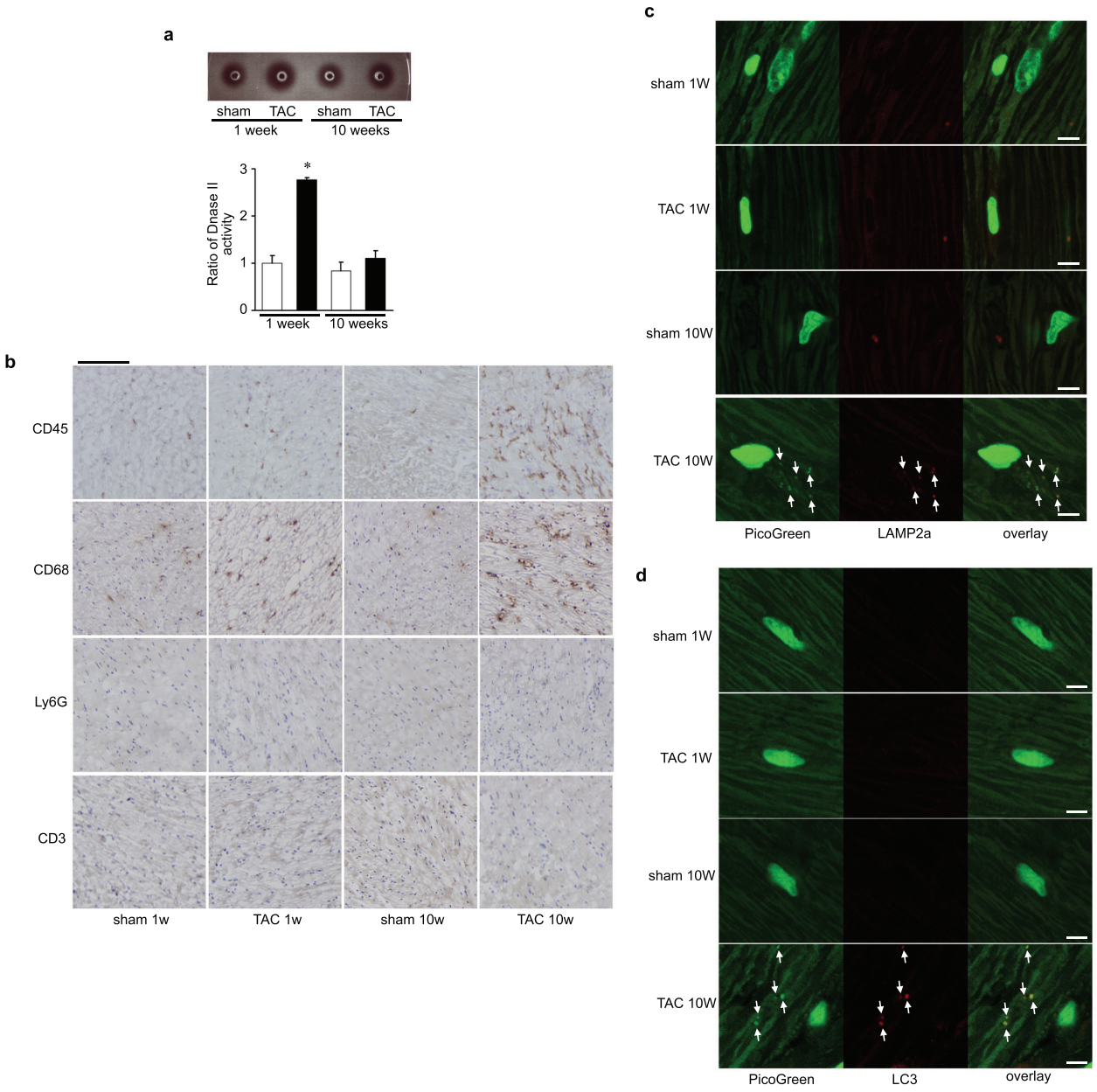
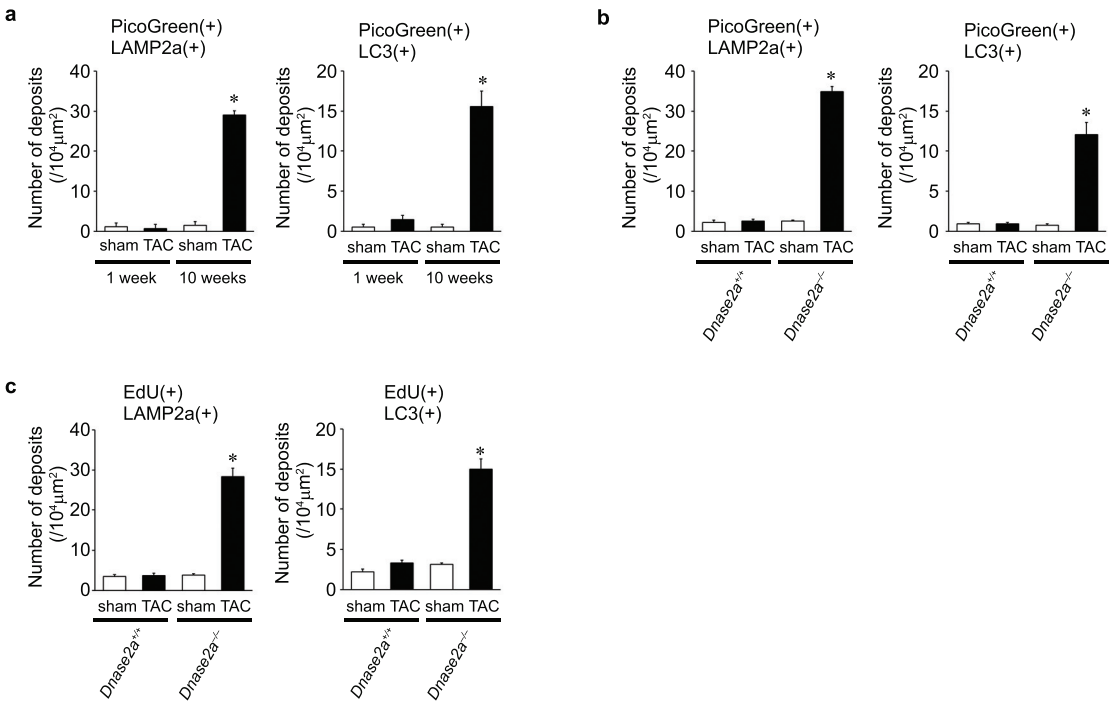
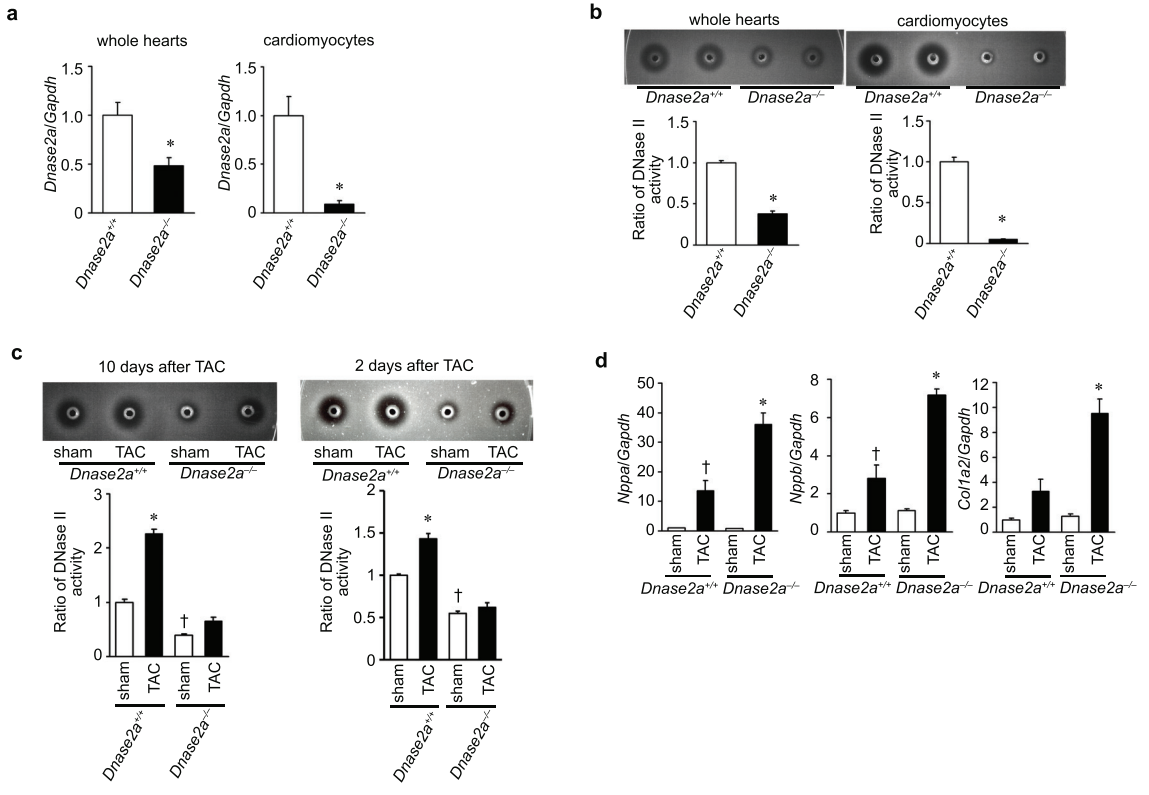


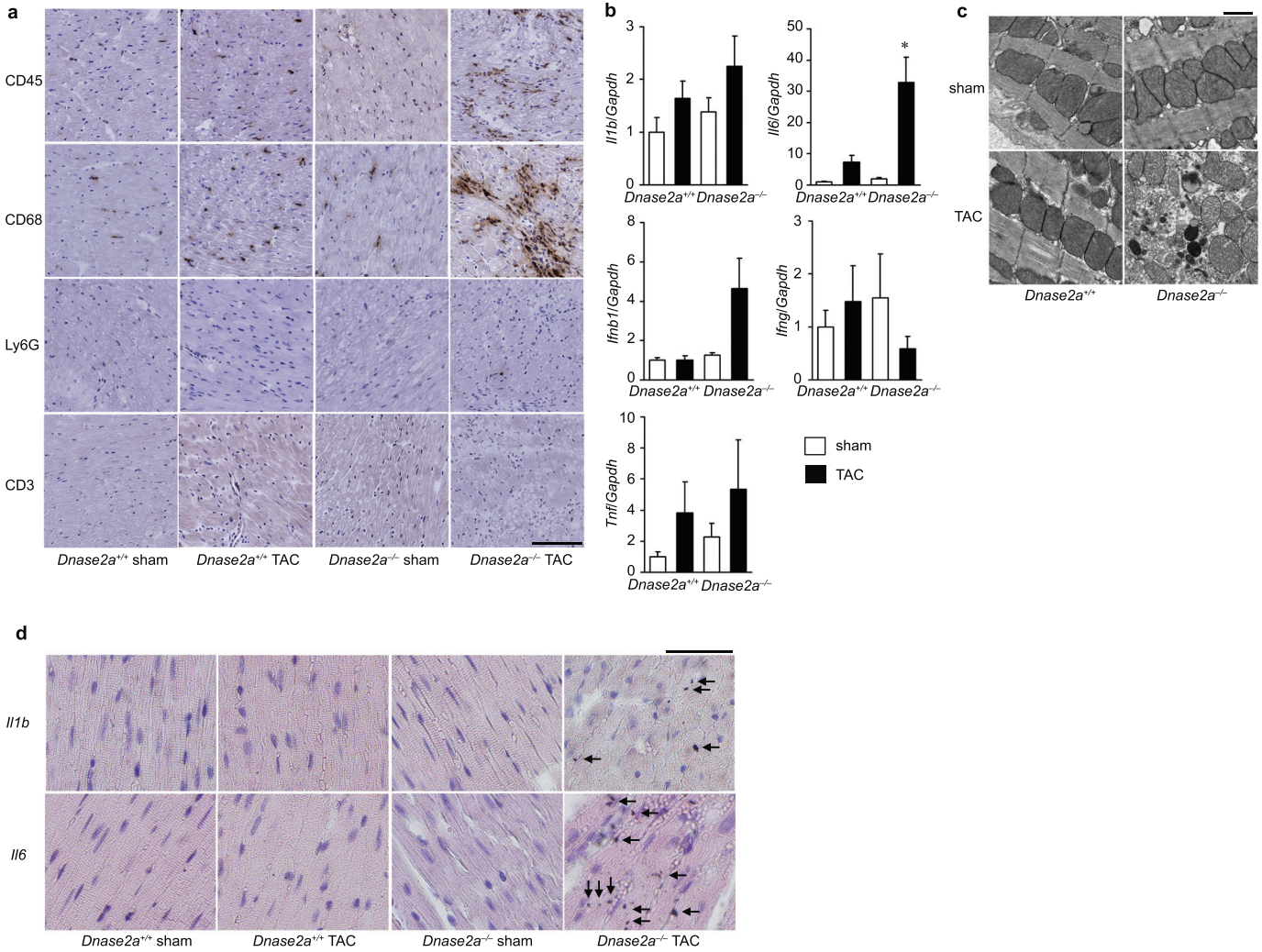
Supplementary Figure 1. DNase II activity, inflammatory response and DNA deposition 1 or 10 weeks after TAC. (**a**) DNase II activity in whole hearts measured by the SRED method. Ratio of DNase II activity on the abscissa represents the averaged value relative to that for sham-operated mice 1 week after operation. Upper panel shows representative image and lower graph shows its quantitative result. Open and closed columns represent sham- and TAC-operated mice, respectively. $^{*}P < 0.05$ versus all other groups. (**b**) Immunohistochemical analysis using antibodies to CD45, CD68, Ly6G and CD3. Scale bar, 100 μm. (**c**) and (**d**) Double staining of heart sections with (**c**) PicoGreen (green) and anti-LAMP2a antibody (red) and (**d**) PicoGreen (green) and anti-LC3 antibody (red). Scale bar, 10 μm. Arrows indicate double positive deposits.



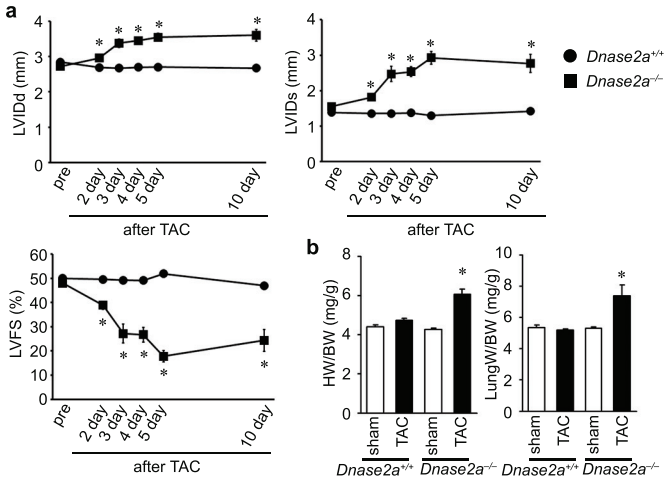
Supplementary Figure 2. Quantification of DNA deposits in autolysosomes. Numbers of PicoGreen- or EdU-positive deposits double-stained with LAMP2a or LC3 were measured on 20 random fields (900 μm^2/field) per mouse (n = 3 - 4). (**a**) DNA deposition in wild-type hearts 1 or 10 weeks after TAC. Numbers of PicoGreen-and LAMP2a-positive deposits (left panel) and PicoGreen- and LC3-positive deposits (right panel). (**b**) DNA deposition in *Dnase2a*$^{-/-}$ hearts 2 days after TAC. Numbers of PicoGreen- and LAMP2a-positive deposits (left panel) and PicoGreen- and LC3-positive deposits (right panel). (**c**) Mitochondrial DNA deposition in *Dnase2a*$^{-/-}$ hearts 2 days after TAC. Numbers of EdU- and LAMP2a-positive deposits (left panel) and EdU- and LC3-positive deposits (right panel). $^{*}P < 0.05$ versus all other groups.



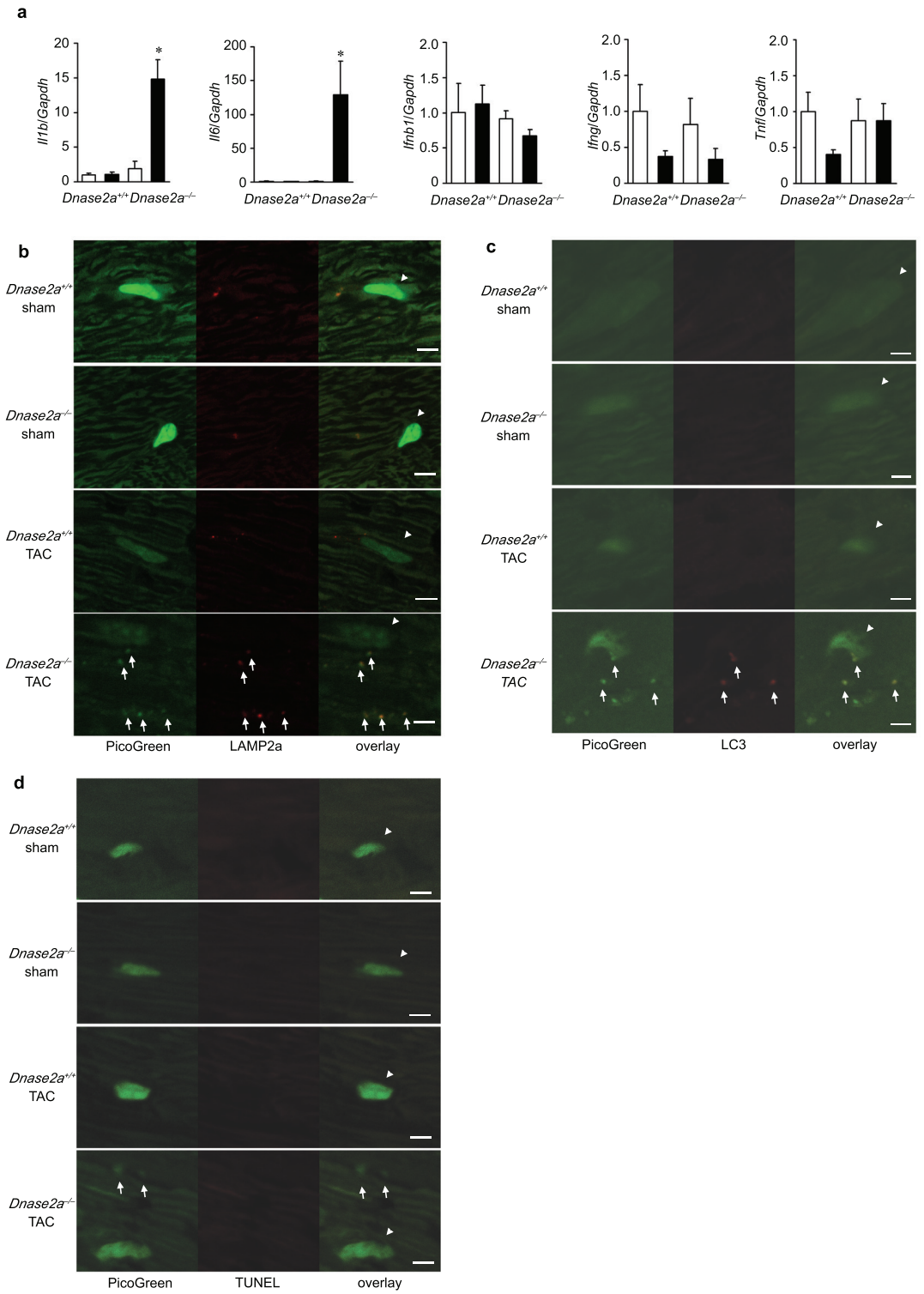
Supplementary Figure 3. Characterization of *Dnase2a*$^{-/-}$ mice. (**a**) mRNA level of *Dnase2a* in whole hearts or isolated cardiomyocytes 12-13 weeks after birth. *Gapdh* was used as the loading control. The averaged value in *Dnase2a*$^{+/+}$ mice was set equal to 1. Values are expressed as the mean ± s.e.m. $^{*}P <$ 0.05 versus *Dnase2a*$^{+/+}$ mice (n = 4 - 7). (**b**) DNase II activity in whole hearts or isolated cardiomyocytes 12-13 weeks after birth measured by the SRED method. DNase II activities for the samples were determined using a standard curve constructed from the serial dilution of porcine DNase II (Sigma). Upper panels show representative images, and lower graphs show their quantitative results. Ratio of DNase II activity on the abscissa represents the averaged value relative to that for *Dnase2a*$^{+/+}$ mice. $^{*}P < 0.05$ versus *Dnase2a*$^{+/+}$ mice (n = 3 - 5). (**c**) DNase II activity in whole hearts 10 days (left panel) and 2 days (right panel) after TAC (n = 3 - 4). Ratio of DNase II activity on the abscissa represents the averaged value relative to that for sham-operated *Dnase2a*$^{+/+}$ mice. $^{*}P < 0.05$ versus all other groups. $^{\dagger}P < 0.05$ versus sham-operated *Dnase2a*$^{+/+}$ mice. In each group, values are expressed as the mean ± s.e.m. (**d**) mRNA expressions of *Nppa*, *Nppb* and *Col1a2* (n = 4 - 5) 10 days after TAC. *Gapdh* was used as the loading control. The averaged value in sham-operated *Dnase2a*$^{+/+}$ mice was set equal to 1. Values are expressed as the mean ± s.e.m. $^{*}P < 0.05$ versus all other groups. $^{\dagger}P < 0.05$ versus sham-operated controls.



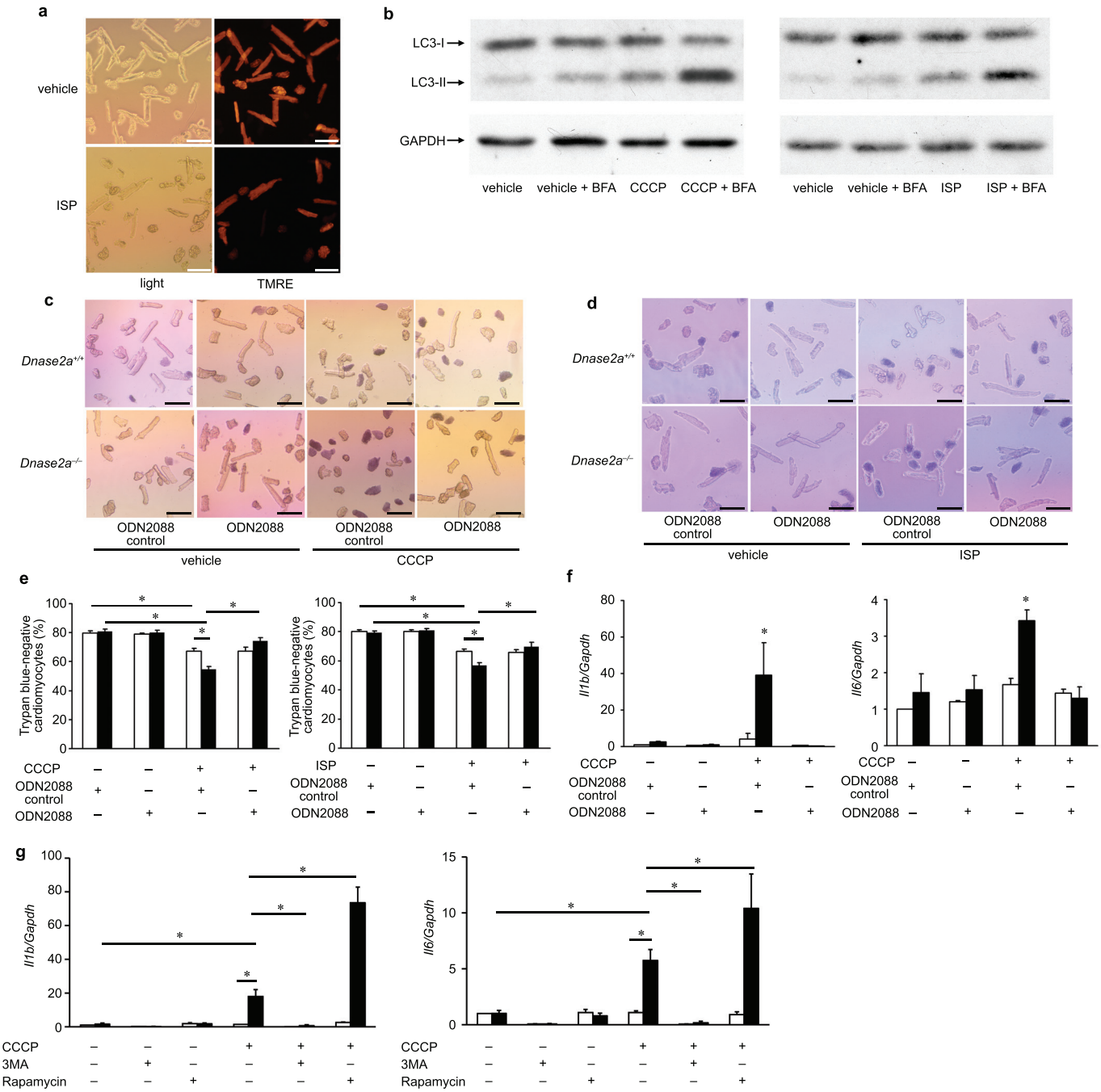
Supplementary Figure 4. Inflammatory responses after TAC. Mice were analyzed 10 days after TAC (**a-c**). (**a**) Immunohistochemical analysis using antibodies to CD45, CD68, Ly6G and CD3. Scale bar, 100 μm. (**b**) mRNA expressions of inflammatory cytokines such as *Il1b*, *Il6*, *Ifnb1*, *Ifng* and *Tnf*. *Gapdh* was used as the loading control. The averaged value in sham-operated *Dnase2a*$^{+/+}$ mice was set equal to 1. Values are expressed as the mean ± s.e.m. (n = 4 - 6). $^{*}P < 0.05$ versus all other groups. (**c**) Electron microscopic images. Scale bar, 1 μm. (**d**) mRNA expressions of *Il1b* and *Il6* were examined in heart sections using *in situ* hybridization 2 days after TAC. Arrows indicate positive signals. Scale bar, 100 μm.



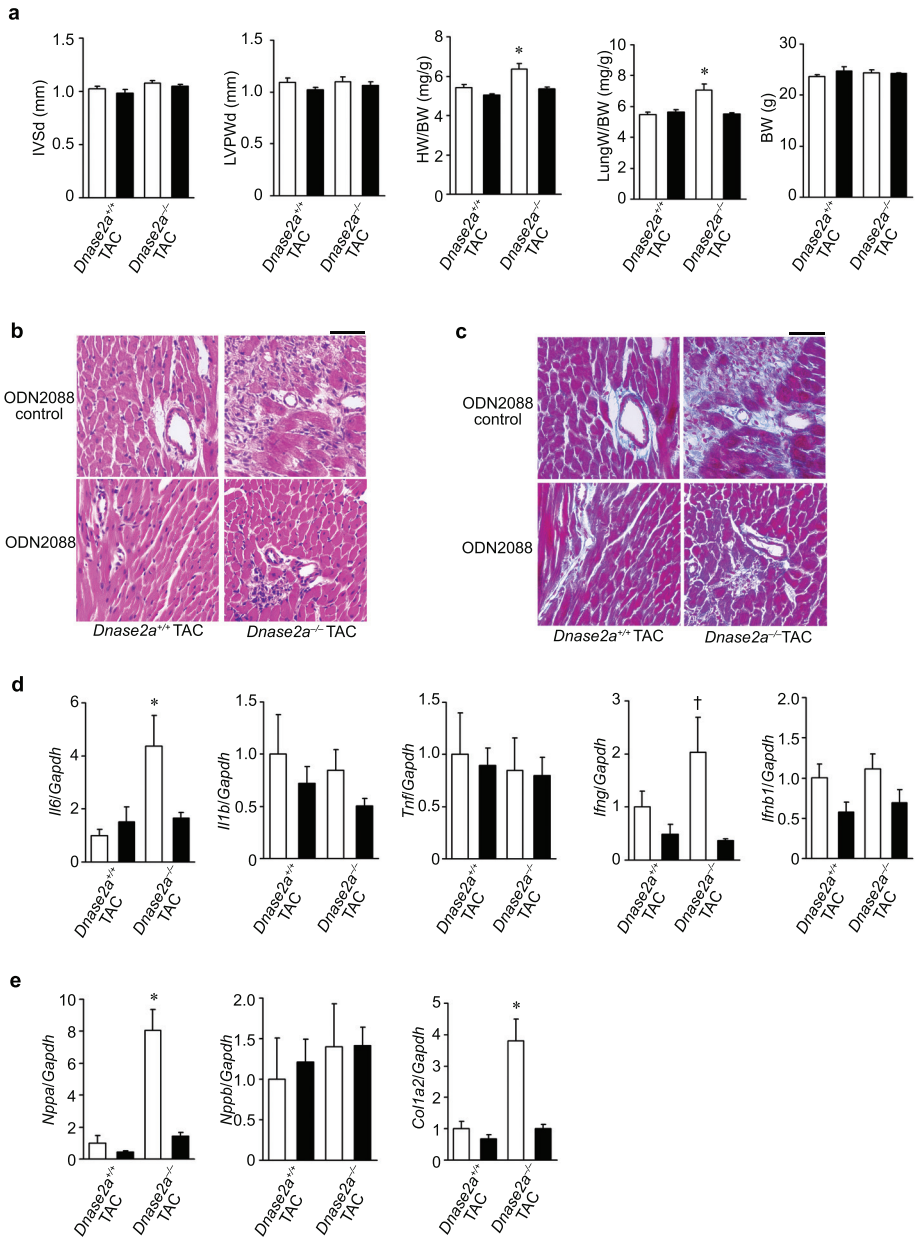
Supplementary Figure 5. Echocardiographic and physiological parameters after TAC. **(a)** Time course of changes in echocardiographic parameters, such as the end-diastolic left ventricle (LV) internal (LVIDd) and end-systolic LV internal dimensions (LVIDs) and LV fractional shortening (LVFS). Echocardiographic analysis was performed before (pre) and 2, 3, 4, 5, and 10 days after TAC (n = 4 - 5). $^{*}P < 0.05$ versus corresponding *Dnase2a*$^{+/+}$ mice. **(b)** Physiological parameters 2 days after TAC. HW/BW, ratio of heart to body weight. LungW/BW, ratio of lung to body weight. Values are expressed as the mean ± s.e.m. (n = 4 - 7). $^{*}P < 0.05$ versus all other groups.



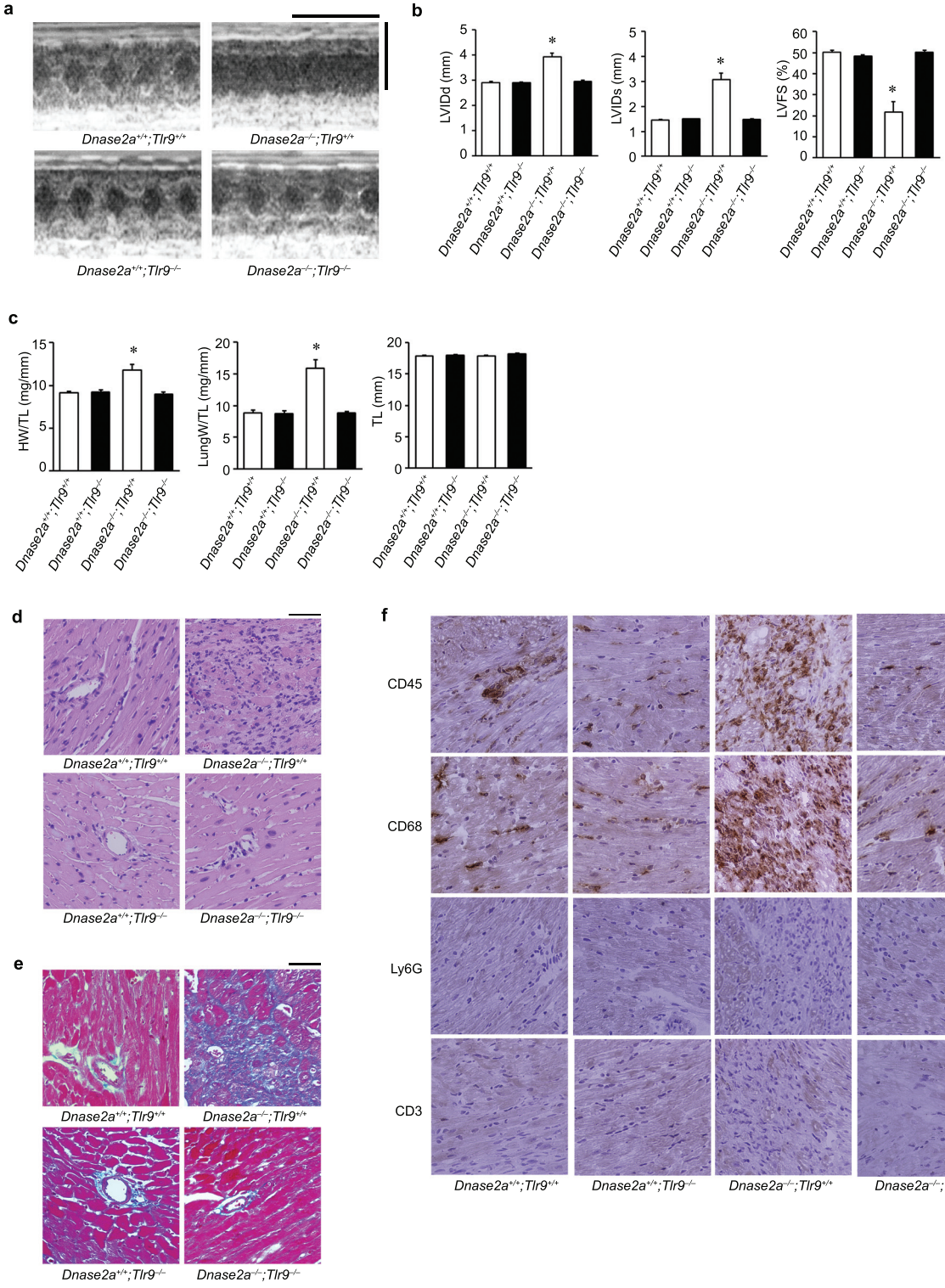
Supplementary Figure 6. Characterization of pressure-overloaded *Dnase2a*$^{-/-}$ hearts. Mice were analyzed 2 days after TAC (**a**-**d**). (**a**) mRNA expressions of inflammatory cytokines. Open and closed bars represent sham- and TAC-operated group, respectively. *Gapdh* was used as the loading control. The averaged value in sham-operated *Dnase2a*$^{+/+}$ mice was set equal to 1. Values are expressed as the mean ± s.e.m. (n = 4 - 7). $^{*}P < 0.05$ versus all other groups. Double staining of heart sections with (**b**) PicoGreen (green) and anti-LAMP2a antibody (red), (**c**) PicoGreen (green) and anti-LC3 antibody (red) or (**d**) PicoGreen (green) and TUNEL (red). Arrows indicate DNA deposits with (**b**) LAMP2a- or (**c**) LC3-positive structures. In (**d**), arrows indicate DNA deposits. Arrow heads indicate nuclei. Scale bar, 10 μm.



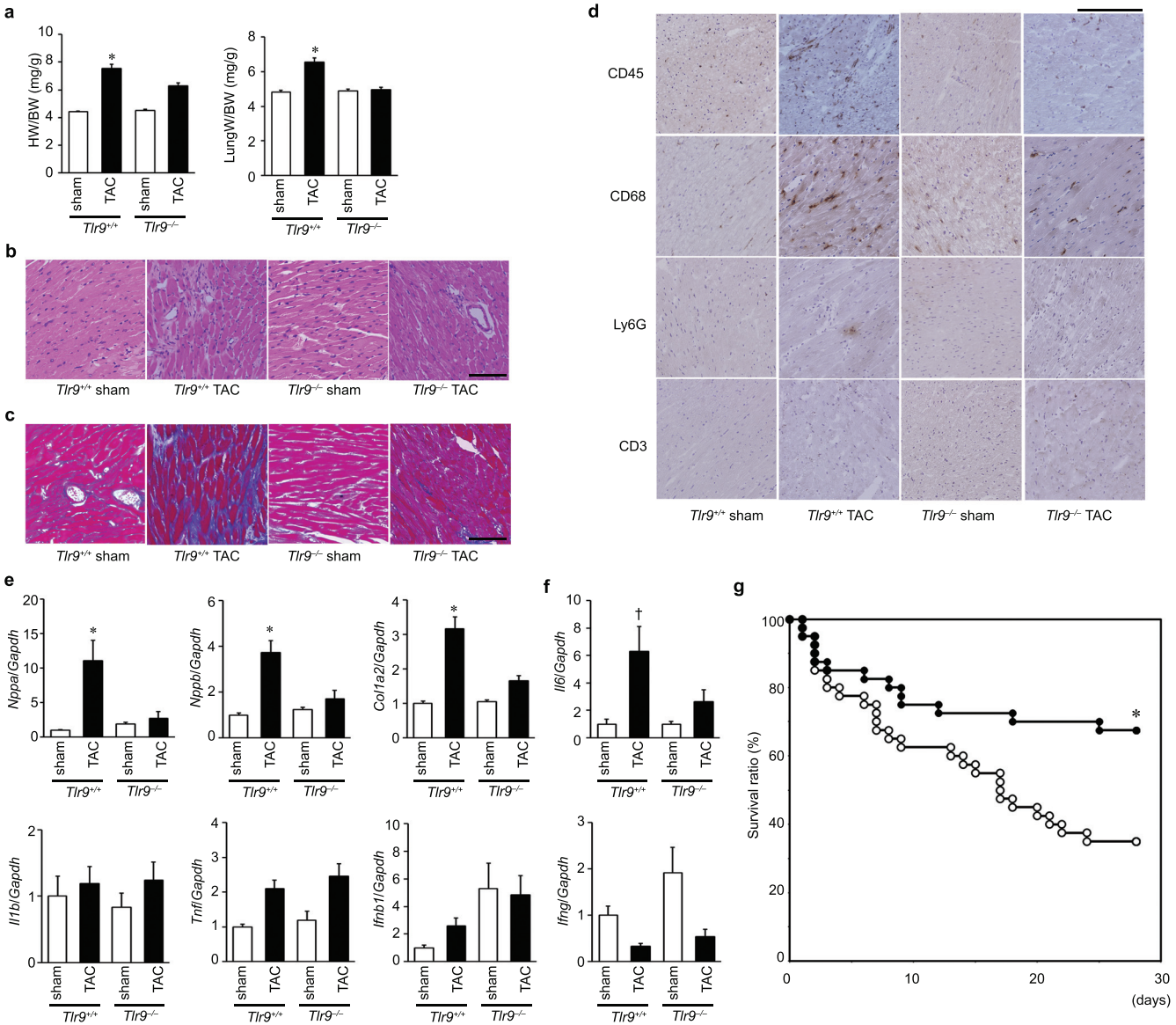
Supplementary Figure 7. TLR9-dependent cell-autonomous induction of cytokine production and cell death in cardiomyocytes. (**a**) Disruption of mitochondrial membrane potential by isoproterenol (ISP). Cardiomyocytes with or without isoproterenol were stained with TMRE, and were observed through light (left panels) or fluorescent microscope (right panels). (**b**) Two hours after treatment with CCCP or ISP, cardiomyocyte lysates were subjected to Western blot analysis using anti-LC3 antibody. To estimate autophagy flux, cardiomyocytes were treated with Bafilomycin A1 (BFA) 1 hour after CCCP or ISP treatment. GAPDH was used as the loading control. (**c**) and (**d**) Representative images of cardiomyocytes stained with trypan blue stimulated with (**c**) CCCP or (**d**) ISP. Mouse adult cardiomyocytes were pretreated with control oligodeoxynucleotides (ODN2088 control) or ODN2088 oligodeoxynucleotides. (**e**) The percentage of trypan blue-negative cardiomyocytes. Values are expressed as the mean ± s.e.m. of 4 independent experiments. Open and closed bars represent cardiomyocytes from *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ mice, respectively. $^{*}P < 0.05$. (**f**) mRNA expression of *Il1b* or *Il6* in CCCP-treated cardiomyocytes. *Gapdh* was used as the loading control. The averaged value in vehicle- and ODN2088 control-treated *Dnase2a*$^{+/+}$ cardiomyocytes was set equal to 1. Values are expressed as the mean ± s.e.m. of 3 independent experiments. $^{*}P < 0.05$ versus all other groups. (**g**) Effect of autophagy activity on *Il1b* or *Il6* mRNA expression in cardiomyocytes. *Gapdh* was used as the loading control. The average value in vehicle-treated *Dnase2a*$^{+/+}$ cardiomyocytes was set equal to 1. Values are expressed as the mean ± s.e.m. of 3 - 4 independent experiments. $^{*}P < 0.05$.



Supplementary Figure 8. Inhibition of TLR9 attenuated pressure overload-induced myocarditis and cardiomyopathy in *Dnase2a*$^{-/-}$ mice. TLR9 inhibitory oligodeoxynucleotides (ODN2088) or inactive control oligodeoxynucleotides (ODN2088 control) were administered before and 2 days after TAC, and analyzed 4 days after TAC (**a**-**e**). Open and closed bars represent ODN2088 control- and ODN2088-treated groups, respectively. (**a**) Echocardiographic and physiological parameters. IVSd indicates diastolic interventricle septum wall thickness; LVPWd, diastolic left ventricle posterior wall thickness; HW, heart weight; BW, body weight; LungW, lung weight, respectively. ODN2088 control-treated (n = 6) or ODN2088-treated (n = 5) TAC-operated *Dnase2a*$^{+/+}$ mice, and ODN2088 control-treated (n = 6) or ODN2088-treated (n = 8) TAC-operated *Dnase2a*$^{-/-}$ mice are shown. (**b**) Hematoxylin-eosin-stained heart sections. Scale bar, 100 μm. (**c**) Azan-Mallory-stained sections. Scale bar, 100 μm. (**d**, **e**) mRNA expressions of inflammatory cytokines, *Nppa*, *Nppb* and *Col1a2* (n = 5 - 8). *Gapdh* was used as the loading control. The averaged value in ODN2088 control-treated *Dnase2a*$^{+/+}$ mice was set equal to 1. Values are expressed as the mean ± s.e.m. $^{*}P < 0.05$ versus all other groups. $^{\dagger}P < 0.05$ versus corresponding controls.



Supplementary Figure 9. Ablation of *Tlr9* attenuated pressure overload-induced myocarditis and dilated cardiomyopathy in TAC-operated *Dnase2a*$^{-/-}$ mice. DNase II and TLR9 double knockout mice were subjected to TAC and analyzed 10 days after operation (**a**-**f**). (**a**) Representative M-mode echocardiographic tracings. Scale bars, 0.2 sec and 5 mm, respectively. Echocardiographic (**b**) and physiological (**c**) parameters. TAC-operated *Dnase2a*$^{+/+}$;*Tlr9*$^{+/+}$ (n = 8), TAC-operated *Dnase2a*$^{+/+}$;*Tlr9*$^{-/-}$ (n = 5), TAC-operated *Dnase2a*$^{-/-}$;*Tlr9*$^{+/+}$ (n = 4 - 5), and TAC-operated *Dnase2a*$^{-/-}$;*Tlr9*$^{-/-}$ mice (n = 8 - 9) are shown. LVIDd indicates end-diastolic left ventricle internal dimension; LVIDs, end-systolic left ventricle internal dimension; LVFS, left ventricle fractional shortening; TL, tibia length; HW, heart weight; LungW, lung weight. Values are expressed as the mean ± s.e.m. *$P < 0.05$ versus all other groups. (**d**) Hematoxylin-eosin-stained heart sections. Scale bar, 100 μm. (**e**) Azan-Mallory-stained heart sections. Scale bar, 100 μm. (**f**) Immunohistochemical analysis using antibodies to CD45, CD68, Ly6G and CD3. Scale bar, 100 μm.



Supplementary Figure 10. Protection from pressure overload-induced heart failure by TLR9 inhibition. *Tlr9*$^{-/-}$ mice were subjected to TAC and analyzed 10 weeks after operation (**a**-**f**). (**a**) Physiological parameters. Sham-operated (n = 8) or TAC-operated *Tlr9*$^{+/+}$ (n = 10) and sham-operated (n = 6) or TAC-operated *Tlr9*$^{-/-}$ (n = 7) mice are shown. HW/BW, ratio of heart to body weight. LungW/BW, ratio of lung to body weight. Values are expressed as the mean ± s.e.m. $^{*}P < 0.05$ versus all other groups. (**b**) Hematoxylin-eosin-stained heart sections. Scale bar, 100 μm. (**c**) Azan-Mallory-stained sections. Scale bar, 100 μm. (**d**) Immunohistochemical analysis. Scale bar, 200 μm. (**e**) mRNA expressions of marker genes of cardiac remodeling or (**f**) inflammatory cytokines in sham-operated (n = 8) or TAC-operated *Tlr9*$^{+/+}$ (n = 10) and sham-operated (n = 5) or TAC-operated *Tlr9*$^{-/-}$ (n = 7) mice. *Gapdh* was used as the loading control. The averaged value in sham-operated *Tlr9*$^{+/+}$ mice was set equal to 1. In panels (**e**) and (**f**), values are expressed as the mean ± s.e.m. $^{*}P < 0.05$ versus all other groups. $^{\dagger}P < 0.05$ versus corresponding sham-operated controls. (**g**) Survival ratio after severe-TAC. Wild-type mice were subjected to severe-TAC. TLR9 inhibitory oligodeoxynucleotides (ODN2088) or inactive control oligodeoxynucleotides (ODN2088 control) were administered before and 2 and 4 days after TAC, and every 3 days thereafter. Open and closed circles indicate TAC-operated ODN2088 control-treated (n = 40) and ODN2088-treated (n = 40) mice, respectively. $^{*}P < 0.05$.

Supplementary Table 1. Physiological and echocardiographic parameters in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ mice at baseline

	Dnase2a$^{+/+}$ (n=8)			Dnase2a$^{-/-}$ (n=8)		
Blood pressure (mmHg)	89	±	2.9	91.4	±	2.5
BW (g)	25.1	±	0.19	25.3	±	0.32
HW (mg)	105.8	±	1.3	107.5	±	1.6
Atrium weight (mg)	12.7	±	0.9	13.2	±	1.1
Right ventricle weight (mg)	13.9	±	1	14.7	±	1
LVW (mg)	79.3	±	1	79.6	±	1.3
Lung weight (mg)	136.7	±	1.9	131.5	±	2.2
Liver weight (mg)	1056.3	±	32.2	1025.7	±	15.5
HW/BW (mg/g)	4.22	±	0.03	4.26	±	0.06
LVW/BW (mg/g)	3.16	±	0.03	3.15	±	0.05
LVIDd (mm)	2.78	±	0.02	2.74	±	0.01
LVIDs (mm)	1.42	±	0.02	1.41	±	0.01
LVFS (%)	49	±	0.71	48.4	±	0.47
IVSd (mm)	0.73	±	0.01	0.74	±	0.01
LVPWd (mm)	0.89	±	0.01	0.88	±	0.01
Heart rate (bpm)	754	±	4.2	759	±	6.8

Mice were 12-14 weeks old. BW indicates body weight; HW, heart weight; LVW, left ventricle weight; LVIDd, end-diastolic left ventricle internal dimension; LVIDs, end-systolic left ventricle internal dimension; LVFS, left ventricle fractional shortening; IVSd, diastolic interventricle septum wall thickness; LVPWd, diastolic left ventricle posterior wall thickness. Data are expressed as the means ± s.e.m. There were no significant differences between *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ mice in any parameters.

Supplementary Table 2. Physiological and echocardiographic parameters in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ mice 10 days after pressure overload.

	Dnase2a$^{+/+}$		*Dnase2a*$^{-/-}$	
	sham (*n*=8)	TAC (*n*=13)	Sham (*n*=10)	TAC (*n*=7)
BW (g)	24.4 ± 0.3	24.5 ± 0.2	24.3 ± 0.2	23.9 ± 0.4
HW (mg)	106.9 ± 1.7	137.2 ± 1.3*	106.8 ± 2.2	171.9 ± 6.3*,†
LVW (mg)	79.6 ± 1.2	109.2 ± 1.4*	81 ± 1.7	136.6 ± 4.0*,†
HW/BW (mg/g)	4.38 ± 0.06	5.6 ± 0.06*	4.4 ± 0.09	7.2 ± 0.27*,†
Lung weight/BW (mg/g)	5.6 ± 0.1	5.8 ± 0.2	5.6 ± 0.1	11.1 ± 1.3*,†
Liver weight/BW (mg/g)	44.9 ± 1.1	43.6 ± 1.1	45.1 ± 1.3	44.7 ± 1.0
LVIDd (mm)	2.76 ± 0.02	2.68 ± 0.02	2.78 ± 0.02	3.6 ± 0.17*,†
LVIDs (mm)	1.45 ± 0.02	1.42 ± 0.02	1.47 ± 0.02	2.77 ± 0.25*,†
LVFS (%)	47.5 ± 0.49	46.9 ± 0.71	47.1 ± 0.64	24.4 ± 4.52*,†
IVSd (mm)	0.74 ± 0.01	0.99 ± 0.02*	0.73 ± 0.02	0.91 ± 0.02*
LVPWd (mm)	0.93 ± 0.01	1.16 ± 0.02*	0.94 ± 0.02	1.01 ± 0.03*,†
Heart rate (bpm)	731.5 ± 8.4	739.9 ± 8.9	738.2 ± 8.1	695.9 ± 14.9*,†

BW, body weight; HW, heart weight; LVW, left ventricle weight; LVIDd, end-diastolic left ventricle internal dimension; LVDs, end-systolic left ventricle internal dimension; LVFS, left ventricle fractional shortening; IVSd, diastolic interventricle septum wall thickness; LVPWd, diastolic left ventricle posterior wall thickness. Data are expressed as the means ± s.e.m. $^{*}P < 0.05$ versus corresponding sham-operated mice, $^{\dagger}P < 0.05$ versus TAC-operated *Dnase2a*$^{+/+}$ mice.