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Supplemental information

lncRNA PRR34-AS1 promotes HCC development via modulating Wnt/β-catenin pathway by absorbing miR-296-5p and upregulating E2F2 and SOX12

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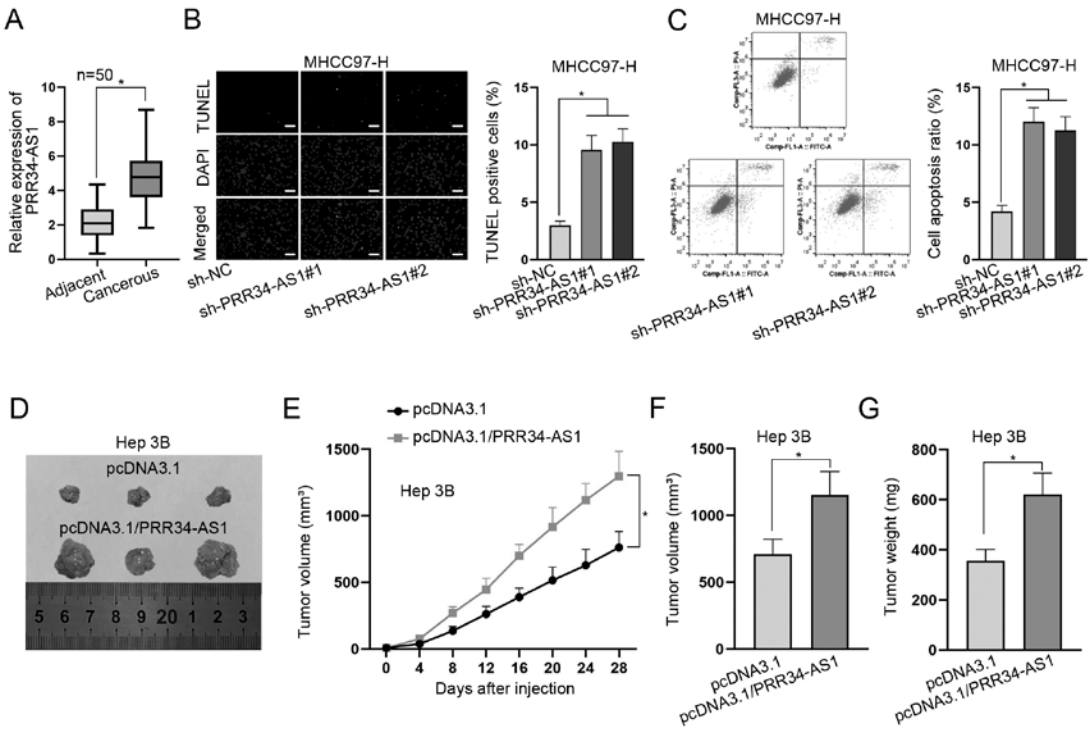

Figure S1 PRR34-AS1 promotes HCC cells biological behaviors and growth

A. RT-qPCR determined PRR34-AS1 expression in 50 HCC samples and paired non-cancerous samples. B-C. The apoptosis rate of PRR34-AS1-silenced MHCC97-H cells was measured via TUNEL assays and flow cytometry analysis. D. Representative images of xenografts originated from Hep 3B cells with or without PRR34-AS1 overexpression. E-G. Tumor growth curves, tumor volume and weight recorded in above two groups. $^{*}P<0.05$.

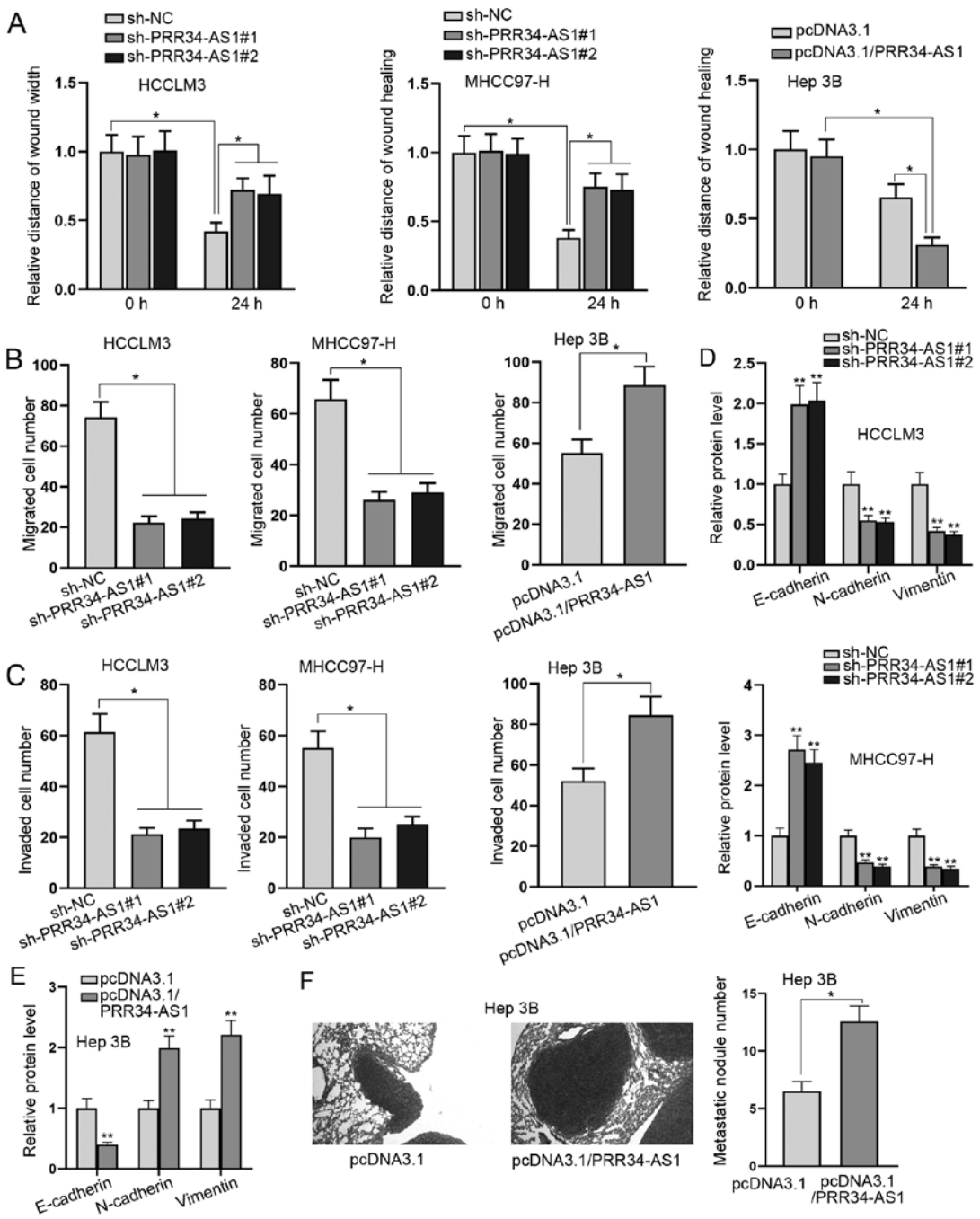

Figure S2 PRR34-AS1 facilitates cell migration, invasion and EMT process *in vitro* and metastasis *in vivo* in HCC

A. Quantification of wound gaps monitored in wound healing assays which were conducted in HCC cells upon PRR34-AS1 silencing or up-regulation. B-C. Quantification of migrated or invaded cell number was made based on the images from Transwell experiments in HCC cells upon PRR34-AS1 silencing or

up-regulation. D-E. Quantification of protein bands was made in the western blot assay of Figure 3D. F. HE staining evaluated the metastatic ability of Hep 3B cells upon PRR34-AS1 overexpression. $^{*}P<0.05$, $^{**}P<0.01$

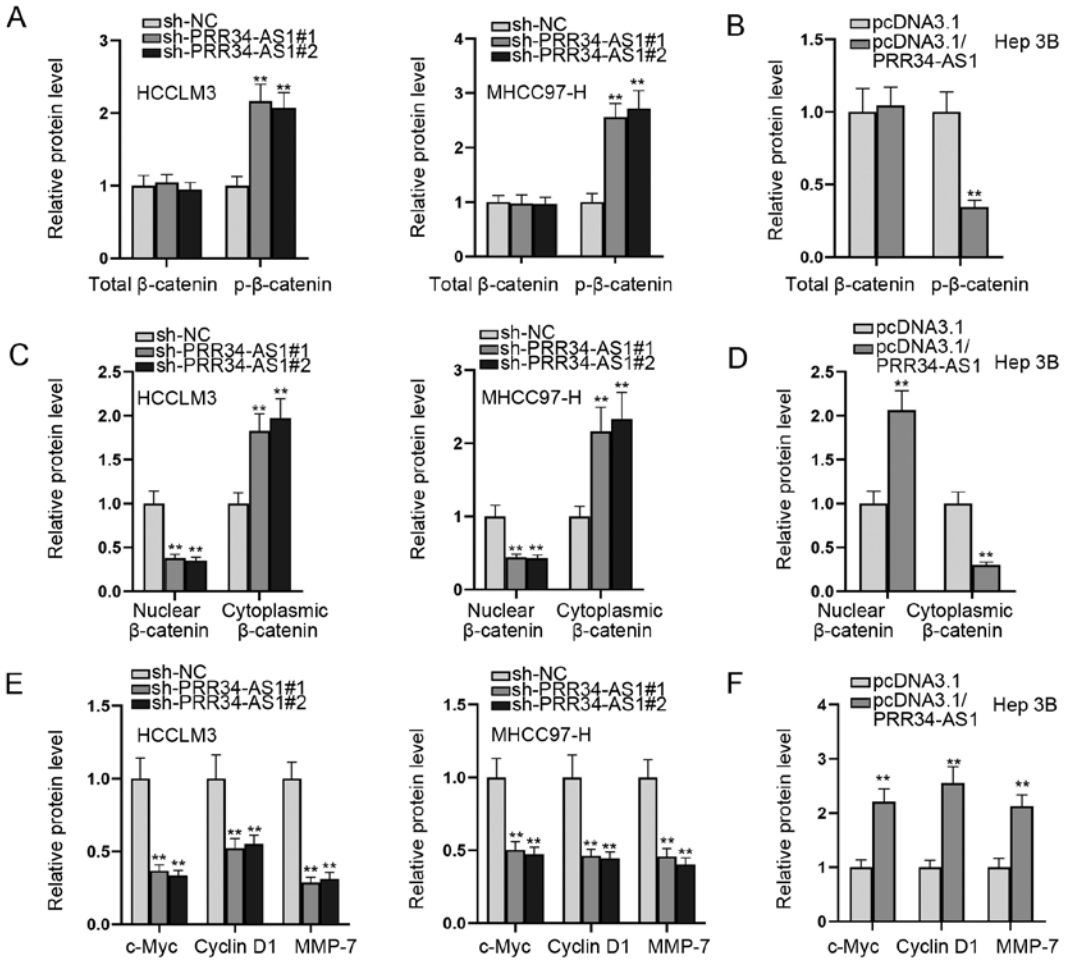

Figure S3 PRR34-AS1 positively regulates the Wnt/β-catenin pathway.

A-B. Quantification of protein bands was made in the western blot assay of Figure 3G.

C-D. Quantification of protein bands was made in the western blot assay of Figure 3H.

E-F. Quantification of protein bands was displayed in the western blot assay of Figure 3I. $^{**}P<0.01$.

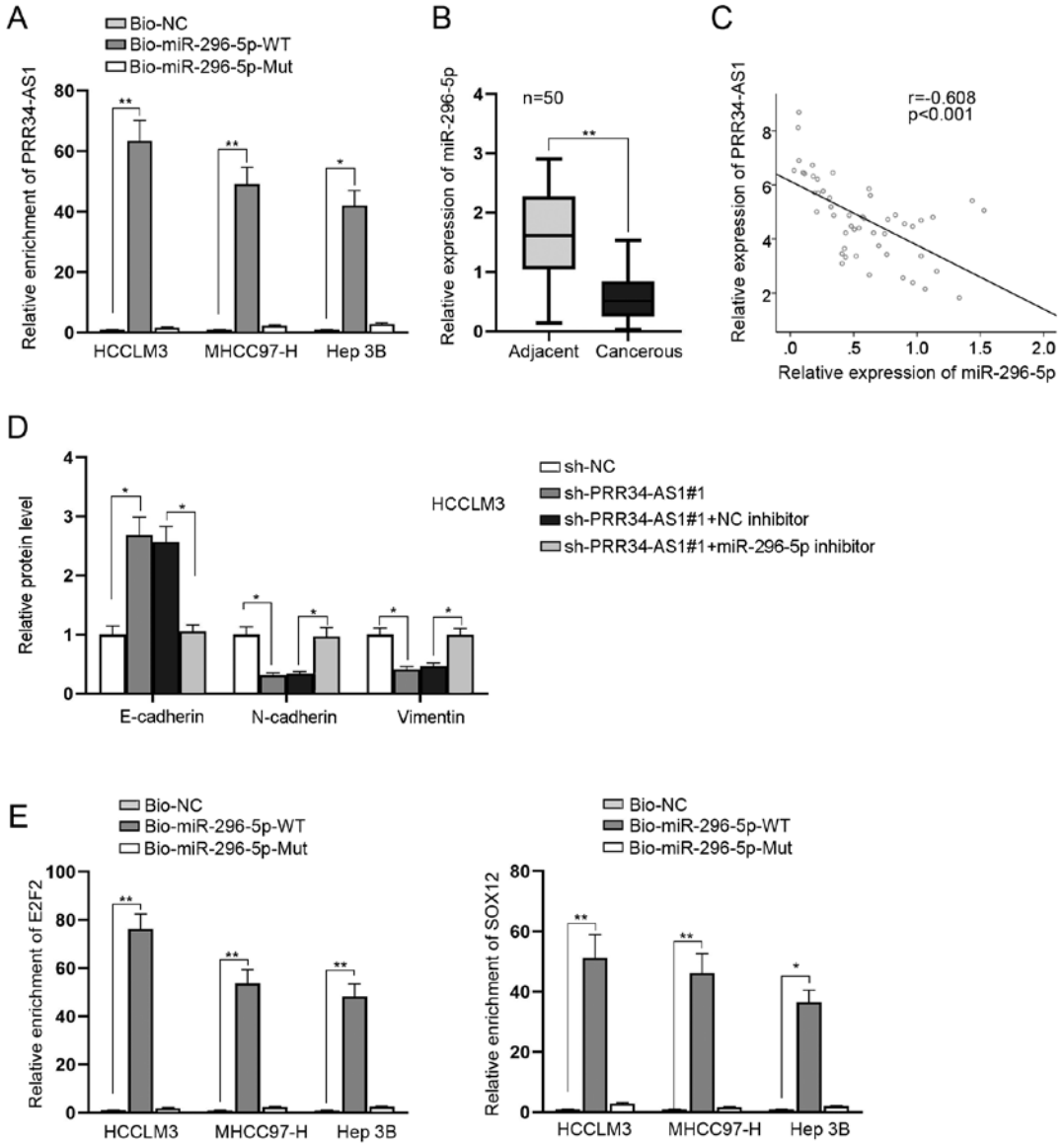

Figure S4 PRR34-AS1 interacts with miR-296-5p to affect E2F2 and SOX12 in HCC cells.

A. RNA pull down experiments examined the enrichments of PRR34-AS1 in Bio-miR-296-5p-WT/Mut groups in HCC cells. B. The expression of miR-296-5p in 50 pairs of clinical samples was tested by RT-qPCR. C. Pearson's correlation analysis determined the relationship between PRR34-AS1 and miR-296-5p in 50 HCC tissues. D. Quantification of protein bands in the western blot assay of Figure 5I. E. RNA pull

down assays confirmed the enrichments of E2F2/SOX12 in Bio-miR-296-5p-WT/Mut groups in HCC cells. $^{*}P<0.05$, $^{**}P<0.01$.

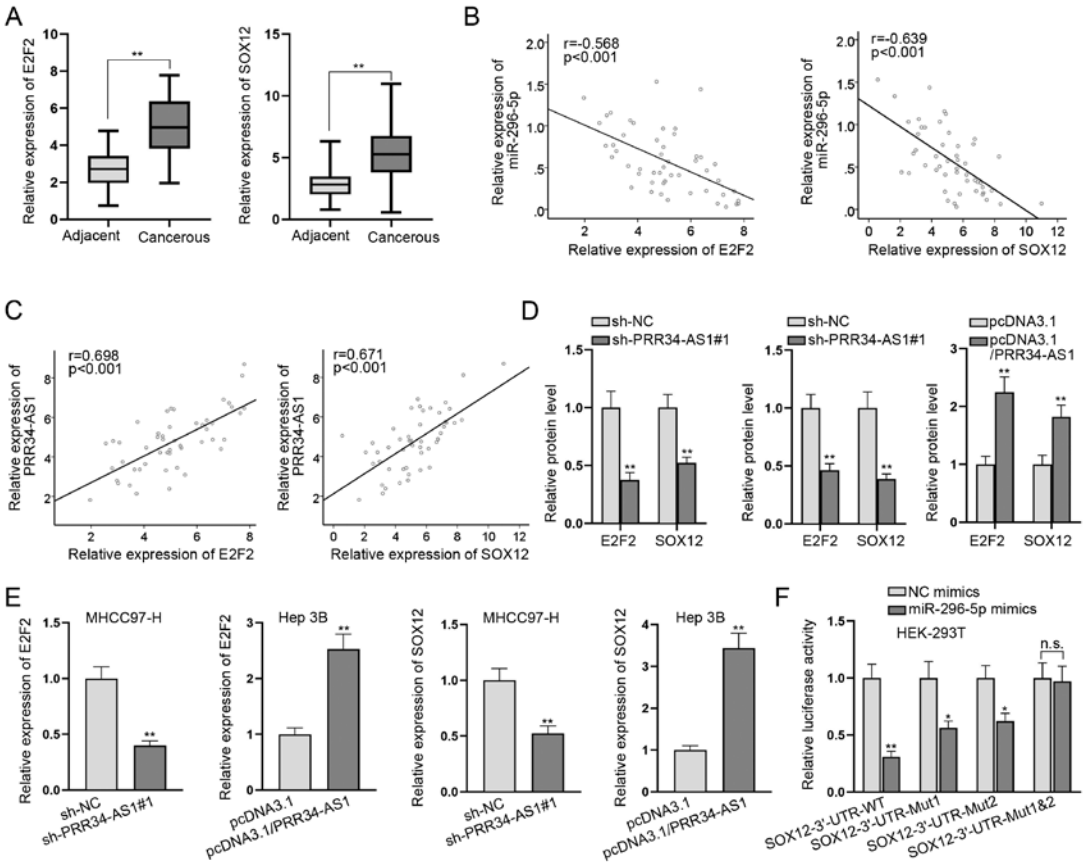

Figure S5 PRR34-AS1 positively modulates E2F2 and SOX12 via sponging miR-296-5p.

A. The expression of E2F2 and SOX12 in 50 pairs of clinical samples was tested by RT-qPCR. B. Pearson's correlation analysis determined the relationship between E2F2/SOX12 and miR-296-5p in 50 HCC tissues. C. Pearson's correlation analysis determined the relationship between E2F2/SOX12 and PRR34-AS1 in 50 HCC tissues. D. Quantification of protein bands in the western blot assay of Figure 6E. E. The impacts of PRR34-AS1 overexpression or silencing on the levels of E2F2 or SOX12 were assessed by RT-qPCR. F. Luciferase reporter experiments examined the binding ability between miR-296-5p and indicated SOX12 3'UTR sequences in HEK-293T cells. $^{*}P<0.05$, $^{**}P<0.01$, n.s.: no significance.

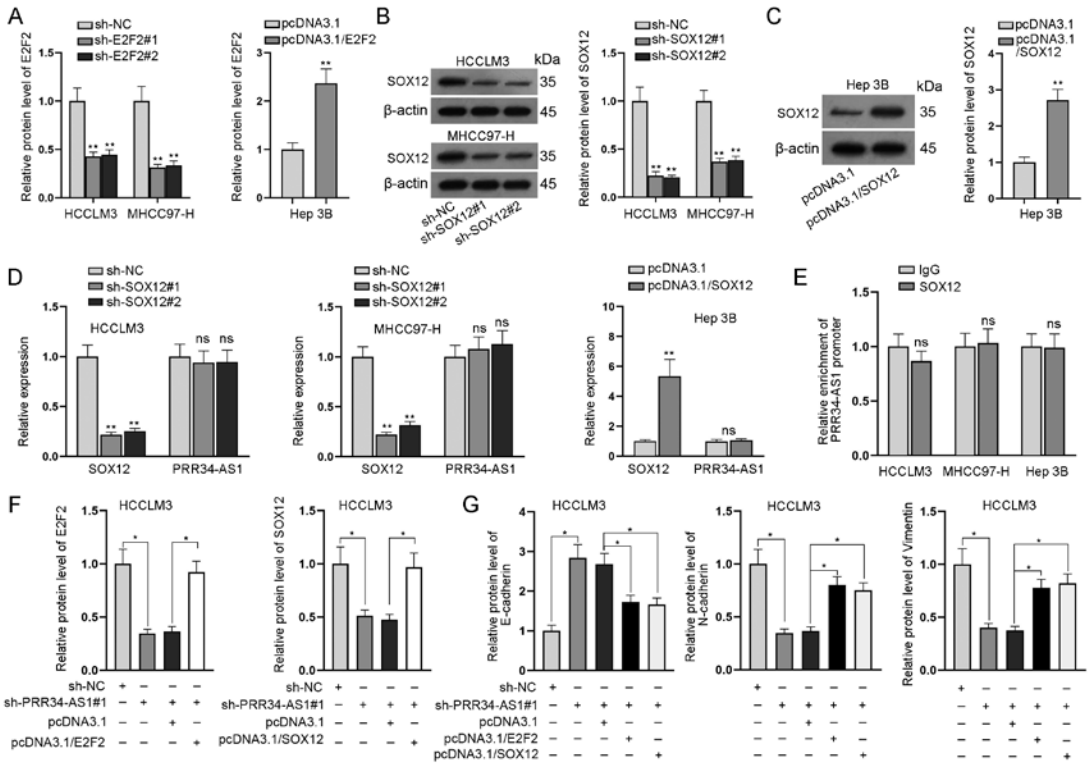

Figure S6 E2F2 regulates PRR34-AS1 in HCC cells

A. Quantification of protein bands in the western blot assay of Figure 7A. B-C. Western blot analysis examined the knockdown or overexpression efficiency of SOX12 in HCC cells, as well as the quantification bar graphs. D. RT-qPCR analyzed the expression levels of SOX12 and PRR34-AS1 in HCC cells after SOX12 was down-regulated or up-regulated. E. The binding capacity between PRR34-AS1 promoter and SOX12 was determined via ChIP assay. F. Quantification of protein bands in the western blot assay of Figure 8B. G. Quantification of protein bands in the western blot assay of Figure 8J. $^{*}P<0.05$, $^{**}P<0.01$, n.s.: no significance.

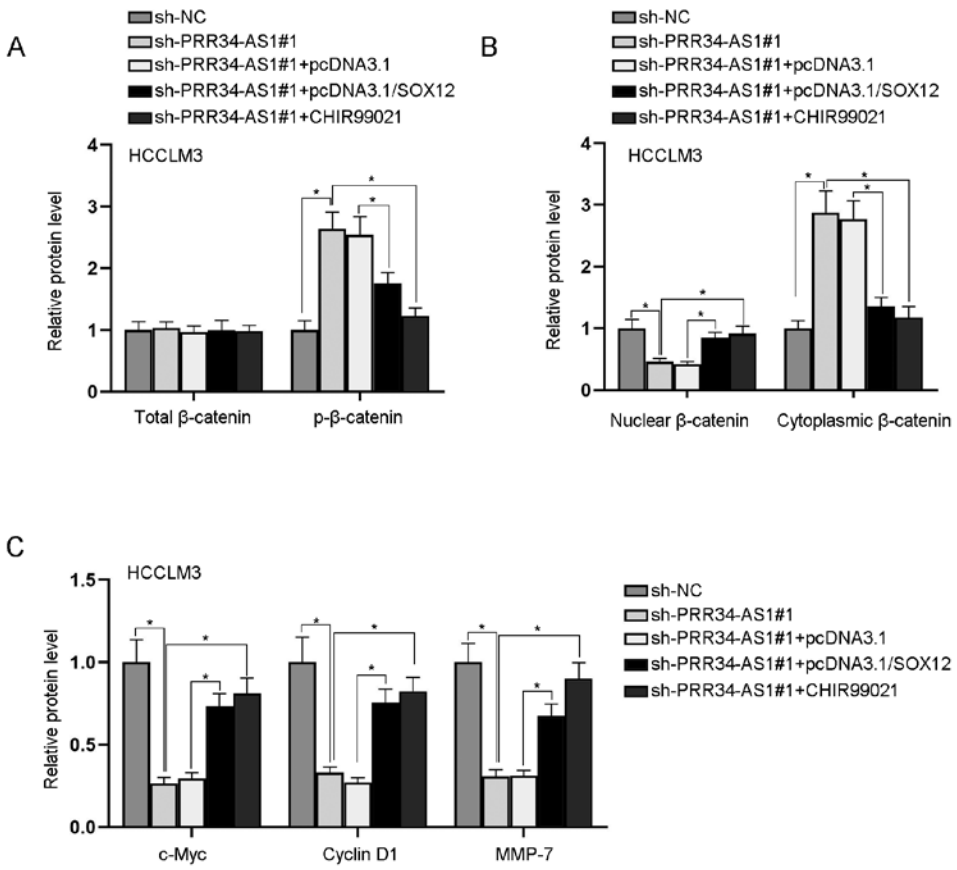

Figure S7 PRR34-AS1 relies on SOX12 to activate Wnt/β-catenin pathway

A. Quantification of protein bands in the western blot assay of Figure 9B. B. Quantification of protein bands in the western blot assay of Figure 9C. C. Quantification of protein bands in the western blot assay of Figure 9D. $^{*}P<0.05$.

Supplementary Table 1 Sequences of transfection plasmids.

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Plasmids	Sequences (5' > 3')
sh-NC (for PRR34-AS1)	CCGGAGTAGCTGAATGACTAGCCACCTCGAGGTGGCTAGTCATTCAGCTACTTTTTTG
sh-PRR34-AS1#1	CCGGTAAGTTAACGCCAAATCGCGGCTCGAGCCGCGATTTGGCGTTAACTTATTTTTG
sh-PRR34-AS1#2	CCGGAGATGAATAAATTGAAGACATCTCGAGATGTCTTCAATTTATTCATCTTTTTTG
sh-PRR34-AS1#3	CCGGAAGAATCGTTCTTTAAAAGTGCTCGAGCACTTTTAAAGAACGATTCTTTTTTTG
sh-NC (for E2F2)	CCGGTATGCGTGCTGTGCATCTACTCTCGAGAGTAGATGCACAGCACGCATATTTTTG
sh-E2F2#1	CCGGTCCGTGCTGTTGGCAACTTTACTCGAGTAAAGTTGCCAACAGCACGGATTTTTG
sh-E2F2#2	CCGGCCACTCTATAAGCAGGGCTAACTCGAGTTAGCCCTGCTTATAGAGTGGTTTTTG
sh-NC (for SOX12)	CCGGGGCAGCACAGGCGGAGAGATACTCGAGTATCTCTCCGCCTGTGCTGCCTTTTTG
sh-SOX12#1	CCGGGCAGCACGAACGGCGGAAGATCTCGAGATCTTCCGCCGTTCGTGCTGCTTTTTG
sh-SOX12#2	CCGGGCCTTTAATGAGGACTAAGAACTCGAGTTCTTAGTCCTCATTAAAGGCTTTTTG
NC mimics	GCCUCACCCGUACCGGAUCCU
miR-296-5p mimics	AGGGCCCCCCCUCAAUCCUGU
NC inhibitors	AGGCGAGGAUGGUGAGGCCCU
miR-296-5p inhibitors	ACAGGAUUGAGGGGGGGCCCU
pcDNA3.1/PRR34-AS1	CGCGGAGCACGGAGGACGGGGCCGGGCGACCTGGGCACCAGCAGGACCCGAGGCCAGGA GCCAGGGGCCCAGAAGATTGAGCTTCTAGAGCCTCAGAGATGGAATTCGCCGTTTTGCCGC GATTTGGCGTTAACTTATTGACCCATGGGGAGGAGGGTCACTTCCCGTGAAAAGAAGGCAG AGATGTTTTGCTGTGCCCAGTGTGAGGAAGCGAAGGAAAAGAAAGATCTTTTGAAAATGTGT ACATCTACTTGCAGCTTGAAAATTCCAAGTGCCAGACATTTTTCTTGGAGGAAATAAATGTCT TCAATTTATTCATCTTACCTGTTGATTATGTTCTGTAATGATTTATTTTATAATTTAATCCTTGTC TAGAACCGAGGCCATCTTTGGAAAGTAAAAGACCTAGGTCTCTTTGGTCCAGGAGACATTGT GGCCCTCTCTTAAAATCATTGACTGCCGAGATCTGGGCCCAGGCGGCTCTCGGACTGAACCG GTCTGTGCTAGGCAGCCTGGCCCACTCGGCCTTCAAAACACAAGGGATGGGAGACATATGC TCGGCTCACATCGTTTTGTTTTGATTTATTTTAAACTGCTTAAGATGATTTTAGCACAAGGAGT GGTGTATCTTACTTGCATTCTGAGAGCCACTGGATTTGCAAGCTAGAGTACAATTTTTTTTTTA ATCCAAATTTAGAAATTGCCTTTTAGAGTAGACAACAGCATGCTACTTTGCCTTTTAACTCACT TTTAAAGAACGATTCTTAAGATCAAGTACATTGAATTTGGAGATTGGTCCCCCTCCGTTATTA GGGGCTGGAAATGGCCCACTTTTCAAAAATAAGGTCAGAAAAGGGGTGTTTCTGACCTAAGA GTTCTTCCAGGCTGGGCTCGTACCACAGAGATTCTTCCACACCCATGTCCTGAGTCCTTGTCC TACACACCATTTTTTCTTCCAAGGAGAAGGAGTGTGGCATTCAGTTCCAGTGTCATCGTGTGG AAAAGAGTCCCTGCCTGGCTGAGAAACTGTCACAGGCAGCCGGGCCACTTCCCAGCATGAC CCACAAGCCCGATGGTGCCCCTCGAATGGTTAAGGGACCCGTACAGATACCATTCTTTTAAA GCAAGCCAATGCAATCTTCACGAATTGCAGTTCCACCCTGGCTGCTGGTACTAACTCTAATAA TGGAAAAAAATTTAAAAGATTGGGGACAACAGGAAACACATTGGATCCCCAGGGGAAACGG CCTGGAAGCTACAGTAGAGACATGGGTGACCCAAGGGCTCTGTTCAAGTCCTGGGGCTGTT CCCTTTATTCCTCCAAGCCTCAGCTCCCCGGATTTAAAGTGAGAACAGCACCCAGCCCAGCC CACTGTCAAGGGCTGTTGCAGGAATATGACAACAGCCACCAATATTTGCATAGCAGAGATGC CCAGTTTCGTTTTCTATTTGAAAGTTTCTCTGAAGGGGGATGTGCTAGAGACACGAGAACAA CTGCTACCATCTTAATAACTTTTCTGGCAATACACGACGATGATTGTTTATGTTAATCTCATAA CTATTAACAATAATTCATTTCATTCTCATAATGAATTATATCTATTTGACTTATAATAAATAGAA TCATATCAGTAAAAAAAAAAAAAAAAAA
pcDNA3.1/SOX12	ATGGTGCAGCAGCGGGGCGCGAGGGCCAAGCGGGACGGCGGGCCGCCGCCCCCGGGACC CGGGCCGGCCGAGGAGGGGGCGCGCGAGCCCGGCTGGTGCAAGACCCCGAGCGGCCACA TCAAGAGGCCGATGAACGCATTCATGGTGTGGTCGCAGCACGAACGGCGGAAGATCATGGA CCAGTGGCCCGACATGCACAACGCCGAGATCTCCAAGCGCCTGGGCCGCCGCTGGCAGCTG CTGCAGGACTCGGAGAAGATCCCGTTCGTGCGGGAGGCGGAGCGGCTGCGGCTCAAGCAC ATGGCGGATTACCCGGACTACAAGTACCGGCCGCGCAAAAAGAGCAAGGGGGCGCCCGCC AAGGCGCGGCCCCGCCCCCCCGGTGGTAGCGGTGGCGGCAGCCGGCTCAAGCCCGGGCC GCAGCTGCCTGGCCGCGGGGGCCGCCGAGCAGCGGGAGGGCCTTTGGGGGGCGGGGCGG CGGCGCCCGAGGACGACGATGAAGACGACGACGAGGAGCTGCTGGAAGTGCGCCTGGTCG AGACCCCGGGGCGGGAGCTGTGGAGGATGGTCCCGGCGGGACGGGCCGCTCGGGGACAA GCGGAGCGCGCCCAAGGGCCGTCGGGCGAGGGGGCGGCCGCCGCCGCCGCCTCCCC GACACCGTCGGAGGACGAGGAGCCGGAGGAAGAGGAGGAGGCGGCAGCGGCTGAG GAAGGTGAAGAGGAGACGGTGGCGTCGGGGGAGGAGTCGCTGGGCTTTCTGTCCAGGCTG CCCCCTGGCCCGGCCGGCCTGGACTGCAGCGCCCTGGATCGCGACCCGGACCTGCAGCCTC CCTCGGGCACGTCGCACTTCGAGTTCCCGGACTACTGCACCCCCGAGGTTACCGAGATGATC GCGGGGGACTGGCGCCCGTCTAGCATCGCAGACCTGGTTTTCACCTACTGA

Supplementary Table 2 Sequences of primers.

A	B	C
Supplementary Table 2 Sequences of primers		
Primers	**Sequences (5' > 3')**	Accession Number
PRR34-AS1-forward (F)	CCGCGATTTGGCGTTAACTT	NR_027034.1
PRR34-AS1-reverse (R)	TCCAAAGATGGCCTCGGTTC	
miR-296-5p-F	ATTAGAGGGCCCCCCCTCAA	MIMAT0000690
miR-296-5p-R	CTCAACTGGTGTCGTGGA	
ZNF76-F	CAAGACCTCAGGAGACCTGC	NM_001292032.2
ZNF76-R	CCGTGCAAACGTATGGCTTC	
HMGA1-F	GCATCCGCATTTGCTACCAGC	NM_001319077.2
HMGA1-R	TCCTTCTGACTCCCTACCAGC	
FAM53B-F	CGCACAGGAGTTGACCACAT	NM_014661.4
FAM53B-R	GGGTGGGTATCAGCCATCTT	
FGFR3-F	ACCGACAAGGAGCTAGAGGT	NM_000142.5
FGFR3-R	TGAACAGGAAGAAGCCCACC	
E2F2-F	ACTCAAGGACTAGAGAGCGAG	NM_004091.4
E2F2-R	TTAGAGATCGCCGCTTGGA	
HIPK1-F	ACAGTTGGATCCCGTACCAC	NM_001369806.1
HIPK1-R	ATGCCATACTGAGGCGGAAG	
SOX12-F	CTGGAGTGGTGGGATTGGTC	NM_006943.4
SOX12-R	GGGTGTCAGAGGGACAAAGG	
CDK16-F	GATGAGAGTGGTGGTGGTGG	NM_001170460.2
CDK16-R	CCTCGTGCACAATCTCTGGT	
BMF-F	CCTCCTTCCCAATCGAGTCTG	NM_001003940.2
BMF-R	CCTCCTTCCCAATCGAGTCTG	
SLC16A3-F	GTCTGAAGGGGGACAGGTGAG	NM_001042422.3
SLC16A3-R	GTGATGACGAAACAGCCGAAG	
GAPDH-F	GGAGCGAGATCCCTCCAAAAT	NM_001256799.3
GAPDH-R	GGCTGTTGTCATACTTCTCATGG	
U6-F	CTCGCTTCGGCAGCACA	NR_004394.1
U6-R	AACGCTTCACGAATTTGCGT	

Supplementary Table 3 Sequences of protein.

Supplementary Table 3 Sequences of Protein		Accession Number
E-cadherin	nfedctgrqrtayfsldtrfkvgtdgvitvkrplrfhnpqihflvyawdstyrkfstkvtlntvghhhrppphqasvsgi qaelltfpnsspglrrqkrdwvippiscpenekgpfpknlvqiksnkdkegkvfysitgqgadtppvgvfiieretgwlk vtepldreriatytlfshavssngnavedpmeilitvtdqndnkpeftqevfkgsvmegalpgtsvmevtatdadddvnt ynaaiaytilsqdpelpdknmftinrntgvisvvttgldresfptytlvvqaadlqgeglsttatavitvtdtndnppi fnpttykgqvpeneanvvittlkvtdadapntpaweavytilnddgggfvvttnpvnndgilktakgldfeakqqyilh vavtnvvpfevslttstatvtvdvldvneapifvppekrvevsedfgvgqeitsytaqepdtfmeqkityriwrdtanw leinpdtgaistraeldredfehvknstytaliiatdngspvatgtgtlllilsdvndnapipeprtiffcernpkpqv iniidadlppntspftaelthgasanwtiqyndptqesiilkpkmalevgdykinlklmdnqnkdqvttlevsvcdceg aagvcrkaqpveaglqipailgilggilallililllflrrravvkepllppeddtrdnvyyydeegggeedqdfdl sqlhrgldarpevtrndvaptlmsvprylprpanpdeignfidenlkaadtdptappydsllvfdyegsgseaaslssl nssesdkdqdydylnewgnrfkkladmygggedd	CAA84586.1
N-cadherin	glkaadndptappydsllvfdyegsgstagslsslnsssggdqdydylndwgprfkkladmygggdd	AAP13881.1
Vimentin	mqlwkklqeemlqrgaqreetqstlgtfrqdvdtvslalldlerkvdslqeetdflkklhndesalcdvrqenes vaaknlqkveeqskskfadlcevan	KAB1275435.1
c-Myc	mplnanfpsknydydydlqpcfffleeenfyhqqsrlqppapsediwkkfellptpplspsrrssqsslfpstadqlemvt eflggdmvnqsficeaddeallksiviqdcmwsgfsaaaklekvvseklasyqasrkesalsssspcqsqpppsplkspsc hgslslggthrsshgflqdpssdcvdpsvvfpyplndsisnasspcqdliletppissnssssesgeepededdcdeeee idvvtvekrqsaskrvessshsqpsrphysplvlkrchvpihqhnyaaspstkvdyvsskraklesnirvlkqisnnrkca sprssdseendkrkthnvlerqrrnelklsffalrdqvpevasnekapkvvilkkateyaislqederrlireteqlkyrk eqlkqrlqqlrnfv	CAA37753.1
Cyclin D1	aeenkqiirkhaqtfvalcatdvkfisnppsmvaagsvvaavqglnlrspnnflsyyrltrflsrvikcdpdclracqeq ieallesslrqaqqnmdpkaaeeeeeeeevdlactptdvrdvdi	CAA54800.1
MMP-7	vpnvaefslfpnhpkwtskvvtyrimsytsdlphitvnqlvakafkiwseaipltfkrlrwgtadimigfarrahgdpypf dgpgatlahafapgpglggdahfdederwtdgigigvnflyvath	AAD30285.1
β-actin	mkiltergytfttaereivrdikeklcyvaldfeqemttaasssleksyelpdgqvitignerfrcpealfqpsflgme scgihettynsimkcdvdirkdlyantvlsggttmypgiadrmqkeitalapstmkikiiapperkysvwiggsilaslst fqqmwiskqeydesgpsivhrkcf	SAY41341.1
E2F2	riyditnvlegvqlirkkskknniqwlmgnvfeggvaggekafalrrelgdleraersldd liqsstt qlkq ltefkdsqslgyvtyqdirsigslrdqtviavkapaetklevpdtagqgslqiylksrngpievylcpeeg ledaspvkslatprkedlkkpvttsmtpqsqavkde	JAP00074.1
SOX12	vwsqiqrakiveeqpnkhnaaiskqlgsewkmlsdearmpyihesqrlkrihkqqypdy	AAX16100.1