Supplementary Figure legends

Supplementary Figure 1. ACE2 expression and blood pressure measurements.

A, Western blot for ACE2 in the hearts of *Ace2*$^{-/y}$ and wild type mice to demonstrate the specificity of anti-ACE2 antibody used in our study. **B-D**, Real-time RT-PCR for baseline mRNA expression of ACE2 (**B**), ACE (**C**) and Renin (**D**) in the indicated organs harvested from wild type and *Apelin*$^{-/y}$mice (n = 4-8 per group). **E-G**, Blood pressure measurements for non-treated wild type, *Apelin*$^{-/y}$, and *Ace2*$^{-/y}$ mice. Systolic (**E**), diastolic (**F**) and mean (**G**) blood pressures were measured by tail-cuff methods (n = 4 per group). **H**, mRNA expression of prolylendopeptidase (Prep), prolylcarboxypeptidase (Prcp), thimet oligopeptidase (Thop1), and neprilysin (Mme) in *Apelin*$^{-/y}$ mouse hearts. Expression was determined by Real-time PCR. All values are means ± SEM. ns; not significant, N.D.; not detectable. † $P < 0.1$.

Supplementary Figure 2. Heart function and blood pressure measurements in AT1R and Apelin double knockout mice.

A, Genotyping PCR and RT-PCRs for Apelin and AT1R genes in *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ mice. In genotyping PCR, *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ mice show only knockout (KO) allele bands for both AT1R and Apelin, and mRNA expression of both AT1R and Apelin are lost in the hearts of *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ mice as shown by RT-PCR. **B-C**, Echocardiography of *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ double knockout rescue of contractility defects in aged *Apelin*$^{-/y}$ mice (12 month old). Representative images of M-mode echocardiography (**B**) and measurements of %Fractional shortening (**C**) (n = 6-7 per group). **D**, Heart weight to body weight ratio. **E-F**, Real-time PCR analysis for *BNP* and *βMHC* mRNA expression in the hearts (n = 7-10 per group). *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ double knockout down-regulates the increased *BNP* and *βMHC* gene expression in aged *Apelin*$^{-/y}$ mice to that in wild type mice. **G-I**, Blood pressure measurements for wild type, *Apelin*$^{-/y}$, *Agtr1a*$^{-/-}$ and *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ mice. Systolic (**G**), diastolic (**H**) and mean (**I**) blood pressures are shown (n = 4-6 per group). All values are means ±

SEM. † $P < 0.1$, *$P < 0.05$, **$P < 0.01$.

Supplementary Figure 3. Histology and gene expression analyses of AT1R and Apelin double knockout mice under TAC.

A, Macroscopic heart images (top panels) and H&E staining (bottom panels) of the *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ double knockout mouse hearts with sham or TAC. Bars indicate 3 mm. **B**, Reduced fibrosis in *Agtr1a*$^{-/-}$*;Apelin*$^{-/y}$ double knockout mouse hearts compared to *Apelin*$^{-/y}$ mice. Histology of hearts stained with Masson-Trichrome (left) and measurements of fibrosis area (right). **C-F**, Real-time PCR analysis for heart failure gene expressions in the hearts; *BNP* (**C**)*, βMHC* (**D**), *ANF* (**E**)*,* and *Tgfb2* (**F**). All values are means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Supplementary Figure 4. ACE2 expression and Angiotensin peptide levels in AT1R knockout mice.

A, Western Blot for ACE2 in wild type and *Agtr1a*$^{-/-}$ mouse hearts. ACE2 expression is up-regulated in *Agtr1a*$^{-/-}$ mice compared to wild type mice. **B**, ACE2 activity in the plasma of *Agtr1a*$^{-/-}$ mice. Hydrolysis rates of the fluorogenic ACE2 substrate Mca-APK(Dnp) in the plasma of *Agtr1a*$^{-/-}$ and wild type mice were measured at baseline. The background hydrolysis rates in the plasma of *Ace2*$^{-/y}$ mice are shown as a dashed line. n = 5-8 per group. All values are means ± SEM. *$P < 0.05$, **$P < 0.01$.

Supplementary Figure 5. Blood pressure and heart morphologies of Ang 1-7-treated Apelin knockout mice under TAC.

A-C, Blood pressure measurements for wild type and *Apelin*$^{-/y}$ mice under TAC treated with Ang 1-7 or vehicle. Systolic (**A**), diastolic (**B**) and mean (**C**) blood pressures are shown (n = 4-5 per group). **D**, Macroscopic heart images of wild type or *Apelin*$^{-/y}$ mouse hearts with sham or TAC treated with Ang

1-7 or vehicle. Bars indicate 3 mm. All values are means ± SEM. $^*P < 0.05$.

Supplementary Figure 6. Apelin-12-induced ACE2 expression *in vivo* and Apelin-13-induced ACE2 promoter activity.

A, Western Blot for ACE2 in Apelin-12 or vehicle-treated wild type mouse hearts. **B**, Echocardiography for Apelin-12 or vehicle-infused wild type mice. %Fractional shortening is elevated in Apelin-12 treated mice compared with vehicle-treated mice. n = 4 per group. **C**, Immunohistochemistry for ACE2 in the frozen heart sections of wild type and *Apelin*$^{-/y}$ mice. ACE2 expression was observed mainly in cardiomyocytes and also less but significantly detected in coronary artery endothelial cells. Blood vessel is pointed by white arrow heads in top panels, and the vascular lumen is marked with white dashed line in the enlarged image in bottom panels. Note that in *Apelin*$^{-/y}$ mouse hearts, ACE2 expression was down-regulated in both cardiomyocytes and endothelial cells. **D**, ACE2 promoter reporter assay with the luciferase reporter plasmids ACE2 (ī 1119/+103)-luc, ACE2 (ī 252/+103)-luc or ACE2 (ī 202/+103)-luc plasmids in combination with APJ expression vector and 1 μM Apelin-13 peptide (Apelin) in HEK293T cells. **E**, Dose-dependency of Apelin on ACE2 promoter activation in HEK293T cells. **F**, Effects of AT1R expression on Apelin-induced activation of ACE2 promoter (ī 252/+103) in Vero E6 cells. Despite antagonistic effect of AT1R, Apelin induces ACE2 promoter activity. All values are means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$.

Supplementary Figure 7. Blood pressure and heart morphologies of Apelin-13-treated AT1R knockout mice.

A-C, Blood pressure measurements for wild type and *Agtr1a*$^{-/-}$ mice under TAC treated with Apelin-13 or vehicle. Systolic (**A**), diastolic (**B**) and mean (**C**) blood pressures are shown. n = 4-5 per group. **D**, Macroscopic appearances of wild type or *Agtr1a*$^{-/-}$ mouse hearts following sham or TAC surgery and administration of Apelin-13 or vehicle. Bars indicate 3 mm. All values are means ±

SEM. ns; not significant, *$P < 0.05$, **$P < 0.01$.

Supplementary Table 1. Echocardiography data of Apelin knockout mice treated with AT1 receptor blocker.

	Wild type + Vehicle	*Apelin*$^{-/y}$ + Vehicle	Wild type +ARB	*Apelin* $^{-/y}$+ ARB
n	3	3	3	4
age (month)	12	12	12	12
HR (bpm)	482.42 ± 27.51	487.42 ± 43.96	516.22 ± 56.47	518.17 ± 19.61
FS (%)	40.75 ± 2	32.62 ± 2.12[A]	39.85 ± 1.99	38.65 ± 0.81[B]
EF (%)	71.45 ± 2.17	60.53 ± 2.92	70.71 ± 2.63	69.34 ± 1.24[B]
LVESD (mm)	2.66 ± 0.27	2.88 ± 0.22	2.55 ± 0.22	2.46 ± 0.33
LVEDD (mm)	4.44 ± 0.4	4.37 ± 0.13	4.23 ± 0.21	4.12 ± 0.44
IVS (mm)	0.76 ± 0.03	0.78 ± 0.03	0.77 ± 0.05	0.77 ± 0.12
PWT (mm)	0.82 ± 0.08	0.93 ± 0.09	0.82 ± 0.2	0.81 ± 0.07

Wild type and *Apelin* $^{-/y}$ mice were treated with AT1 receptor blocker (ARB), Losartan in drinking water for 6 weeks. Values were means ±SEM. Abbreviations; *n*, number of mice per group; HR, heart rate; FS, fractional shortening; EF, ejection fraction; LVESD, left ventricular end systolic diameter; LVEDD, left ventricular end diastolic diameter; IVS, interventricular septum; PWT, posterior wall thickness. [A] $P < 0.05$ compared with control wild type mice. [B] $P < 0.05$ compared to vehicle treated *Apelin*$^{-/y}$ mice.

Supplementary Table 2. Echocardiography data of aged TAC-operated AT1R Apelin double knockout mice

	Wild type + sham	*Apelin*$^{-/y}$ + sham	*Agtr1a*$^{-/-}$ + sham	*Agtr1a*$^{-/-}$;*Apelin*$^{-/y}$ + sham
n	10	8	7	7
age (month)	12	12	12	12
HR (bpm)	499.62 ± 26.90	509.29 ± 45.03	511.62 ± 31.49	519.66 ± 31.73
FS (%)	39.20 ± 2.86	31.43 ± 4.32 ^{A}	38.03 ± 2.10	37.61 ± 4.10
EF (%)	69.69 ± 3.35	59.32 ± 5.90 ^{AA}	68.08 ± 2.87	67.45 ± 5.02
LVESD (mm)	2.64 ± 0.16	2.89 ± 0.29	2.78 ± 0.22	2.77 ± 0.18
LVEDD (mm)	4.32 ± 0.20	4.24 ± 0.30	4.47 ± 0.29	4.36 ± 0.10
IVS (mm)	0.78 ± 0.08	0.81 ± 0.06	0.78 ± 0.09	0.74 ± 0.08
PWT (mm)	0.86 ± 0.12	0.82 ± 0.04	0.82 ± 0.19	0.76 ± 0.05
	Wild type + TAC	***Apelin*$^{-/y}$ + TAC**	***Agtr1a*$^{-/-}$ + TAC**	***Agtr1a*$^{-/-}$;*Apelin*$^{-/y}$ + TAC**
n	14	14	11	5
age (month)	12	12	12	11
HR (bpm)	502.05 ± 45.36	527.22 ± 41.20	501.55 ± 24.39	532.83 ± 33.00
FS (%)	25.75 ± 2.16 ^{AA}	21.53 ± 4.50 ^{BB}	31.44 ± 2.54 ^{BB}	30.13 ± 2.66 ^{CC}
EF (%)	50.17 ± 3.47 ^{AA}	43.01 ± 8.24 ^{BB}	58.87 ± 3.99 ^{BB}	57.29 ± 3.89 ^{CC}
LVESD (mm)	3.61 ± 0.43 ^{AA}	4.01 ± 0.79 ^{B}	3.21 ± 0.54	3.11 ± 0.29 ^{C}
LVEDD (mm)	4.86 ± 0.51 ^{AA}	5.06 ± 0.70	4.64 ± 0.56	4.44 ± 0.33
IVS (mm)	0.97 ± 0.09 ^{AA}	0.95 ± 0.06	0.84 ± 0.08 ^{BB}	0.82 ± 0.07
PWT (mm)	1.02 ± 0.08 ^{AA}	0.97 ± 0.07	0.91 ± 0.07	0.79 ± 0.10

Echocardiography data of Wild type, *Apelin*$^{-/y}$, *Agtr1a*$^{-/-}$, and *Agtr1a*$^{-/-}$; *Apelin*$^{-/y}$ mice 8 weeks after sham or TAC operation. Values were means ±SEM. See Supplementary Table 1 for abbreviations. ^{A} $P < 0.05$, ^{AA} $P < 0.01$ compared with wild type mice with sham. ^{B} $P < 0.05$ and ^{BB} $P < 0.01$ compared with TAC-operated wild type mice. ^{C} $P < 0.05$ and ^{CC} $P < 0.01$ compared with *Apelin*$^{-/y}$ mice with TAC.

Supplementary Table 3. Echocardiography data of TAC-operated AT1R Apelin double knockout mice at the age of 6 months.

	Wild Type + sham	**Wild Type + TAC**	***Apelin$^{-/y}$* + TAC**	***Agtr1a$^{-/-}$* + TAC**	***Agtr1a$^{-/-}$; Apelin$^{-/y}$* + TAC**
n	4	5	9	5	5
age (month)	6	6	6	6	6
HR (bpm)	622.96 ± 25.68	572.55 ± 78.64	606.28 ± 60.82	532.82 ± 28.29	561.55 ± 47.93
FS (%)	41.46 ± 2.98	40.40 ± 6.77	32.50 ± 6.42^{B}	39.36 ± 3.52	44.53 ± 3.45^{CC}
EF (%)	72.87 ± 3.80	69.36 ± 8.54	60.78 ± 9.20^{B}	70.06 ± 4.48	78.30 ± 3.35^{CC}
LVESD (mm)	2.20 ± 0.27	2.40 ± 0.36	2.78 ± 0.41^{B}	2.55 ± 0.23	2.30 ± 0.28^{C}
LVEDD (mm)	3.75 ± 0.28	4.13 ± 0.38	4.15 ± 0.48	4.13 ± 0.32	3.96 ± 0.17
IVS (mm)	0.76 ± 0.09	0.94 ± 0.09^{A}	1.06 ± 0.15	1.01 ± 0.09	0.96 ± 0.03
PWT (mm)	0.70 ± 0.04	0.85 ± 0.04^{AA}	1.02 ± 0.14	1.04 ± 0.12	0.98 ± 0.09

Echocardiography data of Wild type, *Apelin* $^{-/y}$, *Agtr1a*$^{-/-}$, and *Agtr1a*$^{-/-}$; *Apelin*$^{-/y}$ mice at the age of 6 months 2weeks after sham or TAC operation. Values were means ±SEM. See Supplementary Table 1 for abbreviations. ^{A} $P < 0.05$, ^{AA} $P < 0.01$ compared with wild type mice with sham. ^{B} $P < 0.05$ compared with TAC-operated wild type mice. ^{C} $P < 0.05$ and ^{CC} $P < 0.01$ compared with *Apelin*$^{-/y}$ mice with TAC.

Supplementary Table 4. Echocardiography data of TAC-operated Apelin knockout mice with or without Angiotensin 1-7

	Wild type + sham	Wild type + TAC + Vehicle	*Apelin*$^{-/y}$ + TAC + Vehicle	Wild type + TAC + Ang1-7	*Apelin*$^{-/y}$ + TAC + Ang1-7
n	5	5	5	3	4
age (month)	6	6	6	6	6
HR (bpm)	570.05 ± 31.60	579.26 ± 32.85	582.17 ± 18.82	550.17 ± 17.06	543.09 ± 29.98
FS (%)	47.57 ± 2.02	44.70 ± 3.92	25.19 ± 3.15^{BB}	42.01 ± 6.85	40.69 ± 3.47 ^{CC}
EF (%)	79.42 ± 2.15	76.03 ± 4.41	49.43 ± 5.03^{BB}	72.34 ± 7.69	71.26 ± 4.49 ^{CC}
LVESD (mm)	2.08 ± 0.14	2.19 ± 0.17	3.45 ± 0.41^{B}	2.62 ± 0.43	2.47 ± 0.30 ^{C}
LVEDD (mm)	3.97 ± 0.21	3.91 ± 0.16	4.67 ± 0.31^{B}	4.49 ± 0.24	4.14 ± 0.24
IVS (mm)	0.64 ± 0.03	0.86 ± 0.05 ^{AA}	1.12 ± 0.09^{BB}	0.94 ± 0.10	0.81 ± 0.06 ^{C}
PWT (mm)	0.65 ± 0.09	0.86 ± 0.07 ^{A}	1.03 ± 0.10	0.96 ± 0.12	0.80 ± 0.07 ^{C}

Values were means ±SEM. See Supplementary Table 2 for abbreviations. . ^{A} $P < 0.05$, ^{AA} $P < 0.01$ compared with wild type mice with sham. ^{B} $P < 0.05$ and ^{BB} $P < 0.01$ compared with TAC-operated vehicle-treated wild type mice. ^{C} $P < 0.05$ and ^{CC} $P < 0.01$ compared with *Apelin*$^{-/y}$ mice with TAC plus vehicle treatment.

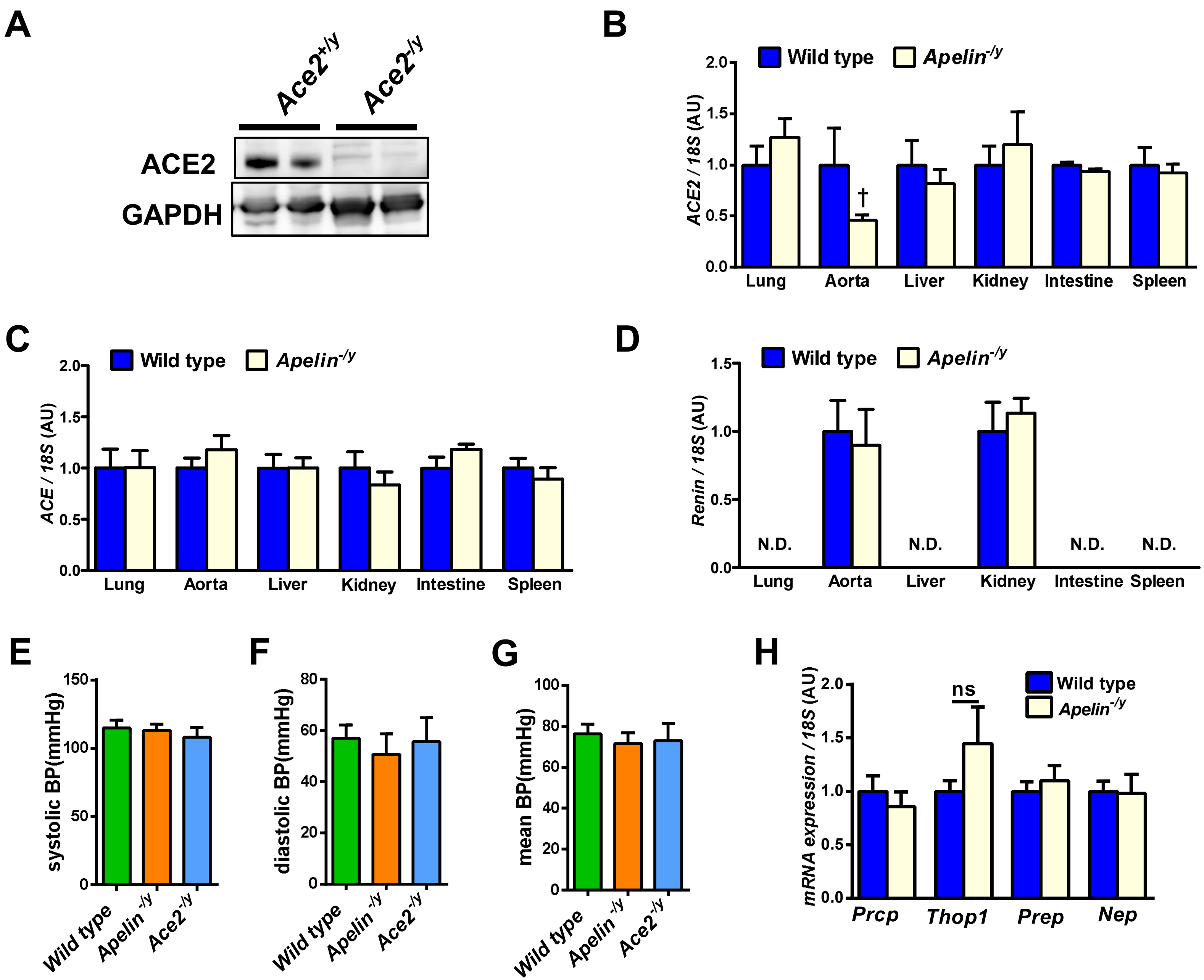
Supplementary Table 5. Echocardiography of TAC-operated AT1R knockout mice with or without Apelin-13

	Wild type + sham	Wild type + TAC + Vehicle	*Agtr1a*$^{-/-}$ + TAC + Vehicle	Wild type + TAC + Apelin	*Agtr1a*$^{-/-}$ + TAC + Apelin
n	4	3	5	5	5
age (month)	6	6	6	5	6
HR (bpm)	622.96 ± 25.68	577.72 ± 8.92	514.25 ± 28.58	630.58 ± 16.36	595.58 ± 36.09
FS (%)	41.46 ± 2.98	39.14 ± 1.33	36.58 ± 2.08	45.48 ± 2.92 ^{B}	43.72 ± 2.35^{C}
EF (%)	72.87 ± 3.80	70.25 ± 1.65	76.70 ± 3.91	67.17 ± 2.27 ^{B}	75.42 ± 2.76 ^{C}
LVESD (mm)	2.20 ± 0.27	2.37 ± 0.17	2.30 ± 0.18	2.17 ± 0.29	2.13 ± 0.28
LVEDD (mm)	3.75 ± 0.28	3.80 ± 0.15	3.62 ± 0.41	3.94 ± 0.31	3.78 ± 0.39
IVS (mm)	0.76 ± 0.09	1.03 ± 0.01 ^{AA}	0.99 ± 0.08	0.81 ± 0.05^{BB}	0.85 ± 0.10 ^{C}
PWT (mm)	0.70 ± 0.04	1.01 ± 0.03 ^{A}	0.93 ± 0.15	0.84 ± 0.05 ^{BB}	0.82 ± 0.07 ^{C}

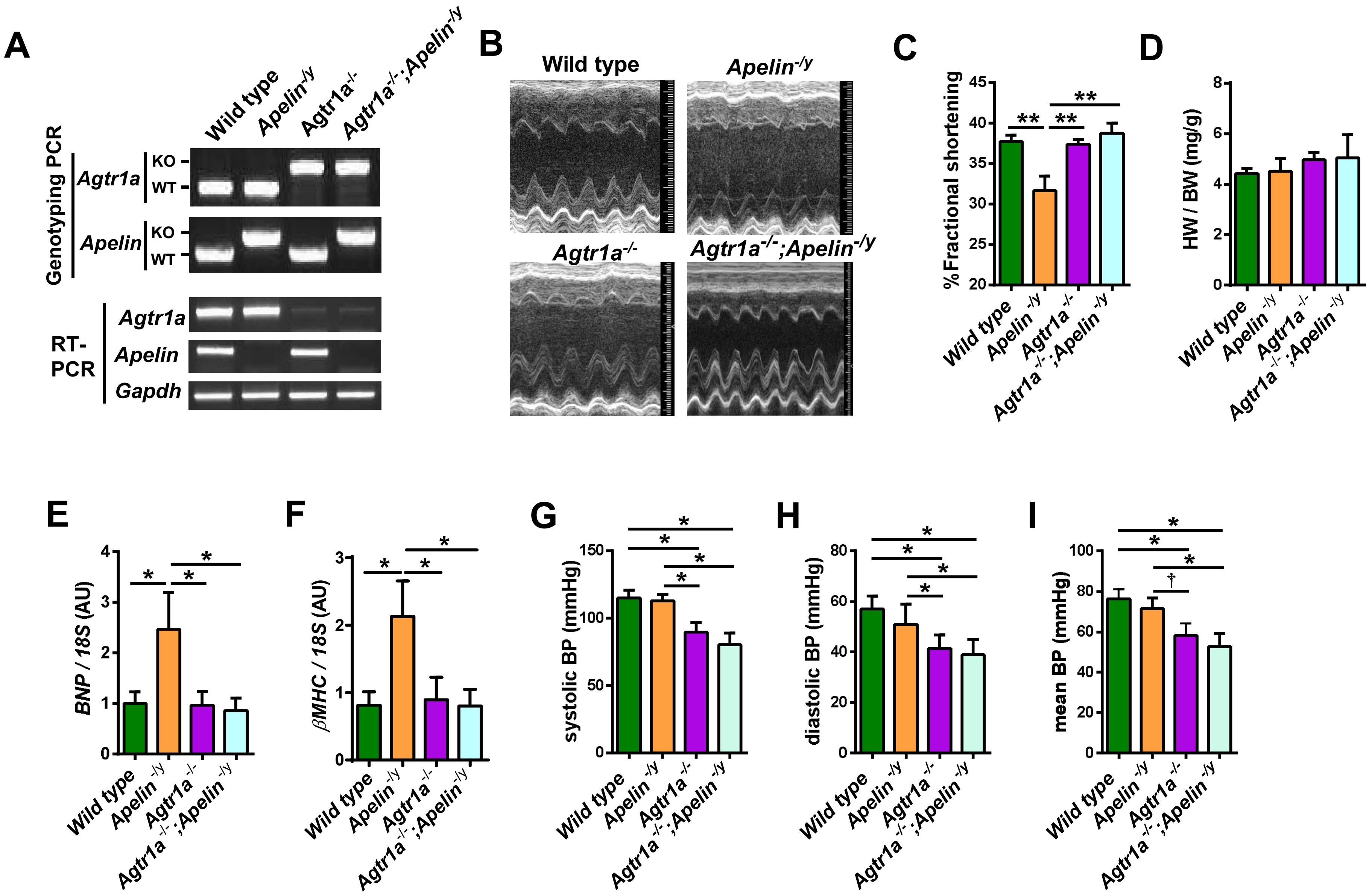
Values were means ±SEM. See Supplementary Table 2 for abbreviations. ^{A} $P < 0.05$ and ^{AA} $P < 0.01$ compared with wild type mice with sham. $^{B}P < 0.05$, $^{BB}P < 0.01$ compared with vehicle treated wild type mice with TAC. ^{C} $P < 0.05$ compared with vehicle treated *Agtr1a*$^{-/-}$ mice with TAC.

Supplementary Table 6.

Gene	Primers	Sequence (5'-3')	Gene	Primers	Sequence (5'-3')
Apelin	Forward	CACTGATGTTGCCTCCAGATGGA	*Tgfb2*	Forward	AGAAGCGCGCTTTGGATGCTGC
	Reverse	ACGCCATTAGACGAACTTGGTGG		Reverse	TGGGACACACAGCAAGGGGAAG
Apj	Forward	CTCAGCAGCTACCTCATCTTTGT	*Ltbp2*	Forward	AAATGGCCAGCTGGAGTGTCCC
	Reverse	TGAAGTGGCCACCATAGAGTAGT		Reverse	TTCACGCACTCCGAGTCCTTGC
Agtr1a	Forward	GCATCATCTTTGTGGTGGG	*Postn*	Forward	TGCTCTGCTGCTGCTGTTCCTG
	Reverse	GAAGAAAGCACAATCGCC		Reverse	TGCTGGAGGGCACAGACGTTTG
Agt	Forward	GAGGCAAATCTGAGCAACATTG	*Mas*	Forward	AGGGTGACTGACTGAGTTTGG
	Reverse	GAGTTCGAGGAGGATGCTATTGA		Reverse	GAAGGTAAGAGGACAGGAGC
Ace	Forward	TGAGAAAAGCACGGAGGTATCC	*18S*	Forward	AAACGGCTACCACATCCAAG
	Reverse	AGAGTTTTGAAAGTTGCTCACATCA		Reverse	CCTCCAATGGATCCTCGTTA
Ace2	Forward	CTACAGGCCCTTCAGCAAAG	*GAPDH*	Forward	CCCATCACCATCTTCCAGGA
	Reverse	TGCCCAGACCCTAGAGTTGT		Reverse	GGGGCCATCCACAGTCTTCT
BNP	Forward	ATGGATCTCCTGAAGGTGCTG	*Prep*	Forward	GGAATCGATGCTGCTGATTA
	Reverse	GTGCTGCCTTGAGACCGAA		Reverse	GGAATCGATGCTGCTGATTA
β–MHC	Forward	GCAGCAGTTGGATGAGCGAC	*Prcp*	Forward	GAACTACCCTTACGCATGCAACT
	Reverse	TGCCTCCTCCAGCCTTTCAC		Reverse	AATATTGGCACACCTCCTTGATG
α–MHC	Forward	CAAGAGGCAACTGGAGGAGGAAG	*Thop1*	Forward	CGTGGAGCGGGACTTTGT
	Reverse	GCCTCTGAGCCAGCTTCTTCTTG		Reverse	CTCCTTTTCCCACACCCAGTT
ANF	Forward	TGAGAAAAGCACGGAGGTATCC	*Nep*	Forward	GCCTCAGCCGAACCTACAAG
	Reverse	AGAGTTTTGAAAGTTGCTCACATCA		Reverse	AATTTGCACAACGTCCTCAAGTT



Supplementary Figure 1



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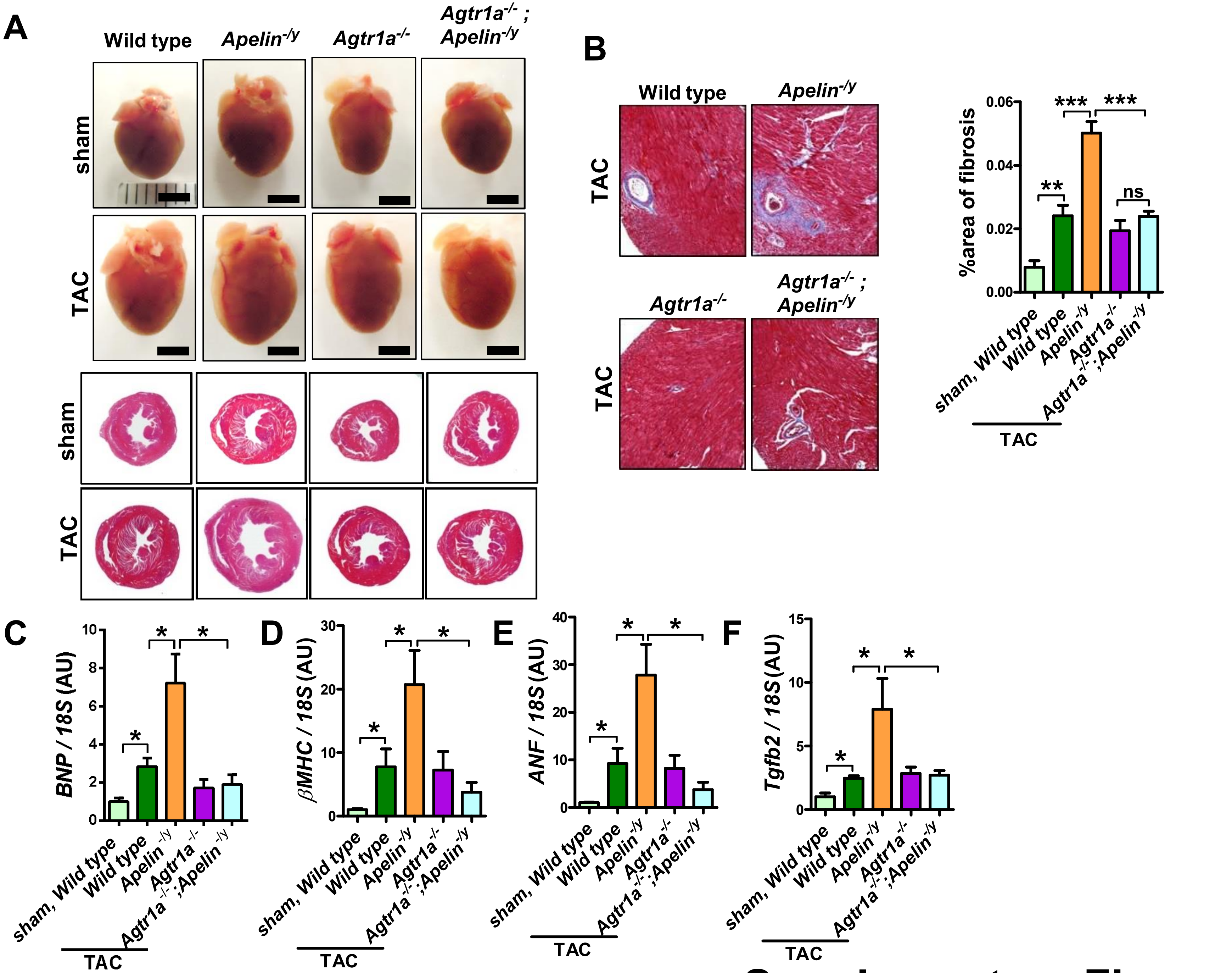

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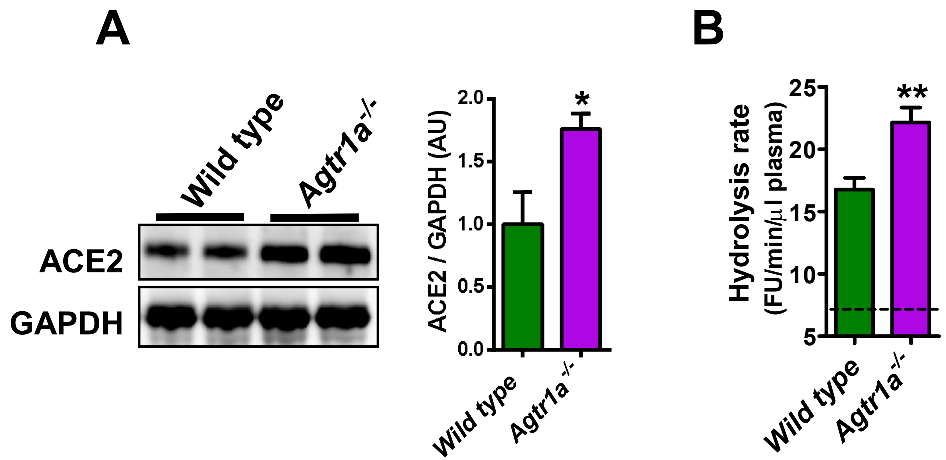

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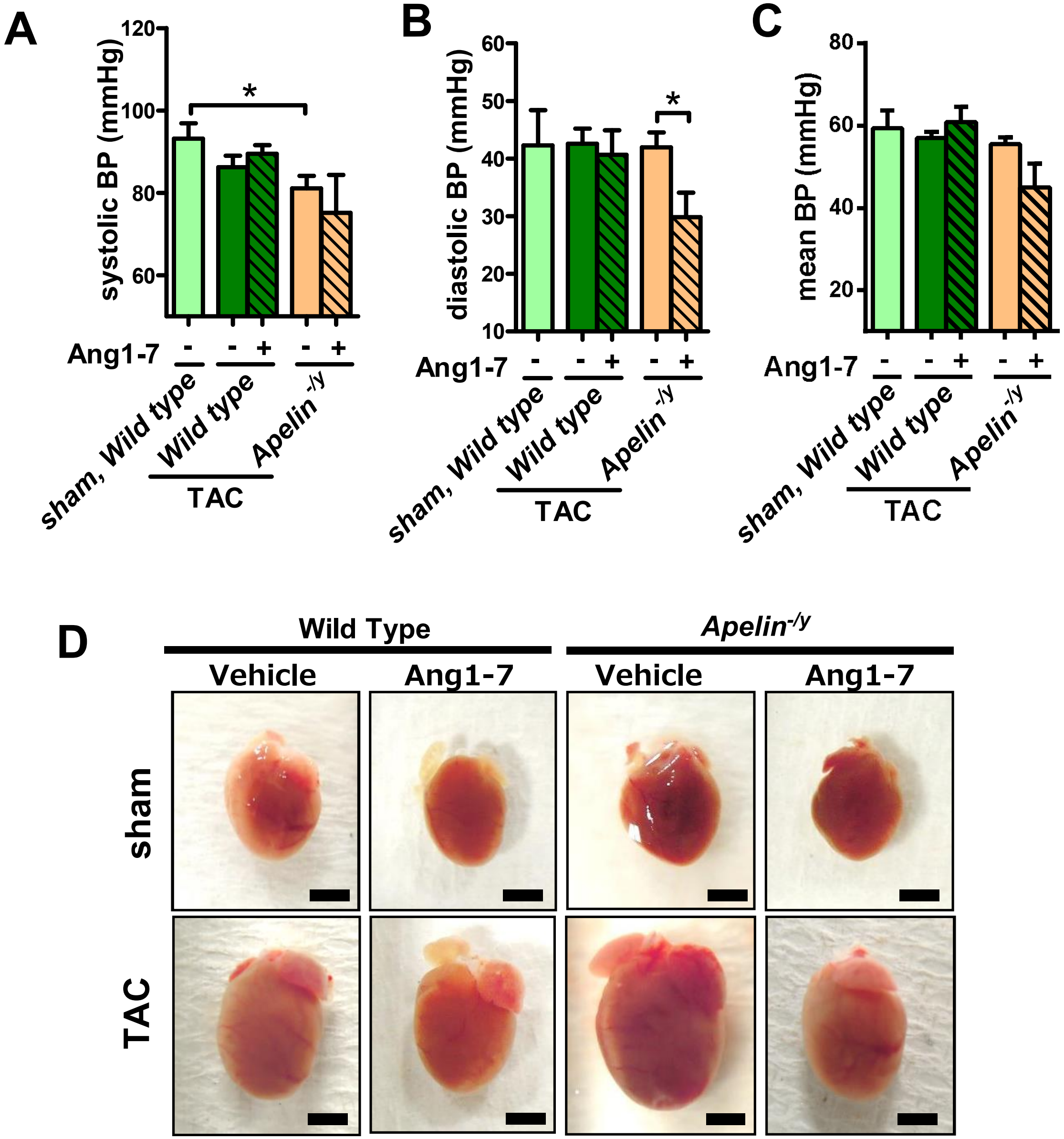

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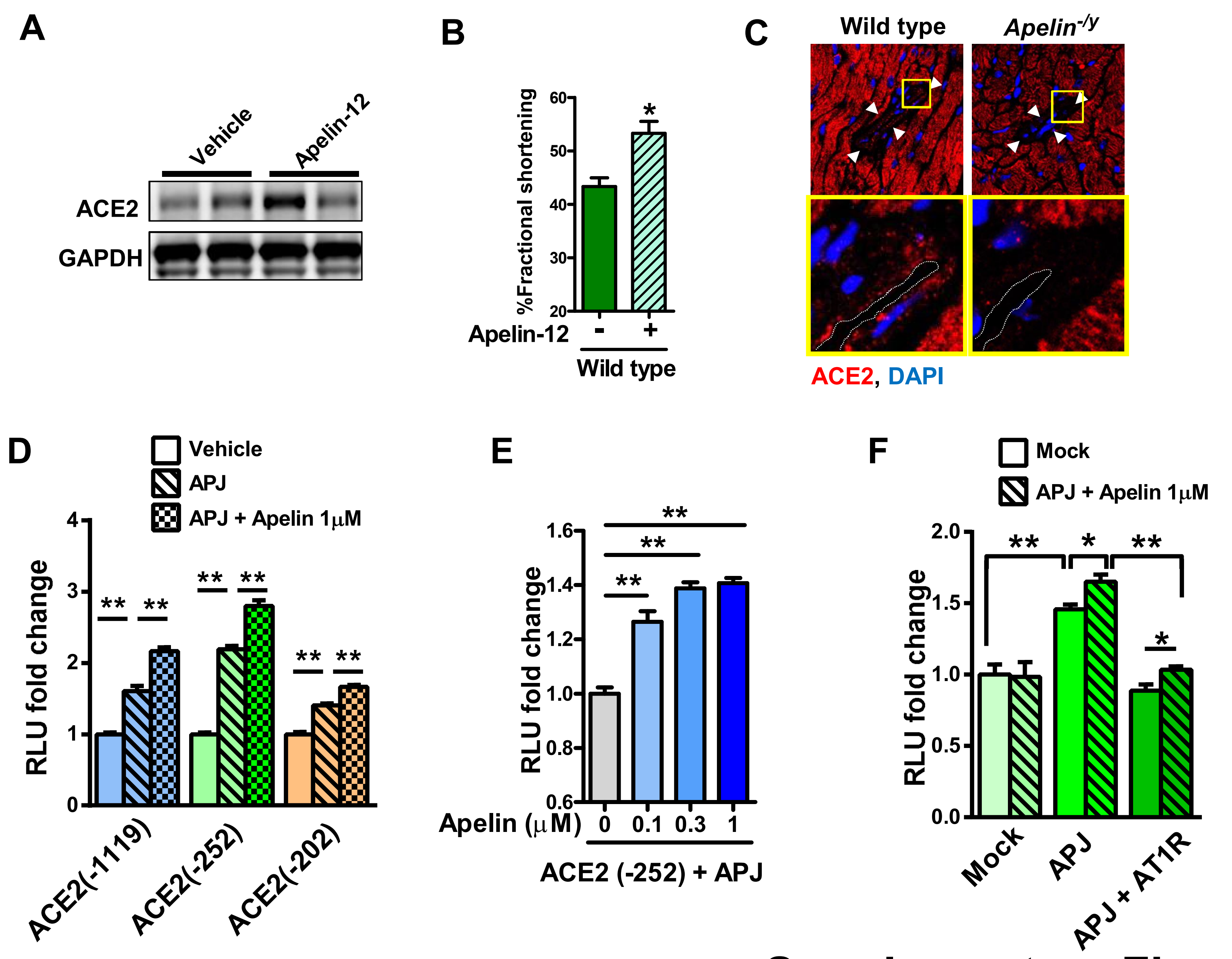

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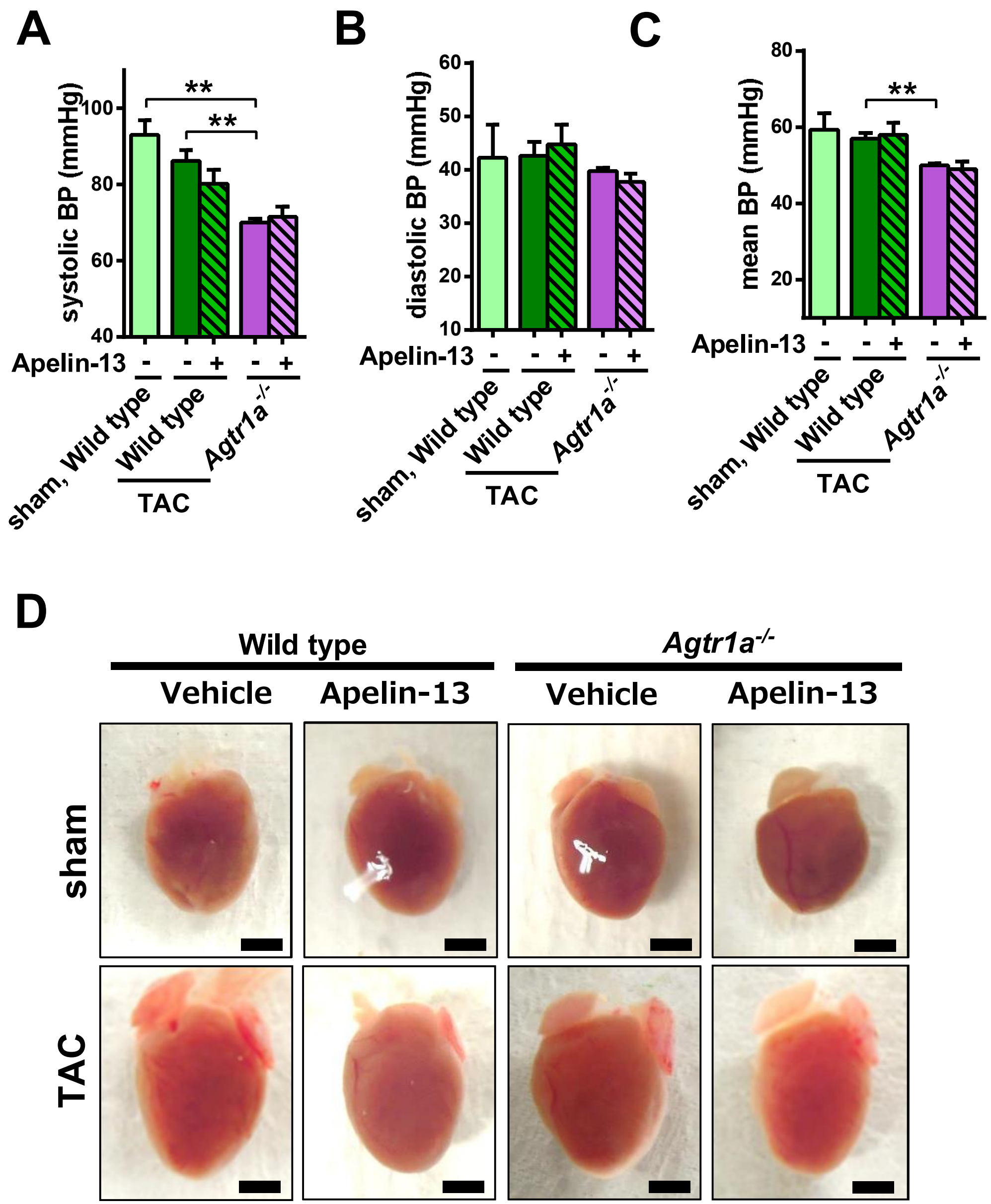

Supplementary Figure 7