Supplementary Information

Manuscript title: The human blood parasite *Schistosoma mansoni* expresses extracellular tegumental calpains that cleave the blood clotting protein fibronectin.

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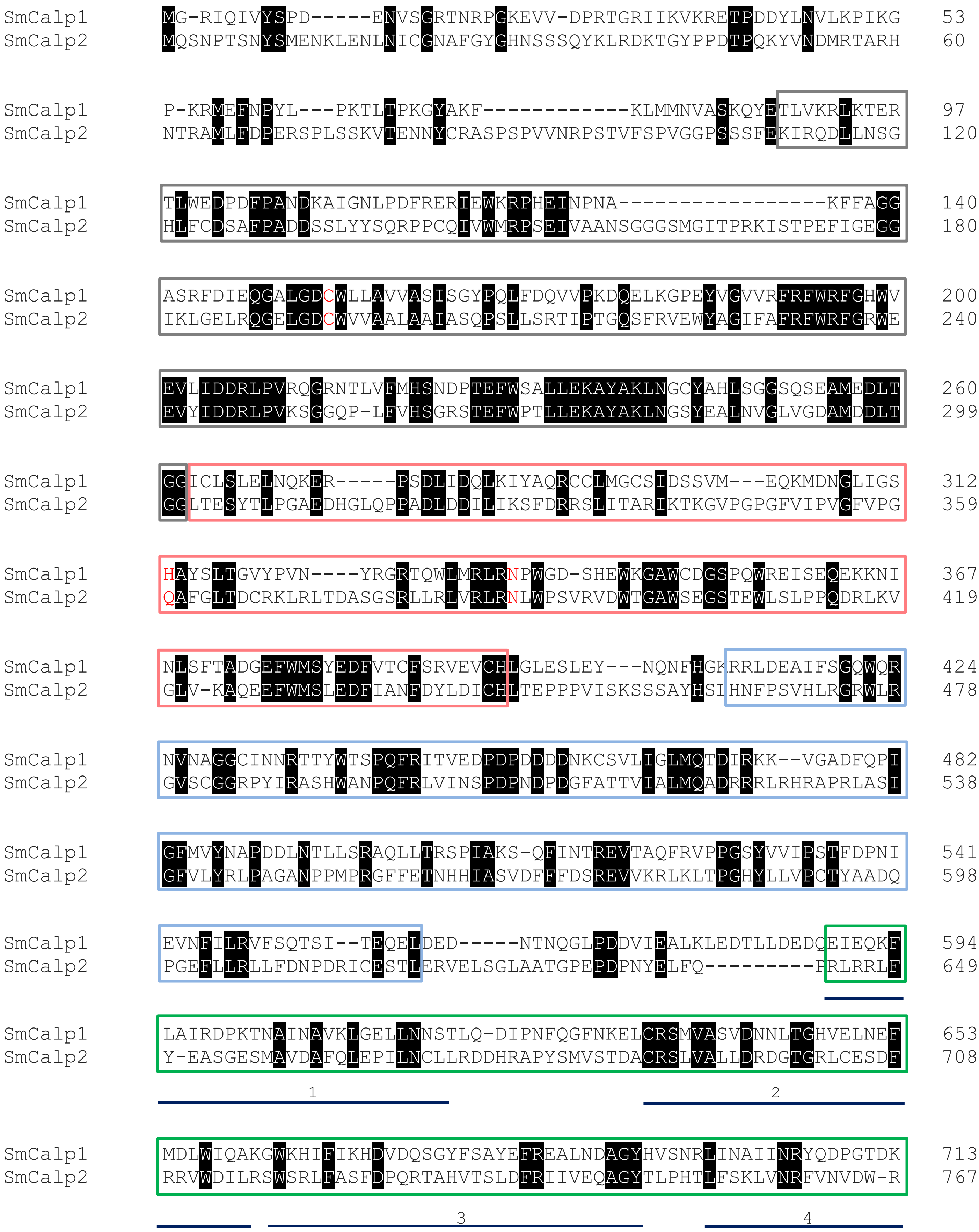

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SmCalp1   MG-RIQIVYSPD-----ENVSGRTNRPGKEVV-DPRTGRIIKVKRETPDDYLNVLKPIKG   53
SmCalp2   MQSNPTSNYSMENKLENLNICGNAFGYGHNSSSQYKLRDKTGYPPDTPQKYVNDMRTARH   60

SmCalp1   P-KRMEFNPYL---PKTLTPKGYAKF------------KLMMNVASKQYETLVKRLKTER   97
SmCalp2   NTRAMLFDPERSPLSSKVTENNYCRASPSPVVNRPSTVFSPVGGPSSSFEKIRQDLLNSG   120

SmCalp1   TLWEDPDFPANDKAIGNLPDFRERIEWKRPHEINPNA-----------------KFFAGG   140
SmCalp2   HLFCDSAFPADDSSLYYSQRPPCQIVWMRPSEIVAANSGGGSMGITPRKISTPEFIGEGG   180

SmCalp1   ASRFDIEQGALGDCWLLAVVASISGYPQLFDQVVPKDQELKGPEYVGVVRFRFWRFGHWV   200
SmCalp2   IKLGELRQGELGDCWVVAALAAIASQPSLLSRTIPTGQSFRVEWYAGIFAFRFWRFGRWE   240

SmCalp1   EVLIDDRLPVRQGRNTLVFMHSNDPTEFWSALLEKAYAKLNGCYAHLSGGSQSEAMEDLT   260
SmCalp2   EVYIDDRLPVKSGGQP-LFVHSGRSTEFWPTLLEKAYAKLNGSYEALNVGLVGDAMDDLT   299

SmCalp1   GGICLSLELNQKER-----PSDLIDQLKIYAQRCCLMGCSIDSSVM---EQKMDNGLIGS   312
SmCalp2   GGLTESYTLPGAEDHGLQPPADLDDILIKSFDRRSLITARIKTKGVPGPGFVIPVGFVPG   359

SmCalp1   HAYSLTGVYPVN----YRGRTQWLMRLRNPWGD-SHEWKGAWCDGSPQWREISEQEKKNI   367
SmCalp2   QAFGLTDCRKLRLTDASGSRLLRLVRLRNLWPSVRVDWTGAWSEGSTEWLSLPPQDRLKV   419

SmCalp1   NLSFTADGEFWMSYEDFVTCFSRVEVCHLGLESLEY---NQNFHGKRRLDEAIFSGQWQR   424
SmCalp2   GLV-KAQEEFWMSLEDFIANFDYLDICHLTEPPPVISKSSSAYHSIHNFPSVHLRGRWLR   478

SmCalp1   NVNAGGCINNRTTYWTSPQFRITVEDPDPDDDDNKCSVLIGLMQTDIRKK--VGADFQPI   482
SmCalp2   GVSCGGRPYIRASHWANPQFRLVINSPDPNDPDGFATTVIALMQADRRRLRHRAPRLASI   538

SmCalp1   GFMVYNAPDDLNTLLSRAQLLTRSPIAKS-QFINTREVTAQFRVPPGSYVVIPSTFDPNI   541
SmCalp2   GFVLYRLPAGANPPMPRGFFETNHHIASVDFFFDSREVVKRLKLTPGHYLLVPCTYAADQ   598

SmCalp1   EVNFILRVFSQTSI--TEQELDED-----NTNQGLPDDVIEALKLEDTLLDEDQEIEQKF   594
SmCalp2   PGEFLLRLLFDNPDRICESTLERVELSGLAATGPEPDPNYELFQ---------PRLRRLF   649

SmCalp1   LAIRDPKTNAINAVKLGELLNNSTLQ-DIPNFQGFNKELCRSMVASVDNNLTGHVELNEF   653
SmCalp2   Y-EASGESMAVDAFQLEPILNCLLRDDHRAPYSMVSTDACRSLVALLDRDGTGRLCESDF   708
                       1                                      2

SmCalp1   MDLWIQAKGWKHIFIKHDVDQSGYFSAYEFREALNDAGYHVSNRLINAIINRYQDPGTDK   713
SmCalp2   RRVWDILRSWSRLFASFDPQRTAHVTSLDFRIIVEQAGYTLPHTLFSKLVNRFVNVDW-R   767
                                   3                             4
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SmCalp1        ISFEDFMLCMVRLKTAFETIEAHPKNIEGTSLFSAEDYLRFSVYI     758
SmCalp2        LDYPHFISAMSLITKAVAIFKN--FDHGGRAVLPLEQFVEIAMTM     810
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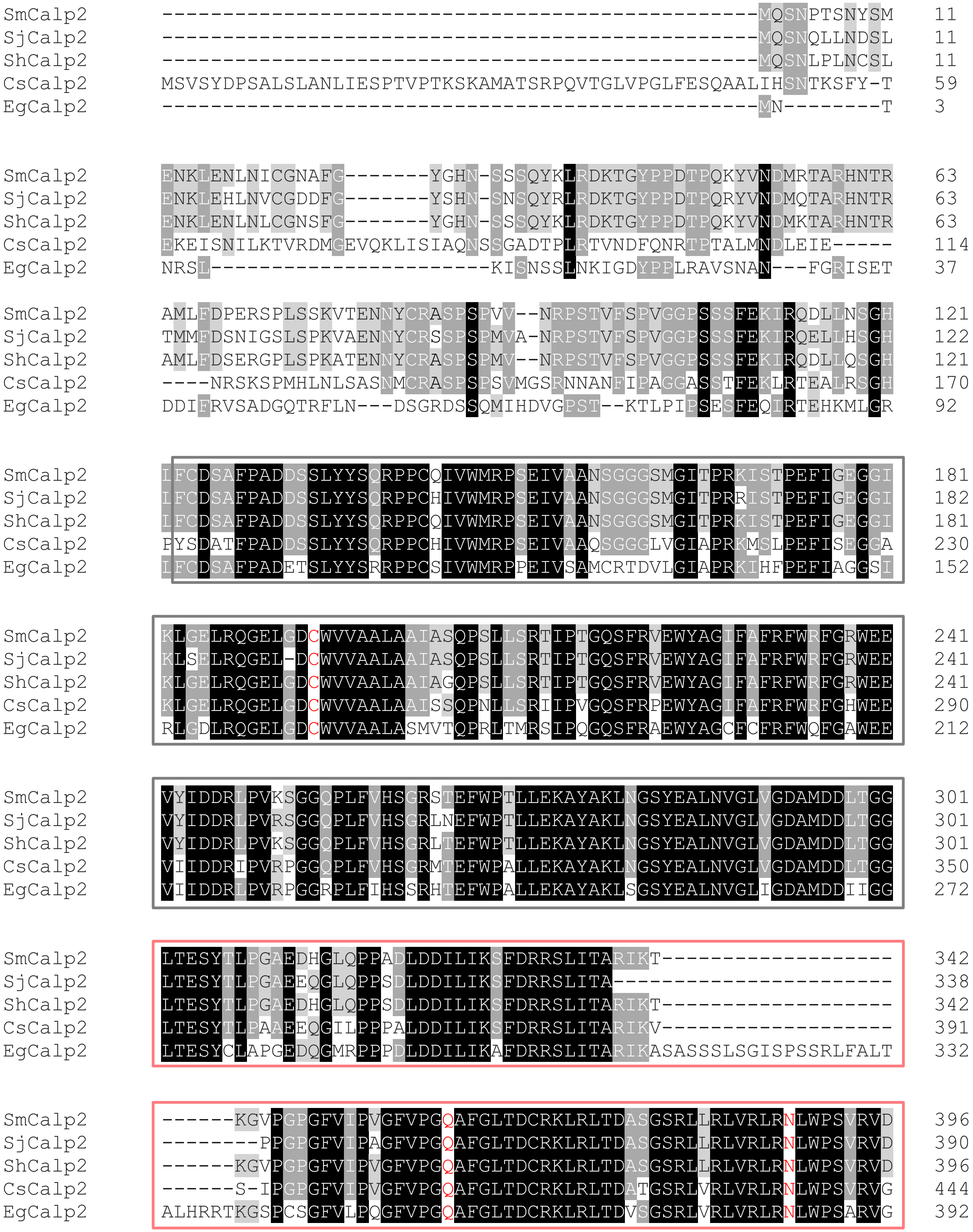
Supplementary figure S1: Alignment of the predicted amino acid sequences of SmCalp1 and SmCalp2 using CLUSTAL. Dashes indicate gaps introduced to maximize matching. Identical amino acids are white on a black background. The catalytic domain (PC1 and PC2), CBSW domain and EF hand domain are indicated with grey (PC1), red (PC2), light blue and green boxes, respectively, corresponding to the diagram in figure 1C. Indicated in red are conserved catalytic residues (C^{154}, H^{313} and N^{337} in SmCalp1 and C^{194}, Q^{330} and N^{296} in SmCalp2). The numbers (1 – 5) below the alignment at the carboxyl end indicate the five EF hand motifs.



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SmCalp2   -------------------------------------------------MQSNPTSNYSM   11
SjCalp2   -------------------------------------------------MQSNQLLNDSL   11
ShCalp2   -------------------------------------------------MQSNLPLNCSL   11
CsCalp2   MSVSYDPSALSLANLIESPTVPTKSKAMATSRPQVTGLVPGLFESQAALIHSNTKSFY-T   59
EgCalp2   -------------------------------------------------MN--------T   3

SmCalp2   ENKLENLNICGNAFG-------YGHN-SSSQYKLRDKTGYPPDTPQKYVNDMRTARHNTR   63
SjCalp2   ENKLEHLNVCGDDFG-------YSHN-SNSQYRLRDKTGYPPDTPQRYVNDMQTARHNTR   63
ShCalp2   ENKLENLNLCGNSFG-------YGHN-SSSQYKLRDKTGYPPDTPQKYVNDMKTARHNTR   63
CsCalp2   EKEISNILKTVRDMGEVQKLISIAQNSSGADTPLRTVNDFQNRTPTALMNDLEIE-----   114
EgCalp2   NRSL-----------------------KISNSSLNKIGDYPPLRAVSNAN---FGRISET   37

SmCalp2   AMLFDPERSPLSSKVTENNYCRASPSPVV--NRPSTVFSPVGGPSSSFEKIRQDLLNSGH   121
SjCalp2   TMMFDSNIGSLSPKVAENNYCRSSPSPMVA-NRPSTVFSPVGGPSSSFEKIRQELLHSGH   122
ShCalp2   AMLFDSERGPLSPKATENNYCRASPSPMV--NRPSTVFSPVGGPSSSFEKIRQDLLQSGH   121
CsCalp2   ----NRSKSPMHLNLSASNMCRASPSPSVMGSRNNANFIPAGGASSTFEKLRTEALRSGH   170
EgCalp2   DDIFRVSADGQTRFLN---DSGRDSSQMIHDVGPST--KTLPIPSESFEQIRTEHKMLGR   92

SmCalp2   IFCDSAFPADDSSLYYSQRPPCQIVWMRPSEIVAANSGGGSMGITPRKISTPEFIGEGGI   181
SjCalp2   IFCDSAFPADDSSLYYSQRPPCHIVWMRPSEIVAANSGGGSMGITPRRISTPEFIGEGGI   182
ShCalp2   IFCDSAFPADDSSLYYSQRPPCQIVWMRPSEIVAANSGGGSMGITPRKISTPEFIGEGGI   181
CsCalp2   PYSDATFPADDSSLYYSQRPPCHIVWMRPSEIVAAQSGGGLVGIAPRKMSLPEFISEGGA   230
EgCalp2   IFCDSAFPADETSLYYSRRPPCSIVWMRPPEIVSAMCRTDVLGIAPRKIHFPEFIAGGSI   152

SmCalp2   KLGELRQGELGDCWVVAALAAIASQPSLLSRTIPTGQSFRVEWYAGIFAFRFWRFGRWEE   241
SjCalp2   KLSELRQGEL-DCWVVAALAAIASQPSLLSRTIPTGQSFRVEWYAGIFAFRFWRFGRWEE   241
ShCalp2   KLGELRQGELGDCWVVAALAAIAGQPSLLSRTIPTGQSFRVEWYAGIFAFRFWRFGRWEE   241
CsCalp2   KLGELRQGELGDCWVVAALAAISSQPNLLSRIIPVGQSFRPEWYAGIFAFRFWRFGHWEE   290
EgCalp2   RLGDLRQGELGDCWVVAALASMVTQPRLTMRSIPQGQSFRAEWYAGCFCFRFWQFGAWEE   212

SmCalp2   VYIDDRLPVKSGGQPLFVHSGRSTEFWPTLLEKAYAKLNGSYEALNVGLVGDAMDDLTGG   301
SjCalp2   VYIDDRLPVRSGGQPLFVHSGRLNEFWPTLLEKAYAKLNGSYEALNVGLVGDAMDDLTGG   301
ShCalp2   VYIDDRLPVKSGGQPLFVHSGRLTEFWPTLLEKAYAKLNGSYEALNVGLVGDAMDDLTGG   301
CsCalp2   VIIDDRIPVRPGGQPLFVHSGRMTEFWPALLEKAYAKLNGSYEALNVGLVGDAMDDLTGG   350
EgCalp2   VIIDDRLPVRPGGRPLFIHSSRHTEFWPALLEKAYAKLSGSYEALNVGLIGDAMDDIIGG   272

SmCalp2   LTESYTLPGAEDHGLQPPADLDDILIKSFDRRSLITARIKT-------------------   342
SjCalp2   LTESYTLPGAEEQGLQPPSDLDDILIKSFDRRSLITA-----------------------   338
ShCalp2   LTESYTLPGAEDHGLQPPSDLDDILIKSFDRRSLITARIKT-------------------   342
CsCalp2   LTESYTLPAAEEQGILPPPALDDILIKSFDRRSLITARIKV-------------------   391
EgCalp2   LTESYCLAPGEDQGMRPPPDLDDILIKAFDRRSLITARIKASASSSLSGISPSSRLFALT   332

SmCalp2   ------KGVPGPGFVIPVGFVPGQAFGLTDCRKLRLTDASGSRLLRLVRLRNLWPSVRVD   396
SjCalp2   --------PPGPGFVIPAGFVPGQAFGLTDCRKLRLTDASGSRLLRLVRLRNLWPSVRVD   390
ShCalp2   ------KGVPGPGFVIPVGFVPGQAFGLTDCRKLRLTDASGSRLLRLVRLRNLWPSVRVD   396
CsCalp2   ------S-IPGPGFVIPVGFVPGQAFGLTDCRKLRLTDATGSRLVRLVRLRNLWPSVRVG   444
EgCalp2   ALHRRTKGSPCSGFVLPQGFVPGQAFGLTDCRKLRLTDVSGSRLVRLVRLRNLWPSARVG   392
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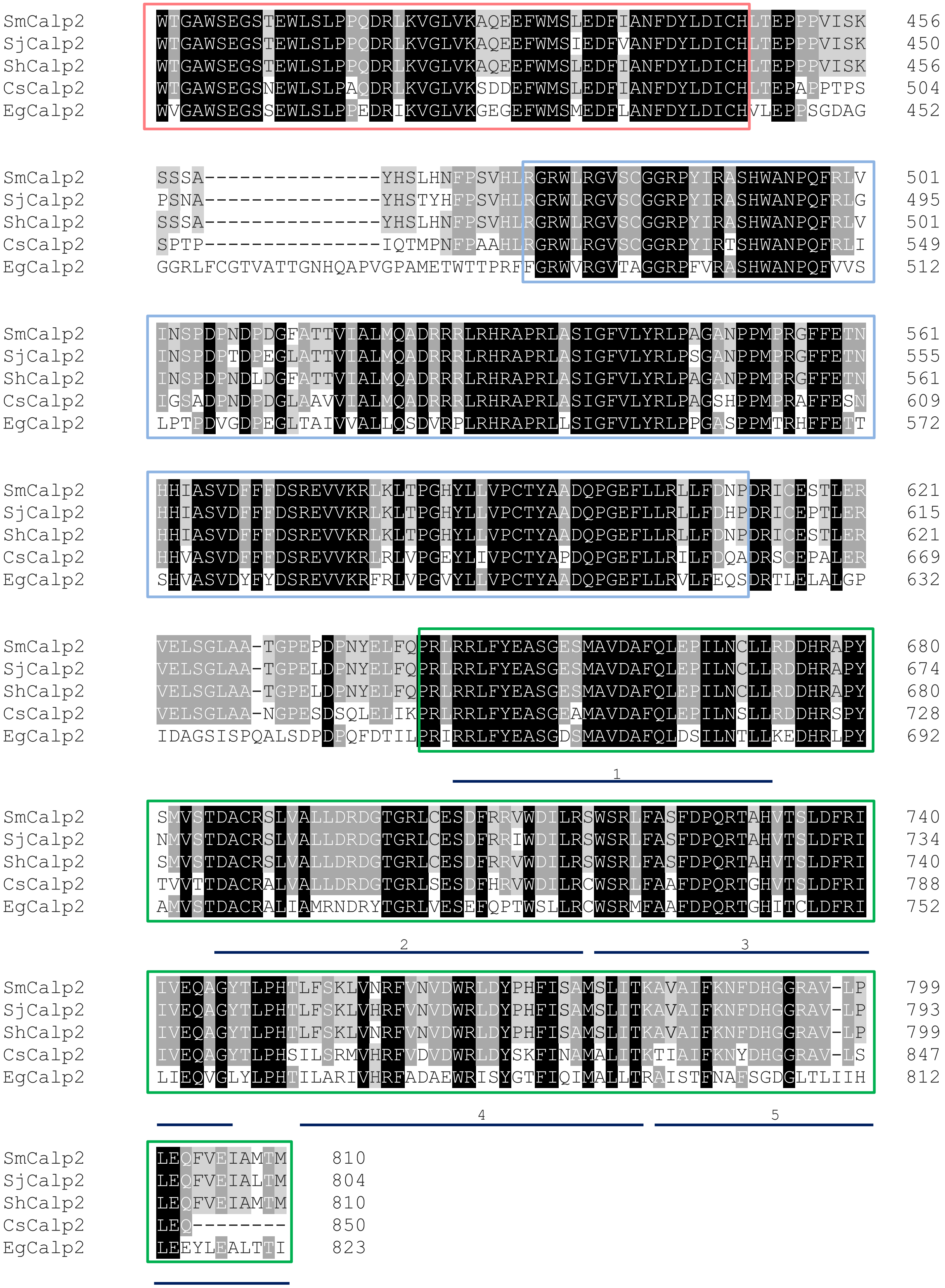

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SmCalp2    WTGAWSEGSTEWLSLPPQDRLKVGLVKAQEEFWMSLEDFIANFDYLDICHLTEPPPVISK    456
SjCalp2    WTGAWSEGSTEWLSLPPQDRLKVGLVKAQEEFWMSIEDFVANFDYLDICHLTEPPPVISK    450
ShCalp2    WTGAWSEGSTEWLSLPPQDRLKVGLVKAQEEFWMSLEDFIANFDYLDICHLTEPPPVISK    456
CsCalp2    WTGAWSEGSNEWLSLPAQDRLKVGLVKSDDEFWMSLEDFIANFDYLDICHLTEPAPPTPS    504
EgCalp2    WVGAWSEGSSEWLSLPPEDRIKVGLVKGEGEFWMSMEDFLANFDYLDICHVLEPPSGDAG    452

SmCalp2    SSSA---------------YHSLHNFPSVHLRGRWLRGVSCGGRPYIRASHWANPQFRLV    501
SjCalp2    PSNA---------------YHSTYHFPSVHLRGRWLRGVSCGGRPYIRASHWANPQFRLG    495
ShCalp2    SSSA---------------YHSLHNFPSVHLRGRWLRGVSCGGRPYIRASHWANPQFRLV    501
CsCalp2    SPTP---------------IQTMPNFPAAHLRGRWLRGVSCGGRPYIRTSHWANPQFRLI    549
EgCalp2    GGRLFCGTVATTGNHQAPVGPAMETWTTPRFFGRWVRGVTAGGRPFVRASHWANPQFVVS    512

SmCalp2    INSPDPNDPDGFATTVIALMQADRRRLRHRAPRLASIGFVLYRLPAGANPPMPRGFFETN    561
SjCalp2    INSPDPTDPEGLATTVIALMQADRRRLRHRAPRLASIGFVLYRLPSGANPPMPRGFFETN    555
ShCalp2    INSPDPNDLDGFATTVIALMQADRRRLRHRAPRLASIGFVLYRLPAGANPPMPRGFFETN    561
CsCalp2    IGSADPNDPDGLAAVVIALMQADRRRLRHRAPRLASIGFVLYRLPAGSHPPMPRAFFESN    609
EgCalp2    LPTPDVGDPEGLTAIVVALLQSDVRPLRHRAPRLLSIGFVLYRLPPGASPPMTRHFFETT    572

SmCalp2    HHIASVDFFFDSREVVKRLKLTPGHYLLVPCTYAADQPGEFLLRLLFDNPDRICESTLER    621
SjCalp2    HHIASVDFFFDSREVVKRLKLTPGHYLLVPCTYAADQPGEFLLRLLFDHPDRICEPTLER    615
ShCalp2    HHIASVDFFFDSREVVKRLKLTPGHYLLVPCTYAADQPGEFLLRLLFDNPDRICESTLER    621
CsCalp2    HHVASVDFFFDSREVVKRLRLVPGEYLIVPCTYAPDQPGEFLLRILFDQADRSCEPALER    669
EgCalp2    SHVASVDYFYDSREVVKRFRLVPGVYLLVPCTYAADQPGEFLLRVLFEQSDRTLELALGP    632

SmCalp2    VELSGLAA-TGPEPDPNYELFQPRLRRLFYEASGESMAVDAFQLEPILNCLLRDDHRAPY    680
SjCalp2    VELSGLAA-TGPELDPNYELFQPRLRRLFYEASGESMAVDAFQLEPILNCLLRDDHRAPY    674
ShCalp2    VELSGLAA-TGPELDPNYELFQPRLRRLFYEASGESMAVDAFQLEPILNCLLRDDHRAPY    680
CsCalp2    VELSGLAA-NGPESDSQLELIKPRLRRLFYEASGEAMAVDAFQLEPILNSLLRDDHRSPY    728
EgCalp2    IDAGSISPQALSDPDPQFDTILPRIRRLFYEASGDSMAVDAFQLDSILNTLLKEDHRLPY    692

SmCalp2    SMVSTDACRSLVALLDRDGTGRLCESDFRRVWDILRSWSRLFASFDPQRTAHVTSLDFRI    740
SjCalp2    NMVSTDACRSLVALLDRDGTGRLCESDFRRIWDILRSWSRLFASFDPQRTAHVTSLDFRI    734
ShCalp2    SMVSTDACRSLVALLDRDGTGRLCESDFRRVWDILRSWSRLFASFDPQRTAHVTSLDFRI    740
CsCalp2    TVVTTDACRALVALLDRDGTGRLSESDFHRVWDILRCWSRLFAAFDPQRTGHVTSLDFRI    788
EgCalp2    AMVSTDACRALIAMRNDRYTGRLVESEFQPTWSLLRCWSRMFAAFDPQRTGHITCLDFRI    752

SmCalp2    IVEQAGYTLPHTLFSKLVNRFVNVDWRLDYPHFISAMSLITKAVAIFKNFDHGGRAV-LP    799
SjCalp2    IVEQAGYTLPHTLFSKLVHRFVNVDWRLDYPHFISAMSLITKAVAIFKNFDHGGRAV-LP    793
ShCalp2    IVEQAGYTLPHTLFSKLVNRFVNVDWRLDYPHFISAMSLITKAVAIFKNFDHGGRAV-LP    799
CsCalp2    IVEQAGYTLPHSILSRMVHRFVDVDWRLDYSKFINAMALITKTIAIFKNYDHGGRAV-LS    847
EgCalp2    LIEQVGLYLPHTILARIVHRFADAEWRISYGTFIQIMALLTRAISTFNAFSGDGLTLIIH    812

SmCalp2    LEQFVEIAMTM    810
SjCalp2    LEQFVEIALTM    804
ShCalp2    LEQFVEIAMTM    810
CsCalp2    LEQ--------    850
EgCalp2    LEEYLEALTTI    823
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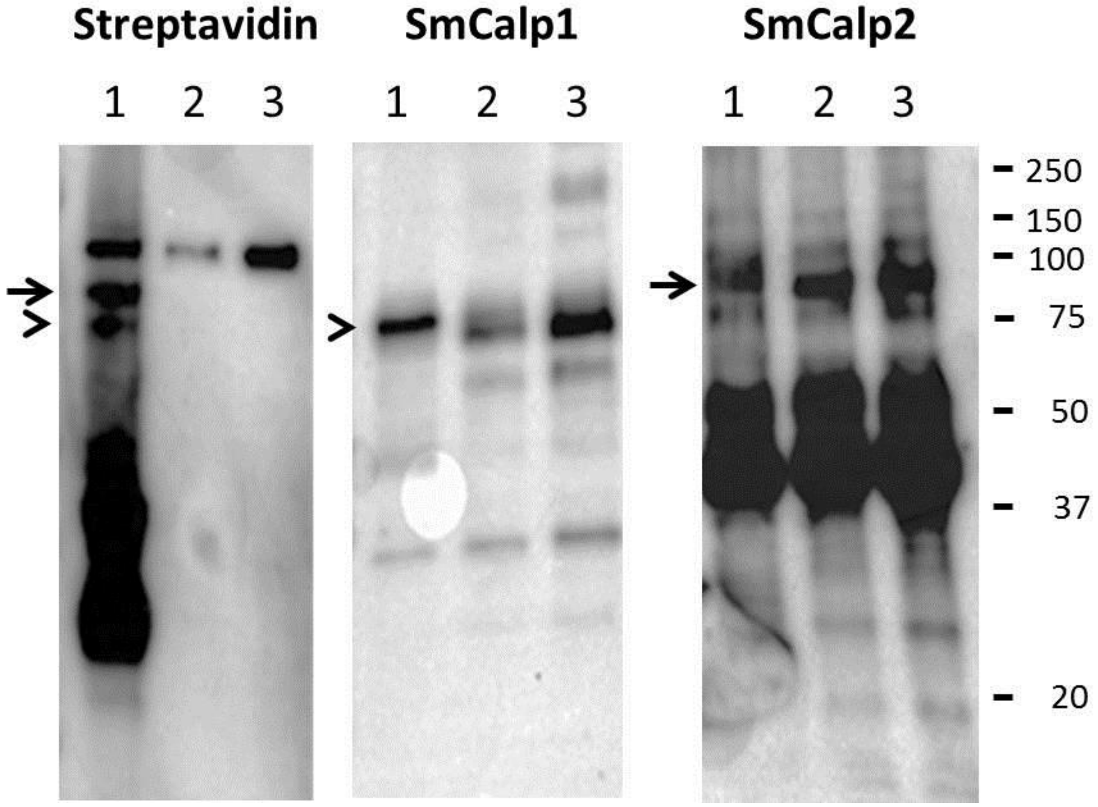
Supplementary figure S2: Alignment of the predicted amino acid sequences of the platyhelminth Calp2 clade 2 proteins using CLUSTAL. Dashes indicate gaps introduced to maximize alignment. Amino acids identical in all five sequences are depicted in white with a black background, amino acids found in 4/5 have a grey background and in 3/5 have a light grey background. The catalytic domain (PC1 and PC2), CBSW domain and EF hand domain are indicated with grey (PC1), red (PC2), blue and green boxes, respectively. Red amino acids indicate conserved catalytic residues. The numbers (1 – 5) below the alignment at the carboxyl end indicate the five EF hand motifs. SmCalp2, *S. mansoni* Calp2 (SmCalp2; MF590064); SjCalp2, *S. japonicum* Calp2 (SjCalp2, MF590065)**;** ShCalp2, *S. haematobium* Calp2 (ShCalp2, XP_012791984) CsCalp2, *Clonorchis sinensis* Calp2 (Cs2, GAA29557.2); EgCalp2, *Echinococcus granulosus* Calp2 (Eg2, CDS20315.1).



Supplementary figure S3: The entire blot from figure 6C - western blot analysis of extracts of adult worms exposed to biotinylated E64c (lanes 1), E64c (lanes 2) or untreated control (lanes 3) worms. The blot was probed with streptavidin-HRP, (left panel) to detect biotinylated proteins or with anti-SmCalp1 antibody (center) to detect SmCalp1 or with anti-SmCalp2 antibody (right panel) to detect SmCalp2. Blots were then probed with HRP-labeled secondary anti-antibody. Lanes 1 contain extracts of biotinylated E64c-treated worms, lanes 2 contain extracts of E64c-treated worms, lanes 3 contain extracts of untreated, control worms, The arrowhead indicates the position of migration of SmCalp1 and the arrow indicates the position of migration of SmCalp2. A non-specific streptavidin binding protein is detected at the top of all lanes in the “Streptavidin” panel (left). Some of the additional lower molecular weight, biotinylated moieties seen in “Streptavidin” lane 1 (left) may represent biotinylated SmCalp2 fragments, also detected by the anti-SmCalp2 antibody in “SmCalp2” lanes 1, 2 and 3 (right panel).