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Supplementary Materials for

β-Catenin/Tcf7l2–dependent transcriptional regulation of GLUT1 gene expression by Zic family proteins in colon cancer

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/7/eaax0698/DC1)

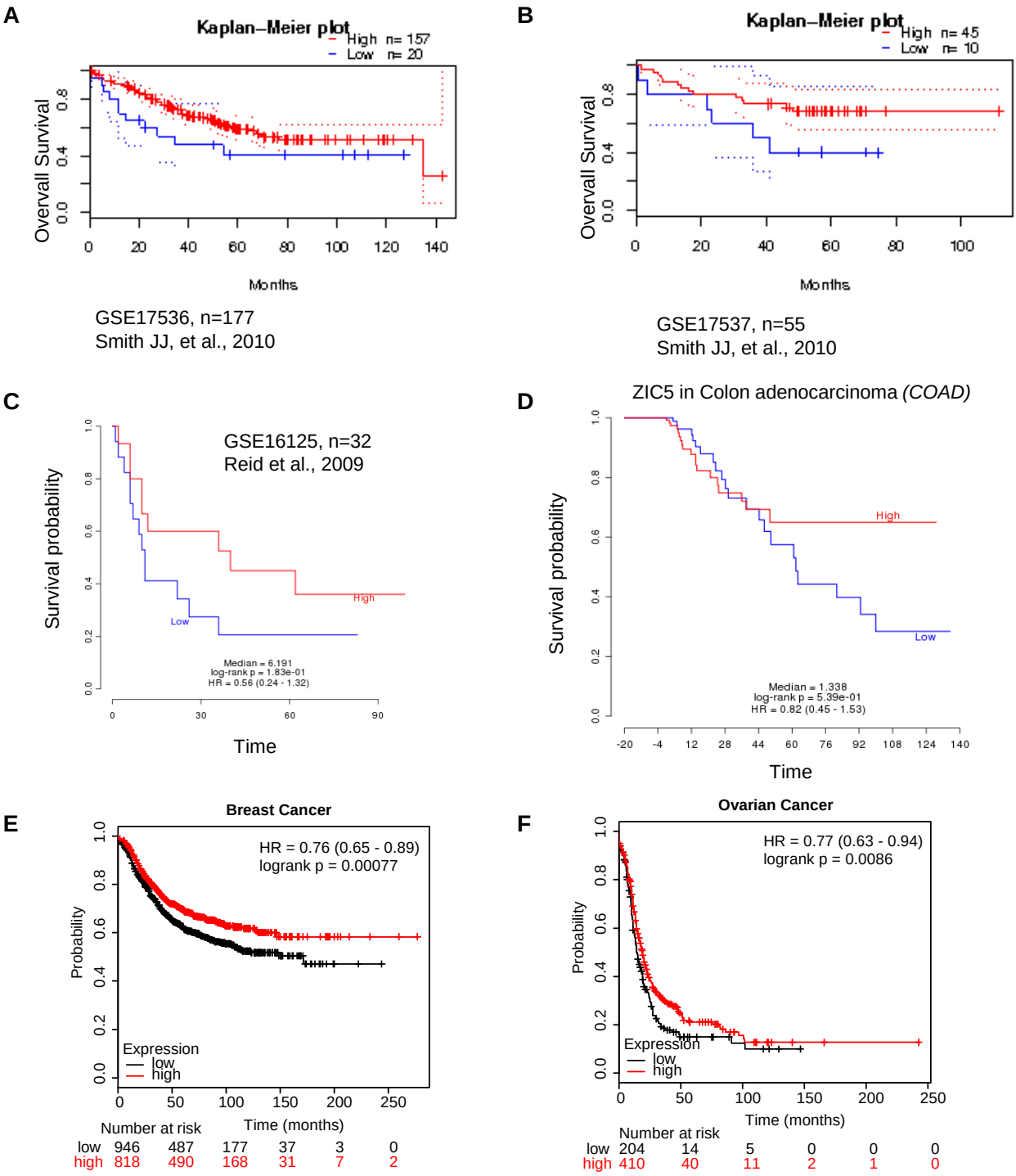

Fig. S1. Potential prognostic value of Zic5 in colon cancer.

A

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mouse   1    MMEPPLSKRNPPALRLADLATAQAQQLQNMTGFPVLVGPPAHSQRRAVAMHLHPRDLGTD  60
                +MEPPLSKRNPPALRLADLATAQ Q LQNMTGFP L GPPAHSQ RA   HL  RDLG D
human   24   LMEPPLSKRNPPALRLADLATAQVQPLQNMTGFPALAGPPAHSQLRAAVAHLRLRDLGAD  83

mouse   61   PGVASTALGPEHMAQASGQGPCPPSQGLPGLSQVPAPAARSVASGTHPGARTHPDGGGSS  120
             PGVA+T LGPEHMAQAS  G  PPSQ  P   + PA AAR+ A   HPGA ++P GGGSS
human   84   PGVATTPLGPEHMAQASTLGLSPPSQAFPAHPEAPAAAARAAALVAHPGAGSYPCGGGSS  143

mouse   121  GAQASAPPPPAPPLPPSQSSSPPPPPPPPPALSGYTATNSGGGSSSGKGHSRDFVLRRDL  180
             GAQ SAPPPPAPPLPP+ S  PPPPPPPPPALSGYT TNSGGG SSGKGHSRDFVLRRDL
human   144  GAQPSAPPPPAPPLPPTPSPPPPPPPPPPPALSGYTTTNSGGGGSSGKGHSRDFVLRRDL  203

mouse   181  SATAPAAAMHGAPLGGEQRSGSSSPQHPTPPPHPAGMFISASGTYAGRDG-GGSALFPAL  239
             SATAPAAAMHGAPLGGEQRSG+ SPQHP PPPH AGMFISASGTYAG DG GG ALFPAL
human   204  SATAPAAAMHGAPLGGEQRSGTGSPQHPAPPPHSAGMFISASGTYAGPDGSGGPALFPAL  263

mouse   240  HDSPGAPGGHP--LNGQMRLGLAAAAAAAA-ELYGRAEPPFAPRSGDAHYGAVAAAAAAA  296
             HD+PGAPGGHP  LNGQMRLGLAAAAAAAA ELYGRAEPPFAPRSGDAHYGAVAAAAAAA
human   264  HDTPGAPGGHPHPLNGQMRLGLAAAAAAAAAELYGRAEPPFAPRSGDAHYGAVAAAAAAA  323

mouse   297  LHGYGAVNLNLNLAAAAAAAAAAGPGPHLQHHAPPPAPPPAPAPHPHHPH----LPGAAG  352
             LHGYGAVNLNLN  AAAAAAAAAGPGPHLQHHAPPPAPPP PAP  H       LPGAAG
human   324  LHGYGAVNLNLN-LAAAAAAAAAGPGPHLQHHAPPPAPPPPPAPAQHPHQHHPHLPGAAG  382

mouse   353  AFLRYMRQPIKRELICKWLDPEELAG----------PPASADSGVKPCSKTFGTMHELV  401
             AFLRYMRQPIK+ELICKW+DP+ELAG          PP     G KPCSKTFGTMHELV
human   383  AFLRYMRQPIKQELICKWIDPDELAGLPPPPPPPPPPPPPPPPAGGAKPCSKTFGTMHELV  442

mouse   402  NHVTVEHVGGPEQSSHVCFWEDCPREGKPFKAKYKLINHIRVHTGEKPFPCPFPGCGKVF  461
             NHVTVEHVGGPEQSSHVCFWEDCPREGKPFKAKYKLINHIRVHTGEKPFPCPFPGCGKVF
human   443  NHVTVEHVGGPEQSSHVCFWEDCPREGKPFKAKYKLINHIRVHTGEKPFPCPFPGCGKVF  502

mouse   462  ARSENLKIHKRTHTGEKPFKCEFDGCDRKFANSSDRKKHSHVHTSDKPYYCKIRGCDKSY  521
             ARSENLKIHKRTHTGEKPFKCEFDGCDRKFANSSDRKKHSHVHTSDKPYYCKIRGCDKSY
human   503  ARSENLKIHKRTHTGEKPFKCEFDGCDRKFANSSDRKKHSHVHTSDKPYYCKIRGCDKSY  562

mouse   522  THPSSLRKHMKIHCKSPPPSPGALGYSSVGTPVGDPLSPVLDPTRSRSSTLSPQVTNLNE  581
             THPSSLRKHMKIHCKSPPPSPG LGYSSVGTPVG PLSPVLDP RS SSTLSPQVTNLNE
human   563  THPSSLRKHMKIHCKSPPPSPGPLGYSSVGTPVGAPLSPVLDPARSHSSTLSPQVTNLNE  622

mouse   582  WYVCQASGAPSHLHTPSSNGTTSESEDEEMYGNPEVMRTIH  622
             WYVCQASGAPSHLHTPSSNGTTSE+EDEE+YGNPEV+RTIH
human   623  WYVCQASGAPSHLHTPSSNGTTSETEDEEIYGNPEVVRTIH  663
```

Zic5CT289: 545 – 622 aa (mouse)
Zic5 FL290: 358 – 622 aa (mouse)

B

	# Peaks
WT.289	10081
WT.289.specific	7040
WT.290	7895
WT.290.specific	3475
WT.289.sp AND WT.290.sp	3475

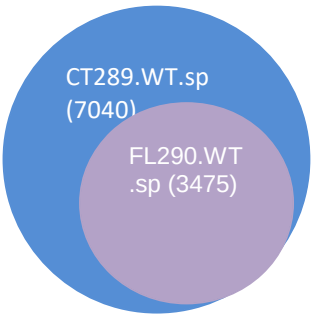
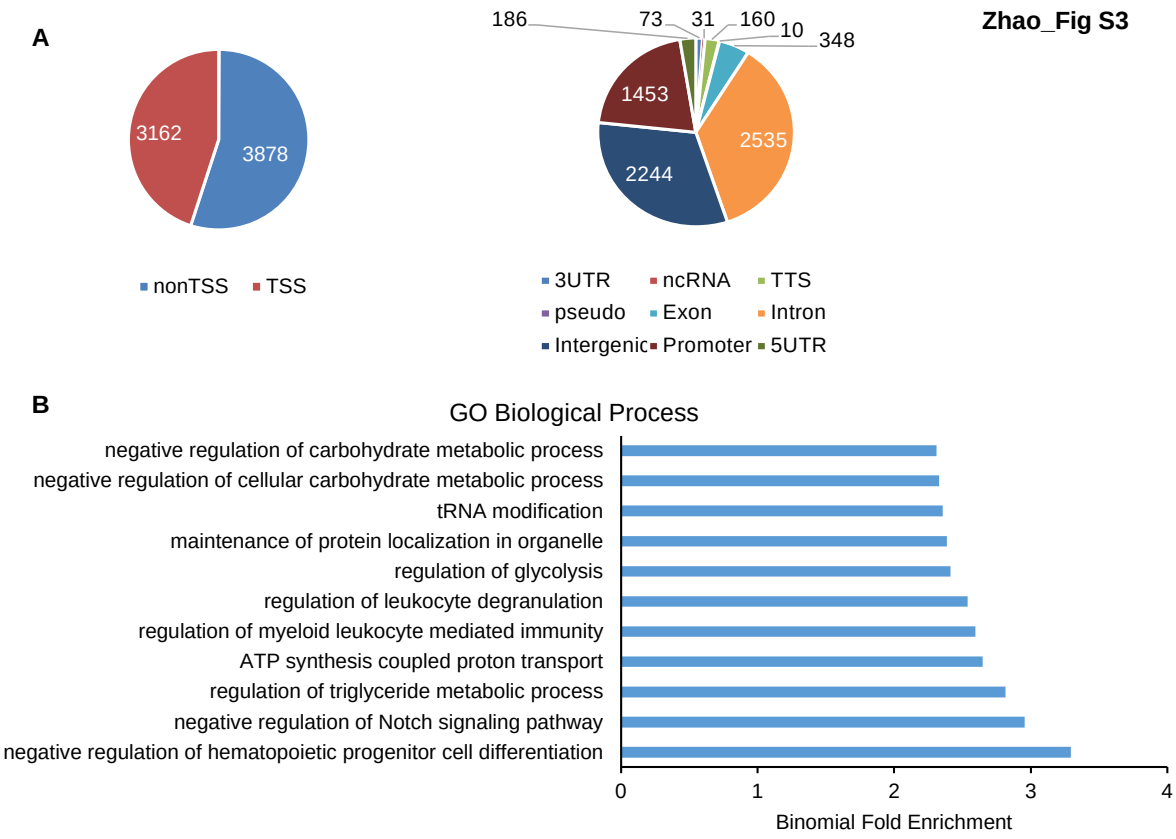
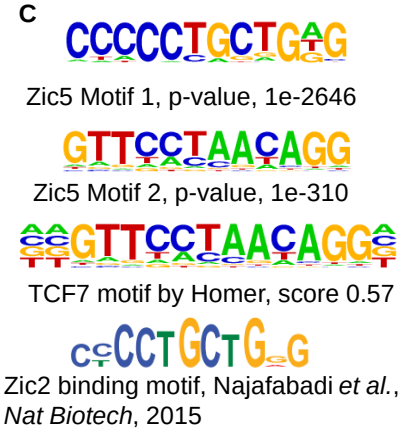


Fig. S2. Generation of our homemade antibodies against Zic5 for ChIP-seq analysis.



C



Zic5 Motif 1, p-value, 1e-2646

Zic5 Motif 2, p-value, 1e-310

TCF7 motif by Homer, score 0.57

Zic2 binding motif, Najafabadi *et al.*, *Nat Biotech*, 2015

D

Motif 1	Score
mES-Nanog-ChIP-Seq(GSE11724)/Homer	0.94
ZIC1/MA0696.1/Jaspar	0.9
ZIC4/MA0751.1/Jaspar	0.89
ZIC3/MA0697.1/Jaspar	0.89
PB0206.1_Zic2_2/Jaspar	0.82
K562-CTCFL-ChIP-Seq(GSE32465)/Homer	0.74
GLIS2/MA0736.1/Jaspar	0.72

E

Motif 2	Score
Ets1-distal(ETS)/CD4+-PolII-ChIP-Seq(Barski_et_al.)/Homer	0.58
POL009.1_DCE_S_II/Jaspar	0.57
PB0187.1_Tcf7_2/Jaspar	0.57
SA0002.1_at_AC_acceptor/Jaspar	0.55
Bcl6/MA0463.1/Jaspar	0.55
SNAI2/MA0745.1/Jaspar	0.54
STAT3/MA0144.2/Jaspar	0.53

Fig. S3. Comparative ChIP-seq analysis of ZIC5 in HCT116 ZIC5 WT and KO cells.

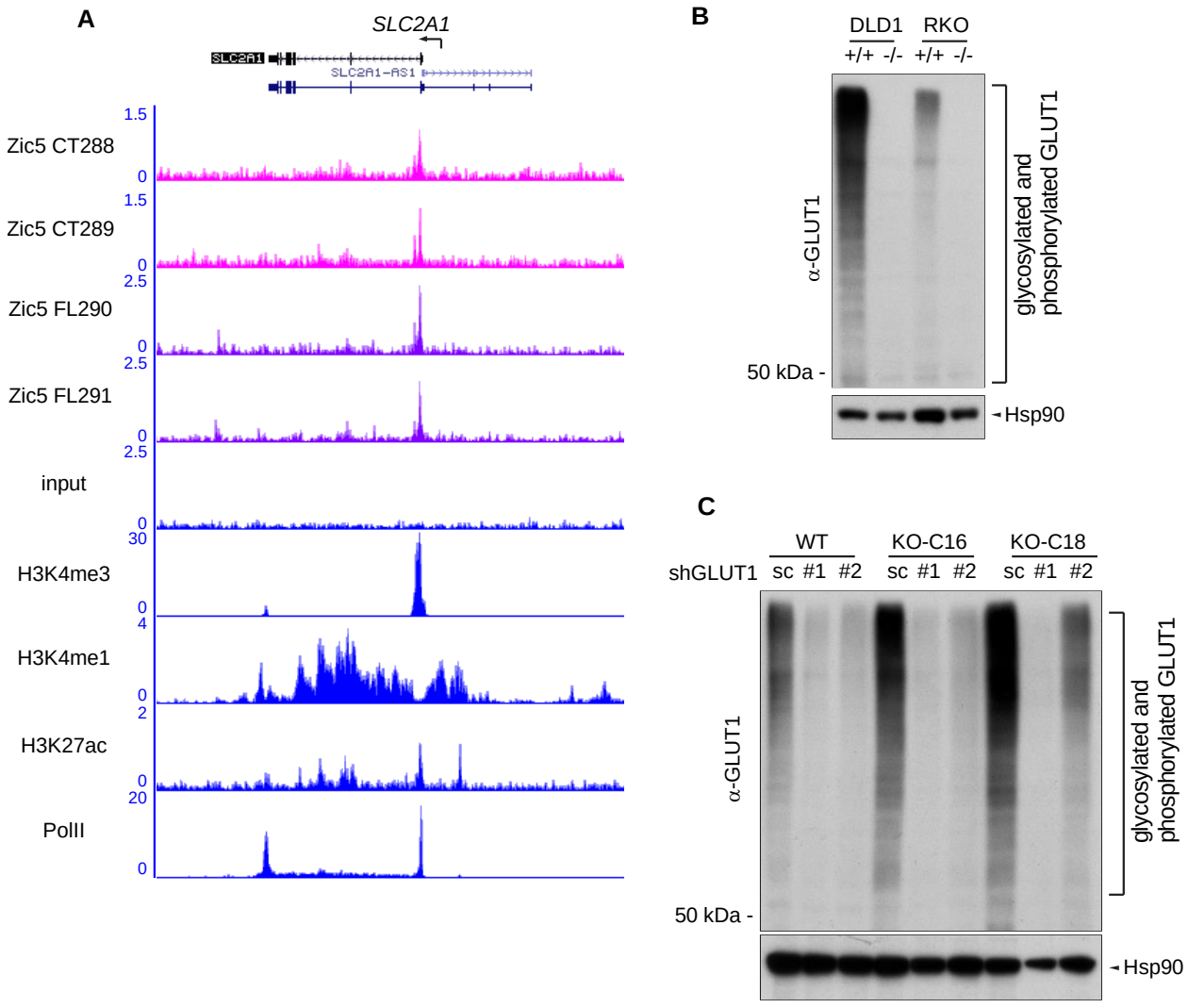


Fig. S4. Loss of Zic5 derepresses *GLUT1/SCL2A1* gene expression.

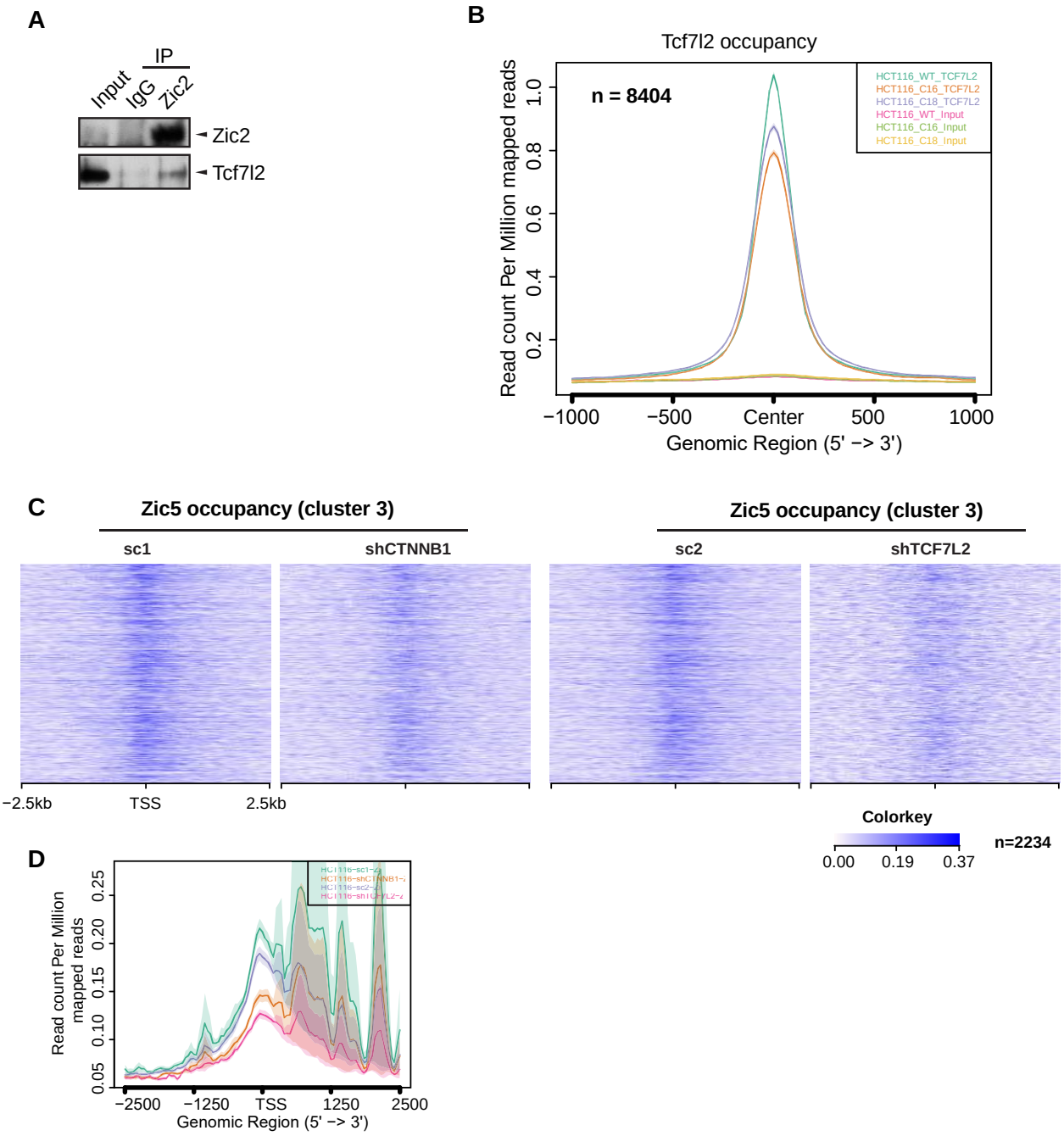
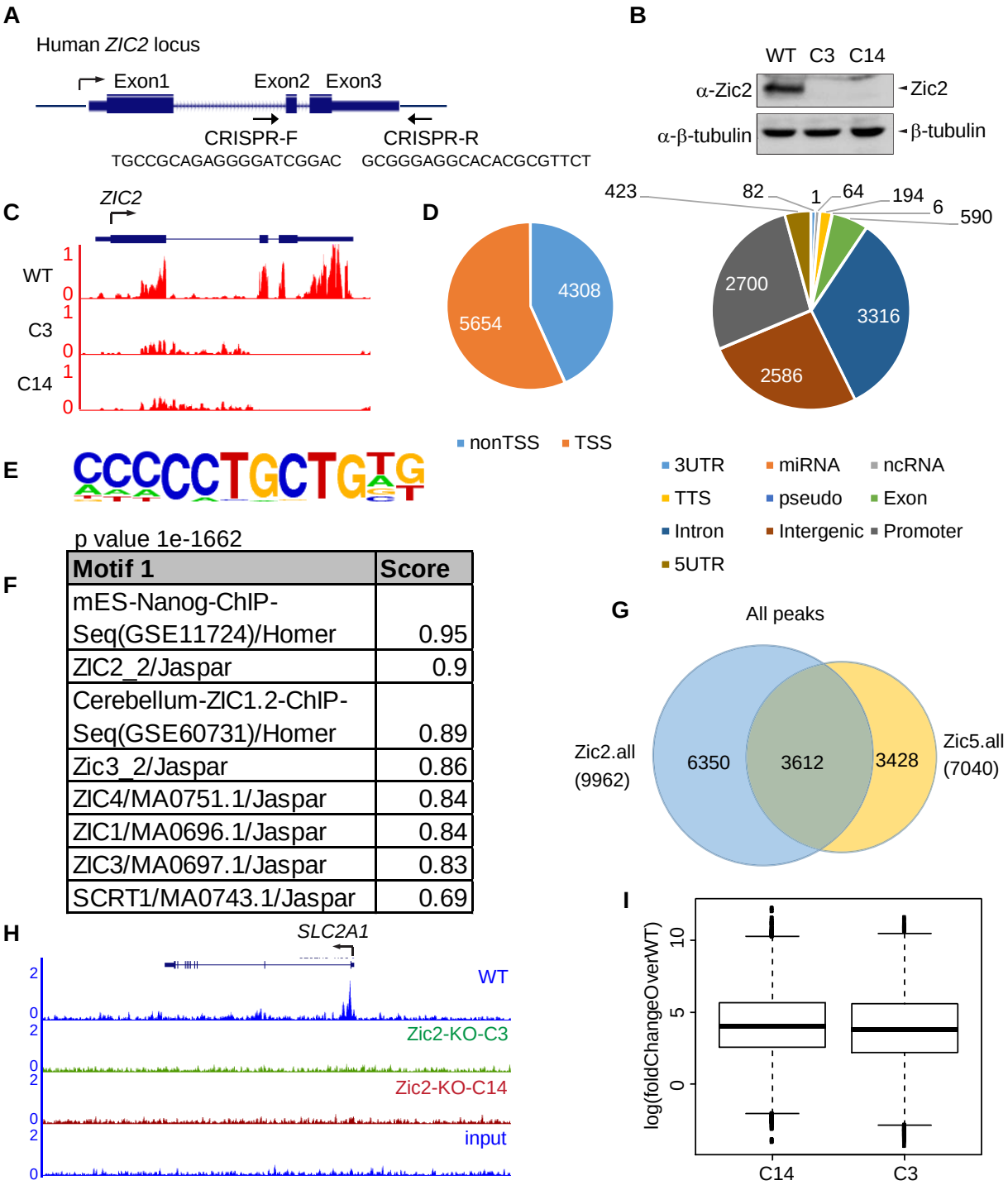


Fig. S5. β-Catenin/Tcf7l2 complex recruits Zic5 to regulate gene expression.



Motif 1	Score
mES-Nanog-ChIP-Seq(GSE11724)/Homer	0.95
ZIC2_2/Jaspar	0.9
Cerebellum-ZIC1.2-ChIP-Seq(GSE60731)/Homer	0.89
Zic3_2/Jaspar	0.86
ZIC4/MA0751.1/Jaspar	0.84
ZIC1/MA0696.1/Jaspar	0.84
ZIC3/MA0697.1/Jaspar	0.83
SCRT1/MA0743.1/Jaspar	0.69

Fig. S6. Comparative ChIP-seq analysis of ZIC2 in HCT116 ZIC2 WT and KO cells.

Table S1. Mass spectrometry data.