

Supporting Information

Table S1 Sequences and MRM^{HR} transitions of target peptides

Name	Alias	Probe peptide sequence	Standard peptide (m/z)		Internal standard (m/z)		CE (V)
			Q1	Fragment	Q1	Fragment	
Equilibrium nucleoside transporter 1	ENT1	WLPSL <u>V</u> LAR	527.8	755.3774 -	762.3774 -		
				755.5774	762.5774		
				658.3246 -	665.3246 -		
				658.5246	665.5246		
				571.2926 -	531.3	578.2926 -	30
				571.4926		578.4926	
Equilibrium nucleoside transporter 2	ENT2	SLTSYFLW <u>P</u> D <u>E</u> DSR	858.4	458.2085 -	465.2085 -		
				458.4085		465.4085	
				718.2002 -	724.2002 -		
				718.4002		724.4002	
				904.2795 -	910.2795 -		
				904.4795		910.4795	32
Concentrative nucleoside transporter 1	CNT1	AGSSFVF <u>G</u> EAL VK	656.4	1164.4320 -	861.4	1170.4320 -	
				1164.6320		1170.6320	
				1017.3636 -	1023.3636 -		
				1017.5636		1023.5636	
				763.3349 -	770.3349 -		
				763.5349		770.5349	
Concentrative nucleoside	CNT2	LAYPE <u>V</u> EESK	582.8	616.2665 -	659.9	623.2665 -	
				616.4665		623.4665	
				862.4033 -	869.4033 -		
				862.6033		869.6033	29
				559.2450 -	569.2450 -		
				559.4450		566.4450	
Concentrative nucleoside	CNT2	LAYPE <u>V</u> EESK	582.8	817.2938 -	585.8	823.2938 -	
				817.4938		823.4938	31
				980.3571 -		986.3571 -	

transporter 2				980.5571		986.5571
				720.2410 -		726.2410 -
				720.4410		726.4410
				591.1984 -		597.1984 -
				591.3984		597.3984
				659.2552 -		669.2552 -
				659.4552		669.4552
Concentrat ive				365.1183 -		375.1183 -
nucleoside transporter 3	CNT3	DHFFA <u>FK</u>	456.2	365.3183		375.3183
				512.1867 -		522.1867 -
				512.3867		522.3867
				765.2355 -		775.2355 -
				765.4355		775.4355
				759.3108 -		766.3108 -
				759.5108		766.5108
Multidrug resistance- associated protein 1	MRP1	TPSGNL <u>VNR</u>	479.3	672.2787 -		679.2787 -
				672.4787		679.4787
				501.2144 -		508.2144 -
				501.4144		508.4144
				615.2573 -		622.2573 -
				615.4573		622.4573
				742.3570 -		749.3570 -
				742.5570		749.5570
Multidrug resistance- associated protein 2	MRP2	QLLNN <u>ILR</u>	492.3	629.2729 -		636.2729 -
				629.4729		636.4729
				515.2300 -		522.2300 -
				515.4300		522.4300
				401.1871 -		408.1871 -
				401.3871		408.3871
				621.1991 -		631.1991 -
				621.3991		631.3991
Multidrug resistance- associated protein 3	MRP3	AEGEISDP <u>FR</u>	560.8	734.2832 -	565.8	744.2832 -
				734.4832		744.4832
				920.3472 -		930.3472 -

				920.5472		930.5472
				534.1671 -		544.1671 -
				534.3671		544.3671
				584.1827 -		584.1827 -
				584.3827		584.3827
Multidrug resistance- associated protein 4	MRP4	AP <u>V</u> LFFDR	482.8	697.2668 - 697.4668 796.3352 - 796.5352	486.3	704.2668 - 704.4668 803.3352 - 803.5352
				893.3880 - 893.5880		900.3880 - 900.5880
Multidrug resistance- associated protein 5	MRP5	SL <u>S</u> EASVAVDR	567.3	646.2519 - 646.4519 717.2890 - 717.4890 933.3636 - 933.5636	570.8	646.2519 - 646.4519 717.2890 - 717.4890 933.3636 - 933.5636
				559.2198 - 559.4198		559.2198 - 559.4198
Multidrug resistance- associated protein 6	MRP6	APETEPFL <u>R</u>	530.3	532.2242 - 532.4242 762.3145 - 762.5145 891.3571 - 891.5571	533.8	539.2242 - 539.4242 769.3145 - 769.5145 898.3571 - 898.5571
				661.2668 - 661.4668		668.2668 - 668.4668
Multidrug resistance protein 1	MDR1	NTTGAL <u>T</u> TR	467.8	618.2570 - 618.4570 719.3046 - 719.5046 490.1984 - 490.3984 561.2355 -	471.3	625.2570 - 625.4570 726.3046 - 726.5046 497.1984 - 497.3984 568.2355 -

			561.4355	568.4355	
Breast cancer resistance protein	BCRP	SSLLDV <u>L</u> AAR	522.8	644.2726 - 644.4726	651.2726 - 651.4726
				757.3567 - 757.5567	764.3567 - 764.5567
				529.2457 - 529.4457	526.3 536.2457 - 536.4457
				430.1772 - 430.3772	437.1772 - 437.3772

Theoretical m/z values of doubly charged ions of intact peptides (Q1) were assumed for precursor ions. Fragment ions from each precursor ion were separated and detected by ToF-MS. Four singly charged fragment ions for each precursor ion were extracted from the ToF-MS spectrum. Underlines indicate amino acid residues labeled with stable isotopes (¹³C and ¹⁵N).

Table S2 HPLC-MS parameters for protein analysis

Parameter	Value
Autosampler	4 °C
Column oven	25 °C
Curtain gas	20 p.s.i
Ion source gas 1	20 p.s.i
Ion source gas 2	0 p.s.i
Ionspray voltage (positive)	2300 V
Ion source temperature	150 °C

Mobile phases A and B consisted of 0.1% formic acid in 98% water and 2% acetonitrile, and 0.1% formic acid in 80% acetonitrile and 20% water, respectively. An aliquot (5 µl) of each sample was injected and loaded on a trap column (C18-3 µm, 200 µm x 0.5 mm, Eksigent) by passing 5 µl/min of mobile phase A for 10 min. Elution was done with a 150-min gradient as follows: 2% B at 0 to 1 min after loading, a linear gradient from 2% B to 10% B at 1 to 6 min, 10% B to 50% B at 6 to 120 min, 50% B to 90% B at 120 to 123 min, 90% B at 123 to 129 min, 90% B to 2% B at 129 to 130 min, and 2% B at 130 to 150 min. The flow rate was 200 nl/min. Trapped samples were separated on a nano column (C18-3 µm, 75 µm x 150 mm, Eksigent). Multiple products derived from single peptide were monitored at specific m/z fragments (Table S1).

Table S3 Protein concentrations of enzymes in cytosolic fraction of pancreatic cancer cell lines

Quantified protein	Protein concentration in cells (fmol / μg cytosolic protein)				
	PK9 [†]	CFPac-1	PK1	SUIT-2	AsPc-1
Enzymes in <i>salvage</i> pathway					
dCK	0.477 \pm 0.047	0.351 \pm 0.016	0.209 \pm 0.006	0.188 \pm 0.040	0.152 \pm 0.017
UMP-CMP kinase	6.17 \pm 0.23	4.46 \pm 0.11	4.00 \pm 0.17	3.72 \pm 0.18	3.73 \pm 0.14
CDA	0.276 \pm 0.018	0.390 \pm 0.040	<0.10	<0.10	1.15 \pm 0.03
DCTD	1.12 \pm 0.04	1.39 \pm 0.07	1.53 \pm 0.07	0.815 \pm 0.055	1.38 \pm 0.08
cN-IA	<0.05	<0.02	<0.02	<0.02	<0.02
cN-IB	<0.05	<0.02	<0.02	<0.02	<0.02
cN-II	2.38 \pm 0.22	2.29 \pm 0.15	3.60 \pm 0.15	0.871 \pm 0.044	1.74 \pm 0.09
cN-III	0.730 \pm 0.083	0.730 \pm 0.076	1.32 \pm 0.11	1.09 \pm 0.06	1.42 \pm 0.23
Enzymes in <i>de novo</i> pathway					
RRM1	1.33 \pm 0.01	1.25 \pm 0.04	2.46 \pm 0.04	0.272 \pm 0.034	1.44 \pm 0.12
RRM2	<0.10	0.787 \pm 0.103	1.34 \pm 0.21	<0.10	1.15 \pm 0.15
CTPS1	2.03 \pm 0.06	1.68 \pm 0.05	2.78 \pm 0.05	1.44 \pm 0.09	1.02 \pm 0.06
CTPS2	0.489 \pm 0.019	0.376 \pm 0.027	0.750 \pm 0.034	0.351 \pm 0.069	0.374 \pm 0.028

[†]Protein concentrations in PK9 cells are cited from Ohmine *et al.*, 2012 (8). Cytosolic fraction from each cell (50 μg protein), spiked with 100 fmol of stable isotope-labeled peptide mixture, was injected into the LC-MS/MS. The protein concentration (fmol/ μg protein) was calculated from the peak area ratio of target and stable isotope-labeled peptide in each MRM transition. The protein concentration of each enzyme was determined as the average from three or four MRM transitions showing signal peaks. The protein concentration of each enzyme in each sample is shown as mean \pm SEM (n=3-4). If the value was below the limit of quantification, the value of the quantification limit was used.

Table S4 Protein concentrations of transporters in membrane fraction of pancreatic cancer cell lines

Quantified protein	Protein concentration in cells (fmol / μg membrane protein)				
	PK9 [†]	CFPac-1	PK1	SUIT-2	AsPc-1
ENT1	1.92 \pm 0.08	2.46 \pm 0.25	3.84 \pm 0.14	4.05 \pm 0.38	6.19 \pm 0.71
ENT2	<2.00	<1.00	<1.00	<1.00	<1.00
CNT1	<0.20	<1.00	<1.00	<1.00	<1.00
CNT2	<0.20	<1.00	<1.00	<1.00	<1.00
CNT3	<0.20	<1.00	<1.00	<1.00	<1.00
MRP1	1.39 \pm 0.16	<1.00	<1.00	<1.00	<1.00
MRP2	0.504 \pm 0.115	<0.10	0.741 \pm 0.219	<0.10	<0.10
MRP3	<0.20	1.20 \pm 0.14	0.690 \pm 0.083	1.21 \pm 0.17	<0.10
MRP4	0.508 \pm 0.055	0.507 \pm 0.081	<0.10	1.90 \pm 0.15	1.84 \pm 0.19
MRP5	<2.00	<1.00	<1.00	<1.00	<1.00
MRP6	<0.20	<0.10	<0.10	<0.10	<0.10
MDR1	<0.20	<1.00	<1.00	<1.00	<1.00
BCRP	0.352 \pm 0.040	<0.20	11.1 \pm 0.6	<0.20	2.49 \pm 0.33

[†]Protein concentrations in PK9 cells are cited from Ohmine *et al.*, 2012 (8). Membrane fraction from each cell (1 μg protein), spiked with 10 fmol stable isotope-labeled peptide mixture, was injected into the LC-MS/MS. The protein concentration (fmol/ μg protein) was calculated from the peak area ratio of target and stable isotope-labeled peptides in each MRM^{HR} transition. The protein concentration of each transporter was determined as the average of three or four MRM^{HR} transitions showing signal peaks. The protein concentration of each transporter in each sample is given as mean \pm SEM (n=3-4). If the value was below the limit of quantification, the value of the quantification limit was used.

Table S5-1 Protein concentrations of enzymes in cytosolic fraction of samples No. 1-5

Quantified protein	Protein concentration in tissues (fmol / µg cytosolic protein)				
	No. 1	No. 2	No. 3	No. 4	No. 5
Enzymes in <i>salvage</i> pathway					
dCK	0.181 ± 0.021	0.294 ± 0.016	0.370 ± 0.026	0.225 ± 0.031	0.366 ± 0.039
UMP-CMP kinase	3.57 ± 0.11	4.85 ± 0.13	16.1 ± 0.6	5.71 ± 0.16	4.50 ± 0.17
CDA	0.279 ± 0.038	0.542 ± 0.071	0.495 ± 0.021	1.45 ± 0.06	0.372 ± 0.025
DCTD	0.648 ± 0.051	1.44 ± 0.10	1.72 ± 0.08	1.18 ± 0.09	1.10 ± 0.11
cN-IA	<1.00	<1.00	<1.00	<1.00	<1.00
cN-IB	<1.00	<1.00	<1.00	<1.00	<1.00
cN-II	0.819 ± 0.036	1.68 ± 0.08	0.663 ± 0.061	1.75 ± 0.10	1.57 ± 0.17
cN-III	0.482 ± 0.073	0.888 ± 0.261	1.66 ± 0.53	1.83 ± 0.29	0.262 ± 0.053
Enzymes in <i>de novo</i> pathway					
RRM1	<0.20	<0.20	<0.20	<0.20	<0.20
RRM2	<0.20	<0.20	<0.20	<0.20	<0.20
CTPS1	0.318 ± 0.03	0.422 ± 0.012	0.593 ± 0.090	0.731 ± 0.033	0.430 ± 0.026
CTPS2	<0.10	<0.10	0.847 ± 0.027	0.385 ± 0.008	0.207 ± 0.031

Table S5-2 Protein concentrations of enzymes in cytosolic fraction of samples No. 6-10

Quantified protein	Protein concentration in tissues (fmol / µg cytosolic protein)				
	No. 6	No. 7	No. 8	No. 9	No. 10
Enzymes in <i>salvage</i> pathway					
dCK	0.479 ± 0.067	0.556 ± 0.055	0.693 ± 0.046	0.544 ± 0.035	0.794 ± 0.032
UMP-CMP kinase	33.0 ± 1.5	14.5 ± 0.8	3.64 ± 0.1	10.0 ± 0.2	19.5 ± 0.3
CDA	2.48 ± 0.03	0.448 ± 0.076	0.233 ± 0.020	<0.20	<0.20
DCTD	1.49 ± 0.21	1.52 ± 0.07	0.804 ± 0.097	1.15 ± 0.08	1.80 ± 0.21
cN-IA	<1.00	<1.00	<1.00	<1.00	<1.00
cN-IB	<1.00	<1.00	<1.00	<1.00	<1.00
cN-II	2.31 ± 0.27	0.563 ± 0.049	0.778 ± 0.082	2.14 ± 0.03	2.23 ± 0.04
cN-III	1.97 ± 0.29	1.49 ± 0.10	0.554 ± 0.053	1.25 ± 0.15	1.43 ± 0.21
Enzymes in <i>de novo</i> pathway					
RRM1	<0.20	<0.20	<0.20	<0.20	<0.20
RRM2	<0.20	<0.20	<0.20	<0.20	<0.20
CTPS1	1.28 ± 0.03	0.400 ± 0.051	0.565 ± 0.024	0.831 ± 0.063	1.34 ± 0.09
CTPS2	0.828 ± 0.005	1.00 ± 0.02	0.248 ± 0.034	0.993 ± 0.047	0.720 ± 0.026

Average, SEM and CV were calculated, excluding samples under the detection limit. Cytosolic fraction from 10 pancreatic cancer tissues (50 µg protein), spiked with 100 fmol of stable isotope-labeled peptide mixture, was injected into the LC-MS/MS. The protein concentration (fmol/µg protein) was calculated from the peak area ratio of target and stable isotope-labeled peptide in each MRM transition. The protein concentration of each enzyme was determined as the average from three or four MRM transitions showing signal peaks. The protein concentration of each enzyme in each sample is shown as mean ± SEM (n=3-4). If the value was below the limit of quantification, the value of the quantification limit was used.

Table S6-1 Protein concentrations of transporters in membrane fraction of samples No. 2-5

Quantified protein	Protein concentration in tissues (fmol / µg membrane protein)			
	No. 2	No. 3	No. 4	No. 5
ENT1	2.76 ± 0.32	3.38 ± 0.04	2.61 ± 0.09	2.55 ± 0.14
ENT2	<1.00	<1.00	<1.00	<1.00
CNT1	<1.00	<1.00	<1.00	<1.00
CNT2	<1.00	<1.00	<1.00	<1.00
CNT3	<1.00	<1.00	<1.00	<1.00
MRP1	<1.00	<1.00	<1.00	<1.00
MRP2	<0.10	<0.10	<0.10	<0.10
MRP3	<0.10	<0.10	<0.10	<0.10
MRP4	<0.50	<0.50	<0.50	<0.50
MRP5	<1.00	<1.00	<1.00	<1.00
MRP6	0.268 ± 0.034	0.299 ± 0.042	<0.10	<0.10
MDR1	<1.00	<1.00	<1.00	<1.00
BCRP	<0.50	<0.50	<0.50	<0.50

Table S6-2 Protein concentrations of transporters in membrane fraction of samples No. 6-10

Quantified protein	Protein concentration in tissues (fmol / µg membrane protein)			
	No. 6	No. 7	No. 9	No. 10
ENT1	3.59 ± 0.16	2.60 ± 0.21	3.97 ± 0.61	3.21 ± 0.17
ENT2	<1.00	<1.00	<1.00	<1.00
CNT1	<1.00	<1.00	<1.00	<1.00
CNT2	<1.00	<1.00	<1.00	<1.00
CNT3	<1.00	<1.00	<1.00	<1.00
MRP1	<1.00	<1.00	<1.00	<1.00
MRP2	<0.10	<0.10	<0.10	<0.10
MRP3	<0.10	<0.10	<0.10	<0.10
MRP4	<0.50	<0.50	<0.50	<0.50
MRP5	<1.00	<1.00	<1.00	<1.00
MRP6	<0.10	<0.10	<0.10	<0.10
MDR1	<1.00	<1.00	<1.00	<1.00
BCRP	<0.50	<0.50	<0.50	<0.50

Average, SEM and CV were calculated, excluding samples under the detection limit.

Membrane fraction from 8 pancreatic cancer tissues (1 µg protein), spiked with 10 fmol stable isotope-labeled peptide mixture, was injected into the LC-MS/MS. The protein concentration (fmol/µg protein) was calculated from the peak area ratio of target and stable isotope-labeled peptides in each MRM^{HR} transition. The protein concentration of each transporter was determined as the average of three or four MRM^{HR} transitions showing signal peaks. The protein concentration of each transporter in each sample is given as mean ± SEM (n=3-4). If the value was below the limit of quantification, the value of the quantification limit was used.