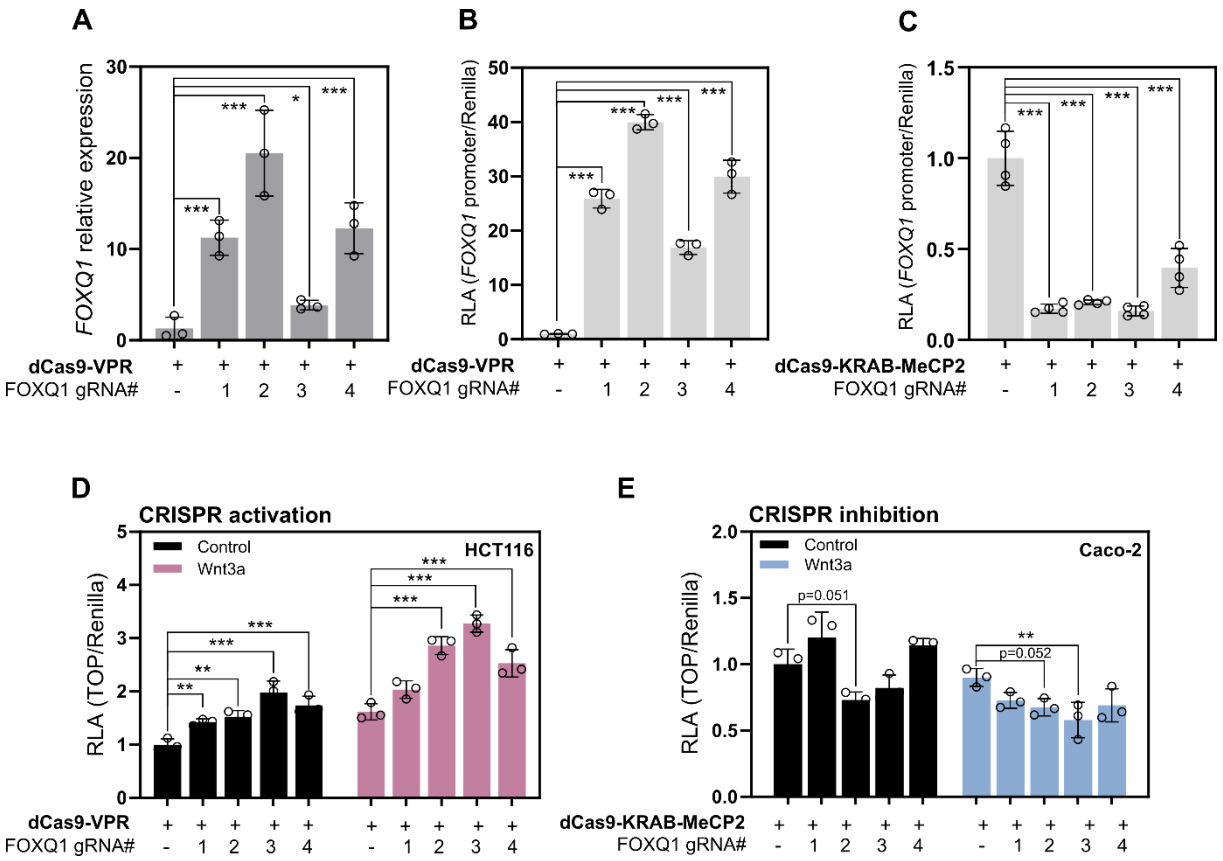


Fig. S1. FOXQ1 is a physiological activator of Wnt/β-catenin signalling.

(A) *FOXQ1* qPCR in 293T cells. *FOXQ1* expression was significantly induced by a dCas9-VPR (CRISPR activation) construct guided to the *FOXQ1* promoter by four non-overlapping guide RNAs. Data are displayed as fold change compared to empty vector control and show biological triplicates. (B-C) Luciferase assay showing that CRISPR-mediated *FOXQ1* induction or inhibition activated (B) or repressed (C) a FOXQ1 promoter reporter construct, respectively. (D) TOPflash reporter assay showing that CRISPR-mediated induction of FOXQ1 activated Wnt/β-catenin signalling in HCT116 cells. Data show one representative of n = 2 independent experiments with biological triplicates. (E) TOPflash reporter assay in Caco-2 cells. CRISPR-mediated inhibition of FOXQ1 decreased the reporter activity. Data represent an independent experiment with biological triplicates.

Data are displayed as mean ± SD. Statistical significance was determined by ANOVA with Dunnett´s post-hoc test (A-E), and defined as **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

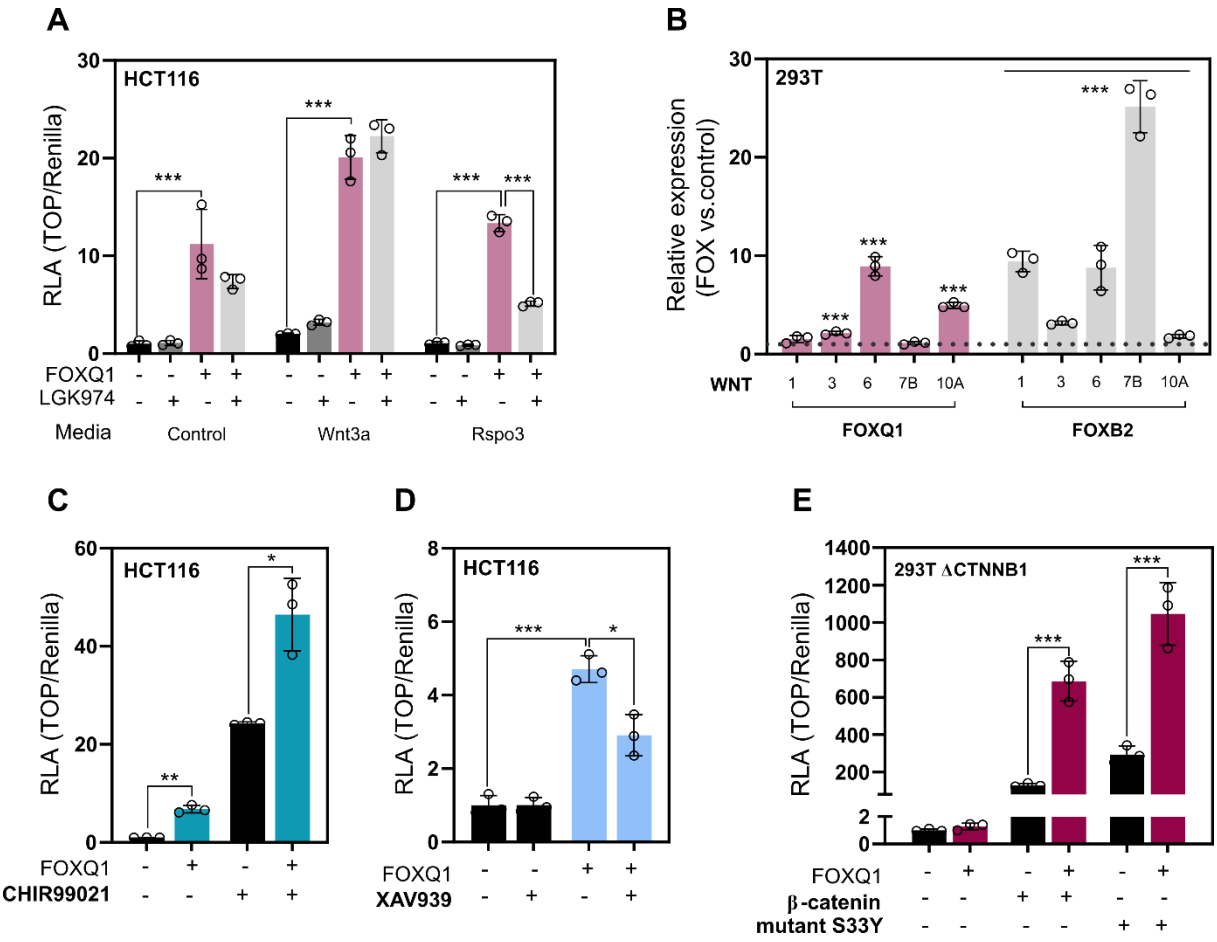


Fig. S2. FOXQ1 requires upstream pathway activation and β-catenin stabilisation to promote Wnt signalling.

(A) TOPflash assay in HCT116 cells in the presence of LGK974 (10 nM). Treatment with LGK974 attenuated FOXQ1-dependent Wnt activation, especially in the presence of exogenous R-spondin 3. Data show one representative of n = 2 independent experiments with biological triplicates. (B) qPCR of relevant Wnt ligands for comparison of FOXQ1 with FOXB2. Data are displayed as fold change compared to empty vector control and show biological triplicates. (C) TOPflash assay in HCT116 cells upon treatment with CHIR99021 (5 μM). FOXQ1 strongly potentiated Wnt signalling in synergy with CHIR99021. Data show one representative of n = 3 independent experiments with biological triplicates. (D) TOPflash assay in HCT116 cells upon treatment with XAV939 (5 μM). FOXQ1-dependent Wnt reporter activity was reduced upon β-catenin de-stabilization by XAV939. Data show one representative of n = 3 independent experiments with biological triplicates. (E) TOPflash assay in 293T cells lacking β-catenin. FOXQ1 did not activate the β-catenin/TCF reporter in the absence of β-catenin. However,

FOXQ1 synergized with exogenous wild-type and constitutively active β-catenin S33Y to activate Wnt signalling. Data show one experiment with biological triplicates.
Data are displayed as mean ± SD. Statistical significance was calculated by ANOVA with Tukey's post-hoc test (A, D, E) or Welch´s t-test (B, C), and defined as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

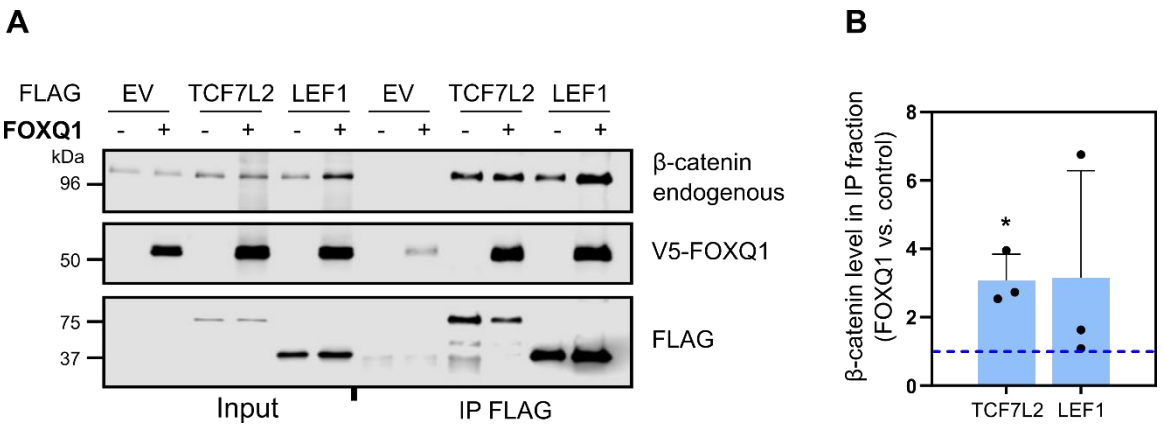
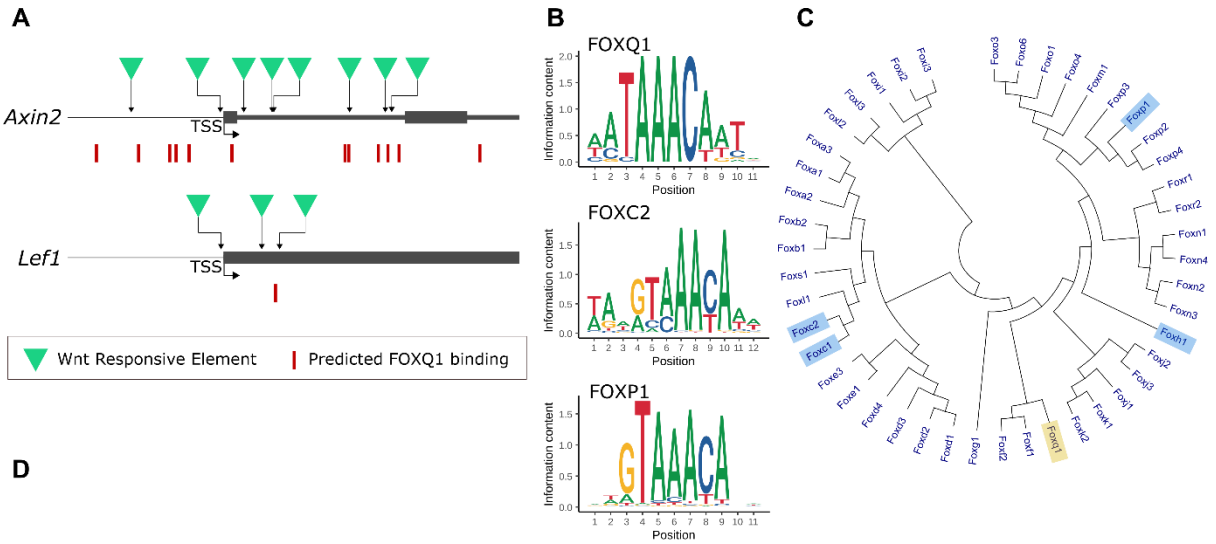


Fig. S3. FOXQ1 interacts with the Wnt transcriptional complex.

(A) Co-immunoprecipitation of Flag-tagged TCF7L2 and LEF1 from nuclear lysates of Wnt3a-treated 293T cells. Where indicated, cells were transfected with V5-FOXQ1. Following Flag pull-down, FOXQ1 and endogenous β-catenin were detected by immunoblotting. Data are representative of n = 3 independent experiments. (B) Relative abundance of β-catenin associated with TCF7L2 and LEF1 from the previous immunoprecipitation experiments. FOXQ1 significantly increased β-catenin association with TCF7L2.

Data are displayed as mean ± SD. Statistical significance was calculated by Welch´s t-test, and defined as $*P < 0.05$.



D

FOX TF	Cell/Tissue	Axin2	Sp5	Lef1	Nkd1	Lgr5	Dkk1	Reference	PubMed	Data available
A1	Mouse embryonic fibroblasts (MEFs)	✗	✗	✗	✗	✗	✗	Horisawa et al. 2020	32755593	GEO:GSE143444
A2	Mouse embryonic fibroblasts (MEFs)	✗	✗	✗	✗	✗	✗	Horisawa et al. 2020	32755593	GEO:GSE143444
A3	Mouse embryonic fibroblasts (MEFs)	✗	✗	✗	✗	✗	✗	Horisawa et al. 2020	32755593	GEO:GSE143444
C1	Adult mouse kidney glomeruli	✓	✗	✓	✗	✗	✗	Yang et al. 2021	33771836	GEO:GSE116302
C2	Adult mouse kidney glomeruli	✓	✗	✗	✗	✗	✗	Yang et al. 2021	33771836	GEO:GSE116302
K1	Myoblasts, cell line C2C12	✗	✗	✓	✗	✗	✗	Bowman et al. 2014	25402684	GEO:GSE56932
F1	Mouse embryonic lung, E18.5	✓	✓	✓	✓	✗	✗	Dharmadhikari et al. 2016	27638768	GEO:GSE77951
F2	Mouse embryonic palate cells, E13.5	✗	✗	✗	✗	✗	✗	Xu et al. 2020	32040930	GEO:GSE137585
G1	Mouse cortex, E14-15	✗	✗	✗	✗	✗	✗	Godbole et al. 2018	29229772	GEO:GSE96070
H1	Mouse embryonic stem cells	✓	✓	✓	✓	✓	✓	Aragón et al. 2019	31582430	GEO:GSE125116
O4	Differentiated TH1 cells	✗	✗	✗	✗	✗	✗	-	-	GEO:GSE133034
P1	Embryonic neural stem cells	✓	✓	✓	✓	✓	✓	Braccioli et al. 2017	29141232	GEO:GSE101632
P3	CD4+ Treg cells	✗	✗	✗	✗	✗	✗	Hayatsu et al. 2017	28778586	GEO:GSE89743
O1	CD4+ cells	✗	✗	✗	✗	✗	✗	Liao et al. 2014	25302145	GEO:GSE60470

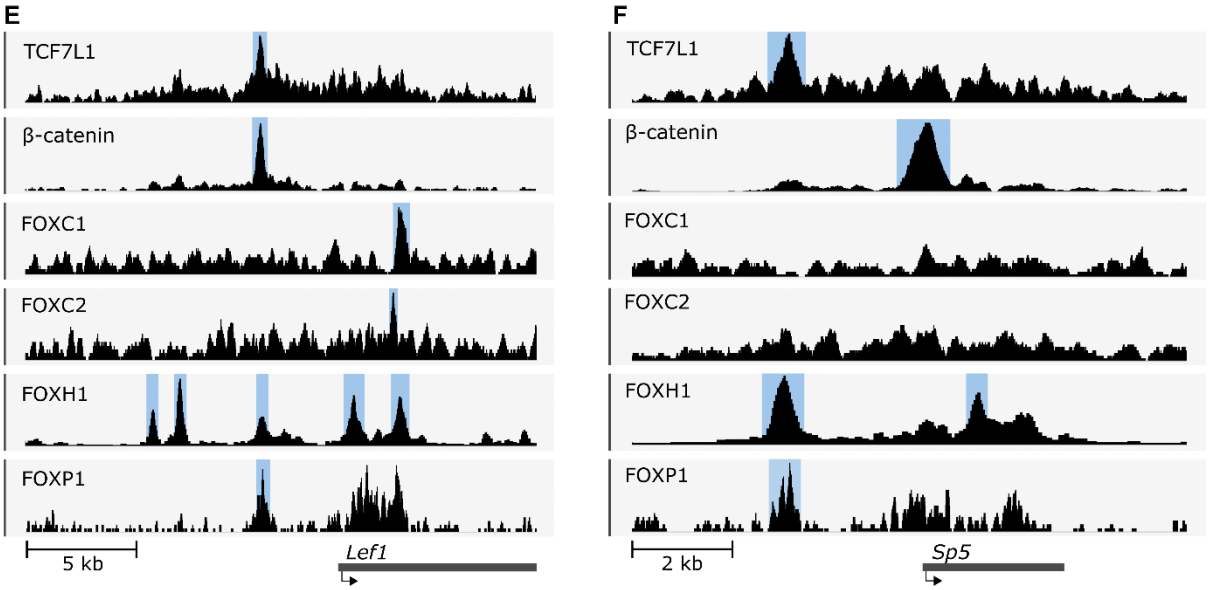


Fig. S4. FOX transcription factors bind at known Wnt target genes.

(A) Prediction of FOXQ1 binding sites at the mouse *Axin2* and *Lef1* loci, based on JASPAR 2022 binding profile data. Green triangles denote Wnt-responsive elements (WREs) required for TCF/LEF binding, as previously identified (Jho et al., 2002; Li et al., 2006). Red rectangles denote predicted FOXQ1 binding sites. (B) Sequence logo displaying the FOXQ1, FOXC2, FOXP1 consensus DNA-binding motif from the JASPAR database. (C) Phylogenetic tree of FOX transcription factors. Phylogenetic relationship between factors were determined based on their Forkhead box sequences. Highlighted in blue are FOX factors for which ChIP-seq genomic tracks are displayed. (D) Table of FOX transcription factors for which ChIP-seq data has been obtained, including references to dataset repositories and associated publications. Blue check symbol denotes the presence of a called ChIP-seq peak at the promoter region of the corresponding gene. Gray cross symbol denotes the absence of a binding event. Note: for FOXH1 and FOXP1, only sequence coverage data (i.e., no peak calling data) were found, and the presence of binding events at gene promoters was assessed by visual inspection of these signalling tracks. (E, F) Genomic tracks showing protein-DNA binding enrichment of TCF7L1, β-catenin, FOXC1, FOXC2, FOXH1 and FOXP1 at the *Lef1* and *Sp5* loci, obtained from publicly available ChIP-seq datasets.

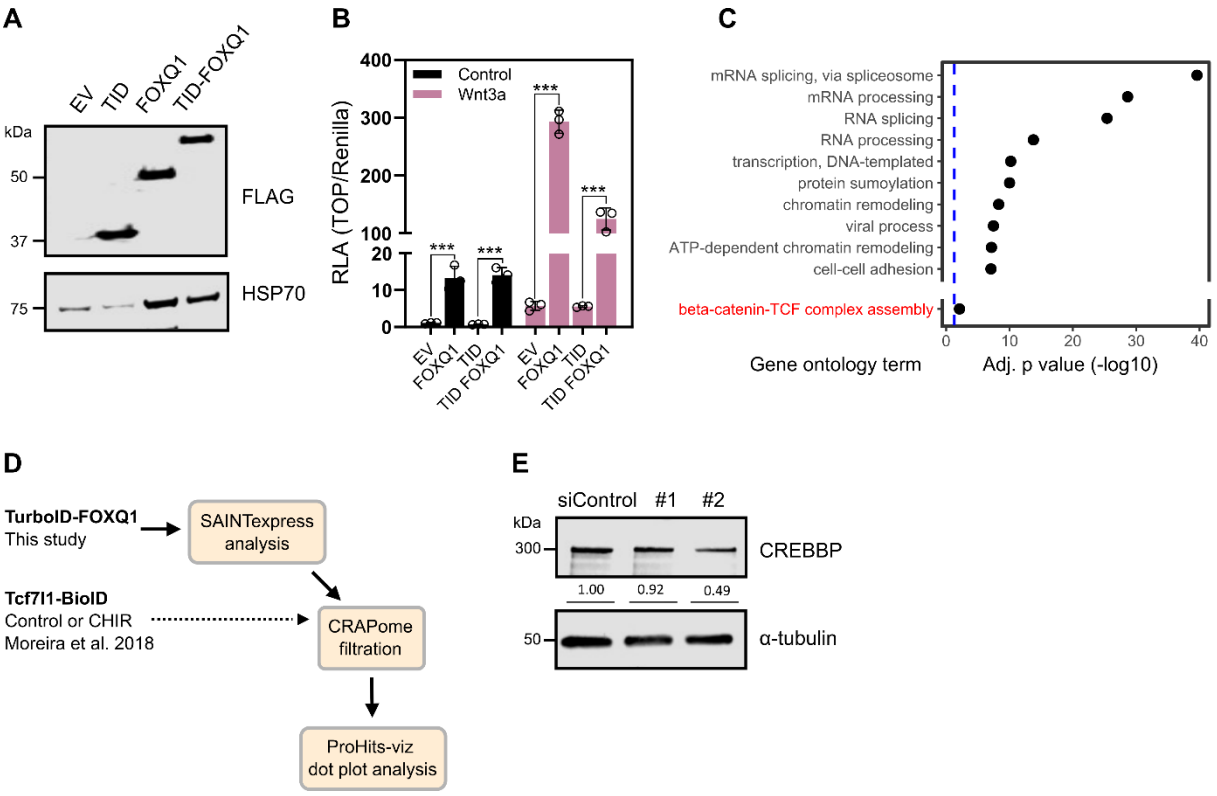
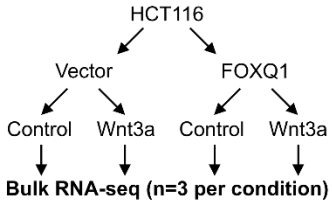


Fig. S5. FOXQ1 shares multiple co-factors with TCF/LEF.

(A) Immunoblot for protein expression of Flag-tagged TurboID-FOXQ1 fusion construct in 293T cells. TID, TurboID. (B) TOPflash assay for functional validation of the TID-FOXQ1 construct in 293T cells. The TID-FOXQ1 construct activated Wnt signalling similarly to wild-type FOXQ1. (C) Gene ontology (GO) analysis of the statistically significant FOXQ1 interactors identified using TurboID. Only the top 10 most significant GO terms are shown in addition to *beta-catenin-TCF complex assembly*. Full results can be found in Table S1. The dashed blue line indicates an adjusted p-value of 0.05. (D) Schematic representation of the workflow used for mass spectrometry data analysis. (E) Immunoblot analysis to confirm CREBBP silencing by RNA interference. Numbers of CREBBP protein quantification relative to the siRNA control are reported.

Data are displayed as mean ± SD. Statistical significance was calculated by ANOVA with Tukey's post-hoc test (B) and defined as ***$P < 0.001$.

A



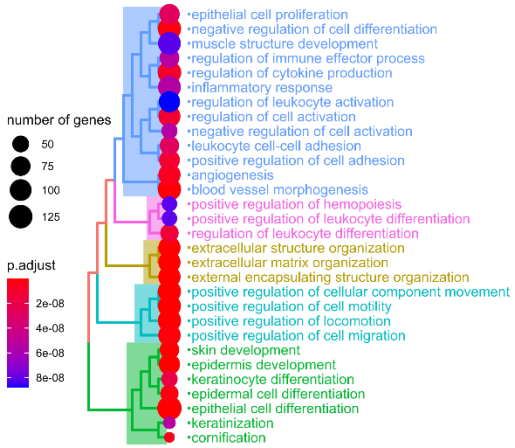
B

Differentially expressed genes (DEGs, FDR-adjusted p value < 0.05)

DEGs (up/down)	Condition: Vector-Control	Vector-Wnt3a	FOXQ1-Control	FOXQ1-Wnt3a
versus Vector-Control	-	166 (96/70)	4097 (1984/2113)	4924 (2150/2774)
versus Vector-Wnt3a		-	4950 (2395/2555)	4948 (2218/2730)
versus FOXQ1-Control			-	137 (74/63)
versus FOXQ1-Wnt3a				-

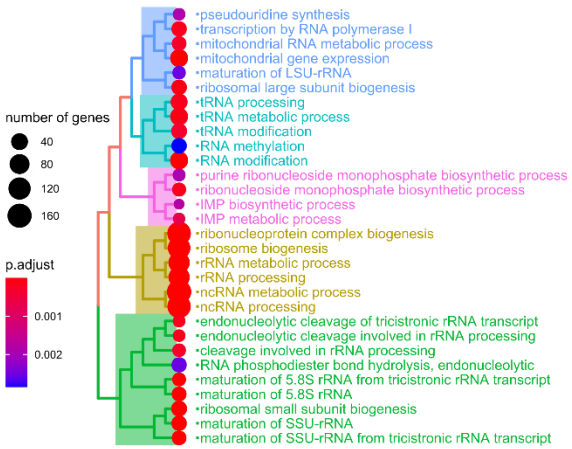
C

Genes upregulated by FOXQ1 (FDR < 0.05, log2 FC > 0)

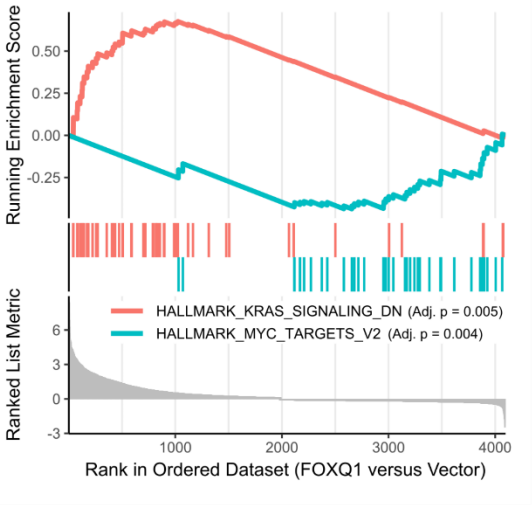


D

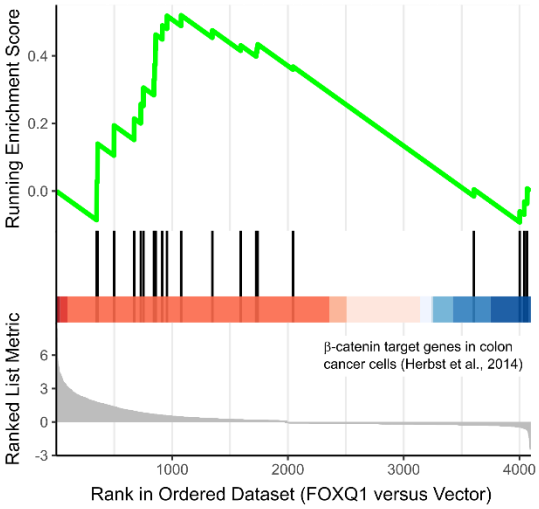
Genes downregulated by FOXQ1 (FDR < 0.05, log2 FC < 0)



E



F



G

Fig. S6. FOXQ1 alters the transcriptome of colorectal cancer cells.

(A) Overview of the bulk RNA sequencing experiment performed in this study. (B) Summary of the number of differentially regulated protein-coding genes (DEGs) when comparing the different conditions. (C, D) Gene ontology analysis of FOXQ1-associated DEGs in untreated cells. Ontology terms were grouped using Ward's clustering algorithm. (E) Gene set enrichment analysis (GSEA) against all hallmark gene sets identified genes downregulated by KRAS and MYC target genes as the only significantly enriched sets in untreated FOXQ1-expressing cells. (F) GSEA using a curated list of β-catenin target genes indicated modest enrichment in untreated FOXQ1-expressing cells. (G) GSEA of 166 Wnt-regulated genes (see panel B) in DLD-1 cells transfected with FOXQ1 shRNA (GSE74223, n = 2 replicates per condition).

Fig. S7. Blot transparency

Fig. 3A

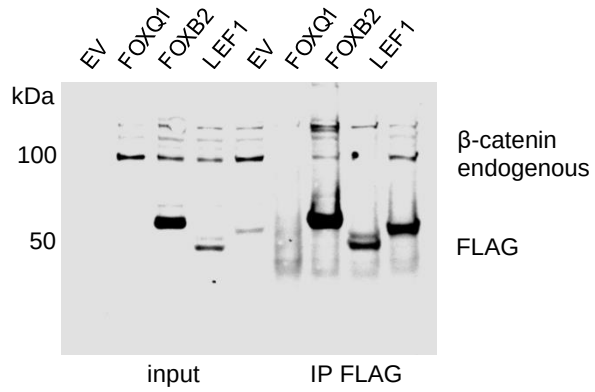


Fig. 3B

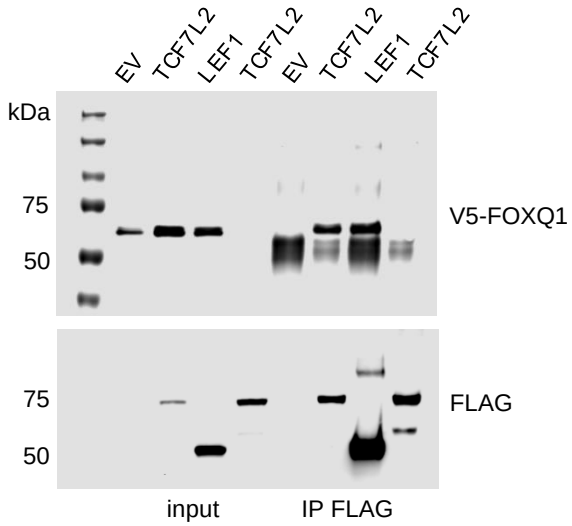


Fig. 3C

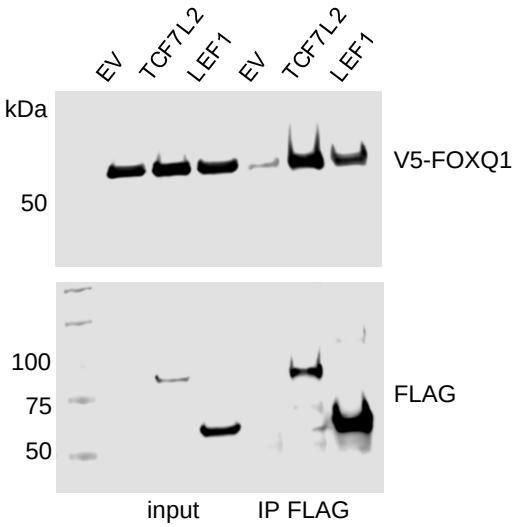


Fig. 3G

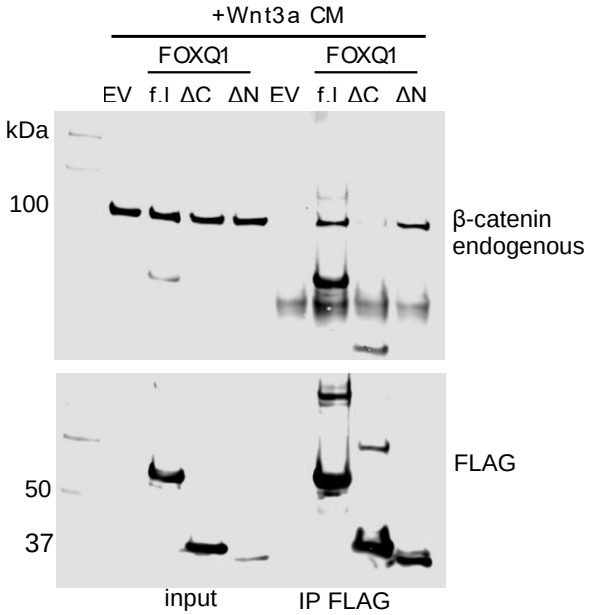


Fig. 3H

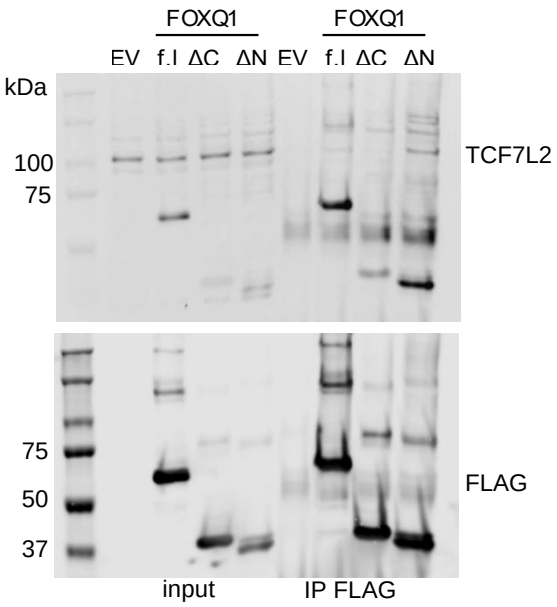


Fig. 5H

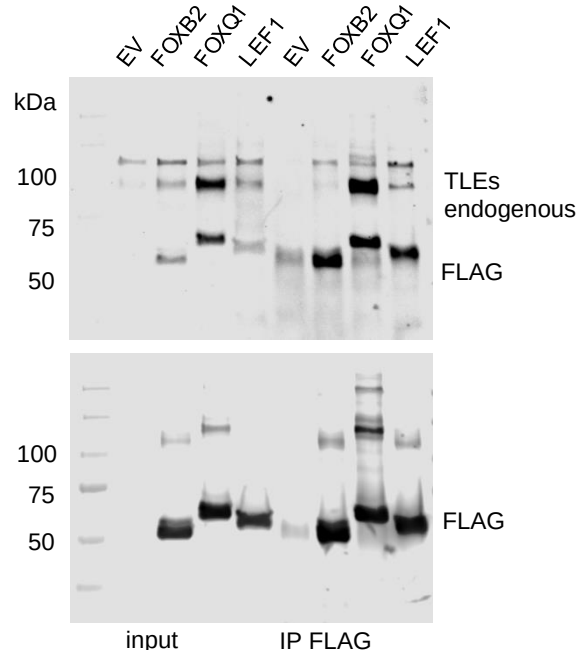


Fig. 5I

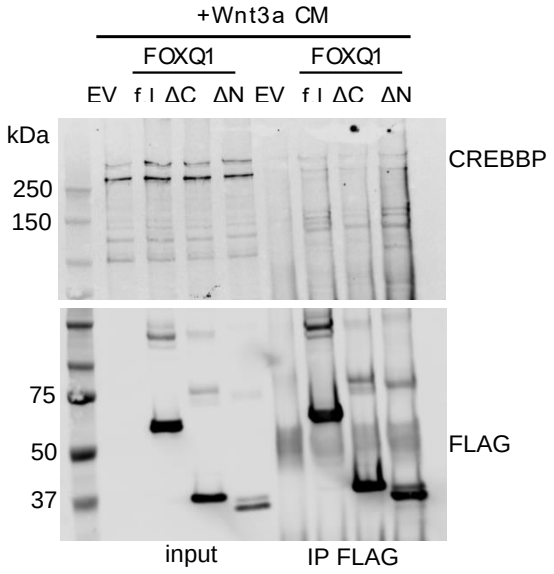


Fig. S3A

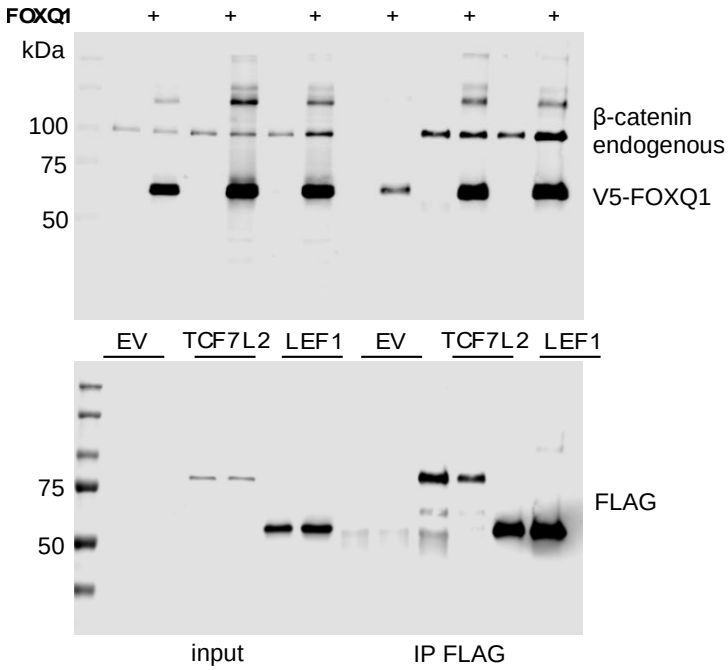


Fig. S5A

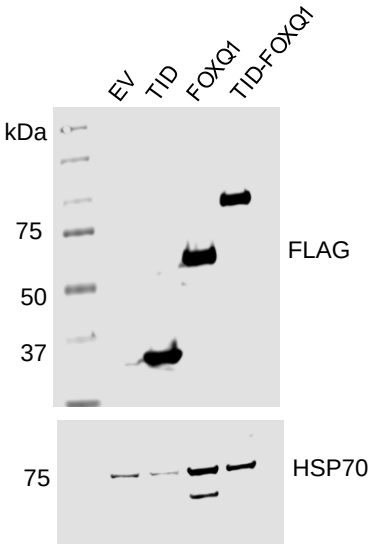


Fig. S5E

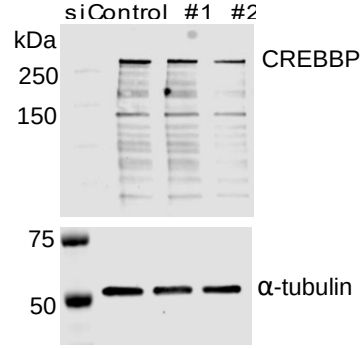


Table S1. Gene Ontology (GO) analysis of FOXQ1 TurboID experiments.

Click here to download Table S1

Table S2. SAINTexpress analysis of FOXQ1 TurboID experiments.

Click here to download Table S2

Table S3. SAINTexpress analysis of FOXQ1 + CHIR TurboID experiments.

Click here to download Table S3

Table S4. Ct values for the qPCR experiments included in the study.

Click here to download Table S4

References

Jho, E. H., Zhang, T., Domon, C., Joo, C. K., Freund, J. N. and Costantini, F. (2002). Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol* **22**, 1172-83.

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