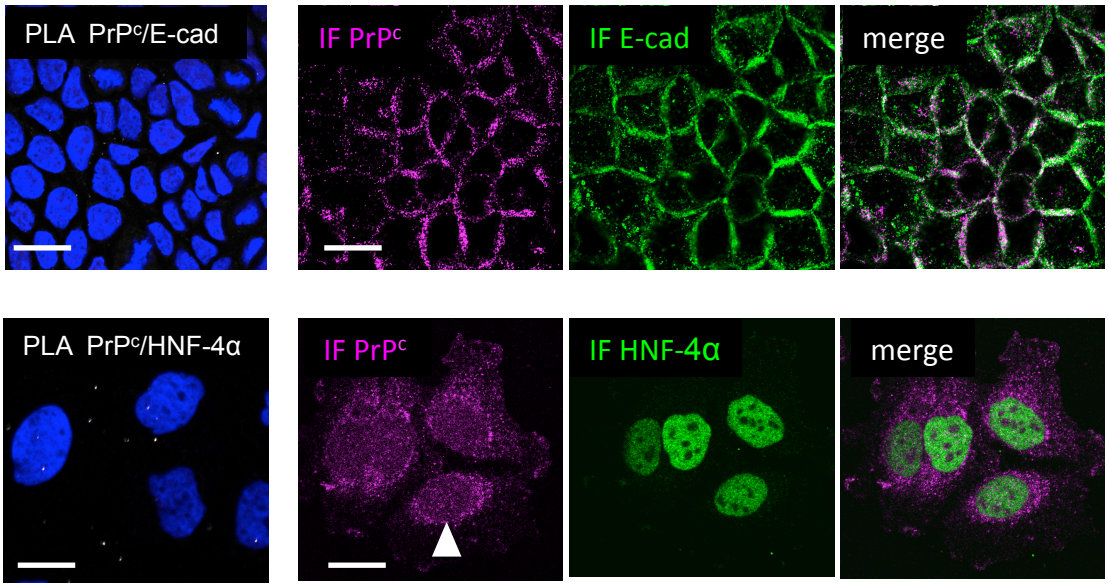
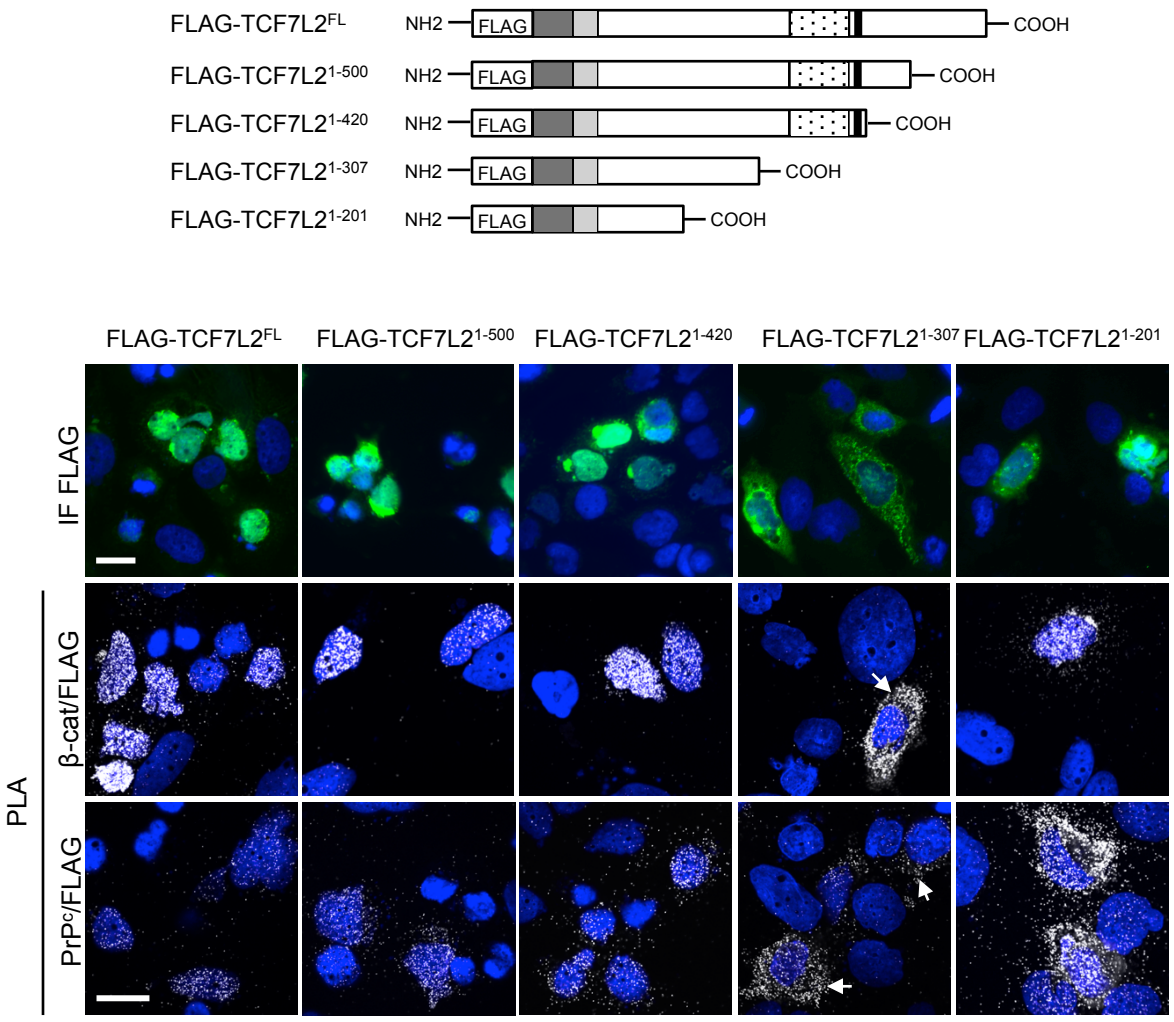
Supplemental Materials

Molecular Biology of the Cell

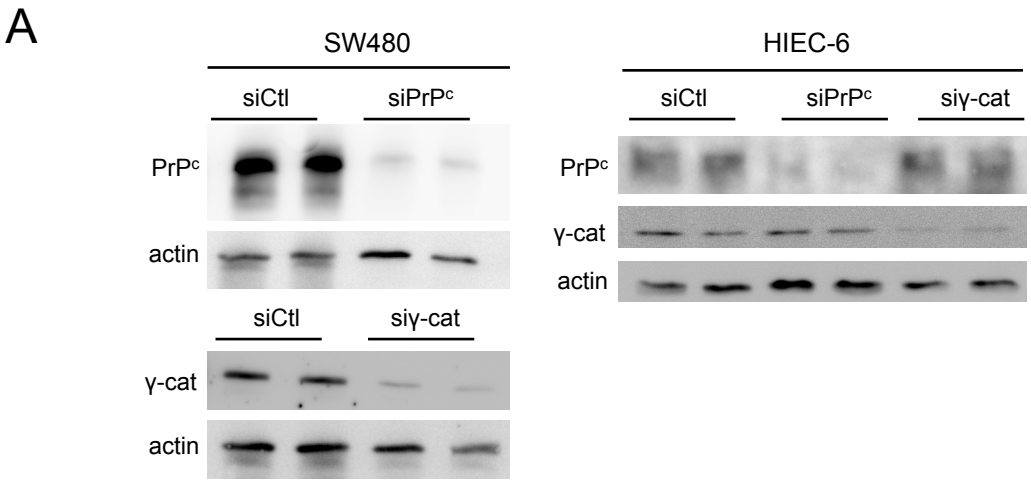
Besnier et al.

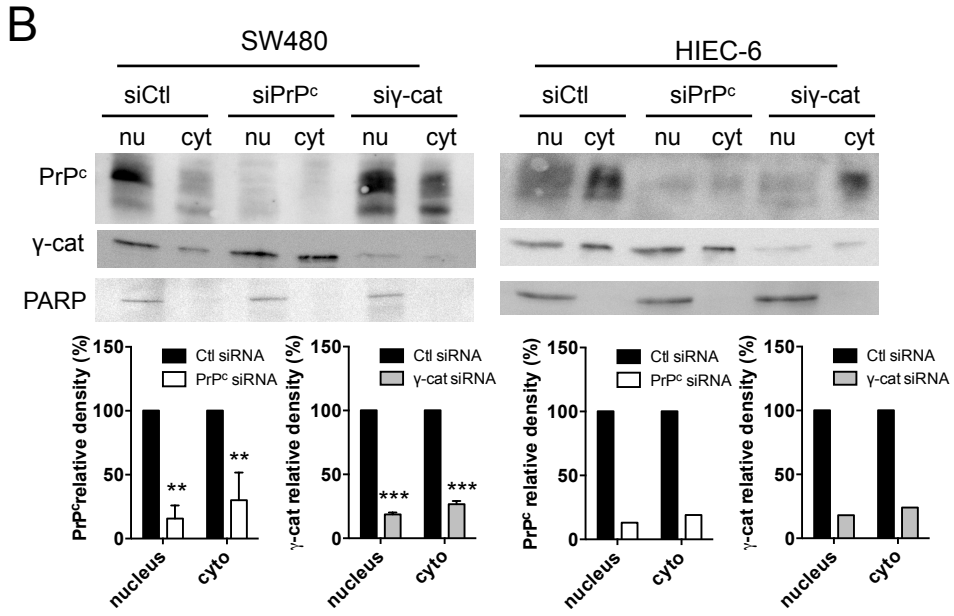


Supplementary Figure 1

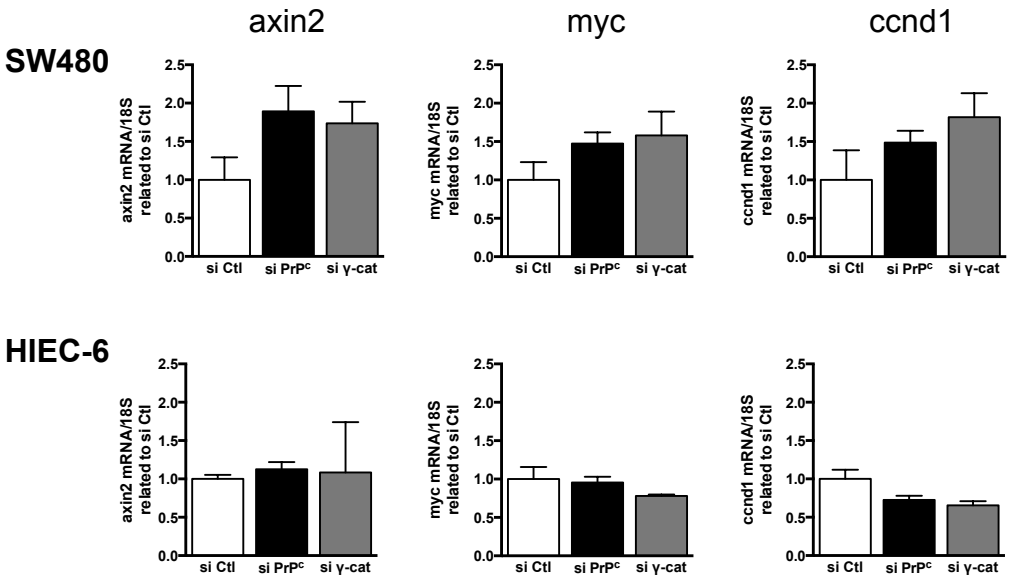


Supplementary Figure 2





Supplementary Figure 3



Supplementary Figure 4

Supplementary Figure 1 : Negative controls for proximity ligation assays (PLA). Caco-2/TC7 cells at confluence (8 days of culture, upper panels) or in exponential phase of growth (2 days of culture, lower panels) were processed for PLA with antibodies against PrP^c, E-cadherin or HNF4-α (PLA PrP^c/E-cad, PLA PrP^c/ HNF4-α) or for classical immunofluorescent labelings (IF PrP^c, IF E-cad, IF HNF4-α) before analysis by confocal microscopy. No PLA signal was obtained in confluent Caco-2/TC7 cells between PrP^c and E-cadherin, which co-localize (see merge IF image) but are not in the same protein complexes (Morel *et al.,* 2004) or in proliferating cells between PrP^c and HNF4-α, which are both nuclear. Immunofluorescence analyses show the junctional and nuclear co-localizations for both couples of proteins in the same cultures of confluent and proliferating cells respectively, revealed with the same antibodies, indicating that the absence of PLA signal cannot be imputed to immunolabeling conditions. The arrowhead points out nuclear PrP^c in proliferating cells. Bars, 20 µm.

Supplementary Figure 2 : Schematic diagram of the FLAG-tagged full length TCF7L2 ($TCF7L2^{FL}$) and different C-terminal-truncated TCF7L2 constructs ($TCF7L2^{1-500}$, $TCF7L2^{1-420}$, $TCF7L2^{1-307}$, $TCF7L2^{1-201}$). Proliferating SW480 cells were transfected with the different FLAG-TCF7L2 constructs. Their expression was assessed by immunofluorescence analysis using an anti-FLAG antibody and PLA were performed to reveal β-cat/FLAG and PrP^c/FLAG interactions. Nuclei were stained by DAPI (Bar, 20 µm). Note that expression of all constructs and interactions were detected mainly in the nucleus, except for $TCF7L2^{1-307}$, and in a lesser extent $TCF7L2^{1-201}$, for which expression and interactions with both β-catenin and PrP^c were detected in both cytoplasm and nucleus, in accordance with the lack of NLS in these constructs. See legend in figure 5 for a description of the different domains.

Supplementary Figure 3 : PrP^c and γ-catenin knockdown in SW480 and HIEC-6 cells upon siRNA transfection. (A) Decrease of total PrP^c or γ-catenin protein levels 24h after transfection with the corresponding siRNAs, as shown by western blot analysis of total cell extracts for each cell line. (B) In the three cell lines, decrease of PrP^c or γ-catenin levels after siRNA transfection occurs in both nucleus and cytoplasmic compartments, as shown by western blot analysis of nuclear (nu) and cytoplasmic (cyt) extracts. Semi-quantitative analysis was performed with Multigauge software (mean ± s.e.m. from 2 independent experiments for SW480 cells; ** $P<.01$ and *** $P<.001$ vs cells transfected with control siRNA). For each cell line and each fraction,

results were expressed as percentage of the corresponding protein level in cells transfected with control siRNA, which was set at 100.

Supplementary Figure 4 : RT-qPCR analysis of the primary Wnt target genes *AXIN2*, *MYC* and *CCND1* upon PrP^c or γ-catenin silencing in SW480 and HIEC-6 cells. mRNA levels were normalized to 18S and reported to mRNA levels in cells transfected with control siRNA, which was set at 1 (mean ± s.e.m from 3 independent experiments for SW480 and 2 independent experiments for HIEC-6 cells). For the three genes, non-parametric Kruskal-Wallis tests indicated no significant differences for each siRNA versus control siRNA

Construct	Sequence
TCF7L2$^{del1-51}$	Fwd CGATAGATCTGATATCGGTACCAATGTCAGAAACGAATCAAAACAGCTCCTCC Rev GGAGGAGCTGTTTTGATTCGTTTCTGACATTGGTACCGATATCAGATCTATCG
TCF7L2$^{del2-100}$	Fwd GATCTGATATCGGTACCAATGTATCCCGGCTACCCCTTC Rev GAAGGGGTAGCCGGGATACATTGGTACCGATATCAGATC
TCF7L2$^{del52-143}$	Fwd CAAATCGTCTCTAGTCAATGAACAGAGTAGACAAGCCCTCAAG Rev CTTGAGGGCTTGTCTACTCTGTTCATTGACTAGAGACGATTTG
TCF7L2$^{del82-143}$	Fwd CAAATCCCGGGAAAGTCAGAGTAGACAAGCCCTCAAGGATG Rev CATCCTTGAGGGCTTGTCTACTCTGACTTTCCCGGGATTTG
TCF7L2$^{1-201}$	Fwd CCACACTTACCAGCCG**G**C**TAGCG**ACCCCAAAACAGGAATCC Rev GGATTCCTGTTTTGGGGT**CGCTA**G**C**CGGCTGGTAAGTGTGG
TCF7L2$^{1-307}$	Fwd CAGAGTGATGTCGGCT**AG**CTCCATAGTTCAAAGC Rev GCTTTGAACTATGGAG**CT**AGCCGACATCACTCTG
TCF7L2$^{1-420}$	Fwd GAGACCAATGAACACAGC**T**A**GC**GTTTCCTAAATCCTTGCC Rev GGCAAGGATTTAGGAAAC**GC**T**A**GCTGTGTTCATTGGTCTC
TCF7L2$^{1-500}$	Fwd CTAGGCTCCCCTCCCCG**CTAGC**CCAAGTCACAGACTGAGCAG Rev CTGCTCAGTCTGTGACTTGG**GCTAG**CGGGGAGGGGAGCCTAG

Supplementary Table 1. Sequences of oligonucleotides used for mutagenesis. For N-terminal deletions (del1-51, del2-100, del52-143 and del82-143), the 5' half of the forward oligonucleotides hybridized with the vector (del 1-51, del 2-100) or the left limit of TCF7L2 (del52 or 82-143) and the 3' half with the TCF7L2 coding sequence starting respectively at codon 52, 101 or 144. For C terminal deletions, a Nhe I site (5' GCTAGC 3') was introduced at the position of the new Stop codon in the truncated C-terminal mutants to facilitate screening. The mutated nucleotides are indicated in bold underlined letters. Five colonies were randomly picked and sequenced before use.

Target	name	sequence
PrP^c	HsPRNP_7	5'-GAGAUUUCAUAGCUAUUUATT-3' 5'-UAAAUAGCUAUGAAAUCUCTA-3'
	HsPRNP_10	5'-GUGACUAUGAGGACCGUUATT-3' 5'-UAACGGUCCUCAUAGUCACTG-3'
γ-catenin (plakoglobin)	HsJUP_1	5'-GCGUAACUACAGUUAUGAATT-3' 5'-UUCAUAACUGUAGUUACGCAT-3'
	HsJUP_2	5'-AGAGCAUGAUUCCAUCAATT-3' 5'-UUGAUGGGAAUCAUGCUCUGG-3'
β-catenin	HsCTNNB1_8	5'-GCGGCUUCUGCGCGACUUATT-3' 5'-UAAGUCGCGCAGAAGCCGCTG-3'
	HsCTNNB1_5	5'-CGGGAUGUUCACAACCGAATT-3' 5'-UUCGGUUGUGAACAUCCCGAG-3'
Control	Negative control siRNA	5'-UUCUCCGAACGUACGUdT dT-3' 5'-ACGUGACACGUUCGGAGAAdT dT-3'

Supplementary Table 2. Sequences of the specific siRNAs used for silencing of human PrP^c or γ-catenin. Two different siRNA sequences were used for each target.

RNA	Primers
Human cell-lines	
PRNP (PrP^C)	5'-GAGCCAGTCGCTGACAGC-3' (forward) 5'-TTCCATCCTCCAGGCTTCGG-3' (reverse)
JUP (γ-catenin)	5'- CTGGTGCAGAACTGCCTGT-3' (forward) 5'-CACCAGAATCTTCAGCACACTC-3' (reverse)
AXIN2	5'-GCTGACGGATGATTCCATGT-3' (forward) 5'-ACTGCCCACACGATAAGGAG-3' (reverse)
CCND1 (Cyclin D1)	5'-GAAGATCGTCGCCACCTG-3' (forward) 5'-GACCTCCTCCTCGCACTTCT-3' (reverse)
MYC	5'-GCTGCTTAGACGCTGGATTT-3' (forward) 5'-TAACGTTGAGGGGCATCG-3' (reverse)
L19	5'- ATGTATCACAGCCTGTACCTG-3' (forward) 5'- CGTGCTTCCTTGGTCTTAGAC-3' (reverse)
18S	5'-GGGAGCCTGAGAAACGGC-3' (forward) 5'-GGGTCGGGAGTGGGTAATTT-3' (reverse)
Mouse	
CCND1 (Cyclin D1)	5' AAGTTCATTTCCAACCCACCC 3' (forward) 3' TGGAAAGAAAGTGCGTTGTGC 5' (reverse)
ETS2	5'AAGCATTCTGGATCTTGCAAC 3' (forward) 3' GATAGGAAAGCTGTAGTCCTGGTC 5' (reverse)
FGF9	5' TGCAGGACTGGATTTCATTTAG 3' (forward) 3' CCAGGCCCACTGCTATACTG5' (reverse)
IGF2	5' CGCTTCAGTTTGTCTGTTCG 3' (forward) 3' GCACACTCTTCCACGATG 5' (reverse)
ID2	5' GACAGAACCAGGCGTCCCA 3' (forward) 3' AGCTCAGAAGGGAATTCAGATG 5' (reverse)
IRS1	5' GCAACTCCCCAAGACGCTCC 3' (forward) 3' CGAGTCTGGGTACCCATGAG 5' (reverse)
GJA1	5' GAGAGCCCGAACTCTCCTTT 3' (forward) 3' CCATGTCTGGGCACCTCT 5' (reverse)
MYC	5' CCTAGTGCTGCATGAGGAGA 3' (forward) 3' TCCACAGACACCACATCAATTT 5' (reverse)
SOX9	5'GTACCCGCATCTGCACAAC 3' (forward) 3' CTCCTCCACGAAGGGTCTCT 5' (reverse)
TCF4	5' CATATTTGTGGCCATTGAAGG 3' (forward) 3' GTCCCTAAGGCAGCCATTC 5' (reverse)
Cyclophilin	5' GCCTTAGCTACAGGAGAGAA 3' (forward) 3' TTTCCTCCTGTGCCATCTC 5' (reverse)

Supplementary Table 3. Sequences of primers used for real-time PCRs.