

## Supplementary Information

### Internalization Studies of Zeolite Nanoparticles by Human Cell

Natália Vilaça,<sup>a</sup> Ricardo Totovao,<sup>b</sup> Eko Adi Prasetyanto,<sup>bc</sup> Vera Miranda-Gonçalves,<sup>d</sup> Filipa Morais-Santos,<sup>ef</sup> Rui Fernandes,<sup>g</sup> Francisco Figueiredo,<sup>g</sup> Manuel Bañobre-López,<sup>h</sup> António M. Fonseca,<sup>ai</sup> Luisa De Cola,<sup>b</sup> Fátima Baltazar\*<sup>ef</sup> and Isabel C. Neves\*<sup>ai</sup>

<sup>a</sup>*Centre of Chemistry, Chemistry Department, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;*

<sup>b</sup>*Institut de Science et Ingénierie Supramoléculaires (ISIS - UMR 7006), Université de Strasbourg & CNRS. 8 Rue Gaspard Monge, 67000 Strasbourg, France;*

<sup>c</sup>*Faculty of Medicine, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia;*

<sup>d</sup>*Cancer Biology & Epigenetics Group – Research Center (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal;*

<sup>e</sup>*Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal;*

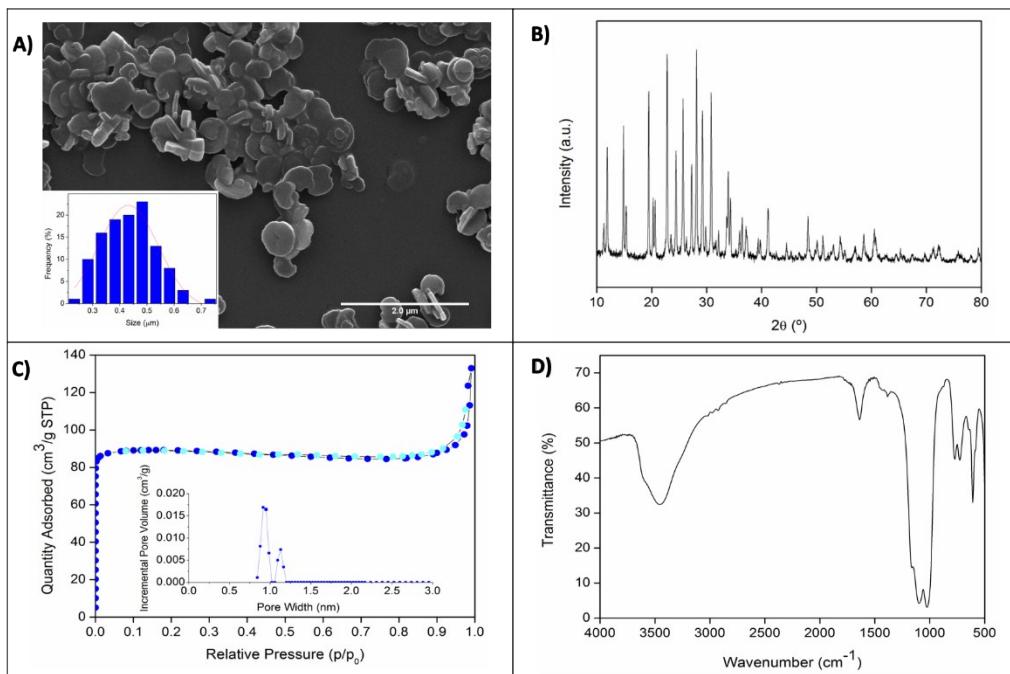
<sup>f</sup>*ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal;*

<sup>g</sup>*i3S - Instituto de Investigação e Inovação em Saúde and HEMS / IBMC - Histology and Electron Microscopy Service, Universidade do Porto, 4200-135 Porto, Portugal;*

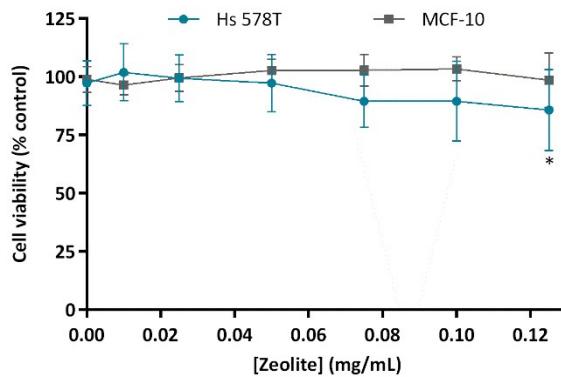
<sup>h</sup>*International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga, 4715-330 Braga, Portugal;*

<sup>i</sup>*CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;*

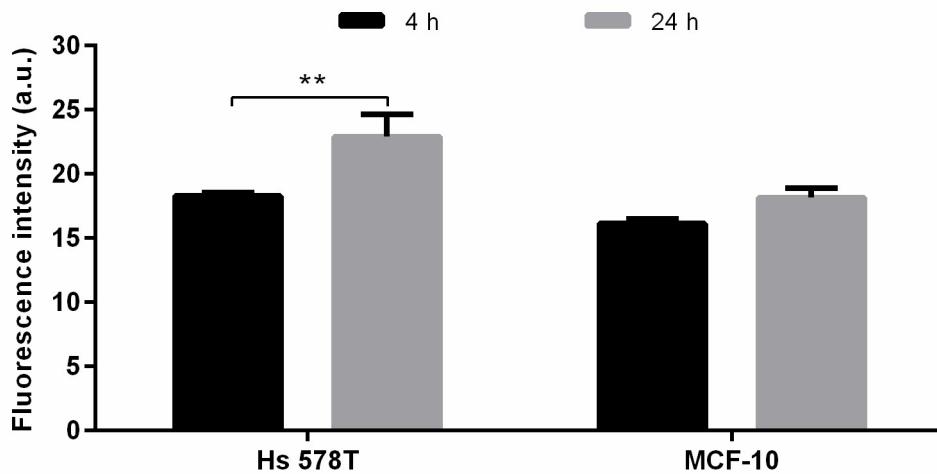
\*Corresponding author: [ineves@quimica.uminho.pt](mailto:ineves@quimica.uminho.pt); [fbaltazar@med.uminho.pt](mailto:fbaltazar@med.uminho.pt)



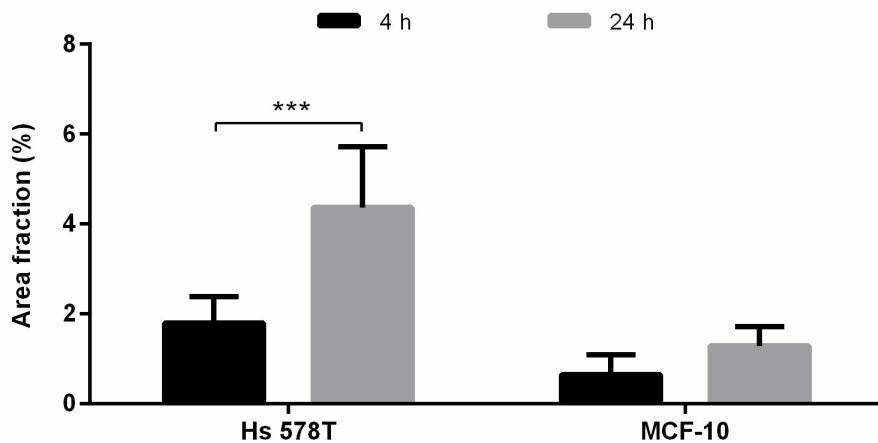
**Figure S1:** Characterization of the synthesized zeolite L. A) SEM micrographs of zeolite L dispersed in ethanol. Inset: Histogram representing statistical size distribution of zeolite L nanoparticles. B) XRD pattern of zeolite L. C) Nitrogen adsorption of zeolite L. Inset: pore size distribution. D) FTIR spectrum of zeolite L.



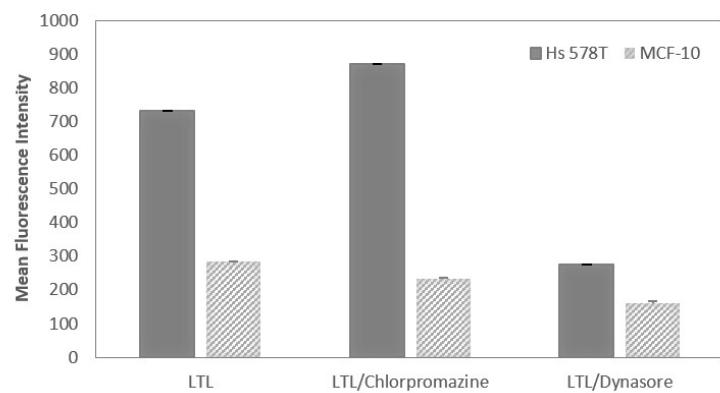
**Figure S2:** Cell viability of Hs 578T and MCF-10 cells, evaluated with SRB assay after 48 h incubation time with increasing concentrations of zeolite. Results are expressed in relation to the control (0 % of zeolite, considered 100 % of viability) as mean  $\pm$  SD of three independent experiments, each performed in triplicate. Differences with a  $p < 0.05$  were considered statistically significant (\*).



**Figure S3:** Fluorescence intensity measured using ImageJ Software. Zeolite L nanoparticles were incubated with Hs 578T and MCF-10 cells for 4 and 24 h incubation times at a concentration of 50  $\mu$ g/mL. Difference between groups were evaluated by Two-way ANOVA followed by Bonferroni post-test. Results are expressed as mean  $\pm$  SD,  $n=3$  for all experiments. \*\* $p<0.01$ .



**Figure S4:** Percentage of zeolite L nanoparticles internalized by Hs 578T and MCF-10 cells. Cells were incubated with 50  $\mu$ g/mL of zeolite L at 4 and 24 h incubation times. Results were analyzed by ImageJ Software. Difference between groups were evaluated by Two-way ANOVA followed by Bonferroni post-test. \*\*\* $p<0.001$ . Mean  $\pm$  SD.  $n=4$  for Hs 578T experiments and  $n=5$  for MCF-10 experiments.



**Figure S5:** Effects of the pharmacological inhibitors on the uptake of zeolite L in Hs 578T and MCF-10 cells. Cells were treated with chlorpromazine (10  $\mu$ g/mL) and dynasore (400  $\mu$ M) for 1 h before incubation with 50  $\mu$ g/mL of zeolite L for 4 h. After incubation cells were collected and analyzed by flow cytometry.