Supplementary Figure 7 Molecular and physiological characterization of PC12 cells. (a) RT-PCR analysis of RIM1 and RIM2 RNA expression in PC12 cells treated with GAPDH siRNA (siControl) and combination of RIM1- and RIM2-specific siRNAs (siRIM1 and siRIM2). Primer sequences are indicated in Supplementary Table 9. RT-PCR was performed using LA-PCR kit (TaKaRa), according to the manufacturer’s information. (b) Transfection levels of cDNAs of RIM constructs are examined using PCR in PC12 cells. Primers are designed to amplify the lactamase gene in the pCI-neo vector (Supplementary Table 9). PCR was carried out using LA-PCR kit. (c) RNA expression analysis of α1 and β subunits using RT-PCR in PC12 cells. (d) Pharmacological dissection of high voltage-activated Ca2+ channel currents in PC12 cells. Left & Middle: time course of blockage of Ca2+ current by serial application of 200 nM ω-Aga IVA, 3 μM ω-Ctx GVIA, 3 μM Nifedipine, and 10 μM Cd2+ in PC12 cell. Right: summary of relative contributions of each current type. (e) Cd2+-sensitive Ca2+ influx pathway is responsible for depolarization-dependent ACh release from ChAT-transfected PC12 cells. Cd2+ is a selective blocker for high voltage-activated Ca2+ channels. ***P < 0.001 versus 2.5 mM Ca2+. 