



**Supplementary Figure 8. The metabolic reprogramming induced by AIF loss was confirmed by confronting the *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocyte compartments.** (a) *Left*, Representative Seahorse OCR assessment of *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes under basal conditions and in response to sequential treatment with Oligomycin, FCCP, and Rotenone/Antimycin A. Arrows indicate the time of the addition of each reagent. *Right*, Basal and maximal OCR of thymocytes expressed as a histogram (n = 3 independent experiments). (b)  $\Delta\Psi_m$  assessment performed in *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes (n = 6 mice per group). (c) Mitochondrial ROS levels recorded in *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes (n = 8 mice per group). (d) Mitochondrial mass of *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes assessed by Mitotracker Green labeling (n = 8 mice per group). (e) Glucose uptake measured by the assimilation of 2-NBDG in *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes (n = 5 mice per group). (f) ATP levels recorded in *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes (n = 5 mice per group). (g) PDK4 mRNA levels determined by qPCR in *AIF*<sup>+/*Y*</sup>, *DN AIF*<sup>+/*Y*</sup>, and *AIF*<sup>-/*Y*</sup> thymocytes (n = 8 mice per group). The 18S mRNA expression was used to normalize the data. Statistical significance was calculated by Mann Whitney test. Bars represent mean  $\pm$  SEM. All tests were performed in 21-day-old animals.