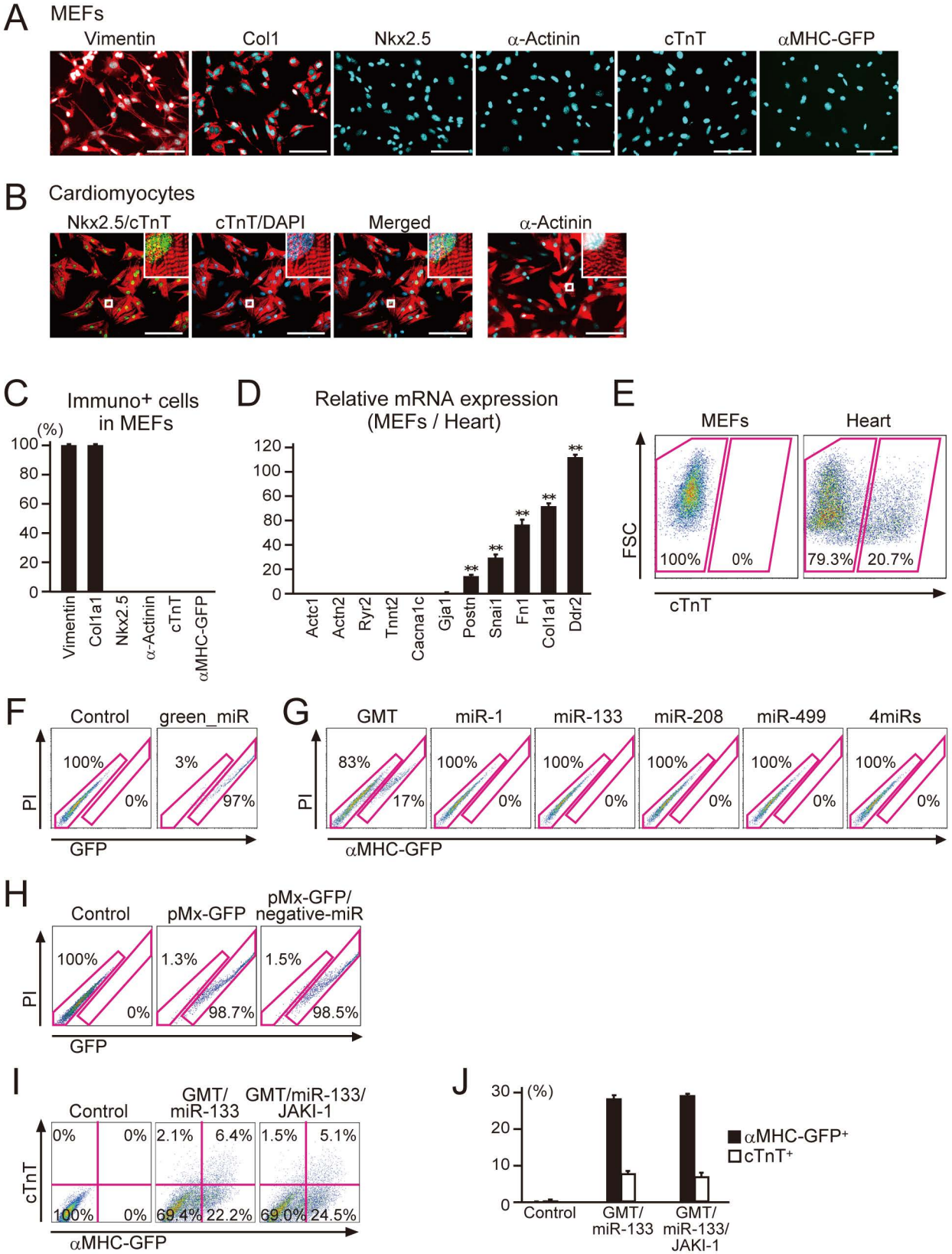
A
MEFs
Vimentin
Col1
Nkx2.5
α-Actinin
cTnT
αMHC-GFP
B
Cardiomyocytes
Nkx2.5/cTnT
cTnT/DAPI
Merged
α-Actinin
C
Immuno+ cells in MEFs
(%)
100
80
60
40
20
0
Vimentin
Col1a1
Nkx2.5
α-Actinin
cTnT
αMHC-GFP
D
Relative mRNA expression (MEFs / Heart)
120
100
80
60
40
20
0
**
Actc1
Actn2
Ryr2
Tnnt2
Cacna1c
Gja1
Postn
Snai1
Fn1
Col1a1
Ddr2
E
MEFs
Heart
FSC
100%
0%
79.3%
20.7%
cTnT
F
Control
green_miR
PI
100%
0%
3%
97%
GFP
G
GMT
miR-1
miR-133
miR-208
miR-499
4miRs
PI
83%
17%
100%
0%
αMHC-GFP
H
Control
pMx-GFP
pMx-GFP/ negative-miR
PI
100%
0%
1.3%
98.7%
1.5%
98.5%
GFP
I
Control
GMT/ miR-133
GMT/miR-133/ JAKI-1
cTnT
0%
0%
100%
0%
2.1%
6.4%
69.4%
22.2%
1.5%
5.1%
69.0%
24.5%
αMHC-GFP
J
30
20
10
0
(%)
αMHC-GFP+
cTnT+
Control
GMT/ miR-133
GMT/ miR-133/ JAKI-1

Figure S1. MEF Cultures Are not Contaminated by Cardiomyocytes or Cardiac Progenitor Cells

(A) Immunocytochemisty for vimentin, collagen1 (Col1), Nkx2.5, α-actinin, cTnT, and α-MHC-GFP, with DAPI staining in MEFs.

(B) Immunostaining for Nkx2.5, cTnT, α-actinin, and DAPI in murine neonatal cardiomyocytes provided a positive control for immunocytochemistry. High-magnification views in insets show sarcomeric organization.

(C) The percentage of vimentin$^+$, Col1$^+$, Nkx2.5$^+$, α-actinin$^+$, cTnT$^+$, and α-MHC-GFP$^+$ cells in MEFs (n = 3).

(D) Relative mRNA expression of *Actc1, Actn2, Ryr2, Tnnt2, Cacna1c, Gja1, Postn, Snai1, Fn1, Col1a1,* and *Ddr2* in MEFs compared to hearts (n = 3).

(E) FACS analyses for cTnT expression in MEFs and mouse hearts. MEFs did not include cTnT$^+$ cardiomyocytes.

(F) FACS analyses for transfection efficiency of miRNA mimics (green-miR).

(G) FACS analyses for αMHC-GFP$^+$ cells. Cells were analyzed 1 week after GMT transduction or miRNA transfection.

(H) FACS analyses for transduction efficiency of pMXs-GFP with and without miRNA mimics in MEFs. Addition of miRNA did not augment the transduction efficiency of pMXs-GFP.

(I, J) FACS analyses for αMHC-GFP$^+$ and cTnT$^+$ cells 1 week after GMT/miR-133 transduction with and without the JAK inhibitor 1 (JAK-I). Quantitative data are shown in (J) (n = 3).

All data are presented as means ± SEM. **, $P < 0.01$ vs. relevant control. Scale bars, 100 μm.