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Supplemental Information

Angiotensin II-induced muscle atrophy via PPARγ suppression is mediated by miR-29b

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Supplemental figures and legends

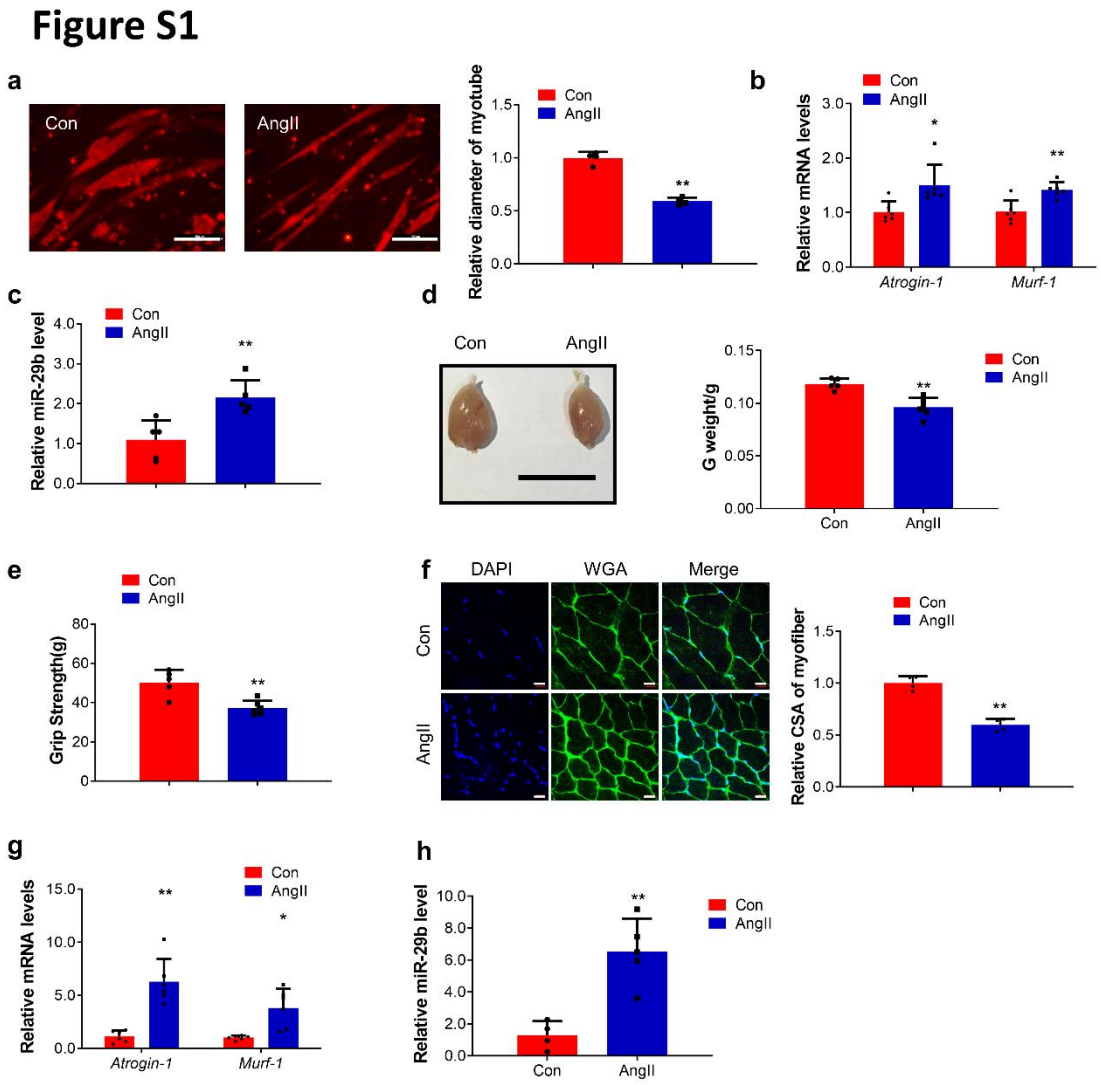

Figure S1. miR-29b is elevated in AngII-induced muscle atrophy. (a) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter in AngII-induced muscle atrophy in *vitro*. Scale bar: 100μm. n=4 per group. (b) qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions in AngII-induced muscle atrophy in *vitro*. n=6 per group. (c) qRT-PCR analysis of miR-29b expression in AngII-induced muscle atrophy in *vitro*. n=5 per group. (d) Gastrocnemius muscle morphology (scale bar: 1 cm) and gastrocnemius weight (GW) of control and AngII treated mice. n=5,6. (e) The grip strength of right hind limb in control and AngII treated mice. n=5,6. (f) Wheat germ agglutinin (WGA) staining was performed to quantify muscle fiber cross sectional area (CSA) from control and AngII treated mice. Scale bar: 20μm. n=4 per group. (g) qRT-PCR analysis of *Atrogin-1* and *Murf-1* expression in gastrocnemius muscles from control and AngII treated mice. n=5, 6. (h) qRT-PCR analysis of miR-29b expression in gastrocnemius muscles from control and AngII treated mice. n=4,5. Con, Control. AngII, Angiotension II. GW, Gastrocnemius weight. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.

Figure S2

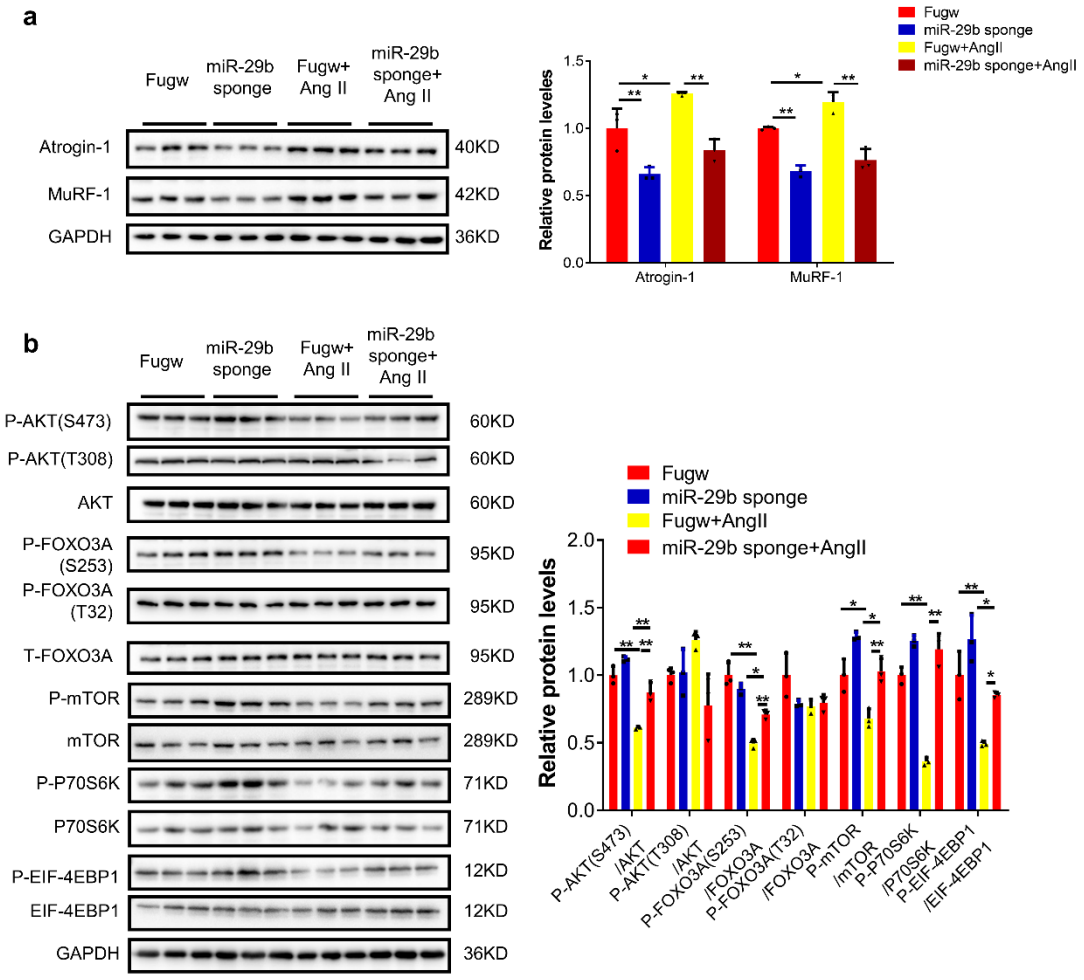

Figure S2. miR-29b inhibition ameliorates AngII-induced muscle atrophy in *vivo*. (a) Western blot analysis of Atrogin-1 and MuRF-1 expressions in control and AngII mice treated with Fugw or miR-29b sponge. n=3 per group. (b) Western blot analysis for the AKT/FOXO3A/mTOR pathway (AKT, FOXO3A, mTOR, P70S6K, 4EBP1) in control and AngII mice treated with Fugw or miR-29b sponge. n=3 per group. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.

Figure S3

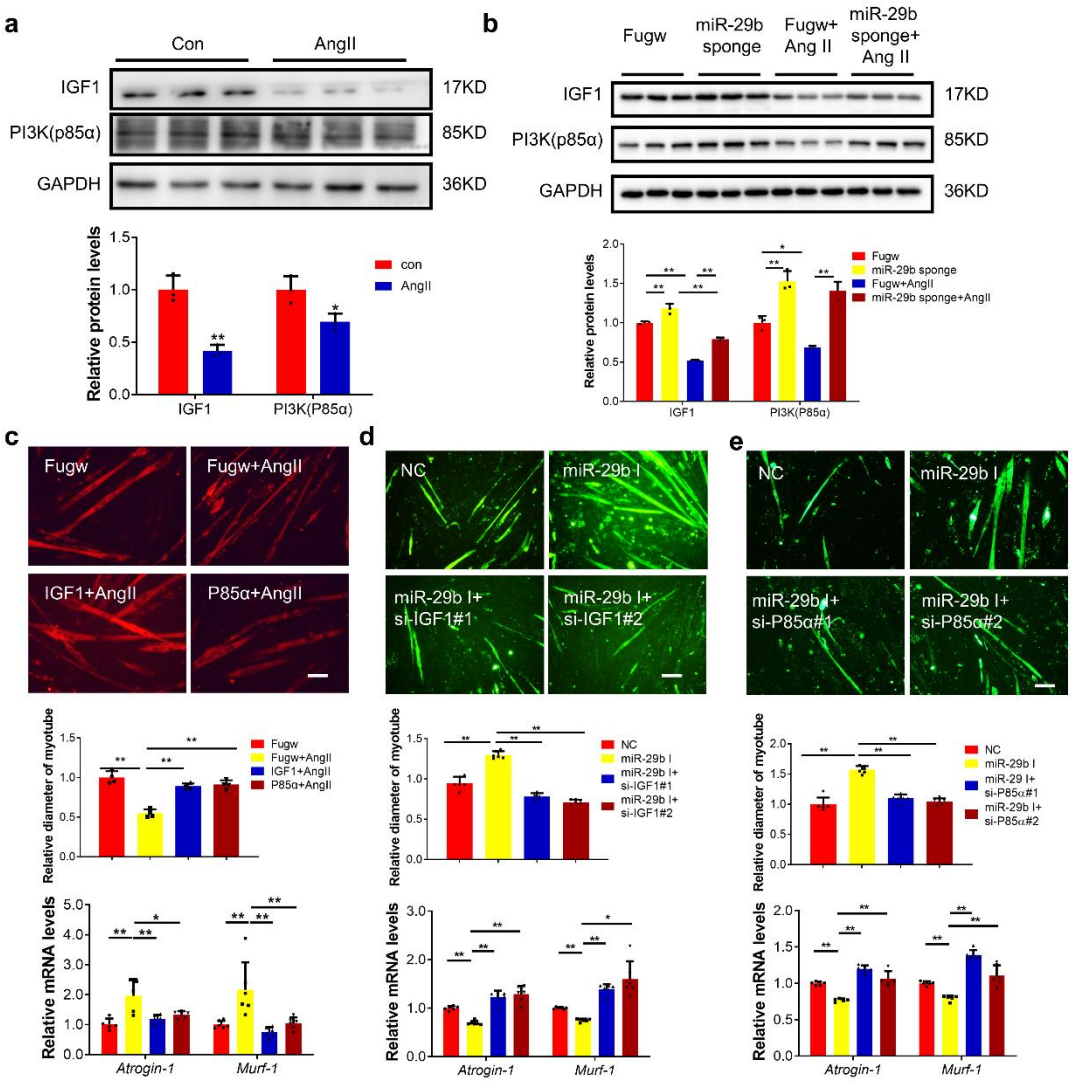

Figure S3. miR-29b mediates AngII-induced muscle atrophy by targeting IGF1, PI3K(p85α). (a)Western blot analysis of IGF1and PI3K(p85α) protein level in control and AngII-induced muscle atrophy cellular model. n=3 per group. (b) Western blot analysis of IGF1 and PI3K(p85α) protein level in the gastrocnemius from control and AngII mice treated with Fugw or miR-29b sponge. n=3 per group. (c) Immunofluorescent staining for C2C12 myotubes and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expression in IGF1 or PI3K(p85α) overexpression in AngII treated C2C12 myotube. Scale bar: 100μm. n=4 per group for staining. n=5-6 per group for qRT-PCR. (d) Immunofluorescent staining for C2C12 myotubes and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expression for C2C12 myotubes when knockdown IGF1 together with miR-29b inhibition in AngII-induced muscle atrophy. Scale bar: 100μm. n=6 per group. (e) Immunofluorescent staining for C2C12 myotubes and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expression for C2C12 myotubes when knockdown P85α together with miR-29b inhibition in AngII-induced muscle atrophy. Scale bar: 100μm. n=6 per group in immunofluorescent staining; n=6,5,6,6 in qRT-PCR. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.

Figure S4

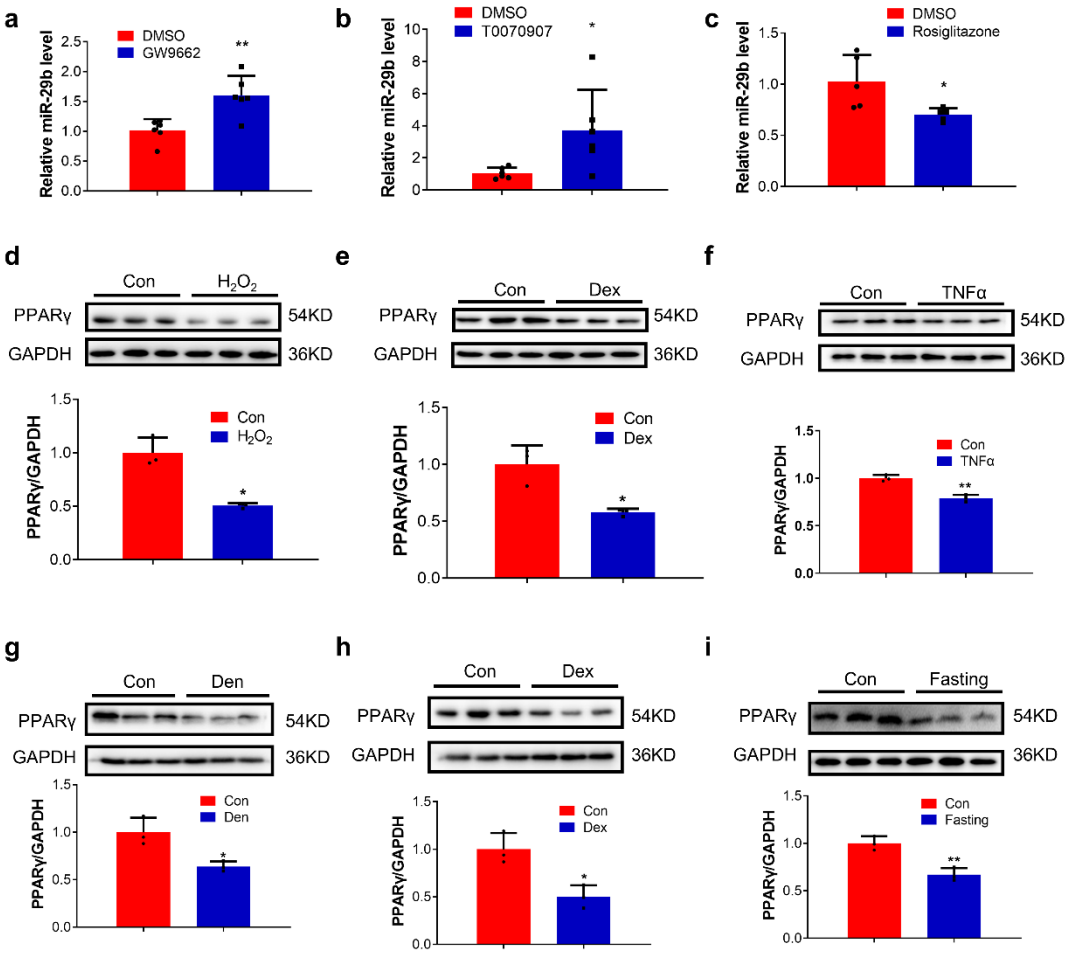

Figure S4. PPARγ acts as an upstream regulator of miR-29b. (a) qRT-PCR analysis of miR-29b expression when C2C12 myotubes were treated with PPARγ antagonist GW9662. n=6 per group. (b) qRT-PCR analysis of miR-29b expression when C2C12 myotubes were treated with PPARγ antagonist T0070907. n=6 per group. (c) qRT-PCR analysis of miR-29b expression when C2C12 myotubes were treated with PPARγ agonist rosiglitazone. n=5 per group. (d) Western blot analysis of PPARγ protein level in H_2O_2 induced muscle atrophy in *vitro*. n=3 per group. (e) Western blot analysis of PPARγ protein level in Dex induced muscle atrophy in *vitro*. n=3 per group. (f) Western blot analysis of PPARγ protein level in TNFα induced muscle atrophy in *vitro*. n=3 per group. (g) Western blot analysis of PPARγ protein level in the gastrocnemius from Den induced muscle atrophy in *vivo*. n=3 per group. (h) Western blot analysis of PPARγ protein level in the gastrocnemius from Dex induced muscle atrophy in *vivo*. n=3 per group. (i) Western blot analysis of PPARγ protein level in the gastrocnemius from Fasting induced muscle atrophy in *vivo*. n=3 per group. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.

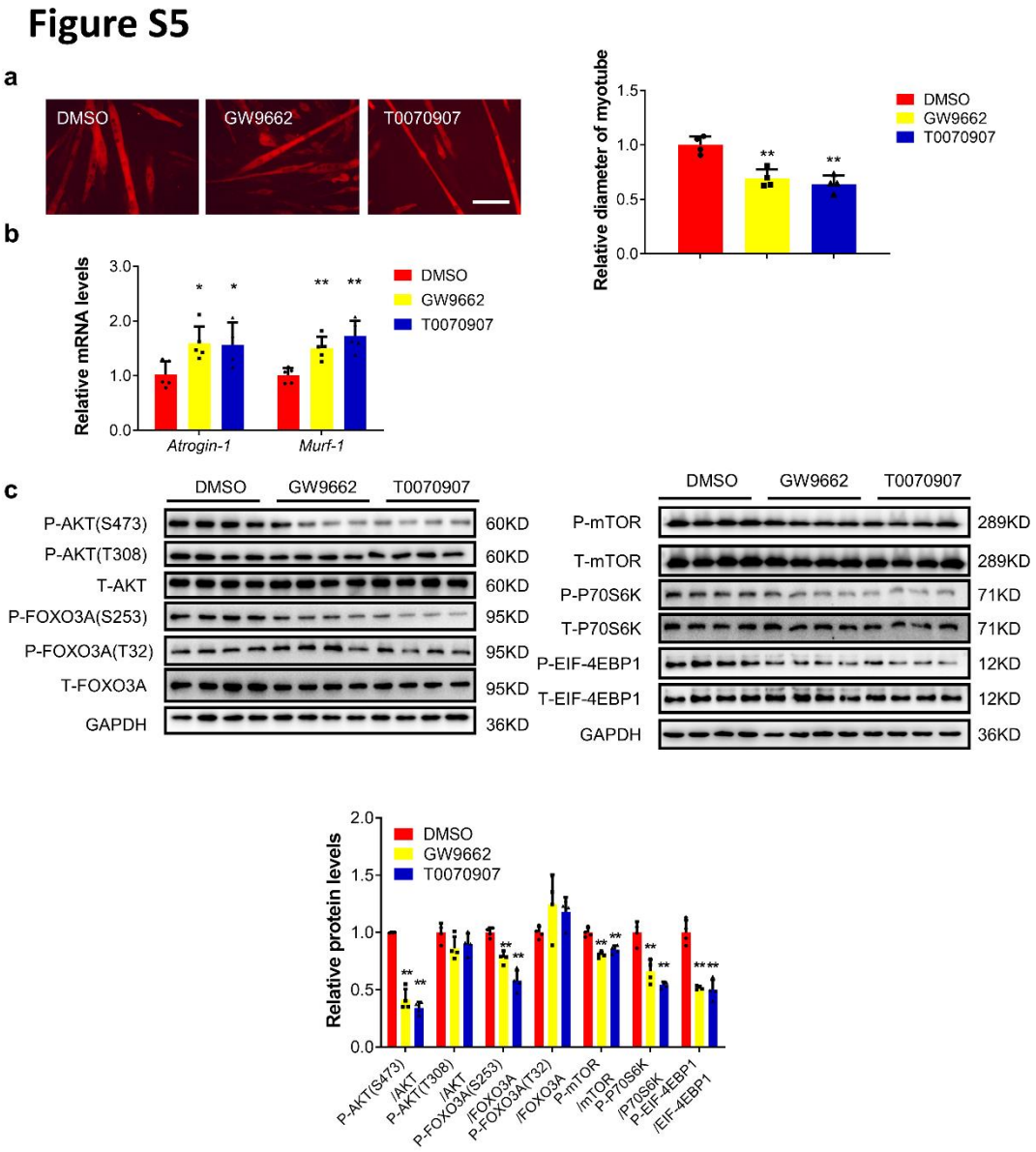

Figure S5. PPARγ inhibition with antagonists promotes muscle atrophy *in vitro*. (a) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter in C2C12 myotubes treated with antagonists. Scale bar: 100μm. n=4 per group. (b) qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions when C2C12 myotubes were treated with antagonists. n=5 per group. (c) Western blot analysis for the AKT/FOXO3A/mTOR pathway (AKT, FOXO3A, mTOR, P70S6K, 4EBP1) when C2C12 myotubes were treated with antagonists. n=4 per group. Data are shown as mean ± SD. GW9662 and T0070907 group were compared with DMSO group. *, P<0.05. **, P<0.01.

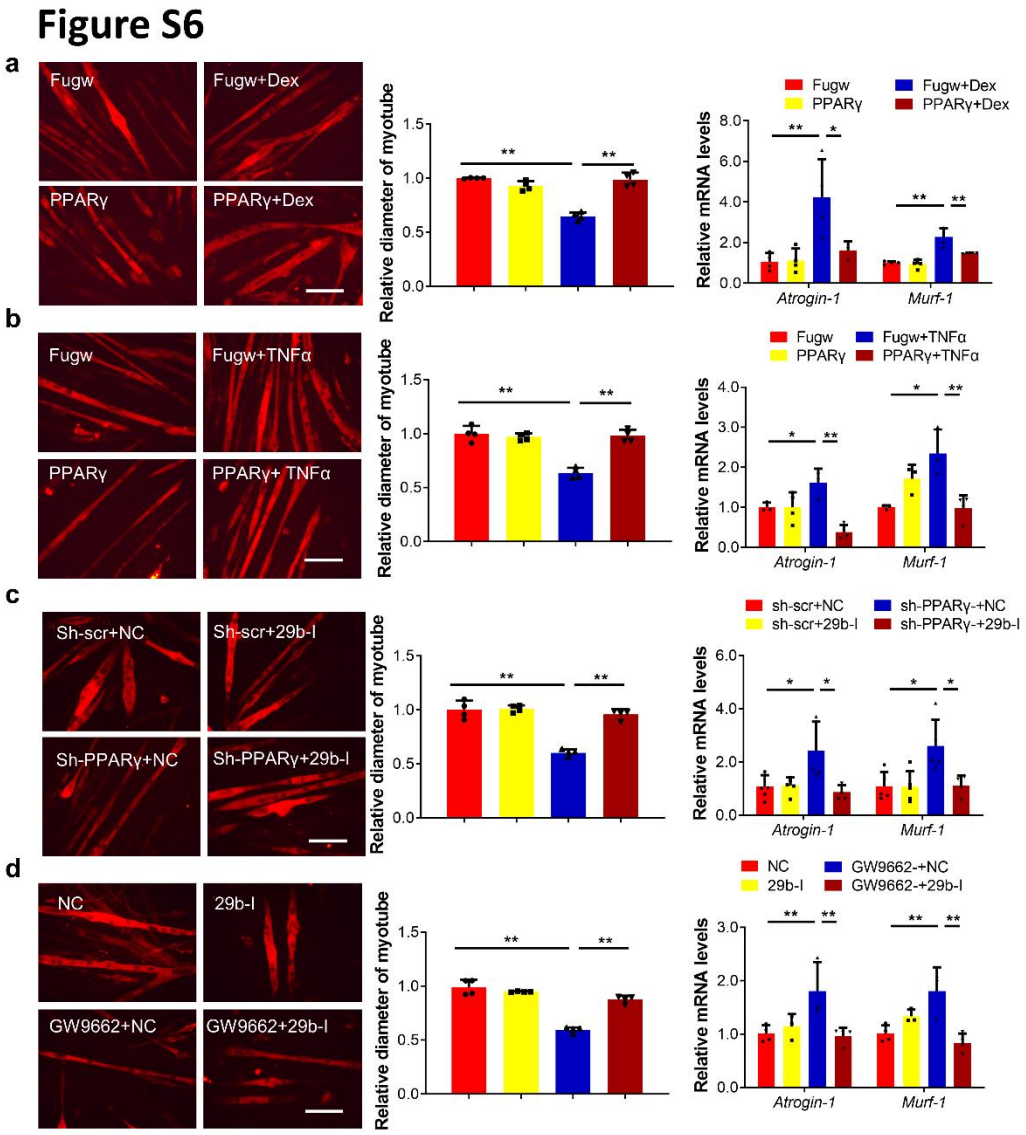

Figure S6. Activation of PPARγ ameliorates AngII-induced muscle atrophy in *vitro*. (a) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions when myotubes were transfected with Fugw and PPARγ overexpression lentivirus in Dex-induced muscle atrophy model. Scale bar: 100μm. n=4 per group for staining. n=3-4 per group for qRT-PCR. (b) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions when myotubes were transfected with Fugw and overexpression lentivirus in TNFα induced muscle atrophy model. Scale bar: 100μm. n=4 per group for staining. n=3-4 per group for qRT-PCR. (c) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions when myotubes were transfected with miR-29b inhibitor in PPARγ shRNA induced muscle atrophy model. Scale bar: 100μm. n=4 per group for staining. n=5-6 per group for qRT-PCR. (d) Immunofluorescent staining for C2C12 myotubes followed by determination of myotube diameter and qRT-PCR analysis of *Atrogin-1* and *Murf-1* expressions when myotubes were transfected with miR-29b

inhibitor in PPARγ antagonist GW9662 induced muscle atrophy model. Scale bar: 100μm. n=4 per group for staining. n=3-4 per group for qRT-PCR. Data are shown as mean ±SD. *, $P<0.05$. **, $P<0.01$.

Figure S7

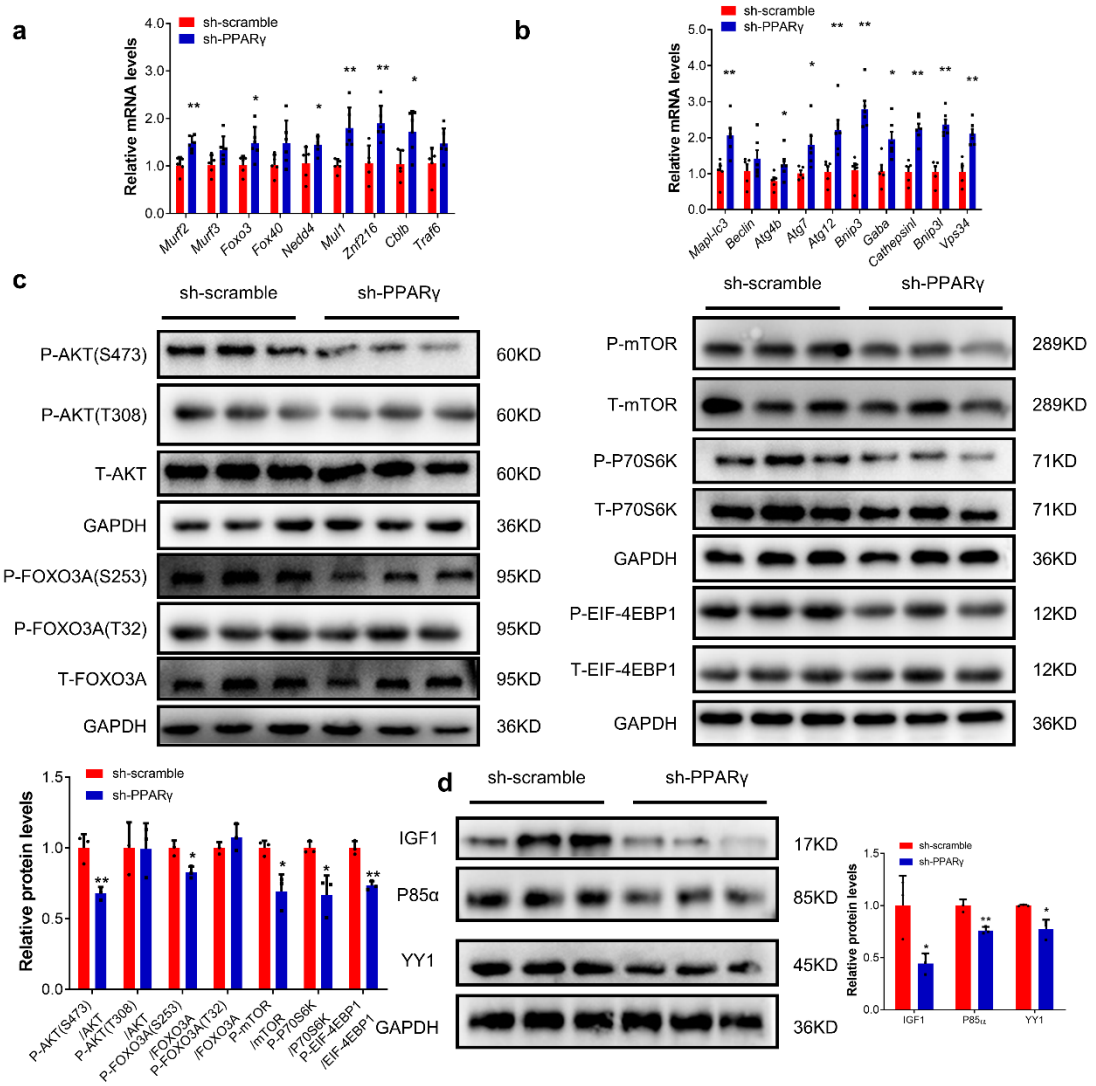

Figure S7. PPARγ inhibition promotes muscle atrophy in *vivo*. (a) qRT-PCR analysis of ubiquitin ligases-related gene expression in sh-scramble and PPARγ shRNA treated mice. n=5,6. (b) qRT-PCR analysis of autophagy-related gene expressions in sh-scramble and PPARγ shRNA treated mice. n=5,6. (c) Western blot analysis for the AKT/FOXO3A/mTOR pathway (AKT, FOXO3A, mTOR, P70S6K, 4EBP1) in sh-scramble and PPARγ shRNA treated mice. n=3 per group. (d) Western blot analysis of IGF1, PI3K(p85α) and YY1 expression in sh-scramble and PPARγ shRNA treated mice. n=3 per group. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.

Figure S8

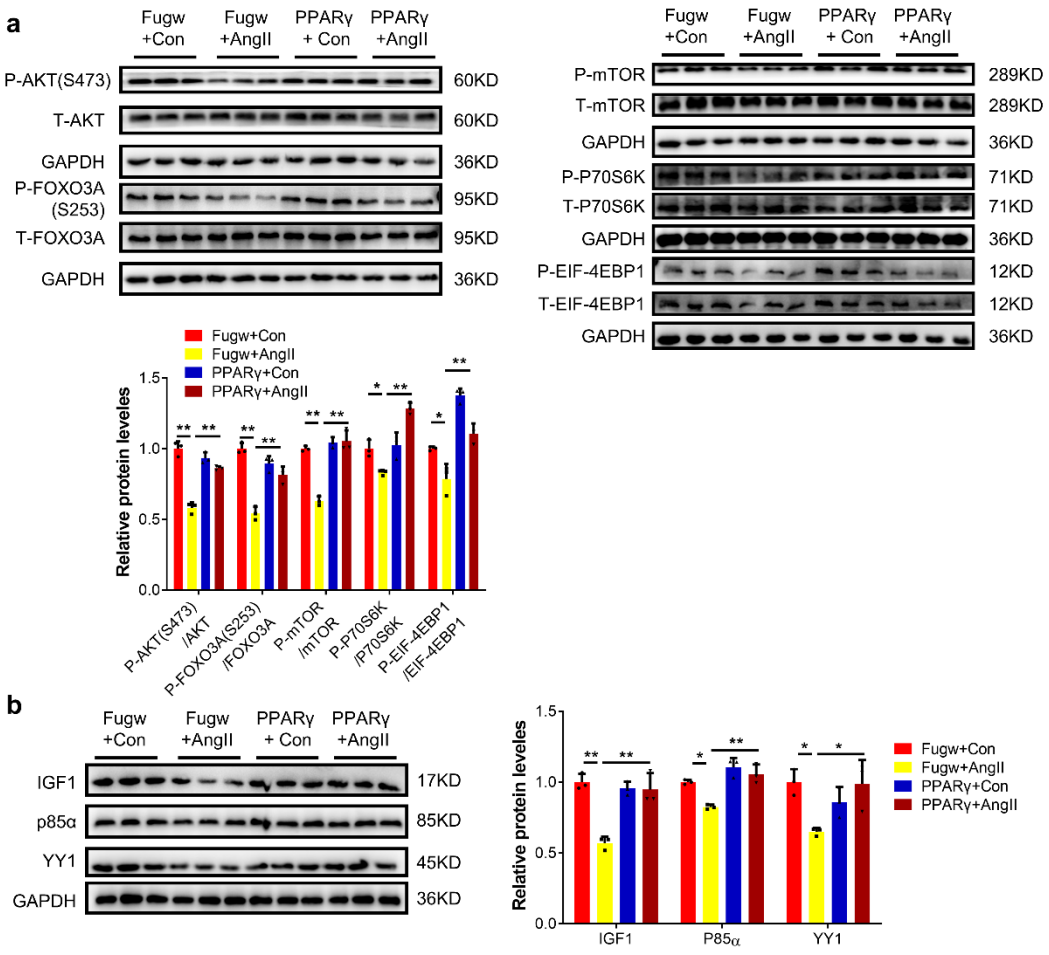

Figure S8. Overexpression of PPARγ ameliorates AngII-induced muscle atrophy in *vivo*. (a) Western blot analysis for the AKT/FOXO3A/mTOR pathway (AKT, FOXO3A, mTOR, P70S6K, 4EBP1) when control and AngII mice treated with Fugw or PPARγ overexpression lentivirus. n=3 per group. (b) Western blot analysis of IGF1, PI3K(p85α) and YY1 expression in control and AngII mice treated with Fugw or PPARγ overexpression lentivirus. n=3 per group. Data are shown as mean ±SD. *, P<0.05. **, P<0.01.