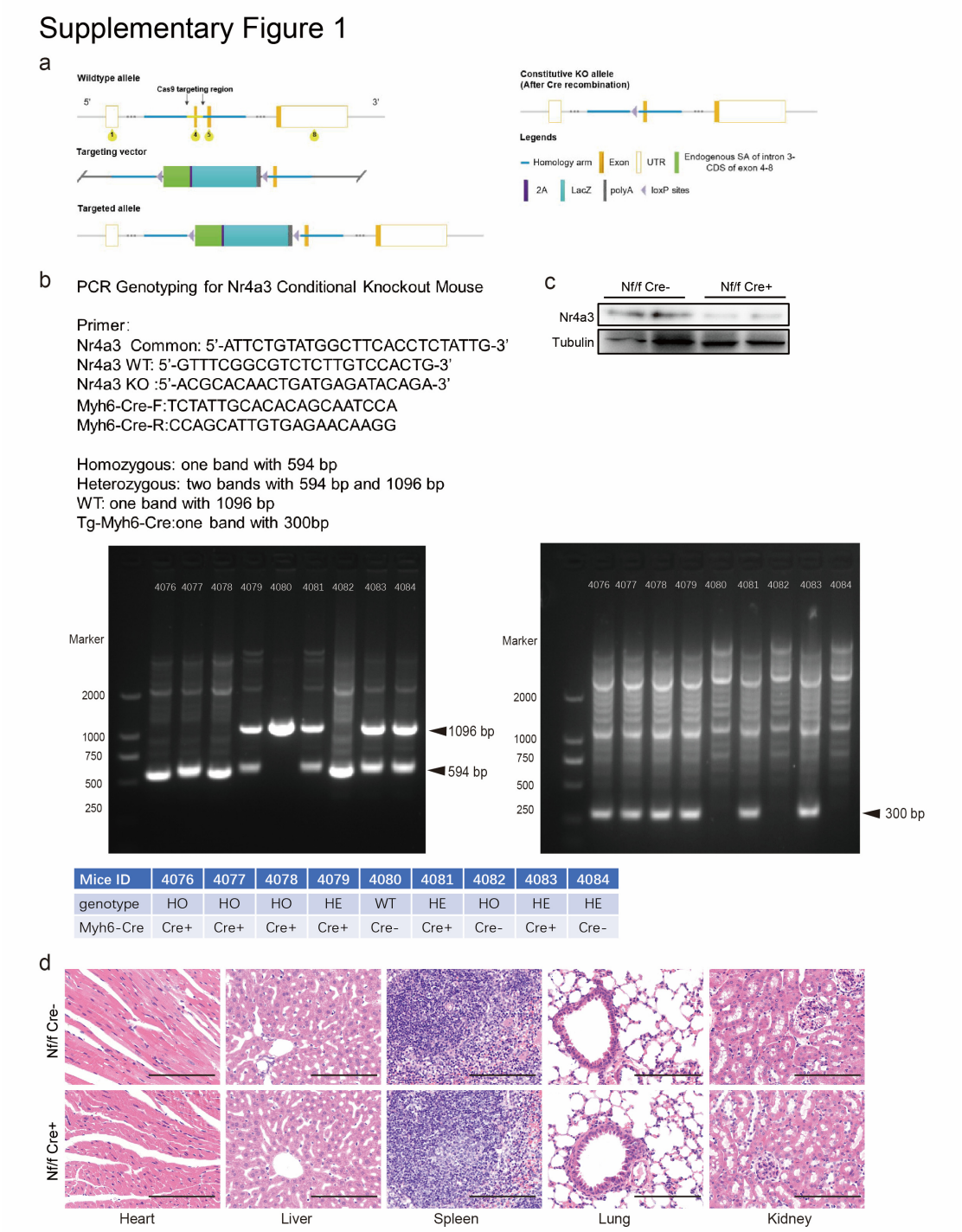
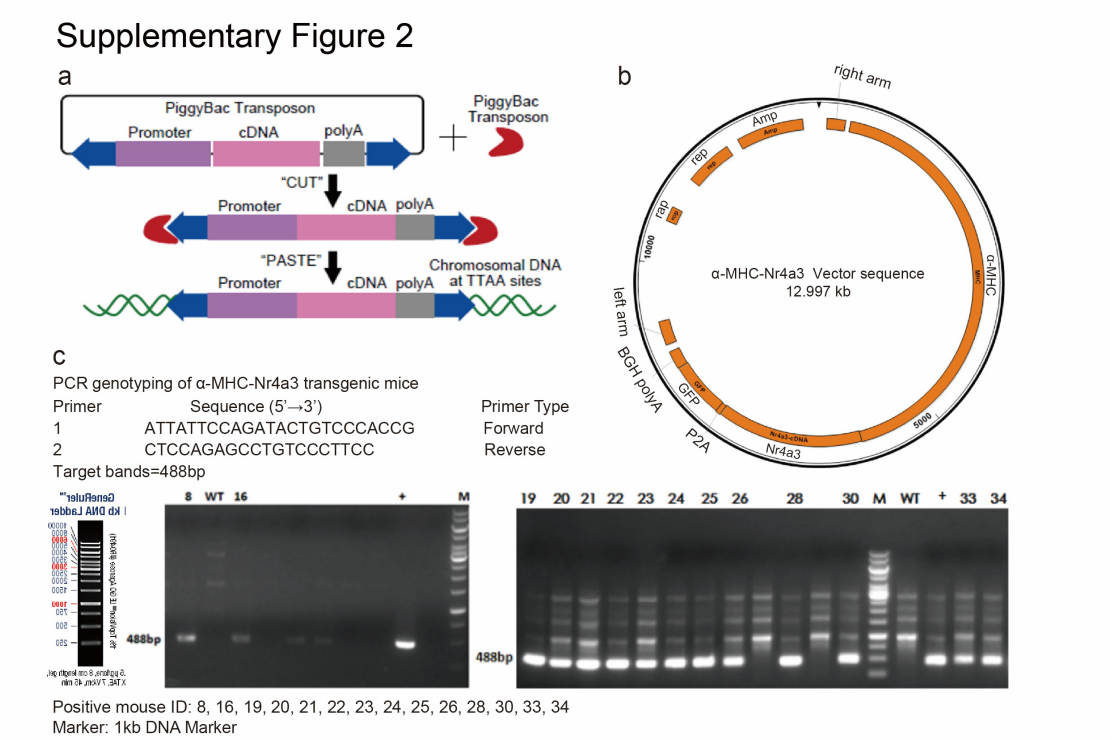


Supplementary Fig. 1. Generation and characterization of cardiac specific Nr4a3 knockout mice

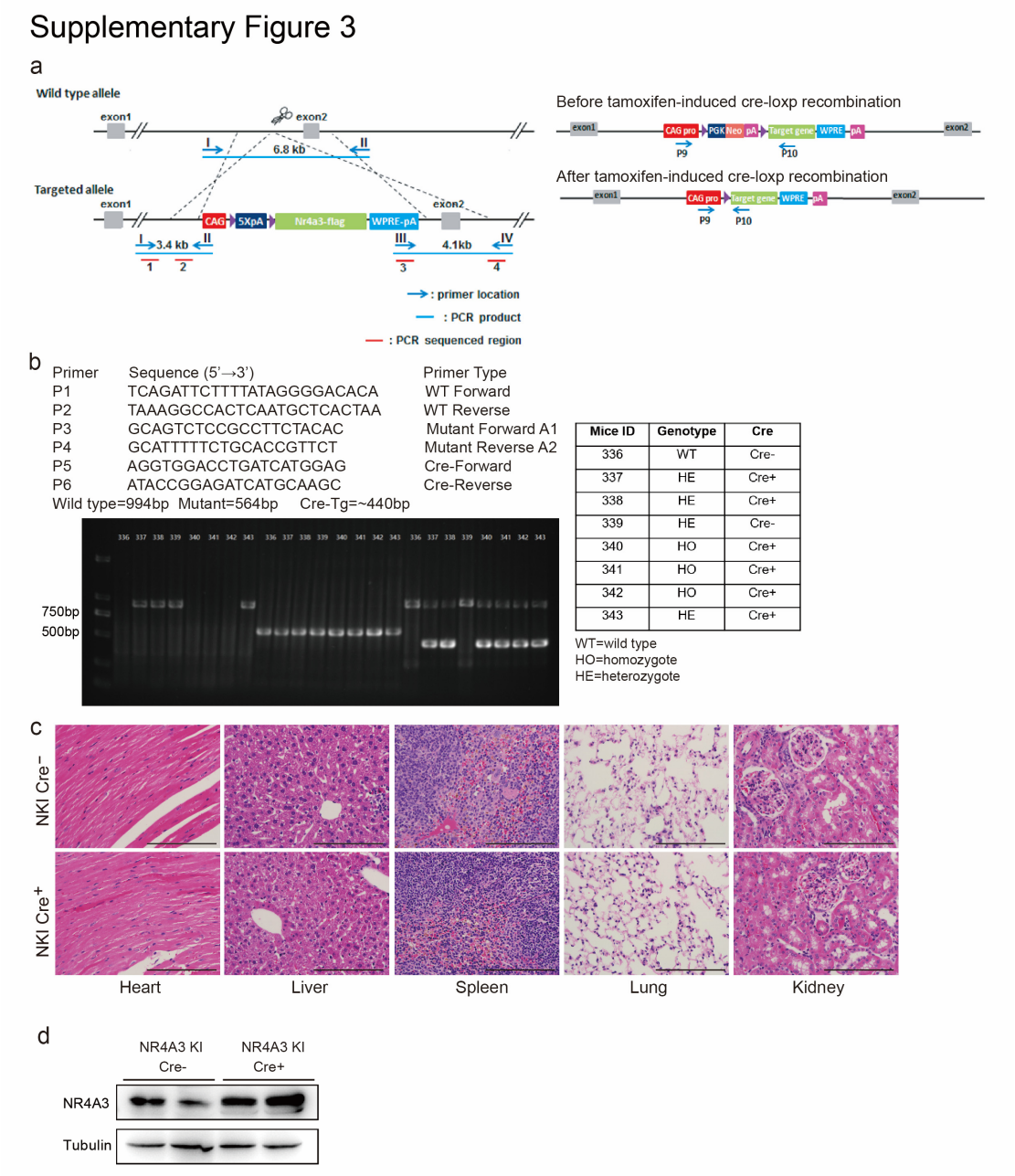
a The strategies of the Nr4a3$^{flox/flox}$ and tissue specific Nr4a3 knockout mice generation. **b** Nr4a3$^{flox/flox}$ mice were crossed with transgenic mice expressing tamoxifen-inducible Cre recombinase protein fused to a mutant estrogen-receptor ligand binding domain driven by α-myosin heavy chain promoter (Myh6-CreERT2, abbreviated as Myh6 Cre), resulting in Nf/f Cre^{+} (Nr4a3$^{flox/flox}$-Myh6-CreERT2^{+}) and Nf/f Cre^{-} mice. Primer sequences and results of the genotyping of tail DNA samples were obtained from the Nf/f Cre^{+} and Nf/f Cre^{-} animals. **c** The expression of Nr4a3 in

the hearts were examined in Nf/f Cre^{+} and Nf/f Cre^{-} mice after tamoxifen treatment. **d** There were no obvious differences in the histological analysis of heart, liver, spleen, lung and kidney tissue between Nf/f Cre^{+} and Nf/f Cre^{-} mice after tamoxifen treatment. Scale bar, 100 μm.



Supplementary Fig. 2. Generation and genotyping of α-MHC-Nr4a3 transgenic mice

a The principle of the PiggyBAC Transposase System. **b** A targeting vector was comprised of right arm, α-MHC promoter, Nr4a3 cDNA, P2A, BGH polyA and left arm sequences. **c** Primer sequences and results of the genotyping wild-type (WT) and α-MHC-Nr4a3 transgenic mice from tail DNA samples.

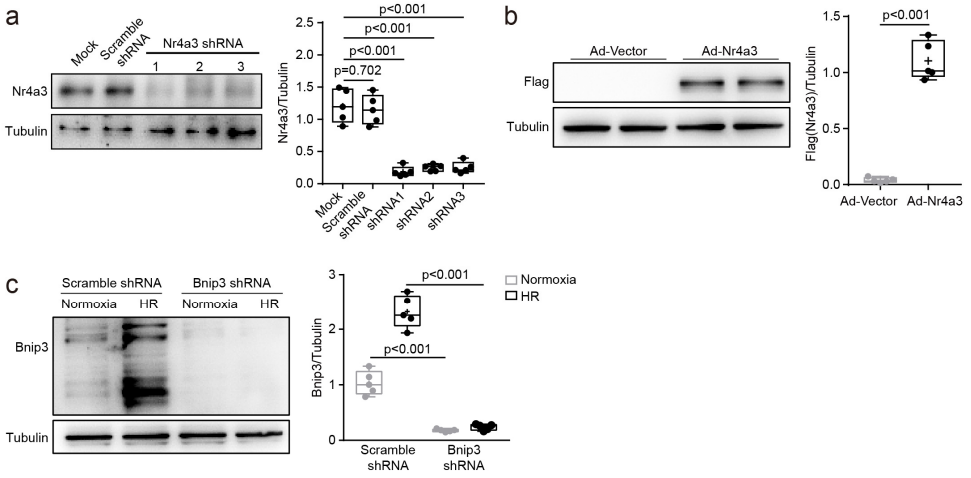


Supplementary Fig. 3. Generation and characterization of conditional Nr4a3 knock-in mice

a This project used CRISPR/Cas9 technology to insert the CAG promoter-loxp-stop-loxp-Nr4a3-flag-WPRE-polyA expression cassette at the *Rosa26* locus by homologous recombination. A targeting vector was constructed by In-Fusion cloning (see Methods). Cas9 mRNA, gRNA and donor vector were microinjected into C57BL/6J mice fertilized eggs to obtain F_0 mice. A purple triangle indicates the inserted loxp sites. The presence of the loxp-stop-loxp expression cassette prevented transcription of the downstream *Nr4a3* target gene. After mating with tissue or cell specific Cre mice, loxp-stop-loxp expression box was knocked out, and high expression of *Nr4a3* was achieved with the CAG promoter. **b** Nr4a3 knock-in

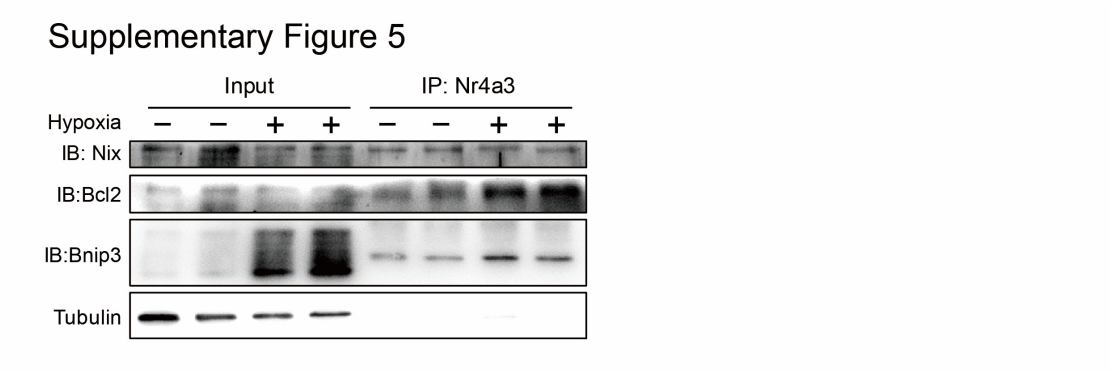
mice (abbreviated as Nr4a3 KI or NKI) were crossed with transgenic mice expressing tamoxifen-inducible Cre recombinase protein fused to a mutant estrogen-receptor ligand binding domain driven by α-myosin heavy chain promoter (Myh6-CreERT2, abbreviated as Myh6 Cre), resulting in NKI Cre^+ (Nr4a3 KI-Myh6-$CreERT2^+$) and NKI Cre^- (Nr4a3 KI) mice. Primer sequences and results of the genotyping of tail DNA samples were obtained from the NKI Cre^+ and NKI Cre^- animals. **c** There were no obvious differences in the histological analysis of heart, liver, spleen, lung and kidney tissue between NKI Cre^+ and NKI Cre^- mice before tamoxifen treatment. Scale bar, 100 μm. **d** The expression of Nr4a3 in the hearts was examined in NKI Cre^+ and NKI Cre^- mice after tamoxifen treatment.

Supplementary Figure 4



Supplementary Fig. 4. Confirm the efficiency of lentivirus-mediated Nr4a3 or Bnip3 knockdown and adenovirus-mediated Nr4a3 overexpression

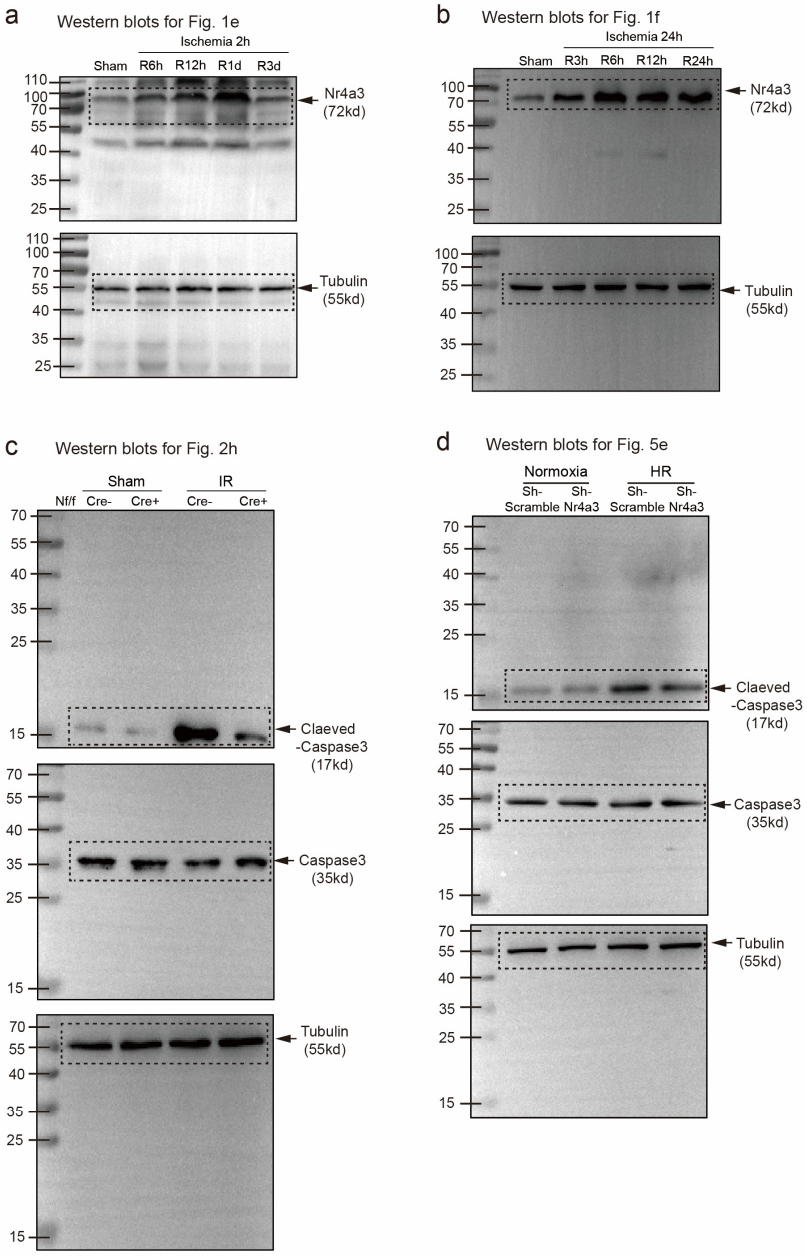
a Lentivirus-mediated shRNA to Nr4a3 was transduced into NRVMs, and the expression of Nr4a3 was examined 24 hours after transfection. **b** NRVMs were transfected with adenovirus-Nr4a3 or its control vector, the expression level of Nr4a3 was examined by western blot 24 hour after transfection. **c** Lentivirus-mediated shRNA to Bnip3 was transduced into NRVMs, which were cultured under normoxic or hypoxia reoxygenation (24 hours hypoxia and 6 hours reoxygenation (HR)) conditions, and then immunoblotted with Bnip3 antibody.



Supplementary Fig. 5. Nr4a3 interacts with the Bcl2 family proteins

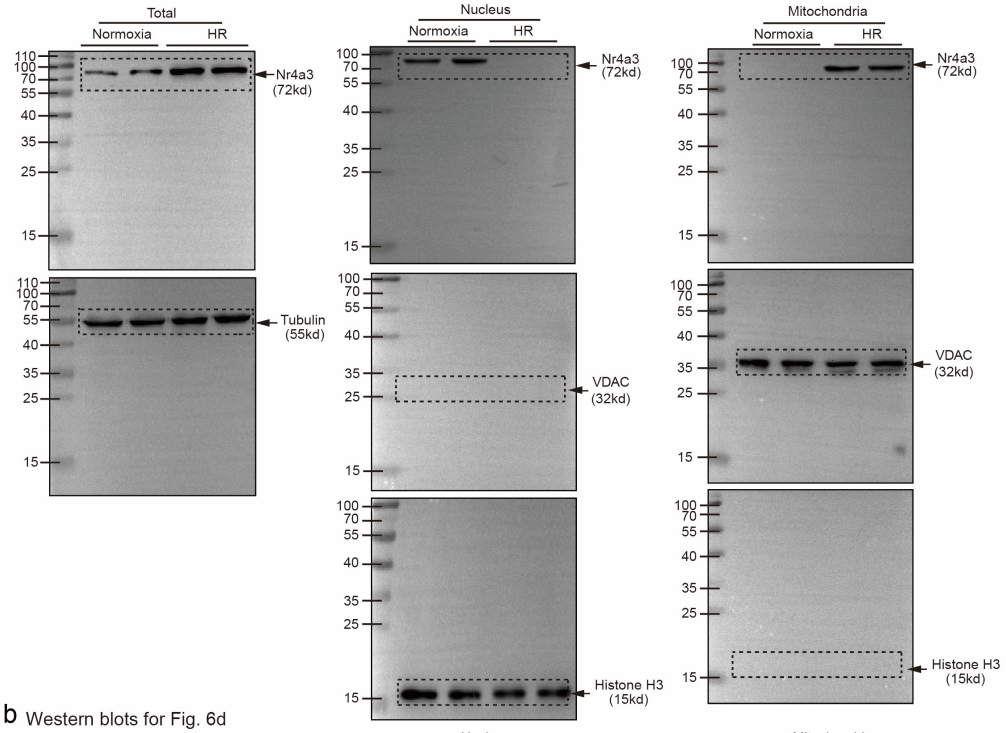
NRVMs were cultured for 24 hours of hypoxia followed by 6 hours of reoxygenation (HR), and then analyzed by immunoprecipitation using an anti-Nr4a3 antibody followed by western blot using antibodies against Nix, Bnip3 and Bcl2, the experiments were repeated for 3 times. NRVMs, neonatal rat ventricular myocytes.

Supplementary Figure 6

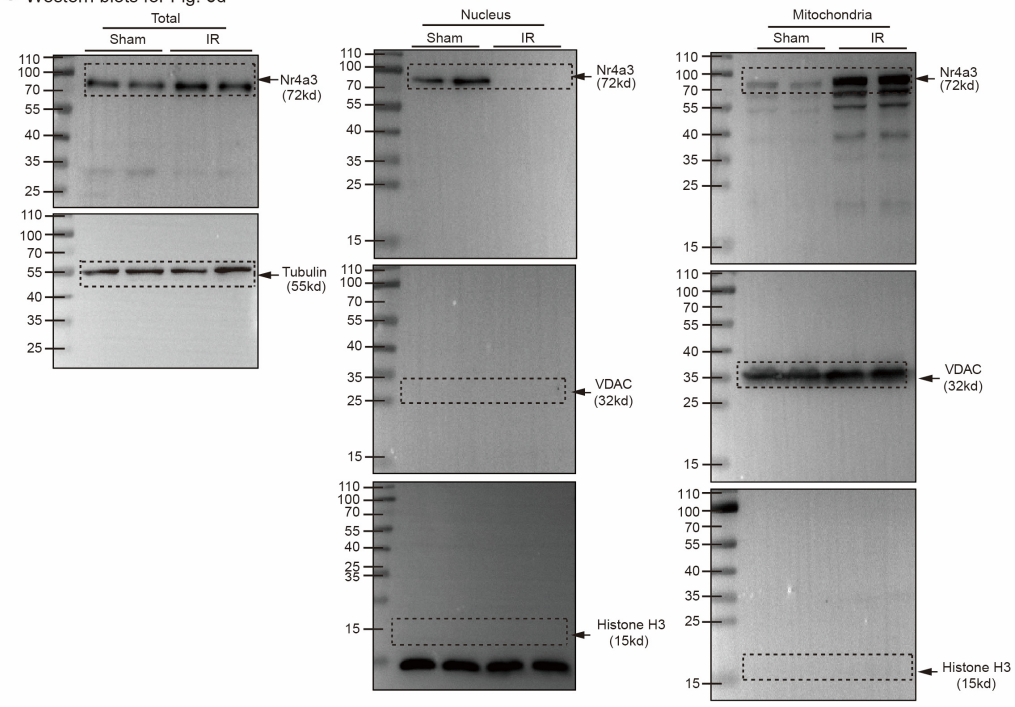


Supplementary Figure 7

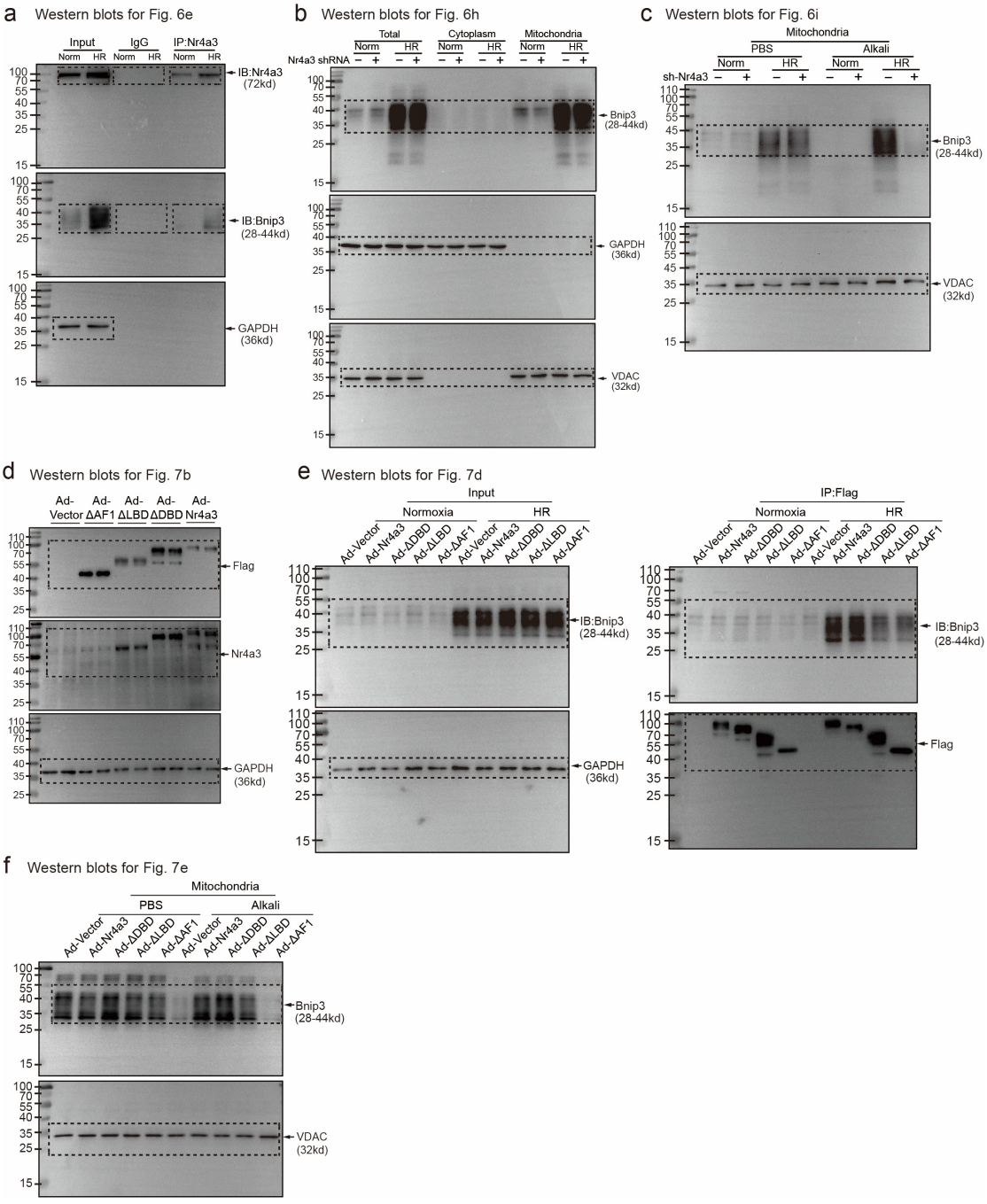
a Western blots for Fig. 6c



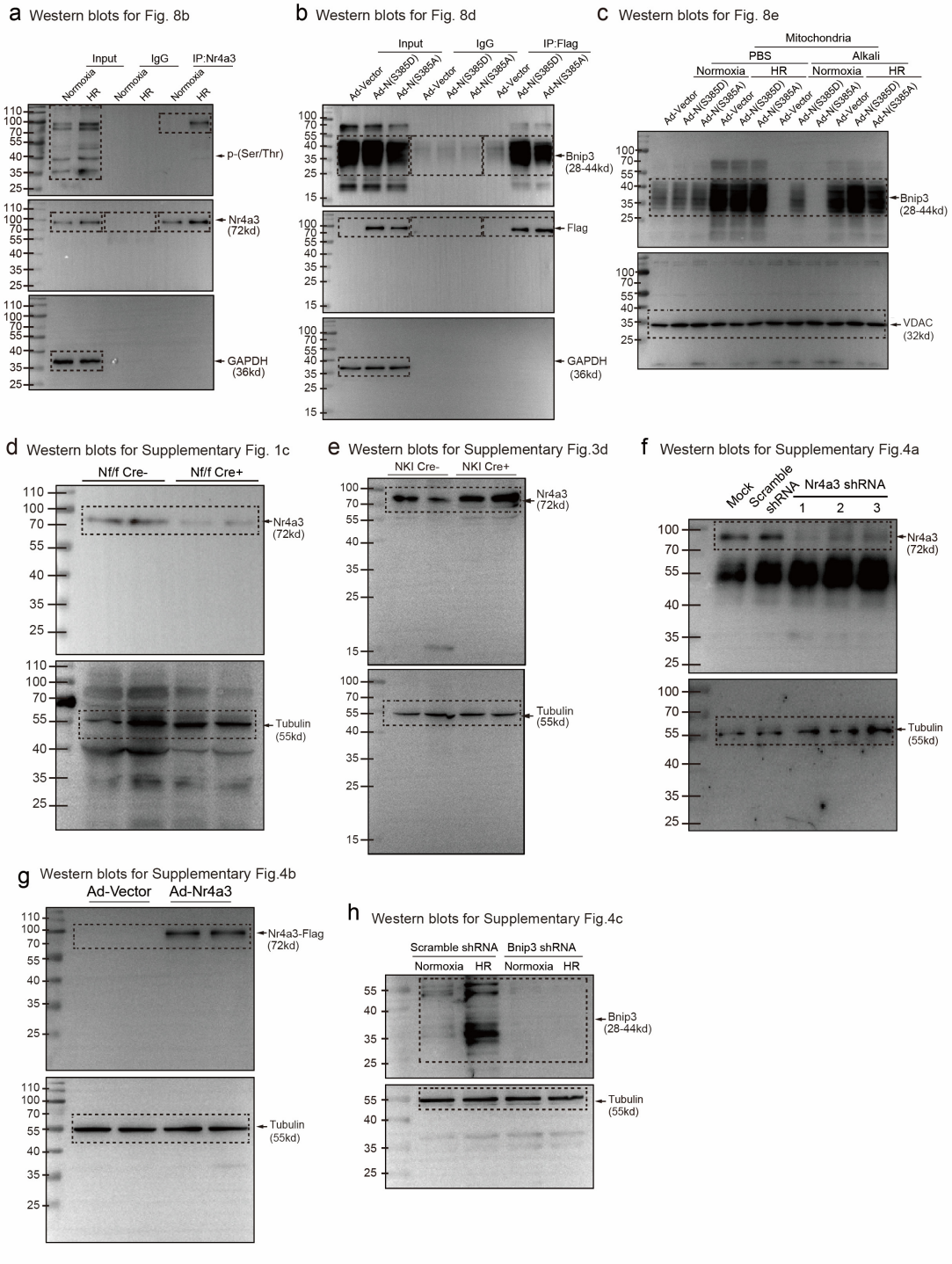
b Western blots for Fig. 6d



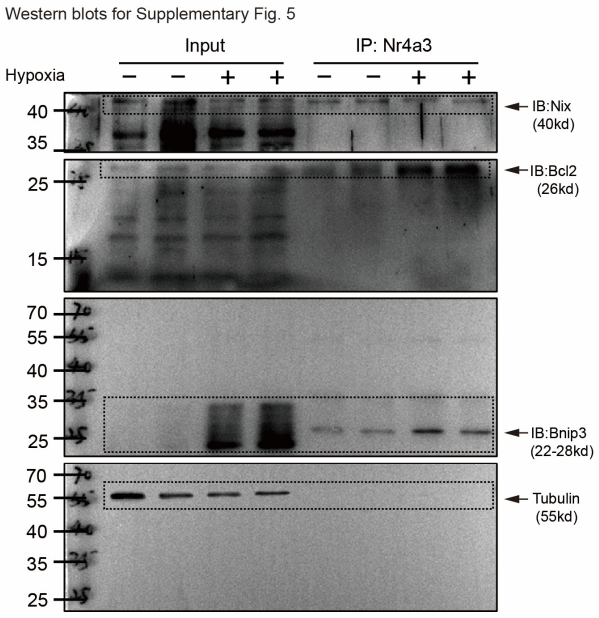
Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10



Supplementary Fig. 6-10. Uncropped gel scans for all presented western blots

Supplementary Table1. List of antibodies and kits used in this study

Antibody specificity	Company	Cat. No.	Application	Dilution
Nr4a3	R&D	#H7833	WB	1:1000
GAPDH	Proteintech	60004-1-1g	WB	1:3000
Tubulin	Sigma	T9026	WB	1:3000
Histone H3	CST	#4499	WB	1:1000
Caspase-3	CST	#9662	WB	1:1000
Cleaved Caspase-3	CST	#9661	WB	1:1000
DYKDDDDK Tag	CST	#14793	WB	1:1000
BNIP3	CST	#3769	WB	1:1000
Nix	CST	#12396	WB	1:1000
BCl-2	CST	#15071	WB	1:1000
VDAC	CST	#4866	WB	1:1000
Anti-DDK	OriGene	TA100011	IP	
Nr4a3	R&D	#H7833	IP	
Nr4a3	Abcam	ab94507	IB	1:1000
Caveolin 3	Abcam	ab2912	IF	1:100
Nr4a3	R&D	#H7833	IF	1:100
Bnip3	Abcam	ab109362	IF	1:100
Hsp60	OriGene	AP22882PU-N	IF	1:100
α-actinin	Sigma	A7811	IF	1:500
TMRM	Invitrogen	T668	IF	1:2000
Calcein, AM	Invitrogen	C3099	IF	1:1000
Mitotracker Red	Invitrogen	M7512	IF	1:1000
Mitotracker Green	Invitrogen	M7514	IF	1:1000
In Situ Cell Death Detection Kit, Fluorescein	Roche	11684795910	IF	
Live/Dead Cell Double Staining Kit	Merck	04511-1KT-F	IF	
CD45-BV421	Biolegend	103134	FACS	1:300
CD31-PE	Biolegend	102419	FACS	1:300
gp38-APC	Biolegend	127410	FACS	1:300
Zombie Aqua™ Fixable Viability Kit	Biolegend	423101	FACS	1:100
Lentiviral Packaging Kits	Origene	TR30037		
Phospho-(Ser/Thr) Akt Substrate Antibody	CST	#9611	WB	1:1000