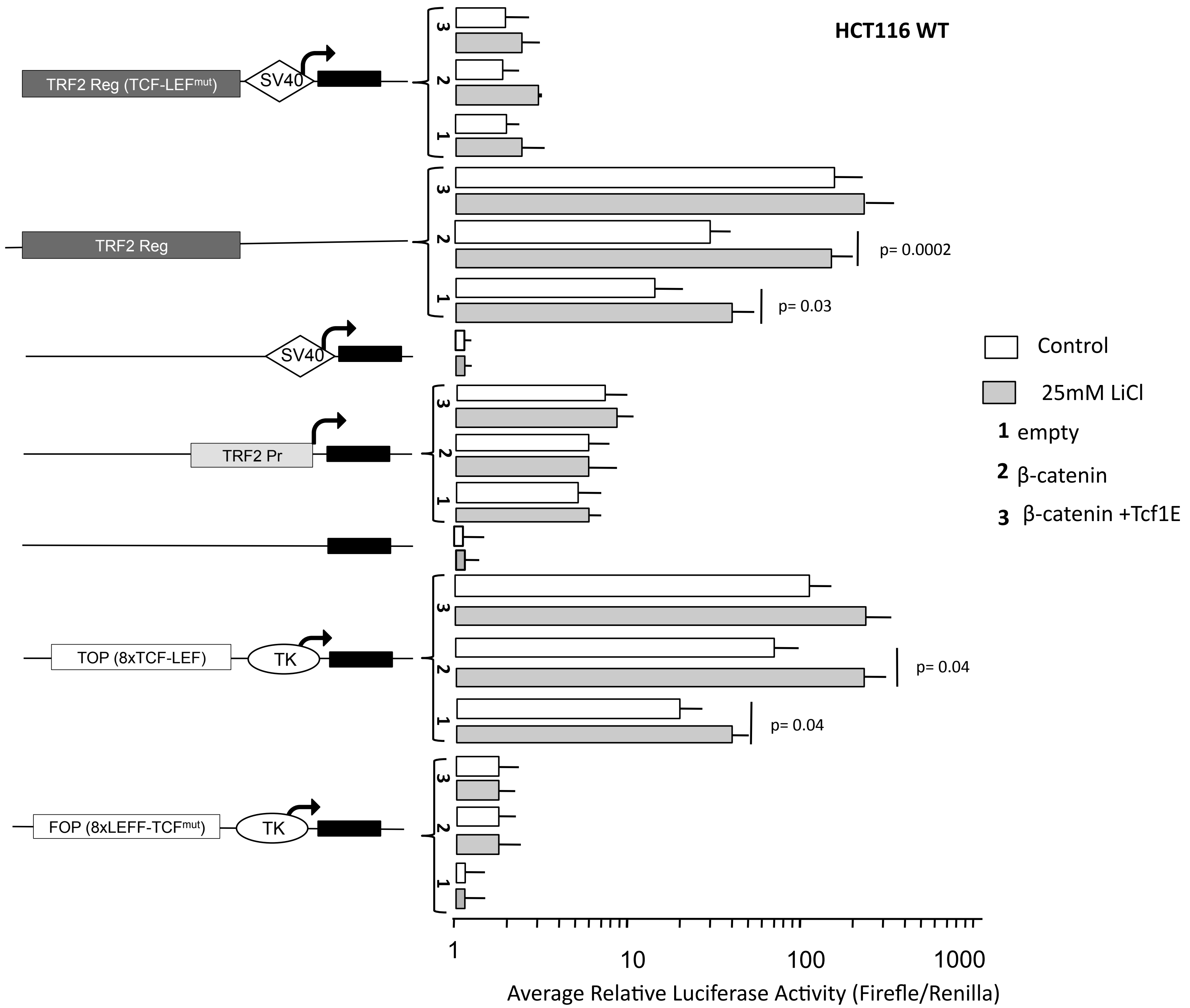
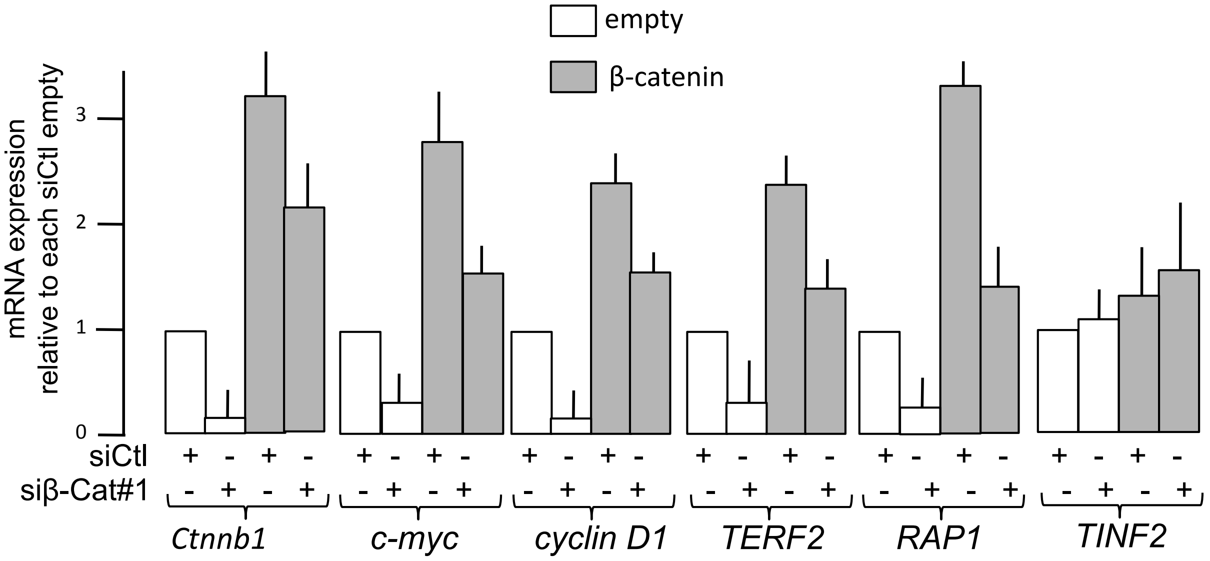

HCT116 WT
TRF2 Reg (TCF-LEF^{mut})
SV40
TRF2 Reg
SV40
TRF2 Pr
TOP (8xTCF-LEF)
TK
FOP (8xLEFF-TCF^{mut})
TK
3
2
1
p= 0.0002
p= 0.03
p= 0.04
p= 0.04
Control
25mM LiCl
1 empty
2 β-catenin
3 β-catenin +Tcf1E
1
10
100
1000
Average Relative Luciferase Activity (Firefle/Renilla)

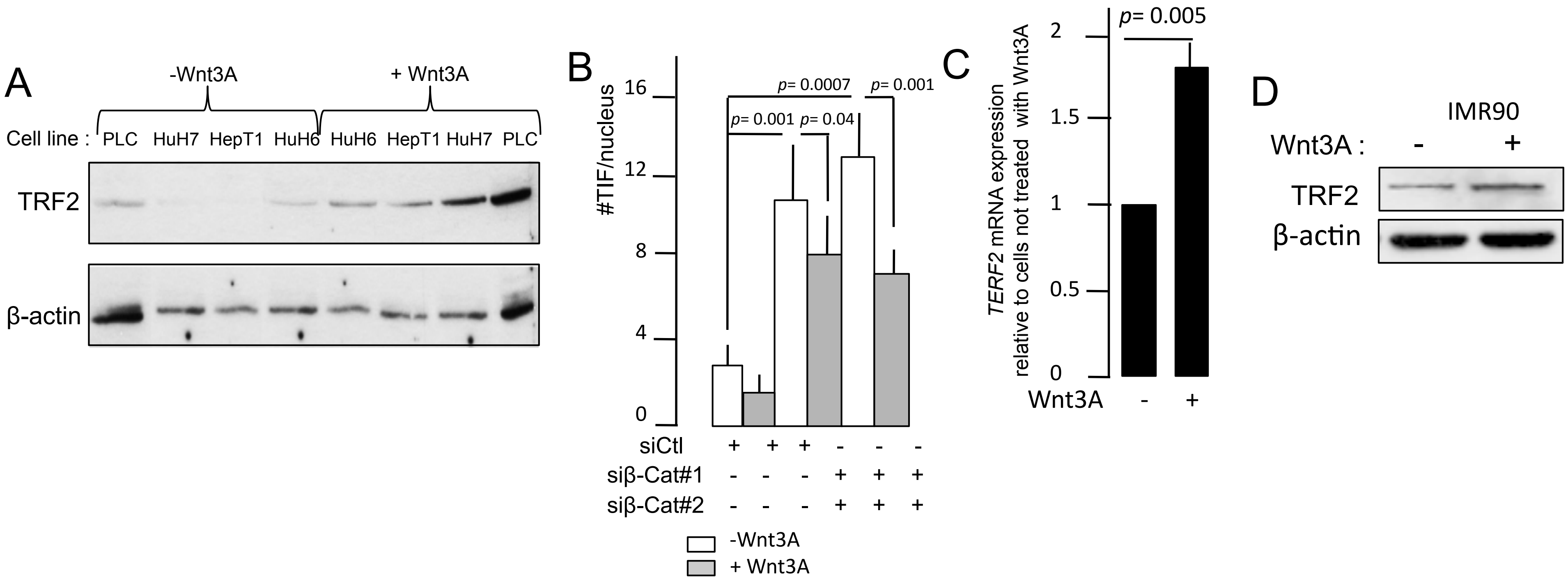
Figure S1

Supplementary Figure S1. Effect of LiCl on the transactivation properties of the Reg region of TRF2. The various reporter constructs derived from the pLG-3/Prom plasmid (for the *TERF2* Reg sequence) and pLG-3/basic (for the *TERF2* Prom sequence). TCF-LEF^{mut}: all the TCF-LEF sites are inactivated by mutation. TOP-Flash and FOP-Flash are positive and negative controls, respectively, for β-catenin/TCF transactivation. Data show average relative luciferase activity in HCT116 cells and represent the mean ± s.e.m. ($n = 6$). Statistical significance of data was assessed using Bonferroni Multiple Comparison Test.

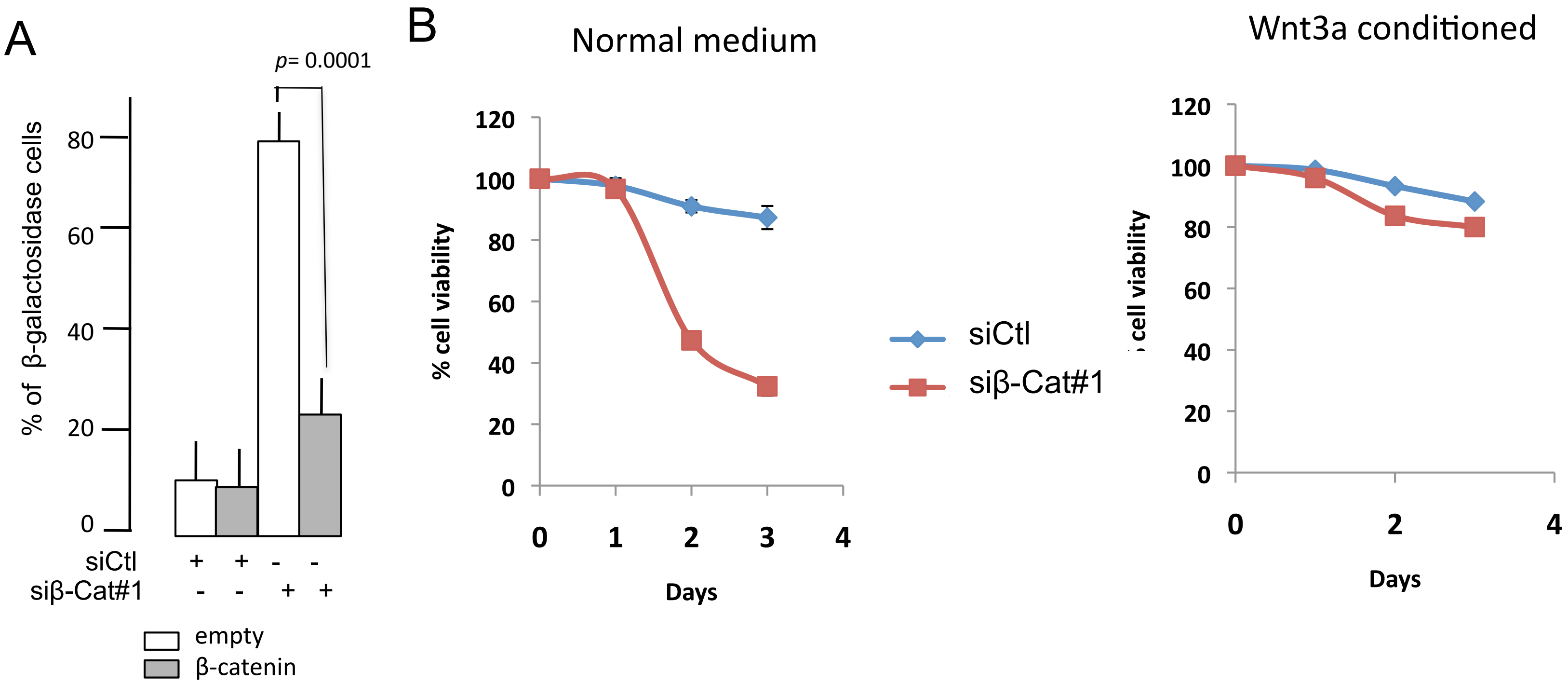


Supplementary Figure S2. β-Catenin regulates the expression of TRF2 and Rap1 in HepG2 cells. After siRNA-mediated β-catenin knockdown, we performed real-time RT-qPCR analysis to evaluate the mRNA expression of *β-catenin*, two known targets of the canonical Wnt signaling (*cyclin D1* and *c-Myc*), *TERF2, RAP1* and *TINF2*. Knocked-down β-catenin expression was rescued by β-catenin overexpression. Error bar represents the mean ± s.e.m for 6 independent experiments.

Figure S2



Supplementary Figure S3. (A) The level of TRF2 protein increases in various hepatoma cell lines upon treatment with Wnt3A. Cell lines without known β-catenin mutation : PLC is for PLC/PRF/5 hepatoma cell line and HuH7; cell lines with known β-catenin mutation : HepT1 and HuH6. (B) shows number of TIFs per nucleus observed for each condition using PLC/PRF/5 cells. p value was determined using student T-test. At least 40 nuclei per condition were analyzed. (C) *TERF2* mRNA level in IMR90 cells with and without Wnt3A. (D) TRF2 level in IMR90 cells with and without Wnt3A. For panels B and C, error bar represents the mean ± s.e.m for 3 independent experiments.



Supplementary Figure S4. (A) The bar graph represents the total count of HCT116 cells that are stained with β-galactosidase. p value was determined using student T-test. Error bar represents the mean ± s.e.m. (B) Data shows cell viability count per day of HCT116 cells upon *Ctnnb1* knock-down as measured using trypan blue staining.

Supplementary Table S1 : mean Ct values (n=4) of the ChIP samples obtained from HCT116 with and without β-catenin and Tcf1E overexpression.

Experiment	ChIP	Ct Pa	Ct H4 pr	$\Delta Ct= Ct_{Pa}-Ct_{H4pr}$	$Pa/H4=1/2^{\Delta Ct}$	Fold enrichment Pa/H4 ,normalized to input	standard error
HCT116 empty	*Input*	23,57	21,84	1,730	0,301		
	Ab beta-cat	28,72	29,44	-0,720	1,647	5,47	1,041
	No Ab	34	32,08	1,920	0,264	0,88	0,85
HCT116 + β-catenin	*Input*	23,34	21,58	1,760	0,295		
	Ab beta-cat	26,40	28,45	-2,050	4,141	14	0,4
	No Ab	32,24	29,07	3,170	0,111	0,37	0,42
HCT116 + β-catenin + Tcf1E	*Input*	23,75	21,52	2,230	0,213		
	Ab beta-cat	28,90	30,83	-1,930	3,811	17,9	0,24
	No Ab	32,72	30,48	2,240	0,212	1	0,12

		Ct Pb	Ct H4 Pr	$\Delta Ct= Ct_{Pb}-Ct_{H4Pr}$	$Pb/H4=1/2^{\Delta Ct}$		
HCT116 empty	*Input*	23,63	21,84	1,790	0,289		
	Ab beta-cat	31,20	29,44	1,760	0,295	1	32
	No Ab	33,78	32,08	1,700	0,308	1	0,225
HCT116 + β-catenin	*Input*	23,87	21,58	2,290	0,204		
	Ab beta-cat	30,67	28,45	2,220	0,215	1,075	0,22
	No Ab	31,45	29,07	2,380	0,192	0,94	0,44
HCT116 + β-catenin + Tcf1E	*Input*	23,88	21,52	2,360	0,195		
	Ab beta-cat	33,00	30,83	2,170	0,222	1,13	0,47
	No Ab	33,67	30,48	3,190	0,110	0,56	0,38

Supplementary Table S2 : Sequence of the qPCR primers used for the ChIP experiment of Figure 2

Pa	F 5'- GTCAGAGCTTCAATGCCAAA - 3'
	R 5'- GAGCAAGCAAGAAAGCACAC - 3'
Pb	F 5' - CTCAAAGTCAGTACCACATTTTCC - 3'
	R 5' - AGGGTTTGGTCACCCTTGTT - 3'
P1	F 5' - CCATTTGGTTTTCCGAAG - 3'
	R 5' - GCCTGAAAGAAGCATCCAGA - 3'
P2	F 5' - TCTTGTCCAGGGAAGACTCG - 3'
	R 5' - CCACACCCAGCTTAAACCAT - 3'
P3	F 5'- TGCACTTTAACCCGGAATCT - 3'
	R 5' - GCGCATTGAAGAAGGAGAAA - 3'
H4 pr	F 5' – TGTGTGATGGGAAGATGGGA – 3'
	R 5' – GAGCAAAATAGCGAGACCCC – 3'
Villin pr	F 5' – CAGACATACATGCAGGCAAAA – 3'
	R 5' – CCAGATCCCTCTTCAGTGTGT – 3'