

Supplementary data

Table S1. Primers used in this study.

Primer	Sequence (5'→3') ^a
pol-miR-novel_547-F	GCGCGTCTACAGCCTCTGAT
pol-miR-novel_547-R	AGTGCAGGGTCCCAGGTATT
PoPTEN-F	TCGAGGGCGGTGTACAGAAAC
PoPTEN-R	CGAAGGGGTACTGTGCAACT
3'UTR-PoPTEN-F	TGTTTAAACGAGCTC <u>GCTAGC</u> ACTCTTTTAAACAGGAA (Nhe I)
3'UTR-PoPTEN-R	CTTGCATGCCTGCAGGTC <u>GACT</u> GGTTTCTTTAAGAAAAA (Sal I)
CDS-PoPTEN-F	GGCTAGCCTCGAGATATCATGGCTGCGATTATAAAA (EcoR V)
CDS-PoPTEN-F	TCCTCCTCCTCCTCCGATACTCACACTTTTGTGATTG (EcoR V)
ATG5-F	AACAGCTGTGGATGGGTCTG
ATG5-R	ACAGGGCGAAACAGTCTCTG
AKT-F	AGCTGATGAAGACGGAACGG
AKT-R	GGGTCTGGAGAGGAGTCCAT
mTOR-F	TCTTCCACAATCGCCAGCTT
mTOR-R	GAGCCAGTCGTCTTTGGACA
beclin 1-F	TCAATGTGCTGGATCGGGTC
beclin 1-R	GCGCTCTCTGTGGACATCAT

Underlined nucleotides are restriction sites.

PoPTEN	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Seriola dumerilii</i> (98.12%)	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Echinacea nausarens</i> (96.96%)	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Cynoglossus semilavis</i> (96.68%)	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Larimichthys crocea</i> (94.16%)	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Xiphophorus maculatus</i> (94.08%)	MAAIKEMVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Homo sapiens</i> (94.83%)	MTAIKEIVSRNKKRYQEDGFDLDLTIVYPNIIAMGFFAERLEGVYRNIDDVRFDSKHNHYKIYNLCAERHYDAKFNCRVAQYFFEDHNPQLEL	100
<i>Mus musculus</i> (78.89%)		
PoPTEN	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Seriola dumerilii</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Echinacea nausarens</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Cynoglossus semilavis</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Larimichthys crocea</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Xiphophorus maculatus</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Homo sapiens</i>	IKPFCELDQWLEDDNHVAAIHCKAGKGRIGVMICAYLLHKGKFLKACQALDFYGEVTRDRKGVTIIPQRRYVYYSLLNCLDLYKFPVALLFHKMVF	200
<i>Mus musculus</i>		67
PoPTEN	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Seriola dumerilii</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Echinacea nausarens</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Cynoglossus semilavis</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Larimichthys crocea</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Xiphophorus maculatus</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Homo sapiens</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	300
<i>Mus musculus</i>	ETTFMFSGGTCNPQFVVYQLKVKIHTSHEATIRREDKRMFEFFPQLFVCGDIKVEFFHQNKMKKDKMFHFVWNTFFIPGEBSSGIRMENGAVNNAAES	167
PoPTEN	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	388
<i>Seriola dumerilii</i>	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	392
<i>Echinacea nausarens</i>	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	394
<i>Cynoglossus semilavis</i>	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	388
<i>Larimichthys crocea</i>	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	388
<i>Xiphophorus maculatus</i>	QQGGPGGQGP..QP...QLQMCTQRAESRSCRS.....DRLDLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	384
<i>Homo sapiens</i>	TS.....ICSIERRADN.....DKEMTLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	369
<i>Mus musculus</i>	TS.....ICSIERRADN.....DKEMTLTLTKNDLDKANKDKANRYFSNFKVKLYFTHVEEPSNPSASTSVTPEDV	236
PoPTEN	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	421
<i>Seriola dumerilii</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	425
<i>Echinacea nausarens</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	427
<i>Cynoglossus semilavis</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	421
<i>Larimichthys crocea</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	421
<i>Xiphophorus maculatus</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	417
<i>Homo sapiens</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	402
<i>Mus musculus</i>	SDNEPCHYRYSSTTSDPENEPFDBEGCHQITK	269

Figure S1. Alignment of the sequences of PoPTEN homologues. Dots denote gaps introduced for maximum matching. Numbers in brackets indicate overall sequence identities between PoPTEN and the compared sequences. The consensus residues are in blue, the residues that are ≥75% identical among the aligned sequences are in pink. The GenBank accession numbers of the aligned sequences are as follows:

Seriola dumerili, XP_022619472.1; *Echeneis naucrates*, XP_029377085.1; *Cynoglossus semilaevis*, XP_008320667.1; *Larimichthys crocea*, XP_010742537.1; *Xiphophorus maculatus*, XP_023183443.1; *Homo sapiens*, NP_000305.3; *Mus musculus*, XP_006526832.1.

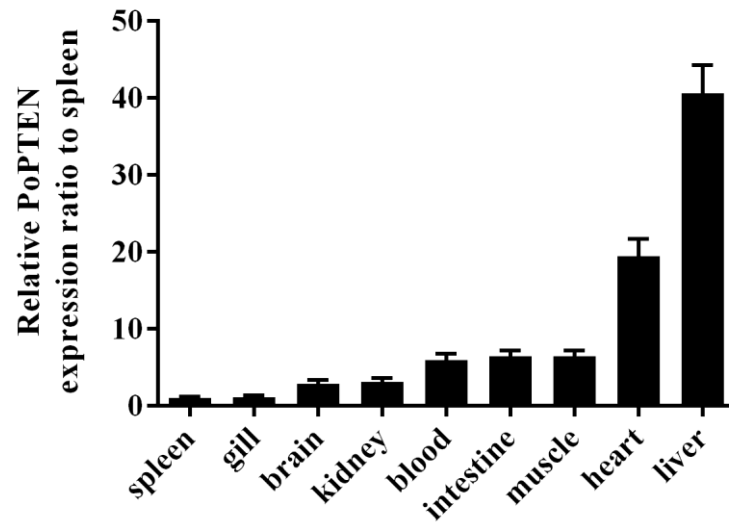


Figure S2. Tissue expression patterns of PoPTEN. PoPTEN expression in the spleen, gill, brain, kidney, blood, intestine, muscle, heart, and liver of flounder was determined by qRT-PCR. For convenience of comparison, the lowest expression level (spleen) was set as 1. Data are the means of triplicate experiments and presented as means $\pm$ SD.

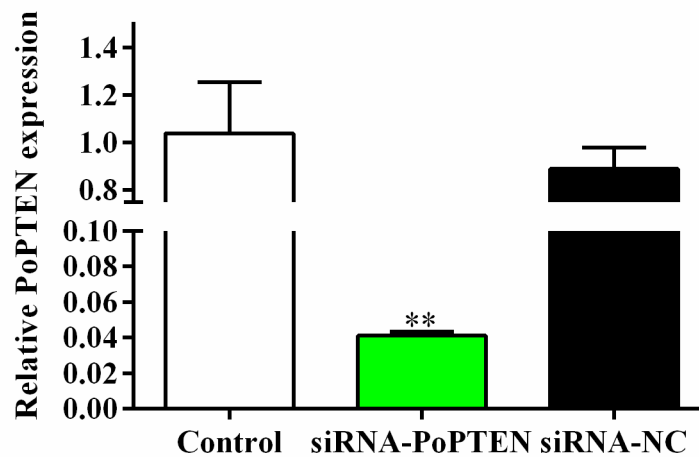


Figure S3. Verification of PoPTEN knockdown in FG-9307 cells. FG-9307 cells were transfected with or without (control) siRNA-PoPTEN, or the control siRNA (siRNA-NC). At 24 h post transfection, PoPTEN expression was measured by qRT-PCR. Values are the means of triplicate experiments and shown as means $\pm$ SEM. ** $p < 0.01$.

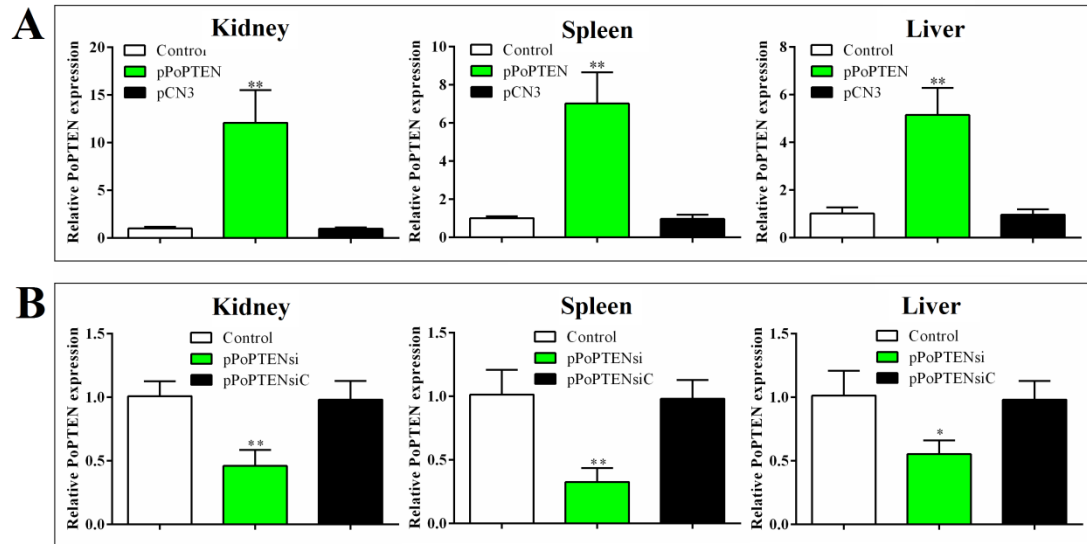


Figure S4. Verification of PoPTEN overexpression (A) and knockdown (B) in flounder. For PoPTEN overexpression, flounder were administered with or without (control) pPoPTEN or the control plasmid pCN3 (A); for PoPTEN knockdown, flounder were administered with or without (control) pPoPTENsi or the control plasmid pPoPTENsiC (B). In both panels, PoPTEN expression in kidney, spleen, and liver was determined by qRT-PCR at 7d post-plasmid injection. In all tissues, the expression level of the control fish was set as 1. Values are the means of triplicate experiments and shown as means $\pm$ SD. ** $p < 0.01$; * $p < 0.05$.

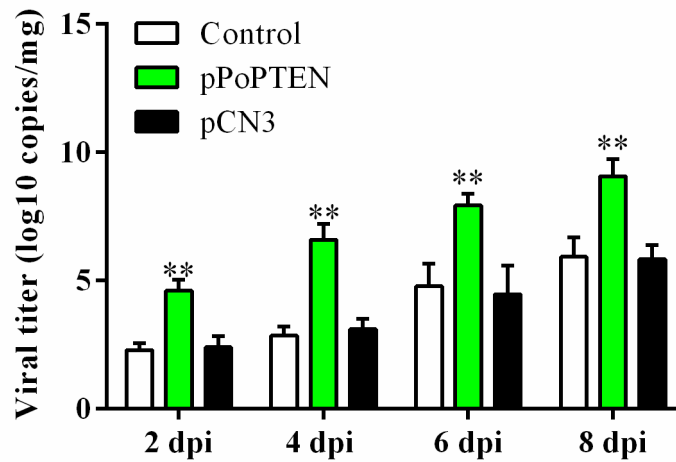


Figure S5. The effect of PoPTEN on megalocytivirus infection. Flounder were administered with or without (control) pPoPTEN or the control plasmid pCN3 and then infected with megalocytivirus. The viral load in kidney was determined at 2, 4, 6, and 8 days post-injection (dpi). Values are shown as means $\pm$ SD ($n = 3$), ** $p < 0.01$.