Supplementary methods

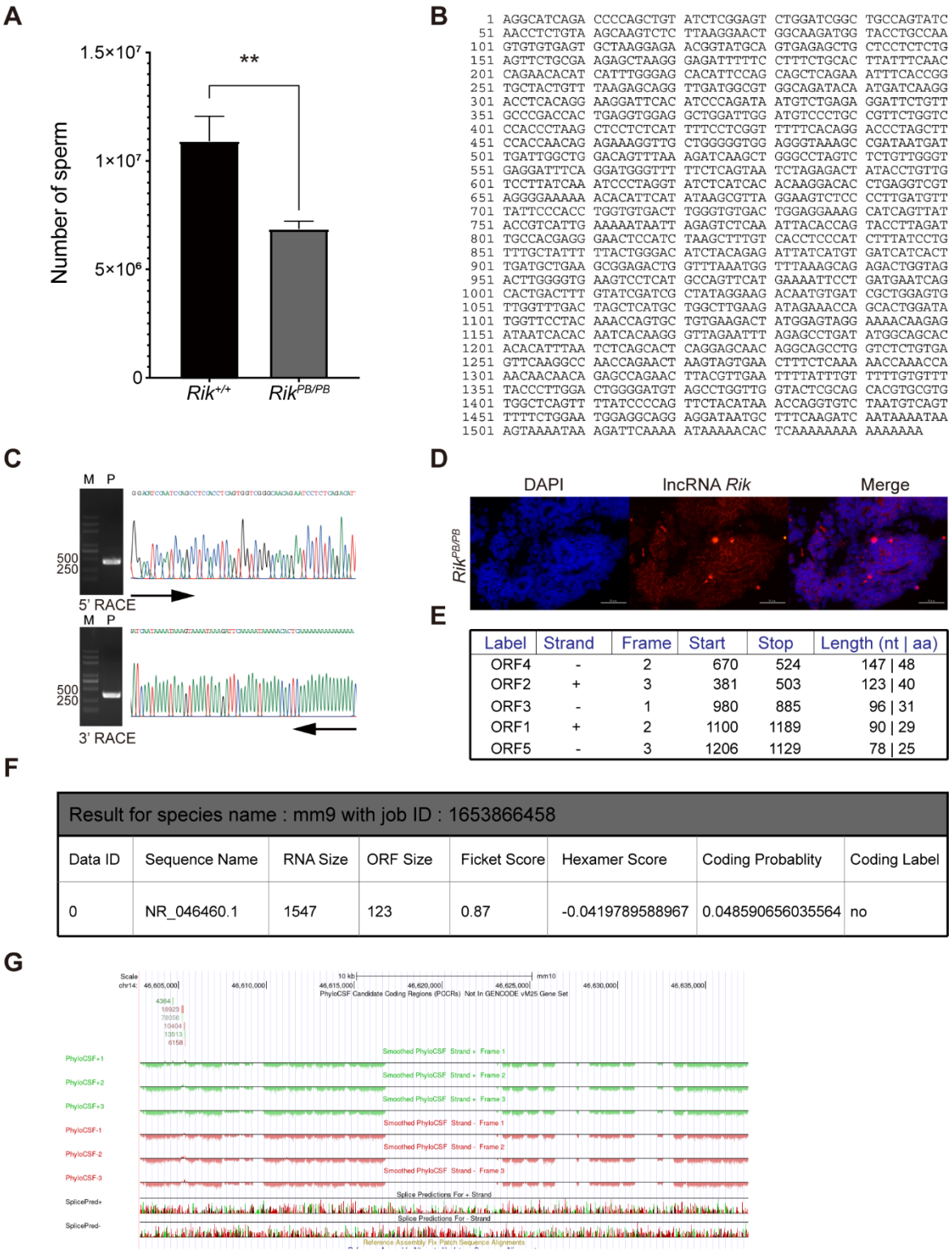
Sperm counting

Sperm counting is the number of sperm in a semen excreted at one time under a microscope. Multiply the sperm count value by the amount of semen to get the amount of ejaculation at one time. The *Rik*$^{+/+}$*;Hoxb7* and *Rik*$^{PB/PB}$*;Hoxb7* male mice at 3 months of age were executed with a broken neck, opened the abdominal cavity layer by layer, removed the epididymis, put it into a dish containing PBS (Sangon Biotech, China), transferred it to the stereoscope (Leica, Germany), peeled off the excess tissue near the epididymis with forceps, and then transferred it to a small dish (Thermo, USA) with 1 ml of PBS, repeatedly poked the epididymis with a 1 ml needle (Kindly Group, China) to help the sperm enter the PBS liquid, and then transferred the sperm to the centrifuge tube (Corning, USA), and then transferred it to the cell microscope (Leica, Germany) for counting.

RNA fluorescent in situ hybridization (RNA-FISH)

Cy3-labeled lncRNA-*Rik* probes were obtained from Sangon Biotech, China. Hybridizations were carried out using a FISH Kit (RiboBio, China) according to the manufacturer's instructions. Briefly, 4% paraformaldehyde (Sangon Biotech, China) was used to fix kidney tissue slices, followed by treatment with 0.5% Triton (Sangon Biotech, China). Then, the slices were cultured with a specific probe overnight. All fluorescence images were captured using a Nikon A1Si Laser Scanning Confocal Microscope (Nikon Instruments Inc., Japan). The sequence for the lncRNA-*Rik* probe was 5′-UGUGCUGCCAUAUCAGGCUCUAAAUUCUAACC-3′.

Supplemental figures and legends



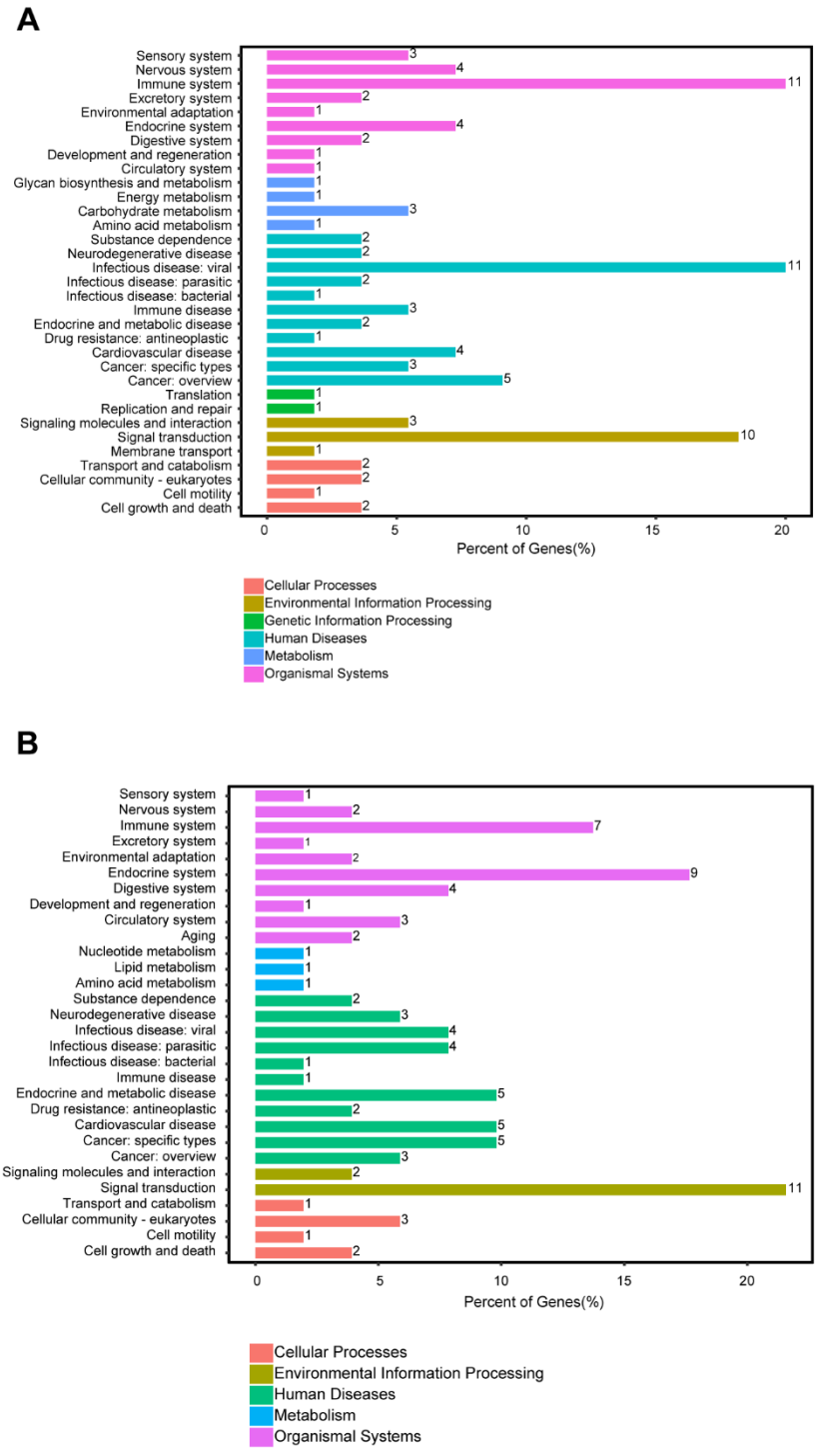
Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF4	-	2	670	524	147 | 48
ORF2	+	3	381	503	123 | 40
ORF3	-	1	980	885	96 | 31
ORF1	+	2	1100	1189	90 | 29
ORF5	-	3	1206	1129	78 | 25

Result for species name : mm9 with job ID : 1653866458

Data ID	Sequence Name	RNA Size	ORF Size	Ficket Score	Hexamer Score	Coding Probablity	Coding Label
0	NR_046460.1	1547	123	0.87	-0.0419789588967	0.048590656035564	no

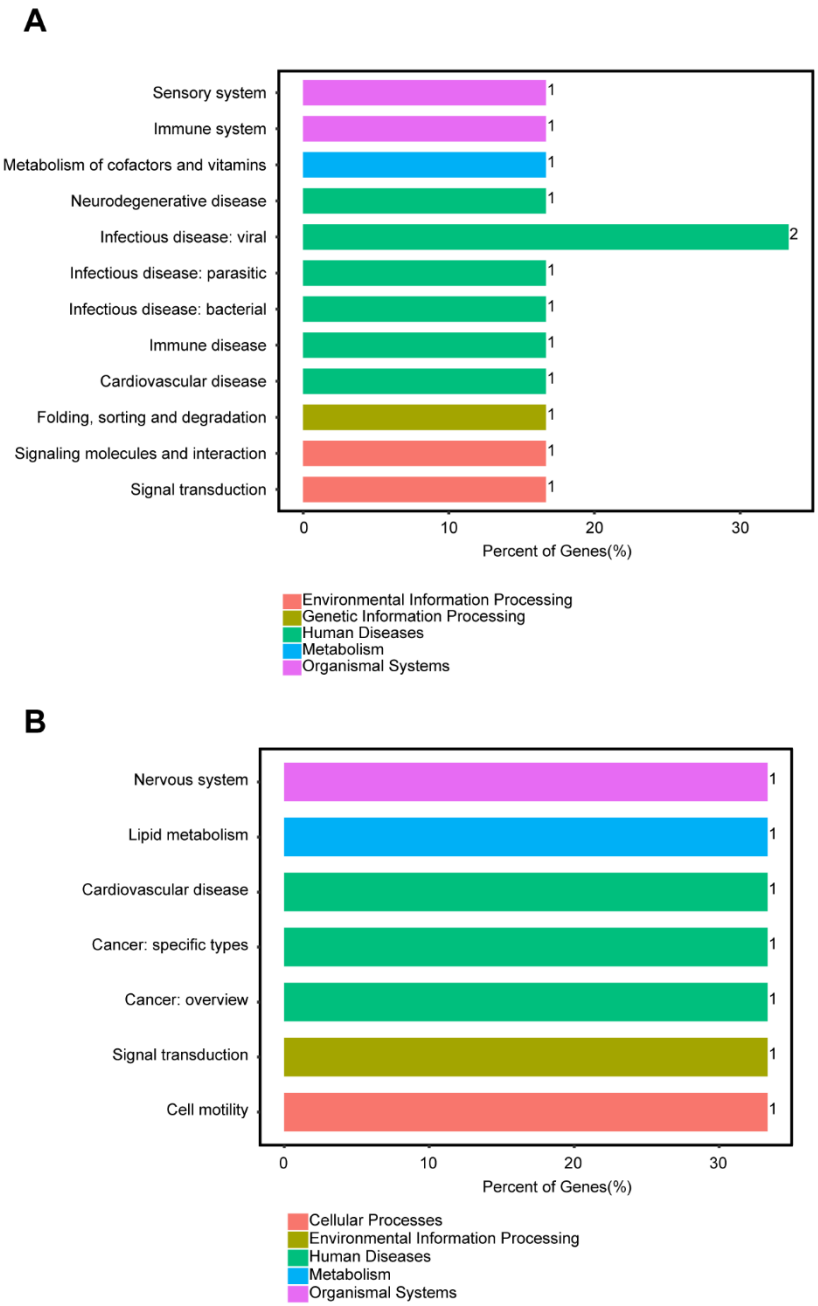
Supplemental Fig. S1 Identification of lncRNA *Rik* and analysis its noncoding nature. (A) The sperm number of *Rik*$^{PB/PB}$;*Hoxb7* male mice (n=5) was significantly lower than that of *Rik*$^{+/+}$;*Hoxb7*mice (n=4) at the same 3 months of age. **, $P < 0.01$. **(B)** The nucleotide sequence

of full-length lncRNA *Rik* determined by rapid amplification of cDNA ends (RACE). **(C)** 5' and 3' RACE assays in testis to detect the whole sequence of lncRNA *Rik*. Left; cropping gel electrophoresis images of PCR products from the 5'-RACE and 3'-RACE assays. M; DL5000 marker. From bottom to top, it is 100，250，500，750，1000，1500，2000，3000，5000 bp. P; PCR products. The lane of 5'-RACE was 403 bp and the lane of 3'-RACE was 298 bp. Right; sequencing of PCR products. The top dosing holes were cropped and the original unprocessed gel electrophoresis images of Supplemental Fig. S1C were list at the end of the supplemental materials. **(D)** Fluorescence in Situ Hybridization (FISH) assay (red) to examine the expression and location of lncRNA *Rik* in $Rik^{PB/PB}$;*Hoxb7* kidney tissue at E12.5. Scale bars, 50μm. **(E)** Putative proteins encoded by lncRNA *Rik* as predicted using ORF Finder. **(F)** Coding potential of lncRNA *Rik* predicted by CPAT. **(G)** The codon substitution frequency scores (CSF) of lncRNA *Rik*.

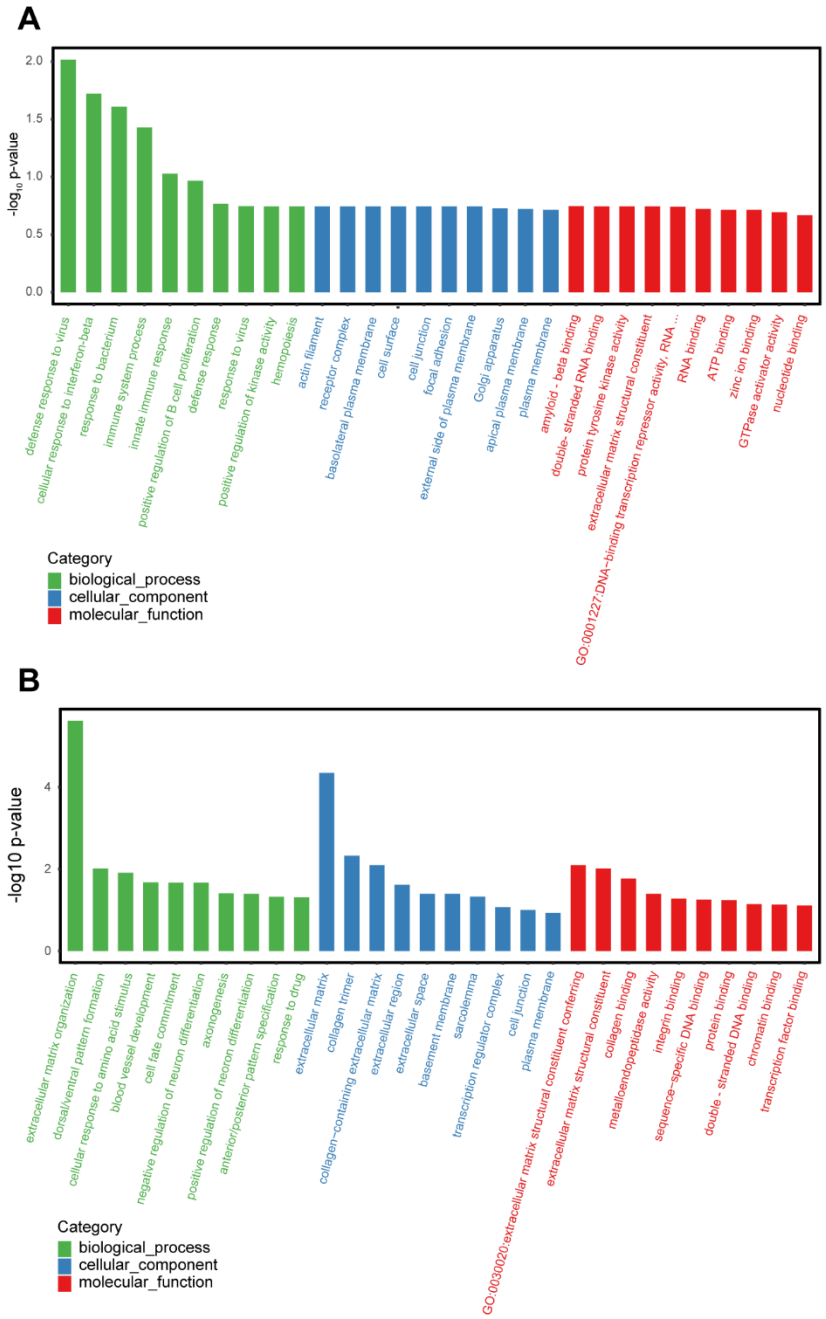


Supplemental Fig. S2 KEGG enrichment analysis of differential genes in *Rik*-overexpressing MPTC cells. (A) KEGG enrichment analysis of upregulated differential genes. **(B)** KEGG enrichment analysis of downregulated differential genes. The horizontal axis is the

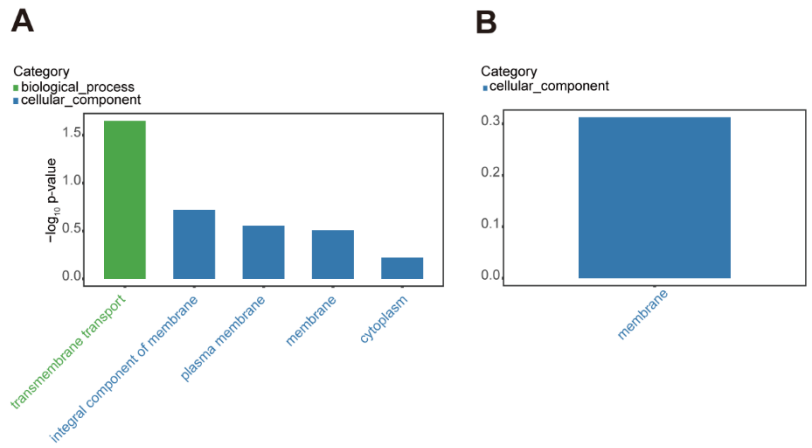
ratio (%) of the total number of genes annotated to each level2 pathway (differentially expressed genes) and all genes annotated to KEGG pathway (differentially expressed genes). The vertical axis represents the name of level2 pathway, and the number on the right side of the column represents the number of differentially expressed genes annotated to this level2 pathway.



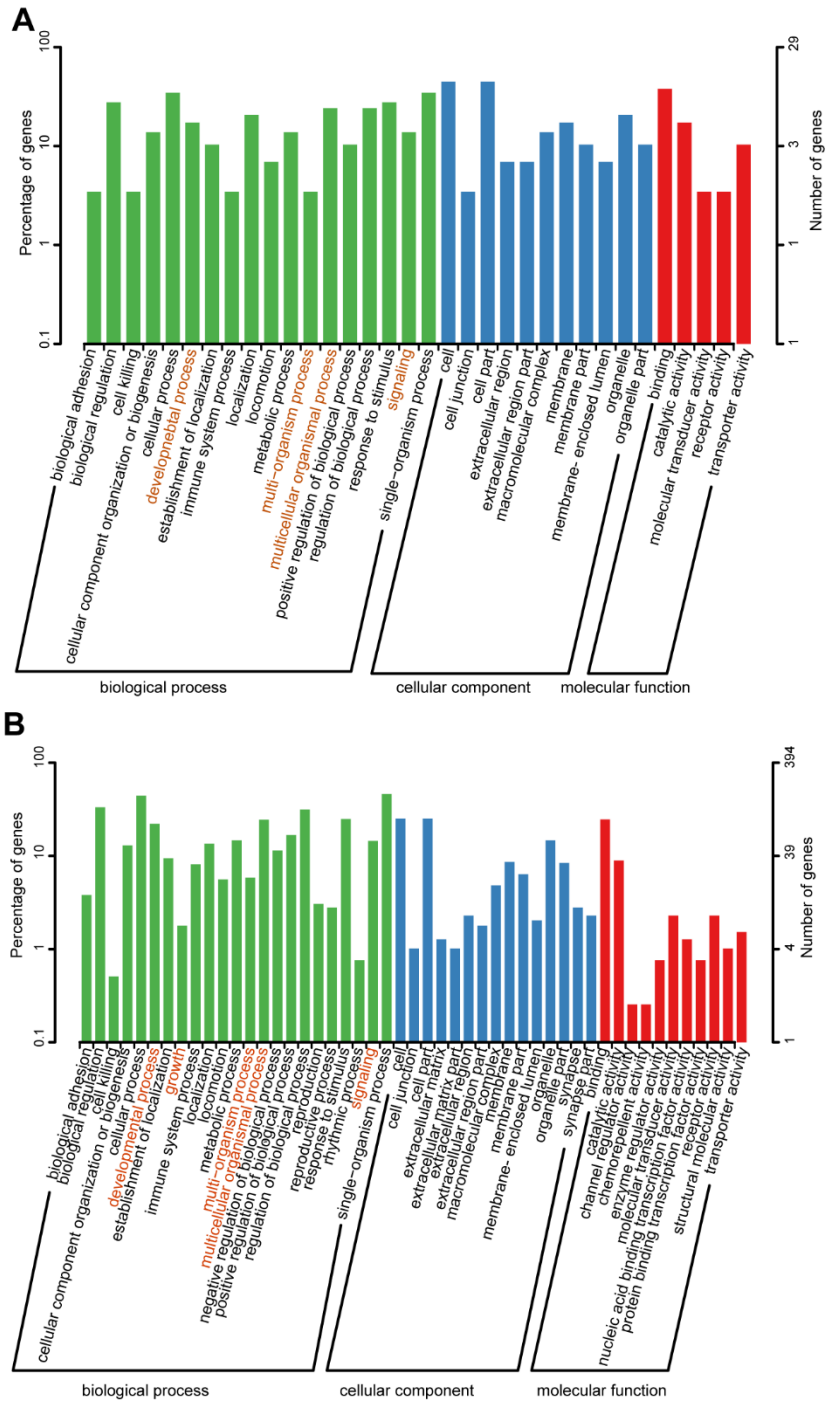
Supplemental Fig. S3 KEGG enrichment analysis of differential genes in *Rik*-overexpressing MK3 cells. (A) KEGG enrichment analysis of upregulated differential genes. **(B)** KEGG enrichment analysis of downregulated differential genes. The horizontal axis is the ratio (%) of the total number of genes annotated to each level2 pathway (differentially expressed genes) and all genes annotated to KEGG pathway (differentially expressed genes). The vertical axis represents the name of level2 pathway, and the number on the right side of the column represents the number of differentially expressed genes annotated to this level2 pathway.



Supplemental Fig. S4 GO enrichment analysis of differential genes in *Rik*-overexpressing MPTC cells. (A) Top 30 GO terms of upregulated differential genes. **(B)** Top 30 GO terms of downregulated differential genes. The GO terms with the number of corresponding differential genes greater than 2 in the three classifications were screened, and 10 terms were sorted from large to small according to the -log$_{10}$ p-value corresponding to each term. The horizontal axis was the name of the GO term, and the vertical axis was -log$_{10}$ p-value.



Supplemental Fig. S5 GO enrichment analysis of differential genes in *Rik*-overexpressing MK3 cells. (A) GO terms of upregulated differential genes. **(B)** GO terms of downregulated differential genes. The GO terms with the number of corresponding differential genes greater than 2 in the three classifications were screened, and 10 terms were sorted from large to small according to the -$\log_{10}$ p-value corresponding to each term. The horizontal axis was the name of the GO term, and the vertical axis was -$\log_{10}$ p-value.



Supplemental Fig. S6 Comparison of the distribution of differentially expressed genes at GO level2 level in *Rik*-overexpressing MPTC and MK3 cell lines. (A) GO classification of total differential genes in *Rik*-overexpressing MK3 cells. **(B)** GO classification of total differential genes in *Rik*-overexpressing MPTC cells. GO level2 included biological process, cellular component and molecular function. The column represented GO level2 terms with rich

differential genes, the horizontal axis was the name of the term, and the vertical axis represented the number and percentage of genes of the corresponding term.

Supplemental tables

Supplemental Table. S1 Genotyping primers.

Gene	**Forward Primer/Reverse Primer**	**WT band size (bp)**	**PB band size (bp)**
Rik	L1A: ACTAATGGTAGTAGTCCTGCTAGGC	535	817
Rik	R1A: ACTCACAGACAATTTACCTTCAGGC		
Rik	LB2: CTGAGATGTCCTAAATGCACAGCG		
Hoxb7	F: AGAGCAAGCCAAAGGTGAG	500	
Hoxb7	R: CTTGATGCCGTTCTTCTGC		

Oligomers listed in 5'- to 3'- orientation.

Supplemental Table. S2 RT-PCR primers.

Gene	**Forward Primer**	**Reverse Primer**
Rik	TTGGGTGTGACTGGAGGAAA	CAAACAGGATAAAGATGGGAGGT
Gapdh	TGTTCCTACCCCCAATGTGTCC	GGAGTTGCTGTTGAAGTCGCAG
Wnt10b	ACTGGTGCTGTTATGTGCTGTGTG	CTTCTCCTCCTCGTCGTGCCTAG
Pax6	AGGGCAATCGGAGGGAGTAAGC	TCTGTCTCGGATTTCCCAAGCAAA

		G
Cxcl12	GGATGGGTTGAAGTAGGATGTGCTC	AGGTCTTGGTGCTCAGGAGGTG
Foxa2	CTGAAGCCCGAGCACCATTACG	GGTGGTGGCTGTGGTGATGTTG
Foxg1	ATCAGGCAGAGTCCCGAGAAGC	CAGGTTGTGGCGGATGGAGTTC
Gata6	CCGTGCGACAGGATTCTTGGTG	GCCATCTGGACTGCTGGACAATATC
Gatm	AACAGCACCCAAGCCCACAATG	CGAATGAAGTCAGCAGCATCAAAGC
Krt8	GAGACCAAGTGGAGCCTGTTGC	CGGCGGAGGTTGTTGATGTAGC
Mmp2	ACCATGCGGAAGCCAAGATGTG	AGGGTCCAGGTCAGGTGTGTAAC
Sema6d	ACGGTGTACGGTGGGAAGTC	GGACATTCATGTGGACCATCTG
Folr1	ACACAAGCCAGGAAGCACATAAGG	ATTCCGATGTCATAGTTCCGCAGTG
Prkcb	ACTCGAACGCAAGGAGATTC	CAGGAGGTGTTAGGACTGGTG
Mmp12	GAGTCCAGCCACCAACATTAC	GCGAAGTGGGTCAAAGAC
Dhx58	TCTGGAGGTGCTGATGGAGAAGG	GTGCTGCCCGCTTAACTAGAGATG
H2-T24	GAACTCAGGACCTTCGGAAGAACG	GCCAGTGCTTAGTTGTGCCTCTC
Plcb2	GTTCAACGGGCAGAGTGGCTAC	CGTAATGGAAAGGGTGGTGGCTAC
Oas2	GAGTTCTCAGACTGCTTCACCACAC	CACTTCTCCTGGCACTGTTCATACC
Slfn8	CGTGCCCAGACTTGACCATC	AACAGAGCACAAGCAGCCTC
Ifit3	GCAGTTCTCCGTGGATGCTC	CTCAGCAGTTTCAGGGCCAG
Ifit3b	AGAAGCCCAAGGACCCAGAG	CCTGCTTCAGAGCATCCACG

Fbln1	TGACGAGGAGGACCAAGAAGACC	AGCCCACAAAGCAAGAGCAGATC
Ifi44	GAGTTCTGCTGCTGGGTCCTATTG	CTGGTGTGTGATGCTGCCCTTG
Rsad2	CCTCTGTGAGCATAGTGAGCAATGG	TGTCGCAGGAGATAGCAAGAATGTC
Slfn2	GCCCTATCGTTCATCCTACCCTTTG	CCCATTCCCGCCAAATCATCCTG
Cd74	TCCCAGAACCTGCAACTGGA	ATTGGACGCATCAGCAAGGG
Rhox5	TCCTGCCTTCCGTGGACAAGAG	ATGCTGTTCTTCCGAGTCTTCCTTG
Trim30d	CTTCAGAGGAGCAGCACAGTTGG	AGAGACCCAGGATTTGTGGAGAGG
Pak6	CTGTACGCTACTGAGGTGGA	GTACCAGCATCCGATCCAGG
Fzd1	CACCTGGATAGGCATCTGGT	CAGAAAGCCAGCGATGTAGG
Fzd2	CCGACGGCTCTATGTTCTTC	TAGCAGCCGGACAGAAAGAT
Fzd3	AGCGTGCCTATAGCGAGTGT	TCTCTGGGACACCAAAAACC
Fzd4	CTGCAGCATGCCTAATGAGA	CGTCTGCCTAGATGCAATCA
Fzd5	TCTTGTCTGCGTGCTACCTG	GGCCATGCCAAAGAAATAGA
Fzd6	TGTTGGGCTGTCTCTCCTCT	TCTCCCAGGTGATCCTGTTC
Fzd7	CCATCCTCTTCATGGTGCTT	TGGCCAAAATGGTGATTGTC
Fzd8	CTGTTCCGAATCCGTTCAGT	CGGTTGTGCTGCTCATAGAA
Fzd9	AGAGCAACCATGTACTGCTG	GCTCTGCCAAGTCTGAAAAG
Lrp5	CAGGTGCTTGTGTGGAGAGA	CATGTTGGTGTCCAGGTCAG
Lrp6	GGTGTCAAAGAAGCCTCTGC	ACCTCAATGCGATTTGTTCC
Dvl2	AGGAGACTCGGATGAGGATGACAC	CGGTGACACTGCTGAAGGATGAG
Dvl3	ATGGAACGCACAGGAGGCATTG	GGTCTCCGTGTCATTGTCCAGATTC

Axin2	GGGTTCTGAAATTCATAGACTAAGA	CGACTGTTCAATAAATATCAGTAA
Gsk3b	GTTCGGCTACCTTCGGCATTCC	CGAGCGACCTGGATAACCAATGAG
β-catenin	GCTGCTGTCCTATTCCGAATGTCTG	GGCACCAATGTCCAGTCCAAGATC
c-myc	GCTCGCCCAAATCCTGTA	AGGACTCGGAGGACAGCA
Cyclin D1	CGTATCTTACTTCAAGTGCGTG	ATGGTCTCCTTCATCTTAGAGG
Runx2	TTTAGGGCGCATTCCTCATC	TGTCCTTGTGGATTAAAAGGACTTG
Lef1	AAATGGGTCCCTTTCTCCAC	TCGTCGCTGTAGGTGATGAG

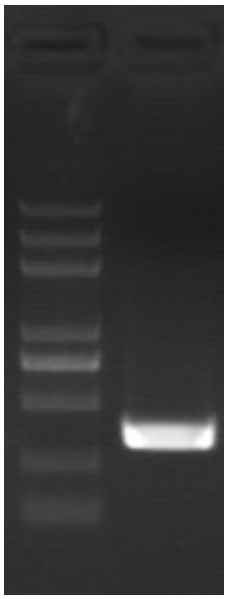
Primers utilized to amplify gene. Oligomers listed in 5'- to 3'- orientation.

Supplemental Table. S3 Knockout cell genotyping primers.

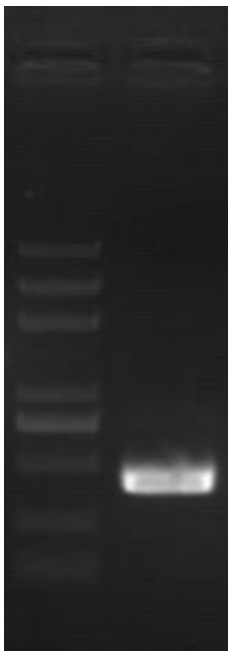
Gene	**Forward Primer/Reverse Primer**	**WT band size (bp)**	**KO band size (bp)**
Rik-F1	GAACTTGTTTGGCATAAACCGAG	3292	2800
Rik-R1	GTTAATGTGAGTAACGGAGTCGG		
Rik-F3	CAGTACCTTAGATTGCCACGAGG	1268	0

Oligomers listed in 5'- to 3'- orientation.

The original unprocessed gel electrophoresis images of Supplemental Fig. S1C:



The original unprocessed gel electrophoresis image of *4933425B07Rik* 3-RACE PCR.



The original unprocessed gel electrophoresis image of *4933425B07Rik* 5-RACE PCR.