# LETTER TO EDITOR

**Letter by Ferté, *et al*. Regarding Article, "Chronic Pressure Overload Induces Cardiac Hypertrophy and Fibrosis via Increases in SGLT1 and IL-18 Gene Expression in Mice"**

*To the Editor,*

Our laboratory has a long-standing interest in understanding the role of sodium-glucose co-transporters (SGLTs) in the heart. It is thus with great attention that we read the report by Matsushita, *et al*,[1] published in International Heart Journal in 2018, where the authors explored the role of SGLT1 in a murine model of cardiac hypertrophy induced by pressure overload. The results reported by Matsushita, *et al* led the authors to conclude that the absence of SGLT1 protects against pressure overload-induced cardiac hypertrophy, mainly by reducing IL-18 gene expression in the heart.

We obtained the $^{\Delta ex1}$KO SGLT1 mouse model used by Matsushita and colleagues from Koepsell's group in 2013, and we attempted to reproduce the protective effect of the KO (referred as $^{\Delta ex1}$KO SGLT1) in cardiac hypertrophy reported by the authors. Despite our best attempts at reproducing their experimental conditions, we failed to replicate their results.

We first evaluated cardiac function in sham and TAC-operated WT and $^{\Delta ex1}$KO SGLT1 animals. Echocardiographic measurements demonstrated the development of cardiac hypertrophy and cardiac dysfunction at 2 weeks and 6 weeks post-TAC in both genotypes. Ejection fraction (EF) and fractional shortening (FS) were significantly reduced in both TAC-operated groups, compared to controls (Figure A). In our hands, FS decreased about 50% at 6 weeks post-TAC, in both WT and $^{\Delta ex1}$KO SGLT1 TAC-operated mice compared to the sham groups. In contrast, Matsushita and colleagues observed FS reduction only in WT animals. We note that their reported reduction in FS was of 30%, suggesting a milder TAC surgery compared to ours. In addition, we found no differences between the two TAC-operated groups in terms of cardiomyocyte area at 6 weeks (Figure B). Finally, mRNA levels of BNP and IL-18 were indistinguishable between WT and $^{\Delta ex1}$KO SGLT1 mice, confirming the lack of a protective effect of $^{\Delta ex1}$KO SGLT1 in TAC-induced cardiac hypertrophy (Figure C). Moreover, we examined the effects of TAC-induced pressure overload on ventricular AMPK phosphorylation. Our results show an increase in AMPK phosphorylation after TAC to the same extent in WT and $^{\Delta ex1}$KO SGLT1 (Figure D). Thus, we observed no protection of $^{\Delta ex1}$KO SGLT1 mice from TAC-induced hypertrophy.

We also evaluated cardiac fibrosis in these animals. In contrast to Matsushita, *et al*, we did not observe any differences between WT and $^{\Delta ex1}$KO SGLT1 at 6 weeks after TAC in collagen deposition (Figure E) and mRNA expression of fibrotic marker Col-1a (Figure F). Therefore, TAC-induced cardiac fibrosis was indistinguishable in $^{\Delta ex1}$KO SGLT1 mice compared to controls.

To further understand the discrepancy between our results and those of Matsushita, *et al*, we decided to deeply characterize SGLT1 in the heart. We recently demonstrated that murine and human hearts exclusively express a SGLT1 transcript variant that lacks glucose-binding domains mandatory for glucose transport.[2] This transcript variant is equally expressed in the heart of WT mice and in transgenic mice with exon 1-invalidated Slc5a1, indicating that this $^{\Delta ex1}$KO SGLT1 model is not suitable for cardiac studies, at least under physiological conditions. In our recent study, we demonstrated that the SGLT1 transcript variant encodes exon 9 to exon 15 in both WT and $^{\Delta ex1}$KO SGLT1 hearts. Indeed, primers targeting exon 1 to 8 did not generate any PCR products in both genotypes, confirming that the mouse heart exclusively expresses this transcript variant.[2] In light of these results, it is surprising that Matsushita, *et al* reported the absence of SGLT1 in $^{\Delta ex1}$KO SGLT1 mouse hearts by qPCR analysis. We could not reproduce this experiment, since the authors did not specify the primer sequences in the methods. However, regardless of the primer sequences, these results are incompatible with our findings.

Matsushita, *et al* used different diets for their SGLT1 WT mice (standard diet) and $^{\Delta ex1}$KO SGLT1 mice (glucose-galactose free diet). The authors do not exclude the possibility that a different diet impacts pressure overload-induced cardiac hypertrophy. To avoid this potential confounding effect, our WT group was fed the same diet as the $^{\Delta ex1}$KO SGLT1 animals. Further work is needed to address the possibility that diet affects TAC-induced hypertrophy in WT animals.[3]

Many studies have shown that SGLT1 is highly expressed in the heart, and it could play a role in cardiac diseases. We can confirm that this total $^{\Delta ex1}$KO SGLT1 model is not suitable for the study of SGLT1 in the myocardium. Indeed, in this model, there is no difference in the main transcript expressed in the mouse heart, therefore, we did not observe any differences in cardiac func-

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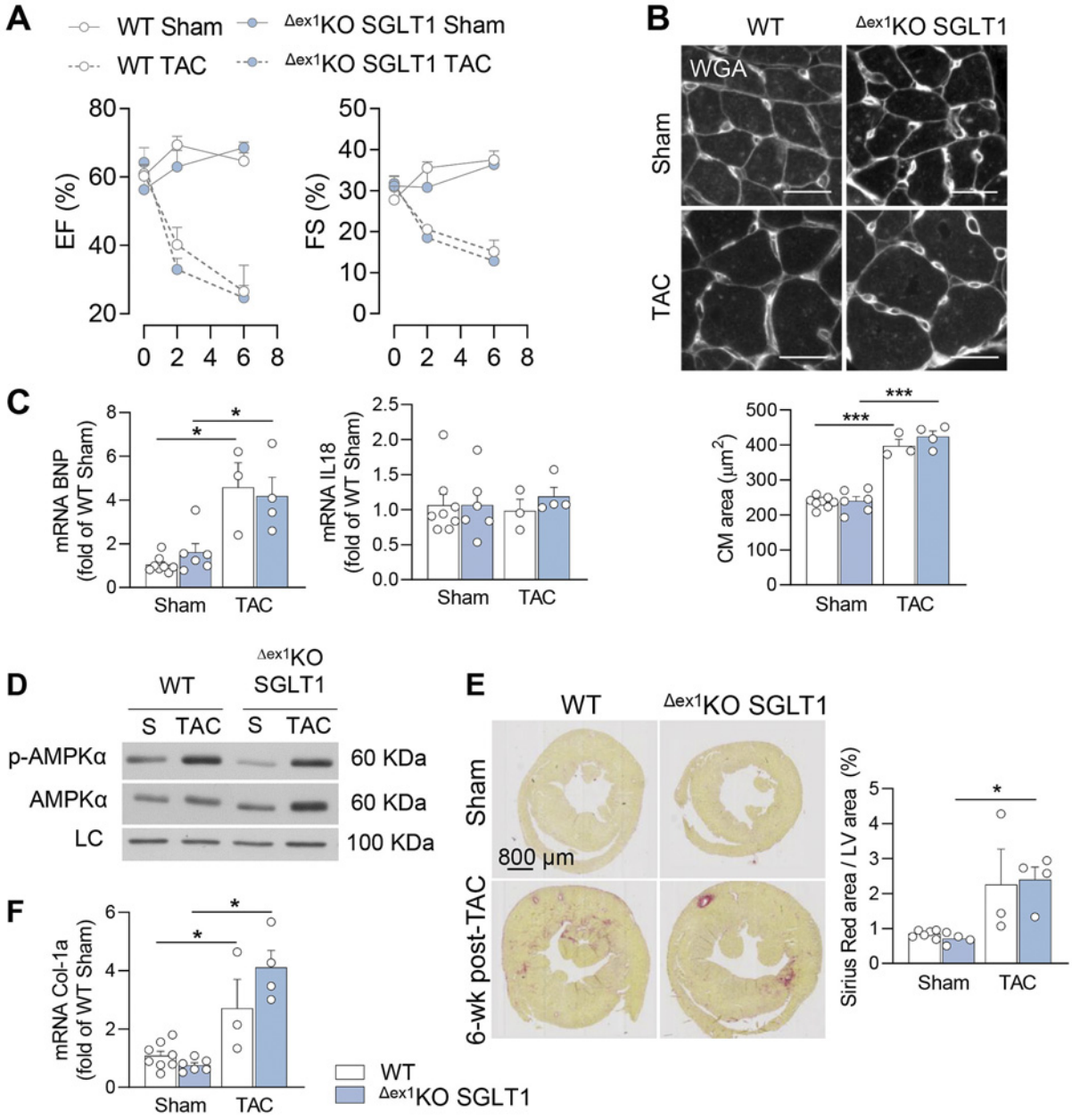


**Figure.** Pressure overload-induced effects on cardiac function, hypertrophy and fibrosis in WT and $^{\Delta ex1}$KO SGLT1 mice. **A:** Ejection fraction (EF), and fractional shortening (FS) measured by echocardiographic analysis in WT and $^{\Delta ex1}$KO SGLT1 mouse hearts at baseline, and 2 and 6 weeks after TAC surgery ($n > 3$ per group). ***$P < 0.001$ by two-way ANOVA. **B:** Representative immunofluorescence staining with wheat germ agglutinin (WGA) and quantification of cardiomyocyte cross-sectional area from sham control and $^{\Delta ex1}$KO SGLT1 mice or mice at 6 weeks after TAC ($n > 3$ per group). Scale bar: 20 μm. ***$P < 0.001$, by one-way ANOVA. **C:** qPCR analyses of hearts from WT and $^{\Delta ex1}$KO SGLT1 sham- and TAC-operated mice of BNP and IL-18 ($n > 3$ per group). *$P < 0.05$ by one-way ANOVA. **D:** Western blot analysis of total and phosphorylated AMPK in heart lysates of control and TAC-operated WT and $^{\Delta ex1}$KO SGLT1 mice ($n > 3$ per group). **E:** Representative images and quantification of fibrosis in sham- and TAC-operated WT and $^{\Delta ex1}$KO SGLT1 hearts ($n > 3$ per group). Scale bar: 200 μm. *$P < 0.05$ by one-way ANOVA. **F:** mRNA levels of Col-1a measured by qPCR analysis in hearts from WT and $^{\Delta ex1}$KO SGLT1 sham- and 6 weeks after TAC ($n > 3$ per group). *$P < 0.05$ by one-way ANOVA.

tion, hypertrophy and fibrosis under hemodynamic stress between the two genotypes. We have not established whether the alternative transcript produces a truncated protein, and we do not know whether this variant would affect the diseased heart (i.e., ischemia/reperfusion[4]). In conclusion, models of cardiac-specific SGLT1 knockout or cardiomyocyte-specific knockdown are needed to investigate the role of SGLT1 in the heart.

## Disclosure

**Conflicts of interest:** None.

## References

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