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 PLCδ1 and PLCβ2 for which the structures are known. Red bars indicate the three loops around the active site. The linkers between the X and Y rgions 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 PLCγ2, the residue mutated in the ALI5 variant.

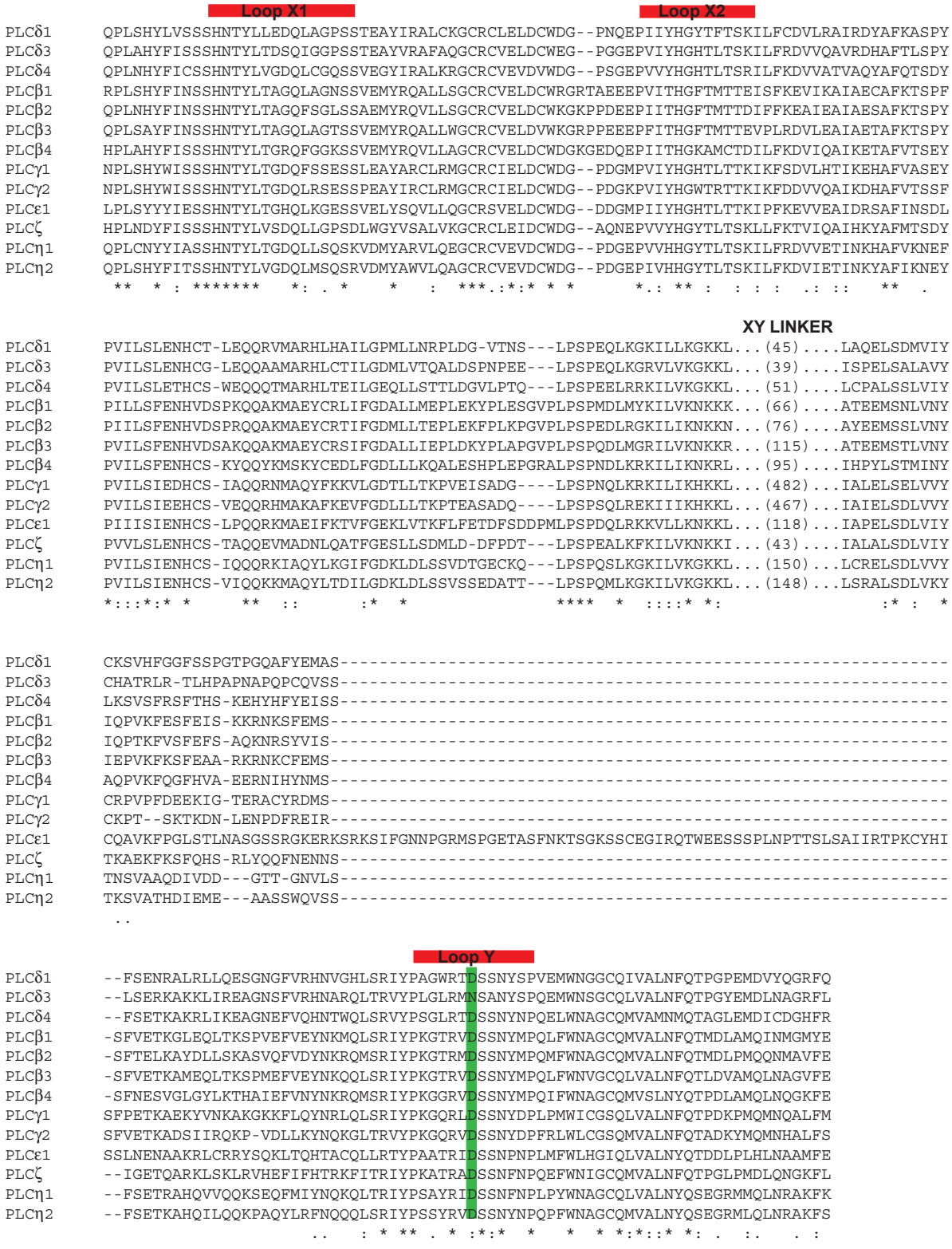
Figure S2. Impact of the selected mutations on PLC activity in COS cell assay
Inositol phosphate production was measured in COS-7 cells transfected with (A) PLCγ1 wild-type and D342 variants, (B) PLCγ1 wild-type and E347 variants and (C) PLCγ1 wild-type and L384 variants. Cells were stimulated with 100 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 PLCγ2 wild-type or Y495F and $Rac2^{G12V}$.
(B) COS-7 cells were transfected with the isolated PLCγ2 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 PLCγ1 and γ2 spPH domains. The ALI14 residue is highlighted in blue. Other conserved residues from the same surface are highlighted. These residues were mutated in PLCγ1.
(D) Structure of the PLCγ1 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 trasfected with PLCγ1 wild-type and spPH mutants. Cells remained unstimulated or were stimulated with 100 ng/ml EGF.

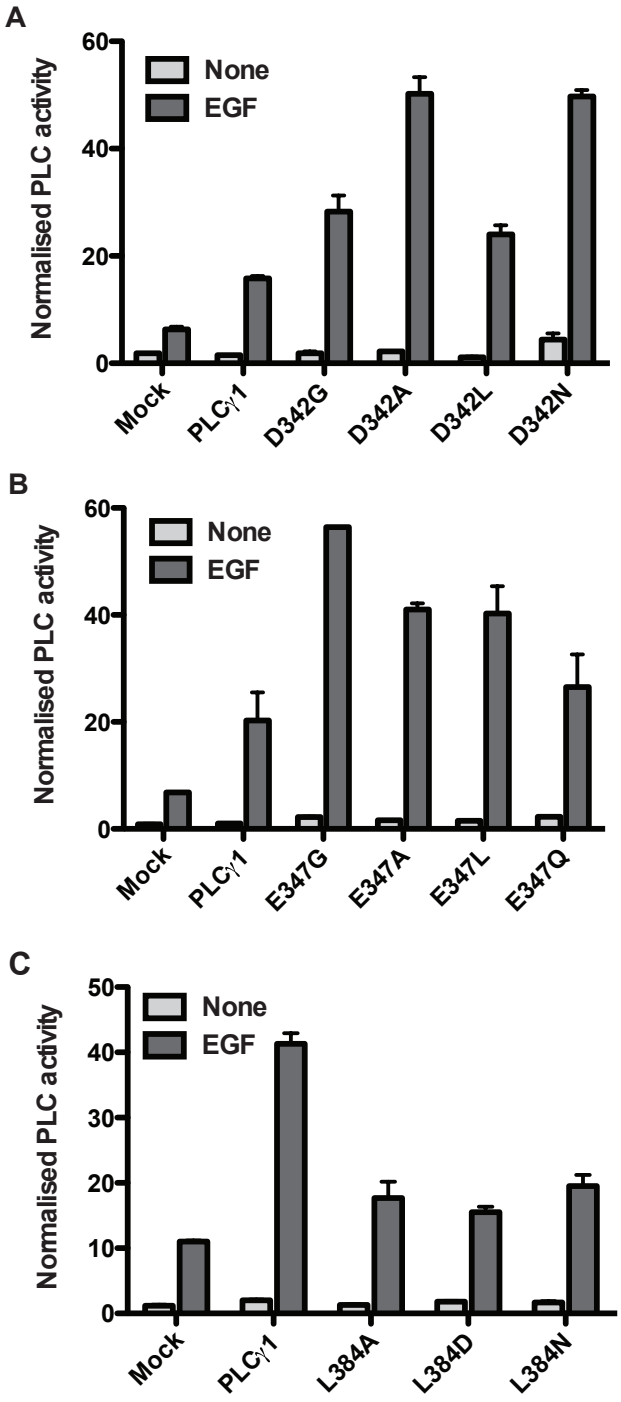
Supplemental Methods

PLCγ2 spPH pull down-COS-7 cells were transfected with pTriEx-4 PLCγ2 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.

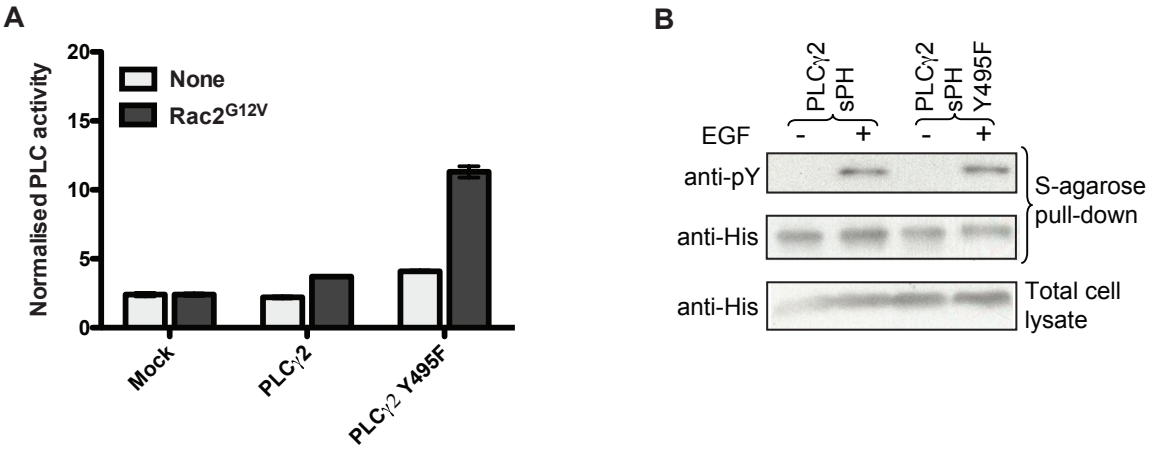
Figure S1


Loop X1
Loop X2
XY LINKER
Loop Y
PLCδ1
PLCδ3
PLCδ4
PLCβ1
PLCβ2
PLCβ3
PLCβ4
PLCγ1
PLCγ2
PLCε1
PLCζ
PLCη1
PLCη2

Supplemental 2



Supplemental 3



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PLCγ1      ----SIKNGILYLEDPVNHEWYPHWFVLTSSKIYYSEETSSD...
PLCγ2      KKDEHKQQGELYMWDSIDQKWTRHICAIADAKLSFSDDIEQT...
                 ::* **: *.::::*  ** .::.:*: :*:: ..

PLCγ1      ..LDENSPLGDLLRGVLDVPACQIAIRPEGKNNRLFVFSISMPSV
PLCγ2      ..MEEDNPLGSLCRGILDLNTYNVVKAPQGKNQKSFVFILEPKQQ
             ::*:.***.* **:**: : ::.  *:***:: *** :.  .

PLCγ1      AQWSLDVAADSQEELQDWVKKIREVAQTA---
PLCγ2      GDPPVEFATDRVEELFEWFQSIREITWKIDTK
           .: .::.*:*  *** :*.:.***:: .
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