High Capacity Nanoporous Silicon Carrier for Systemic Delivery of Gene Silencing Therapeutics

Jianliang Shen, [†,ǂ,§] **Rong Xu,** [†,§] **Junhua Mai,** [†,§] **Han-Cheon Kim,** [†] **Xiaojing Guo,** [†] **Guoting Qin,** [†] **Yong Yang,** [†] **Joy Wolfram,** [†] **Chaofeng Mu,** [†] **Xiaojun Xia,** [†] **Jianhua Gu,** [†] **Xuewu Liu,** [†] **Zong-Wan Mao,** [ǂ] **Mauro Ferrari,** [†,ǁ] **Haifa Shen** [†,ǁ,*]

[†]Department of Nanomedicine, The Methodist Hospital Research Institute, Houston, Texas 77030, United States, [ǂ]MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China, [ǁ]Department of Medicine, [ǁ] Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY10065, United States.

AUTHOR INFORMATION

The authors declare no competing financial interest.

All authors have given approval to the final version of this manuscript.

[§]These authors contributed equally to this work.

*Address correspondence to hshen@houstonmethodist.org

Supporting Information:

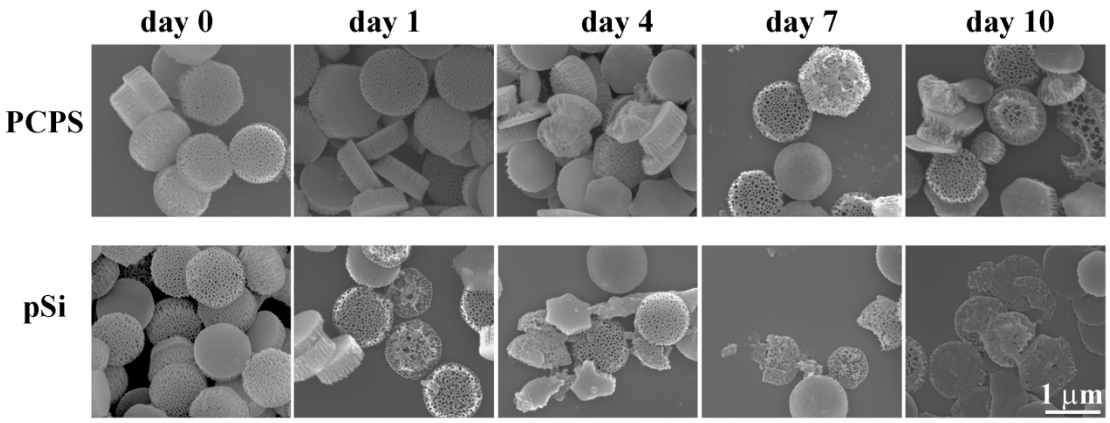


Figure S1. SEM images on degradation of PCPS and the oxidized pSi in PBS at different time points. To prepare the SEM samples, PCPS or oxidize pSi (300×10^6) were incubated in 100 μL PBS at 37°C. They were collected by centrifuged on days 1, 4, 7, and 10, washed 3 times with water, and resuspended in IPA. A drop of IPA particle suspension was directly placed on a clean silicon SEM sample stub and dried. The samples were loaded into the SEM chamber, and SEM images were measured at 7 kV and 5 mm working distance.

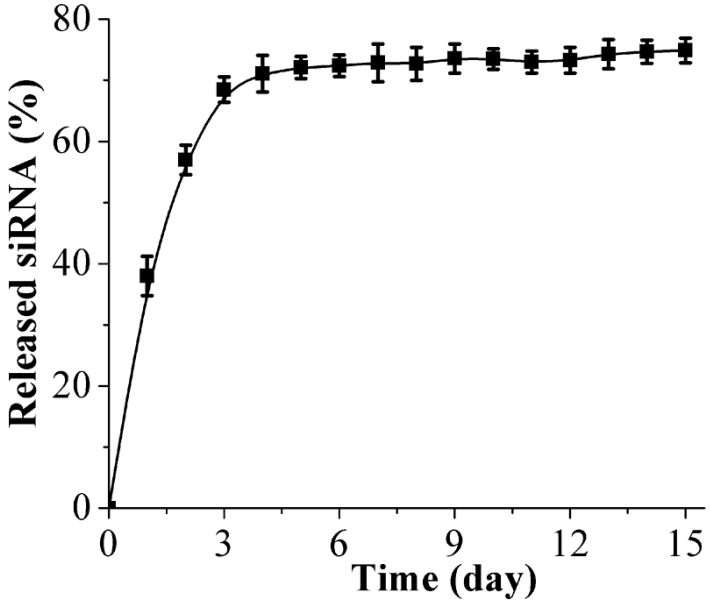


Figure S2. Release profile of siRNA polyplex from a mixture of pSi-APTES-Arg+PEI/siRNA. pSi-APTES-Arg and PEI/siRNA were mixed by sonication for 10 minutes. The particles were then incubated in 10% fetal bovine serum. Supernatant samples were taken at the indicated time points, and fluorescent intensity at Ex543/Em590 was measured. Results are presented as the mean of five measurements ± standard deviation.

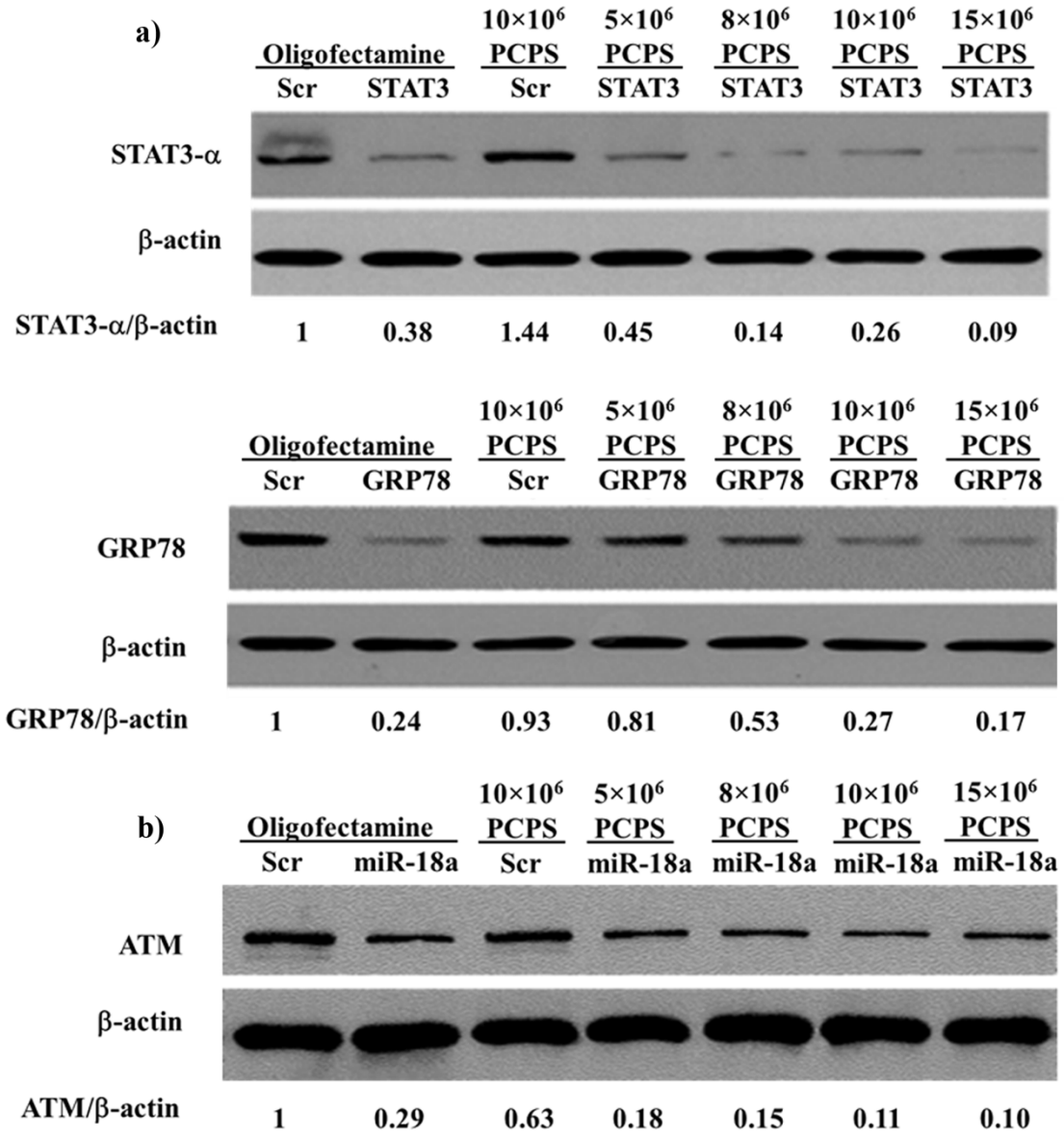


Figure S3. Optimization on knockdown of gene expression *in vitro*. (a) Knockdown of protein expression by PCPS/siRNA in MDA-MB-231 cells. PCPS was used to deliver siRNA oligos specific for STAT3-α and GRP78. Cells in each well were incubated with 1.4 μg siRNA loaded into increasing amount of PCPS ($5x10^6$ - $15x10^6$ PCPS). In the control samples, cells were transfected with 1.4 μg geen-specific siRNA or scramble siRNA with oligofectamine. Cells were harvested 72 hours later, and Western blot analysis was performed to measure expression of STAT3-α and GRP78. (b)

Knockdown of ATM protein expression by PCPS/miR-18a in MCF-7 cells. Total amount of miR-18a or scramble was 1.4 μg/ well of cell culture.

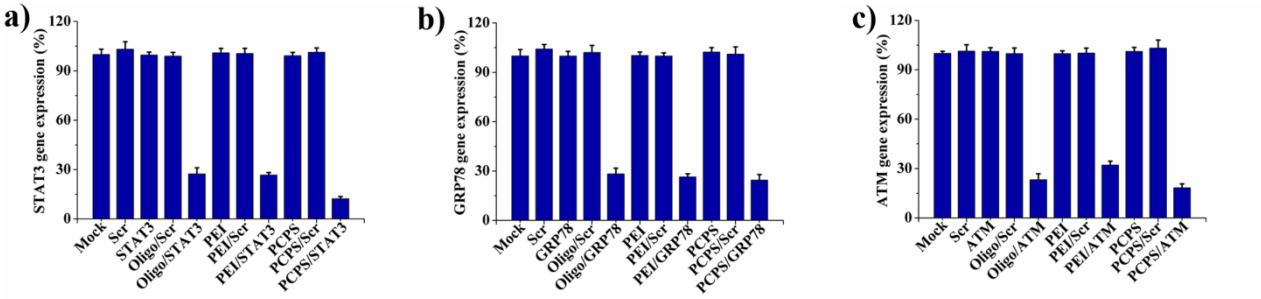


Figure S4. qRT-PCR analysis to evaluate the mRNA level. siRNA or microRNA were either transfected into cells with oligofectamine or PEI, or delivered in PCPS. Cells were harvested 48 h later, and mRNA levels were measured by qRT-PCR and normalized with β-actin. (a) MDA-MB-231 cells were treated with a STAT3 siRNA. (b) MDA-MB-231 cells were treated with a GRP78 siRNA. (c) MCF-7 cells were treated with a miR-18a mimic. All samples were analyzed in triplicates.

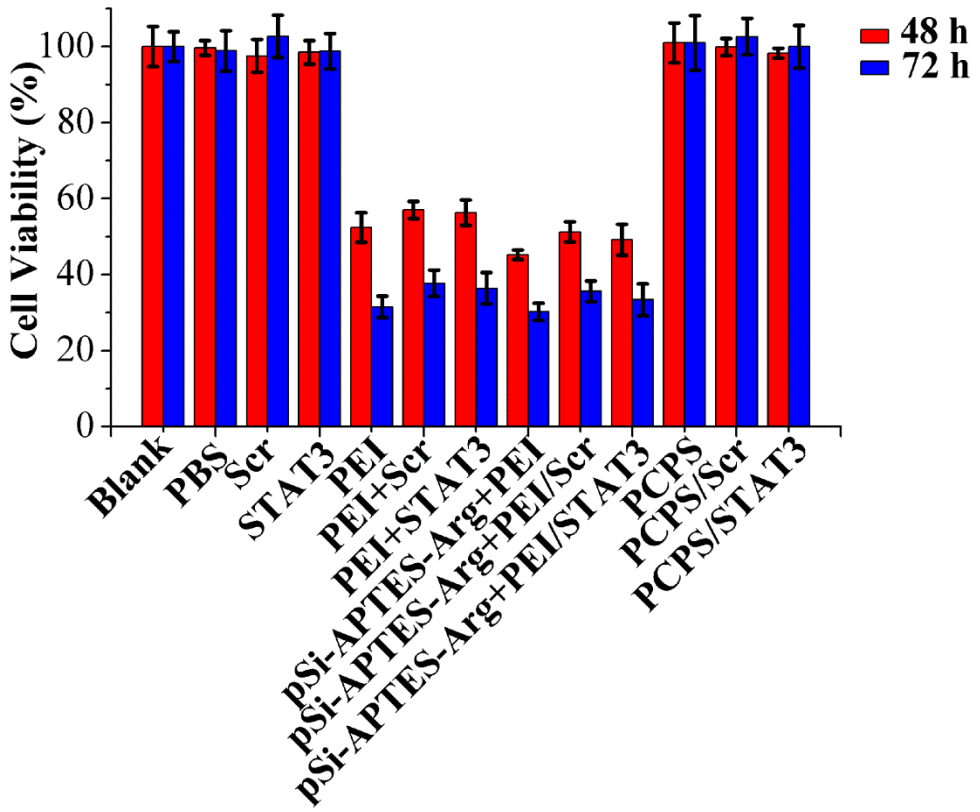
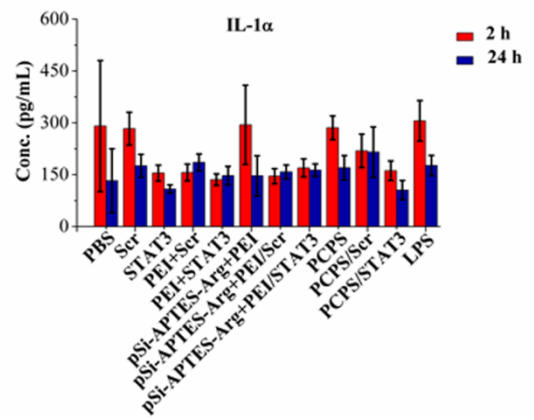
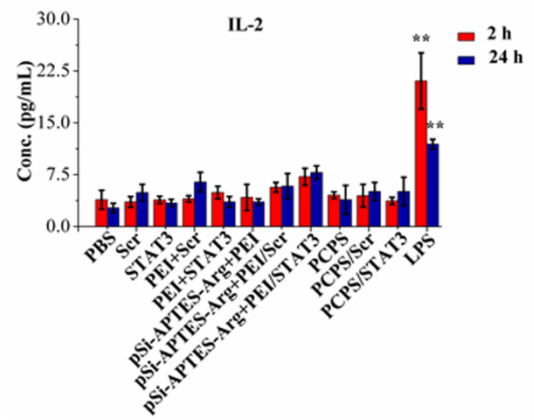


Figure S5. Cell viability assay to evaluate cytotoxicity. MDA-MB-231 cells were seeded in a 96-well tissue culture plate at 2 × 10 ^{4} cells/well with 100 µL DMEM containing 10% FBS. They were treated with the indicated reagents for 48 to 72 hours, and cell viability was evaluated using the Promega's CellTiter 96 aqueous one solution cell proliferation assay kit. Percentage of cell viability was calculated by the ratio of absorbance of treated sample to that of untreated control sample. Results are presented as the mean of three measurements ± standard deviation.


IL-1α
Conc. (pg/mL)
600
450
300
150
0
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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Conc. (pg/mL)
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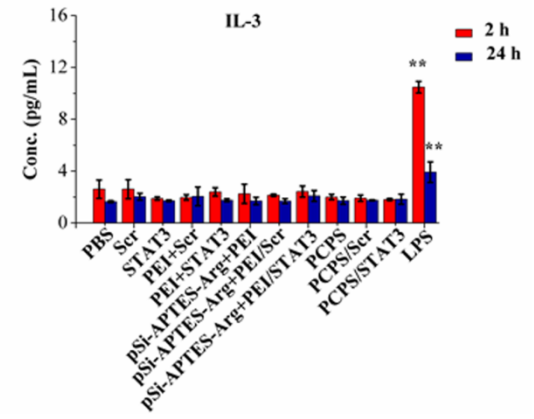
22.5
15.0
7.5
0.0
**
**
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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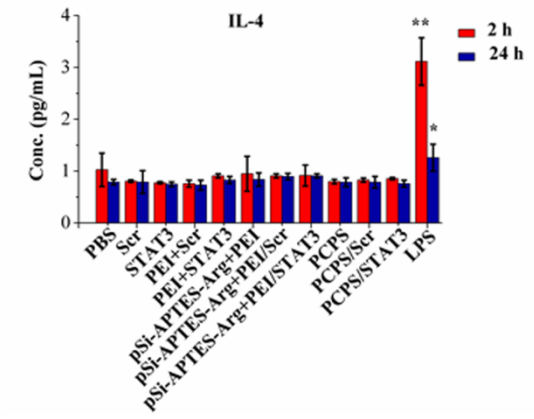
Conc. (pg/mL)
16
12
8
4
0
**
**
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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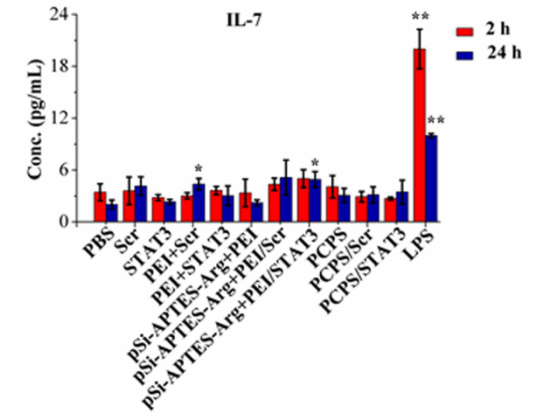
Conc. (pg/mL)
4
3
2
1
0
**
*
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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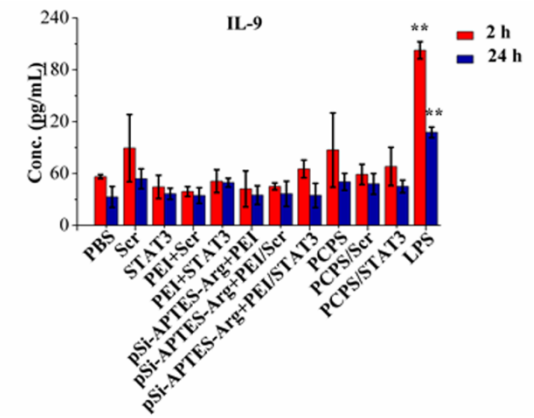
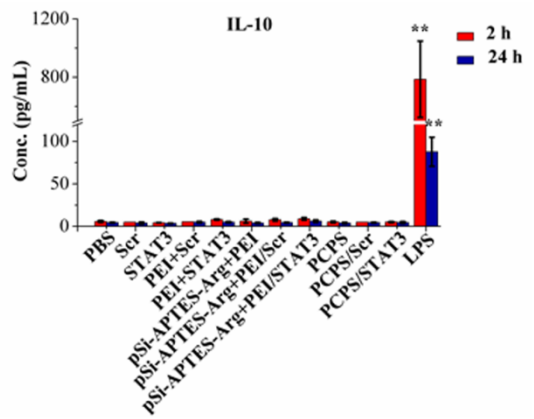
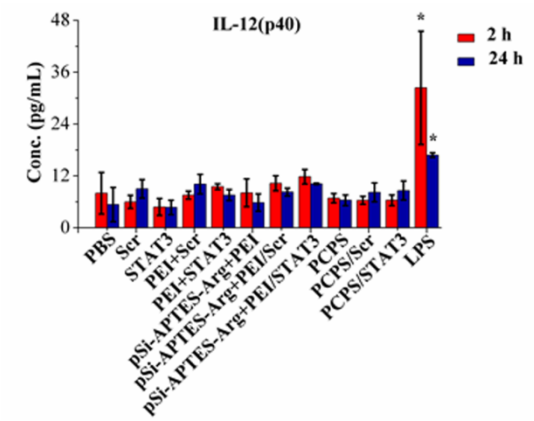
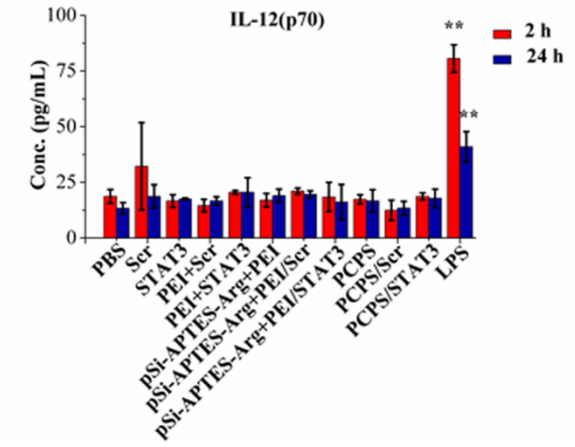
Conc. (pg/mL)
24
18
12
6
0
*
*
**
**
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


IL-9
Conc. (pg/mL)
240
180
120
60
0
**
**
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


IL-10
Conc. (pg/mL)
1200
800
100
50
0
**
**
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


IL-12(p40)
Conc. (pg/mL)
48
36
24
12
0
*
*
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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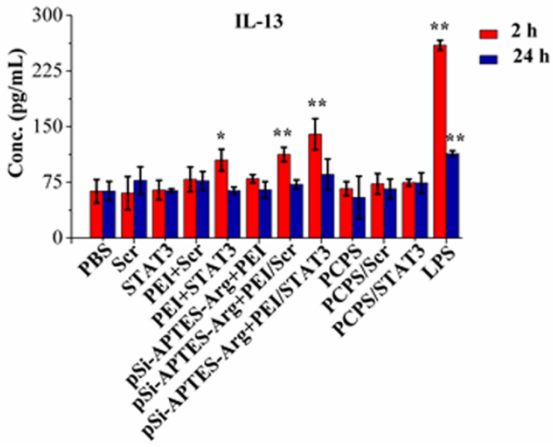
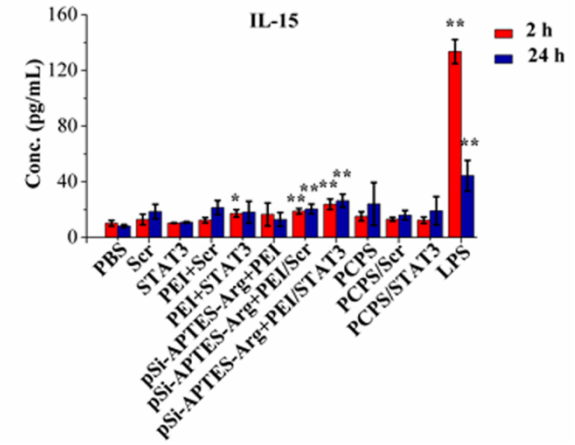
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


IL-13
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS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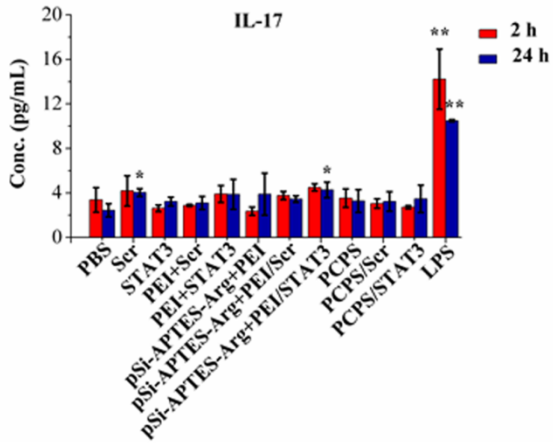
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


IL-17
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
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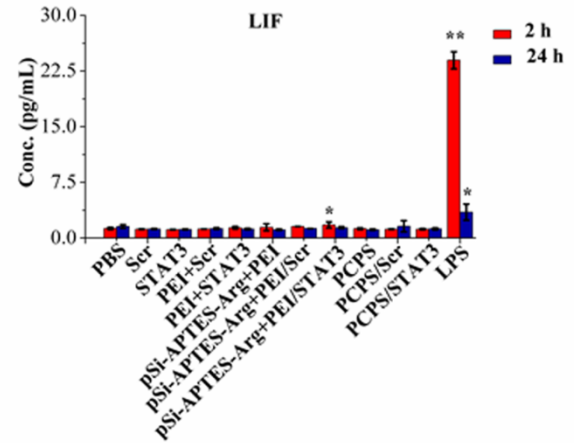
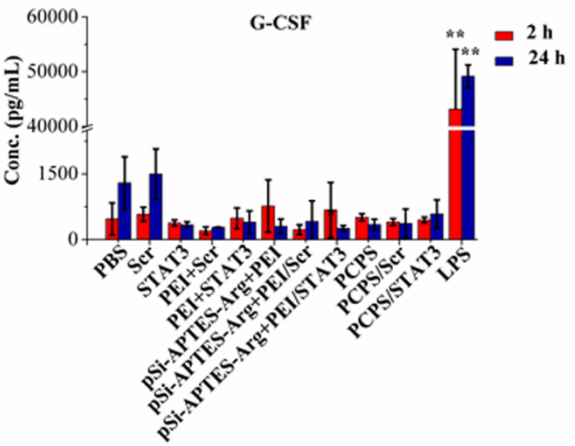
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


LIF
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS


G-CSF
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS

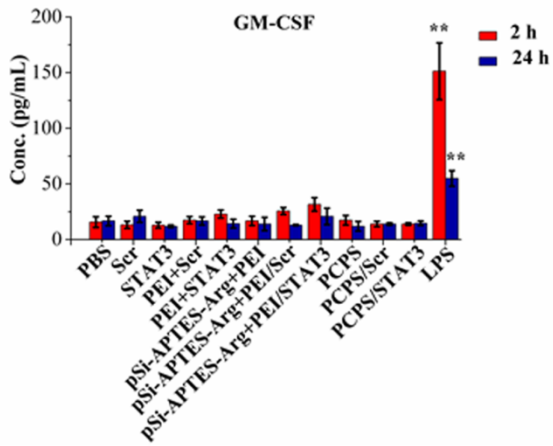

GM-CSF
Conc. (pg/mL)
2 h
24 h
PBS
Scr
STAT3
PEI+Scr
PEI+STAT3
pSi-APTES-Arg+PEI
pSi-APTES-Arg+PEI/Scr
pSi-APTES-Arg+PEI/STAT3
PCPS
PCPS/Scr
PCPS/STAT3
LPS

Figure S6. Changes in levels of selected serum cytokine/chemokine/colony-stimulating factors in post-treatment mice. Blood samples were collected 2 or 24 hours after intravenous dosing of treatment agents. A multiplexed bead-based immunoassay was used to measure levels of the cytokines/chemokines/colony-stimulating factors. Three mice per group was used in the study. Results are presented as the mean of three measurements ± standard deviation. *p <0.05. **p <0.01.