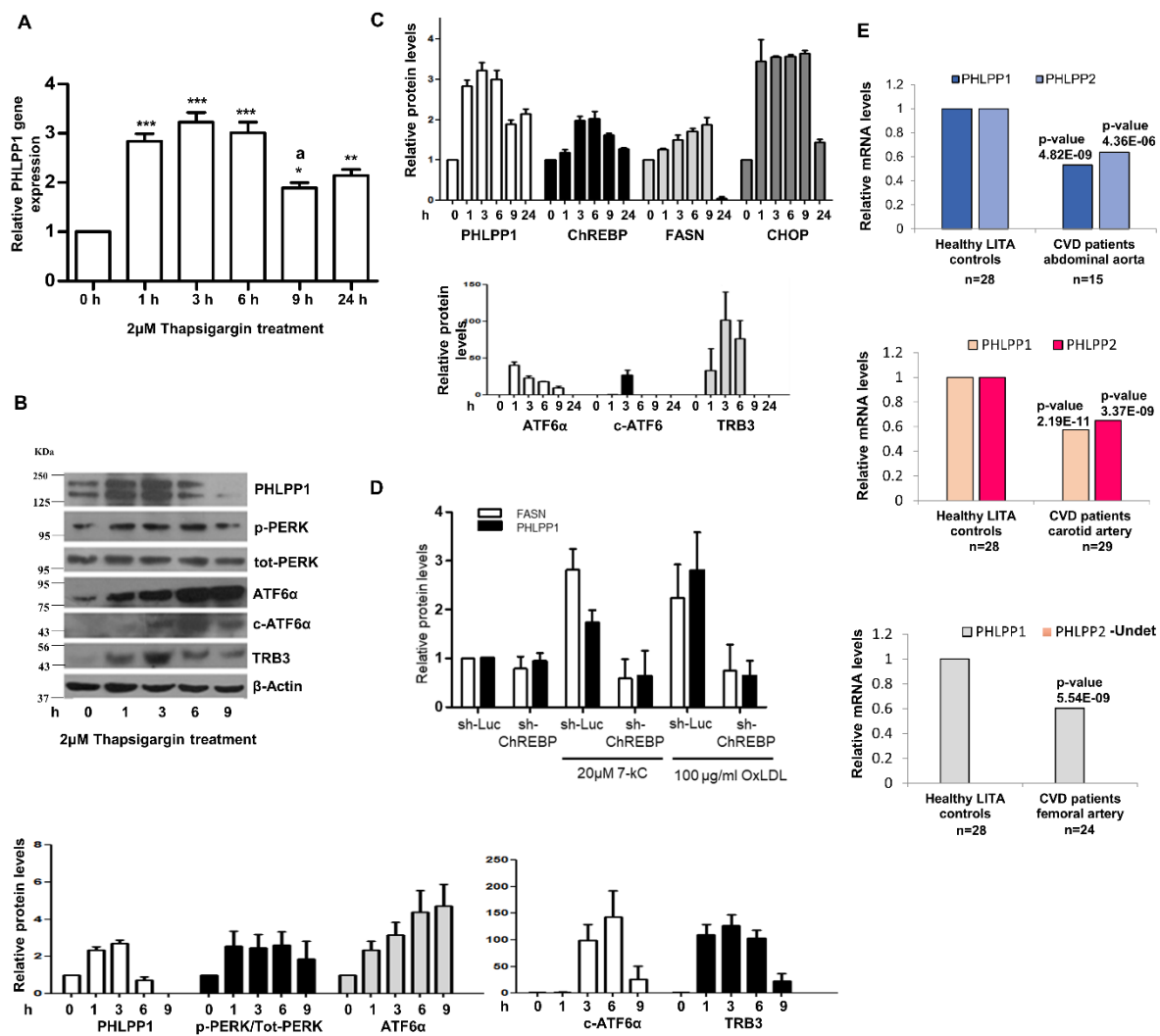


**Supplemental information**

**PHLPP1 promotes neutral lipid accumulation  
through AMPK/ChREBP-dependent lipid uptake  
and fatty acid synthesis pathways**

**Keerthana Balamurugan, Raghavender Medishetti, Jyothi Kotha, Parameshwar Behera, Kanika Chandra, Vijay Aditya Mavuduru, Manjunath B. Joshi, Ramesh Samineni, Madhumohan R. Katika, Writoban Basu Ball, Manjunatha Thondamal, Anil Challa, Kiranam Chatti, and Kishore V.L. Parsa**



**Supplementary Figure S1. PHLPP1 regulation under ER stress conditions and in human atherosclerotic plaques. Related to Figure 1.**

A-B) Time kinetics of PHLPP1 mRNA (A) and protein (B) levels upon treatment with 2 μM thapsigargin along with quantification (bottom); Statistical analysis was performed using one way ANOVA followed by Bonferroni multiple comparisons test (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs 0 h; <sup>a</sup> $p < 0.05$  vs 3 h)

C) Densitometric quantification of Figure 1D: Top panel: PHLPP1 and other non-ER stress proteins; Bottom Panel: ER stress proteins.

D) Densitometric quantification of Figure 1H.

E) PHLPP1 expression levels in human atherosclerotic plaques (Data re-analysed from Sulkava M et al., Sci Rep. 2017 Jan).

Data are a representative of three independent experiments. Numerical data are expressed as mean  $\pm$  SEM.

CLUSTAL O(1.2.4) multiple sequence alignment

```

Danio -548 -----CTACACCACAGCGACGTACATTGATTTCGTTTCGGTT-----T
Homo -691 AGGAGAAGCAGAACCAAAGGACCGCTGGGACTTGTAGTCCACCATGTTTCCCTCGGGATT
Mus -617 -----TCTCTTCTGGGTA
                                         * *

Danio -525 GTGGACACAAA-----ATCTAGAATGGACA-----ATA
Homo -631 GCCGATCCCCTTACGAGAACCACAAC TCCAAGTTTACC GCGCGCGTCGGTCTTCACTG
Mus -604 TCCTCGACACGAAGATGAACTACAACCCCACTGTGCAACGCGAGCCCCTCTCGCCAC--
                      * * * * * **

Danio -497 ATGGATTTGATTTGAGAACAAAAACGTGAAGGTAAATCA-----TAATAAAATAC
Homo -571 CCCTCTCCGAGGCCGGCTCAGGAACCTCCCGGGAAACTGCCCCACCCCGCCCTCGTAC
Mus -548 CTCTCCTCTATGG-CGAGGACAAACCTCTG--GGAAACAGCTCCGCCACCCCGTAC
                      * * * * * * * *

Danio -447 ACACGCGATGCGATTAGTCTCCGAATGCTCCTGCGTCGGGGGCGTTGACTGGTTCTCGT-
Homo -512 GCACGCGTCACGTTTGT TTTACCCTGAAGCTCCGCCTCC-GGCCATCCCCTGGCTCTCCCC
Mus -489 GCACGCGTCACGTTTGT TTTACCCTGAGGCCCCCGCCCC-AGCCTCCCGCTCTCC-----
                      * * * * * * * *

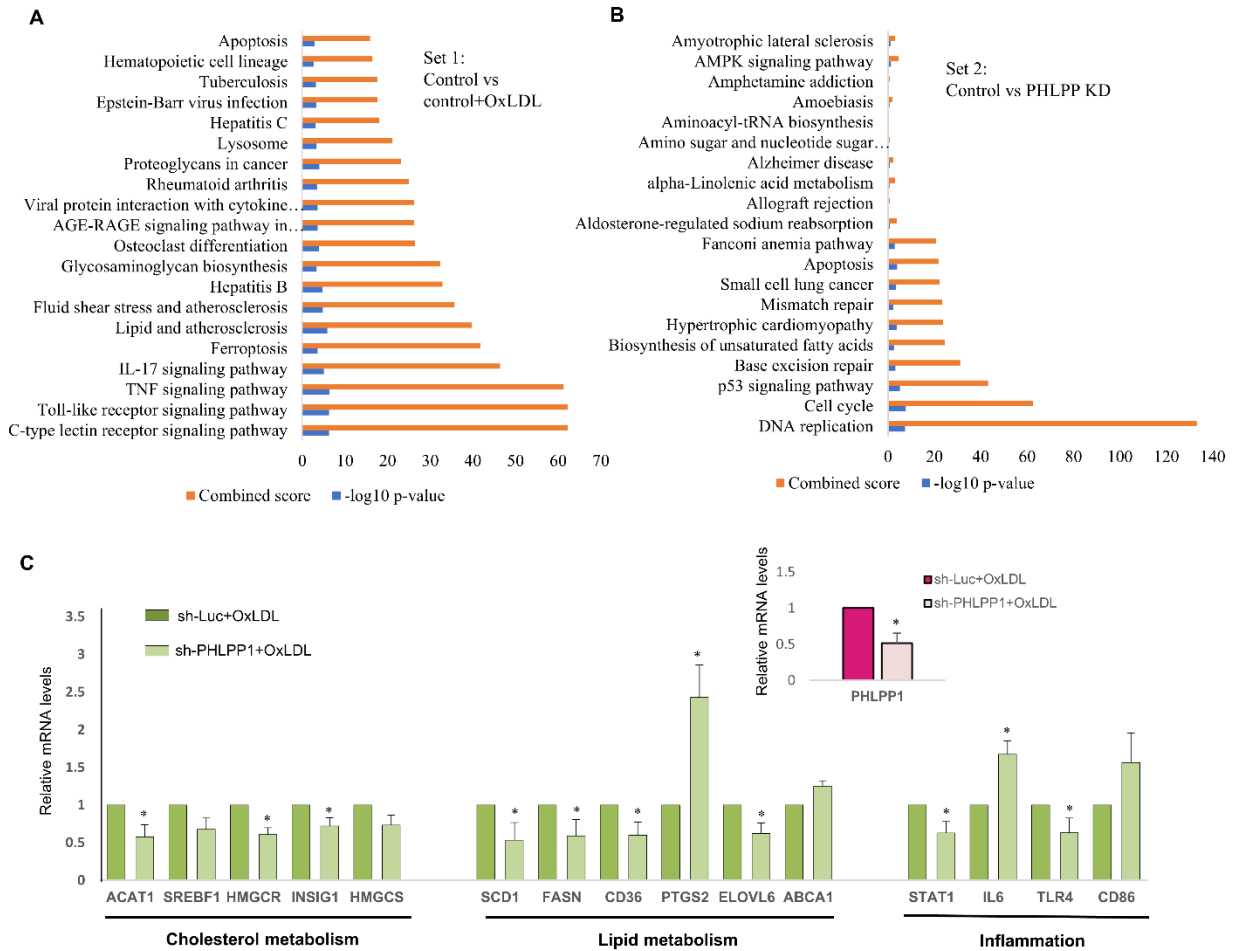
Danio -388 ----CTCGCGAGACTTGCGTGA-----
Homo -460 ACTCCTCCCTGGAATGCCGTGATGCCGGCGATGAATATTTATGAGGCACACG-----
Mus -438 -----GCAGAAATGTCGTGATGCTAACGATGCATATTCATGATCAACTCCGCCCGCC
                      * * * * *

Danio -370 -----AGCGCCGCGCTTCTTG-----
Homo -----
Mus -378 ACCGCCCCGCCTTCCCGCGCTTCATTAATATTCATGAGGCGAGCTCGGGCCGCGAGCTC

```

**Supplementary Figure S2. Multiple sequence alignment of PHLPP1 promoter region. Related to Figure 1.**

Multiple sequence alignment of PHLPP1 promoter region from three different species with ChREBP binding site highlighted



**Supplementary Figure S3. Pathway Analysis of set 1 and 2 RNA-seq data along with set 3 validation. Related to Figure 3.**

**A-B)** Top 20 affected pathways in set 1 (A) and set 2 (B) were identified using Enrichr tool by submitting the differentially altered genes from set 1 and 2 (cut-off: Fold Change > 1.5, FDR: 0.05). KEGG pathway 2021 human pathway tool was used to analyze the possible disease conditions.

**C)** Validation of RNA-seq data in set-3 (control cells treated with OxLDL and PHLPP1 knockdown cells treated with OxLDL (100µg/ml) for 24 h); Statistical analysis was performed using two tailed *t*-test. (\* $p < 0.05$  vs sh-Luc+OxLDL).

Data are a representative of three independent experiments. Numerical data are expressed as mean  $\pm$  SEM.

A

TRANSCRIPTION	
FACTOR	TARGET GENES
<b>HDAC2</b>	CCNE1, NFKB1A, NOS2, SMARCA4
<b>CEBPB</b>	CEBPA, SOCS3, CSF3, MAFB, MYC, ID2, SERPINE1, BCC2, PTGS2, CLU, ATF3
<b>CREB1</b>	LDHA, ENTPD1, NFKB1Z, BCL2, PTGS2, ESR1, NECTIN2
<b>MLXIPL</b>	FASN, FAS, ACACB, ACACA, ELOVL6, GPD1, Cpt1a, Mid1ip1, ACLY, Txnip,
<b>PPARG</b>	CEBPA, CD83, GK, SERPINE1, DB1, PTGS2, KCF4, MMP9, FABP4, CCNE1, MYC, BCL2, RARB, CD36, PDK2
<b>SMAD3</b>	GADD45B, PLAU, MYC, SERPINE1, ANGPTL2, NEDD9, CCL2, MMP9, FBLN5
<b>STAT1</b>	CD40, CEBPD, NOS2, MT1, BACE1, FCGR1, SOCS3, CXCL10, SOCS1, CD36, UPP1, PDCD1, GBP2
<b>NFKB1</b>	CD86, CEBPA, CD40, BMPR2, SRC, NUDT1, SLC2A1, GLRX, PTGS2, PIRB, TNF, CXCL2, CCL9, MYC, ALOX5, NFKB1Z, KDR, PMAIP1, CCL2, UPP1, PRODH, UBE2H, GADD4B, NOS2, IL15, MMP2, LIF, VEGFC, MMP9, NFKBIA, DCSTAMP, CXCL10, EBNA1BP2, IRF4, LCN2, BCL2, FAS, BIRC2, PTGES, BIRC3
<b>XBP1</b>	ERN1, IRF4, FASN, FOXO1
<b>JUN</b>	MSR1, ANGPT2, CEBPD, OS2, SRC, MMP2, SERPINE1, ODC1, SLC2A1, ENO2, PTGS2, TNF, ESR1, CXCL2, MMP9, SLC8A1, SOCS3, DCSTAMP, PLAU, CTSK, CCL2, CD14, CTSE, CD14, CTSE, NECTIN2
<b>IRF1</b>	CXCL10, SOCS1, NOS2, UPP1, GBP2, TNF
<b>ATF4</b>	MAP1LC3B, PLAU, DDIT3, SIGMAR1, CCL2, ASNS, NUPRI, PYGS2, SIRT2, ATF3, DDR2

B

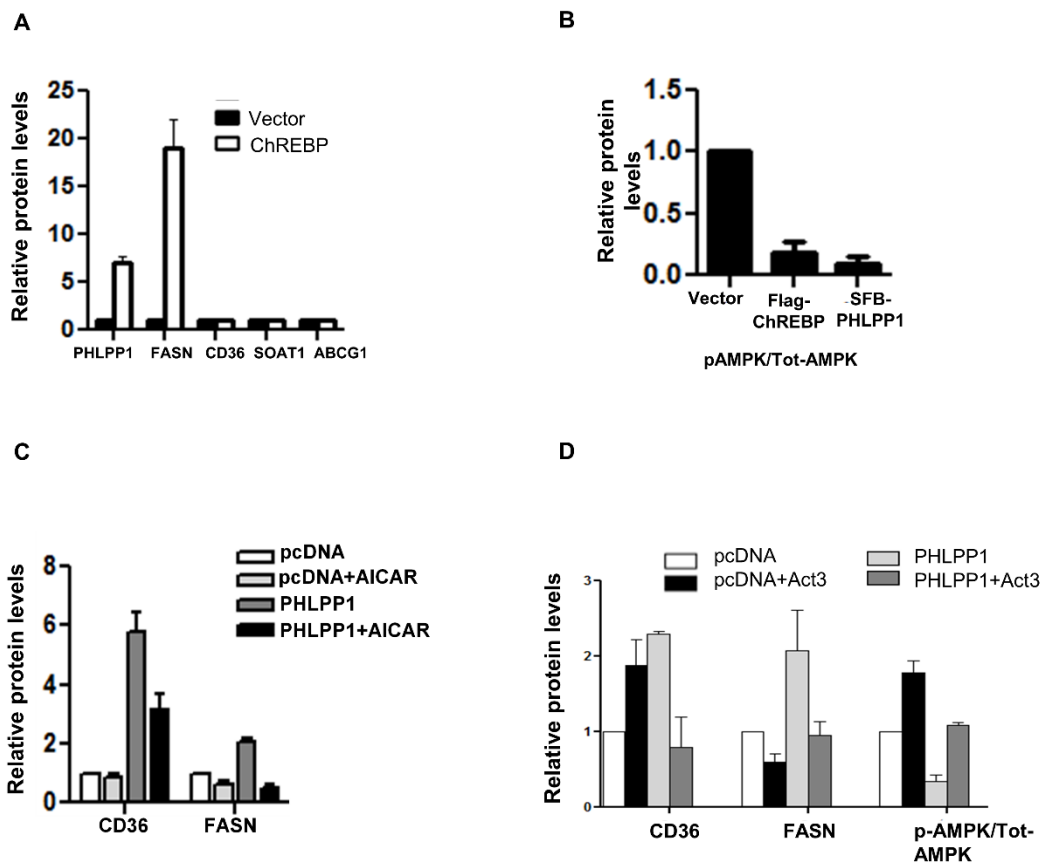
Putative consensus	B	H	X	X	H	pS/pT	P	X	H	P	X
Akt	H	F	P	Q	F	pS	Y	S	A	S	G
Mstl	K	R	R	D	E	pT	M	Q	P	A	K
PKCβII	E	F	E	G	F	pS	F	V	N	S	E
Raf-1	R	G	Q	R	D	pS	S	Y	Y	W	E
p70S6K1	G	T	V	T	H	pT	F	C	G	T	I
AMPKα1/α2	G	E	F	L	R	pT	S	C	G	S	P
Human ChREBP	R	L	R	K	P	pS <sup>196</sup>	R	E	D	D	L
Mouse ChREBP	R	L	R	K	S	pS <sup>196</sup>	R	E	G	D	F
Rat ChREBP	R	L	R	K	S	pS <sup>196</sup>	R	E	G	D	F

B – Basic ; H – Hydrophobic ; X – unknown amino acid; pS/pT – phosphorylated Serine or Threonine; P – Polar

**Supplementary Figure S4. *In silico* analysis of the impact of PHLPP1 on transcription factors highlighting ChREBP as a potential PHLPP1 substrate. Related to Figure 5.**

A) Analysis of target genes of enriched transcription factors.

B) Analysis of PHLPP1 putative target site of ChREBP (pSer<sup>196</sup>).



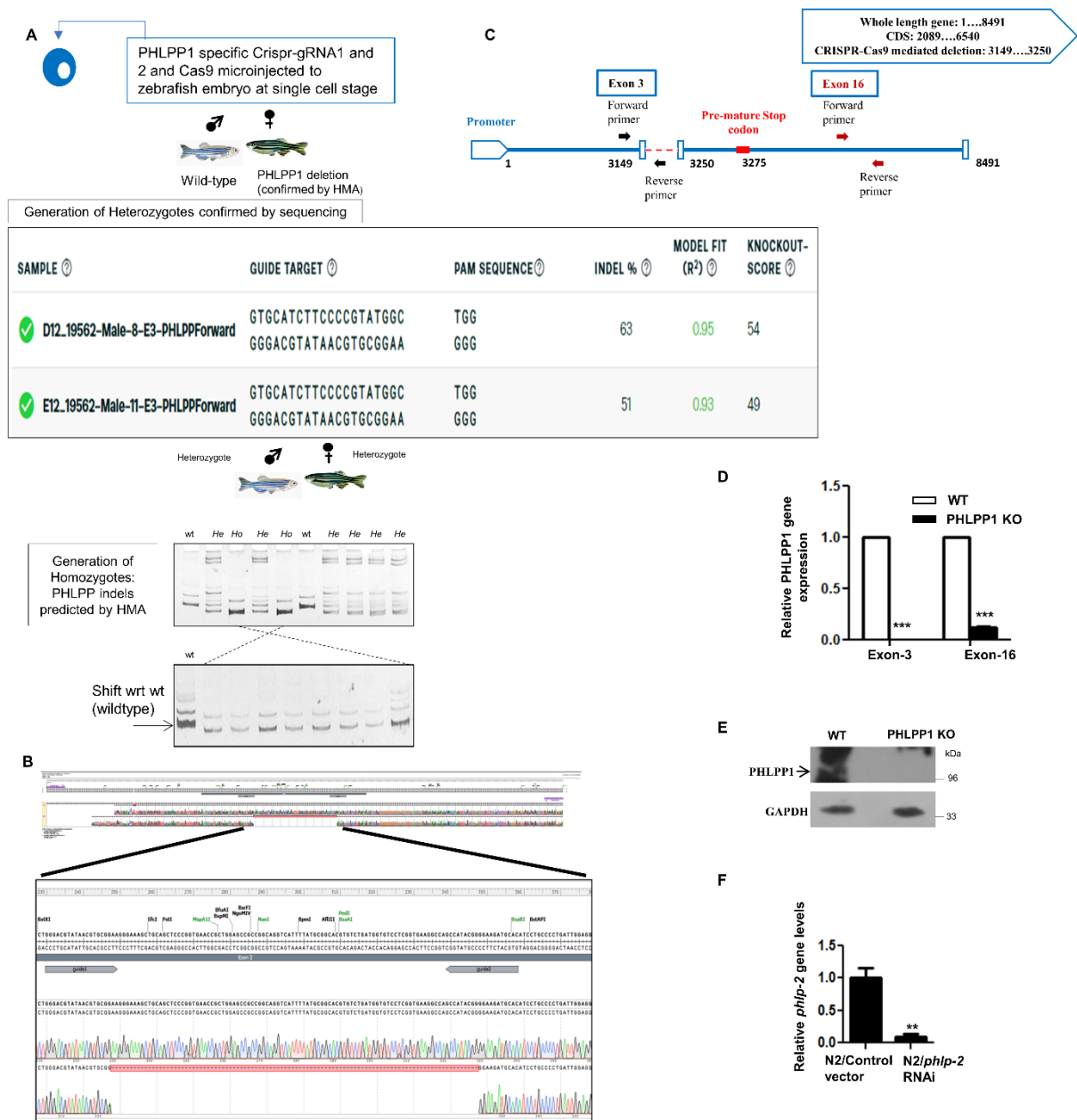
**Supplementary Figure S5. Impact of ChREBP and AMPK on lipid markers. Related to Figure 5.**

A-B) Densitometric quantification of Figure 5G (A) and 5K (B).

C) Densitometric quantification of Figure 5M.

D) Densitometric quantification of CD36 and FASN upon Activator 3 (Act3) treatment for 3 hours in PHLPP1 over-expressed cells (Figure 5M).

Data are a representative of three independent experiments, unless specified. Numerical data are expressed as mean  $\pm$  SEM.



**Supplementary Figure S6. Generation of *PHLPP1*-knockout zebrafish and *phlp-2* deficient *C. elegans*. Related to Figure 7.**

A) Representation of CRISPR-Cas9 mediated generation of *PHLPP1*-knockout zebrafish. Screening for *PHLPP1* gene indels was assessed using Heteroduplex Mobility Assay (HMA).

B-C) Sequencing confirmation (B) and pictorial (C) representation of a 103-bp deletion in the zebrafish *PHLPP1* gene analyzed using Snapgene;



D-E) Confirmation of PHLPP1 loss using qPCR (D) and western blot (E) analysis; Statistical analysis was performed using two tailed t-test (\*\* $p < 0.001$  vs WT).

F) q-PCR analysis of *phlp-2* mRNA levels in *phlp-2* knockdown *C.elegans*; Each data presented are from a representative experiment of two independent trials (n=7-12 per trial); Statistical analysis was performed using Student's *t*-test. (\*\* $p < 0.01$  vs N2/Control vector).

Data are a representative of three independent experiments, unless specified. Numerical data are expressed as mean  $\pm$  SEM.