Silencing human retroviruses

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Fondation Merieux, Annecy
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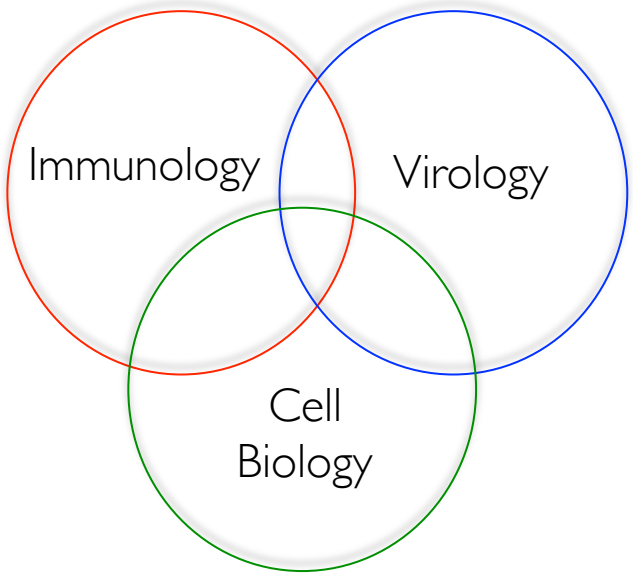
How do viruses avoid recognition by the human immune system

Interface between
3 different disciplines



As intracellular parasites, viruses manipulate host cellular machinery to enable successful replication

This balance between the host response and immune evasion is a key determinant of the outcome of viral infection

How do viruses avoid recognition by the human immune system

Functional proteomic approach 'unbiased view' of how viruses remodel expression of host proteins – emphasis on plasma membrane

Development of forward genetic screens (haploid human cell screens) and now genome-wide CRISPR screens to study viral interactions with the host – example of retroviral silencing

How can we identify novel viral immunoevasion targets?

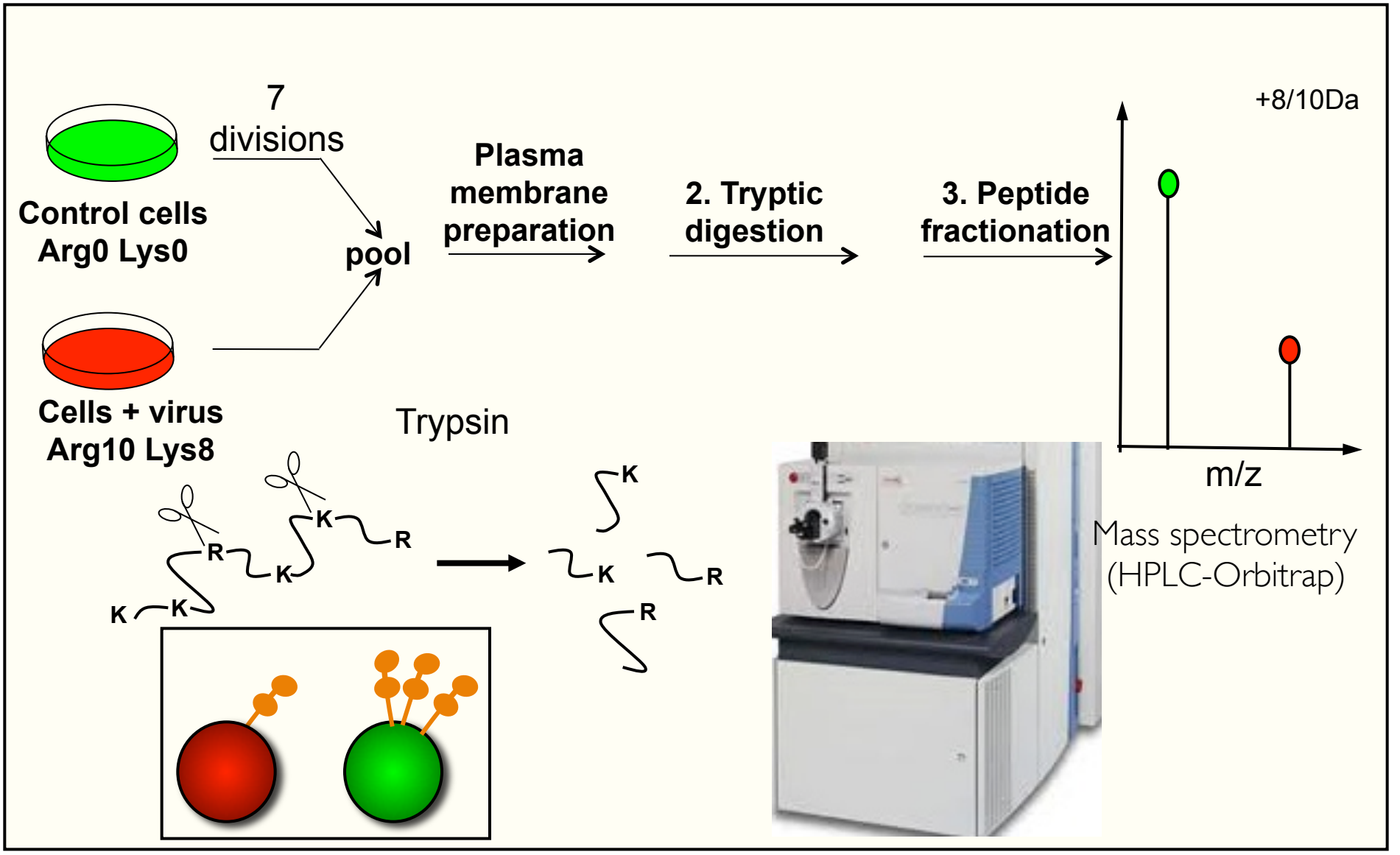
Traditional, target gene approach by flow cytometry to investigate known ligands of critical effector cells eg MHC class I:CTL

It cannot identify the 'Known Unknowns' – unanticipated targets

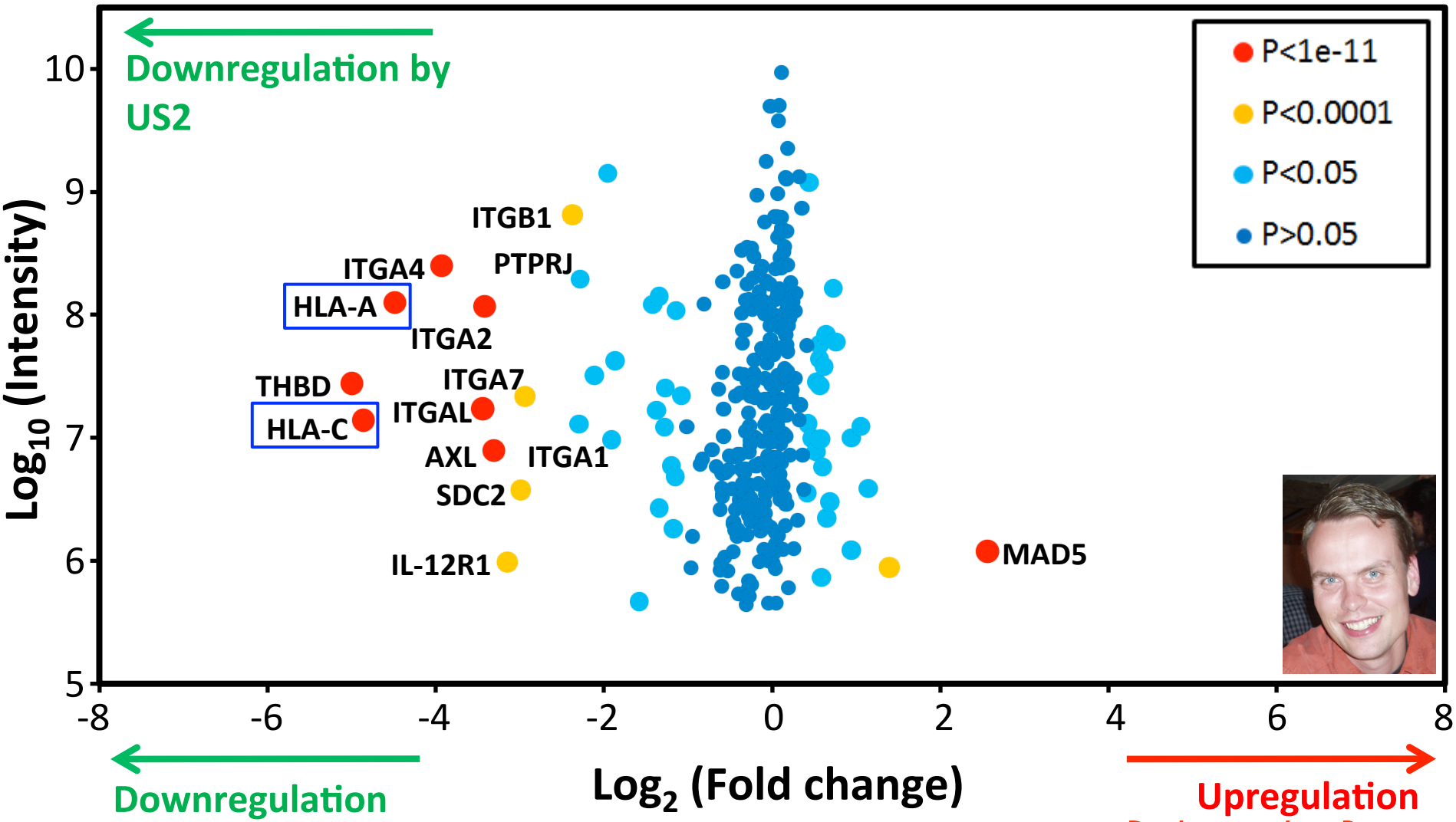
Can we provide an unbiased overview of cell surface proteins whose expression is altered following viral infection?

Combine this knowledge with biology of the virus to try and understand why and how different receptors are manipulated

Plasma membrane protein preparation and SILAC

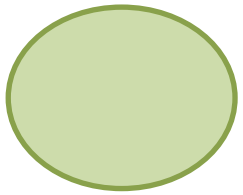


PMP reveals CMV-encoded US2 as a multifunctional modulator of cell surface proteins

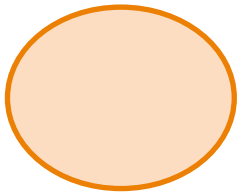


Dick van den Boomen
Plos Path 2015

Can this same approach determine the global effect of HCMV on all cell surface proteins?



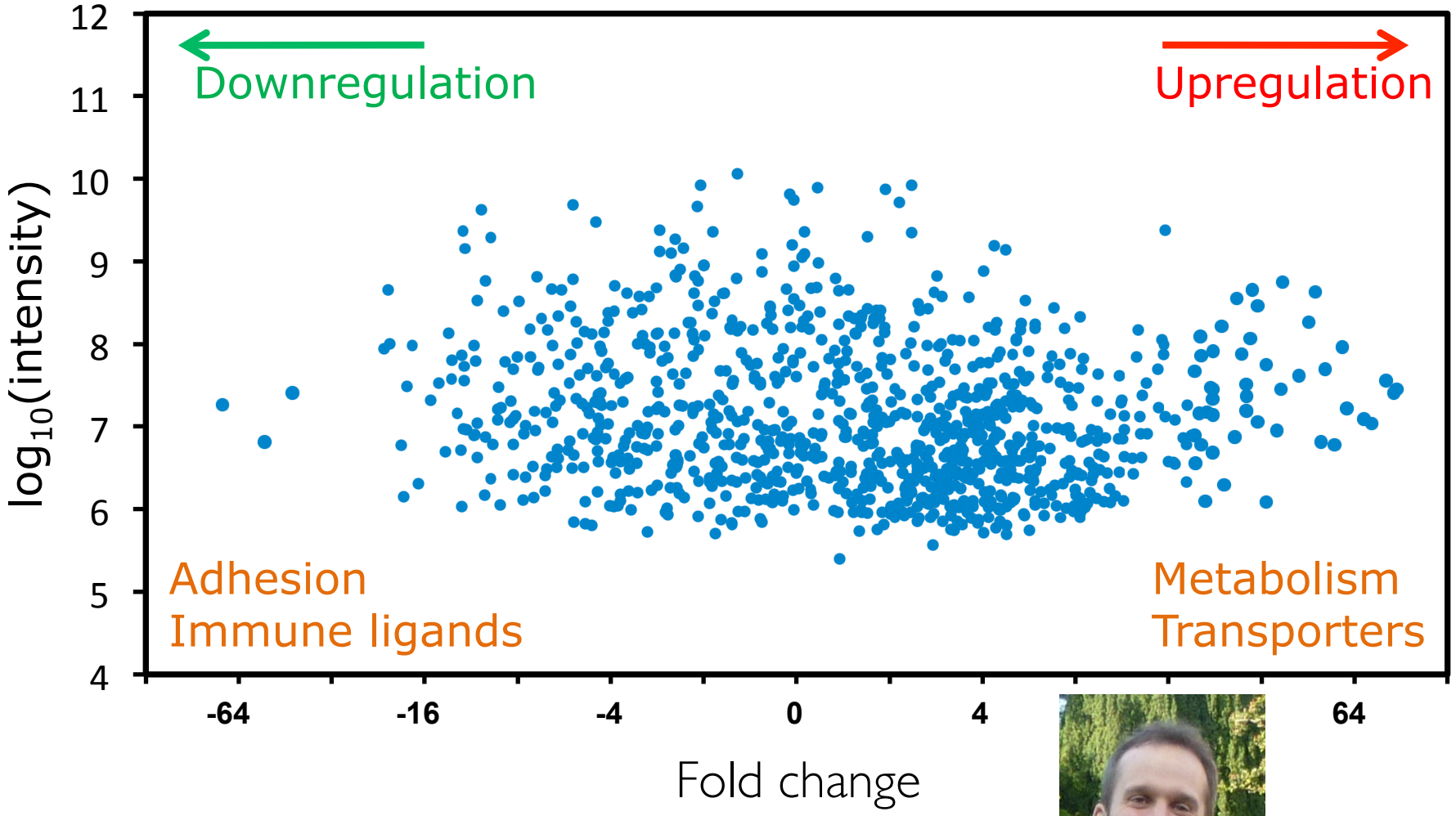
Mock



HCMV

Mike Weekes
Pete Tomasec
Gavin Wilkinson

Can an unbiased screen determine the global effect of HCMV on all cell surface proteins?



Mike Weekes
Pete Tomasec
Gavin Wilkinson

Time resolution can simplify analysis of these changes- Differential labeling – metabolic vs chemical?

SILAC labeling – comparison 3 samples – can increase but complex

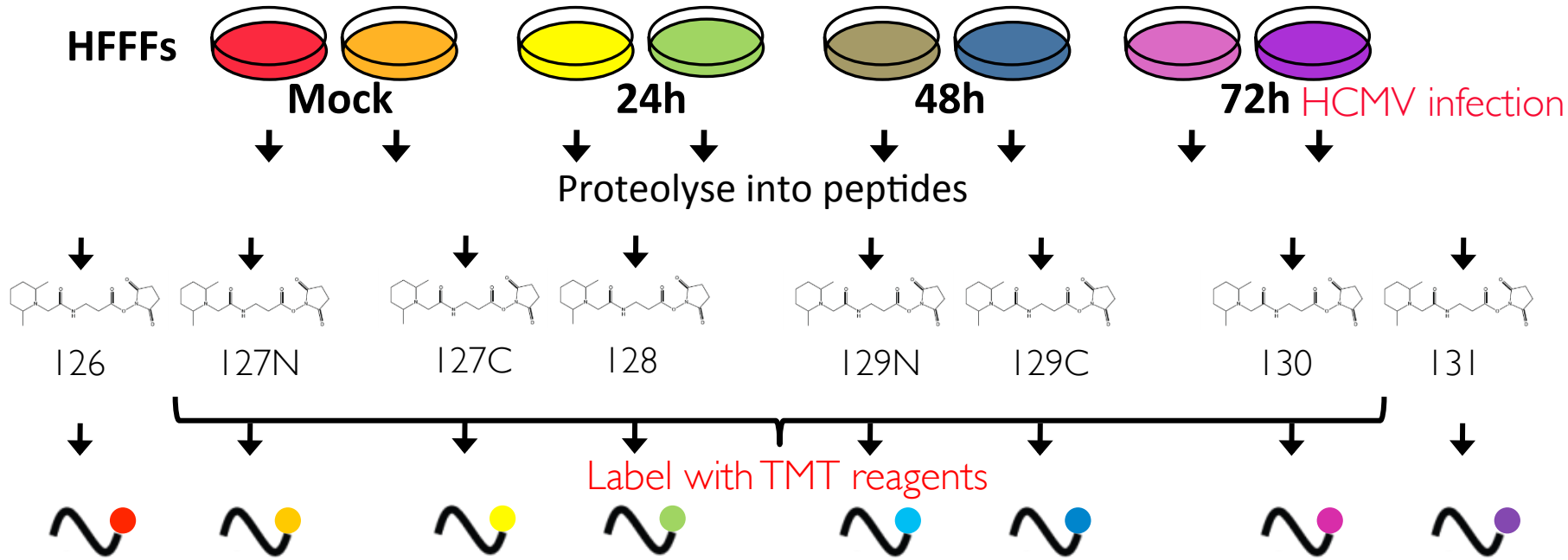
OR

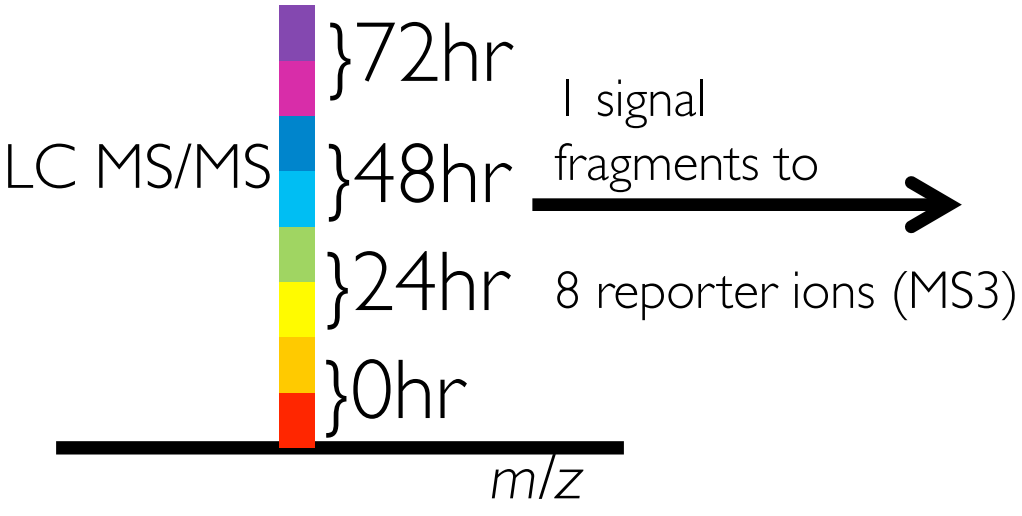
Chemical Labeling – Tandem Mass Tags (TMT) – increases number of samples – up to 10-plex: latest generation orbitrap (Elite/Fusion) resolves ratio compression problems

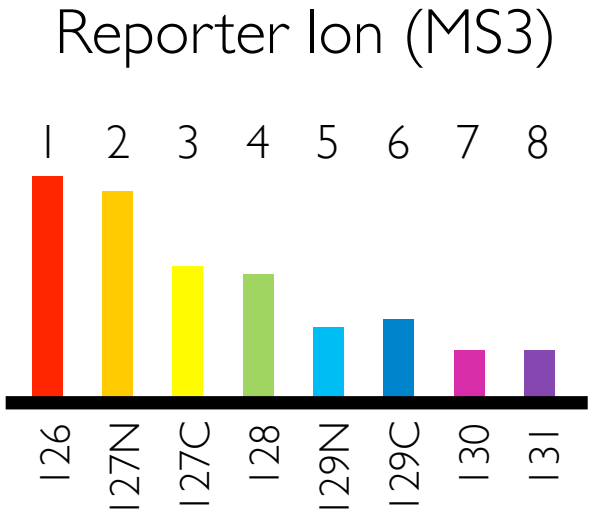
Advantages: No incorporation of label required
Applicable to primary cells (*ex vivo*)
multiple biological samples
temporal resolution

Eg Lytic Phase CMV infection – landscape of plasma membrane over time

Does time resolution help interrogate these changes?

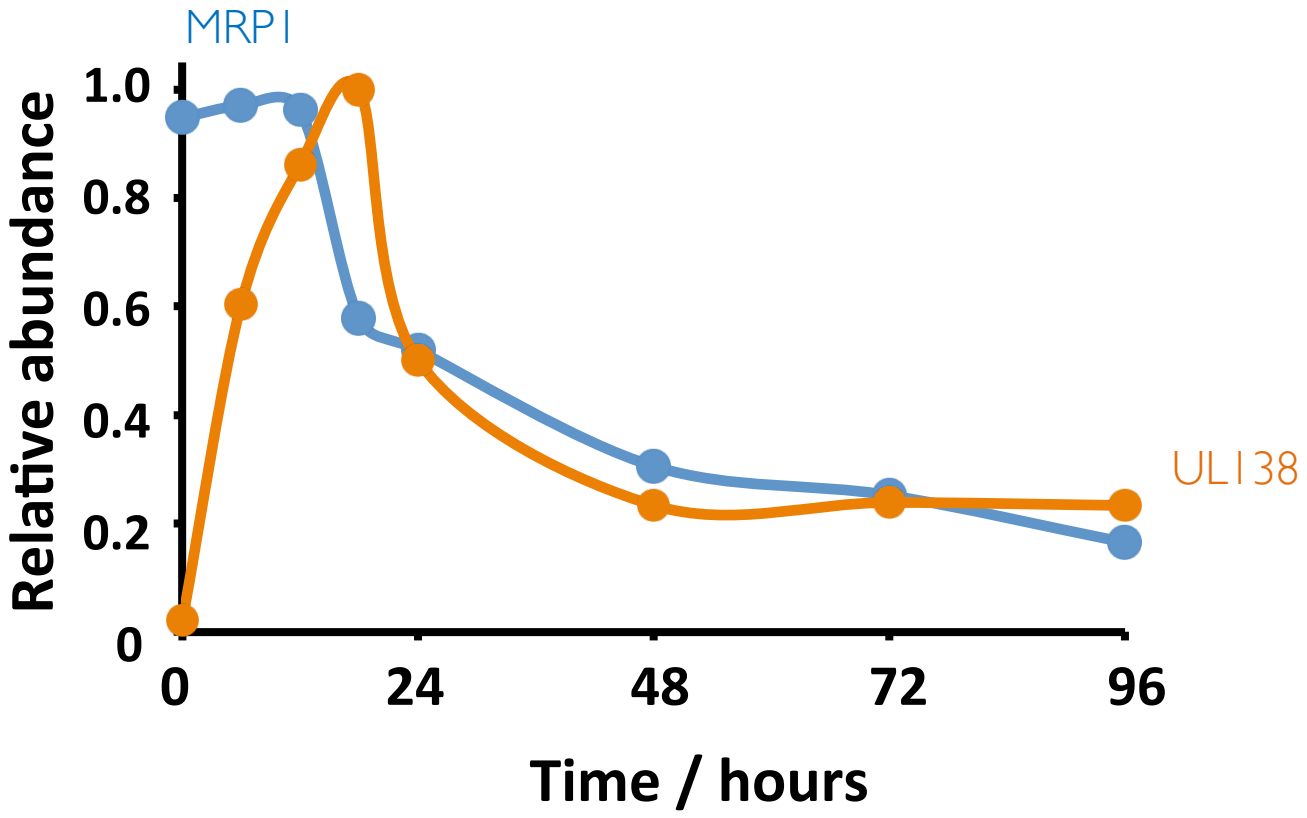






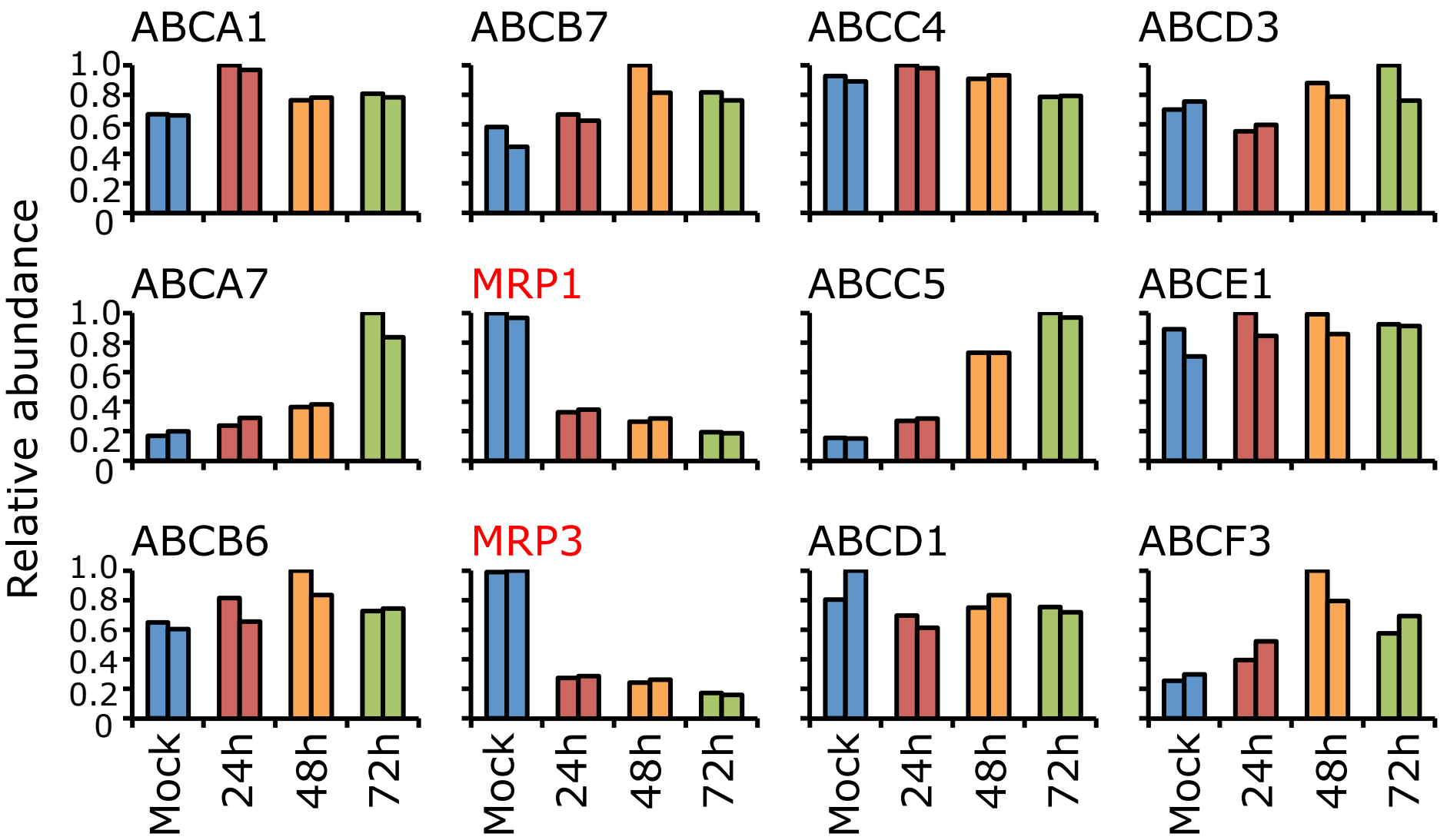
Time resolution helps point to mechanism

Multidrug resistance protein 1
170kDa plasma membrane transporter



Weekes. Tan, Poole et al Science 2013
Weekes et al. Cell 2014

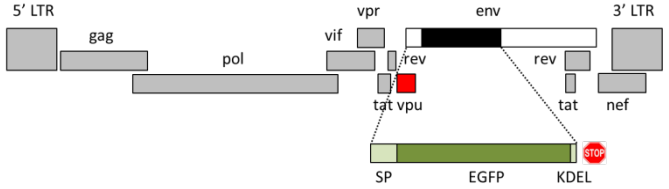
TMT allows us to monitor cell surface changes for any protein

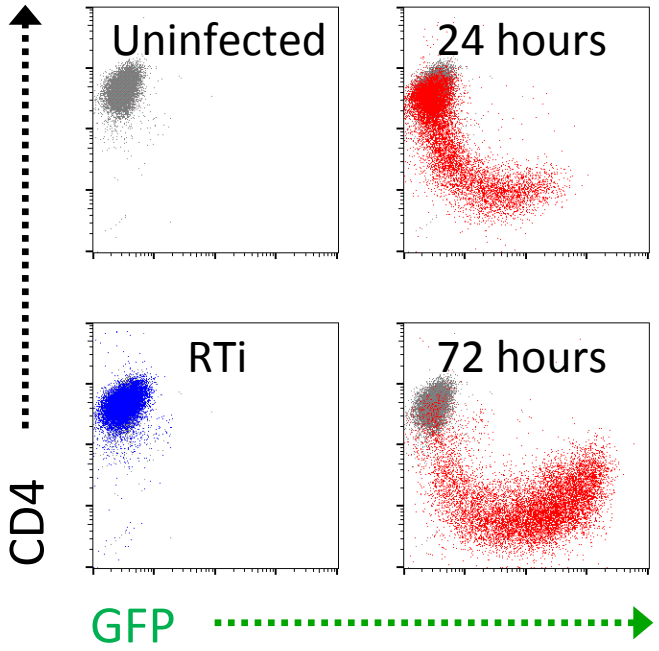


Weekes et al. Cell 2014

Plasma Membrane Profiling of HIV-infected cells

- >30 million people infected with HIV worldwide and almost 2 million AIDS related deaths/year
- Regulation of cell surface proteins by Vpu and Nef
- Known targets CD4 and tetherin critical for viral pathogenesis
- HIV reporter virus encoding GFP

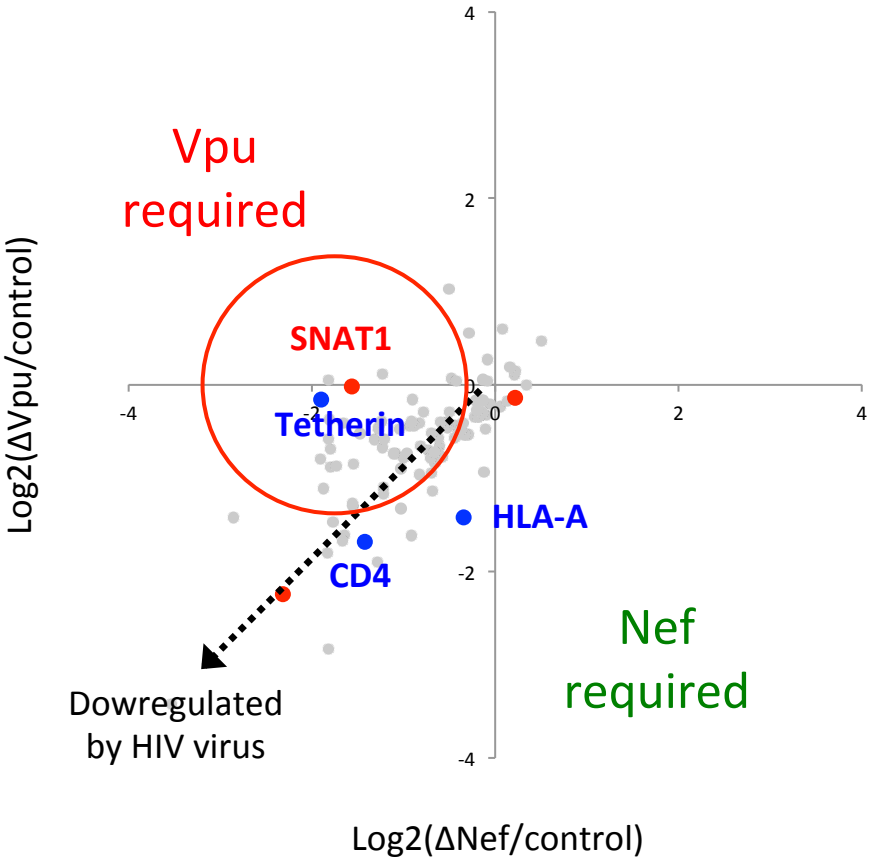




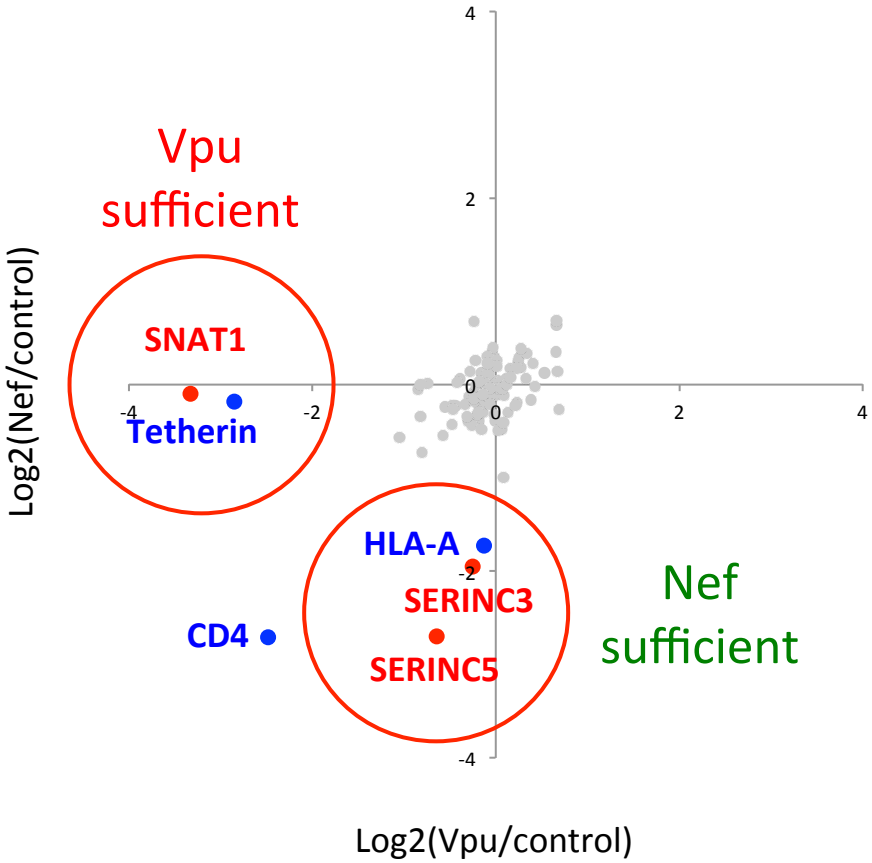
Matheson, N et al. Cell Host & Microbe (2015)
c/w Stuart Neil KCL

SILAC-based proteomic analysis of HIV accessory proteins Vpu and Nef

Δvpu vs Δnef deletion viruses



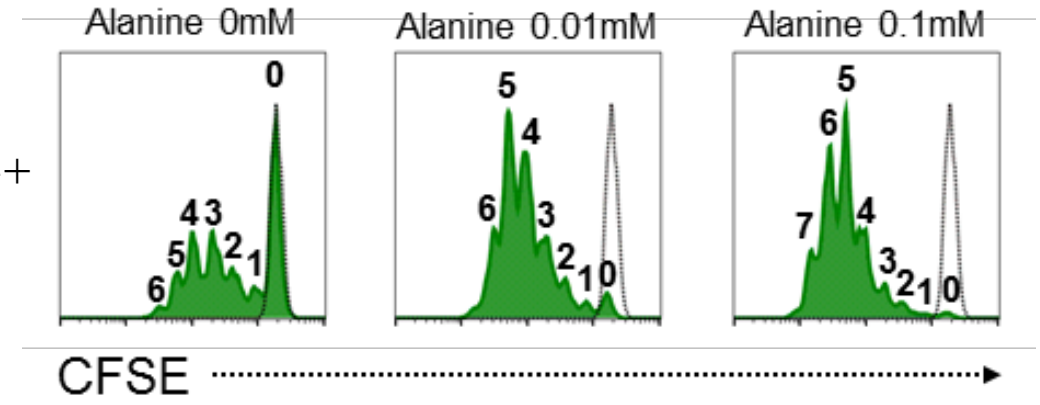
Vpu vs Nef over-expression

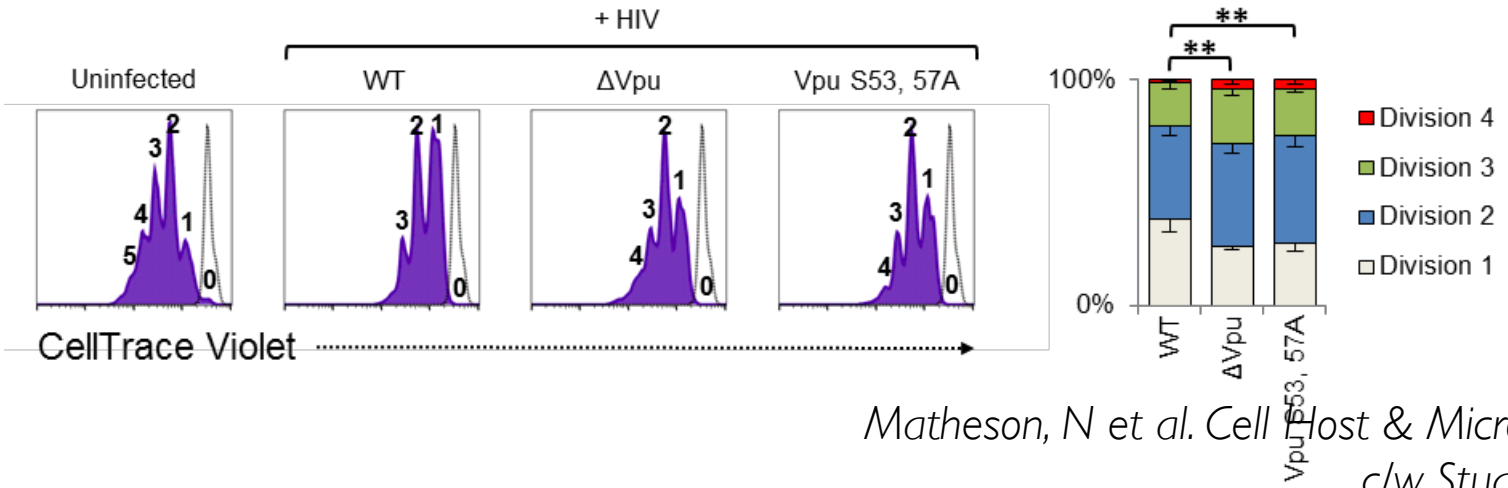


Matheson, N et al. Cell Host & Microbe (2015)
c/w Stuart Neil KCL

SNAT1 downregulation by Vpu impairs proliferation of primary human CD4+ T-cells in HIV infection

Exogenous alanine is critical for proliferation of primary human CD4+ T-cells (*J Immunology 1979*)





Matheson, N et al. Cell Host & Microbe (2015)
c/w Stuart Neil KCL

Our findings uncover a critical and unappreciated role for alanine in T-cell proliferation – and HIV takes advantage of this

Summary

Functional TMT-based proteomic approaches provide a powerful discovery tool to gain an unbiased and uniquely temporal overview of cellular proteins whose abundance is altered upon viral infection

But

Every technique has its limitations:

Proteomics doesn't tell us why or how…

Forward genetic screens in haploid human KBM7 cells
(Insertional gene trap mutagenesis)

KBM7 cells

+

Gene-trap Retrovirus
(insertional mutagenesis)

Phenotypic Selection

Lethal insult

i. Magnetic Sort
ii. Flow sort

Rare resistant cells

Rare cells expressing altered levels of a cell surface marker or a reporter

Map Insertion Sites

Map Insertion Sites

(*Carette & Brummelkamp) Science 2009*)

(Duncan et al Plos One 2011 Van den Boomen PNAS 2014)

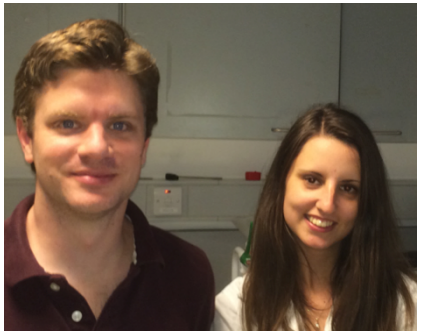
Richard Timms
Lidia Duncan

How is newly integrated retrovirus silenced?

Introduction of viral/foreign DNA: sensed as a 'danger' signal initiates 'alarm response' -control expression and minimize host cell damage:

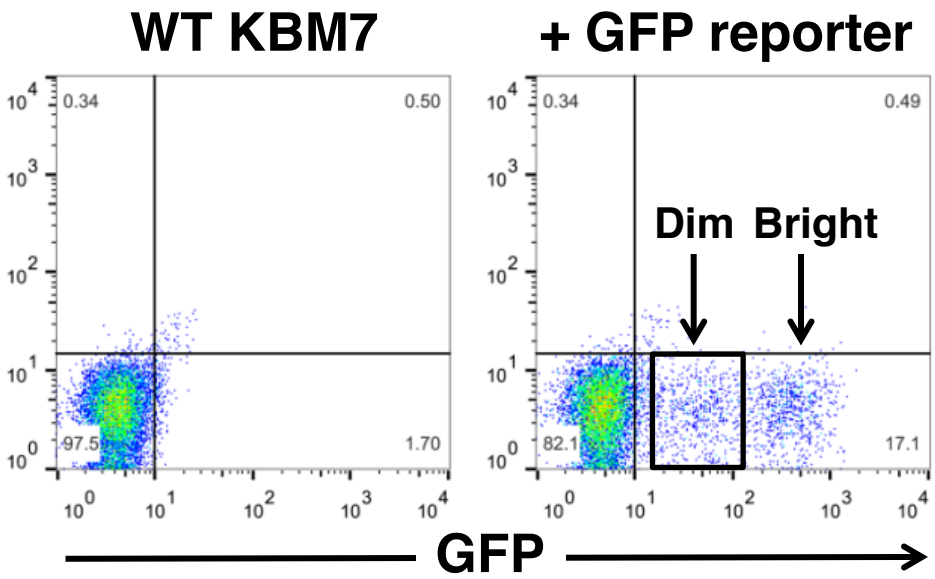
- Induction of innate immune response: STING: IFN response
- Processing and presentation of viral peptides on MHC-I
- Silencing of the DNA to reduce its expression

How does this silencing occur?



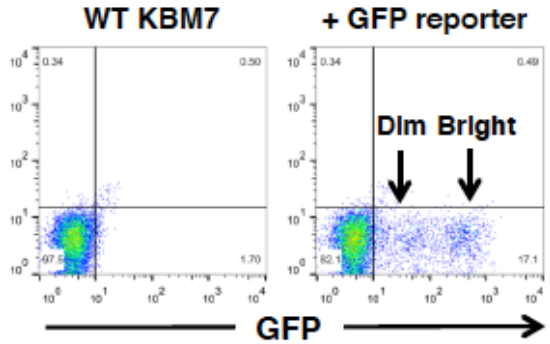
Iva Tchasovnikarova
Richard Timms

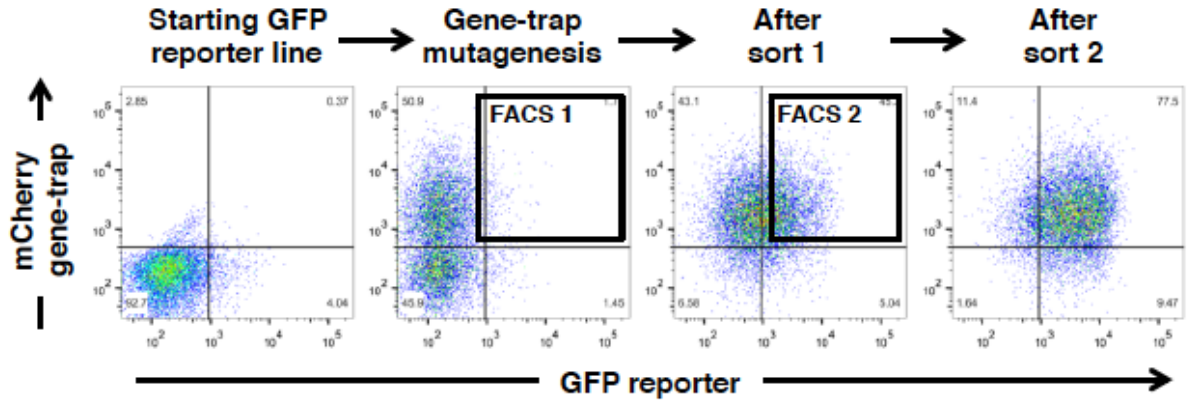
How is newly integrated retrovirus silenced?



Iva Tchasovnikarova
Richard Timms

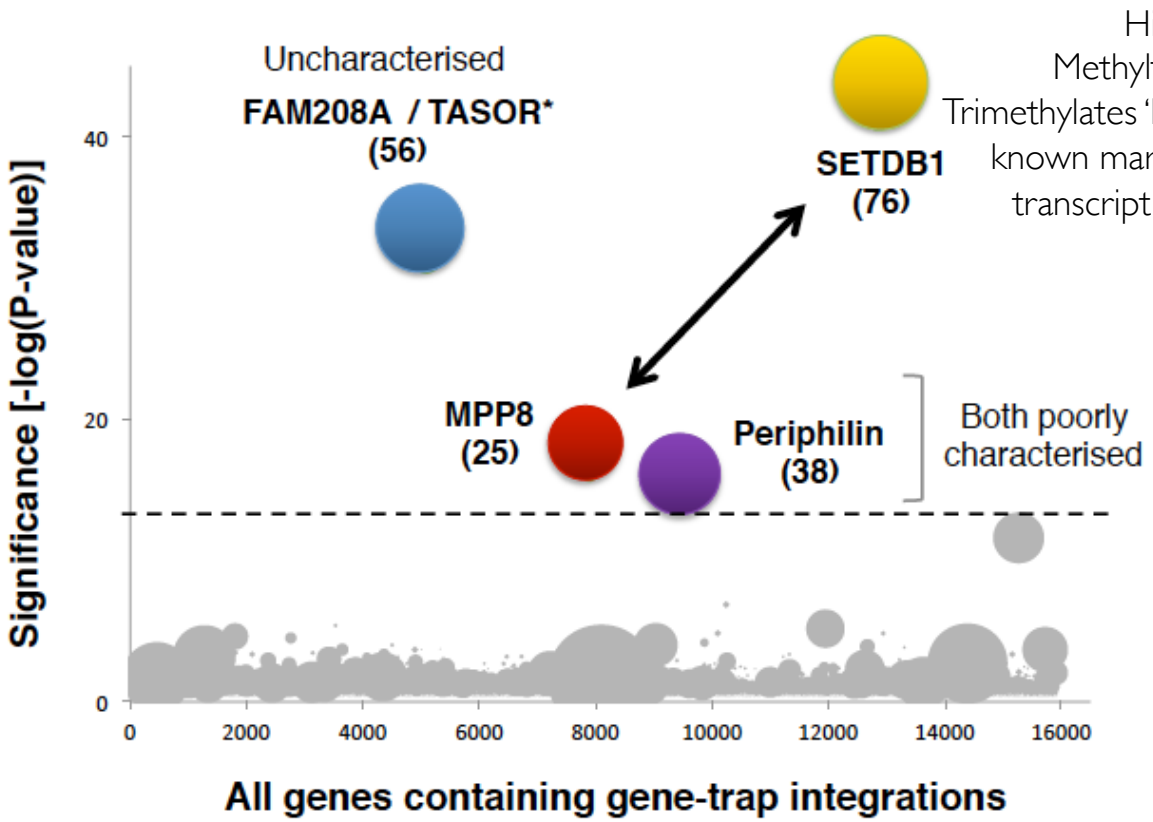
Haploid cell genetic screens to identify transcriptional silencers of retroviral insertion

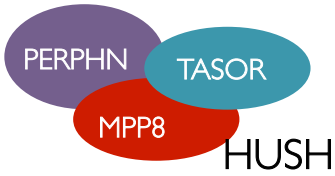




Iva Tchasovnikarova
Richard Timms

Haploid screen for repressors of retroviral integration

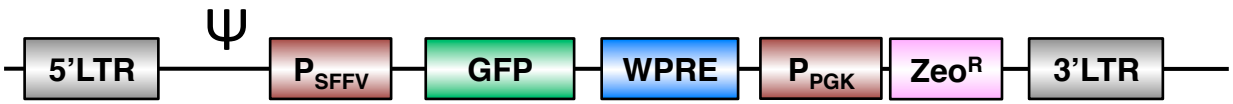


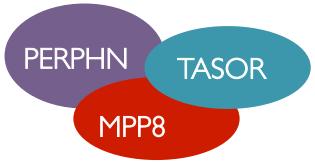


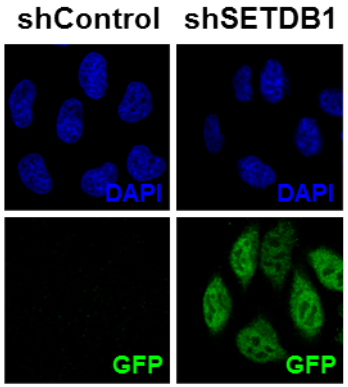
TASOR = Transgene Activation Suppressor
Part of a new complex in humans: HuSH complex
Human Silencing Hub

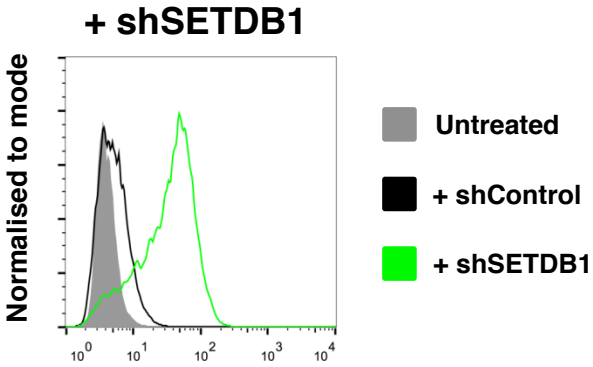
(Tchasovnikarova, Timms et al Science 2015)

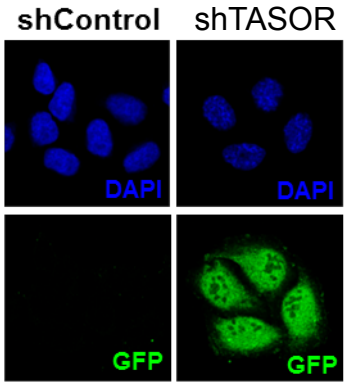
Depletion of HUSH components rescues GFP expression

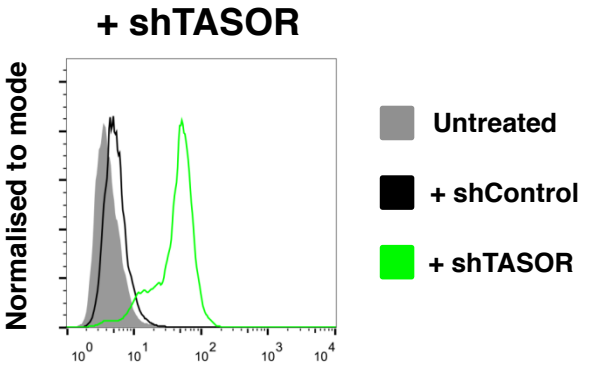








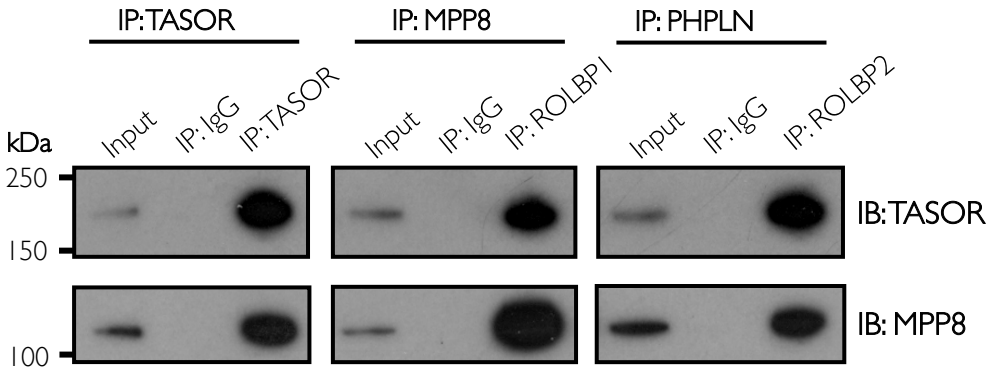




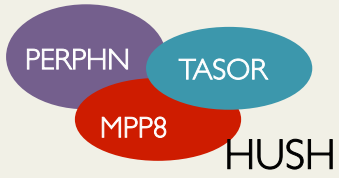
Iva Tchasovnikarova
Richard Timms

TASOR forms a multi-protein complex

Description	Coverage (%)	# Peptides WT	# Peptides Control
TASOR	27.4	35	0
MPP8	18.4	6	0
PERPHLN	11.6	6	0

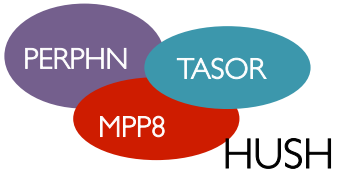


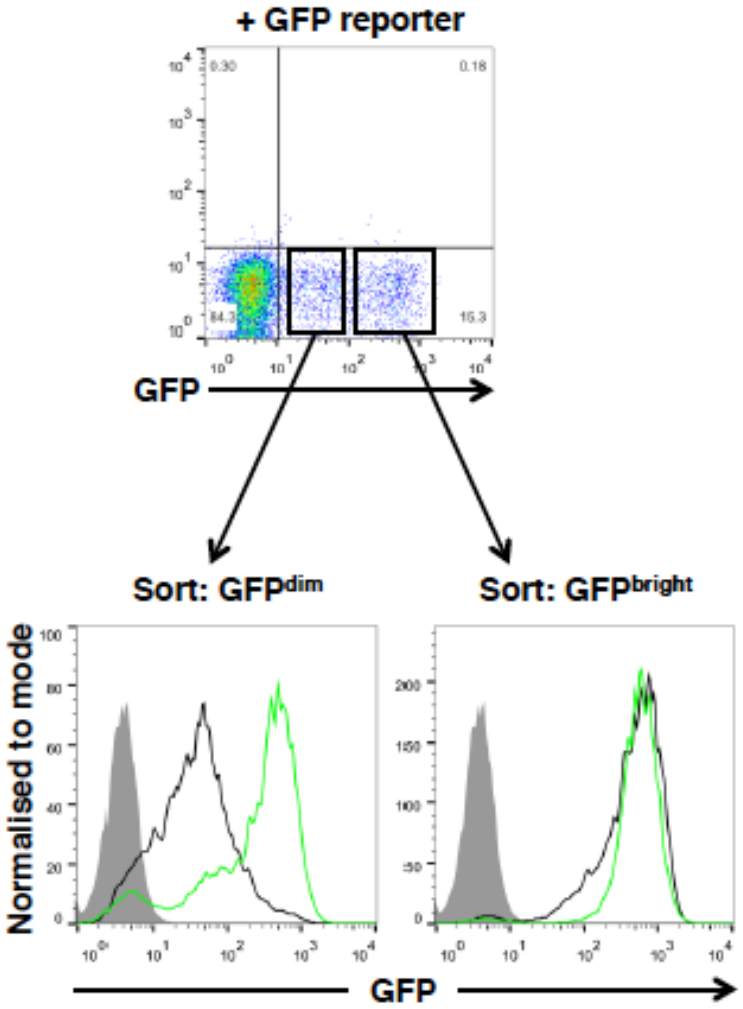
Genetic and Proteomic data converge on a repressive complex comprising TASOR + MPP8 + PHPLN = HUman Silencing Hub = HUSH complex



(Tchasovnikarova, Timms et al Science 2015)

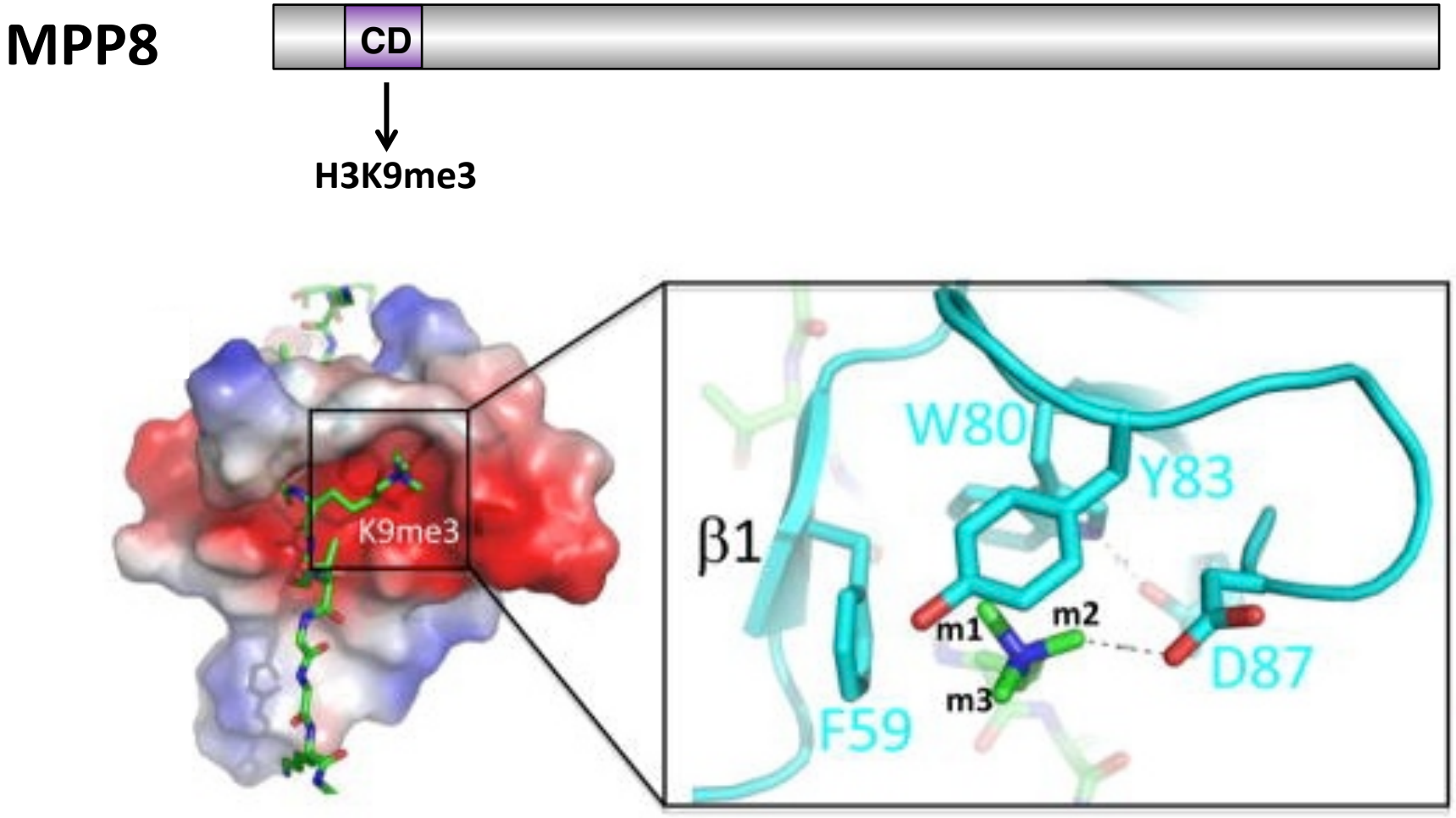
How generalisable are the effects of HUSH?



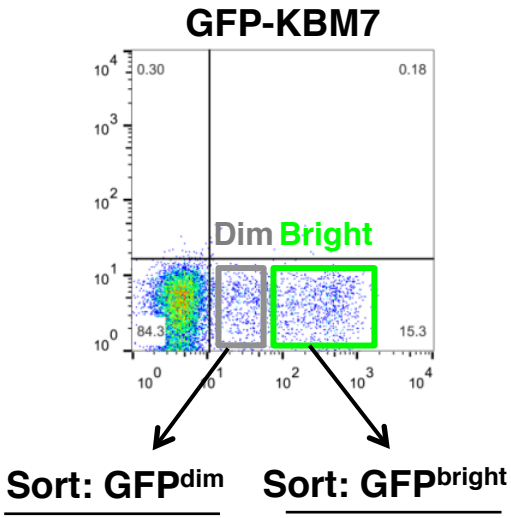


HUSH INHIBITION DE-REPRESSES 95% of all GFPdim integrations

The chromodomain of MPP8 binds H3K9me3

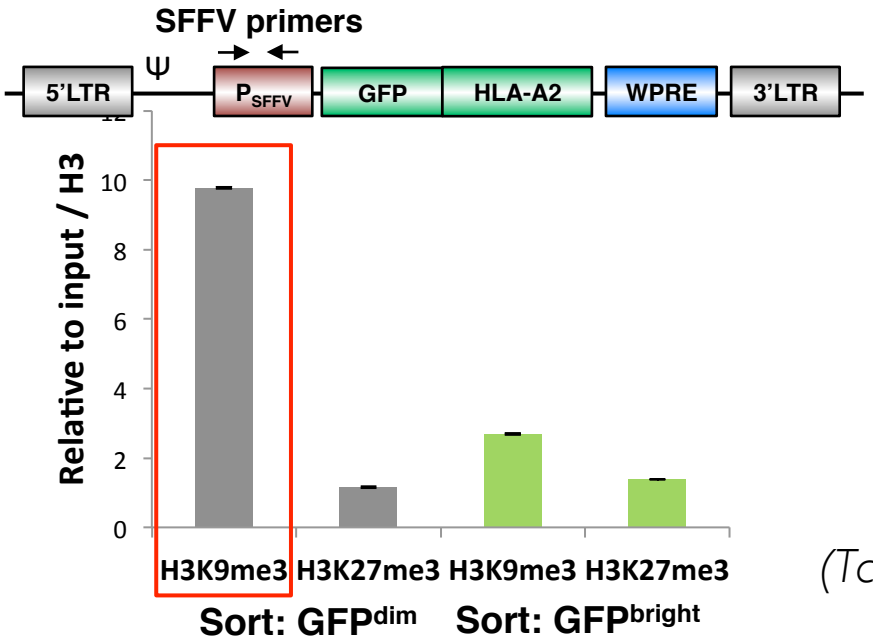


GFP^{dim} lentiviral integrations are packaged into repressive chromatin marked by H3K9me3



CHIP-PCR Dim/Bright Popn.

H3K9me3 is the classic marker of repressive heterochromatin



(Tchasovnikarova, Timms et al Science 2015)

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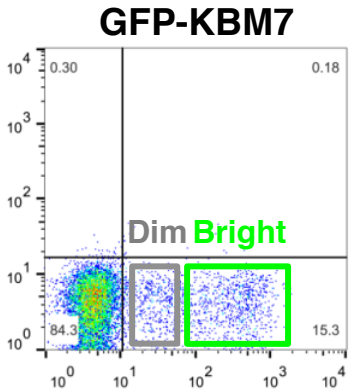
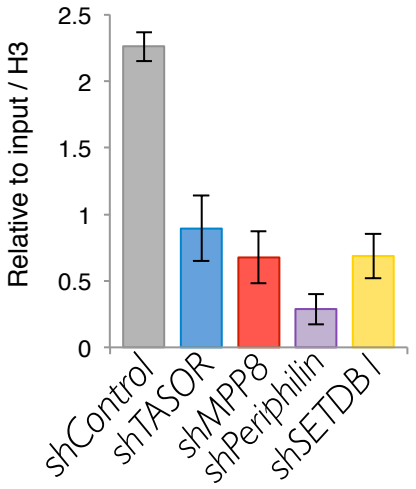
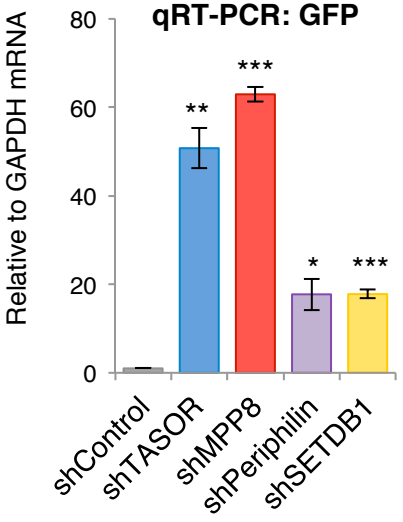


ChIP: H3K9me3
qPCR: GFP reporter



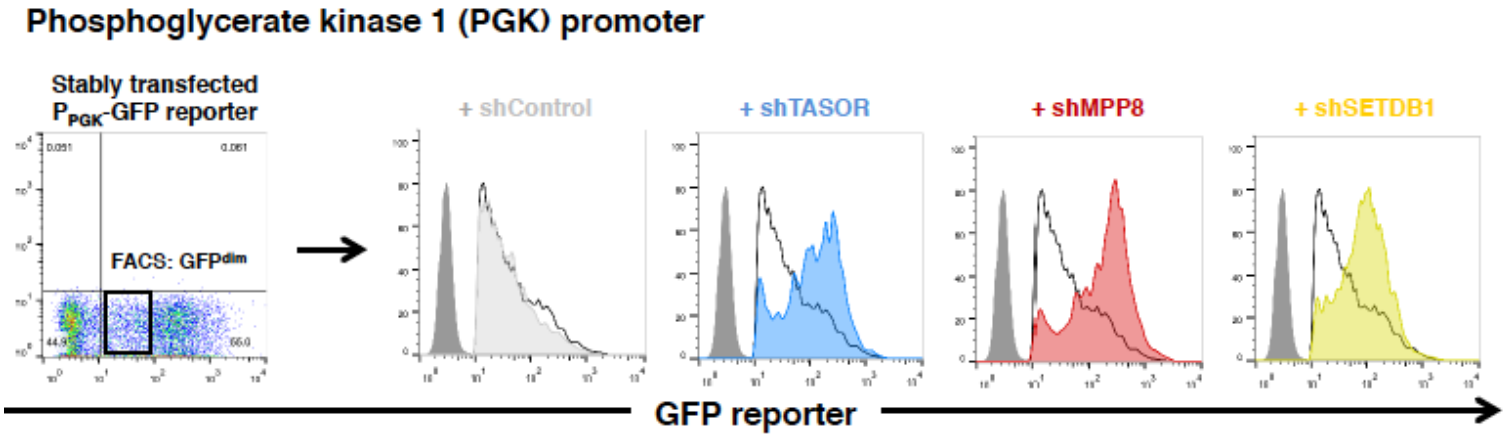
Loss of H3K9me3 across the reporter



Increase in reporter transcription

Is repression by HUSH 'sequence specific' or a 'positional effect' i.e. governed by the genomic landscape?

Genetic screen and validation experiments used lentiviral reporters – is this an antiviral response?
Stable transfection of HeLa cells - GFP expression 3 cellular promoters (SFFV, PGK and eIF4A)

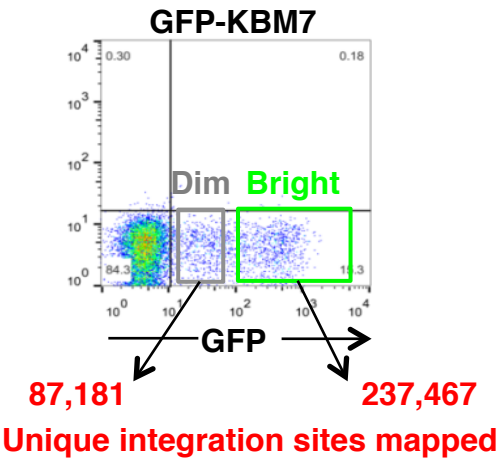


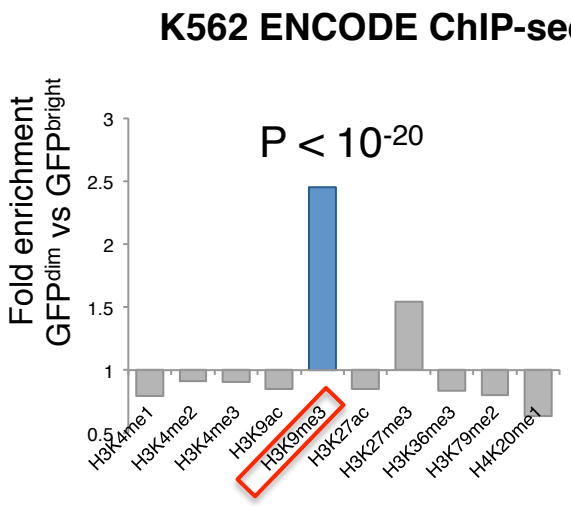
HUSH-mediated repression predominantly governed by genomic landscape surrounding the transgene integration site and NOT dependent on a specific DNA sequence

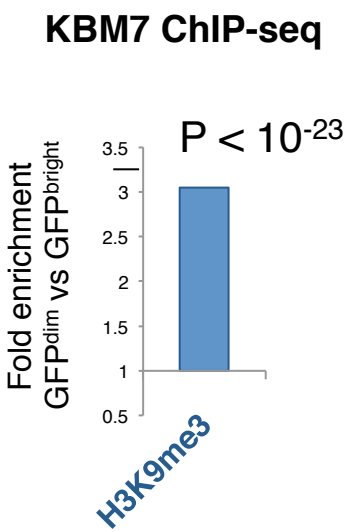
HUSH is responsible for maintenance of H3K9me3 at endogenous genomic loci

compare integration sites of GFP reporter:
GFP^{dim} vs GFP^{bright} popn. to determine where HUSH acts in genome

GFPdim integrations enriched in proximity to H3K9me3

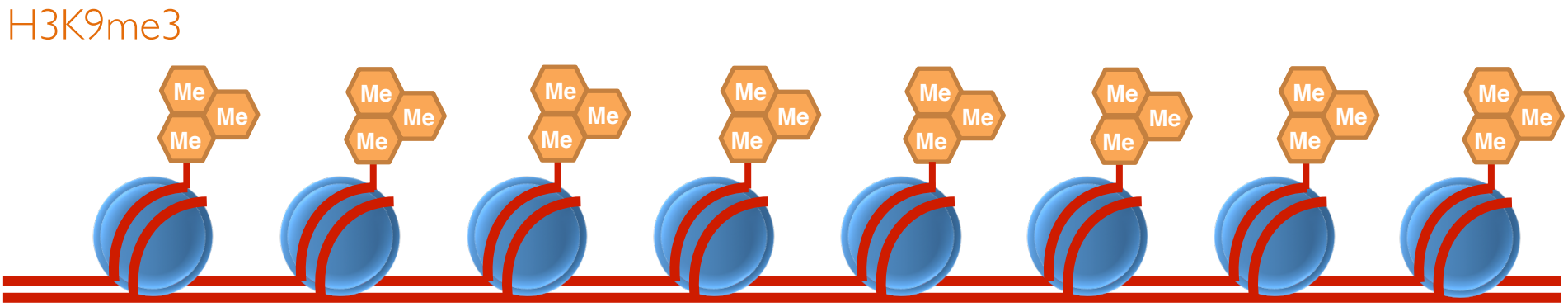






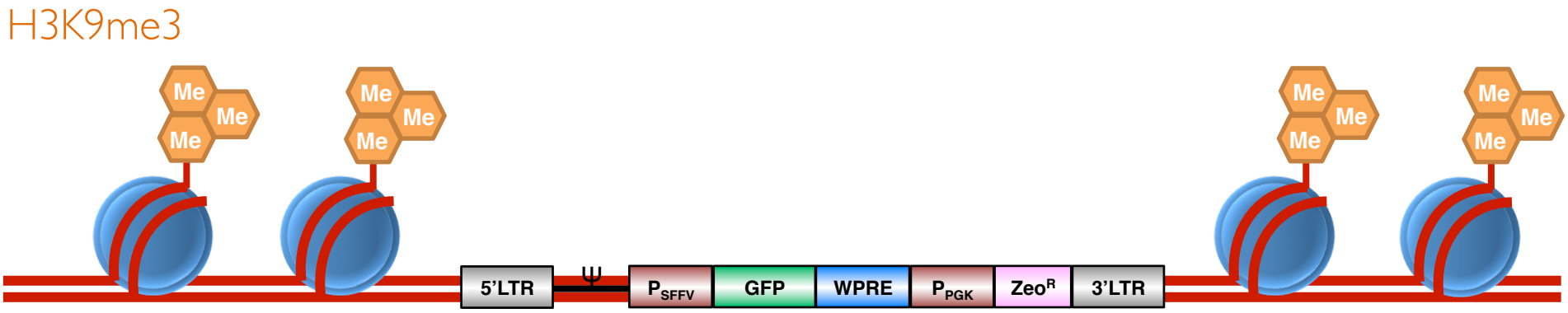
Iva Tchasovnikarova
Richard Timms

Preliminary model for HuSH function



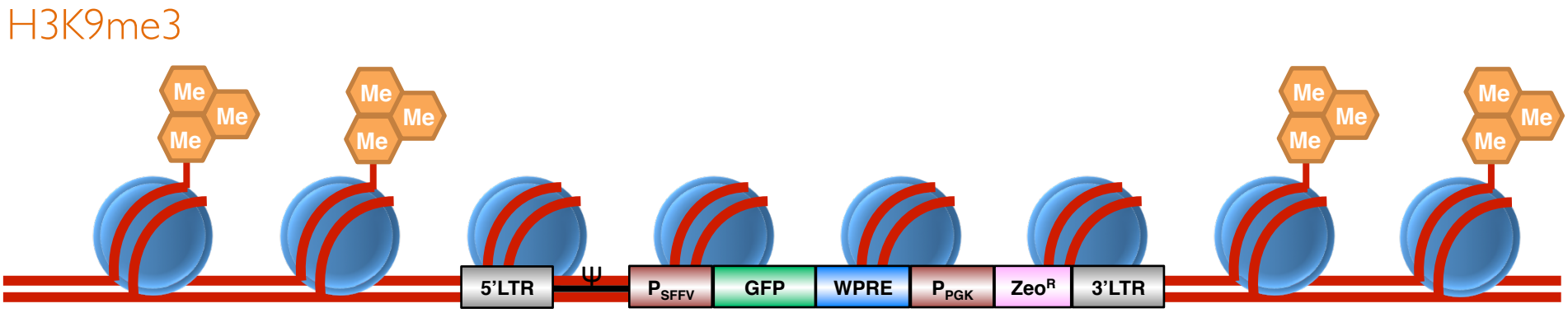
Heterochromatin marked by high H3K9me3 levels

Model for HuSH function



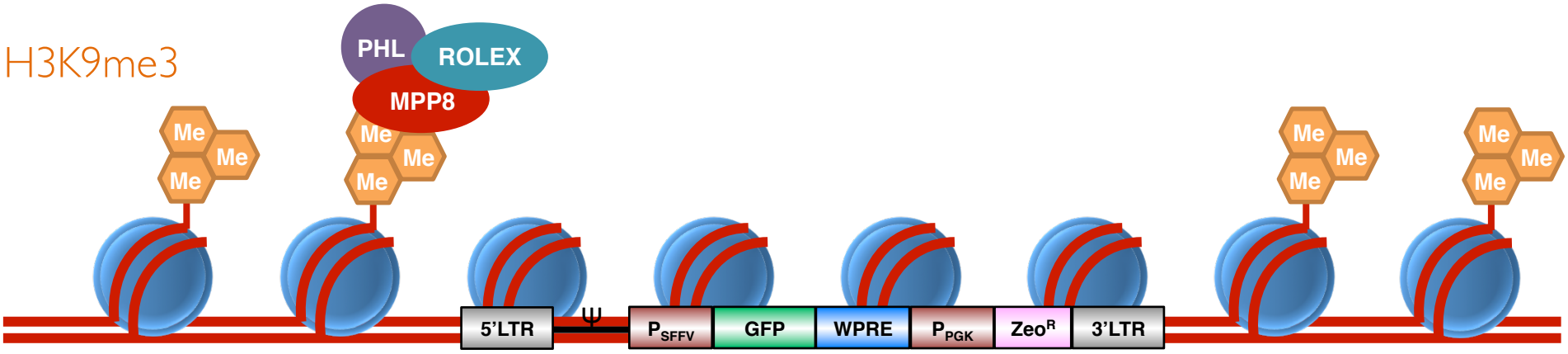
Viral integration into heterochromatic locus

Model for HuSH function



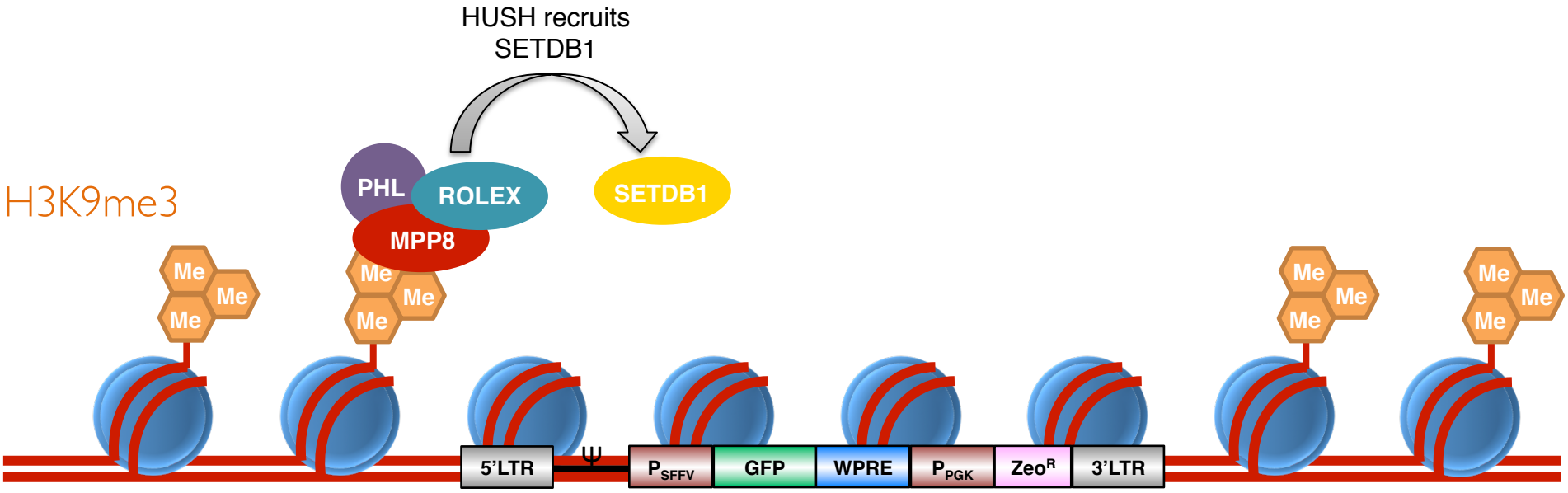
Chromatinisation of the provirus

Model for HuSH function



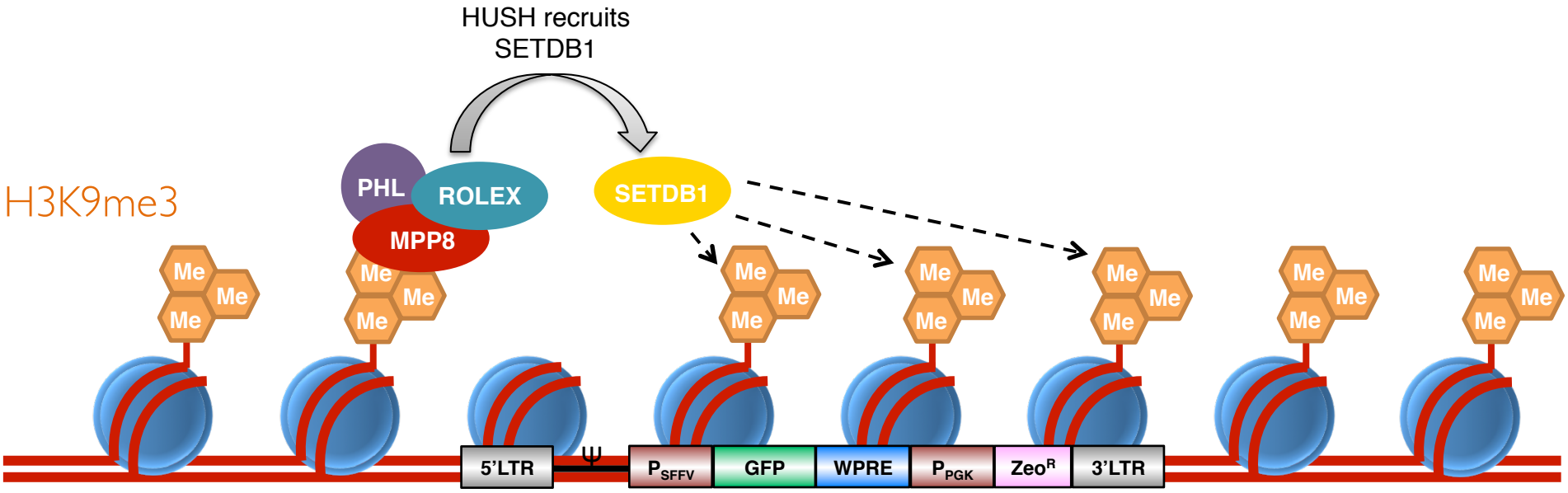
HUSH complex is recruited to flanking regions rich in H3K9me3

Model for HuSH function



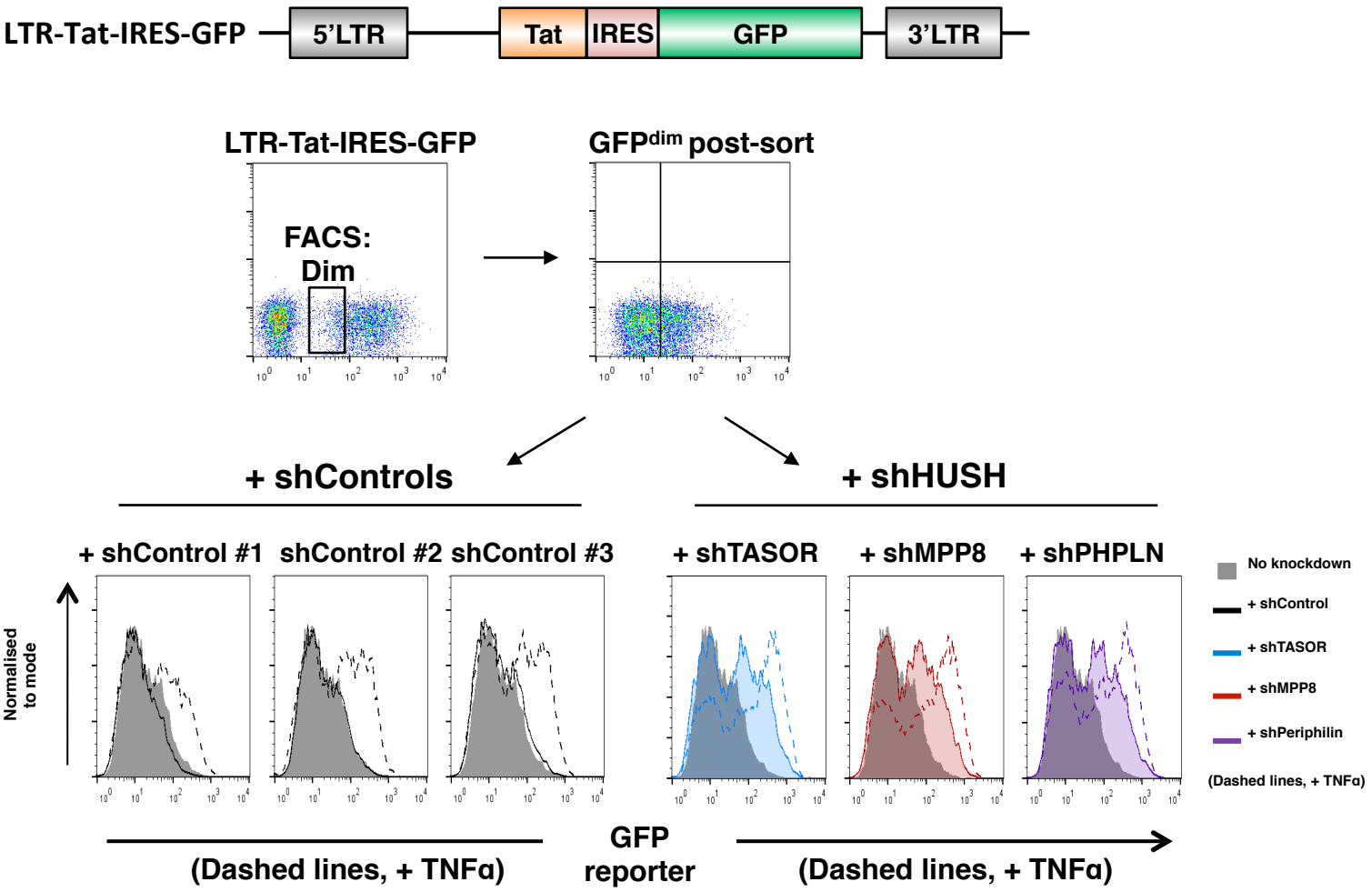
SETDB1 is recruited to desposit H3K9me3 across the provirus

Model for HuSH function



Heterochromatin spreading across the reporter – converts GFP^{bright} to GFP^{dim}

HUSH is required for early silencing with HIV



Iva Tchasovnikarova
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Why have HUSH components not been previously identified?

- Classic position-effect variation screen (PEV) intensely studied in Drosophila
  HUSH is absent from model organisms

- Orthologues first appear in vertebrates: zebrafish – conserved between fish and humans - HUSH represents a novel route to H3K9me3-mediated heterochromatin formation in mammalian cells

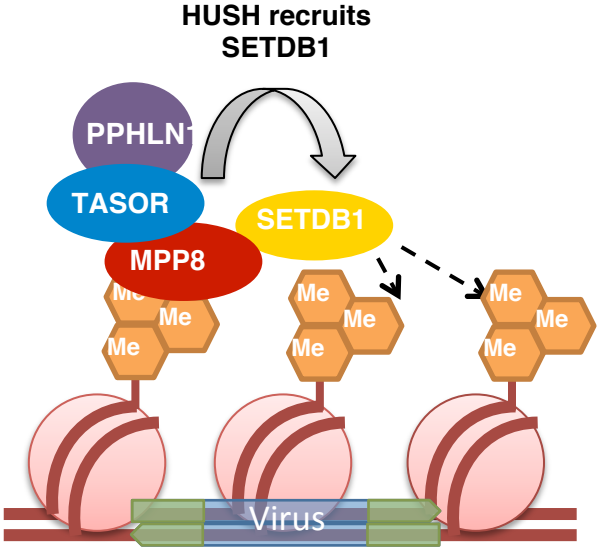
- Emphasizes the power of forward genetic screens in more diverse systems for discovering novel genes and pathways

Unanswered questions:

- Why did our screens not identify known 'canonical regulators' of heterochromatin – HP1 family. ? Redundancy ? Screens not saturating – different mechanisms for silencing at different chromosomal sites.

- How does HUSH fit in/interact with other epigenetic silencers eg KAP1/HP1
- Provide evidence for HUSH-dependent histone-based silencing/methylation – is HUSH also involved in *de novo* DNA methylation?

- Mechanism of silencing?



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