SI Appendix

PACAP is a pathogen-inducible resident antimicrobial neuropeptide affording rapid and contextual molecular host defense of the brain

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Supplemental Figures

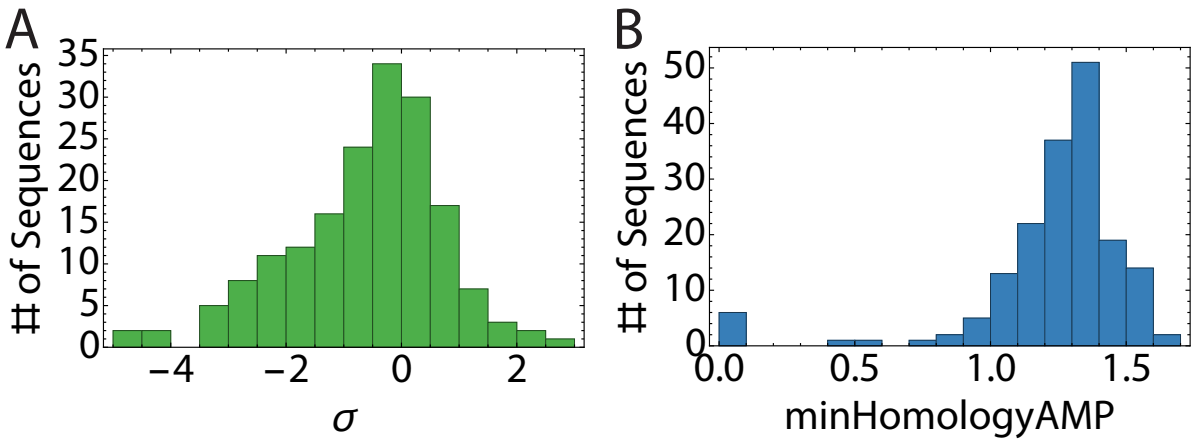

Figure S1: **A comprehensive machine learning screen of a neuropeptide database predicts a number of neuropeptides to have antimicrobial activity**. Distributions of (A) the distance-to-hyperplane metric σ and (B) minHomologyAMP for the screened neuropeptides are shown. The majority neuropeptides have low sequence homology to known AMPs from all kingdoms, but a subset of them are predicted to possess hidden antimicrobial activity indicated by a small number of sequences with scores σ > ~0.8.

Organism		Sequence	% Identity	% Similarity	σ	P(+1)
Human	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK	100	100	2.56	1.00
Mouse	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK	100	100	2.56	1.00
Chicken	1	HTDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK	97	97	2.79	1.00
Frog	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRIKNK	97	100	2.59	1.00
Catfish	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGRRYRQRFRNK	89	97	2.54	1.00
Cockroach	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYRQRYRSK	89	95	1.91	1.00
Tunicate	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYRQRYRNE	89	95	1.35	0.99
Crab	1	HSDGIFTDSYSRYREQMAVKKYLAAVLGKRYRQRYRNK	89	95	2.15	1.00
Planarian	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYRQRYRNK	92	97	2.59	1.00
Squid	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYRQRYRNK	92	97	2.59	1.00
Hydra	1	HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYRQRYRNK	92	97	2.59	1.00
consensus	1	*.************.************.**.**....				

Figure S2: **PACAP is highly conserved across mammals, vertebrates, and invertebrates.** A sequence alignment of PACAP from select organisms spanning vertebrates and invertebrates demonstrates that PACAP retains a high degree of identity and similarity over time. Machine learning results (σ, P(+1)) indicate that differences in the PACAP sequence across multiple species do not appreciably affect its membrane-permeating properties. Notably, the most ancient organism *Hydra* possesses a PACAP isoform with 92% identity and 97% similarity to human PACAP.

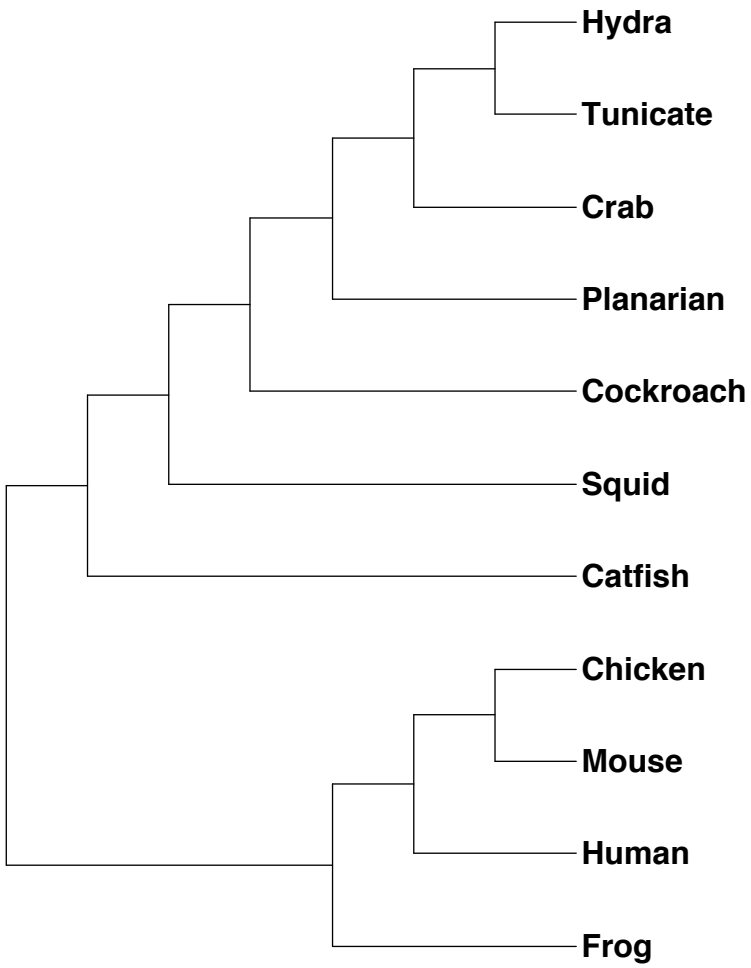

Figure S3: **Phylogenetic reconstruction of selected PACAP sequences from vertebrates and invertebrates**. A neighbor-joining tree visualization of PACAP peptide family was computed from the sequence alignment in Figure S2 using PhyML in SeaView.

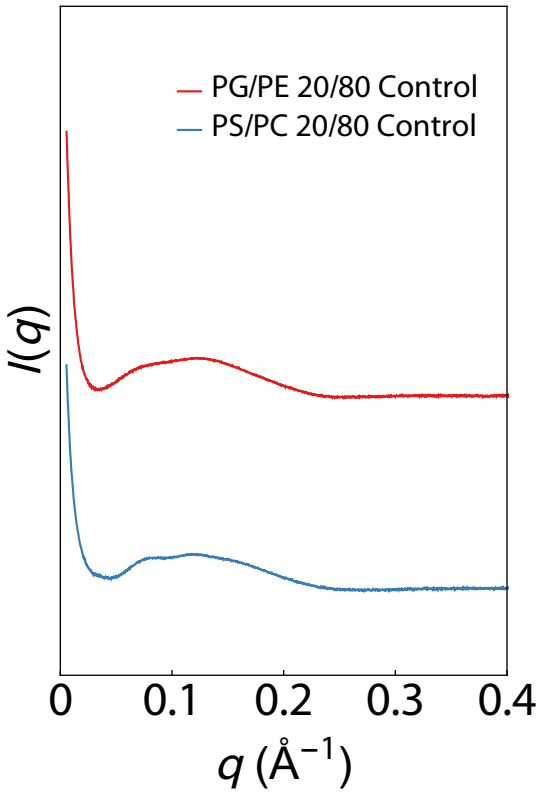

Figure S4: **SAXS spectra of SUV Controls.** Broad scattering form factors are observed for the bacteria-like PG/PE = 20/80 control as well as the eukaryotic-like PS/PC = 20/80 SUVs used to quantify AMP-membrane interactions. No strong scattering was observed from the membranes alone.

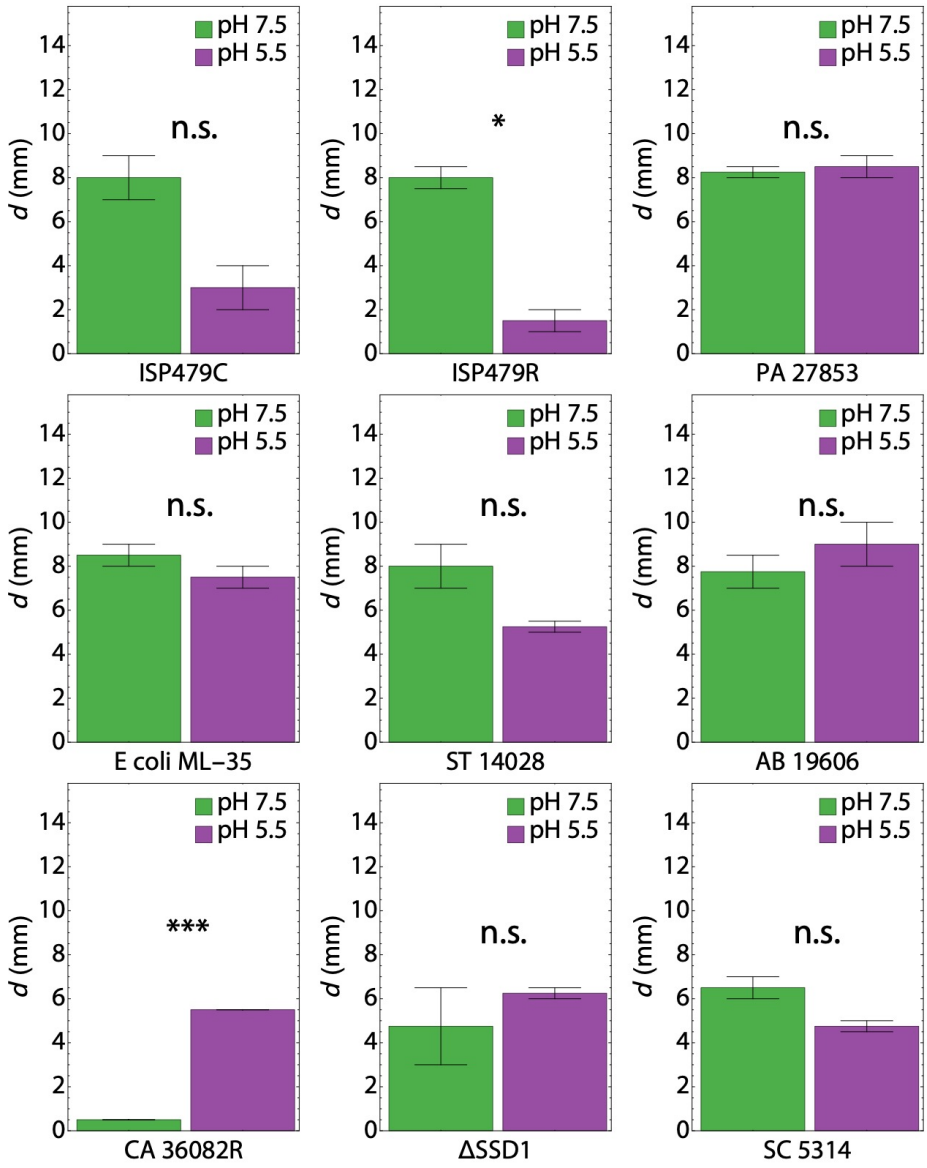

Figure S5: **Context dependent antimicrobial activity of PACAP across a broad range of organisms.** PACAP exhibits antimicrobial activity against sensitive and resistant strains of *S. aureus* better at pH 7.5 vs pH 5.5. Interestingly, PACAP kills certain strains of *C. albicans* better at pH 5.5 than pH 7.5. In other cases, like *P. aeruginosa* and *A. baumanni*, the antimicrobial effect of PACAP is less pH dependent. PACAP exhibits context-dependent antimicrobial activity across various pH conditions. *P < 0.05, **P < 0.01, ***P<0.001, not significant (n.s.). P-values were calculated using a two-tailed Student's t-test.

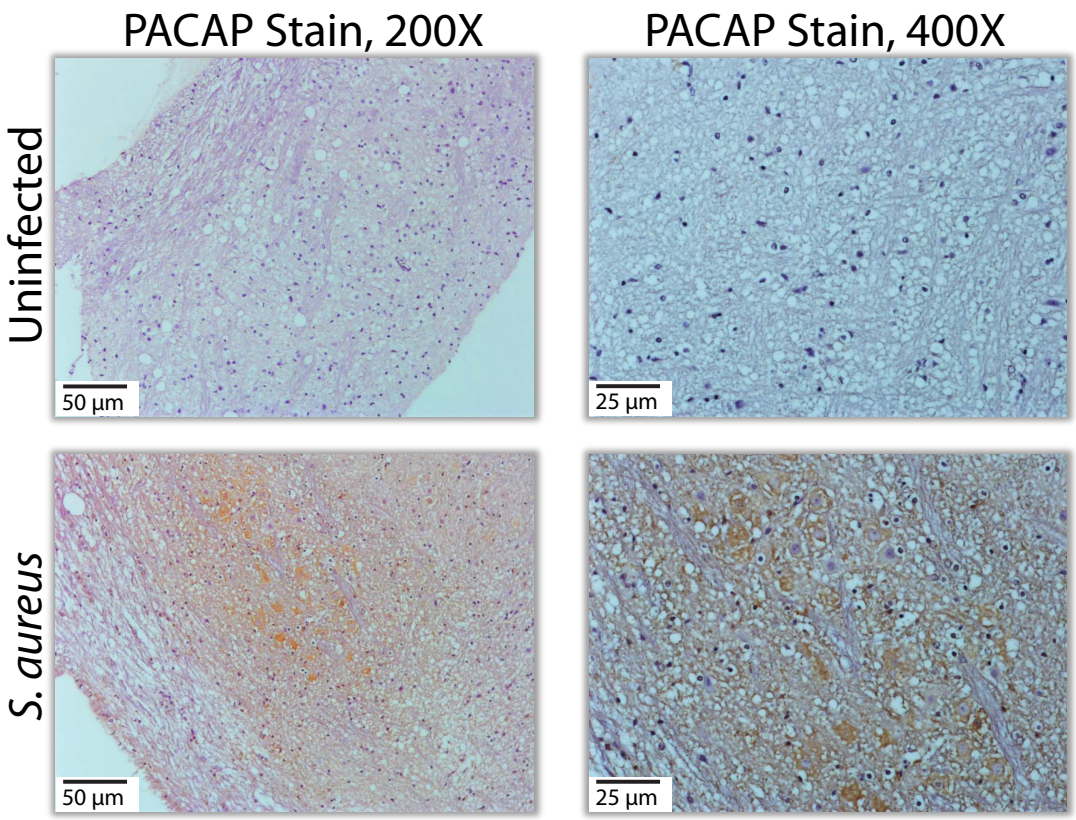

Figure S6: **PACAP is strongly induced in brain tissue in *S. aureus* infection**. PACAP staining shown at 200x and 400x magnification with corresponding scale bars.

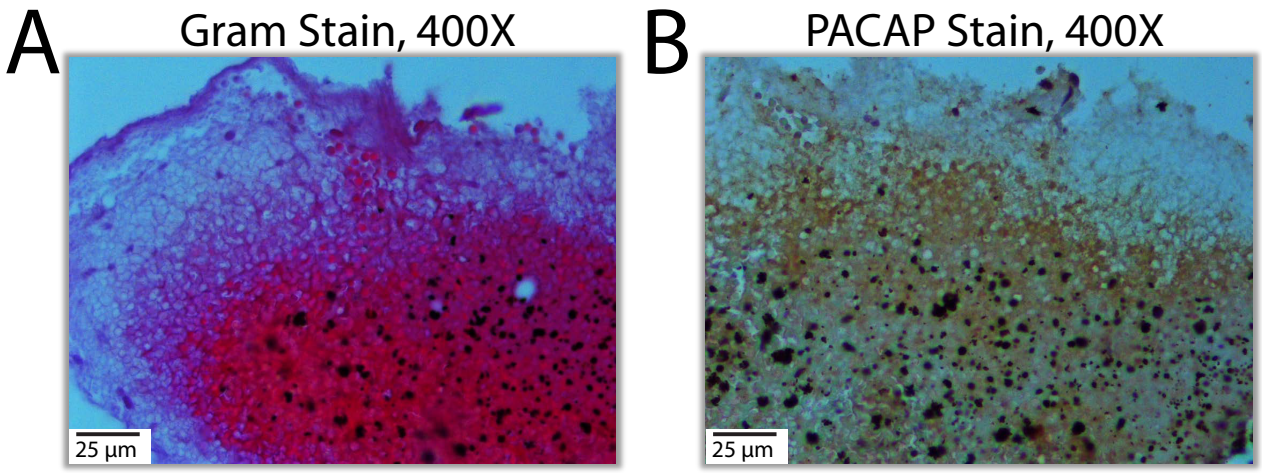

Figure S7: **PACAP colocalizes with bacteria during *S. aureus* brain infection**. Gram stain and PACAP stain of similar brain tissue sections are shown at 400x magnification.

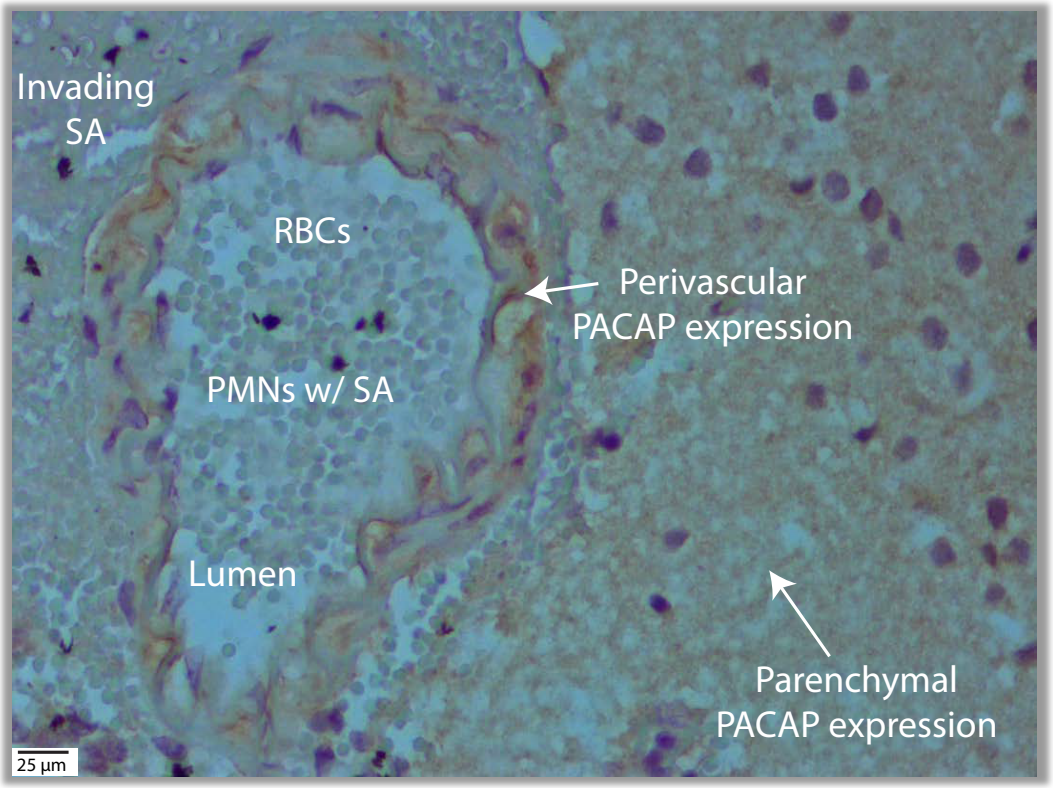

Figure S8: **PACAP is induced in the perivascular and parenchymal brain tissue in response to invading *S. aureus*.**

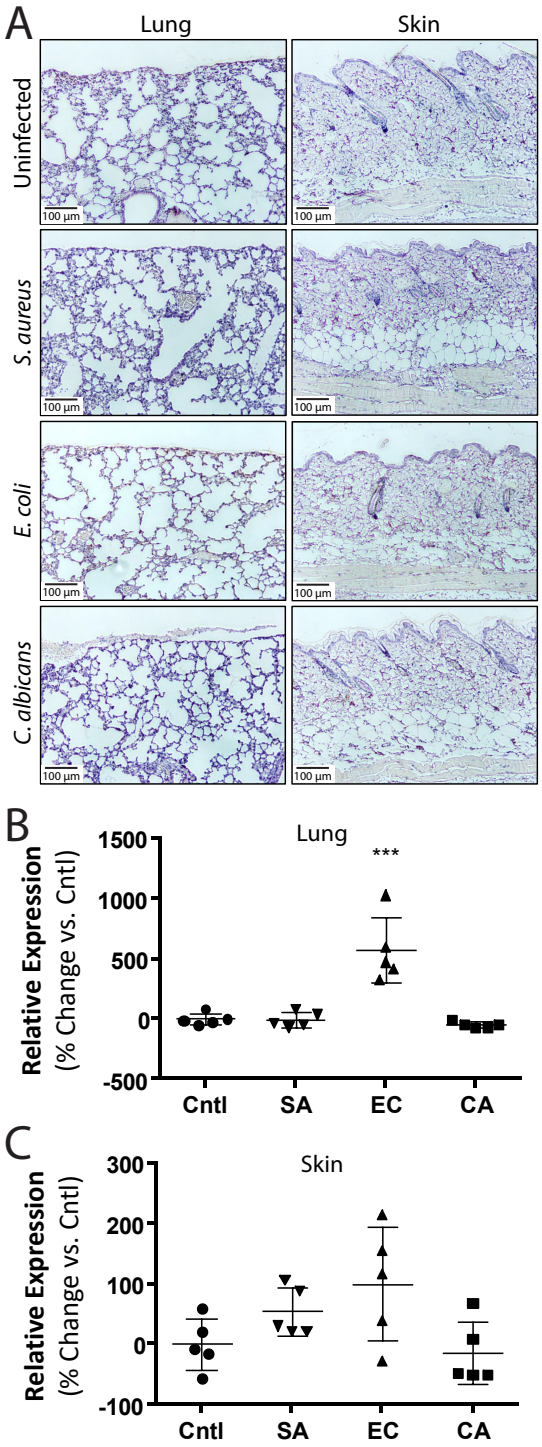

Figure S9: **Additional immunohistochemistry of PACAP in lung and skin tissue in mouse models of bacterial and fungal septicemia.** PACAP is selectively induced in the lung in response to *E. coli* (EC) infection but not to *S. aureus* (SA) or *C. albicans* (CA) compared to control (Cntrl). PACAP is expressed at low levels in the skin but is not appreciably induced in the context of septicemic infection. *P < 0.05, **P < 0.01, ***P<0.001. All other comparisons not significant. P-values were calculated using a two-tailed Student's t-test.