

# In Vivo Analysis of the Replication Capacity and Pathogenic Potential of HIV Primary Isolates from Elite Suppressors

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IAS 2013 Towards an  
HIV Cure Symposium

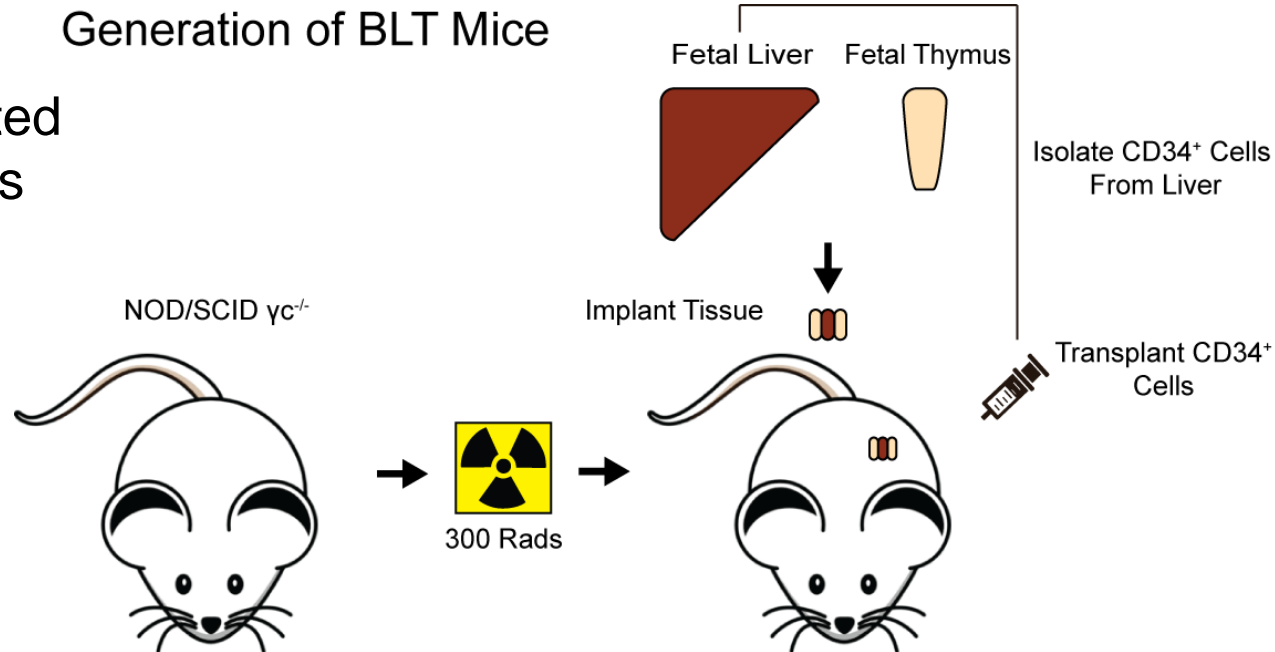
# Elite Suppressors (ES)

- ES= Infected Individuals with viral RNA  $\leq$  50 copies per ml and sustained levels CD4<sup>+</sup> T cells
- Mechanism: Increased immune recognition and/or attenuated virus
  - HLA\*B57
    - Commonly found in ES
  - Mutations resulting in decreased fitness
    - *nef* deletions
  - Few isolates from ES have been characterized (limited *in vivo* studies)

- Is the superior suppression of viral replication by ES attributed to infection with a defective virus?
  - *In vitro* assays may not be representative of *in vivo* results
  - Are HIV isolates from ES replicative and pathogenic *in vivo*?
    - BLT Humanized Mouse Model

# BLT Humanized Mice

- Systemically reconstituted with human immune cells
- Susceptible to natural routes of HIV infection
- Sustain HIV replication
- HIV infection results in CD4<sup>+</sup> T cell depletion



*Nat. Med.* **12**, 1316 (2006)

*Nat. Commun.* **3**, 716 (2012)

- ES38
  - Viral load below 50 copies per mL
  - 935 CD4<sup>+</sup> T cells per  $\mu$ L
  - Not on ART
  - HLA-B\*57:03
  - WT *ccr5*
    - Two HIV isolates: ES38-5 & ES38-9
      - Subtype B
      - No drug resistance mutations, intact *nef* orf

Joel Blankson, Maria Salgado, Christopher Polymeyer, Robert Buckheit III

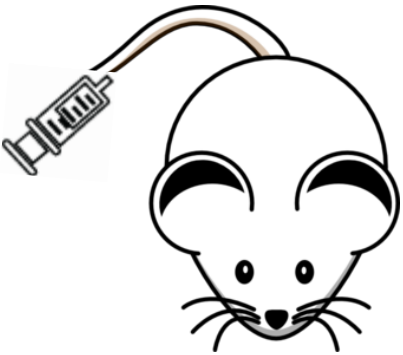
J Virol. 2010 July; 84

- ES8
  - Viral load 26 copies per mL
  - 626CD4<sup>+</sup> T cells per  $\mu$ L
  - Not on ART
  - HLA-B\*57:03
  - WT CCR5
    - HIV isolates: ES8
      - Subtype B
      - No drug resistance mutations, intact *nef* orf

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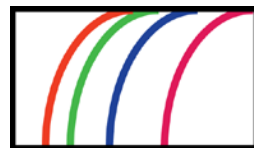
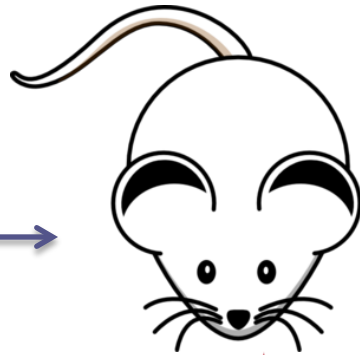
# Experimental Outline

Inject Virus  
Via Tail Vein



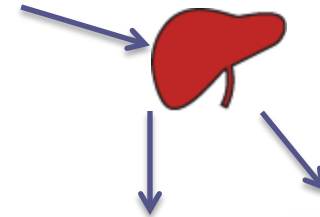
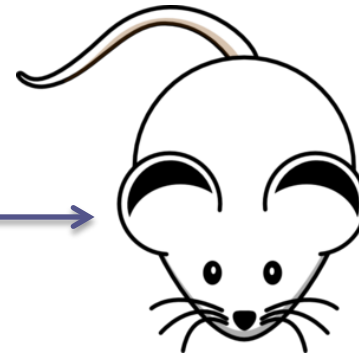
Non-HLA\*B57  
BLT Mice

Obtain Blood/Plasma  
(Several Weeks)



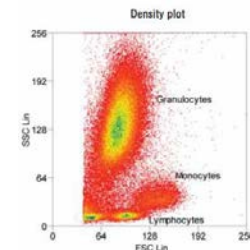
qRT-PCR

Harvest Tissues

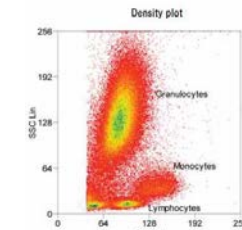


A T C G

Sequencing



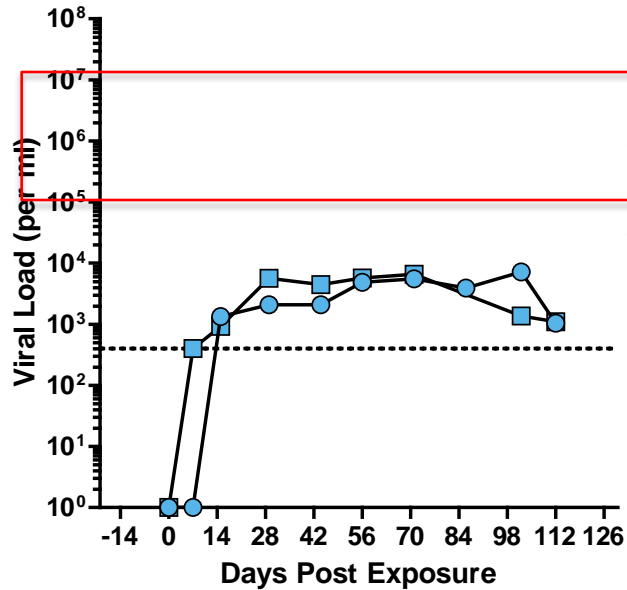
Flow Cytometry



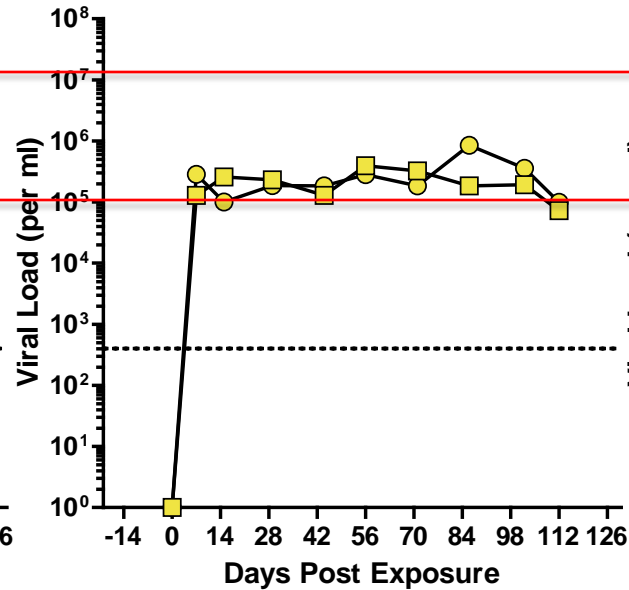
Flow Cytometry

# ES Isolates are Replication Competent In Vivo

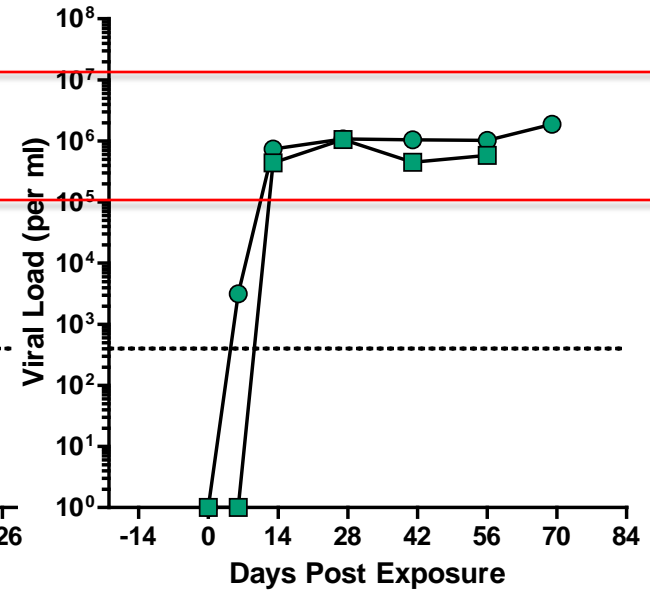
### ES38-5



### ES38-9



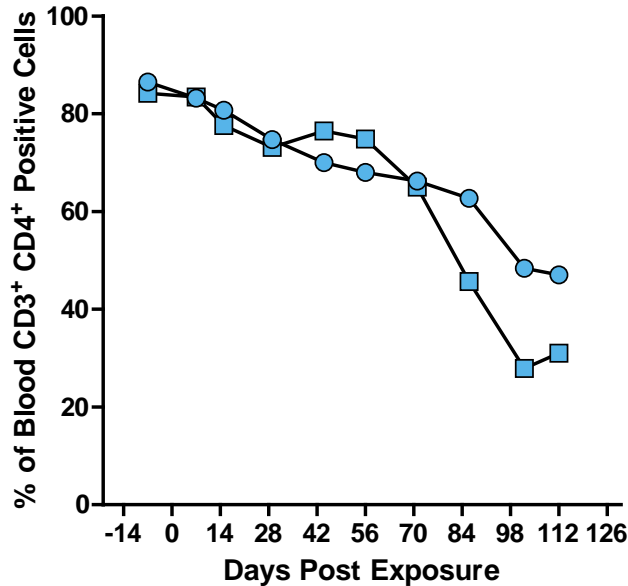
### ES8



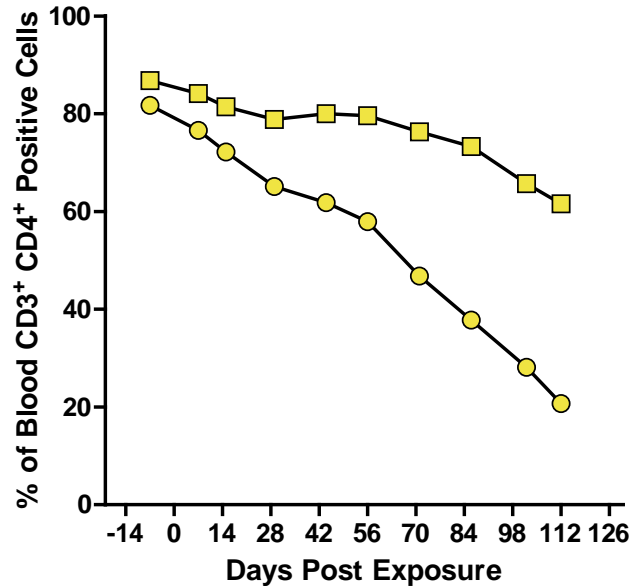


# ES Isolates decrease blood CD4<sup>+</sup> T levels

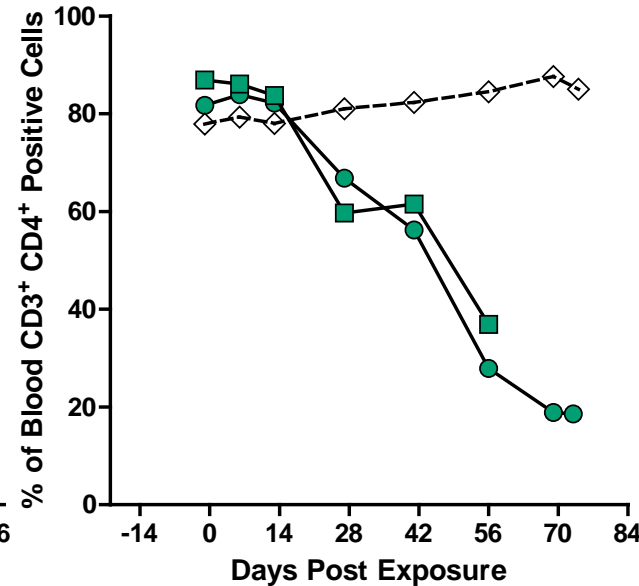
### ES38-5



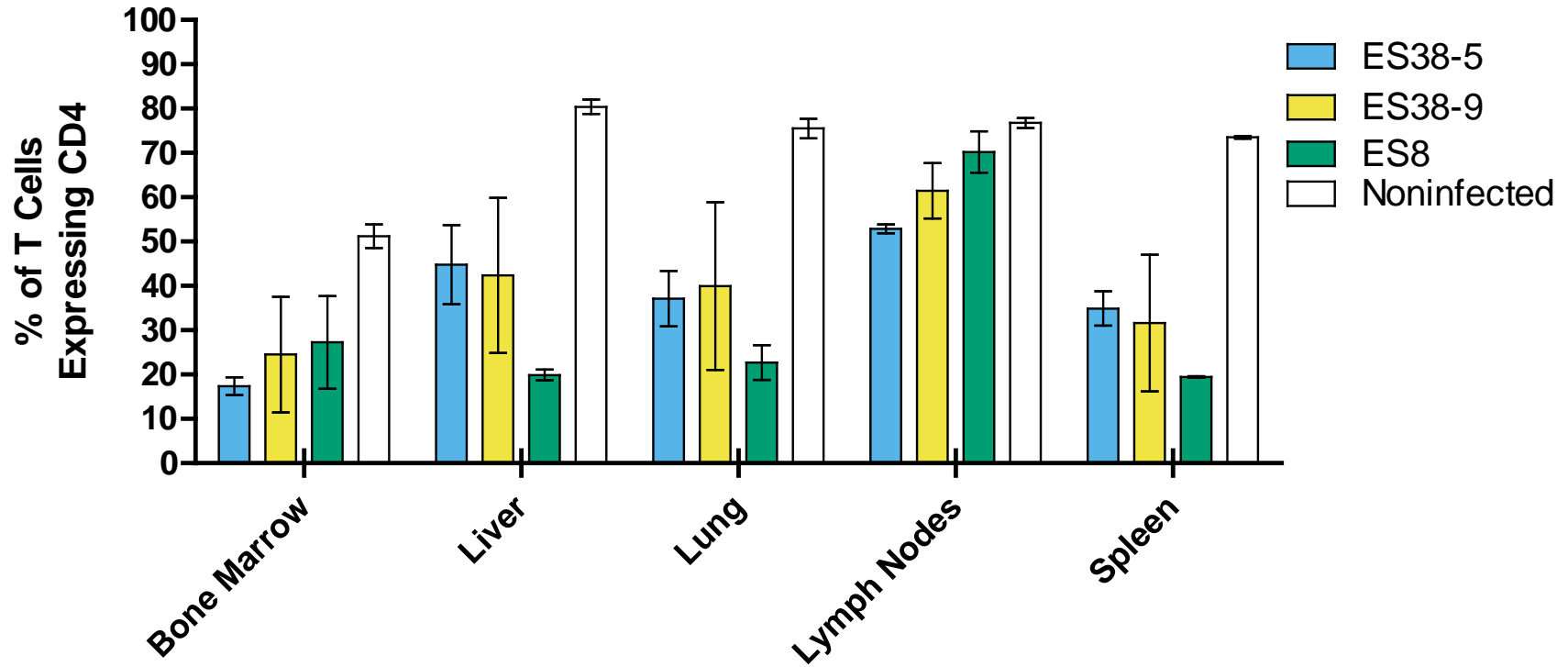
### ES38-9



### ES8



# Systemic Depletion of CD4<sup>+</sup> T cells



# ES Isolates Do Not Mutate to Become Pathogenic and Replicative

## HLA\*B57 epitopes

	Gag			Nef		
	ISW9	KF11	TW10	KF9	HW9	YY8
	150	170	245	100	123	
Subtype B Consensus	... ...  AISPRTLNAW	... ... ... KAFSPPEVIPMF	... ... TSTLQEQIGW	. ... ... KGALDLSHF	... ... HTQGYFPDWQNY	
ES38-5 Virus	.L.....	.....	.....A.	.A.....	N.....	
ES38-5 Mouse #1 T. Org.	.L.....	.....	.....A.	.A.....	N.....	
ES38-5 Mouse #1 Spleen	.L.....	.....	.....A.	.A.....	N.....	
ES38-5 Mouse #2 T. Org.	.L.....	.....	.....A.	.A.....	N.....	
ES38-5 Mouse #2 Spleen	.L.....	.....	.....A.	.A.....	N.....	
ES38-9 Virus	.L.....	.....	.....A.	.A.....	N.....	
ES38-9 Mouse #1 T. Org.	.L.....	.....	.....A.	.A.V.....	N.....	
ES38-9 Mouse #1 Spleen	.L.....	.....	.....A.	.A.V.....	N.....	
ES38-9 Mouse #2 T. Org.	.L.....	.....	.....A.	.A.V.....	N.....	
ES38-9 Mouse #2 Spleen	.L.....	.....	.....A.	.A.V.....	N.....	

ES38-5 and ES38-9 Mice were HLA\*B18 and HLA\*B51







# Acknowledgements

- Johns Hopkins
  - Joel Blankson
  - Robert Siliciano
  - Maria Salgado
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