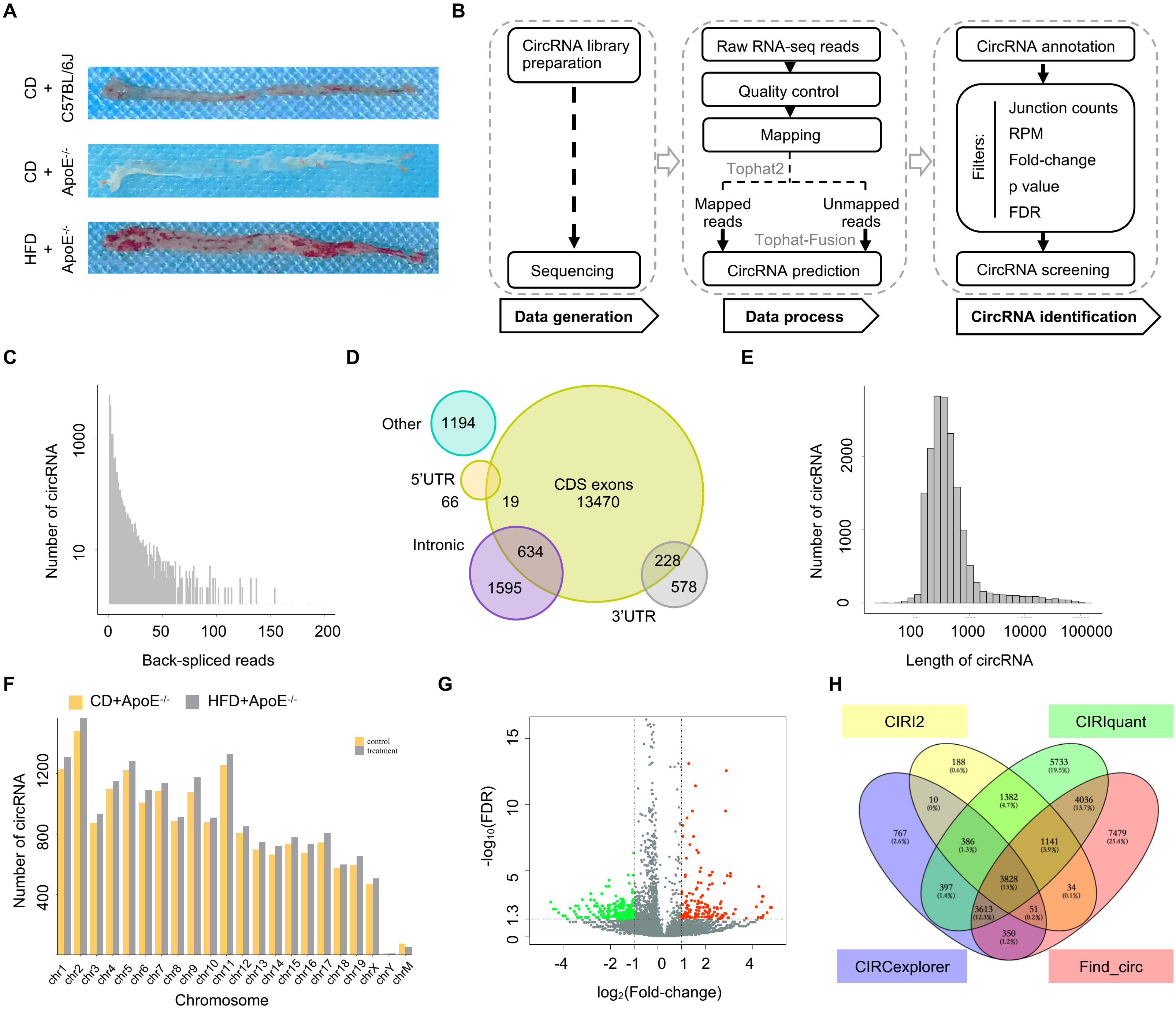
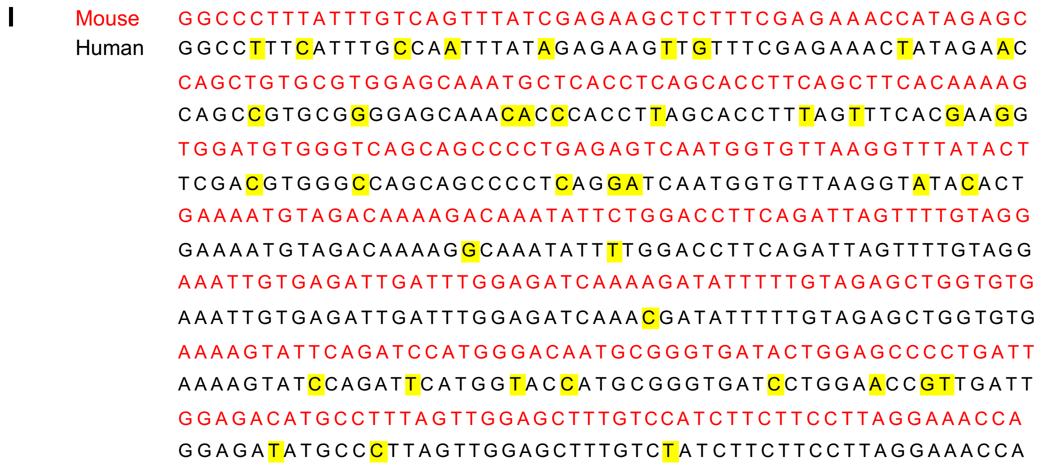


I



Mouse GGCCCTTTATTTGTCAGTTTATCGAGAAGCTCTTTCGAGAAACCATAGAGC
Human GGCCTTTCATTTGCCAATTTATAGAGAAGTTGTTTCGAGAAACTATAGAAC
CAGCTGTGCGTGGAGCAAATGCTCACCTCAGCACCTTCAGCTTCACAAAAG
CAGCCGTGCGGGGAGCAAACACCCACCTTAGCACCTTTAGTTTCACGAAGG
TGGATGTGGGTCAGCAGCCCCTGAGAGTCAATGGTGTTAAGGTTTATACT
TCGACGTGGGCCAGCAGCCCCTCAGGATCAATGGTGTTAAGGTATACACT
GAAAATGTAGACAAAAGACAAATATTCTGGACCTTCAGATTAGTTTTGTAGG
GAAAATGTAGACAAAAGGCAAATATTTTGGACCTTCAGATTAGTTTTGTAGG
AAATTGTGAGATTGATTTGGAGATCAAAAGATATTTTTGTAGAGCTGGTGTG
AAATTGTGAGATTGATTTGGAGATCAAACGATATTTTTGTAGAGCTGGTGTG
AAAAGTATTCAGATCCATGGGACAATGCGGGTGATACTGGAGCCCCTGATT
AAAAGTATCCAGATTCATGGTACCATGCGGGTGATCCTGGAACCGTTGATT
GGAGACATGCCTTTAGTTGGAGCTTTGTCCATCTTCTTCCTTAGGAAACCA
GGAGATATGCCCTTAGTTGGAGCTTTGTCTATCTTCTTCCTTAGGAAACCA

Score	Expect	Gaps	Identities
563 bits(624)	3e-165	0/417(0%)	375/417(90%)

Figure S1

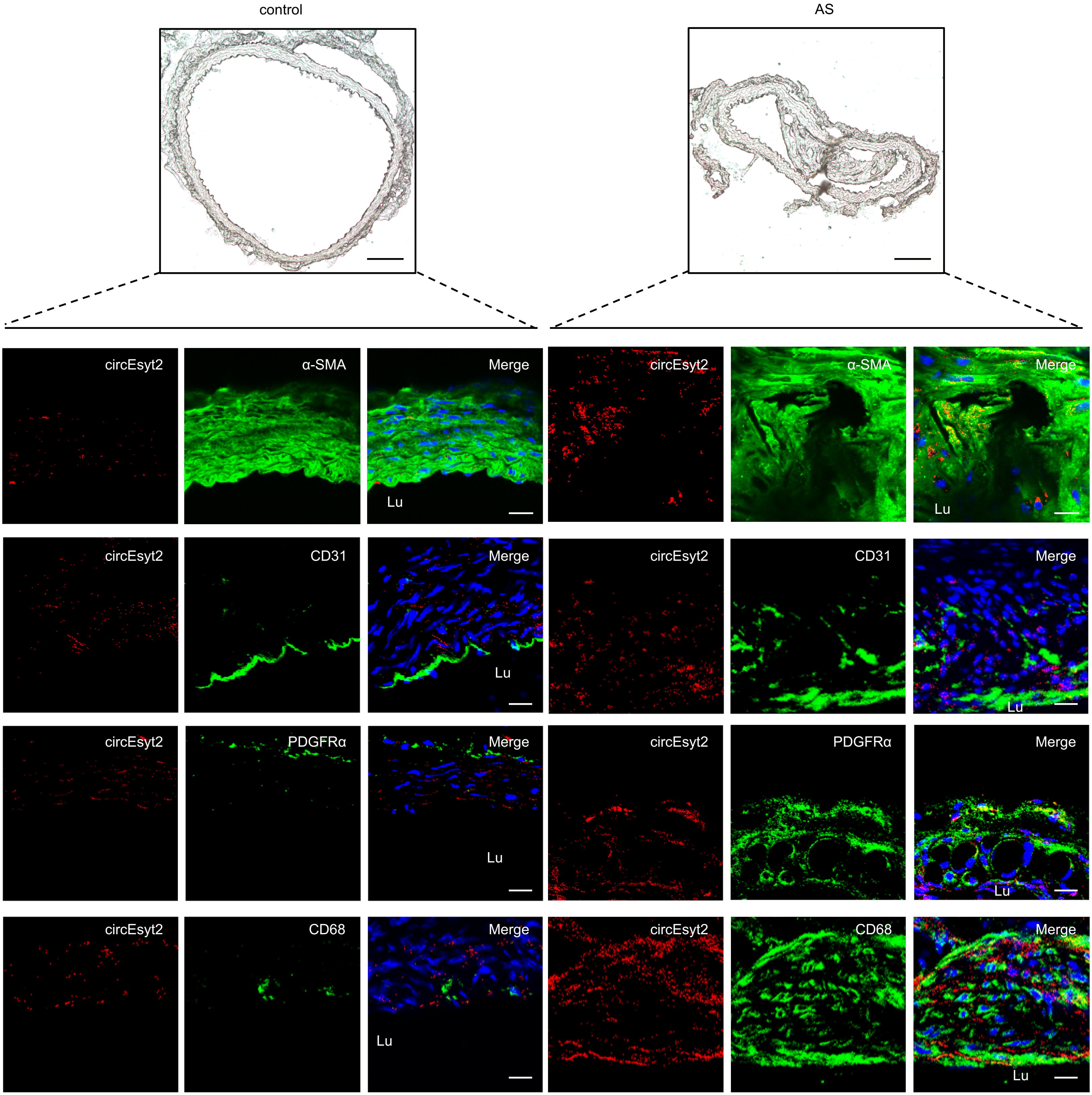

control
AS
circEsyt2
α-SMA
Merge
Lu
circEsyt2
α-SMA
Merge
Lu
circEsyt2
CD31
Merge
Lu
circEsyt2
CD31
Merge
Lu
circEsyt2
PDGFRα
Merge
Lu
circEsyt2
PDGFRα
Merge
Lu
circEsyt2
CD68
Merge
Lu
circEsyt2
CD68
Merge
Lu

Figure S2

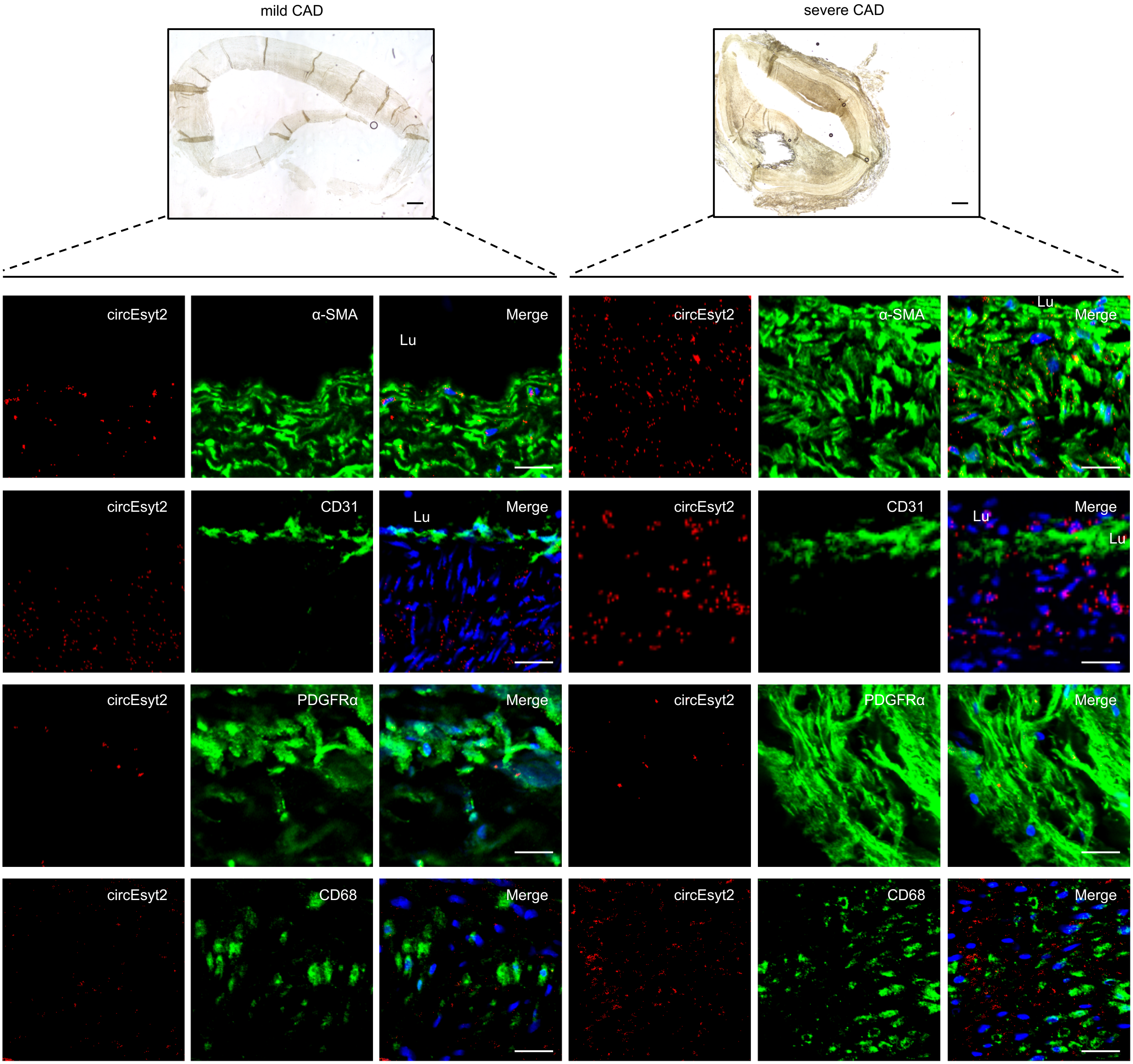

mild CAD
severe CAD
circEsyt2
α-SMA
Merge
Lu
circEsyt2
α-SMA
Lu
Merge
circEsyt2
CD31
Lu
Merge
circEsyt2
CD31
Lu
Merge
Lu
circEsyt2
PDGFRα
Merge
circEsyt2
PDGFRα
Merge
circEsyt2
CD68
Merge
circEsyt2
CD68
Merge

Figure S3

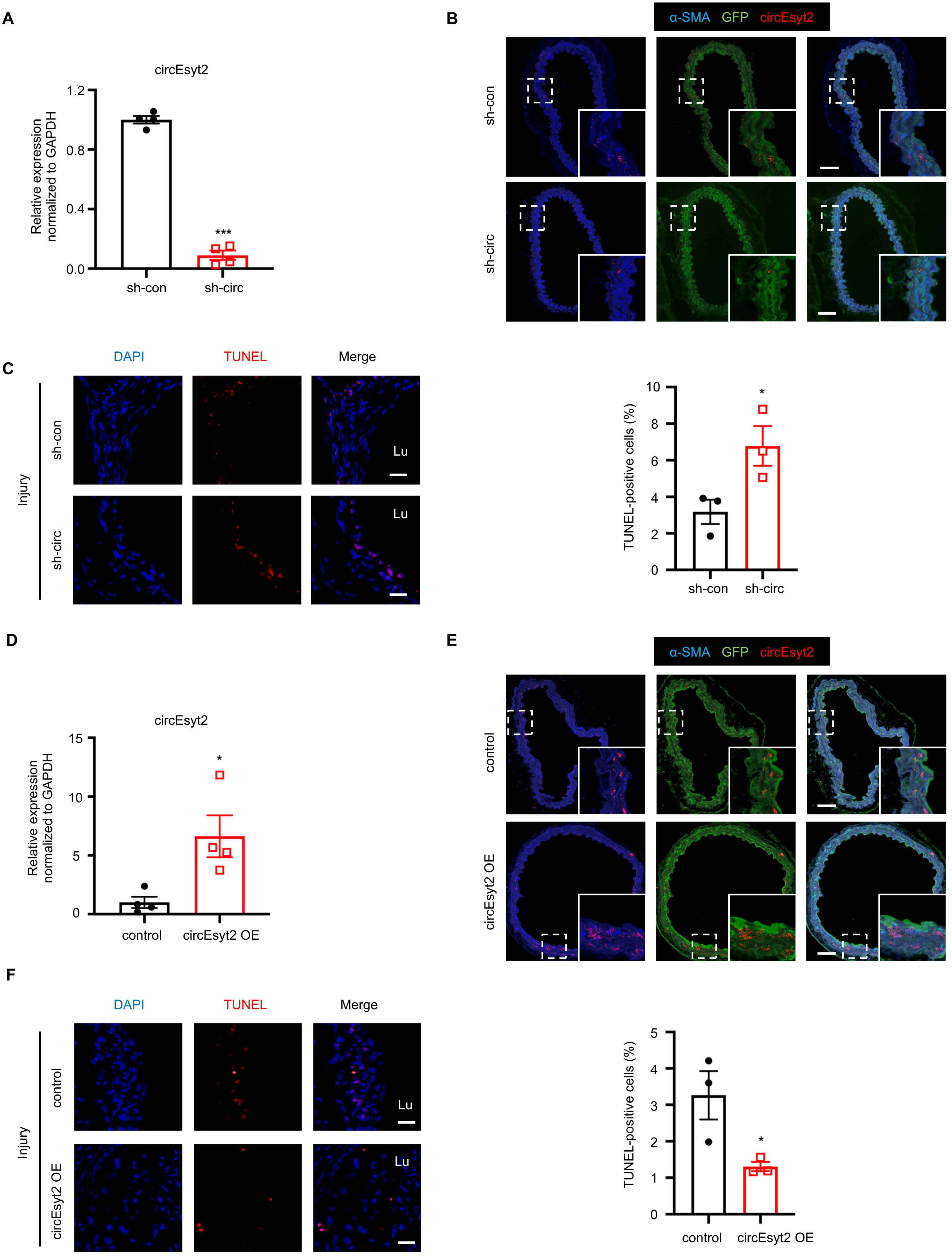

A
circEsyt2
Relative expression normalized to GAPDH
1.2
0.8
0.4
0.0
***
sh-con
sh-circ
B
α-SMA GFP circEsyt2
sh-con
sh-circ
C
DAPI
TUNEL
Merge
Injury
sh-con
sh-circ
Lu
Lu
10
8
6
4
2
0
*
TUNEL-positive cells (%)
sh-con
sh-circ
D
circEsyt2
Relative expression normalized to GAPDH
15
10
5
0
*
control
circEsyt2 OE
E
α-SMA GFP circEsyt2
control
circEsyt2 OE
F
DAPI
TUNEL
Merge
Injury
control
circEsyt2 OE
Lu
Lu
5
4
3
2
1
0
*
TUNEL-positive cells (%)
control
circEsyt2 OE

Figure S4

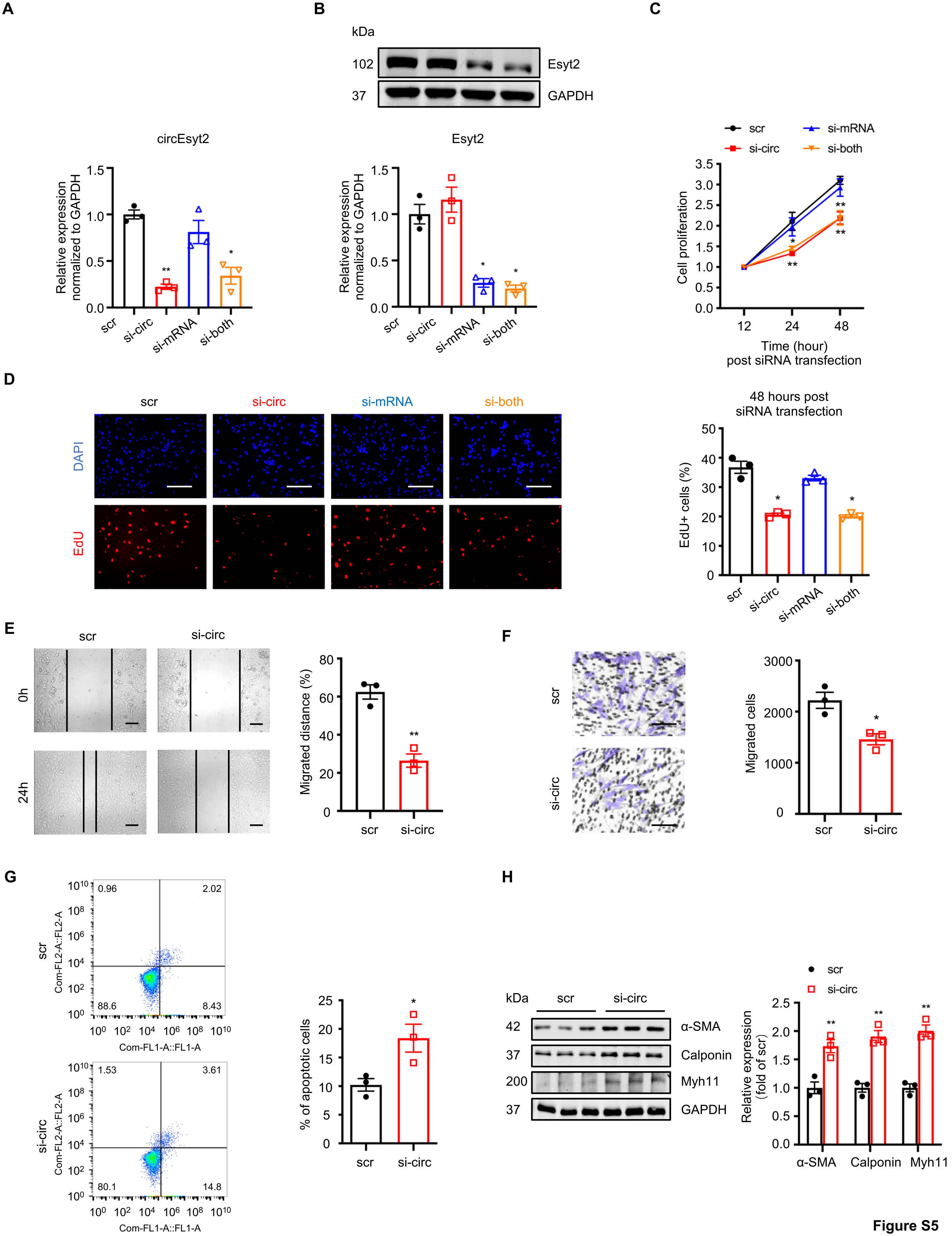

A
circEsyt2
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
**
*
scr
si-circ
si-mRNA
si-both
B
kDa
102
37
Esyt2
GAPDH
Esyt2
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
*
*
scr
si-circ
si-mRNA
si-both
C
scr
si-mRNA
si-circ
si-both
Cell proliferation
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
**
**
12
24
48
Time (hour)
post siRNA transfection
D
scr
si-circ
si-mRNA
si-both
DAPI
EdU
48 hours post siRNA transfection
EdU+ cells (%)
50
40
30
20
10
0
*
*
scr
si-circ
si-mRNA
si-both
E
scr
si-circ
0h
24h
Migrated distance (%)
80
60
40
20
0
**
scr
si-circ
F
scr
si-circ
Migrated cells
3000
2000
1000
0
*
scr
si-circ
G
scr
Com-FL2-A::FL2-A
10^{10}
10^8
10^6
10^4
10^2
10^0
0.96
2.02
88.6
8.43
10^0 10^2 10^4 10^6 10^8 10^{10}
Com-FL1-A::FL1-A
si-circ
Com-FL2-A::FL2-A
1.53
3.61
80.1
14.8
Com-FL1-A::FL1-A
% of apoptotic cells
25
20
15
10
5
0
*
scr
si-circ
H
kDa
scr
si-circ
42
37
200
37
α-SMA
Calponin
Myh11
GAPDH
scr
si-circ
Relative expression (fold of scr)
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
α-SMA
Calponin
Myh11

Figure S5

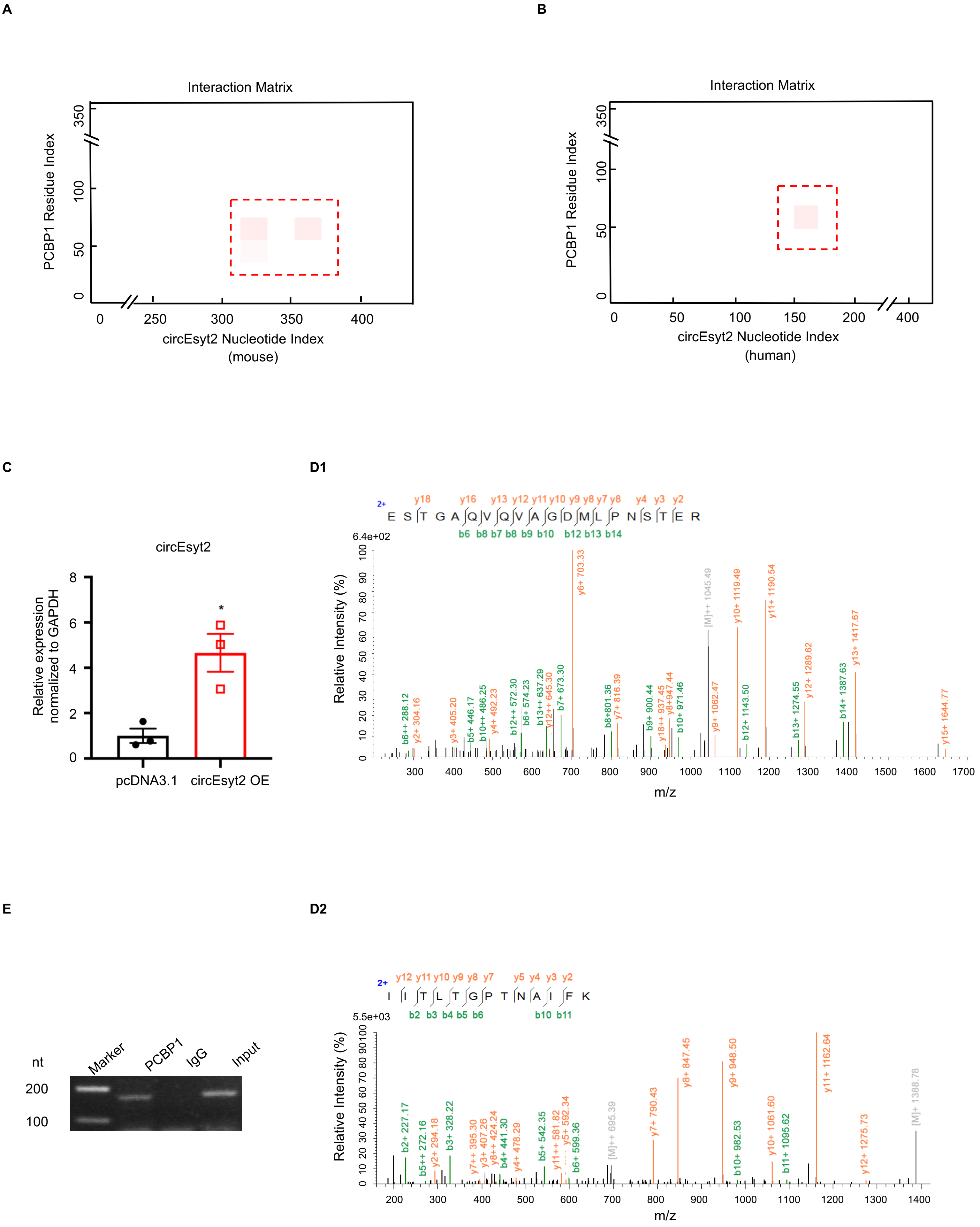

A
Interaction Matrix
PCBP1 Residue Index
350
100
50
0
0
250
300
350
400
circEsyt2 Nucleotide Index
(mouse)
B
Interaction Matrix
PCBP1 Residue Index
350
100
50
0
0
50
100
150
200
400
circEsyt2 Nucleotide Index
(human)
C
circEsyt2
Relative expression normalized to GAPDH
8
6
4
2
0
*
pcDNA3.1
circEsyt2 OE
D1
2+
y18 y16 y13 y12 y11 y10 y9 y8 y7 y8 y4 y3 y2
E S T G A Q V Q V A G D M L P N S T E R
b6 b8 b7 b8 b9 b10 b12 b13 b14
6.4e+02
Relative Intensity (%)
0
10
20
30
40
50
60
70
80
90
100
b6++ 288.12
y2+ 304.16
y3+ 405.20
b5+ 446.17
b10++ 486.25
y4+ 492.23
b12++ 572.30
b6+ 574.23
b13++ 637.29
y12++ 645.30
b7+ 673.30
y6+ 703.33
b8+801.36
y7+ 816.39
b9+ 900.44
y18++ 937.45
y8+947.44
b10+ 971.46
[M]++ 1045.49
y9+ 1062.47
y10+ 1119.49
b12+ 1143.50
y11+ 1190.54
b13+ 1274.55
y12+ 1289.62
b14+ 1387.63
y13+ 1417.67
y15+ 1644.77
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z
E
nt
Marker
PCBP1
IgG
Input
200
100
D2
2+
y12 y11 y10 y9 y8 y7 y5 y4 y3 y2
I I T L T G P T N A I F K
b2 b3 b4 b5 b6 b10 b11
5.5e+03
Relative Intensity (%)
0
10
20
30
40
50
60
70
80
90
100
b2+ 227.17
b5++ 272.16
y2+ 294.18
b3+ 328.22
y7++ 395.30
y3+ 407.26
y8++ 424.24
b4+ 441.30
y4+ 478.29
b5+ 542.35
y11++ 581.82
y5+ 592.34
b6+ 599.36
[M]++ 695.39
y7+ 790.43
y8+ 847.45
y9+ 948.50
b10+ 982.53
y10+ 1061.60
b11+ 1095.62
y11+ 1162.64
y12+ 1275.73
[M]+ 1388.78
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

Figure S6

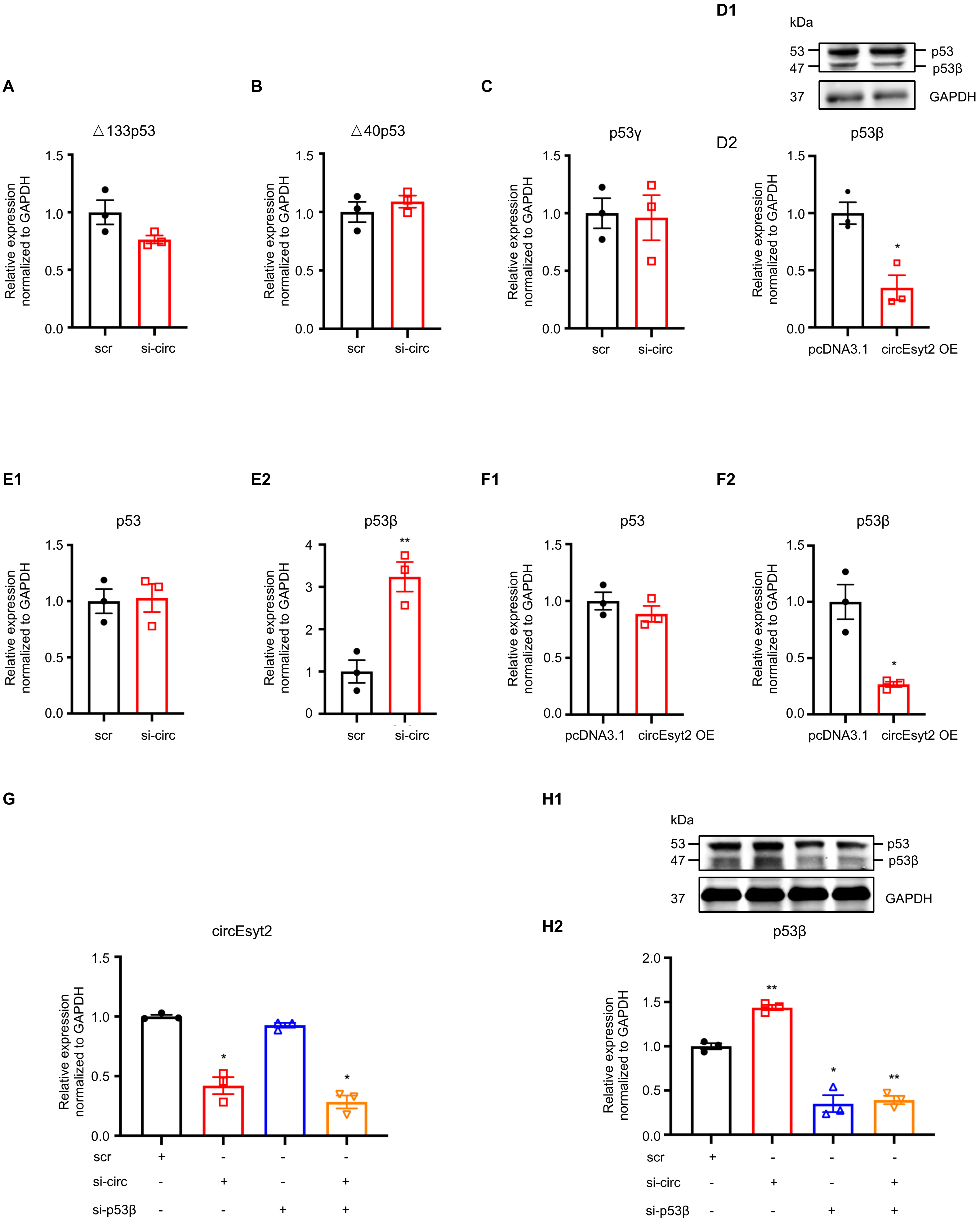
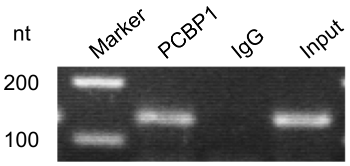
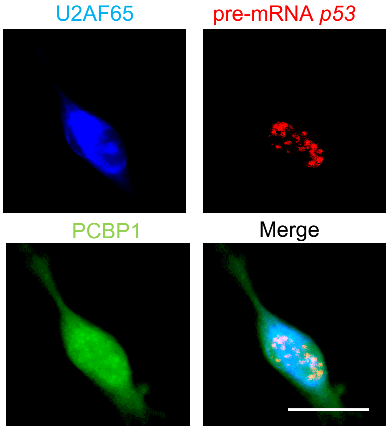

A
△133p53
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
scr
si-circ
B
△40p53
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
scr
si-circ
C
p53γ
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
scr
si-circ
D1
kDa
53
47
37
p53
p53β
GAPDH
D2
p53β
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
*
pcDNA3.1
circEsyt2 OE
E1
p53
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
scr
si-circ
E2
p53β
Relative expression normalized to GAPDH
4
3
2
1
0
**
scr
si-circ
F1
p53
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
pcDNA3.1
circEsyt2 OE
F2
p53β
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
*
pcDNA3.1
circEsyt2 OE
G
circEsyt2
Relative expression normalized to GAPDH
1.5
1.0
0.5
0.0
*
*
scr + - - -
si-circ - + - +
si-p53β - - + +
H1
kDa
53
47
37
p53
p53β
GAPDH
H2
p53β
Relative expression normalized to GAPDH
2.0
1.5
1.0
0.5
0.0
**
*
**
scr + - - -
si-circ - + - +
si-p53β - - + +

Figure S7

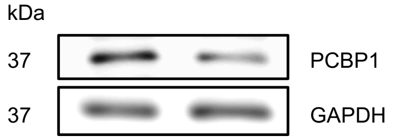
A



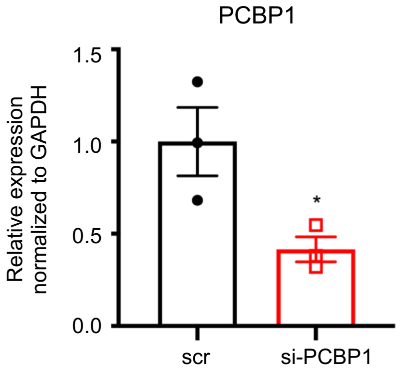
B



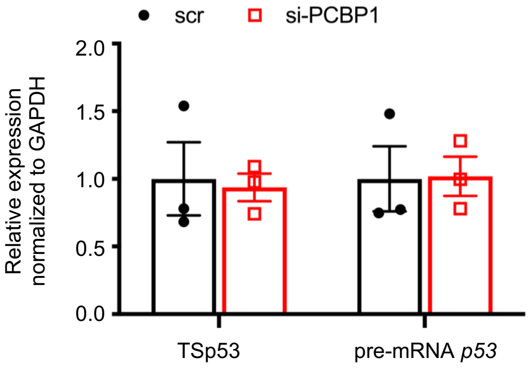
C1



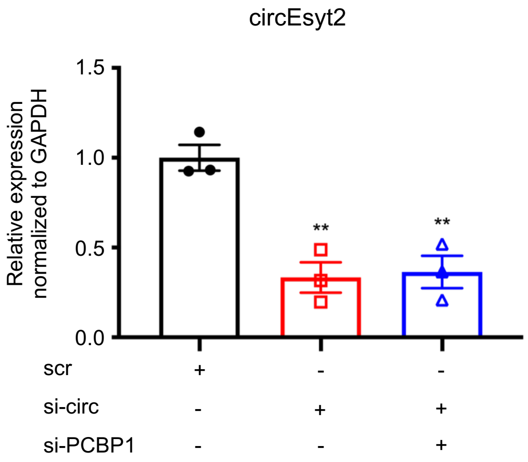
C2



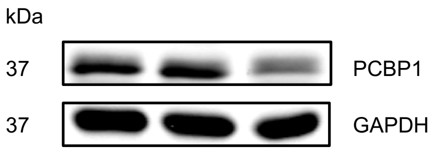
D



E



F1



F2

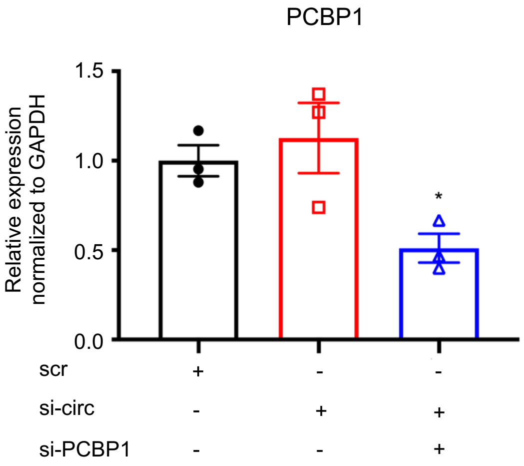


Figure S8

Supplemental figure legends

Figure S1. CircRNA profiling of murine aortae. A) Oil red O staining of atherosclerotic plaques on aortae from atherosclerotic (HFD + *ApoE*$^{-/-}$) and control groups (CD + *ApoE*$^{-/-}$ or CD + C57BL/6J). Negative control: CD + C57BL/6J. CD: chow diet. HFD: high fat diet. *ApoE*$^{-/-}$: *ApoE* knockout mice. **B**) Schema of workflow detailing candidate circRNA selection from sequencing data. RPM, Reads Per Million-mapped reads. **C**-**F**) Distribution of read abundance (C), genomic origin (D), length (E), and circRNAs in chromosomes (F). CDS, coding sequence; UTR, untranslated region. M, mitochondria. **G**) Landscape of circRNA expression. CircRNAs significantly upregulated and downregulated (Fold-change>2.0, $P<0.05$ and FDR value<0.05) are depicted as red and green dots, respectively. **H**) Venn diagram showing the number of circRNAs detected by different algorithms. **I**) Alignment of mouse and human circEsyt2 nucleotide sequences showing high homology (90%).

Figure S2. Characterization of circEsyt2 in mouse atherosclerotic aortae. Upper panel: bright field of frozen transverse sections from C57BL/6J (control) and cholesterol-loaded *ApoE*$^{-/-}$ (AS) mouse aortae. Bar, 100 μm. Lower panel: immunofluorescence staining for α-SMA, CD31, PDGFRα, or CD68, and fluorescence *in situ* hybridization (FISH) for circEsyt2 (red). Lu, lumen. Scale bar, 20 μm.

Figure S3. Characterization of circEsyt2 in coronary arteries of CAD patients. Upper panel: bright field of paraffin-embedded transverse sections of coronary arteries from CAD patients with mild stenosis (≤20%, mild CAD) and severe stenosis (≥70%, severe CAD). Bar, 200 µm. Lower panel: immunofluorescence staining for α-SMA, CD31, PDGFRα or CD68, and FISH for circEsyt2 (red). Scale bar, 50 µm.

Figure S4. Modulation of circEsyt2 expression in vivo and its effects on apoptosis. **A**) Efficacy of enhanced green fluorescent protein (EGFP)-tagged AAV2/8 silencing circEsyt2 determined by qRT-PCR in C57BL/6J aortic arteries 28 days post viral delivery. ****P*<0.001 vs. sh-con. n=4. **B**) Immunofluorescence staining of circEsyt2-silenced (sh-circ) and non-silenced (sh-con) carotid arteries for α-SMA (blue) and GFP (green), and FISH for circEsyt2 (red). Scale bar, 50 µm. **C**) Cell apoptosis detected by TUNEL assay of carotid arteries, treated as in (**A**). Lu, lumen. Scale bar 20 µm. **P*<0.05 vs. sh-con. n=3. **D**) Efficacy of ZsGreen-tagged AAV2/8 overexpressing circEsyt2 determined by qRT-PCR in C57BL/6J aortic arteries, 28 days post viral delivery. **P*<0.05 vs. control. n=4. **E**) Immunofluorescence staining of circEsyt2-overexpressed (circEsyt2 OE) and non-overexpressed (control) carotid arteries for α-SMA (blue) and GFP (green), and FISH for circEsyt2 (red). Scale bar, 50 µm. **F**) Cell apoptosis detected by TUNEL assay of carotid arteries, treated as in (**D**). Lu, lumen. Scale bar 20 µm. *P<0.05 vs. control. n=3. Data are presented as mean±SEM. Two-sided unpaired *t*-test for (**A**), (**C**), (**D**), and (**F**).

Figure S5. Effect of circEsyt2 silencing in mouse VSMCs. A-B) Efficacy of circEsyt2 (si-circ), *Esyt2* (si-mRNA) siRNA, or both (si-both), detected by qRT-PCR and western blotting in VSMCs. GAPDH, protein control. *P<0.05, **P<0.01 vs. scr. n=3. **C**) CCK-8 assay of VSMCs, treated as in (**A**) and (**B**) for the indicated hours. *P<0.05, **P<0.01 vs. scr. n=4. **D**) EdU incorporation assay of VSMCs, treated as in (**A**)-(**C**) for 48 h. Left panel: representative immunofluorescence of EdU (red) and DAPI (blue); Scale bar, 100 μm. Right panel: percentages of EdU-incorporated VSMCs. *P<0.05 vs. scr. n=3. **E-F**) Migratory ability assessed by wound healing (**E**) and Transwell assay (**F**) of circEsyt2-silenced VSMCs. Scale bar, 100 μm. *P<0.05, **P<0.01 vs. scr. n=3. **G**) Annexin-V-conjugated flow cytometry of circEsyt2-silenced VSMCs. *P<0.05 vs. scr. n=3. **H**) Western blotting to check for the expression of α-SMA, Calponin, and Myh11 in circEsyt2-silenced VSMCs. **P<0.01 vs. scr. n=3. Data are presented as mean±SEM. Two-sided unpaired *t*-test for (**E**)-(**H**). Two-way repeated measures ANOVA with LSD's post-hoc test for (**C**). One-way ANOVA test with Dunnett's T3 post-hoc test for (**A**), (**B**), and (**D**).

Figure S6. CircEsyt2 physically binds to PCBP1. A-B) Predicted interaction between mouse (**A**) and human (**B**) circEsyt2 and PCBP1 sequences determined using catRAPID. The binding sites are in red and highlighted with dashed boxes. **C**) Efficacy of circEsyt2 overexpression quantified by qRT-PCR in HEK293T cells. *P<0.05 vs. pcDNA3.1. n=3. **D**) Identification of the circEsyt2-binding protein PCBP1 by mass spectrometry of the proteins pulled down by the circEsyt2 probe. The representative peptides of PCBP1 are displayed as the MS/MS spectra: ESTGAQVQVAGDMLPNSTER (**D1**) and IITLTGPTNAIFK (**D2**). m/z indicates the ratio of mass to charge. E) RT-PCR of circEsyt2 pulled-down by PCBP1 antibody in HASMCs confirms the direct binding of circEsyt2 to PCBP1. Data are presented as mean±SEM. Two-sided unpaired t test for (**C**).

Figure S7. CircEsyt2 functions through regulation of p53β splicing. A-**C**) qRT-PCR of other major p53 isoforms, *△133p53* (**A**), *△40p53* (**B**) and *p53γ* (**C**) in circEsyt2-silenced HASMCs. n=3. **D**) Western blotting (**D1**) and qRT-PCR (**D2**) to detect the expression of p53β in circEsyt2-overexpressed (OE) HASMCs. **P*<0.05 vs. pcDNA3.1. n=3. **E**) Strand-specific reverse transcription-quantitative PCR (RT-qPCR) of *p53* (**E1**) and *p53β* (**E2**) in circEsyt2-silenced (si-circ) HASMCs. ***P*<0.01 vs. pcDNA3.1. n=3. **F**) Strand-specific RT-qPCR of *p53* (**F1**) and *p53β* (**F2**) in circEsyt2-overexpressed (OE) HASMCs. **P*<0.05 vs. pcDNA3.1. n=3. **G**-**H**) Efficacy of siRNA-mediated knockdown of circEsyt2 (si-circ), p53β (si-p53β), or both (si-circ+si-p53β) as measured by qRT-PCR (**G**) and western blotting (**H**) in HASMCs. **P*<0.05, ***P*<0.01 vs. scr. n=3. Data are presented as mean±SEM. Two-sided unpaired *t*-test for (**A**)-(**F1**). Two-sided unpaired *t*-test with Welch's correction for (**F2**). One-way ANOVA test with Dunnett's T3 post-hoc test for (**G**)-(**H**).

Figure S8. CircEsyt2 inhibits p53β splicing via inhibition of PCBP1. A) RT-PCR for pre-mRNA *p53* pulled down by PCBP1 antibody in HASMCs confirms the direct binding of pre-mRNA *p53* to PCBP1 protein. **B**) FISH for U2AF65 (blue), pre-mRNA *p53* (red), and PCBP1 (green) in HASMCs. Scale bar, 25 μm. **C**) Efficacy of silencing of PCBP1 in HASMCs, as quantified by western blotting (**C1**) and qRT-PCR (**C2**). **P*<0.05 vs. scr. n=3. **D**) qRT-PCR to check for the expression of total spliced p53 (TSp53) and precursor RNA p53 (pre-mRNA *p53*) in PCBP1-silenced HASMCs. n=3. **E**-**F**) Efficacy of siRNA-mediated knockdown of circEsyt2 (si-circ), PCBP1 (si-PCBP1), or both (si-circ+si-PCBP1), as quantified by qRT-PCR or western blotting. **P*<0.05, ***P*<0.01 vs. scr. n=3. Data are presented as mean±SEM. Two-sided unpaired *t*-test for (**C**) and (**G**). One-way ANOVA test with Dunnett's T3 post-hoc test for (**E**)-(**F**).

Table S1. Analysis of candidate circRNAs by other algorithms.

(CIRCexplorer)

Location	Host gene	control (RPM*)	AS (RPM*)	Fold-Change	p value	FDR
chr3:51308050-51326035	Elf2	7115.99	11793.93	1.66	1.10E-04	1.06E-02
chr5:3747021-3772787	Ankib1	410.23	642.395	1.57	2.18E-01	5.62E-01
chr12:116317830-116324210	Esyt2	0	2932.25	-	1.04E-12	8.00E-10
chr17:81647809-81649638	Slc8a1	134.92	612.46	4.54	1.15E-01	3.86E-01
chr18:6111685-6115850	Arhgap12	814.98	623.88	0.77	7.59E-01	9.17E-01

*: Reads Per Million mapped reads;

(CIRI2)

Location	Host gene	control (RPM*)	AS (RPM*)	Fold-Change	p value	FDR
chr3:51308050-51326035	Elf2	6295.69	9826.26	1.56	3.07E-05	2.46E-03
chr5:3747021-3772787	Ankib1	258.02	755.925	2.93	1.08E-02	1.59E-01
chr12:116317830-116324210	Esyt2	3489.58	4068.47	1.17	4.14E-02	2.91E-01
chr17:81647809-81649638	Slc8a1	0	405.24	-	1.81E-02	1.98E-01
chr18:6111685-6115850	Arhgap12	697.23	911.47	1.31	2.73E-01	6.03E-01

(CIRIquant)

Location	Host gene	control (RPM*)	AS (RPM*)	Fold-Change	p value	FDR
chr3:51308050-51326035	Elf2	4312.36	8522.03	1.98	7.57E-08	1.06E-05
chr5:3747021-3772787	Ankib1	131.92	677.03	5.13	2.30E-03	5.58E-02
chr12:116317830-116324210	Esyt2	1257.58	2116.70	1.68	5.35E-02	3.28E-01
chr17:81647809-81649638	Slc8a1	70.32	370.57	5.27	6.30E-02	3.35E-01
chr18:6111685-6115850	Arhgap12	545.11	800.815	1.47	2.18E-01	5.69E-01

Table S2. Top 5 upregulated circRNAs enriched in atherosclerotic plaques.

Location	Host gene	control (RPM*)	AS (RPM*)	Fold-Change	p value	FDR
chr3:51308050-51326035	Elf2	1.10	3.30	3.0	1.29E-14	3.88E-12
chr5:3747021-3772787	Ankib1	0.90	2.55	2.83	1.80E-10	2.71E-08
chr12:116317830-116324210	Esyt2	1.18	3.24	2.74	1.42E-12	3.22E-10
chr17:81647809-81649638	Slc8a1	1.40	3.23	2.30	1.18E-08	1.21E-06
chr18:6111685-6115850	Arhgap12	1.24	2.76	2.23	2.87E-07	2.25E-05

*Reads Per Million mapped reads

Table S3. Top 10 predicted circEsyt2-binding proteins.

Ranking	Protein	Z-score	Discriminative Power (%)	Interaction Strength (%)	Domain	Motif
1	PCBP family	-0.02	72	98	+	+
2	TRM1L	0.84	96	88	+	-
3	TDRD3	0.75	95	90	+	-
4	DDX1	0.71	95	89	+	-
5	RAVR1	0.63	93	88	+	-
6	MTER2	0.57	91	96	+	-
7	OAS2	0.52	90	83	+	-
8	RED2	0.5	90	83	+	-
9	PAPOA	0.48	89	80	+	-
10	QKI	0.44	87	93	+	-

Table S4. Oligonucleotides used in this study.

siRNAs used for gene silencing(5'-3')	
mouse and human: circEsyt2 (si-circ)	CCTTAGGAAACCAGTTCAT
mouse: Esyt2 (si-mRNA)	CAAGCATTGCCTCAGACAT
mouse: circEsyt2 and Esyt2 (si-both)	TCAATGGTGTTAAGGTTTA
human: Esyt2 (si-mRNA)	GGACAGGACTGACGAATCT
human: circEsyt2 and Esyt2 (si-both)	CTTCTTCCTTAGGAAACCA
human: PCBP1	GGAGGAAGATATCAACAGC
human: p53β	TCCTGATAAACTCGTCGTA
shRNA used for circEsyt2 knockdown in vivo	
sh-circEsyt2 (sh-circ)	**Top strand:** AATTCGTCCTTAGGAAACCAGTTCATTTTCC ttcaagaga GGAAAATGAACTGGTTTCCTAAGGAtttttg **Bottom strand:** GATCCAAAAAATCCTTAGGAAACCAGTTCA TTTTCCTctcttgaaGGAAAATGAACTGGTTTCC TAAGGACg
biotinylated RNA probes used for RNA pulldown (5’-3’)	
control probe	CAAGGCATTATGGTAGCTAG
circEsyt2 probe	TCTGGAAAATGAACTGGTTT
RNA-FISH probes (5’-3’)	
pre-mRNA p53 probe (Cy3 labelled)	5’CCCACUUAAUGUGUGAUCUCUGACUCCUGUCCCAAAGUUG-3’
circEsyt2 probe (5’-biotin labelled)	5’-TTTCAGTGTCTGGAAAATGAACTGG-3’; 5’-AGTGTCTGGAAAATGAACTGGTTTC-3’; 5’-AAATGAACTGGTTTCCTAAGGAAGA-3’

Table S5. Primers used in this study.

Primers for PCR and real-time PCR		
Genes	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
circEsyt2-divergent (circEsyt2, for mouse and human)	TTTTGTAGAGCTGGTGTGAAAAGTATT	GTGCTTTACAGTCTTATTTAGCCATTC
circEsyt2-convergent (Esyt2, for mouse)	CCCCTGAGAGTCAATGGTGT	TCCAGTATCACCCGCATTGT
circAnkib1-divergent (for mouse)	GGATCTTCGTAGGTTAAAAGATATGCT	CCATGTTTTGTTATTTCTGATAGGCA
circAnkib1-convergent (for mouse)	CCTATCAGCACAACACACCG	AATCATCTTCCACAGGCCGT
circFxr2-divergent (for mouse)	GCAATCCTTACAGCCTCTTGG	CCCTCACCACACCAGACTTAT
circFxr2-convergent (for mouse)	TCCACAGCTTCAGAGACAGAG	CCAAGAGGCTGTAAGGATTGC
circArhgap12-divergent (for mouse)	CCAGAGTTCTTGGACATAGAGAAAA	ATAACACTTGGAAGGCTGTAAACAT
circArhgap12-convergent (for mouse)	TCAGGCCAGACTCTCAACCT	GCCTTCTCCAGCTGAGTCAC
circSlc8a1-divergent (for mouse)	GAGGGGAAGACTTTGAGGACA	TTTCCTCCTGTTTCTGCCTCT
circSlc8a1-convergent (for mouse)	TGTGATCTTCAAACCAGGGGA	GGTGACCCAAGACAAGCAATT
circDlc1-divergent (for mouse)	CCTTCTTCAGCCTCCTTCCT	TTGTCCACTTTCTCTTGCGC
circDlc1-convergent (for mouse)	CCAGCAGAAGGAAGTGGAGA	GCAGTTGTGTCTCAGGTGTG
circRreb1-divergent (for mouse)	GTATCTGTGGGAAGTCGCTGAG	CTCCTTTATGCTGTTTTCTTCGT
circRreb1-convergent (for mouse)	GAAAAGCCCCTGTCTCCTCT	CGGGGAAGTCACTGTACACT
circInsr-divergent (for mouse)	AACGAGGAATGTGGGGATGT	TTCGGGTCTGGTCTTGAACA
circInsr-convergent (for mouse)	TTCCGAGACCTCAGTTTCCC	TTCTTCTCGATGCGGACAGA
circAdcy5-divergent (for mouse)	TGCTGAGTTTCTACCTGTCGT	CGTAAACAGTGATTCTCCGCA
circAdcy5-convergent (for mouse)	CCAACTCCATCGGACACAATC	TCCACTTCATCCTCTGGGTTC
circElf2-divergent (for mouse)	ACAGGAAGTTGAGACGGAGA	GCAACGGAACTAAGGCTCAC
circElf2-convergent (for mouse)	GTGAGCCTTAGTTCCGTTGC	CTCTAACCTCGCACTGGGAA

snoR41 (for mouse)	TGGTCTACAGCTGTCTTATGGT	TCACACATAATCCTCCTCCTGT
Ccnd1 (for mouse)	AGAAGTGCGAAGAGGAGGTC	TTCTCGGCAGTCAAGGGAAT
p21 (for mouse)	TCCCGACTCTTGACATTGCT	TGCAGAAGGGGAAGTATGGG
Noxa (for mouse)	TCGCAAAAGAGCAGGATGAG	CACTTTGTCTCCAATCCTCCG
PUMA (for mouse)	GTACGAGCGGCGGAGACAAG	GCACCTAGTTGGGCTCCATTTCTG
Esyt2 (for human)	GGGCAAAGACACCTACCTTAA	TCTTGTCCAGGGTGTTCATACA
p53 (full length) (for human)	CAGCCAAGTCTGTGACTTGCA	GTGTGGAATCAACCCACAGCT
p53β (for human)	GAGCACTAAGCGAGCACTGCC	TTGAAAGCTGGTCTGGTCCTGA
△133p53 (for human)	ACTCTGTCTCCTTCCTCTTCCTACAG	GTGTGGAATCAACCCACAGCT
△40p53 (for human)	TGAGTGGATCCATTGGAAGG	GTCTGAAAGACAAGAGCAGAAAG
p53γ (for human)	AACCACTGGATGGAGAATATTTCAC	TCAACTTACGACGAGTTTATCAGGAA
TSp53 (for human)	TCACCATCATCACACTGGAAGAC	CACGCACCTCAAAGCTGTTC
pre-mRNA p53 (for human)	CACCTTTCCTTGCCTCTTTCC	CCACTTGATAAGAGGTCCCAAGAC
PCBP1 (for human)	CCTACTCGATTCAAGGACAACAC	GAGTTCATGGGTGGTTTGAGTAG
GAPDH (for mouse and human)	AACGACCCCTTCATTGACCT	TGGAAGATGGTGATGGGCTT

Primers for shRNA plasmid construction

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
circEsyt2	CTCACCTCAGCACCTTCA	AGTATCACCCGCATTGTC
GAPDH	TCAAGGCTGAGAACGGGAAG	TCGCCCCACTTGATTTTGGA

Primers for strand-specific reverse transcription-quantitative PCR

(for RT)

p53	TTTTTGAAAGCTGGTCTGGT
p53β	AGCTCTCGGAACATCTCGAA
GAPDH	GCTAAGCAGTTGGTGGTGCAG

(for qPCR)

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
p53/ p53β	GTCCAGATGAAGCTCCCAGA	GGGACAGAAGATGACAGGGG
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Table S6. Clinical characteristics of coronary artery donors.

Patient Number	Sex	Age	Maximum stenosis	Group	Smoking history	Hypertension	Diabetes mellitus
1	Male	68	22%	Mild CAD*	Yes	None	None
2	Male	63	13%	Mild CAD	Yes	None	None
3	Female	65	10%	Mild CAD	None	None	None
4	Female	75	77%	Severe CAD	None	None	None
5	Male	66	82%	Severe CAD	Yes	None	None
6	Male	70	85%	Severe CAD	Yes	None	None

*CAD: coronary artery disease