SUPPLEMENTARY TABLE AND FIGURE LEGENDS

Supplementary Figure 1. *Fbxl16* restrains differentiation of ESC into cardiomyocytes

(A) UPS genes that restrain cardiomyocyte differentiation. Short interfering RNA pools that target the indicated gene were found in duplicate assays to increase the proportion of differentiating ES cells that express GFP from a cardiac-restricted αMHC promoter by a combined 6 (*Dpf3, Fbxl20*), 5 (*Cul5, Kcns3*), or 4 (*Wdr31, Stam 2, Sumo 2, Fbx116, Amfr, Rcbtb2*) standard deviations above the mean quantified cardiomyocyte differentiation for the same plate. Of the 10 genes shown, four (*Fbxl16*), three (*Wdr31*) or two (*Rcbtb2, Stam2*) of the siRNAs in the pool yielded a positive result. Of the remaining genes, either one or none of the individual siRNAs appeared to promote differentiation. (B) Quantitative impact of *Fbxl16* silencing on cardiogenesis. The mean of the total GFP-positive area from two separate experiments done in the primary screen (n=2) is shown for wells treated with the indicated siRNA pool.

Supplementary Figure 2. Confirmation of *Fbxl16* knockdown

(A) Knockdown of *Fbxl16* was achieved with a second siRNA targeting a distinct region of the gene. siRNA #3 was used for the experiments in the main figures. (B) Knockdown of myc-FBXL16 in stably transfected 3T3 cells was evaluated using both a commercially available antibody targeting the Scirr1 antigen (Millipore) as well as an antibody for the c-myc epitope. The Scirr1 antibody recognizes both overexpressed myc-FBXL16 as well as another protein that is of a similar, but slightly elevated molecular weight, but does not appear to detect the endogenous FBXL16. (C) Effect of the confirmatory siRNA sequence #2 used in panel A on FLK1+ progenitor formation (measured as GFP-positive

cells) were determined by flow cytometry and normalized to control (luciferase) siRNA treatment. Data shown represent the mean of two replicates with very similar results (standard deviation of 0.159 calculated for *Fbxl16* siRNA treated cells). (D) Quantification of viable remaining in culture 4 days after treatment with luciferase (control) or *Fbxl16* siRNAs. Results were normalized to the number of cells in the luciferase siRNA treated samples. Error bars represent +/- one standard deviation, n=3. (E) Quantification of nonadherent, nonviable cells in culture 4 days after treatment with luciferase (control) or *Fbxl16* siRNA. Results were normalized to the number of nonviable cells seen in the control siRNA treated samples. Error bars represent +/- one standard deviation, n=3. (F) Propidium iodide staining was performed on ES cells three days after siRNA treatment. Samples were then analyzed by flow cytometry to assess cell cycle status. Error bars represent +/- one standard deviation, n=2 for each sample. (G) Brdu incorporation into control 3T3 cells was quantitatively assayed using a chemiluminescent assay (Roche) were subject to siRNA treatment with Fbxl16 siRNA. Error bars reflect +/- one standard deviation, n=4. (H) Caspase 3,7 activity was assayed in ES cell cultures 3 days after siRNA treatment. Quantitation was performed using a chemiluminescent Caspase 3,7 activity assay from Promega, and normalized to signal obtained from luciferase siRNA treated controls. Error bars reflect +/- one standard deviation, n=3. (I) Alkaline phosphatase activity was measured using a quantitative colorimetric assay kit 4 days after siRNA treatment. Error bars reflect +/- one standard deviation, n=2. (J) Nanog reporter murine ES cells were used to assay the effects of siRNA treatment on development of Nanog (-) cells 4 days after siRNA treatment of the ES cell culture. *Oct4* siRNA was included as a positive control, and was noted to increase

the fraction of NANOG negative cells. (K) Western blot for OCT4 and NANOG stem cell markers with anti-tubulin blot used as a loading control. Extracts were prepared from mouse ES cells four days after siRNA treatment.

Supplementary Figure 3. HA-B55α and myc-FBXL16 protein analysis

(A) FBXL16 and SKP1 form a complex. FBXL16 and SKP1 were co-expressed in *E. coli*. Cell lysates were then prepared and passed over a strong cation exchange column and fractionated using salt-containing buffer. FBXL-16 and SKP1 eluted in the same fractions (shown as number 1 through 11). B) Overexpressed B55α and FBXL16 interact. 293 cells were transfected with expression vectors that encode HA-B55α or myc-FBXL16, as indicated, and cell lysates were immunoprecipitated with anti-HA. Total cell lysates (input) and anti-HA immunoprecipitates (IP) were fractionated by SDS-PAGE and immunoblotted with anti-myc or anti-HA as indicated. (C) *Fbxl16* suppression does not affect B55α protein levels. Differentiating ESC were harvested 48 hours after treatment with the siRNAs shown. Extracted RNA was subjected to semi-quantitative RT-PCR to evaluate depletion of *Fbxl16* mRNA (*GAPDH* results shown as a control). B55α protein levels were evaluated by SDS-PAGE followed by immunoblotting with antibodies to B55α and α-tubulin.

Supplementary Table 1. Composition of murine UPS siRNA library

Supplementary Table 2. Complete mass spectrometry results for anti-myc immunoprecipitates recovered from 293 cells transiently transfected with myc-FBXL16

or myc-SKP2 expression vectors and anti-myc immunoprecipitates recovered from ES cells that were either mock-transfected or transiently transfected with a vector that expressed myc-FBXL16. This file has five tabs: two tabs for protein and additional peptide data obtained from transfected 293 cells and two additional tabs for protein and additional peptide data obtained from transfected ESC. The PP2A subunits tab lists the number of unique and shared peptides identified for each PP2A subunit in myc-FBXL16 transfected 293 cells and ESC.

Supplementary Table 3. Complete quantitative mass spectrometry results for phosphopeptides recovered from mouse embryonic stem cells transfected with *Fbxl16* siRNA versus control (luciferase) siRNA and control 3T3 cells versus 3T3 cells stably overexpressing myc-FBXL16. This table has six sections: *Fbxl16* suppression localized phosphopeptides (relative levels of enriched phosphopeptides with localized phosphorylation sites in FBXL16-depleted vs. control cells); *Fbxl16* suppression unlocalized phosphopeptides (relative levels of enriched phosphopeptides with unlocalized phosphorylation sites in FBXL16-depleted vs. control cells); *Fbxl16* suppression total protein (relative protein levels in unfractionated lysates of FBXL16-depleted vs. control cells); *Fbxl16* overexpression localized phosphopeptides (relative levels of enriched phosphopeptides with localized phosphorylation sites in FBXL16-overexpressing vs. control cells); *Fbxl16* overexpression unlocalized phosphopeptides (relative levels of enriched phosphopeptides with unlocalized phosphorylation sites in FBXL16-overexpressing vs. control cells); *Fbxl16* overexpression total protein (relative protein levels in unfractionated lysates of FBXL16-overexpressing vs. control cells).