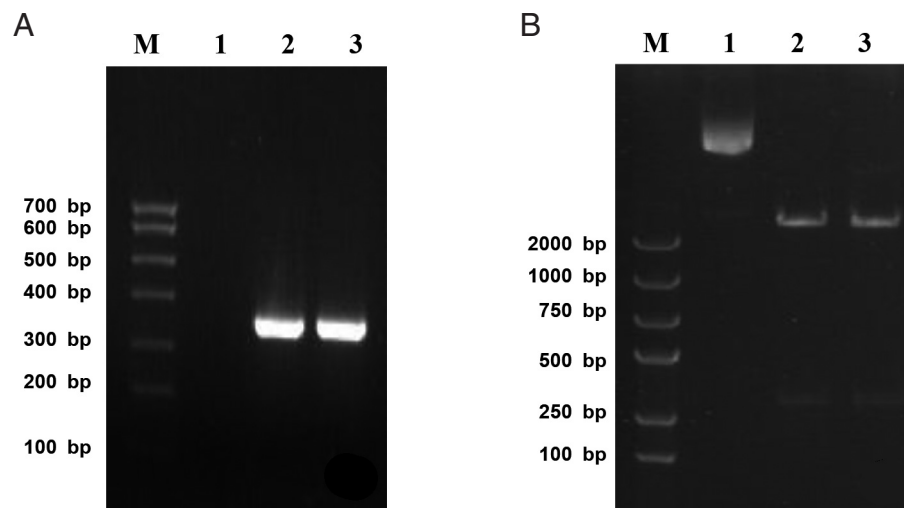


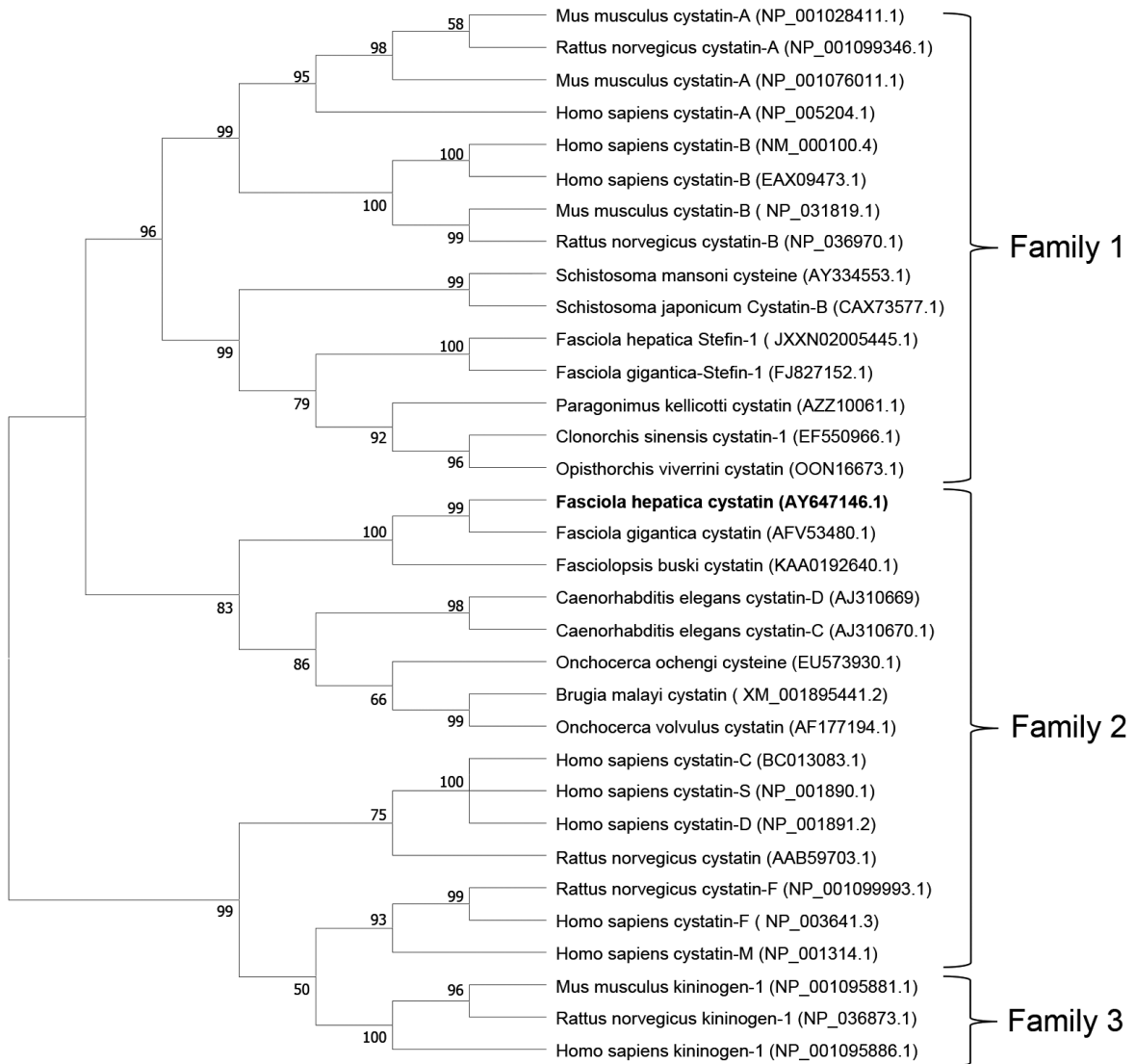
Supplementary Fig. S1. PCR amplification of FhCystatin from cDNA of *F. hepatica* adult worms. M: DNA marker DL700 (700, 600, 500, 400, 300, 200, 100 bp); Lane 1-4: Amplification products of FhCystatin gene.



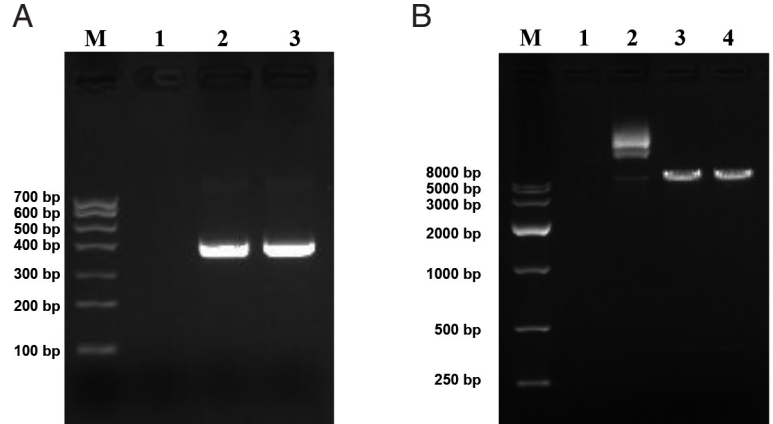
Supplementary Fig. S2. Screening and identification of recombinant plasmid pT-Cystatin by PCR amplification and enzyme digestion. (A) PCR amplification of pMD-Cystatin. M: DL700 DNA marker (700, 600, 500, 400, 300, 200, 100 bp); Lane 1: blank control; Lane 2-3: PCR amplification of pT-Cystatin. (B) Double digestion of the plasmid pT-Cystatin. M: DL2000 DNA marker (2,000, 1,000, 750, 500, 250, 100 bp); Lane 1: plasmids without enzyme digestion; Lane 2-3: enzyme digestion plasmid.

Fbcystatin-1	-----M--ITWVVHGDMLVG-----GYSTPRLLTPEEQTLTKPPVV	34
Fhcystatin	-MLRILLGICILHF--MSCDVFGEMLVG-----GYTEPRSVTPEERSVFPML	46
Fgcystatin-1	-MFRILFGICILHL--MSCDVFGEMLVG-----GYTEPRSVTSEERSVFRPML	46
Rtcystatin-B	-----MHQSTEIFAKMSCGGQQAQQVQRVGGASDARTPTKE--DQAILDVKV	45
Hscystatin	-----MMCG-----APS--ATQPATAETQHIADQVR	24
Sjcystatin-B	-----MPLCCG-----GVGAPREPSVEEKQKLTLE	27
Ovcystatin	MPICGGVAPCLLHLRTNNC--SIMPLCG-----GVGDARTPTPEEKQKLESVLH	47
Pkcystatin-2	-----FRYTSCLLFIMMKCG-----GVGQARAPSAEEKQKLETVLK	36
	* . : : :	
Fbcystatin-1	SRFLTVGVPVNSCHLHLLQVSTQVVAGTNYKLVSDGVTCPGCWEVVVFVPLPSDGTARV	94
Fhcystatin	SKLLTAGSVESSELELLQVSTQVVAGINVKFKVSGGATCPGCWEVVVFVPLYSSKSATS	106
Fgcystatin-1	SKFLTTSVSVESSELELLQVSTQVVAGTNYKFKVSGGATCPGCWEVVVFVPLYSSKAATS	106
Rtcystatin-B	DAFV--KQSATHPSVFKAVLVSTQVVAGKNYFFKVETGANT--YSNVKVFVPLPHENAGPS	102
Hscystatin	SQLE--EKENKFPVFKAVSFKSQVVAGTNYFIKVHVGDED--FVHLRVFQSLPHENKPLT	81
Sjcystatin-B	NNLE--AHIGRKPVPVDIVQVSSQVVAGTNYFVKVHVGDE--YVHARIFEPLPCHGKELQ	84
Ovcystatin	QSLH--THLGSKPDSLEVQVATQVVSQTNYFAKVKLNGDN--YVHARIEKLPCHGGTTE	104
Pkcystatin-2	KALP--AHLGSSPSSLEVQVSTQVVAGTNYFAKVKLP--DE--YIHTRIYEKLPCHGGELE	92
	. : . : . : ***.* ** ** . : : *	
Fbcystatin-1	MGTPQTSCY-----	104
Fhcystatin	VGTPTRVSCT-----	116
Fgcystatin-1	VGTPTRVSCT-----	116
Rtcystatin-B	LVAFQLNKKKDDKLGFF	119
Hscystatin	LSNYQTNKAKHDELT YF	98
Sjcystatin-B	LHSVLDKDKKNDAL EYF	101
Ovcystatin	LHSIQDKKTHADPLGYF	121
Pkcystatin-2	LHSVQRNKTHADPLVYF	109

Supplementary Fig. S3. Multiple sequence alignment amino acid sequences of FhCystatin with homologues from other organisms. The Q-X-V-X-G cystatin motif is indicated as highlighted in the corresponding yellow in the sequence, and the N-terminal region of conserved glycine residues is shown in the corresponding red. *Schistosoma japonicum* (Sjcystatin-B, CAX73577), *Fasciolopsis buski* (Fbcystatin-1, KAA0192640), *Fasciola gigantica* (Fgcystatin-1, AFV53480.1), *Opisthorchis viverrini* (Ovcystatin, OON16673), *Paragonimus kellycotti* (Pkcystatin-2, AZZ10061), *Rana temporaria* (Rtcystatin-B, XP_040195646), and *Homo sapiens* (Hscystatin, EAX09473).



Supplementary Fig. S4. Phylogenetic analysis of cystatin gene families from different organisms. The phylogenetic tree was built by the neighbor-joining method using the MEGA 7 program. Numbers on the branches indicate bootstrap proportions (1,000 replicates).



Supplementary Fig. S5. Identification of recombinant plasmid pPIC9K-Cystatin by PCR amplification and double enzyme digestion. (A) PCR amplification of pPIC9K-Cystatin. M: DL700 DNA marker (700, 600, 500, 400, 300, 200, 100); Lane 1: blank control; Lane 2-3: PCR amplification of pPIC9K-Cystatin. (B) Double digestion of the plasmid pPIC9K-Cystatin. M: DL8000 DNA marker (8,000, 5,000, 3,000, 2,000, 1,000, 500, 250); Lane 1: blank control; Lane 2: plasmids without double enzyme digestion; Lane 3-4: double enzyme digestion plasmid.