Высокоскоростной клеточный сортер FACS Aria III

Лазер	Фильтр детекции
375 и 405 нм	510/50 450/40
488 нм	695/40 530/30 488/10
561 нм	780/60 685 670/14 610/20 582/15
633 нм	780/60 730/45 660/20

Т29а

Обязательный контроль – клетки без флуоресценции

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CRISPR/Cas9 based screening for search of ribophagy regulatory genes

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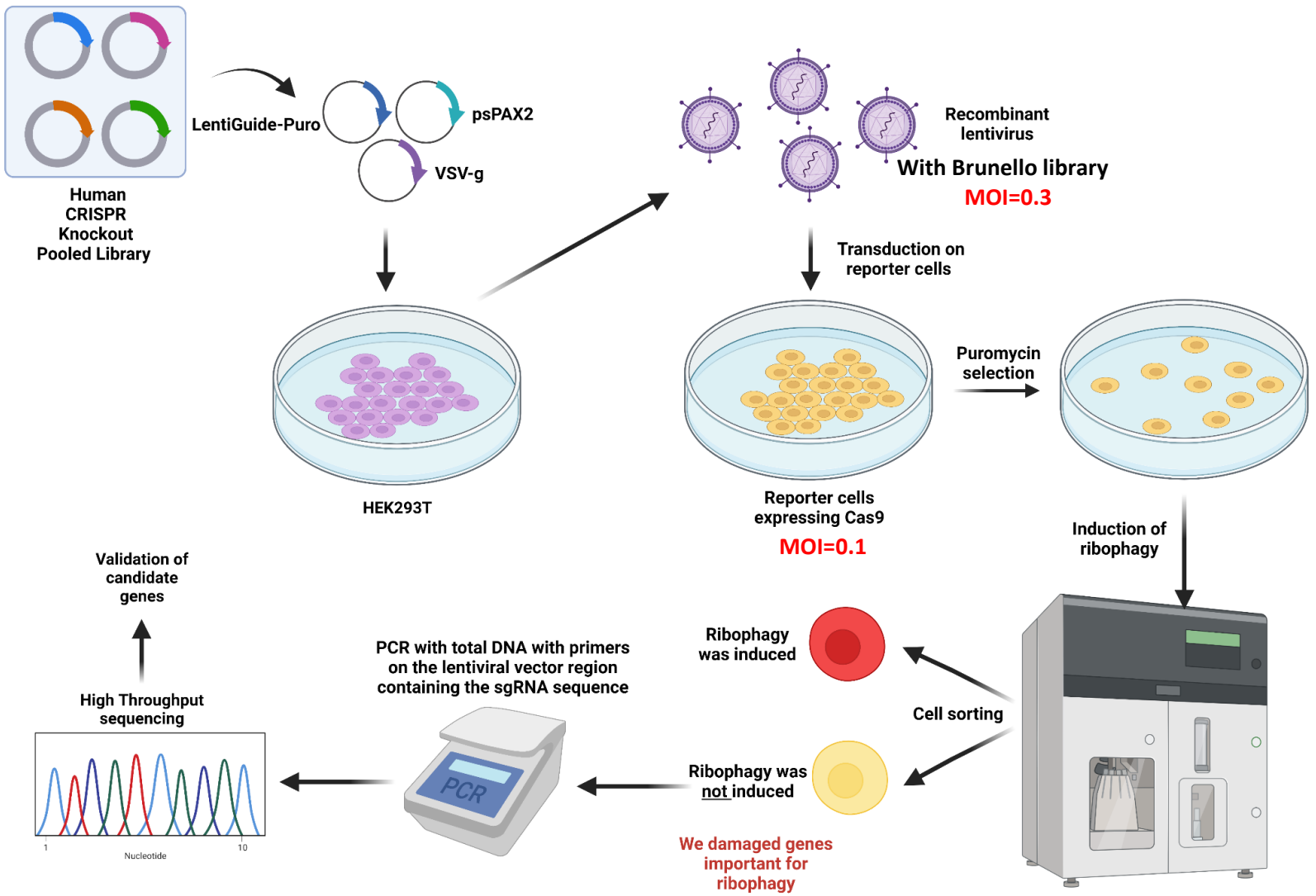
Scientific advisor: Sergiev P.V.

CRISPR/Cas9 based screening using Brunello library



Brunello single guide RNA (sgRNA) library - 76,441 sgRNAs covering 19,114 genes

1. Reporter cells were infected with lentiviruses with Cas9 (MOI<0.1).

Blasticidin selection

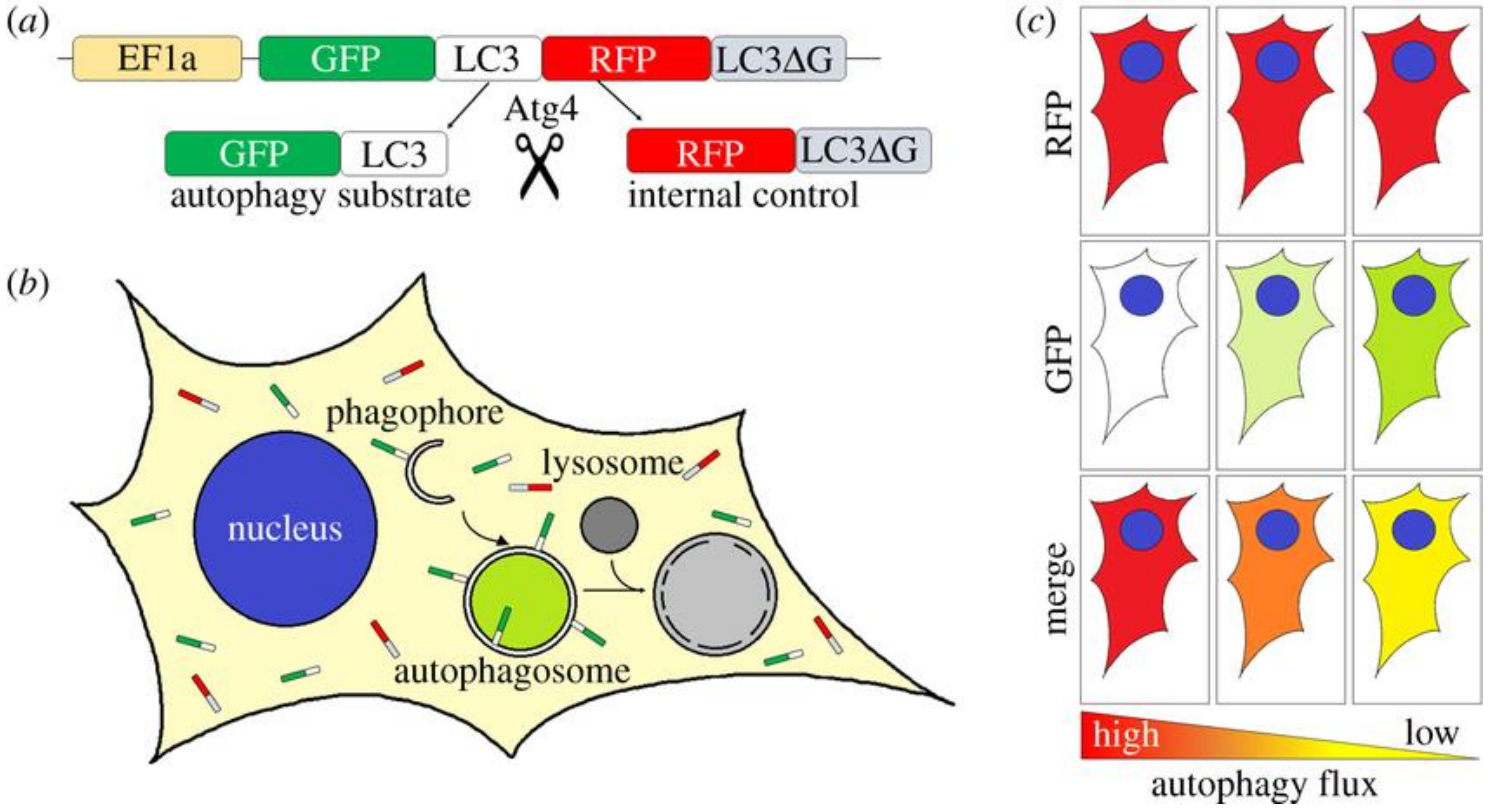
2. **120 million** reporter cells carrying Cas9 were infected with the lentiviral pool containing Brunello library at a MOI of 0.3.

Puromycin selection

3. **40 million** cells were sorted for Red:Green fluorescence.

4. Cells were propagated and subjected to additional rounds of sorting.

CRISPR/Cas9 based screening using Brunello library for determination of the mechanism of action of autophagy activators



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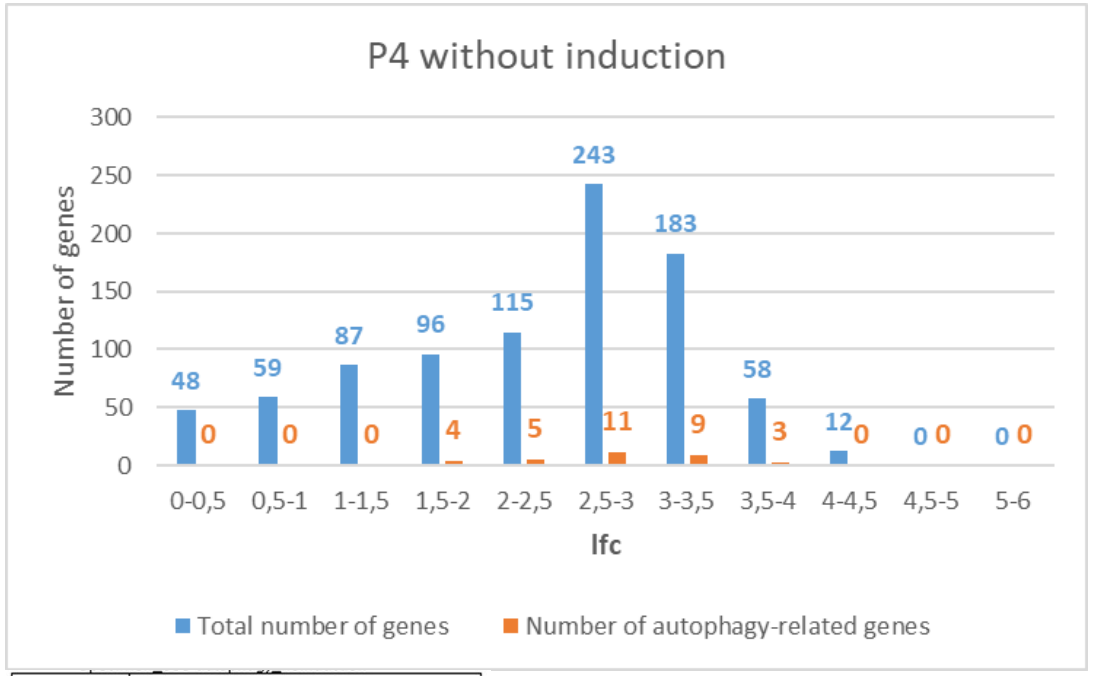
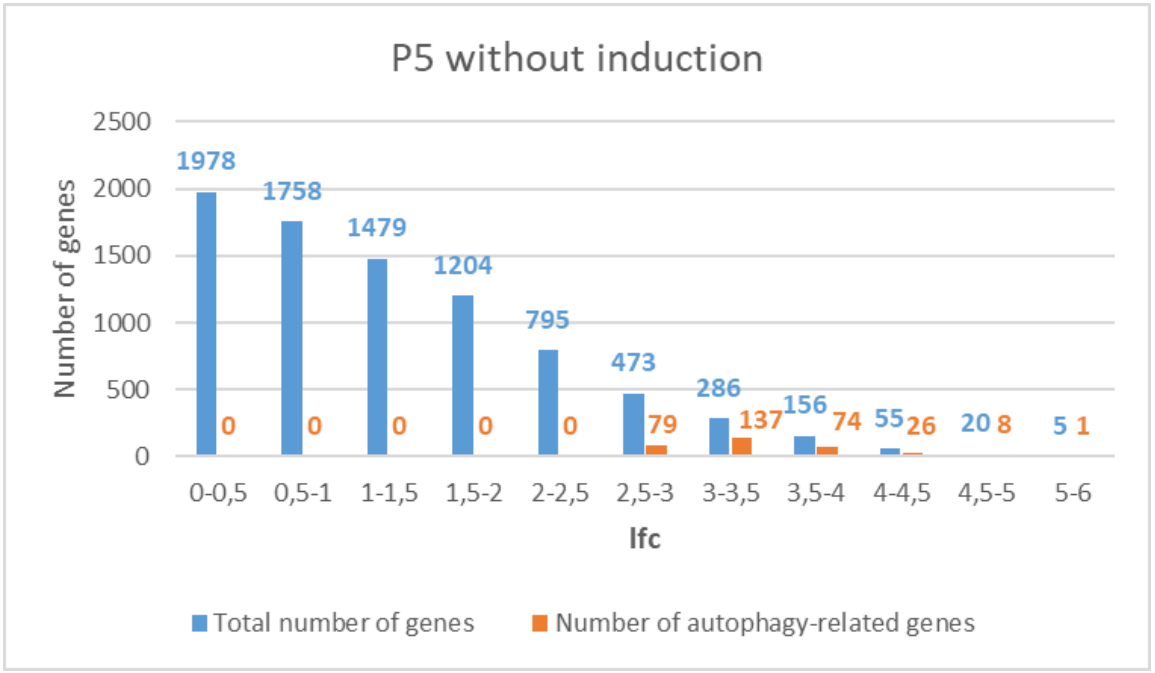
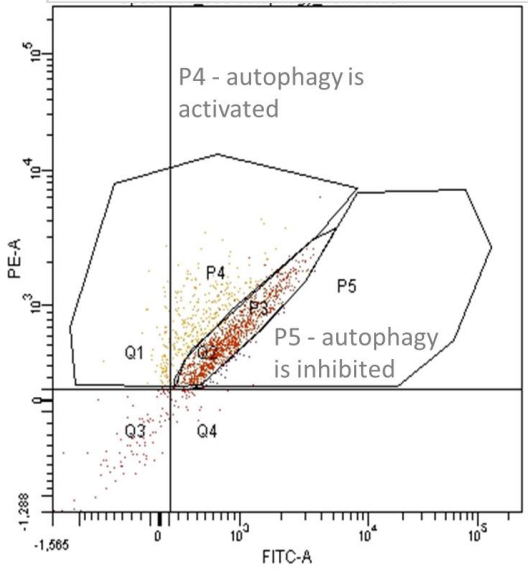
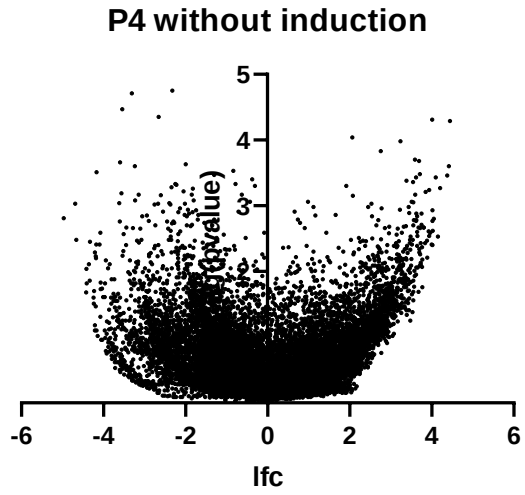
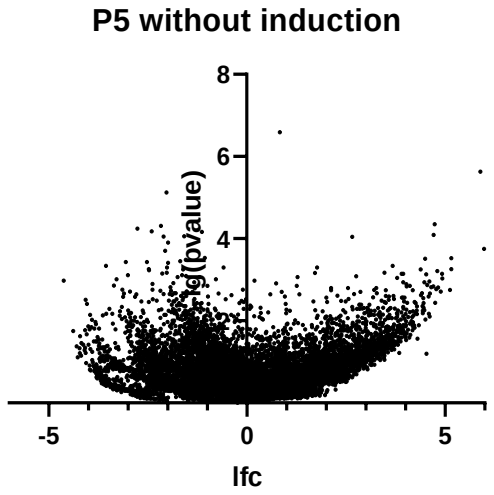
In population defective in autophagy in basal conditions we see accumulation of autophagy-related genes and lysosome-related genes







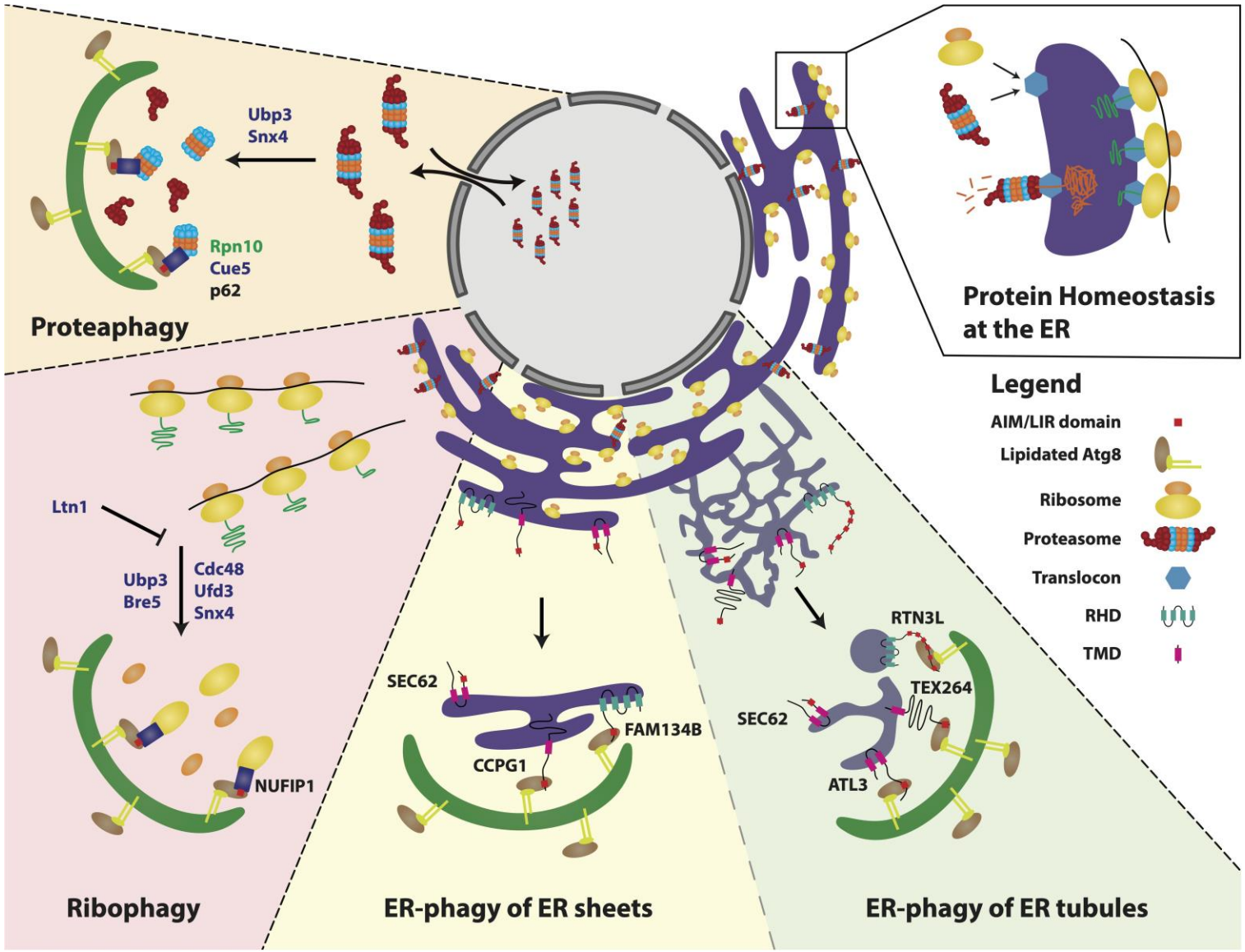




Totally we observe 325 genes related to authophagy, mTOR pathways and lysosome

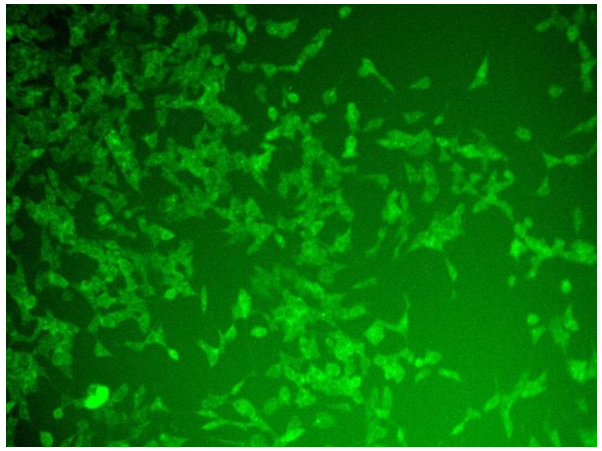
Autophagy core genes – 100
mTOR and upstream pathway - 52
Lysosome related genes – 89

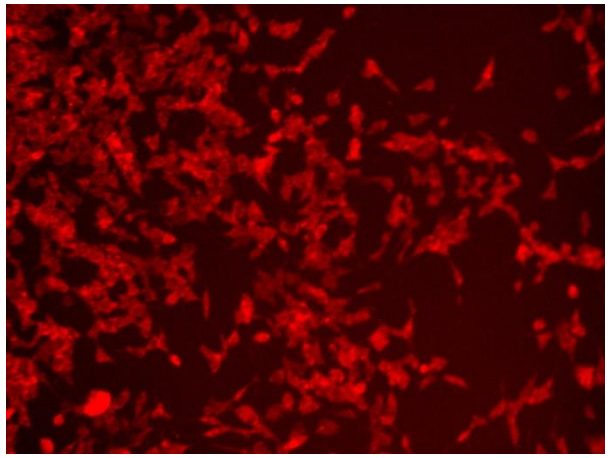
Ribophagy


Ubp3
Snx4
Rpn10
Cue5
p62
Proteaphagy
Protein Homeostasis at the ER
Legend
AIM/LIR domain
Lipidated Atg8
Ribosome
Proteasome
Translocon
RHD
TMD
Ltn1
Ubp3
Bre5
Cdc48
Ufd3
Snx4
NUFIP1
Ribophagy
SEC62
FAM134B
CCPG1
ER-phagy of ER sheets
RTN3L
TEX264
SEC62
ATL3
ER-phagy of ER tubules

Search for conditions that activate ribophagy

Live cell imaging using CelenaX





Fluorescence imaging in RFP and GFP channels

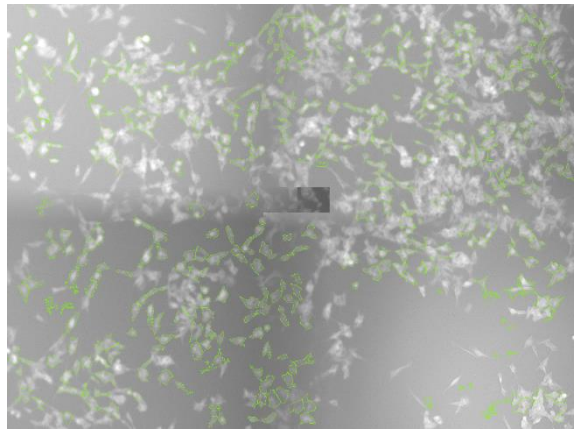
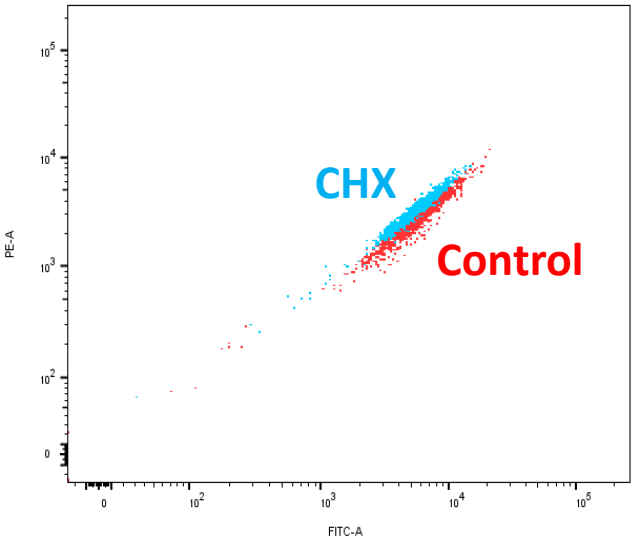


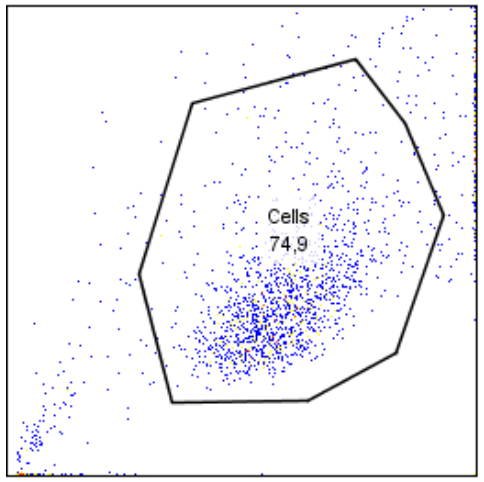
Image Analysis and counting RFP/GFP ratio

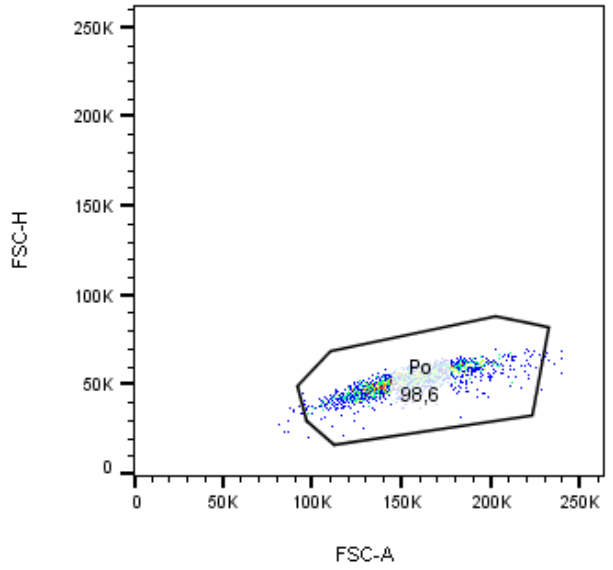
Fluorescence-activated cell sorting for ribophagy

FACS data

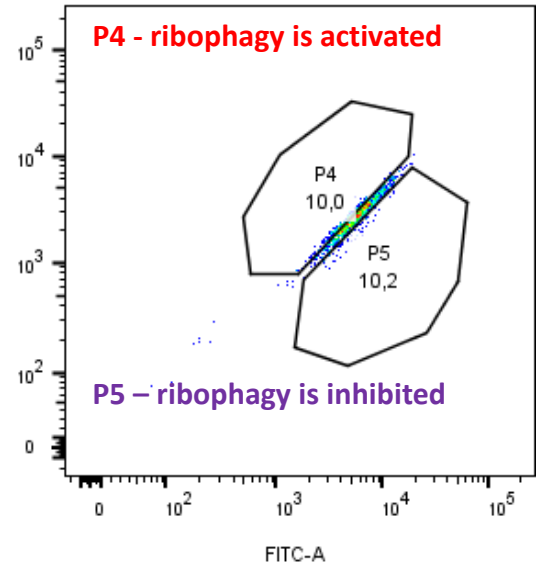


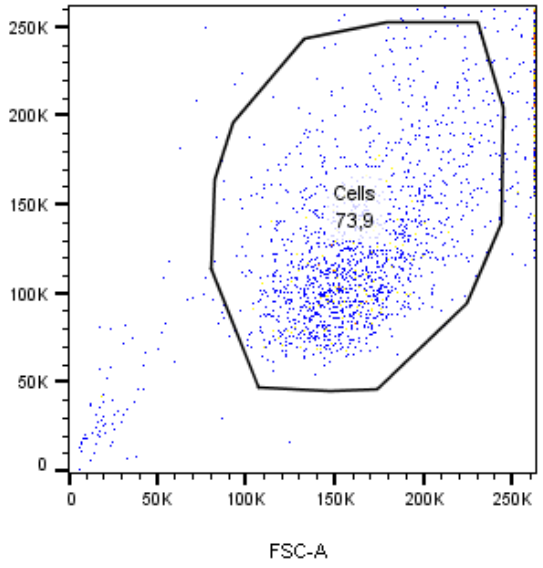
	Sample Name	Subset Name	Count
	Specimen_009_ribophagy_C10_001.fcs	Po	1394
	Specimen_009_ribophagy_C10.fcs	Po	1476

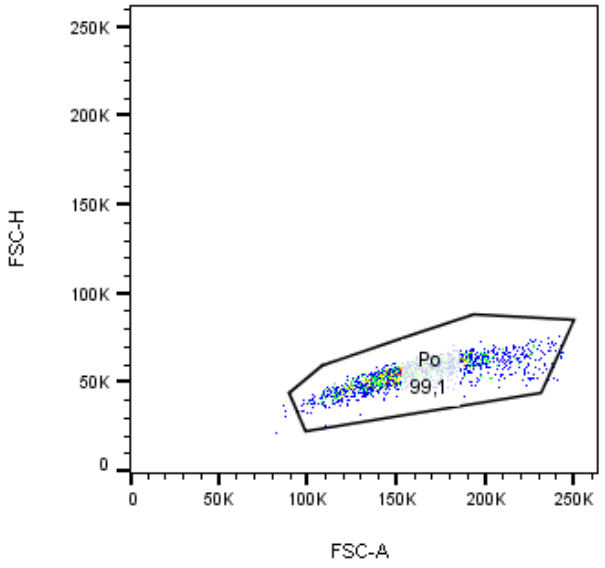




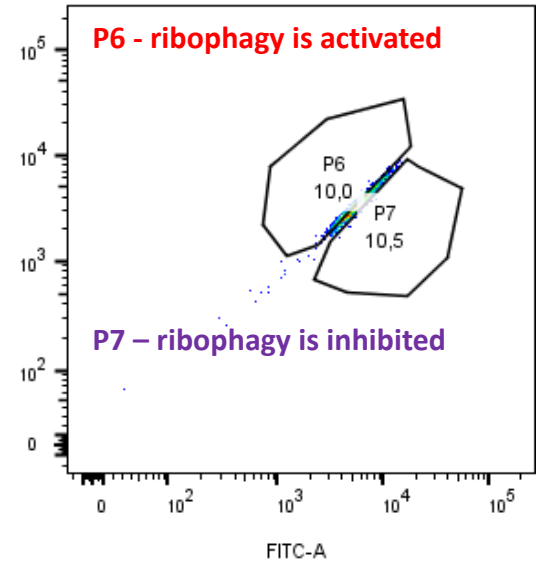
Control





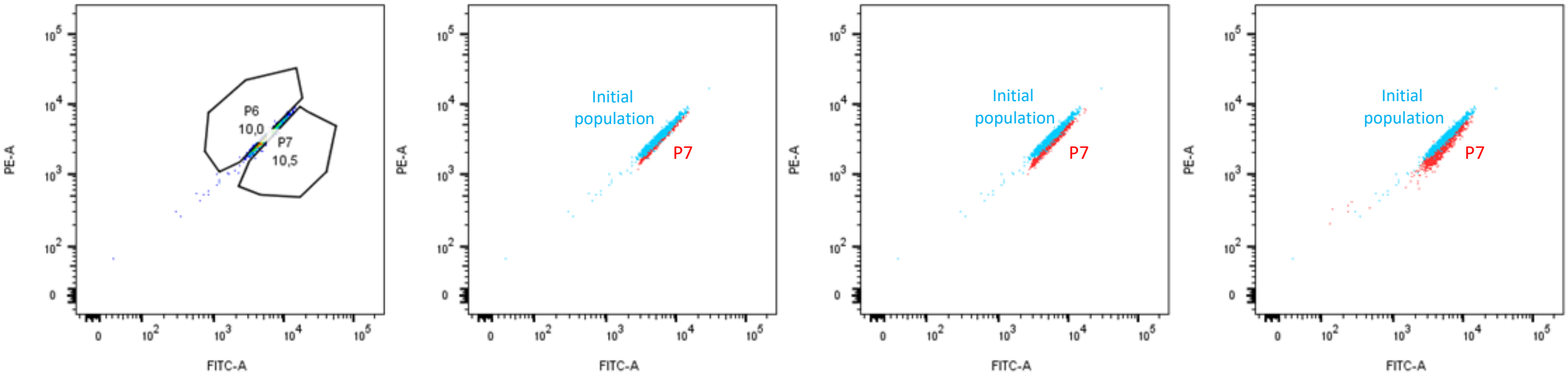


CHX induction



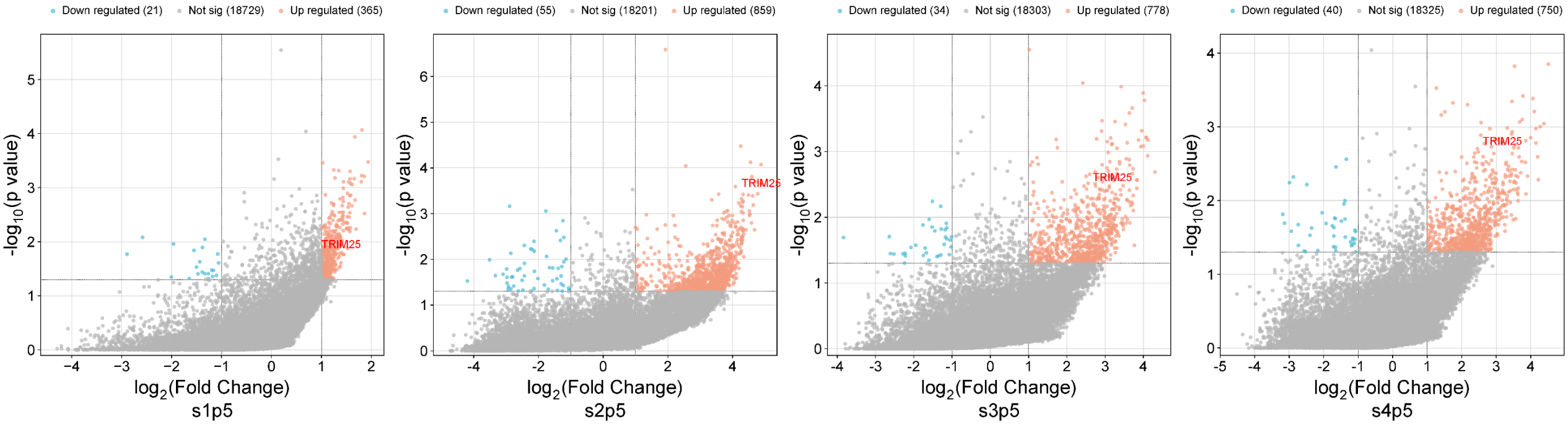
Enrichment of ribophagy-deficient population

Initial population



Round 1 → Round 2 → Round 3 → Round 4

Consistently enrichment of TRIM25 in ribophagy-deficient population



Enrichment

Источники света - лазеры:
- 488 нм, 50 mW
- 640 нм, 80 mW
- 405 нм, 100 mW
- 561 нм, 50 mW

Оптика сбора сигнала.
Регистрируемые оптические параметры - до 30 флуоресцентных параметров и 3 (три) параметра светорассеяния (FSC, SSC и SSC от фиолетового лазера). Эффективная регистрация всего спектра излучения в пределах длин волн 420 - 829 нм без необходимости смены оптических фильтров для регистрации сигналов от отдельных флуорохромов. 48 каналов регистрации флуоресценции.