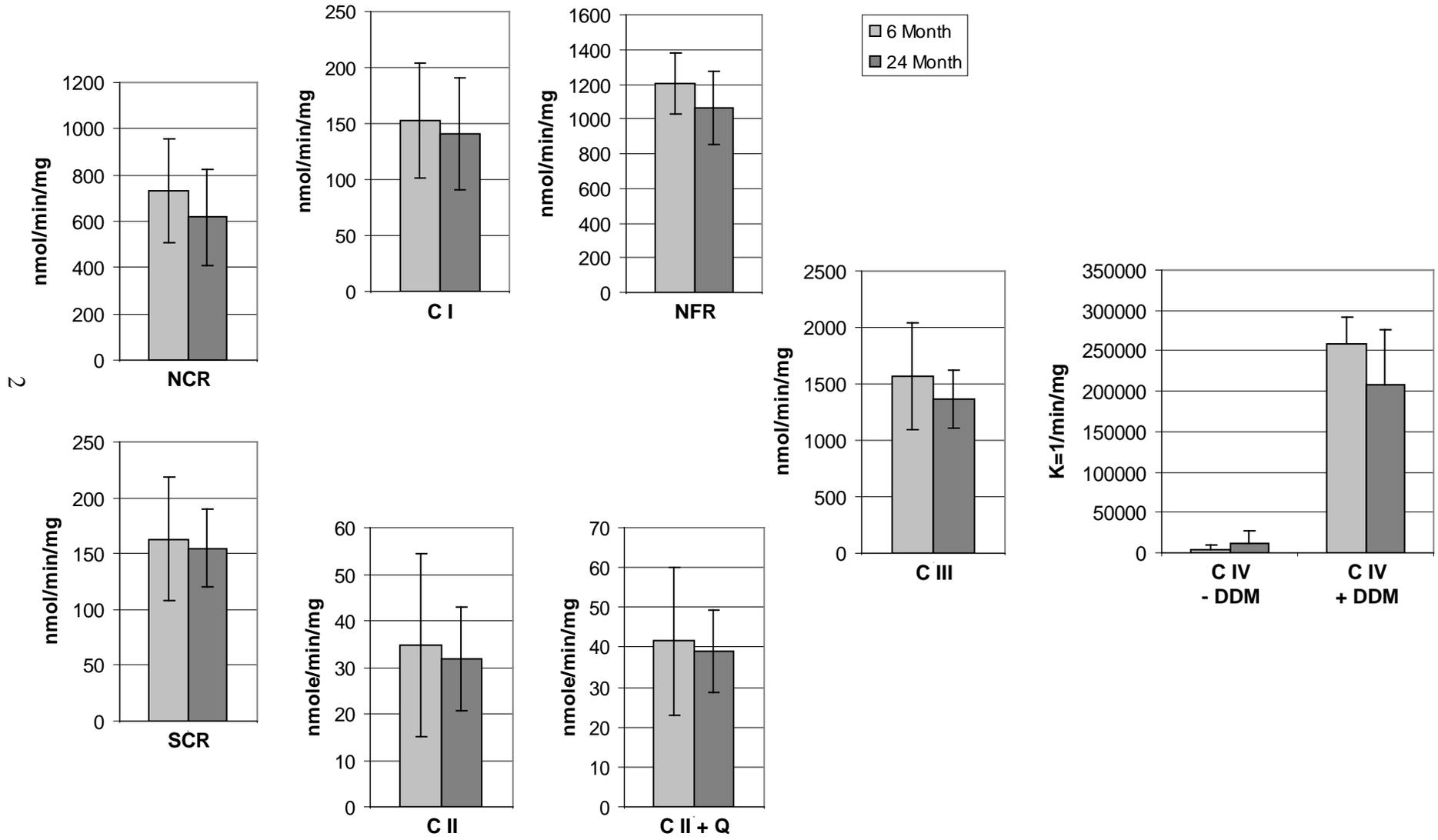


Supplementary Figure 1. Spectrophotometric determination of enzyme rates of the ETC in solubilized mitochondria isolated from 6 and 24 month rats. Freshly isolated mitochondria were solubilized and assayed spectrophotometrically for the activity of complex I (CI), the NADH ferricyanide reductase component of complex I (NFR), TTFA-sensitive complex II (CII), TTFA-sensitive complex II with an exogenous coenzyme Q analog (CII+Q), complexes I-III with rotenone-sensitive NADH-cytochrome c reductase (NCR), complex II-III with antimycin A-sensitive succinate-cytochrome c reductase (SCR), complex III (CIII), and complex IV before (CIV-DDM) and after (CIV+DDM) the addition of dodecyl maltoside. Complex IV activities are expressed as the first order rate constant ($k=1/\text{min}/\text{mg}$ of mitochondrial protein), all other activities are expressed as nmoles/min/mg of mitochondrial protein. Bars represent the mean value of each experimental group ($n=8$ for each group) and the error bars indicate the standard deviation from the mean. P-values were calculated from non-paired two-tailed student t-test, and no comparison was found to have a p-value of <0.05 .

Supplementary Figure 2. Quantitation of respiratory complexes and supercomplexes in mitochondria isolated from 6 and 24 month rats after detergent solubilization and separation using blue-native gel electrophoresis. Mitochondria isolated from 6 month ($n=4$) and 24 month ($n=4$) Fischer 344 rat kidneys were solubilized with dodecyl maltoside (DDM) (**A, C, E, G**) or digitonin (dig) (**B, D, F, H**). Respiratory complexes and supercomplexes were separated using blue native-gel electrophoresis on gradient bis-Tris acrylamide gels. Respiratory complexes from DDM solubilized mitochondria (**A**) separated by blue-native gel electrophoresis are labeled at the left-hand side from top to bottom as complex I (CI), complex V (CV), complex III dimer (CIII₂), complex IV (CIV), and complex II (CII). Supercomplexes and respiratory complexes from dig solubilized mitochondria (**B**) separated by blue-native gel electrophoresis are labeled at the right-hand side from top to bottom as supercomplex (S2), supercomplex (S1), supercomplex (S0), complex I (CI), complex V (CV), complex III dimer (CIII₂), and complex IV (CIV). The blue native gels (**A, B**) were analyzed with band densitometry and the labeled bands are expressed as absolute band density per mg of mitochondrial protein (**C, D**), as band density as a percentage of total band density in that lane (**E, F**) and the ratio of the band density of each component to that of complex V (**G, H**). Comparisons were made between the 6 and 24 month groups. Bars represent the mean value of each group and the error bars indicate the standard deviation. P-values were calculated from non-paired two-tailed student t-test for each group and (*) indicates pairs for which the p-value was <0.05 .

Supplementary Figure 3. Comparable mitochondrial marker protein abundance in mitochondria isolated from 6 and 24 month animals. Mitochondria isolated from the kidneys of 6 and 24 month animals ($n=4$ for each group) were solubilized in loading buffer and 50 μgms of mitochondrial protein/lane were separated on 12.5% SDS-PAGE with size standards (**A** and **B**). Proteins were transferred to PVDF, which was dried then blocked in TBS, pH=7.4, with 5% non-fat dry milk. The blot was then incubated with primary antibody at 1:1,000, (**A**) anti-Smac (Epitomics Inc., Burlingame, CA, USA) or (**B**) anti-VDAC (Mitosciences, Eugene, OR, USA), for 2 hrs at room temperature with rotation in wash solution (TBS, pH=7.4, 1% non-fat dry milk, 0.1% Tween-20). The membrane was washed three times for 10 minutes at room temperature in wash solution, and incubated with the appropriate HRP-conjugated secondary antibody at 1:5,000 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) in wash solution for 1 hr at room temperature. The membrane was washed three times for 10 minutes at room temperature in wash solution and once in TBS, pH=7.4. Membranes were then covered with chemiluminescent HRP detection reagent (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), exposed to film and developed. Film was scanned into TIFF files and band density was quantified (**C** and **D**) using image J software (<http://rsbweb.nih.gov/ij/>). Average band density in arbitrary units is shown in light bars for 6 month animals and dark bars for 24 month animals, error bars represent standard deviation. There was no statistically significant difference between groups for Smac (**C**) or VDAC (**D**).

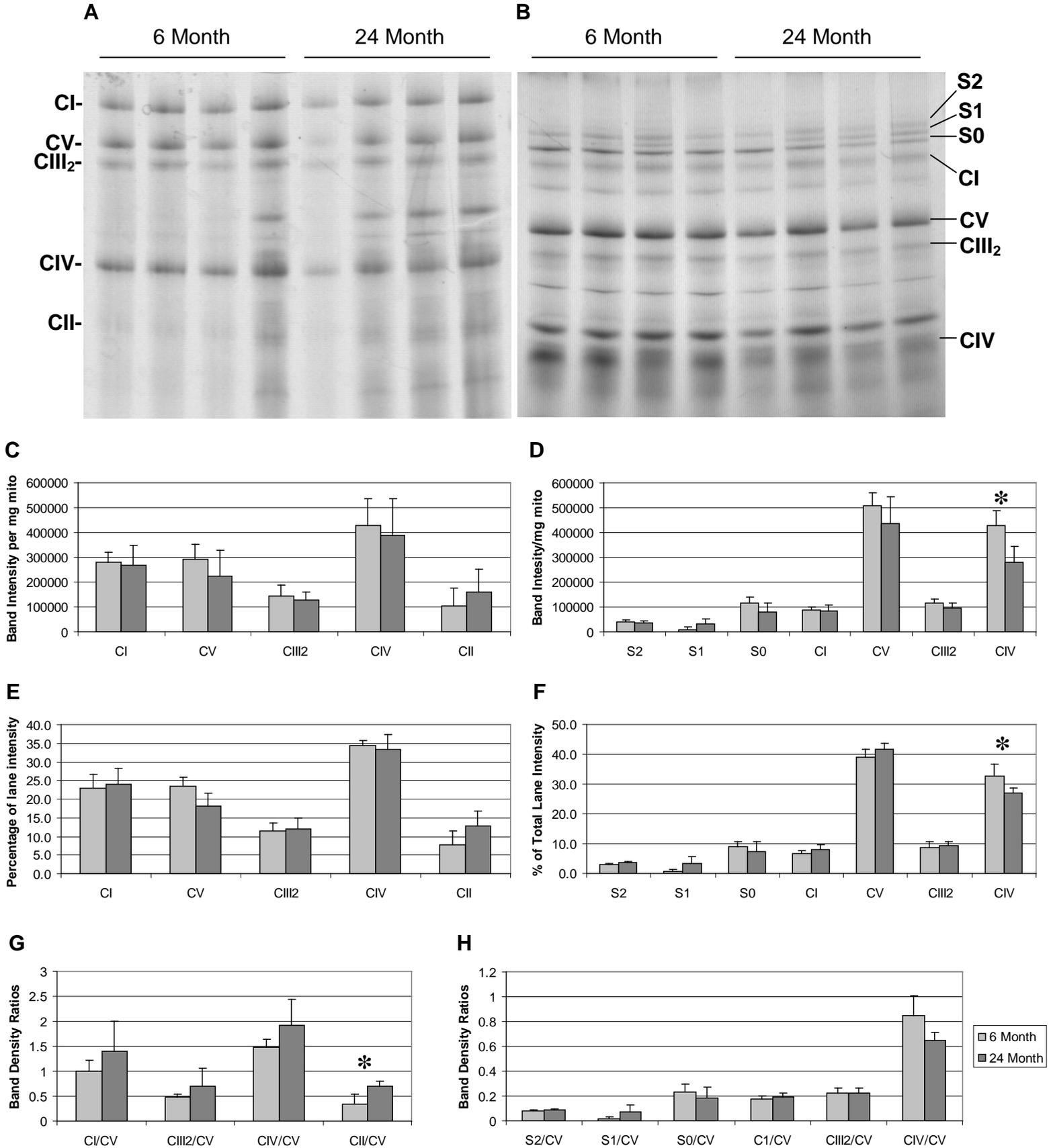
Supplementary Figure 1



2

Aging rat kidney mitochondria

Supplementary Figure 2



Supplementary Figure 3

