The anti-SARS-CoV-2 BNT162b2 vaccine suppresses mithramycin-induced erythroid differentiation and expression of embryo-fetal globin genes in human erythroleukemia K562 cells

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SUPPLEMENTARY MATERIALS

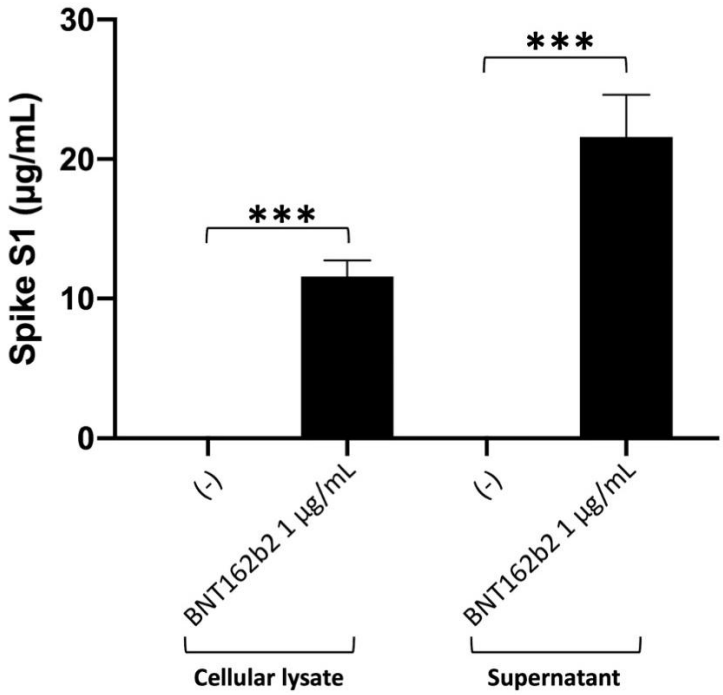

Figure S1. SARS-CoV-2 Spike protein is produced by K562 cells treated with the BNT162b2 vaccine and released in the culture medium. K562 cells were treated for 6 days with 1 µg/mL of the BNT162b2 vaccine, after 6 days of treatment the supernatants were collected and the cellular pellets lysed with water added of protease inhibitors (cat.n. 87786, Thermo Fisher, Waltham, Massachusetts, USA). The production of S-protein in K562 cells cultured in the absence or in the presence of BNT162b2 was assessed by ELISA assay (cat.n. KBVH015-10, KRISHGEN BioSystems, Mumbai, Maharashtra, India) employing cellular lysates (on the left) or supernatant (on the right). Briefly, cellular lysates or supernatant were diluted in the sample diluent and plated together with scalar concentrations of the standard (lyophilized Human SARS-CoV-2 spike protein S1) provided by the kit and following manufacturer's instruction. After 1h of incubation at 37°C shaking at 180 rpm, the plate was washed four times and then incubated with SARS-CoV-2 antibody HRP conjugate. After 1h of incubation at 37°C shaking at 180 rpm, the plate was washed four times and finally incubated for 30 minutes with TMB substrate solution. After the addition of the stop solution, the plate was analyzed using the SUNRISE microplate reader (Tecan Group, Männedorf, Switzerland) and reading the absorbance at 450 nm. Results are presented as mean ± S.E.M; statistical differences between groups were compared using paired t-test. (***): $p < 0.001$ (highly significant).

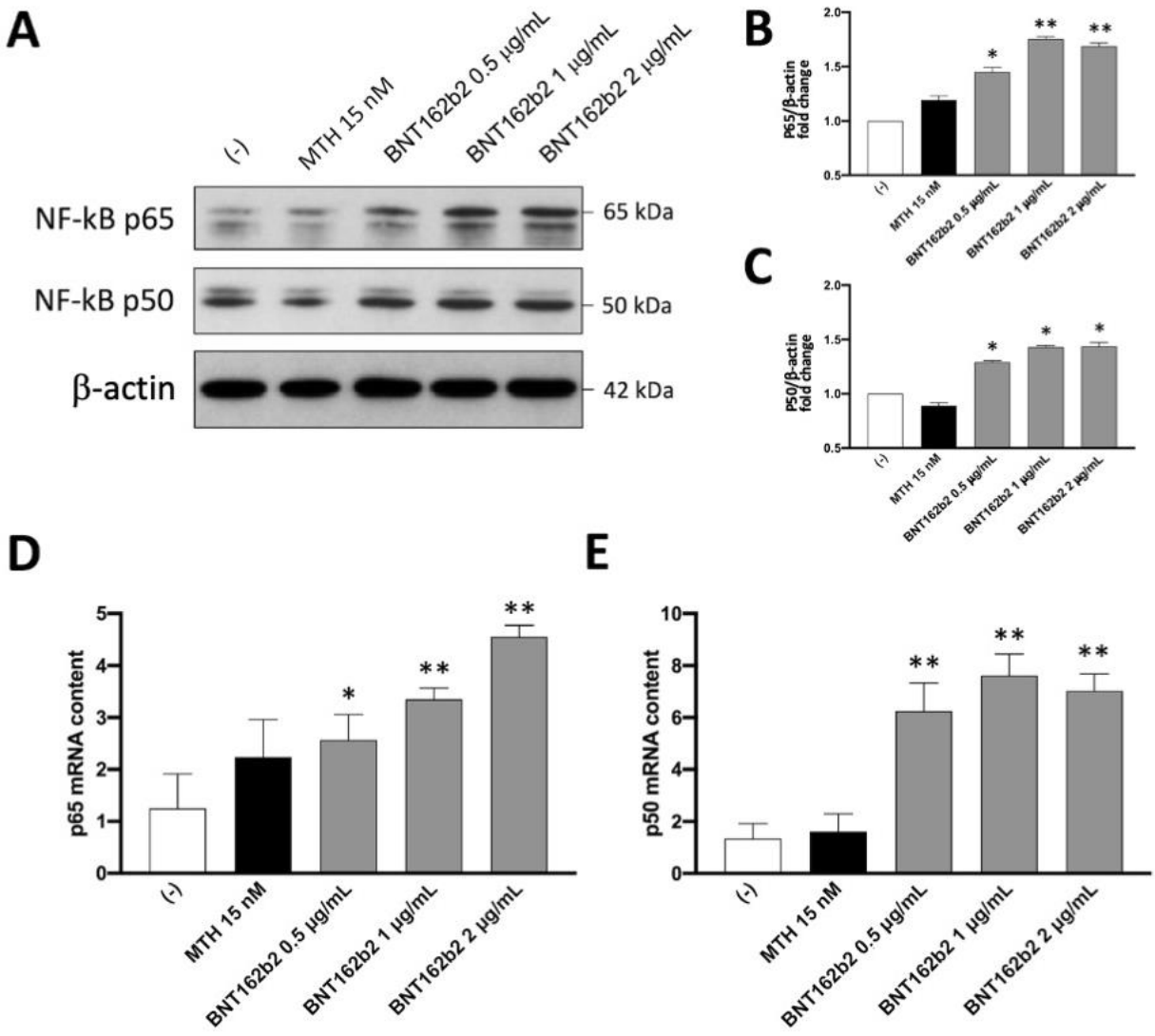

Figure S2. Expression of NF-kB protein in K562 cells treated with the BNT162b2 vaccine. K562 cells were treated for 6 days with 0.5, 1 and 2 µg/mL of the BNT162b2 vaccine. The accumulation of NF-kB p50 and p65 (approximately 50 and 65 kDa, respectively) in K562 cells cultured in the absence or in the presence of BNT162b2 was assessed by Western blotting. Technical details can be found in the legend to Figure S1. After separation by electrophoretic run, the proteins were transferred onto 0.2 µm nitrocellulose paper (Protran®, Cytiva™), and incubated with the primary antibodies against NF-kB p50 (GTX133711, GeneTex, Irvine, CA, USA) or NF-kB p65 (GTX102090, GeneTex, Irvine, CA, USA) (A); the constitutive protein β-actin (4967, Cell Signalling Technology, Danvers, MA, USA) was selected as housekeeping to normalize the quantification of the target proteins (B and C). Obtained data shows a good correlation also analyzing the mRNA content of p65 and p50 by RT-qPCR (D and E respectively); employed primers and probes are listed in Supplementary Table S1. Results are presented as mean ± S.E.M; statistical differences between groups were compared using ANOVA. (*): $p < 0.05$ (significant); (**): $p < 0.01$ (highly significant).

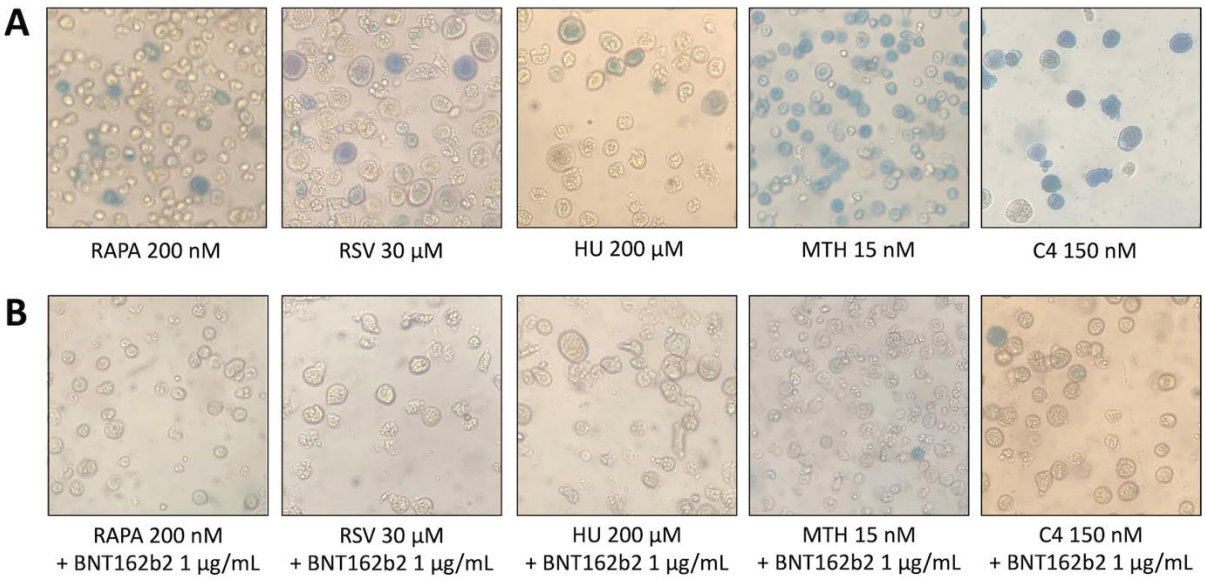

Figure S3. Effects of BNT162b2 vaccine on erythroid differentiation induced in K562 cells by different inducers: benzidine-staining. K562 cells were induced with 200 nM rapamycin (RAPA), 30 µM resveratrol (RSV), 200 µM hydroxyurea (HU), 150 nM isoxazole C4 in the absence (A) or in the presence (B) of 1 µg/mL BNR162b2, as indicated. Benzidine assay was performed after 5 days of treatment with hemoglobin inducers; 15 nM Mithramycin (MTH) was used as a positive control.

Table S1. List of primers and probes used for NF-kB p50 and p65 detection by RT-qPCR.

Primers and probes	**Sequences**
primer forward p50	5'-GGATCTGCACTGTAACTGCT-3'
primer reverse p50	5'-CTCTGTCATTCGTGCTTCCA-3'
probe p50	5'-FAM-TGTCACATGAAGTATACCCAGGTTTGCG-BFQ-3'
primer forward p65	5'-CGAGCTTGTAGGAAAGGACTG-3'
primer reverse p65	5'-TGACTGATAGCCTGCTCCAG-3'
Probe p65	5'-FAM-CGCTGCATCCACAGTTTCCAGAAC-BFQ-3'