Supplemental data

I- Figure Legends:

Supplemental Figure S1: **PG and BMP synthesis in wild type and mutant CHO cells**. Wild type cells (□), PGP synthase- deficient mutants (■), PGP synthase-transfected wild type cells (◪) and PGP synthase-transfected mutants (▨) were labelled at confluence in complete medium containing ^{32}Pi (10μCi/ml) for 3 days at 33^{o}C. Then phospholipids were extracted, purified by DEAE-Sephadex A-25 and separated on 2D-TLC (Method 2). The radioactive spots were quantified with BAS5000 Bioimaging Analyzer as described in Figure 3. Results are expressed in arbitrary units (AU) per μg of proteins.

Supplemental Figure S2: **Kinetic incorporation of ^{32}P into phospholipids of wild type and mutant CHO cells**.

Wild type cells (—◆—), PGP synthase-deficient mutants (—□—) and PGP synthase-transfected mutants (—■—) were prepared as described in Figure 4. Neutral and acidic phospholipids were separated on 1D-TLC (Methods 3 and 4) after DEAE column purification. The radioactive spots were quantified with BAS5000 Bioimaging Analyzer as described in Experimental Procedures. Results are expressed as arbitrary unit (AU) per μg proteins. Values are from one experiment representative of 3 independent experiments.

Supplemental Figure S3: **Turnover of newly synthetized phospholipids in ^{32}Pi-pulse-labeled wild type and mutant CHO cells.**

Wild type cells (left panels) and PGP synthase-deficient mutants (right panels) were prepared as described in Figure 5. At indicated time, phospholipids were extracted and separated on 2D-TLC (method 2) as described in Experimental Procedures. SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine. Results are expressed as arbitrary unit (AU) per μg proteins. Values are from one experiment representative of 2 independent experiments.

Supplemental Figure S4: **Turnover of phospholipids in wild type and mutant CHO cells continuously labelled with ^{32}Pi.** Wild type cells (—◆—), PGP synthase-deficient mutants (—■—) , PGP synthase-transfected wild type cells (—◇—) and PGP synthase-transfected mutants (—□—) were prepared as described in Figure 6. At indicated time, phospholipids were extracted, purified by DEAE column and separated on 2D-TLC (method 2) as described in Experimental Procedures. Results are expressed as arbitrary unit (AU) per μg proteins.

II- Tables:

Supplemental Table S1: Phospholipid composition of wild type and PGP synthase-deficient CHO mutants after 24h ^{32}Pi labeling.

Wild type and mutant cells were cultured to confluence in complete medium containing ^{32}Pi (10 μCi/ml) for 24 h at 33°C, 37°C or 40° C and phospholipids were analyzed as in Table 1. [a] PE spot includes PG in this TLC system. Results are expressed in percentage of total phospholipids and are the mean±sem of 3 (40°C) or 4 (33 et 37°C) experiments (*, $p<0.05$; **, $p< 0.01$).

Phospholipids (%)	Wild type 33°C	Mutants 33°C	Wild type 37°C	Mutants 37°C	Wild type 40°C	Mutants 40°C
SM	5.39±0.54	5.62±0.51	5.32±0.25	5.28±0.40	5.61±0.28	6.26±0.19
PC	52.68±2.21	59.57±1.92*	57.68±2.59	59.37±2.75	60.42±3.83	60.03±1.47
PE +PG[a]	25.98±1.96	21.27±2.15*	22.94±4.05	21.40±3.95	20.59±3.54	20.76±1.85
PI	6.95±1.38	5.73±0.81	6.93±0.97	6.98±1.36	6.76±1.05	6.66±0.85
PS	4.22±0.42	3.97±0.40	3.44±0.53	3.34±0.14	3.18±0.49	3.36±0.13
PA	0.56±0.06	0.72±0.15	0.49±0.14	0.59±0.20	0.58±0.10	0.57±0.11
CL	2.29±0.50	2.38±0.37	1.91±0.36	2.35±0.35	1.58±0.24	1.84±0.09
BMP	1.74±0.24	0.59±0.20**	1.08±0.32	0.56±0.10*	1.13±0.30	0.43±0.10*
X	0.18±0.04	0.15±0.11	0.20±0.10	0.13±0.06	0.15±0.05	0.10±0.03
Neutral Lipids	84.05±1.30	86.46±0.85	85.94±1.51	86.05±1.64	86.61±1.60	87.04±0.83
Acidic Lipids	15.95±1.30	13.54±0.85	14.06±1.51	13.95±1.64	13.39±1.60	12.96±0.83

Supplemental Table S2: Fatty acid composition of BMP in wild type cells, PGP synthase-deficient mutants and PGP synthase-transfected mutants.

Wild type CHO cells, PGP synthase-deficient mutants and stably PGP synthase-transfected mutants were cultured to confluence in complete medium at 33^{o}C and phospholipids were extracted, purified by DEAE-Sephadex A-25 and separated on 2D-TLC. Fatty acid composition of BMP was analyzed by gas chromatography. Results are expressed as mole % and are the mean±sem of 3 independent determinations. MUFA, monounsaturated fatty acids.

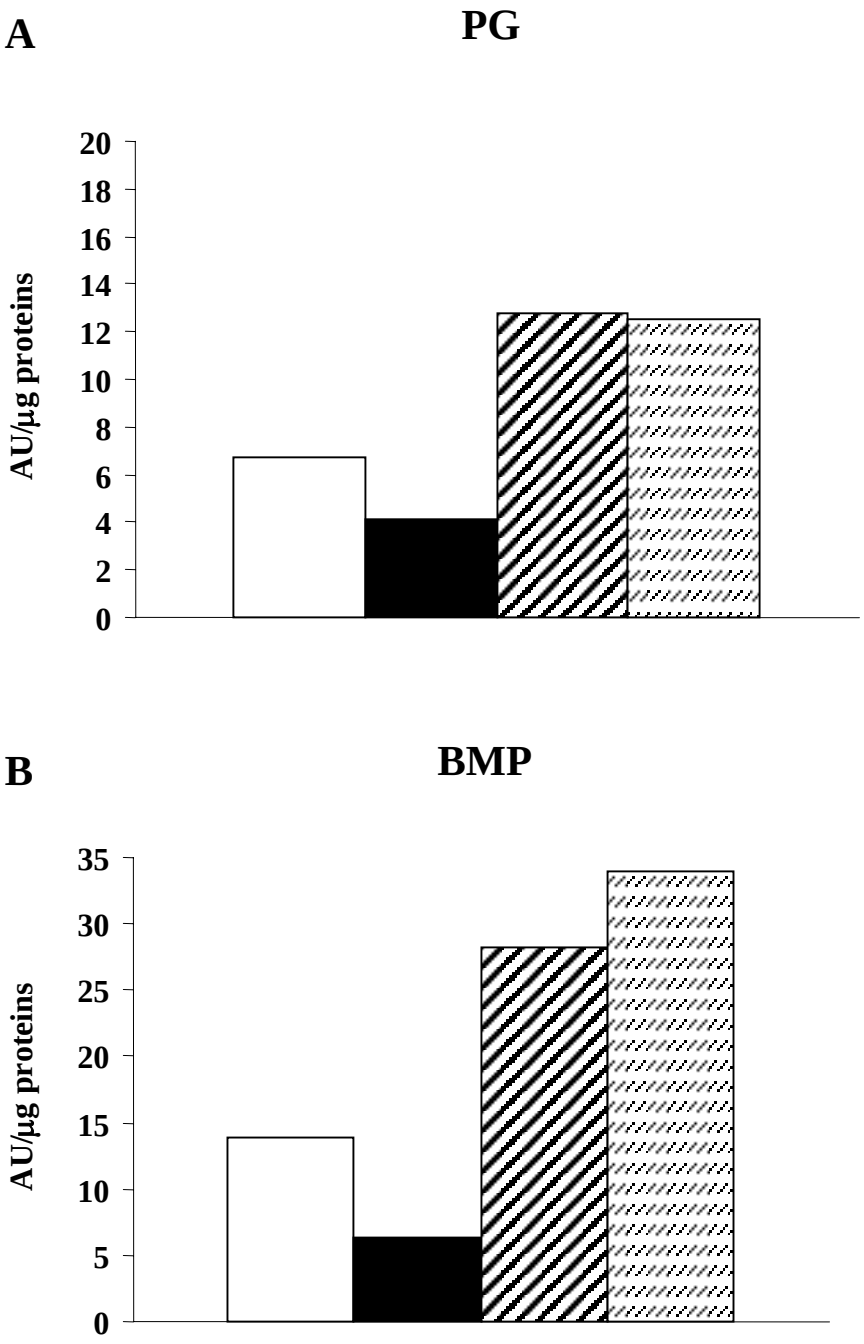
Fatty acids (%)	**Wild type**	**Mutant**	**Transfected mutant**
16:0	7.43 ± 2.98	3.71 ± 0.47	2.65 ± 0.31
16:1	trace	trace	1.39 ± 0.69
18:0	4.83 ± 1.32	4.12 ± 0.27	1.91 ± 0.32
18:1(n-9)	66.34 ± 2.99	68.79 ± 3.00	69.65 ±0.71
18:1 (n-7)	8.95 ±0.53	10.63 ±2.07	7.17 ± 2.23
18:2 (n-6)	12.45±0.91	12.76 ± 1.32	15.94 ± 0.80
saturated	12.26 ± 4.30	7.82 ± 0.20	4.55 ± 0.62
MUFA	75.29 ± 3.41	79.42 ± 1.35	78.21 ± 3.03

Supplemental Table S3: Fatty acid composition of PG in lymphoblasts of control subjects and BTHS patients

Fatty acid composition of PG in lymphoblasts of control subjects (1 and 2) and BTHS patients (3 and 4) was analyzed by gas chromatography. Results are expressed as mole % and are the mean±S.D. of 3 independent determinations. MUFA, monounsaturated fatty acids.

	Control		BTHS	
Fatty acids (%)	**1**	**2**	**3**	**4**
14:0	1.46±0.29	4.73±2.23	1.16±0.74	2.16±2.01
16:0	24.92±3.09	32.87±0.04	28.42±2.20	24.75±1.68
16:1	2.54±0.45	1.71±1.19	1.22±0.06	2.16±1.24
18:0	17.64±3.32	14.33±2.75	12.91±0.87	23.61±6.49
18:1(n-9)	17.53±2.00	8.59±1.83	5.96±0.27	8.36±0.77
18:1 (n-7)	33.21±1.27	33.86±4.43	47.50±2.56	37.15±2.74
18:2 (n-6)	1.10±0.19	0.65±0.32	0.30±0.01	0.31±0.01
20:3 (n-6)	1.60±1.31	3.27±0.36	2.54±0.70	1.50±0.51
saturated	44.02±0.06	51.92±5.02	42.49±2.07	50.52±2.80
MUFA	53.28±1.18	44.15±5.07	54.67±2.77	47.66±2.28

Suppl. Fig. S1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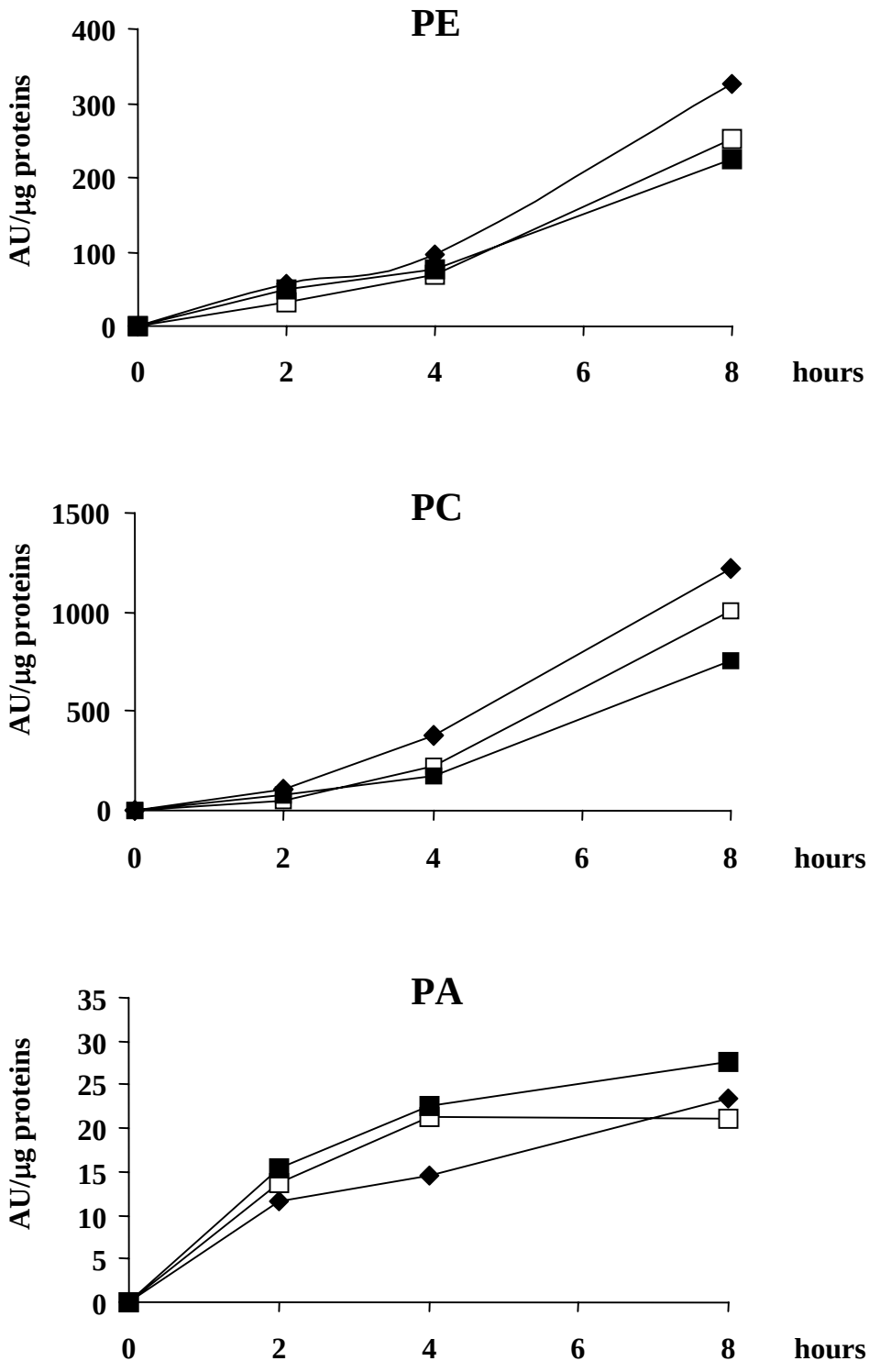
Suppl. Fig. S2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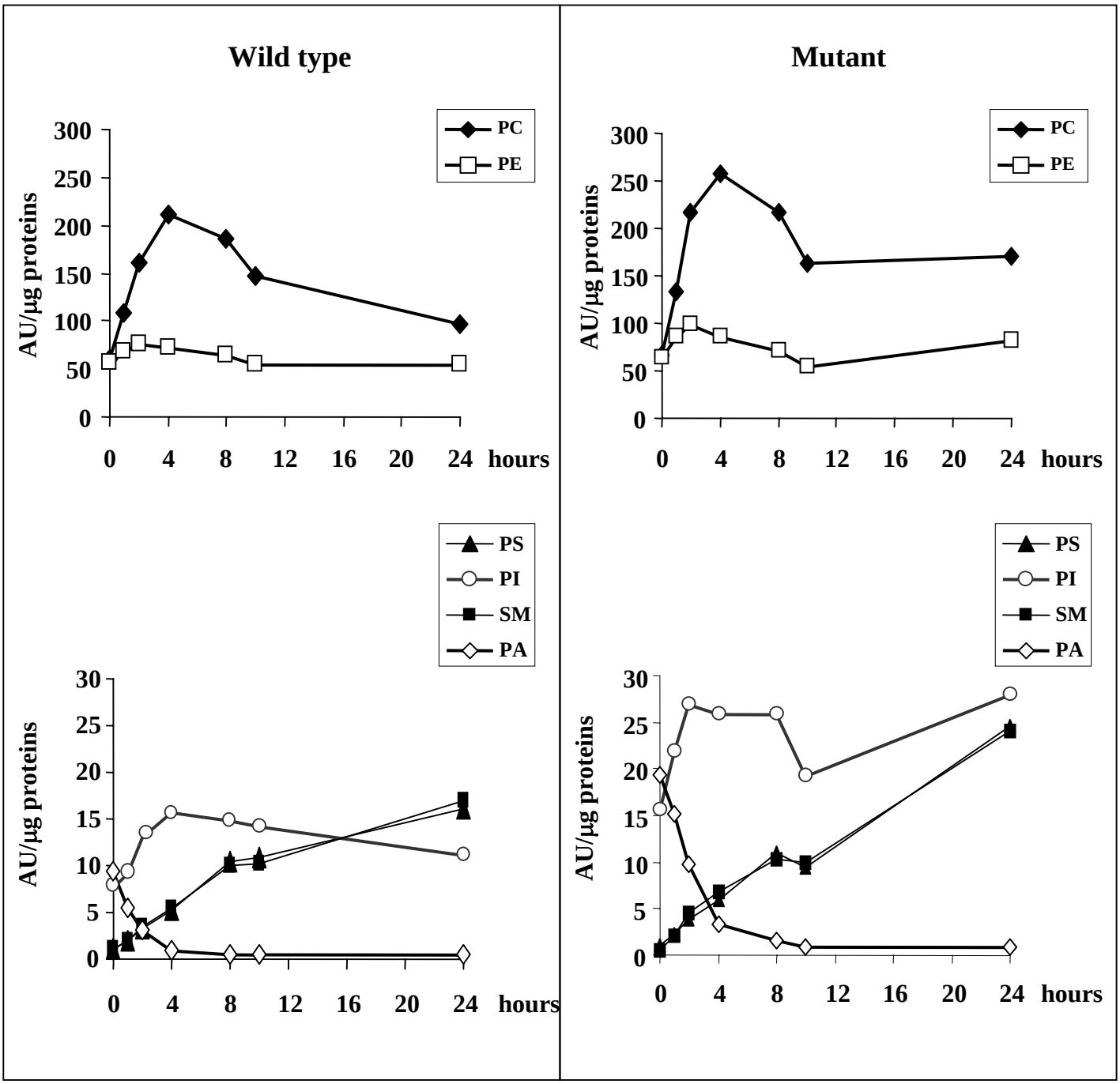
Suppl. Fig. S3


Wild type
Mutant
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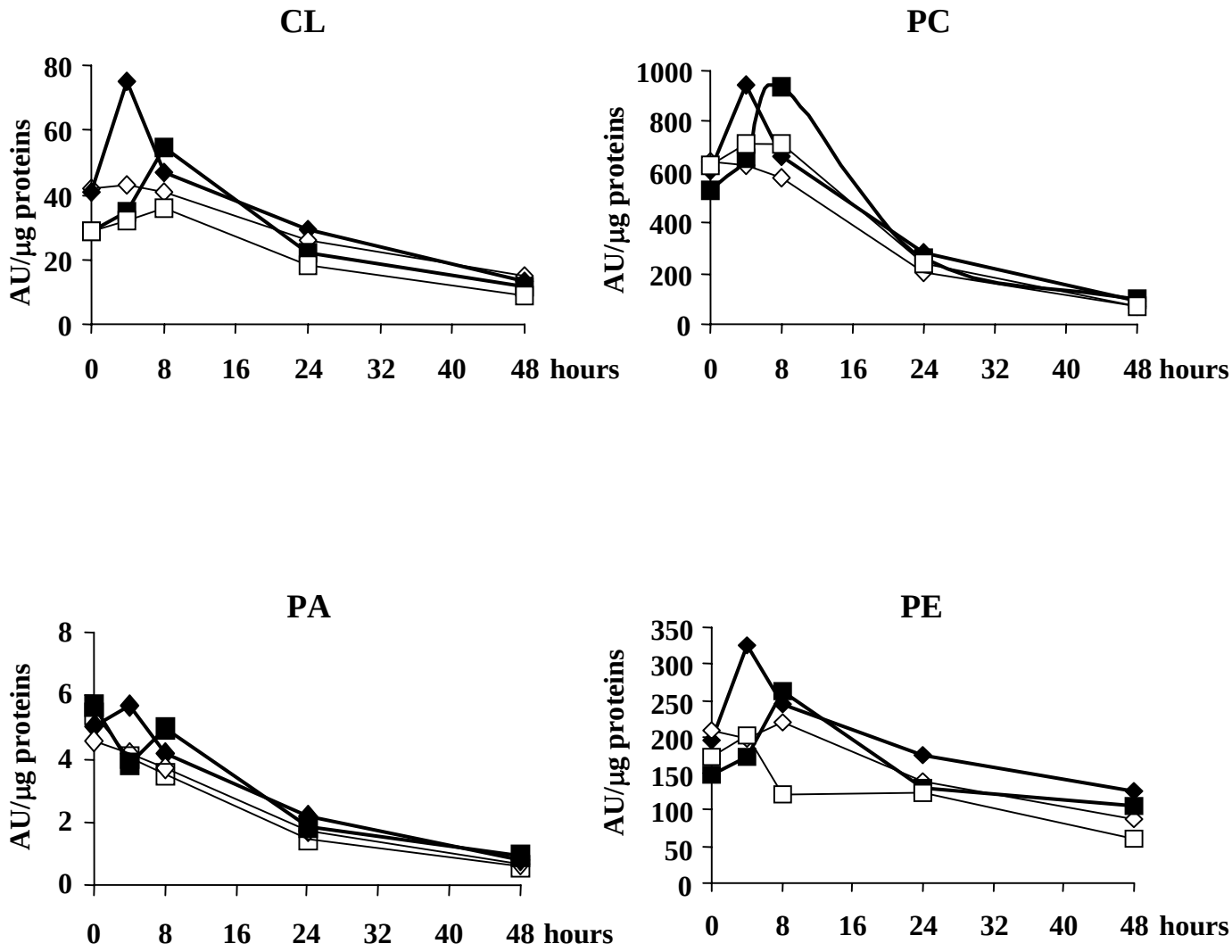
Suppl. Fig. S4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