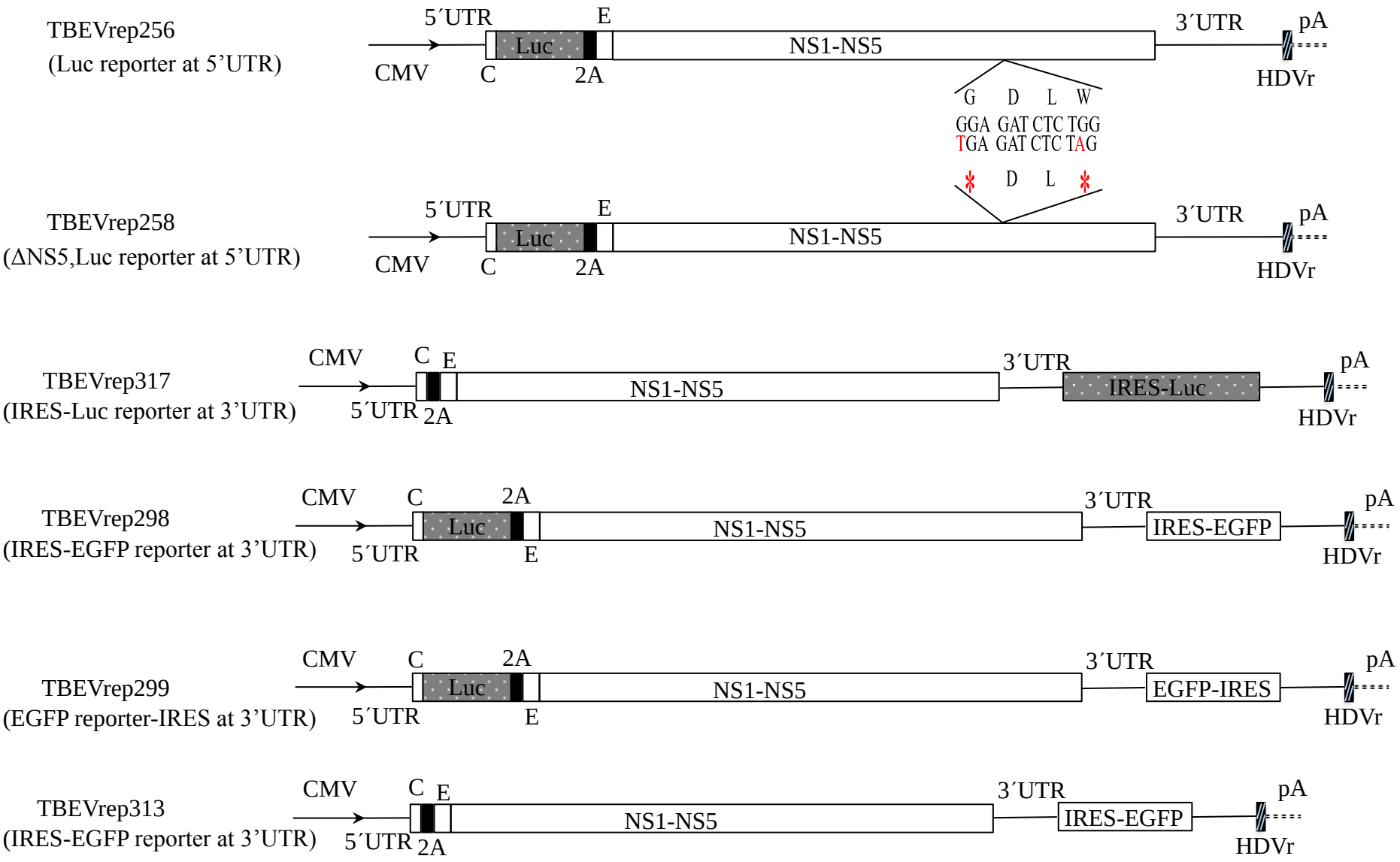
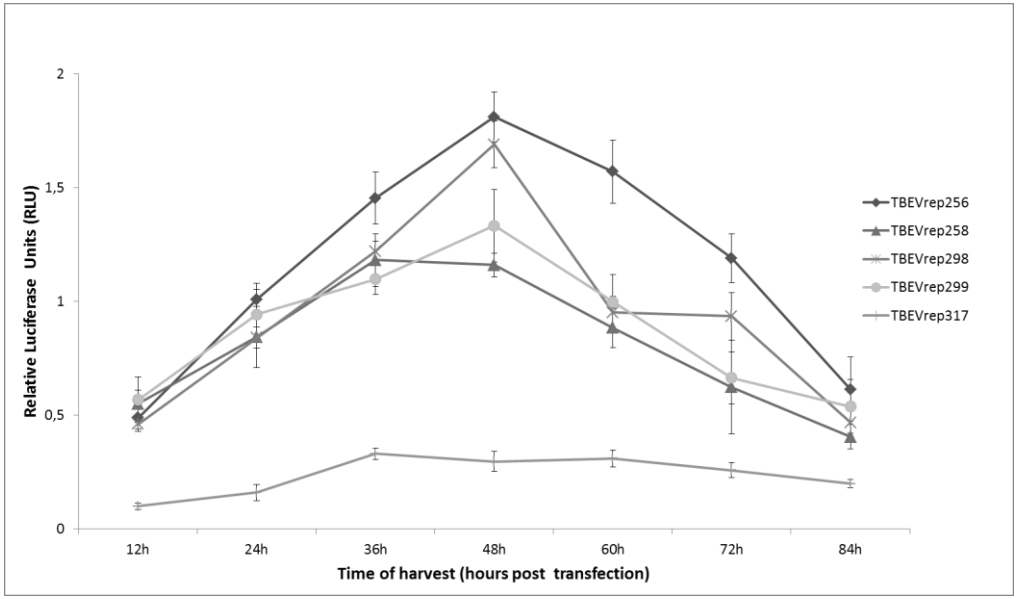
Supplementary Figure a-d

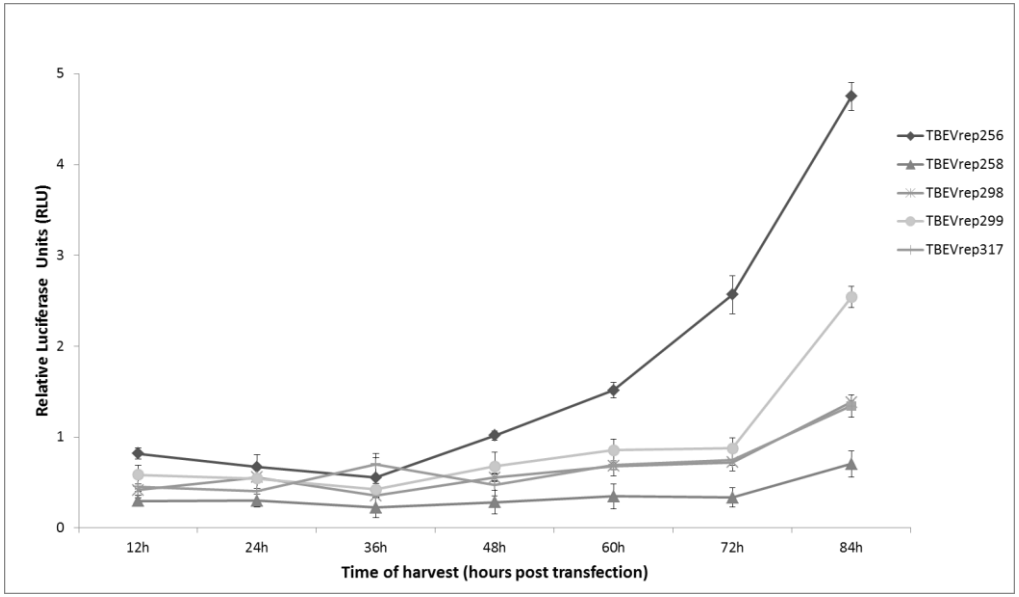
Supplementary Figure (a). Schematic drawing of DNA-based TBEV replicons. All replicons contain in-frame deletion of most of the structural genes sequence. The CMV promoter, the autoprotease of FMDV (2A), the HDVr ribozyme and the SV40 polyA (pA) are indicated. C corresponds to the first 20 amino acids of capsid protein whereas E represents last 28 amino acids of envelope protein. IRES indicates an internal ribosomal entry site sequence. Luciferase (Luc) and/or efficient green fluorescent protein (EGFP) reporters are engineered into two different regions of the TBEV replicons.



Supplementary Figure (b). Evaluation of TBEVrep activity under the various conditions of Luc reporter. COS-1 cells (10^5) were co-transfected with pGL4.74 (10ng) and TBEV rep 256, 258, 298, 299, 317, 337 (350ng respectively) and were lysed for subsequent analysis with the dual luciferase assay (Promega) at the indicated h.p.t.. Diagram shows the ratio of firefly luciferase to renilla luciferase expression with mean ± SD from triplicate experiment.



Supplementary Figure (c). Evaluation of TBEVrep activity under the various conditions of Luc reporter. BHK-21 cells (10^5) were co-transfected with pGL4.74 (10ng) and TBEV rep 256, 258, 298, 299, 317, 337 (350ng respectively) and were lysed for subsequent analysis with the dual luciferase assay (Promega) at the indicated h.p.t.. Diagram shows the ratio of firefly luciferase to renilla luciferase expression with mean ± SD from triplicate experiment.



Supplementary Figure (d). Evaluation of safety of TBEVrep and pCAG-CME *in vitro*. COS-1 cells (10^5) were co-transfected with either (**a**) pCAG-CME (10ng) and TBEV rep 256 (350ng), (**b**) pCAG-CprME (10ng), or (**c**) pCAG. After 48h incubation at 37°C, cell culture supernatants were used to infect new COS-1 cell line. 48 hours post infection, cell culture supernatants of infected cells were used to infect new COS-1 cell line. At each step of experiment, cells were fixed and the presence of TBEV E protein was visualized by immunofluorescence staining using a rabbit polyclonal anti- TBEV E protein serum (red). The nucleus of the cells were also stained in blue by Hoechst stain solution (Sigma-Aldrich). The experiment were performed in triplicate.