

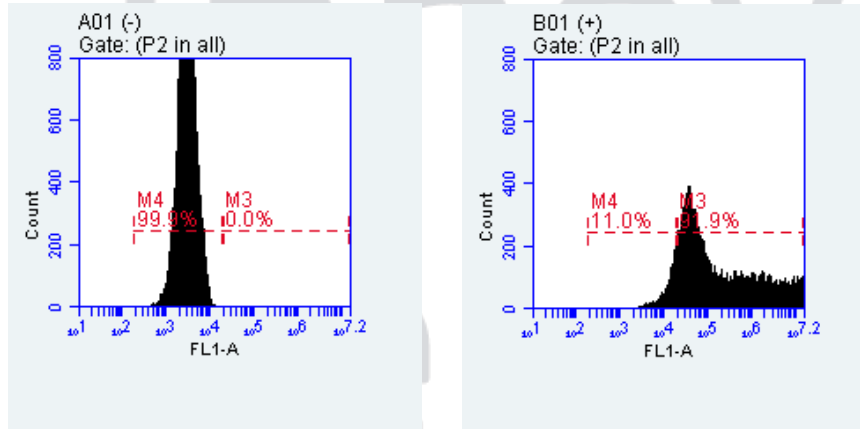
Methods

- All experiments were transient transfections in 293-YFP cells for 72 hours (Mirus TransIT[®]-LT1)
- Cells were maintained and collected at Day 3, 6, 10, 14 for flow cytometry
- T7 Endo 1 assay was performed on samples Day 3 and 14

Innovative
Genomics
Initiative

EGFP as a transfection indicator

~92% efficiency in 293FT cells with Mirus LT-1 transfection reagent



91.88%

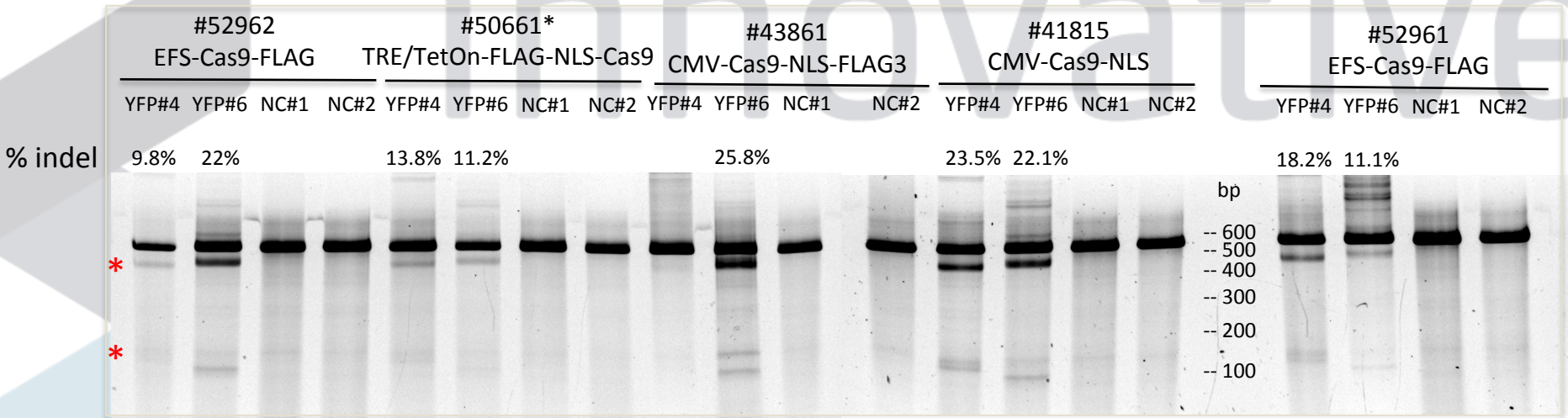
Day 3

#s represent Addgene IDs

T7 endo (60 min)
YFP#1 578 bp
131~143 bp
434~447 bp

Two vectors

One vector



*Dox 25ng/ml for 3 days

YFP = YFP-targeting sgRNA

NC = non-targeting control sgRNA

Pei-Chun Lin

20% TBE gel

Day 3

Two vectors

One vector

#52962
EFS-Cas9-FLAG

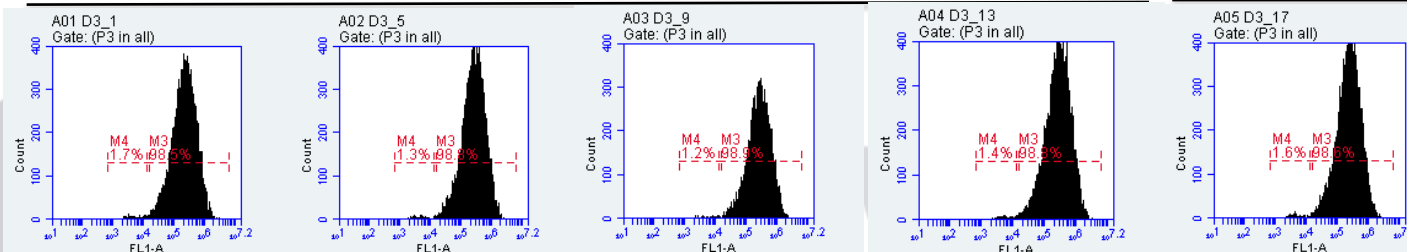
#50661*
TRE/TetOn-FLAG-NLS-Cas9

#43861
CMV-Cas9-NLS-FLAG3

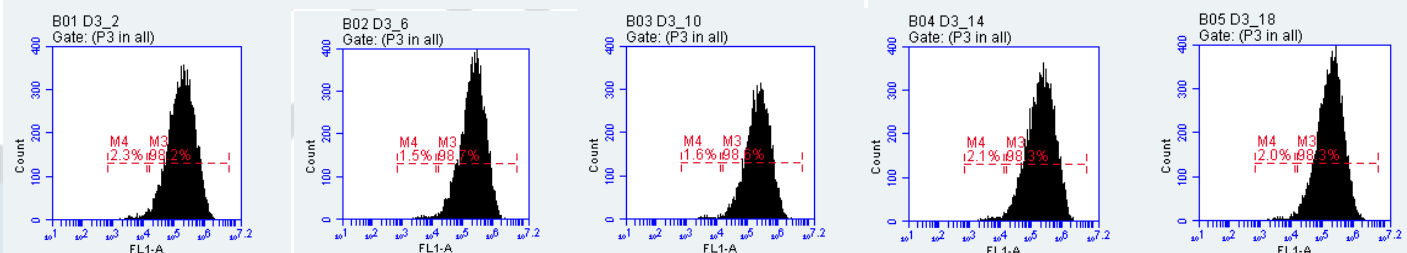
#41815
CMV-Cas9-NLS

#52961
EFS-Cas9-FLAG

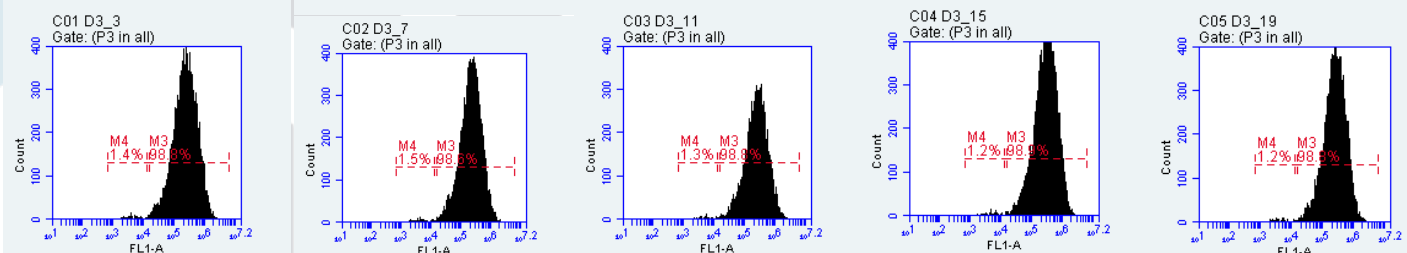
YFPsgRNA#4



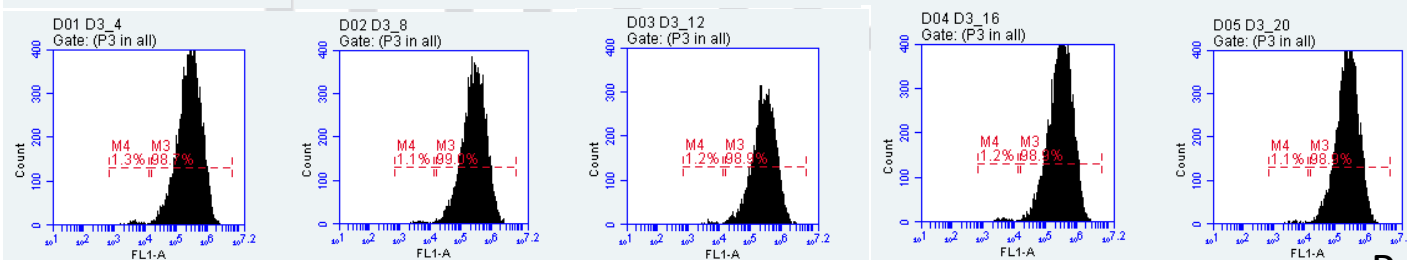
YFPsgRNA#6



NCsgRNA#1



NCsgRNA#2



Day 6

Two vectors

One vector

#52962
EFS-Cas9-FLAG

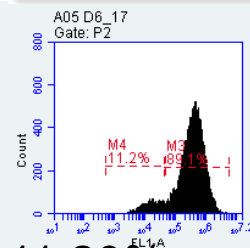
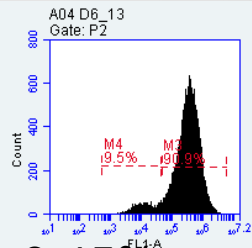
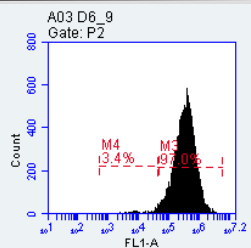
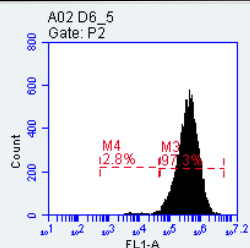
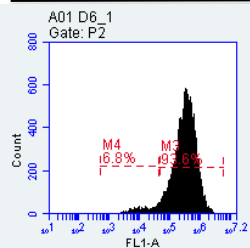
#50661
TRE/TetOn-FLAG-NLS-Cas9

#43861
CMV-Cas9-NLS-FLAG3

#41815
CMV-Cas9-NLS

#52961
EFS-Cas9-FLAG

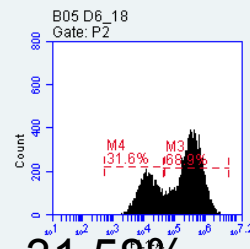
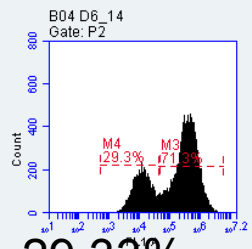
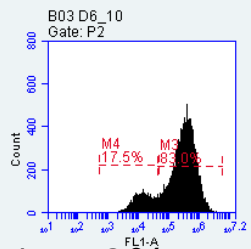
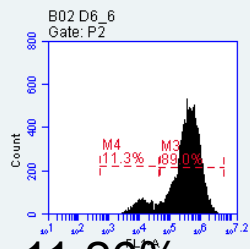
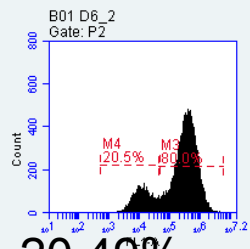
YFPsgRNA#4



9.45%

11.20%

YFPsgRNA#6



20.49%

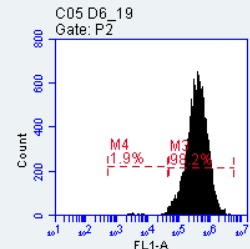
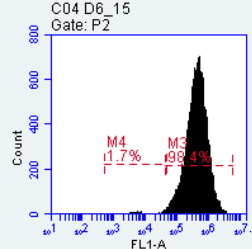
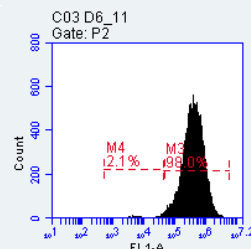
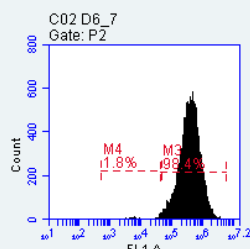
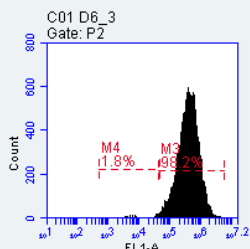
11.26%

17.48%

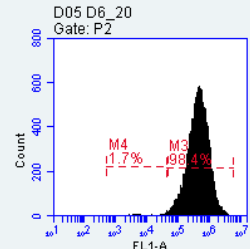
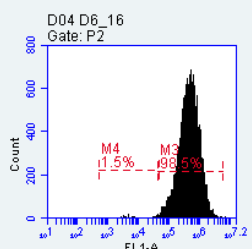
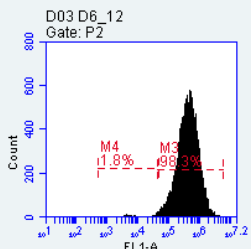
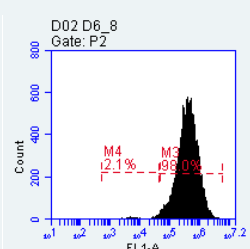
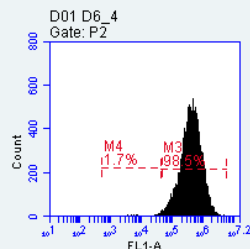
29.33%

31.58%

NCsgRNA#1



NCsgRNA#2



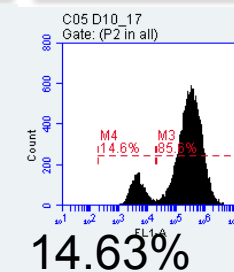
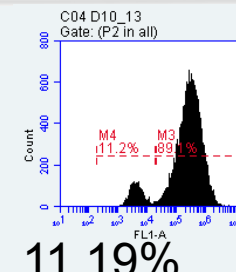
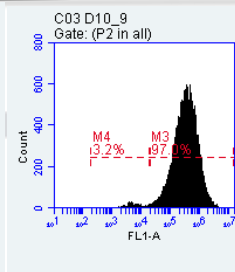
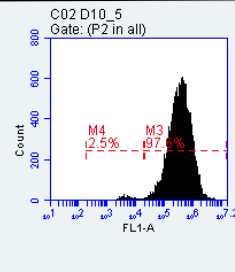
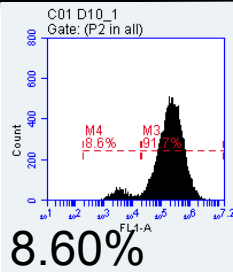
Day 10

Two vectors

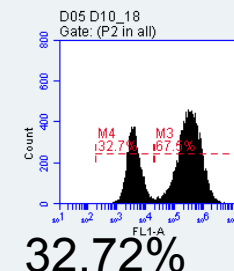
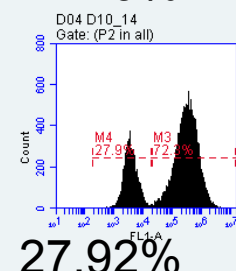
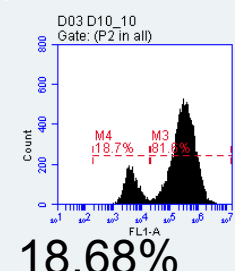
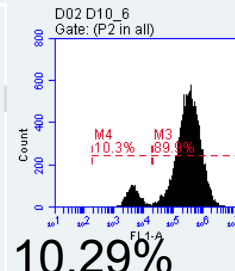
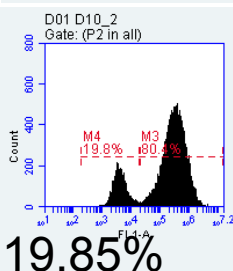
One vector

#52962 EFS-Cas9-FLAG #50661 TRE/TetOn-FLAG-NLS-Cas9 #43861 CMV-Cas9-NLS-FLAG3 #41815 CMV-Cas9-NLS #52961 EFS-Cas9-FLAG

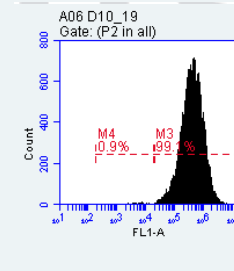
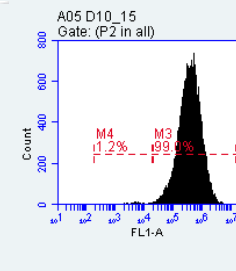
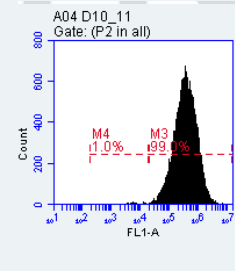
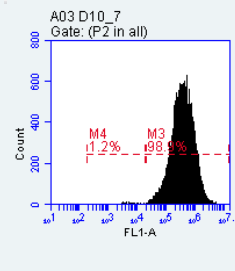
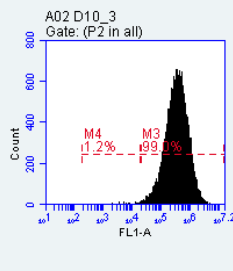
YFPsgRNA#4



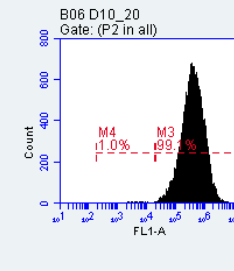
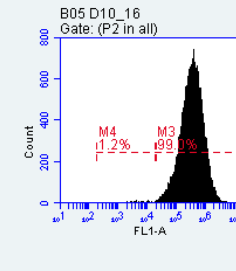
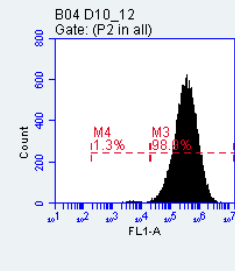
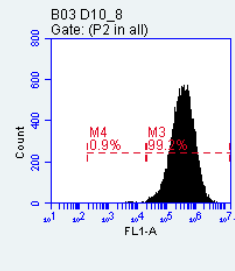
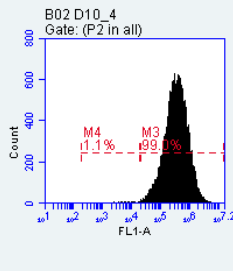
YFPsgRNA#6



NCsgRNA#1



NCsgRNA#2



Day 14

T7 endo (60 min)

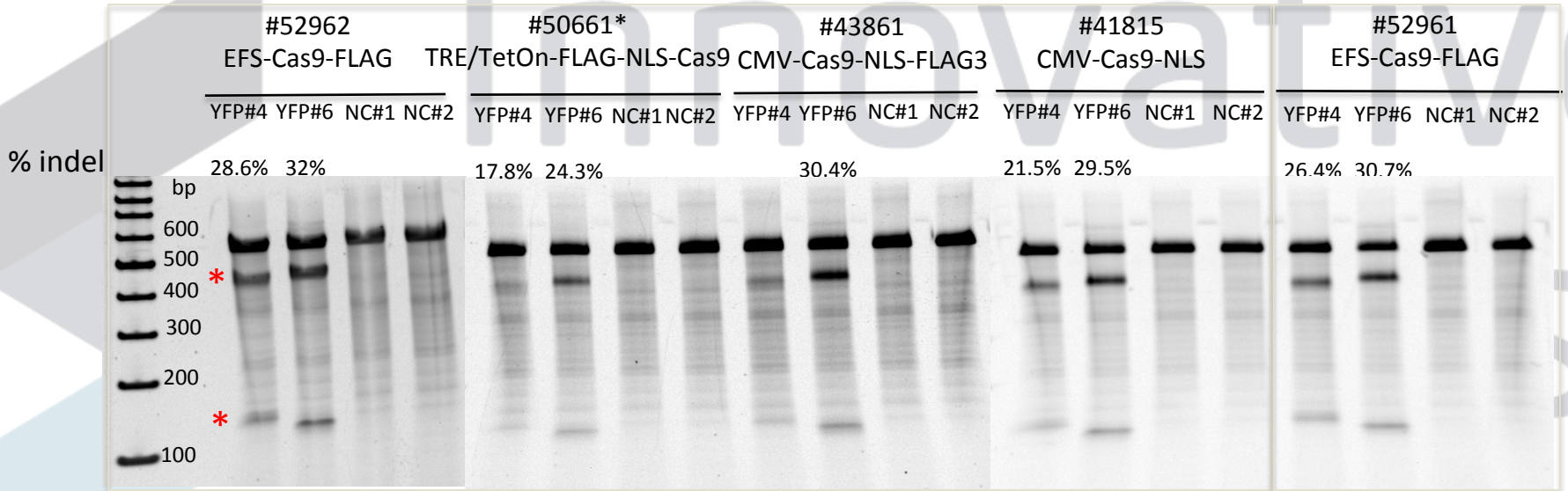
YFP#1 578 bp

131~143 bp

434~447 bp

Two vectors

One vector



*Dox 25ng/ml for 3 days

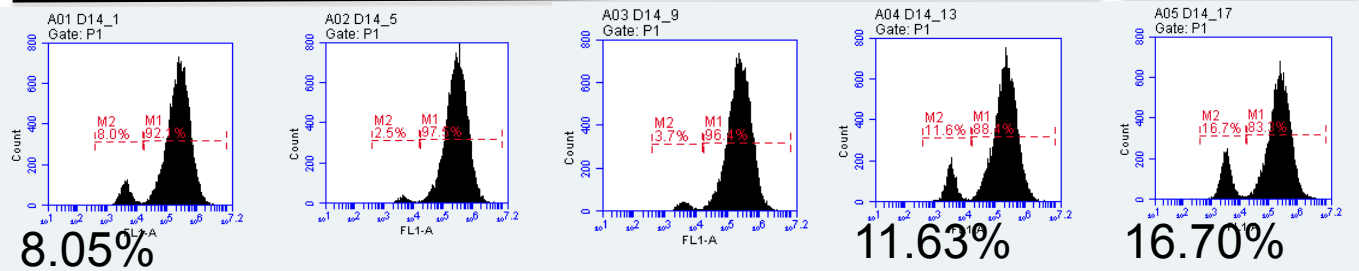
Day 14

Two vectors

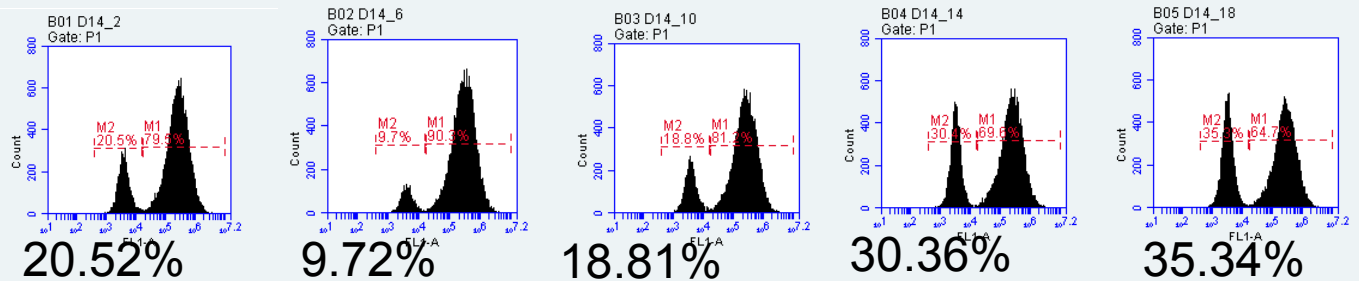
One vector

#52962 EFS-Cas9-FLAG #50661 TRE/TetOn-FLAG-NLS-Cas9 #43861 CMV-Cas9-NLS-FLAG3 #41815 CMV-Cas9-NLS #52961 EFS-Cas9-FLAG

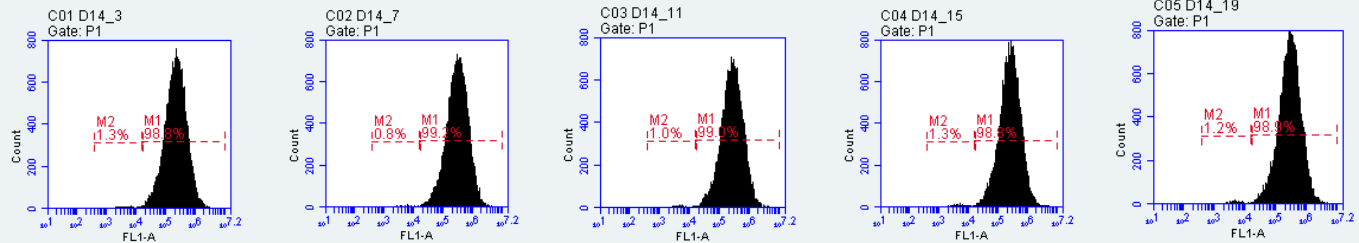
YFPsgRNA#4



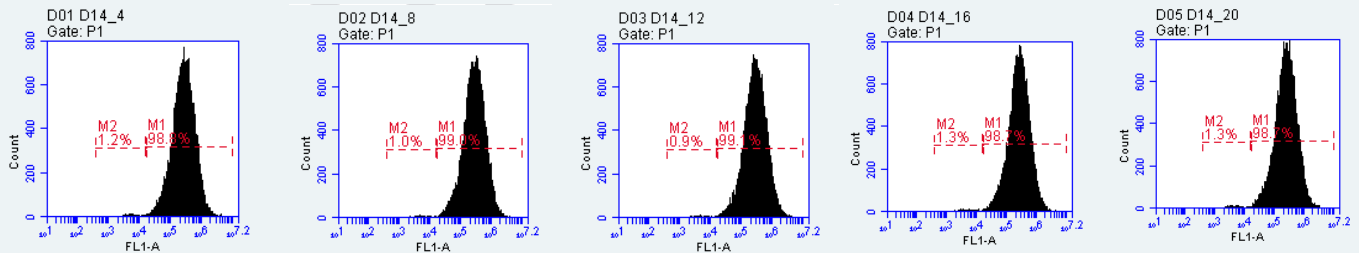
YFPsgRNA#6



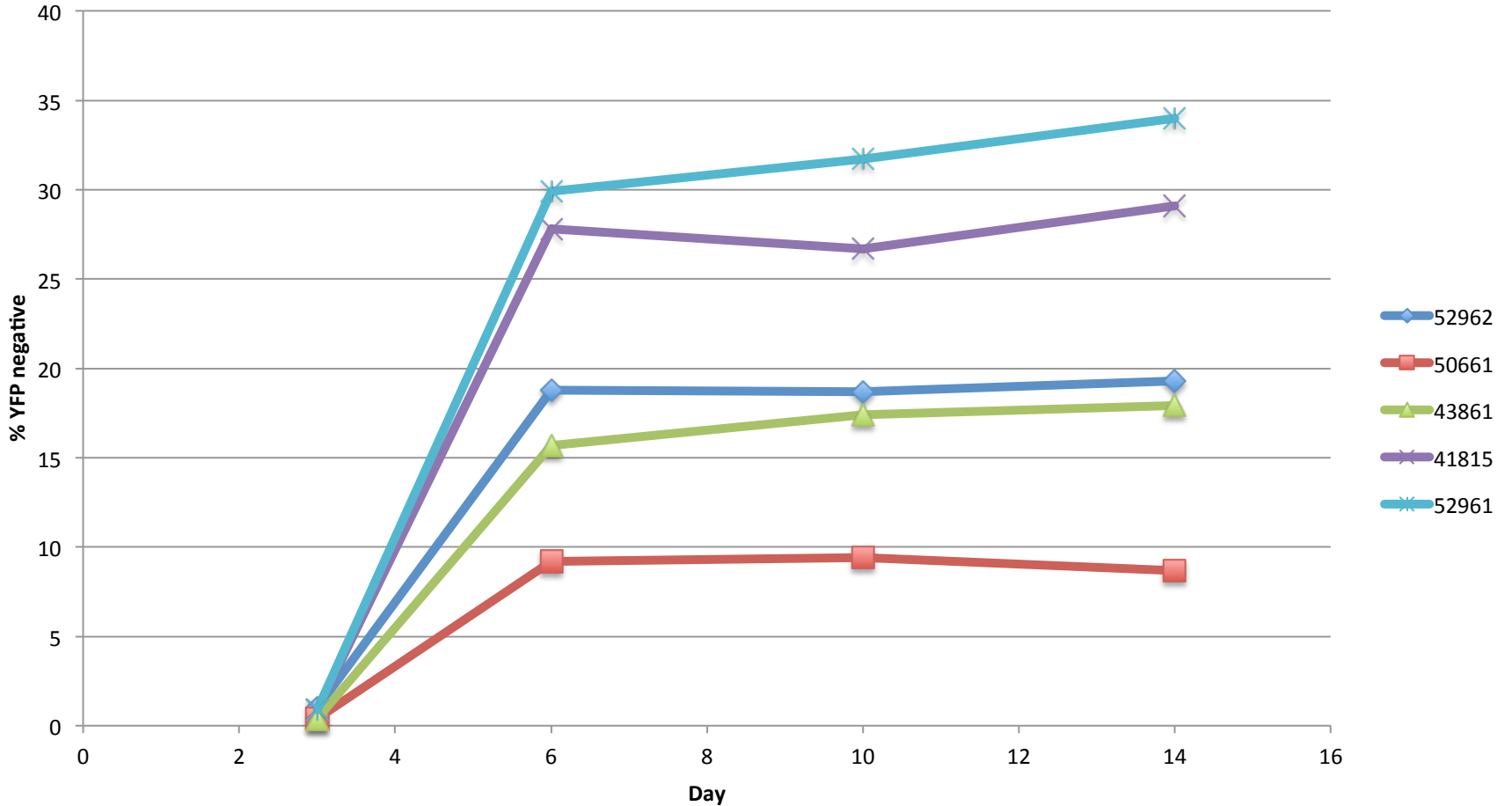
NCsgRNA#1



NCsgRNA#2



YFP FACS negative cells - sgRNA #6



background-corrected for % non-fluorescent cells observed with NC sgRNA