**FIGURE AND TABLE LEGENDS**

**Figure 1. Everolimus treatment activates the canonical TGFβ pathway**

1. Rapalogues enhance phosphorylation of Smad2 and Smad3. BON1 cells were treated with rapamycin or Everolimus (mTORC1 inhibitors) for 3 days. Smad2-pS465/467, Smad3-pS423/425 and p70-S6K-pT389 were monitored by immunoblot. Data represent 3 biological replicates.
2. Quantification of data shown in (A). S6K-pT389, Smad2-pS465/467 and Smad3-pS423/425 levels were compared between control and rapamycin or Everolimus- treated cells using a two-tailed student’s t-test across 3 biological replicates. Data represent the mean ± SEM. \*,p≤0.05; \*\*, p ≤ 0.01; \*\*\*, p ≤ 0.001.
3. mTORC1 inhibition enhances phosphorylation of Smad2 and Smad3. shControl and shRaptor BON1 cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Raptor, Smad2-pS465/467 and Smad3-pS423/425 were monitored by immunoblot. Data represent 3 biological replicates.
4. Quantification of data shown in (C). Raptor, Smad2-pS465/467 and Smad3-pS423/425 levels were compared between shControl and BON1 cells using a two-tailed student’s t-test across 3 biological replicates. Data represent the mean ± SEM. \*,p≤0.05; \*\*, p ≤ 0.01; \*\*\*, p ≤ 0.001.
5. Everolimus enhances the translocation of Smad2 and Smad3 to the nucleus. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days. Samples were separated into cytosolic and nuclear fractions. Smad2-pS465/467, Smad3-pS423/425 and p70-S6K-pT389 were monitored by immunoblot. Data represent 3 biological replicates.
6. Quantification of data shown in (E). Nuclear Smad2 and Smad3 levels were compared between the different conditions using a two-tailed student’s t-test across 3 biological replicates. Data represent the mean ± SEM. \*,p≤0.05.
7. Everolimus enhances phosphorylation and nuclear localization of Smad2. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days. Next, cells were embedded in paraffin and stained against Smad2/ Smad3. Data represent 3 biological replicates. Scale bar represents 100 μm.

**Figure 2. Computational modelling predicts that, upon Everolimus treatment, Akt activates Smad2 while ERK inhibits it**

1. Everolimus enhances Smad2 phsophorylation in BON1 but not in QGP1 cells. BON1 and QGP1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days. Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Smad2-pS465/467, p70-S6K-pT389, Akt-pT308 and ERK-pT202/Y204 were monitored by immunoblot. Data represent 4 biological replicates.
2. Quantification of data shown in (A). Smad2-pS465/467, Akt-pT308 and ERK-pT202/Y204 levels were compared between TGFβ and TGFβ + Everolimus- treated cells using a two-tailed student’s t-test across 4 biological replicates. Data represent the mean ± SEM. ns, not-significant; \*,p≤0.05; \*\*, p ≤ 0.01.
3. Simulated response of Smad2-pS465/467 to Akt or MEK inhibition with the different models generated shown as representative schemes. In the graphs, blue dots represent experimental data from MK2206 (Akt inhibition) time course in the presence of Everolimus (mTORC1 inhibition) (SupplementaryFig. S1A,B) and orange dots represent experimental data from AZD2206 (MEK inhibition) time course in the presence of Everolimus (Supplementary Fig. S1C,D). Data represent the mean ± SEM. Lines represent computational simulation. The corresponding AICc values are indicated at the bottom of each model. Simulations of all used observables are shown in Supplementary Figures S3 and S4 . The corresponding AIC value is indicated at the top of each model.
4. Everolimus-enhanced Smad2 phosphorylation is Akt dependent. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of MK2206 (Akt inhibitor), AZD6244 (MEK inhibitor) or MK2206 + AZD6244 for the indicated time points. Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Smad2-pS465/467, Akt-pT308, TSC2-pT1462, p70-S6K-pT389 and ERK-pT202/Y204 were monitored by immunoblot. Data represent 4 biological replicates.
5. Quantification of data shown in (E). Smad2-pS465/467 levels were compared between the different conditions using a two-tailed student’s t-test across 4 biological replicates. Data represent the mean ± SEM. \*,p≤0.05; \*\*, p ≤ 0.01.
6. Prediction on the effect of Akt and MEK inhibition on Smad2-pS465/467. Dots represent the experimental data (D), shown as mean ± SEM. The line is the prediction performed by model 2.1. Predictions of models 2.2 and 2.3 is shown in Supplementary Figure S5.

**Figure 3. Smad2 phosphorylation is mediated by Akt**

1. Everolimus-enhanced Smad2 phosphorylation is Akt dependent. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of Akt1 and Akt2 knockdown (siAkt1/2). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Akt1, Akt2, Smad2-pS465/467, p70-S6K-pT389 and ERK-pT202/Y204 were monitored by immunoblot. Data represent 4 biological replicates.
2. Quantification of data shown in (A). Akt1, Akt and Smad2-pS465/467 levels were compared between the different conditions using a two-way ANOVA followed by a Bonferroni multiple comparison test across 4 biological replicates. Data represent the mean ± SEM. The p-values for the Bonferroni multiple comparison tests are shown. \*,p≤0.05; \*\*, p ≤ 0.01; \*\*\*, p ≤ 0.001.
3. Everolimus-enhanced Smad2 phosphorylation is PI3K dependent. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of GDC0941 (PI3K inhibitor). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Smad2-pS465/467, Akt-pT308, p70-S6K-pT389 and ERK-pT202/Y204 were monitored by immunoblot. Data represent 5 biological replicates.
4. Quantification of data shown in (C). Akt-pT308 and Smad2-pS465/467 levels were compared between the different conditions using a one-way ANOVA followed by a Bonferroni multiple comparison test across 5 biological replicates. Data represent the mean ± SEM. The p-values for the Bonferroni multiple comparison tests are shown. \*,p≤0.05; \*\*, p ≤ 0.01; \*\*\*, p ≤ 0.001.
5. Everolimus-enhanced Smad2 phosphorylation is TSC2-independent. Control and TSC2 knock out (TSC2 K.O.) BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of MK2206 (Akt inhibitor). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. TSC2, Smad2-pS465/467, Akt-pT308, p70-S6K-pT389 and ERK-pT202/Y204 were monitored by immunoblot. Data represent 3 biological replicates.
6. Quantification of data shown in (E). Akt-pT308 and Smad2-pS465/467 levels were compared between the different conditions using a two-way ANOVA followed by a Bonferroni multiple comparison test across 3 biological replicates. Data represent the mean ± SEM. The p-values for the Bonferroni multiple comparison tests are shown. ns, not-significant.

**Figure 4. Smad3 phosphorylation is mediated by ERK**

1. Everolimus-enhanced Smad3 phosphorylation is Akt independent. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of MK2206 (Akt inhibitor). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Smad3-pS423/425, Akt-pT308, TSC2-pT1462 and p70-S6K-pT389 were monitored by immunoblot. Data represent 4 biological replicates.
2. Quantification of data shown in (A). Smad3-pS423/425 levels were compared with a two-tailed student’s t-test across 4 biological replicates. Data represent the mean ± SEM. \*,p≤0.05.
3. Everolimus-enhanced Smad3 phosphorylation is MEK/ERK dependent. BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of AZD6244 (MEK inhibitor) or SCH772984 (ERK inhibitor). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. Smad3-pS423/425, ERK-pT202/Y204 and p70-S6K-pT389 were monitored by immunoblot. Data represent 4 biological replicates.
4. Quantification of data shown in (C). Smad3-pS423/425 levels were compared with a two-tailed student’s t-test across 4 biological replicates. Data represent the mean ± SEM. \*,p≤0.05.
5. Everolimus-enhanced Smad3 phosphorylation is TSC2-independent. Control and TSC2 knock out (TSC2 K.O.) BON1 cells were treated with Everolimus (mTORC1 inhibitor) for 3 days in the presence or absence of AZD6244 (MEK inhibitor) or SCH772984 (ERK inhibitor). Subsequently, cells were serum starved for one hour and stimulated with TGFβ for 45 minutes. TSC2, Smad3-pS423/425, ERK-pT202/Y204 and S6K-pT389 were monitored by immunoblot. Data represent 3 biological replicates.
6. Quantification of data shown in (E). Akt-pT308 and Smad3-pS423/425 levels were compared between the different conditions using a two-way ANOVA followed by a Bonferroni multiple comparison test across 3 biological replicates. Data represent the mean ± SEM. The p-values for the Bonferroni multiple comparison tests are shown. ns, not-significant.

**Figure 5. Everolimus activates the TGFβ-XXX axis to promote cell growth**

1. Growth analysis of BON1 cells upon mTORC1 and TGFβR inhibition. BON1 NucLight cells were cultured in the presence of Everolimus (mTORC1 inhibitor), LY2157299 (TGFβR inhibitor) or Everolimus + LY2157299. The nuclear signals were measured over 100 hours by live cell imaging. Data are shown as the mean ± SEM of 3 biological replicates.
2. Statistical analysis of data shown in (A). Area under the curves (AUC) were calculated for each biological replicate. The AUC was compared between the different conditions using a two-tailed student’s t-test across 3 biological replicates. Data represent the mean ± SEM. ns, not-significant; \*,p≤0.05; \*\*, p ≤ 0.01.
3. Representative images of cells measured in (A) after 70 hours.
4. Analysis of TGFβ-dependent gene expression upon Everolimus treatment. BON1 cells were treated with Everolimus (mTORC1 inhibitor) and/or LY2157299 (TGFβR inhibitor) for 3 days. Subsequently, RNA was extracted and RNAseq analysis was performed. The genes indicated are the ones which show an effect on the combinatorial treatment (Everolimus + LY2157299) significantly different from the additive effect of the single treatments of Everolimus or LY2157299. Changes in expression are shown as the log2 fold changes to the carrier (DMSO).
5. Analysis of TGFβ-dependent protein expression upon Everolimus treatment. BON1 cells were treated with Everolimus and/or LY2157299 for 3 days. BMPR2, GRID1, ID1, MAP4K4, NOV, PRSS23 and ZDHHC8P1 were monitored by immunoblot. Data represent 5 biological replicates.
6. Quantification of data shown in (E). BMPR2, GRID1, ID1, MAP4K4, NOV, PRSS23 and ZDHHC8P1 levels were compared between the different conditions using a one-way ANOVA followed by a Bonferroni multiple comparison test across 5 biological replicates. Data represent the mean ± SEM. The p-values for the Bonferroni multiple comparison tests are shown. \*\*, p ≤ 0.01.
7. Growth analysis of BON1 cells upon mTORC1 and XXX. BON1 NucLight cells were cultured in the presence of Everolimus (mTORC1 inhibitor), XXXX. The nuclear signals were measured over 100 hours by live cell imaging. Data are shown as the mean ± SEM of 3 biological replicates.
8. Statistical analysis of data shown in (G). Area under the curves (AUC) were calculated for each biological replicate. The AUC was compared between the different conditions using a two-tailed student’s t-test across 3 biological replicates. Data represent the mean ± SEM. ns, not-significant; \*,p≤0.05; \*\*, p ≤ 0.01.
9. Representative images of cells measured in (G) after 70 hours.

**Figure 6. In vivo study shows that Everolimus activates the TGFβ pathway to promote cell growth**