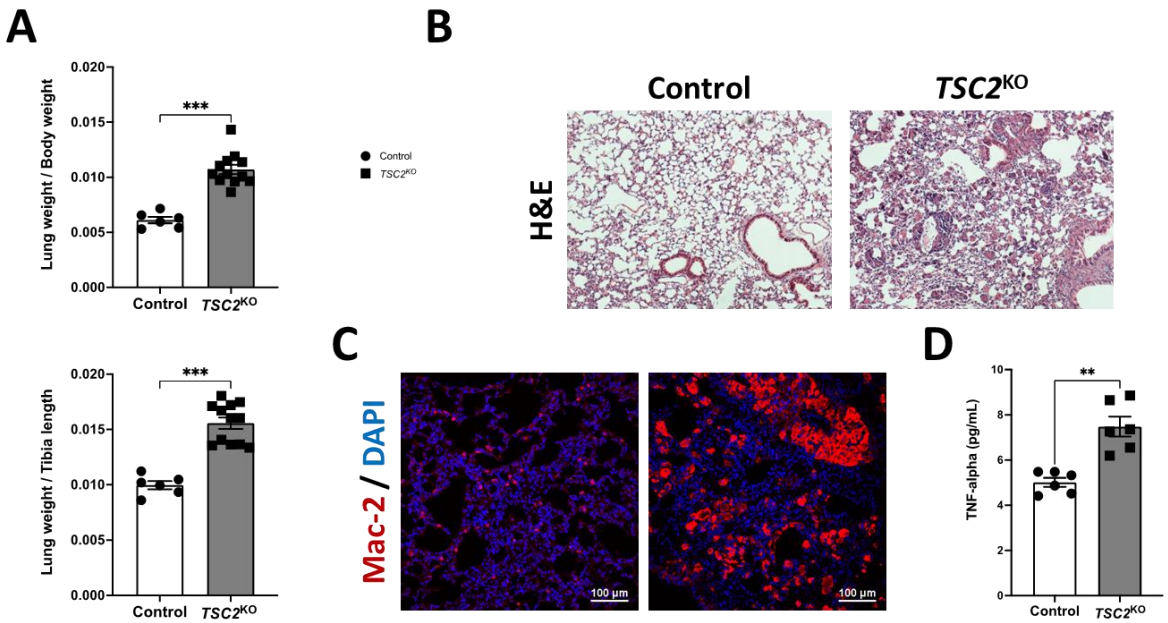
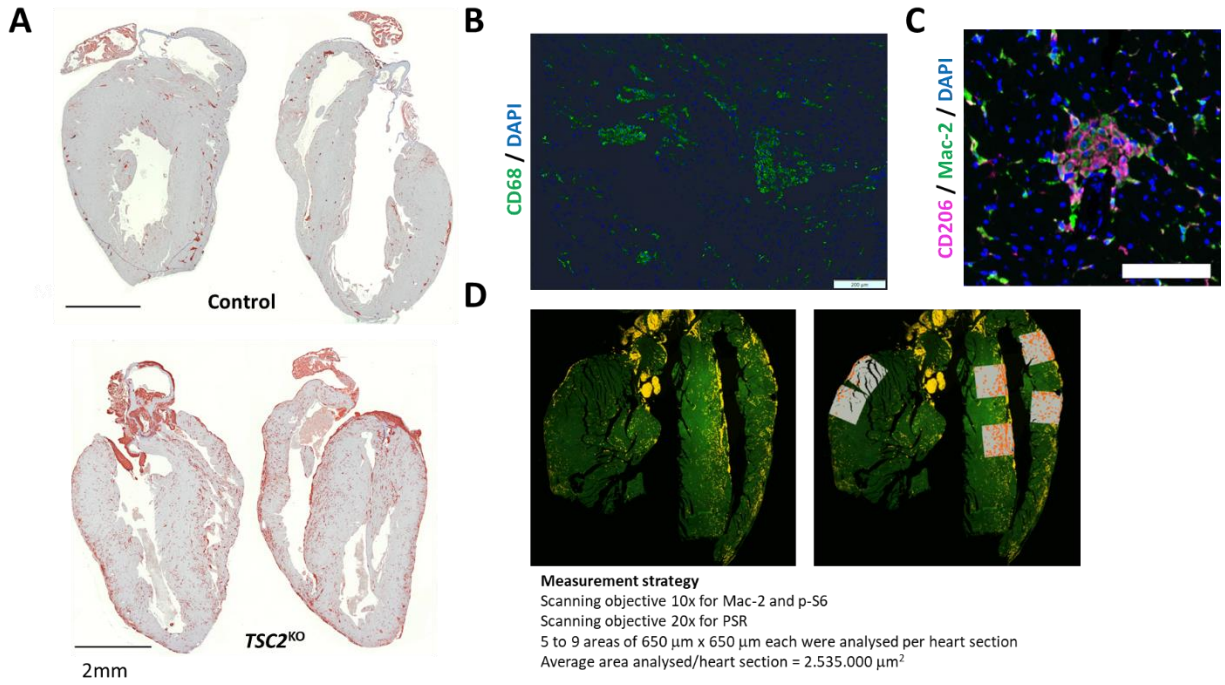
Supplemental Material

Figure S1. Histopathological characterization of the *TSC2*^{KO} mouse lungs.



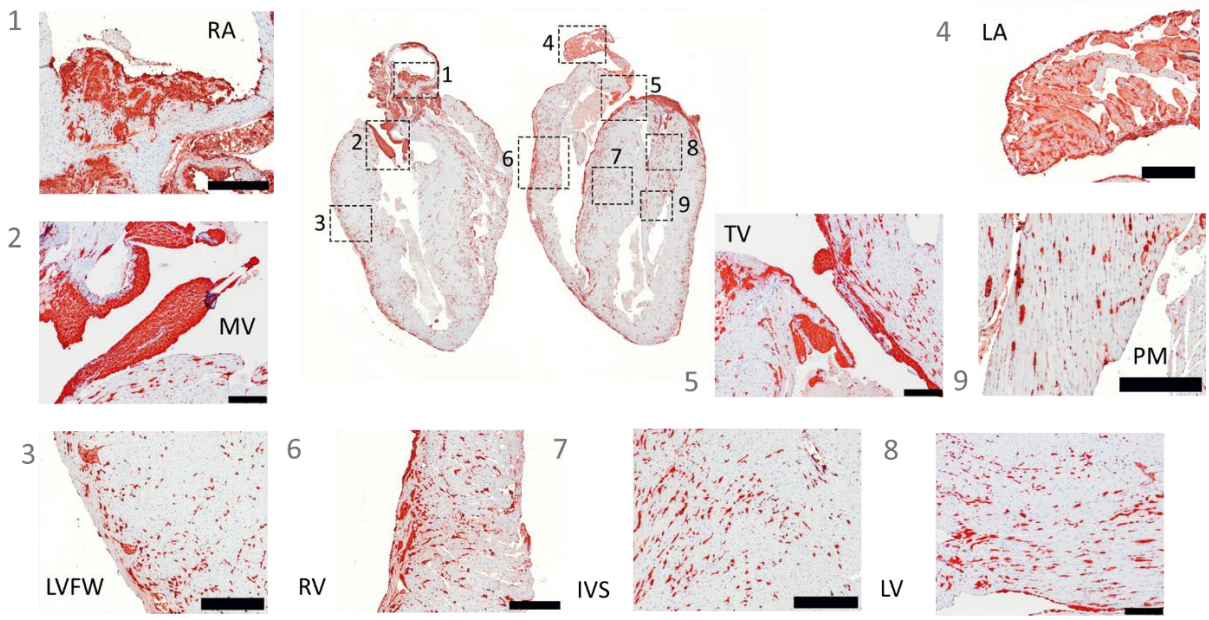
(A) At 34 woa, gross pathology shows increased lung weight to body weight ratio in the *TSC2*^{KO} group when compared to Control group. Lung/ body weight ratios are presented as mean ± SEM (n=6 for the Control group and n = 12 for *TSC2*^{KO} group. *** *P* =0.001 for Controls vs. *TSC2*^{KO} by using a Mann-Whitney test. **(B)** H&E staining (200X magnification). Conditional deletion of *TSC2* in macrophages induces the formation of non-caseating granulomatous aggregates in the lungs of in the *TSC2*^{KO} mice that are absent in the control mice. **(C)** Epithelioid cell clusters in the lungs of *TSC2*^{KO} mice express macrophage marker Mac-2. Immunofluorescence staining against Mac-2 (red); nuclei (blue). Scale bar: 100 µm. **(D)** Concentration of TNF-alpha in the serum of control and *TSC2*^{KO} mice. ** *P* =0.0022 for Controls vs. *TSC2*^{KO} by using a Mann-Whitney test; *TSC2*^{KO}, tuberous sclerosis complex 2 knock out; H&E, haematoxylin and eosin; TNF, tumor necrosis factor; DAPI, 4',6-Diamidin-2-phenylindol.

Figure S2. Histopathological characterization of *TSC2*^{KO} mouse heart at 40 weeks of age.



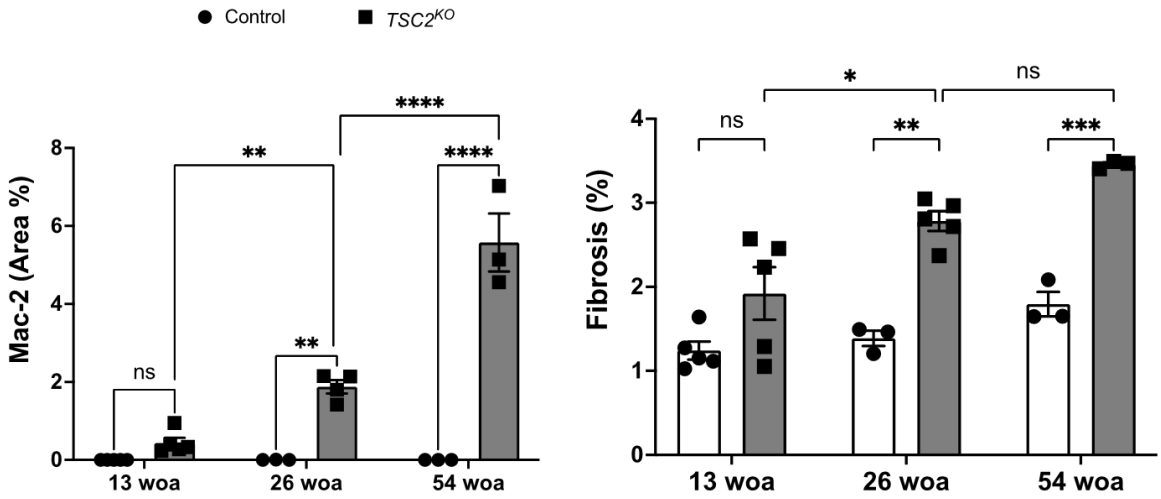
(**A**) Mac-2 staining of whole heart section of control and *TSC2*^{KO} heart (**B**) CD68 and DAPI immunofluorescence staining of left ventricle myocardium from *TSC2*^{KO} heart (**C**) Close-up immunofluorescence image of a granuloma in the perivascular region of the heart. Image stained with DAPI and CD206 and Mac-2 antibodies. Scale bar: 100 µm (**D**) Strategy for measurement of Mac-2, p-S6 expression and picrosirius red (PSR) collagen staining in the hearts of control and sarcoidosis mice; *TSC2*^{KO}, tuberous sclerosis complex 2 knock out.

Figure S3. Granuloma involvement in *TSC2*^{KO} mouse heart at age 40 weeks characterized by Mac-2 staining.



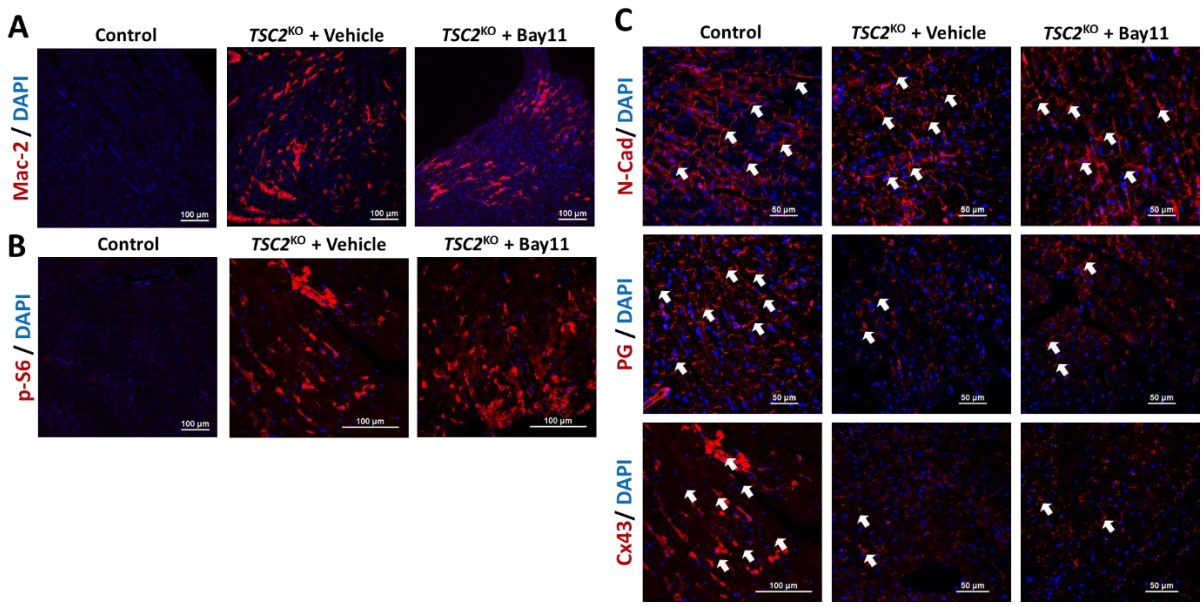
1) RA; right atrium; 2) MV; mitral valve; 3) left ventricle free wall; 4) LA; left atrium; 5) TV; tricuspid valve; 6) RV; right ventricle; 7) IVS; interventricular septum; 8) LV; left ventricle; 9) PM; papillary muscles. Scale bar: 200 µm; *TSC2*^{KO}, tuberous sclerosis complex 2 knock out.

Figure S4. Progressive infiltration of Mac-2 macrophages and fibrotic tissue damage in the hearts of *TSC2*^{KO} mice heart as the mice age.



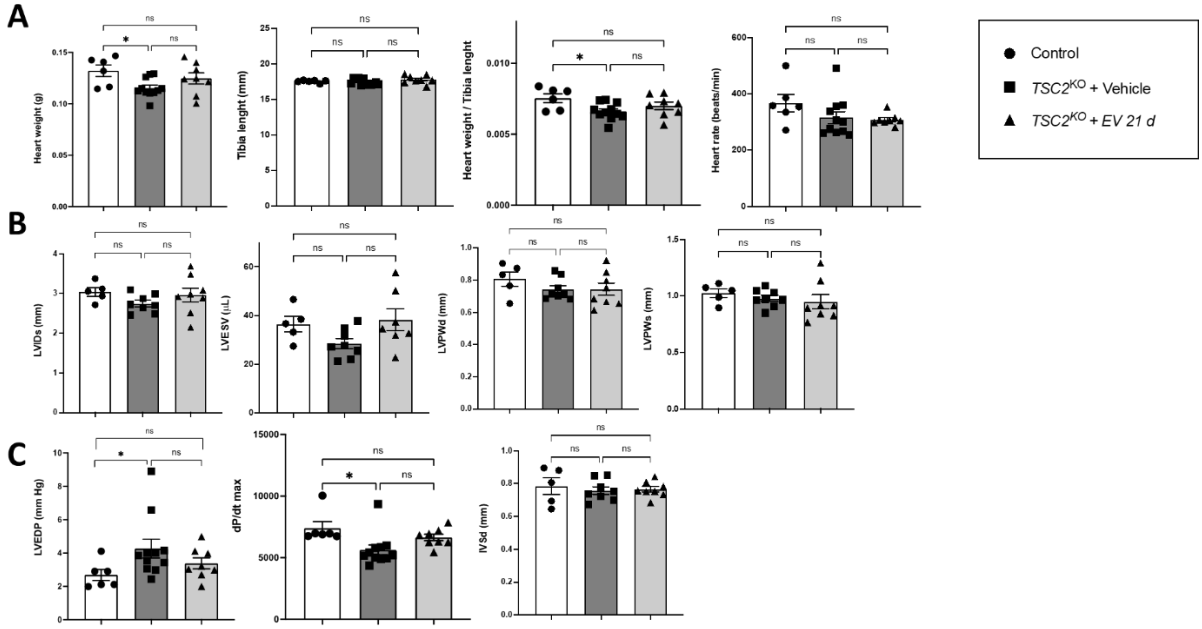
Quantification of Mac-2 and picrosirius red immunohistochemistry stain (for fibrosis) in control *TSC2*^{floxed} and *TSC2*^{KO} mice heart as they age. woa: weeks of age. Data are expressed as mean ± SEM. ns: not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$, by one-way ANOVA with Tukey multiple comparison test; *TSC2*^{KO}, tuberous sclerosis complex 2 knock out; woa, weeks of age.

Figure S5. Inhibition of nuclear factor-κB (NF-κB) with Bay 11-7082 has no effect on cardiac sarcoidosis progression in the *TSC2*^{KO} animal model.



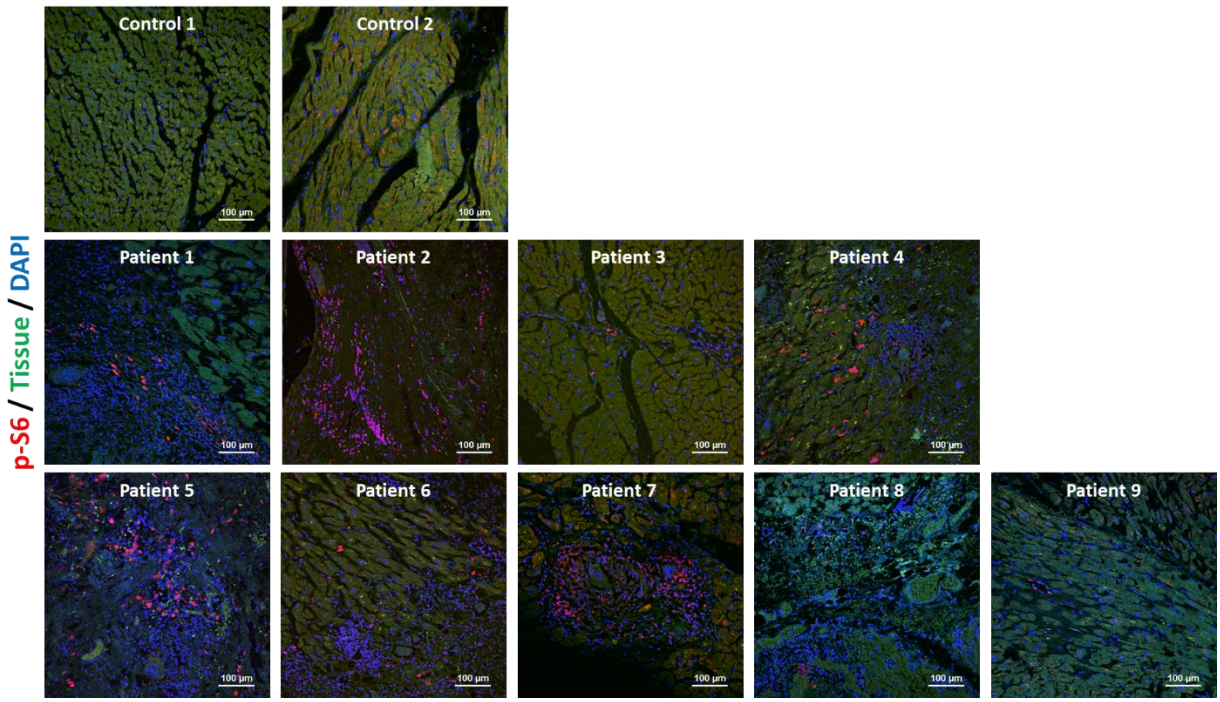
(**A**) Representative confocal images of Control and *TSC2*^{KO} mice hearts treated as previously described immunostained with Mac-2 (red); nuclei were counterstained with DAPI (blue). Scale bar, 100 µm (**B**) Representative confocal images of Control and *TSC2*^{KO} mice hearts treated as previously described immunostained with p-S6 (red); nuclei were counterstained with DAPI (blue). Scale bar, 100 µm. The hearts of *TSC2*^{KO} animals treated with Bay11-7082 for 21 days showed similar numbers of Mac-2+ and p-S6+ cells than *TSC2*^{KO} vehicle-treated group. (**C**) Representative confocal images of Control and *TSC2*^{KO} mice hearts immunostained with N-Cadherin (N-Cad), plakoglobin (PG) and connexin 43 (Cx43) (red); nuclei were counterstained with DAPI (blue). Arrows show the localization of immunoreactive signal at the cardiac intercalated disks. N-cadherin normal distribution in all experimental groups is shown as a tissue quality control. *TSC2*^{KO} animals receiving vehicle showed reduced immunoreactive signal for plakoglobin and connexin 43 when compared to the Control group. No improvement in the expression levels and localization of Plakoglobin and Connexin 43 was observed in *TSC2*^{KO} animals receiving Bay11-7082 for 21 days. Scale bar, 50 µm, (n = 6 for Control, *TSC2*^{KO} + Vehicle, n=4 for Bay11); *TSC2*^{KO}, tuberous sclerosis complex 2 knock out; N-Cad, N-Cadherin; PG, plakoglobin; Cx43, connexin 43; DAPI, 4',6-Diamidin-2-phenylindol.

Figure S6. Quantitative echocardiography and hemodynamic measurements of vehicle- and everolimus-treated *TSC2*^{KO} mice for 21 days and Control mice at 29 weeks of age.



(A) Group data of heart weight, tibia length, ratio heart weight / tibia length, heart rate. **(B)** Group data of left ventricle internal diameter in systole (LVIDs), left ventricle end systolic volume (LVESV), left ventricular posterior wall thickness in diastole (LVPWd), and left ventricular posterior wall thickness in systole (LVPWs). **(C)** Group data of left ventricular end diastolic pressure (LVEDP), dP/dT values from hemodynamic measurements and interventricular septum in diastole (IVSd). Data are expressed as mean ± SEM. (n = 5-6 for Control group, n = 11-12 for *TSC2*^{KO} + vehicle and n = 9 for *TSC2*^{KO} + EV 21 d). ns: not significant, * $P < 0.05$, ** $P < 0.01$ and **** $P < 0.0001$ by 1-way ANOVA with Tukey multiple comparison test except for LVEDP, and LVESV where Kruskal-Wallis test with Dunn's post-hoc (Bonferroni) multiple comparison test was performed; *TSC2*^{KO}, tuberous sclerosis complex 2 knock out; LVIDd, left ventricle internal diameter in diastole; LVESP; ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure; RVESP, right ventricular end systolic pressure; LV, left ventricle; EV, everolimus.

Figure S7. mTOR signaling activation in human cardiac sarcoidosis.



Representative images of hearts immunostained with p-S6 (red); autofluorescence (green) was used to image healthy myocardial tissue; nuclei were counterstained with DAPI (blue); mTOR, mechanistic target of rapamycin; DAPI, 4',6-Diamidin-2-phenylindol.