

Supplementary Information

PRIP/PLCL deficiency activates PI3K-AKT-YAP signaling and promotes organ fibrosis

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Supplementary Table S1. List of antibodies used in this study.

Antibody (Catalog number)	Source
Akt (#9272)	Cell Signaling Technology, Beverly, MA, USA
Phospho-Akt (Ser473) (#9271)	Cell Signaling Technology, Beverly, MA, USA
Phospho-Akt (Thr308) (#13038)	Cell Signaling Technology, Beverly, MA, USA
α -SMA (GTX100034)	GeneTex, Irvine, CA, USA
β -actin (#4967)	Cell Signaling Technology, Beverly, MA, USA
β -actin (#A5441)	Sigma-Aldrich, St. Louis, MO, USA
COL1A1 (#72026)	Cell Signaling Technology, Beverly, MA, USA
Lamin B1 (66095-1-Ig)	Proteintech, Rosemont, IL, USA
MST2 (A9036)	ABclonal, Woburn, MA, USA
Phospho-MST2-T117 (AP0934)	ABclonal, Woburn, MA, USA
PLCL1/PRIP1 (HPA031849)	Sigma-Aldrich, St. Louis, MO, USA
PLCL2/PRIP2 (HPA047616)	Sigma-Aldrich, St. Louis, MO, USA
PLCL2/PRIP2 (17421-1-AP)	Proteintech, Rosemont, IL, USA
Tubulin- β (RB-9249)	Thermo Fisher Scientific, MA, USA
YAP/TAZ (#93622)	Cell Signaling Technology, Beverly, MA, USA
Phospho-YAP (Ser127) (#13008)	Cell Signaling Technology, Beverly, MA, USA
Anti-mouse IgG (AP124P)	Sigma-Aldrich, St. Louis, MO, USA
Anti-rabbit IgG (AP132P)	Sigma-Aldrich, St. Louis, MO, USA
Alexa Fluor 488-conjugated goat anti-rabbit IgG (A31627)	Invitrogen, Carlsbad, CA, USA

α -SMA: alpha smooth muscle actin, COL1A1: collagen type I alpha 1, MST2: mammalian STE20-like kinase 2, PRIP1: phospholipase C like 1/phospholipase C-related catalytically inactive protein 1, PRIP2: phospholipase C like 2/phospholipase C-related catalytically inactive protein 2, YAP/TAZ: yes-associated protein/transcriptional coactivator with PDZ-binding motif

Supplementary Table S2. Sequence of primers used in this study.

Gene	Forward	Reverse
<i>Acta2</i>	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
<i>Actb</i>	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC
<i>Ankrd1</i>	CGACGTCTGCGATGAGTATAAA	CTCCAGCCTCCATTAACCTTCTC
<i>Colla1</i>	GGGTCTAGACATGTTTCAGCTTTGTG	ACCCCTTAGGCCATTGTGTATGC
<i>Gapdh</i>	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
<i>Tgfb1</i>	CAGCCCTGCTCACCGTCGTG	GGTTTGTGGCTCCCGAGGGC

Acta2: actin alpha 2, *Actb*: actin beta, *Ankrd1*: ankyrin repeat domain 1, *Colla1*: collagen type I alpha 1, *Gapdh*: glyceraldehyde-3-phosphate dehydrogenase, *Tgfb1*: transforming growth factor beta 1

Supplementary Table S3. Sample information and grouping metadata used for differential expression analysis in Table S1 and S3 (Data source: GSE76882). (Filename: Supporting Table S1.xlsx)

Supplementary Table S4. Full list of differentially expressed genes (DEGs) identified between fibrosis and non-fibrosis groups (Data source: GSE76882). (Filename: Supporting Table S2.xlsx)

Supplementary Table S5. Statistical comparison of *PLCL1* and *PLCL2* expression levels between fibrosis and non-fibrosis groups (Data source: GSE76882). Shapiro–Wilk tests were used to assess normality, and F-tests to assess variance equality. Due to non-normal distributions, the Wilcoxon rank-sum test was applied. Both *PLCL1* and *PLCL2* showed significantly lower expression in the fibrosis group ($p < 0.001$). (Filename: Supporting Table S3.xlsx)

Supplementary Table S6. Spearman correlation analysis between *PLCL1* and *PLCL2* and a spectrum of key fibrosis-associated genes (Data source: GSE76882). (Filename: Supporting Table S4.xlsx)

Supplementary Table S7. Sample information and grouping metadata based on the percentage of tubulointerstitial fibrosis, obtained from kidney biopsy specimens of patients with chronic kidney disease (CKD). According to the proportion of TIF, the samples were classified into mild, moderate, and severe fibrosis groups (Data source: GSE137570). (Filename: Supporting Table S5.xlsx)

Supplementary Table S8. Full list of DEGs identified by DESeq2 using the likelihood ratio test (LRT) across the three fibrosis severity groups in dataset GSE137570. (Filename: Supporting Table S6.xlsx)

Supplementary Table S9. Full list of DEGs identified from pairwise group comparisons (Severe vs. Mild, Severe vs. Moderate, and Moderate vs. Mild) in dataset GSE137570 using the DESeq2 Wald test. (Filename: Supporting Table S7.xlsx)

Supplementary Table S10. Multivariate linear regression model for TIF including standardized *PLCL_ave*, age, eGFR, and sex. Continuous variables (*PLCL_ave*, age, eGFR) were standardized (mean = 0, SD = 1), so coefficients represent the change in TIF per standard deviation increase. Sex is a categorical variable (Male/Female). Conf.low and conf.high indicate 95% confidence intervals (Data source: GSE137570). (Filename: Supporting Table S8.xlsx)

Supplementary Table S11. Full list of DEGs identified in myocardial tissue between dilated cardiomyopathies (DCM) and non-failing cardiomyopathies (NF) groups from the GSE116250 dataset, using the DESeq2 Wald test. (Filename: Supporting Table S9.xlsx)

Supplementary Table S12. Full list of DEGs identified myocardial tissue between ischemic cardiomyopathies (ICM) and NF groups from the GSE116250 dataset, using the DESeq2 Wald test. (Filename: Supporting Table S10.xlsx)

Supplementary Table S13. Full list of Gene Ontology (GO) enrichment analysis results of the union of genes significantly correlated with *PLCL1* and *PLCL2*. Genes with absolute correlation coefficients ($|\rho|$) > 0.4 and adjusted *P* values (FDR) < 0.05 were selected. GO terms with FDR < 0.05 were considered significantly enriched. (Filename: Supporting Table S11.xlsx)

Supplementary Table S14. Full list of Gene Set Enrichment Analysis (GSEA) results of genes ranked by correlation with *PLCL_ave*. Genes with $|\rho|$ > 0.4 and FDR < 0.05 were selected. Gene sets with FDR < 0.05 were considered significantly enriched. (Filename: Supporting Table S12.xlsx)