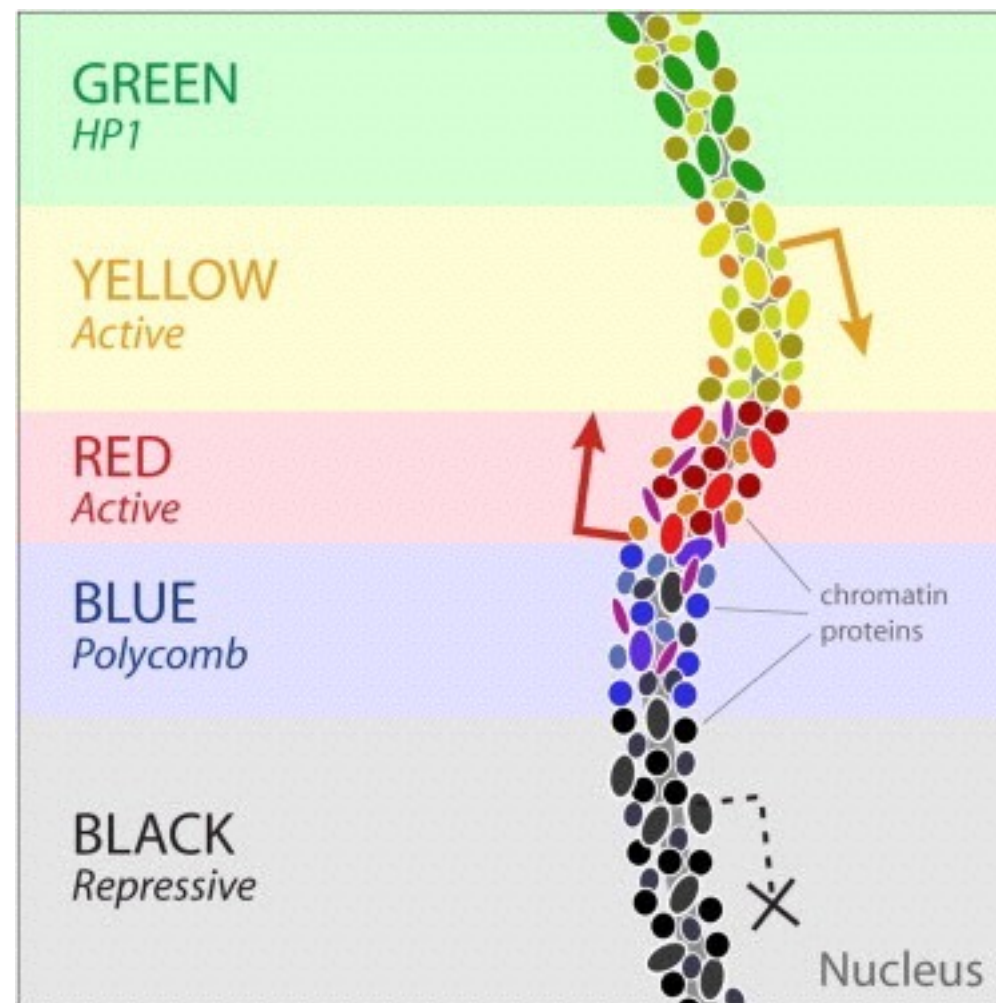


Context-dependent effects of chromatin proteins assessed in high-throughput

Laura Brueckner
B4 meeting 7.3.2016

taking chromatin proteins out of context



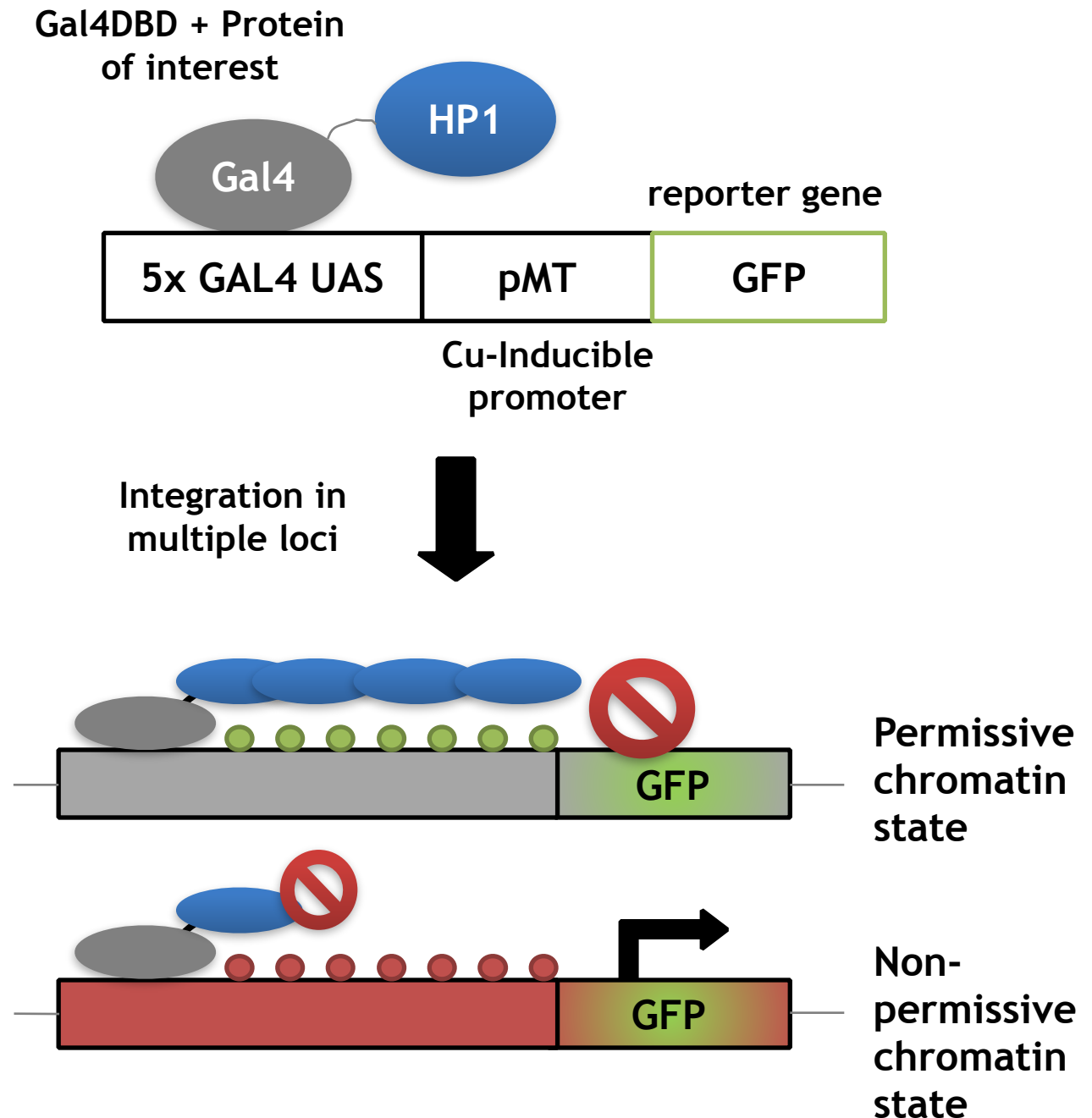
context-dependent effects of HP1

HP1a (drosophila)

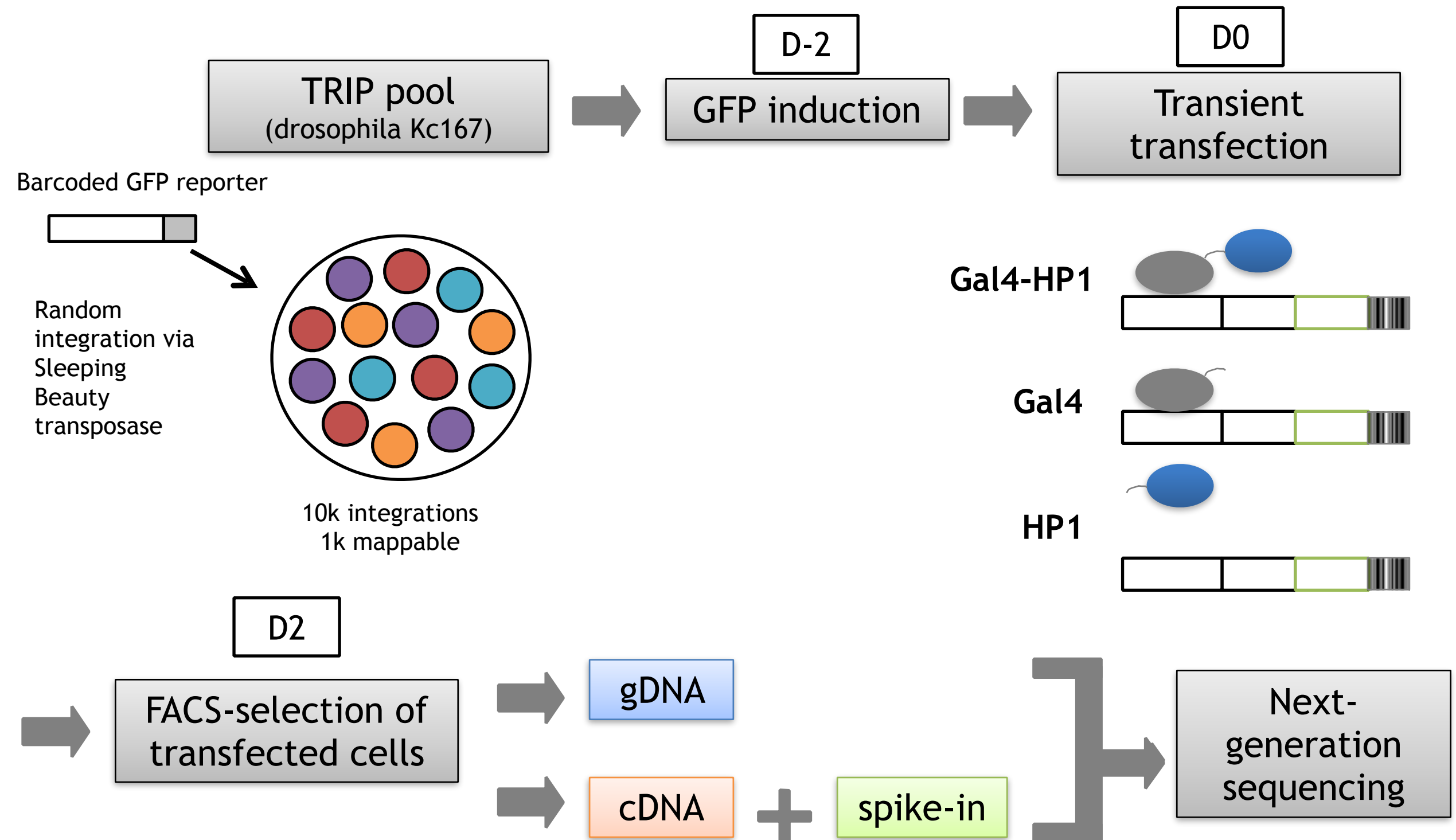
- Nucleates and spreads a heterochromatin state that results in transcriptional silencing
- Associated with euchromatic genes and activating role in their transcription

(d. melanogaster, Piacentini 2003/2009)

How does HP1a function depend on chromatin context?

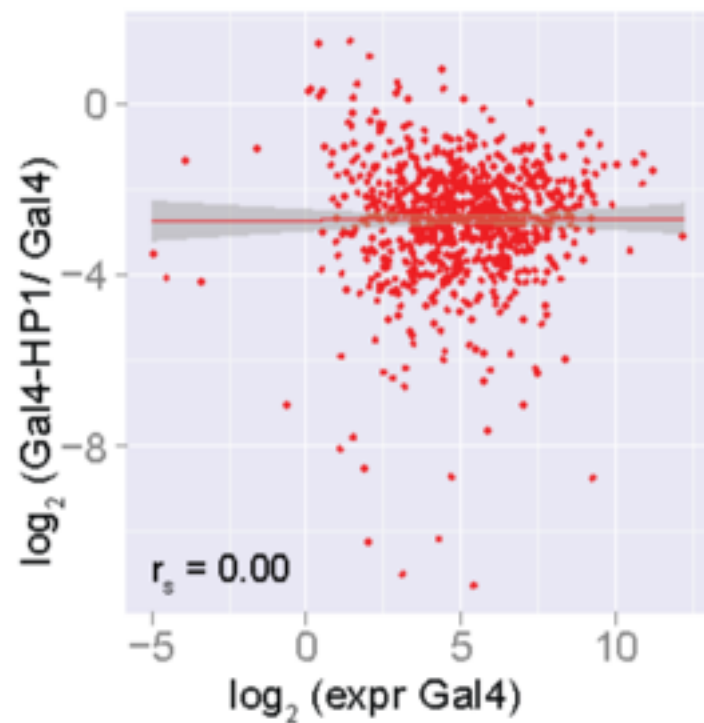


TRIP assay - Assessing chromatin protein effects in multiple genomic contexts

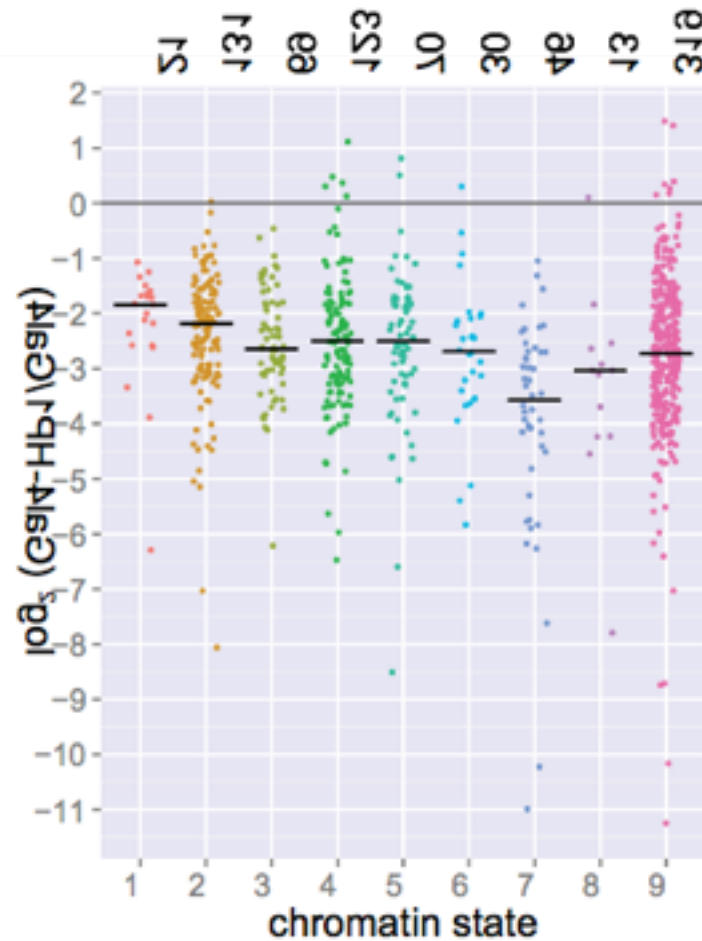


summary TRIP drosophila

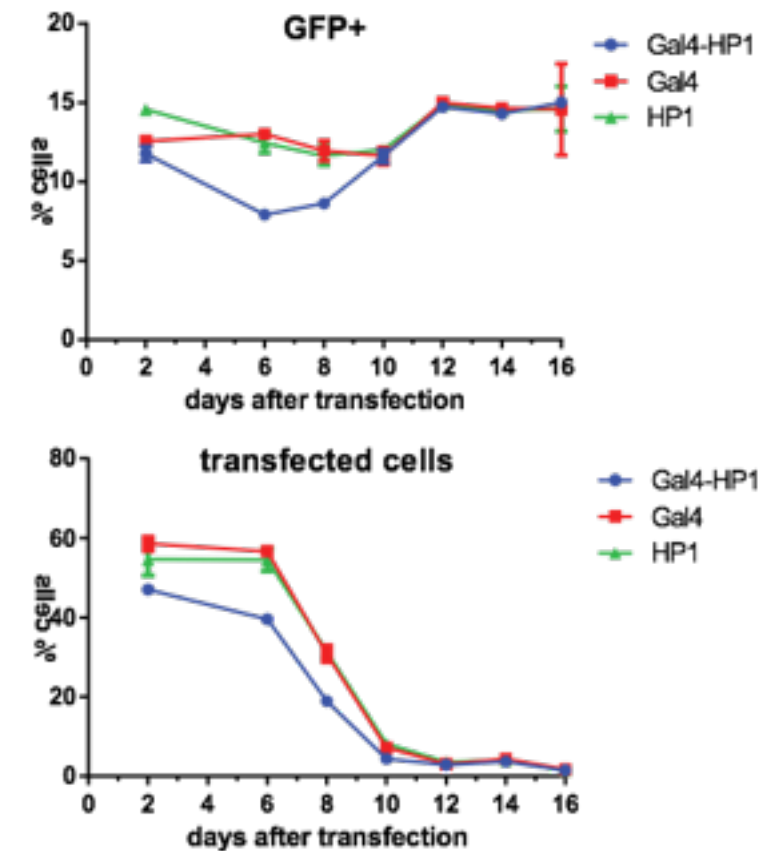
1. HP1a silences even highly expressing loci



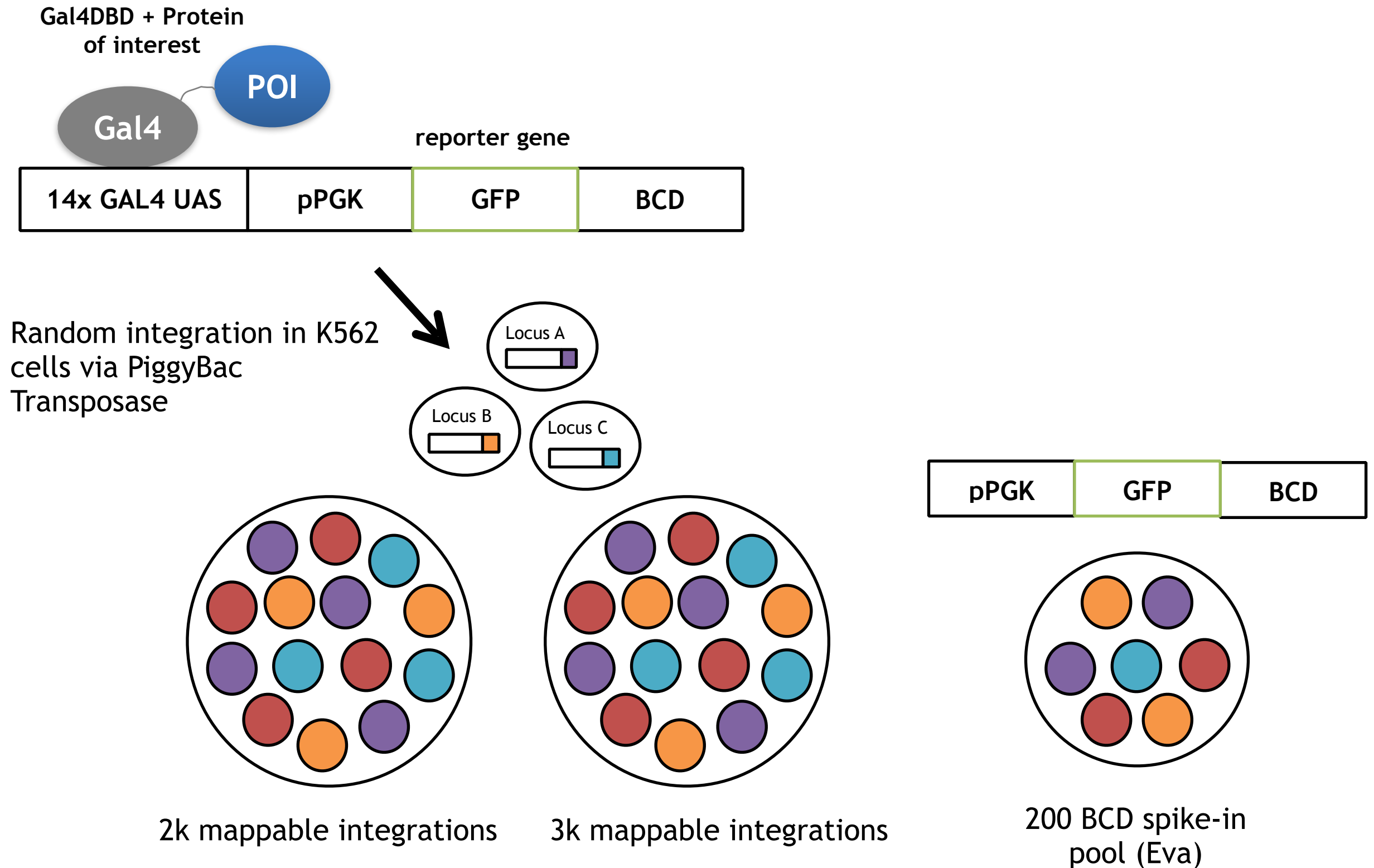
2. HP1a is influenced by chromatin state



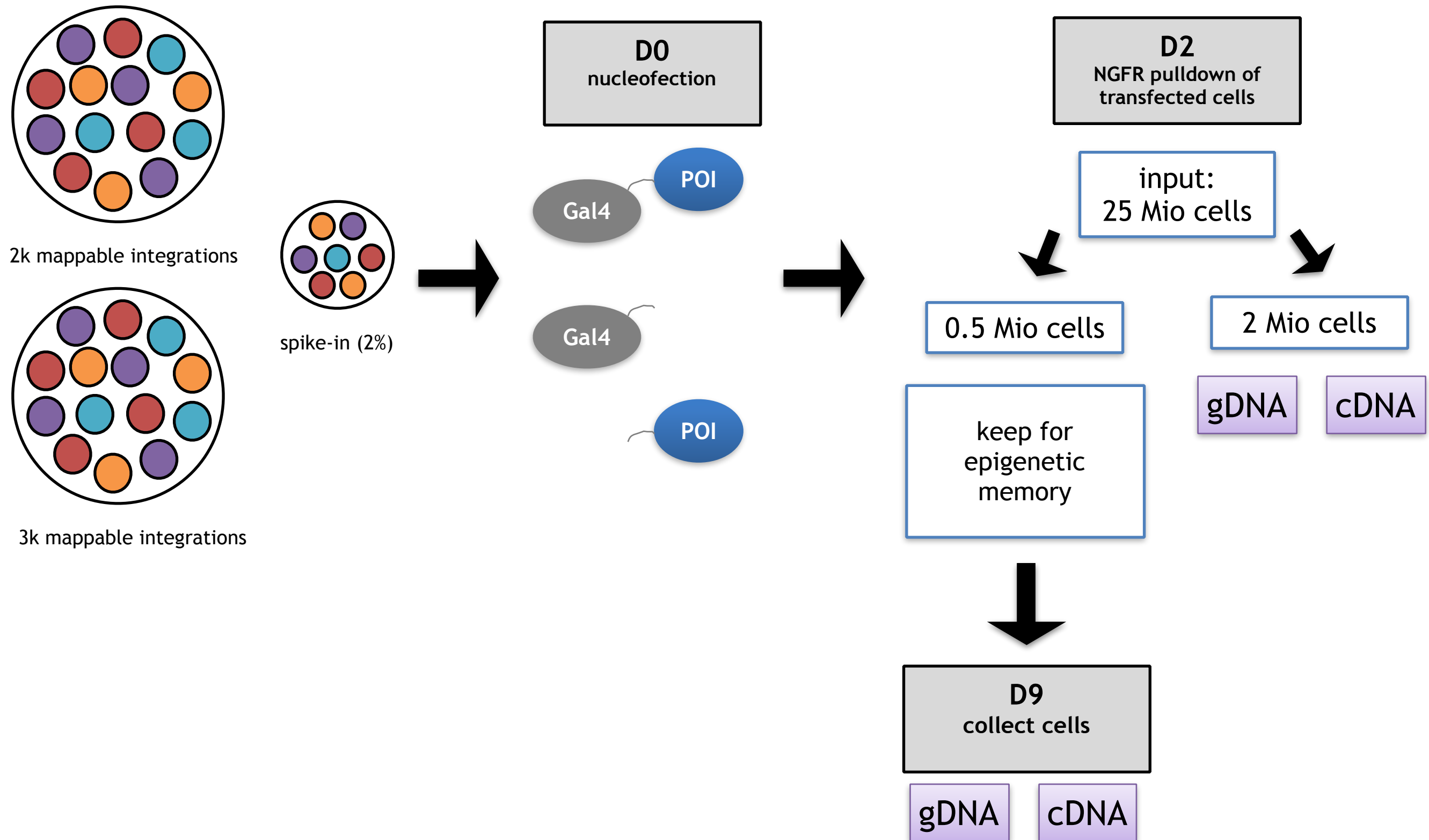
3. no stable memory of silencing



TRIP in human cells (K562)



TRIP in K562 - workflow



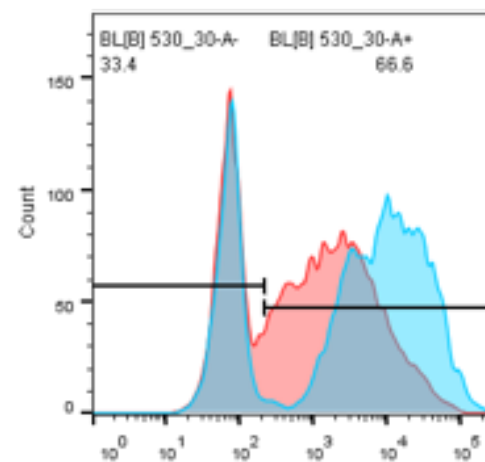
Gal4-fusion proteins for K562 TRIP

D4 after transfection

Mean GFP
POC

CBX5

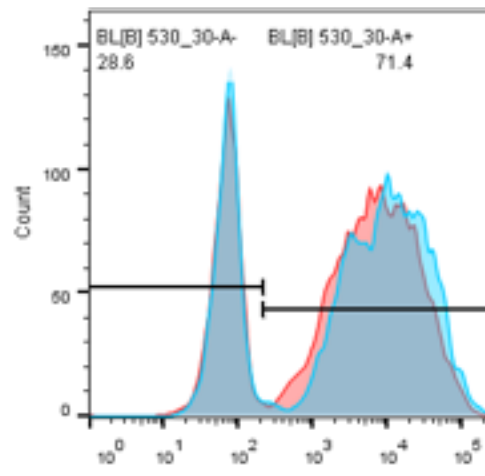
- Aka HP1 α
- Induces heterochromatin and H3K9me



30%

G9a

- H3K9 HMT
- Associated with genome-lamina interactions



65%

- Gal4DBD-POI
- Gal4DBD

Gal4-fusion proteins for K562 TRIP

CBX5

- Aka HP1 α
- Induces heterochromatin and H3K9me

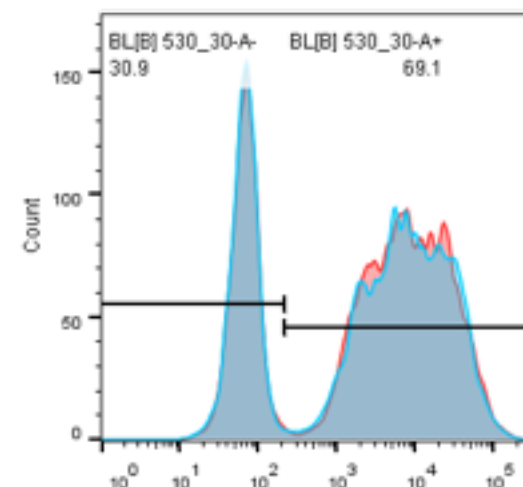
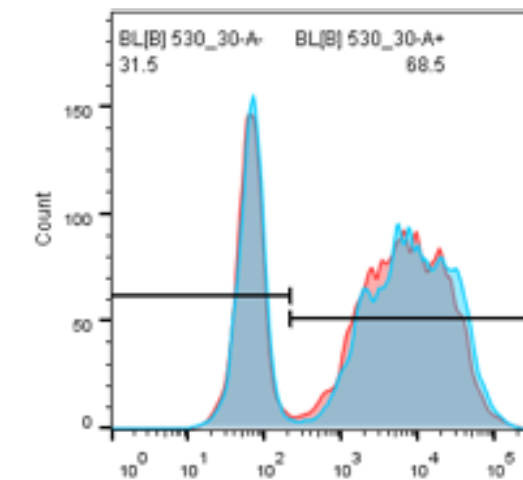
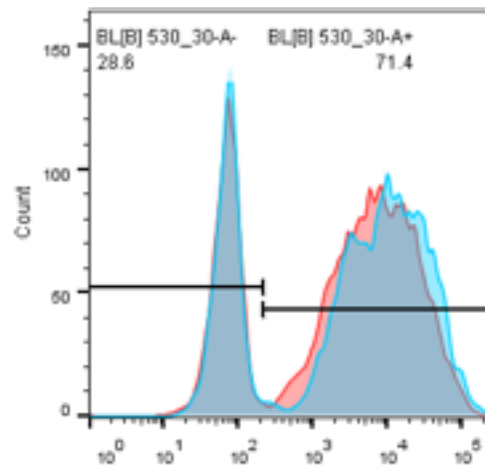
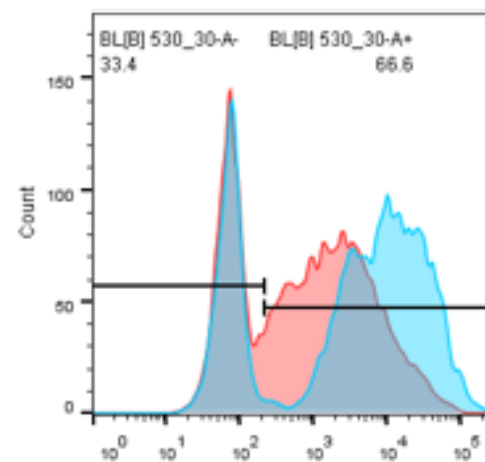
G9a

- H3K9 HMT
- Associated with genome-lamina interactions

D4 after transfection

Mean GFP
POC

D10 after transfection



30%

65%

- Gal4DBD-POI
- Gal4DBD

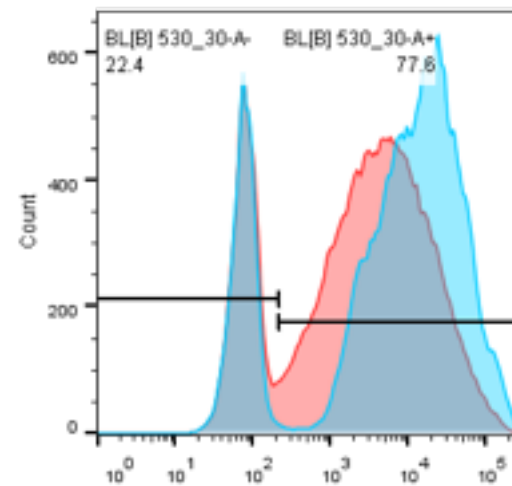
Gal4-fusion proteins for K562 TRIP

D4 after transfection

Mean GFP
POC

KRAB

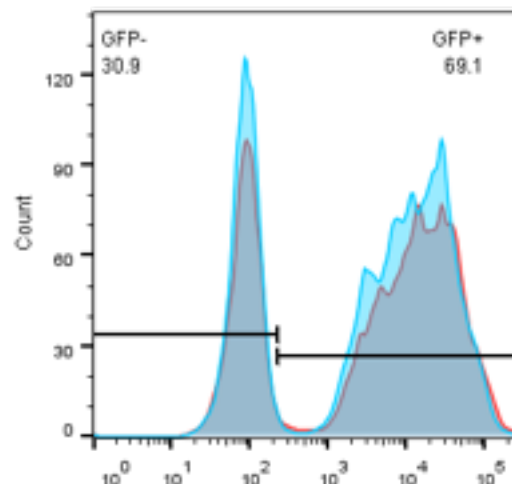
- Recruits KAP1 which targets HP1 and H3K9me3 to retrotransposons



51%

P300 (core)

- H3K27 HAT
- CRISPR-based system can induce various endogenous genes



132%

- Gal4DBD-POI
- Gal4DBD

Gal4-fusion proteins for K562 TRIP

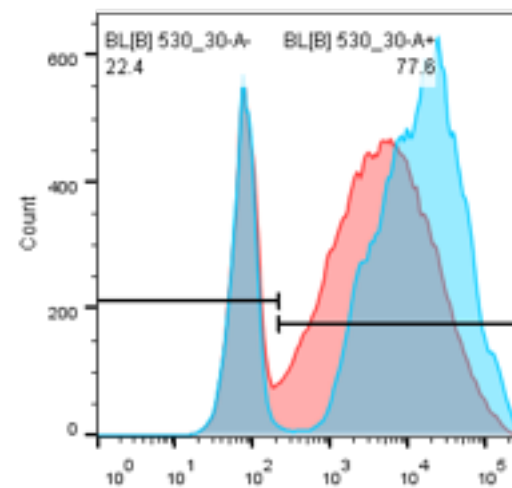
D4 after transfection

Mean GFP
POC

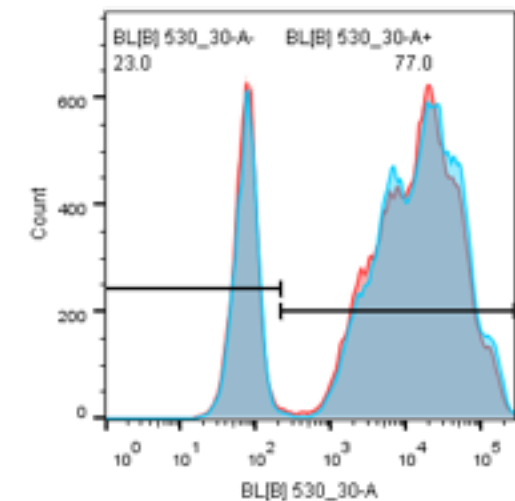
D10 after transfection

KRAB

- Recruits KAP1 which targets HP1 and H3K9me3 to retrotransposons

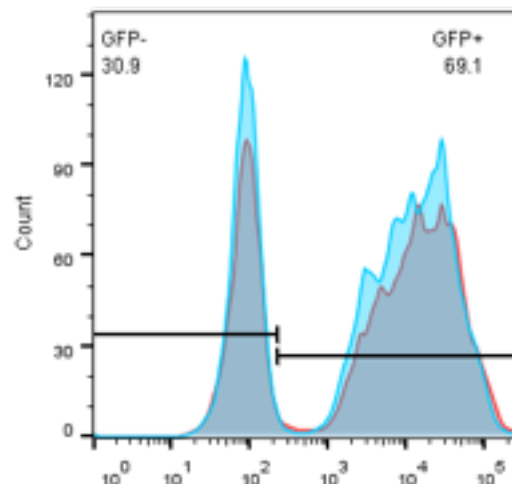


51%

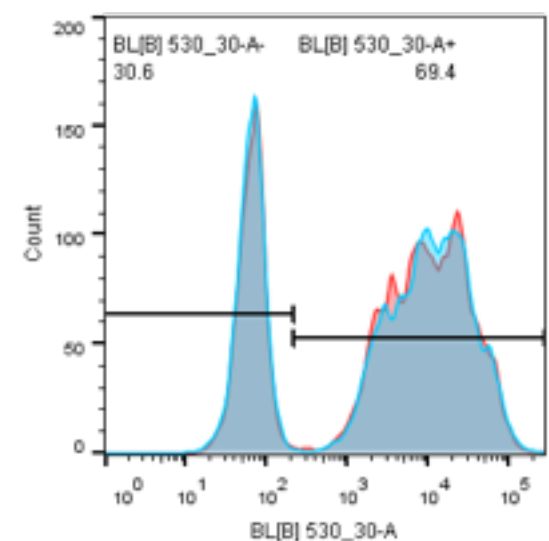


P300 (core)

- H3K27 HAT
- CRISPR-based system can induce various endogenous genes



132%

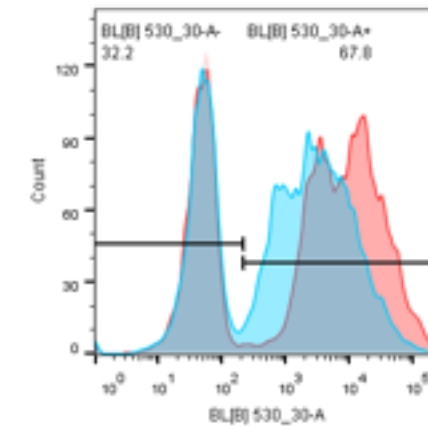


■ Gal4DBD-POI
■ Gal4DBD

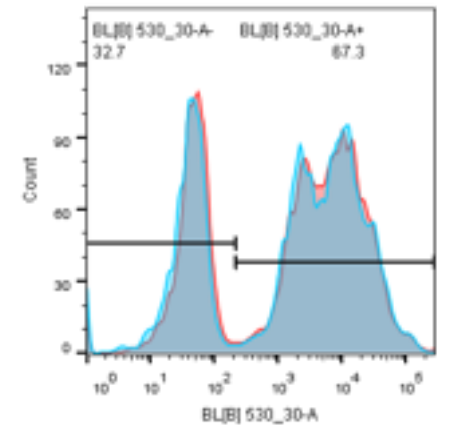
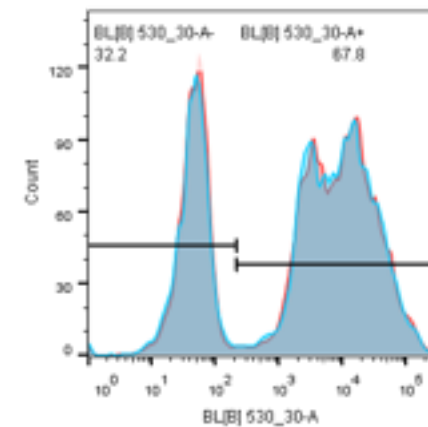
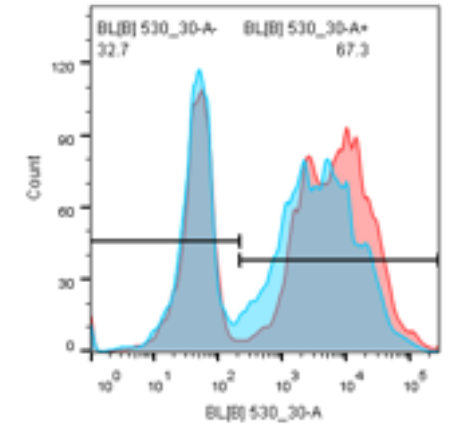
TRIP CBX5

CBX5 r3	Live cells	NGFR+	yield % of max
Gal4-CBX5	70,6	48,1	16
Gal4	80	67,8	27
CBX5	83,1	64,3	34
CBX5 r2	Live cells	NGFR+	
Gal4-CBX5	89,5	42,5	12
Gal4	91,2	70	13
CBX5	89,1	78,3	21

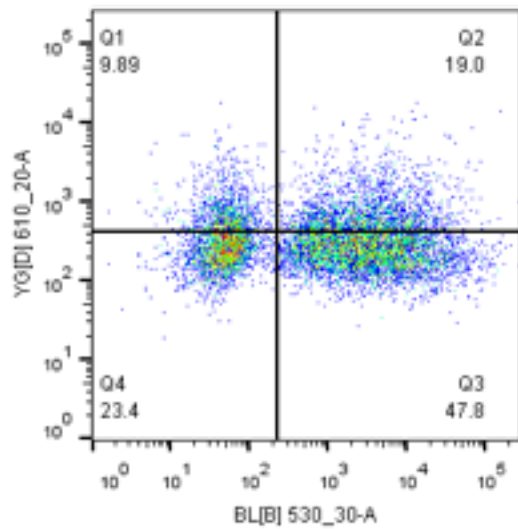
D2



D9

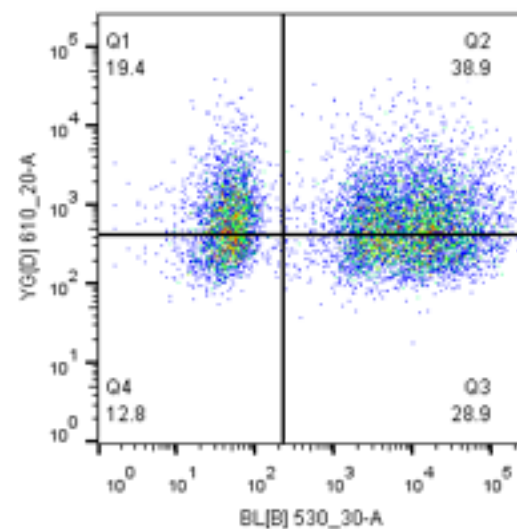


Gal4-CBX5



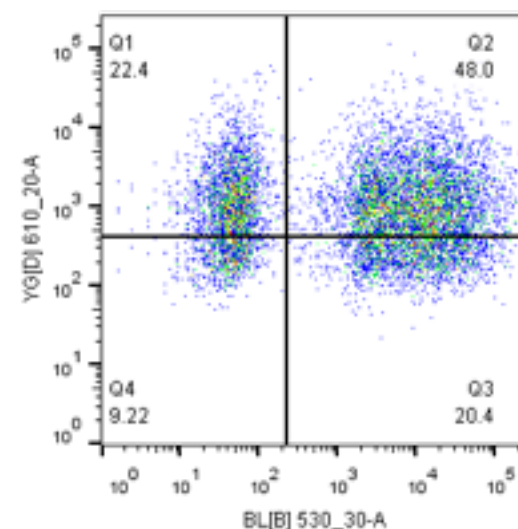
477-20160129 - K562_48.fcs
Single Cells
9620

Gal4



477-20160129 - K562_50.fcs
Single Cells
9652

CBX5



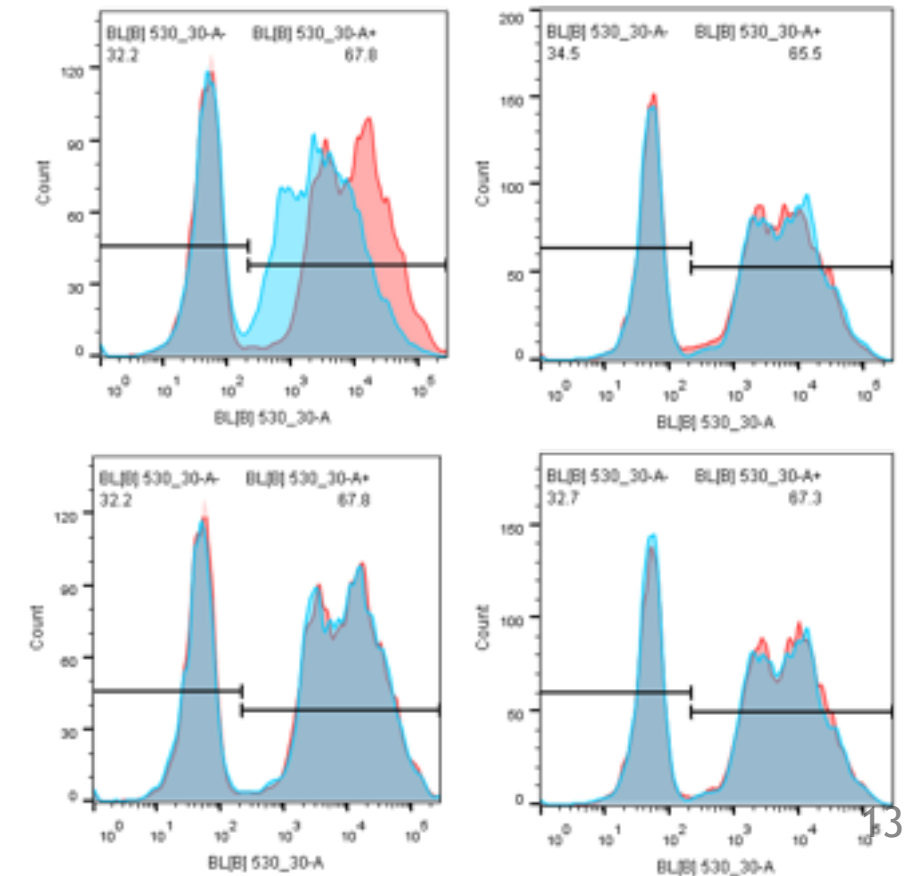
477-20160129 - K562_51.fcs
Single Cells
9614

TRIP CBX5

CBX5 r3	Live cells	NGFR+	yield % of max
Gal4-CBX5	70,6	48,1	16
Gal4	80	67,8	27
CBX5	83,1	64,3	34
CBX5 r2	Live cells	NGFR+	
Gal4-CBX5	89,5	42,5	12
Gal4	91,2	70	13
CBX5	89,1	78,3	21

D2

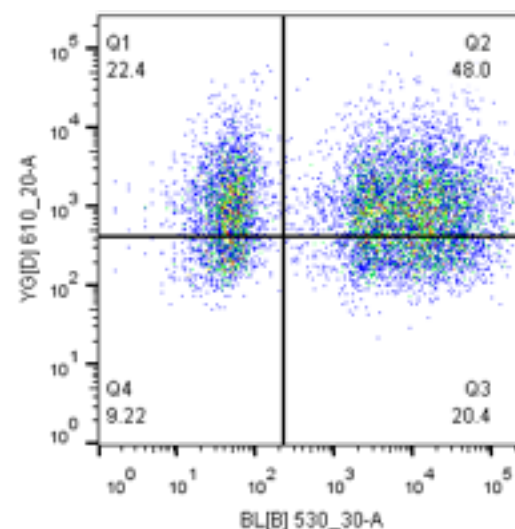
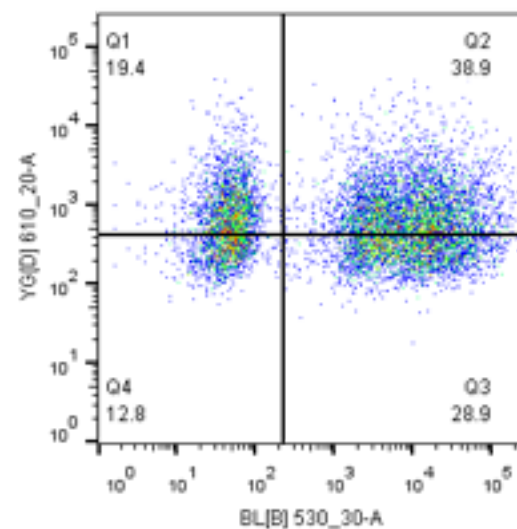
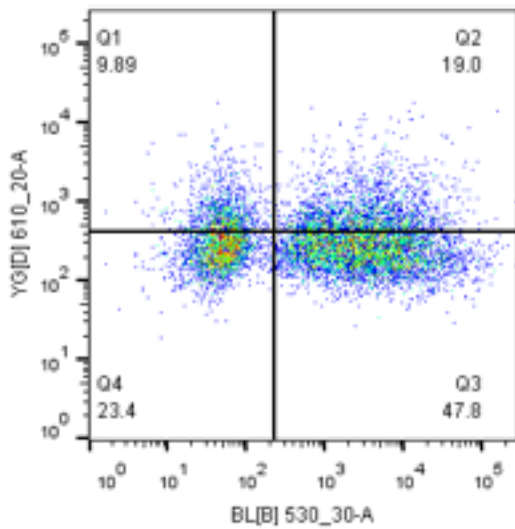
D12



Gal4-CBX5

Gal4

CBX5



477-20160129 - K562_48.fcs
Single Cells
9620

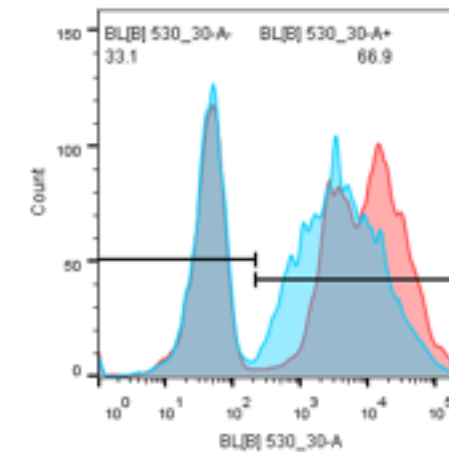
477-20160129 - K562_50.fcs
Single Cells
9652

477-20160129 - K562_51.fcs
Single Cells
9614

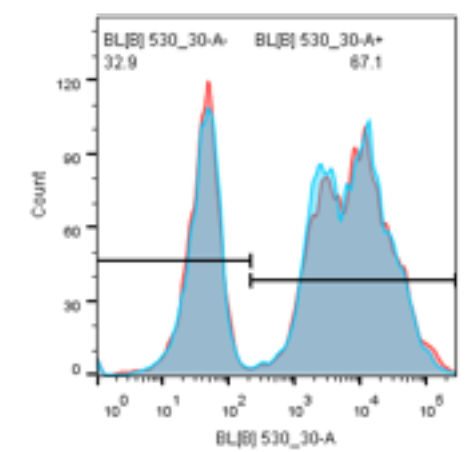
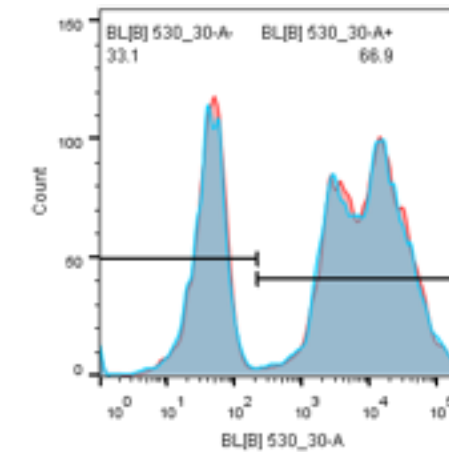
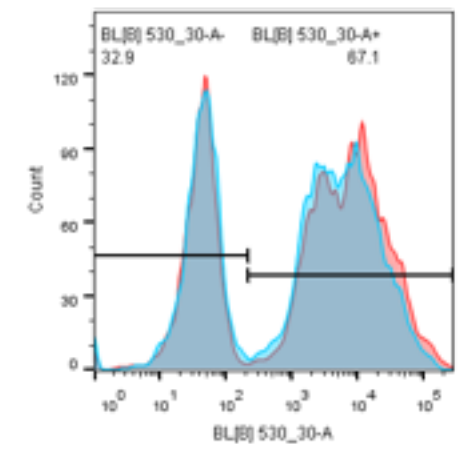
TRIP KRAB

KRAB r1	Live cells	NGFR+	yield % of max
Gal4-KRAB	85,4	42,7	12,3
Gal4	88,5	48,6	9,0
KRAB	86	74,2	18,4
KRAB r2	Live cells	NGFR+	
Gal4-KRAB	86,1	44,5	20
Gal4	90,2	46,7	10
KRAB	87,7	50,2	15

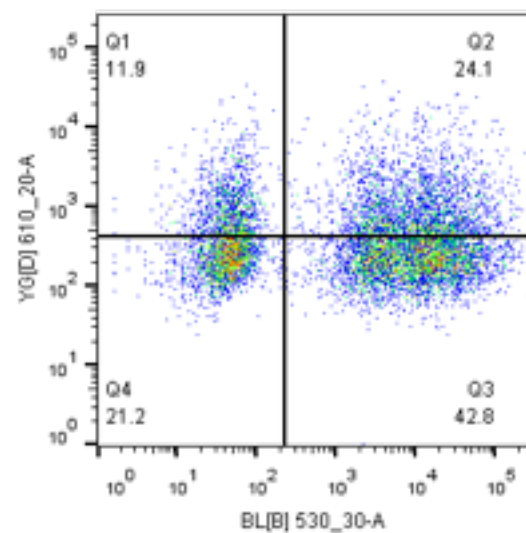
D2



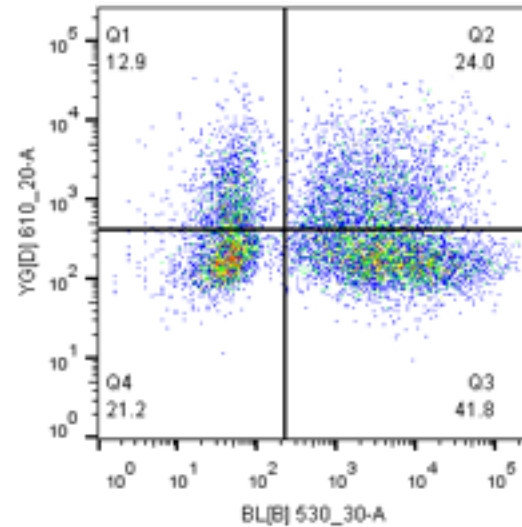
D11



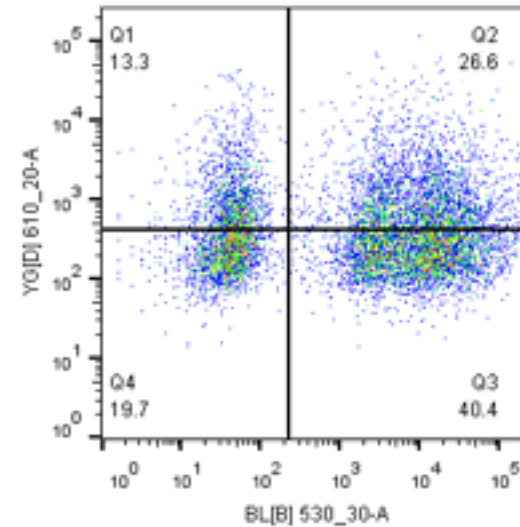
Gal4



Gal4-KRAB



KRAB



477-03022016- K562_50.fcs
Single Cells
9604

477-03022016- K562_74.fcs
Single Cells
9793

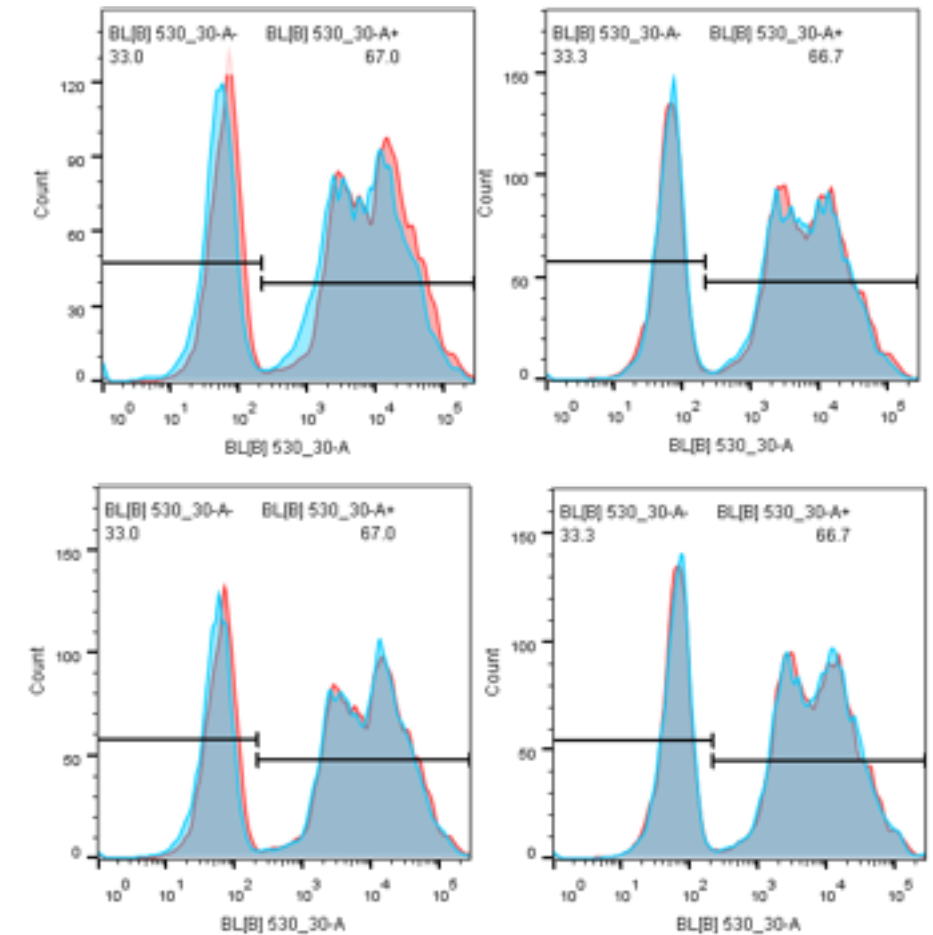
477-03022016- K562_76.fcs
Single Cells
9550

TRIP G9a

G9a r1	Live cells	NGFR+	yield % of max
Gal4-G9a	78,5	27,9	22,4
Gal4	84,5	47,7	16,1
G9a	82,1	40,1	23,0
G9a r2	Live cells	NGFR+	
Gal4-G9a	62,5	34,6	23,7
Gal4	75,5	74,8	13,4
G9a	68,4	76,5	20,4

D2

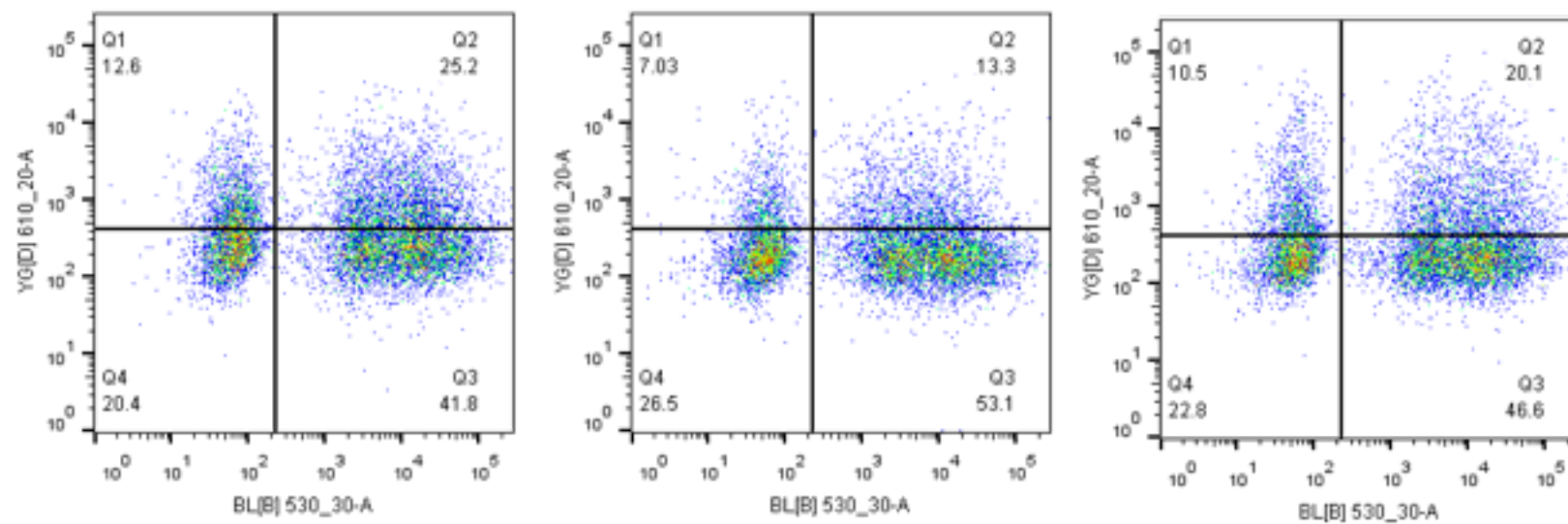
D8



Gal4

Gal4-G9a

G9a



477-20160130 - K562_50.fcs
Single Cells
9486

477-20160130 - K562_53.fcs
Single Cells
9475

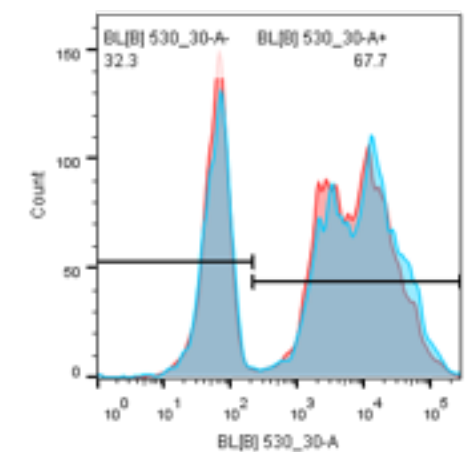
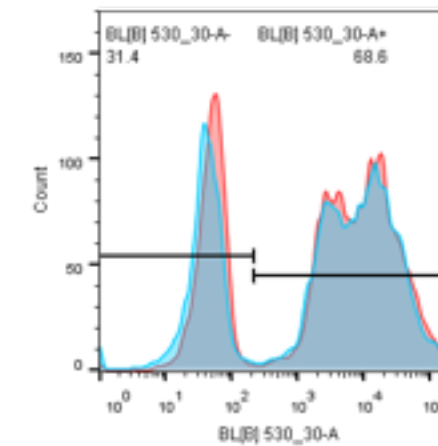
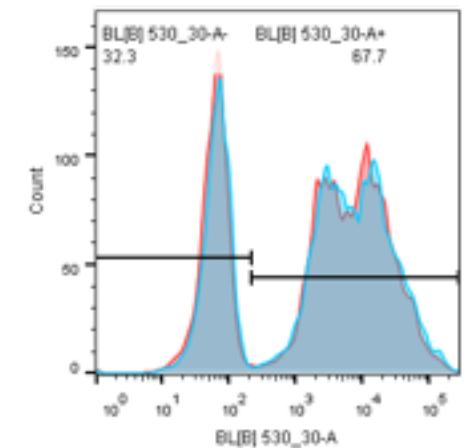
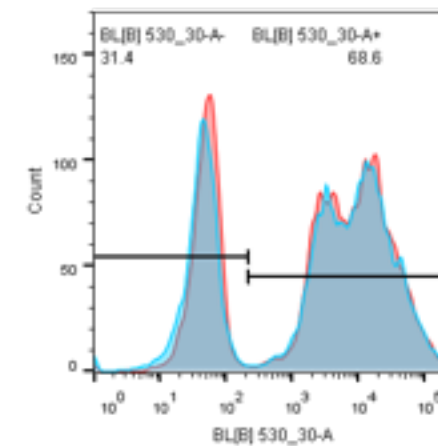
477-20160130 - K562_56.fcs
Single Cells
9510

TRIP p300

p300 r1	Live cells	NGFR+	yield % of max
Gal4-p300	69,6	22	37,2
Gal4	85,2	64,4	18,3
p300	56,9	48	36,6
p300 r2	Live cells	NGFR+	
Gal4-p300	75,9	16,3	36,1
Gal4	90,9	46,9	9,9
p300	70,2	30,8	35,1

D2

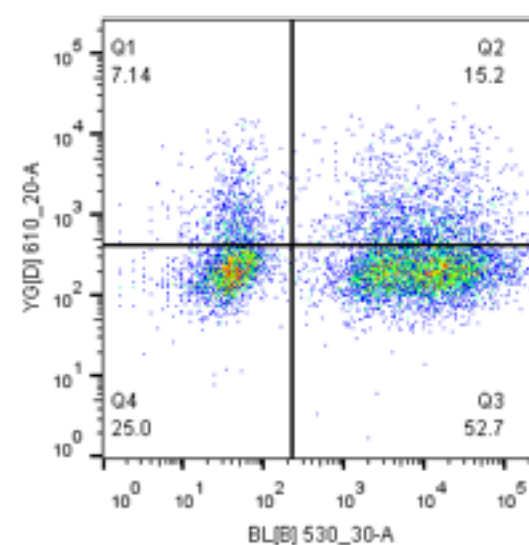
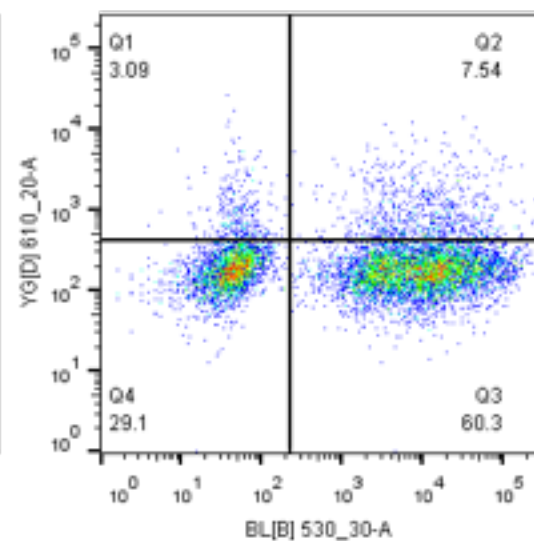
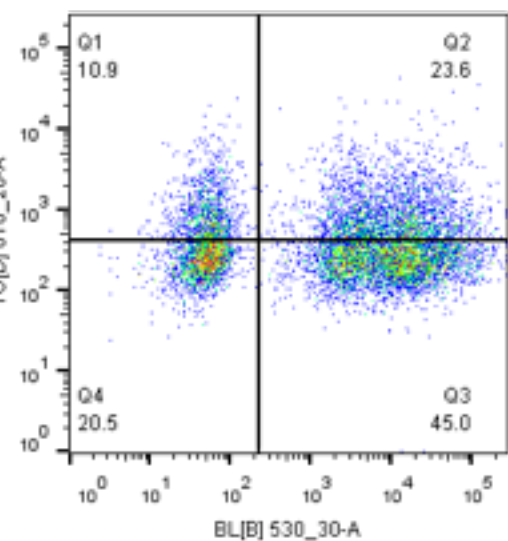
D11



Gal4

Gal4-p300

p300



Summary and future steps

- Good results for K562 TRIP experiments so far, especially CBX5 and KRAB
- Problems with G9a, p300 - other candidate proteins?
- Effects of nuclear organization on TRIP expression: targeting endogenous G9a (compound inhibition, shRNAs)

Thankyous

Van Steensel lab

- Bas van Steensel
- Joris van Arensbergen
- Eva Brinkman
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- Tao Chen
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- Christ Leemans

Van Lohuizen lab

- Waseem Akhtar

Flow Cytometry facility

Genomics Core Facility

NETHERLANDS
CANCER
INSTITUTE



ANTONI VAN LEEUWENHOEK