SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Cell Models and RCAN1-1L Overexpression. Panel A – ENStem Cells. Western analysis illustrates RCAN1-1L expression in dividing cells. Cells were tested at 24 and 48 hours after transfection with different amounts (expressed in moi) of RCAN1-1L adenoviral construct. Control cells were transfected with 15 moi of null construct (vector). Probing with β-tubulin antibody was performed to control protein loading. Cell growth was analyzed in cells transfected with 15 moi of constructs. Cell number was measured using a Coulter Counter (Coulter Corporation). The results represent the means of three experiments ± SE's. **Panel B** – ST14A Cells. Western analysis illustrates RCAN1-1L expression in dividing and differentiating cells. Cells were tested at 24, 48 and 72 hours after transfection with 30 moi of RCAN1-1L adenoviral construct. Control cells were transfected with 30 moi of null construct (vector). Probing with β-tubulin antibody was performed to control protein loading. Cell growth was analyzed in cells transfected with 30 moi of constructs. Cell number was measured using a Coulter Counter (Coulter Corporation). The results represent the means of three independent experiments ± SE's.

Supplemental Figure 2. Representative Photographs of ST14A Cells. Dividing cells were incubated to semiconfluency and RCAN1-1L was overexpressed using our adenoviral construct. Cells were collected at 24 hours, fixed, and analyzed using transmission electron microscopy (TEM) at 5,000 times magnification.

Supplemental Figure 3. Effects of RCAN1-1L on Mitochondrial Membrane Potential. Dividing ST14A cells were analyzed at the indicated time periods of RCAN1-1L overexpression. Cells were labeled with MitoTracker Red CM-H2XRos (Molecular Probes, Inc.), and signals were measured using the FACS analyzer (as described in Experimental Procedures). The membrane potential was evaluated by comparison of the mean fluorescence signals between cells transfected with null vector and vector carrying RCAN1-1L. Results (means ± SE) represent measurements from three experiments. At the 9 hour time point the potential was significantly decreased, while at 24 hour, it was not significantly changed, as evaluated by t-test ($p<0.05$).

Supplemental Figure 4. RCAN1-1L Induces Apoptosis. Dividing ST14A cells were analyzed at the indicated time periods of RCAN1-1L overexpression. Cells were labeled with Alexa Fluor 647-Annexin V (Molecular Probes, Inc.), as described in (45), and signals were measured using the FACS analyzer (as described in Experimental Procedures). Signal intensity depends on number of stained cells, and on the intensity of staining (46); thus, we evaluated apoptosis by overall staining, which reflects both the amount of stained cells and the intensity of staining. Mean values were converted to arbitrary units to make the results from experiments directly comparable to each other. Fluorescence values in samples transfected with null construct (control) were set as 1.0. Results (means ± SE) represent measurements from three experiments. At the 9 hour time point apoptosis was not significantly induced, while at 24 hour, it was significantly increased, as evaluated by t-test ($p<0.05$).