

**Supplementary Table 1.** *Frost* and *regucalcin* primers used for PCR amplification and sequencing

Species	Region analysed	Primers		
<i>Drosophila americana</i>	<i>Frost</i>	FrostvirF	CAATGACCMCYTGCTTTC	
		FrostvirR	TCTATCGGAACTGACRAT	
	<i>frost</i> cDNA	Frost_PEST_F	CAGAAGAGCCCGTAGAAA	
		Frost_PEST_R	GGTGCCTTGGTAGTCTCG	
		Frost_cDNA_F	CGTAATGGGGATAGATGG	
		Frost_cDNA_R	CGTAATGGGGATAGATGG	
		<i>regucalcin</i>	DcaF	ATTTTAGTTGGATTCTCG
			DcaR	TTGTGCGTGTGCTTAGAT
	<i>regucalcin</i> cDNA	Dca_amer_ex1	TGATACCGAGGGCAACAT	
		Dca_amer_ex2	CGAAGGCAACCGAAGTGA	
	<i>RpL32</i> cDNA	RpL32_RT_F	ACAACAGAGTGCGTCGTC	
		RpL32_RT_R	ATCTCCTTGCGTTTCTTC	
	<i>Drosophila melanogaster</i>	<i>frost</i> cDNA	Dmel_Fst_F	TCAGGGTCAGTGGGATGG
			Dmel_Fst_R	CCGTTGGTGGTGGTGGAG
<i>regucalcin</i> cDNA		Dmel_Regucalcin_F	CCTGCTCCGCTACGACTA	
		Dmel_Regucalcin_R	CGAGACGCCATCCCAGTT	
<i>RpL32</i> cDNA		Dmel_RpL32_F	CGTTTACTGCGGCGAGAT	
		Dmel_RpL32_R	CGTTGGGGTTGGTGAGGC	