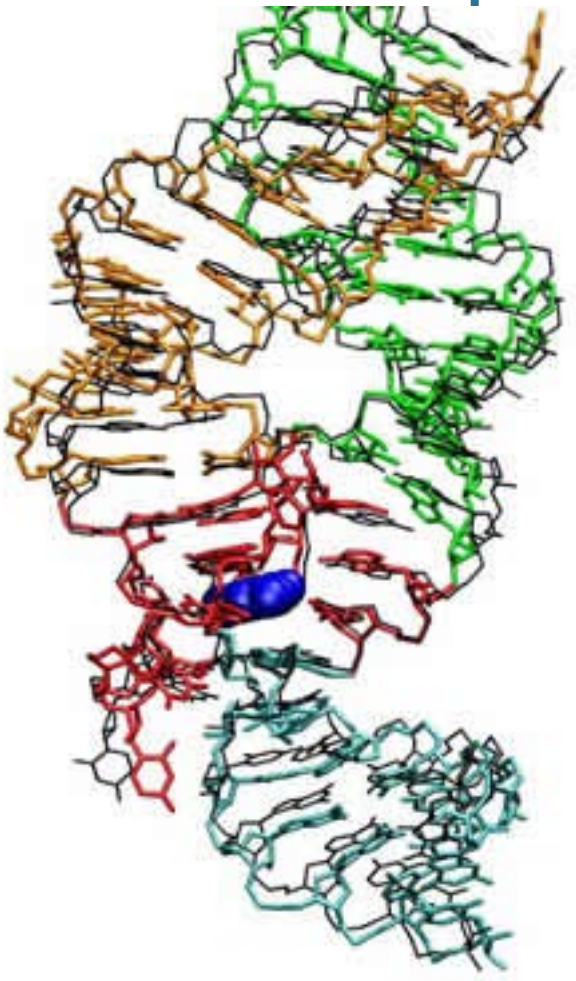
Technical Journal Club 04.11.2014

Engineered Riboswitches and their applications



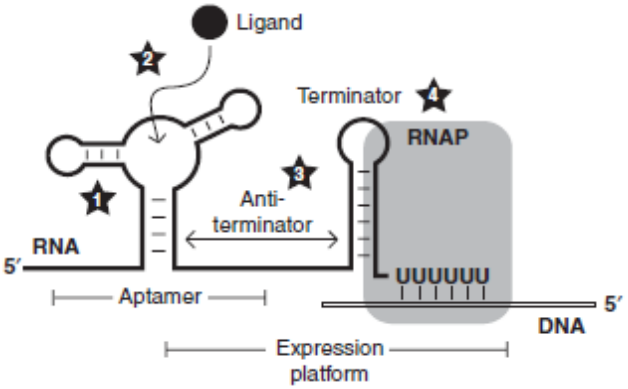
Riboswitches

- Regulatory element of a mRNA, located in the UTR
- Bind a specific small molecule:
  - *coenzymes
  - *nucleobases or derivatives
  - *amino acid
  - *small molecules, vitamins, ions
- Induce a conformation change
- Regulate gene expression in cis
- Requirement: *molecular recognition
  *conformational switching

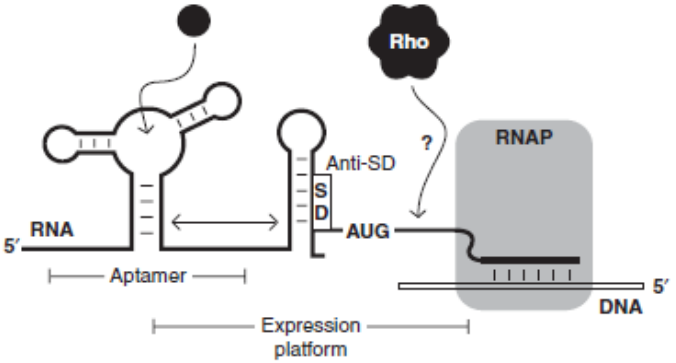
>Ancient sensory and regulatory system

Riboswitch mediated gene control

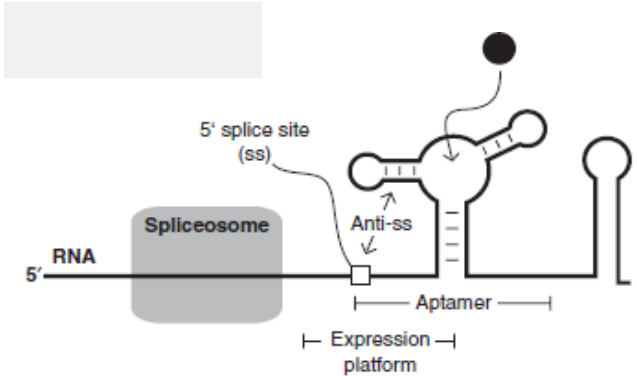
Transcription termination



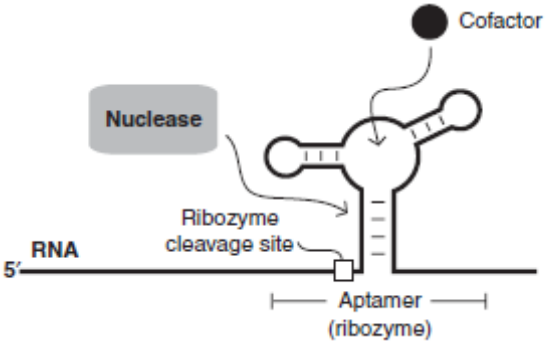
Translation initiation



Splicing eukarya

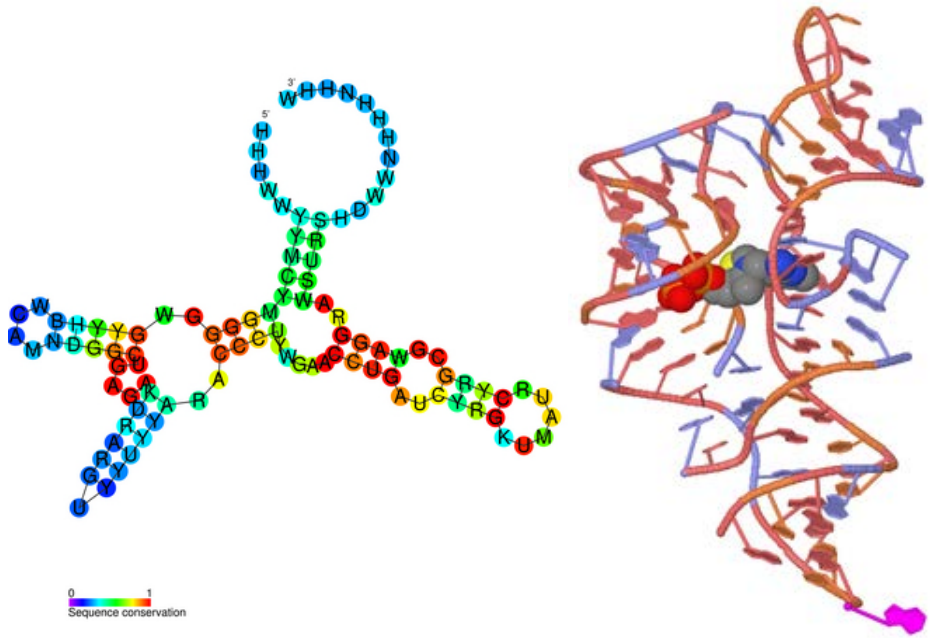


Self-cleaving

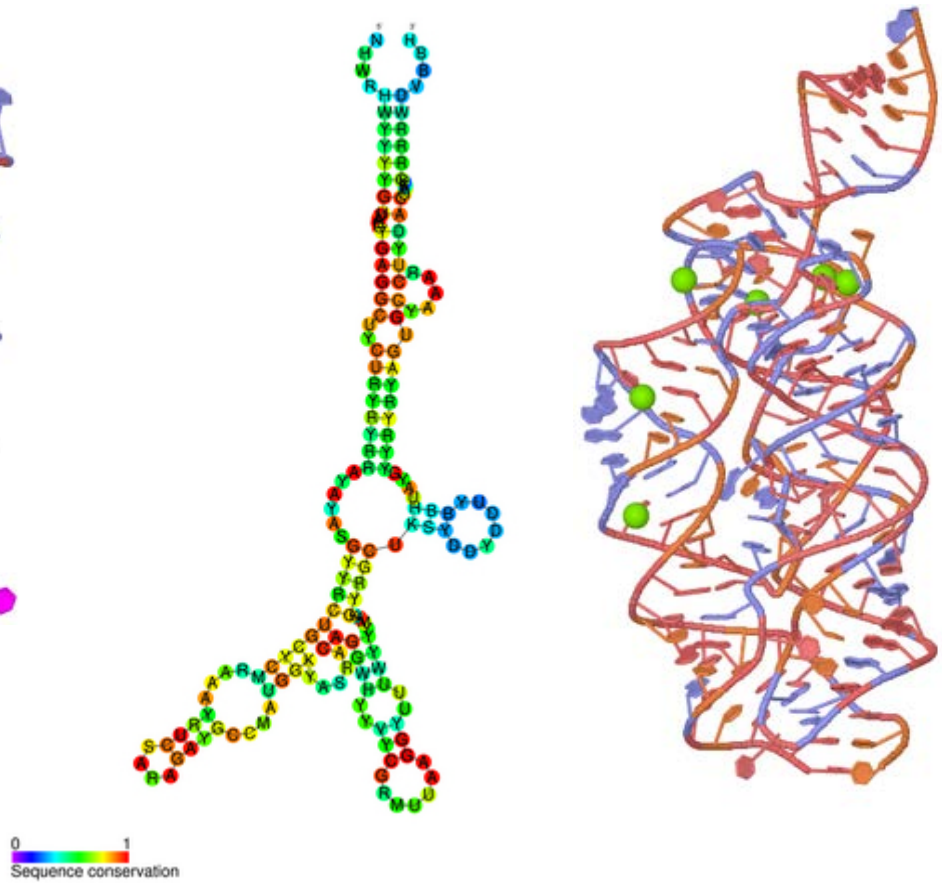


Exemples of Riboswitch

TPP riboswitch



YkoK leader



Engineering Riboswitch

>Chemical simplicity: only 4 ribonucleotides

>Structural flexibility and modularity:

- Secondary and tertiary structure
- Easy design

>Predictable structure-function relationship

>Can be used for monitoring and programing cell behavior

Science
AAAS

Reprogramming Cellular Behavior with RNA Controllers Responsive to Endogenous Proteins

Stephanie J. Culler,[1] Kevin G. Hoff,[1] Christina D. Smolke[1,2]*

26 NOVEMBER 2010

- Aim:

Non-invasive sensing of disease markers and reprogramming cellular fate by pre-mRNA splicing regulation

- Requirements:

1) inputs/outputs functionalities

2) Regulatory properties: able to control the cellular behavior

3) Sensitive to endogenous protein concentration or localization changes

- Approach:

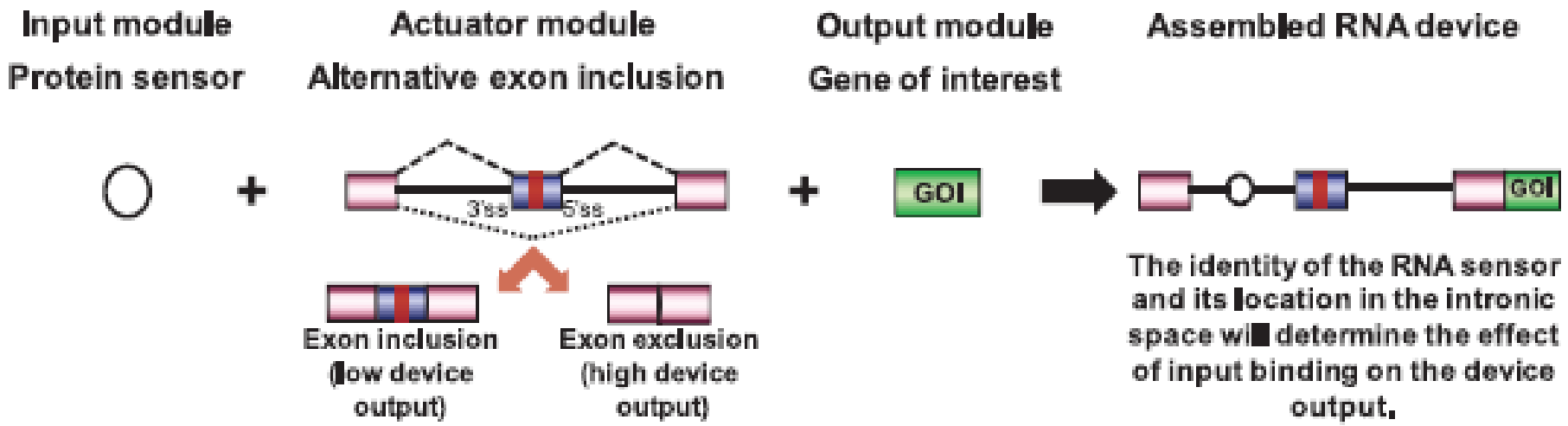
>Couple an increase of protein abundancy with a targeted gene expression through regulation of alternative splicing

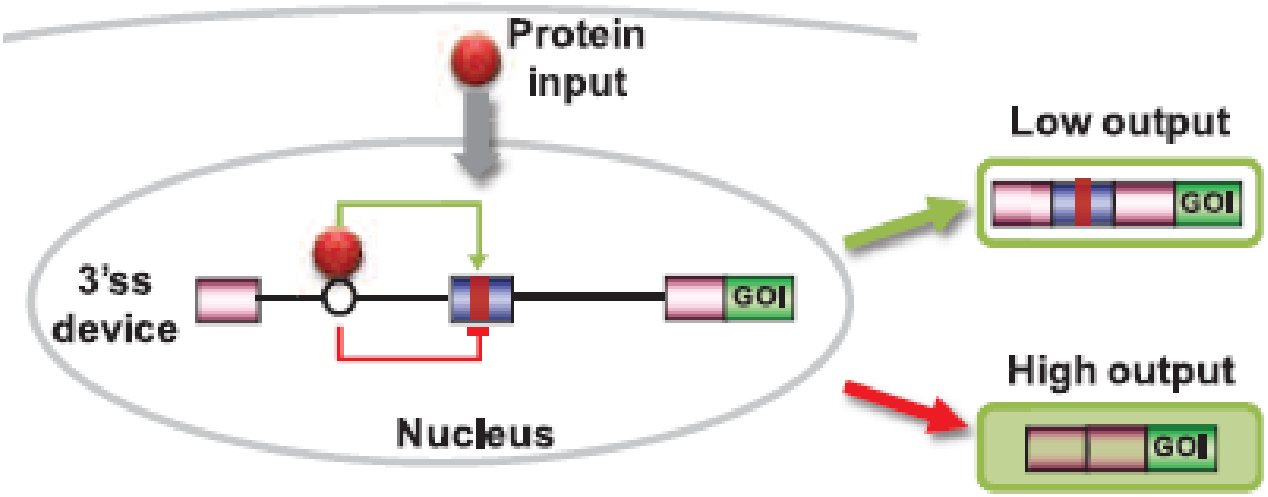
>RNA devices detect signaling through NFkB and Wnt pathways

*Rewire these pathways

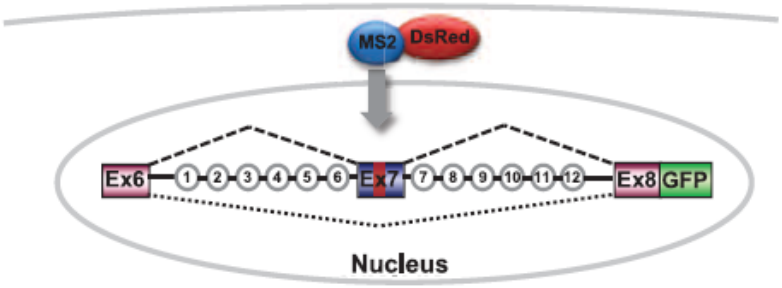
*Create new behaviors

Strategy


Input module
Protein sensor
Actuator module
Alternative exon inclusion
Output module
Gene of interest
Assembled RNA device
3'ss
5'ss
GOI
GOI
Exon inclusion (low device output)
Exon exclusion (high device output)
The identity of the RNA sensor and its location in the intronic space will determine the effect of input binding on the device output.


Protein input
3'ss device
GOI
Nucleus
Low output
GOI
High output
GOI

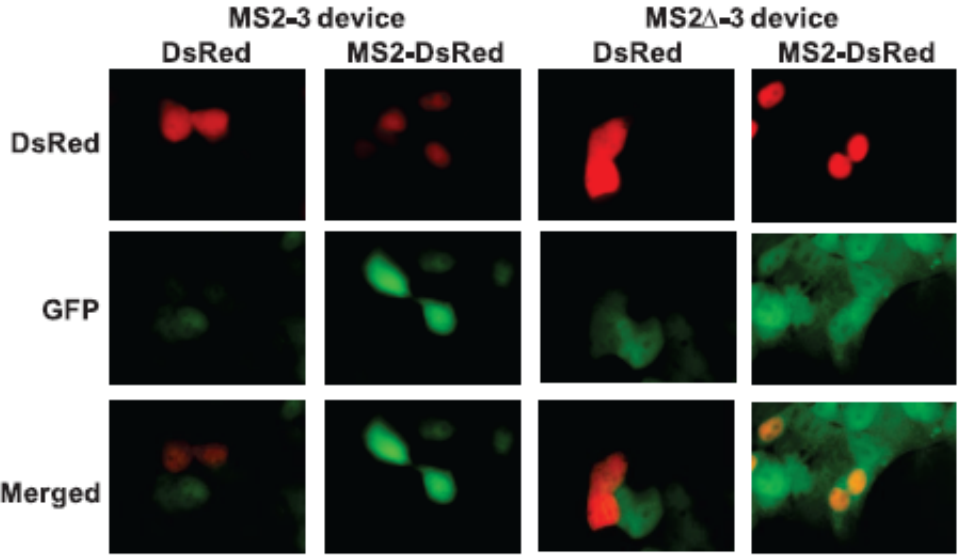
Protein-specific effect on Splicing using MS2 protein

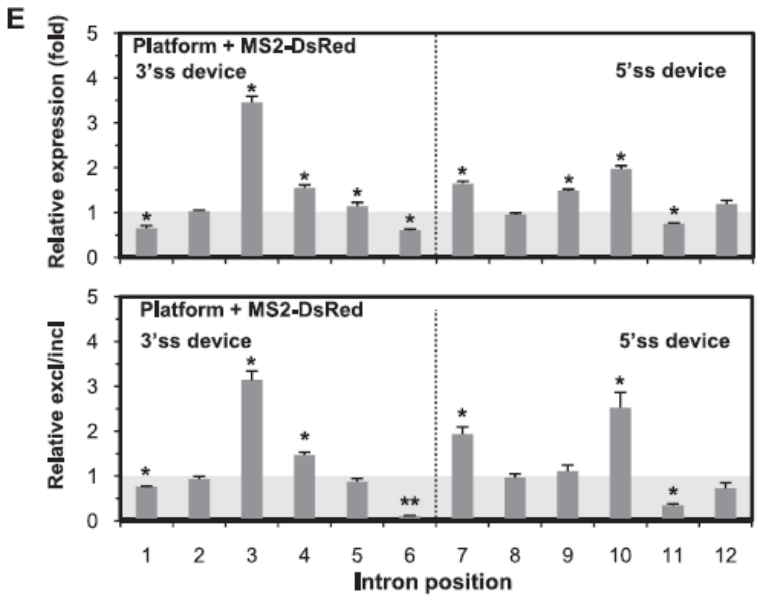


SMN1 gene

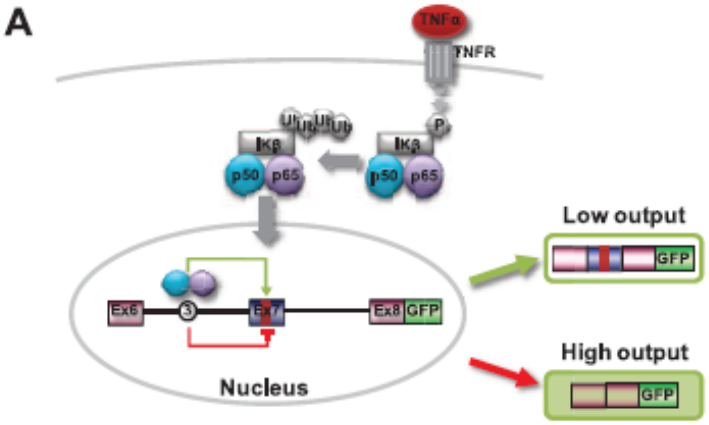
* Aptamers of MS2 inserted in introns
*Transfection of MS2+DS-red plasmid

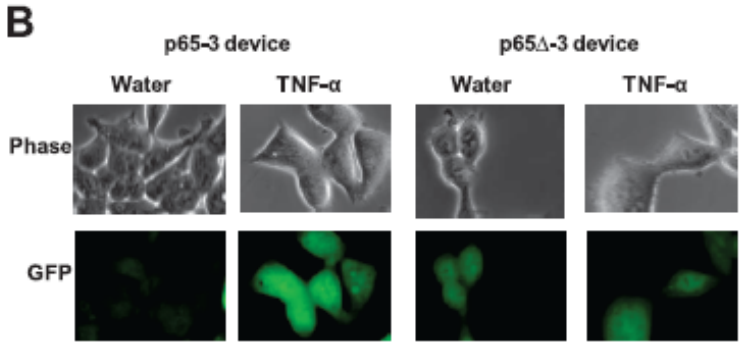
>GFP increase specific to WT aptamer
>Correlation fluorescence, gene expression and splicing pattern
>Position 3,6,10





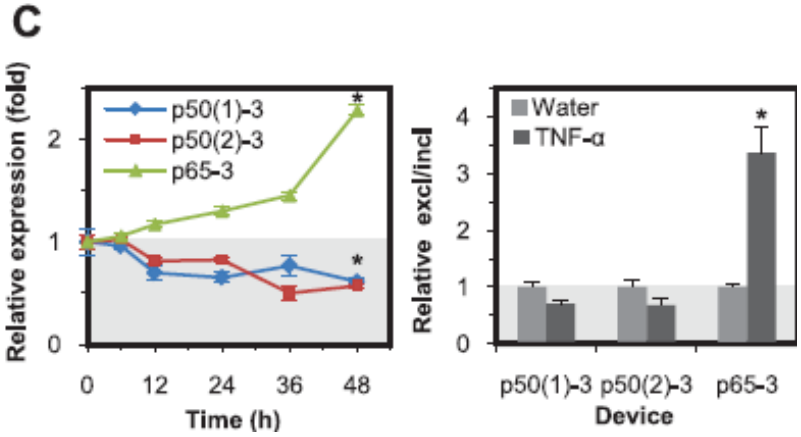
Nuclear detection of NFκB pathway activation

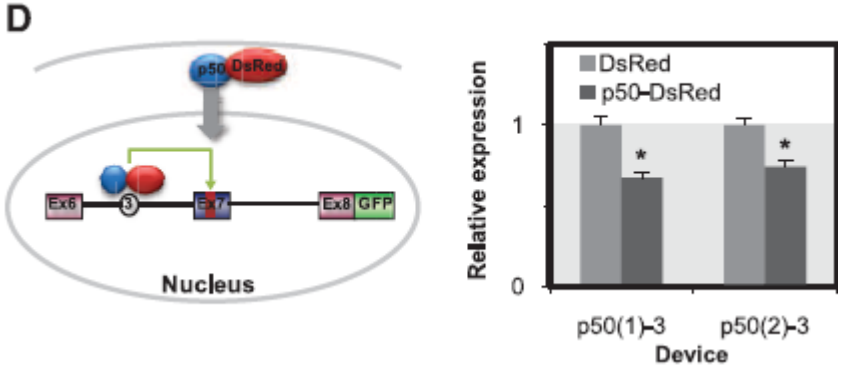




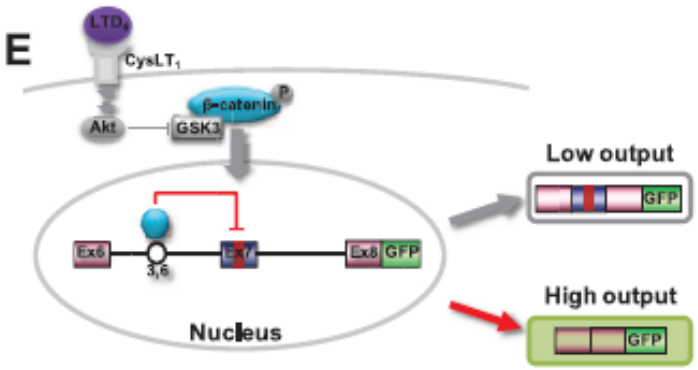
*RNA aptamer binding p50 or p65 subunit in position 3
*TNF stimulation of transfected HEK293

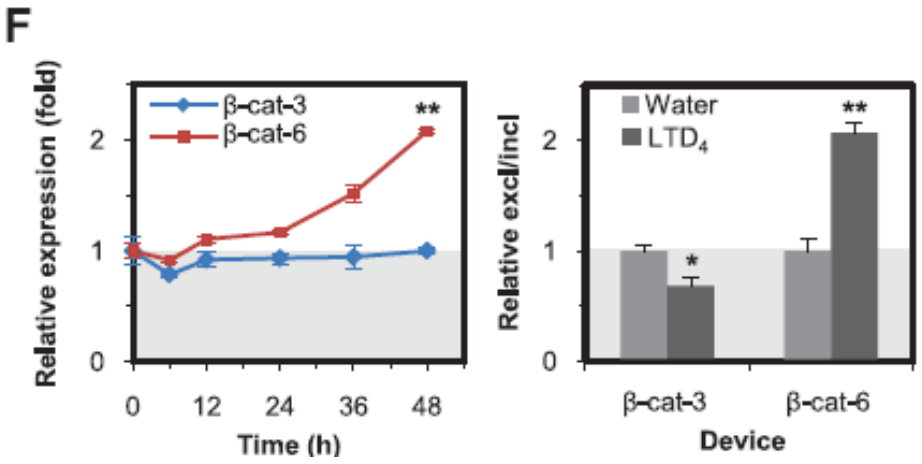
>GFP increase specific to WT aptamer
>Correlation fluorescence, gene expression and splicing pattern
>p50 device reduce GFP expression





Confirmation with Wnt pathway by detecting nuclear β-catenin



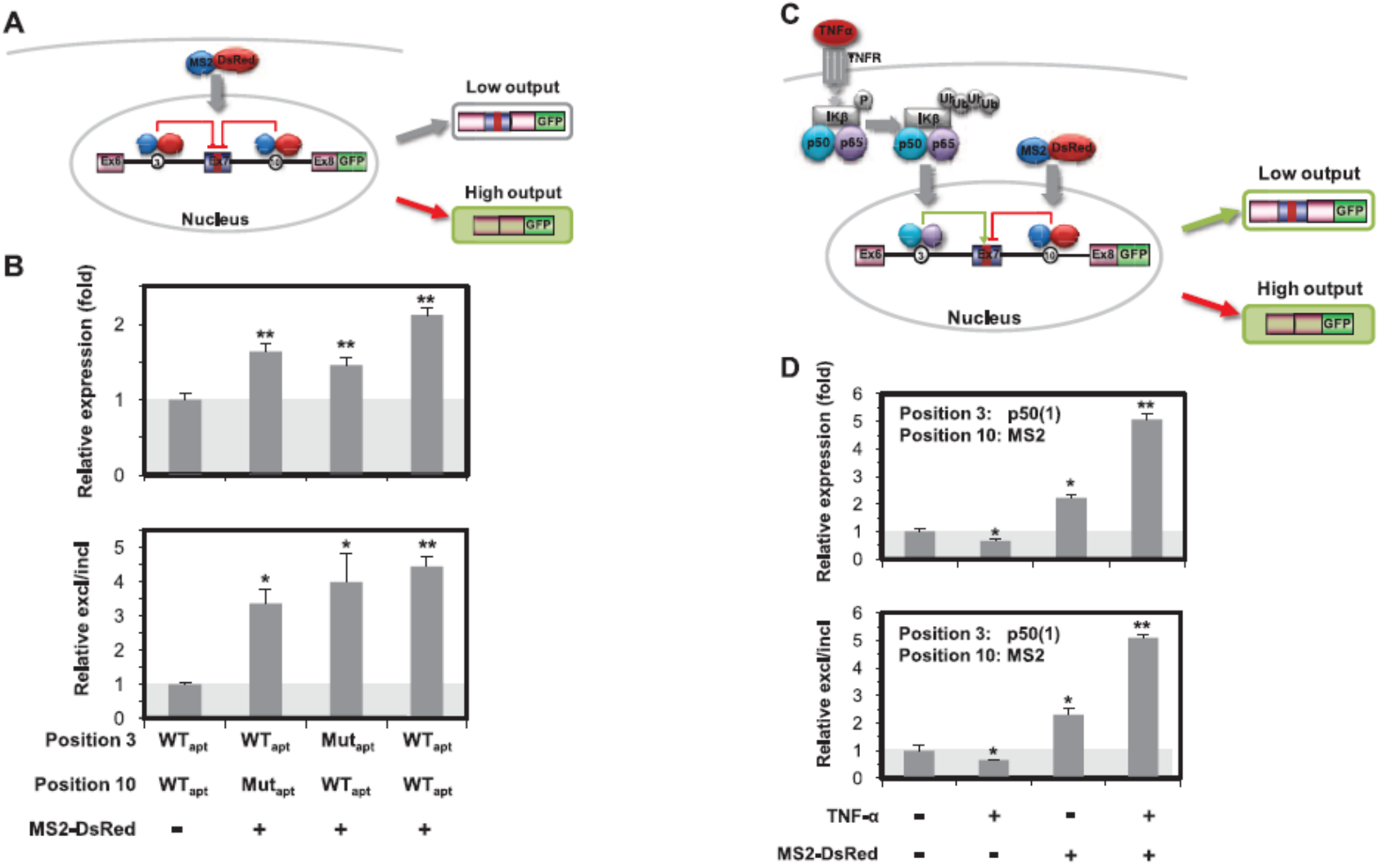


*RNA aptamer in position 3 or 6
*Leukotriene D4 (LTD4) stimulation

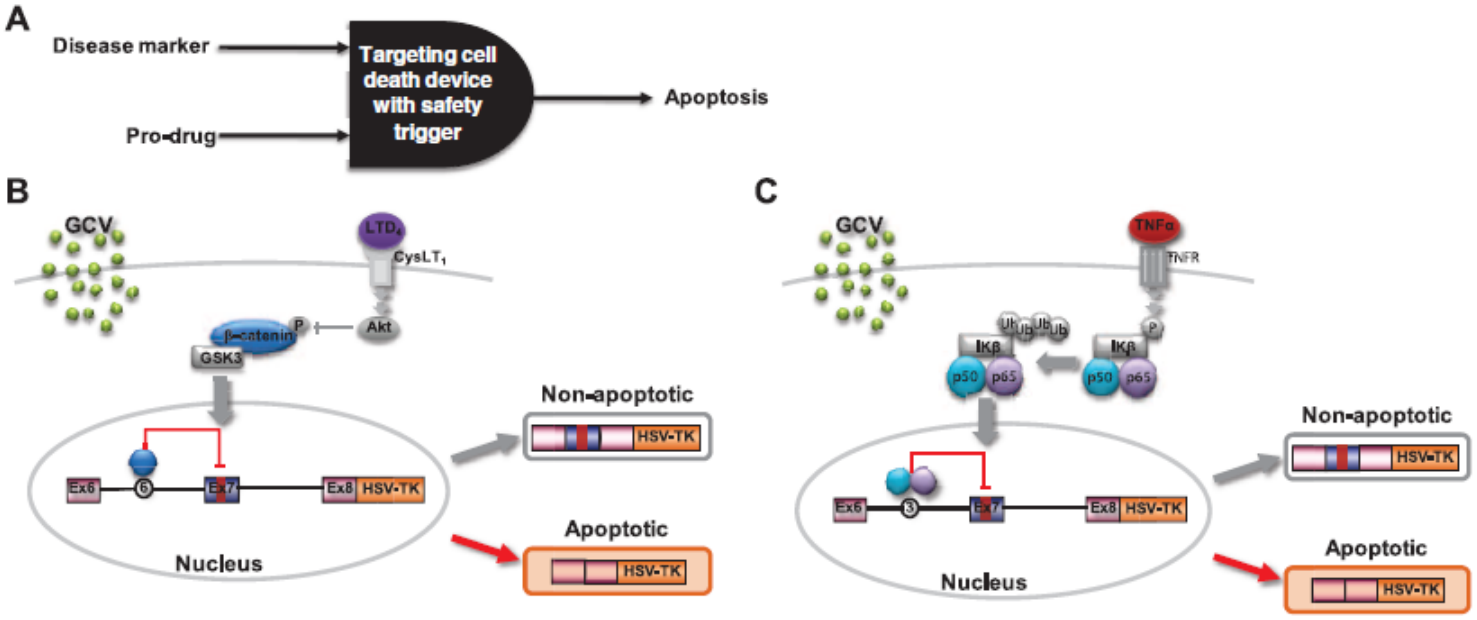
>Increase of GFP and exon exclusion for aptamer in position 6
>No effect in position 3

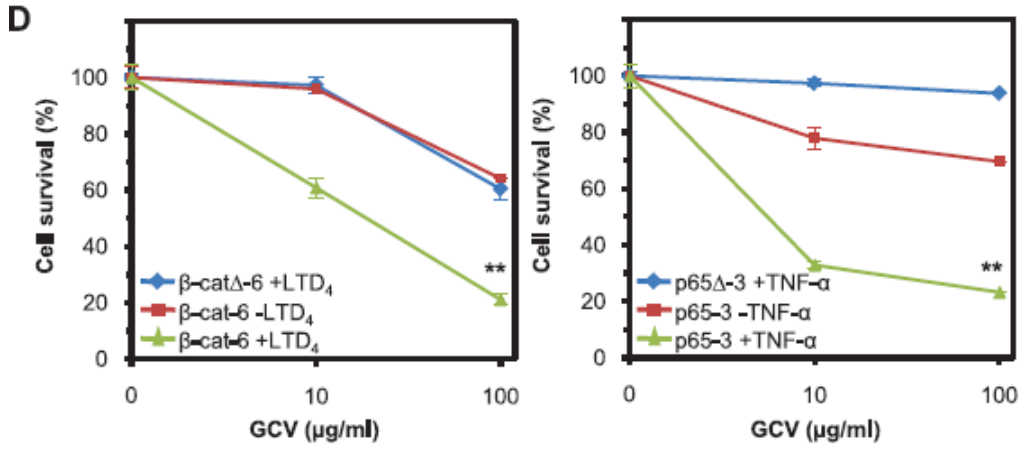
>Distinct positional and functional effects on splicing for a particular protein ligand
>Tuning and flexibility of device
>Able to monitor, detect disease biomarkers

Multiple-inputs-processing increase the overall response



Cell fate regulation upon multiple therapeutic inputs





*Effective cell-killing efficacy in the presence of both inputs
*Minimal background activity

Summary

- Protein can be efficiently directed to alter splicing pattern by aptamers
- Enable response to
- Modularity of the device:

    No re-design needed

    Clinical implementation
- Device able to integrate multiple stimuli
- Synthetic RNA controllers can achieve high alteration in downstream functional behavior

>This technique can be used to build complex regulatory networks to program cell function

Artificial riboswitches for gene expression and replication control of DNA and RNA viruses

Patrick Ketzer[a], Johanna K. Kaufmann[a,1], Sarah Engelhardt[a], Sascha Bossow[b], Christof von Kalle[b], Jörg S. Hartig[c], Guy Ungerechts[b,d], and Dirk M. Nettelbeck[a,2]

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Aim:

>Inhibition of viral replication and pathogenesis in eukaryotic cells

>Validation approach for effective biological outcome control: *Adenovirus

*Measles

Requirements:

>Short sequence

>Applicable for both RNA and DNA virus

>Active at high viral titer

>Simple mode of action

>Aptazyme

Aptazyme

- Short RNA sequence (100bp)
- Self-cleaving ribozyme linked to an aptamer

>ligand-dependant self-cleaving ribozyme

Advantages:

*Inducible by small molecules

*Enable conditional cleavage of RNA

*Acting in cis

*On or Off switch activity

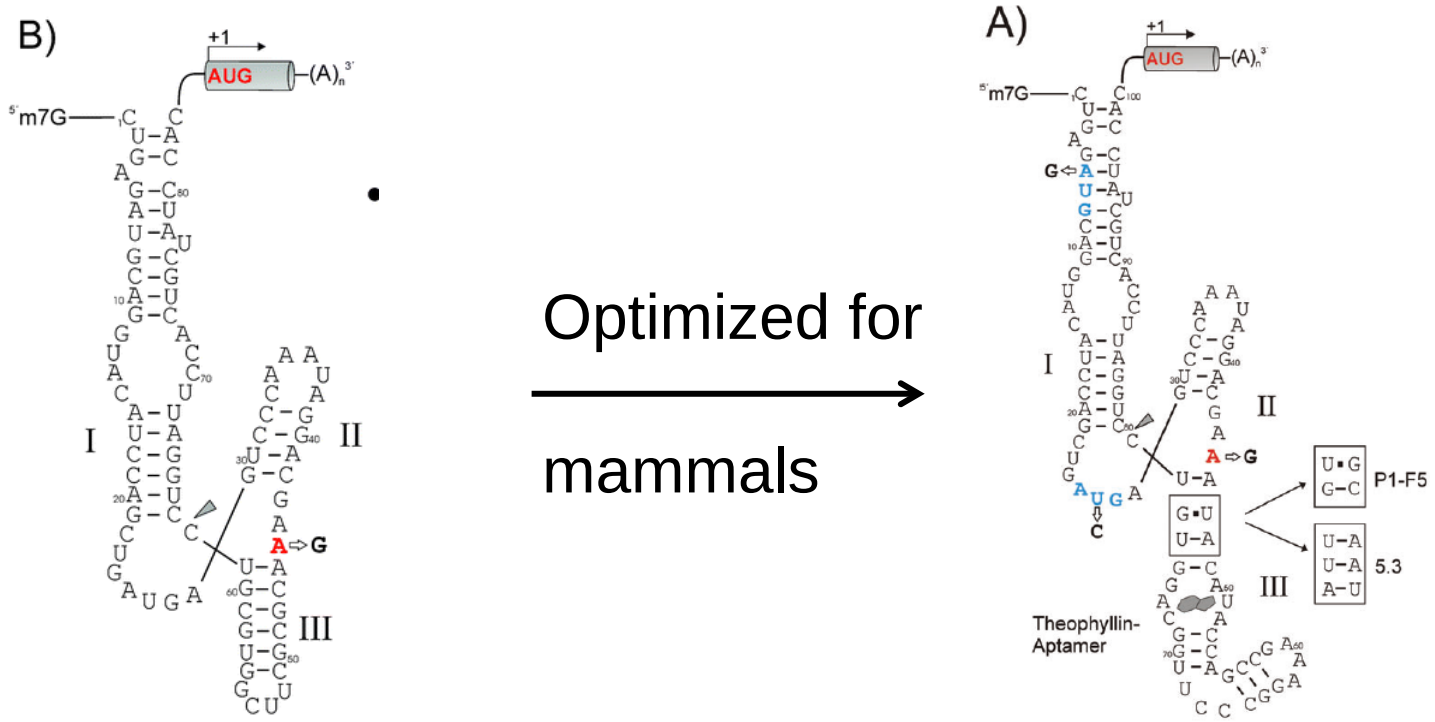
*Easily customized

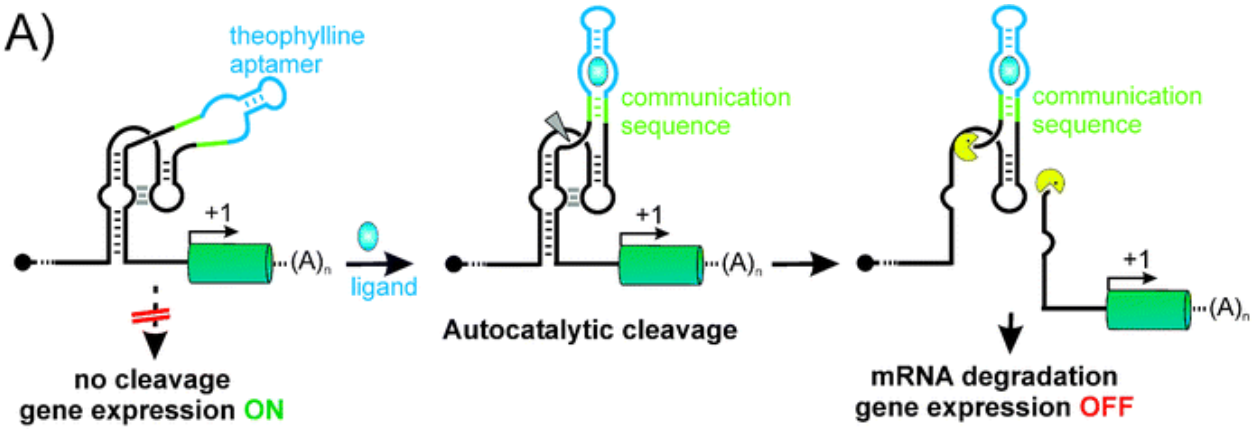
Disavantages:

*No aptazyme reported in mammals

>Challenging application

Principle



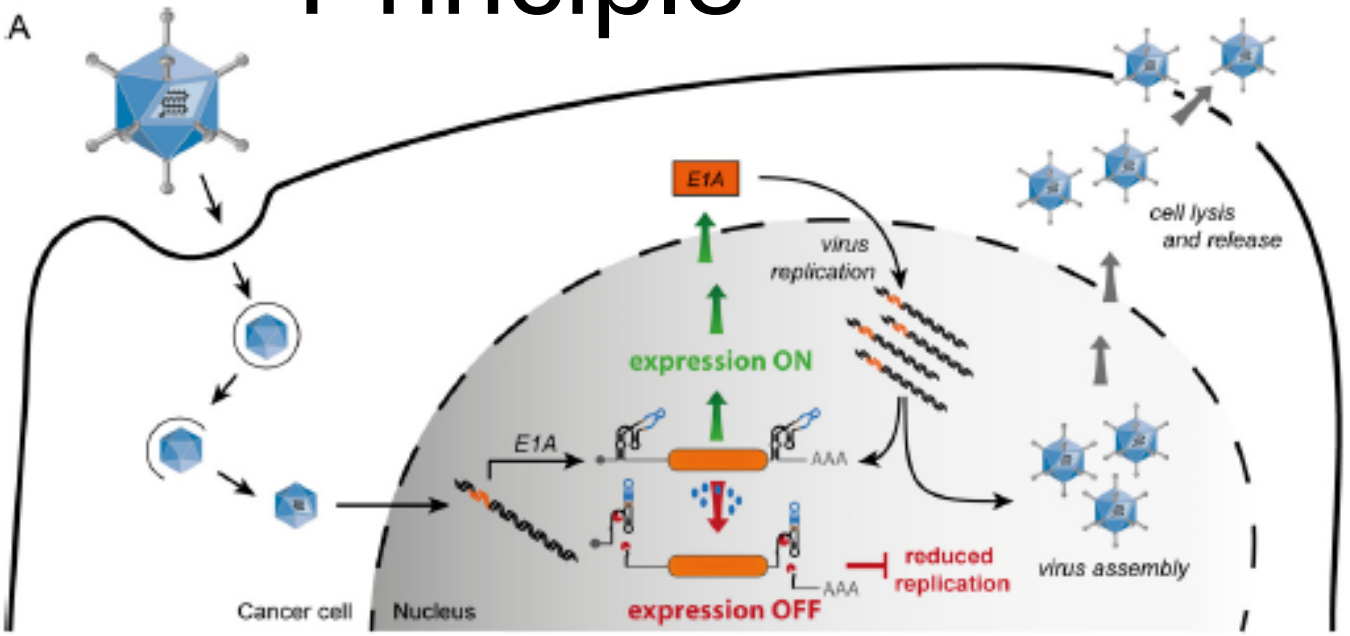


Simon Ausländer,† Patrick Ketzer and Jörg S. Hartig*

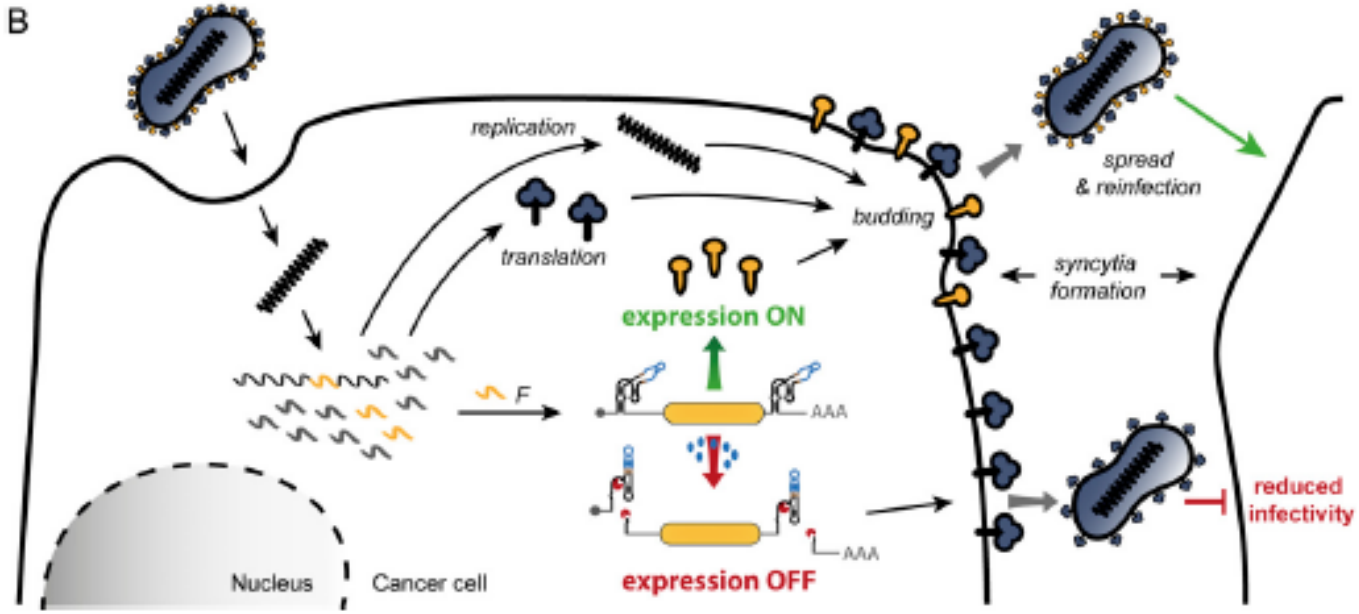
Received 3rd November 2009, Accepted 26th January 2010

Principle

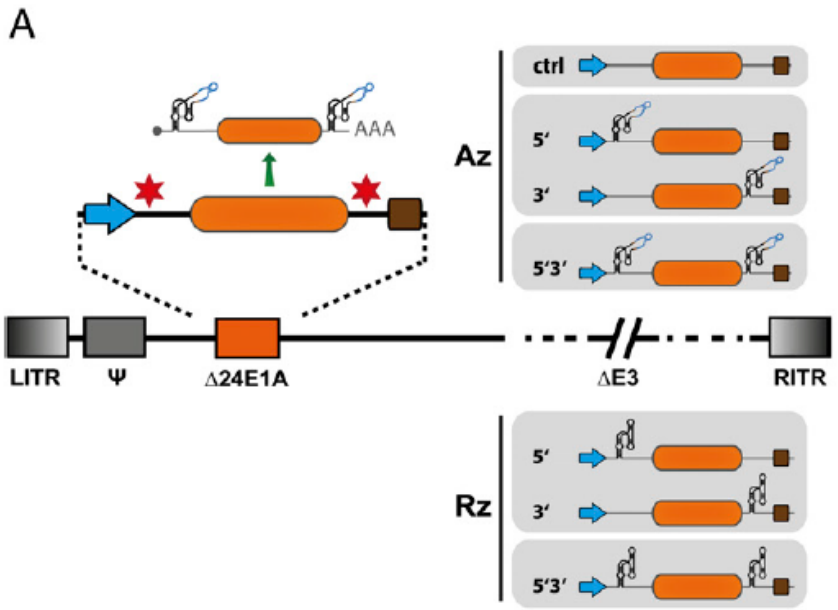
Adenovirus
>dsDNA
>nucleus
>target E1A

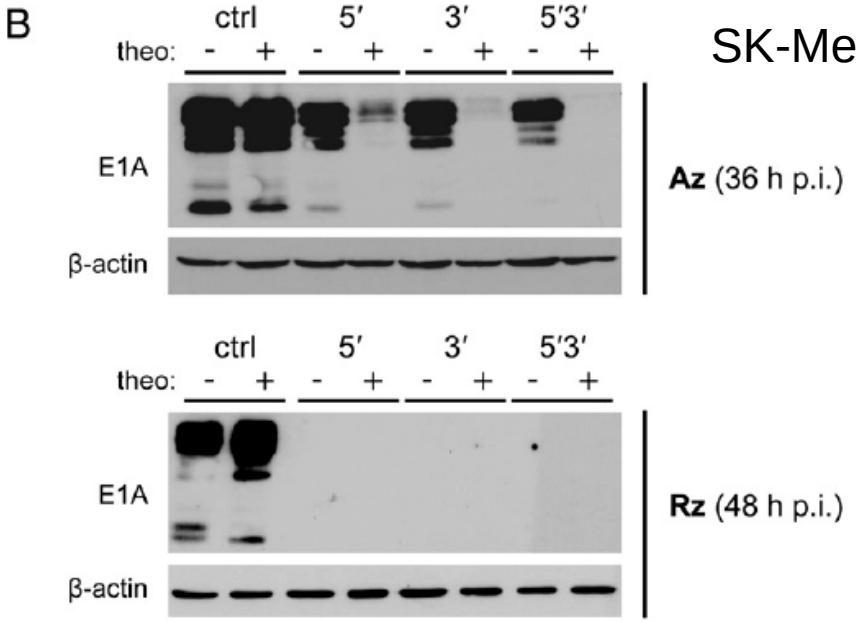


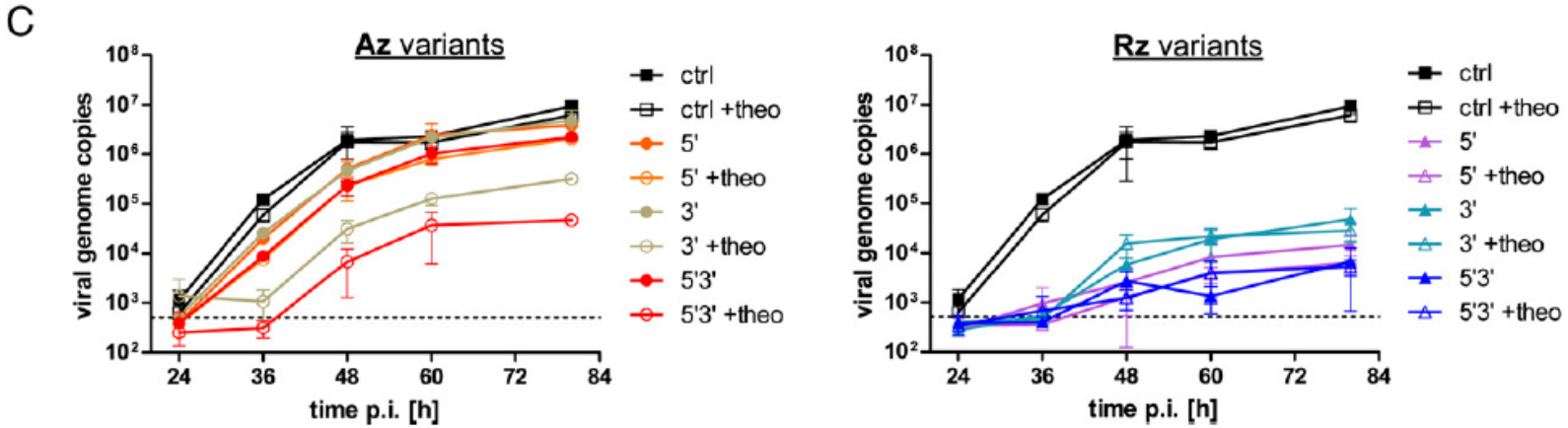
Measles
>ssRNA(-)
>Cytoplasm
>target F protein



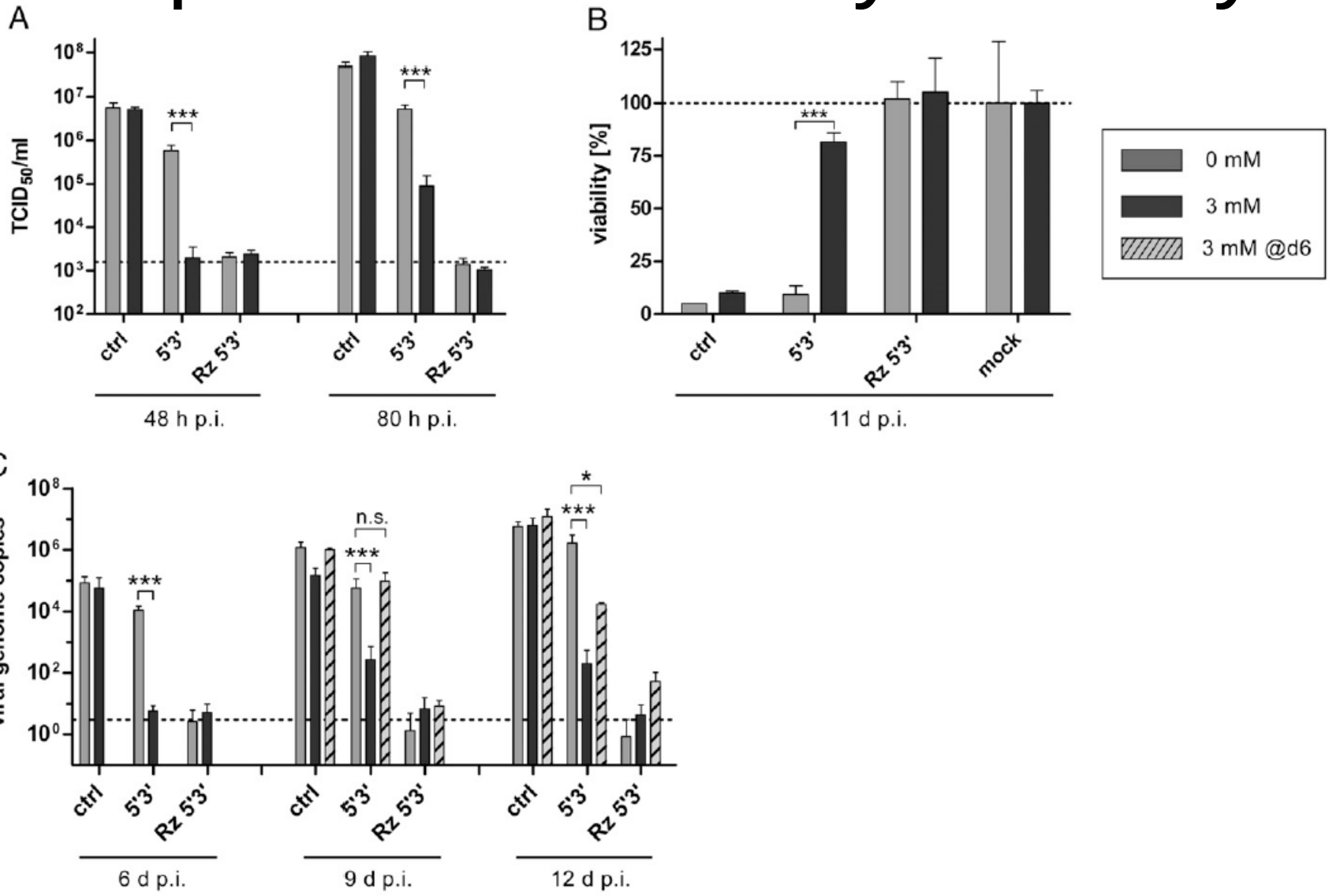
Inducible-shutdown of viral gene expression and replication



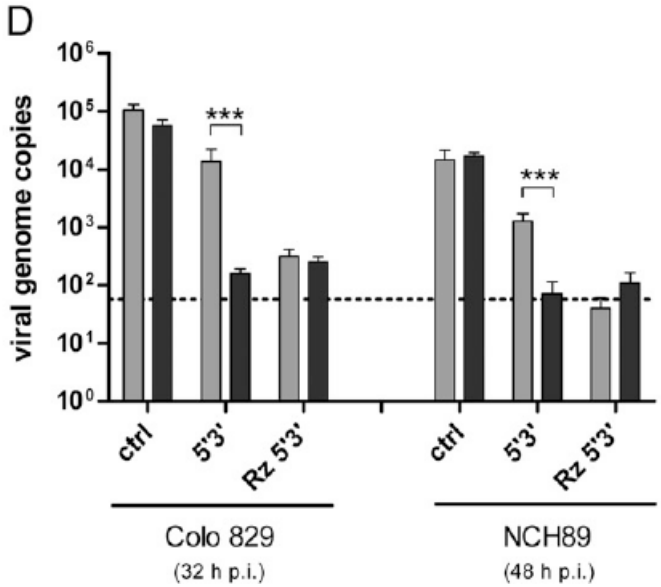


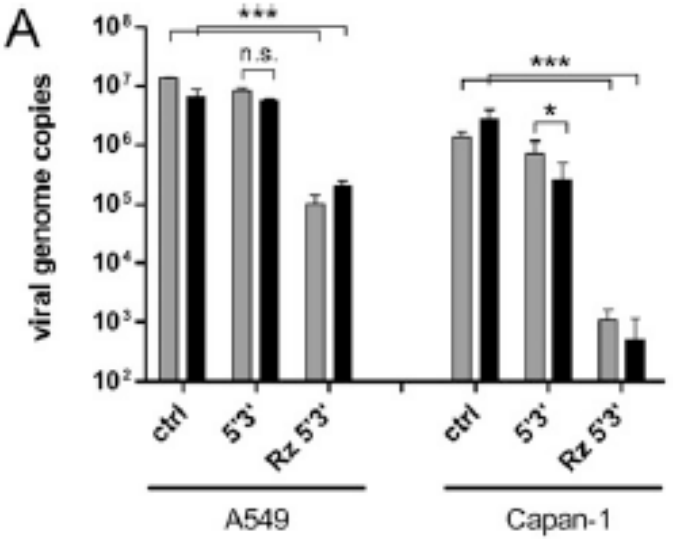


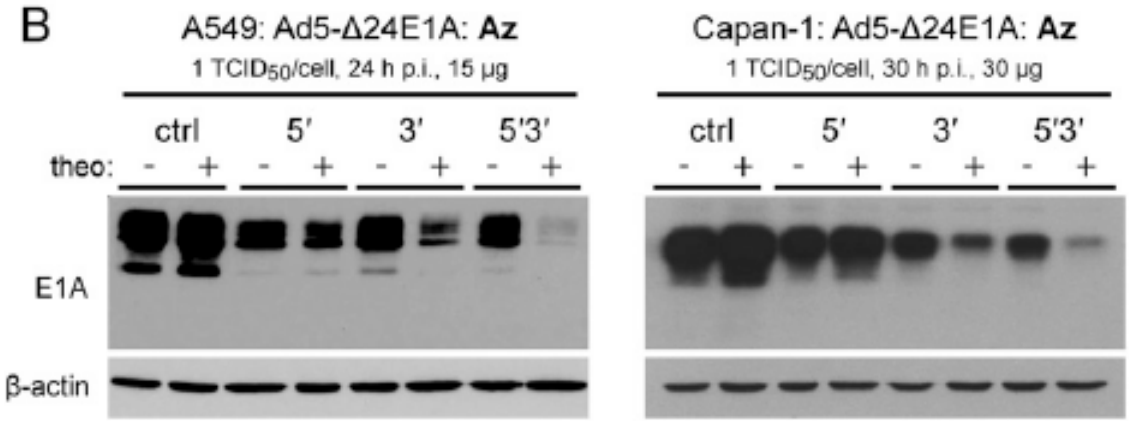
Pre-exposure inhibition of particle production and cytotoxicity



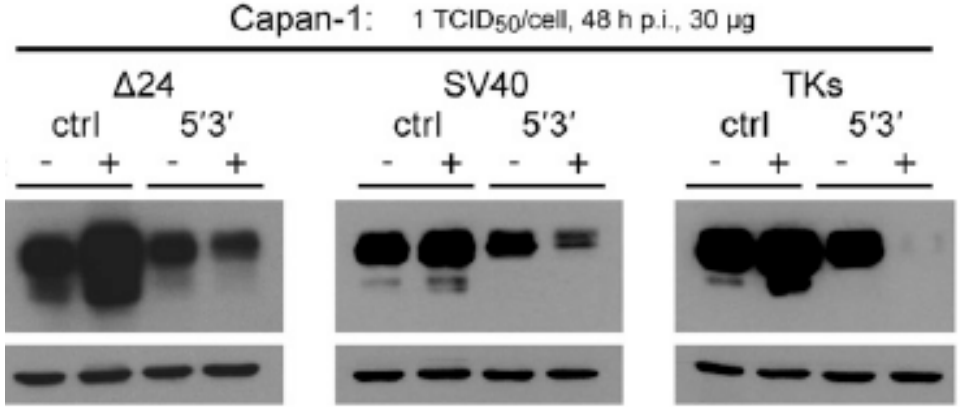
Approach not transposable to all cancerous cells

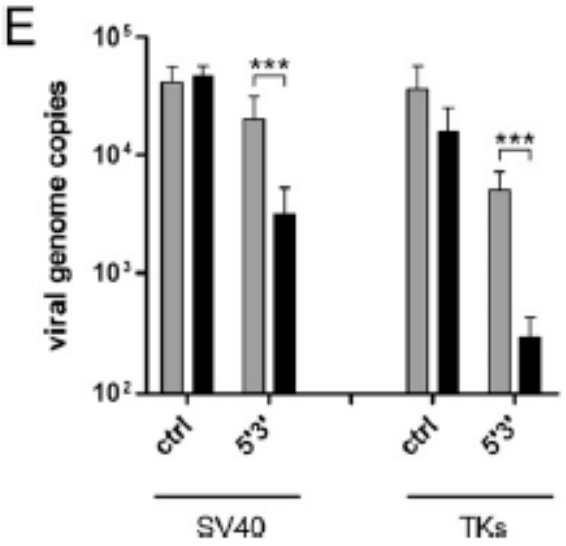


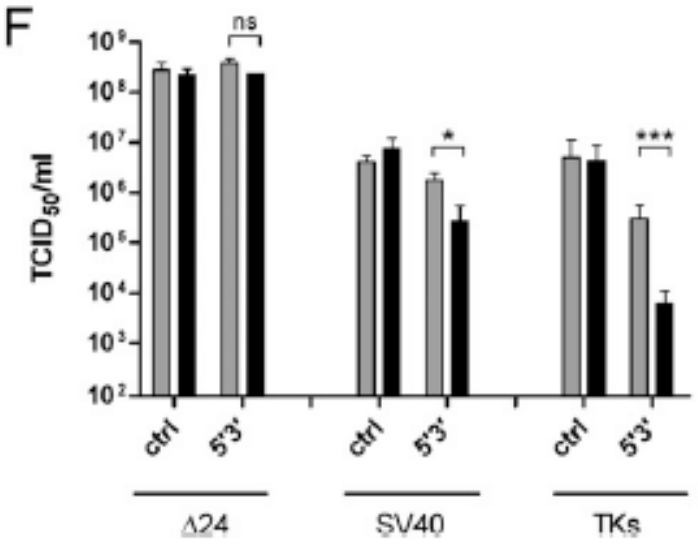




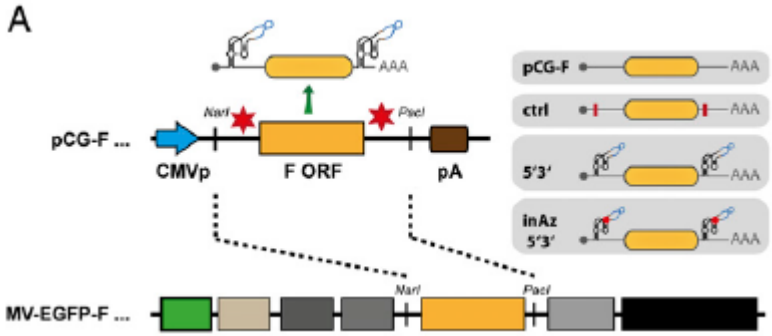
Reducing baseline expression level of E1A

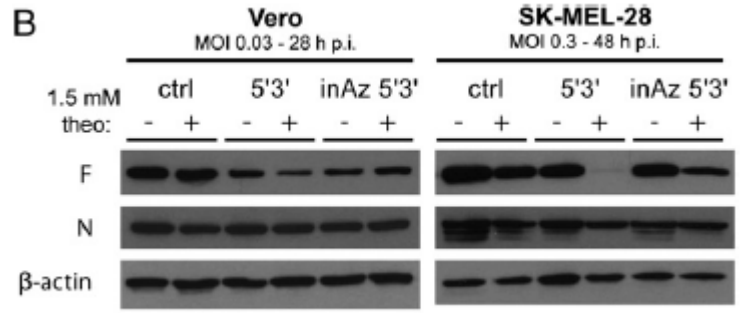


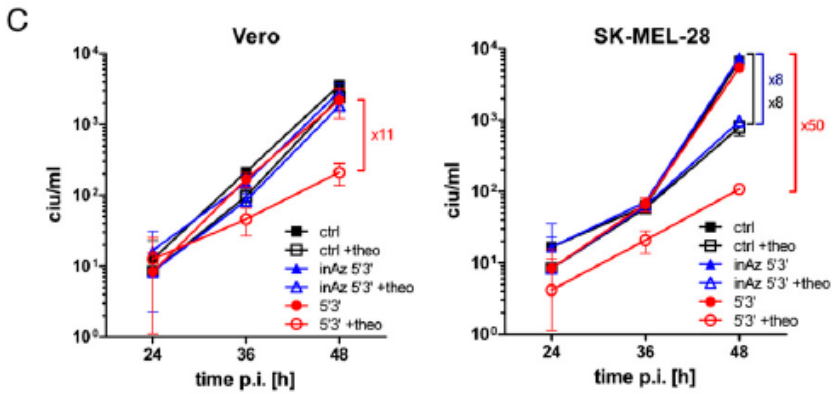


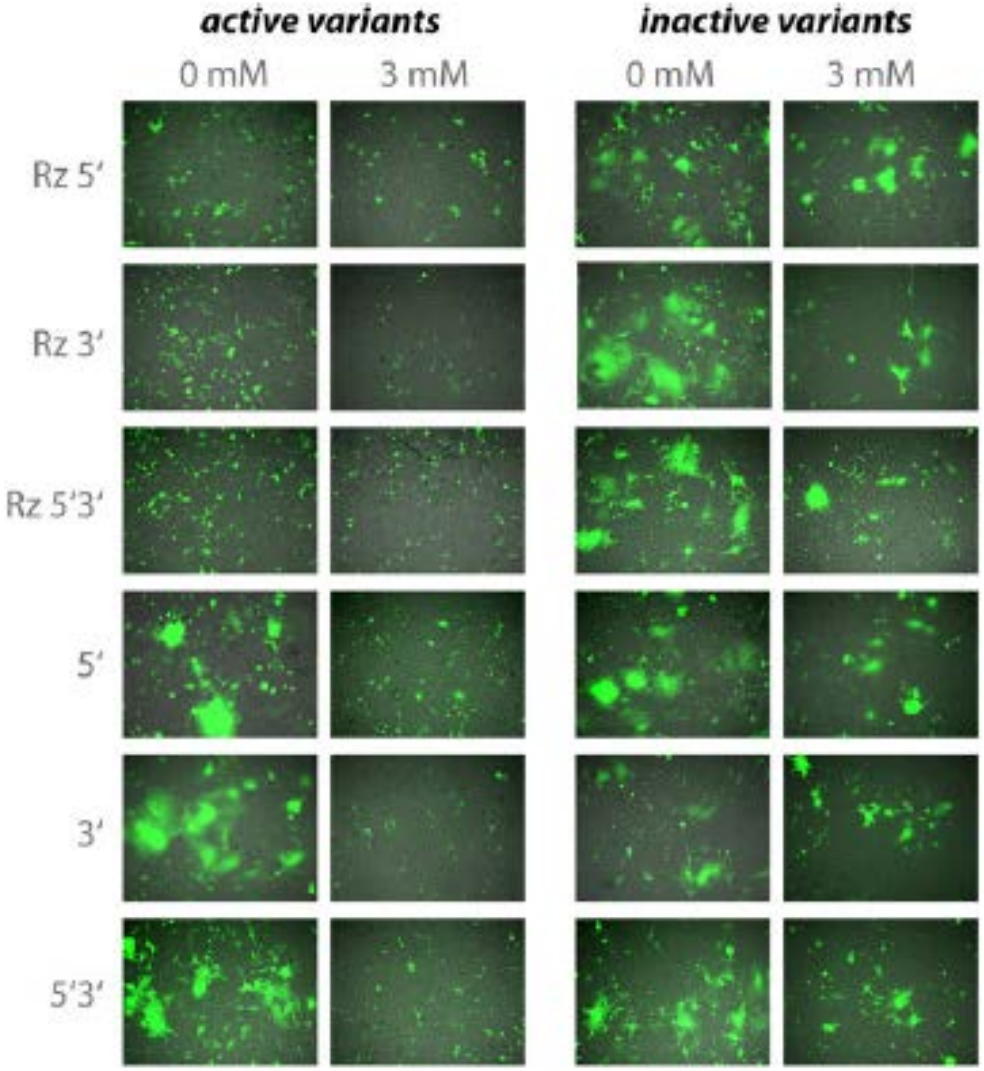


Challenging aptazyme approach on Measle virus









Summary

- Aptazyme enable specific inhibition of viral protein, affecting replication and spread
- 5’3’>3’>5’ UTR regulation efficiency
- Dose-dependant regulation of aptazyme
- Able to target and regulate: DNA/RNA virus
- Able to act in the nucleus or cytoplasm
- Delay in the control of an established infection

>Universal applicability of the aptazyme for gene regulation

Limitations:

- Suboptimal switch
- Not generalizable
- Effect on baseline gene expression

Improvements

Aptazyme insertion into the UTR:

To minimize effects on baseline protein expression without ligand addition

Variability of the aptazyme efficacy:

A) Reducing the baseline level expression

B) Increasing self-cleaving activity (=induction rate)

Targeting crucial viral genes:

>Alone or in combination

Challenges in mammalian cells:

Moderate switching activity

>Integration aptamer into ribozyme impair tertiary interloop structures

NATURE METHODS

A general design strategy for protein-responsive riboswitches in mammalian cells

Simon Ausländer[1], Pascal Stücheli[1], Charlotte Rehm[2], David Ausländer[1], Jörg S Hartig[2] & Martin Fussenegger[1,3]

PUBLISHED ONLINE 5 OCTOBER 2014

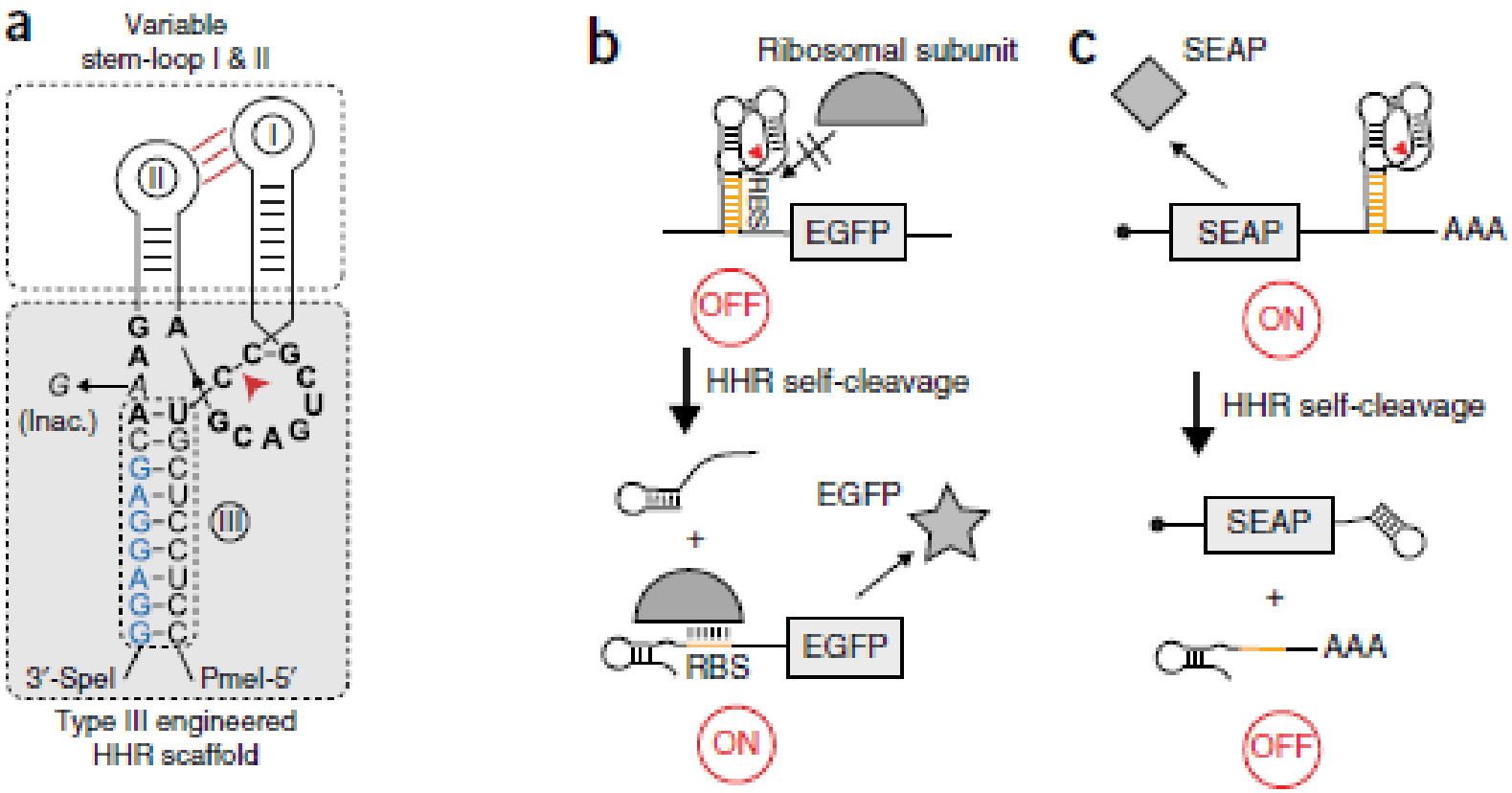
Aim: Elaboration of a versatile system for broad use of ribozyme

Goals:

*Decrease compromising structureal interference

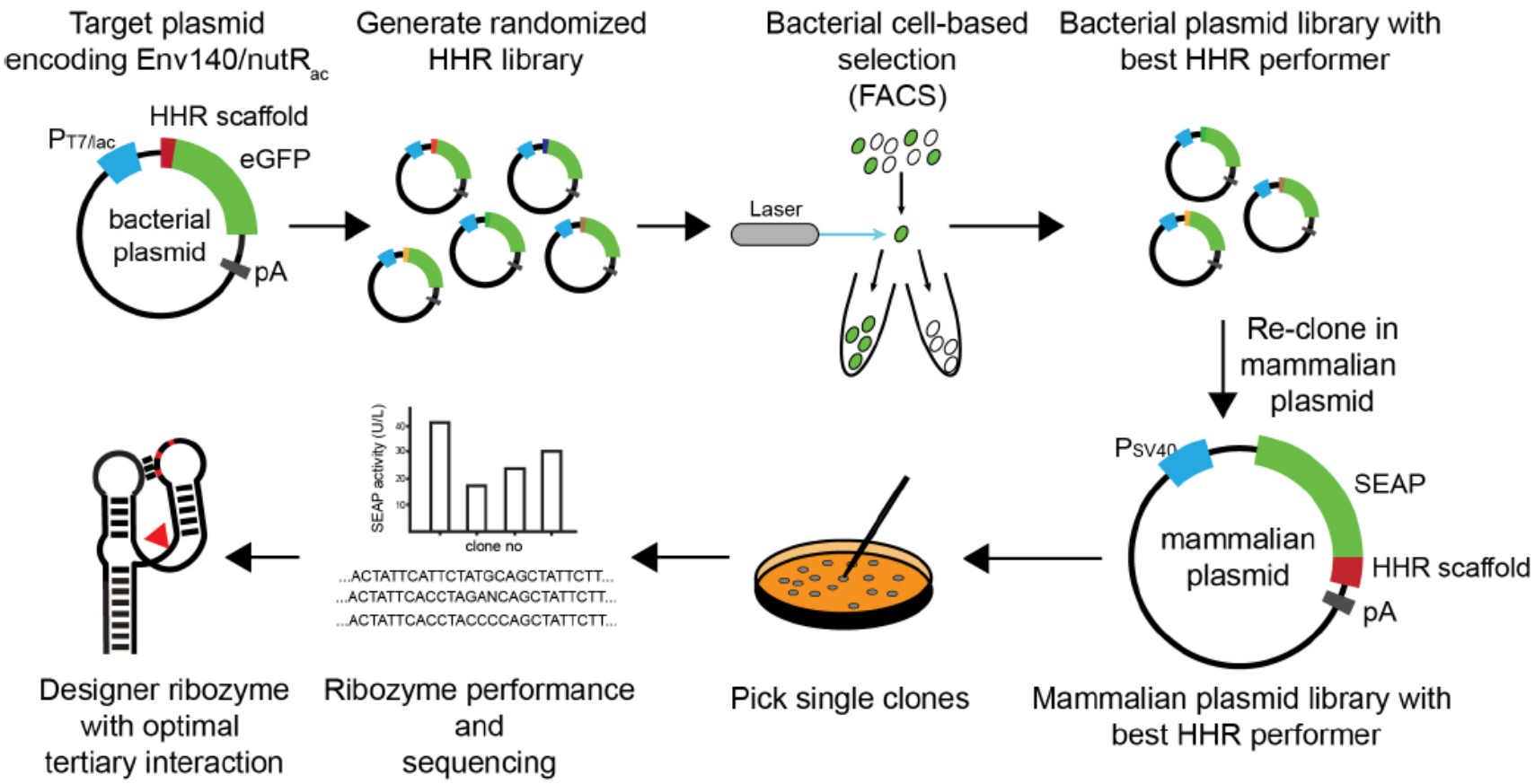
*Preserve optimal cleavage activity

Bimodal expression plateform



Size, seqence identity, structure shape and stability of the stem affect ribozyme performance by influencing the tertiary interloop contact

Screening procedure



Applicability of aptazyme

- Conditional shutdown of viral genes

>In vitro/in vivo

>Efficiency, timing, systemic activation

>Less off-target

- Oncolytic virus:

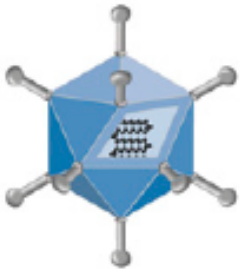
>Drug inducible safety switch

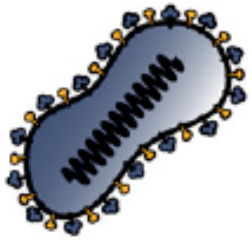
- Live virus vaccine

Thank you for you attention!

Principle

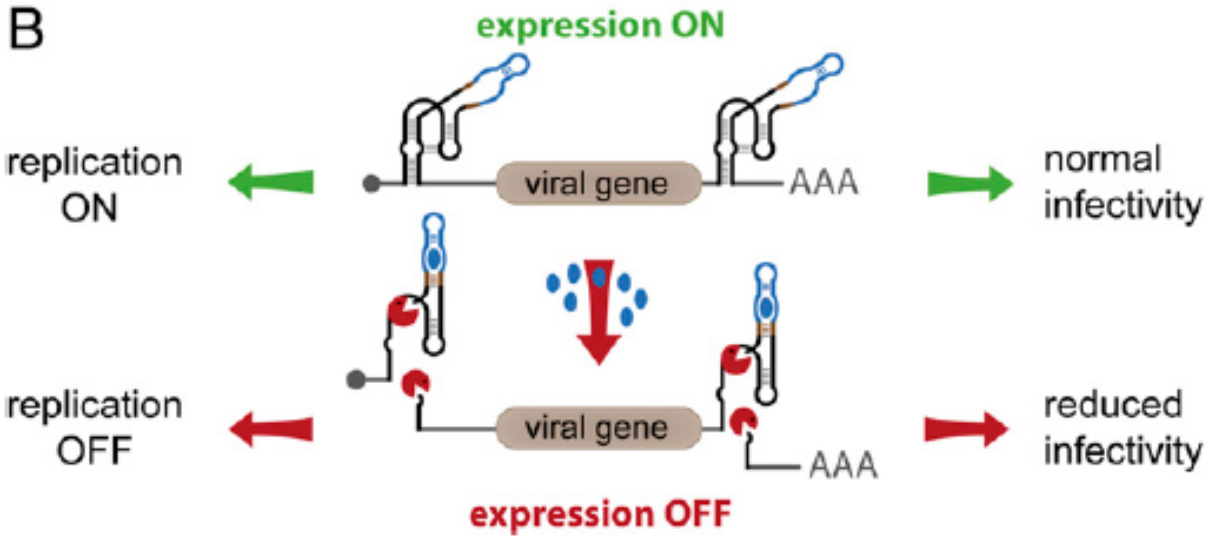
A



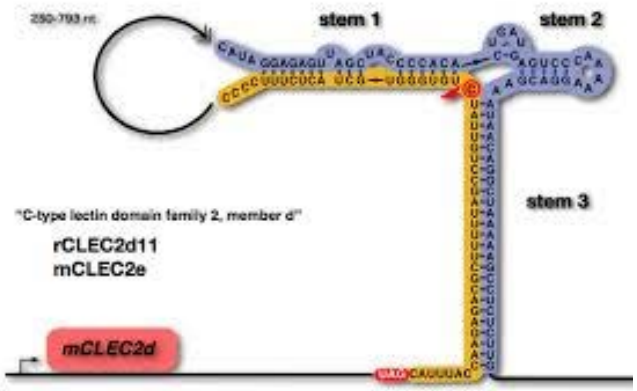


adenovirus	**virus**	measles virus
dsDNA	**genome type**	(-)ssRNA
nucleus	**replication**	cytoplasm
nucleus	**mRNA synthesis**	cytoplasm
E1A	**target gene**	F glycoprotein
replication	**level of control**	infectivity of progeny

B



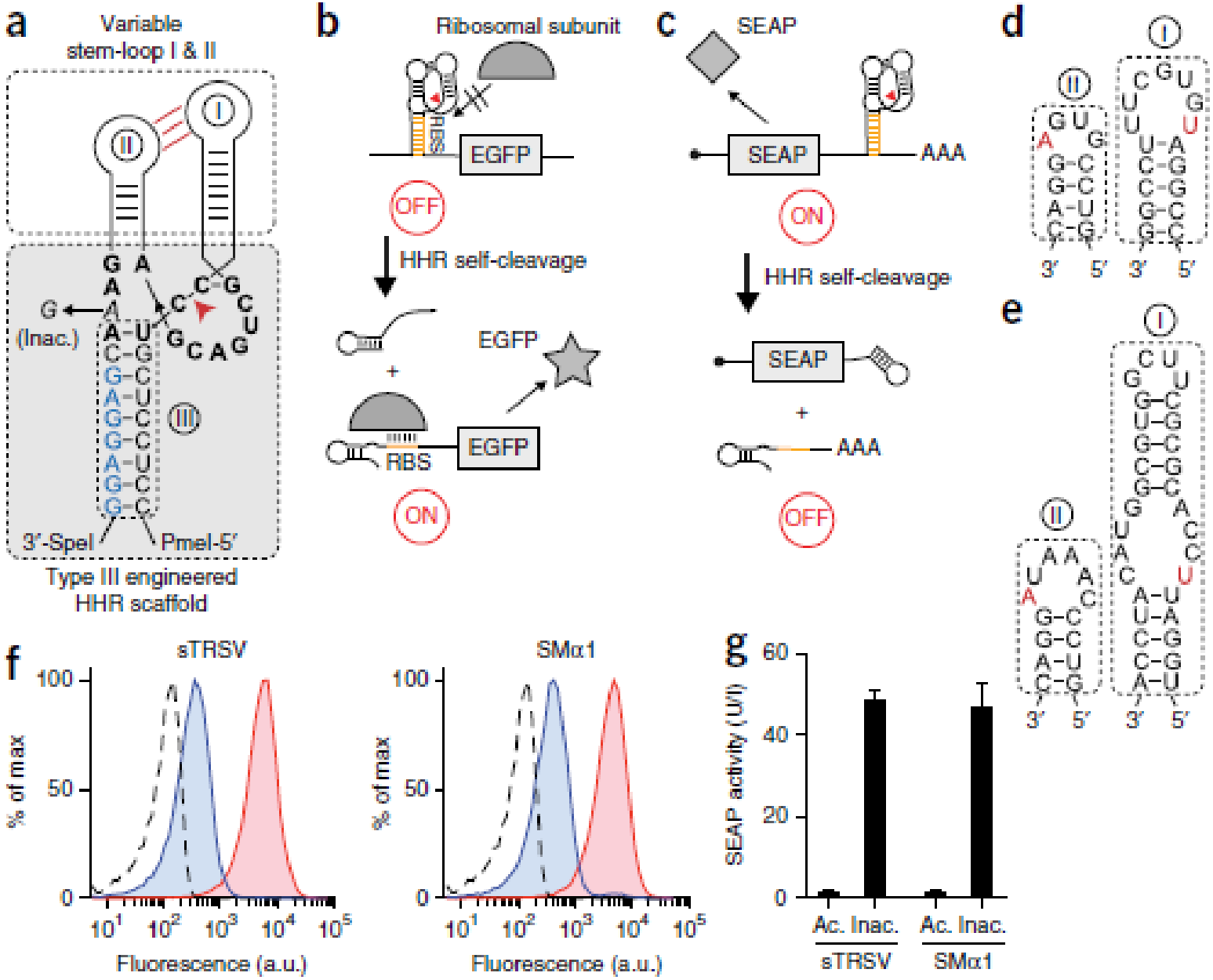
HammerHead Ribozyme



>Ribonucleoprotein
>3stem loop structure
>catalytic core
>Cleave mammalian mRNA
>Irreversibly inhibit translation

Addition of small molecule sensing aptamers:
>control the self cleavage activity

Procedure


a
Variable
stem-loop I & II
(Inac.)
3′-SpeI
PmeI-5′
Type III engineered
HHR scaffold
b
Ribosomal subunit
RBS
EGFP
OFF
HHR self-cleavage
EGFP
RBS
EGFP
ON
c
SEAP
SEAP
AAA
ON
HHR self-cleavage
SEAP
AAA
OFF
d
e
f
sTRSV
SMα1
% of max
Fluorescence (a.u.)
g
SEAP activity (U/l)
Ac. Inac.
Ac. Inac.
sTRSV
SMα1