

SUPPLEMENTAL MATERIAL

Supplemental Figure Legends

Figure S1. Alignment of the catalytic domains from human PLC isozymes

Human PLC isozymes were aligned using ClustalW and the X and Y regions identified by comparison with PLC δ 1 and PLC β 2 for which the structures are known. Red bars indicate the three loops around the active site. The linkers between the X and Y regions are not conserved and are of significantly different lengths in different isoforms. The size of each linker is indicated in parentheses. The residue highlighted in green is equivalent to D993 in PLC γ 2, the residue mutated in the ALI5 variant.

Figure S2. Impact of the selected mutations on PLC activity in COS cell assay

Inositol phosphate production was measured in COS-7 cells transfected with (A) PLC γ 1 wild-type and D342 variants, (B) PLC γ 1 wild-type and E347 variants and (C) PLC γ 1 wild-type and L384 variants. Cells were stimulated with 100 ng/ml EGF as indicated.

Figure S3. Analysis of mutations in the spPH domain

(A) Inositol phosphate production was measured in COS-7 cells co-transfected with PLC γ 2 wild-type or Y495F and Rac2 G^{12V} .

(B) COS-7 cells were transfected with the isolated PLC γ 2 spPH domain (wild-type or Y495F) with an N-terminal S tag and penta-His tag. The cells were stimulated with 100 ng/ml EGF for 10 min. The spPH domain was pulled down using S-agarose beads and Western blotting was performed, probing with antibodies against phosphotyrosine and penta-His.

(C) Sequence alignment of PLC γ 1 and γ 2 spPH domains. The ALI14 residue is highlighted in blue. Other conserved residues from the same surface are highlighted. These residues were mutated in PLC γ 1.

(D) Structure of the PLC γ 1 spPH domain (PDB:2FJL) showing the selected conserved residues. The colouring of the molecular surface indicates that all four residues are on the surface of the domain.

(E) Inositol phosphate production was measured in COS-7 cells transfected with PLC γ 1 wild-type and spPH mutants. Cells remained unstimulated or were stimulated with 100 ng/ml EGF.

Supplemental Methods

PLC γ 2 spPH pull down-COS-7 cells were transfected with pTriEx-4 PLC γ 2 spPH constructs. 24 h after transfection the cells were serum starved in the presence of 0.25 % BSA. After a further 24 h the cells were left unstimulated or stimulated with 100 ng/ml EGF for 10 min. Lysates were produced in the presence of phosphatase inhibitors cocktails 1 and 2 (Sigma). Lysate was added to S-agarose beads (Novagen) and incubated for 1 h at room temperature on a rotating wheel. The beads were washed with lysate buffer and then boiled in sample buffer. The proteins were identified by Western blotting.

Figure S1

XY LINKER

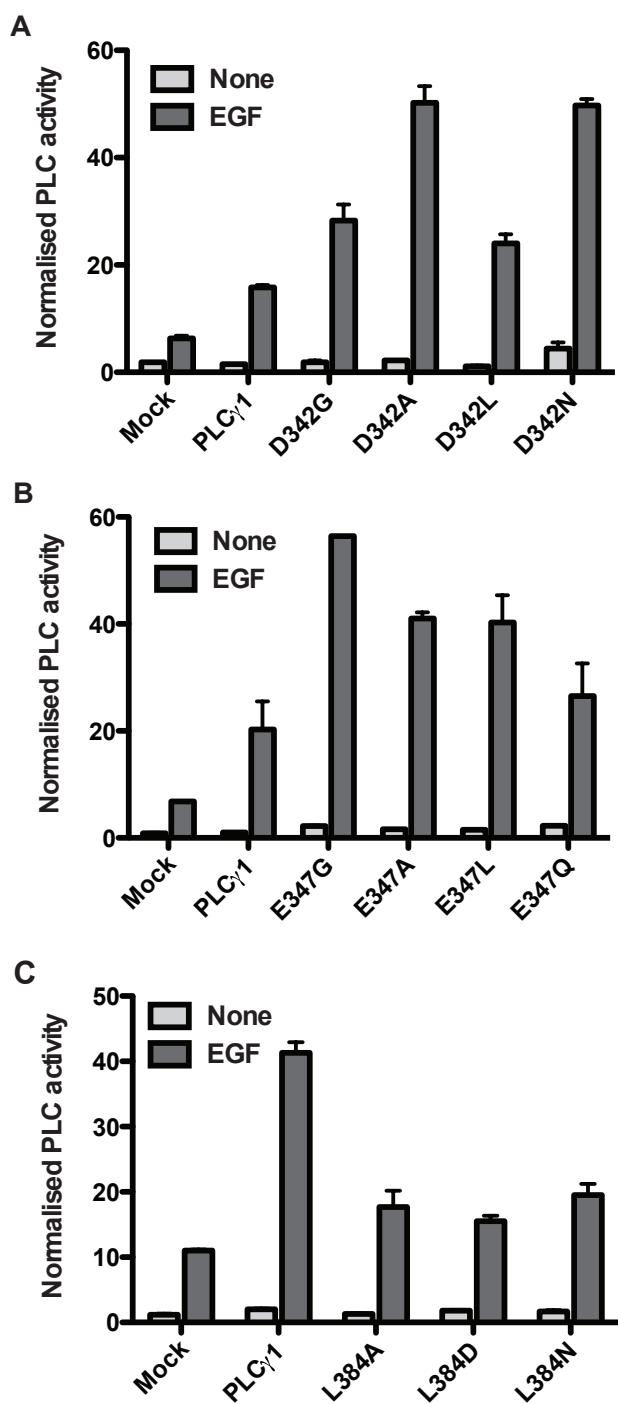
PLCδ1	PVILSLENHCT - LEQQRVMARHLHAILGPMPLLNRPLDG - VTNS - - LPSPEQLKGKILLKGKKL . . . (45) . . . LAQELSDMVY
PLCδ3	PVILSLENHCG - LEQQAAAMARHLCTILGDMLVQTALDSPNPEE - - LPSPEQLKGGRVLVKGGKL . . . (39) . . . ISPELSALAVY
PLCδ4	PVILSLETHCS - WEQQQTMARHLTEILGEQLLSTLDGVLPHQ - - LPSPEELRRKILVKGGKL . . . (51) . . . LCPALSSLVY
PLCβ1	PILLSFENHVDSPKQAKMAEYCRLLFGDALLMEPLEKYPLEGVPLPSPMDLMYKILVKNNKK . . . (66) . . . ATEEMSNLVNY
PLCβ2	PIILSFENHVDSPRQOKMAEYCRTIFGDMLLTEPLEKFPLKPGVPLPSPEDLKVPLIKKNKK . . . (76) . . . AYEEMSLVNY
PLCβ3	PVILSFENHVDSAKQOKMAEYCIRSIFGDALLIEPLDKYPLAPGVPLPSPQDLMGRILVKNNKK . . . (115) . . . ATEEMSLTVY
PLCβ4	PVILSFENHCS - KYQQYKMSKYCEDLFGDLLLKQALESHPLEPGRALPSPNDLKRKILIKKNKL . . . (95) . . . IHPYLSLTMINY
PLCγ1	PVILSIEDHCS - IAQQRNMAQYFKVKGDTLLTKPVEISADG - - LPSPNQLKRKILIKHHKL . . . (482) . . . IALELSELVVY
PLCγ2	PVILSIEEHCS - VEQQRHMAKAFKEVFGDLLLLKPTEASADQ - - LPSPSQLREKIIIKHHKL . . . (467) . . . IAIELSDLVVY
PLCε1	PIIISIENHCS - LPQQRKMAEIFKTVFGEKLVTKFLFETDFSDPMLPSPDQLRKVVLLKNKKL . . . (118) . . . IAPELSDLVY
PLCζ	PVVLSIENHCS - TAQQREVMADNLQATGFESLSDMLD - DFPDT - - LPSPEALKFKGKILVKNNKK . . . (43) . . . IALALSDLVY
PLCη1	PVILSIENHCS - IQQQRKIAQYLGIFGDKLDSLSSVTDGECKQ - - LPSPQSLKGKILVKGGKL . . . (150) . . . LCRELSDLVVY
PLCη2	PVILSIENHCS - VIQQKMAQYLTDLIGDKDGLLSSVSSEDATT - - LPSPQMLKGKILVKGGKL . . . (148) . . . LSRALSDLVY
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PLCδ1	CKSVHFGFSSPGTPGQAFYEMAS-----
PLCδ3	CHATRLR-TLHPAPNAPQPCQVSS-----
PLCδ4	LKSVSFRSFTHS-KEHYHFEISSL-----
PLCβ1	IQPVKFESFEIS-KKRNKSFEMS-----
PLCβ2	IQPTKVFVSFEFS-AQKNRSYVIS-----
PLCβ3	IEPVKFKSFEAA-RKRNKCFEMS-----
PLCβ4	AQPVKFQGFHVA-EERNIHYNMS-----
PLCγ1	CRPVPFDDEEKIG-TERACYRDMS-----
PLCγ2	CKPT--SKTKDN-LENPDFREIR-----
PLCε1	CQAVKFPLSTLNASGSSRGKERKSIFGNNPGRMSPGETASFNKTSGKSCREGIRQTWEESSSPLNPTTSLSAII RTPKCYHI-----
PLCζ	TKAEKFKSFQHS-RLYQQFNEENNNS-----
PLCη1	TNSVAAQDIVDD---GTT-GNVL-----
PLCη2	TKSVATHDIEMB---AASSWVQSS-----

Loop Y

PLCδ1 - FSENRLQESNGFVRHNVGHLRIYPAGWRTRISSNSPVEMWNGGCQIVALNFQTPGPEMDVYQGRFQ
PLCδ3 - LSERAKKLIREAGNSFVRHNRARQLTRVYPLGLRMNSANYSPOEMWNSGCQLVALNFQTPGYEMDLNAGRFL
PLCδ4 - FSETKAKRLIKEAGNEFVQHNTWQLSRVYPSGLRTISSNSNPQELWNAGCQMVA
PLC β 1 - SFVETKGLEQLTKSPVEFVEYNKMQLSRIPKGTRVISNSNYMPQLFWNAGCQMVALNFQTMDLAMQINMGMYE
PLC β 2 - SFTELKAYDLLSKASVQFDYKRNQMSRIPKGTRMDSSNSYMPQMFVNAGCQMVALNFQTMDLPMQQNMAVFE
PLC β 3 - SFVETKAMEQLTKSPMEFVEYNKQQLSRIYPKGTRVISNSYMPQLFWNAGCQLVALNFQTLDVAMQLNAGVFE
PLC β 4 - SFNESVGLGYLKTHIAEFVN
PLC γ 1 - SFPETKA
PLC γ 2 - SFVETKADSIIRQKP - VDLLKYNQKGLTRVY
PLCε1 - SSLNENA
PLCζ - IGETQARKLSKLRLRVHEFIFHTRKFITRIPKATRAD
PLCη1 - FSETRAHVQVQKQSEPMYINQKQLTRIYPSAYRIS
PLCη2 - FSETKAHOILOOKPAOYLRFNQOOLSRIPSYRVISNSNPQPFVNAGCQLVALNYQSEGRMLQINRAKFS

Supplemental 2



Supplemental 3

