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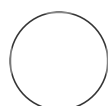
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Dynamin conditional knockout fibroblasts: Tamoxifen inducible Knockout method

Daehun Park^{1,2}, Pietro De Camilli^{1,2}

¹Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815.



daehun.park

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) Catalog #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,  100 U/ml penicillin,  100 mg/mL streptomycin.

- 2 When cells reach 80-90% confluency, detach cells from the dishes using trypsin-EDTA and split the cells 1:4 and add  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.

2d

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

5d

- 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.