

Supplementary information, Figure S3

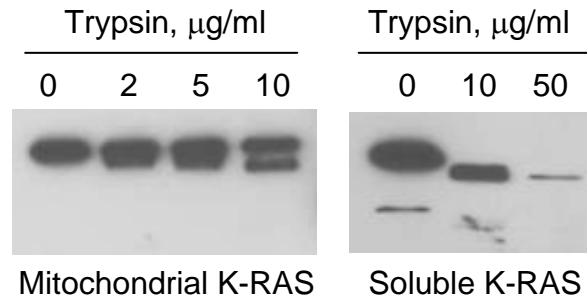


Figure S3 Comparison of trypsin digestion of K-RAS protein in isolated mitochondria and free K-RAS in protein extracts. K-RAS protein expression was induced by doxycycline in T-Rex/K-ras cells for 24 h. Mitochondria were isolated from the cells. The sample was divided into two portions: The first portion was used for direct digestion with trypsin (left panel); the second portion was used for protein extraction using standard RIPA buffer containing detergents (0.1% SDS, 1% Triton X-100) for 30 min (right panel). SDS and salts were removed from the protein extracts by buffer exchange using a YM-10 column (Microcon). Protein concentrations were determined using BCA assay kit. The same amount of protein (15 µg) was then digested with different concentrations of trypsin as indicated for 30 min at 4 °C. The digestion products were analyzed by western blotting using anti-K-ras antibody.