Dynamin conditional knockout fibroblasts: Tamoxifen inducible Knockout method

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Abstract

This cell line was described and characterized in the following paper: Ferguson, S.M., Raimondi, A., Paradise, S., Shen, H., Mesaki, K., Ferguson, A., Destaing, O., Ko, G., Takasaki, J., Cremona, O., O'Toole, E., De Camilli P. Coordinated actions of actin and BAR proteins upstream of dynamin at endocytic clathrin-coated pits. Developmental Cell 17, 811-822, 2009. PMID: 20059951]. This procedure describes tamoxifen-inducible KO method using this cell line.

Solutions to prepare

1. Tamoxifen stock solution (store at -80ºC)

    10 mM 4-hydroxytamoxifen (OHT, Sigma H-6278) in EtOH.

Protocol

1. Cells were cultured at 37ºC and 5% CO_2 in DMEM containing 10%FBS, 100U/ml penicillin, 100mg/mL streptomycin.

2. When cells reached 80-90% confluency, detach cells from the dishes using trypsin-EDTA and split the cells 1:4 and add 2 μM 4-hydroxy-tamoxifen along with fresh culture medium. Incubate the cells for 2 days (48 hours). A dish with no 4-hydroxy-tamoxifen is used as a control.

3. After 2 days, split the cells once again to prevent cell overcrowding. Add 300 nM 4-hydroxy-tamoxifen for 3 days.

4. Check the depletion of dynamin using immunofluorescence or Western blot.

Note 1. Most of the dynamin disappears within the first 3-4 days after starting the 4-hydroxy-tamoxifen treatment but the full phenotype appears after 5-6 days. Thus, experiments should be perfoemd between 5-10 days after adding 4-hydroxy-tamoxifen. No advantage in waiting longer. KO efficiency is around 90%.

Note 2. We used mouse anti-dynamin clone 41 from BD (#610245) to measure the loss of dynamin 1 and 2.