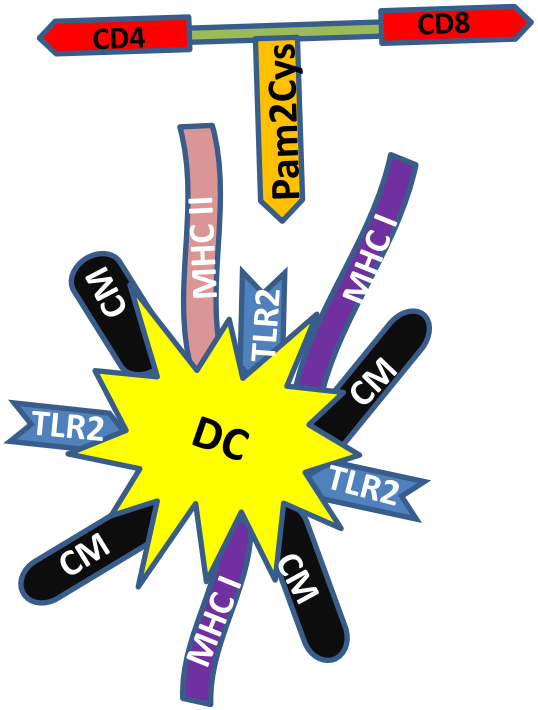
Future peptide vaccine for TB endemic regions: Challenges and solutions



Pradeep K Rai
CSIR-IMTECH, Chandigarh

Epidemiology of Tuberculosis

Best Prophylactic measure

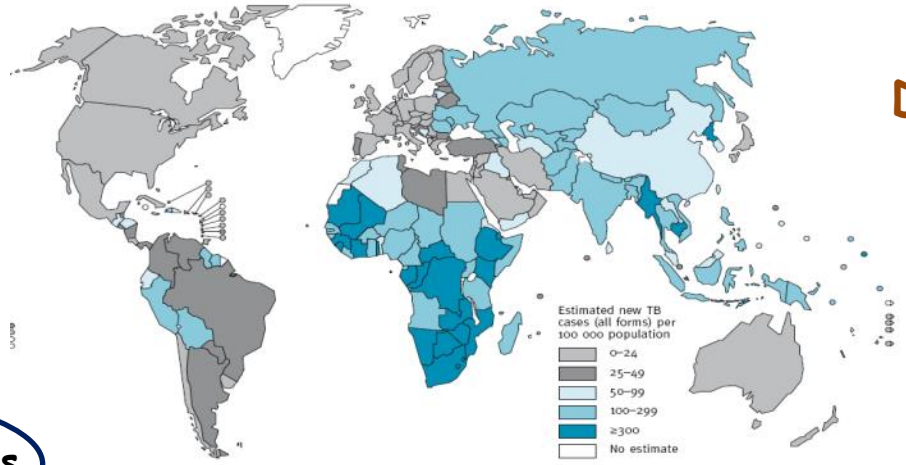
Vaccine

BCG

Total no. of new TB cases
9 million

Total no. of deaths
1.5 million

BCG Failure



Drugs

DOTs Treatment

Side effects

New cases
15%

Previously treated
85%

MDR: Multi drug resistant
480,000

Emergence of drug resistant

WHO Global tuberculosis report , 2014

Reasons for BCG failure

Lack of enduring immunologic memory

(Andersen and Doherty 2005, Narayanan 2006, Orme 2010, Singh et al 2010, 2011)

Interference by environmental mycobacteria (Masking and Blocking hypotheses)

(Andersen and Doherty 2005, Verma and Grover 2009, Flaherty et al 2006, Young et al 2007, Brant et al 2002)

Antigen processing and presentation- Blocking phagosome maturation and export of major histocompatibility complex (MHC) molecules

(Soualhine et al 2007, Pancholi et al 1993, Sendide et al 2005, Sun et al 2007, Fulton et al 2004, Sendide et al 2004)

Helminth infections

(Malhotra et al 1999, Elias et al 2001, 2005, Black et al 2002, Rook et al 2004)

Regulatory T cells

(Hanekom 2005, Ahrens et al 2009, Rook and Kim 2006, Akkoc et al 2010)

Limitations of DOTs

Time consuming (3-12 months), so chances of intermittent therapy increases, resulting in increased risk of drug resistance.

Side Effects.

No cure for latent infection.

Urgent need to develop: A potent construct with two edge sword
(Prophylactic and Therapeutic potential)

Future TB vaccine: Epitope based vaccine

✓ Minimal antigen processing

✓ Minimal presence of mycobacterial components (except antigenic portions)

✓ Robust Th1 response (even in a Th2 bias environment)

✓ Enduring T cell memory

✓ No preformed antibodies in the population

✓ Vaccine candidate with both prophylactic and therapeutic properties

X Poor immunogenicity

X Binds to very few HLA-alleles

Which peptide to choose?
Is promiscuous peptide right choice?



HLA DRB1	Binding Affinity F91-110
0101	++++
0103	++++
0301	++
0401	++++
0701	++++
1101	++++
1301	+++
1501	++++
1601	++++

++++ indicates high-affinity binding (IC_{50}<10μM); +++ intermediate affinity (10 μM<IC_{50}<100μM); and ++ indicates low affinity (100–1000μM).

Agrewala and Wilkinson 1997, 1998, 1999

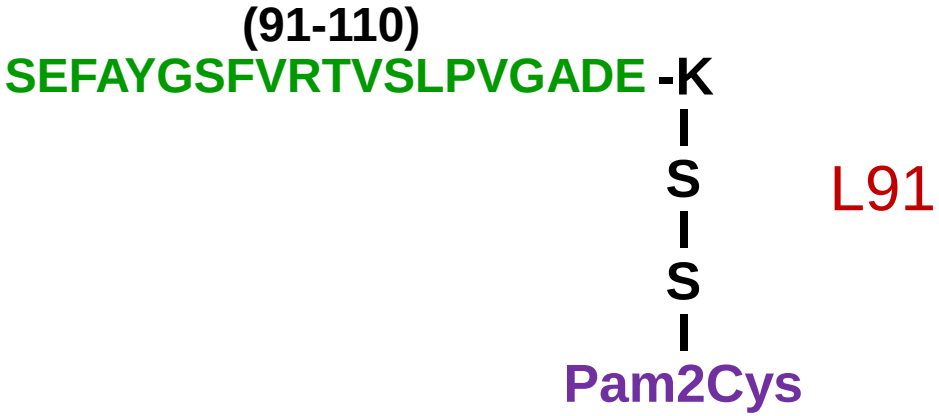
Can coupling promiscuous peptides to Pam2Cys render them immunogenic?

Pam2Cys S-[2,3-is(palmitoyloxy)propyl]cysteine induces effective Th1 immune responses, by evoking DCs to secrete IL-12
(Thoma et al 2000, Jackson et al 2004, Zho et al 2004, Ghosh et al 2006)

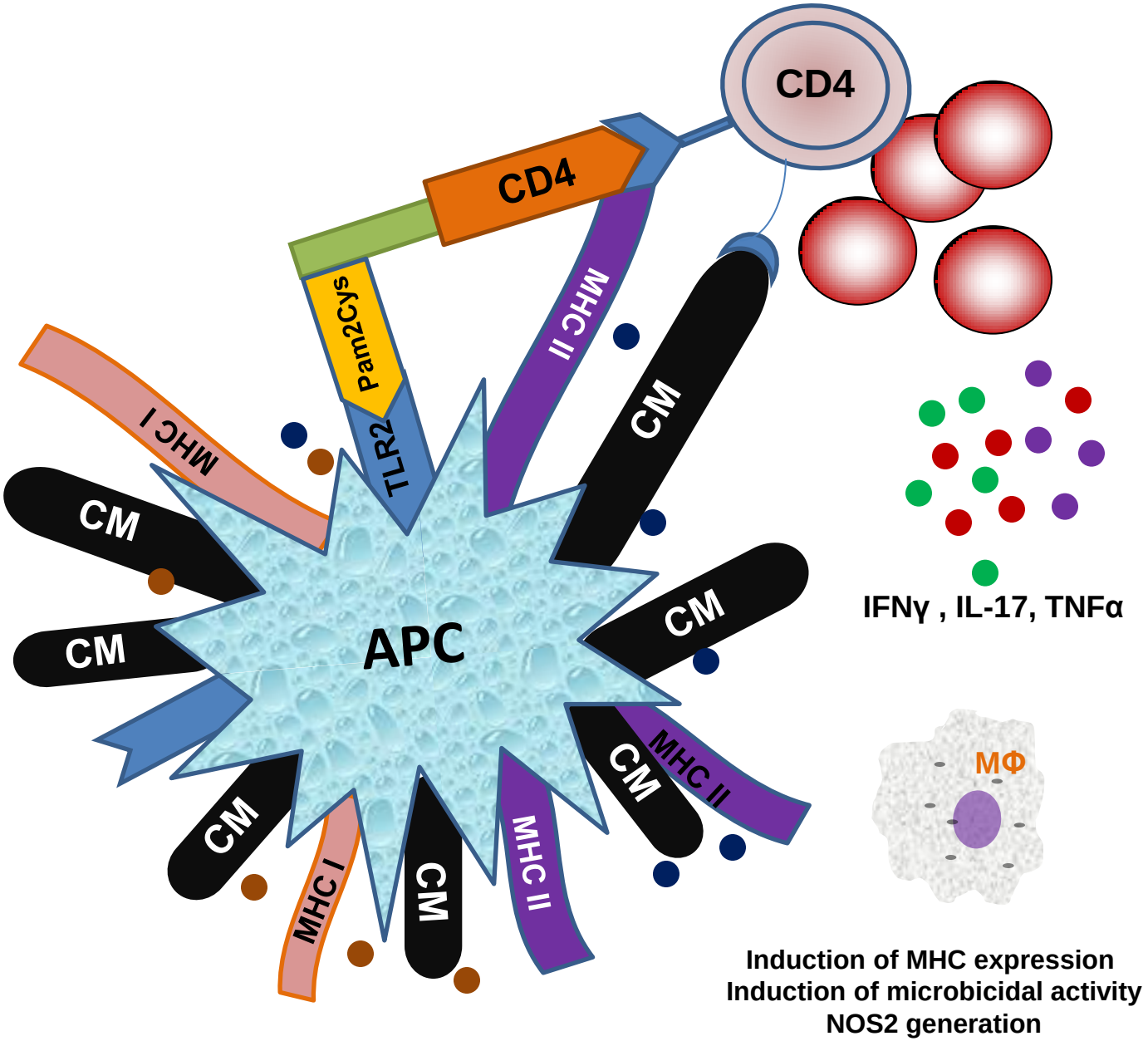
Dendritic cells copiously express TLR2 so Pam2Cys (TLR2 agonist) can be used as vaccine delivery module (Jackson et al 2004, Zho et al 2004)

Pam2Cys has ability to mature and activate DCs
(Hertz et al 2001, Jackson et al 2004, Zho et al 2004)

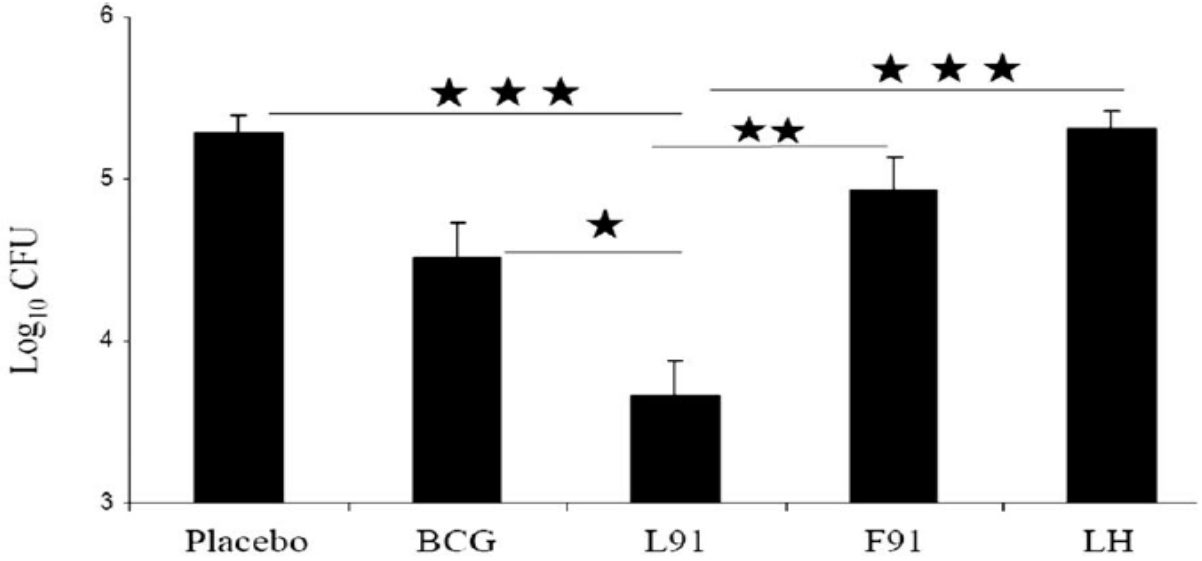
Safe for Human use
(Zeng et al 2002)

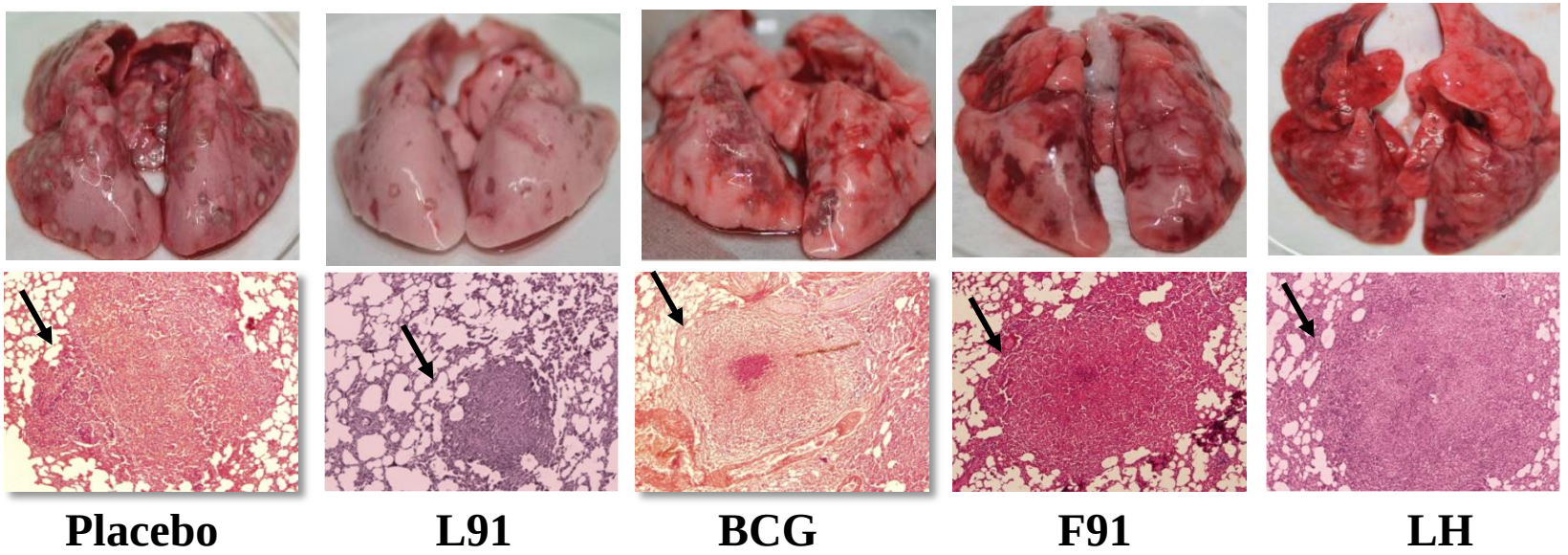


Mechanism of L91



L91 confers better protection than BCG in Guinea pig model of tuberculosis

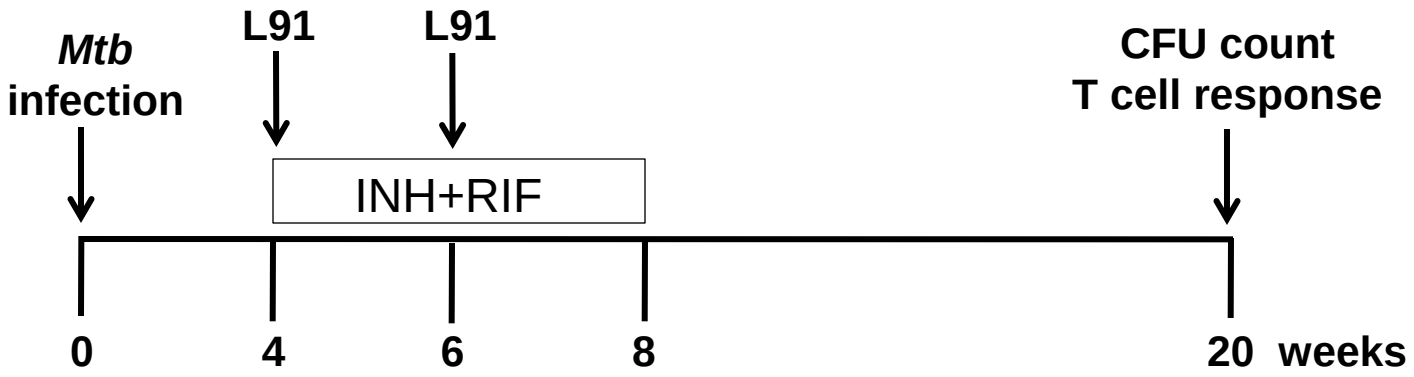


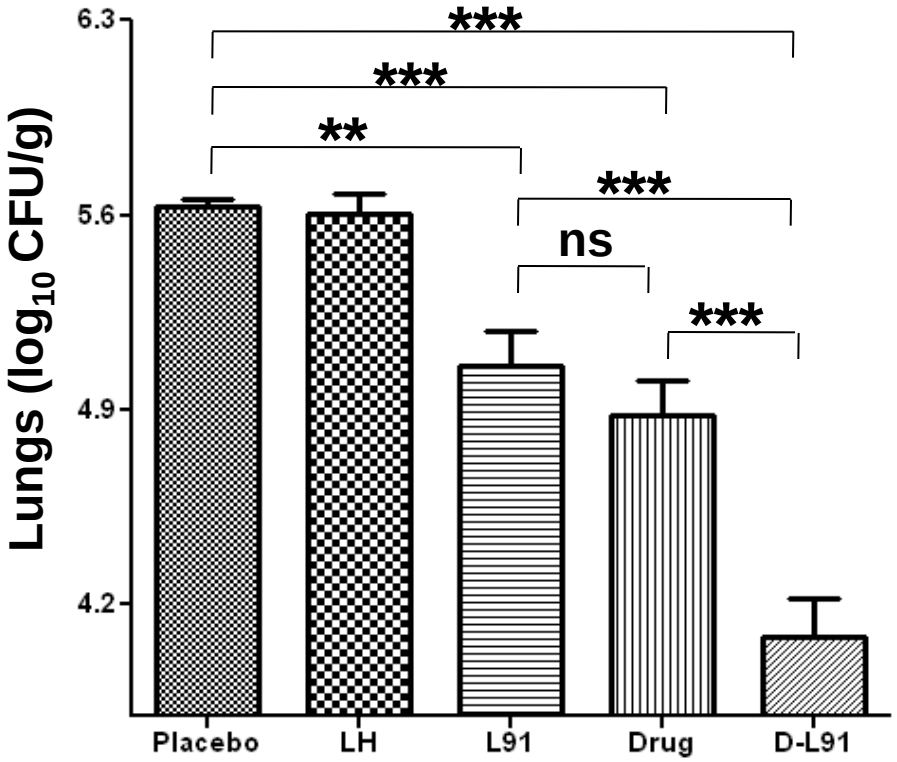


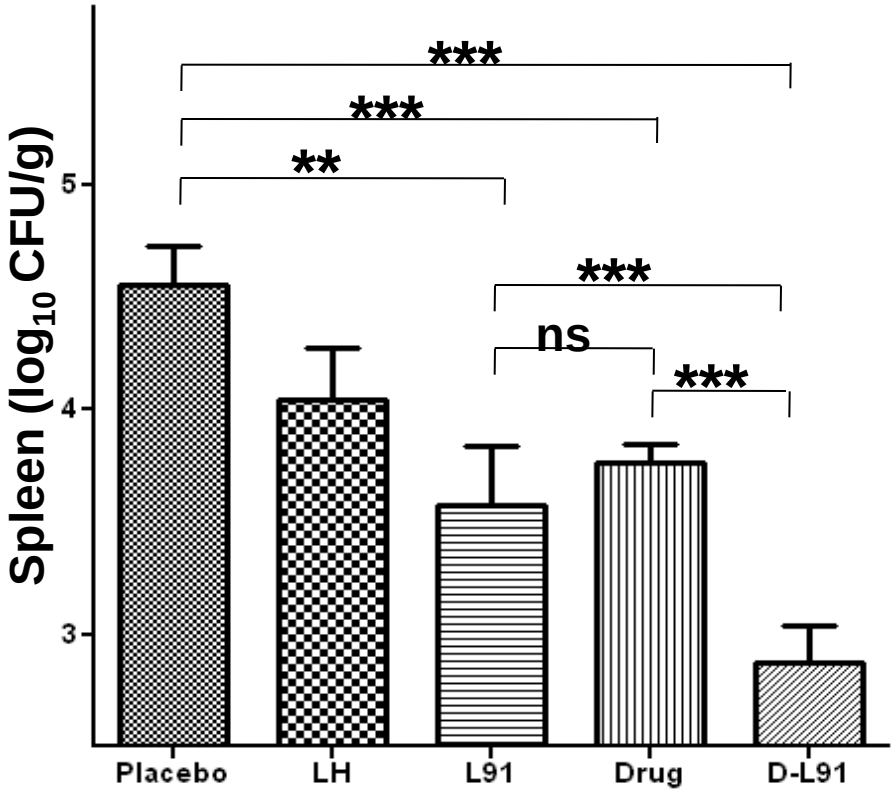
The Journal of Infectious Diseases 2011;204:1328–38

Whether L91 has therapeutic potential?

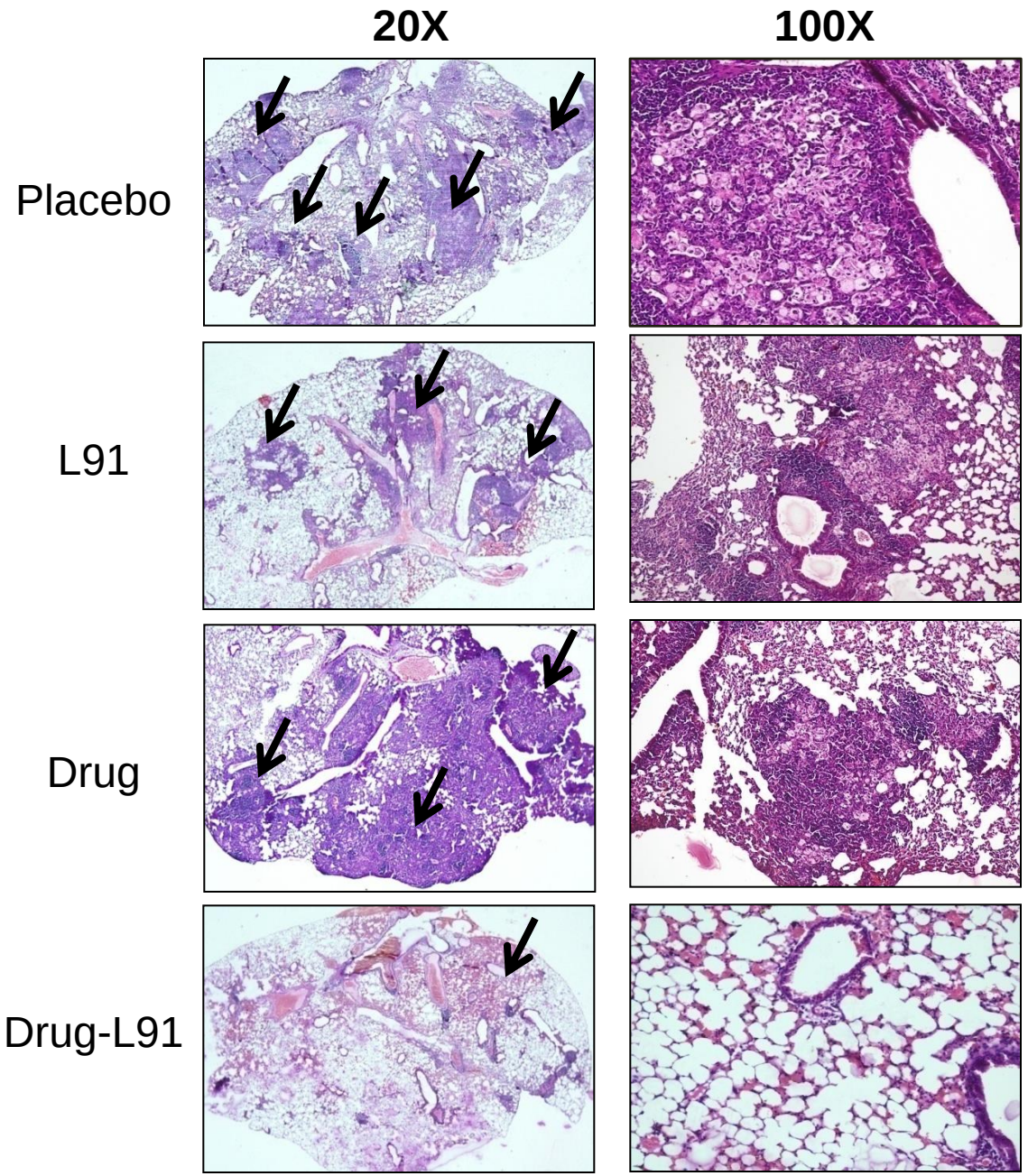
L91 efficiently decreases bacterial burden in the lungs and spleen in conjunction with anti-TB drugs







L91 immunization reduces *Mtb* induced lung pathology

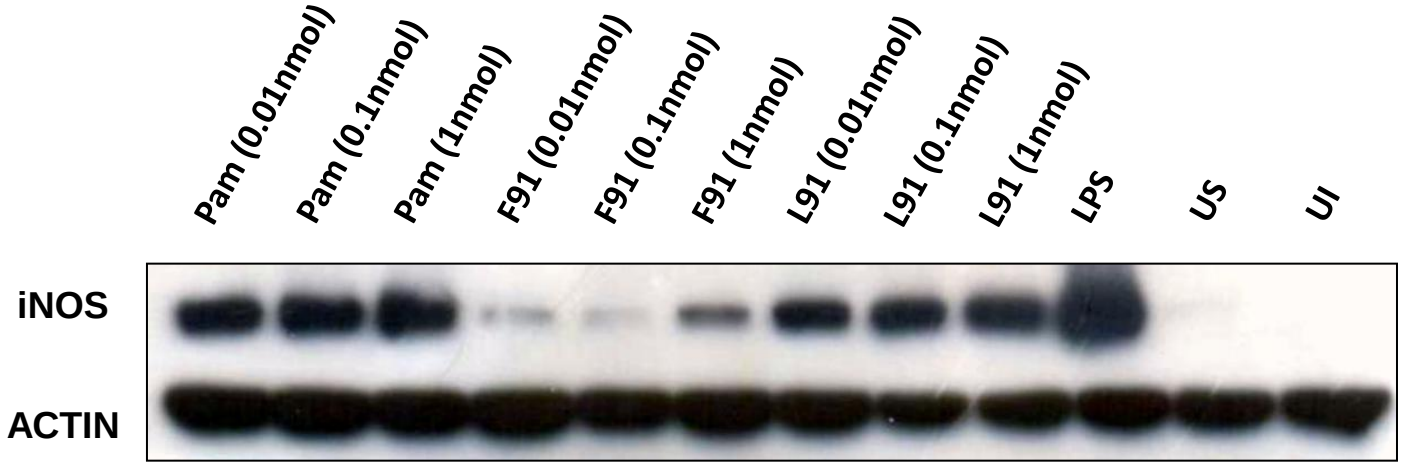


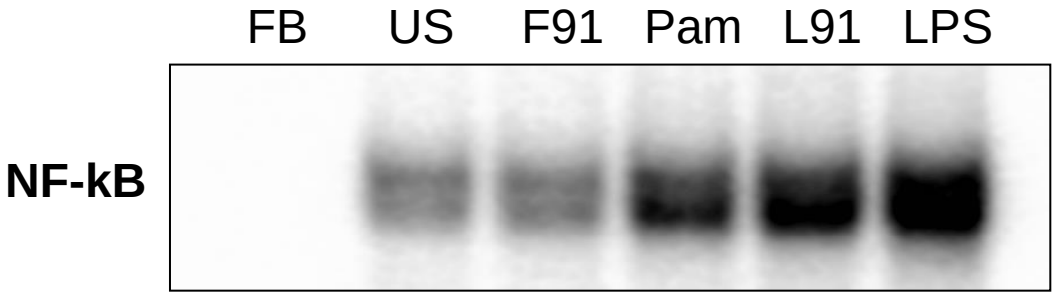
L91 has enough therapeutic potential to confine bacterial growth in lungs

Further, L91 restricts dissemination of bacteria in spleen

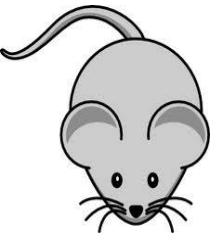
Mechanism of protection?

L91 stimulation elicits innate immunity

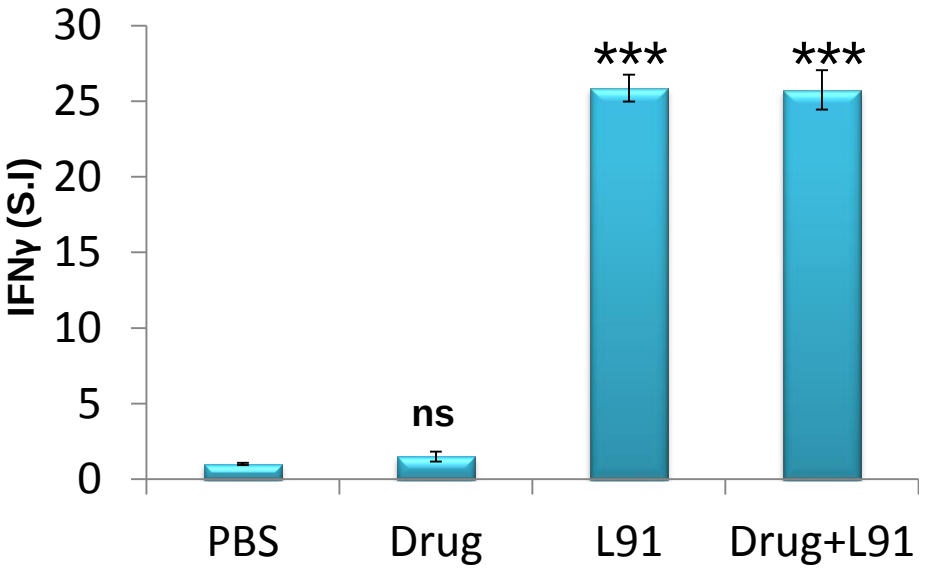


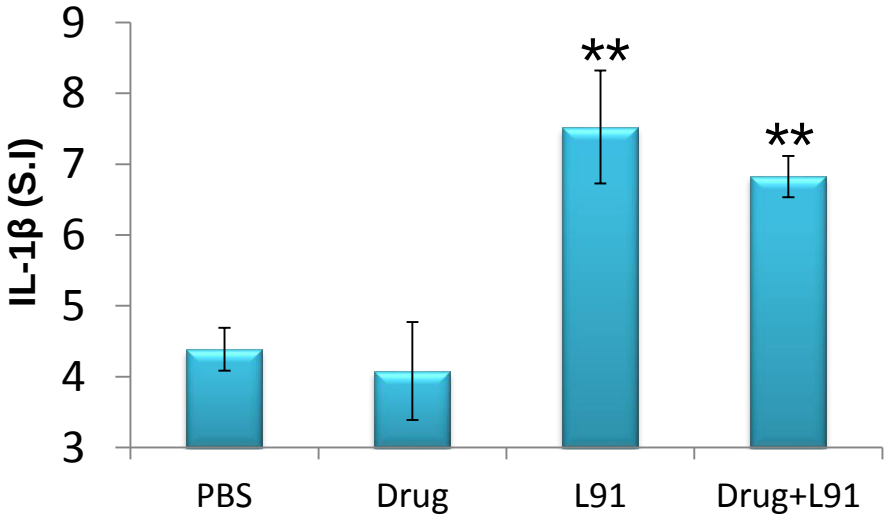


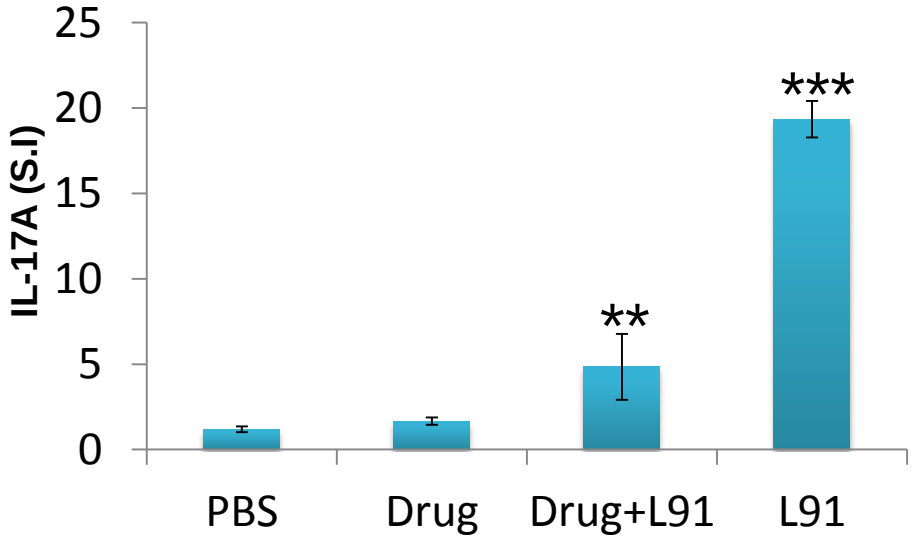
L91 immunization elicits secretion of protective cytokines against *Mtb*



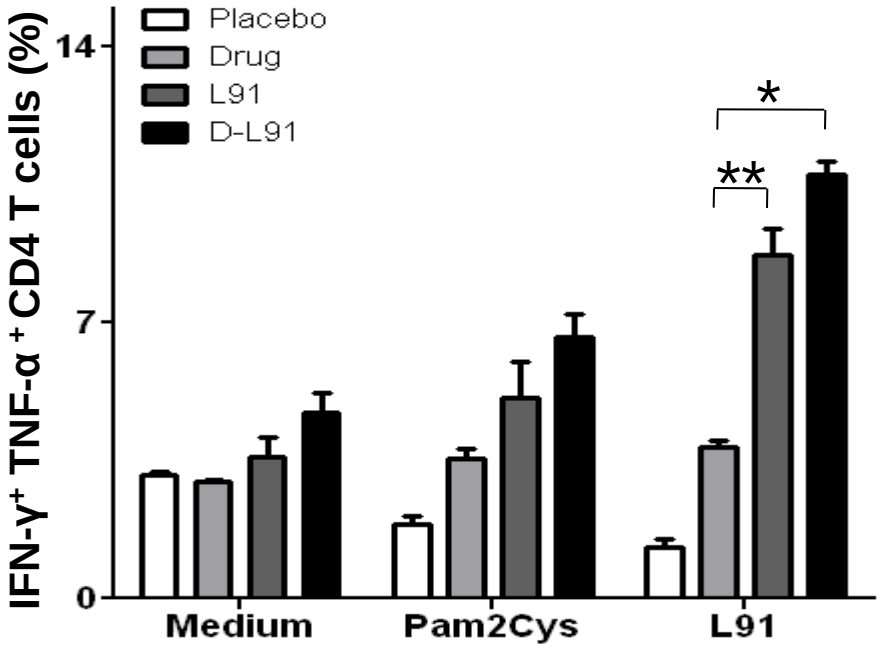
Lungs → Single cell suspension → In vitro stimulation with L91 → 72h → T cell response

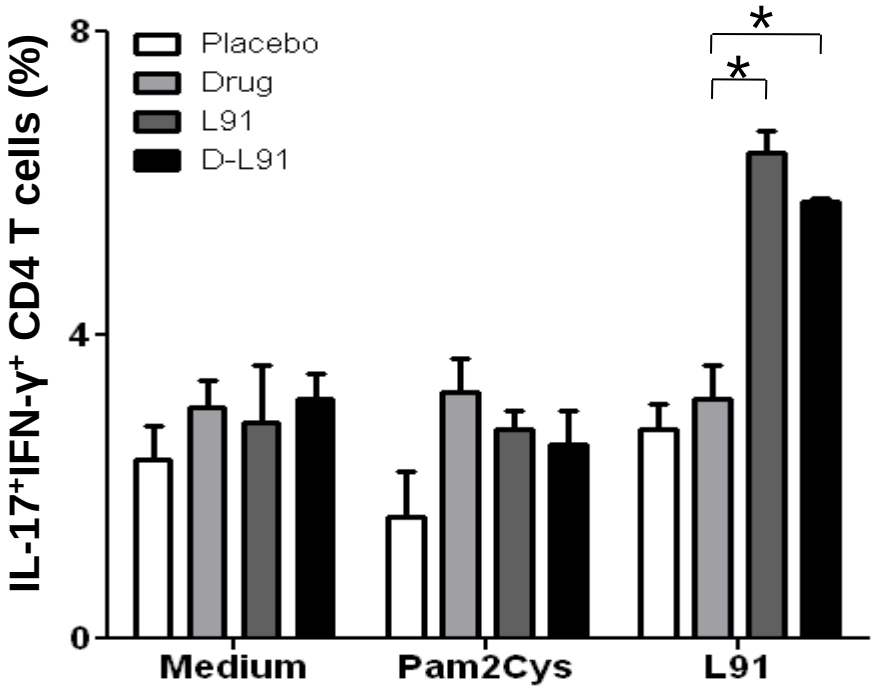




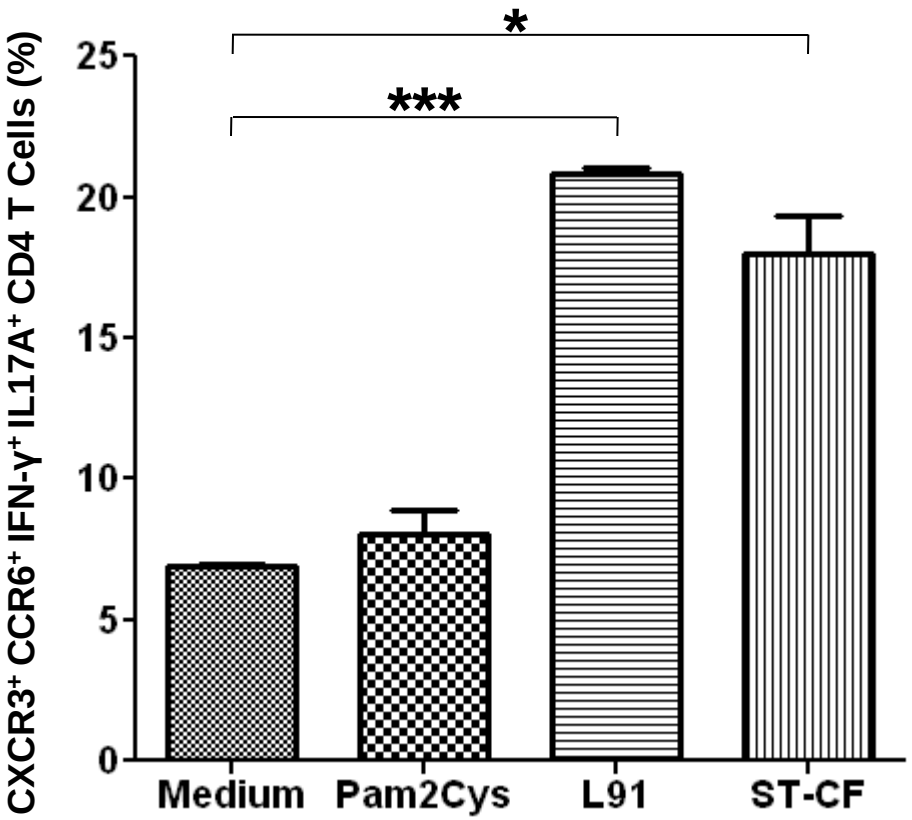


L91 immunization induces generation of multifunctional CD4 T cells

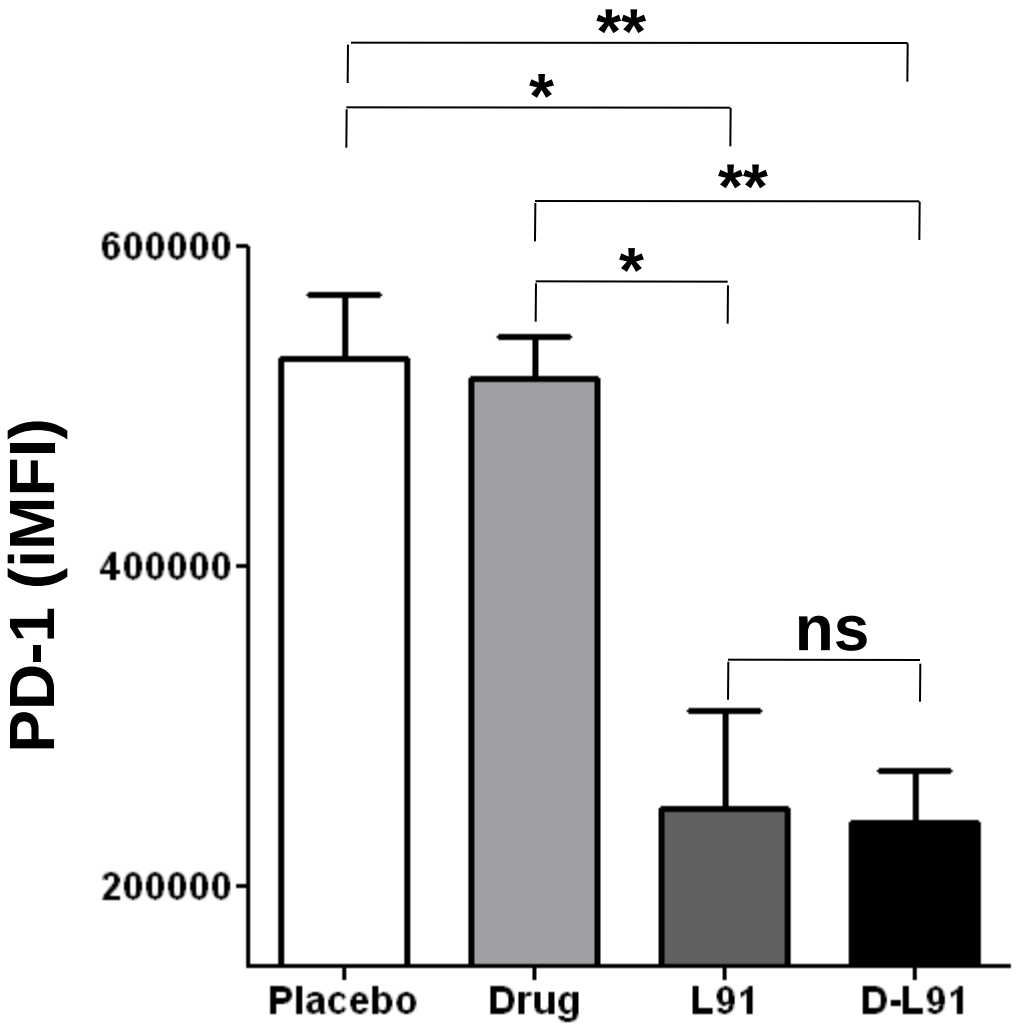




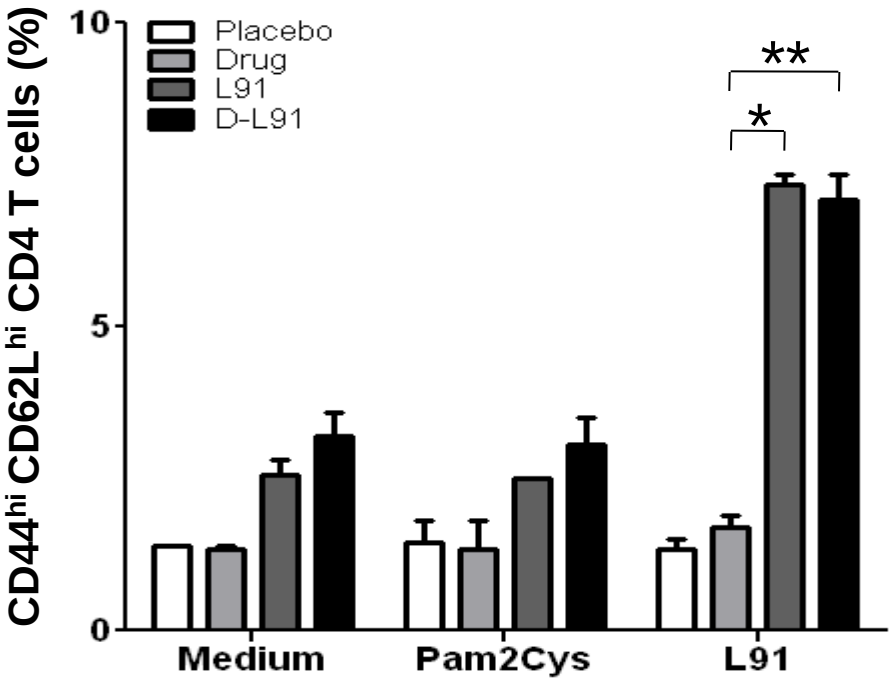
L91 immunization generates specific subtype of Th17 cells

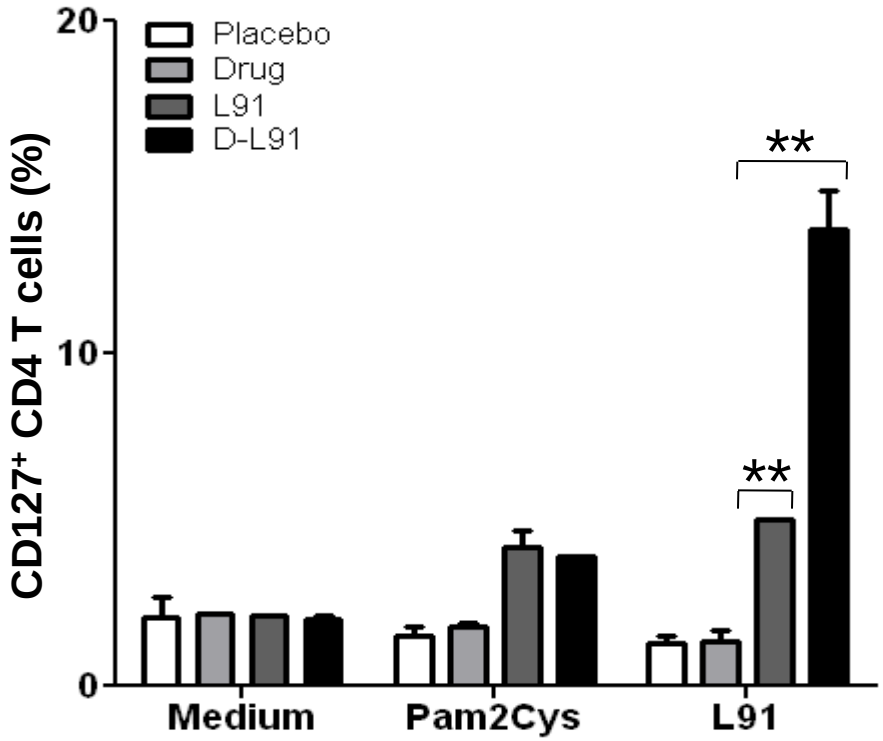


L91 immunization rescues CD4 T cells from exhaustion



L91 immunization engender long lasting memory CD4 T cells





L91 immunization eradicate bacteria by eliciting strong Th1 and Th17 response

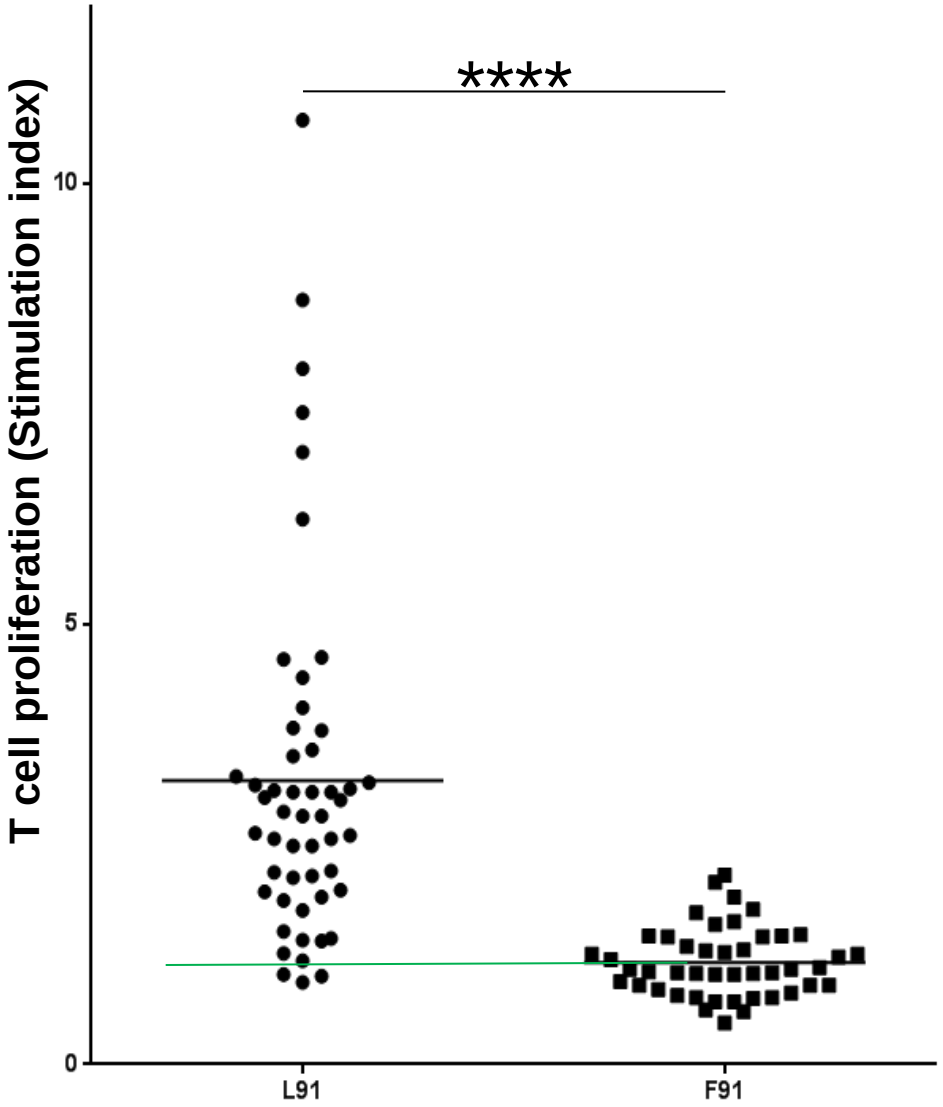
L91 immunization generates unique subset of IFN-γ^{+}IL-17^{+} polyfunctional Th17 cells

L91 engenders memory generation

Whether immunotherapy with L91 elicits immune response in TB patients?

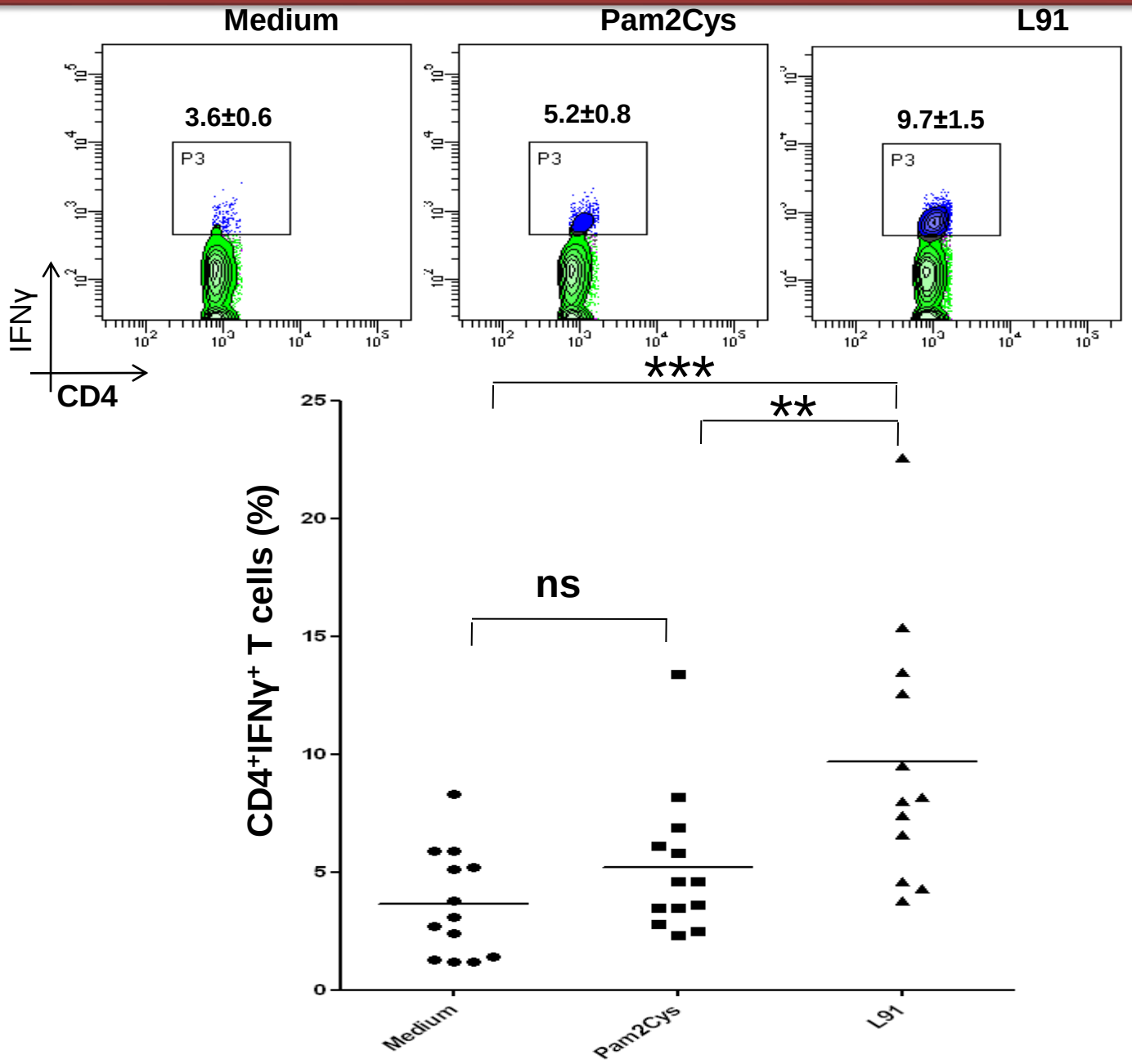
Lipidated peptide exhibits T cell proliferation in PBMCs isolated from TB patients and their close contacts

PBMCs (TB patients) + L91 + IL-2 $\xrightarrow{96h}$ Immune response

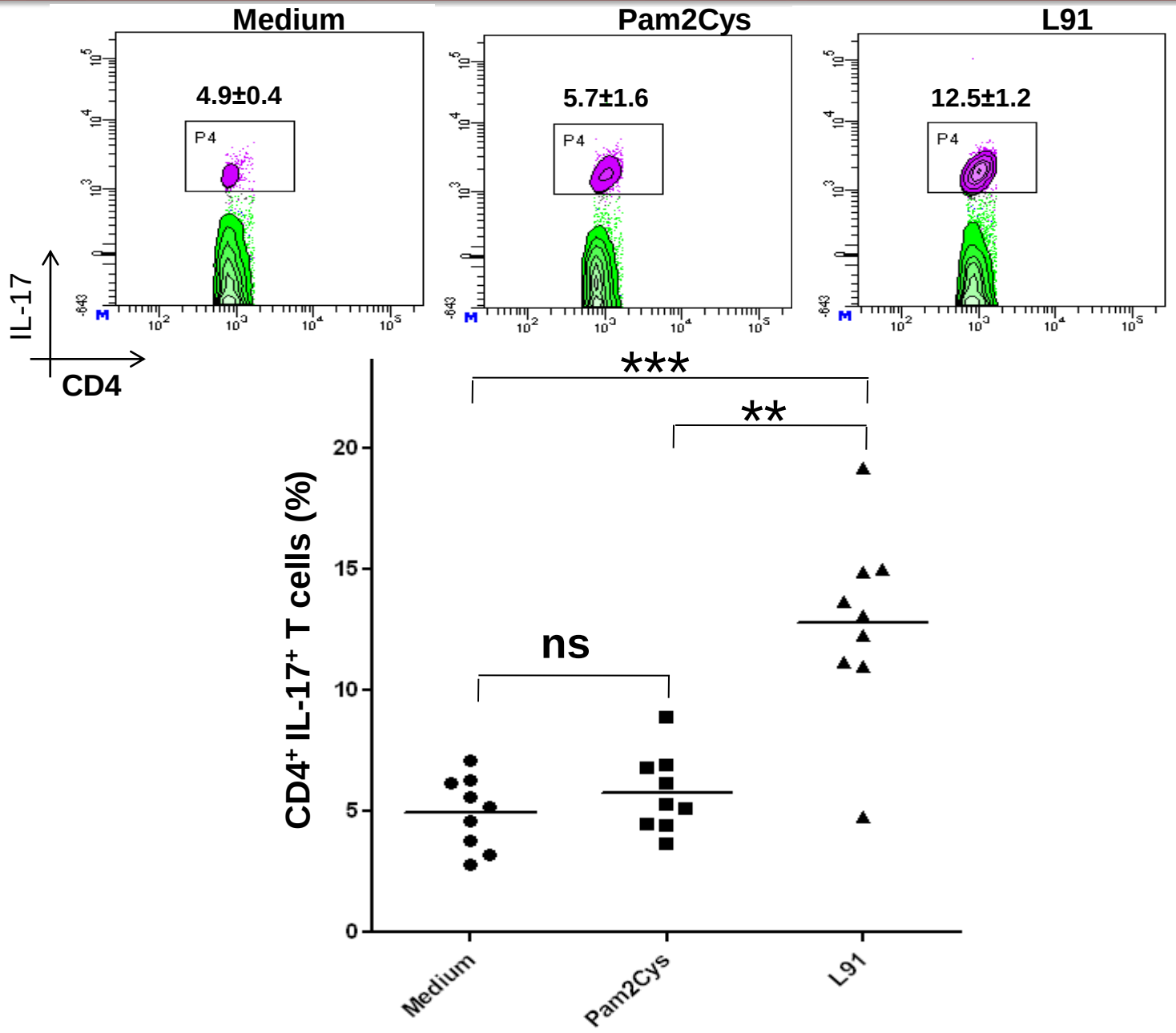


n= 53 (TB patients)

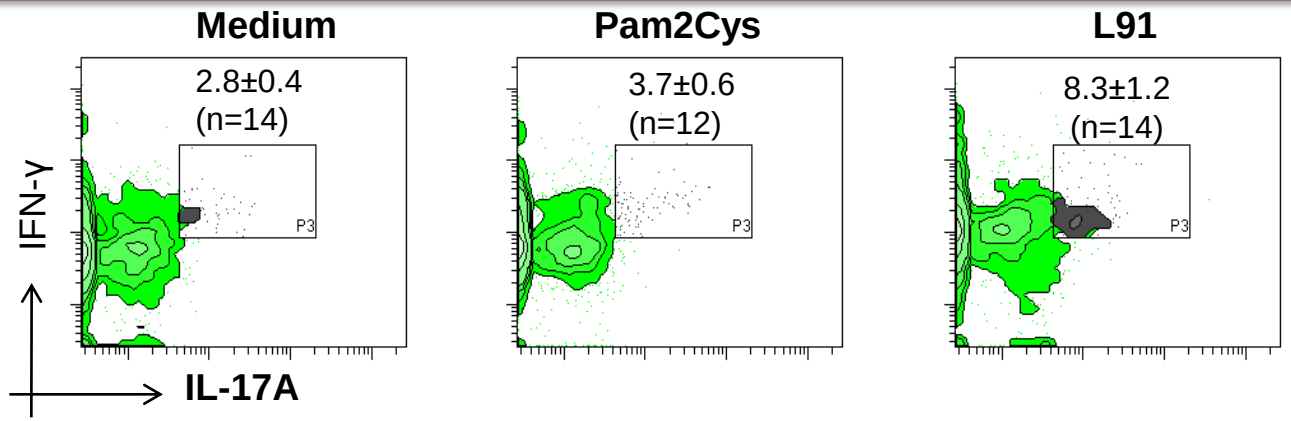
L91 stimulation elicits IFN-γ secretion by the CD4 T cells of TB patients

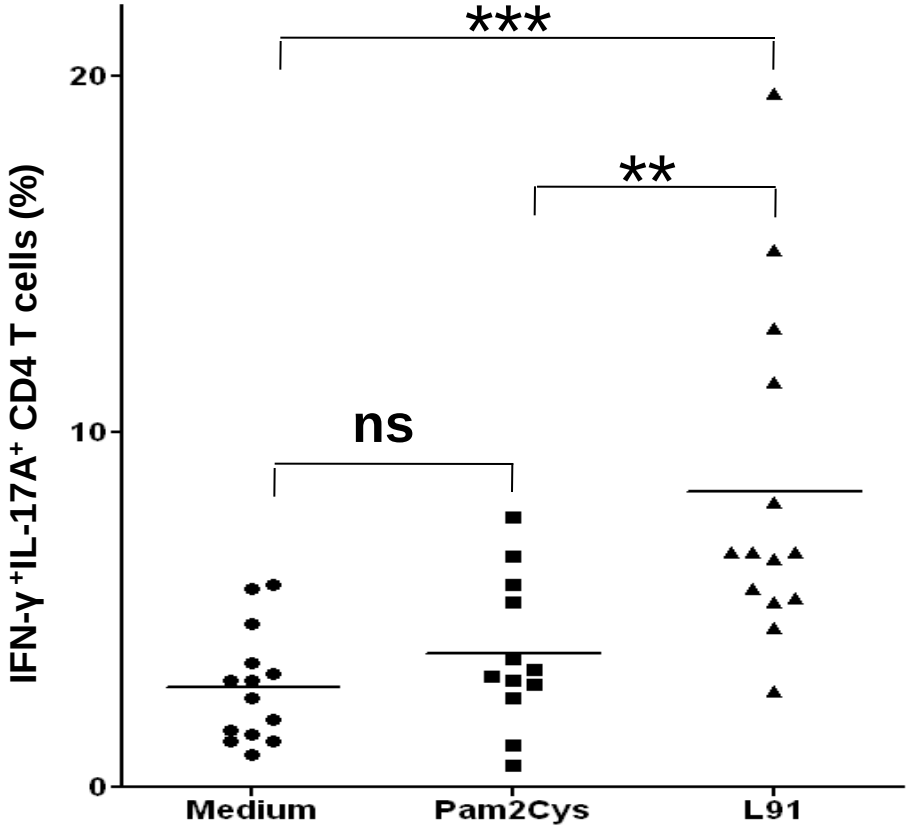


L91 stimulation induces IL-17 secretion by the CD4 T cells of TB patients

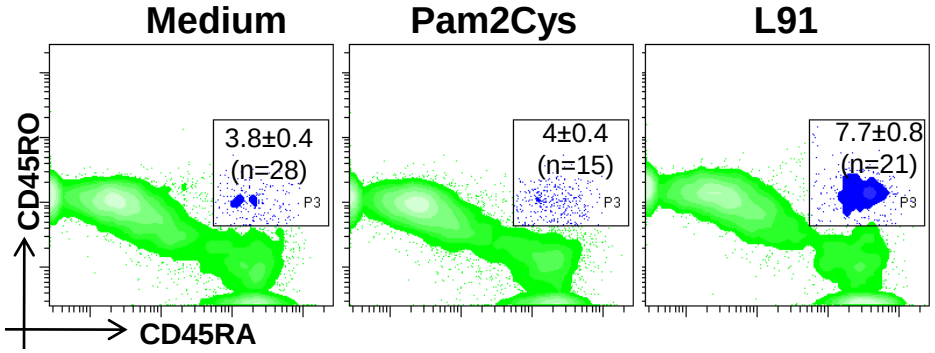


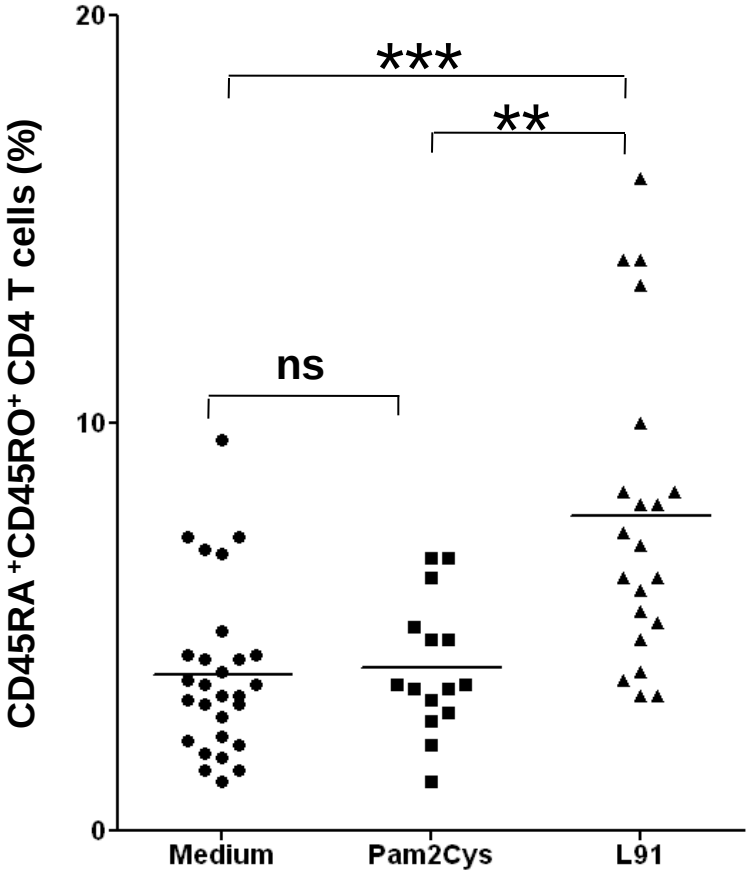
L91 stimulation generates polyfunctional Th17 cells



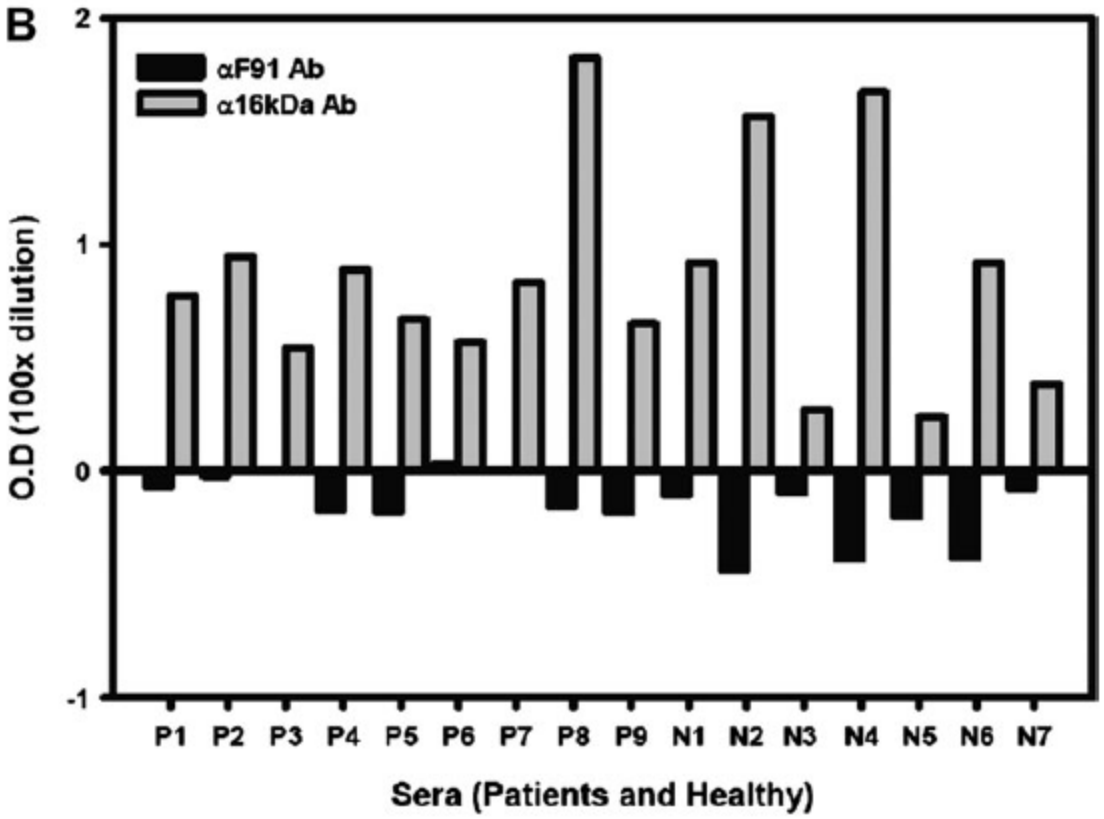


L91 expands the pool of memory T cell





F91 specific antibodies are absent in TB endemic population



Conclusions

- ✓ Promiscuous lipidated peptide can elicit robust Th1 and Th17 response in human PBMCs isolated from TB patients and their closed contacts
- ✓ L91 immunization induces unique subset of co-expressing CCR6 and CXCR3 Th17 cells, which secrets both IFN-γ and IL-17
- ✓ No anti-peptide antibodies in population so L91 has great potential to be a future vaccine candidate for TB endemic area
- ✓ Immunotherapy with L91 may reduces risk of generation of drug resistant *Mtb*

Acknowledgment

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Professor Ashok K Janmeja

CSIR

DBT

Thanks