Supplementary Online Material

Supplementary Figures



Supplementary Figure 1. SDH and COX activity in Rol treated MLM

Succinate dehydrogenase (SDH) (A) and Complex IV (COX) (B) activities measured spectrophotometrically in MLM (n=9). Retinol treatment of MLM did not affect either SDH or COX activity.



Supplementary Figure 2. IEF-2D gel analysis of the kinases and phosphatases involved in PDH regulation

A) IEF (3-10) followed by 2D SDS-PAGE of untreated (n.t) and Retinol (Rol) treatedMLM. Retinol treatment promoted a shift in the migration of PDHE1, PDK1 and PDK2

towards a higher pI, indicating less phosphorylation. PDK3, PDK4, PDP1 and PDP2 migration was not altered upon retinol treatment. B) MLM proteins treated with alkaline phosphatase (CIP) were resolved by IEF (3-10) followed by 2D SDS-PAGE. The shift observed in PDHE1, PDK1 and PDK2 towards a more basic pI in CIP treated MLM confirmed that phosphorylation was responsible for the post-translational modifications revealed by the changes in isoelectric points. The VDAC immunoreactive spot was used as the reference marker for positioning the blots. These blots (A-B) are representative of two independent experiments



Supplementary Figure 3. IEF-2D gel analysis in PKC8 -/- cells expressing PKC8

IEF (7-10) followed by 2D SDS-PAGE of untreated (n.t) and Retinol (Rol) treated PKCδ MIG MEFs. Expression of PKCδ in PKCδ -/- MEFs (PKCδ MIG) restores the Rol mediated de-phosphorylation of PDHE1 and PDK2 observed in WT MEFs. The VDAC immunoreactive spot was used as the reference marker for positioning the blots. These blots are representative of two independent cell lines transfected with two different plasmids expressing PKCδ.