Supplementary Information

IP_6-assisted CSN-COP1 competition regulates a CRL4-ETV5 proteolytic checkpoint to safeguard glucose-induced insulin secretion against hyperinsulinemia

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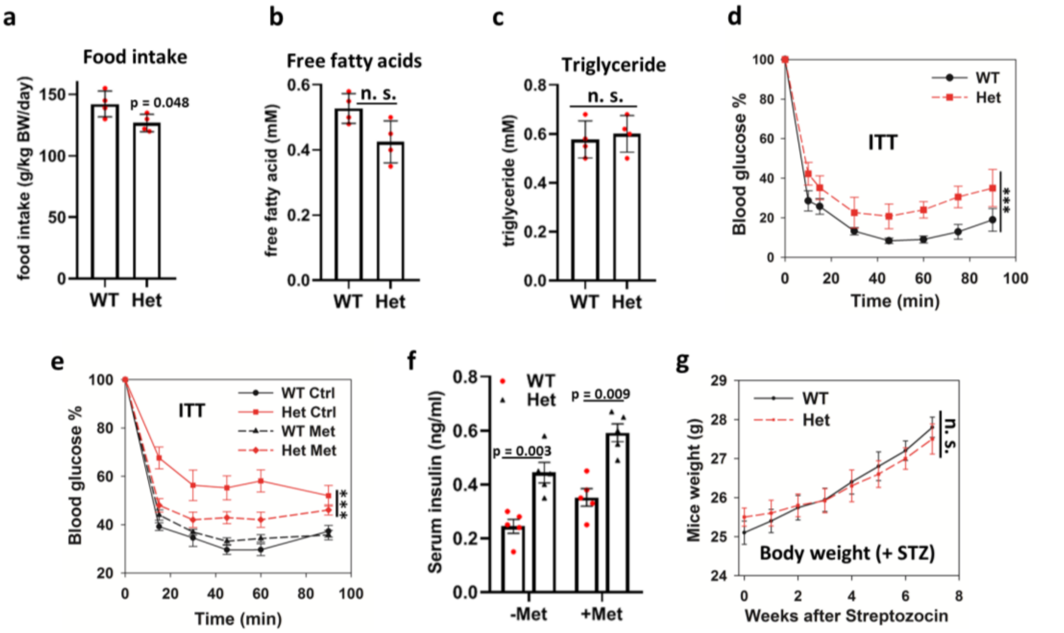
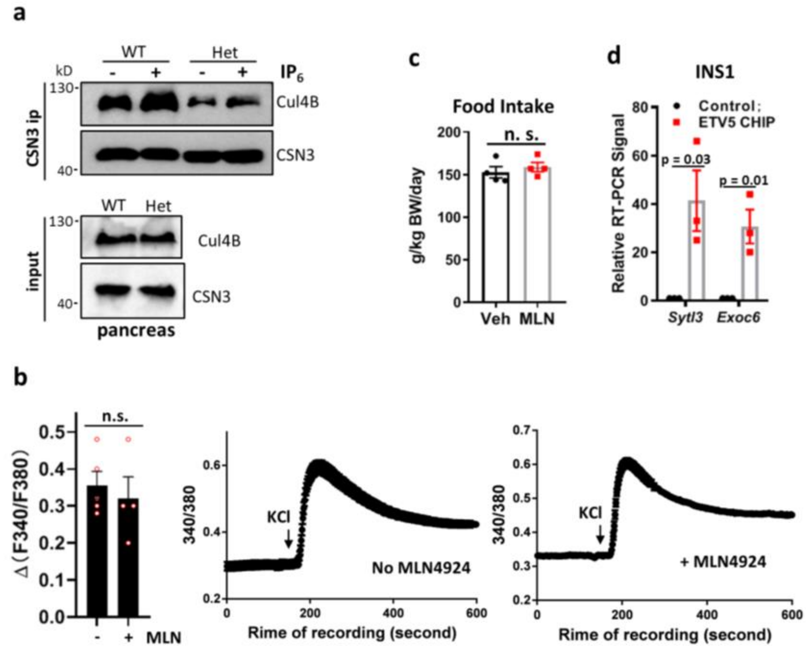
Supplementary Figure 1



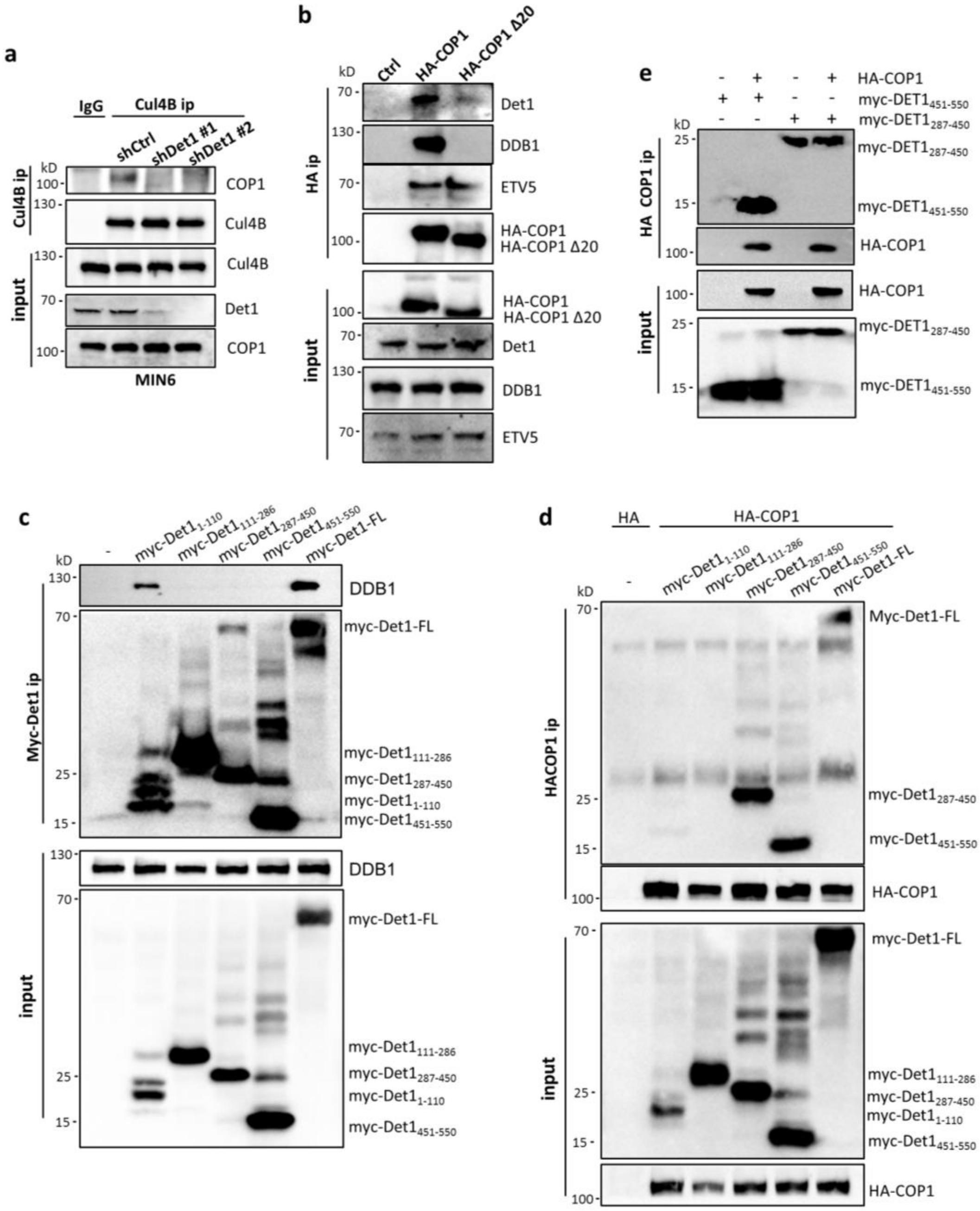
Supplementary Fig. 1. Enhanced insulin secretion and congenital hyperinsulinemia in *Csn2*$^{WT/K70E}$ (Het) mice. (a) Daily food intake of wildtype and Het mice (n = 4). Data are presented as Mean ± SD. P values were calculated by two-tailed Student's t-test. **(b-c)** Serum levels of free fatty acids **(b)** and triglycerides **(c)** after 6-h fasting (n = 4, n.s.: not significant, student's T test). Data are presented as Mean ± SD. **(d)** Intraperitoneal insulin tolerance test (ITT) of wildtype and Het mice at 4 month age (n = 4, ***$p < 0.001$, calculated by two way repeated-measures ANOVA testing Genotype-x-Time effect). Data are presented as Mean ± SD. 100% blood glucose means 9.94 ± 1.3 mM for WT, and 8.3 ± 1.1 mM for Het. **(e)** Effect of metformin (Met) treatment on insulin tolerance test of wildtype and *Csn2*$^{WT/K70E}$ (Het) mice (n = 5, ***$p < 0.001$, calculated by two way repeated-measures ANOVA testing Treatment-x-Time effect). Data are presented as Mean ± SD. 4-month old mice were treated with/without 250 mg/kg Metformin in drinking water for three weeks. 100% blood glucose means 8.8 ± 0.7 mM for WT without Metformin treatment, 8.4 ± 0.8 mM for WT with Metformin treatment, 9.2 ± 0.9 mM for Het without Metformin treatment, and 7.6 ± 0.9 mM for Het with Metformin treatment. **(f)** Effect of metformin (Met) treatment on serum insulin levels of wildtype and *Csn2*$^{WT/K70E}$ (Het) mice (n = 5). Data are presented as Mean ± SEM. P values were calculated by two-tailed Student's t-test. **(g)** Effect of streptozocin (STZ) treatment (i.p., 50 mg/kg for four days) on the body weight of wildtype and *Csn2*$^{WT/K70E}$ (Het) mice (n = 4, n.s.: not significant, calculated by two way ANOVA testing genotype main effect). Data are presented as Mean ± SEM.

Supplementary Figure 2



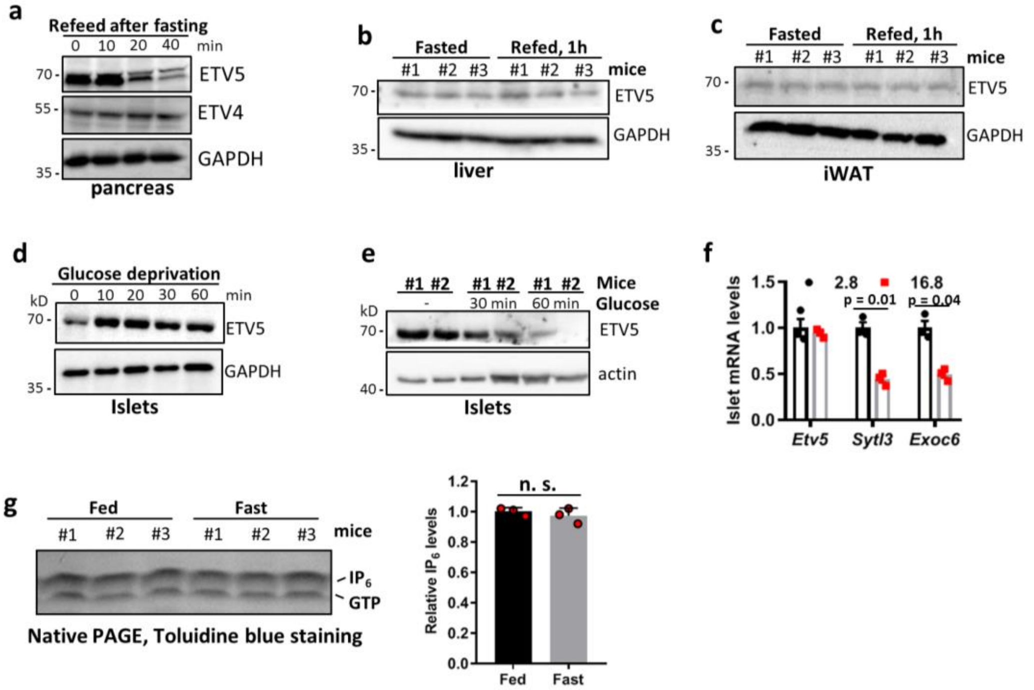
Supplementary Fig. 2. Activation of the $CRL4^{COP1}$-ETV5 degradation axis underlies glucose-induced insulin secretion and is constitutively augmented in *Csn2*$^{WT/K70E}$ (Het) mice. **(a)** Effect of IP_6 (20 μM) addition to pancreatic lysate on CSN3-Cul4B co-immunoprecipitation from wildtype and *Csn2*$^{WT/K70E}$ pancreas. **(b)** Effect of MLN4924 on average islet cytoplasmic Ca^{2+} responses to depolarization induced by 25mM KCl, assayed with Fura2AM [n = 4 (WT), or 5 (Het), n.s.: not significant, student's T test]. Data are presented as Mean ± SEM. **(c)** Daily food intake of mice is not affected by MLN4924 treatment (n = 4, n.s.: not significant, student's T test). Data are presented as Mean ± SEM. P values were calculated by two-tailed Student's t-test. **(d)** ETV5 ChIP on Sytl3 and Exoc6 promoters (n = 3). Data are presented as Mean ± SEM. P values were calculated by two-tailed Student's t-test.

Supplementary Figure 3



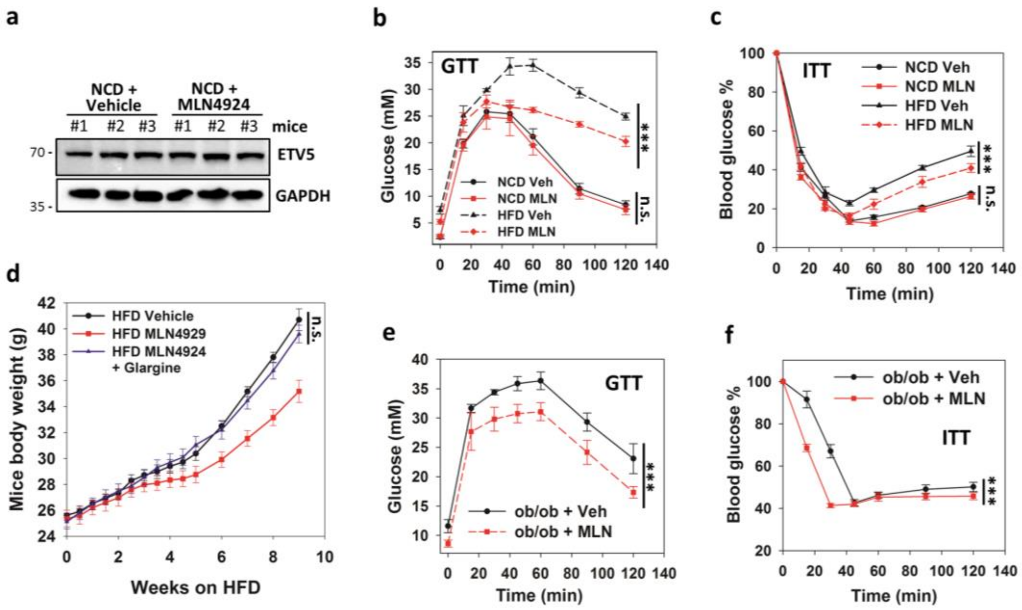
Supplementary Fig. 3. Interaction domain characterization within the $CRL4^{COP1}$ complex. (a) DET1 knockdown abolishes Cul4B immunoprecipitation of COP1. **(b)** Deleting COP1 aa 277-296 (Δ20) abolishes COP1 interaction with DET1 and DDB1, but not ETV5. **(c)** DET1 Fragment mapping experiments showing that DET1 aa 1-110 binds to DDB1. **(d-e)** Reciprocal co-immunoprecipiation experiments showing that DET1 aa 451-500 specifically interacts with COP1.

Supplementary Figure 4



Supplementary Fig. 4. ETV5 as a CRL4[COP1] regulated physiologic checkpoint for nutrient/glucose-induced insulin secretion. (a) Levels of ETV5 in the pancreas of overnight-fasted mice, with/without refeeding for the indicated time periods. **(b-c)** Refeeding does not significantly change Levels of ETV5 in the liver **(b)** and adipose tissue **(c)** of fasted mice**. (d)** Levels of ETV5 in isolated islets cultured in RPMI mediated containing 11 mM glucose, followed by glucose withdrawal at the indicated time points. **(e)** Levels of ETV5 in isolated islets after exposure to 16.8 mM glucose at the indicated time points. **(f)** Transcript levels of Etv5 and its targets in islets incubated with 2.8 mM or 11.8 mM glucose (n = 3). Data are presented as Mean ± SEM. P values were calculated by two-tailed Student's t-test. **(g)** Total levels of cellular IP_6 in the pancreas of mice with/without fasting (6h) (n = 3, n.s.: not significant, student's T test). Data are presented as Mean ± SD.

Supplementary Figure 5



Supplementary Fig. 5. The CRL inhibitor Pevonedistat/MLN4924 stabilizes ETV5 and improves diet-induced or leptin deficiency-induced obesity/diabetes. **(a)** Effect of MLN4924 injection (i.p. 15 mg/kg) on ETV5 levels from mice fed with NCD. **(b-c)** Glucose **(b)** and insulin **(c)** tolerance test in HFD or NCD mice with/without MLN4924 treatment (n = 5, $^{***}p < 0.001$, calculated by two way repeated-measures ANOVA testing Treatment-x-Time effect). 100% blood glucose means 16.3 ± 2.2 mM for WT and 12.2 ± 1.4 mM for Het. 100% blood glucose means 7.2 ± 0.6 mM for NCD without MLN4924, 7.4 ± 0.5 mM for HFD with MLN4924, 9.8 ± 1.4 mM for HFD without MLN4924, and 7.6 ± 0.5 mM for HFD with MLN4924. **(d)** Concurrent treatment with Glargine (Sanofi, 50 IU/Kg, twice weekly), a slow-releasing insulin, reverses the anti-obese effect of MLN4924 (n = 5, n.s.: not significant, two way repeated-measures ANOVA testing Treatment-x-Time effect). Data are presented as Mean ± SD. Note that **(e-f)** Glucose **(e)** and insulin **(f)** tolerance test in ob/ob mice with/without MLN4924 treatment (n = 5, $^{***}p < 0.001$, calculated by two way repeated-measures ANOVA testing Treatment-x-Time effect). Data are presented as Mean ± SEM. 100% blood glucose means 16.3 ± 2.2 mM for ob/ob mice without MLN4924 treatment and 12.2 ± 1.4 mM for ob/ob mice with MLN4924 treatment.

Supplementary Table 1. Primer information

Primers	Primer sequences
A. Primers to generate different constructs	
hCOP1-shRNA1	GCTAACAGTCAGGGTACAATT
hCOP1-shRNA2	GCTGGAGTTACAAAGAAGATT
hETV5-shRNA1	GAGCGATACGTCTACAAATTT
hETV5-shRNA2	TCCATGGCTTTCCCGGATAAC
mCOP1-shRNA1	GCTGTATCAGTTGGAGTAGTT
mCOP1-shRNA2	CCTTGGTATAACAGCACATTA
mDet1-shRNA1	ATCAAGGGCTCTACTTGTATA
mDet1-shRNA2	TCCTTGTTTCTTACGGAAATT
mEtv5-shRNA1	GCCAAAGATGATGCCTGAA
mEtv5-shRNA2	GCCATGAAGGATTCCCGTA
hSytl3-ECOR1-F	CTCGAGCTCAAGCTTCGAATTCATGGCCCAAGAAATAGATC
hSytl3-BamH1-R	CGTCGTCGTCCTTGTAGTCGGATCCTCAGTGCAGGACAAGAGTC
hExoc6-ECOR1-F	CTCGAGCTCAAGCTTCGAATTCATGGCGGAGAACAGCGAGA
hExoc6-BamH1-R	CGTCGTCGTCCTTGTAGTCGGATCCCTACATGTGCTGGGACATA
hDET1-F	AAAAGTCGACcATGGATCATCATGTTTCTACCA
hDET1-R	AAAAGCGGCCGCCTACGTGCAGCAGTGTCGCATAT
hDet1-1-110-F	GCGGAATTCGGTCGACCATGGAT
hDet1-1-110-R	GCGGCGGCCGCCTAGTCATTGCCATTGGACAGGA
hDet1-111-286-F	GCGGAATTCGGTCGACCCAGCGGTCAGTGAATATCC
hDet1-111-286-R	GCGGCGGCCGCCTAGGGATTGGCCATGCCT
hDet1-287-450-F	GCGGAATTCGGTCGACCTTTAGGGATCCTTTCATCAATTCC
hDet1-287-450-R	GCGGCGGCCGCCTAGATGGGGAGCTGACCC
hDet1-451-550-F	GCGGAATTCGGTCGACCAGTGCTCAGTCTTACAGCG
Det1-451-550-R	GCGGCGGCCGCCTACGTG
mDcaf8-shRNA	GCCATACTGGTTGTGTCAATA
mWdr59-shRNA	CTCCCTAGTGATTGCCTTCTT
mWdr51-shRNA	CCAGGTCTAATTGCAGTAATA
mWdr61-shRNA	GATGCCTGGACTTTGGCATTT
mWdr57-shRNA	GCAGGATTTGACTAATA
mDcaf5-shRNA	GAGGAAATGATGAGCAAGTTA
mDcaf9-shRNA	CTTACAAGCAACGGCCATATA
mDcaf14-shRNA	GGAGTCAAAGTTCGATCTTAT
mDcaf16-shRNA	CTCTAAATGGAGCACTGCAAT

mDcaf10-shRNA	TGATACCACAATAGCACTATG
mDcaf2-shRNA	TGATGAAGCTGCCTACATTTG
mDcaf11-shRNA	GAGCGGCCACATTGTTAAGAA
mDcaf4-shRNA	CTTCTCCAGTTATTGCCGTTT
mDcaf7-shRNA	CAACAACAAGAACTCAGACTT
mDcaf19-shRNA	GCAGCATATTTATATGGGATA
mDcaf1-shRNA	TGCAATTGGAAGACCTATTAT
mWdr12-shRNA	CCTACAGATGAAGAAGATGAA
mDcaf13-shRNA	CCCTGTTGAGACATTTCTCTT
mWdr39-shRNA	GCTGGAAATGTATCTGCACTT
mFbxw5-shRNA	GCGGCTCTTTAAGATCCAGAA
mRbbp4-shRNA	ATTTGGGACACTCGTTCAAAC
mWsb2-shRNA	CAATGGTCTTTGCTGCACGTT
mTtrpc4ap-shRNA	CTGATGAAGTTCAACGTCGAT
mEed-shRNA	TCTTGCTAGTAAGGGCACATA
mDdb2-shRNA	ATACCCAGATCCTAATCTTAA
mCsa-shRNA	GAGTTAAACAAAGACAGGGAT
mSMU1-shRNA	CCCATGATTATGTTAAAGCAA
mWsb1-shRNA	ACATGAGCTGCTGCTATATAT
mRbbp7-shRNA	CCTTTGATTCAACTGTCATTT
mDda1-shRNA	CTGCACCAGCAATGGGATAAA
mCrbn-shRNA	CCTACCAAGTTCAAGAGCATA
mDcaf17-shRNA	GAATCTGACTGGATCTATTTC
mWdr75-shRNA	CGAAGTCACAGAGCTTATTAA
B: RT-PCR Primers	
rSytl3-F	ATCCTGCACTGAGGTGAAGC
rSytl3-R	AGGCTCCCCGACAGTAATCT
rExoc6-F	AGATCGAAAGCACGGACACC
rExoc6-R	TCTCCTTGTCATGGTTGCGG
hETV5-F	TAGGCTAAGTTGGGGAGGCT
hETV5-R	GGGTGGTTCCCAAGAGTAGC
rIns1-F	CCAAGTCCCGTCGTGAAGT
rIns1-R	GGTGCAGCACTGATCCACAA
rGapdh-F	ATGACTCTACCCACGGCAAG
rGapdh-R	GGAAGATGGTGATGGGTTTC
mIns1-F	CACTTCCTACCCCTGCTGG
mIns1-R	ACCACAAAGATGCTGTTTGACA
mIns2-F	GCTTCTTCTACACACCCATGTC
mIns2-R	AGCACTGATCTACAATGCCAC
mSytl3-F	AGCTCCTGCAATCTTATCAGAGG
mSytl3-R	GCCAAGTTCCTGTAACCTGC

mExoc6-F	GAAAAGCTAGACGCTTGTATCCG
m Exoc6-R	TGAGGAGTTCCGTAATCGCAT
mGapdh-F	TCAAGAAGGTGGTGAAGCAG
mGapdh-R	AGGTGGAAGAGTGGGAGTTG