Assessing the activities of 2A-creER variants in a cell-based assay

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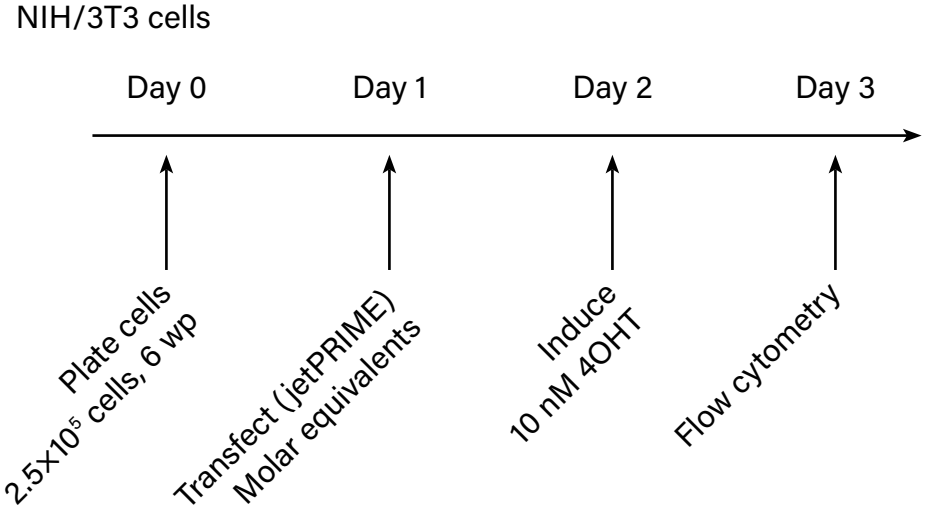
Assay design:



Constructs transfected:

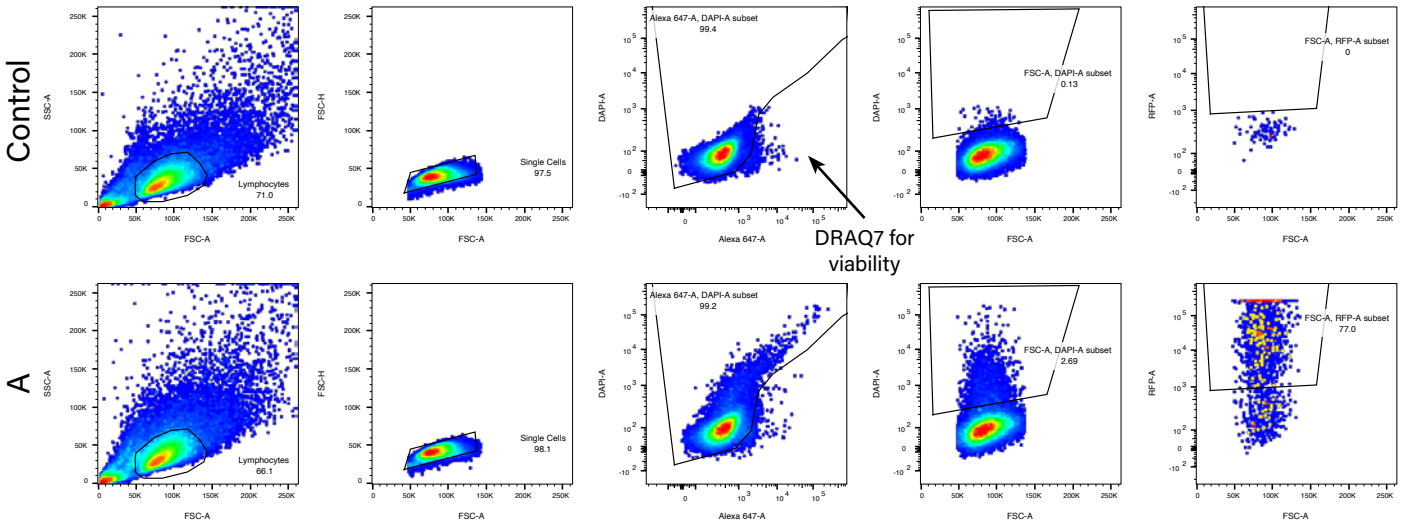
+

pCAG-loxP-3pA-loxP-tdTomato-WPRE-bpA

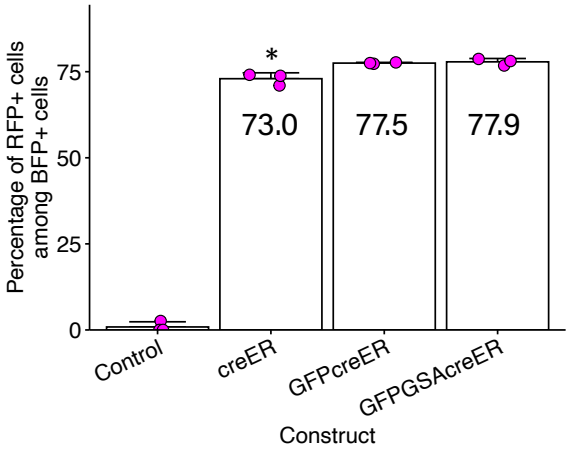
+

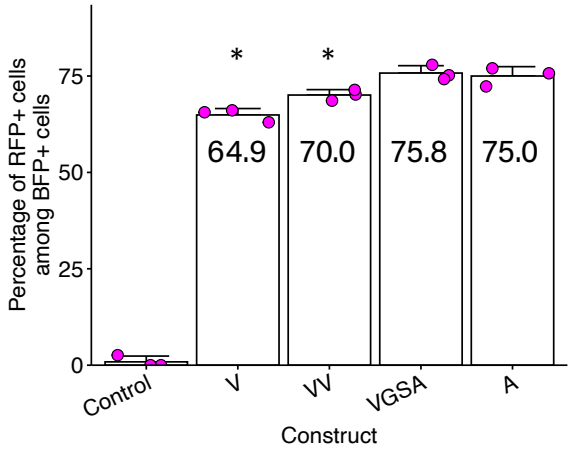
pBluescript II SK- carrier

Flow cytometry gating examples:



Result summary:





From Nam and Capecchi, *Neural Dev*, 15, 3, (2020)

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        Pairwise comparisons using t tests with pooled SD

           A       creER   Control GFPcreER GFPGSAcreER V       VGSA
creER      1.00000 -       -       -        -           -       -
Control    < 2e-16 < 2e-16 -       -        -           -       -
GFPcreER   1.00000 0.08802 < 2e-16 -        -           -       -
GFPGSAcreER 1.00000 0.04864 < 2e-16 1.00000  -           -       -
V          2.4e-05 0.00037 < 2e-16 1.3e-06  8.5e-07     -       -
VGSA       1.00000 1.00000 < 2e-16 1.00000  1.00000     9.3e-06 -
VV         0.04609 1.00000 < 2e-16 0.00093  0.00054     0.03162 0.01345

P value adjustment method: bonferroni
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N-terminal amino acids of 2A-skipped creER seem to modulate recombination efficiency, presumably due to differential stabilities of the proteins

See Kats et al., *Molecular Cell*, 70, 488-501 (2018),
Reinhardt et al., *Stem Cell Reports*, 14, 1-8 (2020)

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