Influence of serotonin 5-HT_4 receptors on responses to cardiac stressors in transgenic mouse models

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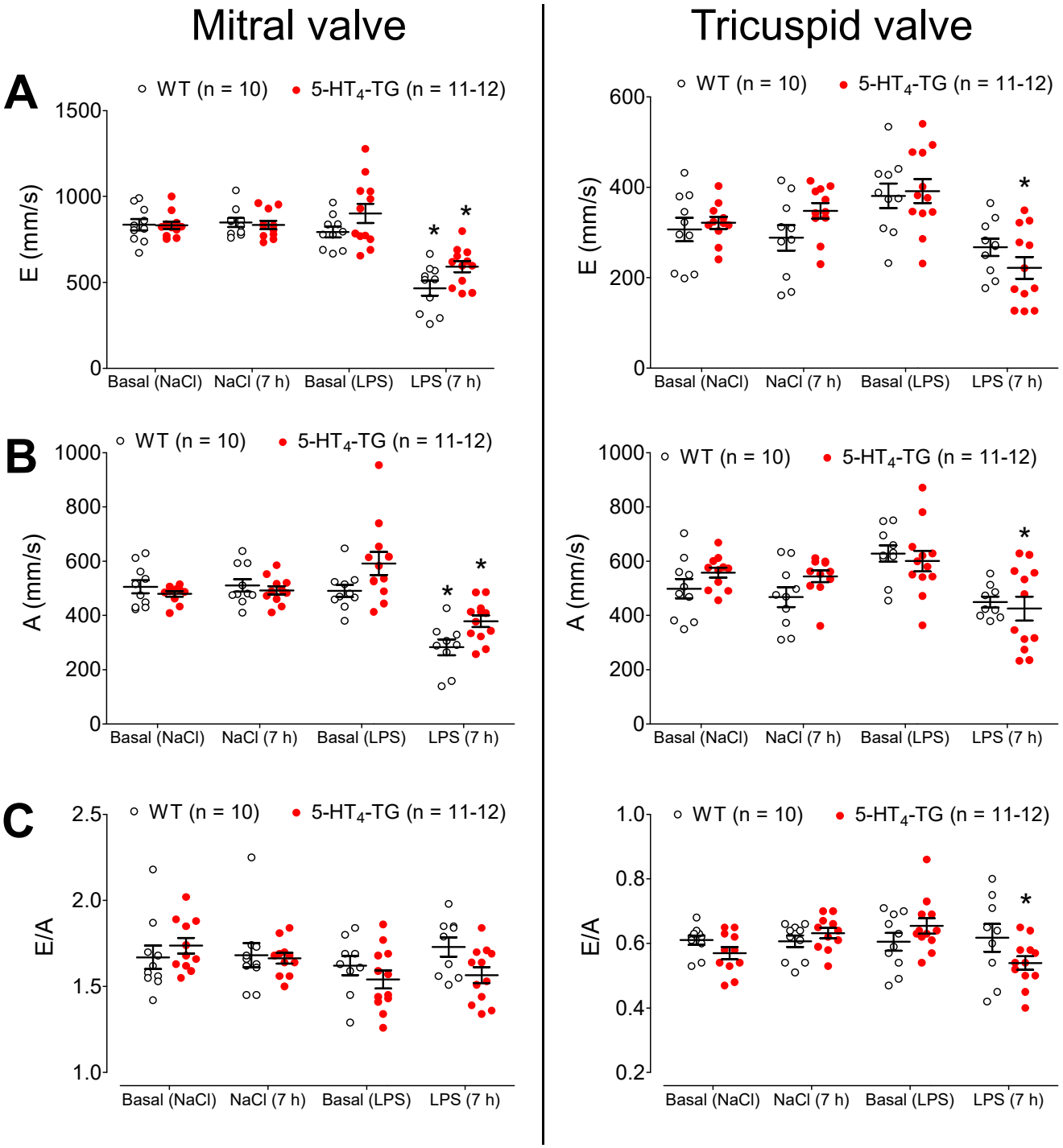
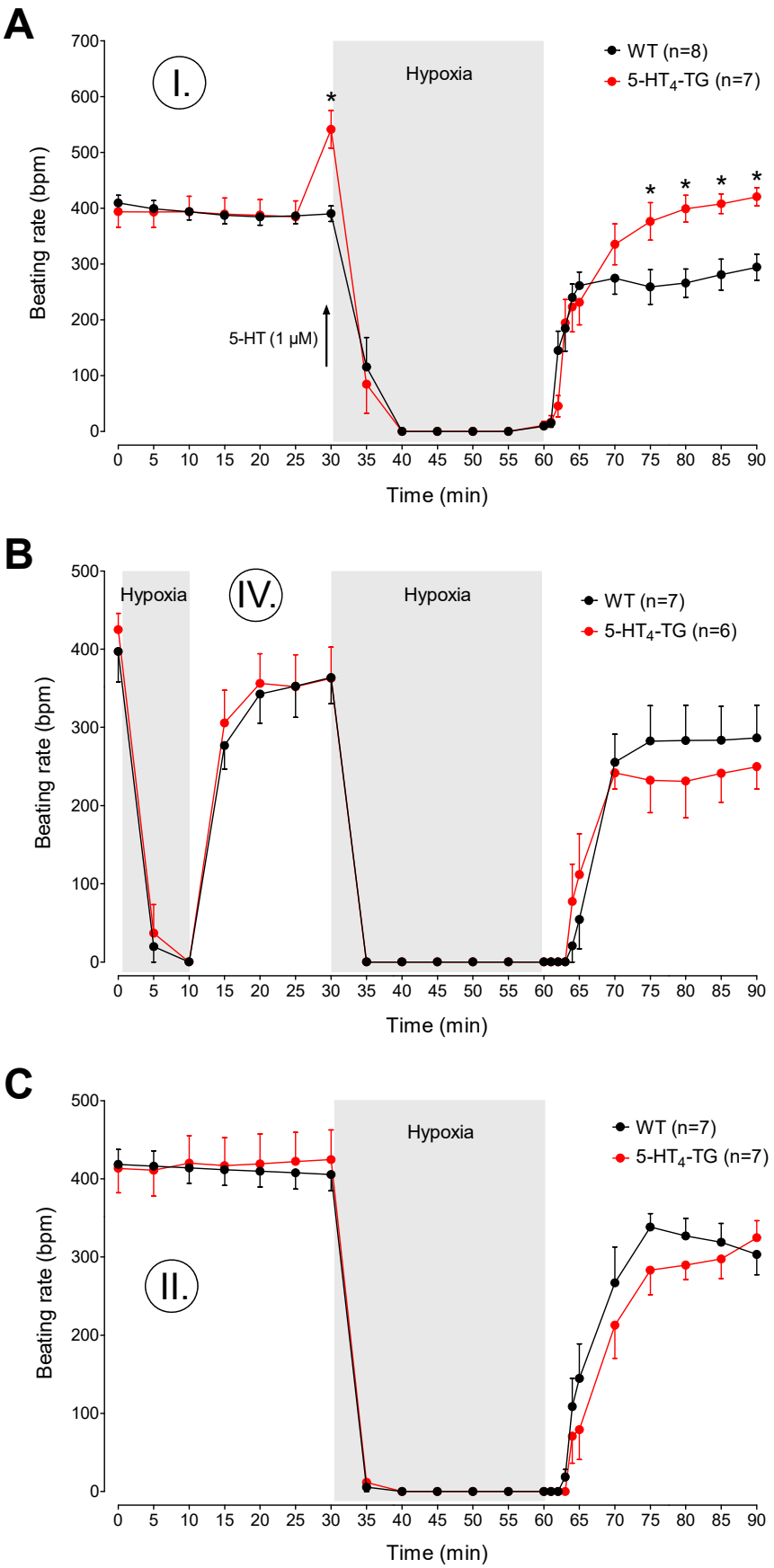
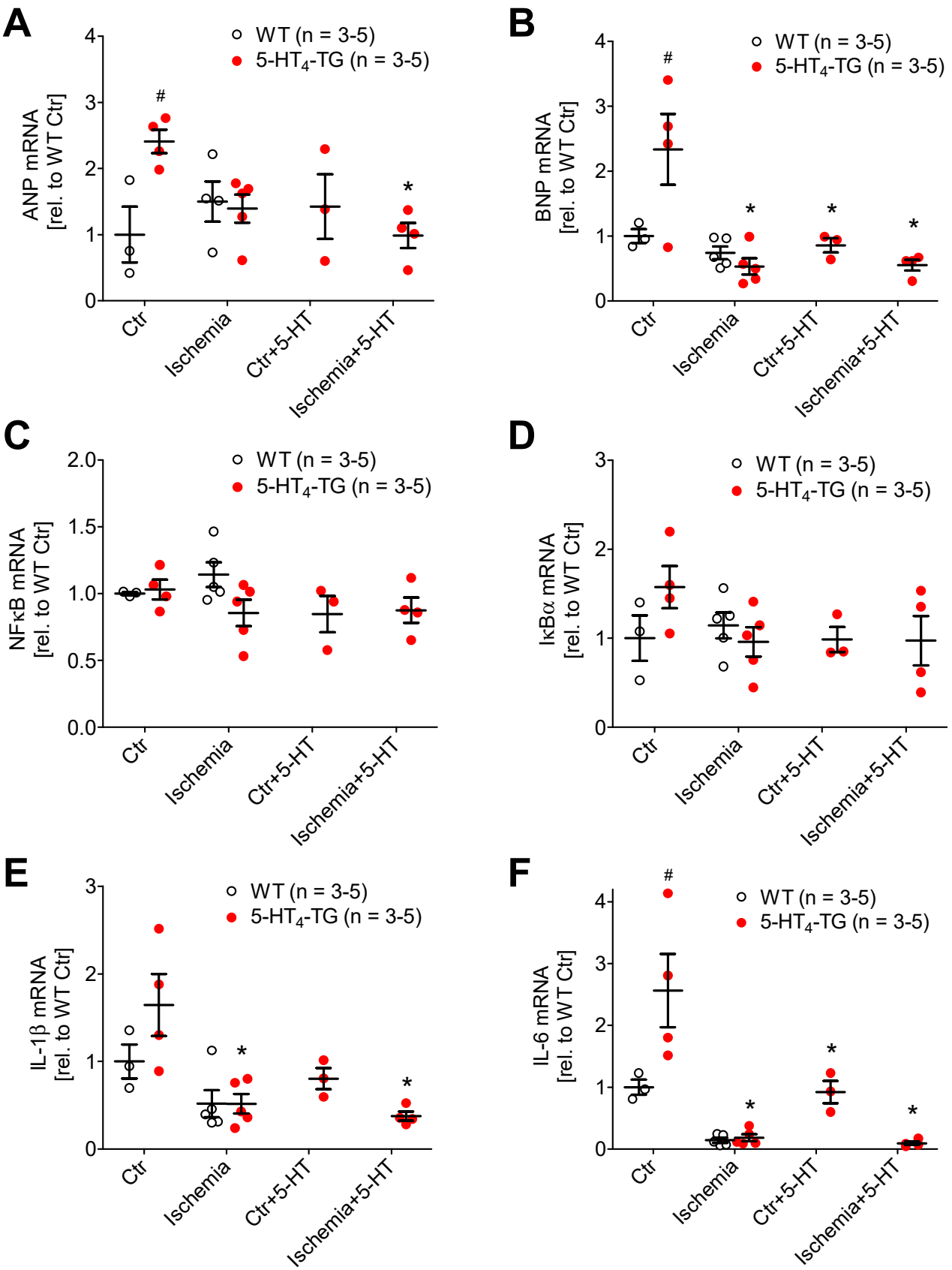
Supplementary Figures



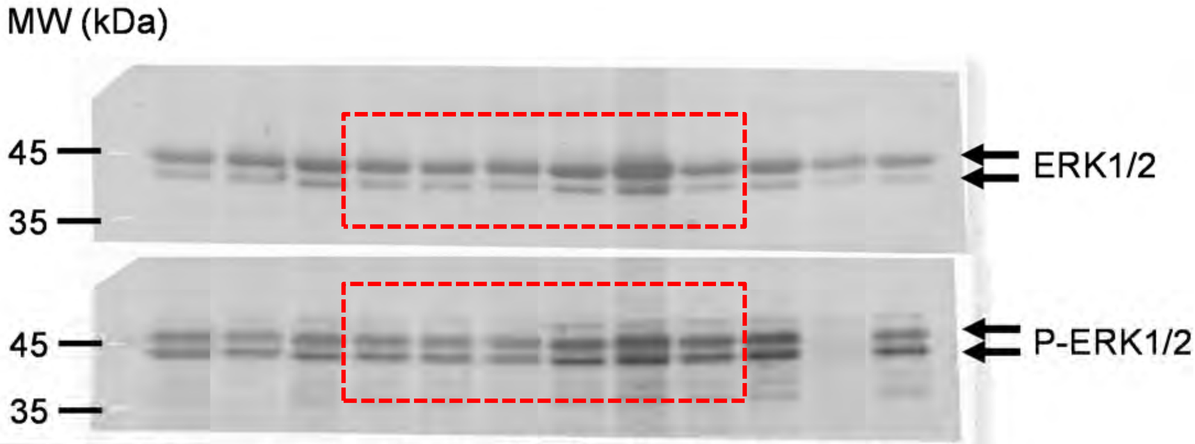
Supplementary Figure S1. Doppler echocardiography of 5-HT_4-TG mice before and after LPS treatment. The flow over the mitral valve (left side) and over the tricuspid valve (right side) was measured before (basal) and 7 h after LPS injection. The injection of a NaCl solution served as control. **(A)** The E wave represents the early filling of the ventricle and **(B)** the A wave represents the atrial contraction. **(C)** The diastolic function expressed as ratio of E and A waves (E/A) was unchanged in all groups. Number in brackets indicates the number of mice studied. WT = wild type mice; *$p < 0.05$ vs. basal.



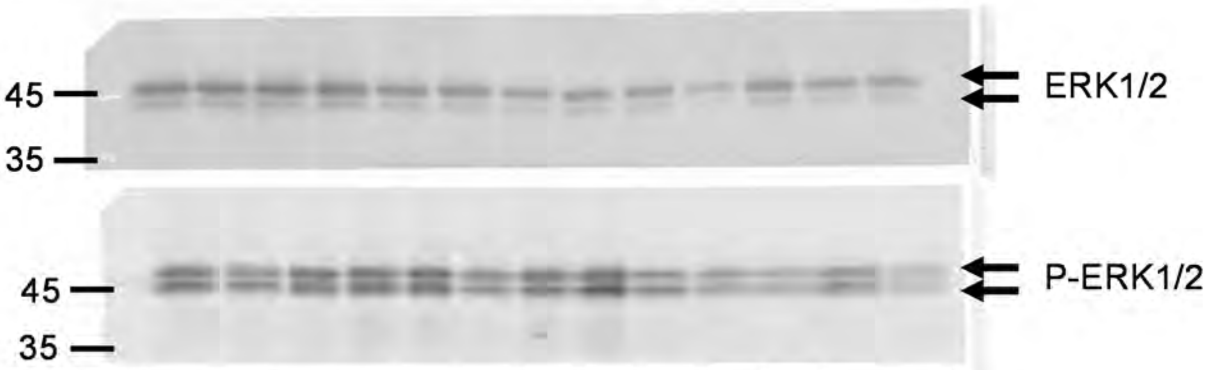
Supplementary Figure S2. Time course of hypoxia in spontaneously beating right atrial preparations. **(A-C)** The beating rate during the time of oxygenation and hypoxia is presented. After the addition of serotonin (5-HT, 1 µM, protocol I) beating rate was increased in 5-HT_4-TG and reached again initial values after hypoxia and reoxygenation **(A)**. Preconditioning (protocol IV) as a short hypoxia for 10 minutes was not beneficial **(B)**. Under the condition of a single hypoxia (protocol II), the beating rate was not different between 5-HT_4-TG and WT right atria **(C)**. WT = wild type mice, 5-HT_4-TG=5-HT_4-transgenic mice. Data shown are means ± SEM. *$p < 0.05$ vs. WT.



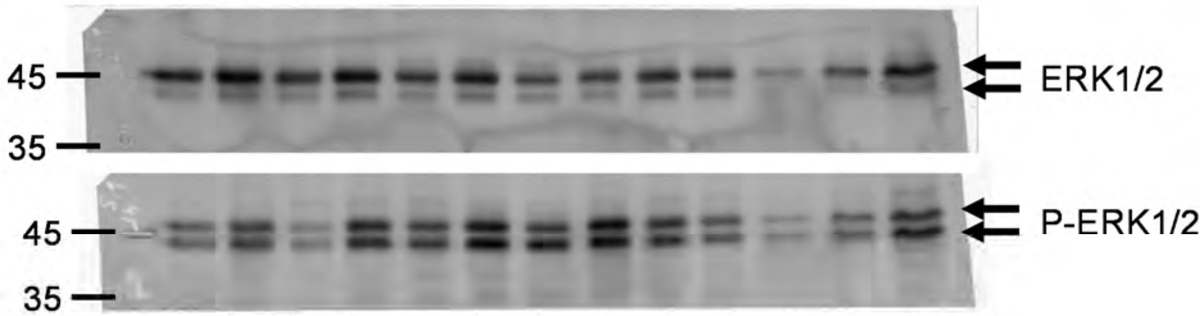
Supplementary Figure S3. mRNA expression after ischemia/reperfusion in isolated perfused hearts. Abundance of mRNAs, measured by quantitative real time PCR (qPCR), after ischemia/reperfusion in isolated perfused hearts of WT und 5-HT_4-TG mice (see Fig. 8A for the protocol). Expression of **(A)** ANP, **(B)** BNP, **(C)** NFκB, **(D)** IκB, **(E)** IL-1β, and **(F)** IL-6 mRNAs in hearts from WT and 5-HT_4-TG mice was normalized to GAPDH expression. Finally, mRNA expression data are presented relative to WT control (Ctr) mice. WT = wild type mice, 5-HT_4-TG=5-HT_4-transgenic mice. Data shown are means ± SEM. *$p < 0.05$ vs. Ctr.; $^{\#}p < 0.05$ vs. WT; numbers in brackets indicate numbers of preparations.



WT	(M)	+		+		+		+				+	+	
5-HT$_4$-TG	(M)		+		+		+		+	+	+			+
Ischemia	(M)			+	+			+	+	+		+		+
5-HT	(M)									+	+			

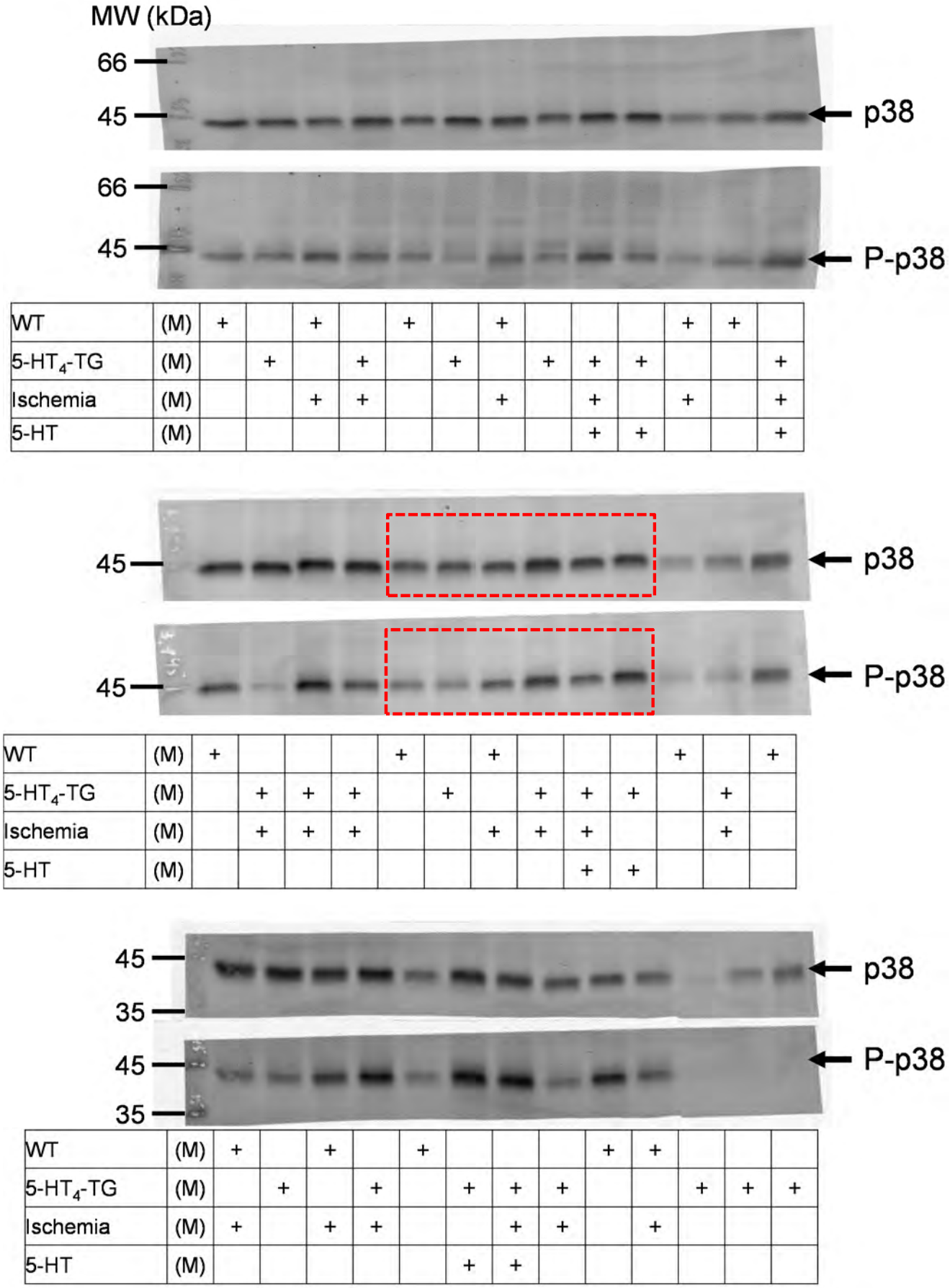


WT	(M)	+		+			+			+		+		+
5-HT$_4$-TG	(M)		+		+	+		+	+		+		+	
Ischemia	(M)			+	+	+			+	+	+			
5-HT	(M)							+	+					

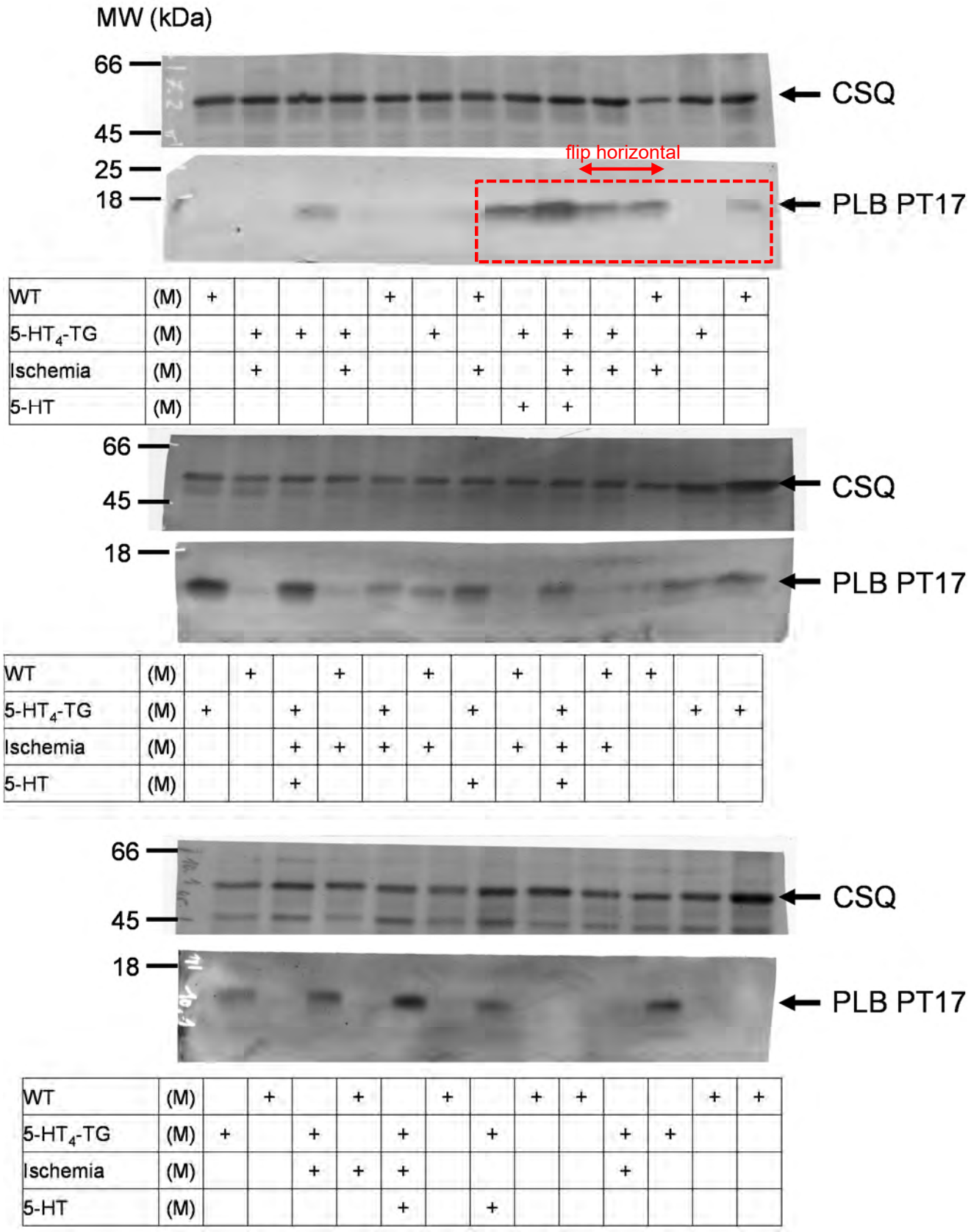


WT	(M)	+		+		+				+	+			
5-HT$_4$-TG	(M)		+		+		+	+	+			+	+	+
Ischemia	(M)	+			+	+	+		+		+			+
5-HT	(M)							+	+					+

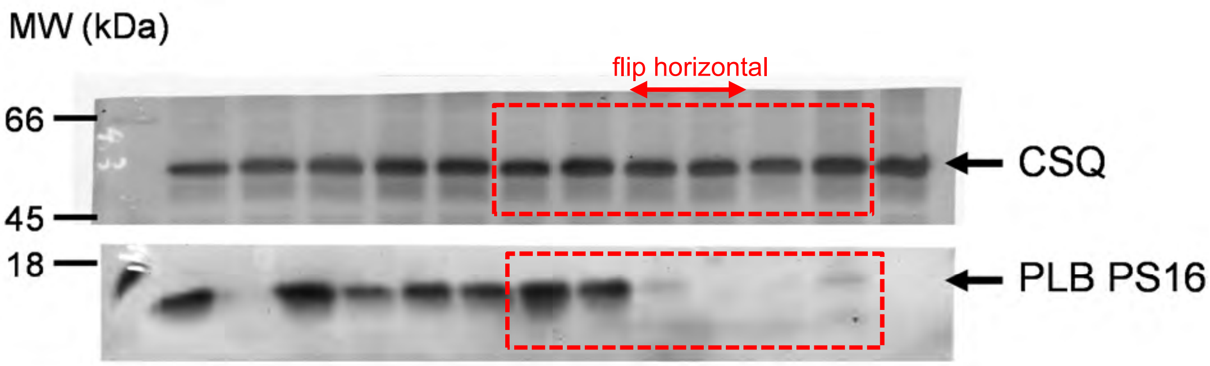
Supplementary Figure S4 (unedited Western blots): Original Western blots of homogenates from isolated perfused hearts with or without ischemia of wild type (WT) and 5-HT$_4$ receptor transgenic (5-HT$_4$-TG) mice. M, molecular weight marker; 5-HT, serotonin (1 µM); ERK1/2, extracellular regulated kinase 1/2; P-ERK1/2, phosphorylated ERK1/2. Not all bands were suitable for quantification (border lanes or duplicates). The red rectangles mark the part of the blot shown in Figure 7.



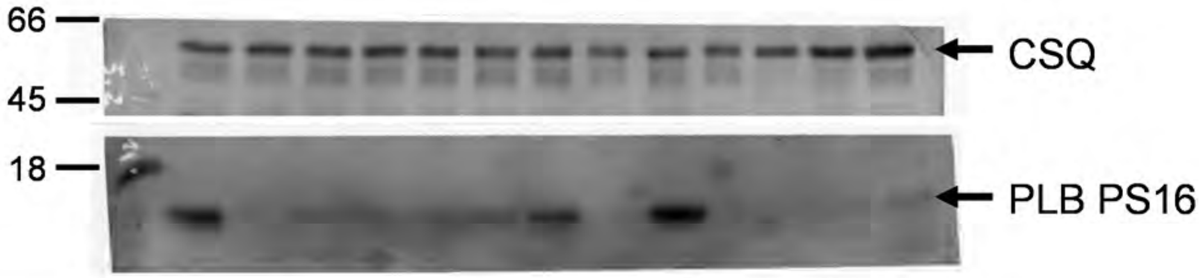
Supplementary Figure S5 (unedited Western blots): Original Western blots of homogenates from isolated perfused hearts with or without ischemia of wild type (WT) and 5-HT_4 receptor transgenic (5-HT_4-TG) mice. M, molecular weight marker; 5-HT, serotonin (1 µM); p38, mitogen activated protein kinase p38; P-p38, phosphorylated p38. Not all bands were suitable for quantification (border lanes or duplicates). The red rectangles mark the part of the blot shown in Figure 7.



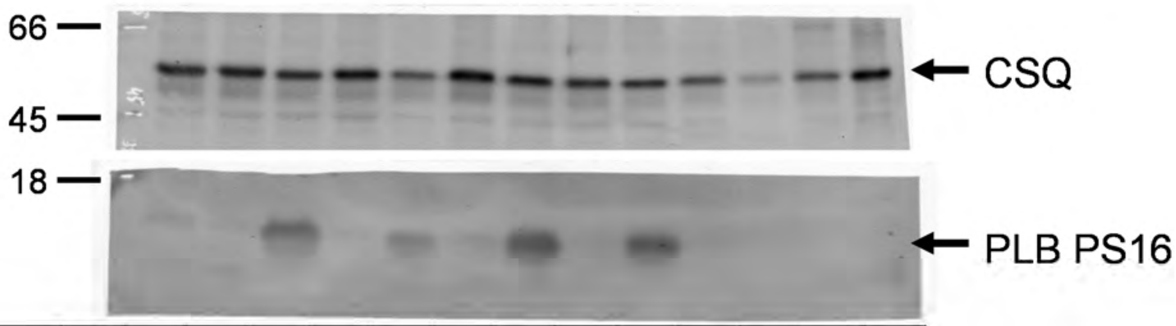
Supplementary Figure S6 (unedited Western blots): Original Western blots of homogenates from isolated perfused hearts with or without ischemia of wild type (WT) and 5-HT_4 receptor transgenic (5-HT_4-TG) mice. M, molecular weight marker; 5-HT, serotonin (1 µM); CSQ, cardiac calsequestrin; PLB PT17, phospholamban phosphorylated at threonine 17. Not all bands were suitable for quantification (border lanes or duplicates). The red rectangle mark the part of the blot shown in Figure 7 after flipping horizontally.



WT	(M)		+		+						+		+	+
5-HT$_4$-TG	(M)	+		+		+	+	+	+	+		+		
Ischemia	(M)		+	+	+				+	+	+			
5-HT	(M)			+				+	+					

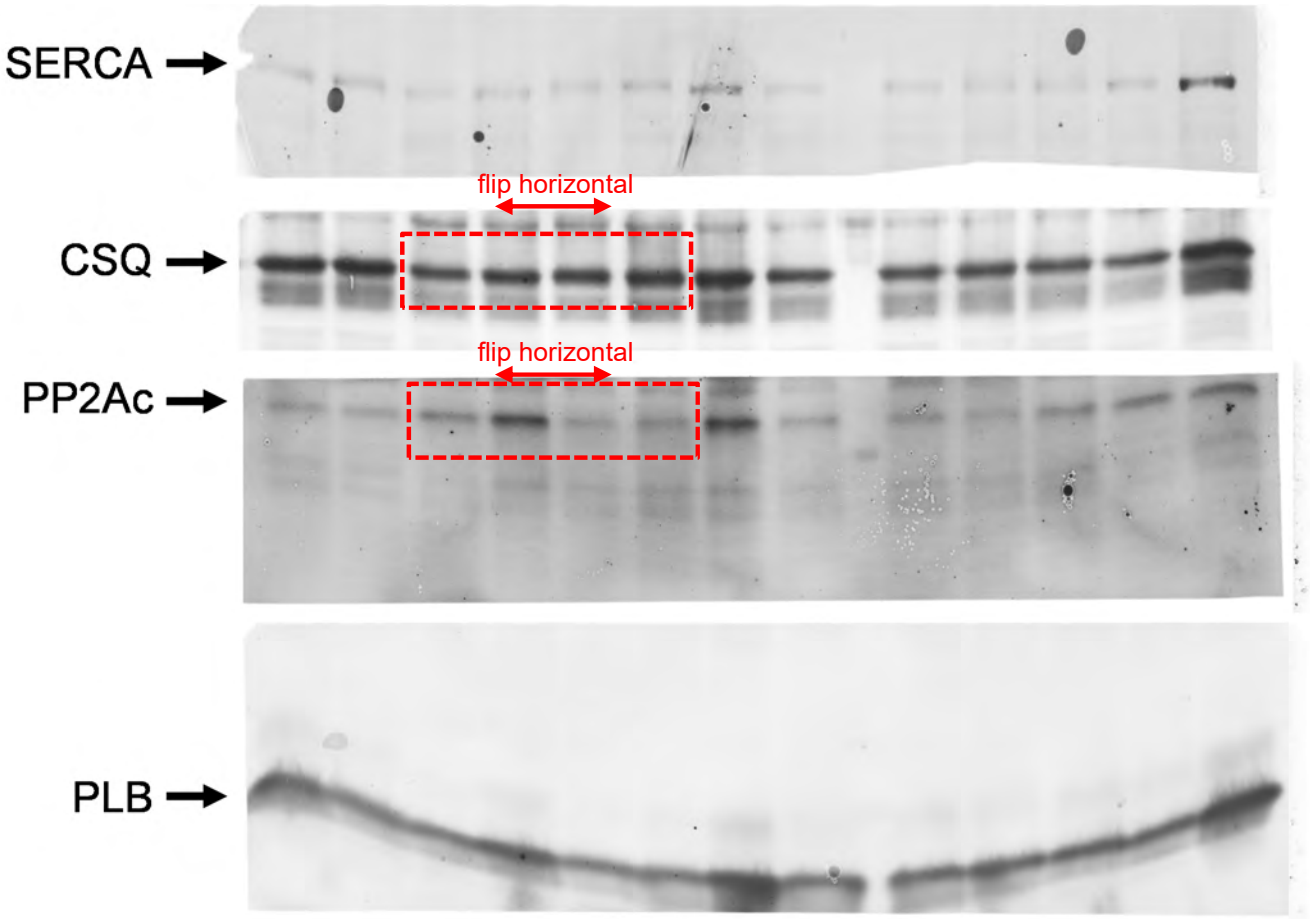


WT	(M)		+		+		+		+		+	+		
5-HT$_4$-TG	(M)	+		+		+		+		+			+	+
Ischemia	(M)			+	+	+	+		+	+	+			
5-HT	(M)			+				+		+				



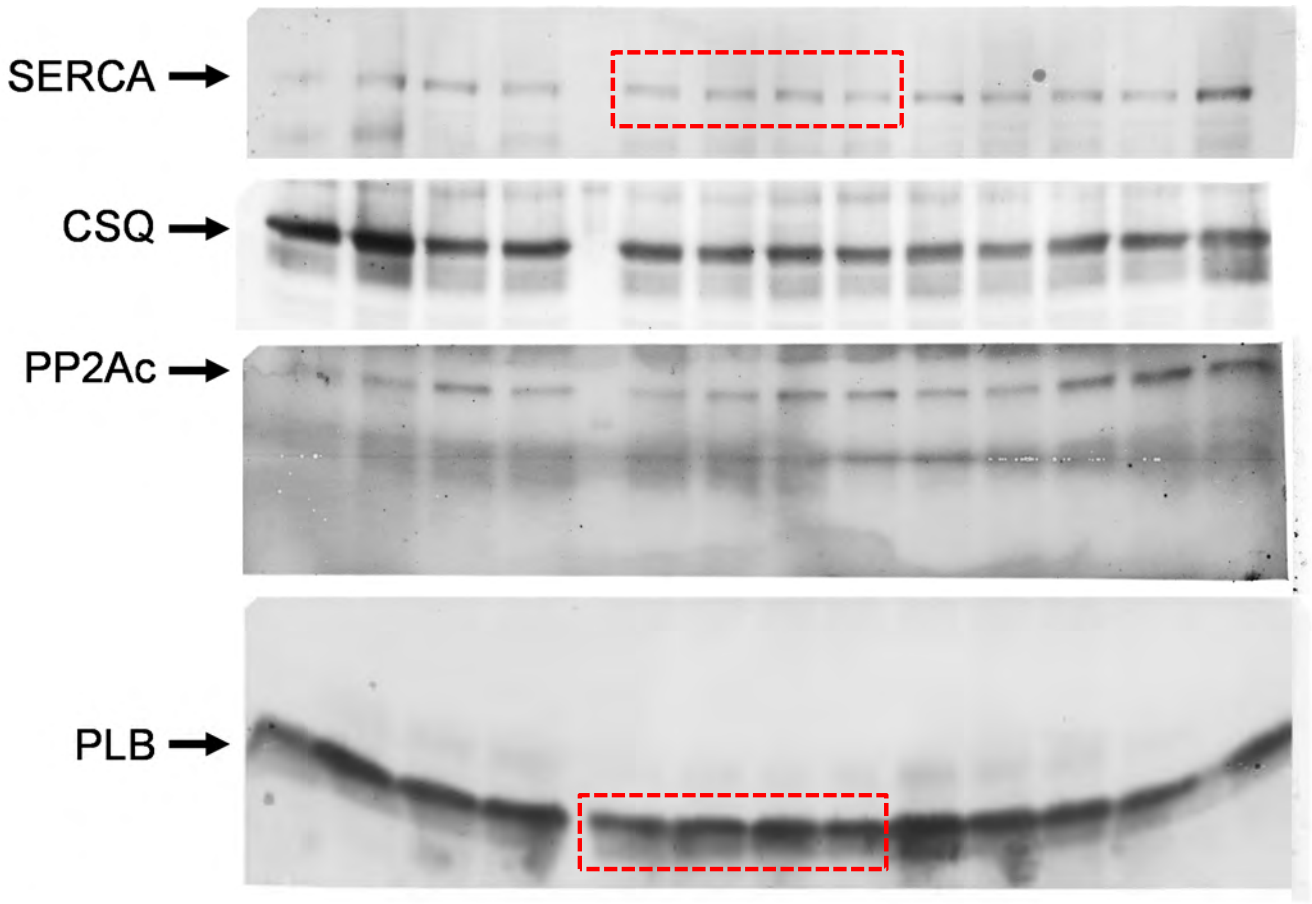
WT	(M)	+	+		+		+		+		+	+	+	+
5-HT$_4$-TG	(M)			+		+		+		+				
Ischemia	(M)	+		+	+					+	+		+	
5-HT	(M)							+		+				

Supplementary Figure S7 (unedited Western blots): Original Western blots of homogenates from isolated perfused hearts with or without ischemia of wild type (WT) and 5-HT$_4$ receptor transgenic (5-HT$_4$-TG) mice. M, molecular weight marker; 5-HT, serotonin (1 µM); CSQ, cardiac calsequestrin; PLB PS16, phospholamban phosphorylated at serine 16. Not all bands were suitable for quantification (border lanes or duplicates). The red rectangles mark the part of the blot shown in Figure 7 after flipping horizontally.



WT	+					+			(M)		+			
5-HT_4-TG		+			+				(M)	+				
PP2A-TG				+			+		(M)				+	+
DT			+					+	(M)			+		

Supplementary Figure S8 (unedited Western blots): Original Western blots (Gel 1) of cardiac homogenates from wild type (WT), 5-HT_4 receptor transgenic (5-HT_4-TG), PP2A transgenic (PP2A-TG), and double transgenic (DT) mice. M, molecular weight marker; CSQ, calsequestrin; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; PLB, phospholamban; PP2Ac, catalytic subunit of protein phosphatase 2A. The red rectangles mark the parts of the blot shown in the Figure 9 after flipping horizontally.



WT	+				(M)	+				+			
5-HT_4-TG		+			(M)		+				+		
PP2A-TG			+		(M)			+				+	
DT				+	(M)				+				+	+

Supplementary Figure S9 (unedited Western blots): Original Western blots (Gel 2) of cardiac homogenates from wild type (WT), 5-HT_4 receptor transgenic (5-HT_4-TG), PP2A transgenic (PP2A-TG), and double transgenic (DT) mice. M, molecular weight marker; CSQ, calsequestrin; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; PLB, phospholamban; PP2Ac, catalytic subunit of protein phosphatase 2A. The red rectangles mark the parts of the blot shown in the Figure 9.