

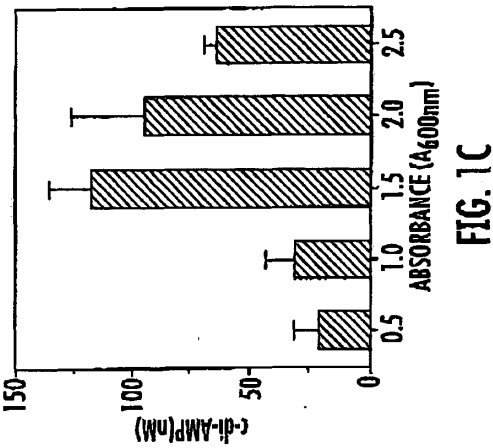
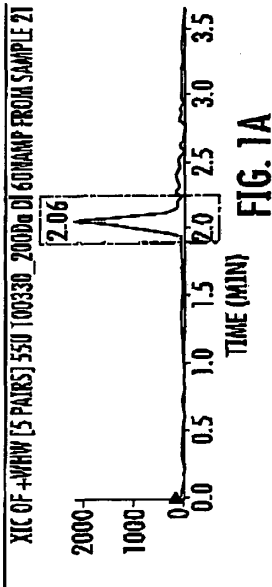
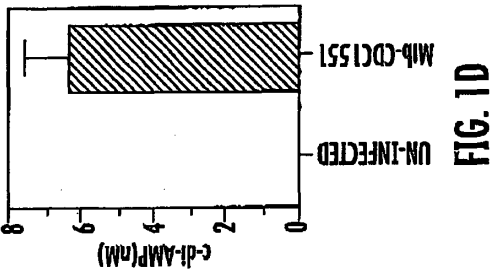
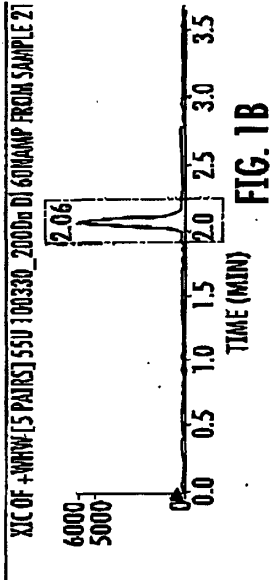
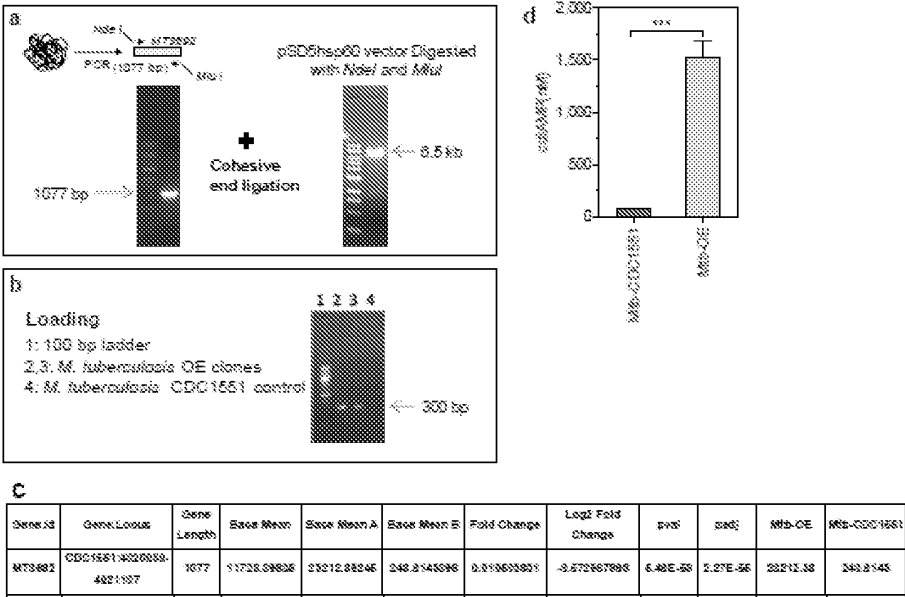
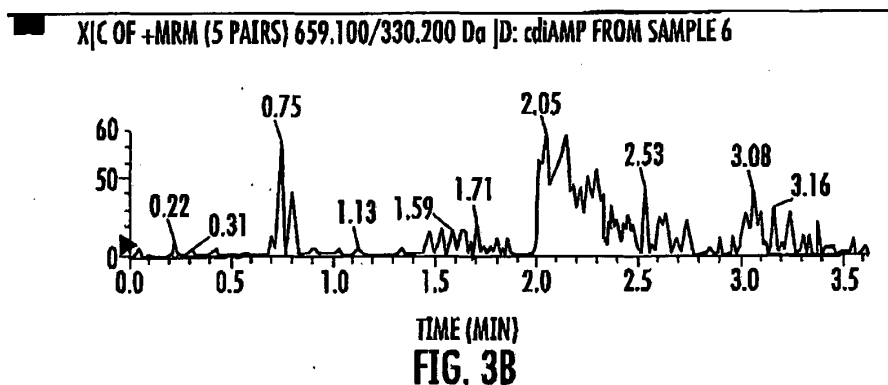
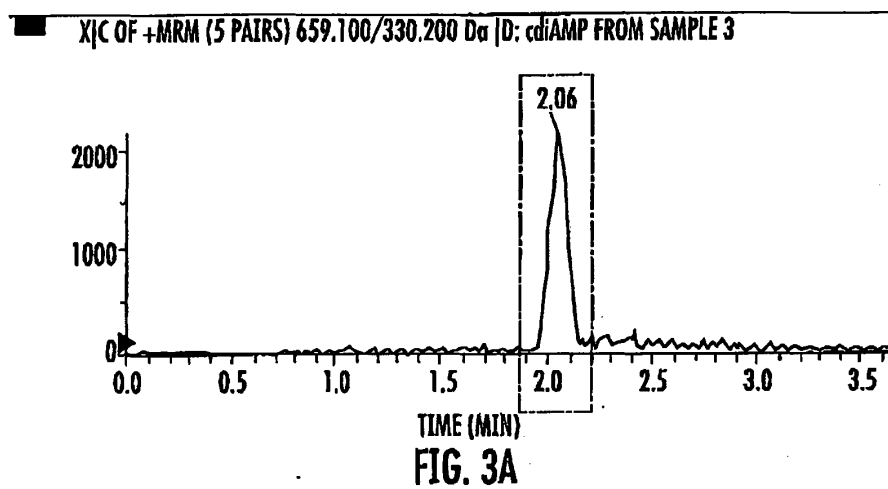


Figure 2



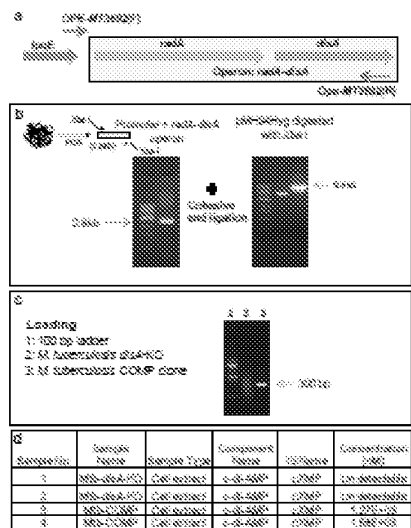
Supplementary Fig. 2. Generation and confirmation of *M. tuberculosis* strain Over-expressing c-di-AMP (Mtb-OE). (a) The *disA* gene of *M. tuberculosis*, MT3692, was PCR-amplified from *M. tuberculosis* CDC1551 genomic DNA using gene-specific primers. The amplicons were cloned into the mycobacterial expression vector pSD5-hsp60 at the *Nde* I and *Mlu* I restriction sites. (b) The resulting construct pSD5-hsp60-MT3692 was sequenced and used to transform *M. tuberculosis* CDC1551 and recombinant clones were selected against Kanamycin (25µg/mL). Colony PCR using Kanamycin gene specific primers confirmed the presence of recombinant plasmid in the Mtb-OE strain. (c) Table depicting the RNAseq result of Mtb-CDC1551 and Mtb-OE strains for the gene MT3692 indicating ~95 fold over-expression of MT3692 in Mtb-OE in comparison to Mtb-CDC1551. For RNA sequencing, total RNA were extracted from *M. tuberculosis* cultures at an O.D. of 1, followed by rRNA depletion and sequencing using Applied Biosystems SOLiD™ Total RNA-Seq Kit protocol on a AB 5500xl SOLiD sequencer. (d) Comparison of the intra-bacterial levels of c-di-AMP extracted from 2X10⁸ cells of Mtb-CDC1551 and Mtb-OE at culture O.D. of 1. Data is Mean ± SD of triplicate samples; ***, p < .001 by Student's t-test (2-tailed).



SAMPLE NO.	SAMPLE NAME	SAMPLE TYPE	COMPONENT NAME	IS NAME	CONCENTRATION (nM)
1	Mib-CDC1551	CELL EXTRACT	c-di-AMP	cXMP	1.27E+02
2	Mib-CDC1551	CELL EXTRACT	c-di-AMP	cXMP	1.29E+02
3	Mib-disA-KO	CELL EXTRACT	c-di-AMP	cXMP	UNDETECTABLE
4	Mib-disA-KO	CELL EXTRACT	c-di-AMP	cXMP	UNDETECTABLE

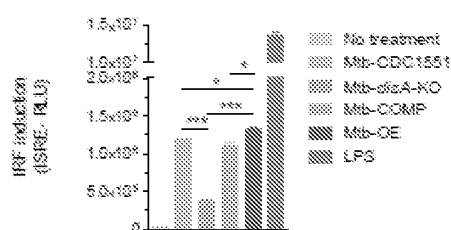
FIG. 3C

Figure 4

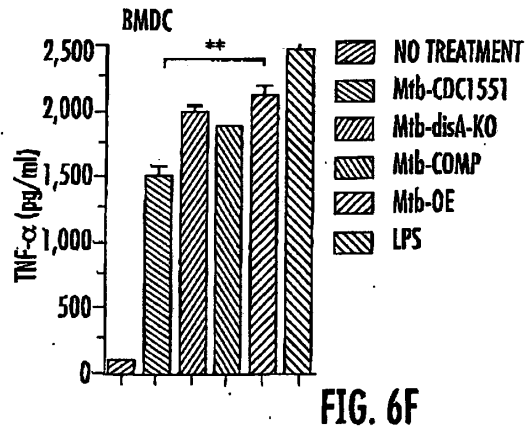
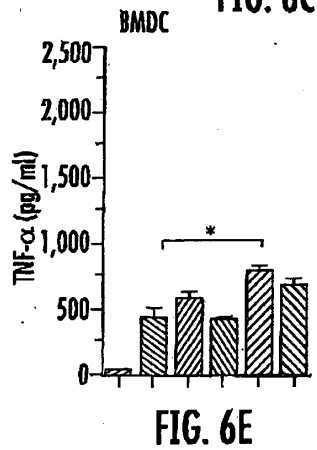
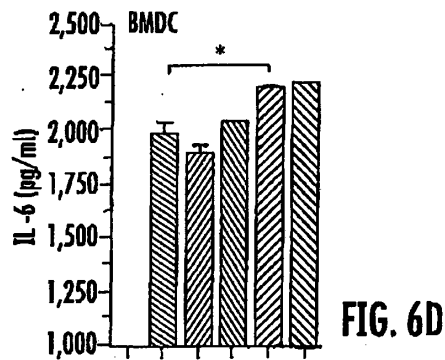
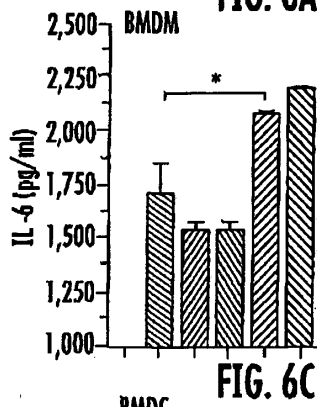
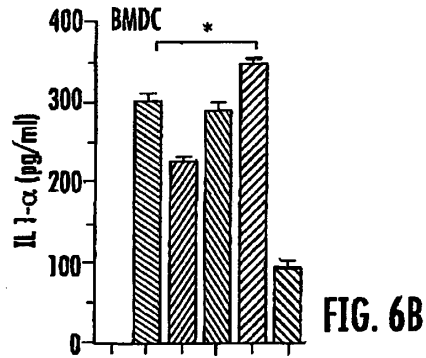
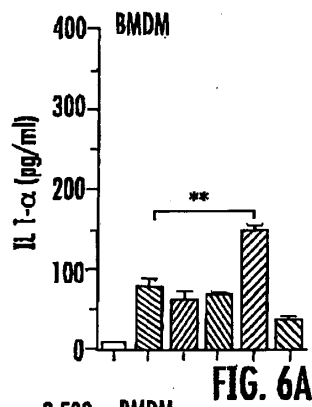


Supplementary Fig. 4. Generation and confirmation of *M. tuberculosis*-CDC1551 MT3692 complemented strain (Mtb-COMP). (a) Organization of *rad4-disA* operon in the genome of *M. tuberculosis* CDC1551. (b) The whole operon of *rad4-disA* including the upstream promoter region was PCR-amplified using *M. tuberculosis* CDC1551 genomic DNA and primers OPE-MT3692(F) and OPE-MT3692(R) and cloned into an integrative vector, pMH94Hyg, at XbaI- restriction site. (c) The resulting construct, pMH94Hyg-MT3692 was used to transform the Mtb-disA-KO strain (JHU-3588). Candidate Hyg^R colonies were selected and genomic DNA from the Mtb-COMP clones were used for PCR based confirmation using Hygromycin gene specific primers. (d) Comparison of the intra-bacterial levels of c-di-AMP extracted from 2X10⁸ cells of Mtb-disA-KO and Mtb-COMP at culture O.D. of 1 by LC-MS-MRM confirms the reconstitution of c-di-AMP production in the Mtb-COMP strain.

Figure 5



Supplementary Fig. 5. Interferon Regulatory Factor (IRF) pathway activation following infection with various *M. tuberculosis* strains with varied levels of c-di-AMP production. THP1-Dual™ cells (InvivoGen) were grown as per the suppliers recommendations. Twenty four hours after infection of PMA activated THP1-Dual™ cells with a pre-calibrated MOI of 1:10, culture supernatants were separated and used for measurement of IRF pathway activation by Quanti-Luc™ assay. Data are mean ± SE of at the least three experiments (n = 3); *, p < 0.05; ***, p < 0.001 by Student's t-test (2-tailed).



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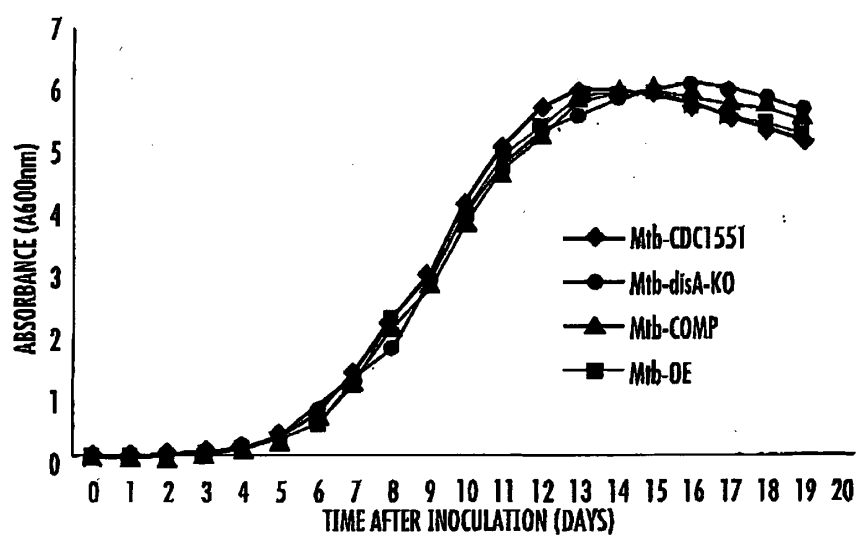


FIG. 7

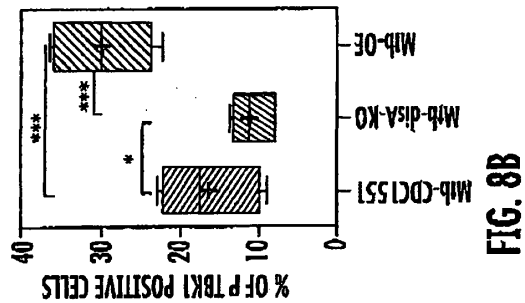


FIG. 8B

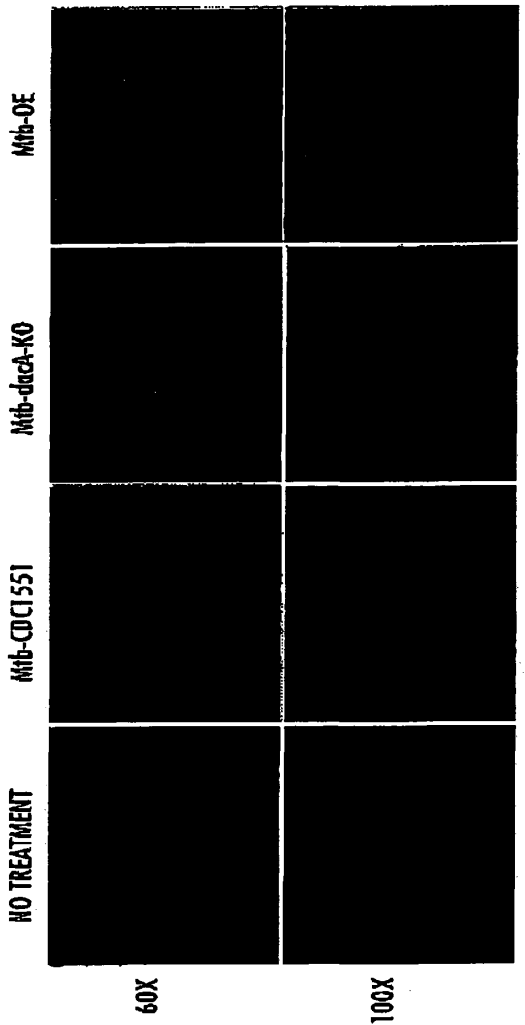
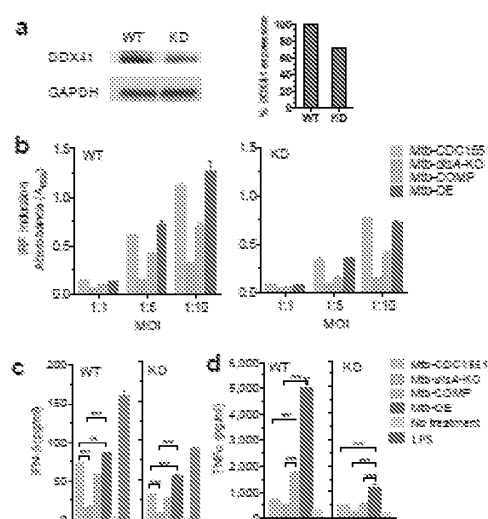


FIG. 8A

Figure 9



Supplementary Fig. 9. Knock down of DDX41 in macrophage leads to reduced induction of type I IFN and TNF- α response. (a) Western blot analysis of DDX41 and GAPDH (loading control) of DDX41 Knock down (KD) and control (WT) RAW-BlueTM ISG cells and densitometry analysis of the western blots showing ~30% reduced DDX41 protein level in the KD cells. Data are mean from duplicate experiments. (b) As an indicator of IRF induction, levels of SEAP in the supernatant of DDX41 Knock down (KD) and control (WT) RAW-BlueTM ISG cells at 18 hr after infection with various *M. tuberculosis* strains were measured by a spectrophotometer following Quanti-BlueTM assay. (c) Levels of IFN- β and (d) TNF- α in culture media of DDX41 Knock down (KD) and control (WT) RAW-BlueTM ISG cells at 18 hr after infection with various *M. tuberculosis* strains at MOI of 1:5 were measured by ELISA. Data are mean $\pm$ SE of at the least three experiments (n = 3). **, p < 0.05 and ***, p < 0.001 by Student's t-test (2-tailed).

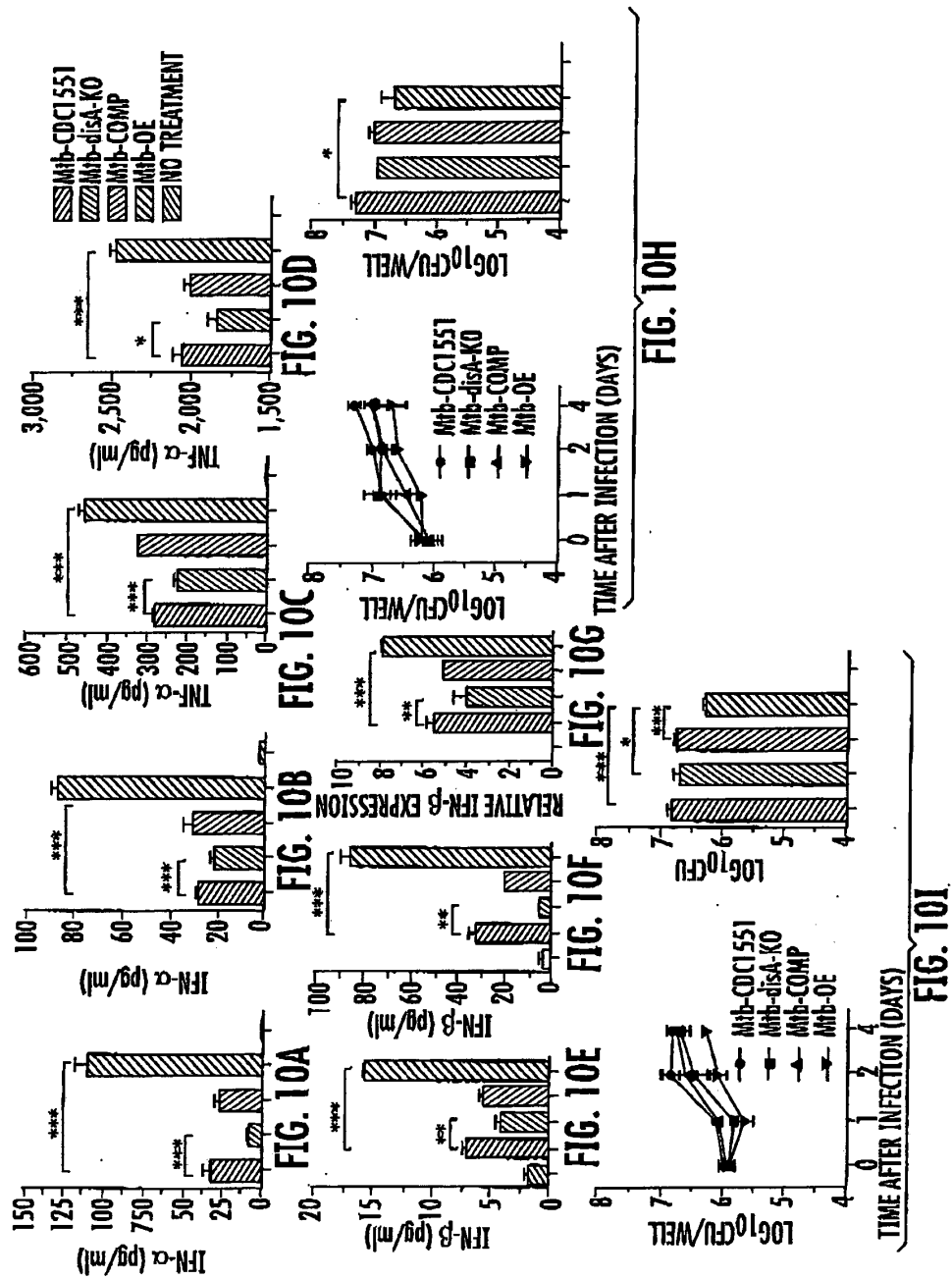


Figure 11. c-di-AMP produced by *M. tuberculosis* induces autophagy in macrophage cells.

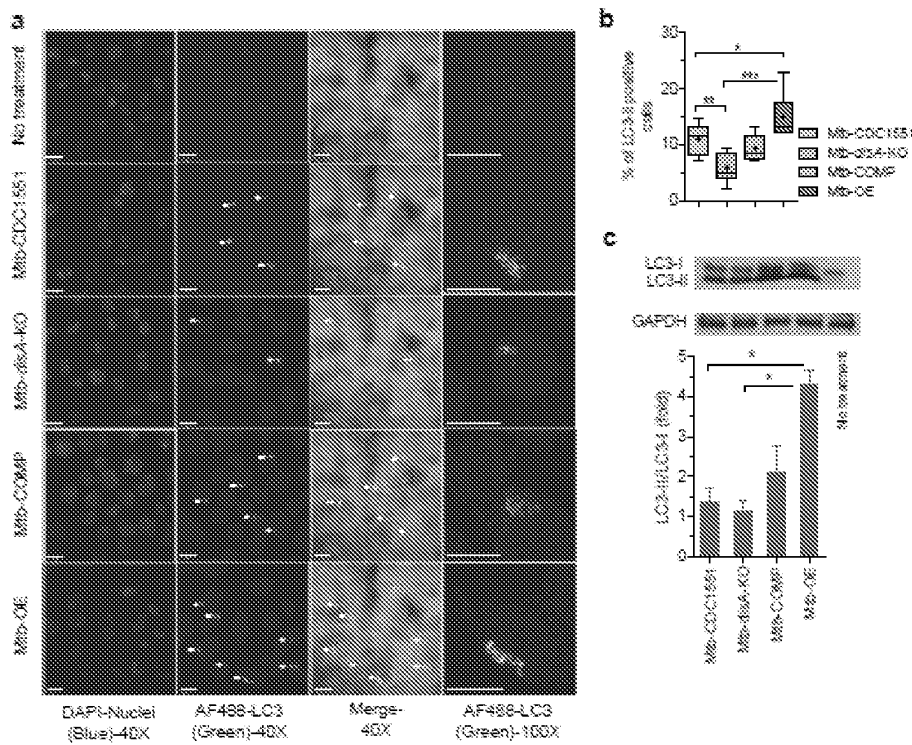
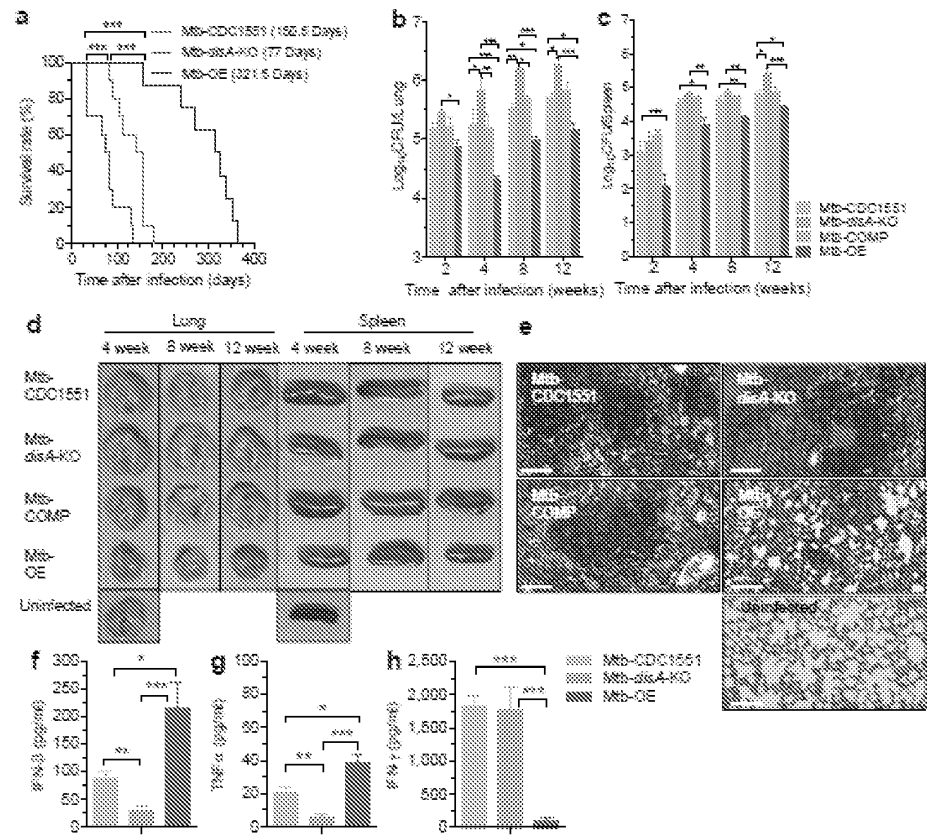
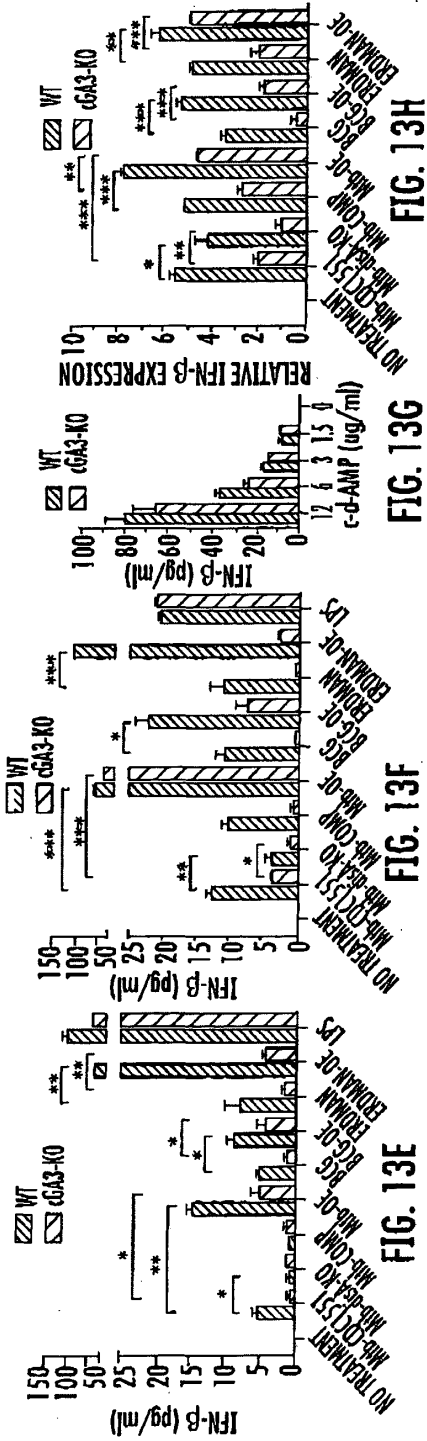
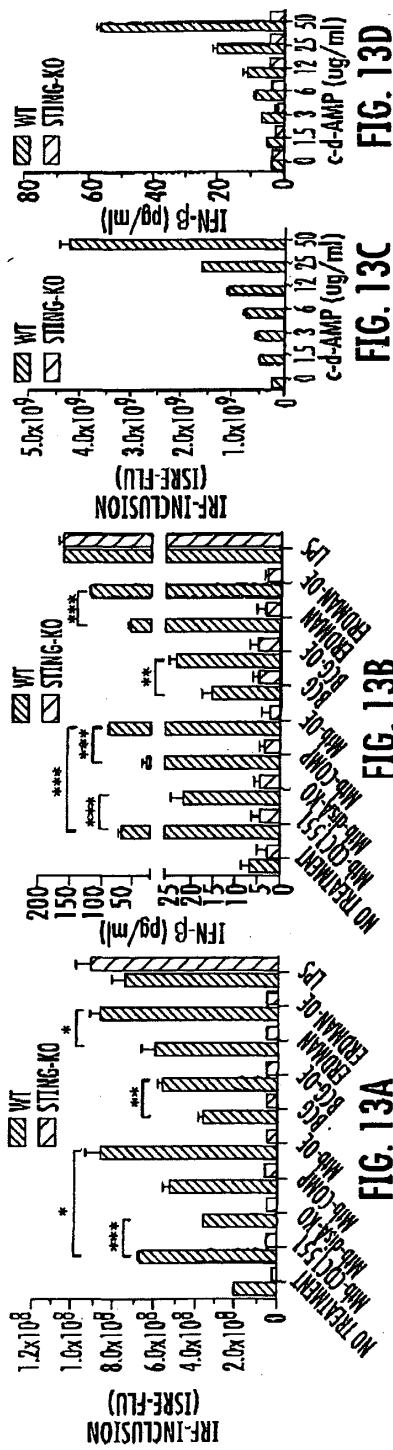


Figure 12. Attenuation of virulence and pathogenicity in c-di-AMP over-producing M. tuberculosis strain.





NAME	DESCRIPTION
PLASMIDS	
pSD5.hsp60	MYCOBACTERIAL EXPRESSION PLASMID WITH hsp60 PROMOTER
pMH94Hyg	INTEGRATIVE MYCOBACTERIAL EXPRESSION PLASMID WITHOUT PROMOTER
pSD5hsp60.MT3692	disA OVER-EXPRESSION PLASMID
pMH94Hyg.MT3692	PLASMID USED FOR COMPLEMENTATION OF Mtb-disA-KO
M. TUBERCULOSIS STRAINS	
Mtb-CDC1551	WILD TYPE M. TUBERCULOSIS
Mtb-disA-KO	M. TUBERCULOSIS TRANSPOSON MUTANT FOR disA GENE (MT3692)
Mtb-COMP	disA COMPLEMENTED Mtb-disA-KO STRAIN
Mtb-OE	M. TUBERCULOSIS CDC1551 STRAIN OVER EXPRESSING disA (MT3692)
ERDMAN	WILD TYPE M. TUBERCULOSIS ERDMAN STRAIN
ERDMAN-OE	M. TUBERCULOSIS ERDMAN STRAIN OVER EXPRESSING disA (MT3692)
M. BOVIS BCG STRAINS	
BCG	M. BOVIS BCG PASTEUR
BCG-OE	BCG STRAIN OVER EXPRESSING disA (MT3692)

FIG. 14

MICE EXPERIMENT

GROUPS	IMMUNIZATION (-42 DAY)	PRE-INFECTION- IMM_ASSAY (DAY-1)	INFECTION (DAY0)	CFU DAY1	CFU/IMM. ASSAY WEEK 10	CFU/IMM. ASSAY WEEK 18
SALINE	25	6	19	3	4	4
BCG	25	6	19	3	4	4
rBCG-dlsA	25	6	19	3	4	4
TOTAL	75	18	57	9	12	12

FIG. 15A

DAY 1 CFU

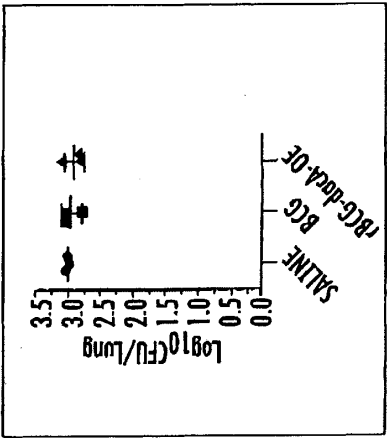
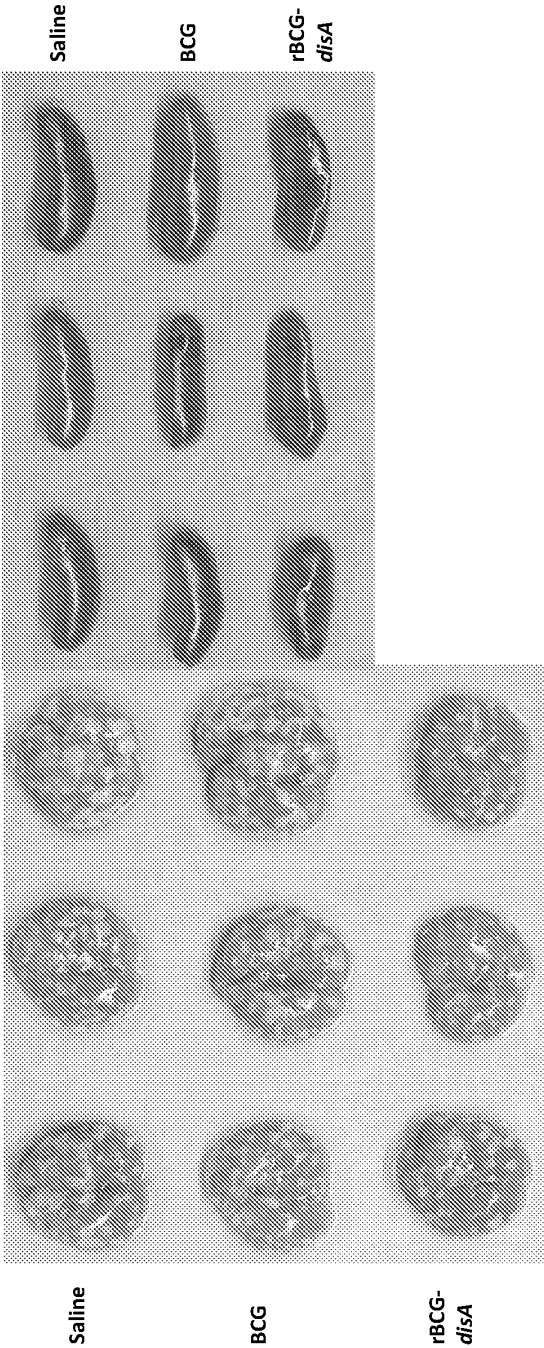


FIG. 15B

Figure 16: Gross pathology [18 week post-infection]



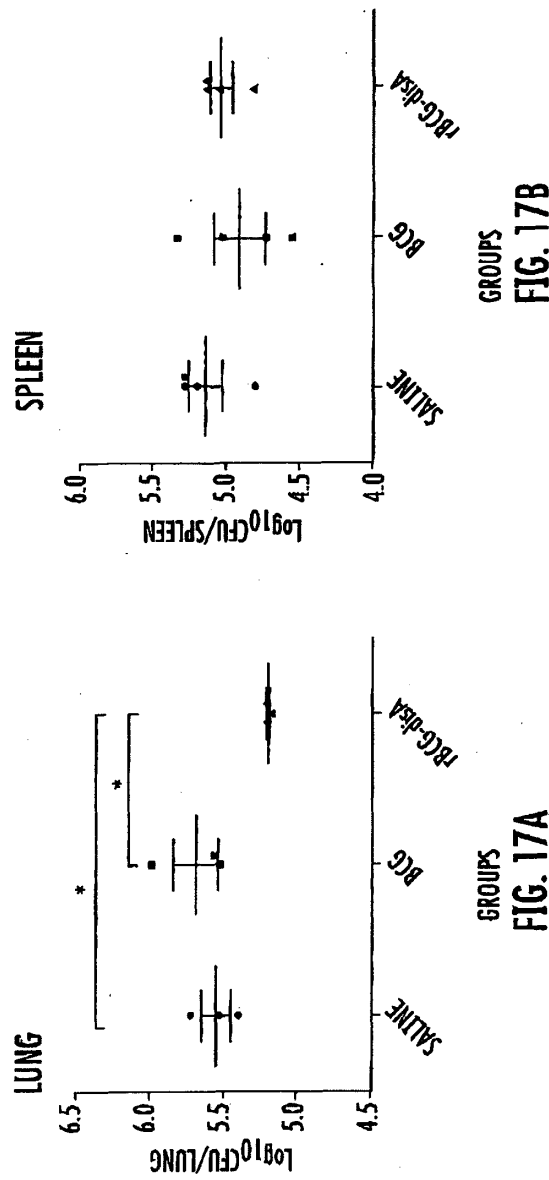


FIG. 17A
FIG. 17B
**p < 0.01; *p < 0.05; ONE WAY ANOVA FOLLOWED BY TURKEY MULTIPLE COMPARISON TEST

GUINEA PIG EXPERIMENT

GROUPS	IMMUNIZATION (-42 DAY)	INFECTION (DAY0)	CFU DAY1	CFU/HISTO/ IMM. ASSAY WEEK 14	CFU/HISTO/ IMM. ASSAY WEEK 18
SALINE	15	12	1	5	6
BCG	15	12	1	5	6
rBCG-dacA	15	12	1	5	6
TOTAL	45	36	3	15	18

FIG. 18A

DAY 1 CFU

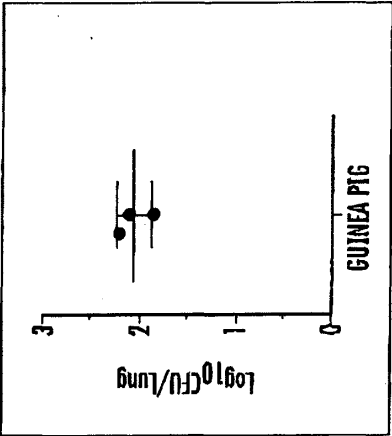
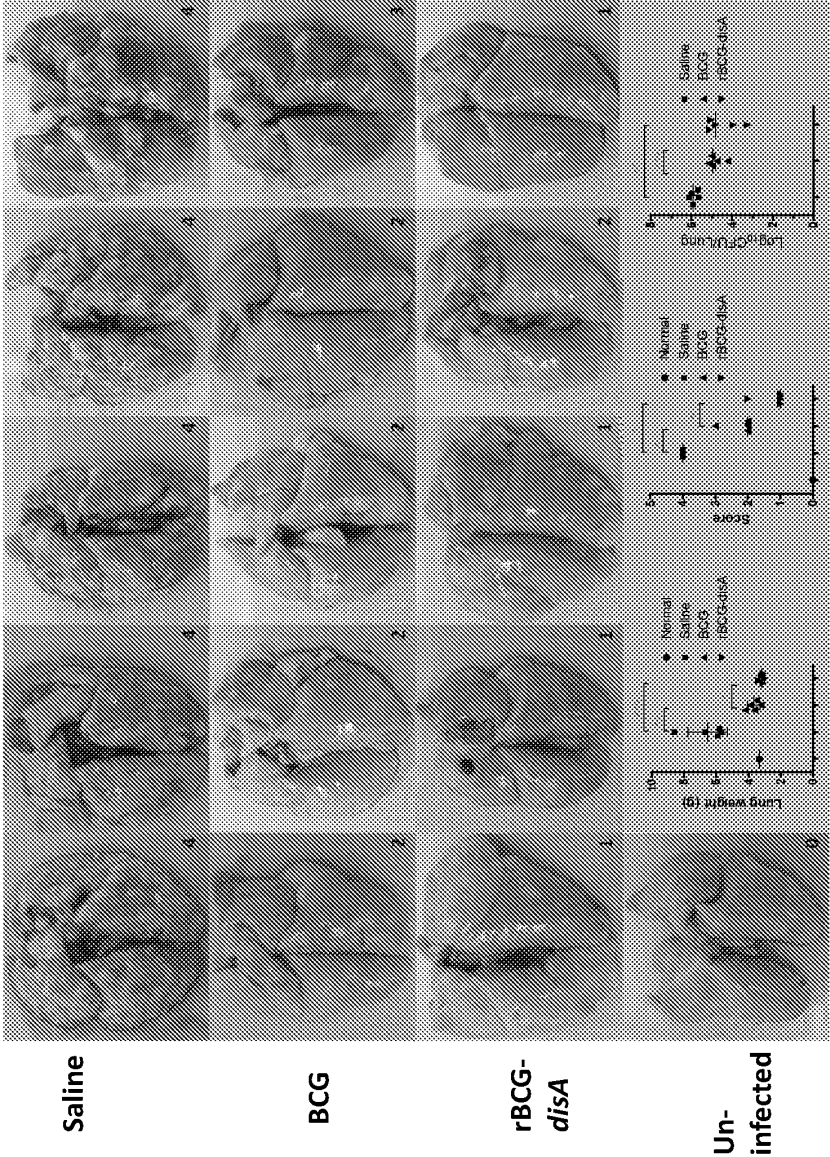


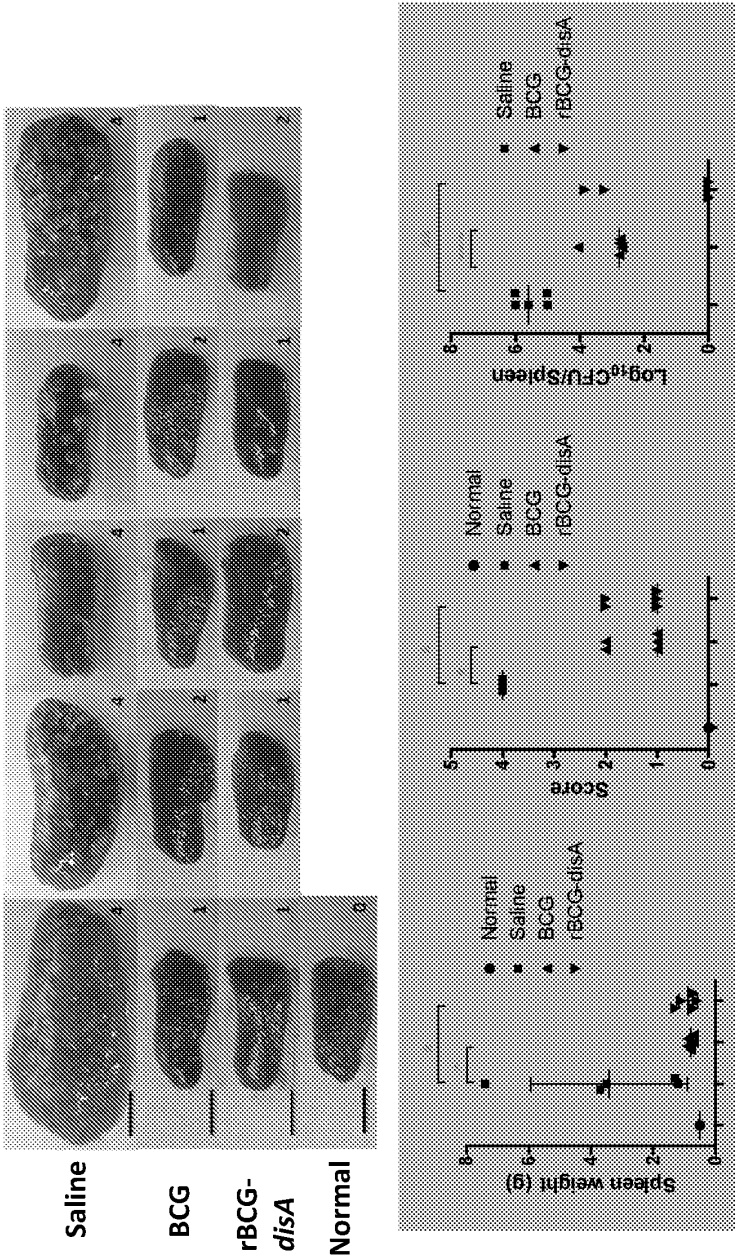
FIG. 18B

Figure 19: Gross pathology and CFU of Lungs [14 week post-infection]



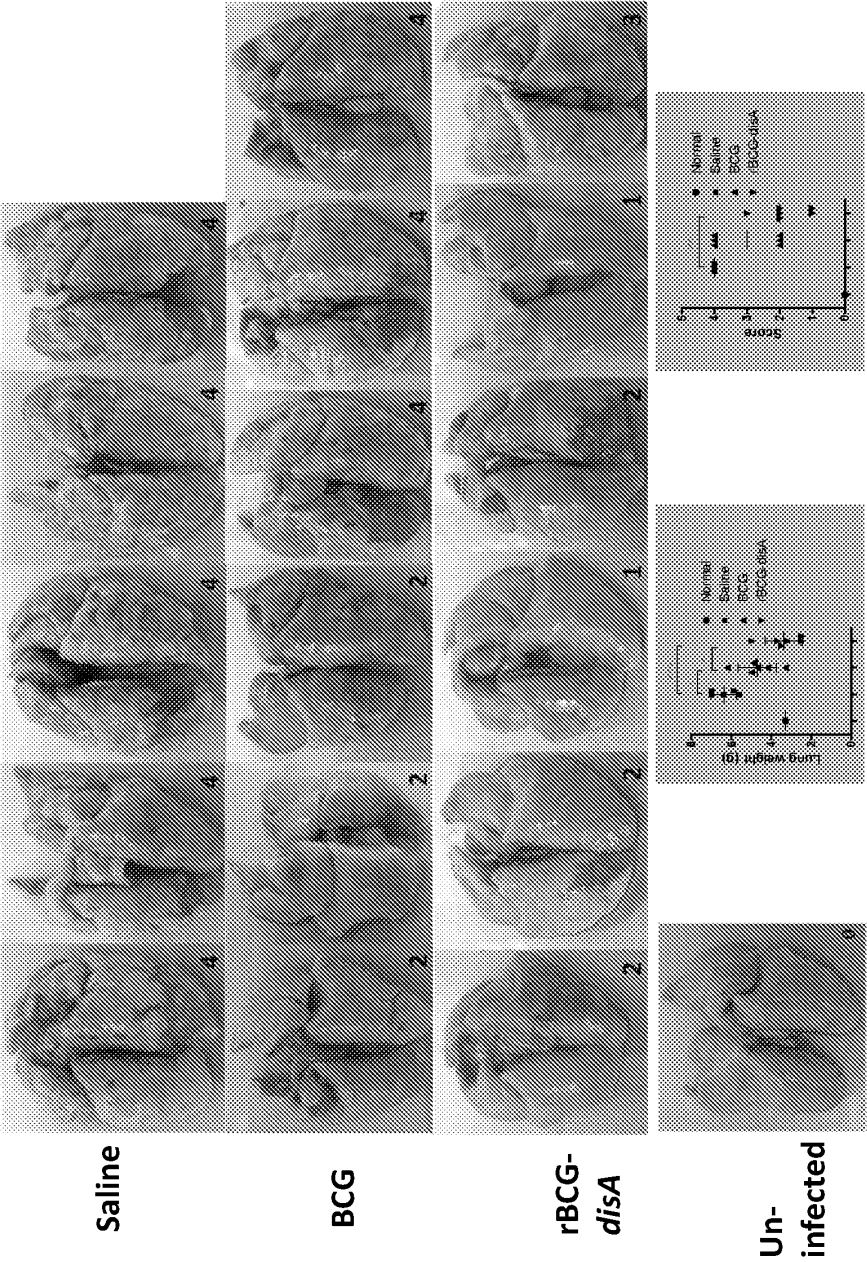
Number in the box is gross pathological score.

Figure 20: Gross pathology and CFU of Spleen [14 week post-infection]



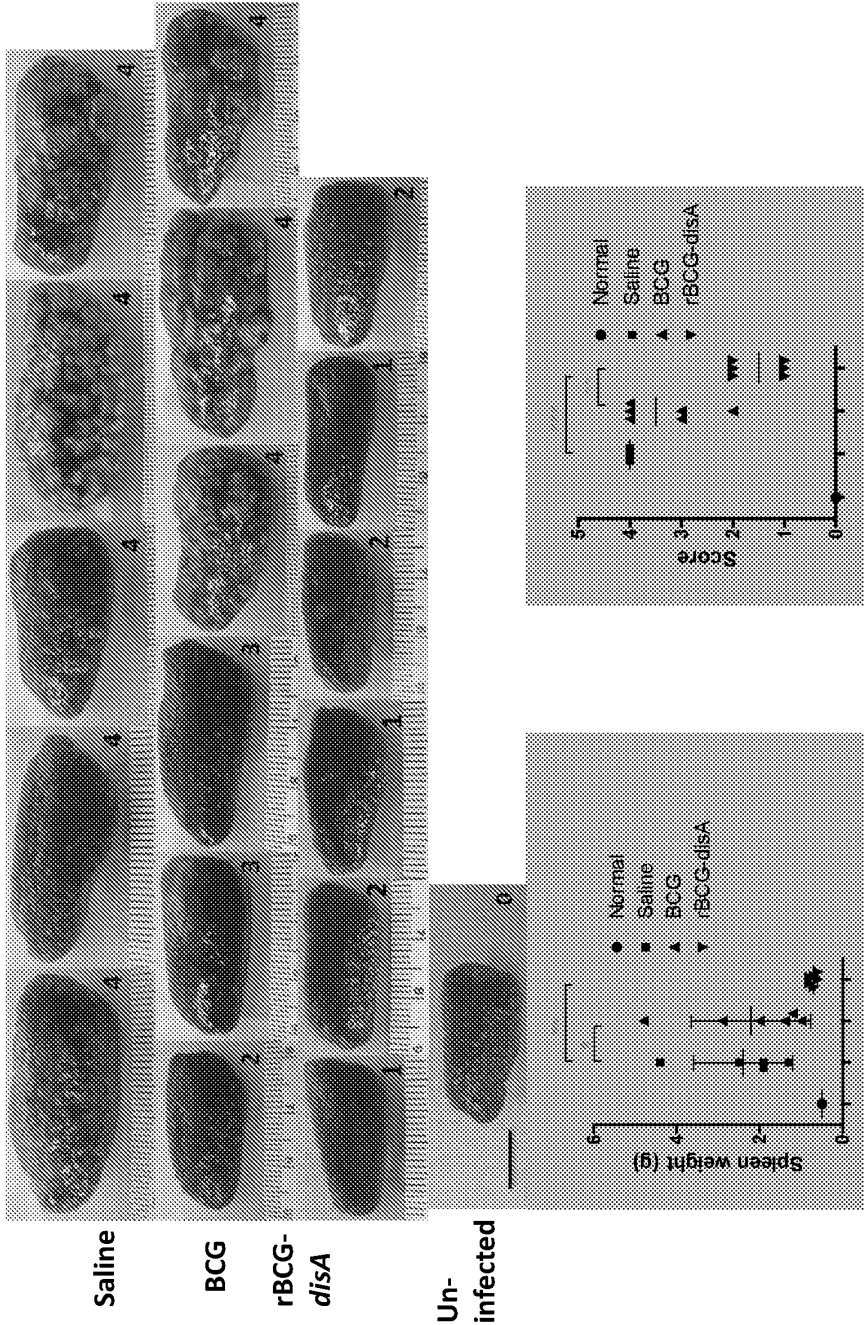
Scale bar = 1 cm, Number in the box is gross pathological score.

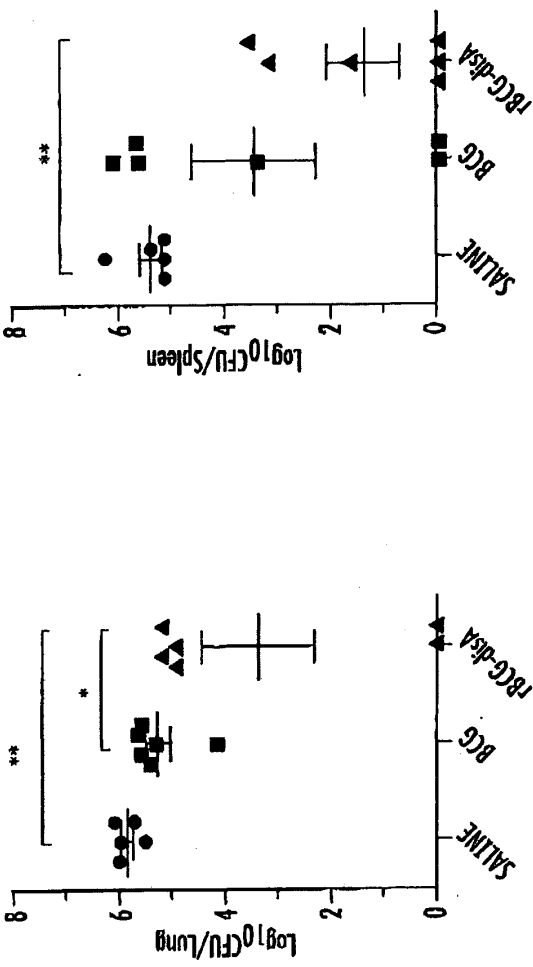
Figure 21: Gross pathology of Lungs [18 week post-infection]



Number in the box is gross pathological score.

Figure 22: Gross pathology of Spleen [18 week post-infection]





- TWO GUINEA PIGS IN rBCG-dsA GROUP HAD LUNG CFU BELOW THE LIMIT OF OUR DETECTION
- THREE GUINEA PIGS IN rBCG-dsA GROUP HAD SPLEEN CFU BELOW THE LIMIT OF OUR DETECTION
- **, p<0.01; *, p<0.05; NON-PARAMETRIC MANN WHITNEY TEST

FIG. 23