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Supporting Information

Self-assembling Ultrashort NSAID-Peptide Nanosponges: Multifunctional Antimicrobial and Anti-inflammatory Materials

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Ibuprofen-L-phenylalanine-L-phenylalanine-L-Lysine-L-Lysine-COOH (IbuFFKK). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.21-8.02 (m, J = 4.06, 4H; NH), 7.66 (s, J = 4.13, 4H; NH_2), 7.25-6.99 (m, J = 14.26, 14H; Ar H), 4.57-4.18 (m, J = 4.40, 4H, C*H*NH), 3.03 (q, J = 1.08, 1H; C*H*CH_3), 2.84-2.68 (m, J = 10.75, 8H; CH_2Ar, 2H; C*H*$_2NH_2$), 2.39 (d, J = 3.23, 3H; CH_3), 1.54-1.07 (m, J = 17.54, 2H; Ar C*H*$_2$CH(CH_3)$_2$, 1H; CH_2C*H*(CH_3)$_2$, 12H; CH_2), 0.86-0.83 (m, J = 6.51, 6H; CH_3). EIMS *m*/*z* (%): 756.46 (100) [M^+], 757.46 (46.5) [M^+ +H]$^+$, 758.46 (10.6) [M^++2H]$^+$; (ESI) *m*/*z*: [M + H]$^+$ calcd for $C_{43}H_{60}N_6O_6$, 756.99; found, 756.46.

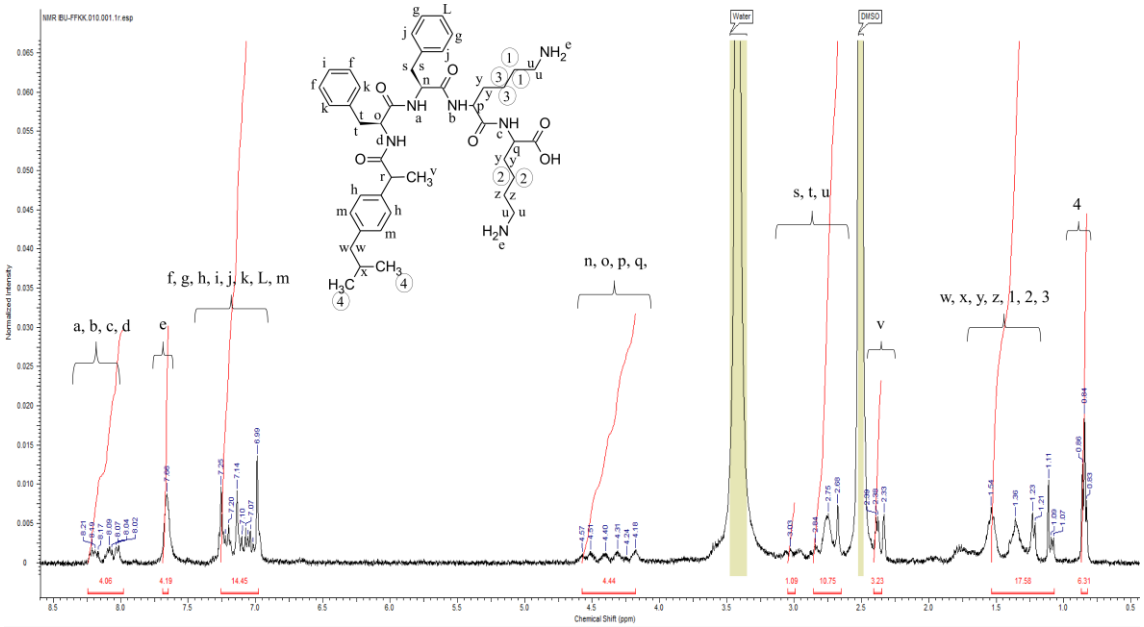

Figure S1. ^{1}H NMR.spectra for IbuFFKK (C_2D_6OS, TMS standard, 400MHZ).

Indomethacin-L-phenylalanine-L-phenylalanine-L-Lysine-L-Lysine-COOH (IndFFKK). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.21-8.16 (m, J = 2.59, 1H; NH, 1H; Ar H), 7.63-7.55 (m, J = 4.16, 1H; Ar H, 2H; NH), 7.22-6.62 (m, J = 17.33, 14H; Ar H, 1H; NH, 4H; NH_2), 4.56-

4.16 (m, *J* = 5.49, 4H; C*H*NH), 3.74-3.67 (m, *J* = 4.41, 3H; CH_3, 2H; CH_2CO), 3.03-2.68 (m, *J* = 14.50, 4H; CH_2NH_2, 4H; CH_2 Ar), 2.33 (s, *J* = 3.02, 3H; CH_3), 1.89-1.24 (m, *J* = 21.08, 12H; CH_2). EIMS *m*/*z* (%): 893.39 (100) [M^+], 894.39 (51.9) [$M^+ - H$], 895.38 (32) [$M^+ - 2H$], 896.39 (16.6); (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{49}H_{58}ClN_7O_8$, 894.47; found, 893.39.

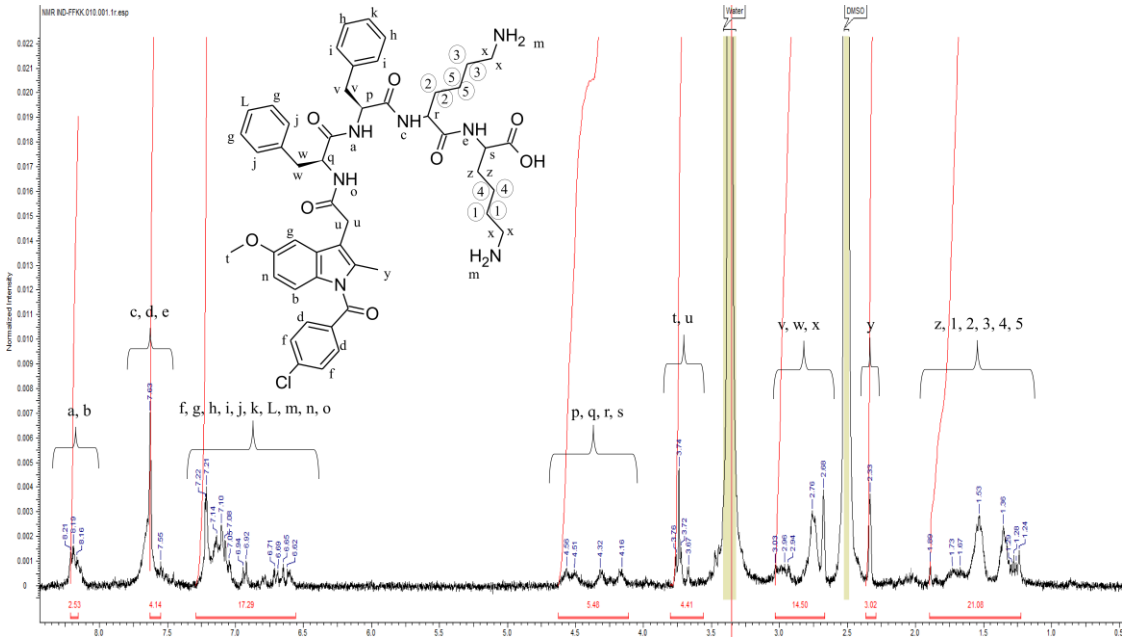

Figure S2. ^{1}H NMR.spectra for IndFFKK (C_2D_6OS, TMS standard, 400MHZ).

Naproxen-L-phenylalanine-L-phenylalanine-L-Lysine-L-Lysine-COOH (NpxFFKK).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.22-8.04 (m, *J* = 5.53, 3H; NH, 2H; Ar H), 7.75-7.05 (m, *J* = 21.43, 14H; Ar H, 4H; NH_2), 4.50-3.71 (m, *J* = 6.20**,** 4H; C*H*NH, 1H; Ar C*H*CH_3), 2.98-2.68 (m, *J* = 9.97 4H; Ar CH_2, 4H; CH_2NH_2), 2.33 (s, *J* = 3.10, 3H; CH_3), 1.73-1.03 (m, *J* = 21.71**,** 12H; CH_2, 3H; CH_3). EIMS *m*/*z* (%): 780.42 (100) [M^+], 781.42 (47.6) [$M^+ - H$], 782.43 (11.1) [$M^+ - 2H$]; (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{44}H_{56}N_6O_7$, 780.97; found, 780.42.

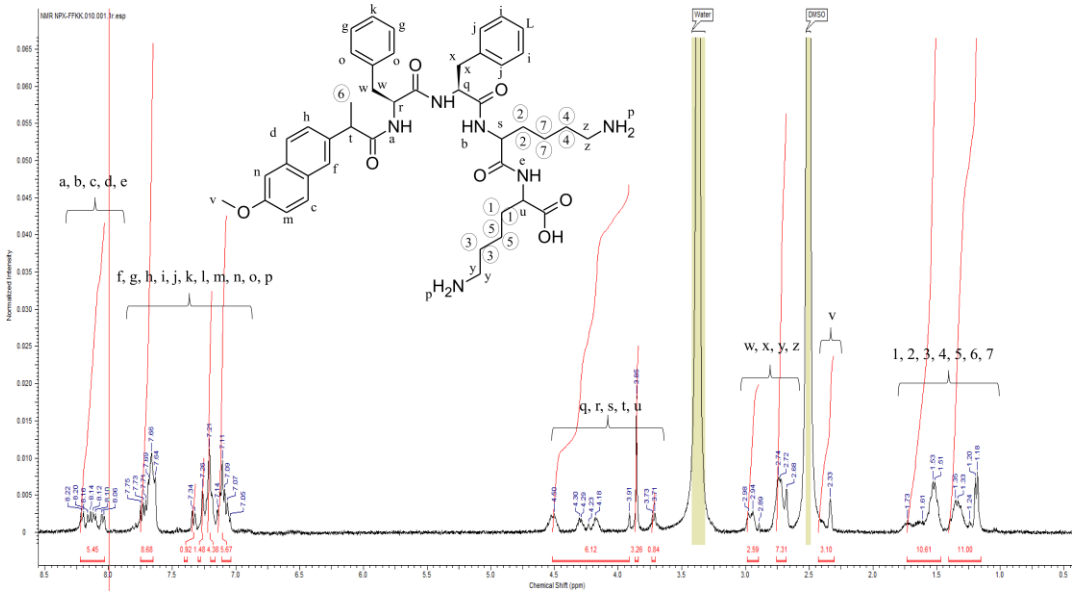

Figure S3. ^{1}H NMR.spectra for NpxFFKK (C_2D_6OS, TMS standard, 400MHZ).

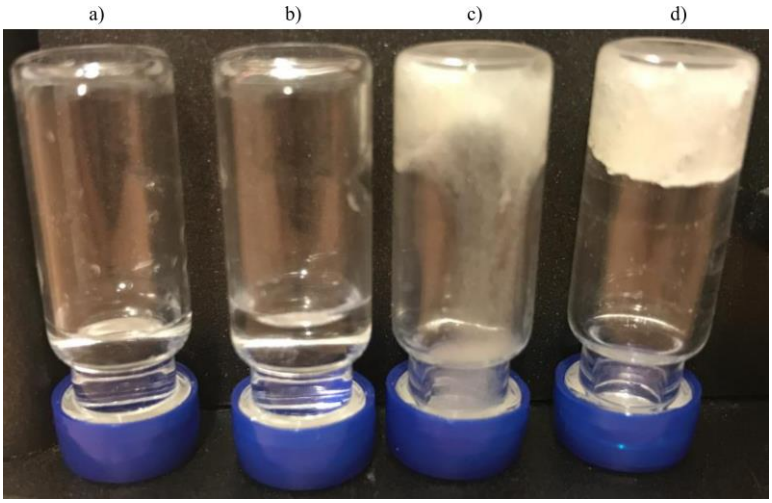

Figure S4. Gel inversion assay for IbuFFKK pH 7.4, H_2O primary vehicle. a) 0.5% w/v, b) 1.0% w/v, c) 1.5% w/v, d) 2.0% w/v.

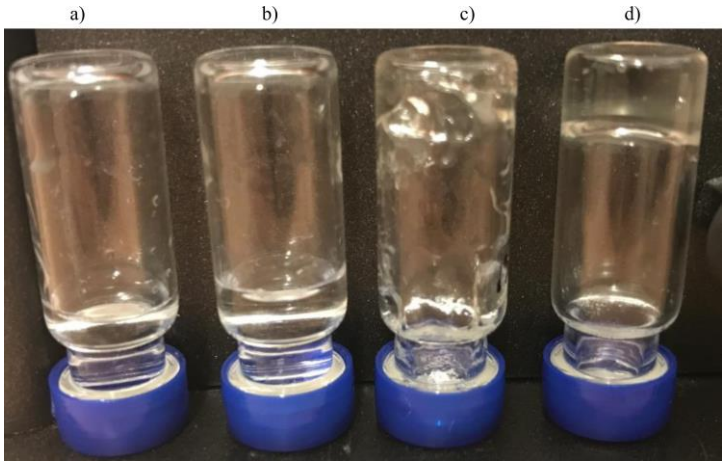

Figure S5. Gel inversion assay for IbuFFKK pH 7.4, D_2O primary vehicle, a) 0.5% w/v, b) 1.0% w/v, c) 1.5% w/v, d) 2.0% w/v.

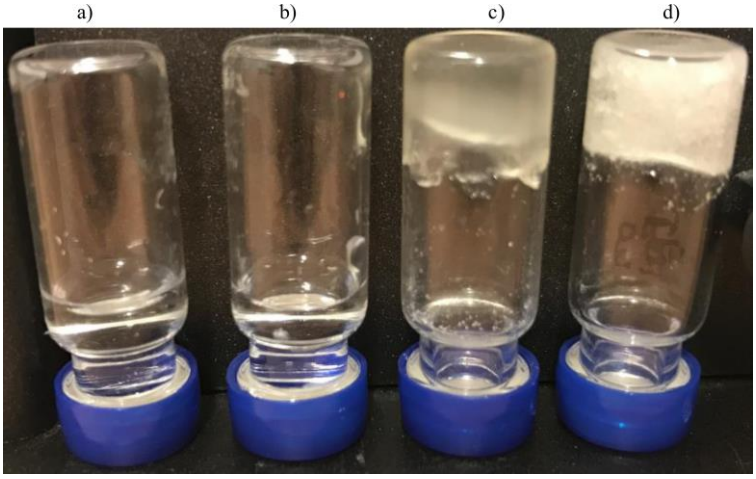

Figure S6. Gel inversion assay for IndFFKK pH 7.4, H_2O primary vehicle, a) 0.5% w/v, b) 1.0% w/v, c) 1.5% w/v, d) 2.0% w/v.

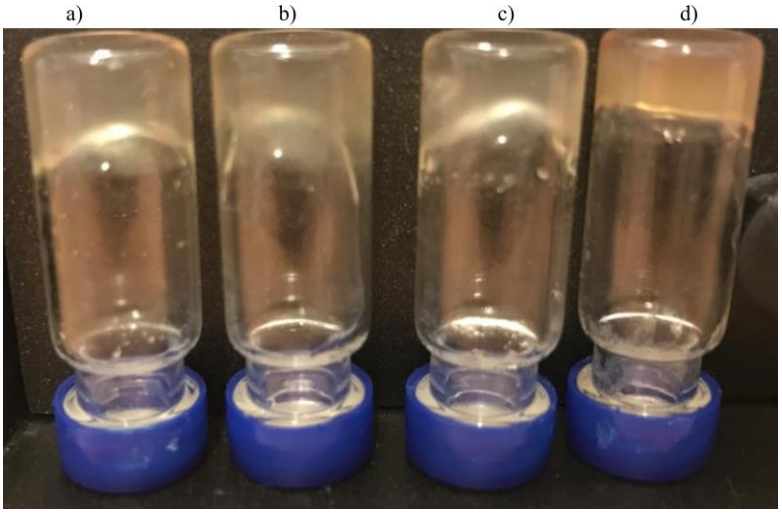

Figure S7. Gel inversion assay for NpxFFKK pH 7.4, H_2O primary vehicle, a) 0.5% w/v, b) 1.0% w/v, c) 1.5% w/v, d) 2.0% w/v

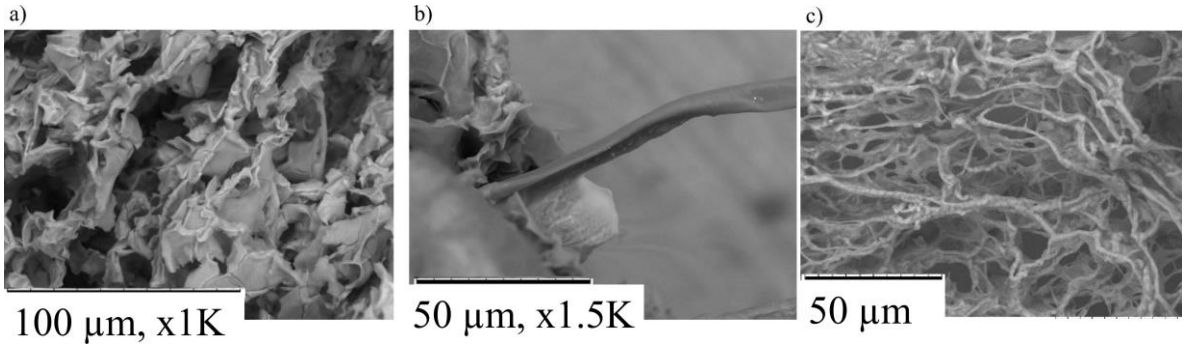

Figure S8. Cryo-SEM images of 2% w/v (a) IbuFFKK (D_2O), (b) IndFFKK (H_2O), (c) NpxFFKK (H_2O).

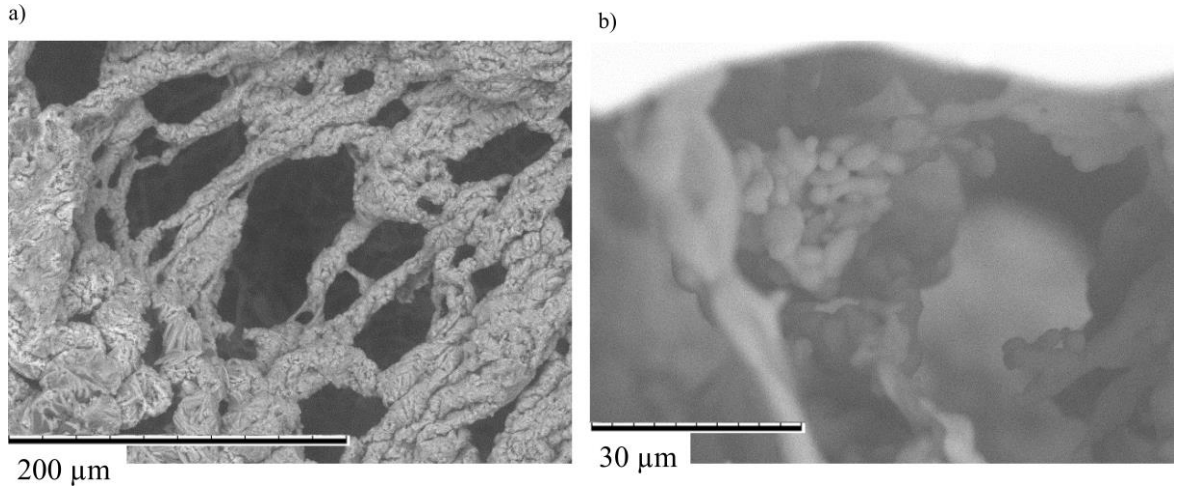

Figure S9. Cryo-SEM images of 2% w/v IbuFFKK (H_2O primary vehicle).

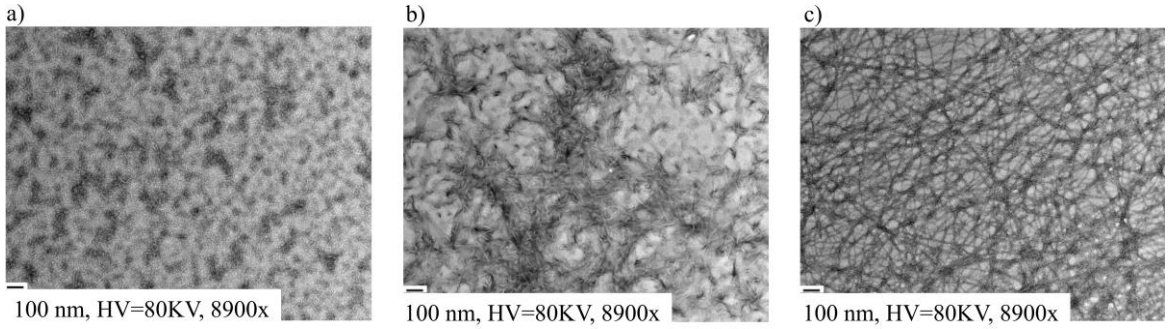

Figure S10. TEM images of 2% w/v (8900x) (a) IbuFFKK (D_2O), (b) IndFFKK (H_2O), (c)NpxFFKK (H_2O).

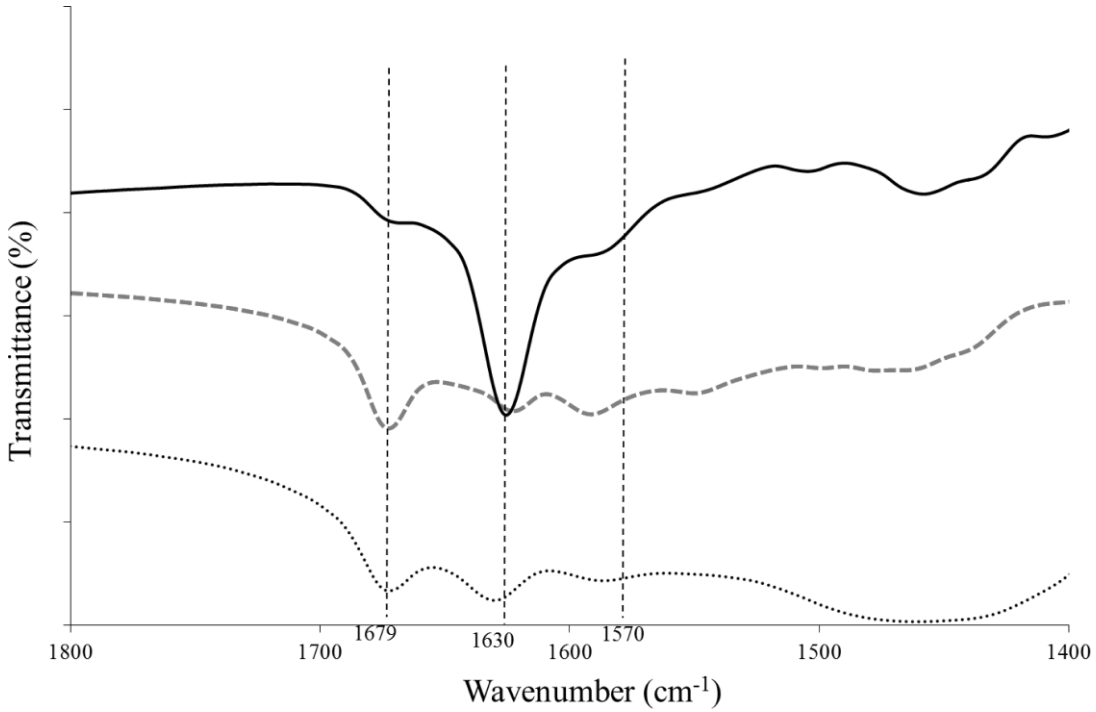

Figure S11. FTIR spectra displaying amide band of 2% w/v NSAID-peptides in deuterated solvents. Key: dotted line: IbuFFKK, dashed line: IndFFKK , full line: NpxFFKK.

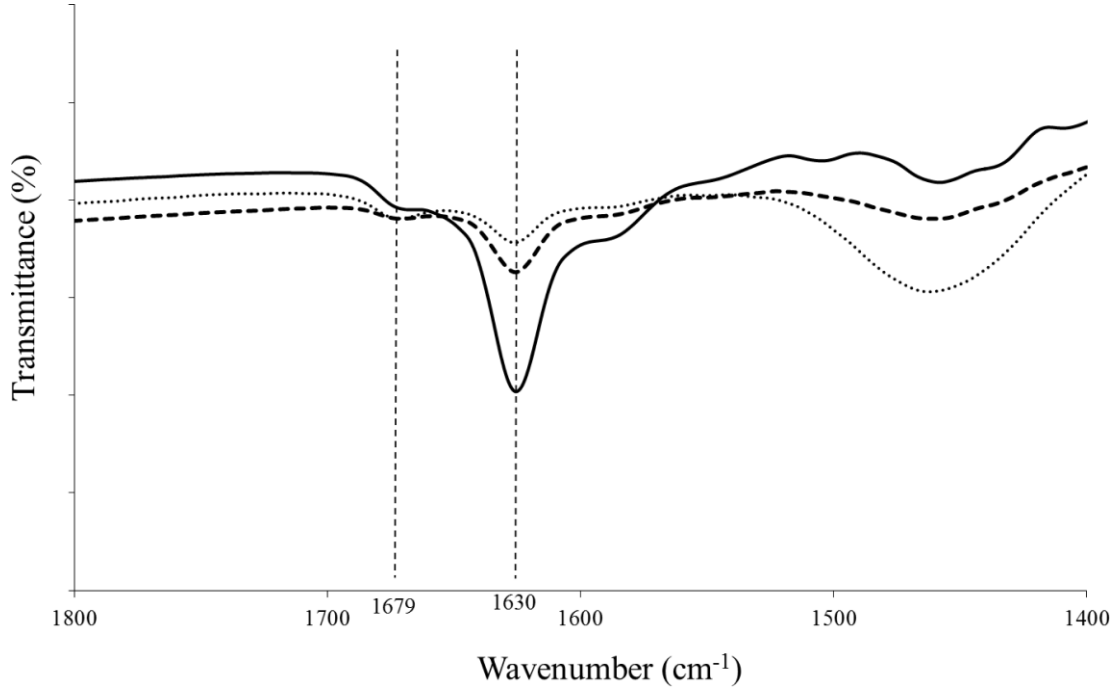

Figure S12. FTIR spectra displaying amide band of 0.5-2% w/v NpxFFKK peptide. Key: dotted line: 0.5% w/v, dashed line: 1.5% w/v, full line: 2% w/v.

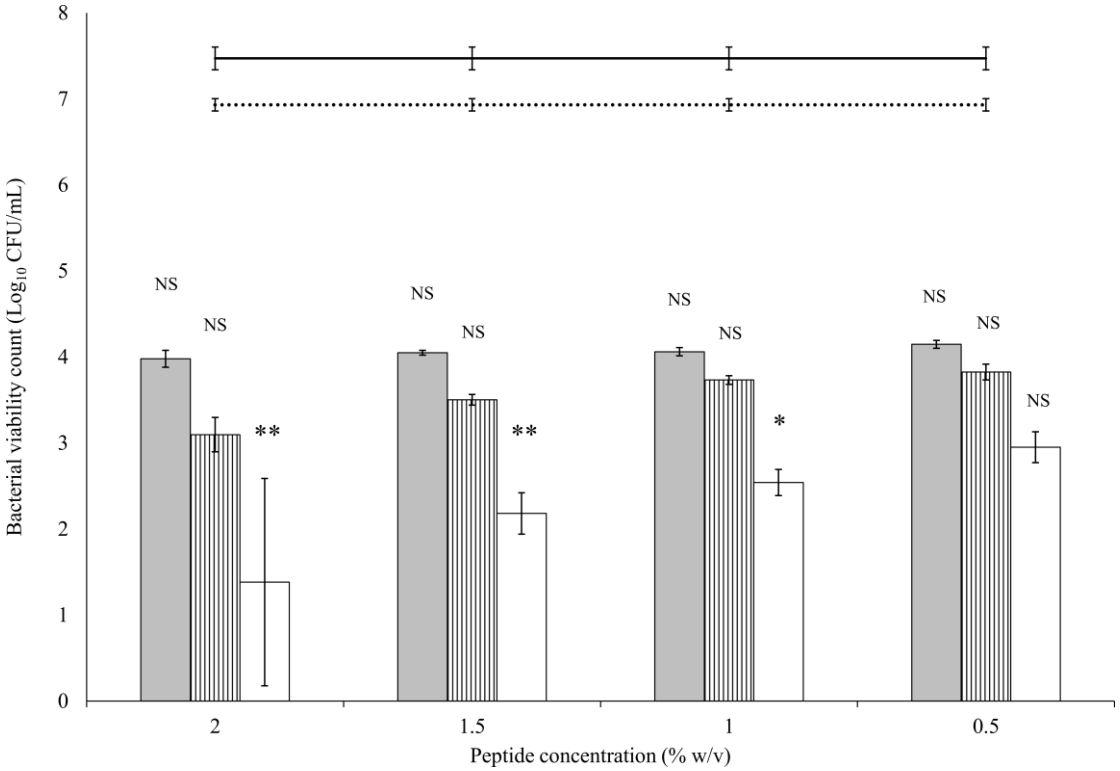

Figure S13. Logarithmic reduction in *S. epidermidis* (ATCC 35984) viable count (Log_{10} CFU/mL) after 24 hour incubation with varying concentrations of NSAID-peptides. Results are displayed as a mean of six replicates. Key: grey column: IbuFFKK, striped column: IndFFKK, white column: NpxFFKK, dotted line: PBS control, black line: 2% w/v HPMC control. NS: no significant difference ($P \geq 0.05$), *: $P < 0.05$, **: $P < 0.01$ significant difference between Log_{10} CFU/mL of NSAID-peptide and the negative control (PBS).

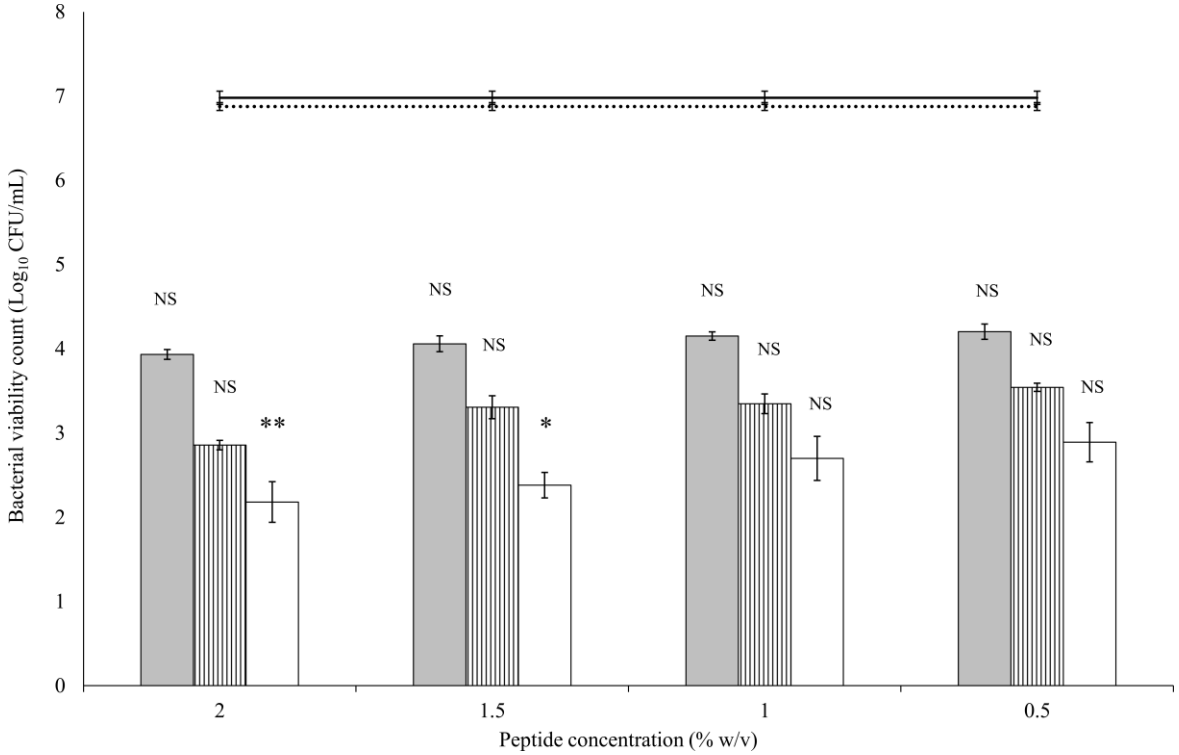

Figure S14. Logarithmic reduction in *E. coli* (ATCC 11303) viable count (Log_{10} CFU/mL) after 24 hour incubation with varying concentrations of NSAID-peptides. Results are displayed as a mean of six replicates. Key: grey column: IbuFFKK, striped column: IndFFKK, white column: NpxFFKK, dotted line: PBS control, black line: 2% w/v HPMC control. NS: no significant difference ($P \geq 0.05$), *: $P < 0.05$, **: $P < 0.01$ significant difference between Log_{10} CFU/mL of NSAID-peptide and the negative control (PBS).

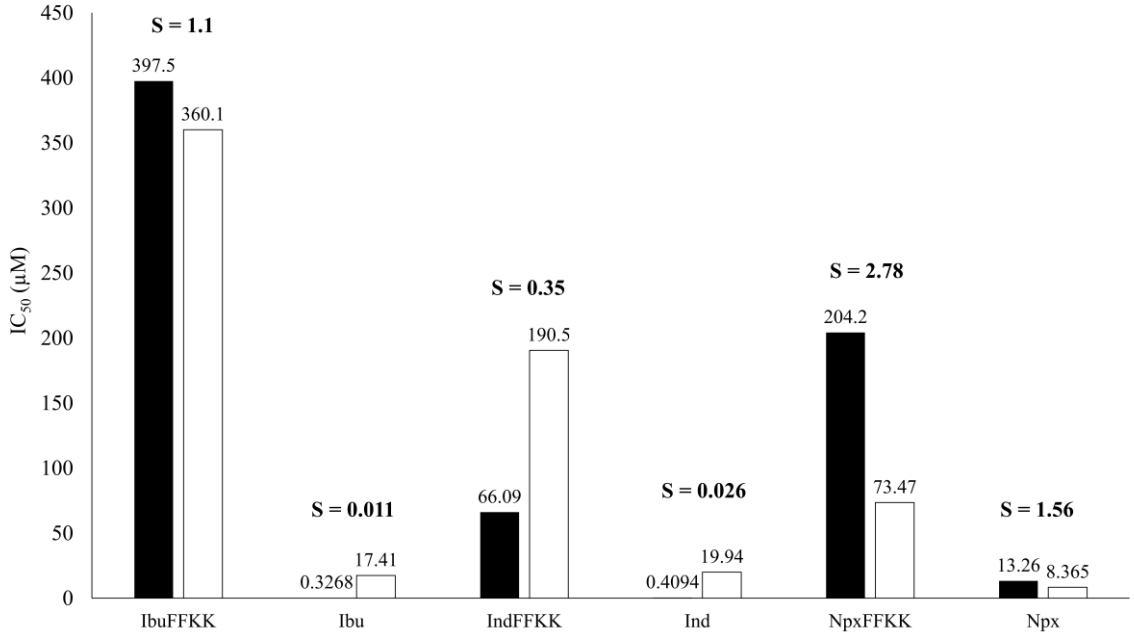

Figure S15. IC_{50} values of NSAID-peptide and NSAIDs only molecules corresponding to inhibition of COX-1 (black column) and COX-2 (white column) enzymes. Selectivity (S) is defined as the ratio of the IC_{50} values relative to COX-1: COX-2.

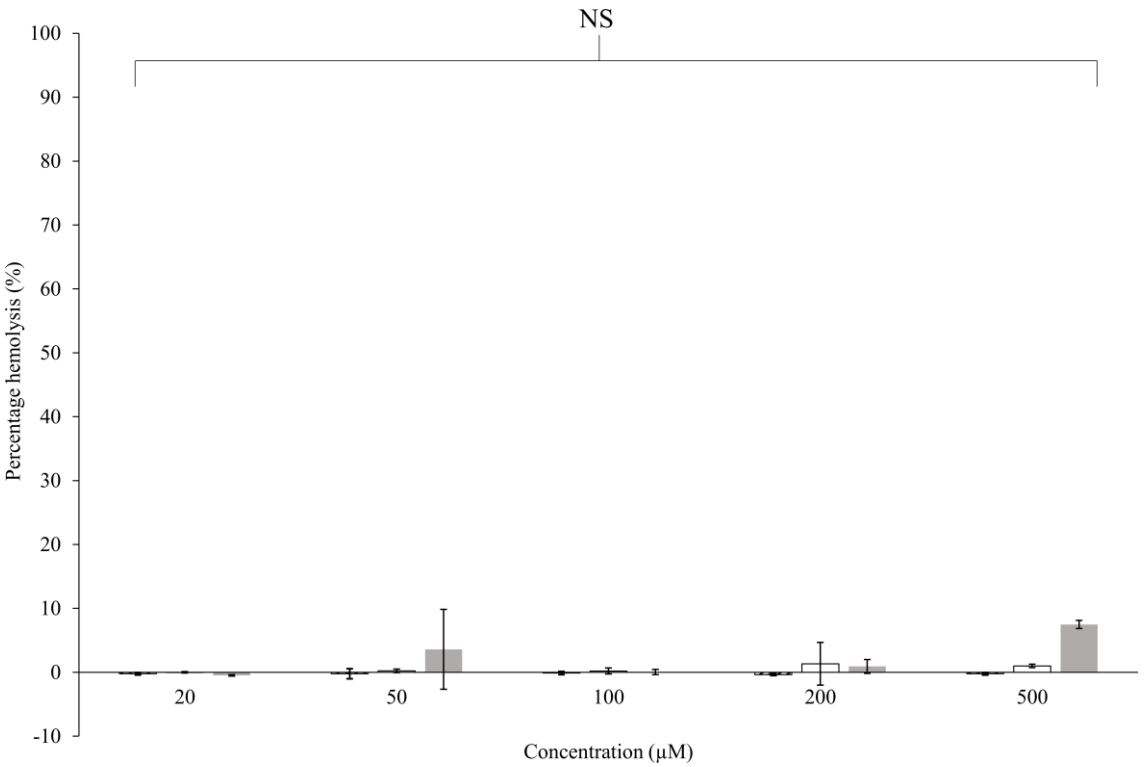

Figure S16. Percentage hemolysis of equine erythrocytes after 1 hour exposure to varying concentrations of NSAID-peptides. Key: striped: IbuFFKK, white: IndFFKK, grey: NpxFFKK, NS: no significant difference (P≥0.05) between the NSAID-peptide and the negative control (PBS).

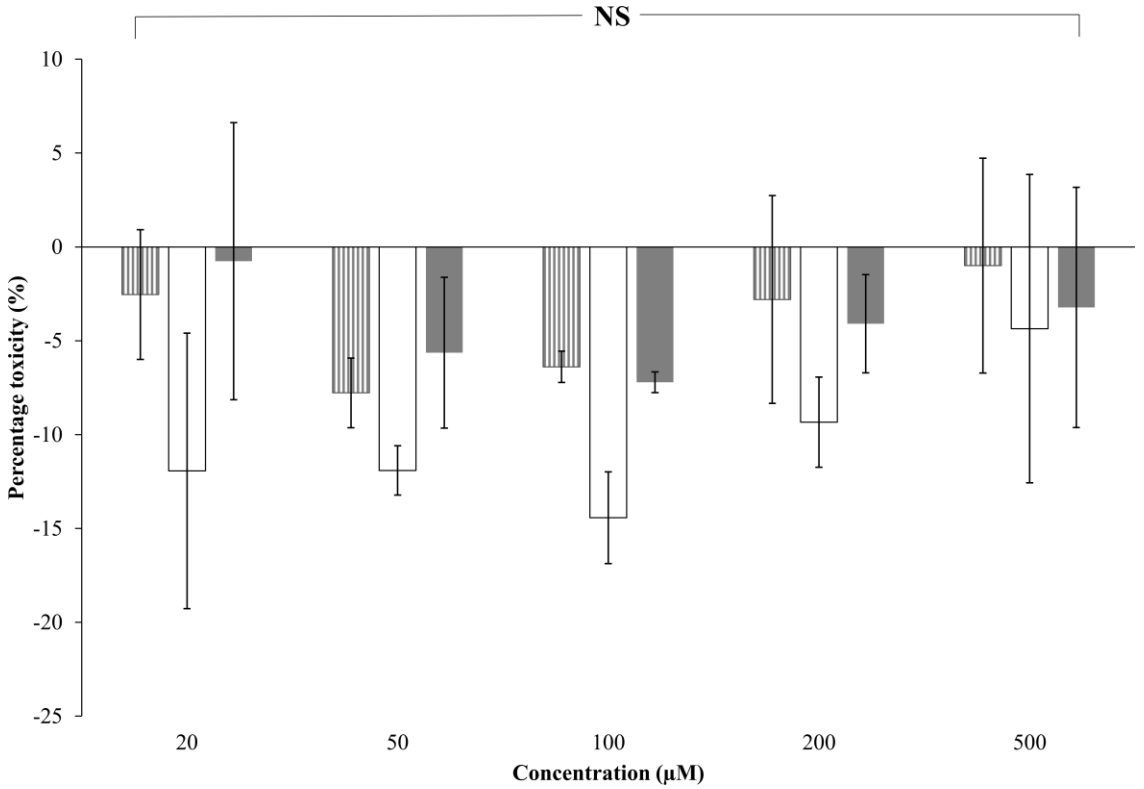

Figure S17. Percentage toxicity of NCTC clone 929 (ATCC CCL 1) cells after 24 hour exposure to varying concentrations of NSAID-peptides. Toxicity is calculated by quantifying

LDH release. Key: striped: IbuFFKK, white: IndFFKK, grey: NpxFFKK, ns: no significant difference (P≥0.05) between the NSAID-peptide and the negative control (PBS).

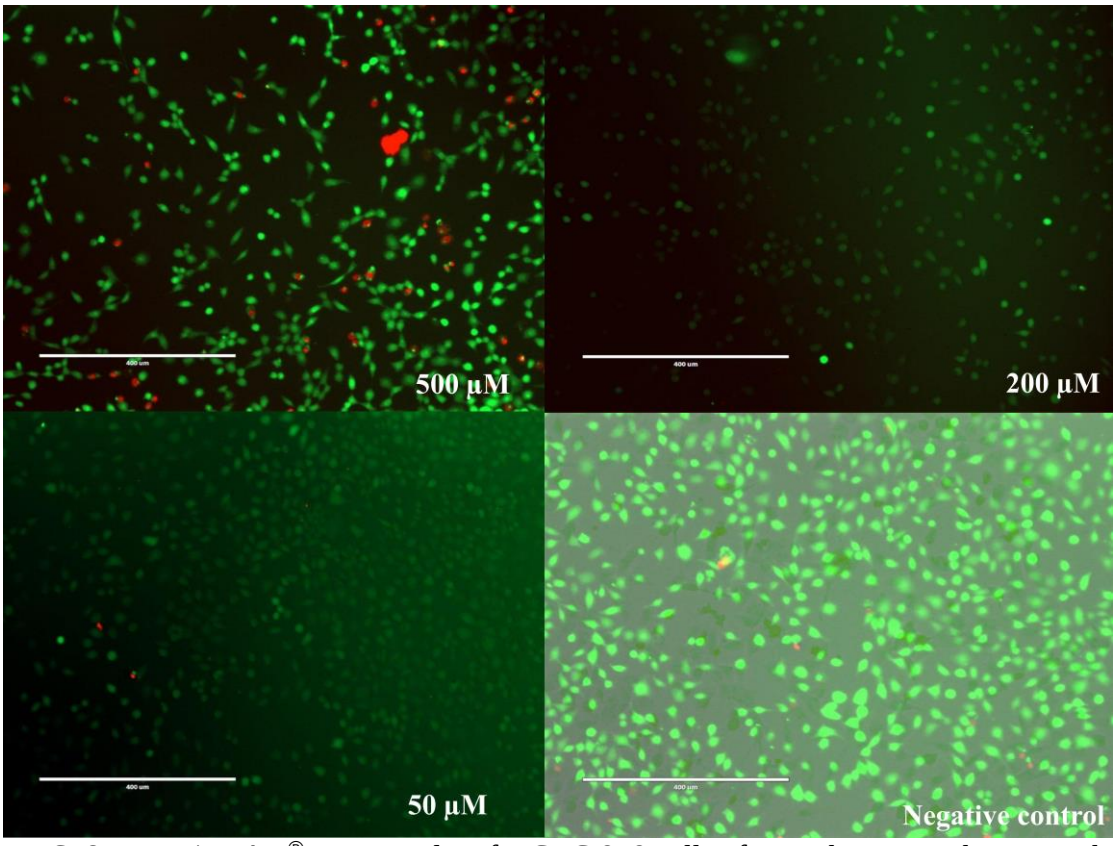

Figure S18. LIVE/DEAD® stain results of NCTC 929 cells after 24 hours incubation with IbuFFKK. Scale bar represents 400 µm, green staining indicates live cells, red staining indicates dead cells.

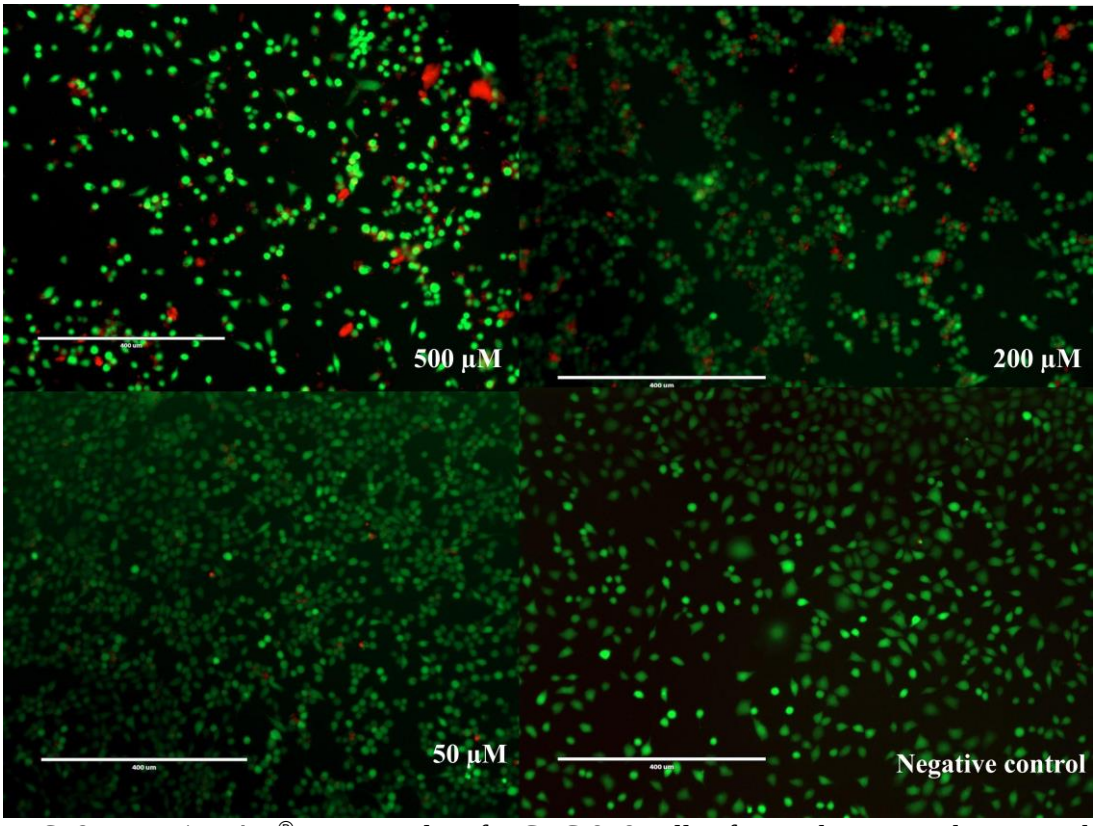

Figure S19. LIVE/DEAD® stain results of NCTC 929 cells after 24 hours incubation with IndFFKK. Scale bar represents 400 µm, green staining indicates live cells, red staining indicates dead cells.

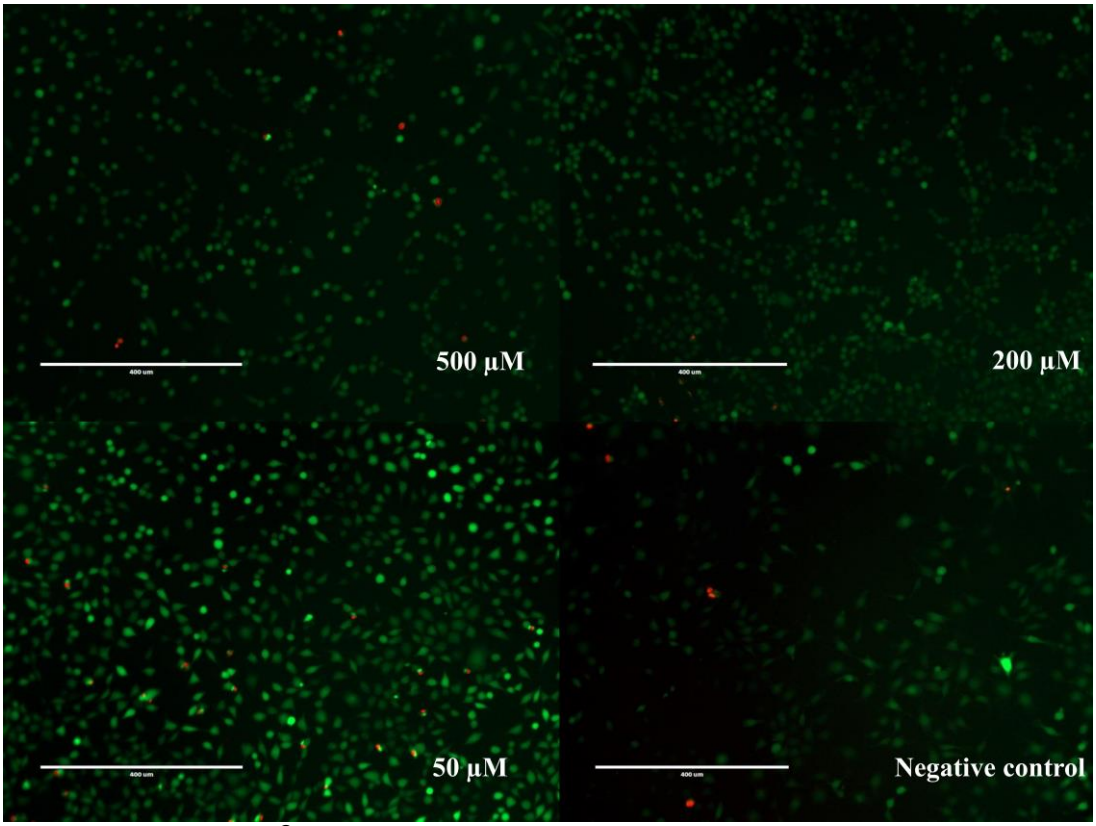

Figure S20. LIVE/DEAD® stain results of NCTC 929 cells after 24 hours incubation with NpxFFKK. Scale bar represents 400 µm, green staining indicates live cells, red staining indicates dead cells.

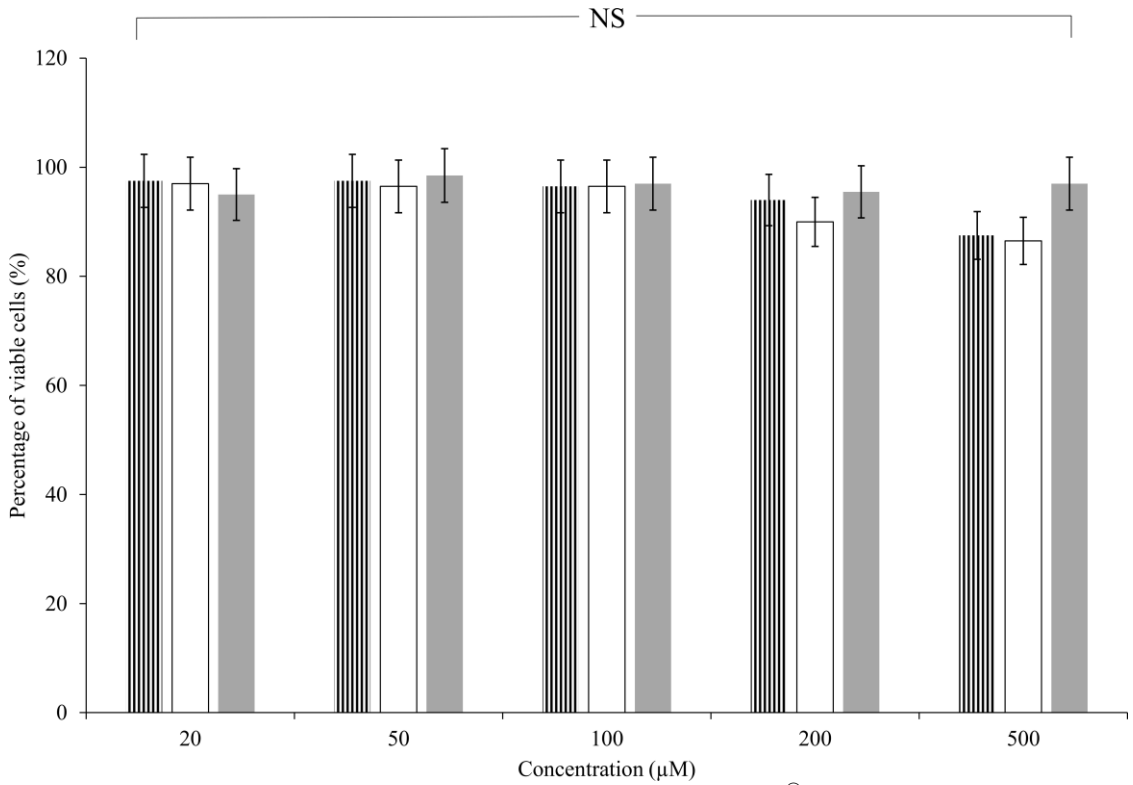

Figure S21. Quantitative cell counting analysis of LIVE/DEAD® stain after 24 hour exposure to varying concentrations of NSAID-peptides. Key: striped: IbuFFKK, white: IndFFKK, grey: NpxFFKK, ns: no significant difference ($P \geq 0.05$) between the NSAID-peptide and the negative control (PBS).