SUPPLEMENTARY MATERIALS

Quantitative Profiling of the Endonuclear Glycero-Phospholipidome of Murine Embryonic Fibroblasts

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LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure S1. Comparative lipidomics. **(A)** Comparison of GPL composition in whole cell WT immortalized and primary MEFs. **(B)** The degree of unsaturation of PtdCho between the iMEFs and primary MEFs showed more polyunsaturated PCs in the primary cells. **(C)** The percentage of PtdEtn that is ether-linked was almost identical between iMEFs and primary MEFs. Abbreviations: PtdOH, PA; PtdCho, PC; PtdEtn, PE; PtdGro, PG; PtdSer, PS; PtdIns, PI.

Supplemental Figure S2. Nuclear localization patterns of PITPα-HA and PtdIns-binding/transfer mutants transiently overexpressed in HeLa cells. **(A)** Cells were stained with anti-HA primary antibodies 16-18 hours after transfection with PITPα-HA. Cells are counterstained with 4',6-diamidino-2-phenylindole (DAPI) to facilitate examination mutant constructs nuclear localization. WT constructs produce nuclear fluorescence that was greater than or equivalent to cytoplasmic fluorescence in the vast majority of expressing cells. PtdIns-binding defective point mutants, Pitpα^{T59D}, Pitpα^{K61A}, Pitpα^{E248K} and the PtdIns/PtdCho-binding defective point mutants and Pitpα^{S166E} were scored for the relative strength of nuclear signal vs. cytoplasmic fluorescence, as judged by coincidence with nuclear DAPI stain. **(B)** Quantitation of nuclear localization defects in cells expressing PITPα-EGFP mutant constructs. Minimally 200 cells were scored for nuclear signal ≥ cytoplasmic signal (nuc ≥ cyt) or if nuclear signal < cytoplasmic signal (nuc < cyt).

Supplemental Figure S3. Quality control analysis of *pitpα*$^{0/0}$ membrane-stripped nuclear fractions. (A) Immunoblot analysis of purified *PITPα*$^{+/+}$ and *pitpα*$^{0/0}$ nuclei. Lysates of membrane free nuclear fractions were compared to whole cell and cytosolic lysates for immunoreactivity to markers for cytoplasmic membrane contaminants. For calnexin and β-tubulin detection, ~30,000 cell equivalents of nuclear and whole cell lysate were loaded. For detection of histone H3 acetylated at lysine 9 (AcK9H3), ~100,000 cell equivalents were loaded. **(B-C)** Electron micrographs of membrane-stripped nuclei purified from *PITPα*$^{+/+}$ and *pitpα*$^{0/0}$ cells. Scale bars are shown: **(B)** 1100x magnification of *PITPα*$^{+/+}$ (left) and *pitpα*$^{0/0}$ (right) nuclei. Nuclei appear as intact particles with minimal background debris. **(C)** 6500x (top) and 15000x (bottom) magnification images of borders of purified *pitpα*$^{0/0}$ nuclei.

Supplemental Figure S4. Comparison of WT and *pitpα*$^{0/0}$ iMEF whole cell and nuclear phospholipidomes. Fractional composition of the indicated phopsholipid classes in *PITPα*$^{+/+}$ (left) and *pitpα*$^{0/0}$ iMEF cells **(A)** and from the corresponding endonuclear fractions **(B)**. For both panels, the values rePresent the averages of three independent experiments for each genotype.

Supplemental Figure S5. Flux of PtdIns and PtdCho into wild-type and PITPα$^{0/0}$ nuclei or whole cells. Deuterated PtdIns **(A)** or PtdCho **(B)** in whole cells as a function of time post-labeling. Data was derived from precursor scans for headgroups of d_6-PtdIns and d_9-PtdCho. The total deuterated PtdIns or PtdCho mass was expressed as a ratio versus total unlabeled PtdIns or PtdCho detected, respectively (triplicate determinations; * $p<0.05$). Deuterated PtdIns **(C)** or PtdCho **(D)** in nuclei as a function of time post-labeling. Data was derived from precursor scans for deuterated headgroups of d_6-PtdIns and d_9-PtdCho. Total resulting deuterated PtdIns or

PtdCho was expressed as a percentage of total non-deuterated PtdIns or PtdCho detected, respectively, and plotted as a function of time (2, 3, 4, 6 hr) and genotype. Data represent measurement from three independent nuclear preparations for each time point combined into a single sample for each time point.

FIG S1:

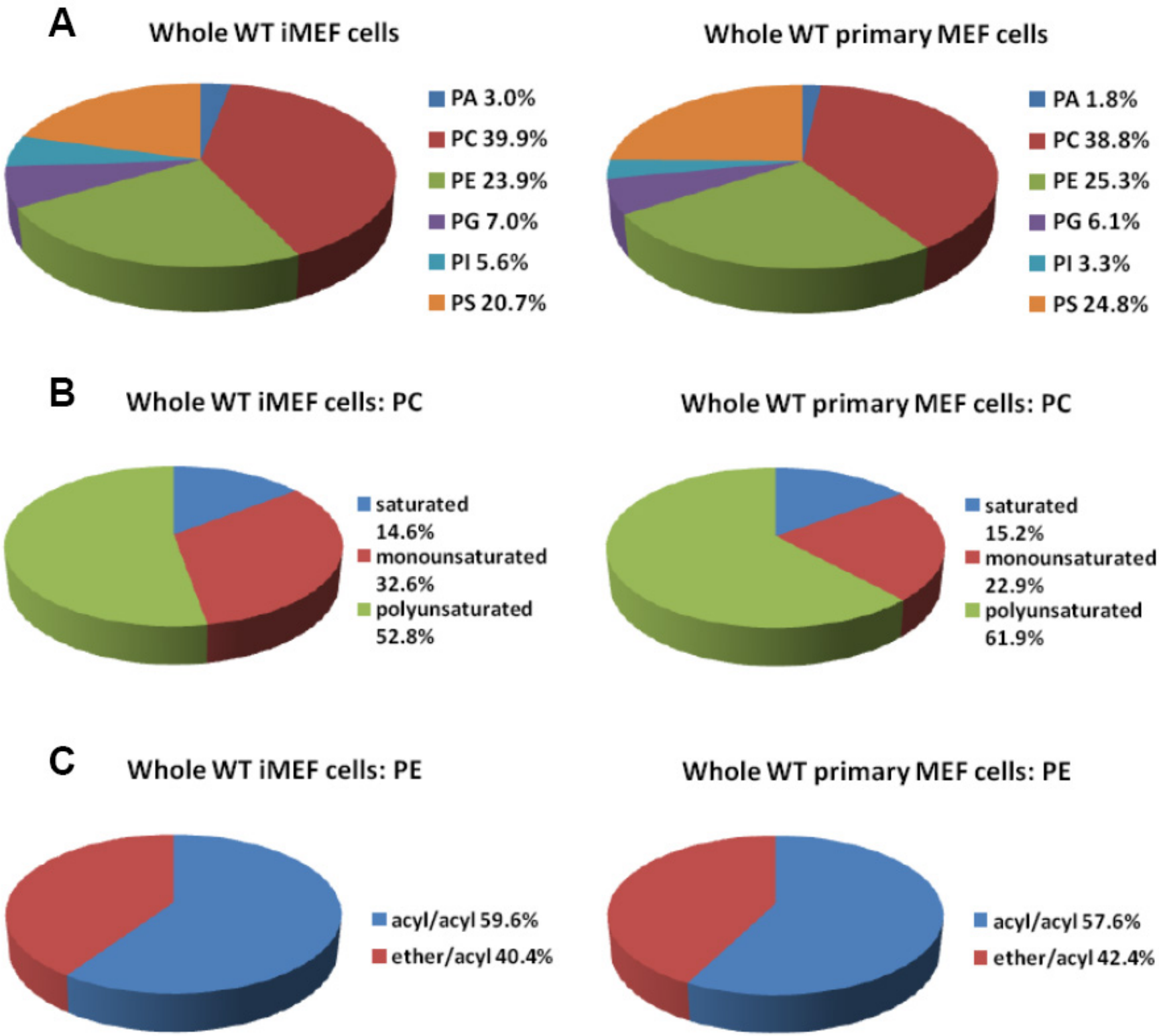

A
Whole WT iMEF cells
PA 3.0%
PC 39.9%
PE 23.9%
PG 7.0%
PI 5.6%
PS 20.7%
Whole WT primary MEF cells
PA 1.8%
PC 38.8%
PE 25.3%
PG 6.1%
PI 3.3%
PS 24.8%
B
Whole WT iMEF cells: PC
saturated 14.6%
monounsaturated 32.6%
polyunsaturated 52.8%
Whole WT primary MEF cells: PC
saturated 15.2%
monounsaturated 22.9%
polyunsaturated 61.9%
C
Whole WT iMEF cells: PE
acyl/acyl 59.6%
ether/acyl 40.4%
Whole WT primary MEF cells: PE
acyl/acyl 57.6%
ether/acyl 42.4%

Fig. S2

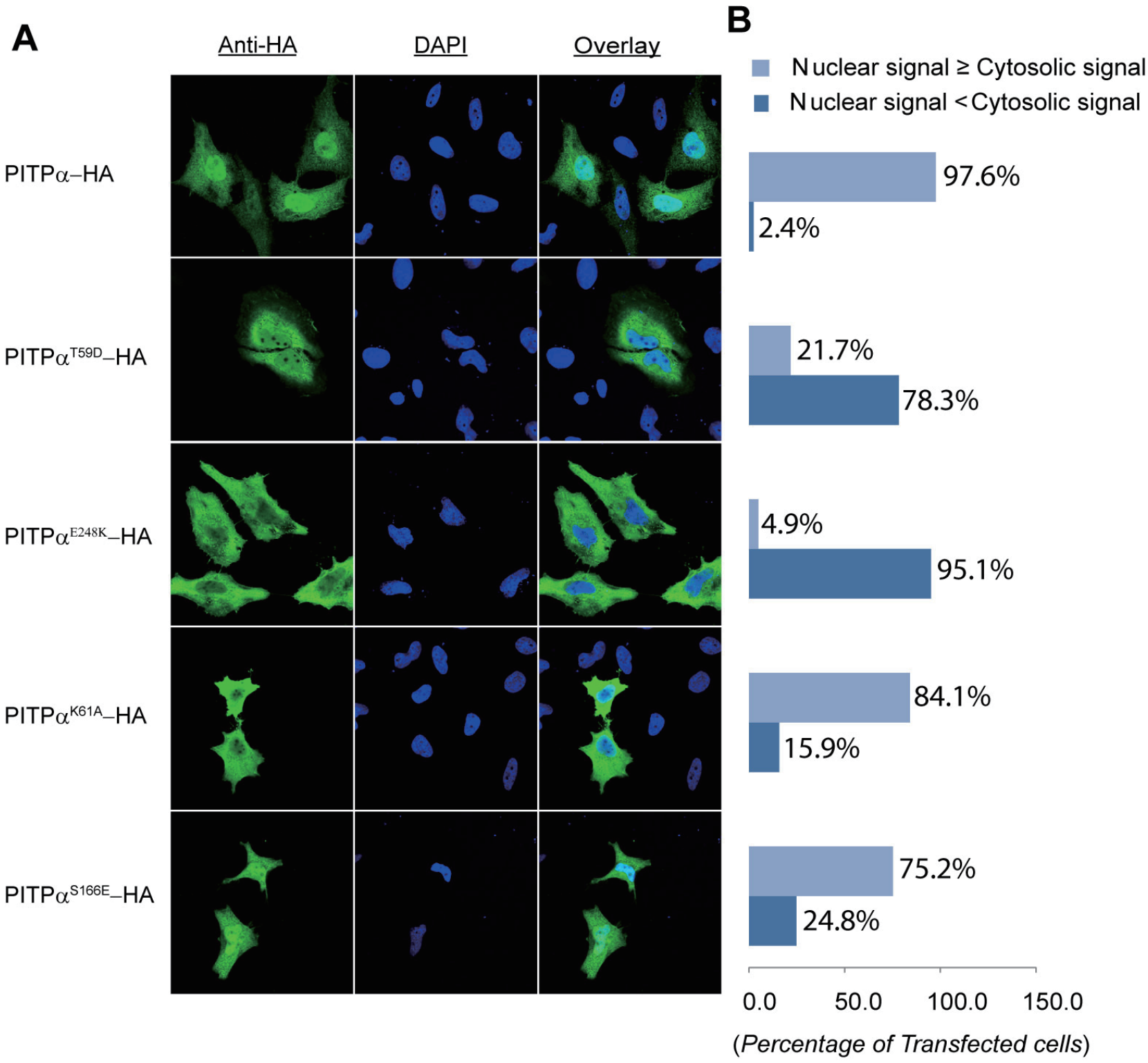

A
Anti-HA
DAPI
Overlay
PITPα–HA
PITPα^{T59D}–HA
PITPα^{E248K}–HA
PITPα^{K61A}–HA
PITPα^{S166E}–HA
B
Nuclear signal ≥ Cytosolic signal
Nuclear signal < Cytosolic signal
97.6%
2.4%
21.7%
78.3%
4.9%
95.1%
84.1%
15.9%
75.2%
24.8%
0.0
50.0
100.0
150.0
(Percentage of Transfected cells)

Fig. S3

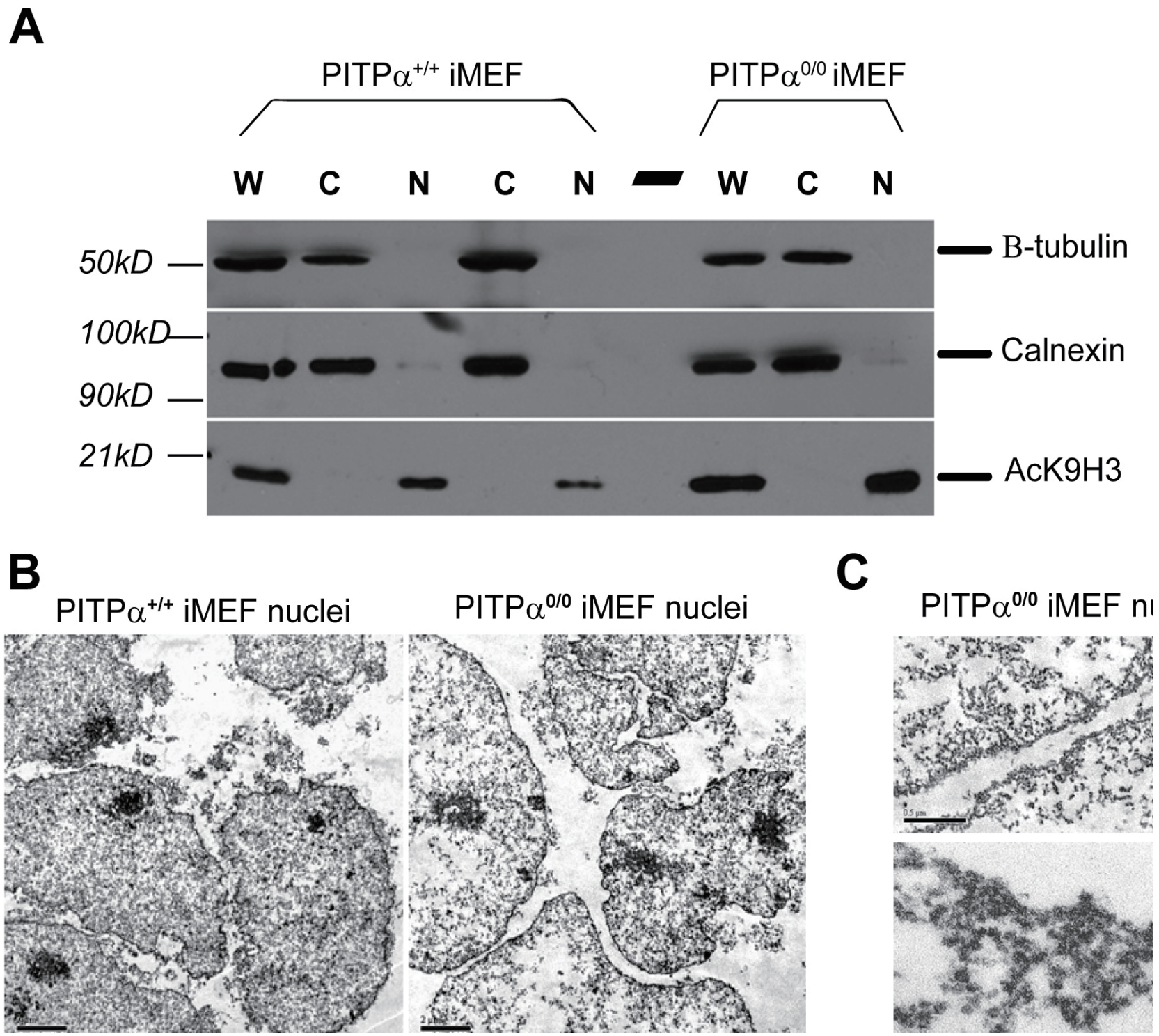
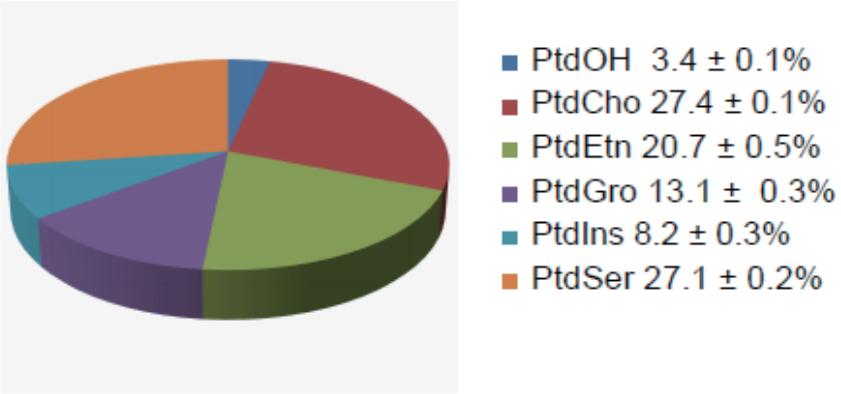

A
PITPα+/+ iMEF
PITPα0/0 iMEF
W C N C N W C N
50kD
100kD
90kD
21kD
B-tubulin
Calnexin
AcK9H3
B
PITPα+/+ iMEF nuclei
PITPα0/0 iMEF nuclei
C
PITPα0/0 iMEF nu

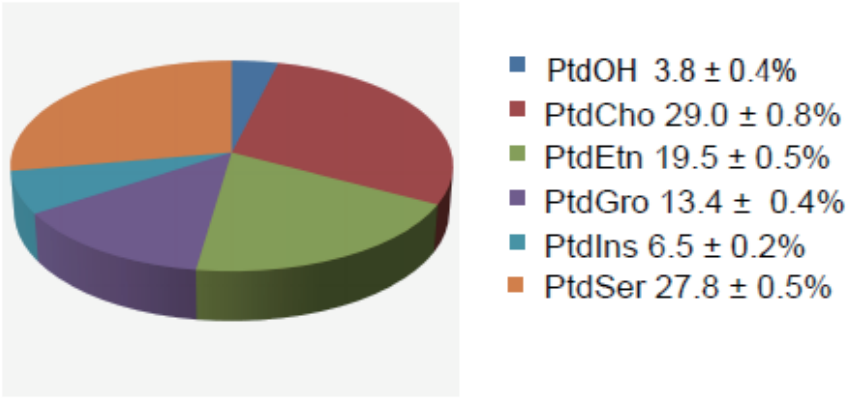
Fig. S4

A

PITPα$^{+/+}$ iMEF Cells

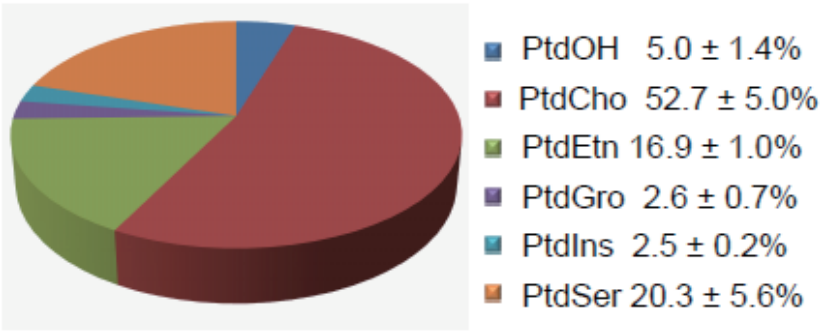


PITPα$^{0/0}$ iMEF cells



B

PITPα$^{+/+}$ iMEF Nuclei



PITPα$^{0/0}$ iMEF Nuclei

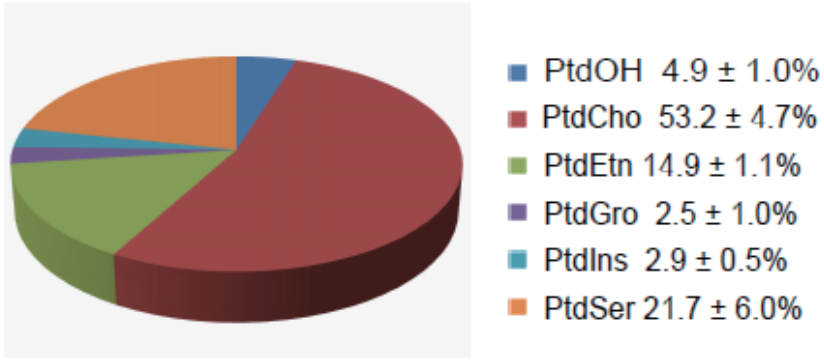
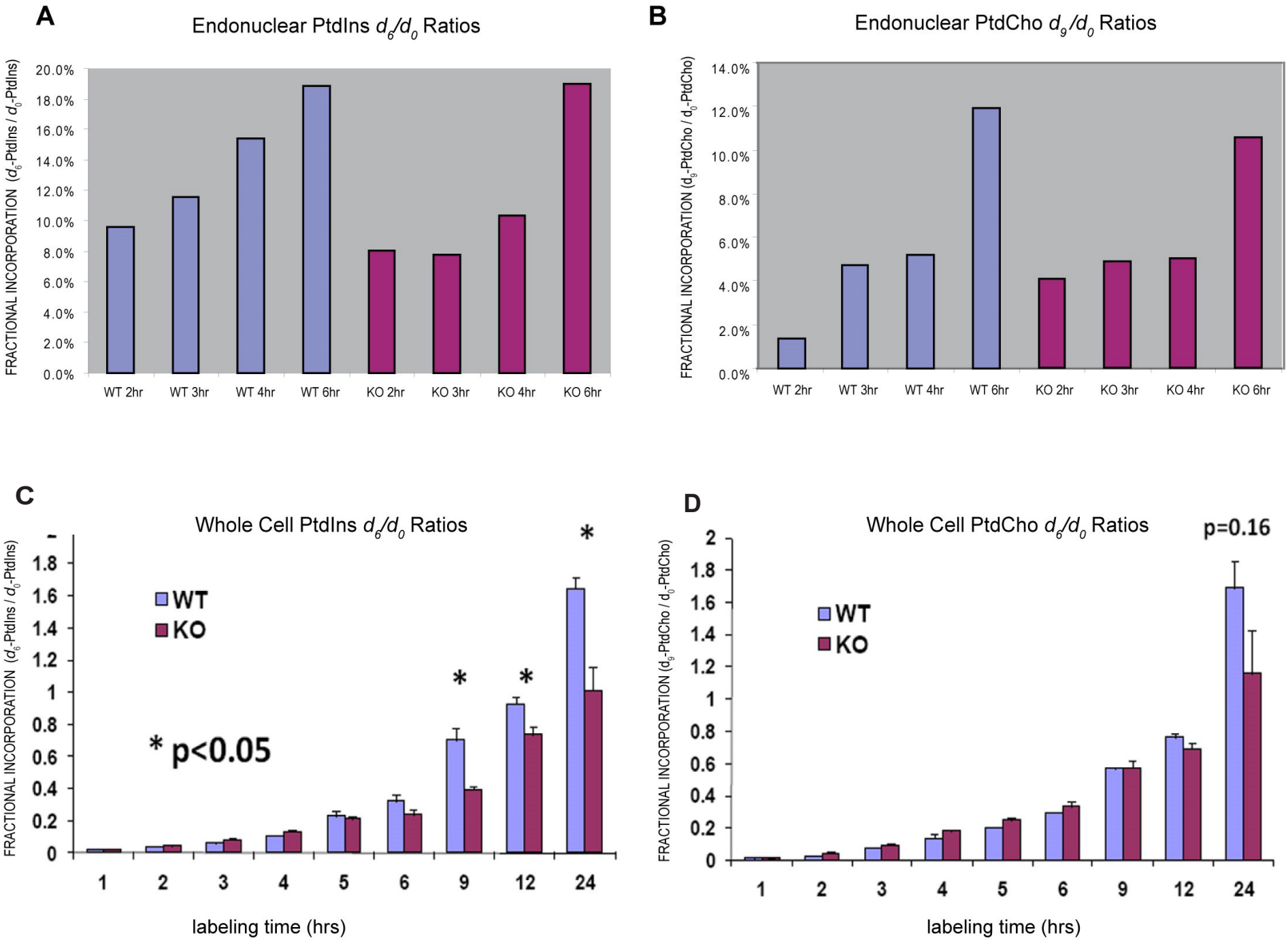


Fig. S5


A
Endonuclear PtdIns d_6/d_0 Ratios
FRACTIONAL INCORPORATION (d_6-PtdIns / d_0-PtdIns)
20.0%
18.0%
16.0%
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
WT 2hr
WT 3hr
WT 4hr
WT 6hr
KO 2hr
KO 3hr
KO 4hr
KO 6hr
B
Endonuclear PtdCho d_9/d_0 Ratios
FRACTIONAL INCORPORATION (d_9-PtdCho / d_0-PtdCho)
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
WT 2hr
WT 3hr
WT 4hr
WT 6hr
KO 2hr
KO 3hr
KO 4hr
KO 6hr
C
Whole Cell PtdIns d_6/d_0 Ratios
FRACTIONAL INCORPORATION (d_6-PtdIns / d_0-PtdIns)
WT
KO
* p<0.05
*
*
*
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
9
12
24
labeling time (hrs)
D
Whole Cell PtdCho d_6/d_0 Ratios
p=0.16
FRACTIONAL INCORPORATION (d_9-PtdCho / d_0-PtdCho)
WT
KO
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
9
12
24
labeling time (hrs)

Supplementary Table S1.

Sample	*# nuclei examined*	*% w/o membrane*	*% w/ 1 membrane patch*	*% w/ > 1 membrane patch*	*# of membrane strands*
1	114	96.5	2.6	0.9	2
2	85	87.1	4.7	9.4	8
3	93	95.7	4.3	0.0	1
4	105	97.1	2.9	0.0	2
5	104	99.0	1.0	0.0	0
6	104	90.4	7.7	1.9	0
7	97	99.0	1.0	0.0	1
8	103	97.1	1.9	1.0	0
9	102	98.0	1.0	0.0	1
AVG	**101**	**95.5**	**3.0**	**1.5**	**2**

Supplementary Table S2.

whole MEFs			
species	mean	sem	rank
PS(36:1)	301.5	44.7	1
PC(36:2)	250.1	17.5	2
PC(32:1)	195.0	12.8	3
PE(38:4)	146.4	14.8	4
PC(34:1)	142.1	8.1	5
PS(34:1)	122.2	10.0	6
PC(38:6)	108.7	14.5	7
PC(36:1)	102.5	25.8	8
PI(38:4)	97.8	18.3	9
PE(38:0)/PE(40:7e)/PE(40:6p)	90.4	16.1	10
PC(38:4)	89.5	10.7	11
PE(38:5e)/PE(38:4p)	89.1	5.4	12
PC(30:1)	86.5	32.8	13
PE(38:5)	84.0	11.2	14
PC(32:0)	83.5	6.0	15
PS(36:2)	82.7	7.4	16
PS(40:5)	82.7	14.7	17
PC(30:0)	81.7	5.1	18
PE(36:2)	81.0	3.8	19
PS(40:6)	80.9	13.0	20
PS(38:4)	78.2	9.6	21
PE(40:6e)/PE(40:5p)	77.6	8.4	22
PE(38:3)	77.2	11.2	23
PC(38:5)	75.2	11.7	24
PC(40:6)	75.2	10.0	25
PE(40:5e)/PE(40:4p)	74.2	11.6	26
PE(36:1)	67.4	4.4	27
PC(38:3)	67.1	8.9	28
PC(34:2)	66.4	4.6	29
PE(34:1)	64.6	5.8	30
PC(38:0)	62.6	15.8	31
PG(36:2)	60.8	18.3	32
PS(38:3)	58.5	12.8	33
PE(40:4e)/PE(40:3p)	58.4	12.1	34
PC(36:3)	56.5	4.4	35
PS(40:4)	56.2	11.4	36
PC(38:6e)/PC(38:5p)	55.9	21.1	37
PC(34:1e)	52.7	10.5	38
PG(40:7)	52.4	15.9	39
PC(36:0)	52.1	7.7	40
PC(38:2)	48.9	6.4	41
PC(38:5e)/PC(38:4p)	46.8	10.5	42
PG(34:1)	46.7	4.6	43
PS(36:0)	45.2	15.8	44
PE(40:6)	44.3	7.7	45
PE(36:5e)/PE(36:4p)	43.4	3.9	46
PE(38:1)	42.7	4.6	47
PC(32:2)	42.3	3.2	48
PI(38:3)	41.7	9.3	49
PI(36:2)	39.9	6.3	50
PC(36:4)	39.3	5.0	51
PE(40:5)	39.2	6.8	52

MEF nuclei			
species	mean	sem	rank
PC(36:2)	5.2	0.7	1
PC(32:1)	5.1	0.5	2
PC(32:0)	5.0	0.5	3
PC(38:6)	4.9	0.5	4
PC(34:1)	4.3	0.5	5
PC(36:1)	4.1	0.5	6
PC(36:4)	3.8	0.6	7
PE(38:4)	3.7	0.5	8
PC(30:0)	3.5	0.4	9
PC(38:4)	3.2	0.7	10
PS(36:1)	2.8	0.7	11
PC(34:2)	2.5	0.5	12
PE(36:1)	2.2	0.2	13
PE(36:2)	2.0	0.3	14
PE(38:5e)/PE(38:4p)	1.6	0.2	15
PE(34:1)	1.6	0.3	16
PE(38:5)	1.5	0.3	17
PS(38:4)	1.5	0.6	18
PS(36:0)	1.3	0.1	19
PS(38:0)	1.3	0.1	20
PS(34:1)	1.2	0.2	21
PE(34:2e)/PE(34:1p)	1.2	0.1	22
PE(36:5e)/PE(36:4p)	1.1	0.1	23
PE(38:6)	1.0	0.2	24
PE(38:0)/PE(40:7e)/PE(40:6p)	1.0	0.1	25
PE(38:3)	1.0	0.2	26
PE(40:6)	0.9	0.1	27
PE(40:6e)/PE(40:5p)	0.9	0.1	28
PA(36:2)	0.9	0.1	29
PS(38:5)	0.8	0.1	30
PI(38:4)	0.8	0.2	31
PS(36:2)	0.8	0.1	32
PE(40:5)	0.7	0.1	33
PS(40:6)	0.7	0.1	34
PS(38:3)	0.7	0.1	35
PG(34:1)	0.7	0.2	36
PS(40:5)	0.6	0.1	37
PS(40:4)	0.6	0.1	38
PE(40:7)	0.6	0.1	39
PE(40:4)	0.5	0.1	40
PI(38:5)	0.3	0.0	41
PI(38:3)	0.2	0.0	42

Table 2, continued.

PI(38:5)	37.8	6.7	53
PG(38:6)	37.1	23.4	54
PG(38:4)	34.4	9.3	55
PC(36:2e)/PC(36:1p)	33.4	5.4	56
PE(36:3e)/PE(36:2p)	32.2	2.4	57
PG(40:6)	31.6	12.3	58
PA(34:1)	31.1	4.6	59
PG(38:3)	30.0	8.4	60
PE(40:4)	29.2	5.0	61
PE(34:2e)/PE(34:1p)	28.6	2.4	62
PS(40:3)	28.0	8.2	63
PS(32:1)	27.6	4.2	64
PA(36:1)	26.3	4.0	65
PC(38:1)	25.9	2.9	66
PG(36:3)	25.7	5.2	67
PE(38:2)	24.2	4.8	68
PS(38:2)	22.6	3.9	69
PG(40:5)	22.2	7.3	70
PG(38:5)	22.0	5.3	71
PG(36:1)	21.7	3.6	72
PS(34:2)	21.4	2.7	73
PE(40:7)	21.4	1.8	74
PA(32:1)	19.7	3.6	75
PA(36:2)	17.3	3.1	76
PC(34:2e)/PC(34:1p)	15.6	3.3	77
PS(34:0)	15.2	4.7	78
PG(34:2)	15.0	2.2	79
PS(36:4)	14.7	1.5	80
PI(36:4)	14.5	1.6	81
PI(36:1)	13.9	3.0	82
PI(36:3)	13.8	2.2	83
PI(34:2)	13.6	1.9	84
PA(32:0)	13.0	2.4	85
PS(36:3)	12.8	1.8	86
PS(42:1)	12.8	2.6	87
PG(42:8)	12.7	4.1	88
PI(34:1)	12.6	1.7	89
PC(36:5)	12.4	0.2	90
PS(40:1)	12.3	2.0	91
PG(40:4)	12.2	3.9	92
PS(40:2)	12.0	2.4	93
PA(40:5)	11.9	2.0	94
PG(38:2)	11.9	3.4	95
PG(42:9)	10.6	3.4	96
PA(40:4)	10.5	2.3	97
PG(42:7)	10.2	3.4	98
PA(34:0)	8.9	1.9	99
PA(38:4)	8.3	1.7	100
PA(40:6)	8.0	2.5	101
PG(40:8)	7.7	2.2	102
PG(42:10)	7.7	2.4	103
PA(34:2)	7.6	1.9	104
PC(36:5e)/PC(36:4p)	7.4	0.9	105
PA(38:3)	6.9	1.3	106
PI(40:5)	6.9	1.5	107
PI(40:6)	5.4	1.2	108
PI(38:2)	5.3	1.1	109
PI(40:4)	4.9	0.8	110
PG(32:1)	3.8	0.3	111