

Supplementary Table 1. Primer sequences**a:Primer sequences used for generating deleted variants**

Primer name	oligos sequences
FlagAXIN1-2_80del-R2	CATAGATCCGCCCGGGCT
FlagAXIN1-2_80del-R3	CATAGATCCGCCCGGGCTCTTAT
AXIN1-delD94Q108-F	GGGCTGTGCCGACTTGCT
AXIN1-delD94Q108-R	TCCAGCAGGGAATGCAGTG
Flag-AXIN1-DELE 65-227-F	CGAGCGCCTCGGAGTGCC
Flag-AXIN1-DELE 65-227-R	AGGGAGATGCAGGAGAGC
Flag-AXIN1-DELE 230-353-F	CCCTGACCCAGAGCTCTG
Flag-AXIN1-DELE 230-353-R	GTGGCACTGGGGGGTGCC
Flag-AXIN1-DELE 434-503-F	GTGGCACTGGGGGGTGCC
Flag-AXIN1-DELE 434-503-R	CCCTGGGGGCCCTGACGA
Flag-AXIN1-DELE 543-676-F	GCTGTGGTGGACGTGGTG
Flag-AXIN1-DELE 543-676-R	CGTCTGGAGGAGGAAGAAAAGAGAGCC
Flag-AXIN1-DELE 680-712-F	GAGCTGAGGGCCGGCCCA
Flag-AXIN1-DELE 680-712-R	TAAGTCGACCTCGAGGGG
Flag-AXIN1-DELE 732-862-F	CACATACCTCTGCTTGAG
Flag-AXIN1-DELE 732-862-R	TGTGTGGACATGGGCTGTGCC
Flag-AXIN1-DELE 373-418-F	GGGAATGTGAGGTAGGGG
Flag-AXIN1-DELE 373-418-R	GTGCAGCGCACGCGGGAGGCCGA
Flag-AXIN1-DELE 373-383-F	GGGAATGTGAGGTAGGGG

Flag-AXIN1-DELE 373-383-R	CACCGCCTGGAGGCTGTG
Flag-AXIN1-DELE 383-393-F	GCGGACCTCCTTCGGCAC
Flag-AXIN1-DELE 383-393-R	CGGGAGGCCGAGGAGAAG
Flag-AXIN1-DELE 393-402-F	GAGCTCCTCCGCGAACTTC
Flag-AXIN1-DELE 393-402-R	GAGGAGGAAGGTGAGGACGG
Flag-AXIN1-DELE 402-418-F	GCGCTGCACAGCCTCCAG
Flag-AXIN1-DELE 402-418-R	GTGGAGCCTCACAAGTTCGCGGAG
Flag-AXIN1-DELE 434-450-F	CCCTGGGGGCCCTGACGA
Flag-AXIN1-DELE 434-450-R	GAGAGCATCCTGGACGAGCAC
Flag-AXIN1-DELE 450-467-F	GGGCGGGAAGTGGTGCCA
Flag-AXIN1-DELE 450-467-R	TCGCCTGGGCCTGGCCAT
Flag-AXIN1-DELE 467-485-F	GTTCTCCTCGTGTGCATCCCGG
Flag-AXIN1-DELE 467-485-R	GTGGCACTGGGGGGTGCC
Flag-AXIN1-DELE 485-503-F	GCGGCCAGGTGTCCTCAG
Flag-AXIN1-DELE 485-503-R	TATCCAGTGATGCGCTGACG
Axin1-del81-212-F	ACAGGCTCGGAGAGCCCC
Axin1-del81-212-R	TGGGGTGGGGGAGGCACT
Axin1-del81-147-F	TACATTCTTGATAACAATGGCATCGTGCCC
Axin1-del147-212-R	TCGGTAGATGGCTCTCGCC
deFlagAxin1-1-430F	CCCCCAGGGCCGTGTCAC
deFlagAxin1-1-430R	CGGGCTTTATCGTCGTCATCC

b:Primers sequences used for generating cancer-related AXIN1 variants

Primer name	oligos sequences
Flag-AXIN1-P385A-F	CTCCTTCGGCACCCGGTA
Flag-AXIN1-P385A-R	AGCTCATCCACTGCCTGGAGGC
Flag-AXIN1-Q386H-F	GCGGACCTCCTTCGGCAC
Flag-AXIN1-Q386H-R	TCAGAAGTTCGTGGAGGAGCTCATCC
Flag-AXIN1-A389V-F	GGCTCCACGCGGACCTCC
Flag-AXIN1-A389V-R	AGTTCGCGGAGAAGCTCATCCAC
Flag-AXIN1-E391K-F	TCTGAGGCTCCACGCGGA
Flag-AXIN1-E391K-R	AGGAGCTCATCAACCGCCTGGAG
Flag-AXIN1-H394N-F	CCGCGAACTTCTGAGGCTC
Flag-AXIN1-H394N-R	GCTCATCCACCACCTGGAGGCTG
Flag-AXIN1-R395H-F	TCCTCCGCGAACTTCTGAG
Flag-AXIN1-R395H-R	GCTCATCCACCCCTGGAGGCTG
Flag-AXIN1-R395P-F	TCCTCCGCGAACTTCTGAG
Flag-AXIN1-R395P-R	AGGCTGTGCAGTGCACGCGGGAG
Flag-AXIN1-R395C-4F	AGCCTCCAGGCAGTGGATGAGCTCCTCCG
Flag-AXIN1-R395C-4R	GAGCCTCAGAAGTTCGCG
Flag-AXIN1-L396M-F	GTCCTCCGCGAACTTCTGAGG
Flag-AXIN1-L396M-R	GCAGCGCACGTGGGAGGCCGA
Flag-AXIN1-R401C-F	CCAGGCGGTGGATGAGCTCC
Flag-AXIN1-R401C-R	GGCTGTGCAGCACACGCGGGAGG
Flag-AXIN1-R401H-F	TCCAGGCGGTGGATGAGCTC

Flag-AXIN1-R401H-R	TGTGCAGCGCAAGCGGGAGGCCG
Flag-AXIN1-T402 K-F	GCCTCCAGGCGGTGGATGAGC
Flag-AXIN1-T402 K-R	TCAGGGACAGGGAAGGGCATATC
Flag-AXIN1-R403W-F	ACAGCCTCCAGGCGGTGGATG
Flag-AXIN1-R403W-R	CCGAGGAGAAGATGGAGGAGCGG
Flag-AXIN1-L409M-F	CCTCCCGCGTGCGCTGCA
Flag-AXIN1-L409M-R	GCTGGAGGAGCAGCTGAAGCGCGT
Flag-AXIN1-R412Q-F	TTCTCCTCGGCCTCCCGC
Flag-AXIN1-R412Q-R	TGGAGGAGCGGATGAAGCGCGTG
Flag-AXIN1-R413M-F	GCTTCTCCTCGGCCTCCCG
Flag-AXIN1-L413M-R	GGAGGAGCGGCGGAAGCGCGTGC
Flag-AXIN1-L413R-F	AGCTTCTCCTCGGCCTCCCG
Flag-AXIN1-L413R-R	GAAGCGCGTGCACATGGAGGAGG
Flag-AXIN1-R417H-F	AGCCGCTCCTCCAGCTTC
Flag-AXIN1-R417H-R	GAGGAGGAAGGTGAGGAC
Flag-AXIN1-R450-R	TGGTGCCAAGCGGGGGCG
Flag-AXIN1-R450H-F	CTTCCCGCCCCACTGTGTGGACATGG
Flag-AXIN1-R450L-F	CTTCCCGCCCCTCTGTGTGGACATGG
Flag-AXIN1-G455V-F	TGTGGACATGGTCTGTGCCGGGC
Flag-AXIN1-G455V-R	CAGCGGGGCGGGAAGTGG
Flag-AXIN1-A457P-F	ACATGGGCTGTCCCGGGCTCCGG
Flag-AXIN1-A457P-R	CCACACAGCGGGGCGGGAAG

Flag-AXIN1-G458R-F	TGGGCTGTGCCAGGCTCCGGGAT
Flag-AXIN1-G458R-R	TGTCCACACAGCGGGGCG
Flag-AXIN1-L459F-F	GCTGTGCCGGGTTCCGGGATGCA
Flag-AXIN1-L459F-R	CCATGTCCACACAGCGGGGC
Flag-AXIN1-D461N-F	CCGGGCTCCGGAATGCACACGAG
Flag-AXIN1-D461N-R	CACAGCCCATGTCCACACAGC
Flag-AXIN1-A462T-F	GGCTCCGGGATACACACGAGGAG
Flag-AXIN1-A462T-R	CGGCACAGCCCATGTCCA
Flag-AXIN1-E464K-F	GGGATGCACACAAGGAGAACCCTGAGAGC
Flag-AXIN1-E464K-R	GGAGCCCGGCACAGCCCA
Flag-AXIN1-E464D-F	GATGCACACGATGAGAACCCTGAGAGCATCCTGG
Flag-AXIN1-E464D-R	CCGGAGCCCGGCACAGCC
Flag-AXIN1-N466Y-F	CACACGAGGAGTACCCTGAGAGCATCC
Flag-AXIN1-N466Y-R	CATCCCGGAGCCCGGCAC
Flag-AXIN1-Q476K-F	ACGAGCACGTAAAGCGTGTGCTG
Flag-AXIN1-Q476K-R	CCAGGATGCTCTCAGGGTTC
Flag-AXIN1-V478G-F2	ACAGCGTGGGCTGAGGACAC
Flag-AXIN1-V478G-R2	ACGTGCTCGTCCAGGATGC
Flag-AXIN1-P482H-F	GCTGAGGACACATGGCCGCCAGT
Flag-AXIN1-P482H-R	ACACGCTGTACGTGCTCGTCC
Flag-AXIN1-G483S-F	TGAGGACACCTAGCCGCCAGTCG
Flag-AXIN1-G483S-R	GCACACGCTGTACGTGCTC

AXIN1-S88P-F	GTGGGCTGAGCCACTGCATTCCC
AXIN1-S88P-R	TTCAAGTATGGTGGGGTGG
AXIN1-H90Y-F	TGAGTCACTGTATTCCCTGCTGG
AXIN1-H90Y-R	GCCCACTTCAAGTATGGTG
AXIN1-S91F-F	GTCACTGCATTTTCTGCTGGATG
AXIN1-S91F-R	TCAGCCCACTTCAAGTATG
AXIN1-D94N-F	TTCCCTGCTGAATGACCAAGATG
AXIN1-D94N-R	TGCAGTGA CT CAGCCAC
AXIN1-G98R-F	TGACCAAGATAGGATAAGCCTGTTC
AXIN1-G98R-R	TCCAGCAGGGAATGCAGT
AXIN1-G98E-F	TGACCAAGATGAGATAAGCCTGTTC
AXIN1-S100N-F	AGATGGGATAAACCTGTT CAGGAC
AXIN1-S100N-R	TGGTCATCCAGCAGGGAA
AXIN1-L101P-F	TGGGATAAGCCCGTT CAGGACTTT
AXIN1-L101P-R	TCTTGGTCATCCAGCAGG
AXIN1-R103M-F	AAGCCTGTT CATGACTTTCCTGAAG
AXIN1-R103M-R	ATCCCATCTTGGTCATCC
AXIN1-T104N-F	CCTGTT CAGGAATTCCTGAAGC
AXIN1-T104N-R	CTTATCCCATCTTGGTCATC
AXIN1-L106R-F	CAGGACTTTCCGGAAGCAGGAGG
AXIN1-L106R-R	AACAGGCTTATCCCATCTTG
AXIN1-L107I-F	GACTTTCCTGATACAGGAGGGCTG

AXIN1-K107I-R	CTGAACAGGCTTATCCCATC
AXIN1-Q108H-F	TTTCCTGAAGCATGAGGGCTGTG
AXIN1-Q108H-R	GTCCTGAACAGGCTTATCC
AXIN1-E109D-F	CCTGAAGCAGGATGGCTGTGCCG
AXIN1-E109D-R	AAAGTCCTGAACAGGCTTATCCC
AXIN1-G110A-F	GAAGCAGGAGGCCTGTGCCGACT
AXIN1-G110A-R	AGGAAAGTCCTGAACAGGCTTATC
AXIN1-A112T-F	GGAGGGCTGTACCGACTTGCTGG
AXIN1-A112T-R	TGCTTCAGGAAAGTCCTGAACAGG
AXIN1_E73K_F2	GTATGAGCCTAAGGGCAGTGCCT
AXIN1_E73K_R2	CCCAGGTCCAGATCCGAGCG
AXIN1_E73_R	CCCAGGTCCAGATCCGAG
AXIN1_G74S_F	TGAGCCTGAGAGCAGTGCCTC
AXIN1_G74S_R	TACCCAGGTCCAGATCC
AXIN1_A76T_F	TGAGGGCAGTACCTCCCCCAC
AXIN1_A76T_R	GGCTCATACCCAGGTCC
AXIN1_A76V_F	GAGGGCAGTGTCTCCCCACC
AXIN1_A76V_R	AGGCTCATACCCAGGTCC
AXIN1_S77F_F	GGCAGTGCCTTCCCCACCCCA
AXIN1_S77F_R	CTCAGGCTCATACCCAGGTCC
AXIN1_D113N_F	GGGCTGTGCCAACTTGCTGGA
AXIN1_D113Y_F	GGGCTGTGCCTACTTGCTGGA

AXIN1_D113_R	TCCTGCTTCAGGAAAGTCCTG
AXIN1_C121F_F	TGGTTTGCCTTCACTGGCTTC
AXIN1_C121F_R	GAAGTCCAGCAAGTCGGC
AXIN1_R125W_F2	CTGCACTGGCTTCTGGAAGCTGGAGC
AXIN1_R125W_R2	GCAAACCAGAAGTCCAGCAAGTC
AXIN1_A143D_F	CTGGCGAGAGACATCTACCGA
AXIN1_A143D_R	CTTCAGCCTCTTCTCCTCG
AXIN1_E188K_F	GGCCCAGACCAAATCCAGGC
AXIN1_E188K_R	TGGTCAAACATGGCAGGATC
AXIN1_L202F_F2	CTATCCCTCCTTCTTTAAGTCTGATA
AXIN1_L202F_R2	GTGTTTTCTCCATAGTG
AXIN1_LK202/3QN_F	CCCTCCTCCAAAATTCTGATATTTATTTG
AXIN1_LK202/3QN_R	ATAGGTGTTTTCTCCATAG
AXIN1_E209Q_F2	TATTTATTTGCAATATACGAGGACAGGC
AXIN1_E209Q_R2	TCAGACTTAAGGAAGGAGGGA
Axin1-I149T-F2	CCGAAAGTACACTCTTGATAACAATGGCA
Axin1-I149T-R2	TAGATGGCTCTCGCCAGCTT
AXIN1_G69E_F	CTGGACCTGGAGTATGAGCCTGAG
AXIN1_G69V_F	CTGGACCTGGTGTATGAGCCTGAG
AXIN1_G69_R	ATCCGAGCGCCTCGGAGT
AXIN1_P72T_F	GGGGTATGAGACTGAGGGCAG
AXIN1_P72T_R	AGGTCCAGATCCGAGCGC

AXIN1_P78S_F	CAGTGCCTCCTCCACCCCACC
AXIN1_P78S_R	CCCTCAGGCTCATACCCCAG
AXIN1_A120D_F	TTCTGGTTTACTGCACTGGC
AXIN1_A120D_R	GTCCAGCAAGTCGGCACA
AXIN1_C121S_F	CTGGTTTGCCAGCACTGGCTT
AXIN1_A141T_F	GCTGAAGCTGACGAGAGCCAT
AXIN1_A141T_R	CTCTTCTCCTCGTTCGAG
AXIN1_V156M_F	CAATGGCATCATGTCCCGGCA
AXIN1_V156M_R	TTATCAAGAATGTACTTTCGGTAGATG
AXIN1_K161T_F	CGGCAGACCACGCCAGCCACC
AXIN1_K161T_R	GGACACGATGCCATTGTTATCAAGAATGTAC
AXIN1_A185D_F	CATGTTTGACCAGGACCAGACCGAAA
AXIN1_A185V_F	CATGTTTGACCAGGTCAGACCGAAA
AXIN1_A185T_F	CATGTTTGACCAGACCCAGACCGAAA
AXIN1_A185_R	GCAGGATCGATCAGCTGC
AXIN1_I206M_F	AGTCTGATATGTATTTGGAATATACG
AXIN1_I206M_R	TAAGGAAGGAGGGATAGG
AXIN1_Y207C_F	TCTGATATTTGTTTGGAAATATACGAG
AXIN1_Y207C_R	CTTAAGGAAGGAGGGATAG

c:Primer sequences to insert and identify RGS at the front of AXIN1

Primer name	oligos sequences
Flag-RGSdel_fwd	atatacaggATGAATATCCAAGAGCAGGGTTTCCC
Flag-RGSdel_rev	tcaagtatggAGATCCGCCCGGGCTCTT
insRGS_fwd	ggcgcatctCCATACTTGAAGTGGGCTG
insRGS_rev	ggatattcatCCTCGTATATCCAAATAAATATCAG
pEN-Tmir-NterMycF	catctcagaagaggatctgAAGACTAATGCGTTTTTCG
pEN-Tmir-NterMycR	agttttgttccatggtggcAGTCGACTGAATTGGTTC
pENTmir-insNMYC_F	AAGACTAATGCGTTTTTCG
pENTmir-insNtMYC_R	CAGATCCTCTTCTGAGATG
FLAG-AXIN1_fwd	tcatctcagaagaggatctgGGCGGATCTATGAATATC
FLAG-AXIN1_rev	ctcgaaaacgcattagtcttTTAGTCCACCTTCTCCAC

d:AXIN1 sgRNAs info

Primer name	oligos sequences
Axin1-intron4-crF	CACCGCACGGGGTTGAAAGGTCAC
Axin1-intron4-crR	AAACGTGACCTTTCAACCCCGTG C
Axin1-intron5-crF	CACCGAAAACAGCACGACACCGACG
Axin1-intron5-crR	AAAC CGTCGGTGTGCTGCTGTTTTTC
Axin1-R395-crF	CACCGGAGGAGCTCATCCACCGCC
Axin1-R395-crR	AAACGGCGGTGGATGAGCTCCTCC
AXIN1-G508-crF	CACCGCCAAGATGCCAGTGGCACT
AXIN1-G508-crR	aaacAGTGCCACTGGCATCTTGGC
AXIN1-R723-crF	CACCGCTGCTTGGAGGGTGCTCGGC

AXIN1-R723-crR	aaacGCCGAGCACCTCCAAGCAGc
AXIN1-R841-crF	caccGGTGTTTGAGGAGGTTTCGAG
AXIN1-R841-crR	aaacCTCGAACCTCCTCAAACACC
JHH7-crF	caccgAGTCGGCACAGCCCTCCAGC
JHH7-crR	aaacGCTGGAGGGCTGTGCCGACTc

e:Primer to identify correctly expected AXIN1 in cell clones

Target	Primer	Oligos sequences
R395 truncated clones , koEXON5, R395P and R395H	AXIN1-Intron4-F1	GACTGGCCGGACACGAAAA
	AXIN1-Intron5-R1	AGAAGTTGAGATCACCCGCTG
G508	Axin1-ex6F	GGCGATCCATCGTCAGGG
	Axin1-ex6R	ACACTCTCTGAGTAGCCTCGG
R723	Axin1-Intron7F	TCTGTGGAGGACGTCCATGT
	Axin1-Intron8R	AACCACGTAGCTTTCCGACC
R841	Axin1-Intron10F	CACAGGCCTCCTTCCCATCT
	Axin1-Intron11R	TGCTATGAGGAGTGGTCCAG

f:Primer sequences for amplifying genomic exon2 of AXIN1 sequences and cloning into TA vector

Target	Primer name	Oligos sequences
AXIN1 exon2	AXIN1-surEX2F	TGCAGCATTCTGACAGAGCAT
	AXIN1-surEX2R	ATGCCCTGAAACGTCCACT
To identify correctly repaired AXIN1 in JHH7	JHH7-ax1-scF	GAGACTTCGACGGCCACT
	Hep3B-AXIN1-scF1	GCTGGATGACCAAGATGGGAT
	Hep3B-AXIN1-scR2	CCAATTCTCCAATTCTTATCGAT GC

g:Primer sequences for amplifying AXIN1 cDNA

Primer name	oligos sequences
AXIN1-cdna3F	AACGACAGCGAGCAGCAGAG
AXIN1-cdna6R	AGCTTGTGACACGGCCCTGG
AXIN1-5UTR-F	GCGCTCATTGTTCTTGACG
Axin1-Intron11R	TGCTATGAGGAGTGGTCCAG

h:HDR sequences for generating knockin clone: R395P and R395H

Name	sequences
WT	CGCGTGGAGCCTCAGAAGTTCGCGGAGGAGCTCATCCAC <u>CGCCTGG</u> AGGCTGTGCAGCGCACGCGGGAGGCCGAGGAGAAG
R395P ssODN	CGCGTGGAGCCTCAGAAGTTCGCGGAGGAGCTCATCCAC <u>CcCCTcG</u> AGGCTGTGCAGCGCACGCGGGAGGCCGAGGAGAAG
R395H ssODN	CGCGTGGAGCCTCAGAAGTTCGCGGAGGAGCTCATCCAC <u>CaCCTcG</u> AGGCTGTGCAGCGCACGCGGGAGGCCGAGGAGAAG

PAM silent: TGG-TcG

i:Primer sequences used for Qpcr

Primer name	oligos sequences
<i>AXIN2</i> -qpcr-EX3F	TATCCAGTGATGCGCTGACG
<i>AXIN2</i> -qpcr-EX4R	TTACTGCCACACGATAAGG
<i>AXIN1</i> -qpcr-ex7F	GTGGCGTGCAAAGAAATGC
<i>AXIN1</i> -qpcr-ex8R	TCTGGAGTTTCATGGGGCT
<i>GAPDH</i> -F	GTCTCCTCTGACTTCAACAGCG
<i>GAPDH</i> -R	ACCACCCTGTTGCTGTAGCCAA