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Constructs and generation of stable cell lines

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We use this protocol and it's working

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Abstract

Protocol used to generate stable Flp-In T-REx-HEK 293 cell lines expressing WT or mutant GCase (E326K or L444P) as
a V5-FLAG-tagged protein using a tetracycline-inducible system.

1 Constructs were purchased from IDT (gBlocks Gene Fragments) and subcloned into pcDNA5/frt/to (Thermo Fisher Scientific, # V652020).

2 For the generation of V5-FLAG-GCase lines, the tag was positioned at the N-terminus, three aa after the cleavage site of the leader sequence. These three amino acids are repeated after the tag to ensure that the GBA1 sequence is intact.

3 To ensure proper cleavage, the V5-FLAG tag was inserted 12 bp after the cleavage site, and this 9 bp were repeated after the tag. To avoid interference with proper protein folding, we employed the short V5 sequence (IPNPLLGLD).

4 Site-directed mutagenesis was performed according to the manufacturer's protocol (Agilent, QuickChange II XL), and base pair exchange was confirmed by Sanger sequencing.

5 Flp-In 293 T-Rex cells(Thermo Fisher Scientific, #R78007) were grown in media composed of Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich), 10% fetal bovine serum
(Gibco), 1% GlutaMax (Gibco) supplemented with 100 µg/ml Zeocin
(InvivoGen) and 15µg/ml blasticidin (InvivoGen).

6 Inducible Flp-In T-Rex 293 cells were generated according to the manufacturer's protocol
(Thermo Fisher Scientific).

7 The selection was performed with DMEM supplemented with 15µg/ml blasticidin and 100 µg/ml hygromycin B Gold (InvivoGen) 48 h after transfection and continued until the expression of the gene of interest was induced by treating the cells with 50 ng/ml doxycycline hyclate (Sigma–Aldrich) for 48 h.