Systems Biology of rBCG-LTAK63 highlights correlation between circadian rhythm, immune response and protection against tuberculosis

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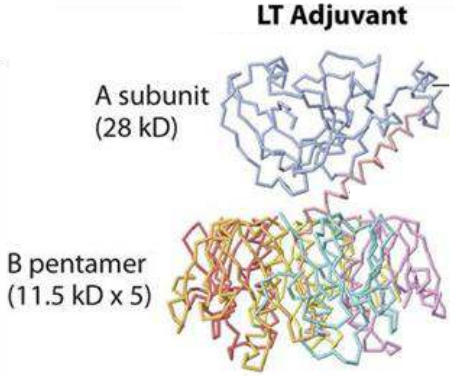
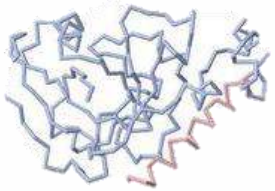
The LT as adjuvant for Tb vaccine



Summary of Main Features

(1) Simple antigen+adjuvant formulation
(2) Enhanced antigen uptake at mucosal and parenteral sites
(3) Multifaceted adjuvant response, including Th17
(4) Promotion of mucosal immunity

"A" subunit



Binds to cytosolic ADP-ribosylates Gsα, resulting in adenylate cyclase activation and accumulation of intracellular cAMP.

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rBCG-LTAK63

LTAK63

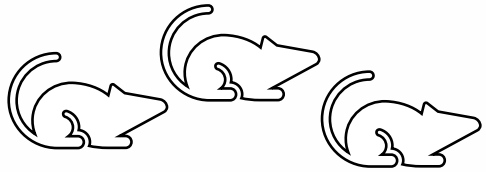
- ✓ Single mutated detoxified subunit A
- ✓ Maintains Th1/Th17 adjuvanticity
- ✓ Minor toxicity

SCIENTIFIC REPORTS

OPEN

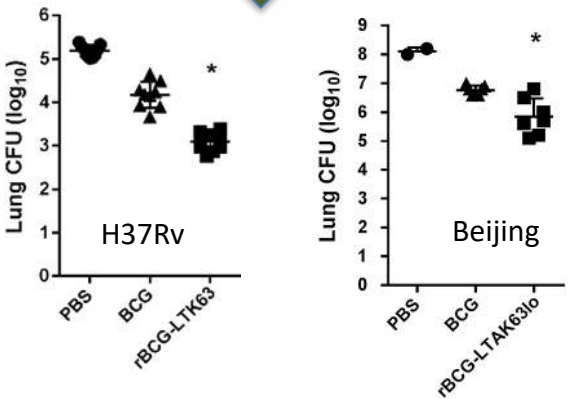
Recombinant BCG Expressing LTAK63 Adjuvant induces Superior Protection against *Mycobacterium tuberculosis*

Ivan P. Nascimento[1], Dunia Rodriguez[1], Carina C. Santos[1,4], Eduardo P. Amaral[2], Henrique K. Rofatto[3], Ana P. Junqueira-Kipnis[5], Eduardo D. C. Gonçalves[6], Maria R. D'Império-Lima[2], Mario H. Hirata[7], Celio L. Silva[8], Nathalie Winter[9], Brigitte Gicquel[10], Kingston H. G. Mills[11], Mariagrazia Pizza[12], Rino Rappuoli[12] & Luciana C. C. Leite[1]



Mice immunized with **BCG-LTAK63** and challenged with Mtb

90 dpi



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- ✓ **Recombinant BCG expressing LTAK63 increases Th1/Th17 response**
- ✓ **Protects against virulent and hypervirulent strain**

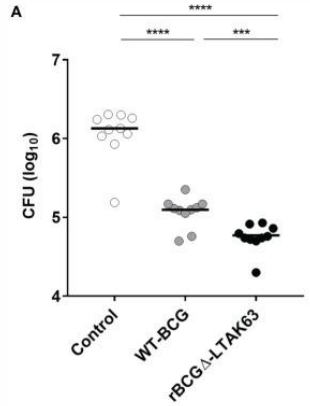
rBCG-LTAK63

frontiers | Frontiers in Immunology

CRISPR/Cas9 Approach to Generate an Auxotrophic BCG Strain for Unmarked Expression of LTAK63 Adjuvant: A Tuberculosis Vaccine Candidate

*Luana Moraes[1,2], Monalisa Martins Trentini[1], Dimitrios Fousteris[1,3], Silas Fernandes Eto[4], Ana Marisa Chudzinski-Tavassi[4,5], Luciana Cezar de Cerqueira Leite[1] and Alex Issamu Kanno[1]**

90 dpi

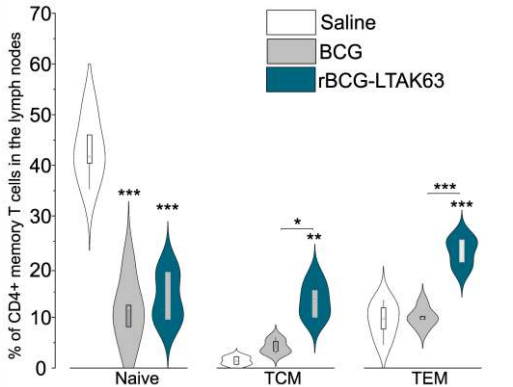


frontiers | Frontiers in Immunology

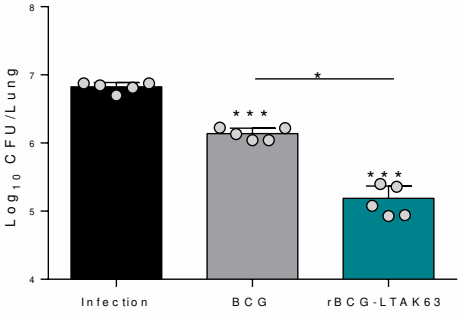
Recombinant BCG expressing the LTAK63 adjuvant increased memory T cells and induced long-lasting protection against *Mycobacterium tuberculosis* challenge in mice

Lázaro Moreira Marques-Neto, Monalisa Martins Trentini, Alex Issamu Kanno, Dunia Rodriguez and Luciana Cezar de Cerqueira Leite*

180 dpi



Lymph node



Lung

- ✓ **Auxotrophic complementation generates unmarked rBCG-LTAK63 appropriate for human use.**
- ✓ **Increased memory T cell development.**
- ✓ **Protects for longer period (up to180 days).**

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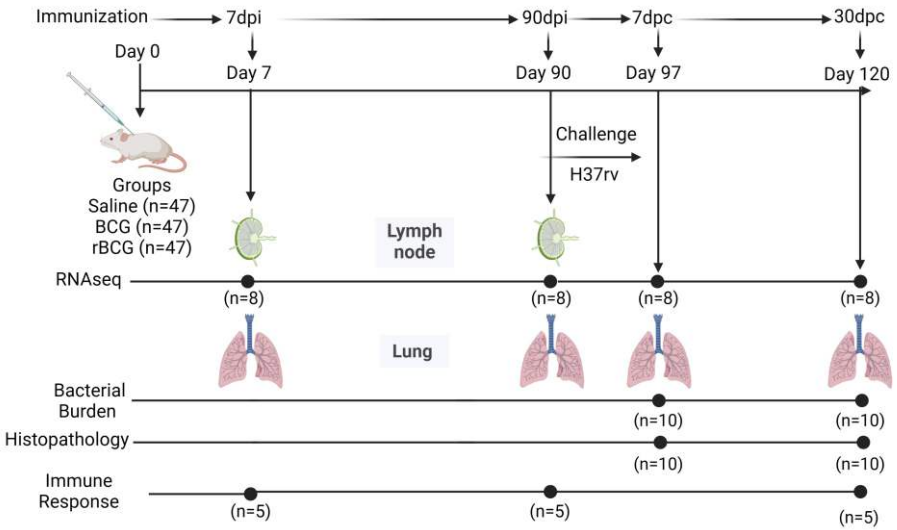
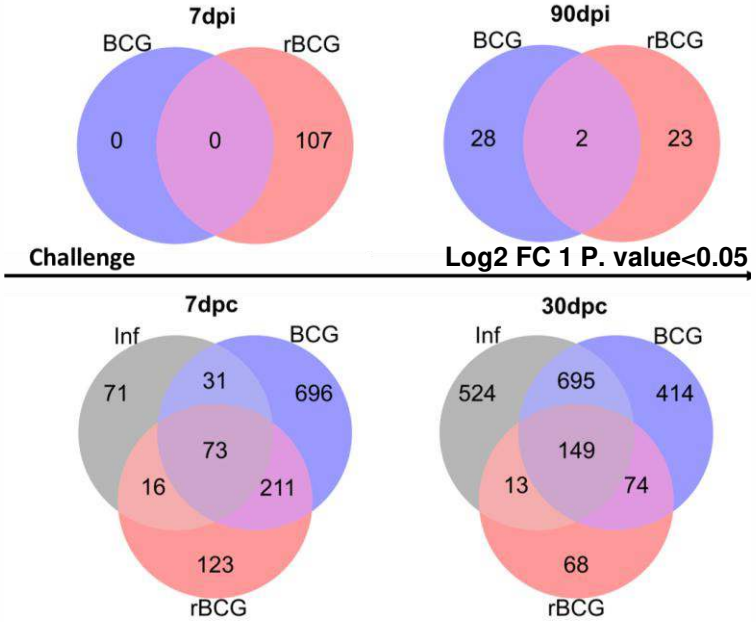
Systems Biology

Experimental design



Number of Differentially Expressed Genes (DEG)



Striking difference in the gene expression profiles between rBCG-LTAK63 and BCG both before and after challenge

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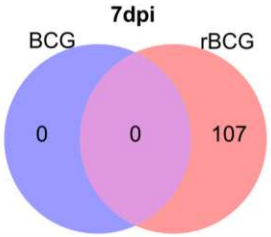
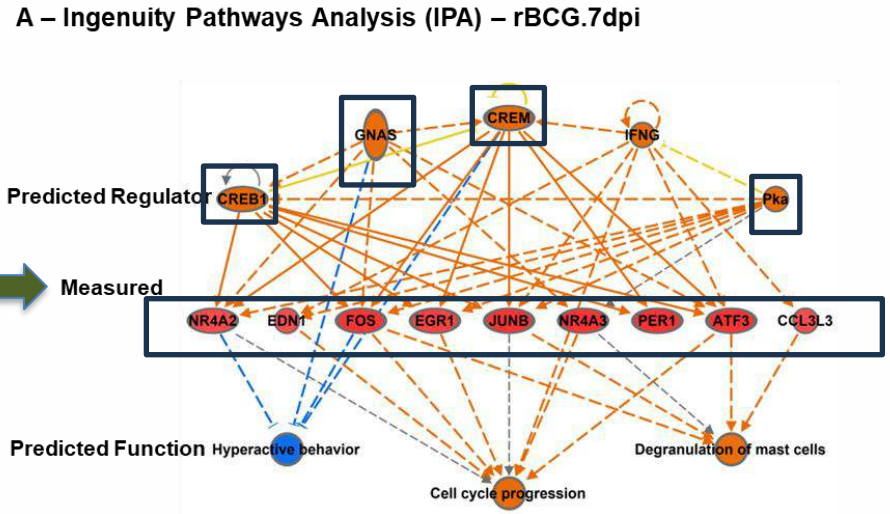
DEGS and Functional analysis (7dpi)



Functional analysis

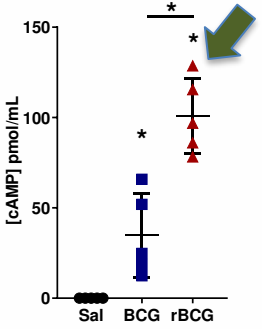
Transcriptional factors associated with cAMP

Upregulated genes



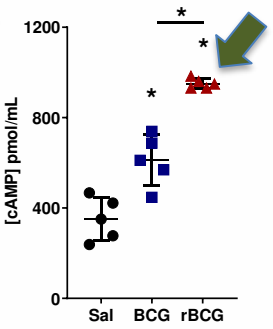
Lung cells

48h - *in vitro*



Immunized animals lung tissue

7dpi - *in vivo*



rBCG-LTAK63 increases cAMP in animals' lungs and induced genes related to cAMP pathway

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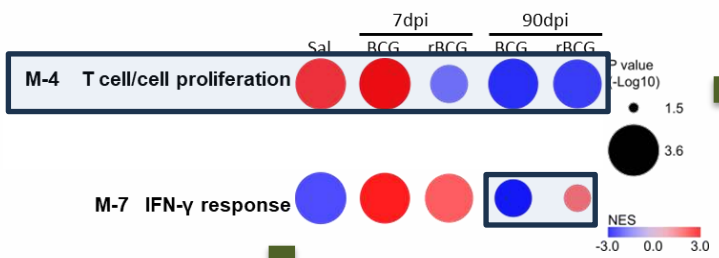
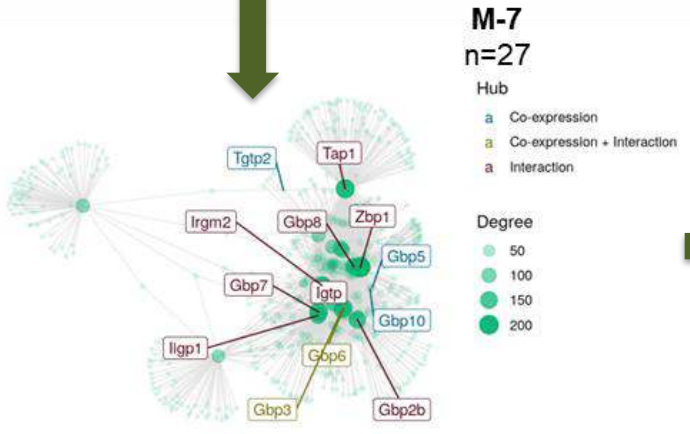
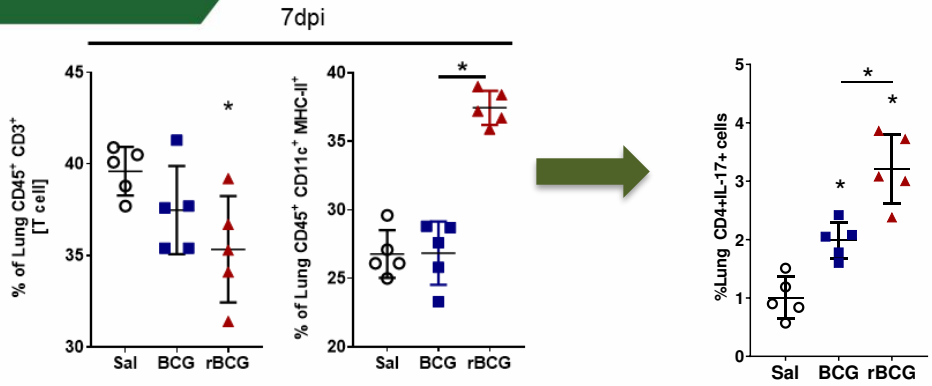
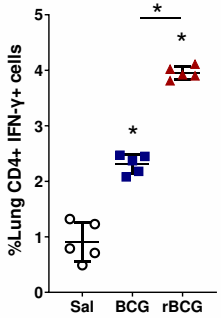
Co-expression Analysis - DPI

A - CEMiTool









Modular transcriptional and phenotypical analysis indicates increase in Th17 and persistent IFN-γ response

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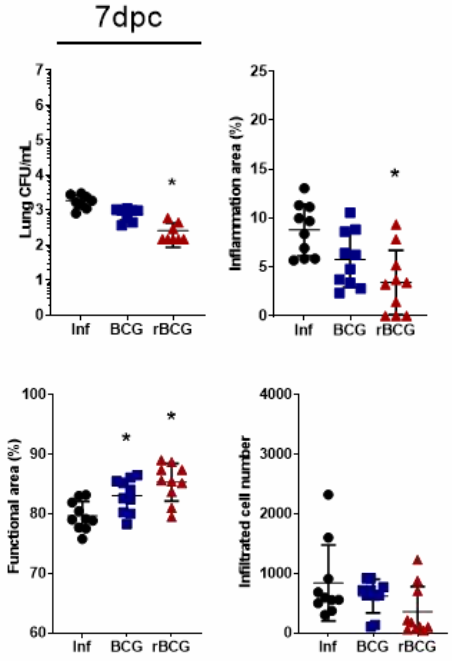
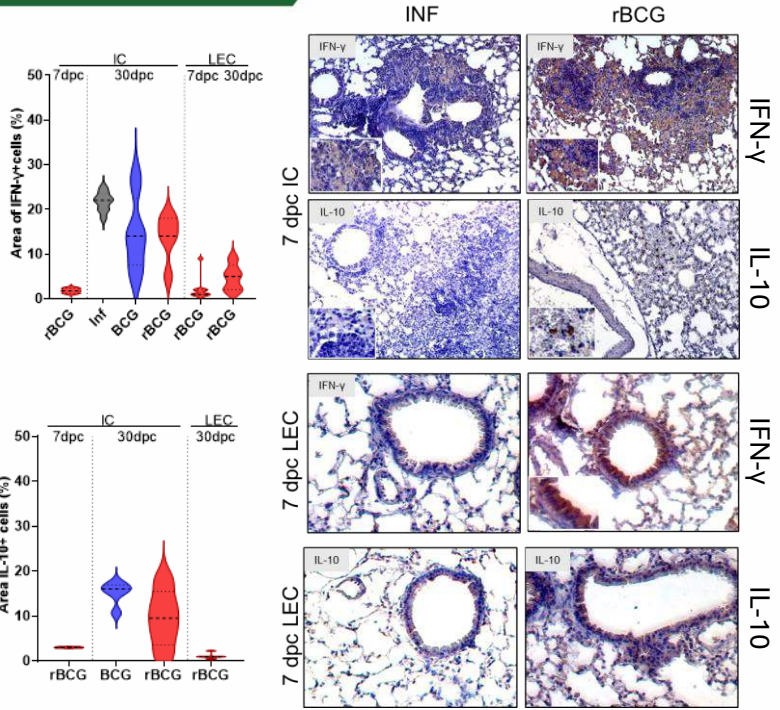
Early protective effect of rBCG-LTAK63





Increased infiltrated cells (IC) producing IFN-g and IL-10

Increased lymphatic endothelial cells (LEC) producing IFN-g and IL-10

rBCG-LTAK63 primes the lung environment for a faster and more coordinated immune response leading to better control of tuberculosis.

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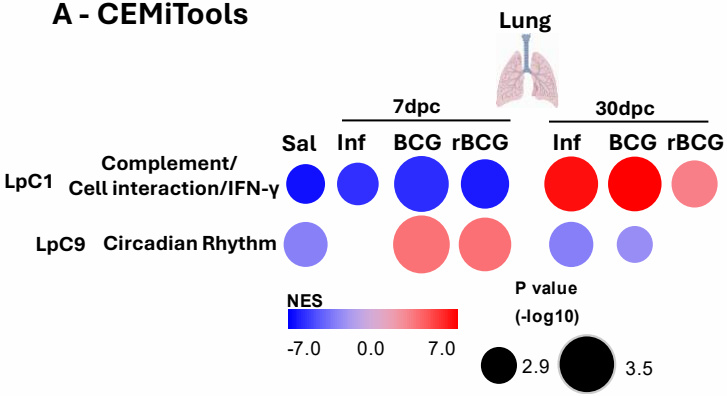
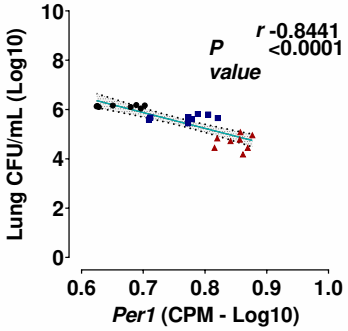
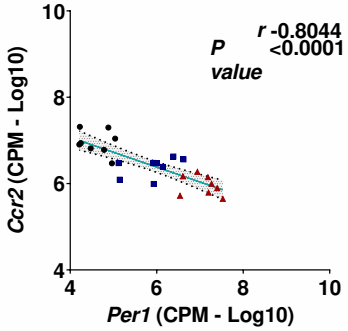
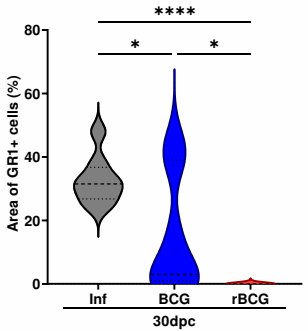
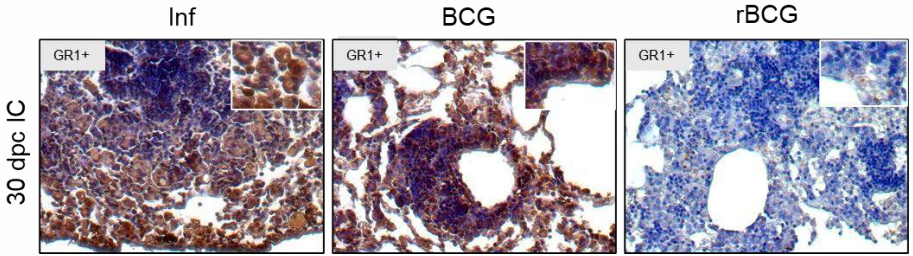
Per1 controls GR1+ recruitment











Immunohistochemistry of lung tissue stained with anti-GR1

rBCG-LTAK63 induces *Per1*, decreases *Ccr2* and GR1 recruitment (susceptible myeloid cells), controlling tissue damage and pathology

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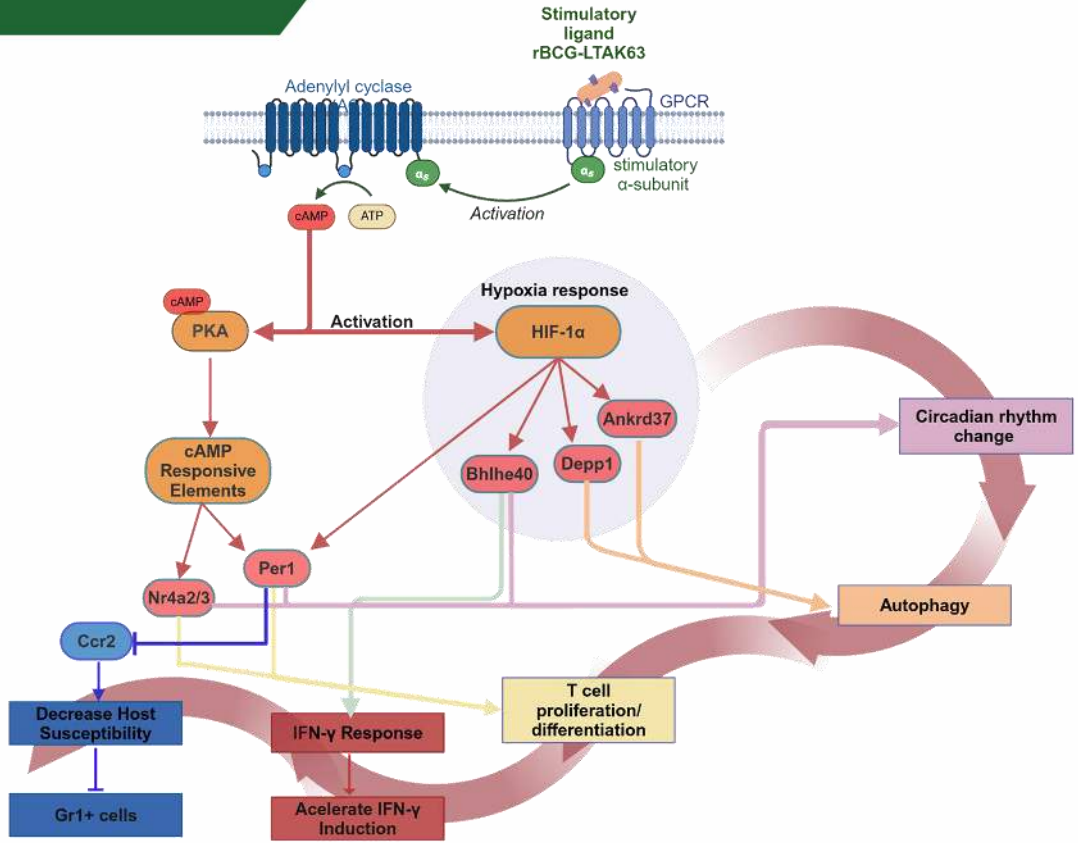
Conclusions

- ✓ **rBCG-LTAK63 induces cAMP production and amplifies Th1 and Th17 responses.**
- ✓ **Upon challenge, IFN is rapidly upregulated accelerating the protective response.**
- ✓ **The circadian rhythm gene, Per1, reduces recruitment of susceptible myeloid cells**
- ✓ **This results in a more balanced inflammatory response, improving pathogen control and reducing tissue damage**



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