Supplementary Figures and Legends

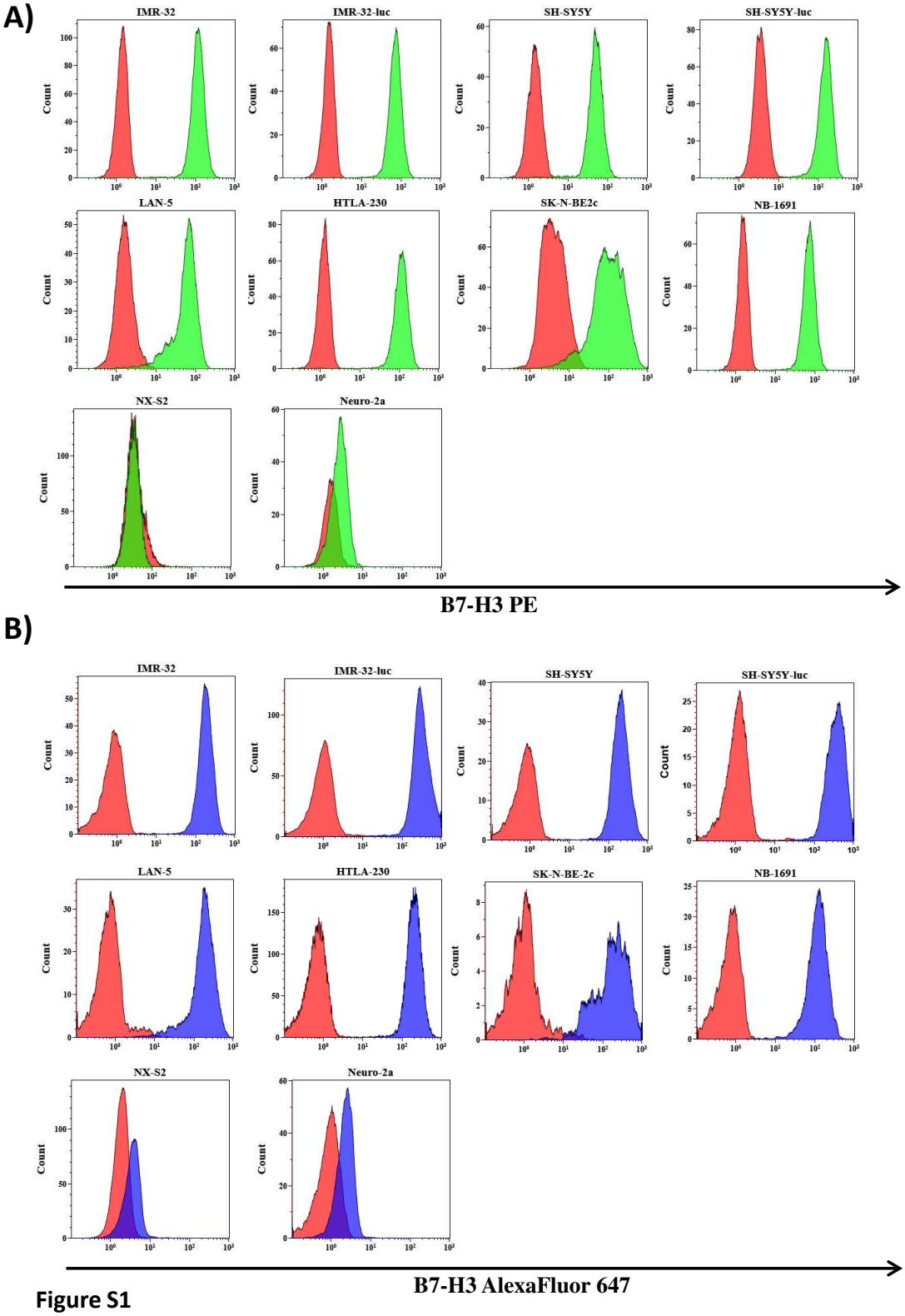


Figure S1

Figure S1: B7-H3 expression by human NB cell lines. A) Representative flow cytometry histograms of B7-H3 expression in human and murine NB cell lines. B7-H3 PE: PE- conjugated

anti-human B7-H3 mAb. Green: cells labeled with PE-conjugated mouse IgG1 anti-B7-H3 mAb. Red: cells labeled with isotype-matched mouse IgG1 control mAb. **B)** Representative flow cytometry histograms of B7-H3 expression in human and murine NB cell lines stained with anti-B7-H3 AlexaFluor 647-conjugated humanized anti-human B7-H3 mAb, MGA017. Blue: cells labeled with AlexaFluor 647-conjugated MGA017 humanized anti-B7-H3 mAb. Red: unstained cells.

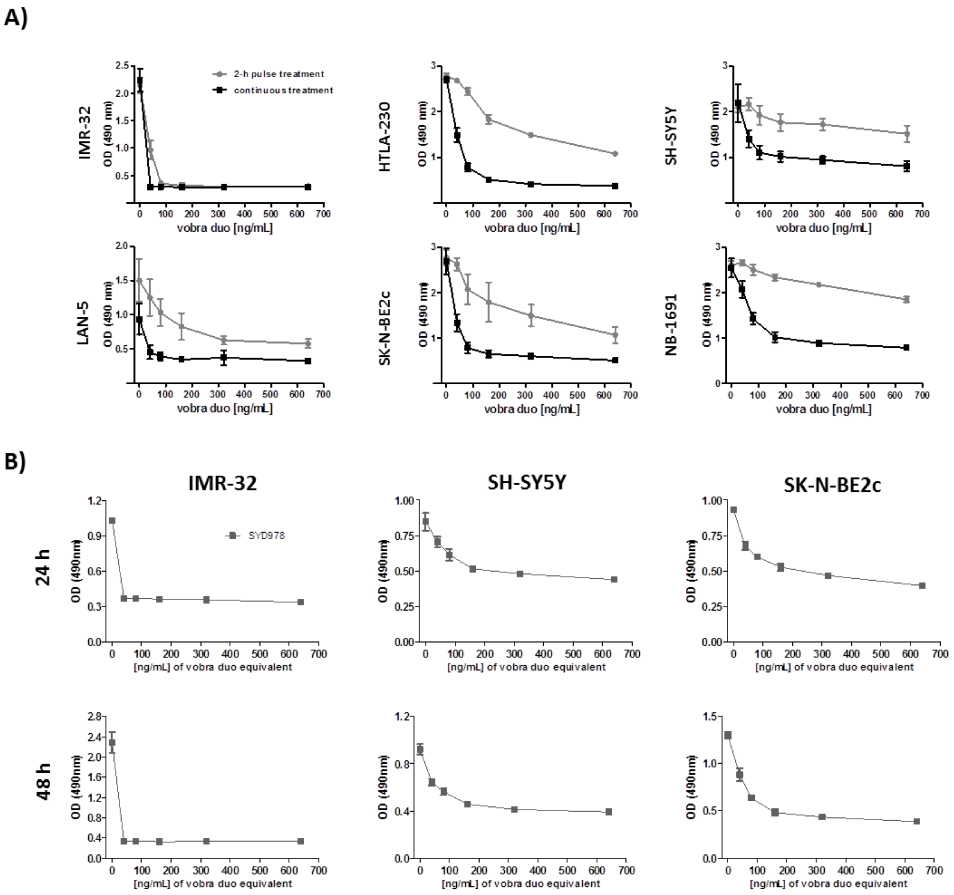


Figure S2

Figure S2: Cytotoxic activity of vobra duo against a panel of human NB cell lines cultured in monolayers. A) NB cell lines were treated with escalating concentrations of vobra duo (40-80-160-320-640 ng/mL) continuously for 5 days (black square) or for a 2h-pulse followed by a washing step and continued incubation (grey circles). **B)** IMR-32, SH-SY5Y, and SK-N-BE2c were treated with escalating concentration of the free payload, SYD978, for a 2h-pulse followed by a washing step and continuous incubation for additional 24 and 48 h. Viability was assessed by the MTS assay. Data are expressed as mean ± SD. OD: optical density.

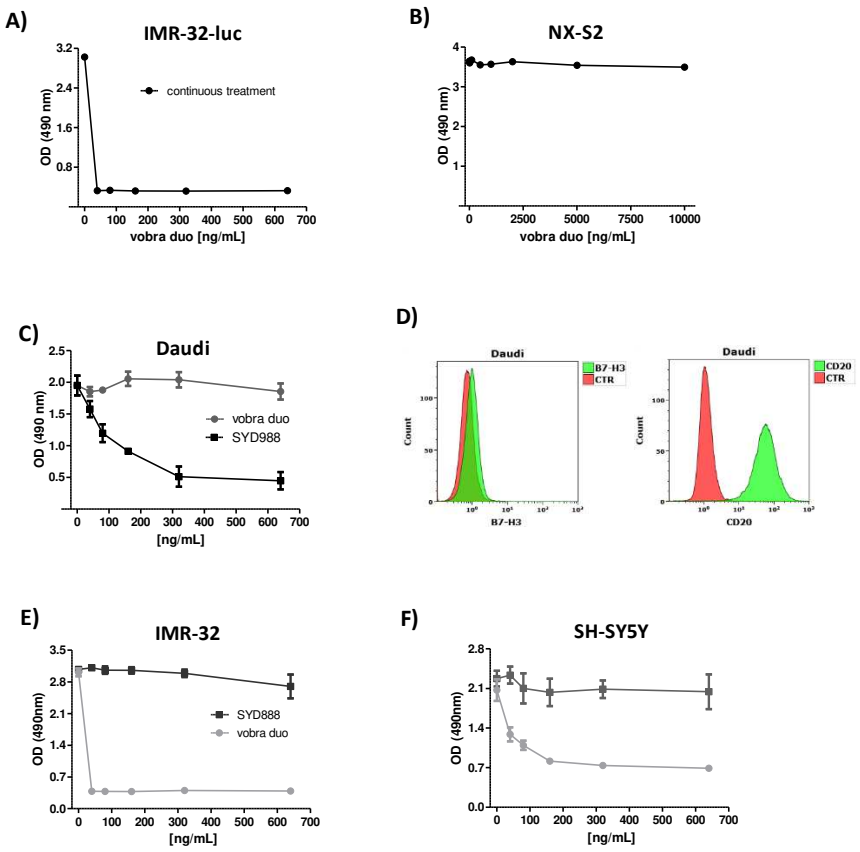


Figure S3

Figure S3: Cytotoxic activity of vobra duo against a stable luciferase-transduced NB cell line that expresses B7-H3 or cell lines not expressing B7-H3. A, B) Graphs showing the cell viability of IMR-32-luc and NX-S2 cells, respectively, after being exposed to vobra duo (A: 40-80-160-320-640 ng/mL; B: 10, 100, 500, 1000, 2000, 5000, 10000 ng/mL) for 7 days. Results of an MTS assay are shown, expressed as mean ± SD. **C)** Cell viability (MTS assay) of Daudi cells treated with increasing doses (40, 80, 160, 320, 640 ng/mL) of anti-CD20-ADC (SYD988, black closed square) or anti-B7-H3-ADC (vobra duo, grey closed circle). Data are expressed as mean ± SD. OD: optical density. **D)** Flow cytometry histograms representing the expression of B7-H3 and CD20 by Daudi cells. **E, F)** Graphs showing the cell viability of IMR-32 and SH-SY5Y cells after being exposed to dose escalation treatment (40, 80, 160, 320, 640 ng/mL) of anti-CD20-ADC (SYD988, black closed square) or anti-B7-H3-ADC (vobra duo, grey closed circle). Data are expressed as mean ± SD. OD: optical density.

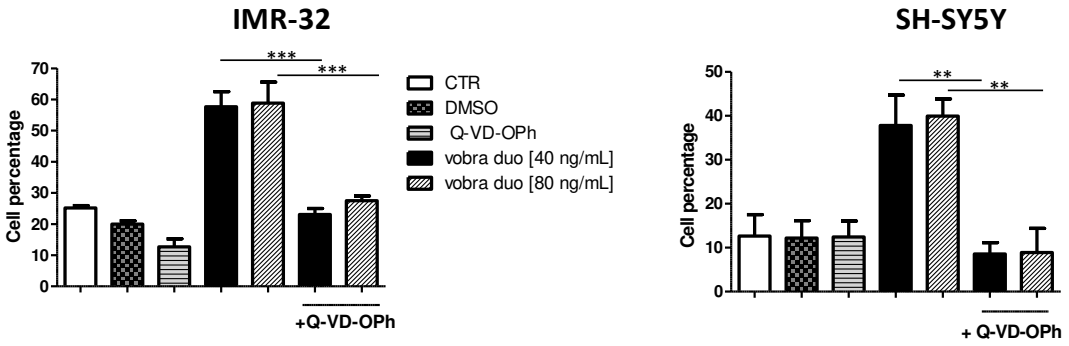


Figure S4

Figure S4: Effect of pre-treatment with the pan-caspase inhibitor, Q-VD-OPh, on vobra duo-induced apoptosis. Cells were preincubated with 30 µM of the pan-caspase inhibitor, Q-VD-OPh, for 30 min prior to exposure to vobra duo. Apoptosis was measured as indicated in M&M. Data are expressed as mean % apoptotic cells ± SD.

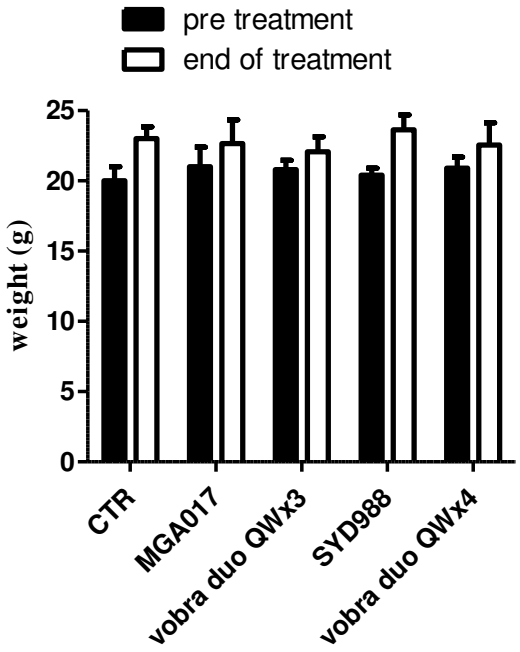


Figure S5

Fig. S5: Mice body weight pre and after the end of treatments. $1x10^6$ IMR-32-luc cells/10 µL medium cells were orthotopically inoculated in the left adrenal gland of mice. Treatments started 7 days post cell inoculation (n=8 mice/group) and were performed as reported in Results. Untreated, control mice (CTR) received PBS. Results are presented as mean ± SD.

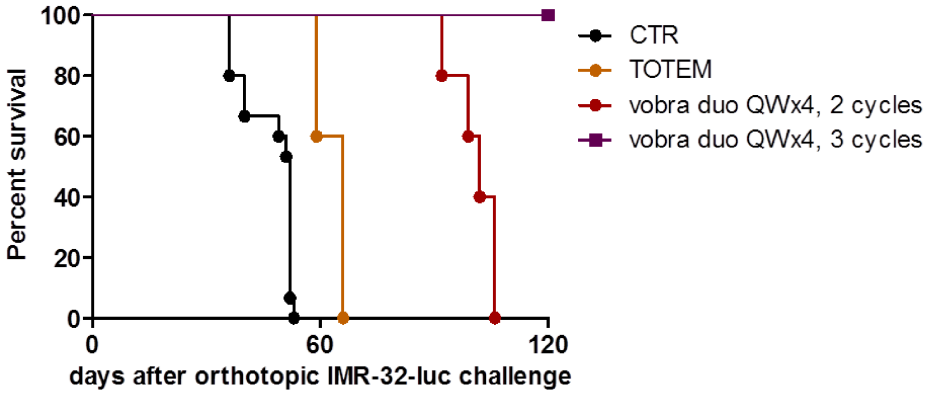
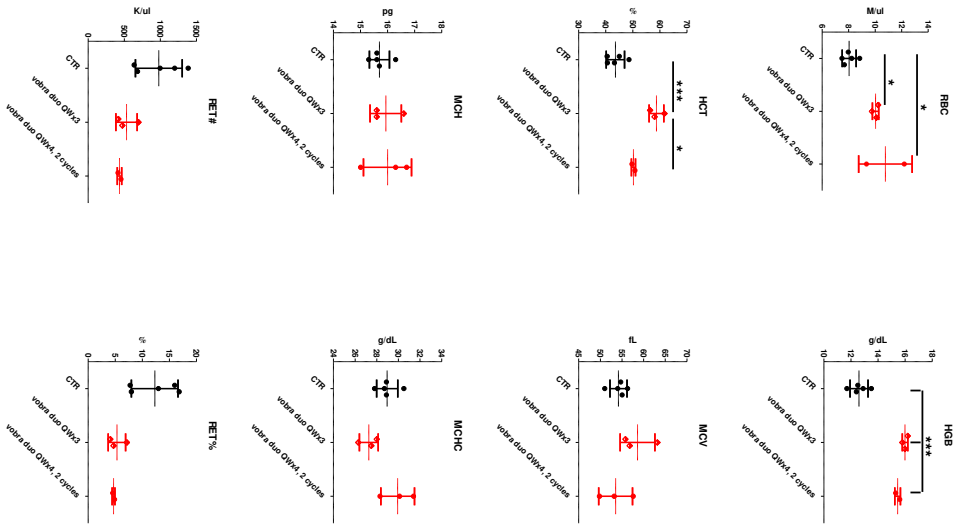


Figure S6

Fig. S6 Treatment of TOTEM and repeat cycles of vobra duo in an orthotopic model of NB. IMR-32-luc cells were orthotopically inoculated in the left adrenal gland of mice. Treatments (CTR, n=13 mice; TOTEM, vobra duo QWx4, 2 cycles and vobra duo QWx4, 3 cycles, n=5 mice/group) started 7 days post cell inoculation. Untreated, control mice (CTR) received PBS. Kaplan-Meier survival curves are shown.

Figure S7 A


RBC
M/ul
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%
MCV
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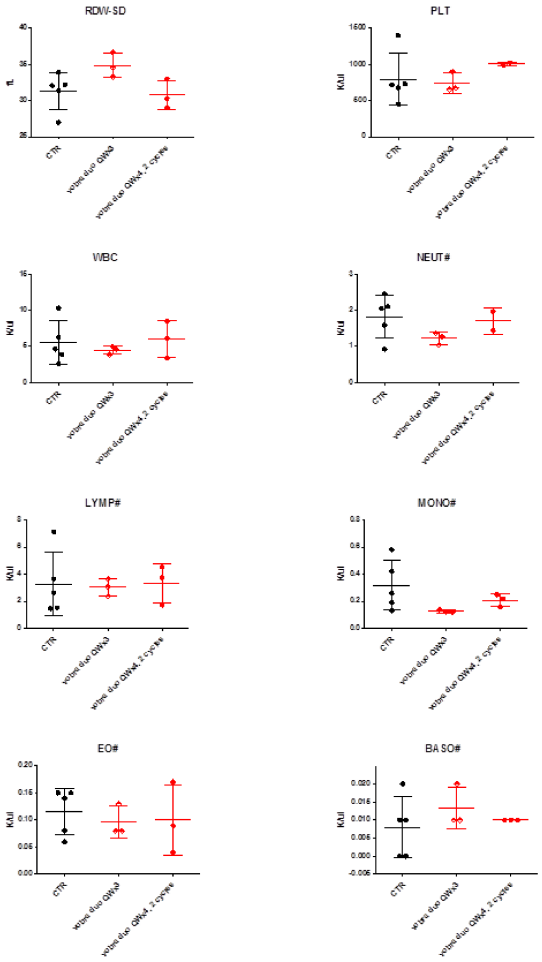

Figure S7 B

Fig. S7A-B: Clinical hematology analyses in IMR-32-luc-bearing mice treated with vobra duo. $1x10^6$ cells/10 µl medium were orthotopically injected in the left adrenal gland of mice (n = 3/group). i.v. treatments (T) started 7 days post cells inoculation. Mice were treated with 1 mg/kg vobra duo QWx3 and vobra duo QWx4, 2 cycles. Hematological levels of red blood cells (RBC), hematocrit (HCT), MCH, reticulocytes (RET), hemoglobin (HGB), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW-SD), white blood cells (WBC) and platelets (PLT) were quantified. Tests were performed 24h after vobra duo QWx3, and 24h after 2 cycles of vobra duo QWx4. * = p<0.05, *** = p<0.001.

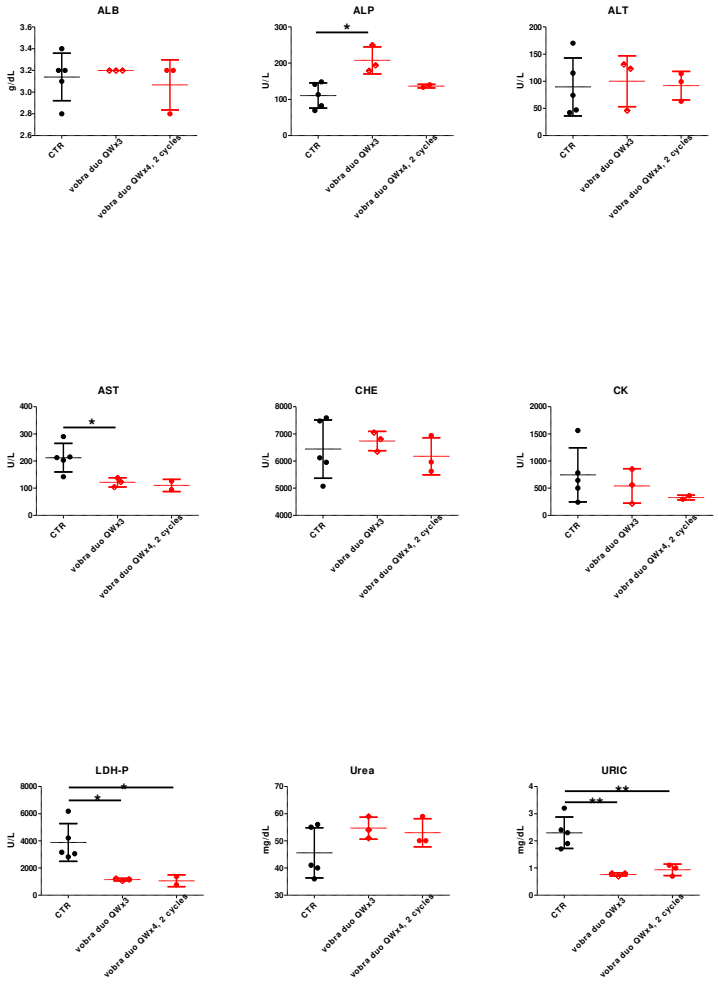

Figure S8

Fig. S8: Clinical chemistry analyses in IMR-32-luc-bearing mice treated with vobra duo. $1x10^6$ cells/10 µl medium were orthotopically injected in the left adrenal gland of mice (n = 3/group). i.v. treatments (Ts) started 7 days post cells inoculation. Mice were treated with 1 mg/kg vobra duo QWx3 and vobra duo QWx4, 2 cycles. Clinical chemistry levels of serum albumin (ALB), phosphatase alkaline (ALP), glutamic-pyruvic transaminase (ALT), glutamic oxaloacetic transaminase (AST), cholinesterase (CHE), creatine phosphokinase (CK), lactate dehydrogenase (LDH-P), urea and uric acid (URIC) were quantified. Tests were performed 24h after vobra duo QWx3, and 24h after 2 cycles of vobra duo QWx4. * = $p<0.05$, ** = $p<0.01$.