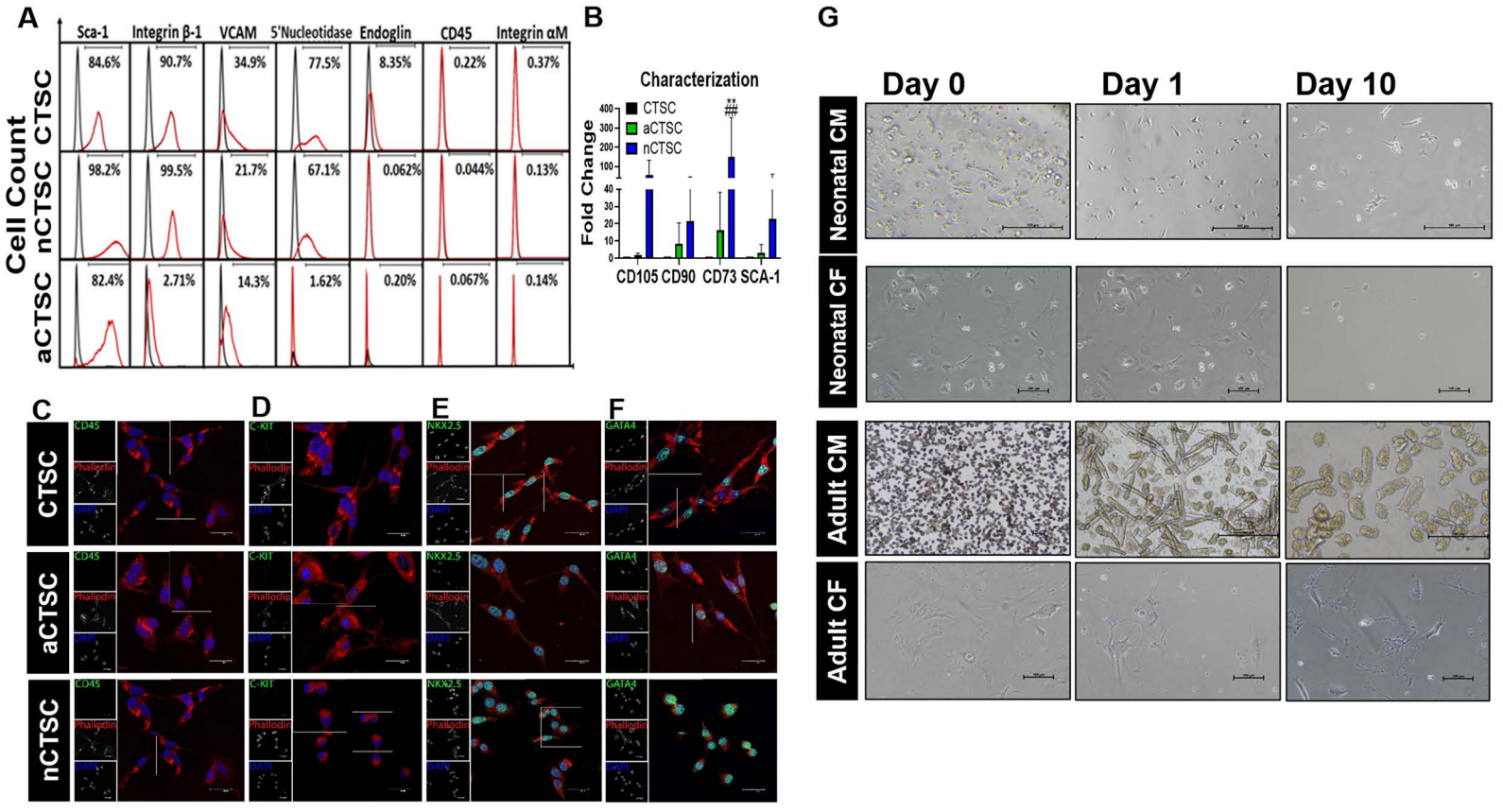
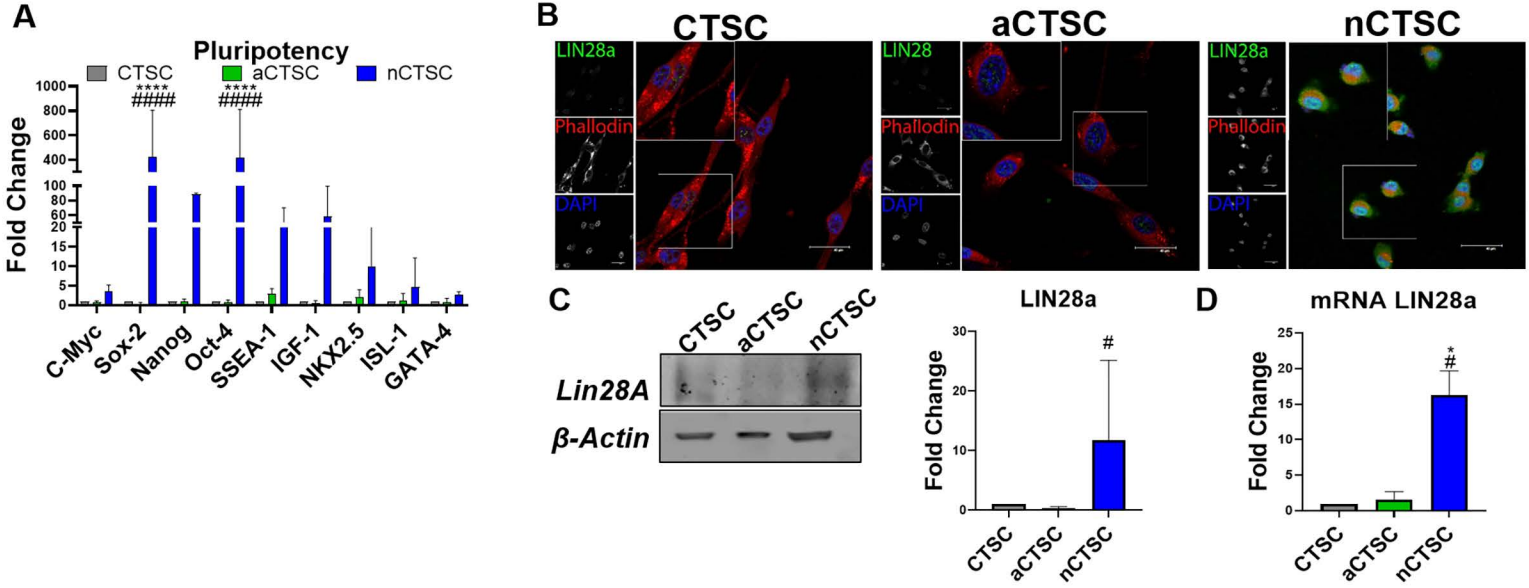
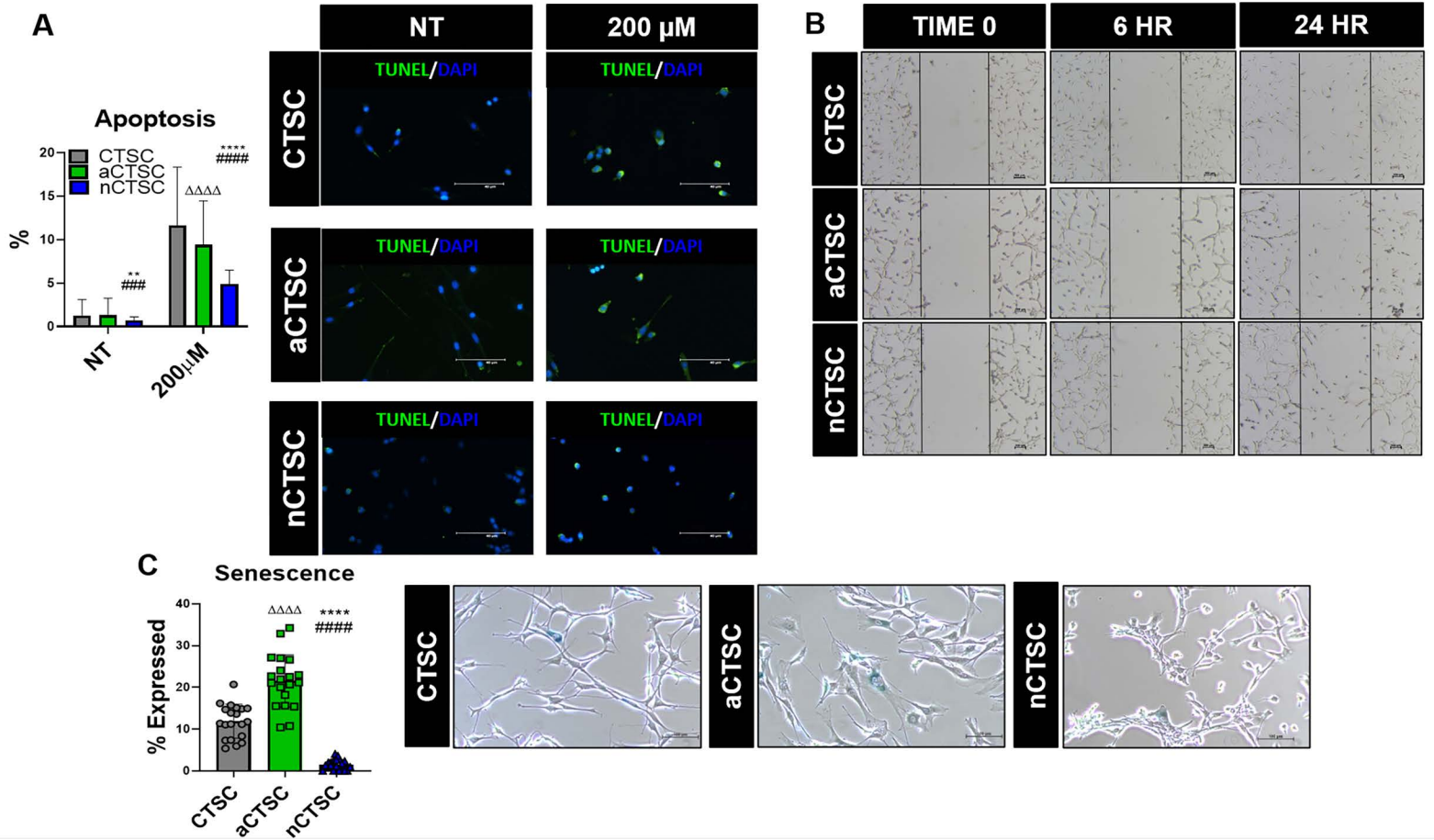
# Figure S1


A
Sca-1
Integrin β-1
VCAM
5'Nucleotidase
Endoglin
CD45
Integrin αM
Cell Count
CTSC
nCTSC
aCTSC
84.6%
90.7%
34.9%
77.5%
8.35%
0.22%
0.37%
98.2%
99.5%
21.7%
67.1%
0.062%
0.044%
0.13%
82.4%
2.71%
14.3%
1.62%
0.20%
0.067%
0.14%
B
Characterization
CTSC
aCTSC
nCTSC
Fold Change
CD105 CD90 CD73 SCA-1
C
D
E
F
CD45
C-KIT
NKX2.5
GATA4
Phallodin
DAPI
G
Day 0
Day 1
Day 10
Neonatal CM
Neonatal CF
Adult CM
Adult CF

# Figure S2


A
Pluripotency
CTSC
aCTSC
nCTSC
Fold Change
C-Myc
Sox-2
Nanog
Oct-4
SSEA-1
IGF-1
NKX2.5
ISL-1
GATA-4
B
CTSC
aCTSC
nCTSC
LIN28a
LIN28
Phallodin
DAPI
C
CTSC
aCTSC
nCTSC
Lin28A
β-Actin
LIN28a
Fold Change
D
mRNA LIN28a
Fold Change

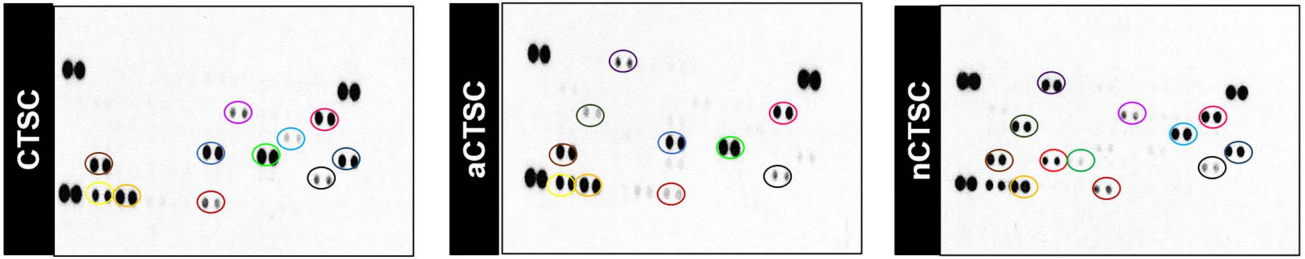
# Figure S3


A
NT
200 μM
Apoptosis
CTSC
aCTSC
nCTSC
%
NT
200μM
TUNEL/DAPI
B
TIME 0
6 HR
24 HR
C
Senescence
% Expressed
CTSC
aCTSC
nCTSC

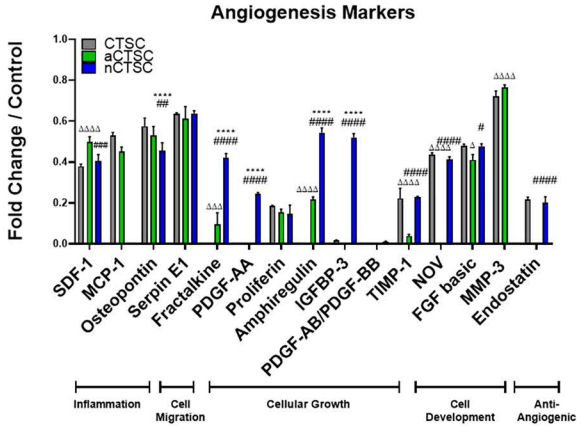
# Figure S4

**A**

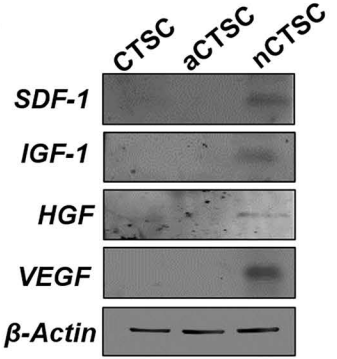
## Proteome Profiler



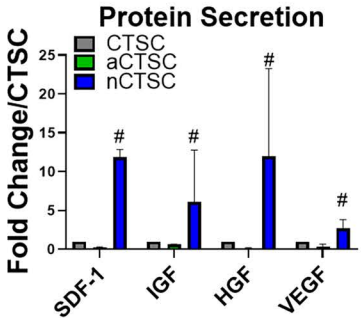

| Identifier | Protein |
|---|---|
| | SDF-1 |
| | MCP-1 |
| | Osteopontin |
| | Serpin E1 |
| | Fracalkine |
| | PDGF-AA |
| | Proliferin |
| | Amphiregulin |
| | IGFBP-3 |
| | PDFG-AB/PDGF-BB |
| | TIMP-1 |
| | NOV |
| | FGF basic |
| | MMP-3 |
| | Endostatin |



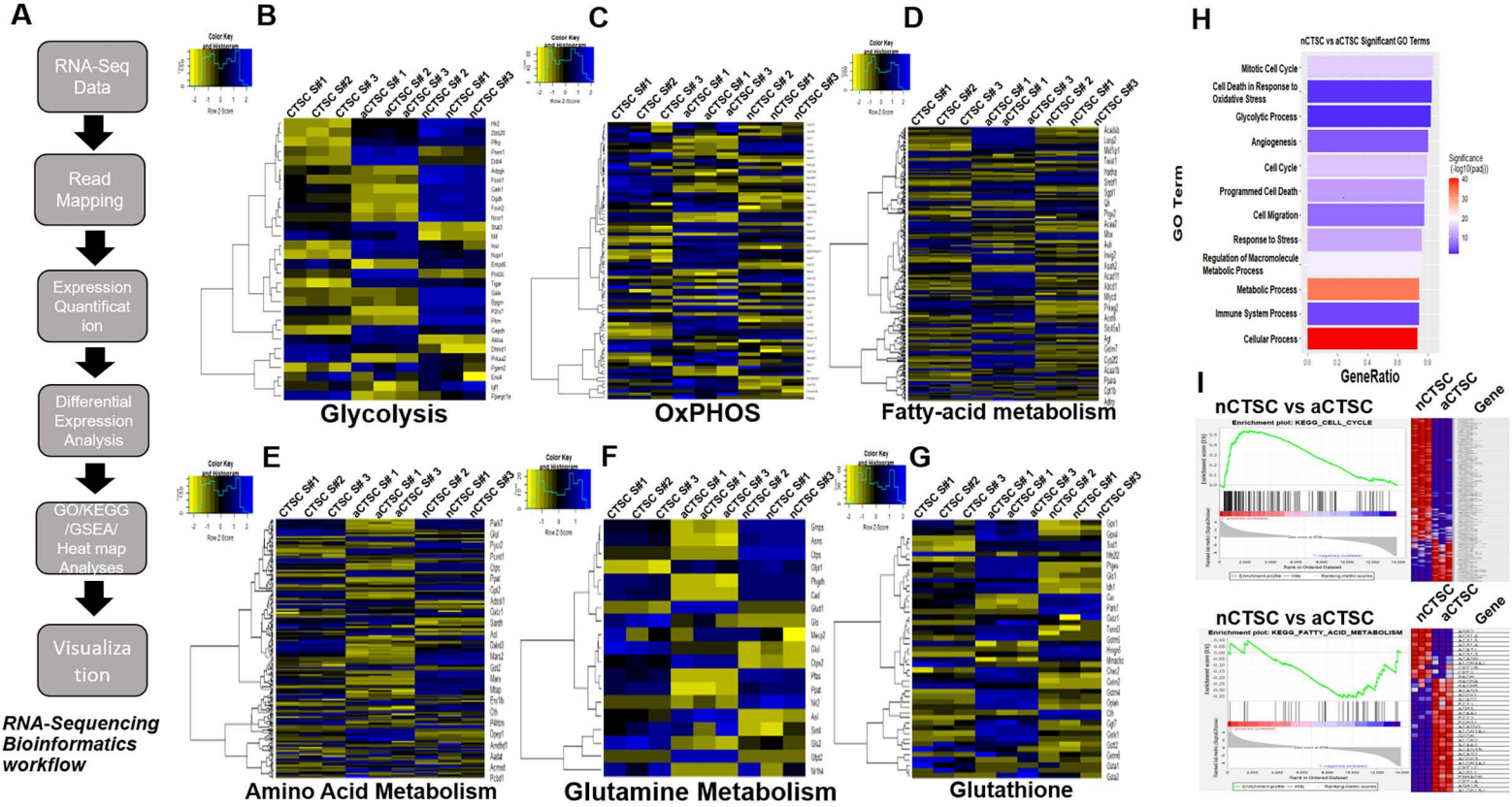

**B**



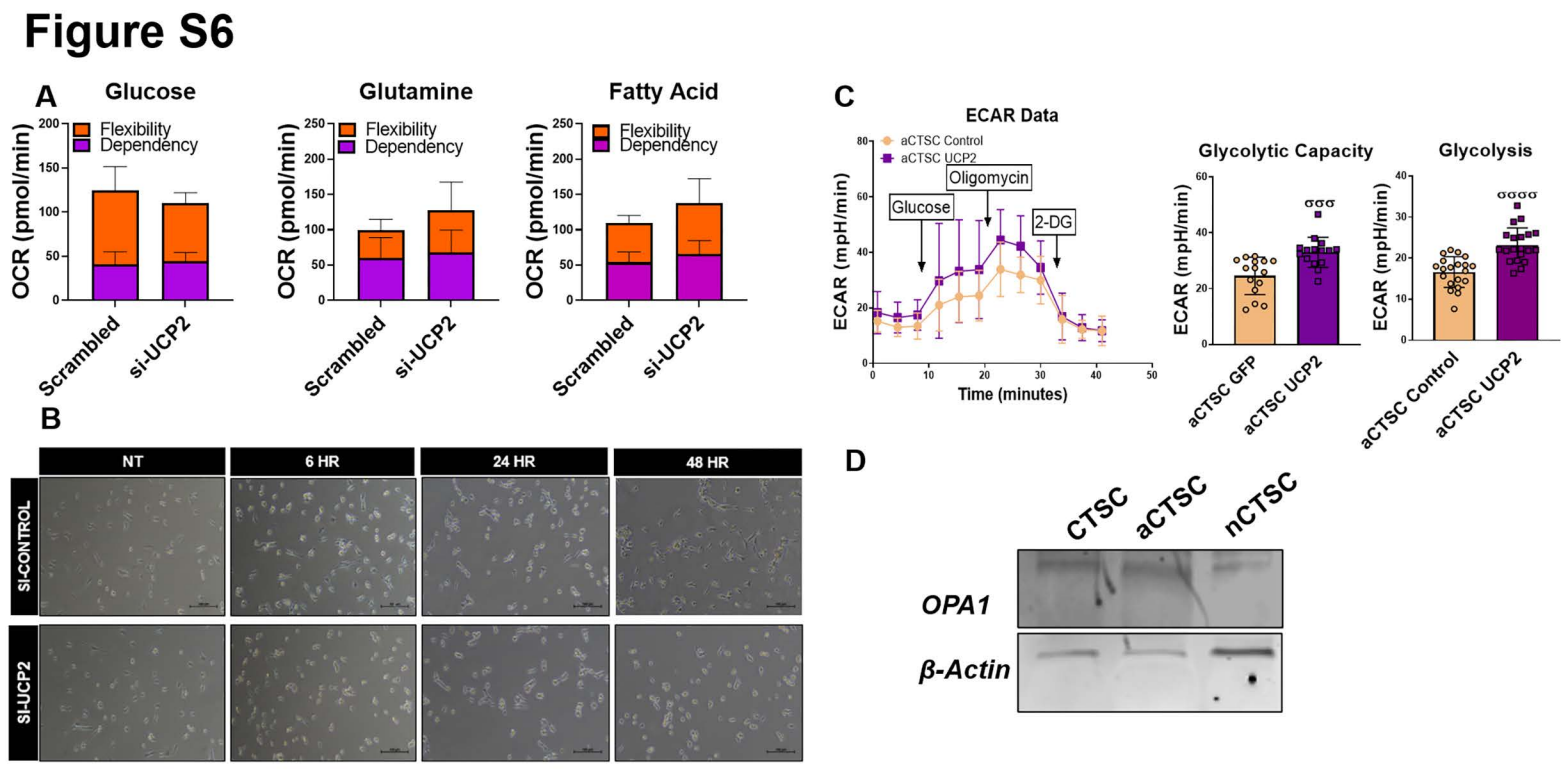

**C**



# Figure S5


A
RNA-Seq Data
Read Mapping
Expression Quantification
Differential Expression Analysis
GO/KEGG /GSEA/ Heat map Analyses
Visualization
RNA-Sequencing Bioinformatics workflow
B
C
D
Glycolysis
OxPHOS
Fatty-acid metabolism
E
F
G
Amino Acid Metabolism
Glutamine Metabolism
Glutathione
H
nCTSC vs aCTSC Significant GO Terms
GO Term
Mitotic Cell Cycle
Cell Death in Response to Oxidative Stress
Glycolytic Process
Angiogenesis
Cell Cycle
Programmed Cell Death
Cell Migration
Response to Stress
Regulation of Macromolecule Metabolic Process
Metabolic Process
Immune System Process
Cellular Process
GeneRatio
Significance (-log10(padj))
I
nCTSC vs aCTSC
Enrichment plot: KEGG_CELL_CYCLE
nCTSC vs aCTSC
Enrichment plot: KEGG_FATTY_ACID_METABOLISM

# Figure S6


A
Glucose
Glutamine
Fatty Acid
Flexibility
Dependency
OCR (pmol/min)
Scrambled
si-UCP2
B
NT
6 HR
24 HR
48 HR
SI-CONTROL
SI-UCP2
C
ECAR Data
aCTSC Control
aCTSC UCP2
Oligomycin
Glucose
2-DG
ECAR (mpH/min)
Time (minutes)
Glycolytic Capacity
Glycolysis
aCTSC GFP
aCTSC UCP2
aCTSC Control
aCTSC UCP2
D
CTSC
aCTSC
nCTSC
OPA1
β-Actin

# Supplemental Methods

## Supplemental Methods

### Morphological Analysis

All 3 cell types were plated in 6 well plate with 50,000cells/well followed by acquisition of brightfield images of the cells using Nikon Eclipse Ts2 microscope. Morphological analysis was conducted using ImageJ software by outlining each cell's structure. For each cell line a total of 100 cells were assessed from 3 separate experiments and data was cumulated.

### Wound Healing Assay

Cells were plated as 100,000 cells/ well of a 6-well plate (BD Falcon). Serum-free media (DMEM/F12 + PSG) was added to cells and kept overnight. Once 95% confluency was achieved in each well, wound was performed by creating a single scrape onto the well using a p200 tip. Images were taken at time 0, 6 hours, and 24 hours using Nikon Eclipse Ts2 microscope.

### Flow cytometry

Cell characterization and cell cycle analysis was done on CTSCs lines by flow cytometry assays as described previously[1]. For cell cycle measurements, CTSCs were serum starved overnight for synchronization and then release by addition of full medium for 6 hrs followed by staining with propidium iodide (BD Biosciences) and assessed on flow cytometer.

### Senescence-Associated β-Galactosidase Staining

Cellular senescence was assessed by staining the cells with senescence associated β-galactosidase kit (Abcam) as described previously[2,3]. Images were taken on Nikon Eclipse Ts2 microscope, 10 images were taken per CTSC slide for 3 independent experiments.

### Cell Apoptosis

For measurement of cell apoptosis, 50,000 cells /wells were plated into 6-well plate. CTSCs growth medium with 2.5% FBS media (12.5mL CTSC Media + 37.5mL F12/DMEM) was added along with 100μM of $H_2O_2$ for assessing cell death in response to stress. Cells were fixed in 4% paraformaldehyde and stained TUNEL cell apoptosis kit (Invitrogen) according to manufacturer's instruction.

### Reverse Transcriptase Polymerase Chain Reaction

CTSCs RNA extraction and purification were done using manufacturer's instruction (Qiagen). After RNA quantification (Nanodrop) cDNA was created using iScript cDNA Synthesis Kit (Bio-Rad). Amplification was conducted using SimpliAmp thermocycler (Applied Biosystems). Real-time polymerase chain reaction (RT-PCR) was done by running samples on StepOnePlus RT-PCR System (Applied Biosystems). Primers were created from Life Technologies and sequences are included in the Table SI.

### Mitochondrial DNA Content Determination

For assessment of Mitochondrial DNA content, cells were centrifuged into a pellet, supernatant was aspirated and DNA lysis buffer (0.5% SDS, 0.1M NaCl, 0.05M Tris (pH 8.0), 3mM EDTA, and $ddH_20$) was added to cells. Proteinase K is added at 100μg/mL to digest any contaminating proteins. Samples were incubated at 60°C overnight and then mixed with 8M Potassium Acetate. Chloroform was added to sample followed by centrifugation for 5 minutes at 9500rpm. Aqueous phase was isolated and 100% EtOH was added and stored in -20°C freezer for 15 minutes. After centrifugation for 5 minutes at 13000 rpm, supernatant was removed and pellet was washed with 75% EtOH. Samples were centrifuged for 2 minutes at 1300 rpm and supernatant was removed. Once air-dried, samples were resuspended in 100 μL $ddH_2O$. DNA

samples were normalized to 100ng/µl. qPCR was performed with primers for β-globin representing nuclear genome and COXII for mitochondrial genome. Mitochondrial genome content was measured after normalizing CTSCs samples.

**Citrate Synthase Activity**

Citrate synthase activity in CTSCs was measured using citrate synthase kit (Biovision) according to manufacturer's protocol. Briefly, CTSCs were grown to confluency followed by lyses using ice-cold CS Assay Buffer. Lysate was kept on ice for 10 minutes then centrifuged at 10,000 RPM for 5 minutes. Once supernatant was collected, samples were added to a 96-well flat bottom plate. Total volume of 50µL was achieved by adding appropriate amount of CS Assay Buffer. Diluted CS Positive Control was added in specific wells and adjusted to total 50µL with CS Assay Buffer. Standard curve preparation involved diluting GSH Standard and adding to wells. Reaction mix consisting of CS Assay Buffer, CS Developer, and CS Substrate Mix was then added to each well containing either sample, Positive Control, or Standards. Absorbance (OD 412 nm) was measured in kinetic mode at 25°C for 20-40 minutes. Calculation of citrate synthase activity was done using manufacturer's equation.

**Measurement of mitochondrial membrane potential by TMRM**

For measurement of mitochondrial membrane potential, 50,000 cells/wells were plated into 2-chamber slide for each CTSC line. Final concentration of 50nM TMRM (Invitrogen) prepared in CTSC growth medium, was added to samples and incubated for 30 minutes at 37°C. Live nuclear dye (Invitrogen) was added to chambers 10 minutes prior to scanning on the microscope. Images were taken on Leica SP8 Confocal Microscope. TMRM intensity quantifications were done through ImageJ.

**Lentivirus Transduction**

nCTSC and aCTSCs were transfected with Lv-CMV-UCP2-GFP (Cyagen) lentivirus while control cells were infected with Lv-GFP to create stable cell lines as described previously [1].

## References


1 Khan M, Mohsin S, Avitabile D et al. beta-Adrenergic regulation of cardiac progenitor cell death versus survival and proliferation. Circ Res 2013;112(3):476-486.

2 Choudhery MS, Khan M, Mahmood R et al. Mesenchymal stem cells conditioned with glucose depletion augments their ability to repair-infarcted myocardium. J Cell Mol Med 2012;16(10):2518-2529.

3 Khan M, Mohsin S, Khan SN et al. Repair of senescent myocardium by mesenchymal stem cells is dependent on the age of donor mice. J Cell Mol Med 2011;15(7):1515-1527.

**Table S1 – List of primers and antibodies**

| Primer Name | Forward/Reverse | Product size | Sequence |
|---|---|---|---|
| *Wee1* | Forward | 363 | TCTTACCGTAGTCGGAGGCA |
| *Wee1* | Reverse | | GCACATGACATTTCTGTTGCGA |
| *Lin28a* | Forward | 78 | TTTGCCTCCGGACTTCTCTG |
| *Lin28a* | Reverse | | CCCATGGTCGTCTGCTGAG |
| *Cyclin B1* | Forward | 134 | GGTGGAACGACTGTTGGTCT |
| *Cyclin B1* | Reverse | | TTTCGTGTTCCTGGTGACCC |
| *Cyclin A2* | Forward | 77 | AGGACAAAGCTGGCCTGAATC |
| *Cyclin A2* | Reverse | | GGTCCATGAGGCAAGGCTTA |
| *Cyclin D1* | Forward | 145 | ATTTCCAACCCGCCTTCCAT |
| *Cyclin D1* | Reverse | | GACAGTCCGCGTCACACTTG |
| *Cyclin D2* | Forward | 165 | GCTCTGTGTGCTACCGACTT |
| *Cyclin D2* | Reverse | | CACATCGGTGTGGGTGATCT |
| *Cyclin E1* | Forward | 243 | TCCAGGAAAAGAAAGGCAAATGT |
| *Cyclin E1* | Reverse | | TGCCCAGTTCAGTATAGGCAG |
| *CDK1* | Forward | 78 | CCTCTAAGCTCCCGGAGTCG |
| *CDK1* | Reverse | | CAACGGACCCTCTCTGTTCC |
| *E2F1* | Forward | 178 | GCCTCGAATAGGCAACCTGA |
| *E2F1* | Reverse | | ACCCTCCTCGAGACCAAAGT |
| *c-myc* | Forward | 187 | ACTCGGTGCAGCCCTATTTC |
| *c-myc* | Reverse | | GTAGCGACCGCAACATAGGA |
| *CDK2* | Forward | 84 | CTTTGCCGAAATGGTGACCC |
| *CDK2* | Reverse | | CCCAGAGTCCGAAAGATCCG |
| *HK1* | Forward | 75 | GATCGTTGGAGCAGACCACA |
| *HK1* | Reverse | | TGTACAAACACCCCGAGACG |
| *ALDO1* | Forward | 80 | CCTTAGTCCTTTCGCCTACCC |
| *ALDO1* | Reverse | | CGTTGCCATGGGTCACCTTG |
| *TPI1* | Forward | 61 | GAGAGCCGTGCGTTTGTACT |
| *TPI1* | Reverse | | CTGGTAGGCGCCATTGTACC |
| *ENO1* | Forward | 61 | TCCTTAAGGCTCTCCTCGGT |
| *ENO1* | Reverse | | AGTAGGATCGCTGCAAAGCA |
| *PGAM* | Forward | 56 | TTGCCAGTGGTCAGGACTTG |
| *PGAM* | Reverse | | CCTGTCAGACCGCCATAGTG |
| *PKM2* | Forward | 135 | CGCCTGGACATTGACTCTG |
| *PKM2* | Reverse | | GAAATTCAGCCGAGCCACATT |
| *PDK4* | Forward | 80 | ACGTCCTTTGCTTTTCTGCG |
| *PDK4* | Reverse | | CGGTCAGGCAGGATGTCAAT |
| *GAPDH* | Forward | 267 | GAAGCTCATTTCCTGGTATGACA |
| *GAPDH* | Reverse | | TATTGATGGTATTCGAGAGAAGGG |
| | | | |
| | | | |

| Application | Antibody | Dilution | Cat No. | Company |
|---|---|---|---|---|
| Western Blot | Wee1 | 1:300 | Ab137377 | Abcam |
| Western Blot | Lin28a | 1:500 | SC-6216 | Santa Cruz |
| Western Blot | Cyclin B1 | 1:1000 | 4138 | Cell Signaling |
| Western Blot | Cyclin D1 | 1:3000 | 2922S | Cell Signaling |
| Western Blot | CDK1 | 1:500 | ab18 | Abcam |
| Western Blot | p-CDK tyr15 | 1:500 | 9114 | Cell signaling |
| Western Blot | βactin | 1:1000 | 9664S | Cell Signaling |
| Western Blot | p-AKT S473 | 1:500 | 4060 | Cell Signaling |
| Western Blot | AKT | 1:500 | 9272 | Cell Signaling |
| ICC | BrdU | 1:100 | Ab6326 | Abcam |
| ICC | Sarcomeric Actin | 1:100 | A2172 | Sigma Aldrich |
| ICC | Aurora B | 1:50 | Ab2254 | Abcam |
| ICC | Phospho Histone 3 | 1:100 | 441190G | Life technologies |
| ICC | Ki67 | 1:100 | Ab15580 | Abcam |
| ICC | SMA | | A2547 | Sigma Aldrich |
| IHC | WGA-488 | 1:200 | W11261 | Life technologies |

## TABLE S2

### A. Comparison Cardiomyocytes (CM) to nCTSC

| No. | Gene | CM | nCTSC |
|---|---|---|---|
| 1. | alpha-Sarcoglycan | ++++ | |
| 2. | Atrial Natriuretic Peptide/ANP | +++ | |
| 3. | beta-Sarcoglycan | ++++ | ++ |
| 4. | BMP-4 | +++ | + |
| 5. | Connexin 37/GJA4 | +++ | |
| 6. | Connexin 40/GJA5 | ++ | |
| 7. | Cripto | | |
| 8. | Desmin | +++++ | |
| 9. | epsilon-Sarcoglycan | +++ | ++ |
| 10. | FABP3/H-FABP | +++++ | + |
| 11. | GATA-4 | ++++ | |
| 12. | GATA-6 | +++ | + |
| 13. | HCN4 | +++ | |
| 14. | Kir2.1 | +++ | + |
| 15. | LRG1 | +++ | |
| 16. | MEF2C | +++ | ++ |
| 17. | MYH6 | +++++ | |
| 18. | MYH7 | +++++ | |
| 19. | TBX5 | +++ | |
| 20. | Troponin T | +++ | |
| 21. | Troponin T2 | +++++ | ++ |

| Quality Score | Normalized read count |
|---|---|
| + | 10s |
| ++ | 100s |
| ++++ | 1000s |
| ++++ | 10000s |
| ++++ | 100000s |

**TABLE S2**

## B. Comparison of Cardiac fibroblast (CF) to nCTSC

| No. | Gene | CF | nCTSC |
|---|---|---|---|
| 1. | WT1 | ++ | |
| 2. | Tcf21 | +++ | |
| 3. | Prolyl-4-hydroxylase | + | + |
| 4. | Vimentin | ++++ | +++++ |
| 5. | aSMA | ++ | + |
| 6. | PDGFR? | +++ | +++ |
| 7. | DDR2 | +++ | +++ |
| 8. | CD90 | +++ | |
| 9. | Sca1 | +++ | |
| 10. | Periostin | +++ | |
| 11. | Fibronectin | +++ | |
| 12. | Collagen type I | ++++ | ++ |
| 13. | Collagen type III | ++++ | ++ |
| 14. | FAP | ++ | |

## TABLE S2

### C. Comparison of Endothelial Cells (ECs) to nCTSCs

| No. | Gene | EC | nCTSC |
|---|---|---|---|
| 1. | ACE/CD143 | ++ | + |
| 2. | C1qR1/CD93 | ++++ | + |
| 3. | VE-Cadherin | ++ | |
| 4. | CC Chemokine Receptor D6 | + | |
| 5. | CD31/PECAM-1 | ++++ | |
| 6. | CD34 | ++++ | ++++ |
| 7. | CD36/SR-B3 | ++++ | |
| 8. | CD151 | ++ | ++++ |
| 9. | CD160 | + | |
| 10. | CD300g/Nepmucin | ++++ | |
| 11. | CL-K1/COLEC11 | + | |
| 12. | CL-P1/COLEC12 | ++ | ++ |
| 13. | Coagulation Factor III/Tissue Factor | | ++ |
| 14. | DCBLD2/ESDN | ++ | ++++ |
| 15. | ECSCR | ++ | |
| 16. | EMMPRIN/CD147 | ++ | ++++ |
| 17. | Endoglin/CD105 | ++ | ++ |
| 18. | Endomucin | ++++ | |
| 19. | Endosialin/CD248 | | ++++ |
| 20. | EPCR | + | ++ |
| 21. | Erythropoietin R | | + |
| 22. | ESAM | ++ | |
| 23. | FABP5/E-FABP | ++ | ++ |
| 24. | ICAM-1/CD54 | ++ | |
| 25. | ICAM-2/CD102 | ++ | |
| 26. | IL-13 R alpha 1 | + | ++++ |
| 27. | Integrin beta 2/CD18 | + | |
| 28. | KLF4 | ++ | ++++ |
| 29. | LYVE-1 | ++ | |
| 30. | MCAM/CD146 | ++ | ++ |
| 31. | Nectin-2/CD112 | + | ++ |
| 32. | PD-ECGF/Thymidine Phosphorylase | + | + |
| 33. | Podocalyxin | ++++ | ++ |
| 34. | Podoplanin | + | + |
| 35. | S1P1/EDG-1 | ++++ | ++ |

| | | | |
|---|---|---|---|
| **36.** | S1P2/EDG-5 | + | ++++ |
| **37.** | S1P3/EDG-3 | + | + |
| **38.** | S1P4/EDG-6 | + | |
| **39.** | E-Selectin/CD62E | + | ++++ |
| **40.** | P-Selectin/CD62P | + | ++++ |
| **41.** | SLAM/CD150 | + | |
| **42.** | Stabilin-1 | ++++ | + |
| **43.** | TEM7/PLXDC1 | + | |
| **44.** | TEM8/ANTXR1 | + | ++++ |
| **45.** | Thrombomodulin/BDCA-3 | ++++ | ++++ |
| **46.** | THSD1 | ++ | + |
| **47.** | THSD7A | ++ | ++ |
| **48.** | Tie-2 | ++++ | |
| **49.** | TNF RI/TNFRSF1A | ++ | ++++ |
| **50.** | TNF RII/TNFRSF1B | ++ | ++ |
| **51.** | TRAIL R2/TNFRSF10B | ++ | ++++ |
| **52.** | TRAILR1/TNFRSF10A | + | ++ |
| **53.** | VCAM-1/CD106 | ++ | ++ |
| **54.** | VE-Statin | ++++ | + |
| **55.** | VEGFR1/Flt-1 | ++++ | ++++ |
| **56.** | VEGFR2/KDR/Flk-1 | ++++ | + |
| **57.** | VEGFR3/Flt-4 | ++++ | + |
| **58.** | VG5Q | ++ | ++ |
| **59.** | vWF-A2 | ++++ | + |

**TABLE S2**

## D. Embryonic stem cell (ESC) comparison to nCTSC

| No. | Gene | ESC | nCTSC |
|---|---|---|---|
| 1. | E-Cadherin | ++++ | |
| 2. | Cbx2 | ++ | ++ |
| 3. | CD9 | +++ | +++ |
| 4. | CD30/TNFRSF8 | + | |
| 5. | CD117/c-kit | +++ | |
| 6. | CHD1 | +++ | +++ |
| 7. | Cripto | ++++ | |
| 8. | DNMT3B | ++ | + |
| 9. | DPPA2 | ++ | |
| 10. | DPPA4 | ++ | |
| 11. | DPPA5/ESG1 | ++++ | |
| 12. | EpCAM/TROP1 | ++ | |
| 13. | F-box protein 15/FBXO15 | +++ | |
| 14. | FGF-4 | +++ | |
| 15. | FGF-5 | + | |
| 16. | FoxD3 | + | |
| 17. | GBX2 | + | |
| 18. | GCNF/NR6A1 | ++ | + |
| 19. | GDF-3 | ++ | |
| 20. | Integrin alpha 6/CD49f | +++ | ++++ |
| 21. | Integrin beta 1/CD29 | +++ | ++++ |
| 22. | KLF4 | +++ | +++ |
| 23. | KLF5 | +++ | ++ |
| 24. | L1TD1 | +++ | |
| 25. | Lefty-1 | | + |
| 26. | Lefty-A | ++ | |
| 27. | LIN-28A | ++ | |
| 28. | LIN-28B | ++ | |
| 29. | LIN-41 | +++ | |
| 30. | c-Maf | +++ | +++ |
| 31. | Nanog | ++++ | |
| 32. | Oct-4. | +++ | |
| 33. | Podocalyxin | ++ | ++ |
| 34. | Rex-1/ZFP42 | +++ | |

| | | | |
|---|---|---|---|
| **35.** | Smad2 | ++ | +++ |
| **36.** | SOX2 | +++ | +++ |
| **37.** | SSEA-1 | | + |
| **38.** | STAT3 | +++ | +++ |
| **39.** | Stella/Dppa3 | + | |
| **40.** | SUZ12 | +++ | +++ |
| **41.** | TBX3 | +++ | +++ |
| **42.** | TBX5 | + | |
| **43.** | TEX19.1 | ++ | |
| **44.** | THAP11 | ++ | ++ |
| **45.** | UTF1 | ++ | |
| **46.** | VISTA/B7-H5/PD-1H | | + |
| **47.** | ZIC3 | ++ | |

## TABLE S2

### E. Cardiac progenitor Cell (CPC) comparison to nCTSC

| No. | Gene | CPC | nCTSC |
|---|---|---|---|
| 1. | ABCG2 | ++ | ++ |
| 2. | CD34 | ++ | +++ |
| 3. | CD117/c-kit | ++ | |
| 4. | ETV2/ER71 | + | |
| 5. | GATA-4 | +++ | |
| 6. | Integrin beta 1/CD29 | +++ | ++++ |
| 7. | Islet-1 | ++ | + |
| 8. | NKX2.5 | +++ | |
| 9. | Sca-1/Ly6 | | ++ |
| 10. | SCF/c-kit Ligand | ++ | ++ |
| 11. | SSEA-1 | + | + |
| 12. | TBX18 | + | + |
| 13. | WT1 | + | |

**TABLE S2**

## F. Comparison of nCTSC to Mesenchymal Stem Cell (MSCs)

| No. | Gene | MSCs | nCTSC |
|---|---|---|---|
| 1. | 5'-Nucleotidase/CD73 | + | +++ |
| 2. | ALCAM/CD166 | ++ | +++ |
| 3. | Aminopeptidase N/CD13 | ++ | + |
| 4. | BMPR-IA/ALK-3 | +++ | +++ |
| 5. | BMPR-IB/ALK-6 | ++ | + |
| 6. | BMPR-II | ++++ | ++++ |
| 7. | N-Cadherin | +++ | +++ |
| 8. | CD44 | ++++ | ++++ |
| 9. | CD45 | ++ | |
| 10. | CD90/Thy1 | +++ | |
| 11. | Endoglin/CD105 | ++ | ++ |
| 12. | Fibronectin | + | |
| 13. | Anastellin | ++++ | ++++ |
| 14. | ICAM-1/CD54 | + | |
| 15. | Integrin alpha 1/CD49a | ++ | + |
| 16. | Integrin alpha 5/CD49e | ++++ | +++ |
| 17. | Integrin alpha V/CD51 | ++++ | +++ |
| 18. | Integrin beta 1/CD29 | ++++ | ++++ |
| 19. | NCAM-1/CD56 | +++ | +++ |
| 20. | Nucleostemin | ++ | +++ |
| 21. | Sca-1/Ly6 | +++ | ++ |
| 22. | SUSD2 | + | |
| 23. | TfR (Transferrin R) | ++ | |
| 24. | VCAM-1/CD106 | +++ | ++ |
| 25. | Vimentin | ++++ | ++++ |

## TABLE S3

Comparison of nCTSC to embryonic CPC populations

| Quality Score | Normalized read count |
|---|---|
| + | 10s |
| ++ | 100s |
| ++++ | 1000s |
| ++++ | 10000s |
| ++++ | 100000s |

| Origin | Subpopulation | Marker | CTSC | aCTSC | nCTSC |
|---|---|---|---|---|---|
| pSHF | A | Foxf1 | ++ | + | ++ |
| pSHF | A | Lefty2 | | | |
| pSHF | A | Pitx2 | | | |
| pSHF | B | Aldh1a2 | | + | |
| pSHF | B | Pbx1 | +++ | +++ | +++ |
| pSHF | C | Gata6 | ++ | + | + |
| pSHF | C | Wnt2 | | | |
| pSHF | C | Nr2f2 | +++ | +++ | +++ |
| pSHF | C | Tnnt2 | | + | ++ |
| pSHF | C | Myl7 | + | ++ | +++ |
| pSHF | C | Sfrp5 | | | |
| pSHF | C | Actc1 | | | |
| pSHF | C | Upp1 | | | |
| *FHF* | D | Phlda2 | | | |
| *FHF* | D | Mab21/2 | | ++ | ++ |
| *FHF* | D | Msx1 | ++ | ++ | ++ |
| *FHF* | D | Krt8 | | | |
| *FHF* | D | Hand1 | | | |
| *FHF* | D | Irx4 | | | |
| **AHF** | E | Schip1 | + | + | ++ |
| **AHF** | E | Fhl1 | | + | + |
| | E | Bambi | ++ | ++ | + |
| **AHF** | E | Nkx2-5 | | | |
| **AHF** | E | Mef2c | ++ | ++ | ++ |
| **AHF** | E | Dkk1 | | | |
| **AHF** | F | Bmp4 | | | + |
| **AHF** | F | Dlk1 | +++ | ++ | + |
| **AHF** | F | Myh10 | +++ | +++ | +++ |
| **AHF** | F | Prrx1 | +++ | +++ | +++ |
| **AHF** | F | Rgs5 | + | ++ | ++ |
| **AHF** | H | Crabp2 | | ++ | |
| **AHF** | H | Vamp8 | ++ | ++ | ++ |
| **AHF** | H | Pdcd4 | ++ | +++ | ++ |
| **AHF** | H | Fgf10 | +++ | +++ | +++ |
| **AHF** | H | Mpped2 | | | |
| **AHF** | H | Igfbpl1 | | | |
| **AHF** | H | Nkx2-6 | | | |
| **AHF** | I | Isl1 | + | | + |
| **AHF** | I | Lefty1 | | + | + |
| **AHF** | I | Fst | ++ | +++ | +++ |
| **AHF** | I | Irx5 | + | ++ | ++ |
| **AHF** | I | Tbx1 | | | |

| | | | | | |
|---|---|---|---|---|---|
| **AHF** | I | Fgf8 | | | |