

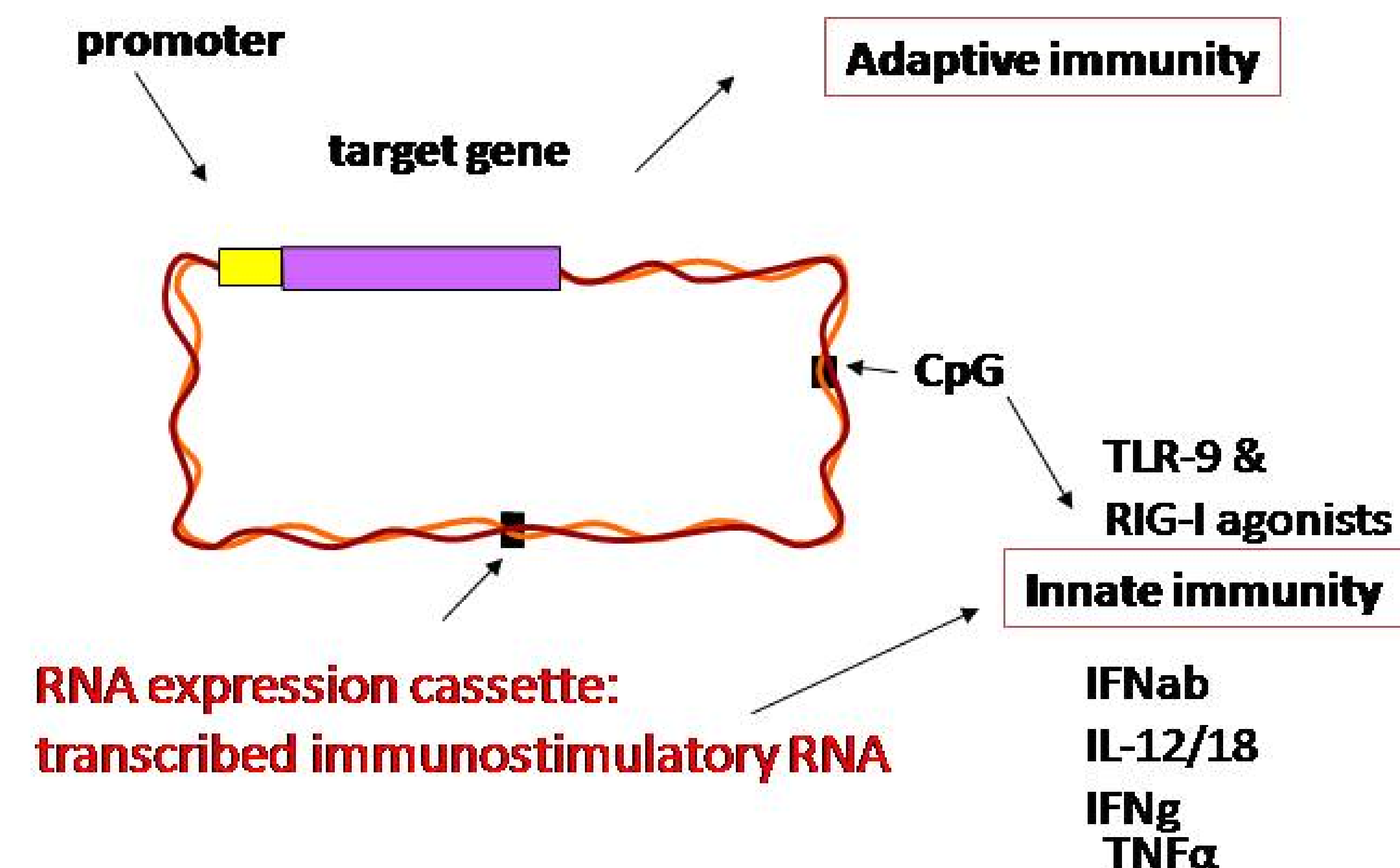
# Coexpressed RIG-I agonist enhances antigen-specific immune response to Influenza DNA Vaccine

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## Abstract

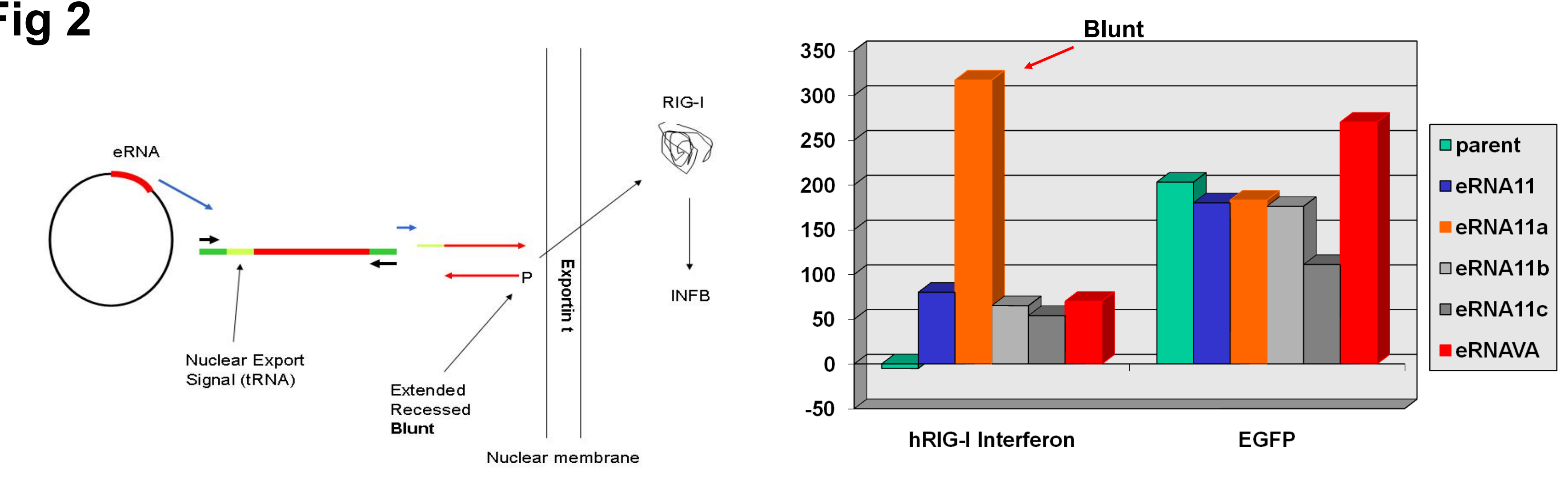
Methods to increase DNA vaccine induced innate immune responses to improve adaptive immunity are needed. Retinoic-acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated gene 5 (mda5) are critical cytoplasmic double stranded RNA (dsRNA) pattern receptors required for innate immune activation in response to viral infection. Activation of RIG-I and mda5 leads to type I interferon (IFN) and inflammatory cytokine production through IPS-1 signaling. With the goal of improving DNA vaccination, we have evaluated the effects on humoral immunity of expression of RIG-I agonist RNA from the vector backbone. Optimized, potent RIG-I agonists (eRNAs) were developed (e.g. eRNA41H) and integrated into the backbone of various DNA vaccine vectors expressing influenza H5N1 hemagglutinin (HA). These vectors potently induced type I IFN production in cell culture through RIG-I activation and combine high level antigen expression with RNA-mediated type I IFN activation. eRNA vectors improved HA-specific serum antibody titers, and HA-specific antibody binding avidity after naked DNA immunization. This demonstrates DNA vaccine potency may be augmented by incorporation of a RIG-I activating agonist into the vector backbone (Fig. 1).

Fig 1



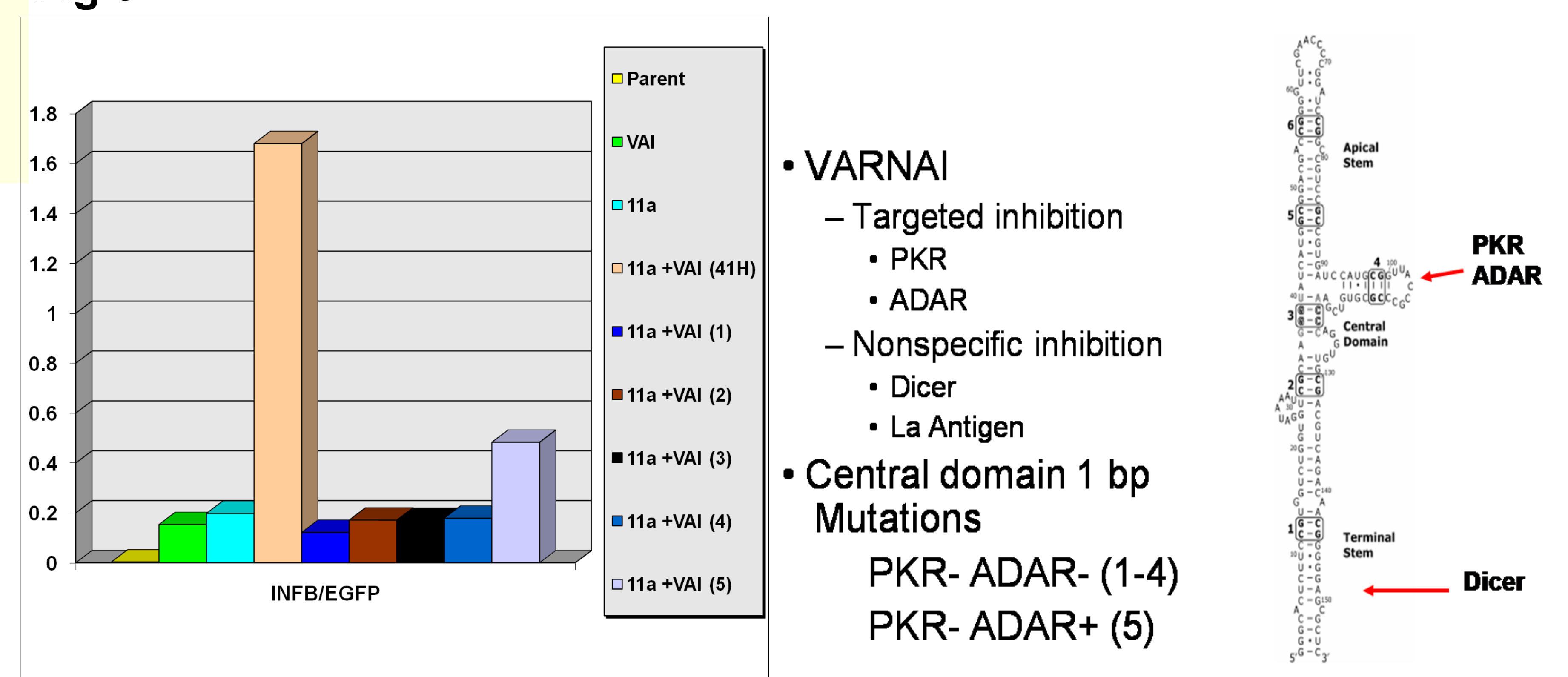
Plasmid vectors expressing single and double stranded RNA's from RNA polymerase III promoters (Williams, 2006) were screened in a human cell culture (HEK293) transfection assay for RIG-I agonist activity (i.e. RIG-I dependent activation of a cotransfected IFN $\beta$  promoter-luciferase reporter). Several RIG-I agonists (eRNAs) were identified and mutagenized versions were screened for improved activity. An optimized convergently transcribed 100 bp RIG-I agonist (eRNA11a) was identified and is shown in Fig. 2.

Fig 2



Plasmid vector backbones expressing various combinations of RIG-I agonists were screened in the HEK293 transfection assay. Synergistic RIG-I activation was obtained with eRNA41H, a combination of adenoviral VA RNAI (VAI) and eRNA11a (Fig. 3). VAI mutagenesis revealed VAI-mediated inhibition of adenosine deaminase acting on RNA (ADAR) is required for eRNA11a potency increasing activity (Williams, 2008).

Fig 3

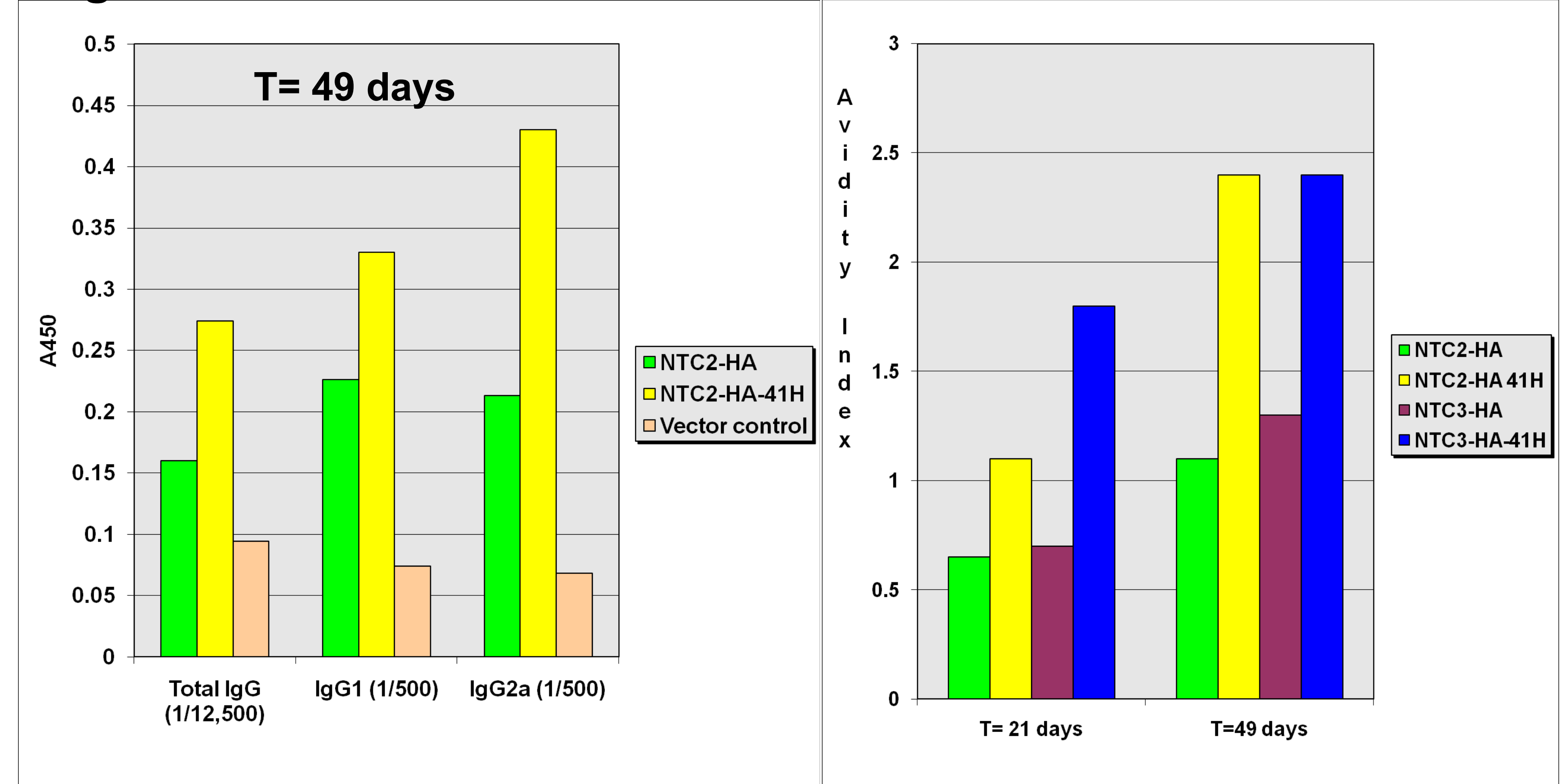


The potent RIG-I agonist eRNA41H was integrated into the backbone of various DNA vaccine vectors (Williams *et al.*, 2006; Luke *et al.*, 2009) expressing detoxified influenza H5N1 A/Vietnam/1203/2004 hemagglutinin (HA). These vectors potently induced type I IFN promoters (IFN  $\alpha$  and  $\beta$ ) in cell culture through RIG-I activation and expressed high levels of HA protein.



Antigen-specific humoral immune responses were evaluated in BALB/C mice after intramuscular delivery of 10  $\mu$ g naked plasmid DNA in prime (day 0) and boost (day 21) injections. The eRNA HA vectors had improved HA-specific serum antibody titers, and improved HA-specific antibody binding avidity, compared to HA vector alone on both day 21 (prime only) and day 49 (prime-boost) with two different vector backbones (NTC2 and NTC3) (Fig. 4).

Fig 4



## Conclusions

- Plasmid encoded RNA polymerase III expressed RIG-I agonists were identified using a cell culture transfection assay
- Combinational RIG-I agonist eRNA41H (eRNA11a and adenoviral RNA VAI) activates a IFN $\beta$  reporter in human (HEK293 and A549) and murine (NIH3T3 and L929) cell lines
- VAI increases eRNA11a potency through ADAR inhibition. We hypothesize that A to I ADAR edited cytoplasmic dsRNA has reduced RIG-I agonist activity
- eRNA41H DNA vaccine vectors combine high level antigen expression with RNA-mediated type I IFN activation
- eRNA41H DNA vaccine vectors have high yield plasmid manufacture up to 2.1 gm/L (Williams *et al.*, 2009)
- eRNA41H HA DNA vaccine vectors improves antibody titer and antibody binding avidity

## Acknowledgements

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## References

Luke J, Carnes AE, Hodgson CP, Williams JA. (2009) Improved antibiotic-free DNA vaccine vectors utilizing a novel RNA based plasmid selection system. *Vaccine*, in press

Williams JA. (2006) Vectors and Methods for Genetic Immunization. World Patent Application WO2006078979

Williams JA. (2008) Vectors and Methods for Genetic Immunization. World Patent Application WO2008153733

Williams JA, Luke J, Johnson L, Hodgson C. (2006) pDNAVACUltra vector family: high throughput intracellular targeting DNA vaccine plasmids. *Vaccine*, 24: 4671-6.

Williams JA, Luke J, Langtry S, Anderson S, Carnes AE, Hodgson CP. (2009) Generic plasmid DNA production platform incorporating low metabolic burden seed-stock and fed-batch fermentation processes. *Biotechnol. Bioeng.*, submitted