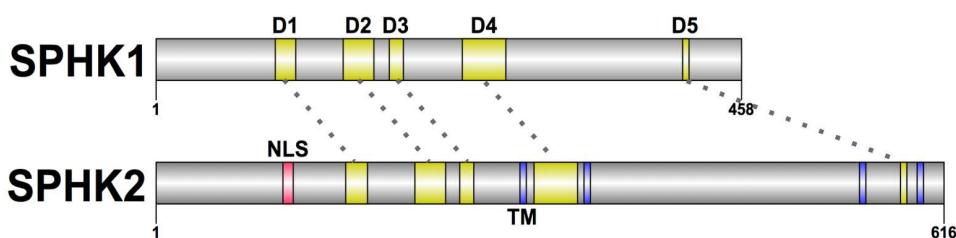


AP-SPHK (VWA) ATGGCGAACTGTGCTTCAACCTGCTTCAGCTCCGAGTAAAGTTATTCAAGTTGGCTTGACGGAGAAACTTTACAGCACGTCATTGATTCTGCTGASAACACTCASAACATCCCATAAATTCGAAGATCTT
 AIPGENE12769 ATGGCTGAAGCTGTGCTTCAAAAGTTGTTCACTTACCGAGTAAATCGTTTGAAGTTCGGTTGACGGAGAAACTTTATCAAAGACATCCATTGATTCTGCTGCAATACATCAAGTCCTCCTTTTCGTTAATCTGGAAGATCTT
 AIPGENE12827 ATGGCTGAAGCTGTGCTTCAAAAGTTGTTCACTTACCGAGTAAATCGTTTGAAGTTCGGTTGACGGAGAAACTTTATCAAAGACATCCATTGATTCTGCTGCAATACATCAAGTCCTCCTTTTCGTTAATCTGGAAGATCTT
 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330
 AP-SPHK (VWA) ATGGTGAAAAGTGTTCAGGAGTACATCAGAAATAATATAATTGACTTGCATATTTCAGTGTCTGGAGAAAAGAACAGATCGAGGAGAAATTCCGGTCAAAGTCGTGGTTGGGAAGATGCGATGCGGCCGAGAATGGCT
 AIPGENE12769 ACGGCTAAACTCTTCAGACGAATTGGAGCCGATTTCATGCTATTGGATTTCACTGCCTCCGTCAGGAAAGAACGCAATCGAAAGAACAAATTCCGGTAAAGTCCTGGATGGGAAGATTAAGCGAAATTCAGAACTGCCGAGCATGGCT
 AIPGENE12827 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490
 AP-SPHK (VWA) GAAGATGATTCTATGGCTTGCAAGAGTTGAGCTTGGAGTGTGAGAAATTAAAGACAGCAAACGCCACCCAGCGTAAGAAATTTGTGTTAAGTCAGGAAAGATGGTCAACCCCTCAGTGGTTCAAGGAAAGAGTTGCAATCTTAA
 AIPGENE12769 TCAGGAAAGAGTTGCAATCTTAAAGAACGCTTAAAGACGATGTTGAGCTTGGTCAACCCCTCAGTGGTTCAAGGAAAGAGTTGCAATCTTAAAGAACGCTTAAAGACGATGTTGAGCTTGGTCAACCCCTCAGTGGTTCAAGGAAAGAGTTGCAATCTTAA
 AIPGENE12827 GAAGATGATTCTATGGCTTGCAAGAGTTGAGCTTGGAGTGTGAGAAATTAAAGACAGCAAACGCCACCCAGCGTAAGAAATTTGTGTTAAGTCAGGAAAGATGGTCAACCCCTCAGTGGTTCAAGGAAAGAGTTGCAATCTTAA
 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660
 AP-SPHK (VWA) GCTTCATAGACTACATTGGCTTACAGAGTATGGCTGGTCATGCAAGAGAATTGGCATGGAGCTTAAATTGAGGAATTGGATGGTCTTGTGATTGGATGGTCTGGAGCTTAAATTGAGGAATTGGATGGPCTTGTGATTGGATGGTCTGGAGCTTAA
 AIPGENE12769 GCTTCATAGACTACATTGGCTTACAGAGTATGGCTGGTCATGCAAGAGAATTGGCATGGAGCTTAAATTGAGGAATTGGATGGPCTTGTGATTGGATGGTCTGGAGCTTAAATTGAGGAATTGGATGGGCTTGTGATTGGACTTGGAGCTTAA
 AIPGENE12827 GCACTGTAGACTATACCTTAATTGTCACAGAAATATGGCTGGTCATGCAAGAGAATTGGCATGGAGCTTAAATTGAGGAATTGGATGGPCTTGTGATTGGATGGGCTTGTGATTGGACTTGGAGCTTAAATTGAGGAATTGGATGGGCTTGTGATTGGAGCTTAA
 670 680 690 700 710 720 730 740 750 760 770 780 790 800 810 820 830
 AP-SPHK (VWA) CAATGACACTACCTATGGGTGATCCCTACTGGCTCAGGGAAATTGCTTGTGTTATTGCGACATATATGCACTGGGGAGCATTGGTCACTGCTGCAGTCTTGTGAGTGGTCTGGGATCTGCGCCATTGAAACACCAAGGCC
 AIPGENE12769 CAATGACACTACCTATGGGTGATCCCTACTGGCTCAGGGAAATTGCTTGTGTTATTGCGACATATATGCACTGGGGAGCATTGGTCACTGCTGCAGTCTTGTGAGTGGTCTGGGATCTGCGCCATTGAAACACCAAGGCC
 AIPGENE12827 CCATACCTACCTCTGGTGTACTCCCTACTGGCTCTGGAAATTGCGCTGTGTTATTGCACTGGGGAGCATTGGTCACTGCTGCAGTCTTGTGAGTGGTCTGGGATCTGCGCCATTGAAACACCAAGGCC
 840 850 860 870 880 890 900 910 920 930 940 950 960 970 980 990 990
 AP-SPHK (VWA) AAAACTCTTCCTTTCTATCTTCTATCTGGGGCATGATATCACTGGTGTATGATGGAAACTACAGACGCCCTGGGAAATGCTAGGTTTACTTGGGGGCCATTATCAGAACCTCTTAACTTCGCTACTTACAGAGGAAACTTCATACTCCACCTGTAT
 AIPGENE12769 AAAACTCTTCCTTTCTCTCTTCTATCTGGGGCATGATATCACTGGTGTATGATGGAAACTACAGACGCCCTGGGAAATGCTAGGTTTACTTGGGGGCCATTATCAGAACCTCTTAACTTCGCTACTTACAGAGGAAACTTCATACTCCACCTGTAT
 AIPGENE12827 AAAATTATCTCTTCTGCTCTGGGGATATTCCTGATGGTGTATGATGGAAACTACAGACGCCCTGGGAAATGCTAGGTTTACTTGGGGGCCATTATCAGAACCTCTTAACTTCGCTACTTACAGAGGAAACTTCATACTCCACCTGTAT
 1,000 1,010 1,020 1,030 1,040 1,050 1,060 1,070 1,080 1,090 1,100 1,110 1,120 1,130 1,140 1,150 1,160
 AP-SPHK (VWA) GAAAGCTG-----BACATCCTACCAAAAGTPTAGATCTGCCAAGGGAAACCAAGAATGAGACGATGATTTAGCAAATTTTATTCTCTACCAGGAAGTCTAGTGGCTGCTTTATTAAC-----ACAAATCTGCAACAGAA
 AIPGENE12769 GAAAGCTG-----BACATCCTACCAAAAGTPTAGATCTGCCAAGGGAAACCAAGAATGAGACGATGATTTAGCAAATTTTATTCTCTACCAGGAAGTCTAGTGGCTGCTTTATTAAC-----ACAAATCTGCAACAGAA
 AIPGENE12827 GATG-----BAAATGATGATTACAGTGTGATGACAGTCATCATCCACRTAAATAGATTTACCAAAACAA-----CAGACTTAAAGGTTTATTCTTCTTAAATGCTTCAAGTGGATTAACATGAAATGAACTGATGAA
 1,170 1,180 1,190 1,200 1,210 1,220 1,230 1,240 1,250 1,260 1,270 1,280 1,290 1,300 1,310 1,320
 AP-SPHK (VWA) AACACCTAAAATTAAT-----TCAAGCCATGAGAAATTGGCATGCTAACAGTGGCATCCATCACTCAATGATATACTCAACAACTGTTAGGAAC-----ACAGAGTACCTCTATGCTCATAGAACG-CATTGCACTCAAACTGGAAAGACAA
 AIPGENE12769 AACACCTAAAATTAAT-----TCAAGCCATGAGAAATTGGCATGCTAACAGTGGCATCCATCACTCAATGATATACTCAACAACTGTTAGGAAC-----ACAGAGTACCTCTATGCTCATAGAACG-CATTGCACTCAAACTGGAAAGACAA
 AIPGENE12827 AGCTCGGGAGTATTGCTSGATTGGAAATGAGGTTATG-----TAAAGCCATTACATCTCACTGATGATATTAATGCAACRARAATATCT-----CCCTCATTAACAAACRAACTGCTCTCAAACACTGGGATCTCAAACACTGGAAAGACTGTC
 1,330 1,340 1,350 1,360 1,370 1,380 1,390 1,400 1,410 1,420 1,430 1,440 1,450 1,460 1,470 1,480 1,490
 AP-SPHK (VWA) GAAAGTGAATTCTAAGCTCTGGCTCTATCTCACATGTTGATGTTATTGCACTGTTGCACTGGCAAGGGAAATTGGCGATGGTTTTTGTACATGGCATACATGCTAACAGTCTGGTGTCTAACACGATGAGATTGCTACATTGAA
 AIPGENE12769 GAAAGTGAATTCTAAGCTCTGGCTCTATCTCACATGTTGATGTTATTGCACTGTTGCACTGGCAAGGGAAATTGGCGATGGTTTTGACATGGCATACATGCTAACAGTCTGGTGTCTAACACGATGAGATTGCTACATTGAA
 AIPGENE12827 GAGGGTGTATTGCTCTGGTCTGGCTCACATGCACTTCTCATTGGGACCAAGATCTTCTCTTCTTCCATCACATGCACTGGCAATGGGGATGGTCTTCAAGGAGAGATCTCATCACATGCACTGGATCAAGGAGATTCTCATCACATGCA
 1,500 1,510 1,520 1,530 1,540 1,550 1,560 1,570 1,580 1,590 1,600 1,610 1,620 1,630 1,640 1,650 1,660
 AP-SPHK (VWA) GCATCAAGTCACAGCAGCTTTTATCTAAAGCGTGTGCAATTAGATTAGAGCCAGATCTCTCACACAGGGCAACATGGCTGTGGATGGAGAAAGACTTCCAATTCTGAGTGGTCAAGGAGAGGTGATAAGGGGTTGGACGAGTAATGTTGGCAG
 AIPGENE12769 GCATCAAGTCACAGCAGCTTTTATCTAAAGCGTGTGCAATTAGATTAGAGCCAGATCTCTCACACAGGGCAACATGGCTGTGGATGGAGAAAGACTTCCAATTCTGAGTGGTCAAGGAGAGGTGATAAGGGGTTGGACGAGTAATGTTGGCAG
 AIPGENE12827 GAGGTCTTGGATTCACAGAATGTTTACCTTAAGGCAACGGCTAACACCTGAGCTAGAACCTGAYCTTCCACACAGGGCAACATGGCAATTAGATGGAGAAAGACTTCCAATTCTGAGTGGAGAGATCTCATCACATGCACTGGATCAAGGAGCTTGGACGAGTAATGTTGGCAG
 1,670 1,680 1,690 1,700 1,710 1,720 1,730 1,740 1,750 1,760 1,770 1,780 1,790 1,800 1,810 1,820
 AP-SPHK (VWA) ACCCT-----ACAGTTTTAAA-----CTGCACAGAGCTGGATAGTA-----ATATTGTTAGAAATA-----GCCCTCTTCTTTC
 AIPGENE12769 ACCCT-----ACAGTTTTGAA-----CTGCACAGAGCTGGATAGTA-----ATATTGTTAGAAATA-----GCCCTCTTCTTTC
 AIPGENE12827 ACCCTATTGAGWTGACAAATTAGATGAGAAATTCTACAGCTTACAGCTTACAGCTTACATCAACAGAACTGCAACGAGGCCGCTCTGCTCTGATCTTCTTGT
 1,830 1,840 1,850 1,860 1,870 1,880 1,890 1,900 1,910 1,920 1,930 1,940 1,950 1,960 1,970 1,980 1,990
 AP-SPHK (VWA) -----CAACTTGAATTC-----AACCTTACACATTTCTTGGAAAGGAG-----GAAAAA
 AIPGENE12769 -----TAACCTCATTC-----AACCTTACACATTTCTTGGAAAGGAG-----GAAAAA
 AIPGENE12827 TGTGACTATTGATGCGTAATTCGCAAGTCAGCTTTAAATGAAATTTCCTAAACATGATATTATAATAACAGGACACATTATGTTATTGAAACATATTAGATAATTGAAATTTCTTAAAGAATGTAATATAGGGTTTTGAAAAA
 2,000 2,010 2,020 2,030 2,040 2,050 2,060 2,070 2,080 2,090 2,100 2,110 2,120 2,130 2,140 2,150
 AP-SPHK (VWA) C-----TCAAGGAGCTGA-----AGACAAAGTCTAGACTATAGATCATT-----GCCTTCA-----TCCCTG-----ATAGTTTTCTGAGCAGCTAAATGAAAGGGAGATGGAA
 AIPGENE12769 C-----TCAAGGAGCTGA-----AGACAAAGTCTAGACTATAGATCATT-----GCCTTCA-----TCCCTG-----ATAGTTTTCTGAGCAGCTAAATGAAAGGGAGATGGAA
 AIPGENE12827 CGCTTCTGACAAGGAGGGCATTTGACACAGYTAAGTGTATAAGGACCATTCAGTTGAGATTAAAGACAAATATGCAACGATAACCGAGGAGCCATCCTGCRCTAACCTGCTCTGATCTTCTTGT
 2,160 2,170 2,180 2,190 2,200 2,210 2,220 2,230 2,240 2,250 2,260 2,270 2,280 2,290 2,300 2,310 2,318
 AP-SPHK (VWA) AAGCGCTTCA-----GGCTGAAGTCAAAGAAAGTCAGA-GGGCTGTAC-----TTTTCCTTCCCCATTATTT-----ATTGTTGTCATTGAG-----ATCAGAAATGCTPTCCGCAGCTGAGCAAA-----AAA
 AIPGENE12769 AAGCGCTTCA-----GGCTGAAGTCAAAGAAAGTCAAATGGCTATAC-----TTTTCCTTCCCCATTATTT-----ATTGTTGTCATTGAG-----ATCAGAAATGCTPTCCGCAGCTGAGCAAACTAAAAGAAA
 AIPGENE12827 AAGCGCTTCAACGAAGGGAGACACAAGAAGAATAGTCCTCTATTCTATATGAAAATAATAATTGTTTTGTTGTATTGTTCAATTATTCAAAAGAGTCATTTTTATTTTAATCAAA-----CTATTGATGCCATCAA-----AAA

Figure S1. Predicted AP-SPHKs from *A. pallida* nucleotide sequence alignment. Nucleotide sequences were aligned using MUSCLE (Edgar, 2004) in Geneious v8.0.3 (Kearse et al., 2012). Sequence similarity was determined using a Blosum62 score matrix (Henikoff and Henikoff, 1992) where > 60% is white, 60-80% is light grey, 80 to 99% is dark grey, and 100% is black.

A



B

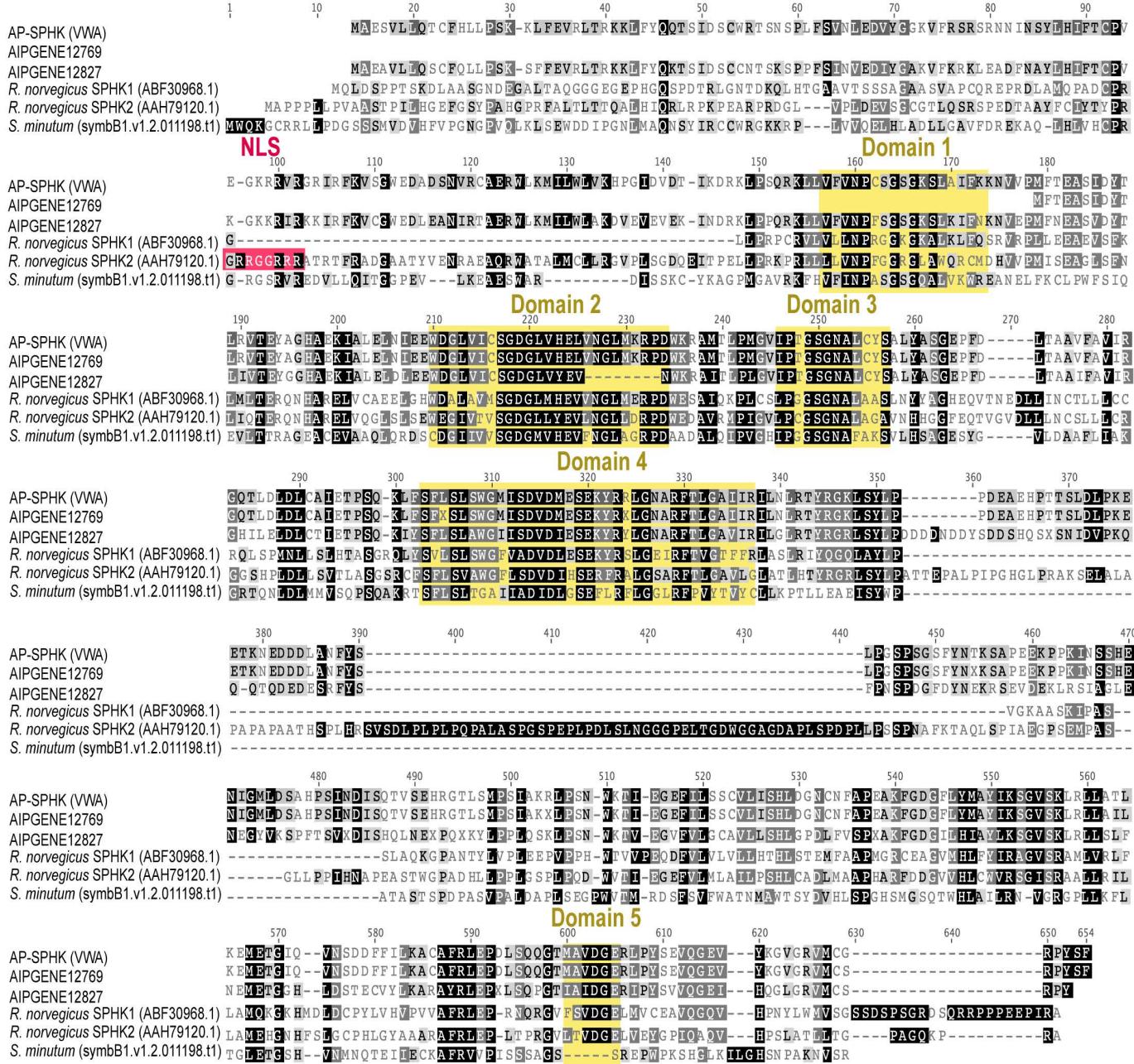


Figure S2. Predicted AP-SPHKs protein sequences from *A. pallida* aligned to characterized *R. norvegicus* SPHKs. Representative *R. norvegicus* SPHK1 (NCBI: ABF30968.1) and SPHK2 (NCBI: AAH79120.1) were compared to the two predicted AP-SPHKs from the *A. pallida* genome. (A) Visualization of protein structure between *R. norvegicus* SPHK1 and SPHK2 was made using Domain Graph (DOG) v2.0 (Ren et al., 2009). Conserved protein domains (D1-5, yellow) the nuclear localization sequence (NLS, red) and transmembrane segments (TM, blue) are highlighted. (B) Multiple sequence alignment of isoenzymes SPHK1 and SPHK2 with predicted AP-SPHK, *A. pallida* and *S. minutum* genome homologs was produced using MUSCLE in Geneious v8.0.3. Sequence similarity was determined using a Blosum62 score matrix (Henikoff and Henikoff, 1992) described in Fig S2.

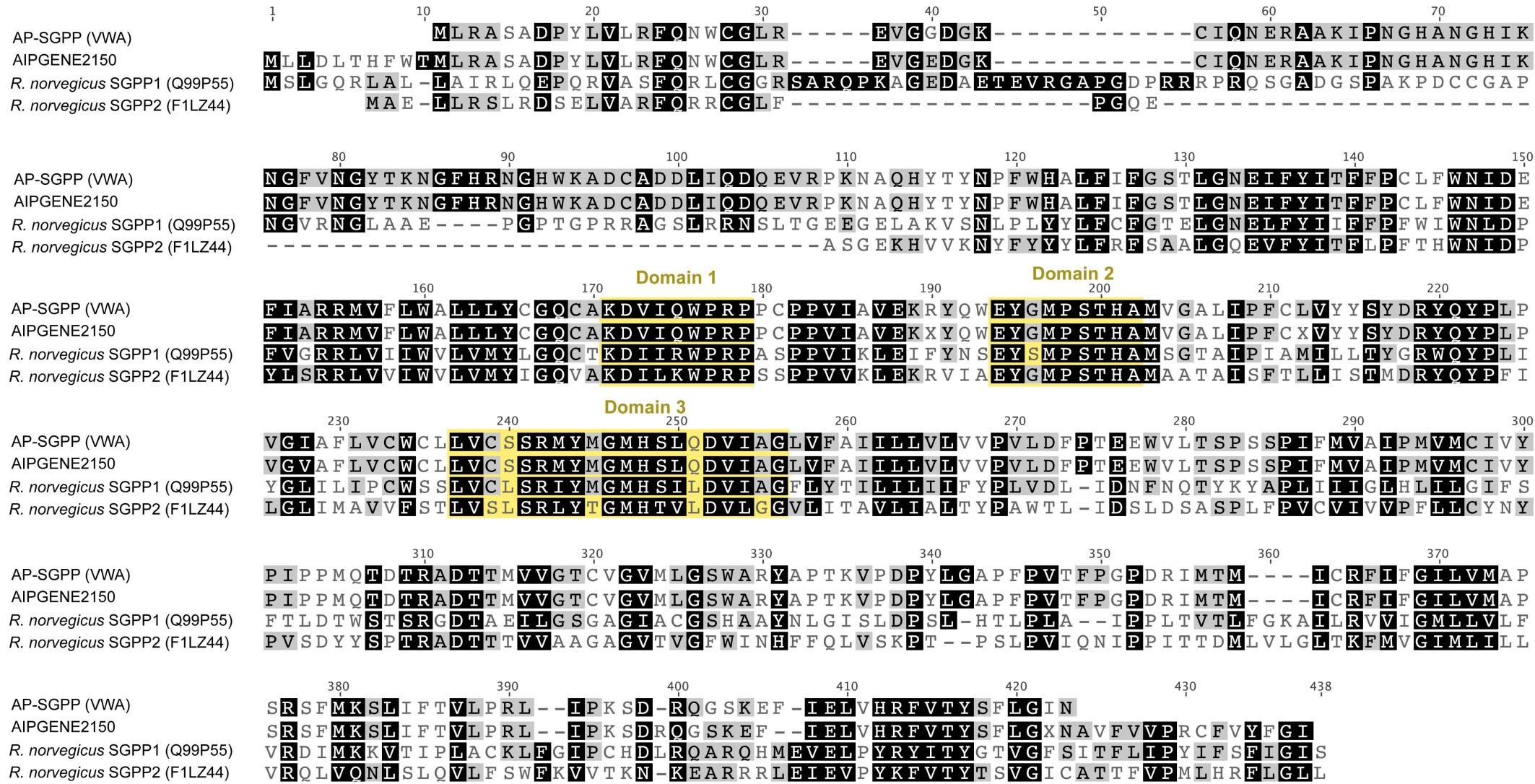


Figure S3. Putative AP-SGPP from *A. pallida* protein sequence alignment. Representative SGPP1 and SGPP2 from *R. norvegicus* were aligned with AP-SGPP using MUSCLE in Geneious v8.0.3. Conserved domains (D1-3) are highlighted yellow. Sequence similarity was determined using a Blosum62 score matrix as described in Fig. S2 (Henikoff and Henikoff, 1992).

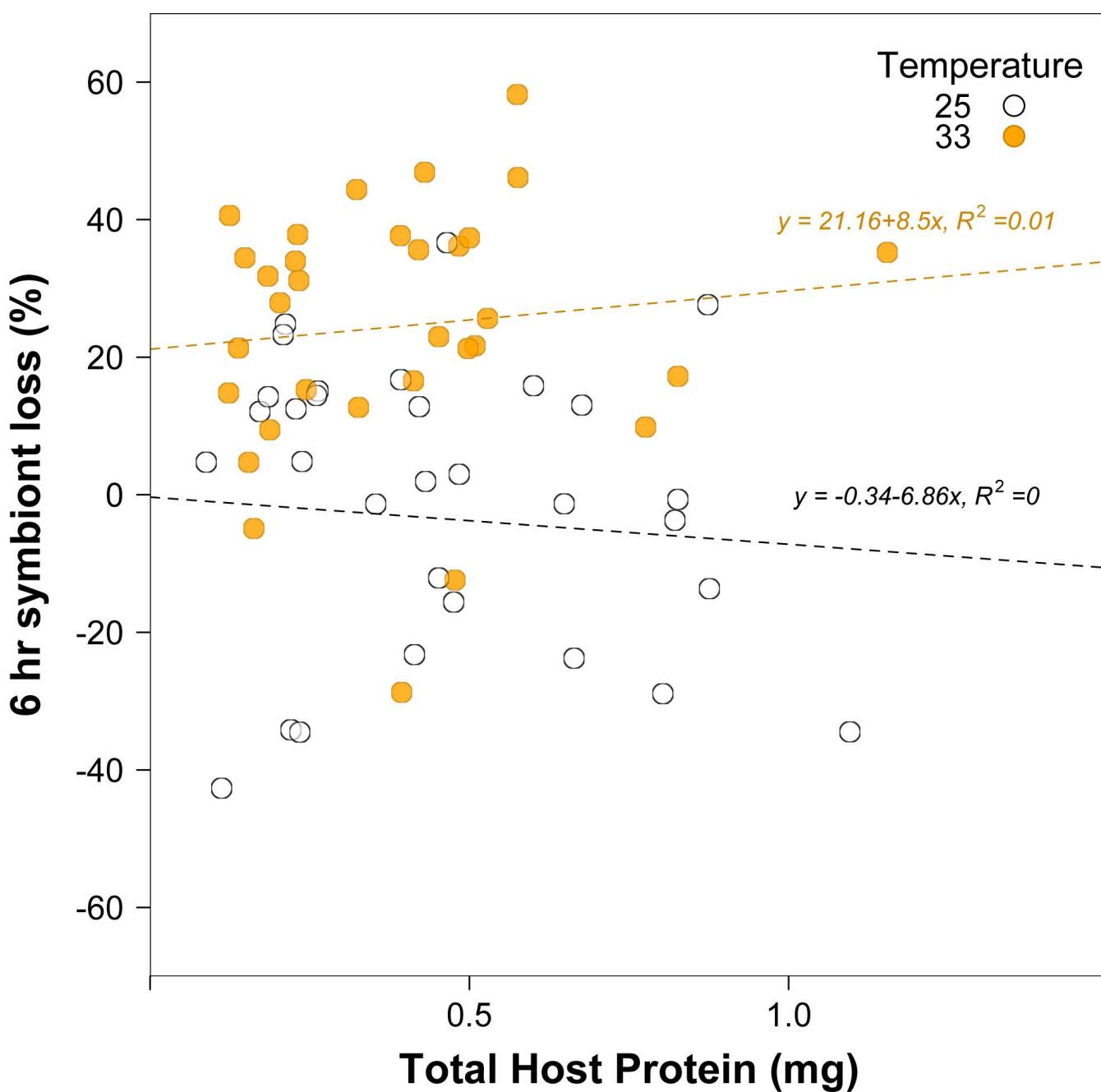


Figure S4. Symbiont loss by 6 h is not correlated with anemone size. The percentage of symbiont loss at 6 h was compared to total anemone protein, a proxy for size, for the two temperatures. Positive values indicate lower autofluorescence at day 0.25. The correlation of symbiont loss to anemone size was tested using Pearson's correlation test. Treatment groups: ambient = ○, and 33 °C = ●.

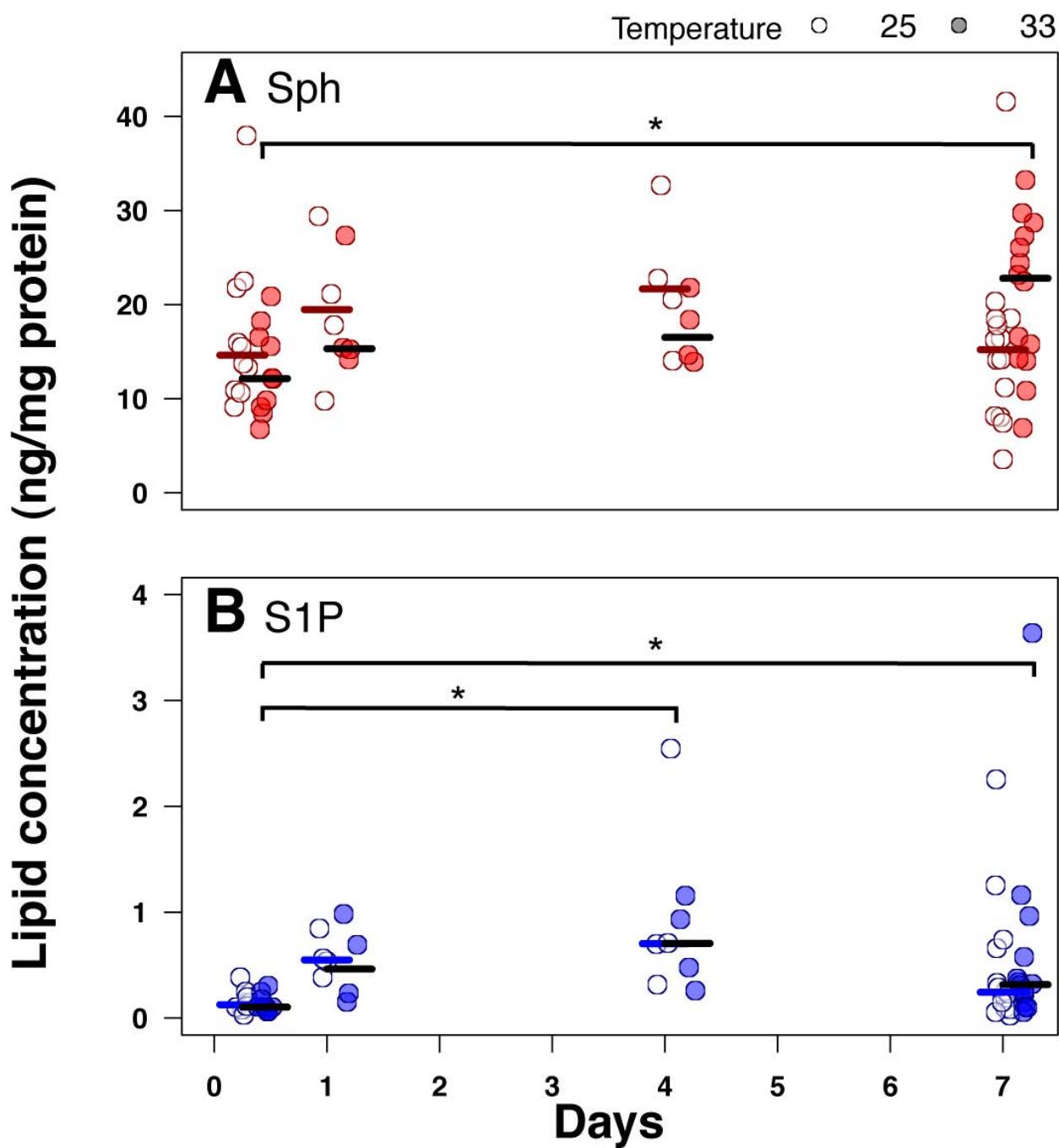


Figure S5. Sph and S1P cellular concentrations at ambient and hyperthermal temperatures. Sphingolipid concentrations of Sph (A, red) and S1P (B, blue) from anemones incubated ambient and hyperthermal temperatures used to calculate the fold change. Concentrations were compared using a non-parametric Kruskal Wallis test. Significance with the Dunn's *post hoc* test is indicated by an asterisk above the bar connecting the two statistically different groups (Benjamini-Hochberg adjusted $p \leq 0.05$). Bars show the median fold change for each metabolite at the separate days. The 33 °C treatment group days were shifted by 0.2 d to improve visualization from the ambient treatment group. Treatment groups: ambient = ○, and 33 °C = ●.

Supplemental Tables:

Table S1. Stability values of reference genes tested for elevated temperature (33 °C) experiment in GeNorm (Vandesompele et al., 2002) and NormFinder (Andersen et al., 2004).

Gene	GeNorm (M)	NormFinder
L10	1.013	0.180
PABP	1.000	0.202
GAPDH	1.202	0.335
L12	1.387	0.580
EEF1A1	2.059	0.752

Table S2. Predicted SPHK homologs identified from publically available cnidarian genome or transcriptomes using *R. norvegicus* SPHK1 (NCBI: ABF30968) as the query.

Class	Species	Accession ID	E value	Resource
Anthozoa	<i>Acropora digitifera</i>	aug_v2a.11507.t1	3e-29	(Shinzato et al., 2011)
	<i>Aiptasia pallida</i>	JV079020.1	3e-43	(Lehnert et al., 2012)
	<i>Anthopleura elegantissima</i>	comp146758_c0_seq1	4e-48	(Kitchen et al., 2015)
	<i>Fungia scutaria</i>	comp154870_c0_seq1	4e-47	(Kitchen et al., 2015)
	<i>Montastrea cavernosa</i>	comp92849_c0_seq1	3e-66	(Kitchen et al., 2015)
	<i>Nematostella vectensis</i>	XP_001632121.1	8e-82	(Putnam et al., 2007)
	<i>Porites australiensis</i>	FX463324.1	1e-58	(Shinzato et al., 2014)
	<i>Seriatopora hystrix</i>	comp210758_c0_seq1	3e-48	(Kitchen et al., 2015)
	<i>Stylophora pistillata</i>	GARY01028221.1	1e-62	(Liew et al., 2014)
Hydrozoa	<i>Hydra vulgaris</i>	GAOL01020445	5e-48	(Juliano et al., 2014)
	<i>Hydractinia symbiolongicarpus</i>	GAWH01039148	2e-51	(Sanders et al., 2014)
Myxozoa	<i>Polypodium hydriforme</i>	GBGH01016640	3e-22	(Shpirer et al., 2014)
	<i>Thelohanellus kitauei</i>	KII70896.1	4e-16	(Yang et al., 2014)
Scyphozoa	<i>Aurelia aurita</i>	GBRG01155343	8e-37	(Brekman et al., 2015)