Supplementary Information

A long non-coding RNA *GATA6-AS1* adjacent to *GATA6* is required for cardiomyocyte differentiation from human pluripotent stem cells

Rajneesh Jha, [*, 1] Dong Li,[*, 1] Qingling Wu,[*, †] Katherine E. Ferguson, [‡] Parvin Forghani,[*] Gregory C. Gibson, [‡] Chunhui Xu[*, †, 2]

[*]Division of Pediatric Cardiology, Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA
[†]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA
[‡]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA

[1]Co-first authors
[2]Correspondence: chunhui.xu@emory.edu

Supplementary Results

Bioinformatic Analysis of *GATA6-AS1*

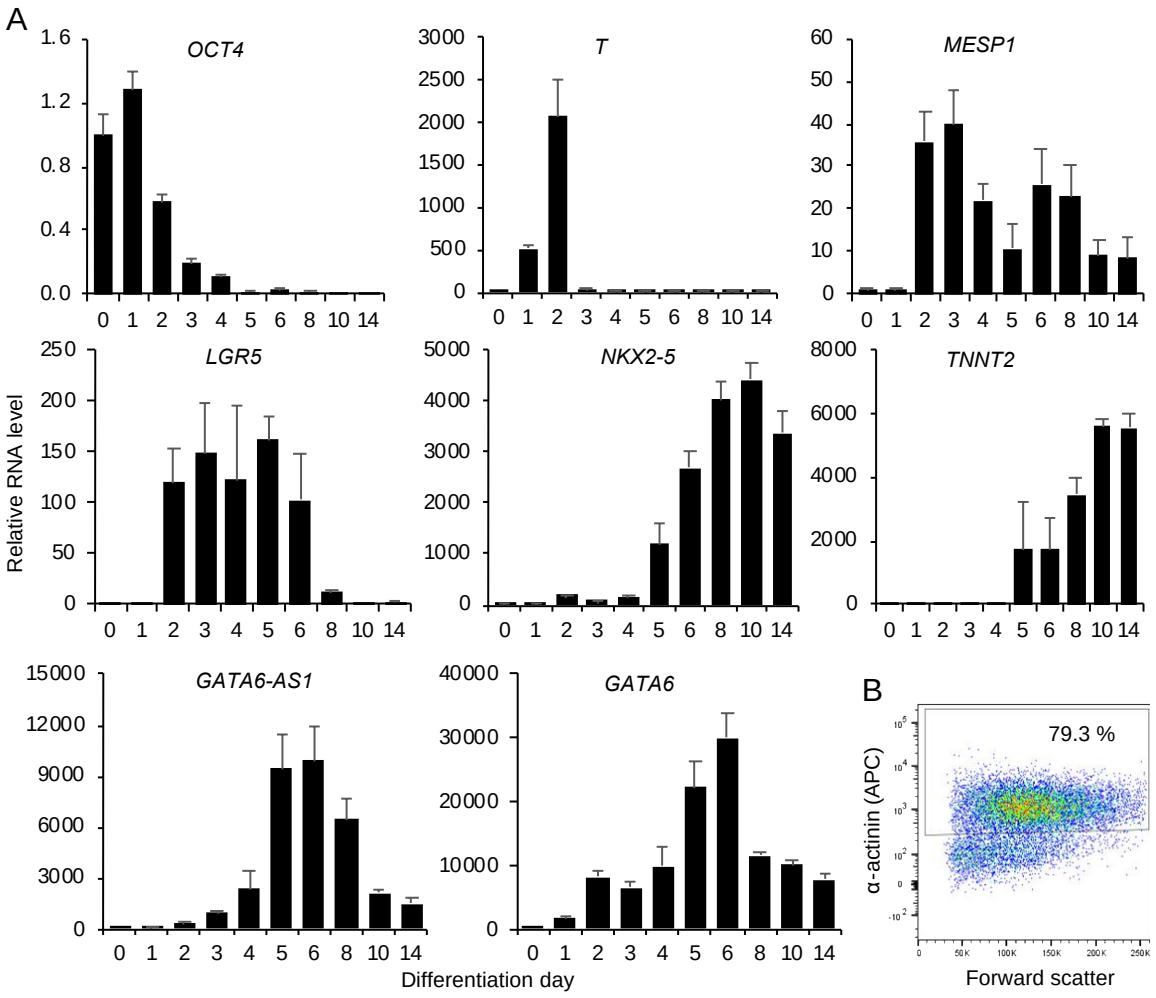
GATA6-AS1 nucleotide sequence was retrieved from National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/gene/100128893) and bioinformatic analysis was performed. No tandem repeats were identified by Tandem Repeat Finder (https://tandem.bu.edu/trf/trf.html). Potential adenylations at nucleotide locations 22166915, 22167044, 22167413, and 22168218 were identified by PolyASite (https://polyasite.unibas.ch/search). The protein coding potential of *GATA6-AS1* was 1.43409 based on Coding Potential Calculator (http://cpc.gao-lab.org).

Supplementary Movies

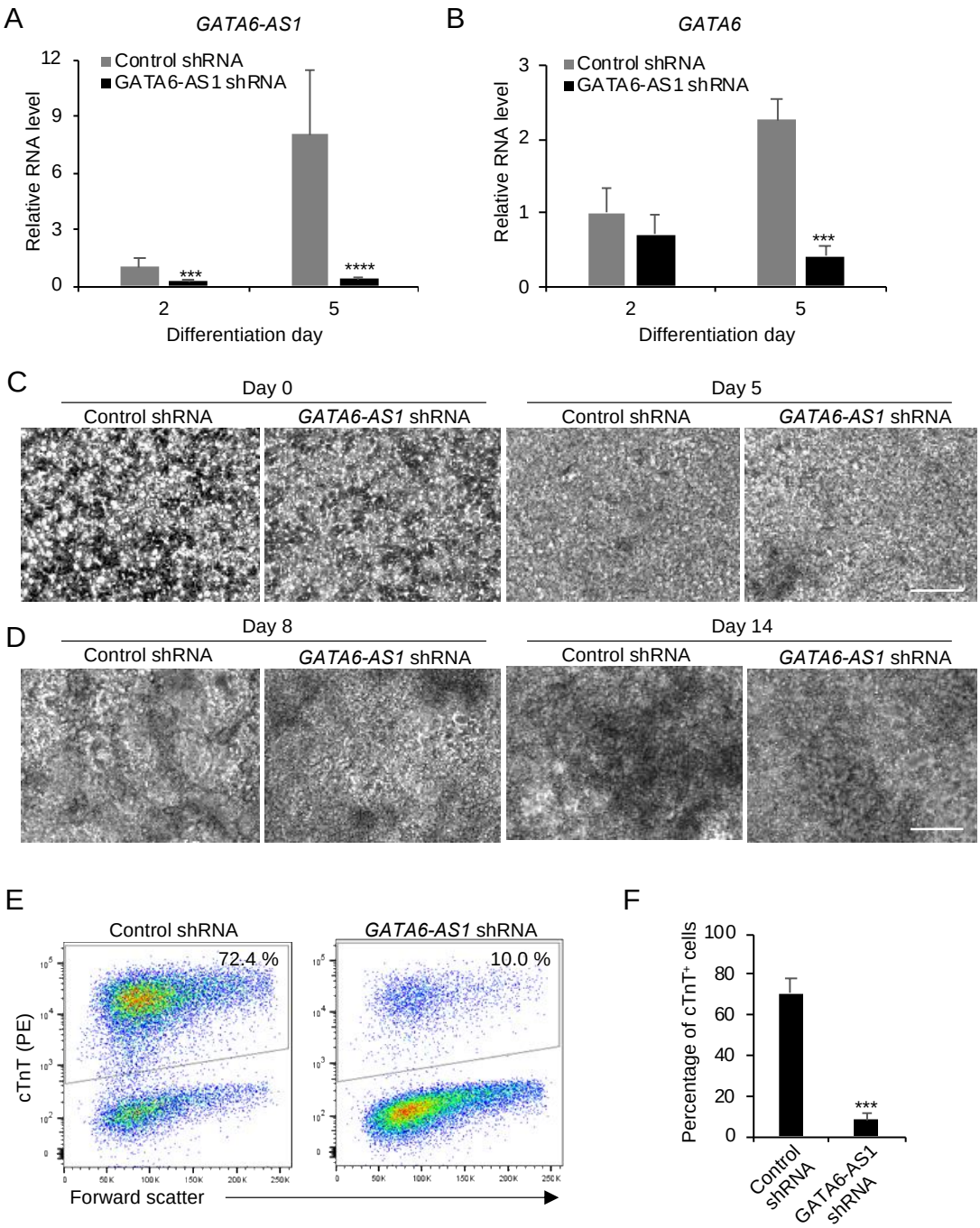
Movie S1. Beating hiPSC-CMs in control shRNA culture on differentiation day 14.
Movie S2. Beating hiPSC-CMs in *GATA6-AS1* shRNA culture on differentiation day 14.

Supplementary Tables

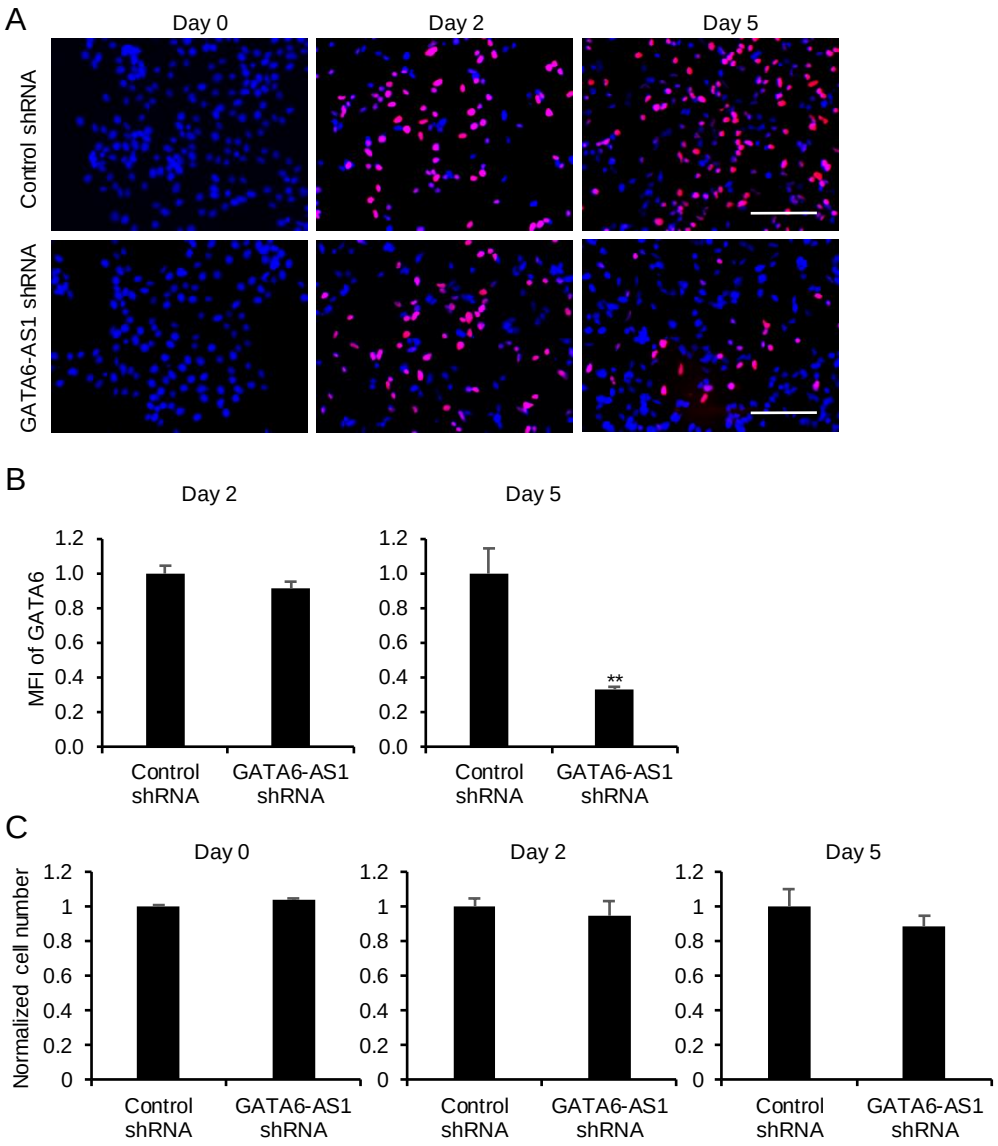
Excel file: Jha_Supplementary Tables



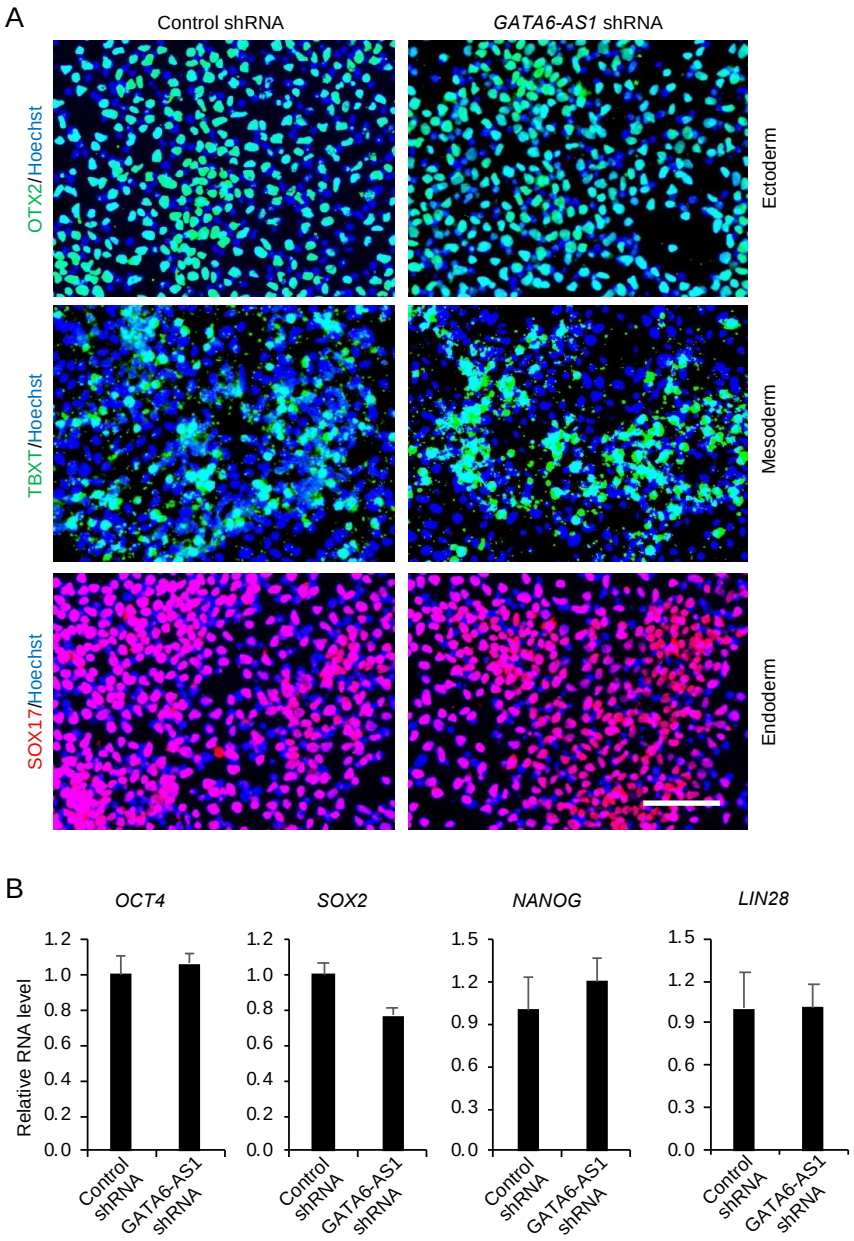
Supplementary Figure 1. *GATA6-AS1* and *GATA6* are transiently upregulated during the early stage of cardiomyocyte differentiation. (**A**) Quantitative reverse transcriptase PCR (qRT-PCR) analysis of genes in differentiating NKX2.5-eGFP hESCs. Expression of genes associated with pluripotent stem cells (*OCT4*), mesoderm (*T* and *MESP1*), and cardiac (*LGR5*, *NKX2-5*, and *cTnT*) along with genes of interest (*GATA6* and lncRNA *GATA6-AS1*) for time points covering differentiation days 0 to 14. Gene expression was normalized to the level of undifferentiated cells on day 0. Standard deviations and mean of fold differences in expression for each time point were calculated using three replicates. (**B**) Flow cytometry analysis of differentiated cells on day 14 showing 79.3% α-actinin positivity.



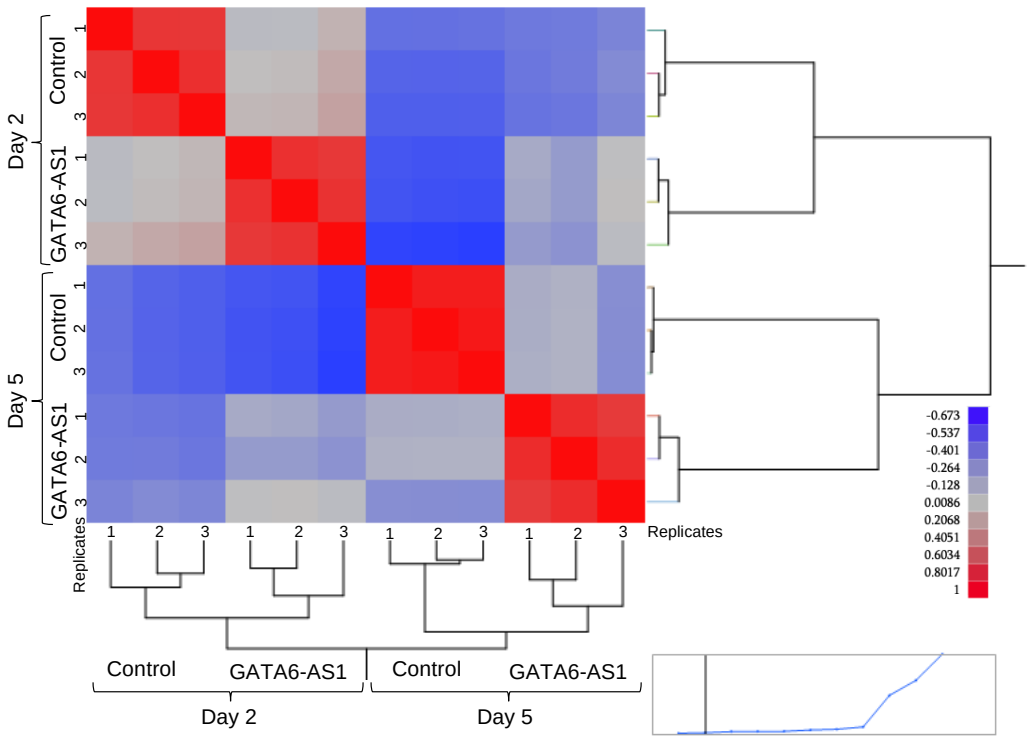
Supplementary Figure 2. Effect of lncRNA *GATA6-AS1* knockdown on the differentiation of cardiomyocytes. Control shRNA or *GATA6-AS1* shRNA IMR90 hiPSCs were induced for cardiomyocyte differentiation using growth factors activin A and BMP4. (A, B) qRT-PCR analysis of *GATA6-AS1* and *GATA6* on differentiation day 2 and day 5. The normalized expression levels (by GAPDH) were relative to the corresponding value for the control shRNA-culture on differentiation day 2. The mean fold change and standard deviation were calculated using three replicates. ***, P<0.001.



Supplementary Figure 3. GATA6 protein level and cell numbers in control shRNA and *GATA6-AS1* shRNA during the cardiomyocyte differentiation. Expression of GATA6 in the control shRNA and *GATA6-AS1* shRNA was evaluated by high-content imaging ArrayScan analysis on differentiation days 0, 2, and 5. (**A**) Represented images of immunocytochemistry for the detection of GATA6 expression in control shRNA and *GATA6-AS1* shRNA cells ondifferentiation day 0, 2, and 5. Scale bars = 50 μm. (**B**) High-content imaging analysis of GATA6 immunostained cells on days 2 and 5. Data was shown as the mean and standard deviation of GATA6 fluorescence intensity at each time point normalized to the level of GATA6 in control shRNA cultures. **, P<0.01. (**C**) Proliferation capacity of control shRNA and *GATA6-AS1* shRNA was evaluated by cell numbers on differentiation days 0, 2, and 5. Data are presented as mean and standard deviation of cell numbers from triplicates normalized to the cell number of control shRNA cultures.



Supplementary Figure 4. Pluripotency of control shRNA and *GATA6-AS1* shRNA hPSCs. NKX2-5-eGFP hESCs treated with control and *GATA6-AS1* shRNA were used for the differentiation of ectoderm, mesoderm, and endoderm using the corresponding medium supplements. (**A**) Cells were fixed, permeabilized and stained with OTX2, TBXT and SOX17 primary antibodies followed by alexa fluor 488 or alexa fluor 594 secondary antibodies. Nuclei were counterstained with Hoechst (blue) and cells positive for OTX2 and TBX2 shown in green, and red for SOX17. (**B**) qRT-PCR analysis of markers of pluripotent stem cells *OCT4*, *SOX2*, *NANOG* and *LIN28*. Scale bar=100 µM.



Supplementary Figure 5. Correlation analysis of all samples for the RNA-seq analysis of control shRNA and *GATA6-AS1* shRNA cultures according to gene expression levels of all genes in each sample.