**ijms-931888 Webb & Sideris Figure Legends**

**Figure 1**. Healthy mitochondria perform a variety of functions including ATP production and synthesis of a variety of metabolites required for cell maintenance. Ageing is associated with an increasing impairment of these functions, manifested as excessive ROS production, oxidative damage to mitochondrial components, reduced ability to supply ATP, dysregulation of the tricarboxylic acid cycle, and an increase in mitochondrially driven inflammation.

**Figure 2.** Mitochondria communicate bidirectionally with the nucleus and cytoplasm. Nuclear gene transcription is required for the synthesis of the vast majority of mitochondrial components, is tightly coordinated with mitochondrial protein synthesis, and is responsive to the energetic requirements of the cell. Small metabolites (e.g. ketoglutarate and succinate) act as ligands for G protein coupled receptors which may in turn affect cellular metabolism and nuclear gene transcription.

**Figure 3.** PGC1a is a transcriptional co-factor that regulates the transcription of nuclearly encoded mitochondrial proteins by interacting with transcription factors such as Nrf1/2, ERRg and the PPAR family . It acts as a hub to integrate signals from several pathways that monitor the energetic and nutritional status of the cell, including AMP-activated protein kinase (AMPK), calcium/calmodulin*-*dependent proteinkinase (CAMK) and cAMP-response element binding protein (CREB).

**Figure 4.** The tricarboxylic acid cycle (TCA), also known as the citric acid cycle of Krebs cycle occurs in the mitochondrial matrix. It functions to supply ATP, NADH and biochemical intermediates for a variety of metabolic pathways. 2-carbon acetyl CoA derived from glycolytic pathways reacts with 4-carbon oxaloacetate in the first step, and a linked series of reactions results in the synthesis of two carbon dioxide molecules, three NADH, one FADH2 and one ATP molecule per cycle, regenerating oxaloacetate as it does so. Several TCA cycle intermediates have potential roles as epigenetic regulators and ligands for G protein coupled receptors. 2-Oxoglutarate is also known as a-ketoglutarate.

**Figure 5.** Overview of NAD synthetic pathways. Three major pathways produce NAD, two from dietary starting points, and one (the salvage pathway) from nicotinamide derived from NAD catabolic pathways.

**Figure 6.** During ageing, upregulation of NAD consuming enzymes such as CD38 and poly (ADP-ribose) polymerase (PARP) and down regulation of the synthetic enzyme nicotinamide phosphor-ribosyltransferase (NAMPT) result in reduced levels of NAD. This is results in lower activity of the NAD–dependent deacylases (sirtuins). SIRT1 and SIRT3 regulate a variety of mitochondrially related functions whose age related change is regarded as deleterious, including decreases in transcription of genes such as PGC1a, PPAR, TFAM and FOXO1.3, and upregulation of NFkB.

**Figure 7.** Complex I and III are the major sites of the electron transfer chain at which reactive oxygen species are released. The superoxide radical (.O2- ) is the initial species generated by reduction of molecular oxygen. It is reduced to hydrogen peroxide by superoxide dismutases 1 or 2, and is further reduced to water by catalase or glutathione peroxidase, these steps constituting the initial anti-oxidant defense mechanisms.

**Figure 8.** Summary of mechanism proposed by the mitochondrial free radical theory of ageing. Reactive oxygen species produced during operation of the electron transfer chain cause damage to mitochondrial components, including mtDNA, proteins and lipids. This results in accumulation of increasingly damaged components of the ETC, resulting in exacerbated production of ROS, which are also able to damage extra-mitochondrial cellular components. Additionally, the increased ROS may trigger epigenetic changes, including telomere shortening, and upregulation of cellular senescence pathways.

**Figure 9**. Induction of cellular senescence programmes by mitochondrial DNA. Under normal healthy conditions, defective mitochondria are removed by mitophagy, and mitochondrial DNA is not released into the cytoplasm. If this process is unable to deal with the increasing load of mitochondrial damage during ageing, mtDNA may be released to the cytoplasm, where it constitutes a damage associated molecular pattern (DAMP). mtDNA is recognised by the enzyme cGAMP, which synthesises the intermediate cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). This activates the transcription factor stimulator of interferon genes (STING), which upregulates transcriptional programmes controlled by IRF3 and NFkB, resulting in the induction of cellular senescence.

**Figure 10. The mitochondrial life cycle.** Newly synthesised components are added to existing mitochondria. These are shared throughout the mitochondrial pool by cycles of fission and fusion which constitute part of the mitochondrial life cycle. Fusion events are critically dependent on mitofusin s 1 and 2, and the inner membrane GTPase Optic atrophy-1 (Opa1). Fission is initiated by recruitment of the cytosolic GTPase dynamin-related protein 1 (DRP1) to the mitochondrial surface, where it docks with one of several receptors, Fis1, mitochondrial fission factor (Mff), Mid 49, or Mid 51. Damaged molecules are segregated and asymmetric fission results on one daughter with normal mitochondrial membrane potential, the other containing the damaged molecular constituents and having a low membrane potential (MMP). This low potential triggers mitophagy by the recruitment of the PTEN-induced kinase 1 (PINK1), which then activates the E3 ubiquitin ligase Parkin. Ubiquitinated mitochondria are then disposed of by mitophagy. Additional pathways to trigger the removal of damaged mitochondria have been described, and these may be initiated by signals other than reduced MMP, such as externalised cardiolipin.