

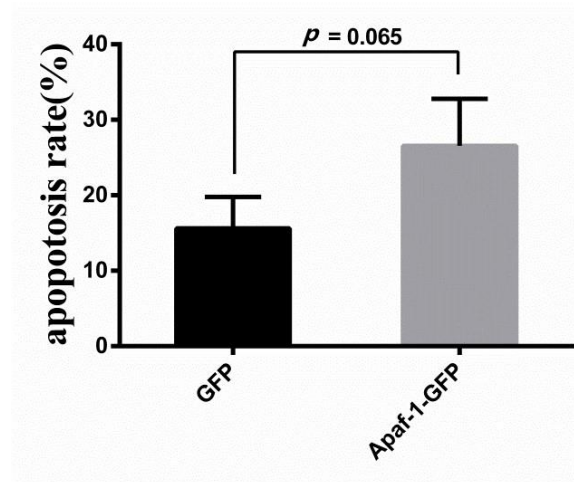
CARD	
Sl-Apaf-1 : ..MDKRRLLQKQCOITIDLDVAVYDELFKRVIS...AEVDVRKSLIIRIDTFRLDLSLRNGTGYCVFVDSLQDKYGLWKEKLAENNNMDDSFEDSL.....	105
Bm-Apaf-1 : ..MDLNRKLLQNOQAIKLDQVYILDELYTKQAISSEVYEHANLVVKNRVSGRYLIDLLRGTGNGHYEFVDSLAKDYNLKEKATCGEMNDSEFEDSL.....	108
Px-Apaf-1 : ..MDIKKCHKRVVVDIVYITDITISKDDHVITSRAR.....CRYITTINGTNSVDTRDRWRWITDMMNN.....DSSES.....	75
Hs-Apaf-1 : ..MDAKARNCILQHEALSKDICTSYMHRMSGDLTISEEVENENHDOOR...AAMLIEMIKRNTSVSEFNALLBEGYDIALDLHGLGPVSESSGSDSVSITSVRTVI.....	114
Dm-DARK : ..MDFETGHOYQKCLDSVFDEAVDNDCKQVQMPSTISSEEDITITKSGSDVRLITMLLSKQCEMVQFEVVLNNYFLMSPITEQPSMIRMRYEORD.....	112
NBD	
Sl-Apaf-1 : ..RGDVPRLPDHVRKRVVEQVASELRLLDARHKVLALHGMSSGSKTCVITSAIRDNDPLVASNFNSVFWLNFGNCKTDDDTICQSKLLRRSSMYTCI..SYMSSSVSSMSIGNSDS.....	222
Bm-Apaf-1 : ..RGDVPRLPDHVRKRVVEVETVLSKIKELRHKILVLHGMSSGSKTCVAINVLRDNEFLNNFNDVFWMNFANCKTDDDTIAQCNLLRKRISMYTCI..SYMSSSVSSMSIGNSDS.....	225
Px-Apaf-1 : ..RGDVR...DHFVR...MAVVAIRKSRHHTHGMSSGSKTCVAINPARN.....IDANGVWIL..GANKSDI.....DRKRRKSSMYTHDSANSSISMSIGNSDS.....	168
Hs-Apaf-1 : ..RGDVPRLPDHVRKRVVETVLSKIKELRHKILVLHGMSSGSKTCVAINPARN.....IDANGVWIL..GANKSDI.....DRKRRKSSMYTHDSANSSISMSIGNSDS.....	210
Dm-DARK : ..RNDNVDVAKNVSRFQDPLTALCLALCLARPNVVDITGVYSSGKIIVALLDGLSKH..VCKGRDFKIFWLNANCSPETVZELCKLGLHFWNTSR.....	211
Sl-Apaf-1 : ..SSSNYDMNDLRDKLKQFSEPLKECLLVLDVENDRKCEAFDICKILVTRDTVVALS...ROIITENSLIEKESLEIFALSLVFPVSKLFOAKMIEELCKGSPFNTLIGA.....	340
Bm-Apaf-1 : ..HSSVYDMNDLRDLKNLSEPLKECLLVMDVENDRKCEAFDICKILVTRDTDVANAH...FOIVTVENSIFERSELELFASSLLFVSKLPSYAKELIELCKGSPFVALIGA.....	343
Px-Apaf-1 : ..HSSVYDMNDLRDKLKVITL.....NKRVMIDIGCKWMTLRTDVANAH..VFNHKSASCLIB.....VSRHKNICKGS.....	243
Hs-Apaf-1 : ..SSSRHPLNIEAKDKRLSLMLKATFPLLLITLVAVTSMVLAFDSCILLITLDRSVSDVDFGVYVAVVSSICRKKLETLHVNMMKADLFOQHEITIELCKGSPFVALIGA.....	329
Dm-DARK : ..DISSNRKLFHIEIDABLRELKSKPFENCLVLIVNNAKAWNAFLNSCKILLITREKOVITBLS...AMTTHSLHSHSMVDPDVSKLLEKLDGPEIDPREVITTN.....	322
Sl-Apaf-1 : ..QLAENRERLIDPRKNWYVVKELKKNIFSEIPINQY...NPMKTIEVCNSLNNQVILFKRILPDKNVKISAKVLSMLNWRPDSQVEIMKELSKSLIEVYDRDQRYMYEIHDL.....	458
Bm-Apaf-1 : ..QLAEDRERKHDHSMYYLKLKLNFFSVKTNND...NPMKTIOVCNSLNNLNMFLKVLDPDVKVTAIVKSLNVDVAEVENIKELSKSLIKKYDRDQRMKLYEIHDL.....	459
Px-Apaf-1 : ..HIAAGCKRTHDTSWNYNIRKAKDSRSDNMATMG...INSRHHRHAD...NVKSAKSKIDKQDQVWMMRSRSHLYNNRYSVVDIMNRYGCSHDKRIVE...KSNRYVHDL.....	356
Hs-Apaf-1 : ..LIRDFEN.....RWLYLKQDNKCKRIRKSSYDHALDASISVENIEDIDVYDLSLQDKVKPKVILQWMEVEEDILAEVWKSLLCGRN..GKSFYVHDL.....	440
Dm-DARK : ..RLSTIASEIDELAVWIDWKEVNCRLTTHIESNVLEHAYVRFKFSVSPSAHIPTLISLITFDVKSQVWVWVKIHRYSVVRKPKESHHSIPSTIMELKVKLEIVYHLS.....	442
Sl-Apaf-1 : ..IMNYLRSE..GEDSKKLHAEFLNSYINTTNEPLEIIDDGYIAYIYGHKHNQVNDMTIEKRLFLNLKFLGNKVLRTGPADVMSDLQNYESVIVKFDKELISNTKAFSLTHGNDI.....	577
Bm-Apaf-1 : ..IMNYLRSCHEEEMKKLHDELNSRYDSFTEPTIEVDGDIAYIYGFHILKRNHKKHSGSKLYLDRFLGNKVLRTGPDVMDLQRYENIVYR.....	557
Px-Apaf-1 : ..VDSVLYIYICHHITADVAVSRYPENVRSGADIDAYIYHARSHDHIHAKSHGQDQVSGDQVLSHSGHLYKACHANCARYNIVYKHS.....	455
Hs-Apaf-1 : ..VDFLDFNCSOLODHRKLTOTOFRTYHPTHTSPDOEL...CMYVYNLIYAHNAKARMDHDEGSDWIKAKTDLGPAHLHDEVEPRLDDEG.....	537
Dm-DARK : ..IVDHNIPKIFISDMLTTPHLQYFYSIGFHURITHESEVMLEKRIEVLDFRGLKRIKSTAWNASGLATITQULKEKRYITNDPYSERLWALIT.....	544
Sl-Apaf-1 : ..PHYCTDIQIISILQNESKGIYKSAQIAQENCNNELYDFDLHECNVDEIKHSTGVKCKTPVFTQYVILSTNGSVKPFHVTNKKCELRGCTSPHKGIVYITKNQVVAAL.....	697
Bm-Apaf-1 :DASCDG.....VTKHKLVLKSGICHS...FENFENKKKELKCTGAATKHWIGVACTKDTATVAALS.....	619
Px-Apaf-1 :DQYVVE.....FHVYRVHILNKSGLIKW...AKSPDILALIT.....	506
Hs-Apaf-1 :CAVSENOPTSLNHLIA.....RQFTFNLQILCEPHESEMYLAPLQAGE.....	588
Dm-DARK :BLKTEENLISAYDUMRIAMAEDEITSEBAKMYEFDLDMETNHGRPHHGRQINLEDG.....	607
WD1	
Sl-Apaf-1 : ..YDGVIKLWYVDMDVEENEDVIEEEPDCYNNIDFANVCH...SSMLNCRWANNEMILMTTSKKILFYIPSRQKNKIKFTIQFDRFEILCCVPCNKDNVIVATIEVKRVVEIT.....	813
Bm-Apaf-1 : ..TDCGLKLVFDDDETDONNNILVEEENDVNNYLSPTTFEPLGILNCRWITSEDLITHTSGHIIYRE...KGARYDVISEFDEKILCCVPCNNDKIVAVASFNAVRYVRLID.....	735
Px-Apaf-1 : ..SDGVVKIWMYHDDHDDSDN...IDASITYDNYIVHLYSG...INCRWANN..MIITHTTKIITIIYSID.....VYKRVSRKILICISND.....	586
Hs-Apaf-1 : ..HGMILHIVNKNINIRSRVIVPHDQVYHACSEIGCS...HSGCADDITQVFASTQDKLEIKR...EDEVLCQAFSTDDETRIANCS...VYKRVKTN.....	685
Dm-DARK : ..NEGRHAVYENDELTALASCHULHVSLEGDTYILHESDSSHILIRVNVNCHSLITDFCNGSVKVMISLPLDGRHRS..GSKQCLVNSVVRIGISYANIKIVAFINDEAGLE.....	727
WD1	
Sl-Apaf-1 : ..LTKREIRITTFDTHIVLDMVTVER...SNKIITLRN...SMHMEHRSHTSTIKCCRRVITSNDIRCNMFIAIAVNSGILLVATDDSRVVCVDLKYTRYFDOLENRRGNVV.....	926
Bm-Apaf-1 : ..IRTKQKVYVETETSAIILMDIVG...TNKIITLQON...SMHMEHRRPASTNR...SGTVISGVKADVRIFAMAVNNSGELIFATDDSRVVCVDLKNRVRFDIENRRGNVV.....	844
Px-Apaf-1 : ..LRHVAVVNNIYIVVDNRGRM...SITIMINIV...TNRVNRVRIETSTKAVNTTSKRIISS...NIRNIAIVNNSG...HISINDSRVICDIT...IYVDNRRGNVV.....	686
Hs-Apaf-1 : ..SVHCEIIVHYDESBVVDNDEHTN...SSHILLIATGSSDCILKLDINDCKGNMVEGHNSVHCRSEPCILKALSCASADILKMDATSANRKSINVKYFFNLNDDPDQDEVI.....	800
Dm-DARK : ..ANIQIVAFINGVSLIANDDQEFKLSIVVVRKPSSTIRSVYDLKRYVYVCENCHIVLMDITNGSSITIDPVENNDTETLAVDEGRKTAIVILFKIKVRLHETLDEAV.....	847
Sl-Apaf-1 : ..A..MAMSE...MMDDEFPSQVLLICTGCSHISKWWILDASYISQICQENRVRITLKEIVSTIANGSEPTNPSPPISNSETVHSTHGHOSFIREVFEVYVNSMSIDPS.....	1038
Bm-Apaf-1 : ..A..MAMSEV...MMDDEFPSQVLLIAGCSHENSKWVWLDASYISQICQENRVRITLKEIVSTIANGSEPTNPSPPISNSETVHSTHGHOSFIREVFEVYVNSMSIDPS.....	956
Px-Apaf-1 : ..A..MAMSEV...MMDDEFPSQVLLIAGCSHENSKWVWLDASYISQICQENRVRITLKEIVSTIANGSEPTNPSPPISNSETVHSTHGHOSFIREVFEVYVNSMSIDPS.....	777
Hs-Apaf-1 : ..VKGGRM...MMDDEFPSQVLLIAGCSHENSKWVWLDASYISQICQENRVRITLKEIVSTIANGSEPTNPSPPISNSETVHSTHGHOSFIREVFEVYVNSMSIDPS.....	905
Dm-DARK : ..SSESEAVLPLEGSTTIGGRKSGDGRMLGTSEGLIYMLKISLIVLNSSEHIEVYVYELTETVYVYVLSGKGVVWVHILKSSSSSHONRREAVHSADESVHTRAGE.....	967
WD2	
Sl-Apaf-1 : ..LKPLNLKIGCINNNEGVQIPLAVVDKNNIQVMRGKRVLTETIERSDKSEITLTKVISPNCNOYIITVLSKGIVK...RYLITHEVIMIMVYSPQVYLN...VNPNLIVAKNNIMAY.....	1154
Bm-Apaf-1 : ..LKPLNLKIGCINNNEGVQIPLAVVDKNNIQVMRGKRVLTETIERSDKSEITLTKVISPNCNOYIITVLSKGIVK...RYLITHEVIMIMVYSPQVYLN...VNPNLIVAKNNIMAY.....	1070
Px-Apaf-1 :KIGCINN...CGSAVDDKNNIIRGRKVLITIS.....DKITTVKSCNVIYVSGGVKIRG...KTKLITIVASTIVSVTVAAKNG...CMYVNSADAWKKSNG.....	878
Hs-Apaf-1 : ..IRIMWRKVCNBAIMLKCEVVVFOENVMVLAVDHIL...RRIOLINGAG...HDLTEQVSCCSHLYOYTFGDNATETIILNRRIS.....	998
Dm-DARK : ..NMYRSTALMTRETR...DOLLAVDSREHRLIKPAISRISESTIPTHANNNKINAISAFNDQIEVSGVGLVDVILHAILOCHSEPIIYLRK..VSNHILVASARSAGKRVIE.....	1082
WD2	
Sl-Apaf-1 : ..KLITIC...DWKPEMMCRNSLGSQE...INDIQIIMKNGHSEILSSSEISLTS..GRIFFCEBKSIKSGSLVCFWVILVITVEFNATIKLWNKINLITINCRQSV.....	1266
Bm-Apaf-1 : ..KLITIC...DWKPEMLQANTHLSQESOLGSDWCKMIRNGHSD..LSTSSSEASVSRNRLFHGEMKELKQSDLVDCFVADTGLITVEFNATIKLWNKINLITINCRQSV.....	1184
Px-Apaf-1 : ..KLITIC...NKKINGDVSRSSESSSI...SHRSAGSGVRCASAVVNNATVQSSMKHIVNG...SDVINGAKNIVIGDYCHIRDSGVSTRTNIVNNKISCSD.....	979
Hs-Apaf-1 : ..RQHRK...VWVHIOFADQKTISS...DDAEIOWWGLDKGILRGHOFYVGR...HLNSRLLSWFDGTVQVWVITIGNKRSLRYCHQK.....	1087
Dm-DARK : ..GLEITIPLOPNDONFIMDYSTRYSISQI...QOYITLSDHGVCILITANPSAFVRRKDSSEYIVGDLINSULFLANNITDOVFRLIESQNLRYEICEEHTVCKM.....	1190
WD2	
Sl-Apaf-1 : ..VITSAALQNNILVICDKNNTFTQFELKINCEVETITIEEYRLNNVITSCDITLGNILALGLDSGNVVMVNGRROLCLKHHSKVSCHSCFSPVPSRNRNMTSSHILQSPYHLS.....	1386
Bm-Apaf-1 : ..VITCAARQNNILVICSENNFQSYELKQNTITLCHIOEYKLNNVVTCIDTDEYILAMGLDGNVVMVNGRROLCLKHHSKVSCHSCFSPVPSRNRNMTSSHILQSPYHLS.....	1295
Px-Apaf-1 : ..HSDGVADESGVAVVNNICFHEKSKV...CSVDRYSNSTSVVGVYSSASVGVVMMHIVVNVVYKHSRNRNMTIRVN...VNASGNRNTITAG.....	1077
Hs-Apaf-1 : ..VUSCDISHPATKSSSFKTKATKQSDLLD...HDLRGHNSGVRCASVSDSTLLAGQNGHETIWRVSGNITLCAPLS.....EDCAPHS.....	1177
Dm-DARK : ..HSLVATIDGTLAMGFENSTIEHANNRVNHSIEEYHEHC...HLSPECLILASQNGELQWNVITRRNITLRRKRSRQ...HKGQSVYEDAVDAS.....	1297
Sl-Apaf-1 : ..TSODDDVDSGPPVLVMTATEIVWNNITHVMKAPKYIKGRNINWMTPLGSPLENRCETPTNTSLHNSATSANSKPSNYFGDGDFLPQCKALWRGKSGCKRGSKRKEILACIKLSG.....	1506
Bm-Apaf-1 : ..TSODDVAVDOPVLVMTASEIVWNNITHVIE.....HASN.....SLWR...CKGSKRKEILACIKLSG.....	1356
Px-Apaf-1 : ..NSRNNNGCNAHGGVWVVRK.....HASN.....CKGSKRKEILACIKLSG.....	1114
Hs-Apaf-1 : ..GWNIDICSPCKMLISAGGYHWN...VAGCSGSG...PEVTE.....	1217
Dm-DARK : ..ATILVDVIEVADDBHVRITATLARN.....KRGNAHRELLACVKE.....	1342
Sl-Apaf-1 : ..MAKRLHDENFSCFVTVDPNGHIIHNMVMSNN.....	1541
Bm-Apaf-1 : ..INAKRIHDENFSCFVTVDPNGHIIHNMVMSNN.....	1391
Px-Apaf-1 : ..MAKKGHDD...CFVTVDPNGHIIHNMVMSNN.....	1145
Hs-Apaf-1 : ..INAKRIHDENFSCFVTVDPNGHIIHNMVMSNN.....	1248
Dm-DARK : ..NBARQFIDAFSHEVADDEGVYHILIRIRIQPPDPVTLDIANGYEDLKNLRILDSPLMQSDSEAGDVGNVLKNGGVARATPILEASS.....	1440

Figure S1. Multiple sequence alignment of Sl-Apaf-1 and other orthologous proteins. The respective positions are indicated on the right. Identical residues between orthologous sequences are shown as white characters against black background, and conservative substitutions are shaded. The CARD, NBD and WD40 domains are highlighted with black lines. GenBank accession numbers: *S. litura* (Sl-Apaf-1, MT793718); *B. mori* (Bm-Apaf-1, XP_012553167); *P. xylostella* (Px-Apaf-1, XP_030028798); *H. sapiens* (Hs-Apaf-1, CAB55588); and *D. melanogaster* (Dm-DARK, AAD45988).

Table S1. Similarity of Sl-Apaf-1 protein with orthologous proteins by BLAST analysis.

Class	Order	Species	Accession	Identities *
Insecta	Lepidoptera	<i>Spodoptera exigua</i>	AYM00395	91.97%
		<i>Spodoptera frugiperda</i>	XP_035450484	89.09%
		<i>Helicoverpa armigera</i>	XP_021181657	80.49%
		<i>Trichoplusia ni</i>	XP_026734098	77.61%
		<i>Manduca sexta</i>	XP_030022507	61.44%
		<i>Chilo suppressalis</i>	RVE48846	61.15%
		<i>Bombyx mori</i>	XP_012553167	59.74%
		<i>Pieris rapae</i>	XP_022124006	59.97%
		<i>Plutella xylostella</i>	AHB86312	58.22%
		<i>Danaus plexippus</i>	XP_032516465	53.41%
	Hemiptera	<i>Acyrtosiphon pisum</i>	XP_008186216	31.04%
		<i>Aphis glycines</i>	KAE9539441	30.20%
		<i>Aphis gossypii</i>	XP_027847974	30.39%
		<i>Myzus persicae</i>	XP_022163751	30.08%
		<i>Cinara cedri</i>	VVC32818	29.59%
	Coleoptera	<i>Anoplophora glabripennis</i>	XP_018564159	28.51%
		<i>Callosobruchus maculatus</i>	VEN55505	28.74%
		<i>Leptinotarsa decemlineata</i>	XP_023029468	28.86%
	Hymenoptera	<i>Solenopsis invicta</i>	XP_025993160	28.44%
		<i>Copidosoma floridanum</i>	XP_023246426	29.43%
		<i>Acromyrmex echinator</i>	XP_011067576	28.30%
		<i>Neodiprion lecontei</i>	XP_015511747	29.91%
	Diptera	<i>Drosophila melanogaster</i>	AAD45988	19.00%
Mammalia	Rodentia	<i>Mus musculus</i>	AAI31684	21.00%
		<i>Rattus norvegicus</i>	NP_076469	21.00%
	Primates	<i>Homo sapiens</i>	CAB55588	20.00%
Osteichthyes	Cypriniformes	<i>Danio rerio</i>	NP_571683	21.00%
Amphibia	Anura	<i>Xenopus laevis</i>	NP_001085834	20.00%
Nematoda	Rhabditida	<i>Caenorhabditis elegans</i>	CAA48781	10.00%

* Amino acid sequence alignment of Sl-Apaf-1 and orthologous proteins from GenBank.

**Figure S2.** Sl-Apaf-1 overexpression induced apoptosis in SL-1 cell line.

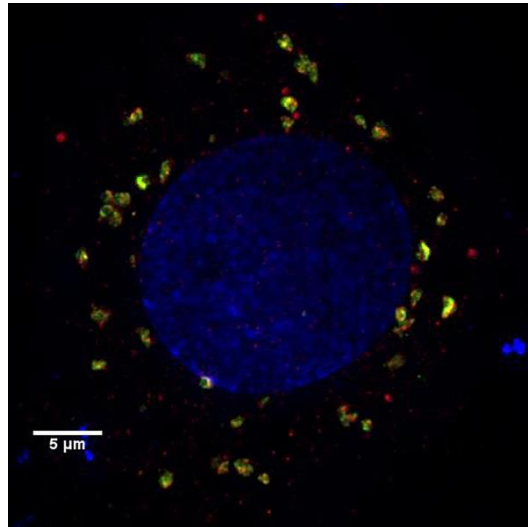


Figure S3. Co-localization of SI-Apaf-1 and SI-cytochrome c in a human U2OS cell. Apaf-1 is expressed with EGFP (green) tag and SI-cytochrome c is expressed with mOrange2 (red) tag. DeltaVision OMX V3 (Marlborough, MA, USA) was used for three-dimensional structured illumination microscopy analysis.

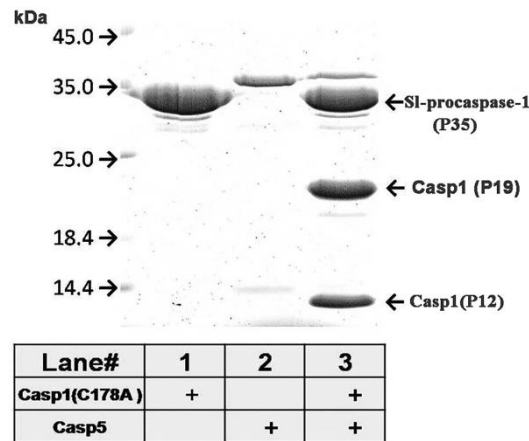


Figure S4. SI-caspase-5 directly cleaves SI-caspase-1. The reaction was performed at 28 °C for 30 min with 2 μM SI-Caspase-5 recombinant protein and 10 μM SI-caspase-1(C178A) protein in the reaction buffer (100 mM KCl, 20 mM HEPES, 5 mM DTT, pH 7.5). The reaction was stopped by adding 5× SDS-loading buffer and boiling at 100 °C for 5min. The reaction products were subjected to 15% SDS-PAGE and visualized by Coomassie blue staining. Lane 1: SI-caspase-1 (C178A); lane2: SI-caspase-5; lane 3: the reaction products of SI-caspase-5 and SI-caspase-1 (C178A).

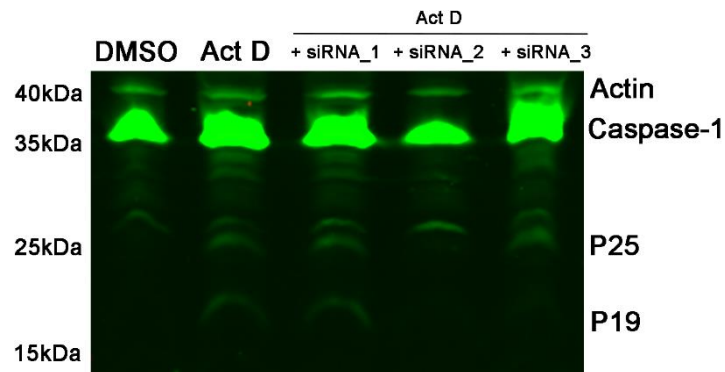


Figure S5. Original western blot figure of Figure 3D (pseudocolor).