



Supplementary Figure S1. Optimization of assay conditions using mouse granulosa cells. (A, B) Optimization of granulosa cell number (plating density) for mitochondrial and glycolytic tests was conducted using 1 µM oligomycin (oligo) or Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and a combination 1 µM antimycin A and rotenone (A/R) as recommended (Agilent Seahorse XF User Guide). (A) Basal oxygen consumption rate (OCR) was measured and compared using cell numbers between 25 000 and 200 000 cells/well. (OCR was virtually undetectable when using 10 000 cells.) As the optimal basal OCR is between 50 and 150 pmol/min, 150 000 cells/well was deemed sufficient. (B) The impact of cell number on extracellular acidification rate (ECAR; milli-pH/minute (mpH/min)) was also assessed, with 150 000 cells/well again providing robust detection. 2DG, 2-deoxyglucose (C) Optimization of oligomycin concentration. OCR and ECAR were measured in 150 000 cells/well before and following treatment with oligomycin at doses ranging from 1.0 to 5.0 µM. Assay conditions require a large difference between OCR and ECAR and thus the optimum concentration of oligomycin was determined to be 1.0 µM. (D) Optimization of FCCP concentration. Maximal respiration (the ratio of OCR after FCCP to basal OCR) was determined using 150 000 cells/well and FCCP concentrations of 0, 2.5, 5.0, and 10 µM. The maximal response to FCCP was achieved using 2.5 µM, with no further increase observed at higher concentrations. Thus, the optimal concentration of FCCP was determined to be 2.5 µM.