SUPPLEMENTARY DATA

In-cell SHAPE uncovers dynamic interactions within distant untranslated regions of the foot-and-mouth disease virus RNA

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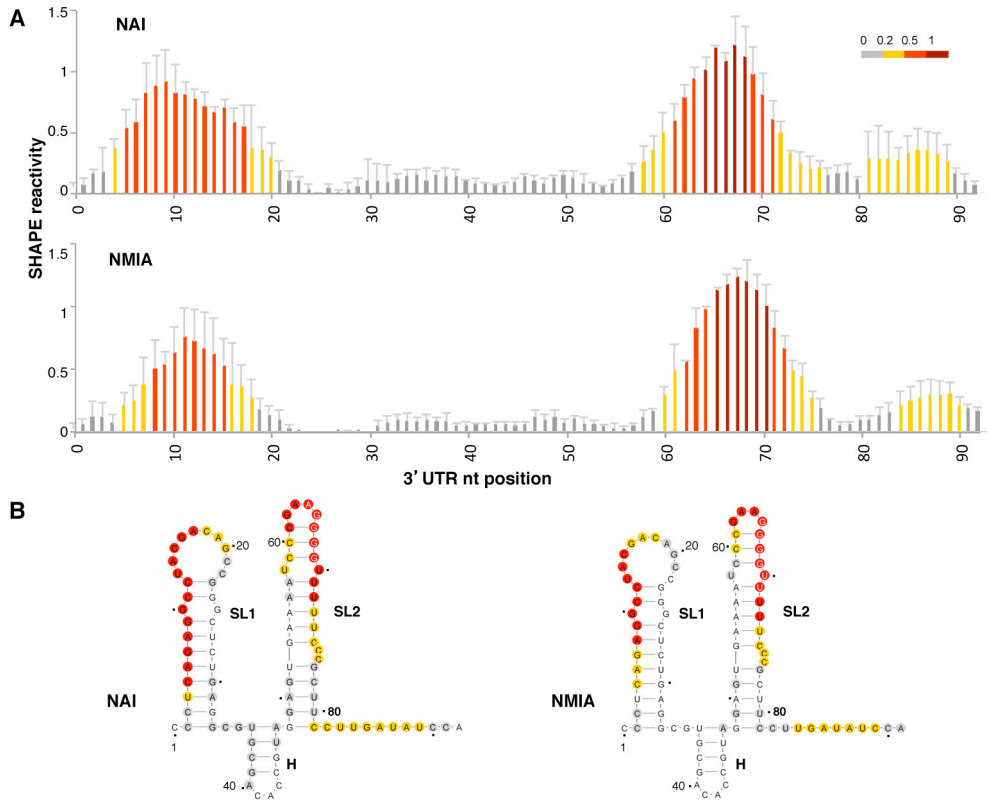
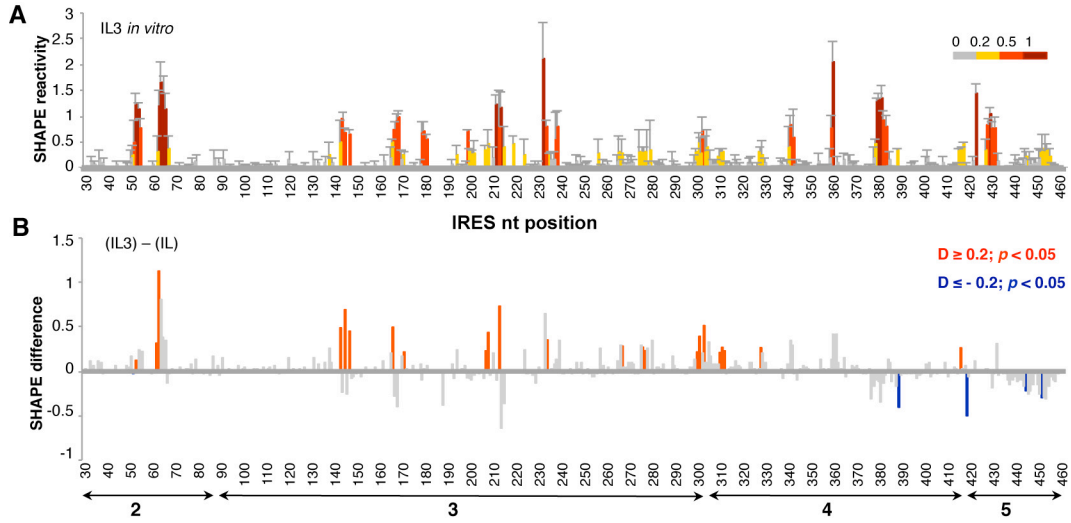
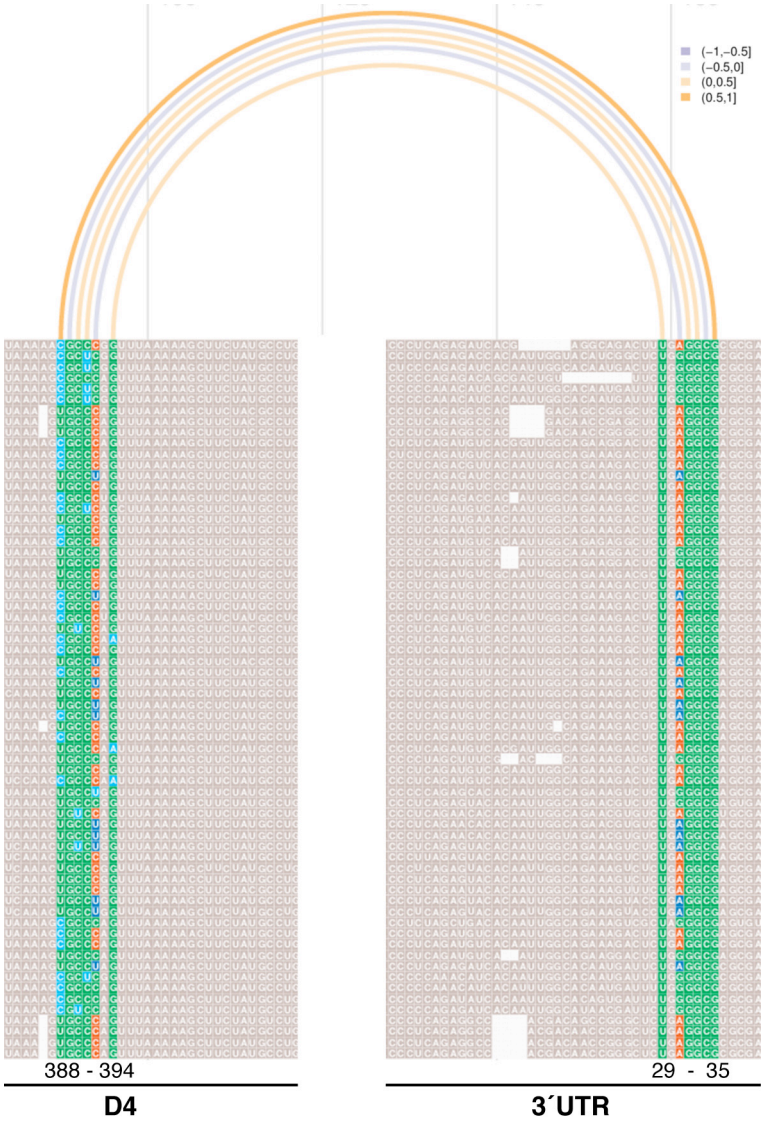
SUPLEMENTARY FIGURES



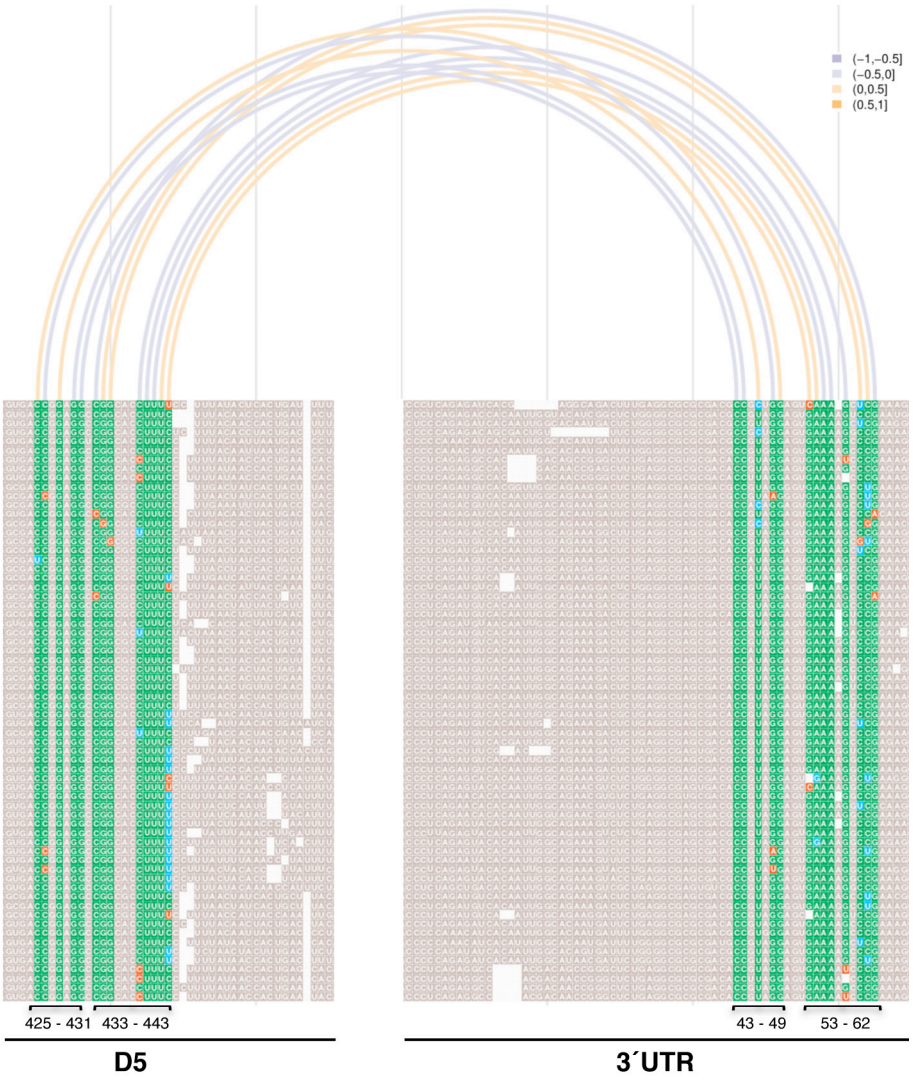
Supplementary Figure S1. SHAPE reactivity of the 3´UTR towards NAI and NMIA. A. Comparison of the SHAPE reactivity towards NAI (top) and NMIA (bottom) *in vitro*. Normalized 3´UTR reactivity (IL3 transcript) determined as a function of the nucleotide position. RNA reactivity is colored according to the scale shown on the right. Values correspond to the mean ± SD of three independent experiments. **B.** 3´UTR RNA secondary structure models predicted by RNAstructure imposing NAI and NMIA reactivity.



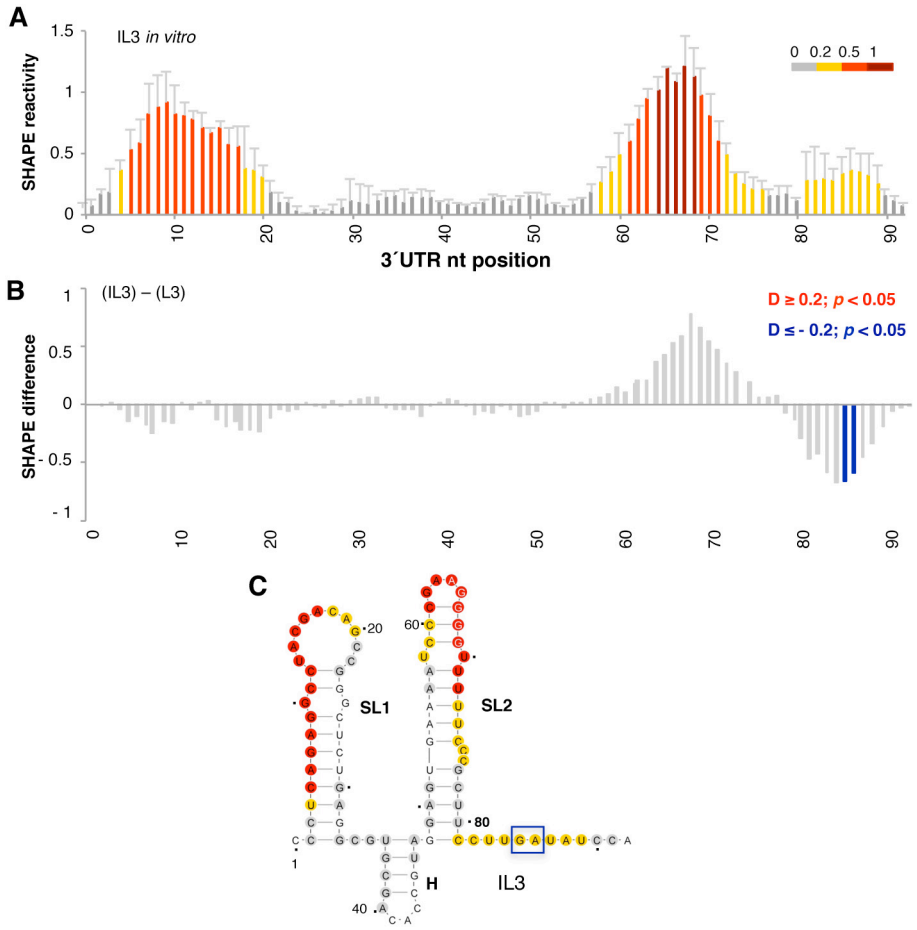
Supplementary Figure S2. Conformational changes on the IRES element induced by the 3´UTR. A. Normalized IRES SHAPE reactivity towards NAI of the IL3 transcript, determined *in vitro*, as a function of the nucleotide position. RNA reactivity is colored according to the scale shown on the right. Values correspond to the mean ± SD of three independent experiments. **B**. Significant SHAPE differences within the IRES region between the transcript IL3 and IL. Red or blue bars depict nucleotides with *p*-values <0.05 and absolute SHAPE reactivity differences (D) higher or lower than 0.2, respectively. Grey bars depict differences which are non statistically significant.



Supplementary Figure S3. Covariant bases involved in long-range interactions between the IRES and the 3´UTR. Covariant positions between domain 4 (subdomain K) and 3'UTR (SL1) were visualized using R-CHIE. Nucleotides are colored by the degree of conservation, green indicates the most frequent canonical base pair; dark blue is used for double sided-mutations and light blue for single-sided mutations; red depicts non-canonical base pairs; grey indicates unpaired nts, and white is used for gaps. Each arc depicts a base pair of the RNA structure. The color of the arc indicates the covariation range. The nt positions involved are indicated (black letters)



Supplementary Figure S4. Covariant bases involved in long-range interactions between the IRES and the 3´UTR. Covariant positions between domain 5 and 3'UTR (H and SL2) were visualized using R-CHIE. Symbols are used as in Figure S3.



Supplementary Figure S5. Conformational changes on the 3´UTR in the presence of the IRES element. **A**. 3´UTR SHAPE reactivity towards NAI in the IL3 transcript. Normalized reactivity determined *in vitro* as a function of the nucleotide position. RNA reactivity is colored according to the scale shown on the right. Values correspond to the mean ± SD of three independent experiments. **B**. Statistically significant SHAPE differences within the 3´UTR region *in vitro* between the transcript IL3 and L3. Red or blue bars depict nucleotides with *p*-values <0.05 and absolute SHAPE reactivity differences (D) higher or lower than 0.2, respectively. Grey bars depict differences which are non-statistically significant. **C**. 3´UTR RNA secondary structure showing NAI reactivity, a blue box denotes protected positions on IL3 relative to the L3 RNA.

arget	Position Reporter	Query	Position 3´UTR	Energy
Luc	301 -- 313	3'UTR	33 -- 45	-6.86014
Luc	264 -- 272	3'UTR	33 -- 41	-4.47202
Luc	1562 -- 1569	3'UTR	34 -- 41	-4.44327
Luc	1175 -- 1183	3'UTR	83 -- 91	-3.95459
Luc	1140 -- 1152	3'UTR	12 -- 21	-3.85188
Luc	1479 -- 1486	3'UTR	14 -- 21	-3.65470
Luc	1001 -- 1008	3'UTR	75 -- 82	-2.84766
Luc	184 -- 190	3'UTR	33 -- 39	-2.62771
Luc	333 -- 339	3'UTR	35 -- 41	-2.01824
Luc	689 -- 695	3'UTR	32 -- 38	-1.95722

Supplementary Figure S6. Prediction of base pairs between the reporter and the 3´UTR. The potential interactions between the target (luciferase reporter) and the query (3'UTR) were predicted using IntaRNA. The nucleotide positions of each region and the energy of the predicted interactions are indicated.