SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Drosophila S2 cells transfected with MTAL-grim on day 0 were treated with double stranded RNAs targeting the indicated caspase on day 1 as described (1). Apoptosis was induced by addition of $CuSO_4$ to the cultures on day 3. Whole cell lysates were prepared 5 hours later and subjected to Immunoblot analysis using anti HSV antibody. P, precursor; N, normal nuclear form; C, caspase-dependent band.

Supplemental Fig. 2. Caspase Sites are highly conserved among SREBPs. Sequences corresponding to the SREBP stalk region (between the transcription factor domain and including the first membrane-spanning helix) were identified by BLAST searches of various databases using Human SREBP-1, -2, and dSREBP as query sequences. Sequences were aligned using the Clustal W algorithm as implemented in Megalign 5.01 (DNAstar , Madison, WI). Gaps were extended or reduced manually to align the sequences to the motifs highlighted in black, which are nearly invariant among all SREBPs. Sites clearly homologous with experimentally-determined sites of caspase cleavage are highlighted in orange, potential caspase sites are highlighted in pale orange. Aspartate residues are indicated in red. Blank space indicates no sequence identified for that region. Dashes (-) indicate introduced gaps. Residues are indicated by the standard single letter code for amino acids. X indicates an undetermined amino acid. For 5 of the 122 sequences (Cele, Dwil, Acar 1, Ogar 1 and Olat 2) no clearly homologous caspase site is present. In the first four cases, the residue aligned with the cleavage site aspartate is a glutamate. In the last case it is an asparagine. At least one caspase, Dronc, can cleave after glutamate as well as aspartate residues (2). For each sequence, the accession number, the organism's common name, generic and specific epithet, and the abbreviation shown in the figure are listed: XM_002118145,Placozoan, *Trichoplax adherens*, Tadh; XP_001636576, Starlet Sea Anemone, *Nematostella vectensis*, Nvec; FC850850, Brown Sea Anemone, *Metridium senile*, Msen; ABEG01008960, Nematode, *Caenorhabditis brenneri*, Cbre; CAAC01000063, Nematode, *Caenorhabditis briggsae*, Cbri; NP_499472, Nematode, *Caenorhabditis elegans*, Cele; AAGD02000060, Nematode, *Caenorhabditis remanei*, Crem; EY566871, Earthworm, *Capitella* sp. Capi; XP_974195, Flour Beetle, *Tribolium castaneum*, Tcas; XP_311076, Mosquito, *Anopheles gambiae*, Agam; XP_001660916, Mosquito, *Aedes aegyptae*, Aaeg; XP_001868890, Mosquito, *Culex quinquefasciatis*, Cqui; DS232014, Mosquito, *Culex pipiens*, Cpip; CG8522-PA Fruit Fly, *Drosophila melanogaster*, Dmel; GD14825-PA Fruit Fly, *Drosophila simulans*, Dsim; GM19644-PA Fruit Fly, *Drosophila sechellia*, Dsec; GE19622-PA Fruit Fly, *Drosophila yakuba*, Dyak; GG16056-PA Fruit Fly, *Drosophila erecta*, Dere; GF23590-PA Fruit Fly, *Drosophila anassae*, Dana; GL15732-PA Fruit Fly, *Drosophila persimilis*, Dper; GA21134-PA Fruit Fly, *Drosophila pseudoobscrura*, Dpse; GH14653-PA Fruit Fly, *Drosophila grimshawi*, Dgri; GJ11320-PA Fruit Fly, *Drosophila virilis*, Dvir; GI11638-PA Fruit Fly, *Drosophila mojavensis*, Dmoj; GK17496-PA Fruit Fly, *Drosophila willistoni*, Dwil; XP_396866, Honey bee, *Apis melifera*, Amel; XP_001608182, Wasp, *Nasonia vitripenis*, Nvit; AAZO01001594,; Louse, *Pediculus humanus*, Phum; CK190716, Spider, *Rhipicephalus microplus*, Rmic; FC578107, Limpet, *Lottia gigantea*, Lgig; XP_001178761, Sea Urchin, *Strongylocentrotus purpuratus*, Spur AM217273, Sea Urchin, *Paracentrotus lividus*, Pliv; NP_001071825, Sea Squirt, *Ciona intestinalis*, Cint; EEA69633, Lancet, *Branchiostoma floridae*, Bflo 1; DV498759, Dogfish Shark, *Squalus acanthias*, Saca 1; DB860411, Cichlid, *Lipochromis sp.*; Lipo 1; CAG03157, Puffer fish, *Tetraodon nigriveridis*, Tnig 1; SINFRUP00000077378, Puffer fish, *Fugu rubripes*, Frub 1; NP_001098599, Zebra fish, *Danio rerio*, Drer 1; DN662194, Stickleback, *Gasterosteus aculeatus*, Gacu 1; CT030351, Frog, *Xenopus tropicalis*, Xtro 1; AAWZ01041754, Lizard, *Anolis corolinensis*, Acar 1; NP_989457, Chicken, *Gallus gallus*, Ggal 1; XP_001510556, Platypus, *Ornithorhynchus anatinus*, Oana 1; AC131895, Short-tailed Opossum, *Didelphis virginiana*, Dvir 1; XP_001379412, Virginia Possum, *Monodelphis domestica*, Mdom 1; 1083186, Hamster, *Cricetulus griseus*, Cgri 1; NP_035610, Mouse, *Mus musculus*, Mmus 1; XP_213329, Rat, *Rattus norvegicus*, Rnor 1; EB366948, Guinea Pig, *Cavia porcellus*, Cpor 1; AANG01607401, Cat, *Felis cattus*, Fcat 1;

XM_536664, Dogfish Shark, *Canis*; *lupus familiaris*, Cfam 1; DQ487874, Goat, *Capra hircus*, Chir 1; XP_001790652, Cow, *Bos taurus*, Btau 1; XP_001918249, Horse, *Equus caballus*, Ecab 1; NP_999322, Pig, *Sus scrofa*, Sscr 1; ABRN01345693, Bottle-nosed Dolphin, *Turciops truncatus*, Ttru 1; ABRQ01039769, Hyrax, *Procavia capensis*, Pcap; AALT01093179, Shrew, *Sorex araneus*, Sara 1; AANN01200410, European Hedgehog, *Erinaceus europaeus*, Eeur 1; ABRP01046613, Vampire Bat, *Pteropus vampyrus*, Pvam 1; AAQR01638909, Bush Baby, *Otolemur garnettii*, Ogar 1; AC141085, Ring-tailed Lemur, *Lemur catta*, Lcat 1; ABDC01168829, Grey Mouse Lemur, *Microcebus murinus*, Mmur 1; AC151885, Squirrel Monkey, *Saimiri boliviensis*, Sbol 1; AC147424, Red-bellied Titi, *Callicebus moloch*, Cmol 1; AC147425, Night Monkey, *Aotus nancymaae*, Anan 1; AC148170, Marmoset, *Callithrix jacchus*, Cjac 1; AC148225, Colobus Monkey, *Colobus guereza*, Cgue 1; BB892046, Crab-eating Macaque, *Macaca fasicularis*, Mfas 1; XP_001095392, Rhesus Macaque, *Macaca mulatta*, Mmul 1; AC147417, Baboon, *Papio hamadryas*, Pham 1; ABGA01117156, Orangutan, *Pongo pygmaeus*, Ppyg 1; CABD01032112, Gorilla, *Gorilla gorilla*, Ggor 1; XR_024614, Chimpanzee, *Pan troglodytes*, Ptro 1; 548977, Human, *Homo sapiens*, Hsap 1; CAG01649, Puffer fish, *Tetraodon nigriveridis*, Tnig 2; SINFRUP00000056455, Puffer fish, *Fugu rubripes*, Frub 2; NP_001082935, Zebra fish, *Danio rerio*, Drer 2; AANH01004216, Stickleback, *Gasterosteus aculeatus*, Gacu 2; DT271409, Minnow, *Pimephales promelas*, Ppro 2; BAAE01000948, Medaka, *Oryzias latipes*, Olat 2; Q6GQ26, Frog, *Xenopus laevis*, Xlae 2; NP_001116910, Frog, *Xenopus tropicalis*, Xtro 2; AAWZ01021152, Lizard, *Anolis carolinensis*, Acar 2; EH284527, Turkey, *Meleagris gallopavo*, Mgal 2; XP_416222, Chicken, *Gallus gallus*, Ggal 2; ABQF01015858, Zebra Finch, *Taeniopygia guttata*, Tgut 2; AAGV020800007, Armadillo, *Dasypus novemcinctum*, Dnov 2; EF640983, Possum, *Monodelphis domestica*, Mdom 2; ABRO01203560, Possum, *Didelphis virginiana*, Dord 2; 1083185, Hamster, *Cricetulus griseus*, Cgri 2; NP_150087, Mouse, *Mus musculus*, Mmus 2; NP_001028866, Rat, *Rattus norvegicus*, Rnor 2; AAQQ01536865, Ground Squirrel, *Spermophilus tridecemlineatus*, Stri 2; AAKN02031303, Guinea Pig, *Cavia porcellus*, Cpor 2; AC145540, Rabbit, *Oryctolagus cuniculus*, Ocun 2; XP_849087, Dog, *Canis*; *lupus familiaris*, Cfam 2; AANG01250199, Cat, *Felis cattus*, Fcat 2; AAY42985, Pig, *Sus scrofa*, Sscr 2; XP_583656, Cow, *Bos taurus*, Btau 2; EE816920, Sheep, *Ovis aries*, Oari 2; AC171147, Muntjak Deer, *Muntiacus reevesi*, Mree 2; ABRR01385837, Llama, *Lama pacos*, Lpac 2; XP_001916701, Horse, *Equus caballus*, Ecab 2; ABRN01225138, Bottle-nosed Dolphin, *Turciops truncatus*, Ttru 2; ABRQ01130997, Hyrax, *Procavia capensis*, Pcap 2; AC145527, African Hedgehog, *Atelerix albiventris*, Atel 2; AANN01302258, European Hedgehog, *Erinaceus europaeus*, Eeur 2; AALT01396400, Shrew, *Sorex araneus*, Sara 2; AAIY01713223, Madagascar Hedgehog, *Echinops telfairi*, Etel 2; AAPY01023878, Tree Shrew, *Tupaia belangeri*, Tbel 2; AAPE01394219, Little Brown Bat, *Myotis lucifugus*, Mluc 2; ABRP01290978, Vampire Bat, *Pteropus vampyrus*, Pvam 2; AAQR01483721, Bush Baby, *Otolemur garnettii*, Ogar 2; AC146284, Ring-tailed Lemur, *Lemur catta*, Lcat 2; ABDC01201703, Grey Mouse Lemur, *Microcebus murinus*, Mmur 2; ABRT010509174, Tarsier, *Tarsius syrichta*, Tsyr 2; AANU01293891, Rhesus Macaque, *Macaca mulatta*, Mmul 2; CABD01633747, Gorilla, *Gorilla gorilla*, Ggor 2; XR_024313, Chimpanzee, *Pan troglodytes*, Ptro 2; NP_004590, Human, *Homo sapiens*, Hsap 2.

Supplemental Fig. 3. Thr385 is required for caspase cleavage of dSREBP. The P4-P1' positions are shown for reference. The down arrow indicates the scissile bond. Cleavage of dSREBP is completely abolished in T385S and T385A mutants (lanes 4 and 6). In the P1' position, the well-conserved Ala residue may be substituted by Phe without preventing cleavage. Other *Drosophila* substrates of Drice, such as the D_{116}TTD site in rep1 (ICAD) and the E_{275}ETD site in CAD, also have a Thr residue at P2 (3,4) and these residues are well conserved among other *Drosophila* species. The experiment was conducted as described for Figure 3B.

SUPPLEMENTAL METHODS

RNAi treatment:

The following oligonucletide sequences were designed from published transcript sequences in Flybase.org and were used to produce double stranded RNAs for RNAi experiments as described previously (1):

Dcp1:	5'-TTAATACGACTCACTATAGGGAGAAAGATGACCGACGAGTGCG-3'
Dcp1R:	5'-TTAATACGACTCACTATAGGGAGAAGAAAGTCGGCGTGTATGG-3'
Drice:	5'-TTAATACGACTCACTATAGGGAGAAACGATCACACAGATG-3'
DriceR:	5'-TTAATACGACTCACTATAGGGAGAGTTCGGCGCACAGGCTC-3'
Dream:	5'-TTAATACGACTCACTATAGGGAGAATGGGTTGGTGGAGCAAGAAAAG-3'
DreamR:	5'-TTAATACGACTCACTATAGGGAGATTTTTGGTGTCTTGATGTTGGG-3'
Decay:	5'-TTAATACGACTCACTATAGGGAGAATGGTCAGCAGCAAAATC-3'
DecayR:	5'-TTAATACGACTCACTATAGGGAGATTTCCTTCATCTGGTTG-3'
Damm:	5'-TTAATACGACTCACTATAGGGAGAATATGACAGCAACAGG-3'
DammR:	5'-TTAATACGACTCACTATAGGGAGATTCGAACCTGTCATAG-3'
Dronc:	5'-TTAATACGACTCACTATAGGGAGACTCGAGATTGGAATG-3'
DroncR:	5'-TTAATACGACTCACTATAGGGAGATATCGACCACAGATC-3'
Dredd:	5'- TTAATACGACTCACTATAGGGAGATCAGCGAGTGCAATTTATCGGC -3'
DreddR:	5'- TTAATACGACTCACTATAGGGAGAGCTCCTTCTGGTCAGCCAGTCC-3'

Double-stranded RNA corresponding to the *Drosophila* caspases was added and the cells incubated as described. Cell lysates and Immunoblot analysis were as described in Materials and Methods.

REFERENCES

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2. Hawkins, C. J., Yoo, S. J., Peterson, E. P., Wang, S. L., Vernooy, S. Y., and Hay, B. A. (2000) *J Biol Chem* **275**, 27084-27093
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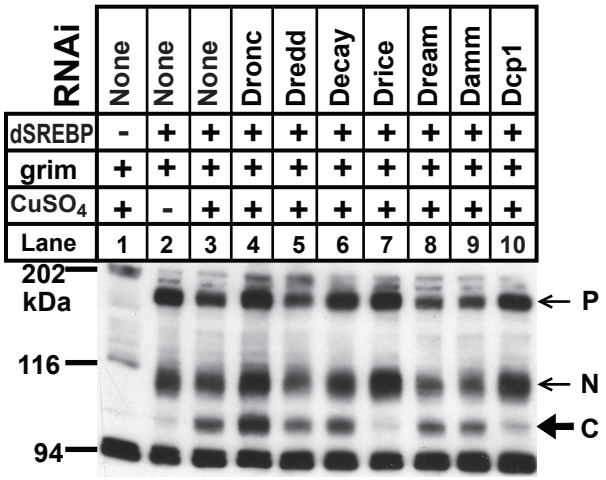
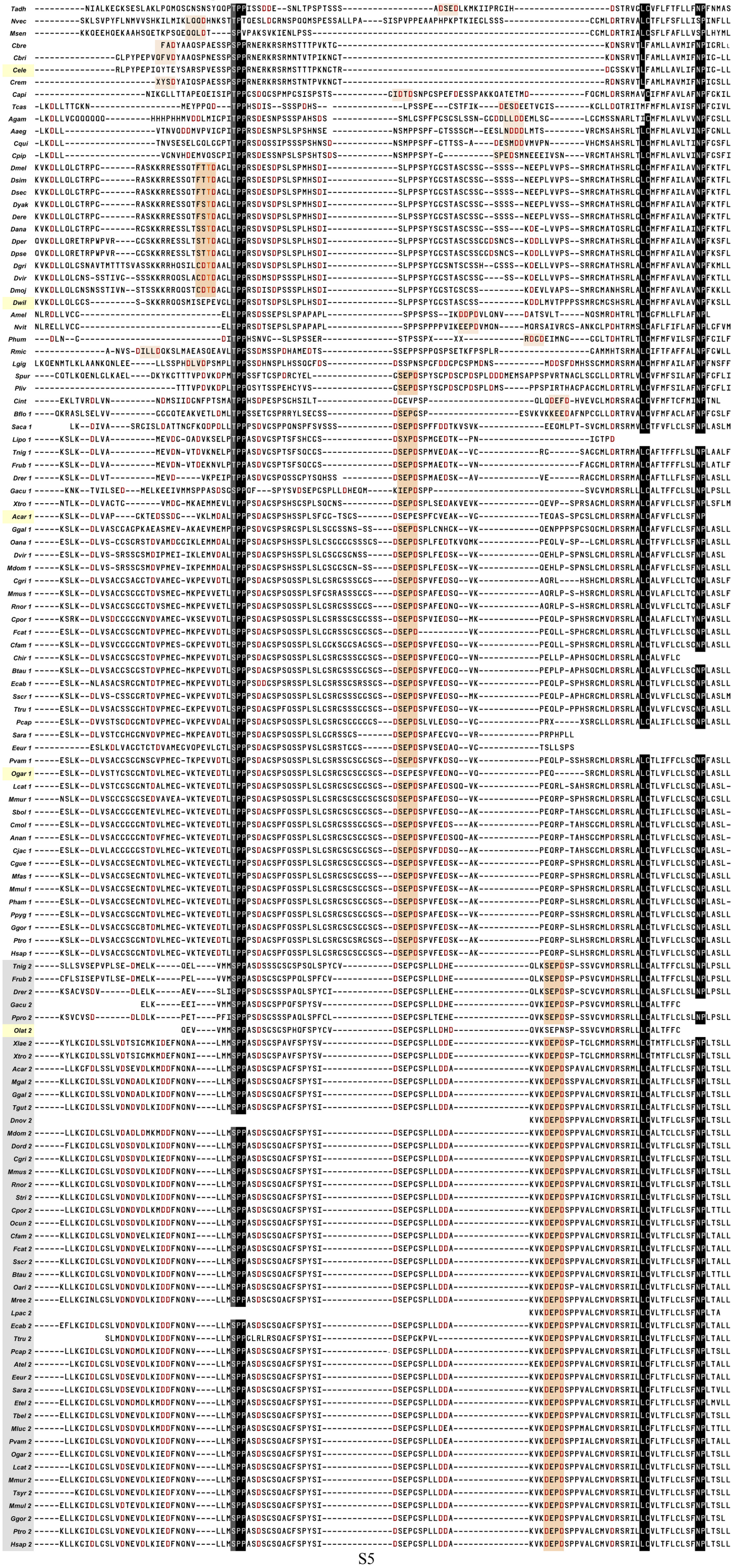
4. Mukae, N., Yokoyama, H., Yokokura, T., Sakoyama, Y., Sakahira, H., and Nagata, S. (2000) *J Biol Chem* **275**, 21402-21408

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3