Immune responses are highly variable among individuals, partially due to genetic variation in immune signaling and in host physiology. However, the underlying causes of the variation are not fully understood. Mitochondria, which produce ATP via oxidative phosphorylation (OXPHOS) and have their own mitochondrial DNA (mtDNA), are suggested to play a role in immunity. We studied the effects of mtDNA variation on innate immunity in the fruit fly *Drosophila melanogaster* model system. We found that flies with the same nuclear genome, but different mitochondrial genomes varied in the efficiency of their response against pathogens. Interestingly, a fly strain that carries mtDNA mutation in OXPHOS complex III (cIII) gene *cytochrome b* (*mtCyt-b*) was particularly immunocompetent. RNA sequencing showed that the immunocompetent fly line had enhanced expression of genes directly involved in OXPHOS and, surprisingly, decreased expression of antimicrobial peptides (AMPs), key factors in humoral immune response. However, we observed an increase in immune cell numbers and activation, which could contribute to the enhanced immunity. We also utilized pharmacological and genetic approaches to study the role of mild mitochondrial dysfunction in immune responses. We fed several fly lines with chloramphenicol, an antibiotic which targets mitochondrial translation, and used RNA interference to silence nuclear encoded OXPHOS cIII gene *UQCR-C1*, which directly interacts with *mtCyt-b* at the active site of cIII. All above-mentioned treatments resulted in activation of cell-mediated immune response. Overall, variation in mitochondrial function creates heterogeneity in infection outcomes and specifically contribute to cell-mediated immune responses via immune cell activation.