

Supplementary Information

**Development of a Dextrin-Vitamin D3 Micelle Nanocarrier for the  
Antimicrobial Peptide LLKKK18 as a potential therapeutic agent for  
Bone Infections**

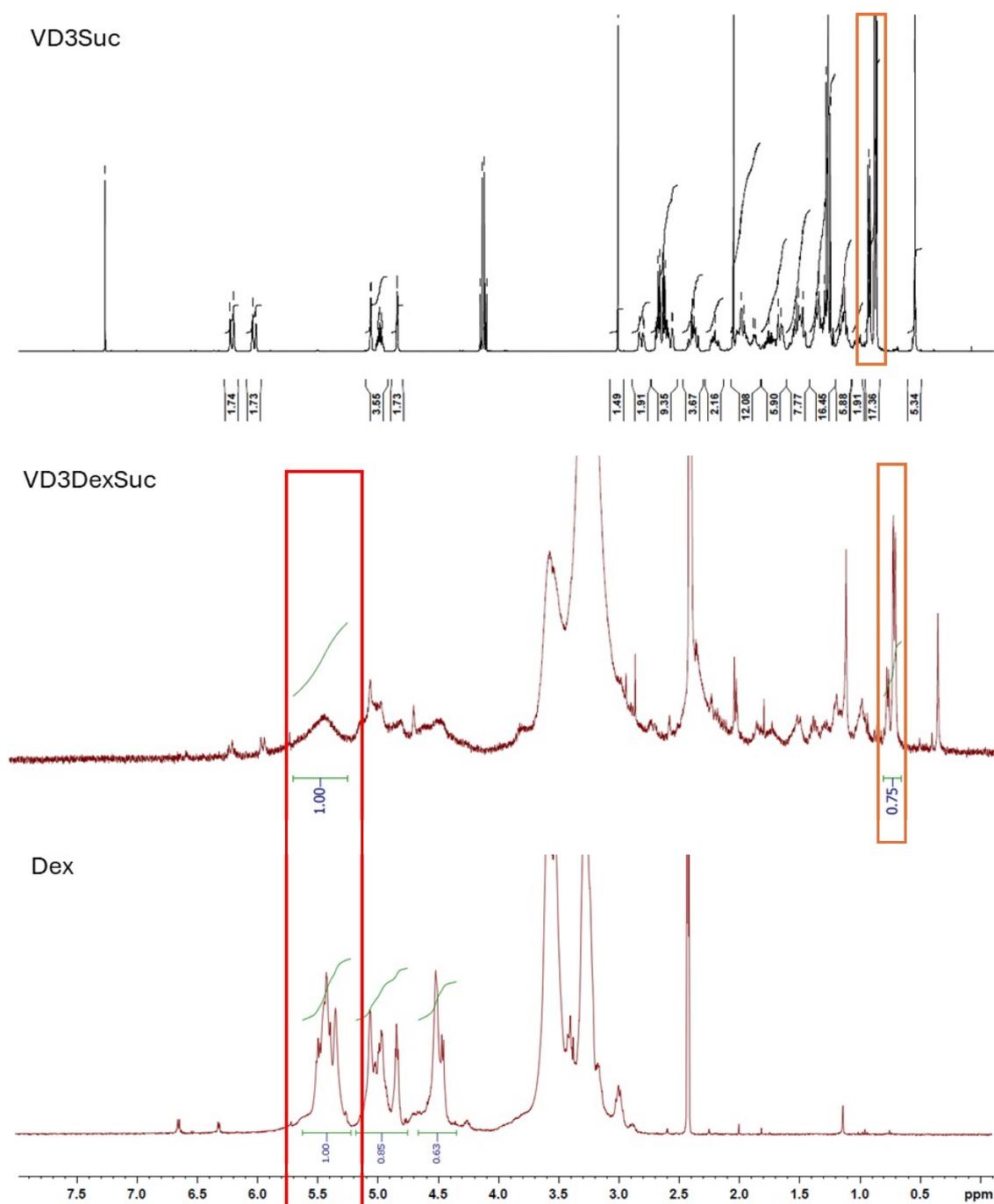
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**Figure SI 1.**  $^1\text{H}$  NMR spectra: SucVD3 (400 MHz,  $\text{CDCl}_3$ ); conjugate VD3DexSuc (400 MHz,  $\text{DMSO}_d_6$ ) and Dextrin (400 MHz,  $\text{DMSO}_d_6$ ).

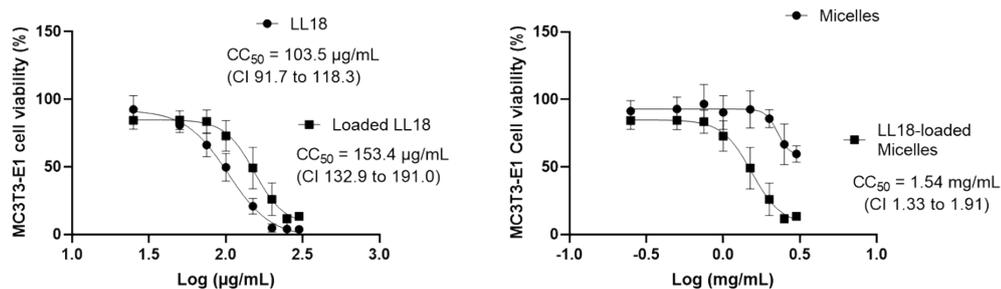
The signal at  $\delta$  5.3-5.8 ppm, can be assigned to the  $\alpha$ -anomeric protons of the glucose units of dextrin. Some signals can be clearly assigned to protons of the SucVD3 moiety, especially the duplet signals at  $\delta$  0.83 and 0.88 ppm, attributed to the isopropyl methyl groups and to the methyl group of the hydrocarbon chain of VD3, respectively. The broad multiplet signal  $\delta$  2.49 ppm, overlapped with the

residual DMSO signal, is assigned to methylene protons of the succinic acid linker of the SucVD3 moiety and to the pendant succinic acid groups (Figure 1 SI). The degree (percentage) of derivatization of the dextrin backbone with SucVD3 (8.3 %) was estimated by equation 1.

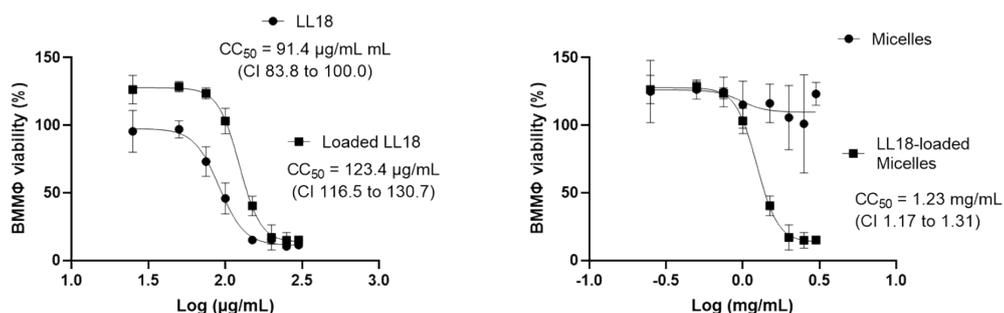
$$\text{Mol\% grafting of Dex with SucVD3} = \frac{\frac{0.75}{9}}{1} \times 100 = 8.3 \text{ mol\% (eq. 1)}$$

$x$  (0.75) represents the aggregated integration of the signals assigned to the methyl protons of the isopropyl group and to the methyl group of the hydrocarbon chain (9 protons) of SucVD3 and  $y$  (1.0) is the integration of the  $\alpha$ -anomeric protons of the glycosidic units (1 proton) of dextrin.

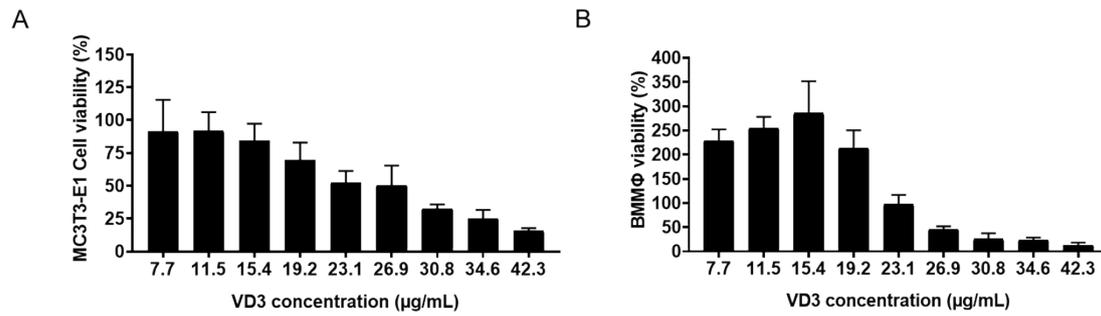
A



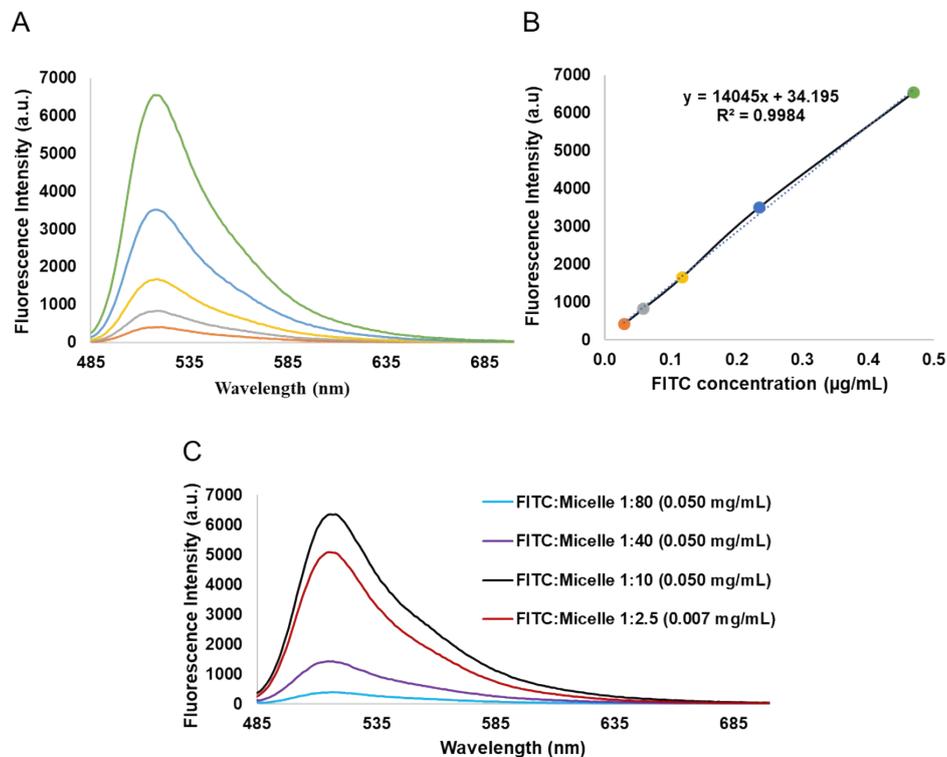
B



**Figure SI 2.** Dose-response (cell viability) curves for cells exposed for 24 h to different concentrations of free LLKKK18 (LL18), loaded-LL18, micelles and LL18-loaded micelles in: (A) MC3T3-E1 and B) BMMΦ, assessed by the MTT reduction assay, for half-maximal inhibitory concentration ( $CC_{50}$ ) calculation. Data are presented as mean  $\pm$  standard deviation ( $n = 3$ ).



**Figure SI 3.** MTT assay dose-dependent cell viability in the MC3T3-E1 cell line (A) and in BMMΦ macrophages (B) after 24 hrs incubation with vitamin D3. Results are expressed as percentage of the negative control and are presented as mean ± SD, n=3.



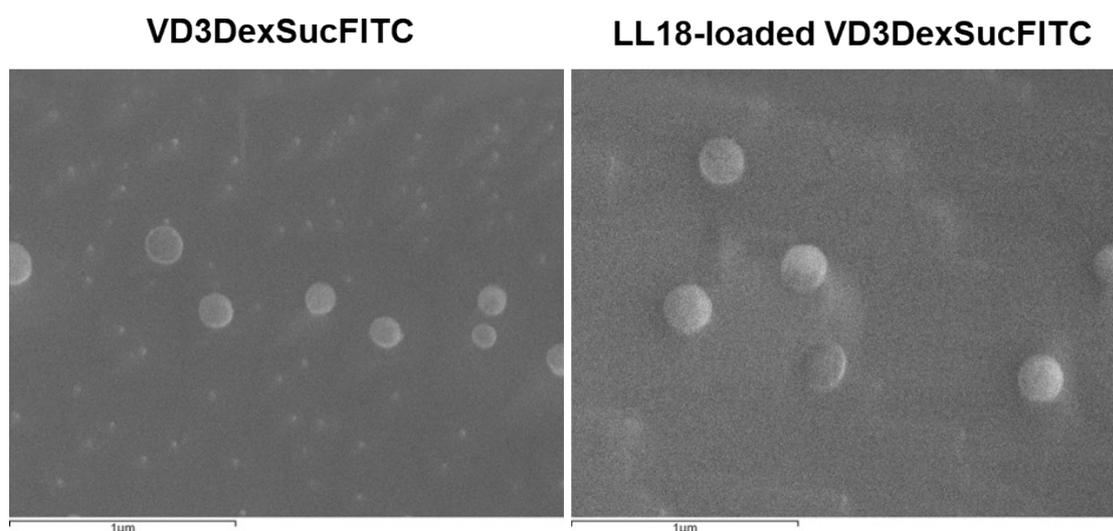
**Figure SI 4.** Micelle-conjugated fluorescein isothiocyanate (FITC) quantification FITC by fluorescence spectroscopy ( $\lambda_{ex477}/\lambda_{em517}$ ): (A) spectral scanning curves of five different pure FITC concentrations, (B) FITC standard curve and (C) spectral scanning curves of four different FITC-VD3-Dex production ratios. Concentrated stock solutions of pure FITC and FITC-VD3-Dex were prepared in DMSO and then serially diluted in PBS solution up to a fluorescent signal that could fit within the calibration curve range.

**Table SI 1.** Dynamic light scattering measurements of unlabeled and FITC-labelled micelles, dissolved in PBS at a constant concentration of 1 mg/mL, and quantification of conjugated FITC by fluorescence spectroscopy ( $\lambda_{ex}477/\lambda_{em}517$ ). Results for LL18 or LL18-TAMRA-loaded micelles are also shown. Results are expressed as mean  $\pm$  SD (n=3).

FITC:VD3DexSuc initial ratio (mg:mg)	Average size (nm)	PDI	$\zeta$ -potential (mV)	FITC ( $\mu$ g)/mg of micelle	Initial LL18 ( $\mu$ g/mL)	LL18 AE (%)
Unlabelled micelles	162.4 $\pm$ 8.9	0.17 $\pm$ 0.02	- 35.2 $\pm$ 2.5			
1:40	124.7 $\pm$ 2.7	0.18 $\pm$ 0.03	- 19.7 $\pm$ 1.8	1.90 $\pm$ 0.08		
Unlabelled micelles	143.1 $\pm$ 4.6	0.21 $\pm$ 0.01	- 26.1 $\pm$ 1.5		100	86.3 $\pm$ 0.4
1:40	147.8 $\pm$ 8.7	0.22 $\pm$ 0.01	- 3.9 $\pm$ 0.1		25	100 $\pm$ 0.0
1:40	204.3 $\pm$ 10.9	0.41 $\pm$ 0.02	- 8.9 $\pm$ 0.3		100	89.7 $\pm$ 0.1
Unlabelled micelles	142.5 $\pm$ 8.5	0.15 $\pm$ 0.03	- 25.8 $\pm$ 2.1		25*	
1:40	96.8 $\pm$ 4.2	0.12 $\pm$ 0.01	- 14.8 $\pm$ 0.1		25*	

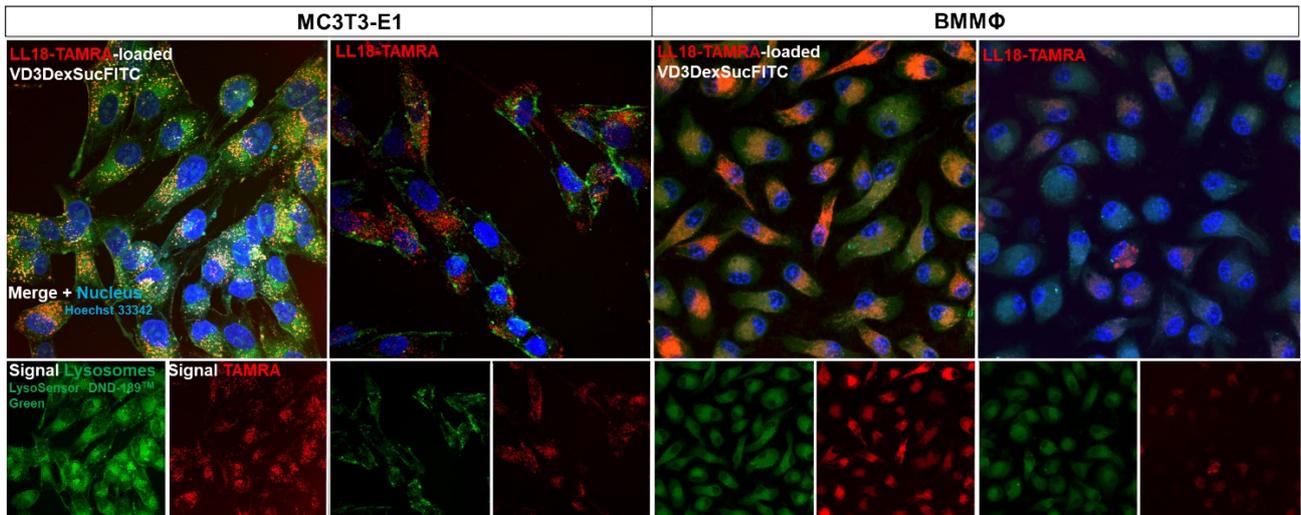
LL18, LLKKK18; FITC, fluorescein isothiocyanate; PDI, polydispersity index; AE, association efficiency.

\*LL18-TAMRA (Based on LL18 AE % results, loading of LL18-TAMRA is assumed as 90 to 100 %).

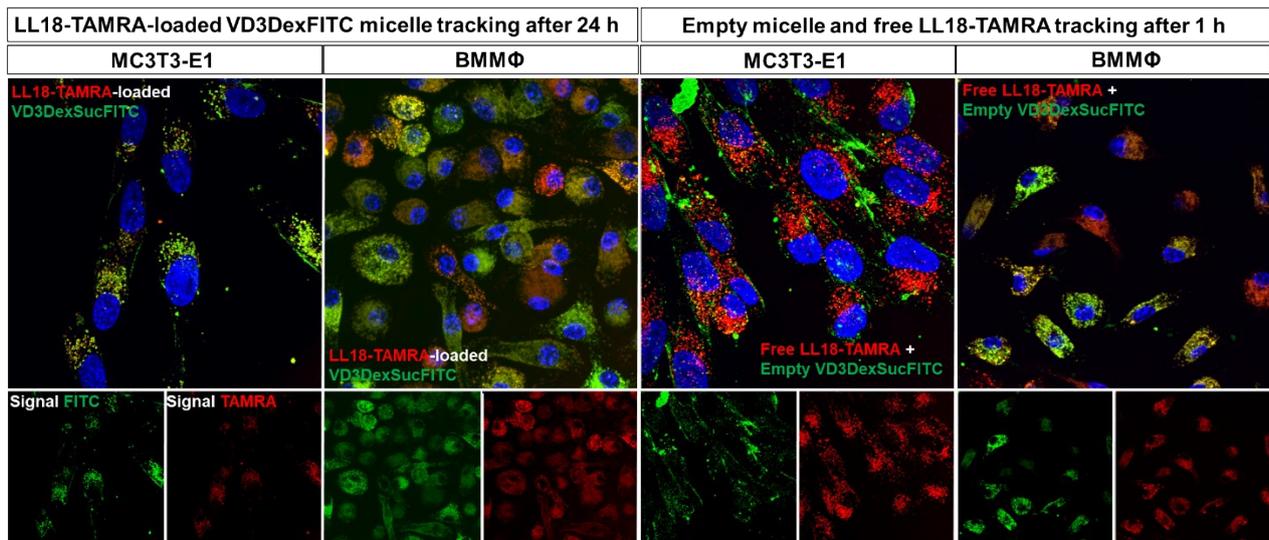


**Figure SI 5.** Representative Cryo-SEM microphotographs of (A) FITC-labelled micelles (1:40 mass ratio) and (B) LL18-loaded FITC-labelled micelles (100  $\mu$ g/mL of LL18/mg micelles), in PBS solution at 1 mg/mL (magnification of 50,000 $\times$ . Scale bars = 1  $\mu$ m).

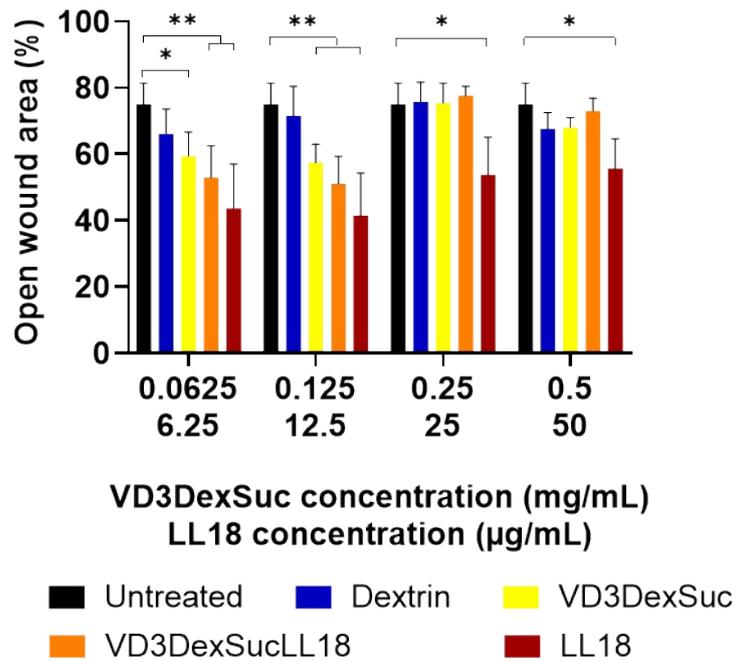
### LL18-TAMRA tracking after 1 h



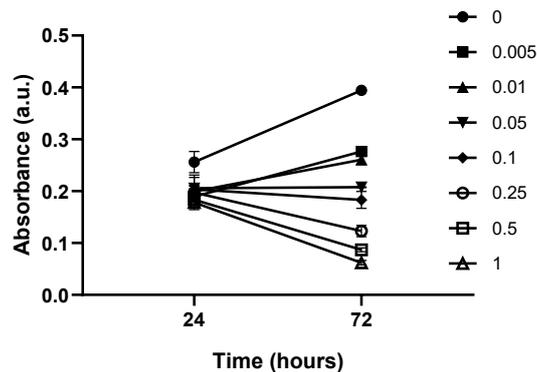
**Figure SI 6.** Subcellular localization analysis by confocal laser scanning microscopy (CLSM) of free and micelle-loaded LL18-TAMRA after 1 h incubation MC3T3-E1 (60x magnification) and BMMΦ (100x magnification). Formulation of 25 µg:1 mg peptide:micelle ratio, incubated at 0.5 mg/mL. Green signal refers to lysosome probe, red signal to TAMRA.



**Figure SI 7.** Subcellular localization analysis using a confocal laser scanning microscope (CLSM) of: (left) FITC-labelled micelles loaded with TAMRA-labelled LL18 after a 24 h incubation; and (right) free TAMRA-labelled LL18 and empty FITC-labelled micelles after 1 h incubation each, separately, in MC3T3-E1 (60x magnification) and BMMΦ (100x magnification). Formulation of 25 µg:1 mg (peptide:micelle ratio), incubated at 0.5 mg/mL. Lysosomes are not marked, green signal refers to FITC, red signal to TAMRA.



**Figure SI 8.** Quantification (%) of open wound area in MC3T3-E1 cells after 24h (n=6). Results presented as mean  $\pm$  SD (\* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001). Formulation of 100 $\mu$ g:1mg peptide:micelle ratio.



**Figure SI 9.** Dose( $\mu$ g/mL)-response results for MC3T3-E1 cells exposed up to 72 h to different concentrations of mitomycin C dissolved in culture medium, assessed by the MTT reduction assay. Typical cell seeding for this assay was reduced to a half ( $0.5 \times 10^5$  cell/mL) in order to provide space for a 72-h proliferation. Results were expressed in comparable absorbance values. A concentration of 0.05  $\mu$ g/mL was shown to inhibit cell proliferation whilst maintaining viability and, therefore, selected for the wound healing assay. Data are presented as mean  $\pm$  SD (n = 3).