



Supporting Information

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**Combinatorial Delivery of miRNA-Nanoparticle Conjugates
in Human Adipose Stem Cells for Amplified Osteogenesis**

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Combinatorial delivery of miRNA-nanoparticle conjugates in human adipose stem cells for amplified osteogenesis

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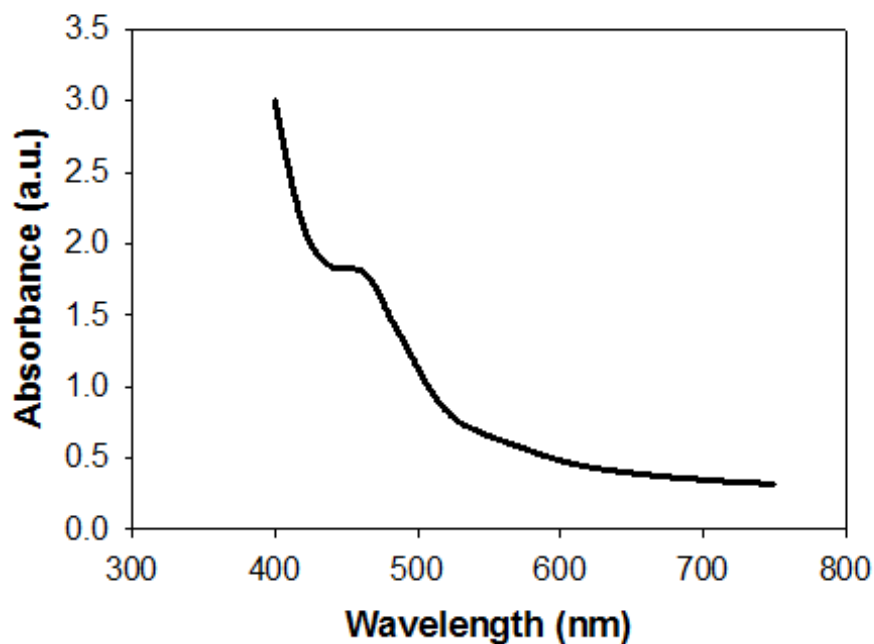


Figure S1. Absorbance spectra of Diels-Alder linker

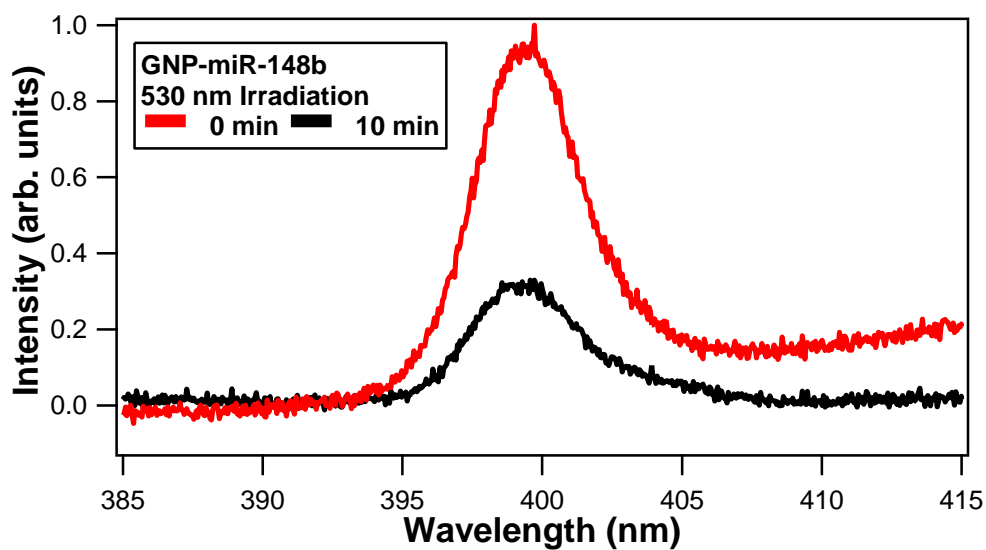


Figure S2. Representative SHG spectra of miRNA-functionalized gold nanoparticles at different wavelengths and irradiation times.

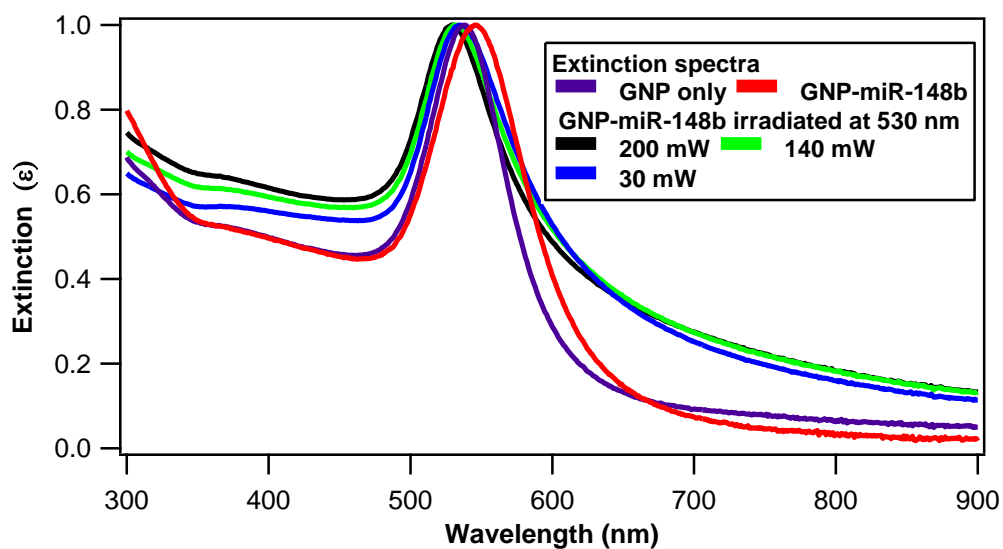


Figure S3. Extinction spectra of the GNPs, the miRNA-functionalized GNPs, and the miRNA-functionalized GNPs after laser irradiation with 530 nm wavelength.

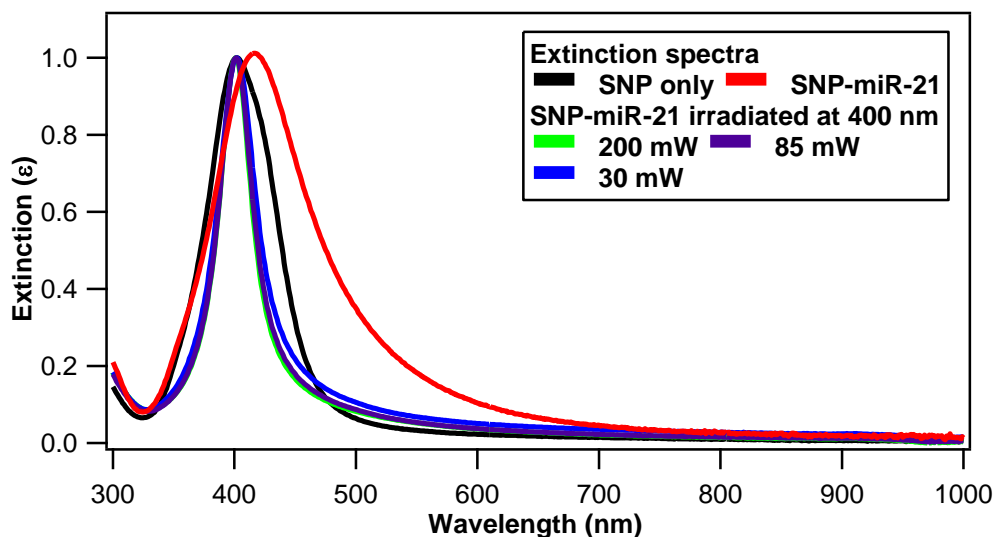


Figure S4. Extinction spectra of the SNPs, the miRNA-functionalized SNPs, and the miRNA-functionalized SNPs after laser irradiation with 400 nm wavelength.

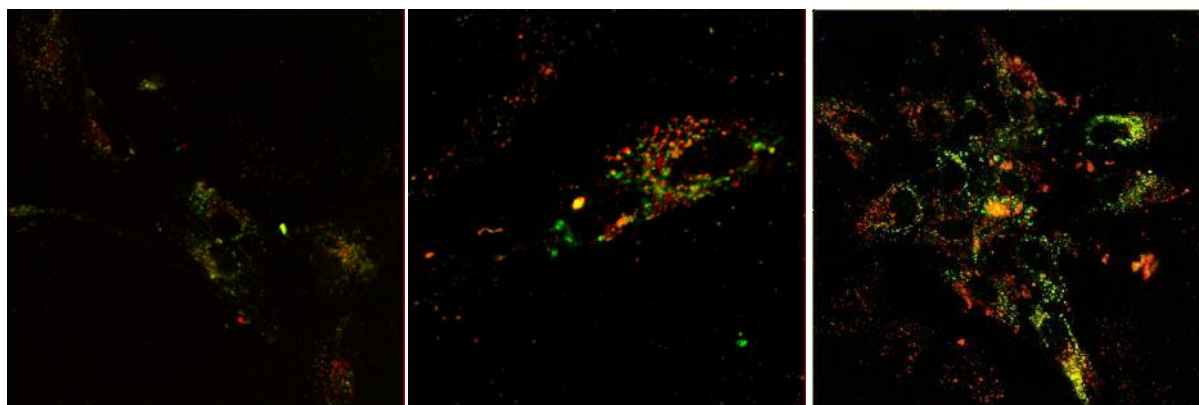
Table S1: Fit parameters obtained for the SNP-miR-21 nanoparticle system at different 400 nm laser powers

Power (mW)	Fit Parameters (SNP-miR-21)		
	A	B	$k (10^{-2} \text{ S}^{-1})$
35	0.652 ± 0.001	0.353 ± 0.005	0.85 ± 0.02
85	0.603 ± 0.001	0.405 ± 0.005	1.23 ± 0.03
140	0.553 ± 0.001	0.432 ± 0.007	1.80 ± 0.05
200	0.504 ± 0.001	0.472 ± 0.012	2.67 ± 0.13

Table S2: Fit parameters obtained for the GNP-miR-148b nanoparticle system at different 530 nm laser powers

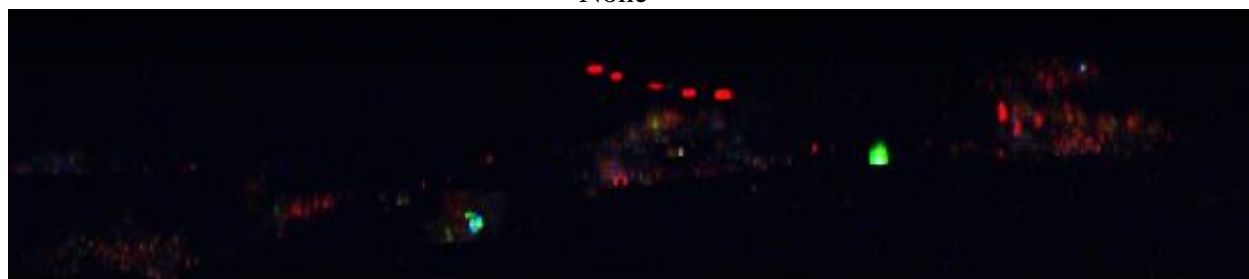
Power (mW)	Fit Parameters (GNP-miR-148b)		
	A	B	$k (10^{-2} \text{ S}^{-1})$
35	0.803 ± 0.001	0.188 ± 0.005	0.82 ± 0.04
85	0.754 ± 0.001	0.242 ± 0.006	1.03 ± 0.04

140	0.699 ± 0.001	0.298 ± 0.005	1.34 ± 0.04
200	0.635 ± 0.001	0.356 ± 0.008	2.04 ± 0.09

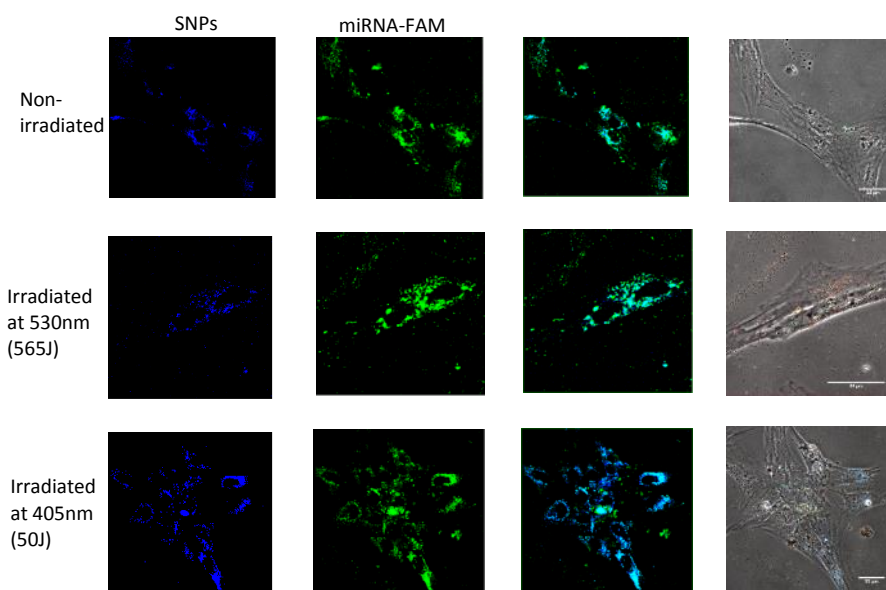
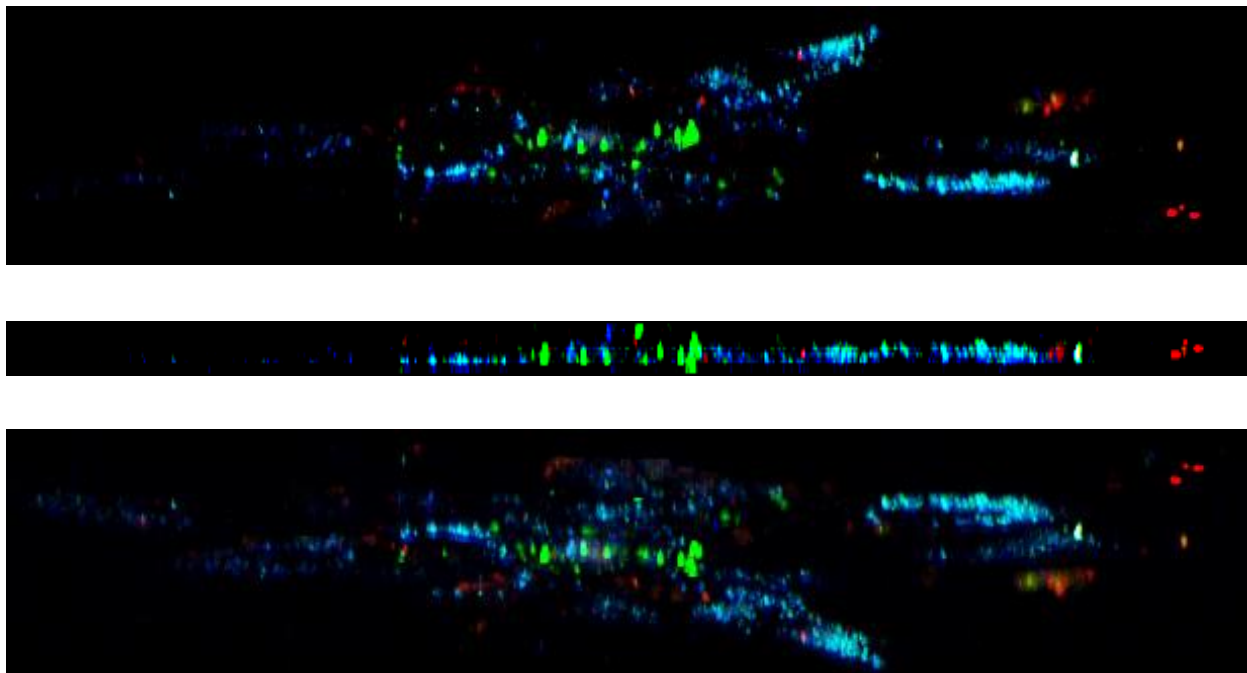


Z Stack images of none irradiated (top) and 405 nm irradiated (bottom) SNP-FAM-transferrin samples

None



405nm



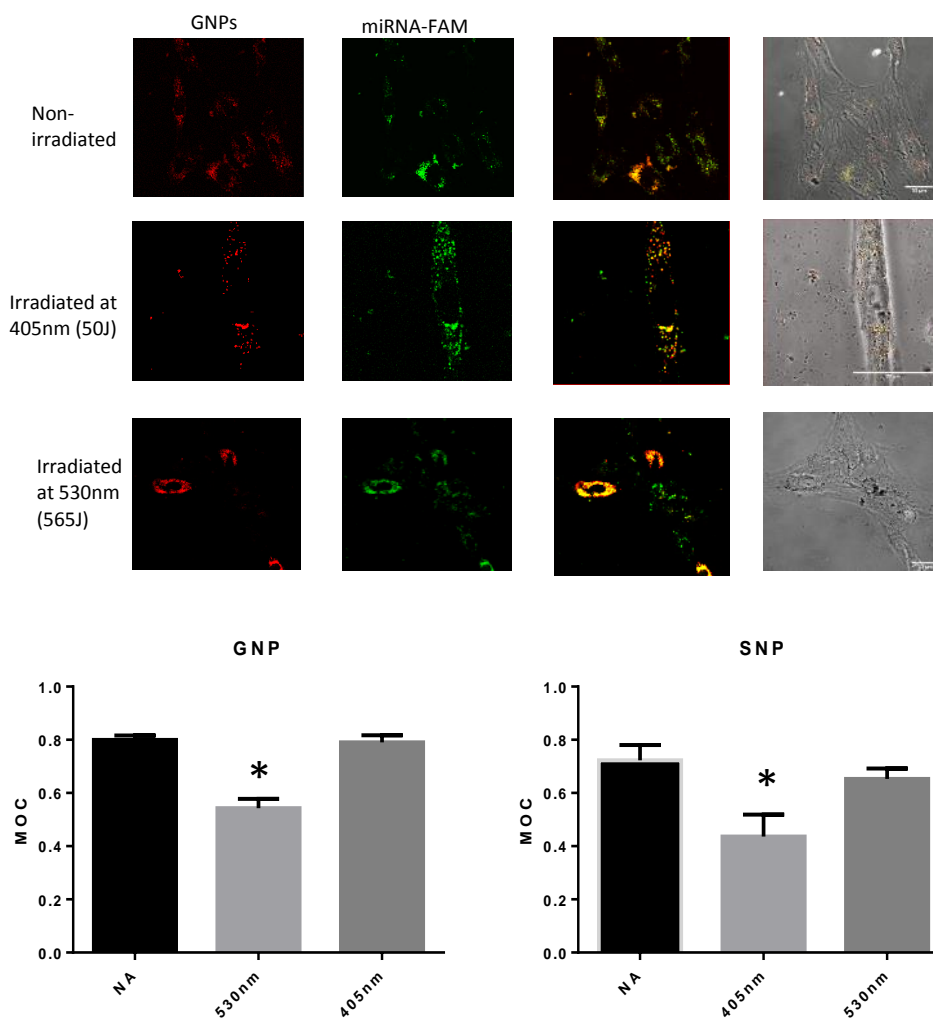


Figure S5. Tansferrin staining of SNPs, z-stack images of SNP/GNP with FAM-miRNA molecules, and plotted Mander's coefficient values for each NP and group.

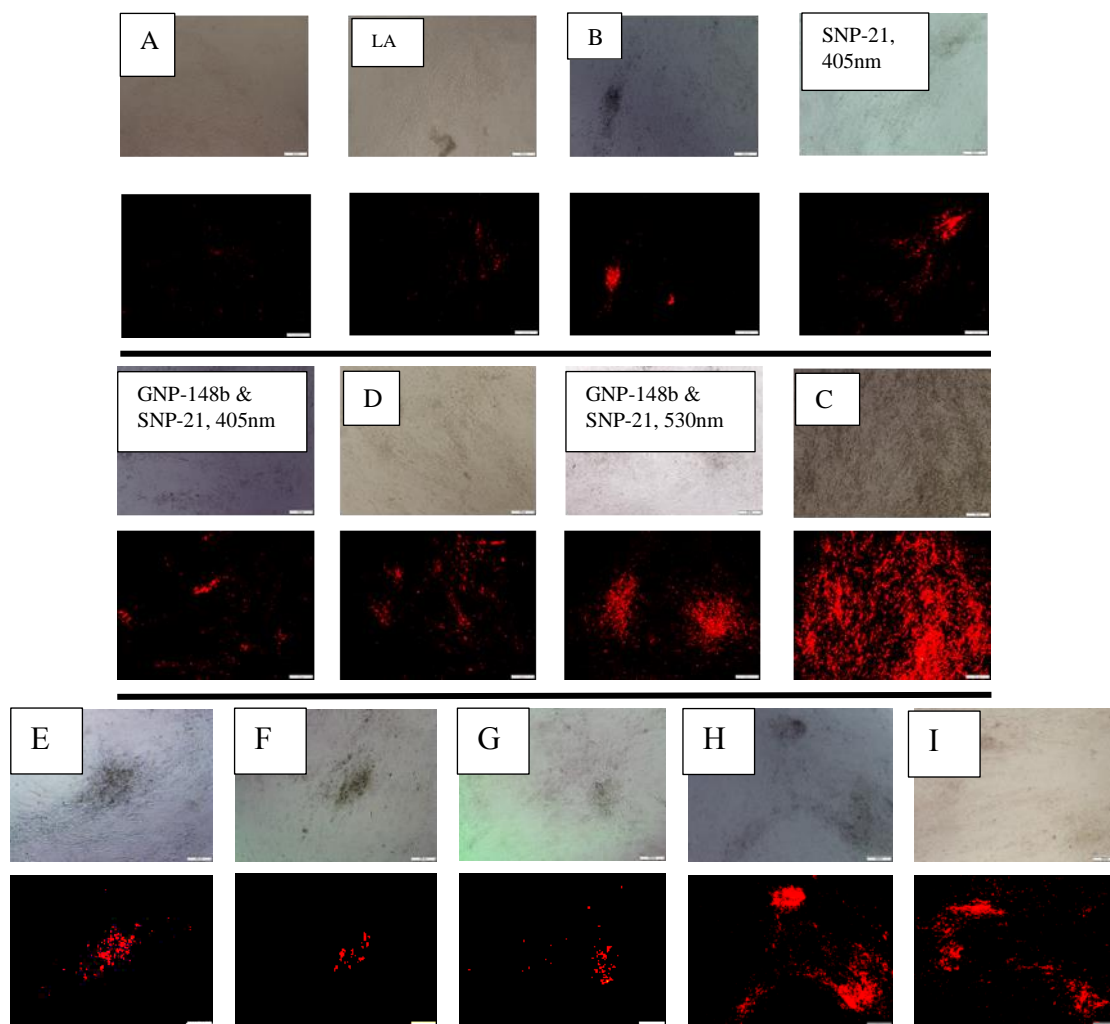
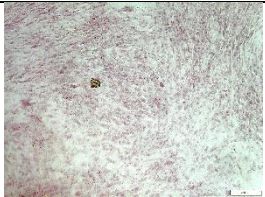
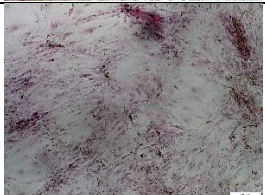
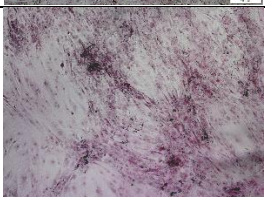

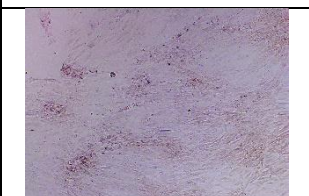

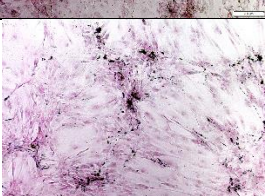
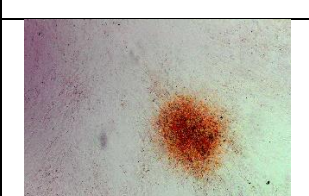
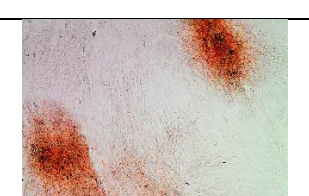
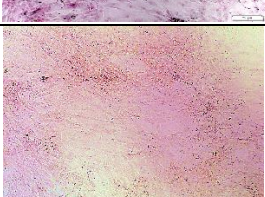
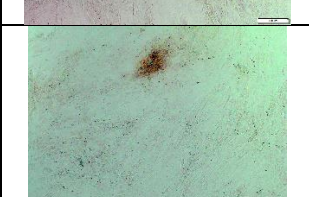
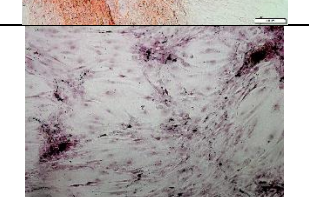
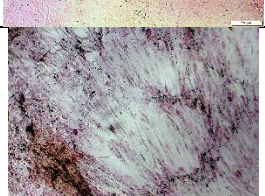
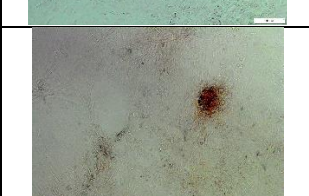
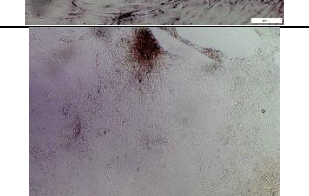

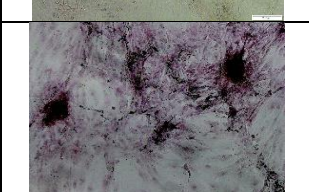


Figure S6. Xylene Orange images for all NP groups tested

Table S3. ARS images for all NP groups tested

Stromal Medium (A)			
Osteogenic Medium (C)			
GNPs & SNPs non-modified, irradiated at 405 nm & 530 nm (LA)			

<p>GNP-miR-148b & SNP-miR-21, non-irradiated (B)</p>			
<p>SNP-miR-21, 405 nm</p>			
<p>GNP-miR-148b, 530 nm (D)</p>			
<p>SNP-miR-21 GNP-miR-148b, 405 nm</p>			
<p>SNP-miR-21 GNP-miR-148b, 530 nm</p>			
<p>SNP-miR-21 GNP-miR-148b, 405 nm & 530 nm 0 h (E)</p>			
<p>SNP-miR-21 GNP-miR-148b, 405 nm at 0 h & 530 nm at 24 h (F)</p>			
<p>SNP-miR-21 GNP-miR-148b, 405 nm at 0 h & 530 nm at 48 h (G)</p>			
<p>SNP-miR-21 GNP-miR-148b, 530 nm at 0 h & 405 nm at 24 h (H)</p>			

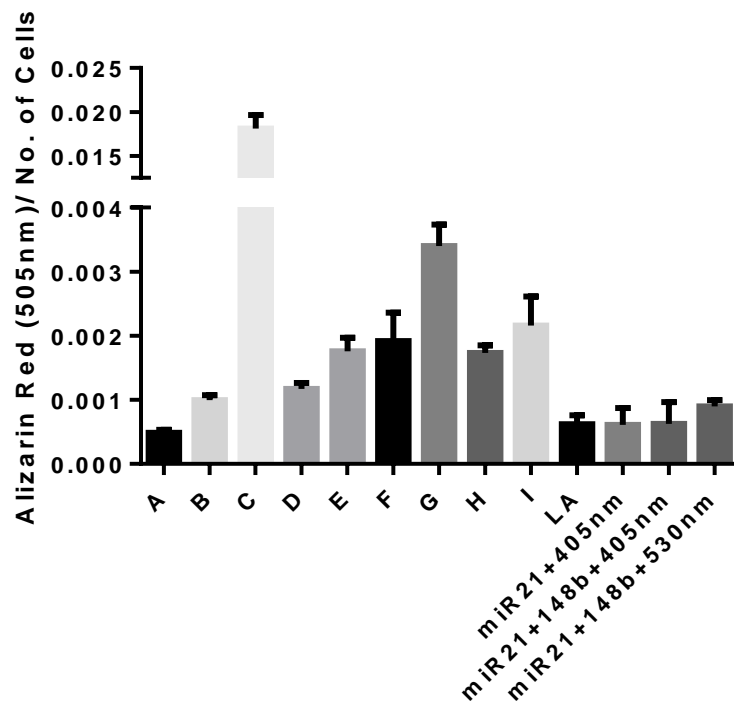
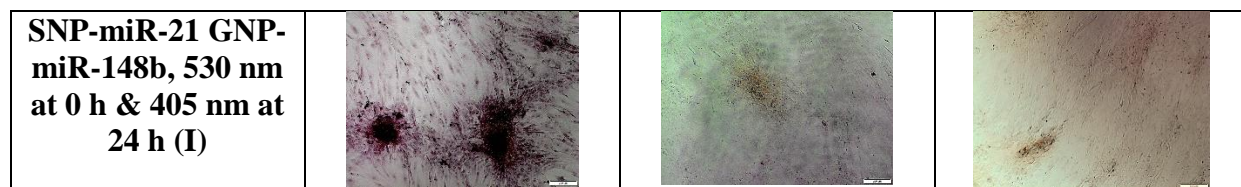


Figure S7. ARS quantification for all NP groups normalized to cell count

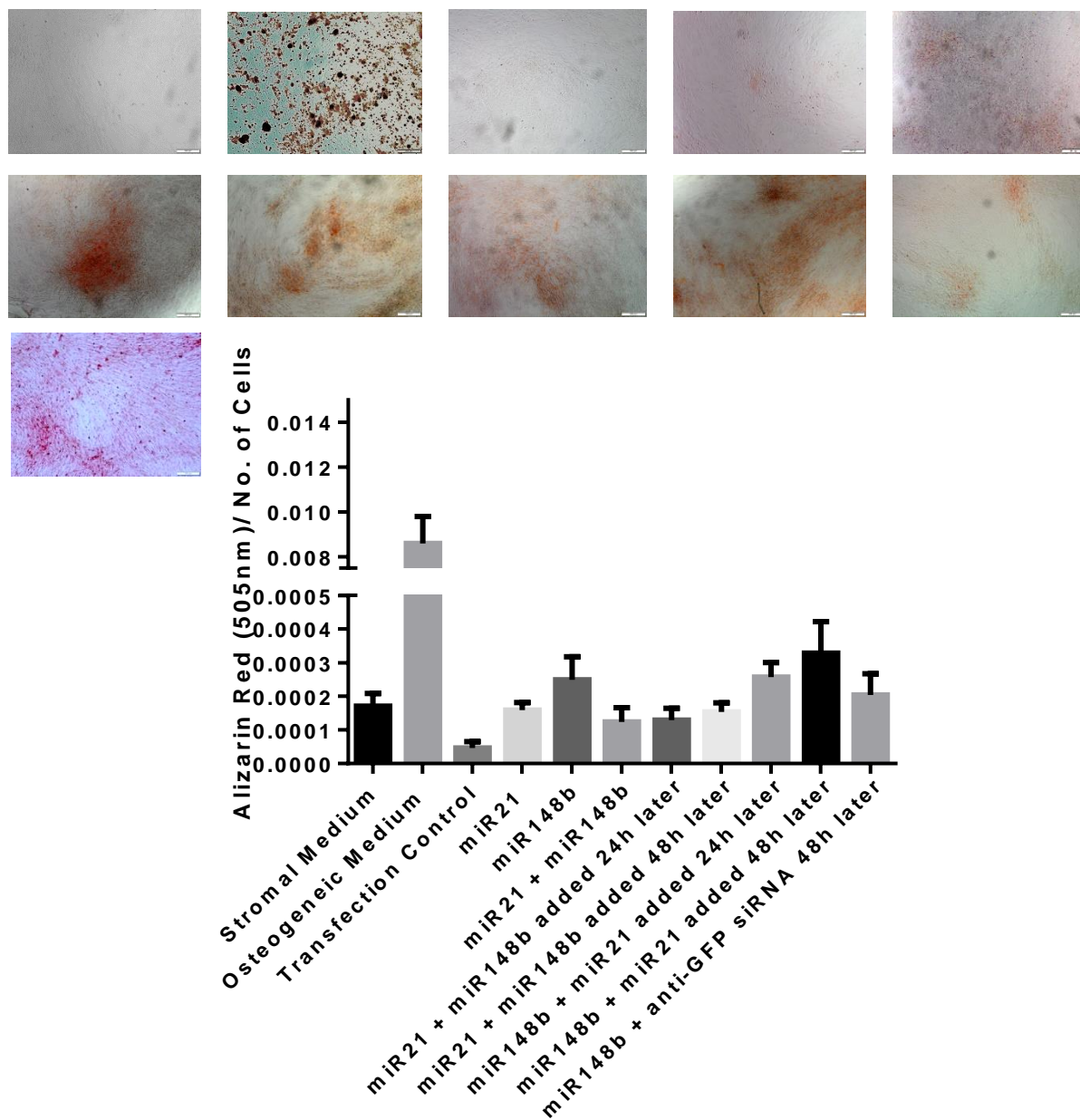


Figure S8. Chemical transfection groups ARS (images presented in same order, left to right/top to bottom, as listed on x-axis of graph)

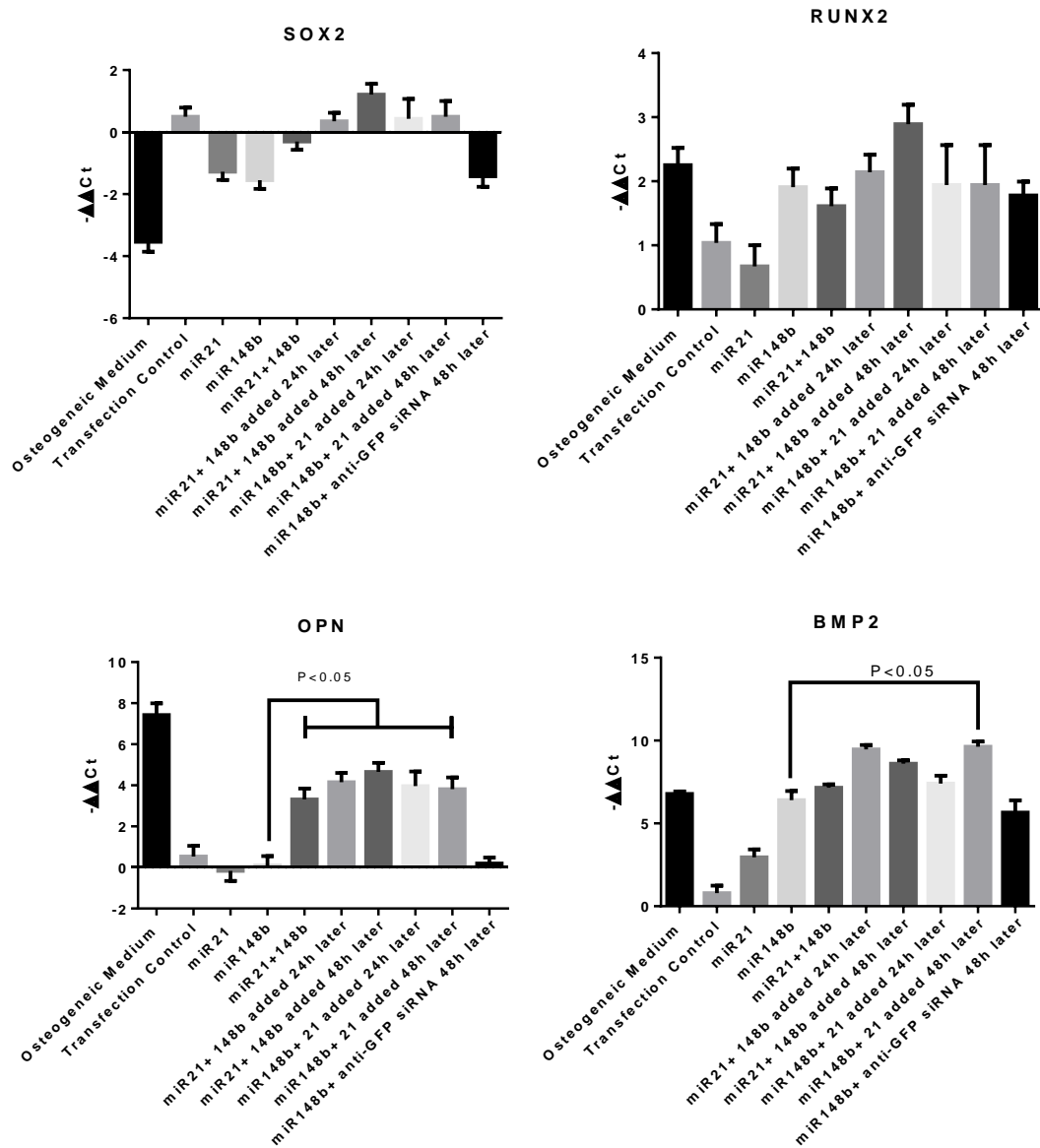


Figure S9. Chemical transfection groups PCR day 21

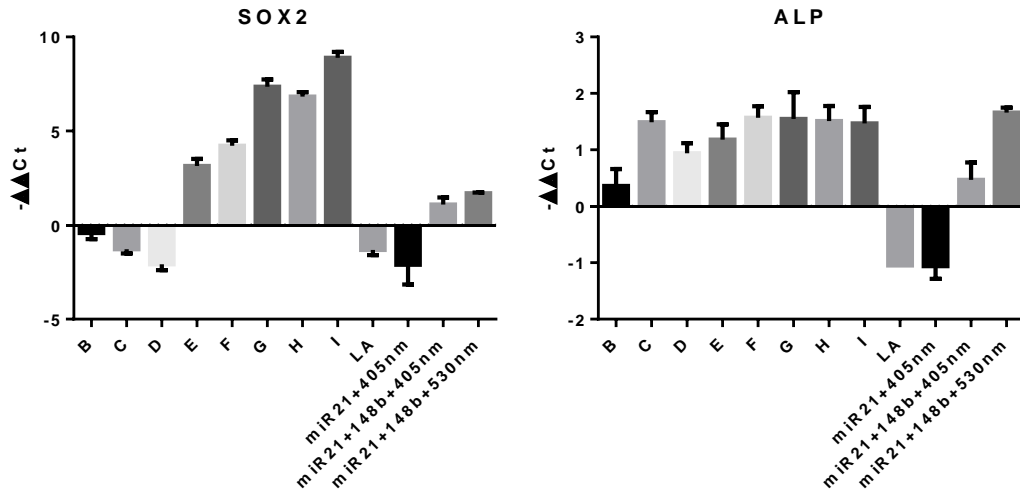


Figure S10. All NP groups PCR day 7

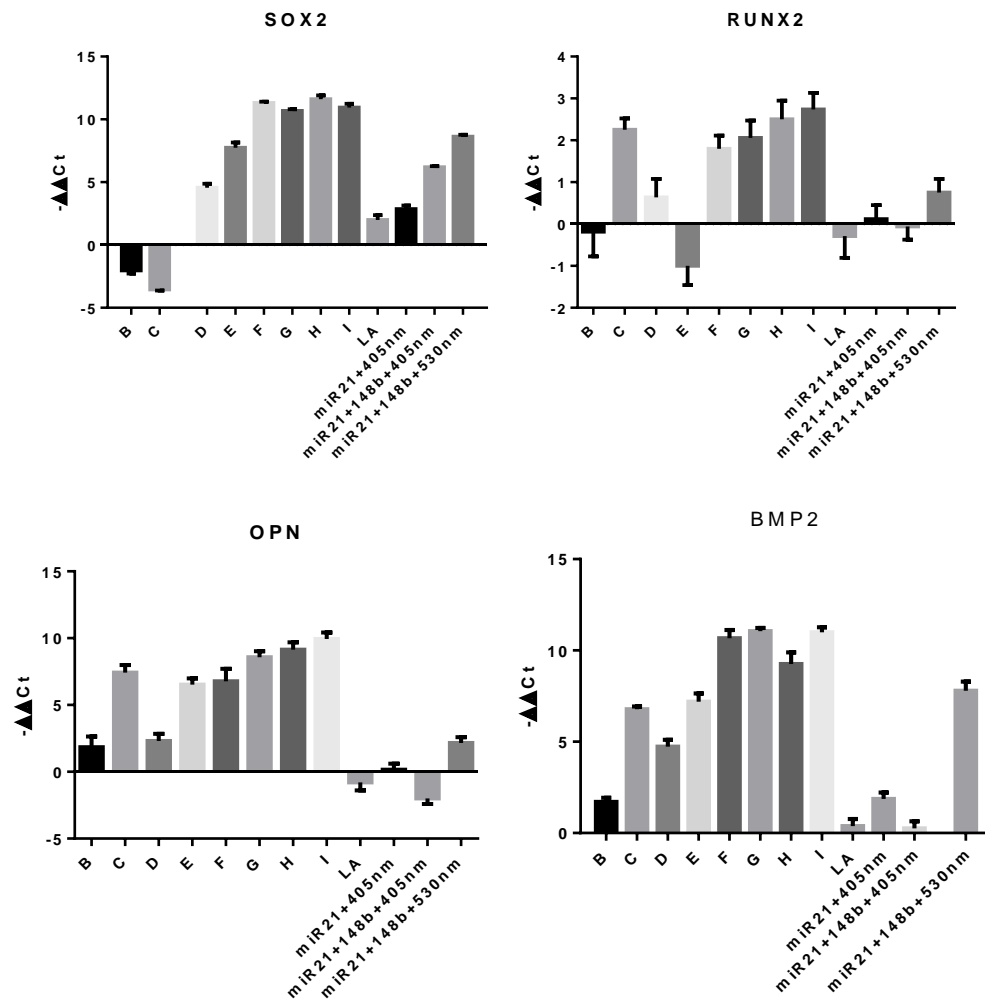


Figure S11. All NP groups PCR day 21

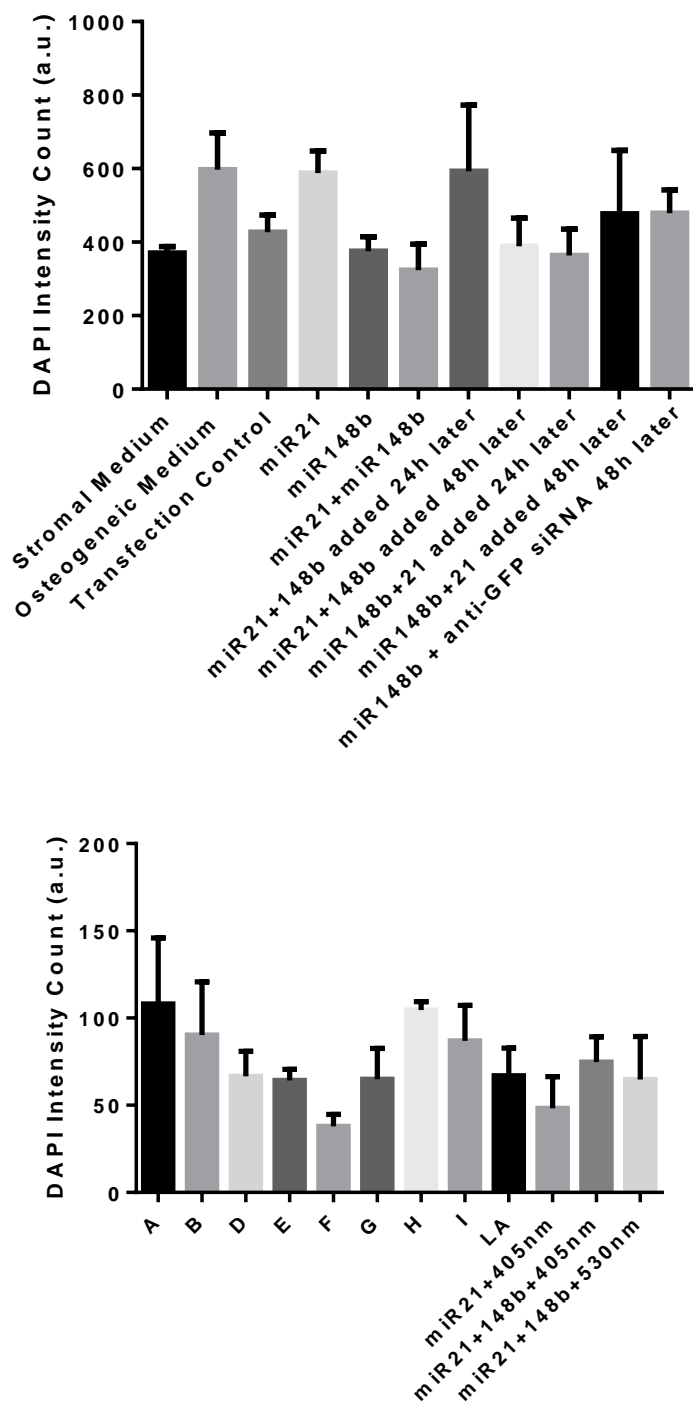


Figure S12. DAPI/Cell count at Day 21 for chemical transfection (top) and NP groups (bottom)

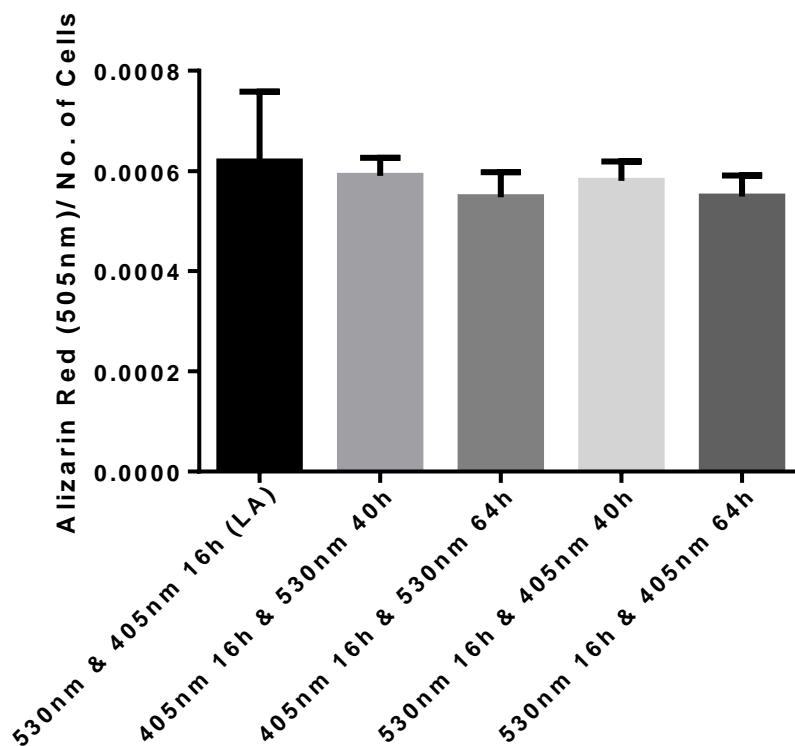
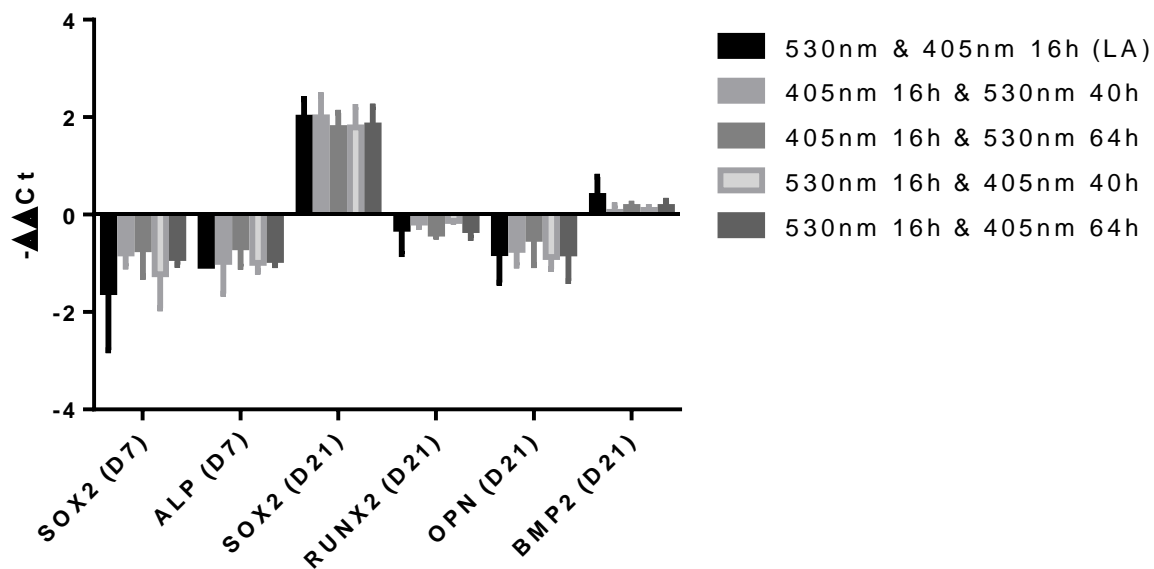


Figure S13. PCR and ARS for light-activated control groups at different timepoints.

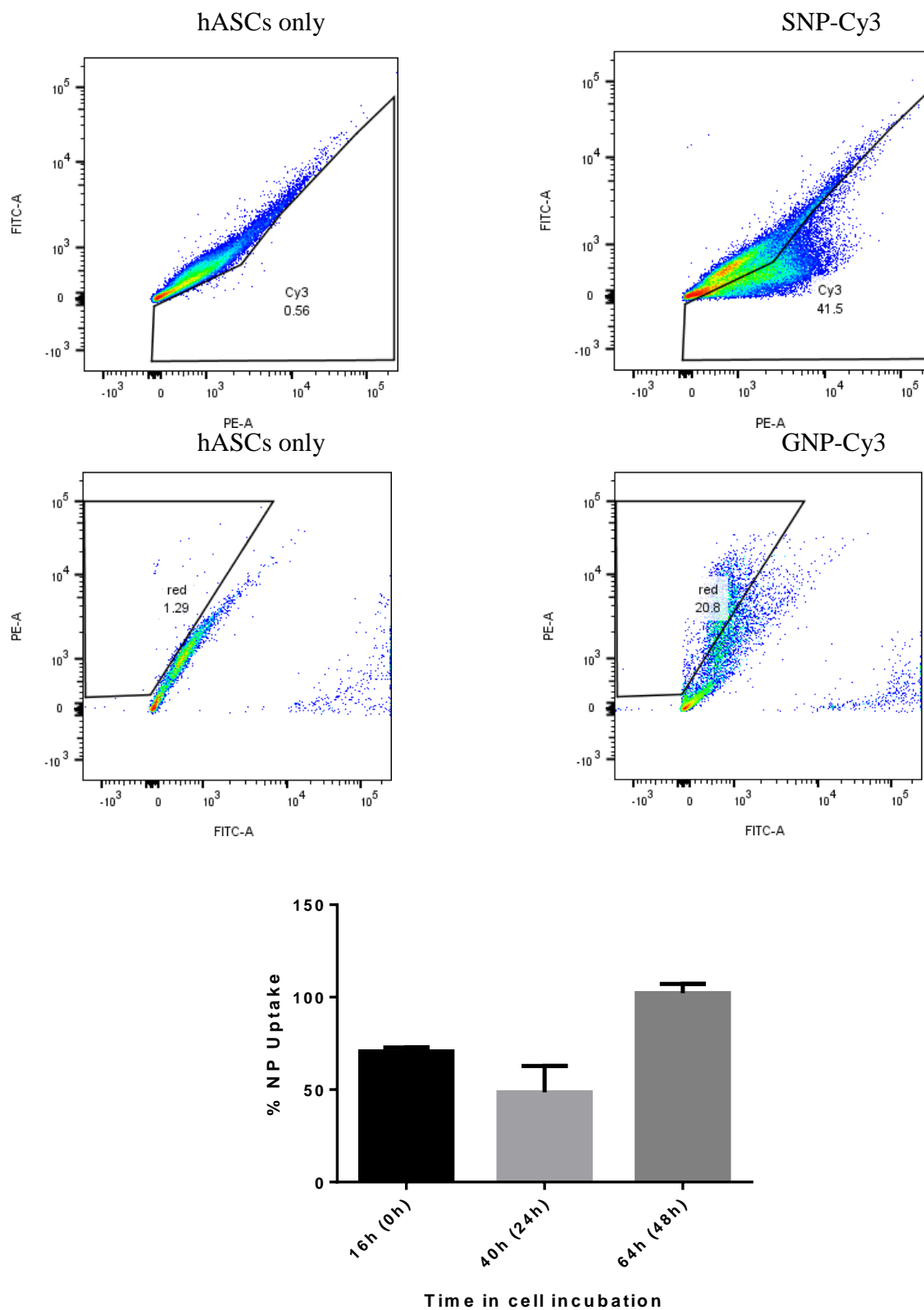


Figure S14. Transfection efficiencies of SNPs and GNPs in hASCs, measured using Flow Cytometry of Cy3 conjugated miRNA mimics, and uptake efficiency of SNPs using ICP-AES.

Table S3. Summary of gene targets for hsa-miR148b-3p, generated from microRNA.org search database. Shown ten results from a total of 700+. Gene function derived from <https://ghr.nlm.nih.gov/gene>

miR148b mRNA target	mirSVR score	Gene function
COL10A1	-1.20	Chondrogenic marker (Transactivated by RUNX2)
NOG	-1.20	BMP down-regulation
TCF12	-1.17	Maintains pluripotency/ high expression in undifferentiated BMSCs
ZFPM2	-1.21	Runx2 antagonist
SNX2	-1.08	Endocytic protein sorting
PIGA	-1.10	Part of membrane anchoring protein
FMR1	-1.23	Transport mRNA between cells
BTAFF1	-1.31	Required for transcription initiation of genes by RNA polymerase II
TAF4	-1.25	Required for transcription initiation of genes by RNA polymerase II
BPY2	-1.24	Male germ cell development and male infertility

Table S4. Summary of gene targets for hsa-miR21b-3p, generated from microRNA.org search database. Shown eight results from a total of 700+. Gene function derived from <https://ghr.nlm.nih.gov/gene>

miR21 mRNA target	mirSVR score	Gene function
TGFBII	-1.10	TGFB receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression
SOX2	-1.17	Pluripotency regulator/self-renewal
SOS2	-1.26	Regulatory protein that is involved in the positive regulation of ras proteins
SOX4	-0.8421	Protein may function in the apoptosis pathway leading to cell death as well as to tumorigenesis
SOX5	-1.14	Endochondral Ossification
OMD	-1.18	Biom mineralization processes / osteoblast binding
FGF7	-1.16	Keratinocyte growth factor
SMAP2	-0.868	Endocytosis