Figure S1

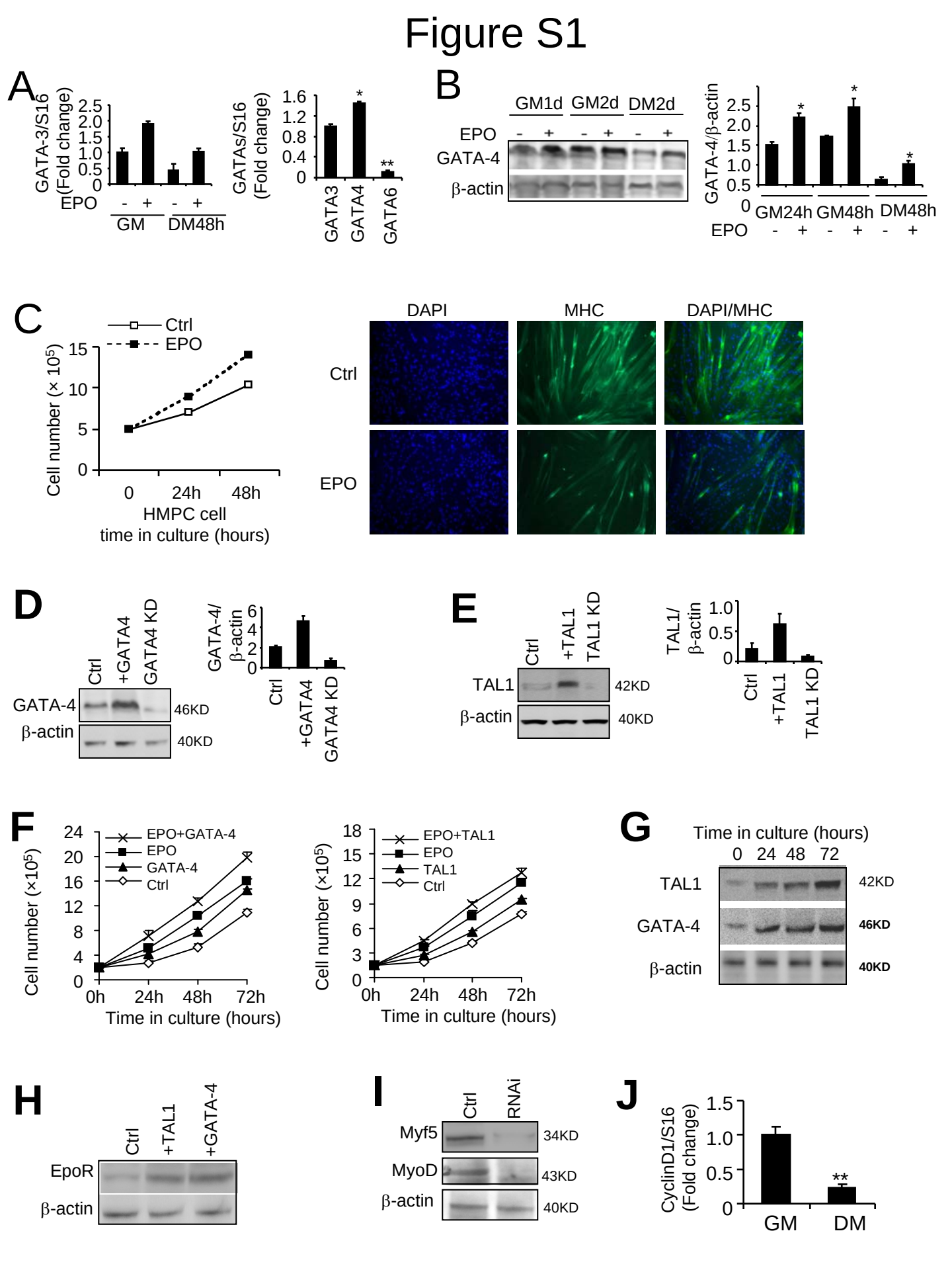
A
GATA-3/S16 (Fold change)
EPO
GM
DM48h
GATAs/S16 (Fold change)
GATA3
GATA4
GATA6
B
GM1d
GM2d
DM2d
EPO
GATA-4
β-actin
GATA-4/β-actin
GM24h
GM48h
DM48h
C
Ctrl
EPO
Cell number (× 10^5)
HMPC cell time in culture (hours)
DAPI
MHC
DAPI/MHC
D
Ctrl
+GATA4
GATA4 KD
GATA-4
β-actin
46KD
40KD
GATA-4/ β-actin
E
TAL1
42KD
40KD
TAL1/ β-actin
F
EPO+GATA-4
EPO
GATA-4
Ctrl
Cell number (×10^5)
Time in culture (hours)
EPO+TAL1
TAL1
G
Time in culture (hours)
H
EpoR
I
Myf5
MyoD
34KD
43KD
RNAi
J
CyclinD1/S16 (Fold change)
GM
DM

Figure S2

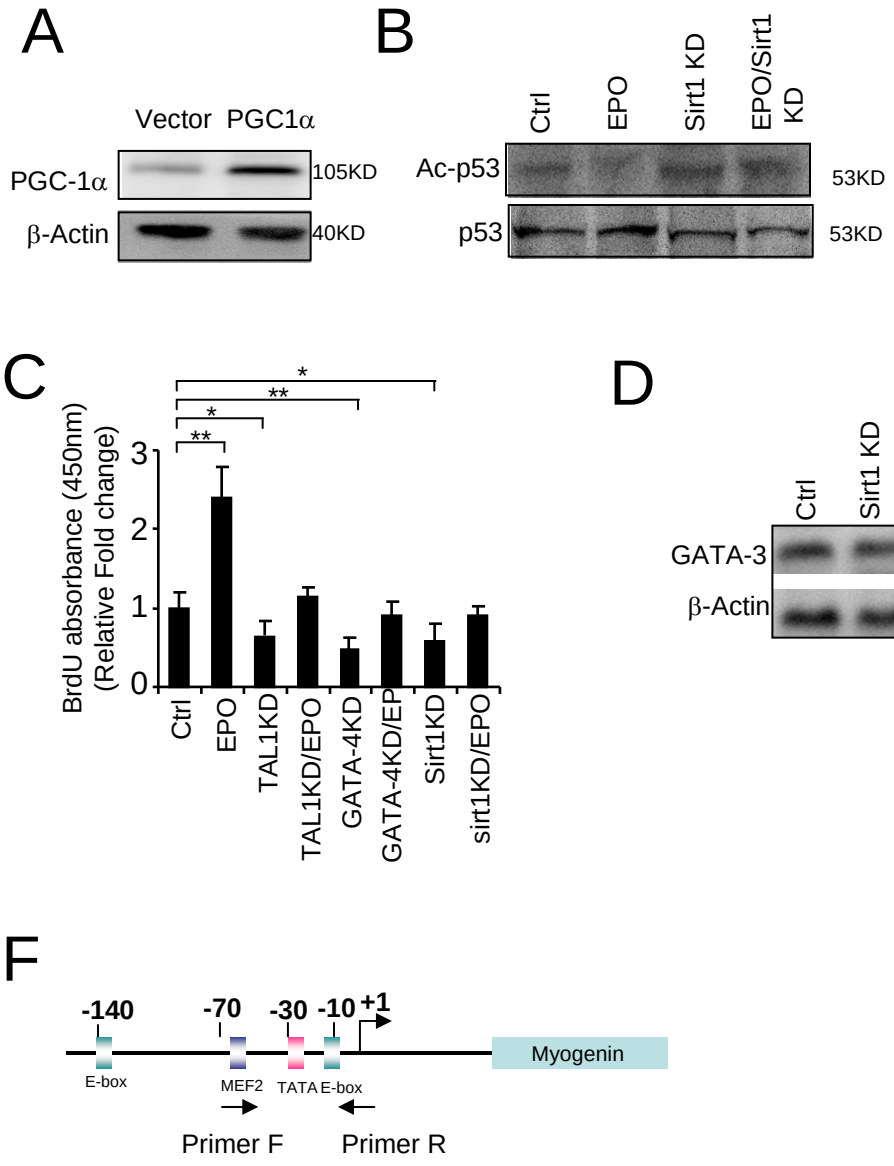
A
Vector
PGC1α
PGC-1α
105KD
β-Actin
40KD
B
Ctrl
EPO
Sirt1 KD
EPO/Sirt1 KD
Ac-p53
53KD
p53
53KD
C
BrdU absorbance (450nm)
(Relative Fold change)
Ctrl
EPO
TAL1KD
TAL1KD/EPO
GATA-4KD
GATA-4KD/EP
Sirt1KD
sirt1KD/EPO
D
Ctrl
Sirt1 KD
GATA-3
48KD
β-Actin
40KD
E
Ctrl
+GATA-3
CyclinD1
36KD
β-Actin
40KD
Myog
34KD
β-Actin
40KD
F
-140
-70
-30
-10
+1
E-box
MEF2
TATA
E-box
Myogenin
Primer F
Primer R

Table S1 Primers for ChIP assays,

Primers	Forward	Reverse
Cyclin D1 prom	AGAACAGGGTGTCCTTGCAC	CGGACTGCTTCTCTCCAAAC
EpoR prom	AAGGGAACAGGGGCCTTCTT	GGCACCCTGAGTTTGTCCAT
TAL1 prom	CAGATCCGTTAGAGGGTTCG	CTGGGAATTACCTCGTGTGC
GATA-4 prom	TCCGCGGACTCACGGAGATC	ACCAGAGCGGCTCCAGCGAA
Myogenin prom	TGGCTATATTTATCTCTGGGTTC	GCTCCCGCAGCCCCT

Supplementary Figure Legends

Supplemental Figure S1. GATA factor and TAL1 expression and myoblast proliferation. A) GATA-3 expression level in C2C12 cultured in growth medium and differentiation medium without or with EPO treatment. GATA factor expression (GATA-3, GATA-4 and GATA-6) in C2C12 cells was determined using quantitative RT-PCR and normalized to S16. B) EPO induction of GATA-4 protein was evident in C2C12 cultures in growth medium (after 1 (GM1) and 2 (GM2) days) and in differentiation medium (after 2 days (DM2)) determined by Western blotting and normalized to β-actin. C) Primary human muscle precursor cells (HMPC) treated without (Ctrl; solid line) or with EPO (5U/ml; dashed line) were assessed by determination of cell number under growth conditions and by MHC staining following myoblast differentiation for 72 hours. D-E). GATA-4 (D) and TAL1 (E) proteins were assessed and quantified using Western blotting after overexpression (+) and knock down (KD) of GATA-4 (D) or TAL1 (E). F) Cell number was determined for C2C12 myoblasts cultured without (control (Ctrl); open diamonds), with GATA-4 (left) or TAL1 (right) over expression (triangles), with EPO treatment (squares) and with EPO treatment plus GATA-4 or TAL1 over expression (X) at times indicated. G) Overexpression of TAL1 and GATA-4 in C2C12 myoblasts were detected by Western blotting at times indicated. H) EpoR protein level was determined in C2C12 cells with overexpression TAL1 and GATA-4 (48h). I) Knocking down of Myf5 and MyoD was confirmed by Western blotting. J) CyclinD1 mRNA level was measured during myogenic differentiation after 2 days. The mean values from 3 experiments are shown. Error bars represent standard deviation; * indicates $p<0.05$ and ** indicates $p<0.01$.

Supplemental Figure S2. EPO and protein acetylation. A) Overexpression of PGC-1α in C1C12 was confirmed by Western blotting. B) The P53 acetylation was determined in the C2C12 with EPO treatment or Sirt1 knocking down by Western blotting. Representative blots are shown. C). Cell proliferation was determined by BrdU cell absorption in C2C12 myoblasts with TAL1, GATA-4 and Sirt1 knock-down without or with EPO treatment. D). GATA-3 protein level was determined by Western blotting with knock down (KD) of Sirt1 in C2C12 cells. E). Cyclin D1 protein level was determined with overexpression of GATA-3 in C2C12 myoblasts cultured in growth medium and myogenin protein level was determined with overexpression of GATA-3 in C2C12 cultured in differentiation medium for 48h. F). The mouse Myogenin promoter consists of a TATA box and is flanked by activator sites (MEF2 and E-box). Arrowheads correspond to the region spanning the Myogenin promoter primer sequences used for analysis in the ChIP assay. * indicates $p<0.05$; ** indicates $p<0.01$;***indicates $p<0.001$.

Supplemental Table S1. The ChIP primer pairs.