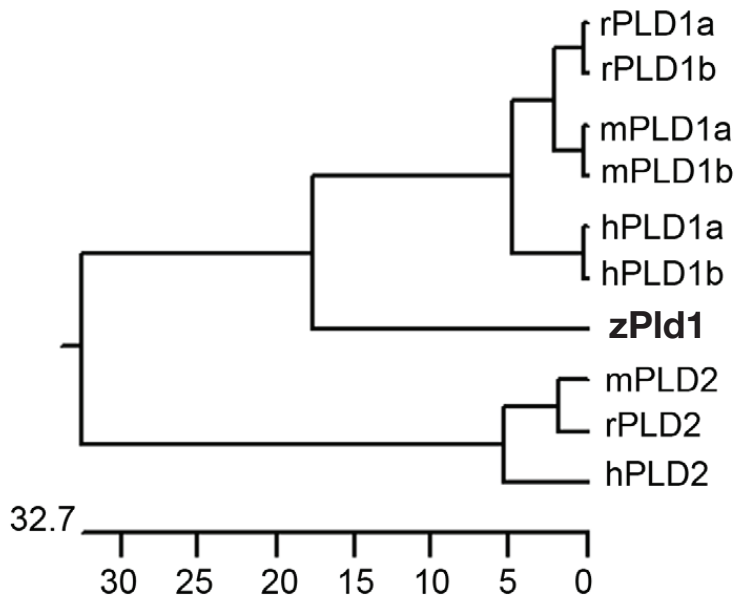


A

	1	10	20	30	40	50	
zPLd1MSD	SVENLDTRE	LGFSDA	DEPEE	VDFDFNPSGASR	IGFSAIYRTVGFKD	
hPLD1	MSLKNEPRVNTS	ALQKIAADMSNI	IENLDTRE	LHFEGE	VDYDVSFSDPKIQE	VYIPFSAIYNTQGFKE	
mPLd1	MSLKSETRVNTS	TLQKIAADMSNI	IENLDTRE	LHFEGE	VEYDA	SPGDPKAOEGCIPFSAIYNTQGFKE	
hPLD2MTATPESLFP	TGDELDSSO	OMESD	EVDTL	KEGEPADRMH	...PFLAIYELQSIK	
	60	70	80	90	100	110	120
zPLd1	TEAHVYLTSL	PITAKILDVER	FTRTQDRFKF	SKHRSV	KAMPA	VFKIELKHG	NFTWIVKRKEKHFLELHR
hPLD1	PNIQTYLSGC	PIKAQVLEVER	FSTST	TRVPSIN	LYTIELTHGEF
mPLd1	PNIQTYLSGC	PIKAQVLEVER	FSTST	SRVPSIN	LYTIELTHGEF
hPLD2	VHPLVFAPGV	PVTAQVVGTER	YTSG	SKVGTCT	LYSVRLTHGDF
	130	140	150	160	170	180	190
zPLd1	ELLKYKTLMRI	PLPTRSHTE	RKRKSIKRS	SEVROMPA	LPRGGDGE	LAREEQVS	SRRKOLEDYLNKLLK
hPLD1	ELLKYKAFIRI	PIPTRRHT	FRRQNVRE	EPREMP	SLPRSEN	MIREEQFLGRRKOLEDY
mPLd1	ELLKYKAFIRI	PIPKRHT	FRRQNVKE	EPREMP	SLPRSEN	AIQEEQFLGRRKOLEDY
hPLD2	DLRHKVAFIMS	LPLARFAVAYS	SPARDAGN	REMP	SLPRAGPEG	STRHAASKOKYLENYLNRL
	200	210	220	230	240	250	260
zPLd1	YRKYYATME	FIDVSQLSF	IQDLGPK	LEGIVY	KRSGGHR	IPGMN	CCGHNEVCYRWSKRWL
hPLD1	YRNYHATTE	FLDISQLSF	IHDLGP	KLEGIM	KRSGGHR	IPGLN	CCGQGRACYRWSKRWL
mPLd1	YRNYHATTE	FLDVSQLSF	IHDLGP	KLEGIM	KRSGGHR	IPGMN	CCGHNEVCYRWSKRWL
hPLD2	YRNYHAMTE	FLEVSQLSF	IPDLGR	KLEGIM	KRSGGHR	IPGLT	CCGRDQVCYRWSKRWL
	270	280	290	300	310	320	330
zPLd1	KPDTGAI	FVLLVDNE	FSIKMDSK	OTETKH	GVRIENLS	RKLVLF	KFTSYRHARWWGQAE
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hPLD2	CLETGAI	FVOLFDPG	FEVQVGR	KSREARHG	GVRI	DTSHRSL	LKCSYRQARWQA
	340	350	360	370	380	390	400
zPLd1	RTHRFGS	FARE	EENIPSK	WYVNGK	TYMEDVA	NALEEA	KEEIFITDWWLSPE
hPLD1	KDHRFGS	YAAIQEN	ALAKWYV	NAKGF	FEDVAN	AMEE	ANEEEIFITDWWLSPE
mPLd1	KDHRFGS	YAAP	HENTLAK	WYVNA	KGF	FEDIAN	AMEEAS
hPLD2	QLHRHDS	YAP	RPGLAR	WFVNG	AGYFA	ADAILRA	QEEEIFITDWWLSPE
	410	420	430	440	450	460	470
zPLd1	KRKAQQ	GVRIFV	MLYKE	VELAL	GINSE	YSKKT	LMHLHPN
hPLD1	KRKAQQ	GVRIFIM	LYKE	VELAL	GINSE	YTKRT	LMRLHPN
mPLd1	KRKAQQ	GVRIFIM	LYKE	VELAL	GINSE	YSKRT	LMRLHPN
hPLD2	KRKAEE	GVRISIL	LK	VELAL	GINSG	YSKRAL	MLHLHPN
	480	490	500	510	520	530	
zPLd1	FVGGI	DLAYGR	WDDREH	RLTDV	GSVTR	SVAA
hPLD1	FVGGI	DLAYGR	WDDNEH	RLTDV	GSVTR	SLGSL
mPLd1	FVGGI	DLAYGR	WDDNEH	RLTDV	GSVTR	SLGSL
hPLD2	FLGGL	DLAYGR	WDDLHY	RLTDL	GD
	540	550	560	570			
zPLd1	LPLKKGIG	RTRK	TRFSLY	HHIQRG	LHHAD	SISSIDS	TNK
hPLD1	SPLKKGIG	KPKR	FKSFLY	KQLHRR	HLHAD	SISSIDS	TSSYF
mPLd1	MPLKKGIG	KPKR	FKSFLY	RQLHRR	HLHAD	SISSIDS	TSSYF
hPLD2	SSESA	SQA	QPP	TP
	580	590	600	610	620	630	
zPLd1	SGSVH	SLOT	GVGEL	MGNTR	FWHG	KDYCNF
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mPLd1	OGLTR	HST	TGS	IRSVOT	GVGEL	LHGETR	FWHG
hPLD2	RPDSP	ATPDL	LSHN	QF	FWL
	640	650	660	670	680	690	700
zPLd1	VHGKA	ARDVAR	HFIQR	WNFTK	IMKPKY	RSLSY	PFLLPK
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mPLd1	VHGKA	ARDVAR	HFIQR	WNFTK	IMKPKY	RSLSY	PFLLPK
hPLD2	VHGLP	ARDLAR	HFIQR	WNFTK	TKAKY	KTP	YPLLPK
	710	720	730	740	750	760	770
zPLd1	IKYHE	ESIHN	AYIQV	IAKSK	HFIYI	ENOFF	FISCAD
hPLD1	IKYHE	ESIHA	AYVHV	IENS	SRHYI	YIEN	OFF
mPLd1	IKYHE	ESIHA	AYIHV	IENS	SKHYI	YIEN	OFF
hPLD2	TL	ENSIL	NAYL	HTIRES	OHFLY	IEN	OFF
	780	790	800	810	820	830	840
zPLd1	PGFEG	DI	TGGGN	ALQAV	MHFN	NYRTM	NRGEYS
hPLD1	PGFEG	DIS	TGGGN	ALQAI	MHFN	NYRTM	CRGENS
mPLd1	PGFEG	DIS	TGGGN	ALQAI	MHFN	NYRTM	CRGESS
hPLD2	PGFEG	DIS	TGGGNS	IQAIL	HFTY	RTL	CRGEYS
	850	860	870	880	890	900	910
zPLd1	VHSKL	LIADD	N	TVI	IGSAN	INDRS	MLGK
hPLD1	VHSKL	LIADD	N	TVI	IGSAN	INDRS	MLGK
mPLd1	VHSKL	LIADD	N	TVI	IGSAN	INDRS	MLGK
hPLD2	IHSKV	LIADD	N	TVI	IGSAN	INDRS	LLGK
	920	930	940	950	960	970	980
zPLd1	GAF	TDP	SID	VDSP	PISNS	FYKDV	WMSV
hPLD1	GYL	DDP	SE	DIQ	DPV	SKF	FKEV
mPLd1	GYL	SDP	SE	LODP	VPV	SKF	FKEI
hPLD2	GAN	TRP	DL	LRDP	PIC	DDFF	QLW
	990	1000	1010	1020			
zPLd1	ELL	KKIR	GFL	VQ	PLDF	LCE	ENL
hPLD1	EEL	KKIR	GFL	VQ	PPY	FL	SE
mPLd1	EEL	LRKIR	GFL	VQ	PPY	FL	SE
hPLD2	SEL	TOV	OGHL	VH	FPL	KFL	E

Fig. S1A

B**C**

	zPld1
hPLD1a	65.6
hPLD1b	67.5
mPLD1a	65.4
mPLD1b	67.8
rPLD1a	64.7
rPLD1b	66.2
hPLD2	50.5
mPLD2	49.6
rPLD2	50.3
dPLD	35.7
yPLD	24.3

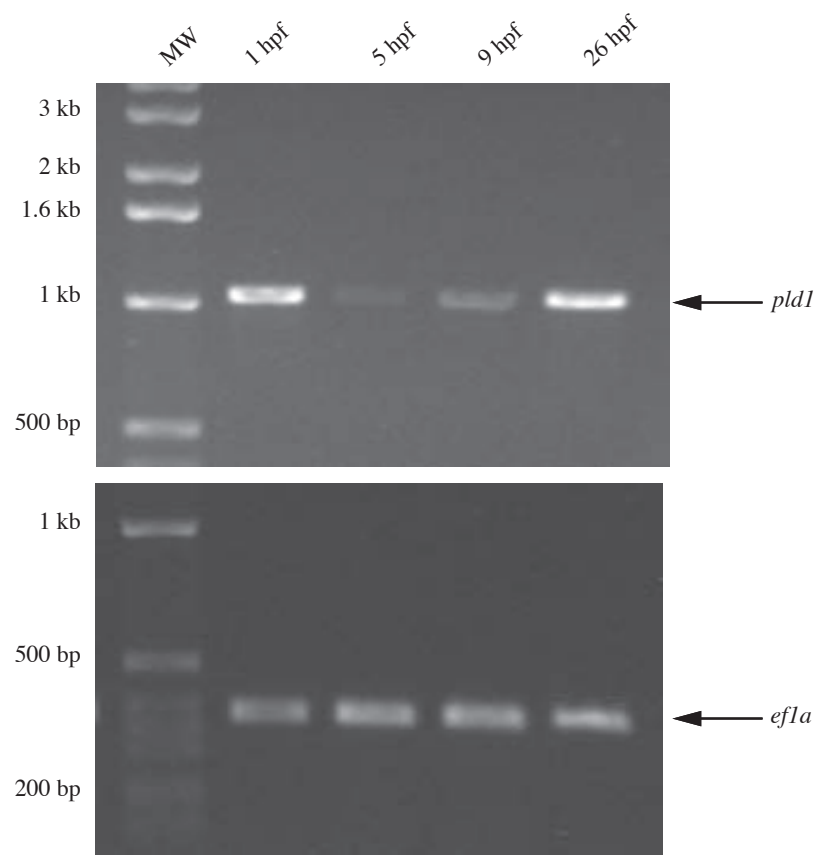


Fig. S2.

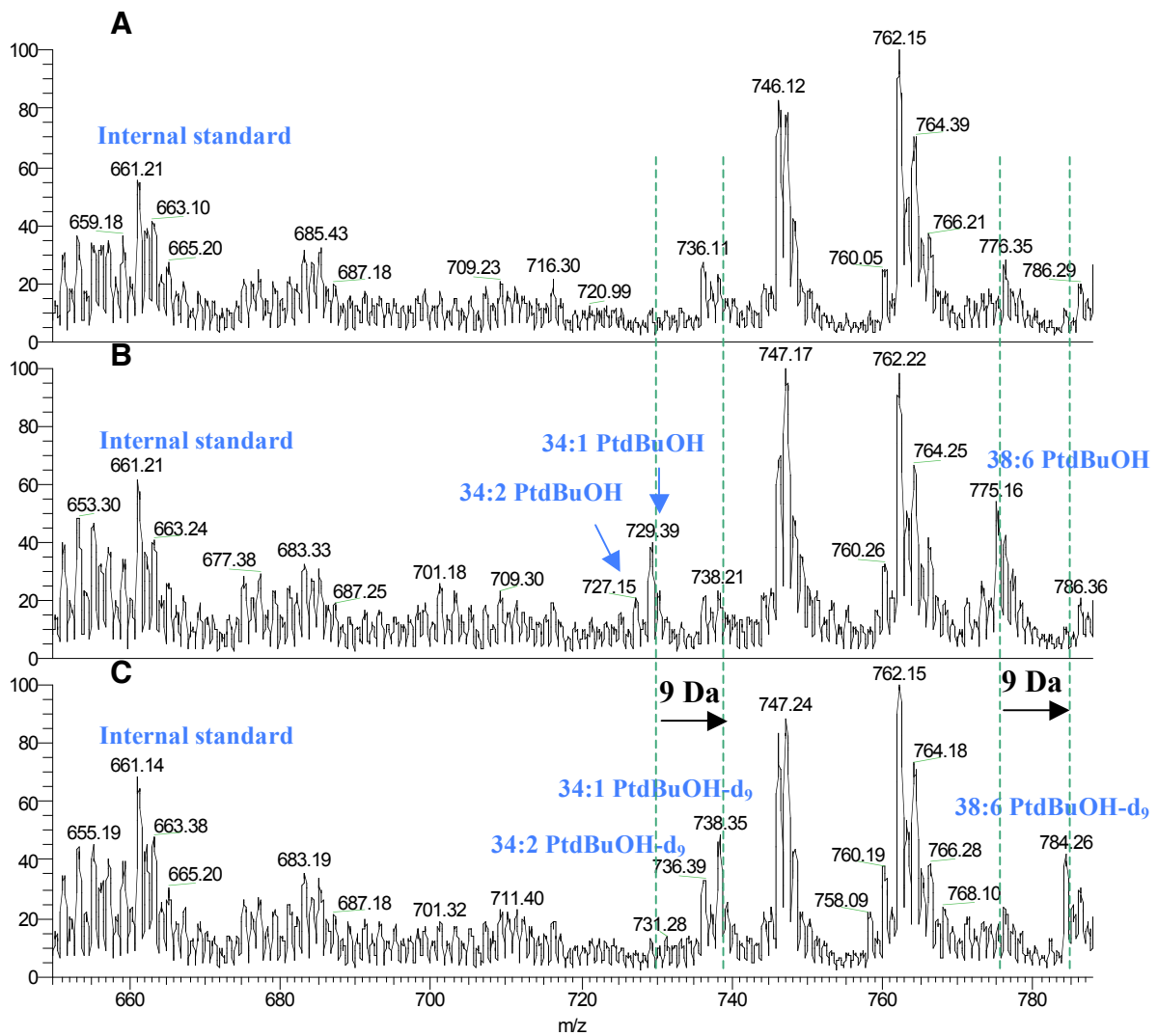


Fig. S3. The endogenous Pld activity assay of the zebrafish embryo.

(A) Mass spectrum of the global phospholipids extracted from the basal (untreated control) zebrafish embryos.

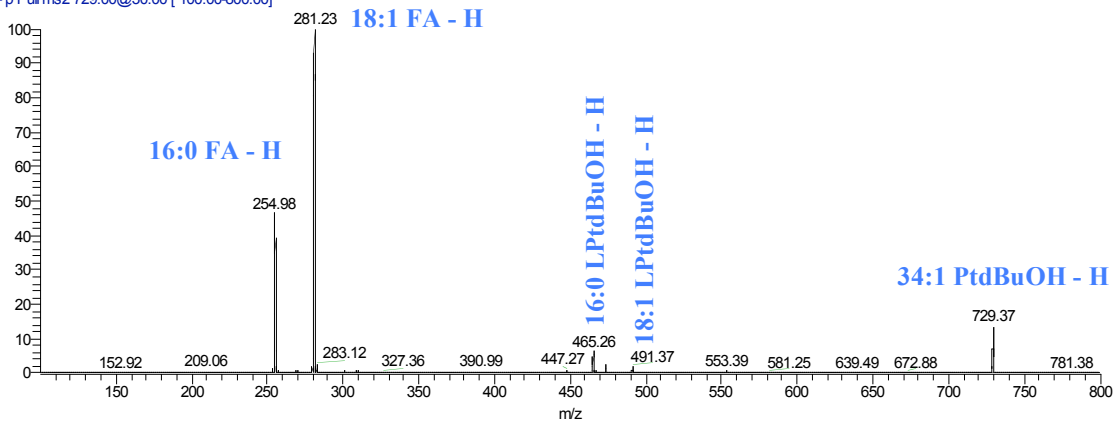
(B) Mass spectrum of the global phospholipids extracted from the embryos treated with PMA and 1-BuOH.

(C) Mass spectrum of the global phospholipids extracted from the embryos treated with PMA and 1-BuOH-d₁₀.

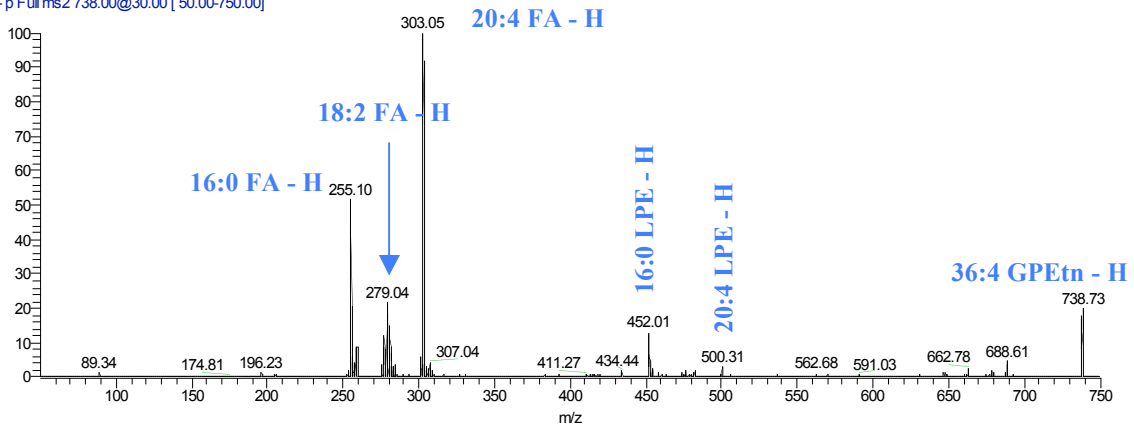
Fig. S4. The MS/MS spectra of the PtdBuOH in the endogenous Pld1 activity assay of the zebrafish embryos.

A

BuOH-1 neg-729 #1-90 RT: 0.01-1.00 AV: 90 NL: 1.97E5
 T: -p Fulms2 729.00@30.00 [100.00-800.00]

**B**

control 2 neg-738 #1-51 RT: 0.01-0.98 AV: 51 NL: 7.62E4
 T: -p Fulms2 738.00@30.00 [50.00-750.00]

**C**

BuOH-d10-1 neg-738 #1-90 RT: 0.01-0.99 AV: 90 NL: 1.45E5
 T: -p Fulms2 737.90@32.00 [100.00-800.00]

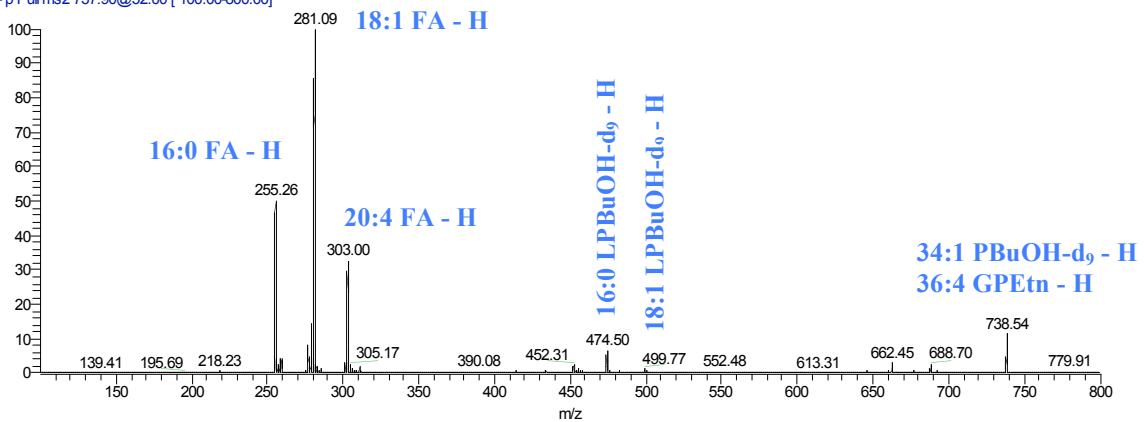


Fig. S4. The MS/MS spectra of the PtdBuOH in the endogenous Pld1 activity assay of the zebrafish embryos.

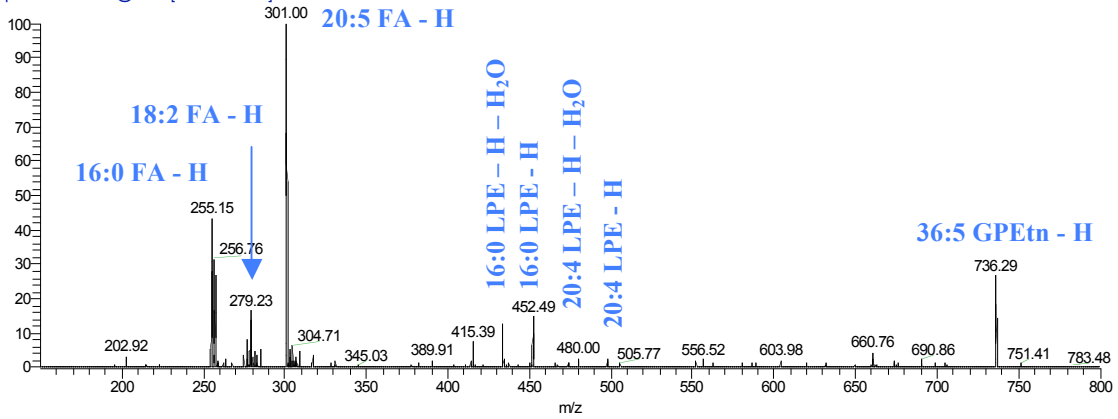
(A) MS/MS spectrum of m/z 729 in the PMA + 1-BuOH treated embryos.

(B) MS/MS spectrum of m/z 738 in control sample.

(C) MS/MS spectrum of m/z 738 in the PMA + 1-BuOH-d₁₀ treated embryos.

D

control neg-736 #1-89 RT: 0.01-0.99 AV: 89 NL: 3.57E4
 T: -p Fullms2 736.50@29.00 [150.00-800.00]

**E**

WT-PMA + 2H-BuOH- 2 neg 736 #1-88 RT: 0.01-0.99 AV: 88 NL: 5.47E4
 T: -p Fullms2 736.30@31.00 [100.00-800.00]

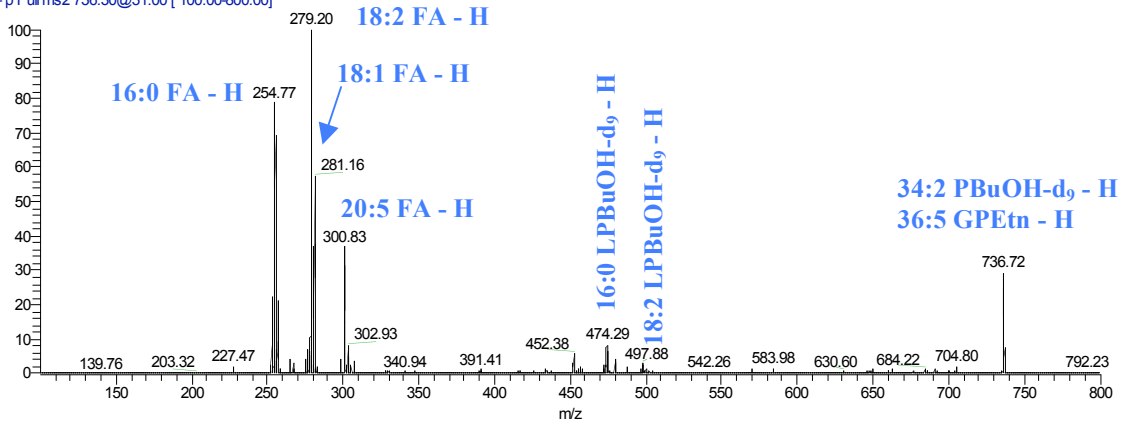


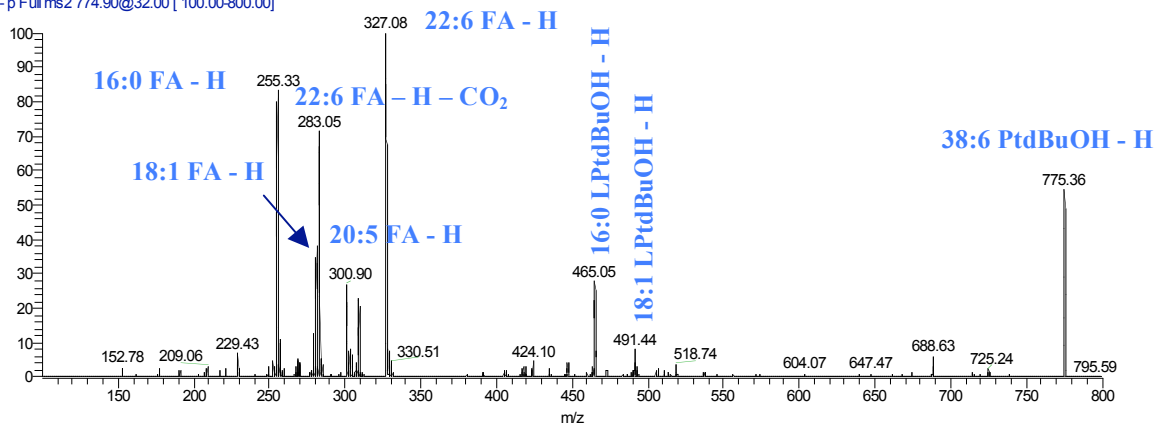
Fig. S4. The MS/MS spectra of the PtdBuOH in the endogenous Pld1 activity assay of the zebrafish embryos.

(D) MS/MS spectrum of m/z 736 in control sample.

(E) MS/MS spectrum of m/z 736 in the PMA + 1-BuOH- d_{10} treated embryos.

F

BuOH-2-neg-775 #1-90 RT: 0.01-1.00 AV: 90 NL: 8.44E4
 T: -p Fullms2 774.90@32.00 [100.00-800.00]

**G**

BuOH-d10-2 neg-784-2 #1-90 RT: 0.00-0.99 AV: 90 NL: 8.10E4
 T: -p Fullms2 783.90@32.00 [100.00-800.00]

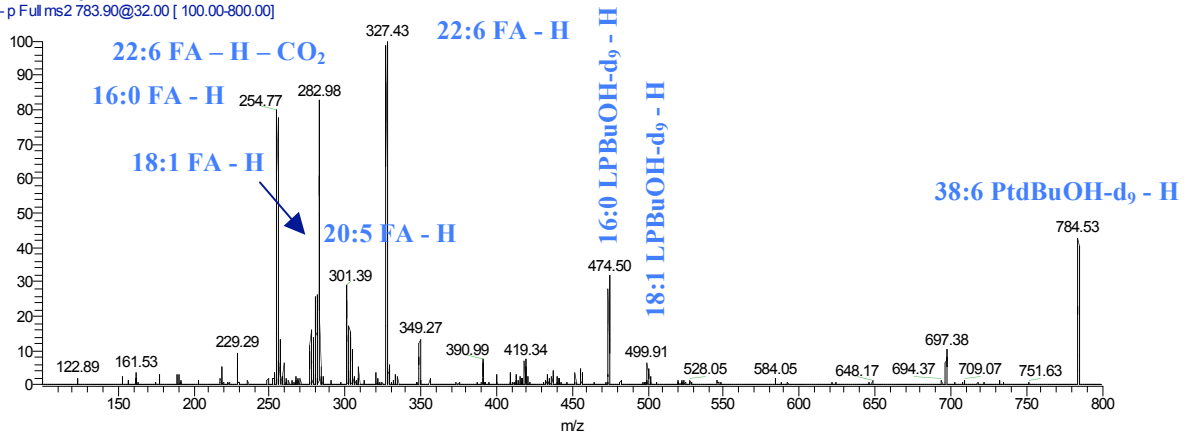


Fig. S4. The MS/MS spectra of the PtdBuOH in the endogenous Pld1 activity assay of the zebrafish embryos.

(F) MS/MS spectrum of m/z 775 in the PMA + 1-BuOH treated embryos.

(G) MS/MS spectrum of m/z 784 in the PMA + 1-BuOH-d₁₀ treated embryos.

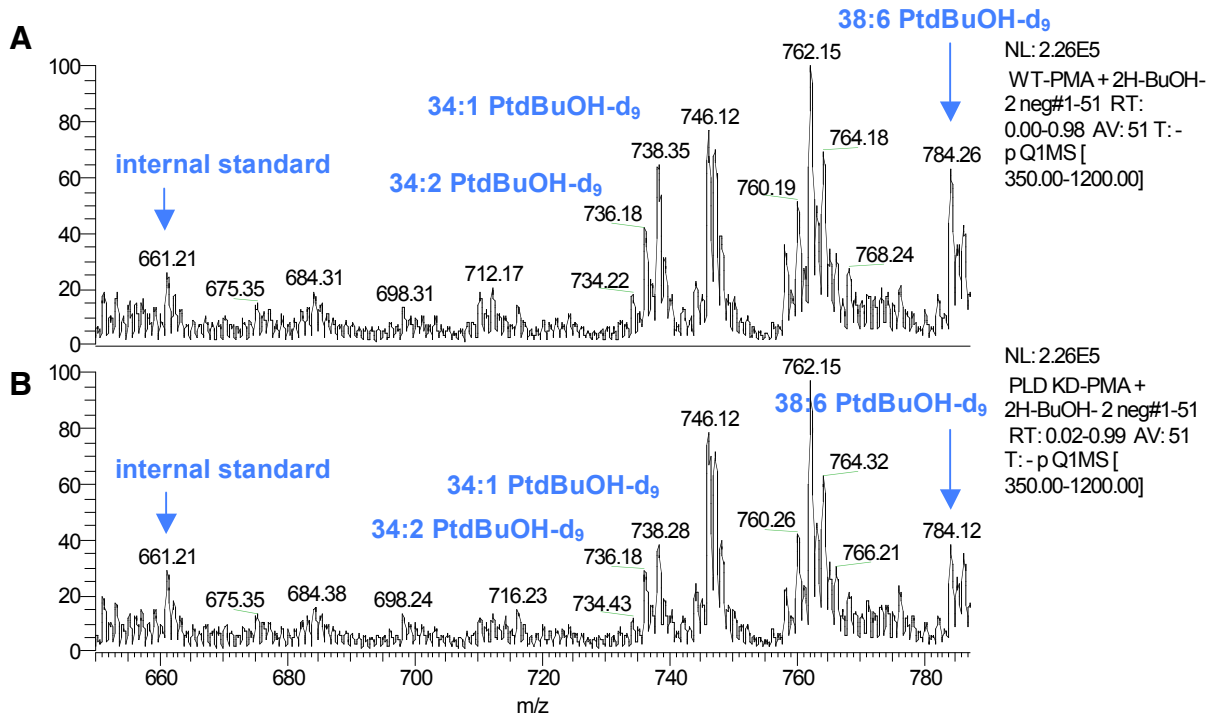
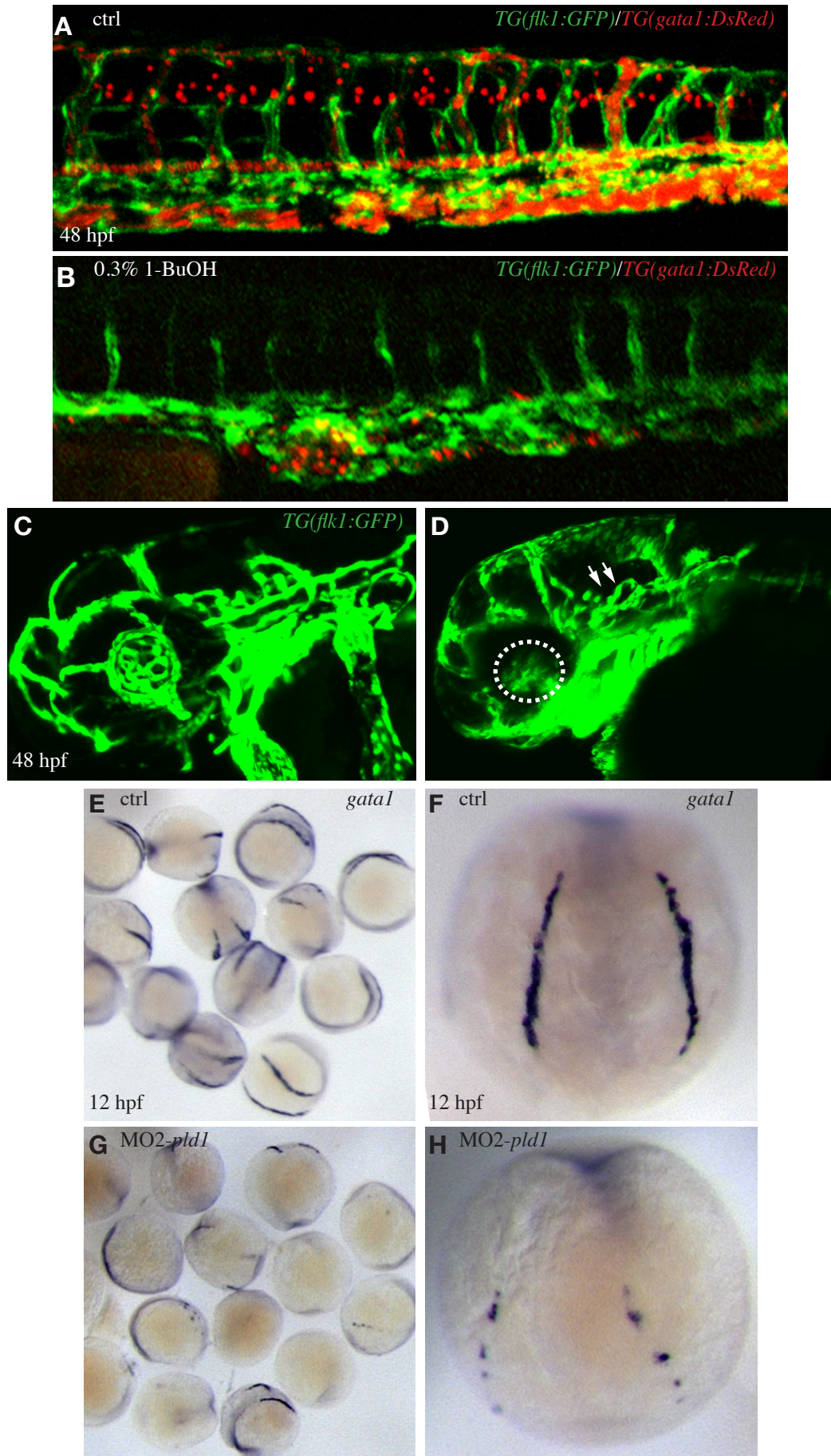


Fig. S5. The endogenous Pld activity assay in *pld1* morphant zebrafish embryos.

(A) The mass spectrum of the global lipids extracted from the PMA + 1-BuOH-d₁₀ treated wild type zebrafish embryos.

(B) The mass spectrum of the global lipids extracted from the PMA + 1-BuOH-d₁₀ treated MO1-*pld1* injected zebrafish embryos.



supplementary Fig. 6