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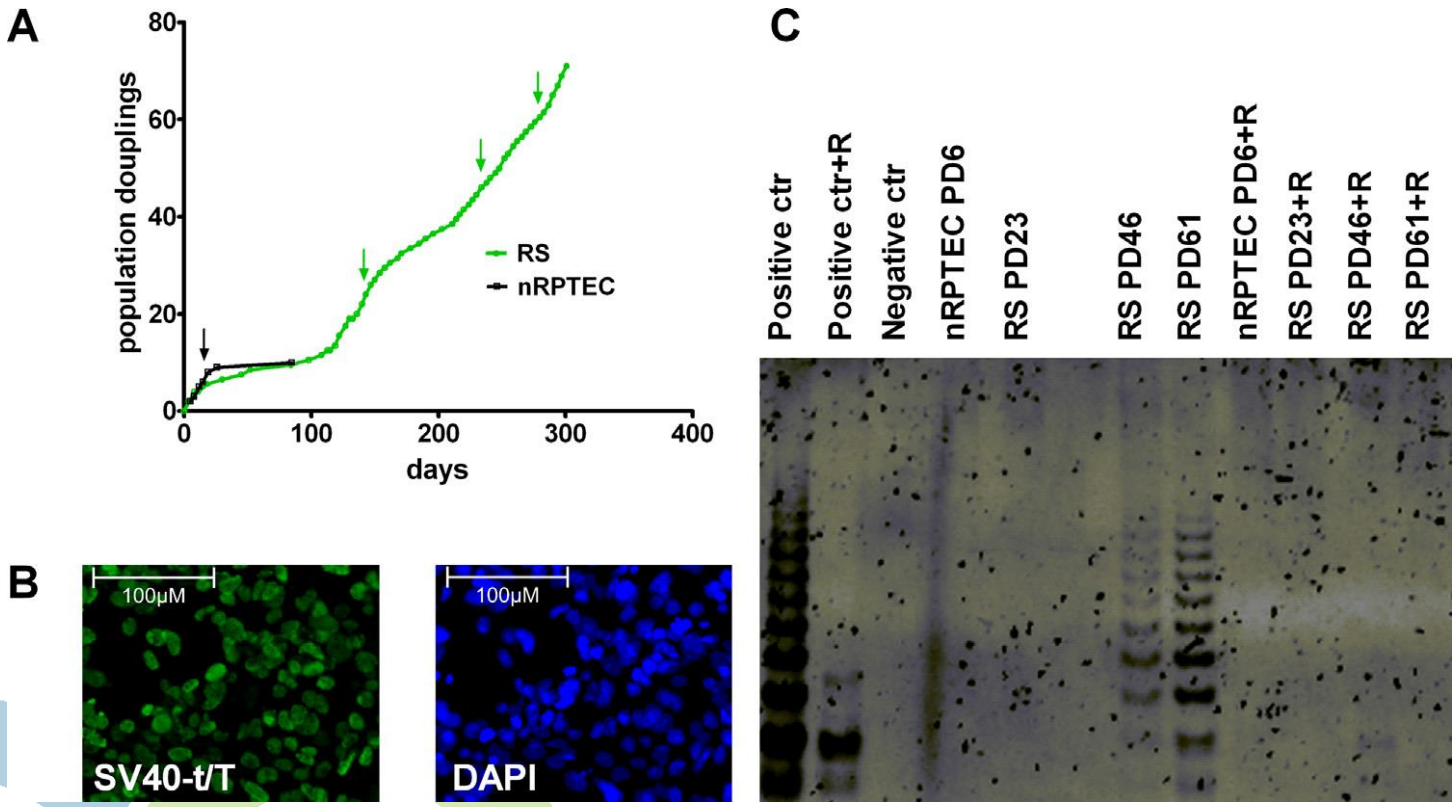
HUMAN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS FOR PRODUCTION OF COMPLEX BIIOPHARMACEUTICALS

„HUMAN PRODUCER SYSTEMS ARE ON THE HORIZON“

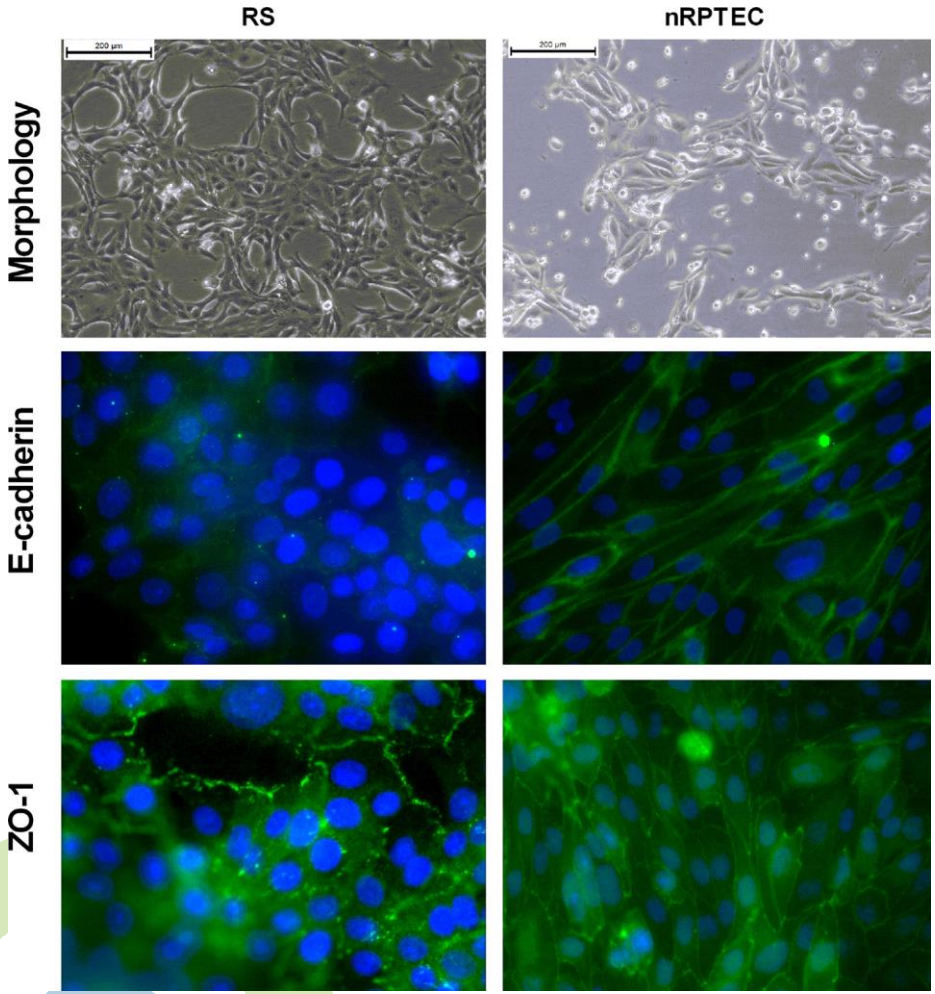
Fliedl et al. N. Biotech 2015

RS cell line
Immortalization of RPTECs with SV40 ER



Fliedl et al. J Biotech 2014

RS cell line
Characterization of cellular phenotype



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RS cell line
Scalable growth

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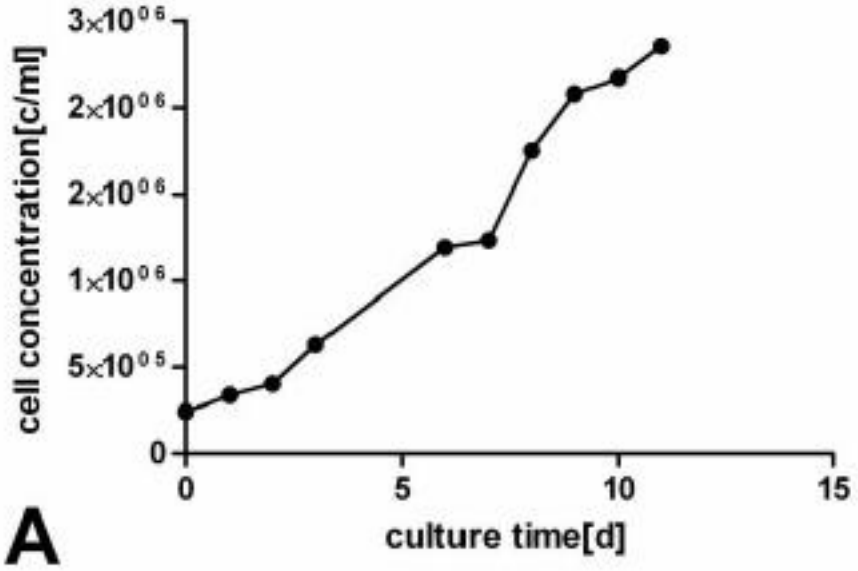
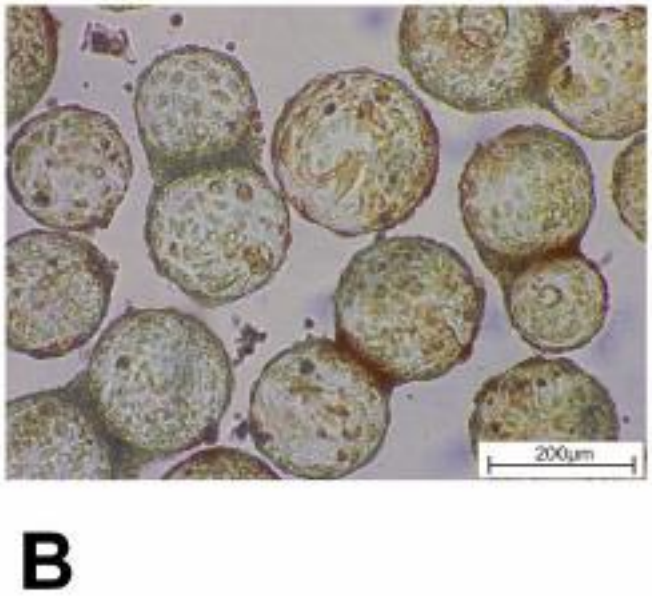
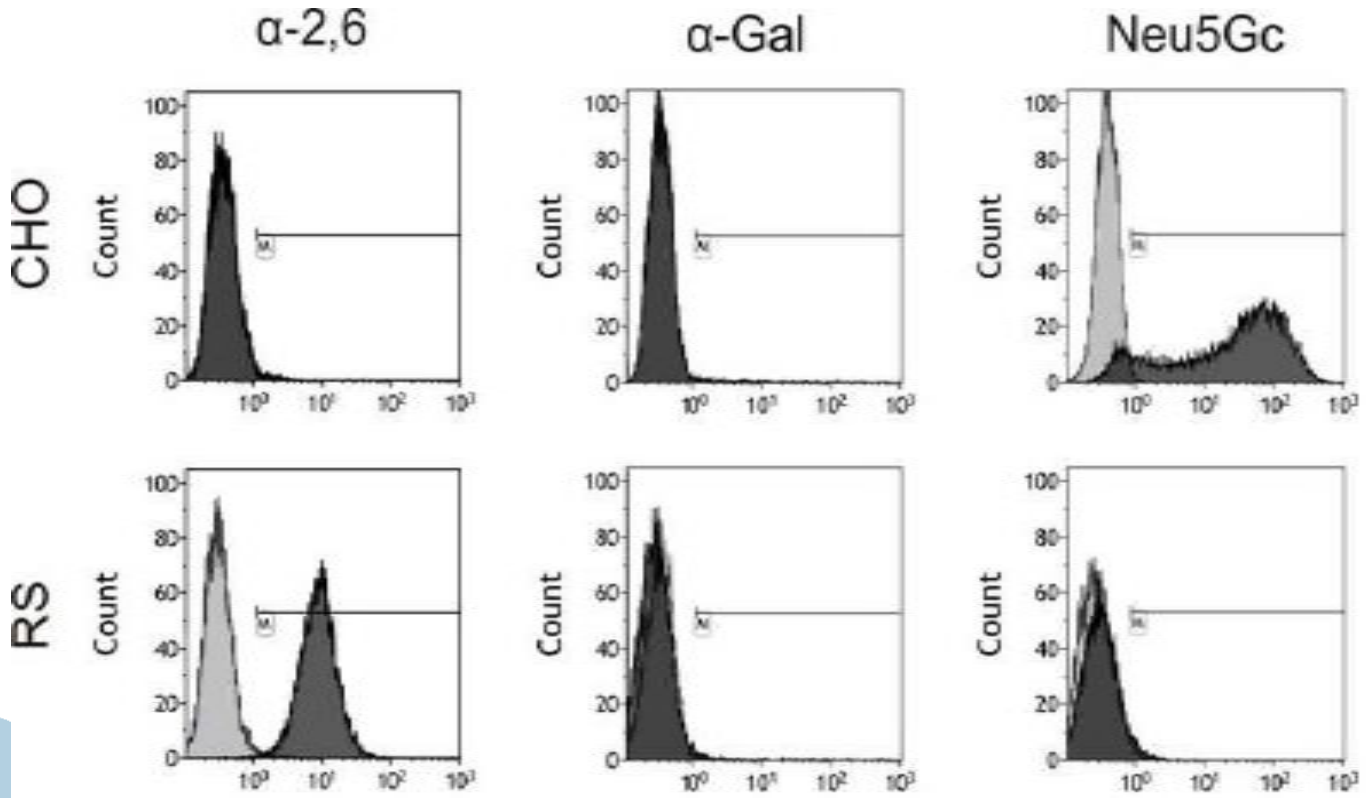




RS cell line
Analysis of specific glycopattern



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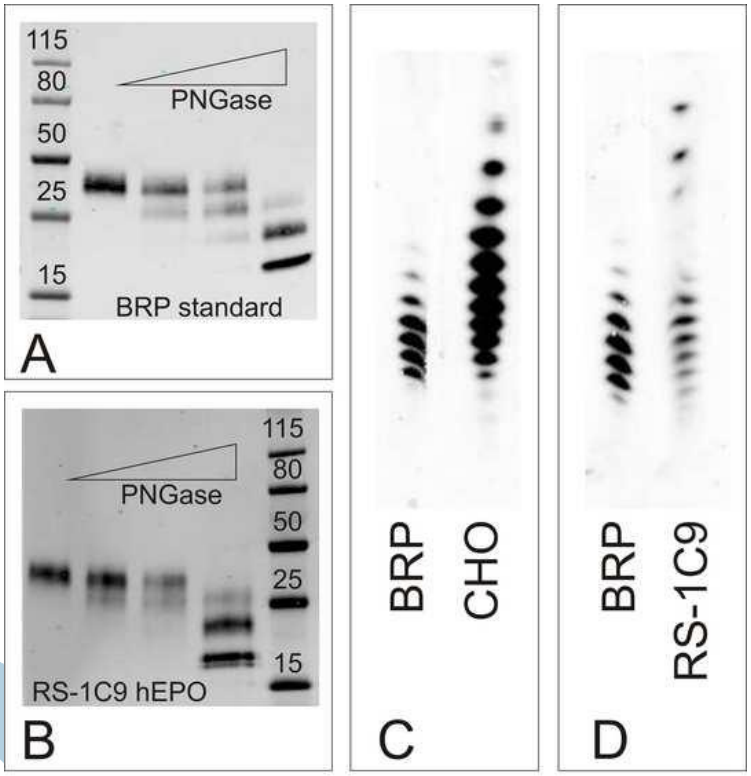
rH EPO produced on RS cells
Characterization of secreted product

Analysis of presence of N-glycans and isoforms



Similar to BRP standard

11% of CHO produced rhEPO
28% of RS produced rhEPO

Fliedl et al. J Biotech 2014

Vaccine production using RS cells

- Cell line characteristics
  - Analysis of cell type specific markers and functions
  - Growth under serum-free culture conditions

- RPTEC-SV40 cell line as host for influenza production
  - Terminal sialic acid linkage – infectability
  - Trypsin tolerance – HA cleavage
  - Proof of concept – with different influenza strains
  - Industrial relevance due to optimal growth characteristics
  - Analysis of produced viral particles
    - Adaptation of (Puerto Rico) PR8 to RPTEC-SV40 cell line
    - Glycoanalysis

Vaccine production
Analysis of terminal sialic acid

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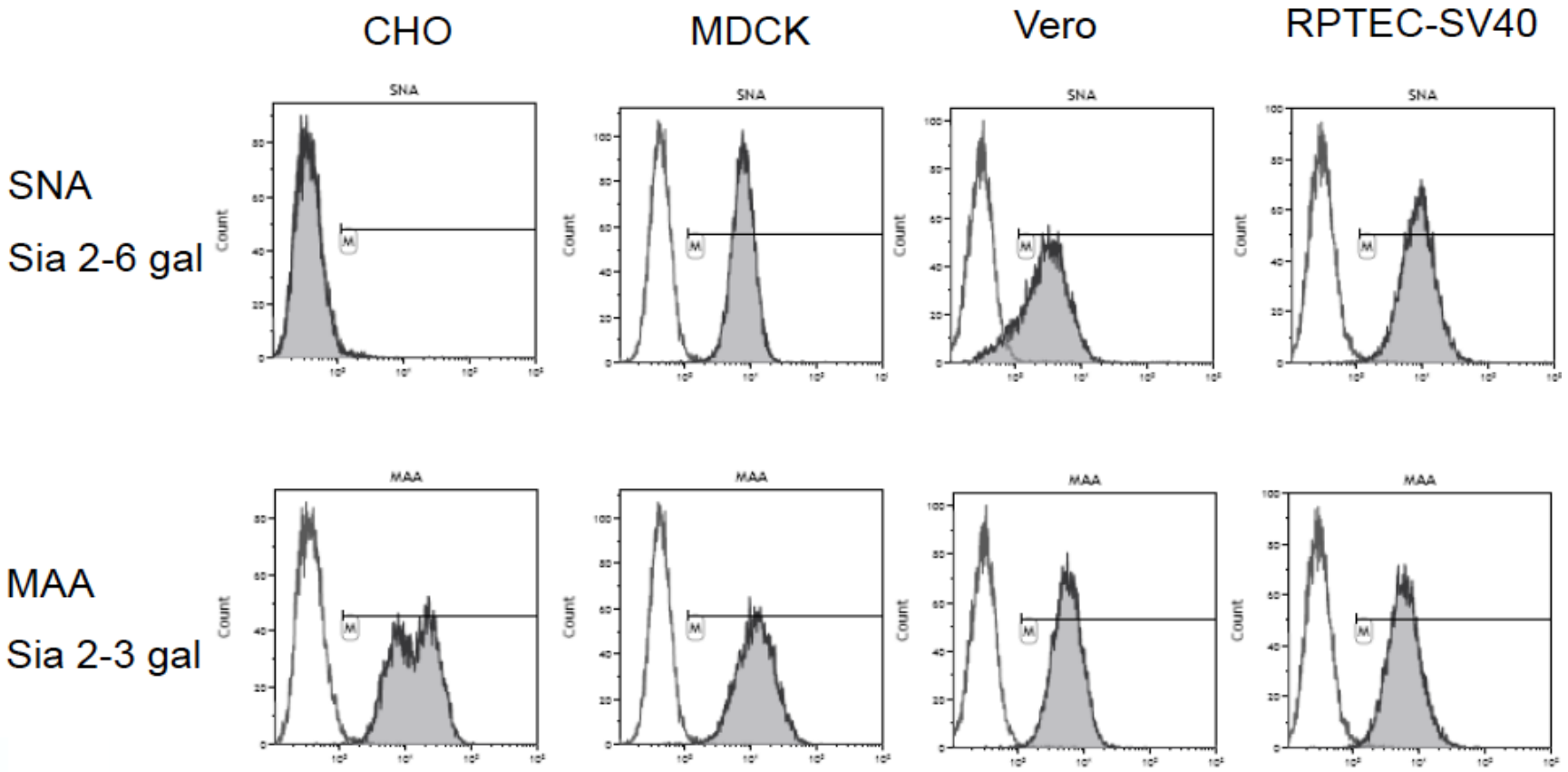

Vaccine production
Analysis of specific glycopattern of RS cells

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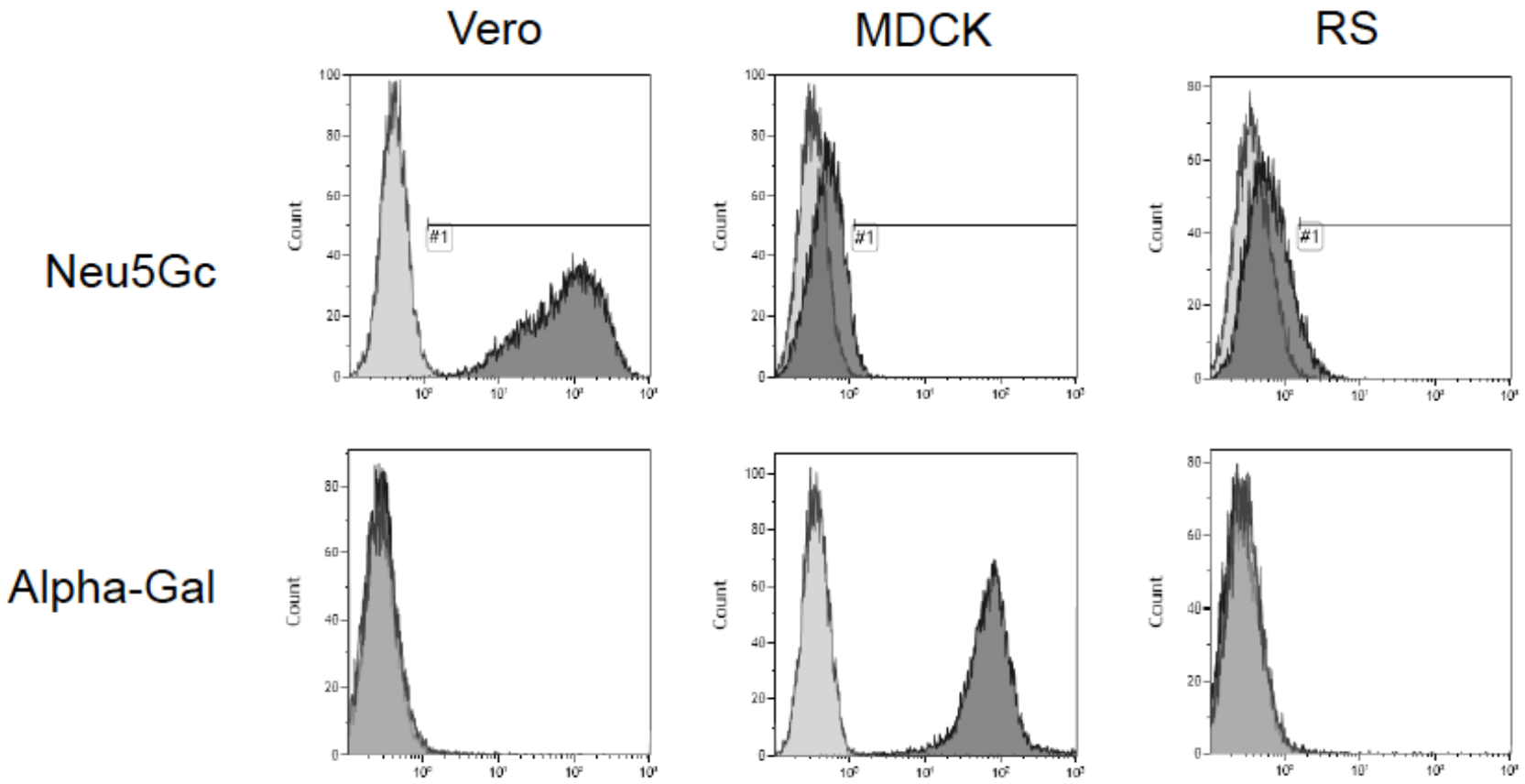

Vaccine production
Trypsin tolerance

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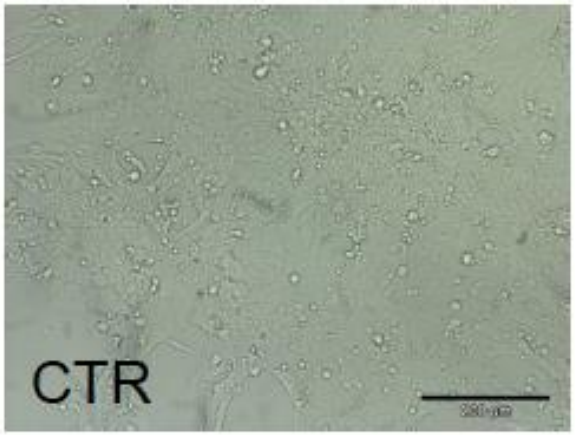

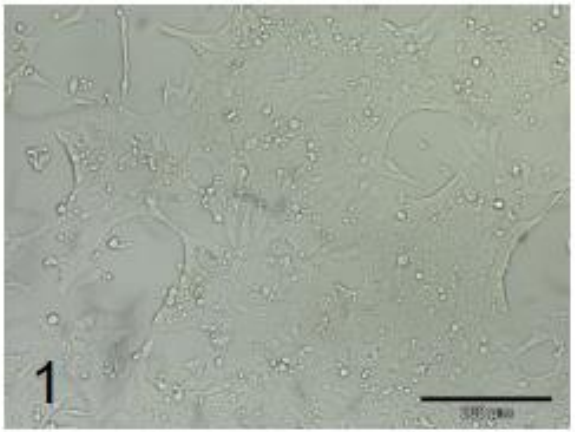

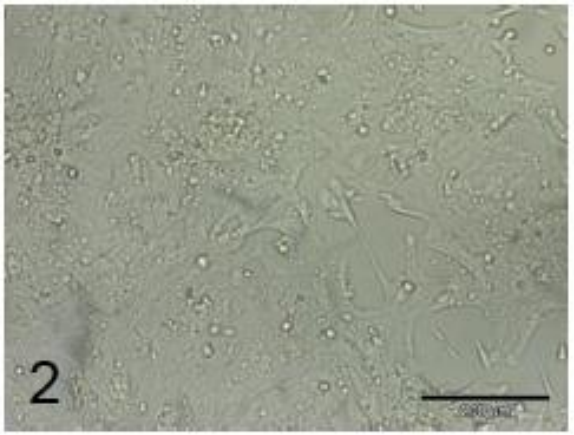

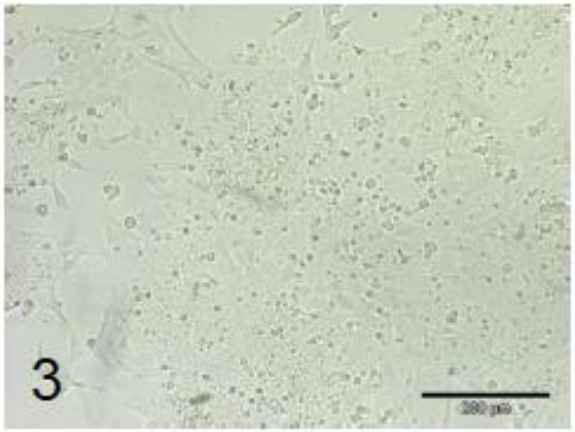

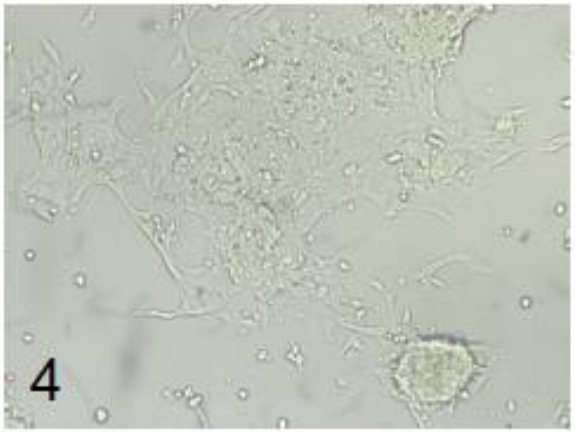

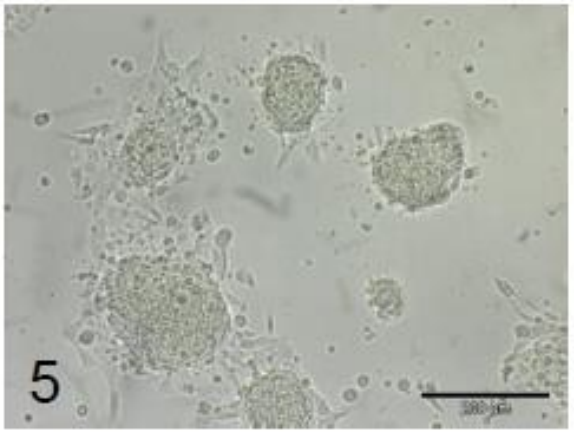

trypsin µg/ml

Vaccine production
Permissive for influenza virus

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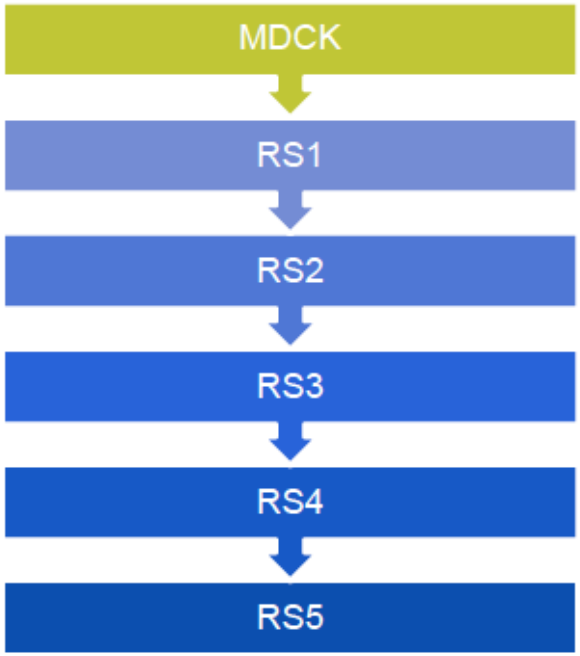
Strain Name	Subtype	NCBI Taxon ID
A/Hiroshima/52/2005	H3N2	487088
A/Moscow/10/1999	H3N2	480019
A/Panama/2007/1999	H3N2	381513
A/Singapore/1/1957	H2N2	382781
A/New Caledonia/20/1999	H1N1	381512
A/Puerto Rico/8/1934	H1N1	211044
B/Panama/45/1990		408929

Vaccine production
Adaptation of PR8



HA - assay

Sample	HAU/ml
AR1	9.39E+03
AR2	2.13E+04
AR3	1.54E+04
AR4	3.58E+04
AR5	1.77E+05

TCID50

Sample	TCID50/ml
AR1	2.72E+06
AR2	1.58E+12
AR3	3.75E+09
AR4	3.75E+09
AR5	2.11E+12

Vaccine production
Detection of HA /WB analysis

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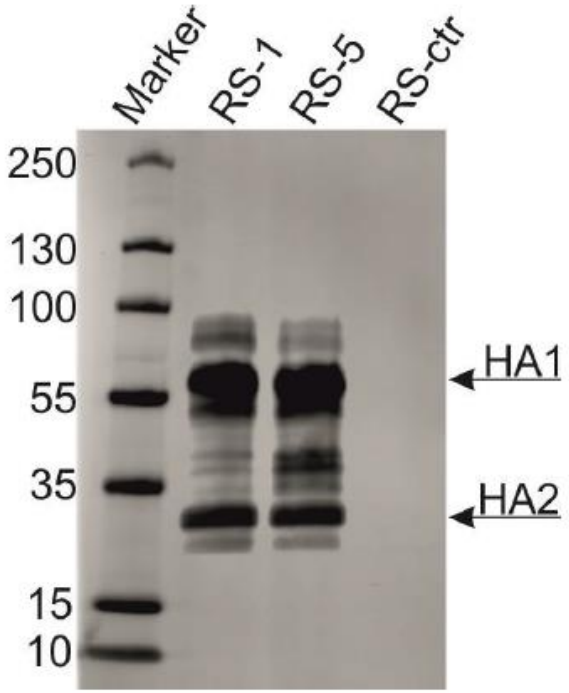

Vaccine production
Glycoanalysis after adaptation

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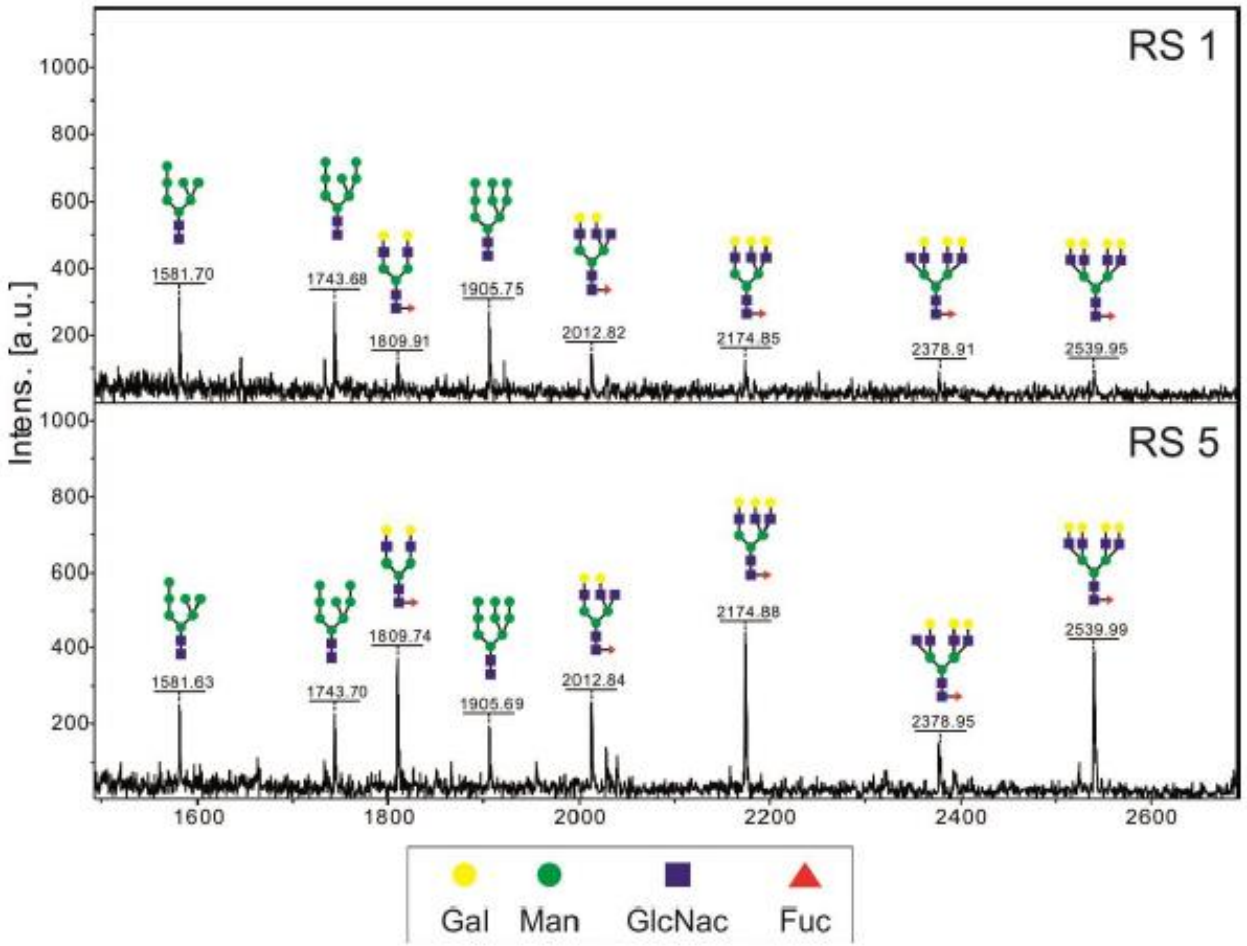

Vaccine production

- Influenza A/PR/8/34 H1N1; MDCK adapted MOI 0.1
  - After 3-4 passages of virus on RS
  - 10^8 CCID50/ml
  - HAU titer 1024/50 µl
  - Compared to:
  - Titer after 3 passages on MDCK, 10^8 DICC50/ml, 64 HAU/50µl
- Influenza A/PR/8/34 H1N1; MOI 0.001
  - After 3-4 passages of virus on RS
  - $3x10^8$ CCID50/ml
  - HAU titer 512/50 µl
  - Compared to MDCK:
  - Titer after 3 passages on MDCK
  - 10^8 DICC50/ml, 64 HAU/50µl
- Similar results with alantanoic fluid adapted virus

Summary

- RS cells are growing
  - Under serum-free conditions
  - Scalable
  - Up to 10^6-10^7 cells/ml in spinner flasks

- RS cells are an option to produce
  - Recombinant proteins
  - Vaccines
  - Helper virus

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Expertise and enthusiasm for your aims

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