Interactions between ovine lentiviral vectors and primary cells

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Livestock vaccines

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- Many infectious diseases of livestock do not yet have an effective vaccine
- Viral vectors
- Lentiviral vaccine vectors
  - Ovine lentivirus

Retroviruses

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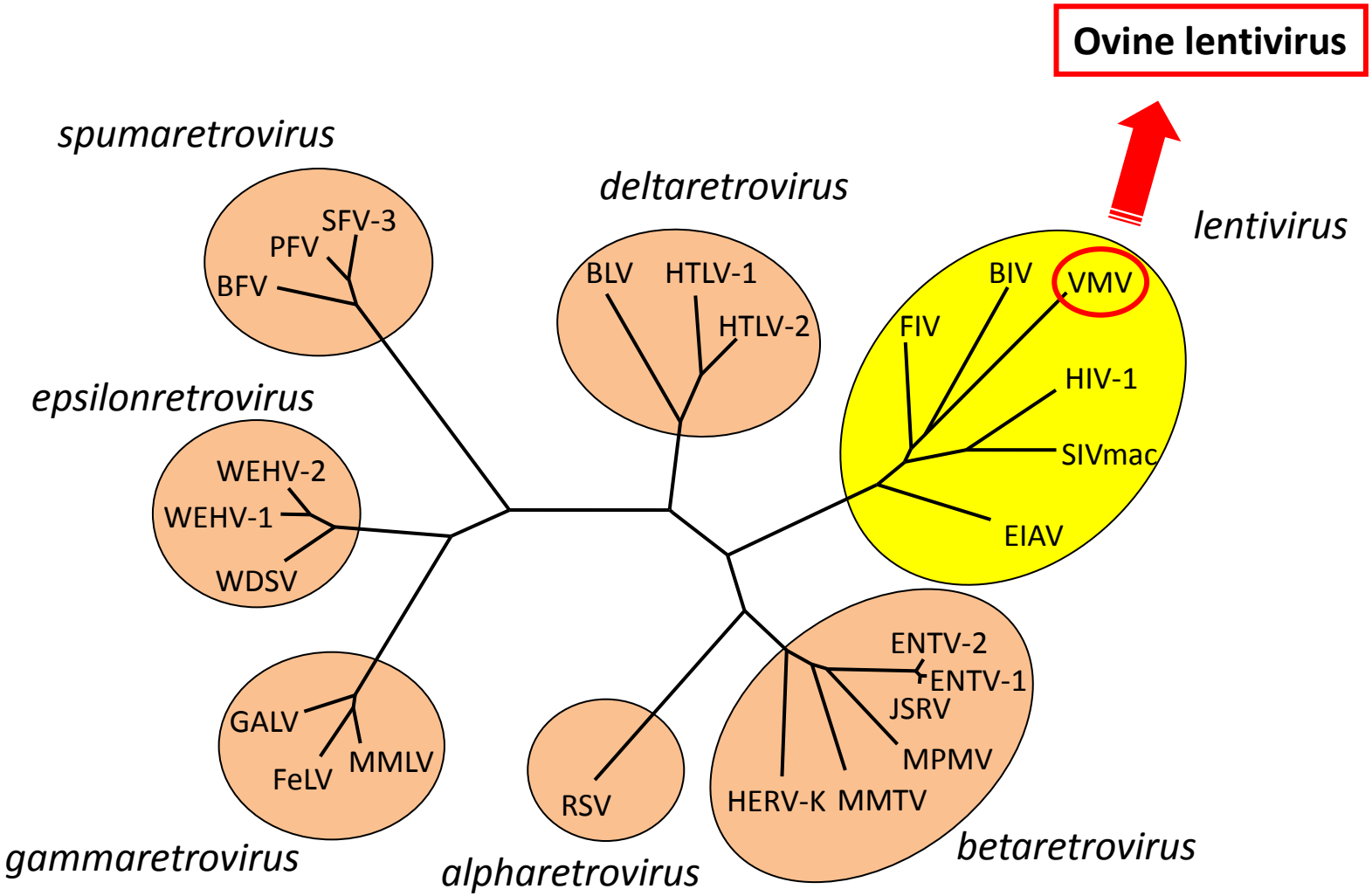

Lentivirus Structure

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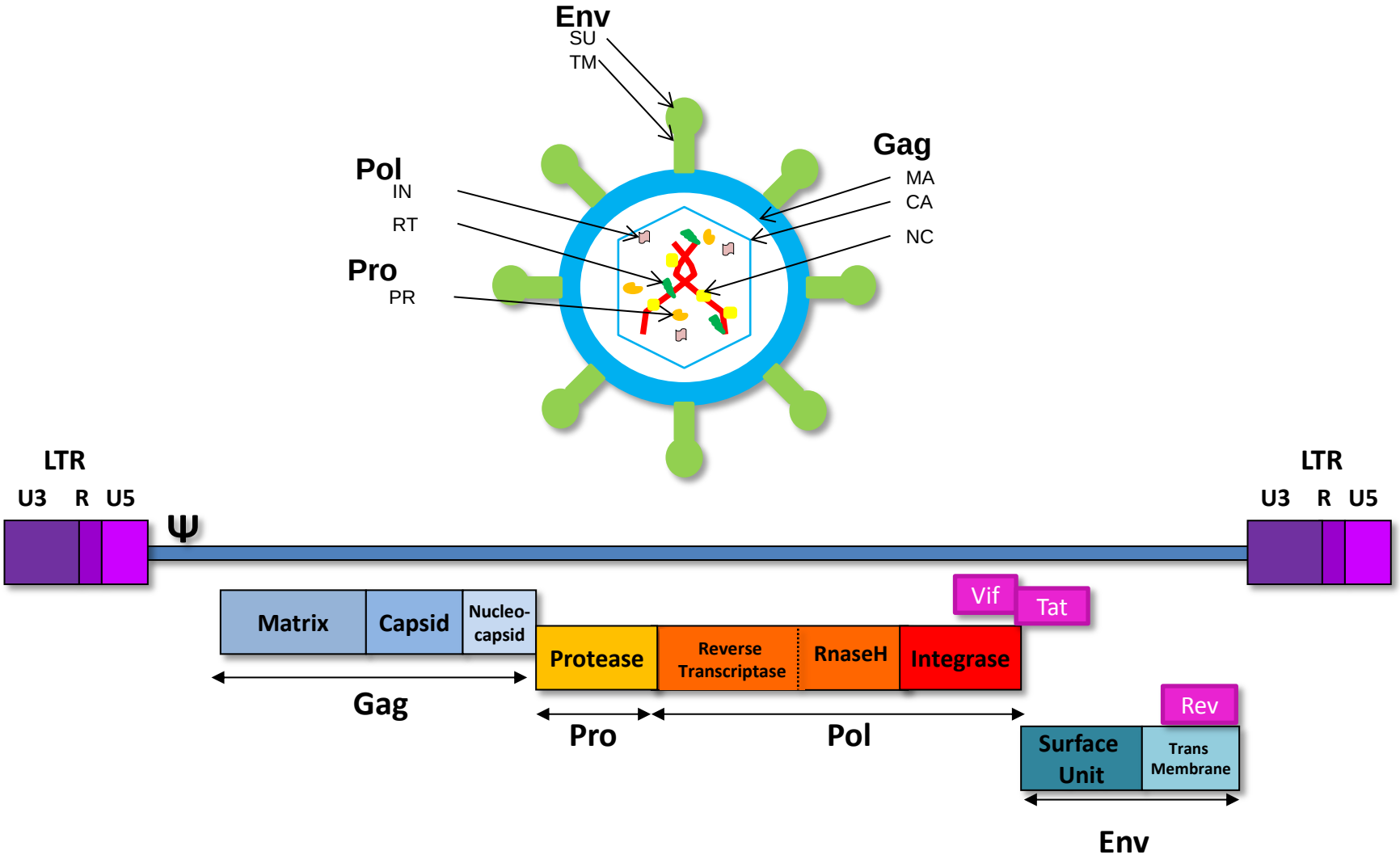
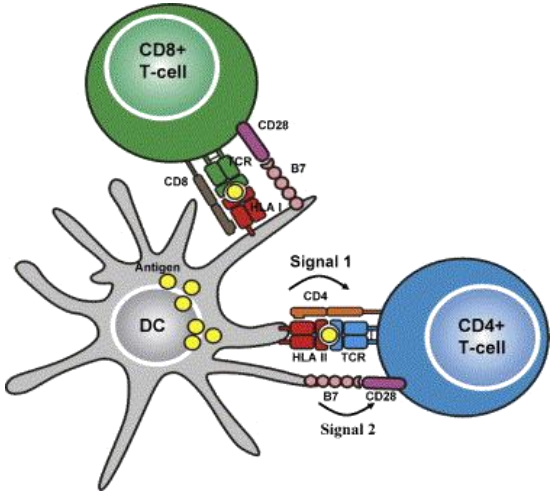


Why lentiviruses?

- Can infect non-dividing antigen presenting cells (APCs)
  - Long lasting, stable transgene expression
- Produce cellular immune responses[1]
  - Infectious disease
- Generate neutralising antibodies[2]



1) Rowe *et al.* 2006. *Mol Ther.* 13.
2) Iglesias *et al.* 2006. *J Gene Med*. 8.

How will it work?

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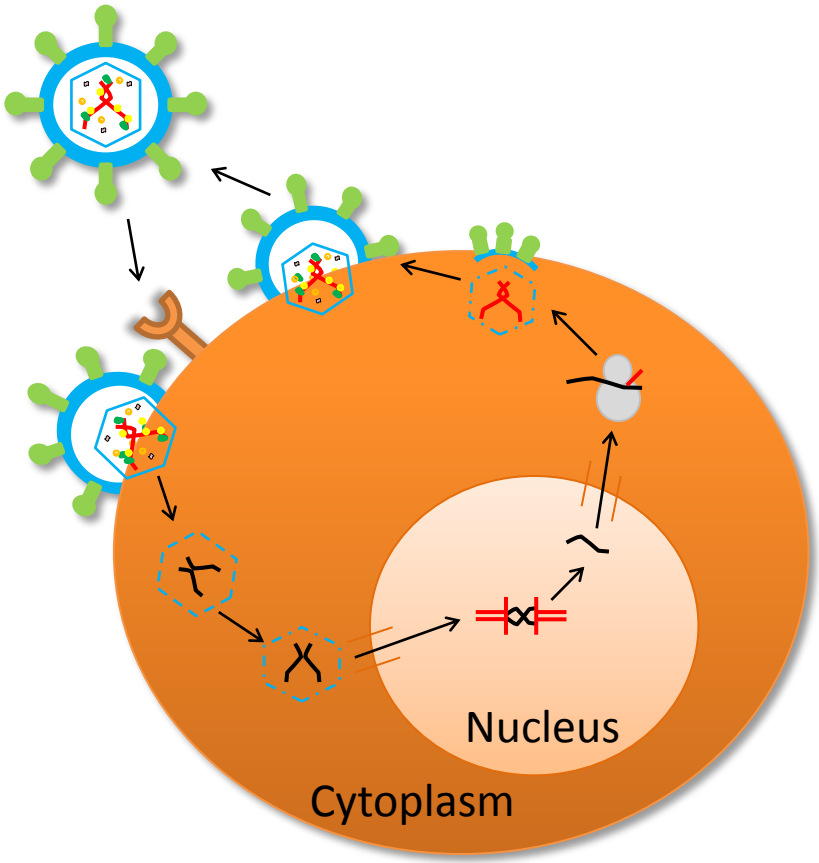
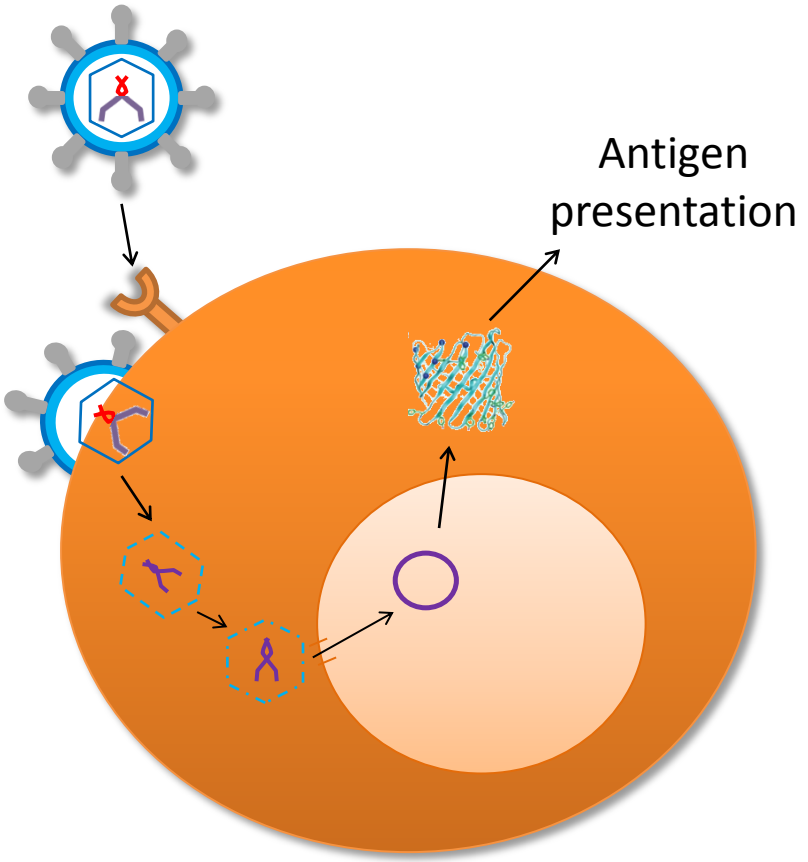


Lentivirus Replication Cycle

- Easily manipulate genome



Lentiviral vaccine vector

- Self-inactivating
- Integration deficient

Aims

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1. Construct and evaluate modified ovine lentiviral vaccine vectors
   - Basic characterisation
   - Improve production efficiency
   - Enhance safety profile
2. Study the innate immune response to ovine lentiviral vectors in primary ruminant dendritic cells and macrophages cultured *in vitro*
   - Efficiency of infection
   - Interactions with primary cells
   - Immune responses

Vector Production

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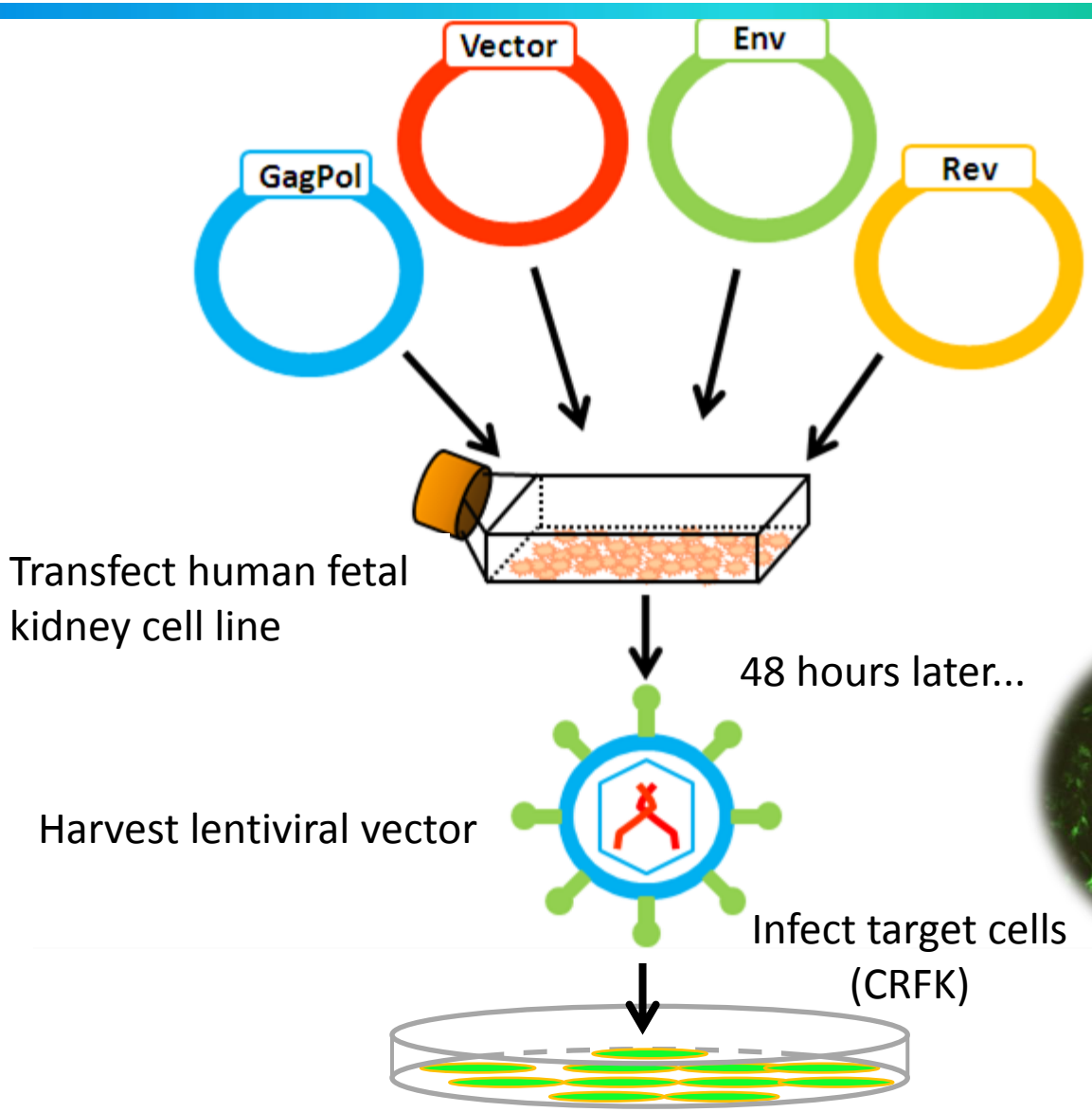

Self-Inactivating

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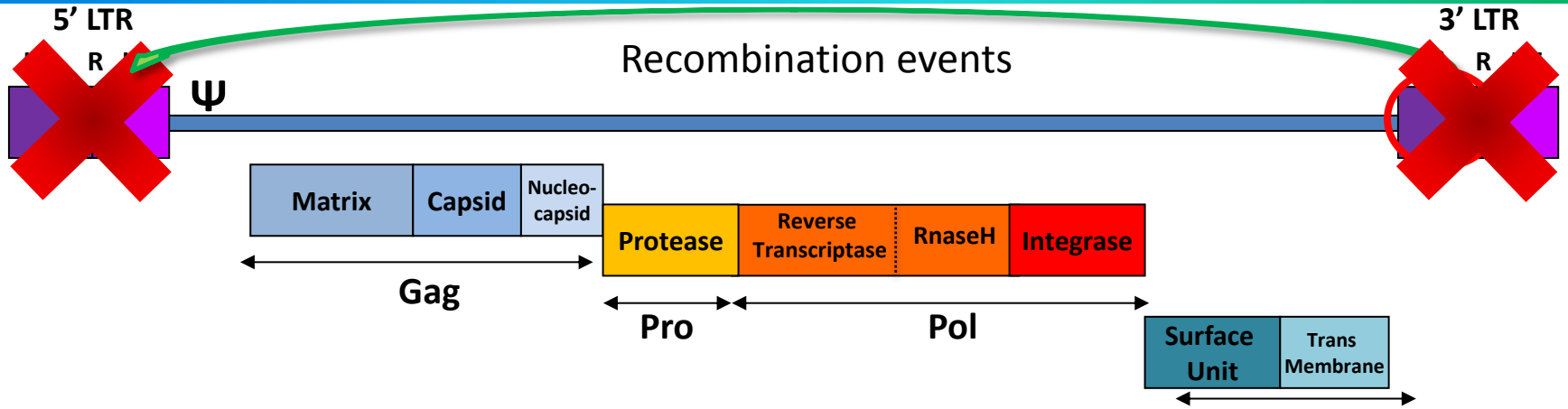

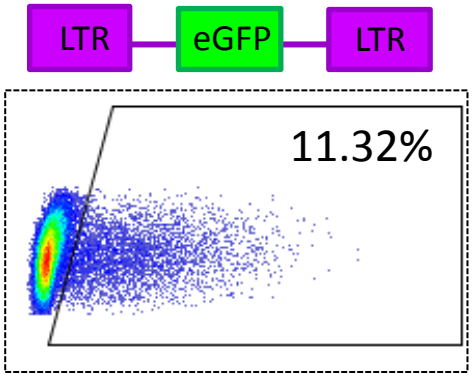

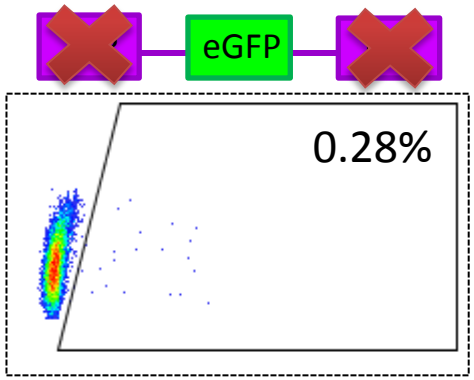

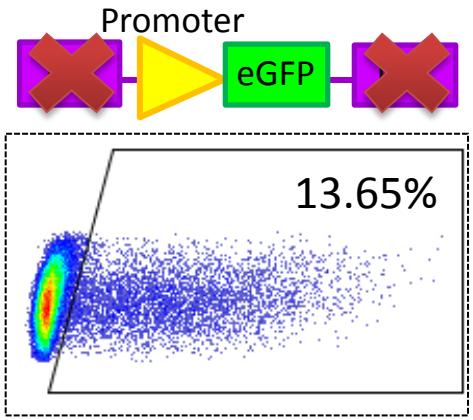

SSC

eGFP

Integration Deficient

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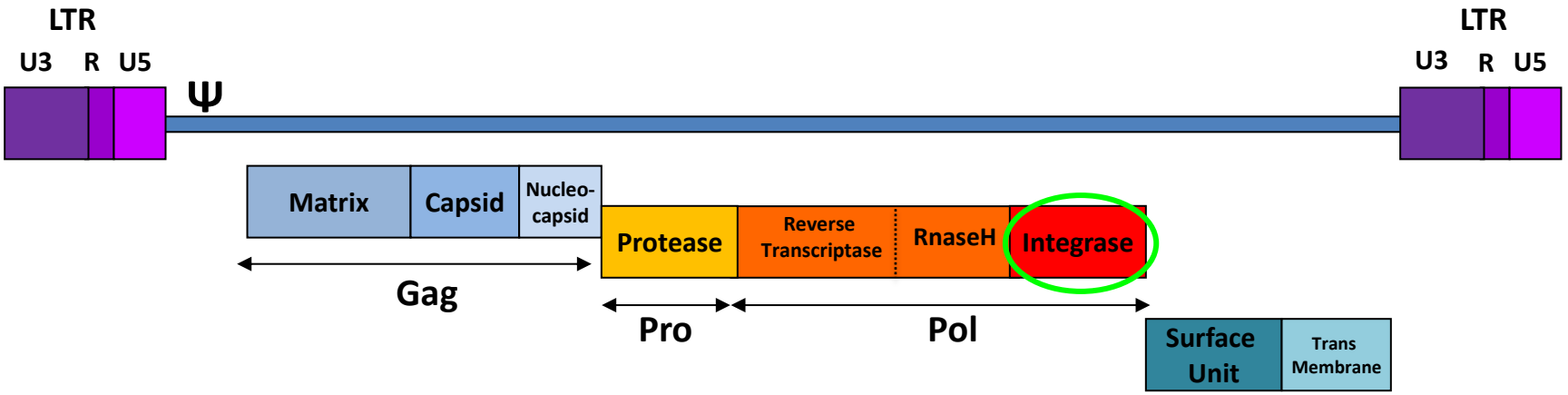


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5751 GAGGGGAATA GATCATTGGC AAGTGGccTA CACTCATTTT GAAGATAAGA
        R   G   I   D   H   W   Q   V   D   Y   T   H   F   E   D   K   I
5801 TATTACTAGT ATGGGTAGAA ACAAATTCGG GATTAATTTA TGCAGAAAGG
        L   L   V   W   V   E   T   N   S   G   L   I   Y   A   E   R
5851 GTGAAAGGGG AGACAGGACA AGAATTTAGA GTAACAGCTA TGAAGTGGTA
        V   K   G   E   T   G   Q   E   F   R   V   T   A   M   K   W   Y
5901 TGCTCTGTTT GCCCCAAAAT CATTGCAATC TGATAATGGG CCAGCATTTG
        A   L   F   A   P   K   S   L   Q   S   D   N   G   P   A   F   V
5951 TAGCAGAAGC AACACAACTG CTAATGAAAT ATTTAGGGAT AATACATACA
        A   E   A   T   Q   L   L   M   K   Y   L   G   I   I   H   T
6001 ACAGGGATAC CTTGGAATCC ACAGTCTCAA GCTCTAGTGG ccAGGGCTCA
        T   G   I   P   W   N   P   Q   S   Q   A   L   V   E   R   A   H
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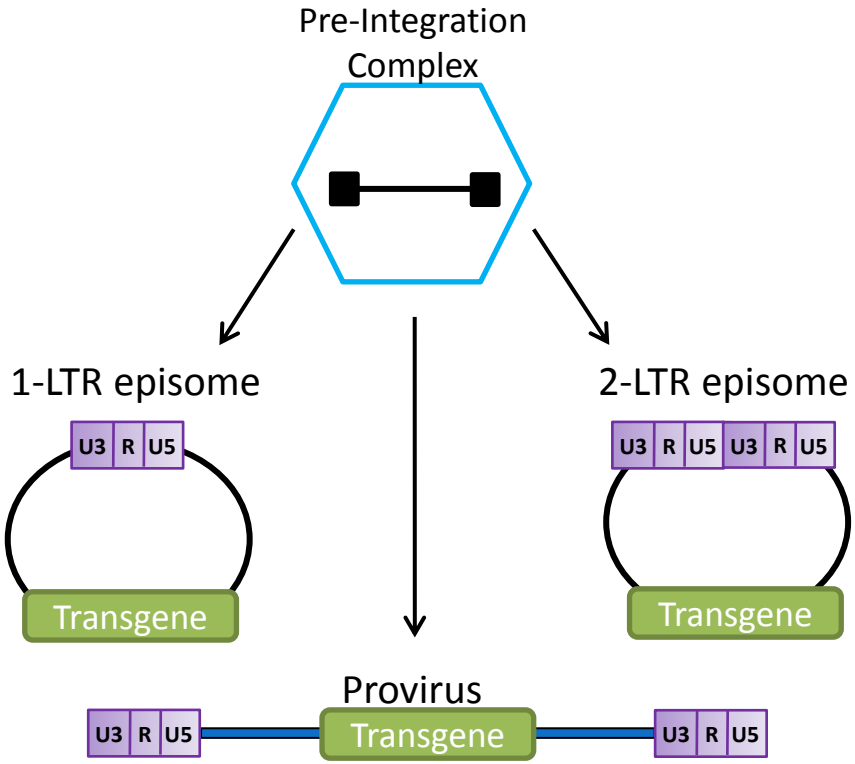
Env

D,D-35-E motif

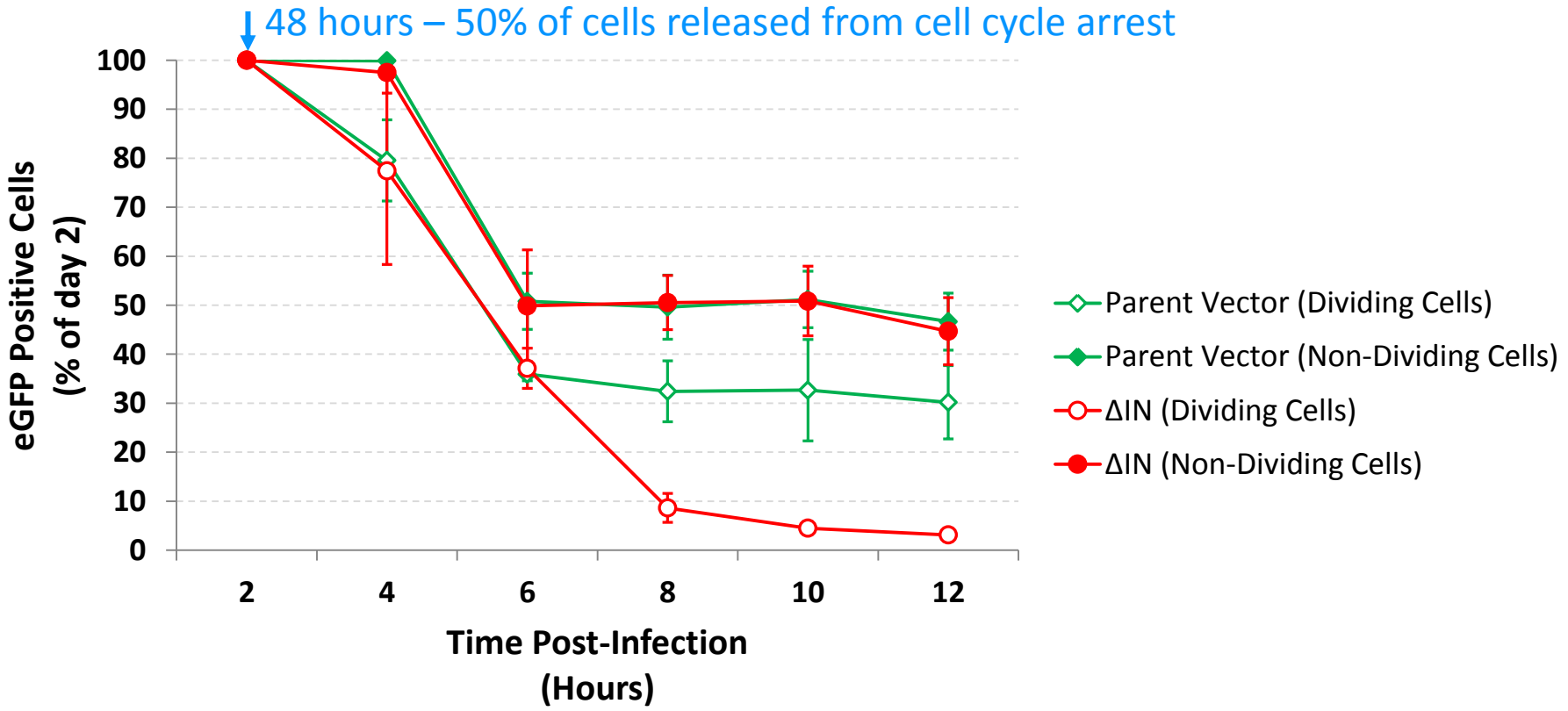
Non-integrated DNA outcome



- Intact viral coding region
  - Transgene expression
- Lack origin of replication (ORI)

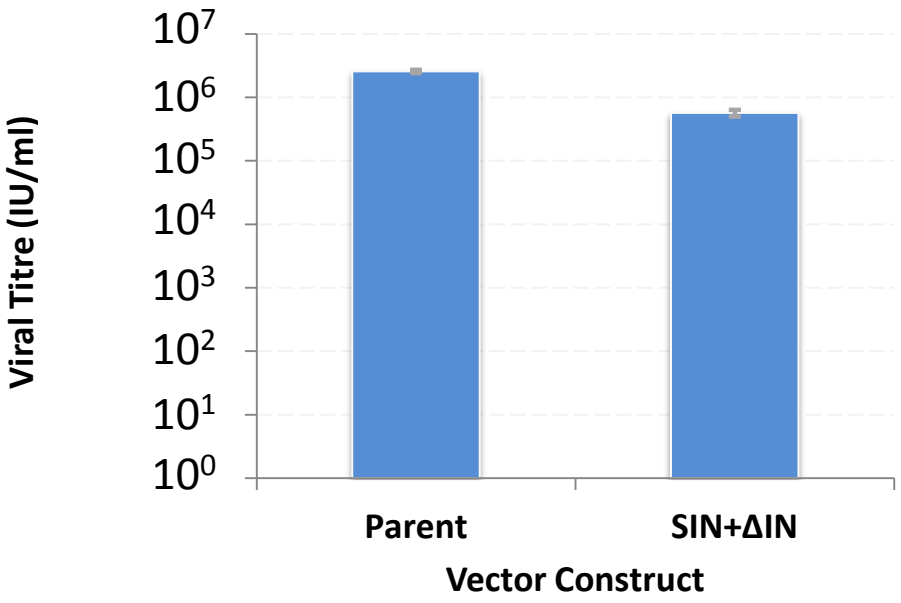
ΔIN Vector - Results

- Dividing and non-dividing CRFK
  - Arrested using aphidicolin
- High multiplicity of infection (MOI=1)
- eGFP-positive cells measured every 2 days for 12 days



Improved Safety

- Self-inactivating (SIN)
- Integration deficient (ΔIN)
- Combined and used to infect CRFK
- Flow analysis at 72 hours post infection



Virus	Construct Type	Viral Titre (IU/ml)	Reduction in titre compared to Parent
HIV-1	Parent	6.2×10^9	270-fold[1]
	SIN+ΔIN	2.3×10^7	
EIAV	Parent	9.3×10^8	290-fold[1]
	SIN+ΔIN	3.2×10^6	
VMV	Parent	2.6×10^6	5-fold
	SIN+ΔIN	5.7×10^5	

1) Ellis *et al.* 2012. Molecular Therapy–Nucleic Acids . 1.

Primary Cells

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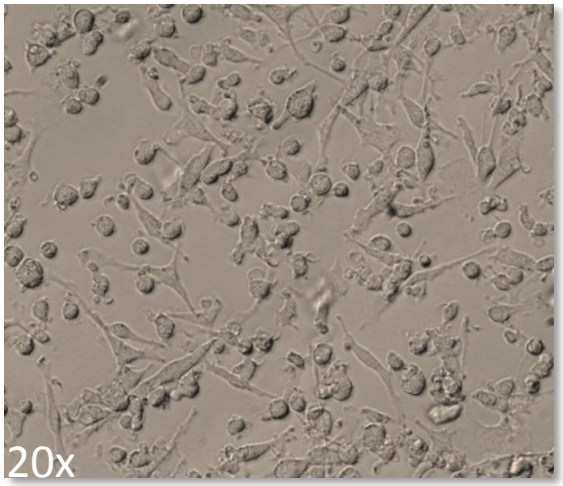
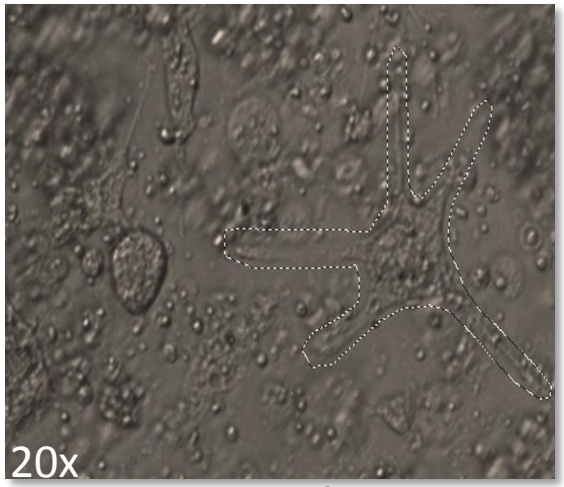
- Ovine peripheral blood
- $CD14^+ \rightarrow$ IL-4 & GM-CSF
- Monocyte derived dendritic cells (DCs)
- Day 6: infect

Day 0



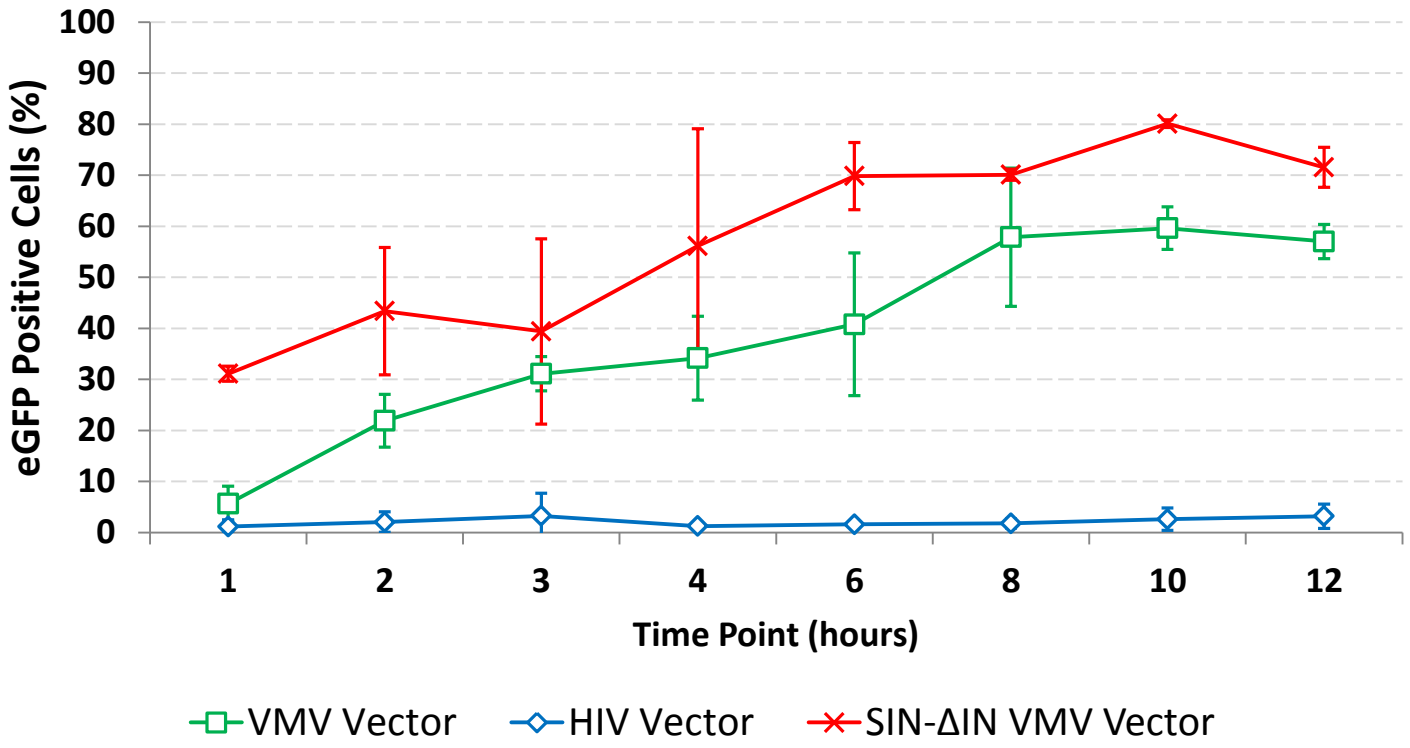
Day 6



Day 9

HIV and VMV Vector comparisons

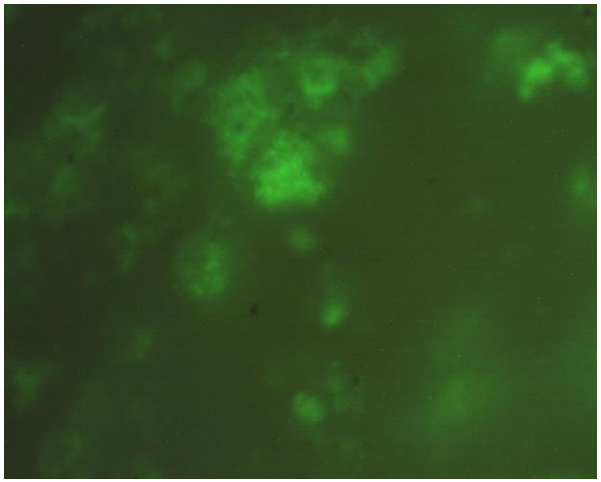
- HIV-1 derived lentiviral vectors commonly used
- MOI=1
- Analysed level of eGFP positive cells over time



High level of cell death

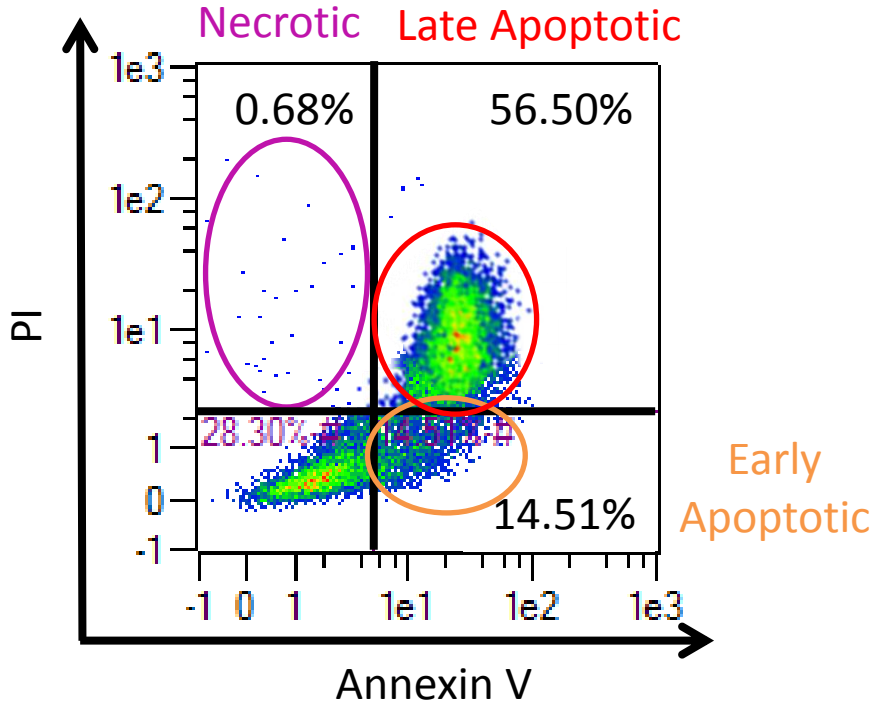
- Flow cytometry analysis
  - 72 hours
  - Only lifted off cells expressing eGFP
  - Large percentage (>90%) of the cells expressing eGFP were dead.

Infected Cells
72 hours post-infection



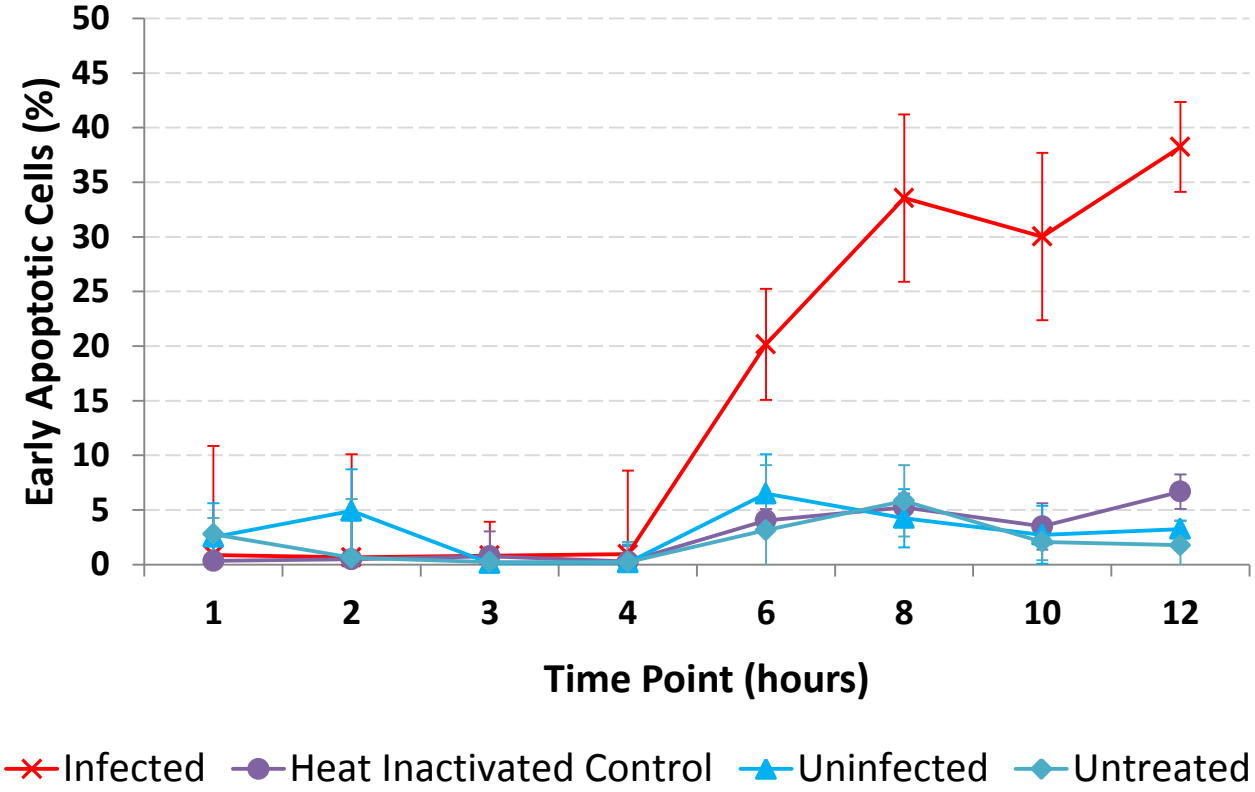
- Mechanism of cell death
- Initial time points – 12 hours and 24 hours

12 hours post-infection

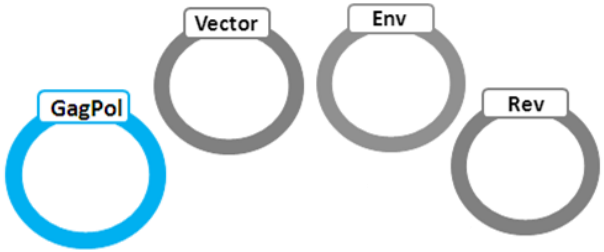


Apoptosis

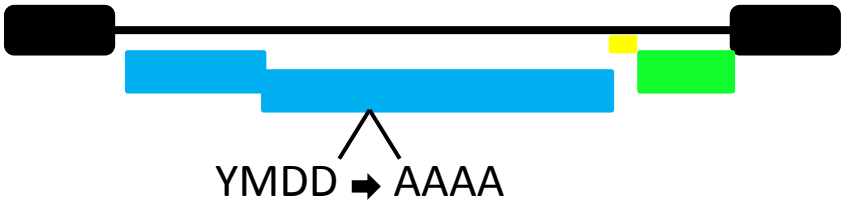
- Time course
- Naturally low levels of apoptosis in all controls
- Infected cells undergo apoptosis due to infection after 4 hours.

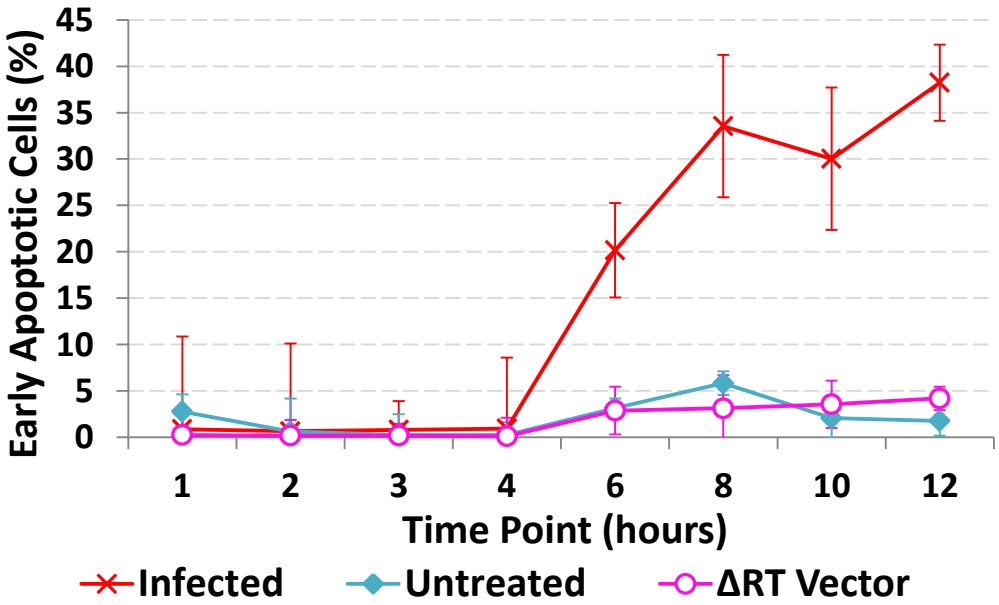


What's inducing cell death?



DNA?



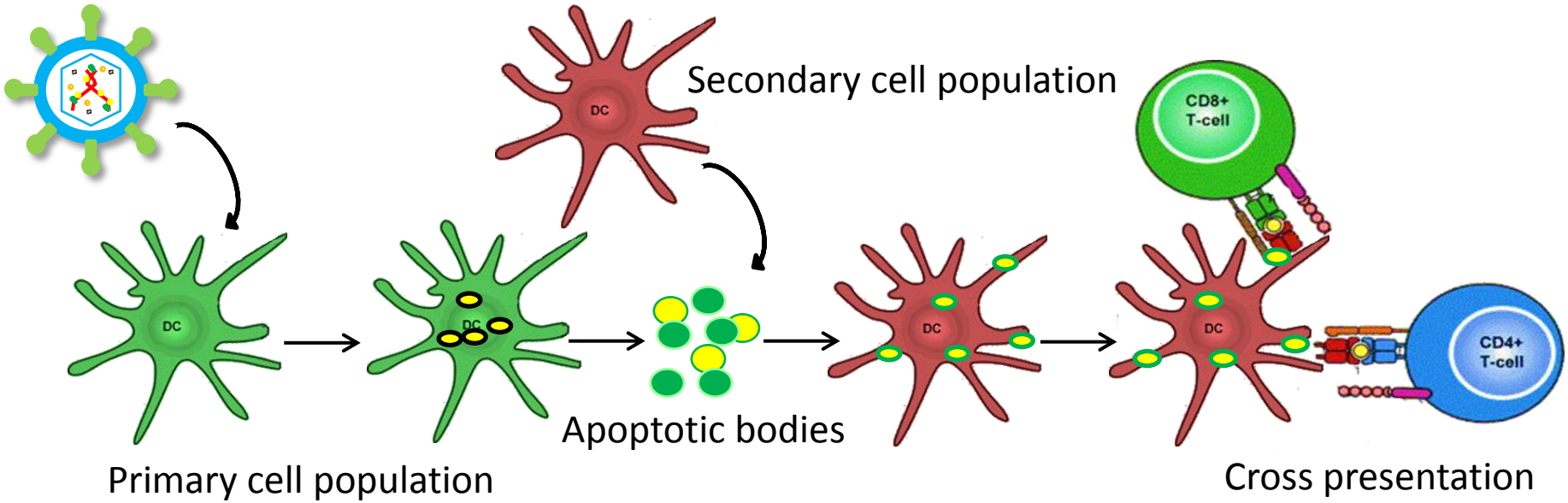


Vector which is unable to reverse transcribe the RNA does not induce apoptosis

Sensing of DNA induces apoptosis

Next Stages

- Some adjuvants induce apoptosis[1]
- Phagocytosis
  - Cross presentation



1) Kool *et al.,* 2008. J Immunology. 181.

Next Stages

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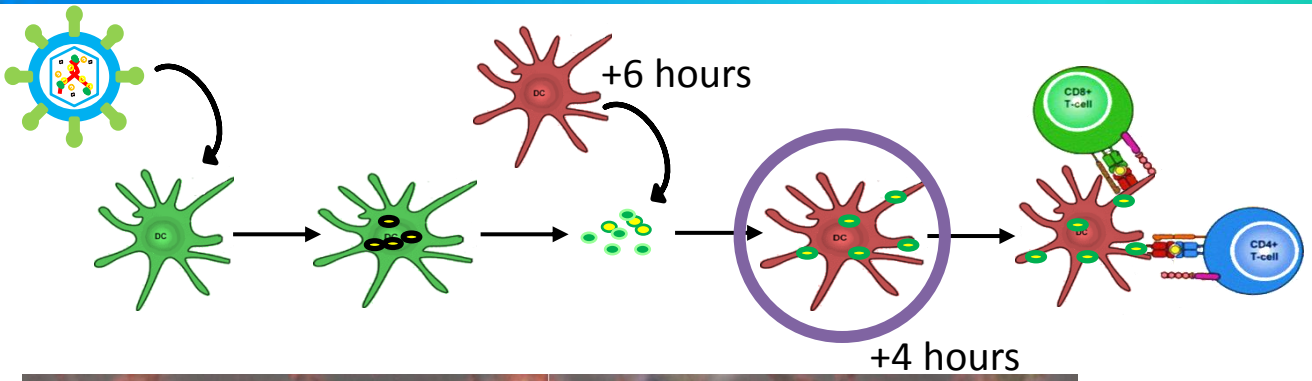

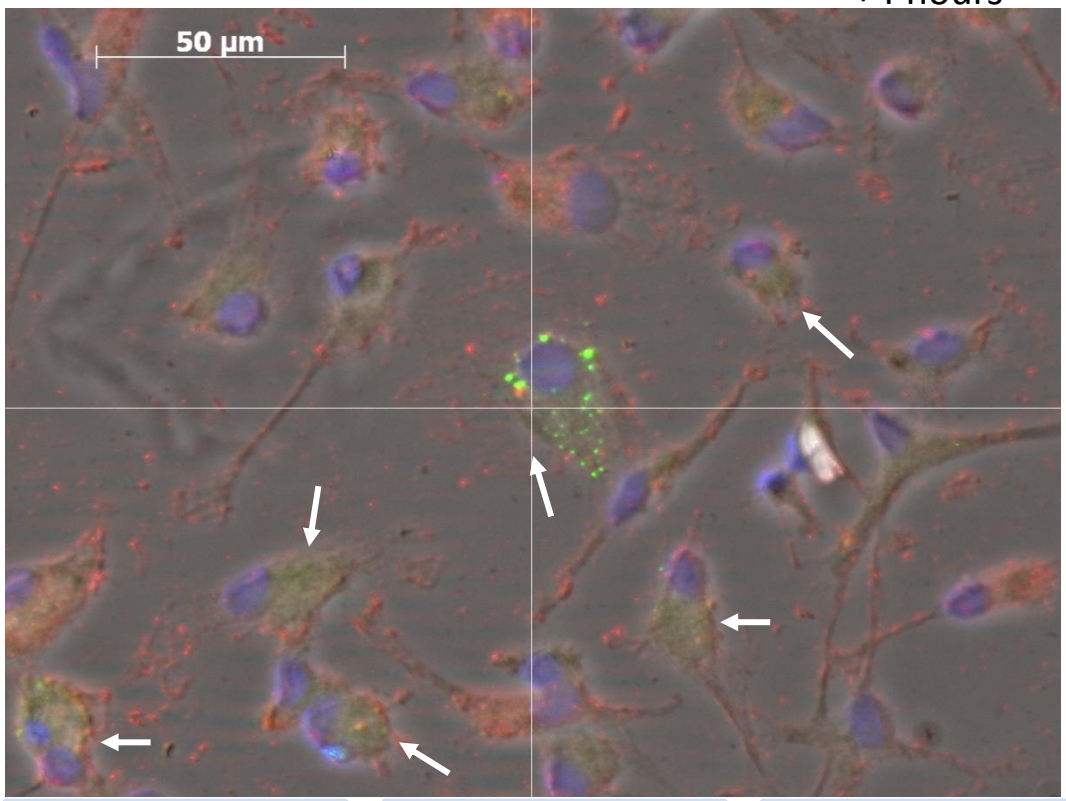

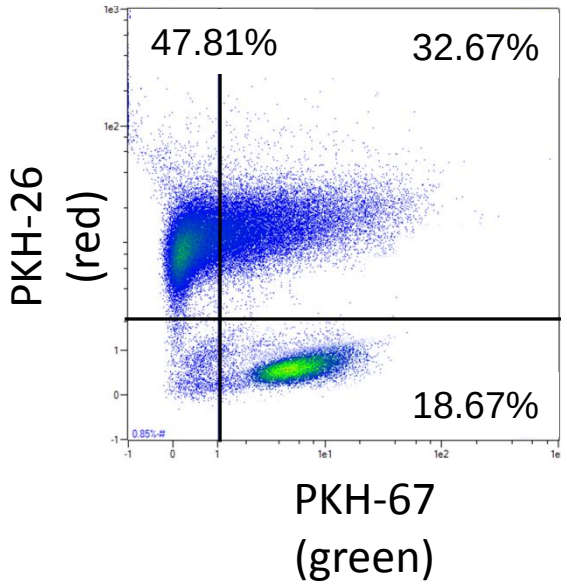

Outputs

- Cloned in a range of pathogen genes:
  - *Chlamydia abortus*
  - *Teladorsagia circumcincta*
  - Louping Ill Virus
  - *Toxoplasma gondii*

- **Improve vaccine efficiency**

Conclusions

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- Ovine lentiviral vectors are promising candidates for gene delivery in ruminants.
  - Integrase deficient and self-inactivating.
- Primary cells can efficiently be infected *in vitro*.
- Infected cells undergo apoptosis after 4 hours of infection.
  - Similar to action of some adjuvants.
- Apoptotic bodies can be phagocytosed.

Acknowledgements

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