Supplementary Material

Differential Changes in Cyclic Adenosine 3′-5′ Monophosphate (cAMP) Effectors And Major Ca^{2+} Handling Proteins During Diabetic Cardiomyopathy

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Supplementary Methods

Animal model

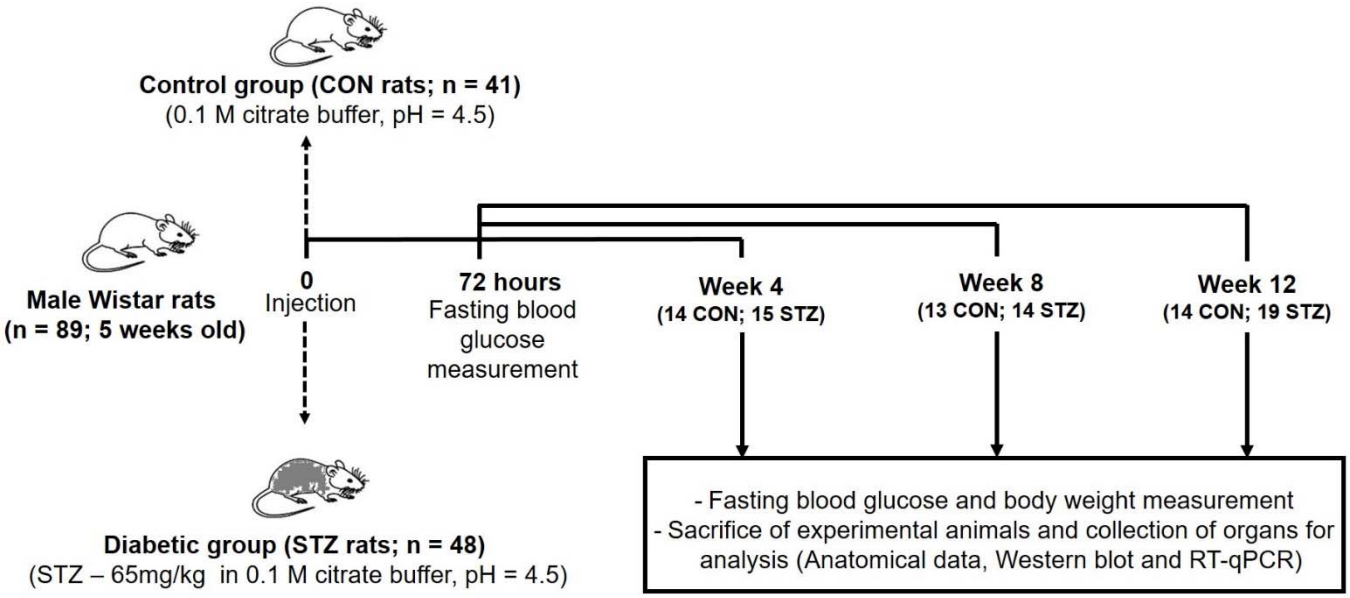

Fig. S1. Experimental design of the study. Five-week-old male Wistar rats (n = 89) were randomized into two groups. After 12 h fasting, one group received one intraperitoneal injection of streptozotocin (STZ, Sigma-Aldrich: 65 mg/kg in 0.1 M citrate buffer, pH = 4.5) to induce type 1 diabetes mellitus while the second age-matched control (CON) group was injected with vehicle (0.1 M citrate buffer, pH = 4.5). After 72 h, fasting blood glucose levels were measured and rats exhibiting blood glucose levels >200 mg/dL following STZ injection were considered diabetic. Four weeks after injection, glycemia and body weight of CON (n = 14) and STZ (n = 15) rats were evaluated. The animals were then sacrificed, and the organs were collected for further analysis. This procedure was repeated on the remaining rats at 8 (13 CON and 14 STZ) and 12 weeks (14 CON and 19 STZ) after STZ or vehicle injection.

Real-time fluorescence quantitative PCR (RT-qPCR).

Table S1. Forward and reverse primers used for quantitative real time PCR.

Gene	*Forward primer (5'-3')*	*Reverse primer (3'-5')*
GAPDH	TGC CAC TCA GAA GAC TGT GG	TTC AGC TCT GGG ATG ACC TT
ANF	ATC TGC CCT CTT GAA AAG CA	AAG CTG TTG CAG CCT AGT CC
Epac1	GAC GTC ACC ACT GCA AAC C	GCT GCC AGC TTG ATG AAC TT
Epac2	CCA CAC ATT TGG AAG GCA TA	GGG AAC AAA GGC AGA TCT CA
PLB	GCA GCT GAG CTC CCA GAC TT	TTT CCA TGA TGC CAG GAA GAC
SERCA2a	AGT GGC TGA TGG TGC TGA AA	GCA CCC GAA CAC CCT TAC AT
TnI	AGA TTG CGA AGC AGG AGA TG	AGC CCA TCC AAC ACC AAG

Supplementary Results

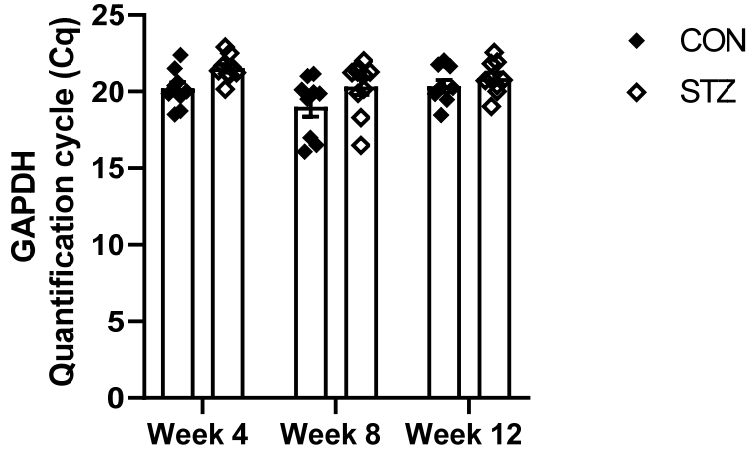

Fig. S2. Quantification cycle (Cq) of GAPDH in hearts from control and diabetic rats at 4, 8 and 12 weeks.

GAPDH quantification cycle in CON (black diamonds; n = 9/9/9 rats) and STZ rats (white diamonds; n= 8/9/8 rats) at 4, 8 and 12 weeks after STZ or vehicle injection. Two-way ANOVA test showed no significant interaction between STZ treatment and time on GAPDH quantification cycle ($p = 0.62$).

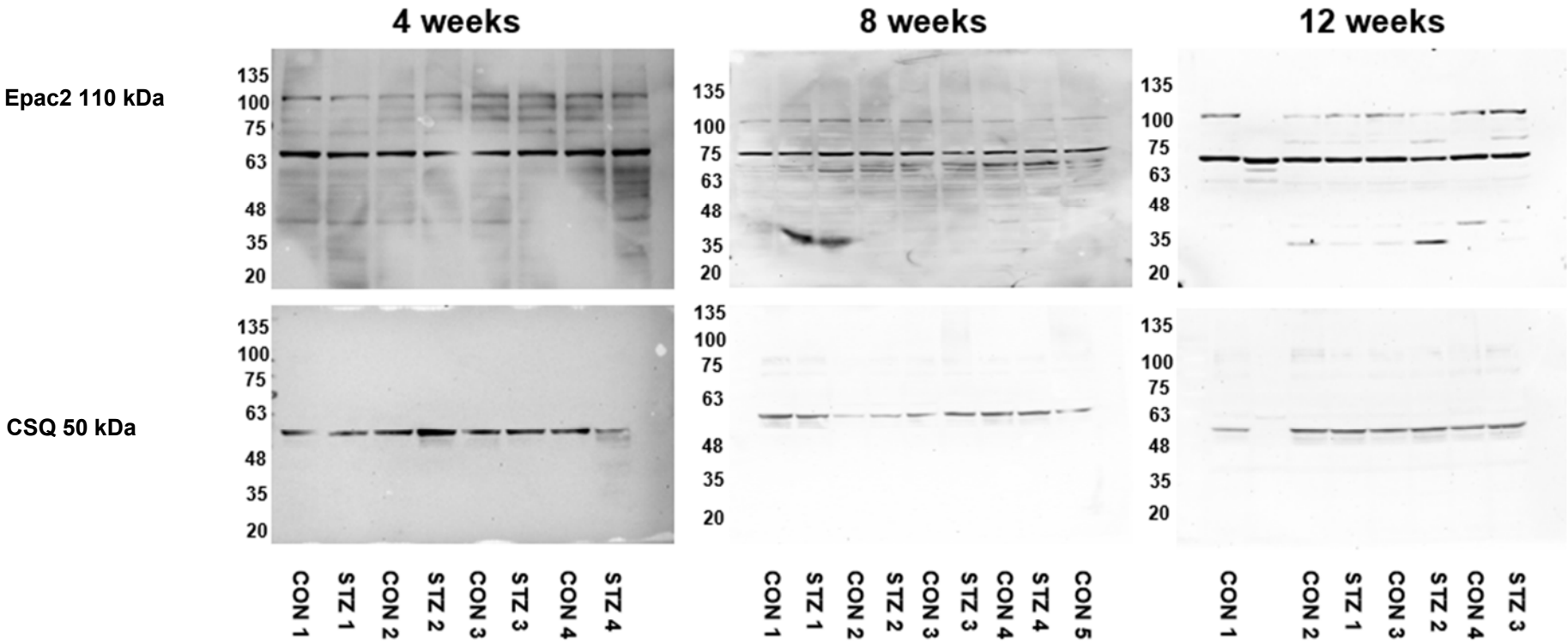

Fig. S3. Original western blot for Epac2 in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with Epac2 specific antibody. Calsequestrin (CSQ) was used as a loading control. Original blots for Epac2 and CSQ in CON (n = 4 to 5 rats) and STZ (n = 3 to 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 110 kDa was detected for Epac2 in both control and diabetic rat hearts. For CSQ, a single band migrating at 50 kDa was detected in rat hearts.

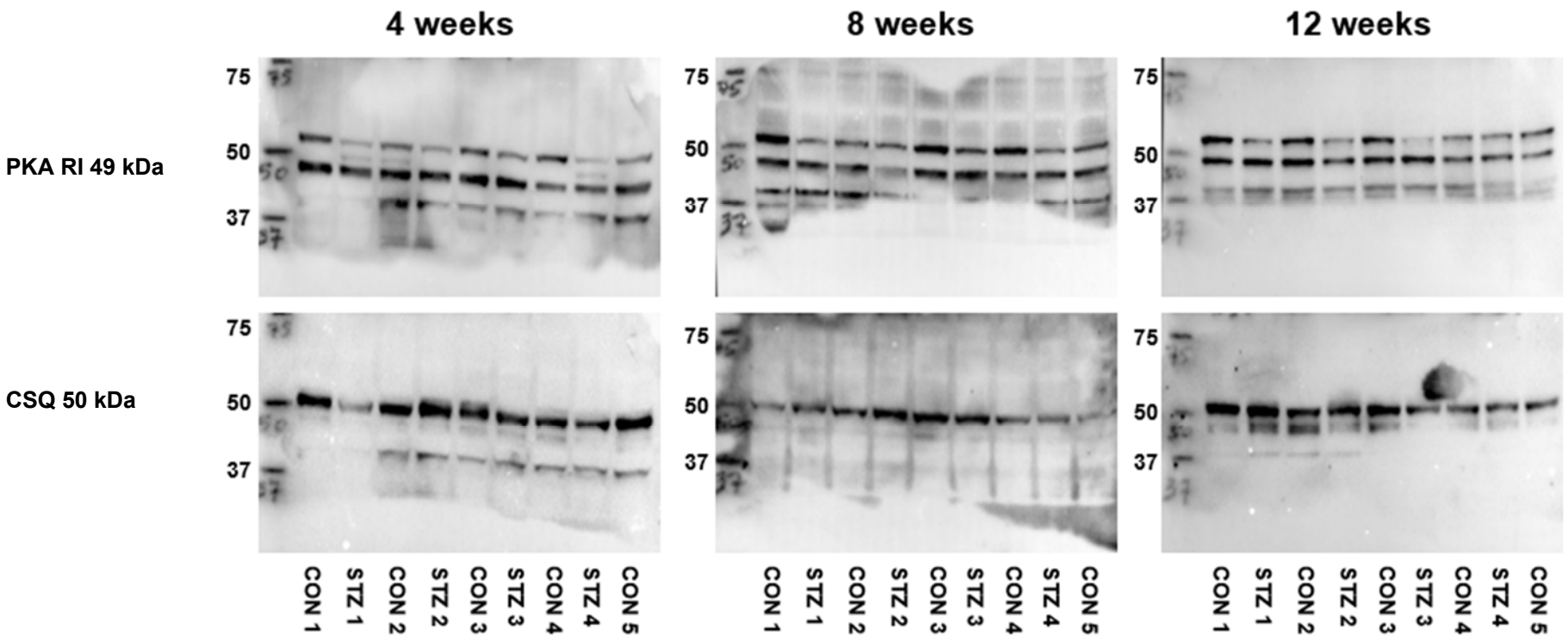

Fig. S4. Original western blot for PKA RI in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with PKA RI specific antibody. Calsequestrin (CSQ) was used as a loading control. Original blots for PKA RI and CSQ in CON (n = 5 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 49 kDa was detected for PKA RI in both control and diabetic rat hearts. For CSQ, a single band migrating at 50 kDa was detected in rat hearts.

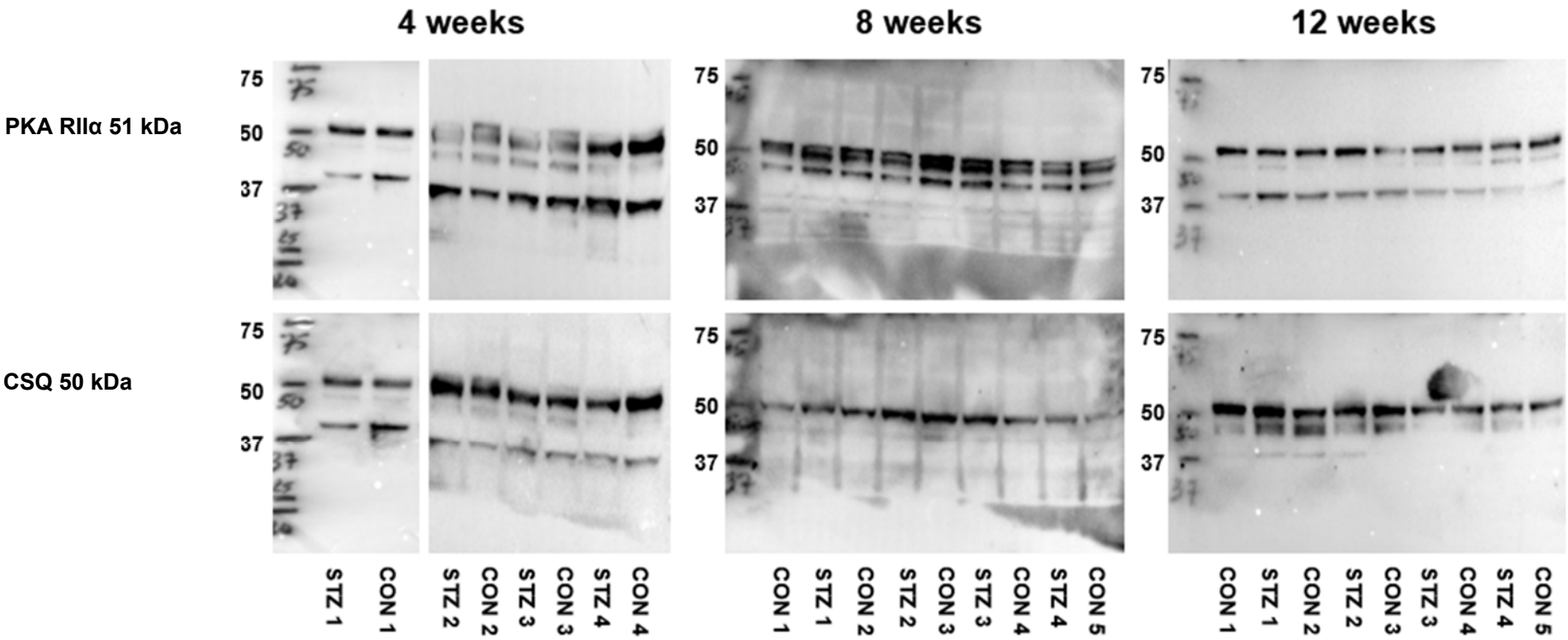

Fig. S5. Original western blot for PKA RII-α in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with PKA RIIα specific antibody. Calsequestrin (CSQ) was used as a loading control. Original blots for PKA RIIα and CSQ in CON (n = 4 to 5 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 51 kDa was detected for PKA RIIα in both control and diabetic rat hearts. For CSQ, a single band migrating at 50 kDa was detected in rat hearts.

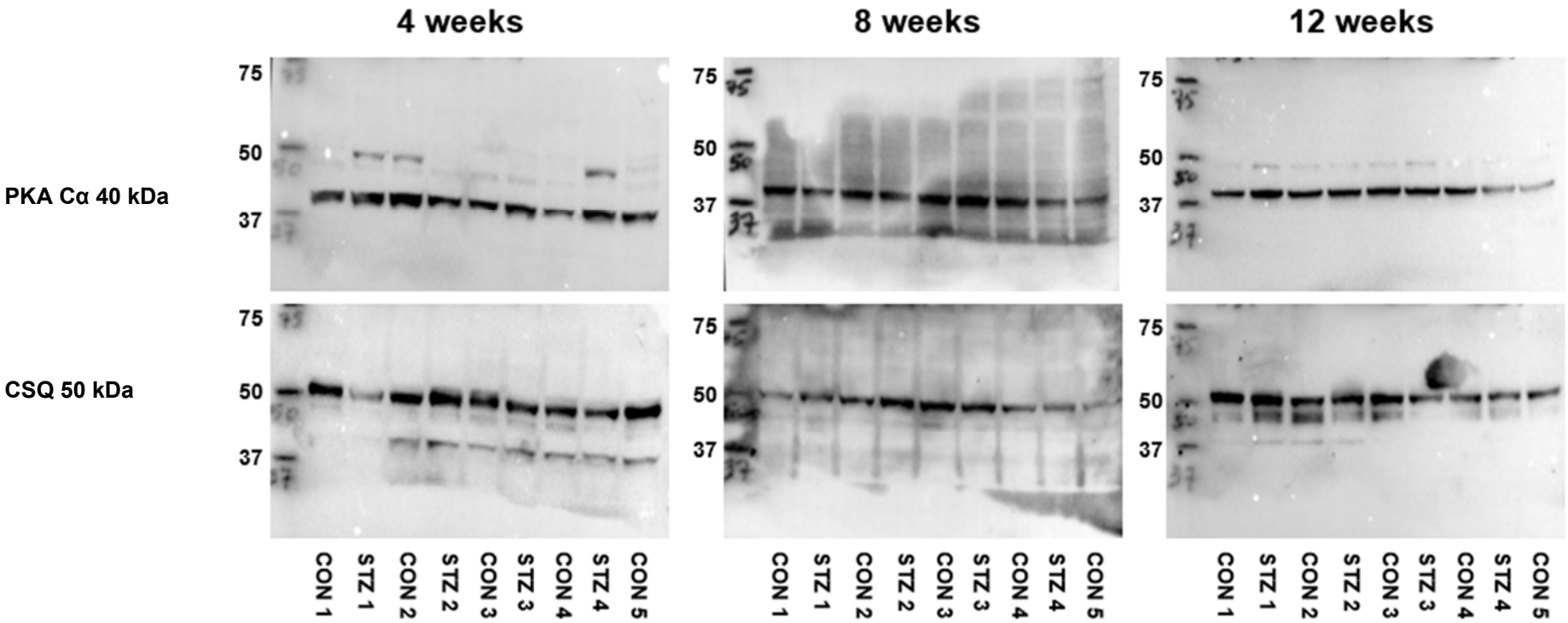

Fig. S6. Original western blot for PKA Ca in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with PKA Cα specific antibody. Calsequestrin (CSQ) was used as a loading control. Original blots for PKA Cα and CSQ in CON (n = 5 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 40 kDa was detected for PKA Cα in both control and diabetic rat hearts. For CSQ, a single band migrating at 50 kDa was detected in rat hearts.

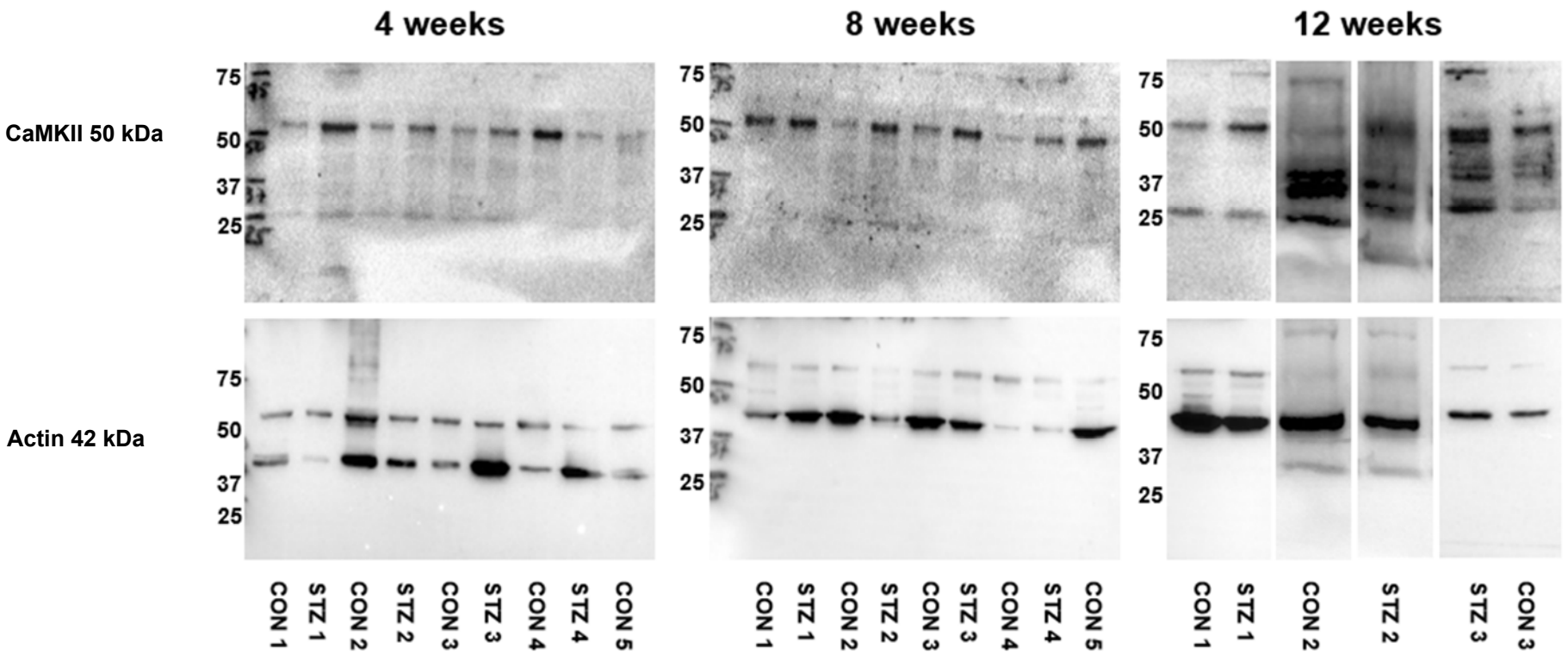

Fig. S7. Original western blot for CaMKII in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with CaMKII specific antibody. Actin was used as a loading control. Original blots for CaMKII and actin in CON (n = 3 to 5 rats) and STZ (n = 3 to 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 50 kDa was detected for CaMKII in both control and diabetic rat hearts. For actin, a single band migrating at approximately 42 kDa was detected in rat hearts.

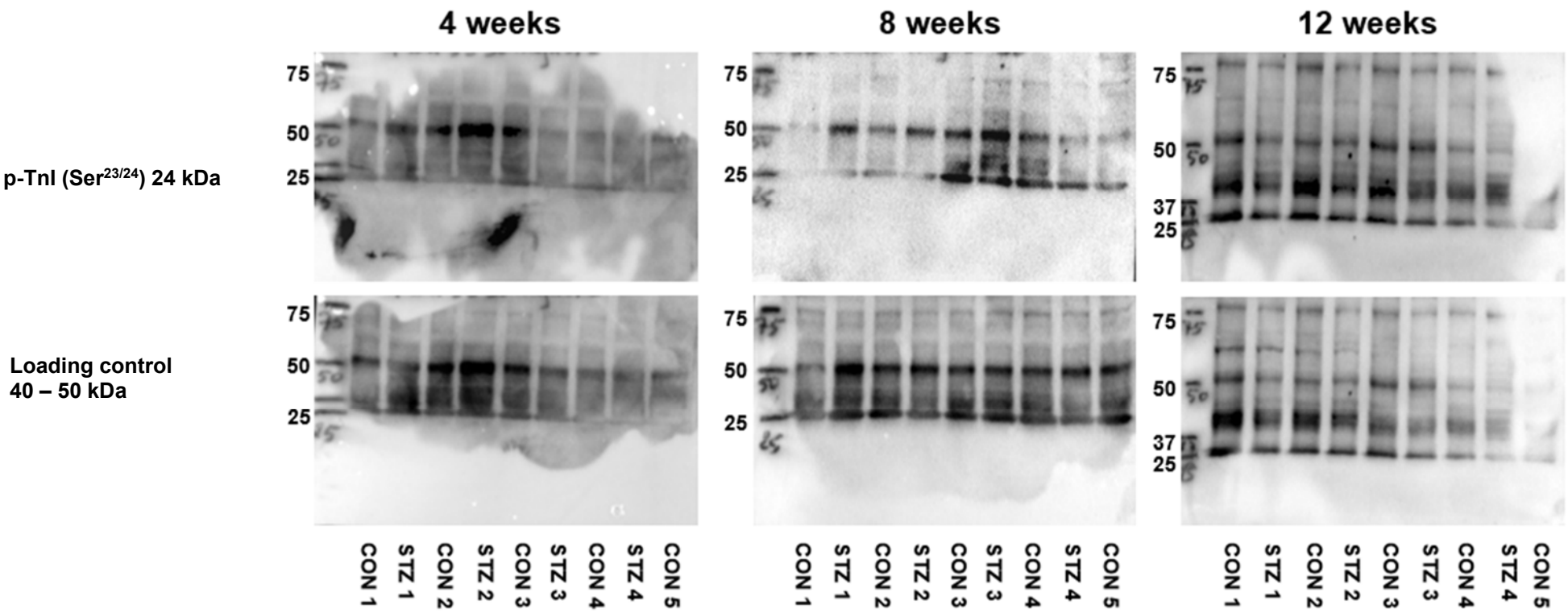

Fig. S8. Original western blot for p-TnI in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with p-TnI ($Ser^{23/24}$) specific antibody. Calsequestrin (CSQ) and actin were used as a loading control for 4/8 weeks and 12 weeks, respectively. Original blots for p-TnI, CSQ and actin in CON (n = 5 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 24 kDa was detected for p-TnI in both control and diabetic rat hearts. For CSQ, a single band migrating at approximately 50 kDa was detected in rat hearts whereas for actin, a single band was detected at 42 kDa.

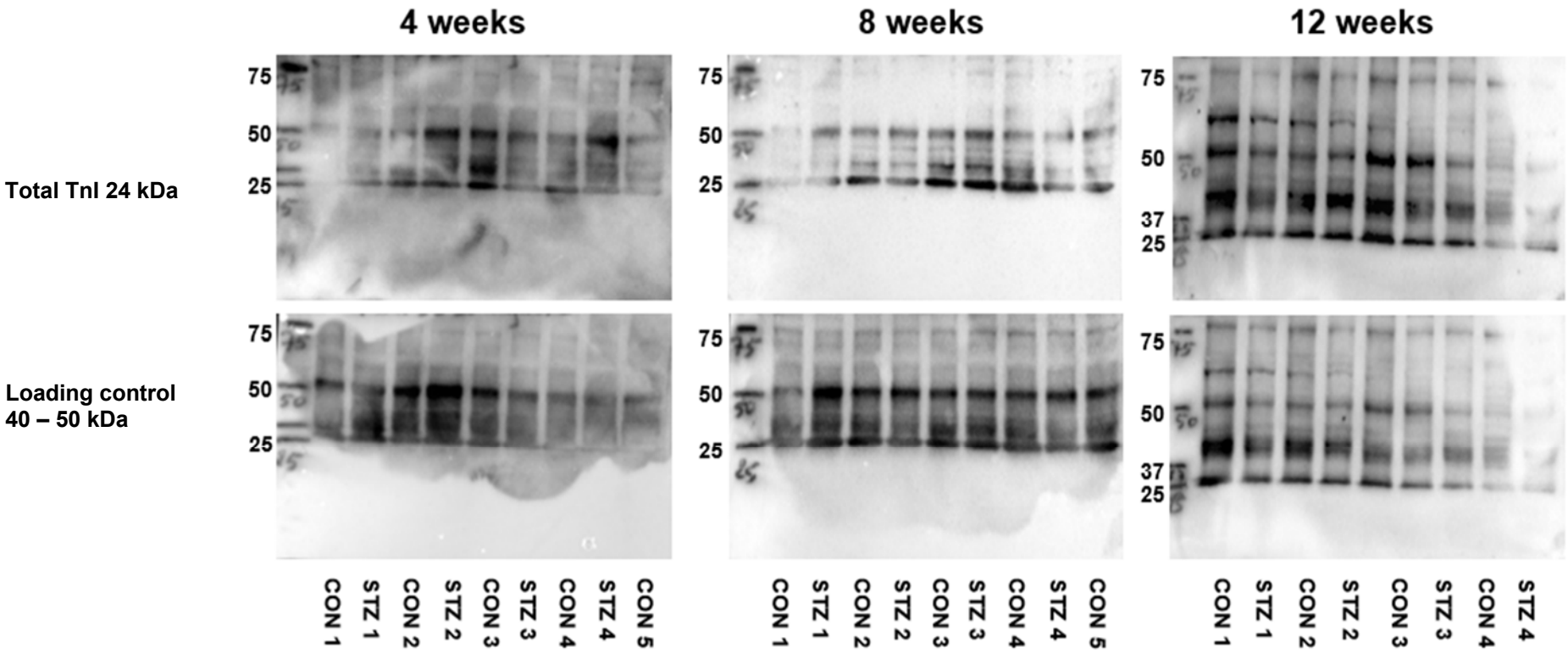

Fig. S9. Original western blot for Total TnI in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with total TnI specific antibody. Calsequestrin (CSQ) and actin were used as a loading control 4/8 weeks and 12 weeks, respectively. Original blots for total TnI, CSQ and actin in CON (n = 4 to 5 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 24 kDa was detected for total TnI in both control and diabetic rat hearts. For CSQ, a single band migrating at approximately 50 kDa was detected in rat hearts whereas for actin, a single band was detected at 42 kDa.

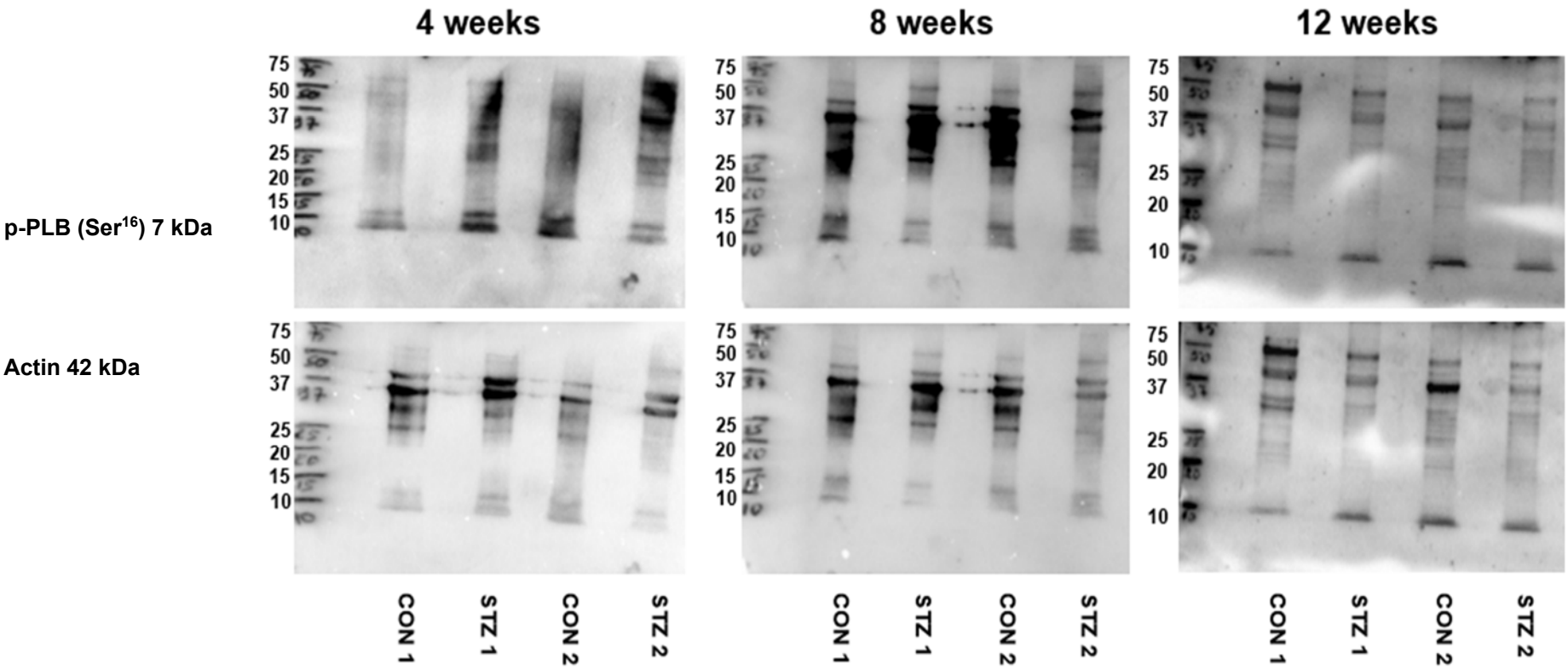

Fig. S10. Original western blot for pPLB-Ser16 in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with p-PLB (Ser^{16}) specific antibody. Actin was used as a loading control. Original blots for p-PLB (Ser^{16}) and actin in CON (n = 2 rats) and STZ (n = 2 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 7 kDa was detected for p-PLB (Ser^{16}) in both control and diabetic rat hearts. For actin, a single band migrating at approximately 42 kDa was detected in rat hearts.

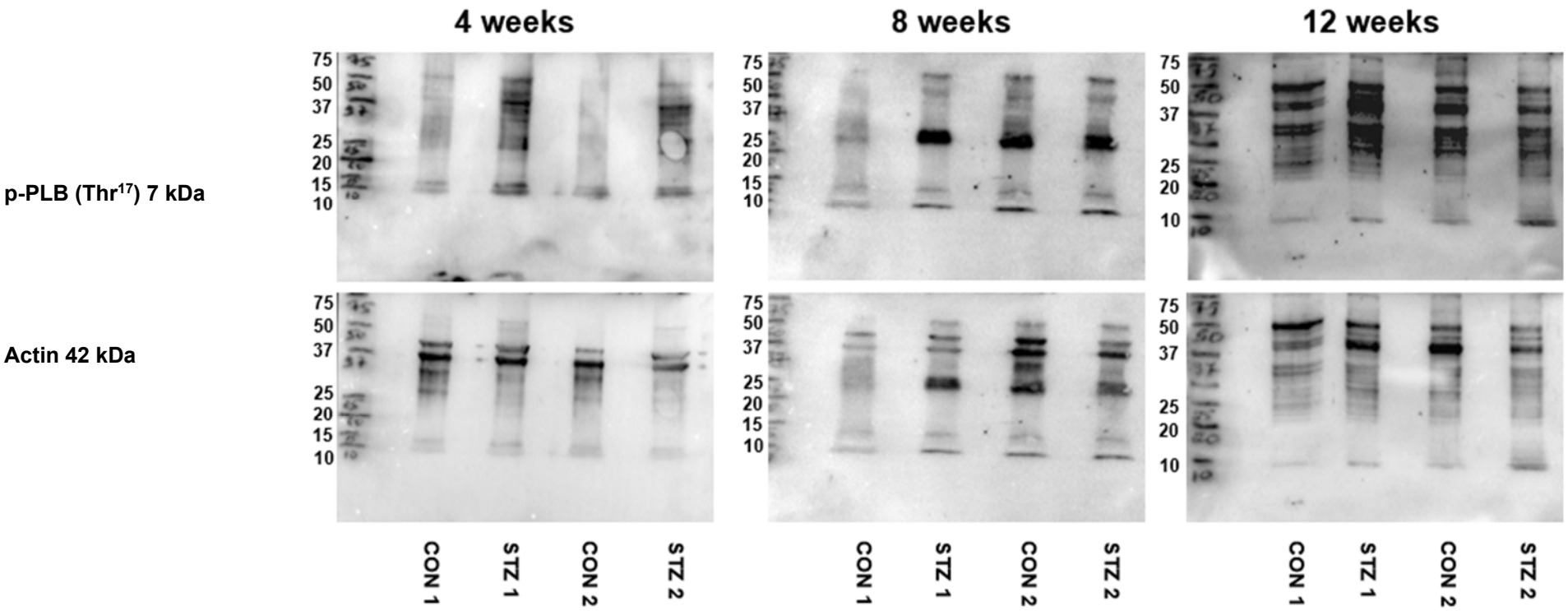

Fig. S11. Original western blot for pPLB-Thr17 in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with p-PLB (Thr^{17}) specific antibody. Actin was used as a loading control. Original blots for p-PLB (Thr^{17}) and actin in CON (n = 2 rats) and STZ (n = 2 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 7 kDa was detected for p-PLB (Thr^{17}) in both control and diabetic rat hearts. For Actin, a single band migrating at approximately 42 kDa was detected in rat hearts.

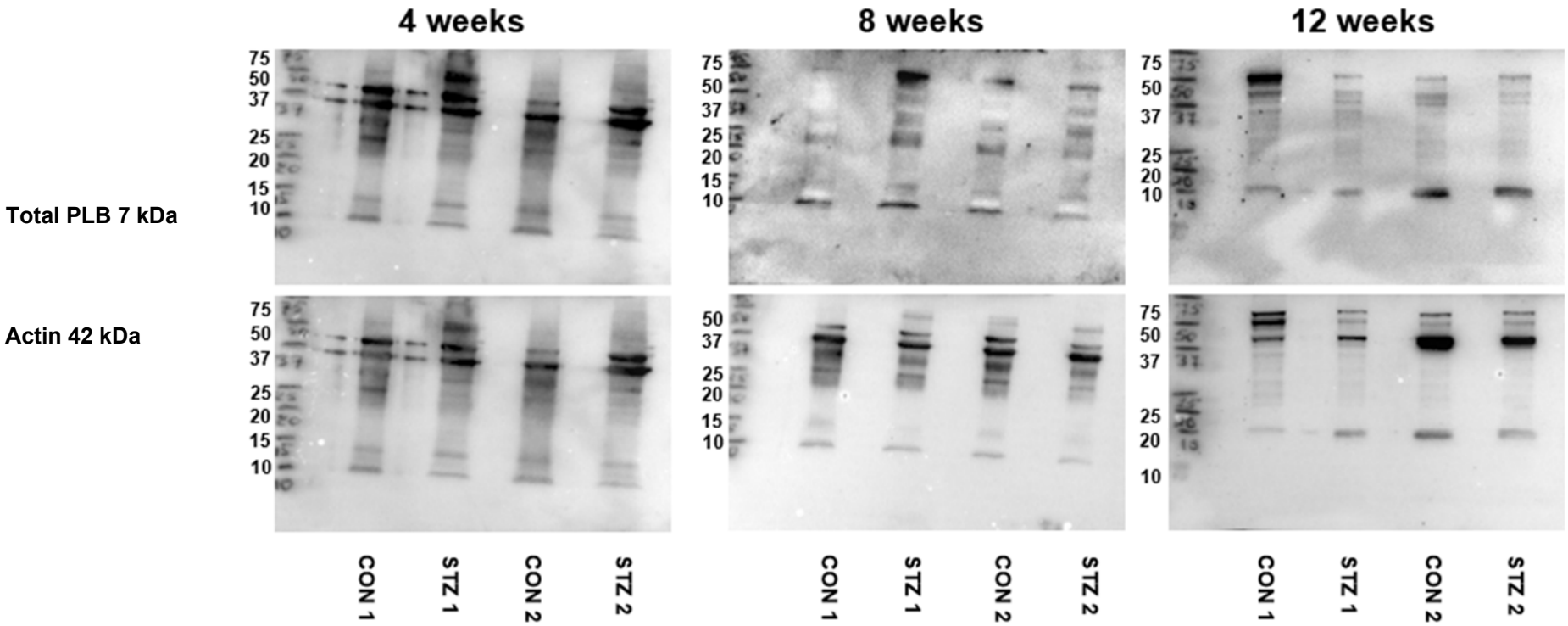

Fig. S12. Original western blot for Total PLB in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with total PLB specific antibody. Actin was used as a loading control. Original blots for total PLB and actin in CON (n = 2 rats) and STZ (n = 2 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 7 kDa was detected for total PLB in both control and diabetic rat hearts. For actin, a single band migrating at approximately 42 kDa was detected in rat hearts.

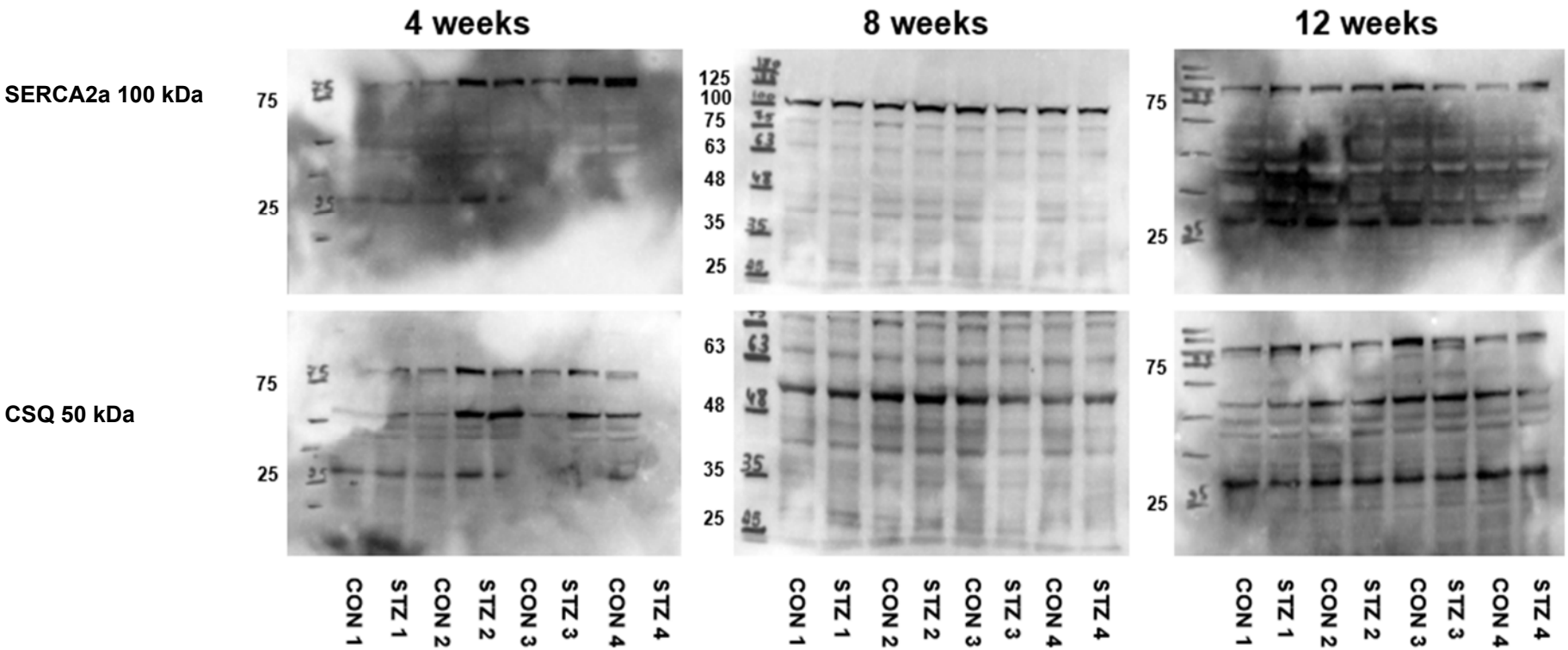

Fig. S13. Original western blot for SERCA2a in hearts from control and diabetic rats at 4, 8, and 12 weeks. Equal amounts of cardiac proteins from control (CON) and diabetic rats (STZ) were separated on SDS/PAGE and revealed with SERCA2a specific antibody. Calsequestrin (CSQ) was used as a loading control. Original blots for SERCA2a and CSQ in CON (n = 4 rats) and STZ (n = 4 rats) at 4, 8 and 12 weeks are shown. A single band migrating at approximately 100 kDa was detected for SERCA2a in both control and diabetic rat hearts. For CSQ, a single band migrating at 50 kDa was detected in rat hearts.