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LONP1 knockdown in iPSC-derived microglia

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

LONP1 knockdown in iPSC-derived microglia

1 Obtain shLONP1 plasmid (target sequence: CCAGTGTTTGAAGAAGACCAA; TRCN0000046793) from the MISSION® shRNA library (Sigma-Aldrich) and clone into the pLKO.1-puro vector backbone.

2 Obtain non-targeting control shRNA from VectorBuilder (VB010000-0005mme)

3 Produce lentiviral particles by transfecting HEK293T cells with shRNA plasmids together with the packaging plasmid psPAX2 (RRID:Addgene_12260) and the envelope plasmid pMD2.G (RRID:Addgene_12259)

4 Use TransIT-X2 (Mirus Bio) for transfection according to the manufacturer's instructions

5 Quantify the concentration of p24 capsid protein using Lenti-X GoStix Plus (Takara Bio)

6 Normalize viral amounts to equal p24 levels

7 Add p24-normalized lentiviral particles directly to iPSC-derived microglial cultures without using transfection reagents

8 Analyze cells at 24, 48, and 72 hours after shRNA treatment