

## Supplementary Material

### **A Bivalent Trans-Amplifying RNA Vaccine Candidate Induces Potent Chikungunya and Ross River Virus Specific Immune Responses**

**Christin Schmidt <sup>1</sup>, Florian D. Hastert <sup>1</sup>, Julia Gerbeth <sup>1</sup>, Tim Beissert <sup>2</sup>, Ugur Sahin <sup>2,3</sup>, Mario Perkovic <sup>2,\*</sup> and Barbara S. Schnierle <sup>1,\*</sup>**

<sup>1</sup> Department of Virology, Paul-Ehrlich-Institut, Paul-Ehrlich Strasse 51-59, 63225 Langen, Germany;

christin.schmidt@pei.de (C.S.); florian.hastert@pei.de (F.H.); julia.gerbeth@pei.de (J.G.)

<sup>2</sup> TRON (Translational Oncology at the University Medical Center), Johannes Gutenberg University Mainz, Freiligrathstraße 12, 55131 Mainz, Germany; tim.beissert@tron-mainz.de (T.B.);

ugur.sahin@tron-mainz.de (U.S.)

<sup>3</sup> Research Center for Immunotherapy (FZI), University Medical Center at the Johannes Gutenberg University, Langenbeckstr. 1, 55131 Mainz, Germany

\* Correspondence: mario.perkovic@tron-mainz.de (M.P.); barbara.schnierle@pei.de (B.S.S.)

TR-R

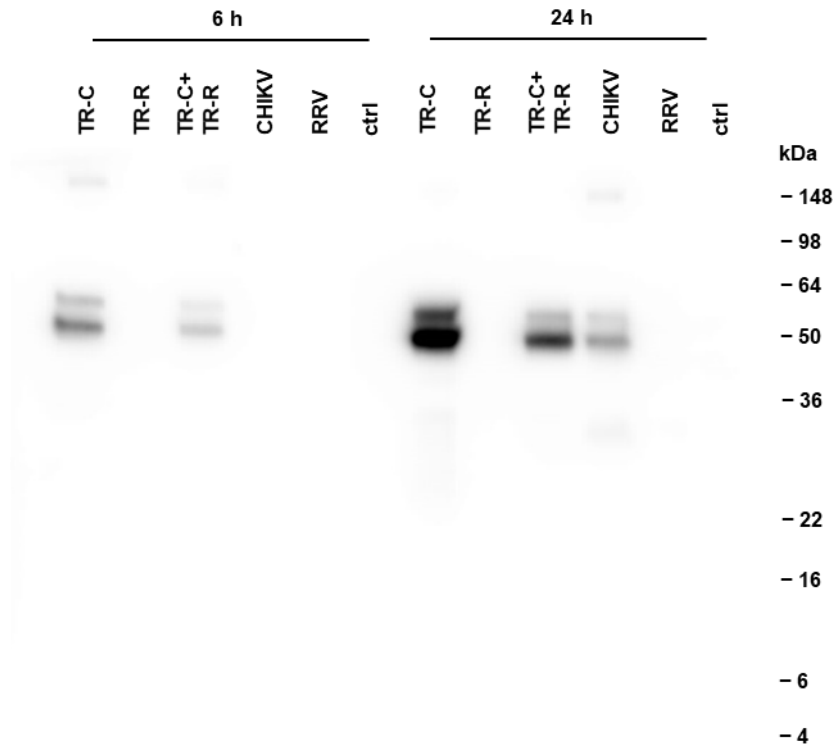
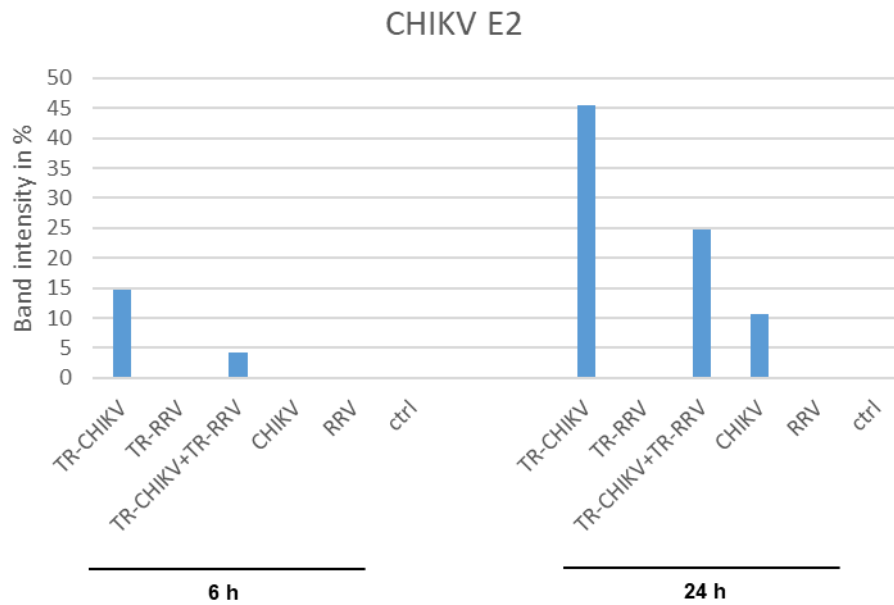
ATGGCTGCGTGAGACACACGTAGCCTACCAGTTTCTTACTGCTCTACTCTGCAAAGCAAG  
AGATTAATAACCCATCATGGATCCTGTGTACGTGGACATAGACGCTGACAGCGCCTTTTT  
GAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGTGGAACCAAGGCAGGTCACACCGAA  
TGACCATGCTAATGCTAGAGCGTTCTCGCATCTAGCTATAAACTAATAGAGCAGGAAAT  
TGACCCCGACTCAACCATCCTGGATATCGGCAGTGCGCCAGCAAGGAGGATGATGTCGGA  
CAGGAAGTAACACCGGTCCAGGTCATTTACAACAGAAGTCAGTACGCCAGTCAGTGCTGC  
CGGTGAACACCCTGGAGGAAGTCCACGAGGAGAAGTGTTACCCACCTAAGCTGGATGAAG  
CAAAGGAGCAACTATTACTTAAGAACTCCAGGAGAGTGCATCCATGGCCAACAGAAGCA  
GGTATCAGTCGCGCAAAGTAGAAAACATGAAAGCAGCAATCATCCAGAGACTAAAGAGAG  
GCTGTAGACTATACTTAATGTCAGAGACCCCCAAAAGTCCCTACTTACCGGACTACATATC  
CGGCGCCTGTGTACTCGCCTCCGATCAACGTCCGATTGTCCAATCCCGAGTCCGCAGTGG  
CAGCATGCAATGAGTTCTTAGCTAGAACTATCCAAGTGTCTCATCATACCAAATTACCG  
ACGAGTATGATGCATATCTAGACATGGTGGACGGGTCGGAGAGTTGCCTGGACCGAGCGA  
CATTCAATCCGTCAAACTCAGGAGCTACCCGAAACAGCACGCTTACCACGCGCCCTCCA  
TCAGAAGCGCTGTACCGTCCCCATTCCAGAACACACTACAGAATGTACTGGCAGCAGCCA  
CGAAAAGAACTGCAACGTCACACAGATGAGGGAATTACCCACTTTGGACTCAGCAGTAT  
TCAACGTGGAGTGTTTCAAAAAATTCGCATGCAACCAAGAATACTGGGAAGAATTTGCTG  
CCAGCCCTATTAGGATAACAACTGAGAATTTAGCAACCTATGTTACTAACTAAAAGGGC  
CAAAAGCAGCAGCGCTATTCGCAAAAACCCATAATCTACTGCCACTACAGGAAGTACCAA  
TGGATAGGTTACAGTAGATATGAAAAGGGACGTAAAGGTGACTCCTGGTACAAAGCATA  
CAGAGGAAAGACCTAAGGTGCAGGTTATACAGGCGGCTGAACCCTTGGCGACAGCATACC  
TATGTGGGATTCACAGAGAGCTGGTTAGGAGGCTGAACGCCGTCCTCCTACCCAATGTAC  
ATACACTATTTGACATGTCTGCCGAGGATTTGATGCCATCATAGCCGCACACTTTAAGC  
CAGGAGACACTGTTTTGGAAACGGACATAGCCTCCTTTGATAAGAGCCAAGATGATTCAC  
TTGCGCTTACTGCTTTGATGCTGTTAGAGGATTTAGGGGTGGATCACTCCCTGCTGGACT  
TGATAGAGGCTGCTTTCGGAGAGATTTCCAGCTGTCACCTACCGACAGGTACGCGCTTCA  
AGTTCGGCGCCATGATGAAATCAGGTATGTTCCCTAACTCTGTTCGTCAACACATTGTAA  
ACATCACCATCGCCAGCCGAGTGCTGGAAGATCGTCTGACAAAATCCGCGTGCGCGGCCT

TCATCGGCGACGACAACATAATACATGGAGTCGTCTCCGATGAATTGATGGCAGCCAGAT  
GTGCCACTTGATGAACATGGAAGTGAAGATCATAGATGCAGTTGTATCCTTGAAAGCCC  
CTTACTTTTGTGGAGGGTTTATACTGCACGATACTGTGACAGGAACAGCTTGCAGAGTGG  
CAGACCCGCTAAAAAGGCTTTTTAAACTGGGCAAACCGCTAGCGGCAGGTGACGAACAAG  
ATGAAGATAGAAGACGAGCGCTGGCTGACGAAGTGATCAGATGGCAACGAACAGGGCTAA  
TTGATGAGCTGGAGAAAGCGGTATACTCTAGGTACGAAGTGCAGGGTATATCAGTTGTGG  
TAATGTCCATGGCCACCTTTGCAAGCTCCAGATCCAACCTTCGAGAAGCTCAGAGGACCCG  
TCATAACTTTGTACGGCGGTCCTAAATAGGTACGCACTACAGCTACCTATTTTGCAGAAG  
CCGACAGCAAGTATCTAAACACTAATCAGCTACACTAGTGCCACCATGTCTGCCGCGCTG  
ATGATGTGTATCCTTGCCAACACCTCTTTCCCCTGCTCATCACCTCCCTGCTACCCCTGC  
TGCTACGAAAAACAGCCAGAACAGACACTGCGGATGCTGGAAGACAATGTGAATAGACCA  
GGGTACTATGAGCTACTGGAAGCGTCCATGACATGCAGAAACAGATCACGCCACCGCCGT  
AGTGTAACAGAGCACTTCAATGTGTATAAGGCTACTAGACCGTACTTAGCGTATTGCGCT  
GACTGTGGGGACGGGTACTTCTGCTATAGCCCAGTTGCTATCGAGAAGATCCGAGATGAG  
GCGTCTGACGGCATGCTCAAGATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAGGT  
ACCCACGCCCCACACGAAGATCCGATATATGGCTGGTCATGATGTTCAAGGAATCTAAGAGA  
GATTCCTTGAGGGTGTACACGTCCGCAGCGTGCTCTATACATGGGACGATGGGACACTTC  
ATCGTCGCACATTGTCCGCCAGGCGACTACCTCAAGGTTTCGTTTCGAGGACGCAGACTCA  
CACGTGAAGGCATGTAAGGTCCAATACAAGCACGACCCATTGCCGGTGGGTAGAGAGAAG  
TTCGTGGTTAGACCCCACCTTTGGCGTAGAGCTGCCATGCACCTCATACCAGCTGACAACA  
GCTCCCACCGACGAGGAGATCGACATGCACACACCGCCAGATATACCGGATCGCACCCCTG  
CTATCACAGACGGCGGGCAACGTCAAAATAACAGCAGGCGGCAGGACTATCAGGTACAAT  
TGTACCTGTGGCCGTGACAACGTAGGCACTACCAGTACTGACAAGACCATCAACACATGC  
AAGATTGACCAATGCCATGCTGCCGTTACCAGCCATGACAAATGGCAATTTACCTCTCCA  
TTTGTTCCCAGGGCTGATCAGACAGCTAGGAGGGGCAAAGTGCATGTTCCATTCCCTTTG  
ACTAACGTCACCTGCCGAGTGCCGTTGGCTCGAGCGCCGGATGTCACCTATGGTAAGAAG  
GAGGTGACCCTGAGATTACACCCAGATCATCCGACGCTGTTCTCCTATAGGAGTTTAGGA  
GCCGAACCGCACCCGTACGAGGAGTGGGTTGACAAGTTCTCTGAGCGCATCATCCCAGTG  
ACGGAAGAAGGGATTGAGTACCAGTGGGGCAACAACCCGCCGGTCCGCCTATGGGCGCAA

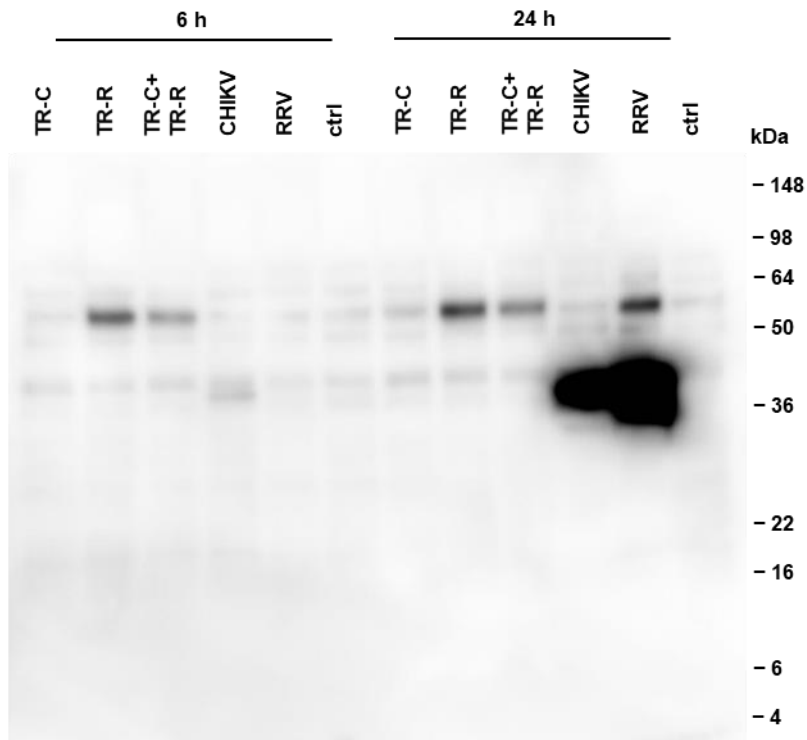
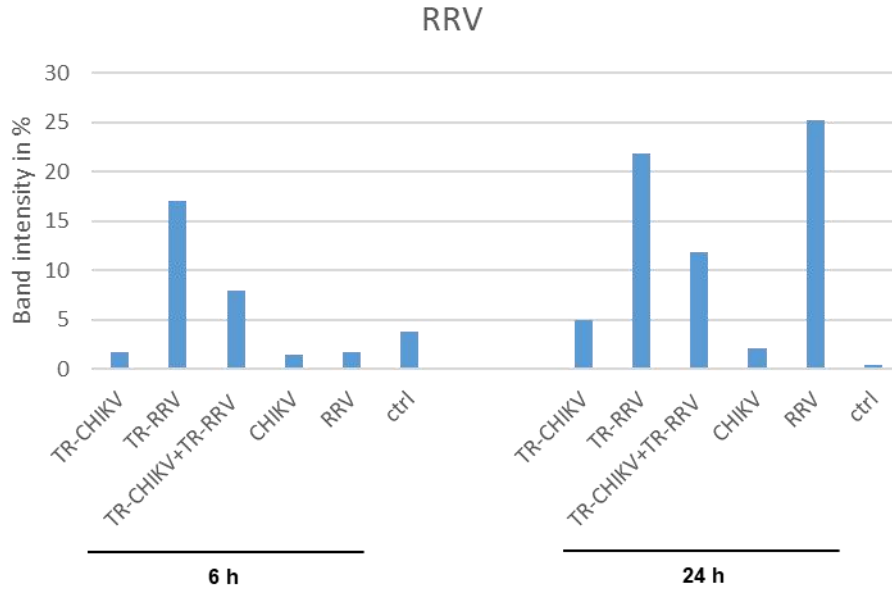
CTGACGACCGAGGGCAAACCCCATGGCTGGCCACATGAAATCATTAGTACTATTATGGA  
CTATACCCCGCCGCCACCATTGCCGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAACT  
CTAGCGGCCACATGCTGCATGCTGGCCACCGCGAGGAGAAAGTGCCTAACACCATACGCC  
TTGACGCCAGGAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCTGCGCACCGAGGGCG  
AACGCAGCATCATTCGCTGAGACTATGGCATATCTGTGGGACGAGAAACAAAACCTCTTT  
TGGATGGAATTCGCGGCCCCAGCCGCAGCGCTTGCTTTGCTGGCATGCTGTATCAAAAGC  
CTGATCTGCTGTTGTAAGCCATTTTCTTTTTTAGTGTTACTGAGCCTGGGAGCCTCCGCA  
AAAGCTTACGAGCACACAGCCACAATTCCGAATGTGGTGGGGTTCCCGTATAAGGCTCAC  
ATTGAAAGGAATGGCTTCTCGCCCATGACTCTGCAGCTTGAAGTGGTGGAGACAAGCTTG  
GAACCCACACTTAACCTGGAGTACATTACCTGCGAATACAAGACGGTGGTCCCTTCGCCA  
TTCATCAAATGTTGCGGAACATCAGAATGCTCATCCAAGGAGCAGCCAGACTACCAATGC  
AAGGTGTACACGGGTGTATACCCATTCATGTGGGGTGGAGCCTACTGTTTCTGCGACTCC  
GAGAACACGCAGCTCAGCGAGGCCTATGTGACAGGTCAGACGTTTGCAAACATGATCAC  
GCATCGGCCTACAAGGCACACACGGCCTCTCTAAAAGCAACAATCAGGATCAGTTATGGC  
ACCATCAACCAGACCACCGAGGCCTTCGTTAATGGTGAACACGCGGTCAACGTGGGCGGA  
AGCAAGTTCATCTTTGGACCGATCTCAACAGCTTGGTCACCGTTCGACAATAAAATTGTC  
GTGTATAAAGATGATGTCTACAACCAGGACTTCCCACCCTACGGATCAGGCCAGCCGGGT  
AGATTCGGAGACATTCAGAGCAGGACAGTGGAGAGCAAAGACTTGTATGCCAACACGGCC  
CTAAAACTCTCAAGACCATCACCCGGGGTTGTGCATGTGCCATACACGCAAACACCATCC  
GGATTAAATATTGGCTGAAGGAGAAAGGATCTTCATTGAATACAAAGGCCCTTTTGGC  
TGCAAGATAAAGACCAATCCAGTCAGAGCCATGGATTGTGCAGTTGGCAGTATACCTGTG  
TCGATGGACATACCTGACAGTGCATTACACGAGTGGTAGATGCCCCGGCTGTAACAGAC  
CTGAGCTGCCAGGTAGTGGTCTGTACACACTCCTCCGATTTCCGAGGAGTTGCCACATTG  
TCTTACAAAACGGACAAACCCGGCAAGTGCGCTGTCCACTCACATTCCAACGTCGCAACG  
TTGCAAGAGGCGACGGTGGATGTCAAGGAGGATGGCAAGGTCACAGTGCACCTTTTCCACG  
GCGTCCGCCTCCCCGGCCTTCAAAGTGTCGTCTGTGACGCAAAAACAACGTGCACGGCG  
GCGTGCGAGCCTCCAAAAGACCACATCGTCCCTTATGGGGCGAGCCATAACAACCAGGTC  
TTCCGGACATGTCAGGAACTGCGATGACGTGGGTGCAGAGGCTGGCCAGTGGGTTAGGT  
GGGCTGGCTCTCATCGCGGTGGTTGTGCTGGTCTTGGTAACTGCATAACAATGCGTCGG

TAACCTCGAGGCGGCCGCTTGACAATTAAGTATGAAGGTATATGTGTCCCCTAAGAGACA  
CACTGTACATAGCAAATAATCTATAGATCAAAGGGCTACGCAACCCCTGAATAGTAACAA  
AATACAAAATCACTAAAAATTATAAAAAACAGAAAAATACATAAATAGGTATACGTGTCCC  
CTAAGAGACACATTGTATGTAGGTGATAAGTATAGATCAAAGGGCCGAATAACCCCTGAA  
TAGTAACAAAATATGAAAATCAATAAAAATCATAAAATAGAAAAACCATAAACAGAAGTA  
GTTCAAAGGGCTATAAAACCCCTGAATAGTAACAAAACATAAAATTAATAAAAATCAAAT  
GAATACCATAATTGGCAAACGGAAGAGATGTAGGTACTTAAGCTTCCTAAAAGCAGCCGA  
ACTCACTTTGAGAAGTAGGCATAGCATACCGAACTCTTCCACGATTCTCCGAACCCACAG  
GGACGTAGGAGATGTTATTTTGTTTTTAATATTC

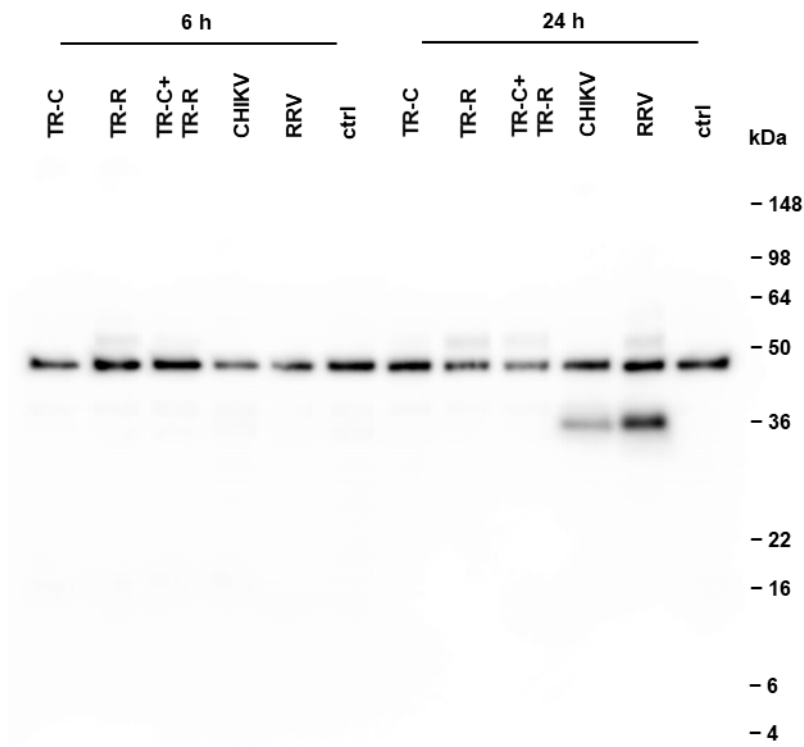
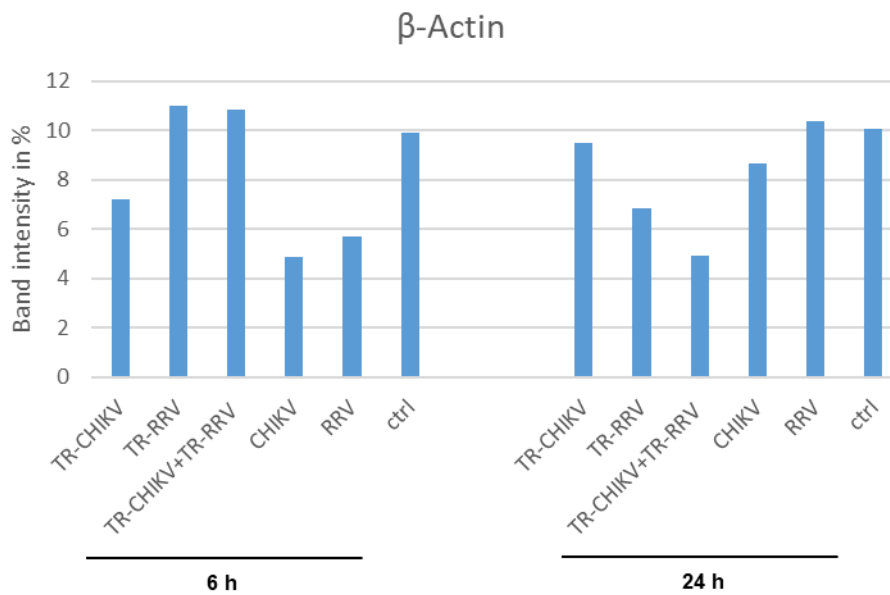
**Figure S1.** Sequence of pTR-R.

**A****B**

**Figure S2.** (A) Uncropped western blot of Figure 3A showing CHIKV E2 protein expression in cellular lysates 6 h and 24 h after transfection. (B) Band intensity of CHIKV E2 protein expression given in relative percentage.

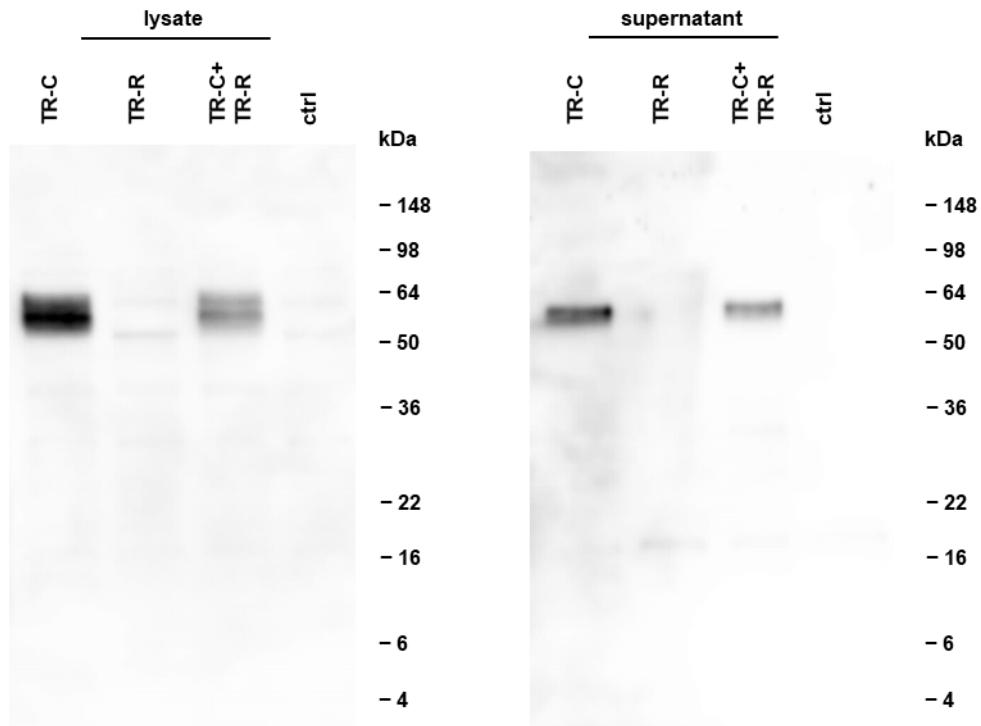
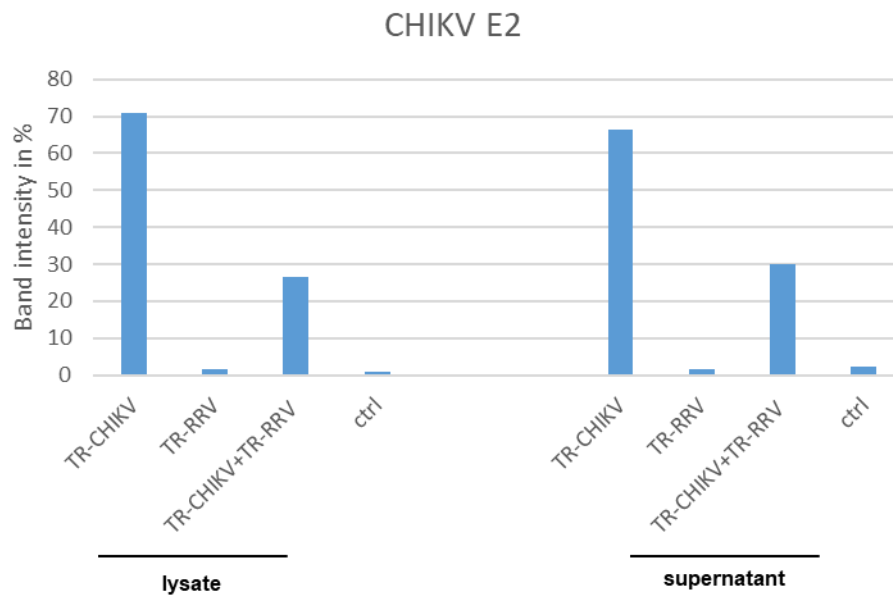
**A****B**

**Figure S3.** (A) Uncropped western blot of Figure 3A showing RRV protein expression in cellular lysates 6 h and 24 h after transfection. (B) Band intensity of RRV protein expression given in relative percentage.

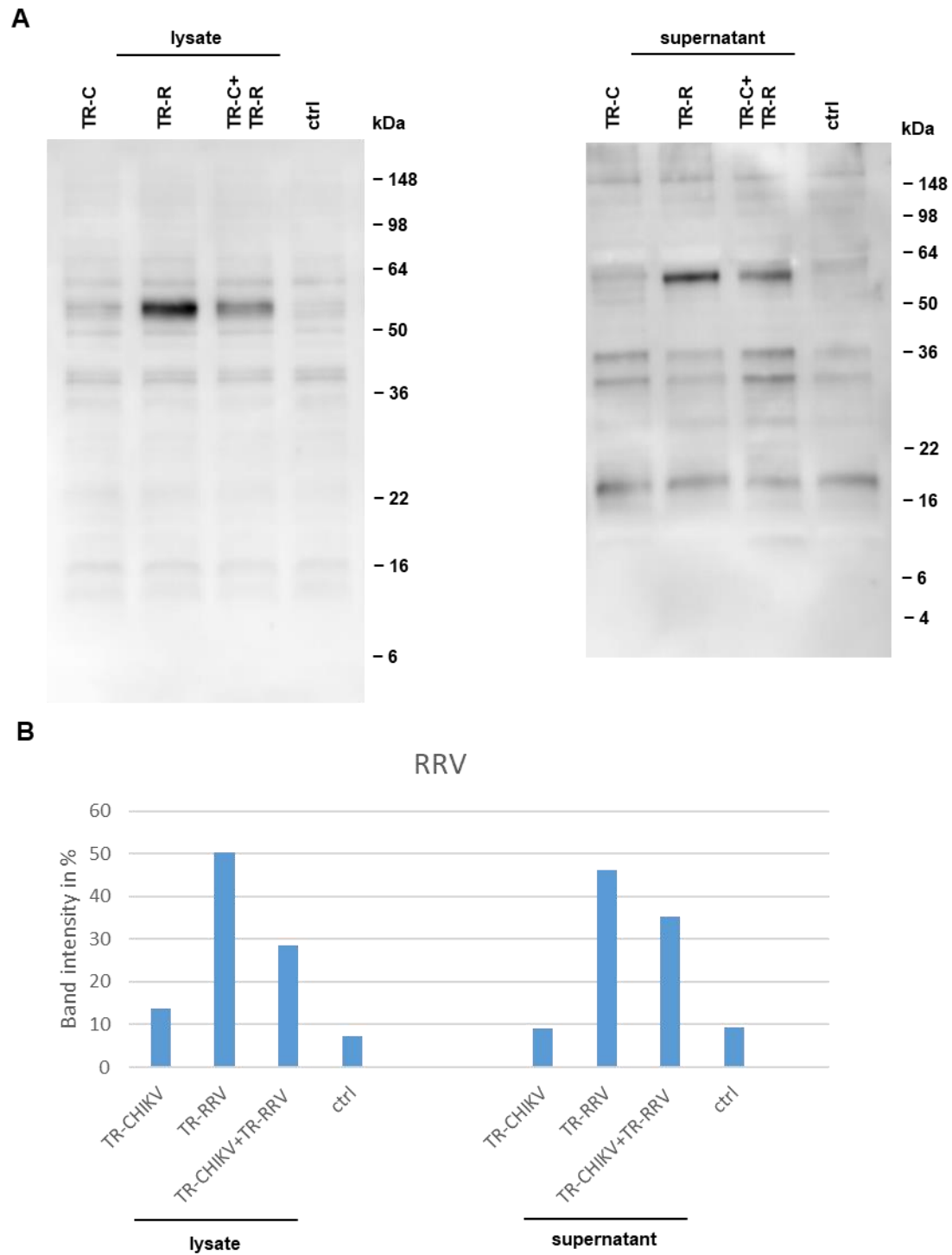
**A****B**

**Figure S4.** (A) Uncropped western blot of Figure 3A showing  $\beta$ -actin protein expression in cellular lysates 6 h and 24 h after transfection. (B) Band intensity of  $\beta$ -actin protein expression given in relative percentage.



**A****B**

**Figure S5.** (A) Uncropped western blot of Figure 3B showing CHIKV E2 protein expression in cellular lysates and concentrated supernatants 48 h after transfection. (B) Band intensity of CHIKV E2 protein expression given in relative percentage.



**Figure S6.** (A) Uncropped western blot of Figure 3B showing RRV protein expression in cellular lysates and concentrated supernatants 48 h after transfection. (B) Band intensity of RRV protein expression given in relative percentage.



**Figure S7.** (A) Uncropped western blot of Figure 3B showing  $\beta$ -actin protein expression in cellular lysates 48 h after transfection. (B) Band intensity of  $\beta$ -actin protein expression given in relative percentage.