SUPPLEMENTAL MATERIAL

Supplemental Figure Legends:

Figure S1. Validation of *REEP5* knockout in rats. **A,** A schematic overview of the CRISPR/Cas9-based genome-editing strategy used to generate the *REEP5* knockout (KO). Bottom, Example chromatogram showing a microdeletion and representative sequences of mutated alleles identified from clonal amplicons. Yellow dashes indicate the deleted bases. **B,** Western blot analysis of REEP5 expression in the hearts of rats carrying homozygous 8-bp deletion mutations. *Left*, representative blots; *right*, pooled data. WT: n=6; *REEP5* KO: n=6. $P < 0.05$ compared with WT. **C,** Analysis of the effects of CRISPR/Cas9-mediated *REEP5* gene editing on the mRNA expression of *REEPs* other than *REEP5*. n=3 for each group. $P < 0.05$

Figure S2. REEP5 deficiency does not affect the expression of SR Ca^{2+}-handling proteins. Analysis of critical Ca^{2+}-handling proteins on the SR. *Left*, typical blots; *Right*, pooled data from three separate experiments.

Figure S3. Measurement of REEP5 protein during the development of heart failure. **A,** Echocardiographic analysis of cardiac function during transverse aortic constriction (TAC) -induced heart failure, as demonstrated by ejection fraction. **B,** Western blotting examination of REEP5 protein in the heart tissues. *Top*, typical blots; *bottom*, pooled data. Three to five

animals were analyzed at indicated time points in WT and REEP5-KO rats.

Figure S1.

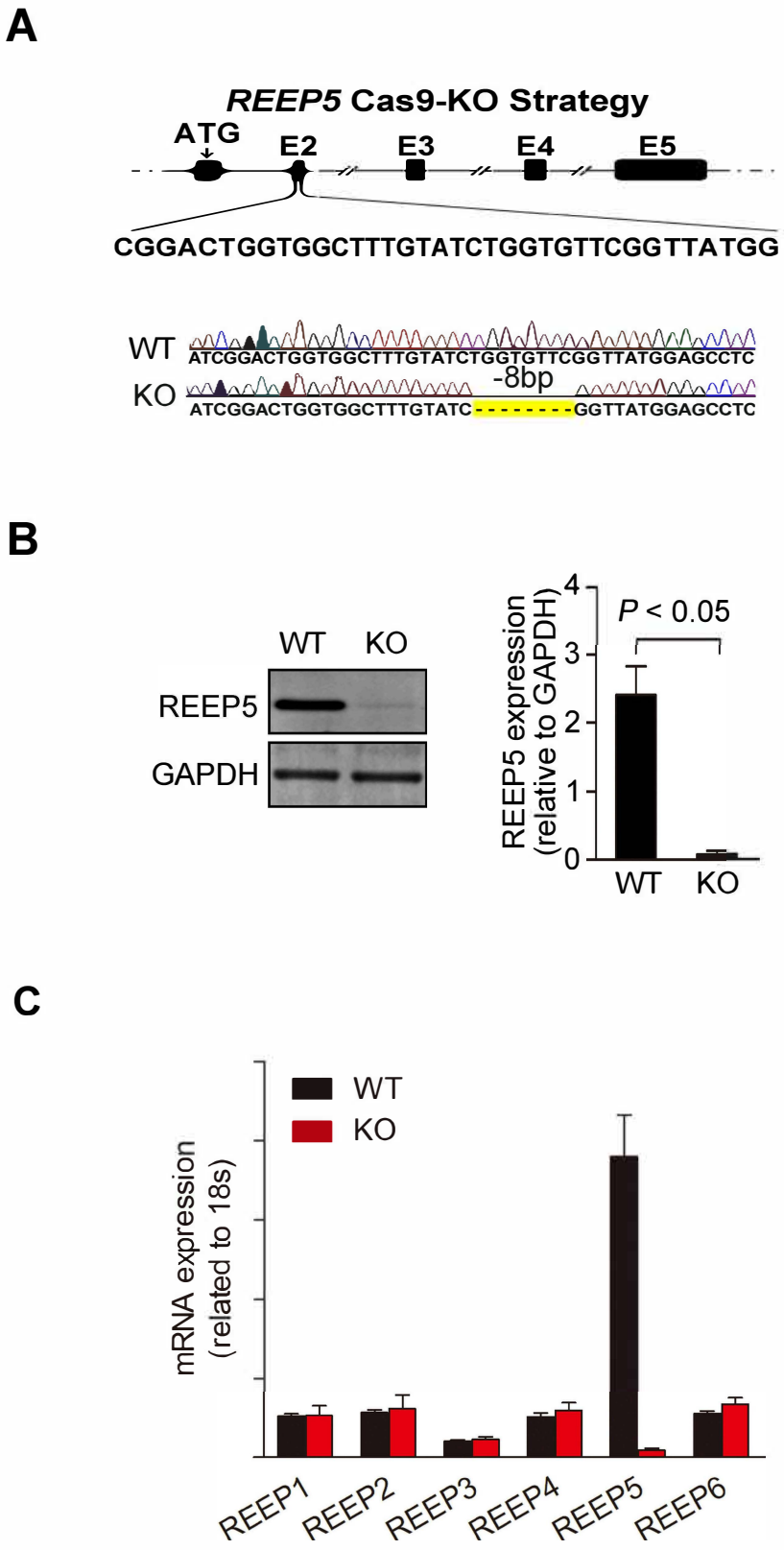

A
REEP5 Cas9-KO Strategy
ATG
E2
E3
E4
E5
CGGACTGGTGGCTTTGTATCTGGTGTTCGGTTATGG
WT
ATCGGACTGGTGGCTTTGTATCTGGTGTTCGGTTATGGAGCCTC
KO
-8bp
ATCGGACTGGTGGCTTTGTATC-------GGTTATGGAGCCTC
B
WT
KO
REEP5
GAPDH
REEP5 expression (relative to GAPDH)
P < 0.05
0
1
2
3
4
WT
KO
C
mRNA expression (related to 18s)
WT
KO
REEP1
REEP2
REEP3
REEP4
REEP5
REEP6

Figure S2.

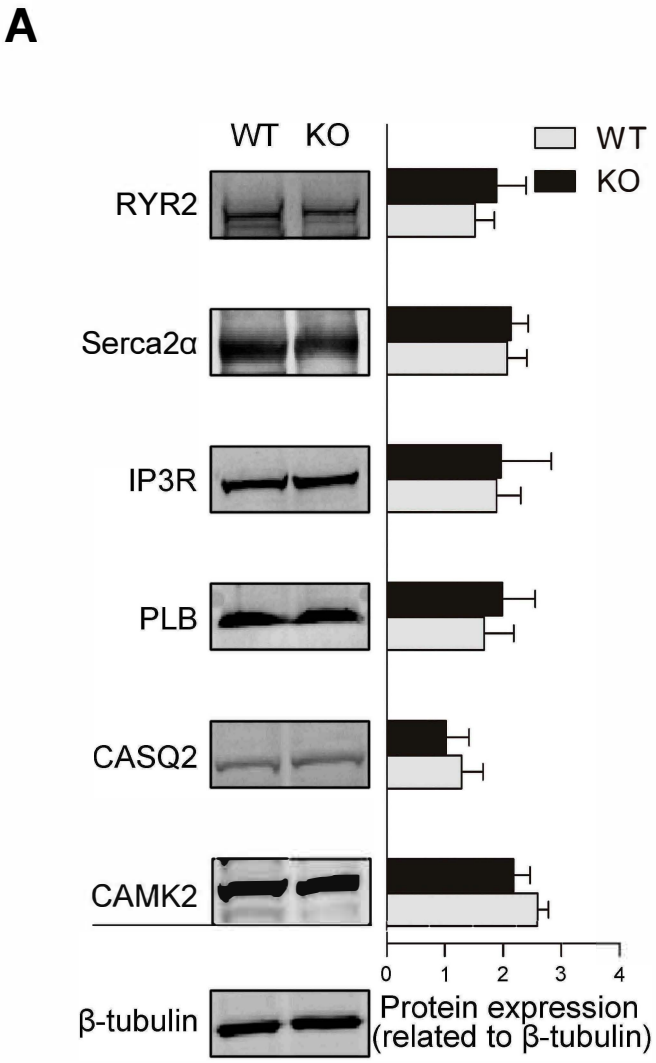
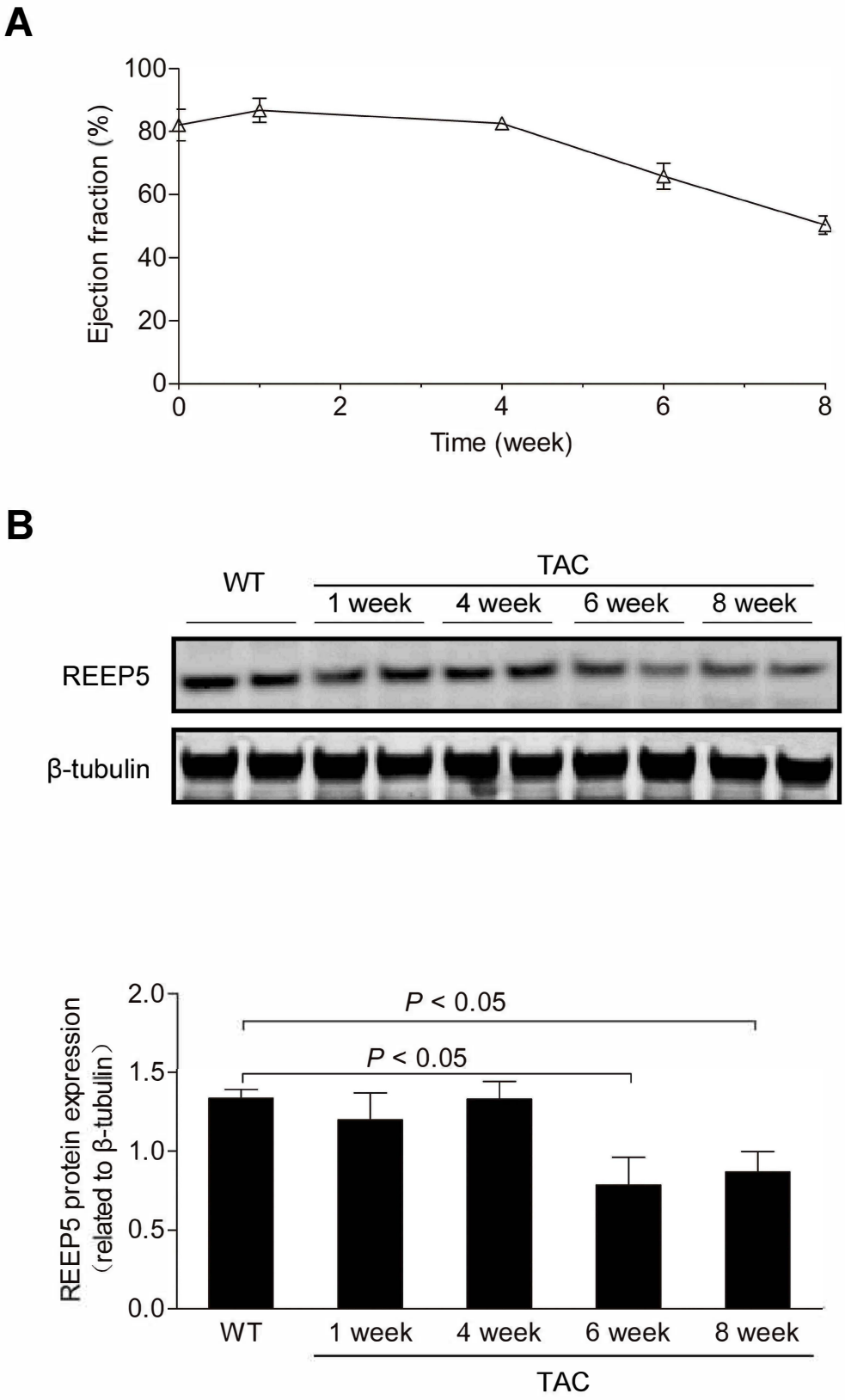

A
WT
KO
WT
KO
RYR2
Serca2α
IP3R
PLB
CASQ2
CAMK2
β-tubulin
0
1
2
3
4
Protein expression
(related to β-tubulin)

Figure S3


A
100
80
60
40
20
0
Ejection fraction (%)
0
2
4
6
8
Time (week)
B
WT
TAC
1 week
4 week
6 week
8 week
REEP5
β-tubulin
2.0
1.5
1.0
0.5
0.0
REEP5 protein expression
(related to β-tubulin)
P < 0.05
P < 0.05
WT
1 week
4 week
6 week
8 week
TAC