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Pneumolysin : Immunogen and adjuvant

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Pneumolysin as a vaccine candidate

- 53kDa cytoplasmic pore forming toxin
- Released during infection
- Conserved
- Expressed by all invasive serotypes
- Protective immunogen

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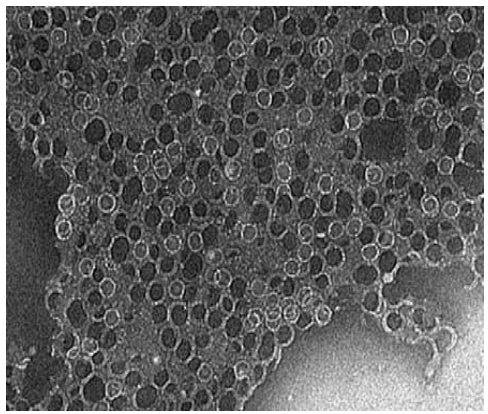
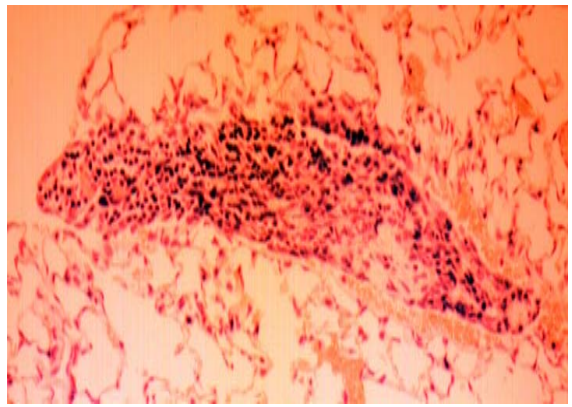
Cytotoxicity of wild type toxin

- Cytotoxic to mammalian cells
- Stimulates inflammatory cytokine release following in vivo treatment
- Reproduces pneumococcal disease symptoms in lungs



Negatively stained PLY treated erythrocyte membrane (0.1mg/ml PLY)



H&E stained lung section x10 mag. (12h post 0.5µg PLY treatment)

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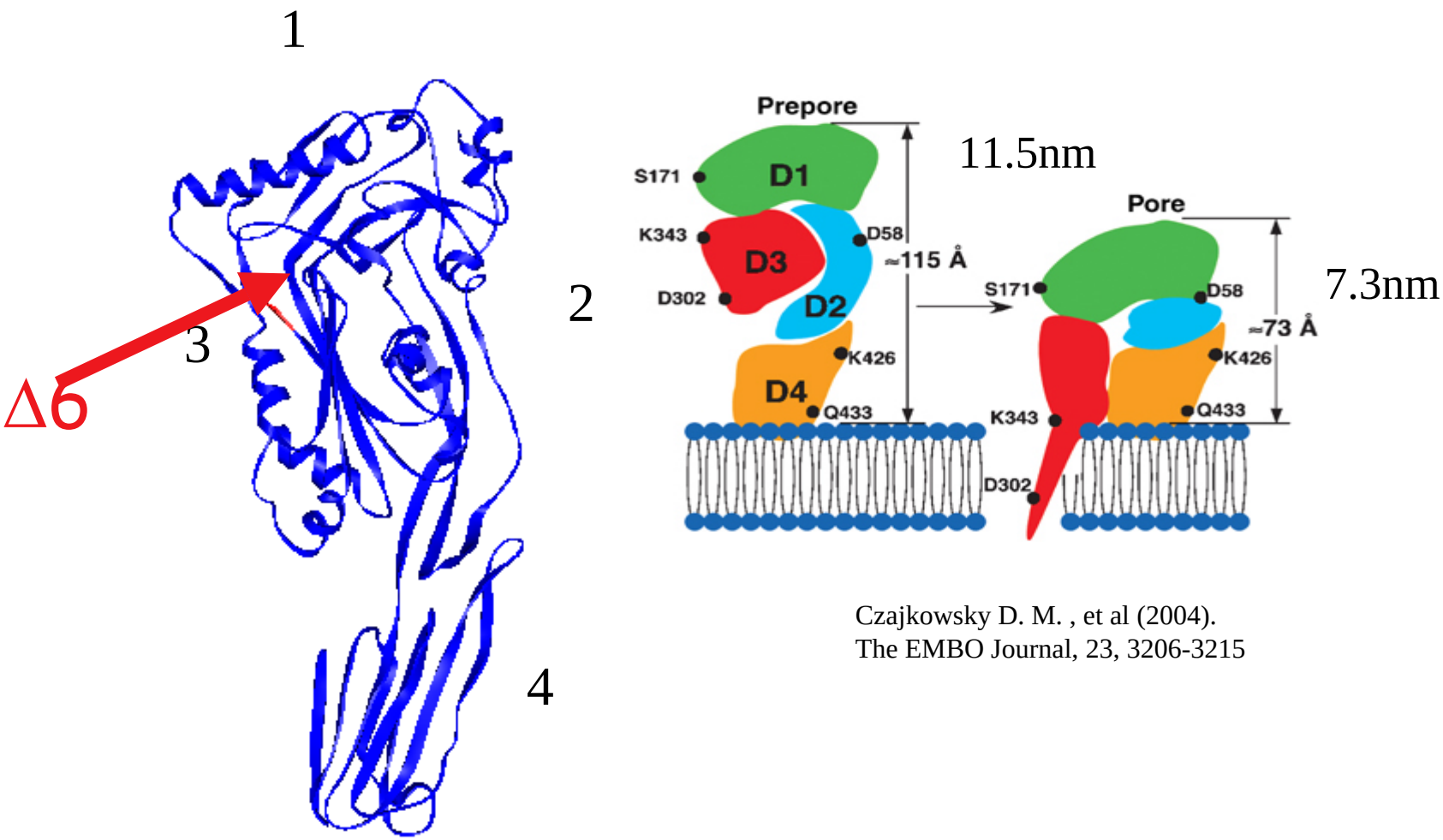
A double deletion (A146R147, Δ6) results in a non-toxic form of PLY



Czajkowsky D. M. , et al (2004).
The EMBO Journal, 23, 3206-3215

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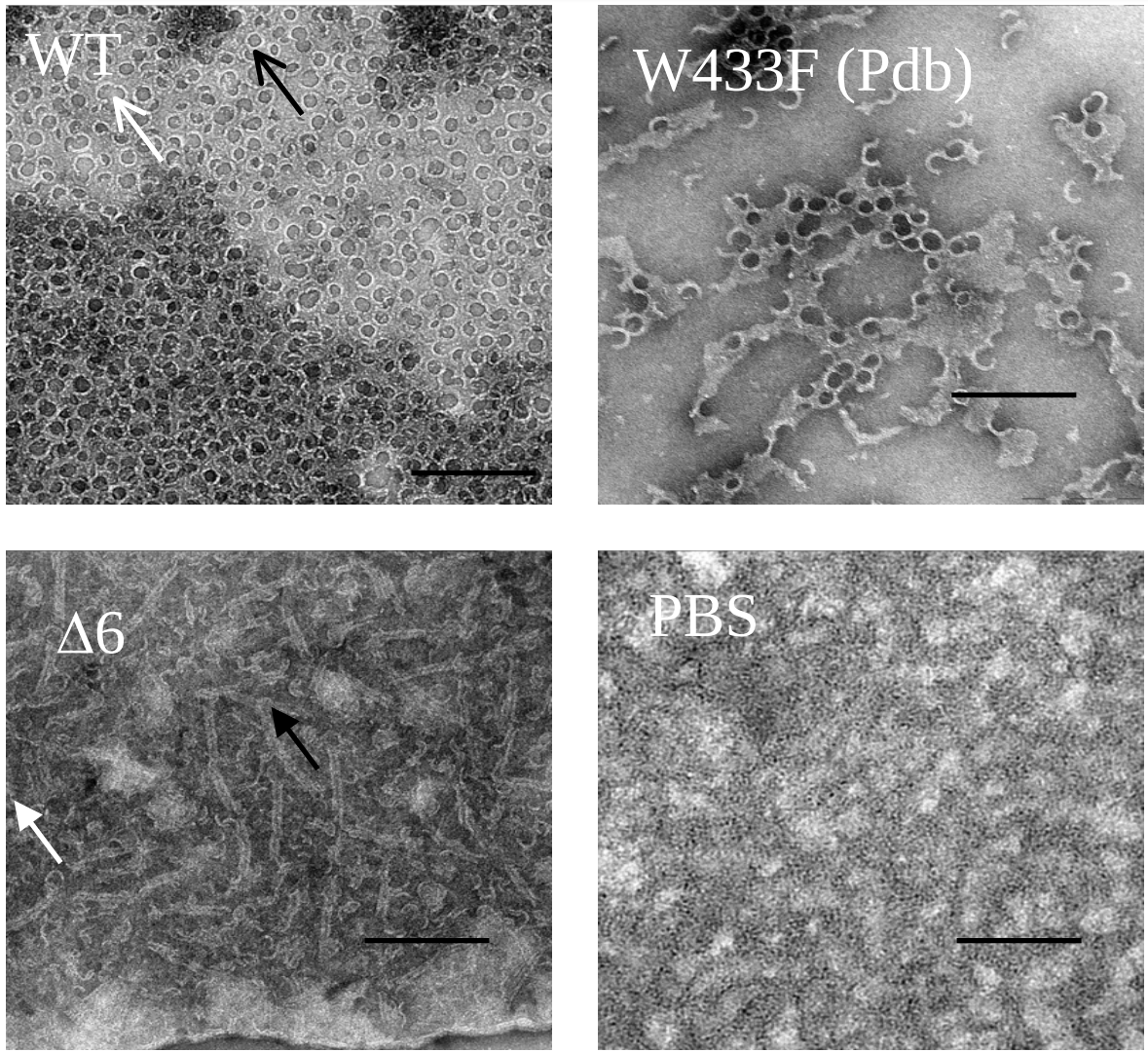
Delta6 does not form pores



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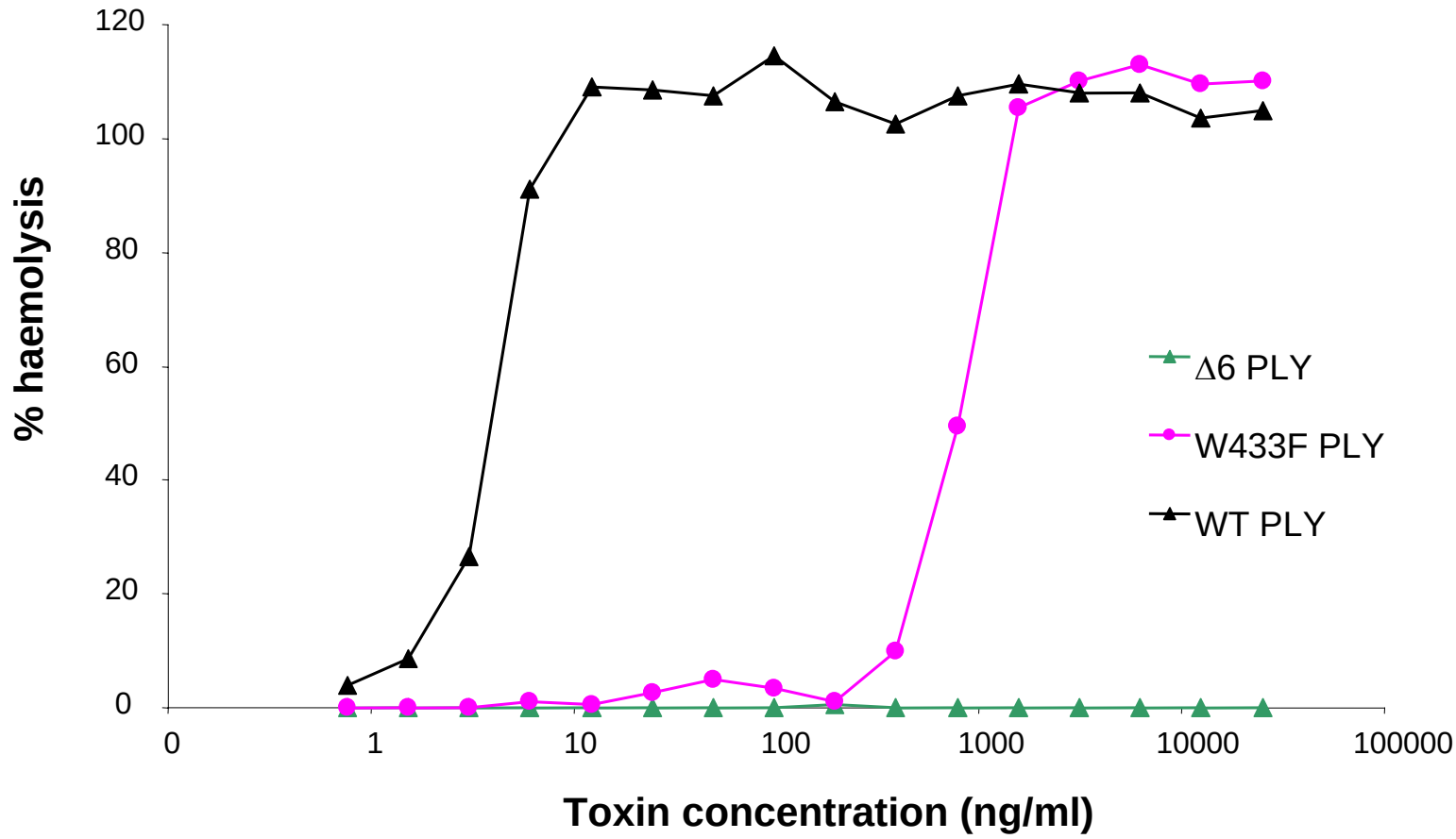
Δ6 PLY does not lyse erythrocytes



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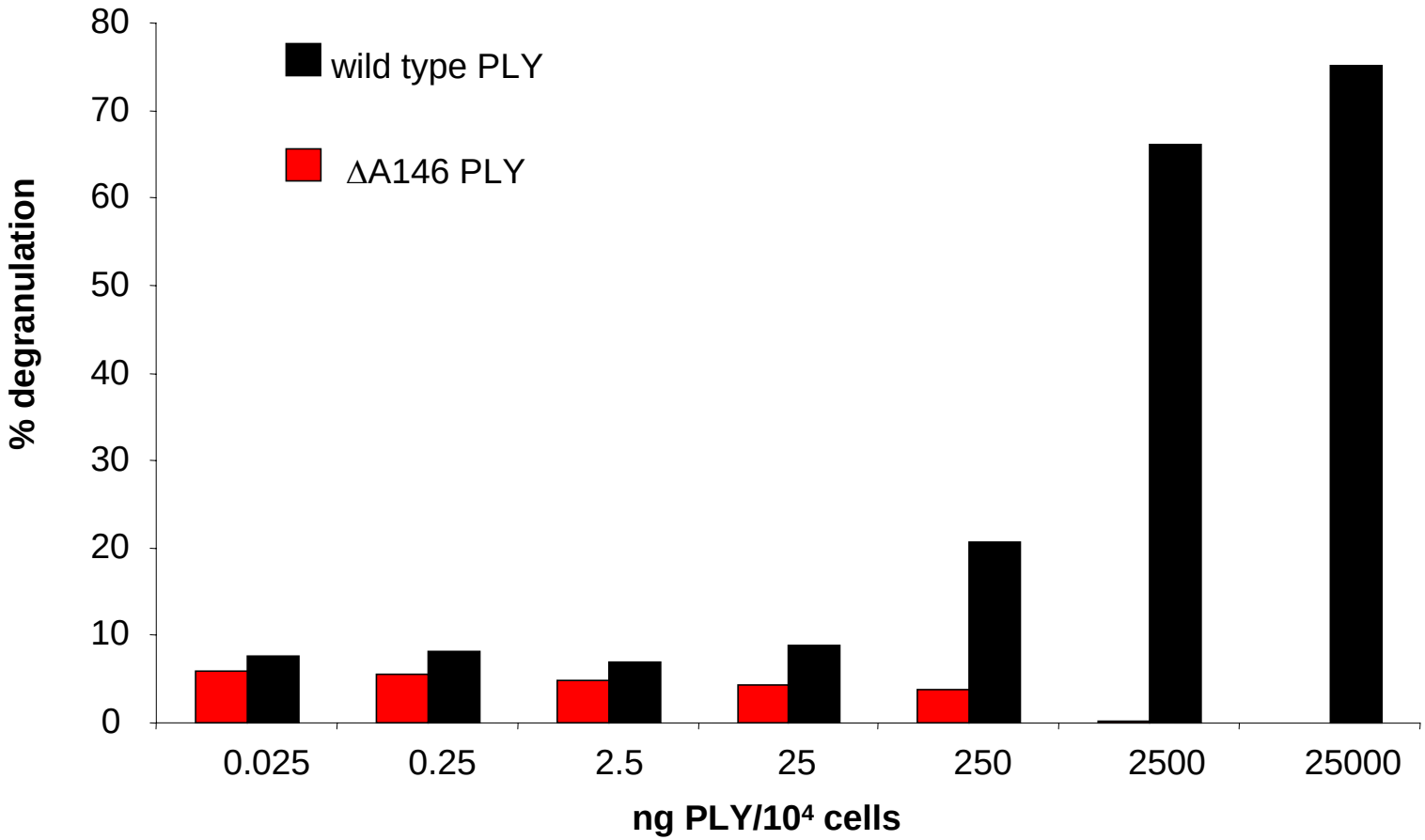
Δ6 PLY does not stimulate degranulation of rat RBL-2H3 mast cells



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In vivo analysis of Δ6 PLY

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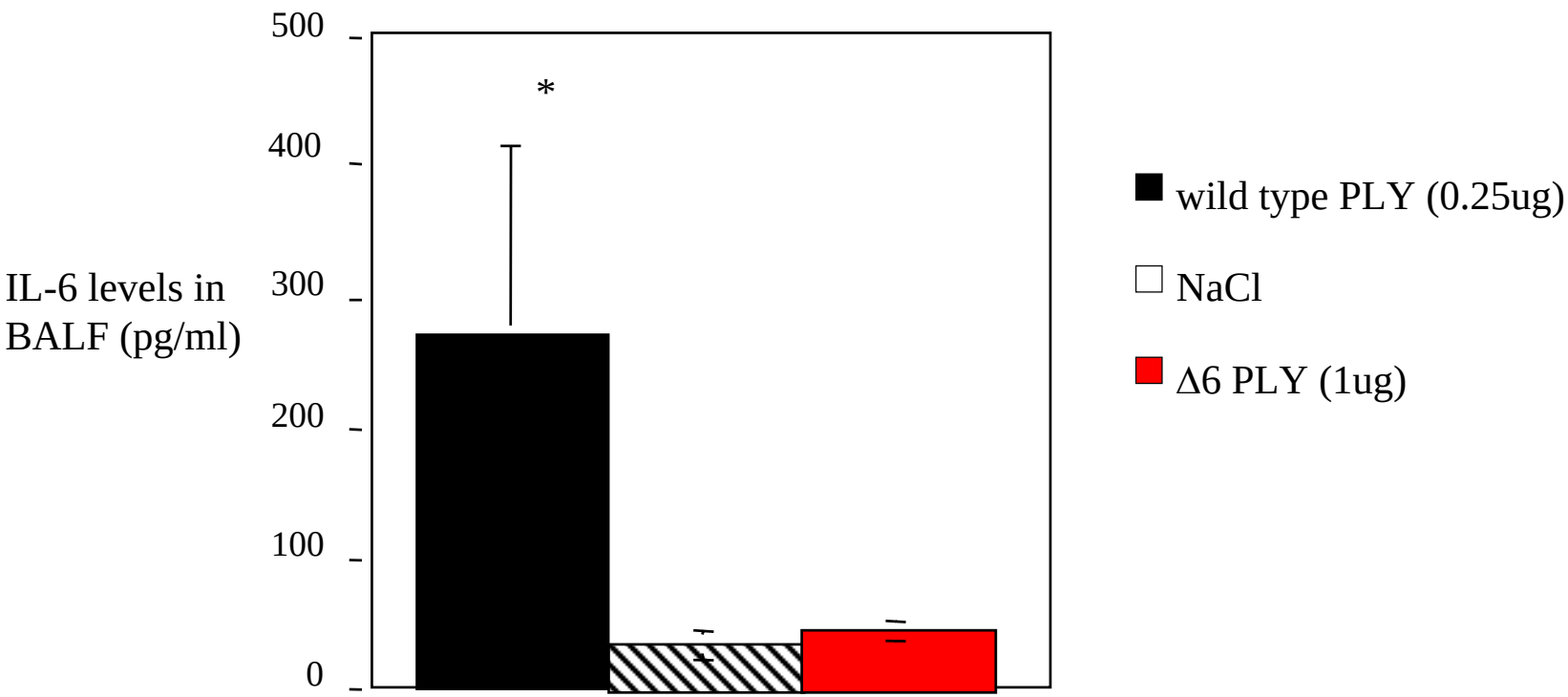
IL-6 Levels in BALF



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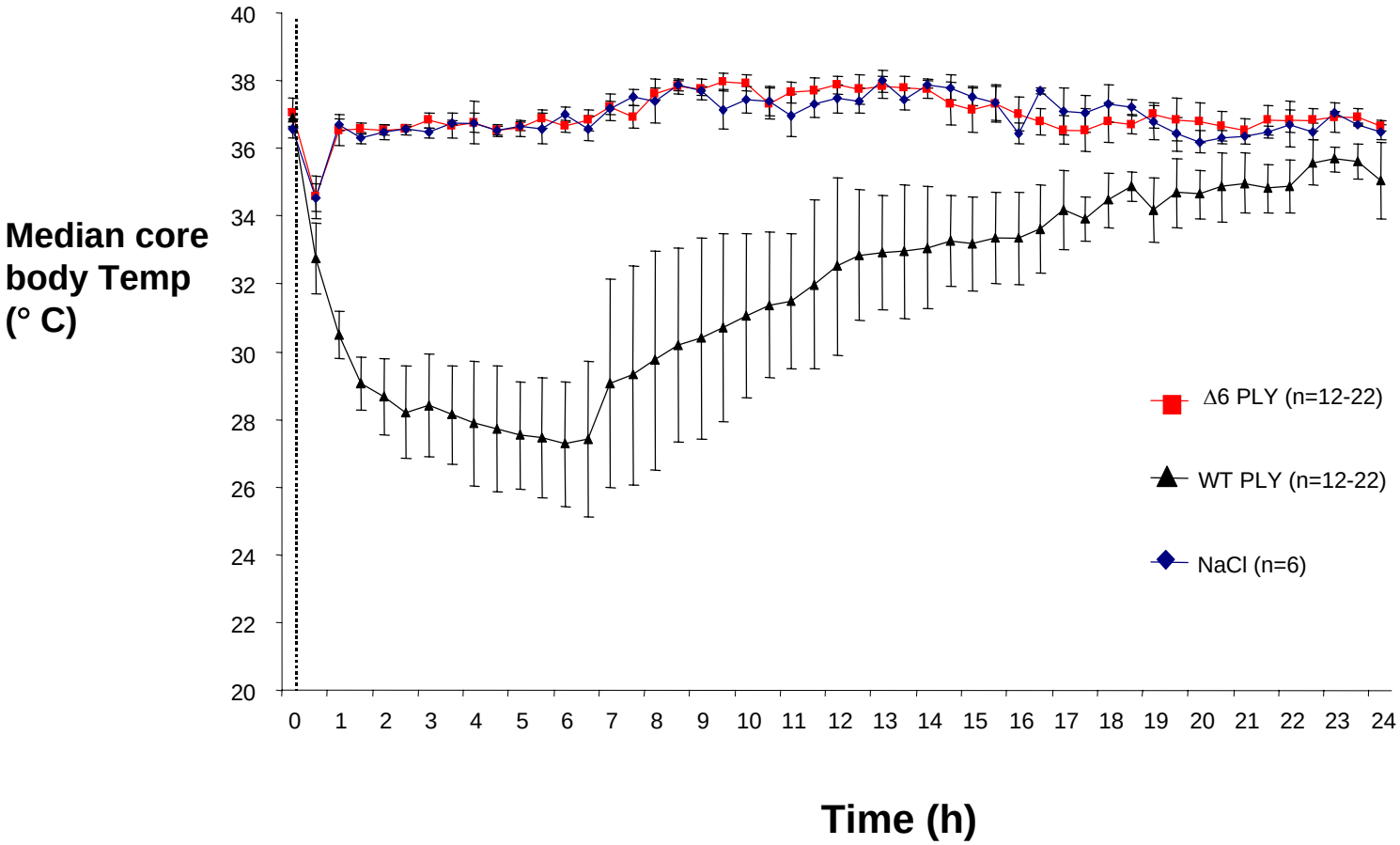
Effect of PLY and d6 PLY on core body temperature



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Vaccination

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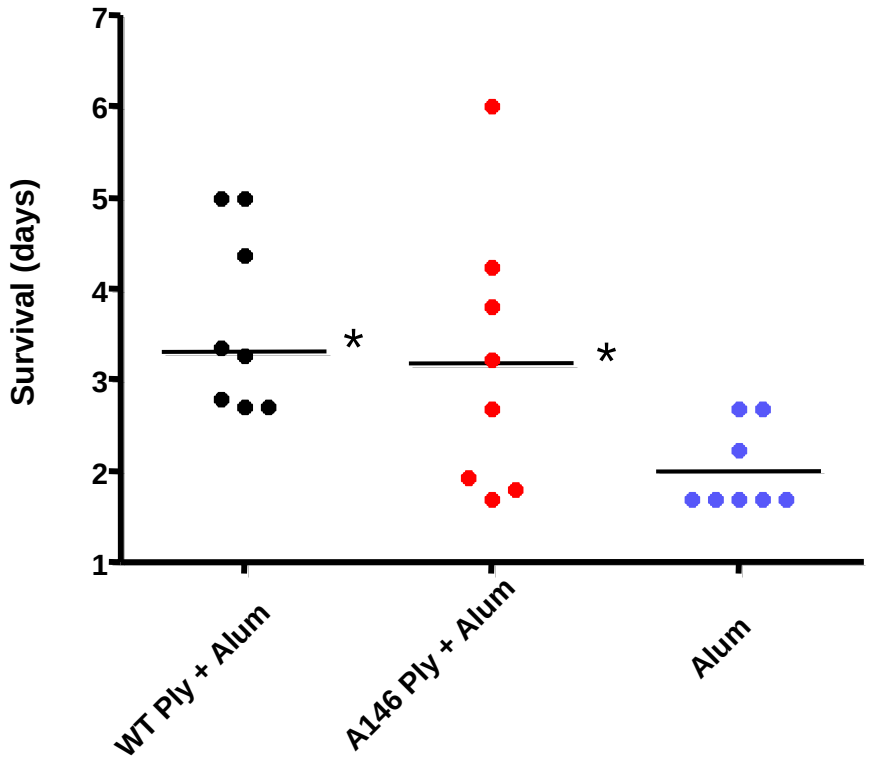
Pneumolysin toxoid is protective (IP, 100 TIGR4)



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Polysaccharide/Protein conjugates

- Δ6 Ply has been conjugated to CPS from type 4 *S.pneumoniae*.
- Mice were immunised with free protein, free CPS, the conjugate, Prevnar and Prevnar mixed with the conjugate (n=10) (and Alum only and PBS controls, all samples corrected to have similar amounts of CPS and Alum)
- Mice were challenged with TIGR4 (10^2 CFU, i.p.)

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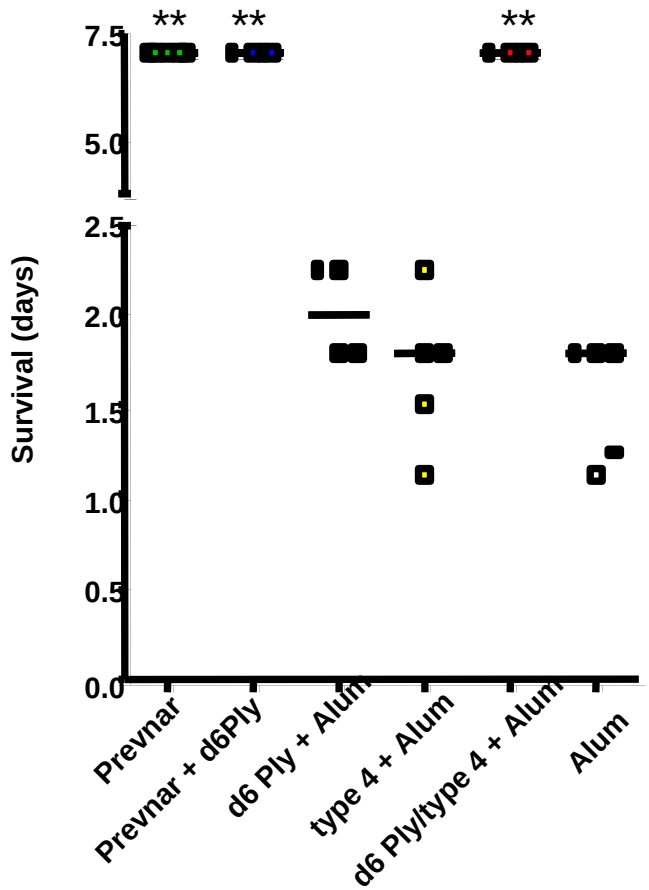
Vaccination with d6PLY conjugate (IP, 100 TIGR4)



**P<0.01, *P<0.05 when compared with Alum group.

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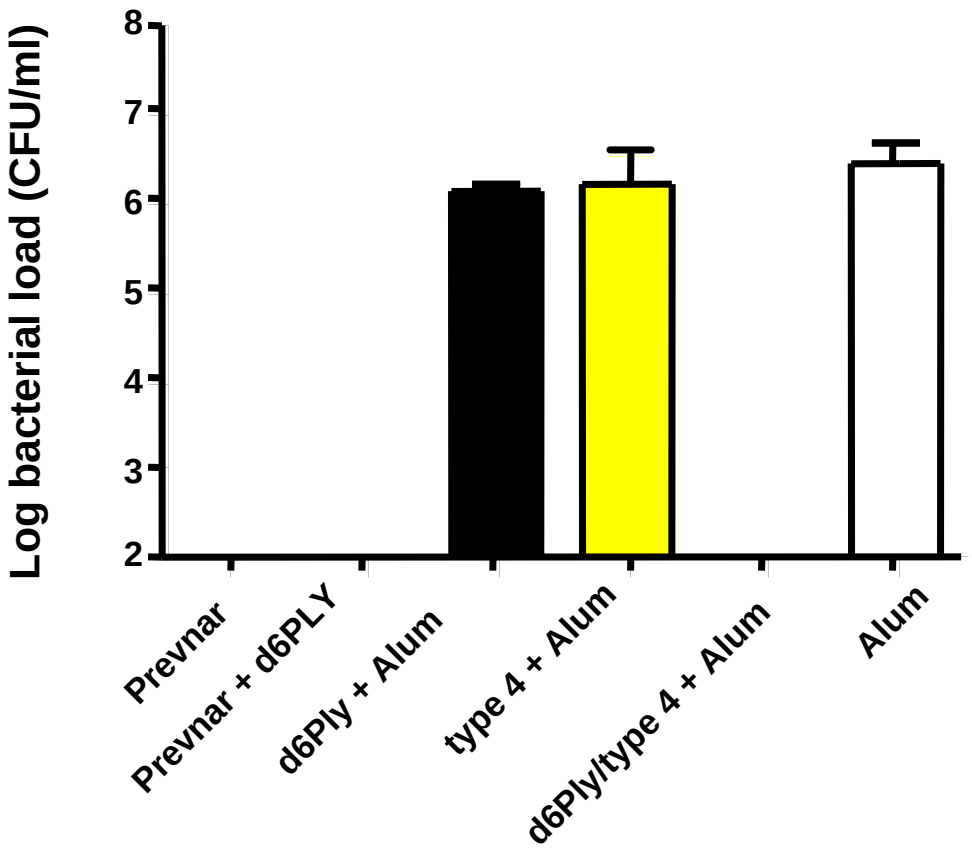
Conjugate protects against invasive disease

TIGR 4 18h bacterial load in blood



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Pneumolysin-protein conjugates

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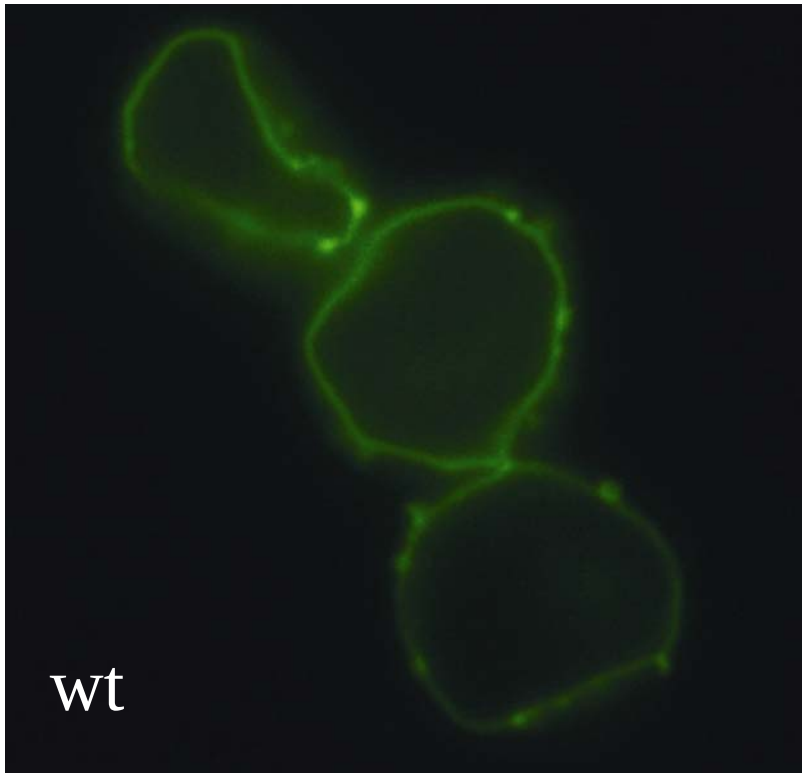
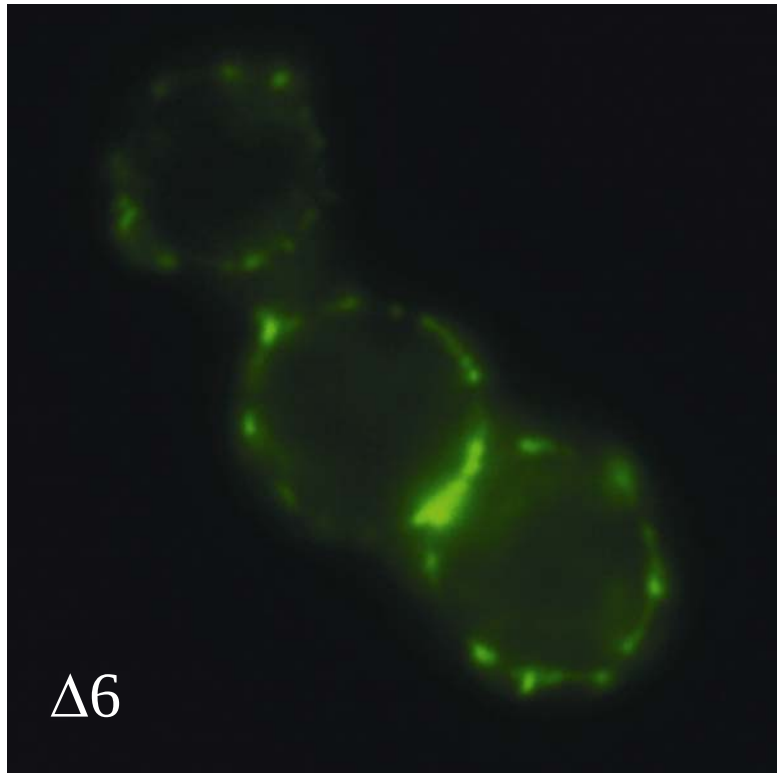
Use of PLY-GFP as model vaccination system





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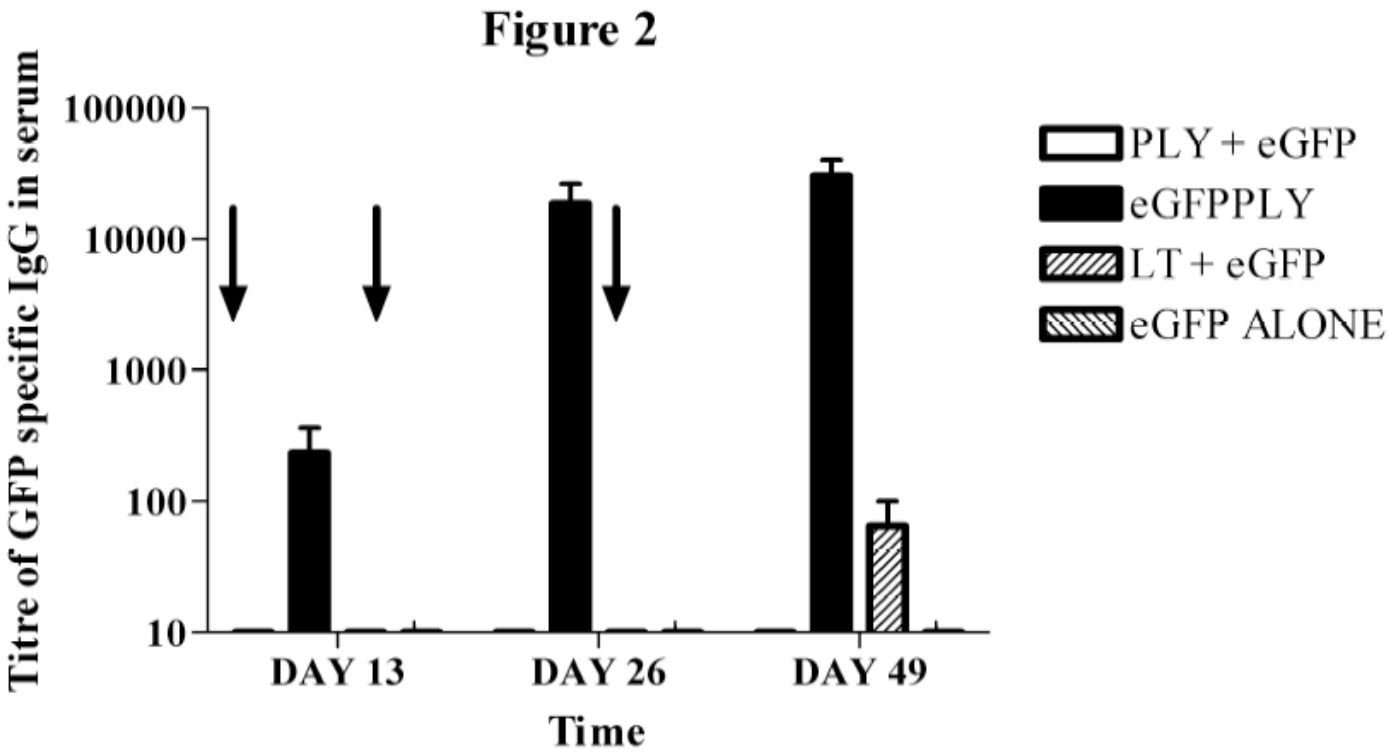
Systemic responses to carried protein following mucosal immunization



NOTE : Requires fusion of the proteins. Just mixing together does not give response

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Advantages

- Response after single mucosal vaccination
- Very small amounts of protein (less than 200ng) required to elicit response
- PLY acts as antigen and adjuvant for carried protein

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Mucosal IgA responses to carried antigen

Figure 3a

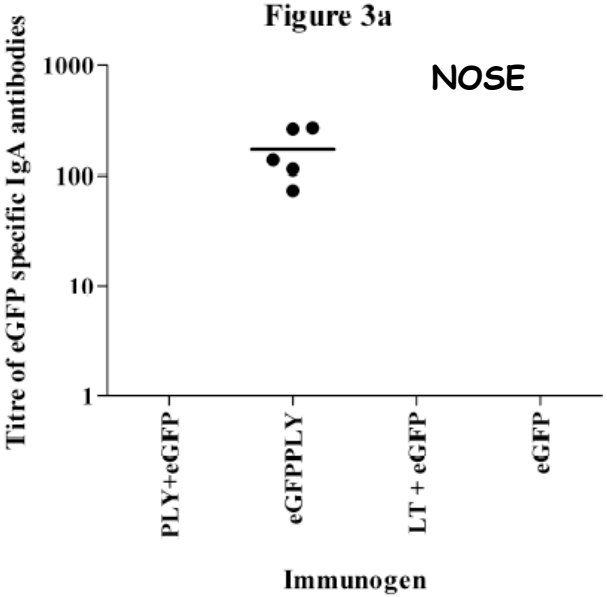
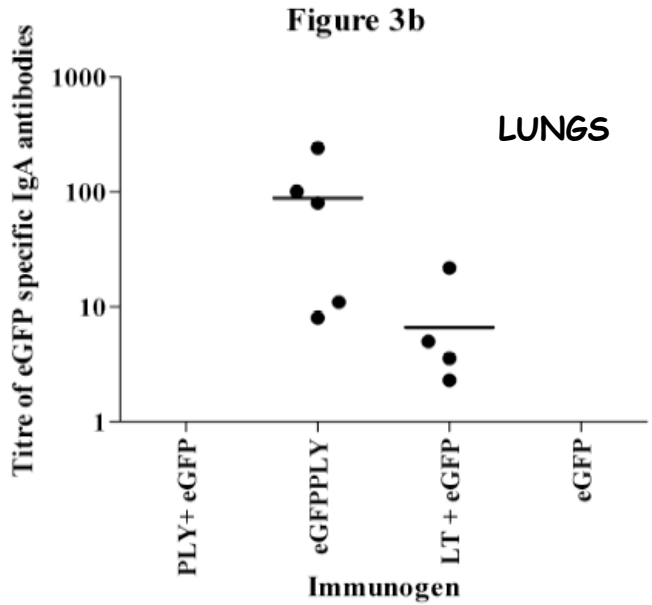


Figure 3b



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Advantages

- Good mucosal as well as systemic responses to intranasal vaccination
- Response better than that achieved with E.coli LT

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PLY fusions for screening for protective antigens

- Currently being evaluated for delivery of other candidate pneumococcal proteins (PsaA, PspA, PspC, PhtD, Mix)

- PATH project : Vaccinate and challenge.
  - 5 antigens
  - 2 routes of vaccination (in and sc)
  - 3 pneumococcal strains (D39, A66, TIGR4)
  - 2 models (colonization and pneumonia)

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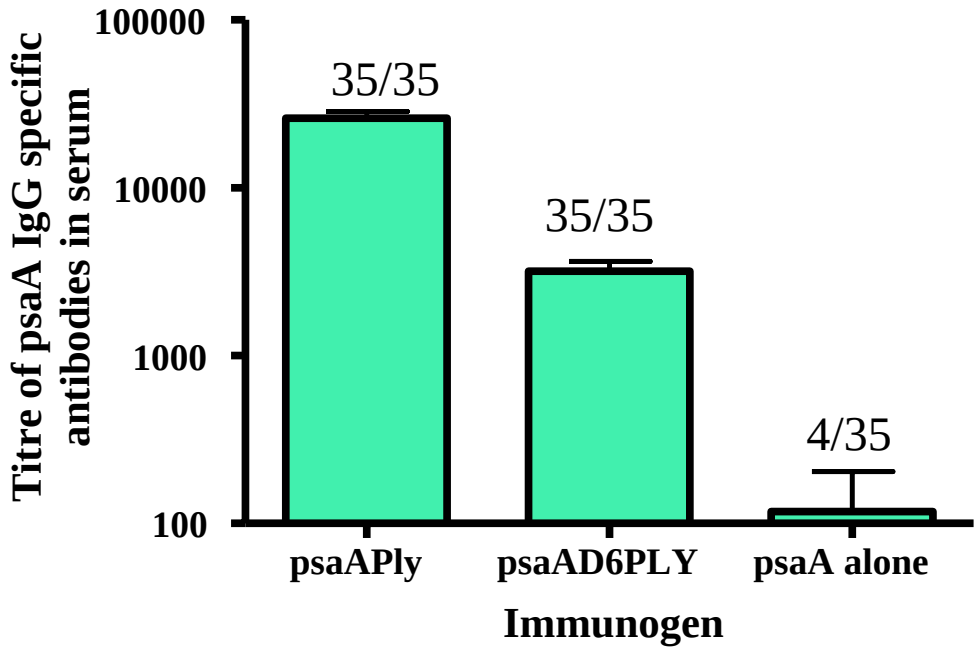
Serum IgG levels to PsaA after mucosal vaccination

Path 1. Titres of anti PsaA in sera following 3 intranasal vaccinations



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Colonisation with Xen35 (TIGR4)

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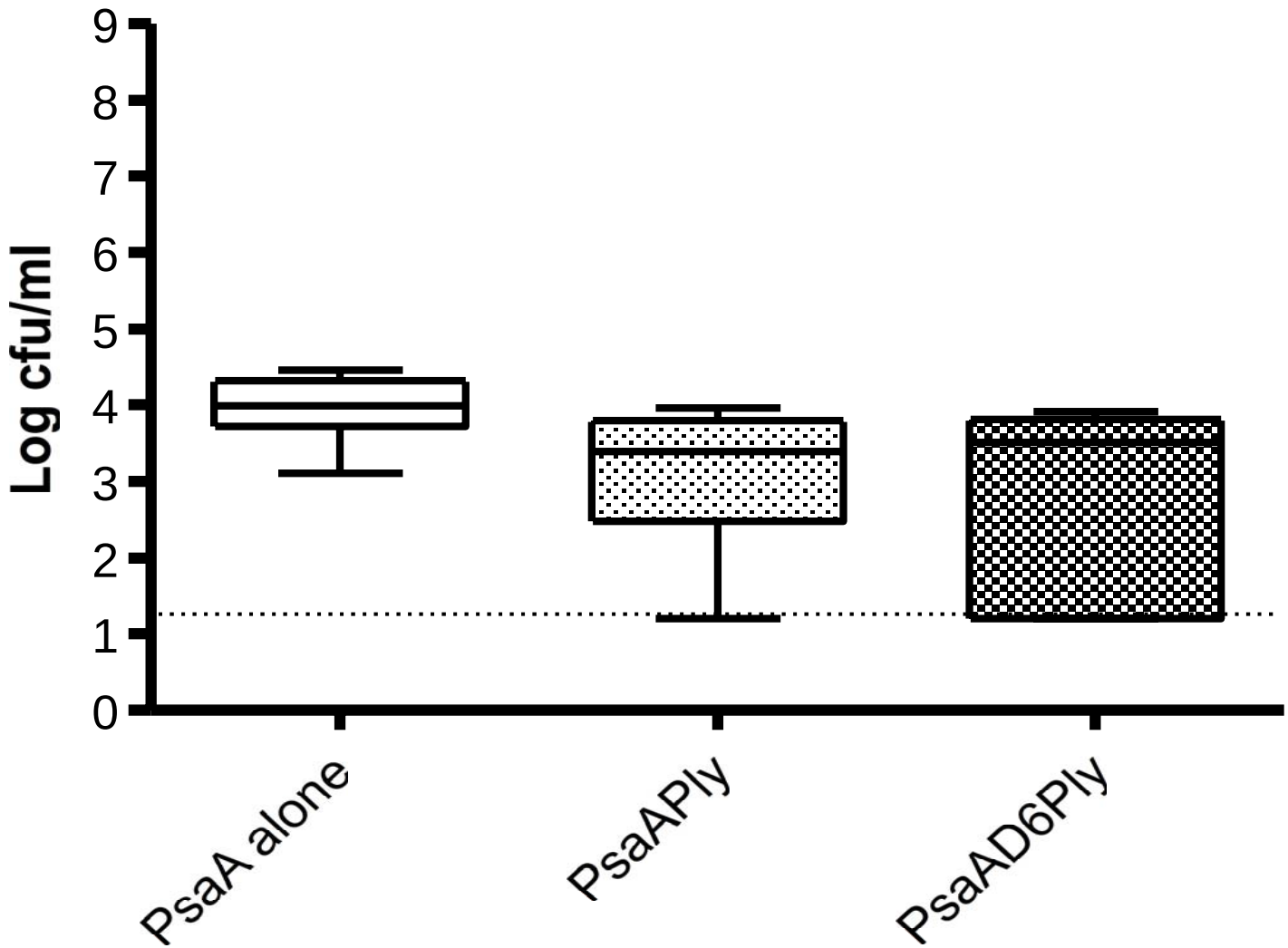
Colonisation with Xenogen 35 TIGR4



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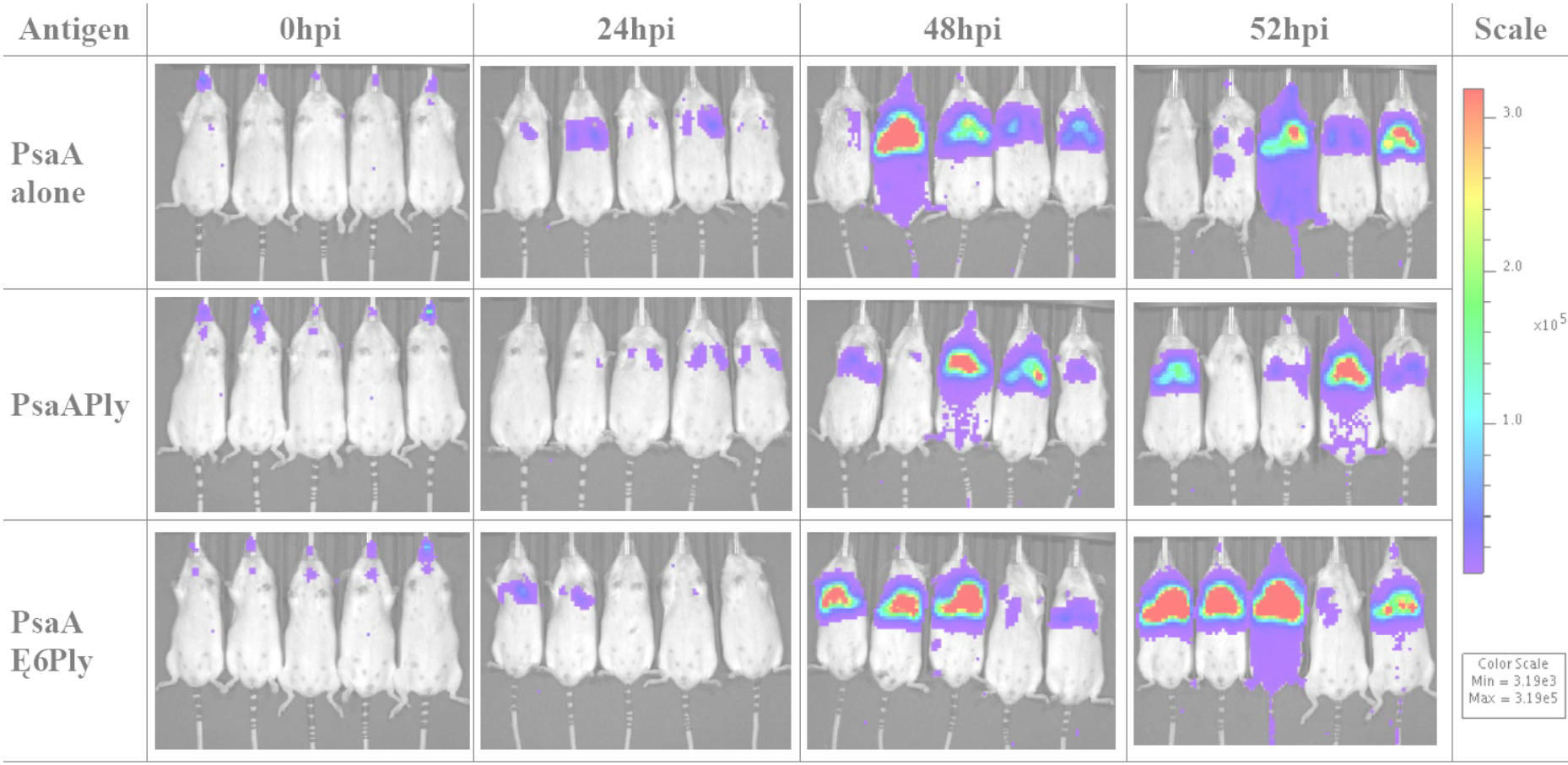
Invasive disease with Xen35 (TIGR4)



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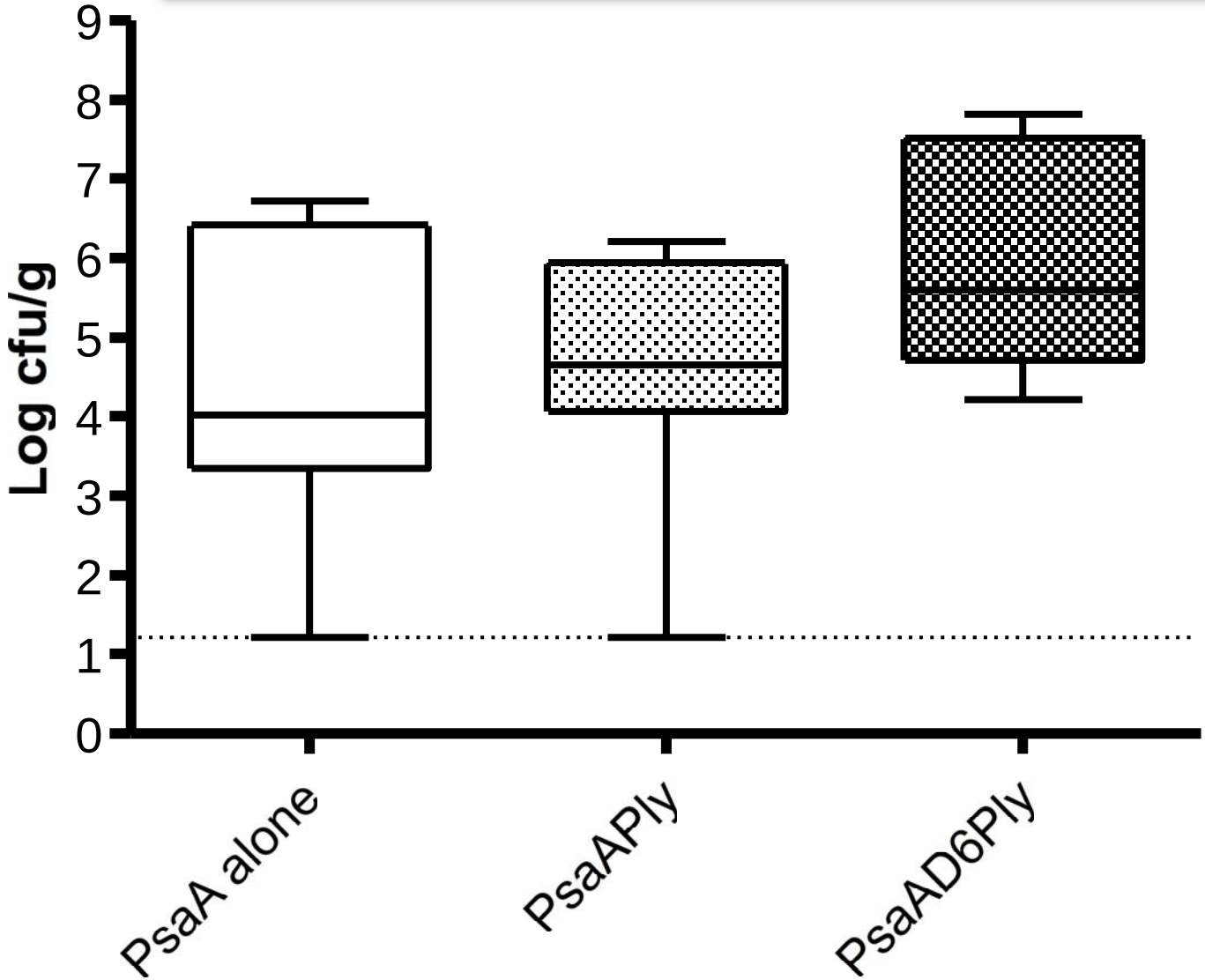
Invasion with Xenogen 35 TIGR4



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PLY as an adjuvant : Acknowledgments

- Previous
  - Graeme Cowan
  - Lea-Ann Kirkham
- Present
  - Gill Douce
  - Kirsty Ross
  - Jiang Tao Ma
  - Carol McInally
- Funding : Wyeth, PATH, MRC

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THE QUESTION(S)

- Which protein(s)?
- What type of response?