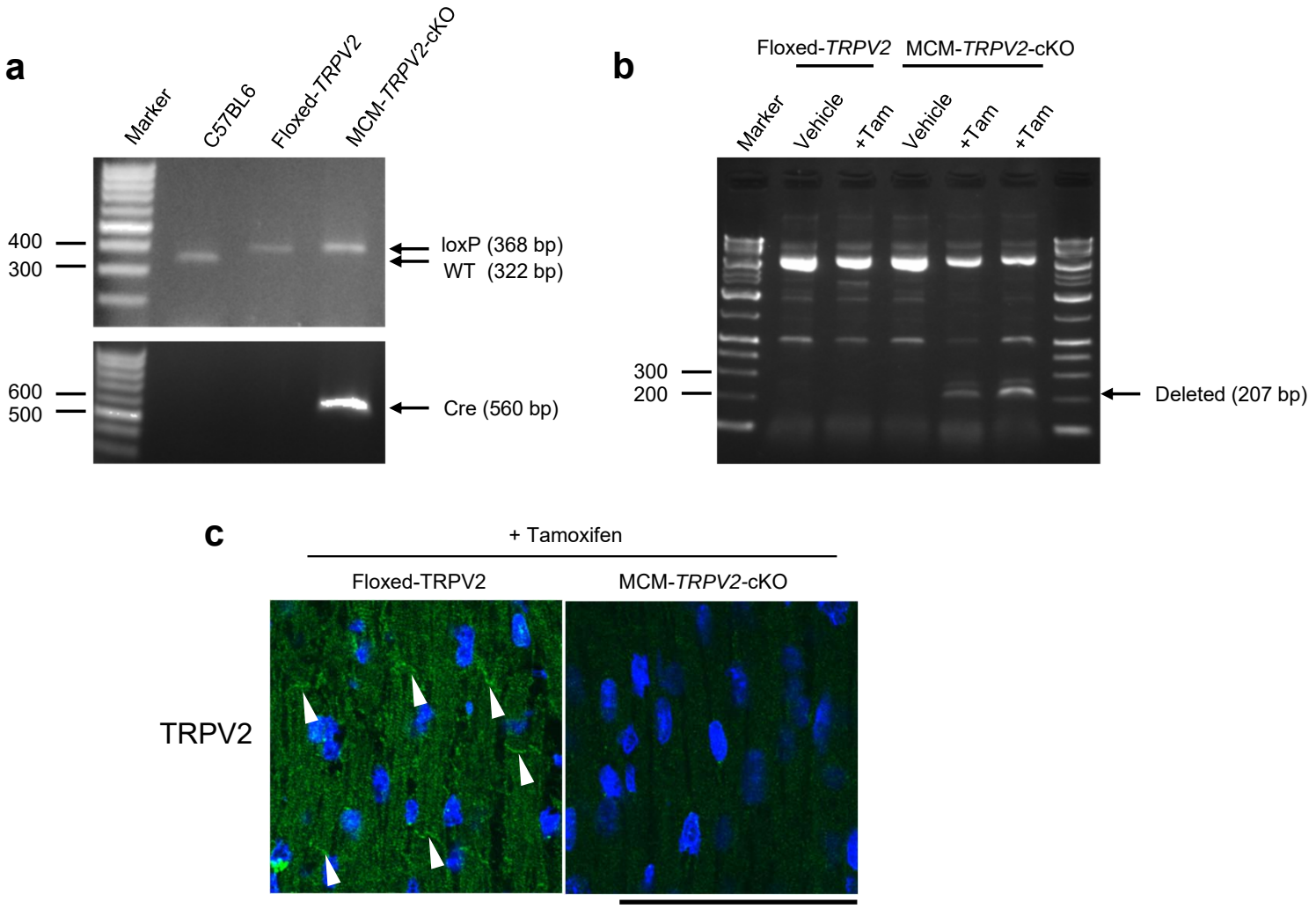
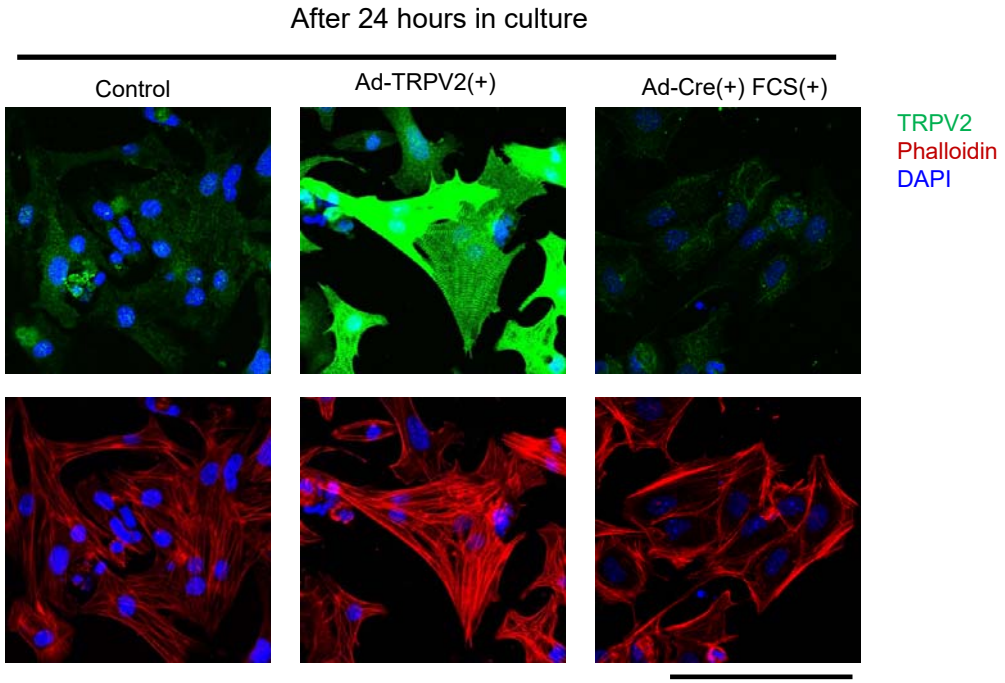
TRPV2 mediates stress resilience in mouse cardiomyocytes

Yubing Dong[1,2], Guohao Wang[1], Yoshihiro Ujihara[3], Yanzhu Chen[1,2], Masashi Yoshida[4], Kazufumi Nakamura[5,6], Kimiaki Katanosaka[7], Keiji Naruse[1], Yuki Katanosaka[1,2]

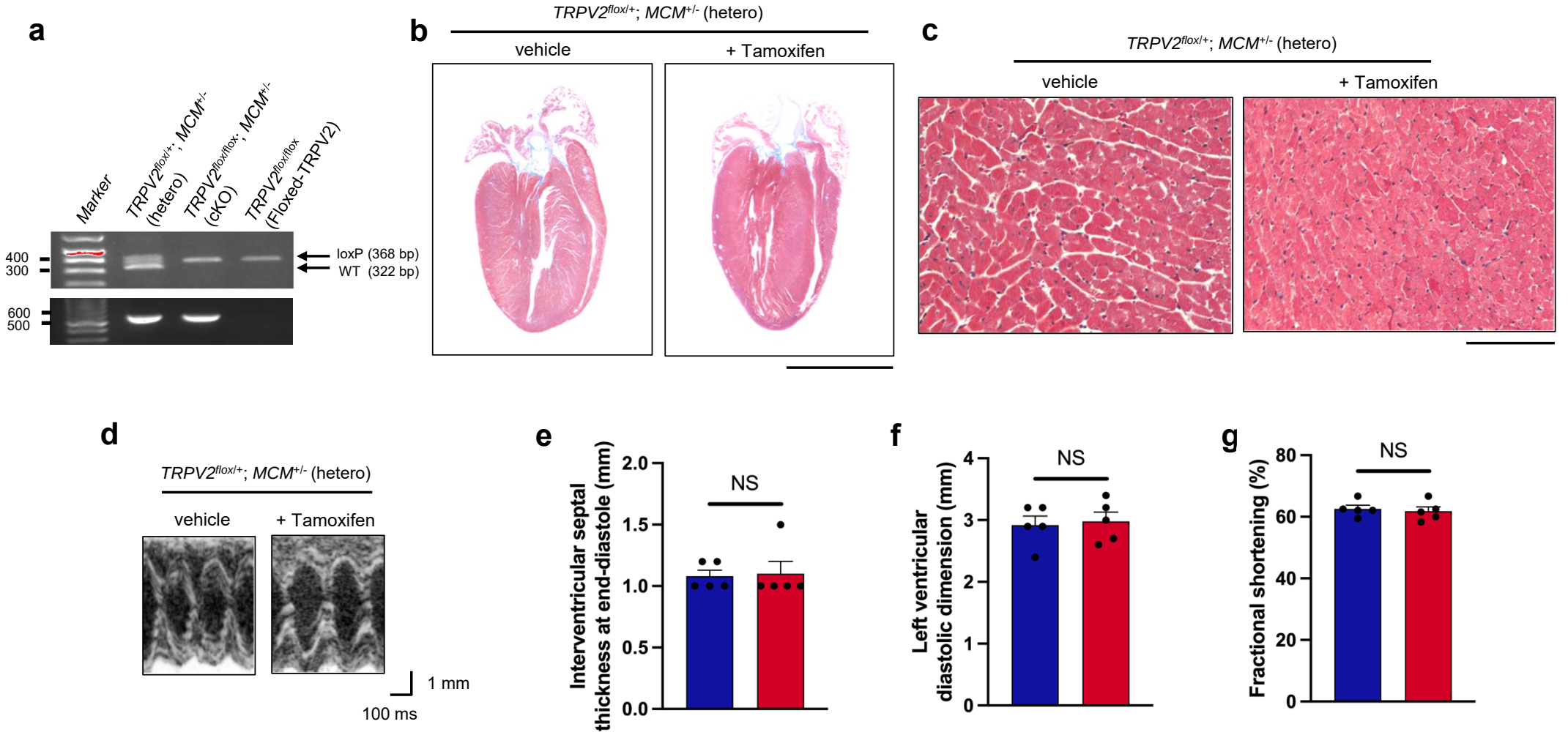
[1]Department of Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[2]Department of Pharmacy, College of Pharmacy, Kinjo Gakuin University, Nagoya, Aichi, Japan
[3]Department of Electrical and Mechanical Engineering, Graduate School of Engineering, Nagoya Institute of Technology, Nagoya, Aichi, Japan
[4]Department of Chronic Kidney Disease and Cardiovascular Disease, Okayama University Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.
[5]Department of Cardiovascular Medicine, Okayama University Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.
[6]Center for Advanced Heart Failure, Okayama University Hospital
[7]Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, Kasugai, Aichi, Japan



Supplementary Figure 1: Genotyping PCR of mouse genomic DNA, *Cre*-recombination, and reduced expression of *TRPV2* in TRPV2-deficient mice. (**a**) Genotyping PCR of mouse genomic DNA. (top) PCR fragments including the loxP insertion site on the *TRPV2* locus: the 322-bp fragment indicates wild-type *TRPV2*, and the 368-bp fragment indicates the insertion of loxP. (bottom) PCR fragments of the Cre recombinase gene. (**b**) Confirmation of *Cre*-mediated recombination by PCR of cardiac genomic DNA. **(c)** Representative immunofluorescence image of anti-TRPV2 antibody in the adult heart. Scale bar, 100 μm.



Supplementary Figure 2: Change in TRPV2 expression induced by adenovirus infection in newborn cardiomyocytes.
Representative immunofluorescence image of anti-TRPV2 antibody. Triple staining with TRPV2 (green), phalloidin (red), and DAPI(blue). Scale bar, 100 μm.



Supplementary Figure 3: No change in cardiac morphology, histology and cardiac function after tamoxifen treatment (i.p. injection for 3 consecutive days at 8 mg/kg body weight) in C57BL/6-congenic *TRPV2*$^{flox/+}$;*MCM*$^{+/-}$ (hetero) mice. Tamoxifen administration was started at 2 weeks of age, and cardiac function and morphology were analyzed at 12 weeks of age.
(a) Representative PCR image of mouse genomic DNA. (top) PCR fragments including the loxP insertion site on the *TRPV2* locus: the 322-bp fragment indicates wild-type *TRPV2*, and the 368-bp fragment indicates the insertion of loxP. (bottom) PCR fragments of the Cre recombinase gene. (b) Cardiac morphology. Scale bar, 5 mm. (c) Masson's trichrome staining of left ventricle. Scale bar, 100 μm. (d) Representative trace by two-dimensional transthoracic M-mode echocardiography. (e) Interventricular septal thickness at endo-diastole. (f) Left ventricular diastolic dimension. (g) Fractional shortening. (N=5 in each group). Data are expressed as means ± s.e.m.

Supplementary Figure 3, Dong et. al.

Figure 1b

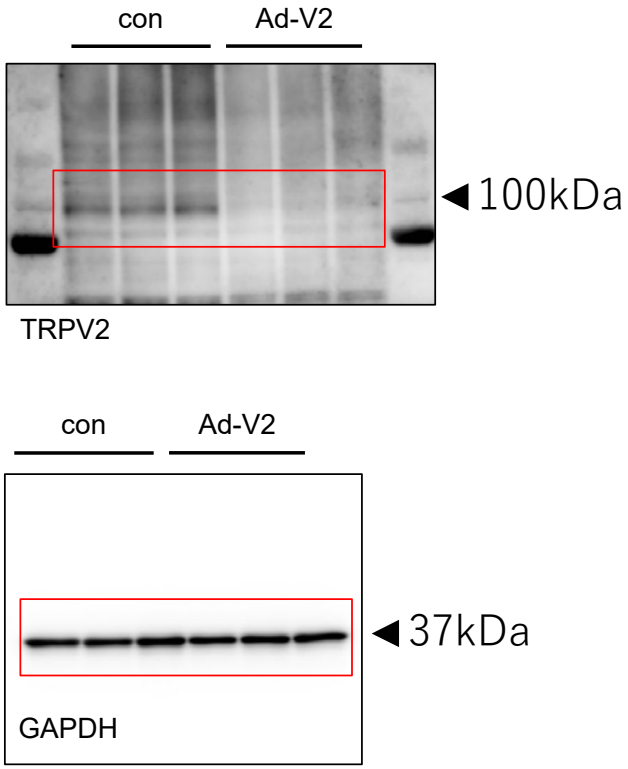
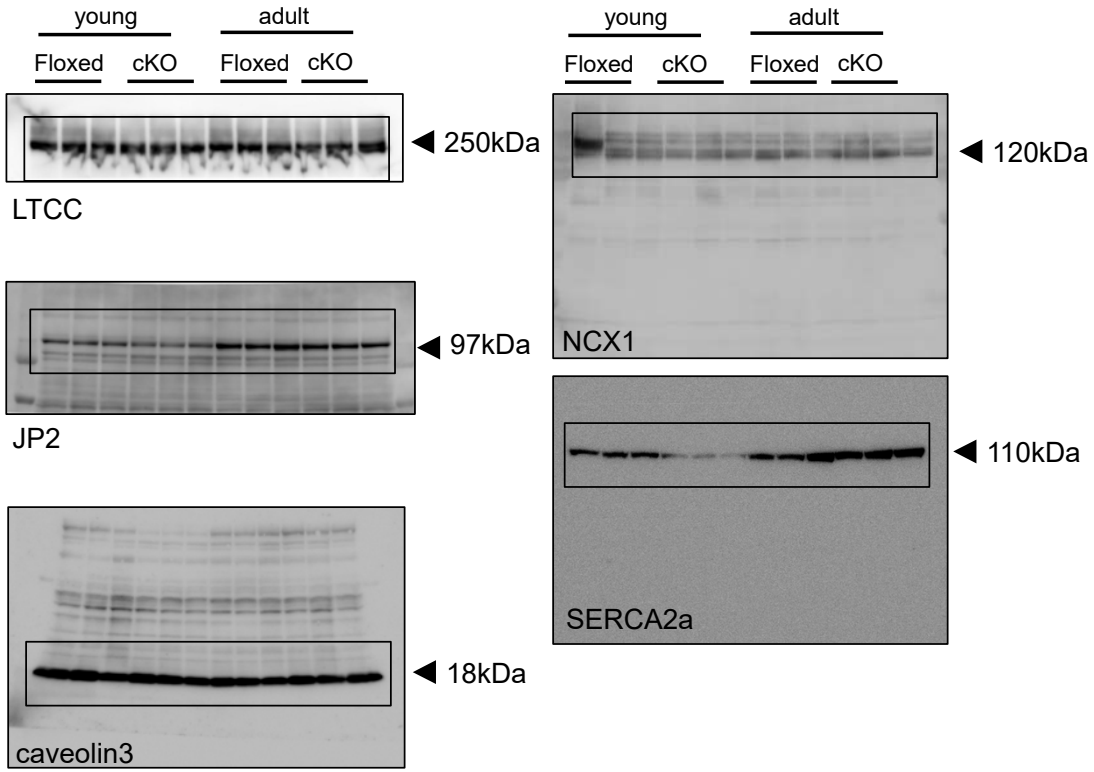
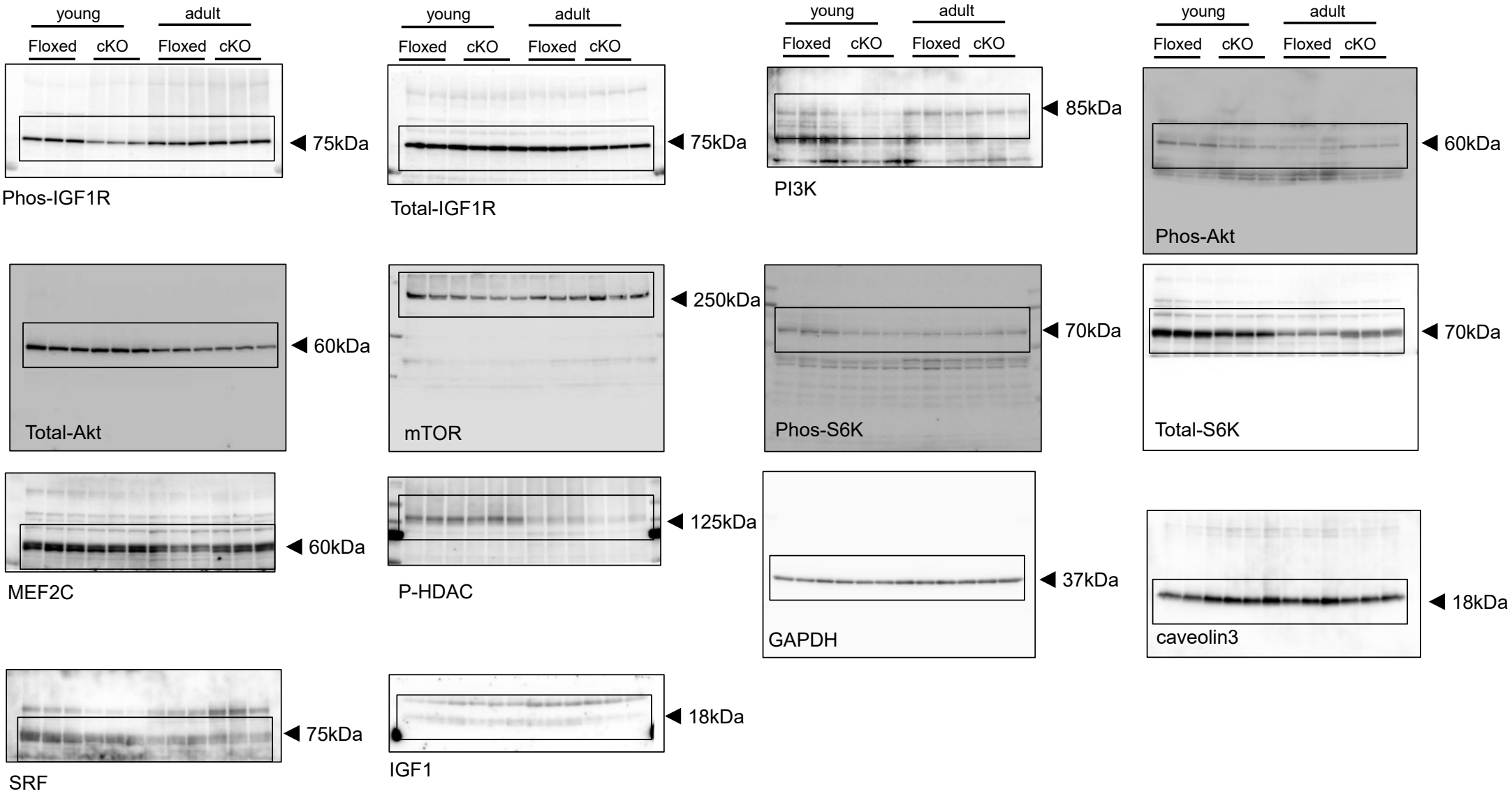


Figure 3b



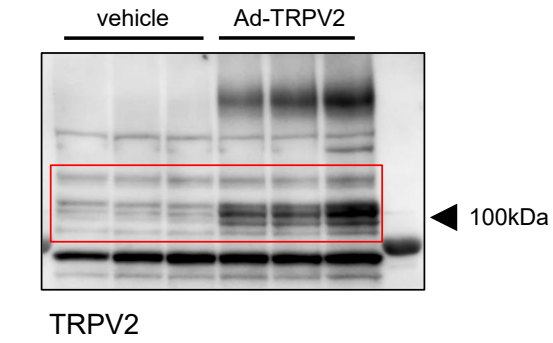
Supplementary Fig. 4-1: Full-length images of immunoblots for Figures 1ba and 3b.
For each lane, 20 μg protein extract was loaded.

Figure 5a



Supplementary Fig. 4-2: Full-length images of immunoblots for Figure 5a.
For each lane, 20 μg protein extracts were loaded.

Figure 7b (upper)



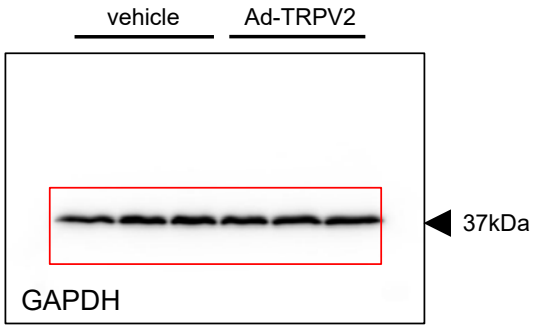
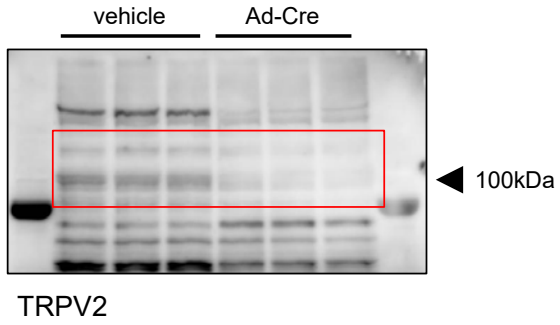


Figure 7b (lower)



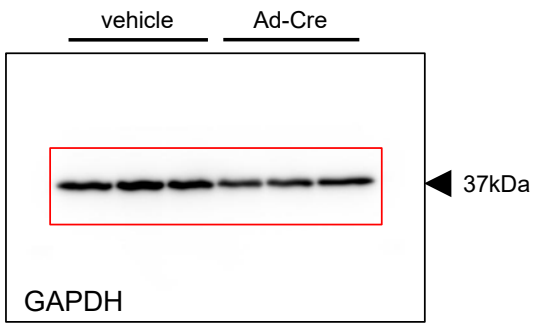
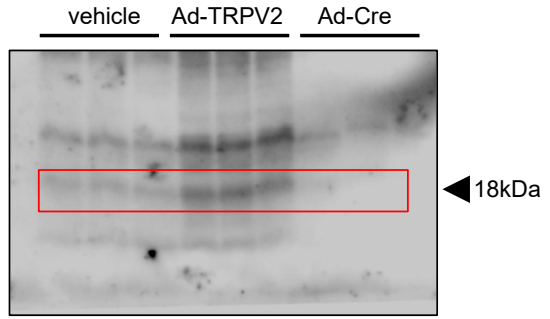
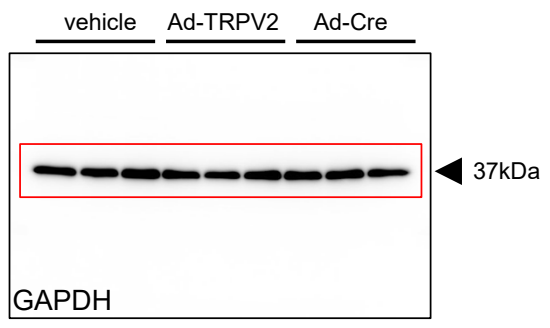


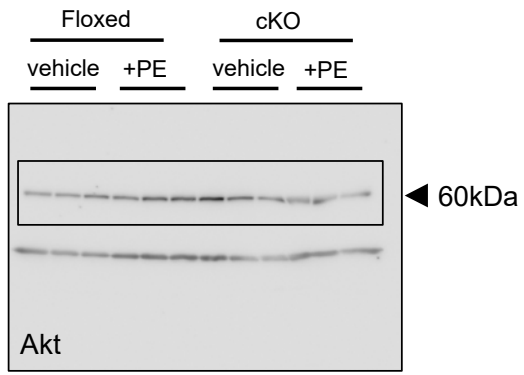
Figure 7c

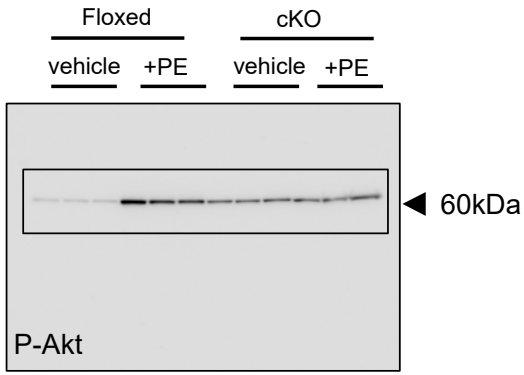


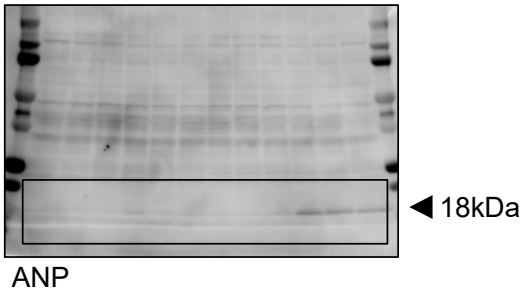


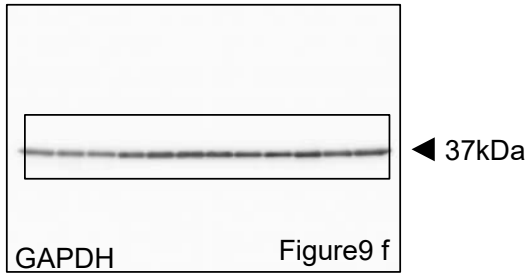
Supplementary Fig. 4-3: Full-length images of immunoblots for Figure 7b and c.
For each lane, 20 μg protein extracts were loaded.

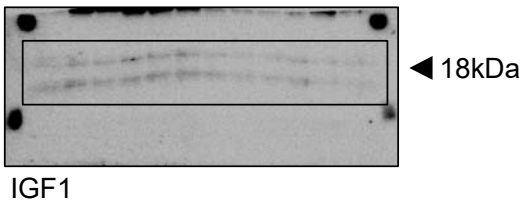
Figure 9f











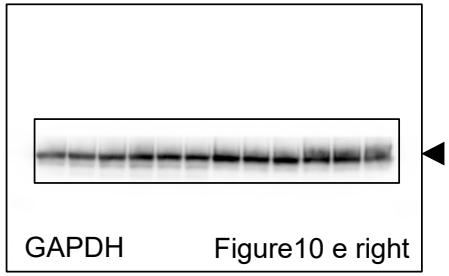
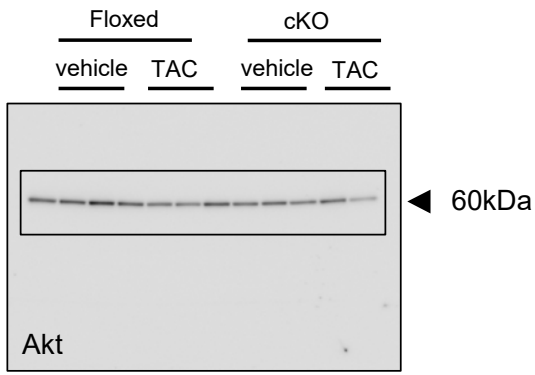
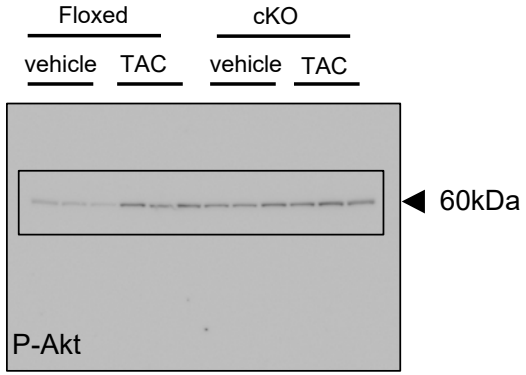
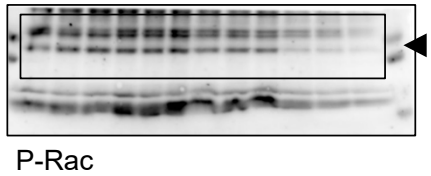
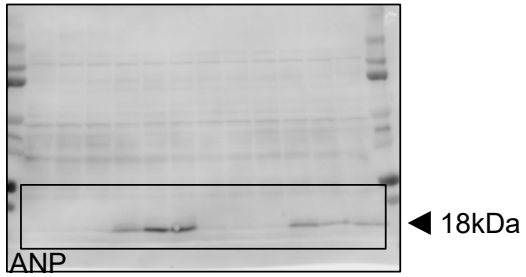


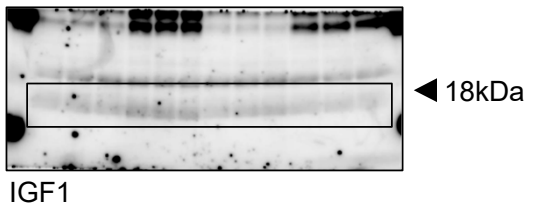
Figure 10e

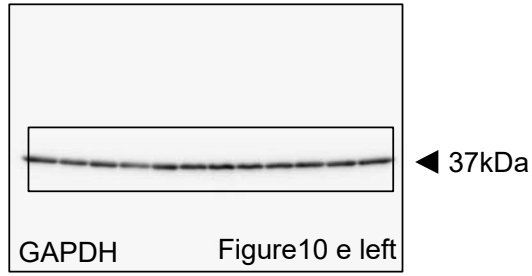












Supplementary Fig. 4-4: Full-length images of immunoblots for Figure 9f and 10e.
For each lane, 20 μg protein extracts were loaded.