatelike2	XP_002933880.2	550
XtMatelike3	XP_012813618.1	565
LcMate1	ENSLACP00000003119	575
LcMate2	ENSLACP00000001877	494
DrMate3	NP_001073648.1	590
DrMate4	NP_001274473.1	586
DrMate5	XP_005157715.1	548
DrMate6	NP_001014332.2	629
DrMate7	NP_001289183.1	607
DrMate8	XP_003201757.2	588
GaMate3	ENSGACP00000027262	566
GaMate4	ENSGACP00000024450	563
GaMate5	ENSGACP00000014451	497
GaMate6	ENSGACP00000027266	577
GaMate7	ENSGACP00000014470	530
GmMate3	ENSGMOP00000015086	454
GmMate5	ENSGMOP00000011176	458
GmMate6	ENSGMOP00000012623	283
OlMate4	ENSORLP00000002448	569
OlMate5	ENSORLP00000006032	566
OlMate6	ENSORLP00000006776	567
OlMate7	ENSORLP00000006011	440
OlMate8	ENSORLP00000005994	576
TrMate3	ENSTRUP00000002595	469
TrMate4	ENSTRUP00000011208	580
TrMate5	ENSTRUP00000007545	524
TrMate6a	ENSTRUP00000000290	570
TrMate6b	ENSTRUP00000003131	560
BfMatelike1	XP_002605941.1	715
BfMatelike2	183877	431
SkMatelike1	XP_002737051.1	647
SkMatelike2	XP_002737052.1	622
SkMatelike3	XP_006820737.1	553
LgMatelike1	XP_009051070.1	659
NvMatelike1	XP_001638537	408
CtMatelike1	164649	427
CtMatelike2	ELU15421.1	612
TaMatelike1	XP_002108992	533
ScMatelike1	NP_011897.1	581
ScMatelike2	NP_010625.1	695
AqMatelike1	XP_003383317.1	671
MbMatelike1	XP_001744290	446
EcNorM	YP_025307.1	457
VcNorM	AAF94694.1	461

Figure S1.

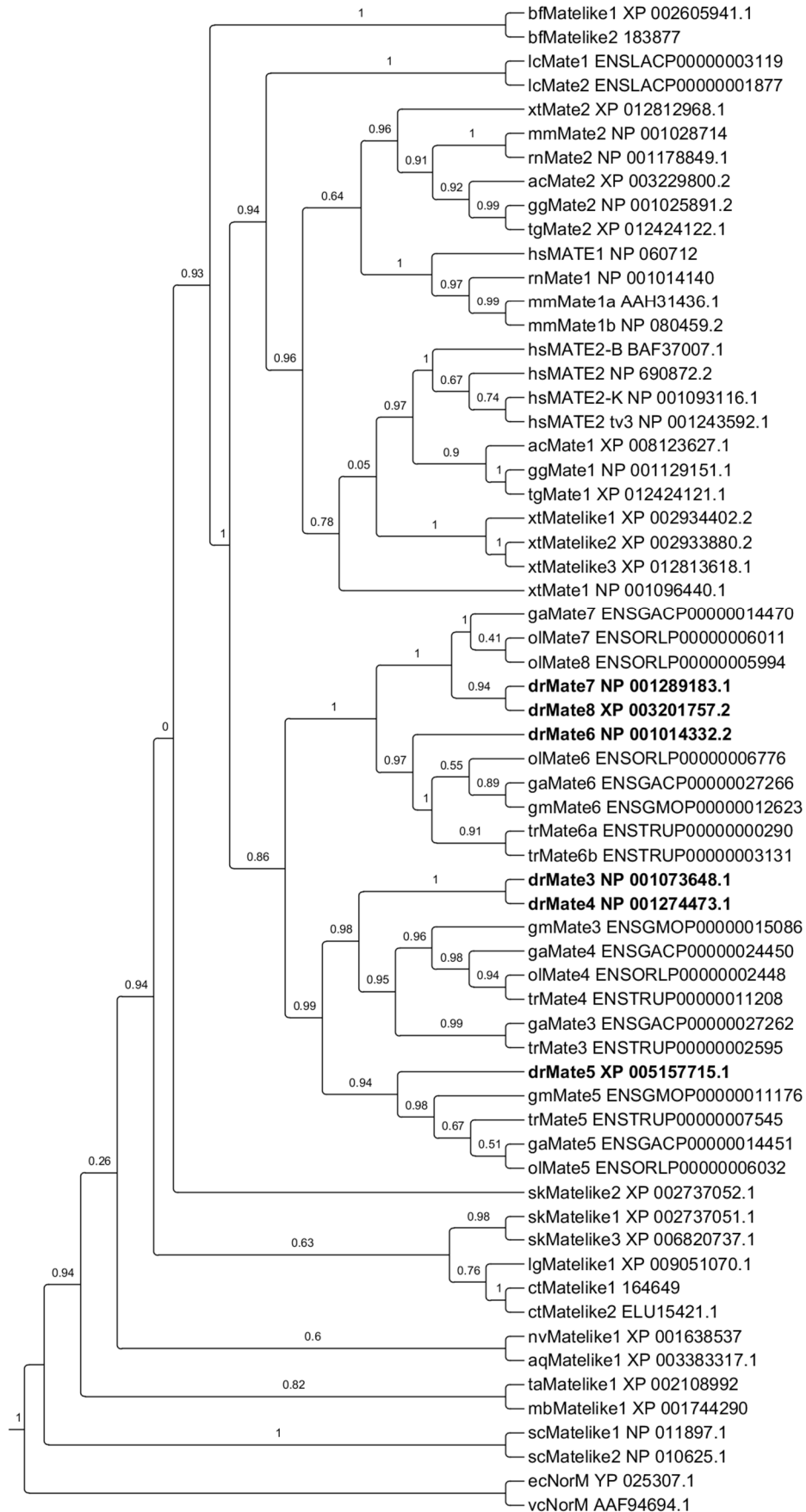
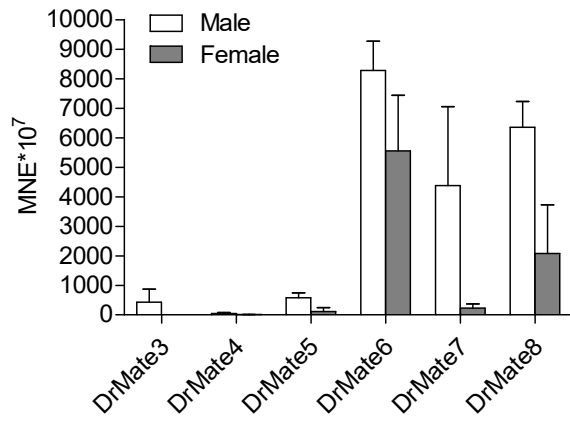


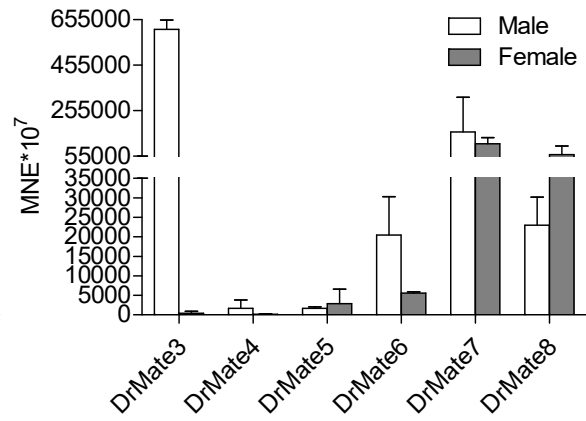
Figure S1. Phylogenetic analysis of MATE/Mate family (Multidrug and toxin extrusion). Phylogenetic tree was constructed with maximum likelihood method. Branch support is estimated with the approximate likelihood ratio test (aLRT). SLC22 gene family was used as an outgroup (not shown in the tree). Species abbreviations: Hs, *Homo sapiens*; Rn, *Rattus norvegicus*; Mm, *Mus musculus*; Gg, *Gallus gallus*; Tg, *Taeniopygia guttata*; Ac, *Anolis carolinensis*; Xt, *Xenopus tropicalis*; Dr, *Danio rerio*; Tn, *Tetraodon nigroviridis*; Tr, *Takifugu rubripes*; Ga, *Gasterosteus aculeatus*; Ol, *Oryzias latipes*; Gm, *Gadus morhua*; Lc, *Latimeria chalumnae*; Le, *Leucoraja erinacea*; Bf, *Branchiostoma floridae*; Pm, *Petromyzon marinus*; Od, *Oikopleura dioica*; Ci, *Ciona intestinalis*; Sk, *Saccoglossus kowalevskii*; Sp, *Strongylocentrotus purpuratus*; Ct, *Capitella teleta*; Nv, *Nematostella vectensis*; Aq, *Amphimedon queenslandica*; Ta, *Trichoplax adhaerens*; Mb, *Monosiga brevicollis*; Sc, *Saccharomyces cerevisiae*; Ec, *Escherichia coli*; Vc, *Vibrio cholera*.

Figure S2.

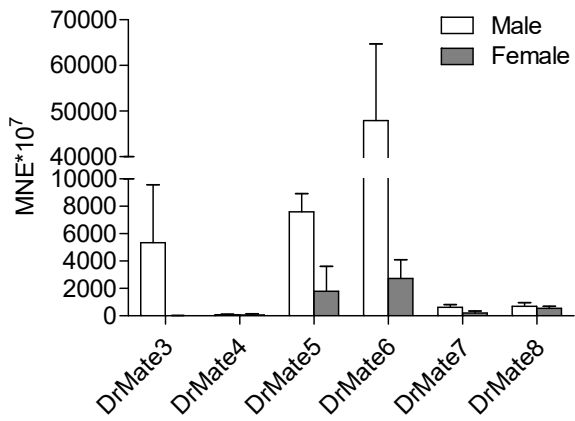
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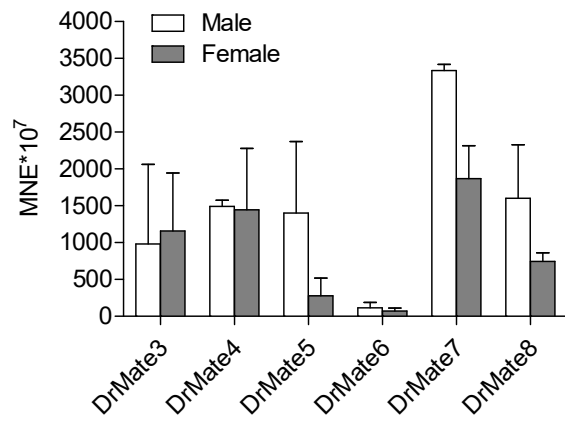
B. kidney



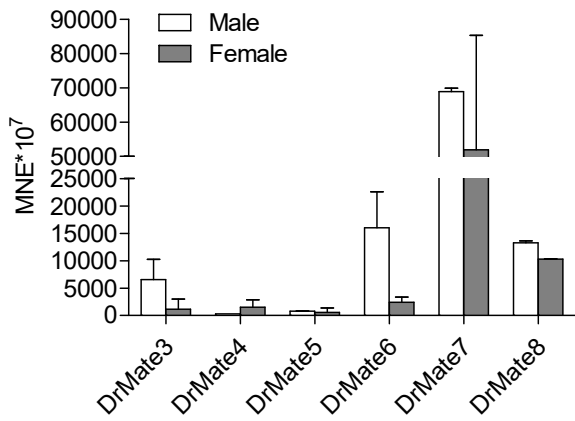
C. intestine



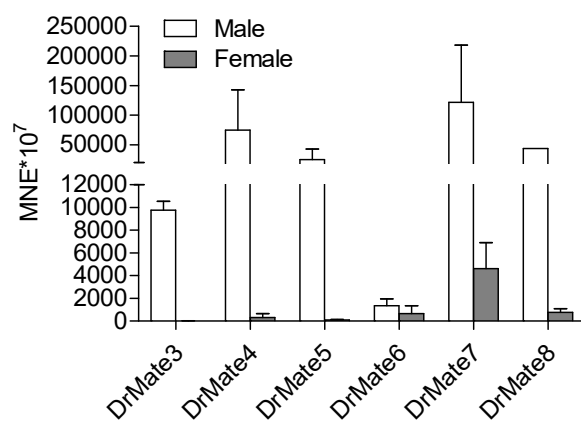
D. gills



E. brain



F. gonads



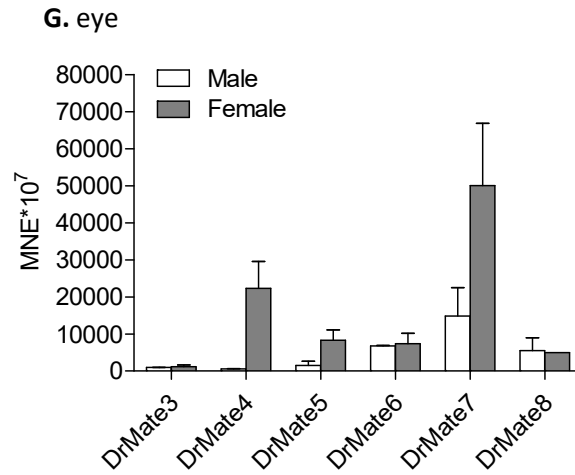
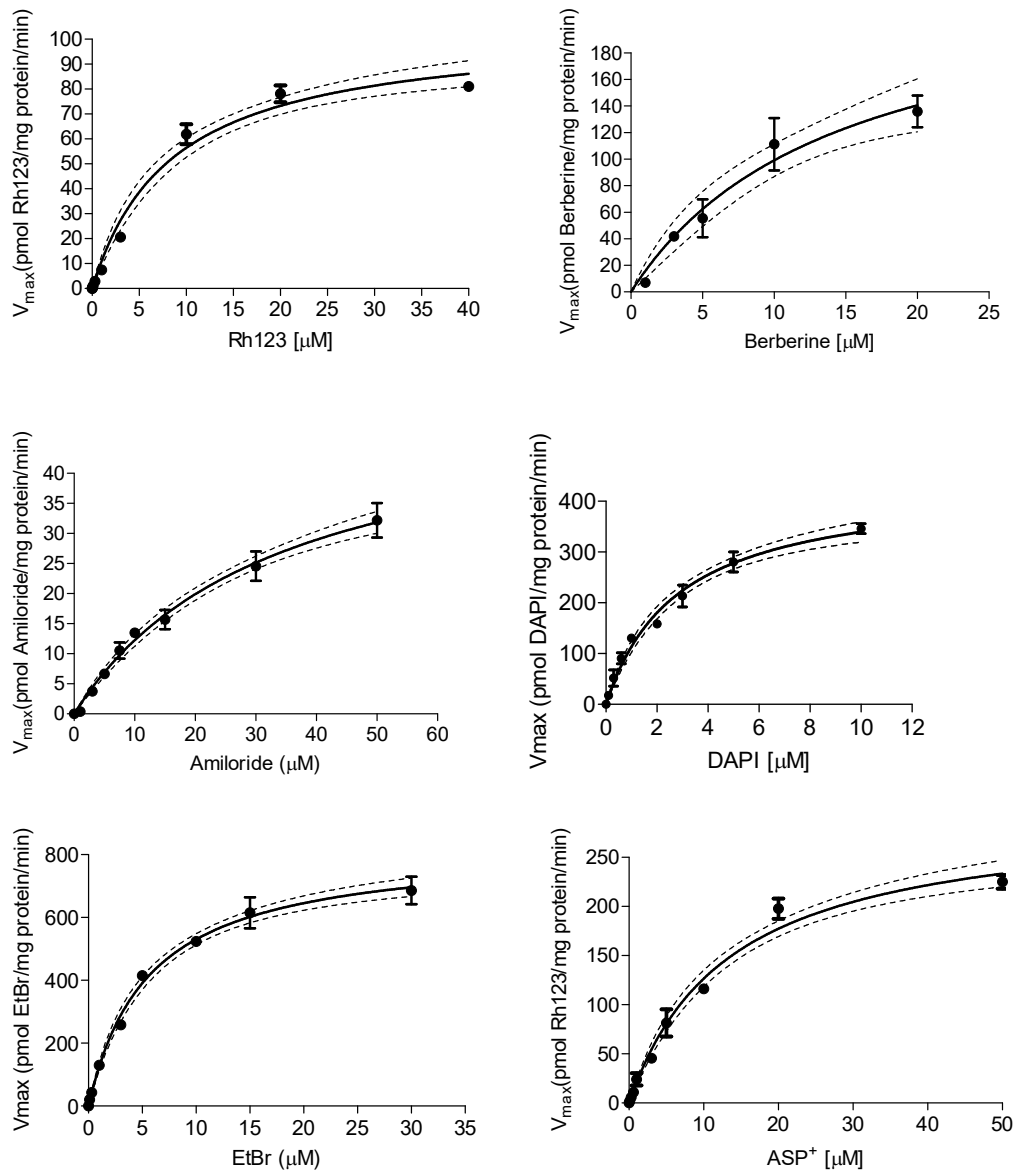


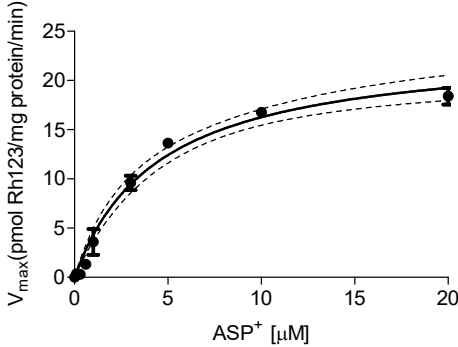
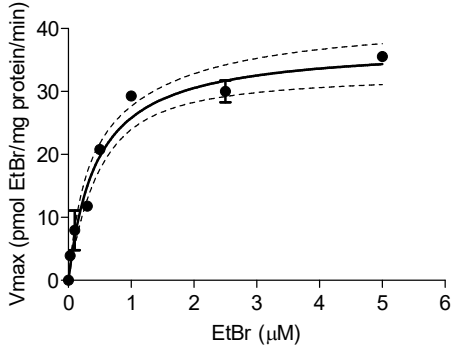
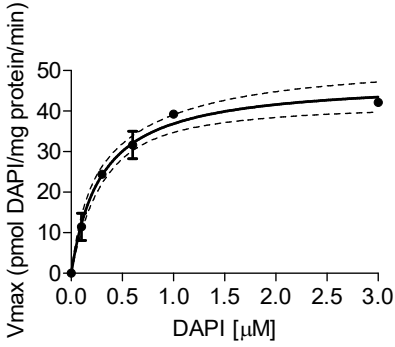
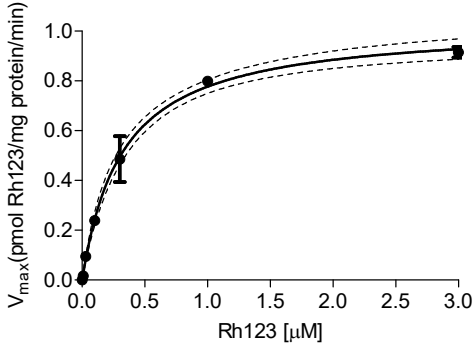
Figure S2. Tissue expression pattern of zebrafish *mate* genes: **A**, liver; **B**, kidney; **C**, intestine; **D**, gills; **E**, brain; **F**, gonads; and **G**, eye in female and male zebrafish, quantified with qRT-PCR. Results of two to three independent experiments for females and males (two to three pools of five individuals) are given, while the kidney expression data results are from two pools of 15 individuals. Data represent MNE (mean normalized expression) \pm SE normalized to the Ef1 α .

Figure S3.

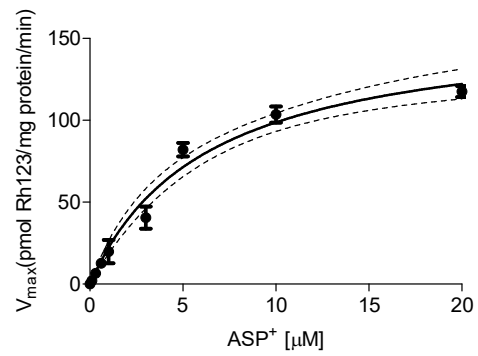
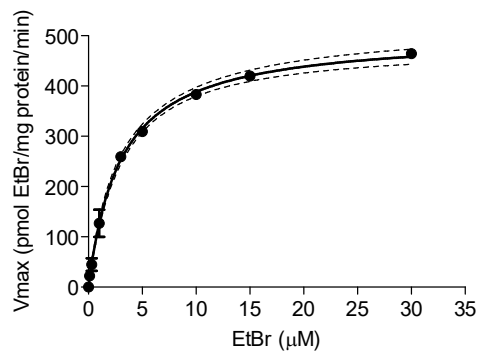
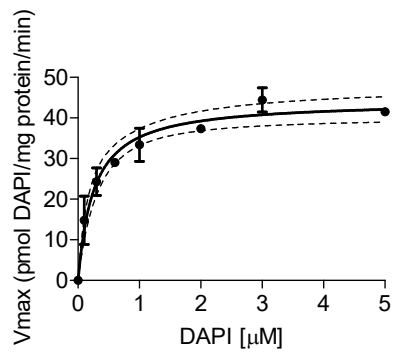
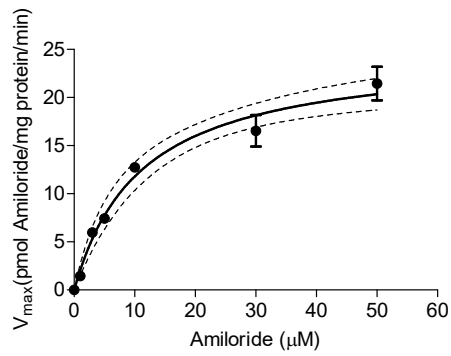
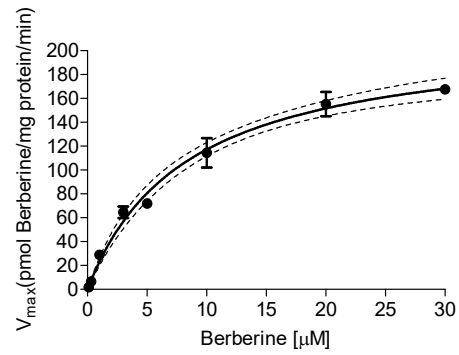
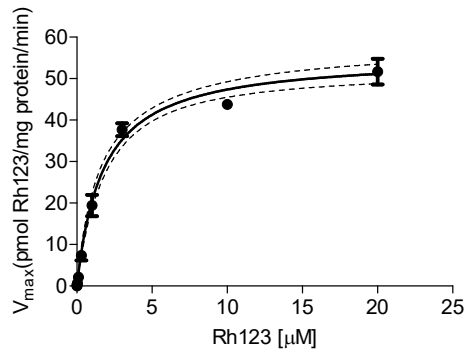
A. Mate3



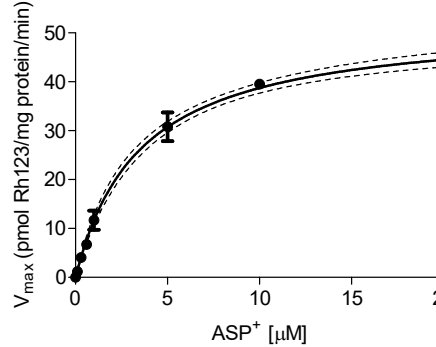
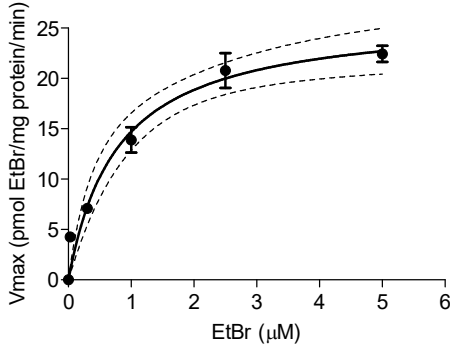
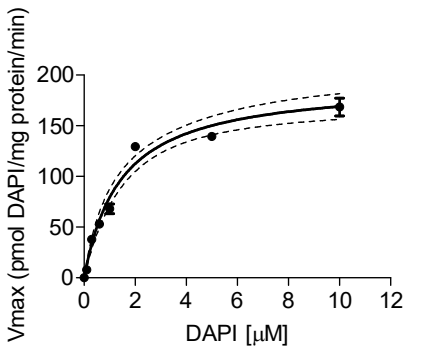
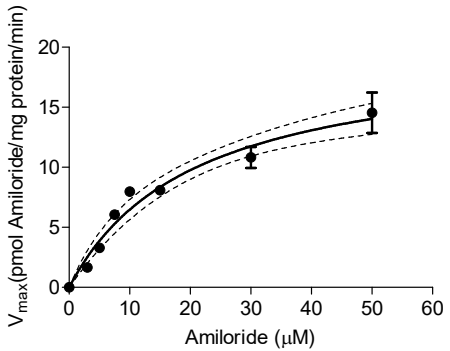
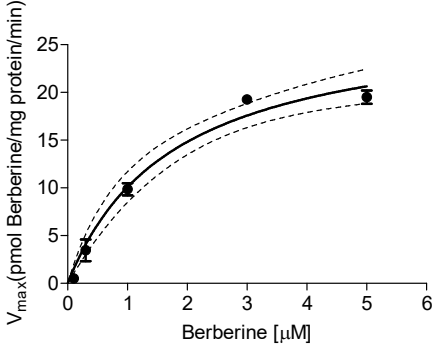
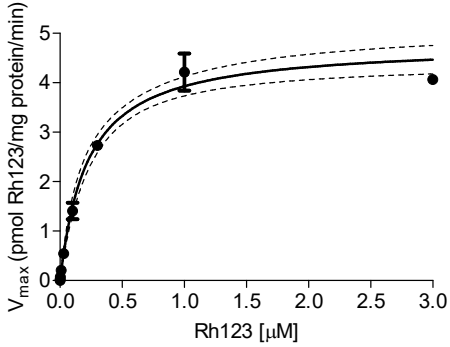
B. Mate4



C. Mate6



D. Mate7



E. Mate8

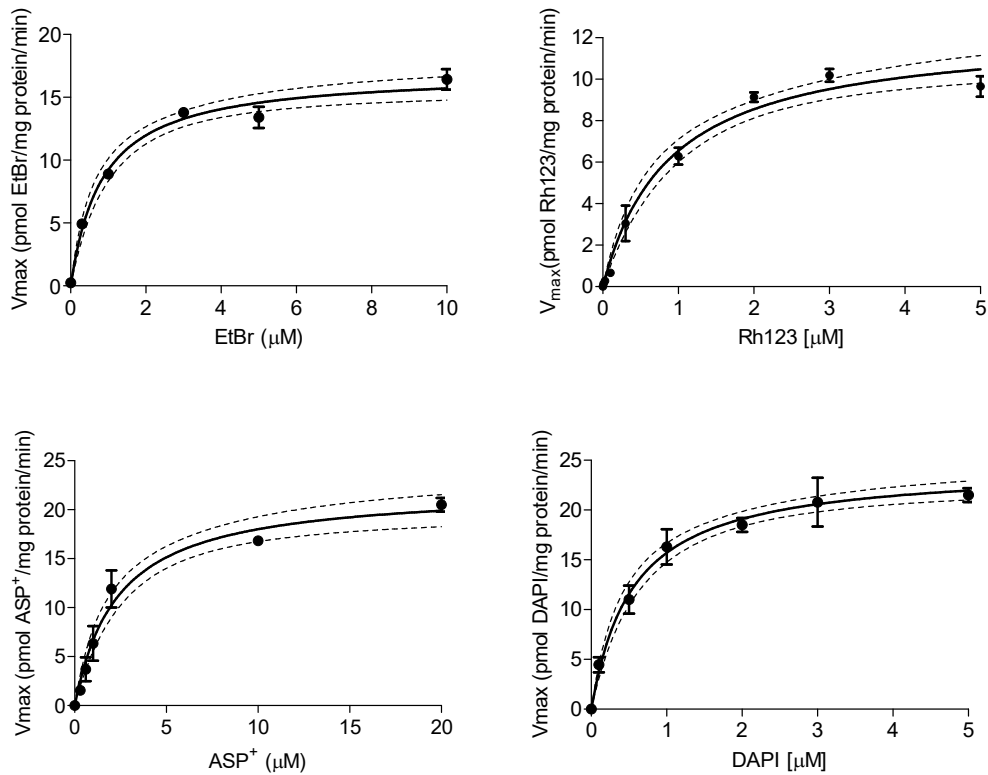


Figure S3. Michaelis-Menten kinetics of the uptake of fluorescent dyes: 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP⁺), 4',6-diamidino-2-phenylindole (DAPI), rhodamine 123 (Rh123), ethidium bromide (EtBr), berberine and amiloride by zebrafish Mates: **A**, Mate3; **B**, Mate4; **C**, Mate6; **D**, Mate7 and **E**, Mate8. Concentration dependence of dye uptake expressed as transport rate (pmol/mg protein/min) over dye concentration (μM) after/during 5 min incubation with the dye except with the DAPI where kinetics for 5-10 min was measured. The uptake into vector-transfected HEK293 cells (mock cells) was subtracted to obtain transporter-specific uptake and data were fitted in the GraphPad Prism 5. Each value represents the mean \pm SD from triplicate determinations of a representative experiment.

Figure S4.

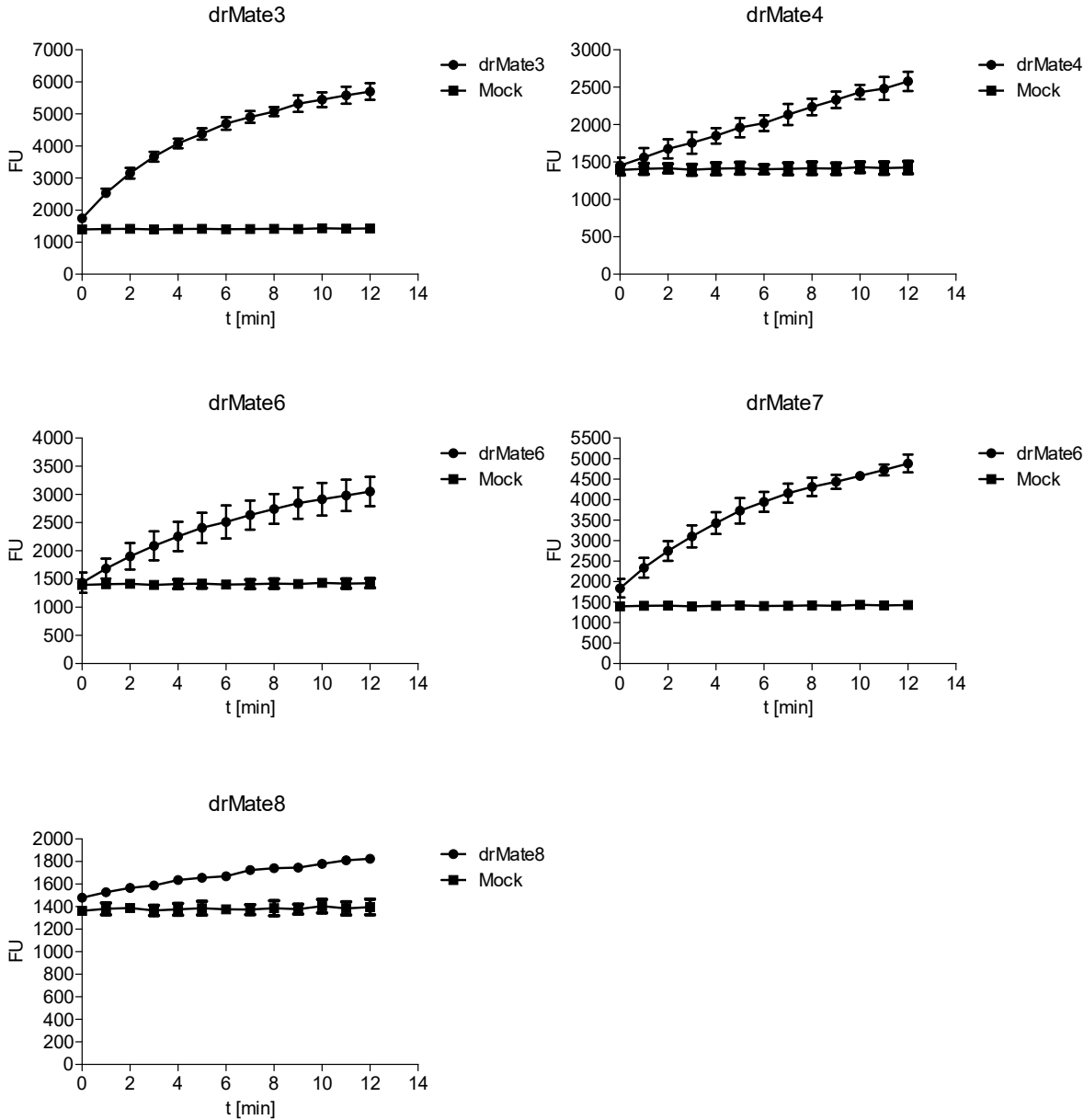
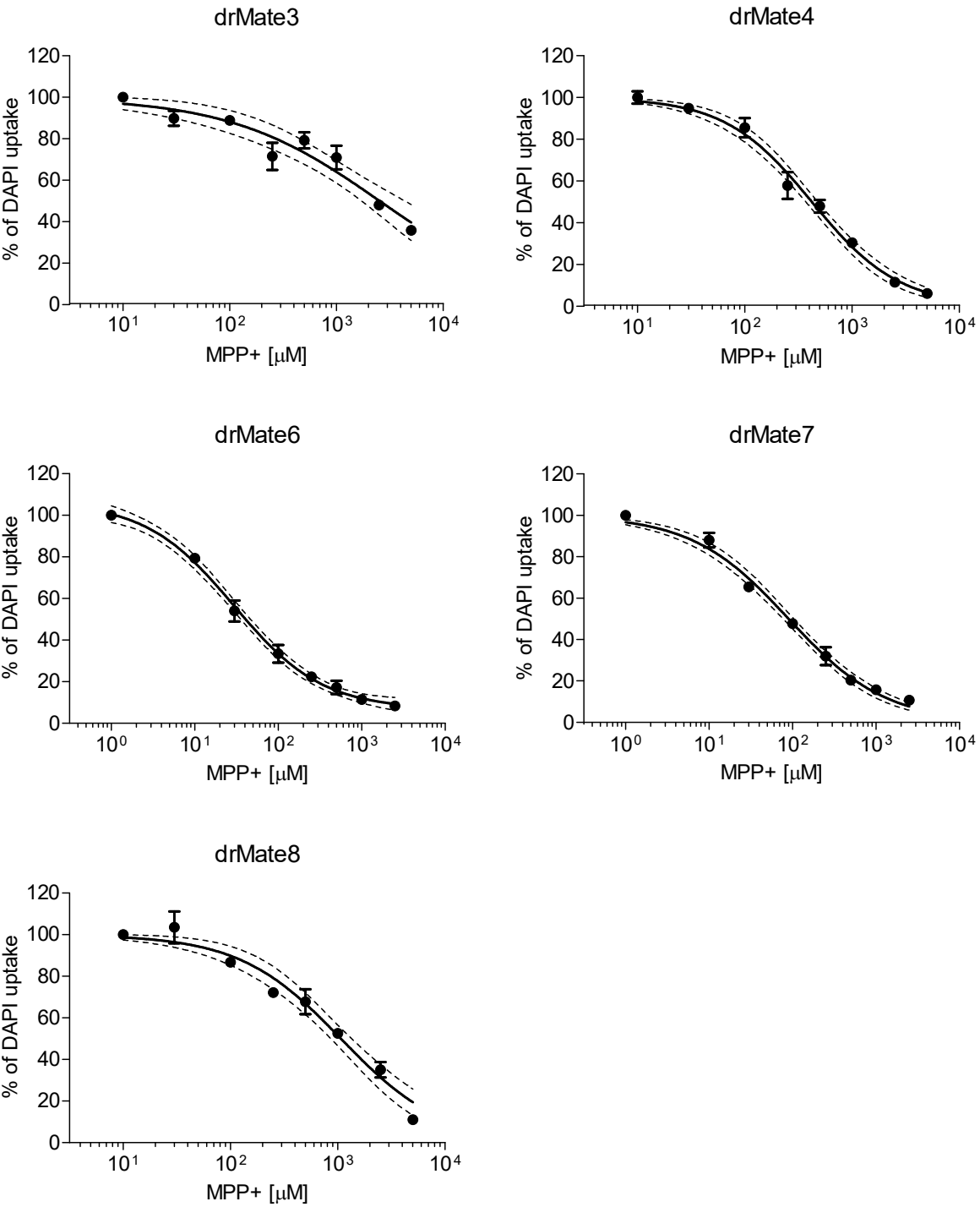


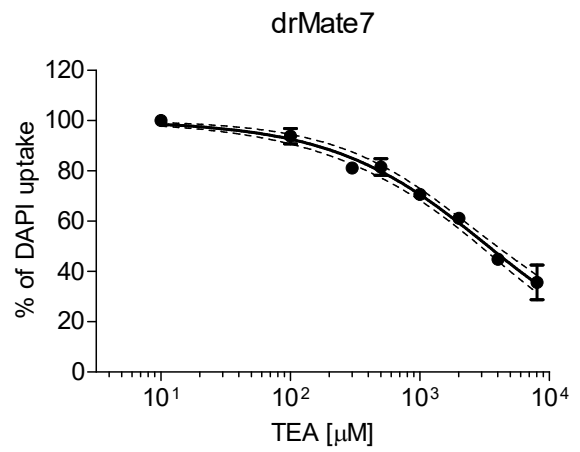
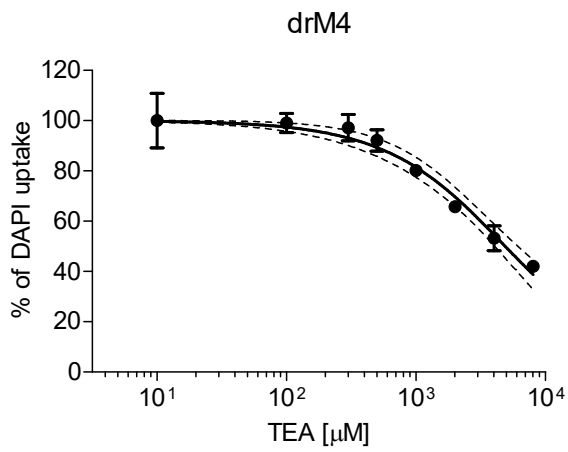
Figure S4. Time response curves for 1.0 μ M DAPI (Mate4, Mate6 and Mate8) and 2.5 μ M DAPI (Mate3 and Mate7).

Figure S5.

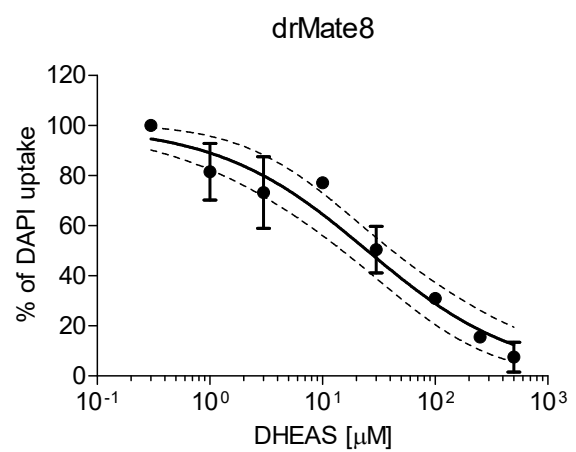
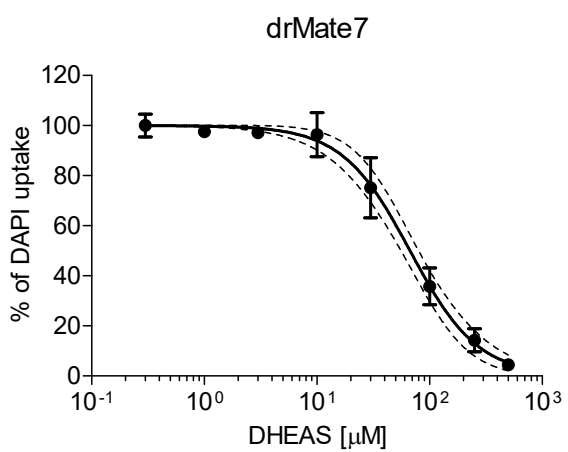
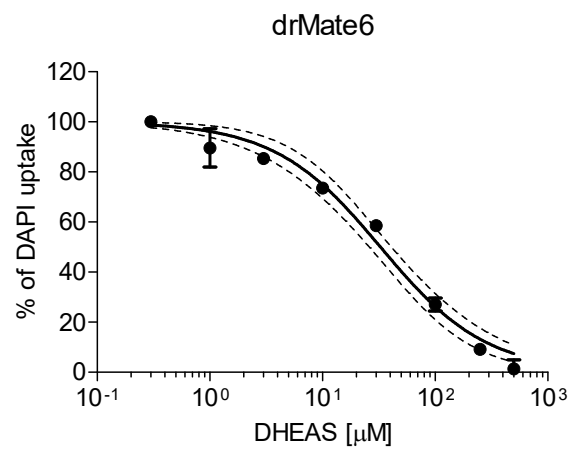
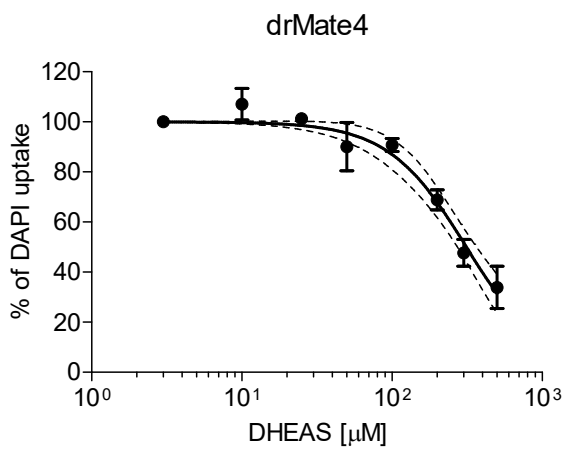
A. MPP+



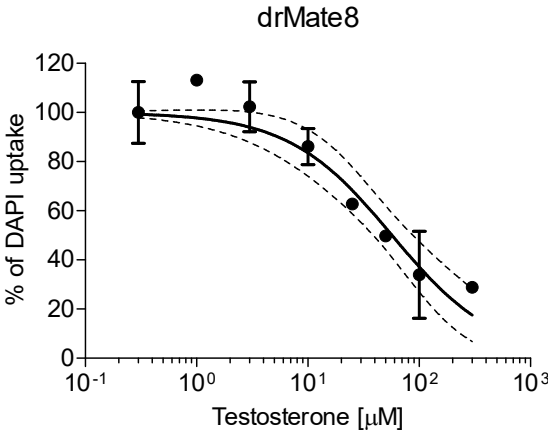
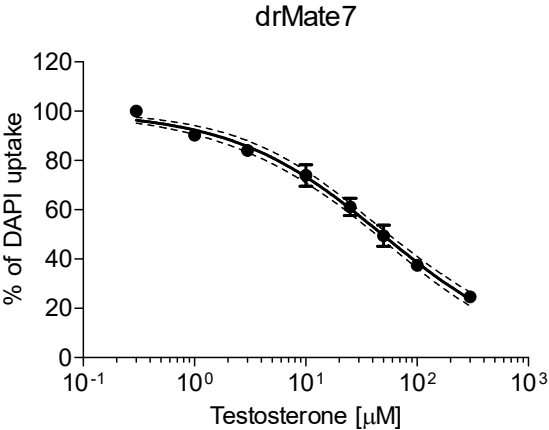
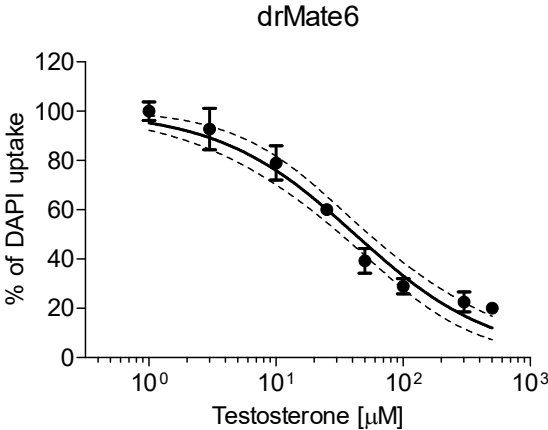
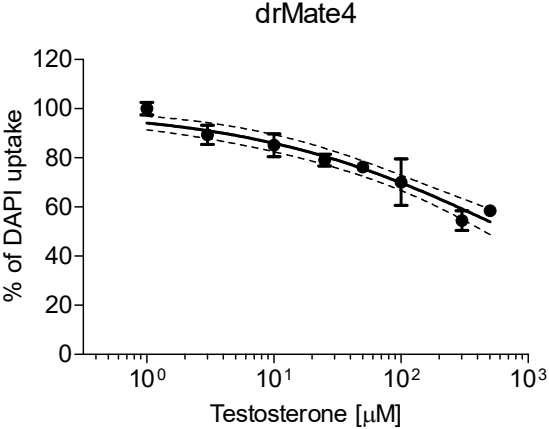
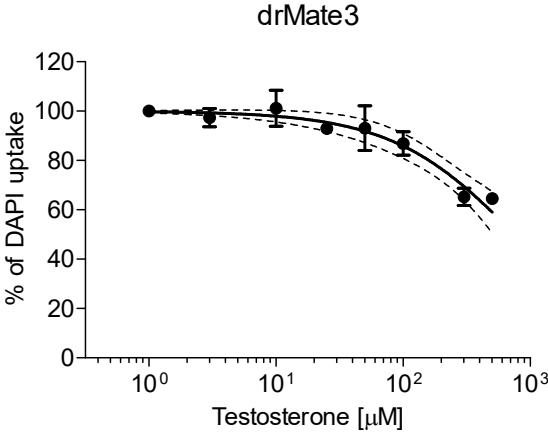
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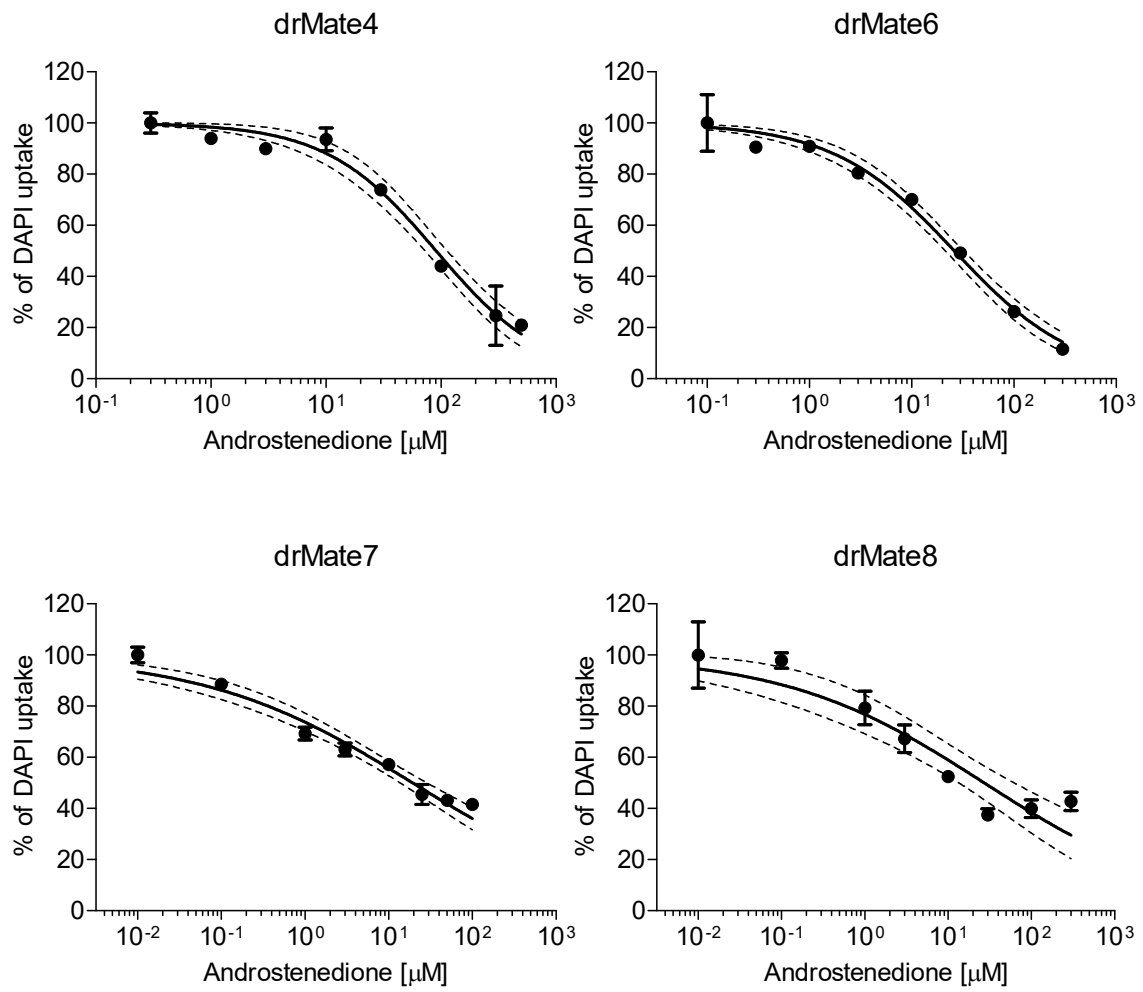
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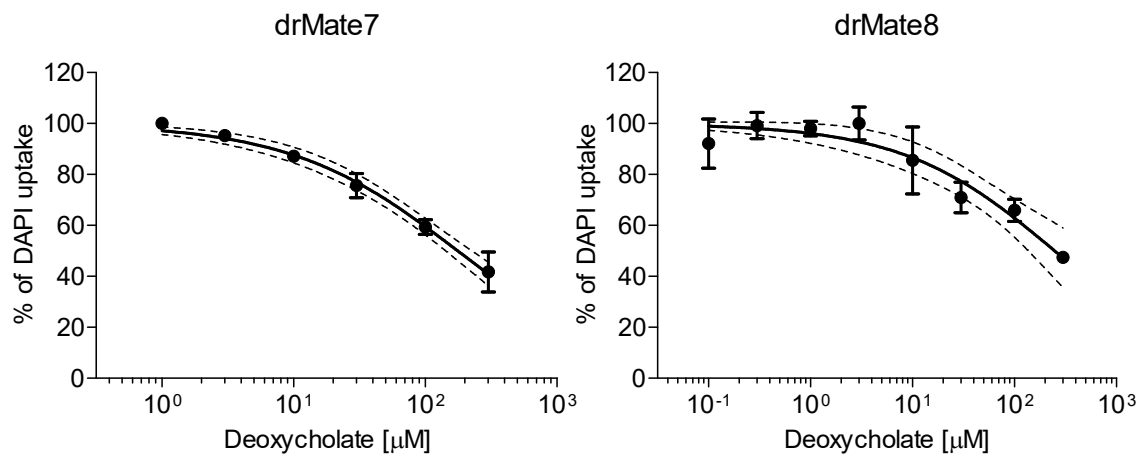
D. Testosterone



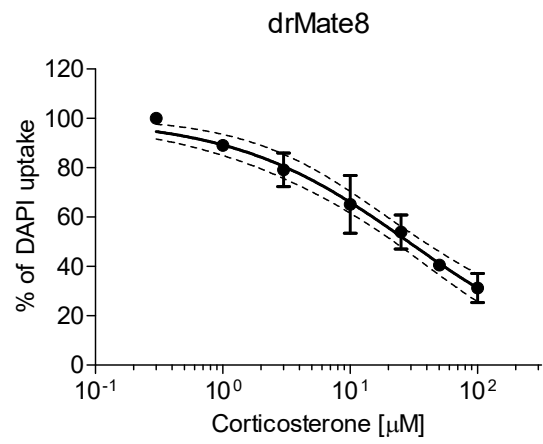
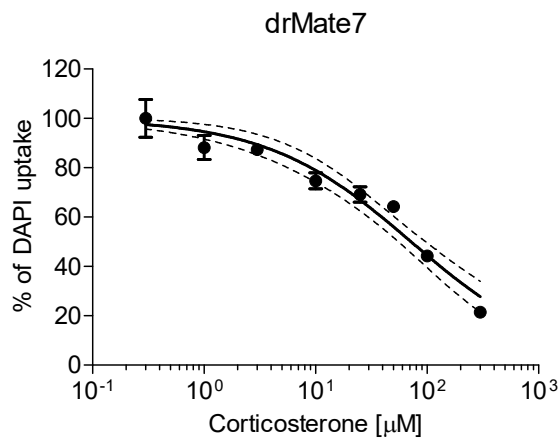
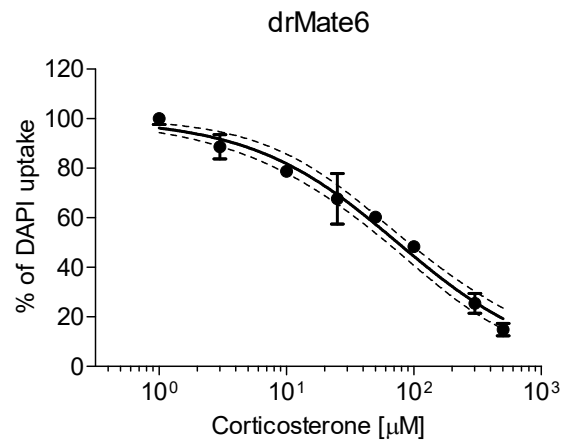
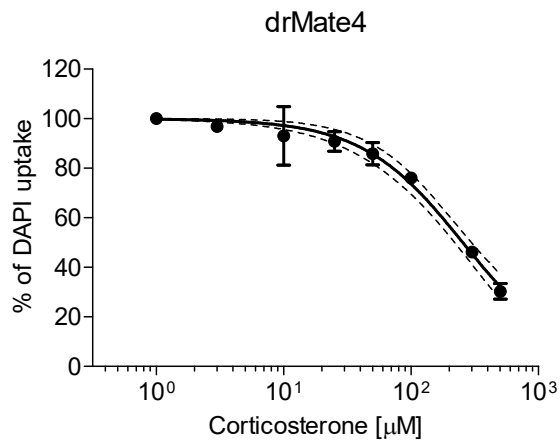
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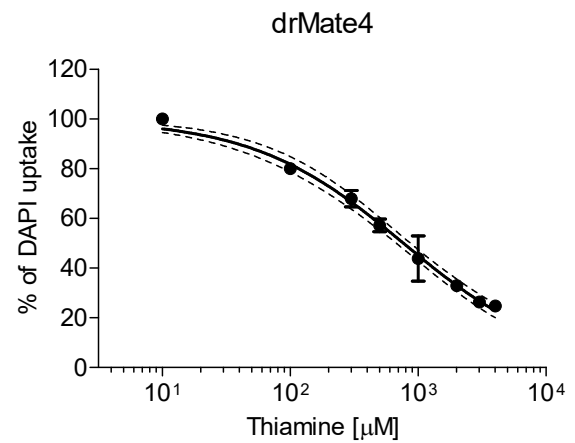
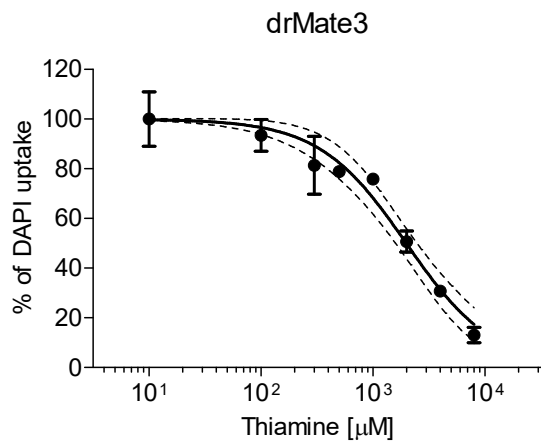
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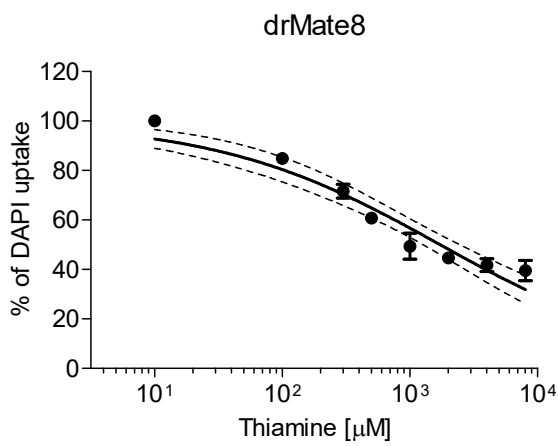
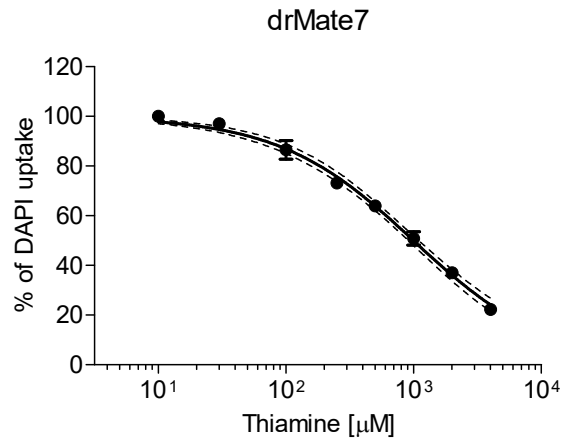
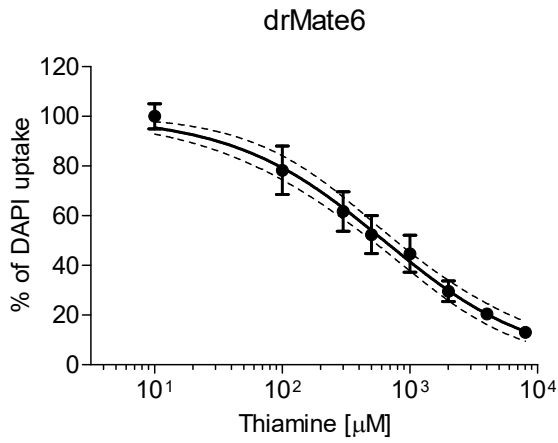


G. Corticosterone

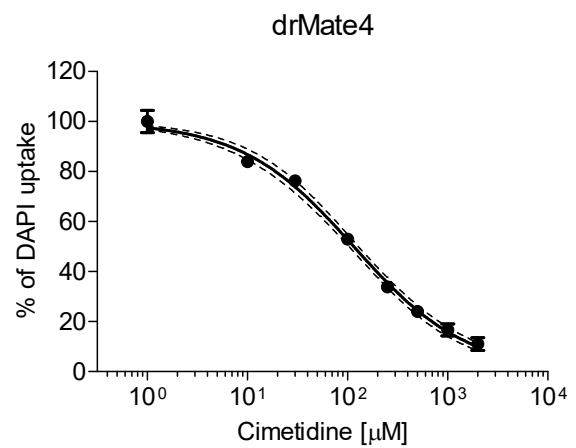
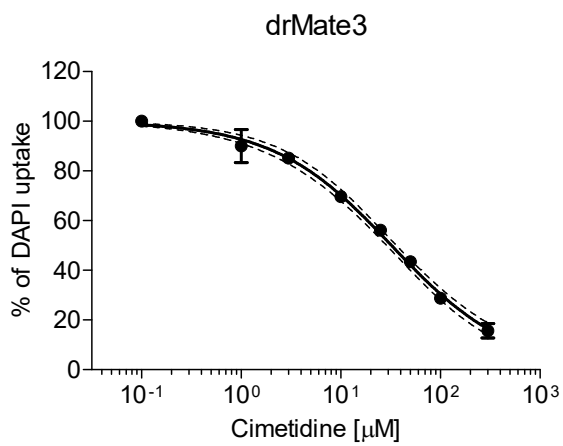


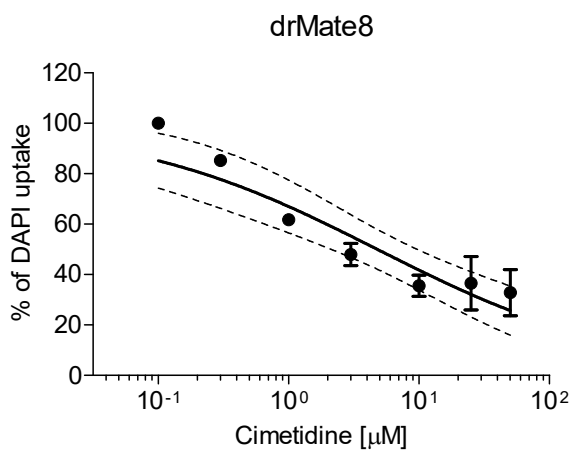
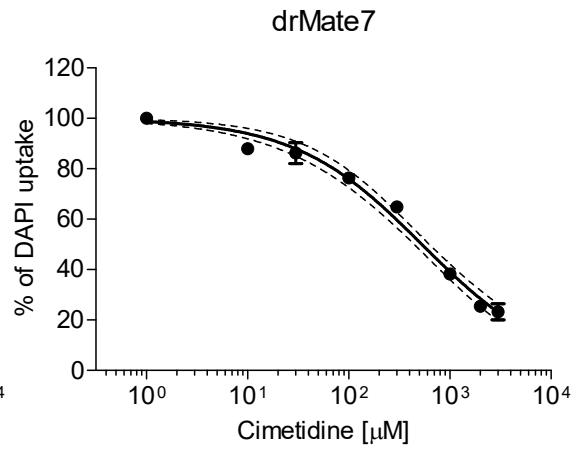
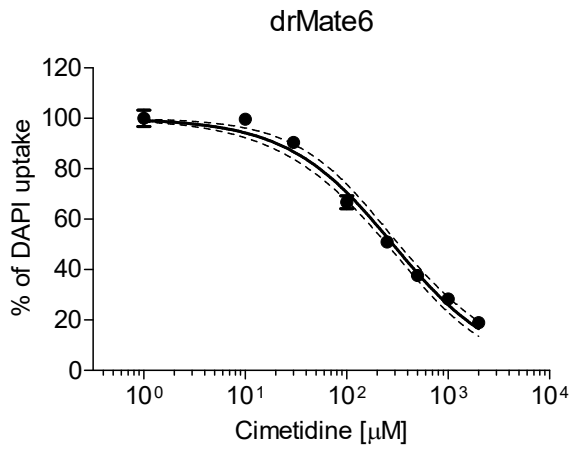
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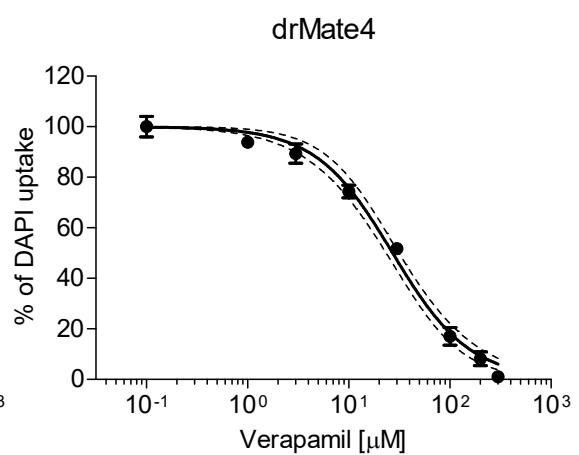
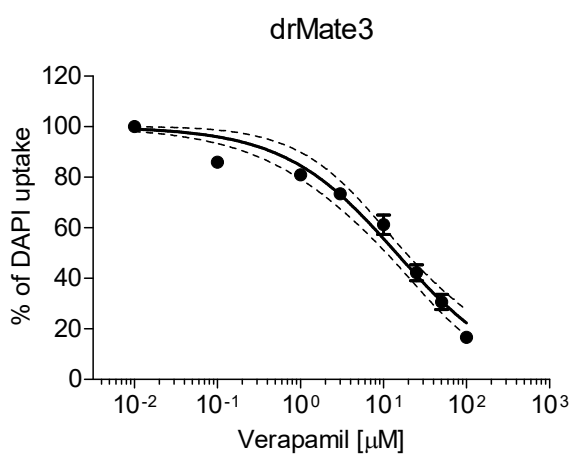


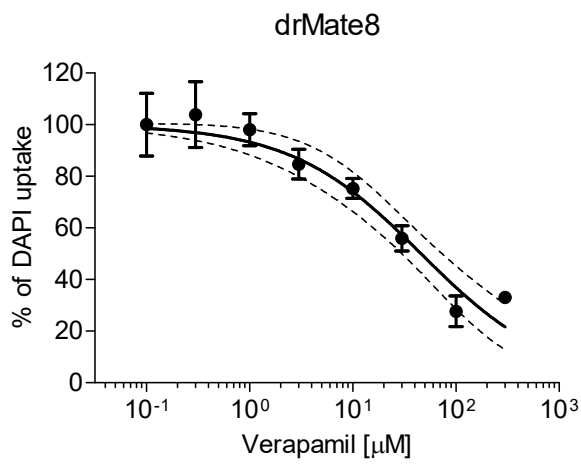
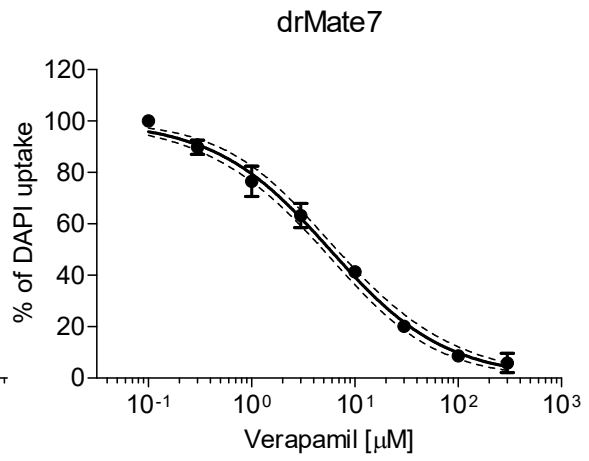
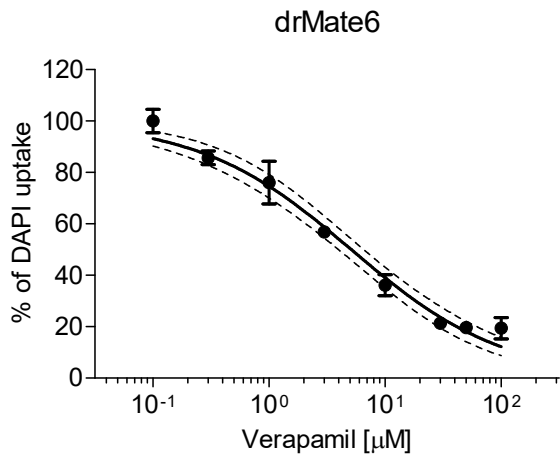
I. Cimetidine



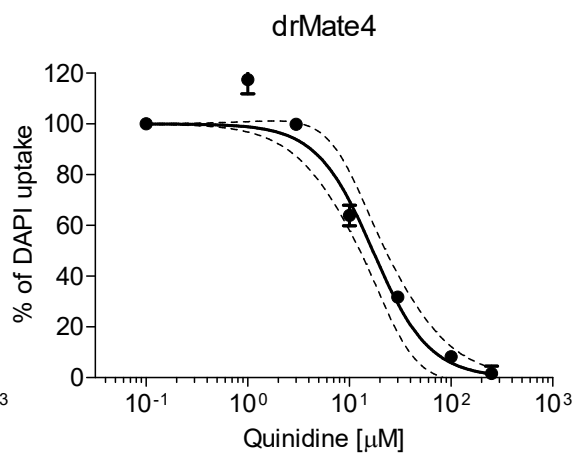
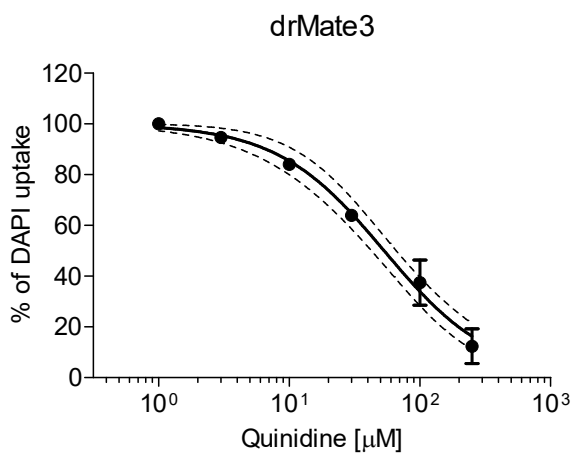


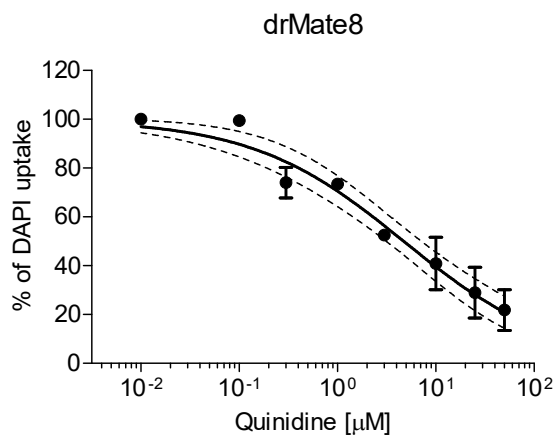
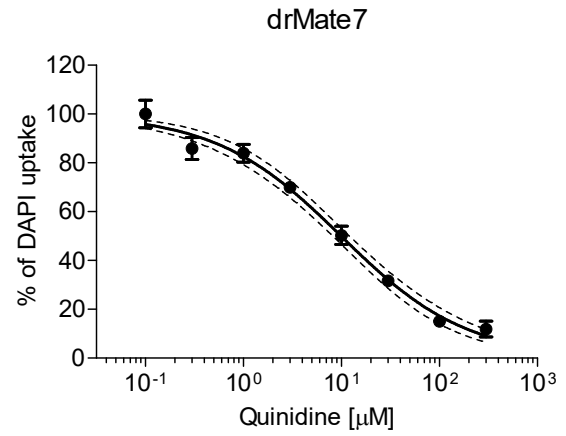
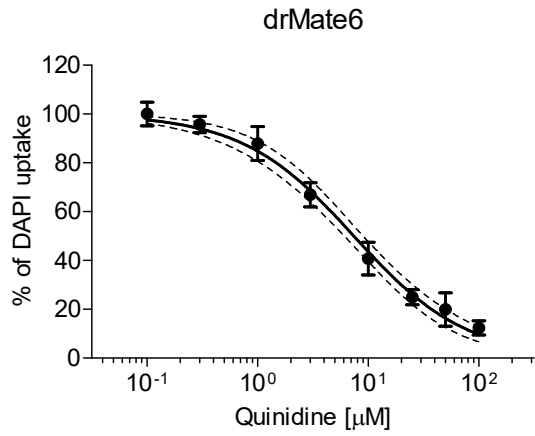
J. Verapamil



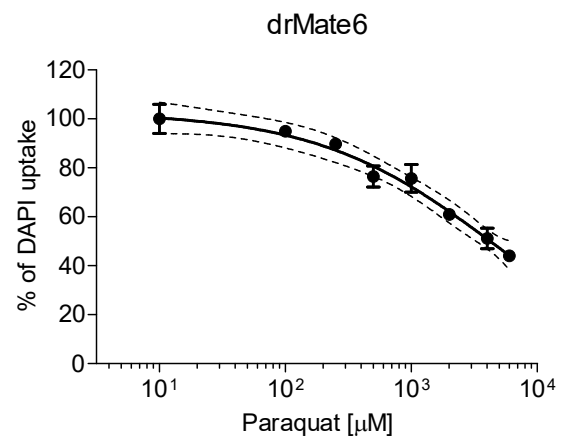
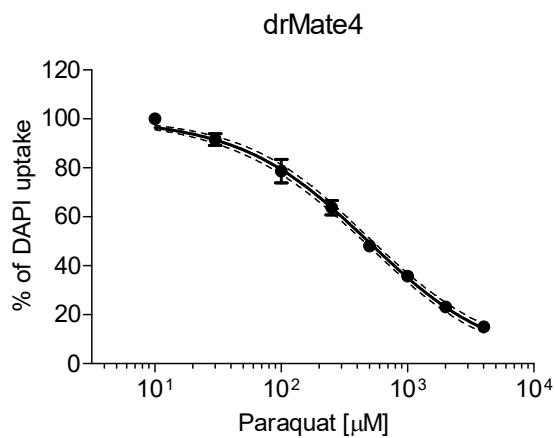


K. Quinidine





L. Paraquat



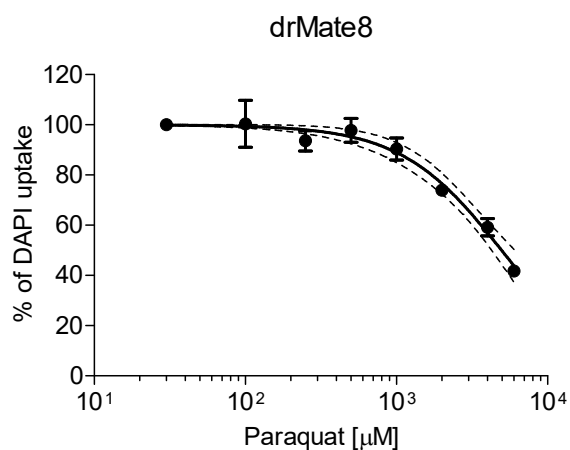


Figure S5. Concentration dependent inhibition of Mate mediated 4',6-diamidino-2-phenylindole (DAPI) uptake by typical interactors of mammalian MATEs: **A**, MPP+; **B**, TEA; **C**, DHEAS; **D**, Testosterone; **E**, Androstenedione; **F**, Deoxycholate; **G**, Corticosterone; **H**, Thiamine; **I**, Cimetidine; **J**, Veramil; **K**, Quinidine; **L**, Paraquat. The specific uptake of DAPI is expressed as a percentage relative to the DAPI uptake in the absence of an interactor (which is set to 100 %). For the IC_{50} calculations data were fitted to the sigmoidal four parameters dose–response model (variable slope) in the GraphPad Prism 5. Each data point represents the mean \pm SD from triplicate determinations of a representative experiment.