

In Vitro Assessment of Tick-Borne Encephalitis Vaccine: Suitable Human Cell Platforms and Potential Biomarkers

Supplementary Data

Tab. S1: Primer list for the genes assessed through qPCR

Gene	Forward	Reverse	Source
<i>IL-12p40</i>	CTGCCAGAGCAAGATGTGTC	CATTCTCCAGGGGCATCCG	Own design
<i>TNF-α</i>	ATGAGCACTGAAAGCATGATCC	GAGGGCTGATTAGAGAGAGGTC	Rajput et al. (2013)
<i>ISG56</i>	CCTGGAGTACTATGAGCGGGC	TGGGTGCCTAAGGACCTTGTC	Holzinger et al. (2007)
<i>MxA</i>	TTCAGCACCTGATGGCCTATC	TGGATGATCAAAGGGATGTGG	Holzinger et al. (2007)
<i>GAPDH</i>	AGGGCTGCTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA	Abubaker et al. (2013)

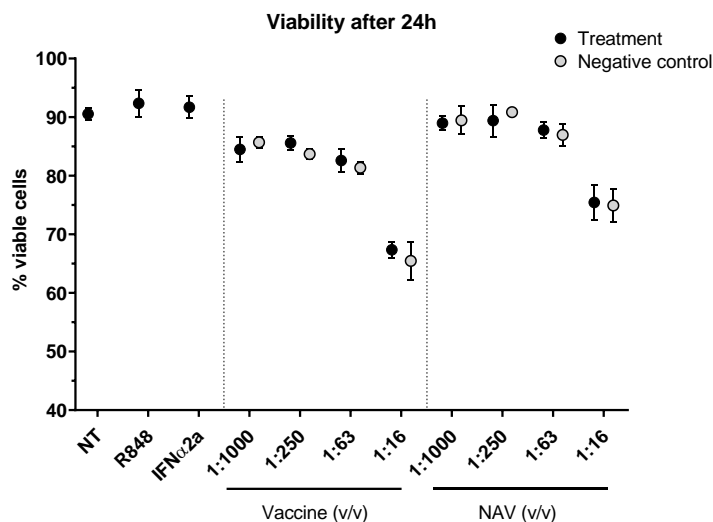


Fig. S1: Viability of vaccine-stimulated THP-1 cells

THP-1 cells were assessed for their viability following 24 h incubation with TBE vaccine or non-adjuvanted TBE vaccine (and their respective controls, excipient and matrix) by flow cytometry using a Viability Fixable Dye. Concentration of vaccine and NAV is indicated as the dilution factor (v/v) in the medium and corresponds to 3, 12, 48 and 192 ng/mL for the vaccine and to 0.06, 0.24, 1 and 4 μ g/mL for the NAV. Results represent the mean percentage of viable cells \pm SD of 4 independent experiments.

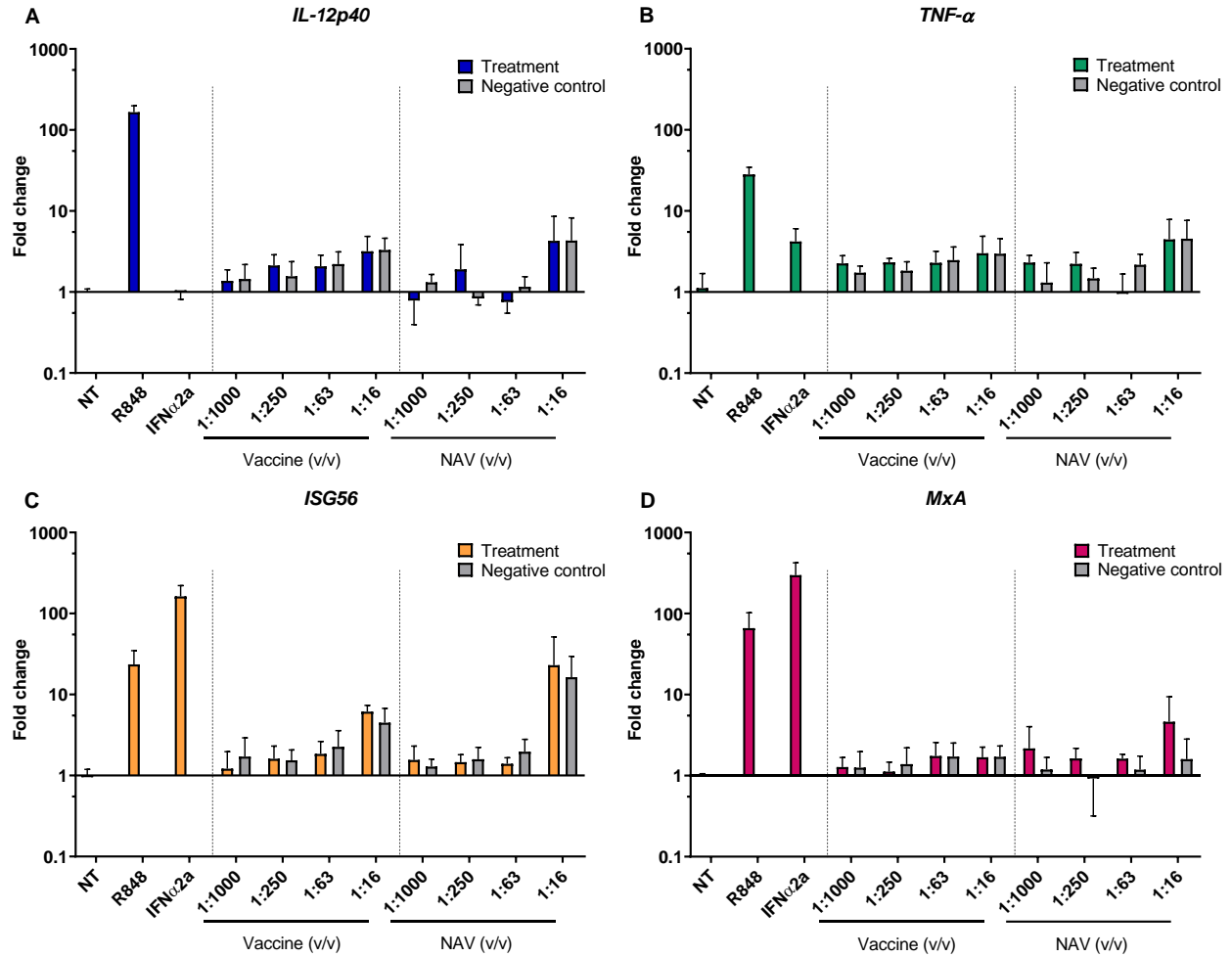


Fig. S2: Gene expression levels in THP-1 cells exposed to TBE final vaccine or non-adjuvanted vaccine (NAV)
 (A-D) THP-1 cells were exposed to the indicated stimuli for 24 h, and expression levels of 4 genes representative of inflammatory and antiviral responses (*IL-12p40*, *TNF- α* , *ISG56* and *MxA*) were analyzed by RT-qPCR. Concentration of vaccine and NAV is indicated as the dilution factor (v/v) in the medium and corresponds to 3, 12, 48 and 192 ng/mL for the vaccine and to 0.06, 0.24, 1 and 4 μ g/mL for the NAV. Bars represent the mean fold change in gene expression as compared to the non-treated (NT) control \pm SD of 4 independent experiments.

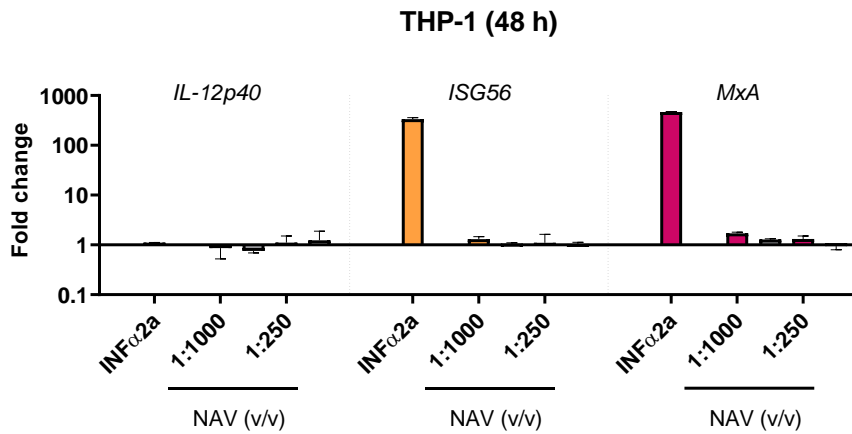


Fig. S3: Gene expression levels of THP-1 cells following 24 h stimulation

Expression levels of *IL-12p40*, *ISG56* and *MxA* were analyzed by RT-qPCR following 48 h incubation with the indicated stimuli. Concentrations of vaccine and NAV are indicated as the dilution factor (v/v) in the medium and correspond to 0.06 and 0.24 μ g/mL. Bars represent the mean fold change in gene expression as compared to the non-treated control \pm SD of 3 independent experiments. Pastel-colored bars represent the responses to positive controls and NAV, grey bars to the matrix.

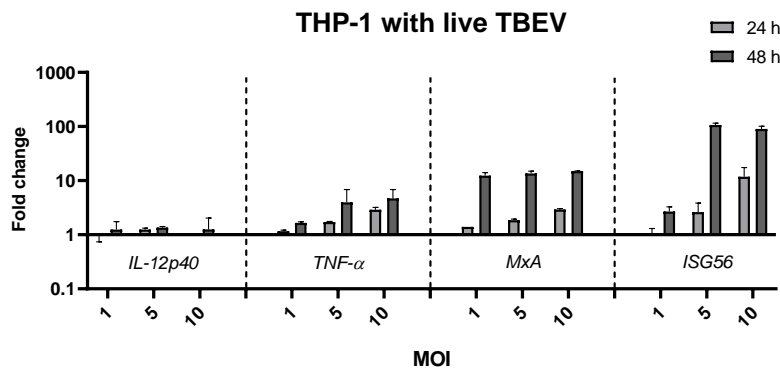


Fig. S4: Gene expression levels of THP-1 cells stimulated with live TBEV

Expression levels of *IL-12p40*, *TNF- α* , *MxA* and *ISG56* were analyzed by RT-qPCR following 24 and 48 h incubation with live TBEV at different multiplicities of infection (MOI). Bars represent the mean fold change in gene expression as compared to the non-treated control \pm SD of 3 independent experiments.

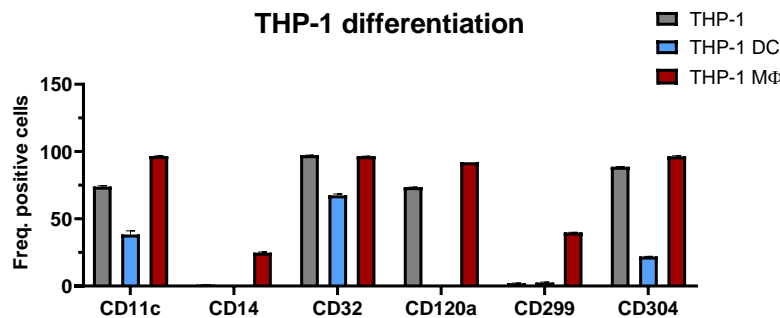


Fig. S5: Differentiation of THP-1 cells

THP-1 cells were differentiated to DC-like and M Φ -like cells. Differentiation was assessed via flow cytometry by analyzing the expression of the surface markers associated with the DC-or M Φ -like phenotype. The graph depicts the mean percentages of positive cells for the different markers \pm SD of 3 independent experiments.

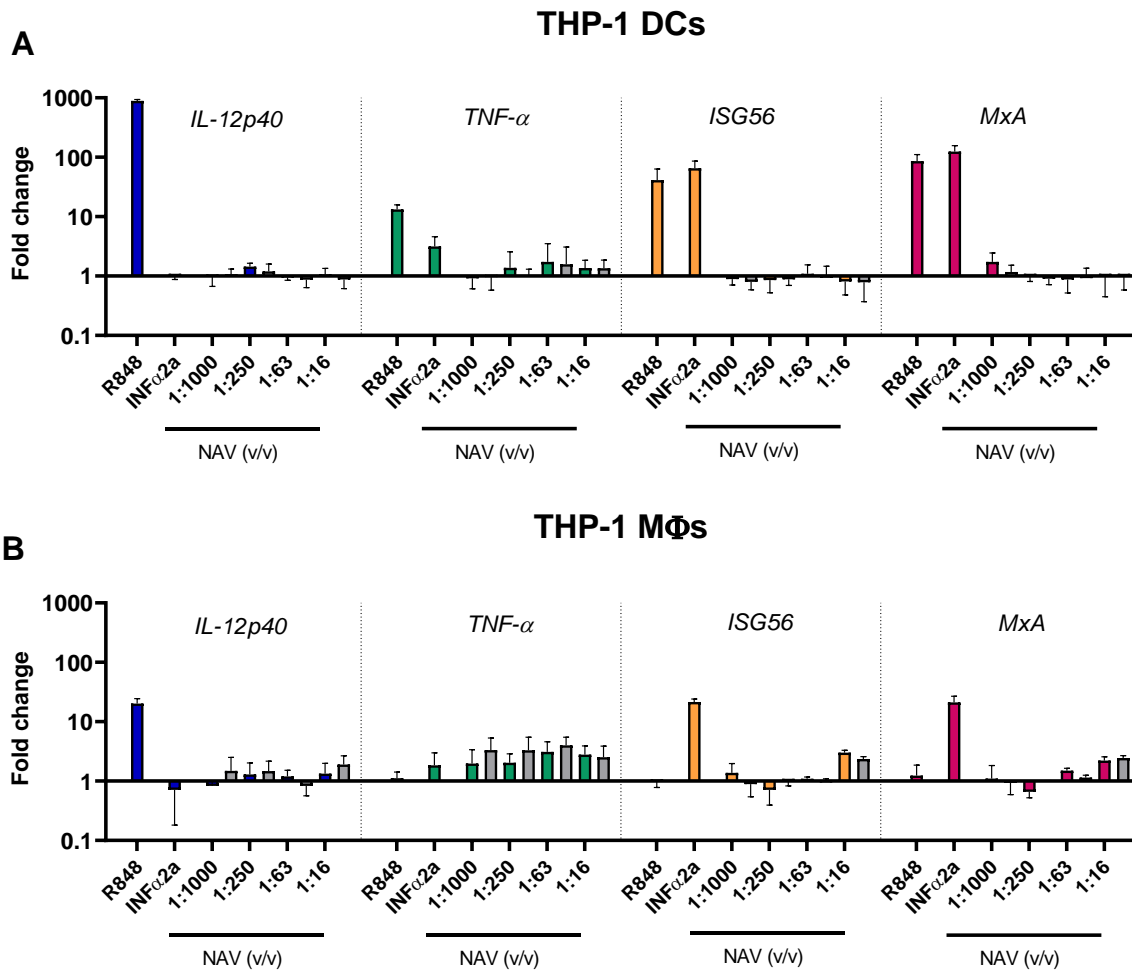


Fig. S6: Gene expression levels of NAV-stimulated dendritic-like and macrophage-like THP-1 cells
 (A, B). Differentiated THP-1 cells were exposed to the indicated stimuli for 24 h. Expression levels *IL-12p40*, *TNF- α* , *ISG56* and *MxA* were analyzed by RT-qPCR following 24 h incubation. Concentration of NAV is indicated as the dilution factor (v/v) in the medium, where 1:1000, 1:250, 1:63 and 1:16 correspond to 0.06, 0.24, 1 and 4 $\mu\text{g/mL}$. Bars represent the mean fold change in gene expression as compared to the non-treated control \pm SD of 3 independent experiments. Pastel-colored bars represent the responses to positive controls and NAV, grey bars to the matrix.

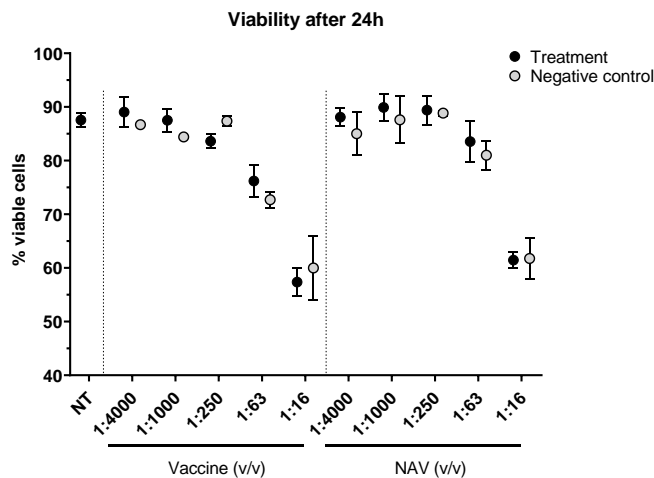


Fig. S7: Viability of TBE vaccine-stimulated human PBMCs
 Cells were assessed for their viability following 24 h incubation with TBE vaccine or NAV (and their respective negative controls, excipient and matrix) by flow cytometry. Concentration of vaccine and NAV is indicated as the dilution factor (v/v) in the medium and corresponds to 0.75, 3, 12, 48 and 192 ng/mL for the vaccine and to 0.015, 0.06, 0.24, 1 and 4 $\mu\text{g/mL}$ for the NAV. Results represent the mean percentage of viable cells \pm SD of 3 independent experiments.

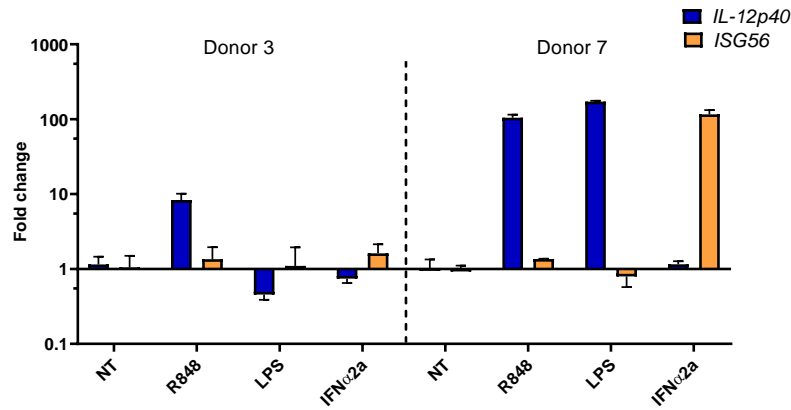


Fig. S8: Gene expression levels in PBMCs stimulated with positive controls PBMCs from a non-responder (donor 3) and a responder (donor 7) were analyzed for changes in expressions of *IL-12p40* and *ISG56* by RT-qPCR following 24 h incubation with the positive controls R848, LPS and IFNα2a. Bars represent the mean fold change in gene expression as compared to the non-treated (NT) control \pm SD of 3 independent experiments.

Tab. S2: Tukey's multiple comparisons test for assessing the sensitivity of the assay using *ISG56* as biomarker

Tukey's multiple comparisons test	Mean diff.	95% CI of diff.	Significance	Summary	Adjusted p value
NT vs. 0%	-2	-8.418 to 4.418	No	ns	0.9194
NT vs. 20%	-6.74	-13.16 to -0.3222	Yes	*	0.0374
NT vs. 40%	-12.5	-18.92 to -6.085	Yes	***	0.0003
NT vs. 60%	-16.43	-22.85 to -10.01	Yes	****	<0.0001
NT vs. 80%	-25.25	-31.67 to -18.83	Yes	****	<0.0001
NT vs. 100%	-32.03	-38.44 to -25.61	Yes	****	<0.0001
0% vs. 20%	-4.74	-11.16 to 1.678	No	ns	0.2127
0% vs. 40%	-10.5	-16.92 to -4.085	Yes	**	0.0014
0% vs. 60%	-14.43	-20.85 to -8.012	Yes	****	<0.0001
0% vs. 80%	-23.25	-29.67 to -16.83	Yes	****	<0.0001
0% vs. 100%	-30.03	-36.44 to -23.61	Yes	****	<0.0001
20% vs. 40%	-5.763	-12.18 to 0.6545	No	ns	0.0896
20% vs. 60%	-9.69	-16.11 to -3.272	Yes	**	0.0027
20% vs. 80%	-18.51	-24.93 to -12.09	Yes	****	<0.0001
20% vs. 100%	-25.29	-31.70 to -18.87	Yes	****	<0.0001
40% vs. 60%	-3.927	-10.34 to 2.491	No	ns	0.3891
40% vs. 80%	-12.75	-19.16 to -6.329	Yes	***	0.0002
40% vs. 100%	-19.52	-25.94 to -13.11	Yes	****	<0.0001
60% vs. 80%	-8.82	-15.24 to -2.402	Yes	**	0.0057
60% vs. 100%	-15.6	-22.01 to -9.179	Yes	****	<0.0001
80% vs. 100%	-6.777	-13.19 to -0.3588	Yes	*	0.0361

Tab. S3: Tukey's multiple comparisons test for assessing the sensitivity of the assay using CXCL10 as biomarker

Tukey's multiple comparisons test	Mean diff.	95% CI of diff.	Significance	Summary	Adjusted p value
NT vs. 0%	-7.617	-38.01 to 22.78	No	ns	0.9696
NT vs. 20%	-31.10	-61.49 to -0.7025	Yes	*	0.0437
NT vs. 40%	-55.08	-85.48 to -24.69	Yes	***	0.0005
NT vs. 60%	-87.05	-117.4 to -56.66	Yes	****	<0.0001
NT vs. 80%	-129.8	-160.2 to -99.42	Yes	****	<0.0001
NT vs. 100%	-184.9	-215.3 to -154.5	Yes	****	<0.0001
0% vs. 20%	-23.48	-53.87 to 6.914	No	ns	0.1782
0% vs. 40%	-47.47	-77.86 to -17.07	Yes	**	0.0020
0% vs. 60%	-79.44	-109.8 to -49.04	Yes	****	<0.0001
0% vs. 80%	-122.2	-152.6 to -91.81	Yes	****	<0.0001
0% vs. 100%	-177.3	-207.6 to -146.9	Yes	****	<0.0001
20% vs. 40%	-23.99	-54.38 to 6.408	No	ns	0.1632
20% vs. 60%	-55.96	-86.35 to -25.56	Yes	***	0.0005
20% vs. 80%	-98.72	-129.1 to -68.33	Yes	****	<0.0001
20% vs. 100%	-153.8	-184.2 to -123.4	Yes	****	<0.0001
40% vs. 60%	-31.97	-62.36 to -1.576	Yes	*	0.0370
40% vs. 80%	-74.73	-105.1 to -44.34	Yes	****	<0.0001
40% vs. 100%	-129.8	-160.2 to -99.39	Yes	****	<0.0001
60% vs. 80%	-42.76	-73.16 to -12.37	Yes	**	0.0048
60% vs. 100%	-97.82	-128.2 to -67.42	Yes	****	<0.0001
80% vs. 100%	-55.05	-85.45 to -24.66	Yes	***	0.0005

References

Abubaker, J., Tiss, A., Abu-Farha, M. et al. (2013). DNAJB3/HSP-40 cochaperone is downregulated in obese humans and is restored by physical exercise. *PLoS One* 8, e69217. doi:10.1371/journal.pone.0069217

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