Overview of Models of CNS TB and TB Meningitis

Tuberculosis Meningitis: Advancing Immunopathogenesis, Diagnosis, and Treatment
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High rates of TB meningitis in mostly HIV-negative young children in India

- 223 children ≤5 years (median age 31 months), with suspected TB were enrolled prospectively at BJGMC (Pune, India)
- 86% had received BCG, 57% were malnourished (WAZ ≤ 2 SD), and 10% were HIV-positive
- 12% (n = 26) had active TB (definite or probable) and extra-pulmonary disease was noted in 46% (n = 12), which was predominantly meningeal (75%; n = 9)
- 57% (4 of 7) of children with culture-confirmed TB, harbored drug-resistant (DR) strains of which 2 (50%) were multi-DR (MDR)
- Whole genome sequencing was performed on isolates from children with definite TB. *M. tuberculosis* strains causing TB meningitis clustered together, suggesting that *M. tuberculosis* possess virulence factors that promote the development of CNS disease.

Jain, S.K., et al., Biomed Res Int. 2013
Shaikh et al. Int J Tuberc Lung Dis. 2017

Current Understanding of CNS Invasion by *Mycobacterium tuberculosis*

THE PATHOGENESIS OF TUBERCULOUS MENINGITIS

1933

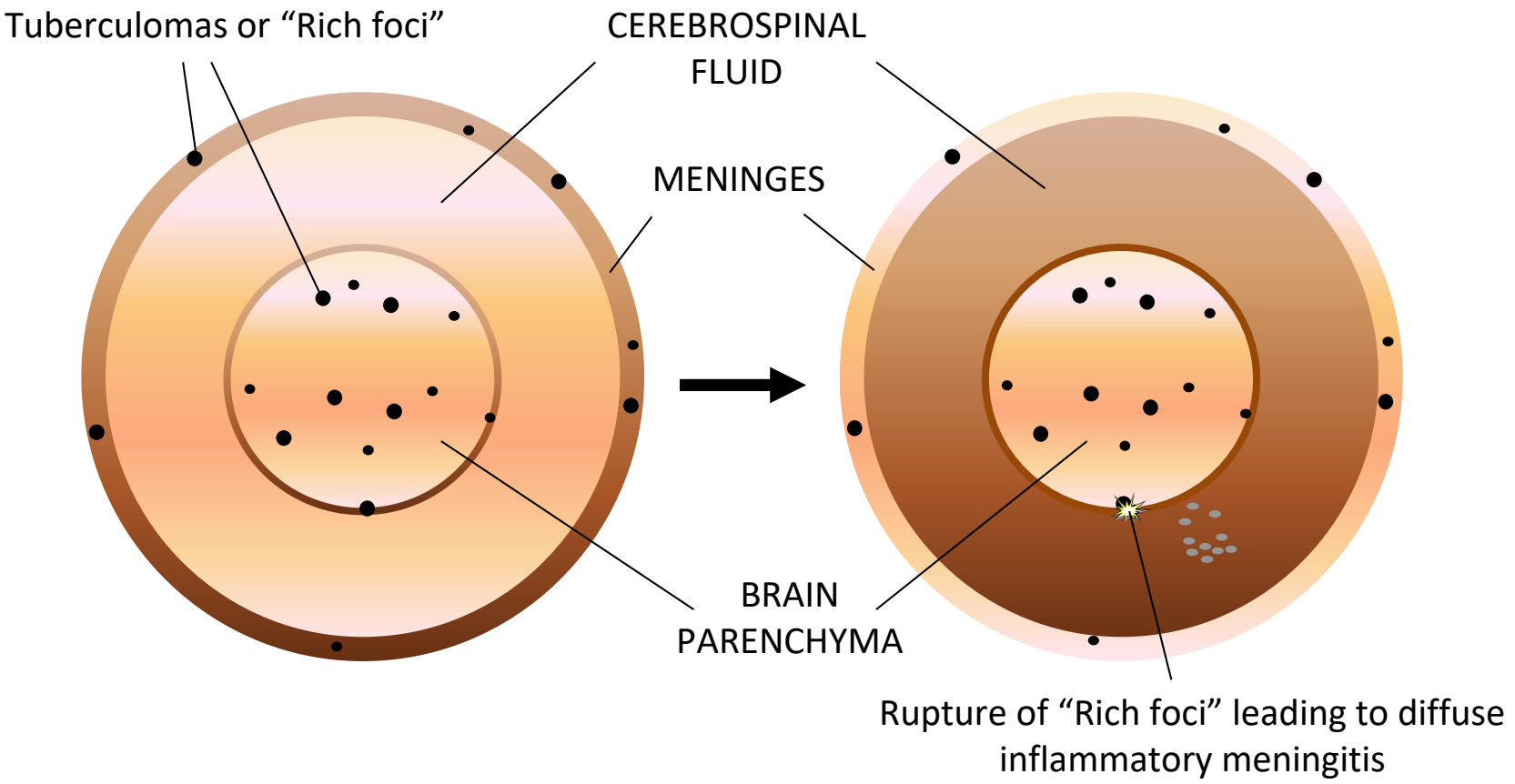
ARNOLD R. RICH AND HOWARD A. McCORDOCK

From the Department of Pathology, Johns Hopkins Medical School, Baltimore, Md.

Acute diffuse tuberculous meningitis is characterized especially by its exudative inflammatory nature, and by the tendency to widespread necrosis of the inflammatory exudate and of contiguous meningeal tissues. Even in cases in which the duration has been sufficiently long to permit the appearance of a proliferative reaction leading to the formation of true tubercles or of a compact tissue composed of epithelioid cells and fibroblasts, one usually finds the remains of an earlier exudative-necrotic process. Since extensive exudative inflammatory reactions with prominent necrosis in tuberculosis are well known to be expressions of the allergic state, and since tuberculous meningitis develops only in persons carrying an older sensitizing infection somewhere

Rich, A.R., et al. *Bull. Johns Hopkins Hosp.* 1933.

Current Understanding of CNS Invasion by *Mycobacterium tuberculosis*



Rich, A.R., et al. ***Bull. Johns Hopkins Hosp.*** **1933.**

Animal models of CNS TB and TB meningitis

- Several animal models have employed ***direct*** intracisternal or intracerebral infection:
  - Tsenova *et al*. (rabbit)
  - van Well *et al*. (mouse)
  - Tucker *et al*. (baby rabbit)
- *Useful for studying pathogenesis and antibiotic or host-directed treatments after established disease.*

- Other models have utilized ***intravenous or aerosol*** infections to study the **initial stage** of invasion from the lung / bloodstream to the CNS:
  - Rich *et al*. (guinea pig, rabbit)
  - Be *et al*. (mouse, guinea pig – intravenous)
  - Skerry *et al*. (guinea pig – aerosol)
  - Zebrafish model
- *Useful for studying early pathogenesis and studying microbial factors needed for translocation to the brain and for developing preventive strategies.*

Tsenova, L., et al. J Infect Dis, 1998
Rich, A.R. and H.A. McCordock. Bull. Johns Hopkins Hosp, 1933
Be, N.A., et al. BMC Micro. 2012
van Well, G.T.J., et al. J Infect Dis, 2007
Be, N.A., et al. J Infect Dis, 2008
Skerry, C., et al. PLoS ONE 2013

Bacterial traversal across the Blood-Brain Barrier (BBB)?

- *M. tuberculosis* can initiate CNS TB by crossing the BBB as free (extracellular) organisms or via infected leukocytes
- Leukocyte trafficking could be restricted across the BBB, prior to the onset of TB meningitis
- Data from Rich et al, and later confirmed by MacGregor et al, demonstrate that *free bacteria* can invade the CNS
- Data utilizing CD18-/- (leukocyte adhesion deficient) mice suggest that free mycobacteria can traverse the BBB independent of leukocytes or macrophages

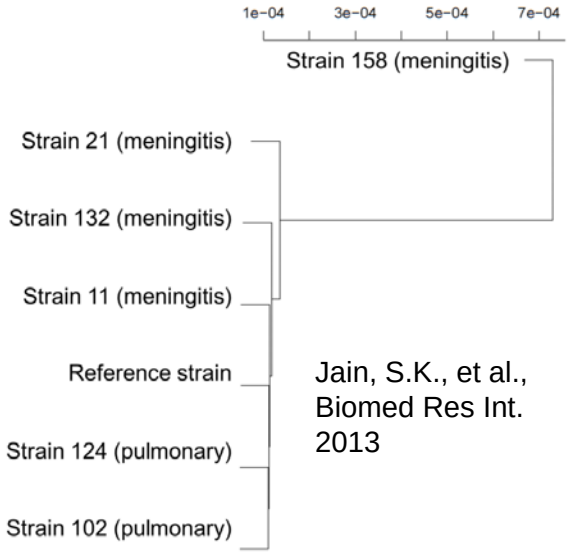
Be, N.A., et al. Curr Mol Med, 2009
Ransohoff, R.M., et al. Nat Rev Immunol, 2003
Wu, H.S., et al. Infect Immun, 2000

Rich, A.R. and H.A. McCordock. Bull. Johns Hopkins Hosp, 1933
MacGregor, A.R. and C.A. Green. J. Path. Bact., 1937

Microbial factors may be associated with TB meningitis

- Multiple reports have shown the association of distinct *M. tuberculosis* strains with extra-pulmonary and / or CNS dissemination
  - Compartmentalization of strains in distinct physiological sites
  - Association of certain lineages with dissemination and meningeal disease

Whole genome sequencing performed on 6 of the 7 isolates using the Genome Analyzer IIx (Illumina, USA). *M. tuberculosis* strains causing TB meningitis cluster together.



Jain, S.K., et al., Biomed Res Int. 2013

Relationship between *Mycobacterium tuberculosis* Genotype and the Clinical Phenotype of Pulmonary and Meningeal Tuberculosis ▽

Guy Thwaites,[1,2*] Maxine Caws,[2,3] Tran Thi Hong Chau,[2,4] Anthony D'Sa,[5] Nguyen Thi Ngoc Lan,[6] Mai Nguyet Thu Huyen,[6] Sebastien Gagneux,[7†] Phan Thi Hoang Anh,[6] Dau Quang Tho,[2] Estee Torok,[2,3] Nguyen Thi Quynh Nhu,[2] Nguyen Thi Hong Duyen,[2] Phan Minh Duy,[2] Jonathan Richenberg,[5] Cameron Simmons,[2,3] Tran Tinh Hien,[4] and Jeremy Farrar[2,3]

Hesseling, A.C., et al., Int J Tuberc Lung Dis, 2010.
Hernandez Pando, R., et al., Tuberculosis (Edinb), 2010.

Caws, M., et al., PLoS Pathog, 2008.
Jain, S.K., et al., Biomed Res Int. 2013

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- Data from Rich et al, and later confirmed by MacGregor et al, demonstrate that *free bacteria* can invade the CNS.
- A study utilizing CD18-/- (leukocyte adhesion deficient) mice suggest that free mycobacteria can traverse the BBB independent of leukocytes or macrophages.
- Zebra fish model studies have shown traversal of bacteria within leukocytes.

Be, N.A., et al. Curr Mol Med, 2009
Ransohoff, R.M., et al. Nat Rev Immunol, 2003
Wu, H.S., et al. Infect Immun, 2000

Rich, A.R. and H.A. McCordock. Bull. Johns Hopkins Hosp, 1933
MacGregor, A.R. and C.A. Green. J. Path. Bact., 1937
Davis, J.M., et al. Immunity, 2002

Immune response to foreign antigens is different in the brain parenchyma

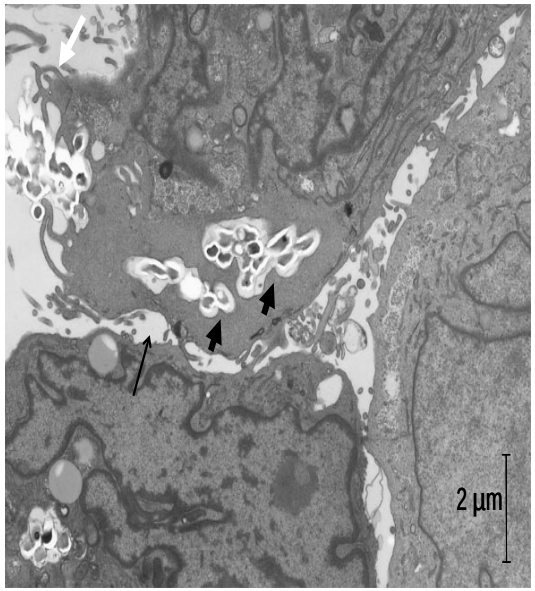
- Intracranial injection of BCG in rats resulted in a rapid response which persisted for approximately two weeks, but disappeared by four weeks.
- However, at 4 weeks, staining demonstrated the presence of BCG at the site of the original intracranial injection, suggesting that the inflammatory response had not cleared the bacteria completely.
- No T-cell proliferation responses (to PPD) were noted from splenic or lymph nodes of animals injected with BCG intracranially.
- Animals that were subsequently peripherally sensitized (subcutaneous injection of BCG) developed a strong delayed-type hypersensitivity response with extensive inflammatory lesions at the site of BCG injection in the CNS.
- Delayed-type hypersensitivity responses could be detected for several months in the CNS in animals that underwent peripheral sensitization.

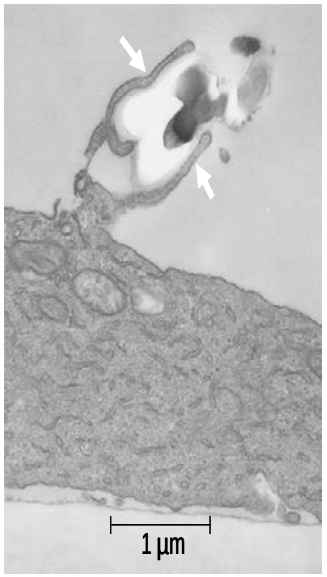
Be, N.A., et al. Curr Mol Med, 2009
Ford, A.L., et al., J Exp Med, 1996.
Liu, Y., et al., Nat Med, 2006.

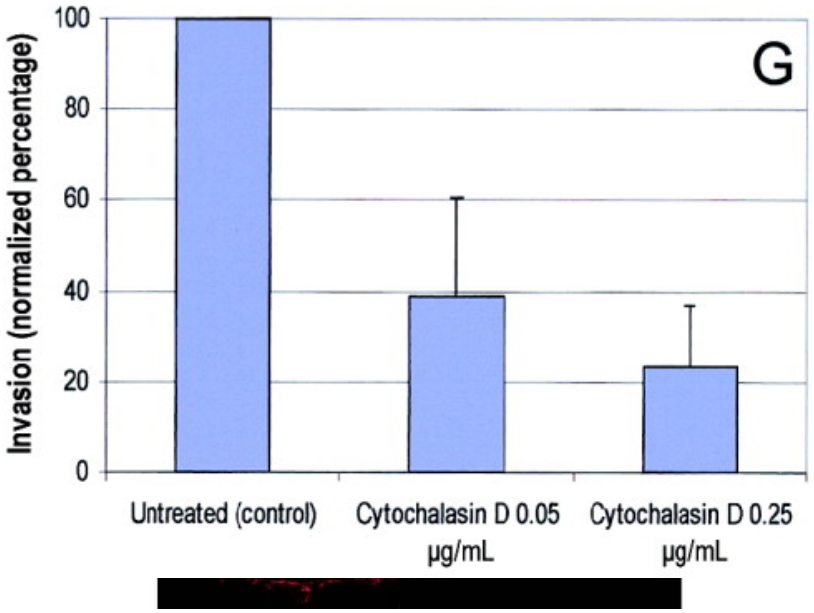
Matyszak, M.K. et al. J Neuroimmunol, 1998.
Matyszak, M.K. et al. Neuroscience, 1995.

Invasion by *M. tuberculosis* may be an Active Process

- *M. tuberculosis* invade and traverse an *in vitro* blood-brain barrier
- Non-pathogenic mycobacteria do not stimulate internalization
- Bacterial internalization is partially dependent on actin polymerization







Jain SK *et al.*, *J Infect Dis*. 2006.

Are there specific ***microbial*** factors which promote invasion / survival in the host CNS?

Aim: To identify *M. tuberculosis* genes involved in CNS TB using a screen in an animal model

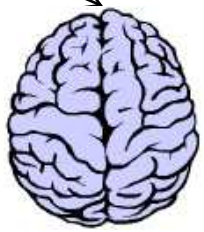
Nick Be

N = 398

Mutant 1

Mutant 2

Mutant 3



Input pool

Output pool

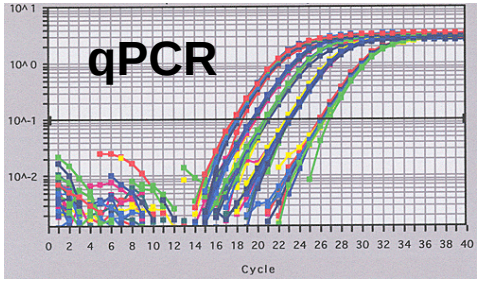
Mutant 1

Mutant 1

Mutant 1

Mutant 1

Mutant 1



Mutant 1 is attenuated in the CNS

Murine CNS TB model used to screen mutants for attenuation

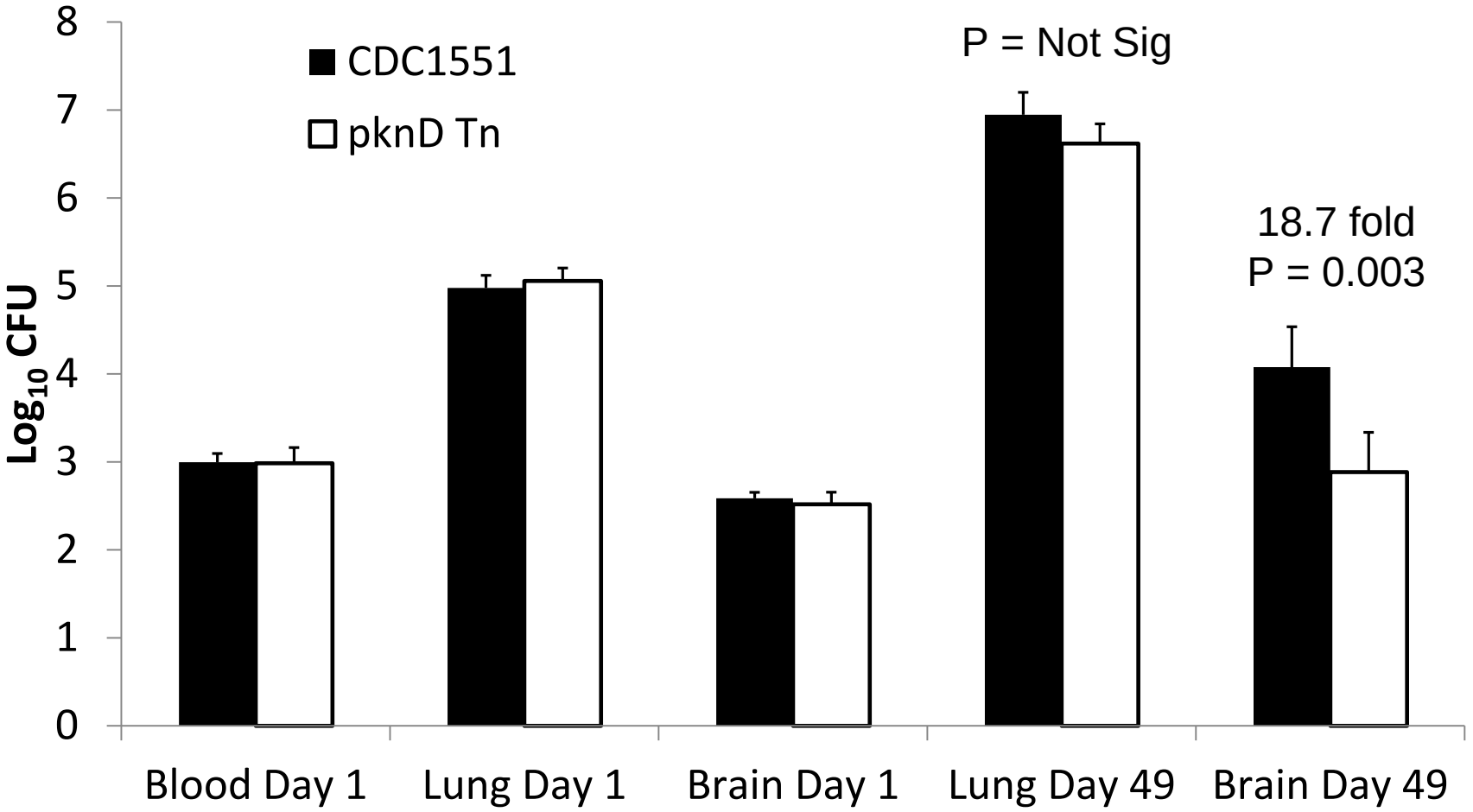
Be, N.A., et al. J Infect Dis, 2008

Rv#	MT#	ORF description	Fold attenuation in brain relative to lung
0311	0324	Hypothetical protein	17.70
0805	0825	Phosphodiesterase	17.08
0931c	0958	Serine/threonine protein kinase (*pknD*)	431.25
0986	1014	ABC transporter	35.03
N/A	3280	Hypothetical protein	5.75
		Control	1.03

MT0752	Possible Aldolase	4987	5.07E-04
MT0779	PPE Family Protein	5969	1.25E-04
MT0958	Ser-Thr Protein Kinase (*pknD*)	1089	1.65E-03
MT1311	Probable Drug-Tra	0	2.71E-04
MT1711	Conserved Hypothe	Detected on D21	
MT1965	Conserved Hypothe	Detected on D21	
MT1982	Probable Thiol Perc		4.47E-05
MT2456	Conserved Hypothetical Protein	5806	2.53E-04
MT3178	Conserved Hypothetical Protein	Not Detected on D21	
MT3247	PPE Family Protein	15626	5.87E-05
MT3321	Iron-Regulated Dehydrogenase/Reductase	293907	2.77E-03
MT3461	Conserved Hypothetical Protein	895	9.12E-03

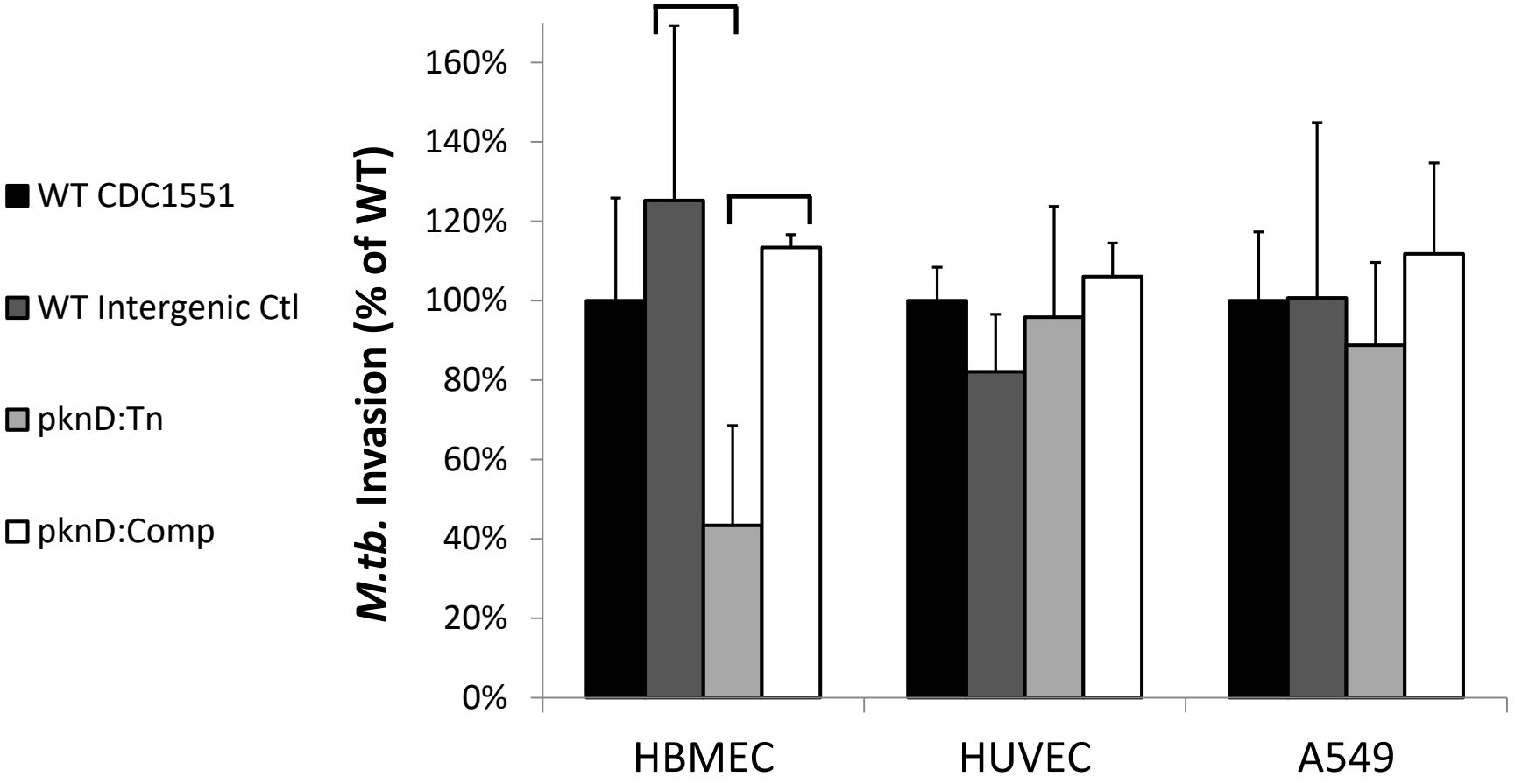
Also found in mouse model screen

PknD deficient *M. tuberculosis* Mutant is Attenuated for CNS Survival in the Mouse



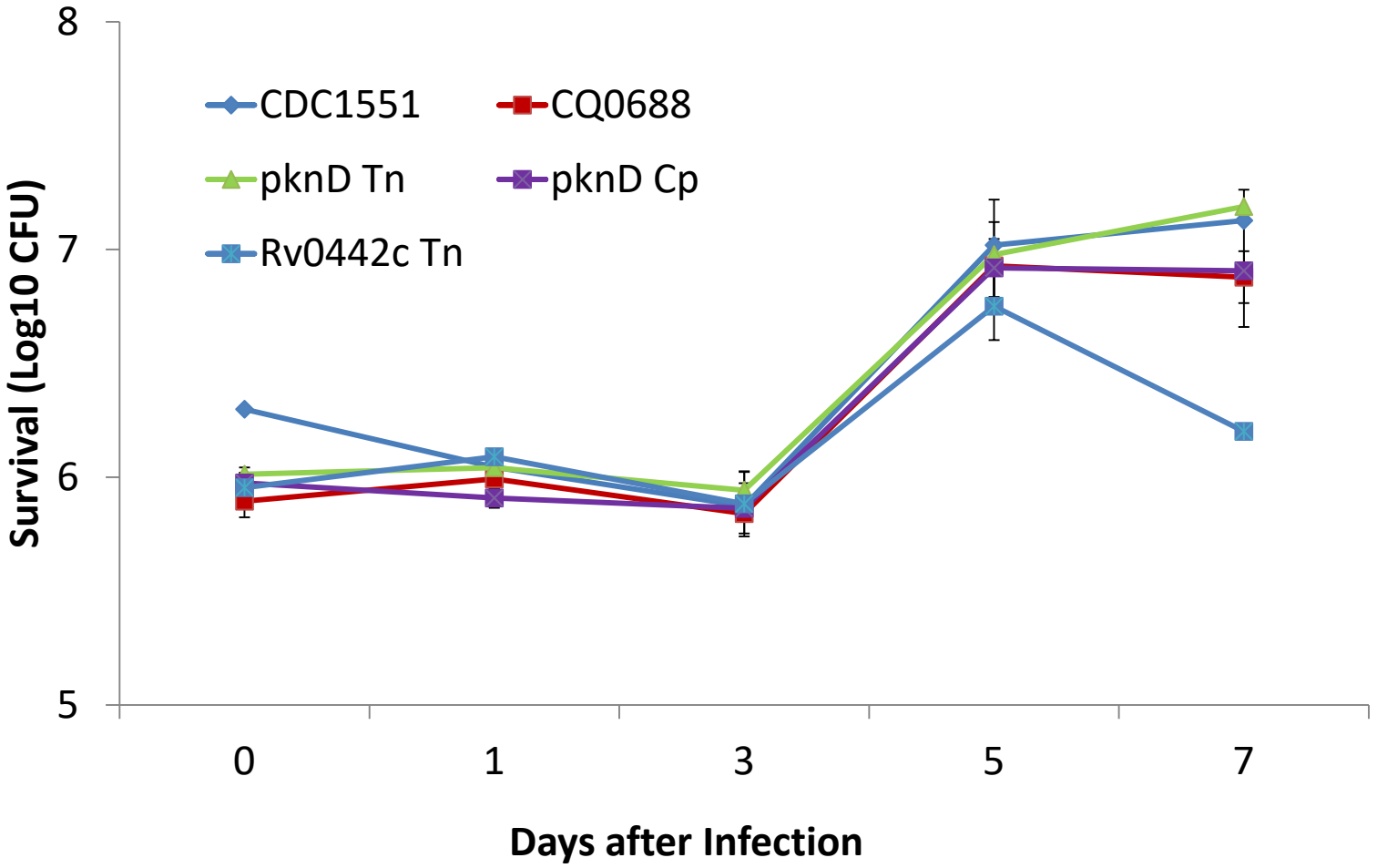
Be, N.A., et al. BMC Micro. 2012

PknD deficient *M. tuberculosis* Mutant has defective invasion into brain endothelia



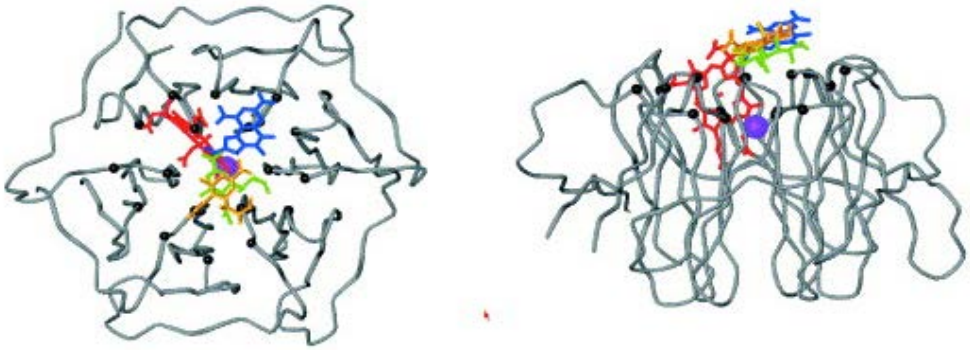
- No difference in invasion was observed in HUVEC or A549
- Significant reduction in invasive capacity, observed in HBMEC (p=0.02)
- Defect was restored by genetic complementation with the native *pknD* gene

PknD deficient *M. tuberculosis* Mutant is NOT Attenuated in Macrophages



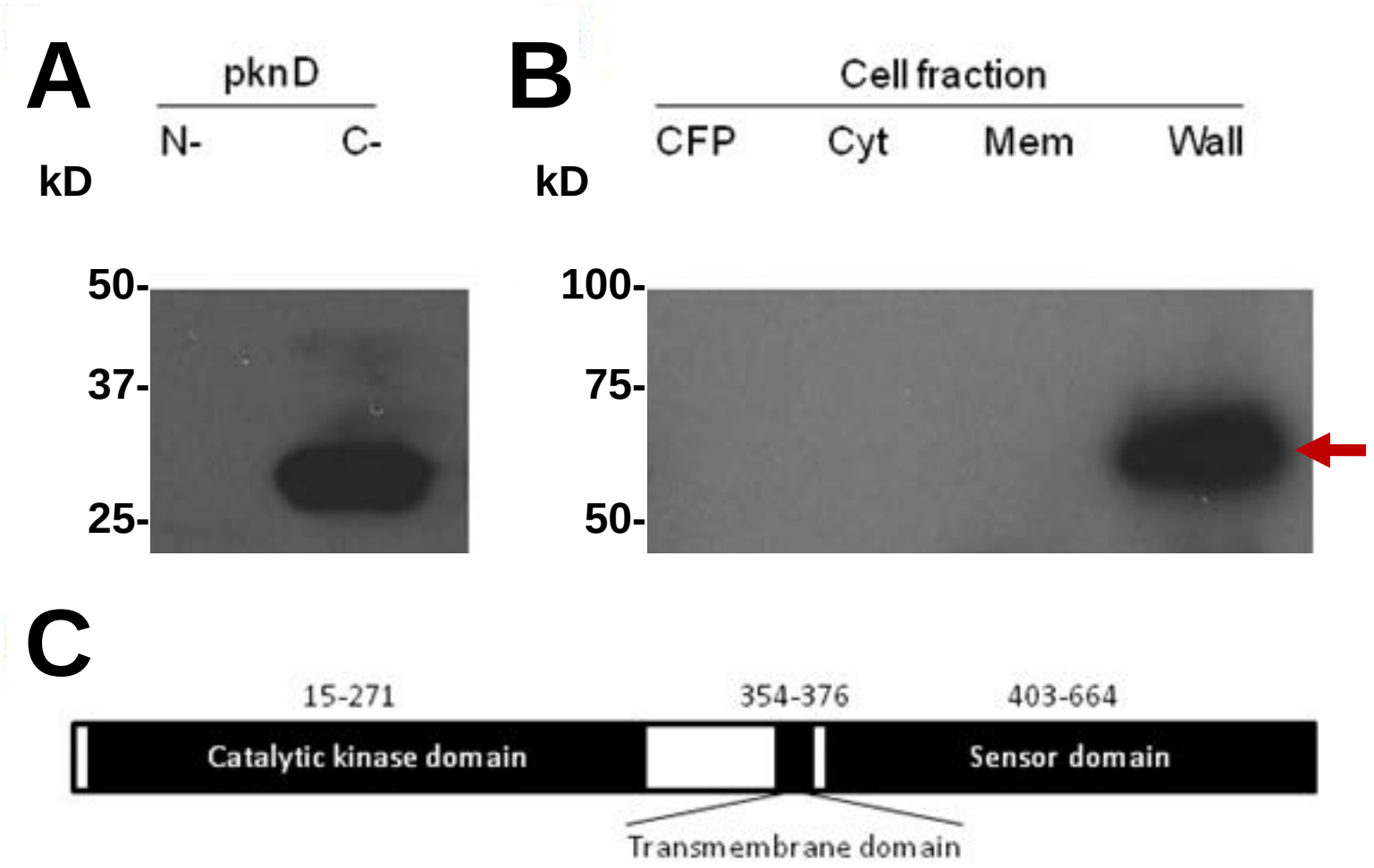
PknD sensor domain: A role in host-pathogen interaction?

- The PknD C-terminal sensor domain forms a symmetric β-propeller
- Cup of β-propeller exhibits polar and non-polar patches, contains residues unique to individual blades of the propeller
  - Indicates a functional binding surface
- Similar structures found in multiple proteins in other organisms which mediate adhesion
- **Could the pknD sensor domain assist in bacterial adherence to host cells in the CNS?**



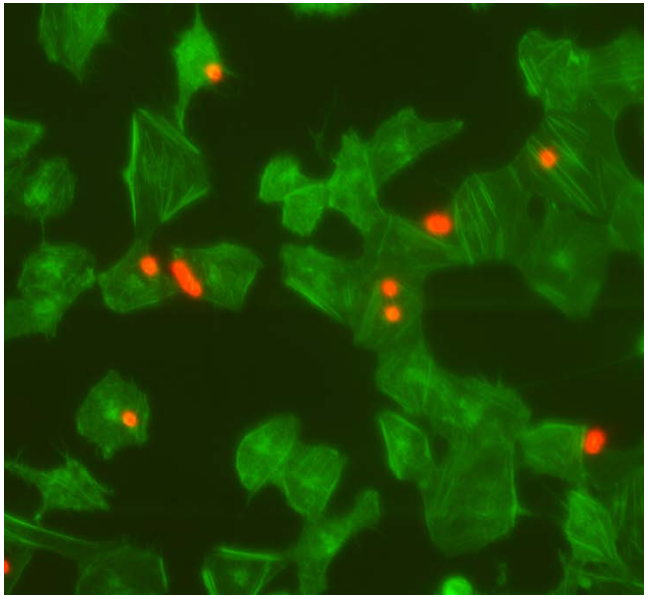
Good, et al. *JMB.* 2009.

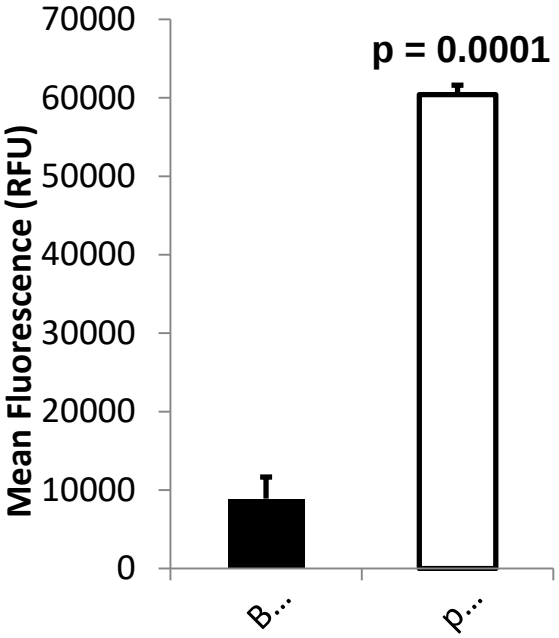
PknD sensor domain localizes to the cell wall fraction *M. tuberculosis*



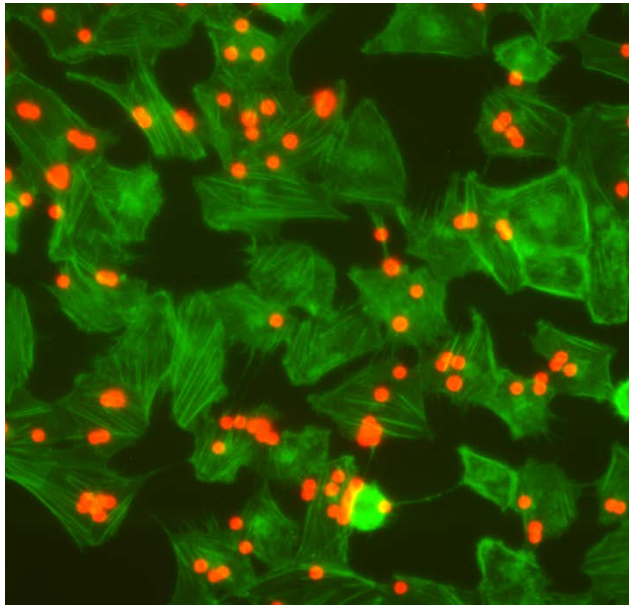
PknD sensor domain is sufficient to trigger association with brain endothelial cells

BSA-coated beads



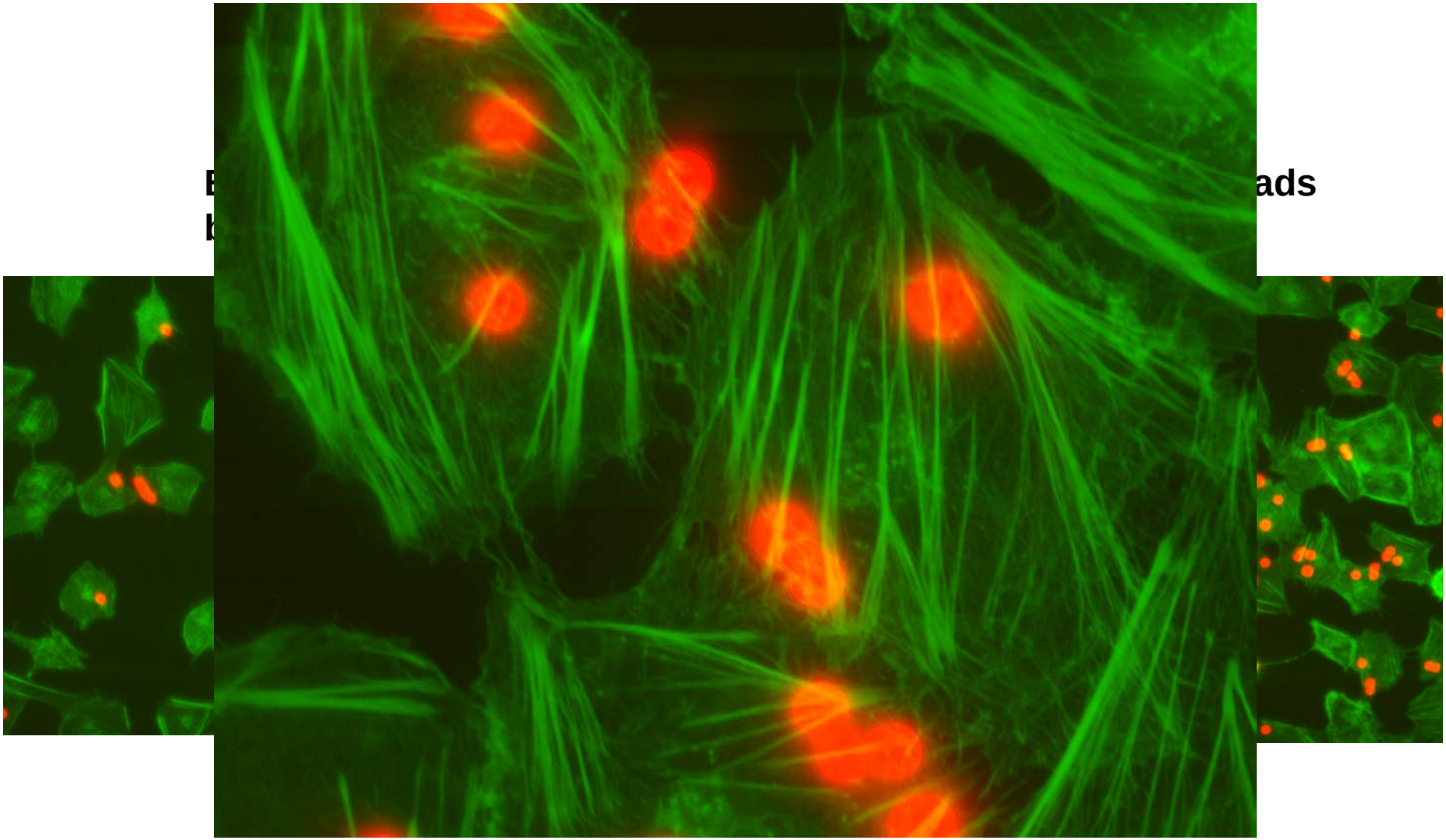


pknD-coated beads



Red: coated beads
Green: actin

PknD sensor domain is sufficient to trigger association with brain endothelial cells



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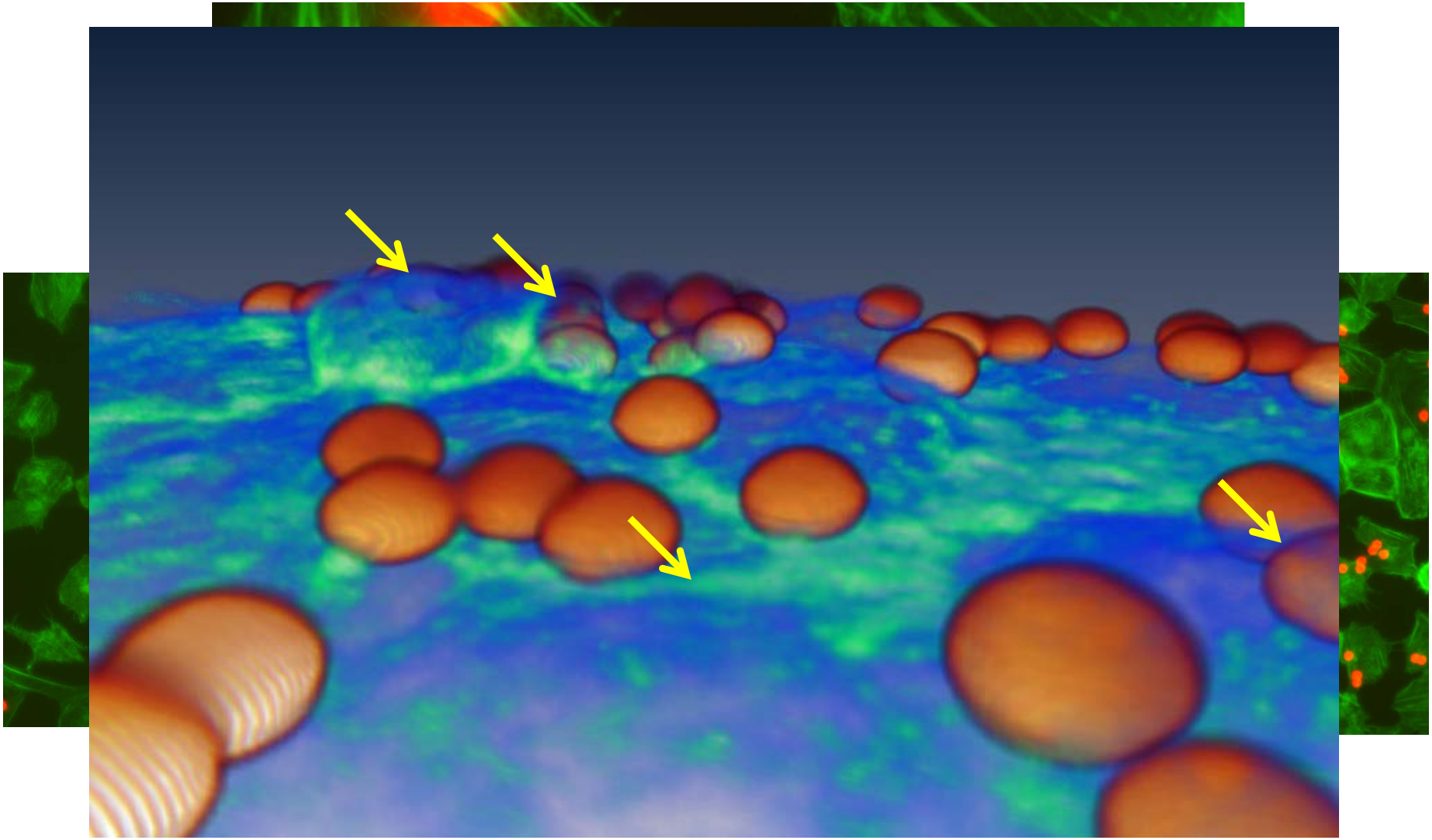

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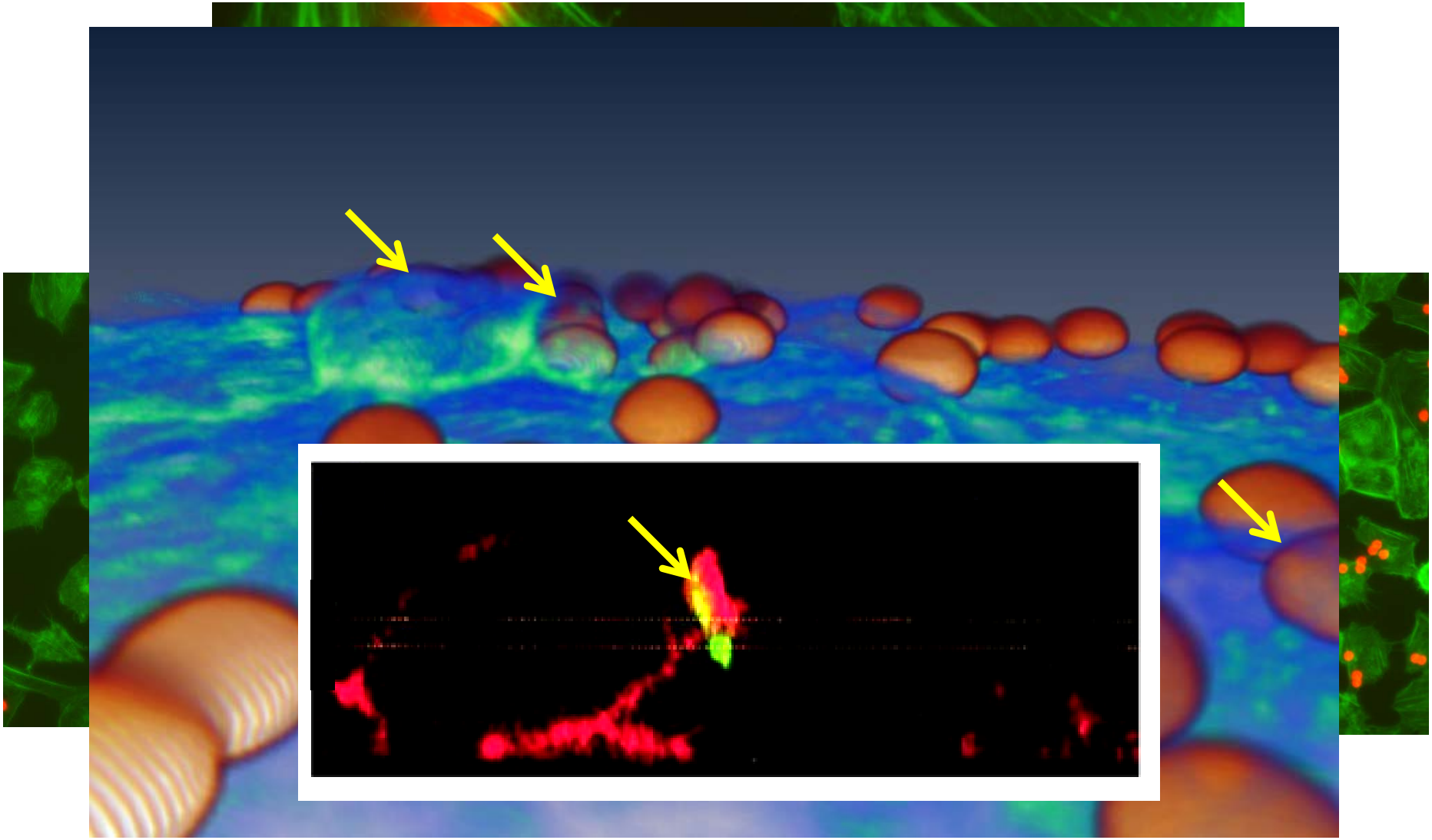
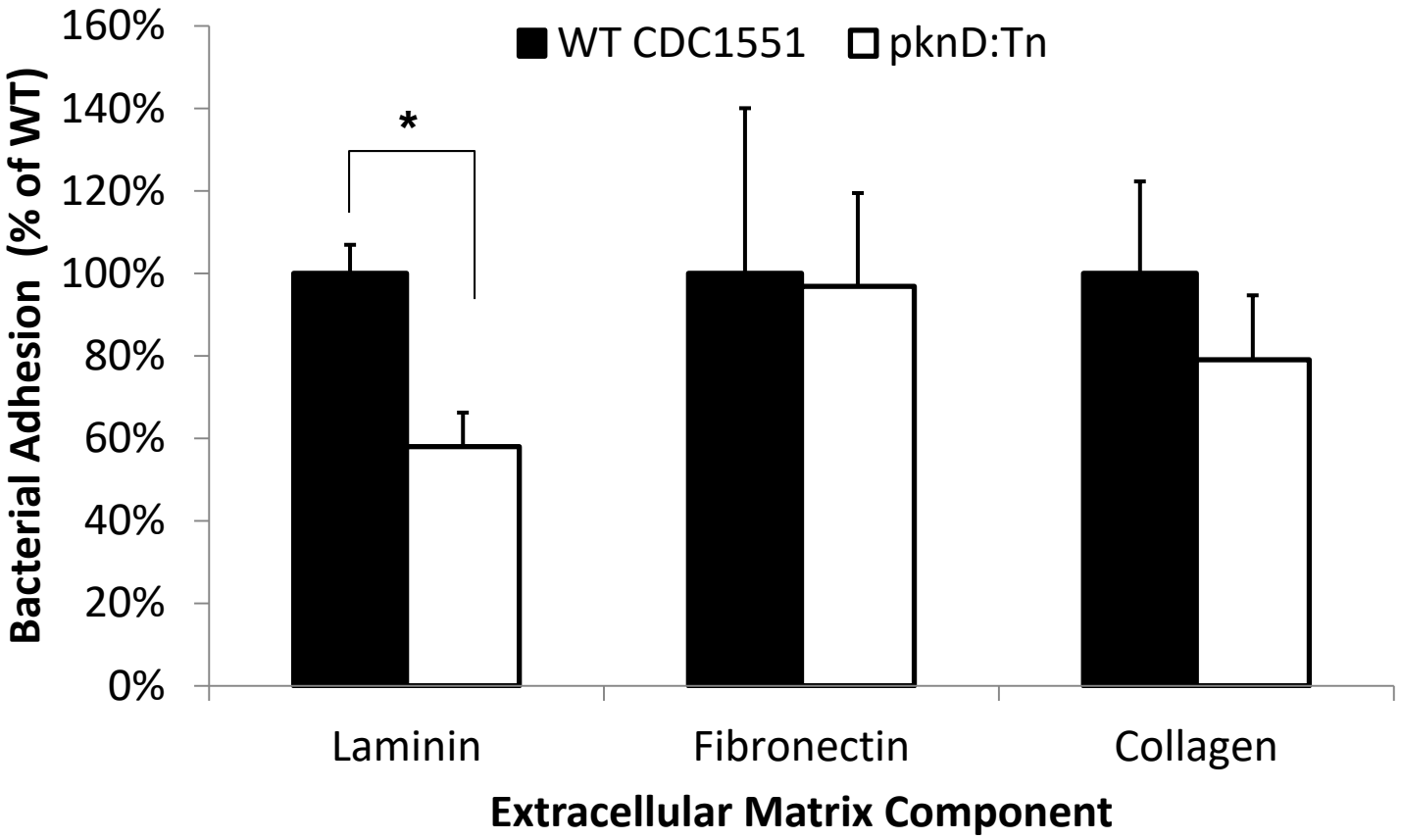


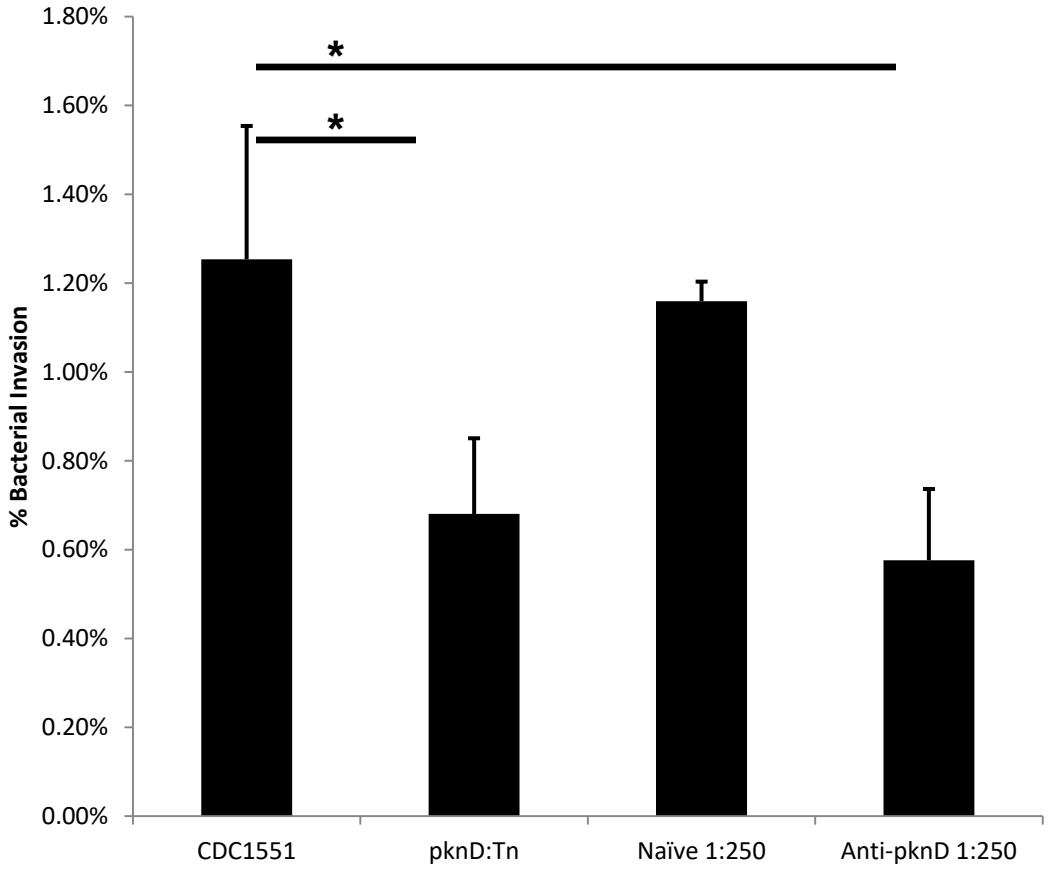
Red: coated beads
Green: actin

PknD deficient *M. tuberculosis* Mutant exhibits reduced binding to laminin



Incubation of PknD sensor with brain endothelial lysates and immobilization on affinity column demonstrated interaction with laminin

Antibody specific for PknD reduced invasion of brain endothelia by *M. tuberculosis*



TB meningitis develops subsequent to hematogenous dissemination of bacteria.

The surface exposed PknD sensor is required for invading the BBB that protects the CNS from the systemic circulation.

We therefore hypothesized that antibody-mediated humoral immunity against PknD could be utilized as a strategy to protect against CNS TB in the guinea pig model.

BCG and TB

- BCG is the only licensed vaccine against TB and recommended (WHO policy) at birth to newborns in high TB burden countries.
- Protection by BCG vaccination is highly variable (0-80%), and its use is mainly guided by its ability to prevent TB meningitis and miliary TB in young children by decreasing the bacterial burden in lung tissues.
- Nonetheless, protection by BCG vaccination against TB meningitis and military TB is also inconsistent and only ~50%.

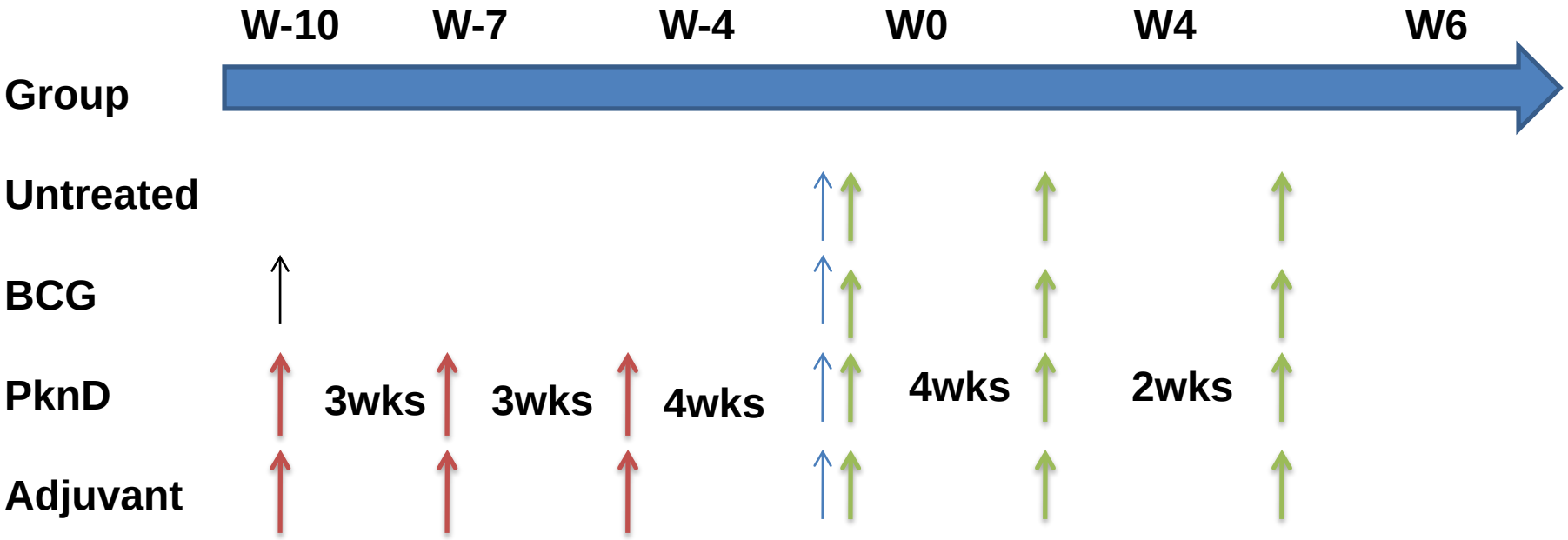
Rosenthal, S.R. et al. Am Rev Respir Dis. 1961
Rosenthal, S.R. et al. Pediatrics. 1961
Colditz, G.A. et al. JAMA. 1994

Hypothesis: Vaccination with the *M. tuberculosis* PknD extra-cellular subunit protein will provide protection against CNS TB.

Aim: To use the guinea pig aerosol infection model to test the efficacy of the *M. tuberculosis* PknD sensor (extra-cellular subunit protein) against CNS TB and compare it with BCG.

Work led by Ciaran Skerry

PknD sensor as a Vaccine-target to prevent CNS TB: Methodology in the Guinea Pig model



BCG Danish strain 1331
s.c 5x10^4 CFU

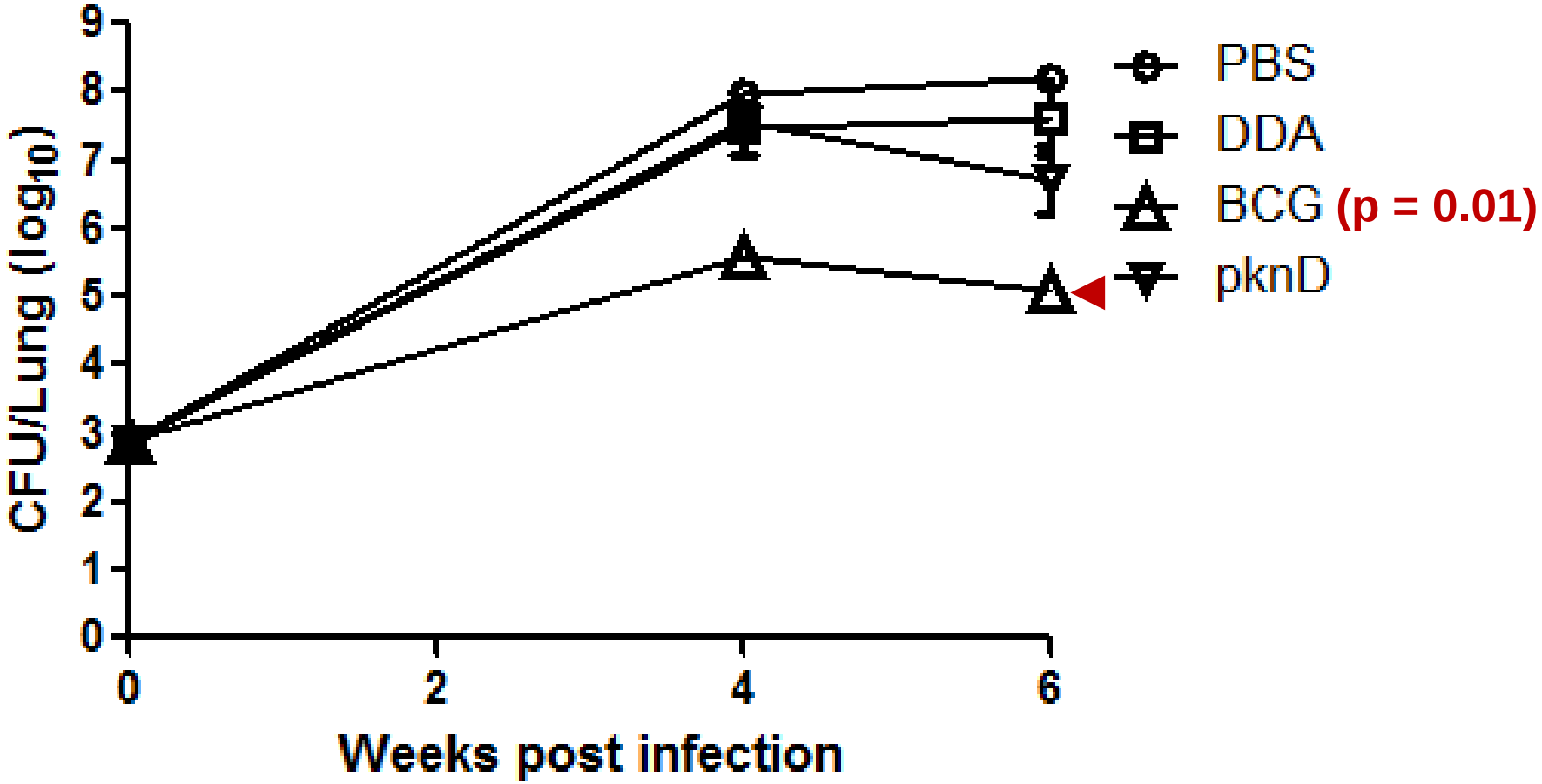
Harvest organs

PknD 20ug+20ug DDA s.c

M. tuberculosis CDC1551 challenge via aerosol (day 1: ~3 $\log_{10}$)

4 animals per group per time-point;
TCH plates also used for BCG infected animals

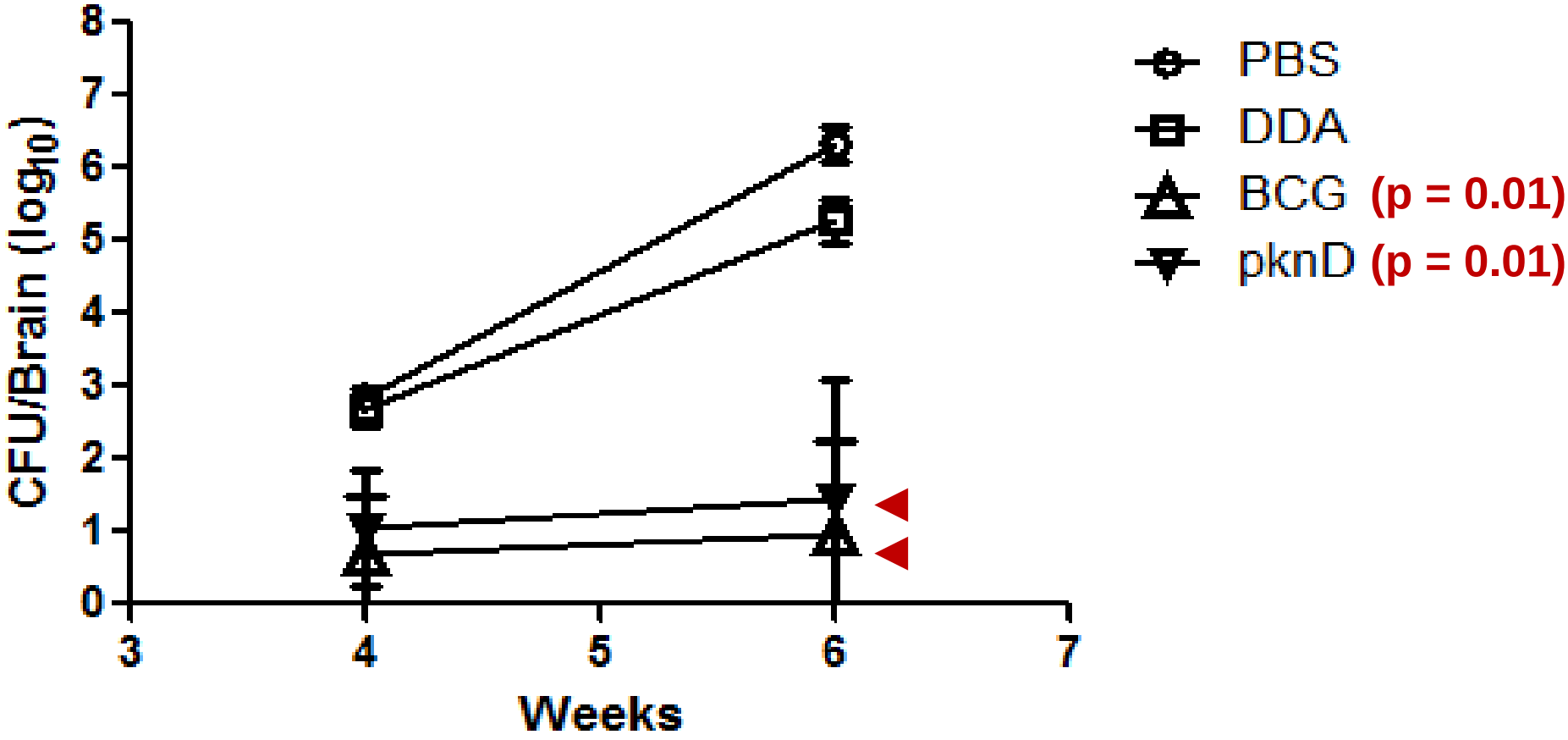
Vaccination with BCG limits *M. tuberculosis* burden at site of primary infection (Lungs)



Results are shown as mean CFU per lung (±SD). Animals vaccinated with BCG show significantly lower CFU than those treated with pknD or controls.

Skerry C, et al. PLoS ONE 2013

Vaccination with *M. tuberculosis* PknD protects against CNS TB



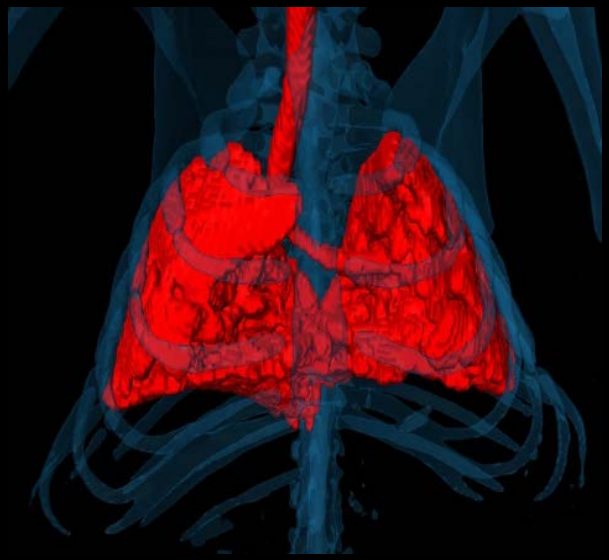
Results are shown as mean CFU per brain (±SD). The brains from PknD- and BCG-treated animals show significantly lower bacterial burden than those treated with PBS or adjuvant control.

Guinea pig survival

Group	Deaths
PBS	5/7 (71%)
DDA	4/4 (100%)
BCG	1/4 (25%)
PknD	1/5 (20%)

Lung pathology (computed tomography) in BCG, PknD and PBS vaccinated animals 6 weeks after aerosol challenge

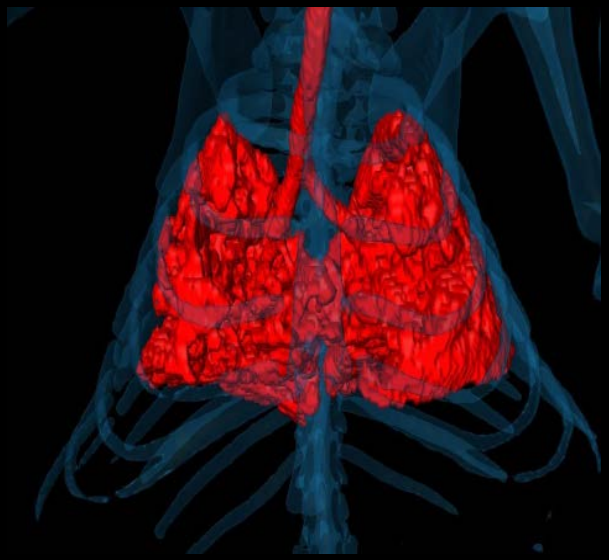
5.09 ± 0.16
log_{10} CFU



BCG

Remaining air volume
1.0061 ml

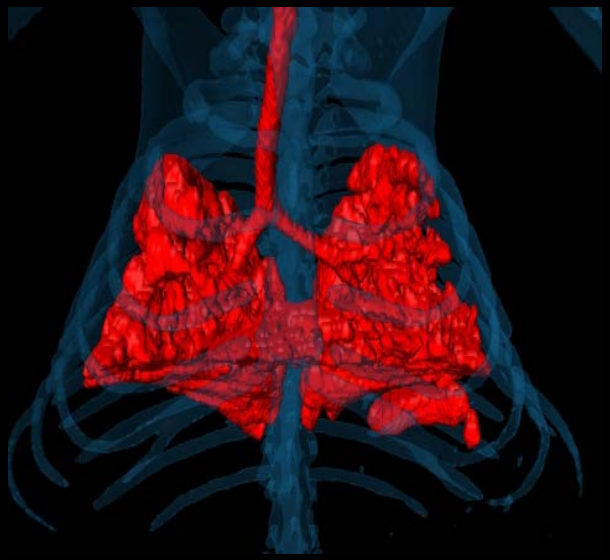
6.71 ± 0.50
log_{10} CFU



PknD

Remaining air volume
0.6696 ml
33.44% reduction

8.19 ± 0.17
log_{10} CFU



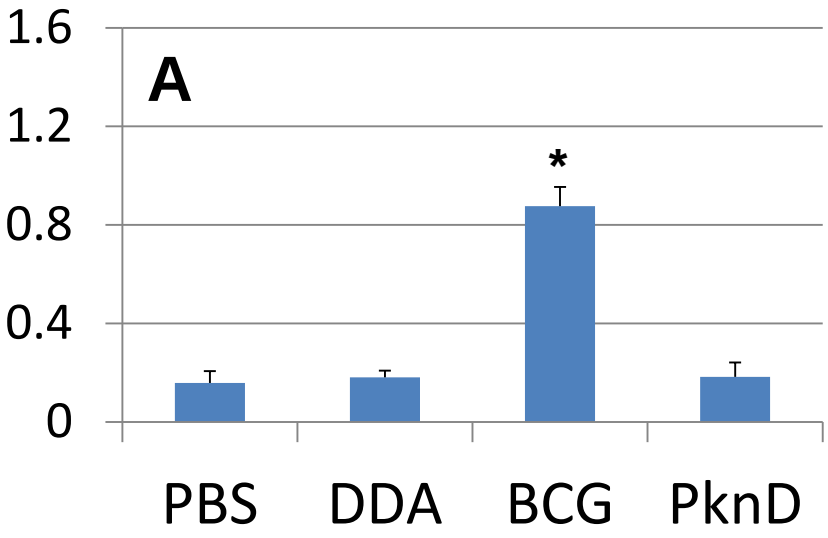
PBS

Remaining air volume
0.5939 ml
40.97% reduction

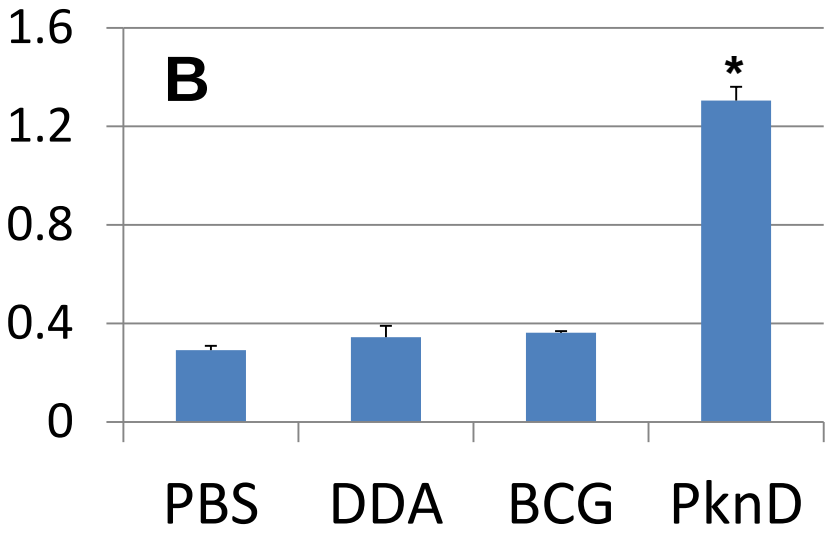
Integrated over the whole lung (every slice), so more accurate than histopathology

IgG levels in vaccinated guinea pigs

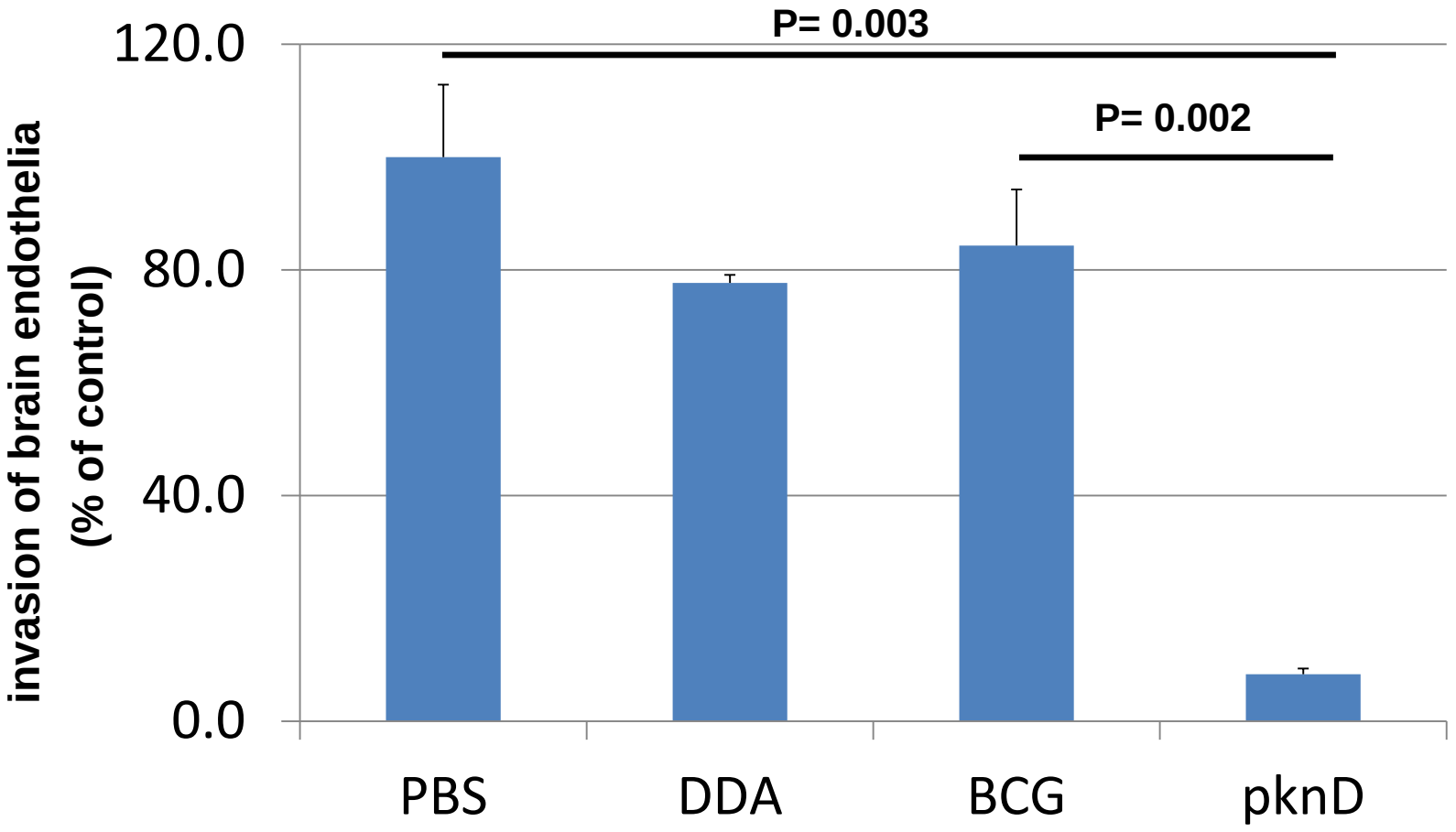
M.tb.-specific (bacterial lysates)



M.tb. PknD-specific



Pre-incubation of *M. tuberculosis* with sera from PknD-vaccinated guinea pigs reduces invasion of brain endothelia



Skerry C, et al. PLoS ONE 2013

Summary

- Pathogenesis of TB meningitis remains poorly understood and this field of research remains under-studied.
- Animal models utilizing direct (intracisternal / intracerebral) or intravenous challenge have been described and could be useful to study the pathogenesis of TB meningitis as well as development of novel therapeutics – antibiotic, host-directed treatments and vaccines.
- *M. tuberculosis* PknD *may be a key microbial factor with a role in CNS invasion.*
- Vaccination with the *M. tuberculosis* PknD sensor domain can offer protection against CNS TB in the guinea pig model.
- Has a potentially a unique mechanism of protection (serological):
  - BCG does not make the extracellular portion of PknD
- Vaccination strategies against *M. tuberculosis* PknD could also protect against other forms of extra-pulmonary TB.
- Study limitations
  - Did not test protection in other extra-pulmonary sites
  - Protection could also be by mediated by additional mechanisms which were not fully evaluated

Acknowledgments

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Lucia Donatelli
Kimberly Townsend

Jain Lab
Alvaro Ordonez
Ed Weinstein
Liz Tucker
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