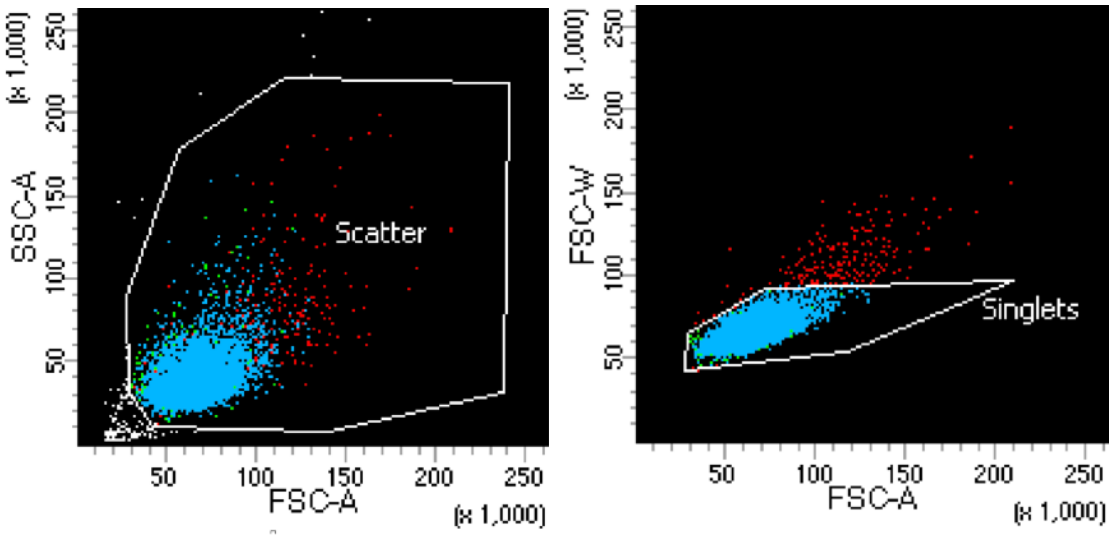
Supplementary file 1

FACS gating strategy of the screen

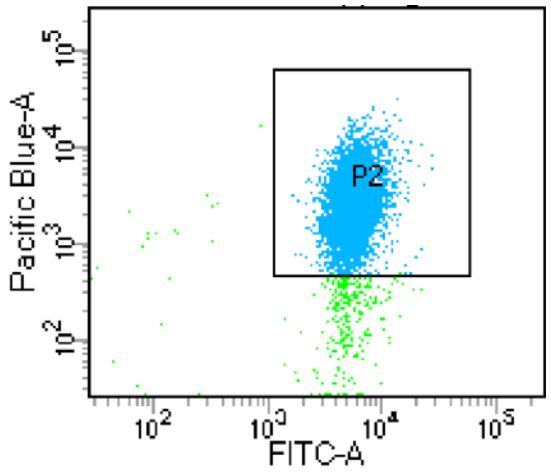
1. Cell gate and single cell gate

For all FACS experiments, forward versus side scatter (FSC-A vs. SSC-A) gating was used to identify cells of interest based on size and granularity; forward scatter width (FSC-W) vs. forward scatter area (FSC-A) density plot was used to exclude doublets as shown below.



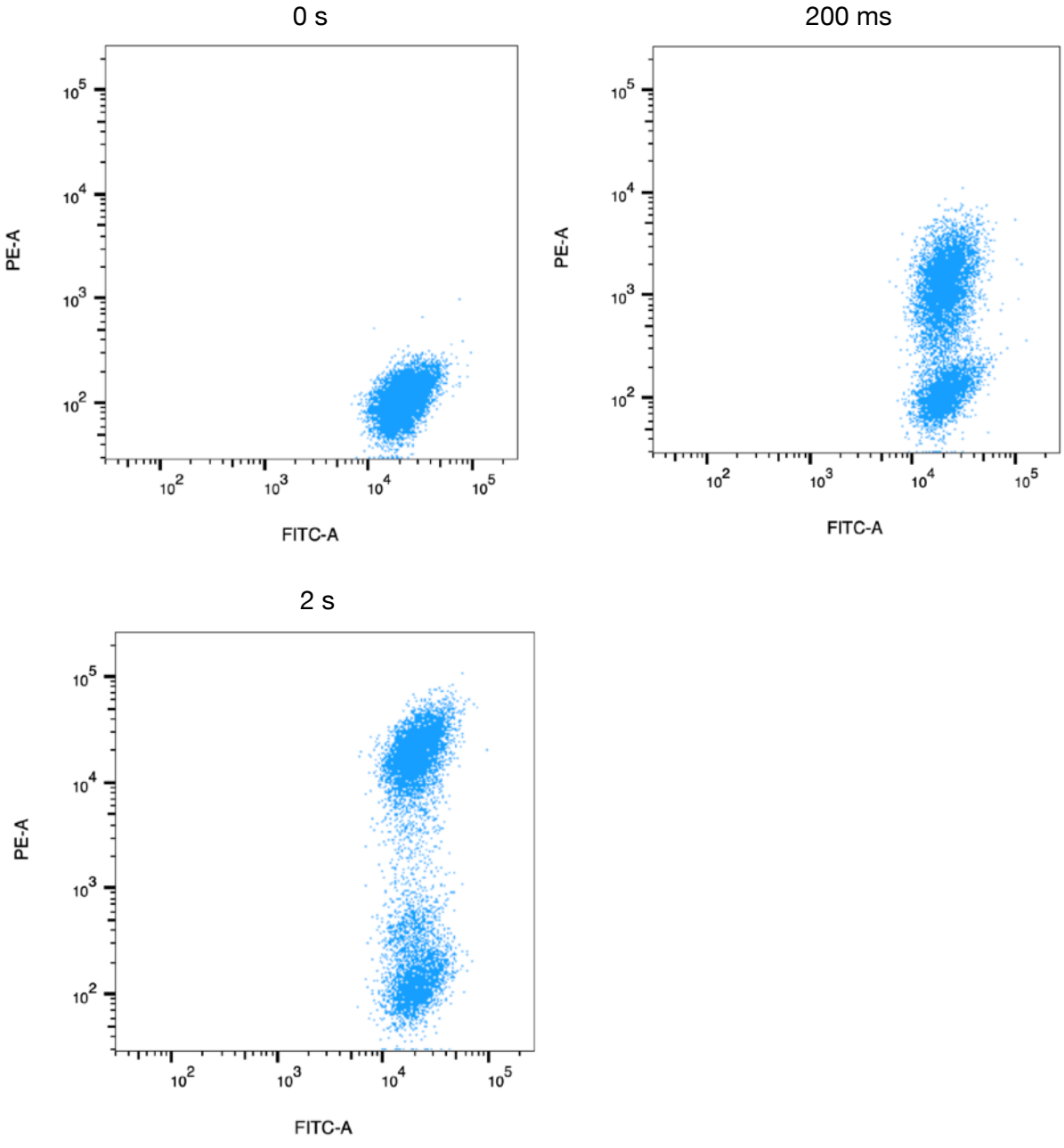
2. sgRNA gate (gate for sgRNA infected cells)

BFP (Pacific Blue-A) vs. GFP (FITC-A) density plot was used to gate H2B-mGFP cells successfully receiving sgRNAs (high BFP high GFP group). For single activation experiment (single activation mIFP proof-of-principle screen and nuclear size screen), unanalyzed samples were collected using sgRNA gate (P2 shown below).



3. mCherry gate (gate for different intensity mCherry cells)

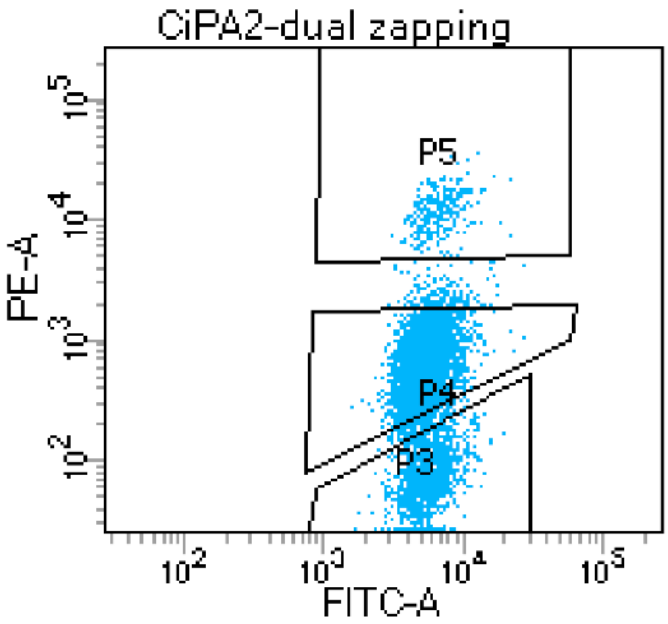
mCherry (PE-A) vs. GFP (FITC-A) density plot was used to gate for photo-activated cells. Cells activated with different photo-activation times were used to determine corresponding gates (Examples shown below). Cells in dual-activation experiments were collected with mCherry gates of corresponding photo-activation time.



For example, in dual-activation mIFP proof-of-principle screening experiments, three distinct populations were separately sorted based on FITC-A/PE-A:

P5: positively sorted sample (2 s photo-activation)

P4: negatively sorted sample (100 ms photo-activation)
P3: unanalyzed sample (0 s photo-activation)



Note: laser and filters used for different channel

Channel	Laser	Filter	Measured Fluorophore
Pacific Blue	Violet 407 nm	450/50	BFP
FITC	Blue 488 nm	530/30 - 502LP	GFP
PE	Green 561 nm	582/15	mCherry
APC-Cy5.5	Red 633 nm	710/50 - 685LP	mIFP