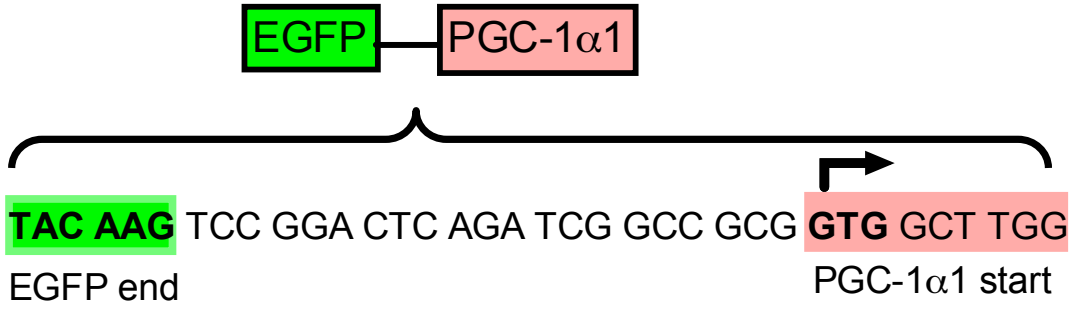
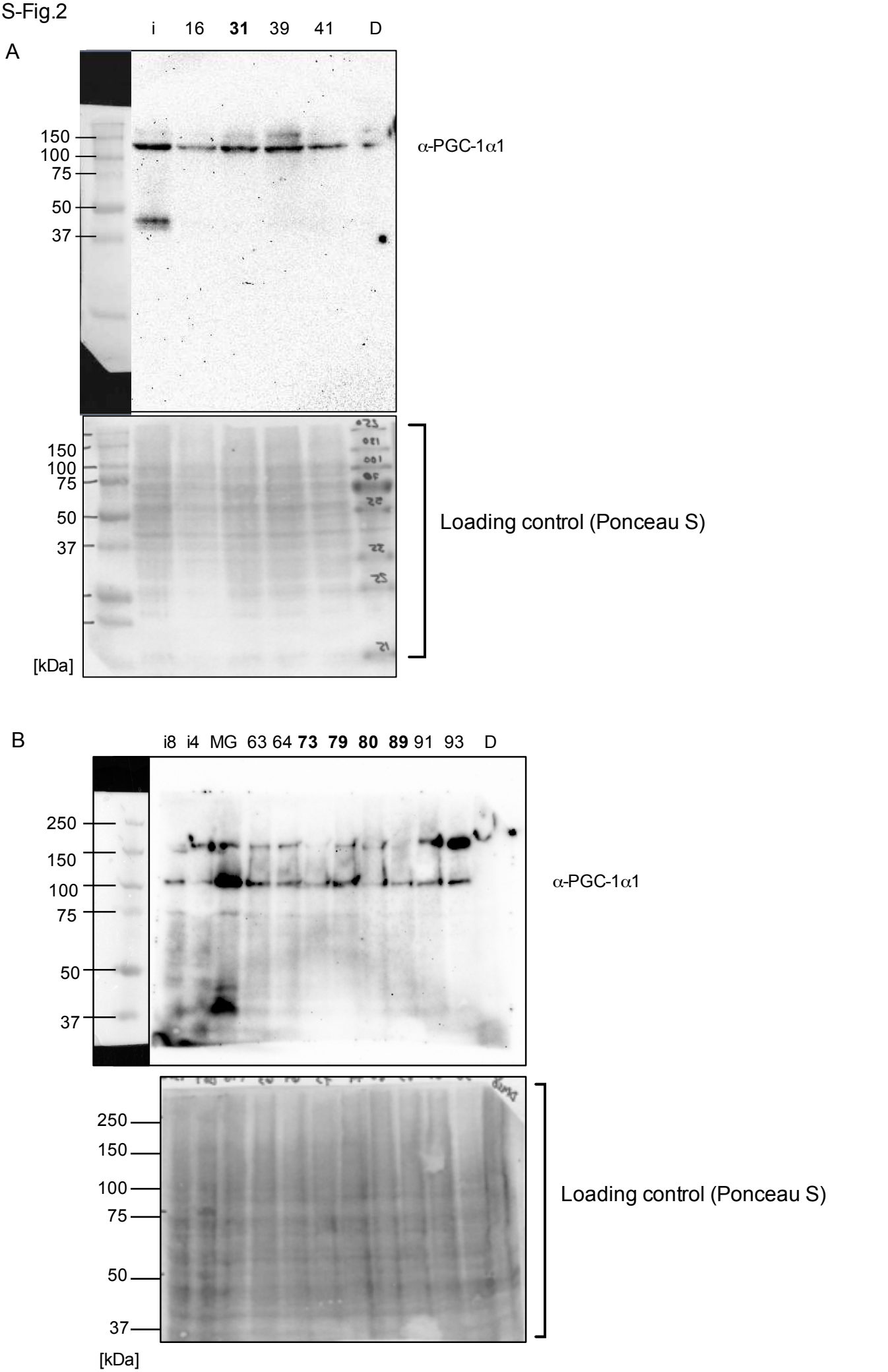
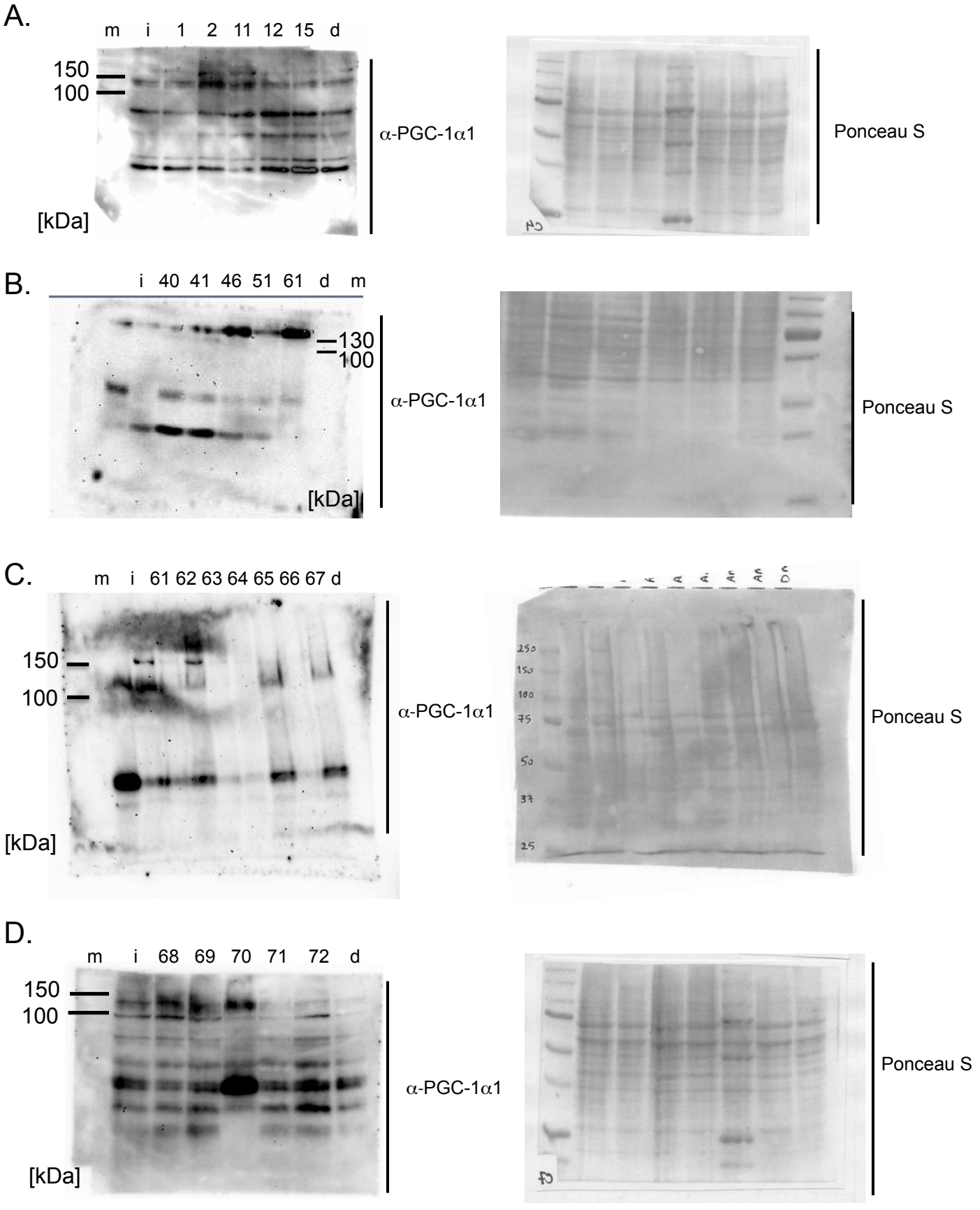
S-Fig.1


EGFP
PGC-1α1
TAC AAG TCC GGA CTC AGA TCG GCC GCG GTG GCT TGG
EGFP end
PGC-1α1 start

S-Fig.2

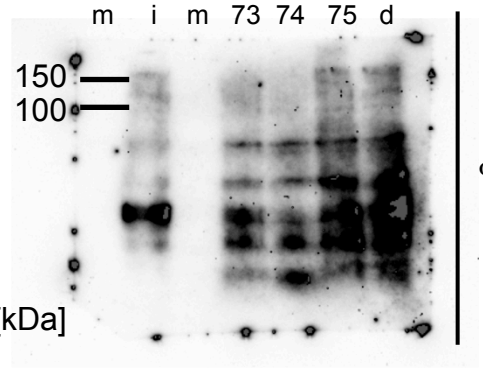

A
i 16 31 39 41 D
150
100
75
50
37
α-PGC-1α1
150
100
75
50
37
[kDa]
Loading control (Ponceau S)
B
i8 i4 MG 63 64 73 79 80 89 91 93 D
250
150
100
75
50
37
α-PGC-1α1
250
150
100
75
50
37
[kDa]
Loading control (Ponceau S)

S-Fig.3

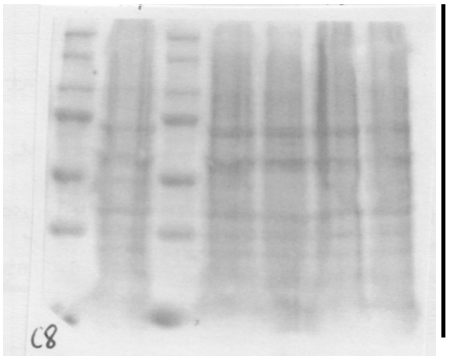

A.
m i 1 2 11 12 15 d
150
100
[kDa]
α-PGC-1α1
Ponceau S
B.
i 40 41 46 51 61 d m
130
100
[kDa]
α-PGC-1α1
Ponceau S
C.
m i 61 62 63 64 65 66 67 d
150
100
[kDa]
α-PGC-1α1
250
150
100
75
50
37
25
Ponceau S
D.
m i 68 69 70 71 72 d
150
100
[kDa]
α-PGC-1α1
Ponceau S

S-Fig.4

A.

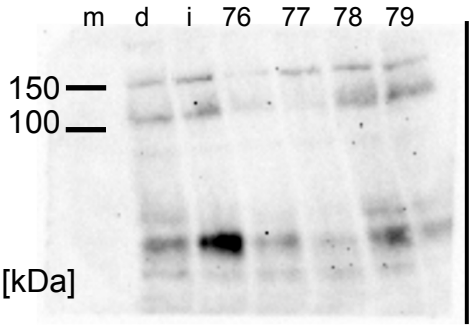


α-PGC-1α1

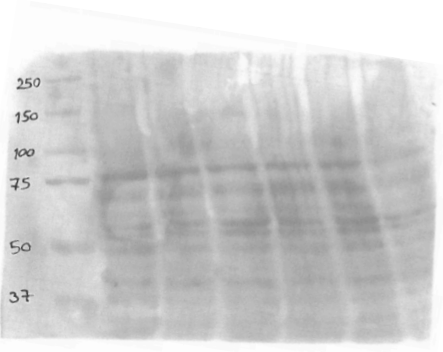


Ponceau S

B.

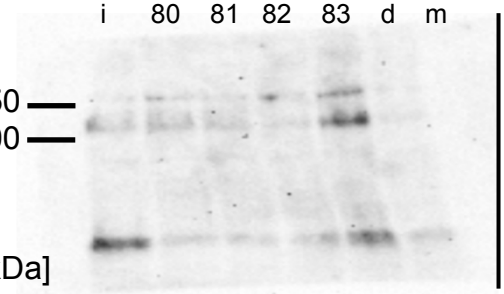


α-PGC-1α1

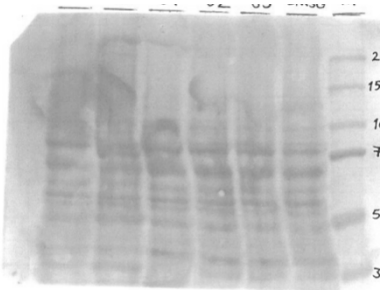


Ponceau S

C.

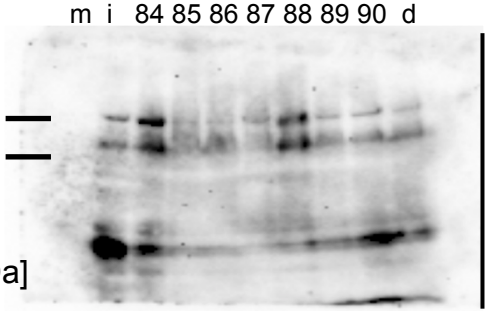


α-PGC-1α1

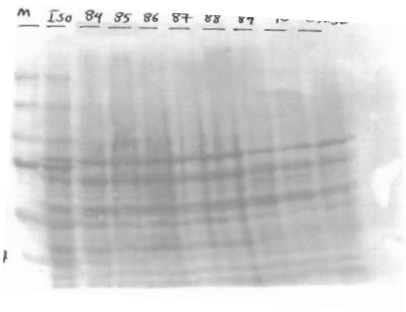


Ponceau S

D.

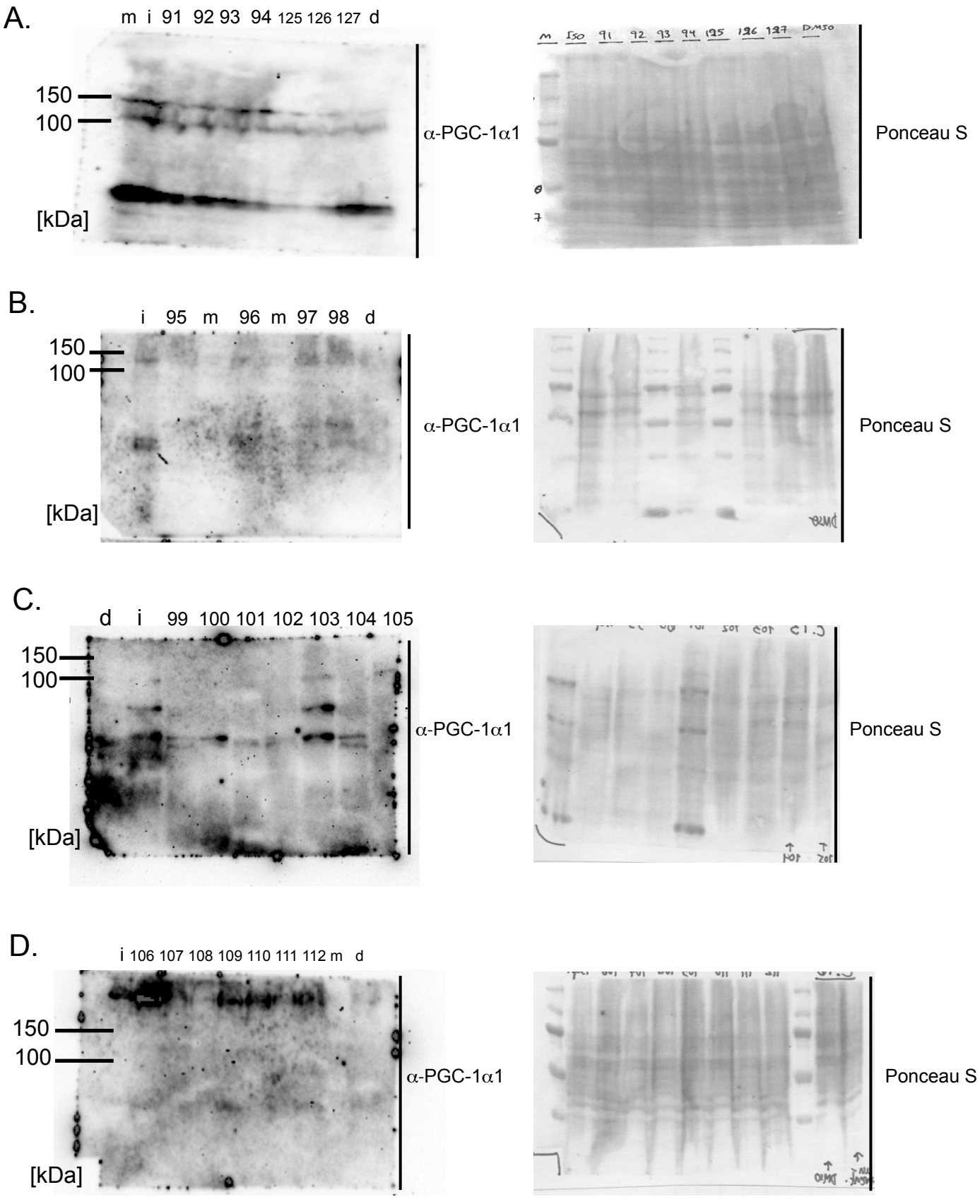


α-PGC-1α1



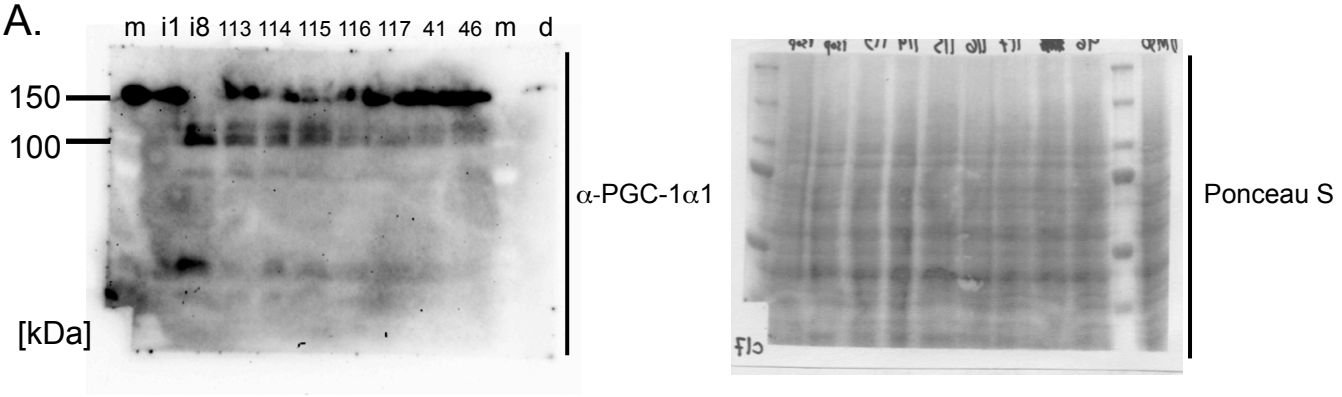
Ponceau S

S-Fig.5

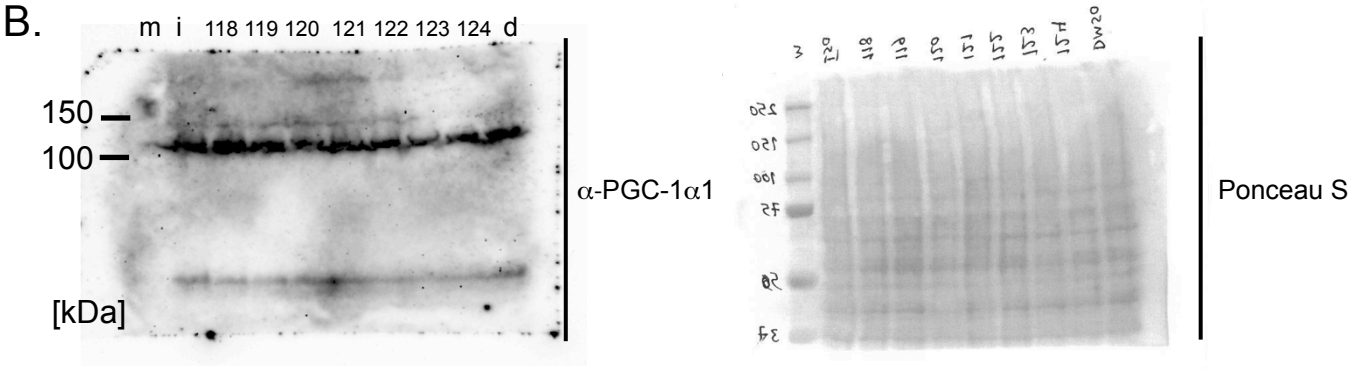

A.
m i 91 92 93 94 125 126 127 d
150
100
[kDa]
α-PGC-1α1
Ponceau S
B.
i 95 m 96 m 97 98 d
150
100
[kDa]
α-PGC-1α1
Ponceau S
C.
d i 99 100 101 102 103 104 105
150
100
[kDa]
α-PGC-1α1
Ponceau S
D.
i 106 107 108 109 110 111 112 m d
150
100
[kDa]
α-PGC-1α1
Ponceau S

S-Fig.6

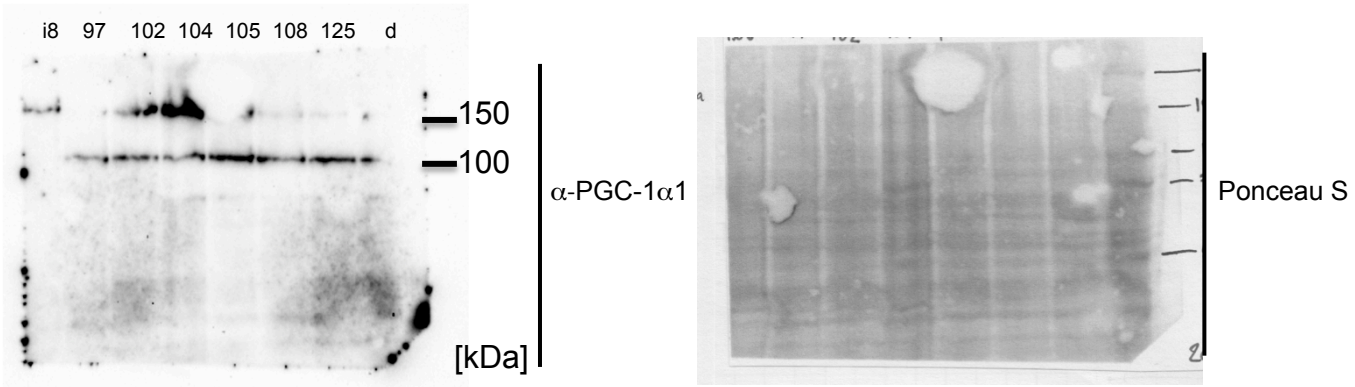
A.


m i1 i8 113 114 115 116 117 41 46 m d
150
100
[kDa]
α-PGC-1α1
Ponceau S

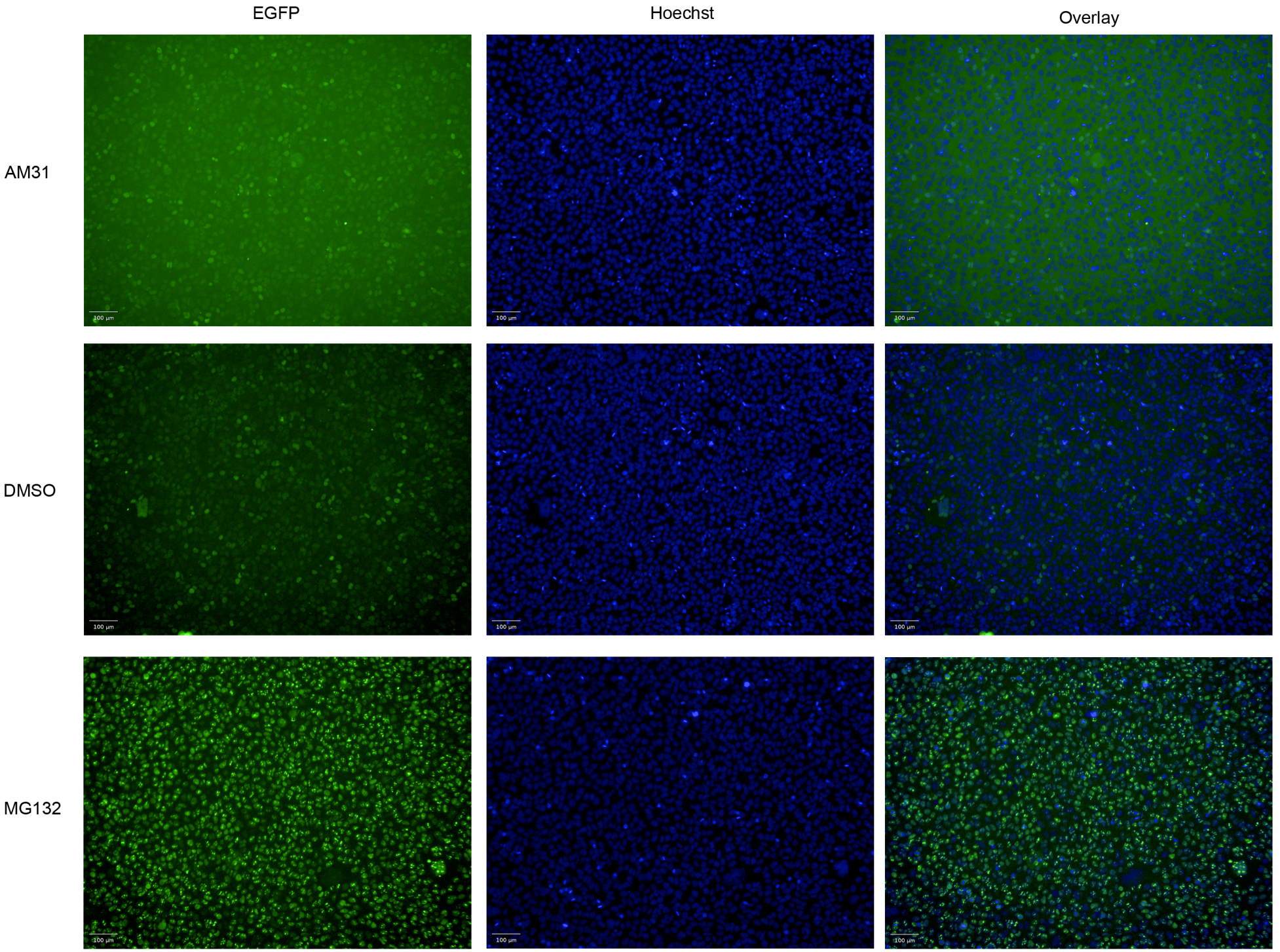
B.


m i 118 119 120 121 122 123 124 d
150
100
[kDa]
α-PGC-1α1
Ponceau S

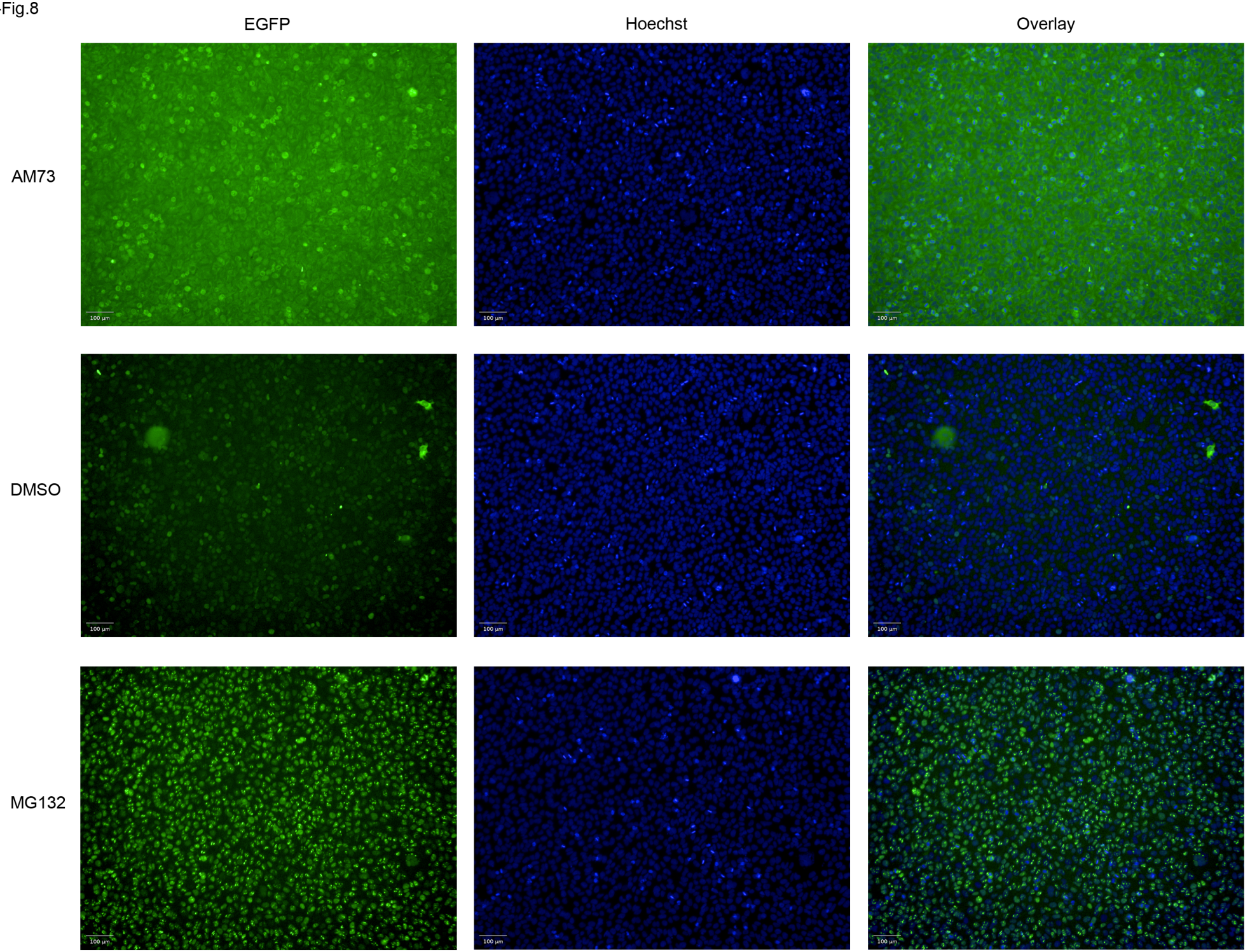
C.


i8 97 102 104 105 108 125 d
150
100
[kDa]
α-PGC-1α1
Ponceau S

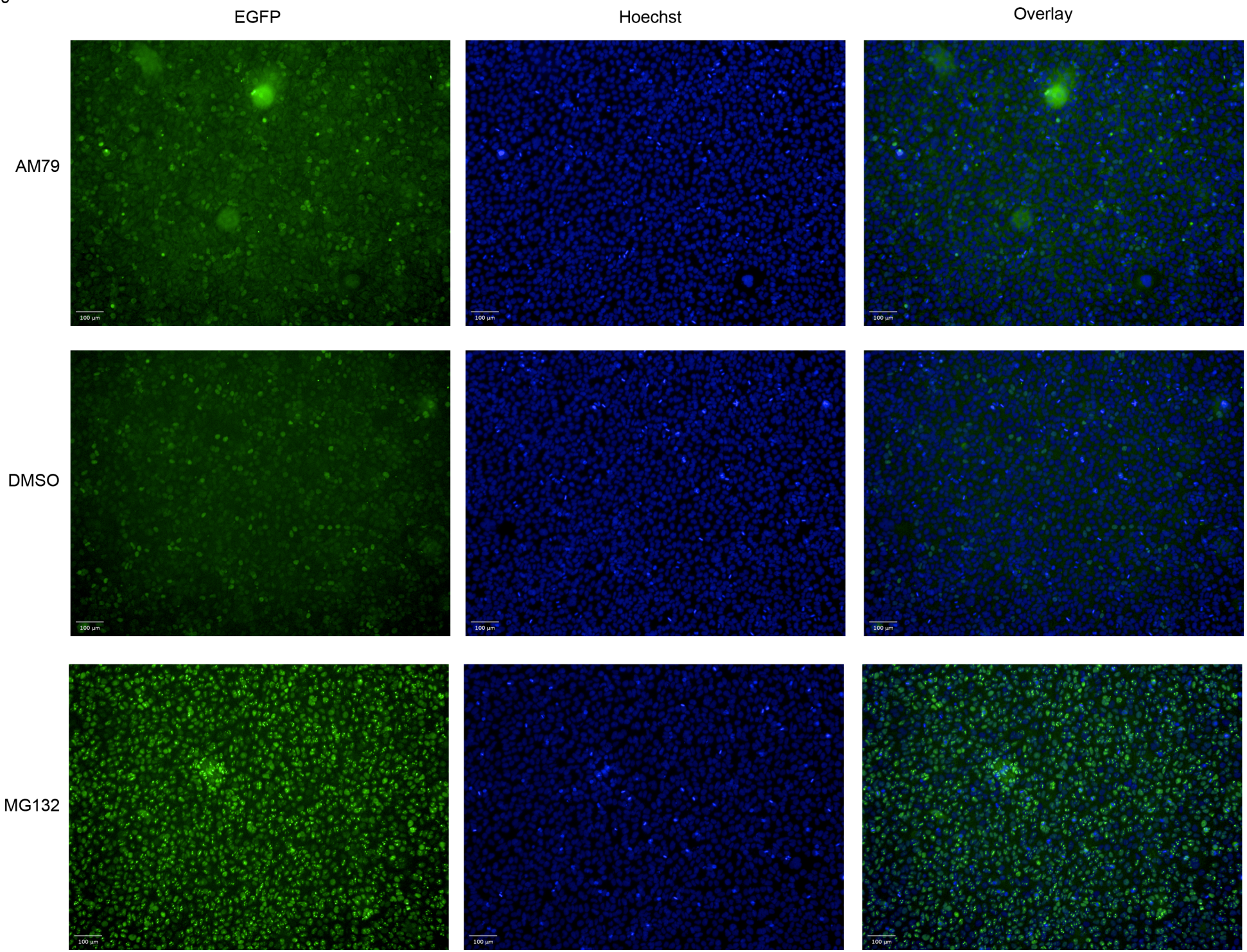
S-Fig.7



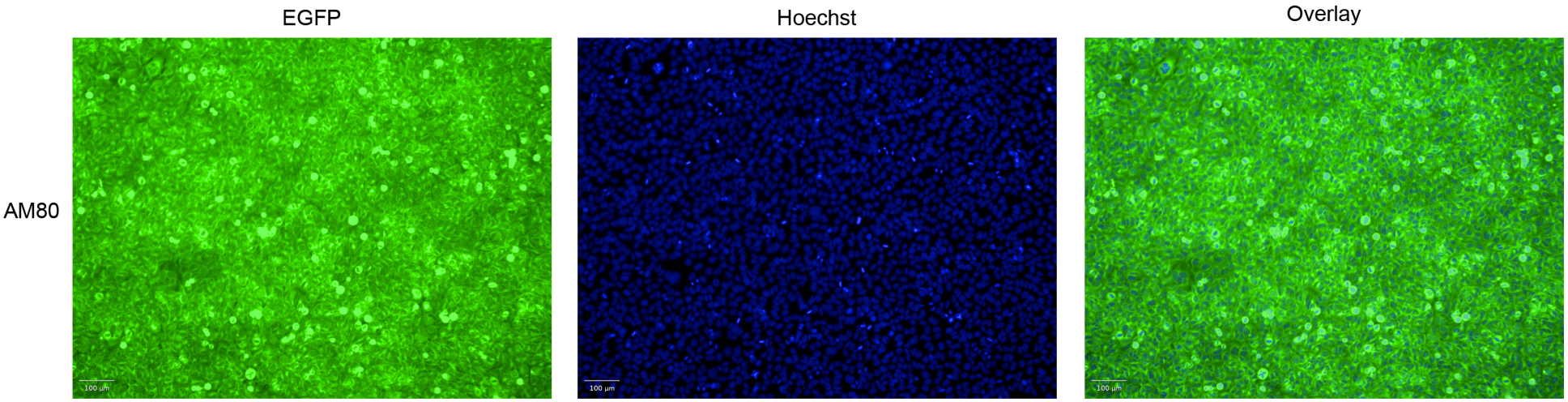
S-Fig.8



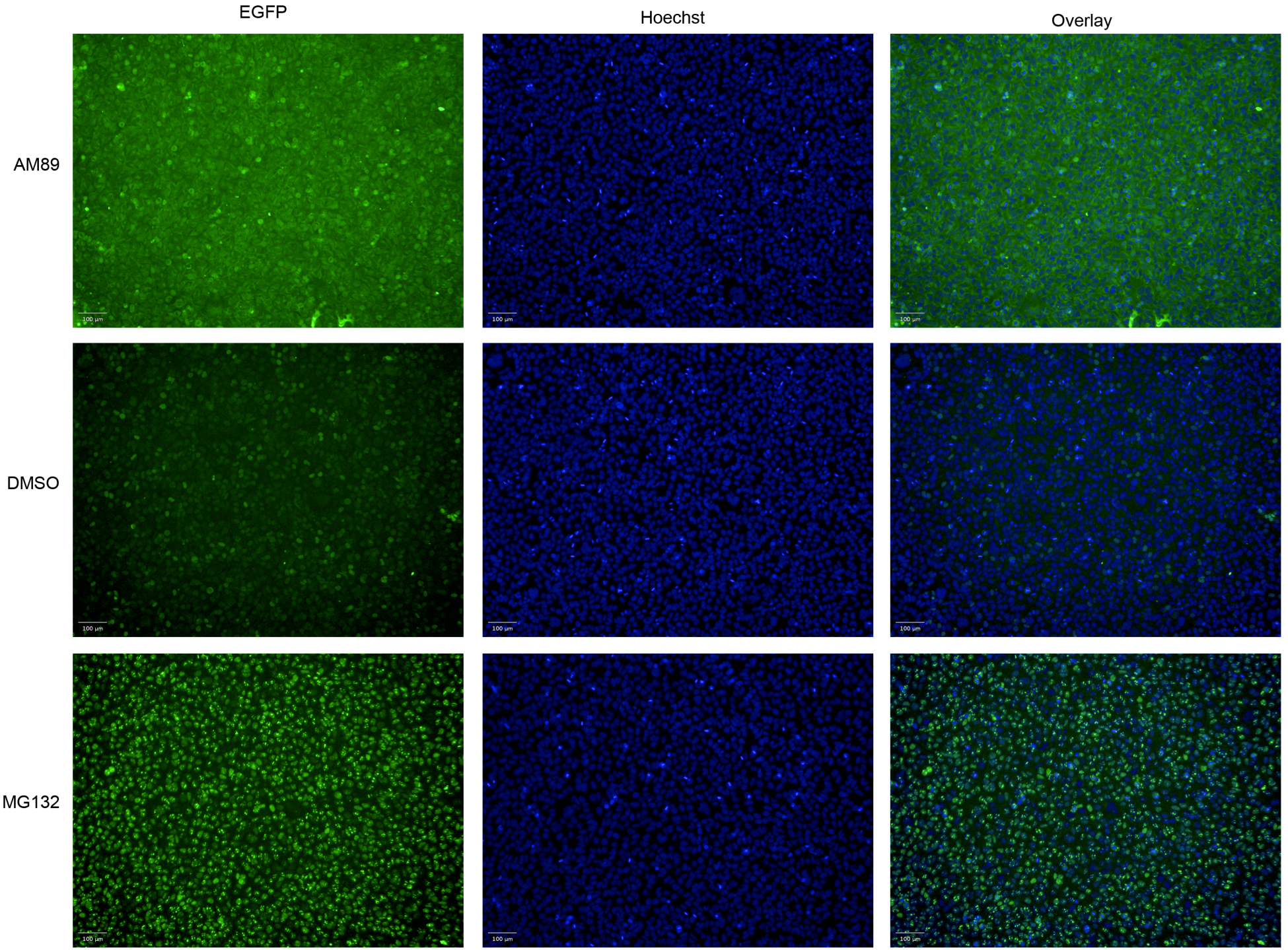
S-Fig.9



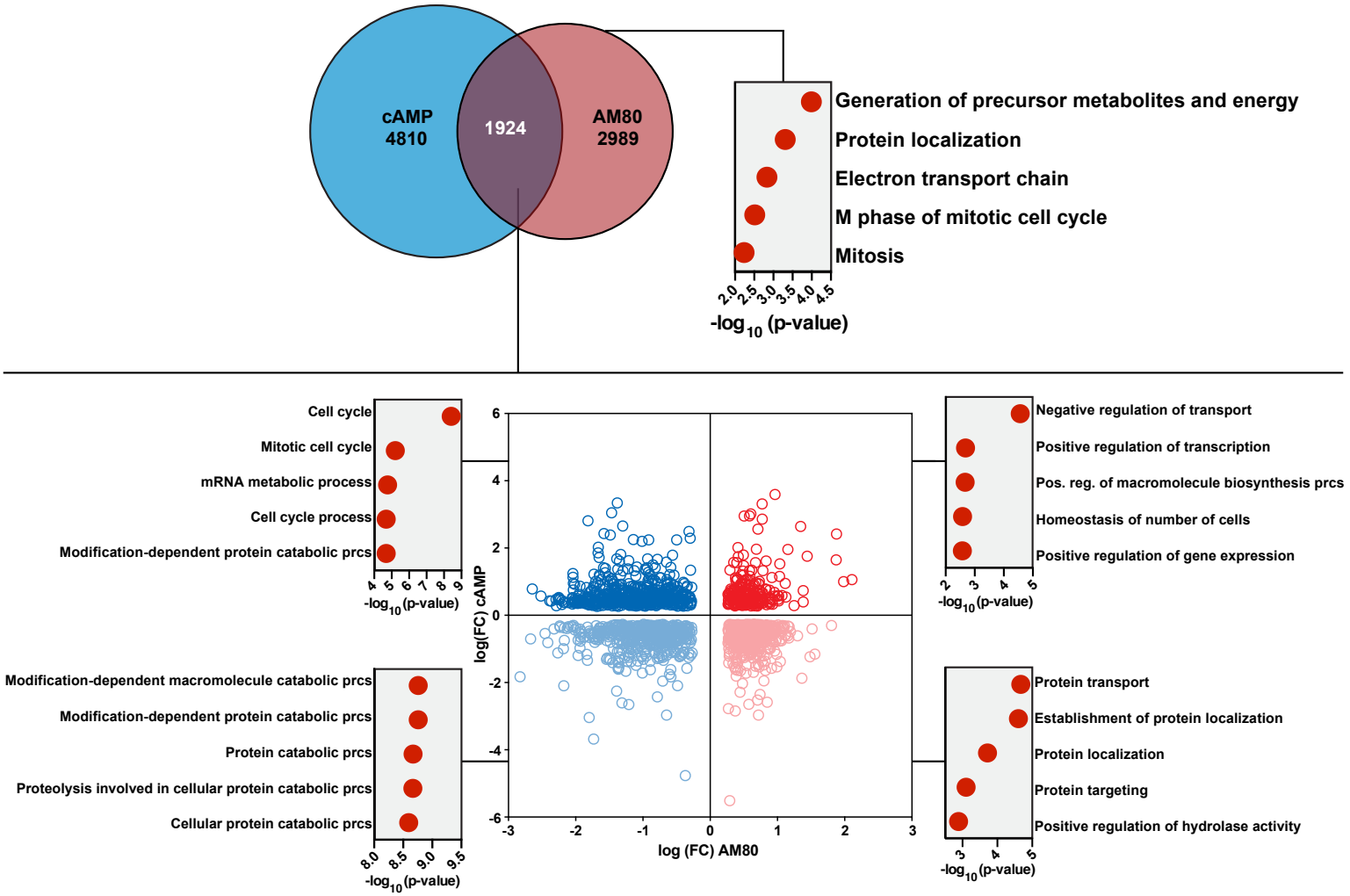
S-Fig.10



S-Fig.11



S-Fig.12


cAMP
4810
1924
AM80
2989
Generation of precursor metabolites and energy
Protein localization
Electron transport chain
M phase of mitotic cell cycle
Mitosis
2.0 2.5 3.0 3.5 4.0 4.5
-log10 (p-value)
Cell cycle
Mitotic cell cycle
mRNA metabolic process
Cell cycle process
Modification-dependent protein catabolic prcs
4 5 6 7 8 9
-log10(p-value)
Modification-dependent macromolecule catabolic prcs
Modification-dependent protein catabolic prcs
Protein catabolic prcs
Proteolysis involved in cellular protein catabolic prcs
Cellular protein catabolic prcs
8.0 8.5 9.0 9.5
-log10(p-value)
log(FC) cAMP
6 4 2 0 -2 -4 -6
-3 -2 -1 0 1 2 3
log (FC) AM80
Negative regulation of transport
Positive regulation of transcription
Pos. reg. of macromolecule biosynthesis prcs
Homeostasis of number of cells
Positive regulation of gene expression
2 3 4 5
-log10(p-value)
Protein transport
Establishment of protein localization
Protein localization
Protein targeting
Positive regulation of hydrolase activity
3 4 5
-log10(p-value)

Supplementary Data – Captions

Supplementary figure 1. Mutagenesis of the first ATG codon in PGC-1α1 to generate a fusion protein with an EGFP tag.

Supplementary figure 2. **Uncropped pictures of western blots and protein loading control.** Protein lysates from fully differentiated brown adipocytes treated for 8h with indicated compounds (10 uM), positive controls, (i=isoproterenol, 4 or 8h) and MG132 or a negative control (D=DMSO) (m= marker for western blot). PVDF membranes were stained with Ponceau S to control for protein loading and transfer efficiency (A-B.).

Supplementary figure 3. Validation of PGC-1α1 protein stabilization by western blot. Fully differentiated brown adipocytes were treated for 8h with 10μM compounds, 10μM isoproterenol (i) or a negative control d=DMSO and harvested for protein (m=indicates a marker for western blot). Right panel of (**A**-**D**) shows Ponceau S stained PVDF membranes.

Supplementary figure 4. Validation of PGC-1α1 protein stabilization by western blot. Fully differentiated brown adipocytes were treated for 8h with 10μM compounds, 10μM isoproterenol (i) or a negative control d=DMSO and harvested for protein (m=indicates a marker for western blot). Right panel of (**A**-**D**) shows Ponceau S stained PVDF membranes.

Supplementary figure 5. Validation of PGC-1α1 protein stabilization by western blot. Fully differentiated brown adipocytes were treated for 8h with 10μM compounds, 10μM isoproterenol (i) or a negative control d=DMSO and harvested for protein (m=indicates a marker for western blot). Right panel of (**A**-**D**) shows Ponceau S stained PVDF membranes.

Supplementary figure 6. Validation of PGC-1α1 protein stabilization by western blot. Fully differentiated brown adipocytes were treated for 8h with 10μM compounds, 10μM isoproterenol (i) for 1h (i1) or 8h (i8) or a negative control

d=DMSO and harvested for protein (m=indicates a marker for western blot). Right panel of (**A-C**) shows Ponceau S stained PVDF membranes.

Supplementary figure 7. **Screening cell line 293-EGFPmPGC-1α1 treated with compound AM31 or controls (DMSO and MG132).** Images were captured by a 10X objective in the Operetta® High Content Imaging System as described in Online Methods.

Supplementary figure 8. **Screening cell line 293-EGFPmPGC-1α1 treated with compound AM73 or controls (DMSO and MG132).** Images were captured by a 10X objective in the Operetta® High Content Imaging System as described in Online Methods.

Supplementary figure 9. **Screening cell line 293-EGFPmPGC-1α1 treated with compound AM79 or controls (DMSO and MG132).** Images were captured by a 10X objective in the Operetta® High Content Imaging System as described in Online Methods.

Supplementary figure 10. Screening cell line 293-EGFPmPGC-1α1 treated with compound AM80 or controls (DMSO and MG132). Control pictures of DMSO and MG132-treated cells are the same as for AM79 and are shown in Supplementary figure 5. Images were captured by a 10X objective in the Operetta® High Content Imaging System as described in Online Methods.

Supplementary figure 11. Screening cell line 293-EGFPmPGC-1α1 treated with compound AM89 or controls (DMSO and MG132). Images were captured by a 10X objective in the Operetta® High Content Imaging System as described in Online Methods.

Supplementary figure 12. Comparison of global gene expression changes following cAMP or AM80 treatment of murine brown adipocytes.
RNA from brown adipocytes treated with 10 μM AM80 for 8h was used for global gene expression analysis by microarray. All data was normalized for a negative control (DMSO). Gene lists from AM80 were compared to previously published results from the same cell line treated with cAMP for 4h. Overlap of hits between cAMP 4h versus AM80. Top right panel of shows Gene Ontology (GO) pathways significantly changed by AM80 alone and top left panel shows overlapping genes between AM80 and cAMP treatment. The lower panel shows pathways changed in the same direction; both up (upper right) or both down (lower left); or in opposite direction; up with cAMP 4h and down with AM80 (upper left) or down with cAMP 4h and up with AM80 (lower right).

Supplementary Table 1. Primer sequences. Primer sequences listed are for murine transcript except when marked with h=human. Tot Ppargc1a-primers targets all described isoforms of Ppargc1a.

Supplementary Table 2. Regulatory elements predicted (by DiRE) in the genes regulated by AM73 in brown adipocytes. RNA from brown adipocytes treated with 10 μM AM73 for 8h was used for global gene expression analysis by microarray. All data was normalized for a negative control (DMSO). To predict common regulators of gene expression, microarray data was analyzed by DiRE (distant regulatory elements of co-regulated genes).

Supplementary Table 3. Regulatory elements predicted (by DiRE) in the genes regulated by AM80 in brown adipocytes. RNA from brown adipocytes treated with 10 μM AM80 for 8h was used for global gene expression analysis by microarray. All data was normalized for a negative control (DMSO). To predict common regulators of gene expression, microarray data was analyzed by DiRE (distant regulatory elements of co-regulated genes).