

Supplementary figure legends

Supplementary Figure 1 : IRS1 expression in colorectal carcinomas.

A Immunohistochemical staining for IRS1 was performed on tissue microarrays containing normal colonic mucosa and colorectal carcinomas. Representative samples for weakly, moderately and strongly staining colorectal carcinoma specimens, as well as moderately staining colorectal mucosa are shown.

B Analysis of IRS1 expression by IHC in colorectal carcinoma specimens scored in a semiquantitative scale of weak, moderate and strong.

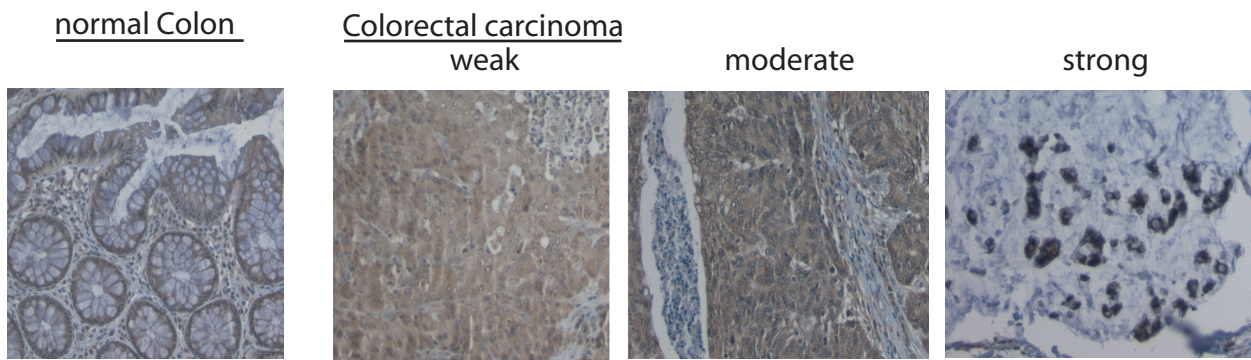
Supplementary Figure 2 : IRS1 knockdown reduces focus formation in RK3E

A Representative images of methylene blue stained plates after focus formation assay in RK3E cell lines expressing control shRNA and two different shRNAs targeting the IRS1 transcript, upon transduction with recombinant retroviruses driving expression of LacZ, wildtype β -catenin and S33Y β -catenin.

Supplementary Figure 3 : Genomic loci analyzed by chromatin immunoprecipitation.

Predicted TCF binding sites are highlighted (CTTTGAT) or underlined (CWTTGWW). Mutations introduced into the downstream site 2 reporter construct are indicated in red.

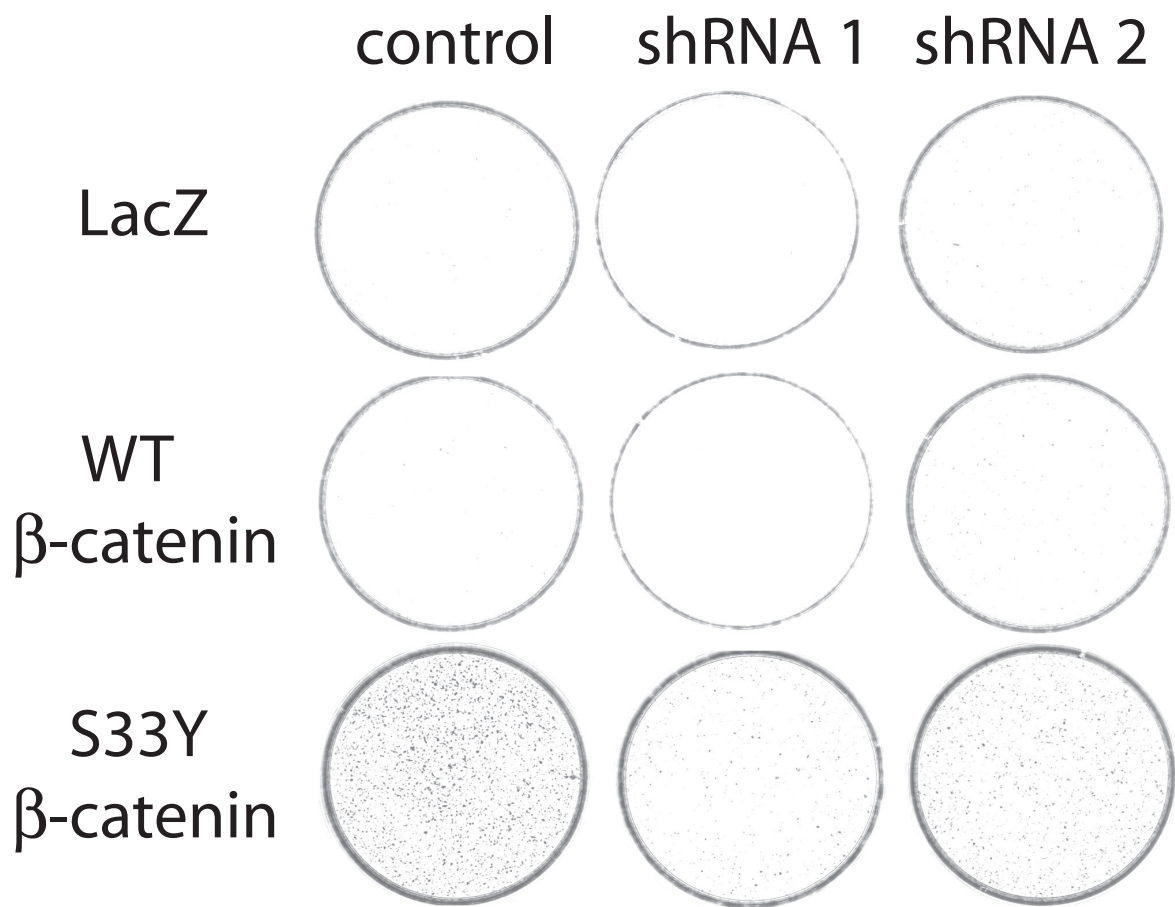
A



B

	weak	moderate	strong
colorectal cancer	16	21	7
normal mucosa	1	2	0

Bommer et al Supplementary figure 1:



Bommer et al Supplementary Figure 2

Supplementary figure 3

Genomic fragments encompassing the ChIP sites in the IRS1 locus

CTTTGAT sites are shaded and underlined

CWTTGWW sites are underlined

Intronic site

AGAATACCGTAGAGGTCTGTAGCTAATGACCTATGTAAGTCTAGCTGGACTATTTCTGGAAA
CTTTGAAACAAAGGCCATTGGCAGATATATATTCCTGTTTTTTTAGTATCAGTTTATAAAGGA
ACATAAGGTTTTGTTTCAGGTTAGTTAATATTTAAACAATTAAAAACAACCTTTTTTGCAAAGA
TTCATTTTGGTGAATAGGATGTATATATAGAAACAGCTGGGTTCTCTGTTTGAAACAACCTCT
TCTGGGGCCCTTCACAGTCAACAAGTACAGACAGCCAAACTGATTATAAGGGAAGGTGTTG
AAATATTACACATGAAACTCTAAATATATGGAGAAAACACATACATACACACTGGTATAAAA
GAAGCCTCACTGTAATCTGTATGATCTAGCATGTCAGGAATTCTACTGCAAGAATTATACAA
CAAGCTGAGACAATTTTCGGTGAGAGAGAAAGAAAA**ATCAATG**TCTGTGTTTTTAAAGAAA

downstream site 1

AAGCCATCTATTAAATTTGGTATCCCAGTAAGTGTTTTTATTAAATGTGAGTTGAGCTGCCT
TTCACTTAACTCTACCATGAGCTTATTAAATTAATAAAAATTGTAAAGAAG**ATCAAAG**TTC
CTTCTTTTAATTCCTTTAGATCCTTTGGGGAGACCACTGTTTATCTCTGTAAAGTTCAAGTC
TGCGTAACTCTAAGTCAGCCAAATCTGACATCCAACCATGGAATCTTGCGGAGACCATTTCC
TCCTCCTTTCTCAGTCTTCATATTCCCATTCCCTGCCAGACTACAGTTTGGAAGTTGTTGA
ATGAATTCACAAGTACTACCATTTGCACTTTCACACACTGTTTAAAGGCAGAAGTATCAGAT
TTCTCAGCACTGTGAAGAAGTTGGCAACTAC**TTCAAAG**CAATTTTGAAGCATCTATTACATT
ATTAAGCAAGGGCAATAATGATCTACAAACTGCCAACCAGCTCATG

Downstream site 2

GGCCATATGCCAAGAATAACAGATTGAACTTTAACTCCAAGTCTGAAAAGTTTCCTTTTAC
TGACTAGAGTTCAACCCAGTCTATTATCCTAACTAATGATAAAGCAATC**CTTTGAT**GCTG
TC**CTTTGAA**CAAGTTGTGTAAGCTAAAACCTCAGTGAGAAAGAAAAAGCAGCATATAAAGGA
AGCTTTTGGAGTTAGCAGCTATGCTAATTCCTAATTGCAGACATAGTACAAACATTTACACCC
TTAAGGTTGCAATAACAAGACCCA

Mutant construct for site 2 :

GGCCATATGCCAAGAATAACAGATTGAACTTTAACTCCAAGTCTGAAAAGTTTCCTTTTAC
TGACTAGAGTTCAACCCAGTCTATTATCCTAACTAATGATAAAGCAATC**CTGCGAT**GCTG
TC**CTTTGAA**CAAGTTGTGTAAGCTAAAACCTCAGTGAGAAAGAAAAAGCAGCATATAAAGGA
AGCTTTTGGAGTTAGCAGCTATGCTAATTCCTAATTGCAGACATAGTACAAACATTTACACCC
TTAAGGTTGCAATAACAAGACCCA

Downstream site 3

TATGTAAGGAAAGAATCACAAAGTGCCTTTATCAAGCACCTGCAATTAACACACAGCCAACG
TATATTGCAAAAGAGCATCTTTATTACAAGGATACAGATACTTATCTTTTAAATTTAGTAGT
TATTCTAAACATACTTCCCTAACTGCCTGCATTCCAAATTCCAAACTGTATTG**CTTTGAT**CC
ACTTAAAAGAGAAAGCATTCTACTCTGAATTAGAACAAGGAAACAGGACTGCAAAACAGCCT
AGAGACTCCTGAGCATACTCTAGGAGAAATTTTAAAGCTTTTATTTTACCTGAAAATACTG
ACAACAAAAGCCAATAACCTGCTGTATTACTGAGTAGTTACAATTATATAGAAAAACAATT
TTTACATAATGATTTGGTAAATGTCATCAT**CTTTGTT**TATATTTTACTTTTCGCCCTATATTGT
ACAAGGGCACTAAGTATTAATAAACCCAATCACAGACTTTCTTATTGTTAAATCATTCAATT

Downstream site 4

TGTGGCTATGACTTGGAATTATTGGTATCACTTAGTAAACACTGTTTGATCTAGCATTGCC
TTCTCTCCCAATTA AAAATAGAGTCTGGCC**AACAATG**ACAGGTCCCCACCACCAGGTATTGG
CCTCAGTCTGTTTCAGTGA AAAGCATTACAGCCTTCTAATTGGCATTCTCTTCAGAATTTGG
CAAATTTGGTCAAAGCAGACCAGCACTATAGTAACCTTT**CATTATCAATG**CAAATCCTAACA
GAAAAGCATTCCATTCTTCTTA**CATTGTA**CGTCATAAATGAAACACAATAAATTTTATTGTG
AAACCTCCTAGGAAGACATTCCCTGAATAGACAAAGCAGAAAATGCTTGCA

Downstream site 5

AGCTTGTGATCTGAGGTGAACGACTTTATCTCCCTATTTCTGTCCTTTCTCAGCTATGAAAG
ACCTAATACACTTCTAAAAGTGAACAGGCACACATCC**CTTTGATA**ACCTGTTCTCAGTTTTA
GCTTGCCAGAAGTTTATACTTTATTTTTTTACTACAATTAATATCTTTTTCTTGTGGTAGTG
GGAGGAGAAACATAATGATGTACATGCAAACCAAATATGCCAAAAGAATGCATGTATTTTC
CCTCTGGAAGGAGTGCATGAAAGTTAGGCATGGTGAGTAACCCCGACTGATTA ACTCTACCA
CTGATTAGAACACTGACTCACCATATGACTAACCACACATCAGATATTTATCTTGGGCCAAC
CCTGGTTGACTCATAATCTATGGTTTAAAGATGAAGATCGGAGCTACTTTTGATCTTCTTCTT
GCAAGCAT**TACAAAG**AAGATAATACCCTGTGTACTAGCAGAGCCAAC TTTACTGCCACAGAG