ATP-induced increase in intracellular calcium levels and subsequent activation of mTOR as regulators of skeletal muscle hypertrophy Naoki Ito, Urs T. Ruegg and Shin'ichi Takeda



Supplementary figure 1. ATP induces increase in $[Ca^{2+}]_i$ by activation of the P2Y₂ receptor/PLC/IP₃R pathway in C2C12 myotubes related to Figure 2. (A) Quantitative analysis of the peak magnitudes of Fluo-4 intensity in ATP or ATP analog-treated C2C12 myotubes (n = 4). (**B** and **C**) Concentration-dependent effects of ATP (B) or UTP (C) on the peak magnitude of the Ca²⁺ response (n = 4). (**D**) Western blot analysis of the P2Y₂ receptor in siRNA-transfected C2C12 myotubes. (**E**) Quantitative analysis showing the effect of P2Y₂ gene knockdown on ATP- or UTP-induced increases in $[Ca^{2+}]_i$ (n = 4). (**F**) Quantitative analysis for the peak magnitudes of Fluo-4 intensity in suramin- or U73122-treated C2C12 myotubes with ATP or UTP (100 μ M) (n = 4). (**G**) Quantitative analysis of the peak magnitudes of Fluo-4 intensity in thapsigargin-treated C2C12 myotubes, or C2C12 myotubes in 0 Ca²⁺ buffer with ATP, UTP or ATP γ S (100 μ M) (n = 4). (**H**) Quantitative analysis showing the effect of overexpression of an IP₃-sponge-eGFP on ATP- or UTP-induced increases of $[Ca^{2+}]_i$ (n = 4). (**I**) Quantitative analysis of the peak magnitudes of Fluo-4 intensity in XeC-treated C2C12 myotubes (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001 by ANOVA with Tukey-Kramer test. Error bars indicate S.E.M.