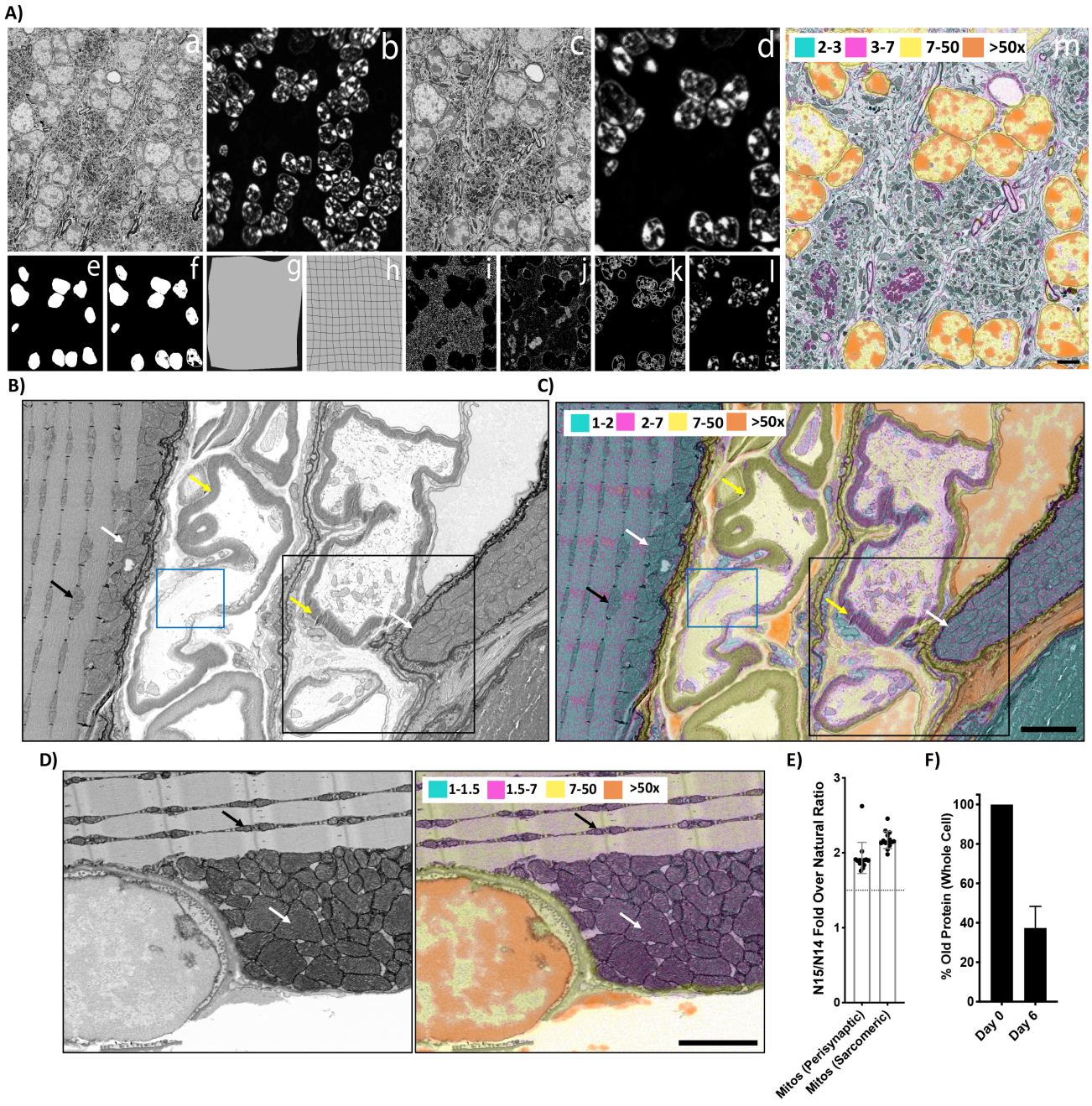

Figure S1. MIMS-EM imaging of cerebellum, neuromuscular junction and intercostal muscles in 6-month old mice, Related to Figure 1.

A) Alignment of SEM and MIMS images using the Mesofusion Plugin (See methods). **a)** SEM and **b)** MIMS uncropped images of cerebellar neurons from a 6-month chase mouse. **c)** SEM and **d)** MIMS images after the ReScale and ReSize tool. **e)** SEM and **f)** MIMS barebones images

after the Create Layout tool. **g-h)** Images containing warping information after the UnwarpJ tool. Categorization of N^{15}/N^{14} ratio signals from **i)** 74-111 x 10^{-4} **j)** 111-259 x 10^{-4} **k)** 259-1850 x 10^{-4} **l)** > 1850 x 10^{-4} using Categorize Ratio Map tool. **m)** Aligned, categorized and colorized final image using the Colorize Ratio Map tool. **B)** SEM and **C)** MIMS overlay of myelinated axons near a neuromuscular junction in intercostal muscle from a 6-month chase mouse. Myelinated axons (yellow arrows) with a Node of Ranvier (blue rectangle) are seen next to a large intercostal muscle fiber with perisynpatic mitochondria aggregates at the edge of the fiber (white arrow, left) and sarcomeric mitochondria in the fiber (black arrow). The inset (black rectangle) containing two myelinated axons with mitochondria (myelin, yellow arrow) and another perisynaptic mitochondria aggregate in the muscle fiber (white arrow, right) is magnified in Figure 1D. $^{15}N/^{14}N$ signal thresholds: 1-2x (cyan), 2-7x (magenta), 7-50x (yellow), >50x (vermillion). **D)** SEM (left image) and MIMS Overlay (right image) of intercostal muscle fiber containing perisynaptic mitochondria aggregates (white arrow) next to a nucleus and sarcomeric mitochondria within the muscle fiber (black arrow). $^{15}N/^{14}N$ signal thresholds: 1-1.5x (cyan), 2-7x (magenta), 7-50x (yellow), >50x (vermillion). **E)** Graph shows $^{15}N/^{14}N$ ratio for both perisynaptic and sarcomeric mitochondria (Mitos perisynaptic, n=14, Mitos Sarcomeric, n=14). A dotted line is drawn at the background $^{15}N/^{14}N$ signal of 1.5xNR. **F)** Graph shows %old protein in whole neurons at Day 0 (n=5 cells) and Day 6 (n=6 cells) as measured by RITE tag turnover in Fig 1H.

For all graphs, error bars indicate standard deviation. Scale bars are 5μm.

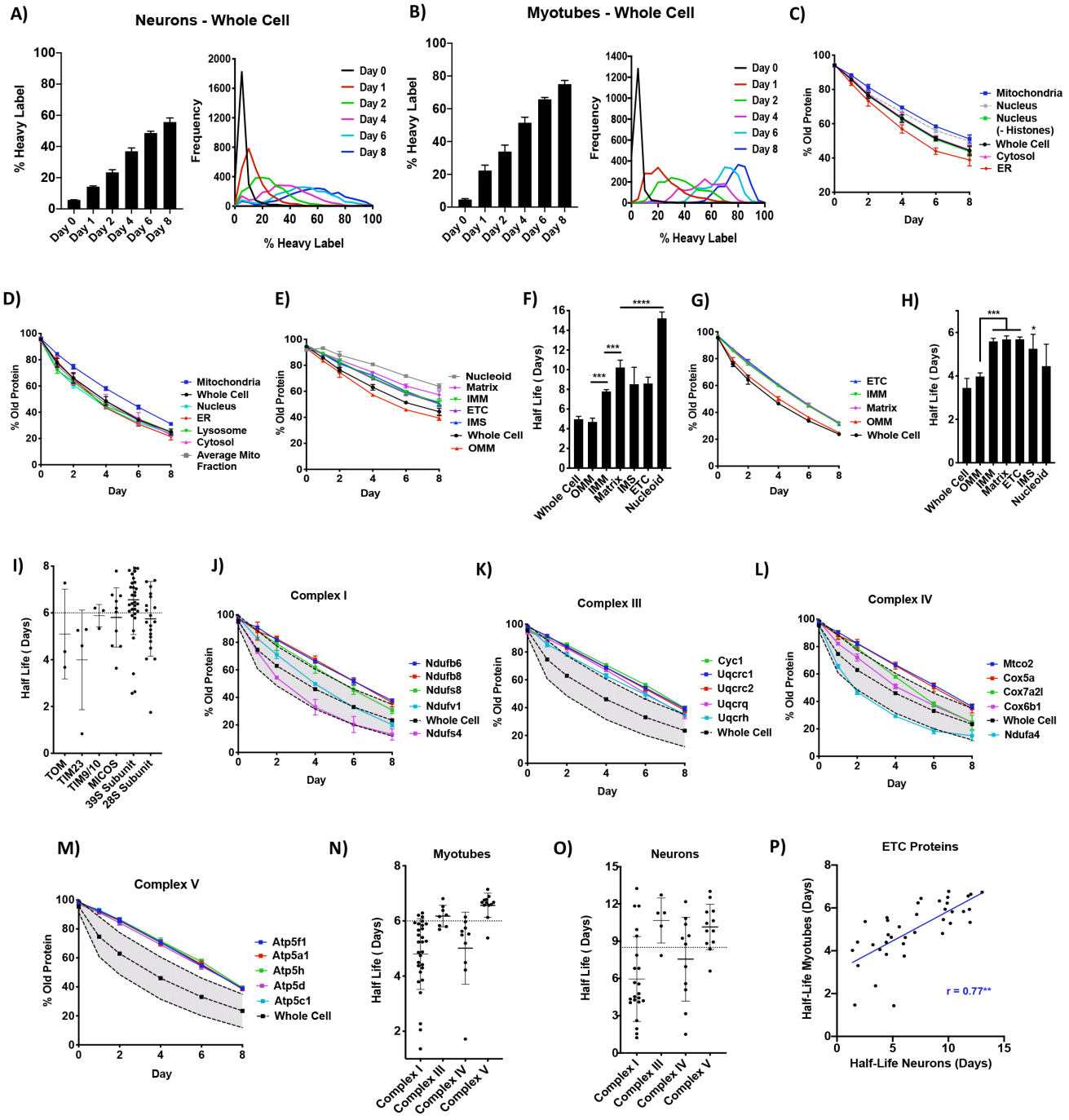

Figure S2. Protein turnover in subcellular organelles and ETC complexes in neurons and myotubes, Related to Figure 2.

A-B) Incorporation of heavy amino acids in the whole cell proteome of **A)** neurons and **B)** myotubes during the SILAC time-course. Graph on left shows %heavy label from Day 0-8, and histogram on right shows the frequency of proteins plotted against the %heavy label over the

time-course. **C)** Protein turnover in different subcellular organelles in neurons. Graph shows %old protein for Day 0-8 for the Whole Cell, Nucleus, Nucleus (-Histones), Mitochondria, ER and Cytosol. **D)** Protein turnover in different subcellular organelles in myotubes. Graph shows %old protein for Day 0-8 for the Whole Cell, Nucleus, Average Mito fraction, Mitochondria, ER, Lysosome and Cytosol. **E-F)** Protein turnover in different mitochondrial subcompartments in neurons. Graph shows **E)** % old protein for Day 0-8 and **F)** Half-lives for the Whole Cell, outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), Matrix, intermembrane space (IMS), electron transport chain (ETC) and Nucleoid proteins. **G-H)** Protein turnover in different mitochondrial subcompartments in myotubes. Graph shows **G)** %old protein for Day 0-8 and **H)** Half-lives for Whole cell, OMM, IMM, Matrix, IMS, ETC and nucleoid proteins **I)** Dot plot representing half-lives of the different proteins in the TOM, TIM23, TIM9/10, MICOS Complex and the 28S and 39S ribosomal subunits in myotubes. **J)** Protein turnover in the ETC Complex I in myotubes. Graph shows %old protein for Day 0-8 for the different CI subunits. **K)** Protein turnover in the ETC Complex III in myotubes. Graph shows %old protein for Day 0-8 for the different CIII subunits. **L)** Protein turnover in the CIV in myotubes. Graph shows %old protein for Day 0-8 for the different CIV subunits **M)** Protein turnover in the CV in myotubes. Graph shows %old protein for Day 0-8 for the different CV subunits. **N-O)** Dot plot representing half-lives of the different proteins in the CI, CIII, CIV and CV in **N)** myotubes and **O)** neurons. **P)** Scatter Plot showing half-lives of ETC proteins in neurons vs myotubes with a spearman correlation coefficient of r=0.77.

For all graphs, the average whole cell is the dashed black line and the shaded grey area within dotted lines is the standard deviation for the fraction. For all graphs, error bars indicate standard deviation. For all half-life dot plots, a dotted line is indicated for a half-life of 6 days for myotubes and 8.5 days for neurons, the cut-off used for mitoLLPs. Data are from n=3. *p <0.05, **p<0.001, ***p<0.0001

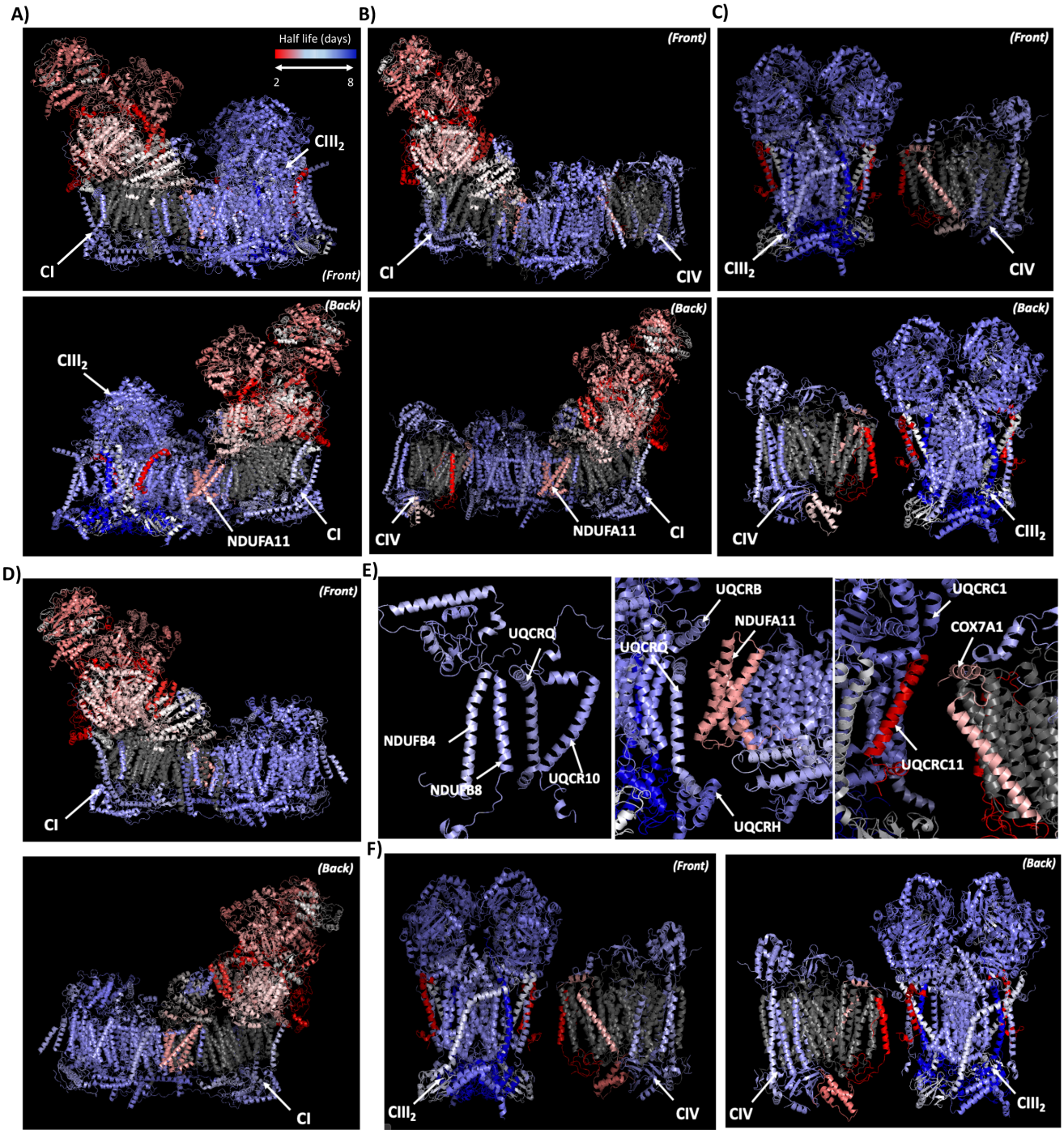

Figure S3. Protein turnover of ETC subunits in respiratory supercomplexes in myotubes, Related to Figure 4.

A,B,C) Turnover of subunits in the respirasome supercomplex with individual complexes removed for clarity. Images show **A)** CI and $CIII_2$ **B)** CI and CIV, **C)** $CIII_2$ and CIV of the porcine respirasome structure from the front (top) and back (bottom). CI, $CIII_2$, CIV and subunit

NDUFA11 are indicated. **D)** Images show turnover of subunits in CI from the porcine respirasome ($CIII_2$ and CIV removed) from the front (top) and back (bottom). **E)** Turnover of contact sites between the different complexes within the porcine respirasome. Contact site between CI and $CIII_2$ containing NDUFB4, NDUFB8 from CI and UQCRQ, UQCR10 from $CIII_2$ (left image), between CI and $CIII_2$ containing UQCRB, UQCRQ, UQCRH from $CIII_2$ and NDUFA11 from CI (middle image) and between $CIII_2$ and CIV containing UQCRC1, UQCRC11 from $CIII_2$ and COX7A1 from CIV (right image). **F)** Image shows turnover of subunits in the intermediate supercomplex using the porcine respirasome structure with CI removed from the front (left) and back (right). $CIII_2$ and CIV are indicated.

The scale of half-lives for all images is 2 to 8 days color-coded from dark red to white to dark blue. The proteins with no coverage in mass spectrometry are colored grey. Data are from an n=3.

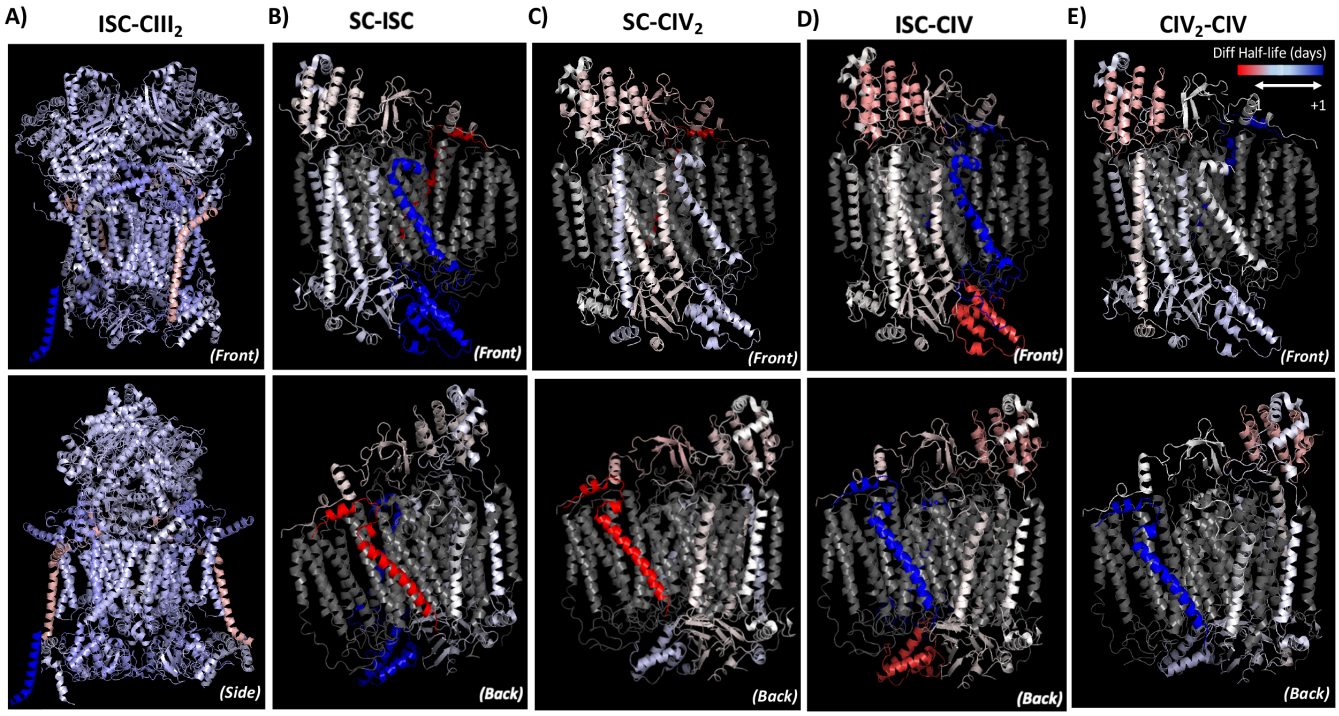

Figure S4. Differential protein turnover of ETC subunits between supercomplexes and individual complexes in myotubes, Related to Figure 4.

Images show differential turnover between **A)** the intermediate supercomplex (ISC) and $CIII_2$, **B)** supercomplexes (SC) and the intermediate supercomplex (ISC) for CIV, **C)** supercomplexes (SC) and CIV_2 (dimer), **D)** intermediate complex (ISC) and CIV (individual complex), **E)** CIV_2 (dimer) and CIV (individual). The top panel represents the front, and the bottom panel represents the side for CIII and back for CIV. The scale for differential turnover structures is in the range of -1 to +1 days from dark red to white to dark blue representing faster turnover in SCs to slower turnover in SCs. The proteins with no coverage in mass spectrometry are colored grey. Data are an n=3.

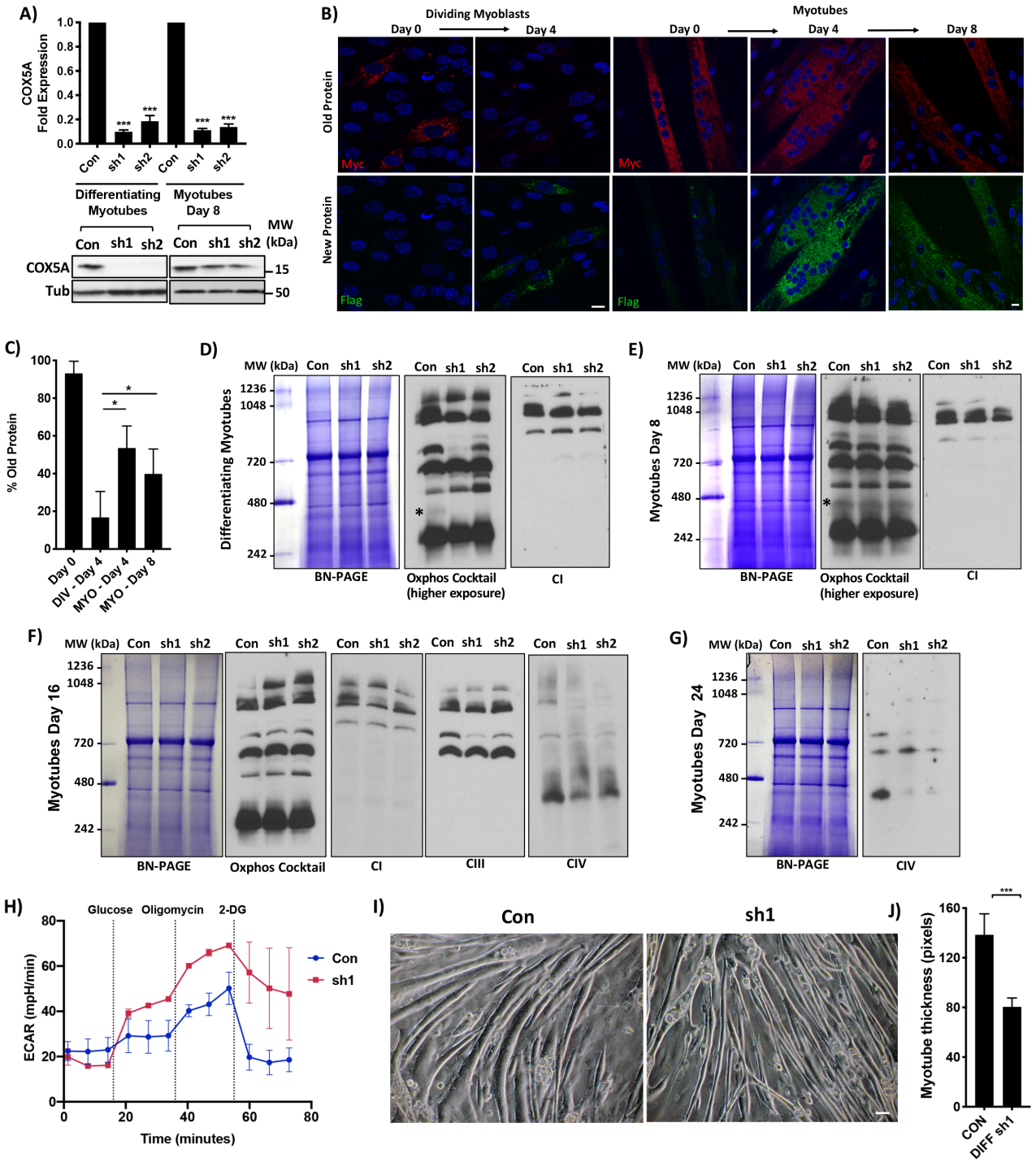

Figure S5. Role of COX7C turnover on ETC complexes, mitochondrial function and myotube size, Related to Figure 5.

A) COX5A mRNA levels (qPCR, top panel) and protein levels (western blot, bottom panels) with control and COX5A-hairpins (sh1, sh2) in differentiating myotubes and fully differentiated

myotubes at Day 8. Western blot shows Tubulin as loading control. **B)** Images with lower magnification showing COX7C turnover as measured by RITE. Images show Myc tag (old protein, top panel) and Flag tag (new protein, bottom panel) at Day 0 and 4 in dividing myoblasts (left) and Day 0, 4 and 8 in myotubes (right). **C)** Graph shows %old protein in dividing myoblasts at Day 0 and Day 4, and fully differentiated myotubes at Day 0, Day 4 and Day 8 as measured by RITE tag turnover in Fig 5D& S5B. **D-E)** BN-PAGE (left gel) and western blots (right) show levels of all the complexes (Oxphos cocktail, higher exposure) and CI containing complexes in **D)** differentiating myotubes and **E)** Day 8 myotubes. Asterisk indicates the individual CIV complex band in the higher exposure of the Oxphos cocktail blot. **F)** BN-PAGE (left gel) and western blots (right) show levels of all the complexes (Oxphos cocktail) and CI, CIII and CIV containing complexes in Day 16 myotubes **G)** BN-PAGE (left gel) and western blot (right) show levels CIV containing complexes in Day 24 myotubes. **H)** Extracellular Acidification Rates (ECAR) in mpH/min over a seahorse assay of 80 minutes and in control and sh-1mediated knockdowns in differentiating myotubes. Injection timing of Glucose, Oligomycin and 2-DG is indicated with dotted lines. **I-J)** Myotube size with COX7C knockdowns. **I)** Images and **J)** graph show myotube thickness in control and sh-1 mediated knockdowns in differentiating myotubes.

For all graphs, error bars represent standard deviation from an n=3. *$p<0.05$,**$p<0.01$, ***$p<0.001$. Scale bars are 10μm (B) and 50μm (I). Blot markers are indicated.