

Supplementary Figure S2. Vti1a deletion leads to a reduction of voltage gated  $Ca^{2+}$ channels the plasma membrane. A. Examples of whole-cell voltage clamp experiments to test the current-to-voltage (I-V) relationship in wildtype (black) and vtila null (blue) chromaffin cells are shown on the left. Cells were clamped at -70 mV at rest and depolarized for 5 ms from -80 mV to +80 mV in 10 mV intervals. Channel activation was determined by analysis of tail currents and the average plotted against the membrane potential (error bars too small to be visible). This relationship was fit with a Boltzmann function to determine the membrane potential at which 50 % of the channels activated ( $V_{50}$ ). These values are shown in the right hand histogram. B. Non-stationary fluctuation analysis on repetitive depolarizations to 20 mV was performed. The two graphs on the left show two examples of the variance (var) in wildtype (black) and vtila null (blue) cells plotted against the average current (I) obtained during the activation-phase at the beginning of depolarizations. The inserts show 50 sweeps of the measured current (note that the variation in the current increases as the currents increase). The red lines show the best fit of a parabola to the data. The offset of the fit was fixed to match the variance measured in the currents prior to depolarization. The right hand histograms summarize the quantification of the fluctuation analysis. "i" signifies the singlechannel current. The number of channels was divided by the cellular capacitance to compare the density of channels on the cell surface. Scale bars in B: 50 pA (vertical) and 1 ms (horizontal). Histograms show means, error bars are SEM, except for the histogram in panel A where the error of the fit is displayed. Number of cells (n): wildtype: n=11; vti1a null: n=11; \*: p=0.04.