Supporting information

Gold Nanorod-Photothermal Therapy Alters Cell Junctions and Actin Network in Inhibiting Cancer Cell Collective Migration

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Figures

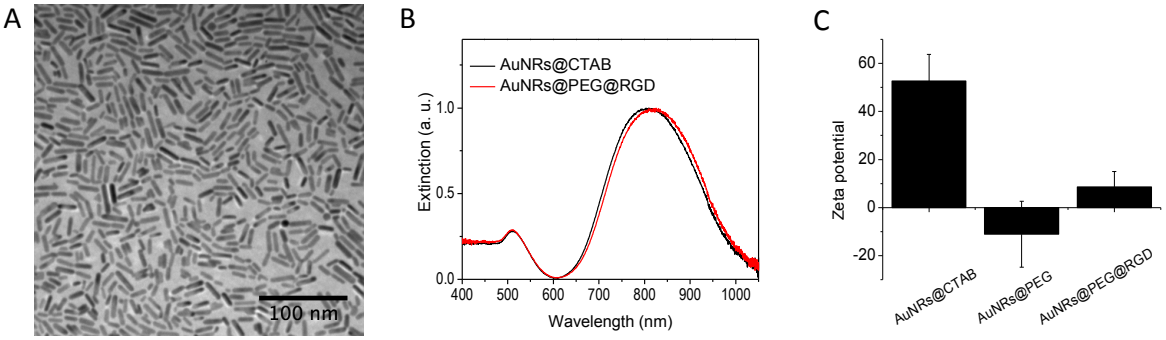
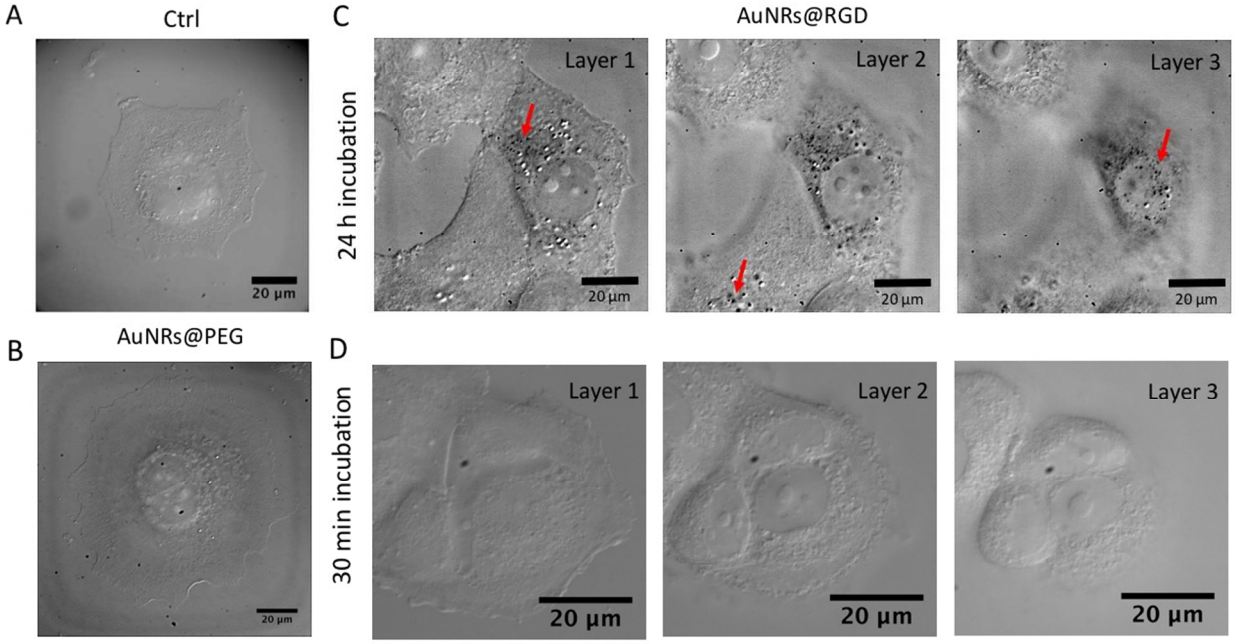


Figure S1. AuNR synthesis and characterization. (A) TEM image of AuNRs. (B) UV-Vis spectra of AuNRs with different surface ligands. Black, the as-synthesized AuNRs with CTAB on the surface; red, AuNRs conjugated with PEG and RGD. (C) Zeta potential of AuNRs before/after conjugations (n=3).


A
Ctrl
20 μm
B
AuNRs@PEG
20 μm
C
AuNRs@RGD
24 h incubation
Layer 1
20 μm
Layer 2
20 μm
Layer 3
20 μm
D
30 min incubation
Layer 1
20 μm
Layer 2
20 μm
Layer 3
20 μm

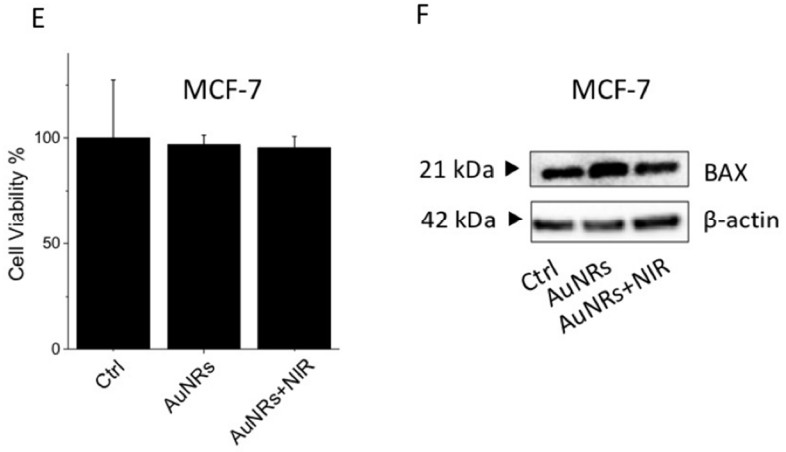

E
MCF-7
Cell Viability %
100
50
0
Ctrl
AuNRs
AuNRs+NIR
F
MCF-7
21 kDa
BAX
42 kDa
β-actin
Ctrl
AuNRs
AuNRs+NIR

Figure S2. Cellular uptake and cytotoxicity of AuNRs treatments of MCF-7 cells. (A-D) Differential interference contrast (DIC) microscopic images of MCF-7 cells without AuNRs (A) and with AuNRs@PEG (B), or with AuNRs@RGD for 24 h (C) and 30 min (D) with Z-scanning. The red arrow indicates the locations of AuNRs. Three layers (layer 1 locates close to the bottom (surface), layer 2 locates in the middle of cells, layer 3 locates in the top of cells) indicate clearly the internalization of AuNRs. (E) Cell viability of MCF-7 cells after 24 h AuNRs and AuNRs+NIR treatments (n=3). (F) Western blotting for the BAX protein upon different treatments (after 24 h).

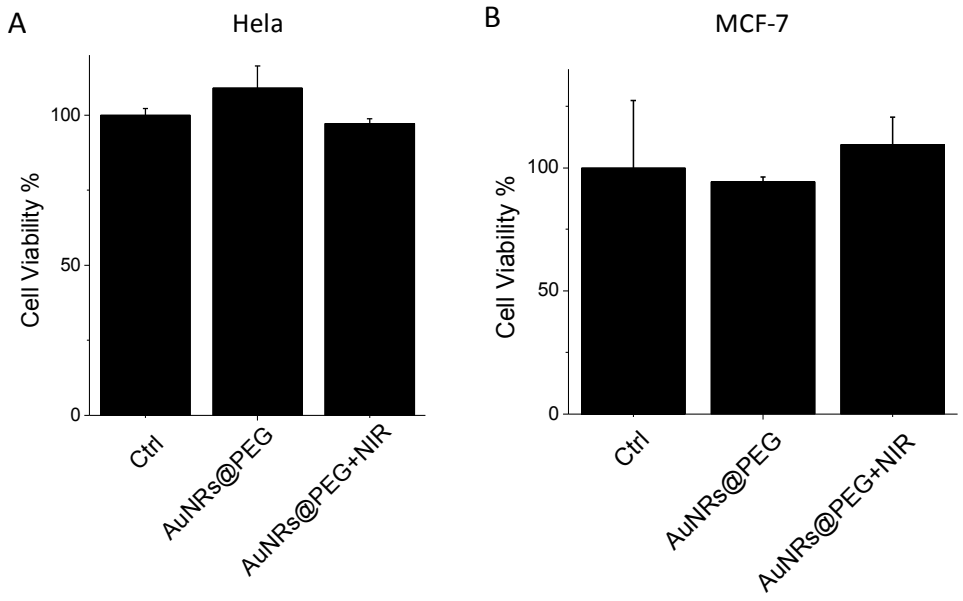


Figure S3. Cytotoxicity of non-specifically targeted AuNRs (AuNRs@PEG) on HeLa and MCF-7 cells (n=3).

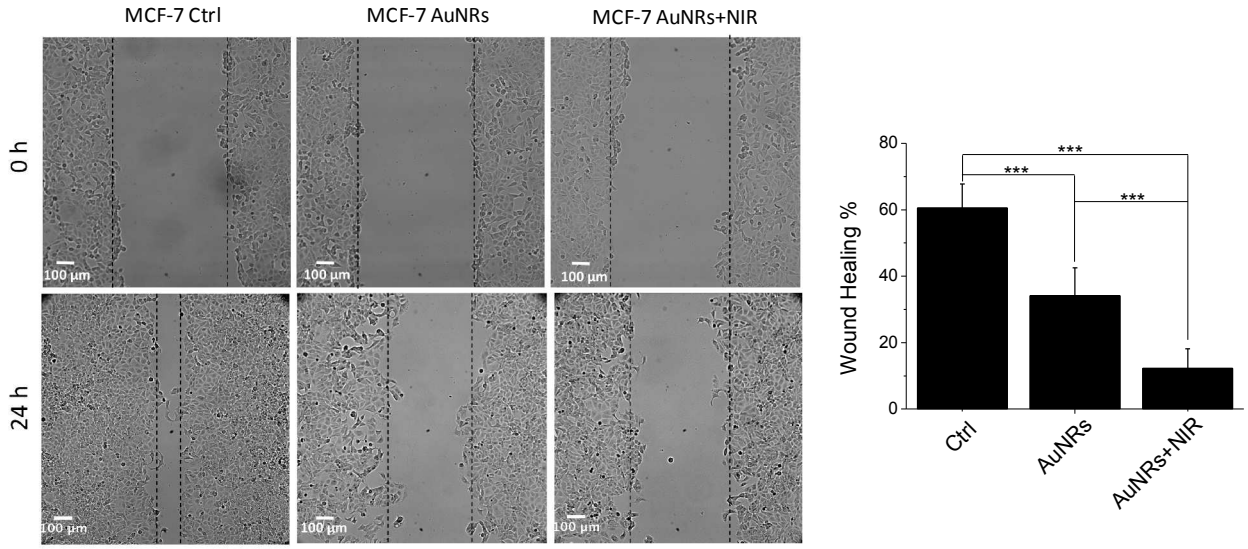


Figure S4. Scratch assay images of MCF-7 cells (control, AuNRs treatment, and AuNRs/PPTT treatment) at 0 and 24 h (n=6). Student's t test was used for statistical analysis. All values are expressed as means ± standard errors of the mean (SEM). ***$p < 0.001$, **$p < 0.01$, *$p<0.05$.

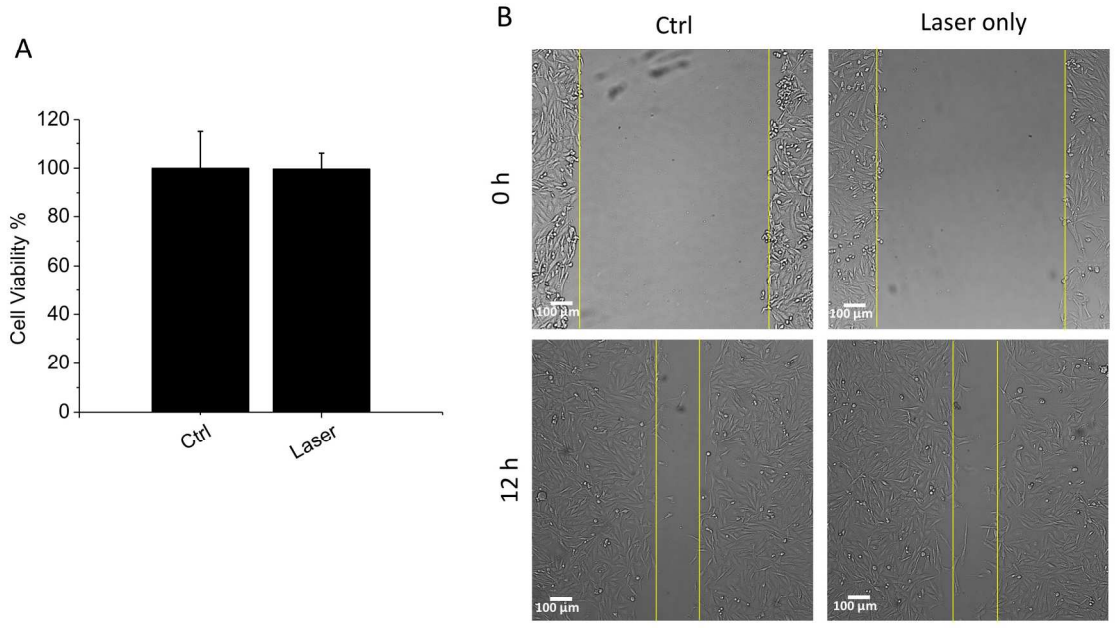


Figure S5. (A) HeLa cell viability comparing Ctrl (no treatment) and Laser treatment (no AuNRs added, n=3). (B) Scratch assay images of HeLa cells (Ctrl and Laser treatment) at 0 and 12 h.

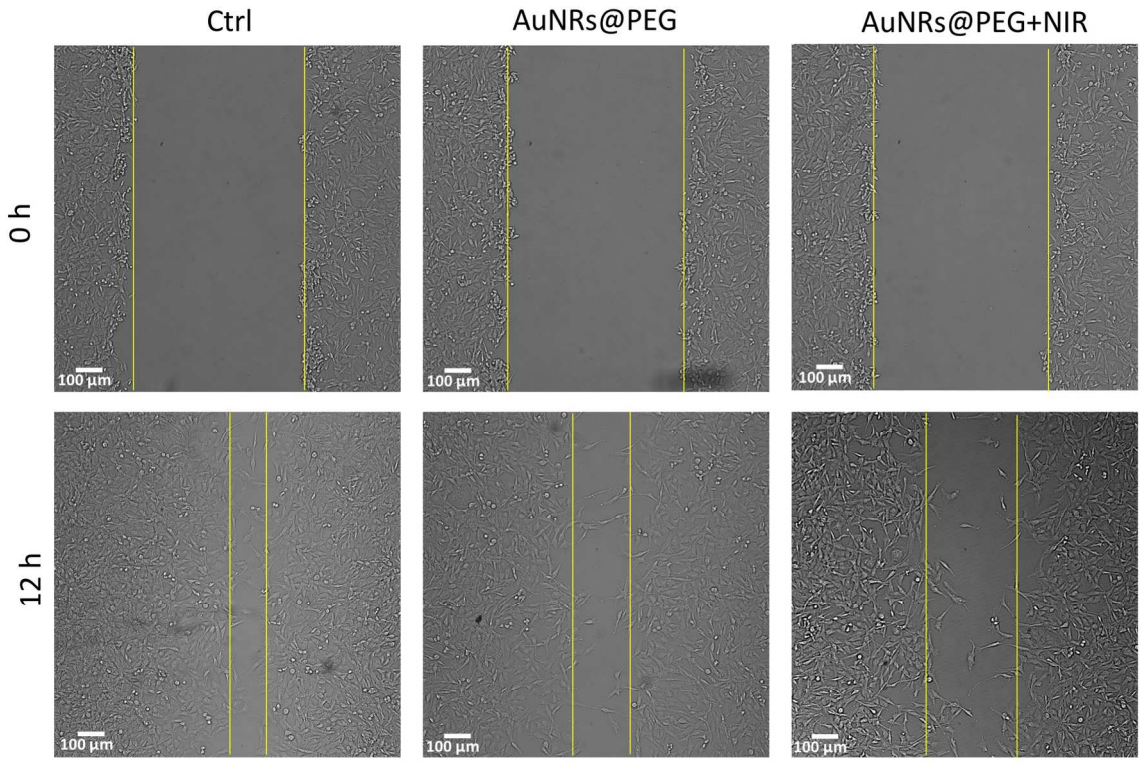


Figure S6. Scratch assay images of HeLa cells (Ctrl, AuNRs@PEG, AuNRs@PEG+NIR treatments) at 0 and 12 h.

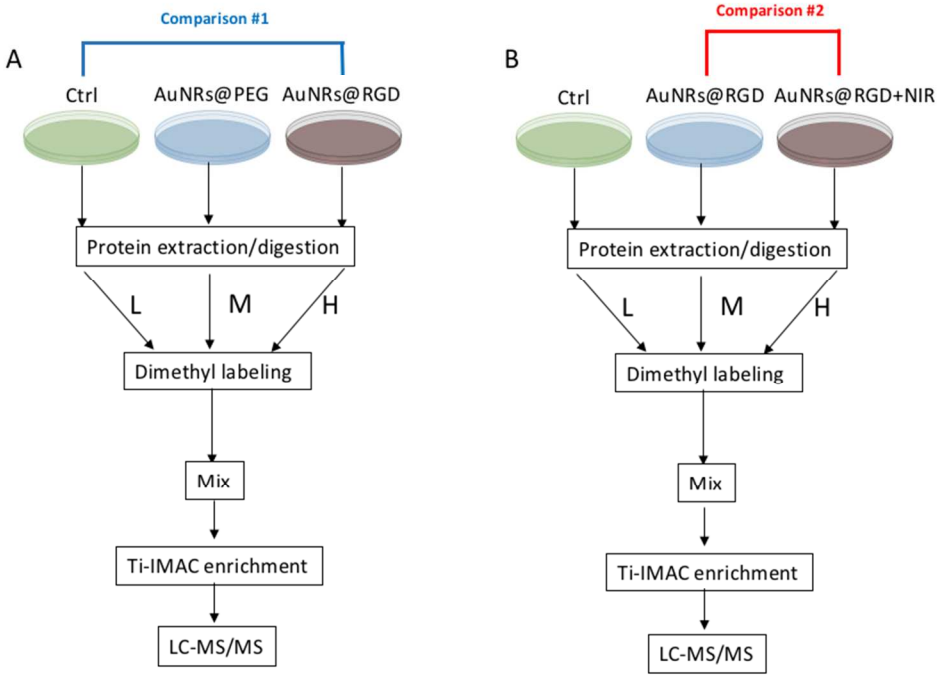


Figure S7. Experimental design of quantitative phosphoproteomics. Two sets of experiments were performed to examine the AuNRs and the photothermal effects separately. (A) Studying the protein phosphorylation upon treatments of AuNRs@PEG (30 min stimulation) and AuNRs@RGD (30 min stimulation). (B) Studying the protein phosphorylation upon photothermal effects (30 min stimulation) after overnight incubating the cells with AuNRs@RGD. The comparisons #1 and #2 are indicated in Figure 2.

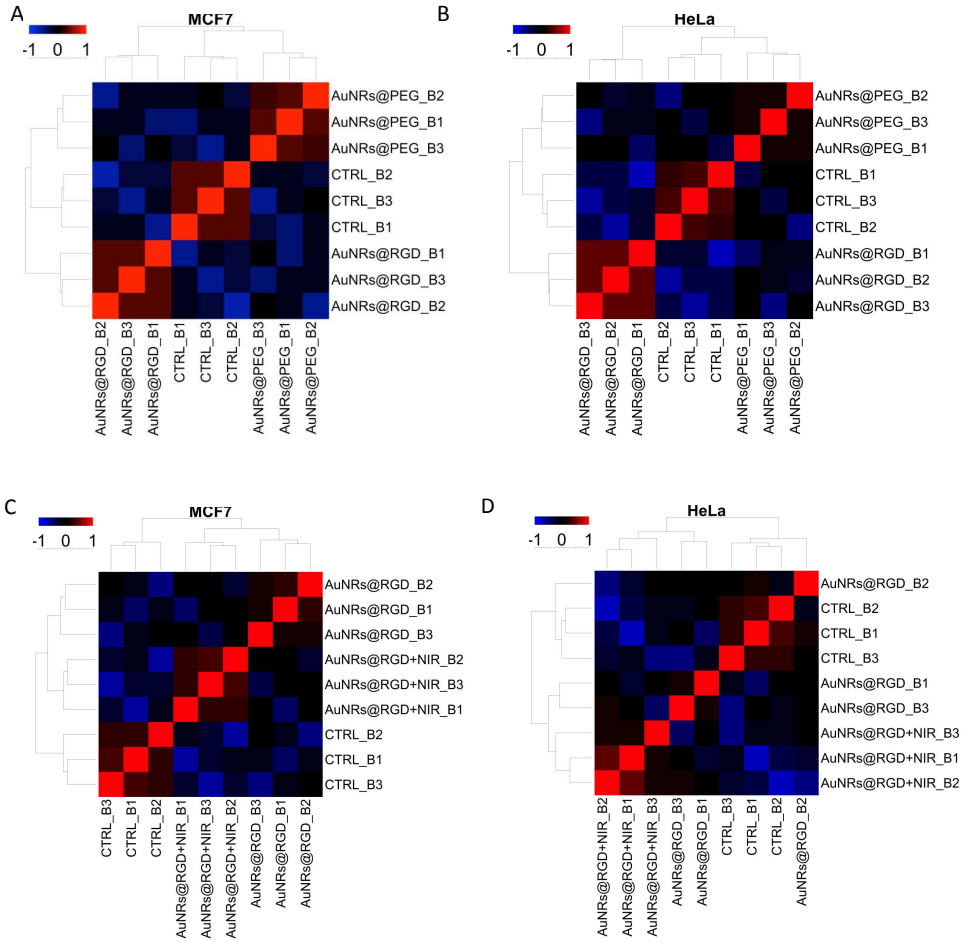


Figure S8. Clustering analysis of the samples. (A) AuNRs@PEG, AuNRs@RGD and control for MCF-7. (B) AuNRs@PEG, AuNRs@RGD and control for HeLa. (C) AuNRs@RGD, AuNRs@RGD+NIR, and control for MCF-7. (D) AuNRs@RGD, AuNRs@RGD+NIR, and control for HeLa. B1, B2, and B3 in the figures indicate the three biological replications.

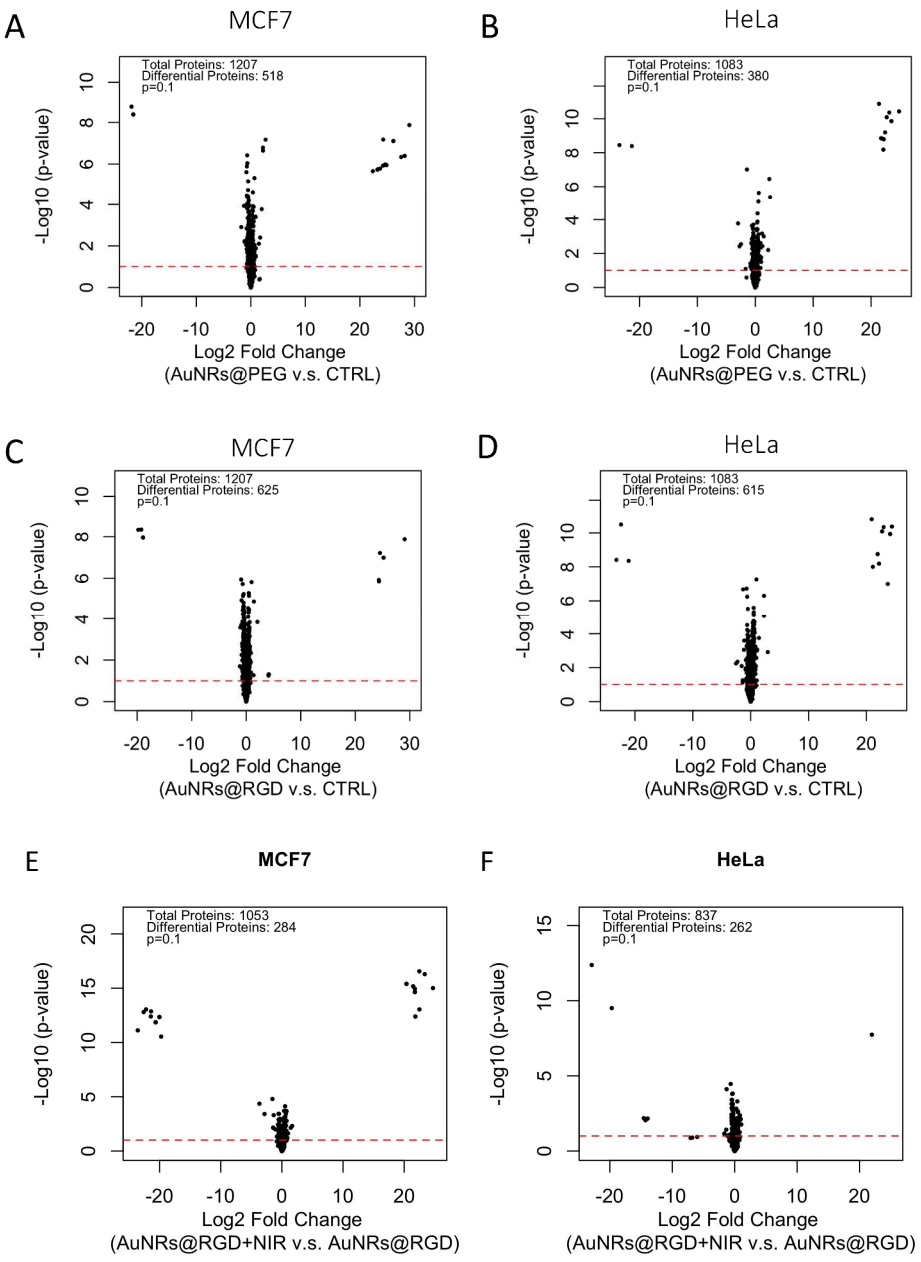


Figure S9. Volcano plots of proteins under perturbation by (A) AuNRs@PEG for MCF-7, (B) AuNRs@PEG for HeLa, (C) AuNRs@RGD for MCF-7, (D) AuNRs@RGD for HeLa, (E) AuNRs@RGD+NIR for MCF-7 and (F) AuNRs@RGD+NIR for HeLa.

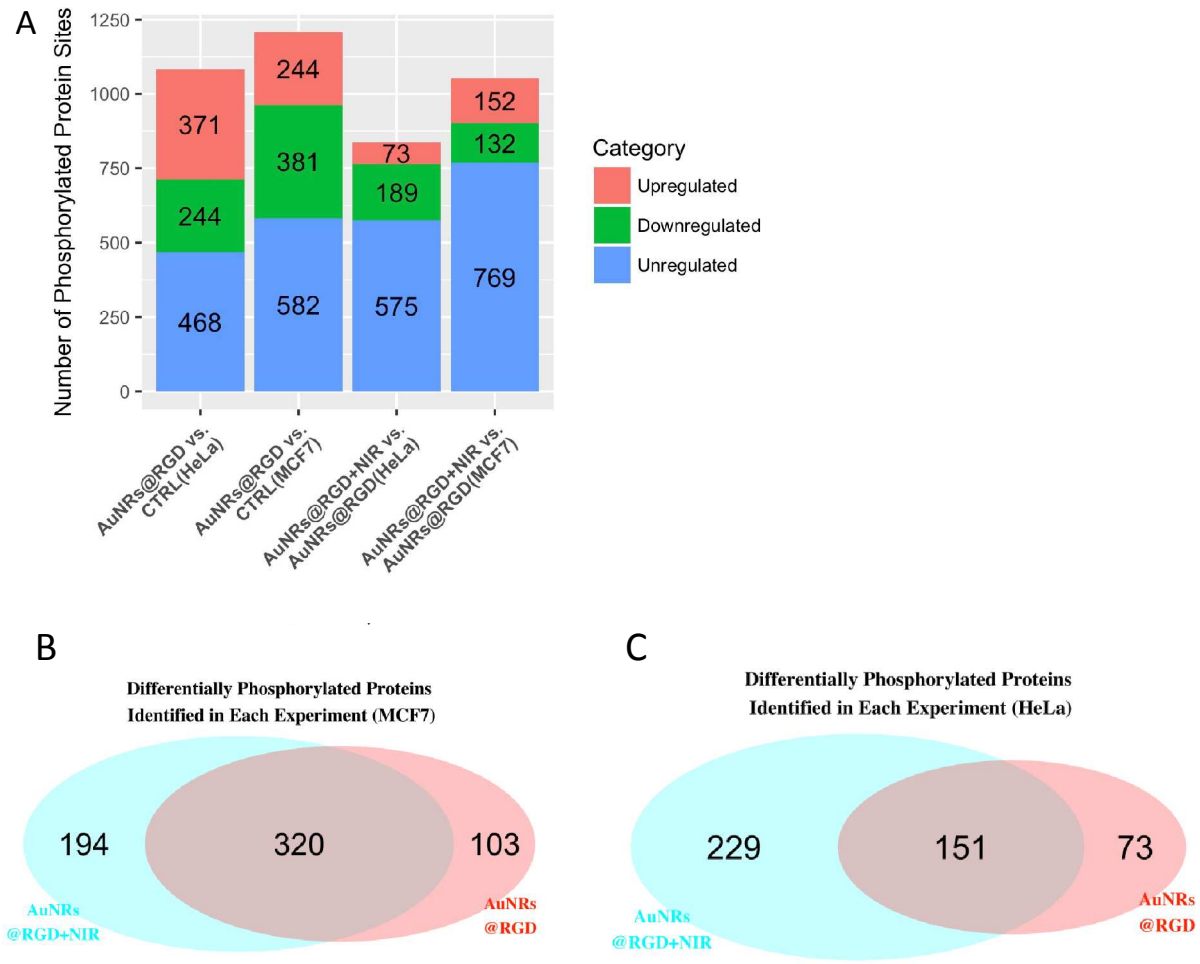
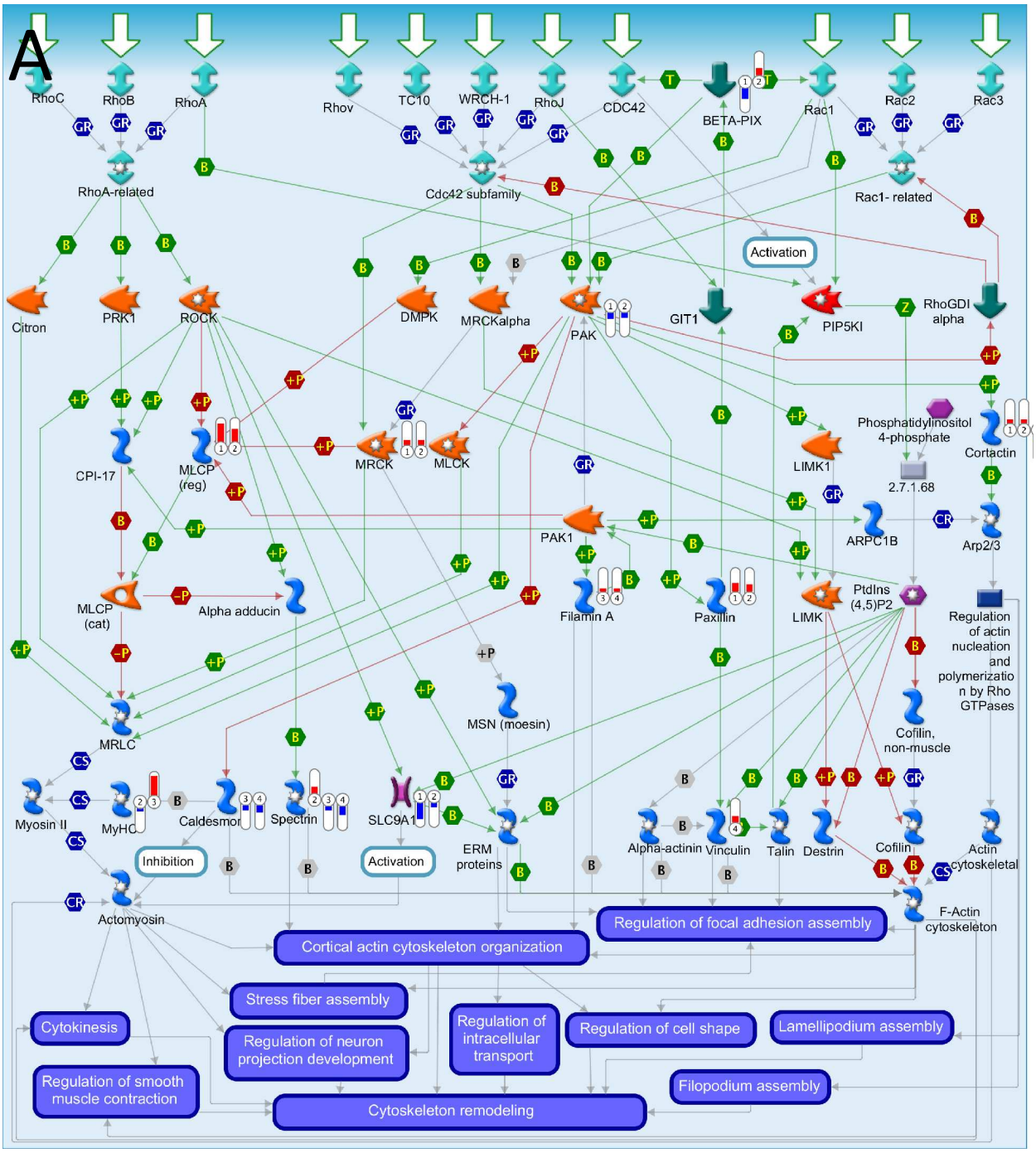
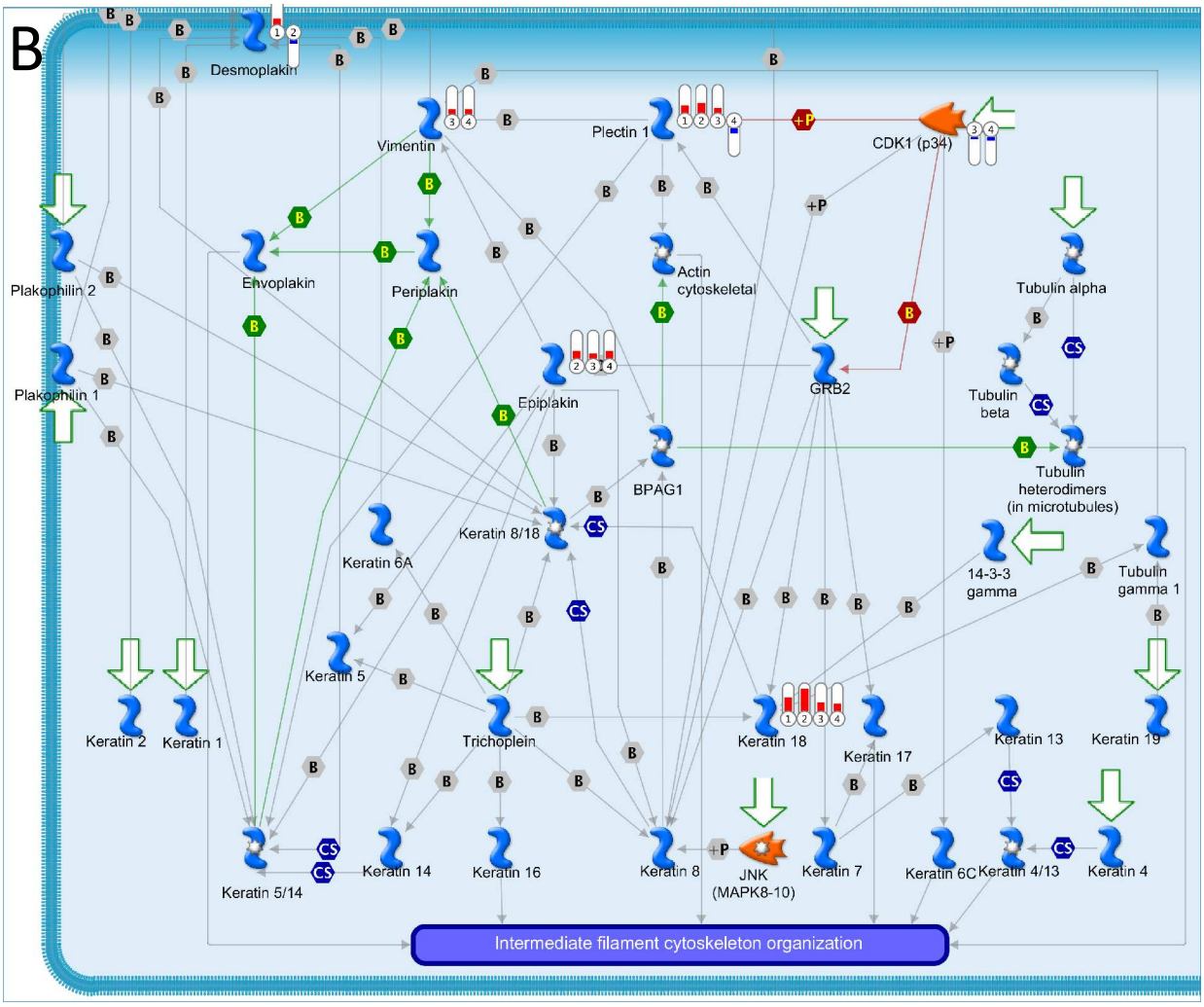
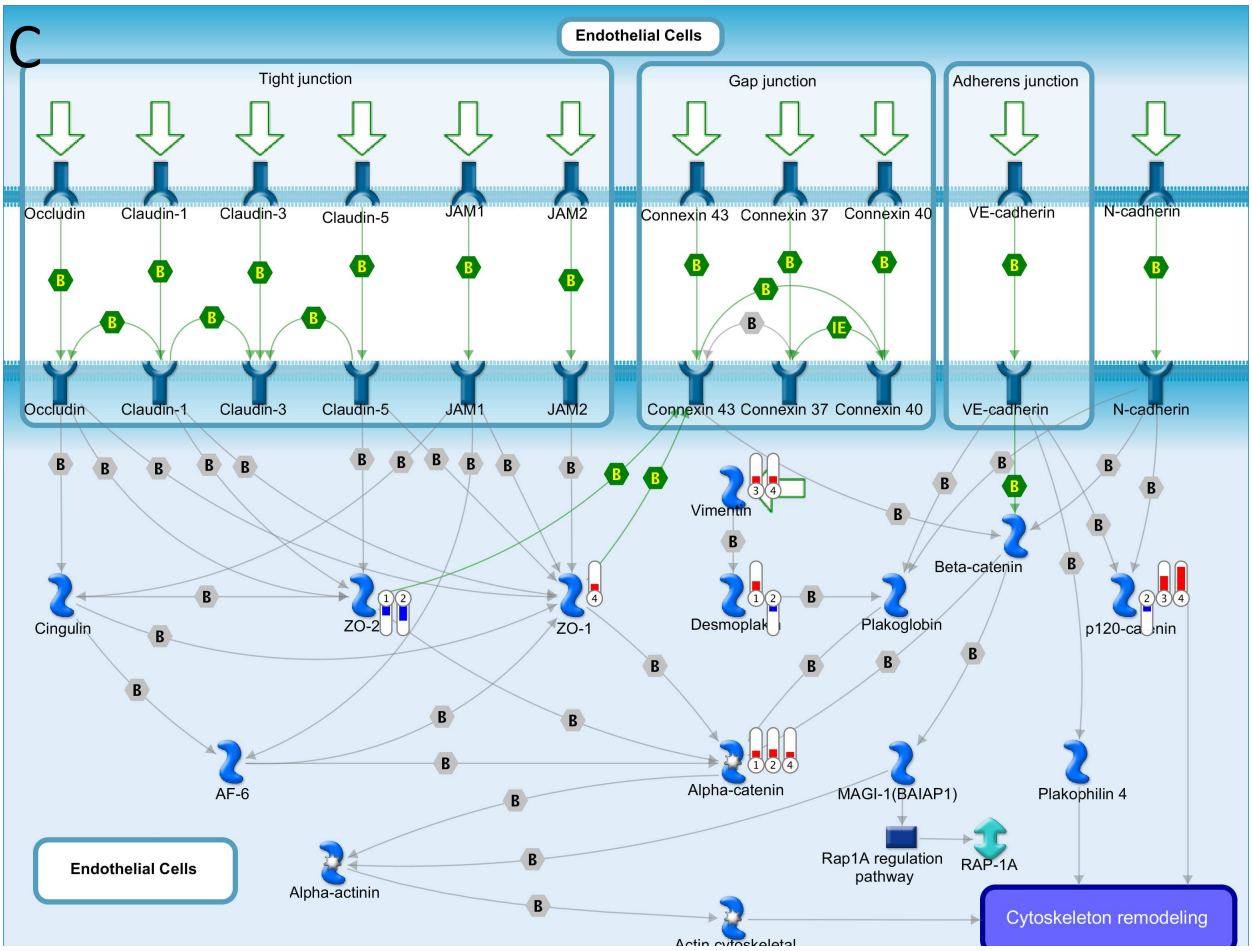


Figure S10. (A) Numbers of regulated/unregulated phosphorylated sites identified in each experiment. (B-C) Venn diagram showing the comparison of differentially phosphorylated sites identified in each experiment.


A
RhoC
RhoB
RhoA
Rhov
TC10
WRCH-1
RhoJ
CDC42
BETA-PIX
Rac1
Rac2
Rac3
RhoA-related
Cdc42 subfamily
Rac1- related
Activation
Citron
PRK1
ROCK
DMPK
MRCKalpha
PAK
GIT1
PIP5KI
RhoGDI alpha
Phosphatidylinositol 4-phosphate
Cortactin
CPI-17
MLCP (reg)
MRCK
MLCK
LIMK1
2.7.1.68
PAK1
ARPC1B
Arp2/3
MLCP (cat)
Alpha adducin
Filamin A
Paxillin
LIMK
PtdIns (4,5)P2
Regulation of actin nucleation and polymerization by Rho GTPases
MSN (moesin)
Cofilin, non-muscle
MRLC
Myosin II
MyHC
Caldesmon
Spectrin
SLC9A1
ERM proteins
Alpha-actinin
Vinculin
Talin
Destrin
Cofilin
Actin cytoskeletal
Inhibition
Activation
Actomyosin
F-Actin cytoskeleton
Regulation of focal adhesion assembly
Cortical actin cytoskeleton organization
Stress fiber assembly
Cytokinesis
Regulation of neuron projection development
Regulation of intracellular transport
Regulation of cell shape
Lamellipodium assembly
Regulation of smooth muscle contraction
Filopodium assembly
Cytoskeleton remodeling


B
Desmoplakin
Vimentin
Plectin 1
CDK1 (p34)
Plakophilin 2
Envoplakin
Periplakin
Actin cytoskeletal
Tubulin alpha
Plakophilin 1
Epiplakin
GRB2
Tubulin beta
BPAG1
Tubulin heterodimers (in microtubules)
Keratin 8/18
Keratin 6A
14-3-3 gamma
Tubulin gamma 1
Keratin 5
Keratin 2
Keratin 1
Trichoplein
Keratin 18
Keratin 17
Keratin 13
Keratin 19
Keratin 5/14
Keratin 14
Keratin 16
Keratin 8
JNK (MAPK8-10)
Keratin 7
Keratin 6C
Keratin 4/13
Keratin 4
Intermediate filament cytoskeleton organization


C
Endothelial Cells
Tight junction
Gap junction
Adherens junction
Occludin
Claudin-1
Claudin-3
Claudin-5
JAM1
JAM2
Connexin 43
Connexin 37
Connexin 40
VE-cadherin
N-cadherin
Vimentin
Beta-catenin
Cingulin
ZO-2
ZO-1
Desmoplak
Plakoglobin
p120-ca nin
AF-6
Alpha-catenin
MAGI-1(BAIAP1)
Plakophilin 4
Rap1A regulation pathway
RAP-1A
Alpha-actinin
Actin cytoskeletal
Cytoskeleton remodeling
Endothelial Cells

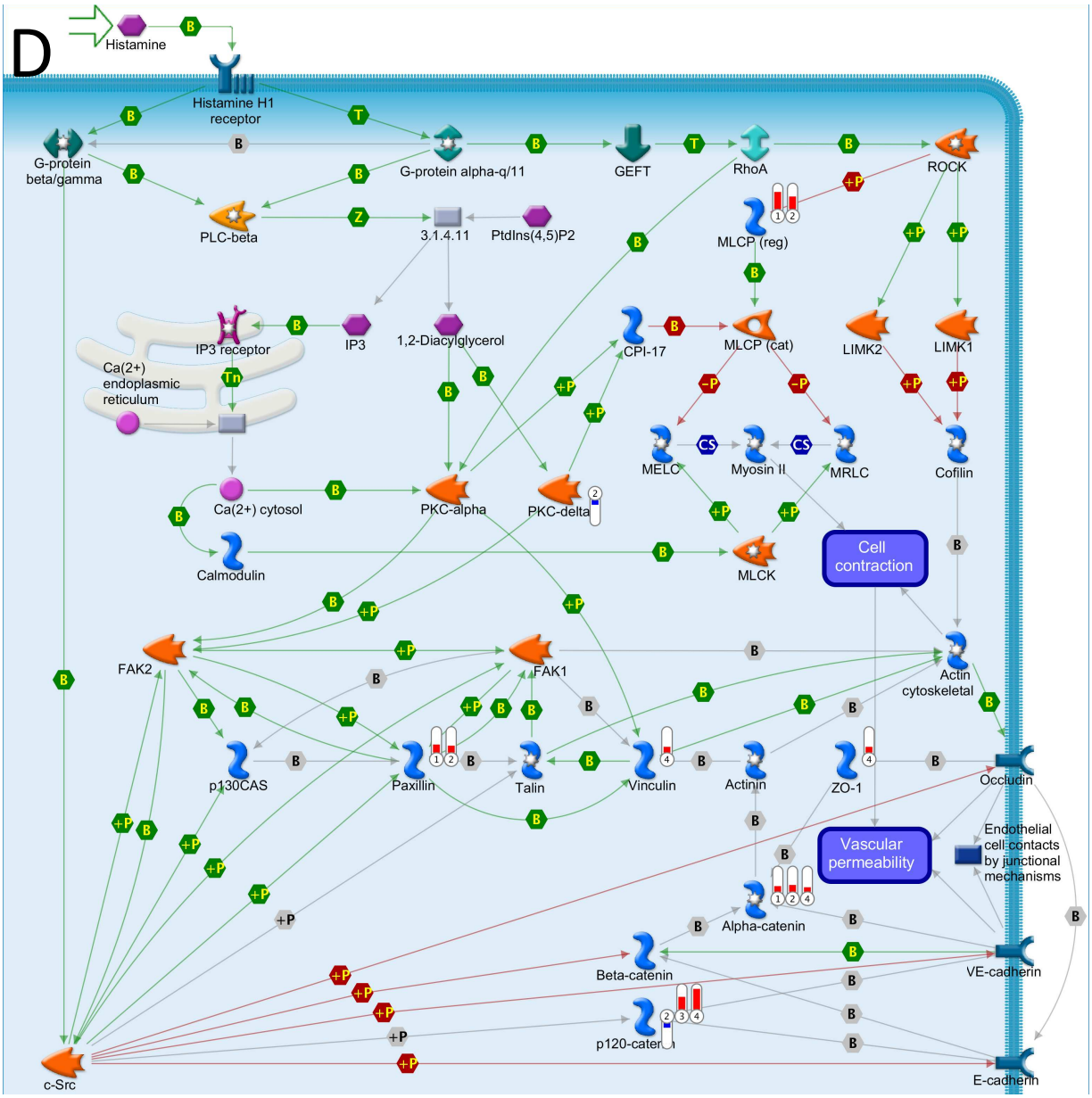
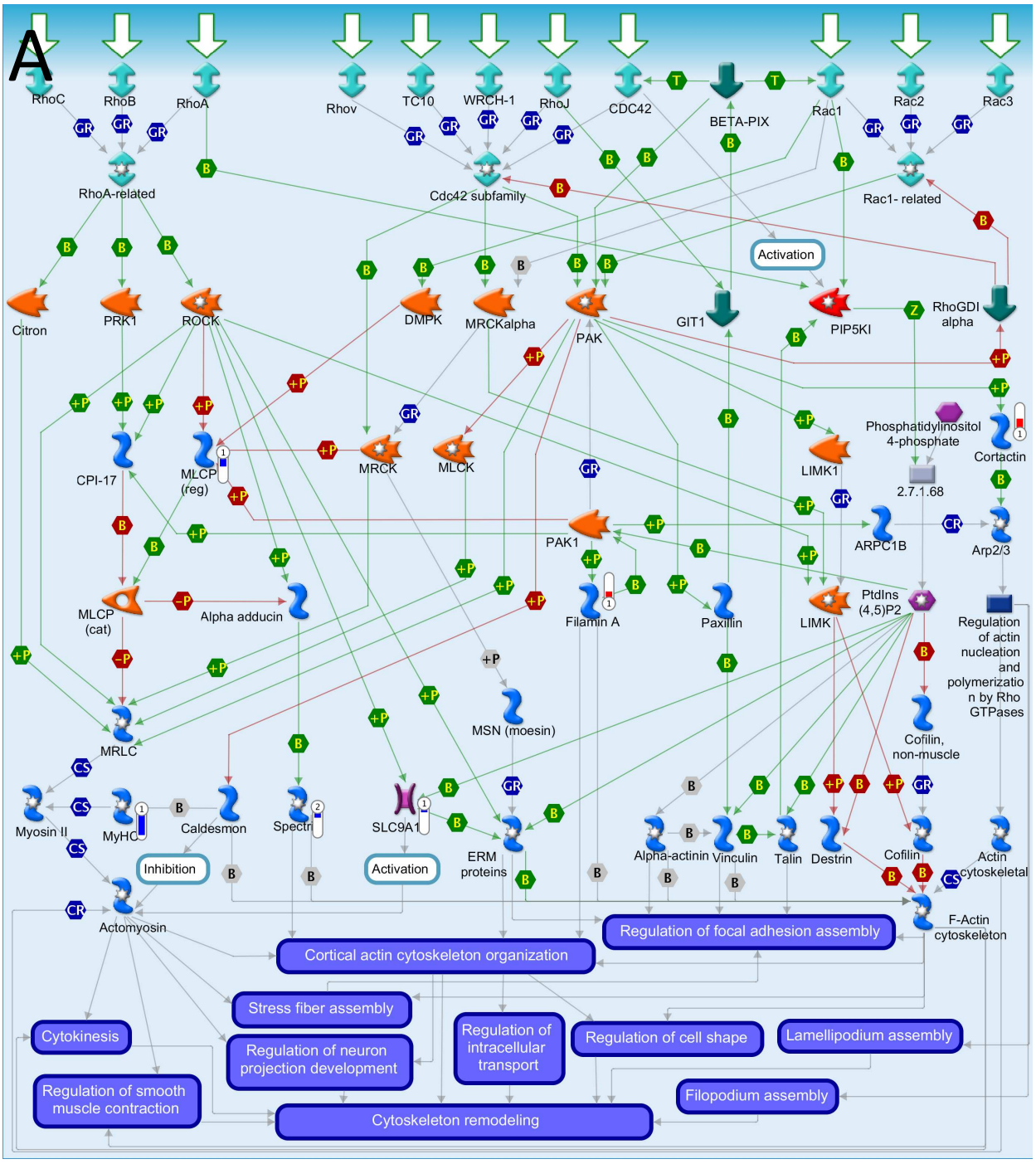


Figure S11. Key pathways perturbed by AuNRs (*vs* control group) identified with MetaCore from Thomson Reuters. In the thermometer sign, red means up-regulation and blue means down-regulation.1 refers to AuNRs@PEG (MCF-7), 2 refers to AuNRs@RGD (MCF-7), 3 refers to AuNRs@PEG (HeLa) and 4 refers to AuNRs@RGD (HeLa). The thermometers are filled to various degrees, corresponding to the amount by which the markers were up-regulated or down-regulated. (A) Pathway map of "Cytoskeleton remodeling_Regulation of actin cytoskeleton organization by the kinase effectors of Rho GTPases" (B) Pathway map of "Cytoskeleton remodeling_Keratin filaments." (C) Pathway map of "Cell adhesion_Endothelial cell contacts by junctional mechanisms." (D) Pathway map of "Cell adhesion_Histamine H1 receptor signaling in the interruption of cell barrier integrity."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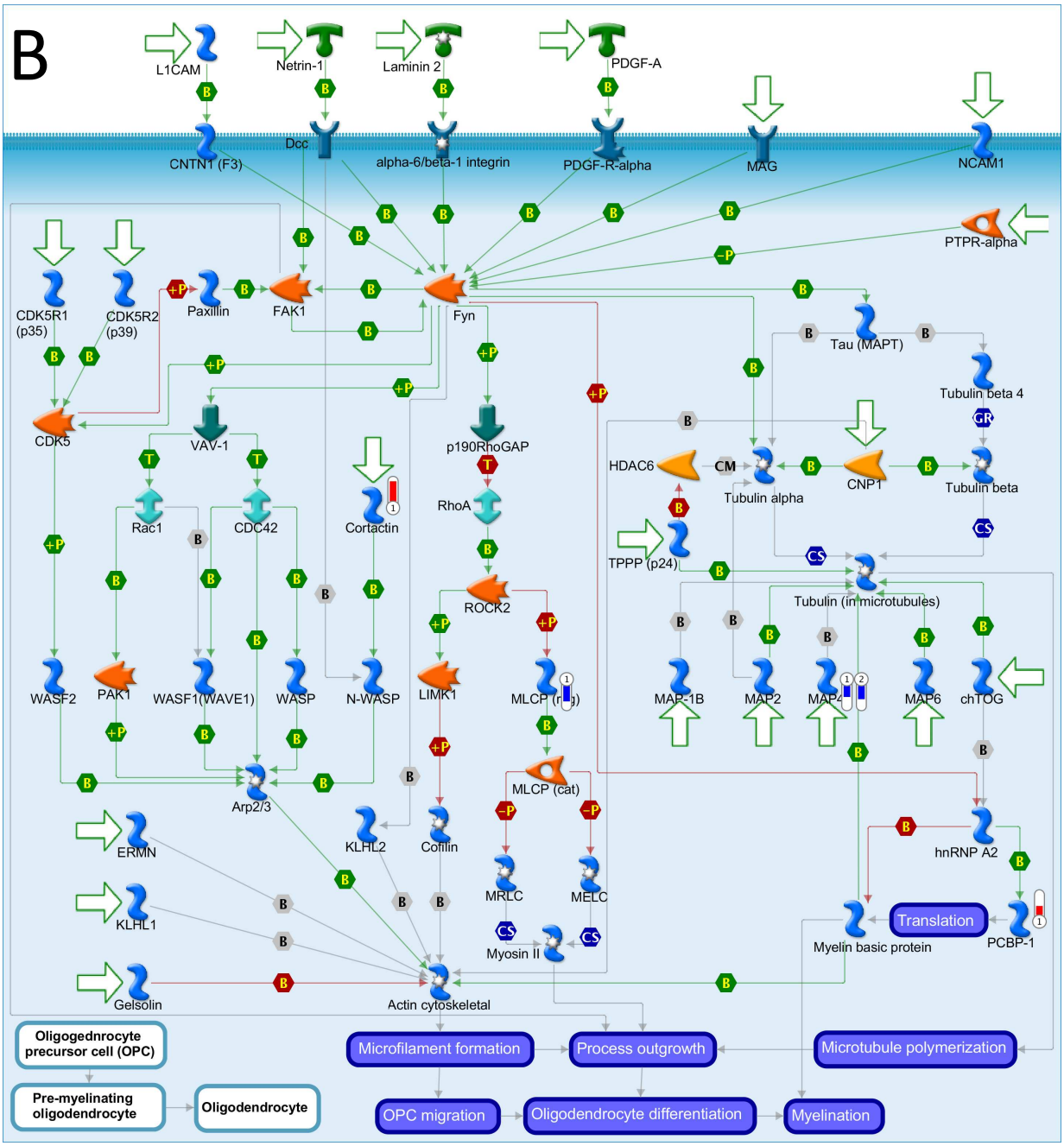
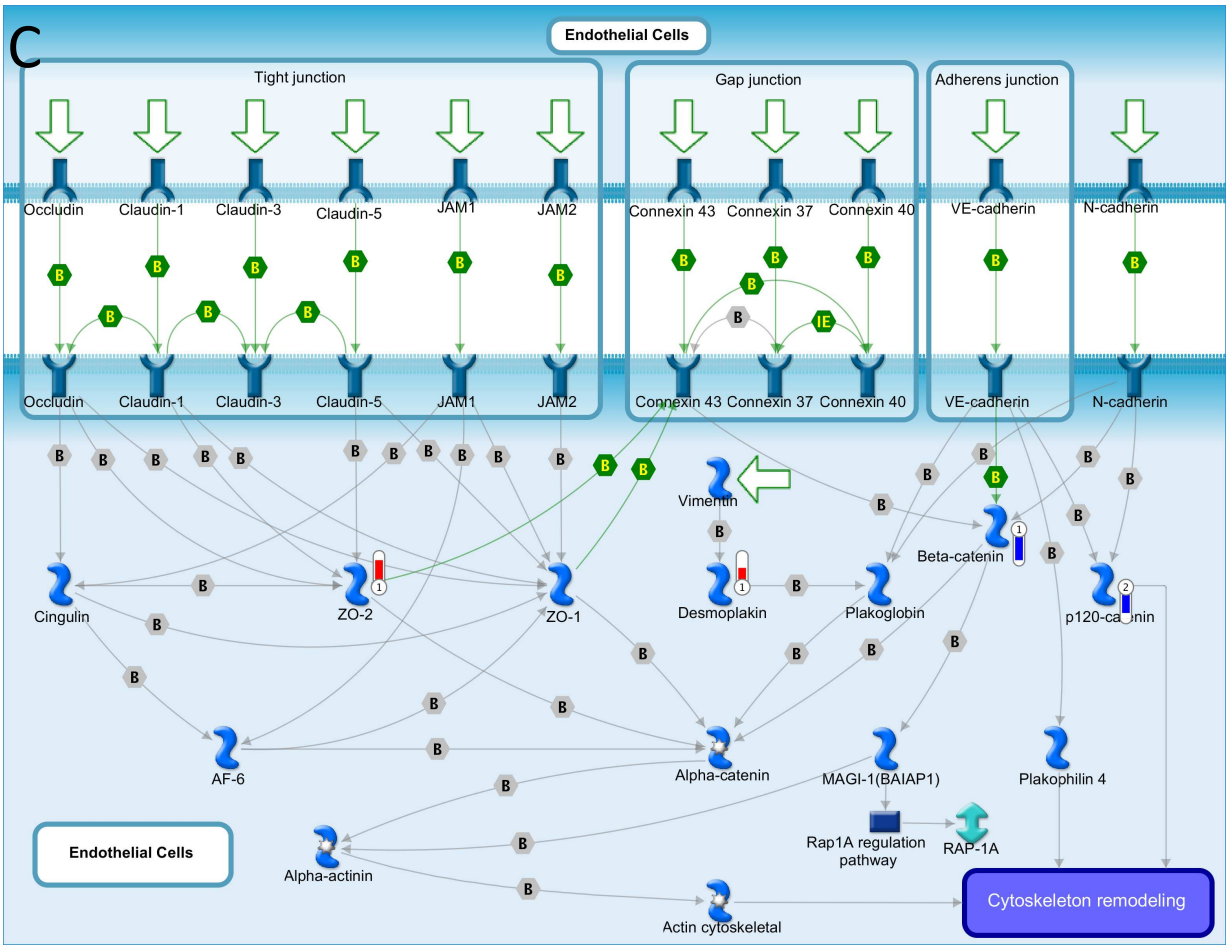
RhoC
RhoB
RhoA
Rhov
TC10
WRCH-1
RhoJ
CDC42
BETA-PIX
Rac1
Rac2
Rac3
RhoA-related
Cdc42 subfamily
Rac1- related
Activation
Citron
PRK1
ROCK
DMPK
MRCKalpha
PAK
GIT1
PIP5KI
RhoGDI alpha
CPI-17
MLCP (reg)
MRCK
MLCK
LIMK1
Phosphatidylinositol 4-phosphate
2.7.1.68
Cortactin
PAK1
ARPC1B
Arp2/3
MLCP (cat)
Alpha adducin
Filamin A
Paxillin
LIMK
PtdIns (4,5)P2
Regulation of actin nucleation and polymerization by Rho GTPases
MRLC
MSN (moesin)
Cofilin, non-muscle
Myosin II
MyHC
Caldesmon
Spectrin
SLC9A1
ERM proteins
Alpha-actinin
Vinculin
Talin
Destrin
Cofilin
Actin cytoskeletal
Inhibition
Activation
Actomyosin
F-Actin cytoskeleton
Regulation of focal adhesion assembly
Cortical actin cytoskeleton organization
Stress fiber assembly
Cytokinesis
Regulation of neuron projection development
Regulation of intracellular transport
Regulation of cell shape
Lamellipodium assembly
Regulation of smooth muscle contraction
Filopodium assembly
Cytoskeleton remodeling


B
L1CAM
Netrin-1
Laminin 2
PDGF-A
CNTN1 (F3)
Dcc
alpha-6/beta-1 integrin
PDGF-R-alpha
MAG
NCAM1
PTPR-alpha
CDK5R1 (p35)
CDK5R2 (p39)
Paxillin
FAK1
Fyn
Tau (MAPT)
Tubulin beta 4
CDK5
VAV-1
p190RhoGAP
HDAC6
CM
Tubulin alpha
CNP1
Tubulin beta
Rac1
CDC42
Cortactin
RhoA
TPPP (p24)
Tubulin (in microtubules)
ROCK2
WASF2
PAK1
WASF1(WAVE1)
WASP
N-WASP
LIMK1
MLCP (r
MAP-1B
MAP2
MAP4
MAP6
chTOG
Arp2/3
MLCP (cat)
ERMN
KLHL2
Cofilin
hnRNP A2
MRLC
MELC
KLHL1
Translation
Myosin II
Myelin basic protein
PCBP-1
Gelsolin
Actin cytoskeletal
Oligogednrocyte precursor cell (OPC)
Microfilament formation
Process outgrowth
Microtubule polymerization
Pre-myelinating oligodendrocyte
Oligodendrocyte
OPC migration
Oligodendrocyte differentiation
Myelination


C
Endothelial Cells
Tight junction
Gap junction
Adherens junction
Occludin
Claudin-1
Claudin-3
Claudin-5
JAM1
JAM2
Connexin 43
Connexin 37
Connexin 40
VE-cadherin
N-cadherin
Vimentin
Beta-catenin
Cingulin
ZO-2
ZO-1
Desmoplakin
Plakoglobin
p120-catenin
AF-6
Alpha-catenin
MAGI-1(BAIAP1)
Plakophilin 4
Rap1A regulation pathway
RAP-1A
Alpha-actinin
Actin cytoskeletal
Cytoskeleton remodeling
Endothelial Cells

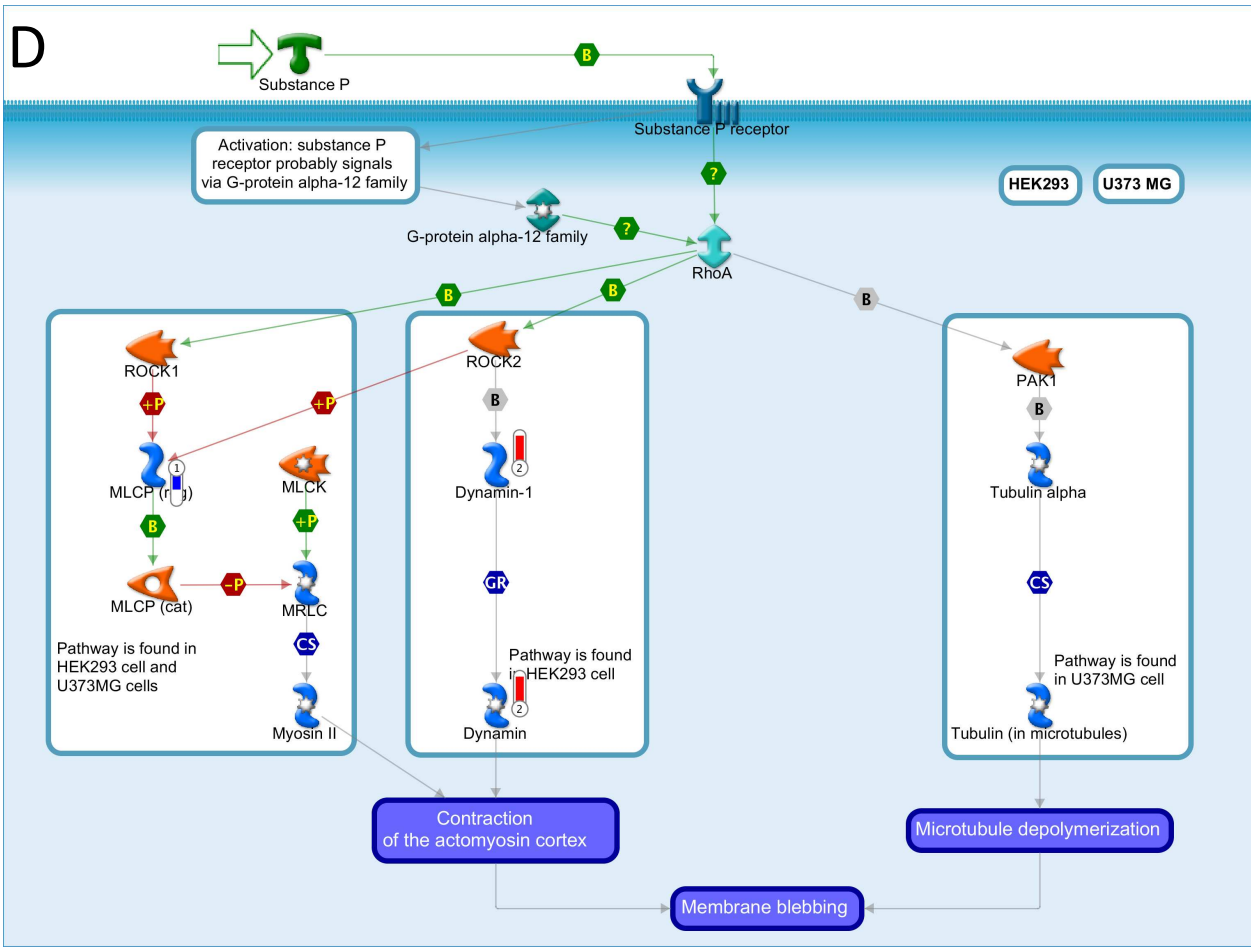


Figure S12. Key pathways perturbed by AuNRs+PPTT (*vs* AuNRs group). 1 refers to AuNRs-PPTT (MCF-7), 2 refers to AuNRs-PPTT (HeLa). (*A*) Pathway map of "Cytoskeleton remodeling_Regulation of actin cytoskeleton organization by the kinase effectors of Rho GTPases" (*B*) Pathway map of "Development_Regulation of cytoskeleton proteins in oligodendrocyte differentiation and myelination" (*C*) Pathway map of "Cell adhesion_Endothelial cell contacts by junctional mechanisms" (*D*) Pathway map of "Cytoskeleton remodeling_Substance P mediated membrane blebbing".

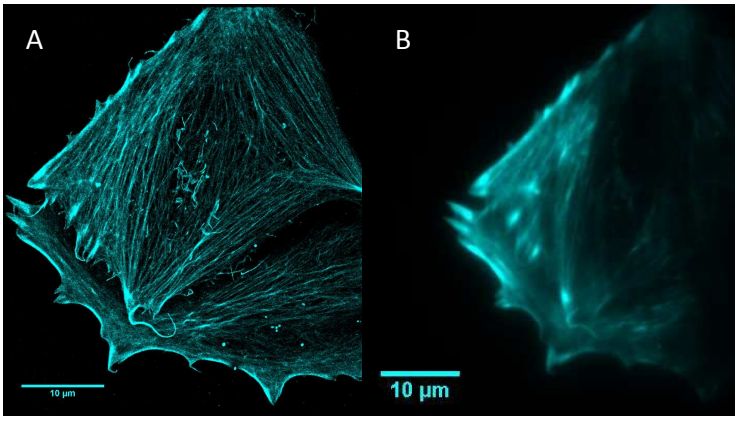


Figure S13. Comparison of the resolution of STORM (A) and conventional fluorescence microscopy imaging (B) for actin filaments.

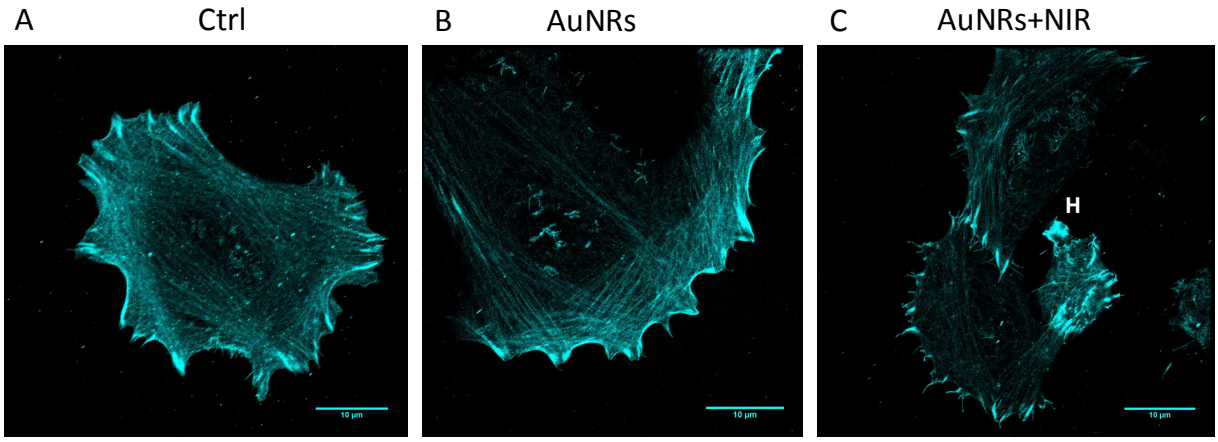


Figure S14. STORM images of actin filaments in individual HeLa cells.

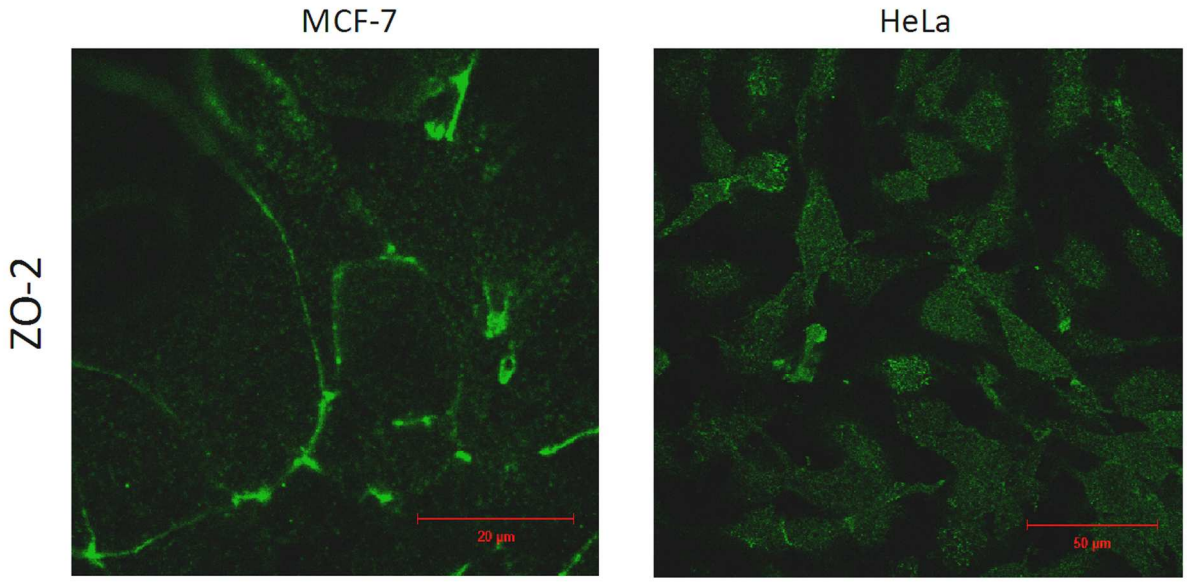


Figure S15. Low expression of tight junctions in HeLa cells compared with MCF-7 cells.

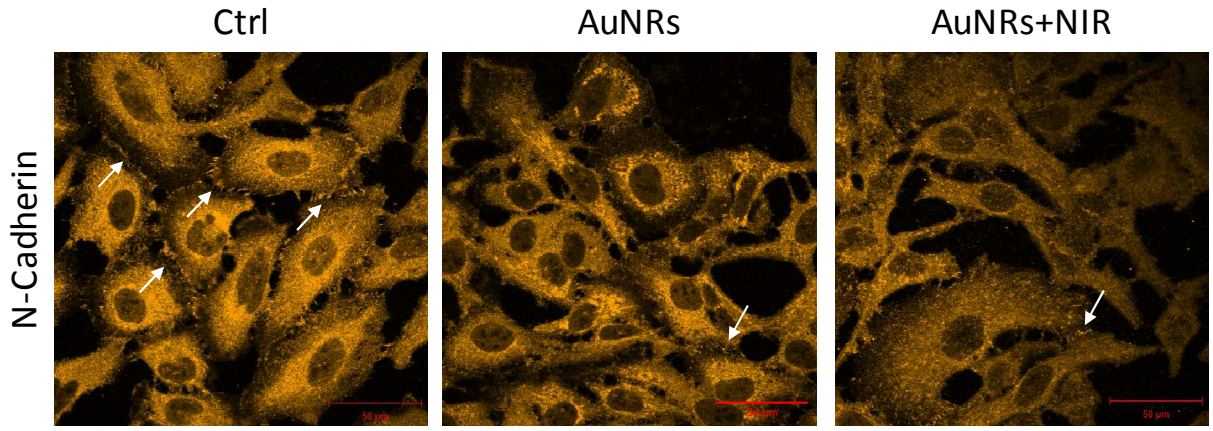


Figure S16. Immunofluorescence images of N-cadherin in HeLa cells before and after AuNRs or AuNRs/PPTT treatments. The arrows indicate the N-cadherin junctions.

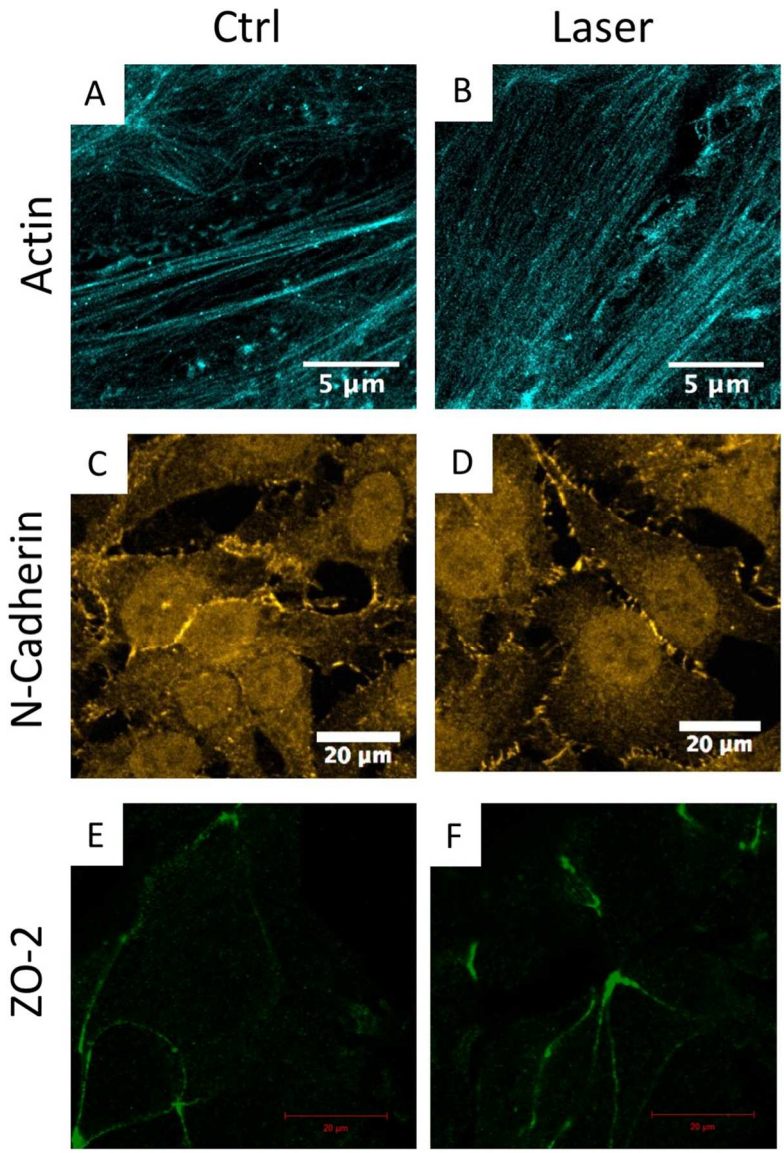


Figure S17. STORM images of actin filaments in the cell-cell junction for control (A) and laser control (no AuNRs) (B). Scale bar = 5 µm. Immunofluorescence images of N-cadherin in HeLa cells for control (C) and laser control (no AuNRs) (D). Scale bar = 20 µm. Immunofluorescence images of tight junction protein ZO-2 in MCF-7 cells, for control (E) and laser control (no AuNRs) (F). Scale bar = 20 µm.

Table S1. List of selected proteins with their altered phosphorylation sites.

Category		Protein	Protein Function	Phosphorylation sites altered	Phosphorylation sites function
Actin	Focal adhesions	Paxillin	Form focal adhesions	pS303, pS302, pS106, pS85	Increase of pS85 of paxillin could have an important function in cell adhesion [1, 2]
		Zyxin		pS258, pS288, and pS267	--
		Vinculin		pS290	--
	Myosin related proteins	MYH9	Form stress fibers and create a contraction force in cell migration [3]	pS1943	Phosphorylation status of MYH9 at Ser 1943 could alter cell motility , relating to cell junction [4]
		MLCP		pS299, pS445, pS871	The Ser 445 of MLCP is closely related to cell adhesion [5]
	Actin-binding proteins	Filamin	Actin filament crosslinking protein [6]	pS1084, pS1459, pS1432, pS2112	--
		Cortactin	Actin-nucleation-promoting factor[7]	pS39, pS52, pT33, pT35, pT45	--
		Drebrin	Induce stabilization of actin filaments [8]	pS142	--
Microtubule		MAP4	Promoting microtubule assembly, regulating cell invasion/migration [9]	pS1073, pS787, pS280, pS789	MAP4 pS1073 is related to cancer cell metastasis potential [10] and pS787 could promote tubulin polymerization [11] thereby changing the MT organization.
		MAP1B	Microtubule assembly [12]	pS248, pS250, pS345, pS352, pS367, pS552	--
		GSK3	Regulate microtubule dynamics [13]	pY279	--
Desmosome related intermediate filaments		Keratin 18	Keratin 18 and its filament partner keratin 8 are regarded as the most commonly found members of the intermediate filament family.	pS34, pT65, pS420, pS42	--

		Vimentin	A hallmark protein of epithelial to mesenchymal transition (EMT), which is related to the increase of migration and invasive properties [14, 15]	pS459, pS56, pT458	pS56 was reported with the function of cytoskeleton reorganization [16]
Kinases		Raf1	A MAP kinase kinase kinase (MAP3K), egulates Rho signaling and cell migration [17]	pS621	--
		MAP2K2	MAP kinase kinase family	pT394	--
		CDK1	Regulation of cell cycle progression and greatly related to cancer development [18]	pT14, pY15	--
Junction proteins	Tight junction proteins	ZO-1	Connect cytoskeletons of adjacent cells and act as barriers for the passage of molecules and ions [19]	pS125, pS131	--
		ZO-2		pS966, pS986, pS978, pS266, pS986, pS1159, pS130	--
	Catenins	α-Catenin	Form cell-cell adhesion complexes, anchoring actin cytoskeleton and interacting with cadherins [20].	pT654, pS641, pT634, pS652, pS655	S641 affects cell motility [21]
		β-Catenin		pT551 or pT556	--
		p120-Catenin		pS230, pS268, pS349, pS352, pS252	--
	Desmosomes	Desmoplakin	Desmosome protein, confer strong cell–cell adhesion,	pS22, pY172, pT173, pS166, pS176, pS2821, pS2825	--
		Epiplakin	Epiplakin, and plectin connect and reorganize the intermediate filaments, such as keratins [22-24], to the desmosome, which are also closely related to cell motility [25 26]	pS2716	--
		Plectin		pS4386, pS4385, pS4616, pS4396, pS4389	--

-- No information

Supplementary References

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