Generation of ATG3 KO Hela cells stably expressing HaloTag-LC3B

Xuefeng Ren (Laboratory of James H. Hurley, University of California, Berkeley, CA)
(snowren@berkeley.edu)

Buffers and reagents:

- pVSV-G (Addgene #138479)
- pBS-CMV-gagpol (Addgene #35614)
- pMRX-IP-HaloTag7-LC3 (Addgene #184899)
- Stbl3 competent E. coli (MacroLab, UC Berkeley)
- HiSpeed Plasmid Midi Kit (Qiagen #12643)
- Growth broth: LB broth
- DMEM medium with GlutaMAX (Gibco #10566016) containing 10% FBS (Gibco #A5256801) and 10% Pen-Strep (Gibco #15140122).
- Opti-MEM reduced serum medium (Gibco #31985070)
- TransIT-LT1 (Mirus #MIR2300)
- Lenti-X concentrator (Takara Bio #631231)
- polybrene (Sigma-Aldrich #H9268)
- puromycin (GoldBio #P-600)

Procedures:

1. Each plasmid is transformed into Stbl3 competent cells for the propagation. Next day, pick up one colony to inoculate overnight culture in LB medium with ampicillin.
2. Midi prep the cultures to purify plasmids (Qiagen).
3. Plate 5x 10^6 HEK 293T cells on a 10 cm plate in DMEM medium.
4. HEK293T transfection:
   - Add retroviral packaging plasmids (pVSV-G, pBS-CMV-gagpol) and pMRX-IP-HaloTag7-LC3, 5 µg each in 1.5 ml warm Opti-MEM medium.
   - Add 45 µl of TransIT-LT1 transfection reagent (Mirus) and swirl.
   - Incubate at room temperature for 15 minutes.
   - Add 1.5ml dropwise into 10 cm HEK293T plate.
   - At 72 hours post-transfection, collect retroviral supernatant into a falcon tube.
5. Concentrate retroviral supernatant to 1 ml using Lenti-X concentrator (Takara) with the manufacturer instruction.
6. Retroviral transduction:
   - Plate 1x 10^5 ATG3 KO HeLa cells into 12-well plate one day before.
   - Next day, titrate 100, 200, 400 µl of concentrated retroviral solution with 8 µg/ml Polybrene (Sigma) into target HeLa cells.
   - At 24 hours post-transduction, remove retroviral supernatant and replace with fresh DMEM complete medium with 2 µg/mL puromycin (GoldBio).
   - Puromycin selection was performed for two weeks. Halo-LC3B positive cells were confirmed by FACS sorting.