

Supplemental Information

Translation of Angiotensin-Converting Enzyme 2 upon Liver- and Lung-Targeted Delivery of Optimized Chemically Modified mRNA

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S1

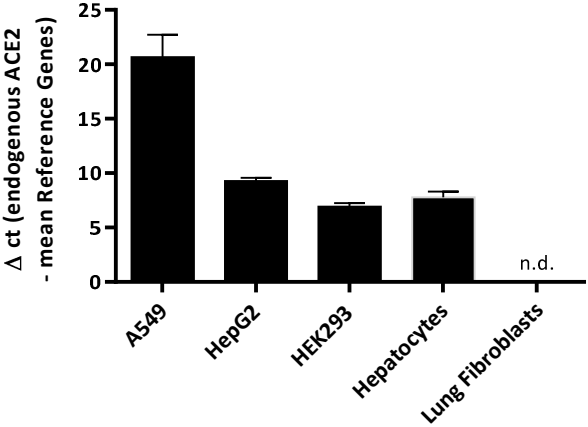


Figure S1. Levels of endogenous ACE2 mRNA. Total RNA of untreated cells of A549, HepG2 and HEK293 was collected and transcribed into first-strand cDNA. Real-time PCR was performed and delta ct values were calculated against a panel of reference genes. Reference genes for human cells: β -2-microglobulin, MRPL19 and SDHA. Reference genes for murine cells: MRPL19, GusB and HPRT.

S2

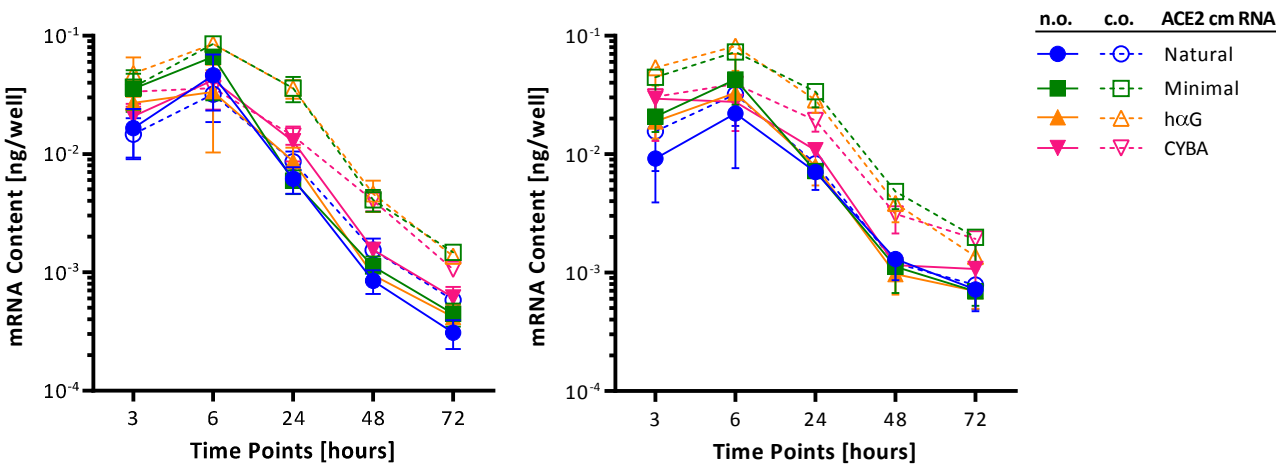


Figure S2. Kinetics of ACE2 cmRNA constructs in A549 and HepG2. A549 (left panel) and HepG2 (right panel) were lysed at time points indicated after transfection. Total RNA was collected and transcribed into first-strand cDNA. The amount of ACE2 cmRNA was quantified by real-time PCR.

S3

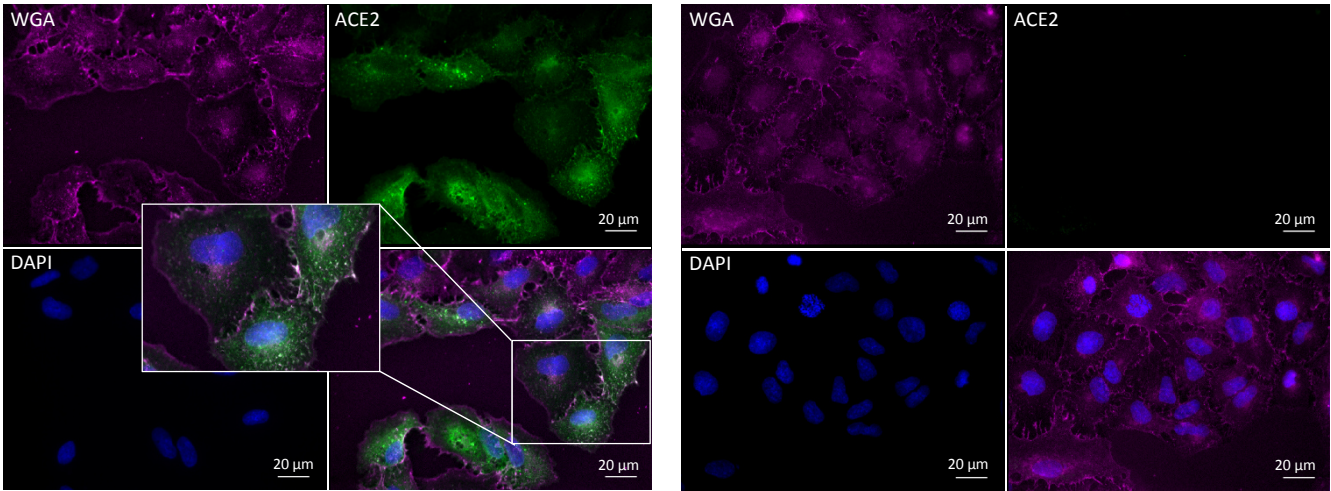


Figure S3. Immunofluorescent staining for ACE2 protein in A549. 24 h after transfection cells were incubated with tetramethylrhodamine conjugated wheat germ agglutinin for membrane staining, fixed and then stained with anti-ACE2 antibody (R&D Systems, 5 μ g/ml, AF933). Finally, cell nuclei were stained with DAPI and ACE2 was visualized by addition of secondary anti-goat AF488 antibody (Thermo Fisher Scientific, 1:400, A11087). ACE2 cmRNA transfected cells (left panel) and control cmRNA transfected cells (right panel). green: ACE2, blue: nucleus, violet: cell membrane.

S4

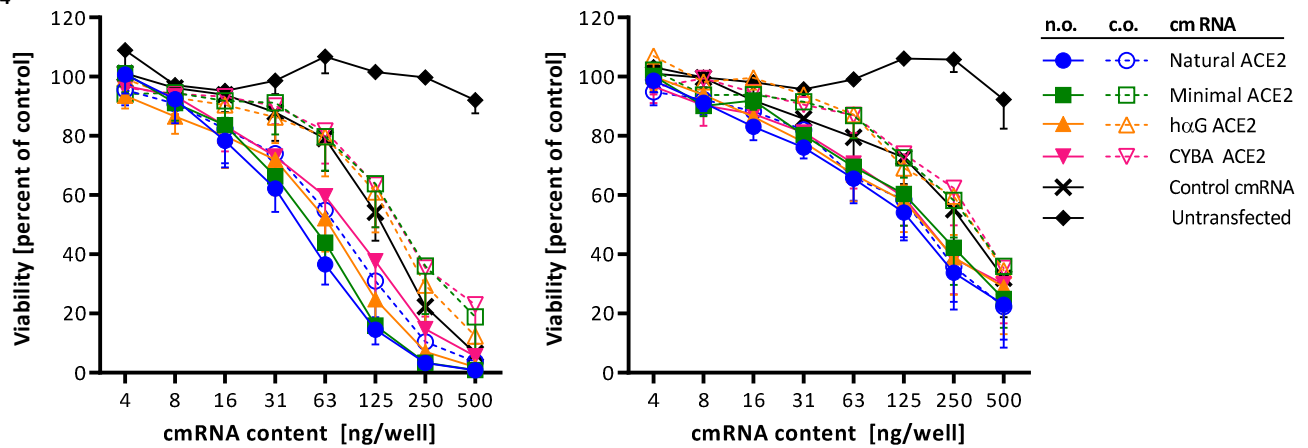


Figure S4. Viability of cells 72 h post cmRNA transfection. A549 (left panel) and HepG2 (right panel) were transfected with a decreasing series of metridia luciferase cmRNA. 72 h post transfection, cell supernatant was collected and luciferase activity was detected by addition of coelenterazine buffer (Synchem) and luminescence measurement on a Tecan Infinite 200 PRO plate reader.

S5

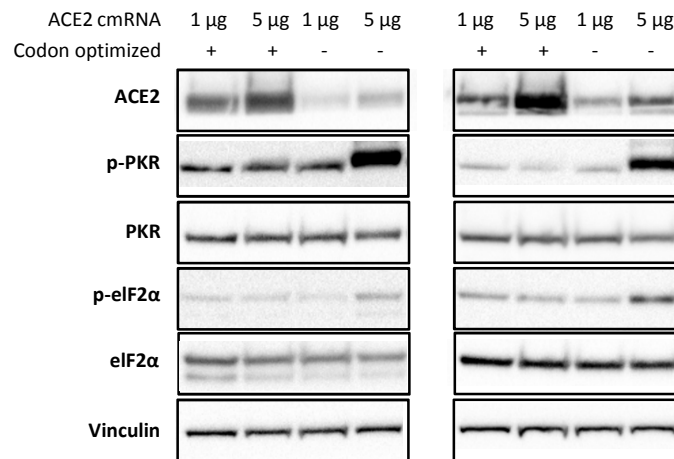


Figure S5. Effects of codon optimization on PKR activation. A549 (left panel) and HEK293 (right panel) were transfected with 1 or 5 μ g of native or codon optimized hαG ACE2 cmRNA. 24 h after transfection, cells were lysed and cell lysate was analyzed by Western Blot with Vincullin as loading control. The following primary antibodies were used: anti-ACE2 (R&D systems, 0.1 μ g/ml, AF933), anti – phospho eIF2 α (Cell Signaling, 1:3000, #3398, detects phosphorylation on Ser51), anti-eIF2 α (Cell Signaling, 1:3000, #2103), anti – phospho PKR (abcam, 1:3000, ab32036, detects phosphorylation on Thr446), anti-PKR (Cell Signaling, 1:3000, #12297), anti-Vinculin (abcam, 1:10000, ab91459). Secondary antibodies were used as previously described.

S6

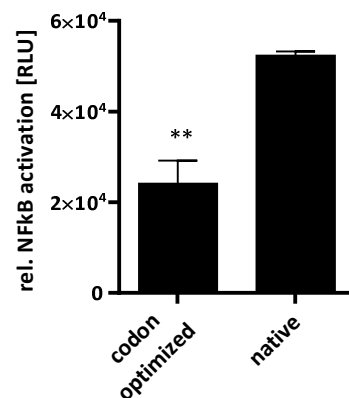


Figure S6. Effects of codon optimization on NFκB activation. NFκB activation was studied in HEK293 NFκB-reporter cells, which contain a luciferase gene under the control of a promoter with multiple Nuclear Factor-κB response elements. Upon binding of NFκB to the response elements, these cells express luciferase which can be detected by a luciferase activity assay. Cells were transfected with native or codon optimized hαG ACE2 cmRNA and lysed 24 h after transfection. Luciferase activity was then determined for the cell lysates, which correlates to NFκB activation in these cells.

S7

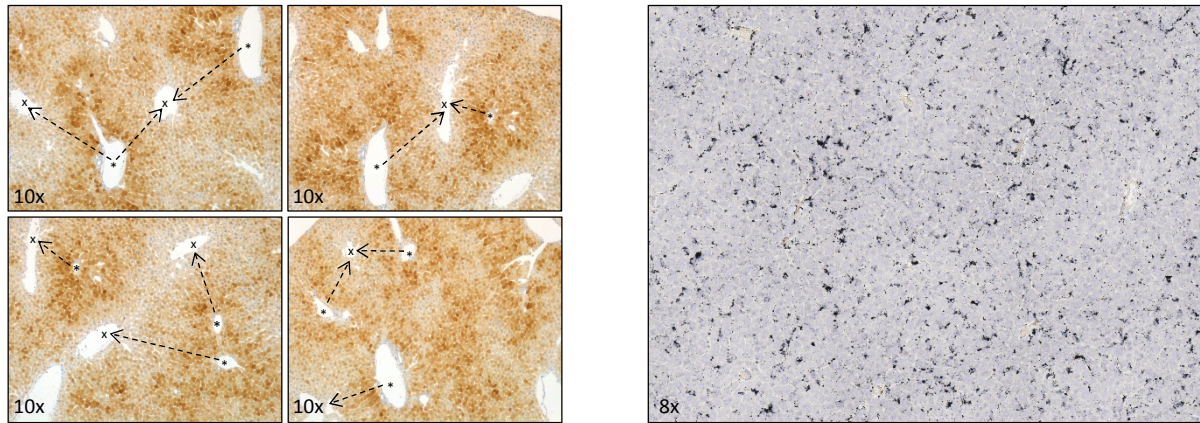


Figure S7. Left panel: Mice were intravenously injected 1 mg/kg of luciferase cmRNA in LLF. 6 h after injection, animals were sacrificed and parts of the liver was embedded in paraffin and stained for luciferase protein with anti-luciferase antibody. 4 representative images of luciferase cmRNA treated animals. * portal vessel, x central vein. Right panel: Mice received 4 mg/kg codon-optimized hG ACE2 cmRNA in LLF by intravenous injection. 6 h after transfection, animals were sacrificed, liver excised and embedded in paraffin. In situ hybridisation was performed for detection of ACE2 cmRNA (black signal).

S8

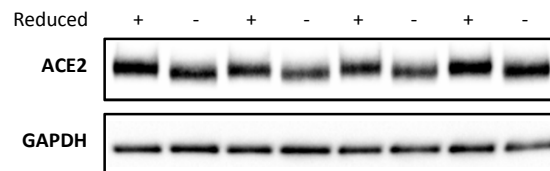


Figure S8. Disulfide bridge formation during posttranslational modification. A549 were transfected with 2 µg ACE2 cmRNA. 24 h after transfection, cells were lysed and cell lysate was analyzed by Western Blot under reducing and non-reducing conditions with GAPDH as loading control. The following primary antibodies were used: anti-ACE2 (R&D systems, 0.1 µg/ml, AF933), anti – GAPDH (Cell Signaling, 1:10000, #5174). Secondary antibodies were used as previously described. The same sample was applied repeatedly.