Figure S1 – P120 catenin (and ARVCF) associates with REST/CoREST.

A. Bacterial MBP, MBP-ARVCF, GST-CoREST, and REST were purified conventionally. GST was cleaved from GST-REST using thrombin. The indicated proteins were co-incubated, and MBP or MBP-ARVCF precipitated using amylose beads, followed by immunoblotting.

B. P120-catenin associates with REST in HEK293 cells. P120-flag and REST-HA were transiently co-expressed in HEK293 cells. Forty-eight hrs post transfection, cells were lysed, and REST-HA precipitated , followed by immunoblotting for p120-flag.

C. P120-catenin associates with CoREST in pluripotent mESCs. Endogenous CoREST was precipitated, with REST and p120 co-precipitating from nuclear-fractionated pluripotent mESCs. mESCs were differentiated via retinoic acid addition for 2 days after embryoid body formation (Note: Plu.=pluripotent ESCs; Dif.= differentiating mESCs).

D. Endogenous p120 associates with endogenous REST and CoREST in HEK293 cells. Using nuclear extracts, CoREST or REST proteins were precipitated and p120 was detected by immuno-blotting.

E. Kaiso does not associate with REST. The indicated proteins were overexpressed in AB1 cells using liposome-mediated transfection. 48hrs post transfection, whole cell lysates were used and REST was precipitated by anti-HA antibody, followed by immuno-blotting with anti-CoREST or anti-Kaiso antibodies.

F. P120 colocalizes with CoREST and REST in AB1 cells. 24hrs post transfection of the indicated cDNA expressing vectors, cells were fixed with 4% formaldehyde, and incubated with 0.3% TritonX100 to improve antibody permeability. Anti-HA and anti-myc antibodies were used for immuno-staining, and DAPI was used for counter-staining. Scale bar represents 30μm.

Figure S2 – P120 can form a trimetric complex with REST/ CoREST, and p120 promotes REST protein destabilization, likely through a proteasome-dependent mechanism.

A. CoREST:p120-catenin binding domain mapping. The indicated protein fragments of CoREST were expressed using an in vitro transcription and translation system, as indicated in Figure 2B, where the results for constructs #1 and #5 are displayed. The

CoREST-myc and flag-p120-HA proteins were incubated together, and CoREST-myc or flag-p120-HA was precipitated.

B. The indicated proteins were expressed using an in vitro transcription and translation system. RESTΔC represents a carboxyl-terminal deleted mutant of REST (1-983 amino acids) in which the putative CoREST binding region is removed. CoREST-myc was precipitated by anti-myc antibody.

C. The indicated protein expressing constructs were transfected using lipofectamine2000 into AB1 cells and 48hrs post transfection, MG132 was added at 5μM final concentration for 2hrs. REST and p120 proteins were detected by immunoblotting using anti-HA and anti-myc antibodies, respectively.

Figure S3 - P120 modulates REST/CoREST gene targets in different contexts, as shown in mouse ESCs, NIH3T3 cells and in Xenopus laevis embryos.

A. REST/CoREST gene targets in AB1 mESCs are repressed upon depletion of p120 by either of two independent shRNAs. P120 knockdown was confirmed by immuno-blotting, and the level of REST target transcripts was assessed by real-time PCR. GAPDH was used as an internal control. Error bars represent SD.

B. Non-targetable p120 expression rescues p120 depletion effect on REST gene targets. Non-targetable p120 cDNA (lacking UTR region) plasmid was transfected to shRNA-mediated control and p120 depleted AB1 cells. 48hrs after incubation, cells were harvested and the indicated REST gene targets were tested by real-time PCR. Transcripts were normalized to GAPDH. Error bars represent SD.

C. P120 knockdown decreases known REST/CoREST gene targets in NIH3T3 cells. P120-depleted NIH3T3 cells were generated by shRNA-mediated RNAi. Whole cell lysates were used for immunoblotting. The REST/CoREST gene target transcripts (mash1 and syt4) were tested by semi-qRT-PCR. GAPDH was used as an internal control.

D. CoREST knockdown rescues p120 depletion effects. CoREST was knocked down by siRNA in p120-depleted NIH3T3 cells, and p120 was expressed after liposome-mediated transfection in p120-depleted NIH3T3 cells. Seventy-two hrs later, the indicated transcript levels were measured by semi-qRT-PCR. Mash1 and syt4 transcripts were

normalized by GAPDH and quantified relative to parental values. Whole cell lysates were used for immunoblotting to confirm CoREST knockdown.

E. CoREST expression rescues p120 overexpression effects. P120 was co-expressed with CoREST or REST in NIH3T3 cells for 48 hrs, and cells were lysed for immunoblotting or RT-PCR. Mash1 and syt4 transcripts were normalized to GAPDH and quantified relative to parental values.

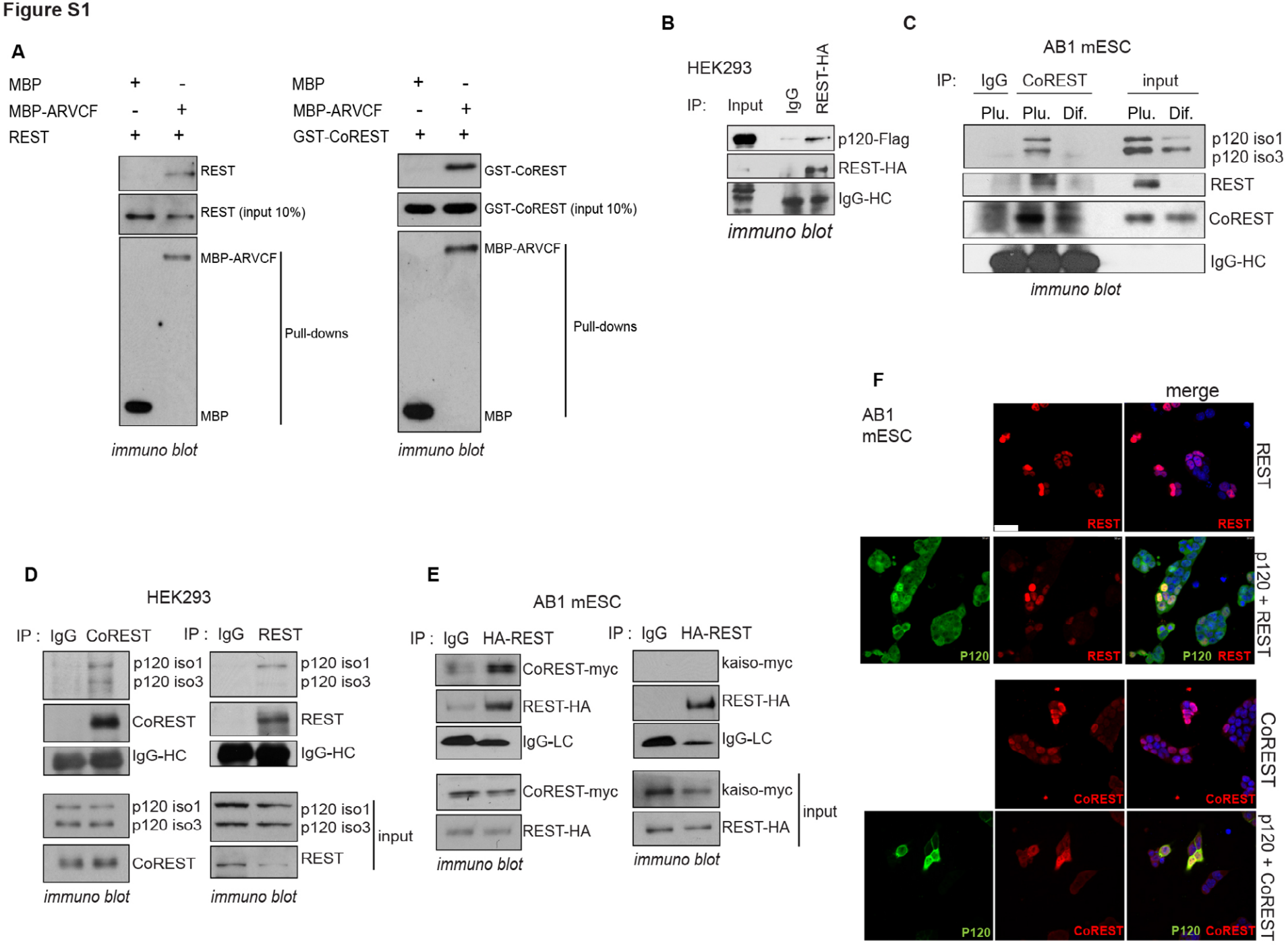
F. P120 mutant (N478A), unable to bind E-cadherin, remains capable of partially rescuing, REST/CoREST gene targets in p120 depleted AB1 cells. P120 (wild-type and N478A) cDNAs were transfected into shP120 AB1 cells, and 48hrs post-transfection, cells were harvested to measure the transcript levels of REST/CoREST gene targets via real-time PCR. MiR124 and miR132 transcripts were normalized to GAPDH and error bars represent SD.

G. P120 knockdown leads to increased repression of REST/CoREST gene targets in *Xenopus laevis* embryos. We micro-injected 10 ng of morpholino antisense-oligonucleotide directed against xp120-catenin (p120MO) or standard-control morpholino (ConMO) into both embryonic cells at the 2-cell stage and harvested the cells at the gastrula stage (stage 11). *Xenopus* calbindin and *Xenopus* mash1 transcripts were measured by RT-PCR, and *Xenopus* histone H4 was used as an internal control.

Figure S4 - P120 modulates the neuronal differentiation (retinoic acid promoted) of mESCs.

A and B. We used a standard retinoic acid (RA) mediated neuronal differentiation method. In brief, p120-depleted and control-depleted mESCs were plated on bacterial culture dishes in differentiation media (DMEM with 10% FBS, not including LIF and beta-mercaptoethanol). Four days later, embryoid bodies that had formed were incubated with RA for an additional 2 days before being harvested. The neural stem/progenitor markers (sox1 and nestin) (A) and the transcripts of three REST/CoREST gene-targets (calbindin, mash1, and miR-124) (B) were measured using real-time PCR. All transcripts were normalized to GAPDH. (Note: RA0 = 4-day-old embryoid bodies; RA2 = 4-day-old embryoid bodies + 2-days RA treatment)

Figure S1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MBP-ARVCF - +
GST-CoREST + +
GST-CoREST
GST-CoREST (input 10%)
MBP-ARVCF
MBP
Pull-downs
immuno blot
B
HEK293
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p120-Flag
REST-HA
IgG-HC
immuno blot
C
AB1 mESC
IP: IgG CoREST input
Plu. Plu. Dif. Plu. Dif.
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p120 iso3
REST
CoREST
IgG-HC
immuno blot
D
HEK293
IP : IgG CoREST
IP : IgG REST
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p120 iso3
CoREST
REST
IgG-HC
input
immuno blot
immuno blot
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AB1 mESC
IP : IgG HA-REST
IP : IgG HA-REST
CoREST-myc
REST-HA
IgG-LC
kaiso-myc
REST-HA
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immuno blot
F
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merge
REST
REST
REST
P120
REST
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P120
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P120 CoREST
p120 + CoREST

Figure S2. P120-catenin associates with REST and CoREST

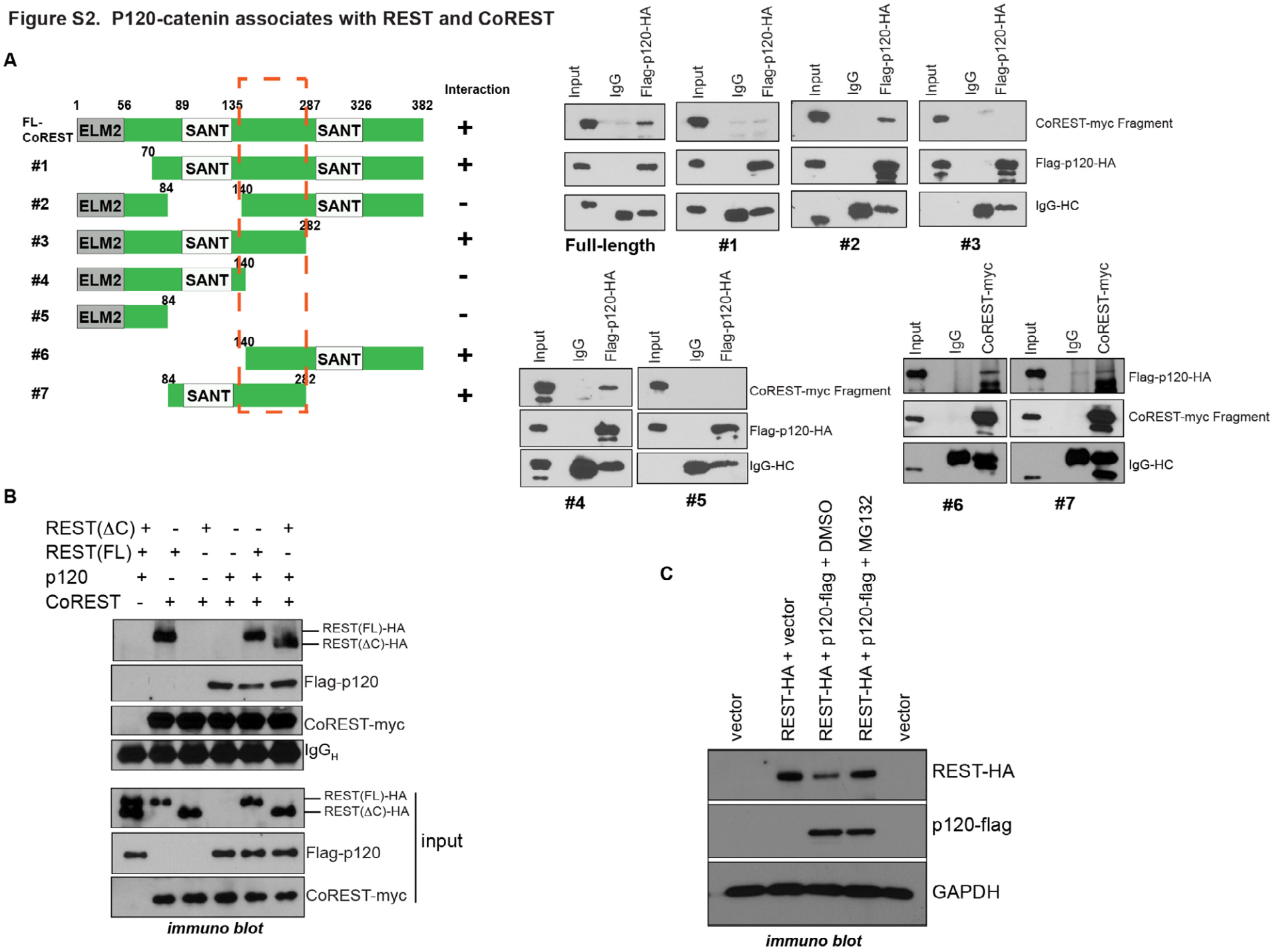
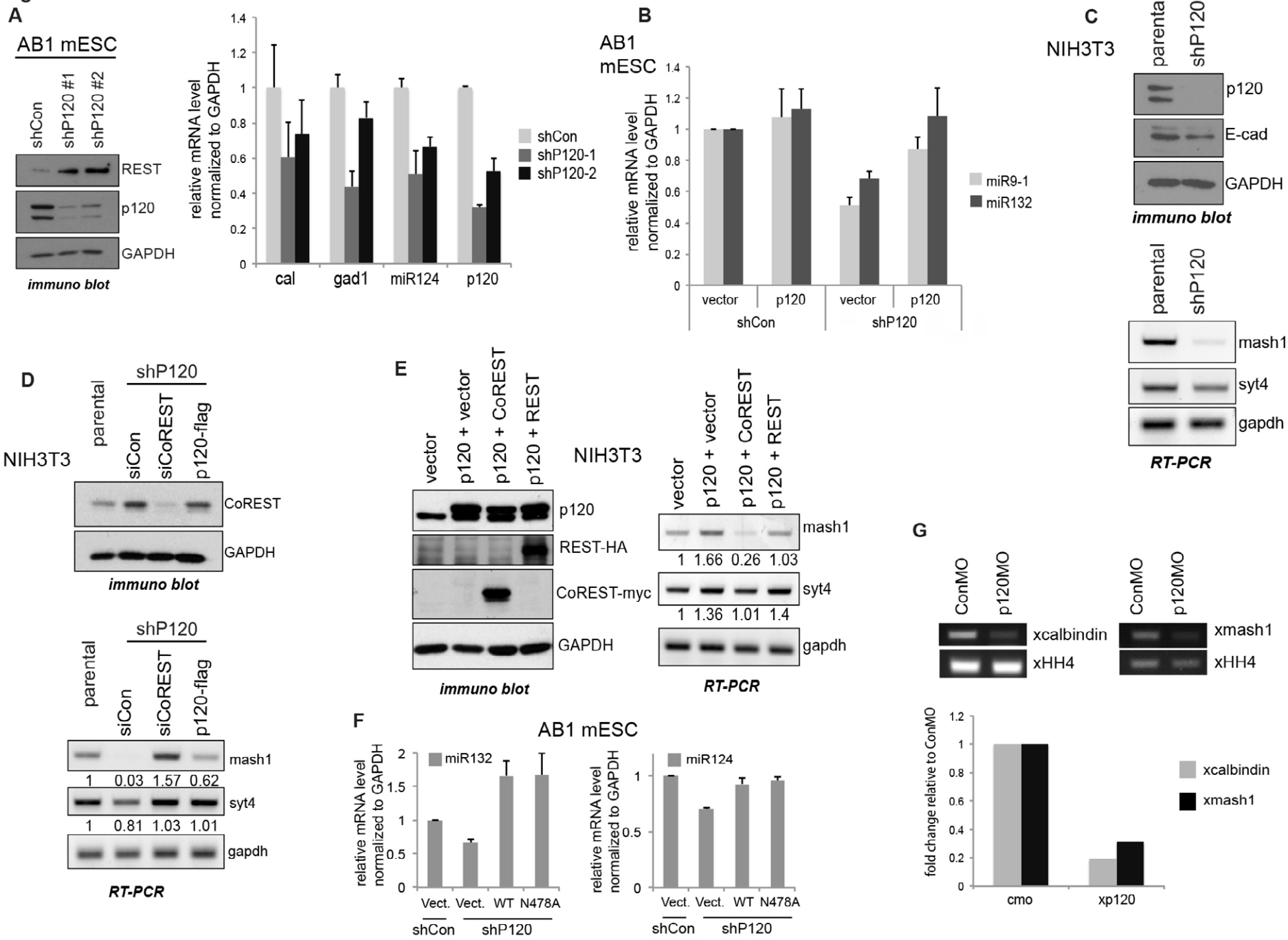


Figure S3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shP120 #1
shP120 #2
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GAPDH
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shP120-2
cal
gad1
miR124
p120
B
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miR9-1
miR132
vector
p120
shCon
shP120
C
NIH3T3
parental
shP120
p120
E-cad
GAPDH
immuno blot
mash1
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gapdh
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syt4
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p120 + CoREST
p120 + REST
p120
REST-HA
CoREST-myc
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miR124
Vect.
Vect.
WT
N478A
shCon
shP120
G
ConMO
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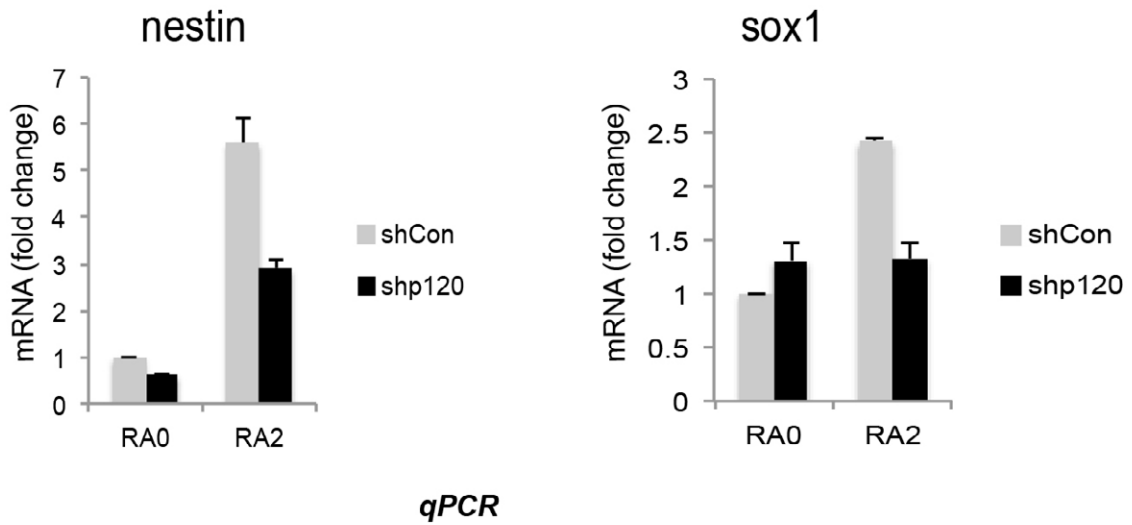
xp120

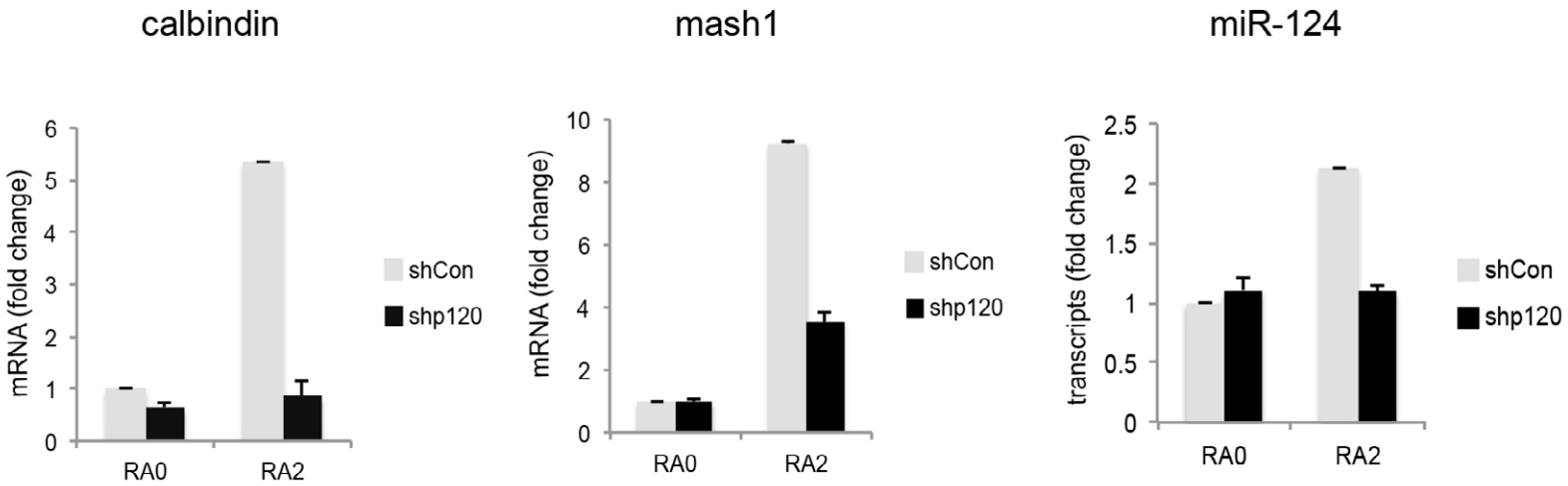
Figure S4

A



qPCR

B



qPCR

Table S1. Primer sequences.

qPCR primers

mGAPDH-F	TCGTCCCGTAGACAAAATGG
mGAPDH-R	TTGAGGTCAATGAAGGGGTC
mNeuro D1 -F	GAGGCTCCAGGGTTATGAGA
mNeuro D1 -R	ACTCATCTGTCCAGCTTGGG
q-mSyt4-F	AATGAGGTGATTGGACGGTTG
q-mSyt4-R	AGTGCCCCCCACCGC
q-mMash1-F	TCGTCCTCTCCGGAACTG AT
q-mMash1-R	TAGCCGAAGCCGCTGAAG
mNanog-F	GGTTGAAGACTAGCAATGGTCTGA
mNanog-R	TCCAGATGCGTTCACCAGATAG
mOct4-F	TGCTGAAGCAGAAGAGGATCAC
mOct4-R	CAGATGGTGGTCTGGCTGAA
mSox2-F	AGATGCACAACTCGGAGATCAG
mSox2-R	TCATGAGCGTCTTGGTTTTCC
mFoxA2-F	GGCACCTTGAGAAAGCAGTC
mFoxA2-R	GACATACCGACGCAGCTACA
mSox1-F	AGATGCACAACTCGGAGATCAG
mSox1-R	GAGTACTTGTCCTTCTTGAGCAGC
miR-9-1-F	GGGTTGGTTGTTATCTTTGGTTATC
miR-9-1-R	AGACTCCACACCACTCATACAGC
miR-124a-F	CTCTGCGTGTTCACAGCGG
miR-124a-R	CTCTTGGCATTCACCGCGTG
miR-132-F	CTCCAGGGCAACCGTGGCTTTC
miR-132-R	TGGCTGTAGACTGTTACCTCCGGTTC
mGAD1-F	AACAAACACGGGTGCAATTT
mGAD1-R	TCACCCTCGATTTTTCAACC
mSyn1-F	CCACAGGGTATGTTGTGCTG

mSyn1-R	GCCCAGATGGTTCGACTACA
mMap2-F	GCTGGTGGTATGTTCTGGCT
mMap2-R	TACCGGTTCCTCAGCTTGTC
mDcx-F	TTCAGGACCACAAGCAATGA
mDcx-R	GGAAACCGGAGTTGTCAAAA
mBra-F	GAGCCTCGAAAGAACTGAGC
mBra-R	CAGCCCACCTACTGGCTCTA
mTubb3-F	AGTCCCCTACATAGTTGCCG
mTubb3-R	AGTCAGCATGAGGGAGATCG

RT-PCR primers

mMash1 RT-F	GGAACTGATGCGCTGCAAACGCCG
mMash1 RT-R	GTTGGTAAAGTCCAGCAGCTCTTGTT
mCalbindin RT-F	GTTTCGTGTATCCTTTAGCTAGTGTGT
mCalbindin RT-R	TCTAAAGTCACTGCTTCCAAATACGTGC
mSyt4 RT-F	GGTGTTGGCCAAGTTTTCATAAGATATTC
mSyt4 RT-R	GCTACCCTTCTTATGATGAGACTGTATC

ChIP-qPCR primers

ChIP-mSyt4-3UTR-F	CAAACAACCCCCAAAACAAC
ChIP-mSyt4-3UTR-R	CAAGGAGACACAGCCTCACA
ChIP-mCal-3UTR-F	GGGGAAACTGGGTAGATGGT
ChIP-mCal-3UTR-R	GCCTGCCTCTGTTTTCCATA
ChIP-miR124a3 5UTR-F	CCCTTTCTGGAGGAATGACA
ChIP-miR124a3 5UTR-R	ATCAACAGAAACCCGTGGAG
ChIP-miR124a3-F	ACCCCAGAGAAATGGGGTAG
ChIP-miR124a3-R	AAAGTGATCACCGCCTTCAC
ChIP-mGAPDH-F	CTGCAGTACTGTGGGGAGGT
ChIP-mGAPDH-R	CAAAGGCGGAGTTACCAGAG
ChIP-mGAD1-F	CGCACCTGCAGTGAACACC

ChIP-mGAD1-R	AAGACTTCAGCACCGAGGACA
ChIP-mSyt4-F	ACTTGCTCACCGAATTCCAC
ChIP-mSyt4-R	GAAGAGCCAACAGGAACAGG
ChIP-mCal-F	CCACCTGCTGCTTCCTAGAC
ChIP-mCal-R	CCGCACCCAGTTCTCTGTAT

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