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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 tamoxifeninducible KO method using this cell line.

ATTACHMENTS

fy7bbpdyf.pdf

MATERIALS

Solutions to prepare

1. Tamoxifen stock solution (store at -80 °C)

10 mM 4-hydroxytamoxifen **Merck MilliporeSigma (Sigma-Aldrich) Catalo #H-6278**
in EtOH.

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Protocol

1w

1 Culture the cells at 37 °C and 5% CO2 in DMEM containing 10% FBS, [M] 100 U/ml penicillin, [M] 100 mg/mL streptomycin.

2 2d

When cells reach 80-90% confluency, detach cells from the dishes using trypsin-EDTA and split the cells 1:4 and add [M] 2 micromolar (µM) 4-hydroxy-tamoxifen along with fresh culture medium. Incubate the cells for 48:00:00 (48 hours). A dish with no 4- hydroxy-tamoxifen is used as a control.

3 5d

After 48:00:00, split the cells once again to prevent cell overcrowding. Add [M] 300 nanomolar (nM) 4-hydroxy-tamoxifen for 72:00:00.

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

Note

Most of the dynamin disappears within the first 72:00:00 - 96:00:00 after starting the 4- hydroxy-tamoxifen treatment but the full phenotype appears after 120:00:00 - 144:00:00. Thus, perform the experiments between 120:00:00 - 240:00:00 after adding 4-hydroxy-tamoxifen. No advantage in waiting longer. KO efficiency is around 90%.

Note

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