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SUPPLEMENTARY DATA

Postnatal cardiomyocyte growth and mitochondrial reorganization cause multiple changes in the proteome of human cardiomyocytes

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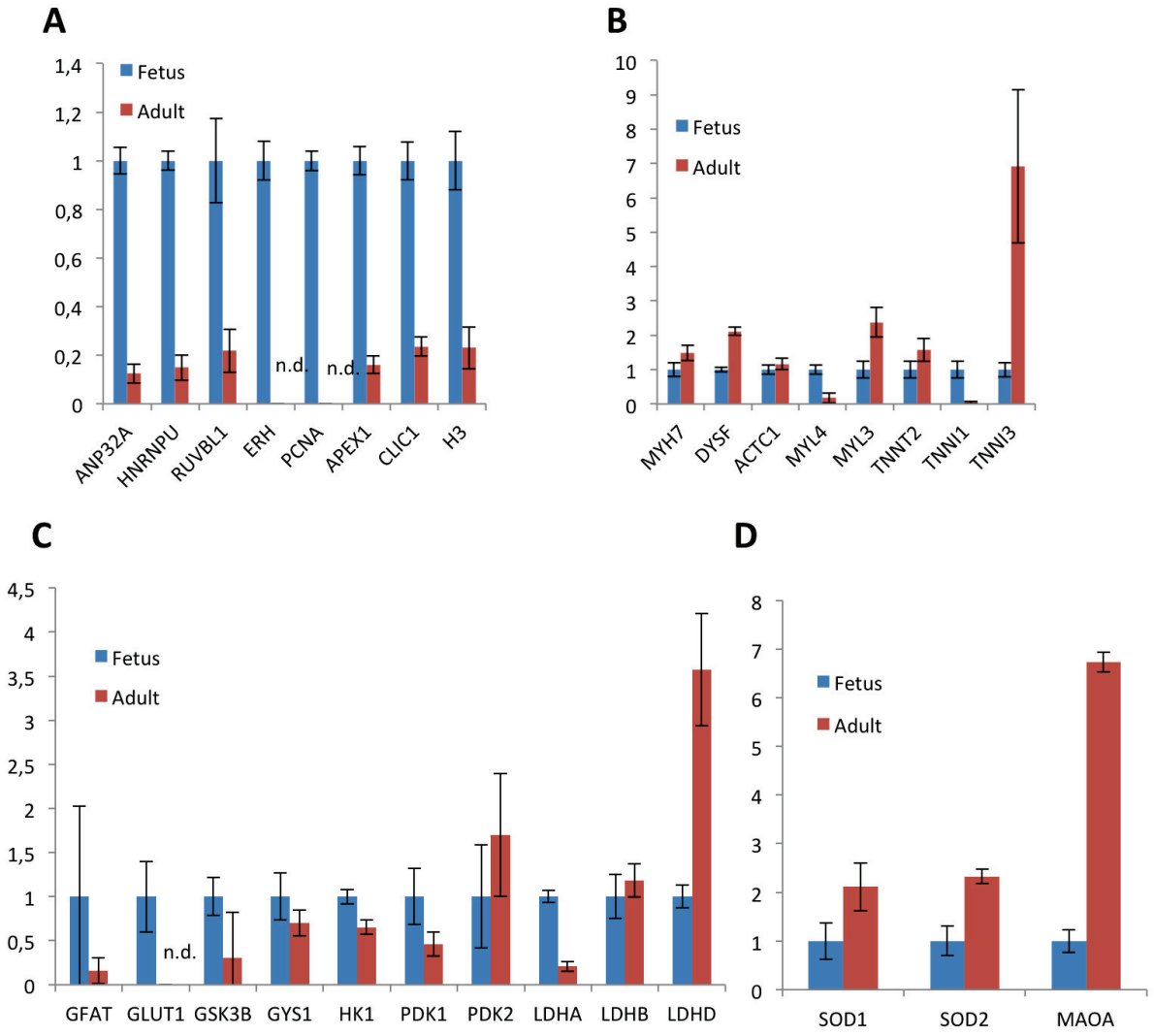
Supplementary figures



S-Fig. 1. Examples of proteins differentially expressed in fetal and adult human heart. (A) All proteins of the nuclear compartment were much more abundant in fetal heart, partially resulting from the fact that adult cardiomyocytes have less nuclei compared to total cell mass (as seen from histone H3 and heterogenous nuclear ribonucleoprotein HNRNPU) and partially because of the post-mitotic nature of adult heart (eg. Proliferating cell nuclear antigen, PCNA). (B) The sarcomeric proteins showed expected isoform switch from fetal forms (such as myosin light chain 4, MYL4 and troponin 1, TNNI1) to adult forms (MYL3,

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TNNI3). Some, like myocin heavy chain 7, MYH) show little or no (a-cardiac muscle actin, ACTC1) changes in their abundance from fetal to adult stage. (C) As a sign of metabolic remodeling, all proteins central to glucose metabolism are downregulated in the adult heart. These include proteins involved in glucose transporter (GLUT1), glycogen synthesis (GYS1) and glycolysis (HK1). Pyruvate dehydrogenase kinase 2 (PDK2) is a dowregulator of puryvate dehydrogenase and thus required for preference of fatty acid derived CoA for TCA-cycle substrates, with the expense of carbohydrate utilization. D-lactate dehydrogenase (LDHD) is a poorly characterized mitochondrial enzyme and is highly abundant in adult heart1. This is somewhat remarkable as the main muscle specific lactate dehydrogenase A (LDHA) is significantly downregulated in adults compared to fetal stage. (D) As a sign of adaptation against increased oxidative stress, antioxidant enzymes such as superoxide dismutases 1 and 2 (SOD1, SOD2). Monoamine oxidase A (MAOA) is a mitochondrial outer membrane enzyme required for oxidative deamination of monoamines. Its biological significance in heart is still obscure, but its activity has been linked with heart pathology2. All values normalized against the mean fetal protein intensity. T-test p-values for each protein are given in S-table 2.

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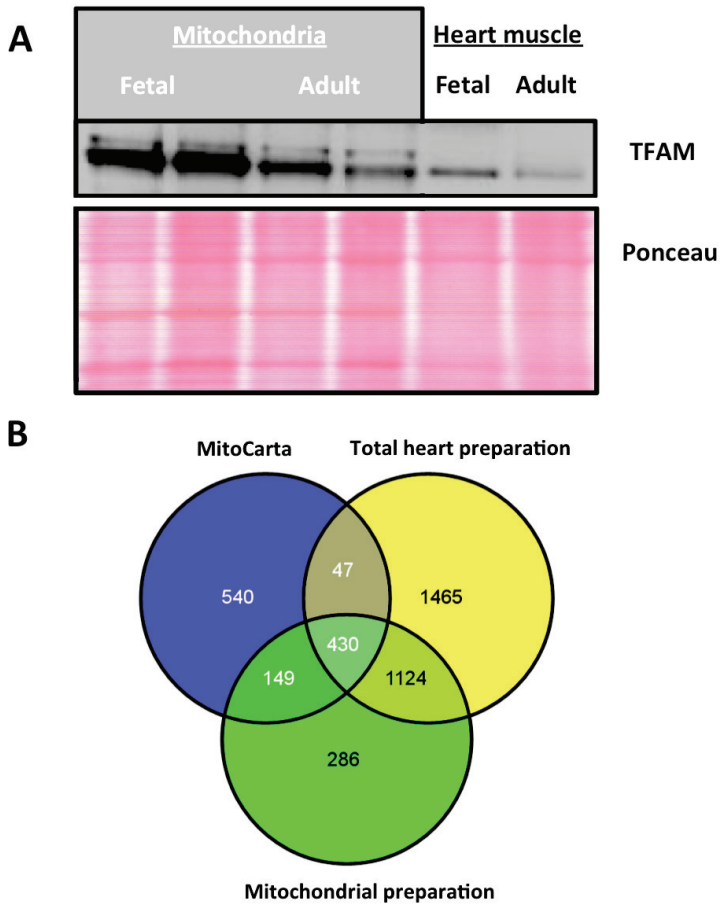

S-Fig. 2. Enrichment of mitochondrial proteins in the sucrose gradient purified mitochondrial preparations. (A) Western blot showing the enrichment of mitochondrial TFAM-protein in the mitochondrial preparations equaling 4.78±0.52 –fold increase in the quantified signal. Ponceau staining of the membrane included to show equal protein loading on the lanes. (B) A Venn diagram showing the increase in the number of quantified mitochondrial proteins in the mitochondrial preparation when compared to the total heart lysate.

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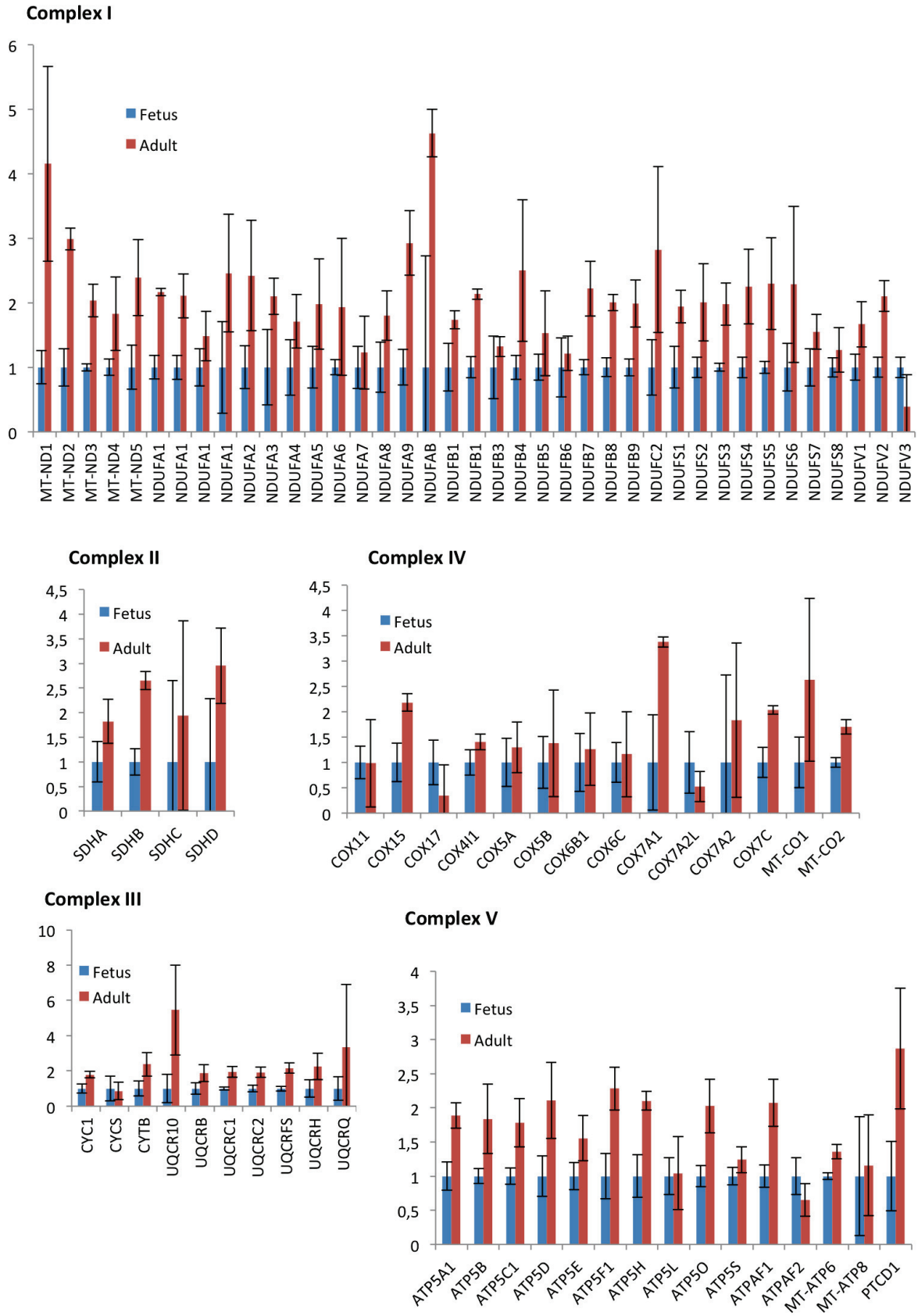

S-Fig. 3.

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S-Fig. 3. Relative levels of proteins involved in mitochondrial oxidative phosphorylation (OXPHOS) complexes as measured from the mitochondrial preparations of fetal and adult human heart. OXPHOS complexes I, II, III and V display a 2-fold or larger increase of individual subunits in adult compared to fetal hearts whereas expression of complex IV shows only minor changes. Some subunits (NDUFV3, COX17 and ATPF2) are expressed at lower levels in the adult heart suggesting a role mainly during development. All values are normalized against the mean fetal protein intensity. T-test *p*-values for each protein are given in S-table 2.

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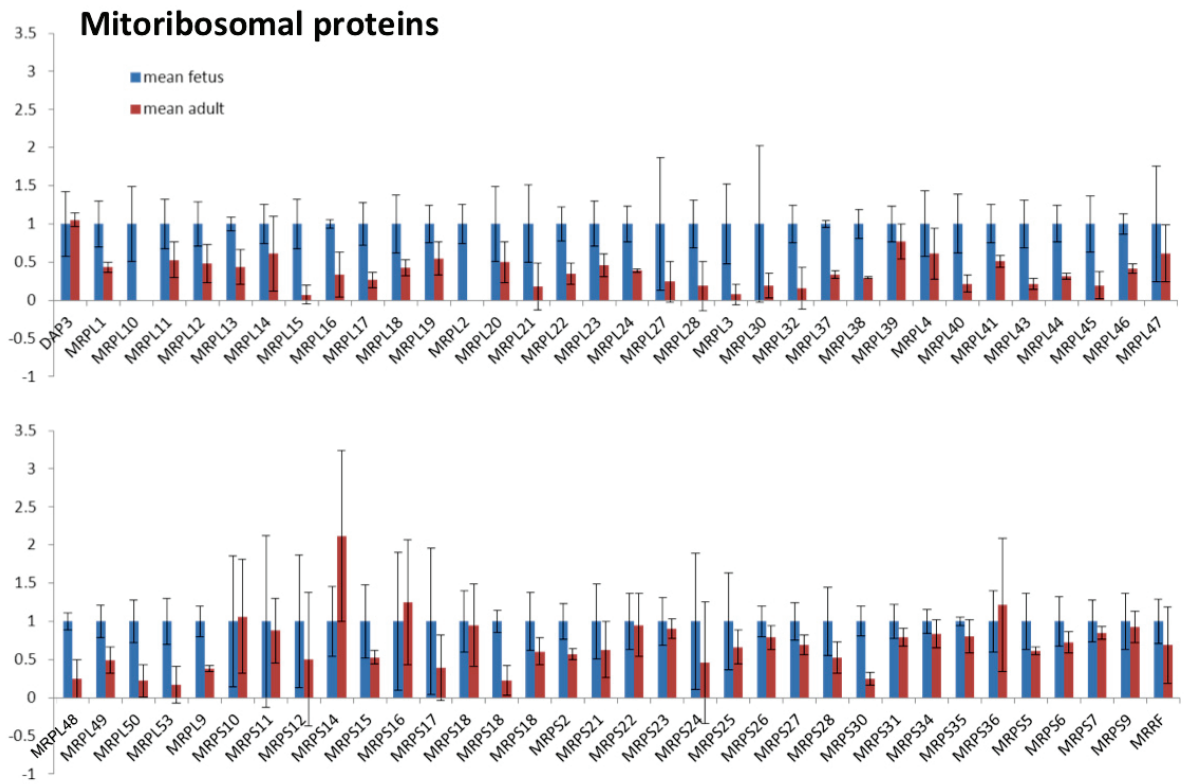

S-Fig. 4. Relative levels of mitoribosomal proteins in fetal and adult heart mitochondrial preparations. In contrast to OXPHOS proteins, most mitoribosomal proteins are significantly more abundant in fetal samples. Exceptions include death associated protein 3 (DAP3, also known as the mitoribosomal protein S29), MRPS10, MRPS14, and MRPS36, which are all parts of the smaller ribosomal subunit. All values were normalized against the mean fetal protein intensity. T-test *p*-values for each protein are given in S-table 2.

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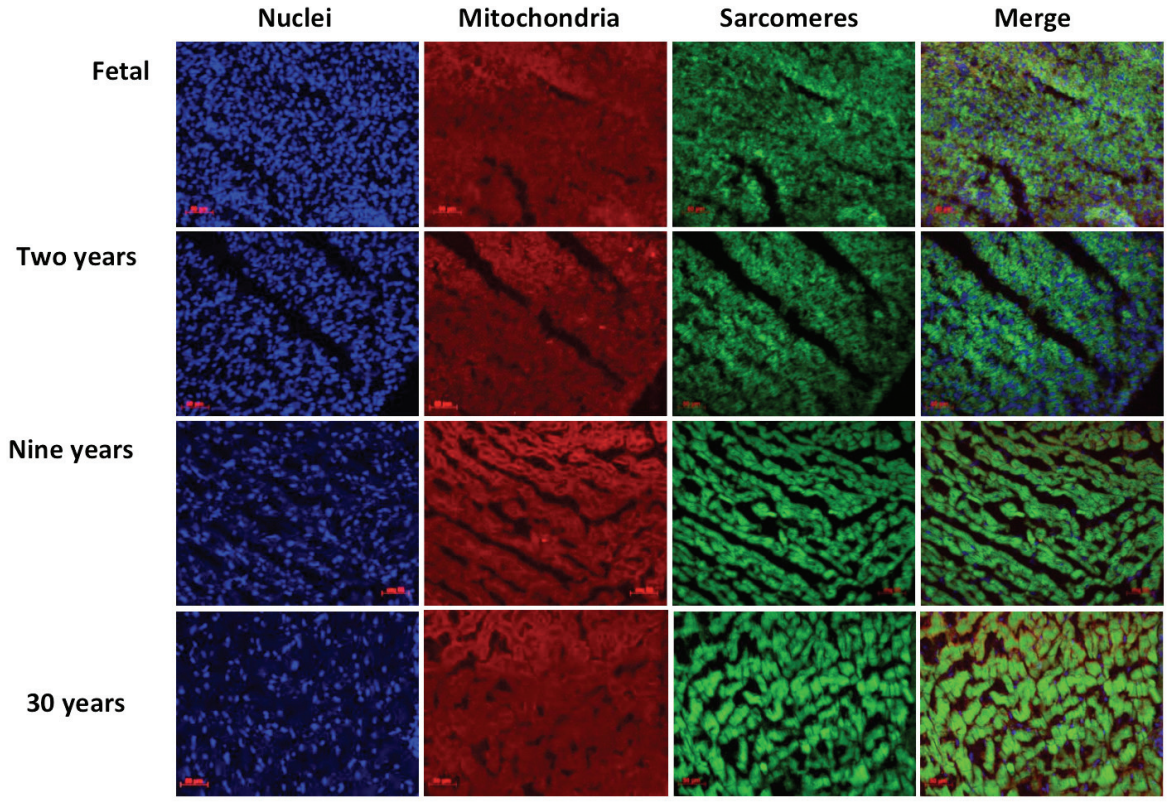

S-Fig. 5. Post-natal hypertrophy of the human heart. The postnatal increase in cardiomyocyte cell size results in 5-fold reduction of the density of nuclei, concomitant with an increase in mitochondrial mass and enhanced organization of sarcomeric structures.

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References

1. M. J. Flick and S. F. Konieczny, *Biochem Biophys Res Commun*, 2002, **295**, 910-916.

2. J. Petrak, J. Pospisilova, M. Sedinova, P. Jedelsky, L. Lorkova, O. Vit, M. Kolar, H. Strnad, J. Benes, D. Sedmera, L. Cervenka and V. Melenovsky, *Proteome Sci*, 2011, **9**, 69.

S-Table 1: Relative mean levels of all quantified proteins in total heart lysates, together with standard deviations and t-test *p*-values. (See separate supplementary excel file)

S-Table 2: Relative mean values for the quantified proteins present in mitochondrial preparations, standard deviations and t-test *p*-values. (See separate supplementary excel file)