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Supplementary information

CRISPR-based engineering of gene knockout cells by homology-directed insertion in polyploid Drosophila S2R+ cells

In the format provided by the authors and unedited

Supplementary Data 1. Genomic sequences of *Tnks* and *Apc*.

Tnks genomic sequence

TTCTTTTTTTTTTTAAGTTTTGAGCACTTTTTATTATCTGGCGGTGGGTCTTTCTTTCTTTCTATAAACT
GGTTTTCCATTATTATGTTCGTCTGTCTTTTTGTCACGTTCTGTGCACAAAAAGTGAGATCTTCGAGG
GGTCTGTTATTGTCGGGTGGCGTTTAAGTTATATATATTTTATTGCGGCTGCTTTTCACCTCGTTGAAT
GTATGACATTTTTGGCCACTCGAAGTTTTACAATACCTGTTGCGGTGGAAACCAAAACGGAGCTCAC
GTTTCCTCCTTCCTTTCCCGCAATCGTGACACATAGTAAAACGCAATCGCTTTACTTCGCTTCTTTGC
GACTATCAGATTTTCTGACATTGGCCCGTTATCATGGCATTTATGTGTGTGTGTGTACATATATATAGT
AAATATAGTCGATAAACCCGGAGAGACCTTCCCCTCCATATCTTGGCCATATTCGAGATACTTAAAAC
ATACGTTTATCGCTGGCCAAAAAGTTGTGCAAATTAACCTTCGGGGTGGTAGTTGCAAATCAAGTTGA
AATTGCATACATATATCTCAGTTTTTATTCATATATTGTTCACAGTCCCAAGGATCACACTTCCAAGTG
GCGAGCAATTGGCGCTTTGACGCTCCAAGTGCAACAGTTGGAATTAAGTTTTCCATTCTATTAATTTT
TAATGCAAAACTTTCGTAATTGTTTGCCCACAGAAAAGTGAACGGCACAAACGTTTCGCAAACCACTT
TTTGGTGGGTGGCTCAAGCTTTTGTTTGCCCAGATTTGTAATTCGATCCGGTTCTCTTTGCAGTTCAT
TATAAACGTAGCAATAAGTACGATCATAAACATTTATTTTATGTTGATCATCTCTGGTGTGACCAATTA
CGGTACCCTATTAAGATGACTCAGATTTCTGGCATTGACAATGCAAAAGTTGCCAAAAGTATGTTTAT
AAATCGGGGGCTCGGTTGGATCATCACAACTGTCATCCGTTTATATTGATTATTATGCGACTTATTTT
GATTATTTATCATTTAATTGTAAGGTTTACTTTCAACTGAACATAAGCTAATGTAAACTTTTTTGAGATT
TCCTTGGGCAACAGTTCTGCAAATGCGGAAAACATATCTTTCTTTTCGTATGCCATGGACAAGGTTGA
AATCCCCGAAGGCAAGACCTTCTCGCTCATTTGTAGGGCTTTCCCATCGACCAATACATGACATTCAT
CTAATAACCTATTCGTTTTTATTTTACCAGGTTATGGACGCCGGGAAGTGGTTGAATTCCTGCTGAAC
AGCGGCGCCTCCATACAGGCGTGTGACGAGGGTGGGCTGCACCCGCTGCACAACTGTTGCTCCTTTT
GGCCACGCCGAGGTAGTTCGATTGTTGCTGAAGGCAGGTGCCAGTCCAAACACCACCGACAACTGG
AACTACACGCCATTGCACGAGGCGGCCAGCAAGGGCAAGGTGGATGTGTGCCTGGCTCTGTTGCA
GCATGGCGCAAACCATACGATCCGCAACTCGGAGCAGAAGACACCACTGGAGCTGGCGGACGAGG
CGACGCGTCCCGTATTGACCGGCGAATATCGAAAGGATGAGCTGCTTGAAGCCGCACGCTCGGGG
GCCGAGGATCGCCTGCTGGCCCTACTCACGCCACTCAATGTCAACTGTCATGCCAGCGATGGACGA
CGCTCAACGCCGCTCCATCTGGCAGCGGGCTACAATCGGATCGGCATCGTGGAAATTCTGCTGGCC
AACGGAGCGGATGTACATGCTAAGGACAAGGGCGGTCTGGTGCCGCTGCACAATGCCTGCTCCTAC
GGACACTTCGATGTGACCAAGCTGCTTATCCAGGCGGGCGCCAATGTCAACGCCAACGATCTGTGG
GCCTTTACGCCGCTCCACGAGGCCGCCTCCAAAAGTCGCGTCGAGGTCTGCAGCCTGCTGCTCAGT
CGTGGAGCGGATCCCACCCTCCTAAACTGCCACAGCAAGTCGGCCATCGATGCGGCGCCCACCAG
GGAGCTGAGAGAGCGGATTGCCTGTGAGTTATAAGCTGTCTGCGTTTTAAATATCTTAGCTCTAACG
TCATTCATATTACTTTTCAGTTGAATACAAGGGTCACTGCCTGCTGGACGCCTGTCGAAAGTGTGATG
TGTCCCGTGCCAAGAAGCTGGTATGCGCAGAGATTGTTAACTTCGTGCATCCATATACAGGAGACAC
TCCGCTCCACCTGGCCGTTGTCAGTCCGGATGGGAAGCGCAAGCAGCTGATGGAACTGCTGACCAG
AAAGGGATCCTTGCTGAACGAGAAAAACAAGGCTTTCCTCACGCCCCTGCATTTGGCTGCCGAGCT
GCTTCACTACGATGCCATGGAGGTGCTGCTAAAGCAGGGCGCCAAGGTTAATGCATTGGACAGTCT
TGGACAAACGCCACTGCATCGGTGCGCCCGTGATGAGCAAGCGGTGCGACTGCTGCTCTCGTACG
CAGCGGACACGAATATCGTTTCCCTTGAGGGACTTACGGCCGCTCAATTGGCCTCGGACAGCGTGC
TGAAGCTGCTCAAGAATCCTCCGGACAGTGAGACACATTTACTGGAGGCAGCCAAGGCGGGAGATC
TGGACACTGTGCGCCGTATAGTGCTCAACAATCCGATTTCGGTCAATTGCCGGGATTTGGACGGAC
GACATTCCACACCTTTGCACTTTGCTGCTGGGTTTAATAGAGTGCCAGTGGTTCAGTTTCTTTTGGAA
CACGGCGCCGAGGTTTATGCGGCTGACAAGGGCGGACTGGTGCCCCTGCACAATGCCTGCTCTTA

sgRNA sequences are marked with underlines. PAM sequences are marked in red. Homology arms are highlighted as blue.

Apc genomic sequence

AACAGCTGAAAAAAAAGAAGTGCAGCACCAAATTGCATTTGCACATAATTCAACGGTAGCTCAAATGA
GAAATATGTCTTGTGCTTATGGAGTGTGTGTAGCAACAACAATGAGAGCCGAGGCAATATAATATATC
TGCTGAAAACGCATCTGTATCTTTCTTCATGCACTGGAAATTCCGCAAAGACGACGCCGTGTGCCGC
TAACTTTCTAAAAAGATGCAGCGCCAGCTAATCATGATAATGACTTAGCTGCTCATTTGCTATTGTGG
CTGCCGCCACCTGAATGTTGGCCGATGATTAAGTTACAGAGCGATAGAGCAGGCAATGCACAGAAC
CTGCCACAATAGGCGAATTCTTCTGGGATACAACAGCATCCAAGCATCTGCTTAGAAAGTCGATCCT
ACCGAGCAACCTTATAGATAGATAGGGGTCGGCCAATTAAAAAGTGAAAAGCTATTAAATTGGTTTCC
CATTAAGATGAATTTATATATTTCGCTTTTATATTTTATGTGGTGTTCTAAAATAAAACAACAAGAACTA
CAAGAAACTTGAGGCTTTGGATCATTTCCTTAAAATTTGGCGCAATGACTTCACCGCTTAAGATAGCA
TTTATCTTGGCTGGATTTTCATATCATAAAGCTGACATACATAGTTTCCATCGATCATAAAGCGAGTCA
TATGCTCATGATGAGTTAAGTGTAGGCACTAAACTGACGACTGCCAATATTTTCCCATGCTTAACTTG
CTGTTTTAACTTTCCTCAGTTAAATAAACTATAATTAAGTTGAGCTTCGAATGAGTGTTAAGAAAAAAG
AAATATATACATTCAATATCATCTTTTTCGAGACACACGCCTATGCCTTTTTACATTTTTGCTATATTTT
CCATCGAATCACCTTTTAAACTTAATACTGTTGCCTTTCTTAGCGCTGCCCACATCTAATATGTCTTAT
ATGCATCCATGCAACTAGCTGACTCGTCTCTGTTTGAGTTTTGCCCATCGGATTACTACAGAGATTGG
CGGGCAGAGTGGTGGTGGTGGTGGTGCGTCTATAATTAGCCCACGCCCTGGTCCGGCACTAAATTT
ATAGCAATCTAGAGTCTCAATTGTGCCTTCGCTCACTGTGTTTTCCTATCTGCTTTCCTTGCAGGCCA
TGGAGCATTGACTTGCACCACTGAGACCCCGAATCCCTAGTCCCCGATTCCCGCCAGGCCATGTTG
GAGCCAACGCTGACGCCCGATCTGGAGGAGGGCATCGCCGAGCTCAGTCTGGAGGACGTGGATGT
GGACTCGGTGCCAGAGCGTAAGCCGCACTTCCTCGACTACGACGCAGTGCCCCCGCCAGACTATGA
AGAAGGCTTTGGGGCCAGTTCCGGGGAGCAGCTAAAGATGGACAAGAAGTACGAGCGGGATGGCG
AAGTGAGCGACTACGAACTGGCCGCCAGTGGCTATACCAAGAAGGAGTTCACCCAGGACGACAACA
CGCTGCACTTCACCCAGAGCGCAGTCGGACTGGGATCCGGAGCCGTTGGTAAAAAACCAGCAGCCA
AGCATTTTCTGGACGAGAACCCCATTCCGCCGGACTACATGTTGGCCCAGGAGCTGCGGGAGATGA
GAGAACATCGCAGCCTGGACAGGAACTTCGAGCGACAGTCGGCGCAGCAGCAGCAGCTGGATGAA
TTGCCTCCGAGGAATGGAGGCGGCTCTCCAGCCAGCGCAGGTCGTCCATCGCGCAGCAAGGAACC
ATCTTACACGCTCTCCCGATTTCTGGACGGAGATGCACCTGCCCCTGCTCCAAGGCTGCCCAAGGG
AGCTGCCTGGACGACCAGCTTCGATGAGCGATACACCTCCTCGGCAGTGGAAGCCACCTTGGGATC
CAAGGTGGAGTGTGTTTACTCACTGCTCTCCATGTTGGGCTCAAATGATCCTCTCGAAATGGCCAAA
AAGTTCCTGGAGCTGTCCGGAAATGCTCAAAGTTGTGCAACTCTTAGACGCTCTGGTTGCATGCCGC
TTCTGGTGCAGATGATGCATGCACCGGACAACGACCAGGAGGTGAGGAAGTGTGCTGGACAGGCG
CTGCACAATGTGGTGCACAGCCATCCGGACGAGAAGGCTGGCAGAAGGGAAGCCAAGGTGCTCCG
ACTGCTTGACCAGATCGTTGACTACTGCTCCTTCCTGAAGACGCTGCTCCAGAGTGGCGGAGAGGC
TATTGCAGATGACTCCGACCGTCATCCGCTGGCCGCCATCTCTTCACTGATGAAGGTGAGCTTCGAT
GAGGAGCACCGACACGCTATGTGCGAGCTGGGGGCATTGCACGCGATCCCCAATCTGGTTCATTTG
GATCACGCCGTTCATGGACCCAAACCGGAGGATCAGTGCTGCAATTCTCTGCGAAGATACGCTTTGA
TGGCCCTGACCAATCTGACCTTTGGGGATGAGAACAACAAGGCGCTGCTGTGTGGTCAAAAGCAGT
TCATGGAGGCCCTGGTGGCCCAATTGGACTCGGCCCCGGACGATCTGCTCCAGGTGACGGCCAGC
GTGCTGCGCAATCTCTCCTGGCGAGCGGACAGCAACATGAAGGCAGTGCTGAATGAGATTGGAACA
GTGACAGCTCTGGCCCTGGCGGCCATGCGGAATAGGAGTGAGAACACCCTGAAGGCCATACTCTCG
GCCCTGTGGAATCTCTCGGCGCACTGCAGCACCAACAAGGCGGAGTTC

sgRNA sequences are marked with underlines. PAM sequences are marked in red. Homology arms are highlighted as blue.

Supplementary Data 2. Next generation sequencing results showing Tnks loci and Apc loci in 3 *Tnks* KO and 3 *Apc* KO cells.

The next generation sequencing results show the cassette is correctly inserted into the target locus in the knockout cell lines. We highlighted the flanking genomic sequence as blue, the homology arm sequence as yellow, the insertion cassette sequence as green.

Upstream sequence of insertion cassette of *Tnks* KO clone1

ATGACATTTTTGGCCACTCGAAGTTTTACAATACCTGTTGCGGTGGAAACCAAAACGGAGCTCACGTT
TCCTCCTTCCTTTCCCGCAATCGTGACACATAGTAAAACGCAATCGCTTTACTTCGCTTCTTTGCGAC
TATCAGATTTTCTGACATTGGCCCGTTATCATGGCATTTATGTGTGTGTGTGTACATATATATAGTAAA
TATAGTCGATAAACCCGGAGAGACCTTCCCCTCCATATCTTGGCCATATTCGAGATACTTAAAACATA
CGTTTATCGCTGGCCAAAAAGTTGGGCAAATTAACCTTCGGGGCGGTAGTTGCAAATCAAGTTGAAA
TTGCATACATATATCTCAGTTTTTATTCATATATTGTTCACAGTCCCAAGGATCACACTTCCAAGTGGC
GAGCAATTGGCGCTTTGACGCTCCAAGTGCAACAGTTGGAATTAAGTTTTCCATTCTATTAATTTTTAA
TGCAAAACTTTCGTAATTGTTTGCACACAGAAAAGTGAACGGCACAAACGTTTCGCAAACCACTTTTT
GGTGGGTGGCTCAAGCTTTTGTTTGCCCAGATTTGTAATTCGATCCGGTTCTCTTTGCAGTTCATTAT
AAACGTAGCAATAAGTACGATCATAAACATTTATTTTATGTTGATCATCTCTGGTGTGACCAATTACGG
TACCCTATTAAGATGACTCAGATTTCTGGCATTGACAATGCAAAAGTTGCCAAAAGTATGTTTATAAAT
CGGGGGCTCGGTTGGATCATCACAACTGTCATCCGTTTATATTGATTATTATGCGACTTATTTTGATT
ATTTATCATTTAATTGTAAGGTTTACTTTCAACTGAACATAAGCTAATGTAAACTTTTTTGAGATTTCCT
TGGGCAACAGTTCTGCAAATGCGGAAAACATATCTTTCTTTTCGTATGCCATGGACAAGGTTGAAATC
CCCGAAGGCAAGACCTTCTCGCTCATTTGTAGGGCTTTCCCATCGACCAATACATGACATTCATCTAA
TAACCTATTCGTTTTTATTTTACCAGGTTATGGACGCCGGGAAGTGGTTGAATTCCTGCTGAACAGCG
GCGCCTCCATACAGGCGTGTGAGGGCAGAGGAAGCCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTGCTAGCGGTAGCGGCAGCGGTAGCATGACCGAGTACAAGCCCACGGTGCGCCTCGC
CACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCG

Downstream sequence of insertion cassette of *Tnks* KO clone1

GACAATGGTTTTCCGTTGACGTACATACATCTGACGTGTGTTTATTTAGACATAATAGTTATGTTTTCA
CATCTTTTTAATGTTCGCTTAATGCGTATGCATTCTAGACAATTGTGCTCGGCAACAGCCATACGATC
CGCAACTCGGAGCAGAAGACACCACTGGAGCTGGCGGACGAGGCGACGCGTCCCGTATTGACCGG
CGAATATCGAAAGGATGAGCTGCTTGAAGCCGCACGCTCGGGGGCCGAGGATCGCCTGCTGGCCC
TACTCACGCCACTCAATGTCAACTGTCATGCCAGCGATGGACGACGCTCAACGCCGCTCCATCTTGC
AGCGGGCTACAATCGGATCGGCATCGTGGAAATTCTGCTGGCCAACGGAGCGGATGTACATGCTAA
GGACAAGGGCGGTCTGGTGCCGCTGCACAATGCCTGCTCCTACGGACACTTCGATGTGACCAAGCT
GCTTATCCAGGCGGGCGCTAATGTTAACGCCAACGATCTGTGGGCCTTTACGCCGCTCCACGAGGC
CGCCTCCAAAAGTCGCGTCGAGGTCTGCAGCCTGCTGCTCAGTCGTGGAGCGGATCCCACCCTCCT
AAACTGCCACAGCAAGTCGGCCATCGATGCGGCGCCCACCAGGGAGCTGAGAGAGCGGATTGCCT
GTGAGTTATAAGCTGTCTGCGTTTTAAATATCTTAGCTCTAACGTCATTCATATTACTTTTCAGTTGAA
TACAAGGGTCACTGCCTGCTGGACGCCTGTCGAAAGTGTGATGTGTCCCGTGCCAAGAAGCTGGTA
TGCGCAGAGATTGTTAACTTCGTGCATCCATATACAGGAGACACTCCGCTCCACCTGGCCGTTGTCA
GTCCGGATGGGAAGCGCAAGCAGCTGATGGAACTGCTGACCAGAAAGGGATCCTTGCTGAACGAGA
AAAACAAGGCTTTCCTCACGCCCCTGCATTTGGCTGCCGAGCTGCTTCACTACGATGCCATGGAGGT
GCTGCTAAAGCAGGGCGCCAAGGTTAATGCATTGGACAGTCTTGGACAAACGCCACTGCATCGGTG
CGCCCGTGATGAGCAAGCGGTGCGACTGCTGCTCTCGTACGCAGCGGACACGAATATCGTTTCCCT
TGAGGGACTTACGGCCGCTCAATTGGCCTCGGACAGCGTGCTGAAGCTGCTCAAGAATCCTCCGGA
CAGTGAGACACATTTACTGGAGGCAGCCAAGGCGGGAGATCTGGACACTGTGCGCCGTATAGTGCT
CAACAATCCGATTTC

Upstream sequence of insertion cassette of *Tnks* KO clone2

ATGACATTTTTGGCCACTCGAAGTTTTACAATACCTGTTGCGGTGGAAACCAAAACGGAGCTCACGTT
TCCTCCTTCCTTTCCCGCAATCGTGACACATAGTAAAACGCAATCGCTTTACTTCGCTTCTTTGCGAC
TATCAGATTTTCTGACATTGGCCCGTTATCATGGCATTTATGTGTGTGTGTGTACATATATATAGTAAA
TATAGTCGATAAACCCGGAGAGACCTTCCCCTCCATATCTTGGCCATATTCGAGATACTTAAAACATA
CGTTTATCGCTGGCCAAAAAGTTGGGCAAATTAACCTTCGGGGCGGTAGTTGCAAATCAAGTTGAAA
TTGCATACATATATCTCAGTTTTTATTCATATATTGTTCACAGTCCCAAGGATCACACTTCCAAGTGGC
GAGCAATTGGCGCTTTGACGCTCCAAGTGCAACAGTTGGAATTAAGTTTTCCATTCTATTAATTTTTAA
TGCAAAACTTTCGTAATTGTTTGCACACAGAAAAGTGAACGGCACAAACGTTTCGCAAACCACTTTTT
GGTGGGTGGCTCAAGCTTTTGTTTGCCCAGATTTGTAATTCGATCCGGTTCTCTTTGCAGTTCATTAT
AAACGTAGCAATAAGTACGATCATAAACATTTATTTTATGTTGATCATCTCTGGTGTGACCAATTACGG
TACCCTATTAAGATGACTCAGATTTCTGGCATTGACAATGCAAAAGTTGCCAAAAGTATGTTTATAAAT
CGGGGGCTCGGTTGGATCATCACAACTGTCATCCGTTTATATTGATTATTATGCGACTTATTTTGATT
ATTTATCATTTAATTGTAAGGTTTACTTTCAACTGAACATAAGCTAATGTAAACTTTTTTGAGATTTCCT
TGGGCAACAGTTCTGCAAATGCGGAAAACATATCTTTCTTTTCGTATGCCATGGACAAGGTTGAAATC
CCCGAAGGCAAGACCTTCTCGCTCATTTGTAGGGCTTTCCCATCGACCAATACATGACATTCATCTAA
TAACCTATTCGTTTTTATTTTACCAGGTTATGGACGCCGGGAAGTGGTTGAATTCCTGCTGAACAGCG
GCGCCTCCATACAGGCGTGTGAGGGCAGAGGAAGCCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTGCTAGCGGTAGCGGCAGCGGTAGCATGACCGAGTACAAGCCCACGGTGCGCCTCGC
CACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCG

Downstream sequence of insertion cassette of *Tnks* KO clone2

GACAATGGTTTTCCGTTGACGTACATACATCTGACGTGTGTTTATTTAGACATAATAGTTATGTTTTCA
CATCTTTTTAATGTTCGCTTAATGCGTATGCATTCTAGACAATTGTGCTCGGCAACAGCCATACGATC
CGCAACTCGGAGCAGAAGACACCACTGGAGCTGGCGGACGAGGCGACGCGTCCCGTATTGACCGG
CGAATATCGAAAGGATGAGCTGCTTGAAGCCGCACGCTCGGGGGCCGAGGATCGCCTGCTGGCCC
TACTCACGCCACTCAATGTCAACTGTCATGCCAGCGATGGACGACGCTCAACGCCGCTCCATCTTGC
AGCGGGCTACAATCGGATCGGCATCGTGGAAATTCTGCTGGCCAACGGAGCGGATGTACATGCTAA
GGACAAGGGCGGTCTGGTGCCGCTGCACAATGCCTGCTCCTACGGACACTTCGATGTGACCAAGCT
GCTTATCCAGGCGGGCGCTAATGTTAACGCCAACGATCTGTGGGCCTTTACGCCGCTCCACGAGGC
CGCCTCCAAAAGTCGCGTCGAGGTCTGCAGCCTGCTGCTCAGTCGTGGAGCGGATCCCACCCTCCT
AAACTGCCACAGCAAGTCGGCCATCGATGCGGCGCCCACCAGGGAGCTGAGAGAGCGGATTGCCT
GTGAGTTATAAGCTGTCTGCGTTTTAAATATCTTAGCTCTAACGTCATTCATATTACTTTTCAGTTGAA
TACAAGGGTCACTGCCTGCTGGACGCCTGTCGAAAGTGTGATGTGTCCCGTGCCAAGAAGCTGGTA
TGCGCAGAGATTGTTAACTTCGTGCATCCATATACAGGAGACACTCCGCTCCACCTGGCCGTTGTCA
GTCCGGATGGGAAGCGCAAGCAGCTGATGGAACTGCTGACCAGAAAGGGATCCTTGCTGAACGAGA
AAAACAAGGCTTTCCTCACGCCCCTGCATTTGGCTGCCGAGCTGCTTCACTACGATGCCATGGAGGT
GCTGCTAAAGCAGGGCGCCAAGGTTAATGCATTGGACAGTCTTGGACAAACGCCACTGCATCGGTG
CGCCCGTGATGAGCAAGCGGTGCGACTGCTGCTCTCGTACGCAGCGGACACGAATATCGTTTCCCT
TGAGGGACTTACGGCCGCTCAATTGGCCTCGGACAGCGTGCTGAAGCTGCTCAAGAATCCTCCGGA
CAGTGAGACACATTTACTGGAGGCAGCCAAGGCGGGAGATCTGGACACTGTGCGCCGTATAGTGCT
CAACAATCCGATTTC

Upstream sequence of insertion cassette of *Tnks* KO clone3

ATGACATTTTTGGCCACTCGAAGTTTTACAATACCTGTTGCGGTGGAAACCAAAACGGAGCTCACGTT
TCCTCCTTCCTTTCCCGCAATCGTGACACATAGTAAAACGCAATCGCTTTACTTCGCTTCTTTGCGAC
TATCAGATTTTCTGACATTGGCCCGTTATCATGGCATTTATGTGTGTGTGTGTACATATATATAGTAAA
TATAGTCGATAAACCCGGAGAGACCTTCCCCTCCATATCTTGGCCATATTCGAGATACTTAAAACATA
CGTTTATCGCTGGCCAAAAAGTTGGGCAAATTAACCTTCGGGGCGGTAGTTGCAAATCAAGTTGAAA
TTGCATACATATATCTCAGTTTTTATTCATATATTGTTCACAGTCCCAAGGATCACACTTCCAAGTGGC
GAGCAATTGGCGCTTTGACGCTCCAAGTGCAACAGTTGGAATTAAGTTTTCCATTCTATTAATTTTTAA
TGCAAAACTTTCGTAATTGTTTGCACACAGAAAAGTGAACGGCACAAACGTTTCGCAAACCACTTTTT

GGTGGGTGGCTCAAGCTTTTGTTTGCCCAGATTTGTAATTCGATCCGGTTCTCTTTGCAGTTCATTAT
AAACGTAGCAATAAGTACGATCATAAACATTTATTTTATGTTGATCATCTCTGGTGTGACCAATTACGG
TACCCTATTAAGATGACTCAGATTTCTGGCATTGACAATGCAAAAGTTGCCAAAAGTATGTTTATAAAT
CGGGGGCTCGGTTGGATCATCACAACTGTCATCCGTTTATATTGATTATTATGCGACTTATTTTGATT
ATTTATCATTTAATTGTAAGGTTTACTTTCAACTGAACATAAGCTAATGTAAACTTTTTTGAGATTTCCT
TGGGCAACAGTTCTGCAAATGCGGAAAACATATCTTTCTTTTCGTATGCCATGGACAAGGTTGAAATC
CCCGAAGGCAAGACCTTCTCGCTCATTTGTAGGGCTTTCCCATCGACCAATACATGACATTCATCTAA
TAACCTATTCGTTTTTATTTTACCAGGTTATGGACGCCGGGAAGTGGTTGAATTCCTGCTGAACAGCG
GCGCCTCCATACAGGCGTGTGAGGGCAGAGGAAGCCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTGCTAGCGGTAGCGGCAGCGGTAGCATGACCGAGTACAAGCCCACGGTGCGCCTCGC
CACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCG

Downstream sequence of insertion cassette of *Tnks* KO clone3

GACAATGGTTTTCCGTTGACGTACATACATCTGACGTGTGTTTATTTAGACATAATAGTTATGTTTTCA
CATCTTTTTAATGTTCGCTTAATGCGTATGCATTCTAGACAATTGTGCTCGGCAACAGCCATACGATC
CGCAACTCGGAGCAGAAGACACCACTGGAGCTGGCGGACGAGGCGACGCGTCCCGTATTGACCGG
CGAATATCGAAAGGATGAGCTGCTTGAAGCCGCACGCTCGGGGGCCGAGGATCGCCTGCTGGCCC
TACTCACGCCACTCAATGTCAACTGTCATGCCAGCGATGGACGACGCTCAACGCCGCTCCATCTTGC
AGCGGGCTACAATCGGATCGGCATCGTGGAAATTCTGCTGGCCAACGGAGCGGATGTACATGCTAA
GGACAAGGGCGGTCTGGTGCCGCTGCACAATGCCTGCTCCTACGGACACTTCGATGTGACCAAGCT
GCTTATCCAGGCGGGCGCTAATGTTAACGCCAACGATCTGTGGGCCTTTACGCCGCTCCACGAGGC
CGCCTCCAAAAGTCGCGTCGAGGTCTGCAGCCTGCTGCTCAGTCGTGGAGCGGATCCCACCCTCCT
AAACTGCCACAGCAAGTCGGCCATCGATGCGGCGCCCACCAGGGAGCTGAGAGAGCGGATTGCCT
GTGAGTTATAAGCTGTCTGCGTTTTAAATATCTTAGCTCTAACGTCATTCATATTACTTTTCAGTTGAA
TACAAGGGTCACTGCCTGCTGGACGCCTGTCGAAAGTGTGATGTGTCCCGTGCCAAGAAGCTGGTA
TGCGCAGAGATTGTTAACTTCGTGCATCCATATACAGGAGACACTCCGCTCCACCTGGCCGTTGTCA
GTCCGGATGGGAAGCGCAAGCAGCTGATGGAACTGCTGACCAGAAAGGGATCCTTGCTGAACGAGA
AAAACAAGGCTTTCCTCACGCCCCTGCATTTGGCTGCCGAGCTGCTTCACTACGATGCCATGGAGGT
GCTGCTAAAGCAGGGCGCCAAGGTTAATGCATTGGACAGTCTTGGACAAACGCCACTGCATCGGTG
CGCCCGTGATGAGCAAGCGGTGCGACTGCTGCTCTCGTACGCAGCGGACACGAATATCGTTTCCCT
TGAGGGACTTACGGCCGCTCAATTGGCCTCGGACAGCGTGCTGAAGCTGCTCAAGAATCCTCCGGA
CAGTGAGACACATTTACTGGAGGCAGCCAAGGCGGGAGATCTGGACACTGTGCGCCGTATAGTGCT
CAACAATCCGATTTC

Upstream sequence of insertion cassette of *Apc* KO clone1

GTATCTTTCTTCATGCACTGGAAATTCCGCAAAGACGACGCCGTGTGCCGCTAACTTTCTAAAAGATG
CAGCGCCAGCTAATCATGATAATGACTTAGCTGCTCATTTGCTATTGTGGCTGCCGCCACCTGAATG
TTGGCCGATGATTAAGTTACAGAGCGATAGAGCAGGCAATGCACAGAACCTGCCACAATAGGCGAAT
TCTTCTGGGATACAACAGCATCCACGCATCTGCTTAGAAAGTCGATCCTACCGAGCAACCTTATAGAT
AGATAGGGGTCGGCCAATTAAAAAGTGAAAAGCTATTAAATTGGTTTCCCATTAAGATCAATTTATATA
TTTCGCTTTTATATTTTATGTGGTGTTATAAAATAAAACAACAAGAACTACAAGAAACTTGAGGCTTGG
ATCATTTCCTTAAAATTTGGCGCAATGACTTCACCGCTTAAGATAGCATTTATCTTGGCTGGATTTTCA
TATCATAAAGCTGACATACATAGTTTCCATCGATCATAAAGCGAGTCATATGCTCATGATGAGTTAAG
TGTAGGCACTAAACTGACGACTGCCAATATTTTCCCATGCTTAACTTGCTGTTTTAACTTTCCTCAGTT
AAATAAACTATAATTAAGTTGAGCTTCGAATGAGTGTTAAGAAAAAAGAAATATATACATTCAATATCA
TCTTTTTCGAGACACACGCCTATGCCTTTTTACATTTTTGCTATATTTTCCATCGAATCACCTTTTAAAC
TTAATACTGTTGCCTTTCTTAGCGCTGCCCACATCTAATATGTCTTATATGCATCCATGCAACTAGCTG
ACTCGTCTCTGTTTGAGTTTTGCCCATCGGATTACTACAGAGATTGGCGGGCAGAGTGGTGGTGGTG
GTGGTGCGTCTATAATTAGCCCACGCCCTGGTCCGGCACTAAATTTATAGCAATCTAGAGTCTCAATT
GTGCCTTCGCTCACTGTGTTTTCCTATCTGCTTTCCTTGCAGGCCATGGAGCATTGACTTGCACCACT
GAGACCCCGAATCCCTAGTCCCCGATTCCCGCCAGGCCATGTTGGAGCCAACGCTGACGCCCGATC

TGGAGGAGGGCATCGCCGAGGAGGGCAGAGGAAGCCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTGCTAGCGGTAGCGGCAGCGGTAGCATGACCGAGTACAAGCCCACGGTGCGCCTCGC
CACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCG

Downstream sequence of insertion cassette of *Apc* KO clone1

GACAATGGTTTTCCGTTGACGTACATACATCTGACGTGTGTTTATTTAGACATAATAGTTATGTTTTCA
CATCTTTTTAATGTTCGCTTAATGCGTATGCATTCTAGACAATTGTGCTCGGCAACAGGGATGGCGAA
GTGAGCGACTACGAACTGGCCGCCAGTGGCTATACCAAGAAGGAGTTCACCCAGGACGACAACACG
CTGCACTTCACCCAGAGCGCAGTCGGACTGGGATCCGGAGCCGTTGGTAAAAAACCAGCAGCCAAG
CATTTTCTGGACGAGAACCCCATTCCGCCGGACTACATGTTGGCCCAGGAGCTGCGGGAGATGAGA
GAACATCGCAGCCTGGACAGGAACTTCGAGCGACAGTCGGCGCAGCAGCAGCAGCTGGATGAATT
GCCTCCGAGGAATGGAGGCGGCTCTCCAGCCAGCGCAGGTCGTCCATCGCGCAGCAAGGAACCAT
CTTACACGCTCTCCCGATTTCTGGACGGAGATGCACCTGCCCCTGCTCCAAGGCTGCCCAAGGGAG
CTGCCTGGACGACCAGCTTCGATGAGCGATACACCTCCTCGGCAGTGGAAGCCACCTTGGGATCCA
AGGTGGAGTGTGTTTACTCACTGCTCTCCATGTTGGGCTCAAATGATCCTCTCGAAATGGCCAAAAA
GTTCCTGGAGCTGTCCGGAAATGCTCAAAGTTGTGCAACTCTTAGACGCTCTGGTTGCATGCCGCTT
CTGGTGCAGATGATGCATGCACCGGACAACGACCAGGAGGTGAGGAAGTGTGCTGGACAGGCGCT
GCACAATGTGGTGCACAGCCATCCGGACGAGAAGGCTGGCAGAAGGGAAGCCAAGGTGCTCCGAC
TGCTTGACCAGATCGTTGACTACTGCTCCTTCCTGAAGACGCTGCTCCAGAGTGGCGGAGAGGCTA
TTGCAGATGACTCCGACCGTCATCCGCTGGCCGCCATCTCTTCACTGATGAAGGTGAGCTTCGATGA
GGAGCACCGACACGCTATGTGCGAGCTGGGGGCATTGCACGCGATCCCCAATCTGGTTCATTTGGA
TCACGCCGTTCATGGACCCAAACCGGAGGATCAGTGCTGCAATTCTCTGCGAAGATACGCTTTGATG
GCCCTGACCAATCTGACCTTTGGGGATGAGAACAACAAGGCGCTGCTGTGTGGTCAAAAGCAGTTC
ATGGAGGCCCTGGTGGCCCAATTGGACTCGGCCCCG

Upstream sequence of insertion cassette of *Apc* KO clone2

GTATCTTTCTTCATGCACTGGAAATTCCGCAAAGACGACGCCGTGTGCCGCTAACTTTCTAAAAGATG
CAGCGCCAGCTAATCATGATAATGACTTAGCTGCTCATTTGCTATTGTGGCTGCCGCCACCTGAATG
TTGGCCGATGATTAAGTTACAGAGCGATAGAGCAGGCAATGCACAGAACCTGCCACAATAGGCGAAT
TCTTCTGGGATACAACAGCATCCACGCATCTGCTTAGAAAGTCGATCCTACCGAGCAACCTTATAGAT
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TGGAGGAGGGCATCGCCGAGGAGGGCAGAGGAAGCCTTCTAACATGCGGTGACGTGGAGGAGAAT
CCCGGCCCTGCTAGCGGTAGCGGCAGCGGTAGCATGACCGAGTACAAGCCCACGGTGCGCCTCGC
CACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCG

Downstream sequence of insertion cassette of *Apc* KO clone2

GACAATGGTTTTCCGTTGACGTACATACATCTGACGTGTGTTTATTTAGACATAATAGTTATGTTTTCA
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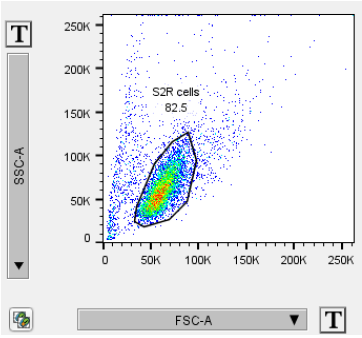
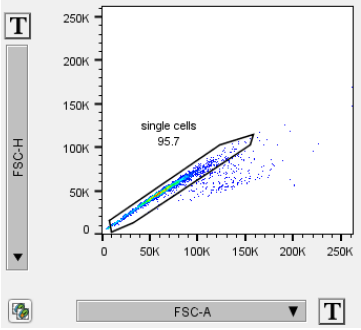
Supplementary Figure 1. Single cell gating in flow cytometry.

FSC vs SSC

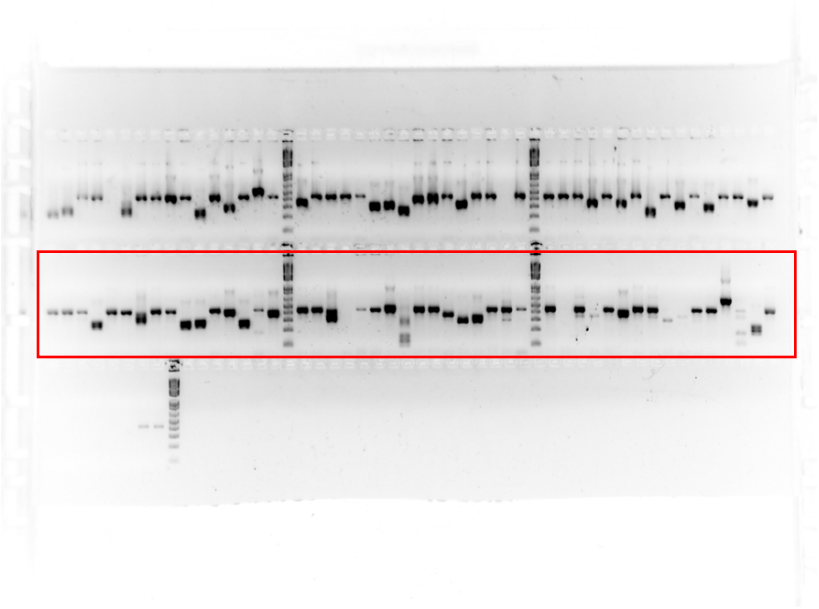


FSC-A vs FSC-H

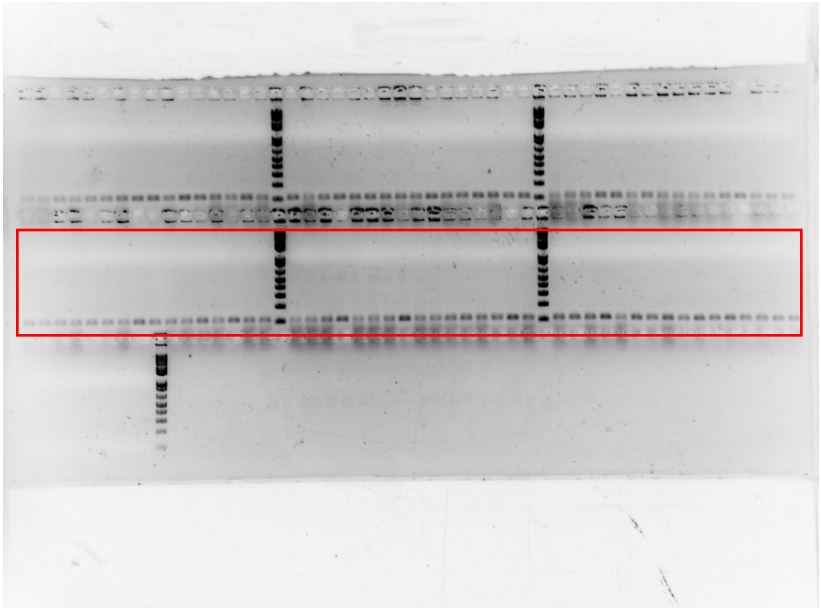


Supplementary Figure 2

Tnks nested PCR

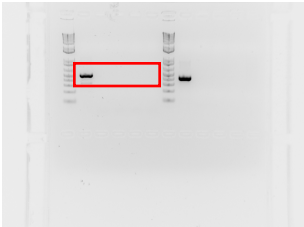


Act5c nested PCR

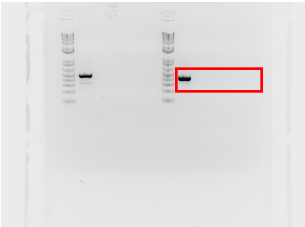


Supplementary Figure 3

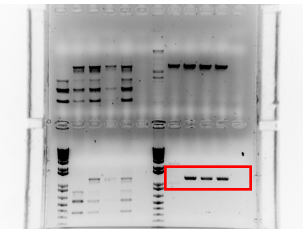
Tnks 1st round PCR



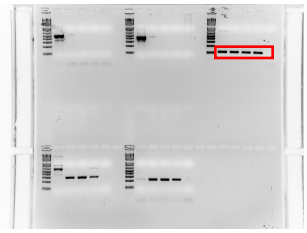
Tnks 2nd round PCR



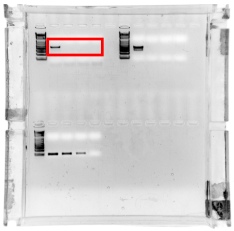
HDR test of *Tnks* loci



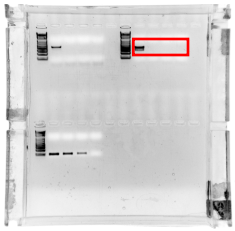
Act5c PCR of *Tnks* KO cells



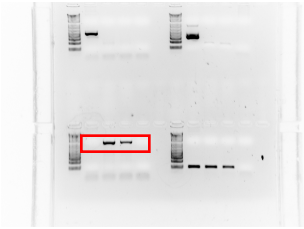
Apc 1st round PCR



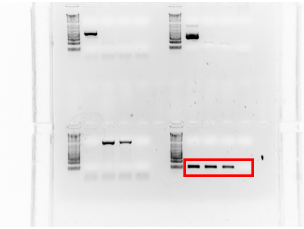
Apc 2nd round PCR



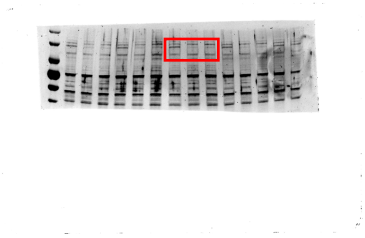
HDR test of *Apc* loci



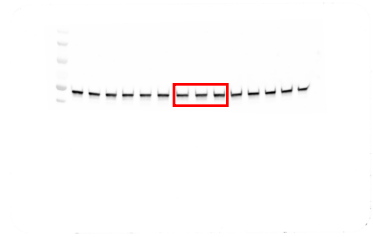
Act5c PCR of *Apc* KO cells



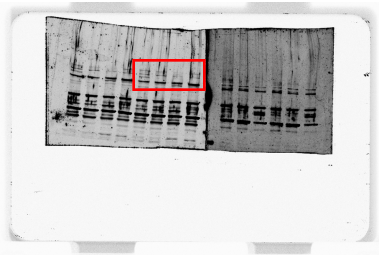
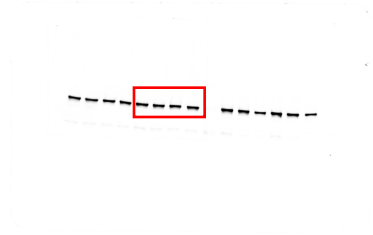
Western blot of Tnks in Bulk population



Western blot of alpha-Tubulin in Bulk population



Western blot of Tnks in KO cells



Western blot of alpha-Tubulin in KO cells