SUPPORTING INFORMATION

Neonatal Fc receptor-targeted lignin-encapsulated porous silicon nanoparticles for enhanced cellular interactions and insulin permeation across the intestinal epithelium

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1. Experimental Section

Materials: ChromPure Human IgG, Fc fragment was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Baltimore Pike, West Grove, PA, USA). BioPiva™ softwood kraft lignin was obtained from UPM Biochemicals (Helsinki, Finland). N-Mal-N-bis(PEG2-acid) was purchased from BroadPharm® (San Diego, CA, USA). CellMask™ DeepRed, Hank's Balanced Salt Solution (10× HBSS), fetal bovine serum (FBS), ethylenediaminetetraacetic acid (EDTA) and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Life Technologies Gibco® (Waltham, MA, USA). CellTiter-Glo® reagent assay was purchased from Promega Corporation (Fitchburg, WI, USA). Polycarbonate Transwell™ filters (0.4 μm pore size, 6-well plate), 96-well microplates, 25 cm^2 and 75 cm^2 cell culture flasks were purchased from Corning® Inc. (New York, NY, USA). Human recombinant Insulin (MW 5800 Da), 2-(*N*-morpholino-ethanesulfonic acid (MES), tetrahydrofuran (THF), N,N′-Dicyclohexylcarbodiimide (DCC), 4-(2-hydroxyethyl)-1-pipezarinethanesulfonic acid (HEPES), deuterated dimethyl sulfoxide (DMSO-d_6), Tween® 20, monoclonal anti-α-tubulin antibody, trifluoroacetic acid (TFA), acetonitrile (ACN), 4-dimethylaminopyridine (DMAP), bovine serum albumin (BSA), ethanolamine (MEA), hydrogen peroxide, ammonia hydroxide, sodium chloride (NaCl), sodium hydroxide pellets (NaOH) and monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Phosphate-buffered saline (10× PBS), non-essential aminoacids (NEEA), L-glutamine 200 mM, penicillin-streptomycin (Pen-Strep, 100 U/mL), sodium pyruvate (100 mM) and trypsin 2.5% were purchased from HyClone™, GE Healthcare Lifesciences (Logan, UT, USA). Human FCRN Antibody (MAB8639) was purchased from R&D Systems (Minneapolis, MN, USA). Anti-mouse IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 conjugate) was purchased from Cell Signaling Technology (Danvers, MA, USA). Mini-PROTEAN® TGX™ gels (#456-1096, 4–20%) and Trans-Blot Turbo Mini 0.2 μm Nitrocellulose Transfer Packs (#1704158) were purchased from Bio-Rad (Hercules, CA, USA). Triton X-100 was purchased from Merck Millipore (Darmstadt, Germany). Tris Buffered Saline

(TBS) was purchased from Media Kitchen (Helsinki, Finland). Oxygen plasma was purchased from Harrick Scientific Products Inc. (Pleasantville, NY, USA). Dithiobis(succinimidyl propionate) (DSP) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) was purchased from VWR International (Radnor, PA, USA). All materials were used as received.

Preparation of undecylenic acid modified thermally hydrocarbonized porous silicon (UnTHCPSi) nanoparticles: UnTHCPSi NP were prepared as reported elsewhere [1, 2]. Monocrystalline, boron-doped p+-type Si ⟨100⟩ wafers with a resistivity of 0.01–0.02 Ω·cm were electrochemically anodized in a 1:1 (v/v) hydrofluoric acid (38%)–ethanol electrolyte. At desired intervals, a repeated pulsed low/high etching profile was applied to create fracture planes on the Si wafers, forming a multilayered structure. After the etching, the multilayer film was detached from the substrate by abruptly increasing the current to the electropolishing region. For the next 30 min, residual moisture and oxygen were removed by exposing the fresh PSi multilayer films to N_2 flow (1 mL/min). Then, the PSi films were thermally hydrocarbonized with acetylene (C_2H_2, 1 L/min), added to a 1 L/min N_2 for 15 min at room temperature (RT) and heat treated for 15 min at 500 ºC under the 1:1 (vol.) N_2/C_2H_2 flow. When completing the thermal hydrocarbonization, the films were let to cool down to RT under N_2 flow. To render the films with –COOH termination, they were immersed into undecylenic acid for 16 h at 120 ºC. The undecylenic acid modified thermally hydrocarbonized porous silicon (UnTHCPSi) films were wet milled in a 5% vol undecylenic acid-dodecane using a high energy ball mill and separated by centrifugation to obtain UnTHCPSi NPs. The physical properties of the NPs were evaluated by N_2 adsorption/desorption measurements on a Tristar 3000 (Micromeritics) instrument at –196 °C. Results of the particle characterization are presented in **Table S1**. The specific surface area was calculated using the Brunauer–Emmett–Teller (BET) equation. The total pore

volume was taken as the total amount of adsorbed N_2 at a relative pressure of 0.97, and the average pore diameter was estimated by assuming that the pores are cylindrical.

Transepithelial electrical resistance (TEER) measurements: the measurements of electrical resistance across the Caco-2/HT29-MTX co-culture monolayers were used to assess the establishment of tight junctions over the culture period. The measurements were performed using a Millicell® ERS-2 volt/ohm meter with STX01 electrodes (Millipore, MA, USA). TEER values were calculated by subtracting the TEER of the blank Transwell™ filters, in which no cells were cultured, from the values measured on the filters in which the co-cultures were grown. These values were then multiplied by the surface area of the insert and reported as $\Omega \cdot cm^2$.

2. Results and discussion

Table S1. N_2 sorption isotherms for surface area, total pore volume and estimated pore diameter of UnTHCPSi NPs. Results are presented as mean ± s.d. ($n \geq 3$).

Parameter	**Value**
Specific surface area	282 ± 8 m^2/g
Total pore volume	0.62 ± 0.01 cm^3/g
Average pore diameter	8.8 ± 0.2 nm

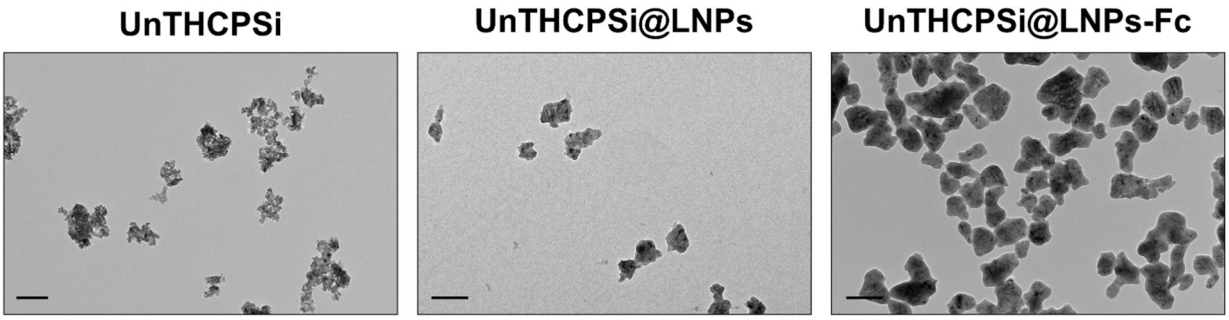

Figure S1. Morphological characterization of the NPs. a) TEM images of UnTHCPSi, UnTHCPSi@LNPs, and UnTHCPSi@LNPs-Fc (scale bars represent 200 nm).

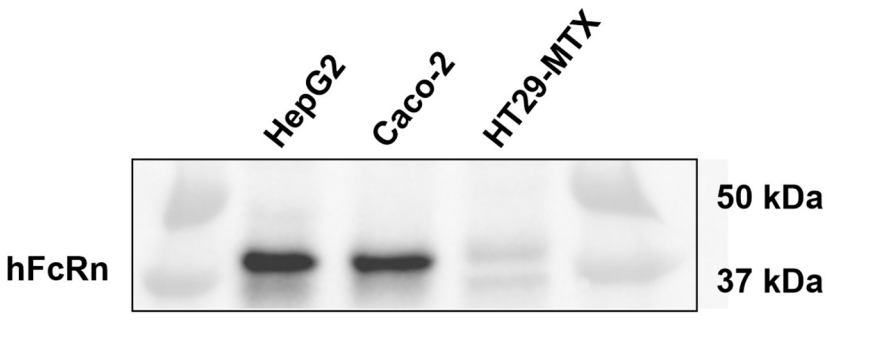

Figure S2. Detection of Human FcRn by Western Blot. Western blot shows lysates of HepG2, Caco-2 and HT29-MTX cells cultured for 7 days, probed with 5 µg/mL of human FcRn, followed by HRP-conjugated anti-mouse IgG Secondary Antibody.

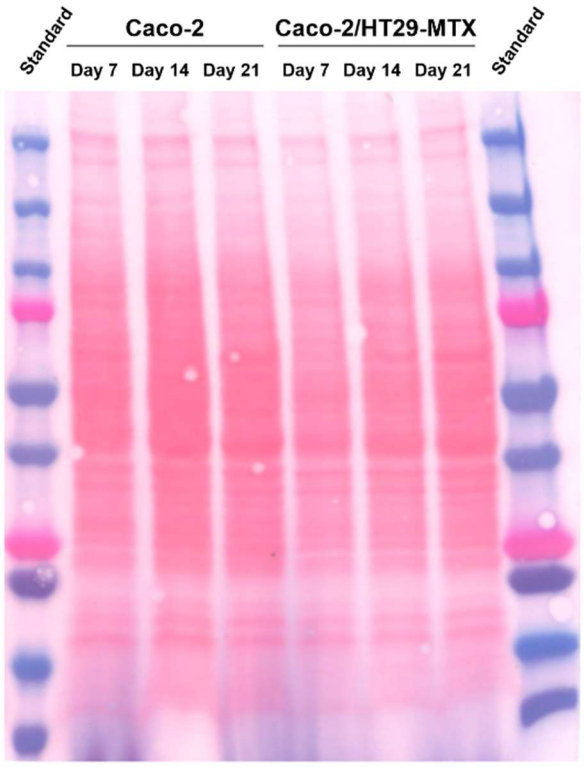

Figure S3. Ponceau S staining. Ponceau S staining to identify and locate proteins on Caco-2 and Caco-2/HT29-MTX cultures grown for 7, 14 and 21 days, after electroblotting of SDS-PAGE gels onto a polyvinylidene difluoride membrane.

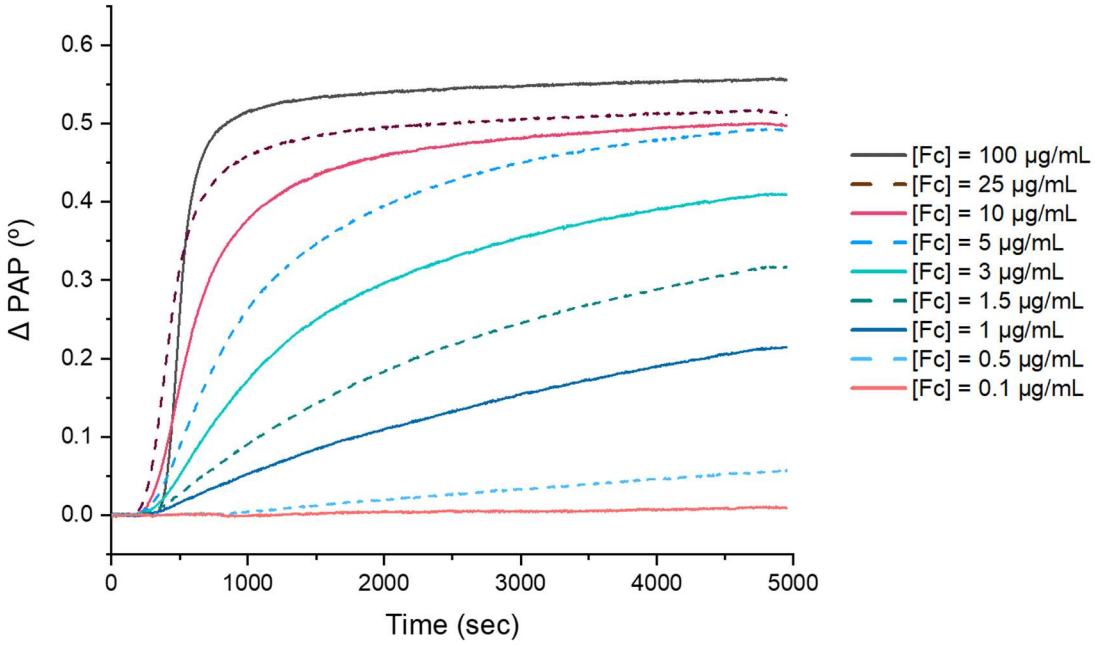

Figure S4. SPR analysis of the interaction between different concentrations of Fc with Protein A. Representative SPR peak angular position (PAP) responses to different concentrations of Fc fragment (0.1–100 µg/mL) with protein A.

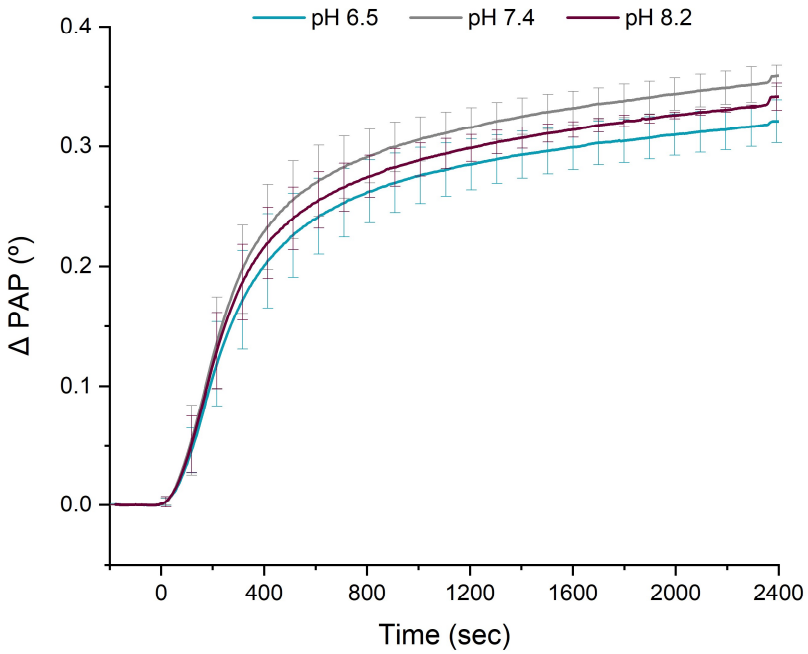

Figure S5. SPR analysis of Fc fragment interactions with protein A at different pH conditions. Representative SPR PAP responses to Fc fragment (10 μg/mL) with protein A at different pH conditions (6.5, 7.4 and 8.2).

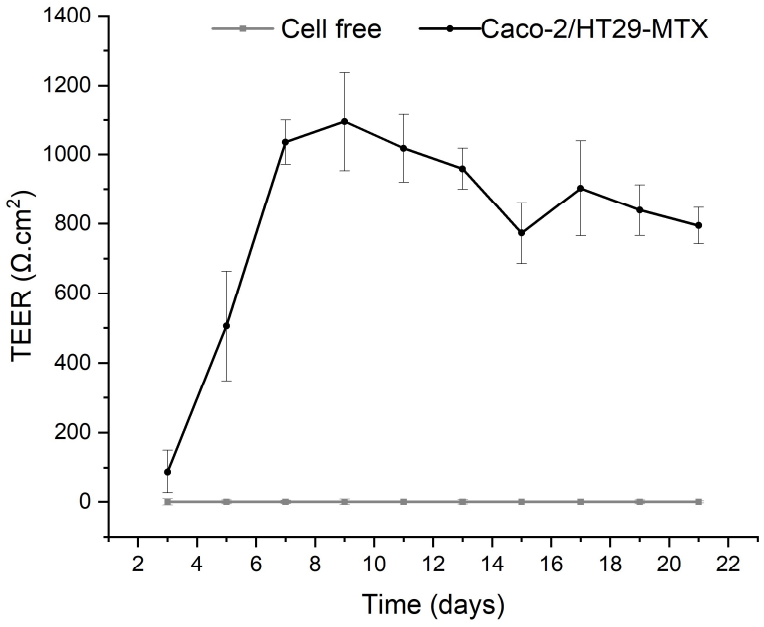

Figure S6. Monitoring of the TEER values. TEER measurements of Caco-2/HT29-MTX co-cultures grown in Transwell® permeable supports over 21 days. Results are presented as mean ± *s.d.* ($n \geq 3$).

References

[1] H.A. Santos, J. Riikonen, J. Salonen, E. Makila, T. Heikkila, T. Laaksonen, L. Peltonen, V.P. Lehto, J. Hirvonen, In vitro cytotoxicity of porous silicon microparticles: effect of the particle concentration, surface chemistry and size, Acta Biomater. 6(7) (2010) 2721-31.

[2] L.M. Bimbo, E. Mäkilä, T. Laaksonen, V.-P. Lehto, J. Salonen, J. Hirvonen, H.A. Santos, Drug permeation across intestinal epithelial cells using porous silicon nanoparticles, Biomaterials 32(10) (2011) 2625-2633.