

## Supplementary information

### Controlled release of human growth hormone fused with a human hybrid Fc fragment through nanoporous polymer membrane

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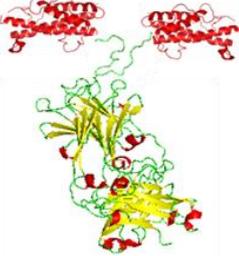
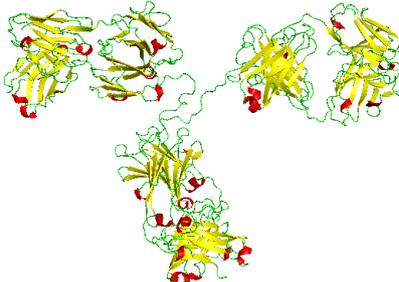
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**Table S1. Comparison of molecular features of rhGH, hGH-hyFc and IgG antibody.** The 3D-structure of hGH and IgG antibody was reconstructed with the PyMol Viewer program (PyMOL Molecular Graphics System, Version 1.2.8, DeLano Scientific LLC) by accessing the DPB file for rhGH (1HGU) and mouse IgG2a antibody (1IGT), respectively, from the Protein Data Bank (<http://www.rcsb.org/pdb>). The tentative structure of hGH-hyFc was graphically represented by fusing the two available structures for rhGH and IgG antibody.

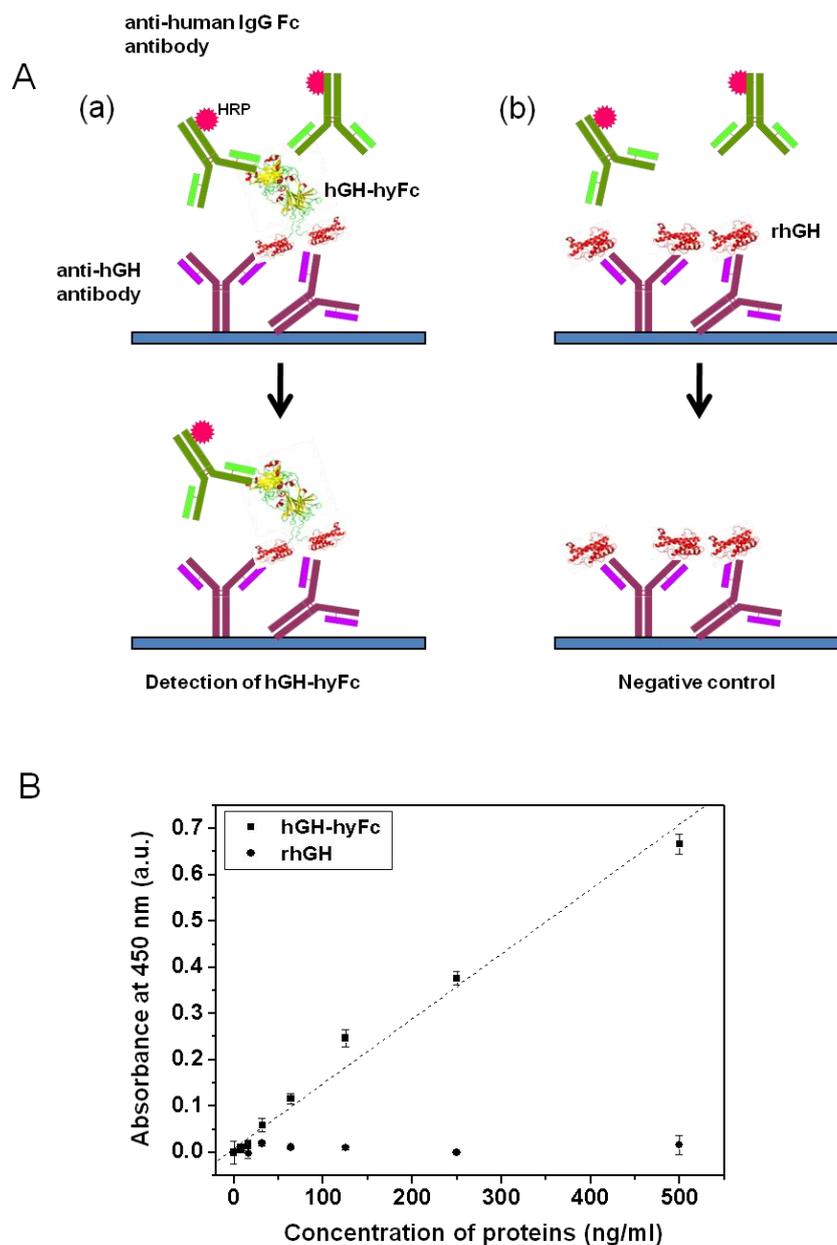
	rhGH	hGH-hyFc	IgG Antibody
3D structure			
PCB ID	1HGU <sup>a</sup> Human growth hormone	NA <sup>b</sup>	1IGT <sup>c</sup> Mouse IgG <sub>2a</sub>
Feature size	Width: 5.3 nm Height: 3.1 nm four-helix bundle	Radius of gyration: 6.5 nm (from Small-Angle X-ray Scattering)	Width: 16 nm Height: 11 nm
Molecular weight <sup>d</sup>	22 kDa	100 kDa	160 kDa

<sup>a</sup> Chantalat, L., Jones, N.D., Korber, F., Navaza, J., Pavlovsky, A.G., The crystal structure of wild type growth hormone at 2.5 angstrom resolution. *Protein Pept. Lett.* 2 (1995) 333-340.

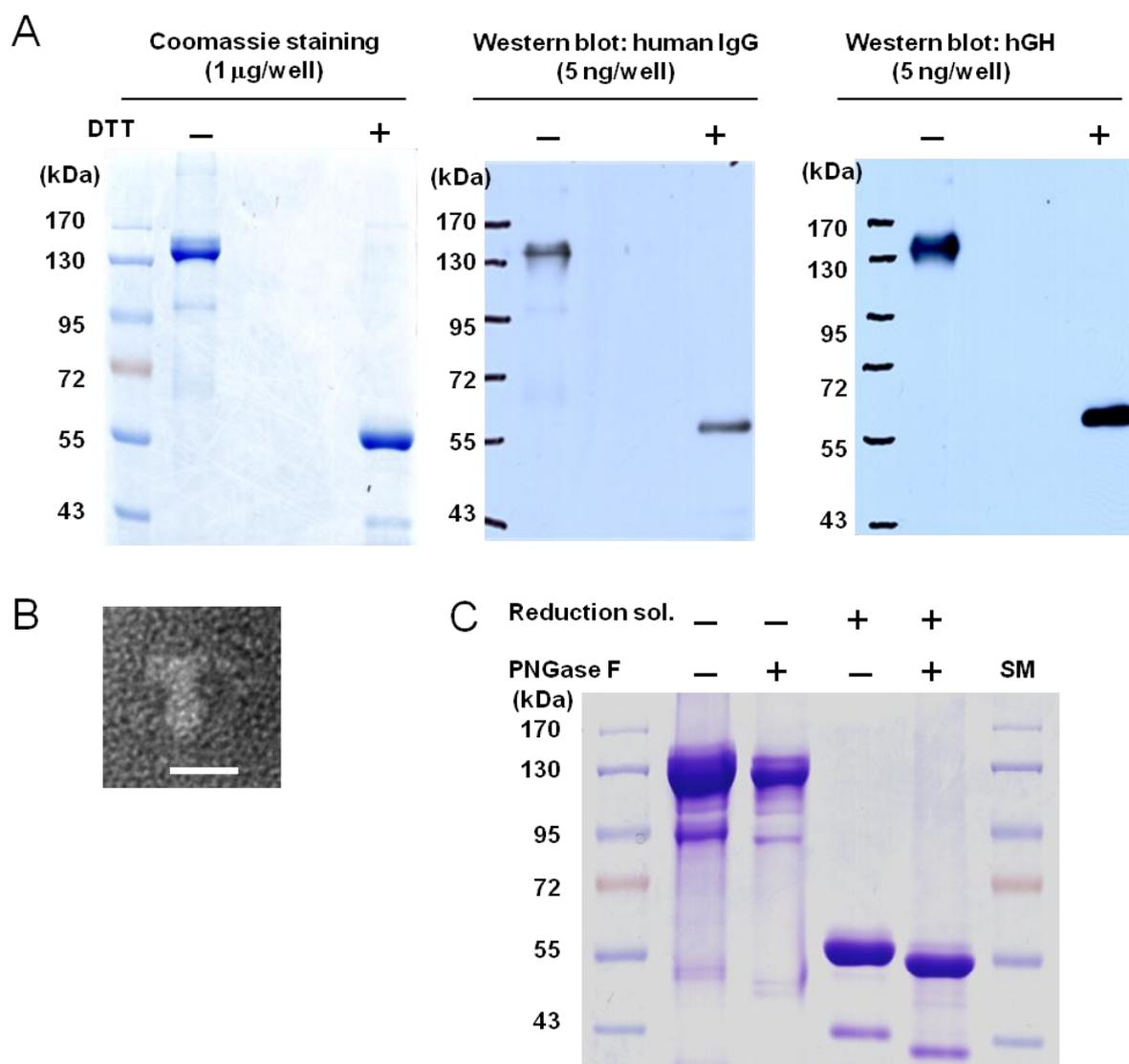
<sup>b</sup> Not available yet.

<sup>c</sup> Lisa J. Harris, Steven B. Larson, Karl W. Hasel, Alexander McPherson, Refined structure of an intact IgG2a monoclonal antibody. *Biochemistry* 36 (1997) 1581-1597.

<sup>d</sup> Based on the constituent amino acids only.

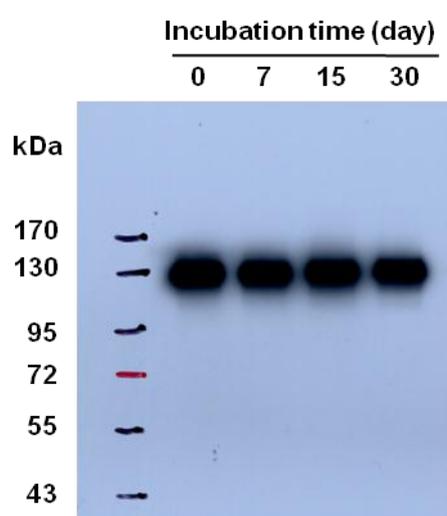


**Figure S1. Scheme for sandwich-type ELISA for hGH-hyFc.** (A) The anti-hGH antibody (in crimson) coated on the bottom of a well can interact with the binding site of hGH and hGH-hyFc. The anti-human IgG Fc antibody (in green) conjugated by HRP (in red) was allowed to specifically bind to the Fc region of the fusion protein (a), whereas the detection antibody was not able to bind to rhGH captured by the anti-hGH antibody since rhGH has no Fc domain (b). (B) The standard curve for hGH-hyFc in the culture medium was obtained by the sandwich ELISA ( $R^2=0.97$  for three independent assays).

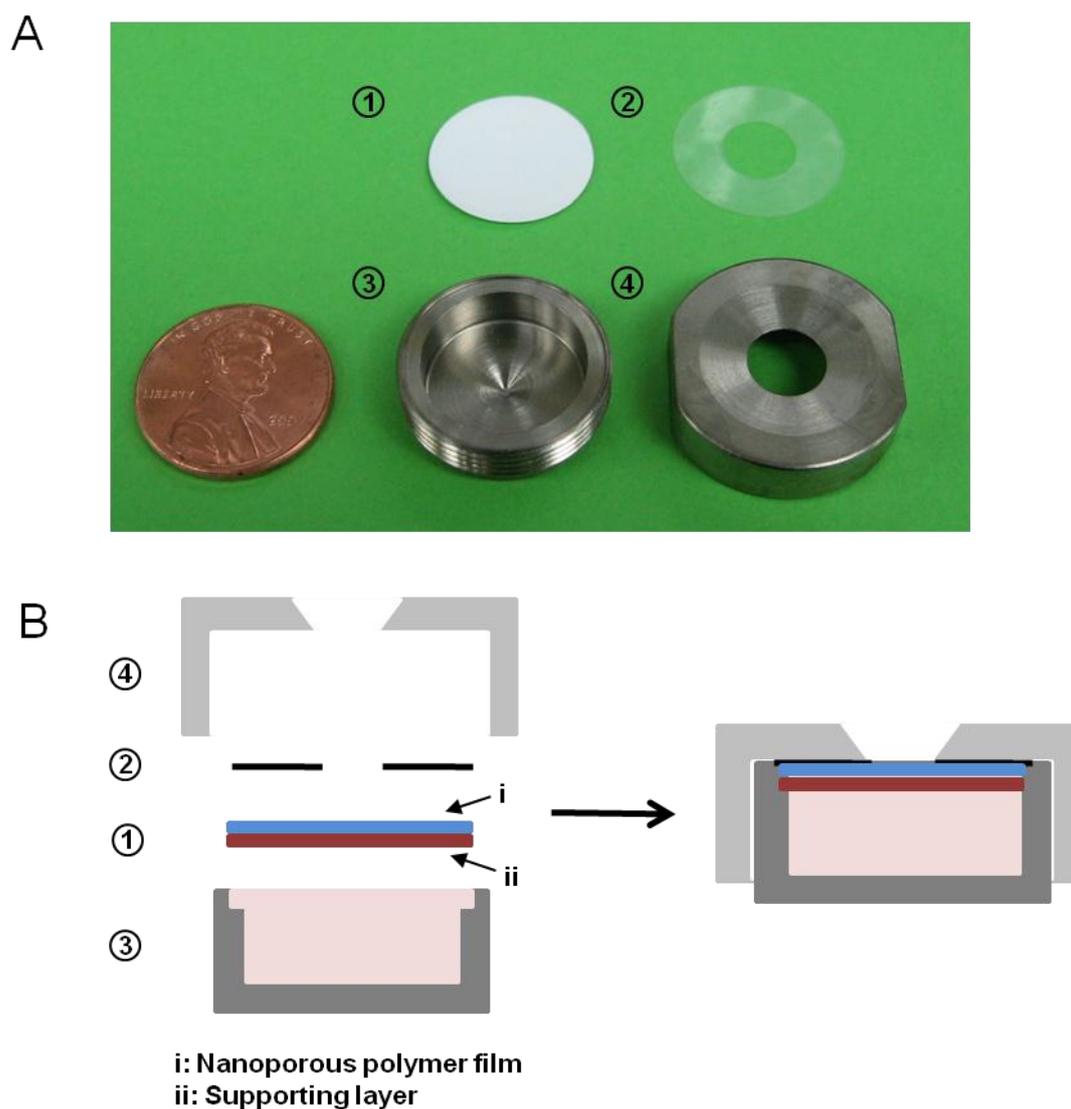


**Figure S2. Other molecular features of hGH-hyFc.** (A) The hGH-hyFc was treated by 100 mM dithiothreitol (DTT) for 1 h at 30°C prior to gel electrophoresis and run on a 8% gel followed by either Coomassie blue staining or the antibody probing. For the test of specificity in the western blotting, the protein sample was added to 0.5% (v/v) FBS solution prior to the gel electrophoresis. The hGH-hyFc proteins were probed by either anti-human IgG or anti-hGH antibody for immuno-blotting. (B) The transmission electron micrograph of hGH-hyFc was obtained at 500,000x magnification. The scale bar represents 10 nm. (C) The hGH-hyFc of 7 µg was prepared in the reduction solution (0.5% SDS, 40 mM DTT) followed by the heating at 90 °C for 10 min. The addition of the reaction solution (50 mM sodium phosphate,

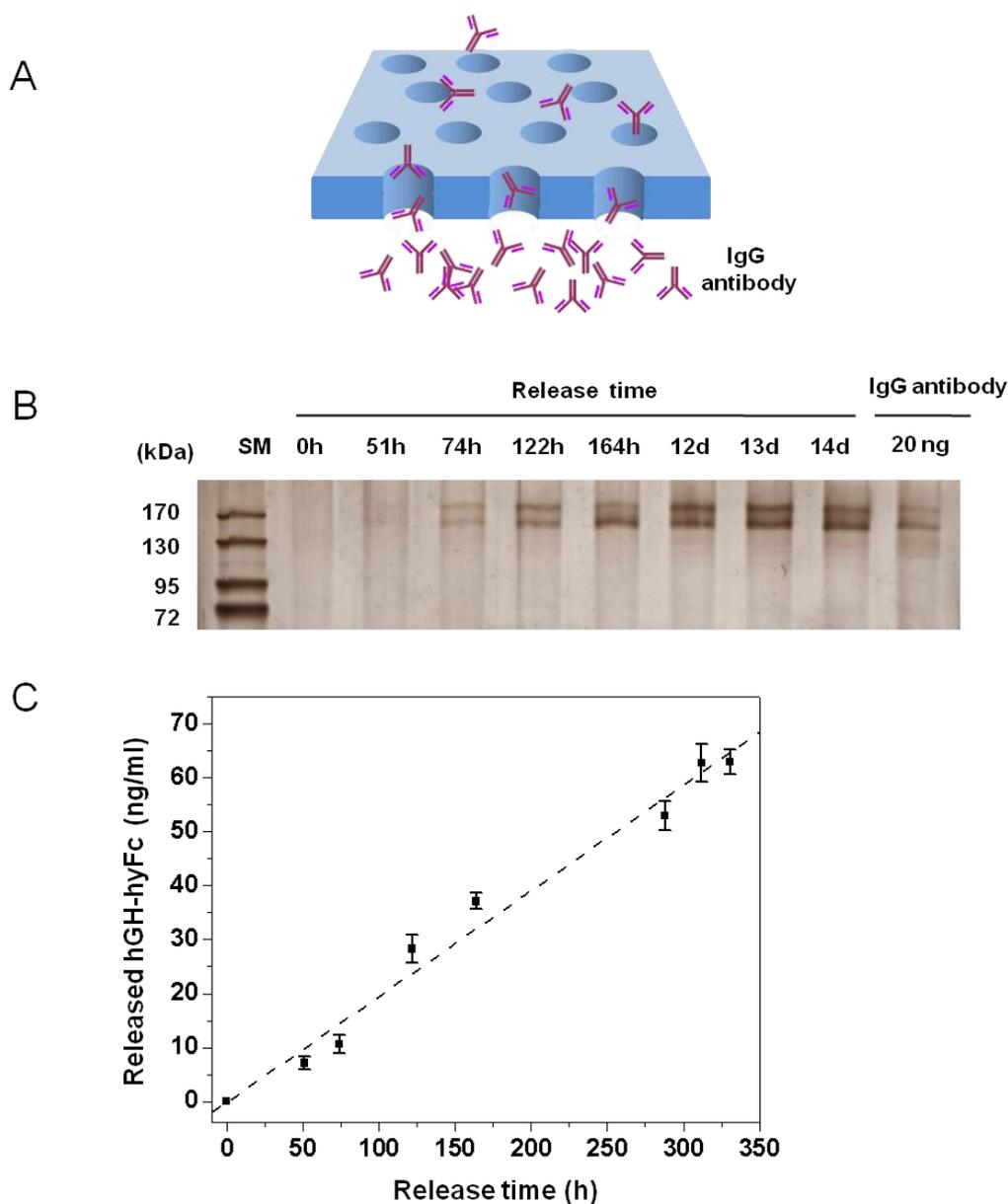
pH 7.5, 1% NP-40 solution and PNGase F (New England BioLabs) of 500 units to the native or reduced hGH-hyFc resulted in the downward shift of the band in an 8% SDS-PAGE compared to the glycosylated ones. The 3kDa difference of bands for PNGase F-treated and untreated hGH-hyFc was more apparent in the reduced hGH-hyFc than in the native one.



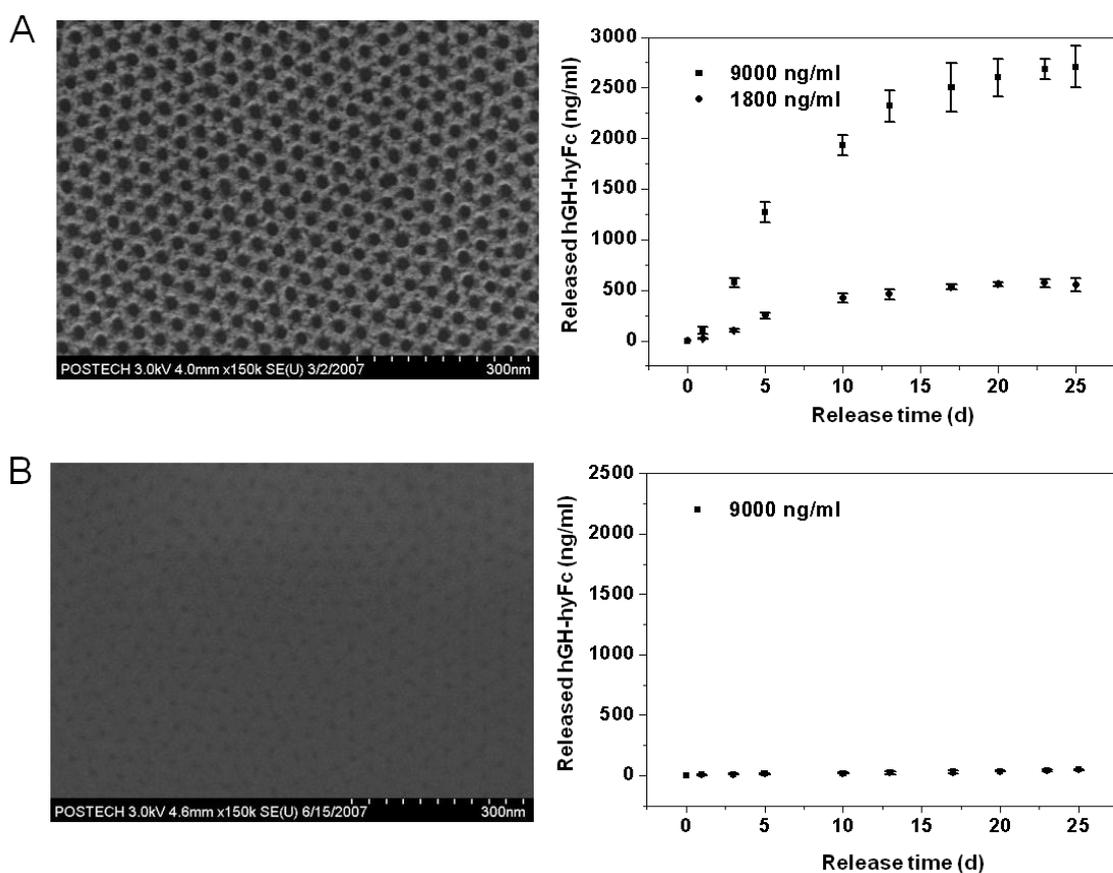
**Figure S3. Stability of hGH-hyFc in the culture medium.** The hGH-hyFc (1,800 ng/ml) was incubated in the culture medium supplemented with 0.5% (v/v) FBS at 37 °C. The same volume of hGH-hyFc was sampled out up to 30 days to be analyzed by the western blot with the anti-hGH antibody.



**Figure S4. Assembly of titanium reservoir for controlled release of hGH-hyFc.** (A) Components for titanium reservoir: nanoporous polymer membrane on a microfiltration supporting layer (1), polymer elastic gasket (2), lower titanium case (3), and titanium cover (4). The US one-cent coin was pictured together for scale reference. (B) The nanoporous polymer film on the supporting layer was placed on the rim of the lower titanium case that was filled with the protein solution. The annular elastic gasket was aligned along the central axis of the titanium cover before screwing the titanium cover on the lower case.

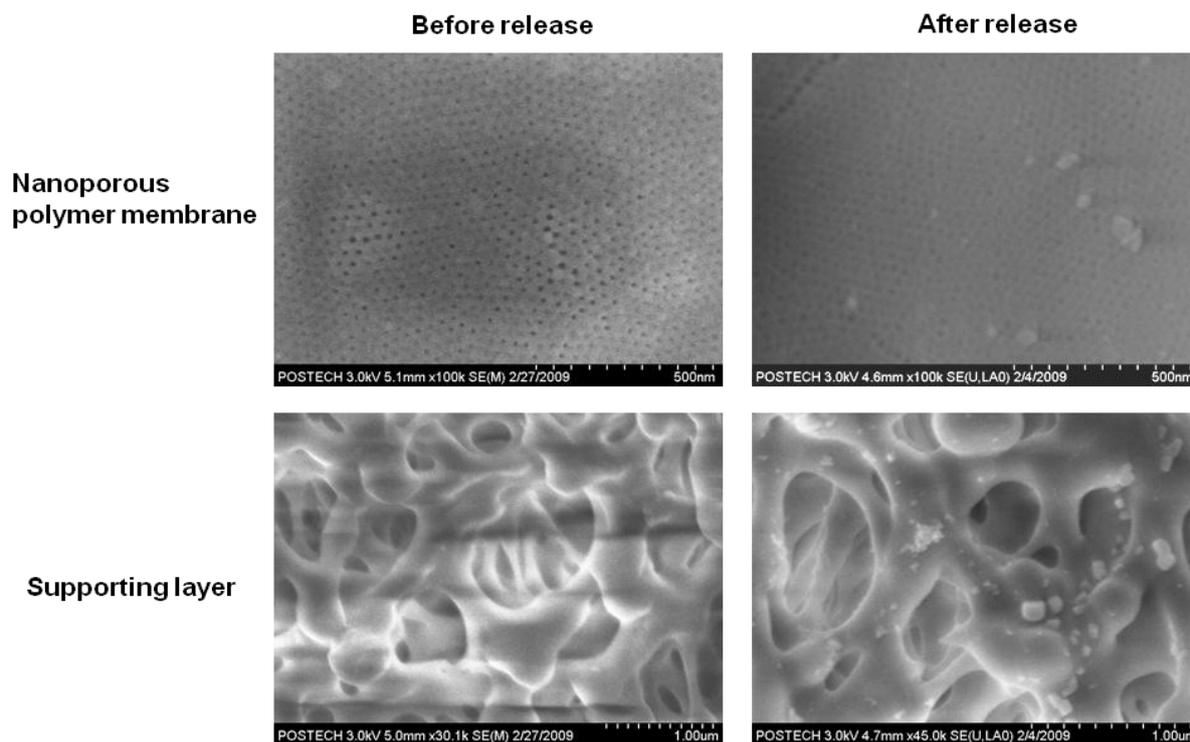


**Figure S5. Controlled release of rat IgG antibody through a nanoporous polymer membrane.** (A) Schematic diagram for controlled release of IgG antibody with passing through the cylindrical nanochannels. (B) Silver staining for released IgG antibody up to 14 days along the original IgG antibody for the standard of quantification. (C) Quantification of released IgG antibody at the different time points and the regression line ( $R^2 = 0.95$ ) to estimate the release rate from the three independent release trials.



**Figure S6. Release profiles of hGH-hyFc throughout nanopores with different diameters.**

hGH-hyFc was released through the nanopores with either  $27.0 \pm 3.2$  (A) nm or  $7.4 \pm 1.4$  nm (B) in diameter. The polymer membranes with different sizes of nanopores were imaged by SEM. The released hGH-hyFc from the assembled titanium reservoir was quantified by ELISA and each release profile up to 25 days was obtained and plotted as a function of release time.



**Figure S7. SEM images of the nanoporous polymer film and supporting layer before and after the controlled release of hGH-hyFc.** The hGH-hyFc was released from the titanium reservoir containing the hGH-hyFc with the initial concentration of 9,000 ng/ml in the culture media supplemented with 0.5% FBS at 37 °C. After 25 days of release, the surfaces of the nanoporous film and its supporting layer were compared with their initial surfaces using scanning electron microscopy.