Supporting information

1. Synthesis of Materials

Nanoparticles of MIL-88A, MIL-100 and MIL-88B_4CH_3 were synthesized as previously described.[1] XRPD patterns were collected in a SIEMENS D5000 (θ-2θ) using Cu Kα1 radiation (lambda = 1.54056 angstroms) from 5 to 16 º (2θ) using a step size of 0.04 º and 3 s per step in continuous mode

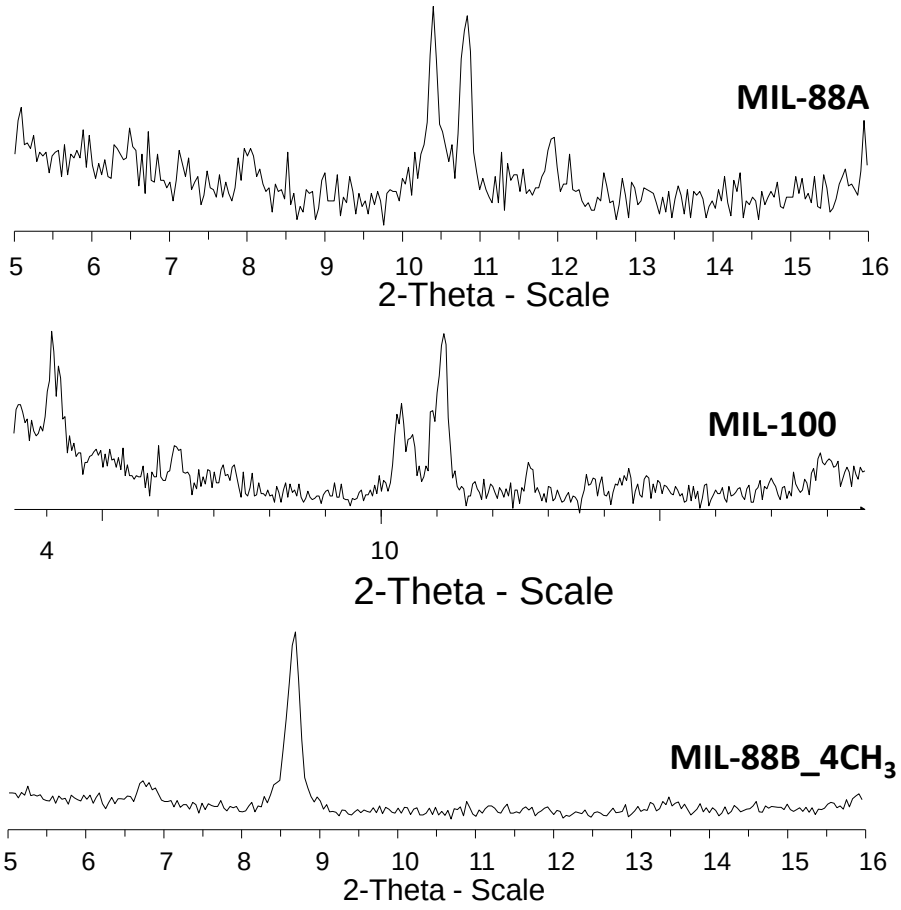

Figure S1. X ray powder diffraction patterns of MIL-88A (on the top), MIL-100 (center) and MIL-88B_4CH_3 nanoparticles (on the bottom).

2. Animals.

All experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the Faculty of Pharmacie of Paris-Sud 11 University. Wistar female rats (4-weeks-old, 100 ± 20 g) were obtained from the central animal care facilities, Janvier R Centre d'Elevage, France.

Rats were randomly divided into 7 groups of 6 animals and were housed in individual metabolic cages. They were maintained in an air-conditioned room (22-25 °C) on a 12 h light/dark cycle with water and food available.

In order to study the toxicity of the different nanoparticles, 220 $mg.kg^{-1}$ of nanoMIL-88A or nanoMIL100 or 110 $mg.kg^{-1}$ of nanoMIL88-4CH_3 (doses corresponding to the dry state of the MOF) were suspended in a 0.5 mL aqueous solution of glucose (10%) and intravenously perfused in the jugular vein under isofluorane anesthesia. Nanoparticles suspensions were dispersed by ultrasounds and then, slightly settled in order to remove the bigger particles. Likewise, a control group (Control) was perfused in the jugular vein with 0.5 mL of a glucose solution 10%. 1, 7 and 30 days after administration, all animal groups were sacrificed under isofluorane anesthesia. Spleen, heart, kidney, lungs and liver were extracted, washed with a NaCl 0.9% solution at 4°C, then immerged in liquid nitrogen and stored at -80°C until analysis. Urines samples, collected during the whole experiment, were centrifuged (8000 g/15 min) and stored with 0.1 mL of H_2SO_4 0.01M per mL of urine at -20°C until analysis.

Toxicity was assessed by animal growth, anatomic aspect and histology of organs, antioxidant status, cytochrome activity, inflammatory reaction, iron and linker biodistribution, as well as different hepatic and serum parameters.

3. Antioxidant enzymes assay

- Reduced and oxidized glutathione (GSH, GSSG) were spectrophotometrically quantified in deproteinized supernatant fractions from the liver and the blood by the Akerboom and Sies method,[2] using 5,5-dithiobis(2-nitrobenzoic acid). Absorbance values were compared with standard curves generated from known amounts of GSH standards.

- CuZn superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund[3] using pyrogallol as substrate. This method is based on pyrogallol oxidation by the superoxide anion ($O_2^{\bullet -}$) and its dismutation by the enzyme. One unit (U) of CuZn SOD is defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%.

- Catalase activity (CAT) was determined by measuring hydrogen peroxide decomposition at 240 nm as described previously by Beers and Sizer.[4]

4. Proteins assay

- The protein content in the supernatant of homogenate tissue was estimated by the Biuret method using bovine serum albumin as standard.[5]

- Microsomal protein concentration weas determined by a BCA Protein Assay Kit (PIERCE, France) using bovine serum albumin as standard. Analysis was carried out on a Shimadzu 160A UV-visible spectrophotometer and absorbance was measured at 560 nm.

5. Plasma iron assay

- Plasma iron concentration was measured using the reported assay based on ferrozine.[6]

6. Biochemical parameters

- Plasma aspartate aminotransferase (AST), alanine aminotransaminase (ALT) and creatine phosphokinase (CPK) activities were spectrophotometrically determined using commercial diagnostics kits (Biomaghreb, Tunisia; Randox, United Kingdom).

7. Ligands determination

Trimesic acid, fumaric acid and tetramethylterephthalic acid were determined by HPLC in deferent biological matrices as previously described.[7, 8, 9]

8. CYP2E1 and CYP3A4 activities

2E1 (CYP2E1) and 3A4 (CYP3A4) isoforms of the cytochrome P450 activities were determined in the hepatic microsomal fractions. The hepatic microsomal fractions were prepared by differential centrifugation, as described previously,[10] and stored at -80°C until analysis. Microsomal protein concentration was determined by the Biuret method using bovine serum albumin as standard.[5] The CYP2E1 and the CYP3A4 activities were determined by HPLC as previously reported.[10, 11]

9. Statistics

The normality of data distribution was tested by Shapiro-Wilk test. Data are shown as the mean and the standard deviation. Comparisons with control were performed by using Student test, according to the homogeneity of variances determined by Fisher or by Mann-Whitney test. A value of $P < 0.05$ was considered statistically significant.

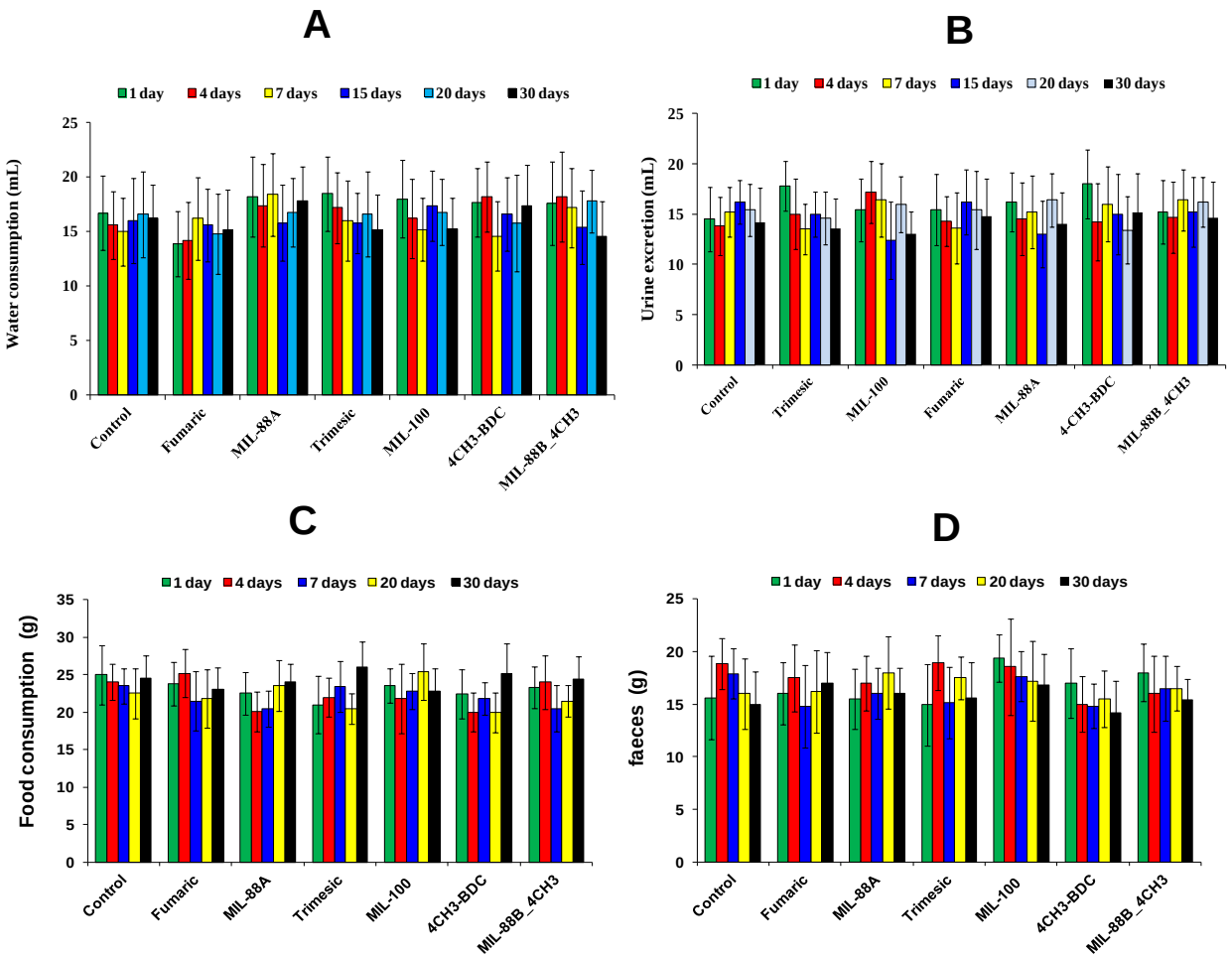

Figure S2. (A) Water consumption, (B) urine excretion, (C) food consumption and (D) dejection excrection.

No significant differences were monitored in food consumption or water intake, as well as in urine volume or dejection weight between treated and control groups (Fig. S2).

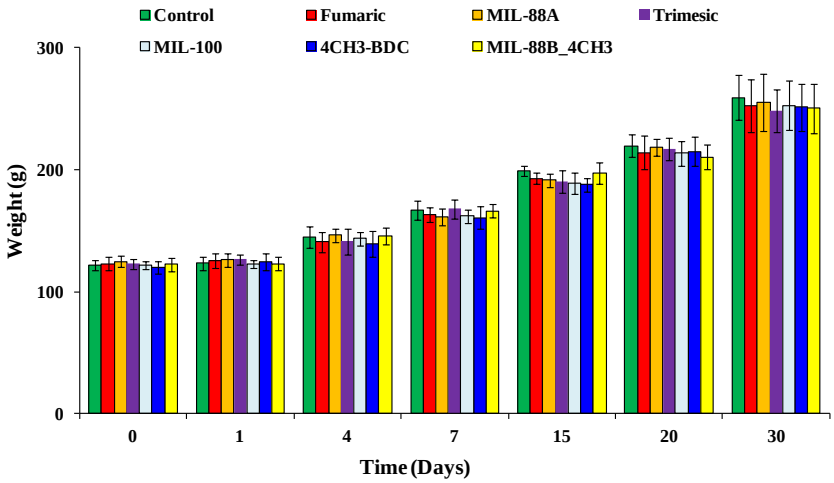

Figure S3. Evolution of the mean body weight of rats measured during 30 days after administration of nanoMOFs or their linkers.

Results showed a normal corporal weight increases with no statistically significant differences between nanoMOFs or linkers treated groups and control group (Fig. S3).

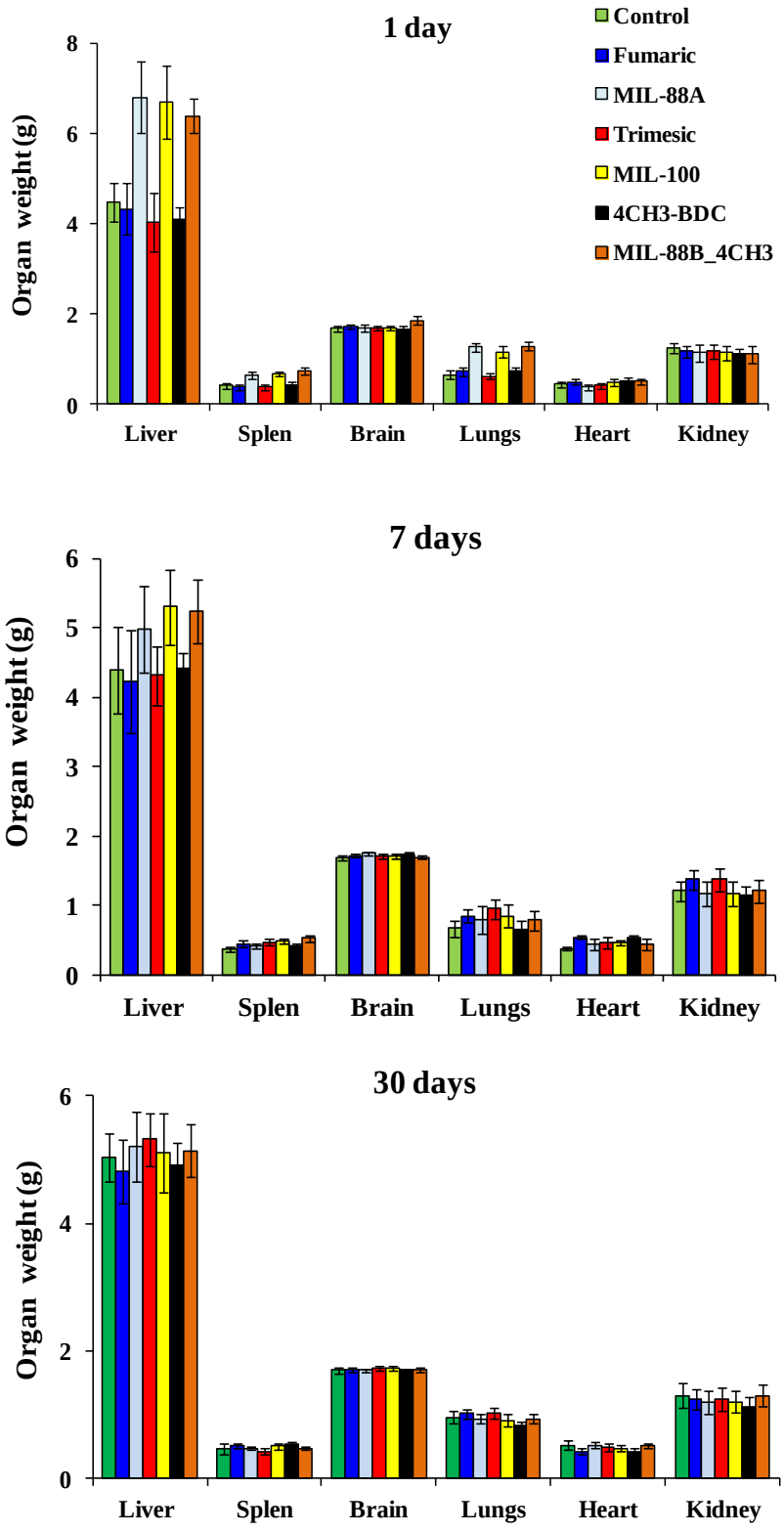

Figure S4. Organs weight of different rat groups sacrificed at 1, 7 and 30 days after treatment.

Organ weight comparison between treated and control groups did not show any significant difference, except a slight increase in the spleen, liver and lung weights, attributed to the sequestration of nanoMOFs by the reticulo-endothelial system and nanoparticle aggregation in small pulmonary capillaries. Then, weight of these organs decreased progressively up to normal values after 7 days (Fig. S4).

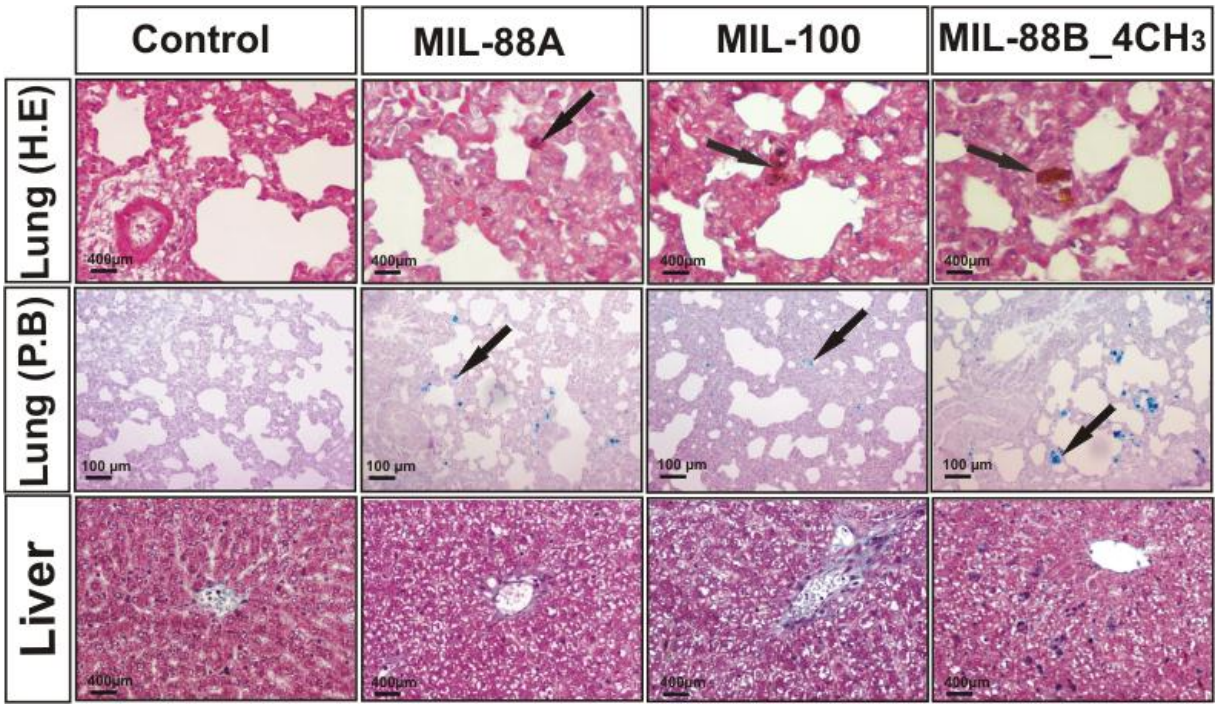

Figure S5. Histological sections of lungs (H.E: Hematoxylin-eosin staingin, P.B: Prussian blue staining,) and liver (trichrome staining) after 1 day of the i.v. administration of MIL-88A, MIL-100 and MIL-88B_4CH3 nanoparticles in comparison with the control group. Black arrows indicate the particle aggregates.

Lungs sections were stained with hematoxylin-eosin staining and with Prussian blue staining, which colors specifically the iron in blue. NanoMOFs aggregates were revealed in some of the pulmonary capillaries (hematoxylin-eosin). Livers sections were stained with trichromic staining and did not show any fibrosis in the hepatic parenchyma due to the accumulation of nanoMOFs in kupffer cells.

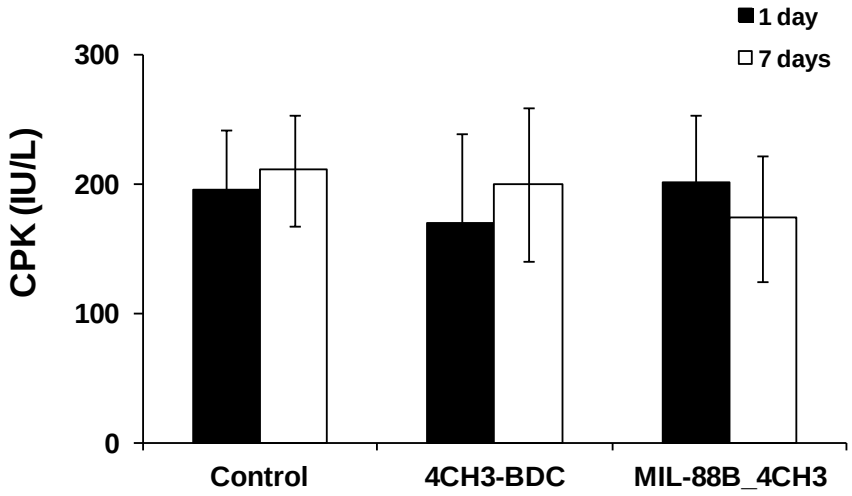

Figure S6. Total creatine phosphokinase (CPK) activity determined after 1 and 7 days following the i.v administration of nanoMIL-88B_4CH_3 and their linker 4CH_3-H_2BDC. (*n=6*, data are the mean ± SD, p≤0.5).

The CPK is commonly used as biochemical marker for the heart, the muscles and the brain function. It was impossible to specifically determine the CPK-BB (specific for the brain function) due to the lack of compatibility of rat antigen to human antibody. Therefore, the assay of CPK implied the assay of the three forms; CPK-BB (for brain function), CPK-MM (for muscles dysfunction) and CPK-MB (for heart function).

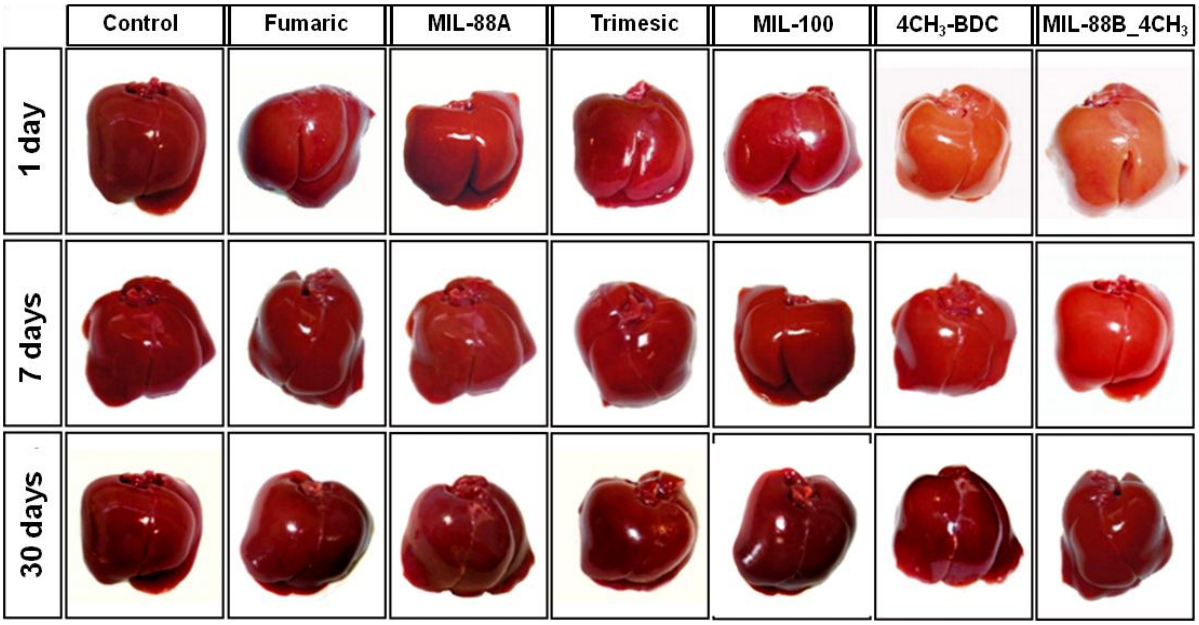

Figure S7. Macroscopic observation of livers after 1, 7 and 30 days of the *i.v.* administration of MIL-88A, MIL-100 and MIL-88B_4CH_3 nanoparticles and their corresponding linkers in comparison with the control group.

The livers of all animals exhibit an ordinary color and morphology without adherent lobes except those of rats injected with MIL88-4CH_3 and 4CH_3- H_2BDC. They exhibited a normal morphology but with a slight clarification which was progressively reversible at 7 days to be normal at 30 days.

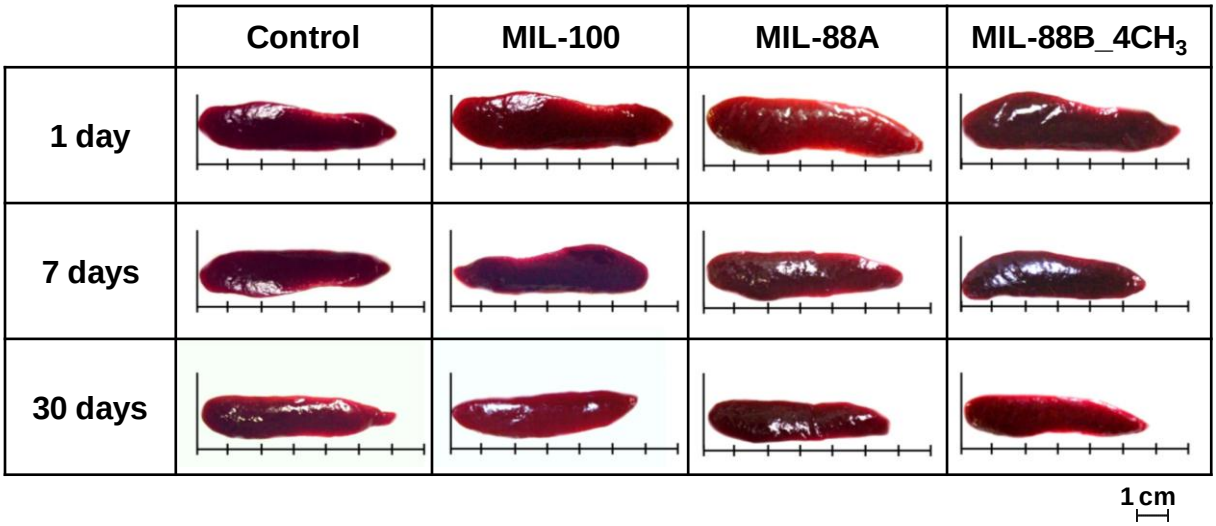

Figure S8. Macroscopic observation of spleens after 1, 7 and 30 days of the *i.v.* administration of MIL-88A, MIL-100 and MIL-88B_4CH_3 nanoparticles and their corresponding linkers in comparison with the control group.

A slight hypertrophy was observed in spleens 1 day after the administration of nanoparticles. This hypertrophy decreased progressively after 7 days to completely disappear 30 days after the injection.

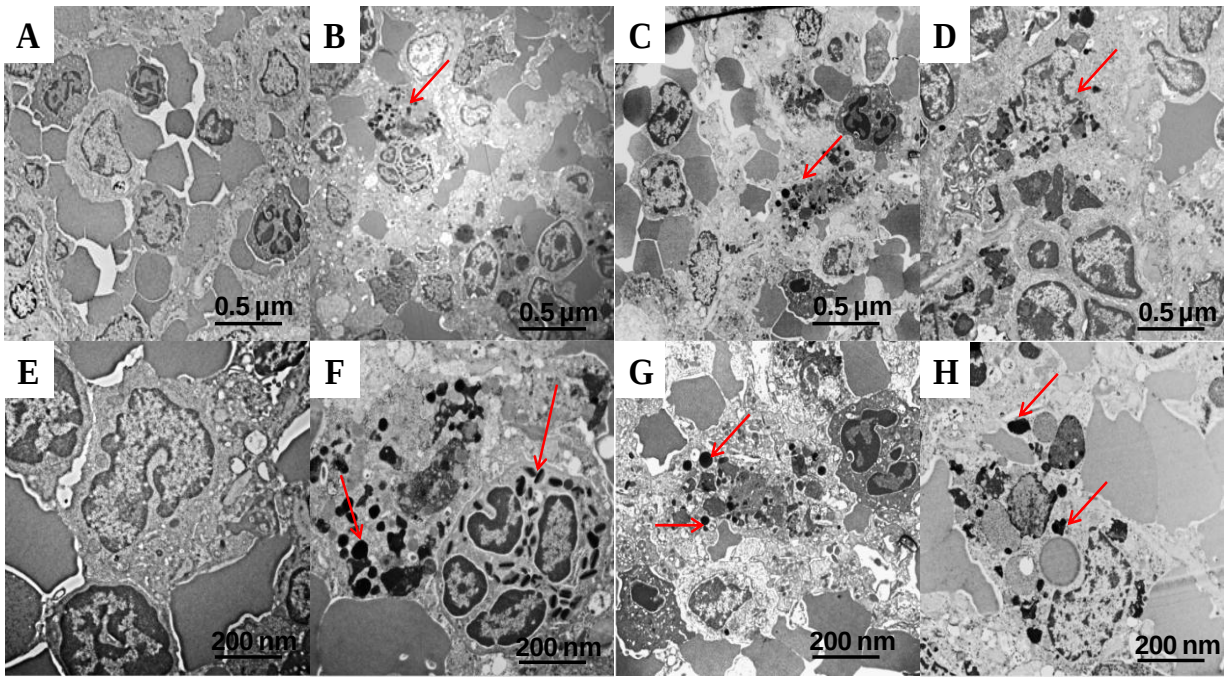

Figure S9. TEM images of spleen after 1 day of the *i.v.* injection of (A) Control, (B) MIL-88A, (C) MIL-100 and (D) MIL-88B_4CH_3 nanoparticles. Red arrows remark the presence of nanoparticles inside macrophages. (E,F,G,H) are the magnification of A, B, C, D respectively.

MOFs nanoparticles were located exclusively into the splenic macrophages without inducing hyperactivity compared to the control macrophages which exhibit the same size.

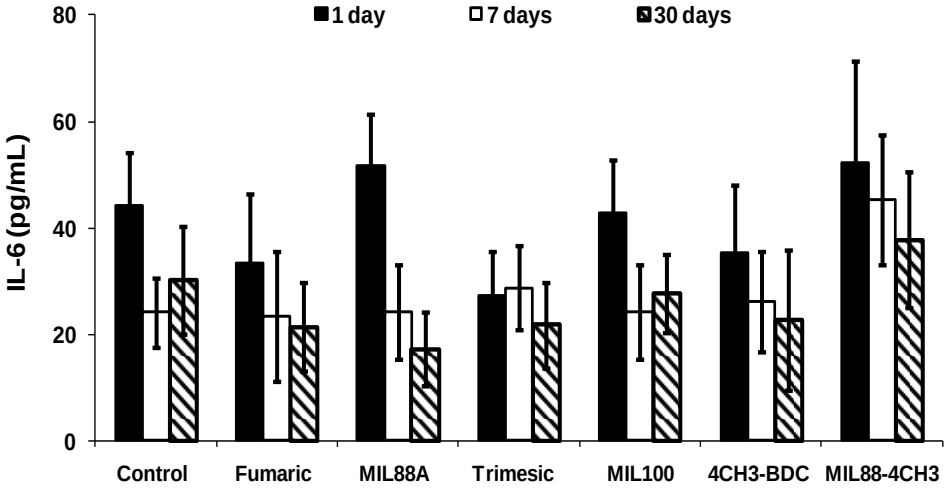

Figure S10. Serum IL-6 levels determined after 1, 7 and 30 days following the i.v administration of nanoMOFs and their corresponding linkers. (*n=6*, data are the mean ± SD, p≤0.5).

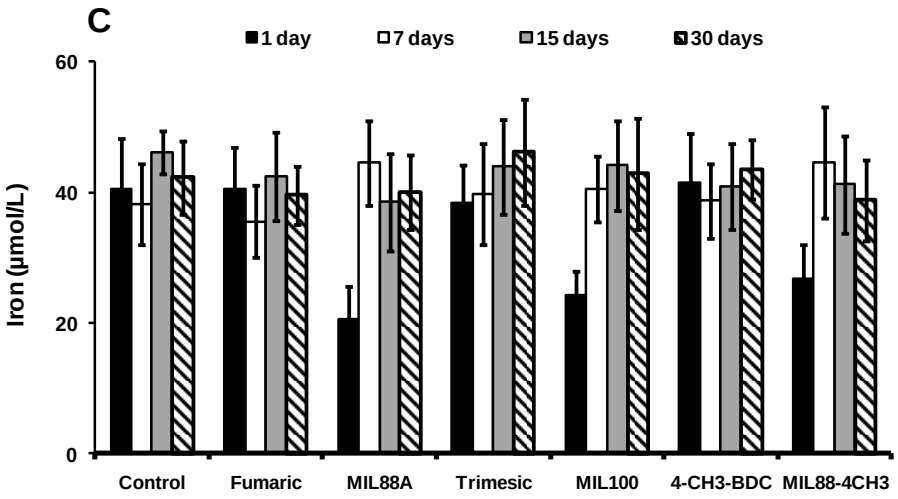

Figure S11. Serum iron levels determined after 1, 7, 15 and 30 days following the i.v administration of nanoMOFs and their corresponding linkers. (*n=6*, data are the mean ± SD, p≤0.5).

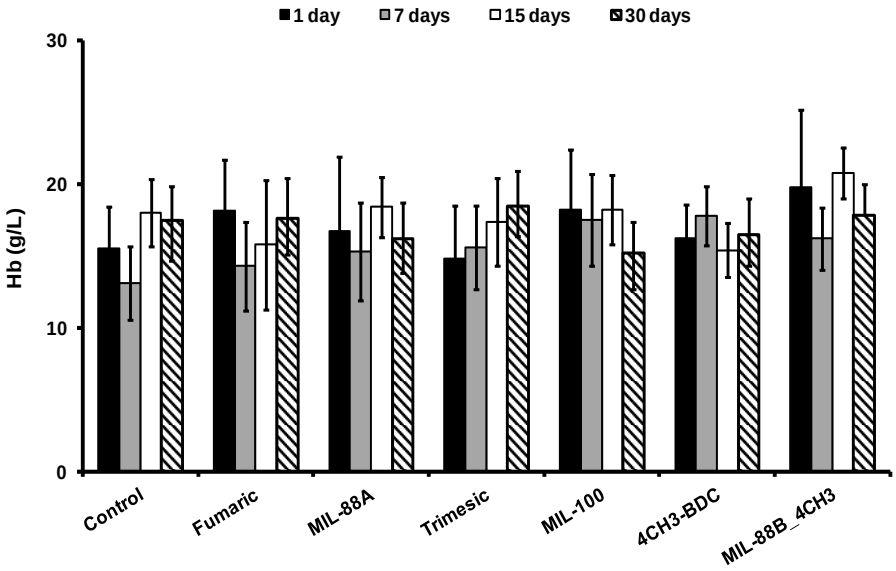

Figure S12. Hemoglobin levels determined after 1, 7, 15 and 30 days following the i.v administration of nanoMOFs and their corresponding linkers. (*n=6*, data are the mean ± SD, $p \leq 0.5$).

The determination of the hemoglobin concentration in all treated animals did not show any significant difference compared to the control group.

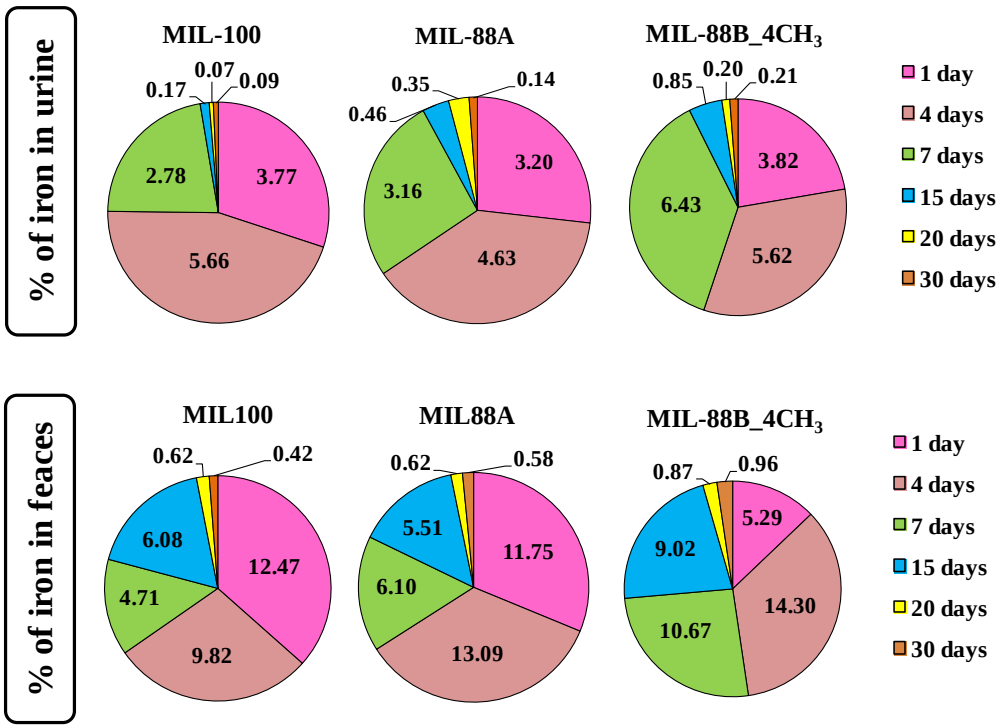

Figure S13. Iron excreted in urine and feaces after 1, 4, 7, 15, 20 and 30 days of the injection of MIL-88A, MIL-100 (220 mg/kg) and MIL-88B_4CH_3 nanoparticles (110 mg/kg). (*n=6*, data are the mean ± SD)

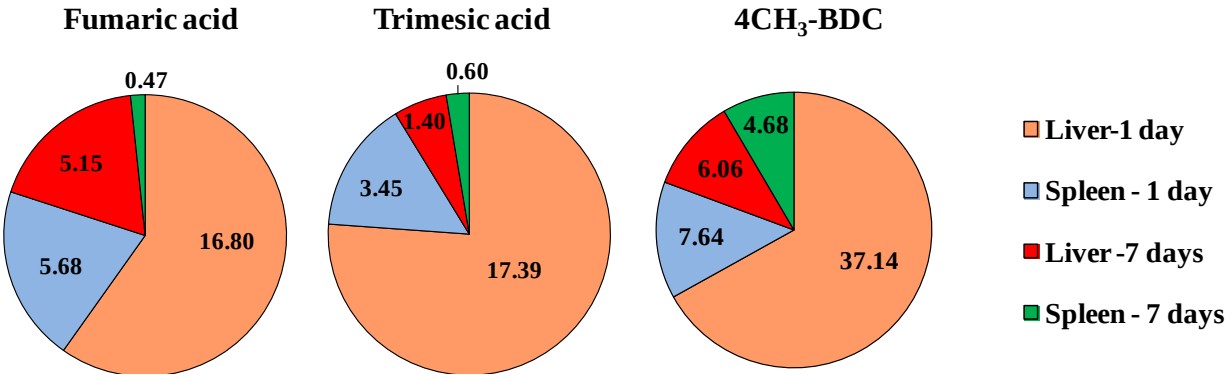

Figure S14. Linker content in liver and spleen after i.v administration of MIL-88A, MIL-100 (220 mg/kg) and MIL-88_4CH_3 (110 mg/kg) (*n=6*, data are the mean ± SD, p≤0.5).

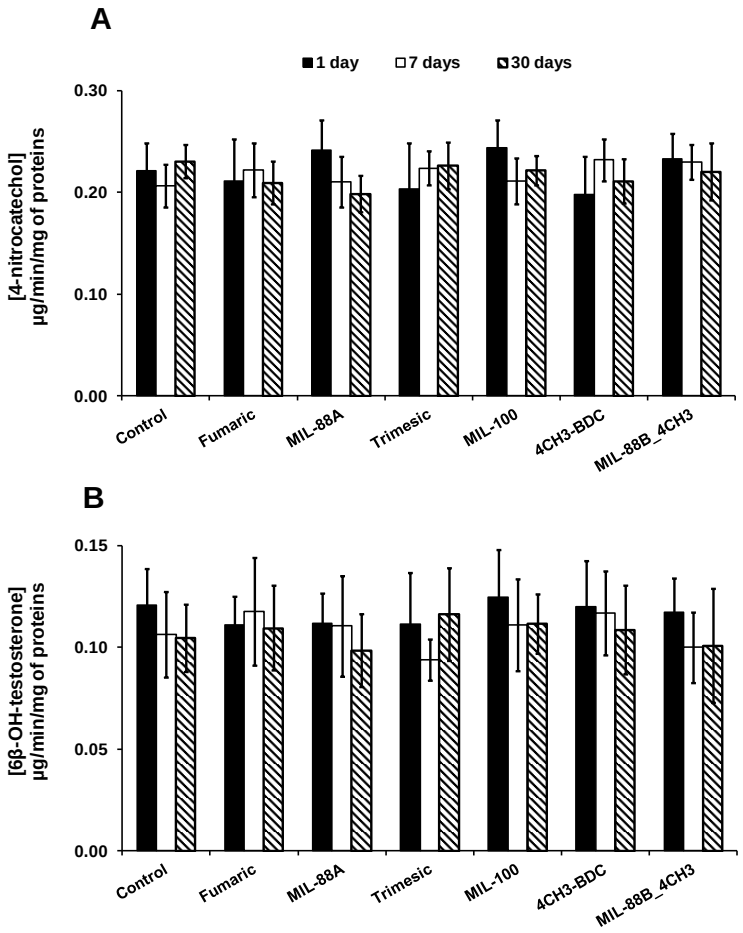

Figure S15. (**A**) CYP2E1 and (**B**) CYP3A4 isoenzymes activities quantified in liver microsomes after 1, 7 and 30 days following the i.v administration of nanoMOFs and their corresponding linkers. (*n=6*, data are the mean ± SD, p≤0.5).

Compared to the control group, CYP2E1 and CYP3A4 of all other groups exhibited normal activities.

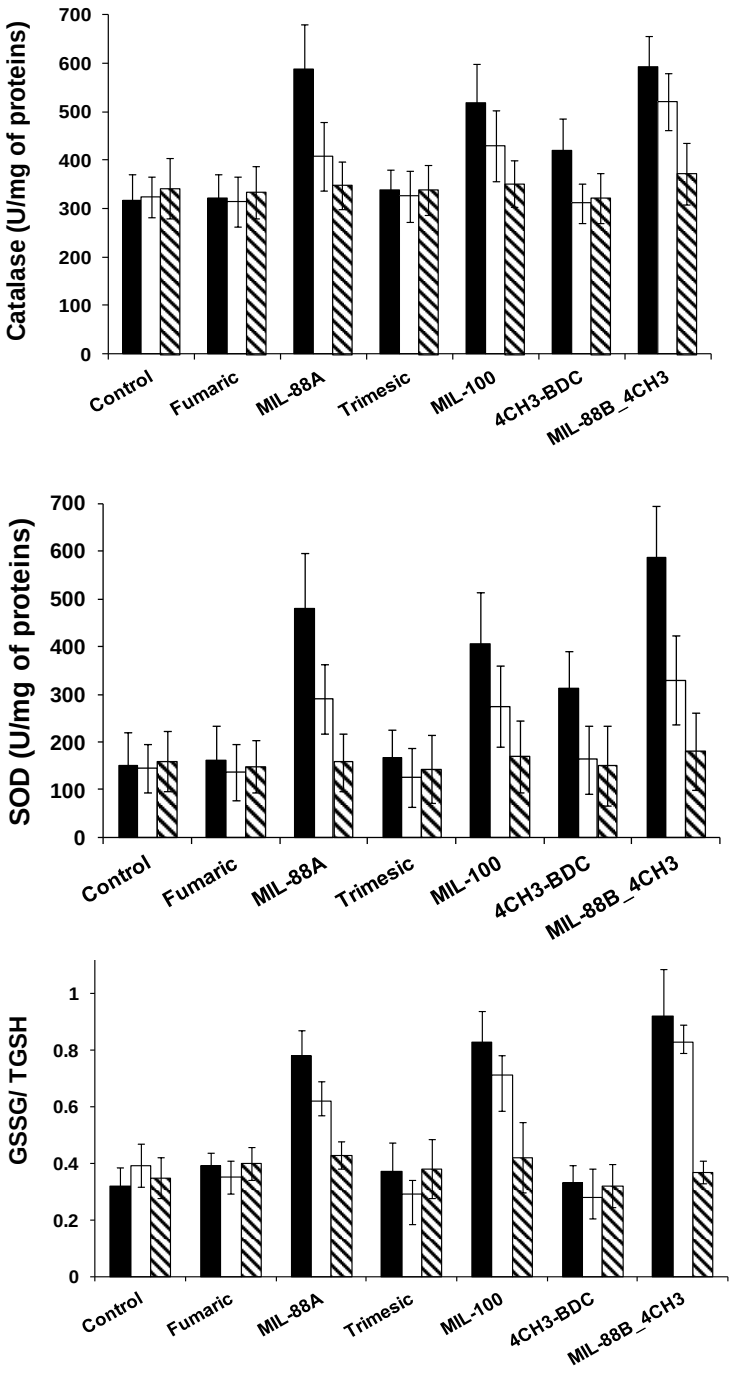

Figure S16. The activities of hepatic catalase (CAT) and superoxide dismutase (SOD), as well as the reduced oxidized glutathione/total glutathione ratio (GSSG/TGSH) determined after the nanoMOFs administration and their corresponding linkers.

While the organic linkers alone did not cause any modification on the antioxidant level, injection of nanoMOFs led to a reversible increase in the GSSG/TGSH ratio and SOD and

CAT activities. SOD, CAT and GSSG/TGSH values increased up to 7 days and then decreased up to normal values at 15 days.

References supporting information

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