Supplemental Figure Legends

Figure S1. Specificity of adiponectin-mediated inhibition of LRP6 phosphorylation. Mouse embryonic fibroblasts were grown to confluency and then serum-starved overnight. Cells were then treated with recombinant adiponectin (APN; 5 μg/ml) or bovine serum albumin (BSA) as an abundant protein control in the following amounts: equimolar to APN (+), two times molar excess (++), or three times molar excess (+++). Two hours later, cells were treated with Wnt3a (100ng/ml). Lysates were collected 30 minutes after Wnt3a treatment, and immunoblot analyses were conducted to assess levels of phospho-LRP6. Tubulin served as a loading control.

Figure S2. Verification of the loss of AdipoR1 and AdipoR2 in AdipoR1/R2 double-knockout cells. Genomic DNA was isolated from wild-type MEFs (WT) and AdipoR1/R2 knockout MEFs (KO). PCR analysis was conducted using primers specific for WT and KO alleles for both AdipoR1 and AdipoR2.

Figure S3. AMPK signaling is not required for adiponectin to inhibit LRP6 phosphorylation and Wnt/β-catenin target gene expression. A-B) Mouse embryonic fibroblasts (WT or AMPK KO) were grown to confluency and serum-starved overnight. Cells were then treated with recombinant adiponectin (APN; 5 μg/ml). Two hours later, cells were treated with recombinant Wnt3a (100ng/ml). Thirty minutes after Wnt3a treatment, lysates were collected and immunoblot analyses were used to assess levels of phosho- and total LRP6. GAPDH or tubulin served as a loading control. The bar graph in B depicts the average pLRP6 levels relative to GAPDH or tubulin from three independent experiments, including that shown in A. Error bars represent SD. In C, primary dermal fibroblasts were grown to confluency and then serum-starved overnight. Cells were then treated with the AMPK inhibitor Compound C (CompC). One-hour later, cells were treated with recombinant adiponectin (APN; 5 μg/ml); two hours later, cells were treated with recombinant Wnt3a (100ng/ml); twenty-four hours after

Wnt3a treatment, RNA was isolated and qRT-PCR was conducted to measure expression of Wnt target genes including Axin2 (left). Expression of the AMPK target gene MMP1 (right) was assessed to demonstrate effectiveness of Compound C at inhibiting AMPK signaling. Bar graphs depict averages of two independent experiments. Error bars represent SD.

Supplemental Figure 1

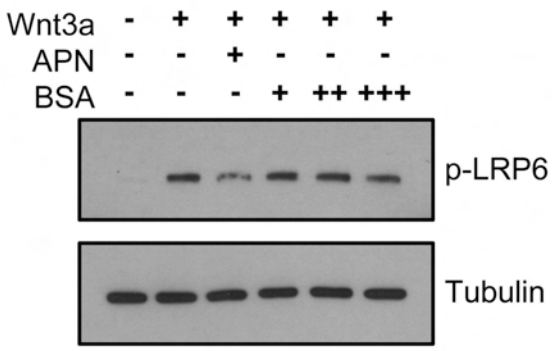
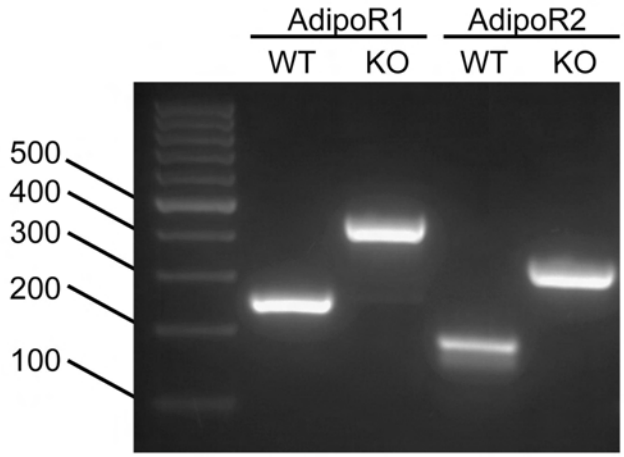


Figure S1. Adiponectin's inhibitory effect on LRP6 phosphorylation is specific to adiponectin. Mouse embryonic fibroblasts were grown to confluency and then serum-starved overnight. Cells were then treated with recombinant adiponectin (APN; 5 µg/ml) or bovine serum albumin (BSA) in the following amounts: equimolar to APN (+), two times molar excess (++), or three times molar excess (+++) . 2hr later, cells were treated with Wnt3a (100ng/ml). Lysates were collected 30 minutes after Wnt3a treatment, and immunoblot analyses were conducted to asses levels of phospho-LRP6. Tubuin served as a loading control.

Supplemental Figure 2



Expected PCR Product Sizes (bp)

	WT	KO
AdipoR1	253	469
AdipoR2	185	391

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Fig S3

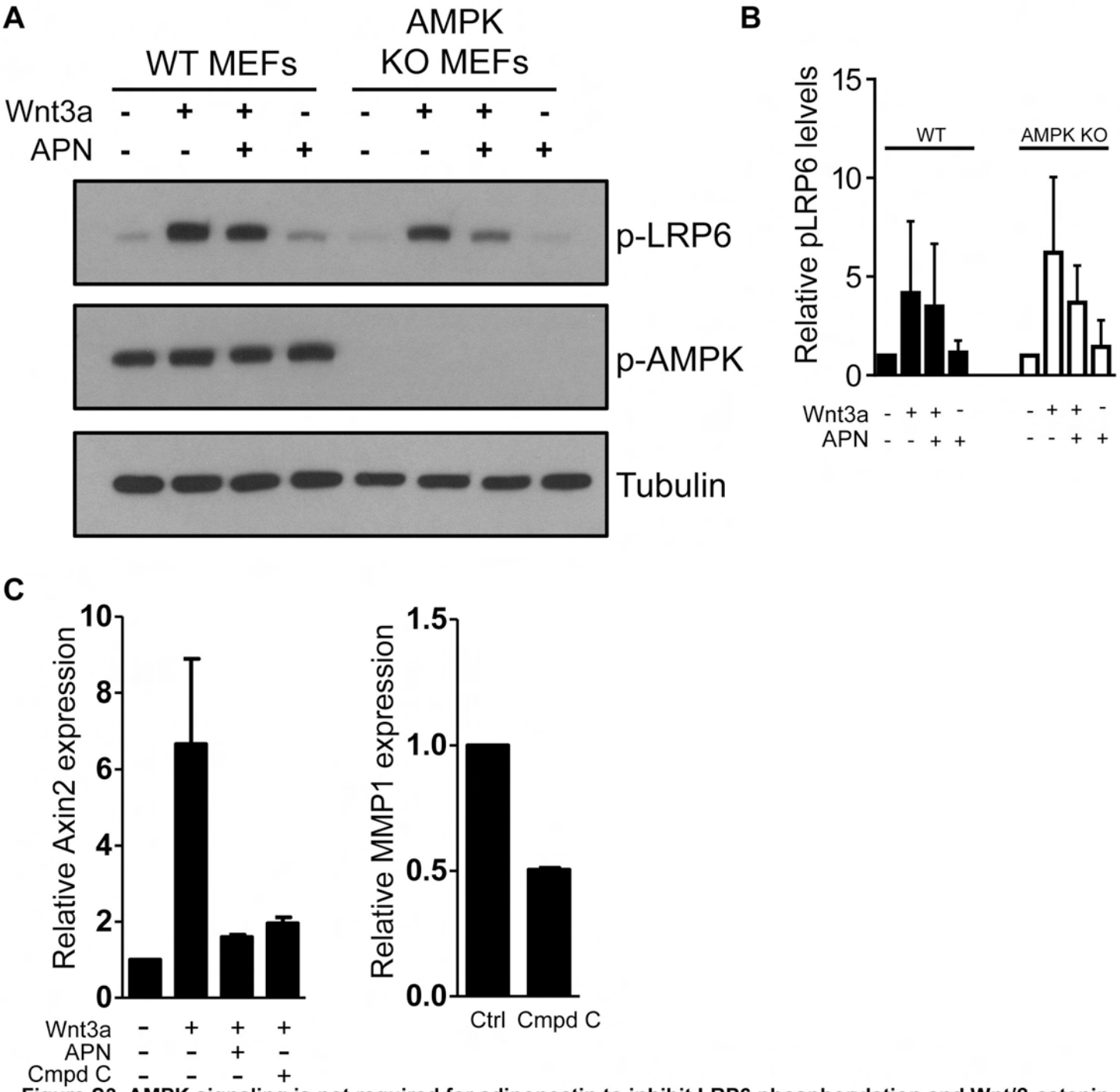

Figure S3. AMPK signaling is not required for adiponectin to inhibit LRP6 phosphorylation and Wnt/β-catenin target gene expression.(A-B) Mouse embryonic fibroblasts (WT or AMPK KO) were grown to confluency and then serum-starved overnight. Cells were then treated with recombinant adiponectin (APN; 5 μg/ml). Two hours later, cells were treated with recombinant Wnt3a (100ng/ml). 30 minutes after Wnt3a treatments, lysates were collected and immunoblot analyses were used to assess levels of phosho- and total LRP6. GAPDH or Tubuin served as a loading control. The bar graph in B depicts the average pLRP6 levels relative to GAPDH or Tubulin from three independent experiments, including that shown in A. Error bars represent SD. In C, primary dermal fibroblasts were grown to confluency and then serum-starved overnight. Cells were then treated with the AMPK inhibitor Compound C (Cmpd C). One hour later, cells were treated with recombinant adiponectin (APN; 5 μg/ml). Two hours later, cells were treated with recombinant Wnt3a (100ng/ml). 24 hours after Wnt3a treatment, RNA was isolated and qRT-PCR was conducted to measure expression of Wnt target genes including Axin2 (left). Expression of the AMPK target gene MMP1 (right) was assessed to demonstrate effectiveness of Compound C at inhibiting AMPK signaling. Bar graphs depict averages of two independent experiments. Error bars represent SD.