

Supplementary Information

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RNF2 regulates Wnt/ β -catenin signaling via TCF7L1 destabilization

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Table 1

Genes	Full name	Role	Rank
TRIM33	E3 ubiquitin-protein ligase TRIM33	E3 ligase	854
RNF2	E3 ubiquitin-protein ligase RING2	E3 ligase	39
RNF169	E3 ubiquitin-protein ligase RNF169	E3 ligase	203
CBLL1	E3 ubiquitin-protein ligase Hakai	E3 ligase	980
RING1	Isoform 2 of E3 ubiquitin-protein ligase RING1	E3 ligase	29
STUB1	E3 ubiquitin-protein ligase CHIP	E3 ligase	1014
USP7	Ubiquitin carboxyl-terminal hydrolase 7	DUB	134
USP14	Isoform 3 of Ubiquitin carboxyl-terminal hydrolase 14	DUB	1126
OTULIN	Ubiquitin thioesterase otulin	DUB	1390
UBA1	Ubiquitin-like modifier-activating enzyme 1	E1 enzyme	585
HERPUD1	Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein	ER protein with Ubiquitin like domain	16
UBAP2	Ubiquitin-associated protein 2	Ubiquitin associated protein2	509
UBAP2L	Isoform 5 of Ubiquitin-associated protein 2-like	Ubiquitin associated protein2 Like	1459
URM1	Ubiquitin-related modifier 1	Ubiquitin like protein	1356
ADRM1	Proteasomal ubiquitin receptor ADRM1	Ubiquitin receptor	269

Table 2

Candidate	Role	Rank (1470)
RING1	E3 ligase	29
RNF2	E3 ligase	39
RNF169	E3 ligase	203
TRIM33	E3 ligase	854
CBLL1	E3 ligase	980
STUB1	E3 ligase	1014

Table 3

Genes	Target sequence	Primer sequences (5'→3')
STUB1	GAAGAGGAAGAAGCGAGACAT	CCGGGAAGAGGAAGAAGCGAGACATCTCGAGATGTCTCGCTTCTTCTCTCTTTTG
		AATTCAAAAAGAAGAGGAAGAAGCGAGACATCTCGAGATGTCTCGCTTCTTCTCTCTC
URM1	GCTCCTGTTTGACGGTATTAA	CCGGGCTCCTGTTTGACGGTATTAACCTCGAGTTAATACCGTCAAACAGGAGCTTTTTG
		AATTCAAAAAGCTCCTGTTTGACGGTATTAACCTCGAGTTAATACCGTCAAACAGGAGC
UBAP2L	GCCAATACTGATGATAACTAT	CCGGGCCAATACTGATGATAACTATCTCGAGATAGTTATCATCAGTATGGCTTTTTG
		AATTCAAAAAGCCAATACTGATGATAACTATCTCGAGATAGTTATCATCAGTATGGC
UBAP2	CGTGGAACAACAACCGGAAA	CCGGCGTGGAACAACAACCGGAAACTCGAGTTCCGGTTGTTGTTCCACGTTTTTG
		AATTCAAAAACGTGGAAACAACAACCGGAAACTCGAGTTCCGGTTGTTGTTCCACG
RNF169	ATCAACTCCACGCAACCTAAA	CCGGATCAACTCCACGCAACCTAACTCGAGTTAGGTTGCGTGGAGTTGATTTTTG
		AATTCAAAAATCAACTCCACGCAACCTAACTCGAGTTAGGTTGCGTGGAGTTGAT
ADRM1	CAGACGGACGACTCGCTTATT	CCGGCAGACGGACGACTCGCTTATTCTCGAGAATAAGCGAGTCGTCCTGTTTTG
		AATTCAAAAACAGACGGACGACTCGCTTATTCTCGAGAATAAGCGAGTCGTCCTG
UBA1	CCACTGCCTTCTACCTTGTTT	CCGGCCACTGCCTTCTACCTTGTTTCTCGAGAAACAAGGTAGAAGGCAGTGGTTTTG
		AATTCAAAAACCACTGCCTTCTACCTTGTTTCTCGAGAAACAAGGTAGAAGGCAGTGG
RNF2	GCCAGGATCAACAAGCACAAT	CCGGGCCAGGATCAACAAGCACAATCTCGAGATTGTGCTTGTGATCCTGGCTTTTTG
		AATTCAAAAAGCCAGGATCAACAAGCACAATCTCGAGATTGTGCTTGTGATCCTGGC
RING1	CTGGAGCTGGTGAATGAGAAA	CCGGCTGGAGCTGGTGAATGAGAAAACCTCGAGTTTCTATTACCAGCTCCAGTTTTG
		AATTCAAAAACCTGGAGCTGGTGAATGAGAAAACCTCGAGTTTCTATTACCAGCTCCAG
HERPUD1	CCTCCTCTGACGTTGTAAAT	CCGGCCTCCTCTGACGTTGTAAATCTCGAGATTACAACGTCAGGAGGAGTTTTG
		AATTCAAAAACCTCCTCTGACGTTGTAAATCTCGAGATTACAACGTCAGGAGGAGG
OTULIN	CCCTTAGTAGTAACGGGTTT	CCGGCCTTAGTAGTAACGGGTTTCTCGAGAAACCGTTACTACTAAAGGTTTTG
		AATTCAAAAACCTTTAGTAGTAACGGGTTTCTCGAGAAACCGTTACTACTAAAGGG
USP14	CCCAAGATTCAGCAGTCAGAT	CCGGCCCAAGATTCAGCAGTCAGATCTCGAGATCTGACTGCTGAATCTGGGTTTTG
		AATTCAAAAACCCAAGATTCAGCAGTCAGATCTCGAGATCTGACTGCTGAATCTGGG
CBLL1	TCATATCAACCATCGCCATAT	CCGGTCATATCAACCATCGCCATATCTCGAGATATGGCGATGGTTGATATGTTTTG
		AATTCAAAAATCATATCAACCATCGCCATATCTCGAGATATGGCGATGGTTGATATGA
TRIM33	TACTTTCCAGTTGCGTCATAT	CCGGTACTTTCCAGTTGCGTCATATCTCGAGATATGACGCAACTGGAAAGTATTTTTG
		AATTCAAAAATACTTTCCAGTTGCGTCATATCTCGAGATATGACGCAACTGGAAAGTA
USP7	CCTGGATTTGTGGTTACGTTA	CCGGCCTGGATTTGTGGTTACGTTACTCGAGTAACGTAACCACAAATCCAGTTTTG
		AATTCAAAAACCTGGATTTGTGGTTACGTTACTCGAGTAACGTAACCACAAATCCAGG

Table 4

Genes	Primer sequences (5'→3')
UBAP2L	TGAGCCACAGTGAGGAGATTCC
	AGATTCGCCTCACTCTCCACAC
OTULIN	GACAGCTTCTGAGGAACACCT
	TCCGTGTTGTACTTGGAGAGCC
URM1	CATCGAGTCACTTTGCCTGGAC
	ACGCTGTCTCCCTGGATGAACA
USP14	GGGAAATGGCTTCAGCGCAGTA
	CACCTTTCTCGGCAAACCTGTGG
HERPUD1	CCAATGTCTCAGGGACTTGCTTC
	CGATTAGAACCAGCAGGCTCCT
CBLL1	AACAGGATGCCTGCAAAGGCTC
	GGTGTCCAGGAAATCTTCGCTG
ADRM1	AAGCGGAAAGGGCTGGTGTACA
	CCGCTTGAACCTCACAGTCGTCA
UBA1	TCCTCACAGAGGACAAGTGCCCT
	CTTGAGCAGCTCACAGCCAATG
RNF2	CAGTCACAGCATTGAGGAAGGAC
	GCTTCTGATTGCTATGTGTGGA

Table 5

Genes	Target sequence	Primer sequences (5'→3')
RNF2	TGAGTTACAACGAACACCTC	CACCGTGAGTTACAACGAACACCTC
		AAACGAGGTGTTTCGTTGTAACCTAC
RNF169	GAGTACTCGGGCCTCTTCCG	CACCGGAGTACTCGGGCCTCTTCCG
		AAACCGGAAGAGGCCCGAGTACTCC
STUB1	GAATCGCGAAGAAGAAGCGC	CACCGGAATCGCGAAGAAGAAGCGC
		AAACGCGCTTCTTCTTCGCGATTCC
TRIM33	GTGTTGTGTACCGATACTAC	CACCG CATCATAGCCATGTGTTGTG
		AAACCACAACACATGGCTATGATGC
USP7	AGATGTATGATCCCAAACG	CACCG AGATGTATGATCCCAAACG
		AAACCGTTTGGGATCATACTCTC
RING1	TAAGATCTATCCTAGCCGGG	CACCG TAAGATCTATCCTAGCCGGG
		AAACCCCGGCTAGGATAGATCTTAC

Table 6

Genes	Primer sequences (5'→3')
RNF2	AAACGGAACTCAACCATTAAGC
	TTATTCACCACTGTTACCCAG
RNF169	AGGTCTGAGGAGAACCCTCTTT
	CAACAGCGAAGGTCCAGAAG
STUB1	GCTGGAGATGGAGAGCTATGAT
	ATGAGCCTGGAGAGGTAGGAGT
TRIM33	GCCCACTTCTTTGTTTCTTCTG
	GCATTTGCCATAGTTGCTGTAG
USP7	TTGTGCTGAGTGTGGATCTT
	GAAGGTAAGTGCACGAAGGACT
RING1	CAGGAACAAGGAGTGTCTTACC
	CCATCAGTTCTGCTTCTCTCCT

Table 7

Antibody	Catalog	Company
β-Actin(C4)	sc-47778	Santa cruz
TCF3(E-2)	sc-166411	Santa cruz
TCF4(H-7)	sc-271287	Santa cruz
c-myc(9E10)	sc-40	Santa cruz
HA-tag(F-7)	sc-7392	Santa cruz
β-Catenin(H-102)	sc-7199	Santa cruz
Lamin B1(B-10)	sc-374015	Santa cruz
OctA(H-5)	sc-166355	Santa cruz
Active-β-Catenin(8E7)	05-665	Sigma-Aldrich
K48-linkage Specific Polyubiquitin	#4289	Cell Signaling Technology
RING1B (D22F2)	#5694	Cell Signaling Technology

Table 8

Genes	Primer sequences (5'→3')
RNF2	GTCGTGATGAGTATGAAGCTC
	TTGTTACTAGGGCCTGCTTC
TCF7L1	AGAACCAGAGCAGCAGCTC
	TTCATCTGCAGGTAGGTGCG
c-myc	AGAAGCTGGCCTCCTACCA
	GTCGAGGAGAGCAGAGAATC
Axin2	GGCATCAAGAAGCAGCAGAT
	TTTGCACTTGAAGTCGGCAC
TCF1	CCGTCTACTCCGCCTTCAAT
	AGGTCAGGGAGTAGAAGCCA
LEF1	ACGGAAAGCATCCAGATGGA
	ATGTGTGACGGGTGTGATCC
GAPDH	GTCTCCTCTGACTTCAACAGCG
	ACCACCCTGTTGCTGTAGCCAA

Table 9

Genes	Primer sequences (5'→3')
USP7	CATTGACTATGTAGCAGTGGAG
	TTTGCCTTCTCCTTAGGATTCAA
RNF169	CCAAGTAGTGAATATGCAAAATGG
	CTAATTTGCGCCGGCCGTG
RNF2	GGCGCAAGCAGTGCAGAC
	CATCAAAGTTCGGATCTGGCC
USP14	TGCCCCTCTACTCAGTGAAC
	GTGTTACCGAGGTTTGTGAGG
ADRM1	GTCATCTGGAGCGCTGTTTC
	TCAGTCTTGGGCTCCTGCAT
TRIM33	GGCGGATAACAAAGGAGGAG
	GCTTGGGCTCAGTGTCCC
HERPUD1	ATACGGAGCCCTAGCCTTAG
	CTAATGCCATCAGCCTCAGG
CBLL1	GATAATGATCTACAAGGCACTAAC
	AGGCAGGCCACATTTGTCAC
RING1	CGCCAGTCTCGCGTGATG
	AATCTGTGGAGACATTCCTTGG
UBA1	GTCCAGCTCTCCGCTGTC
	GAAAGATCAGTCCACTCCGTG
UBAP2	ATGATGACTTCGGTGGGAAATG
	GGAAGTCTCTTTGCCAATGCTT
STUB1	GAAAGGAAAGGAGGAGGGTG
	TCTCTAGTTCCATCTGACACTG
OTULIN1	CACCAGAGTAGCTAAAATGAAAATC
	GCTGAGAGCACTCTATGGAAC

Table 10

Genes	Primer sequences (5'→3')
TCF7L1	ATCTTGGAGGGCATTACTTGCC
	TGGGACTAATGTTCCCAGAGGA
RNF2	CAACGAAAGAGTGCTTGCATCG
	TGTATCGAGTCTGTGCACTGTC

Table 1. Candidate genes from mass spectrometry result

Table 2. Six E3 ligase candidate genes from the mass spectrometry data

Table 3. Primers used for shRNA

Table 4. Primers used for knockdown validation

Table 5. Primers used for making gRNA included PX459 vector

Table 6. Primers used for mutation detection

Table 7. Antibody information used for western blot

Table 8-9. Primers used for RT-PCR

Table 10. Primers used for cloning probe