Supporting Information

Gold Nanoparticle Reprograms Pancreatic Tumor Microenvironment and Inhibits Tumor Growth

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SUPPLEMENTARY MATERIALS AND METHODS

Chemicals and Media

Tetrachloroauric acid ($HAuCl_4.3H_2O$) and Sodium Citrate tribasic trihydrate were purchased from Sigma-Aldrich. Cell culture media DMEM (10-014-CV), DMEM/Ham's F12 (10-090) and RPMI1640 (10-040-CV) were obtained from Corning Inc. (Corning, NY, USA). Fetal Bovine Serum (16000-044) and Penn-Strep (15140-122) were purchased from Life technologies (Grand Island, NY, USA).

Synthesis and characterization of 20 nm AuNP

20 nm AuNPs were synthesized as described previously in endotoxin-free water from G Biosciences (St. Louis, MO, USA).[1] Further testing for the presence of endotoxin was carried out with Pyrosate Kit (CAPE COD Inc., East Falmouth, MA, USA). Nanoparticles were characterized using UV-Visible spectroscopy (Spectrostar Nano, BMG Labtech), dynamic light scattering (DLS) and zeta potential measurements (Malvern Zetasizer Nano ZS). The nanoparticles were concentrated by centrifugation before *in vitro* and *in vivo* treatments and gold content in the preparations were ascertained from the absorbance of the SPR band centered at 522 nm and by Instrumental Neutron Activation Analysis (INAA).

Cell lines and culture

Patient-derived pancreatic stellate cells (iTAF, PTAF2, PTAF3 and PTAF4) were isolated by the out-growth method [2] from freshly resected pancreatic tissue from patients undergoing surgical resection at the University of Oklahoma Health Sciences Center with approved IRB protocol.

CAF19 cells were a kind gift from Prof. Michael Goggins (Johns Hopkins, Baltimore, USA). CAF19 and iTAF were grown in DMEM supplemented with 10% FBS and 1% Penn-Strep. PTAF2, PTAF3 and PTAF4 were grown in DMEM/Ham's F12 media supplemented with 10%FBS and 1% Penn-Strep. AsPc1 (ATCC CRL-1482) and Panc-1 (ATCC CRL-1469) cells were grown in RPMI1640 and DMEM respectively, supplemented with 10% FBS and 1% Penn-Strep.

Conditioned media experiments

Conditioned media (CM) from untreated or AuNP-treated cells were generated as follows: On day 0, 2 x 10^6 cells of PCCs or PSCs were plated in 150 mm culture dishes. After 24 h, on day 1, the media was replaced with serum-free media. On day 2 (24 h interval), the media was again replaced with fresh serum-free media and treated with various doses of freshly prepared 20 nm AuNP. After 48 h treatment, on day 4, the media was collected, spun down at 3500 rpm for 15 mins to remove cell debris, filtered through a 0.22 μm filter and diluted with an equal volume of fresh serum-free media before subsequent experiments as reported previously [3].

^{3}H-thymidine incorporation assay

1.5 x 10^4/ml of PCCs/PSCs were plated per well in a 24 well plate and allowed to grow for 24 h in serum-supplemented media in a 5% CO_2 incubator. After 24 h, the media was replaced with serum-free media and allowed to grow for an additional 24 h. At the end of this starvation period, the media was replaced with fresh serum-free media and treated with various doses of AuNP for 48 h. Post 48 h treatment, 1 μCi of ^{3}H-thymidine in 1 ml of fresh serum-free media was added per well and incubated for 4 h. After 4 h, cells were washed with cold PBS, fixed with 100% cold methanol at room temperature (RT) for 20 min, lysed with 0.1 N NaOH for 30 min at

RT and the lysate was collected in Biosafe II scintillation liquid for measurement of radioactivity. Experiments were repeated at least three times and each time in triplicate.

Immunoblotting

PCCs or PSCs, both AuNP treated and non-treated, were washed twice with ice-cold PBS and lysed in ice-cold radioimmunoprecipitation (RIPA) buffer with freshly added 1X protease-phosphatase inhibitor cocktail and incubated on ice for 30 min with occasional shaking. The cells were then harvested using cell scrapper and centrifuged at 14000 rpm for 10 min at 4°C to remove the cellular debris and the supernatant was collected. Protein concentration in the supernatant was estimated using BCA assay. 10 µg of protein was boiled with SDS-PAGE loading buffer at 95 ºC for 5 min and separated on 4-15% SDS-PAGE gel. The separated proteins were transferred onto a PVDF membrane, blocked with 10% milk in TBST for 1 h at RT and incubated in diluted primary antibodies in 3% milk in TBST overnight at 4°C. After overnight incubation with primary antibodies, the membranes were washed thrice with TBST for 10 min each at RT and incubated with HRP-conjugated secondary antibody (1:10000 dilution) in TBST for 1 h at RT. After three washes with TBST, the proteins were detected using HRP-catalyzed luminol reaction. The details of all antibodies used are provided in Supplementary Table S7.

Real-time PCR (qRT-PCR)

Total RNA was isolated from untreated/AuNP-treated PSCs or PCCs using RNeasy Plus Mini kit (QIAGEN) according to manufacturers' protocol. Isolated RNA (500-1000 ng) was then retrotranscribed using iScript cDNA Synthesis kit (Bio-Rad) and then real time PCR (qRT-PCR)

was performed using iTaq Universal SYBR Green Supermix (Biorad) using gene specific primers. The details of the primers used are provided in Supplementary Table S8.

Cell viability assay for conditioned media treatment

2.5 x 10^3 PCCs/200 µl were plated per well in a 96 well plate with/without the presence of serum or conditioned media of AuNP-treated CAF19 or iTAF cells. Cells were then allowed to grow for 24 h. After 24 h, the media was replaced with the corresponding growth media and allowed to grow for an additional 48 h before assessing cellular viability by MTS assay with Cell Titer 96® (Promega) according to the manufacturer's protocol. The cell viability was expressed as a percentage ratio of the absorbance of the serum or conditioned media treated cells to the cells grown in serum free media. A similar method was adopted for viability studies of PSCs with CM of PCCs

Antibody Arrays

2 × 10^5 PCCs/PSCs in 3 ml media were plated onto 60 mm dishes and allowed to grow for 24 h. The following day, the media was replaced with serum-free media and the cells further grown for another 24 h after which the media was replaced with fresh serum free media and the cells were treated with 25 µg/mL of 20 nm AuNPs. After 48 h treatment with/without AuNPs, the media was purified by centrifugation at 14,000 rpm for 10 min and the resulting supernatant was used to analyze expression of human angiogenic cytokine using a commercially available kit according to the manufacturer's instructions (R&D Systems, ARY007, R&D Systems). Intensity of the signals was quantified using NIH Image J. Each experiment is independently repeated at least thrice.

Transmission Electron Microscopy.

Cells were treated with AuNPs for 48 h in starving conditions. After the incubation, cells were washed thrice in PBS and cell pellets collected after trypsinization and centrifugation at 14,000×g for 10 min. Cell pellets were fixed with 4% Paraformaldehyde (EM grade), 2% Gluteraldehyde (EM grade), in 0.1M Sodium Cacodylate buffer overnight at 4^{o}C. Samples were then post fixed for 90 minutes in 1% Osmium tetroxide (OsO_4) in Sodium Cacodylate, and rinsed three times for five minutes each in 0.1M Sodium Cacodylate buffer. The samples were then dehydrated in a graded acetone series. The acetone gradient was as follows; 50%, 60%, 75%, 85%, 95%, 100%. The samples were in each concentration for 15 minutes on a rocker. Then the cells had two 15 minute treatments in 100% Propylene Oxide. Following dehydration, the samples were infiltrated in a graded Epon/Araldite (EMS) resin /Propylene Oxide series (1:3, 1:1, 3:1) for 60 minutes,120 minutes, and overnight respectfully. The following day samples were further infiltrated with pure resin for 45 minutes, 90 minutes, and then overnight. The cells were then embedded in resin plus BDMA (accelerator) and polymerized at 60°C for 48 hours. Ultrathin sections were stained with Lead Citrate and Uranyl Acetate before viewing on a Hitachi H7600 Transmission Electron Microscope at 80 kV equipped with a 2k X 2k AMT digital camera.

Animal studies

Female athymic nude mice (NCrnu; 5–6 wk old) were purchased from the National Cancer Institute– Frederick Cancer Research and Development Center. All mice were housed and maintained under specific pathogen-free conditions in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current

regulations and standards of the US Department of Agriculture, US Department of Health and Human Services, and National Institutes of Health. All studies were approved and supervised by the OUHSC Institutional Animal Care and Use Committee. A total of 48 animals were divided into 3 groups receiving $1x10^6$ CAF19 cells or $1x10^6$ AsPc1 cells only or a mixture of $1x10^6$ AsPc1 and $1x10^6$ CAF19 cells in 100 µl PBS into the head of the pancreas. 4 days post implantation, the animals in each group were randomized and divided into 2 subgroups (8 animals each), receiving daily intraperitoneal injections of 100 µl HBSS or 100 µg of AuNP in 100 µl volume. Treatment was continued for 21 days. After 21 days, the animals were euthanized and the tumors and tissues were collected for further analyses.

Immunocytochemistry/Immunohistochemistry

Tumor tissues collected were fixed in formalin solution, embedded in paraffin and 4 µm sections were made. The slides were deparaffinized through a series of solutions (100% xylene through 100% ethanol to 100% water) and then stained for H&E, Ki67, or CD31 using Leica multistainer (ST5020) and Leica Bond III. For staining of αSMA the tissues were deparaffinized as described above, antigen-retrival performed with Tris-EDTA (pH 9.0) for 40 min at 95 ºC, blocked with Protein Block (ab64261) followed by staining with αSMA antibody (Abcam ab5694, 1:400 dilution in Protein block) overnight at 4 ºC. αSMA stained tissues were imaged after staining with goat anti-rabbit Alexa 568 in Zeiss Axiovert microscope after DAPI counterstaining. For fibronectin staining, the tissues were being deparaffinized as above, antigen retrival was performed with Trypsin Enzymatic Antigen Retrieval Solution (ab970, Abcam), blocked with Protein block (ab64261, Abcam) for 10 min at RT and then incubated with Anti-fibronectin antibody (1:200, Abcam ab2413) overnight at 4 ºC and further processed for DAB staining using rabbit-specific detection kit (ab64261, Abcam) as per manufacturers' instructions

Counterstaining was performed by incubating the slides in Hematoxylin for 5 min. Sirius red staining was carried out on 7 µm sections using Picro Sirius Red (Abcam ab150681) as per manufacturers' protocol. Tunnel staining was performed after deparafinization of 4 µm sections using In situ Cell Death Detection Kit, AP (Roche Diagnostics GmbH, Manheim, Germany) following manufacturers' protocol.

Statistical Analysis

For *in vitro* experiments, comparison between two groups were carried out using two-sided Students *t*-test and for comparison between three or more groups One-way ANOVA followed by Newman-Keuls' multiple comparison test was performed. For statistical analysis of *in vivo* data, One-way ANOVA followed by Newman-Keuls' multiple comparison test was used. p-value ≤ 0.05 was deemed significant.

iTAF and AsPc1 network construction

iTAF network was constructed using a list of compiled from 22 proteins using GeneMania database. AsPc1 network was constructed using a list of 16 proteins using GeneMania and STRING databases. Nodes and edges and source of interactions used to derive networks were provided in supplementary data file (See AsPc1_iTAF_NetworkAnalysis.xlsx).

Network analysis

We used quantitative network assessment parameters closeness centrality [4] and radiality [5], to identify hub proteins[6] from the iTAF and AsPc1 protein networks. We used nodes with higher degree of centrality and radiality measures for prioritizing proteins for downstream functional and validation studies. Centrality *Cc(n)* of a node *n* in the protein network is defined as the

reciprocal of the average shortest path length in the given network and was computed as follows:

$Cc(n) = 1 / avg(L(n,m))$

Here, $L(n,m)$ is the length of the shortest path between two nodes n and m. Radiality was computed by subtracting the average shortest path length of a node *n* from the diameter of the connected component (all nodes that are connected pairwise) plus 1 and divided by the diameter of the connected component in the network. Both of these parameters range from 0 to 1 and provides a quantitative estimate of the influence and dependency of a node with the rest of the network. [7] Network analyses were performed and visualized using NetworkAnalyzer and Cytoscape. [8]

Enrichment analysis of proteins in iTAF and AsPc1 networks

Gene Ontology, pathway and protein domain enrichment analyses of proteins from iTAF network and AsPc1 network were performed using Enrichr using default settings [9]. Pathway analyses were performed using annotations from KEGG [10], Panther [11] and Reactome [12]. Background for statistical assessment were defined using 179 pathways and 3800 genes from KEGG; 104 pathways and 1918 genes from Panther and 1389 pathways and 6768 genes from Reactome. Gene Ontology annotations from the 2014 release of Gene Ontology Annotation data for the human genome were used to identify significantly enriched biological processes, molecular function and cellular components [13]. Background for GO term enrichment tests were defined using annotation database of 5192 terms and 14264 genes for biological processes; 641 terms and 13236 genes for cellular components and 1136 terms and 12753 genes for molecular functions. Evolutionarily conserved sequence domains associated with enriched with the proteins in iTAF and AsPc1 networks were identified using annotations from Pfam [14] and Interpro [15]

database and background was defined using 311 protein domains and 7588 genes. GO terms, pathways, protein domains enriched among iTAF and AsPc1 networks, associated proteins and *P*-values adjusted after multiple testing corrections are provided in supplementary data file (See AsPc1_iTAF_NetworkAnalysis.xlsx).

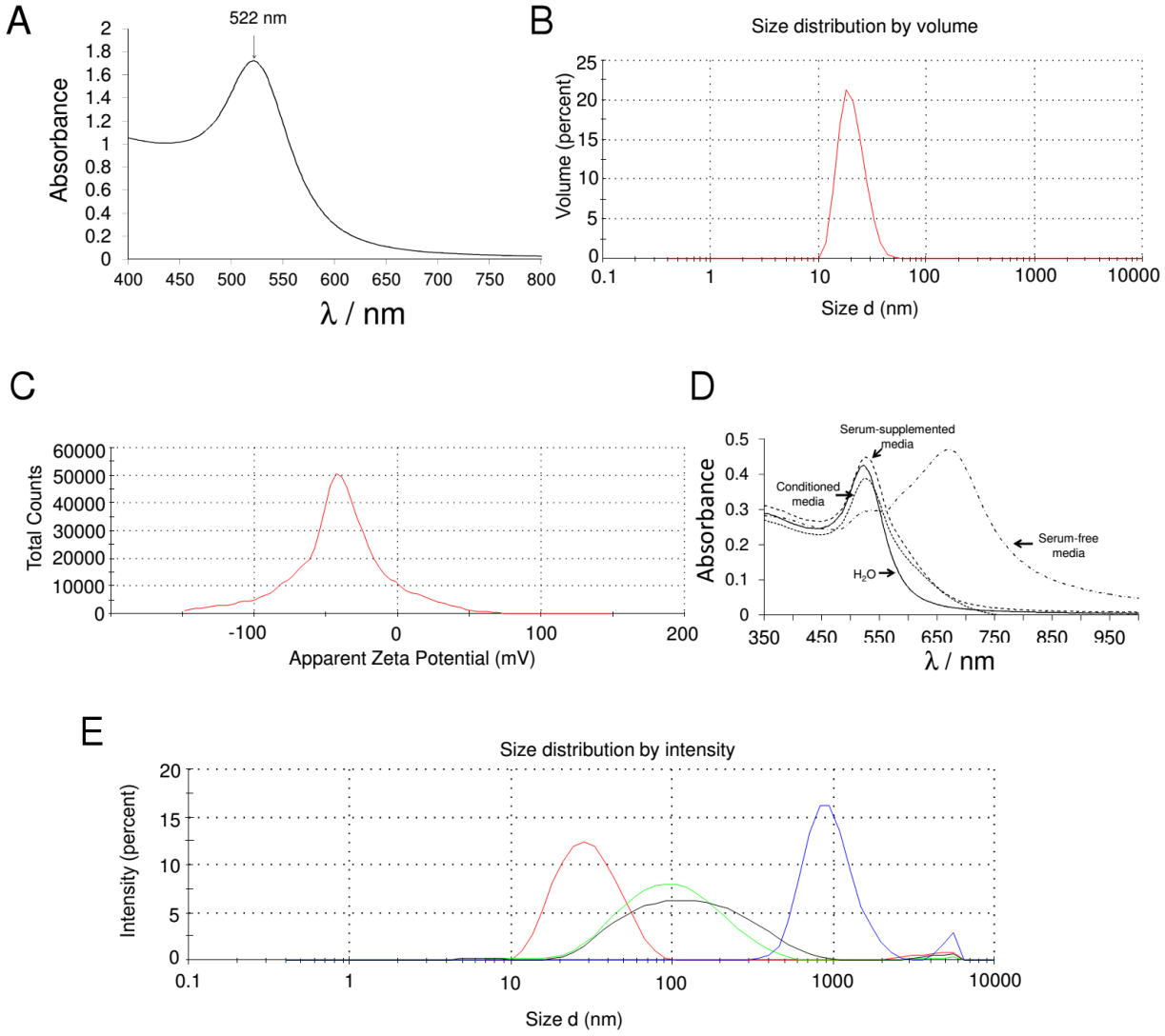


Fig. S1. (A) UV-visible spectra of 20 nm AuNP in H_2O. (B) Volume size distribution of 20 nm AuNP determined by DLS in H_2O. (C) Zeta potential measurement of 20 nm AuNP in H_2O. Average Zeta potential is -44.3 mV (D) UV-visible spectra of 20 nm AuNP in H_2O, serum free cell culture media, conditioned media from iTAF cells and 10% serum-supplemented media. (E) Intensity size distribution of 20 nm AuNP determined by DLS in H_2O (red), serum free cell culture media (blue), conditioned media from iTAF cells (black) and 10% serum-supplemented media (green) having charge of -8.6±0.3 mV. 25 µg of AuNP was mixed with 1 ml of the media as stated above and subjected to end-to-end rotation for 24 h at RT before analysis.

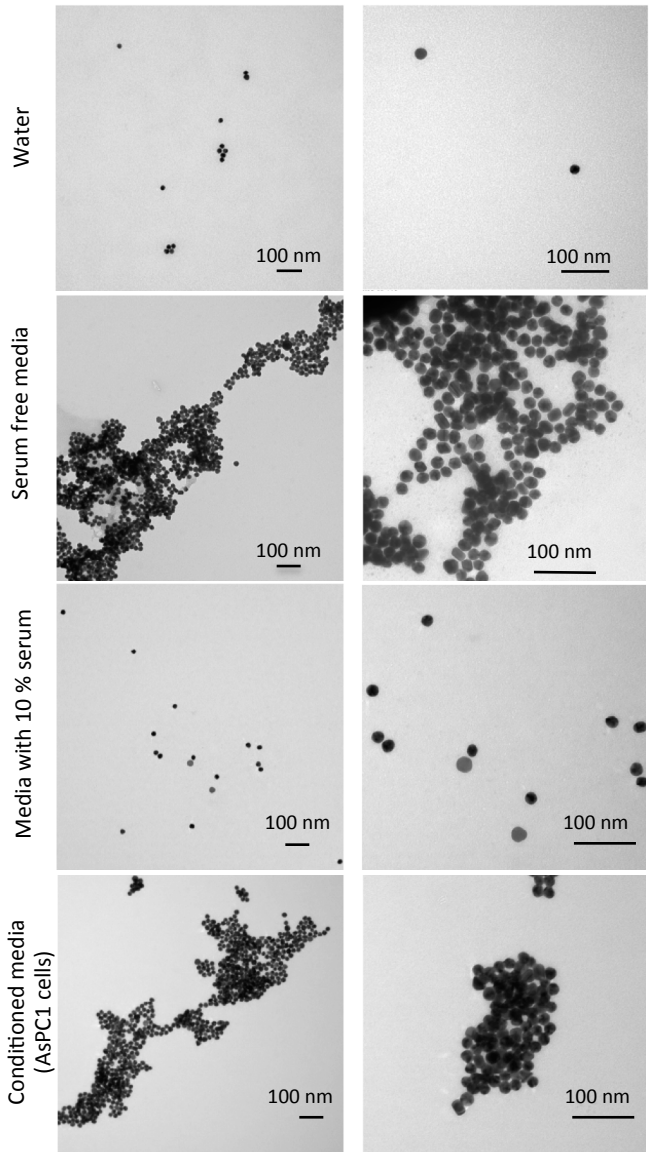


Fig. S2. Transmission electron microscopy of 20 nm citrate-capped negatively charged AuNPs post-treatment with serum-free conditioned media, serum-supplemented media and conditioned media from iTAF cells. 25 µg of AuNP was mixed with 1 ml of the media as stated above and subjected to end-to-end rotation for 24 h at RT before TEM analysis.

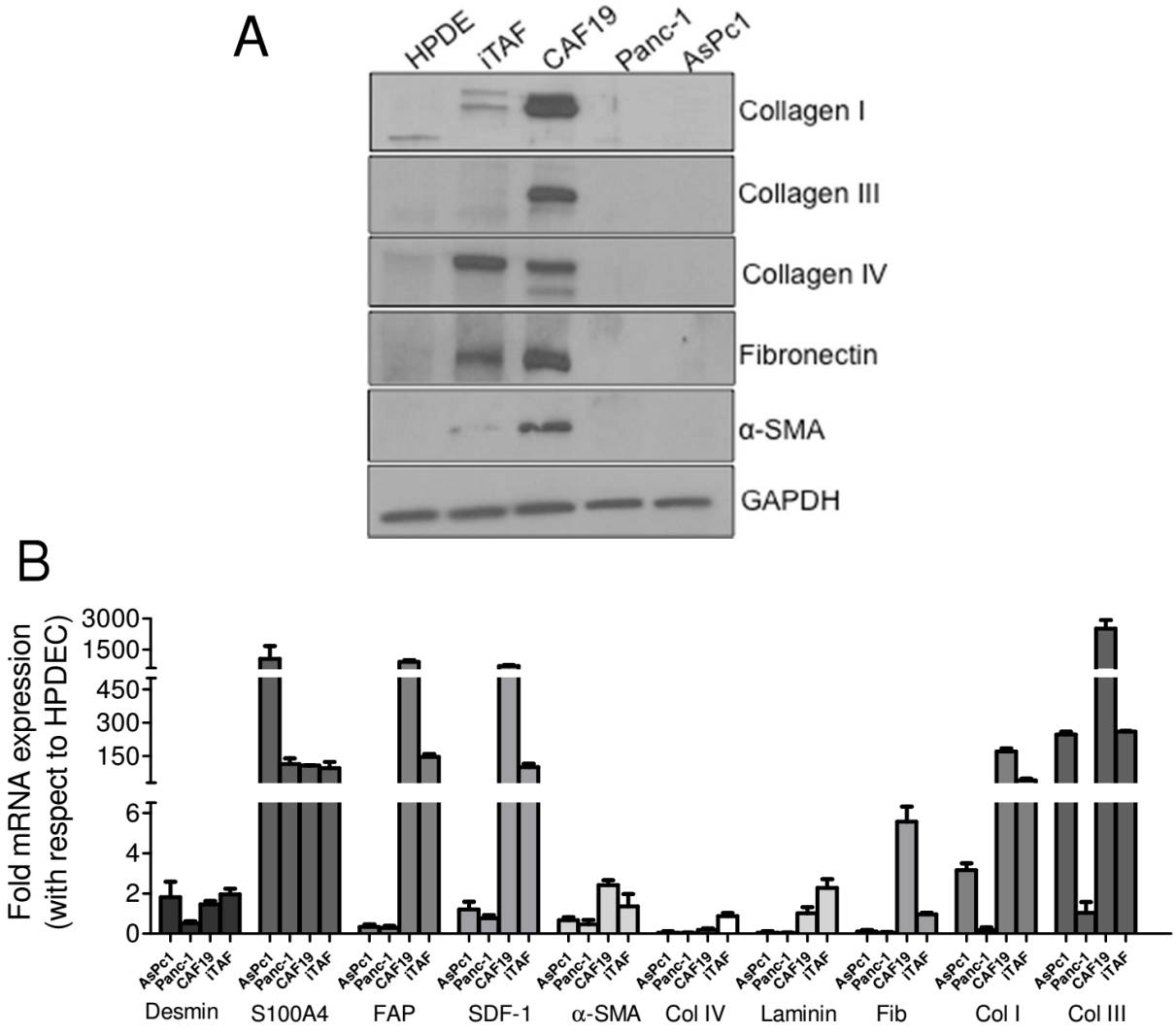
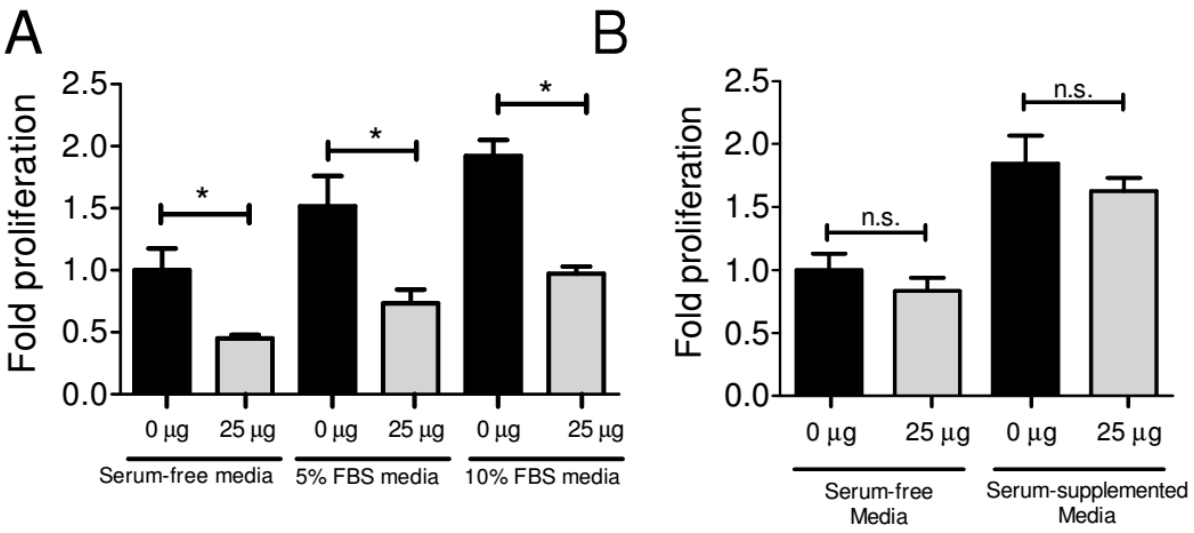


Fig. S3. (A) Expression of various ECM components and α-SMA in HPDE, Panc-1, AsPc1, CAF19 and iTAF cells by immunoblotting. (B) mRNA expression of important fibroblast marker genes determined by qRT-PCR in HPDE, Panc-1, AsPc1, CAF19 and iTAF cells. HPDE set to 1 for all genes. β-Actin was used as internal normalization control.



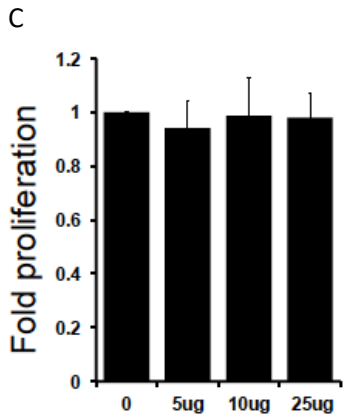
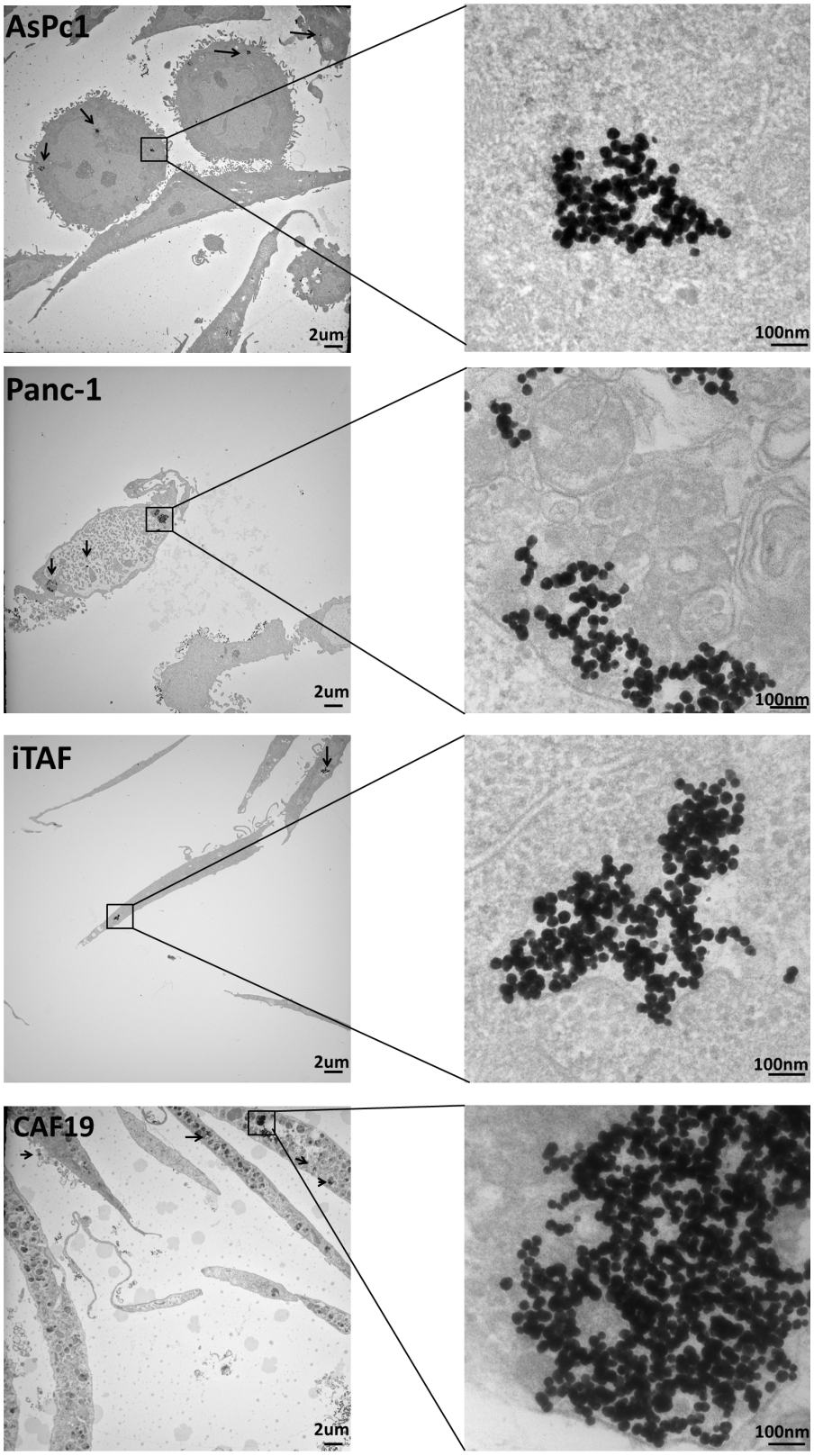


Fig. S4. (A) Proliferation of AsPc1 cells assessed by thymidine incorporation assay post-treatment with 25 µg of AuNPs for 48 h under varied level of serum supplementation. The error bars represent mean±s.d. (n=3), *$p<0.05$. (**B**) Proliferation of HPDEC cells in serum-free and serum-supplemented media post treatment with 20 nm AuNPs for 48 h ascertained by thymidine incorporation assay. The error bars represent mean±s.d. (n=3), n.s. $p>0.05$. **(C)** Proliferation of NIH3T3 cells in serum-free media post treatment with 20 nm AuNPs for 48 h ascertained by CyQuant assay. The error bars represent mean±s.d. (n=3), n.s. $p>0.05$.

A



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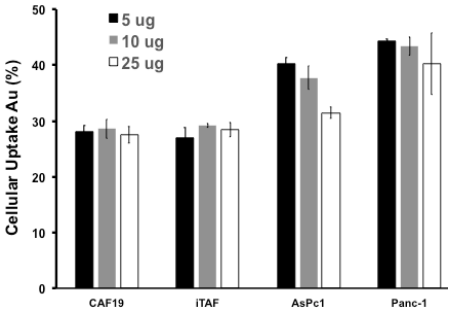

Fig. S5: (A) Representative TEM showing internalization of AuNPs in PCCs and PSCs treated with 25 µg of 20 nm AuNPs for 48h post serum starvation for 24h. (B) Cellular uptake of AuNPs into cells. The uptake was determined by measuring the gold concentration in cells using INAA. The *y* axis represents gold concentrations as percentage of microgram of total dry mass of cells.

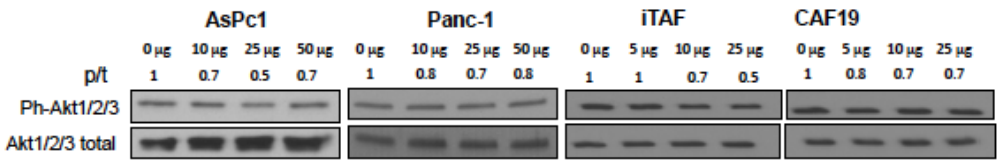

Fig. S6. Effect of various doses of 20 nm AuNP treatment for 48 h on AKT1/2/3 phosphorylation levels in AsPc1, Panc-1, iTAF and CAF19 cells. The images were quantified using NIH Image J. p/t indicates ratio of phosphorylated AKT to total AKT ratio.

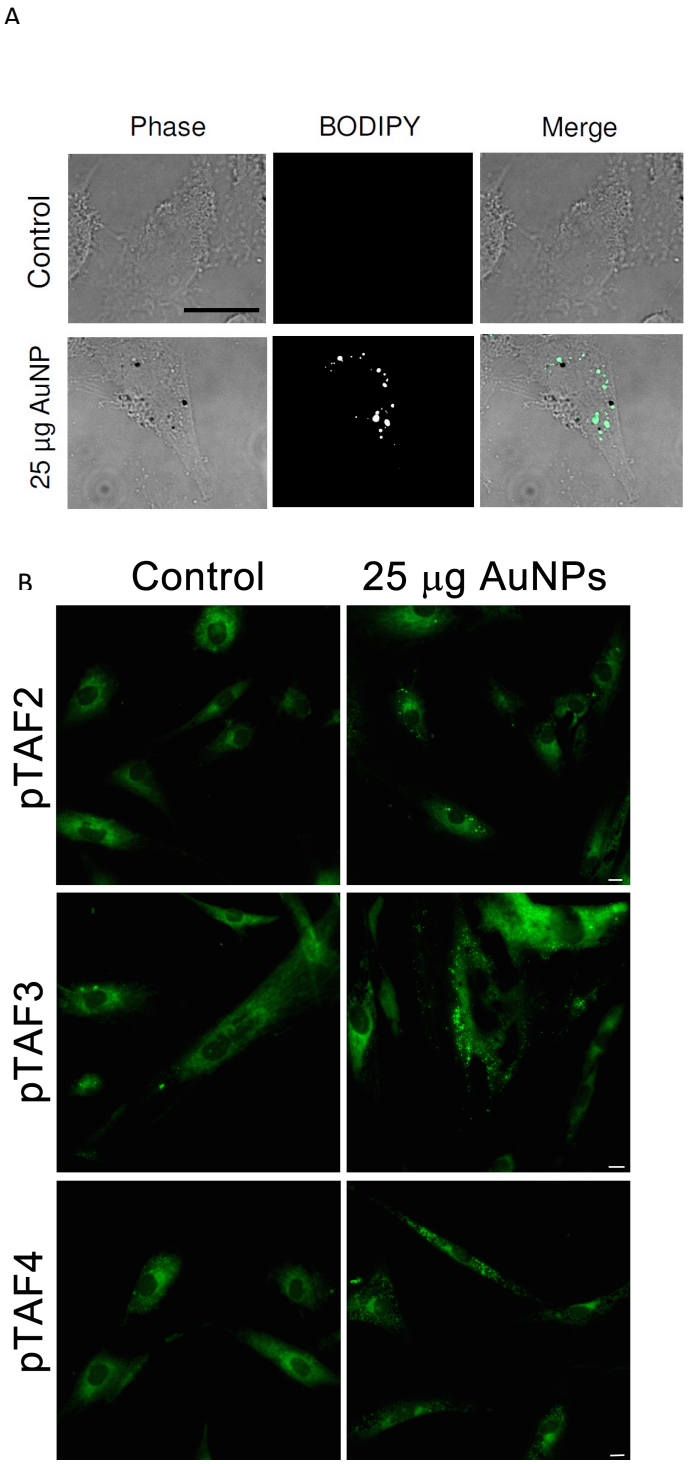

Fig. S7. BODIPY493/503 staining of iTAF cells treated with 20 nm AuNP (25 µg/ml) for 48 h to detect lipid droplets. Post-AuNP treatment, cells were washed twice with pre-warmed PBS (37 °C) and incubated with 10 µg/ml of BODIPY493/503 in serum-free media for 20 mins incubation at 37 °C. The cells were then given two washes of pre-warmed PBS, overlaid with

prewarmed PBS and imaged live. The scale bar represents 10 μm. (B) Showing lipid droplets in primary patient derived fibroblast after similar treatment with 20 nm AuNP.

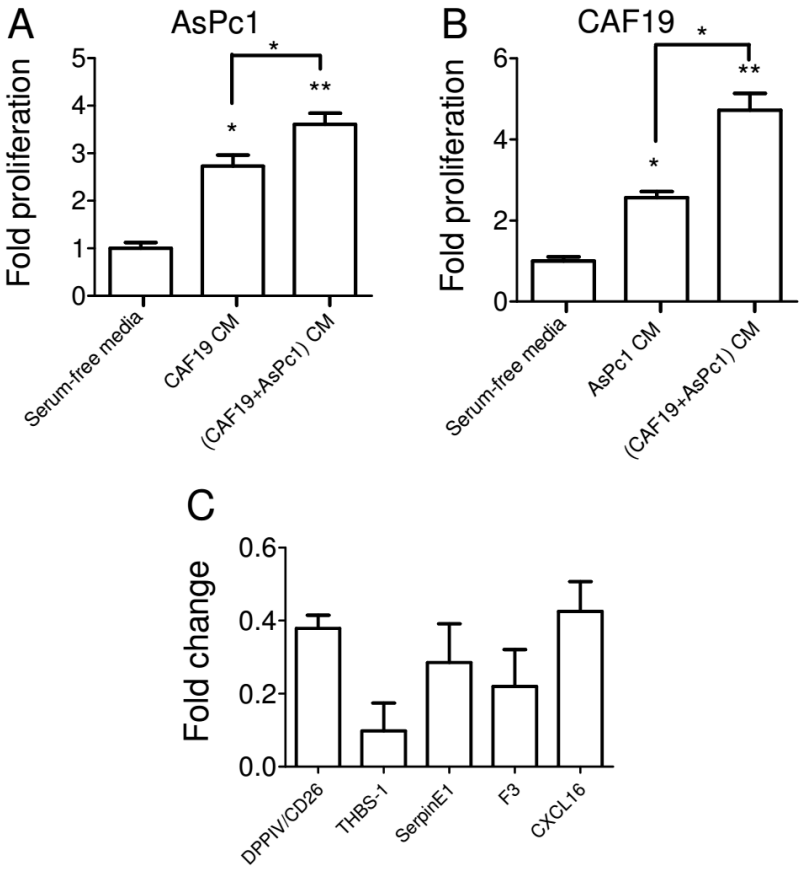

Fig. S8: (A) Effect of CAF19 conditioned media and conditioned media derived from AsPc1/CAF19 cells on proliferation of AsPc1 cells ascertained by thymidine incorporation assay post 48 h incubation at 37 °C. (**B**) Effect of AsPc1 conditioned media and conditioned media derived from AsPc1/CAF19 cells on proliferation of CAF19 cells ascertained by thymidine incorporation assay post 48 h incubation at 37 °C. (**C**) Levels of altered cytokines in AsPc1 cell conditioned media post 6h incubation with AuNPs. The conditioned media was collected from AsPc1 cell cultures and then incubated for 6 h with 25 μg AuNP with end-to-end rotation at RT and analyzed by Angiogenesis array kit.

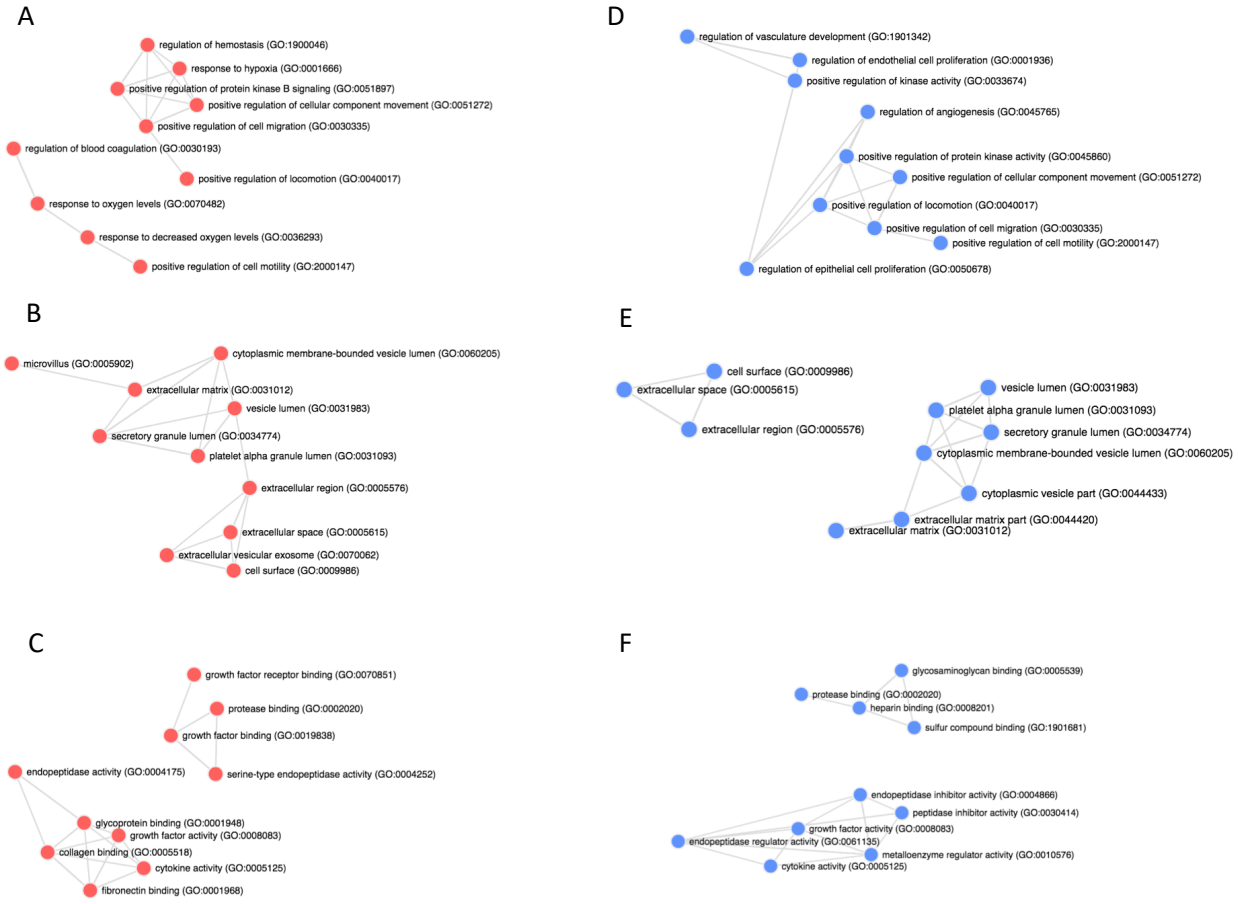

Fig. S9: Gene ontology enrichment analyses of proteins in iTAF and AsPc1 networks whose levels were altered significantly upon 25 µg AuNP treatment for 48 h as determined using angiogenesis array kit. Proteins in iTAF network are enriched for (A) biological processes, (B) cellular components and (C) molecular functions. Proteins in AsPc1 network are enriched for (D) biological processes, (E) cellular components and (F) molecular functions. Full list of terms are provided in supplementary data file (See SuppNetworkAnalysis.xlsx).

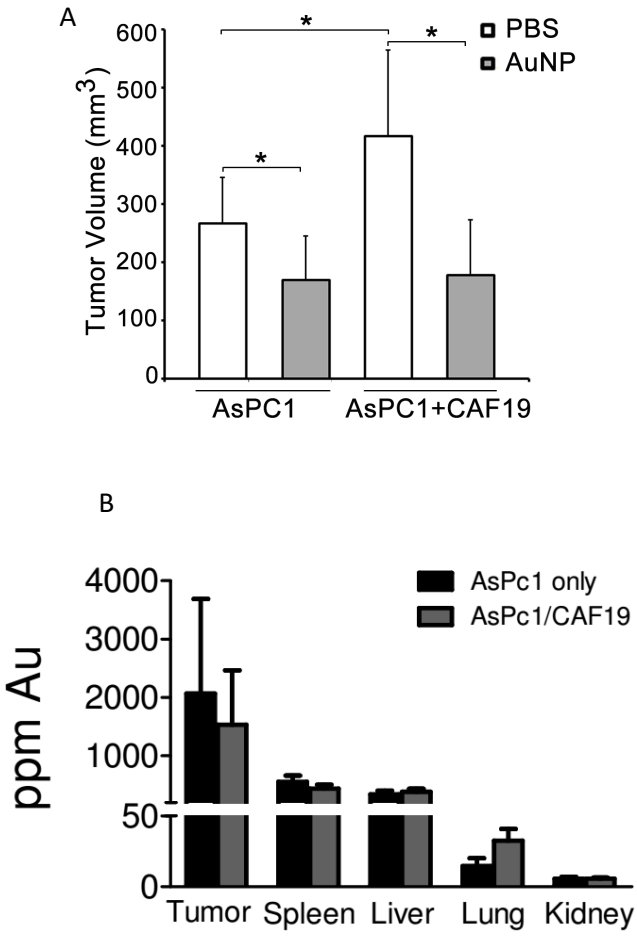

Fig. S10. (A) Change in tumor volume in HBSS-treated (sham) or 100 μg/daily i.p. 20 nm AuNP-treated animals post 21 days of treatment. Statistical analysis was performed using One-way ANOVA followed by Newman-Keuls multiple comparison test. **(B)** Instrumental Neutron Activation Analysis (INAA) of tumor and major organs for gold uptake from athymic Nu/Nu mice post 21 days treatment with 100 μg AuNP i.p. daily.

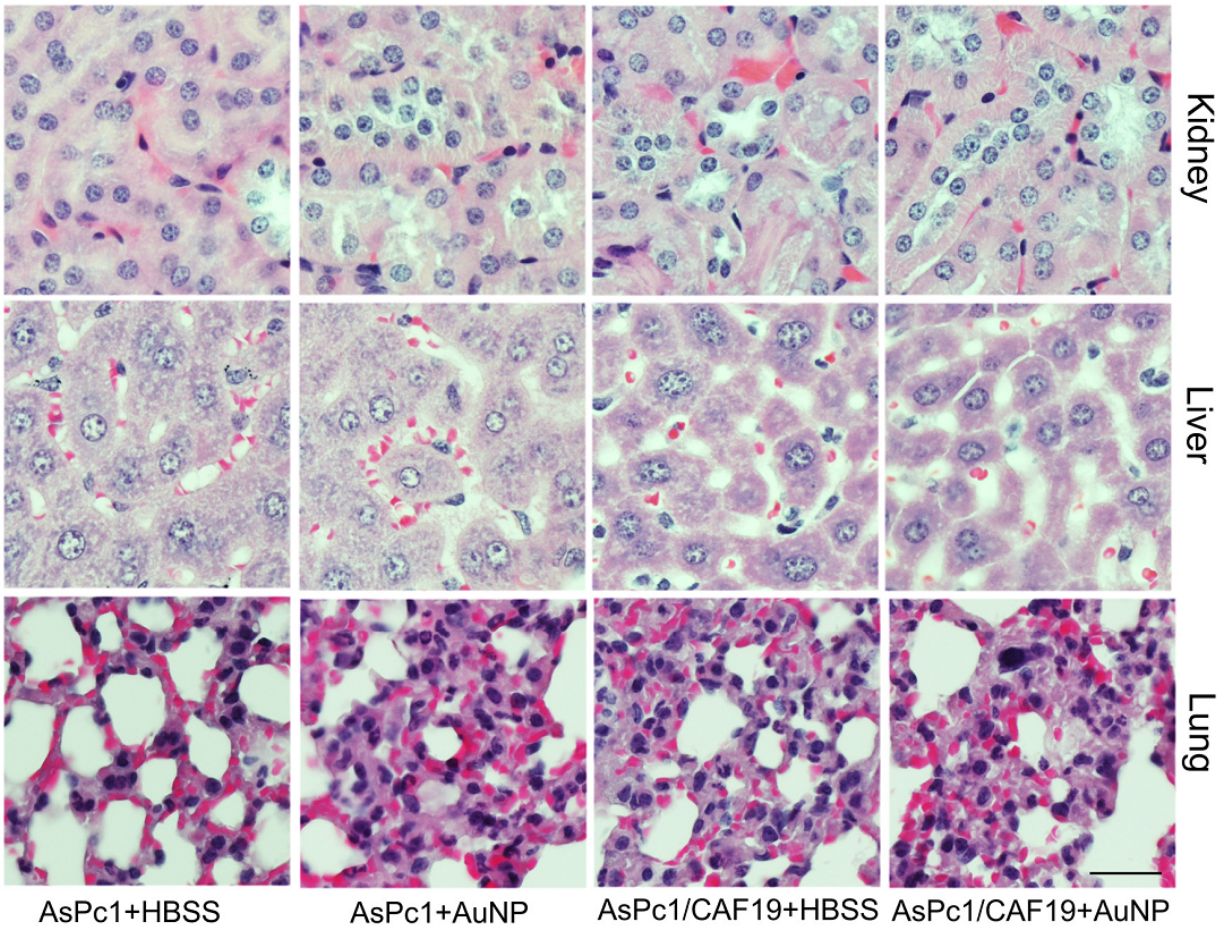

Fig. S11: Immunohistochemical H&E staining for assessment of toxicity of liver, lung and kidney from athymic Nu/Nu mice post treatment for 21 days with 100 μg AuNP i.p. daily. The scale bar represents 100 μm.

Table S1: List of all growth factors altered in AsPc1 cells by 25 µg AuNP treatment for 48 h in serum free culture media			
Sl. No.	**Growth Factor/Cytokine**	**Fold change**	**St.Dev.**
1	Amphiregulin	0.50310559	0.06832298
2	DPPIV / CD26	0.5174792	0.02306134
3	EG-VEGF	0.38726287	0.05718157
4	Endostatin/Collagen XVIII	0.24117647	0.06617647
5	TGF beta 1 (LAP)	0.36923077	0.03076923
6	MMP-9	0.42583732	0.15311005
7	PIGF	0.22922824	0.09589491
8	Endothelin-1	0.83780904	0.05349531
9	Coagulation Factor F3	0.73055556	0.13055556
10	IGFBP3	0.75711939	0.106516977
11	PDGFAA	0.79388715	0.06974922
12	THBS1	0.56888889	1.04888889
13	uPA	0.82121212	0.08787879
14	IL8	0.61384111	0.007780508
15	Angiogenin	0.67775314	0.019216556
16	CXCL16	0.62068966	0.321947104

Table S2: List of all growth factors altered in iTAF cells by 25 µg AuNP treatment for 48 h in serum free culture media

Sl. No.	Growth Factor/Cytokine	Fold change	St.Dev.
1	Angiopoietin-1	0.485294	0.033230402
2	Coagulation Factor III	0.452381	0.00622195
3	bFGF	0.197531	0.02654545
4	GDNF	0.365079	0.03086577
5	GM-CSF	0.356037	0.07711216
6	IGFBP-1	0.090909	0.10163574
7	IGFBP-2	0.292035	0.00806265
8	IL-8	0.632266	0.13651116
9	Serpin E1	0.880823	0.15998642
10	Thrombospondin-1 (THBS1)	0.610136	0.09786468
11	uPA (PLAU)	0.800664	0.18752266
12	endothelin 1 (ET1)	0.636804	0.08327475
13	DPPIV/CD26	0.688109	0.02343239
14	Pentraxin (PTX3)	0.803922	0.02761786
15	EG-VEGF	0.58042	0.0099971
16	Serpin F1	0.532379	0.0163883
17	TIMP-4	1.289906	0.55854796
18	VEGFA	0.385965	0.41875429
19	MCP-1 (CCL2)	0.442256	0.23829014
20	Angiogenin (ANG)	0.624145	0.130210233
21	Activin A (INHBA)	0.357143	0.213254426
22	TIMP-1	0.96	0.171028478

Table S3: Topological properties of altered proteins in iTAF upon 25 µg AuNP treatment for 48 h in serum free culture media computed using the network derived from GeneMANIA

Protein name	**Degree**	**Closeness Centrality**	**Radiality**
PROK1	1	0.344	0.523
ANG	2	0.375	0.583
GDNF	2	0.446	0.690
IGFBP1	5	0.477	0.726
TIMP4	4	0.5	0.75
DPP4	4	0.512	0.761
VEGFA	6	0.512	0.761
ANGPT1	6	0.512	0.761
FGF2	8	0.525	0.773
CCL2	13	0.538	0.785
IGFBP2	6	0.538	0.785
CSF2	7	0.552	0.797
EDN1	9	0.552	0.797
THBS1	12	0.6	0.833
PTX3	11	0.617	0.845
SERPINF1	9	0.617	0.845
F3	12	0.636	0.857
PLAU	13	0.636	0.857
INHBA	11	0.656	0.869
TIMP1	15	0.656	0.869
SERPINE1	16	0.677	0.881
IL8	16	0.677	0.881

Table S4: Biological pathways enriched in iTAF and AsPc1 networks of altered proteins upon 25 µg AuNP treatment for 48 h in serum free culture media		
KEGG-iTAF_Network		
Pathways	***P*^**	**Genes**
complement and coagulation cascades	0.012	PLAU;SERPINE1;F3
cytokine cytokine receptor interaction	0.023	CSF2;CCL2;INHBA;VEGFA
bladder cancer	0.028	THBS1;VEGFA
PantheriTAF_Network		
Blood coagulation	0.003	PLAU;SERPINE1;F3
Angiogenesis	0.034	ANGPT1;F3;VEGFA
Plasminogen activating cascade	0.005	PLAU;SERPINE1
Panther-AsPc1_Network		
Plasminogen activating cascade	0.004	MMP9;PLAU
Blood coagulation	0.012	F3;PLAU
Reactome-iTAF_Network		
Platelet degranulation	0.003	SERPINE1;TIMP1;THBS1;VEGFA
Response to elevated platelet cytosolic Ca2+	0.003	SERPINE1;TIMP1;THBS1;VEGFA
Platelet activation, signaling and aggregation	0.007	CSF2;SERPINE1;TIMP1;THBS1;VEGFA
Dissolution of Fibrin Clot	0.019	PLAU;SERPINE1
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	0.031	IGFBP1;IGFBP2
Syndecan interactions	0.031	THBS1;FGF2
ATF4 activates genes	0.037	IGFBP1;CCL2
PERK regulates gene expression	0.040	IGFBP1;CCL2
Reactome-AsPc1_Network		
Extracellular matrix organization	0.001	COL18A1;PDGFA;MMP9;THBS1;TGFB1
Non-integrin membrane-ECM interactions	0.001	PDGFA;THBS1;TGFB1
Platelet degranulation	0.006	PDGFA;THBS1;TGFB1
Response to elevated platelet cytosolic Ca2+	0.006	PDGFA;THBS1;TGFB1
Syndecan interactions	0.009	THBS1;TGFB1
Activation of Matrix Metalloproteinases	0.018	COL18A1;MMP9
Collagen degradation	0.021	COL18A1;MMP9
Platelet activation, signaling and aggregation	0.043	PDGFA;THBS1;TGFB1
Assembly of collagen fibrils and other multimeric structures	0.036	COL18A1;MMP9

Integrin cell surface interactions	0.043	COL18A1;THBS1

^ After adjusting for multiple-testing correction

Table S5: Evolutionarily conserved protein domains enriched among altered proteins in iTAF and AsPc1 network upon 25 µg AuNP treatment for 48 h in serum free culture media

iTAF_Network			
Term	***P*^**	**Proteins**	**Curated function(s) of domains**
Thyroglobulin_1	0.0005	IGFBP1;IGFBP2	Control of proteolytic degradation
IGFBP_like	0.0005	IGFBP1;IGFBP2	Insulin-like growth factor binding
TGFb	0.0014	GDNF;INHBA	Growth factor activity
TGFb_N	0.0328	INHBA	Growth factor activity
VWF_C	0.0328	THBS1	Protein binding
Fibrinogen_a/b/g_C	0.0328	ANGPT1	Protein binding, bridging
Kringle	0.0328	PLAU	Binding mediators (e.g., membranes, other proteins or phospholipids)
AsPc1_Network			
IGFBP_like	0.02	IGFBP3	Insulin-like growth factor binding
FN_type2_col_bd	0.02	MMP9	Binds cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin
Kringle	0.02	PLAU	Binding mediators (e.g., membranes, other proteins or phospholipids)
Thyroglobulin_1	0.02	IGFBP3	Control of proteolytic degradation
TGFb_N	0.02	TGFB1	Growth factor activity
Hemopexin	0.02	MMP9	Prevents haem-mediated oxidative stress
PGBD_1	0.02	MMP9	--
Fz_domain	0.02	COL18A1	Protein binding
Pept_M10A_M12B	0.02	MMP9	--
VWF_C	0.02	THBS1	Protein binding
TGFβ	0.03	TGFB1	Growth factor activity

^ After adjusting for multiple-testing correction

Table S6: Topological properties of altered AsPc1 proteins upon 25 μg AuNP treatment for 48 h in serum free culture media computed using the network derived from GeneMANIA			
Protein name	**Degree**	**Closeness Centrality**	**Radiality**
MMP9	12	0.682	0.883
THBS1	11	0.652	0.867
PLAU	8	0.652	0.867
IL8	9	0.625	0.850
IGFBP3	6	0.600	0.833
COL18A1	6	0.600	0.833
AREG	3	0.517	0.767
F3	4	0.517	0.767
CXCL16	4	0.517	0.767
TGFB1	4	0.500	0.750
DPP4	4	0.500	0.750
PDGFA	3	0.469	0.717
PGF	2	0.441	0.683
EDN1	2	0.417	0.650
ANG	2	0.405	0.633
PROK1	2	0.357	0.550

Table S7: List of antibodies used in the study			
Antibody against	**Source**	**Company**	**Cat #**
p38 MAPK	Rabbit	Cell Signalling	9212S
Phospho p38 MAPK	Rabbit	Cell Signalling	9215S
Phospho p44/42 MAPK	Mouse	Cell Signalling	9106S
p44/42 MAPK	Rabbit	Cell Signalling	9102S
Akt1/2/3	Rabbit	Santa Cruz	sc-8312
Phospho Akt1/2/3	Rabbit	Santa Cruz	sc-7985-R
Fibronectin	Mouse	BD Biosciences	610077
Collagen I	Rabbit	Rockland	600-401-103
Collagen III	Rabbit	Rockland	600-401-105
Collagen IV	Rabbit	Rockland	600-401-106
α-SMA	Mouse	Abcam	ab7817
ER stress markers		Cell Signalling	9956
IRE1 (phospho S724)	Rabbit	Abcam	ab48187

Table S8: List of qRT-PCR primers used in this study	
Gene name	**Primer sequence**
b-actin FW	TGCACTGTGCGGCGAAGC
b-actin-RV	TCGAGCCATAAAAGGCAA
GDNF-FW	TTATGGGATGTCGTGGCTGT
GDNF-RV	ACACCGTTTAGCGGAATGCT
THBS1-FW	CTCCCCTATGCTATCACAACG
THBS1-RV	AGGAACTGTGGCATTGGAG
IL8-FW	ATACTCCAAACCTTTCCACCC
IL8-RV	TCTGCACCCAGTTTTCCTTG
IGFBP2-FW	ACATCCCCAACTGTGACAAG
IGFBP2-RV	ATCAGCTTCCCGGTGTTG
IGFBP1-FW	CACAGGAGACATCAGGAGAAG
IGFBP1-RV	GATCCTCTTCCCATTCCAAGG
F3-FW	CCAGAGTTCACACCTTACCTG
F3-RV	CATTCACTTTTGTTCCCACCTG
ANGPT1-FW	AACCGAGCCTATTCACAGTATG
ANGP1-Rev	ATCAGCACCGTGTAAGATCAG
PIGF-FW	CTGTTAGGCGTGGGTCTC
PIGF-RV	CATGGTGTTTTCTTGGATGGC
MMP9-FW	CGAACTTTGACAGCGACAAG
MMP9-RV	CACTGAGGAATGATCTAAGCCC
Endostatin-FW	CAATGTGTTTGCTGAGTCCAG
Endostatin-RV	GAAAGTCAAACGGAAACTGCC
EG-VEGF-FW	GTTTTGCCTTCACCCCAAG
EG-VEGF-RV	AGCACAGTCAGACACAGTTAC
CD26-FW	CCAAAGACTGTACGGGTTCC
CD26-RV	TCAACATAGAAGCAGGAGCAG
Amphiregulin-FW	GCTGTCGCTCTTGATACTCG
Amphiregulin-RV	CTTCCCAGAGTAGGTGTCATTG
PSPN-FW	TCTGAACAGGTGGCAAAGG
PSPN-FW	AGGGTCAGGCTCCACAG
Platelet Factor 4-FW	CCCACTGCCCAACTGATAG
Platelet Factor 4-RV	GCAAATGCACACACGTAGG
PTX3-FW	TCCAGCCTCTCACTCTCAC
PTX3-FW	CACAAAACAGAATCGCAAGGAG
FGF7-FW	CCTGAGGATCGATAAAAGAGGC
FGF7-RV	CACTTTCCACCCCTTTGATTG
TGFB1-FW	AGGACCTCAGCTTTCCCT
TGFB1-RV	CGAACAGGGCTGGTGTG
INHBA-FW	ACGGGTATGTGGAGATAGAGG
INHBA-RV	TGGAAATCTCGAAGTGCAGC
CSF2-FW	ACTTTCTGCTTGTCATCCCC
CSF2-RV	CCATCCTGAGTTTCTAGCTCTTG

SDF-1-FW	GATTGTAGCCCGGCTGAAGA
SDF-1-RV	TTCGGGTCAATGCACACTTGT
FAP-FW	CCAAAGACCCAGGAGCATATA
FAP-RV	GTTTGTAGCCATCCTTGTCAC
Desmin-FW	GATCAATCTCCCCATCCAGAC
Desmin-RV	GACCTCAGAACCCCTTTGC
S100A4-FW	TTGGTGCTTCTGAGATGTGG
S100A4-RV	TCTTTGCCCGAGTACTTGTG
COLIII-FW	AAGTCAAGGAGAAAGTGGTCG
COLIII-RV	CTCGTTCTCCATTCTTACCAGG
bFGF-FW	ACCCTCACATCAAGCTACAAC
bFGF-RV	AAAAGAAACACTCATCCGTAACAC
Fib-FW	GTGGCAGAAGGAATATCTCGG
Fib-RV	GAGAATACTGGTTGTAGGACTGG
COLI-FW	CCCCTGGAAAGAATGGAGATG
COLI-RV	CATCCAAACCACTGAAACCTC
HGF-FW	GCTATACTCTTGACCCTCACAC
HGF-RV	GTAGCCTTCTCCTTGACCTTG
PDGFA-FW	GATACCTCGCCCATGTTCTG
PDGFA-RV	CAAAGAATCCTCACTCCCTACG
LamininA1-FW	AATGGAGTGAGACAGGAACAAG
LamininA1-RV	TGACCATACGATGCCTTGATG
COLIVA1-FW	TGTGGATCGGCTACTCTTTTG
COLIVA1-RV	TAGTAATTGCAGGTCCCACG
α-SMA-FW	CTATGCCTCTGGACGCACAACT
α-SMA-RV	CAGATCCAGACGCATGATGGCA
N.B. FW= forward, RV- reverse. All primers were designed using IDT-DNA RT-PCR tool and synthesized from Integrated DNA Technologies Inc.	

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