

mouse

CRP1 LRDLVCYCRSRGCKGRERMNGTCKGHLLYTLCCR
CRP2 LRDLVCYCRTRGCKRRERMNGTCKGHLMYTLCCR
CRP3 LRDLVCYCRKRGCKRRERMNGTCKGHLMYTLCCR
CRP4 GLLCYCRKGHCCKGERVVGTC-G--IRFLYCCPRR
CRP5 LSKKLIICYCRIRGCKRRERMVFGTCNLFLLTFVFCSS
CRP6 LRDLVCYCRARGCKGRERMNGTCKGHLLYMLCCR
B6a LHEKSSRDLIYCRKGGCNRGEQVYGTC-S--GRLLFCCRRRH
B6b LSRDLICLCRNRRCNRGELFYGTC-A--GPFLRCCRRR

human

HNP-1 ACYCRIPACIAGERRYGTCTIYQGRLWAFCC
HNP-2 CYCRIPACIAGERRYGTCTIYQGRLWAFCC
HNP-3 DCYCRIPACIAGERRYGTCTIYQGRLWAFCC
HNP-4 VCSCRLVFCRRTELRVGNCLIGVVSFTYCTTRVD
HD-5 ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
HD-6 AFTCHCRRS-CYSTEYSYGTCTVMGINHRFCL

rat

RD-5 LRDLKCFCKRRKSCNWEGIMGICKKRYGSPILCCR
NP-1 VTCYCRRTRCGFRERLSGACGYRGRIYRLCCR
NP-2 VTCYCRSTRCGFRERLSGACGYRGRIYRLCCR
NP-3 CSCRTSSCFRGERLSGACRLNGRIYRLCC
NP-4 ACYCRIGACVSGERLTGACGLNGRIYRLCCR

rabbit

NP-1 VVCACRRALCLPERRRAGFCRIRGRIHPLCCR
NP-2 VVCACRRALCLPLERRRAGFCRIRGRIHPLCCR
NP-3A GICACRRRFPNSERFSGYCRVNGARYVRCSSR
NP-3B GRCVCRRTSSCFRGERLSGACRLNGRIYRLCC
NP-4 VSCTCRRFSCGFRERASGSCTVNGVRHTLCCR
NP-5 VFCTCRGFLCGSGERASGSCTINGVRHTLCCR

monkey

RMAD-1 ACYCRIPACLAGEERRYGTCTFYLRVWAFCC
RMAD-2 ACYCRIPACLAGEERRYGTCTFYMRVWAFCC
RMAD-3 ACYCRIPACLAGEERRYGTCTFYRRRVWAFCC
RMAD-4 RRTCRCRFRGRCFRRESYSGSCNINGRIFSLCCR
RMAD-5 RRTCRCRFRGRCFRRESYSGSCNINGRIFSLCCR
RMAD-6 RRTCRCRFRGRCFRRESYSGSCNINGRISSLCCR
RMAD-7 RRTCRCRFRGRCFRRESYSGSCNINGRISSLCCR
RMAD-8 ACYCRIPACLAGEERRYGTCTFYLRVWAFCC
RED-1 RRTCRCRIRRCRGLLESSFGNCILHGQFAKLCR
RED-2 FTCHCRIGRCSWFETRFRSCTLLGLAANLCCR
RED-3 HTCYCRNRCFTPEFHAGCKVEGRITYKLCR
RED-4 RTCYCRTGRCYTPEFHSGKCVFNGRITYKLCR
RED-5 MICLCRIGRCSWREAHFGSCTKMGQFAKICRRAS
RED-6 RNCHCRIGHCRPAPPMGVCIHGQFGKLCR

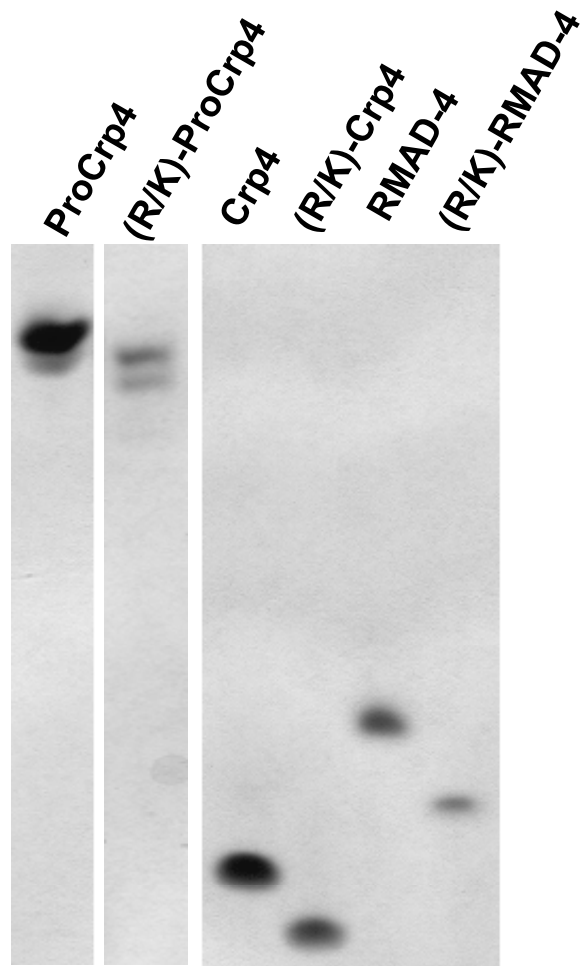
hamster

HANP-4 VTCFCRKPVCDSGETQIGYCRLGNTFYRLCCRQ
HANP-3 VTCFCRRRGCASRERLIGYCRFGNTIYGLCCR
HANP-2 CFCKRPVCDSGETQIGYCRLGNTFYRLCCRQ
HANP-1 VTCFCRRRGCASRERHIGYCRFGNTIYRLCCR

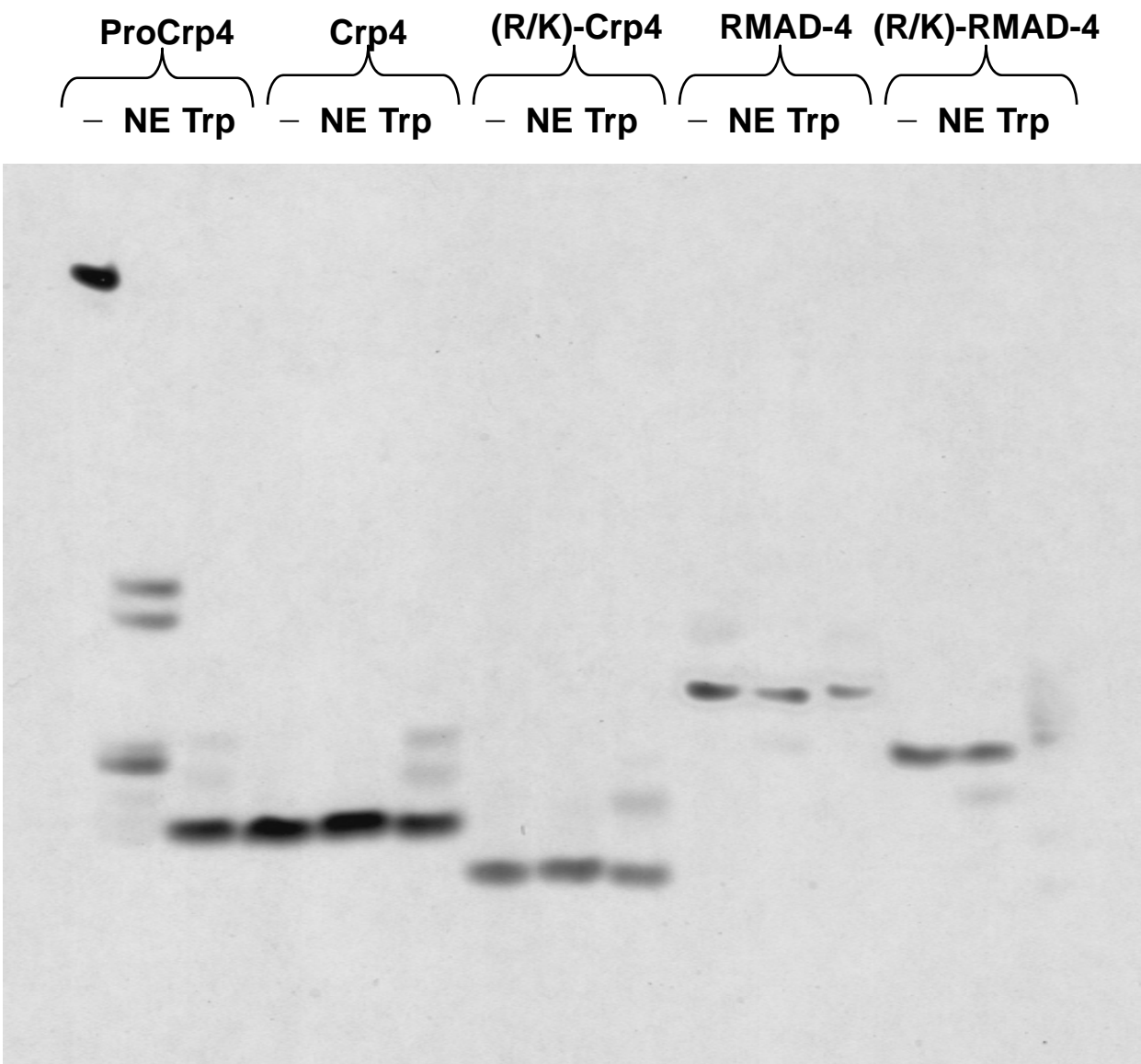
guinea pig

GNP-1 RRCICTTRTCRFPYRRLGTCIFQNRVYTFCC

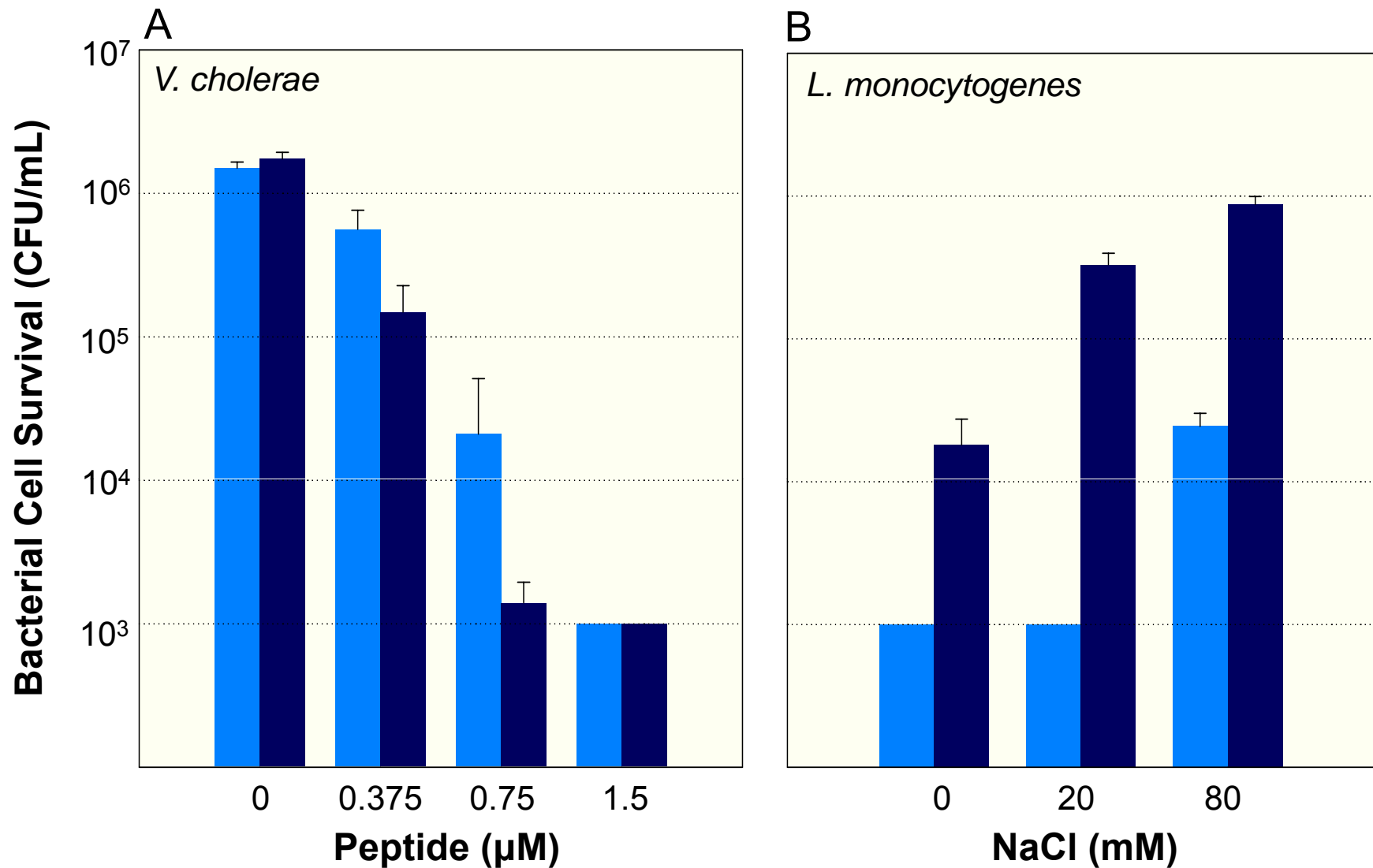
Supplementary Figure S1. An alignment of representative known mammalian α -defensins illustrates an Arg:Lys ratio of 9:1. Arg residue positions are highlighted in black and Lys positions in blue.



Supplementary Figure S2. Twelve micrograms proCrp4 and (R/K)-proCrp4 and 6 μ g samples of Crp4, (R/K)-Crp4, RMAD-4, and (R/K)-RMAD-4 were resolved by acid-urea-PAGE and stained with Coomassie Blue.



Supplementary Figure S3. Sensitivity of Lys-substituted α -defensins to neutrophil elastase (NE) and trypsin (Trp) proteolysis. Twelve micrograms of proCrp4 and 6 μ g of Crp4, (R/K)-Crp4, RMAD-4 and (R/K)-RMAD-4 were incubated in 50 mM ammonium bicarbonate alone (-), with NE in 50 mM Tris, 150 mM NaCl (pH 7.5) at 37 $^{\circ}$ C for 2 h at a substrate:enzyme ratio of 40:1 NE, or with Trp in 50 mM ammonium bicarbonate at a 50:1 substrate:enzyme ratio for 2 h (see "Materials and Methods").



Supplementary Figure S4. In panel A, five replicate bactericidal peptide assays were performed using RMAD-4 (light blue) and (R/K)-RMAD-4 (dark blue) at 1.5 μM , 0.75 μM , 0.375 μM and 0 μM against *V. cholerae*. Log-phase bacteria were exposed to peptide for 1 h in PIPES-TSB buffer (see “Materials and Methods”). Following peptide exposure, the bacteria were plated on TSB-agar plates and surviving bacteria were counted as CFU/ml after growth overnight at 37 °C. (B) Five replicate bactericidal peptide assays of RMAD-4 (light blue) and (R/K)-RMAD4 (dark blue) activity at 1.5 μM concentration against *L. monocytogenes*. Log-phase bacteria were exposed to peptides for 1 h in PIPES-TSB buffer, or in PIPES-TSB supplemented with 20 mM or 80 mM NaCl for 1 h. Following peptide exposure, the bacterial cell survival was determined as in panel A (see “Material and Methods”). Error bars denote standard deviation from the mean.