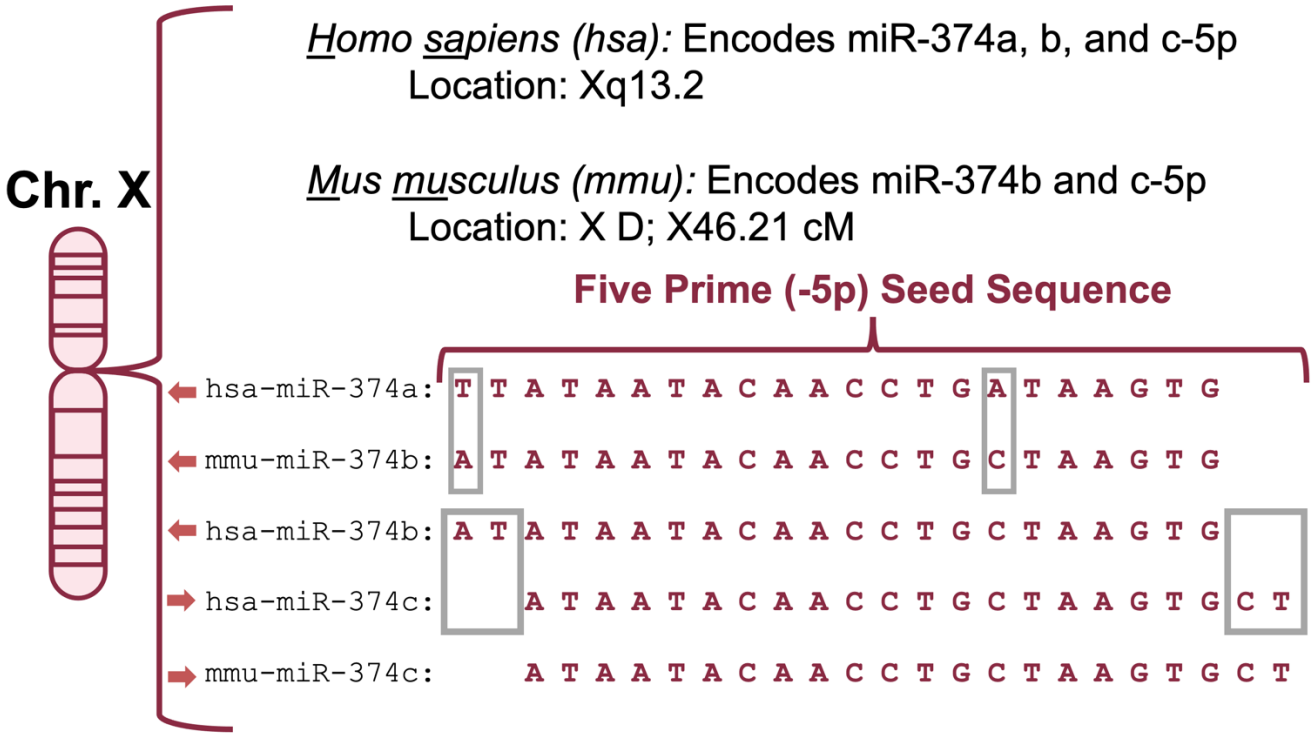
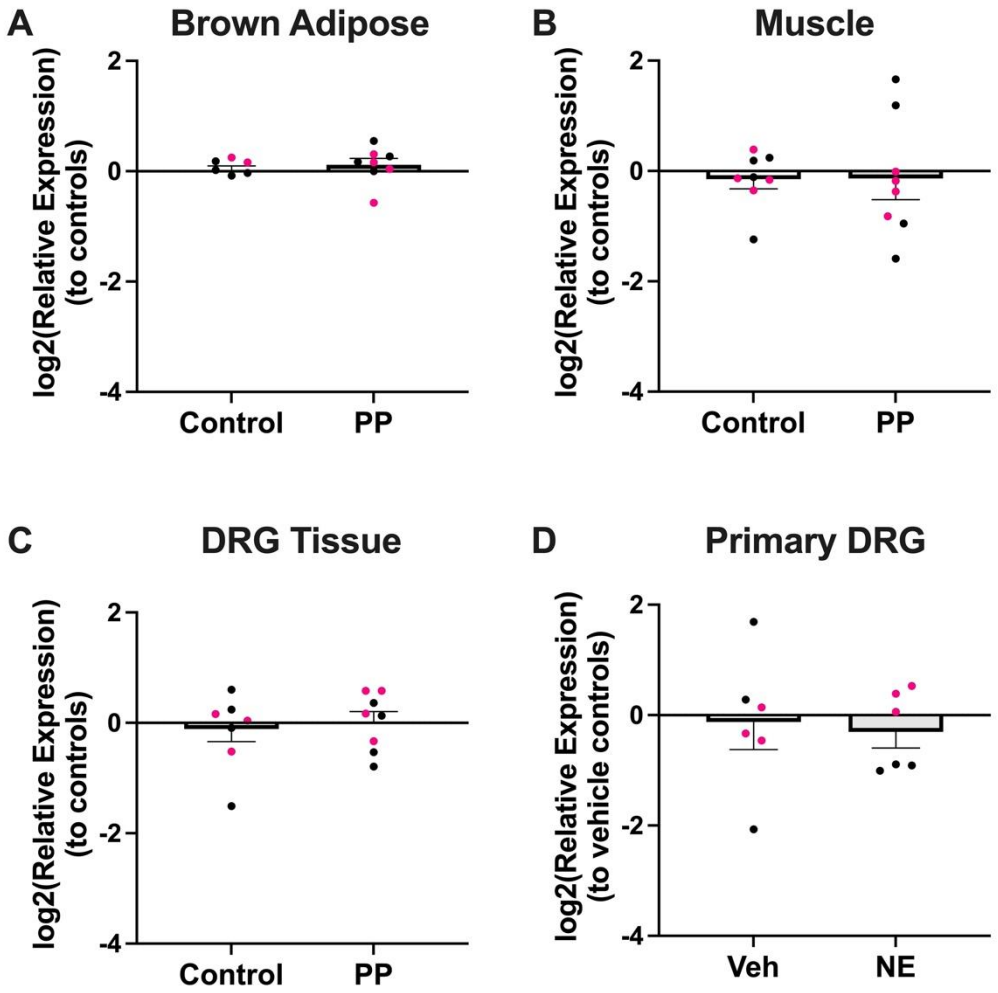
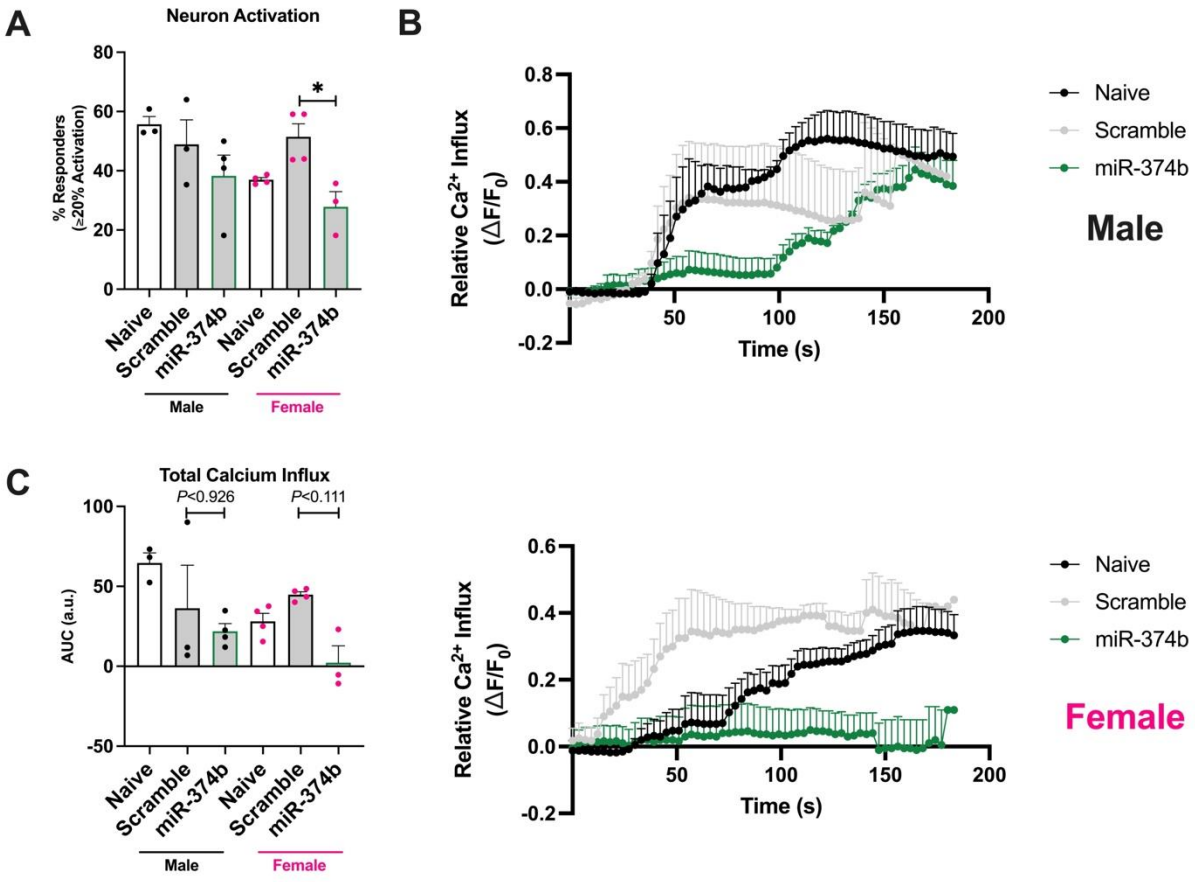


Supplementary Figure 1. Map of miR-374 family. Adapted from Bian et al. (*J Cell Mol Med*, 2019), the miR-374 family is located within and cleaved from the long noncoding RNA Five prime to X-inactive specific transcript (*FTX*), which is situated in the X-chromosome in both humans and mice. Arrows indicate direction of transcription. Across species and within subtype, miR-374 members are 100% homologous in the 22 base pair sequence. Across subtypes, miR-374 members are 91% homologous with 20/22 identical base pairs. In fact, miR-374b-5p and miR-374c-5p are isomiRs of each other since they are derived from the same precursor miRNA.



Supplementary Figure 2. miR-374 is not globally downregulated. While differential expression of miR-374 was observed in other tissues during PP onset, no differential expression of this miRNA was observed in either brown adipose (**A**), muscle (**B)**, or dorsal root ganglion (DRG) tissue (**C**), suggesting tissue-specific regulation of miR-374. miR-374 expression is not significantly changed by stimulating primary DRG cultures from either male or female mice with the pan-adrenergic endogenous agonist norepinephrine ("NE") (**D**). Data from females and their derived cultures are shown in **pink**, while males are shown in **black**. Data are mean + SEM.



Supplementary Figure 3. Emerging sex differences in miR-374b-5p's role in neuron activation. Although no measurement of calcium influx showed a significant group*sex interaction, percent responders and total calcium influx (area under the curve, AUC) did feature a main effect of sex (P<0.05). For measures of percent responders (**A**), relative calcium influx (**B**), and total calcium influx (**C**), female-derived neurons showed greater inhibition than male-derived neurons. Data from female-derived cultures are shown in **pink**, while male-derived cultures are shown in **black**. Data are mean + SEM, and asterisk indicates a Tukey's HSD P<0.05.

Supplemental Table 1. Demographics for TMD Study Participants

	Pain-free Controls N=283	TMD N=205	P-value
Other CPPCs, N (%)			<0.001
No	107 (37.81%)	25 (15.12%)	
Yes	184 (62.19%)	174 (84.88%)	
Age, N (%)			0.03*
18-24	6 (2.12%)	25 (12.20%)	
25-34	109 (38.52%)	78 (38.05%)	
35-44	84 (29.68%)	51 (24.88%)	
45-54	86 (30.39%)	38 (18.54%)	
55-64	4 (1.41%)	9 (4.39%)	
65-74	4 (1.41%)	2 (0.98%)	
Mean (SD)	38.6 (9.2)	36.5 (11.34)	
Gender, N (%)			<0.001*
Women	189 (66.78%)	153 (74.63)	
Men	94 (33.22%)	52 (25.37%)	
Race/Ethnicity, N (%)			<0.001
White	192 (67.84%)	144 (70.24%)	
African American	79 (27.92%)	30 (14.63%)	
Asian	6 (2.12%)	8 (3.90%)	
Native American	0 (0.00%)	0 (0.00%)	
Multiple	1 (0.35%)	8 (3.90%)	
Unknown	3 (1.06%)	2 (0.98%)	
Hispanic/Latino	11 (3.89%)	14 (6.83%)	

Supplemental Table 2. miRNA Sequence and Chromosome Location

mb22_NAME	mb22_ID	hg38_LOCUS	Sequence
hsa-miR-197-3p	MIMAT0000227	1:109598940-109598961	UUCACCACCUUCUCCACCCAGC
hsa-miR-6734-5p	MIMAT0027369	1:43364688-43364710	UUGAGGGGAGAAUGAGGUGGAGA
hsa-miR-1248	MIMAT0005900	3:186786675-186786701	ACCUUCUUGUAUAAGCACUGUGCUAAA
hsa-miR-1226-3p	MIMAT0005577	3:47849608-47849629	UCACCAGCCCUGUGUUCCCUAG
hsa-miR-1229-3p	MIMAT0005584	5:179798278-179798300	CUCUCACCACUGCCCUCCCACAG
hsa-miR-4646-5p	MIMAT0019707	6:31701070-31701091	ACUGGGAAGAGGAGCUGAGGGA
hsa-miR-3609	MIMAT0017986	7:98881700-98881723	CAAAGUGAUGAGUAAUACUGGCUG
hsa-miR-671-5p	MIMAT0003880	7:151238449-151238471	AGGAAGCCCUGGAGGGGCUGGAG
hsa-miR-146b-3p	MIMAT0004766	10:102436557-102436578	GCCCUGUGGACUCAGUUCUGGU
hsa-miR-6743-3p	MIMAT0027388	11:209383-209405	AGCCGCUCUUCUCCCUGCCCACA
hsa-miR-127-3p	MIMAT0000446	14:100883035-100883056	CUGAAGCUCAGAGGGCUCUGAU
hsa-miR-433-3p	MIMAT0001627	14:100881949-100881970	AUCAUGAUGGGCUCCUCGGUGU
hsa-miR-485-3p	MIMAT0002176	14:101055464-101055485	GUCAUACACGGCUCUCCUCUCU
hsa-miR-1249-3p	MIMAT0005901	22:45200958-45200979	ACGCCCUUCCCCCCCUUCUUCA
hsa-miR-374a-5p	MIMAT0000727	X:74287325-74287346	UUAUAAUACAACCUGAUAAGUG
hsa-miR-766-3p	MIMAT0003888	X:119646763-119646784	AGGAGGAAUUGGUGCUGGUCUU

Supplemental Table 3. Demographics for FMS Study Participants

	Pain-free Controls N=24	FMS N=24	P-value
Age, N (%)			0.06
40-50	8 (33.33%)	5 (20.83%)	
51-60	8 (33.33%)	16 (66.67%)	
61-74	8 (33.33%)	3 (12.50%)	
Mean (SD)	56.00 (8.15)	54.00 (7.36)	
Gender, N (%)			0.70
Women	19 (79.17%)	23 (87.50%)	
Men	5 (20.83%)	1 (4.17%)	
Race/Ethnicity, N (%)			0.16
White	17 (70.83%)	21 (70.90%)	
Black	0 (0.00%)	0 (0.00%)	
Arab	0 (0.00%)	0 (0.00%)	
Latino/Hispanic	1 (4.17%)	2 (8.33%)	
Filipino	0 (0.00%)	0 (0.00%)	
South Asian	0 (0.00%)	0 (0.00%)	
West Asian	2 (8.33%)	0 (0.00%)	
East Asian	0 (0.00%)	0 (0.00%)	
Southeast Asian	1 (4.17%)	0 (0.00%)	
Jewish	1 (4.17%)	0 (0.00%)	
Aboriginal	0 (0.00%)	1 (4.17%)	
Other	1 (4.17%)	0 (0.00%)	
Prefer Not to Answer	0 (0.00%)	0 (0.00%)	
I Don't Know	1 (4.17%)	0 (0.00%)	
BMI, Mean (SD)	28.80 (5.30)	31.50 (8.25)	0.20
FMS Score, Mean (SD)	5 (5.00)	22 (6.00)	<0.001*

Supplemental Table 4. Demographics for the MVC Study Participants

	Low Pain N=81	High Pain N=86	P-value
Age, Mean (SD)	34.80 (12.30)	34.90 (11.80)	0.63
Gender, N (%)			0.89
Women	51 (63.00%)	55 (64.00%)	
Men	30 (37.00%)	31 (36.00%)	
BMI, Mean (SD)	28.90 (6.00)	29.70 (7.20)	0.44
Pain Severity, Mean (SD)	5.52 (1.54)	8.95 (0.89)	<0.001*
Stress Level (PDI Score), Mean (SD)	20.40 (11.10)	26.30 (11.40)	<0.001*

Supplemental Table 5. Mimics, primers and kits used for experiments

Item Name	Company	Cat No.	Assay ID	Sequence
mmu-miR-374b-5p Mimic	ThermoFisher	4464066	*MH11339*	AUAUAAUACAACCUGCUAAGUG
mirVana™ miRNA Inhibitor, Negative Control #1	ThermoFisher	4464076	N/A	Proprietary
mmu-miR-374b-5p	Qiagen	339306	YP00204608	AUAUAAUACAACCUGCUAAGUG
cel-miR-39-3p	Qiagen	339306	YP00203952	UAUACCGAGAGCCCAGCUGAUU UCGUCUUGGUAAUAAGCUCGUC AUUGAGAUUAUCACCGGGUGUA AAUCAGCUUGGCUCUGGUGUC
UniSp6	Qiagen	339306	YP00203954	Proprietary
ATXN7	ThermoFisher	4331182	Mm01315281_m1	NM_139227.4
CBL	ThermoFisher	431182	Mm00483069_m1	NM_007619.2
CRK, Variant II	ThermoFisher	4331182	Mm00467065_m1	NM_133656.5
CRKL	ThermoFisher	4331182	Mm00464462_m1	NM_007764.5
EP300	ThermoFisher	4331182	Mm00625535_m1	NM_177821.6
HIF1A	ThermoFisher	4331182	Mm00468869_m1	NM_010431.2
KIDINS220	ThermoFisher	4331182	Mm01287158_m1	NM_001081378.1
NUMB	ThermoFisher	4331182	Mm00477927_m1	NM_001272056.1
PTPN11	ThermoFisher	4331182	Mm00448434_m1	NM_001109992.1
TGFBR2	ThermoFisher	4331182	Mm00436976_m1	NM_009371.3
ACTB	ThermoFisher	4331182	Mm02619580_g1	GenBank: AK075973.1
18S	ThermoFisher	4331182	Mm03928990_g1	NR_003278.3
PAXgene Blood miRNA Kit	Qiagen	763134	N/A	N/A
miRCURY LNA RT	Qiagen	339340	N/A	N/A
miRNA Spike In	Qiagen	339390	N/A	N/A
miRCURY LNA SYBR Green PCR	Qiagen	339345	N/A	N/A
High-capacity cDNA Reverse Transcription Kit	ThermoFisher	4368813	N/A	N/A

TaqMan Fast Advanced Master Mix	ThermoFisher	4444557	N/A	N/A

Supplemental Table 6. Reactome Analysis

Rank	Unique Enriched Pathway	Observed Genes (of 69)	Strength	False Discovery Rate
1	Signal Transduction	34	0.58	2.60E-10
2	Disease	26	0.64	1.24E-08
3	Membrane Trafficking	21	0.98	6.69E-12
4	Protein Metabolism	19	0.45	1.10E-03
5	Immune System	19	0.44	1.40E-03
6	Post-Translational Protein Modification	18	0.56	9.25E-05
7	Generic Transcription Pathway	16	0.58	2.60E-04
8	Developmental Biology	14	0.56	1.20E-03
9	Chromatin Modifying Enzymes	13	1.19	1.92E-09
10	Hemostasis	9	0.63	5.90E-03

Supplementary File 1. Additional RNA-seq Methods

OPPERA: Blood samples were collected at the time of enrollment in PaxGene tubes and total RNA isolated using Qiacube column-based or Chemagic magnetic bead separation. For small RNA-seq, approximately 75ng total RNA was prepared using the TriLink Cleantag small RNA kit, small RNA-seq libraries were generated and quantified with Qubit reading and Bioanlyzer size checking, and then pooled for cBot amplification and sequencing on the Illumina HiSeq 3000 platform with 50bp single read sequencing. Demultiplexing with Bcl2fastq2 was employed to generate the fastq file for each sample, the 3' adapters were trimmed, and sequencing reads aligned with their reference genome.

For mRNA-seq, approximately 250ng total RNA was prepared using the Illumina TruSeq stranded mRNA kit. The first step in the workflow involves purifying the poly-A containing mRNA molecules using poly-T oligo-attached magnetic beads. Following purification, the mRNA is fragmented into small pieces using divalent cautions under elevated temperature. The cleaved RNA fragments are copied into first strand cDNA using reverse transcriptase and random primers. This is followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. In stranded RNA-seq protocol, Strand specificity is achieved by replacing dTTP with dUTP in the Second Strand Marking Mix (SMM). The incorporation of dUTP in second strand synthesis effectively quenches the second strand during amplification since the polymerase used in the assay will not incorporate past this nucleotide. Further specificity is achieved by addition of Actinomycin D to the First Strand Master Mix Act D (FSA). Actinomycin prevents spurious DNA dependent synthesis during first strand synthesis, while allowing RNA dependent synthesis. These cDNA fragments then go through an end repair process, the addition of a single 'A' base, and then ligation of the adapters. The products are then purified and enriched with PCR to create the final RNA-seq library. After RNA-seq libraries were subjected to quantification process, pooled for cBot amplification and subsequent sequencing run with Illumina HiSeq 3000 platform with 50bp single read sequencing. After the sequencing run, demultiplexing with Bcl2fastq2 was employed to generate the fastq file for each sample.

For both miRNA- and mRNA-seq, Deep-sequencing reads were aligned on the human genome version hg38/GRCh38 using Bowtie2 v2.3.4 with very sensitive local alignment preset parameters, after trimming adaptor sequences with cutadapt v1.15. Quantification of miRNAs were obtained using ShortStack v3.8.5 against miRbase release 22 annotation with hg38 reference genome, and output as count matrix.

AA CRASH: Blood samples were collected in the ED at the time of enrollment using PAXgene RNA tubes and total RNA isolated using the PAXgene blood miRNA kit. Template libraries for small RNA sequencing were produced from 1.0 µg total RNA. Using an in-house adaptation of published protocols (TruSeq Small RNA library prep kits according to Illumina's manufacturer specifications), 12 barcoded libraries were combined per lane and sequenced on a HiSeq 2000 at the University of North Carolina at Chapel Hill High Throughput Sequencing Facility. Reads were demultiplexed (bcl2fastq), had barcode and adapter sequences removed, and then fastq files were generated. Mature miRNA sequences were obtained from miRbase v22.1 and genomic extensions were added before aligning with sequencing reads. Reads were aligned on human genome version hg19, and quantification of miRNAs were obtained using RSEM. Total read counts were generated including isomir and non-templated nucleotide addition.