Supplementary File 4: Genotyping protocol for *Tcte1* knockout model.

PCR primers:

First reaction:

Identification of wildtype and positives:

Mouse Tcte1-F: CGTTTTAAGAATGTATTGAGGGTTGGG

Mouse Tcte1-R: CTCCGTAGGCTCCTGCCAATATG

Product Size: WT: 1915 bp; MT: ~630 bp, delete~1280 bp

Annealing Temp: 59℃

Second reaction:

Identification of heterozygotes and homozygotes:

(to confirm whether the positive founders were homozygotes or not, one primer located inside the deletion sequence and other located outside)

Mouse Tcte1-Wt/He-F: AGCTTGCCACACCCTCAAGGTACTA

Mouse Tcte1-R: CTCCGTAGGCTCCTGCCAATATG

Product Size: 658 bp or 0 bp

Annealing Temp: 59℃

PCR mixture (per 1 sample, each volume in μl):

ddH_2O	21.85
dNTP Mix	1.50
10×buffer	3.00
HS Taq	0.15
primer-F	1.00
primer-R	1.00
DNA Sample	1.50
Total	*30.00*

PCR reaction conditions:

Initial denaturation	94℃	5 min	
Denaturation	94℃	30 s	35cycles
Annealing	59℃	30 s	
Extension	72℃	2 min	
Additional extension	72℃	5 min	
Keeping temperature	12℃		

PCR result analysis – first reaction:

One band: 630 bp; heterozygotes and homozygotes

One band: 1915 bp or 0 bp; wildtype

PCR result analysis – second reaction:

One band: 658 bp; heterozygotes

No band: 0 bp; homozygotes