3p10LA9 was 199nM, 3p10L was 108nM, B) RNA was transfected at a fixed concentration of 100nM in HEK-Lucia™ RIG-I and luminescence was measured 4, 6, 8, 10, 12, 24, 48 and 72 hours post-transfection. C) RNA was transfected in THP1-Dual™ at a different concentration range of RNA and luminescence was measured 48 hours post-transfection. Results were measured in triplicate and presented as percentage of the maximum activity vs log concentration. The EC_{50} for 3p10L G9 was 91nM, 3p10LA9 was 51nM, 3p10L was 226nM D) RNA was transfected at a fixed concentration of 100nM in THP1-Dual™ and luminescence was measured 4, 6, 8, 10, 12, 24, 48 and 72 hours post-transfection.

Figure 4. Michaelis-Menten plot showing ATPase activity of mus musculus RIG-I (mmRIG-I) and mmRIG-I Δ CARDs binding to different RNA molecules (A) Rate of catalysis over ATP concentration ranging from 0 to 5mM - for mmRIG-I stimulated by short RNA hairpins with different stem modification (B) Rate of catalysis over ATP concentration ranging from 0 to 5mM for mmRIG-I stimulated by RNA hairpins of different base composition (C) Rate of catalysis over ATP concentration ranging from 0 to 5mM for mmRIG-I stimulated by RNA hairpins of different length.

Figure 5. Screening of immRNA constructs for their capacity to induce Type I IFN. A) RIG-I ligand immunomodulating RNA (immRNA) was transfected into HEK-293T cells that contain a stably integrated luciferase reporter driven by a MX1 promoter (MX1P-luc). RNA was transfected at various concentrations achieved by serial dilution (2x) and luminescence was measured 24h after transfection. Results are represented as a percentage of the RLU measured for 312nM treatment of OHYr23 (parental construct; SEQ ID NO:37). B) HEK293T cells expressing the MX1 promoter driving luciferase was transfected with OHYr23 at 10nM. These cells were co-transfected with either OHYr10 (SEQ ID NO:30) or OHYr09 (SEQ ID NO:34) in a range of concentrations (0nM - 312nM). Cells were lysed with Brightglo and luminescence was read. Error bars are for triplicate transfections per condition

Figure 6. Screening of immRNA constructs for their capacity to induce Type I IFN. A), B) and C) ImmRNA was transfected into THP1-Dual™ cells which were derived from the human THP-1 monocyte cell line by stable integration of the lucia luciferase reporter driven by ISG 54. The luciferase reporter response was normalized to 100% and plotted against the log concentration of RNA. The EC50 value for OHYr05 (SEQ ID NO:26) was determined to be 3.47nM as compared to OHYr23 (SEQ ID NO:37) with EC50 of 14nM.

Figure 7. Cell based assay with different ImmRNA in HEK-Lucia™ RIG-I and HEK-Lucia™ null. A) ImmRNA of different length, B) ImmRNA with different guanosine insertion. C) ImmRNA with different nucleotide at position 9. RNA of different length was transfected at a fixed concentration

of 100nM and luminescence was measured 24 hours post-transfection. Results are measured in triplicate and presented as RLU.

Figure 8. Comparison between the activity of commercial RNA and ImmRNA OHYr16 (3p10LA9; SEQ ID NO:25) in A) THP1-Dual™ cells and B) HEK-Lucia™ RIG-I. RNAs of different concentration were transfected into both cells and were serially diluted 2x. The luminescence values were recorded 24 hours post transfection.

Figure 9. Stability test for ImmRNA OHYr16 (3p10LA9; SEQ ID NO:25) and commercial RNAs in A) THP1-Dual™ cells and B) HEK-Lucia™ RIG-I. RNAs were incubated in serum free medium for 24, 48, 72 and 96 hours prior to transfection into cells. The luminescence values were measured 24 hours post transfection.

Figure 10. Kink position of immRNA influences biological activity. A) A kink at nucleotide position 5 (OHYr10), position 19 (OHYr11) and position 21 (OHYr01) abrogates IFN production. % luciferace activity was normalized to the value detected from OHYr23 used at 312nM. B) A kink on the 5' side of immRNA at position 9 from the stem (OHYr05) improved biological activity compare to no kink (OHYr23), whereas a kink at the 3' side of immRNA at position 9 counted from the stem (OHYr02) abrogated biological activity. % luciferace activity was normalized to the value detected from OHYr23 used at 100nM. C) A purine at kink position 9 (Guanine in OHYr05 and Adenine in OHYr16) has a higher biological activity than a pyrimidine at kink position 9 (Uracil in OHYr17 and a Cytosine OHYr18). Directly transfected HEK293T MX1P-luc reporter cells were used as a readout in A-C.

Figure 11. Length of stem influences biological activity of immRNA. OHYr23 (length 10 nucleotides) and OHYr08 (length 30 nucleotides) have high activity in a HEK293T MX1P-luc reporter assay. In contrast, OHYr12 (length 6 nucleotides), OHYr20 (length 9 nucleotides), OHYr03 (length 11 nucleotides), OHYr07 (length 12 nucleotides) and OHYr13 (length 14 nucleotides) showed low or no biological activity. % luciferace activity was normalized to the value detected from OHYr23 used at 100nM. Directly transfected HEK293T MX1P-luc reporter cells were used as a readout in A-C.

Figure 12. 3p10LG9 (SEQ ID NO:26) induces an interferon response and is effective as prophylaxis against DENV-2 infection in both U937-DC-SIGN cells and A549 cells. (A) Structure and sequence of 3p10L (SEQ ID NO:37) and 3p10LG9 (SEQ ID NO:26) are as shown in the table. (B) 3p10L, 3p10LG9 (SEQ ID NO:26) or G9neg (SEQ ID NO:26 without 5' phosphorylation) was transfected into U937-DC-SIGN or A549 cells. Supernatants were harvested and incubated on ISRE-luc HEK-293T reporter cells. Luminescence was measured 6h after incubation with the supernatant. Tables show EC50 values (nM) for 3p10L (SEQ ID NO:37) and 3p10LG9 (SEQ ID