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Pardons Marion, HIV Cure Research Center (Belgium)
Session 3: Cure advances globally

HIV reactivation from latency using a Tat compound

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AIDS 2022

Affiliated Independent Event

Conflict of interest disclosure

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Shock-and-kill strategy

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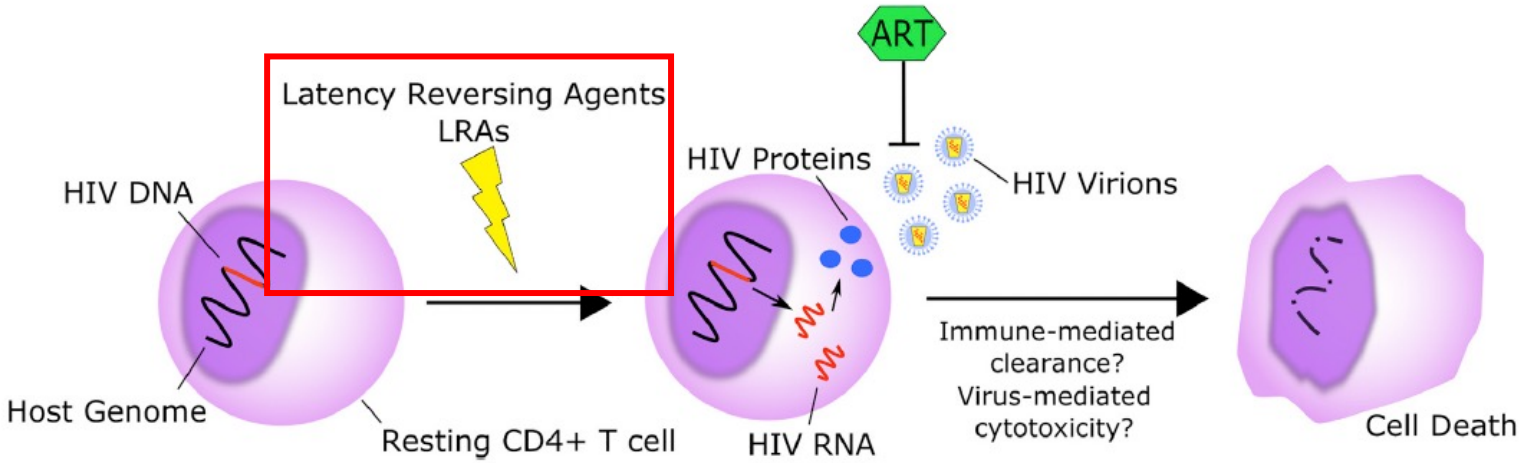

Mitogens	Other classes of LRAs
PMA, PHA, CD3/CD28	HDACi, PKC agonists, etc
Gold standard for *in vitro* assays	Not as potent as mitogens to reactivate HIV
Highly toxic → Not in the clinic	Safe to be used *in vivo*
Induces global T cell activation	Some classes do not induce global activation

Identifying compounds that reactivate HIV efficiently **without modifying the transcriptome/phenotype of the cells** is of interest to study the profile of latently infected cells

Clinical characteristics of the participants

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Patient ID	Age	Gender	CD4/CD8 ratio	NADIR	Subtype	VL	Time since infection	Time to ART	ART duration
MRC01	55	M	0.3	44	B	<20	NA	NA	18.5
MRC02	62	M	1.2	484	B	<20	NA	NA	4.4
MRC03	33	M	0.7	114	F1	<20	30.7	16.0	14.6
MRC04	51	M	1.0	171	B	<20	NA	NA	18.4
MRC05	42	M	1.3	492	B	<20	13.0	6.9	6.1
MRC06	50	F	0.9	226	C	<20	NA	NA	3.7
MRC07	37	M	1.1	382	B	<20	7.5	2.4	5.1
MRC08	50	M	0.5	488	B	<20	16.5	15.2	1.3
MRC09	53	F	0.8	102	A1	<20	NA	NA	17.1
MRC11	40	M	1.3	350	B	<20	8.4	1.1	7.3
MRC12	31	M	1.0	NA	B	<20	11.7	4.2	7.5
MRC13	26	M	1.3	601	Recomb B/F1	<20	NA	NA	7.2
MRC14	61	M	0.9	211	CRF02_AG	<20	11.0	5.1	5.9
MRC15	56	M	0.6	98	B	<20	NA	NA	14.7
MRC19	32	M	0.8	395	B	<20	NA	NA	1.4
MRC20	49	M	0.6	294	B	<20	19.8	3.4	16.4
MRC21	61	M	0.9	179	B	<20	30.6	5.8	24.8
MRC22	62	M	0.8	196	B	<20	11.6	3.0	8.7
MRC23	58	M	0.8	182	B	<20	17.0	0.7	16.3
MRC24	54	M	0.9	231	B	<20	15.6	2.8	12.8
MRC25	48	M	0.7	361	B	<20	14.1	2.6	11.5
UZG3034	39	M	0.7	356	B	<20	3.0	0.5	2.4
STAR10	55	M	0.7	327	B	<20	21.3	3.0	18.3

Viability of the cells

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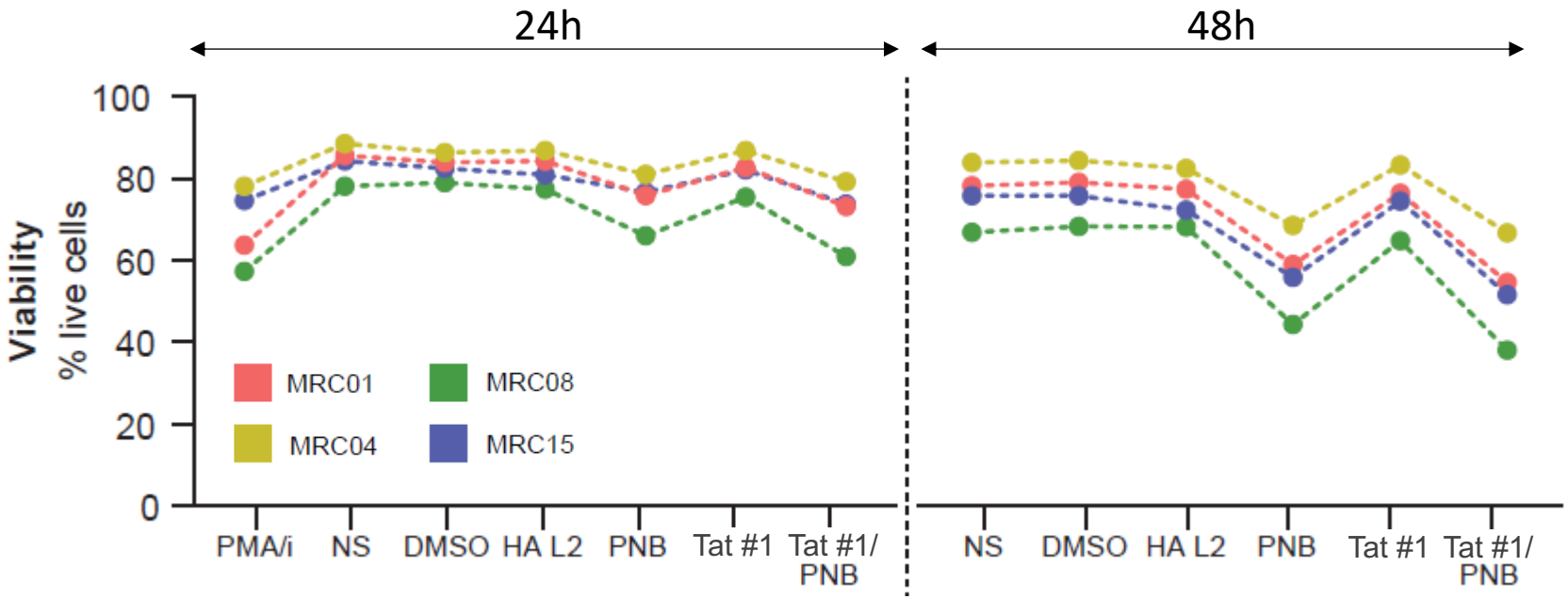

PNB = Panobinostat (HDAC inhibitor)

- Viability of CD4 T cells is not affected by Tat #1 treatment (both at 24h and 48h)
- Cell viability is impaired following PNB treatment at 48h

Frequency of p24+ cells following latency reversal

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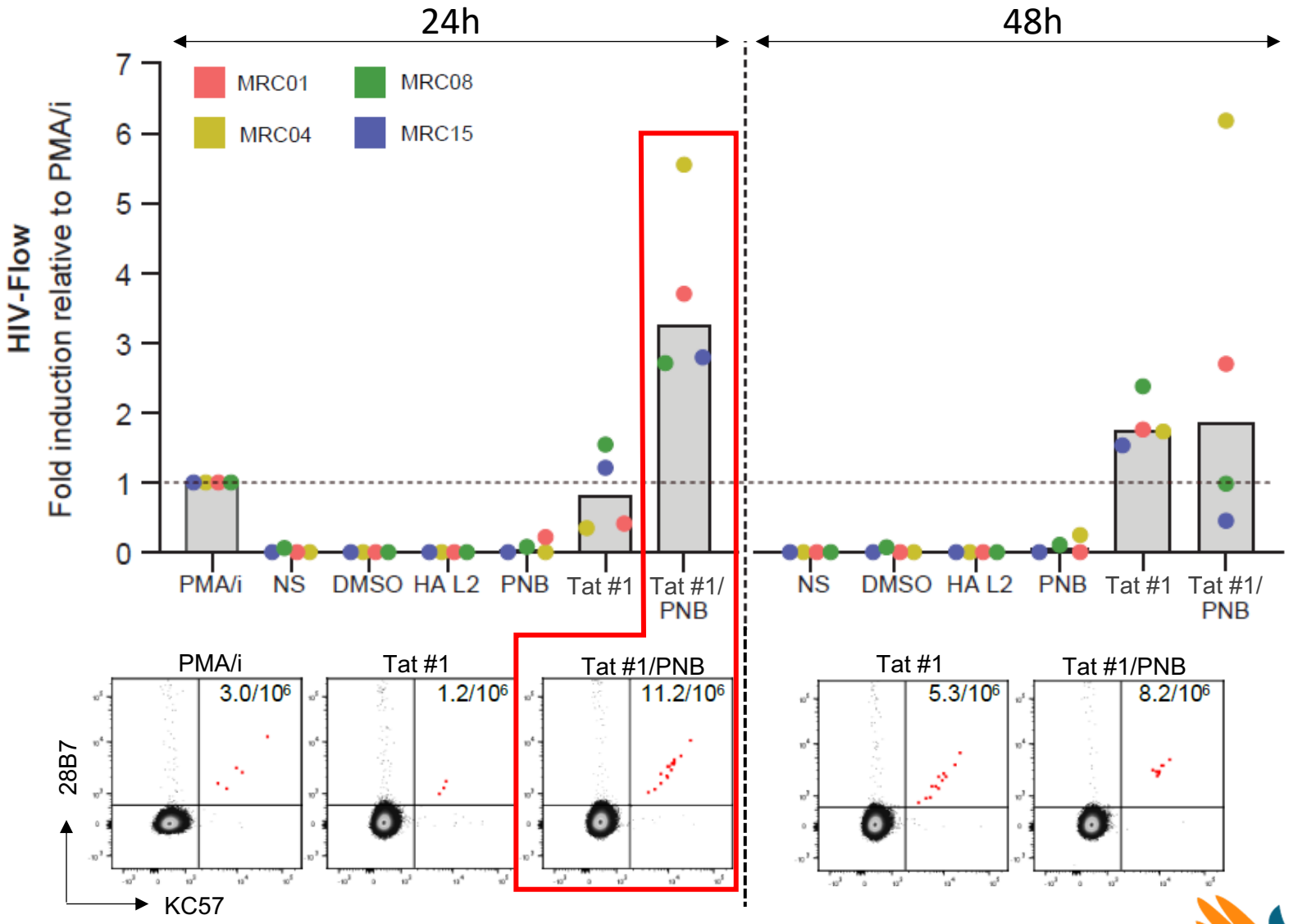

PNB = Panobinostat (HDAC inhibitor)

- The highest fold induction relative to PMA/i is observed at 24h post-stim with the combination Tat #1/PNB

P24 release in the supernatant following latency reversal

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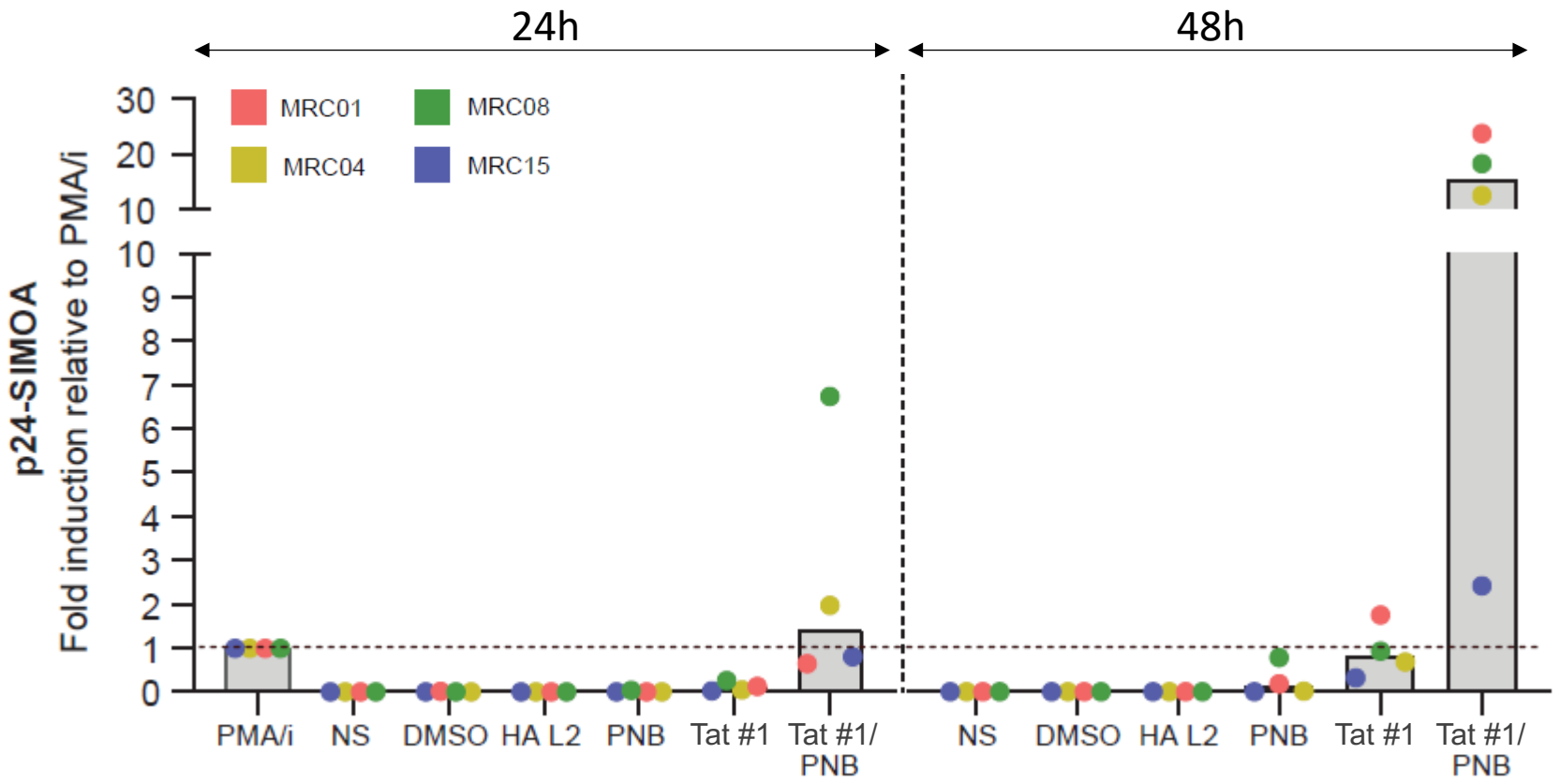

- Stimulation with Tat #1 leads to viral particles release in the culture supernatant

Frequencies of p24+ cells following stimulation with PMA/i vs Tat #1/PNB

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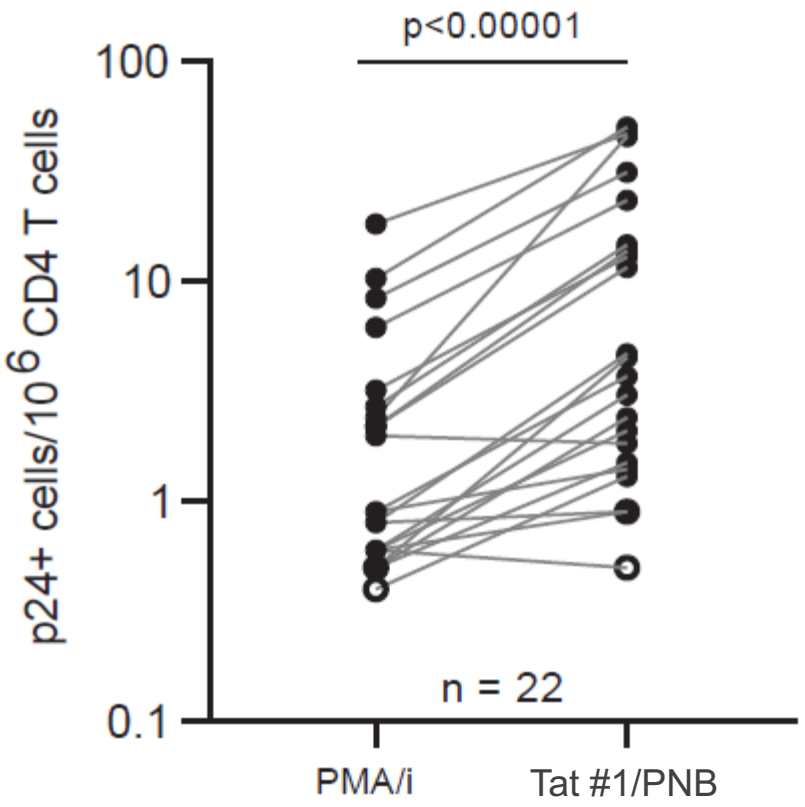
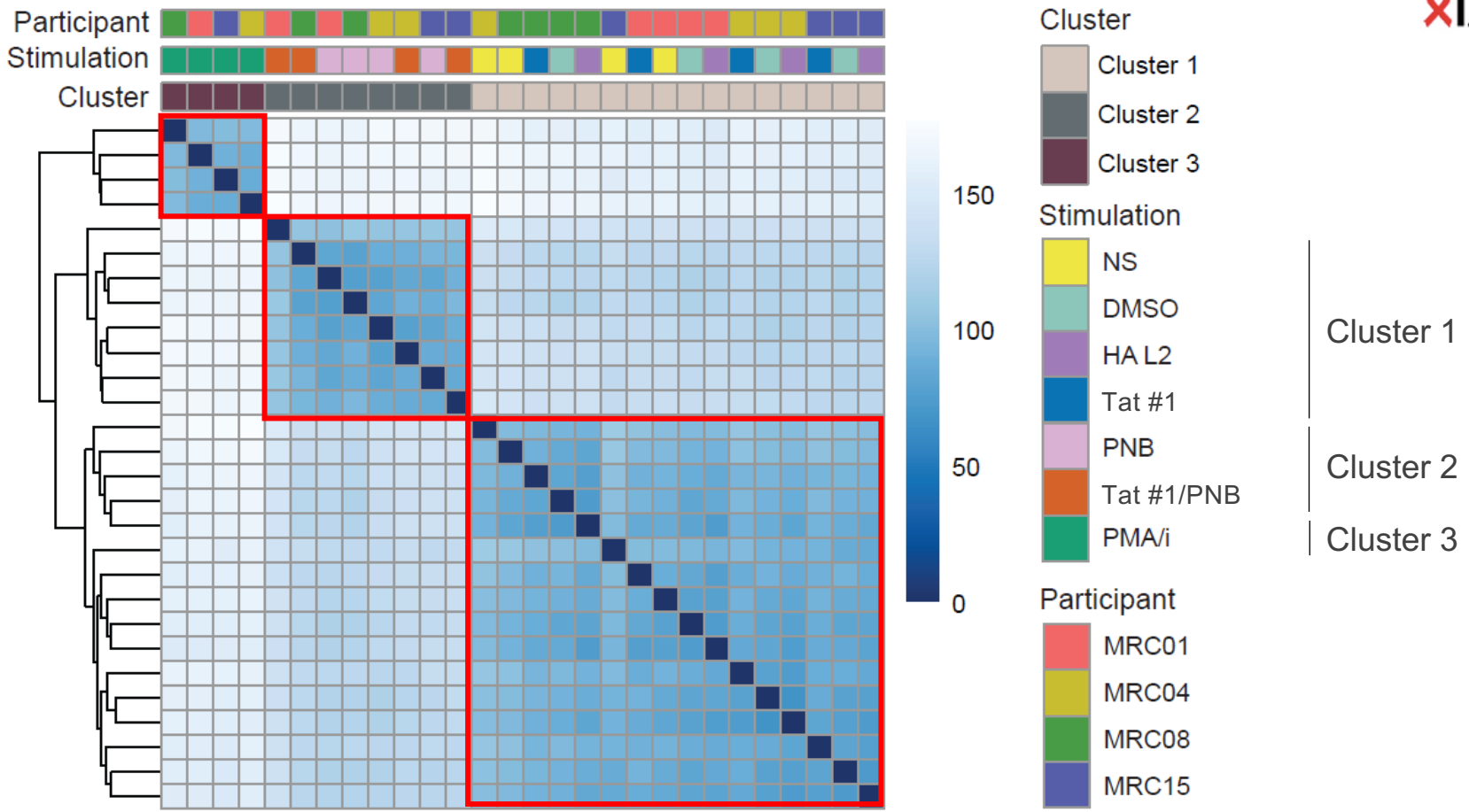


- Significantly higher frequencies of p24+ cells are observed following Tat #1/PNB stimulation when compared to PMA/i (median fold increase = 3.9)

Microarray analyses on bulk CD4 T cells



- Tat #1 has a minimal impact on the transcriptomic profile of the cells (clusters with the negative controls)

Phenotype of p24+ cells following Tat #1/PNB treatment

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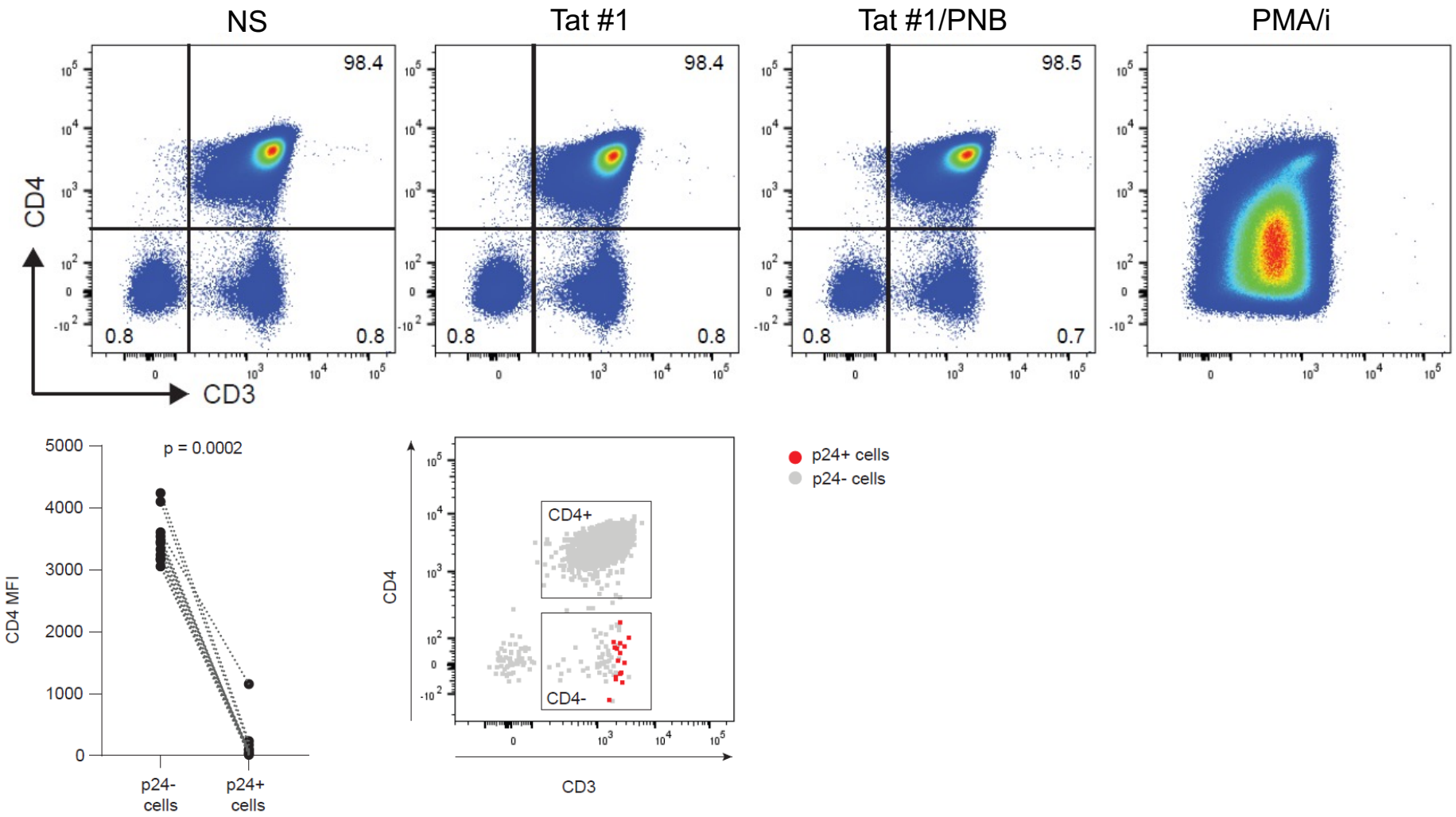
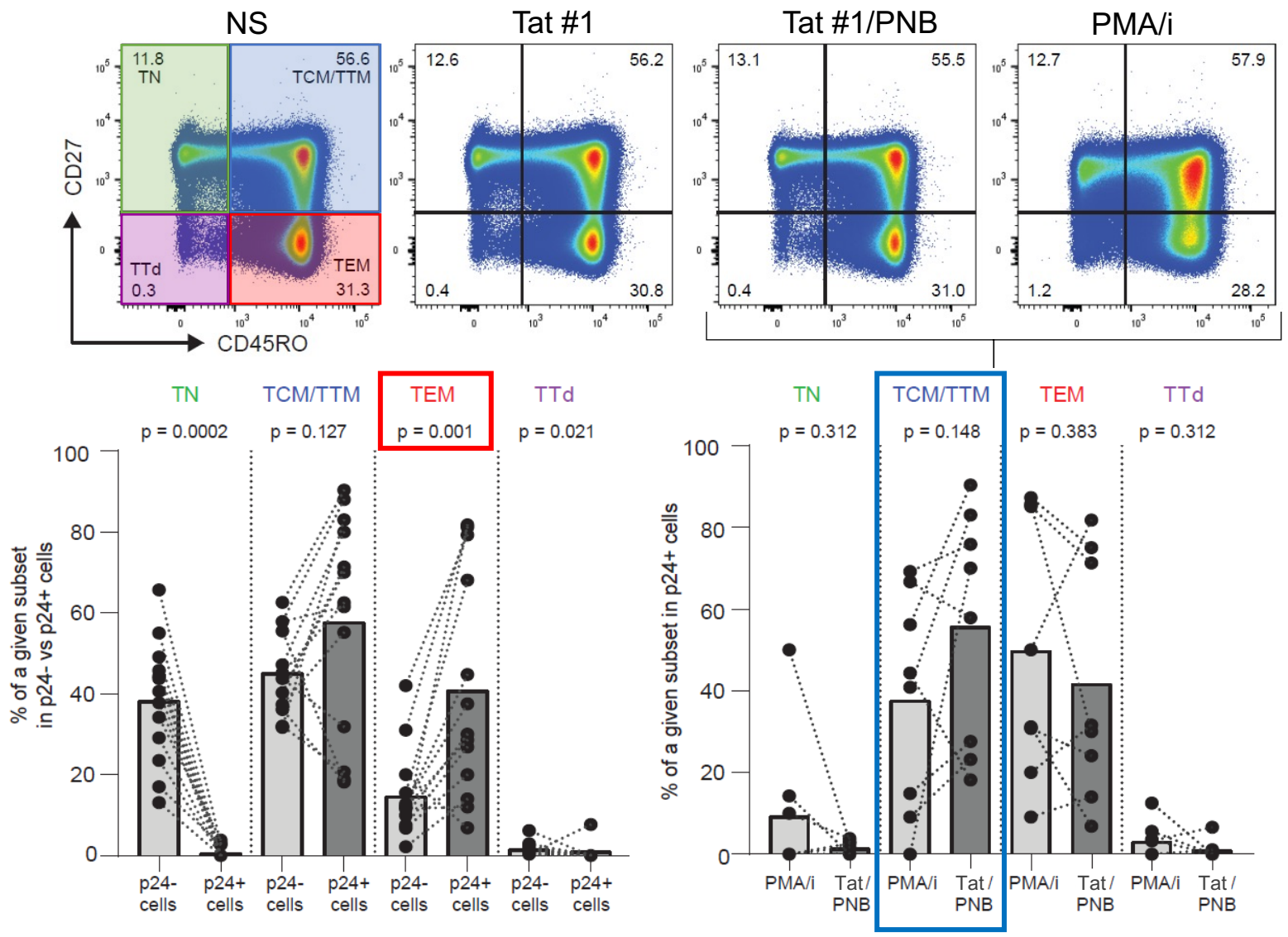


- CD4 expression is downregulated in p24+ cells

Phenotype of p24+ cells following Tat #1/PNB treatment



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Studying the genetic and transcriptional environment of proviruses in p24+ cells

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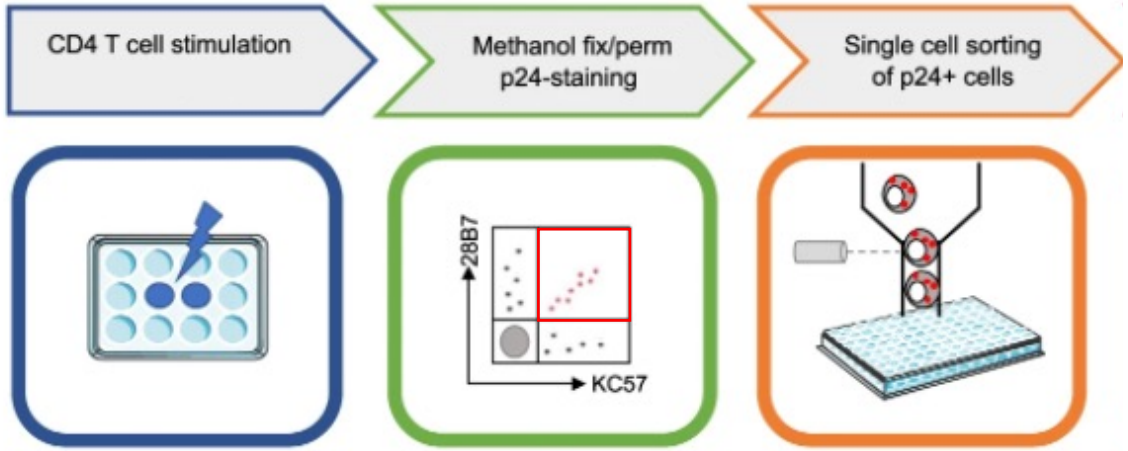
STIP-Seq
Cole et al, Nat Com, 2021



scRNA-seq
(Smart-seq2)

Talk on the 2nd of August
Shake and bake session

Analysis of near-full length sequences in p24+ cells following latency reversal

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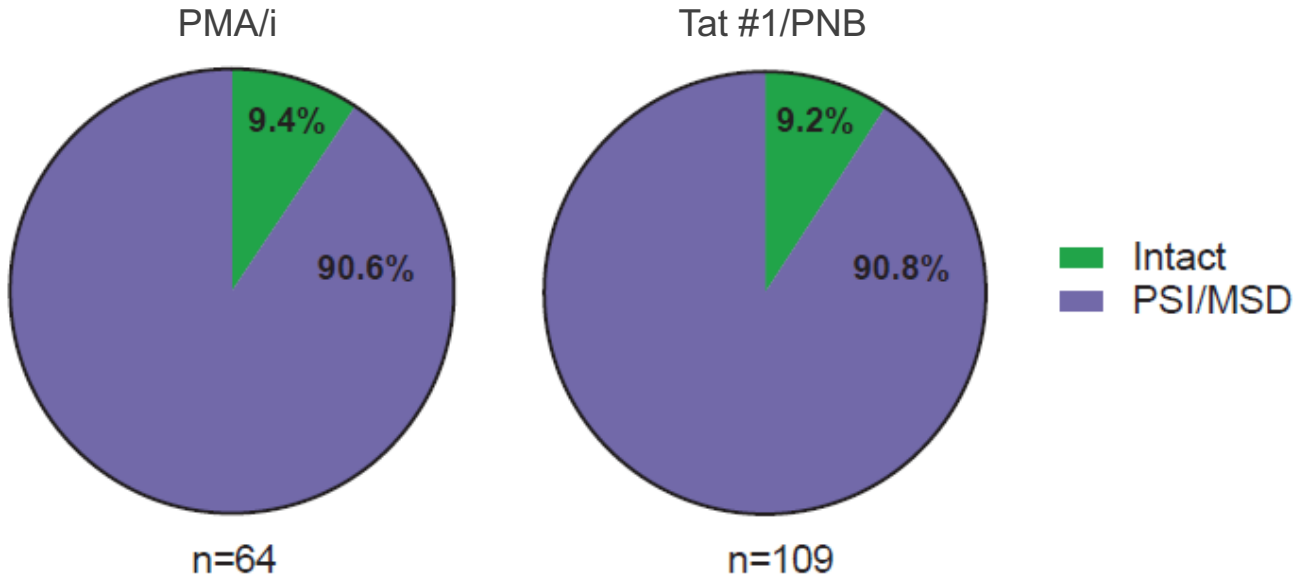

- Most of the p24+ cells harbor PSI/MSD defective proviruses
- Proportions of genome-intact or PSI/MSD-defective proviruses do not differ significantly after PMA/i or Tat #1/PNB stimulation

Analysis of the integration sites in p24+ cells following latency reversal

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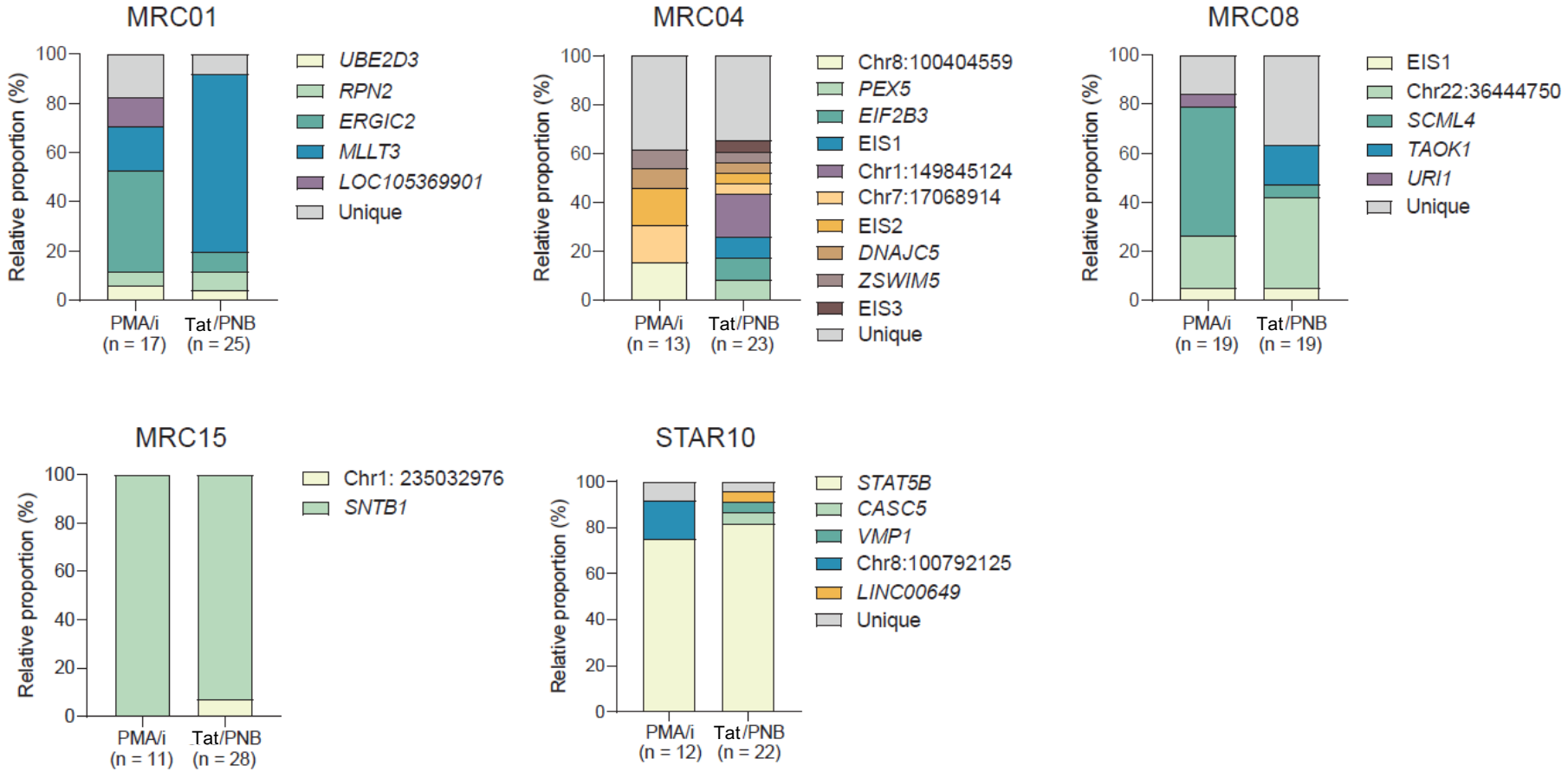

- Tat #1/PNB globally reactivates the same clones as PMA/i
- Some rare and minor clones were detected only in one of the two conditions
- Some clones are represented in different proportions between the two conditions (e.g. MLLT3, SCML4)

Conclusions

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- Tat : physiologically relevant, not toxic *in vitro*
- Does not modify the transcriptome/phenotype of the cells

- In combination with PNB:
  - It induces latency reversal in a higher proportion of latently infected cells than PMA/i
  - Reactivates the same clones than PMA/i, with some exceptions

- Can be used as a tool to study:
  - The proviral sequence and integration site in p24+ cells
  - Phenotype of p24+ cells

Acknowledgements

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