

SUPPLEMENTARY FIG. S3. Loss of *Sirt2* changes the morphology of mitochondria in MEFs. (A) Sirt2^{+/+} and Sirt2^{-/-} MEFs were stained with MitoTracker (*green*) and To-pro3 (*blue*) and subjected to immunofluorescence microscopy. *White arrowheads* point to condensed mitochondria. Scale bar: 40 μ m. (B) Sirt2^{+/+} and Sirt2^{-/-} MEFs were transfected with Flag-tagged IDH2 and stained with anti-IDH2. Mitochondria were visualized by anti-Flag staining. *White arrows* point to swollen mitochondria. Scale bar: 8 μ m. (C) Wild-type and Sirt2^{-/-} MEFs were harvested and, subsequently, immunoblotted with anti-COX-IV, cytochrome C (Cyto C), and SIRT2 antibodies. β -Actin and α -tubulin were used as the loading control. The Western blot shows that lower levels of COX-IV and cytochrome C were found in Sirt2^{-/-} MEFs compared with controls. (D) Bar graph quantifies the relative expression levels of COX-IV in homogenates of wild-type and Sirt2^{-/-} MEFs. Data are presented as the mean ± SEM (n=5). ***p < 0.001. (E) Western blot shows the expression level of PGC-1 α in 24 month-old Sirt2^{+/+} and Sirt2^{-/-} mouse brains. (G) Bar graph quantifies the relative expression level of PGC-1 α in homogenates of 24 month-old Sirt2^{+/+} and Sirt2^{+/-} mouse brains. Data are presented as the mean ± SEM (n=3). IDH2, isocitrate dehydrogenase 2 (NADP4); MEF, mouse embryonic fibroblast; SEM, standard error of the mean.