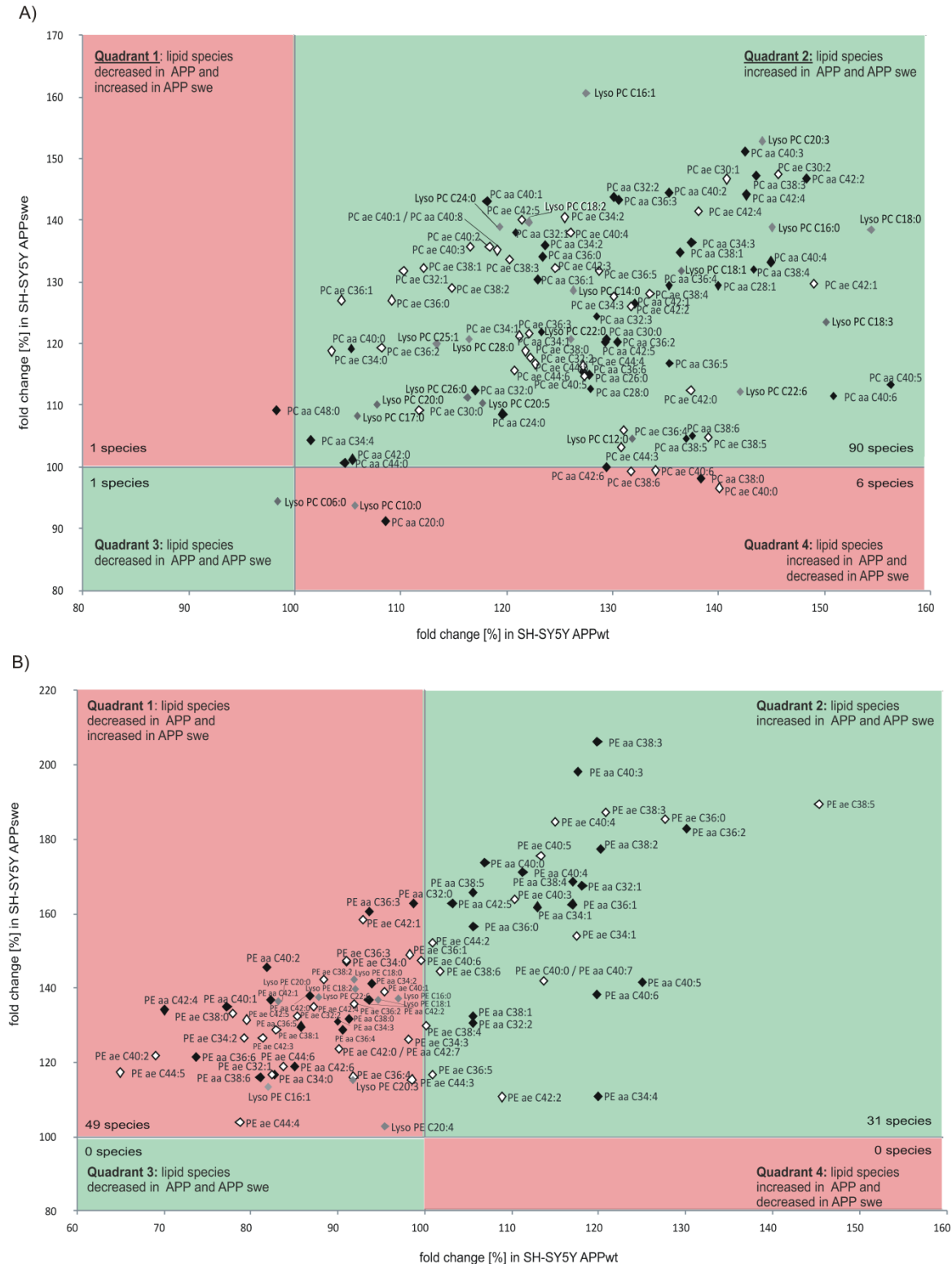


Supplementary Materials

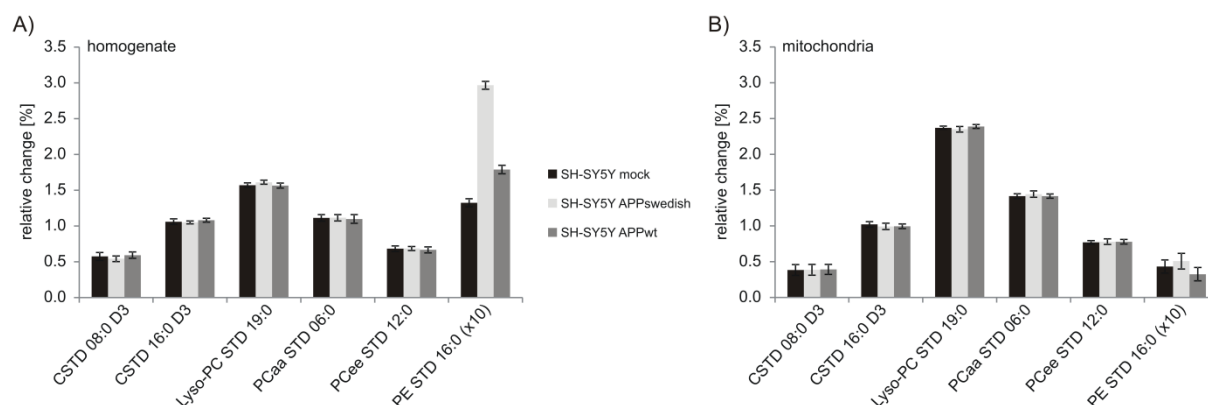
Targeted lipidomics of mitochondria in a cellular Alzheimer's disease model

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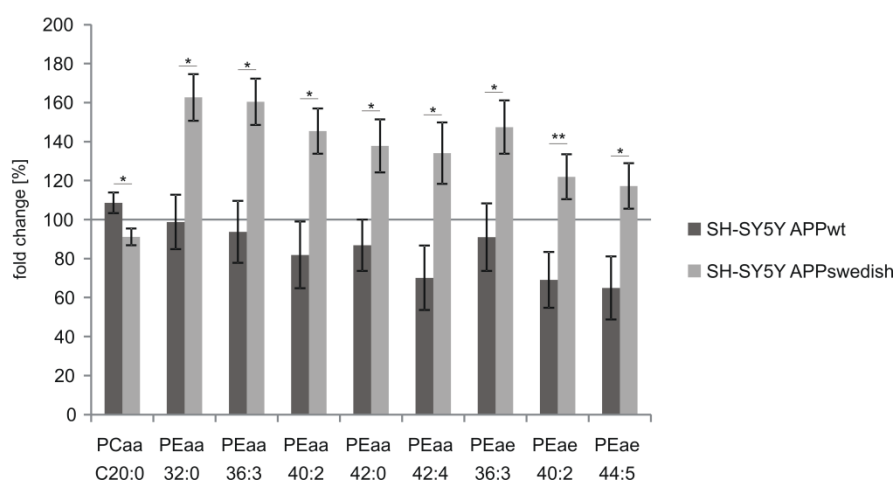


Supplementary Figure S1: Comparison of lipid changes observed in SH-SY5Y APPswedish cells to SH-SY5Y APPwt overexpressing cells. (A) Fold changes of all measured PC species (PCaa, PCae and lyso-PC) in SH-SY5Y APPwt cells (abscissa axis) plotted against fold changes of these species in SH-SY5Y APPswedish cells (ordinate

axis). Quadrants two and three (green) represent PC species that are consistently de- or increased in both cell lines while quadrants one and four (red) include those with different effects in the compared neuroblastoma cell lines. **(B)** Fold changes of all measured PE species (PEaa, PEae and lyso-PE) in SH-SY5Y APPwt cells (abscissa axis) plotted against fold changes of these species in SH-SY5Y APPswedish cells (ordinate axis). Quadrants two and three (green) represent PC species that are consistently de- or increased in both cell lines while quadrants one and four (red) include those with different effects in the compared neuroblastoma cell lines.



Supplementary Figure S2: Matrix effects. Calculated matrix effects of the measured parameters carnitine, lyso-phosphatidylcholines (lyso-PC), phosphatidylcholines (PCaa), phosphatidylcholines plasmalogens (PCae) and phosphatidylethanolamines (PE) in homogenates (A) and mitochondria (B).



Supplementary Figure S3: Alterations in lipid homeostasis in mitochondrial fractions of SH-SY5Y APPwt cells compared to cells overexpressing APPswedish. Significant differences in PEaa and PEae species are shown as bar charts. Error bars represent the standard error of the mean (SEM). Statistical significance was calculated using two-tailed student's t-test with * $p \leq 0.05$ and ** $p \leq 0.01$.

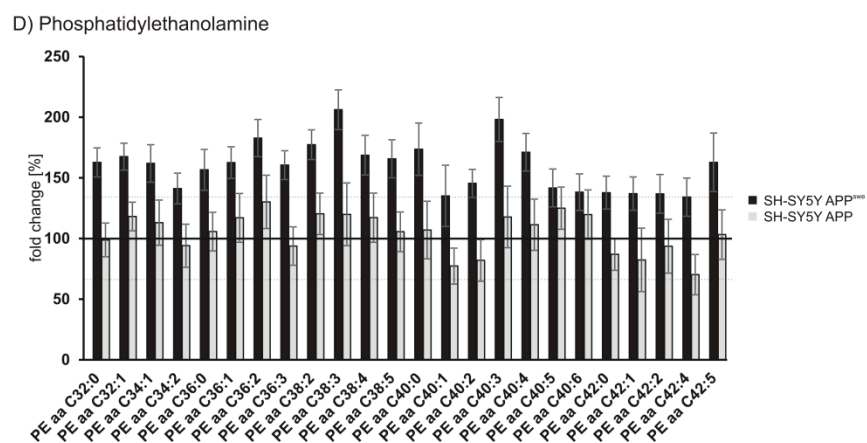
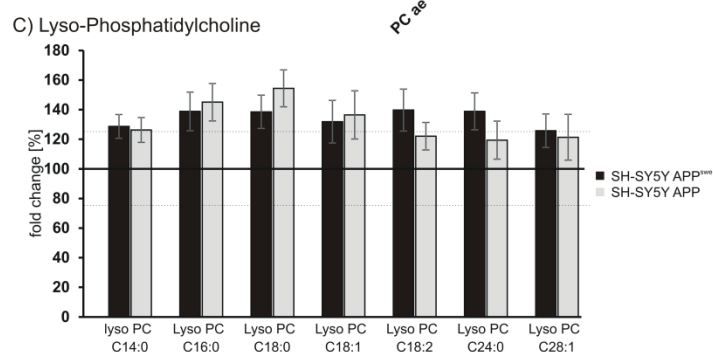
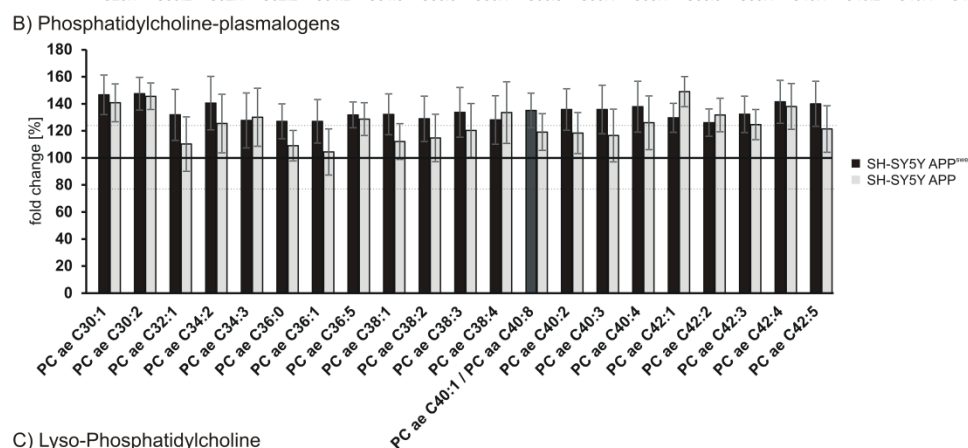
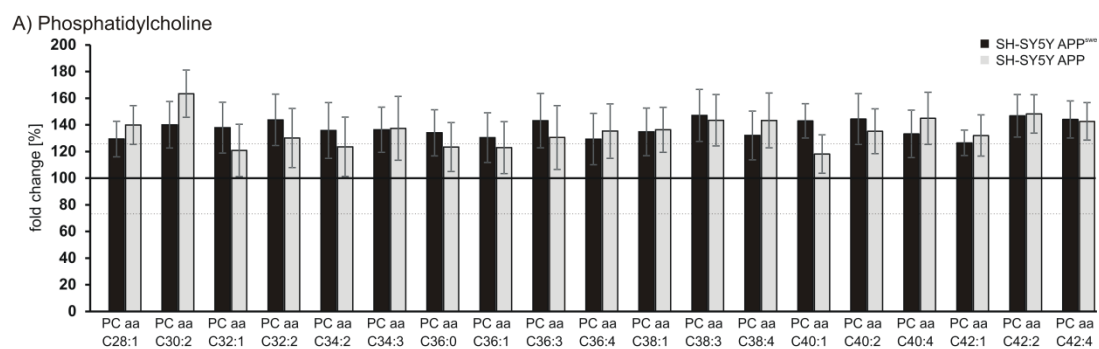
A)

Lipid	mitochondria				homogenate			
	PC aa	PC ae	PE aa	PE ae	PC aa	PC ae	PE aa	PE ae
C32:1	137.9	131.9	167.4	116.6	111.3	95.5	106.5	93.8
C32:2	143.8	117.7	130.4	132.5	115.4	95.8	114.3	103.1
C34:1	121.8	121.3	161.8	153.8	103.6	89.0	99.5	99.1
C34:2	135.9	140.5	141.2	126.7	114.4	101.9	117.4	99.2
C34:3	136.4	127.7	131.0	126.3	120.1	99.7	110.0	98.7
C36:0	134.1	127.1	156.6	185.2	107.5	100.7	95.8	112.2
C36:1	130.4	127.1	162.5	149.0	103.6	98.1	96.8	98.0
C36:2	120.3	119.3	182.7	135.6	102.8	91.1	111.1	101.6
C36:3	143.2	121.5	160.5	147.4	121.7	89.4	122.2	96.6
C36:4	129.4	105.9	128.8	116.1	110.3	78.6	94.5	88.1
C36:5	116.9	131.8	129.7	116.7	109.5	99.7	92.6	85.9
C38:0	98.2	118.8	131.7	133.2	76.8	92.6	70.4	84.3
C38:1	134.8	132.2	132.3	128.6	104.7	101.5	103.5	98.3
C38:3	147.2	133.7	206.1	187.3	123.4	96.2	122.7	99.7
C38:4	132.1	128.1	168.7	129.8	117.1	92.3	95.2	92.5
C38:5	104.6	104.7	165.6	189.4	102.3	81.0	89.9	76.2
C38:6	105.1	99.4	116.0	144.6	93.4	78.9	73.9	74.7
C40:0	119.2	96.6	173.6	142.0	90.8	79.7	84.4	72.3
C40:1	143.1	135.2	135.1	139.1	113.7	103.9	109.0	104.1
C40:2	144.4	135.8	145.4	121.9	122.7	107.8	123.3	107.6
C40:3	151.0	135.8	198.1	163.8	128.4	104.8	131.7	107.9
C40:4	133.3	137.9	171.1	184.7	112.6	97.5	95.5	99.0
C40:5	113.2	114.8	141.6	175.7	106.6	82.9	78.2	83.0
C40:6	111.5	99.4	138.2	147.4	98.0	72.8	70.2	70.9
C42:0	101.2	112.4	137.8	123.7	110.1	108.0	71.7	83.8
C42:1	126.5	129.6	137.0	158.3	116.5	112.0	91.8	79.0
C42:2	146.8	126.1	136.8	110.7	123.1	106.3	106.3	84.2
C42:4	144.1	141.5	134.1	134.8	122.1	104.3	95.8	100.3
C42:5	120.2	140.0	162.7	131.5	113.3	96.3	96.3	98.9

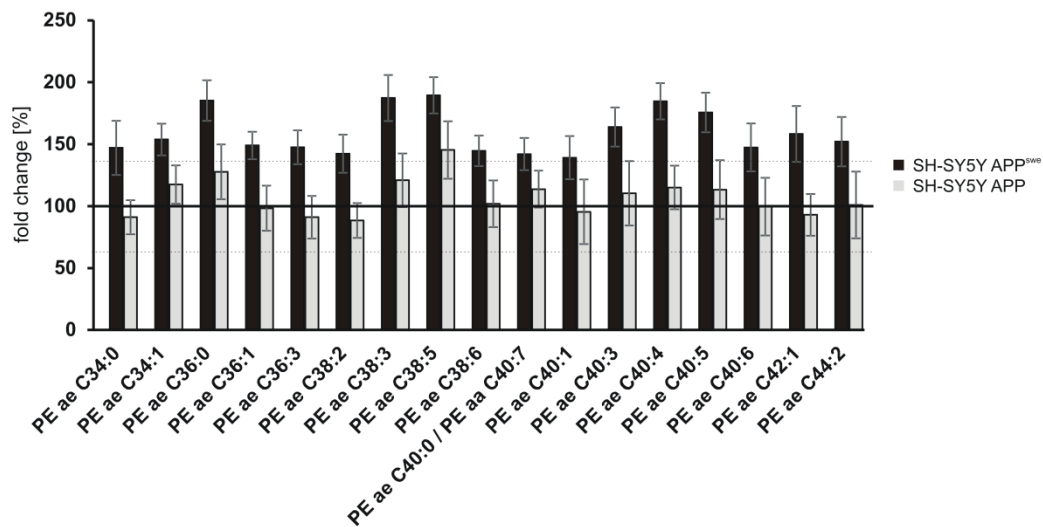
B)

Lipid	mitochondria		homogenate	
	Lyso PC	Lyso PE	Lyso PC	Lyso PE
C16:0	138.8	137.2	112.9	114.4
C16:1	160.6	113.5	140.4	135.4
C18:0	138.5	142.4	111.7	87.5
C18:1	131.9	136.8	116.1	125.5
C18:2	139.7	139.8	123.9	125.1
C20:0	110.2	136.5	96.5	109.4
C20:3	152.9	115.2	143.4	159.9
C20:4	122.6	102.8	111.0	125.0
C22:6	112.1	137.4	90.7	97.0

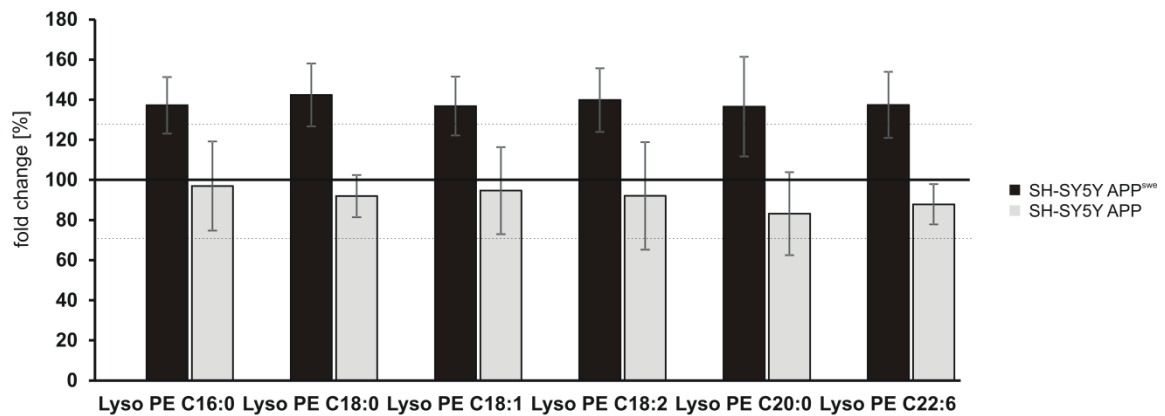
Supplementary Figure S4: Alterations in lipid homeostasis in mitochondrial fractions of SH-SY5Y APPswedish cells compared to those in homogenates. (A) Heat map listing the fold changes of the measured PCaa, PCae, PEaa and PEae species in mitochondria compared to homogenate. The lipid classes are arranged that every line contains the same fatty acids. Therefore effects due to the fatty acid composition can be illustrated more clearly. Green color indicates elevating effects and red color reducing effects. **(B)** Heat map listing the fold changes of the measured lyso-PC and lyso-PE species in mitochondria compared to homogenate. Green color indicates elevating effects and red color reducing effects.



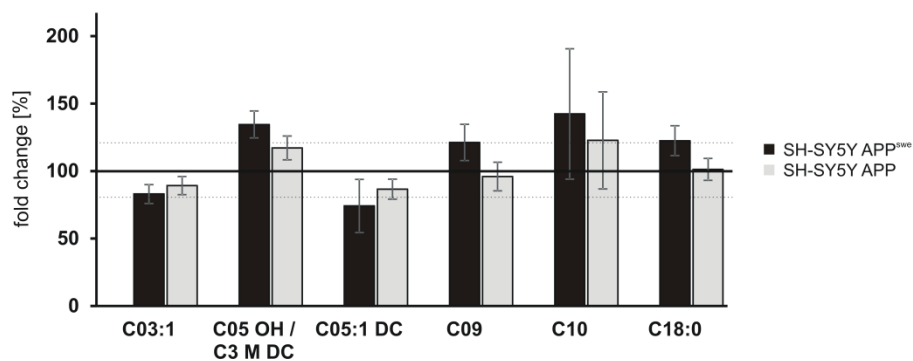
E) Phosphatidylethanolamine-Plasmalogens



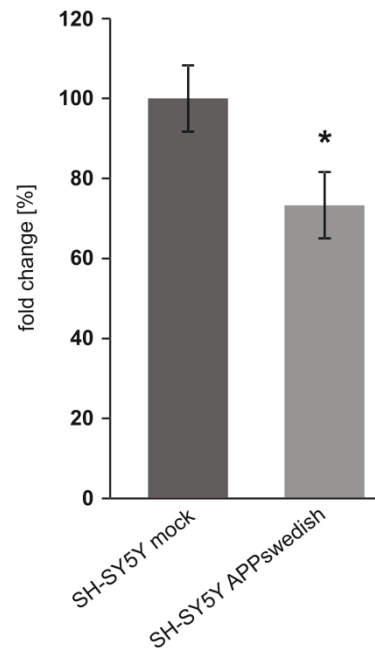
F) Lyso-Phosphatidylethanolamines



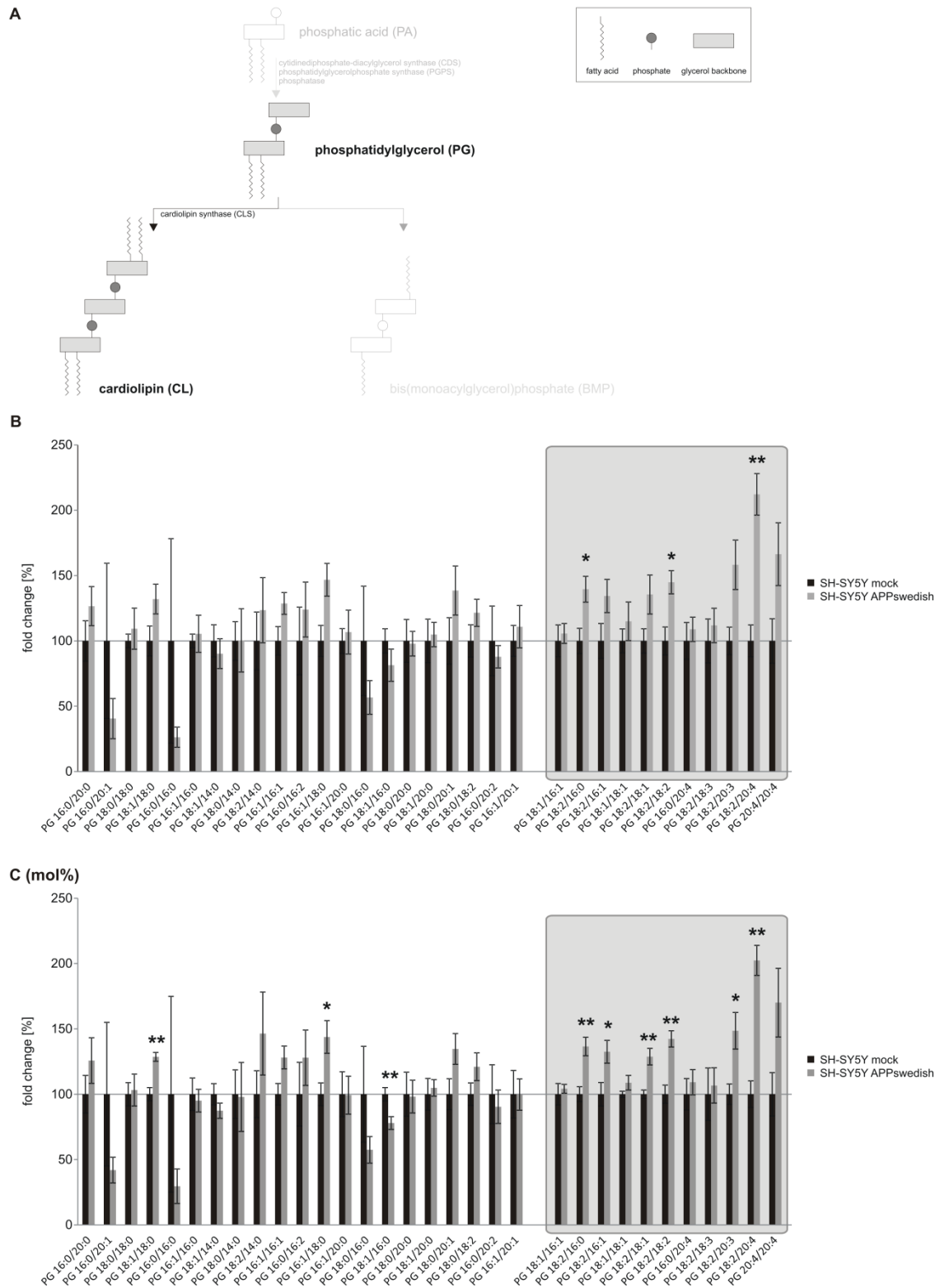
G) Carnitine



Supplementary Figure S5: Comparison of lipid changes observed in SH-SY5Y APP^{swd} cells to SH-SY5Y APP^wt overexpressing cells. The fold changes of those single phosphatidylcholine (A), phosphatidylcholine-plasmalogens (B), lyso-phosphatidylcholine (C), phosphatidylethanolamine (D), phosphatidylethanolamine-plasmalogens (E), lyso-phosphatidylethanolamine (F) and carnitine (G) species that are exclusively changed in mitochondria of APP^{swd} overexpressing cells in comparison to SH-SY5Y APP^wt cells. Changes in SH-SY5Y mock cells were set as 100 % and are marked as the corresponding line in the bar chart. Error bars represent the standard error of the mean (SEM). Average SEM was shown as two grey lines in each diagram, respectively. Statistical significance was calculated using two-tailed student's t-test.



Supplementary Figure S6: Comparison of cardiolipin levels in SH-SY5Y APPswedish overexpressing cells to SH-SY5Y mock transfected cells. The levels of cardiolipin were detected using the cardiolipin assay kit from abcam (ab241036). Changes in SH-SY5Y mock cells were set as 100 %. Error bars represent the standard error of the mean (SEM). Statistical significance was calculated using two-tailed student's t-test with * $p \leq 0.05$.



Supplementary Figure S7: Analysis of phosphatidylglycerol (PG) levels in homogenates of SH-SY5Y APPswedish overexpressing cells in comparison to SH-SY5Y mock transfected cells. (A) Schematic overview of the cardiolipin synthesis. The cytidinediphosphate-diacylglycerol synthase (CDS) catalyzes the formation of cytidinediphosphate-diacylglycerol (CDP-DAG) by condensation of CTP and phosphatidic acid (PA). The phosphatidylglycerolphosphate synthase (PGPS) converted CDP-DAG to phosphatidylglycerolphosphate (PGP), which is dephosphorylated by a phosphatase resulting in phosphatidylglycerol (PG). Condensation of one molecule PG and CDP-DAG, respectively, by the cardiolipin synthase leads to the formation of cardiolipin. **(B)** The fold changes of single PG

species in homogenates are shown as bar charts. (C) The effects of single PG species independent of PG lipid class effect are presented as bar chart. Changes in SH-SY5Y mock cells were set as 100 %. Error bars represent the standard error of the mean (SEM). Statistical significance was calculated using two-tailed student's t-test with * $p \leq 0.05$ and ** $p \leq 0.01$.

Supplementary table S1: Q1/Q3 masses, declustering potentials (DP) and collision energy (CE) for the analyzed metabolites.

metabolite	Q1 mass (Da)	Q3 mass (Da)	DP (volts)	CE (volts)
C0	162.1	85.1	61	27
C2	204.1	85.1	41	27
C3	218.1	85.1	46	29
C03 OH	234.1	85.1	53	30
C3:1	216.1	85.1	49	27
C4	232.2	85.1	46	29
C4 OH	248.1	85.1	55	32
C4:1	230.1	85.1	52	29
C5	246.2	85.1	46	29
C5 M DC	290.2	85.1	63	37
C5 OH	262.2	85.1	58	33
C5:1	244.2	85.1	55	31
C5:1 DC	274.1	85.1	60	35
C6	260.2	85.1	56	27
C6 OH	276.2	85.1	61	35
C6:1	258.2	85.1	57	33
C7 DC	304.2	85.1	66	39
C8	288.2	85.1	66	33
C8:1	286.2	85.1	63	37
C9	302.2	85.1	66	39
C10	316.2	85.1	56	37
C10:1	314.2	85.1	68	40
C10:2	312.2	85.1	67	40
C12	344.3	85.1	73	44
C12 DC	374.3	85.1	86	45
C12:1	342.3	85.1	73	44
C14	372.3	85.1	86	45
C14:1	370.3	85.1	78	47
C14:1 OH	386.3	85.1	81	50
C14:2	368.3	85.1	78	47
C14:2 OH	384.3	85.1	81	49
C16	400.3	85.1	84	51
C16 OH	416.3	85.1	87	53
C16:1	398.3	85.1	84	51
C16:1 OH	414.3	85.1	87	53
C16:2	396.3	85.1	83	51
C16:2 OH	412.3	85.1	86	53
C18:0	428.4	85.1	96	63
C18:1	426.4	85.1	89	55
C18:1 OH	442.4	85.1	92	57
C18:2	424.3	85.1	89	54

Carnitine Std 1	291.2	85.1	66	33
Carnitine Std 2	403.3	85.1	84	51
Lyso PC 06:0	356.2	184	72	31
Lyso PC 10:0	412.3	184	76	30
Lyso PC 12:0	440.4	184	78	30
Lyso PC 14:0	468.3	184	79	30
Lyso PC 16:0	496.3	184	81	31
Lyso PC 16:1	494.3	184	79	30
Lyso PC 17:0	510.3	184	82	31
Lyso PC 18:0	524.3	184	85	31
Lyso PC 18:1	522.3	184	82	31
Lyso PC 18:2	520.3	184	82	31
Lyso PC 18:3	518.4	184	82	31
Lyso PC 20:0	552.4	184	89	31
Lyso PC 20:3	546.3	184	87	31
Lyso PC 20:4	544.3	184	85	31
Lyso PC 20:5	542.4	184	84	31
Lyso PC 22:0	580.5	184	93	32
Lyso PC 22:6	568.4	184	90	32
Lyso PC 24:0	608.4	184	96	33
Lyso PC 26:0	636.5	184	101	34
Lyso PC 26:1	634.4	184	98	33
Lyso PC 28:0	664.5	184	105	35
Lyso PC 28:1	662.5	184	103	35
Lyso PC Std	538.6	184	96	40
PC aa C20:0	566.2	184	94	31
PC aa C24:0	622.4	184	98	33
PC aa C26:0	650.5	184	103	35
PC aa C28:0	678.5	184	106	35
PC aa C28:1	676.5	184	106	35
PC aa C30:0	706.5	184	114	37
PC aa C30:2	702.5	184	111	37
PC aa C32:0	734.6	184	119	39
PC aa C32:1	732.6	184	118	39
PC aa C32:2	730.5	184	117	38
PC aa C32:3	728.5	184	117	38
PC aa C34:1	760.6	184	125	41
PC aa C34:2	758.6	184	123	40
PC aa C34:3	756.6	184	123	40
PC aa C34:4	754.5	184	122	40
PC aa C36:0	790.6	184	131	43
PC aa C36:1	788.6	184	131	43
PC aa C36:2	786.6	184	130	42
PC aa C36:3	784.6	184	130	42

PC aa C36:4	782.6	184	129	42
PC aa C36:5	780.6	184	128	42
PC aa C36:6	778.5	184	128	42
PC aa C38:0	818.7	184	138	45
PC aa C38:1	816.7	184	138	45
PC aa C38:3	812.6	184	136	44
PC aa C38:4	810.6	184	136	44
PC aa C38:5	808.6	184	136	44
PC aa C38:6	806.6	184	135	44
PC aa C40:0	846.7	184	145	47
PC aa C40:1	844.7	184	145	47
PC aa C40:2	842.7	184	144	47
PC aa C40:3	840.7	184	144	47
PC aa C40:4	838.6	184	144	47
PC aa C40:5	836.6	184	143	47
PC aa C40:6	834.6	184	143	46
PC aa C42:0	874.7	184	154	50
PC aa C42:1	872.7	184	153	50
PC aa C42:2	870.7	184	153	50
PC aa C42:4	866.7	184	152	50
PC aa C42:5	864.7	184	151	49
PC aa C42:6	862.6	184	150	49
PC aa C44:0	902.7	184	163	50
PC aa C48:0	958.8	184	170	50
PC aa Std	454.4	184	76	39
PC ae C30:0	692.6	184	111	37
PC ae C30:1	690.5	184	109	36
PC ae C30:2	688.5	184	108	36
PC ae C32:1	718.6	184	115	38
PC ae C32:2	716.6	184	114	37
PC ae C34:0	748.6	184	122	40
PC ae C34:1	746.6	184	121	39
PC ae C34:2	744.6	184	120	39
PC ae C34:3	742.6	184	120	39
PC ae C36:0	776.7	184	128	42
PC ae C36:1	774.6	184	127	41
PC ae C36:2	772.6	184	127	41
PC ae C36:3	770.6	184	126	41
PC ae C36:4	768.6	184	126	41
PC ae C36:5	766.6	184	125	41
PC ae C38:0	804.7	184	135	44
PC ae C38:1	802.7	184	134	44
PC ae C38:2	800.7	184	133	43
PC ae C38:3	798.6	184	133	43

PC ae C38:4	796.6	184	133	43
PC ae C38:5	794.6	184	132	43
PC ae C38:6	792.6	184	132	43
PC ae C40:0	832.7	184	142	46
PC ae C40:1	830.7	184	141	46
PC ae C40:2	828.7	184	141	46
PC ae C40:3	826.7	184	140	46
PC ae C40:4	824.7	184	140	45
PC ae C40:5	822.6	184	139	45
PC ae C40:6	820.6	184	139	45
PC ae C42:0	860.8	184	150	49
PC ae C42:1	858.7	184	141	46
PC ae C42:2	856.7	184	149	48
PC ae C42:3	854.7	184	148	48
PC ae C42:4	852.7	184	148	48
PC ae C42:5	850.7	184	147	48
PC ae C44:3	882.7	184	156	51
PC ae C44:4	880.7	184	156	51
PC ae C44:5	878.7	184	155	51
PC ae C44:6	876.7	184	154	50
PC ee Std	594.6	184	74	57
Lyso PE 16:1	452.3	311.2	54	23
Lyso PE 16:0	454.3	313.2	54	23
Lyso PE 18:2	478.3	337.3	54	24
Lyso PE 18:1	480.3	339.3	54	24
Lyso PE 18:0	482.3	341.3	55	24
Lyso PE 20:4	502.4	361.3	55	24
Lyso PE 20:3	504.4	363.2	55	25
Lyso PE 20:0	510.4	369.4	55	25
Lyso PE 22:6	526.4	385.2	56	25
PE ae 32:2	674.6	533.5	61	27
PE ae 32:1	676.6	535.5	61	27
PE aa 32:2	688.5	547.5	64	29
PE aa 32:1	690.5	549.5	64	29
PE aa 32:0	692.5	551.5	64	29
PE ae 34:3	700.6	559.5	64	28
PE ae 34:2	702.6	561.5	64	28
PE ae 34:1	704.7	563.6	64	28
PE ae 34:0	706.6	565.5	64	28
PE aa 34:4	712.5	571.5	66	30
PE aa 34:3	714.5	573.5	66	30
PE aa 34:2	716.5	575.5	66	30
PE aa 34:1	718.5	577.5	66	30
PE aa 34:0	720.5	579.5	67	30

PE ae 36:5	724.7	583.6	66	29
PE ae 36:4	726.6	585.5	66	29
PE ae 36:3	728.6	587.6	66	29
PE ae 36:2	730.6	589.5	67	29
PE ae 36:1	732.6	591.5	67	29
PE ae 36:0	734.6	593.5	67	29
PE aa 36:6	736.6	595.6	68	31
PE aa 36:5	738.6	597.6	68	31
PE aa 36:4	740.6	599.6	68	31
PE aa 36:3	742.6	601.6	68	31
PE aa 36:2	744.6	603.6	68	31
PE aa 36:1	746.6	605.6	69	31
PE aa 36:0	748.6	607.6	69	31
PE ae 38:6	750.6	609.6	69	30
PE ae 38:5	752.6	611.6	69	30
PE ae 38:4	754.6	613.6	69	30
PE ae 38:3	756.6	615.6	69	30
PE ae 38:2	758.6	617.6	69	30
PE ae 38:1	760.6	619.6	70	30
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PE aa 38:4	768.6	627.6	70	32
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PE aa 38:2	772.6	631.6	71	32
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PE aa 38:0	776.7	635.6	71	32
PE ae 40:6	778.6	637.6	72	31
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PEae 40:0/PEaa 40:7	790.6	649.6	73	31
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PE aa 40:5	794.6	653.6	73	33
PE aa 40:4	796.7	655.6	73	33
PE aa 40:3	798.6	657.6	73	33
PE aa 40:2	800.7	659.7	73	33
PE aa 40:1	802.7	661.7	73	33
PE aa 40:0	804.6	663.6	74	33
PE ae 42:5	808.7	667.6	75	32
PE ae 42:4	810.7	669.6	75	32
PE ae 42:3	812.7	671.6	75	32

PE ae 42:2	814.7	673.6	75	32
PE ae 42:1	816.7	675.6	76	32
PEae 42:0/PEaa 42:7	818.7	677.6	76	32
PE aa 42:6	820.7	679.6	75	33
PE aa 42:5	822.7	681.6	75	34
PE aa 42:4	824.7	683.6	75	34
PE aa 42:2	828.7	687.6	76	34
PE aa 42:1	830.7	689.6	76	34
PE aa 42:0	832.7	691.6	76	34
PE ae 44:6	834.7	693.6	78	32
PE ae 44:5	836.7	695.6	78	33
PE ae 44:4	838.7	697.6	78	33
PE ae 44:3	840.7	699.6	78	33
PE ae 44:2	842.7	701.6	79	33
Std PE aa 16:0	468.3	327.2	53	21
PG 16:0/20:0	777.6	255.2	-146	-64
PG 16:0/20:1	775.7	255.2	-156	-55
PG 18:0/18:0	777.6	283.2	-146	-59
PG 18:1/18:0	775.7	281.1	-156	-54
PG 16:0/16:0	721.6	255.1	-139	-50
PG 16:1/16:0	719.6	253.1	-133	-51
PG 18:1/14:0	719.6	281.1	-133	-51
PG 18:0/14:0	721.6	283.2	-139	-49
PG 18:2/14:0	719.6	279.1	-133	-57
PG 16:1/16:1	717.6	253.1	-130	-45
PG 16:0/16:2	717.6	251.1	-130	-46
PG 16:1/18:0	747.6	253.1	-145	-55
PG 16:1/20:0	775.7	253.1	-156	-47
PG 18:0/16:0	749.6	255.2	-147	-56
PG 18:1/16:0	747.6	281.1	-145	-55
PG 18:2/16:0	745.6	279.1	-146	-53
PG 18:1/16:1	745.6	281.1	-146	-54
PG 18:0/20:0	805.6	283.2	-148	-64
PG 18:1/20:0	803.6	281.1	-146	-61
PG 18:0/20:1	803.6	283.2	-146	-66
PG 18:1/18:1	773.6	281.1	-149	-58
PG 18:0/18:2	773.6	283.1	-149	-59
PG 16:0/20:2	773.6	255.2	-149	-56
PG 16:1/20:1	773.6	253.1	-149	-55
PG Std 17:0/14:1	705.5	269.2	-160	-54