Cardiovascular Research Advance Access published January 18, 2010

Elevated expression of the metabolic regulator RIP140 results in cardiac hypertrophy and impaired cardiac function

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ABSTRACT

Aims: Receptor-interacting protein 140 (RIP140) is a ligand-dependent cofactor for nuclear receptors that regulate networks of genes involved in cellular processes, including metabolism. An important role for RIP140 in metabolic control has been identified in RIP140 null mice, whose phenotypes include derepression of genes involved in energy mobilisation or catabolism in adipocytes and a switch to more oxidative fibres in skeletal muscle. We hypothesized that ubiquitous expression of RIP140 would suppress metabolic processes, leading to defects in development or cellular function.

Methods and Results: The primary effect of exogenous expression of RIP140 mRNA (real-time PCR) and protein (Western blotting) in transgenic mice is impaired postnatal heart function. There was rapid onset of cardiac hypertrophy and ventricular fibrosis, detected microscopically, in male RIP140 transgenic mice from 4 weeks of age, resulting in 25% mortality by 5 months. RIP140 exogenous expression in the heart leads to decreased mitochondria state III and state IV membrane potential and oxygen consumption. Quantitative PCR showed more than 50% reduced expression of genes involved in mitochondrial activity and fatty acid metabolism, including mitochondrial transcription factor A, cytochrome oxidase VIIa, cytochrome XII, CD36, medium chain acyl dehydrogenase and fatty acid transport protein, many of which are known targets for nuclear receptors, including peroxisome proliferator activated receptors PPARα and PPARδ and estrogen-related receptors ERRα and ERRγ.

Conclusion: This study demonstrates that RIP140 is an important cofactor in postnatal cardiac function and that inhibition of the action of RIP140 may provide a model system

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to investigate specific interventions designed to prevent or delay the onset of cardiac disease.

INTRODUCTION

RIP140 is a corepressor that is recruited to nuclear receptors primarily in the presence of agonists and thereby reduces the expression of target genes[1-3]. Phenotypic analysis of RIP140 null mice[4] has shown that while the repressor is not essential for embryonic or tissue development it has profound effects on cell function, in particular with regard to the physiological activity of metabolic tissues including adipose and skeletal muscle[5,6]. In the absence of RIP140, white adipocytes express elevated levels of genes that promote energy mobilisation rather than storage[7,8] while in skeletal muscle the absence of RIP140 results in alterations in fibre type towards more oxidative ones with increased mitochondrial number[6]. In addition to its repressive action, RIP140 has also been shown to act as a cofactor for other transcription factors including AP1[9] and as an activator of Sp1[10], AhR[11] and more recently NFkB[12] in macrophages, where reduction in the level of RIP140 leads to inhibition of the pro-inflammatory gene expression program. Thus RIP140 expression has been identified as an important factor in many metabolic and cellular processes.

While the consequences of RIP140 depletion have been well characterised the effect of overexpression of RIP140 *in vivo* is unknown. The CAGGS promoter system has been well characterised and used extensively to assess the consequences of ubiquitous transgene expression *in vivo*[13], efficiently producing elevated levels of gene expression in many different cell types[14]. In view of the effect of RIP140 on metabolic gene

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regulation, the use of a ubiquitous transgenic approach is appropriate to determine the functional consequences of overexpressing RIP140 *in vivo*.

Many studies have shown the importance of nuclear receptors regulating cardiac metabolic gene expression[15-20]. Nuclear receptor coactivators are also important factors in transcriptional control of metabolic genes with both peroxisome-proliferator-activated receptor-gamma coactivator (PGC)-1α[21-23] and PGC-1β[24,25] identified as important regulators of cardiac function. The primary contractile function of the heart in early postnatal development and in adults is fuelled by adenosine triphosphate generated mainly from oxidation of fatty acids in the mitochondrion. Because the heart has a limited capacity to store or synthesise fatty acids, cellular fatty acid uptake and oxidation are tightly coupled and factors that control these gene networks are important regulators of cardiac function[26]. Animal models and *in vitro* work using cardiomyocytes have shown that peroxisome-proliferator-activated receptor (PPAR)α[27,28], PPARδ[20,29] and estrogen-related receptor (ERR)α[16,30] are all important in controlling fatty acid metabolism and they share a number of overlapping targets. Recently it was shown that ERRγ and ERRα target a common set of promoters in the heart[16] indicating that ERRγ is also an important regulatory factor of cardiac gene expression. Deregulated expression or alteration in the activity of these factors can potentially lead to cardiac hypertrophy and pathological changes characterised by myocyte disarray and/or increased ventricular fibrosis. This in turn may result in impaired cardiac function and decreased survival. These changes may be accompanied by up-regulation of markers of heart failure and cardiac pathology such as atrial natriuretic factor and myosin heavy chain β[31-34]. In this

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study we demonstrate a crucial role for RIP140 as a controlling factor in gene networks essential for normal postnatal cardiac function.

MATERIALS AND METHODS.

Generation and maintenance of RIP140 transgenic mice.

Human RIP140 was expressed in the FVB/N mouse strain using a pCAGGS-hRIP construct (see Supplementary Methods and Supplementary Figure 1A).

Real-Time PCR.

RNA was extracted from the left atrium and ventricle of the heart (n=5 per group) using Trizol as described by manufacturer and 1µg was reverse transcribed using Superscript First Strand Synthesis Kit (Invitrogen). Real-time PCR was carried out with ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The ribosomal coding gene, L19, was used to normalise cDNA levels.

Protein analysis by Western blot.

50 µg of proteins extracted from 2 week-old WT and transgenic hearts (n=4/group) was used for SDS-PAGE and immunoblot analysis. Primary antibodies against FABP3 (sc-58274), CD36 (sc-9154), α-tubulin (sc-8035) (Santa-Cruz Biotechnologies) and ERRα (Novus Biologicals) were purchased and mouse monoclonal anti-RIP140 (6D7) was made in house. Quantification was made using NIH Image software.

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Histology.

Hearts were dissected from animals aged 1 to 8 weeks and older. Animals were weighed prior to dissection and the hearts were bisected and weighed (n=5-15 animals per group). Heart weight/body weight ratio was then calculated. The left side of each heart (4-8 weeks) was frozen for expression analysis and the right side was fixed in neutral buffered formalin. Paraffin sections (5 µm) were stained using haematoxylin and eosin, Masson's Trichrome, Alizarin Red or Alcian Blue-Van Gieson. Collagen staining was quantified using image analysis on sections stained with Van Gieson (supplementary methods). Immunohistochemistry was performed using F4/80 anti-murine macrophage marker (Serotec). Frozen sections were stained for cytochrome c oxidase (COX) and succinate dehydrogenase (SDH). Electron microscopy was performed on some samples (see supplementary methods).

Magnetic resonance imaging (MRI).

Male and female WT and transgenic mice aged 2-5 months (n= 20 in total) were scanned using non-invasive Cine MRI. Dynamic cine images are available to view as supplementary material.

Mitochondrial oxygen consumption and membrane potential measurements.

Oxygen consumption rate and membrane potential were measured simultaneously using electrodes sensitive to oxygen or the membrane potential-dependent probe, triphenylmethylphosphonium ($TPMP^+$) as described previously[35]. Isolated heart mitochondria (0.15mg/ml) were energised with 4mM succinate. State III respiration was

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determined with 0.3 mM ADP. State IV respiration was determined following the addition of oligomycin (1 μg/mg protein).

Statistical analysis.

Data were analysed using Student's two-tailed *t*-test, and reported as mean + SEM. Survival data were analysed using a log rank test. *P*-values of less than 0.05 were considered significant.

RESULTS

Characterisation of the cardiac phenotype in RIP140 transgenic mice.

Eleven separate RIP140 transgenic founders were generated of which six lines (27, 34, 40, 45, 49 and 51) gave rise to litters. All transgenic lines were maintained by crossing heterozygous transgenic males with WT females. Transgenic progeny were born at a normal Mendelian ratio and there was no evidence of growth retardation. From six independent transgenic mouse lines, four showed elevated expression of human RIP140 mRNA in hearts from mice aged 4-5 months (Supplementary Figure 1B). Two lines (lines 34 and 51) were studied in detail. The phenotype was similar in both lines and data presented are primarily from line 51.

Initial observations showed reduced rates of survival of both male and female transgenic mice. Macroscopic pathology was observed in the hearts of transgenic mice aged 4 months showing evidence of hypertrophy: the ventricles were distorted in shape and atria were enlarged and rigid with areas of calcification (Figure 1A). With the exception of line 27, the line which did not express human RIP140 transgene in the heart

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(Supplementary Figure 1B), all lines showed elevated RIP140 expression and cardiac pathology suggesting that exogenous expression of RIP140 in the heart may participate at the development of cardiac hypertrophy.

Exogenous expression of RIP140 in transgenic mice results in reduced survival.

Death from heart failure occurred by 20 weeks of age in 24% and 20% of transgenic males and females respectively (Figure 1B). Furthermore, a significantly greater number of males over females died prior to 8 weeks. Some mice had pleural effusion and in some cases ascites. Microscopy revealed signs of inflammation in the lungs in some animals, with congestion and fibrosis (data not shown). Reduced survival in male transgenic mice compared to females could be due to a more pronounced decrease in cardiac function. However, it is notable that transgenic females failed to survive after parturition which may reflect an inability to respond to elevated stress.

Exogenous expression of RIP140 in transgenic mice results in cardiac hypertrophy.

Heart mass progressively increased from about 4 weeks of age in transgenic males and from 8 weeks of age for the females as shown by the heart weight/body weight ratio (Figure 1C), with no differences in body weight between age-matched WT and transgenic mice (Supplementary Figure 2). In several models of cardiac hypertrophy, an increase in myocardial mass is often associated with a shift in myosin heavy chain (MyHC) protein and mRNA from the α to the β isoform. Analysis of cardiac MyHC from 8 week-old mice showed that both WT and transgenic hearts expressed α-MyHC as the predominant isoform with no differences in expression between WT and

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transgenic (Figure 1D); no appearance in β-MyHC was observed in the hearts of transgenic mice. These data suggest that alteration in cardiac MyHC profile is not implicated in the cardiomyopathy observed in the RIP140 transgenic mice.

Non-invasive Cine MRI revealed evidence of substantial cardiomyopathy in RIP140 transgenic mice. This was characterised by ventricular wall thinning and increase in size of the ventricular cavity leading to decreased ejection fraction (Supplementary Figure 3). Altogether, these data suggest that exogenous expression of RIP140 impairs cardiac function.

Early onset of cardiac fibrosis and atrial defects in RIP140 transgenic mice.

Ventricles of transgenic mice showed evidence of myocyte disarray and variable amounts of interstitial fibrosis, in all transgenic lines which overexpressed RIP140 (Supplementary Figure 4). The fibrosis was detectable histologically as early as 2 weeks of age. In 4 week-old mice the fibrosis had increased in localised areas of the ventricle, and by 8 weeks of age, fibrosis was severe in a proportion of transgenic animals. Collagen was localised around groups of small cardiomyocytes with perinuclear spaces, as a result of myofibril loss and myocytolysis (Figure 2A-C).

Atria of RIP140 transgenic mice also showed increased collagen deposition, and thrombus formation in one or both atria was seen in about 75% of males and 30% of females from 6 weeks of age onwards (Figure 2D-I). In younger animals, macrophages and leukocytes could be seen in the atrium adhering to the endothelial cells lining the lumen (Figure 2E, J). In animals over 8 weeks old, thrombi were sometimes large with

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compacted blood cells, fibrin and cartilage forming at the edges (Figure 2I) with evidence of calcification (Figure 2K-L).

Quantification of the area of representative ventricular sections occupied by collagen staining showed that at 4 weeks of age both males and females had equal amounts of fibrosis, while 8 week-old males had significantly more fibrosis than 4 week-old males and females of the same age (Figure 2M). WT ventricles showed no fibrosis, and collagen staining occupied approximately 1% of ventricular section area, mainly around blood vessels.

Endogenous and overexpressed RIP140 levels in transgenic hearts.

In view of the rapid appearance and development of cardiomyopathy, expression of transgenic and endogenous levels of RIP140 was determined by real-time PCR. RIP140 mRNA level was increased in transgenic mice compared to WT at all ages analysed (Figure 3A) with the amount of exogenous RIP140 level 5-10 fold higher than endogenous. RIP140 expression level was unchanged at 1 and 4 weeks of age in both sexes although it increased between 4 and 8 weeks of age both in WT and transgenic mice. The elevated expression of RIP140 at the mRNA level was confirmed by detection of increased RIP140 protein levels in hearts from 2 week-old transgenic mice compared with WT littermates (Figure 3B). The level of RIP140 protein was at least 3-fold higher in transgenic hearts than WT with some variation between individual transgenic hearts in the degree of expression (data not shown).

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Elevated levels of cardiac RIP140 alter metabolic gene expression.

Previously, we have shown that RIP140 acts as a transcriptional corepressor for nuclear receptors, such as ERRs and PPARs, implicated in the control of fatty acid and glucose metabolism in the white adipose tissue and skeletal muscle. Similarly, ERRs and PPARs are important factors controlling cardiac function. In order to understand better how RIP140 increased expression impacts heart function, we investigated the changes in metabolic gene expression in transgenic and WT mice at 4 and 8 weeks of age (Figure 4).

Expression of factors involved in regulating beta-oxidation was lower in hearts of male transgenic mice (Figure 4A). The expression of transporters such as fatty acid binding protein 3 (FABP3), CD36 and fatty acid transport protein (FATP) was decreased as were enzymes involved in fatty acid oxidation, including lipoprotein lipase (LPL), medium chain acyl dehydrogenase (MCAD) and trifunctional protein α (TFα), in both 4 and 8 week old males (Figure 4A). Many of these factors were also decreased in female transgenic mice although the reduction was only apparent in adults at 8 weeks of age (Supplementary Figure 5). The downregulation of genes implicated in fatty acid handling suggests that beta-oxidation should be decreased. A reduction in beta-oxidation was observed in transgenic hearts compared to WT but it did not reach statistical significance (Supplementary Figure 6). Additionally, expression of PPARα, PPARδ and ERRα was reduced in both 4 and 8 week old male transgenic mice compared to WT (Figure 4B) while expression of ERRγ was reduced in 8 week old transgenic mice. In the heart of transgenic females of the same age, only PPARα expression was decreased

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(Table 1). Protein levels for selected key target genes were analysed (Figure 4C) and their decrease confirms the changes observed at the mRNA level.

The levels of members of the electron transport chain including cytochrome oxidase (Cox)IV, CoxVIIa, cytochrome C and NADH dehydrogenase subunit 1 (ND1) were reduced in the hearts of male transgenic mice, in agreement with RIP140 acting as a transcriptional corepressor (Figure 4D). Likewise, the expression of mitochondrial transcription factor A (mtTFAM), which controls the expression of genes implicated in mitochondrial function and biogenesis, was decreased in transgenic males compared to WT (Figure 4D). A summary of gene expression changes is shown in Table 1. These data suggest that mitochondrial function is impaired in the hearts of RIP140 transgenic mice.

Mitochondrial structure and function are altered in RIP140 transgenic mice.

Ultrastructural analysis showed that in some cardiomyocytes, mitochondria were smaller with evidence of disorganisation of mitochondria and myofibrils in RIP140 transgenic mice (Figure 5A). Mitochondrial defects included breakage and deterioration of cristae and the presence of intramitochondrial lamellar bodies. Abnormal cardiomyocytes containing degenerating mitochondria and surrounded by collagen fibres were present in both atria and ventricles. In contrast, WT hearts showed no evidence of these abnormal features. Notably, atrial-specific granules, which contain the atrial natriuretic peptide (ANP), were more numerous in transgenic atrial cardiomyocytes than in the WT (Figure 5A). The increased storage of ANP in the atria of transgenic mice correlated with

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increased level of expression in RIP140 transgenic hearts earlier in males compared with females (Supplemental Figure 7B).

Frozen sections of adult hearts were stained for SDH as a marker for mitochondrial activity. There was intense labelling in the WT, but reduced staining in the transgenic, with spaces in the tissue caused by cell loss (Figure 5B). There was no evidence of COX–deficient/ SDH-positive cardiomyocytes in 1 or 2 week-old transgenic mouse hearts indicating no significant alteration in mitochondrial function during the early post-natal period of development (Supplementary Figure 7A).

A decrease in the number of total mitochondria per cell and/or decreased mitochondrial activity per se can explain the decreased SDH staining observed in the transgenic hearts. The mitochondria/cell ratio was lower in the transgenic hearts compared to WT (Figure 5C). To test mitochondrial function, oxygen consumption and steady state membrane potential were measured in mitochondria isolated from WT or RIP140 transgenic hearts (Figure 5D). During state III (ADP phosphorylation), state IV (mitochondrial proton leak) and maximal capacity (fully uncoupled) steady state respiration, both oxygen consumption and membrane potential were reduced in mitochondria isolated from transgenic hearts compared to WT. These results suggest that the activity of the electron transport chain in RIP140 transgenic mitochondria is compromised. Thus, in addition to the lower number of mitochondria, reduced electron transport chain activity is likely to contribute to reduced heart substrate usage and respiration.

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DISCUSSION

In this study we report that RIP140 transgenic mice, expressing increased levels of RIP140 mRNA and protein in the heart, exhibit rapid and progressive postnatal cardiomyopathy leading to premature death predominantly due to compromised energy production. The effect of elevated RIP140 expression in the transgenic mouse heart suggests that this co-repressor acts as a feed-forward and feedback regulator in cardiac function.

Cardiac hypertrophy, compensating for increased work load and loss of contractile function, nearly always results in cardiac insufficiency and heart failure[36] and takes place without any division of muscle cells[37,38]. Mitochondrial biogenesis is important in maintaining the balance of energy production and use and is one of the key determining factors in cardiomyocyte hypertrophy and heart failure[26,39-41]. Cardiomyocyte mitochondria in RIP140 transgenic mice are less numerous, show ultrastructural signs of degeneration, as described in other transgenic models with cardiomyopathy[42], accompanied by a concomitant reduction in electron transport chain activity.

Nuclear receptors play a key role in mitochondrial activity with respect to cardiac function[16,18,28-30]. Our finding that a reduction in expression of PPARα, PPARδ and ERRα accompanied the increased expression of RIP140 provides an explanation for the reduced expression of mitochondrial genes and a number of metabolic gene networks. Previous work has shown that the down-regulation of PPARα results in a shift in substrate preference from fatty acids to glucose leading to cardiac hypertrophy and heart failure[43]. While GLUT1 and GLUT4 glucose transporter expression are unchanged in PPAR-α null mice glucose utilisation is increased and fatty acid β-oxidation reduced[44]. In

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RIP140 transgenic mice, however, both PPAR-α and GLUT4 (Table 1) are repressed suggesting that an increase in glucose utilisation as compensation for reduced fatty acid metabolism may be impaired in these mice. Repression by RIP140 also leads to reduced expression of PPAR-δ, which activates targets in the glucose utilisation pathway[15], maintaining energy balance[45]. Cardiac-specific PPAR-δ null mice show cardiac hypertrophy which causes heart failure in 4 month-old animals[20]. Activators of both PPAR-α and PPAR-δ have been used to treat diabetes and reduce fibrosis in animal models of hypertension and cardiac hypertrophy[46,47]. In addition, RIP140 has been shown to act as a corepressor for these nuclear receptors. According to the level of overexpression of RIP140 in the heart, the severity of the symptoms observed in the transgenic mice may vary in individuals and determine the viability of the animals. Therefore, in the RIP140 transgenic mice, the relatively more severe phenotype could result from simultaneous down-regulation of nuclear receptors level and activity leading to reduced glucose utilisation and fatty acid oxidation.

Overexpression of RIP140 had a greater impact on the heart in male RIP140 transgenic mice, with more severe fibrosis, atrial thrombosis and cardiac hypertrophy, than that observed in females from about 4 weeks of age. It has been shown that estrogen treatment can result in the prevention of cardiac hypertrophy[48] and that this effect is primarily targeted through the ERβ isoform[49]. Interestingly initial studies identified RIP140 overexpression as a factor in the repression of estrogen receptor signalling[1,50]. Whether this pathway is relevant to the development of the cardiac phenotype described in this study is still to be determined.

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RIP140 has been shown to be a cofactor for ERRα and ERRγ, both of which have been identified as important transcriptional regulators of cardiac function[16-17]. The activation of these nuclear receptors has been shown to be mediated by cofactors including PGC1α and PGC1β, both of which in turn act through nuclear receptor dependent and independent pathways to regulate the expression of metabolic genes[23,30,51,52]. In this study, we have identified RIP140 as an important factor in the early development of cardiac hypertrophy and the onset of disease. As a result of the ability of RIP140 to target many different transcription factors and regulate a number of signalling pathways, the RIP140 transgenic mouse may provide a valuable model system to investigate specific or combinations of treatments designed to prevent or delay the onset of cardiac dysfunction.

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FUNDING

This work was supported by grants from the Wellcome Trust, Reference 079200/Z/06/Z (J.H.S and A.F); and the Biotechnology and Biological Sciences Research Council (02/B1/S/08214 to D.N).

ACKNOWLEDGEMENTS

We acknowledge Nina Messner and Heli Niittymäki for transgenic mouse production, Erja Mäntysalo for animal husbandry and colony management in Turku, Finland, and Michael Schneider for advice and comments on the manuscript.

CONFLICT OF INTEREST

None declared

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FIGURE LEGENDS.

Figure 1: A. Representative macroscopic images of hearts (4-5 months old) from RIP140 transgenic lines 34, 45 and 51 compared to WT littermates. Size marker = 1mm. **B.** Survival curve showing the percentage of male and female transgenic mice (n= 512), $p<0.0001$ for male versus female transgenics. **C.** Heart weight/body weight ratio for 4 and 8 weeks old WT and transgenic males (n=5-15/group, $p<0.05$ compared to WT). **D.** Silver stained SDS-PAGE gel showing MyHCα proteins in WT and transgenic mice.

Figure 2: A-C. Comparison of healthy WT ventricle **(A)** with typical appearance of fibrosis and perinuclear myofibril loss in RIP140 transgenic ventricle at 8 week-old **(B-C)**, stained with Masson's trichrome. **D-I**. Atria stained with H&E **(D-F)** or Masson's trichrome **(G-I)**, showing thrombus formation. **D.** WT atrium; **E.** Transgenic with macrophages adhering to endothelium (arrow); **F.** small thrombus in the transgenic atrial lumen. **G.** WT atrium; **H.** thrombus accumulating in the atrium; **I.** reorganising thrombus (T) with cartilage. **J**. Macrophages (arrows) in transgenic atrium immunostained with F4/80 antibody. Cartilage and calcification in RIP140 transgenic atrial thrombus. **K.** Alcian Blue/Van Gieson staining; **L.** Alizarin red staining showing calcified tissue. Size markers: A-C = 50 µm, D-L = 100 µm.

M. Quantification of collagen staining in 4- and 8 week-old RIP140 transgenic male and female ventricles. *$p<0.05$, for 8 week-old male versus 4 week-old male and all females.

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Figure 3: A. Total RIP140 mRNA was measured by real-time PCR at 1, 4 and 8 week-old WT and transgenics. n=5. **B.** Western blot of RIP140 proteins in heart extracts from 2 week-old male WT and RIP140 Transgenic mice and corresponding quantification, **p*<0.05, WT *versus* TG.

Figure 4: Metabolic gene expression in WT and RIP140 Transgenic (TG) mice (n=5/group), **p*<0.05, ***p*<0.01. **A-B, D.** Fatty acid oxidation **(A)**, nuclear receptor **(B)** and mitochondrial **(D)** gene expression was measured in 4 and 8 week-old WT and RIP140 TG males by real-time PCR. **C.** Protein extracts isolated from WT and TG 2 week-old male hearts were assayed for ERRα, FABP3, CD36 and α-tubulin by Western blot.

Figure 5: A. Electron microscopy of 6 week-old WT and RIP140 transgenic (TG) hearts. WT ventricular mitochondria are organised in a regular pattern compared with small, disorganised mitochondria showing evidence of degeneration in the transgenic. Increased number of atrial-specific granules (arrows) in transgenic atrium compared to WT. **B.** Succinate dehydrogenase staining in WT and transgenic ventricles. Size marker is 100 µm. **C.** Mitochondrial number/cell ratio was measured as the ratio between mitochondrial DNA (coxII) normalised to nuclear DNA (RPL7). **D.** State III and state IV mitochondrial respiration and membrane potential were measured using succinate as a substrate following addition of ADP and oligomycin, respectively, (n=4/group), *p<0.05, **p<0.01, ***p<0.001.

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TABLE 1 Summary of gene expression analysis

↓ = significantly reduced expression compared to WT.
↑ = significantly increased expression compared to WT.
● = no change

Gene	4 week male	4 week female	8 week male	8 week female
PPAR-α	↓	●	↓	↓
PPAR-δ	↓	●	↓	●
ERRα	↓	●	↓	●
ERRγ	●	●	↓	●
PGC-1α	●	●	↓	●
PGC-1β	●	●	↓	↓
FABP3	↓	●	↓	↓
Tfα	↓	●	↓	●
FATP	↓	●	↓	↓
LPL	↓	●	↓	↓
MCAD	↓	●	↓	↓
CD36	↓	●	↓	●
VLCAD	●	●	●	↓
SCAD	●	●	●	↓
CPT-1	●	●	●	●
COX IV	↓	●	↓	↓
CIDEA	↑	●	●	●
UCP3	●	●	↓	↓
Cytochrome C	●	●	↓	↓
Cyto XII	●	●	↓	↓
COX VIIA	●	●	↓	↓
mtFAM	●	●	↓	↓
NRF1	●	●	●	●
ND1	●	●	↓	↓
Citrate synthase	●	●	↓	●
Glut4	●	●	↓	↓
PDK4	↓	●	●	●
ANP	↑	●	↑	↑

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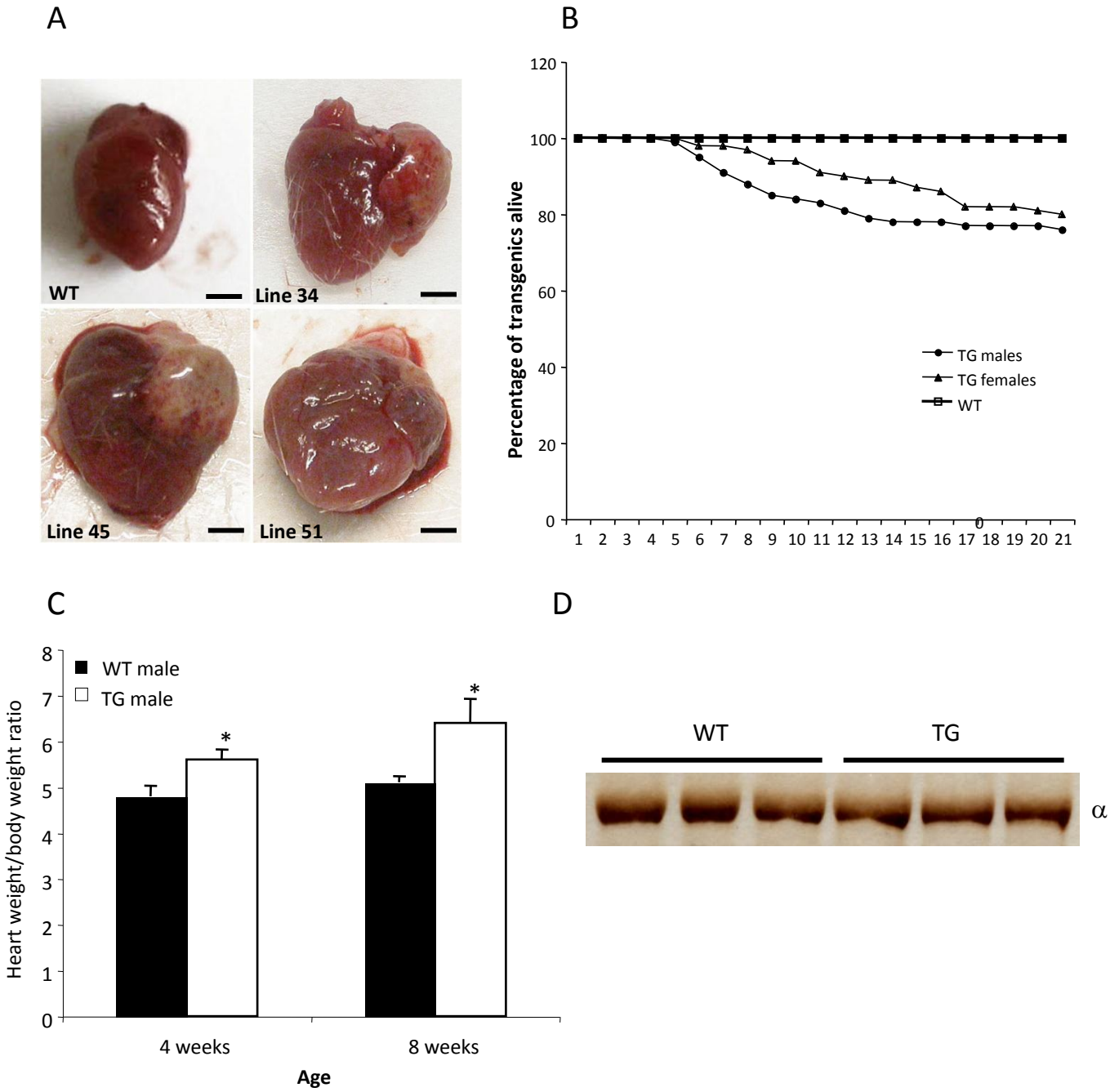
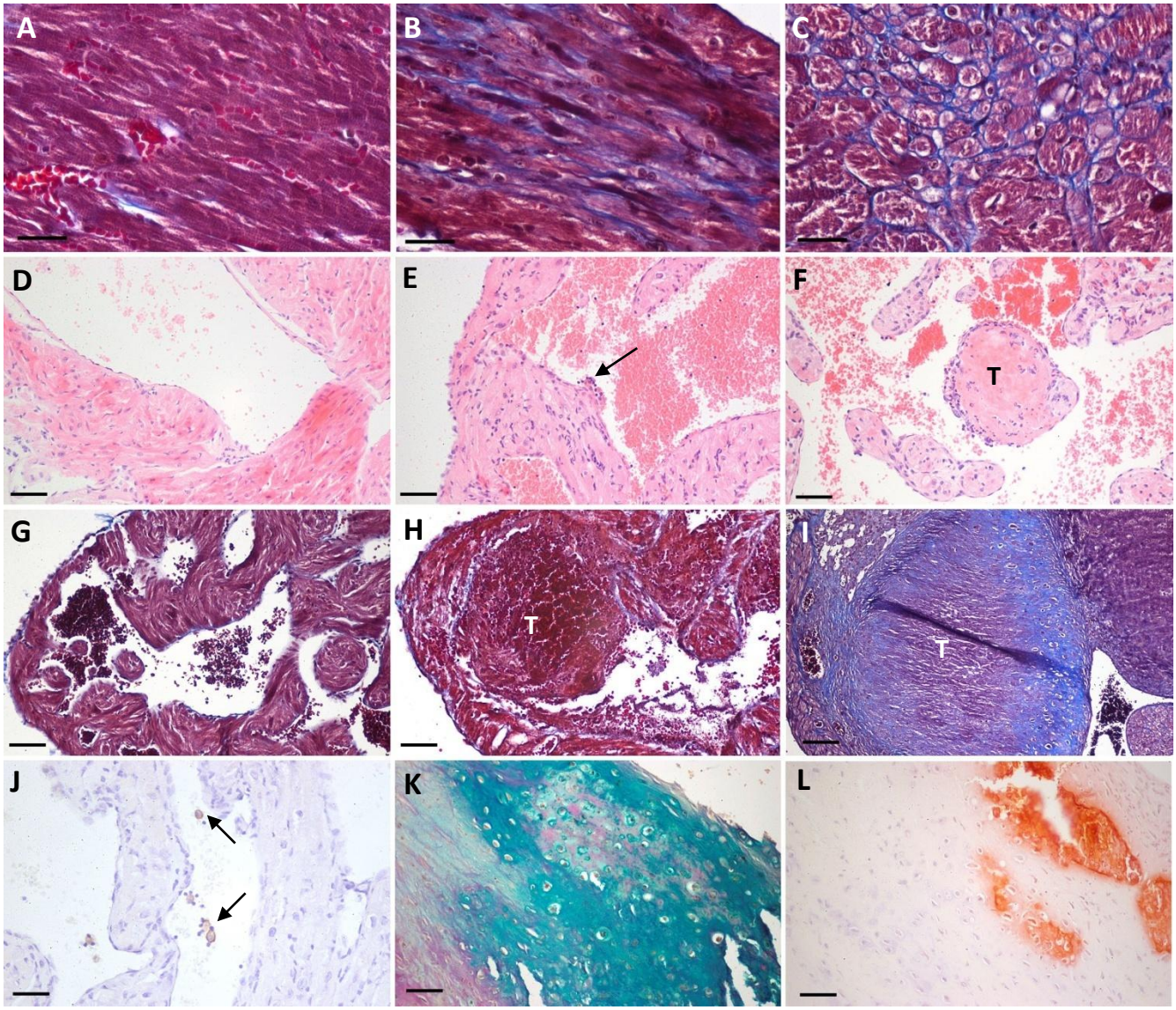

A
WT
Line 34
Line 45
Line 51
B
Percentage of transgenics alive
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
TG males
TG females
WT
C
WT male
TG male
Heart weight/body weight ratio
8
7
6
5
4
3
2
1
0
*
*
4 weeks
8 weeks
Age
D
WT
TG
α

Figure 1


A
B
C
D
E
F
T
G
H
T
I
T
J
K
L

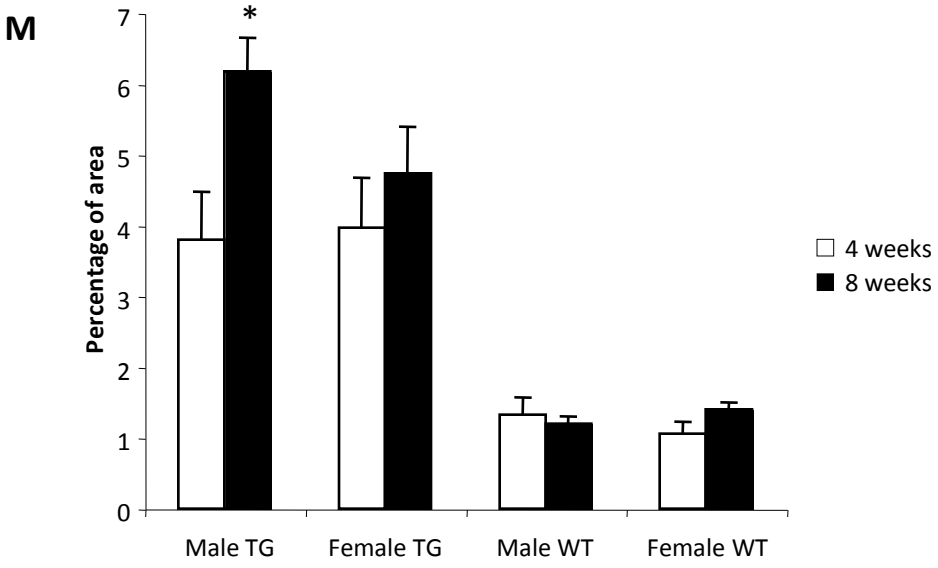
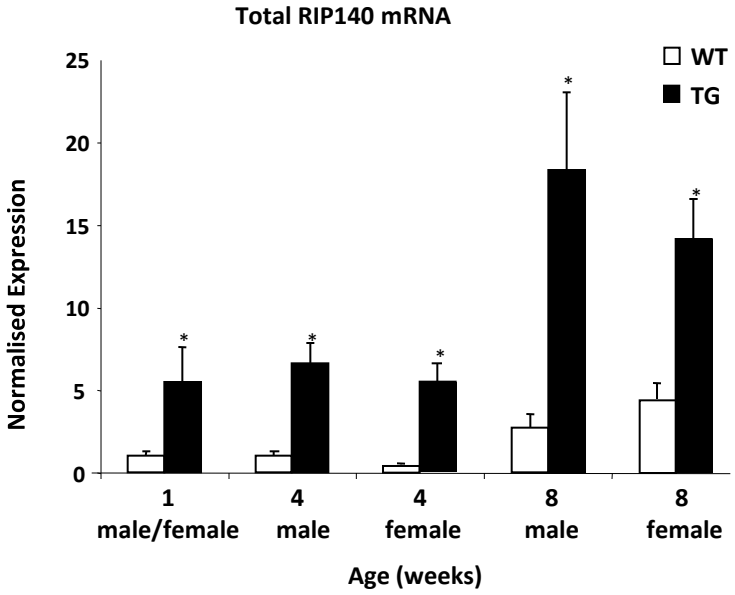
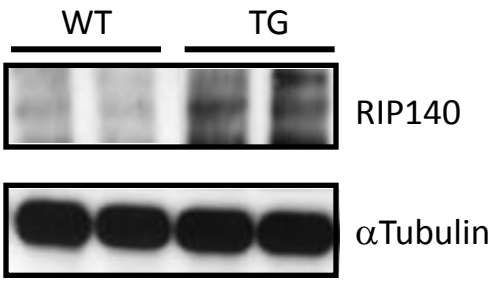

M
*
Percentage of area
0
1
2
3
4
5
6
7
Male TG
Female TG
Male WT
Female WT
4 weeks
8 weeks

Figure 2

A


Total RIP140 mRNA
WT
TG
Normalised Expression
25
20
15
10
5
0
*
1 male/female
4 male
4 female
8 male
8 female
Age (weeks)

B


WT
TG
RIP140
αTubulin

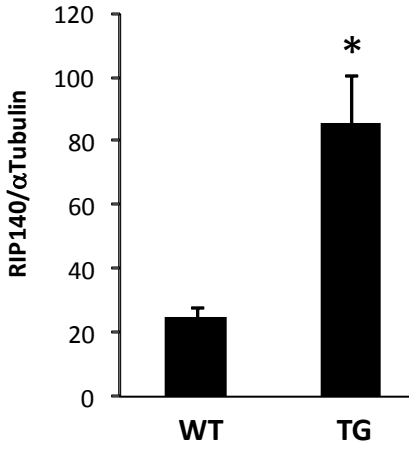

RIP140/αTubulin
120
100
80
60
40
20
0
*
WT
TG

Figure 3

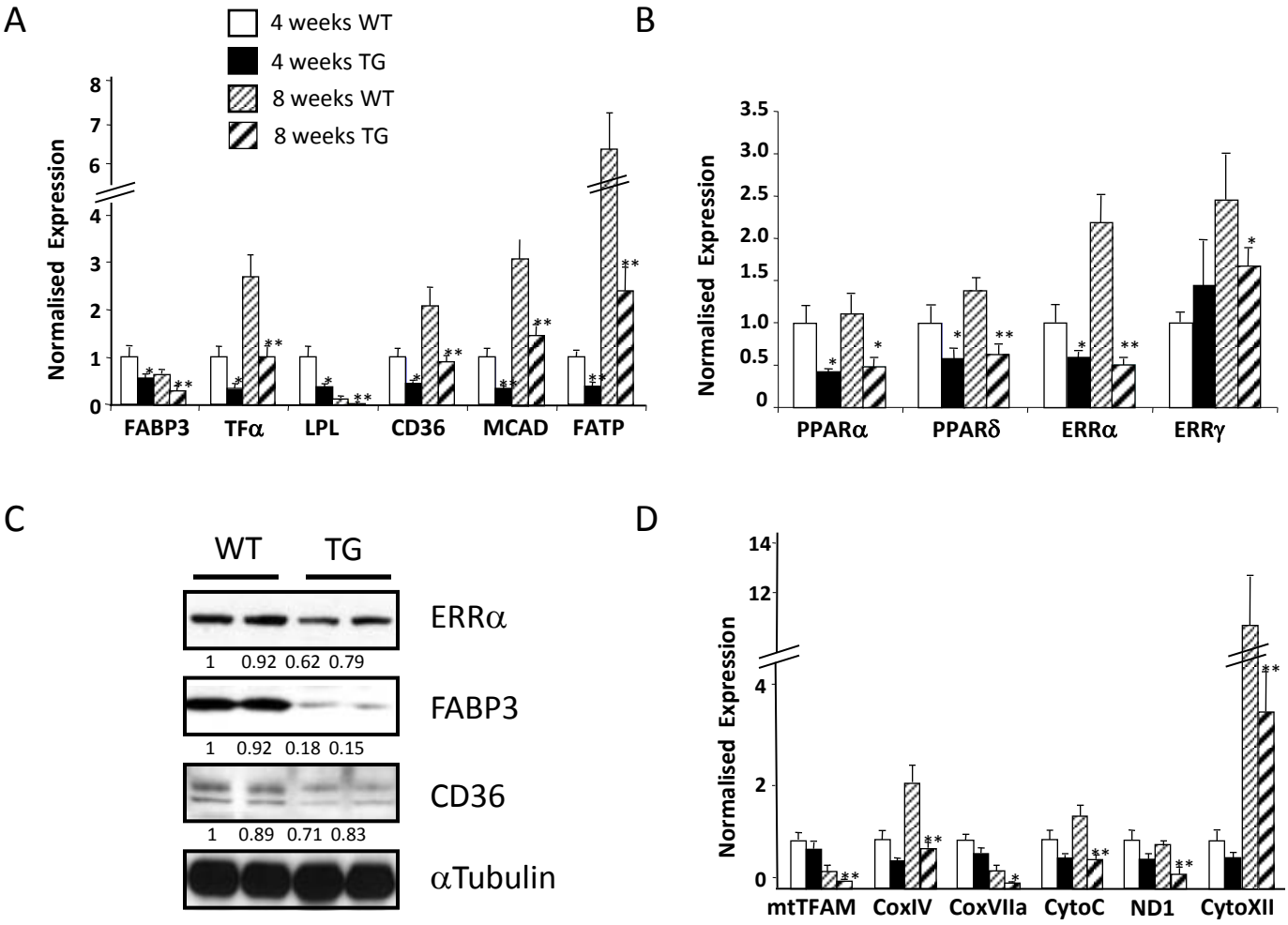

A
4 weeks WT
4 weeks TG
8 weeks WT
8 weeks TG
Normalised Expression
FABP3
TFα
LPL
CD36
MCAD
FATP
B
Normalised Expression
PPARα
PPARδ
ERRα
ERRγ
C
WT
TG
ERRα
1 0.92 0.62 0.79
FABP3
1 0.92 0.18 0.15
CD36
1 0.89 0.71 0.83
αTubulin
D
Normalised Expression
mtTFAM
CoxIV
CoxVIIa
CytoC
ND1
CytoXII

Figure 4

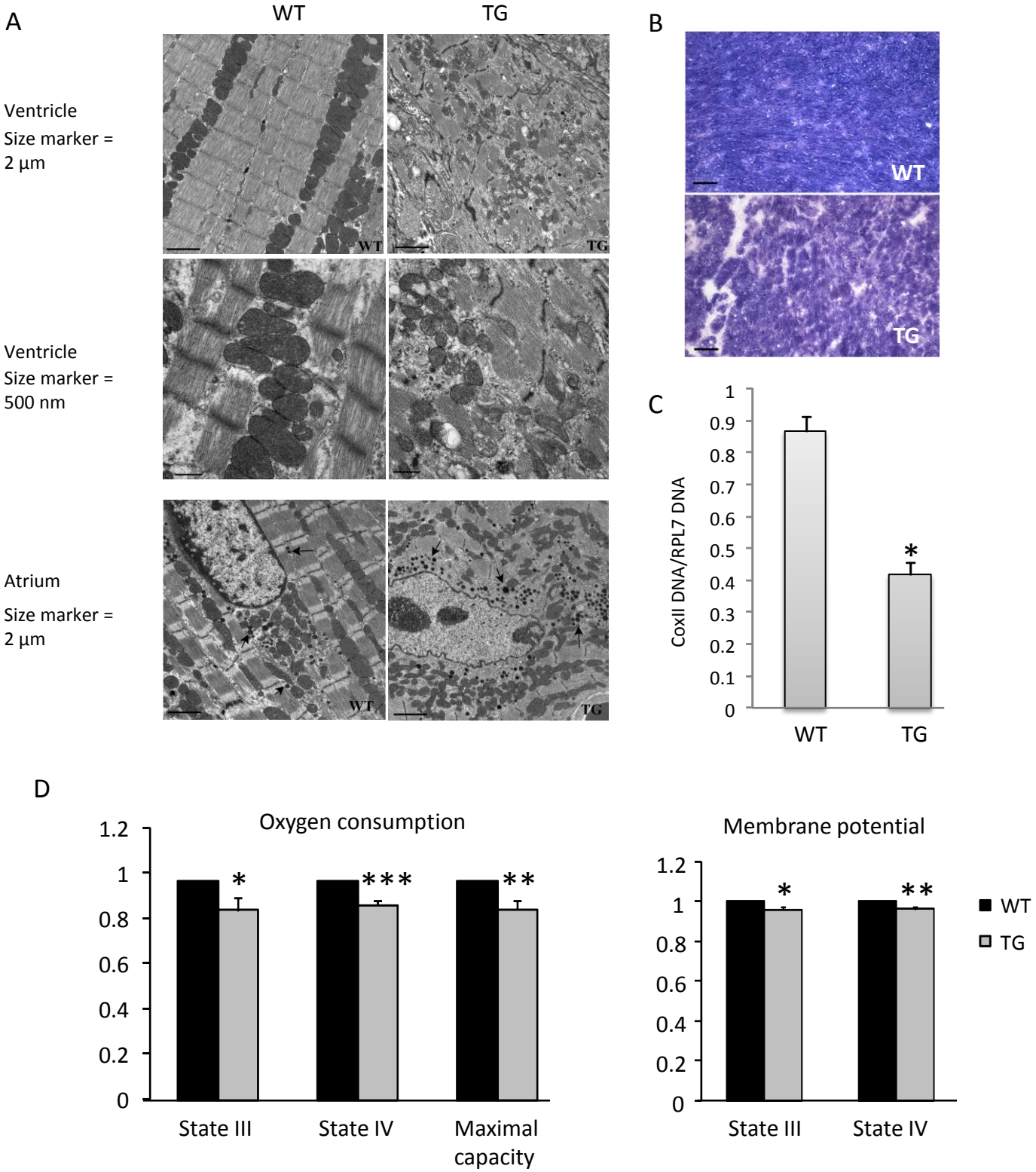

A
WT
TG
Ventricle
Size marker = 2 µm
Ventricle
Size marker = 500 nm
Atrium
Size marker = 2 µm
WT
TG
B
WT
TG
C
CoxII DNA/RPL7 DNA
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
WT
TG
D
Oxygen consumption
1.2
1
0.8
0.6
0.4
0.2
0
*
***
**
State III
State IV
Maximal capacity
Membrane potential
1.2
1
0.8
0.6
0.4
0.2
0
*
**
State III
State IV
WT
TG

Figure 5