## SUPPLEMENTAL INFORMATION: Investigation of the Structure-Activity Relationship in Ponericin L1 from *Neoponera goeldii*

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Supplemental Figure 1: Normalized Trp emission spectra for L1 peptide before (red) and after (blue) interacting with lipid vesicles. Data are representative spectra after subtraction of a background spectrum. All samples contained 2  $\mu$ M peptide and 300  $\mu$ M lipid – (A) PC, (B) PC:PG, (C) PC:Chol, (D) PE:PG.



Supplemental Figure 2: Acrylamide Quenching of L1 Peptides in (A) PBS buffer, (B) PC vesicles, (C) PC:PG vesicles, (D) PC:Chol vesicles or (E) PE:PG vesicles. All samples contained 2  $\mu$ M peptide and 250  $\mu$ M lipid (for those with vesicles). Data are corrected for inner filter effects and are background subtracted. Data represent the average and standard deviation of 3-6 samples.



Supplemental Figure 3: *E. coli* outer membrane permeabilization. Absorbance at 486 nm represents the breakdown of the nitrocefin substrate by the periplasmic enzyme  $\beta$ -lactamase. Bacteria were exposed to varying concentrations of peptide and the corresponding colors for each panel are shown in the legend. (A) L1, (B) L1-Q,(C) L1-K, (D) L1-R, (E) L1-O, (F) L1-X, (G) Polymyxin B. Data represent the average and standard deviation of 3-6 samples.



Supplemental Figure 4: *E. coli* inner membrane permeabilization. Absorbance at 420 nm represents the breakdown of the ONPG substrate by the periplasmic enzyme  $\beta$ -galactosidase. Bacteria were exposed to varying concentrations of peptide and the corresponding colors for each panel are shown in the legend. (A) L1, (B) L1-Q,(C) L1-K, (D) L1-R, (E) L1-O, (F) L1-X, (G) cetyltrimethylammonium bromide (CTAB). Data represent the average and standard deviation of 3-6 samples.

	Table S1			
	Ponericin Peptide Family Sequence C	Comparison	1	
Name	Amino Acid Sequnce <sup>a</sup>	Length	$MW^{b}$	Net Charge <sup>c</sup>
L1	LLKELWTKMKGAGKAVLGKIKGLL	24	2596	+5
L2	LLKELWTKIKGAGKAVLGKIKGLL	24	2578	+5
G1	GWKDWAKKAGGWLKKKGPGMAKAALKAAMQ	30	3214	+7
G2	GWKDWLKKGKEWLKAKGPGIVKAALQAATQ	30	3308	+5
G3	GWKDWLNKGKEWLKKKGPGIMKAALKAATQ	30	3383	+6
G4	DFKDWMKTAGEWLKKKGPGILKAAMAAAT	29	3165	+3
G5	GLKDWVKIAGGWLKKKGPGILKAAMAAATQ	30	3109	+5
G6	GLVDVLGKVGGLIKKLLP	18	1819	+2
G7	GLVDVLGKVGGLIKKLLPG	19	1876	+2
W1	WLGSALKIGAKLLPSVVGLFKKKKQ	25	2710	+6
W2	WLGSALKIGAKLLPSVVGLFQKKKK	25	2710	+6
W3	GIWGTLAKIGIKAVPRVISMLKKKKQ	26	2864	+7
W4	GIWGTALKWGVKLLPKLVGMAQTKKQ	26	2853	+5
W5	FWGALIKGAAKLIPSVVGLFKKKQ	24	2600	+5
W6	FIGTALGIASAIPAIVKLFK	20	2031	+2

a Sequences are from NCBI annotated under old classification Pachycondyla goeldii

b MW calculated using Expasy ProtParam and rounded to nearest whole digit

c charge assuming pH 7 and free N/C termini

Ksv (M <sup>-1</sup> )           L1 $0.30$ $\pm$ $0.04$ L1-O $15.35$ $\pm$ $3.72$
L1 0.30 $\pm$ 0.04 L1-O 15.35 $\pm$ 3.75
L1-O 15.35 ± 3.75
L1-K 17.63 ± 1.2
L1-R 4.23 ± 2.78
L1-O 10.97 ± 1.25
L1-X 10.50 ± 2.52