



Supplementary Figure 9. The metabolic changes associated to AIF deficiency in thymocytes are caspase-independent. (a) *Left*, Representative Seahorse OCR assessment of $AIF^{+/Y}$ and $AIF^{-/Y}$ thymocytes pretreated or not during 30 min with the caspase inhibitor Q-VD-OPh (QVD, 1 μ M) under basal conditions (initial rates) 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 \pm QVD expressed as a histogram ($n = 3$ independent experiments). (b) $\Delta\Psi_m$ assessment performed in $AIF^{+/Y}$ and $AIF^{-/Y}$ thymocytes \pm QVD ($n = 6$ mice per group). (c) Mitochondrial ROS levels recorded in $AIF^{+/Y}$ and $AIF^{-/Y}$ thymocytes \pm QVD ($n = 4$ mice per group). (d) Glucose uptake measured by the assimilation of 2-NBDG in $AIF^{+/Y}$ and $AIF^{-/Y}$ thymocytes \pm QVD ($n = 5$ mice per group). (e) Representative Seahorse OCR assessment in thymocytes from $AIF^{-/Y}$ 21-day-old animals pretreated or not with QVD. After measurement of basal OCR, BSA control or Palmitate-BSA FAO Substrate (17 μ M) were injected and maximal OCR was measured and expressed as a histogram ($n = 3$ independent experiments). Statistical significance was calculated by Mann Whitney test. Bars represent mean \pm SEM. All tests were performed in thymocytes from 21-day-old animals.