SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

Figure S1: A novel mouse model for CIN: Cre-inducible Mps1 Knock-in (CiMKi)

(A) Genomic PCR of targeted ES cells confirming presence of the *CiMKi* allele. Shown here specifically is the *CiMKi*-T649A ES clone that was used for blastocyst injection. **(B)** Confirmation of correct integration of the CiMKi alleles by Southern blot. Shown here specifically is the *CiMKi*-T649A ES clone that was used for blastocyst injection. Lower schematic shows EcoRV restriction sites used for Southern blot, indicated 5' of exon 15 and in exon 20. **(C)** Targeted sequencing confirming presence of *Mps1* mutations in mouse ear genomic DNA. **(D)** RT-PCR followed by targeted sequencing of cDNA from *CiMKi* MEF lines 56 hours after 4-OHT addition shows hetero- or homozygous expression of both D637A (A→C) or T649A (A→G) alleles. **(E)** Western blot of Mps1 protein expression in *CiMKi;Rosa26-CreER^{T2}* MEFs 56 hours after 4-OHT addition. Intensity is normalized over α-tubulin. **(F)** Examples and quantification of Mad1 localization on kinetochores as a proxy for Mps1 activity in *CiMKi;Rosa26-CreER^{T2}* MEFs 72 hours after 4-OHT addition. Cells were blocked in mitosis by nocodazole and MG132 for 30 minutes. Graph shows quantifications of kinetochore signals as ratios over ACA signals. Data represents mean ± SD of at least 20 cells per condition. **(G)** Examples and quantification of diploid and aneuploid cells on metaphase spreads (DAPI) of *CiMKi;R26CreER^{T2}* primary MEFS 56 hours after 4-OHT addition. MEFS were blocked in mitosis by 4 hours treatment with nocodazole. Ploidy was assessed by counting the number of chromosomes per cell, percentage of diploid cells is given. **(H)** RT-PCR followed by targeted sequencing on cDNA from *CiMKi;R26CreER^{T2}* small intestine tissue one week after tamoxifen injection confirms effective recombination and expression of the mutant alleles. Hetero- or homozygous expressions of both D637A (A→C) or T649A (A→G) alleles are shown.

Figure S2: CIN leads to spontaneous tumorigenesis in the intestine

(A) Relative bodyweight of *CiMKi;R26CreER^{T2}* mice after three consecutive days of intraperitoneal tamoxifen injection. Lines represent change in bodyweight per group as fraction of their weight at the start of the experiment (mean ± SD). **(B)** Relative body weight in male (left) and female (right) *CiMKi;VillinCre* mice of all genotypes. Increase in weight is shown as percentage from start of the experiment (4 weeks) to end (8 months). Data is shown as mean percentage increase ± SD.

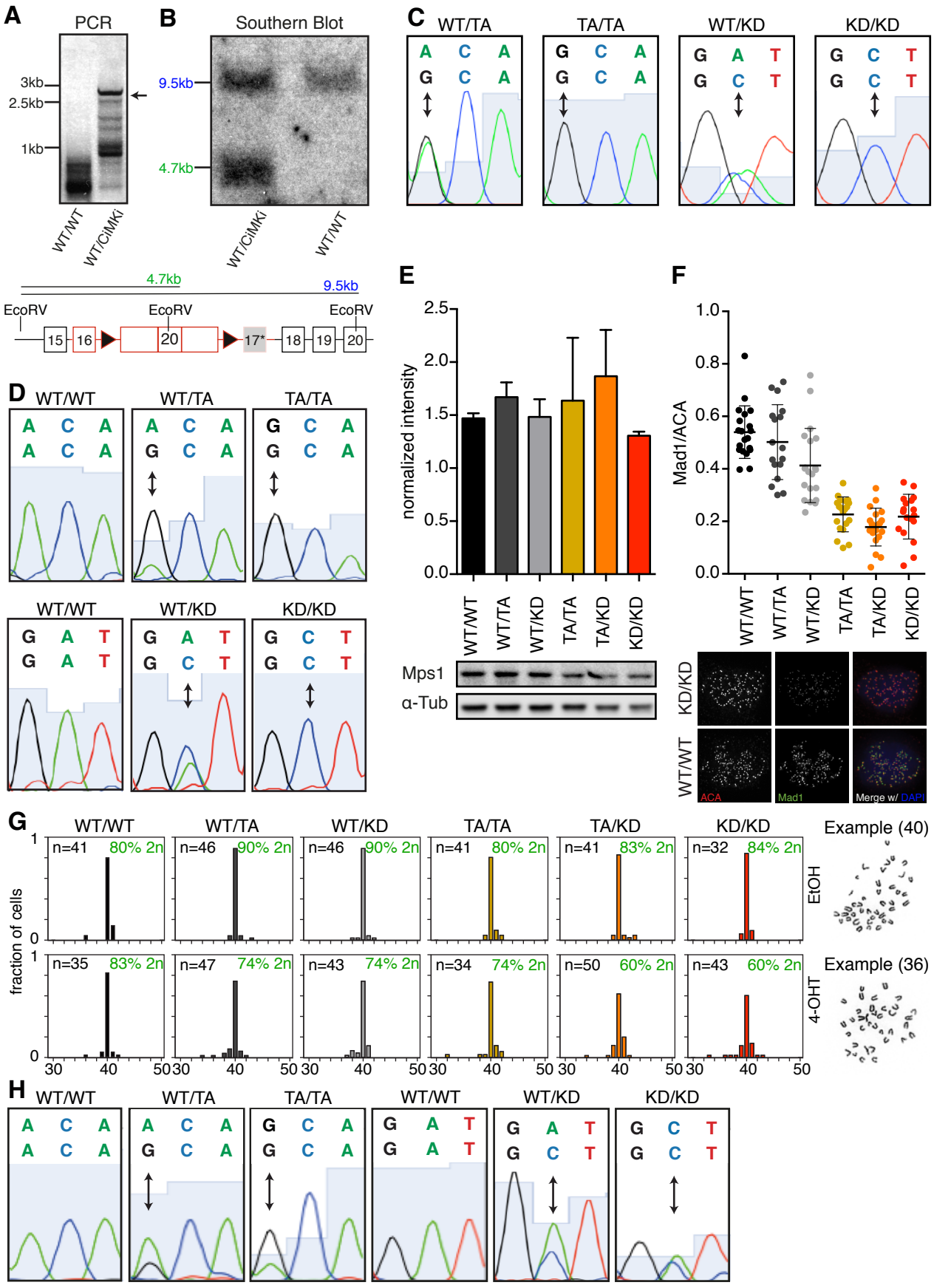
Figure S3. CIN differently affects small intestine and colon tumor formation in *Apc$^{min/+}$* mice

(A) Quantification of small intestine adenomas on H&E sections of *CiMKi;Apc$^{Min/+}$;VillinCre* mice. Each mouse is represented by an individual dot (n=4-15 mice per group), data represents mean ± SD, asterisk indicate significance (one-tailed t-test, comparing each group to WT/WT, $p<0.001$ (***)). Open dots represent mice euthanatized at 6-8 weeks of age, closed dots represent mice euthanatized at 12 weeks of age. **(B)** Average size of small intestine adenoma for each mouse was measured by taking the diameter of the lesion on H&E slides of *CiMKi;Apc$^{Min/+}$;VillinCre* mice. Data represents mean ± SD. **(C)** Total tumor burden in small intestine as the sum of all tumor diameters per mouse. Data represents mean ± SD, asterisk indicate significance (one-tailed t-test, $p<0.001$ (***). **(D)** Quantification of colon adenomas on H&E sections of *CiMKi;Apc$^{Min/+}$;VillinCre* mice. Each mouse is represented by an individual dot (n=4-15 mice per group), data represents mean ± SD, asterisk indicate significance (one-tailed t-test, comparing each group to wild-type, $p<0.001$ (***), $p<0.05$ (*)). Open dots represent mice euthanatized at 6-8 weeks of age, closed dots represent mice euthanatized at 12 weeks of age. **(E)** Average size of colon adenoma for each mouse was measured by taking the diameter of the lesion on H&E slides of *CiMKi;Apc$^{Min/+}$;VillinCre* mice. Data represents mean ± SD. **(F)** Total tumor burden in colon as the sum of all tumor diameters per mouse. Data represents mean ± SD, asterisk indicate significance (one-tailed t-

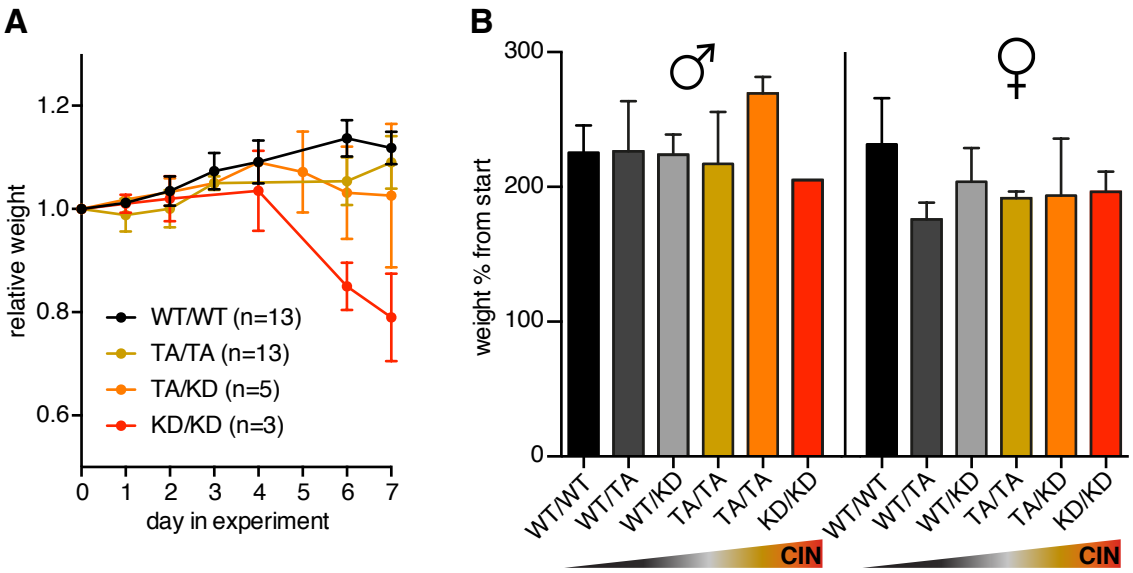
test, p<0.001 (***), p<0.05 (*)). **(G)** Colon tumor incidence in *CiMKi;Apc$^{Min/+}$;VillinCre* mice of the indicated genotypes. **(H)** Targeted sequencing confirming absence of wild-type *Apc* genomic DNA in tumor organoids. *Apc$^{Min/+}$* mouse ear genomic DNA is given as reference.

Figure S4: Colonic crypts retain proliferating CIN cells more readily than small intestinal crypts

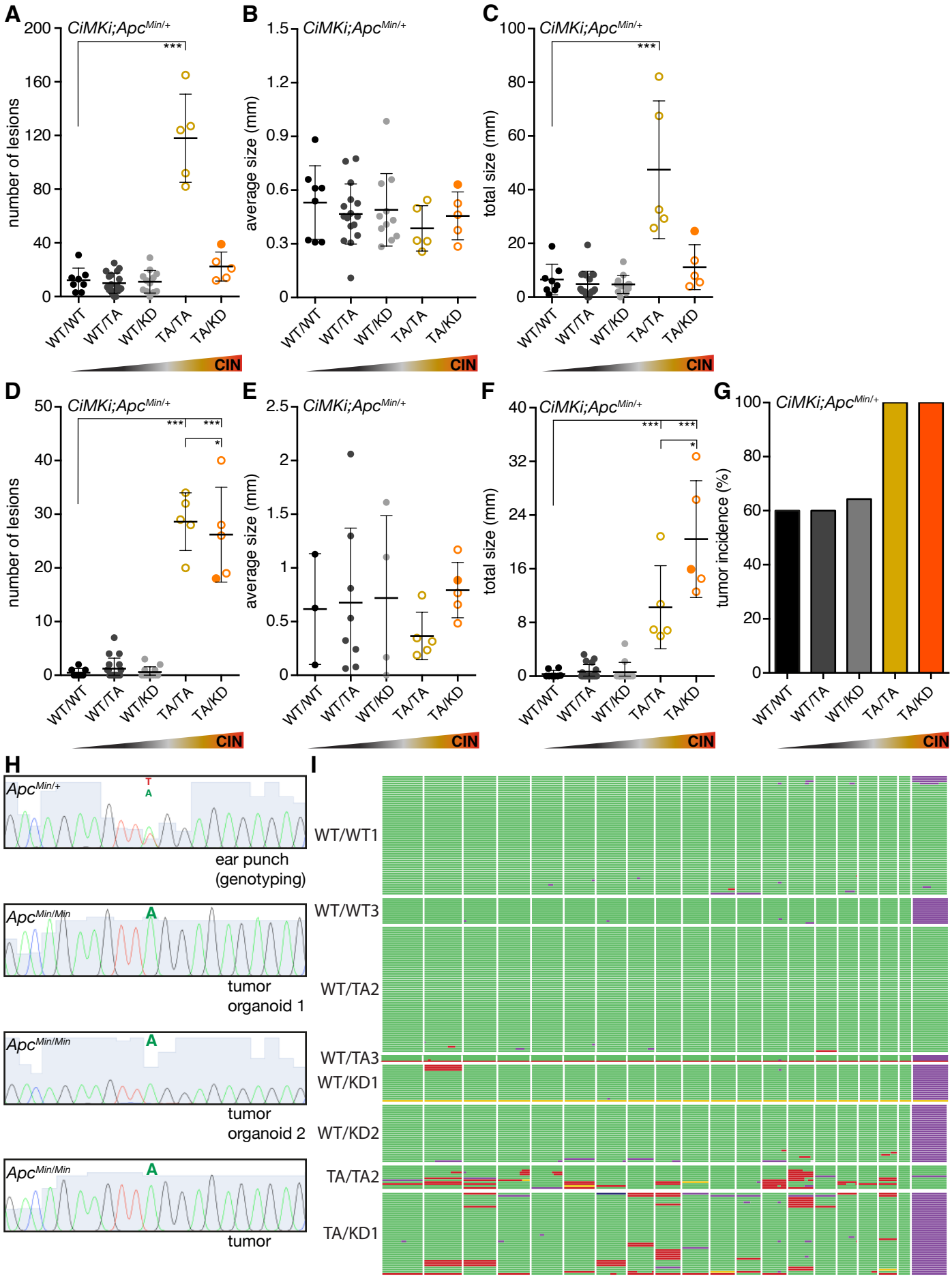
(A, B) Proliferative index in colon (**A**) and small intestine (**B**) of 4-week old *CiMKi;Apc$^{Min/+}$;VillinCre* mice as determined on Ki-67 stained FFPE slides by calculating the percentage of Ki-67 positive cells within the proliferative compartment. Data represents mean ± SD, n=3-4 mice per genotype. **(C, D)** Number of Ki-67 positive cells in proliferative compartment I colon (**C**) and small intestine (**D**) of 4-week old *CiMKi;Apc$^{Min/+}$;VillinCre* mice. Data represents mean ± SD, n=3-4 mice per genotype. Asterisk indicate significance (one-tailed t-test, p<0.01 (**), p<0.05 (*)).



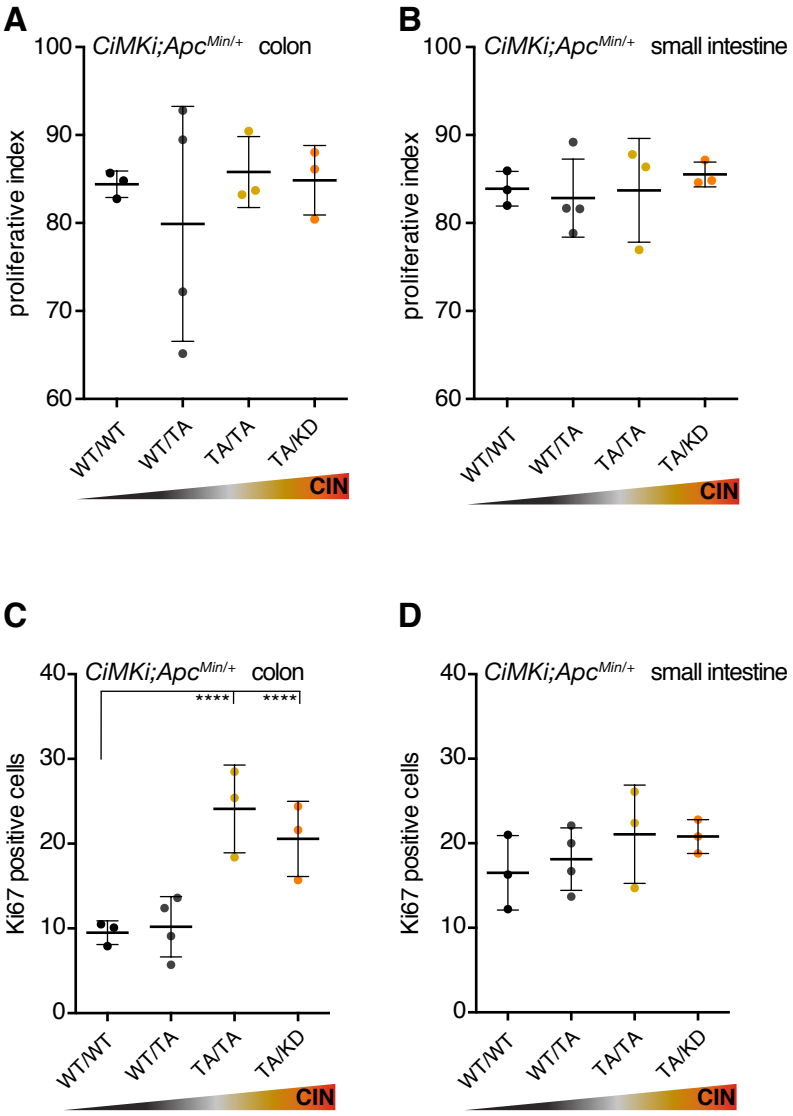
Hoevenaar et al., Figure S1



Hoevenaar et al., Figure S2



Hoevenaar et al., Figure S3



Hoevenaar et al., Figure S4

Supplementary Movie Legends

Movies S1: Increased missegregation rates in *CiMKi;Rosa26-CreER^{T2}* MEFs.

(A) Time lapse imaging of *CiMKi$^{WT/WT}$;R26CreER^{T2}* immortalised MEFs expressing H2B-mNeon, 56 hours after 4-OHT addition. **(B)** As **A**, but for *CiMKi$^{KD/KD}$;R26CreER^{T2}*.

Movies S2: Increase in missegregation rates in *CiMKi;Apc$^{Min/+}$;Villin-Cre* colon tumor organoids.

(A) Time lapse imaging of *CiMKi$^{WT/WT}$;Apc$^{Min/+}$;Villin-Cre* colon tumor organoids. Colour dept-coding (purple is bottom of organoid, red is top) was used to identify the position of the cells, left panel) and maximum projections are depicted in the right panel. **(B)** As **A**, but for *CiMKi$^{TA/TA}$;Apc$^{Min/+}$;Villin-Cre* colon tumor organoids.

Table 1: Primers for genotyping and cDNA analysis

Gene	Forward primer	Reverse primer	Expected band size	Sequence primer	Mutation sequence
CiMKi	*CCAAATGGCTAGGGGAGCCACTGATG*	*GGTGAGGTTGTTTCCAACTGGTAG*	**Mutant 250 bp**	NA	NA
CiMKi (mutation)	GTGTCCTCACCCTGAAAATG	CAAAGCACAGCTGGGCTGTAGAG	**~1000 bp**	CGGATTTTATTTTGAAGGTATTG	T649A ACAA/GCA D637A TTGGA/CT
CiMKi cDNA (mutation)	CCTAGAAGACGCCGATAGCC	GTCTCTGATTGCTTCTGGGGC	**~400 bp**	GATAAGATCATCCGCCTCTATG	T649A ACAA/GCA D637A TTGGA/CT
Apc$^{Min/+}$	*CCGGAGTAAGCAGAGACACAAG*	*CTGTCGTCTGCCACACAATG*	**~400 bp**	*CATGACTGTTCTTTCACC*	*GACAGAAGTTT/A*
Rosa26-CreER^{T2} (mutant)	GGCAGGAAGCACTTGCTCTCCC	CCTGATCCTGGCAATTTCG	**~825 bp**	NA	NA
Rosa26-CreER^{T2} (wild-type)	GGCAGGAAGCACTTGCTCTCCC	GGAGCGGGAGAAATGGATATG	**~650 bp**	NA	NA
Villin-Cre(ER^{T2})	*CAAGCCTGGCTCGACGGCC*	*CCTGATCCTGGCAATTTCG*	**~220 bp**	NA	NA

Extended Methods

Cloning of CiMKi targeting vectors

CiMKi alleles were cloned into the pAC targeting vector (based on pFlexible(Van Der Weyden et al., 2005)) (kind gift from Jos Jonkers). Three fragments were ligated separately into the vector as follows:

1) Conditional fragment into HindIII-PacI (between loxP sites)

2) 5' Recombination arm into PmeI-AscI (upstream of 5' loxP site)

3) 3' Recombination arm, including either point mutation, into SbfI-NotI (downstream of 3' loxP site).

Details for the three fragments:

1) Conditional fragment

The conditional fragment was obtained by assembling three separate PCR products by ligation into pCDNA3 (see details below):

#1=*HindIII*-(last part of)intron16-*XhoI* (~600 bp)

#2=*XhoI*-**exon17-exon18-exon19-exon20-exon21-exon22**-*BamHI* (~1000 bp)

#3=*BamHI*-intron22(polyAsignal)-*PacI* (~600 bp)

This resulted in the following complete sequence of the conditional fragment (exon17-22 part (PCR product #2) in bold):

*AAGCTT*ATGGCCAGTTTTTCAGTCTCCAAAGGATTTTCTCTTTGGAGCCCGGTGTGAGAGT
TGGTCTGTTCTTGCTGTTTTCTGTTTCATTATTTGTTTGTTCTTGTTCTCTCCAGTTTCCTCT
GCCTTTCCCTTGTCTTTTAAACAGATGTGTTCAATCTCATGCGCTTCCTCTGCTTCTCCTTT
TAGAAGCAGTAACTAAGCAGAGGATAGGACTCTTTGGGGGTGAGGGAGGCCTTGCGATC
TTATTGTCTGACAGTTCTTGGTGGTGCTTTCCTGGCCTGTGCATGGCAGTGTGCAGCACAC
ACTGACAGGCTAGGCCTGGAATCCTGAACAGTTCACTTCAACTCAAATCACATTGAGTGT
CCTCACCCTGAAAATGATGATGATAATGGGACTTATTTGCTATGCATTGCTATAGAATGG
TACCTGGCCCACAACATTTGCTAAGAAAGTGTAGAATGTATAATAACTAAATATTAATGT
GTTGCCTAATTGAAAGGATAAGCCTGACCTAAAGATTATGAAATGCTTTTTTGTCCCCTG
GCCATAGAATGTTTTTCTTCATCTAACAGAACGGATTTTATTTTGAAG**GTATTGTTCATA**

GTGATCTGAAGCCTGCTAACTTTGTGATAGTGGATGGAATGCTAAAGCTAATTGATT
TTGGGATTGCAAACCAAATGCAGCCAGACACAACAAGCATTGTTAAAGATTCTCAG
GTTGGCACAGTTAACTATATGGCCCCAGAAGCAATCAGAGACATGTCTTCTTCAAG
AGAAAATTCGAAAATCAGAACCAAGGTAAGTCCCAGAAGTGATGTCTGGTCCTTGG
GGTGCATTTTGTACTACATGACTTATGGGAGGACGCCATTTCAGCACATCATCAATC
AGGTCTCTAAACTGCACGCCATAATCAACCCTGCTCATGAGATTGAATTTCCCGAGA
TTTCGGAAAAAGATCTTCGAGACGTGCTAAAGTGCTGTTTAGTGAGGAACCCTAAA
GAGAGGATATCTATCCCTGAGCTCCTCACACATCCGTATGTTCAAATTCAGCCCCAT
CCAGGCAGCCAAATGGCTAGGGGAGCCACTGATGAAATGAAATATGTGTTGGGTCA
ACTTGTTGGTCTGAATTCTCCTAACTCCATCTTGAAAACTGCAAAAACTTTGTATGA
ACGTTATAATTGTGGTGAAGGTCAAGATTCTTCGTCATCCAAGACTTTTGACAAAAA
GAGAGAAAGAAAGTGATGCACAGCTACGTACAAACCAAGAACACTAGATTGTTTCC
TCTGCCATACTCTTGAATCTCTGAGGAAATCTACCAGTTGGAAACAACCTCACCTGG
ATTTTATCAGTTAAAAAAACAAACAAACAAAACTTCAGTAGATTATCCTCAAAAGAA
AGCTGTAAAGTTAACCACTCATAGCACTGTGTATATTAAATTATAGAGTTGTGCTTT
TCTTTTATGCTTTTCTGTAAATCTGCTAATGTTTTACGTTTGGAACAGTGAATGATA
GCTGGAATGTTGAAGAGCTCTGTAAATAAAGCGTCACCACAGTTCCAGAACTGTACA
GTGGTCAGTTTCTTCAATCAAATGTGTTCTTGGCATGATAGCAAAATTTTTAGAAAAACG
GGATTAAGAATAGACCGTAGTAAATAAAGTTTAACAATTAAATTTCCAAAGGATTTAGG
ACTGTAAACAGGCTCACACCTTTAGTCCCAGCACTTGGGAGGCAGAGGCAGGCAGATTT
CTGAGTTCGAGGCCAGCCTGGTCTACAGAGTGAGTTCCAGGACAGCCAGGGCTACACAG
AGAAACCCTGTCTTGGCGGGGAGGGGCCGGGGAGGGGGGGAGATCCAAAGGATGTAGG
AGGCAGAGACAGGTGGATCTCTGTGATTCTGAAGCCAGCCTGGTCTACAGAACTAGTTCT
AGGATAGCCAAGGCTATACAGACACTTTCTCCCCACCACCATCCTGCCCTCAAAAAAATT
GTAAATAAATTTCCTAATTGTGTACAGCCATGATACCATCTATAGTATTTGGTCTGCAAG
TGGCTTTTTCAGTTCTCCCTTTGACTCTTCAAAGTACATATGGGGTTTGGTGTTCTAAATA
TTGTGCTGGAGTTTGTGATTTAATGTCTATAGTTAATACATGCCATTATTGAG*TTAATTAA*

Product #1 was obtained by standard PCR using genomic DNA from 129/Ola-derived IB10 ES cells (kind gift from Hans Clevers). Primers used (*Italic* is overhang, in *Capitals/Italic* the restriction sites):

#1 Forward: *cggcgAAGCTT*ATGGCCAGTTTTTCAG

#1 Reverse: *cgccgCTCGAG*CTTCAAAATAAAATCCGTC

Product #2 was obtained by standard PCR using mouse cDNA (Imagenes, Germany, BC 058851, ID: 30023533). Primers used (*Italic* is overhang, in *Capitals/Italic* the restriction sites):

#2 Forward: *ccggcCTCGAGgcgctactctggtg*GTATTGTTCATAGTG

#2 Reverse: *ccgcgGGATCCgcgctactctggtg*CTGGAACTGTGGTGAC

Product #3 was obtained by standard PCR using genomic DNA from 129/Ola-derived IB10 ES cells (kind gift from Hans Clevers). Primers used (*Italic* is overhang, in *Capitals/Italic* the restriction sites:

#3 Forward: *ccgccGGATCC*AACTGTACAGTGGTC

#3 Reverse: *ccgccgTTAATTAA*CTCAATAATGGCATG

First, products #1 and #2 were ligated by standard methods into pCDNA3 simultaneously. XhoI site was removed using site-directed mutagenesis. Primers used:

(XhoI)loop Forward: GACGGATTTTATTTTGAAGGTATTGTTCATAGTGATC

(XhoI)loop Reverse: GATCACTATGAACAATACCTTCAAAATAAAATCCGTC

Second, product #3 was ligated into the pCDNA containing #1 and #2. BamHI site was removed using site-directed mutagenesis. Primers used:

(BamHI)loop Forward: GCGTCACCACAGTTCCAGAACTGTACAGTGGTCAG

(BamHI)loop Reverse: CTGACCACTGTACAGTTCTGGAACTGTGGTGACGC

This resulted is completion of the conditional fragment. Third, the conditional fragment was digested from pCDNA and ligated into pAC16 (using *HindIII and PacI restriction sites*).

2) 5' Recombination arm

The 5' recombination arm fragment was obtained by standard PCR using genomic DNA (from 129/Ola-derived IB10 ES cells (kind gift from Hans Clevers). Primers used (*Italic* is overhang, in *Capitals/Italic* the restriction sites):

5'arm Forward: c*tagcgGTTTAAAC*TCGAAGGCCTCAACCTCACAGAGATCTTTC

5'arm Reverse: *atcttaGGCGCGCCGGGCCC*TCTCCTCCTATCTGTAGGATG

This resulted in the following complete sequence of the 5' recombination arm:

(*PmeI*-(lastpartof)intron14-**exon15**-intron15-**exon16**-(firstpartof)intron16-*ApaI-AscI* (~2kb):

*GTTTAAAC*TCGAAGGCCTCAACCTCACAGAGATCTTTCTGCTTCTGCCTCTCTCCTGAGTG
CTGAGATTAAAGGTGTGTGCAGCCATGCCTAGCTGTGTGTGGCTTTCCTGTTACCACATT
CTGTGGATAGCTTATCTGTTGTTTGTGCCCCACCTTGTACTTAATATCAAGATGAGATGTT
TGGCTGTTCGGTGATACTAGCTCTGGTGACTAAAACTGAATAGGACCTTTATTCCTTTGCT
GAGTGTTCCTCCTTGGCTATCAGGCTGTTTTATGTTATGGTGATACATGAACAGAGATAA
AGGGCTTATTTTAAGTTTTACTGTAATTCTCTAAGCCAGCTAGCTGTAATTAGACTTGCTT
CTGGCTATTTTATTAACTTATAGCAAGTAATTGGAAAGCATCCCATCAGACCCATTTGTA
TAATTCCTGCACACAGTACGCTGAGGCGGGATGATTGCCTGGGCTACATACATGCACTGT
ATAGGCTGCTGCTATGGCTTTGGTGCAGGGCCCTGGCTTTAATCTCTTAACATAAAAACC
ATAAGCAAAAGACAAAATAGGTAGGAGTGTATATTTCCACATGGAGCATGTCTTCCCAT
AAATATTTTCCTTTTCACGCTCCCCTTATTAGATTTTCAGTTATGAGCATAAGGAAGAAGG
TGGGGTAAGAGTGATTGAACAAGAGTGACAGGGAAGAGGATTCGTGCAGGGGAGGGAA
GTGCATGCATGTTCTAGGCACTGAGTGATGGGTGTGCTGGAAGCTGTAAACTGCGTGGG
GGCCTCTTCCTCAGTGCTTTAAGAAATTGATTCATAGGAACATCATTGCTCCTGCCAACCT
AACTCAACTGTGACTTGCGCTGCTTTCCACAAATGAATGTAGTGATGGCTTACAATTACT
GTGATTTTTAAAAATATTCCCTATCAGAGAAATGAATTGGTTGATAGTAGGCACAATGAA
AAGGTGGGAGTTGGTGGGGAGAGGGGTCTAGGAAGTGAACTGTCAAATCACAGCCTTAG
TCATCCTTATCGTCTTCGAATGTCTTTTCCTTGATGTTTTCTCCCTGGATAAGAAAGGCAT
CCCTAGAATTTTGTGGATATAGCAACATCATATTTAAGTTGGTTTTCTTAGACACTGATGT
AGAAAACCTTTGAATTATTTGAATGTCCATTGTTATAGGGGCTGGAAATGGATACTTAGC
TTCTCATGTTGGTATTTCTTTAGGAATATCCTCAGCCTGAGACTGTTAGTGTTAAATGGAA
AGGTACTGCTCCAGTTTTCAGAGGGAGACATGTCCTAAGCTCTTTCTCCACTTTTTATGTA
GGTGTTTCAGGTATTGAATGAGAAAAAACAGATAAACGCTATCAAATATGTGAACCT
AGAAGACGCCGATAGCCAAACTATTGAGAGCTACCGCAACGAGATAGCGTTTTTGA
ACAAACTACAGCAACACAGTGATAAGATCATCCGCCTCTATGATTAGTATGAATTCA
TTTTTATTTTAAAAATAAAAGTTTGTTCTTGCCATAATTCTTAGGCAAAGAGTAAATCCTT
AATGACATAATGTGGGCATTTATTGTTTTGTTGTGTCTGTTTATCTTTAATTGCAG**TGAAA**
TCACCGAGCAGTACATCTACATGGTAATGGAATGTGGAAACATTGACCTAAATAGT
TGGCTTAAAAAGAAAAAATCCATCAATCCATGGGAACGCAAGAGCTACTGGAAAAA
CATGTTGGAGGCAGTACACATAATCCATCAGCATGGTATTTTCATATCTCTTCATACA
CGTAAAGTTAAAATAGTTGTTAATTGTGCCATTTTAGAAACATACCCTTAACTGGAAGTT
CATTAGAGGTGAAGGCACTCTTAAGAGTGGTTATACACAGGCTACAGAACACAAACAAG
CACAGGATGTAGAACAGAAATGGCCACATGTACAATGTAAACTTACCCTCCTCTGGTACC
TGGGGATTCCTATCTTCAAGTCCTGAGGATTTGGACATCCTACAGATAGGAGGAGA*GGG*
CCCGGCGCGCC

This fragment was ligated into pAC16 containing the conditional fragment (using *PmeI and AscI restriction sites*, upstream of 5' loxP site).

3) 3' Recombination arm

The 3' recombination arm fragment was obtained by standard PCR using genomic DNA (from 129/Ola-derived IB10 ES cells (kind gift from Hans Clevers)). Primers used (*Italic* is overhang, in *Capitals/Italic* the restriction sites):

3'arm Forward: *ccgccCCTGCAGG*ATGGCCAGTTTTTCAG

3'arm Reverse: *ctagcgGCGGCCGC*CTATTTGCAAATCACAAAG

This fragment was ligated into pAC16 (using *SbfI and NotI restriction sites*, downstream of 3' loxP site). CiMKi point mutations were introduced using site-directed mutagenesis.

Primers used for T649A mutation:

mMps1-T649A-F: CAAATGCAGCCAGACACA***GCA****AGCATTGTTAAAGATTC

mMps1-T649A-R: GAATCTTTAACAATGCTTGCTGTGTCTGGCTGCATTTG

Primers used for D637A mutation:

mMps1-D637A(KD)-F: GAATGCTAAAGCTAATT***GCT****TTTGGGATTGCAAAC

mMps1-D637A(KD)-R: GTTTGCAATCCCAAAAGCAATTAGCTTTAGCATTC

This resulted in the following complete sequence of the 3' recombination arm (*SbfI*-(lastpartof)intron16-**exon17***-intron17-*NotI* (~2.2kb), containing either T649A or D637A point mutation in exon 17):

*CCTGCAGG*ATGGCCAGTTTTTCAGTCTCCAAAGGATTTTCTCTTTGGAGCCCGGTGTGAGA
GTTGGTCTGTTCTTGCTGTTTTCTGTTTCATTATTTGTTTGTTCTTGTTCTCTCCAGTTTCCT
CTGCCTTTCCCTTGTCTTTTAAACAGATGTGTTCAATCTCATGCGCTTCCTCTGCTTCTCCT
TTTAGAAGCAGTAACTAAGCAGAGGATAGGACTCTTTGGGGGTGAGGGAGGCCTTGCGA
TCTTATTGTCTGACAGTTCTTGGTGGTGCTTTCCTGGCCTGTGCATGGCAGTGTGCAGCAC
ACACTGACAGGCTAGGCCTGGAATCCTGAACAGTTCACTTCAACTCAAATCACATTGAGT
GTCCTCACCCTGAAAATGATGATGATAATGGGACTTATTTGCTATGCATTGCTATAGAAT
GGTACCTGGCCCACAACATTTGCTAAGAAAGTGTAGAATGTATAATAACTAAATATTAAT
GTGTTGCCTAATTGAAAGGATAAGCCTGACCTAAAGATTATGAAATGCTTTTTTGTCCCC
TGGCCATAGAATGTTTTTCTTCATCTAACAGAACGGATTTTATTTTGAAG**GTATTGTTCA**
TAGTGATCTGAAGCCTGCTAACTTTGTGATAGTGGATGGAATGCTAAAGCTAATT*G
CT*TTTGGGATTGCAAACCAAATGCAGCCAGACACA*GCA*AGCATTGTTAAAGATT
CTCAGGTAGGAGTTTTGCTGTCTTGGTTGTATTTTAGTGTTTTGAACCAGGGTTTTGCATC
AGGGTTTTGCATAACCTAGAATGCTCTTGACTTTGATCAGTGGCCTTCAGCTCCTGATCCT
GCTGCCTGTGCATCCCAGGTGTGGGCTTATAGGTGTCAGCCCCGACACCCGACTTCAGGT
AGGATTTTAATGATGGCTGGTTACTACAAGGCTTAGTTCATTTTTATCTGTTAAATATGTT
GCCAATATTATATTTTTACCAACCATGTTATTCCAAAAATTTGAAGTCTTTTTAAAGAATA
GAAACTATGTTTATAAAAGACCATGGTCAAAGCCATGGTCAATTTGATTTATAAAAGCAG
TTCAAGATCAGACAAGTATATTTATGAATTTTGGATGATTTTCTCATAGCTGAGGCAGGG
CAGAGAGTAATTGCACCTTCATGTTCTCCACTGTCCTGTTTCTTTTTCTTACTGCTTAAATT
TGGGAGAAAGTTTTAAGAGAGCCTTATTGGGAATACTGAAGCGTTCCACTCAGCTACGC
GTTAAAAAGGAAATATTTTACTTACTGTTTGGGGGGCGGTGTGCGGATTCCAGATGTAAG
TGTGCCATTGTGGAGGTGTGGGGATGGCTTCGGTAAACCACTTCTCTCCTACTGTGGGCC
CTGGGAGCCAAAGTCAGATTGTGTGTGCAGCAAGCTCTACAGCCCAGCTGTGCTTTGTAG
TAACATTTGCTGTGGTAAATCTCATGAAGCTGAAGTAGTGAGGGGAAAACAGAGCTGAA
AGGTGATGTCGACTGCACCTCGCAGGCTGTGTCCAGGGATGGAGATAAATCAGAAGATA
AATTACCATGCACGTAGAAAGTCATTCTTCTTGACAGCCATTCATTGTTTTTTGTTTCAGA
AGTACAGATGATGAACAGTGAGTGTAGATGAGACTGAAGTTTTCTATGGCAAGGTCTTA
GCAGGCCGACATTTTGTTACCTTAGAACTAAAGGATTTTGCGTATTATCTCCATGCCCAG

CTAGAGAAGCTCTTCTCTGATATAGGTTTCCAACCCATCTTGATCTGCACATGGAGCCGA
AGAATATTGGGAGATAAAGCTAGCTGGTTCCTTTTATTCATGTATTAATTTGTTGCTTGGT
TTATTGAGGGAAGAATATGTTGAATTTATTGGAACATGAAAGTGAATGAAAGGCCAAGT
TCAGAATCCGCCTACGCAGTTGTAAAGACTTAGTACTTAGTACTTAGTACTTAGCACTAG
CTCTCCAGCACAGCTGCAGACAGCACAGTGCTCCCTGTGCTCCAGACGGAGCCCGTTCAT
TCTCAGCCCAGCTCATCTGATTGTACCTGGGATGGGATAGTACATACATTCTTATATTGTT
AGCAGTTATTTGAATTTTTCAAGTCTGTCATTTAAATCATTAGTTATTCAAATTTCCAAGA
ATCTGACATTTACATATTTACAAATCTAGAAAGATATTCTCATTGATTTCTTTGTGATTTG
CAAATAG*GCGGCCGC*
(*GCT* =D637A, *GCA* =T649A)

These fragments were ligated separately into pAC16 containing the conditional fragment and the 5' recombination arm (using *SbfI and NotI restriction sites*, downstream of 3' loxP site), to obtain the two separate complete targeting vectors containing CiMKi-T649A or CiMKi-D637A.

Fidelity of all PCR products, site-directed mutagenesis steps, and ligation steps were verified by sequencing.