Supporting Information

# Hsp90 chaperones PPARγ and regulates differentiation and survival of 3T3-L1 adipocytes

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This file includes: Supplementary Table S1, Supplementary Figures S1 through S7 and Legends.

# Supplementary Table

## Table S1

### Statistical comparison of IC50 values of geldanamycin treatment

| Figure | Parameter studied | Mean IC50 | Mean LogIC50 | SEM LogIC50 | n | p *vs.* differentiation | p *vs.* PPARγ2 protein |
|---|---|---|---|---|---|---|---|
| 1 | Differentiation | 16.38 | 1.214 | 0.080 | 3 | -- | 0.0908 (ns) |
| 1 | Viability | 163.60 | 2.214 | 1.111 | 3 | 0.0005 (***) | 0.0026 (**) |
| 1 | Differentiation/viable cells | 20.45 | 1.311 | 0.100 | 3 | 0.0777 (ns) | 0.3249 (ns) |
| 2 | PPARγ1 protein | 50.99 | 1.708 | 0.053 | 3 | 0.0413 (*) | 0.5457 (ns) |
| 2 | PPARγ2 protein | 40.78 | 1.610 | 0.062 | 3 | 0.0908 (ns) | -- |
| 2 | Akt protein | 53.74 | 1.730 | 0.094 | 3 | 0.0339 (*) | 0.3356 (ns) |
| 4 | PPARγ2 mRNA | 39.06 | 1.592 | 0.103 | 3 | 0.1498 (ns) | 0.8533 (ns) |
| 4 | GLUT4 mRNA | 41.93 | 1.622 | 0.113 | 3 | 0.0999 (ns) | 0.9936 (ns) |
| 4 | aP2 mRNA | 85.49 | 1.932 | 0.082 | 3 | 0.0096 (**) | 0.1032 (ns) |
| 4 | adiponectin mRNA | 43.82 | 1.642 | 0.087 | 3 | 0.0688 (ns) | 0.8044 (ns) |

3T3-L1 cells were treated by geldanamycin for 20 hours on day 3 of differentiation. Differentiation and viability were assayed on day 14, all the other parameters were obtained on day 4.

SEM = standard error of the mean

n = number of experiments

p = p value, *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired *t* test

# Supplementary Figures

## Figure S1

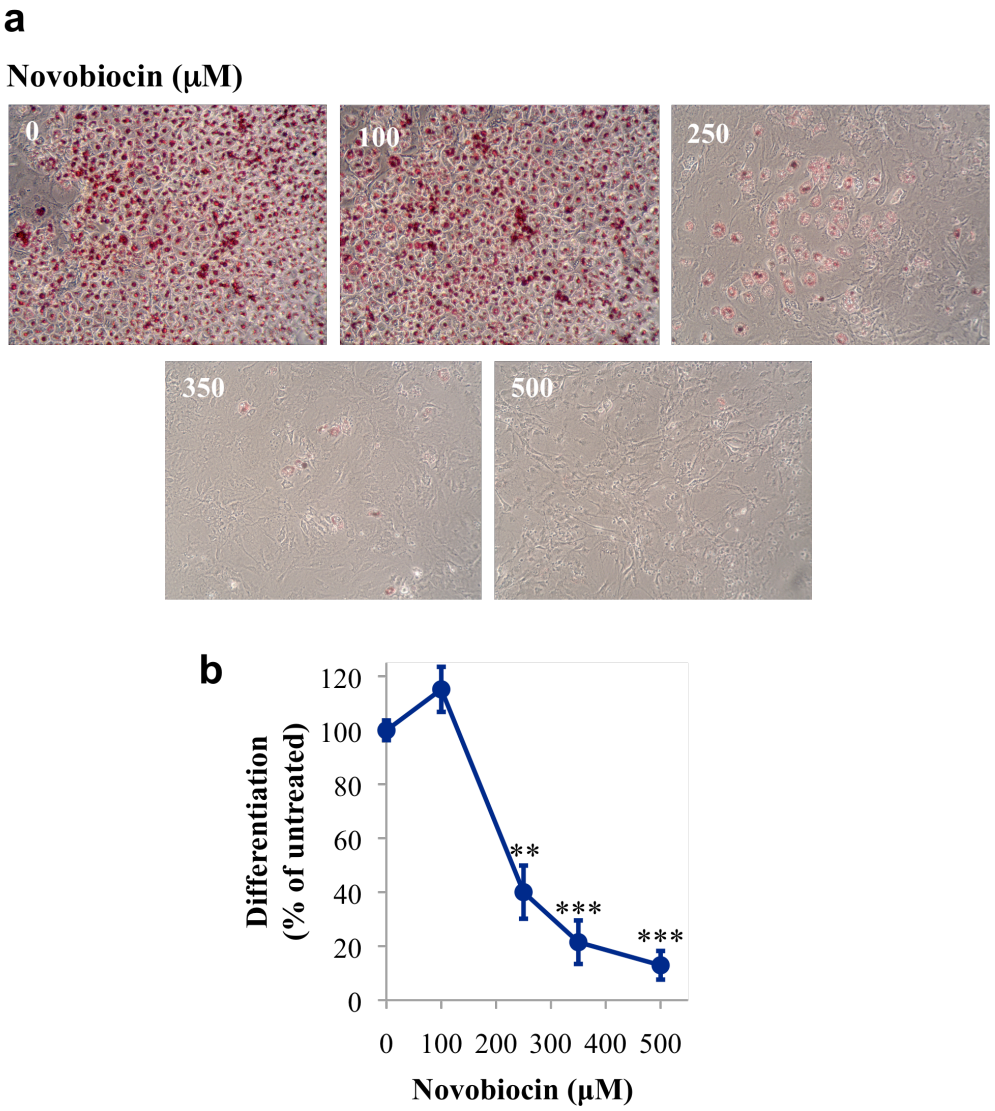



**Figure S1** **Novobiocin inhibits adipocyte differentiation. (a)** Oil Red O staining of 3T3-L1 cells on day 14, treated by various concentrations of novobiocin for 20 hours on day 3. Note the presence of unstained undifferentiated attached preadipocytes in novobiocin-treated samples. Images are representatives of 3 experiments. **(b)** Quantification of Oil Red O absorption. Values are means ± SD-s of 3 experiments and statistically compared to the untreated control. *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired *t* test.

## Figure S2

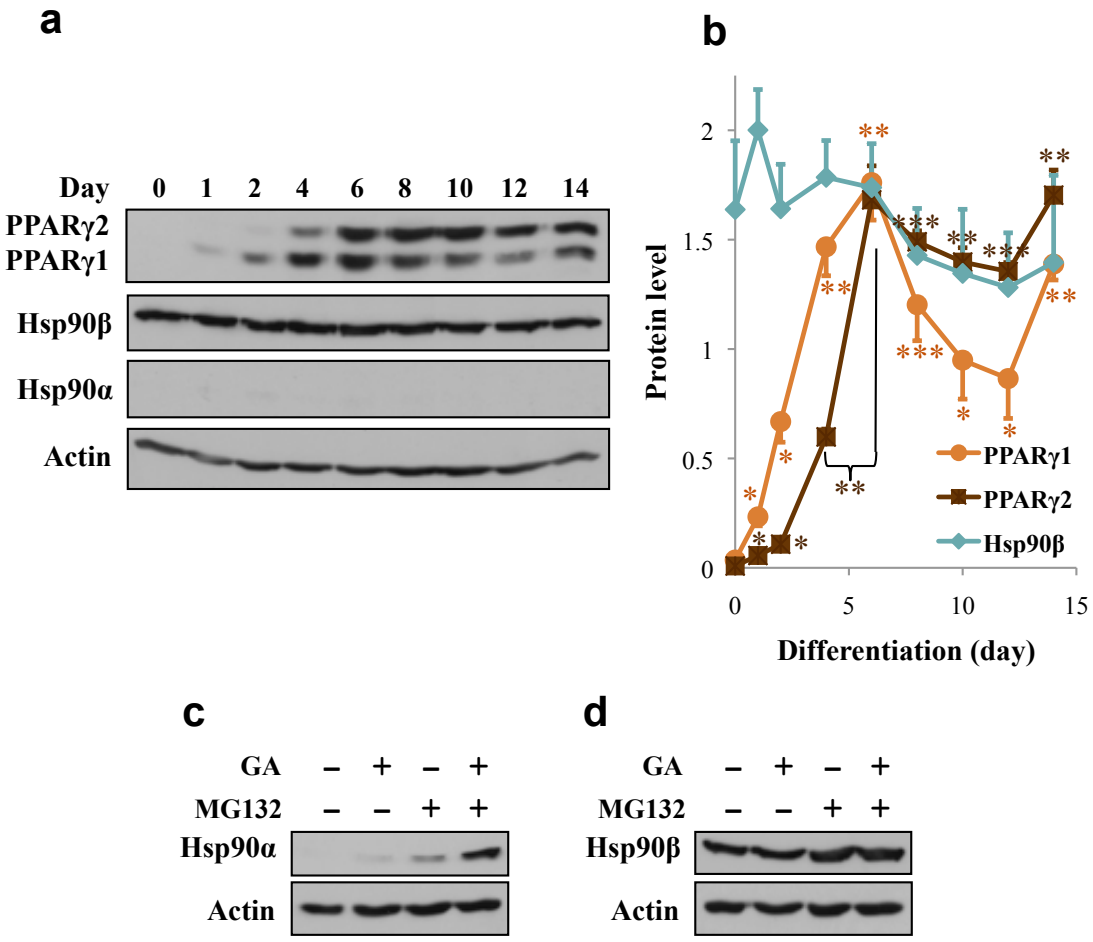



**Figure S2** **Expression of PPARγ, Hsp90α and β in 3T3-L1 cells. (a)** Western blots showing PPARγ, Hsp90α and β protein levels during adipogenesis. Images are representatives of 2 independent experiments. **(b)** Densitometric analysis of protein levels shown on panel (a) normalized to β-actin. Values are means ± SD-s of 2 experiments and statistically compared to the untreated control. *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired t test. **(c)** Anti-Hsp90α and **(d)** anti-Hsp90β Western blots of lysates from cells treated by 446 nM (0.25 μg/ml) GA and/or 20 μM MG132 for 20 hours on day 3. Images are representatives of 2 independent experiments.

## Figure S3

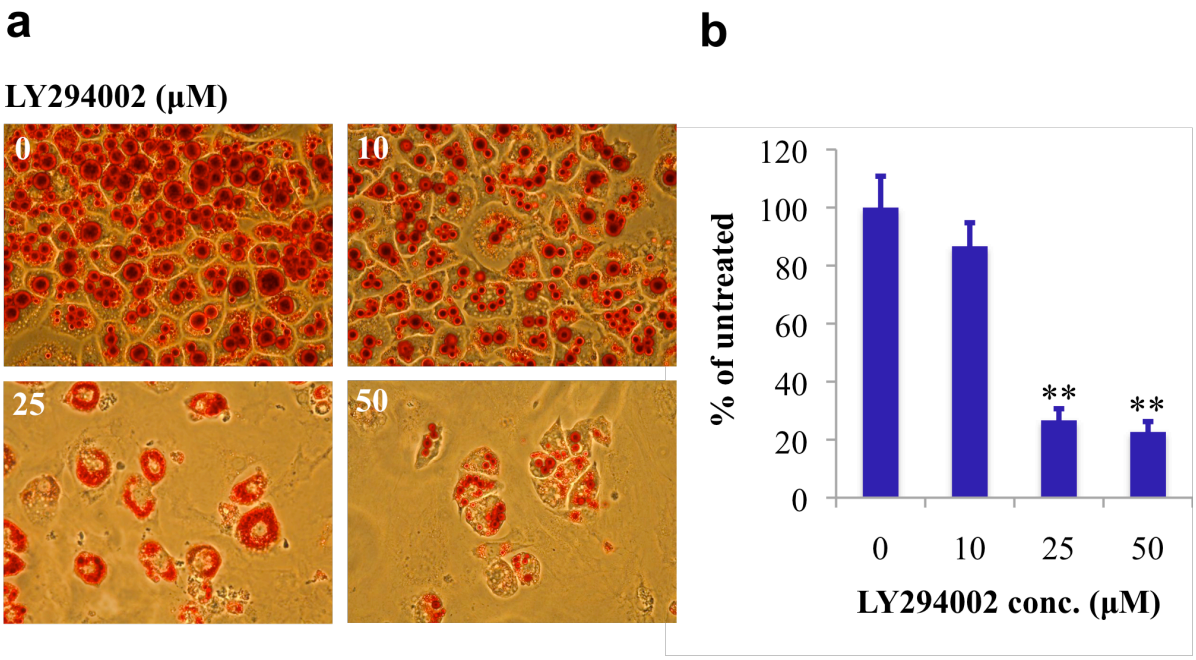



**Figure S3 Phosphatidyl-inositol-3-kinase inhibition compromises adipogenesis in 3T3-L1 cells. (a)** Oil Red O staining of 3T3-L1 cells on day 14 treated by various concentrations of PI3K inhibitor LY294002 for 20 hours on day 3. Images are representatives of 2 independent experiments. **(b)** Quantification of Oil Red O absorption by photometry. Values are means ± SD-s of 2 experiments and statistically compared to the untreated control. *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired *t* test.

## Figure S4

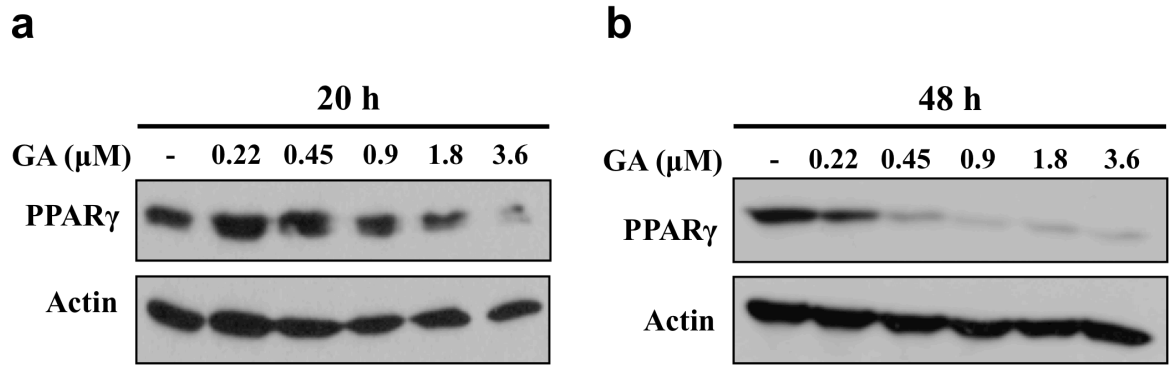



**Figure S4 Hsp90 inhibition depletes PPARγ protein in HepG2 human hepatoma cells.** Western blots of lysates from HepG2 cells treated by GA for 20 **(a)** or 48 **(b)** hours. Images are representatives of 2 independent experiments.

**Figure S5**

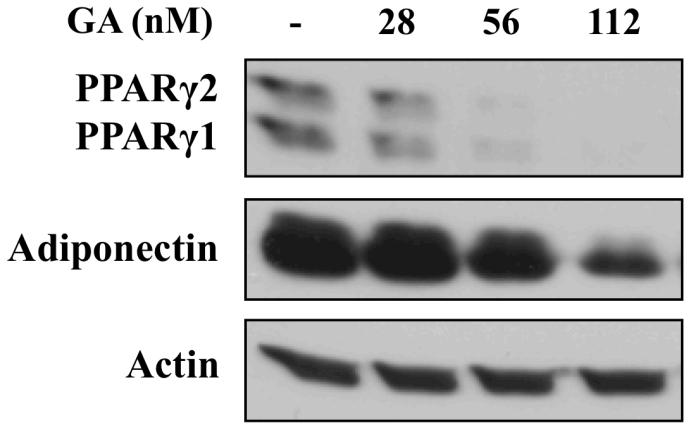



**Figure S5 Geldanamycin treatment down-regulates adiponectin protein in parallel with PPARγ in differentiated 3T3-L1 cells.** Western blots of lysates from cells treated by various concentrations of geldanamycin (GA) for 20 hours on day 13. Images are representatives of 2 independent experiments.

**Figure S6**

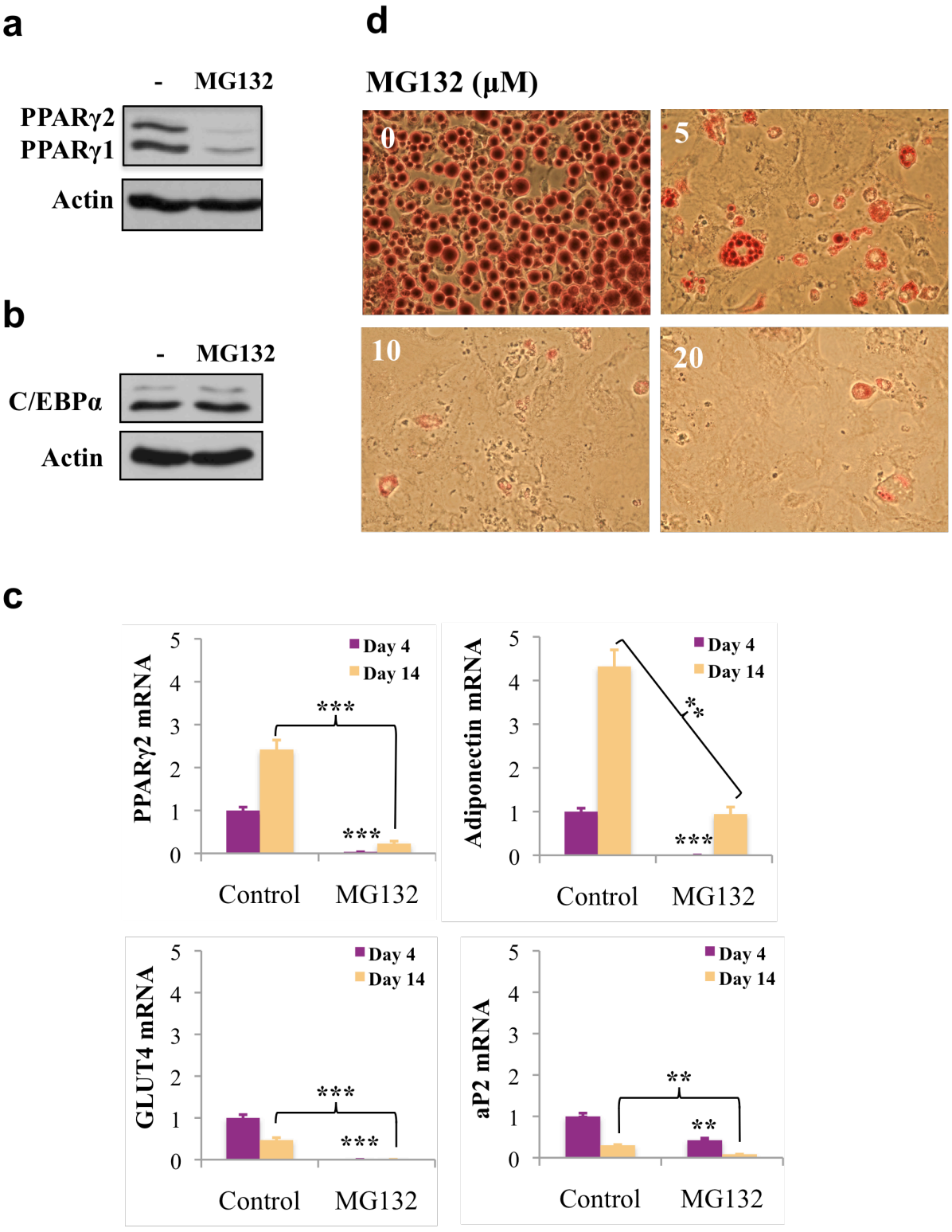
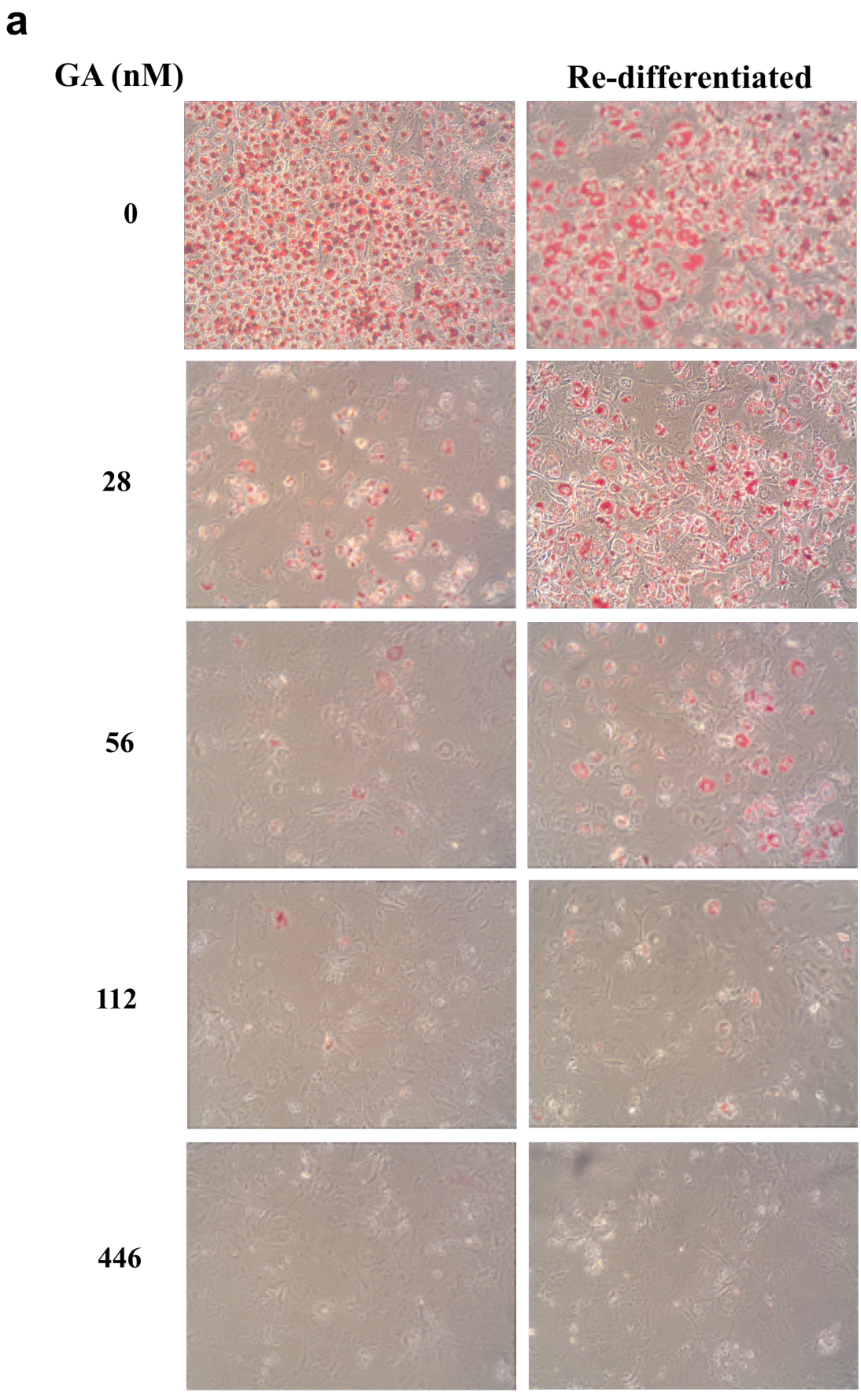
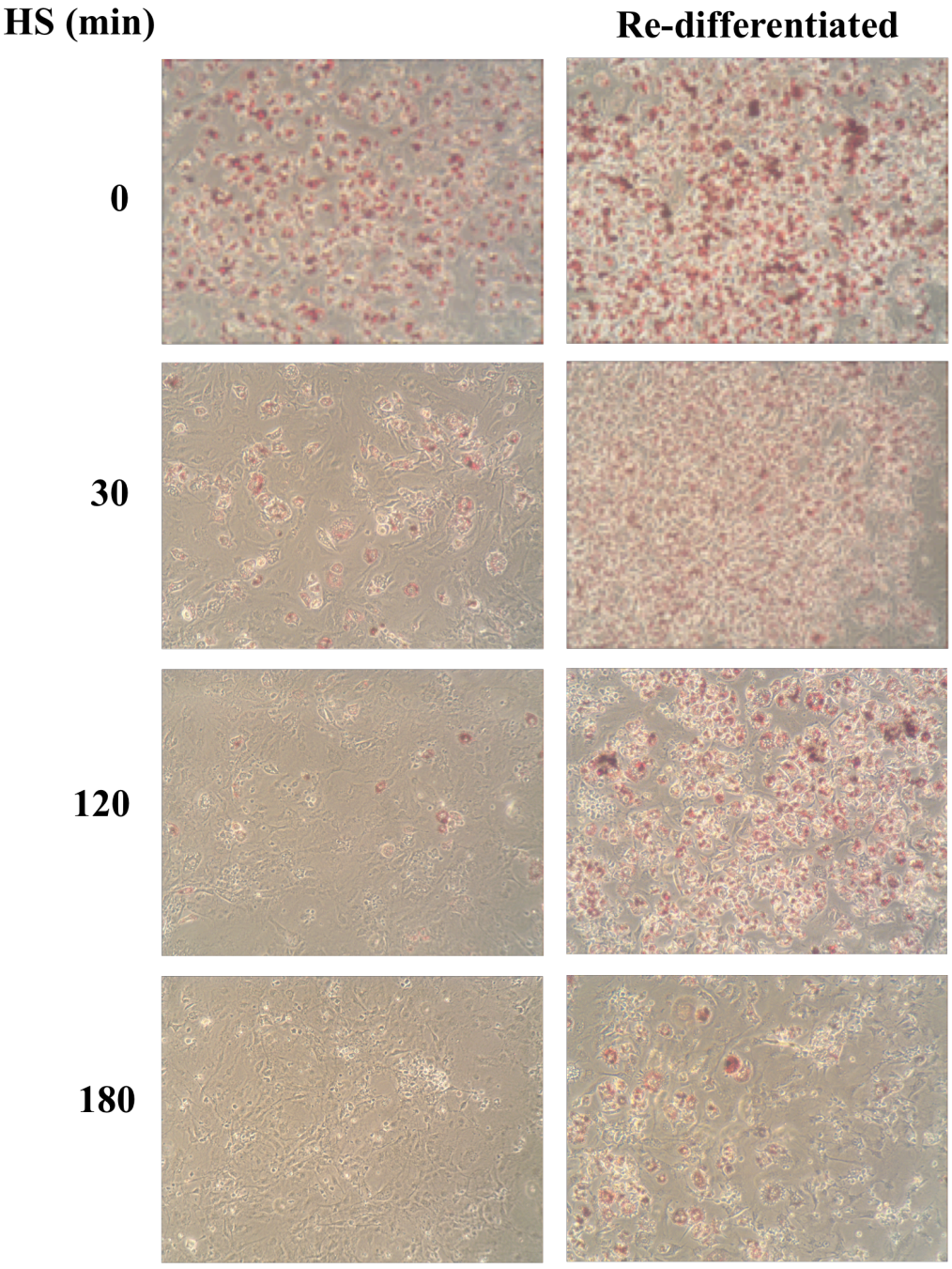

a
- MG132
PPARγ2
PPARγ1
Actin
b
- MG132
C/EBPα
Actin
d
MG132 (μM)
0
5
10
20
c
PPARγ2 mRNA
Adiponectin mRNA
GLUT4 mRNA
aP2 mRNA
Day 4
Day 14
Control
MG132

**Figure S6 Proteasome inhibition impairs PPARγ folding, transcriptional output and adipocyte differentiation.** Effect of proteasome inhibition on PPARγ **(a)** and on C/EBPα p42 **(b)** protein levels. Western blots of lysates from cells subjected to 5 μM MG132 treatment for 20 hours on day 3. **(c)** Effect of 10 μM MG132 treatment on day 3 on PPARγ2, adiponectin, GLUT4 and aP2 mRNA levels. mRNA expression data assayed by qRT-PCR from cells on day 4 and 14, normalized to 28S rRNA and expressed relative to the respective (day 4 and day 14) untreated controls. Data are means ± SD-s of 2 experiments and statistically compared to the respective untreated controls. *$p<0.05$, **$p<0.01$, ***$p<0.001$ by two-tailed unpaired *t* test. **(d)** Proteasome inhibition compromises adipocyte differentiation. Oil Red O staining of differentiated cells on day 14 treated by MG132 at the indicated concentrations for 20 hours on day 3. Images are representatives of 3 independent experiments.

**Figure S7**



**b**



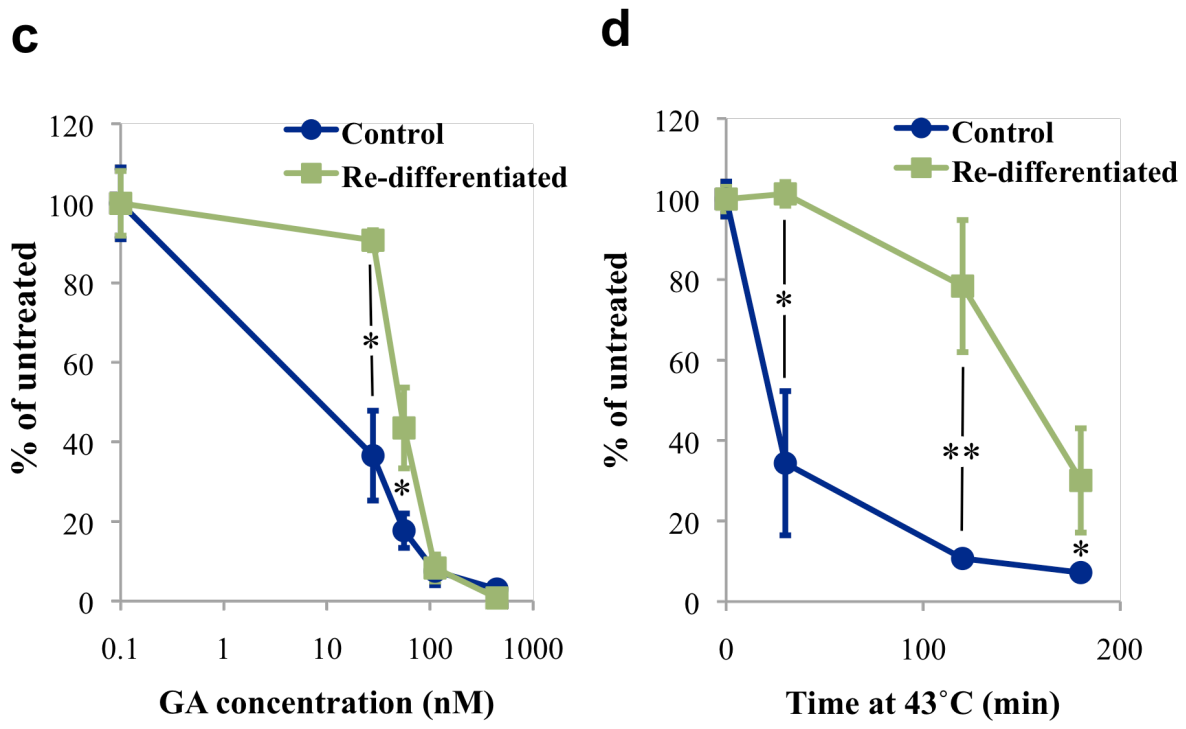



**Figure S7 Effect of geldanamycin and heat shock treatments on the re-differentiation of 3T3-L1 cells. (a)** Oil Red O staining of 3T3-L1 cells on day 14, treated by various concentrations of geldanamycin (GA) for 24 hours on day 3, with or without re-differentiation. Images are representatives of 2 experiments. **(b)** Oil Red O staining of 3T3-L1 cells on day 14, treated by various durations of heat shock (HS) at 43ºC on day 3, with or without re-differentiation. Images are representatives of 2 experiments. **(c** and **d)** Quantification of Oil Red O absorption of viable cells subjected to GA (**c**) or HS (**d**) treatments with or without re-differentiation, from the experiments shown in panel (a) and (b). Values are means ± SD-s of 2 experiments, normalized to viability and expressed relative to the respective untreated (control and re-differentiated) samples. Statistical pairwise comparison is made between similar treatments with and without re-differentiation. *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired *t* test.