# Supplementary Materials

**T helper cells with specificity for an antigen in cardiomyocytes promote pressure overload-induced progression from hypertrophy to heart failure**

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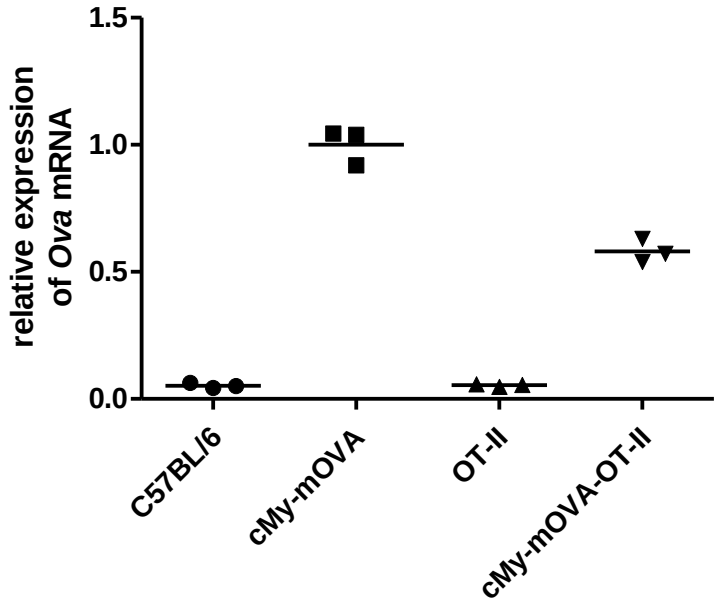
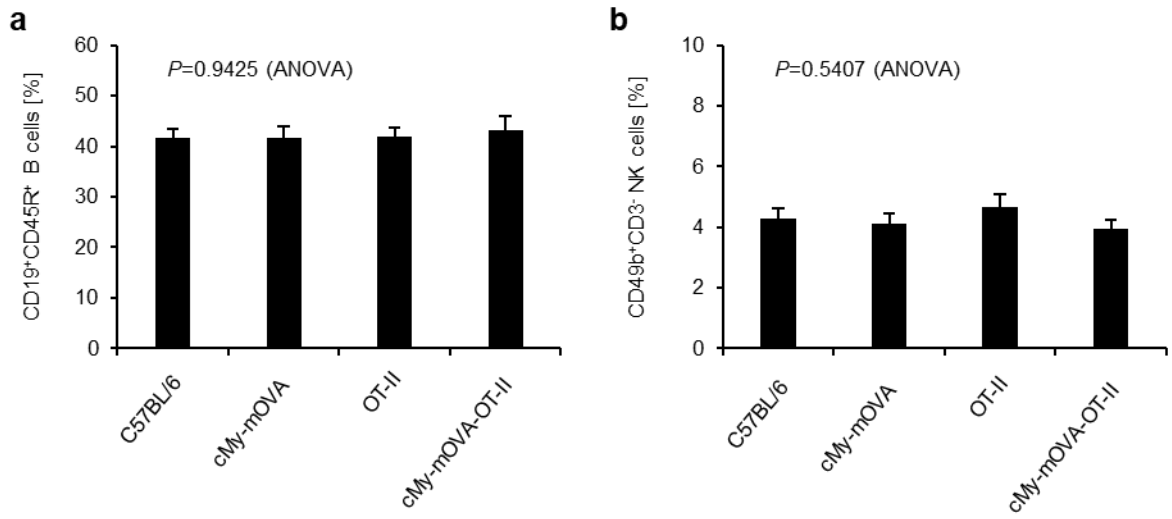
Supplementary Figures 1 to 8

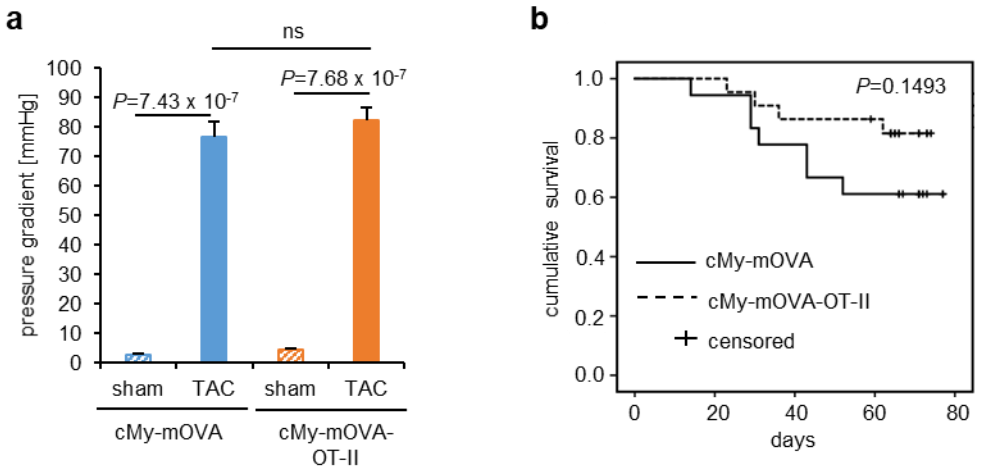
Supplementary Table 1



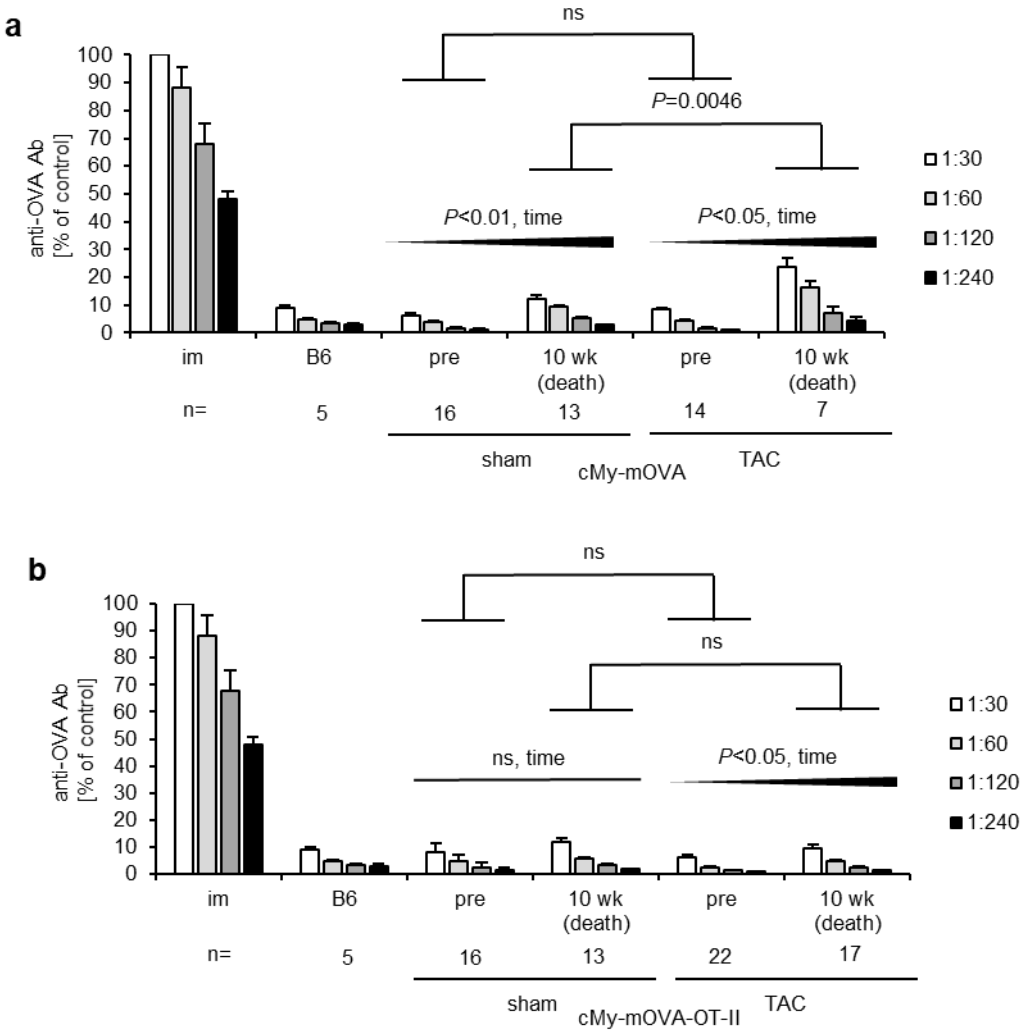

**Supplementary Figure 1.** *Ova* mRNA is expressed in the hearts of cMy-mOVA and cMy-mOVA-OT-II but not in C57BL/6 and OT-II mice. The mRNA expression was determined in hearts of mice of these strains (n=3) by qPCR in comparison to the house keeping gene *Gapdh*. The Δct values (ct *Ova* minus ct *Gapdh*) were used to calculate the relative expression of *Ova* in single heart samples in reference to the mean of the Δct values for the hearts of cMy-mOVA mice (Δct reference) ($2^{\Delta ct\ Ova}/2^{\Delta ct\ reference}$). Single data points and their means are shown. An ANOVA indicated significant differences between the strains ($P$=8.03 x $10^{-9}$) and Bonferroni post hoc tests revealed differences ($P$<0.001) between all strains with exception of the comparison of C57BL/6 and OT-II mice. The cMy-mOVA mice were homozygous for the *Ova* transgene whereas cMy-mOVA-OT-II were hemizygous explaining the difference in *Ova* expression levels between these strains.



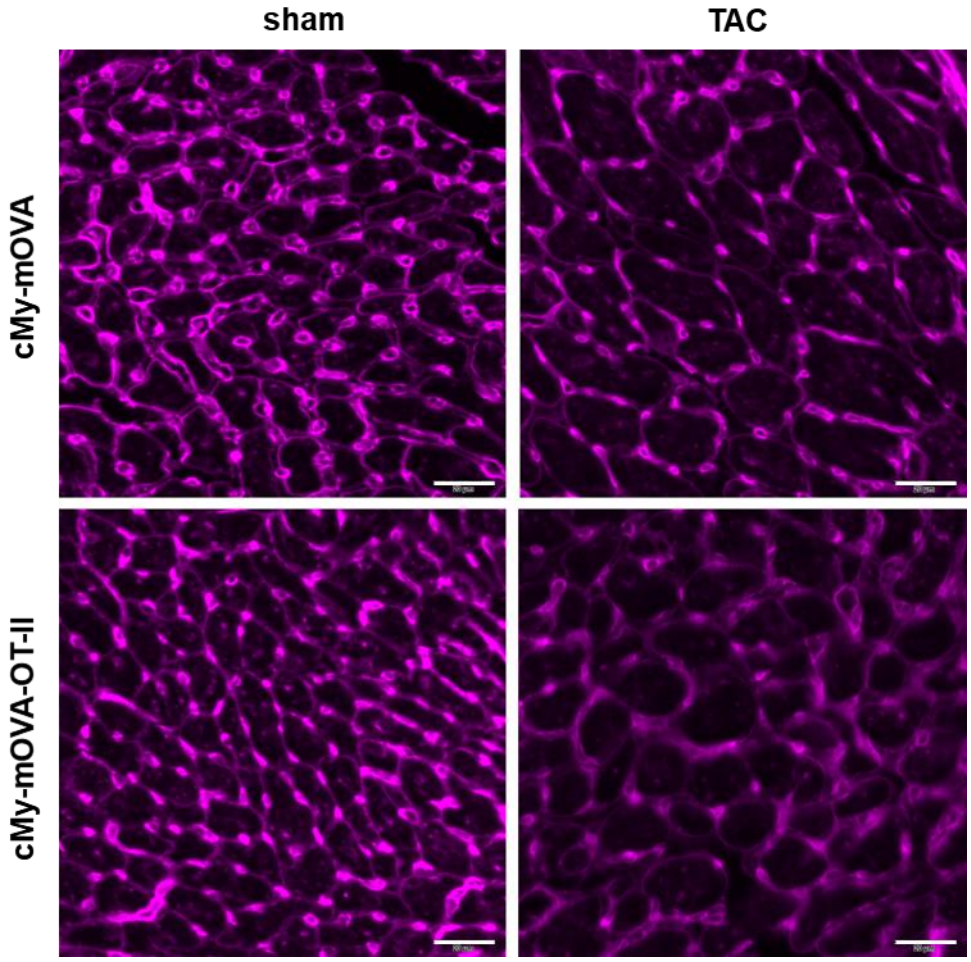

**Supplementary Figure 2.** The frequency of B cells and NK cells is not altered in the spleen of cMy-mOVA-OT-II mice. Splenocytes derived from 8 to 12 weeks old C57BL/6, cMy-mOVA, OT-II, and cMy-mOVA-OT-II mice (n=8 for each strain) were analyzed by flow cytometry for $CD19^+CD45R^+$ B cells (**a**) and $CD49b^+CD3^-$ NK cells (**b**), Means and SEM are shown. The data were analyzed by ANOVA and the *P*-values are given in the figure.



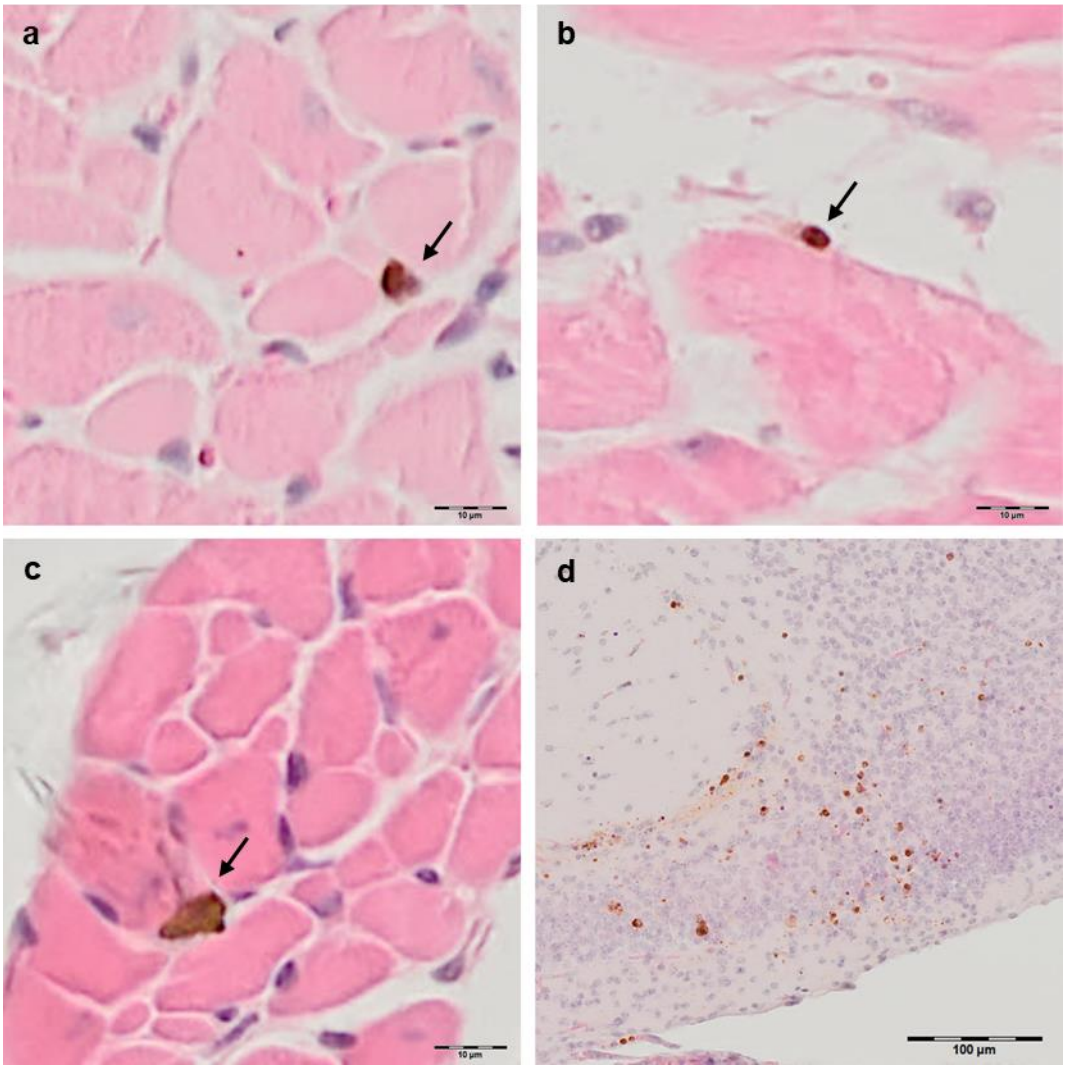

**Supplementary Figure 3.** The aortic stenosis after TAC operation was similar in cMy-mOVA and cMy-mOVA-OT-II mice and the survival of the animals was not different. (**a**) The pressure gradient over the aortic ligature was determined 3 days after surgery using pulsed wave Doppler in sham (n=22) and TAC-operated (n=18) cMy-mOVA mice as well as in sham (n=15) and TAC-operated (n=22) cMy-mOVA-OT-II mice. Means plus SEM are shown. Differences between two groups were analyzed by U-tests and significant *P*-values are indicated (ns, non-significant). (**b**) Kaplan-Meier survival curves for TAC-operated cMy-mOVA and cMy-mOVA-OT-II mice are displayed. A log-rank test (Mantel-Cox) did not indicate a significant difference between the strains. All sham-operated mice of both strains survived until the end of the experiment and are therefore not displayed in the figure.



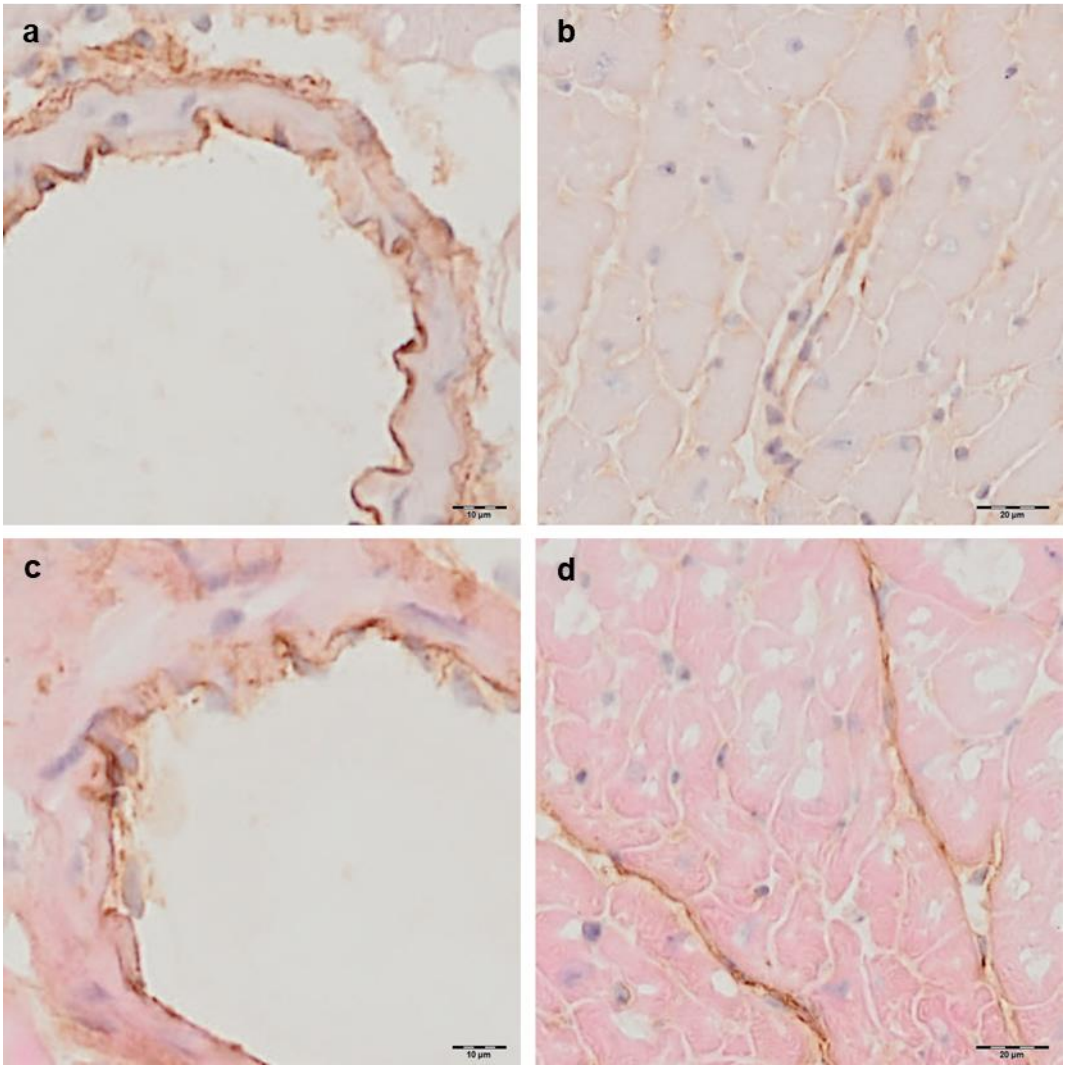

**Supplementary Figure 4.** Slight induction of OVA-specific autoantibodies in cMy-mOVA mice after TAC. OVA-specific autoantibodies were determined by ELISA in serial-diluted (1:30, 1:60, 1:120, 1:240) sera of (**a**) cMy-mOVA and (**b**) cMy-mOVA-OT-II mice before (pre) and at 10 weeks after sham or TAC surgery when the mice were sacrificed. Pooled serum (pre-diluted 1:10000) of 4 FVB mice immunized with OVA (im) served as positive control on each plate and the OD at the highest dilution was set to 100 % to adjust the other results and means plus SEM are shown. Sera of C57BL/6 (B6) mice (n=5) served as negative control. The numbers of analyzed animals are indicated. The increase of anti-OVA antibodies over time (pre compared to 10 weeks) and differences between sham and TAC-operated mice have been analyzed by repeated measures ANOVA and significant *P*-values are indicated (ns, non-significant).



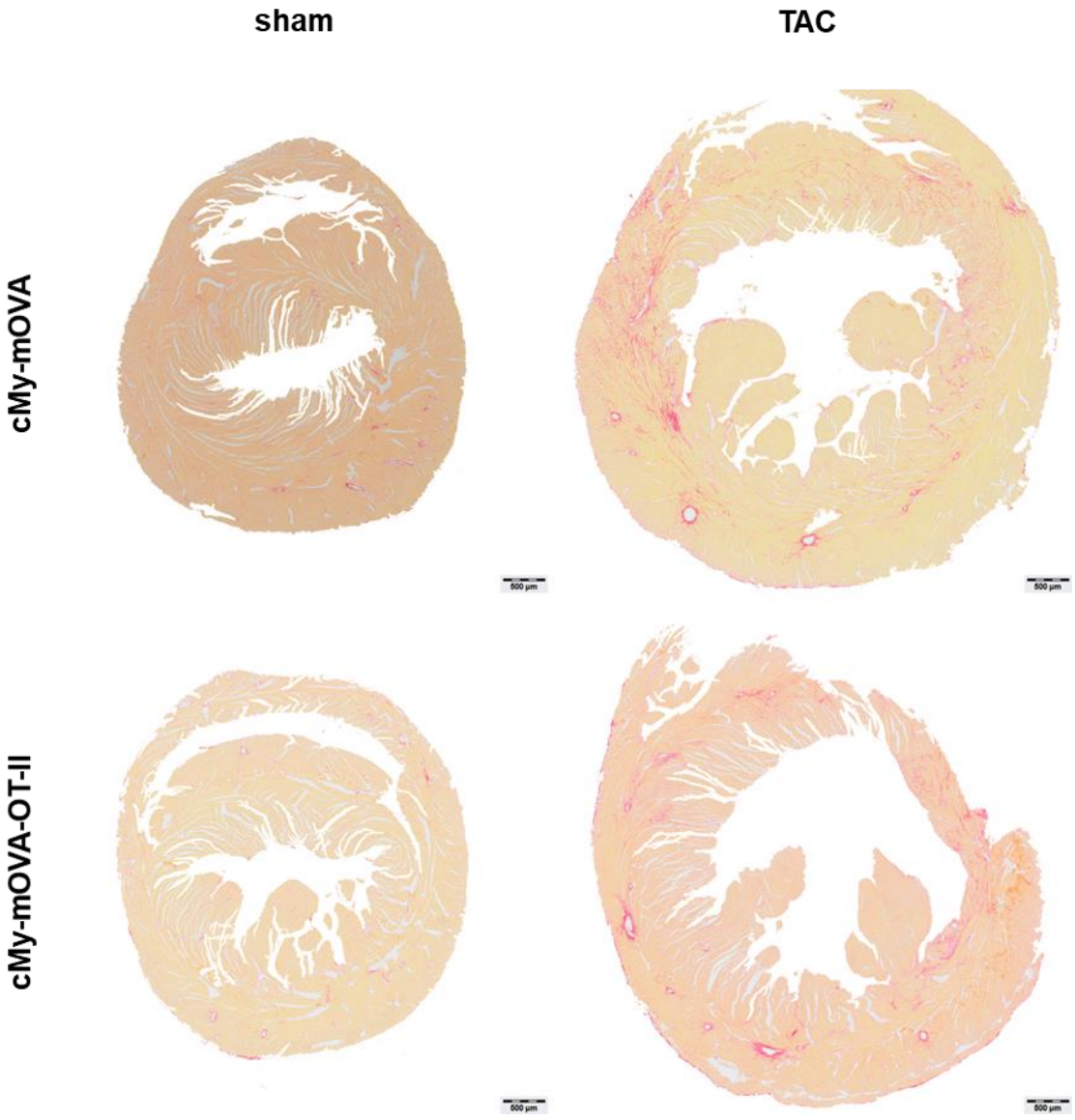

**Supplementary Figure 5.** Heart sections were stained as exemplified here with Alexa680-labeled WGA, which stains plasma membranes, to determine the CSA of cardiomyocytes in ventricles of sham and TAC-operated cMy-mOVA and cMy-mOVA-OT-II mice. The bars indicate 20 µm.




**Supplementary Figure 6.** In heart sections of TAC-operated mice (**a**-**c**), apoptotic cells expressing cleaved caspase 3 were detected by immunohistochemistry and are marked by an arrow. Whereas most apoptotic cells were non-cardiomyocytes (**a**, **b**), an apoptotic cardiomyocyte (**c**) was detected in a cMy-mOVA mouse. A section of a hypocampus of a 7 days old mouse, which contains several apoptotic cells, is shown as positive control (**d**). The bars indicate 10 µm in panels (**a**-**c**) and 100 µm in panel **d**.




**Supplementary Figure 7.** In heart sections of cMy-mOVA (**a**, **b**) and cMy-mOVA-OT-II mice (**c**, **d**), endothelial cells expressing CD31 (PECAM-1) were detected by immunohistochemistry. In panels **a** and **c**, the endothelial cells of larger vessels are stained whereas panels **b** and **d** display capillaries in the myocardium. The bars indicate 10 µm in panels **a** and **c** and 20 µm in panel **b** and **d**.




**Supplementary Figure 8.** Heart sections were stained as exemplified here with Sirius Red, which stains collagen, to determine the fibrotic areas in the ventricles of sham and TAC-operated cMy-mOVA and cMy-mOVA-OT-II mice. Fibrotic areas directly associated with vessels with a diameter of more than 100 µm were considered as perivascular fibrosis. The bars indicate 500 µm.

**Supplementary Tab. 1** Antibodies and isotype controls used for flow cytometry

| Antigen | Isotype | Clone | Label | Supplier |
|---|---|---|---|---|
| CD3 | rat $IgG_{2b}$ | 17A2 | FITC<br>PE | Biolegend, Fell, Germany |
| CD4 | rat $IgG_{2a}$ | RM4-5 | PE<br>PE/Cy5 | Biolegend, Fell, Germany |
| CD8 | rat $IgG_{2a}$ | 53-6.7 | PE/Cy5 | Biolegend, Fell, Germany |
| CD19 | rat $IgG_{2a}$ | 6D5 | FITC | Biolegend, Fell, Germany |
| CD25 | rat $IgG_1$ | PC61 | FITC | Biolegend, Fell, Germany |
| CD45R/B220 | rat $IgG_{2a}$ | RA3-6B2 | PE | Biolegend, Fell, Germany |
| CD49b | rat IgM | DX5 | PE | BioLegend, Fell, Germany |
| CD69 | hamster IgG | H1.2F3 | PE | BioLegend, Fell, Germany |
| FOXP3 | mouse $IgG_1$ | 150D | PE | BioLegend, Fell, Germany |
| IL-2 | rat $IgG_{2b}$ | JES6-5H4 | PE | Biolegend, Fell, Germany |
| IL-4 | rat $IgG_1$ | 11B11 | PE | Biolegend, Fell, Germany |
| IL-6 | rat $IgG_1$ | MP5-20F3 | PE | Biolegend, Fell, Germany |
| IL-17A | rat $IgG_1$ | TC11-18H10.1 | PE | Biolegend, Fell, Germany |
| IL-17F | mouse $IgG_1$ | 9D3.1C8 | PE | BioLegend, Fell, Germany |
| IL-10 | rat $IgG_{2b}$ | JES5-16E3 | PE | Biolegend, Fell, Germany |
| IFN-γ | rat $IgG_1$ | XMG1.2 | PE | Biolegend, Fell, Germany |
| TCRγδ | hamster IgG | GL3 | PE | BioLegend, Fell, Germany |
| TCRVβ5.1/5.2 | mouse $IgG_1$ | MR9-4 | FITC | BioLegend, Fell, Germany |
| TNF-α | rat $IgG_1$ | MP6-XT22 | PE | Biolegend, Fell, Germany |
| TGF-β | mouse $IgG_1$ | TW7-20B9 | PE | BioLegend, Fell, Germany |
| - | hamster IgG | HTK888 | PE | BioLegend, Fell, Germany |
| - | mouse $IgG_1$ | MOPC-21 | FITC<br>PE | BioLegend, Fell, Germany |
| - | rat $IgG_1$ | RTK2071 | FITC<br>PE | BioLegend, Fell, Germany |

| Antigen | Isotype | Clone | Label | Supplier |
|---|---|---|---|---|
| - | rat $IgG_{2a}$ | RTK2758 | FITC<br>PE | BioLegend, Fell, Germany |
| - | rat $IgG_{2b}$ | RTK4530 | FITC<br>PE | BioLegend, Fell, Germany |
| - | rat IgM | RTK2118 | PE | BioLegend, Fell, Germany |

The following abbreviations are used: FITC, fluorescein isothiocyanate, PE, phycoerythrin; PE/Cy5, phycoerythrin/cyanine 5.