AF Peptide	Amino Acid Sequence	Transcript				
AF1	KNEFIRFa	afp-7				
AF2	KHEYLRFa	afp-4				
AF3	AVPGVLRFa	afp-1				
AF4	GDVPGVLRFa	afp-1				
AF5	SGKPTFIRFa	afp-2				
AF6	FIRFa					
AF7	AGPRFIRFa					
AF8	KSAYMRFa	afp-3				
AF9	GLGPRPLRFa					
AF10	GFGDEMSMPGVLRFa	afp-1				
AF11	SDIGISEPNFLRFa	afp-11				
AF13	SDMPGVLRFa	afp-1				
AF14	SMPGVLRFa	afp-1				
AF15	AQTFVRFa	afp-9				
AF16	ILMRFa	afp-10				
AF17	DFDRDFMHFa	afp-5				
AF18	NKFFLRKP					
AF19	AEGLSSPLIRFa	afp-13				
AF20	GMPGVLRFa	afp-1				
AF21	AMRNALVRFa	afp-6				
AF22	NGAPQPFVRFa	afp-6				
AF23	SGMRNALVRFa	afp-6				
AF24	RNKFEFIRFa	afp-8				
AF25	NNFLRFa	afp-11				
AF26	KPNFLRFa	afp-11				
AF27	PADPNFLRFa	afp-11				
AF28	SAEPNFLRFa	afp-11				
AF29	NAEPNFLRFa	afp-11				
AF30	APNKILMRFa	afp-10				
AF31	TPSNNFLRFa	afp-11				
AF32	GSDPNFLRFa	afp-11				
AF33	SNQAQNFLRFa	afp-11				
AF34	DSKLMDPLIRFa	afp-13				
AF35	DPQQRIVTDETVLRFa	afp-13				
AF36	VPSAADMMIRFa	afp-12				
AF37	FRGEPIRFa					
AF38	AQREPIRFa					
AF39	SPKQKFIRFa					
AF41	KPNFIRFa	afp-11				

Supplementary Table S1. *Ascaris* FMRFamide-like peptides (AF peptides). When known, peptide transcripts are given in the 3rd column. (*1-11*)

	AVK1	AVK2	АVКЗ	AVK4	AVK5	AVK+a1	AVK+b1	AVK+b2	AVK+b3	AVK+b4	AVK+b5	AVK+b6
	450.4					450.2						
	462.1											
	466.0	465.8				465.9	466.0	466.0	465.4		465.5	466.0
										467.4		
										473.2	473.0	473.3
									473.9		474.1	474.2
										475.1	475.2	475.2
										476.2		
							477.0					
	479.0		478.9									
	480.2		480.5			480.0		480.2				
		481.2	481.2		481.2			481.3	481.3	481.3	481.3	481.3
	482.0					482.0						
	483.1					483.0	483.1	483.1				
			484.9									485.0
									491.4		491.4	
			494.5									
									502.9		502.9	503.0
									504.9		504.9	505.0
						506.0	506.2	506.2				
										507.2		
							520.2	520.2				
			522.5	522.5					522.1		522.1	522.1
			523.0									523.1
	524.2	524.1				524.0	524.2	524.2			524.1	524.2
	526.2					526.1	526.2	526.2	500.0	500.0	500.0	
									530.2	530.2	530.2	
									534.7	500.1	534.8	500.0
									536.7	536.1	536.1	536.2
			E 40.4	E 40 4						038.1	538.1	538.2
			542.4	542.4				542.0				
	550.7		550.6	550.6		550.0	550.7	550.7				
	000.7		556.4	000.0		- 550.0	- 550.7					
	568.2	568.1	568.1			568.1	568.2	568.2	568.1			568.2
	000.2	000.1	570.4			000.1	000.2	000.2	000.1			000.2
			570.4								572.2	
	580.4										01 2.2	
	000.4	582.3										
		002.0										586.1
							587.2	587.2				000.1
			590.5				007.2	007.2				
			604.6					604 1				
	606.1		001.0			606.0		501.1				
			610.5	610.5								

			612.5					612.3				
	617.1						617.1	617.1				
	618.1											
	622.1	622.0	622.0			622.0	622.1	622.1				622.1
						628.0						
	630.1	630.0	630.0			630.0		630.1	630.1		630.1	630.1
		632.0	632.0			632.0			632.1		632.1	632.1
			638.6									
			640.5	640.5								
	644.1					644.0						
	647.9								648.0		648.0	648.1
	01110								650.0		650.0	650.1
									654.2		654.2	656.1
			CEC O						656.0		004.0	660.2
			0.000						606.2			660.3
	660.0									660.2	660.2	666.1
			666.6									
			668.6	668.6								
								676.1				676.1
							678.6				678.5	678.5
							682.1					
									692.0		692.0	692.1
			693.9						694.0		694.0	694.1
	695.4	695.4	695.4	695.4	695.3	695.4			695.4	695.4	695.4	695.5
					696.3					696.4		696.1
							698.1					
								706.6				
												746.0
												740.0
							7047					740.0
							784.7			700.0	700.0	
							/86./			786.6	786.6	
							788.7			788.6	788.6	
	792.5	792.4			792.4	792.4				792.4		
AF25	809.5	809.4	809.4	809.4	809.4	809.4	809.6	809.6	809.4	809.4	809.5	809.5
								810.5				
									817.8			
AF25Na		831.4	831.4			831.4			831.4			831.5
	833.1					833.1						
					839.4					839.5		
	844.1											
						851.4						
	855,1					855.0						
	856.1											
	857.4											
	960.1											
	000.1											961.0
		0000				000						001.2
		863.3				863.4						

	1133.6	1133.5	1137.5			1133.6		1133.7		1133.5		
					1132.5		1132.7					
	1129.6	1129.5				1129.5		1129.7				
AF29Na		1128.6	1128.6	1128.6	1128.6	1128.5			1128.5		1128.6	1128.7
						1124.4						
					1121.6	1121.6						
											1118.6	
	1117.6				1117.5	1117.6	1117.7					
AF31Na		1116.6	1116.6	1116.6	1116.6				1116.5		1116.6	1116.7
AF32Cu		1113.5			1113.5	1113.5				1113.4	1113.5	
AF29	1106.6	1106.6	1106.6	1106.6	1106.6	1106.6	1106.7	1106.7	1106.6	1106.6	1106.6	1106.7
AF28Na			1101.6	1101.6					1101.5			
			1097.6									
AF31	1094.6	1094.6	1094.6	1094.6	1094.6	1094.6	1094.7	1094.7	1094.6	1094.6	1094.6	1094.7
PepNY	1086.5	1086.5	1086.5	1086.5	1086.5	1086.5	1086.6	1086.6	1086.4	1086.4	1086.5	1086.5
AF28	1079.6	1079.6	1079.6	1079.6	1079.6	1079.6	1079.7	1079.7	1079.5	1079.5	1079.6	1079.7
AF27	1075.6	1075.6	1075.6	1075.6	1075.6	1075.6	1075.7	1075.7	1075.6	1075.5	1075.6	1075.7
	1068.5	1068.5			1068.4	1068.5			1068.4	1068.4		
					1063.6							
	1060.1											
	1056.5				1056.5	1056.5				1056.5	1056.5	
AF32	1051.6	1051.5	1051.5	1051.6	1051.5	1051.5	1051.7	1051.7	1051.5	1051.5	1051.5	1051.6
											1046.4	
	1044.1											
					1024.6	1024.6				1024.5		1024,7
						1004.6						
AF2	991.6	991.6	991.6	L	991.5	991.6		991.7	00110	991.5	00110	991.6
		984.5	984.5		984.5	984.5			984.5	984.5	984.5	984.6
AF26/41Cu	982.5	982.5	982.5		982.5	982.5		5/0./	982.5	982.5	982.5	982.6
	974.5	9/4.5			974.5	974.5		974.6		979.6		9/4.0
	074 5	074 5			962.5	962.6		074.6				074.6
	958.5	958.5			958.5	958.5	958.7					
AF26/41Na	942.6	942.5	942.5	942.5	942.5	942.5	942.7		942.5	942.5	942.5	942.6
AF26/41	920.6	920.5	920.6	920.6	920.5	920.5	920.7	920.7	920.5	920.5	920.6	920.6
					913.5	913.5						
	905.7	905.5			905.5	905.5				905.7		
		902.5	902.5		902.5	902.5			902.5		902.5	
					898.5							
AF31 ⁻	896.5	896.5	896.5	896.5	896.5	896.5		896.6	896.5	896.5	896.5	896.6
							893.2					
			877.0									877.1
	873.1	873.3							873.3	873.4		
						872.5					873.4	
AF25Cu	871.1	871.4	871.4			871.3			871.4	871.4	871.4	871.5
								867.6		867.4		
								865.2				

						1136.6						
AF27Cu		1137.5						1137.7		1137.5	1137.5	1137.6
		1139.5										
		1140.4				1140.4		1140.5				
AF28Cu		1141.5				1141.5				1141.5	1141.5	1141.6
										1143.5	1143.5	
	1144.6	1144.5			1144.5	1144.6						
PepNYCu		1148.4				1148.6					1148.4	
								1152.8		1152.6		
					1155.6							
AF31Cu		1156.5	1156.5			1156.5			1156.5	1156.5	1156.5	1156.6
	1157.0											
		1158.5							1158.5	1158.5	1158.5	
			1159.5									
		1160.5				1160.5		1160.7				1160.6
		1162.1				1162.6						
								1164.8		1164.6		
AF29Cu		1168.5			1168.5	1168.5				1168.5	1168.5	1168.6
		1170.5								1170.5	1170.5	
						1171.5						
						1175.6						
					1183.6							
	1206.7	1206.6			1206.6	1206.6				1206.6		
					1210.6							
AF33	1223.7	1223.6	1223.7	1223.7	1223.6	1223.7	1223.8	1223.8	1223.6	1223.6	1223.6	1223.7
											1235.6	
AF33Na	1245.7	1245.6	1245.6	1245.6	1245.6	1245.6			1245.6	1245.6	1245.6	1245.7
	1261.7				1261.6		1261.8			1261.6		
					1265.6	1265.7						
						1277.6						
										1280.6		
								1281.8				
AF33Cu		1285.6	1285.6			1285.6			1285.5	1285.5	1285.5	1285.7
		1287.6								1287.5	1287.5	
	1300.1											
					1327.7	1327.7				1327.6		
AF11I3V						1479.8						
AF11	1493.8	1493.8	1493.8	1493.8	1493.8	1493.8	1494.0	1494.0	1493.8	1493.7	1493.8	1493.9
AF11I3VNa						1501.8						
										1505.7		
AF11Na		1515.8	1515.8	1515.8	1515.8					1515.7		
						1517.8						
						1521.8						
		1531.7			1531.8	1531.8						
						1535.8						

		1547.7								
							1552.0			
AF11Cu		1555.7						1555.7		1555.8
				1597.8						
AF32*	2092.4	2092.2	2092.2	2092.2	2092.2	2092.3	2092.3	2092.2	2092.2	2092.4
					2134.2					
AF26*	2144.3	2144.1		2144.1	2144.1			2144.1		
								2226.3		
				2289.2				2289.2		
		2303.2								
	2305.5	2305.3		2305.2	2305.3			2305.3		
	2321.5	2321.2	2321.2	2321.2	2321.2	2321.3	2321.4	2321.2		2321.5
		2375.1			2375.1					
		2383.1			2383.1			2383.2		
				2457.3	2457.4			2457.4		
AF33*	2485.7	2485.4	2485.4	2485.4	2485.4			2485.5		2485.7
		2810.4		2810.4	2810.4					2810.9
		2832.4	2832.5	2832.4						
		2872.4			2872.4					

Supplemental Table S2. Peak lists from 5 unmodified, 1 acetylated, and 6 methylene blue-treated AVKs. Relative peak height is indicated by 3 intensities of color; the highest intensity peaks are indicated by the darkest color, and lowest intensity peaks are indicated by the lightest color. O, oxygen; Na, sodium; Cu, copper; *, extended form of peptide. a: acetylated; b: treated with Methylene Blue



Supplemental Figure S1. Mass spectrum of oxidized AVK neuron. a) Spectrum from an individual AVK stained with methylene blue, which oxidizes methionines and adds 16 mass units. b) Expansion of (a) from m/z 1000-2000. X axis: m/z is mass-to-charge ratio. Y axis is intensity of MS signal in arbitrary units, a.u.





Supplemental Figure S2. MS/MS of previously identified AF peptides from AVK. Peaks representing a (green), b (blue), and y (red) ions are labeled, and b and y ions are summarized in the sequence at the top of each spectrum. a) MS/MS spectrum of AF25, m/z 809.4. b) MS/MS spectrum of AF26, m/z 920.5. c) MS/MS spectrum of AF2, m/z 991.5. Insets in a and c show the same spectra with condensed X axis and lower scale Y axis. X axis: m/z is mass-to-charge ratio. Y axis is intensity of MS signal in arbitrary units, a.u.



Supplemental Figure S3. Structure of the *afp-11* gene. There are 4 exons of 48, 184, 154 and 100 bp, separated by introns of 726, 1477, and 1313 bp. The 3'UTR is 125 bp in length.







Supplemental Figure S4. MS/MS of minor peaks. Peaks representing b (blue) and y (red) ions are labeled, and b and y ions are summarized in the sequence at the top of each spectrum. *, elongated form of predicted peptide; ⁻, truncated form of predicted peptide. a) MS/MS spectrum of PepNY*, m/z 1086.4. b) MS/MS spectrum of AF31⁻, m/z 896.5. c) MS/MS spectrum of AF26*, m/z 2144.1. d) MS/MS spectrum of AF32*, m/z 2092.1. Inset in d shows the same spectra with condensed X axis and lower scale Y axis. X axis: m/z is massto-charge ratio. Y axis is intensity of MS signal in arbitrary units, a.u.









Supplemental Figure S5. MS/MS of synthetic novel peptides. Peaks representing b (blue) and y (red) ions are labeled, and b and y ions are summarized in the sequence at the top of each spectrum. a) MS/MS spectrum of AF27, m/z 1075.6. b) MS/MS spectrum of AF28, m/z 1079.6. c) MS/MS spectrum of AF29, m/z 1106.6. d) MS/MS spectrum of AF31, m/z 1094.6. e) MS/MS spectrum of AF32, m/z 1051.5. f) MS/MS spectrum of AF33, m/z 1223.6. g) MS/MS spectrum of PepNY, m/z 1086.4. Insets in a - e show the same spectra with condensed X axis and lower scale Y axis. X axis: m/z is mass-to-charge ratio. Y axis is intensity of MS signal in arbitrary units, a.u.

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