Supplementary information

An injectable supramolecular hydrogel as self-drug-delivery system for local chemoimmunotherapy against melanoma

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Experimental Section:

1. Materials and Methods for Physical Measurements:

All the reagents and chemicals used herein were purchased from commercial sources and used without further purification. 5-amino-1,10-phenanthroline (5-AP), NSAIDs, Dyes for biological experiments i.e., Hoechst 33342 (HO), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), JC-1, 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA), Hematoxylin and, eosin were purchased from Sigma-Aldrich. PE-CF594 Rat Anti-Mouse CD31 was gifted by Dr. Amitava Sengupta, CSIR-IICB, Kolkata. Annexin V-FITC apoptosis kit was obtained from Invitrogen (Thermo Fisher Scientific). PGE_2 ELISA assay kit was obtained from Cayman chemical. Verapamil was purchased from TCI Chemicals. Glutaraldehyde solution (25% w/w) was purchased from HiMedia. Liberase^{TM}, Mitotracker^{TM}, Lysotracker^{TM}, anti Ki-67 antibody, Alexa Fluor plus 488 tagged anti rabbit secondary antibody and RNase A were purchased from Thermo Fischer Scientific. Antibodies for cytokines were obtained from BD Biosciences. Perkin Elmer, FT-IR Spectrometer, Spectrometer Two instrument was used to record FT-IR spectra. Elemental analysis was carried on PerkinElmer Precisely, Series-II, CHNO/S Analyser-2400 instrument. Powder X-ray diffraction patterns were recorded on Bruker AXS D8 Advance Powder (CuKα1 radiation, λ=1.5406 Å) X-ray diffractometer and Rigaku SmartLab (40 kV, 110 mA) equipped with an 0D detector (HyPix-3000) using CuKα1 radiation (λ=1.5406Å) at a scan speed 50 °/min. TEM photographs were taken using a JEOL JEM 2100F instrument with 300 mesh copper TEM grid. SEM images were captured in a GeminiSEM 500 ZEISS instrument. Cryo-TEM images were taken in a JEOL JEM-2100Plus instrument operated at 120 kV. UV/Vis spectroscopic measurements were performed on a Hewlett–Packard 8453 diode array spectrophotometer equipped with a Peltier temperature controller. Fluorescence spectroscopic measurements were carried out on a FluoroMax-3 spectrometer from Horiba Jobin Yvon. MCR 102 Anton Paar modular compact rheometer was used to perform rheological experiments. Mice melanoma cell (B16-F10), mice macrophage cell (RAW 264.7), mice muscle cell (C2C12) and horse dermis cell (E. Derm) were purchased from National Centre for Cell Sciences, Pune, India. Multi-plate ELISA reader (Varioskan Flash Elisa Reader, Thermo Fisher) was used to carry MTT assay. CARL-ZEISS inverted laser scanning confocal microscope (LSM880) was used to perform cell imaging experiments. BD LSR Fortessa flow cytometer was used to collect FACS data. Still images of the cells were captured in an OLYMPUS CKX31 microscope. Still images of tissues were captured in an

OLYMPUS BX53M microscope equipped with an optical polariser. Animal experiments were performed under the ethical permission of Institutional Animal Ethics Committee (IAEC) having approval no. IACS/IAEC/2021-02 and maintained following the IAEC guideline. LC-MS data were recorded using the Agilent 6560 ion mobility quadrupole time-of-flight (IM-QTOF) mass spectrometer connected to an HPLC system (Agilent 1290 UHPLC). The HPLC was performed using a C18 column (ZORBAX Eclipse Plus C18 RRHD, 1.8 μm, 2.1 × 100 mm^2) in presence of methanol/H_2O (1:1) with a flow rate of 1 mL/min.

2. General procedure for synthesis of sodium salt of NSAIDs:

100 mg of NSAID was dispersed in a beaker containing 20 mL of distilled water. To this dispersion NaOH solution (1 M) was added dropwise with constant stirring and monitoring of pH. The neutral solution was filtered before it was completely dissolved. The solution was evaporated to get the sodium salt of NSAID as white powder.

3. General procedure for synthesis of Coordination Complexes (CCs):

Sodium salt of NSAIDs (0.05 mmol) in H_2O (1 mL) was taken in layering tube (diameter: 0.8 cm and length: 20 cm). The co-ligand 5-AP (0.025 mmol, 4.9 mg) in MeOH (4 mL) was layered slowly over the aqueous phase followed by layering of $Zn(NO_3)_2.6H_2O$ (0.025 mmol, 7.5 mg) in EtOH (4 mL) in the layering tube. The tubes were then kept for slow evaporation. Yellow crystals were obtained at the junction of the layer after one week, which were harvested for characterization.

➢ *Synthesis of CC-1:*

CC-1 was synthesized following the general procedure. Na-MEC (0.05 mmol, 16 mg) was taken as NSAID. Yield ≈ 15%. Elemental analysis calcd for $C_{40}H_{33}Cl_4N_5O_5Zn$ (%): C 55.16, H 3.82, N 8.04; found: C 55.05, H 3.44, N 7.72. FT-IR ($\bar{\upsilon}$, cm^{-1}): 3353, 3230, 1615, 1579, 1493, 1455, 1403, 1286, 1151, 1037, 871, 844, 812, 750, 728.

➢ *Synthesis of CC-2:*

CC-2 was synthesized following the general procedure. Na-DIC (0.05 mmol, 15.9 mg) was taken as NSAID. Yield ≈ 27%. Elemental analysis calcd for $C_{40}H_{33}Cl_4N_5O_5Zn$ (%): C 55.16, H 3.82, N 8.04; found: C 55.31, H 3.76, N 7.83. FT-IR ($\bar{\upsilon}$, cm^{-1}): 3238, 1615, 1575, 1505, 1452, 1372, 1302, 850, 766, 749, 727, 664.

➢ *Synthesis of CC-3:*

CC-3 was synthesized following the general procedure. Na-MEF (0.05 mmol, 13.1 mg) was taken as NSAID. Yield ≈ 31%. Elemental analysis calcd for $C_{44}H_{45}N_5O_5Zn$ (%): C 66.96, H

5.75, N 8.87; found: C 67.20, H 5.66, N 8.45. FT-IR ($\bar{\upsilon}$, cm^{-1}): 3346, 3239, 1614, 1576, 1494, 1462, 1393, 1279, 1155, 1042, 861, 750, 730.

- ***Synthesis of CC-4:***

CC-4 was synthesized following the general procedure. Na-FLU (0.05 mmol, 15.1 mg) was taken as NSAID. Yield ≈ 18%. Elemental analysis calcd for $C_{40}H_{29}F_6N_5O_4Zn$ (%): C 58.37, H 3.55, N 8.51; found: C 58.18, H 3.99, N 8.95. FT-IR ($\bar{\upsilon}$, cm^{-1}): 3403, 3323, 3223, 1605, 1576, 1489, 1379, 1329, 1275, 1231, 1160, 1120, 850, 751, 729, 698.

4. Metallo-hydrogelation experiment:

Metallo-hydrogelation experiment was performed in water. 5-AP and $Zn(NO_3)_2.6H_2O$ were taken in a glass vial (3 mL) containing H_2O (500 μL) and shaken by hand to obtain a clear yellow solution. Na-NSAID in water (500 μL) was added to it and mechanically stirred to obtain yellow gel. Gelation was confirmed by both tube inversion method and dynamic rheology experiments. Na-NSAID, 5-AP and $Zn(NO_3)_2.6H_2O$ were taken in 2:1:1 ratio as per the SXRD.

5. Rheological studies:

In a glass vial (15 mL), 3 mL gel (8 wt %, w/v) was prepared. Small amount of gel was scooped out and placed on the stationary plate of the rheometer and rheological experiments were carried out by using parallel plate geometry (diameter - 25 mm, 1 mm gap).

6. TEM:

Small amount of gel sample prepared at MGC was carefully smeared on a 300-mesh carbon coated Cu grid and dried at room temperature for overnight. Then TEM images were recorded.

7. SXRD:

After carefully isolating a suitable single crystal in paratone oil, Single crystal X-ray data were collected by using Mokα (λ=0.7107Å) radiation on a BRUKER APEX II diffractometer equipped with CCD area detector. APEX III software package was used for data collection, data reduction and structure solution. The structures of the CCs were solved by using direct method and refined by full-matrix least squares based on F^2 against all reflections in the SHELXL-2014[1] in routine manner. Final refinement, disorder treatment and CIF finalization were carried out by using OLEX2 version 1.2.10 software.[2] In all cases, the non-hydrogen atoms were treated anisotropically whereas most of the hydrogen atoms were geometrically fixed. Final CIF files were deposited to The Cambridge Crystallographic Data Centre

(CCDC); CCDC 1918450 (CC-1), 1918448 (CC-2), 1918451 (CC-3), 1918449 (CC-4). These data can be obtained free of charge from CCDC.

8. PXRD:

Thin films of finely ground bulk crystal of coordination complexes (~15 mg) were made on quartz slide. PXRD data were collected by placing the slides on a Rigaku/Bruker powder X-ray diffractometer with a scan range of 2θ (4˚-35˚).

9. UV-vis and fluorescence spectroscopy:

$3.5x10^{-5}$ (M) solution of CC-1 in DMSO (3 mL) was used to record absorption and emission (excited at 300 nm with slit 3/3) spectra.

10. Drug release experiment:

Release of gelator molecules from the hydrogel (MHG-1) was performed under physiological condition (in phosphate-buffered saline (PBS)(1X) of pH 7.4 at 37 °C) to establish the self-delivery ability of the MHG-1. For that experiment, MHG-1 (1 mL) was prepared at MGC in a glass vial (15 mL). Then PBS (1X)(2 mL; pH 7.4) was carefully added on the top of MHG-1 and 200 μL of aliquot was taken out at different time interval followed by addition of 200 μL of fresh PBS (1X) in order to maintain the PBS amount constant. The collected aliquot was subjected to UV-vis to measure the released amount of 5-AP. Note that, 200 μL of the aliquot was diluted to 3 mL with PBS (1X) prior to recording the spectra.

11. Cell culture:

The cells (B16-F10, RAW 264.7, C2C12 and E. Derm) were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a humidified incubator at 37 °C and 5% CO_2.

12. Mice model:

~8-12 weeks old C57BL/6 mice having weight of 18-22 gm were used for *in vivo* experiments. Mice were kept under pathogen free condition at Indian Association for the Cultivation of Science (IACS) animal house facility. All mice were quarantined for one week before experiments. All animal experiments were conducted under the ethical guidelines of institutional ethical committee (approval no.: IACS/IAEC/2021-02).

13. In vivo tumour model:

Almost confluent (60 %) B16-F10 cells were treated with trypsin-EDTA (1X) (0.05 %) and centrifuged at 2000 rpm for 2 min. The supernatant was discarded and the cell pellet then made to a suspension in ice cold PBS (1X) (1 mL) and centrifuged (2000 rpm, 2 min) and the supernatant was discarded. This process was repeated thrice to get rid of trypsin. After that, the cells were suspended in ice cold PBS (1X) (100 μL) maintaining a concentration of ~$1x10^6$ cells/mL. Dorsal fur of the mice was removed using a trimmer. After wiping the dorsal skin with ethanol (70%), 100 μL of the suspended cells as prepared above was injected intradermally in the flank region and the bleb formation was observed. Tumour was formed after one week of cell injection. The diameter of the tumour was measured using a slide calipers. Dosing was started when the tumour volume (mm^3) reached ~ 60-80.

14. Subcutaneous injection of the hydrogel:

After one week of B16-F10 cell injection, tumour bearing mice were divided into five groups. Each group contained three mice. The hydrogel MHG-1 (200 μL) was injected subcutaneously to three groups with varying concentration (2, 4 and 8 wt%). The control group was treated with PBS (1X) (200 μL) via subcutaneous route. The fifth group was a positive control; the group was administered with a well-known anti-melanoma drug i.e., 5-fluorouracil (5-FU) (20 mg/kg body weight) as PBS (1X) suspension through subcutaneous route. Then mice were carefully maintained for three weeks (21 day) continuously measuring the tumour size and body weight. After three weeks, mice were sacrificed and tumours were collected for weight measurement, histopathological and immunohistochemistry.

Groups	Treatment	Injection volume	No. of mice (n)
Group-1	Control (PBS)	200 μL	3
Group-2	MHG-1 (2 wt%)	200 μL	3
Group-3	MHG-1 (4 wt%)	200 μL	3
Group-4	MHG-1 (8 wt%)	200 μL	3
Group-5	5-FU (20 mg/kg body weight)	200 μL	3

15. Subcutaneous injection of the components of the hydrogel:

The individual components i.e., 5-AP, $Zn(NO_3)_2.6H_2O$ and Na-MEC present in 4 wt % hydrogel were calculated to be 1.5, 2.2 and 4.8 mg per 200μL. The same subcutaneous route was followed as mentioned above on four groups of mice including the control group that

received 200 µL PBS (1X) only. Then mice were carefully maintained for three weeks (21 day) continuously measuring the tumour size and body weight. After three weeks, mice were sacrificed and tumours were collected.

Groups	Treatment	Injection volume	No. of mice (n)
Group-1	Control (PBS)	200 µL	3
Group-2	$Zn(NO_3)_2.6H_2O$ (2.2 mg)	200 µL	3
Group-3	Na-MEC (4.8 mg)	200 µL	3
Group-4	5-AP (1.5 mg)	200 µL	3

16. MTT assay:

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay of the **CCs** were carried out on different cell lines to evaluate the cytotoxicity. Approximately $1x10^4$ cells per well were seeded in 96-well plates and incubated at 37 °C and 5 % CO_2 for 24 h in a humidified incubator. After 24 h, the old media in the wells were replaced by DMEM containing CCs at different concentration (150 µL) and 1% DMSO containing DMEM was used as control set and kept in a humidified incubator at 37 °C and 5% CO_2 for 48 h. After 48 h, the drug containing culture medium was discarded and fresh media containing MTT (1 mg in 2 mL) was added (100 µL per well) to the wells and kept for 4 h at 37 °C in 5 % CO_2. After 4 h, MTT containing media was replaced with DMSO (100 µL per well) to dissolve the formazan produced by mitochondrial reductase from live cells. The absorbance of formazan was recorded at λ=570 nm by using a multiplate ELISA reader. The percentage of live cells in the coordination complexes treated sample was calculated by considering the 1 % DMSO containing DMEM-treated sample to be 100%.

17. Cell Migration:

The cells were seeded in a 6-well plate and kept in a humidified incubator until the plates become almost confluent. Then the wells were scratched with a 20 µL sterile pipette tip to create a narrow path in the middle of the wells. The wells were then treated with CC-1 (0.025 mM, 2 mL), Na-MEC (0.049 mM, 2 mL) and control (1% DMSO in DMEM). Still images were captured under an optical microscope at different time intervals for 24 h to measure the migration speed in each case.

18. In vitro conditions for apoptosis, side population and ROS:

In *in vitro* condition, apoptosis, side population and ROS were determined by using flow cytometry. Several experiments (annexin V-FITC/PI and MMP) were carried out for the detection of apoptosis in *in vitro* condition. For these purposes seeded cells (~ $2x10^6$ cells in 35 mm culture plates) were treated with different concentrations of CC-1 (0.025, 0.05 and 0.075 mM) and control (1% DMSO containing DMEM) for 48 h in a humidified incubator at 37 °C in 5% CO_2. Side population and ROS were determined by Hoechst efflux assay and DCFDA staining respectively.

For the confirmation of *in vitro* flow cytometric data optical microscope, Cryo-TEM, SEM and confocal imaging were performed. For these purposes seeded cells (~$1x10^4$ cells in 10 mm culture plates) were treated with CC-1 (0.025 mM) for 48 h in a humidified incubator at 37 °C in 5 % CO_2.

19. Preparation of single cell suspension from solid tumour:

The hydrogel treated tumour bearing mice as mentioned above in section 14, were sacrificed and the tumours were collected. Single cell suspensions from the solid tumours were prepared by treating with Liberase™ (following manufacturer instruction) at 37 °C for 1 h.

20. Hemolysis assay on murine blood:

Blood collected by cardiac puncture method from healthy C57BL/6 mice was kept in a EDTA coated vial in order to prevent blood coagulation. The blood sample was then diluted with PBS (1X) (5 mL) and RBC was collected by centrifugation at 4000 rpm for 10 min. Supernatant PBS was discarded and RBC was suspended in PBS (1X) (5 mL). RBC containing solution (1 mL) was distributed in different Eppendorf tube (1.5 mL) and treated with different concentration of CC-1 (0.025 mM, 0.05 mM and 0.075 mM), SDS (0.1%), DMSO (1%) and control (untreated). The sets were then incubated at 37 °C for 3 h. % Hemolysis was determined by measuring the absorbance at λ= 540 nm and considering the absorbance of SDS treated cells as 100 % Hemolysis.

Following the same procedure hemolysis assay of individual components of CC-1 was performed. For this purpose, 0.2 mM of each individual components (i.e., 5-AP, $Zn(NO_3)$ and Na-MEC) were taken.

21. Cell cycle:

During cell cycle analysis, roughly 1 x 10^6 B16-F10 cells were seeded in eight tissue culture plates (10 mm). Plates were then divided into two sets (control and treatment). In treatment set, the cells were incubated with CC-1 (0.025 mM, 1 mL) and in control set, DMEM containing 1% DMSO (1 mL) was used. Each set was incubated for 4, 8, 12 and 16 h respectively at 37 ºC, 5% CO_2. After the incubation period, cells were collected using scrapper followed by centrifugation. After removing the supernatant media, cells were fixed with 1 mL of ice cold 70% ethanol. Then the cells were kept in -20 °C for overnight. After that, ethanol was removed and the cells were washed with PBS (1X). RNase A (final concentration: 100 µg/mL) in PBS (1X) was added to the cells and incubated for 4 h at 37 ºC. After 4 h, the cells were again washed with PBS (1X) and incubated for 10 min with PI (final concentration: 50 µg/mL) at 37 °C. the cells were suspended in PBS (1X, 1 mL) after washing and % of cell arrest in different cell cycle phases were determined by flow cytometry.

22. Drug internalization:

For drug internalization study using flow cytometry, the treated cell suspensions (both *in vitro* and *in vivo*) were washed three times with fresh PBS (1X) to remove trace of CC-1. The cells were then suspended in PBS (1X) and internalization was measured using blue filter.

To know the subcellular localization in *in vitro* condition, the cells were treated with CC-1 (0.025 mM) for 2 h. After that, localization of CC-1 was visualized by staining the cells with MitoTracker™ green and LysoTracker™ red (final concentrations: as per protocol provided by manufacturer) under LSCM.

23. Flow cytometry:

Flow cytometry investigation was carried out in both *in vitro* and *in vivo* model system. For *in vivo* model system single cell suspension was prepared from solid tumour (Please see section 19).

- ***Annexin V-FITC/PI study:***

In vitro and *in vivo* cell apoptosis was determined by following standard Annexin V-FITC/PI method provided by the manufacturer. Briefly, the cell suspensions were thoroughly washed

thrice with PBS (1X). The cells were suspended in 1X binding buffer (1 mL) (provided by manufacturer) and incubated for 10 min at 37 °C with annexin V-FITC (5 µL) and PI (Final concentration: 5 µg/mL). % of cells in different quadrant was determined by using flow cytometry.

- ***Mitochondrial Membrane Potential (MMP) study:***

Mitochondrial Membrane Potential (MMP) was determined in both *in vitro* as well as *in vivo* conditions. Treated cell suspensions were washed thrice with PBS (1X). After that PBS (1X, 1 mL) suspended cells were stained with JC-1 (final concentration: 5 µg/mL) and depolarization of MMP was determined by using flow cytometry.

- ***Hoechst efflux study:***

Hoechst efflux assay was performed following a previously reported procedure.[3] Briefly, the treated cells (*in vitro* and *in vivo*) were washed thrice with PBS (1X). For positive control, cells were treated with verapamil (50 and 100 µg/mL) and incubated at 37 °C for 15 min. After that, PBS (1X) suspended cells were incubated at 37 °C with Hoechst 33342 (final concentration: 5 µg/mL) for 45 min (efflux period). Data were recorded using a FACS instrument.

- ***Determination of intracellular ROS:***

Both *in vitro* and *in vivo* treated cells were suspended in PBS (1X, 1 mL) and incubated at 37 °C with 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) (final concentration: 5 µM) for 15 min. Cells were then again washed with PBS (1X) to remove excess DCFDA and suspended in PBS (1X, 1 mL). Cells were then analysed in a flow cytometer instrument.

24. In vitro SEM, cryo-TEM and optical microscope images of cells:

For SEM, cells were cultured in tissue culture plate (10 mm) containing small coverslips. Plates were then treated with CC-1 (0.025 µM) and 1% DMSO containing DMEM for 48 h. After that, supernatant DMEM was discarded and the cells were fixed with 4 % glutaraldehyde solution in PBS (1X). After fixation, the cell containing coverslips were washed with different alcohol gradient (30%, 50%, 70%, 80%, 90% and 100%) and dried in air. The coverslip containing fixed cells were then placed on a SEM stub and coated with platinum. SEM images of the cells were captured in a SEM instrument.

For cryo-TEM, the live cells [control (1 % DMSO in DMEM) and treated (CC-1; 0.025 mM)] were suspended in ultrapure water and directly drop casted on TEM grid. The TEM grids were immediately snap frozen in liquid ethane and the sample was observed under cryo-TEM.

For optical microscope images, seeded cells were treated with CC-1 (0.025 μM) and 1% DMSO containing DMEM (control). Change in morphology of the cells was captured at different time intervals under optical microscope.

25. Cell imaging:

For cell imaging, approximately 1×10^6 B16-F10 cells were seeded in confocal plates. After 24 h of seeding, the cells were treated with CC-1 (0.025 mM) and 1% DMSO in DMEM (control) for 48 h.

- ***Nuclear condensation:***

After treatment, the cells were stained with Hoechst 33342 and PI (final concentration for the both dyes: 5 μg/mL) and incubated for 15 min in a humidified incubator at 37 °C in 5% CO_2. After that, the plates were washed three times with fresh DMEM to remove excess CC-1 and dyes. Finally, confocal images were captured after adding DMEM (1 mL) to the plates.

- ***Annexin-V FITC/PI:***

After treatment, the cells were stained with Hoechst 33342 (final concentration: 5 μg/mL), Annexin-V FITC (concentration: as per protocol provided by manufacturer) and PI (final concentration: 5 μg/mL) and incubated for 15 min in a humidified incubator at 37 °C in 5% CO_2. After that, the plates were washed three times with fresh DMEM to remove excess CC-1 and dyes. Finally, the confocal images were captured by adding DMEM (1 mL) to the plates.

- ***Mitochondrial Membrane Potential:***

After treatment, the cells were stained with Hoechst 33342 and JC-1 (final concentration for both dyes: 5 μg/mL) and incubated for 15 min in a humidified incubator at 37 °C in 5% CO_2. After that, the plates were washed three times with fresh DMEM to remove excess CC-1 and dyes. Finally, the confocal images were captured by adding DMEM (1 mL) to the plates.

- ***In vitro membrane permeability:***

The seeded cells were treated with CC-1 (0.025 mM) and 1% DMSO in DMEM (control) for 3 h in a humidified incubator at 37 °C in 5% CO_2. After 3h, the cells were stained with Hoechst 33342 and PI (final concentration for both dyes: 5 μg/mL) and incubated for 15 min in a humidified incubator at 37 °C in 5% CO_2. After that, the plates were washed three times with fresh DMEM to remove excess CC-1 and dyes. Finally, confocal images were captured after adding DMEM (1 mL) to the plates.

- ***Determination of apoptosis in tumour tissue*:**

For annexin V-FITC study in *in vivo*, slides containing paraffin embedded fixed tumours tissue sections were used (control and 4 wt% hydrogel treated). Firstly, paraffin was removed by washing the slides with o-xylene for three times followed by dipping into o-xylene for 2 h in order to remove trace amount of paraffin. Paraffin free slides were then rehydrated by dipping into different alcohol gradient (100, 80, 50, 30 and 10 %) for 15 min each respectively and finally in H_2O for 1 h. The hydrated slides were then trypsinized (0.1 %) for 10 min. The trypsinized slides were then washed thrice with fresh PBS (1X) in order to remove trace amount of trypsin. After that, the slides were dipped into H_2O_2 solution (3%) for 5 min. The H_2O_2 solution was removed after 5 min and washed three times with fresh PBS (1X). The slides were then dipped into BSA solution (5%) for 4 h at 4 °C in order to avoid nonspecific binding of antibody. After 4 h, the slides were again washed thrice with PBS (1X) (contained 0.5% BSA) to remove excess BSA. Finally, BSA treated slides were incubated with annexin V-FITC containing 1X binding buffer for 6 h at 4 °C. After 6 h of labeling with antibody, the slides were washed thrice with 1X binding buffer to remove unbound antibody and confocal images were captured.

- ***Determination of Ki-67 in tumour tissue:***

For Ki-67, staining was carried out following the above-mentioned protocol. After BSA treatment, the tissue was incubated with Rabbit anti Ki-67 primary antibody for 6 h at 4 ^oC. After 6h, the tissue was washed with PBS (1X) (contained 0.5% BSA) and further incubated with Alexa Fluor plus 488 tagged anti rabbit secondary antibody for another 3 h at 4 ^oC. Before imaging, the tissue was washed with PBS (1X). Images were captured under LSCM.

- ***Determination of endothelial cells in tumour tissue:***

For CD31 expression determination, staining was carried out following the above-mentioned protocol. After BSA treatment, the tissue was incubated with PE-CF594 Rat Anti-Mouse CD31 monoclonal antibody for 6 h at 4 ^{o}C. After 6h, the tissue was washed with PBS (1X). Images were captured under LSCM.

26. PGE_2 assay:

- ***On RAW 264.7 cell line in in vitro:***

PGE2 assay was carried out following a standard protocol provided by the manufacturer. Approximately 1×10^6 RAW 264.7 cells/well were seeded in 10 mm tissue culture plates and kept at 37 ^{o}C in 5 % CO_2 in a humidified incubator for 24 h. After that, the plates were treated with lipopolysaccharide (LPS) (final concentration: 5 μg/mL) in order to get maximum PGE_2 production. A plate containing 1% DMSO in DMEM was left untreated with LPS and termed as control. Rest of the plates were treated with CC-1 (0.025 mM) and Na-MEC (0.049 mM) respectively for 48 h in a humidified incubator at 37 °C and 5% CO_2. After that, the supernatant media was collected and presence of PGE_2 was determined in two sets by diluting it in 1:100 and 1:500 ratio using ELLMAN's reagent.

- ***In in vivo tumour cells:***

After collecting the tumour from hydrogel (MHG-1, 4 wt%) and Na-MEC (4.8 mg/ 200 μL) treated mice respectively, tumour was smashed and sonicated. After that, cell lysate was collected by centrifugation and used for PGE_2 determination as described above.

- ***On tumour associated skin cells in in vivo:***

For PGE_2 determination from tumour associated skin, same procedure followed as described for PGE_2 determination from tumour in *in vivo*.

27. Histopathology:

After 3 weeks of hydrogel treatment, mice were sacrificed and tumours, tumour adjacent skins and organs (heart, kidney, liver, spleen and lung) were collected in a formaldehyde solution. After that, they were fixed using xylene and embedded in paraffin. Paraffin embedded blocks were then sectioned in a microtome instrument and fixed in a glass slide.

After that, paraffin was removed from the glass slide using xylene and paraffin free sections were stained with hematoxylin and eosin dye. Images of the sections were captured in an optical microscope.

28. Flow cytometric investigation at Tumour Microenvironment (TME):

The antibodies used herein were: 1) APC Rat Anti-Mouse IL-6, 2) APC Rat Anti-Mouse IL-2, 3) PerCP Hamster Anti-Mouse CD-3e, 4) APC Rat Anti-Mouse IFN-γ, 5) PE Rat Anti-Mouse IL-12, 6) PE-Cy 7 Rat Anti-Mouse TNF-α, 7) APC Rat Anti-Mouse CD-8a.

Single cell suspension of tumour was prepared following the above-described procedure and cells were permeabilized by cell permeabilization buffer (0.3% Triton X-100 in antibody dilution buffer) (except CD3 and CD8). The cells were further incubated with corresponding fluorophore tagged antibody in antibody dilution buffer (0.5% BSA containing PBS (1X)) at 4 ^{o}C for 6 h. After incubation, the cells were washed with PBS (1X) and data were recorded in flow cytometry.

29. Statistical analysis:

All experiments mentioned herein were individually performed for three times (n=3) and plotted by taking mean values of them. Columns represented mean values and SD of the replicates was presented as bar or line. One way ANOVA analysis was carried out on the results and differences at $p<0.05$ were considered as statistically significant by Dunnett's/Tukey's Multiple Comparison Test. t-tests and two-way ANOVA were used whenever applicable. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and ns represent non-significant. GraphPad PRISM (ver. 5.03) software was used to analyse all data.

30. References:

1. G. M. Sheldrick, *Acta Cryst.*, 2015, **71**, 3-8.
2. O.V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Cryst.*, 2009, **42**, 339-341.
3. C.W. Scharenberg, M. A. Harkey, B. Torok-Storb, *Blood*, 2002, **99**, 507−512.

31. FT-IR spectra of the coordination complexes (CCs):

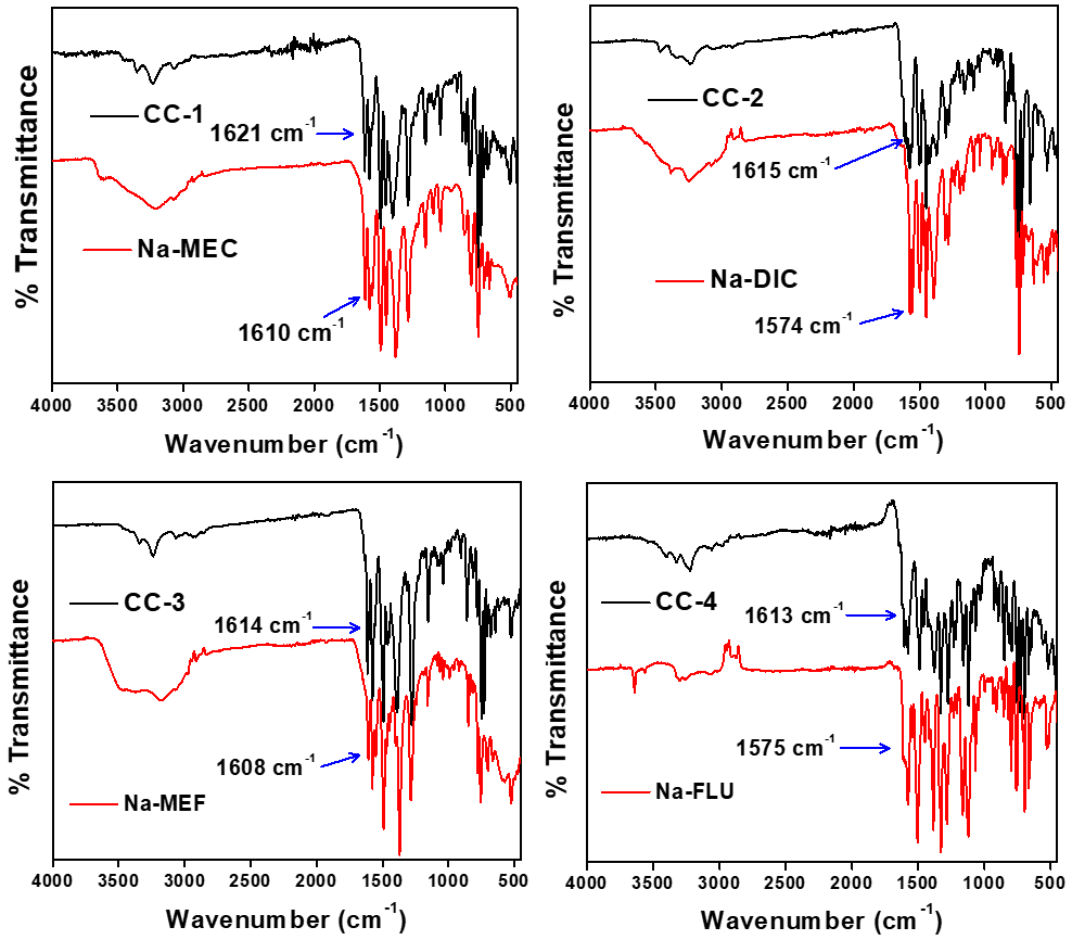

Fig. S1: FT-IR spectra of CCs (Blue arrow indicated the stretching frequency of >C=O of carboxylate).

Table S1: Tabular representation showing $C{=}O_{str}$ frequency (cm^{-1}) in different CCs and corresponding Na-NSAID.

Compounds	$\nu_{C=O}$ (cm^{-1})	$\Delta\nu$ (cm^{-1})
Na-MEC	1610	11
CC-1	1621	
Na-DIC	1574	41
CC-2	1615	
Na-MEF	1608	6
CC-3	1614	
Na-FLU	1575	38
CC-4	1613	

32. SXRD data of the coordination complexes:

Table S2: Crystallographic table of CCs.

Identification code	CC-1	CC-2	CC-3	CC-4
CCDC No.	1918450	1918448	1918451	1918449
Empirical formula	$C_{40}H_{29}Cl_4N_5O_4Zn$	$C_{40}H_{29.92}Cl_4N_5O_4Zn$	$C_{44}H_{43}N_5O_5Zn$	$C_{40}H_{27}F_6N_5O_4Zn$
Formula weight	850.85	851.78	787.2	821.03
Temperature/K	110.07	150	150	296.15
Crystal system	triclinic	monoclinic	monoclinic	triclinic
Space group	*P*-1	*P*2/c	*P*c	*P*-1
a/Å	10.336(3)	12.7178(3)	14.0202(11)	10.3330(5)
b/Å	11.317(3)	10.0179(2)	8.7730(7)	12.5987(6)
c/Å	17.692(5)	15.7221(3)	16.5619(13)	16.5506(8)
α/°	100.996(7)	90	90	67.806(4)
β/°	103.957(7)	113.853(2)	109.5330(10)	87.904(4)
γ/°	106.496(8)	90	90	65.842(3)
Volume/Å^3	1849.5(8)	1831.99(7)	1919.9(3)	1802.40(16)
Z	2	2	2	2
ρ_{calc}g/cm^3	1.528	1.544	1.362	1.513
μ/mm^{-1}	1.003	1.013	0.693	0.763
F(000)	868	870	824	836
Crystal size/mm^3	0.25 × 0.25 × 0.12	0.35 × 0.18 × 0.12	0.23 × 0.18 × 0.08	0.35 × 0.25 × 0.08
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.722 to 50.698	3.502 to 50.964	3.082 to 50.814	2.684 to 50.872
Index ranges	-12 ≤ h ≤ 12, -11 ≤ k ≤ 13, -21 ≤ l ≤ 21	-15 ≤ h ≤ 15, -12 ≤ k ≤ 12, -19 ≤ l ≤ 18	-16 ≤ h ≤ 16, -10 ≤ k ≤ 10, -19 ≤ l ≤ 19	-12 ≤ h ≤ 12, -15 ≤ k ≤ 15, -19 ≤ l ≤ 19
Reflections collected	18124	33245	29032	21541
Independent reflections	6652 [R_{int} = 0.1056, R_{sigma} = 0.1560]	3401 [R_{int} = 0.0516, R_{sigma} = 0.0258]	7044 [R_{int} = 0.1022, R_{sigma} = 0.1009]	6590 [R_{int} = 0.1454, R_{sigma} = 0.1789]
Data/restraints/parameters	6652/152/493	3401/3/291	7044/2/504	6590/0/510
Goodness-of-fit on F^2	1.041	1.022	0.992	0.948
Final R indexes [I>=2σ (I)]	R_1 = 0.1042, wR_2 = 0.2516	R_1 = 0.0401, wR_2 = 0.0927	R_1 = 0.0492, wR_2 = 0.0719	R_1 = 0.0792, wR_2 = 0.1385
Final R indexes [all data]	R_1 = 0.2281, wR_2 = 0.3210	R_1 = 0.0562, wR_2 = 0.1007	R_1 = 0.0917, wR_2 = 0.0825	R_1 = 0.2054, wR_2 = 0.1740
Largest diff. peak/hole / e Å^{-3}	1.15/-0.72	0.53/-0.87	0.38/-0.40	0.48/-0.35
Flack parameter	-	-	0.109(15)	-

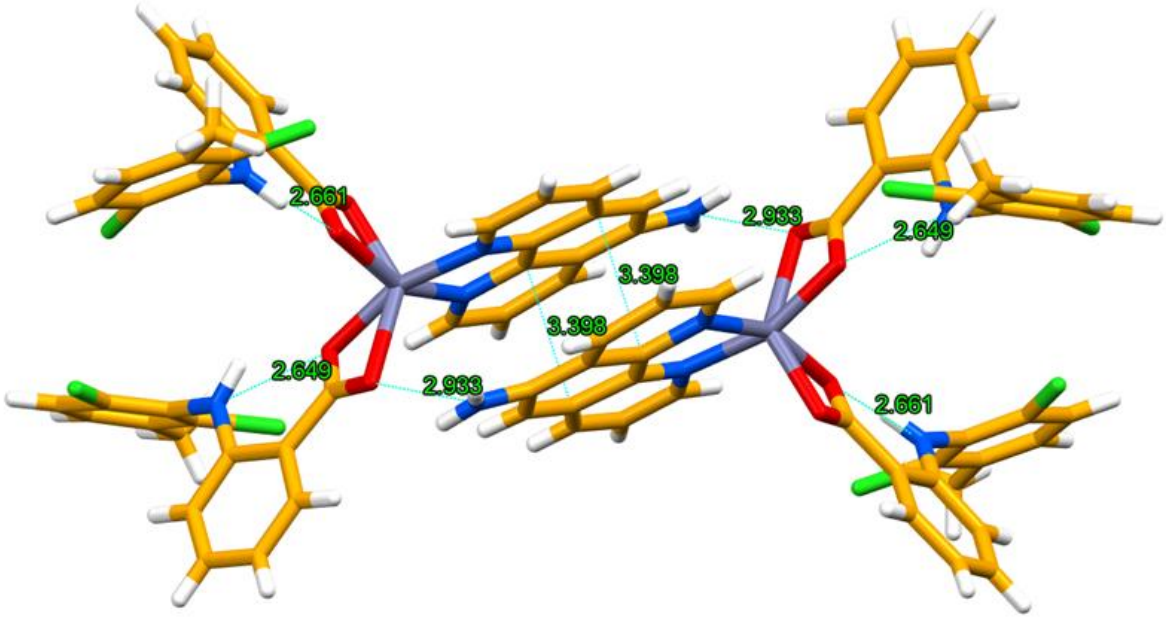

Fig. S2: H-bonding and π-π stacking interactions present in CC-1.

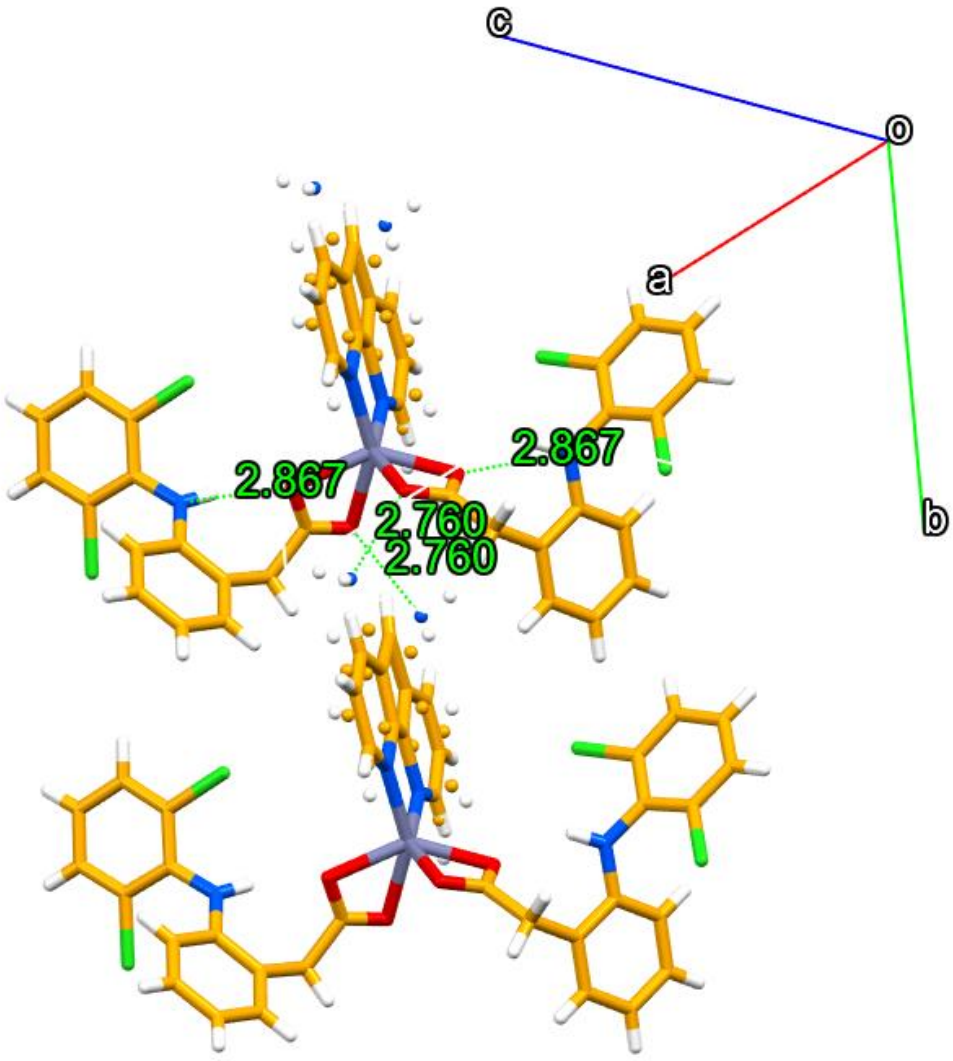

Fig. S3: H-bonding interactions present in CC-2.

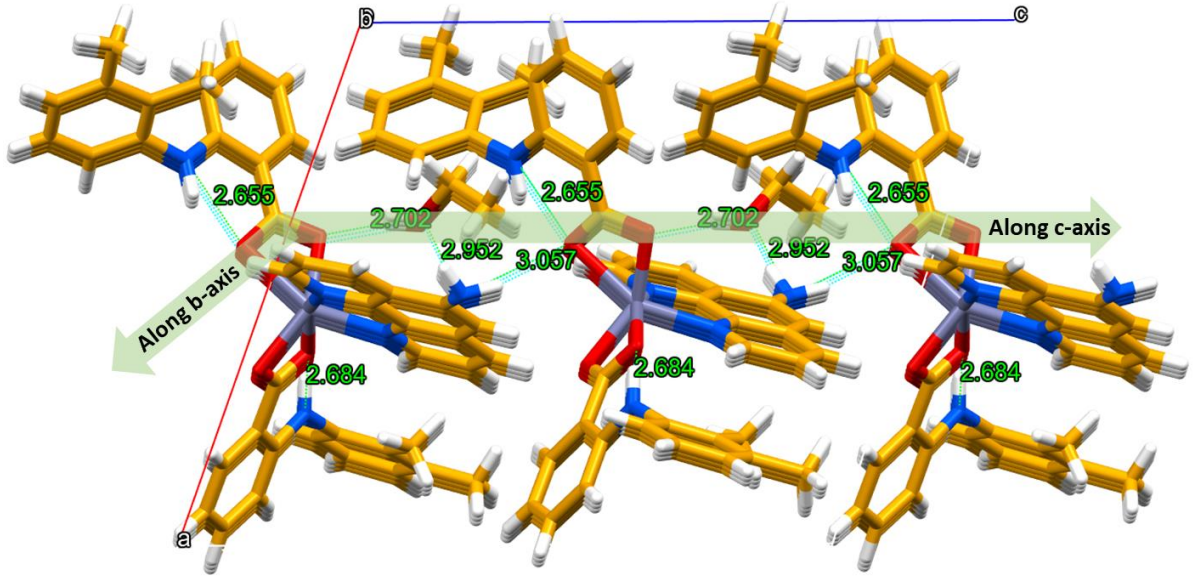

Fig. S4: H-bonding interactions present in CC-3.

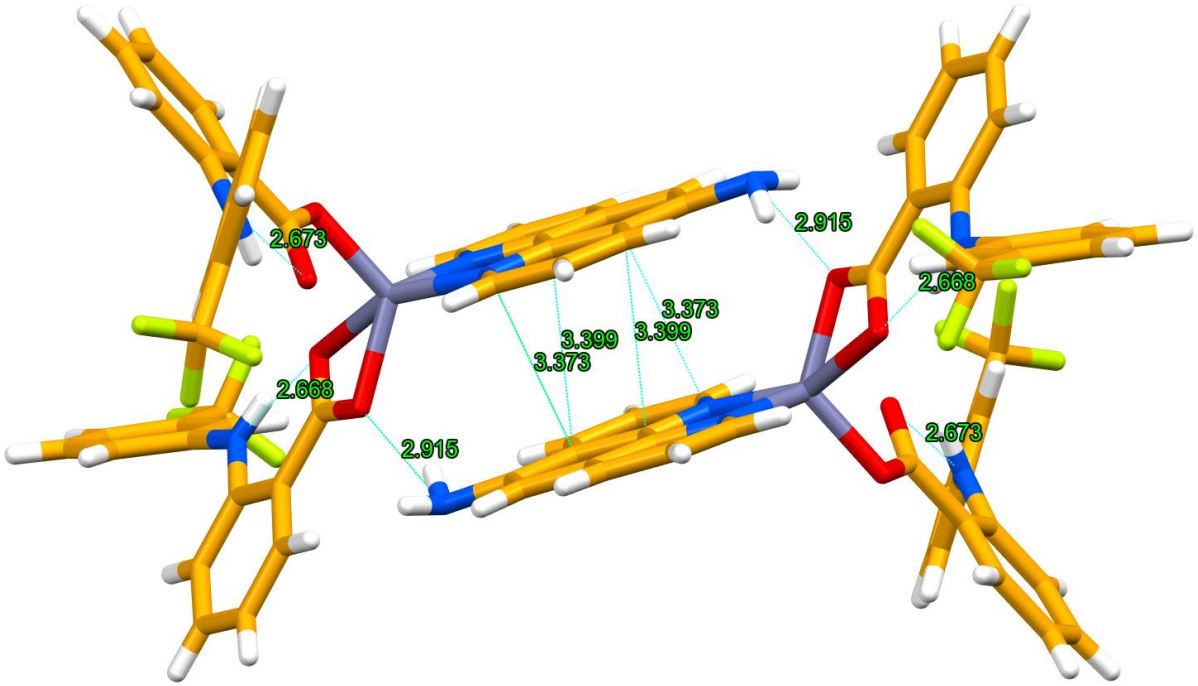

Fig. S5: H-bonding and π-π stacking interactions present in CC-4.

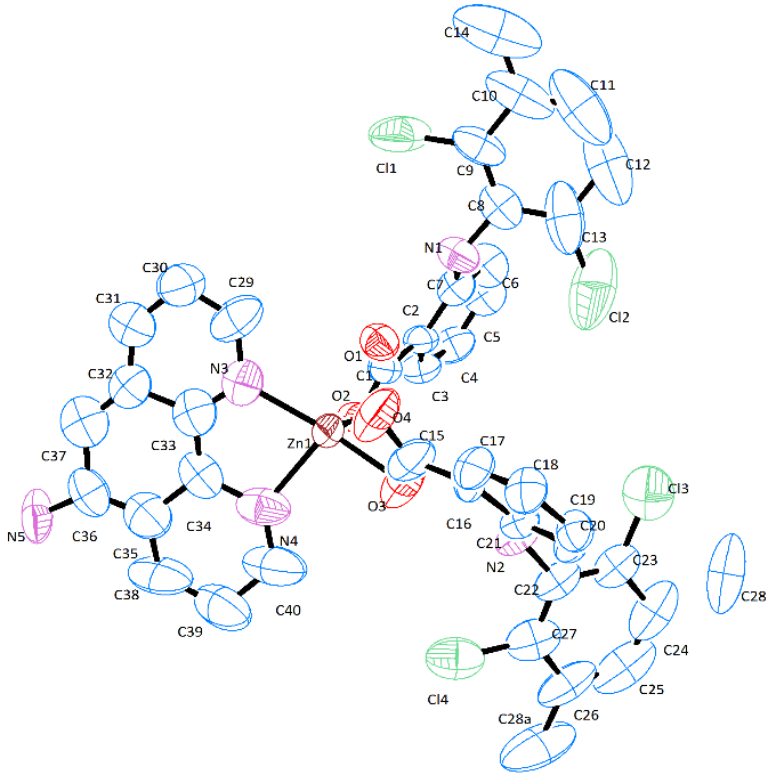

Fig. S6: ORTEP plot of CC-1 (50% probability).

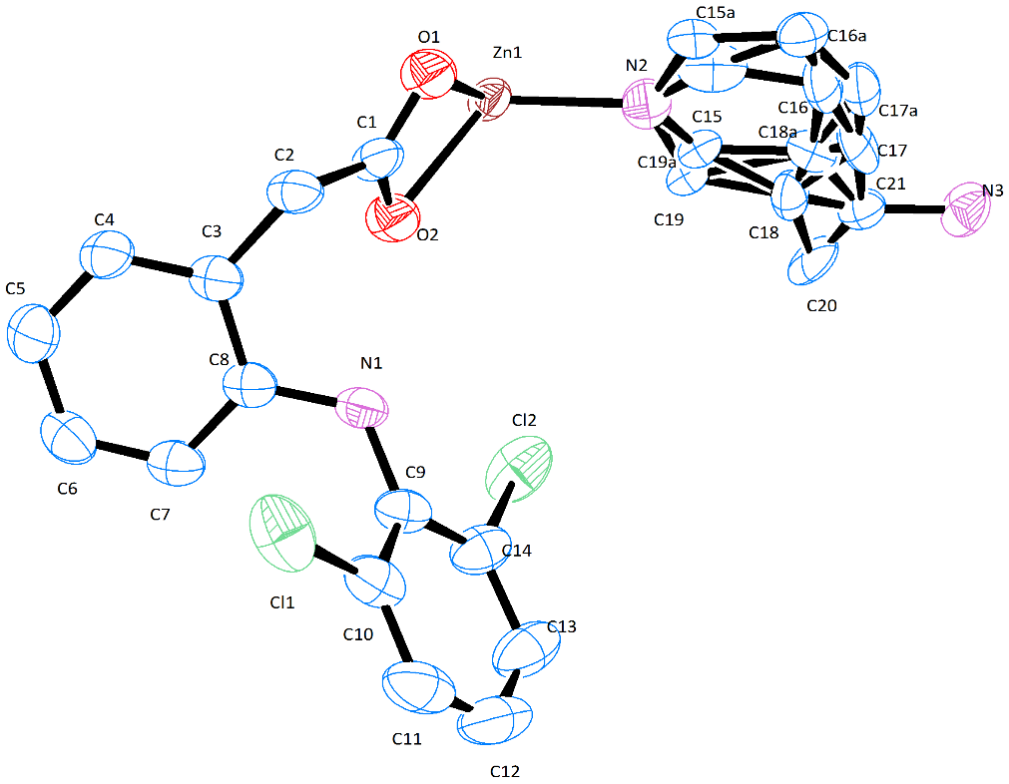

Fig. S7: ORTEP plot of CC-2 (50% probability).

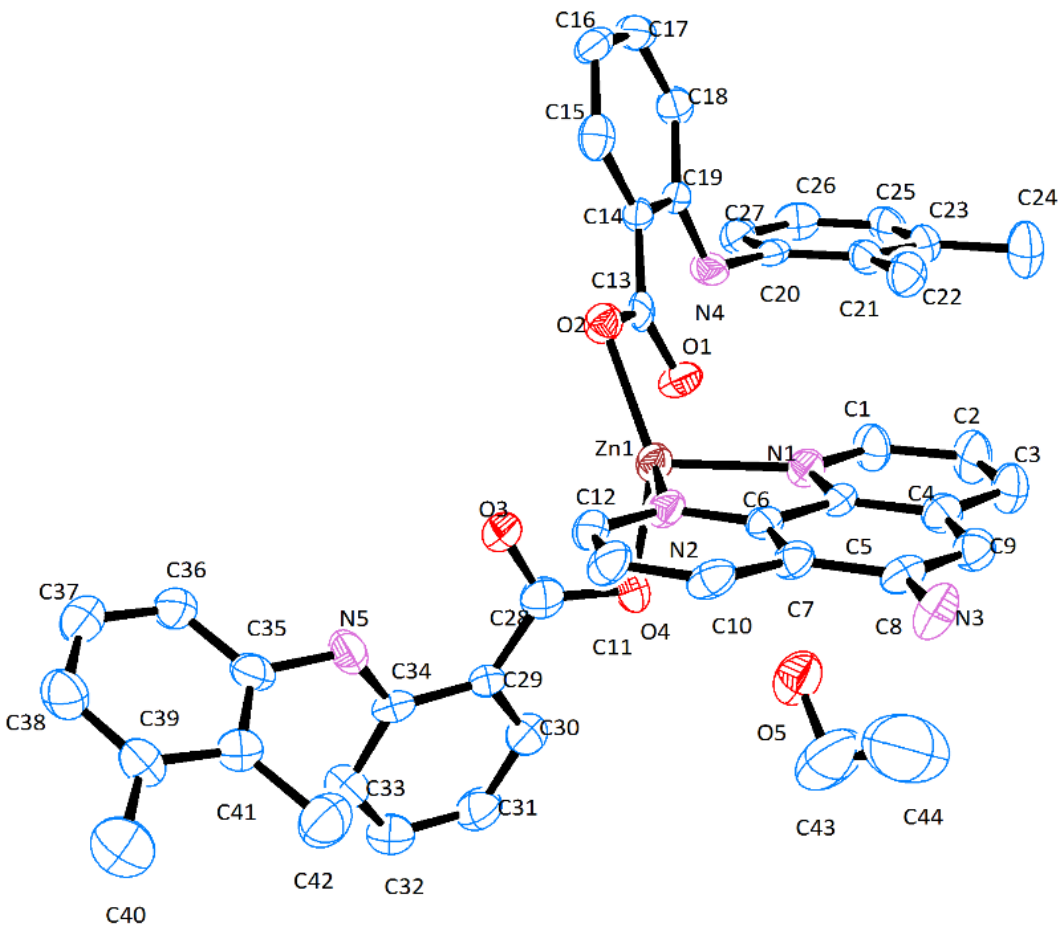

Fig. S8: ORTEP plot of CC-3 (50% probability).

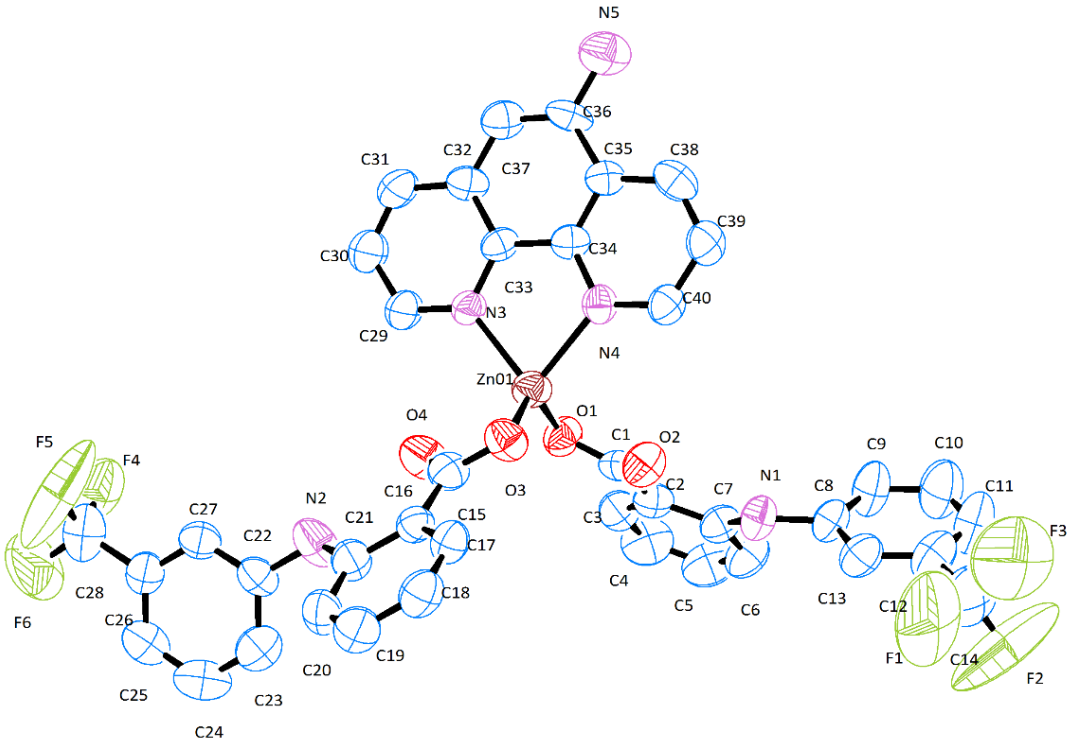

Fig. S9: ORTEP plot of CC-4 (50% probability).

Table S3: H-bond table of CC-1.

D-H	d(D-H) (Å)	d(H···A) (Å)	∠D-H···A (°)	d(D···A)	A	Symmetry
N1-H1	0.88	1.99	132	2.661(13)	O1	x,y,z
N2-H2	0.88	2.01	129	2.650(15)	O3	x,y,z
N5-H5A	0.88	2.53	158	2.362(17)	O4	-1+x,y,z
N5-H5B	0.88	2.30	129	2.933(13)	O4	-x,1-y,2-z

Table S4: H-bond table of CC-2.

D-H	d(D-H) (Å)	d(H···A) (Å)	∠D-H···A (°)	d(D···A)	A	Symmetry
N1-H1	0.86	2.27	126	2.867(3)	O2	x,y,z
N3-H3A	0.88	3.07	130	3.700(6)	O2	-x,y,1/2-z

Table S5: H-bond table of CC-3.

D-H	d(D-H) (Å)	d(H···A) (Å)	∠D-H···A (°)	d(D···A)	A	Symmetry
N3-H3A	0.88	2.12	157	2.952(7)	O5	x,1+y,z
N3-H3B	0.88	2.21	162	3.057(8)	O3	x,2-y,1/2+z
N4-H4	0.88	2.13	120	2.684(7)	O1	x,y,z
N5-H5	0.88	1.94	137	2.655(7)	O3	x,y,z
O5-H5A	0.84	1.89	162	2.702(7)	O4	x,y,z

Table S6: H-bond table of CC-4.

D-H	d(D-H) (Å)	d(H···A) (Å)	∠D-H···A (°)	d(D···A)	A	Symmetry
N1-H1	0.86	2.03	131	2.673(7)	O2	x,y,z
N2-H2	0.82(7)	1.96(7)	145(7)	2.668(9)	O4	x,y,z
N5-H5A	0.86	2.61	115	3.073(7)	O2	x,y,z
N5-H5B	0.86	2.10	158	2.915(8)	O3	1-x,2-y,1-z

33. PXRD data of CCs:

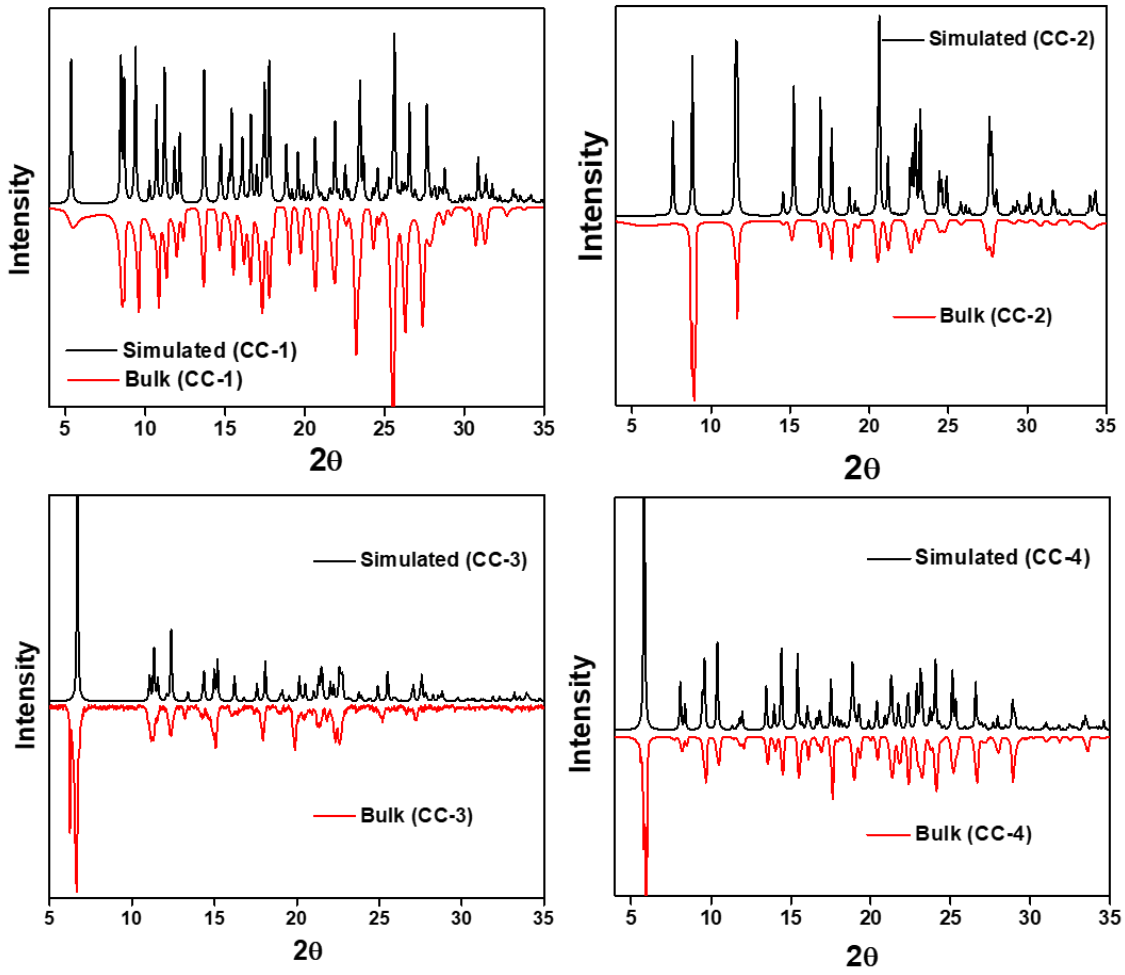

Fig. S10: PXRD plot of CCs. A good match between bulk and simulated PXRD pattern was observed suggesting bulk phase purity of the CCs.

34. Characterization of the hydrogels:

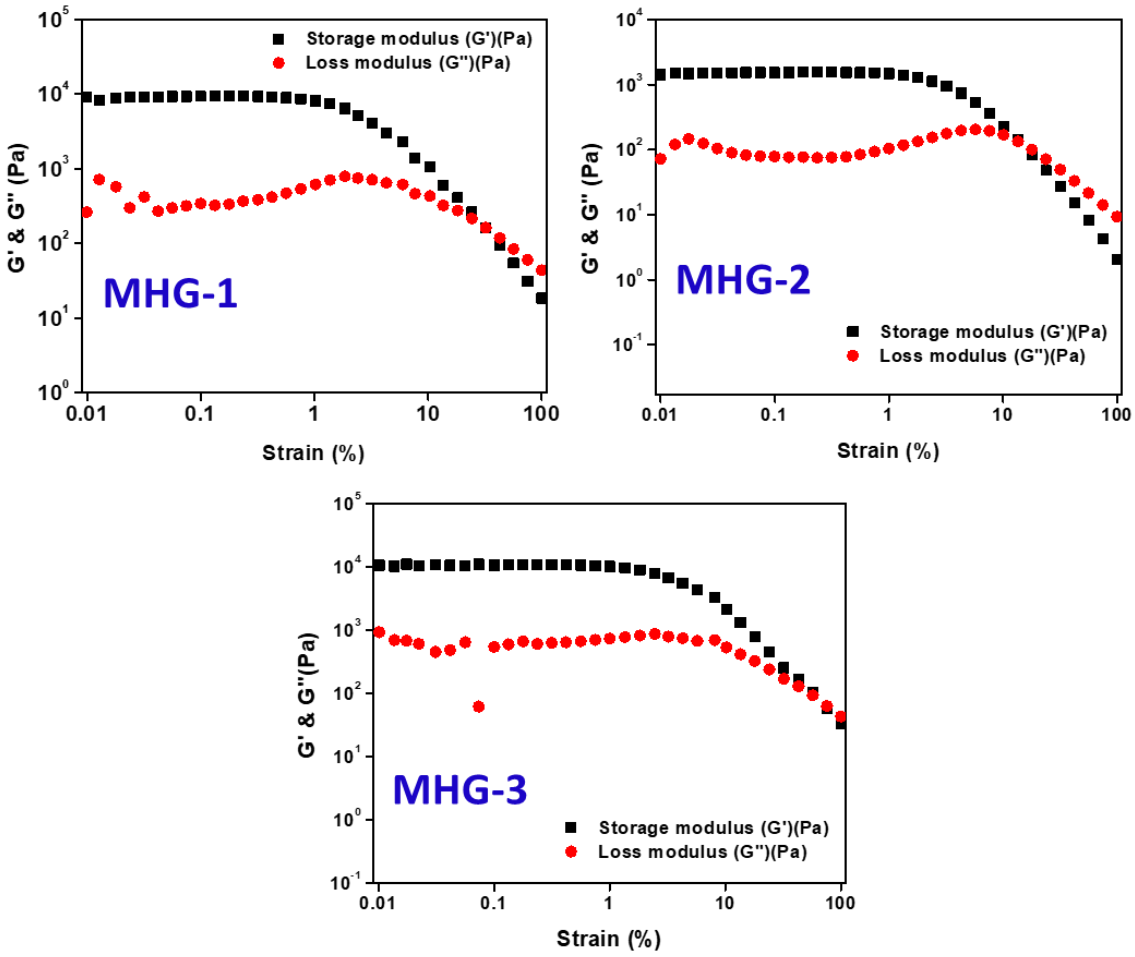

Fig. S11: Amplitude sweep data of the hydrogels.

Table S7: tan δ table.

Hydrogel	G' (kPa)	G'' (kPa)	tan δ (G''/G')
MHG-1	10.4	0.588	0.0563
MHG-2	2.33	0.167	0.0717
MHG-3	9.12	0.797	0.0873

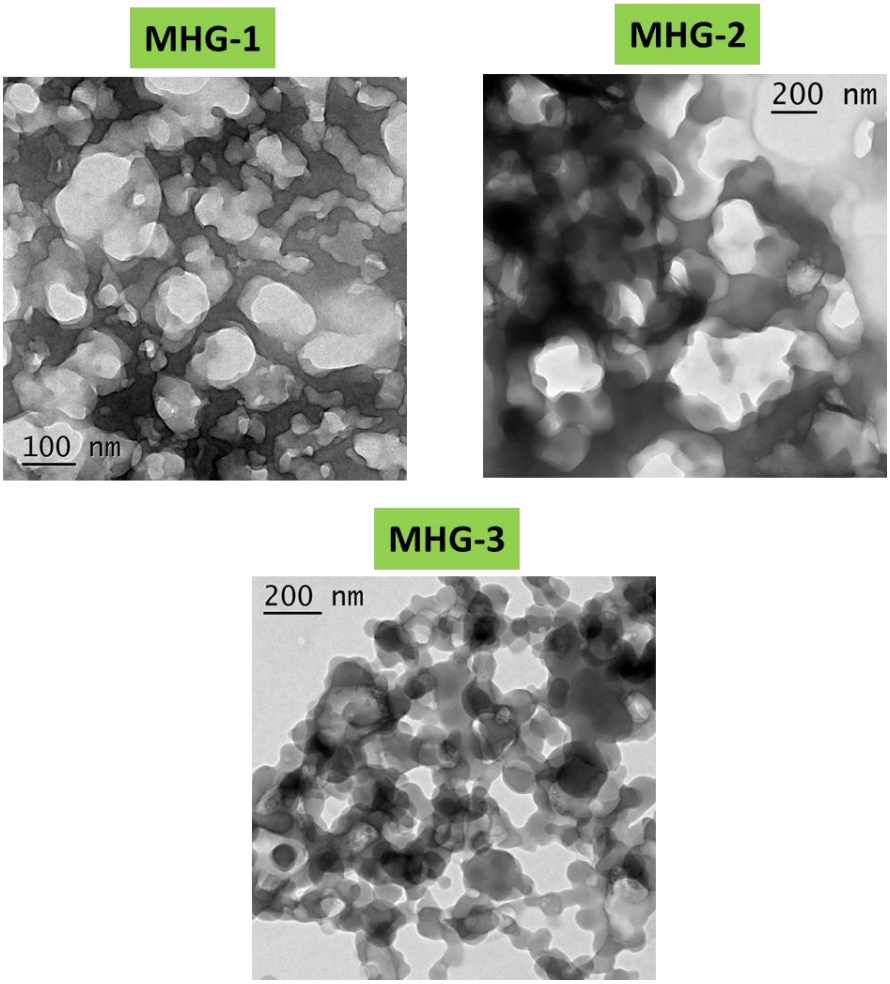

Fig. S12: TEM images of the xerogels (MHG-1, MHG-2 and MHG-3).

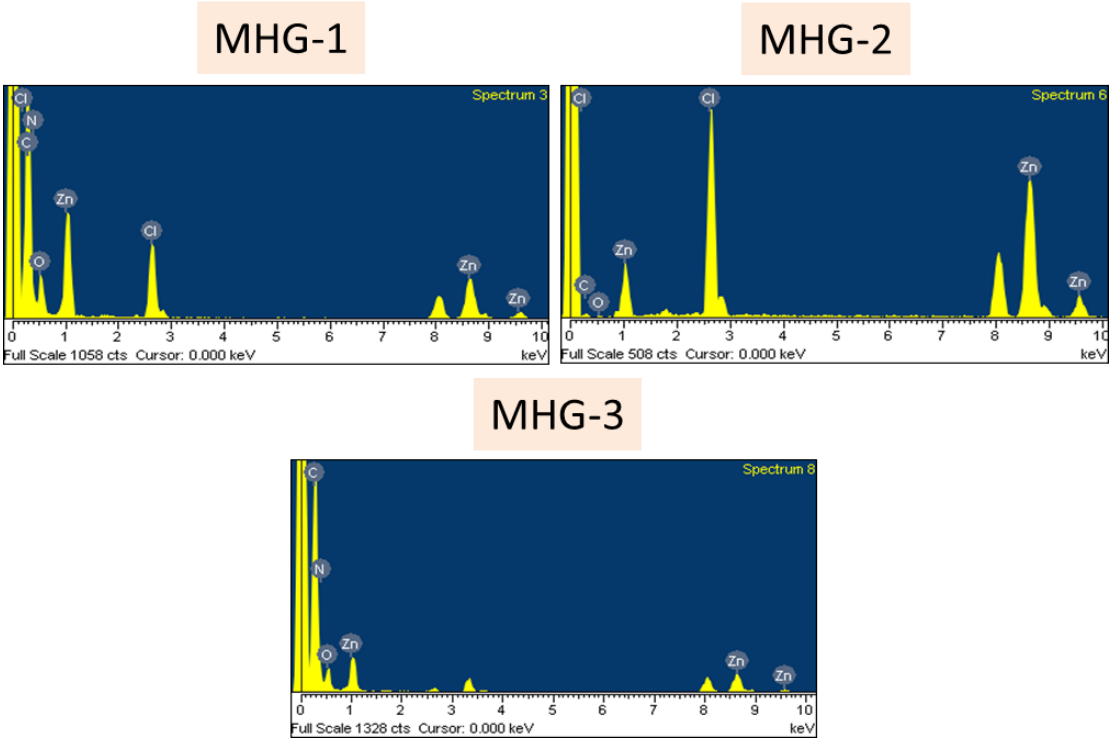

Fig. S13: EDS images of the xerogels (MHG-1, MHG-2 and MHG-3).

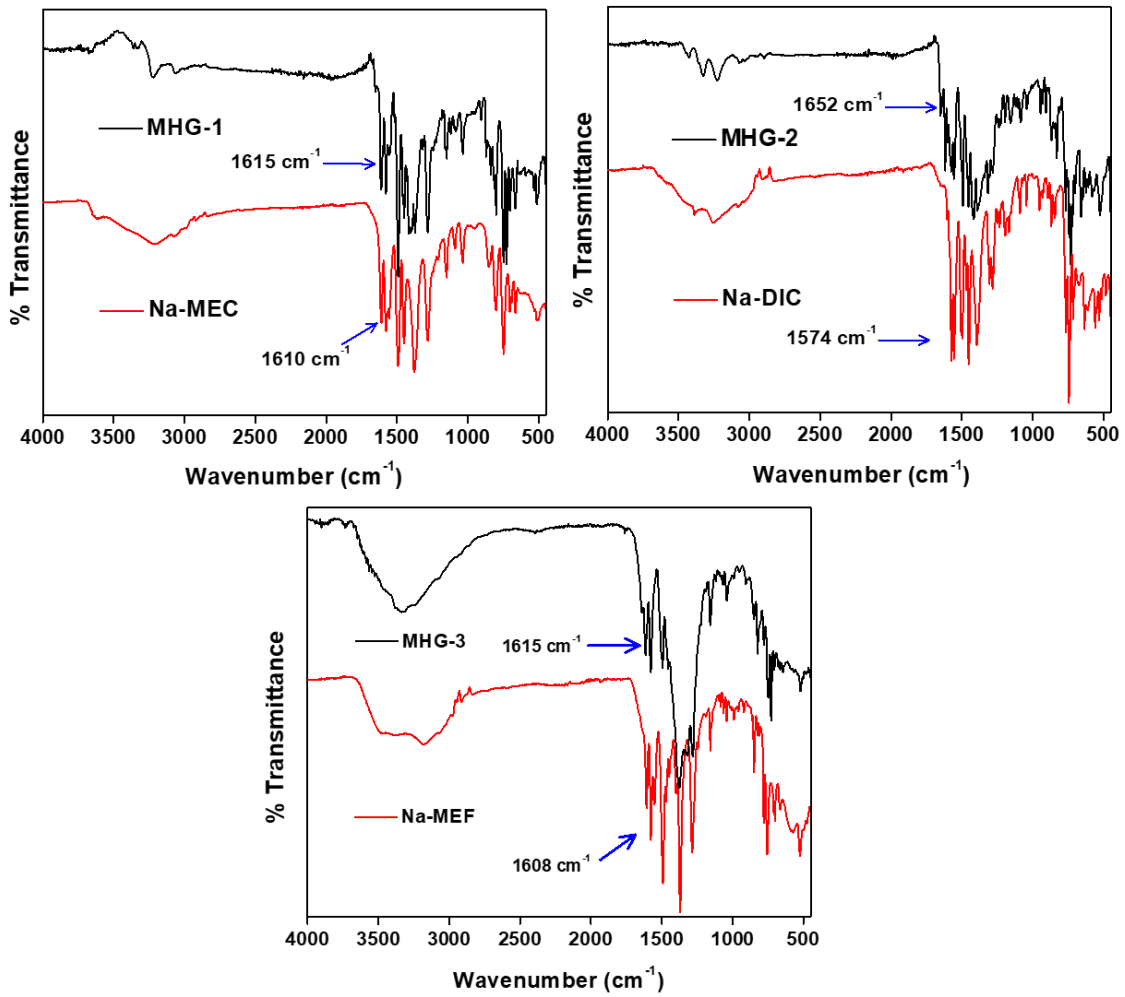

Fig. S14: FT-IR spectra of the xerogels (blue arrow indicated peak of C=O stretching).

Table S8: Tabular representation showing $C=O_{str}$ frequency (cm^{-1}) in different xerogels and corresponding Na-NSAIDs.

Compounds	$\nu_{C=O}$ (cm^{-1})	$\Delta\nu$ (cm^{-1})
Na-MEC	1610	5
MHG-1	1615	
Na-DIC	1574	78
MHG-2	1652	
Na-MEF	1608	7
MHG-3	1615	

35. MTT assay:

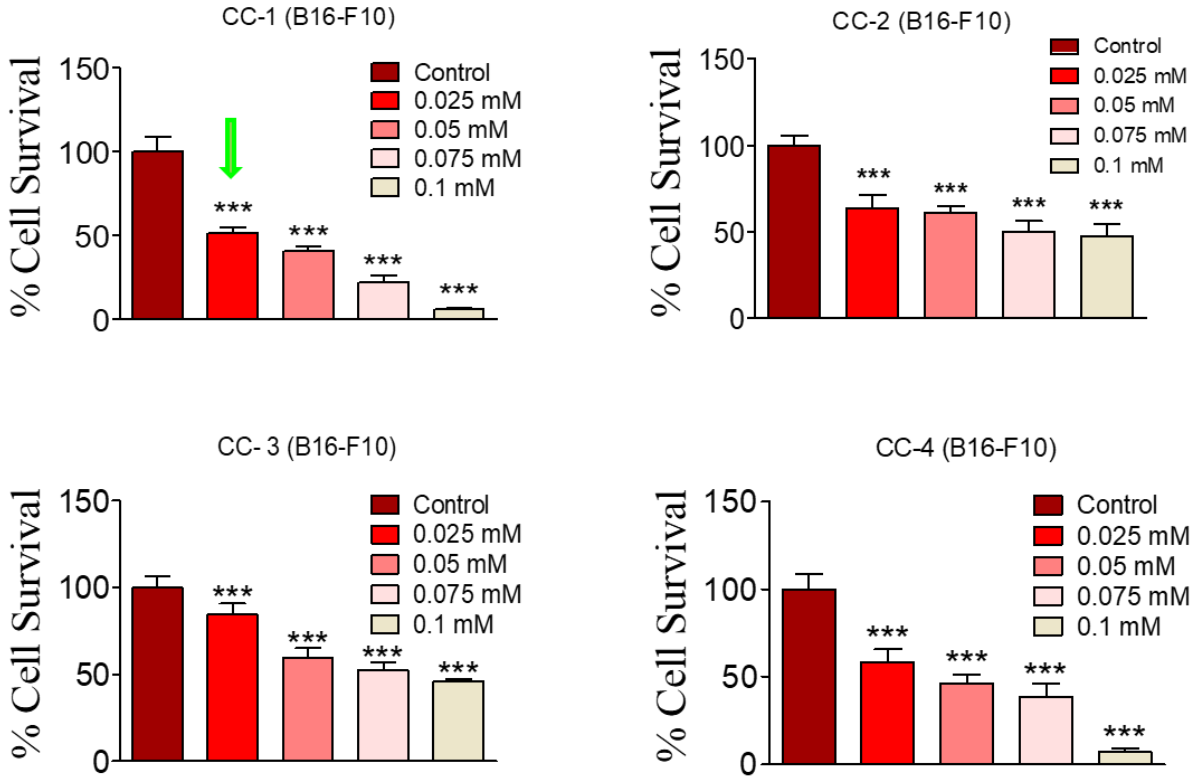

Fig. S15: MTT assay of the CCs on B16-F10 cells after 48 h of incubation (Green arrow indicated IC_{50} value of CC-1 which was lowest among others). In graphs, data were represented as mean+SD (n=3); where ***p<0.001.

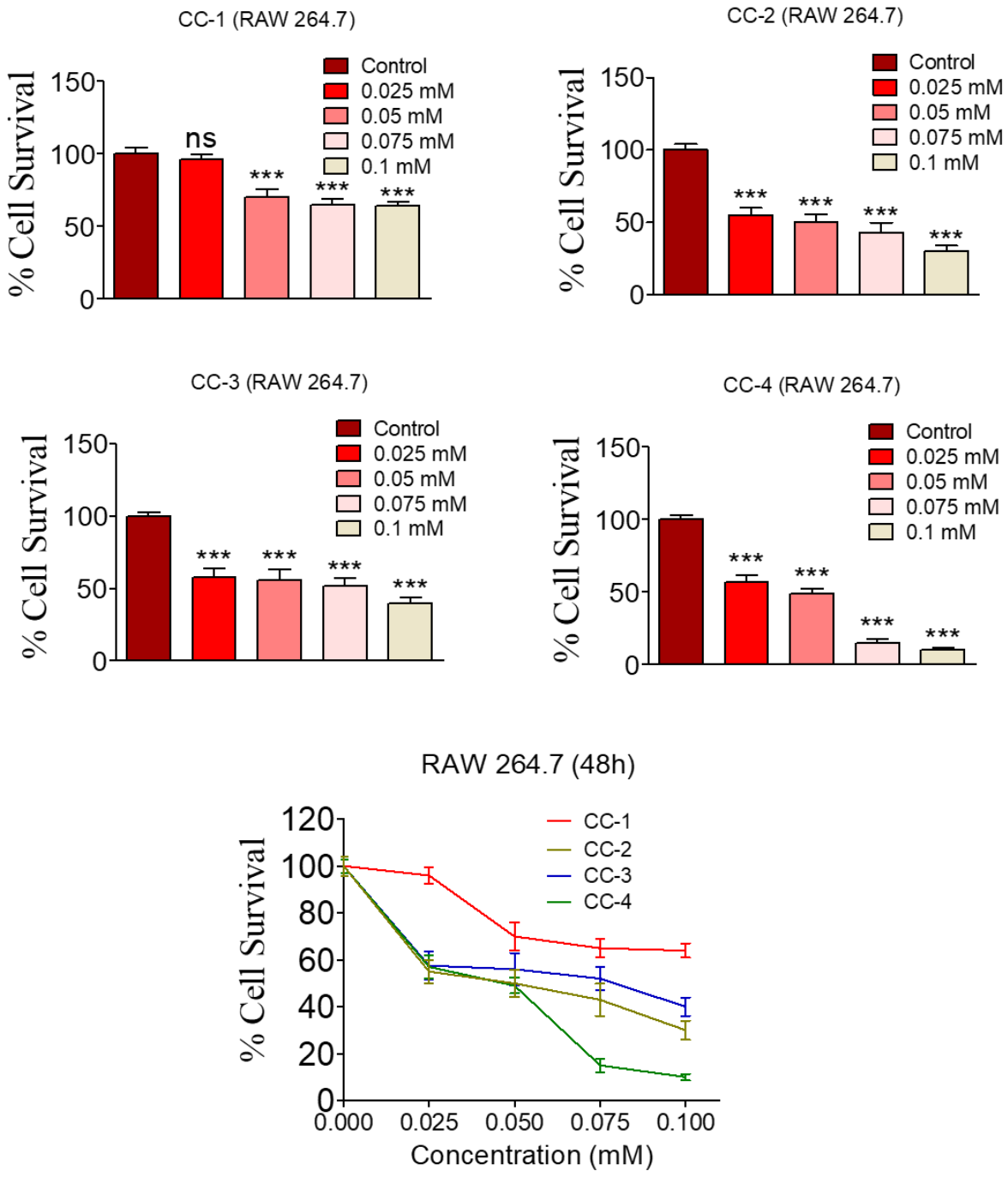

Fig. S16: MTT assay of the CCs on RAW 264.7 cells after 48 h of incubation. In bar graphs, data were represented as mean+SD (n=3); where ***p<0.001 and ns is nonsignificant and in line graphs data were represented as mean±SD (n=3).

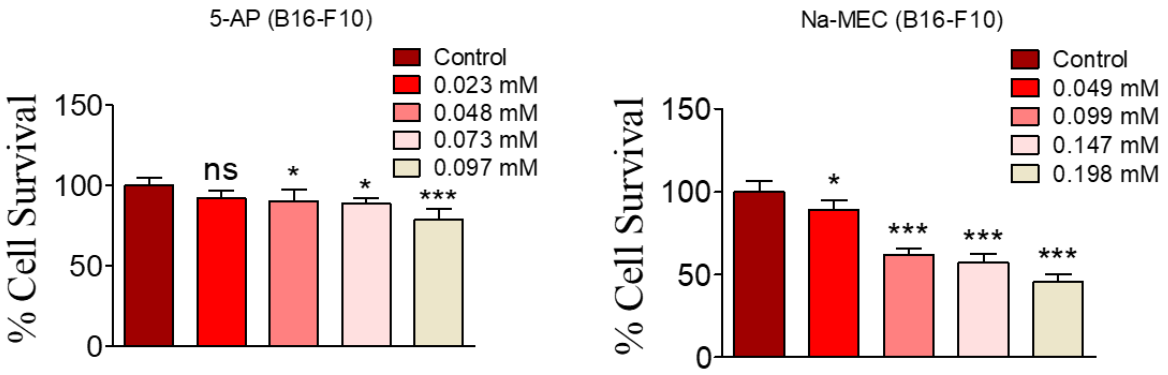

Fig. S17: MTT assay of the components of CC-1 (5-AP and Na-MEC) on B16-F10 cells after 48 h of incubation. The concentrations were taken according to the equivalent amount present in 0.025, 0.05, 0.075, 0.1 mM of CC-1. In graphs, data were represented as mean+SD (n=3); where *p<0.05, ***p<0.001 and ns is nonsignificant.

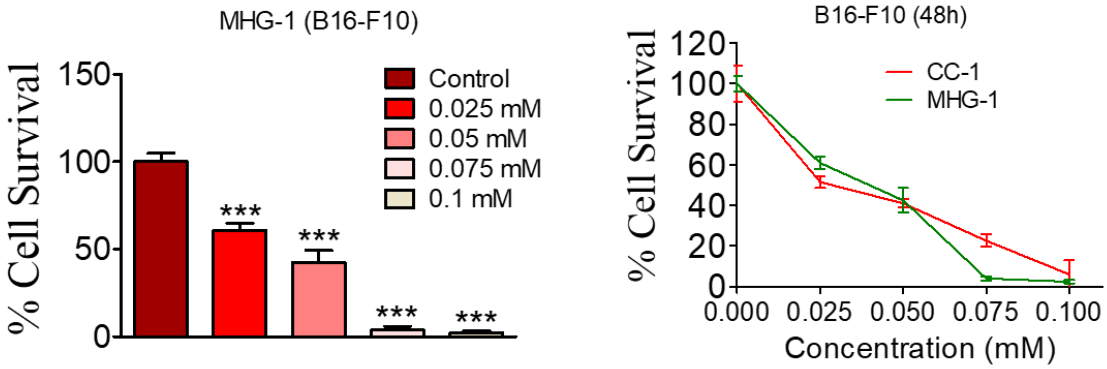

Fig. S18: MTT assay of xerogel of MHG-1 and its comparison with CC-1 against B16-F10 cells (Incubation time 48 h). In bar graphs, data were represented as mean+SD (n=3); where ***p<0.001 and in line graphs data were represented as mean±SD (n=3).

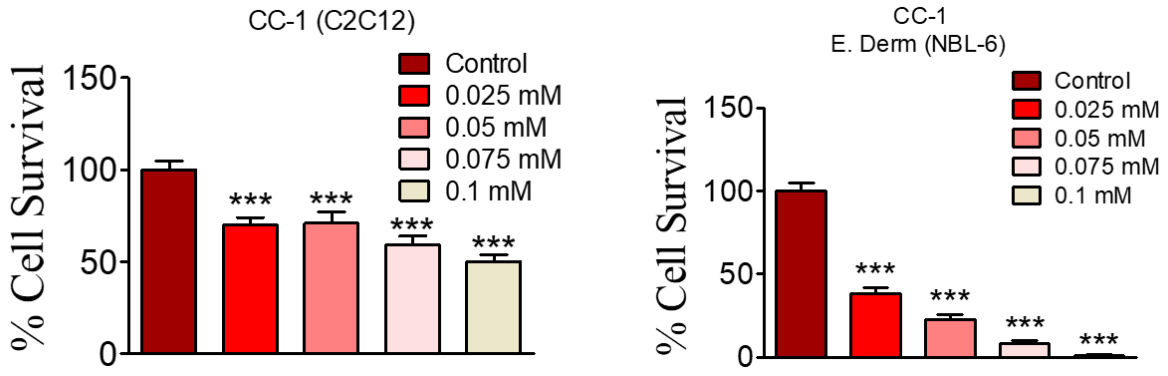

Fig. S19: MTT assay of CC-1 against murine muscle cell (C2C12) and horse skin cell (E. Derm (NBL-6)) (Incubation time 48 h). In graphs, data were represented as mean+SD (n=3); where ***p<0.001.

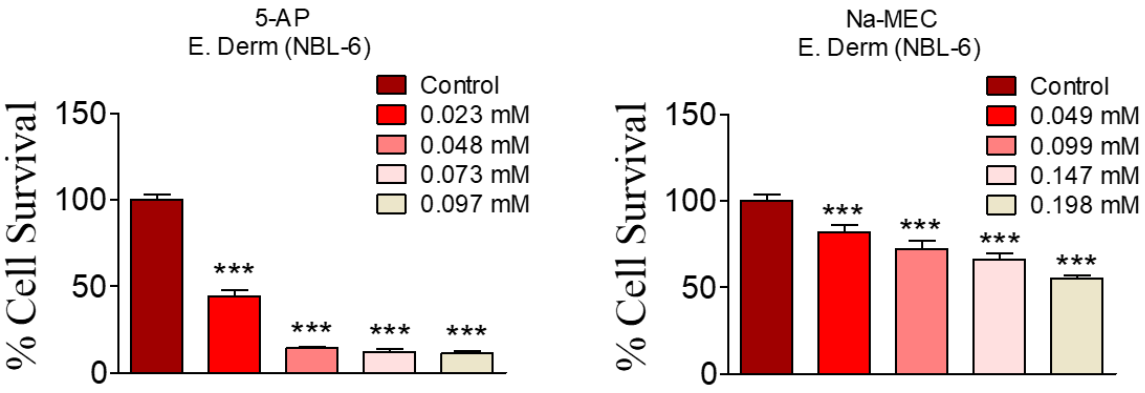

Fig. S20: MTT assay of the components of CC-1 (5-AP and Na-MEC) on E. Derm (NBL-6) after 48 h of incubation, showing 5-AP was responsible for killing skin cells. The concentrations were taken according to the equivalent amount present in 0.025, 0.05, 0.075, 0.1 mM of CC-1. In graphs, data were represented as mean+SD (n=3); where ***p<0.001.

36. Hemolysis study on murine blood:

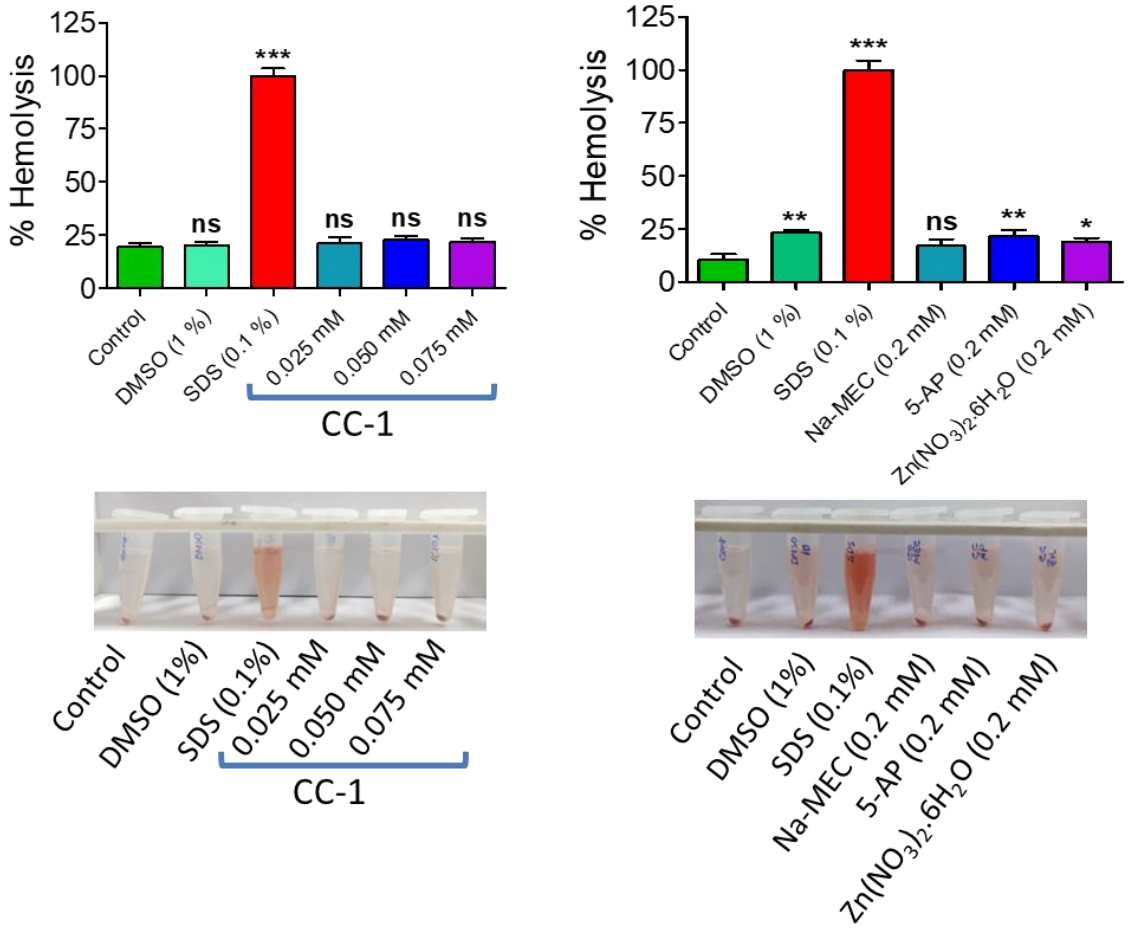

Fig. S21: Hemolytic assay of CC-1 (0.025, 0.05, 0.075 mM) and components of CC-1 (5-AP, Na-MEC and $Zn(NO_3)_2.6H_2O$) showing good biocompatibility. The concentrations of the components were taken nearly 3-fold higher than 0.075 mM of CC-1. % Hemolysis was calculated by considering SDS (0.1 %) treated set as 100 %. In graphs, data were represented as mean+SD (n=3); where *p<0.05, **p<0.01, ***p<0.001 and ns is nonsignificant.

37. UV-vis and Fluorescence spectra of CC-1:

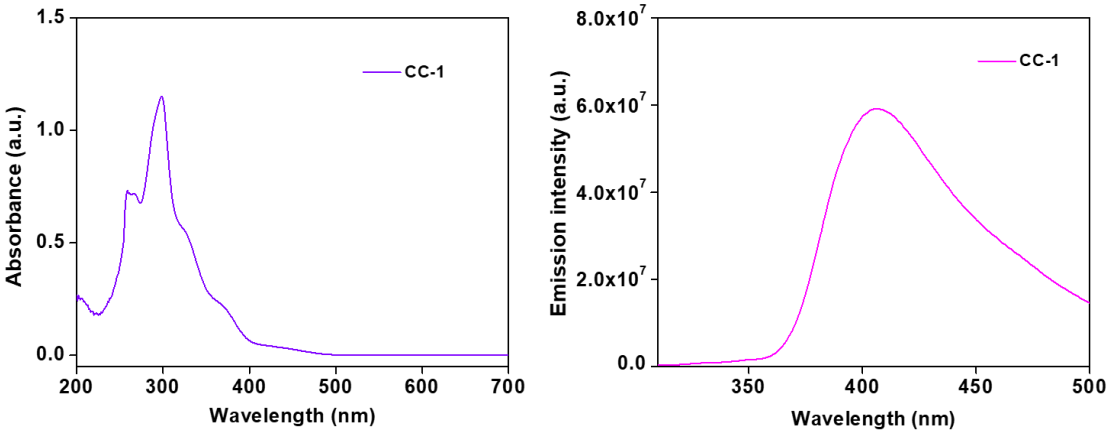

Fig. S22: UV-vis and fluorescence spectra of CC-1 (concentration: ~$3.5x10^{-5}$ (M); excitation wavelength: 300 nm; slit: 3/3). Fluorescence spectra showing its emission in the blue region (emission maxima: 406 nm).

38. In vitro anticancer activity study:

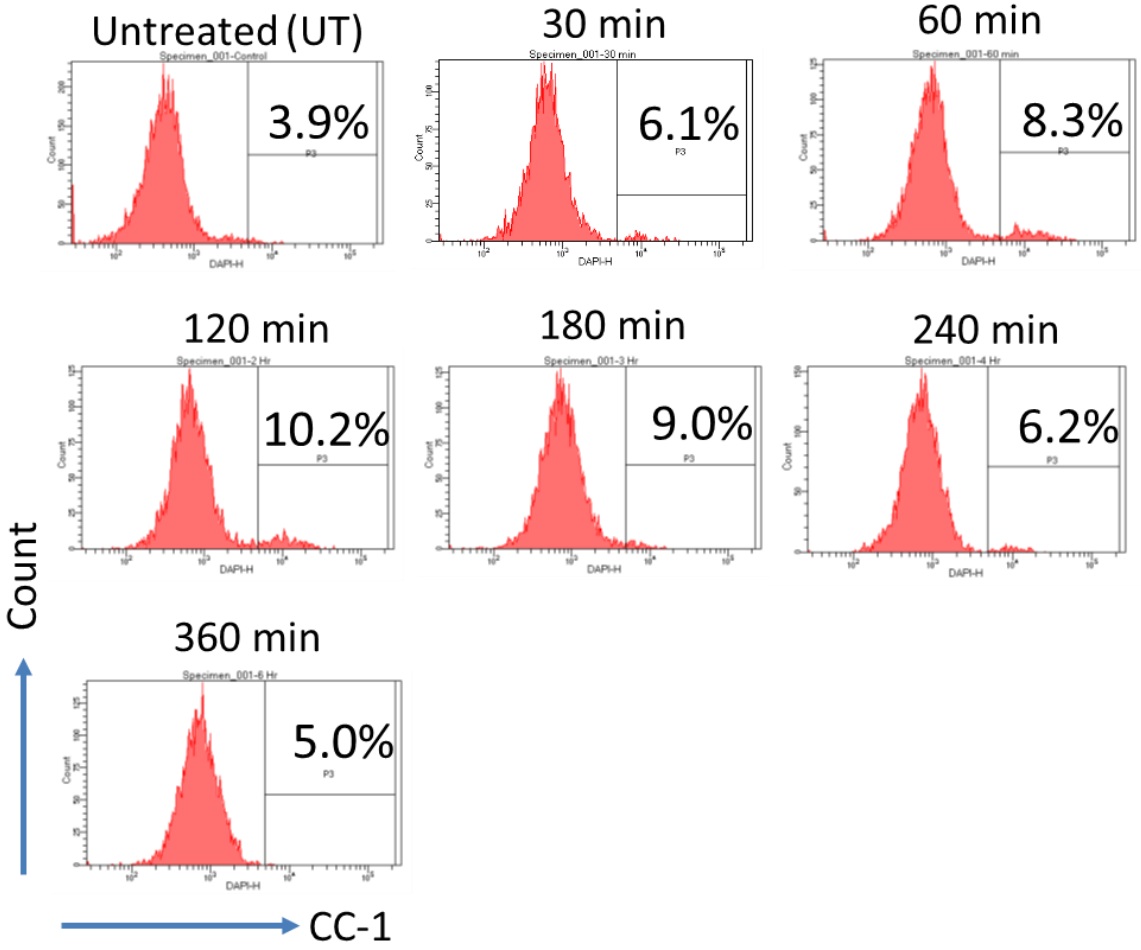

Fig. S23: Flow cytometric data of CC-1 (0.025 mM) treated cells under blue filter at different time intervals showing internalization of CC-1 within B16-F10 cells and maximum internalization occurred at 120 min.

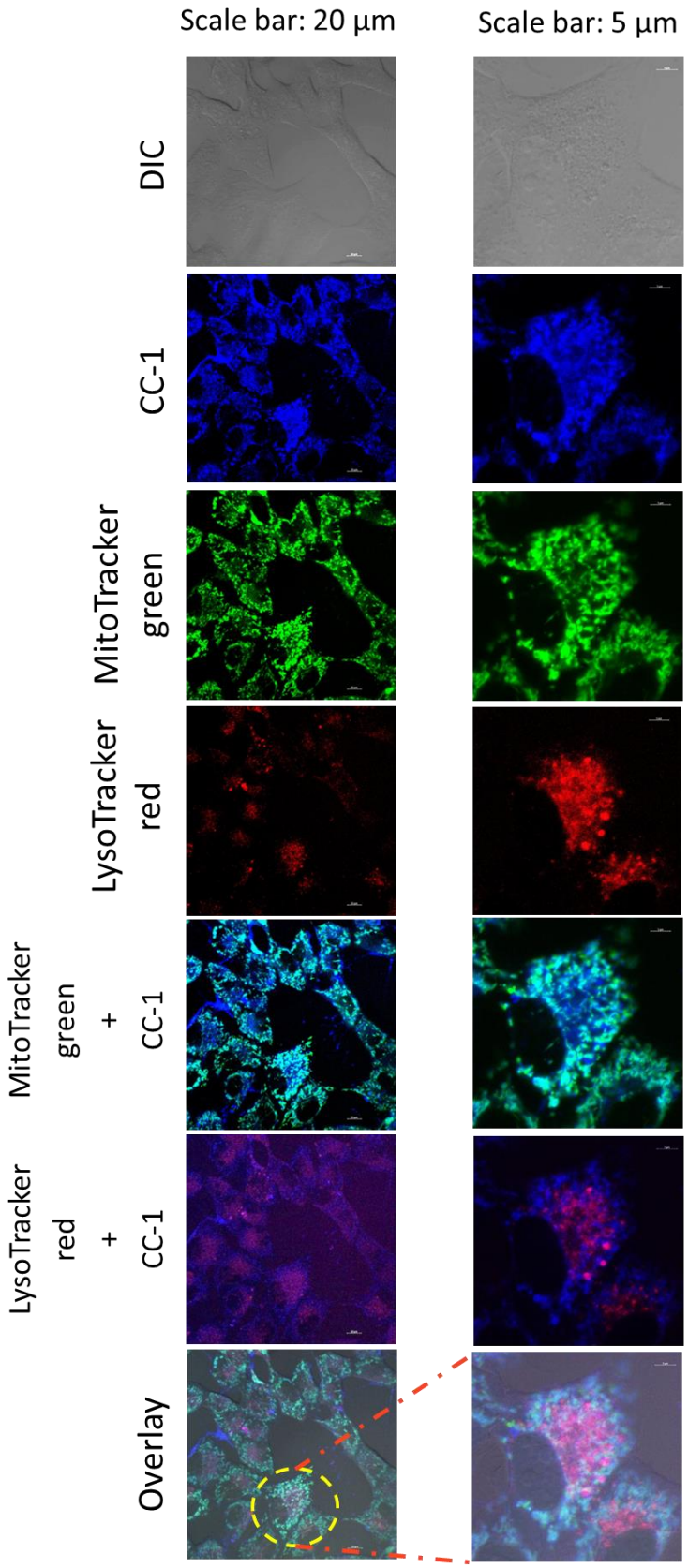

Fig. S24: LSCM images of B16-F10 cells treated with CC-1 (0.025 mM) for 120 min. Various staining (MitoTracker green for mitochondria and LysoTracker red for Lysosome) showed sub-cellular mitochondrial localization of CC-1 (Blue) (Scale bar= 10 µm).

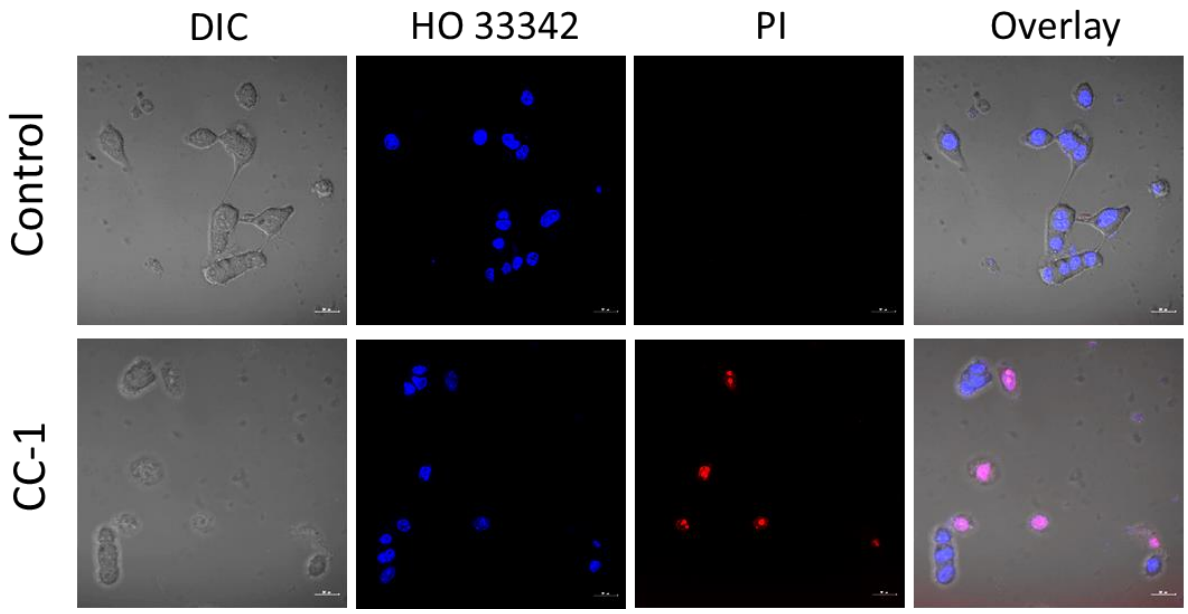

Fig. S25: LSCM images of B16-F10 cells treated with CC-1 (0.025 mM) for 180 min (Scale bar = 20 µm). Various staining (HO 33342 for blue and PI for red) showed internalization of PI (red) due to leaky cell membrane.

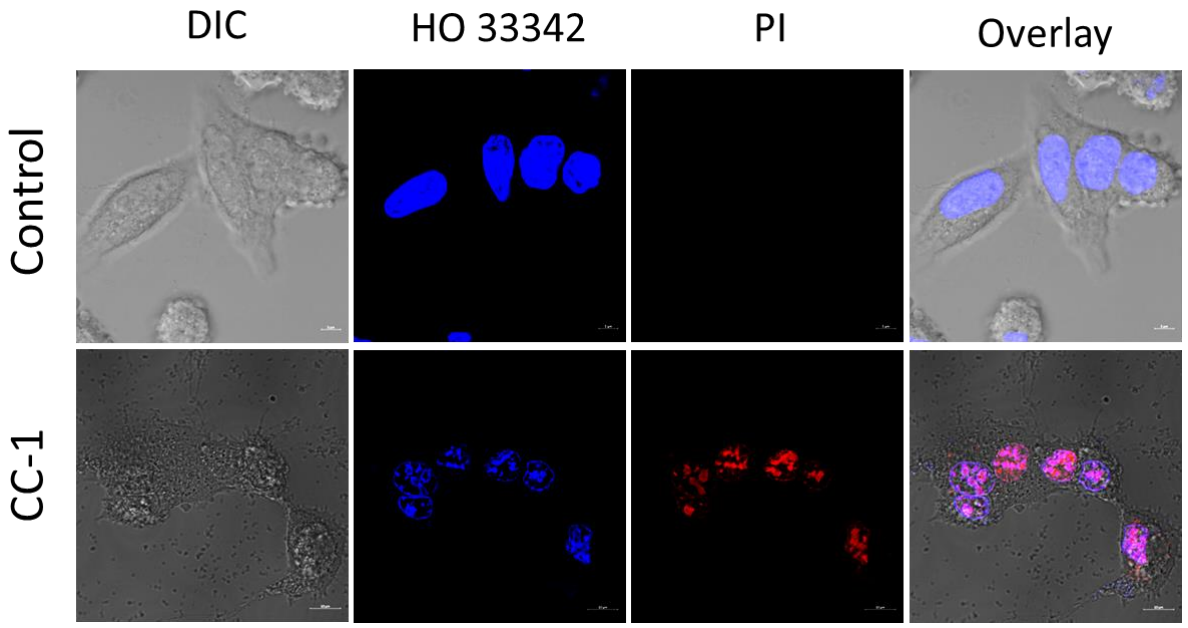

Fig. S26: LSCM images of B16-F10 cells treated with CC-1 (0.025 mM) for 48 h (Scale bar = 10 µm). Various staining (HO 33342 for blue and PI for red) showed nuclear condensation.

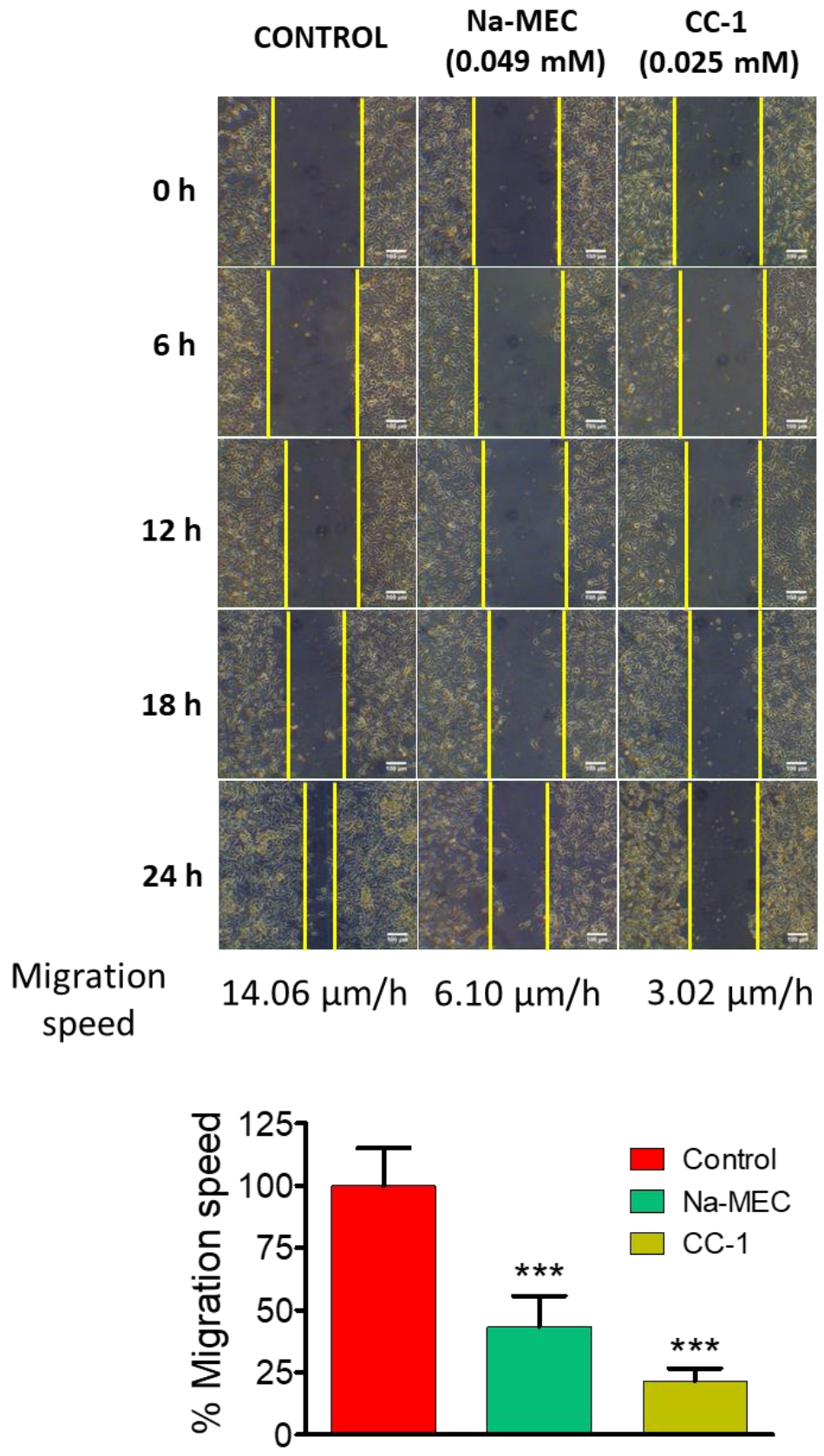

Fig. S27: Migration assay on B16-F10 cells treated with CC-1 (0.025 mM) and Na-MEC (equivalent amount of Na-MEC present in 0.025 mM CC-1) up to 24 h (Scale bar = 100 µm). In graphs, data were represented as mean+SD (n=3); where ***p<0.001.

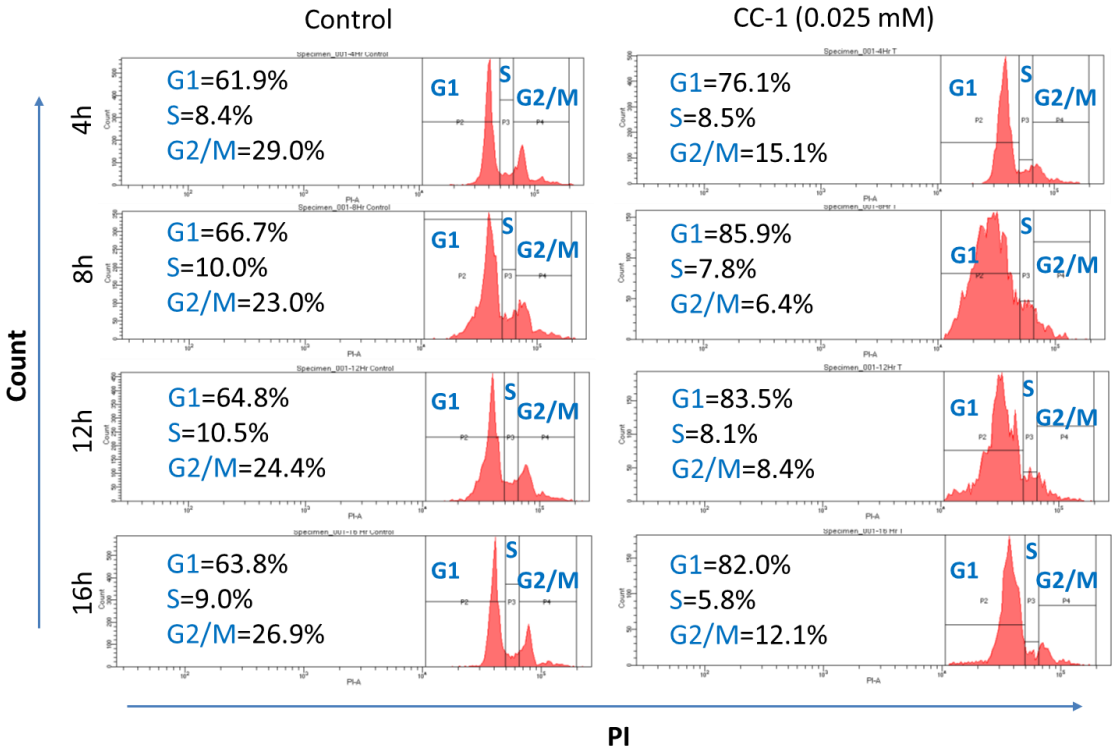

Fig. S28: Cell cycle analysis of CC-1 (0.025 mM) treated B16-F10 cells at different time intervals. Flow cytometric data showing cells were arrested at G1 phase.

39. In vivo anti-cancer activity study:

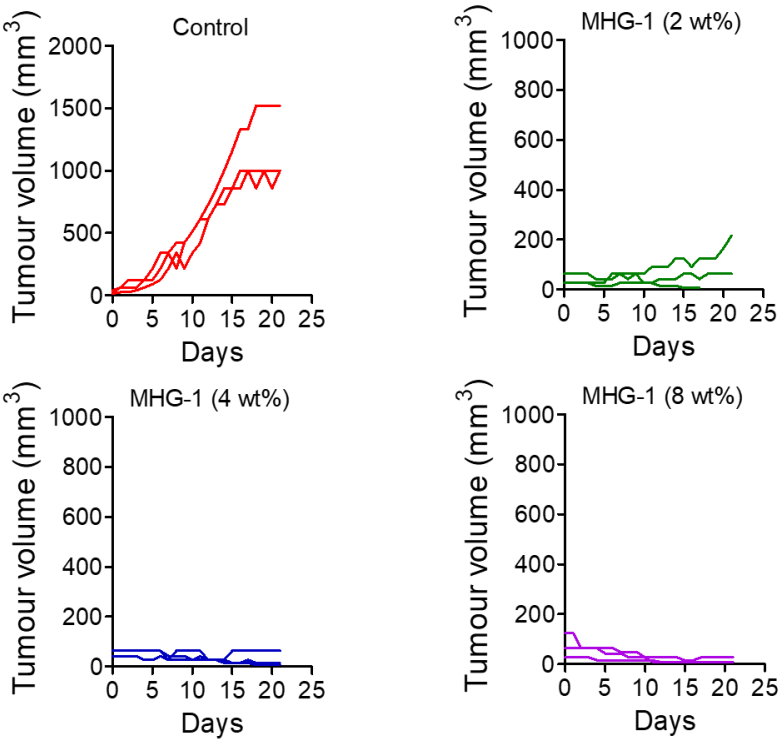

Fig. S29: Measurement of tumour volume (mm^3) during the treatment period (21 days). Different treatment conditions: MHG-1 (2, 4 and 8 wt%). Each group contained three mice (n=3).

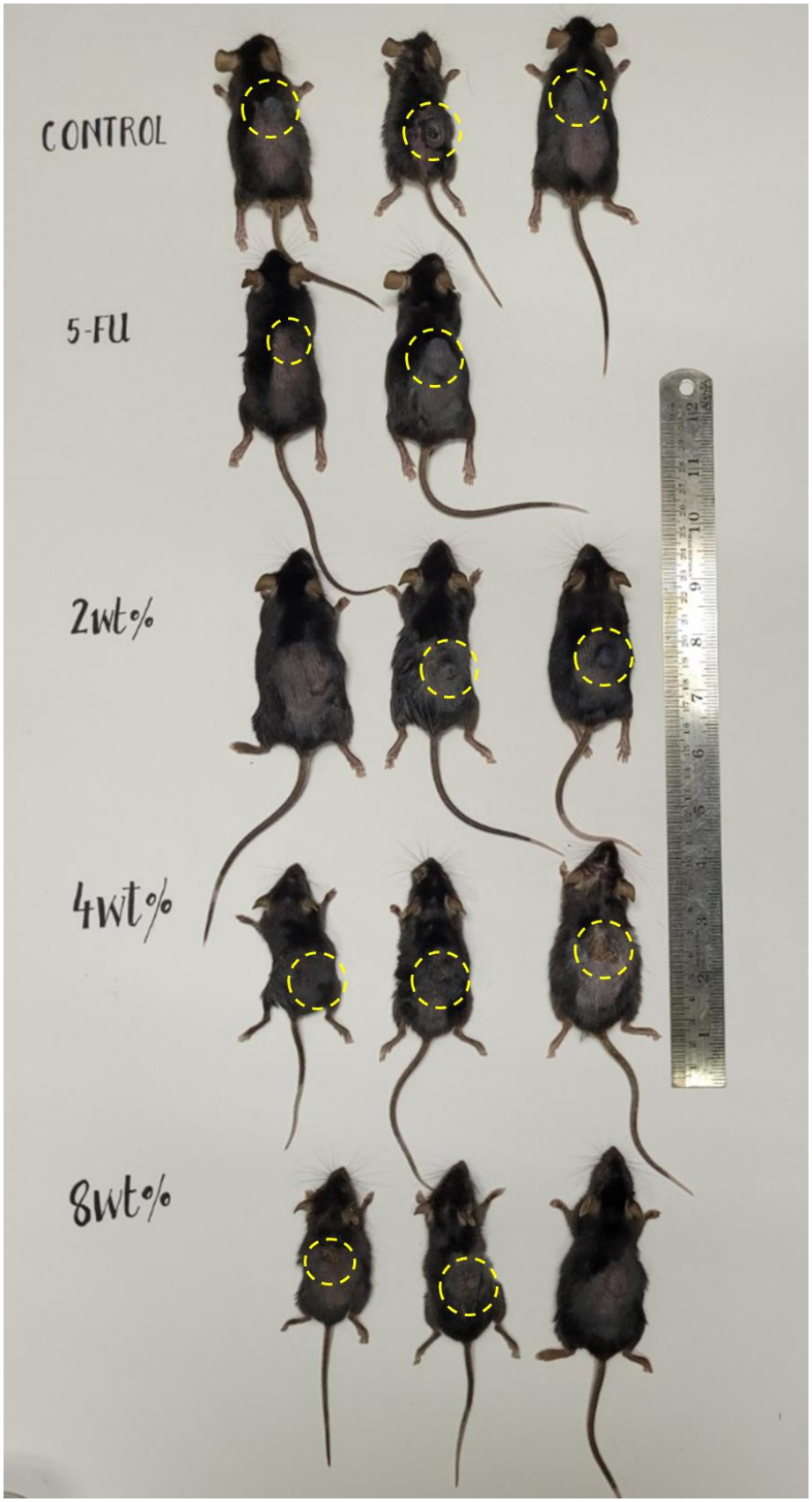

Fig. S30: Representative images of tumour bearing mice in different groups (Control: PBS (1x, 200 µL); 5-FU: 5-fluorouracil (20 mg/kg body weight); MHG-1 (2, 4, 8 wt%; 200 µL)). Each group contained three mice (n=3).

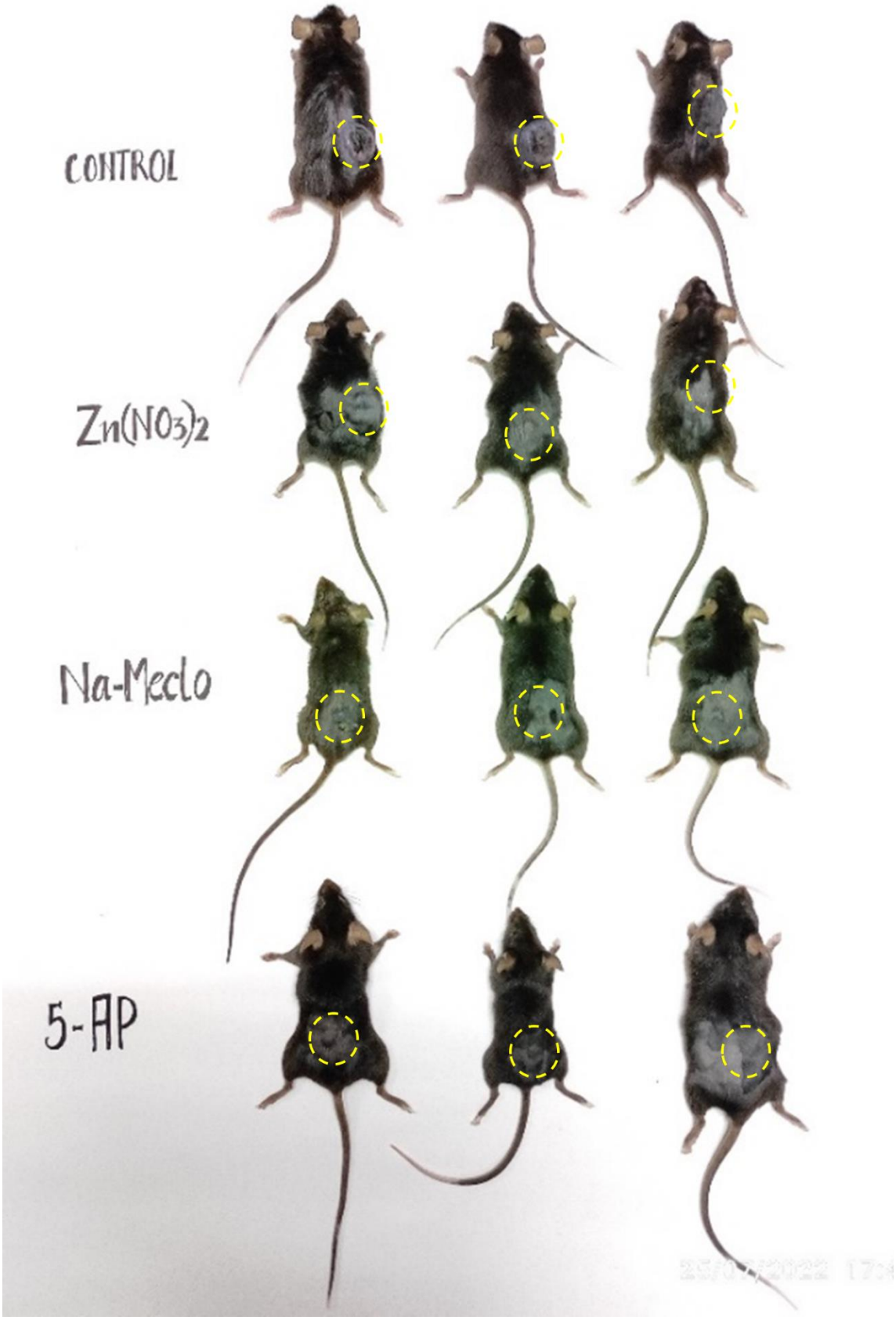

Fig. S31: Representative images of tumour bearing mice in different groups (Control: PBS (1x, 200 µL); $Zn(NO_3)_2$: 2.2 mg in 200 µL; Na-MEC or Na-MECLO: 4.8 mg in 200 µL; 5-AP: 1.5 mg in 200 µL). Each group contained three mice (n=3).

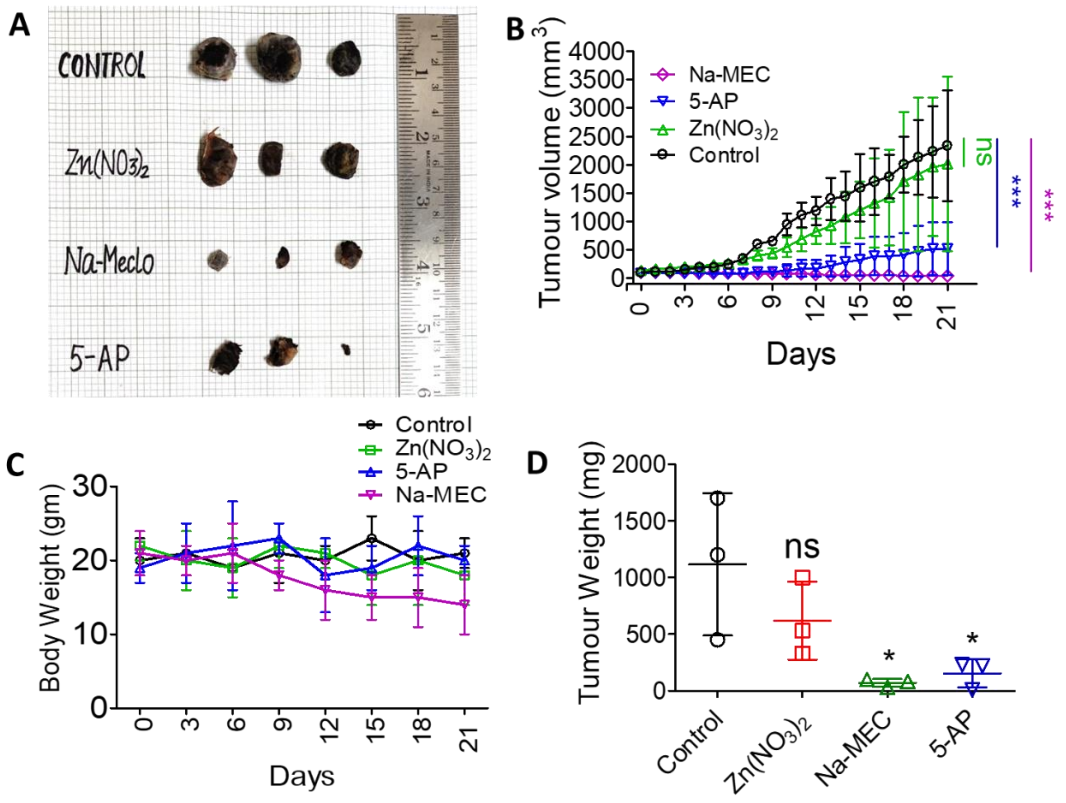

Fig. S32: *In vivo* experiment of individual components: A) tumour images of different components treated set; B) tumour volume; C) mice body weight and D) tumour weight after 21 days of treatment. In graphs, data were represented as mean±SD (n=3); where *p<0.05, ***p<0.001 and ns is nonsignificant.

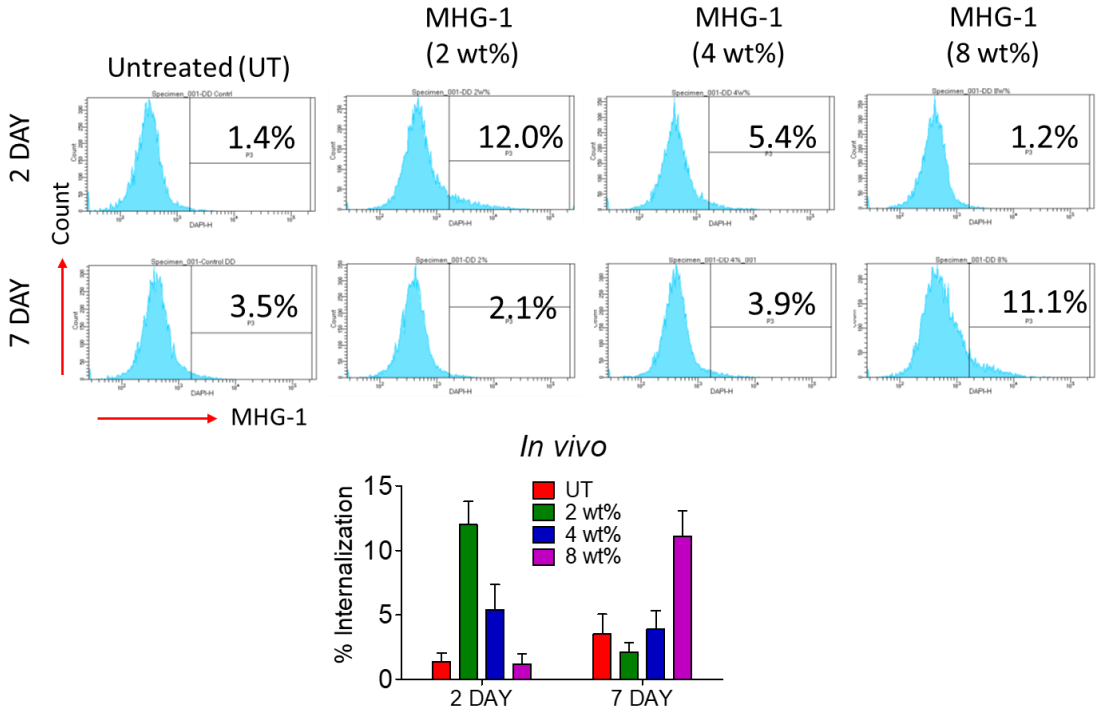

Fig. S33: *In vivo* drug internalization with respect to 5-AP after MHG-1 (4 wt%) hydrogel treatment at 2 and 7 days.

40. In vitro apoptosis study:

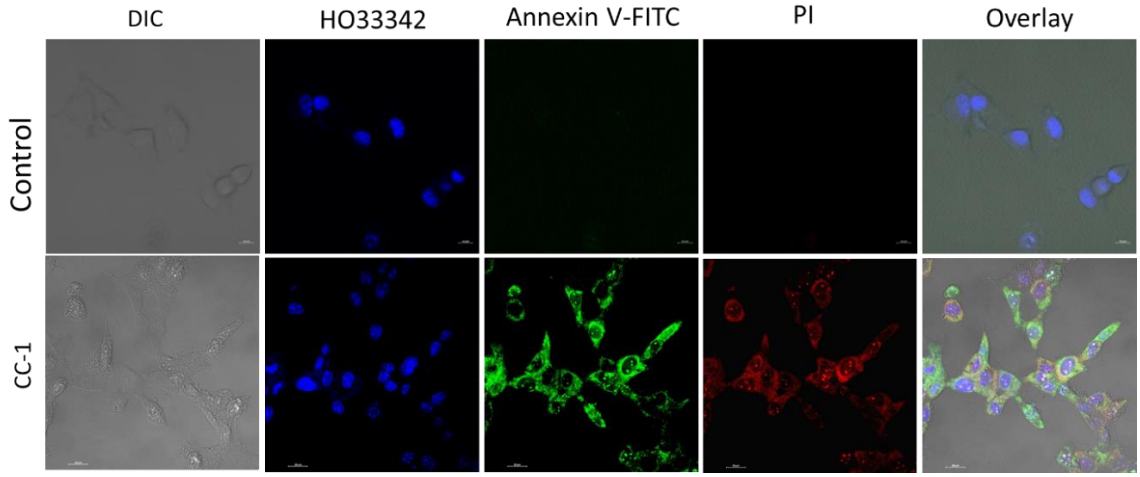

Fig. S34: *In vitro* apoptosis study by LSCM imaging under different staining conditions (CC-1 treatment concentration: 0.025 mM) (Scale bar= 20 µm).

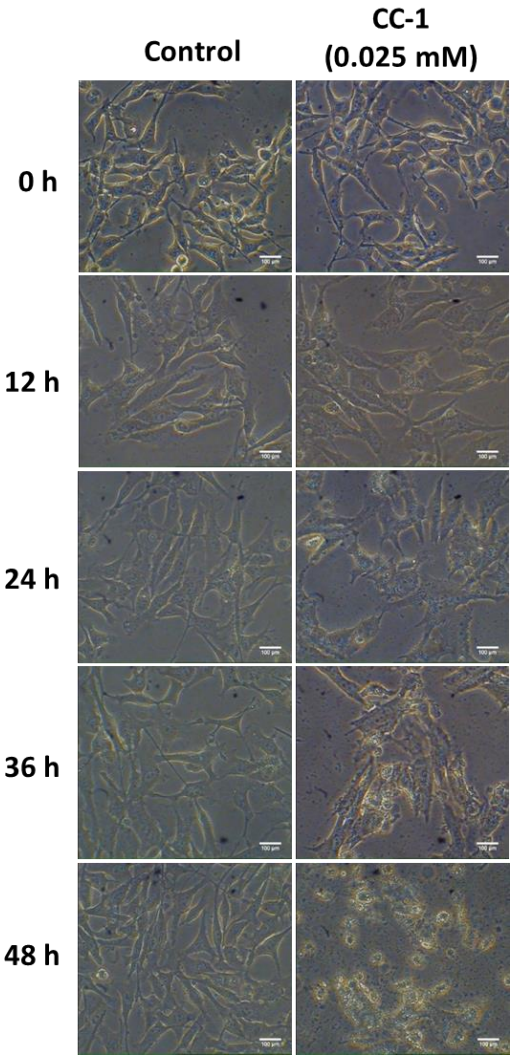

Fig. S35: Optical microscope images of CC-1 treated B16-F10 cells at different time intervals in *in vitro* condition (Scale bar= 100 µm).

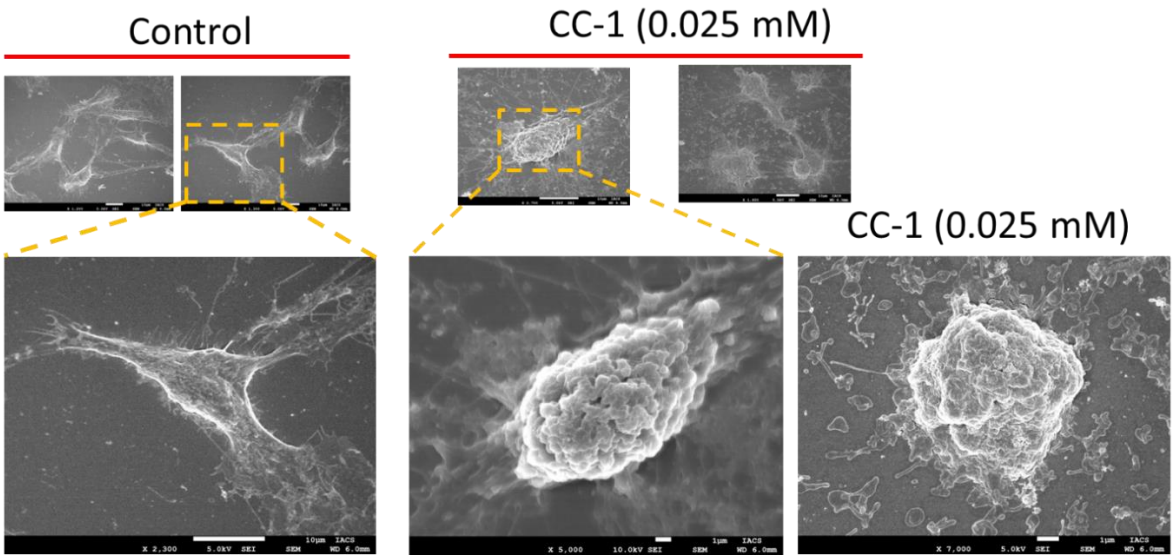

Fig. S36: SEM images of CC-1 treated B16-F10 cells after 48 h in *in vitro* condition (Scale bar= 10 µm). Cell shrinkage along with bleb formation was observed after treatment supporting apoptosis.

41. In vivo apoptosis study:

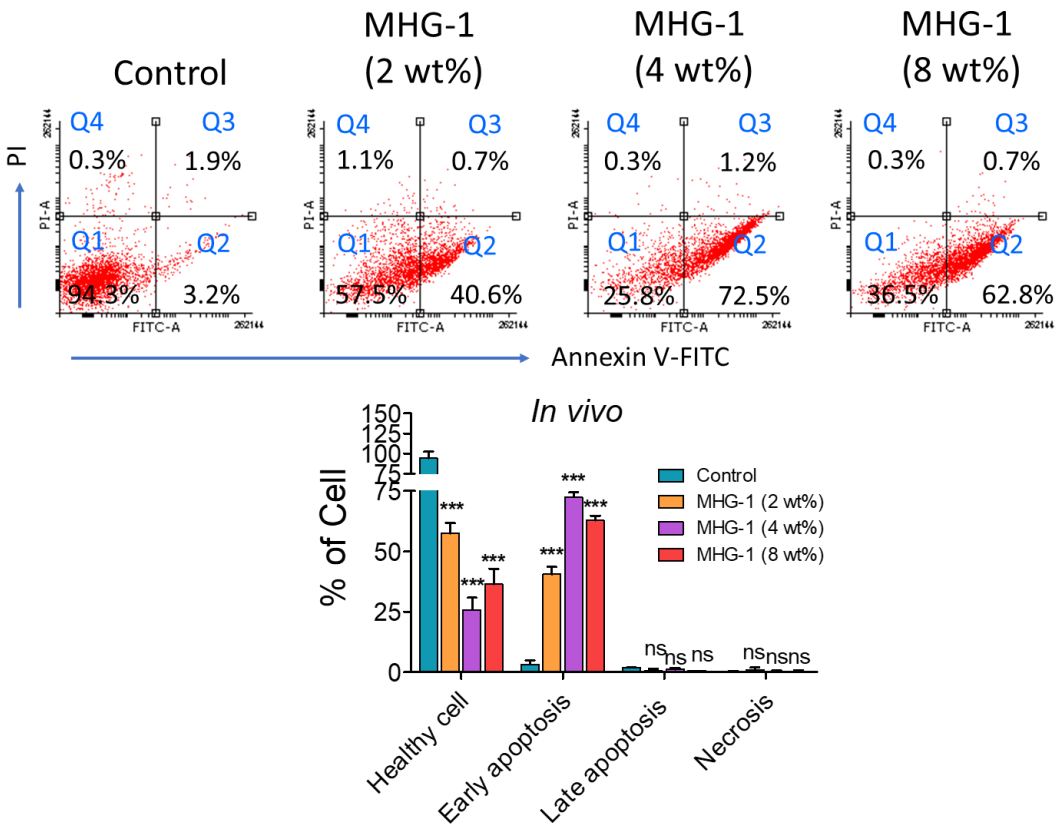

Fig. S37: *In vivo* apoptosis study using flow cytometry after 7 days of MHG-1 (2, 4 and 8 wt%) treatment. % of cells in different quadrant was determined by staining with annexin V-FITC and PI (Q1-healthy cells; Q2-early apoptosis; Q3-late apoptosis; Q4-necrosis). In graphs, data were represented as mean+SD (n=3); where ***p*<0.01, ****p*<0.001.

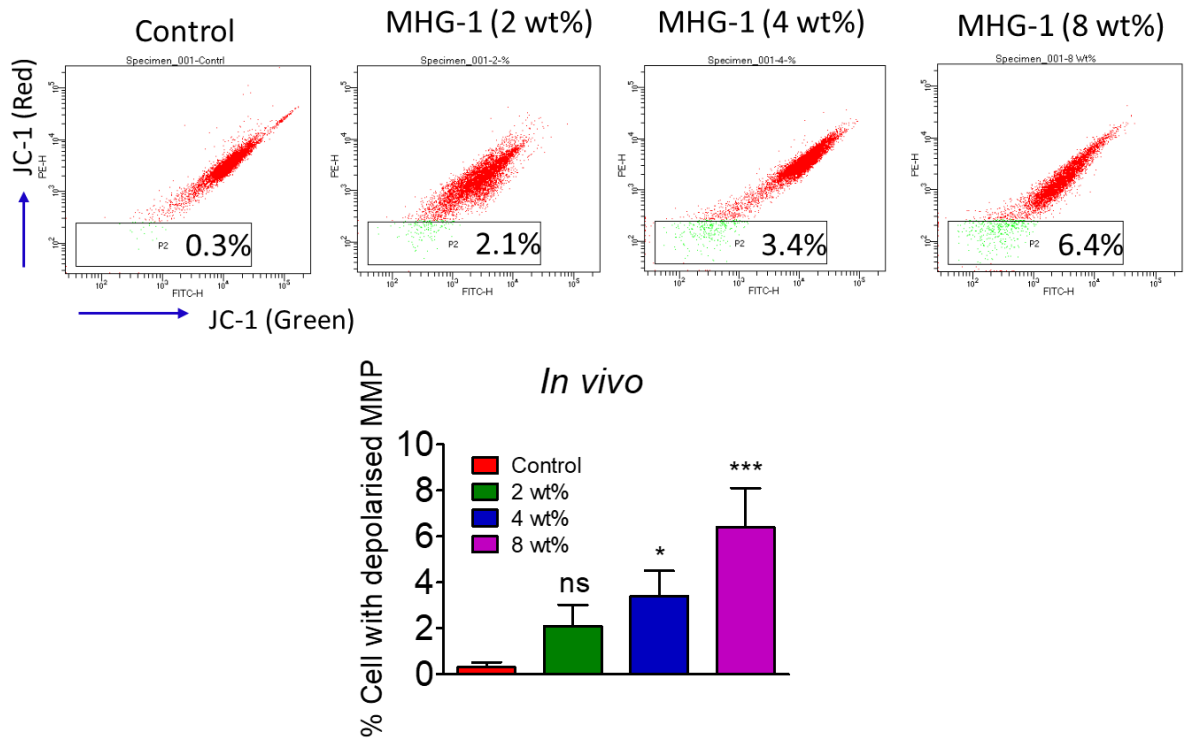

Fig. S38: *In vivo* mitochondrial membrane potential (MMP) study using flow cytometry after 7 days of MHG-1 (2, 4 and 8 wt%) treatment. % of MMP depolarisation was determined by staining with JC-1. In graphs, data were represented as mean+SD (n=3); where *p<0.05, ***p<0.001 and ns is nonsignificant.

42. In vitro side population (SP) study:

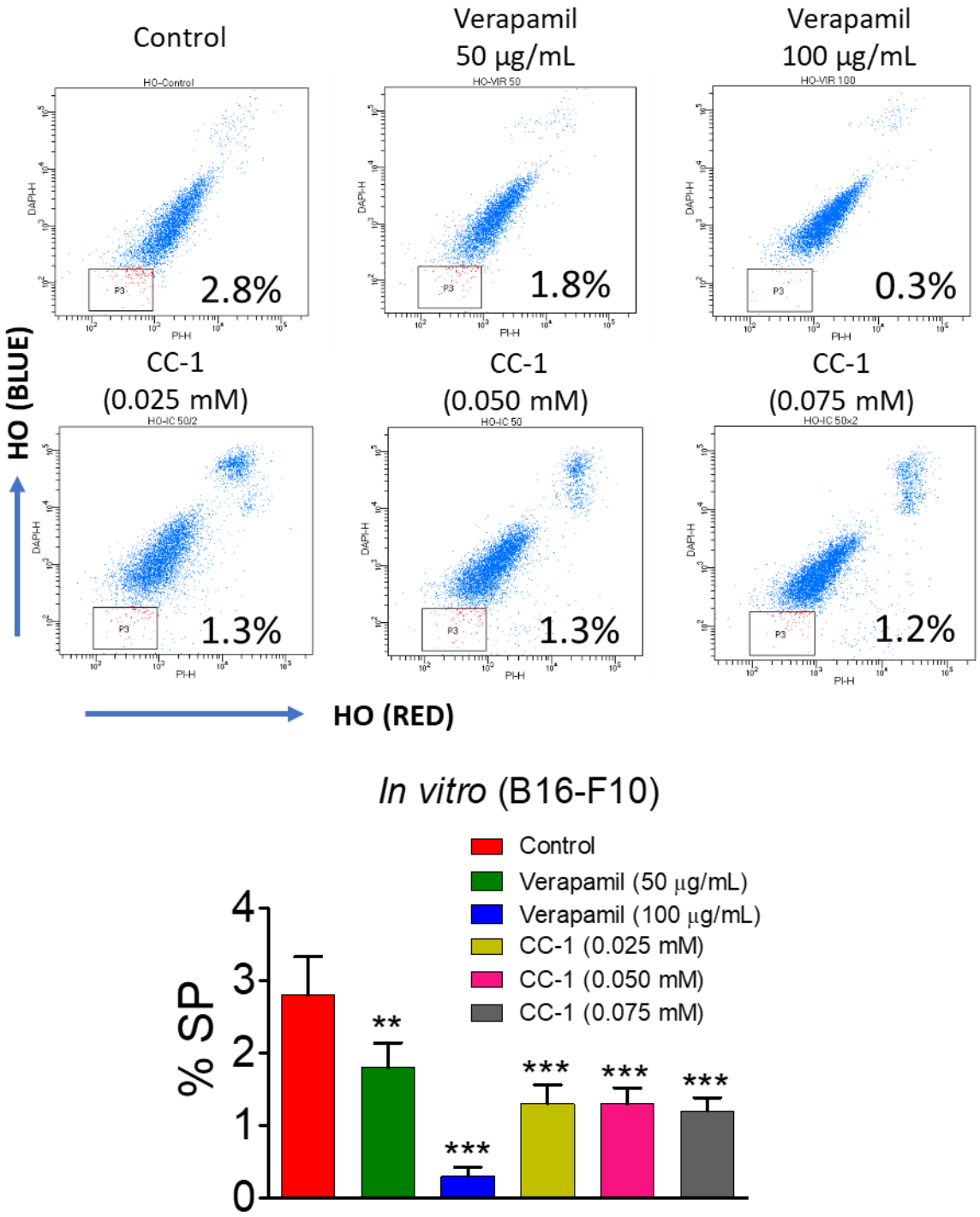

Fig. S39: *In vitro* Hoechst efflux assay using flow cytometry after CC-1 (0.025, 0.05 and 0.075 mM) treatment for 48 h. Verapamil (50 and 100 µg/mL) was taken as positive control. In graphs, data were represented as mean+SD (n=3); where $^{**}p<0.01$ and $^{***}p<0.001$.

43. In vivo side population (SP) study:

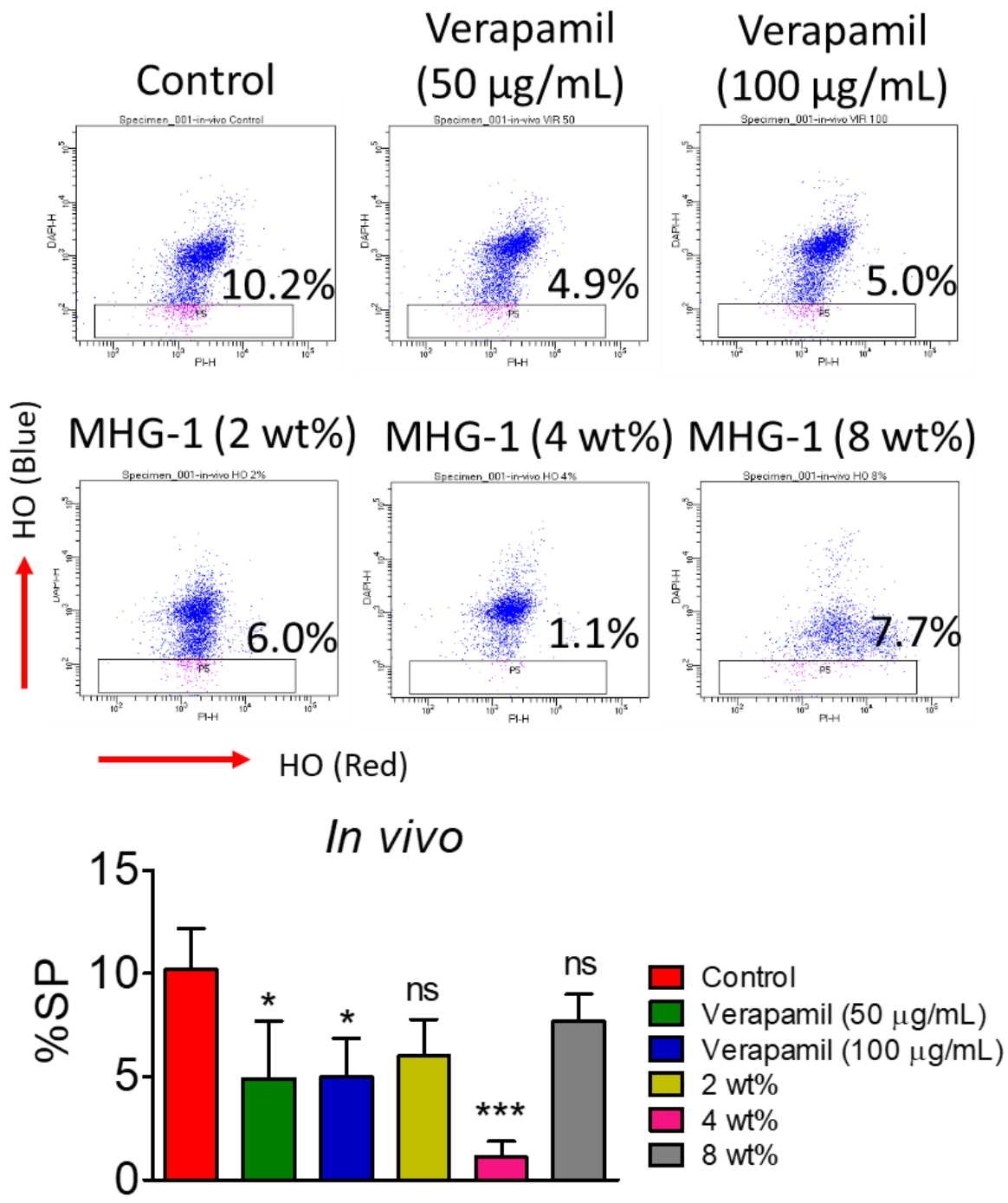

Fig. S40: *In vivo* Hoechst efflux study using flow cytometry after MHG-1 (2, 4 and 8 wt%) treatment for 7 days. Verapamil (50, 100 µg/mL) was taken as positive control. In graphs, data were represented as mean+SD (n=3); where *p<0.05, ***p<0.001 and ns is nonsignificant.

44. In vitro reactive oxygen species (ROS) determination:

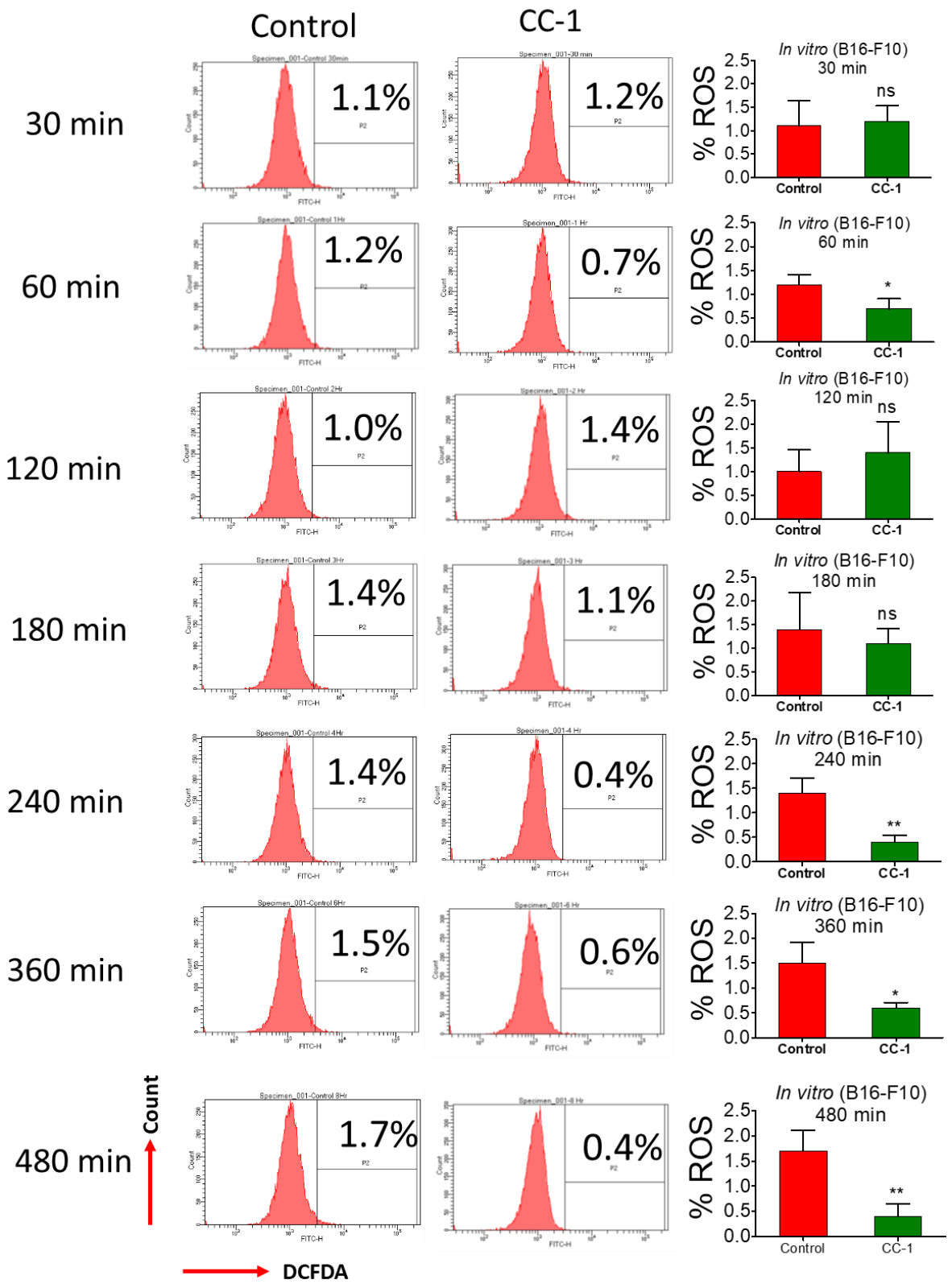

Fig. S41: *In vitro* ROS determination using flow cytometry after CC-1 (0.025 mM) treatment for 48 h. ROS was determined by staining with DCFDA. In graphs, data were represented as mean+SD (n=3); where *p<0.05, **p<0.01 and ns is nonsignificant.

45. In vivo reactive oxygen species (ROS) determination:

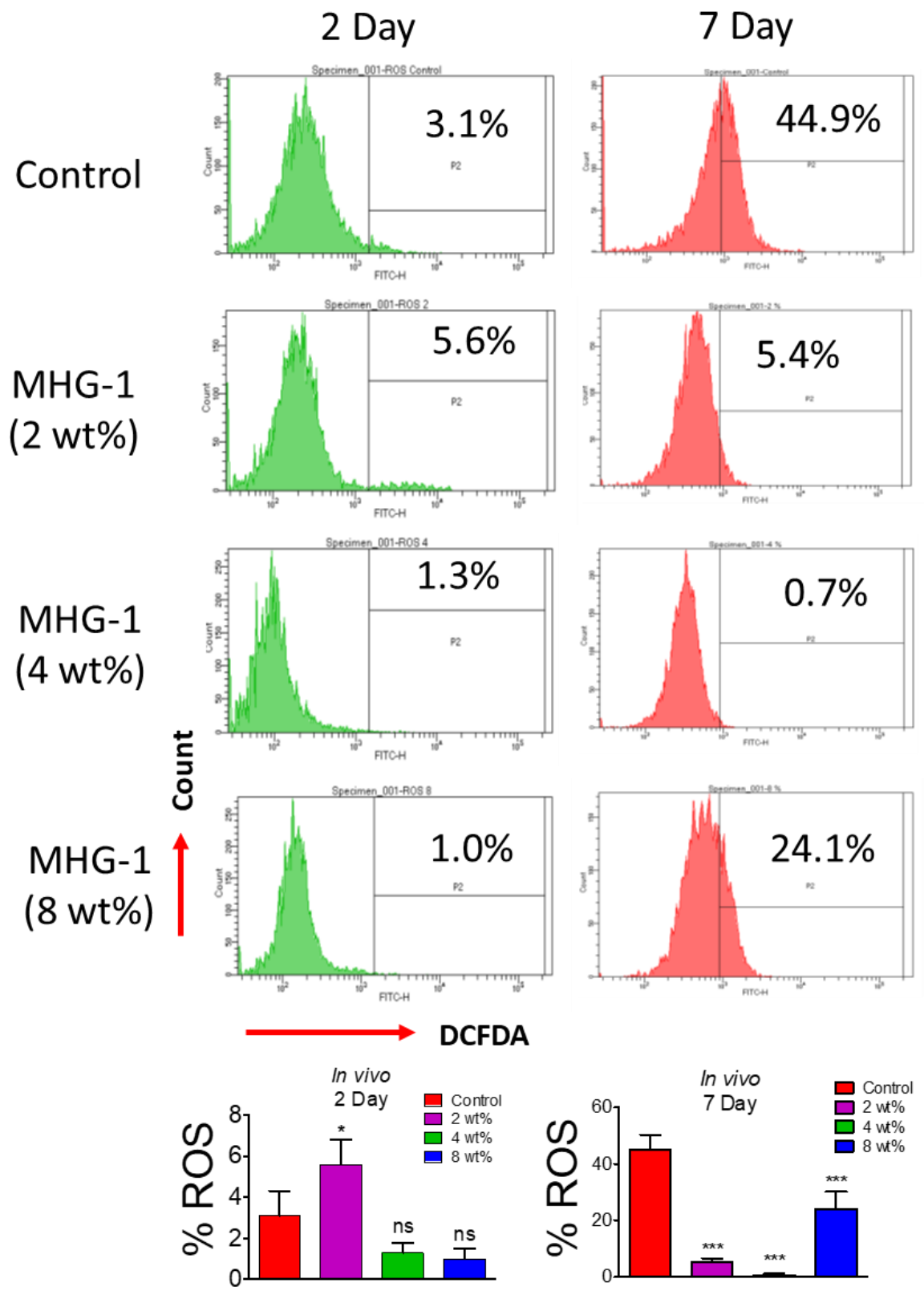

Fig. S42: *In vivo* ROS determination using flow cytometry after MHG-1 (2, 4, 8 wt%) treatment for 2 and 7 days. ROS was determined by staining with DCFDA. In graphs, data were represented as mean+SD (n=3); where *p<0.05, ***p<0.001 and ns is nonsignificant.

46. Histopthology:

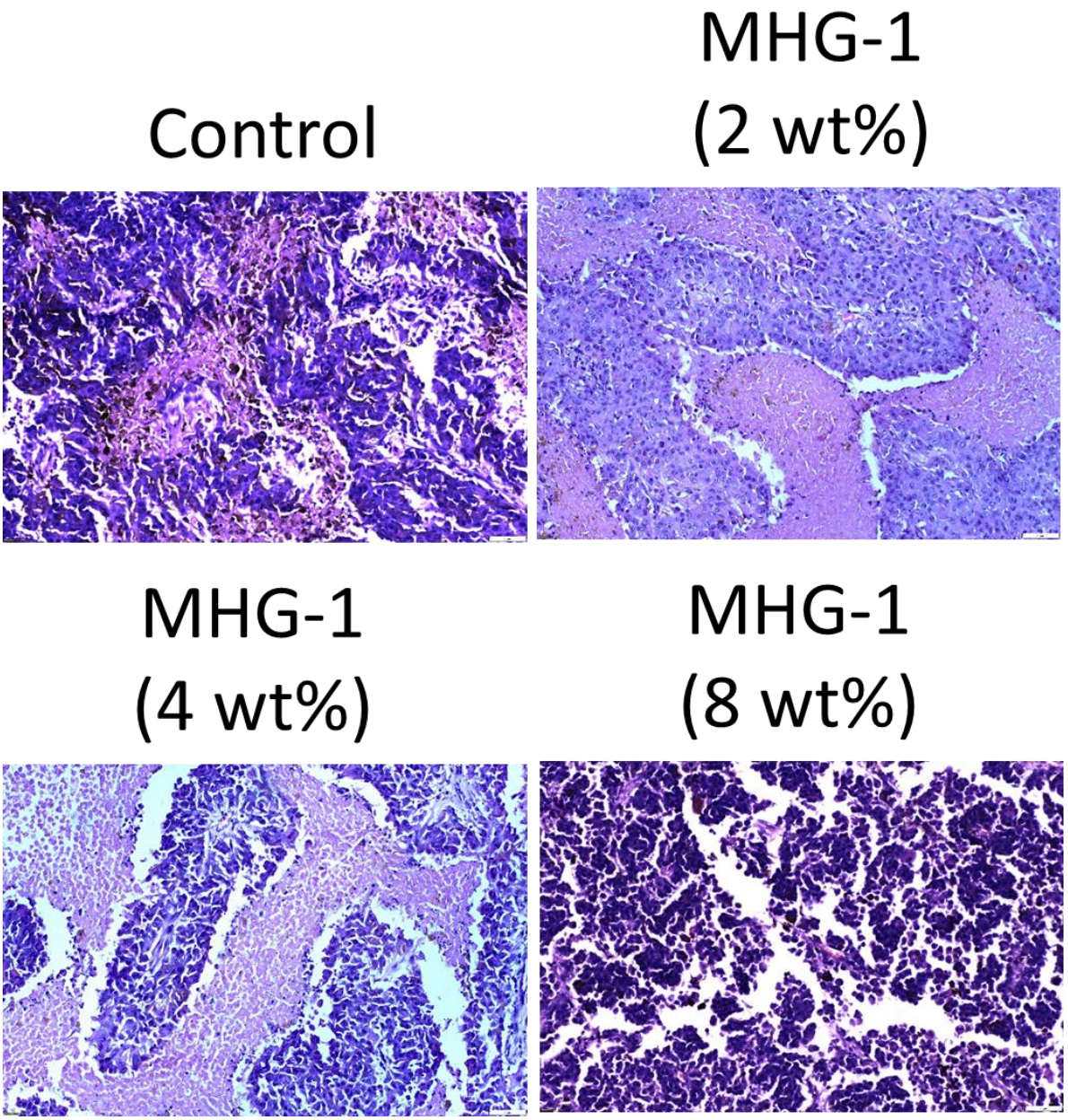

Fig. S43: Hematoxylin–Eosin (H&E) stained tumour tissue sections after different concentrations of gel treatment for 21 days (scale bar= 100 µm). Various treatment conditions: MHG-1 (2, 4 and 8 wt%).

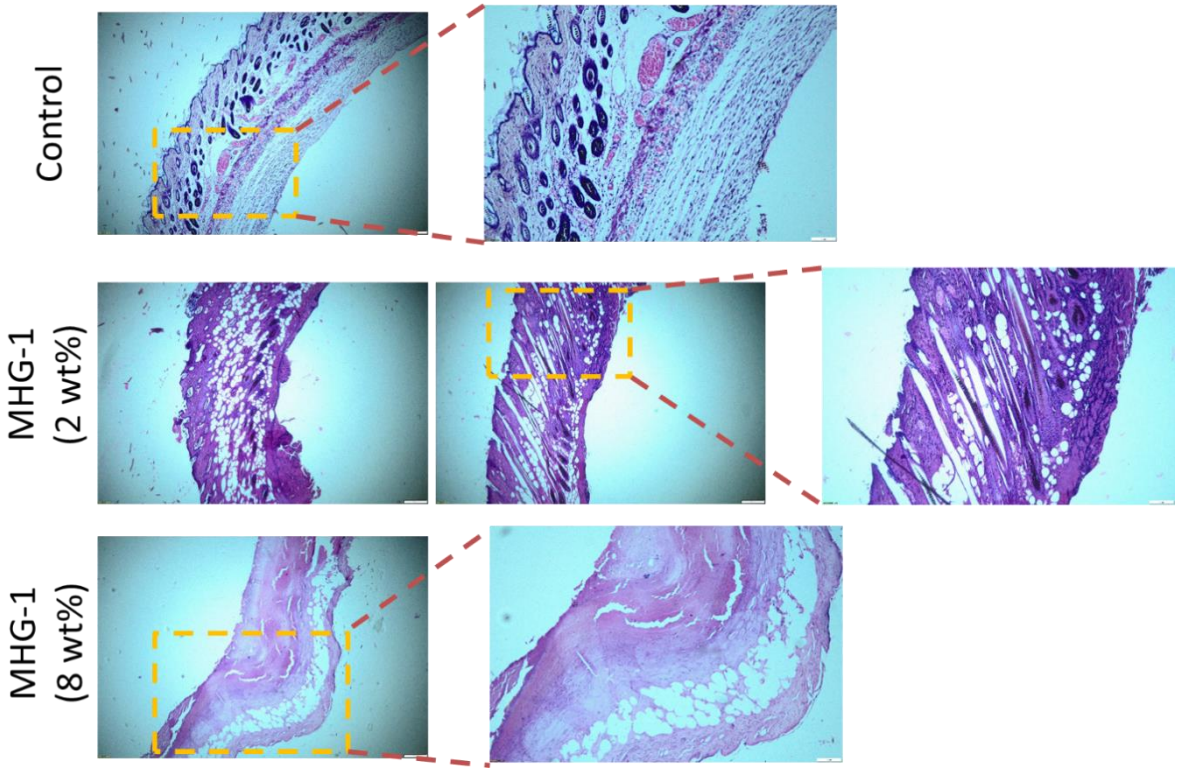

Fig. S44: Hematoxylin–Eosin (H&E) stained skin tissue sections after different concentrations of gel treatment for 21 days. Various treatment conditions: MHG-1 (2 and 8 wt%) (Scale bar= 200 µm).

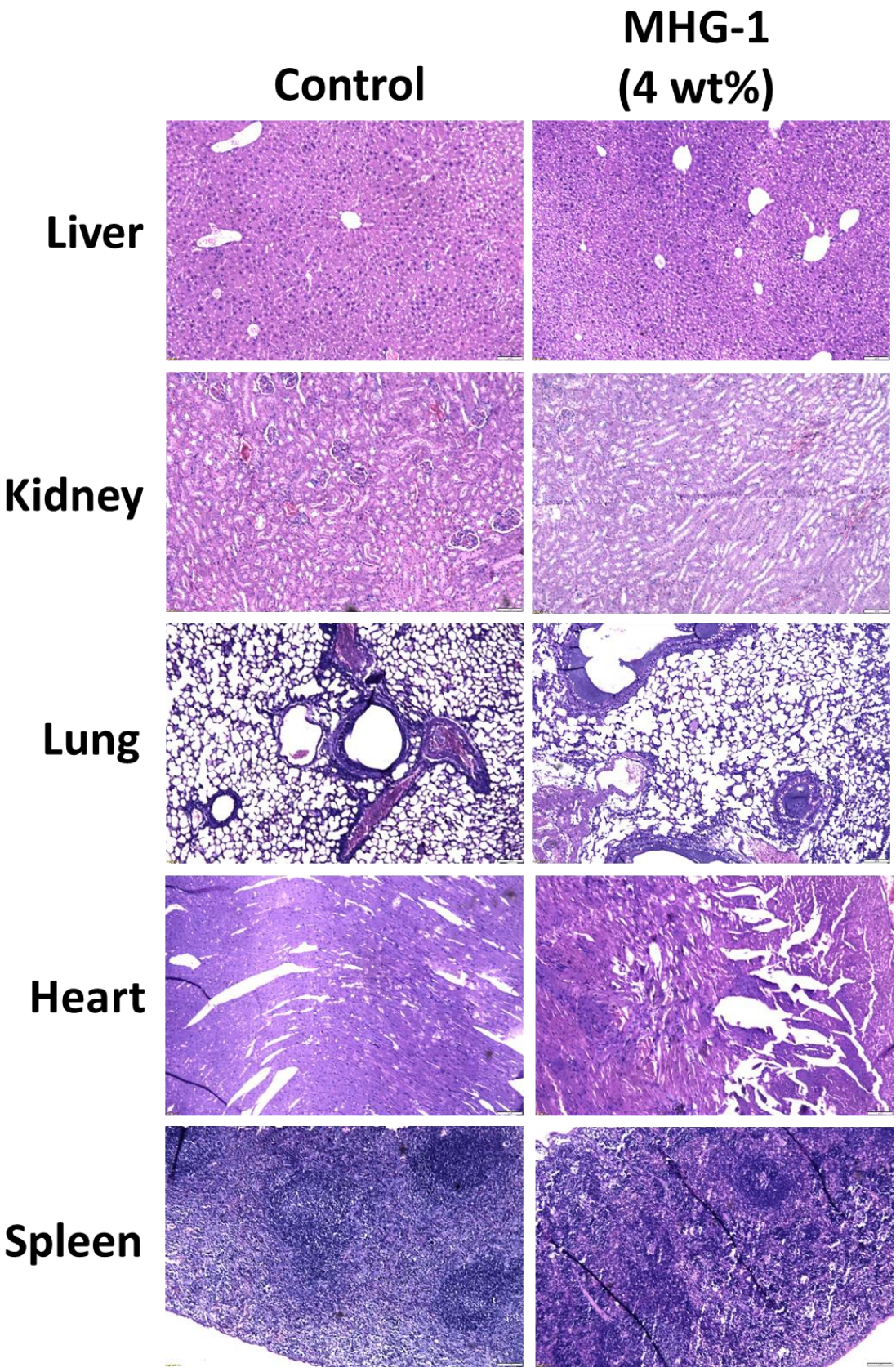

Fig. S45: Hematoxylin–Eosin (H&E) stained different organ sections after gel treatment for 21 days. Treatment condition: MHG-1 (4 wt%) (Scale bar= 100 µm).

47. In vitro PGE_2 assay:

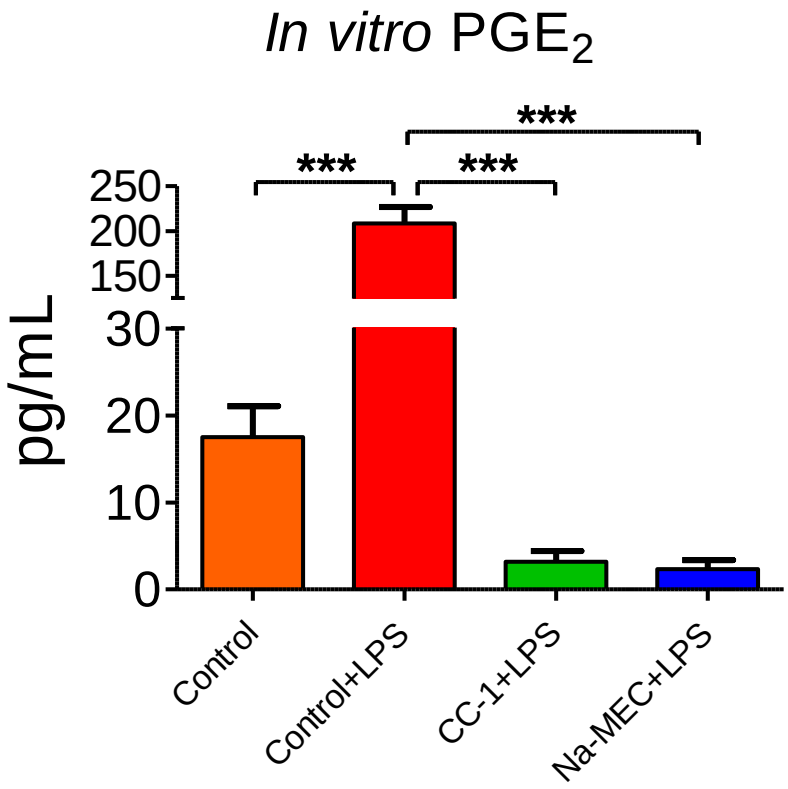

Fig. S46: *In vitro* PGE_2 assay on Raw 264.7 cells showing decrease in PGE_2 level after CC-1 treatment. LPS was used to increase the PGE_2 level in the cells. Various treatment conditions: CC-1 (0.025 mM), Na-MEC (0.049 mM), LPS (5 µg/mL). In graph, data were represented as mean+SD (n=3); where ***p<0.001.

48. In vivo PGE_2 assay:

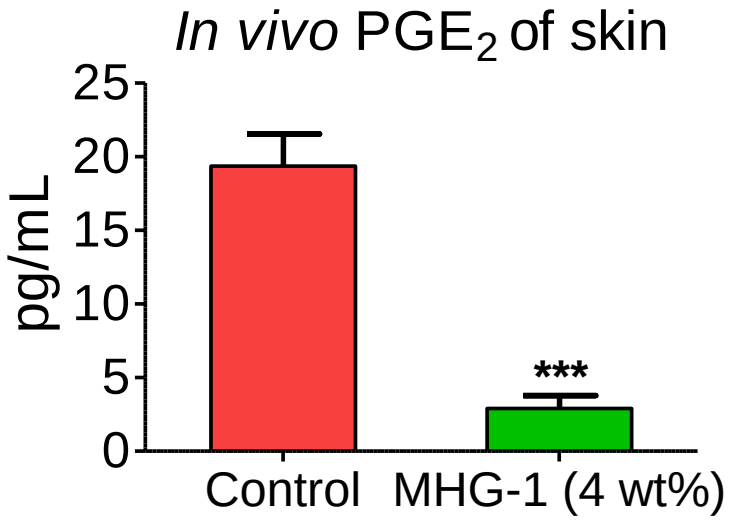

Fig. S47: *In vivo* PGE_2 assay on skin tissue showing decrease in PGE_2 level after MHG-1 (4 wt%) treatment. LPS was used to increase the PGE_2 level in the cells. Treatment condition: MHG-1 (4 wt%). In graph, data were represented as mean+SD (n=3); where ***p<0.001.

49. In vivo immunomodulation study:

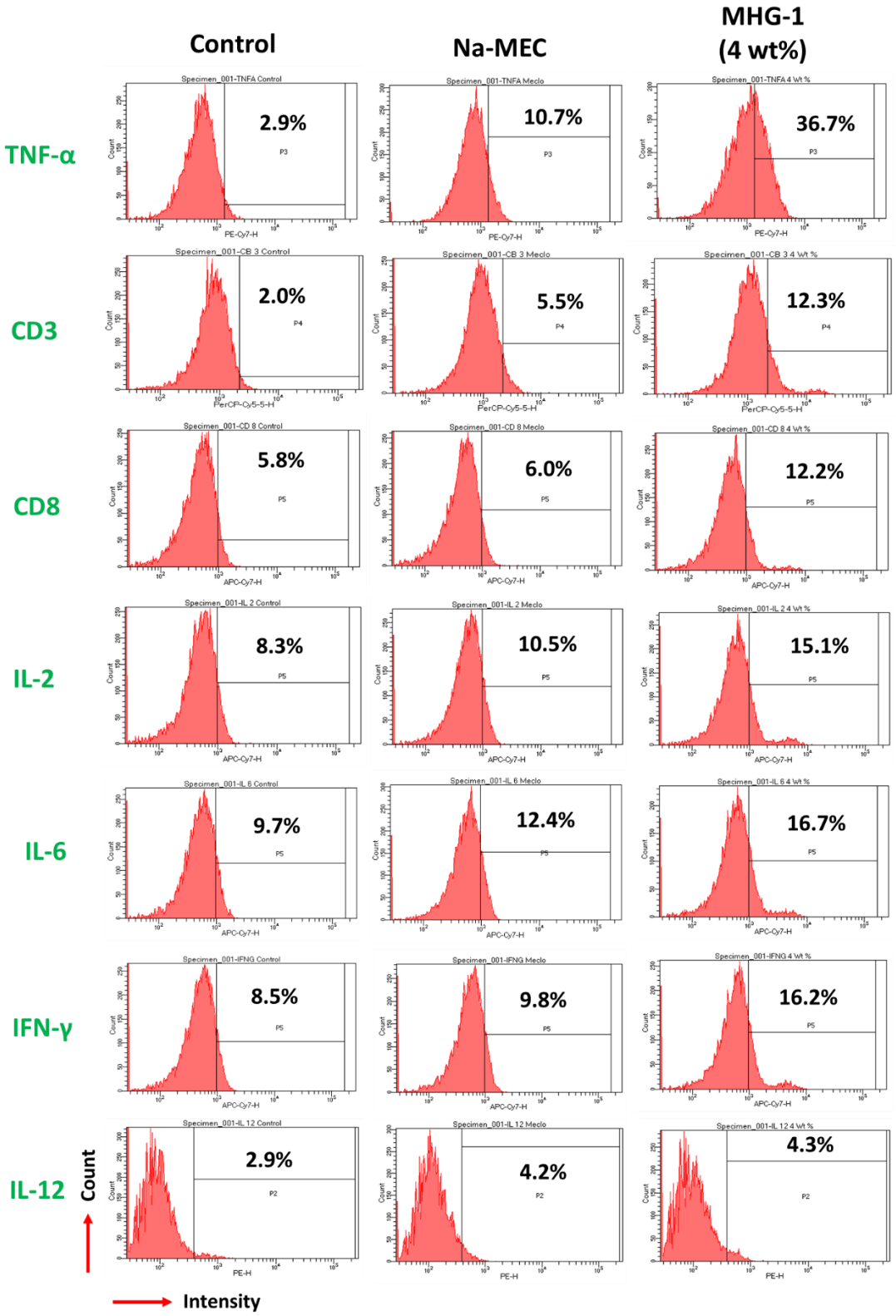

Fig. S48: *In vivo* flow cytometry for determination of different cytokine expressions and T cell populations after 9 days of treatment. Different treatment conditions: MHG-1 (4 wt%), Na-MEC (4.8 mg/200 µL).

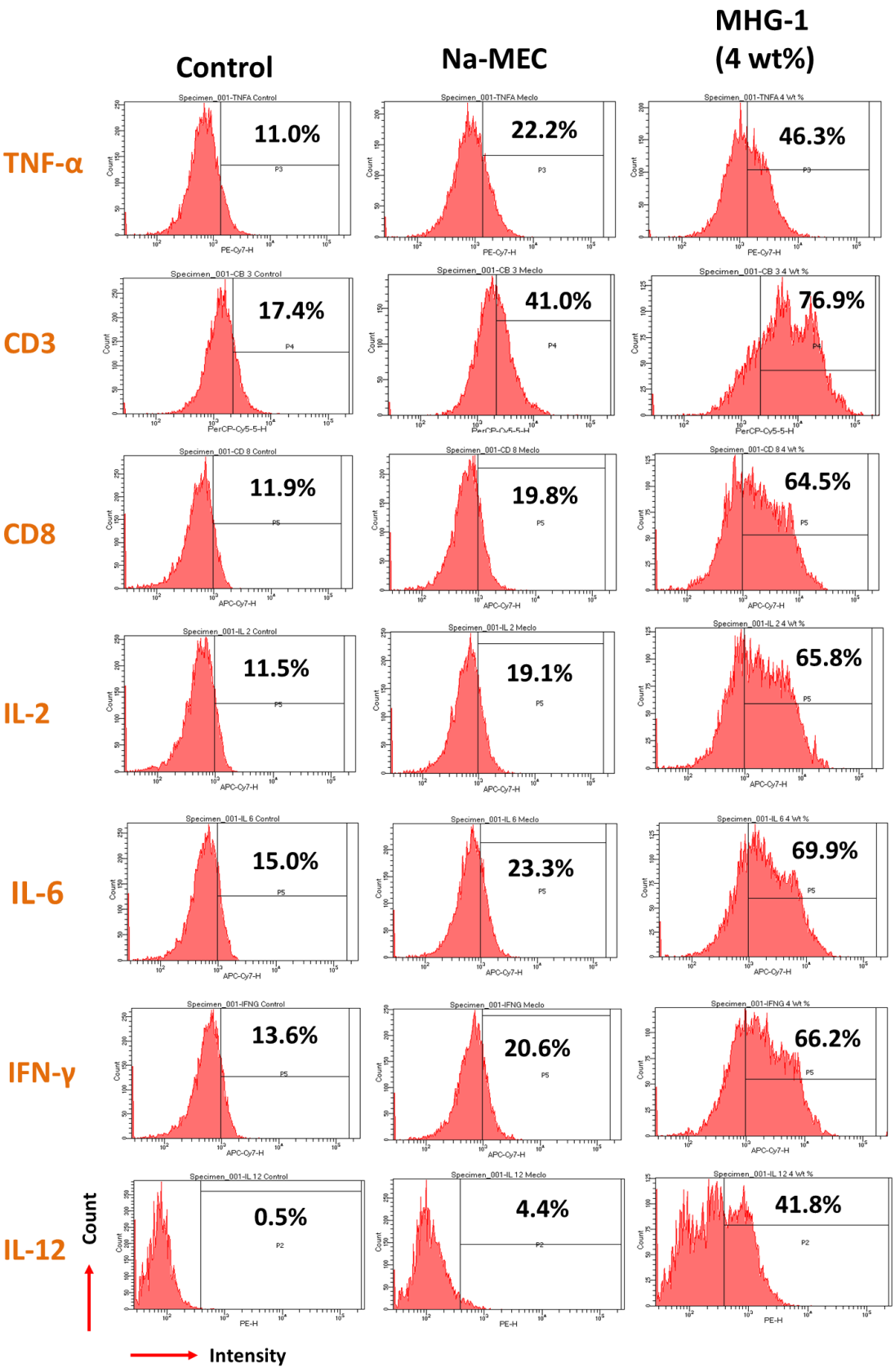

Fig. S49: *In vivo* flow cytometry for determination of different cytokine expressions and T cell populations after 18 days of treatment. Different treatment conditions: MHG-1 (4 wt%), Na-MEC (4.8 mg/200 µL).

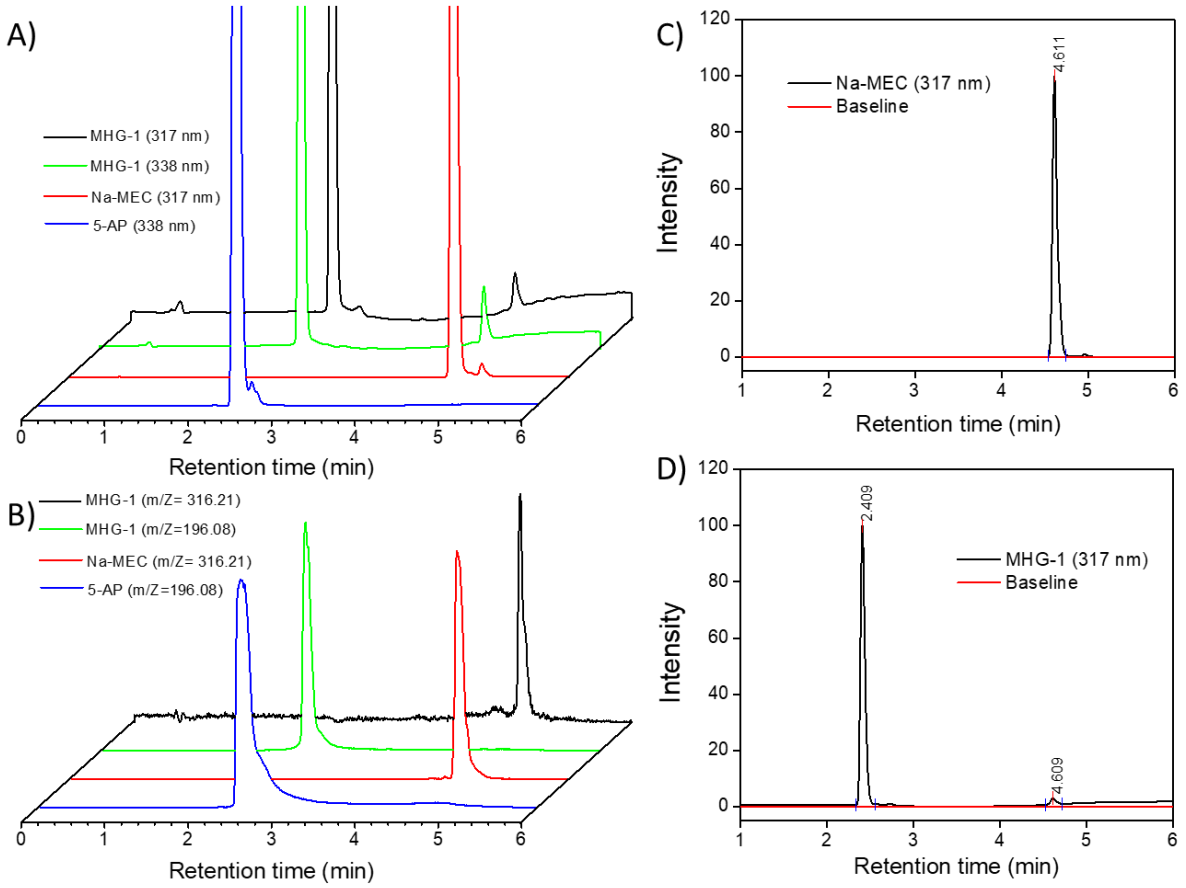

Fig. S50: LC-MS study of the supernatant of MHG-1: A) chromatograph detected by UV-vis detector and B) extracted ion-chromatograph of MHG-1 supernatant and its components; C) and D) UV-vis chromatographs for calculating area under the curve.

Drug release:

1 mL of MHG-1 was prepared at MGC and 1 mL of PBS(1X) was placed on the top of the gel. The setup was left undisturbed for 48 h at 37 ^{o}C. After 48 h supernatant was collected and LC-MS was performed to determine MEC release.

LC-MS experiment:

For Na-MEC, 1 µL of 2 mg/mL solution was injected in LC-MS.

For MHG-1, supernatant solution was diluted to 1/25 times (40 µL of supernatant solution added in 960 µL PBS(1X)) and then 1 µL was injected in LC-MS.

Peak area of Na-MEC in Fig. S50C = 6.69

Peak area of MHG-1 in Fig. S50D = 0.32

Thus, 6.69 peak area is due to 2 µg of Na-MEC.

And, 0.32 peak area is due to 0.1 µg of MEC.

Thus, 1 µL of diluted supernatant solution contains 0.1 µg of MEC.

Therefore, 1 mL of diluted supernatant solution contains 100 µg of MEC.

So, 1 mL of supernatant solution contains 100x25 = 2500 µg of MEC

= 2.5 mg of MEC

Hydrogel MHG-1 (MGC) contains 6.4 mg/mL of MEC.

Thus, % release of MEC after 48 h = (2.5/6.4)x100

= 39.06 %

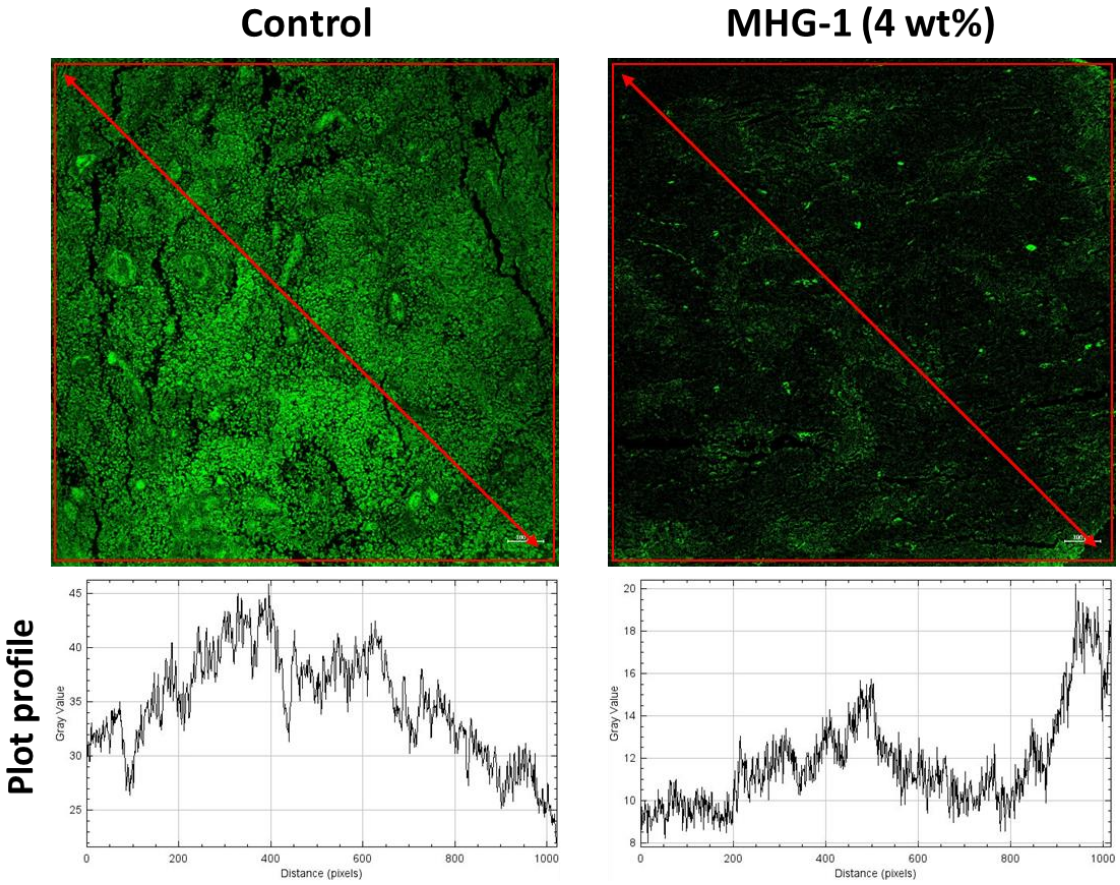

Fig. S51: LSCM images and plot profile of anti-Ki-67 monoclonal antibody stained untreated (control) and MHG-1 (4 wt%) treated tumor tissue (Scale bar= 100 µm).

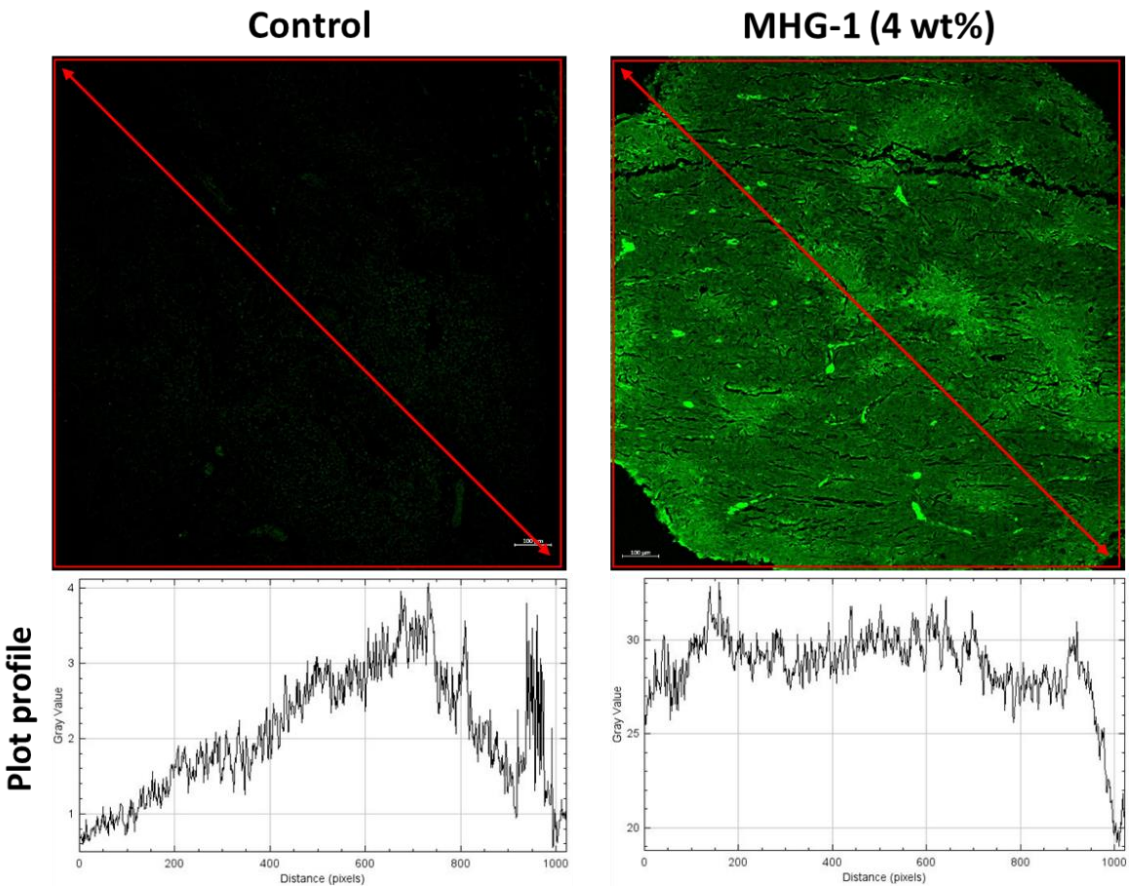

Fig. S52: LSCM images and plot profile of annexin V-FITC stained untreated (control) and MHG-1 (4 wt%) treated tumor tissue (Scale bar= 100 µm).

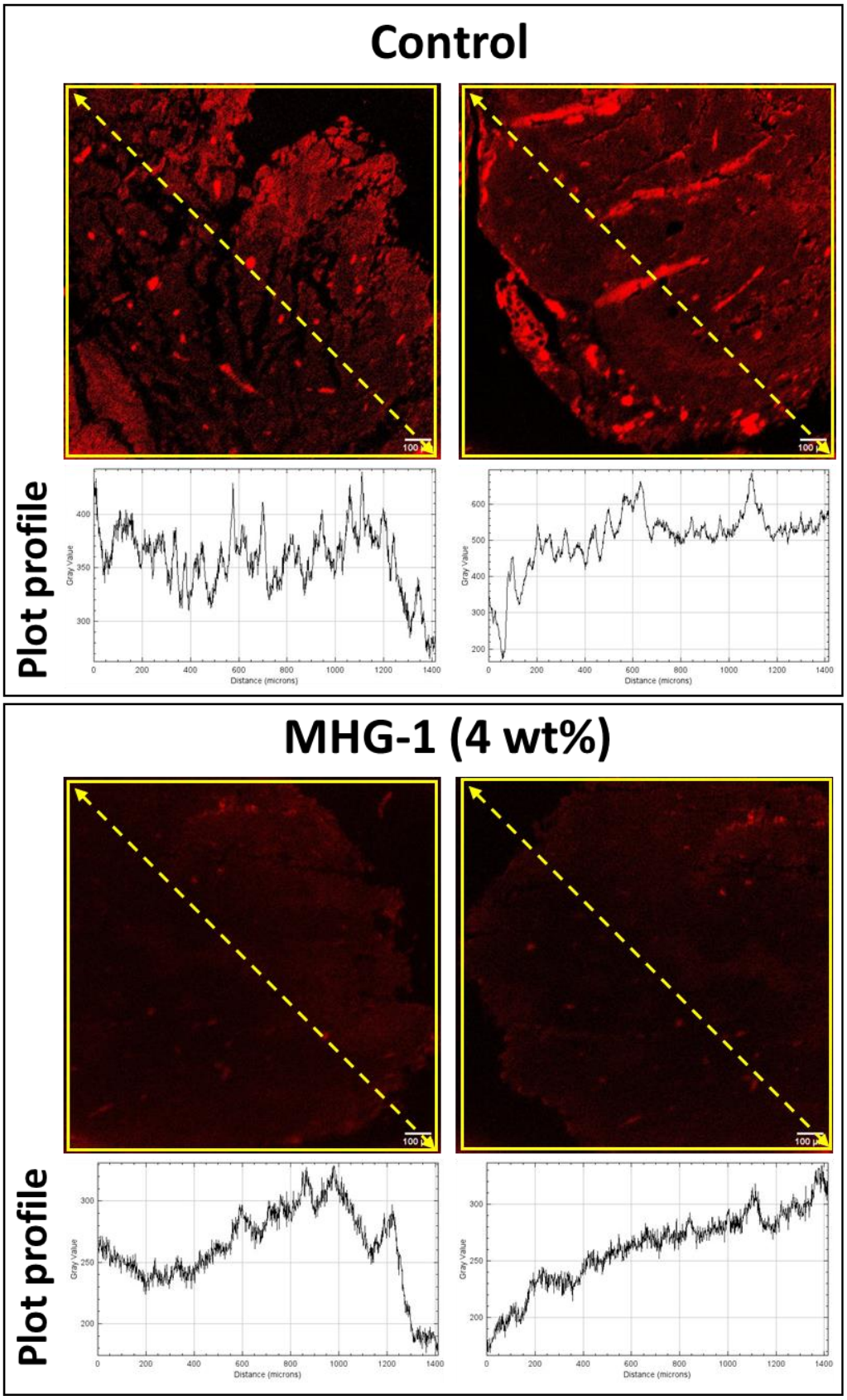

Fig. S53: LSCM images and plot profile of anti-CD31 antibody stained untreated (control) and MHG-1 (4 wt%) treated tumor tissue (Scale bar= 100 µm). Two images in each case are provided.

50. Flow chart of the overall work:

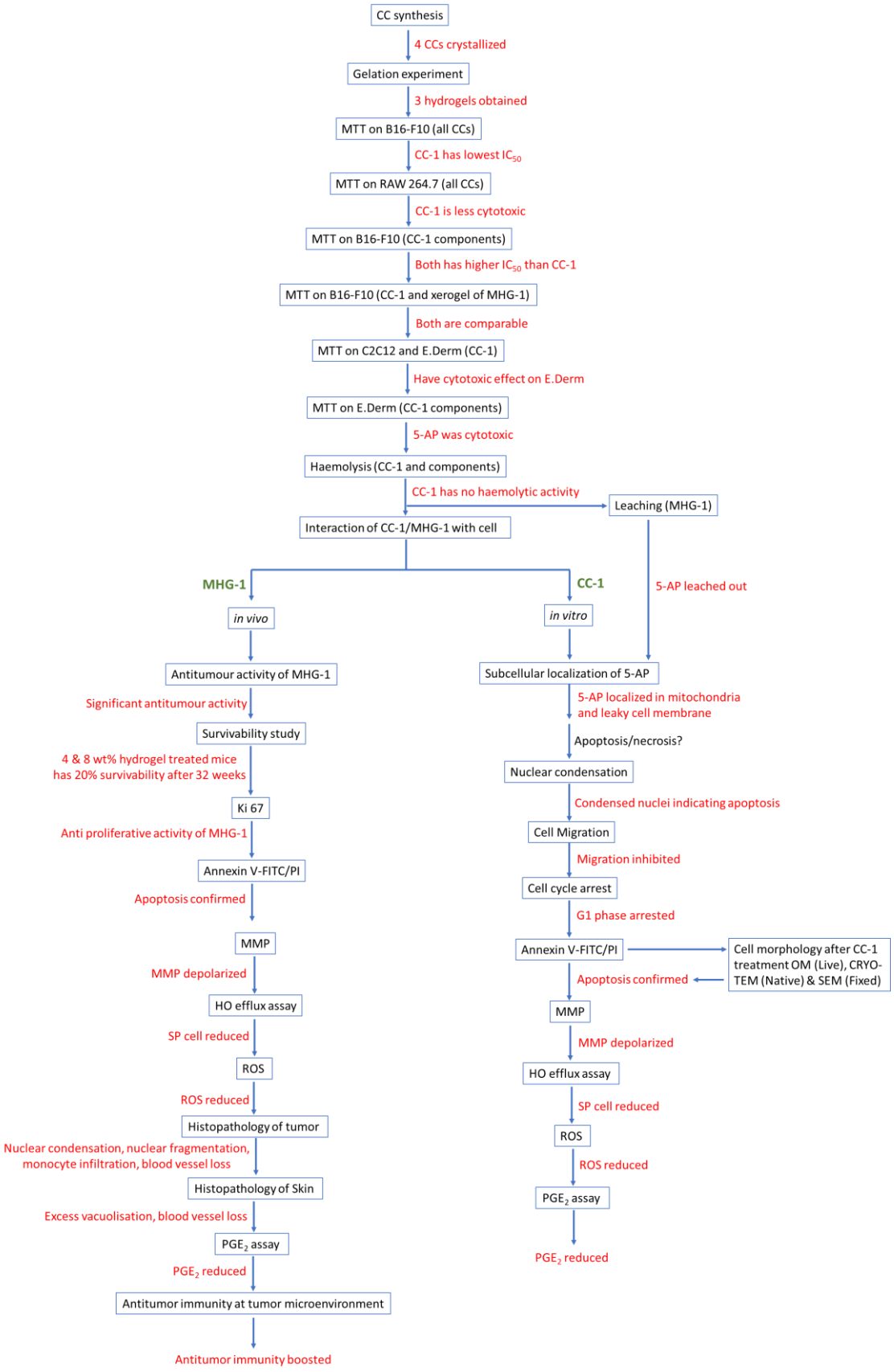

Fig. S54: Flow chart showing the overall work and major findings obtained here.