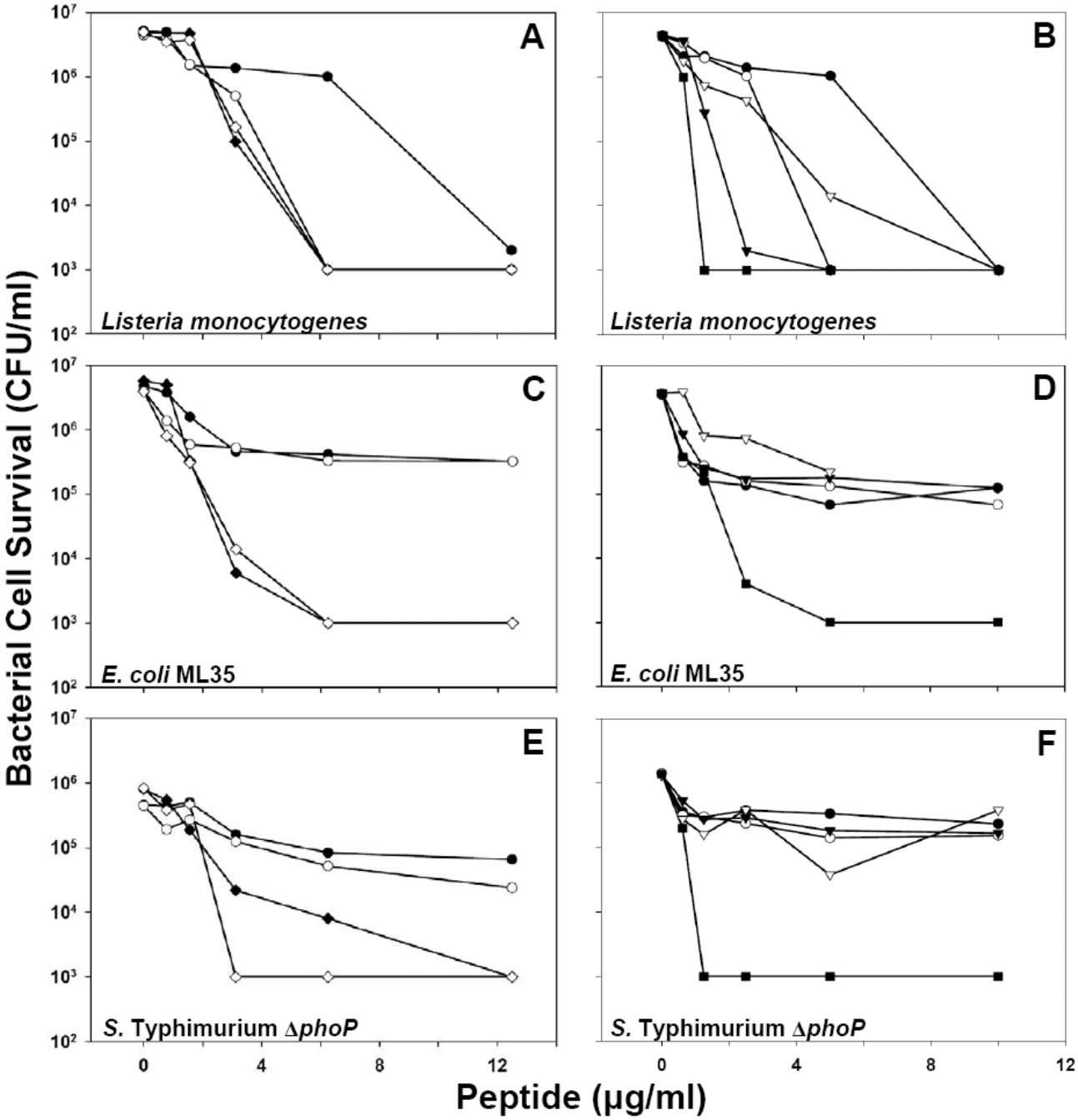

Figure S1. Native α-defensin preparations retain bactericidal activity. Data from bactericidal peptide assays shown in Fig. 4 are depicted here as CFU/ml of surviving bacteria. Assays were performed against *Listeria monocytogenes* (panels A and B), *E. coli* ML35 (panels C and D), and *Salmonella enterica* serovar Typhimurium *ΔphoP* (panels E and F). Symbols: (-■-) Crp3, (-◆-) Crp4, (-◇-) Crp Mix, (-●-) α-defensins from complete ileum, (-○-) α-defensins from complete colon, (-▼-) α-defensins from ileal tissue, (-▽-) α-defensins from colonic lumen. Slight differences in bactericidal peptide activities between control α-defensins and α-defensin preparations from mouse sources are evident on the log scale. These differences could be a result of the extraction procedure, because the positive control ileal tissue α-defensins showed similar activity when compared with other samples extracted from mice.

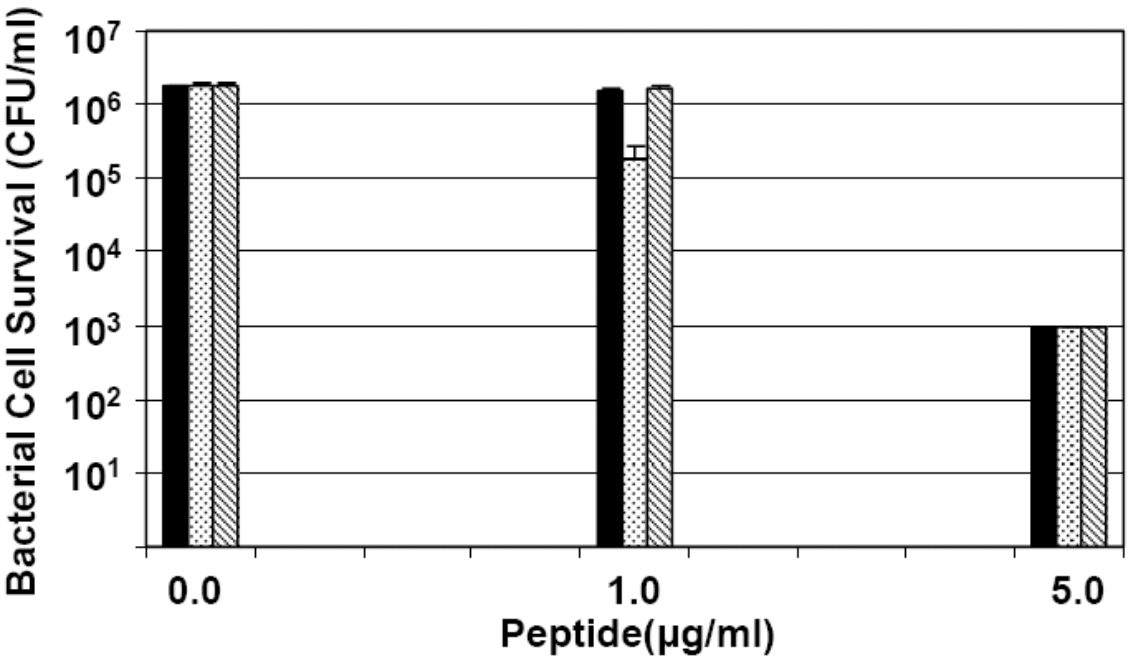

Figure S2. Replicate bactericidal peptide assays with α-defensin preparations demonstrate the reproducibility of the assay. A bactericidal peptide assay was performed against *Listeria monocytogenes* to test the efficacy of Crp3 (solid bar), complete ileum α-defensins (dotted bar), and complete colon α-defensins (striped bar) in triplicate at the concentrations shown. Standard deviation was calculated for each peptide at each concentration.

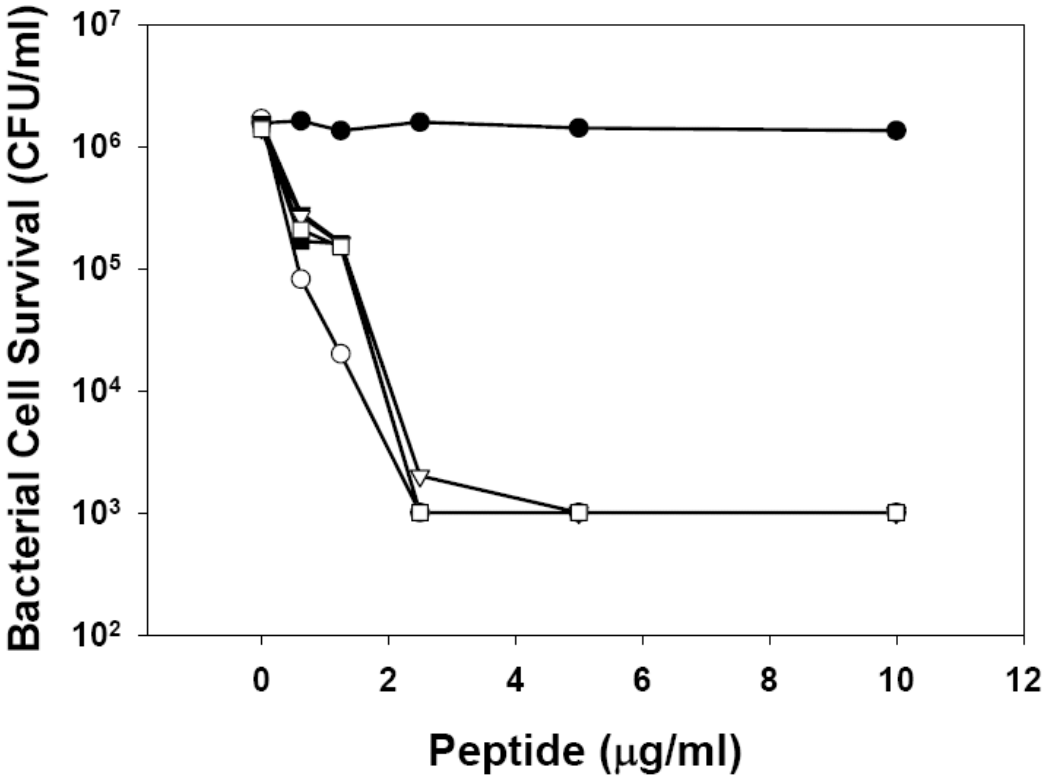

Figure S3. Bactericidal activity of Crp controls. A bactericidal peptide assay was performed against *E. coli* ML35 to confirm that all recombinant and synthetic Crp control peptides used in these studies are functional. Symbols: (-●-) ProCrp4, (-○-) (M19L)-Crp1, (-▼-) Crp2, (-▽-) Crp3, (-■-) Crp4, (-□-) (M19L)-Crp6.

Table S1. Performic acid oxidation of colonic α-defensins.[1]

Peptide		Theoretical Mass (A.M.U.)	Experimental Mass (A.M.U.)	PFA Oxidized Mass (A.M.U.)	Mass Shift (A.M.U.)	# of O's	Corresponds to Cys	Corresponds to Met
(des-Leu-Arg)-Cryptdin-1		3846.6	3853.1	4174.1	321.0	20	6	1
(des-Leu)-Cryptdin-1	*	4002.8	4007.4	4330.9	323.5	20	6	1
Cryptdin-1		4115.9	4120.4	4445.1	324.7	20	6	1
(des-Leu)-Cryptdin-2	^	4134.0	4138.8	4492.2	353.4	22	6	2
			4136.7	4420.8	284.1	18	6	0
				4456.5	319.8	18	6	1
				4486.7	350.0	22	6	2
(des-Leu-Arg)-Cryptdin-3	*	4002.8	4007.4	4330.9	323.5	20	6	1
(des-Leu)-Cryptdin-3		4161.0	4166.8	4521.0	354.2	22	6	2
Cryptdin-3		4274.2	4277.1	4551.3	274.2	18	6	0
(des-Gly-Leu)-Cryptdin-4		3585.3	3589.6	3878.2	288.6	18	6	0
(des-Leu)-Cryptdin-6		4016.9	4018.1	4375.1	357.0	22	6	2
Cryptdin-6	^	4130.0	4138.8	4492.2	353.4	22	6	2
			4136.7	4420.8	284.1	18	6	0
				4456.5	319.8	18	6	1
				4486.7	350.0	22	6	2

[1]Colonic α-defensin identities were deduced by comparing experimental masses (see column "Experimental Mass") with theoretical masses (see column "Theoretical Mass") of previously characterized Crps. Peptides were modified with performic acid, resulting in an increase in molecular weight (see column "PFA Oxidized Mass") due to the addition of oxygen atoms to Cys and Met residues. This increase in mass (see column "Mass Shift") varies based on the number of oxygen atoms added to the peptide (see column "# of O's"), which depends on the number of Cys and Met residues that are oxidized. α-Defensins are defined by the presence of six Cys residues, and they can contain zero, one, or two Met's. Therefore, the number of oxygens added to a peptide can be used to infer how many Cys and Met residues are present in that peptide (see column "Corresponds to"). As oxidation of Cys's occurs more readily than oxidation of Met's, for some peptides masses corresponding to oxidation of zero, one, or two Met's was seen. Due to highly similar theoretical masses, it was not possible to differentiate between the two peptides with a * next to them or the two peptides with a ^ next to them.