



# Persistent Gene Expression in the Murine Lung is Dependent on Transgene CpG Content

**Cathy Oliveira**

**D.Phil Student**

**Gene Medicine Research Group**

**University of Oxford**

**& United Kingdom Cystic Fibrosis Gene Therapy Consortium**



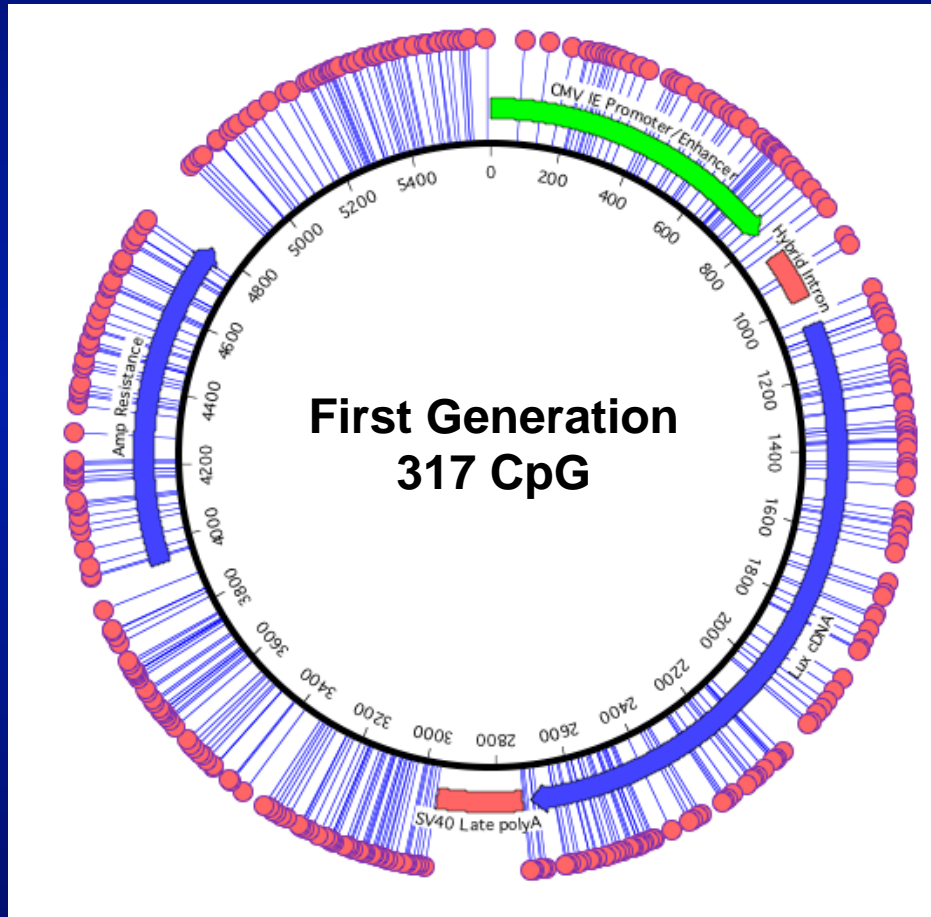
# Introduction



- Lung gene therapy is being investigated for a number of diseases
  - Relatively accessible organ via aerosolisation
  - Strategy is dependent on the type of disease (acute vs chronic)
  - Chronic conditions will probably require multiple doses (turnover of lung cells every ~17 months; Rawlins *et al.*, 2008)
- Cystic Fibrosis:-
  - CFTR gene affects chloride channels in epithelial cells
  - Chronic lung disease is the primary cause of mortality (~25 years)
- Multiple phase I gene therapy clinical trials have been conducted
  - Repeat administration is required
  - Viral vectors - Lack of successful repeat administration
  - Non-viral - Transient expression; flu-like symptoms

# Plasmid Design

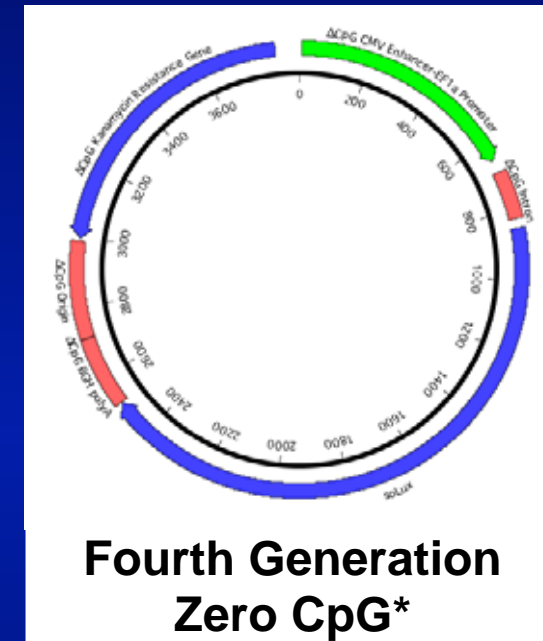
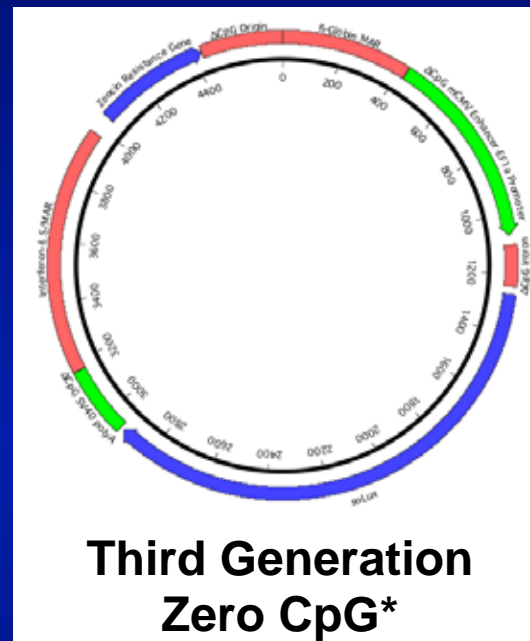
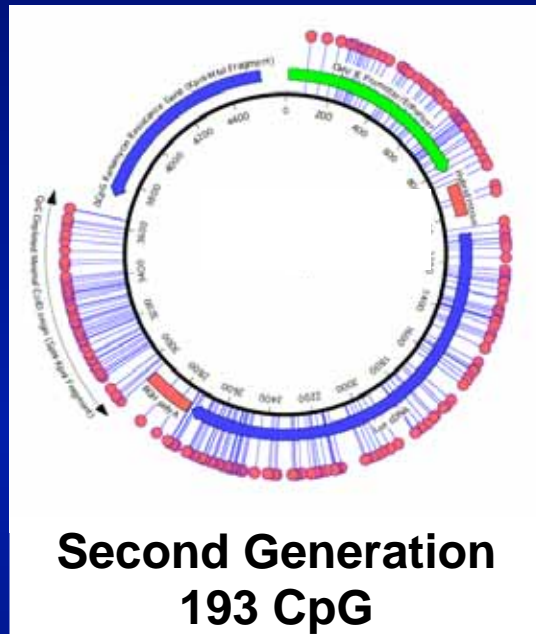
## First Generation plasmids have many CpGs



- Hypothesis:  
CpGs (CG Dinucleotides)  
Recognised by TLR-9  
Activates inflammatory cascade  
Leading to flu-like symptoms

**Remove CpGs**

# Plasmid Design: Fourth Generation have zero CpGs and are regulator-compliant



Less inflammation & better transgene expression levels

\*Utilising CpG-free plasmid technology developed by Cayla Invivogen (Toulouse, France)

# Mouse Aerosol Model for Lung Gene Delivery



**Aerosol delivery to mouse lung**



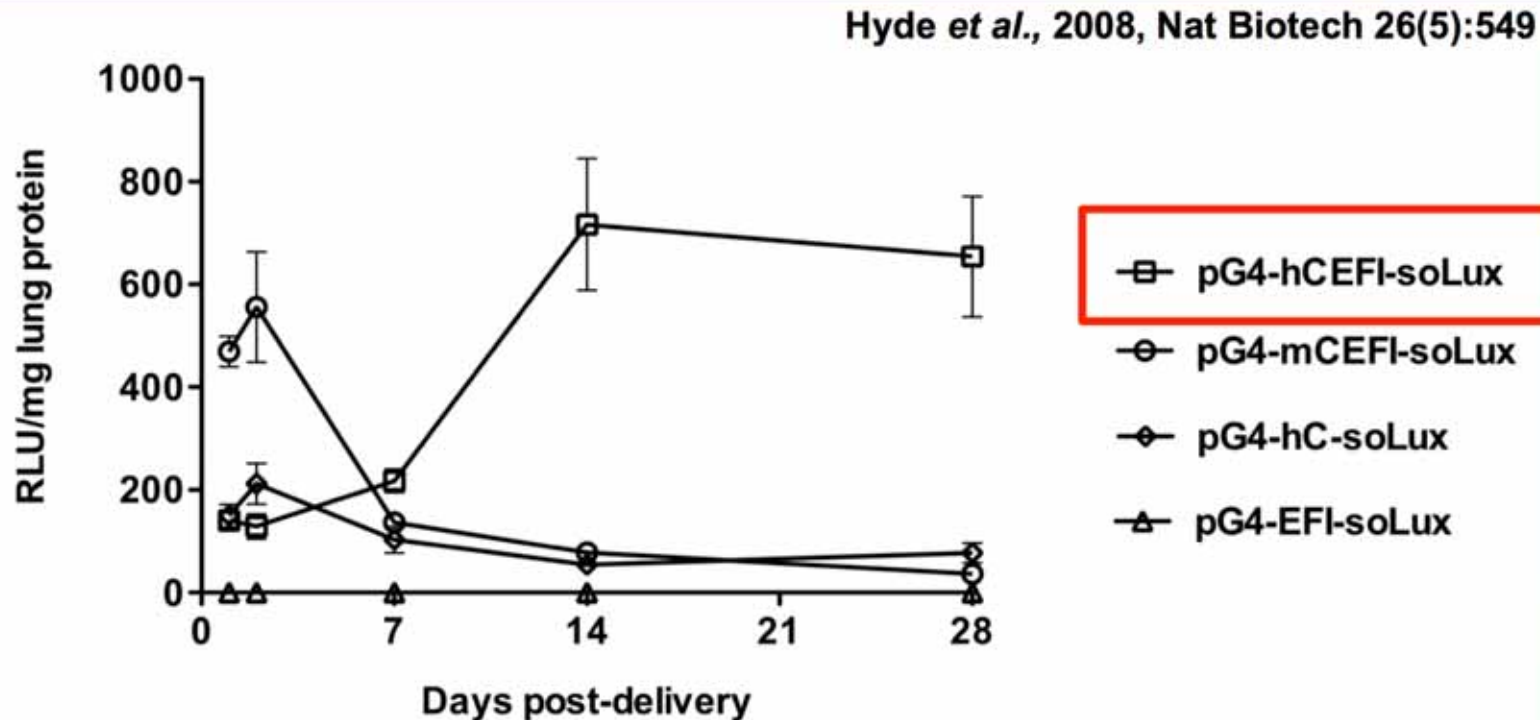
**Up to 30 mice dosed simultaneously**

**Aerosolised pDNA and GTA**



**10 ml total volume**

# Choice of enhancer/promoter is critical for persistent, high-level expression



n=6  
BALB/c  
Aerosol  
GL67A/pDNA

**hCEFI – a synthetic, hybrid enhancer/promoter consisting of:  
CpG-free versions of human CMV enhancer and human Elongation Factor 1a promoter**



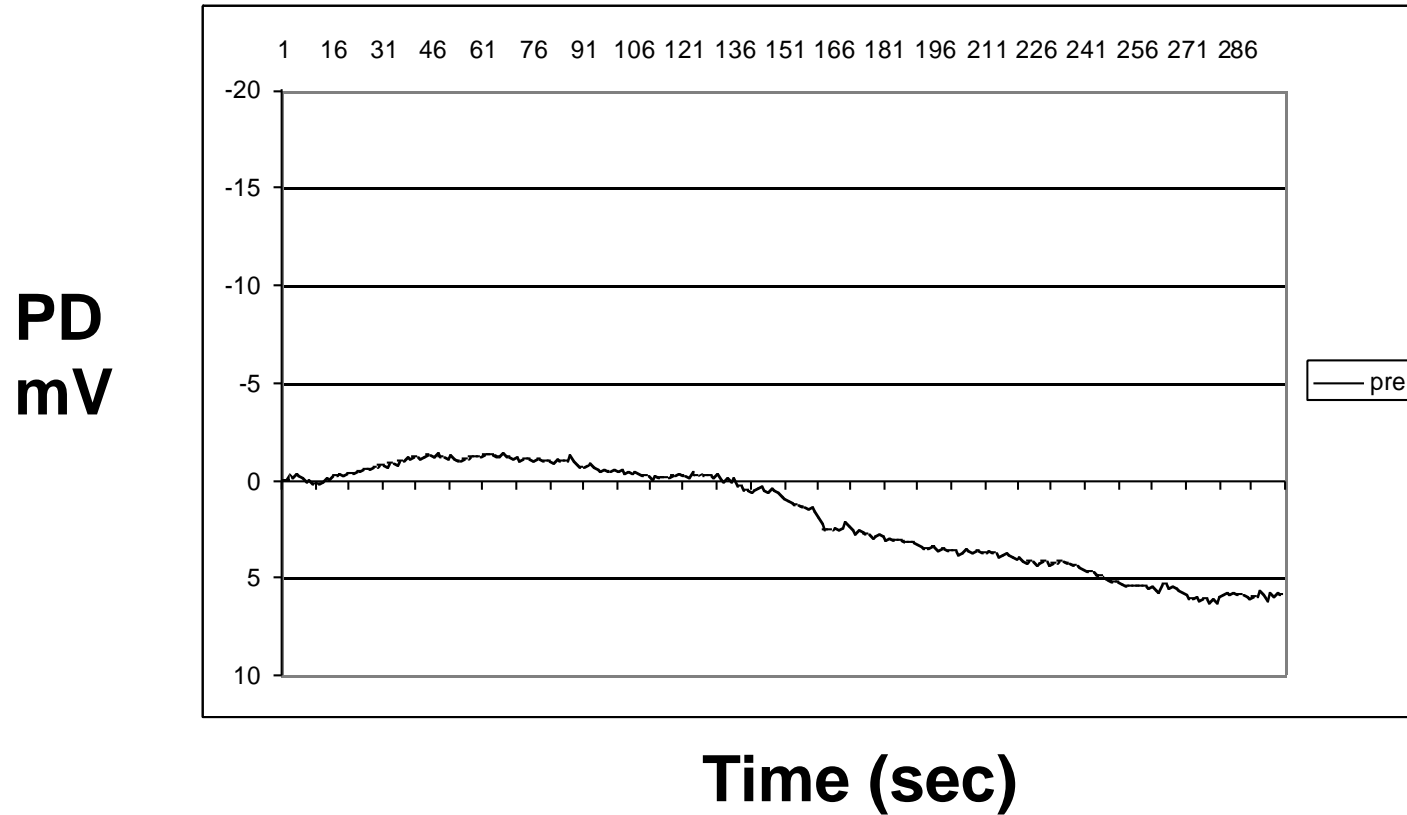
# UKCFGTC – Clinical Studies in CF Patients

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- UK CF Gene Therapy Consortium
  - Phase 1/2a **Single Dose** clinical study (pG4-hCEFI-soCFTR/GL67A)
    - so = synthetic, codon optimised
    - safety & dose selection
    - **completed 2011**

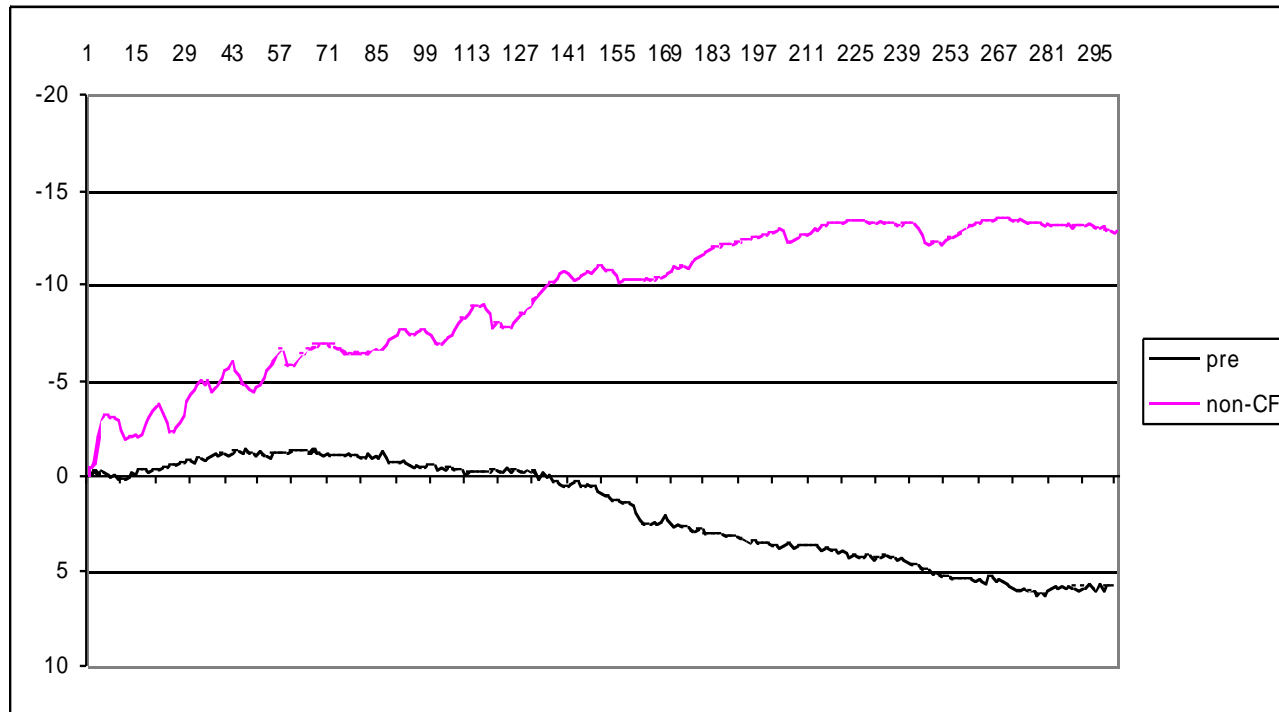
# CF: Pre-Treatment (mean of 3)





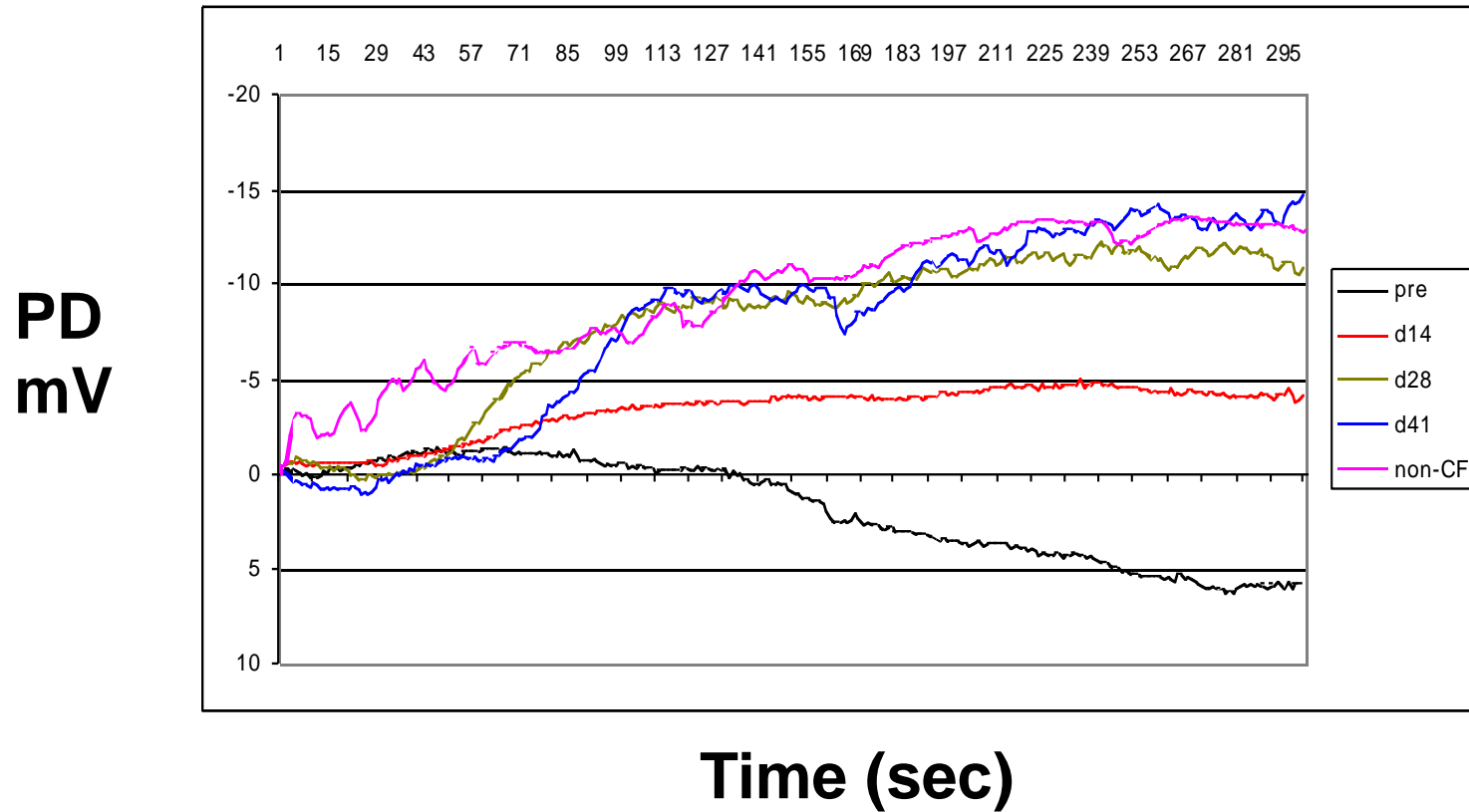
# Non-CF

PD  
mV



Time (sec)

# CF Post-Treatment: 6 Weeks



# UKCFGTC – Clinical Studies in CF Patients



- UK CF Gene Therapy Consortium
  - Phase 1/2a **Single Dose** clinical study (pG4-hCEFI-soCFTR/GL67A)
    - so = synthetic, codon optimised
    - safety & dose selection
    - **completed 2011**
  - Phase 2b **Multiple Dose** clinical study
    - 12 monthly doses
    - look for clinical benefit
    - **initiated 2012**
    - results available end 2014

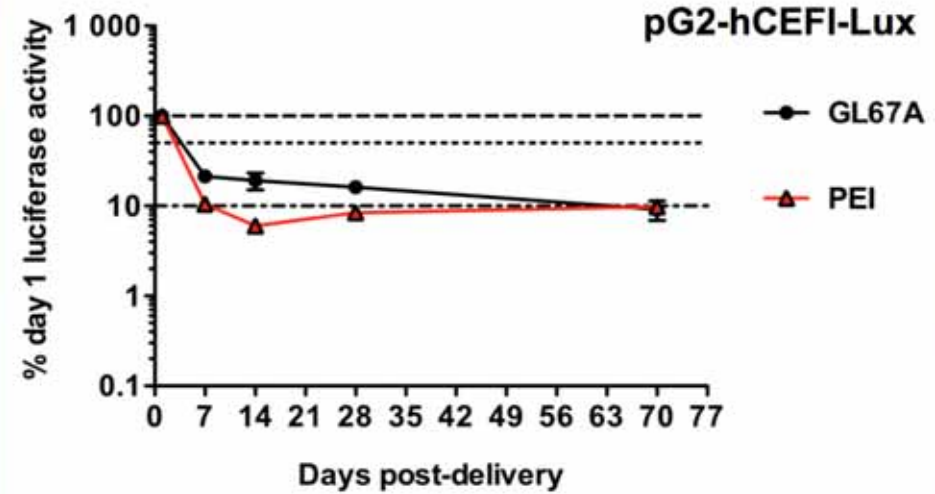
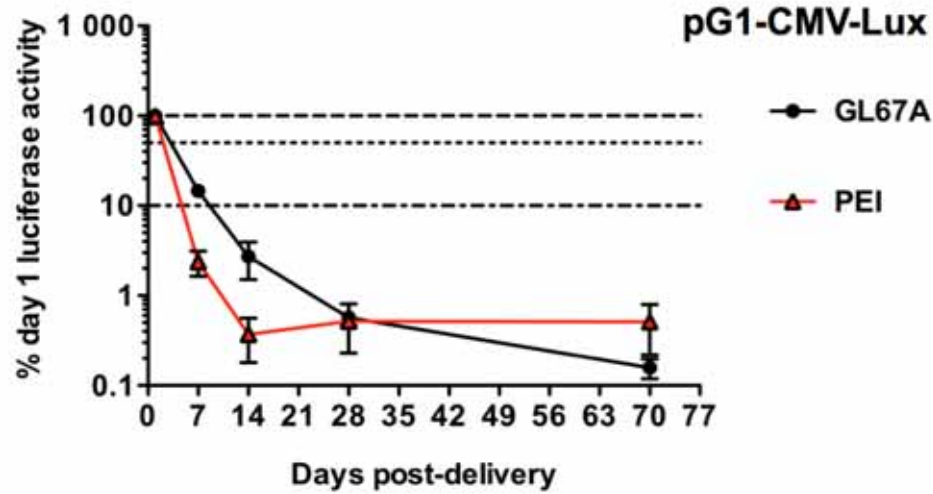
# What factors affect persistent transgene expression in the mouse aerosol model?

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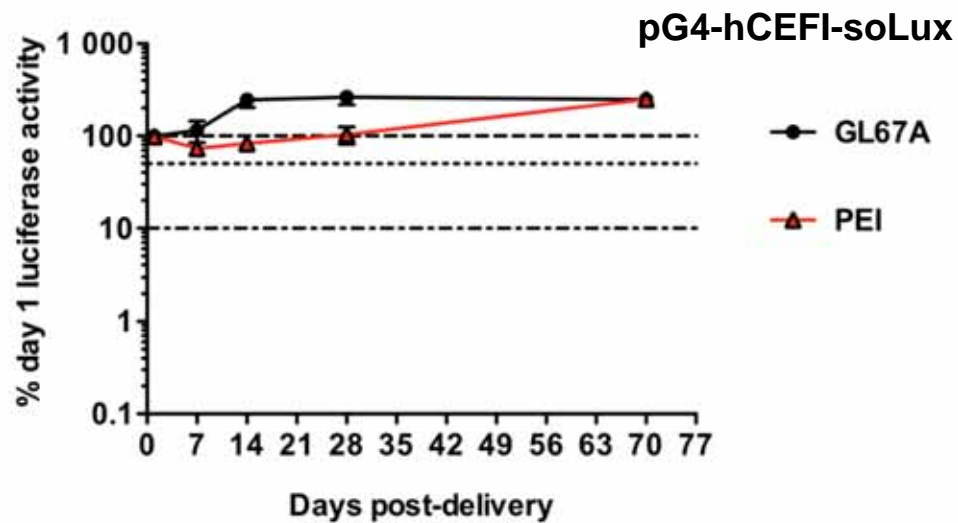


- **CpG-free vs CpG-rich transgenes:**
  1. **Gene transfer agents (GL67A vs PEI)**
  2. **Different plasmid backbones (G1 vs G2 vs G4)**
  3. **Minicircle DNA vs conventional plasmids**

# 1. Effect of GTA on transgene expression



n=6  
BALB/c  
Aerosol  
PEI/pDNA  
or  
GL67A/  
pDNA



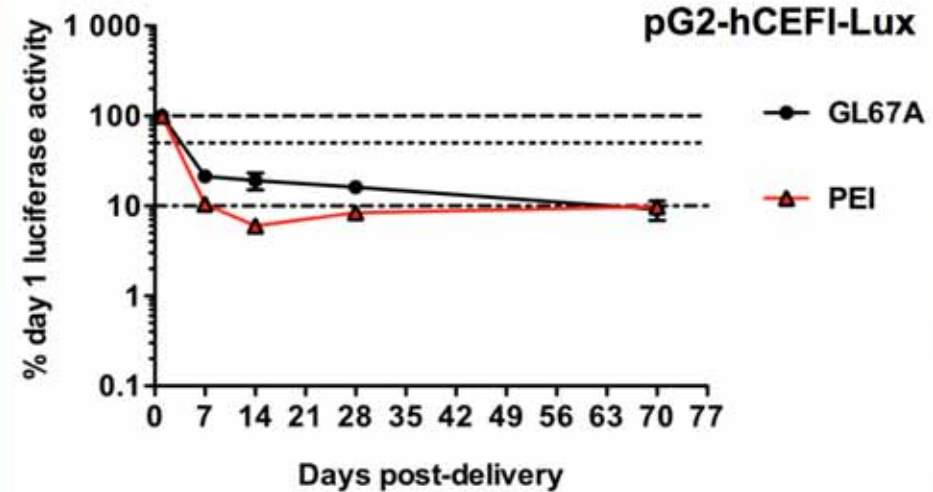
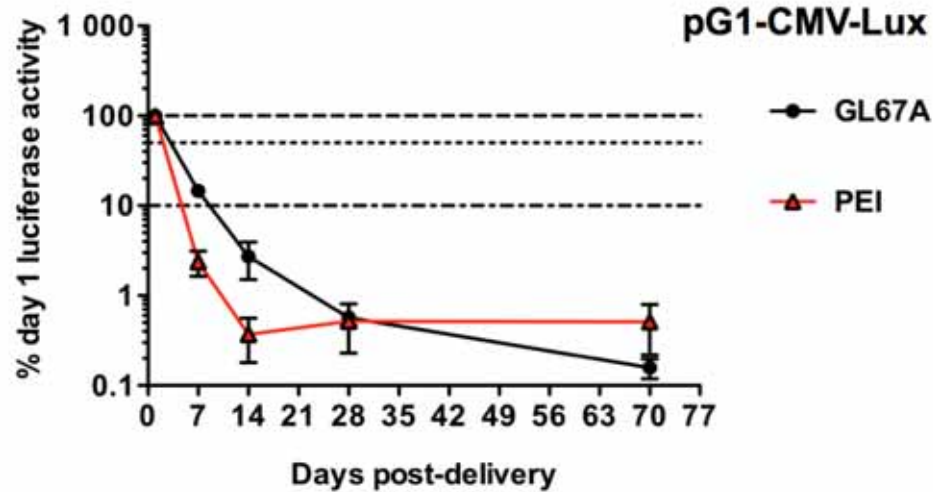
PEI = 25kDa polyethyleneimine

--- = 100%

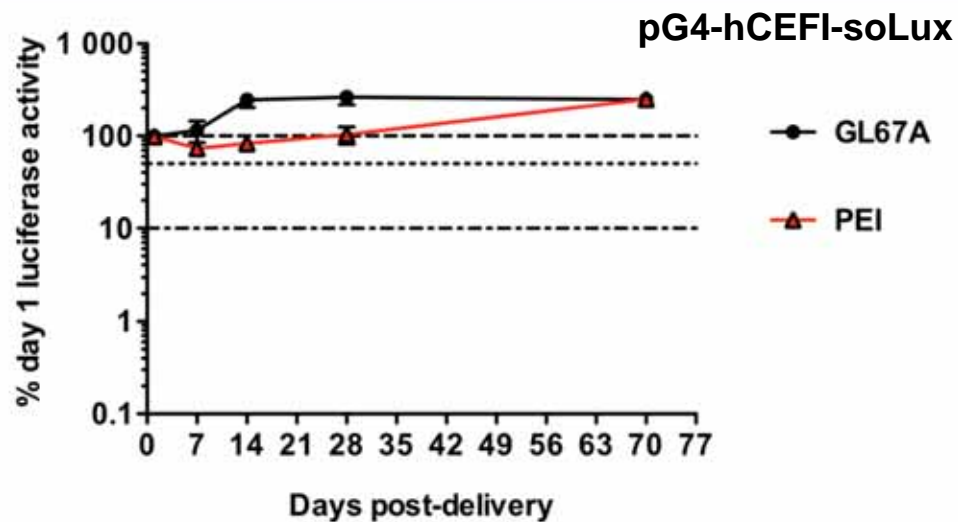
••• = 50%

•-• = 10%

# 1. Effect of GTA on transgene expression



n=6  
BALB/c  
Aerosol  
PEI/pDNA  
or  
GL67A/  
pDNA

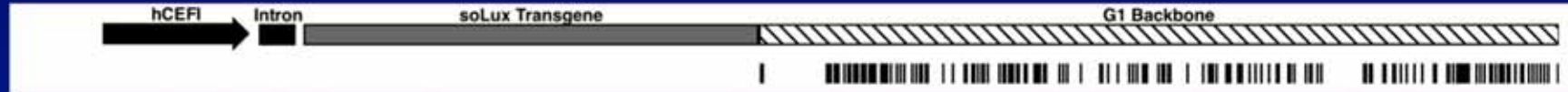


➤ Results are independent of the GTA

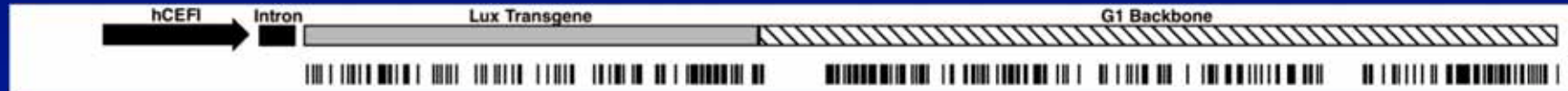
## 2. Effect of plasmid backbone on transgene expression



pG1-hCEFI-soLux  
(176 CpGs)



pG1-hCEFI-Lux  
(273 CpGs)



pG2-hCEFI-soLux  
(51 CpGs)



pG2-hCEFI-Lux  
(149 CpGs)



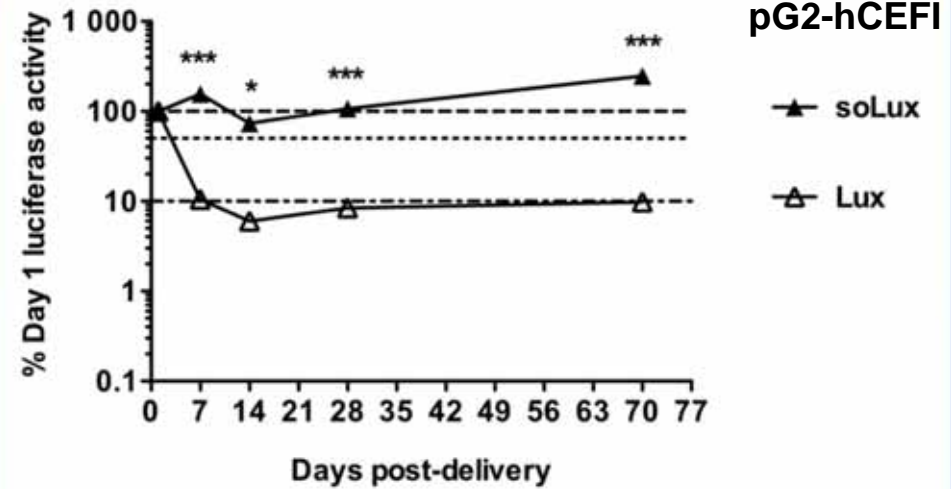
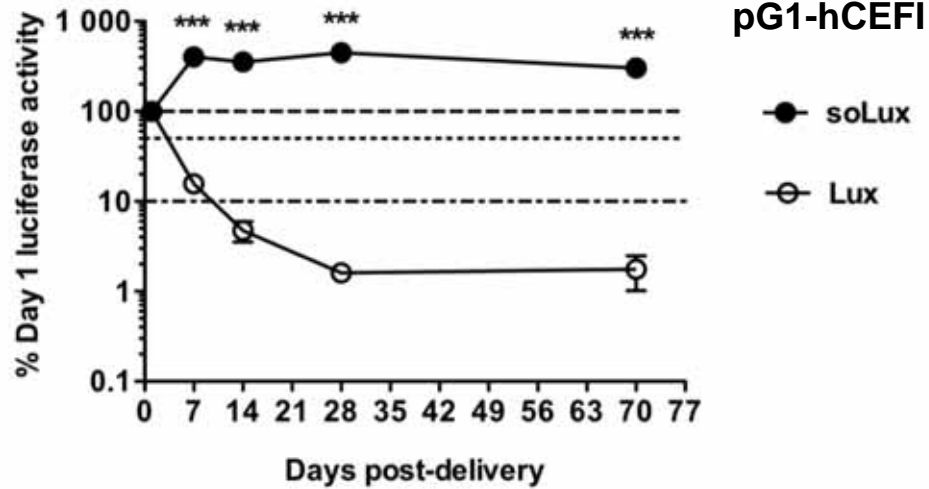
pG4-hCEFI-soLux  
(Zero CpGs)



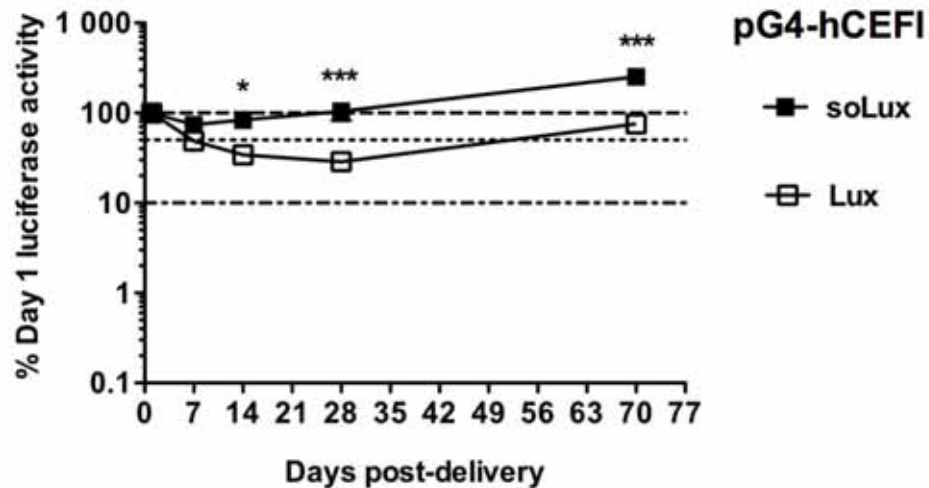
pG4-hCEFI-Lux  
(98 CpGs)



## 2. Effect of plasmid backbone on transgene expression

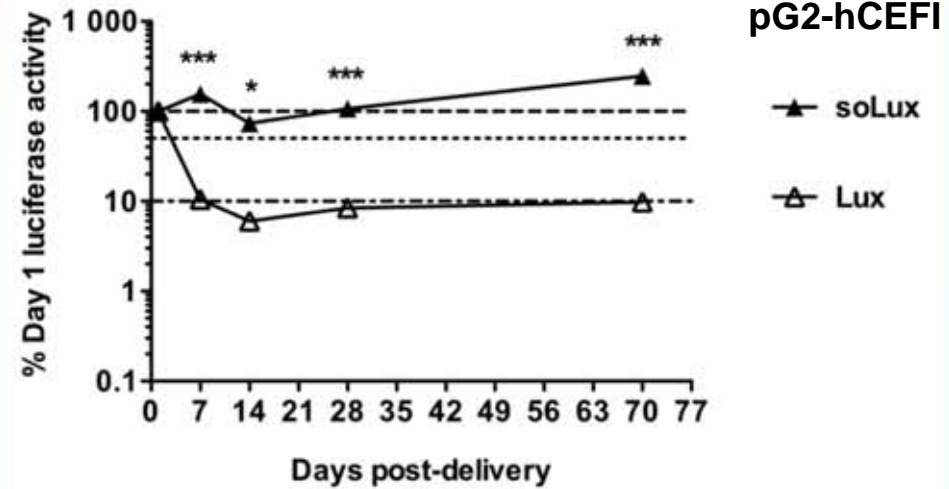
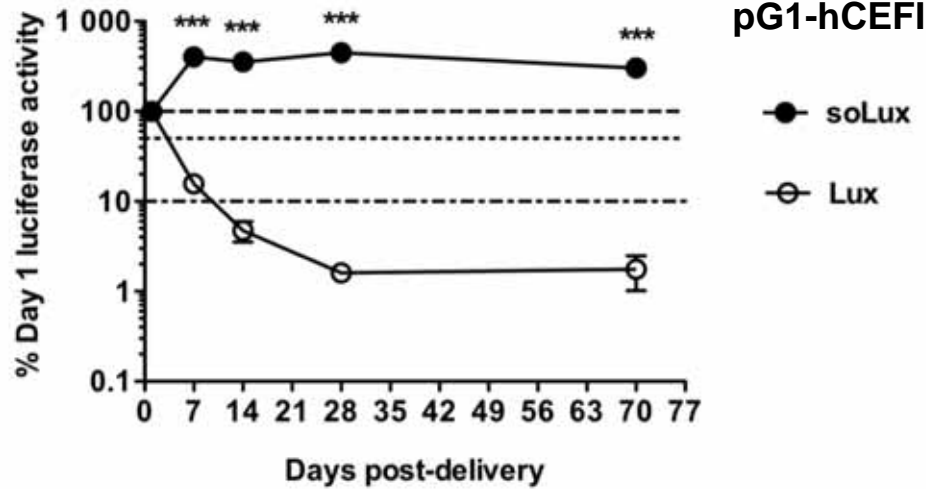


n=6  
BALB/c  
Aerosol  
PEI/pDNA

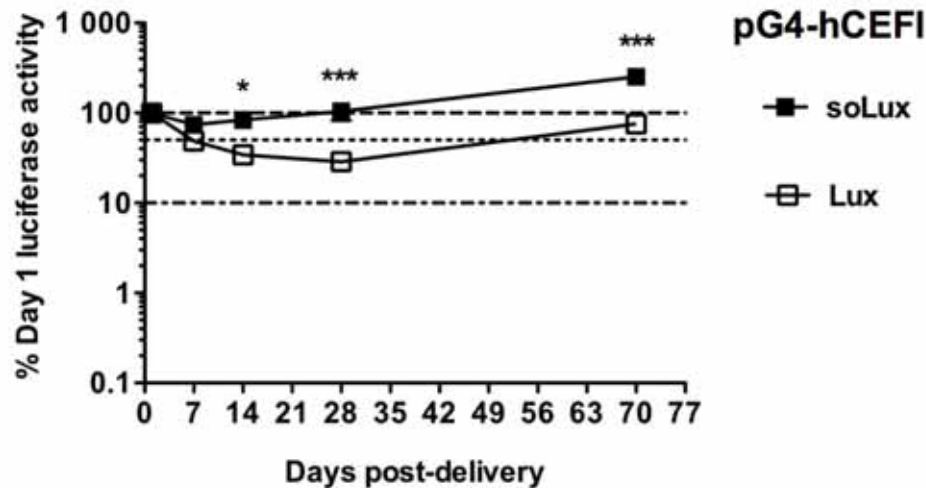




## 2. Effect of plasmid backbone on transgene expression



n=6  
BALB/c  
Aerosol  
PEI/pDNA



➤  $\Delta G1 > G2 > G4$

➤ Results are:

➤ Independent of the backbone

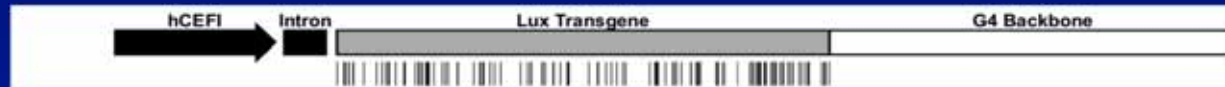
➤ Independent of TLR-9 status

➤ Other pairs of transgenes yield similar results

### 3. Effect of backbone linked to expression cassette



**pG4-hCEFI-Lux**  
(98 CpGs)



**MC-hCEFI-Lux**  
(109 CpGs)



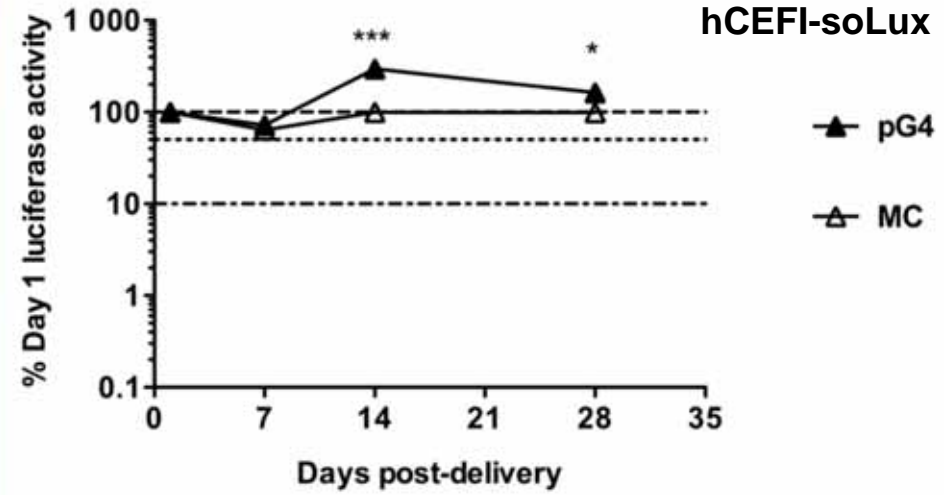
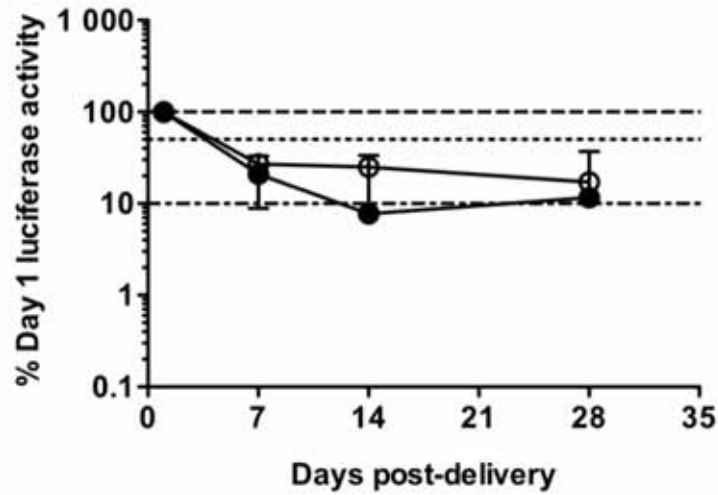
**pG4-hCEFI-soLux**  
(Zero CpGs)



**MC-hCEFI-soLux**  
(11 CpGs)

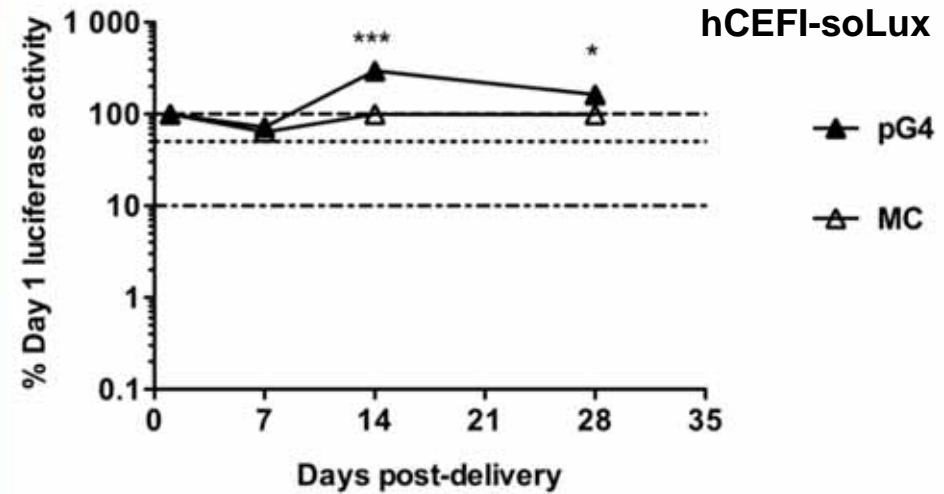
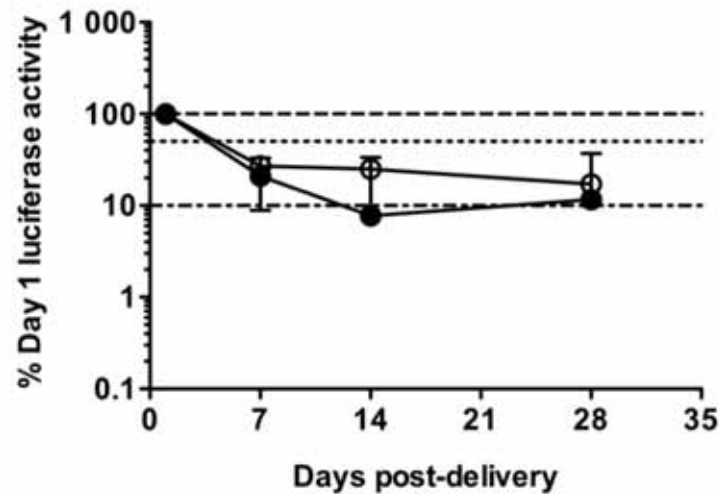


### 3. Effect of backbone linked to expression cassette



n=5-7  
BALB/c  
Aerosol  
PEI/pDNA

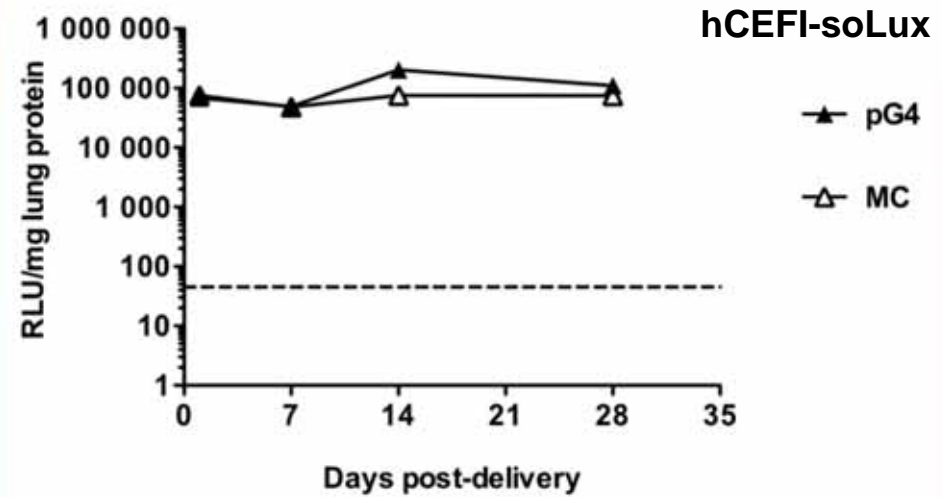
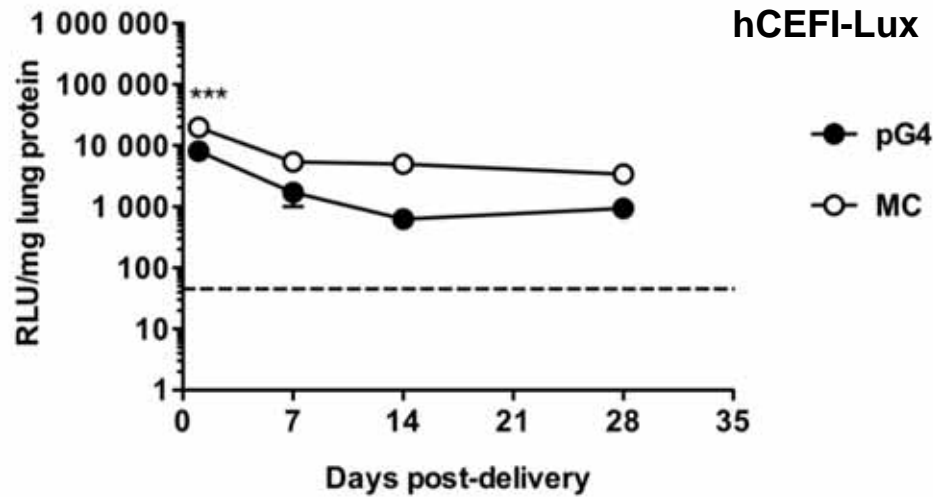
### 3. Effect of backbone linked to expression cassette



n=5-7  
BALB/c  
Aerosol  
PEI/pDNA

- Persistence observed with CpG-free or Minicircle plasmids with soLux
- First time that Minicircles have been assessed in the mouse aerosol model
- Results are independent of backbone

### 3. Effect of backbone linked to expression cassette



n=5-7  
BALB/c  
Aerosol  
PEI/pDNA

- Actual RLU values are higher in Minicircles when using Lux transgene
- This may indicate that Minicircles can lead to higher expression when using CpG-rich transgenes

## Interim Conclusions

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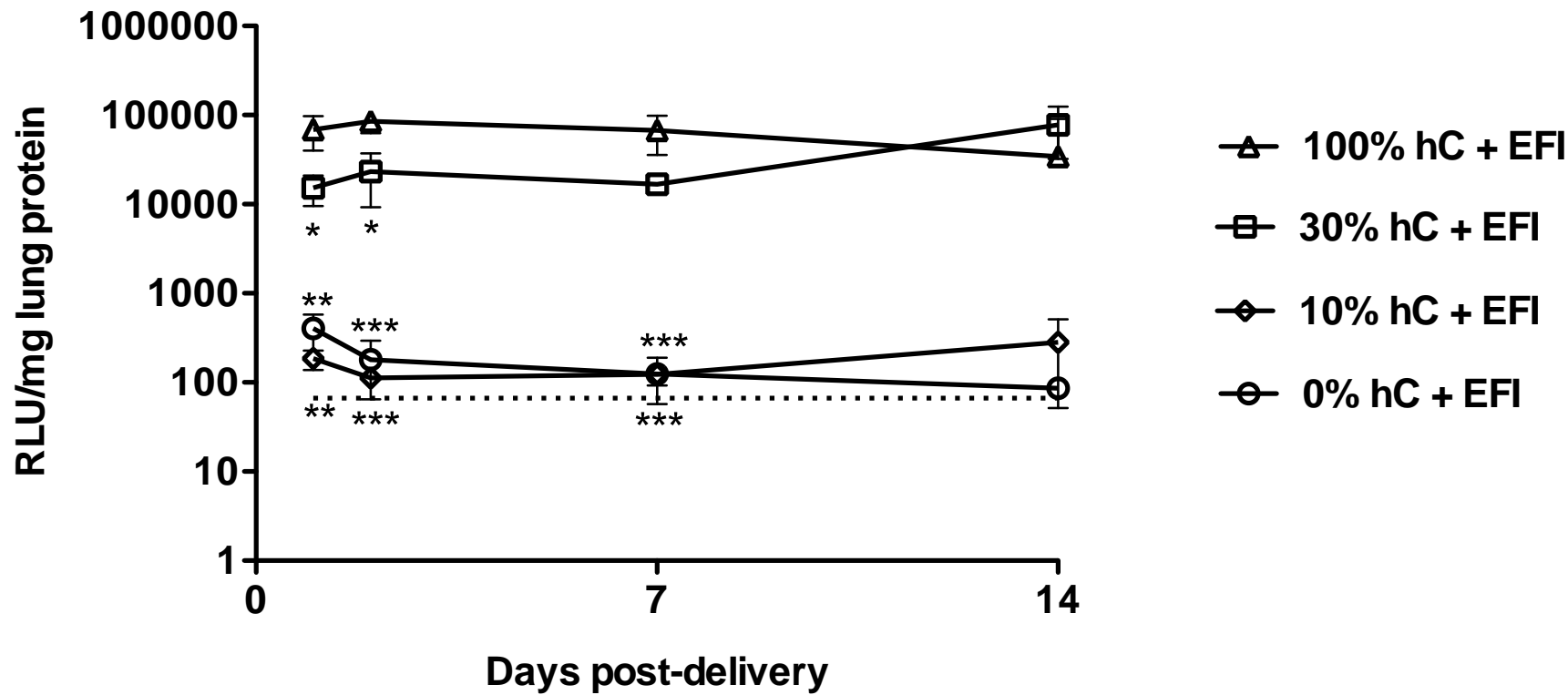
- **Expression observed in the context of CpG content is:**
  - **Independent of GTA, TLR-9 status or backbone**
  - **Dependent on number and location of CpGs**
    - **CpGs in the sequence closest to the promoter (i.e. the transgene) have a significant effect on persistence of expression**
- **hCEFI is still the most successful promoter in the mouse lung and is the most important element in these studies**

# Analysis of the hCEFI promoter



Plasmid Map- Enhancer, Promoter, TSS and Intron Only	% of hC enhancer
	100%
	80%
	50%
	30%
	10%
	0%

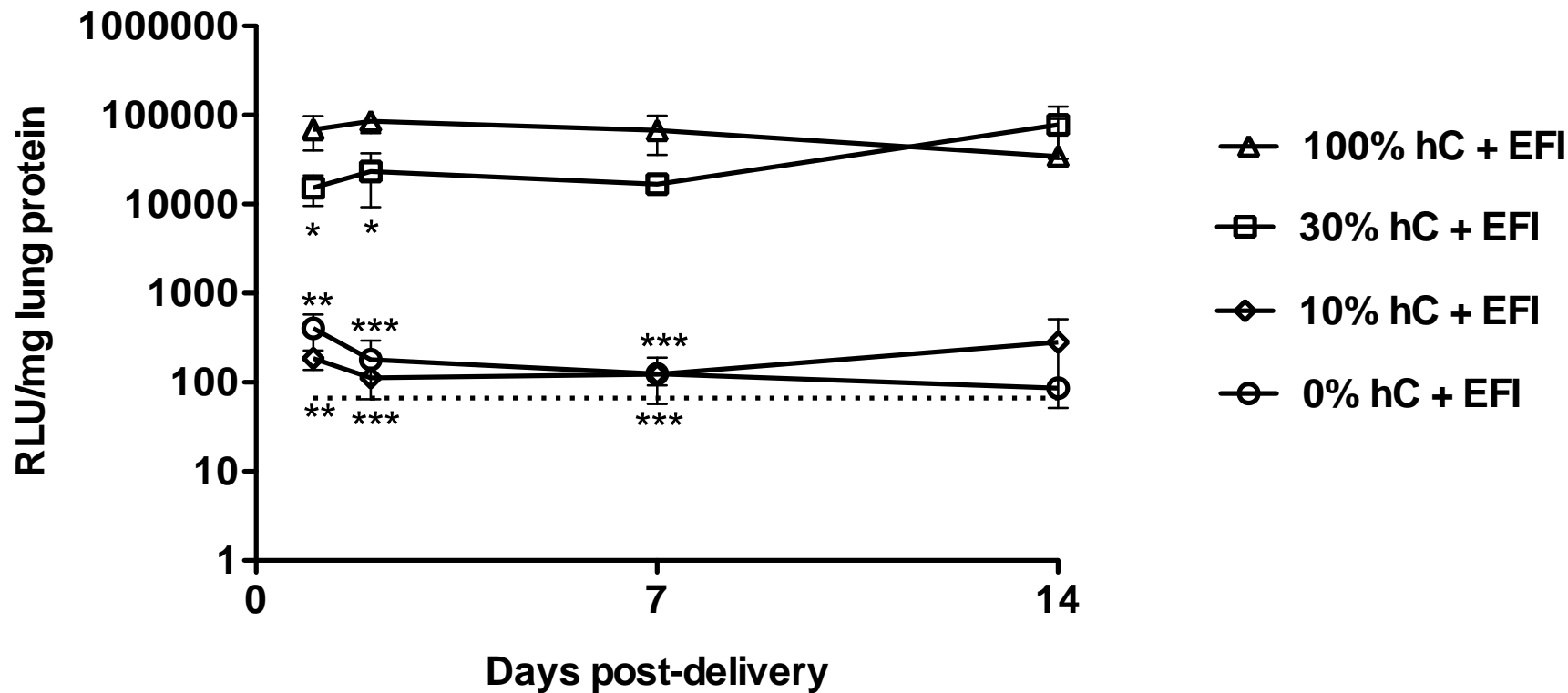
# Only 30% of the hC enhancer is required



n=3  
BALB/c  
Lung Aerosol  
PEI/pDNA



## Only 30% of the hC enhancer is required



n=3  
BALB/c  
Lung Aerosol  
PEI/pDNA

➤ What is the specific sequence or transcription factor binding site(s) required for long-term expression in the mouse lung?

## Conclusions



- The number of CpGs has been implicated in lower levels of transgene expression in the mouse lung (Hyde *et al.*, 2008, Nat Biotech 26(5):549)
- These studies highlight that it is not only the number of CpGs, but their location and density which is important
  - Transgenes rich in CpGs result in lower levels of expression when compared with matched transgenes free of CpGs
  - This observation is independent of TLR-9 status, GTA or whether Minicircles are used
- The portion of hCEFI which is required for duration in the mouse lung is contained in the final 30% of the hC enhancer

## Acknowledgements



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Thank you for your attention!



Edinburgh University  
Imperial College

[cathy.oliveira@linacre.ox.ac.uk](mailto:cathy.oliveira@linacre.ox.ac.uk)