Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Network-based analysis implies critical roles of microRNAs in the long-term cellular responses to gold nanoparticles

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Additional materials

Data file S1 (.xlsx format). List of miRNAs by module

Data file S2 (.xlsx format). Hub miRNAs by module

Data file S3 (.xlsx format). miRNA target genes by module

Data file S4 (.xlsx format). Inversely correlated miRNA-mRNA matched pairs

Data file S5 (.xlsx format). Enriched REACTOME biological pathways by module

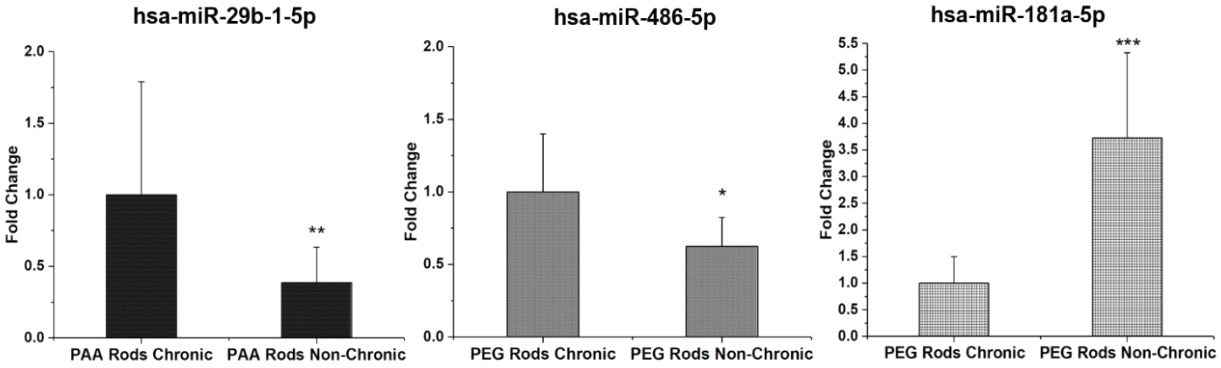

Fig. S1. **Validation of differentially expressed miRNAs by qPCR**. Difference in miRNA expression between two samples was assessed by t-test. Significant differences were indicated by *(*P* < 0.05), **(*P* < 0.01) and ***(*P* < 0.001). Samples were run in triplicate for each miRNA along with no-template controls.

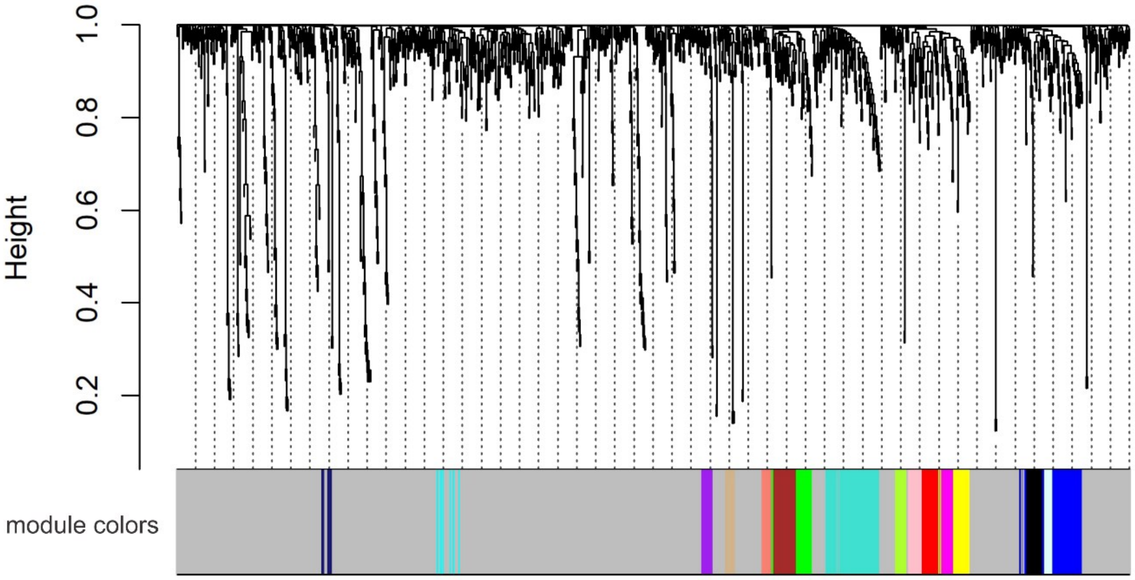

Fig. S2. Hierarchical clustering dendrogram showing miRNA co-expression modules defined by WGCNA. Each leaf (short vertical line) in the dendrogram corresponds to a miRNA and the branches represent expression modules of highly interconnected miRNAs with a color to indicate the module assignment. miRNAs not assigned to any of the modules are colored grey.

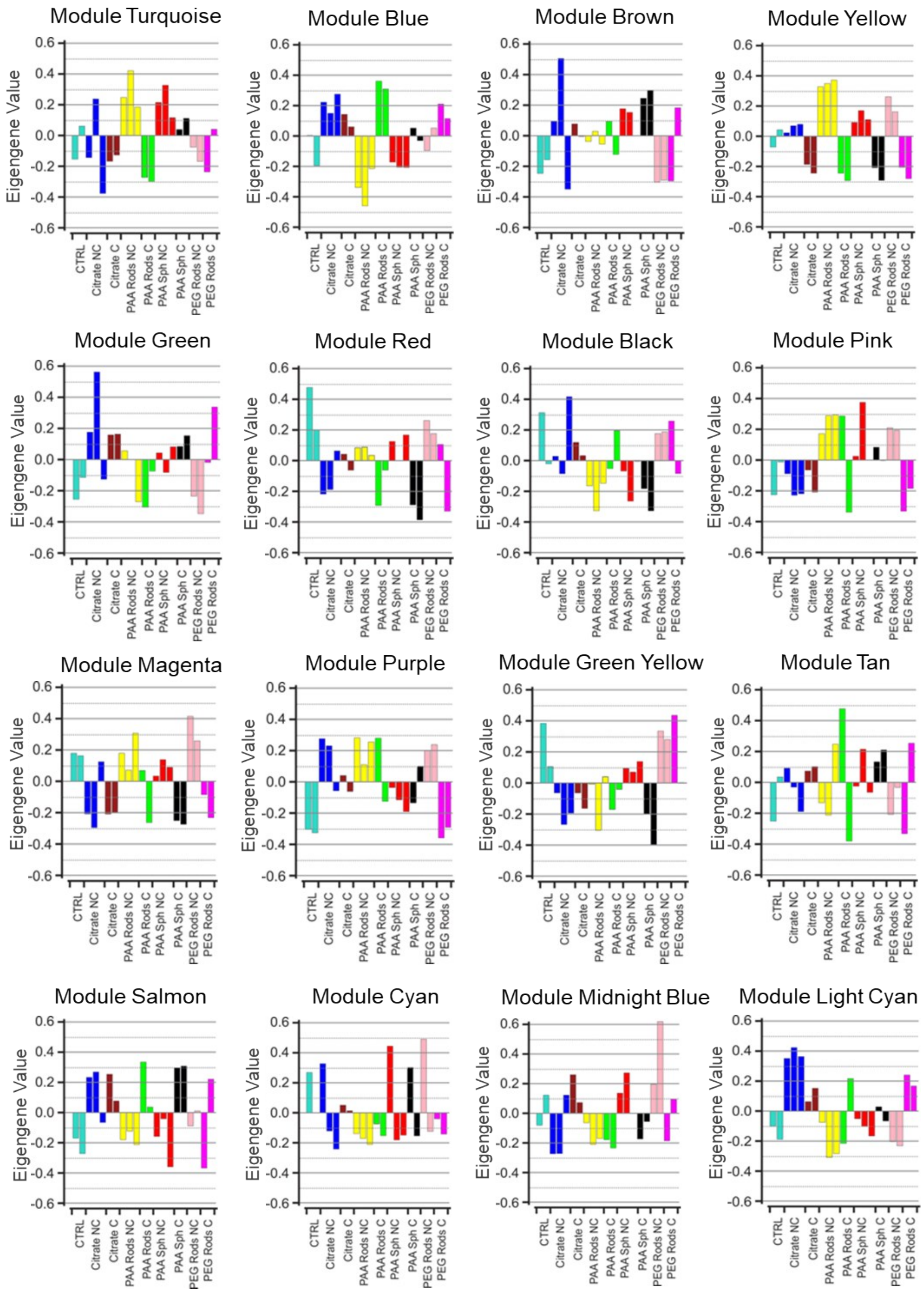

Fig. S3. Eigengene expression levels (y-axis) across samples treated with AuNPs and controls (x-axis) by module. C – chronic; NC - non-chronic.

Table S1. Characterization of AuNPs - Spheres

NP Type	Coating	Solvent	UV-Vis peak (nm)	DLS (nm)	TEM (nm)	Zeta potential (mV)
CIT Spheres	CIT	Water	522	31.2 ± 0.2	18.4 ± 2.0	-31.7 ± 1.5
	CIT	Media	527	65.4 ± 0.5	18.4 ± 2.0	-0.1 ± 6.1
PAA Spheres	PAA	Water	526	54.6 ± 0.2	18.4 ± 2.0	-33.2 ± 1.1
	PAA	Media	528	56.1 ± 0.3	18.4 ± 2.0	-14.1 ± 3.3

Table S2. Characterization of AuNPs - Rods

NP Type	Coating	Solvent	UV-Vis peak (nm)	TEM length (nm)	TEM width (nm)	Zeta potential (mV)
PAA Rods	PAA	Water	700	49.2 ± 5.0	17.0 ± 3.0	-53.8 ± 1.2
	PAA	Media	702	49.2 ± 5.0	17.0 ± 3.0	-18.0 ± 2.1
PEG Rods	PEG	Water	689	46.0 ± 4.1	15.9 ± 2.6	-7.2 ± 0.8
	PEG	Media	680	46.0 ± 4.1	15.9 ± 2.6	4.7 ± 8.3

Table S3. miRNAs differentially expressed in HDF cells exposed to AuNPs compared to controls.

miRNA ID	Fold Change	*P* value[a]	FDR[b]
PAA Spheres Chronic x CTRL			
hsa-let-7a-5p	-2.57	1.83E-05	0.018
Citrate Non-Chronic x CTRL			
hsa-let-7c-5p	-2.50	1.18E-05	0.006
hsa-miR-424-3p	-2.39	4.15E-05	0.014
hsa-miR-660-5p	-2.88	1.00E-04	0.021
hsa-miR-625-3p	4.08	2.37E-06	0.002
hsa-miR-146a-5p	3.34	6.81E-05	0.017
hsa-miR-369-5p	3.07	1.30E-04	0.021
hsa-miR-126-3p	3.34	2.00E-04	0.029
PAA Rods Non-Chronic x CTRL			
hsa-let-7c-5p	-2.62	3.91E-06	0.004
hsa-miR-29b-1-5p	-2.56	2.53E-05	0.013
hsa-miR-486-5p	-2.56	5.34E-05	0.018
hsa-let-7b-5p	-2.25	1.60E-04	0.032
hsa-miR-193b-3p	2.99	1.63E-04	0.032

[a]One-way ANOVA test. [b]*P* values from one-way ANOVA were FDR adjusted using the Benjamini-Hochberg method. miRNAs presenting fold change (FC) ≥ 2 or ≤ -2 and FDR < 0.05 were considered differentially expressed.

Table S4. miRNAs differentially expressed in HDF cells exposed to the same AuNP type under different exposure condition (chronic or non-chronic).

miRNA ID	Fold Change	*P* value[a]	FDR[b]
Citrate Chronic x Non-Chronic			
hsa-miR-4497	-5.53	6.12E-06	0.006
PAA Spheres Chronic x Non-Chronic			
hsa-miR-4488	-10.18	4.36E-05	0.043
PAA Rods Chronic x Non-Chronic			
hsa-miR-1260b	-3.98	1.17E-11	1.16E-08
hsa-miR-1260a	-3.76	8.95E-11	3.24E-08
hsa-miR-486-5p	-4.58	9.81E-11	3.24E-08
hsa-miR-7977	-3.45	2.51E-06	4.98E-04
hsa-miR-15b-5p	-2.41	5.69E-06	9.23 E-04
hsa-miR-324-3p	-3.15	1.41E-05	0.001
hsa-miR-491-5p	-9.83	1.44E-05	0.001
hsa-miR-29b-1-5p	-2.57	2.30E-05	0.002
hsa-miR-34b-3p	-2.71	2.66E-05	0.002
hsa-miR-3129-3p	-9.95	3.13E-05	0.002
hsa-miR-4488	-10.00	4.79E-05	0.003
hsa-miR-197-3p	-2.59	5.76E-05	0.003
hsa-miR-532-3p	-4.25	6.99E-05	0.003
hsa-miR-191-5p	-2.15	1.10E-04	0.004
hsa-miR-4634	-12.94	1.18E-04	0.004
hsa-miR-3960	-4.79	4.57E-04	0.015
hsa-miR-486-3p	-4.55	4.75E-04	0.015
hsa-miR-339-5p	-2.43	7.55E-04	0.029
hsa-miR-138-5p	-3.75	8.55E-04	0.024
hsa-miR-34c-3p	-2.52	9.10E-04	0.024
hsa-miR-17-5p	-2.54	0.002	0.037
hsa-let-7c-3p	-4.58	0.002	0.038
hsa-miR-23b-5p	-2.90	0.002	0.038

hsa-miR-143-3p	3.08	4.37E-07	1.00E-04
hsa-miR-542-3p	4.93	6.78E-06	9.00E-04
hsa-miR-148a-3p	2.74	7.45E-06	9.00E-04
hsa-miR-146b-5p	2.69	9.29E-06	0.001
hsa-miR-30a-5p	2.42	9.76E-06	0.001
hsa-miR-98-5p	2.39	1.64E-05	0.001
hsa-let-7i-5p	2.40	1.88E-05	0.001
hsa-miR-374a-3p	4.41	4.34E-05	0.002
hsa-miR-379-5p	2.79	6.65E-05	0.003
hsa-miR-101-3p	2.67	6.83E-05	0.003
hsa-miR-181c-5p	5.38	8.36E-05	0.004
hsa-miR-369-3p	2.20	1.13E-04	0.004
hsa-miR-21-5p	2.15	1.65E-04	0.006
hsa-miR-3529-3p	2.40	4.94E-04	0.015
hsa-miR-21-3p	2.62	7.70E-04	0.022
hsa-miR-381-3p	2.26	0.001	0.030
hsa-miR-181b-5p	2.04	0.001	0.036
hsa-miR-889-3p	2.72	0.002	0.037
hsa-miR-136-3p	2.14	0.002	0.049
hsa-miR-452-5p	2.14	0.002	0.049
hsa-miR-340-5p	2.76	0.002	0.049

PEG Rods Chronic x Non-Chronic

hsa-miR-486-5p	-4.10	1.45E-07	1.43E-04
hsa-miR-24-3p	-3.75	1.02E-05	0.002
hsa-miR-99b-5p	-2.62	2.05E-04	0.029
hsa-miR-22-3p	-3.00	3.19E-04	0.040
hsa-miR-320a	3.67	2.49E-06	0.001
hsa-miR-335-3p	4.40	3.20E-06	0.001
hsa-miR-181a-5p	2.74	7.29E-06	0.002
hsa-miR-320b	3.48	3.74E-05	0.006

[a] One-way ANOVA test. [b] *P* values from one-way ANOVA were FDR adjusted using the Benjamini-Hochberg method. miRNAs presenting fold change (FC) ≥ 2 or ≤ -2 and FDR < 0.05 were considered differentially expressed.