Supporting Information

Prostaglandin A_2 Enhances Cellular Insulin Sensitivity via a Mechanism that Involves the Orphan Nuclear Receptor NR4A3

Authors

Xiaolin Zhu[1], R GracevWalton[2], Ling Tian[1], Nanlan Luo[2], Shiuh-Rong Ho[1], Yuchang Fu[2], W. Timothy Garvey[2,3]

Affiliations

[1]Department of Cell Biology, University of Alabama at Birmingham, Birmingham, USA

[2]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, USA

[3]the Birmingham Veterans Affairs Medical Center, Birmingham, USA

Correspondence

Xiaolin Zhu

Department of Nutrition Sciences

The University of Alabama at Birmingham

1675 University Boulevard,

Birmingham, AL 35294-3360

Tel.: +1/205/996 2660

Fax: +1/205/934 7049

E-mail: xiaolin@uab.edu

© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S1A

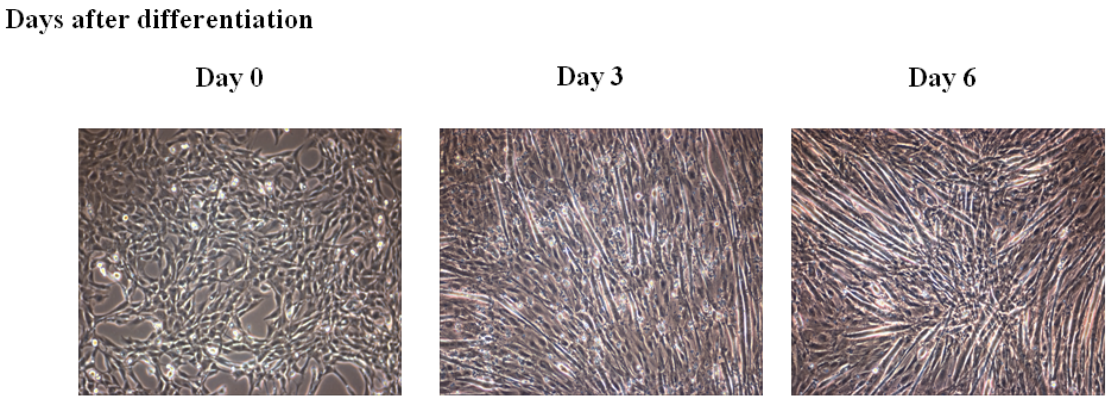
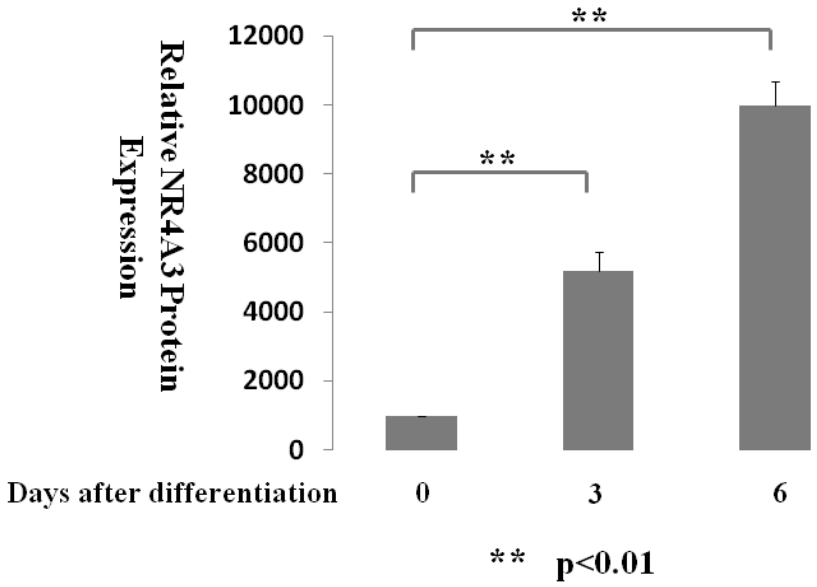


Figure S1B



© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S1C

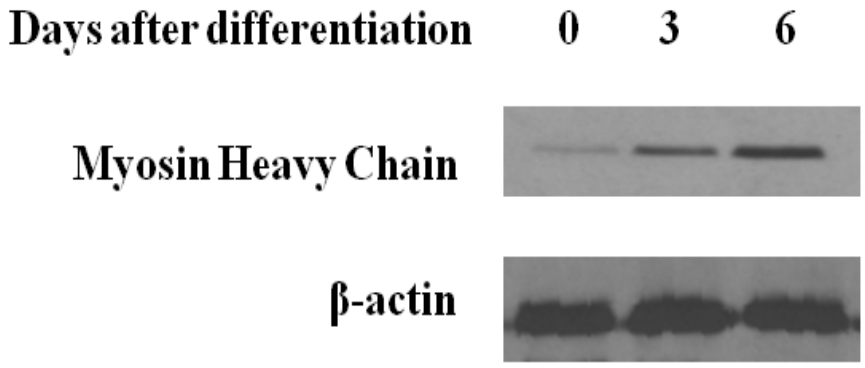
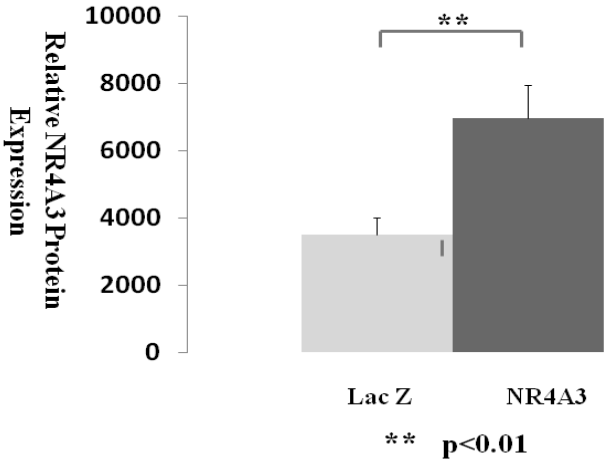


Figure S1: The proper differentiation of wild type C2C12 cells. A) Images of C2C12 myoblasts at basal level, 3 days after differentiation and 6 days of differentiation. B) Quantification of NR4A3 protein expression at day 0, 3 days after differentiation and 6 days after differentiation. C) The myosin heavy chain protein expression in C2C12 cells before and after differentiation. All data are representatives over three time of independent experiments.

Figure S2A



© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S2B

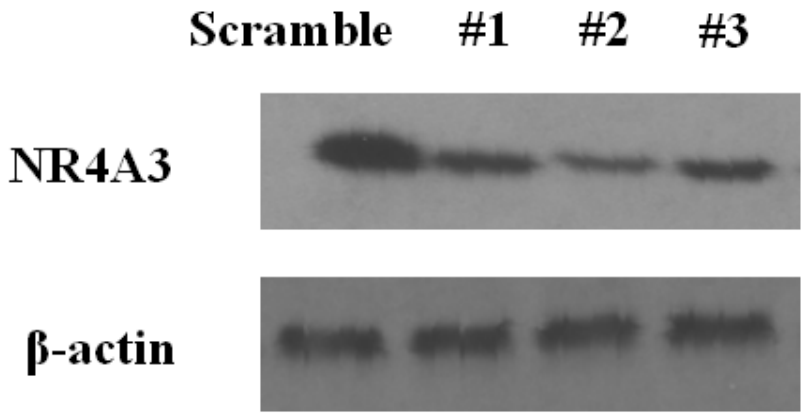
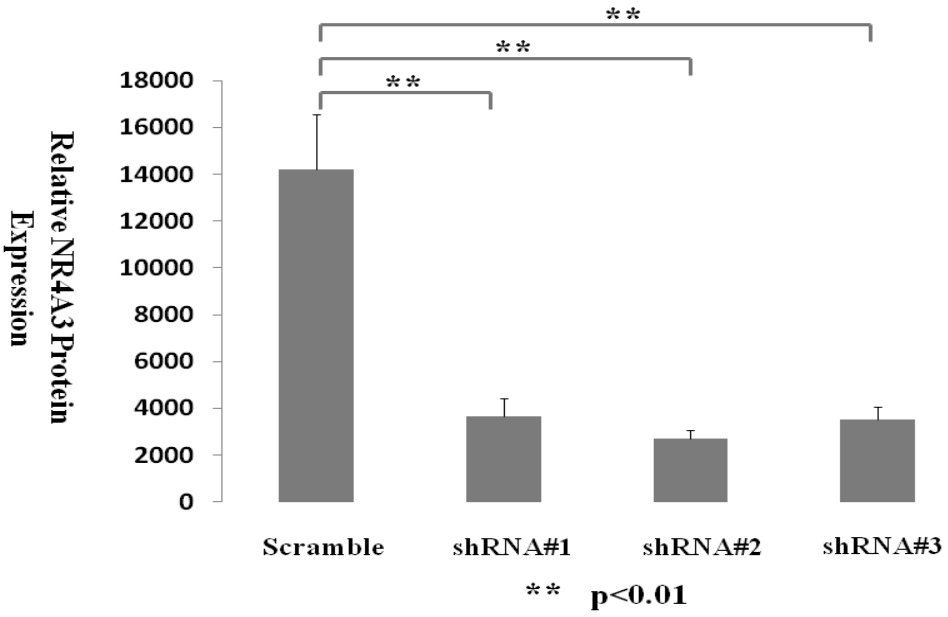


Figure S2C



© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S2D

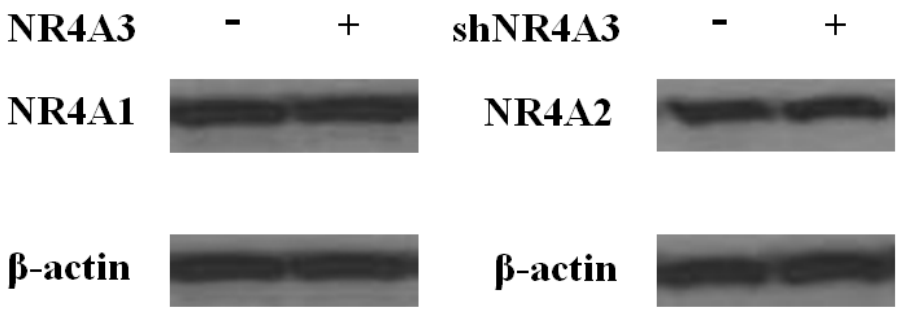


Figure S2E

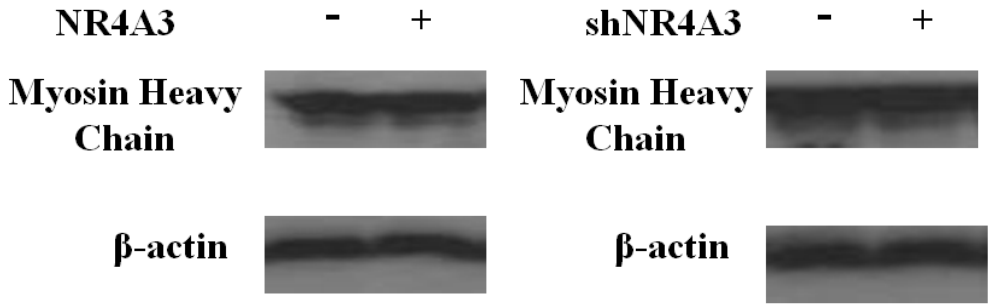


Figure S2F

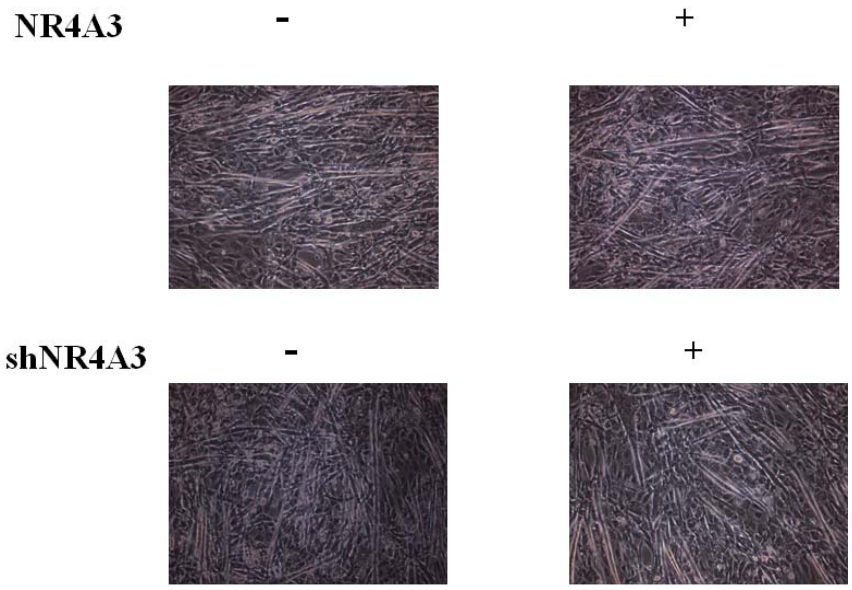


Figure S2: Characterization of NR4A3 hyperexpression and knock-down cell line. A) Quantification of NR4A3 protein expression in NR4A3 hyperexpressing C2C12 myocytes. B) NR4A3 knockdown by three different shRNAs. C) Quantification of NR4A3 knockdown effects by 3 shRNAs. D) NR4A1 and NR4A2 protein expression were not changed after NR4A3 over-expression or knock-down. E) Myosin Heavy Chain Protein expression were not changed after NR4A3 over-expression or knock-down. F) The gross morphology of differentiated C2C12 cells were not changed after NR4A3 over-expression or knock-down. All data are representatives over three time of independent experiments.

© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S3A

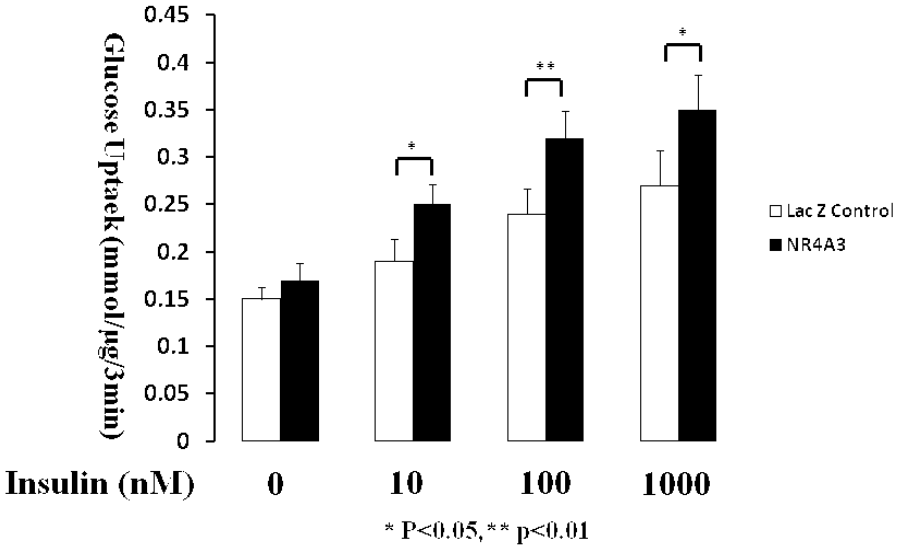
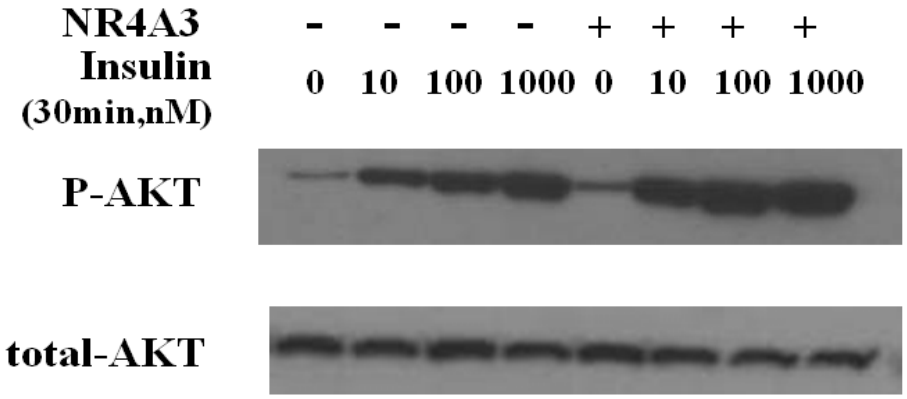


Figure S3B



© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S3C

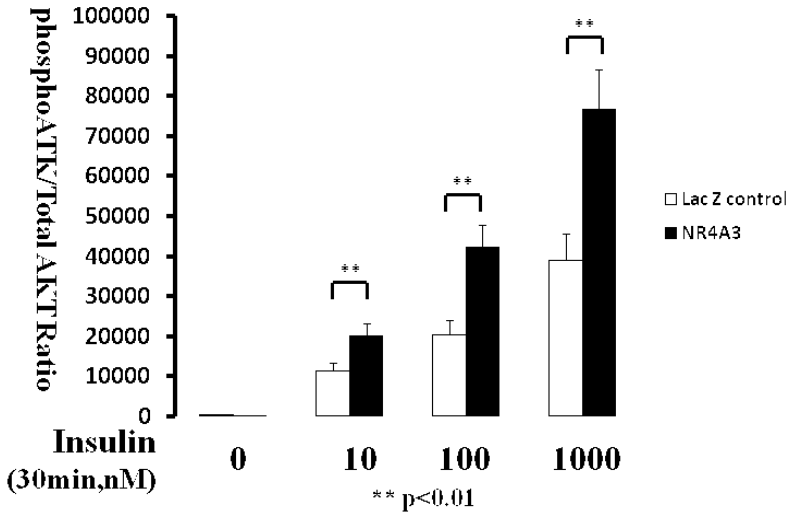
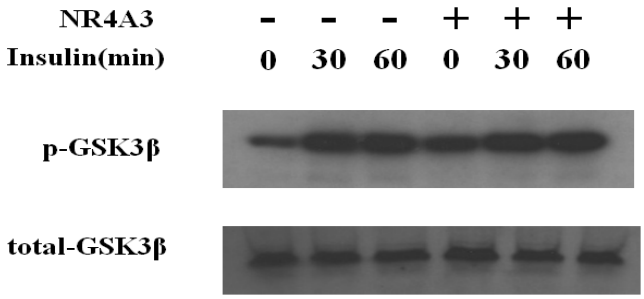


Figure S3D



© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S3E

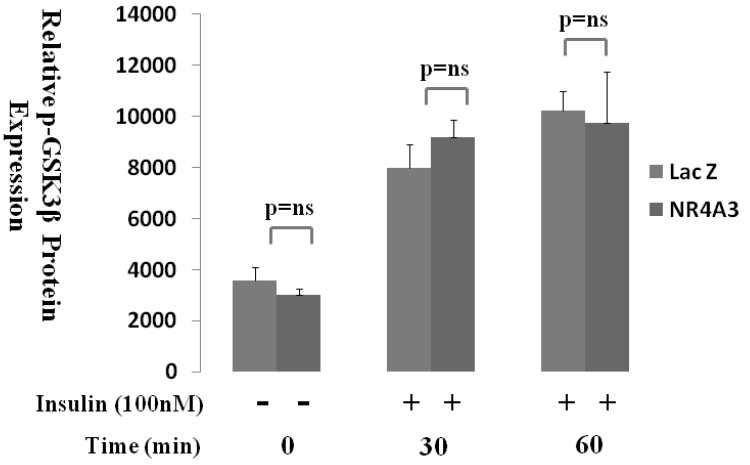


Figure S3: Function phenotypes of NR4A3 hyperexpression cell line. A) Quantification of Glucose Uptake in NR4A3 over-expressed C2C12 cell after different concentrations of insulin stimulation. B) NR4A3 hyperexpression results in enhanced insulin stimulated Akt phosphorylation in C2C12 cells at different insulin concentrations. C) Quantification of Akt Phosphorylation in C2C12 cells after different concentrations of insulin treatment. D) The insulin stimulated serine-9 GSK-3β phosphorylation in NR4A3 hyperexpressing C2C12 myocytes. E) Quantifiation of serine-9 GSK-3β/total GSK-3β ratio in NR4A3 hyperexpressing C2C12 myocytes. All data are representatives over three time of independent experiments.

© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S4A

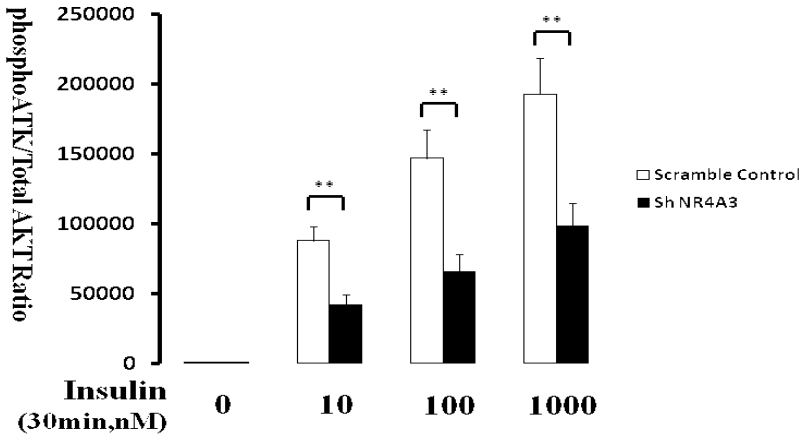


Figure S4B

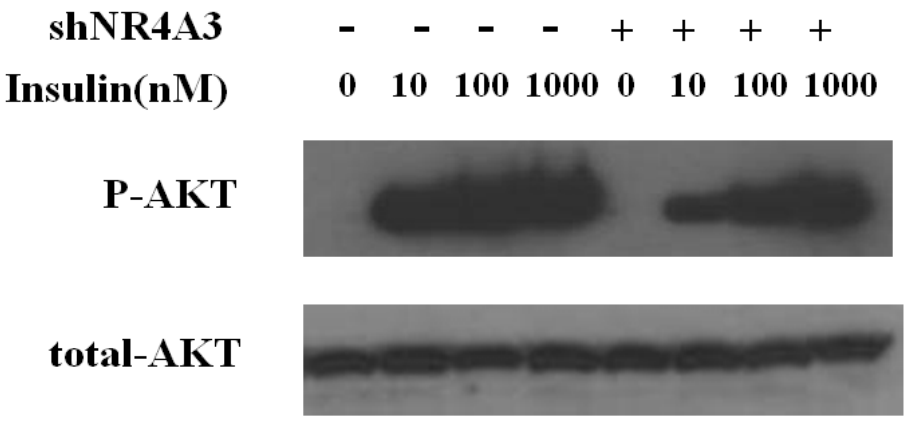


Figure S4C

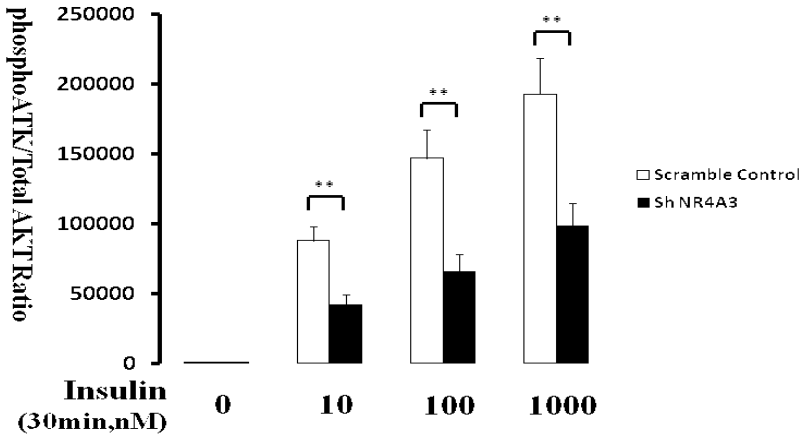


Figure S4D

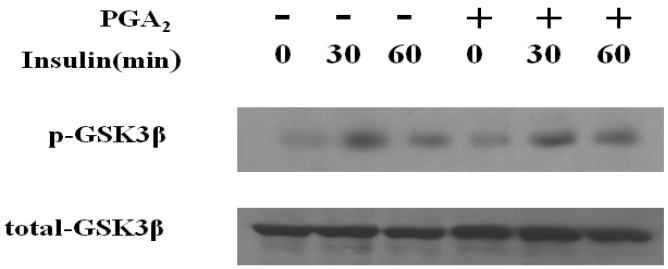


Figure S4E

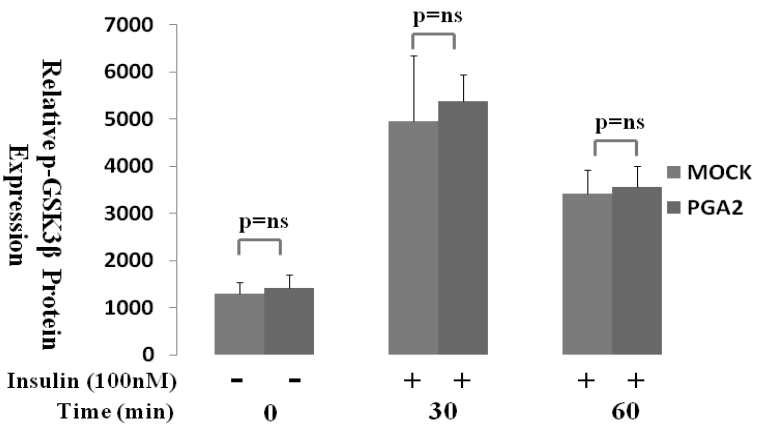


Figure S4: Function phenotypes of NR4A3 knock-down cell lines and the insulin stimulated serine-9 GSK-3β phosphorylation in PGA_2 treated C2C12 myocytes. A) Quantification of Glucose Uptake in NR4A3 knock-down C2C12 cell after different concentrations of insulin stimulation. B) NR4A3 knockdown results in decreased insulin stimulated Akt phosphorylation in C2C12 cells at different insulin concentrations. C) Quantification of Akt Phosphorylation in C2C12 cells after different concentrations of insulin treatment.. D) The insulin stimulated serine-9 GSK-3β phosphorylation in PGA_2 treated C2C12 myocytes. E) Quantifiation of serine-9 GSK-3β/total GSK-3β ratio in PGA_2 treated C2C12 myocytes. All data are representatives over three time of independent experiments.

© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.

Figure S5A

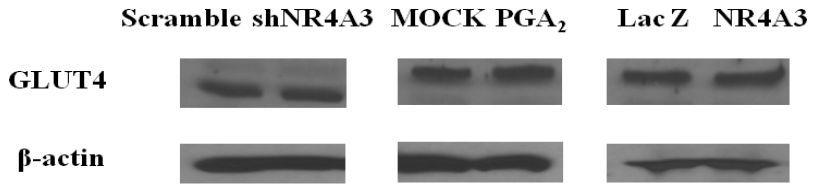


Figure S5B

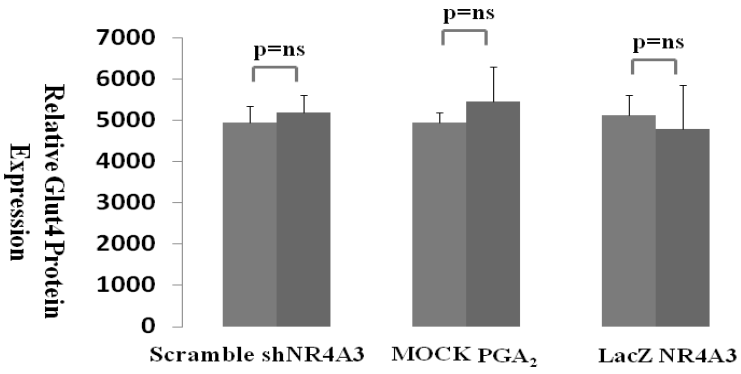


Figure S5: The Glut4 protein expression. A) The Glut4 protein expression in NR4A3 hyperexpressing, knockdown and PGA_2 treated C2C12 myocytes. B) Quantifiation of Glut4 protein expression in NR4A3 hyperexpressing, knockdown and PGA_2 treated C2C12 myocytes. All data are representatives over three time of independent experiments.

© Georg Thieme Verlag KG · DOI: 10.1055/s-0032-1327619 · Horm Metab Res 2012; 44: e1–e13 · Zhu X et al.