

**Long noncoding RNAs (lncRNA) MALAT1 in regulating osteogenic and adipogenic differentiation using a novel molecular nanobiosensor**

Samantha Fasciano<sup>1,2</sup>, Shuai Luo<sup>1</sup>, Shue Wang<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, Chemical and Biomedical Engineering, Tagliatela College of Engineering, University of New Haven, West Haven, CT, 06516, USA

<sup>2</sup>Department of Cellular and Molecular Biology, College of Art and Science, University of New Haven, West Haven, CT, 06516, USA

Corresponding author: Dr. Shue Wang (e-mail: [swang@newhaven.edu](mailto:swang@newhaven.edu))

**Fig. S1.** Stability of ds-GapM-LNA nanobiosensor.

**Fig. S2.** siRNA silencing efficiency.

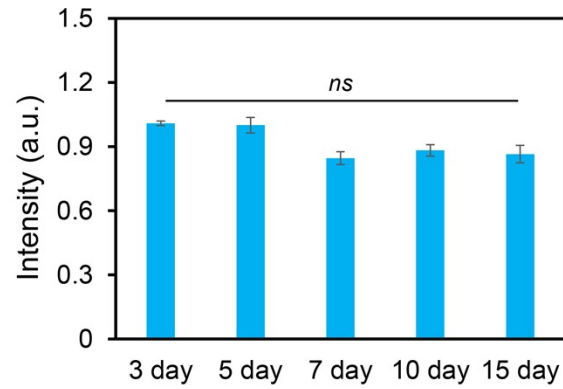
**Fig. S2.** Dynamic tracking of MALAT1 expression during osteogenic differentiation.

**Fig. S3.** Dynamic tracking of MALAT1 expression during adipogenic differentiation.

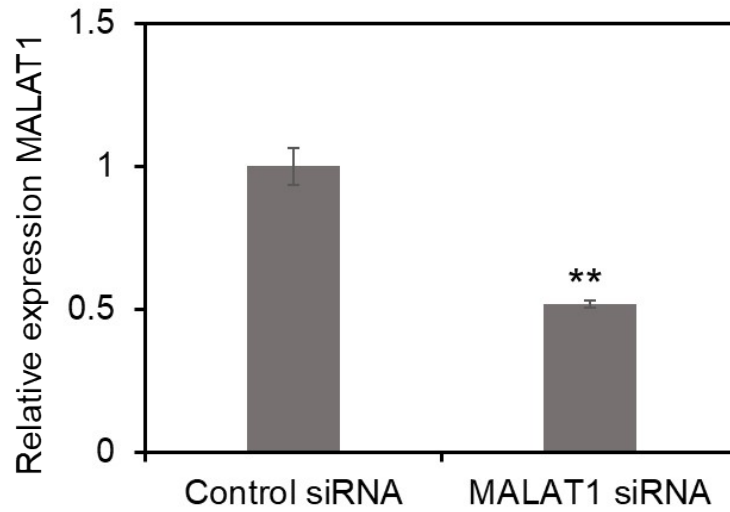
**Fig. S4.** Representative bright field and fluorescence images of hMSCs after 15 days under different treatments.

**Fig. S5.** Comparison of cell proliferation of hMSCs under control siRNA and MALAT1 siRNA treatments during osteogenic and adipogenic induction.

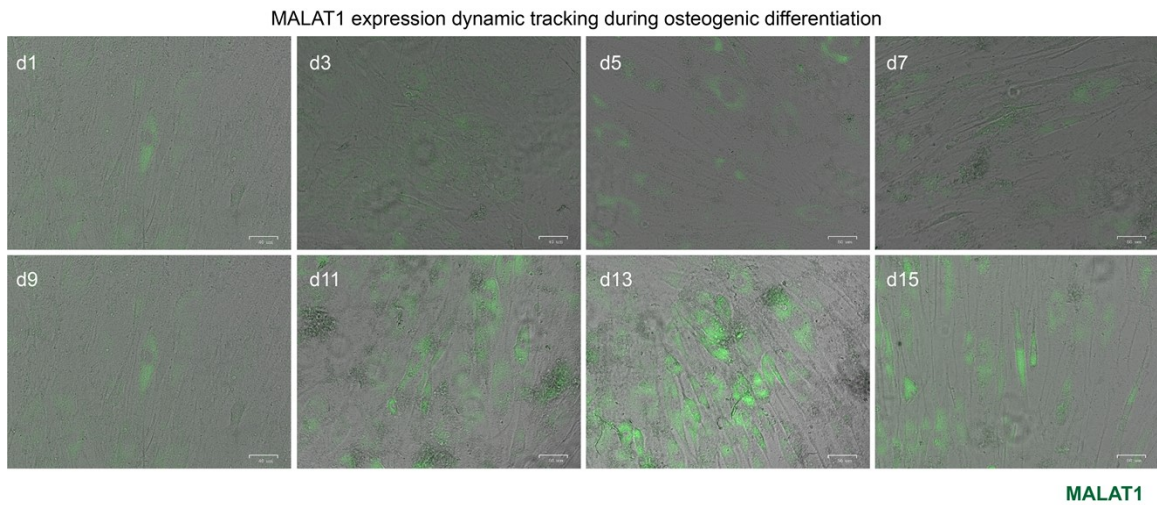
**Tab. S1.** ds-GapM-LNA probes and quencher sequences



**Fig. S1. Stability of ds-GapM-LNA nanobiosensor.** Comparison of fluorescence intensity of ds-GapM-LNA nanobiosensor in the presence of target sequence. All the concentrations were set to 100 nM. Data are expressed as mean  $\pm$ SEM. p-Values were calculated using a two-sample t-test within groups. *ns*, not significant.

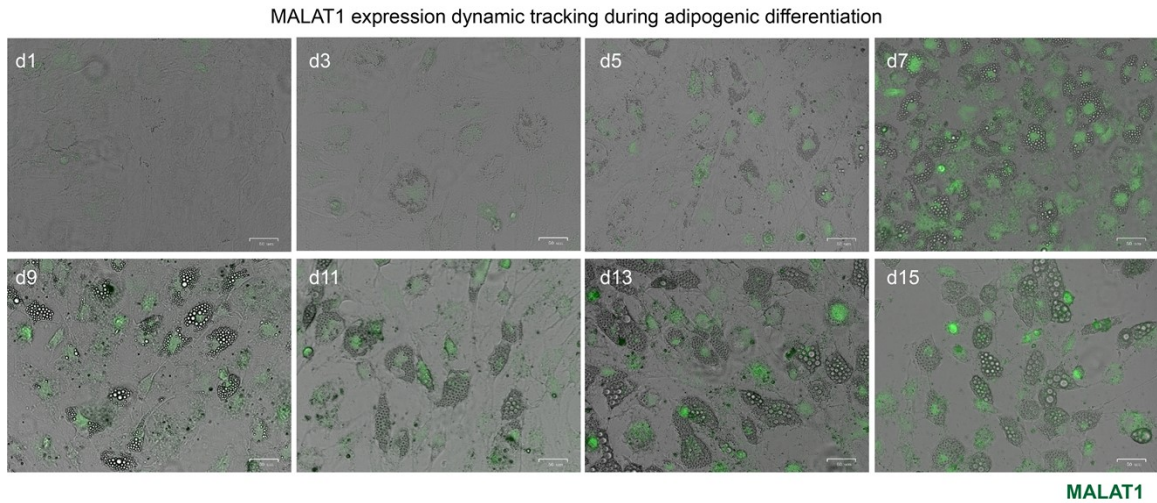


**Fig. S2. siRNA silencing efficiency.** The expression of lncRNA MALAT1 was evaluated and analyzed using RT-PCR assay. Experiments were performed at least three times. The relative expression levels of lncRNAs were determined by the equation  $2^{-\Delta\Delta C_t}$ . Data are expressed as mean  $\pm$  s.e.m. (n = 3). A two-tailed t-test was used to analyze differences between control siRNA and MALAT1 siRNA. \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.005.



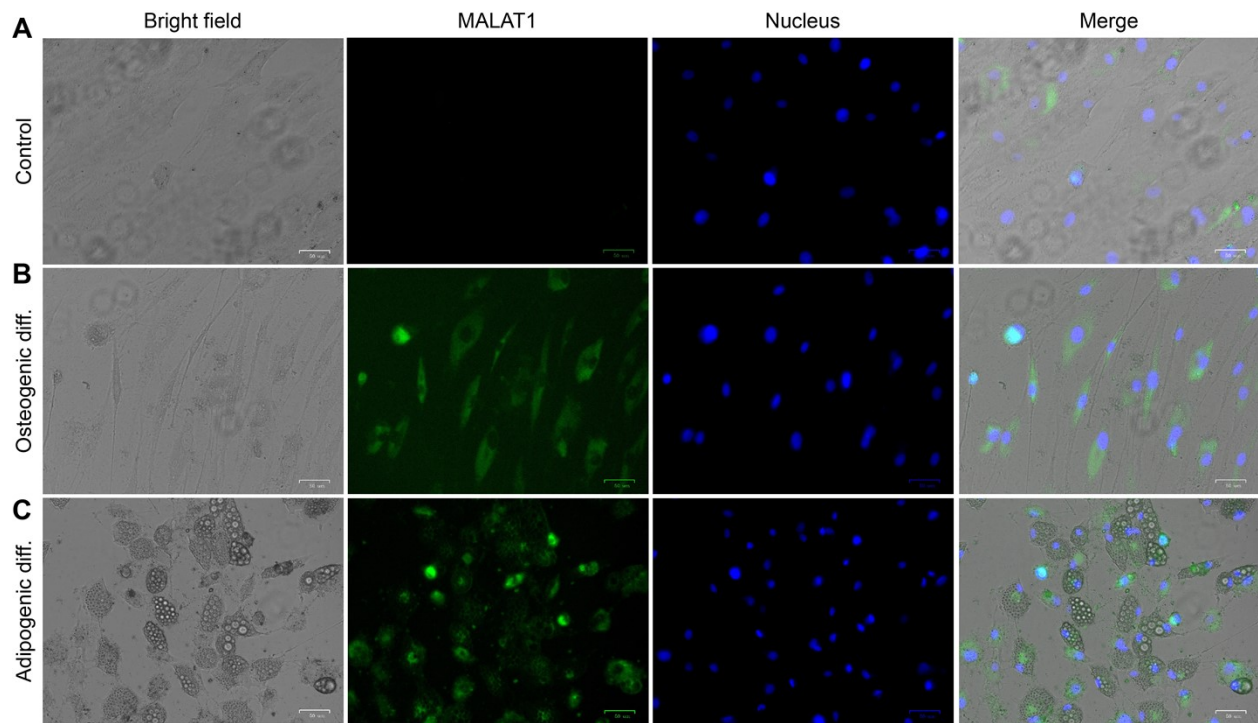
**Fig. S3. Dynamic tracking of MALAT1 expression during osteogenic differentiation.**

Representative merged images of hMSCs during osteogenic differentiation. Images were taken every two days until 15 days of differentiation. Green fluorescence indicates MALAT1 expression. Scale bar: 100  $\mu$ m.

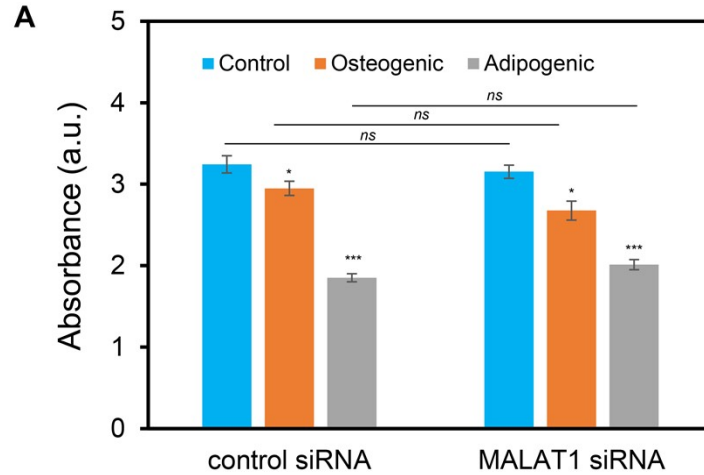


**Fig. S4. Dynamic tracking of MALAT1 expression during adipogenic differentiation.**

Representative merged images of hMSCs during adipogenic differentiation. Images were taken every two days until 15 days of differentiation. Green fluorescence indicates MALAT1 expression. Scale bar: 100  $\mu$ m.



**Fig. S5. Representative bright field and fluorescence images of hMSCs after 15 days under different treatments.** (A) Control group; hMSCs were cultured in the basal medium without treatments. (B) Osteogenic induction group; hMSCs were cultured in osteogenic induction medium. (C) Adipogenic induction group, hMSCs were cultured in adipogenic induction medium. Green: MALAT1; Blue: Nucleus. Scale bar: 100  $\mu$ m.



**Fig. S6. Comparison of cell proliferation of hMSCs under control siRNA and MALAT1 siRNA treatments during osteogenic and adipogenic induction.** For the control group, hMSCs were maintained in basal culture medium. hMSCs were seeded in 96-well plates with 2000 cells per well. After cells reached 80% confluency, hMSCs were treated with control siRNA and MALAT1 siRNAs. After 24 hours of silencing, hMSCs were induced to osteogenic or adipogenic differentiation. The cell proliferation was evaluated using a cell counting kit (CCK-8) assay. Absorbance was measured and compared at 450 nm. Experiments were repeated independently at least three times. Data are expressed as mean  $\pm$  s.e.m. (n=3, \*\*\*,  $p < 0.001$ , \*\*,  $p < 0.01$ )



**Tab. S1.** ds-GapM-LNA probes and quencher sequences

Name		Sequence (5'-3')	Fluorophore
DII4 mRNA	Donor	+T+C+G+C+A TACGT GTGTC TGCTG AGTGT +T+C+C+T+G	/56-FAM
	Quencher	+G+A+C+A+C ACGTA TGCGA	/3-lowa BlackFQ
	Target	CAGGA ACACT CAGCA GACAC ACGTA TGCGA	
Random	Donor	+T+A+C+A+G TATCT CGAAG ACCAG TAG GG +C+A+C+C+T	/56-FAM
	Quencher	+C+T+T+C+G AGATA CTGTA	/3-lowa BlackFQ
	Target	AGGTG CCCTA CTGGT CTTCTG AGATA CTGTA	

\* + represents LNA monomer