Supplemental Figure Legends

Fig. S1. Wnt7a specifically induces myotube hypertrophy. (a) C2C12 cells were either stably transfected with a CMV-Wnt7a-HA expression plasmid and differentiated for 5 days or differentiated C2C12 cells (at day three of differentiation) were treated with recombinant Wnt7a (50 ng/ml), n=5, *** p< 0.0001. Fusion index was determined by counting Dapi positive nuclei per fiber. (b) Application of 50 ng/ml Wnt7a recombinant protein at day 0 and day 3 resulted in significantly increased fiber diameters in C2C12 cells. n=3, *** p< 0.0001. (c) Differentiation of a coculture of Wnt7a-HA expressing T10 ½ cells with C2C12 cells resulted in increased fibre diameters, while coculture of T10 ½ control cells and C2C12 cells did not lead to changes in fiber diameter compared to C2C12 cells alone. n=3, *** p< 0.0001. (d) C2C12 cells expressing Wnt7a-HA show a similar proliferation rate as C2C12 control cells. (e-h) Immunofluorescence analysis of C2C12 cells treated with different recombinant Wnt proteins (50 ng/ml each). The Wnt recombinant proteins were applied at day 0 of differentiation. Staining for myosin heavy chain (MyHC, in green) marks differentiated cells. Nuclei were counterstained with Dapi (in blue). Scale bar: 50um. (i) Immunoblot analysis of myogenin and MHC reveal no changes in the kinetics of differentiation between CMV-Wnt7a-HA expressing cells and control cells during differentiation. (j) Quantitative Real-time PCR of C2C12 cells during differentiation does not show differences in myogenic markers between CMV-Wnt7a-HA expressing cells and control cells. n=3. (k) C2C12 cells were differentiated for 2 days, then AraC was applied for one day. At day three of differentiation cells were either treated with Wnt7a or BSA as a control and differentiated for 2 additional days. The fibre

diameter was measured at day 5 of differentiation. Application of Wnt7a to AraC treated C2C12 cells resulted in a similar fiber diameter compared to non-treated C2C12 cells (no AraC, but Wnt7a). n=3, ***p<0.001. (l,m) representative images of C2C12 cells after application of Wnt7a with (l) and without (m) treatment with AraC. Staining for myosin heavy chain in green, nuclei are counterstained with Dapi (in blue). Error bars represent SEM

Fig. S2. Wnt7a evoked hypertrophy is Fzd7 dependent.

(a) Immunoprecipitation analyses using GFP coupled beads demonstrated that Fzd7YFP binds Wnt7a-HA in C2C12 cells. (b) In COS cells Wnt7a-HA also coimmunoprecipitates with Fzd7YFP while it does not bind to Fzd3YFP as well as YFP alone. (c) Knockdown of Fzd3 using specific siRNA did not result in changes in the fiber diameter in Wnt7a-HA expressing cells after 5 days of differentiation. n=3. (d) Knockdown of Vangl2 using siRNA did not change the fiber diameter of Wnt7a-HA expressing C2C12 cells 5 days after differentiation. n=3 (e) Transfection of siRNA directed to Fzd7 resulted in a knockdown efficiency of 50% shown by quantitative real-time PCR. (f) Transfection of siRNA directed to Fzd3 yielded in a 56 percent reduction of Fzd3 expression. n=3. (g) Wnt7a-HA expressing cells transfected with siRNA directed to Vangl-2 displayed a reduction in Vangl-2 mRNA expression of 64%, n=3. (h) Immunoblot analyses revealed no changes in the amount of phosphorylated or total GSK3beta and the amount of active beta-catenin or total beta-catenin in Wnt7a-HA expressing C2C12 cells compared to control cells suggesting that Wnt7a does not act through the canonical Wnt pathway. (i) Immunoblot analyses of Wnt7a-HA expressing C2C12 cells demonstrate the activation of canonical Wnt signalling in these cells by application of LiCl or BIO. (j) Inhibition of GSK3beta by LiCl did not alter the fiber diameter of C2C12 cells expressing Wnt7a-HA or control cells at 5 days after differentiation, n=4. (k) Application of BIO did not result in changes in the average fiber diameter after 5 days of differentiation of C2C12 cells or Wnt7a-HA expressing cells, Error bars represent SEM ,n=3.

Fig. S3: Densitometric analyses of Wnt7a inducing phosphorylation of components of the Akt/mTOR hypertrophy pathway.

(a) Densitometric analysis of active beta catenin. (b) Densitometric analysis of total beta catenin. (c) Densitometric analysis of phosphorylated GSK3beta. (d) Densitometric analysis of total GSK3beta. **(**e) Densitometric analysis of phosphorylated PI3Kinase. (f) Densitometric analysis of total p85alpha subunit of PI3K. (g) Densitometric analysis of phosphorylated Akt. (h) Densitometric analysis of total Akt. (i) Densitometric analysis of phosphorylated S6. (j) Densitometric analysis of total S6. All values are normalized to tubulin levels. Error bars represent SEM; n=3; * $p<0.05$, *** $p<0.001$.

Fig. S4 Rapamycin blocks Wnt7a induced hypertrophy but not formation of myotubes.

(a,b) Immunofluorescence analyses using antibodies directed to MyHC (in green) showing C2C12-Wnt7a-HA cells after 5 days of differentitation under control condition and in the presence of rapamycin. Nuclei are counterstained with Dapi (in blue). Scale bar: 50 um. (c) Immunoblot analyses using lysates from differentiated C2C12 cells expressing Wnt7a-HA demonstrate the functionality of rapamycin as well as of the PI3kinase inhibitor LY294002. (d) Immunoblot analyses of C2C12 cells differentitated in the presence of different Wnt proteins (50ng/ul each, differentiation for 5 days) reveal that only Wnt7a induces phosphorylation of Akt and S6. (e) Immunoblot analysis of pc-Jun and pCamKII in TA muscles after Wnt7a injections. (f). Densitometric analysis of the amount of phosphorylated IGFR, n=3. (g) Densitometric analysis of the amount of phosphorylated IRS-1, n=3. (h) Quantitiative Real time PCR using primers specific for GNAS1 revealed its expression in myoblasts and myotubes of C2C12 cells. (i) The G protein alpha S is expressed in myoblasts and myotubes of C2C12 cells. (i) Application of suramin inhibited Wnt7a but not IGF-1 evoked hypertrophy. (k) C2C12 cells

transfected with siRNA directed to GNAS1 displayed a reduction in GNAS1 mRNA expression of 70%, n=3, ***<0.001. Error bars represent SEM

Figure S5. Dual function of Wnt7a in adult skeletal muscle. (**a**) In satellite stem cells, Wnt7a signals through its receptor Fzd7 to activate the PCP pathway and drives the symmetric expansion of stem cells. (**b**) In differentiated myofibres, Wnt7a signals through Fzd7 to activate the PI3K/Akt/mTOR growth pathway to induce myofibre hypertrophy.