

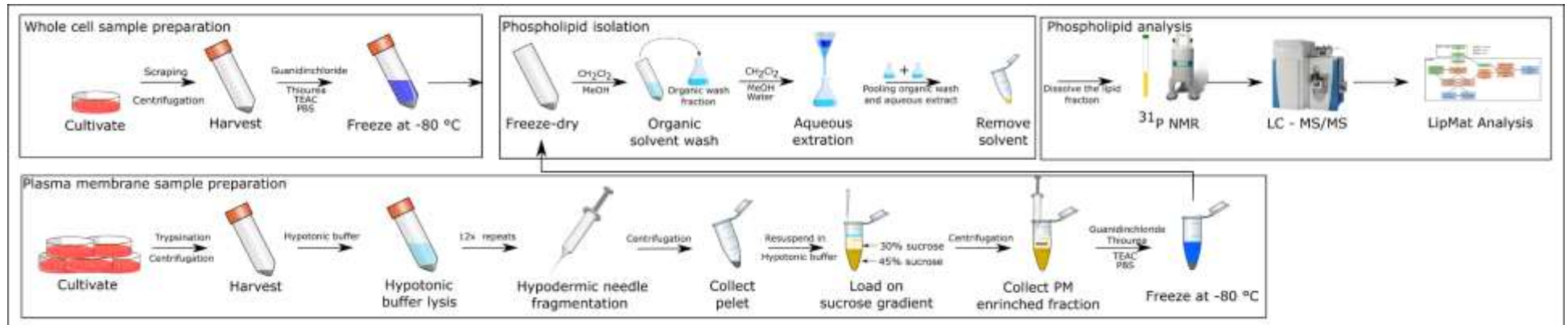
## Supplementary information

For: Fast and quantitative phospholipidomic analysis of SH-SY5Y neuroblastoma cell cultures using LC-MS/MS and  $^{31}\text{P}$  NMR  
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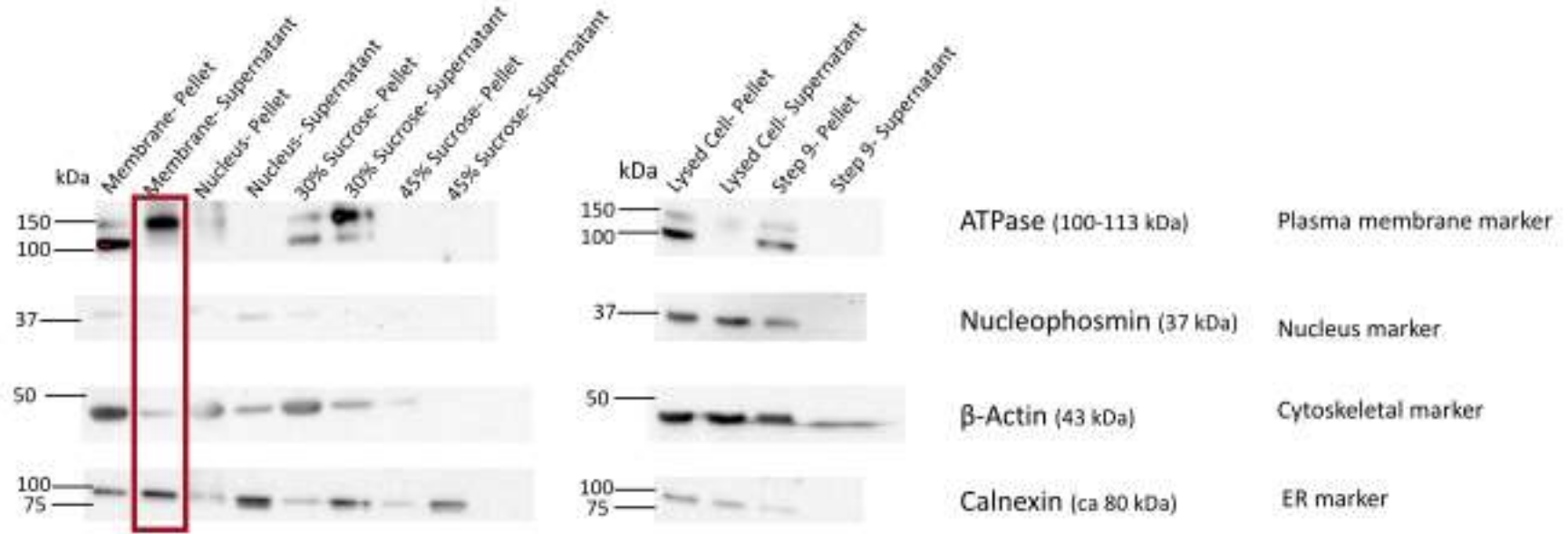
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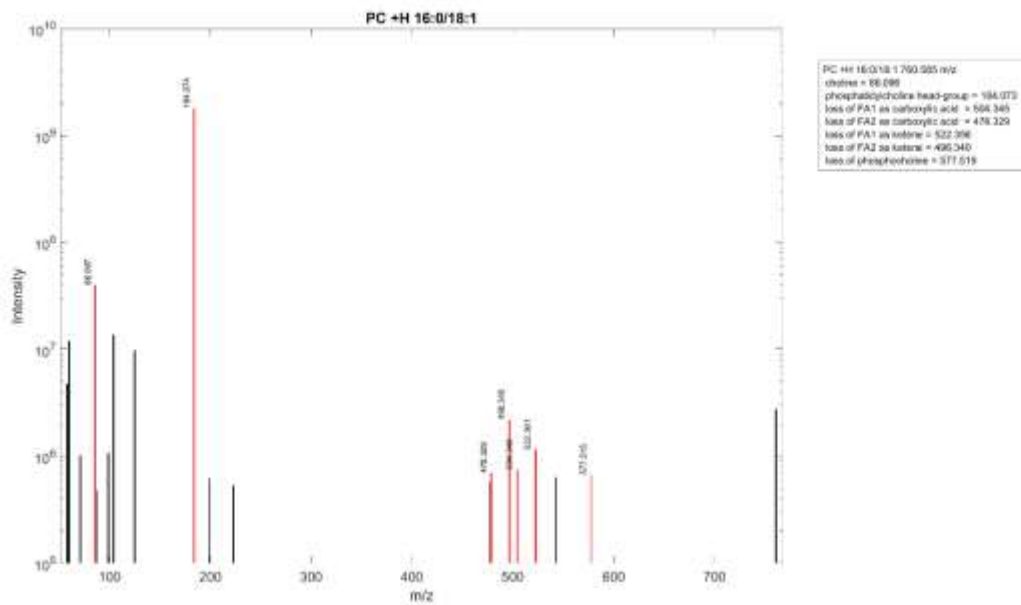
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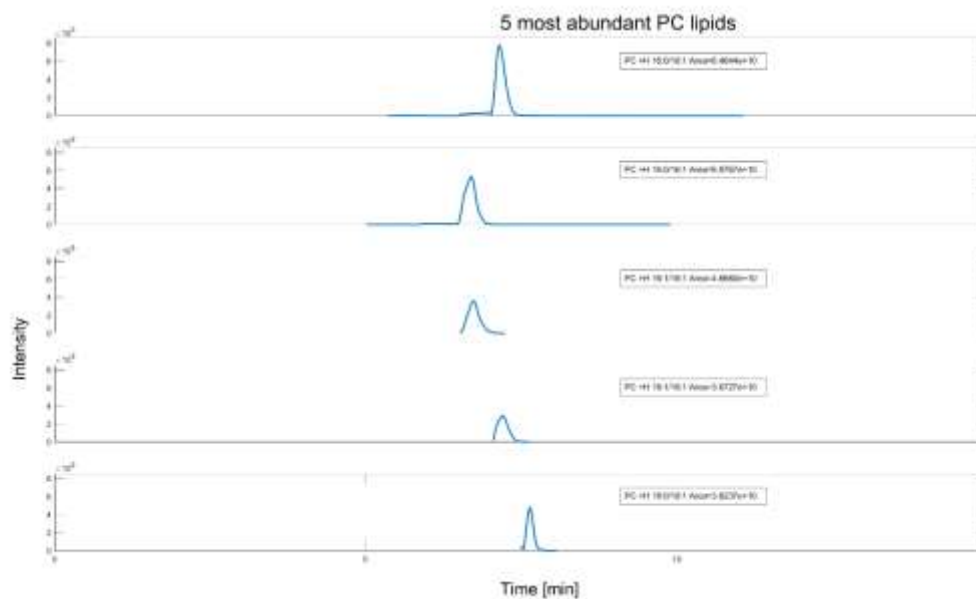
**Figure S1:** Graphical representation of methodology used for the whole cell and plasma membrane phospholipid isolation. For plasma membrane isolation modified sucrose gradient protocol has been used (see Methodology in main text).



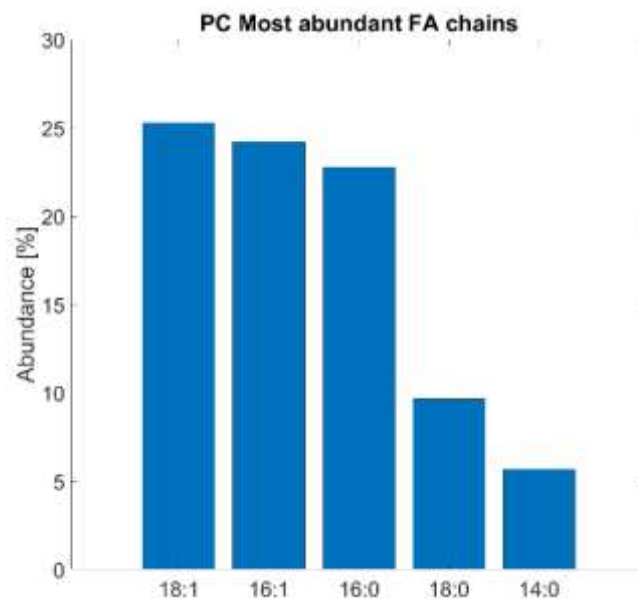
**Figure S2:** Western blot analysis of the purity of isolated plasma membrane fractions. The samples were fractionated on a 10 % SDS-PAGE. Antibodies for Na<sup>+</sup>/K<sup>+</sup>-ATPase (plasma membrane marker), Nucleophosmin (Nucleus marker), Calnexin (ER marker) and β-actin (cytosolic marker) were used to assess the purity of each fraction isolated. The plasma membrane enriched sample is the supernatant from the last centrifugation step and is indicated by a red rectangle. For comparison, results from other purification steps are included.



**Figure S3:** Example of LipMat fragmentation output. MS/MS fragmentation of PC +H 16:0/18:1 from whole cell SH-SY5Y samples. Observed peaks which correspond with predicted fragments are marked in red and their m/z values are listed in the figure. The table on the right lists the names of identified fragments and their corresponding m/z values.



**Figure S4:** Example of LipMat output. LC Chromatogram output of the five most abundant PC lipid species. The chromatogram is scaled to the highest intensity of most abundant lipid species. The area for each lipid is plotted against the retention time where the lipid was identified,  $\pm$  a user-defined retention offset time.



**Figure S5:** Example of LipMat output. The abundance of FA chains of PC headgroups detected in whole cell SH-SY5Y lipid samples. From chromatogram intensities, LipMat assigns abundance for each monitored FA and create a bar plot for the five most abundant FA for each monitored lipid headgroup.

	Retention [min]	Flow [ml/min]	%A	%B	Curve type
<b>1</b>	0	0.4	60	40	5
<b>2</b>	3	0.4	30	70	5
<b>3</b>	12	0.4	20	80	5
<b>4</b>	15	0.4	0	100	5
<b>5</b>	17	0.4	0	100	5
<b>6</b>	17	0.4	60	40	5
<b>7</b>	20	0.4	60	40	5

**Table S1:** Optimized multi-step gradient for LC settings during LC-MS/MS runs. Settings are valid for Dionex Ultimate 3000 UPLC using an Acquity UPLC HSS C18 column, 2.1 mm x 100 mm (Waters, USA). Buffer A (40% acetonitrile, 60% water), and of buffer B (10% acetonitrile, 90% isopropanol) were both supplemented with 10 mM ammonium acetate.

Lipid_species	Mean_mz	ppm_of_mz	Number_of_hits	Mean_score	STD_Score	Sum_of_score	Mean_of_retention_time	STD_of_retention_time	Lipid_headgroup
PC Lyso +H 16:0/0:0	496.342907	1.53835194	24	28.67932944	2.642840992	688.3039066	2.009775694	0.247818648	PC
PC Lyso +H 18:1/0:0	522.3590078	0.974157412	22	30.87219062	3.168932211	679.1881937	2.169001515	0.221999771	PC
PC +H 16:0/18:1	760.5867456	0.956078041	12	30.07056284	9.417942066	360.8467541	8.215118056	2.373897092	PC
PC +H 16:0/16:1	732.5555074	1.807295292	10	31.46908132	8.78208572	314.6908132	7.392963333	2.045586071	PC
PC +H 16:1/16:1	730.540441	1.048192152	9	30.79034663	5.259627589	277.1131197	6.211035185	0.112971467	PC
PC +H 18:1/18:1	786.6012104	1.572863445	8	31.86024269	5.042466847	254.8819415	7.231804167	0.105816086	PC
PC +H 16:1/18:1	758.5693459	0.56573319	7	37.84106527	11.33469794	264.8874569	6.684759524	0.080731171	PC
PC +H 14:0/16:1	704.5250816	0.389549711	7	36.74751835	8.937795471	257.2326285	6.052940476	0.105664891	PC
PC +H 18:0/18:1	788.6198698	1.972241597	6	26.53068153	3.061259378	159.1840892	7.689277778	0.078547915	PC
PC +H 16:0/16:0	734.5711249	0.621000872	5	33.83771426	3.791874529	169.1885713	7.150163333	0.052844865	PC
PC +H 16:0/17:1	746.5722697	0.5263939	5	30.64144137	7.369723608	153.2072068	6.918266667	0.068256914	PC
PC +H 17:1/18:1	772.5869026	0.652062716	5	30.00759539	6.107289706	150.037977	6.955803333	0.063807338	PC
SM +H d18:1/24:1	813.6906786	1.030316929	5	29.6473189	4.531820771	148.2365945	8.09453	0.052573096	SM
PC +H 14:0/16:0	706.5406102	2.112613634	5	28.89937251	12.48517782	144.4968626	6.575413333	0.06858462	PC
PC +H 15:0/18:1	746.575039	2.513935817	5	24.04735095	3.195346999	120.2367548	7.60878	0.399661749	PC
PC +H 18:1/18:2	784.5848904	0.285096432	4	35.22308757	14.1505026	140.8923503	6.7480875	0.041455432	PC
PC +H 14:0/14:0	678.5097961	0.416195585	4	34.3359483	3.204391353	137.3437932	5.931216667	0.044065948	PC
PC +H 16:0/18:0	762.6036814	0.492333797	4	34.09527812	6.417566159	136.3811125	7.635520833	0.041700556	PC
PC +H 18:1/20:1	814.6333708	0.981297234	4	26.08475545	6.142532472	104.3390218	7.635591667	0.044124853	PC
SM +H d18:1/24:0	815.7074718	0.488827031	4	24.90188345	1.173226951	99.6075338	8.556258333	0.044864565	SM
PC +H 16:0/20:3	784.5873742	0.122556685	3	33.83476767	10.00347802	101.504303	7.033227778	0.03359477	PC
PC +H 16:0/16:2	730.5422277	0.486176005	3	29.72204825	5.869125638	89.16614475	6.441394444	0.034133335	PC
PC +H 14:0/18:1	732.5557526	1.875970957	3	29.5567686	5.760505905	88.6703058	6.679716667	0.20713456	PC
SM +Na d14:0/26:0	811.6716198	3.067374554	3	24.69679935	0.934580921	74.09039804	7.71705	0.051779922	SM
PE +H 18:0/18:1	746.5754442	0.537645194	3	24.59394146	0.47941201	73.78182438	7.769483333	0.032596425	PE
PC +Na 16:0/18:1	782.5671098	0.783984629	2	39.07852593	1.804038367	78.15705187	7.170833333	0.022603847	PC
PC +Na 18:0/18:1	810.5991174	0.25394568	2	37.61863166	0.156318003	75.23726332	7.652591667	0.024029845	PC
PC +H 16:0/19:1	774.6035542	0.643079126	2	35.79536193	0.064500006	71.59072386	7.366683333	0.022273864	PC
PC +H 16:0/18:4	754.5363656	0.563350114	2	34.39936194	0.810794741	68.79872388	6.734625	0.024949084	PC
PC Lyso +H 18:0/0:0	524.3751071	0.823744275	2	28.06032148	0.196620403	56.12064297	3.215033333	0.025337993	PC
PC +H 17:0/18:1	774.6031033	1.438154204	2	26.46392069	4.061873433	52.92784139	7.358533333	0.077381052	PC
PS +H 18:0/18:1	790.5610198	1.395734612	2	25.1120339	0.209615771	50.2240678	7.068108333	0.024807663	PS
PC +H 18:1/19:1	800.6186608	0.10274194	2	24.12718839	0.371199509	48.25437678	7.401375	0.047741493	PC
PC Lyso +H 30:0/0:0	692.5614558	0.152770297	2	24.00812369	0.07443844	48.01624738	6.932425	0.022521351	PC
PC +H 16:1/18:2	756.556524	0.555021435	2	23.84694133	0.099302124	47.69388267	6.323466667	0.024159482	PC
PC +H 16:0/22:3	812.6183208	0.55825885	2	23.615976	1.98649962	47.23195201	7.492066667	0.133383909	PC
PC +H 18:1/20:2	812.6169462	0	1	36.29976185	0	36.29976185	7.23905	0	PC

**Table S2:** Example of LipMat table output. Results are for lipid identification of positive LC-MS/MS data-dependent analysis of a whole cell SH-SYS5 sample, iterative exclusion run 1. All uniquely identified lipid species are listed with additional information, including a number of hits in spectra, mean score for all hits and the mean retention time, where hits were observed. For scores <30, the lipid presence should be critically checked against generated fragmentation plots (Figure S3).