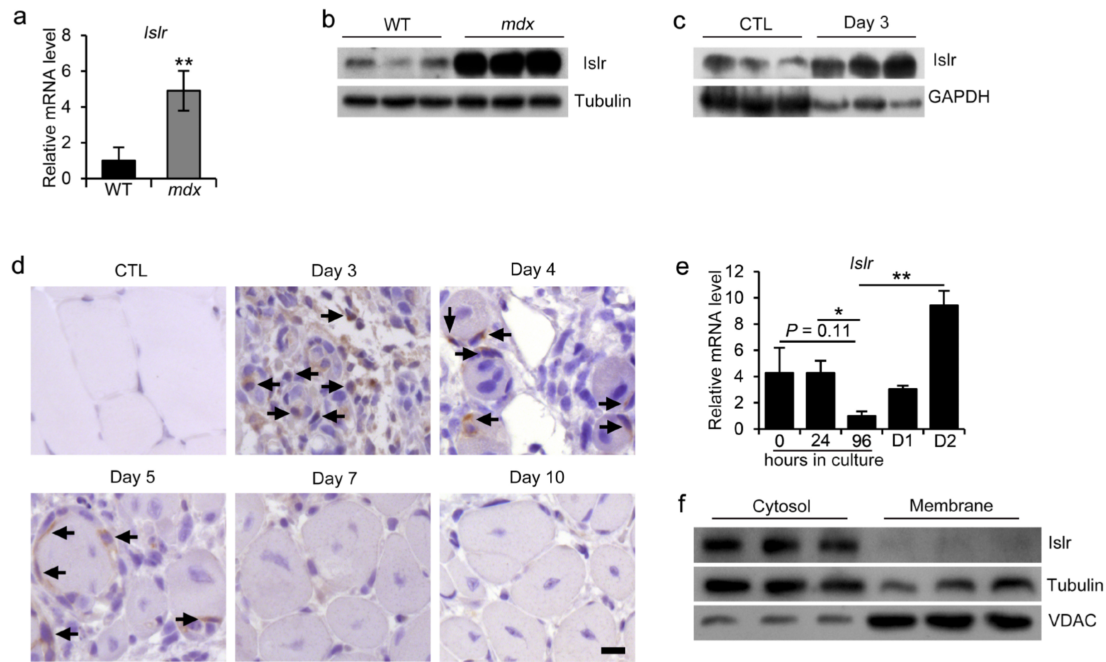
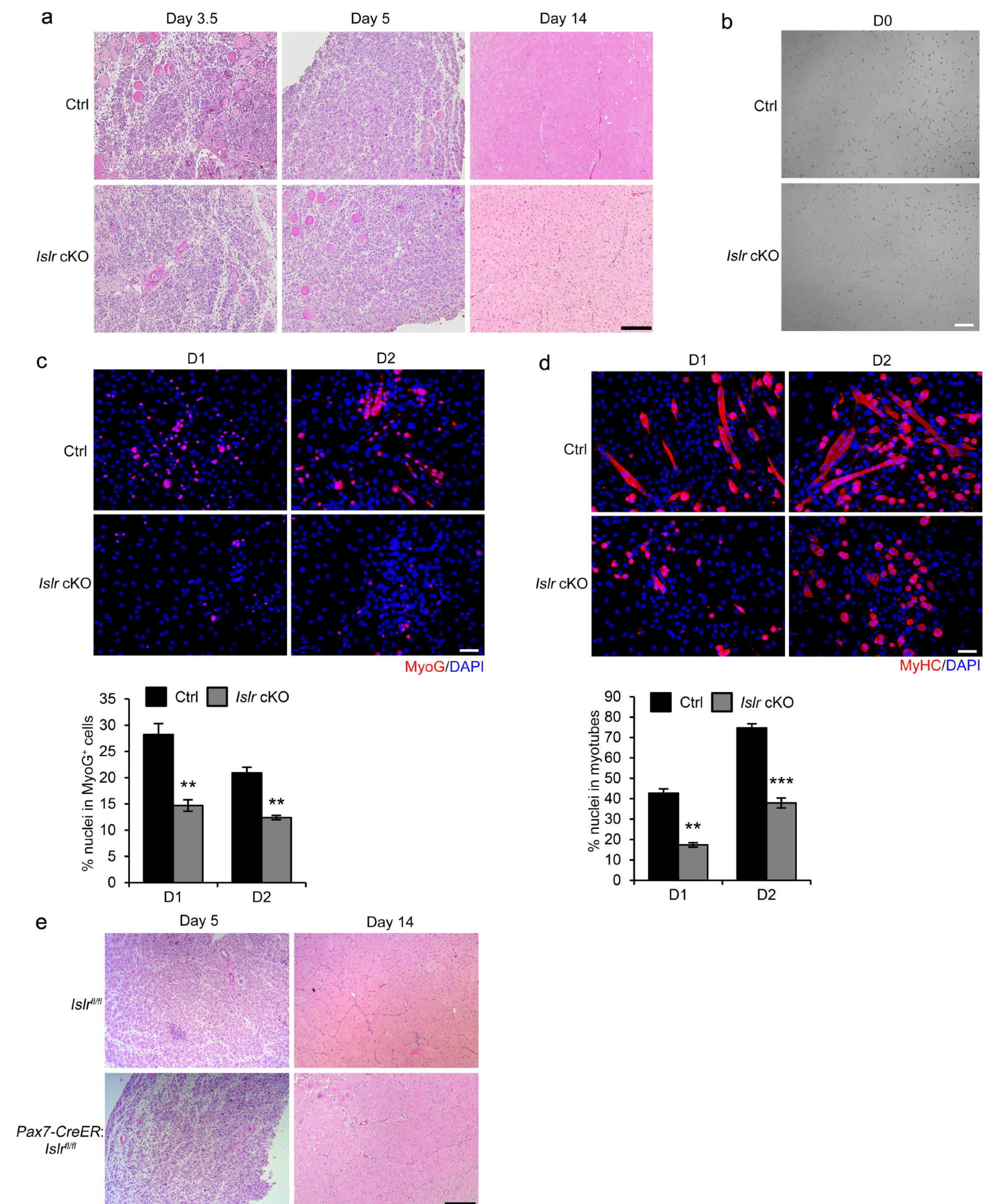
Zhang et al., Islr regulates canonical Wnt signaling-mediated skeletal muscle regeneration by stabilizing Dishevelled-2 and preventing autophagy

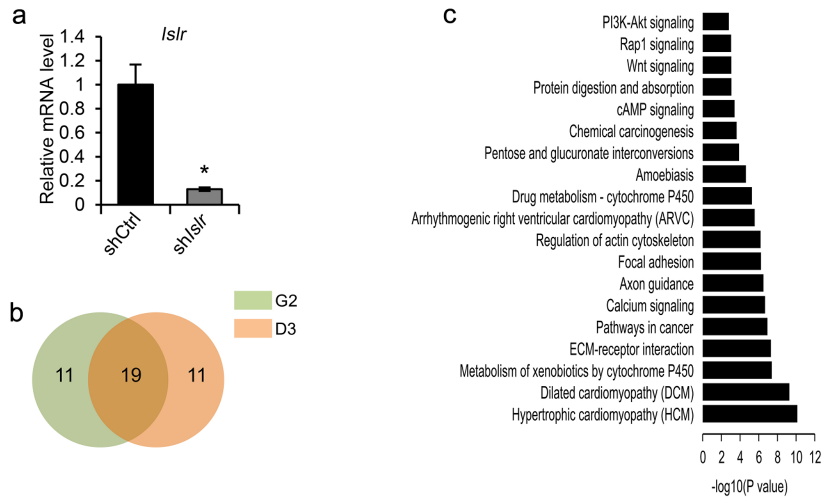


Supplemental Figure 1. Islr participates in adult skeletal muscle regeneration and satellite cell differentiation. (**a**) Expression analysis of *Islr* in wild type (WT) and *mdx* mice at the age of 9 months using quantitative real-time PCR (qRT-PCR). $N = 3$ in each group. (**b**) Western blot analysis of Islr protein levels in WT and *mdx* mice at the age of 9 months. $N = 3$ in each group. (**c**) Western blot analysis of Islr protein levels in injured and contralateral TA muscles (CTL) at 3 d post injury. (**d**) Immunohistochemistry analysis of Islr in injured TA muscles of WT mice at 3, 4, 5, 7, and 10 d post injury. Scale bar = 50 μm. (**e**) Expression analysis of *Islr* in freshly isolated satellite cells, activated satellite cells cultured for 24 h in proliferation medium, proliferating cells cultured for 96 h in proliferation medium, differentiating cells cultured for 4 d in proliferation medium followed by 1 d in differentiation medium (D1), and differentiating cells cultured for 4 d in proliferation medium followed by 2 d in differentiation medium (D2) using qRT-PCR. (**f**) Western blot analysis of Islr in cytosol and membrane fractions of differentiating cells cultured for 3 d in differentiation medium. Error bars represent the means ± s.d. $^{*}P < 0.05$, $^{**}P < 0.01$; Student's *t* test.

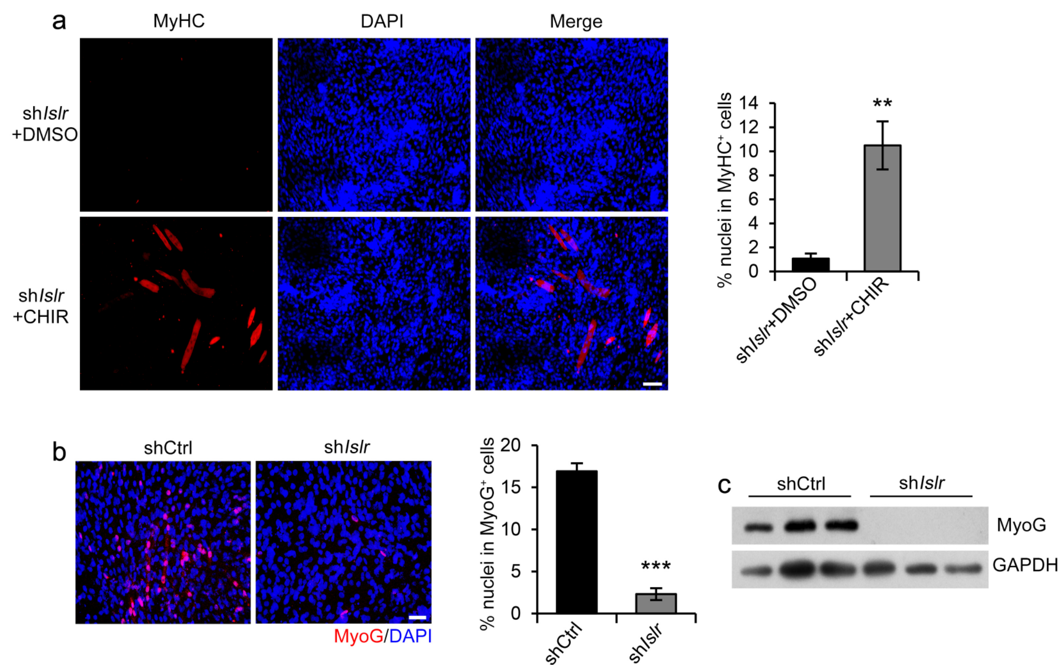


Supplemental Figure 2. Islr-deficiency delayed the progression of myogenesis. (**a**) Low magnification photos of **Fig. 2e**. $N = 3$ in each group. Scale bar = 200 μm. (**b**) Photos of isolated satellite cells from control and *Islr* cKO mice cultured for 4 d in proliferation medium before starting differentiation. $N = 3$ cell cultures in each group. Scale bar = 250 μm. (**c**) Immunofluorescence analysis of MyoG^{+} cells in primary myoblasts from control and *Islr* cKO mice after 1 ($N = 3$) and 2 ($N = 3$) d in differentiation medium (D1, D2). Scale bar = 50 μm. The percentages of MyoG^{+} cells are shown on the

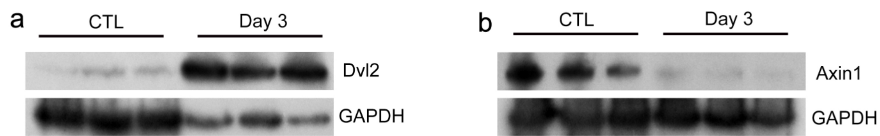
below. (**d**) Immunofluorescence analysis of $MyHC^+$ cells in primary myoblasts from control and *Islr* cKO mice after 1 ($N = 3$) and 2 ($N = 3$) d in differentiation medium. Scale bar = 50 μm. The percentages of nuclei contained in the myotubes (a $MyHC^+$ cell with at least 2 nuclei) are shown on the right. (**e**) Low magnification photos of **Fig. 5d**. $N = 3$ in each group. Scale bar = 200 μm. Error bars represent the means ± s.d. $^{**}P < 0.01$, $^{***}P < 0.001$; Student's *t* test.



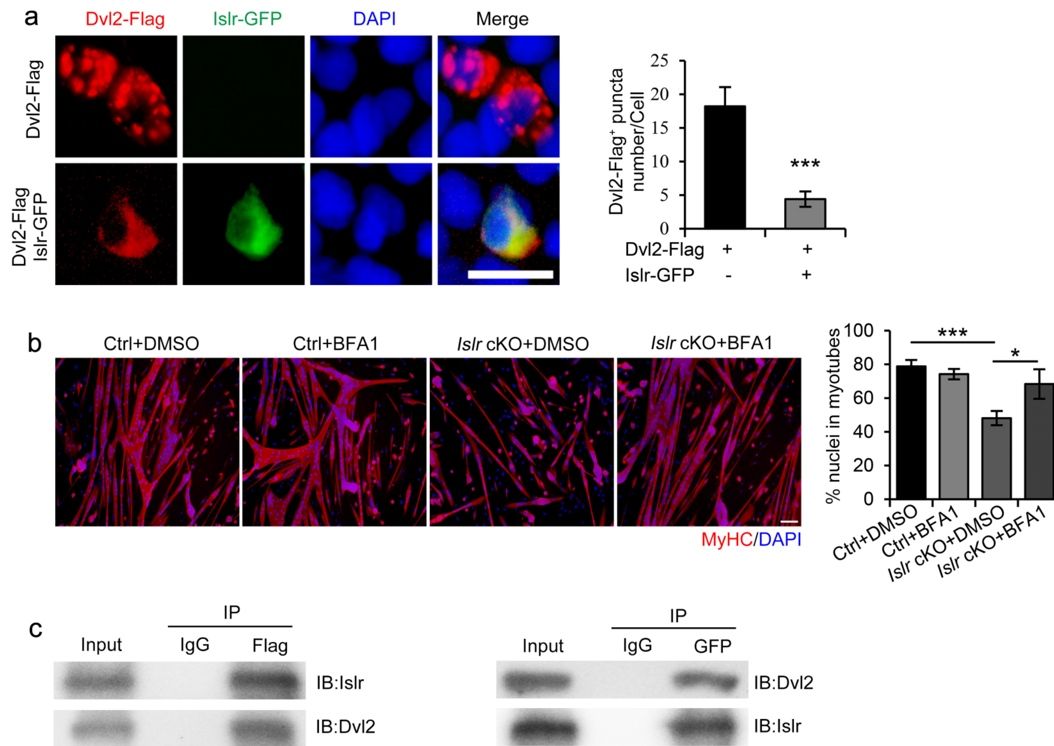
Supplemental Figure 3. Gene expression analysis of sh*Islr* C2C12 cells by RNA sequencing (RNA-seq). (**a**) Expression analysis of *Islr* in control shRNA (shCtrl) and *Islr* shRNA stable (sh*Islr*) C2C12 cells after 2 d in growth medium (G2) using qRT-PCR. (**b**) Gene pathways enriched in sh*Islr* C2C12 cells relative to shCtrl C2C12 cells at both G2 and D3. $N = 3$ cell cultures in each group. (**c**) The significance of 19 pathways in sh*Islr* C2C12 cells relative to shCtrl C2C12 cells at D3. $N = 3$ cell cultures in each group. Error bars represent the means ± s.d. $^{*}P < 0.05$; Student's t test.



Supplemental Figure 4. CHIR rescues the failure of sh*Islr* C2C12 cells to differentiate, and the numbers of MyoG⁺ cells are decreased in sh*Islr* C2C12 cells. (**a**) Immunofluorescence staining for MyHC in sh*Islr* C2C12 cells treated with CHIR or DMSO after 3 d in differentiation medium. $N = 3$ cell cultures in each group. Scale bar = 100 μm. The percentages of MyHC⁺ cells are shown on the right. (**b**) Immunofluorescence staining for MyoG in shCtrl and sh*Islr* C2C12 cells after 3 d in differentiation medium. $N = 3$ cell cultures in each group. The percentages of MyoG⁺ cells are shown on the right. Scale bar = 50 μm. (**c**) Western blot analysis of MyoG protein levels in shCtrl and sh*Islr* C2C12 cells after 3 d in differentiation medium. Error bars represent the means ± s.d. $^{**}P < 0.01$, $^{***}P < 0.001$; Student's *t* test.



Supplemental Figure 5. The canonical Wnt signaling pathway is activated during skeletal muscle regeneration. (**a**) Western blot analysis of Dvl2 protein levels in injured and contralateral TA muscles at 3 d post injury. (**b**) Western blot analysis of Axin1 protein levels in injured and contralateral TA muscles at 3 d post injury.



Supplemental Figure 6. Islr interacts with Dvl2 in HEK293T cells, and BFA1 rescued the phenotype of primary myoblasts of *Islr* cKO mice. (**a**) Immunofluorescence analysis of Dvl2-Flag^{+} puncta in HEK293T cells transfected with Dvl2-Flag plasmid or Dvl2-Flag and Islr-GFP plasmids. N = 3 cell cultures in each group. Approximately 20 cells total in each group. Scale bar = 25 µm. The numbers of Dvl2-Flag^{+} puncta per cell are shown on the right. (**b**) Immunofluorescence staining for MyHC in primary myoblasts of control and *Islr* cKO mice treated with BFA1 or DMSO for 24 h after 3 d in differentiation medium. N = 3 cell cultures in each group. Scale bar = 100 µm. The percentages of nuclei contained in the myotubes (a MyHC^{+} cell with at least 2 nuclei) are shown on the right. (**c**) Reciprocal co-immunoprecipitation analysis between GFP-tagged Islr and Flag-tagged Dvl2 in HEK293T cells. IB: immunoblotting; IP: immunoprecipitation. Error bars represent the means ± s.d. $^{*}P < 0.05$, $^{***}P < 0.001$; Student's t test.

Figure 1e

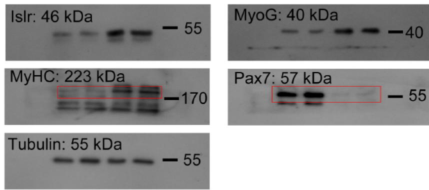


Figure 1k

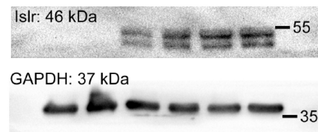


Figure 2b

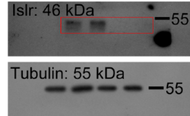
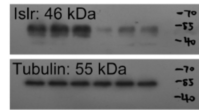


Figure 2d



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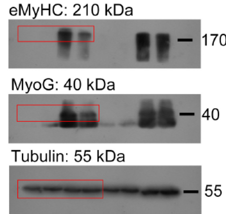

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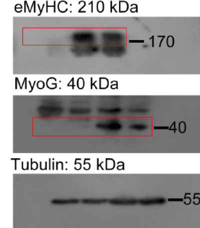

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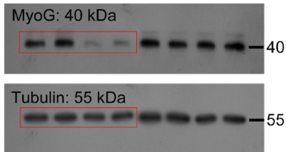

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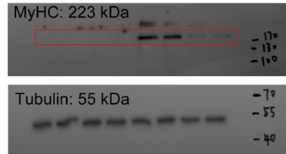

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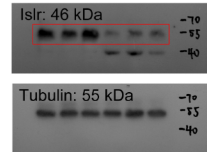

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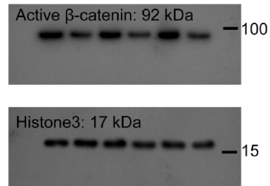

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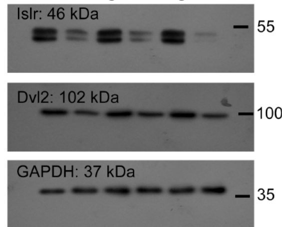

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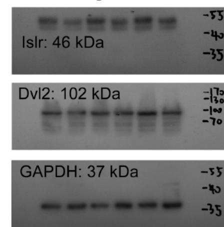


Figure 6i

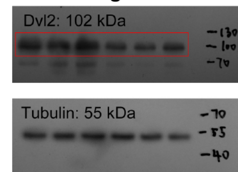


Figure 7a

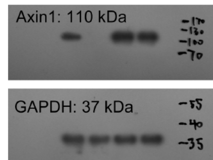
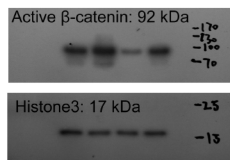


Figure 7b



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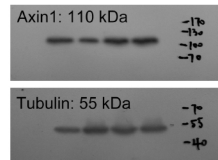


Figure 7d

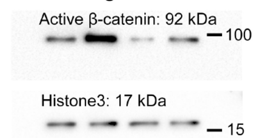
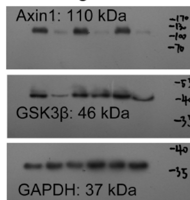
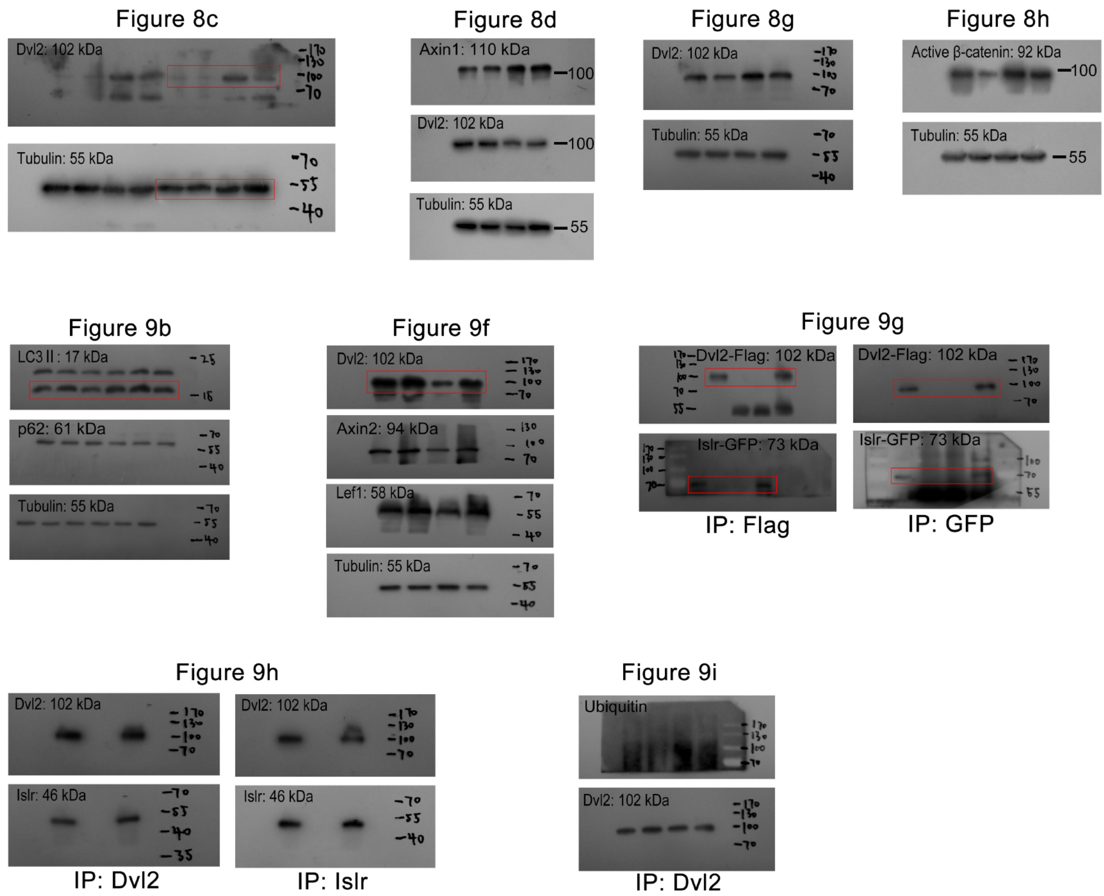


Figure 7e





Supplemental Figure 7. Full scans of immunoblots shown in Figs 1-9.