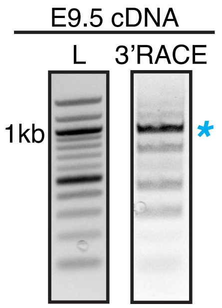
**A**



**B**

## *Predominant Handlr Isoform*

***Exon 1***
TCTTCTCTCTGCACTGTCTCTCCTTTGTGCATCTTGGACTCCTGAAGGCCACTCAGAGCATA
GATGCG
***Exon 2***
CCAGCTTAGTTCTTTGGAGAAGCAGTGTGGAGTCCTAAAAAGGAGGCTGAGTCTTACCGT
TGAGGTCGGTGCCGGTAGAGAATGGGAGGGAGTCTGCAGGAGCCAAGCACCCTAAAGAAG
AGGAAGAGAGCACGTTGCTGAGATTTGAACAGCGACCTGAGAGGTCAGACTGGACCCAGG
CCTCCTCAAG
***Exon 3***
AAGGAACGGAGATGGAGATTCTTCCCTTTTCCCGGCCCTTCGGTCCTTCTCCGGAAGAGCT
ACTCTGGCCAGCTCTGGGCTAGGGGCAGTGGCCGAACAGAAGGGGAAA
***Exon 4***
GTTTTTAGGTTTTTGCCTGTTGCTCTTAGGGATCCAGGGGCAGAGTTTTGCCTTAGGTGCTG
AAG
***Exon 5***
ACAGCTGCTGGAGCCTGTTGCTTTTTCACACCACAGATACTTCATGCTGCCTAATATTCTACC
GAGCCAGCCTAGGGGACTTGGATCTGTCTCGCCTGGGGCTAAGAGAATAGTTTCTCTAAGA
AGAAAACAGTTCTAATTCAAAGATGCTGGATCTGGCTTCCTTTAAAGCCTGAAGTCGCTGAA
GTGGCCATAGAACTATGGGGTCCAGAGGACTGCCAGTCTCAGCCAGCCTTTAGGCTGGAGA
GAGGCTGTGGGCCTGCTTGGGGACTAAAATGGATGAATAAGAAACCTGTAAGAAATGTAAAC
AGATGGAATGGCAAGGAGGAGAGCGCTGGAGATTGTTCAGGTGTGAGCTGAGGTTTCTGA
GTTTGTACTGGGGATGCCTTTGCACACAGTGAGGTGCCCCCTCCCTAAGTGGCTCTTGTTTT
GCACAAACAGCCCCACCCTGCGCACACACTGTGGGTCTCAGTAGGGACTGAGGGTGTAGG
TGCTCAGGTTTTGGGGGTGGTGGTGAAGGGGCACTCAGAATCAACAAGACAGGGCGGGGA
AAATCAAAATCAACCAAGGAACCAGCCTTCCATTTCTGAAAAGCCGCGCTCTGNNCCCGTG
CTTCAGTTCTGAAATACGAATAAATGGNGTCAGATCCCGTACTACAAAAAAAAAAAAAAAAA

**Figure S1. Predominant *Handlr* transcript *in vivo*.** A. Electrophoresed Handlr 3' RACE PCR products from E9.5 mouse cDNA. B. Sanger sequence of predominant RNA transcript, polyA tail highlighted; T in place of U due to DNA sequencing; arrow, location of 3'RACE *Handlr*-specific primer.

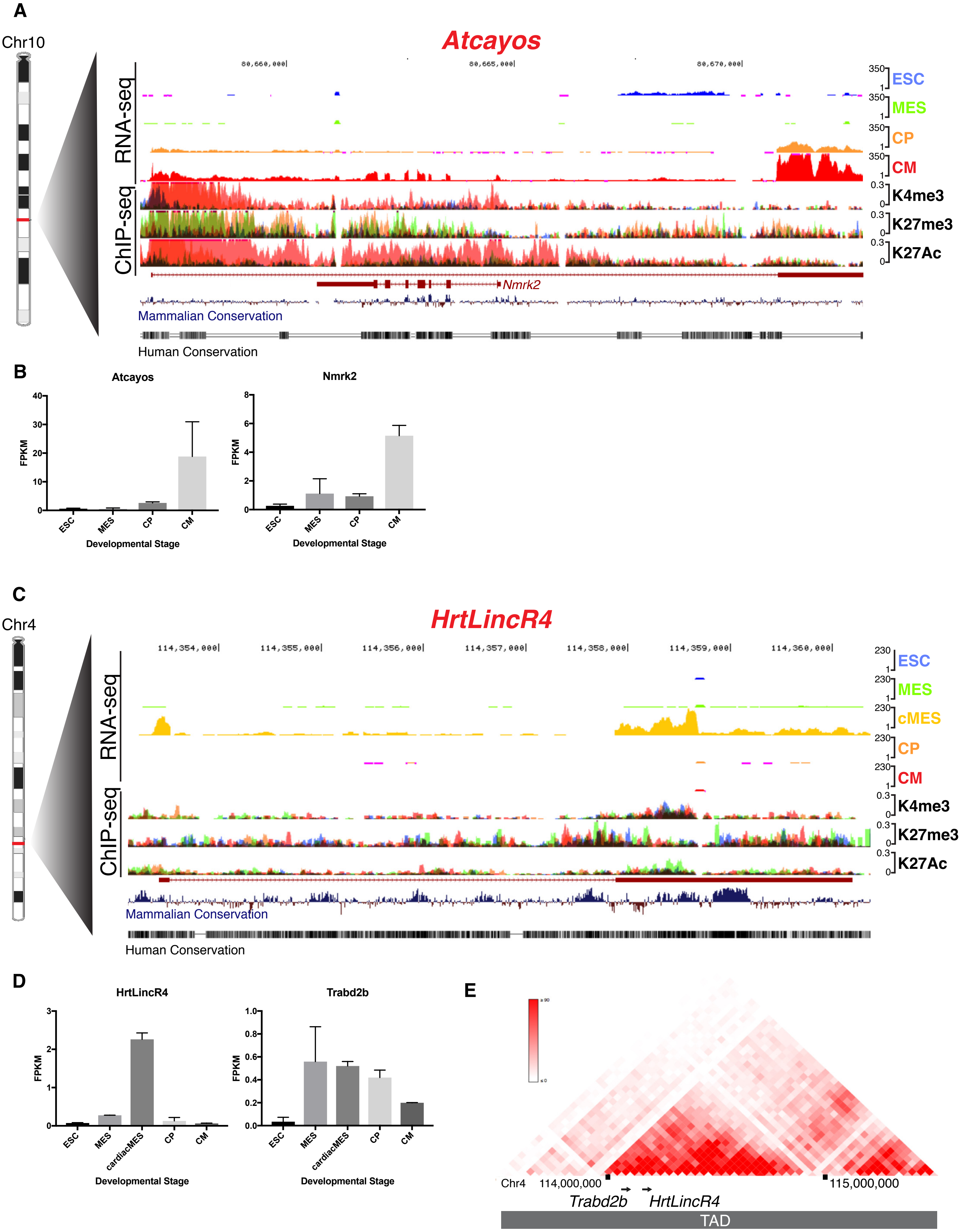

A
Chr10
Atcayos
80,660,000|
80,665,000|
80,670,000|
RNA-seq
ChIP-seq
350
1
ESC
350
1
MES
350
1
CP
350
1
CM
0.3
0
K4me3
0.3
0
K27me3
0.3
0
K27Ac
Nmrk2
Mammalian Conservation
Human Conservation
B
Atcayos
FPKM
40
30
20
10
0
ESC
MES
CP
CM
Developmental Stage
Nmrk2
FPKM
8
6
4
2
0
ESC
MES
CP
CM
Developmental Stage
C
Chr4
HrtLincR4
114,354,000|
114,355,000|
114,356,000|
114,357,000|
114,358,000|
114,359,000|
114,360,000|
RNA-seq
ChIP-seq
230
1
ESC
230
1
MES
230
1
cMES
230
1
CP
230
1
CM
0.3
0
K4me3
0.3
0
K27me3
0.3
0
K27Ac
Mammalian Conservation
Human Conservation
D
HrtLincR4
FPKM
3
2
1
0
ESC
MES
cardiacMES
CP
CM
Developmental Stage
Trabd2b
FPKM
1.0
0.8
0.6
0.4
0.2
0.0
ESC
MES
cardiacMES
CP
CM
Developmental Stage
E
≥ 90
≤ 0
Chr4
114,000,000
115,000,000
Trabd2b
HrtLincR4
TAD

**Figure S2. Genomic characterization of *Atcayos* and *HrtLincR4 in vitro*.** A. UCSC Genome Browser tracks of *Atcayos* and *Nmrk2* RNA-seq and overlaid histone H3 ChIP-seq at ESC, MES, CP, and CM stages of *in vitro* differentiation. B. Quantified expression of Atcayos and Nmrk2 at each differentiation stage. C. UCSC Genome Browser tracks of HrtLincR4 RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*; D. Quantified expression of *HrtlincR4* and *Trabd2b* at each differentiation stage. E. 3D Genome Browser Hi-C heatmap of chromosome interactions around *HrtlincR4* and *Trabd2b* loci. ESC, embryonic stem cell, MES, mesoderm, CP, cardiac progenitor, CM, cardiomyocyte; blue, ESC, green, MES, orange, CP, red, CM; K4me3, histone H3 lysine 4 trimethylation; K27me3, histone H3 lysine 27 trimethylation; K27Ac, histone H3 lysine 27 acetylation; TAD, topologically associated domain. Ensembl annotations in red. Data presented as mean +/- S.E.M.

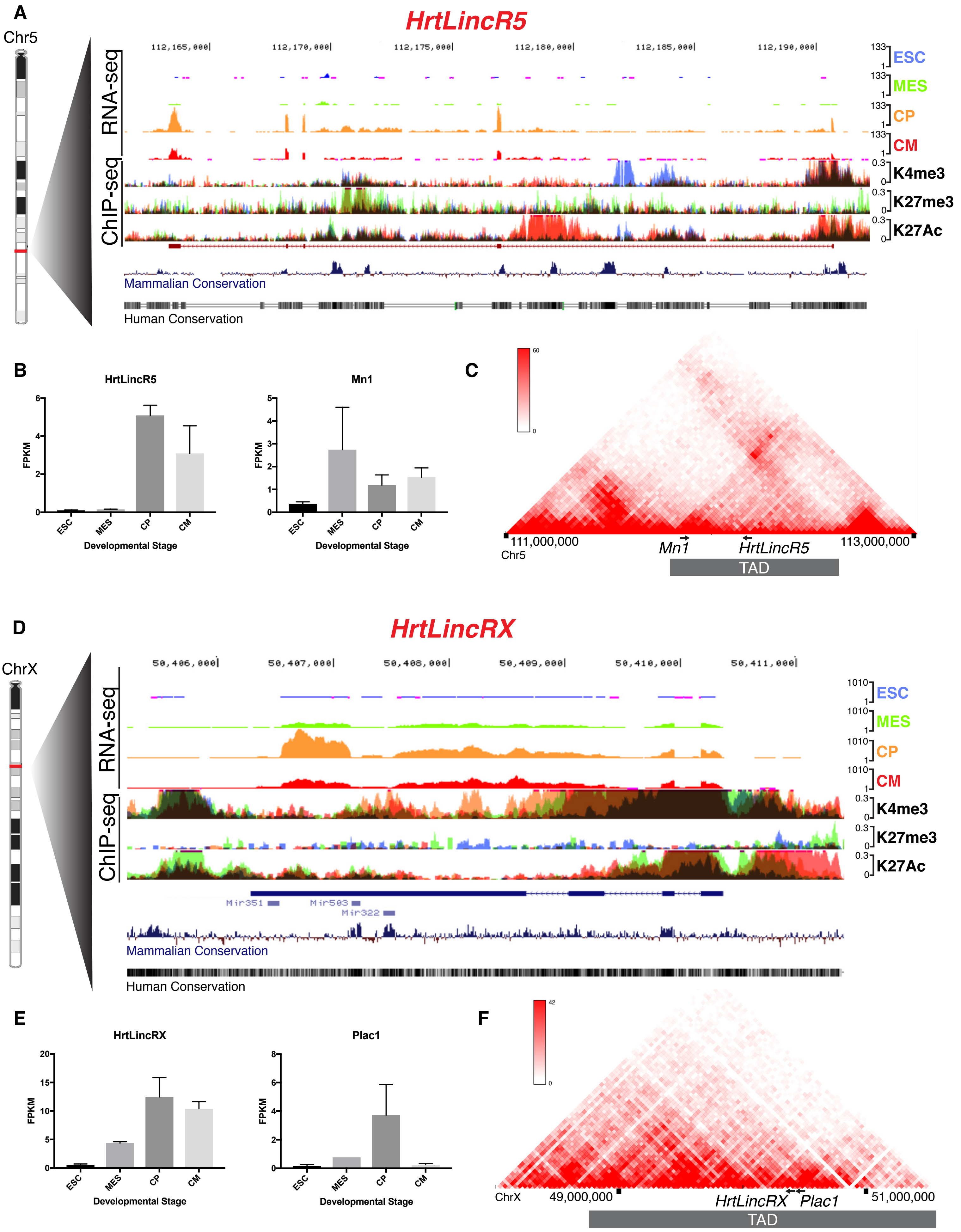
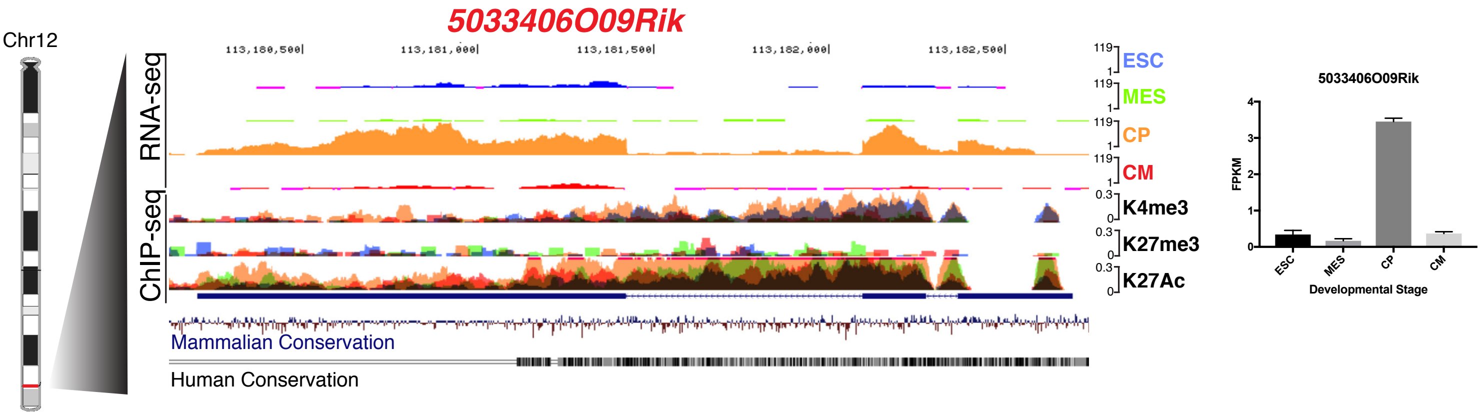
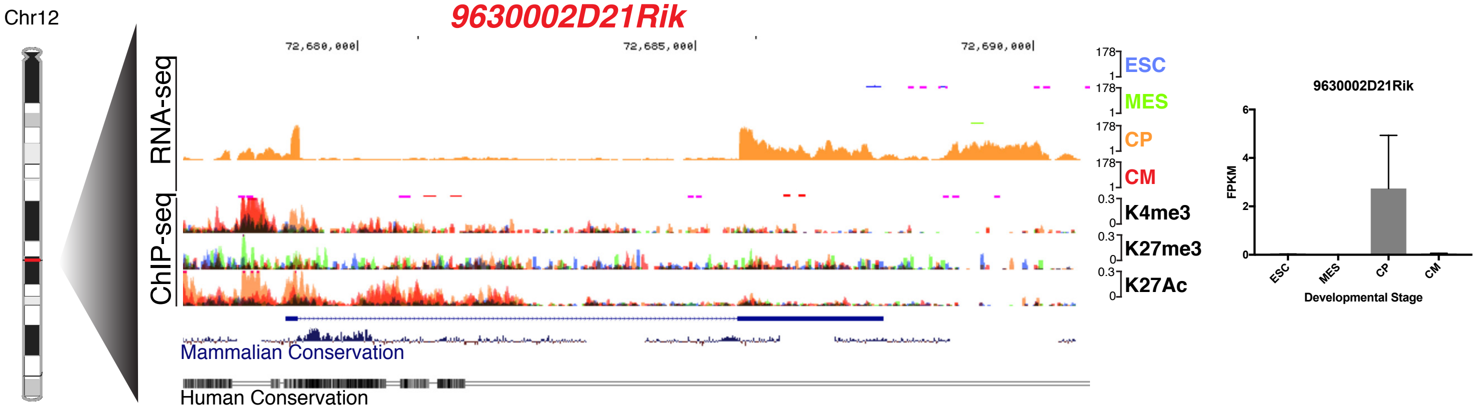

A
HrtLincR5
Chr5
112,165,000|
112,170,000|
112,175,000|
112,180,000|
112,185,000|
112,190,000|
RNA-seq
ChIP-seq
133
1
ESC
MES
CP
CM
0.3
0
K4me3
K27me3
K27Ac
Mammalian Conservation
Human Conservation
B
HrtLincR5
Mn1
FPKM
0
2
4
6
1
3
5
ESC
MES
CP
CM
Developmental Stage
C
60
0
111,000,000
Chr5
Mn1
HrtLincR5
113,000,000
TAD
D
HrtLincRX
ChrX
50,406,000|
50,407,000|
50,408,000|
50,409,000|
50,410,000|
50,411,000|
1010
Mir351
Mir503
Mir322
E
HrtLincRX
Plac1
20
15
10
8
F
42
ChrX
49,000,000
HrtLincRX
Plac1
51,000,000
TAD

**Figure S3. Genomic characterization of *HrtLincR5* and *HrtLincRX in vitro*.** A. UCSC Genome Browser tracks of *HrtLincR5* RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*. Ensembl annotation in red. B. Quantified expression of HrtlincR5 and Mn1 at each differentiation stage. C. 3D Genome Browser Hi-C heatmap (of chromosome interactions around *HrtlincR5* and *Mn1* loci. D. Genome Browser tracks of *HrtLincRX* RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*; E. Quantified expression of *HrtLincRX* and *Plac1* at each differentiation stage. F. 3D Genome Browser Hi-C heatmap of chromosome interactions around *HrtlincRX* and *Plac1* loci. ESC, embryonic stem cell, MES, mesoderm, cMES, cardiac mesoderm; CP, cardiac progenitor, CM, cardiomyocyte; blue, ESC; green, MES; orange, CP; red, CM; K4me3, histone H3 lysine 4 trimethylation; K27me3, histone H3 lysine 27 trimethylation; K27Ac, histone H3 lysine 27 acetylation; RefSeq annotation in blue. RefSeq annotations, including 3’ miRNA cluster, in blue. Data presented as mean +/- S.E.M.

**A**


5033406O09Rik
Chr12
113,180,500
113,181,000
113,181,500
113,182,000
113,182,500
RNA-seq
ChIP-seq
119
1
ESC
MES
CP
CM
0.3
0
K4me3
K27me3
K27Ac
Mammalian Conservation
Human Conservation
5033406O09Rik
FPKM
4
3
2
1
0
ESC
MES
CP
CM
Developmental Stage

**B**


9630002D21Rik
Chr12
72,680,000
72,685,000
72,690,000
RNA-seq
ChIP-seq
178
1
ESC
MES
CP
CM
0.3
0
K4me3
K27me3
K27Ac
Mammalian Conservation
Human Conservation
9630002D21Rik
FPKM
6
4
2
0
ESC
MES
CP
CM
Developmental Stage

**C**

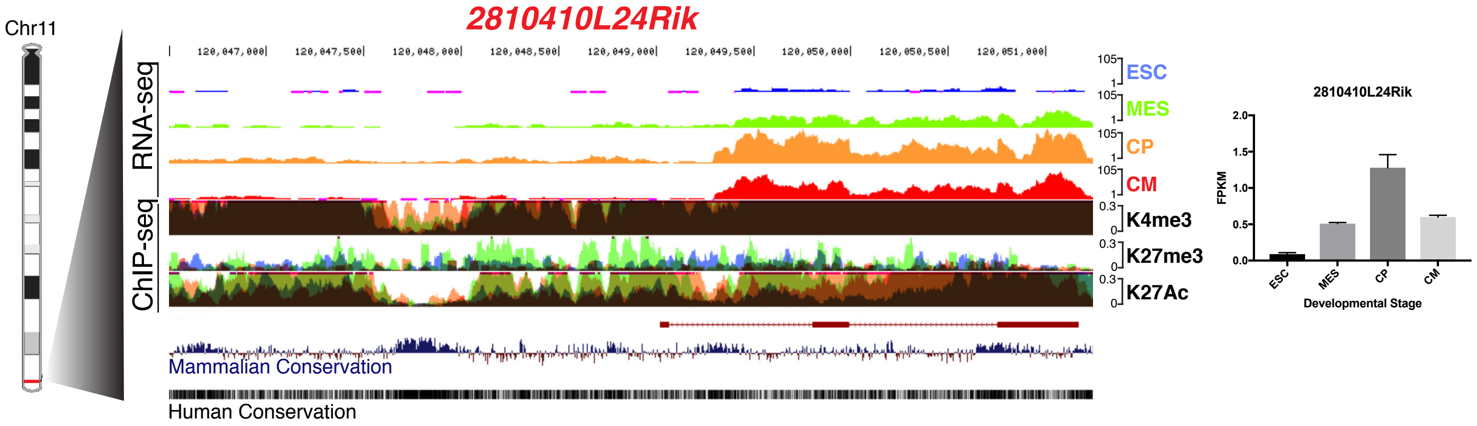

2810410L24Rik
Chr11
120,047,000
120,047,500
120,048,000
120,048,500
120,049,000
120,049,500
120,050,000
120,050,500
120,051,000
RNA-seq
ChIP-seq
105
1
ESC
MES
CP
CM
0.3
0
K4me3
K27me3
K27Ac
Mammalian Conservation
Human Conservation
2810410L24Rik
FPKM
2.0
1.5
1.0
0.5
0.0
ESC
MES
CP
CM
Developmental Stage

**Figure S4. Genomic characterization of *5033406O09Rik*, *9630002D21Rik*, and *2810410L24Rik in vitro*.** A. UCSC Genome Browser tracks of *5033406O09Rik* RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*, as well as quantified expression at each differentiation stage. B. UCSC Genome Browser tracks of *9630002D21Rik* RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*, as well as quantified expression at each differentiation stage. C. UCSC Genome Browser tracks of *2810410L24Rik* RNA-seq and overlaid histone H3 ChIP-seq during cardiac differentiation *in vitro*, as well as quantified expression at each differentiation stage. ESC, embryonic stem cell, MES, mesoderm, cMES, cardiac mesoderm; CP, cardiac progenitor, CM, cardiomyocyte; blue, ESC; green, MES; orange, CP; red, CM; K4me3, histone H3 lysine 4 trimethylation; K27me3, histone H3 lysine 27 trimethylation; K27Ac, histone H3 lysine 27 acetylation; Ensembl annotation in red, RefSeq annotations in blue. Data presented as mean +/- S.E.M.

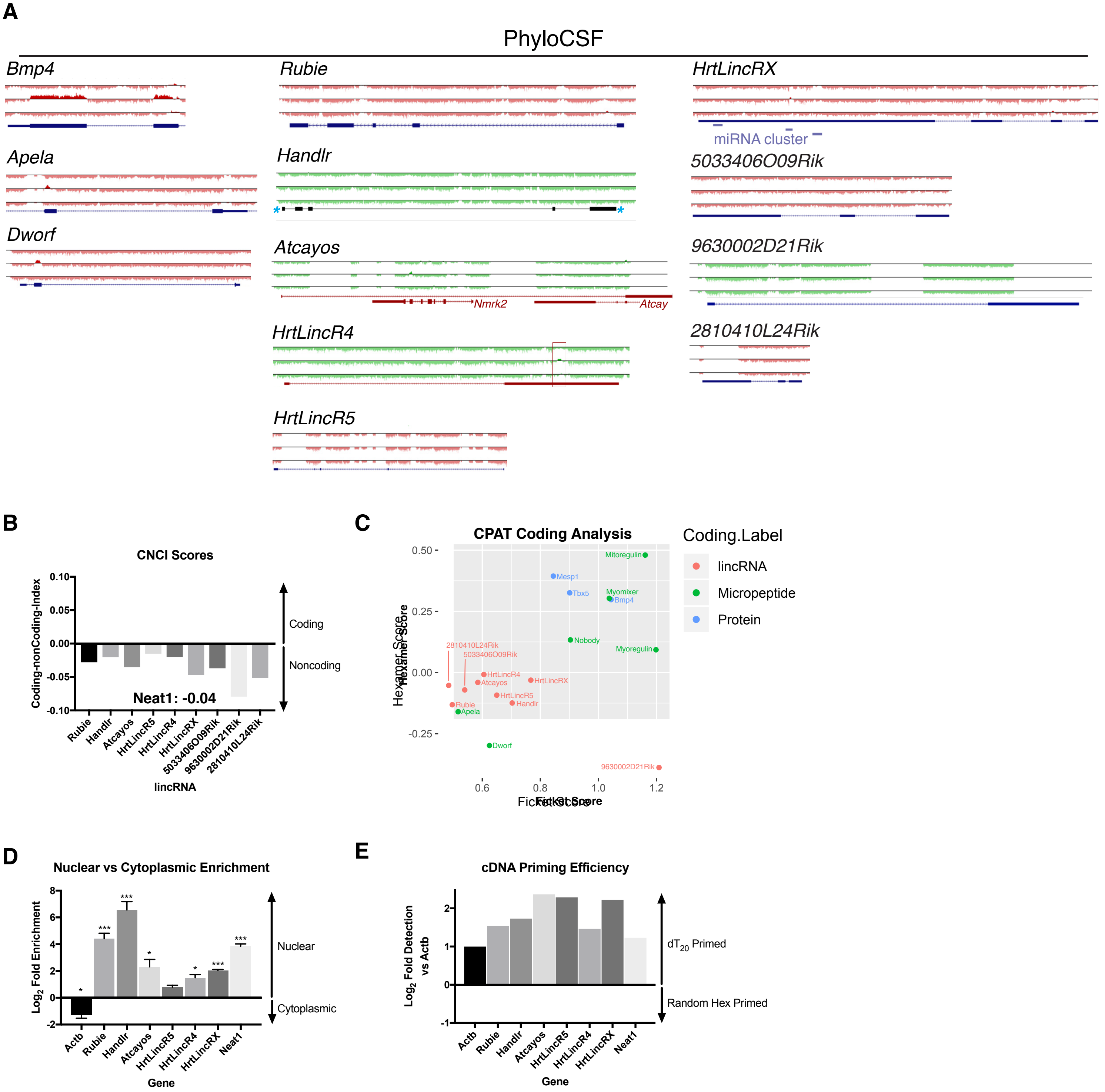

A
PhyloCSF
Bmp4
Rubie
HrtLincRX
miRNA cluster
Apela
Handlr
5033406O09Rik
Dworf
Atcayos
Nmrk2
Atcay
9630002D21Rik
HrtLincR4
2810410L24Rik
HrtLincR5
B
CNCI Scores
Coding-nonCoding-Index
0.10
0.05
0.00
-0.05
-0.10
Coding
Noncoding
Neat1: -0.04
Rubie
Handlr
Atcayos
HrtLincR5
HrtLincR4
HrtLincRX
5033406O09Rik
9630002D21Rik
2810410L24Rik
lincRNA
C
CPAT Coding Analysis
Hexamer Score
0.50
0.25
0.00
-0.25
Fickett Score
0.6
0.8
1.0
1.2
Mitoregulin
Mesp1
Tbx5
Myomixer
Bmp4
Nobody
Myoregulin
2810410L24Rik
5033406O09Rik
HrtLincR4
Atcayos
HrtLincRX
HrtLincR5
Handlr
Rubie
Apela
Dworf
9630002D21Rik
Coding.Label
lincRNA
Micropeptide
Protein
D
Nuclear vs Cytoplasmic Enrichment
$Log_2$ Fold Enrichment
8
6
4
2
0
-2
*
***
***
*
*
***
***
Nuclear
Cytoplasmic
Actb
Rubie
Handlr
Atcayos
HrtLincR5
HrtLincR4
HrtLincRX
Neat1
Gene
E
cDNA Priming Efficiency
$Log_2$ Fold Detection vs Actb
2
1
0
-1
$dT_{20}$ Primed
Random Hex Primed
Actb
Rubie
Handlr
Atcayos
HrtLincR5
HrtLincR4
HrtLincRX
Neat1
Gene

**Figure S5. Molecular characterization of lincRNA cohort.** A. UCSC Genome Browser tracks of PhyloCSF codon scores for all three frames of known protein coding gene *Bmp4*, micropeptide coding genes *Apela* and *Dworf,* and lincRNA cohort; red box, potential 28 amino acid-coding open reading frame in *HrtLincR4*; scale, -15 to +15; positive score indicates higher coding potential; green, (+) strand; red, (-) strand. B. Coding-non-Coding-Index (CNCI) scores for lincRNA cohort compared to known lincRNA Neat1. C. Comparison of CPAT algorithm calculations of Ficket and hexamer scores for known protein coding genes, micropeptide coding genes, and our lincRNA cohort. D. Nuclear vs cytoplasmic enrichment of lincRNA cohort compared to Actb and known nuclear-enriched lincRNA Neat1; *, $p < 0.05$; ***, $p < 0.005$; n.s., not significant; Student's 2-tailed t-test. E. Efficiency of RT-qPCR amplification from $dT_{20}$- or random hexamer-primed cDNA for lincRNA cohort compared to *Actb* and known polyadenylated lincRNA *Neat1*. Data presented as mean +/- S.E.M.

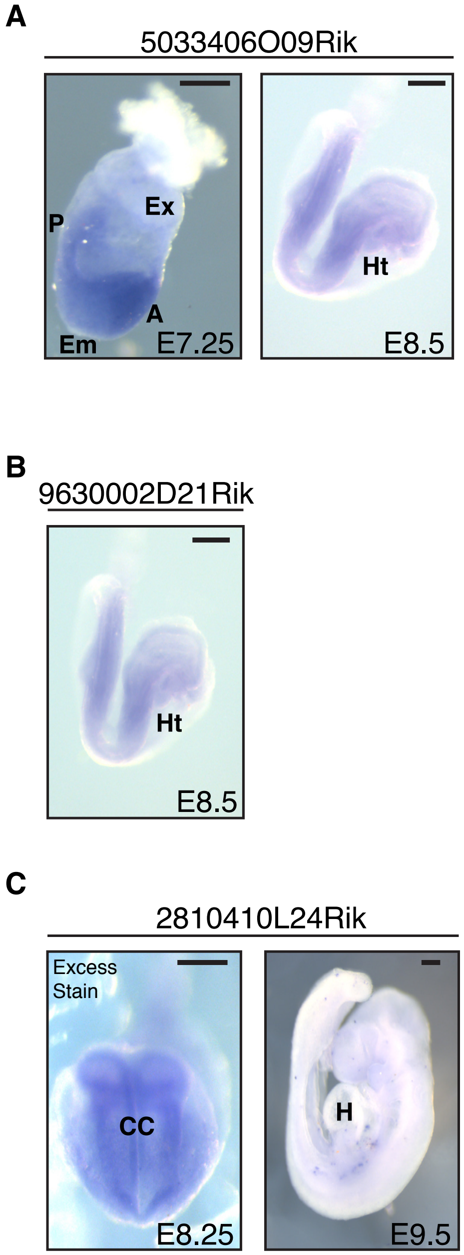


**Figure S6. lincRNA expression patterns *in vivo*.** A. *in situ* hybridization staining for *5033406O09Rik* at E7.25 and E7.5. B. *in situ* hybridization staining for *9630002D21Rik* at E8.5. C. *in situ* hybridization staining for *2810410L24Rik* at E8.25 and E9.5. A, anterior; Em, embryonic region; Ex, extraembryonic region; P, posterior; CC, cardiac crescent; Ht, heart tube; H, heart.

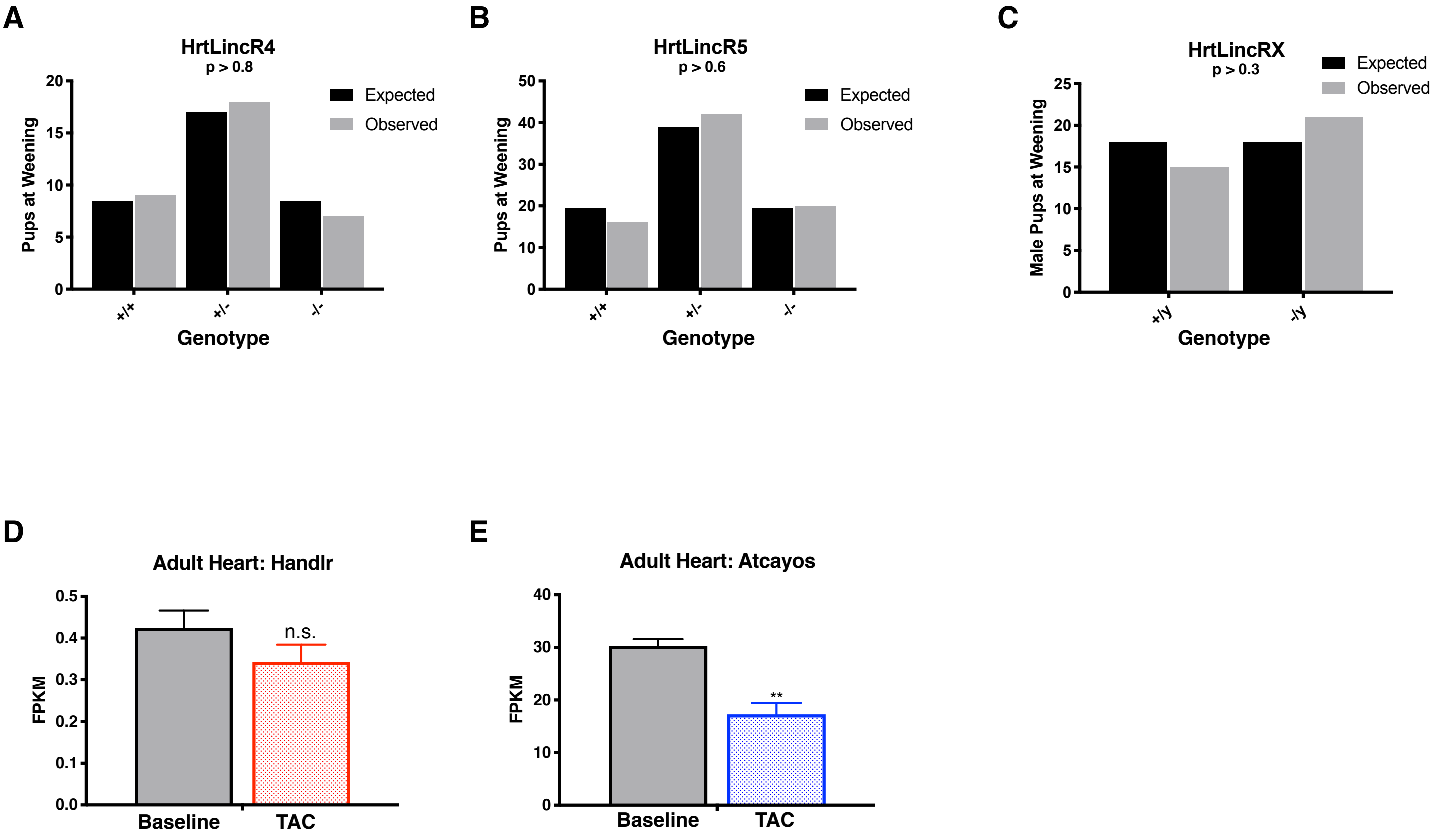


**Figure S7. Viability and phenotypic effects after lincRNA knockout.** A. Offspring recovered at weaning from *HrtLincR4*$^{+/-}$ x *HrtLincR4*$^{+/-}$ cross vs expected Mendelian ratios. B. Offspring recovered at weaning from *HrtLincR5*$^{+/-}$ x *HrtLincR5*$^{+/-}$ cross vs expected Mendelian ratios. C. Male offspring recovered at weaning from *HrtLincRX*$^{+/-}$ x *HrtLincRX*$^{+/y}$ cross vs expected Mendelian ratios; p-values in panels A-C derived from Chi-Square test. D. RNA-seq expression of Handlr in adult heart before and after TAC. E. RNA-seq expression of Atcayos in adult heart before and after TAC from Duan et al, 2017. **, $p < 0.01$, Student's 2-tailed t-test; TAC, transverse aortic constriction; WT, wild type.

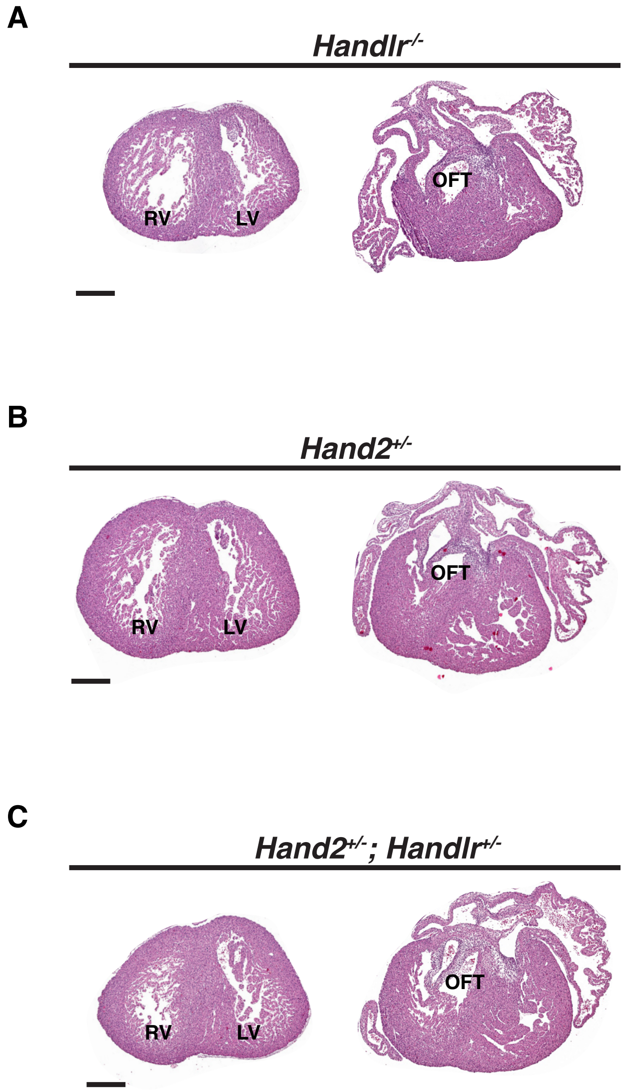


**Figure S8. Effect of *Handlr* ablation on heart development at E15.5.** A-C. Oblique transverse hematoxylin and eosin histological sections of cardiac ventricular and OFT morphogenesis, respectively, at E15.5. A. Representative *Handlr*$^{-/-}$ morphology. B. Representative *Hand2*$^{+/-}$ morphology. C. Representative *Hand2*$^{+/-}$; *Handlr*$^{+/-}$ morphology. RV, right ventricle; LV, left ventricle; OFT, outflow tract; WT, wild type; scale bar, 300$\mu$m.

| Target | Forward Primer | Reverse Primer | Product Size (bp) |
|---|---|---|---|
| *Handlr* | CTGTGGGTCTCAGTAGGGAC | TTTTGATTTTCCCCGCCCTG | 101 |
| *Rubie* | CTGCTTCCTCCTCTTCGTGT | CCAAATGCTTCAGTTCCCCT | 90 |
| *Atcayos* | CCCAGTCGTTGAAGAGGAGT | TTCACTTCCCACCCCATCTC | 126 |
| *HrtLincR4* | TCTACAACCACAGTCAGCCC | CTGAGATATCCCCGCCTGAG | 113 |
| *HrtLincR5* | CCAGCAGCACCAACCAAATA | AGACGGTGGACCCTTTTCTT | 94 |
| *HrtLincRX* | CTTAAGGAGTGGGGCTGTTAT | AATGATCCAGTGCTAACATATGC | 78 |
| *Hand2* | CAGCTACATCGCCTACCTCA | CTCCTCTTTCACGTCGGTCT | 103 |
| *Bmp4* | AGCCAACACTGTGAGGAGTT | GGATGCTGCTGAGGTTGAAG | 104 |
| *Nmrk2* | GAAGACCACCCTGACCAACA | CCCACTGTTTAAAGCCGTCC | 122 |
| *Trabd2b* | TCGAGGACAGCACTACGATC | CTGGAGTTGGTGTGAGGACT | 101 |
| *Mn1* | TGCCAGAACATGATTGCCAG | GCTCAGTTTCCTCTTCCCCT | 90 |
| *Plac1* | TACCAGTTTCACTACCGCGT | ACAGGACACGGGAATCACAT | 135 |
| *Neat1* | CTGCACTGTAGATCGGGACC | CTTTCCCCAACACCCACAAG | 117 |

**Table S1. RT-qPCR primers.** Primers utilized to amplify gene or lincRNA cDNA. Oligomers listed in 5’- to 3’- orientation.

| Target | Fwd Primer | Rev Primer |
|---|---|---|
| *Handlr* | GACTTGGATCTGTCTCGCCT | TCTGTCCCCGGAGTGTAGTA |
| *Rubie* | CTCCCTGCTTCCTCCTCTTC | GTCCTGGTCATGGTCTGTCA |
| *Atcayos* | GAGATGGGGTGGGAAGTGAA | ACCTTTACCTGCTGAGCCAT |
| *HrtLincR4* | ACCGAGAGACAGCAGTTGAA | CCGCGAGTCCCTTTGTAATG |
| *HrtLincR5* | TATGGGGATGGGAACTCAGC | AGGATGAACGGAGAAGCACA |
| *HrtLincRX* | ACCGCTCCAACCCGATTC | GCTCCCACTGTTGCTTTTCA |
| *5033406O09Rik* | CCCACTCTCTGCTGATCCAA | AGCCCCGTTTCTTCTTCTGA |
| *9630002D21Rik* | AGGATGAACGGAGAAGCACA | GCCTTCTTCCTCCTCTGGTT |
| *2810410L24Rik* | CTCACATCCTGTTCTGGGGT | AACATCCCGTGCTGTAAAGC |

**Table S2. Primers for *in situ* hybridization probe generation.** Oligomers listed in 5'- to 3'-orientation.

| Target | Left Cut | Right Cut |
|---|---|---|
| *Handlr* | AATGATGGTCATCCACCG<br>TTAAAAAGAAGGCCCCGG | CCTAATAAGATGCCTGAC<br>CCTTCTCATTGAAACCCC |
| *Rubie* | GGGCTAAATAACCCATTA | GATTAATTCATGCCCCGG |
| *Atcayos* | CAACCCGCTTTAAACTCC<br>CTCATAACTTGGGGTGTC | GACCAGCATCAGAGTCAC<br>ACCCGCCTGGTGAGCCGA |
| *HrtLincR4* | GGTTCAGGCAGGAATAC | GGGGACAGCCTTTACAA |
| *HrtLincR5* | GTGATAGACCACTTCTTG | GCGCCCCATCCGTATGC |
| *HrtLincRX* | GGCCATTTTGACCCGTCG | GGCGGTCCACTTTAGGGG |

**Table S3. tru-sgRNA crRNA oligomers.** Cas9-targeting oligomers cloned into pX330 for lincRNA knockout. Sequences are truncated by 2-3 nucleotides to diminish off-target effects. Oligomers listed in 5’- to 3’- orientation. Genomic PAM sequence located immediately 3’- to each target sequence. Left and right cuts relative to *mus musculus* mm9 assembly.

| Locus | Fwd Primer | Rev Primer | WT band size (bp) | KO band size (bp) |
|---|---|---|---|---|
| *Handlr* | TGGATCCAACTAACCTAATGCTT | ACGCGCAGGAAAGGTAAAT<br>CCGCATTAGTTTCCCTTCCC | 600 | 400 |
| *Rubie* | CCTGGTTGCCGTCATTACTG | GTGTATGTGGGCAGAGAGGT<br>AGTCACTGTTTCGCATGAGA | 617 | 680 |
| *Atcayos* | TGACGACAGCTGATAGGGC | TGCTTGCCCTAGTATGGTCA<br>AGTTCCATCTTCAAGGCCCT | 443 | 280 |
| *HrtLincR4* | ATCTTGGGGCCATCTCCAAA | GACAATTGGCTGTGGTGGTT<br>TTTCTCTGGACCCGTGTGAG | 448 | 500 |
| *HrtLincR5* | GTAAAGGCTCTTGTCACAGGG | TTTTCCTTGCAACTCCCAGC<br>TAACTAGAGGGAGGGGAGGG | 255 | 180 |
| *HrtLincRX* | AGGAGAGAGAAAAGCCGTGT | CGCGGTGCAATGAAAGACTA<br>GGGCCACTCATTTGTAAGCA | 784 | 855 |
| *Bmp4 (loxP)* | GAGCTAAGTTTTGCTGGTTTGC | GCCCATGAGCTTTTCTGAGA | 200 | 250 (loxP) |
| *Cre Transgene* | ATGCTTCTGTCCGTTTGCCG | CCTGTTTTGCACGTTCACCG | 280 | |
| *Hand2 (loxP)* | AAAGAGTCAGTGGTTCTCATCC<br>GAAGTTCCTATTCTCTAGAAA | AATCTAACCCAACCCCTCGC | 600 | 634 (loxP)<br>300 (KO) |

**Table S4. Genotyping primers.** 3-primer genotyping oligomers used for embryo genotyping. Wild type (WT) and knockout (KO) alleles amplified independently, resulting in variable gDNA amplified products of variable lengths (larger band sizes in KO reactions do not represent DNA insertions).

| | ***Handlr*$^{+/+}$** | | | | | ***Handlr*$^{-/-}$** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time Point** | **Baseline** | **Week 1** | **Week 4** | **Week 6** | **Week 8** | **Baseline** | **Week 1** | **Week 4** | **Week 6** | **Week 8** |
| **ENDOarea;d (mm2)** | 16.21+/-0.71 | 16.26+/-1.19 | 16.65+/-1.42 | 16.19+/-1.58 | 17.35+/-1.63 | 14.00+/-0.70 | 14.63+/-0.29 | 15.39+/-1.16 | 15.65+/-0.49 | 16.05+/-1.09 |
| **ENDOarea;s (mm2)** | 10.07+/-0.45 | 11.82+/-1.15 | 12.59+/-1.10 | 12.30+/-1.17 | 13.35+/-1.36 | 7.51+/-0.53 | 9.72+/-0.35 | 10.68+/-1.25 | 11.38+/-0.27 | 12.45+/-1.04 |
| **IVS;d (mm)** | 0.64+/-0.04 | 1.02+/-0.05 | 0.90+/-0.06 | 1.12+/-0.06 | 1.20+/-0.08 | 0.63+/-0.04 | 0.93+/-0.04 | 1.13+/-0.04 | 1.05+/-0.07 | 1.29+/-0.06 |
| **IVS;s (mm)** | 0.96+/-0.04 | 1.38+/-0.04 | 1.16+/-0.08 | 1.25+/-0.05 | 1.39+/-0.05 | 0.91+/-0.02 | 1.28+/-0.07 | 1.33+/-0.06 | 1.28+/-0.05 | 1.46+/-0.11 |
| **LVID;d (mm)** | 4.54+/-0.10 | 4.38+/-0.16 | 4.65+/-0.19 | 4.58+/-0.24 | 4.68+/-0.19 | 4.19+/-0.09 | 4.25+/-0.04 | 4.49+/-0.18 | 4.57+/-0.06 | 4.43+/-0.13 |
| **LVPW;d (mm)** | 0.66+/-0.05 | 1.00+/-0.05 | 1.03+/-0.11 | 1.22+/-0.07 | 1.13+/-0.04 | 0.61+/-0.04 | 0.89+/-0.09 | 0.93+/-0.09 | 1.03+/-0.05 | 1.11+/-0.05 |
| **LVPW;s (mm)** | 0.82+/-0.06 | 0.97+/-0.06 | 1.26+/-0.10 | 1.52+/-0.07 | 1.37+/-0.07 | 0.87+/-0.05 | 1.09+/-0.12 | 1.32+/-0.22 | 1.37+/-0.12 | 1.25+/-0.06 |
| **Doppler (mm/s)** | | -3665+/-206 | | | | | -3671+/-209 | | | |
| **HR (bpm)** | 511.23+/-16.33 | 513.64+/-11.34 | 512.55+/-28.10 | 506.11+/-26.13 | 545.55+/-29.02 | 470.08+/-12.68 | 493.20+/-21.93 | 492.34+/-24.46 | 476.12+/-30.90 | 498.91+/-19.11 |
| **Body Weight (g)** | 28.30+/-0.76 | 26.70+/-0.41 | 28.57+/-0.70 | 29.87+/-0.73 | 31.00+/-0.53 | 28.70+/-1.49 | 27.96+/-1.74 | 28.44+/-1.19 | 30.00+/-1.52 | 29.60+/-1.03 |
| **LV Mass (mg)** | 110.68+/-6.06 | 191.11+/-17.75 | 198.85+/-27.89 | 252.14+/-24.46 | 257.60+/-24.23 | 90.33+/-4.89 | 156.06+/-12.16 | 201.37+/-11.15 | 209.86+/-11.13 | 245.80+/-19.90 |
| **FAC (%)** | 37.78+/-1.55 | 27.93+/-3.16 | 24.25+/-2.52 | 23.85+/-1.96 | 23.38+/-1.37 | 46.50+/-1.70 | 33.50+/-2.68 | 31.45+/-4.94 | 27.06+/-2.83 | 22.50+/-3.65 |

**Table S5. *Handlr* KO TAC echocardiography.** Echocardiographic measurements in 12-20 week-old litter-matched adult males at baseline and after transverse aortic constriction (TAC). d, diastolic; s, systolic; IVS, interventricular septum; LVID, left ventricular internal diameter; LVPW; left ventricular posterior wall; HR, heart rate; LV, left ventricle; FAC, fractional area contractility. Data presented as mean +/- S.E.M. N = 4-9 for all measurements.

| | ***Atcayos*$^{+/+}$** | | | | | ***Atcayos*$^{-/-}$** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time Point** | **Baseline** | **Week 1** | **Week 4** | **Week 6** | **Week 8** | **Baseline** | **Week 1** | **Week 4** | **Week 6** | **Week 8** |
| **ENDOarea;d (mm2)** | 12.95+/-0.65 | 15.66+/-1.10 | 14.89+/-0.89 | 15.09+/-1.57 | 16.15+/-1.51 | 14.95+/-1.70 | 15.21+/-0.94 | 14.44+/-0.97 | 14.17+/-1.26 | 15.74+/-1.20 |
| **ENDOarea;s (mm2)** | 8.11+/-0.43 | 10.15+/-0.94 | 10.71+/-0.84 | 11.02+/-1.52 | 12.47+/-1.38 | 8.90+/-0.86 | 9.95+/-0.81 | 10.22+/-0.87 | 10.65+/-1.25 | 12.27+/-1.30 |
| **IVS;d (mm)** | 0.77+/-0.03 | 1.05+/-0.06 | 1.08+/-0.05 | 1.03+/-0.04 | 1.08+/-0.05 | 0.62+/-0.04 | 0.90+/-0.09 | 1.01+/-0.04 | 0.99+/-0.05 | 1.14+/-0.07 |
| **IVS;s (mm)** | 1.01+/-0.06 | 1.42+/-0.07 | 1.32+/-0.06 | 1.30+/-0.04 | 1.39+/-0.06 | 0.83+/-0.04 | 1.33+/-0.05 | 1.25+/-0.07 | 1.18+/-0.05 | 1.31+/-0.05 |
| **LVID;d (mm)** | 4.01+/-0.09 | 4.32+/-0.15 | 4.47+/-0.14 | 4.47+/-0.19 | 4.45+/-0.20 | 4.25+/-0.09 | 4.22+/-0.15 | 4.33+/-0.16 | 4.24+/-0.17 | 4.52+/-0.16 |
| **LVPW;d (mm)** | 0.82+/-0.04 | 1.05+/-0.05 | 1.09+/-0.04 | 1.17+/-0.07 | 1.21+/-0.06 | 0.60+/-0.04 | 0.93+/-0.05 | 1.14+/-0.04 | 1.21+/-0.09 | 1.12+/-0.07 |
| **LVPW;s (mm)** | 0.99+/-0.06 | 1.24+/-0.06 | 1.36+/-0.06 | 1.36+/-0.03 | 1.37+/-0.05 | 0.77+/-0.05 | 1.14+/-0.07 | 1.35+/-0.06 | 1.35+/-0.08 | 1.33+/-0.05 |
| **Doppler (mm/s)** | | -3457+/-174 | | | | | -3509+/-210 | | | |
| **HR (bpm)** | 485.01+/-22.77 | 513.39+/-13.83 | 520.69+/-15.65 | 510.88+/-18.73 | 538.68+/-13.28 | 451.29+/-34.10 | 515.49+/-7.01 | 555.15+/-17.93 | 513.05+/-19.75 | 527.46+/-29.90 |
| **Body Weight (g)** | 28.37+/-0.77 | 27.40+/-0.51 | 28.50+/-0.74 | 29.31+/-0.59 | 29.44+/-0.47 | 27.36+/-0.81 | 26.41+/-0.63 | 27.26+/-0.74 | 28.31+/-0.82 | 29.29+/-0.84 |
| **LV Mass (mg)** | 117.88+/-7.48 | 195.39+/-16.13 | 216.98+/-14.07 | 221.22+/-19.53 | 234.12+/-20.87 | 91.61+/-3.64 | 154.58+/-11.35 | 202.07+/-11.93 | 203.23+/-18.00 | 234.41+/-22.46 |
| **FAC (%)** | 37.41+/-0.66 | 35.60+/-1.93 | 29.18+/-2.09 | 27.98+/-2.40 | 23.47+/-2.09 | 40.13+/-1.73 | 34.66+/-2.63 | 29.59+/-2.08 | 25.66+/-3.29 | 22.75+/-3.03 |

**Table S6. *Atcayos* KO TAC echocardiography.** Echocardiographic measurements in 12-20 week-old litter-matched adult males at baseline and after transverse aortic constriction (TAC). d, diastolic; s, systolic; IVS, interventricular septum; LVID, left ventricular internal diameter; LVPW; left ventricular posterior wall; HR, heart rate; LV, left ventricle; FAC, fractional area contractility. Data presented as mean +/- S.E.M. N = 4-9 for all measurements.