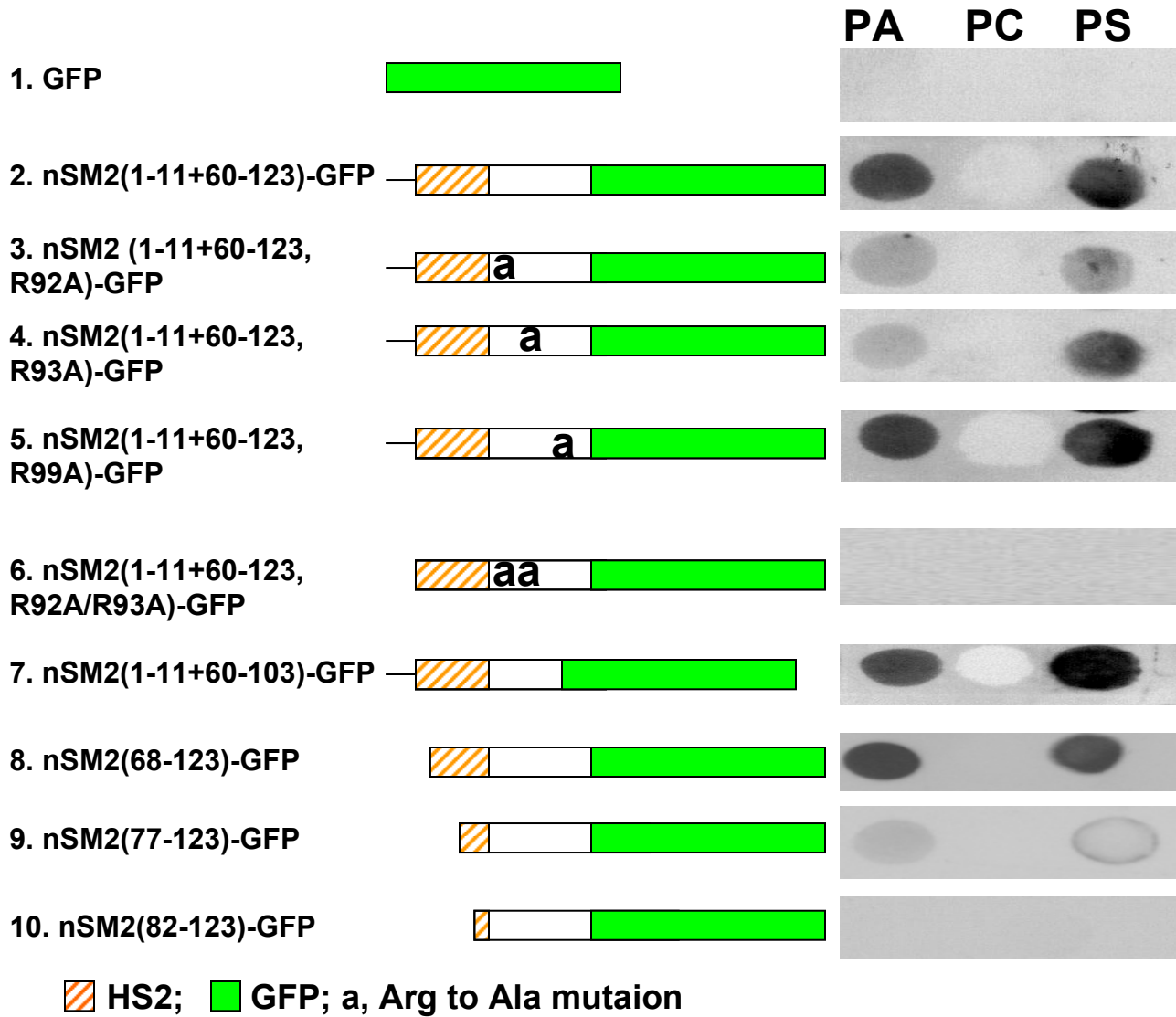
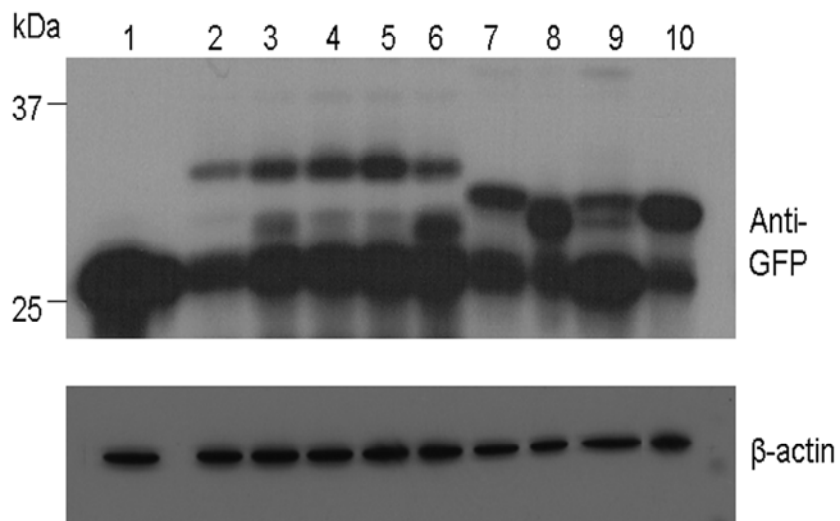


Supplemental Table 1. PCR primers utilized for mutagenesis

<i>Mutation</i>	<i>PCR Primers (5'-3')</i>
<i>R33A</i>	F: CCATGTTACTGGCTGGTGGACGCGCTGTTGGCCTCTTTCATACC R: GGTATGAAAGAGGCCAACAGCGCGTCCACCAGCCAGTAACATGG
<i>R92A</i>	F: CCACTGCAGTCTGCCGCCCGCCCTACTCCTACTCC R: GGAGTAGGAGTAGGGCCGGCGGCAGACTGCAGTGG
<i>R93A</i>	F: CACTGCAGTCTGCCCGCGGCCCTACTCCTACTCC R: GGAGTAGGAGTAGGGCGCGCGGCAGACTGCAGTG
<i>R92A/R93A</i>	F: TCTCCACTGCAGTCTGCCGCCCGCCCTACTCCTACTCCC R: CGGGAGTAGGAGTAGGGCGCGGCAGACTGCAGTGGAGA
<i>R99A</i>	F: CCTACTCCTACTCCGCGCTAGAAGACAAGAAC R: GTTCTTGTCTTCTAGCGCGAGTAGGAGTAGG
<i>Delete aa45-48</i>	F: CTTTCATACCCACCACCTATGAGGCAGATGACCCCTGCTGC R: GCAGCAGGGGTTCATCTGCCTCATAGGTGGTGGGTATGAAAG
<i>Delete aa2-81*</i>	F: GACTCAGATCTCGAGAATGTTTCATCTTCTGGTCTCCAC R: GTGGAGACCAGAAGATGAACATTCTCGAGATCTGAGTC
<i>Delete aa104-123*</i>	F: CTCCC GGCTAGAAGACAAGGGGATCCACCGCCGGTTCGCC R: GGCGACCGGCCGGTGGATCCCCTTGTCTTCTAGCCGGGAG
<i>Delete aa2-24</i>	F: GACTCAGATCTCGAGAA TGTTCATGTTACTGGCTGGTG R: CACCAGCCAGTAACATGGGAACATTCTCGAGATCTGAGTC
<i>Delete aa2-59</i>	F: GACTCAGATCTCGAGAATGACGGTCTTTCACGCCAG R: CTGGCGTGAAGAGGACCGTCATTCTCGAGATCTGAGTC
<i>Delete aa2-67:</i>	F: GACTCAGATCTCGAGAATGCTGGCTCTGCTTGTGGCTG R: CAGCCACAAGCAGAGCCAGCATTCTCGAGATCTGAGTC
<i>Delete aa2-76</i>	F: GACTCAGATCTCGAGAATGTTTGCCTTCTCGGGTTCATC R: GATGAACCCGAGAAAGGCAAACATTCTCGAGATCTGAGTC
<i>nSMase2 (aal-40)-GFP *</i>	F: GTTGGCCTCTTTCATACCCGGGATCCACCGGCCG R: CGGCCGGTGGATCCC GGGTATGAAAGAGGCCAAC
<i>nSMase2 (aal-59)-GFP *</i>	F: CTGCCTGCAGCTGTTCTGCGGGATCCACCGGCCG R: CGGCCGGTGGATCCC GCGAGAACAGCTGCAGGCAG
<i>Delete aa12-123</i>	F: ACGACCCCTTTTCCTAATAGCTTTTGTCTCGCCACGGCCAATG R: CATTGGCCGTGGCGAAGCAAAGCTATTAGGAAAGGGGGTTCGT

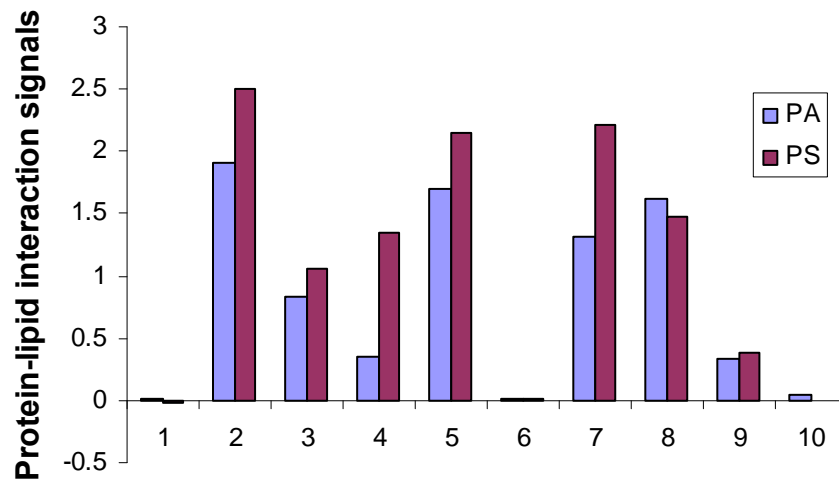
* Vector *nSMase2(1-123)-GFP* was used as PCR template for these mutagenesis experiments.

“F” and “R” refer to the forward and reverse primers, respectively.

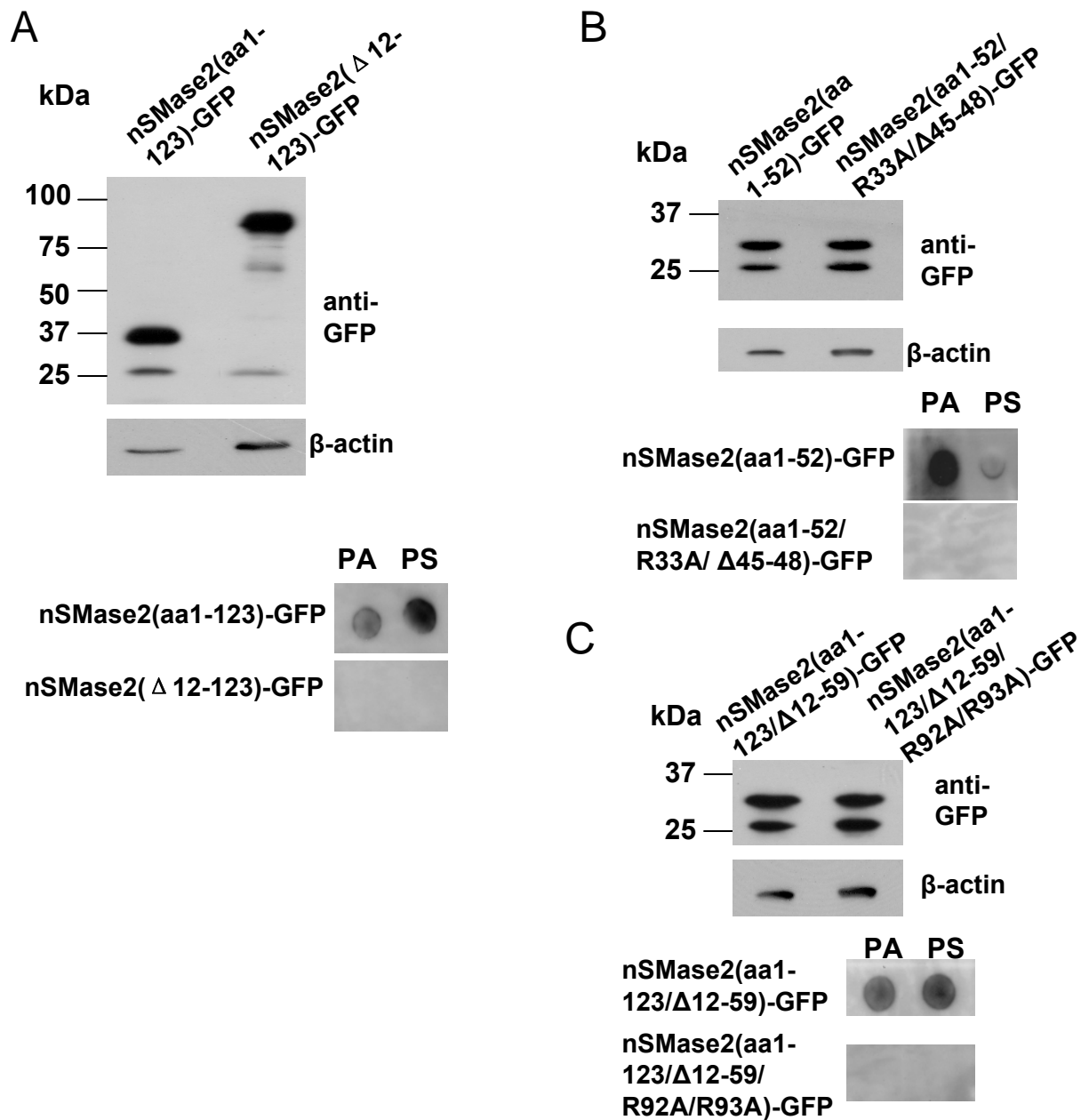
A**B**

Supplemental Figure 1

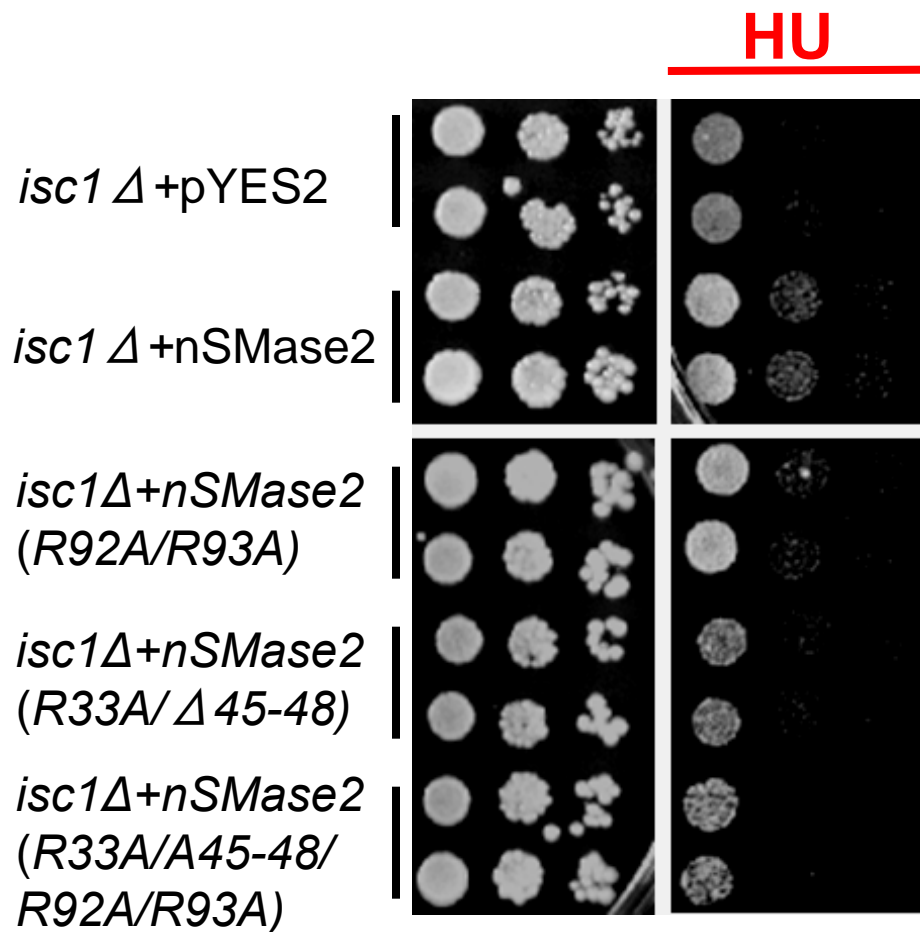
C



Supplemental Figure 1. Identification of the second APL binding domain in the N-terminus of nSMase2. *A*, a series of GFP-tagged deletion or point mutants were generated from mutant nSMase2(aa1-123/ Δ 12-59)-GFP (which contains the second HS) and overexpressed in HEK293 cells. The cell lysates were subjected to lipid-protein overlay assays. *B*, immunoblot analysis of expression of deletion mutants of nSMase2 with anti-GFP antibody. *C*, densitometry analyses were performed using ImageQuant TL software (GE health care). The relative value of each lipid-protein interaction signal was calculated by subtracting the background and normalized to its western signals from the upper band (the correct sized band). Similar results were obtained in two separate experiments.



Supplemental Figure 2. Comparison of lipid-protein interaction using similar amount of mutant nSMase2-GFP protein. HEK293 cells were transfected using vector with nSMase2-GFP mutants. Different amount for protein lysates were used for western blot analysis for acquiring similar level of mutant nSMase2 protein (the upper band on Western blot results). The cell lysates were then used for lipid-protein overlay assays. *A*, 10 μ g nSMase2(aa1-123)-GFP and 50 μ g of nSMase2(Δ 12-123)-GFP cell lysates were used for western blot analysis (upper panel). 10 μ g/ml nSMase2(1-123)-GFP and 50 μ g/ml of nSMase2(Δ 12-123)-GFP cell lysates were used for lipid-protein overlay assays (lower panel). *B*, 10 μ g nSMase2(aa1-52)-GFP and 25 μ g nSMase2(1-52/R33A/ Δ 45-48)-GFP cell lysates were used for western blot analysis (upper panel). 10 μ g/ml nSMase2(1-52)-GFP and 25 μ g/ml of nSMase2(aa1-52/R33A/ Δ 45-48)-GFP cell lysates were used for lipid-protein overlay assays (lower panel). *C*, 10 μ g nSMase2(aa1-123/ Δ 12-59)-GFP and 25 μ g nSMase2(aa1-123/ Δ 12-59/R92A/R93A)-GFP cell lysates were used for western blot analysis (upper panel). 10 μ g/ml nSMase2(aa1-123/ Δ 12-59)-GFP and 25 μ g/ml nSMase2(aa1-123/ Δ 12-59/R92A/R93A)-GFP cell lysates were used for lipid-protein overlay assays (lower panel). Similar results were obtained in two separate experiments.



Supplemental Figure 3. Effects of nSMase2 and APL binding domain mutants on rescuing the defect of *isc1*Δ *S. cerevisiae* cells. Different vectors, including pYES2 control, wildtype nSMase2-pYES2 or various nSMase2-pYES2 mutants, were transformed and expressed in *isc1*Δ *S. cerevisiae* cells, respectively. Exponentially growing cells were first diluted to an A600 of 0.3, and then serially diluted and spotted onto agar Raf/Galactose uracil minus plates with or without 10 mg/ml hydroxyurea (HU). The plates were incubated for 3-4 days at 30°C. This experiment was performed two times with similar results.

A

	1					50
Cf_nSMase2	MVLYTAPFPN	SCLSALHAVS	WALLFPCYWL	ADRLVASFIP	TTYEKQRAD	
Am_nSMase2	MVLYTAPFPN	SCLSALHAVS	WALLFPCYWL	ADRLVASFLP	TTYEKQRAD	
Bt_nSMase2	MVLYTTPFPN	SCLSALHAVS	WALIFPCYWL	ADRLLASFIP	TTYEKQRAD	
Mm_nSMase2	MVLYTTPFPN	SCLSALHAVS	WALIFPCYWL	VDRLLASFIP	TTYEKQRAD	
Rn_nSMase2	MVLYTTPFPN	SCLSALHAVS	WALIFPCYWL	VDRLVASFIP	TTYEKQRAD	
Pt_nSMase2	MVLYTTPFPN	SCLSALHCVS	WALIFPCYWL	VDRLAASFIP	TTYEKQRAD	
Hs_nSMase2	MVLYTTPFPN	SCLSALHCVS	WALIFPCYWL	VDRLAASFIP	TTYEKQRAD	
Cj_nSMase2	MVLYTTPFPN	SCLSALHCVS	WALIFPCYWL	VDRLAASFIP	TTYEKQRAD	
Oc_nSMase2	MVLYTTPFPT	SCLSALHAVS	WALIFPCYWL	VDRLAASFRP	TTYEKQRAD	
Xl_nSMase2	MVLYTTPFPS	SFLSSLHSL	WALIFPCYWL	GDRLLASFLP	TTYEKQRSD	
Gg_nSMase2	MVLYTSPFPN	SFLSVLHSFS	WGLIFPCYWF	ADRLVASFVP	TTYEKQRAD	
Dr_nSMase2	MVLHTSPYPS	AFLSFLSGLS	WAFVFPYWL	LDRLLASCV	TSLEKRRRSQ	
Consensus	MVL-T-P-P-	--LS-L----	W----FPCYW-	-DRL-AS---	T--EKR-R-	
			FPCYWX	XDRLXASXXX	TXXEKRXR	
			nSM2-APLB1			
	51					100
Cf_nSMase2	DPCYLQLLCT	VLFTPVIYAL	LVASLPFAFV	GFLWSPLOQ	ARRPYIYSR.	
Am_nSMase2	DPCYLQLLCT	LLFTPVIYAL	LVASLPFAFV	GFLWSPLOQ	ARRPYIYSR.	
Bt_nSMase2	DPCYLQLLCT	VLFTPVIYAL	LVASLPFAFL	GFLWSPLOQ	ARRPYVYSR.	
Mm_nSMase2	DPCCLQLFCT	VLFTPVIYAL	LVAALPFAFL	GFIFWSPLOQ	ARRPYSYSR.	
Rn_nSMase2	DPCYLQLFCT	VLFTPVIYAL	LVAALPFAFL	GFIFWSPLOQ	ARRPYSYSR.	
Pt_nSMase2	DPCCLQLLCT	ALFTPVIYAL	LVASLPFAFL	GFLWSPLOQ	ARRPYIYSR.	
Hs_nSMase2	DPCCLQLLCT	ALFTPVIYAL	LVASLPFAFL	GFLWSPLOQ	ARRPYIYSR.	
Cj_nSMase2	DPCCLQLLCT	ALFTPVIYAL	LVASLPFAFL	GFLWSPLOQ	ARRPYIYSR.	
Oc_nSMase2	DPCCLQLLCT	VLFTPVIYAL	LVAALPFAFL	GFLWSPLOQ	VRRPYVYSR.	
Xl_nSMase2	DPCYFQALCI	IITTPVIYAL	LVSSLPFAFI	GFLVWSP IQA	ARRPYVYSH.	
Gg_nSMase2	DPCYFHALCI	IITTPVIYAL	LVASLPFALI	GFLWSPLOQ	ARRPYIYSR.	
Dr_nSMase2	DPCSFLALGV	LISTPLYLVL	LLASLPFAFI	GFLWAPLOQ	IRKPYIYSHQ	
Consensus	DPC-----	---TP-YL-L	L---LPFA--	GF--W-P-Q-	-R-PY-YS--	
			LXL	LXXXLPFAXX	GFXXWXPXQX	XRR/K
			nSM2-APLB2			
	101					150
Cf_nSMase2LEDQ	GSASGAALLS	EWKGTGPGKS	FCFATANLCL	LPDSLARLNN	
Am_nSMase2LEDK	GPAGGAALLS	EWKGTGPGKS	FCFATANLCL	LPDSLARLNN	
Bt_nSMase2LEDK	GPTGGAALLS	EWKGTGPGKS	FCFATANLCL	LPDSLARLNN	
Mm_nSMase2LEDK	NPAGGAALLS	EWKGTGAGKS	FCFATANVCL	LPDSLARLNN	
Rn_nSMase2LEDK	SPAGGAALLS	EWKGTGAGKS	FCFATANVCL	LPDSLARLNN	
Pt_nSMase2LEDK	GLAGGAALLS	EWKGTGPGKS	FCFATANVCL	LPDSLARVNN	
Hs_nSMase2LEDK	GLAGGAALLS	EWKGTGPGKS	FCFATANVCL	LPDSLARVNN	
Cj_nSMase2LEDK	GLASGAALLS	EWKGTGPGKS	FCFATANVCL	LPDSLARVNN	
Oc_nSMase2LGDK	GAAGGAALLG	EWKGTGTGKS	FCFATANLCL	LPDSLARLNN	
Xl_nSMase2IQEK	ARATEAKLLA	EWRATSSGKS	FCFGTANVCL	LPDSIARFNN	
Gg_nSMase2LQDK	SHANGAALLS	EWKAAGNGKS	FCFGSANVCL	LPDSLARFNN	
Dr_nSMase2	KPDKHGVEQG	SAAGTGAALA	EWRP..QGRS	FCFGSANVCL	LPDSLARFNN	
Consensus	-----	-----L-	EW-----G-S	FCF--AN-CL	LPDS-AR-NN	

Supplemental figure 4A

	151					200
Cf_nSMase2	VFNTQARARE	IGQRIRNGAS	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGS	
Am_nSMase2	VFNTQARAKE	IGQRIRNGAS	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGS	
Bt_nSMase2	VFNTQARAKE	IGQRIRNGAS	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGS	
Mm_nSMase2	VFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Rn_nSMase2	VFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGS	
Pt_nSMase2	LFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Hs_nSMase2	LFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Cj_nSMase2	LFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Oc_nSMase2	LFNTQARAKE	IGQRIRNGAA	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Xl_nSMase2	LFNTQARAKE	IGRRIRNGAS	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Gg_nSMase2	VFNTQARAKE	IGRRIRNGAS	RPQIKIYIDS	PTNTSISAAS	FSSLVSPQGG	
Dr_nSMase2	LADTQRRARE	VGKRIRNGAS	RPQIKIYIDS	PTNTSISAVS	FCSL.STQG.	
Consensus	---TQ-RA-E	-G-RIRNGA-	RPQIKIYIDS	PTNTSISA-S	F-SL-S-QG-	

	201					250
Cf_nSMase2	DGVARAVPGS	IKRTASVEYK	GD.GGRHPSE	EAA.....	.NGPA.SGDP	
Am_nSMase2	DGVARAVPGS	IKRTASVEYK	GD.GGRHPSD	EAA.....	.NGLA.SGDP	
Bt_nSMase2	DGVPRAVPGS	IKRTASVEYK	GD.GGRHPSD	EAA.....	.NGLA.SGDP	
Mm_nSMase2	DG.SRAVPGS	IKRTASVEYK	GD.GGRHPSD	EAA.....	.NGPA.SGEQ	
Rn_nSMase2	DG.ARAVPGS	IKRTASVEYK	GD.GGRHPSD	EAA.....	.NGPA.SGEQ	
Pt_nSMase2	DGVARAVPGS	IKRTASVEYK	GD.GGRHPGD	EAA.....	.NGPA.SGDP	
Hs_nSMase2	DGVARAVPGS	IKRTASVEYK	GD.GGRHPGD	EAA.....	.NGPA.SGDP	
Cj_nSMase2	DGVARAVSGS	IKRTASVEYK	GD.GGRHPSE	EAA.....	.NGPA.SGDP	
Oc_nSMase2	DGGARVVPGS	IKRTASVEYK	GD.GGRH.GD	EAA.....	.NGPA.PADP	
Xl_nSMase2	DTSHRAVNVG	MKRTTSMEYK	GENSGENSSE	ENR.....	.DGRNG.GDT	
Gg_nSMase2	DSTNRTVNAG	MKRTTSMEYK	GDNSGSNNSE	DGR.....	.DDKAGSGEL	
Dr_nSMase2	FRRTSSLDHR	PDPVVTTENE	TEALTECPIH	TSGGTSSDCP	
Consensus	-----	--RT-S----	-----	-----	-----	

	251					300
Cf_nSMase2	AEGVGLEDA.	.CIVRIGGEE	GG.....RP	PEADDPAAGG	QTRNGA....	
Am_nSMase2	AEGGSLEDA.	.CIVRIGGDE	GG.....RP	PEADDATAGG	QARNGA....	
Bt_nSMase2	ADGGNLEDA.	.CIVRISGDE	GG.....RP	PEAGDPANGG	QARNGA....	
Mm_nSMase2	ADGSL.EDS.	.CIVRIGGEE	GG.....RP	QEADDPAAGS	QARNGA....	
Rn_nSMase2	ADGSL.EDS.	.CIVRIGGEE	GG.....RA	QEADDPAPGS	QARNGA....	
Pt_nSMase2	VDSSSPEDA.	.CIVRISGEE	GG.....RP	PEADDPAPGG	QARNGA....	
Hs_nSMase2	VDSSSPEDA.	.CIVRIGGEE	GG.....RP	PEADDPVPGG	QARNGA....	
Cj_nSMase2	ADGSSPEDS.	.CIVRISGEE	GG.....RP	PEAEDPAPGG	QARNGA....	
Oc_nSMase2	TDGGGLEET.	.CIVRIGGEE	GG.....RP	PEADDPAPGS	QARNGA....	
Xl_nSMase2	ADGE..DCP.	.CIIRISGED	SG.....HI	SDTETDAISG	HVNVACLAE.	
Gg_nSMase2	NEGE..ENT.	.CIVRINGED	NG.....HV	SDIETDVVNG	QANASGTAEH	
Dr_nSMase2	IHSTGVQNSS	ECPLHPNEEE	HAPDCAMHQS	SDSECPVHSN	IMQNSEDCPL	
Consensus	-----	-C-----	-----	-----	-----	

Supplemental figure 4A (cont'd)

	301				350
Cf_nSMase2	...G.....G	SP.....QGQ	TPNHSQRDGD	SGSLGSPSAS	RESLVKTRAA
Am_nSMase2	...G.....G	AP.....QGQ	TPNHSQRDGD	SGSLGSPSAS	RESLVKVRRAA
Bt_nSMase2	...G.....G	GP.....RGQ	TPNHSQQDGD	SGSLGSPSAS	RESLVKGRSG
Mm_nSMase2	...G.....G	TP.....KGQ	TPNHNQRDGD	SGSLGSPSAS	RESLVKARAG
Rn_nSMase2	...G.....G	TP.....KGQ	TPNHNQRDGD	SGSLGSPSAS	RESLVKARAG
Pt_nSMase2	...G.....G	GP.....RGQ	TPNHNQQDGD	SGSLGSPSAS	RESLVKGRAG
Hs_nSMase2	...G.....G	GP.....RGQ	TPNHNQQDGD	SGSLGSPSAS	RESLVKGRAG
Cj_nSMase2	...G.....R	GP.....RGQ	TPNHNQRDGD	SGSLGSPSAS	RESLVKGRAG
Oc_nSMase2	...G.....G	GP.....RGQ	TPNHSQRDGD	SGSLGSPSAS	RESLVKARAG
Xl_nSMase2	.IEP.....D	FQ.....KSP	TANHKNKDAD	GGSLDSYTAS	RESLVKVRMA
Gg_nSMase2	SLEG.....D	TE.....ISK	TPNHKQKDGD	SGSLDSYSAS	RESLVKVRVG
Dr_nSMase2	HPSGVQISIT	SPDSEPAEAE	KGNHQTGDGD	TGSLDSRTAS	RESLVRFHAA
Consensus	-----	-----	--NH---D-D	-GSL-S--AS	RESLV-----
	351				400
Cf_nSMase2	AD.GGGSGEP	GATNSKFPYK	ASGVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Am_nSMase2	AD.SGGSGEP	GATNSKLPYK	ASGVKKAAR	KRRHPDEAFD	HEVSAFFPAN
Bt_nSMase2	AD..GGSGEP	GA.NSKLPYK	ASVVKKAAMR	RRRHPDEAFD	HEVSAFFPAN
Mm_nSMase2	QD.SGGSGEP	GA.NSKLLYK	TSVVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Rn_nSMase2	QD.SGGSGEP	GS.NSKLLYK	TSVVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Pt_nSMase2	PD.TSASGEP	GA.NSKLLYK	ASVVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Hs_nSMase2	PD.TSASGEP	GA.NSKLLYK	ASVVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Cj_nSMase2	PD.AGAGGEP	GA.NSKLLYR	ASMVKKAAR	RRRHPDEAFD	HEVSAFFPAN
Oc_nSMase2	PDGSSGSTEP	GS.NSKLLYK	ASVVKKAATR	RRRHPDEAFD	YEVSAFFPAN
Xl_nSMase2DGAADQ	SSINTKLLHK	TSIIKKATAR	KKKHTDDILD	HEISAFFPAN
Gg_nSMase2DGTVDQ	STVNNKLLYK	ASIMKTSVR	KKKHTDETFD	HEISAFFPAN
Dr_nSMase2DGGISP	N..NTLSQHR	TSVFKRPIGR	KRRHGDDGFD	HEISAFFPAN
Consensus	-----	---N-----	-S--K----R	---H-D---D	-E-SAFFPAN
	401				450
Cf_nSMase2	LDFLCLQEVF	DKRAAAKLKD	QLHGYFEYIL	YDVG VYGCQG	CCS.FKFLNS
Am_nSMase2	LDFLCLQEVF	DKRAAAKLKD	QLHGYFEYIL	YDVG VYGCQG	CCS.FKCLNS
Bt_nSMase2	LDFLCLQEVF	DKRAAAKLKD	QLHSYFEYIL	YDVG VYGCHG	CCS.FKCLNS
Mm_nSMase2	LDFLCLQEVF	DKRAAAKLKE	QLHGYFEYIL	YDVG VYGCHG	CCN.FKCLNS
Rn_nSMase2	LDFLCLQEVF	DKRAAAKLKE	QLHGYFEYIL	YDVG VYGCHG	CCN.FKCLNS
Pt_nSMase2	LDFLCLQEVF	DKRAATKLKE	QLHGYFEYIL	YDVG VYGCQG	CCS.FKCLNS
Hs_nSMase2	LDFLCLQEVF	DKRAATKLKE	QLHGYFEYIL	YDVG VYGCQG	CCS.FKCLNS
Cj_nSMase2	LDFLCLQEVF	DKRAATKLKD	QLHGYFEYIL	YDVG VYGCQG	CCS.FKCLNS
Oc_nSMase2	LDFLCLQEVF	DKRAAAKLKD	QLHGYFEYIL	YDVG VYGCHG	CCG.FKFLNS
Xl_nSMase2	LDFLCLQEVF	DKRAAEKLKE	QLHYFFEYVL	YDVG VYSCHG	CCG.FKFLNS
Gg_nSMase2	LDFLCLQEVF	DKRAAEKLKE	QLHHYFEYIV	YDVG VYGCHA	CCS.FK FVNS
Dr_nSMase2	LDFLCLQEVF	DRRAADRLRR	QLHRYFPFVL	SDVGRYGWKG	CCSRFKFLNS
Consensus	LDFLCLQEVF	D-RAA--L--	QLH-YF----	-DVG-Y----	CC--FK--NS

Supplemental figure 4A (cont'd)

	451				500
Cf_nSMase2	GLFFASRYPI	MDVAYHCYPN	GRRSDGLASK	GALFLKVQVG	STPQDQRIVG
Am_nSMase2	GLFFASRYPI	MDVAYHCYPN	GRRSDGLASK	GALFLKVQVG	STPQDQRIVG
Bt_nSMase2	GLFFASRYPI	MDVAYHCYPN	GRFSDSLASK	GALYLKVQVG	STPQDQRIVG
Mm_nSMase2	GLFFASRYPV	MDVAYHCYPN	GCSFDALASK	GALFLKVQVG	STPQDQRIVG
Rn_nSMase2	GLFFASRYPV	MDVAYHCYPN	GCSFDALASK	GALFLKVQVG	STPQDQRIVG
Pt_nSMase2	GLLFASRYPI	MDVAYHCYPN	KCNDDALASK	GALFLKVQVG	STPQDQRIVG
Hs_nSMase2	GLLFASRYPI	MDVAYHCYPN	KCNDDALASK	GALFLKVQVG	STPQDQRIVG
Cj_nSMase2	GLLFASRYPI	MDVAYHCYPN	KCNDDALASK	GALFLKVQVG	STPQDQRIVG
Oc_nSMase2	GLFFASRYPV	MDVAYHCYPN	GRGFDNLASK	GALFLKVQVG	STPQDQRIVG
Xl_nSMase2	GLLFASRYPI	LDATYHCYPN	GRGIDALAAK	GALFIKVQVG	TPPQDQRIVG
Gg_nSMase2	GLLFASRYPV	MDAAYHCYPN	GKGTDSLASK	GALFLKVQVG	STPQDQRIVG
Dr_nSMase2	GLLLASRYPI	LDAHYECPN	GRSEDALAAK	GVLFAKVQVG	SSAQDQRVVG
Consensus	GL--ASRYP-	-D--Y-C-PN	----D-LA-K	G-L--KVQVG	---QDQR-VG

	501				550
Cf_nSMase2	YISCTHLHAL	PEDSAIRCEQ	LDLLQDWLAD	FRKSTSSSST	ANP..EELVA
Am_nSMase2	YISCTHLHAL	PEDSAIRCEQ	LDLLQDWLAD	FRKSTSSSST	ANP..EELVA
Bt_nSMase2	YISCTHLHAL	SESDIRCEQ	LNMLQDWLAD	FRKSTSSSSA	ANP..EELVA
Mm_nSMase2	YIACTHLHAP	PEDSAVRCEQ	LDLLQDWLAD	FRKSTSSTST	ANP..EELVV
Rn_nSMase2	YIACTHLHAP	PEDSAIRCEQ	LDLLQDWLAD	FRKSTSSTST	ANP..EELVV
Pt_nSMase2	YIACTHLHAP	QEDSAIRCGQ	LDLLQDWLAD	FRKSTSSSSA	ANP..EELVA
Hs_nSMase2	YIACTHLHAP	QEDSAIRCGQ	LDLLQDWLAD	FRKSTSSSSA	ANP..EELVA
Cj_nSMase2	YIACTHLHAP	QEDSAIRCGQ	LDLLQDWLAD	FRKSTSSSSA	ANP..EELVA
Oc_nSMase2	YIACTHLHAP	PEDSAVRCEQ	LDLLQDWLAE	FRKSTSSSST	ANP..EELVA
Xl_nSMase2	YISCTHLHAL	AGDAVTRCEQ	LDMLQEWVSE	FRKSTSSSSA	ANP..EELVA
Gg_nSMase2	YISCTHLQAI	AGDTTVRCEQ	LDMLQDWLSE	FRKSTSSSST	ANP..EELVA
Dr_nSMase2	YITCTHLHAI	EGDAAVRCEQ	LDMLLEWGAE	FRRVTSCPAD	EEKVLEDQVA
Consensus	YI-CTHL-A-	--D---RC-Q	L--L--W---	FR--TS----	-----E--V-

	551				600
Cf_nSMase2	FDVICGDLNF	DNCSSDDKLE	QQHSLFTHYK	DPCRLGPGE	KSWAIGTLLD
Am_nSMase2	FDVICGDFNF	DNCSSDDKLE	QQHSLFTHYK	DPCRLGPGE	KSWVIGTLLD
Bt_nSMase2	FDIICGDFNF	DNCSSDDKLE	QQHSLFTRYK	DPCRLGPGE	KPWAIGTLLD
Mm_nSMase2	FDVICGDLNF	DNCSSDDKLE	QQHSLFTRYK	DPCRLGPGE	KPWAIGTLLD
Rn_nSMase2	FDVICGDLNF	DNCSSDDKLE	QQHSLFTRYK	DPCRLGPGE	KPWAIGTLLD
Pt_nSMase2	FDVVCGDFNF	DNCSSDDKLE	QQHSLFTHYR	DPCRLGPGE	KPWAIGTLLD
Hs_nSMase2	FDVVCGDFNF	DNCSSDDKLE	QQHSLFTHYR	DPCRLGPGE	KPWAIGTLLD
Cj_nSMase2	FDVVCGDFNF	DNCSSDDKLE	QQHSLFTHYR	DPCRLGPGE	KPWAIGTLLD
Oc_nSMase2	FDVVCGDLNF	DNCSSDDKLE	QQHALFTRYK	DPCRLGPGE	KPWAIGTLLD
Xl_nSMase2	FDVICGDLNF	DNCSSDDKLE	QQHSLFTHYK	DPCRLSPGEE	KPWAIGTLLD
Gg_nSMase2	FDVICGDLNF	DNCSSDDKLE	QQHSLFTHYK	DPCRLGPGE	KPWAIGTLLD
Dr_nSMase2	FDVILGDLNF	DNCSSDDKLE	QQHSLFTHYK	DPCRLGPGE	KPWALGTLYD
Consensus	FD---GD-NF	DNCSS--DKLE	QQH-LFT-Y-	DPCR--PGE-	K-W--GT-LD

Supplemental figure 4A (cont'd)

	601				650
Cf_nSMase2	TDNLYDEEVC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGAG	QKGRKDLLKG
Am_nSMase2	TDNLYDEEVC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGAG	QKGRKDLLKG
Bt_nSMase2	QDGLYDEEVC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGGG	QKGRKELLKG
Mm_nSMase2	TNGLYDEDVC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGAG	QKGRKDLLKG
Rn_nSMase2	INGLYDEDVC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGAG	QKGRKDLLKG
Pt_nSMase2	TNGLYDEDVC	TPDNLQKVLE	SEEGRREYLA	FPTSKS..SG	QKGRKELLKG
Hs_nSMase2	TNGLYDEDVC	TPDNLQKVLE	SEEGRREYLA	FPTSKS..SG	QKGRKELLKG
Cj_nSMase2	TNGLYDEDVC	TPDNLQKVLE	SEEGRREYLA	FPTSKS..SG	QKGRKELLKG
Oc_nSMase2	INGLYDEDAC	TPDNLQKVLE	SEEGRREYLA	FPTSKSPGGG	QKGRKDLLKG
Xl_nSMase2	PEGLYDEEVC	TPDNLQKVLE	NEEGRKEYIV	YPTSKNHCPN	QKGRKVP LKG
Gg_nSMase2	PEGLYDEEVF	TPDNLQKVLE	SEEGRKGYLV	YPTSKNHSSS	QKGRKASLKG
Dr_nSMase2	TSGIYDEEVS	SPESLQKVM	NEEGRKEYLV	FPTSKNHCPN	QKGRKIPLKG
Consensus	----YDE---	-P--LQKV-E	-EEGR--Y--	-PTSK-----	QKGRK--LKG

	651				700
Cf_nSMase2	NGRRIDYILY	AEEGLWPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSAG
Am_nSMase2	NGRRIDYMLY	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSTG
Bt_nSMase2	NGRRIDYMLH	GEEGLYPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSAG
Mm_nSMase2	NGRRIDYMLH	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSAG
Rn_nSMase2	NGRRIDYMLH	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSAG
Pt_nSMase2	NGRRIDYMLH	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSSG
Hs_nSMase2	NGRRIDYMLH	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSSG
Cj_nSMase2	NGRRIDYMLH	AEEGLCPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSSG
Oc_nSMase2	SGRRIDYMLH	AEDGLGPDWK	AEVEEFSFIT	QLSGLTDHLP	VAMRLMVSSG
Xl_nSMase2	SGRRVDYMLY	TEEGLNMEWK	VDVEEYSFIT	QLAGLTDHVP	VAFRLTVSTG
Gg_nSMase2	NGRRIDYMLY	TEEGLYLEWK	VEVEEFSFIT	QLAGLTDHLP	VAMRLTVSTG
Dr_nSMase2	NGRRIDYILY	KEEGLHQDWK	VDIEEFSFIT	QLAGLTDHLS	VAARLVVSTG
Consensus	-GRR-DY-L-	-E-GL---WK	---EE-SFIT	QL-GLTDH--	VA-RL-VS-G

	701
Cf_nSMase2	EEEEA
Am_nSMase2	EEEEA
Bt_nSMase2	DDEA
Mm_nSMase2	EEEEA
Rn_nSMase2	EEEEA
Pt_nSMase2	EEEEA
Hs_nSMase2	EEEEA
Cj_nSMase2	EEEEA
Oc_nSMase2	EEEEA
Xl_nSMase2	EEES
Gg_nSMase2	EDDP
Dr_nSMase2	EEET
Consensus	----

Supplemental figure 4A (cont'd)

