A Light-Driven Therapy of Pancreatic Adenocarcinoma Using Gold Nanorods-Based Nanocarriers for Co-Delivery of Doxorubicin and siRNA

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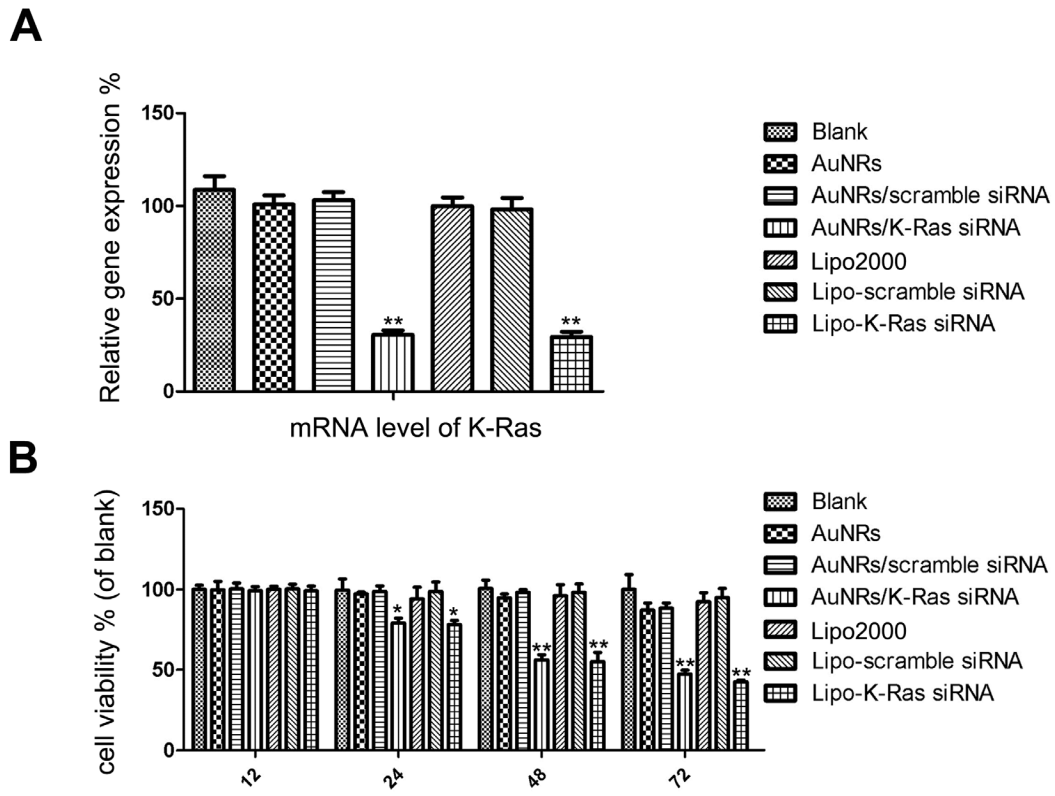
Supplementary Information

Materials and Methods

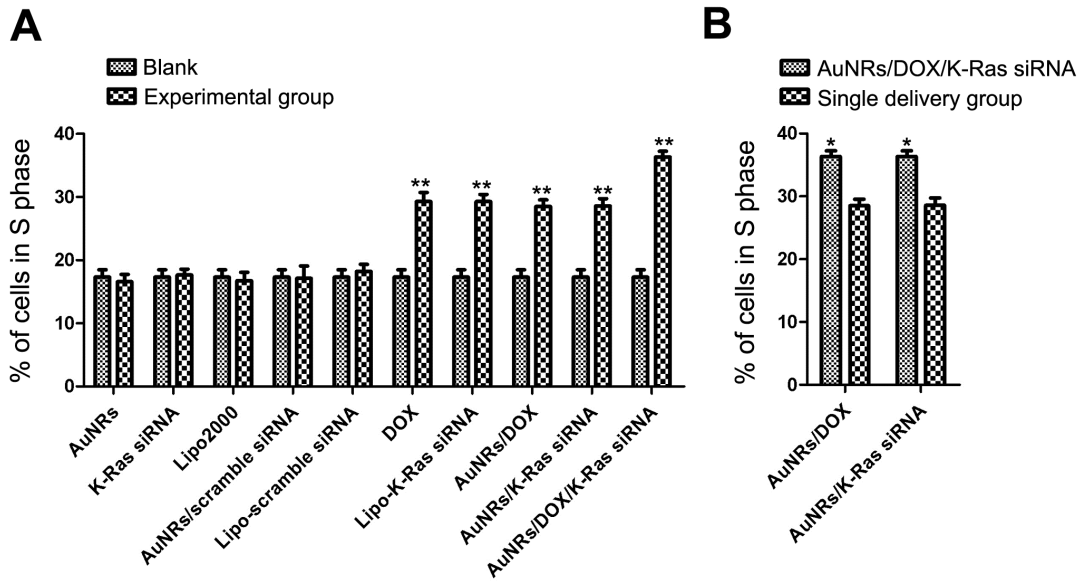
Paraffin section and histology

For histological experiments, tumor tissues were collected on Day 25 and the samples are fixed with 4% buffered formalin-saline at room temperature for 24 hours. Next, these fixed tissue samples were embedded in paraffin blocks and they were sliced into 4 μm-thick sections using a microtome. The paraffin sections were then mounted on glass slides for hematoxylin and eosin (H&E) staining. The H&E staining slices were examined using an Olympus BX51 light microscope.

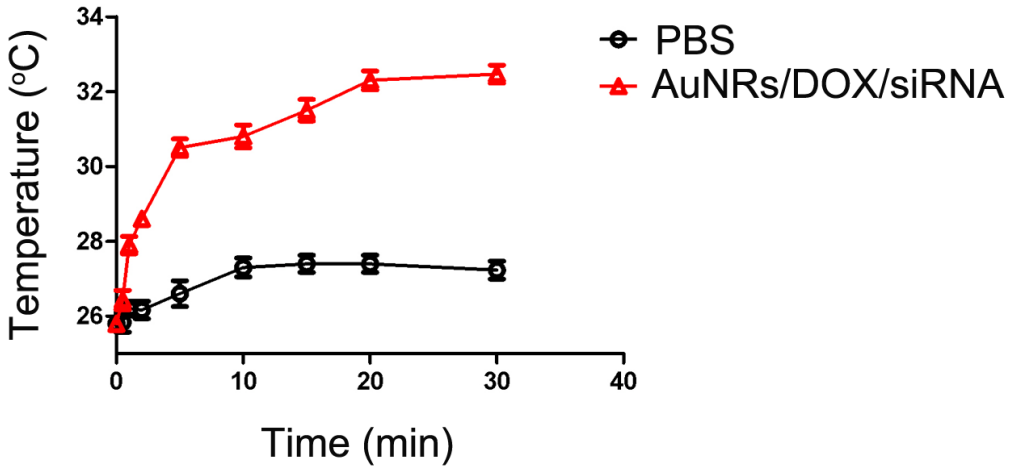
Figures and Figure legends



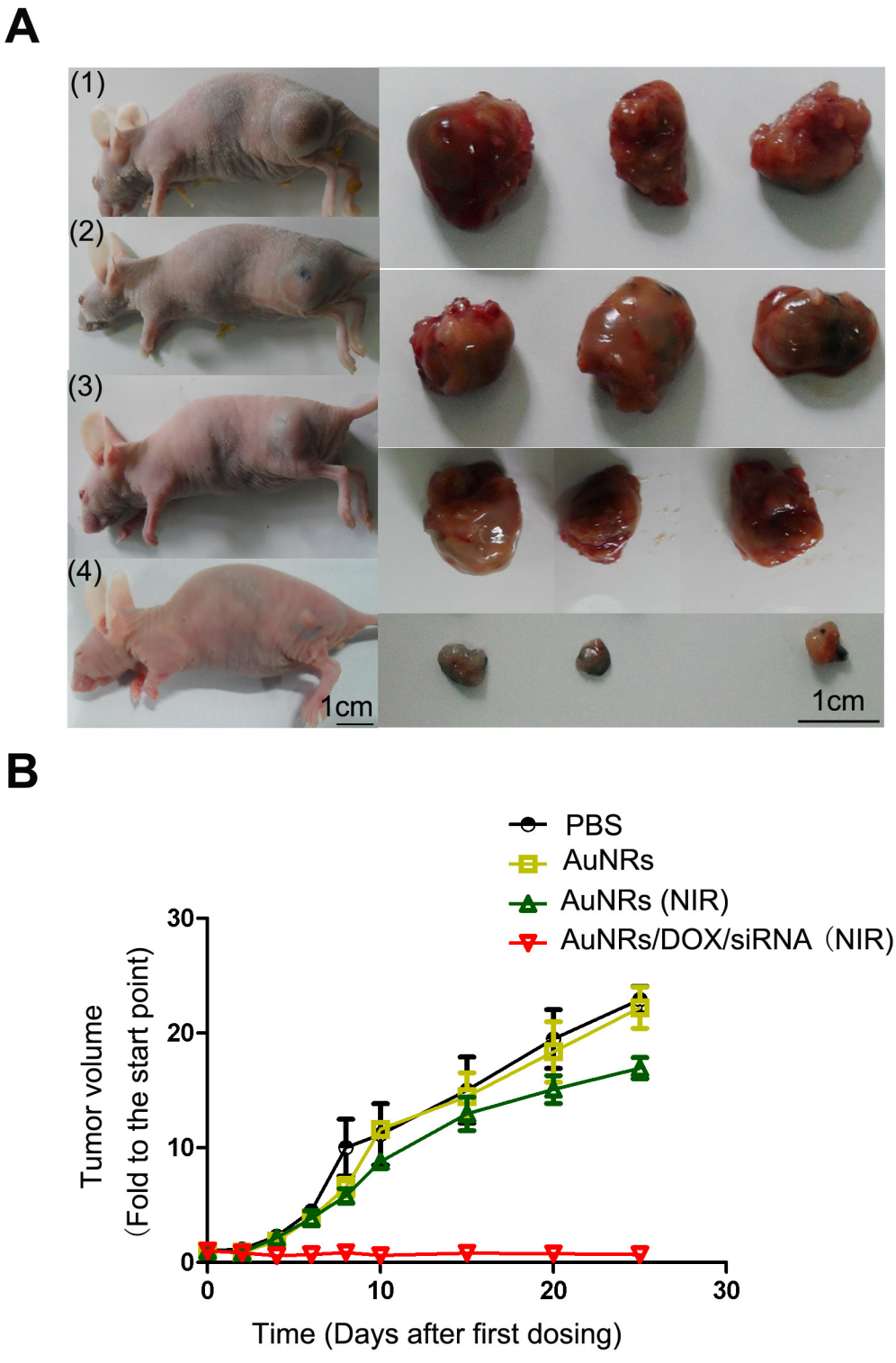
Supplementary Figure S1: The exclusion of the off-target effect of siRNA. Panc-1 cells were treated with Blank, AuNRs, AuNRs/scramble siRNA, AuNRs/K-Ras siRNA, Lipo2000, Lipo-scramble siRNA and Lipo-K-Ras siRNA. (A) After 48 hours treatment, mRNA relative expression levels detected by RT-PCR. (B) Cell viability test of Panc-1 cells after 12, 24, 48 and 72 hours treatment. Data are presented as the mean±SEM of triplicate experiments.*, $P < 0.05$, **, $P < 0.01$ vs blank, AuNRs and Lipofectamine.



Supplementary Figure S2: Statistical data of cell cycle analysis. The change of S phase were monitored by fluorescence activated cell sorting (FACS) analysis and quantified by GraphPad Prism (version 5) software. Data are presented as the mean±SEM of triplicate experiments. **, $P < 0.01$ vs blank; *, $P < 0.05$, the comparison between single delivery group and co-delivery group.

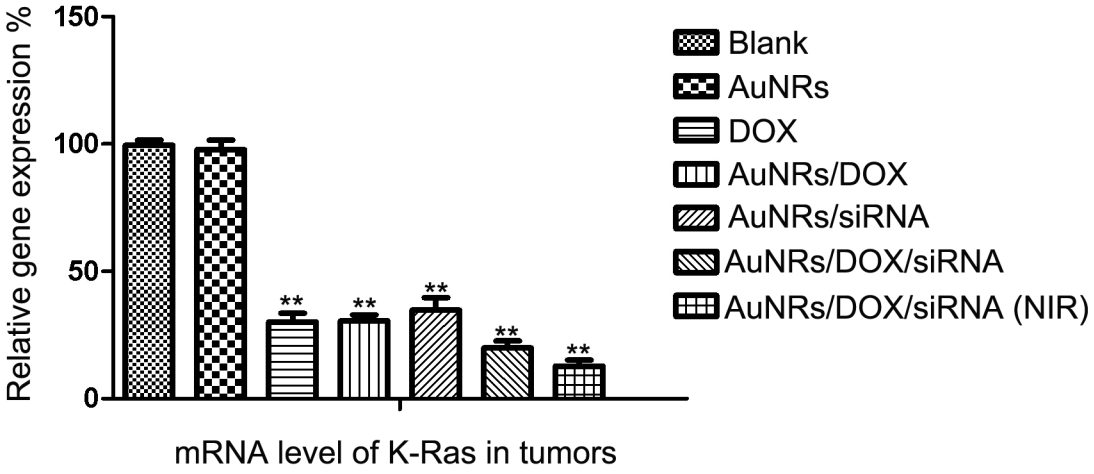


Supplementary Figure S3: Temperature dependence of AuNRs/DOX/siRNA nanoplex under 665 nm light irradiation. AuNRs/DOX/siRNA nanoplex was dispersed in PBS and the temperatures of the samples were measured by Thermometer (T208, Digitron). Within the first 30 minutes of the 665 nm light exposure, a 7 °C increment was observed for the AuNRs/DOX/siRNA nanoplex (from 26 °C to 33 °C). On the other hand, only 1 °C increment was observed for PBS buffer solution (from 26°C to 27 °C). Error bars represent SEMs for triplicate experiments.

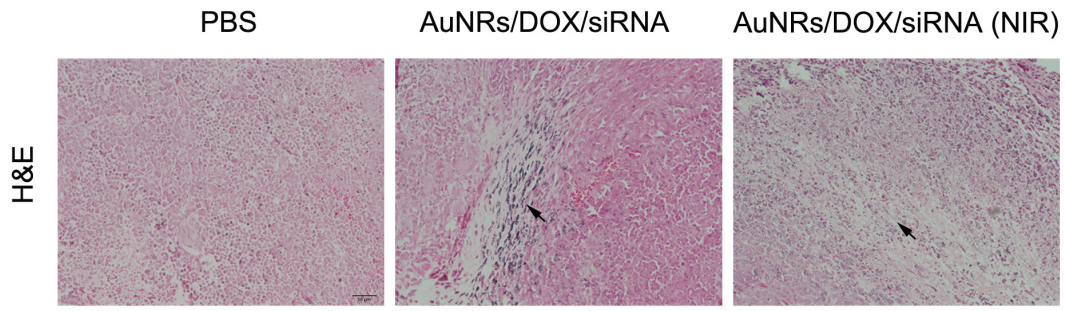


Supplementary Figure S4: Comparison of the antitumor activity of AuNRs and AuNRs/DOX/siRNA nanoplex in a Panc-1 xenograft animal model. In both cases, the experimental subjects were exposed to 665 nm light. (**A**) Representative images of mouse and tumor tissues treated with (1) PBS, (2) free AuNRs, (3) combination of AuNRs and 665 nm light irradiation or (4) combination of AuNRs/DOX/siRNA and

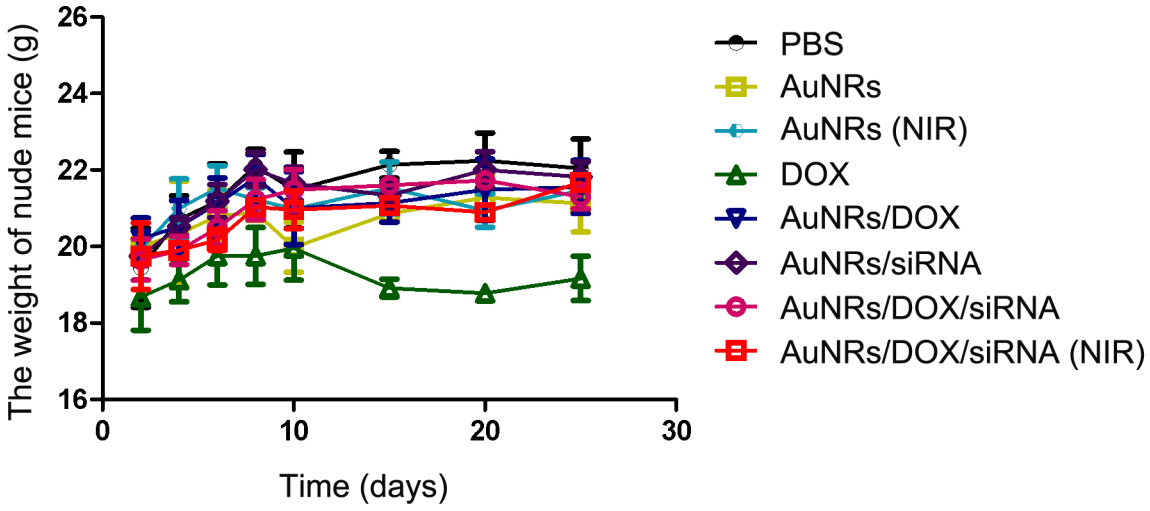
665 nm light irradiation. The mice treated with combination of AuNRs/DOX/siRNA and 665 nm light exhibits the strongest tumor inhibition rate when compare to the other 3 control groups. (**B**) Relative changes in tumor volume versus time of mice treated by PBS, free AuNRs, free AuNRs and 665 nm light or AuNRs/DOX/siRNA and 665 nm light, respectively. Relative tumor volume was defined as $(V-V_0)/ V_0$, where V and V_0 indicate the tumor volume on a particular day and day 0, respectively. Error bars represent SEMs for triplicate data. Mean tumor volumes were analyzed using one-way ANOVA. *, $P < 0.05$, **, $P < 0.01$ (n=5-7 tumors).



Supplementary Figure S5: K-Ras mRNA relative expression levels in pancreatic tumors were detected by RT-PCR. Values are means ± SEM, n = 3; **, $P < 0.01$ vs Control, AuNRs and siRNA.



Supplementary Figure S6: Representative histological images from the major tumors of the mice treated by AuNRs/DOX/siRNA with or without 665 nm light, comparing with the untreated control at day 25. The arrows indicate the AuNRs nanoplex.



Supplementary Figure S7: The body weights of nude mice were measured every other day treated by PBS, AuNRs, AuNRs with 665 nm light, DOX, AuNRs/DOX, AuNRs/siRNA, AuNRs/DOX/siRNA or AuNRs/DOX/siRNA with 665 nm light. Values are means ± SEM, n = 5.

Supplementary Table S1: Zeta potential and hydrodynamic size of different AuNRs-based nanoplex.

Name of nanoplex	Grafted polymers	Zeta potential	Hydrodynamic size
AuNRs		+33.33 mV	24.90±2.15 nm
AuNRs/PSS	PSS	-33.92 mV	29.50±1.83 nm
AuNRs/PSS/PAH	PSS & PAH	+35.50 mV	34.52±1.67 nm
AuNRs/PSS/DOX/PAH	PSS & PAH	+37.88 mV	59.20±1.78 nm
AuNRs/PSS/PAH/siRNA	PSS & PAH	+29.90 mV	44.73±3.57 nm
AuNRs/PSS/DOX/PAH/siRNA	PSS & PAH	+31.34 mV	67.59±1.85 nm

Supplementary Table S2: Transfection efficiency and average fluorescence intensity.

Name of reagent	Transfection efficiency (DOX and $siRNA^{FAM}$)	Average fluorescence intensity (DOX and $siRNA^{FAM}$)	SSC signal intensity (10^4)	FSC signal intensity (10^4)
Blank	0.35% and 0.69%	0.05×10^3 and 0.15×10^3	6.61	5.08
AuNRs	0.72% and 0.54%	0.15×10^3 and 0.08×10^3	7.34	6.02
$siRNA^{FAM}$	0.51% and 3.30%	0.13×10^3 and 0.12×10^3	6.69	5.12
DOX	94.6% and 2.57%	8.93×10^3 and 0.09×10^3	7.02	5.43
AuNRs/DOX/ $siRNA^{FAM}$	94.3% and 85.8%	8.31×10^3 and 3.13×10^3	7.65	6.09
Lipo- $siRNA^{FAM}$	3.52% and 87.2%	0.56×10^3 and 3.20×10^3	7.57	5.91