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Cytoplasmic and Nuclear TAZ Exert Distinct Functions in Regulating Primed Pluripotency

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SUMMARY

Mouse epiblast stem cells (mEpiSCs) and human embryonic stem cells (hESCs) are primed pluripotent stem cells whose self-renewal can be maintained through cytoplasmic stabilization and retention of β-catenin. The underlying mechanism, however, remains largely unknown. Here, we show that cytoplasmic β-catenin interacts with and retains TAZ, a Hippo pathway effector, in the cytoplasm. Cytoplasmic retention of TAZ promotes mEpiSC self-renewal in the absence of nuclear β-catenin, whereas nuclear translocation of TAZ induces mEpiSC differentiation. TAZ is dispensable for naive mouse embryonic stem cell (mESC) self-renewal but required for the proper conversion of mESCs to mEpiSCs. The self-renewal of hESCs, like that of mEpiSCs, can also be maintained through the cytoplasmic retention of β-catenin and TAZ. Our study indicates that how TAZ regulates cell fate depends on not only the cell type but also its subcellular localization.

INTRODUCTION

Mouse epiblast stem cells (mEpiSCs) and human embryonic stem cells (hESCs) share common molecular and cellular characteristics that define the primed pluripotent state (Nichols and Smith, 2009). For example, both mEpiSC and hESC self-renewal can be maintained through the activation of basic fibroblast growth factor (bFGF) and transforming growth factor β (TGFβ) signaling pathways (Zhou et al., 2015). Recently, we developed a culture condition for mEpiSCs and hESCs that does not require any exogenous supplement of cytokines but relies on combined administration of two small-molecule inhibitors: CHIR99021 (CHIR) and IWR-1 (Kim et al., 2013). CHIR is a selective glycogen synthase kinase 3 (GSK3) inhibitor that promotes the stabilization of β-catenin (Ring et al., 2003). IWR-1 is a tankyrase inhibitor that stabilizes AXIN1/2 and increases their protein levels in the cytoplasm (Chen et al., 2009). AXIN2, a negative feedback regulator of the canonical Wnt signaling pathway, binds to β-catenin in the cytoplasm and blocks its nuclear translocation (Jho et al., 2002). Thus, the combined use of CHIR and IWR-1 stabilizes and retains β-catenin in the cytoplasm (Kim et al., 2013). As a transcriptional co-activator, β-catenin is well documented in regulating gene expression in the nucleus (Clevers and Nusse, 2012). How cytoplasmic β-catenin controls stem cell fate choice, however, is currently unknown.

Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are well-known transcriptional co-activators in the Hippo signaling pathway (Varelas, 2014). Their roles in pluripotent stem cell maintenance, however, remain unclear. Several reports have suggested that YAP contributes to the maintenance of naive pluripotency (Lian et al., 2010; Qin et al., 2016), while others have shown that YAP/TAZ contribute to the maintenance of primed pluripotency (Beyer et al., 2013; Ohgushi et al., 2015; Varelas et al., 2008). Interestingly, a recent paper reported that YAP is dispensable for mESC self-renewal but required for proper differentiation (Chung et al., 2016). These findings indicate that the role of YAP/TAZ in pluripotency maintenance is complex and potentially context dependent. Whether YAP/TAZ are involved in β-catenin-dependent pluripotency maintenance, however, remains elusive.

Here, we report that cytoplasmic β-catenin promotes mEpiSC and hESC self-renewal through interaction with and retention of cytoplasmic TAZ. Nuclear translocation of either β-catenin or TAZ induces mEpiSC and hESC differentiation. TAZ is dispensable for mESC self-renewal but required for proper mESCs-to-mEpiSCs conversion. Cytoplasmic retention of TAZ promotes mEpiSC and hESC self-renewal in the absence of nuclear β-catenin.

RESULTS

Cytoplasmic Retention of β-Catenin Maintains mEpiSC Self-Renewal

Previously, we demonstrated that the combined use of CHIR and IWR-1 maintains mEpiSC self-renewal (Kim et al., 2013). To determine whether β-catenin is necessary for mEpiSC self-renewal under CHIR/IWR-1 condition, we generated β-catenin knockout (*Ctnnb1*$^{-/-}$) mEpiSCs under bFGF/Activin A (ActA) condition. The loss of β-catenin protein in *Ctnnb1*$^{-/-}$ mEpiSCs was confirmed by western

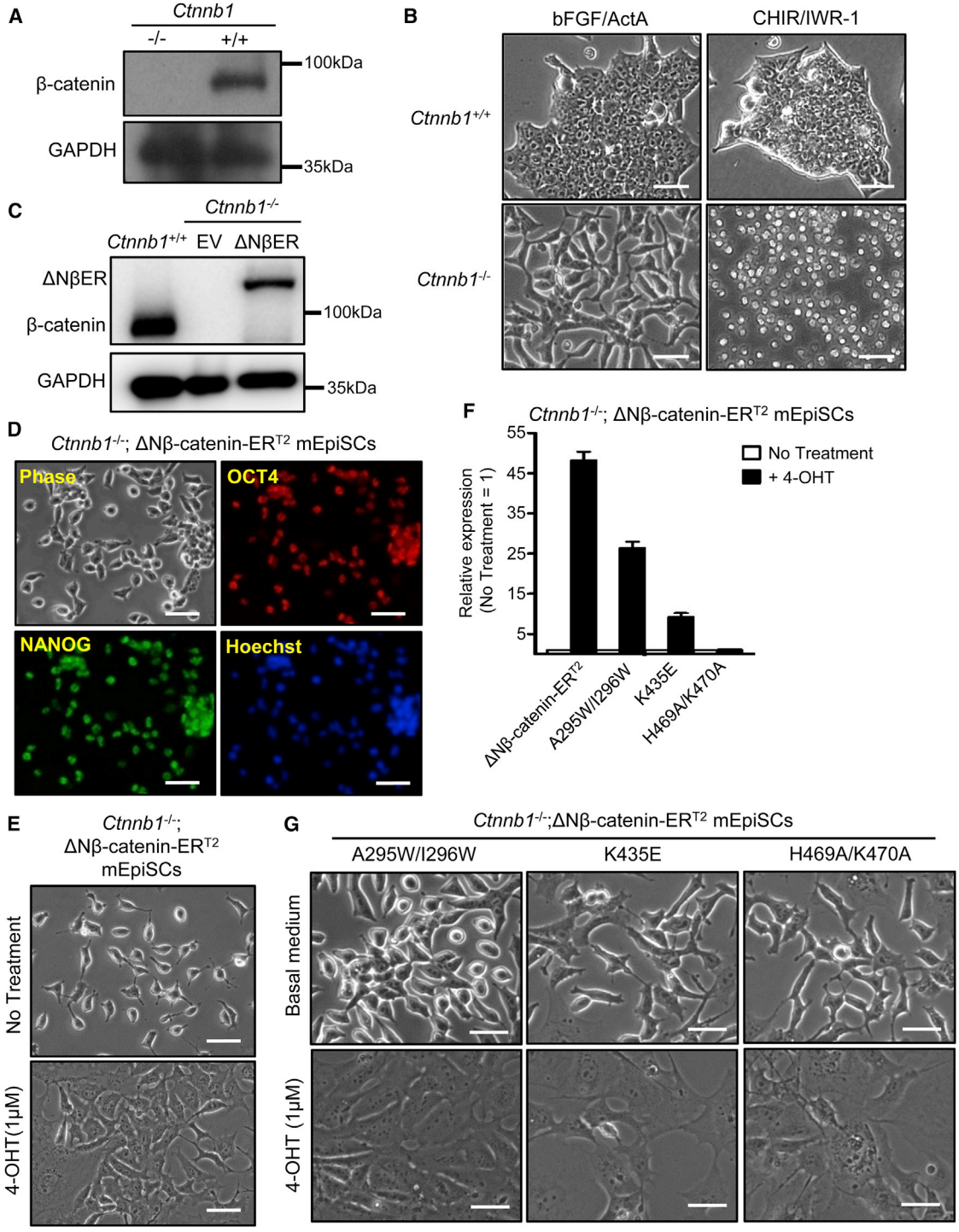

Figure 1. Cytoplasmic Retention of β-Catenin Is Both Required and Sufficient for CHIR/IWR-1-Mediated mEpiSC Self-Renewal

(A) Western blot analysis of β-catenin expression in *Ctnnb1*$^{+/+}$ and *Ctnnb1*$^{-/-}$ mEpiSCs.

(B) Representative images of *Ctnnb1*$^{+/+}$ and *Ctnnb1*$^{-/-}$ mEpiSCs cultured in bFGF/ActA or CHIR/IWR-1 for 7 days. Scale bars, 200 μm.

(C) Western blot analysis of β-catenin and ΔNβ-catenin-ER^{T2} (ΔNβER) expression in *Ctnnb1*$^{+/+}$ mEpiSCs and *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing empty vector (EV) or ΔNβ-catenin-ER^{T2} (ΔNβER).

(D) Representative phase contrast and immunofluorescent images of *Ctnnb1*$^{-/-}$; ΔNβ-catenin-ER^{T2} mEpiSCs cultured in basal medium for 47 days. Hoechst stains nuclei. Scale bars, 200 μm.

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blot (Figure 1A). *Ctnnb1*$^{-/-}$ mEpiSCs could be maintained in bFGF/ActA (Figure S1A) but failed to expand under CHIR/IWR-1 condition (Figure 1B), indicating that β-catenin is dispensable for mEpiSCs self-renewal under bFGF/ActA condition but required under CHIR/IWR-1 condition. Next, we overexpressed ΔNβ-catenin-ER^{T2} in *Ctnnb1*$^{-/-}$ mEpiSCs. Degradation of β-catenin is controlled by casein kinase 1α (CK1α) and GSK3β phosphorylation sites in its N terminus; therefore, truncation of the N terminus (ΔNβ-catenin) stabilizes β-catenin (Zhu and Watt, 1999). The fusion of ΔNβ-catenin with the mutant estrogen receptor α (ERα) ligand-binding domain (ER^{T2}) gives 4-hydroxytamoxifen (4-OHT), a synthetic ERα ligand, control of subcellular localization of the fusion protein (Feil et al., 1997). The expression level of ΔNβ-catenin-ER^{T2} in *Ctnnb1*$^{-/-}$;ΔNβ-catenin-ER^{T2} mEpiSCs was comparable with that of endogenous β-catenin in *Ctnnb1*$^{+/+}$ mEpiSCs (Figure 1C). Under bFGF/ActA condition without 4-OHT, ΔNβ-catenin-ER^{T2} remained in the cytoplasm (Kim et al., 2013). Upon 4-OHT treatment, ΔNβ-catenin-ER^{T2} translocated into the nucleus and activated β-catenin target genes (Figure S1B). Undifferentiated *Ctnnb1*$^{-/-}$;ΔNβ-catenin-ER^{T2} mEpiSCs were expanded for more than 20 passages without any exogenous cytokines or small molecules (Figures 1D and S1C). When treated with 4-OHT, these cells differentiated rapidly even with bFGF/ActA (Figure 1E). Taken together, these results indicate that cytoplasmic stabilization and retention of β-catenin is both necessary and sufficient to maintain mEpiSC self-renewal under CHIR/IWR-1.

Nuclear translocation and subsequent interaction of β-catenin with TCF/LEF1 transcription factors are the two critical steps in the activation of the canonical Wnt signaling pathway (Clevers and Nusse, 2012). Many Wnt target genes have been implicated in lineage commitment (Davidson et al., 2012). To investigate whether the interaction between β-catenin and TCF/LEF1 transcription factors is involved in nuclear β-catenin-induced mEpiSC differentiation, we generated ΔNβ-catenin-ER^{T2} mutants carrying point mutations that interrupt the interaction between β-catenin and TCF/LEF1 transcription factors. Specifically, we introduced A295W/I296W, K435E, or H469A/K470A mutation to abrogate the ability of β-catenin to interact with TCF3 (Graham et al., 2000), TCF4 (Graham et al., 2001), and TCF3/TCF4/LEF1 (Jin et al., 2008), respectively. We introduced these mutants into *Ctnnb1*$^{-/-}$ mEpiSCs (Figure S1D) and assessed the transactivation activity of these mutants by qPCR analysis of *Axin2* expression before and after 4-OHT treatment. In the absence of 4-OHT, *Ctnnb1*$^{-/-}$ mEpiSCs expressing ΔNβ-catenin-ER^{T2} or its mutants showed negligible expression levels of *Axin2,* a well-known β-catenin target gene (Jho et al., 2002). Treatment of 4-OHT in *Ctnnb1*$^{-/-}$;ΔNβ-catenin-ER^{T2} mEpiSCs for 4 hr induced 48.28 ± 1.99-fold increase of *Axin2* expression. The introduction of A295W/I296W or K435E mutation into ΔNβ-catenin-ER^{T2} reduced *Axin2* induction by 45.28% ± 0.03% and 80.92% ± 0.02%, respectively, while the introduction of H469A/K470A mutation essentially abolished its transactivation activity (Figure 1F). *Ctnnb1*$^{-/-}$ mEpiSCs expressing ΔNβ-catenin-ER^{T2} or any of its mutants remained undifferentiated in basal medium only (Figures S1C and S1E). The treatment of 4-OHT, surprisingly, resulted in rapid differentiation of cell lines expressing ΔNβ-catenin-ER^{T2} or its mutants, including the H469A/K470A mutant that lacks transactivation activity (Figures 1F and 1G). These results indicate that mEpiSC differentiation induced by nuclear translocation of β-catenin is independent of β-catenin's transactivation activity. This further implies that retention of β-catenin in the cytoplasm is the key for β-catenin-mediated mEpiSCs self-renewal.

TAZ Is a β-Catenin Binding Partner in the Cytoplasm

β-Catenin acts as a cytoskeletal protein on the cell membrane and a transcriptional co-activator in the nucleus (Clevers and Nusse, 2012). The function of β-catenin in the cytoplasm, however, remains largely unknown. We reasoned that cytoplasmic β-catenin might promote mEpiSC self-renewal by interacting with and/or modulating proteins that regulate self-renewal and differentiation. TGFβ/SMAD- and FGF/MAPK-mediated signals contribute to mEpiSC self-renewal. We sought to determine whether cytoplasmic β-catenin interacts with regulatory proteins in these two pathways to promote mEpiSC self-renewal. To this end, we investigated whether these two signaling pathways are activated by CHIR/IWR-1 in CD1 mEpiSCs (Kim et al., 2013). Interestingly, stimulation of CD1 mEpiSCs with CHIR and/or IWR-1 did not increase p-SMAD2 or p-ERK1/2 levels (Figure S2A), suggesting that

(E) Representative images of *Ctnnb1*$^{-/-}$; ΔNβ-catenin-ER^{T2} mEpiSCs cultured in bFGF/ActA (No Treatment) or bFGF/ActA plus 1 μM 4-OHT for 3 days. Scale bars, 200 μm.

(F) qPCR analysis of *Axin2* expression in *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing ΔNβ-catenin-ER^{T2} or ΔNβ-catenin-ER^{T2} carrying the indicated mutations. Cells were cultured in bFGF/ActA for 24 hr before treatment with or without 1 μM 4-OHT for 4 hr. Data represent means ± SEM of three independent experiments.

(G) Representative images of *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing ΔNβ-catenin-ER^{T2} carrying the indicated mutations and cultured in basal medium with or without 1 μM 4-OHT for 3 days. Scale bars, 200 μm.

See also Figure S1.

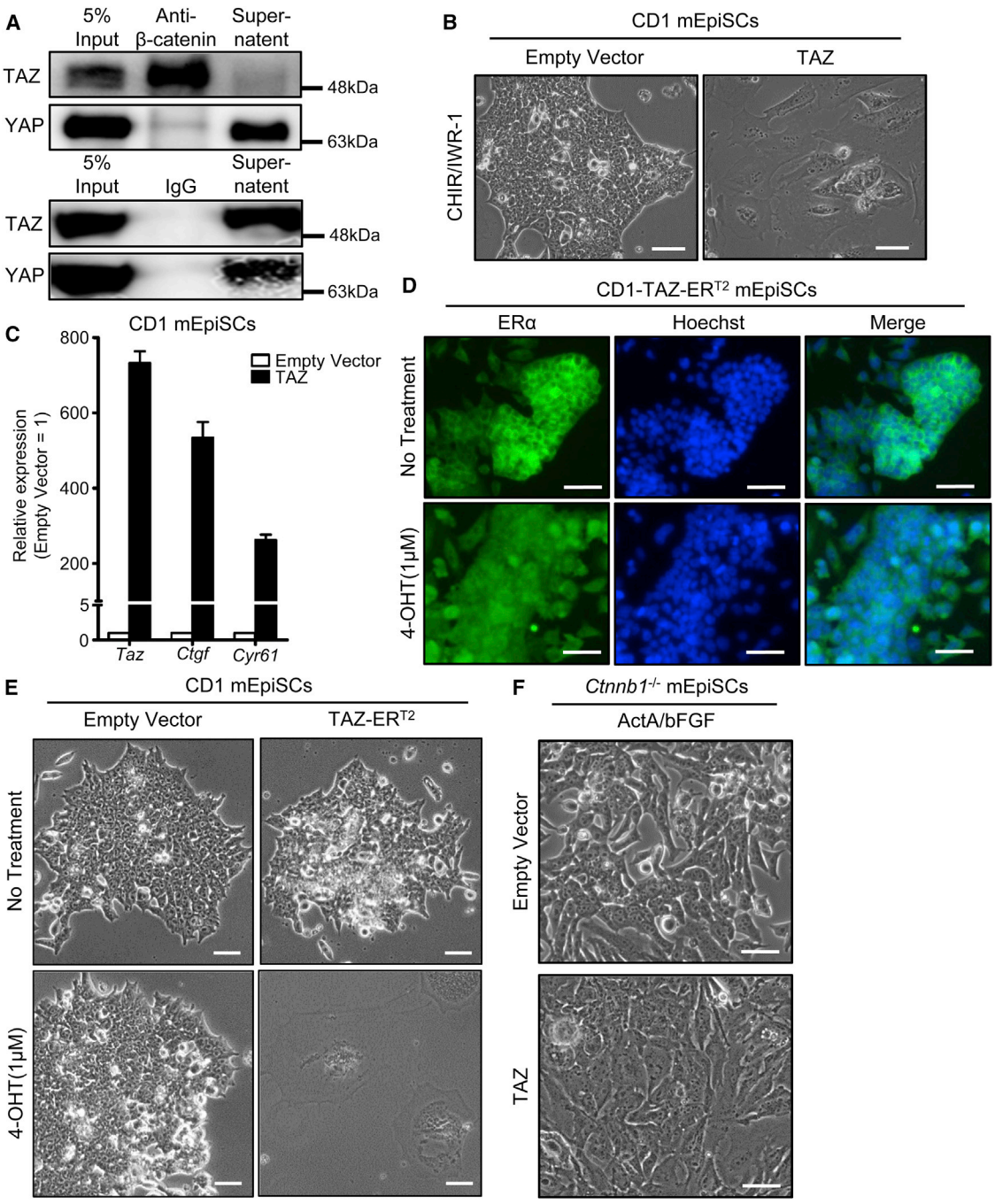

Figure 2. Nuclear Translocation of TAZ Induces mEpiSC Differentiation

(A) CoIP analysis of the interaction between β-catenin and TAZ/YAP in CD1 mEpiSCs cultured in CHIR/IWR-1. Whole-cell lysate was collected and incubated with β-catenin antibody or control IgG before western blot analysis.

(B) Representative images of CD1 mEpiSCs transfected with empty vector or TAZ transgene and cultured in CHIR/IWR-1 plus selection with 1 μg/mL puromycin for 3 days. Scale bars, 200 μm.

(C) qPCR analysis of *Taz*, *Ctgf*, and *Cyr61* expression in CD1 mEpiSCs transfected with empty vector or TAZ transgene. Data represent means ± SEM of three independent experiments.

(D) Representative immunofluorescent images of CD1-TAZ-ER^{T2} mEpiSCs cultured in CHIR/IWR-1 in the absence (No Treatment) or presence of 1 μM 4-OHT for 24 hr. Scale bars, 200 μm.

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TGFβ/SMAD and FGF/MAPK signaling pathways are unlikely the direct targets of cytoplasmic β-catenin. Next, we focused on YAP and TAZ, which have been shown to interact with β-catenin in the cytoplasm (Azzolin et al., 2012, 2014) and regulate the fate choice of various types of stem cells, including mESCs and hESCs (Chung et al., 2016; Lian et al., 2010; Ohgushi et al., 2015; Varelas et al., 2008). To determine whether YAP and TAZ are associated with cytoplasmic β-catenin, we performed co-immunoprecipitation (coIP) of β-catenin in CD1 mEpiSCs cultured in CHIR/IWR-1. The coIP result (Figure 2A) indicated that β-catenin formed a protein complex with TAZ, but not YAP. Interestingly, the association between β-catenin and TAZ was not detected in CD1 mEpiSCs cultured in bFGF/ActA (Figure S2B). Together, our results suggest that TAZ is a potential β-catenin binding partner in the cytoplasm of mEpiSCs under CHIR/IWR-1.

Nuclear Translocation of TAZ Induces mEpiSC Differentiation

Next, we investigated the function of TAZ in mEpiSC self-renewal. CD1 mEpiSC overexpressing TAZ differentiated rapidly so that no stable cell line could be established (Figure 2B). The expression levels of TAZ and its direct downstream targets, *Ctgf* and *Cyr61* (Lai et al., 2011), increased significantly in TAZ-transfected cells (Figure 2C). Similarly, overexpressing TAZ in 46C mEpiSCs converted from 46C mESCs (Ying et al., 2003) induced rapid differentiation under both CHIR/IWR-1 and bFGF/ActA conditions (Figure S2C).

To determine whether mEpiSC differentiation is induced by cytoplasmic or nuclear TAZ, we overexpressed TAZ-ER^{T2} in CD1 mEpiSCs (Figure S2D). TAZ-ER^{T2} fusion protein localized in the cytoplasm, and translocated into the nucleus to activate its downstream targets only in the presence of 4-OHT (Figures 2D and S2E). CD1 mEpiSCs overexpressing TAZ-ER^{T2} could be maintained in CHIR/IWR-1 but underwent rapid differentiation in the presence of 4-OHT (Figure 2E), indicating that nuclear translocation of TAZ induces mEpiSC differentiation.

Since β-catenin is a binding partner of TAZ (Figure 2A) and nuclear translocation of β-catenin induces mEpiSC differentiation (Figure 1E), we investigated whether β-catenin is necessary for TAZ-induced mEpiSC differentiation. We overexpressed TAZ in *Ctnnb1*$^{-/-}$ mEpiSCs and detected increased expression of both TAZ and its target genes, *Ctgf* and *Cyr61* (Figure S2F). Interestingly, *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing TAZ differentiated rapidly (Figure 2F), suggesting that TAZ-induced mEpiSC differentiation is independent of β-catenin.

TAZ Is Essential for the Conversion of mESCs to mEpiSCs

To further investigate the role of TAZ in mEpiSC maintenance, we generated TAZ knockout (*Wwtr1*$^{-/-}$) mESCs by CRISPR/Cas9. The loss of TAZ protein in *Wwtr1*$^{-/-}$ mESCs was confirmed by western blot (Figure 3A) and sequencing analysis (Figure S3A). *Wwtr1*$^{-/-}$ mESCs expanded efficiently under 2i (CHIR and PD0325901, a MEK inhibitor) plus leukemia inhibitory factor (LIF) condition (Wray et al., 2010; Ying et al., 2008) (Figure 3B). To convert *Wwtr1*$^{-/-}$ mESCs into mEpiSCs, we transferred these cells to bFGF/ActA plus CHIR/IWR-1 condition, following a standard protocol (Kim et al., 2013). Surprisingly, all transferred *Wwtr1*$^{-/-}$ mESCs died within 5 days (Figure 3B). In contrast, *Wwtr1*$^{-/-}$ mESCs overexpressing TAZ-ER^{T2} could be efficiently converted to mEpiSCs (Figures 3C, 3D, and S3B). Converted *Wwtr1*$^{-/-}$;TAZ-ER^{T2} mEpiSCs expanded robustly and remained undifferentiated in CHIR/IWR-1 (Figure S3C). These results indicate that TAZ is essential for the conversion of mESCs to mEpiSCs.

Cytoplasmic Retention of TAZ Promotes mEpiSC Self-Renewal in the Absence of Nuclear β-Catenin

To determine whether cytoplasmic retention of TAZ is sufficient for the maintenance of mEpiSCs, we cultured *Wwtr1*$^{-/-}$;TAZ-ER^{T2} mEpiSCs in basal medium with or without CHIR/IWR-1. *Wwtr1*$^{-/-}$;TAZ-ER^{T2} mEpiSCs remained undifferentiated in CHIR/IWR-1 but gradually differentiated after the removal of CHIR/IWR-1 (Figure S3C), suggesting that cytoplasmic retention of TAZ alone is not sufficient for the maintenance of mEpiSCs.

Since nuclear translocation of β-catenin is sufficient to induce mEpiSC differentiation, we investigated whether cytoplasmic retention of TAZ can promote mEpiSC self-renewal in the absence of nuclear β-catenin. First, we overexpressed TAZ-ER^{T2} in *Ctnnb1*$^{-/-}$ mEpiSCs (Figure 3E). *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing TAZ-ER^{T2} remained undifferentiated in basal medium only, whereas *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing empty vector rapidly differentiated (Figures 3F and S3D). *Ctnnb1*$^{-/-}$ mEpiSCs overexpressing either empty vector or TAZ-ER^{T2} rapidly differentiated in the presence of 4-OHT (Figure 3F). Together, these results indicate that cytoplasmic retention

(E) Representative images of CD1 mEpiSCs overexpressing empty vector or TAZ-ER^{T2} and cultured in CHIR/IWR-1 (No Treatment) or CHIR/IWR-1 plus 1 μM 4-OHT for 3 days. Scale bars, 200 μm.

(F) Representative images of *Ctnnb1*$^{-/-}$ mEpiSCs transfected with empty vector or TAZ transgene and cultured in bFGF/ActA plus selection with 1 μg/mL puromycin for 3 days. Scale bars, 200 μm.

See also Figure S2.

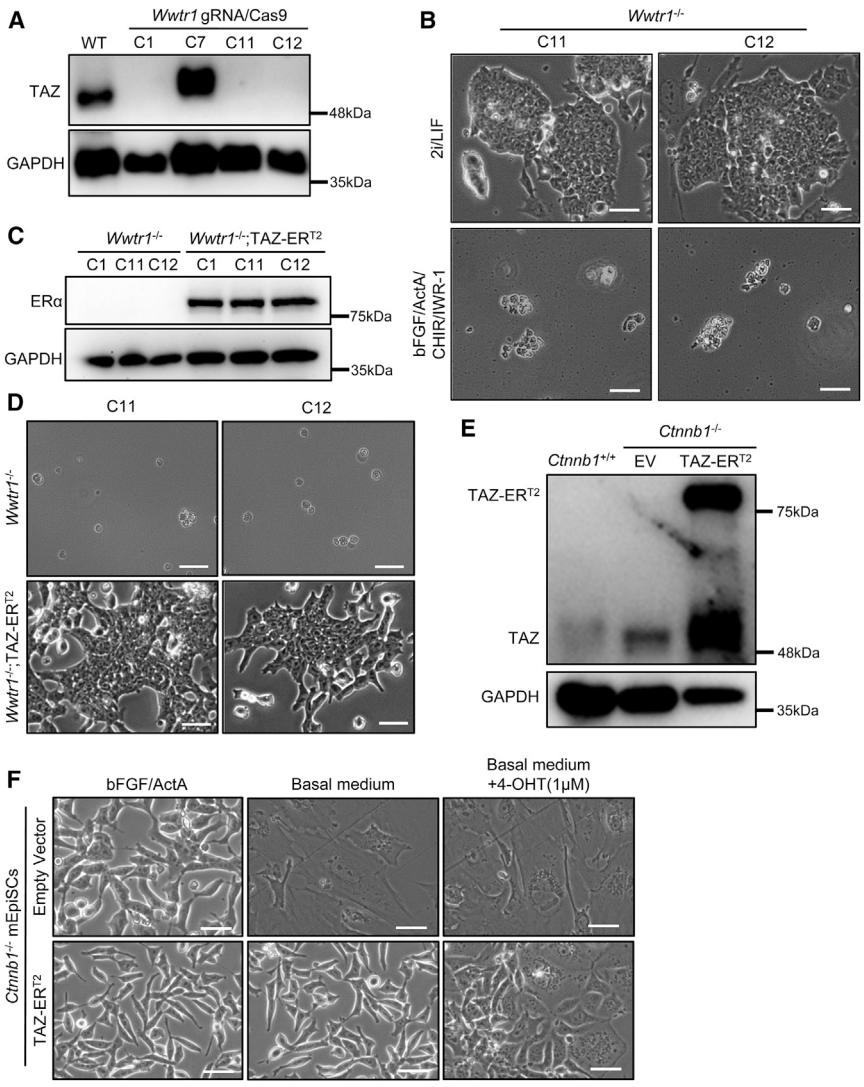

Figure 3. Cytoplasmic Retention of TAZ Promotes mEpiSC Self-Renewal

(A) Western blot analysis of TAZ expression in wild-type (WT) and *Wwtr1* gRNA/Cas9 transfected 46C mESCs. C1, C7, C11, and C12 were four individual clones. Clones C1, C11, and C12 were used for further experiments.

(B) Representative images of $Wwtr1^{-/-}$ mESCs cultured in 2i/LIF or bFGF/ActA/CHIR/IWR-1 for 5 days. Scale bars, 200 μm.

(C) Western blot analysis of TAZ-ER^{T2} expression in $Wwtr1^{-/-}$ and $Wwtr1^{-/-}$;TAZ-ER^{T2} mESCs.

(D) Representative images of $Wwtr1^{-/-}$ and $Wwtr1^{-/-}$;TAZ-ER^{T2} mESCs cultured in bFGF/ActA/CHIR/IWR-1 for 5 days. Scale bars, 200 μm.

(E) Western blot analysis of TAZ and TAZ-ER^{T2} expression in $Ctnnb1^{+/+}$ mEpiSCs and $Ctnnb1^{-/-}$ mEpiSCs overexpressing empty vector or TAZ-ER^{T2}.

(F) Representative images of $Ctnnb1^{-/-}$ mEpiSCs overexpressing empty vector or TAZ-ER^{T2} and cultured in bFGF/ActA, basal medium, or basal medium plus 1 μM 4-OHT for 14 days. Scale bars, 200 μm.

See also Figure S3.

of TAZ promotes mEpiSC self-renewal in the absence of β-catenin.

Next, we investigated whether cytoplasmic retention of TAZ can promote mEpiSC self-renewal when nuclear translocation of β-catenin is blocked by IWR-1. To this end, we cultured CD1-TAZ-ER^{T2} mEpiSCs in basal medium supplemented with either CHIR/IWR-1 or IWR-1 alone. Both CD1 mEpiSCs and CD1-TAZ-ER^{T2} mEpiSCs remained undifferentiated in CHIR/IWR-1 and rapidly differentiated after the removal of CHIR/IWR-1 (Figure S3E). Interestingly, in the presence of IWR-1 only, CD1-TAZ-ER^{T2} mEpiSCs expanded and remained undifferentiated, while CD1 mEpiSCs differentiated after passaging (Figure S3E). We also observed the same phenotype in 46C mEpiSCs and 46C-TAZ-ER^{T2} mEpiSCs (Figure S3F). Taken together, these results indicate that cytoplasmic retention of TAZ promotes mEpiSC self-renewal in the absence of nuclear β-catenin.

Cytoplasmic Retention of β-Catenin and TAZ Promotes hESC Self-Renewal

Previously, we demonstrated that hESC self-renewal can also be maintained through cytoplasmic stabilization and retention of β-catenin (Kim et al., 2013). We investigated whether cytoplasmic β-catenin promotes hESC self-renewal through modulation of TAZ. First, we overexpressed ΔNβ-catenin-ER^{T2} carrying A295W/I296W, K435E, or H469A/K470A mutation in HES2 hESCs (Reubinoff et al., 2000). HES2 hESCs overexpressing any of the ΔNβ-catenin-ER^{T2} mutants could be expanded continuously in basal medium only, and differentiated rapidly in the presence of 4-OHT (Figure 4A).

Next, we determined whether YAP/TAZ are β-catenin's binding partners in hESCs cultured in CHIR/IWR-1. In H9 hESCs (Thomson et al., 1998) cultured in CHIR/IWR-1, β-catenin was associated with TAZ, but not YAP, as

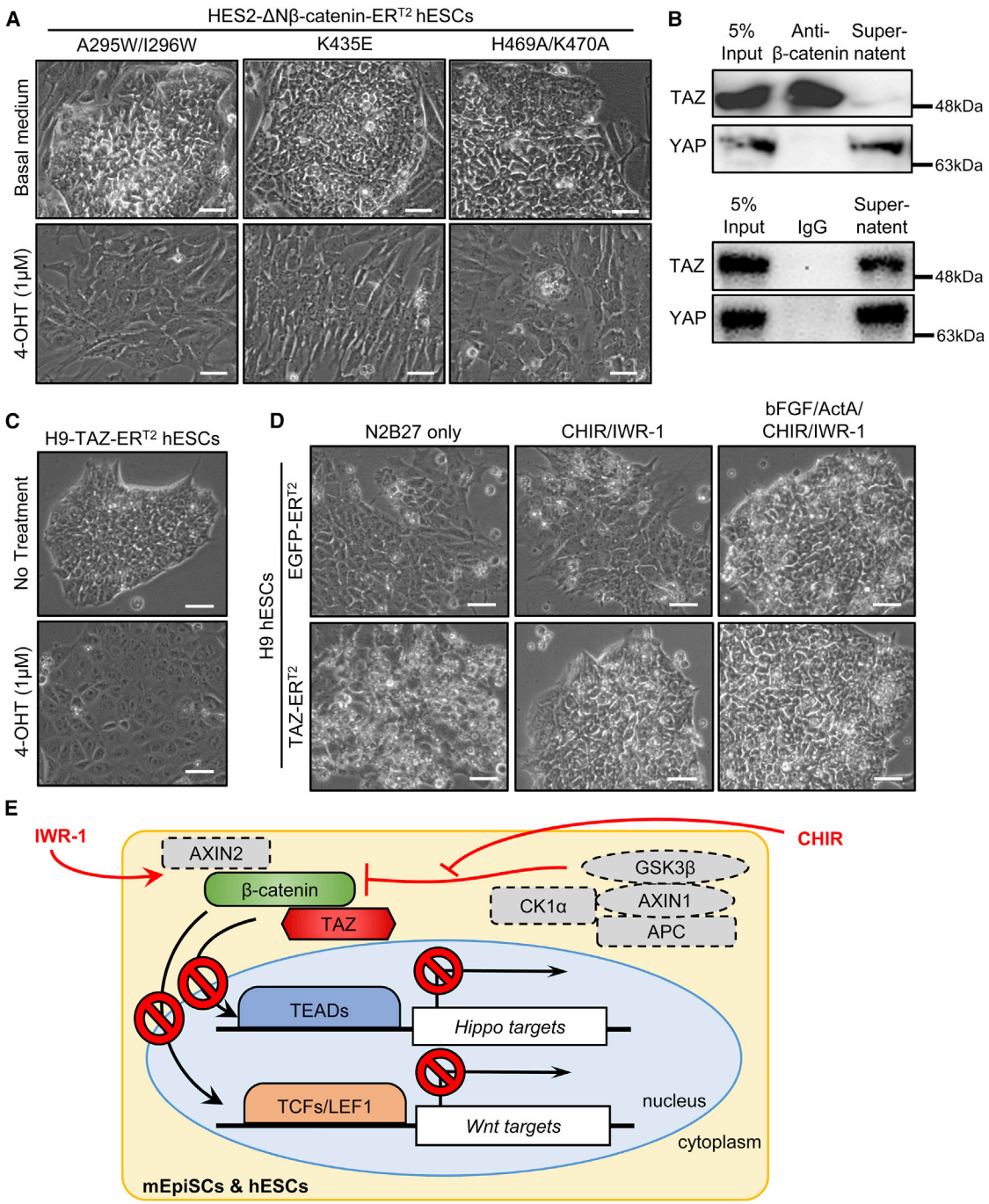

Figure 4. Cytoplasmic Retention of TAZ Promotes hESC Self-Renewal

(A) Representative images of HES2 hESCs overexpressing ΔN-β-catenin-ER^{T2} carrying the indicated mutations and cultured in basal medium or basal medium plus 1 μM 4-OHT for 7 days. Scale bars, 200 μm.

(B) CoIP analysis of the interaction between β-catenin and TAZ/YAP in H9 hESCs cultured in CHIR/IWR-1. Whole-cell lysate was collected and incubated with β-catenin antibody or control IgG before western blot analysis.

(C) Representative images of H9-TAZ-ER^{T2} hESCs cultured in mTeSR-1 (No Treatment) or mTeSR-1 plus 1 μM 4-OHT for 3 days. Scale bars, 200 μm.

(D) Representative images of H9 hESCs overexpressing EGFP-ER^{T2} or TAZ-ER^{T2} and cultured in the indicated conditions for 12 days. Scale bars, 200 μm.

(legend continued on next page)

demonstrated by coIP (Figure 4B). In H9 hESCs cultured in bFGF/ActA, association between β-catenin and TAZ was not detected (Figure S4A). These results are consistent with our findings in mEpiSCs (Figures 2A and S2B).

To define the role of TAZ in hESC self-renewal, we overexpressed EGFP-ER^{T2} or TAZ-ER^{T2} in H9 hESCs. In the absence of 4-OHT, both EGFP-ER^{T2} and TAZ-ER^{T2} localized in the cytoplasm, while 4-OHT treatment induced their nuclear translocation (Figure S4B). H9-EGFP-ER^{T2} hESCs remained undifferentiated in mTeSR-1 medium in the absence or presence of 4-OHT (Figure S4C). mTeSR-1 is a serum-free hESC culture medium supplemented with bFGF and TGFβ (Ludwig et al., 2006). H9-TAZ-ER^{T2} hESCs remained undifferentiated in mTeSR-1 but rapidly differentiated in the presence of 4-OHT (Figure 4C), suggesting that nuclear translocation of TAZ induces hESC differentiation. We also investigated whether cytoplasmic retention of TAZ can promote hESC self-renewal. To this end, H9-TAZ-ER^{T2} and H9-EGFP-ER^{T2} hESCs were cultured in serum-free N2B27 medium (Ying et al., 2003). In N2B27 medium only, both H9-EGFP-ER^{T2} and H9-TAZ-ER^{T2} hESCs rapidly differentiated (Figure 4D). In the presence of CHIR/IWR-1 and bFGF/ActA, both H9-EGFP-ER^{T2} and H9-TAZ-ER^{T2} hESCs remained undifferentiated (Figure 4D). In the presence of CHIR/IWR-1 alone, H9-TAZ-ER^{T2} hESCs could be expanded while remaining undifferentiated, whereas H9-EGFP-ER^{T2} hESCs differentiated after passaging (Figure 4D). These results suggest that cytoplasmic retention of TAZ also promotes hESC self-renewal when nuclear translocation of β-catenin is blocked.

DISCUSSION

In this study, we demonstrated that cytoplasmic retention of TAZ promotes mEpiSC and hESC self-renewal in the absence of nuclear β-catenin, whereas nuclear translocation of TAZ induces differentiation (Figure 4E). The role of TAZ in pluripotent stem cell maintenance has been controversial (Beyer et al., 2013; Ohgushi et al., 2015; Varelas et al., 2008). Our findings that the function of TAZ is dictated by its subcellular localization and also dependent on cell types provide a rational explanation for the diverse and sometimes opposite effects of TAZ observed in different contexts.

Several reports (Beyer et al., 2013; Varelas et al., 2008) claimed that nuclear TAZ promotes hESC self-renewal through interaction with p-SMAD2/SMAD4 and OCT4. In our study, however, p-SMAD2 was not detectable in mEpiSCs under CHIR/IWR-1 condition (Figure S2A). We did detect an interaction between β-catenin and TAZ by coIP in mEpiSCs and hESCs cultured in CHIR/IWR-1 (Figures 2A, S2B, 4B, and S4A). How this interaction happens, however, remains unknown. Previously, it has been shown that AXIN1 forms a protein complex with both β-catenin and TAZ (Azzolin et al., 2014; Li et al., 2012). In mEpiSCs, AXIN2 acts to anchor and retain β-catenin in the cytoplasm (Kim et al., 2013). It would be of great interest to investigate whether AXIN1/AXIN2 serve as a scaffold protein to mediate β-catenin-TAZ interaction in mEpiSC and hESCs cultured in CHIR/IWR-1.

The role of β-catenin localized on the cell membrane or in the nucleus has been well documented (Clevers and Nusse, 2012). Our study reveals a function of cytoplasmic β-catenin: regulating primed pluripotent stem cell fate through interaction with and retention of TAZ in the cytoplasm. The roles of β-catenin and TAZ in mESCs and mEpiSCs are distinct, even opposite, under certain conditions. For instance, nuclear translocation of β-catenin promotes mESC self-renewal (Wray et al., 2010; Ying et al., 2008) but induces differentiation in mEpiSCs (Figures 1E and 1G). Similarly, TAZ is not required for mESC self-renewal but is essential for ESC-to-EpiSC conversion (Figures 3B and 3D). Given the similarities between the recently established naive hESCs (Takashima et al., 2014; Theunissen et al., 2014; Zhou et al., 2015) and mESCs, it would be of great interest to investigate whether TAZ's role in regulating naive-to-primed pluripotency conversion is conserved between mouse and human.

Our finding that cytoplasmic retention of TAZ and β-catenin promotes mEpiSC and hESC self-renewal extends our understanding of the molecular mechanisms underlying pluripotency maintenance. This might advance our efforts in better controlling stem cell fate for future applications in regenerative medicine. Furthermore, our finding that cytoplasmic β-catenin and TAZ exerts functional roles opens a new research avenue that can lead to a paradigm shift in studying signal transduction.

EXPERIMENTAL PROCEDURES

Ctnnb1$^{-/-}$ mESCs were maintained on mouse embryonic fibroblasts (MEFs) in serum-containing medium supplemented with LIF (10 ng/mL, prepared in house) and PD0325901 (1 μM, synthesized in the Division of Signal Transduction Therapy, University of Dundee, UK). mEpiSCs were cultured in serum-containing

(E) Working model for cytoplasmic β-catenin and TAZ-mediated mEpiSC and hESC self-renewal. TAZ is a binding partner of cytoplasmic β-catenin in mEpiSCs and hESCs cultured in CHIR/IWR-1. Nuclear translocation of either β-catenin or TAZ induces mEpiSC and hESC differentiation. Cytoplasmic retention of TAZ promotes mEpiSC and hESC self-renewal in the absence of nuclear β-catenin.
See also Figure S4.

medium supplemented with CHIR (1.5 μM, synthesized in the Division of Signal Transduction Therapy, University of Dundee, UK) and IWR-1 (2.5 μM, Sigma). mESCs were converted to mEpiSCs in serum-containing medium supplemented with basic fibroblast growth factor (bFGF, 20 ng/mL; PeproTech), ActA (20 ng/mL, PeproTech), CHIR, and IWR-1. *Ctnnb1*$^{-/-}$ mESCs were converted to mEpiSCs on MEFs in serum-containing medium supplemented with bFGF, ActA, and Y-27632 (10 μM, Sigma). hESCs were propagated on MEFs in bFGF-supplemented medium or on Matrigel (Corning)-coated plates in mTeSR-1 medium (STEMCELL Technologies).

Additional experimental procedures are provided in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2017.07.019.

AUTHOR CONTRIBUTIONS

X.Z. designed the study, performed the experiments and data analysis, and prepared the manuscript. J.P.C. constructed plasmids and prepared the manuscript. B.R. constructed plasmids. Q.-L.Y. conceived the study, prepared the manuscript, and supervised the study.

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