

## **Supplementary Information**

### **Disrupting Sympathetic Nerve-Tumor Crosstalk via Biomimetic Nanovesicles to Augment Chemotherapy Efficacy Under Chronic Stress**

Jingjie Liu<sup>1,2,3,4,#</sup>, Jiaqi Qin<sup>1,5,#</sup>, Wenxia Zheng<sup>1</sup>, Xiaojuan Wang<sup>1</sup>, Zhaoan Wei<sup>1</sup>, Zixiang Xie<sup>1</sup>, Tingwei Yan<sup>1</sup>, Xin Li<sup>1</sup>, Ziqiao Ding<sup>1</sup>, Haojie Liu<sup>1</sup>, Zhiheng Cai<sup>1</sup>, Shanmiao Gou<sup>6</sup>, Xiangliang Yang<sup>1,2,3,4,\*</sup>, Tuying Yong<sup>1,2,3,4,\*</sup>, Lu Gan<sup>1,2,3,4,\*</sup>

<sup>1</sup>Department of Nanomedicine and Biopharmaceuticals, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, P. R. China.

<sup>2</sup>National Engineering Research Center for Nanomedicine, Huazhong University of Science and Technology, Wuhan, P. R. China.

<sup>3</sup>Key Laboratory of Molecular Biophysics of Ministry of Education, Huazhong University of Science and Technology, Wuhan, P. R. China.

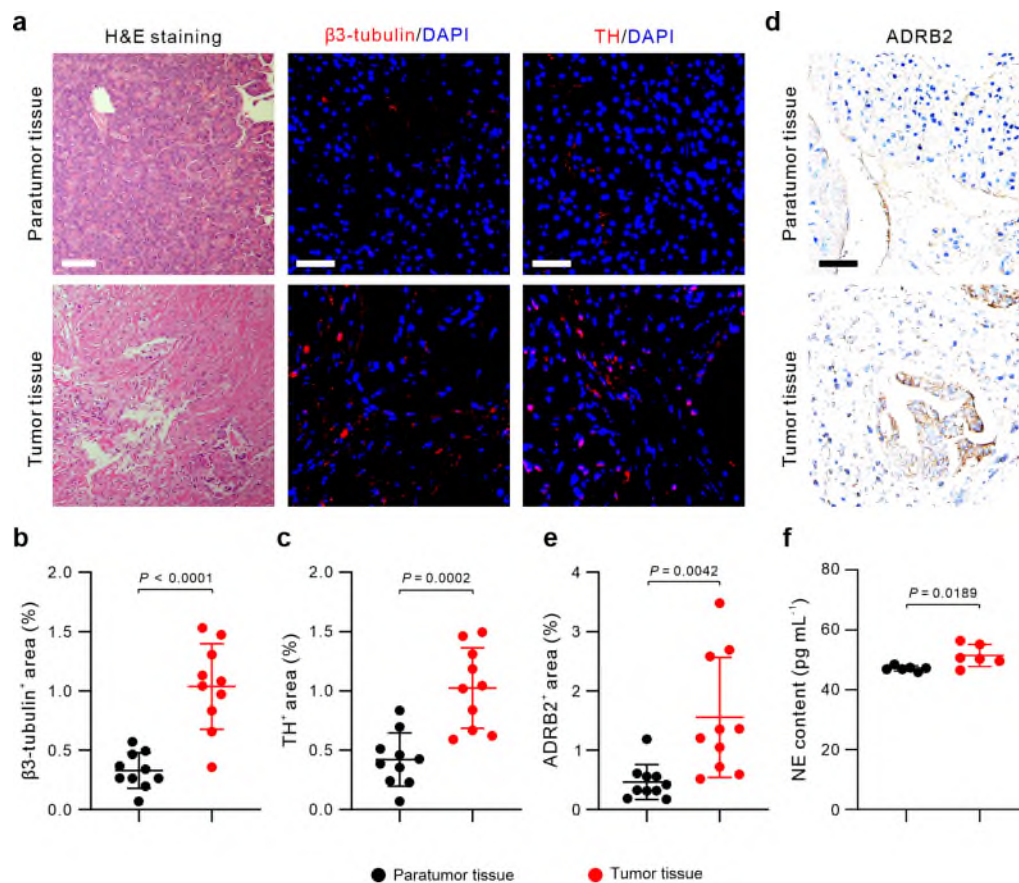
<sup>4</sup>Hubei Key Laboratory of Bioinorganic Chemistry and Materia Medical, Huazhong University of Science and Technology, Wuhan, P. R. China.

<sup>5</sup>School of Biomedical Engineering, Anhui Medical University, Hefei, P. R. China.

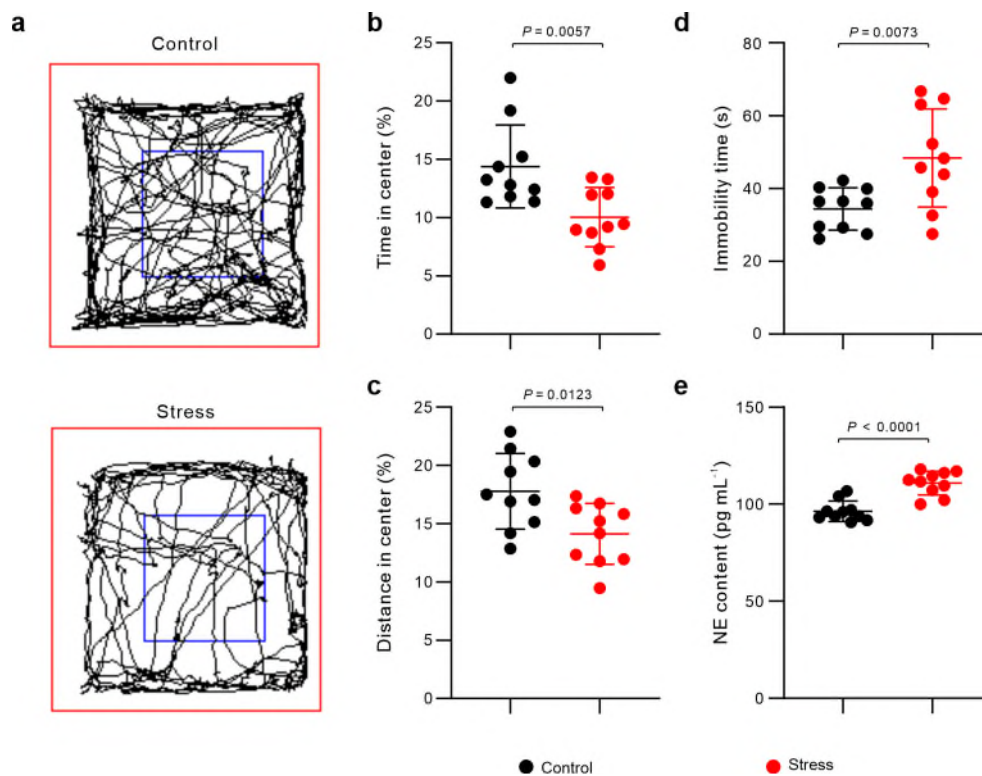
<sup>6</sup>Department of Pancreatic Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P. R. China.

<sup>#</sup>Jingjie Liu and Jiaqi Qin contributed equally to this work.

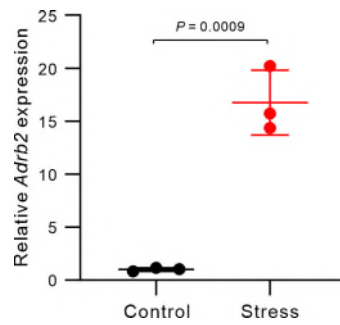
<sup>\*</sup>Correspondence should be addressed to: yangxl@mail.hust.edu.cn, yongty2018@hust.edu.cn, lugan@mail.hust.edu.cn.



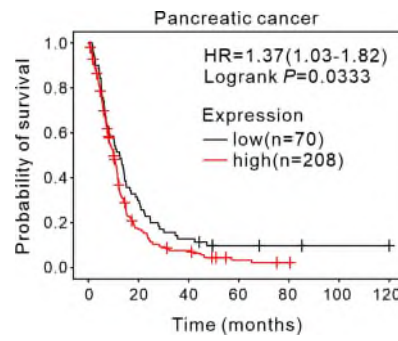
**Supplementary Fig. 1 Sympathetic nerve innervation within human pancreatic tumors.** **a** Representative immunofluorescence staining for  $\beta 3$ -tubulin and TH in human pancreatic tumor tissues and adjacent paratumor tissues. The cell nuclei were stained with DAPI (blue). **b, c** Quantification of  $\beta 3$ -tubulin<sup>+</sup> (**b**) and TH<sup>+</sup> (**c**) areas in human pancreatic tumor tissues and adjacent paratumor tissues. **d, e** Representative immunohistochemistry staining (**d**) and quantification (**e**) of ADRB2<sup>+</sup> areas in human pancreatic tumor tissues and adjacent paratumor tissues. **f** NE contents in human pancreatic tumor tissues and adjacent paratumor tissues. Scale bar: 50  $\mu$ m for **a** and **d**. Images in **a** and **d** are representative of three independent samples. Data are means  $\pm$  s.d. ( $n = 10$  fields in total from 3 biologically independent samples for **b, c** and **e**, and  $n = 6$  biologically independent samples for **f**). The  $P$  values in **b, c, e** and **f** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.



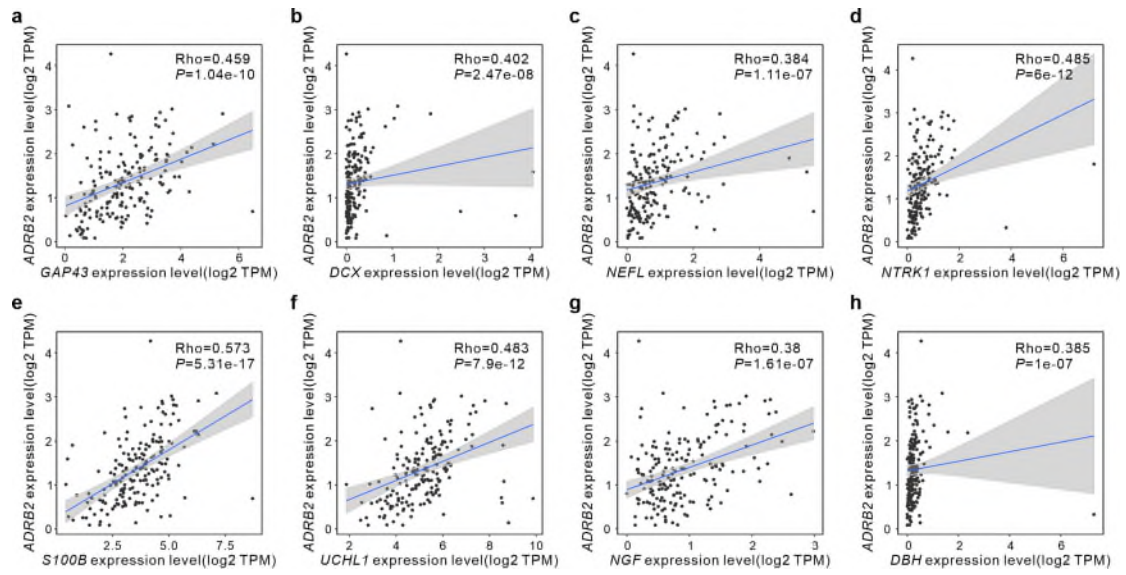
**Supplementary Fig. 2 Characterization of the CRS model in mice bearing orthotopic Panc02 tumors.** **a** Representative trajectory plots of mice in the open field test on day 17 day of CRS. Images are representative of ten biologically independent mice. **b, c** Center area activity time (**b**) and distance (**c**) of mice in the open field test on day 17 of CRS. **d** Immobility time of mice in the forced swim test on day 17 of CRS. **e** Serum NE concentration in mice on day 17 of CRS. Data are means  $\pm$  s.d. ( $n = 10$  mice per group for **b-e**). The  $P$  values in **b-e** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.



**Supplementary Fig. 3 Significant upregulation of *Adrb2* expression by chronic stress in orthotopic Panc02 tumors.** The mRNA levels of *Adrb2* in tumor tissues of orthotopic Panc02 tumor-bearing mice at 17 days under CRS or normal conditions by real time RT-PCR. Data are means  $\pm$  s.d. ( $n = 3$  mice per group). The  $P$  value was determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.



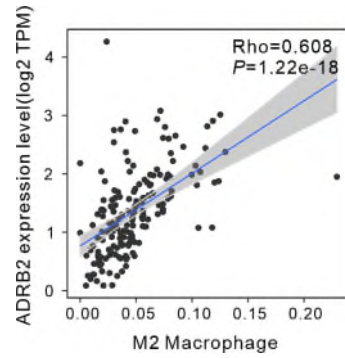
**Supplementary Fig. 4 Correlation of *ADRB2* with poor prognosis in pancreatic cancer.** The association between *ADRB2* expression and disease-free survival in pancreatic cancer patients analyzed by Kaplan-Meier plotter (<http://kmplot.com>). The *P* value (log-rank, Mantel Cox test), the hazard ratio (HR) and number of patients were indicated.



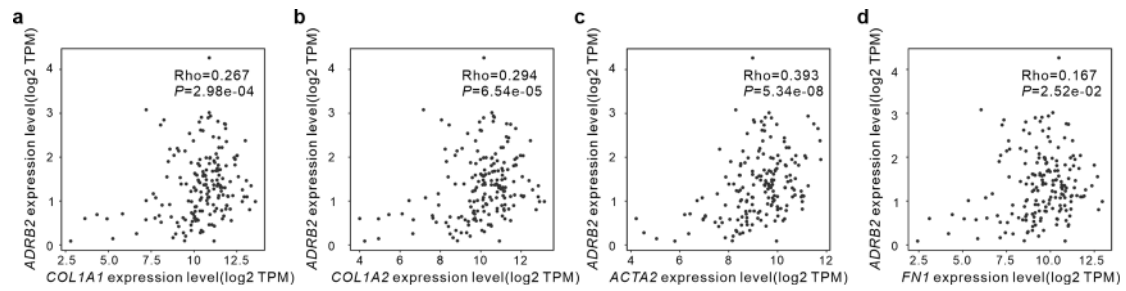
**Supplementary Fig. 5 Correlation of *ADRB2* with nerves in pancreatic tumors. a-**

**h** The association between *ADRB2* expression and the expression of nerve markers *GAP43* (a), *DCX* (b), *NEFL* (c), *NTRK1* (d), *S100B* (e), *UCHL1* (f), *NGF* (g) and *DBH* (h) in tumor tissues of pancreatic cancer patients analyzed by TIMER2.0 ( $n = 179$ ).

TPM, transcripts per million. The best-fitting line with 95% confidence interval (highlighted in grey) is represented. The Spearman's rank correlation coefficients and  $P$  values (two-tailed) were shown in the respective figures.

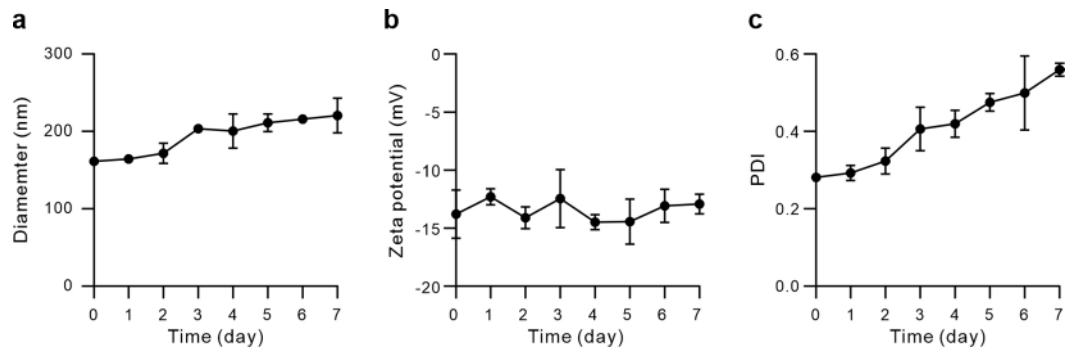


**Supplementary Fig. 6 Correlation of *ADRB2* expression with M2-like macrophage infiltration in pancreatic tumors.** The association between *ADRB2* expression and the infiltration levels of M2-like macrophages in tumor tissues of pancreatic cancer patients analyzed by TIMER2.0 ( $n = 179$ ). The best-fitting line with 95% confidence interval (highlighted in grey) is represented. The Spearman's rank correlation coefficients and  $P$  values (two-tailed) were shown in the figures.

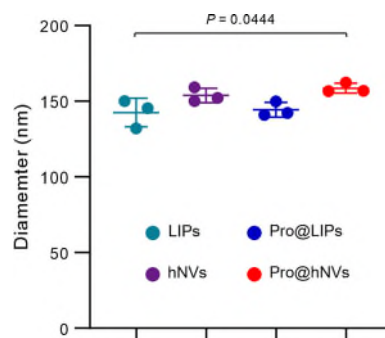


**Supplementary Fig. 7 Correlation of *ADRB2* with stromal formation in pancreatic**

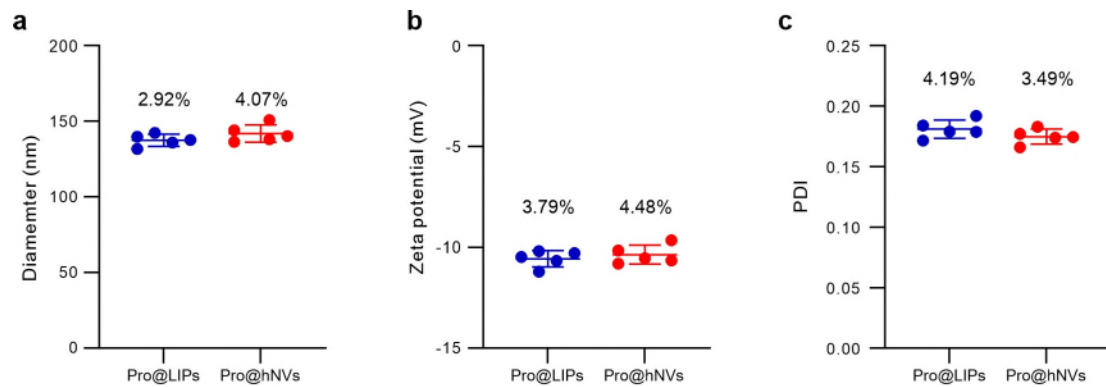
**tumors. a-d** The association between *ADRB2* expression and the expression of stromal markers *COL1A1* (a), *COL1A2* (b), *ACTA2* (c) and *FN1* (d) in tumor tissues of pancreatic cancer patients analyzed by TIMER2.0 ( $n = 179$ ). The Spearman's rank correlation coefficients and  $P$  values (two-tailed) were shown in the respective figures.



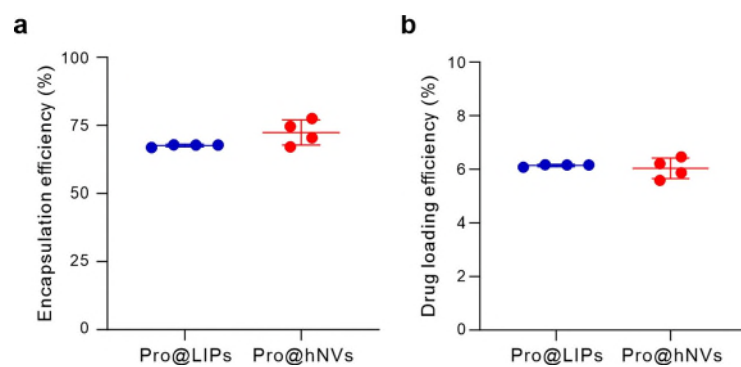
**Supplementary Fig. 8 Characterization of M1-NVs.** **a-c** Hydrodynamic diameter (**a**), zeta potential (**b**) and PDI (**c**) of M1-NVs in PBS over time by DLS analysis. Data are means  $\pm$  s.d. ( $n = 3$  independent samples for **a-c**). Source data are provided as a Source Data file.



**Supplementary Fig. 9** Hydrodynamic diameter of LIPs, hNVs, Pro@LIPs and Pro@hNVs by DLS analysis. Data are means  $\pm$  s.d. ( $n = 3$  independent samples). The  $P$  value was determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.

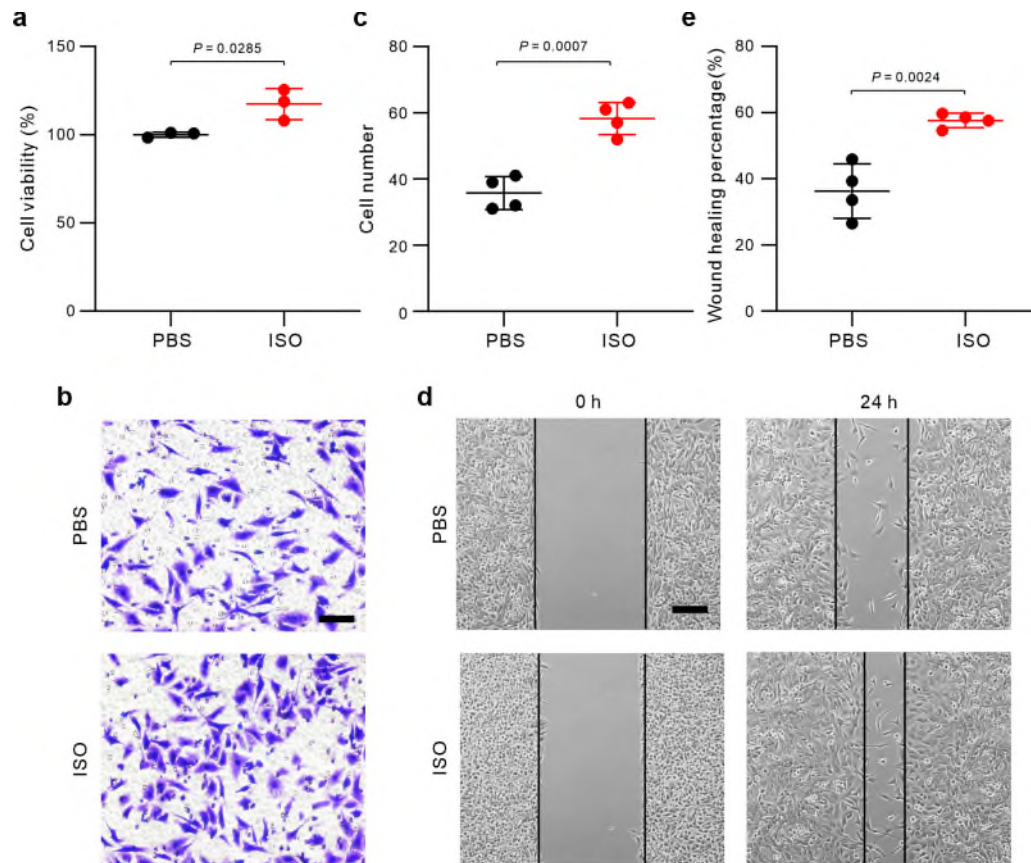


**Supplementary Fig. 10 Batch-to-batch consistency of Pro@hNVs. a-c** Hydrodynamic diameter (a), zeta potential (b), and PDI (c) of Pro@LIPs and Pro@hNVs from five batches by DLS analysis. Data are presented as mean  $\pm$  s.d. ( $n = 5$  independent batches for a-c). The CV for each parameter was shown in the respective figures. Source data are provided as a Source Data file.



**Supplementary Fig. 11 Drug loading property of Pro@hNVs. a, b** Drug encapsulation efficiency (**a**) and loading efficiency (**b**) of Pro@LIPs and Pro@hNVs by HPLC analysis. Data are means  $\pm$  s.d. ( $n = 4$  independent samples for **a** and **b**).

Source data are provided as a Source Data file.

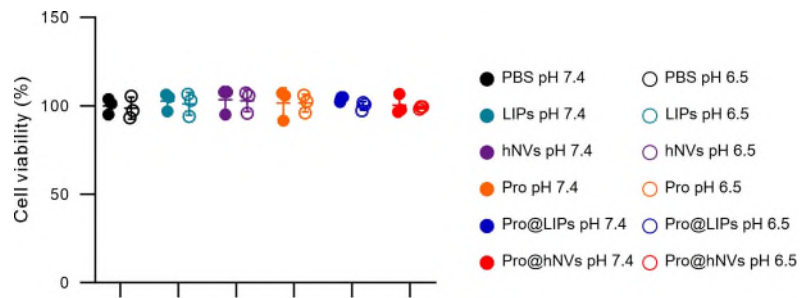


**Supplementary Fig. 12 ISO-induced promotion of cancer cell proliferation and**

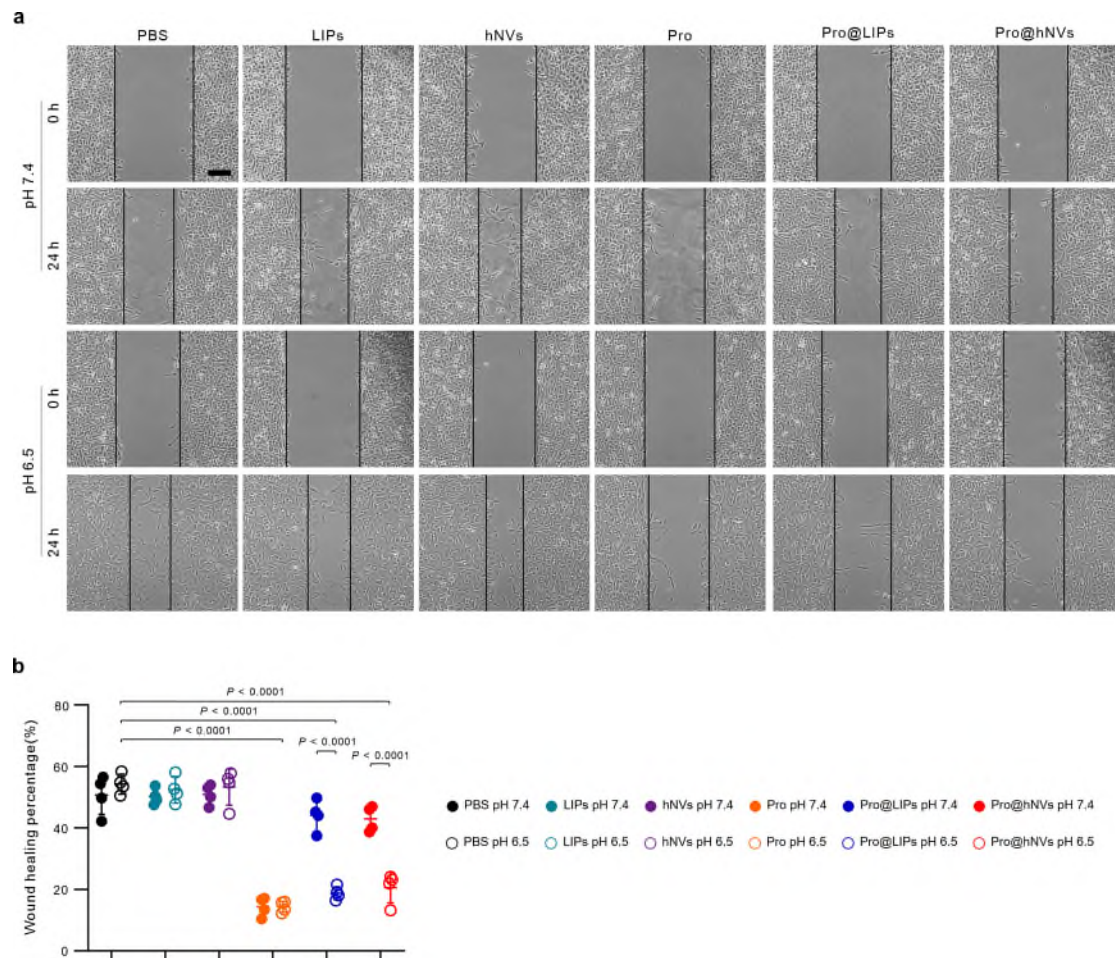
**migration in vitro. a** Viability of Panc02 cells treated with PBS or 5  $\mu$ M ISO for 24 h.

**b, c** Representative images (**b**) and quantification (**c**) of migratory Panc02 cells stained with crystal violet at 12 h after pretreatment as indicated in **a** by transwell assay. **d, e** Representative images (**d**) and quantification (**e**) of migratory Panc02 cells at 24 h after pretreatment as indicated in **a** by wound healing assay. Scale bar: 100  $\mu$ m for **b** and **d**.

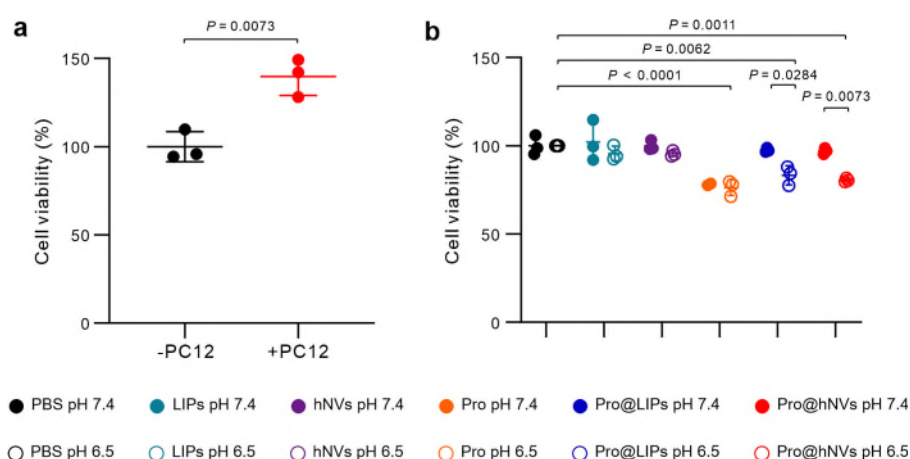
Images in **b** and **d** are representative of three independent experiments. Data are means  $\pm$  s.d. ( $n = 3$  biologically independent samples for **a**, and  $n = 4$  biologically independent samples for **c** and **e**). The  $P$  values in **a**, **c** and **e** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.



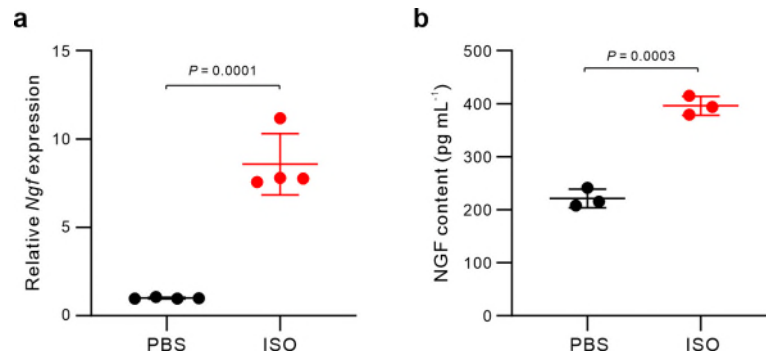
**Supplementary Fig. 13 Lack of direct inhibitory effects of Pro@hNVs on cancer cell proliferation.** Viability of Panc02 cells after treatment with PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at Pro concentration of 5  $\mu$ M for 24 h at pH 7.4 or 6.5. Data are means  $\pm$  s.d. ( $n = 3$  biologically independent samples). Source data are provided as a Source Data file.



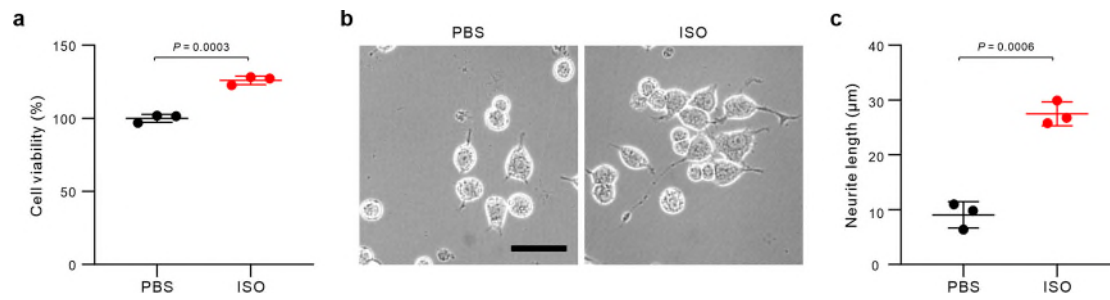
**Supplementary Fig. 14 Effective inhibition of cancer cell migration by Pro@hNVs under chronic stress in vitro. a, b** Representative images (**a**) and the quantification (**b**) of migratory Panc02 cells at 24 h after pretreatment with PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at Pro concentration of 5  $\mu$ M in the presence of 5  $\mu$ M ISO at pH 7.4 or 6.5 for 24 h. Scale bar: 100  $\mu$ m for **a**. Images are representative of three independent experiments for **a**. Data are means  $\pm$  s.d. ( $n = 4$  biologically independent samples for **b**). The  $P$  values in **b** were determined using two-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



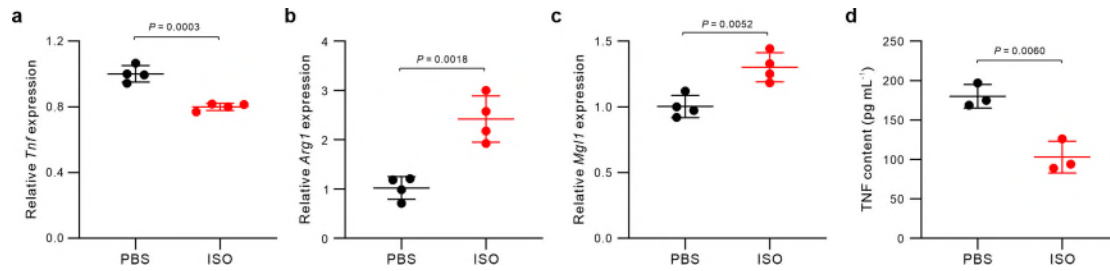
**Supplementary Fig. 15 Inhibition of PC12 cell supernatant-stimulated cancer cell proliferation by Pro@hNVs. a** Viability of Panc02 cells after incubation with basic culture medium or the supernatants of PC12 cells for 24 h. **b** Viability of PC12 cell supernatant-stimulated Panc02 cells after treatment with PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at Pro concentration of 5  $\mu$ M for 24 h at pH 7.4 or 6.5. Data are means  $\pm$  s.d. ( $n = 3$  biologically independent samples for **a** and **b**). The  $P$  values were determined using unpaired two-tailed Student's  $t$ -test for **a**, and two-way ANOVA followed by Tukey's multiple comparisons test for **b**. Source data are provided as a Source Data file.



**Supplementary Fig. 16 ISO-induced upregulation of NGF expression and secretion in tumor cells in vitro. a** *Ngf* mRNA levels in Panc02 cells after treatment with PBS or 5  $\mu$ M ISO for 12 h by real time RT-PCR. **b** NGF contents in Panc02 cells after treatment with PBS or 5  $\mu$ M ISO for 24 h by ELISA kit. Data are means  $\pm$  s.d. ( $n$  = 4 biologically independent samples for **a**, and  $n$  = 3 biologically independent samples for **b**). The  $P$  values in **a** and **b** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.

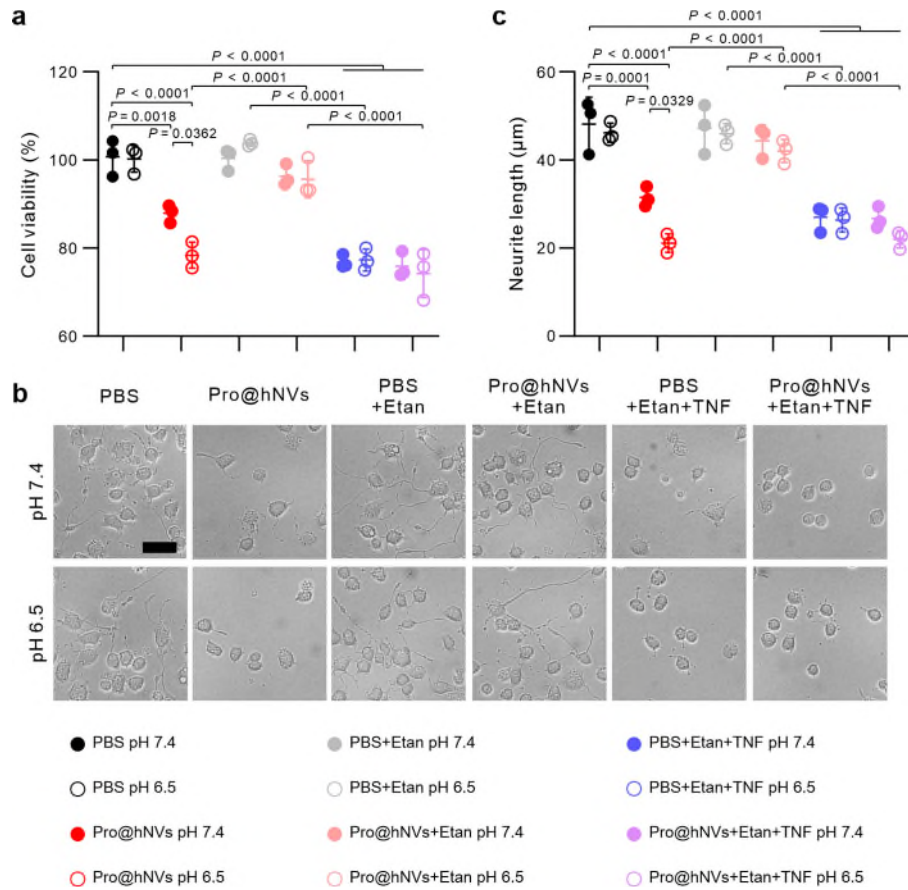


**Supplementary Fig. 17 Enhanced proliferation and neurite outgrowth of PC12 cells by the supernatants from ISO-treated Panc02 cells.** **a** Viability of PC12 cells at 48 h after treatment with the supernatants from Panc02 cells pretreated with PBS or 5 μM ISO for 24 h. **b, c** Representative neurite outgrowth images (**b**) and average neurite length (**c**) in PC12 cells at 72 h after treatment with the supernatants from Panc02 cells pretreated as indicated in **a** for 24 h. Scale bar: 25 μm for **b**. Images are representative of three independent experiments for **b**. Data are means ± s.d. ( $n = 3$  biologically independent samples for **a** and **c**). The  $P$  values in **a** and **c** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.

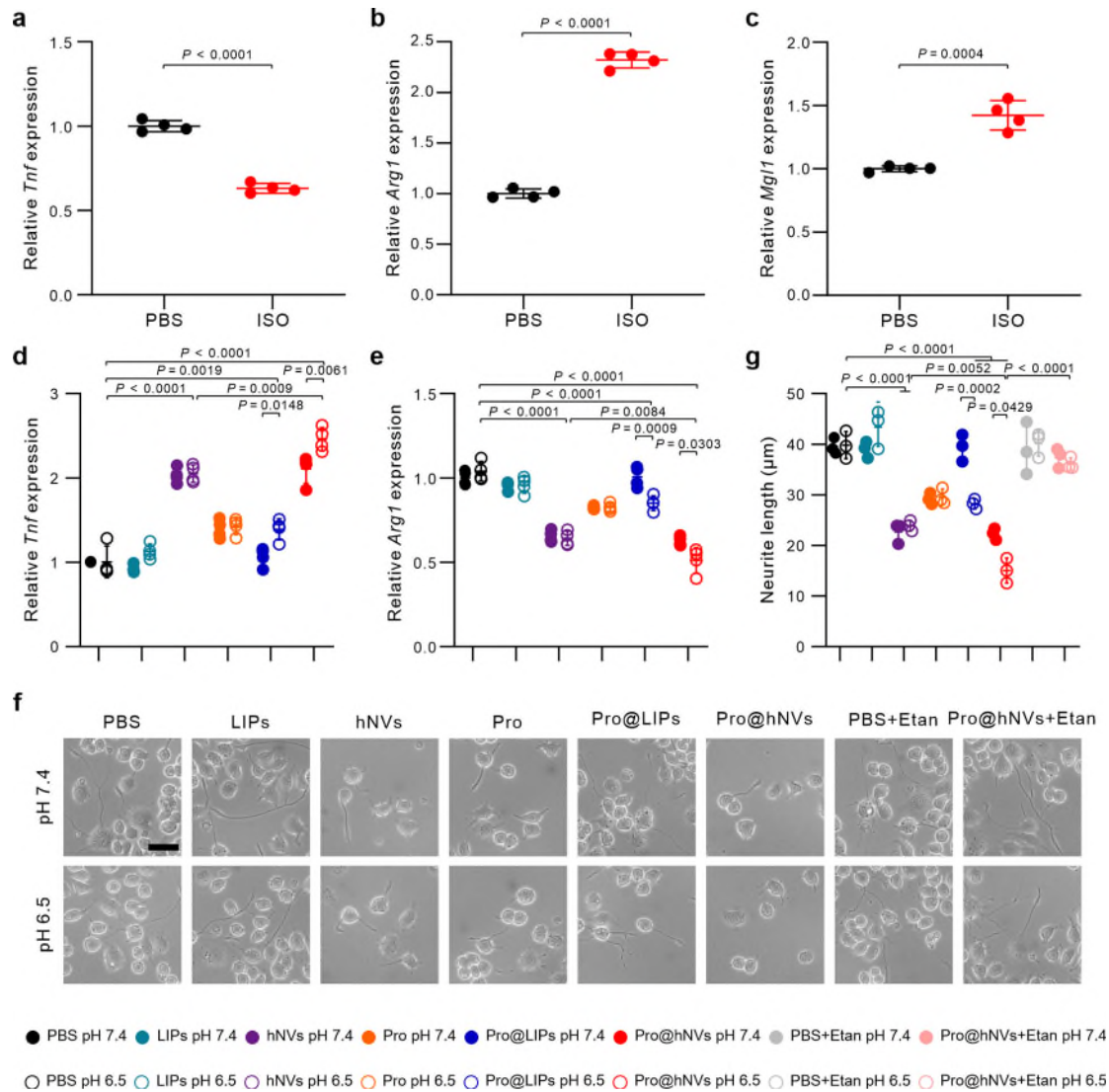


**Supplementary Fig. 18 ISO-induced macrophage polarization into M2 phenotype.**

**a-c** mRNA expression of *Tnf* (**a**), *Arg1* (**b**) and *Mgl1* (**c**) in RAW264.7 cells after treatment with PBS or 5  $\mu$ M ISO for 12 h. **d** TNF contents in RAW264.7 cells after treatment indicated in **a** for 24 h. Data are means  $\pm$  s.d. ( $n = 4$  biologically independent samples for **a-c**, and  $n = 3$  biologically independent samples for **d**). The  $P$  values in **a-d** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.

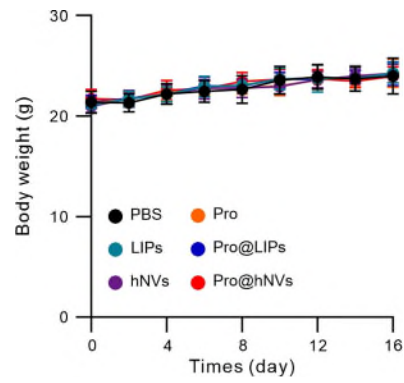


**Supplementary Fig. 19 TNF-mediated neurotoxicity by Pro@hNVs-repolarized macrophages.** **a** Viability of PC12 cells at 48 h after treatment with conditioned media from ISO-stimulated RAW264.7 cells pretreated with PBS or Pro@hNVs at Pro concentration of 5  $\mu\text{M}$  for 24 h at pH 7.4 or 6.5 in the presence or absence of 0.5  $\mu\text{g mL}^{-1}$  Etan and/or 1  $\text{ng mL}^{-1}$  TNF. **b, c** Representative neurite outgrowth images (**b**) and average neurite length (**c**) of PC12 cells at 72 h after treatment with conditioned media from ISO-stimulated RAW264.7 cells pretreated as indicated in **a** for 24 h. Scale bar: 25  $\mu\text{m}$  for **b**. Images are representative of three independent experiments for **b**. Data are means  $\pm$  s.d. ( $n = 3$  biologically independent samples for **a** and **c**). The  $P$  values in **a** and **c** were determined using two-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.

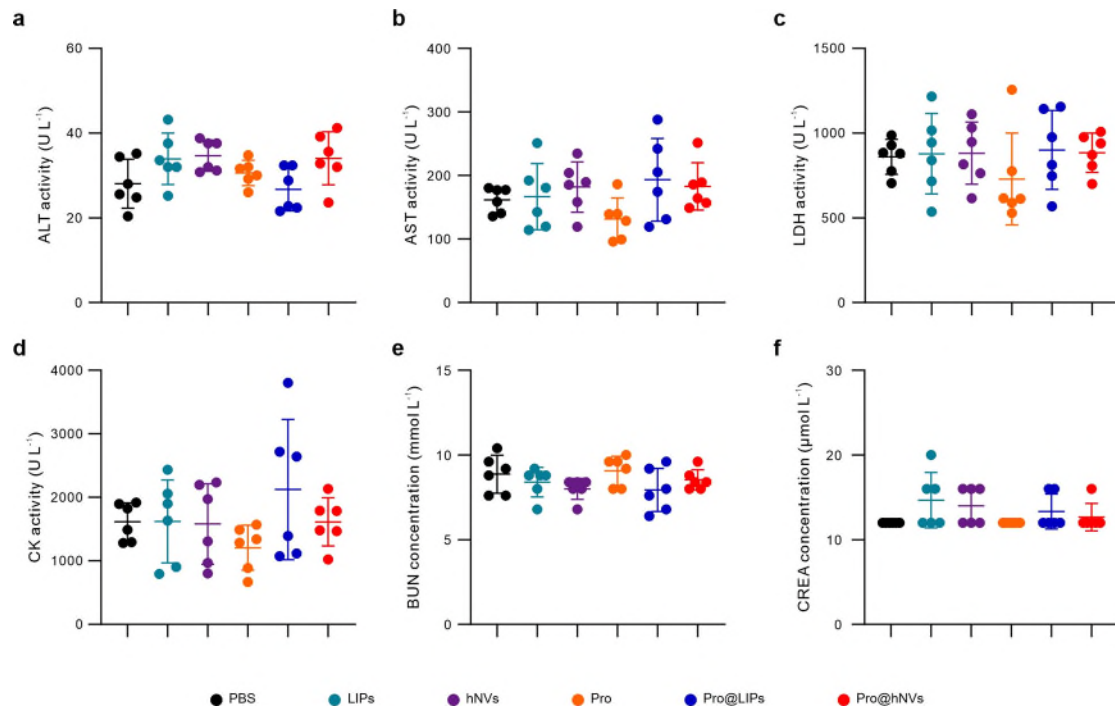


**Supplementary Fig. 20 Efficient inhibition of sympathetic nerve-macrophage crosstalk by Pro@hNVs under chronic stress in vitro.** **a-c** mRNA expression of *Tnf* (**a**), *Arg1* (**b**) and *Mgl1* (**c**) in BMDMs after treatment with PBS or 5  $\mu\text{M}$  ISO for 12 h. **d, e** mRNA levels of *Tnf* (**d**) and *Arg1* (**e**) in BMDMs treated with PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at Pro concentration of 5  $\mu\text{M}$  for 12 h in the presence of 5  $\mu\text{M}$  ISO at pH 7.4 or 6.5. **f, g** Representative neurite outgrowth images (**f**) and average neurite length (**g**) of PC12 cells at 72 h after treatment with the supernatants from BMDMs pretreated as indicated in **d** for 24 h in the presence or absence of 0.5  $\mu\text{g mL}^{-1}$

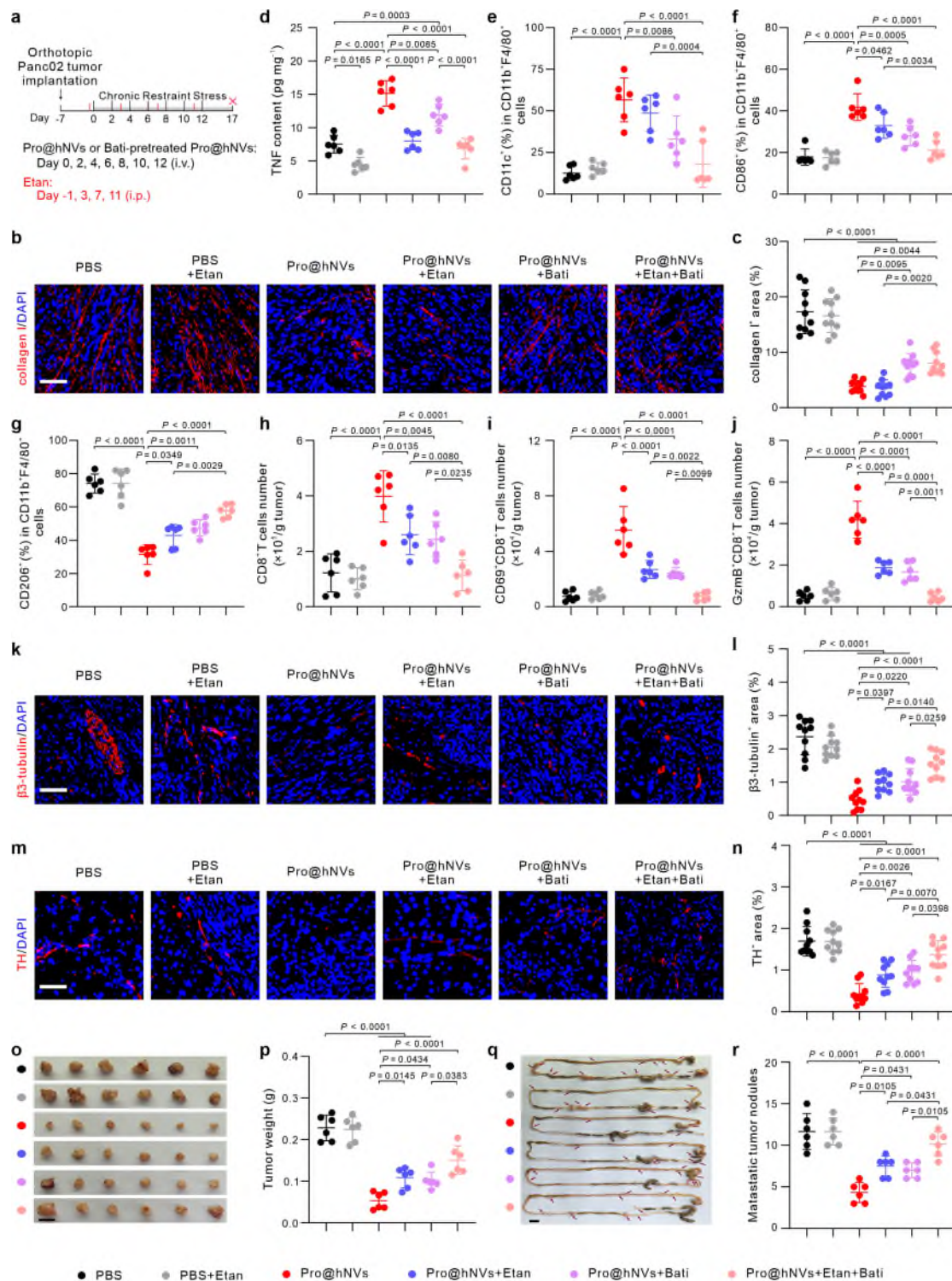
<sup>1</sup> Etan. Scale bar: 25  $\mu\text{m}$  for **f**. Images are representative of three independent experiments for **f**. Data are means  $\pm$  s.d. ( $n = 4$  biologically independent samples for **a-e**, and  $n = 3$  biologically independent samples for **g**). The *P* values were determined using unpaired two-tailed Student's *t*-test for **a-c**, and two-way ANOVA followed by Tukey's multiple comparisons for **d**, **e** and **g**. Source data are provided as a Source Data file.



**Supplementary Fig. 21** Body weight of orthotopic Panc02 tumor-bearing mice after intravenous injection of PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> after CRS exposure as indicated in Fig. 6a. Data are means  $\pm$  s.d. ( $n = 6$  mice per group). Source data are provided as a Source Data file.

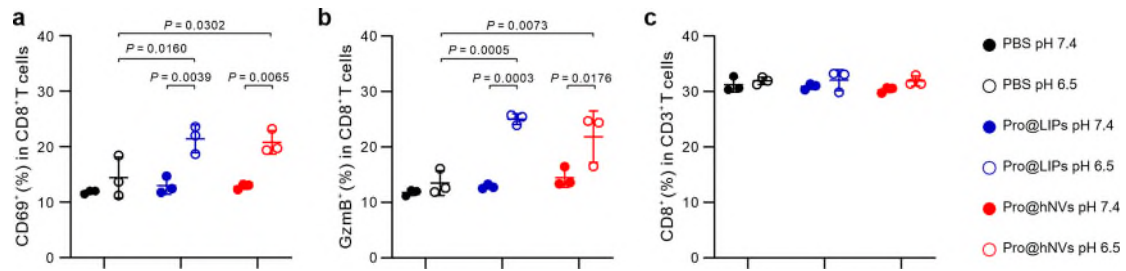


**Supplementary Fig. 22 Blood biochemical analysis of Pro@hNVs in orthotopic Panc02 tumor-bearing mice under CRS exposure.** **a-f** Contents of alanine aminotransferase (ALT, **a**), aspartate aminotransferase (AST, **b**), lactate dehydrogenase (LDH, **c**), creatine kinase (CK, **d**), blood urea nitrogen (BUN, **e**) and creatinine (CREA, **f**) in serum of orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS, LIPs, hNVs, Pro, Pro@LIPs or Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> after CRS exposure as indicated in Fig. 6a. Data are means ± s.d. (*n* = 6 mice per group for **a-f**). Source data are provided as a Source Data file.

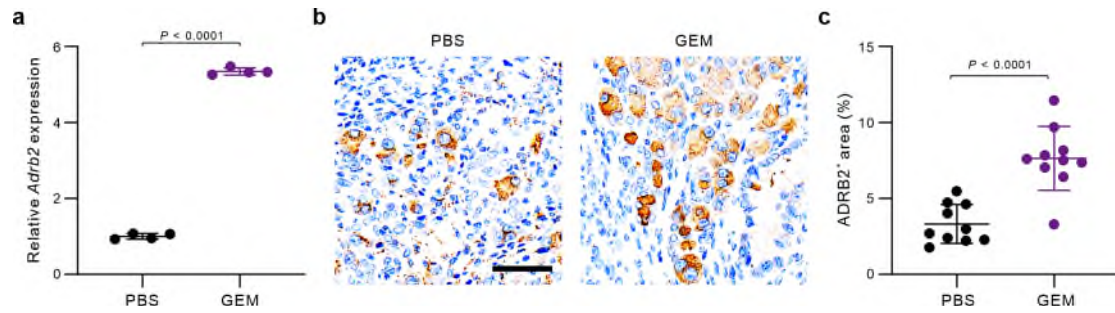


**Supplementary Fig. 23 Involvement of Pro@hNVs-mediated ECM degradation and TNF secretion in anticancer activity in orthotopic Panc02 tumor-bearing mice upon CRS exposure.** **a** Schematic illustration of the experimental design for anticancer efficacy in mice bearing orthotopic Panc02 tumors following intravenous injection of

PBS or Pro@hNVs pretreated with or without  $0.6 \text{ mg mL}^{-1}$  Bati or/and intraperitoneal injection of  $5 \text{ mg kg}^{-1}$  Etan at the Pro dosage of  $7 \text{ mg kg}^{-1}$  after CRS exposure. **b, c, k-n** Representative immunofluorescence staining and quantification of collagen I (**b, c**),  $\beta 3$ -tubulin (**k, l**) and TH (**m, n**) in tumors at 17 days post-treatment. The cell nuclei were stained with DAPI (blue). **d** TNF contents in tumors at 17 days post-treatment. **e-g** Percentages of  $\text{CD11c}^+$  TAMs (**e**),  $\text{CD86}^+$  TAMs (**f**) and  $\text{CD206}^+$  TAMs (**g**) in tumors at 17 days post-treatment. **h-j** Numbers of  $\text{CD8}^+$  T (**h**),  $\text{CD69}^+\text{CD8}^+$  T (**i**), and  $\text{GzmB}^+\text{CD8}^+$  T (**j**) cells in tumors at 17 days post-treatment. **o, p** Tumor images (**o**) and tumor weight (**p**) of mice at 17 days post-treatment. **q, r** Representative images of intestines (**q**) and number of metastatic tumor nodules in the intestines (**r**) at 17 days post-treatment. Scale bar:  $50 \mu\text{m}$  for **b, k** and **m** and  $1 \text{ cm}$  for **o** and **q**. Images in **b, k** and **m** are representative of three biologically independent mice. Data are means  $\pm$  s.d. ( $n = 10$  fields in total from 3 mice for **c, l** and **n**, and  $n = 6$  mice per group for **d-j, p** and **r**). The  $P$  values in **c-j, l, n, p** and **r** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



**Supplementary Fig. 24 Inhibition of ISO-suppressed T cell activation by Pro@hNVs.** **a-c** Flow cytometric analysis of CD69<sup>+</sup>CD8<sup>+</sup> (**a**), GzmB<sup>+</sup>CD8<sup>+</sup> (**b**) and CD8<sup>+</sup> (**c**) ratios in CD3<sup>+</sup> T cells after treatment with PBS, Pro@LIPs or Pro@hNVs at Pro concentration of 5  $\mu$ M for 4 days in the presence of plate-bound anti-CD3 $\epsilon$  antibodies (5  $\mu$ g mL<sup>-1</sup>), soluble anti-CD28 antibodies (2  $\mu$ g mL<sup>-1</sup>) and ISO (5  $\mu$ M) at pH 7.4 or 6.5. Data are means  $\pm$  s.d. ( $n$  = 3 biologically independent samples for **a-c**). The  $P$  values in **a** and **b** were determined using two-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



**Supplementary Fig. 25 Effective upregulation of ADRB2 expression in pancreatic**

**cancer by GEM. a** *Adrb2* mRNA levels in Panc02 cells after treatment with PBS or

0.03  $\mu\text{g mL}^{-1}$  GEM for 12 h by real time RT-PCR. **b, c** Representative

immunohistochemistry images (**b**) and quantification (**c**) of ADRB2<sup>+</sup> area in tumors of

orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS or

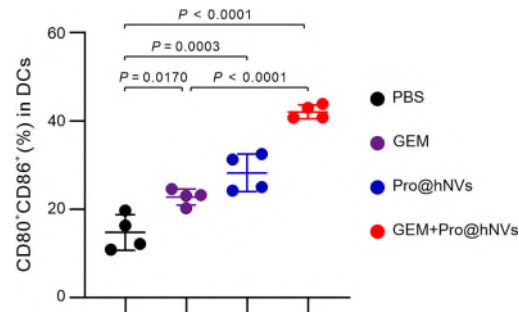
GEM at the dosage of 20 mg kg<sup>-1</sup> under CRS exposure. Scale bar: 50  $\mu\text{m}$  for **b**. Images

in **b** are representative of three biologically independent mice. Data are means  $\pm$  s.d. ( $n$

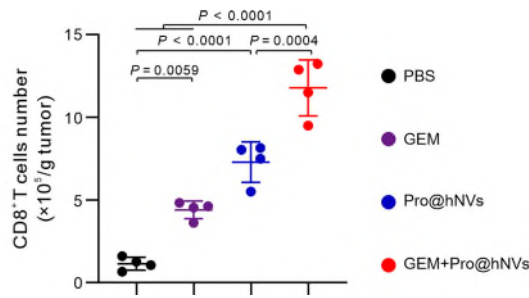
= 4 biologically independent samples for **a**, and  $n = 10$  fields in total from 3 mice for

**c**). The  $P$  values in **a** and **c** were determined using unpaired two-tailed Student's  $t$ -test.

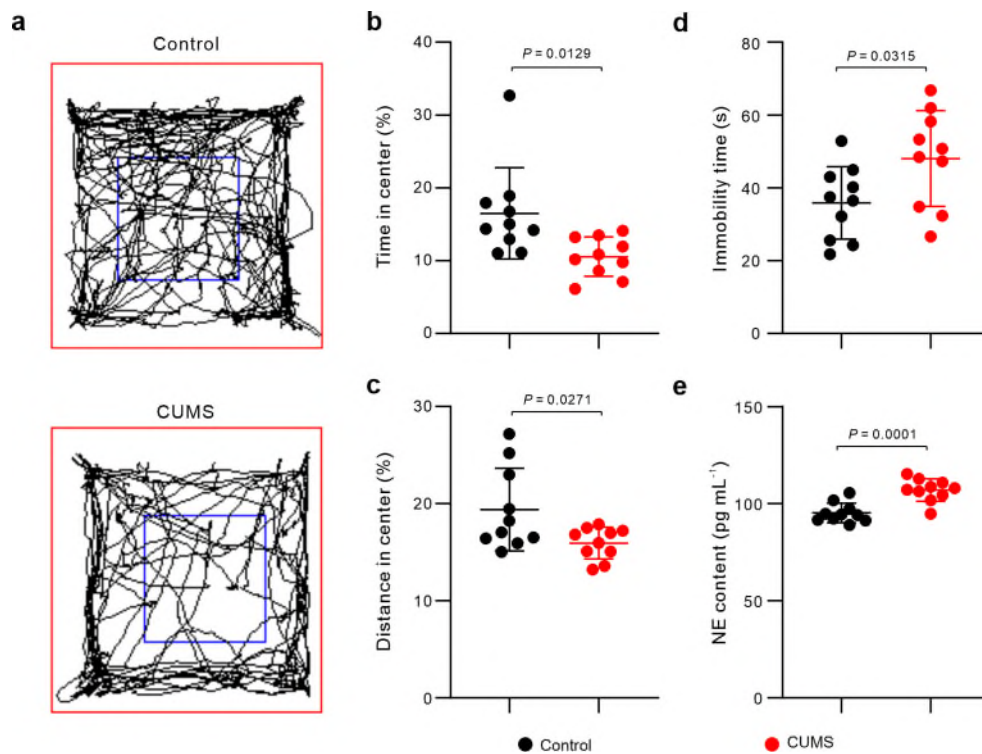
Source data are provided as a Source Data file.



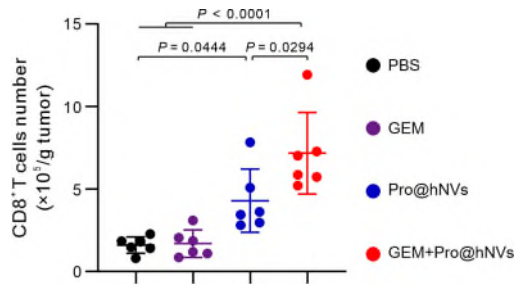
**Supplementary Fig. 26 Promoted DC maturation by GEM+Pro@hNVs in orthotopic Panc02 tumor-bearing mice under CRS exposure.** The percentages of CD80<sup>+</sup>CD86<sup>+</sup> DCs in orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> after CRS exposure as indicated in Fig. 7a. Data are means  $\pm$  s.d. ( $n = 4$  mice per group). The  $P$  values were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



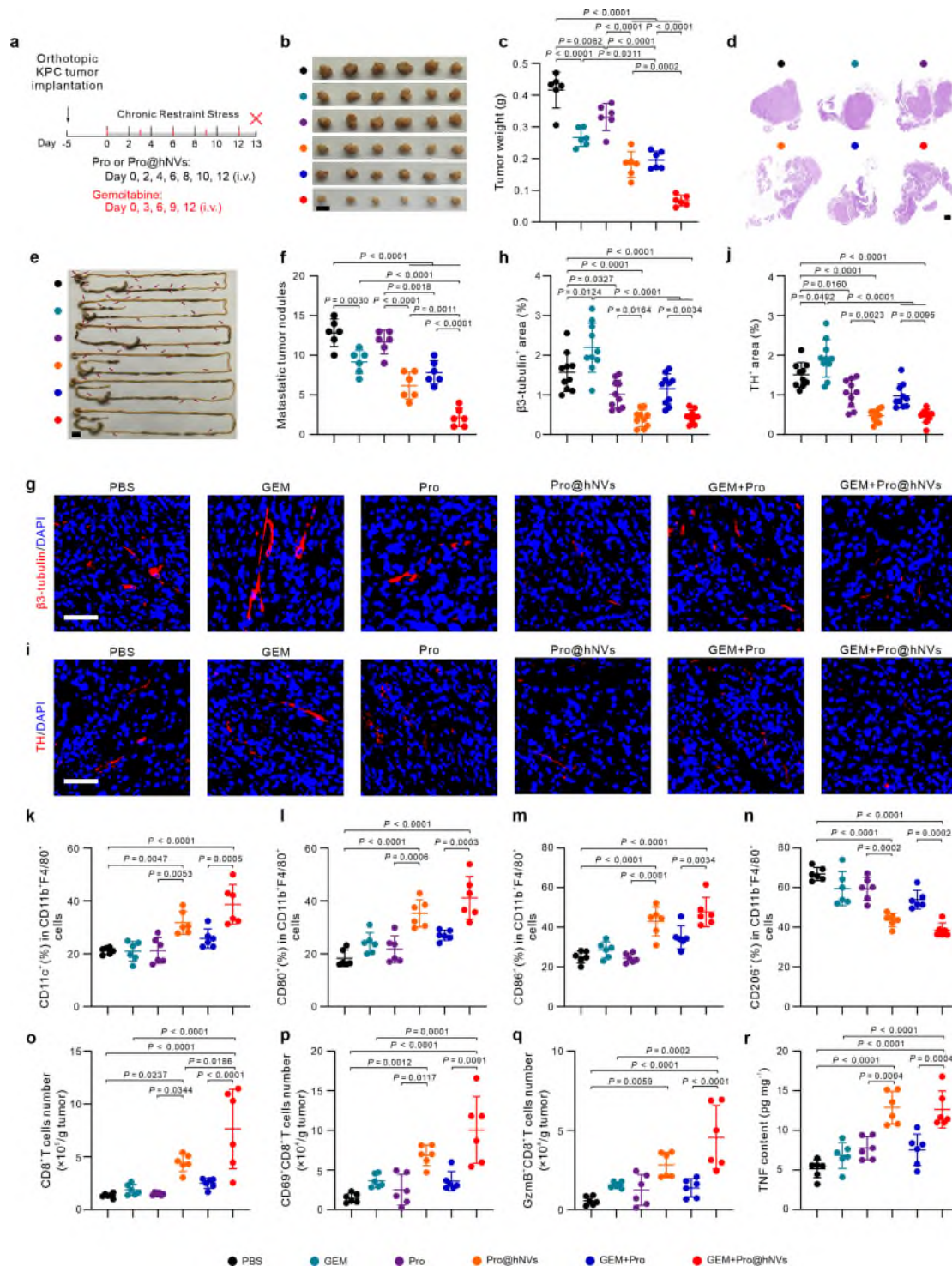
**Supplementary Fig. 27 Increased number of CD8<sup>+</sup> T cells by GEM+Pro@hNVs in orthotopic Panc02 tumor-bearing mice under CRS exposure.** The number of CD8<sup>+</sup> T cells in orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> after CRS exposure as indicated in Fig. 7a. Data are means  $\pm$  s.d. ( $n = 4$  mice per group). The  $P$  values were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



**Supplementary Fig. 28 Characterization of the CUMS model in mice bearing orthotopic Panc02 tumors.** **a** Representative trajectory plots of mice in the open field test on day 17 day of CUMS. Images are representative of ten biologically independent mice. **b, c** Center area activity time (**b**) and distance (**c**) of mice in the open field test on day 17 of CUMS. **d** Immobility time of mice in the forced swim test on day 17 of CUMS. **e** Serum NE concentration in mice on day 17 of CUMS. Data are means  $\pm$  s.d. ( $n = 10$  mice per group for **b-e**). The  $P$  values in **b-e** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.

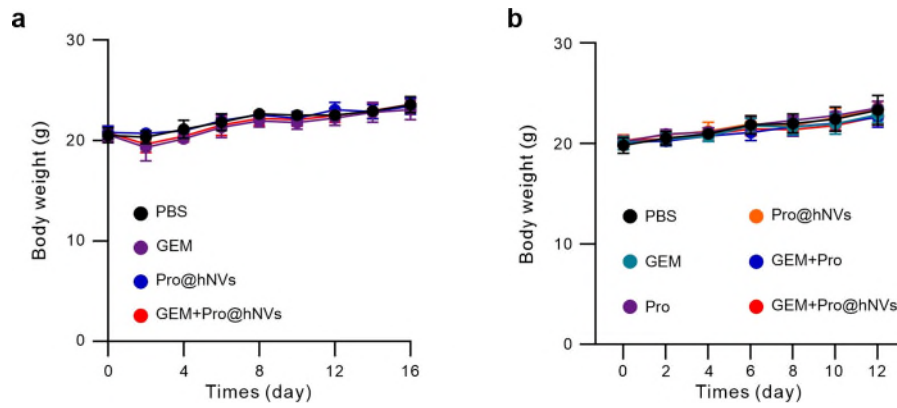


**Supplementary Fig. 29 Increased number of CD8<sup>+</sup> T cells by GEM+Pro@hNVs in orthotopic Panc02 tumor-bearing mice under CUMS exposure.** The number of CD8<sup>+</sup> T cells in orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS, GEM, Pro@hNVs or combination of GEM and Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> upon CUMS exposure as indicated in Fig. 8a. Data are means ± s.d. (*n* = 6 mice per group). The *P* values were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.

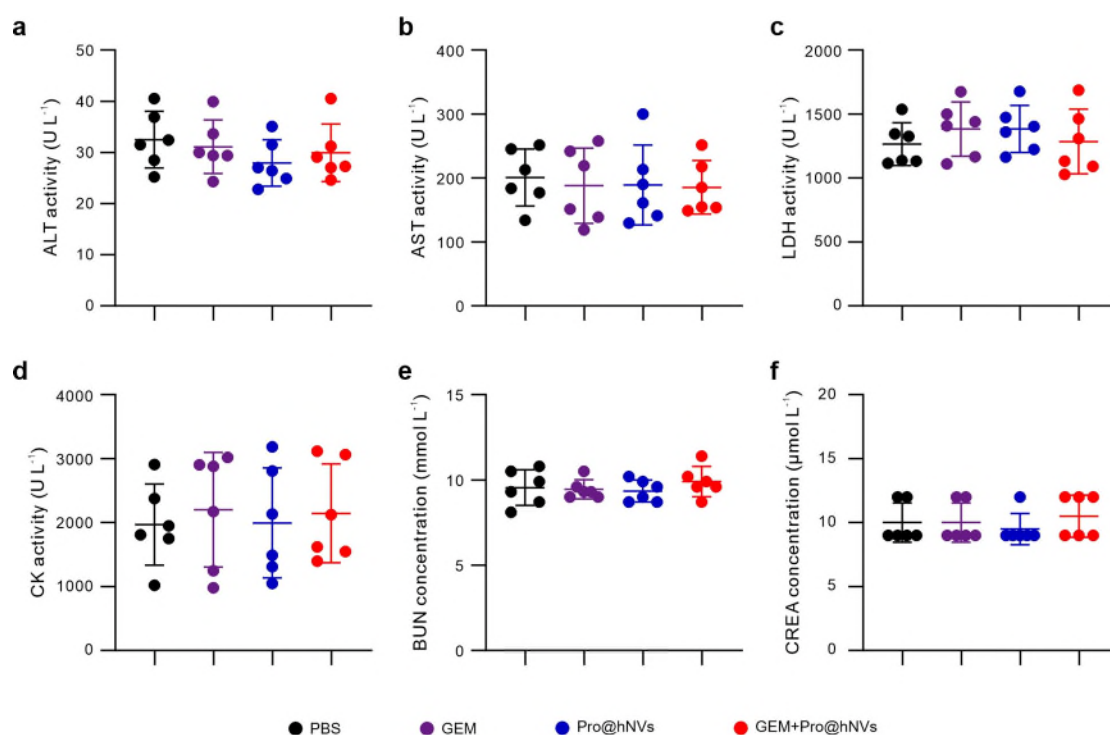


**Supplementary Fig. 30 Efficient tumor inhibition and improved tumor microenvironment in mice bearing orthotopic KPC tumors by GEM+Pro@hNVs after CRS exposure.** **a** Schematic illustration of the experimental design for the anticancer study in mice bearing orthotopic KPC tumors following intravenous

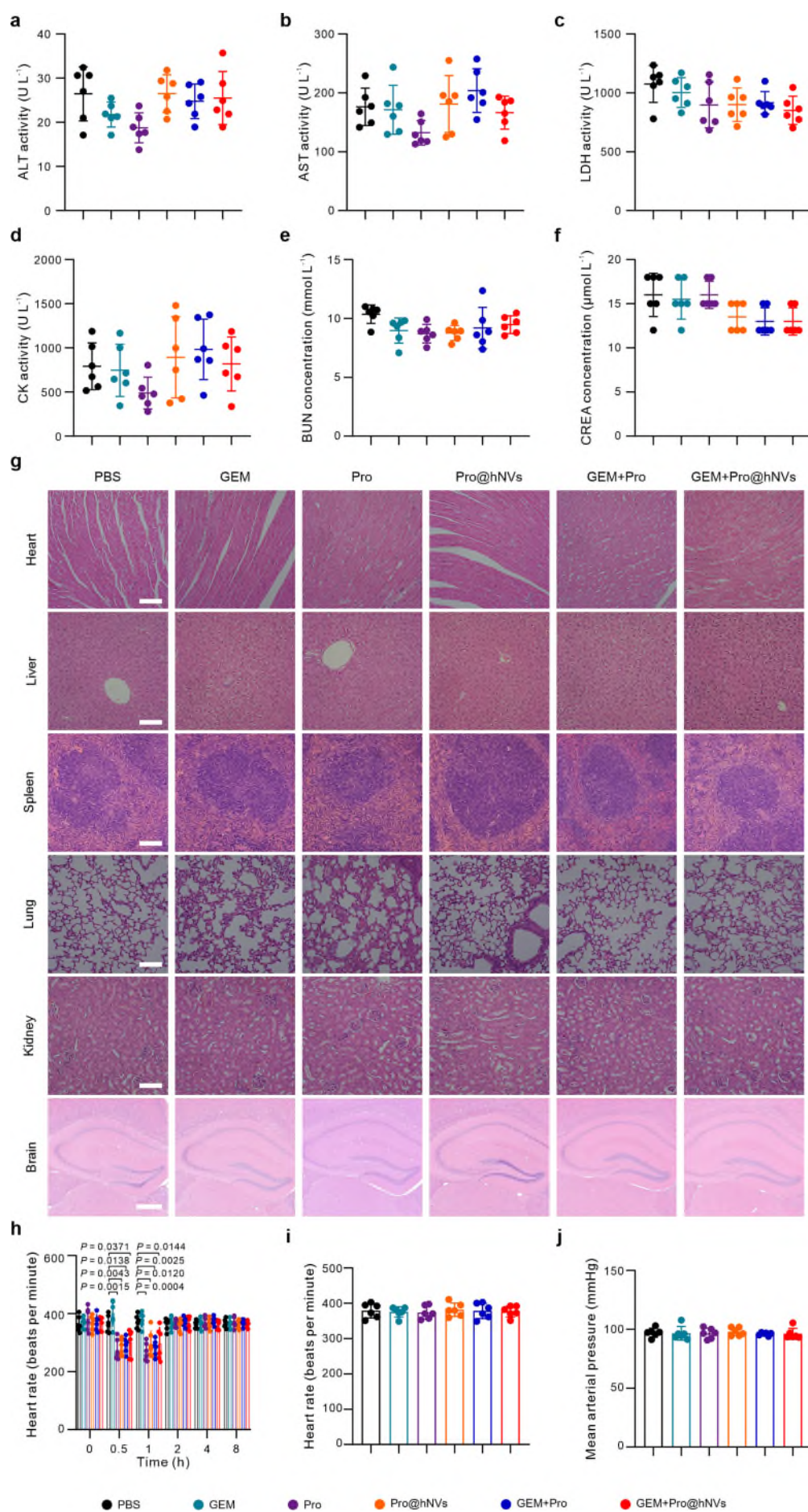
injection of PBS, GEM, Pro, Pro@hNVs, GEM+Pro, or GEM+Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> after CRS exposure. **b, c** Tumor images (**b**) and tumor weight (**c**) of mice at 13 days post-treatment. **d** Representative H&E images of tumors at 13 days post-treatment. **e, f** Representative images of intestines (**e**) and number of metastatic tumor nodules in the intestines (**f**) at 13 days post-treatment. **g-j** Representative immunofluorescence staining and quantification of  $\beta$ 3-tubulin (**g, h**) and TH (**i, j**) in tumors at 13 days post-treatment. The cell nuclei were stained with DAPI (blue). **k-n** Percentages of CD11c<sup>+</sup> TAMs (**k**), CD80<sup>+</sup> TAMs (**l**), CD86<sup>+</sup> TAMs (**m**), CD206<sup>+</sup> TAMs (**n**) in tumors at 13 days post-treatment. **o-q** Numbers of CD8<sup>+</sup> T (**o**), CD69<sup>+</sup>CD8<sup>+</sup> T (**p**), and GzmB<sup>+</sup>CD8<sup>+</sup> T (**q**) cells in tumors at 13 days post-treatment. **r** TNF content in tumors at 13 days post-treatment. Scale bar: 1 cm for **b** and **e**, 1 mm for **d** and 50  $\mu$ m for **g** and **i**. Images in **d, g** and **i** are representative of three biologically independent mice. Data are means  $\pm$  s.d. ( $n = 6$  mice per group for **c, f** and **k-r**, and  $n = 10$  fields in total from 3 mice for **h** and **j**). The  $P$  values in **c, f, h** and **j-r** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



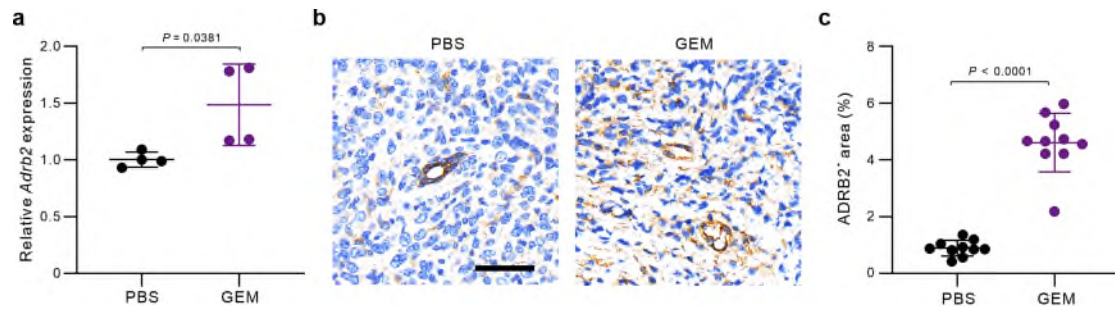
**Supplementary Fig. 31 Biosafety of GEM+Pro@hNVs in tumor-bearing mice under CRS exposure.** **a** Body weight of orthotopic Panc02 tumor-bearing mice during the treatment indicated in Fig. 7a. **b** Body weight of orthotopic KPC tumor-bearing mice during the treatment indicated in Supplementary Fig. 30a. Data are means  $\pm$  s.d. ( $n = 6$  mice per group for **a** and **b**). Source data are provided as a Source Data file.



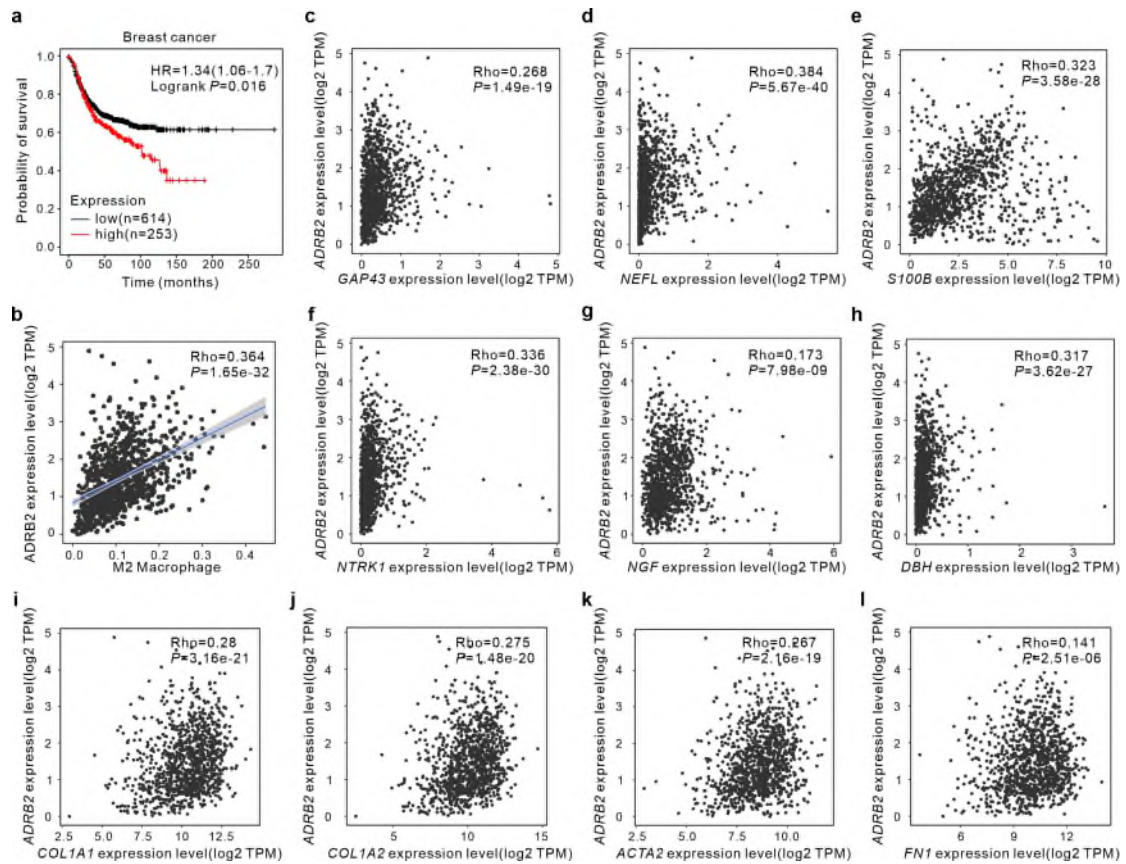
**Supplementary Fig. 32 Blood biochemical analysis of the combination of Pro@hNVs and GEM in orthotopic Panc02 tumor-bearing mice under CRS exposure. a-f** Contents of ALT (a), AST (b), LDH (c), CK (d), BUN (e) and CREA (f) in serum of orthotopic Panc02 tumor-bearing mice at 17 days after intravenous injection of PBS, GEM, Pro@hNVs or combination of GEM and Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> after CRS exposure as indicated in Fig. 7a. Data are means ± s.d. (*n* = 6 mice per group for a-f). Source data are provided as a Source Data file.



**Supplementary Fig. 33 Biosafety of GEM+Pro@hNVs in orthotopic KPC tumor-bearing mice under CRS exposure. a-f** Contents of ALT (**a**), AST (**b**), LDH (**c**), CK (**d**), BUN (**e**) and CREA (**f**) in serum of orthotopic KPC tumor-bearing mice at 13 days after intravenous injection of PBS, GEM, Pro, Pro@hNVs, GEM+Pro, or GEM+Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 20 mg kg<sup>-1</sup> after CRS exposure indicated in Supplementary Fig. **30a**. **g** Histological observation of major organs in orthotopic KPC tumor bearing mice after treatment indicated in Supplementary Fig. **30a**. Scale bar: 50 μm for heart, liver, spleen, lung and kidney, and 100 μm for brain. Images are representative of three biologically independent mice. **h** Heart rate changes over time in orthotopic KPC tumor-bearing mice following the first intravenous injection of the above formulations as indicated in **a**. **i, j** Heart rate (**i**) and mean arterial pressure (**j**) of orthotopic KPC tumor-bearing mice at 13 days after treatment indicated in Supplementary Fig. **30a**. Data are means ± s.d. (*n* = 6 mice per group for **a-f** and **h-j**). The *P* values in **h** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



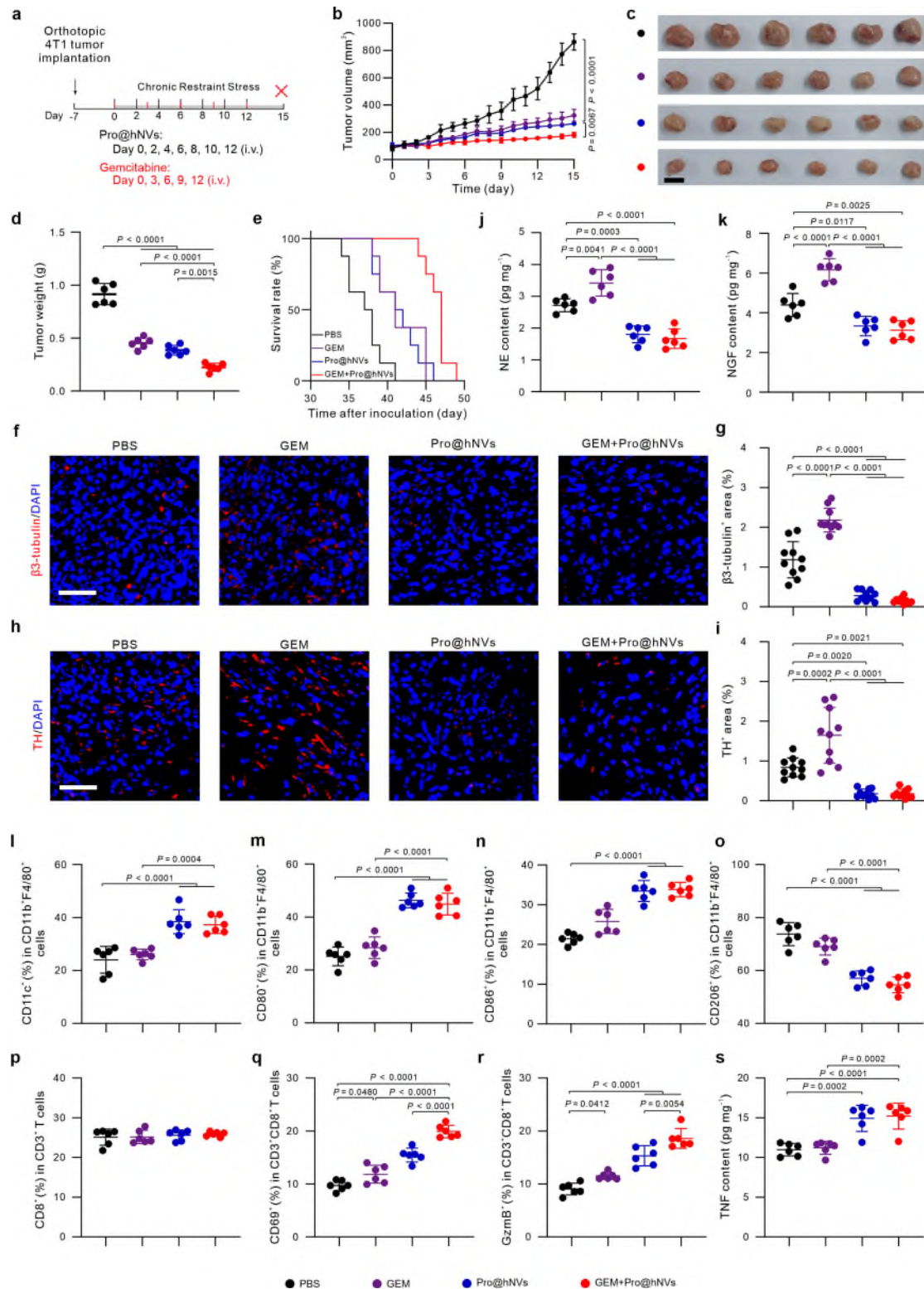
**Supplementary Fig. 34 Effective upregulation of ADRB2 expression in breast cancer by GEM.** **a** *Adrb2* mRNA levels in 4T1 cells after treatment with PBS or 0.03  $\mu\text{g mL}^{-1}$  GEM for 12 h by real time RT-PCR. **b**, **c** Representative immunohistochemistry images (**b**) and quantification (**c**) of ADRB2<sup>+</sup> area in tumors of orthotopic 4T1 tumor-bearing mice at 15 days after intravenous injection of PBS or GEM at the dosage of 10 mg kg<sup>-1</sup> under CRS exposure. Scale bar: 50  $\mu\text{m}$  for **b**. Images are representative of three biologically independent mice for **b**. Data are means  $\pm$  s.d. ( $n = 4$  biologically independent samples for **a**, and  $n = 10$  fields in total from 3 mice for **c**). The  $P$  values in **a** and **c** were determined using unpaired two-tailed Student's  $t$ -test. Source data are provided as a Source Data file.



**Supplementary Fig. 35 Correlation of *ADRB2* with poor prognosis in breast cancer.**

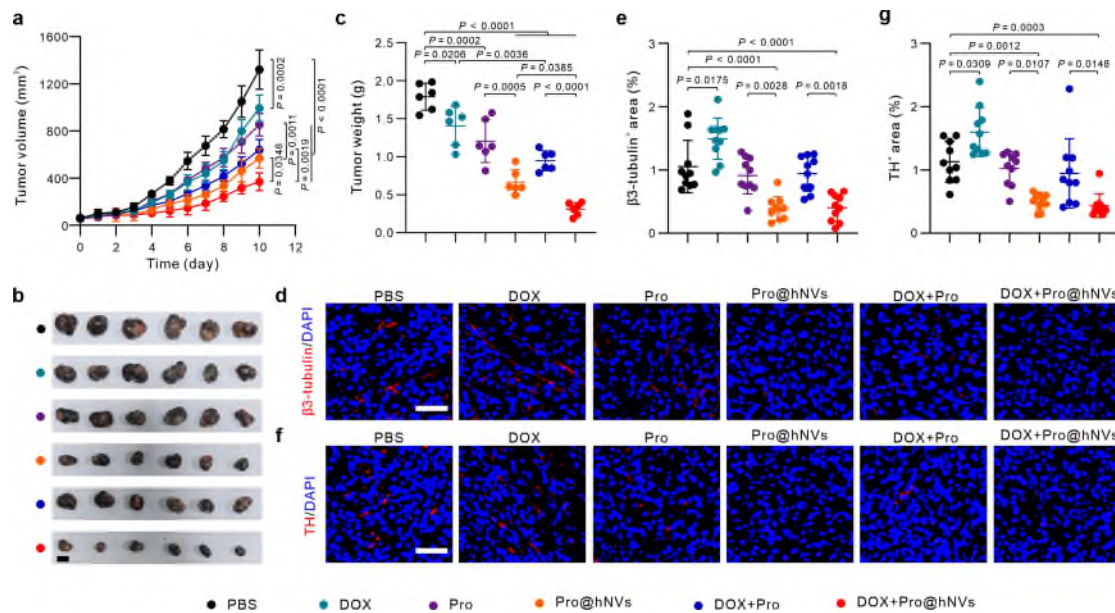
**a** Correlation between expressions of *ADRB2* and recurrence-free survival in patients with breast cancer analyzed by Kaplan-Meier plotter (<http://kmplot.com>). The *P* value (log-rank, Mantel Cox test), the hazard ratio (HR) and number of patients were indicated. **b** Correlation between the expression of *ADRB2* and the infiltration levels of M2-like macrophages in tumors of breast cancer patients analyzed by TIMER2.0 ( $n = 1100$ ). The best-fitting line with 95% confidence interval (highlighted in grey) is represented. **c-h** The association between *ADRB2* expression and the expression of nerve markers *GAP43* (**c**), *NEFL* (**d**), *S100B* (**e**), *NTRK1* (**f**), *NGF* (**g**) and *DBH* (**h**) in tumors of breast cancer patients analyzed by TIMER2.0 ( $n = 1100$ ). **i-l** The association between *ADRB2* expression and the expression of stromal markers *COL1A1* (**i**),

*COL1A2* (**j**), *ACTA2* (**k**) and *FN1* (**l**) in tumors of breast cancer patients analyzed by TIMER2.0 ( $n = 1100$ ). The Spearman's rank correlation coefficients and  $P$  values (two-tailed) were shown in the respective figures.

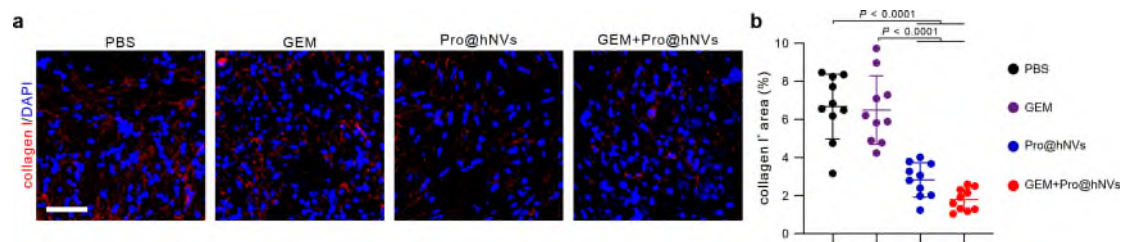


**Supplementary Fig. 36 Efficient tumor inhibition and improved tumor microenvironment in mice bearing orthotopic 4T1 tumors by the combination of Pro@hNVs and GEM after CRS exposure.** a Schematic illustration of the

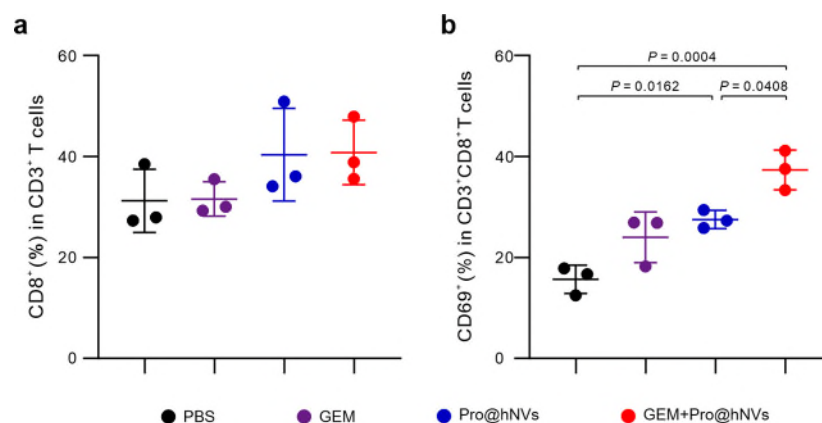
experimental design for the anticancer study in mice bearing orthotopic 4T1 tumors following intravenous injection of PBS, GEM, Pro@hNVs or the combination of GEM and Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and GEM dosage of 10 mg kg<sup>-1</sup> after CRS exposure. **b** Tumor growth curves of mice after treatment indicated in **a**. **c**, **d** Tumor images (**c**) and tumor weight (**d**) at 15 days post-treatment. **e** Kaplan-Meier survival plot of mice post-treatment. **f-i** Representative immunofluorescence staining and quantification of  $\beta$ 3-tubulin (**f**, **g**) and TH (**h**, **i**) in tumors at 15 days post-treatment. The cell nuclei were stained with DAPI (blue). **j**, **k**, **s** NE (**j**), NGF (**k**) and TNF (**s**) contents in tumors at 15 days post-treatment. **l-r** Percentages of CD11c<sup>+</sup> TAMs (**l**), CD80<sup>+</sup> TAMs (**m**), CD86<sup>+</sup> TAMs (**n**), CD206<sup>+</sup> TAMs (**o**), CD8<sup>+</sup> T cells (**p**), CD69<sup>+</sup>CD8<sup>+</sup> T cells (**q**) and GzmB<sup>+</sup>CD8<sup>+</sup> T cells (**r**) in tumors at 15 days post-treatment. Scale bar: 1 cm for **c** and 50  $\mu$ m for **f** and **h**. Images in **f** and **h** are representative of three biologically independent mice. Data are means  $\pm$  s.d. ( $n = 6$  mice per group for **b**, **d** and **j-s**,  $n = 8$  mice per group for **e**, and  $n = 10$  fields in total from 3 mice for **g** and **i**). The *P* values in **b**, **d**, **g** and **i-s** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



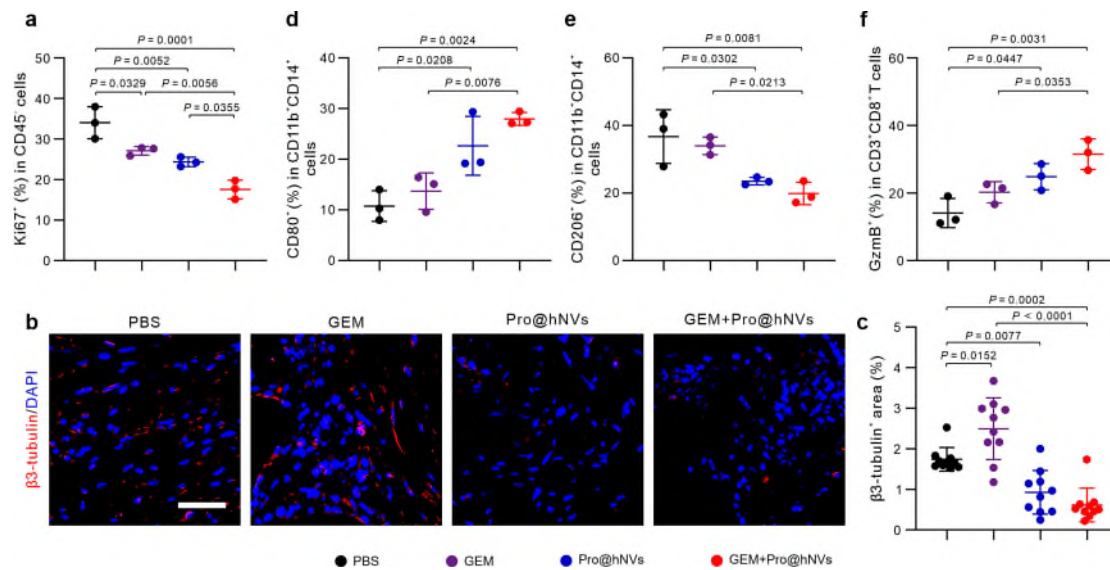
**Supplementary Fig. 37 Efficient tumor inhibition in mice bearing B16F10 melanoma tumors by DOX+Pro@hNVs after CRS exposure.** **a** Tumor growth curves of B16F10 tumor-bearing mice after intravenous injection of PBS, DOX, Pro, Pro@hNVs, DOX+Pro, or DOX+Pro@hNVs at the Pro dosage of 7 mg kg<sup>-1</sup> and DOX dosage of 2 mg kg<sup>-1</sup> every other day for a total of six doses under CRS exposure. **b**, **c** Tumor images (**b**) and tumor weight (**c**) at 12 days post-treatment. **d-g** Representative immunofluorescence staining and quantification of β3-tubulin (**d**, **e**) and TH (**f**, **g**) in tumors at 12 days post-treatment. The cell nuclei were stained with DAPI (blue). Scale bar: 1 cm for **b** and 50 μm for **d** and **f**. Images in **d** and **f** are representative of three biologically independent mice. Data are means ± s.d. ( $n = 6$  mice per group for **a** and **c**, and  $n = 10$  fields in total from 3 mice for **e** and **g**). The  $P$  values in **a**, **c**, **e** and **g** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



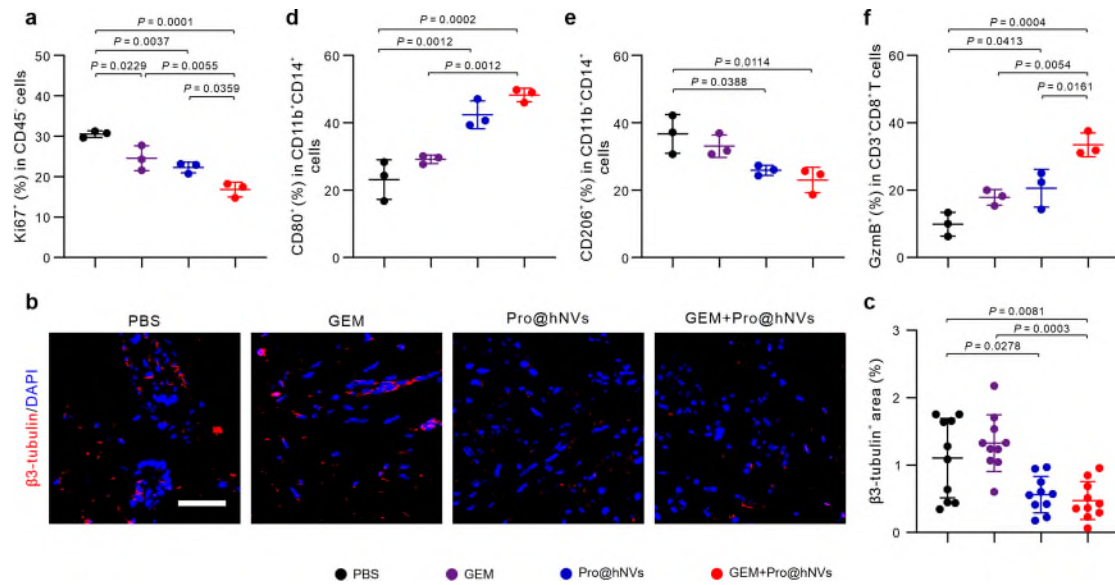
**Supplementary Fig. 38 Effects of GEM+Pro@hNVs on tumor ECM in pancreatic cancer patient-derived tumor slices (Patient 1).** **a** Representative immunofluorescence images of collagen I in tumor slices after treatment with PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at GEM concentration of  $1 \mu\text{g mL}^{-1}$  and Pro concentration of  $5 \mu\text{M}$  for 4 days. The cell nuclei were stained with DAPI (blue). Scale bar:  $50 \mu\text{m}$ . **b** Quantification of collagen I<sup>+</sup> areas in tumor slices post-treatments indicated in **a**. Images in **a** are representative of three independent samples. Data are means  $\pm$  s.d. ( $n = 10$  fields in total from 3 biologically independent samples for **b**). The  $P$  values in **b** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



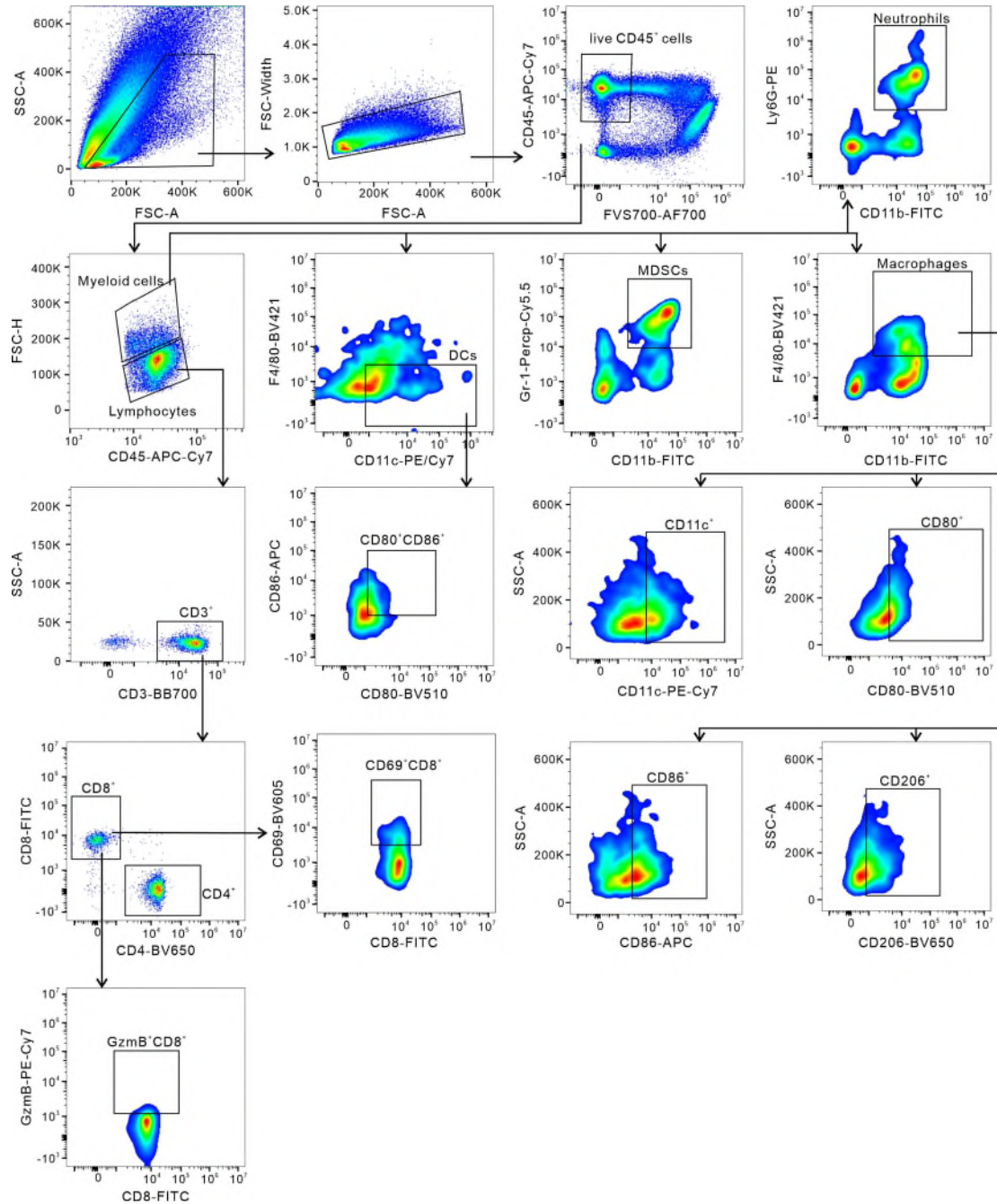
**Supplementary Fig. 39 Effects of GEM+Pro@hNVs on tumor immune microenvironment in pancreatic cancer patient-derived tumor slices (Patient 1).** **a**, **b** The percentages of CD8<sup>+</sup> T cells (**a**) and CD69<sup>+</sup>CD8<sup>+</sup> T cells (**b**) in pancreatic cancer patient-derived tumor slices after treatment with PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at GEM concentration of 1  $\mu\text{g mL}^{-1}$  and Pro concentration of 5  $\mu\text{M}$  for 4 days. Data are means  $\pm$  s.d. ( $n = 3$  biologically independent samples for **a** and **b**). The  $P$  values in **b** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



**Supplementary Fig. 40 Efficient inhibition of cancer cell proliferation and improved tumor microenvironment by GEM+Pro@hNVs in another fresh tumor slices from pancreatic cancer patient (Patient 2).** **a** Percentages of Ki67<sup>+</sup>CD45<sup>-</sup> cells in tumor slices from pancreatic cancer patient after treatment with PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at GEM concentration of 1 μg mL<sup>-1</sup> and Pro concentration of 5 μM for 4 days. **b, c** Representative immunofluorescence images (**b**) and quantification (**c**) of β3-tubulin in tumor slices post-treatments indicated in **a**. The cell nuclei were stained with DAPI (blue). Images in **b** are representative of three independent samples. **d-f** Percentages of CD80<sup>+</sup> TAMs (**d**), CD206<sup>+</sup> TAMs (**e**) and GzmB<sup>+</sup>CD8<sup>+</sup> T cells (**f**) in tumor slices post-treatments indicated in **a**. Scale bar: 50 μm for **b**. Data are means ± s.d. (*n* = 3 biologically independent samples for **a** and **d-f**, and *n* = 10 fields in total from 3 biologically independent samples for **c**). The *P* values in **a**, **c** and **d-f** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.

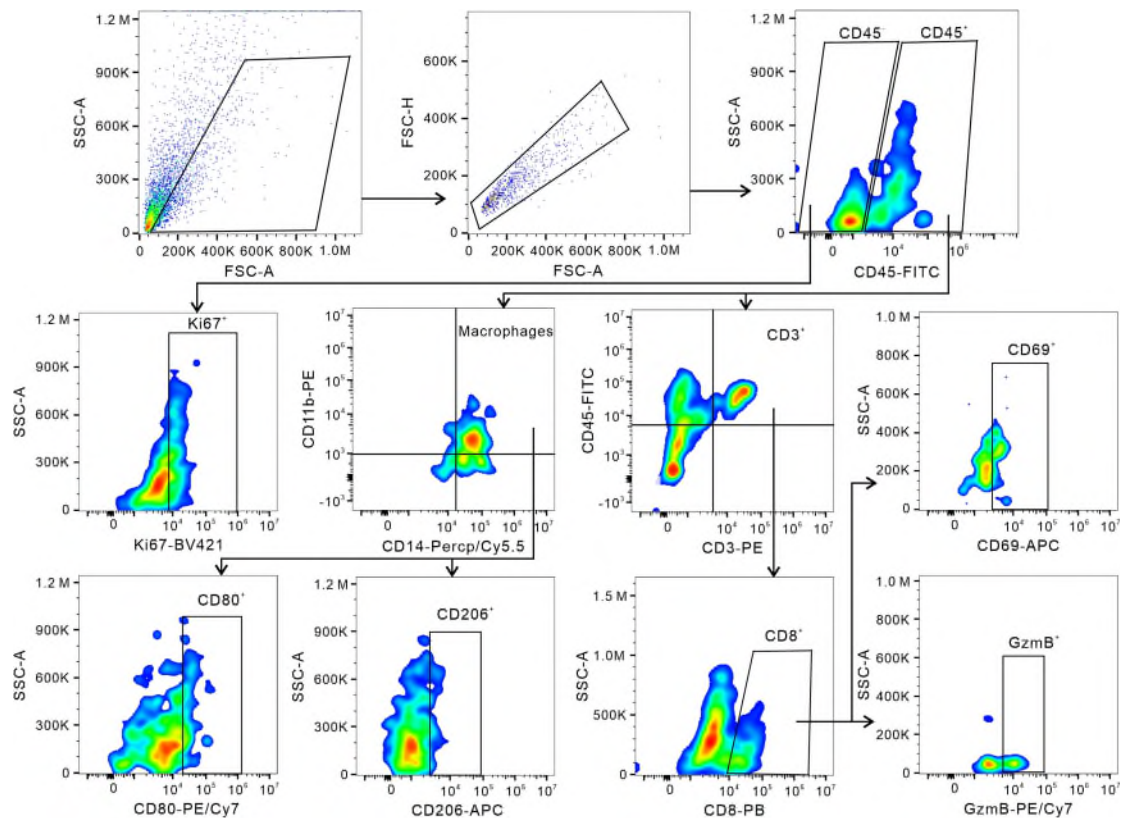


**Supplementary Fig. 41 Efficient inhibition of cancer cell proliferation and improved tumor microenvironment by GEM+Pro@hNVs in another fresh tumor slices from pancreatic cancer patient (Patient 3).** **a** Percentages of Ki67<sup>+</sup>CD45<sup>-</sup> cells in tumor slices from pancreatic cancer patient after treatment with PBS, GEM, Pro@hNVs or GEM+Pro@hNVs at GEM concentration of 1 μg mL<sup>-1</sup> and Pro concentration of 5 μM for 4 days. **b, c** Representative immunofluorescence images (**b**) and quantification (**c**) of β3-tubulin in tumor slices post-treatments indicated in **a**. The cell nuclei were stained with DAPI (blue). Images in **b** are representative of three independent samples. **d-f** Percentages of CD80<sup>+</sup> TAMs (**d**), CD206<sup>+</sup> TAMs (**e**) and GzmB<sup>+</sup>CD8<sup>+</sup> T cells (**f**) in tumor slices post-treatments indicated in **a**. Scale bar: 50 μm for **b**. Data are means ± s.d. (*n* = 3 biologically independent samples for **a** and **d-f**, and *n* = 10 fields in total from 3 biologically independent samples for **c**). The *P* values in **a**, **c** and **d-f** were determined using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



**Supplementary Fig. 42 Gating Strategy for identifying major immune cell populations in tumor tissues of tumor-bearing mice.** Gating strategy for identifying DCs (CD45<sup>+</sup>CD11c<sup>+</sup>F4/80<sup>-</sup>), MDSCs (CD45<sup>+</sup>CD11b<sup>+</sup>Gr-1<sup>+</sup>), Neutrophils (CD45<sup>+</sup>CD11b<sup>+</sup>Ly6G<sup>+</sup>), Macrophages (CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup>), T cells (CD45<sup>+</sup>CD3<sup>+</sup>), CD80<sup>+</sup>CD86<sup>+</sup>DCs (CD45<sup>+</sup>CD11c<sup>+</sup>F4/80<sup>-</sup>CD80<sup>+</sup>CD86<sup>+</sup>), M1-like TAMs (CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup>CD11c<sup>+</sup>, CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup>CD80<sup>+</sup>,

CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup>CD86<sup>+</sup>), M2-like TAMs (CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup>CD206<sup>+</sup>), CD69<sup>+</sup>CD8<sup>+</sup>T cells (CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>CD69<sup>+</sup>) and GzmB<sup>+</sup>CD8<sup>+</sup>T cells (CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>GzmB<sup>+</sup>) in tumor tissues of tumor-bearing mice presented on Fig. **1j, k**, Fig. **6g-j**, Fig. **7m-s**, Fig. **8m-s**, Supplementary Fig. **23e-j**, Supplementary Fig. **26**, Supplementary Fig. **27**, Supplementary Fig. **29**, Supplementary Fig. **30k-q**, and Supplementary Fig. **36l-r**.



**Supplementary Fig. 43** Gating strategy for identifying Ki67<sup>+</sup> tumor cells (CD45<sup>-</sup> Ki67<sup>+</sup>), M1-like TAM (CD45<sup>+</sup>CD11b<sup>+</sup>CD14<sup>+</sup>CD80<sup>+</sup>), M2-like TAMs (CD45<sup>+</sup>CD11b<sup>+</sup>CD14<sup>+</sup>CD206<sup>+</sup>), CD8<sup>+</sup>T cells (CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>), CD69<sup>+</sup>CD8<sup>+</sup>T cells (CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>CD69<sup>+</sup>) and GzmB<sup>+</sup>CD8<sup>+</sup>T cells (CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>GzmB<sup>+</sup>) in slices from the pancreatic cancer patient-derived tumor presented on Fig. 9a, h-j, Supplementary Fig. 39, Supplementary Fig. 40a, d-f and Supplementary Fig. 41a, d-f.

**Supplementary table 1** Antibodies used for the flow cytometric analysis

| Antibodies                            | Company        | Cat. No | Clone    | Dilution |
|---------------------------------------|----------------|---------|----------|----------|
| APC-Cyanine7 anti-mouse CD45          | BD Biosciences | 557659  | 30-F11   | 1/20     |
| FITC anti-mouse/human CD11b           | Biolegend      | 101206  | M1/70    | 1/200    |
| PE/Cyanine7 anti-mouse CD11c          | Biolegend      | 117318  | N418     | 1/80     |
| Brilliant Violet 421 anti-mouse F4/80 | Biolegend      | 123137  | BM8      | 1/80     |
| Brilliant Violet 510 anti-mouse CD80  | Biolegend      | 104741  | 16-10A1  | 1/40     |
| APC anti-mouse CD86                   | Biolegend      | 105012  | GL-1     | 1/80     |
| Brilliant Violet 650 anti-mouse CD206 | Biolegend      | 141723  | C068C2   | 1/30     |
| PerCP/Cyanine5.5 anti-mouse Gr-1      | Biolegend      | 108428  | RB6-8C5  | 1/80     |
| PE anti-mouse Ly-6G                   | Biolegend      | 127607  | 1A8      | 1/80     |
| BB700 anti-mouse CD3e                 | BD Biosciences | 566494  | 145-2C11 | 1/20     |
| Brilliant Violet 650 anti-mouse CD4   | BD Biosciences | 563232  | GK1.5    | 1/20     |
| FITC anti-mouse CD8a                  | Biolegend      | 100706  | 53-6.7   | 1/50     |
| Brilliant Violet 605 anti-mouse CD69  | Biolegend      | 104530  | H1.2F3   | 1/80     |
| FITC anti-human CD45                  | Biolegend      | 304006  | HI30     | 1/20     |
| PE anti-mouse/human CD11b             | Biolegend      | 101207  | M1/70    | 1/80     |
| PerCP/Cyanine5.5 anti-human CD14      | Biolegend      | 325622  | HCD14    | 1/20     |
| PE/Cyanine7 anti-human CD80           | Biolegend      | 305218  | 2D10     | 1/20     |
| APC anti-human CD206                  | Biolegend      | 321110  | 15-2     | 1/20     |
| Brilliant Violet 421 anti-human Ki-67 | Biolegend      | 350505  | Ki-67    | 1/20     |
| PE anti-human CD3                     | Biolegend      | 300307  | HIT3a    | 1/20     |
| Pacific Blue anti-human CD8           | Biolegend      | 344717  | SK1      | 1/20     |
| APC anti-human CD69                   | Biolegend      | 310909  | FN50     | 1/20     |
| PE/Cyanine7 anti-human/mouse          | Biolegend      | 372214  | QA16A02  | 1/20     |
| Granzyme B                            |                |         |          |          |