**Supplementary Data for**

# Formation of HERV-K and HERV-Fc1 envelope family members is suppressed on transcriptional and translational level

Victoria Gröger, Lisa Wieland, Marcel Naumann, Ann-Christin Meinecke, Beate Meinhardt, Steffen Rossner, Christian Ihling, Alexander Emmer, Martin S. Staege and Holger Cynis

This file contains:

- Supplementary Table S1-S4
- Supplementary Fig. S1-S11

**Supplementary Table S1.** List of Primers for cloning HERV sequences. The primer sequences show restriction sites in bold and the respective tag underlined.

| **Primer name** | **Sequence 5'-3'** | **Restriction site** |
|---|---|---|
| *Fc1_mut_C705T_for*<br>*Fc1_mut_C705T_rev* | CCTAGTCCGCCACGGGGCTCGCCTTG<br>CAAGGCGAGCCCCGTGGCGGACTAGG | |
| *Fc1_mut_A438T_for*<br>*Fc1_ mut_A438T_rev* | CCTAGTCCGCCACGGGGCTCGCCTTG<br>CAAGGCGAGCCCCGTGGCGGACTAGG | |
| *pcDNA_Fc1-Flag_for;*<br>*pcDNA_Fc1-EGFP_for* | TATA**GGATCC**ATGGGCAGACCTTCCCCAC | *Bam*HI |
| *pcDNA_Fc1-Flag_rev;*<br>*pcDNA_coFc1-Flag_rev* | TATA**GCGGCCGC**TCACTTGTCGTCATCGTCTT<br>TGTAGTCTCTGGCTGCTTCCTGCTG | *Not*I |
| *pcDNA_Fc1-EGFP_rev* | TATA**GCGGCCGC**TTTCTGGCTGCTTCCTGCTG | *Not*I |
| *pcDNA_coFc1-Flag_for* | TATA**GGATCC**ATGGCCCGCCCTTCAC | *Bam*HI |
| *pcDNA_K18-Flag_for* | TATA**AAGCTT**ATGGTAACACCAGTCACATG | *Hind*III |
| *pcDNA_K18-Flag_rev* | TATA**GCGGCCGC**TCACTTGTCGTCATCGTCTT<br>TGTAGTCTGGCCCGTTCTCGATGTC | *Not*I |
| *pcDNA_coK18-Flag_for* | ATAT**AAGCTT**GCCGCCACCATGGTGACACCT<br>GTGACTTG | *Hind*III |
| *pcDNA_coK113-Flag_for;*<br>*pcDNA_coK18*-Flag_for* | ATGCATCTAGAT**AAGCTT**GCCGCCACCATG | *Hind*III |
| *pcDNA_coK113-Flag_rev* | CGAT**GCGGCCGC**TTACTTATCGTCATC | *Not*I |
| *pcDNA_coK18*-Flag_rev;*<br>*pcDNA_coK18-Flag_rev* | CGAT**GCGGCCGC**TTATTTATCGTCATC | *Not*I |

**Supplementary Table S2.** Antibodies used for Western Blot analysis

| Name | Type | Organism | Dilution | Manufacturer |
|---|---|---|---|---|
| anti-DYKDDDDK (FLAG) tag | primary | rabbit | 1:1000 | Cell signaling, Cambridge, Great Britain |
| anti-HERV-K TM HERM1811-5 | primary | mouse | 1:1000 | Austral Biologicals, San Ramon, CA, USA |
| Anti-HERV-K SU HERM1821-5 | primary | mouse | 1:1000 | Austral Biologicals, San Ramon, CA, USA |
| anti-β-Aktin | primary | rabbit | 1:1000 | Cell signaling, Cambridge, Great Britain |
| anti-mouse IgG-HRP | secondary | horse | 1:2000 | Cell signaling, Cambridge, Great Britain |
| anti-rabbit IgG-HRP | secondary | goat | 1:2000 | Cell signaling, Cambridge, Great Britain |

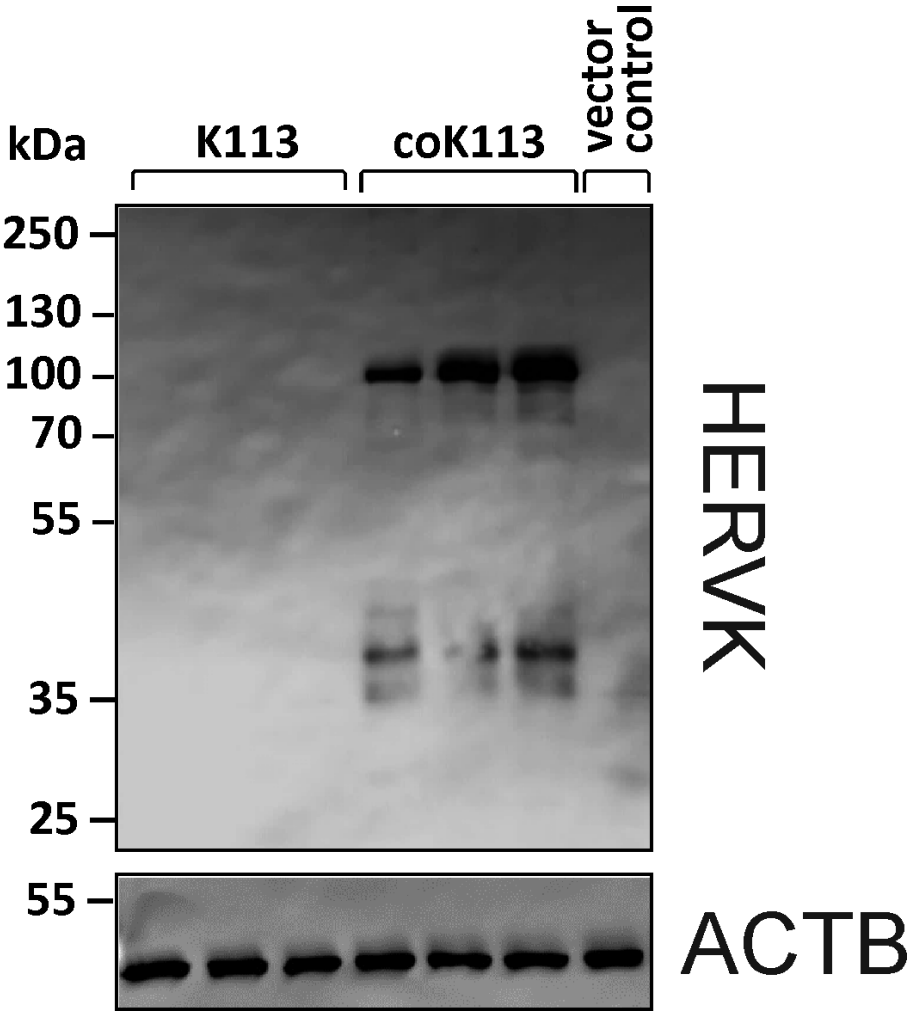
**Supplementary Table S3.** Antibodies used for immunofluorescence and FACS analysis

| **Antibody** | **Dilution** | **Manufacturer** |
|---|---|---|
| anti-DYKDDDDK (FLAG) tag | 1:800 | Cell signaling, Cambridge, Great Britain |
| anti-HERV-K TM HERM1811-5 | 1:500 | Austral Biologicals, San Ramon, CA, USA |
| anti-calnexin | 1:50 | Cell signaling, Cambridge, Great Britain |
| anti-golgin-97 | 1:100 | Cell signaling, Cambridge, Great Britain |
| anti-GP73 | 1:50 | Santa Cruz, Heidelberg, Germany |
| anti-tubulin-α | 1:250 | BioLegend, San Diego, USA |
| Cy™2 anti-rabbit IgG | 1:200 | Dianova, Hamburg, Germany |
| Cy™2 anti-mouse IgG | 1:200 | Dianova, Hamburg, Germany |
| Cy™3 anti-rabbit IgG | 1:400 | Dianova, Hamburg, Germany |
| Cy™3 anti-mouse IgG | 1:200 | Dianova, Hamburg, Germany |

**Supplementary Table S4.** Primer sequences for qRT PCR

| **Target gene** | **Sequence of forward primer 5‘-3‘** | **Sequence of reverse primer 5‘-3‘** |
|---|---|---|
| *DDIT3* | TTGCCTTTCTCCTTCGGGAC | TGATTCTTCCTCTTCATTTCC |
| *APOBEC3B* | CCATCCTCTATGGTCGGAGC | GAGGCTTGAAATACACCTGGC |
| *APOBEC3G* | GCATCGTGACCAGGAGTATGA | GTCAGGGTAACCTTCGGGT |
| *MOV10* | GGGCCAGTGTTTCGAGAGTTT | TCTTGGTGACGTAGGCCAGA |
| *HSPA5* | GAACGTCTGATTGGCGATGC | TCAACCACCTTGAACGGCAA |
| *sXBP1* | CTGAGTCCGCAGCAGGTG | ATGACTGGGTCCAAGTTGTCC |
| *ATF4* | TCCAACAACAGCAAGGAGGAT | TCCAACGTGGTCAGAAGGTC |
| *GAPDH* | ACCCAGAAGACTGTGGATGG | TTCTAGACGGCAGGTCAGGT |
| *K113 ENV* | CCTTGTGTGCCTGTTTTGTC | ATCTCTCTTGCTTTTCCCCACA |
| *coK113 ENV* | TGTCTGCTGCTGGTGTATAGG | ATCTGGTCCCTTTTGCTTTTGC |
| *Neo$^{R}$* | AGACAATCGGCTGCTCTGAT | AGTGACAACGTCGAGCACAG |
| *HPRT1* | ACCAGTCAACAGGGGACATAA | CTTCGTGGGGTCCTTTTCACC |

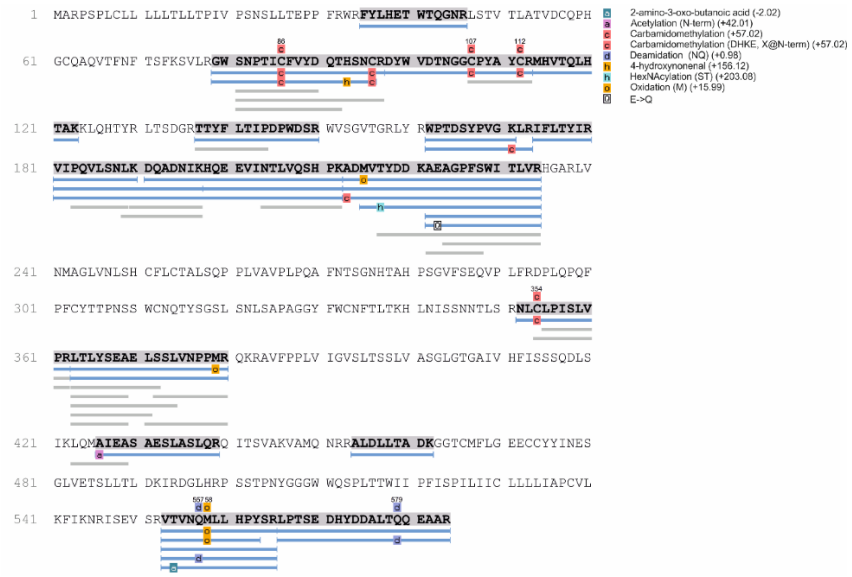
**Supplementary Figure S1**



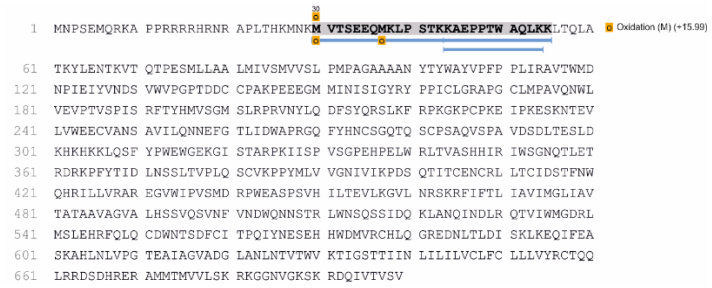

**Supplementary Figure S1.** Representative Western Blot Image showing the expression of K113 and codon-optimized coK113 in HEK 293 cells in relation to the loading control ACTB (beta-Actin).

## Supplementary Figure S2

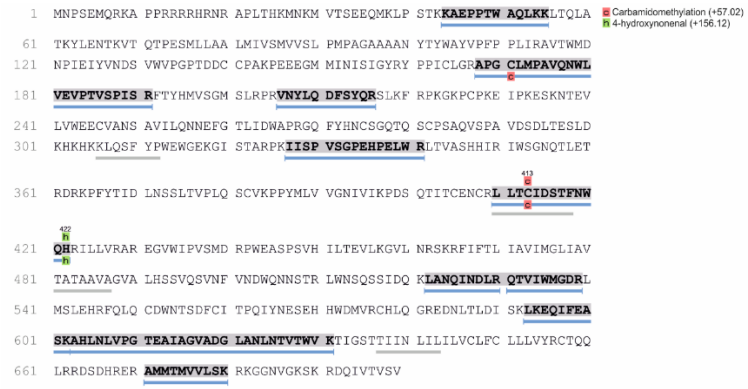
**a**




**b**

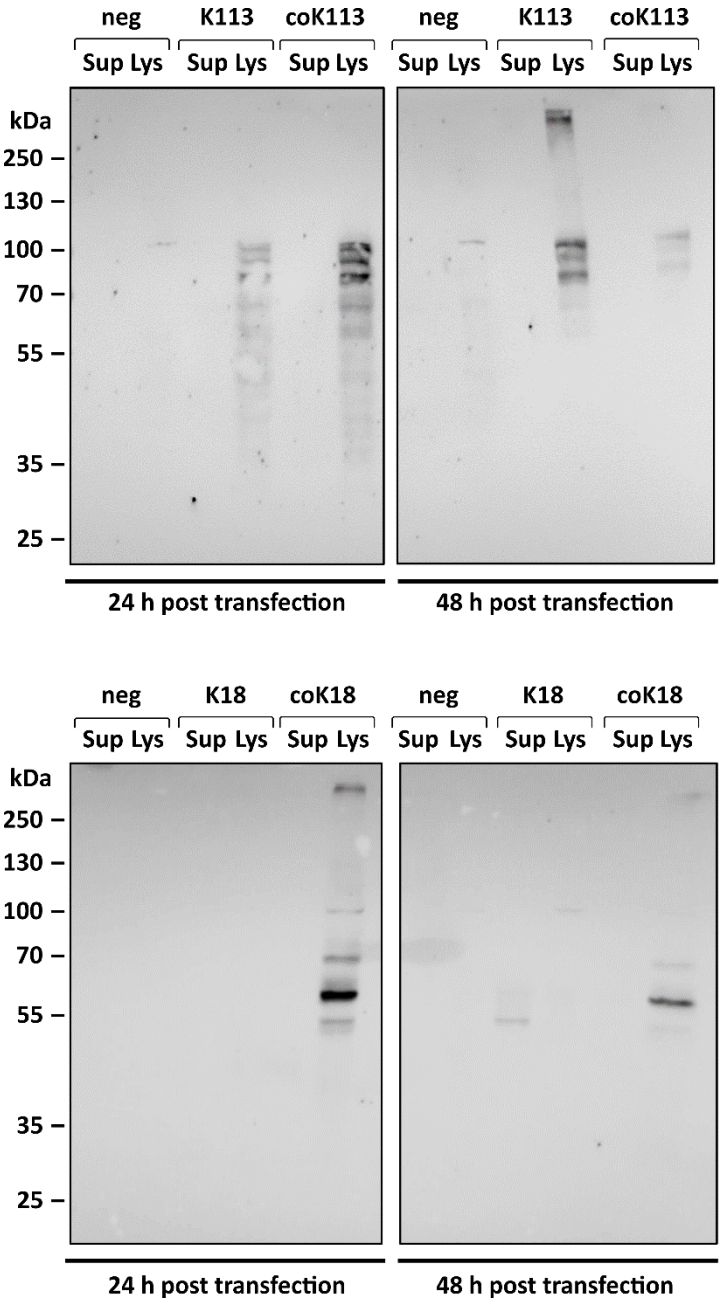



**c**



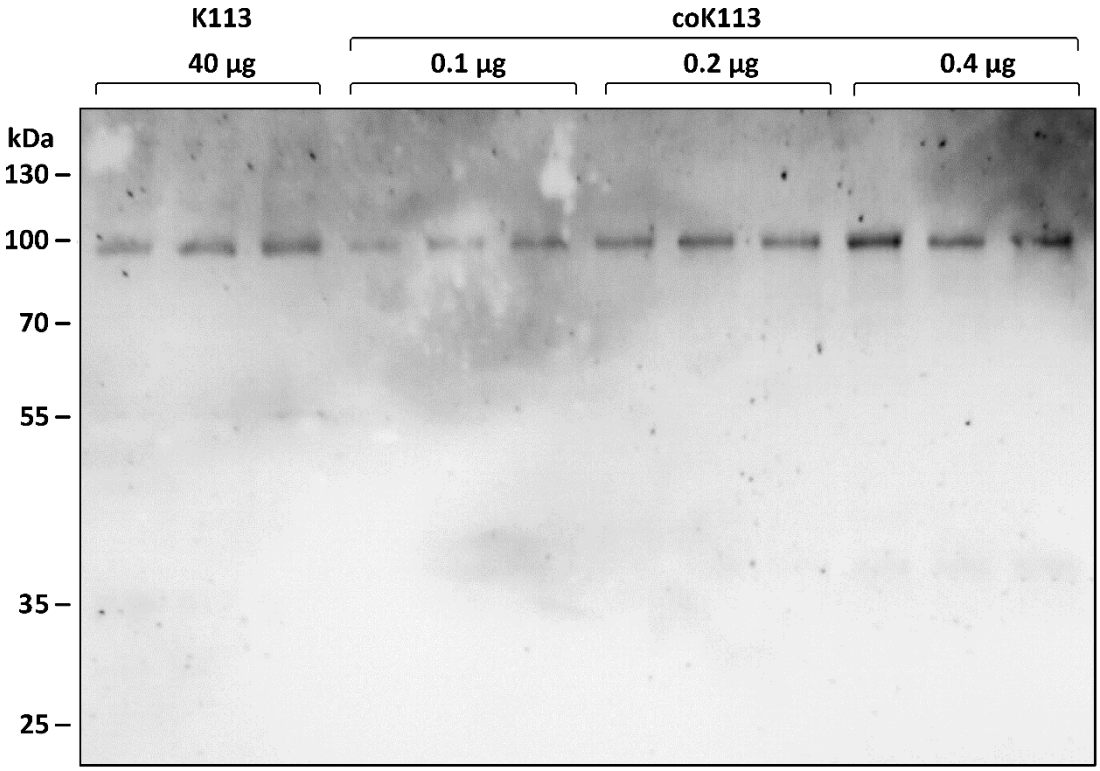

**Supplementary Figure S2.** Sequence coverage for the envelope proteins of the (a) endogenous retrovirus group Fc1 (UniProt: P60507) identified in HEK293 cells expressing coFc1, (b) endogenous retrovirus group K member 113 (UniProt: Q902F9) identified in HEK293 cells expressing K113 and (c) endogenous retrovirus group K member 113 (UniProt: Q902F9) identified in HEK293 cells expressing coK113.

## Supplementary Figure S3



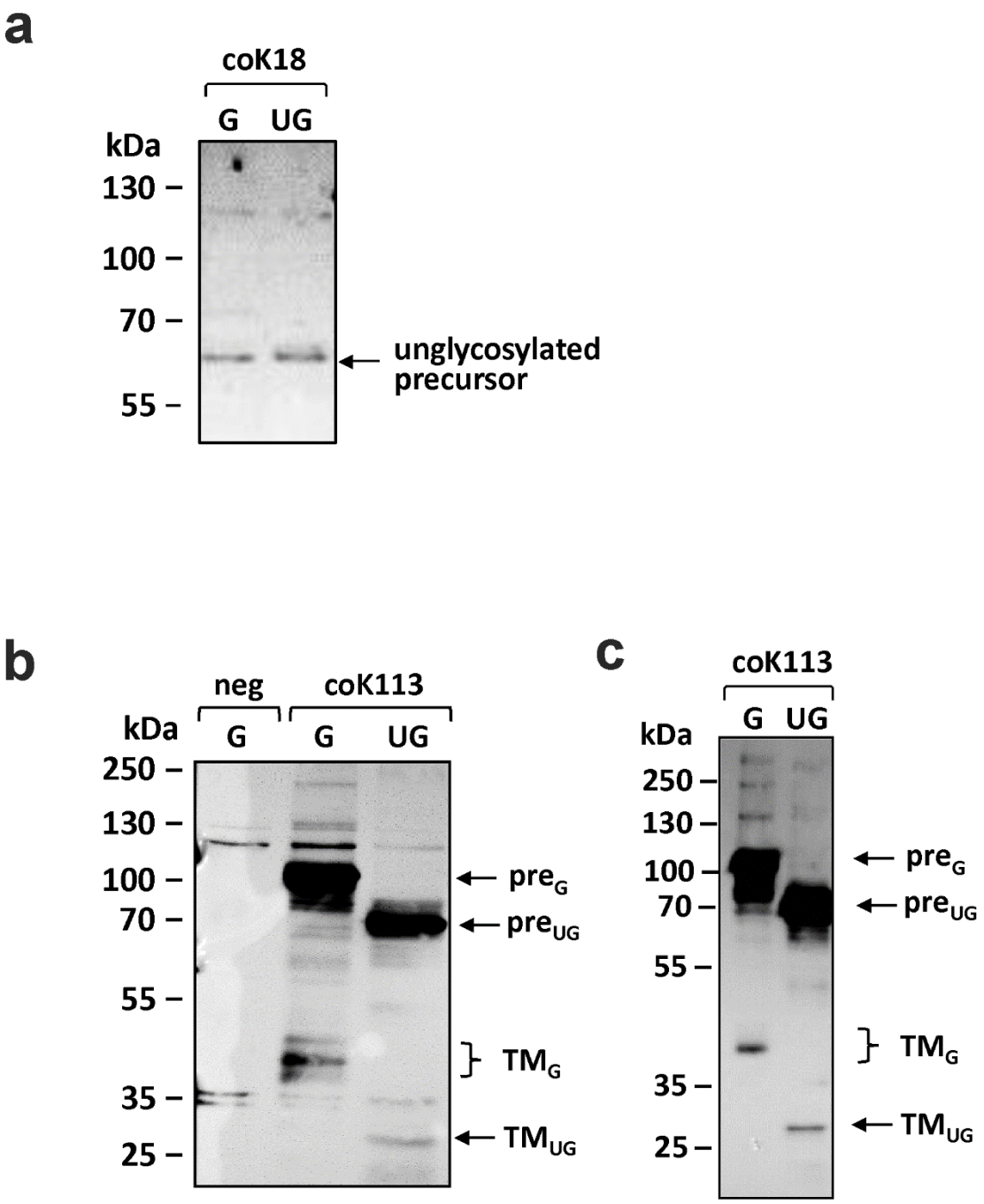

**Supplementary Figure S3.** Time-dependent expression of WT and codon-optimized variants of K113 and K18. Cell harvest was 24h and 48h post-transfection. For each time-point, 40 µg of cell lysate (Lys) and 20 µl of cell culture supernatant (Sup) were analyzed using a HERV-K antibody specific for the surface unit. HERV expression was compared to empty vector control (neg).

**Supplementary Figure S4**



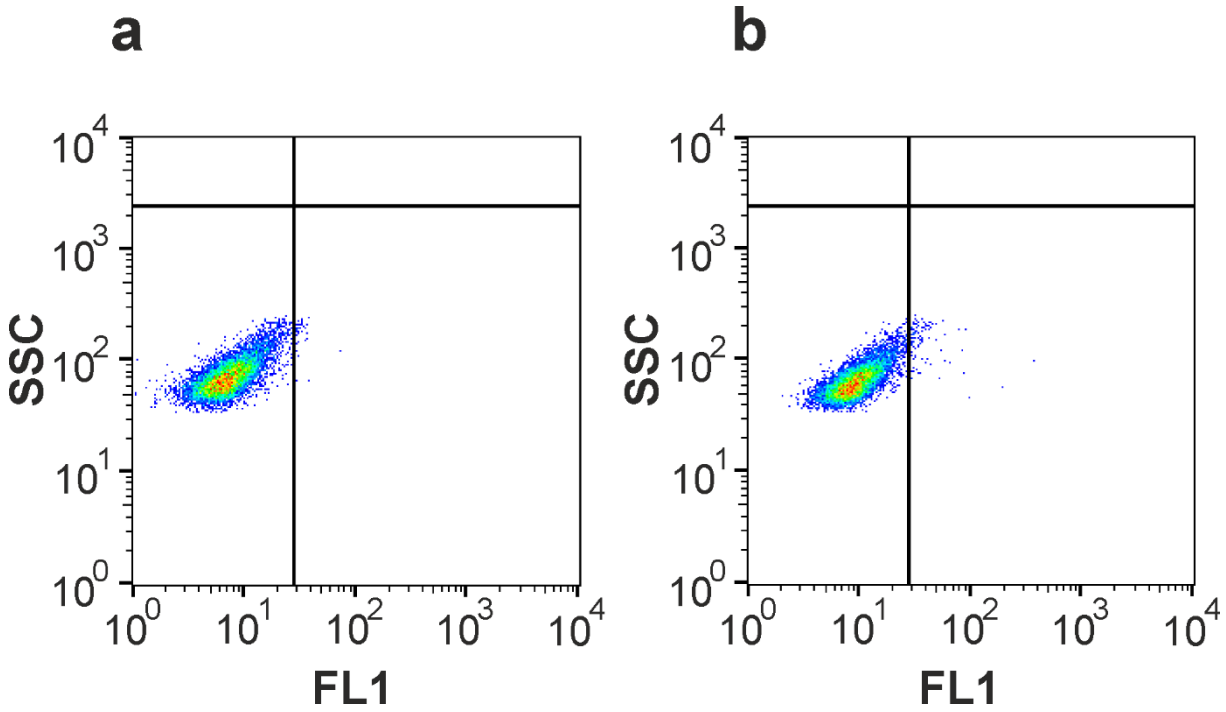

**Supplementary Figure S4.** Entire image of the blot used for densitometric quantification of expression increase caused by codon-optimization and depicted as Fig. 1d in the main body of the manuscript.

**Supplementary Figure S5**



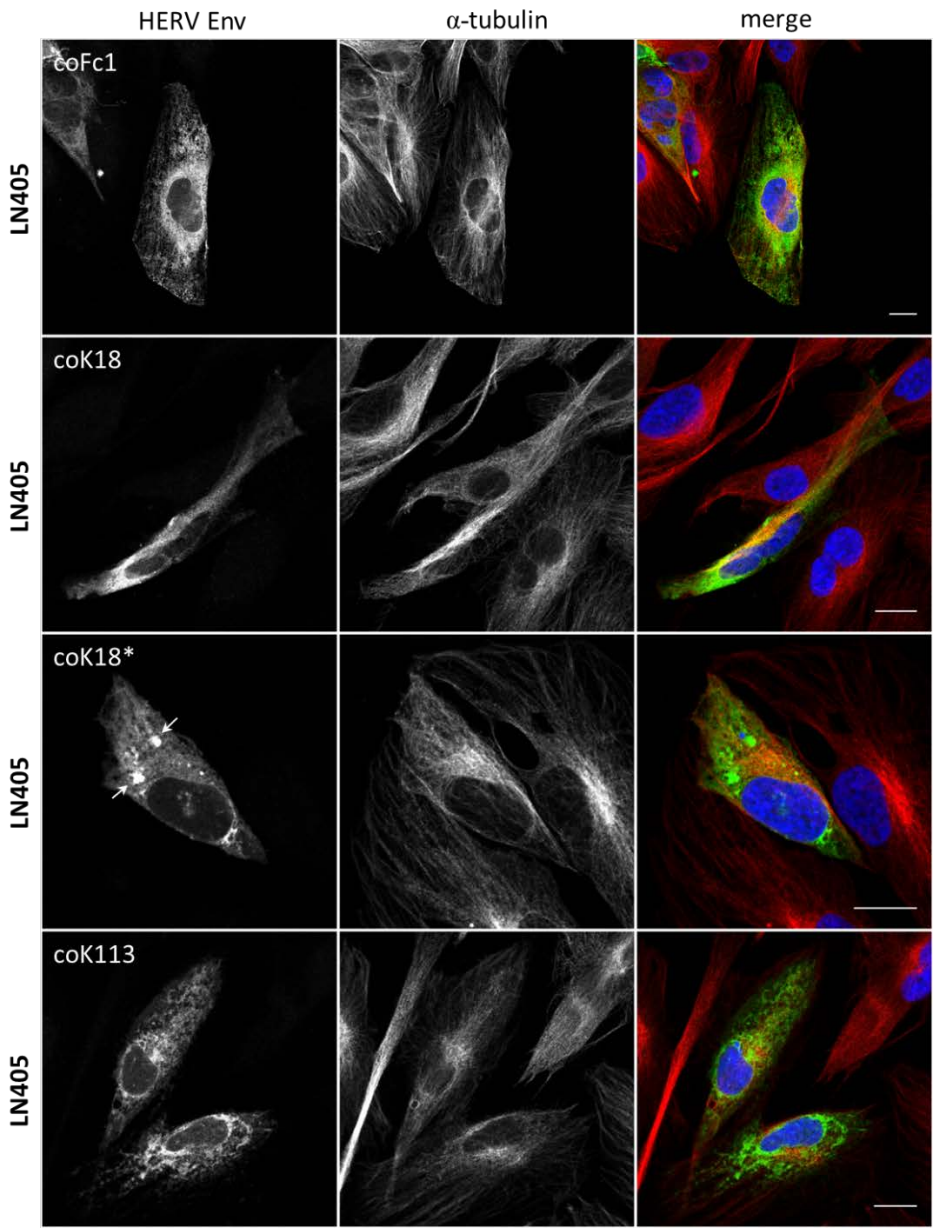

**Supplementary Figure S5.** (a) No glycosylation of HERV-K18 ENV without signal peptide in transfected HEK293 cells. Cell lysates were analyzed via Western blot 24 h after transient transfection of HEK293 cells with expression plasmids containing codon-optimized (co) sequence of the envelope proteins of HERV-K18 and subsequent treatment with (UG) or without (G) de-glycosylating agent PNGase F. Each lane was loaded with 20 µg total protein. (b) and (c) The cleavage of the precursor (pre) of coK113 into SU and TM is visible, whereby only the precursor and TM were detected by the anti-HERV-K TM HERM1811-5 antibody (two independent experiments). In (b) empty vector-transfected cells served as negative control (neg).

**Supplementary Figure S6**




**Supplementary Figure S6.** Dot plots of HEK293 as determined by FACS analysis. (a) Native HEK293 cells were compared to (b) HEK293 cells incubated with Cy2-labled secondary antibody goat anti-mouse IgG. No unspecific adhesion to HEK293 cells was observed.

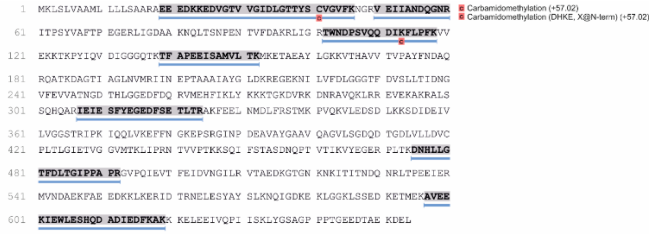
**Supplementary Figure S7**



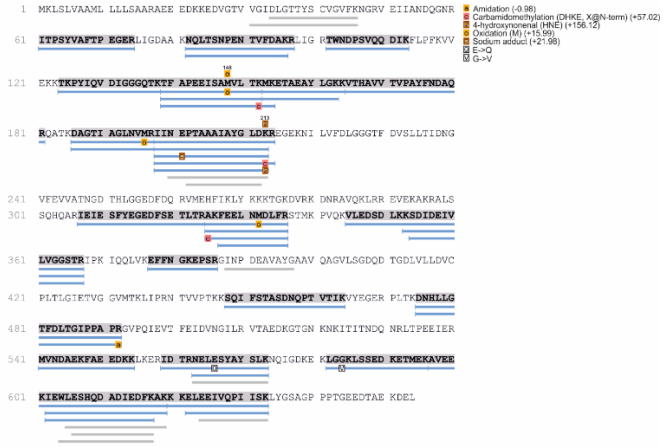

**Supplementary Figure S7.** Localization of HERV ENV with α-tubulin in transfected LN405 cells. Fluorescence microscopic images showing the subcellular localization of specified HERV ENV and α-tubulin in transiently transfected LN405 cells. The merge image is a z-projection (maximum intensity) of the 3 recorded channels (green: HERV ENV, red: α-tubulin, blue (DAPI): cell nucleus). Pixels with red and green fluorescence appear yellow. The detection of the envelope proteins was performed using anti-HERV-K-TM antibody HERM1811-5 (coK18, coK18*, coK113) or EGFP tag (Fc1). The α-tubulin was visualized by an anti-tubulin-α antibody. Scale bar: 20 µm.

## Supplementary Figure S8

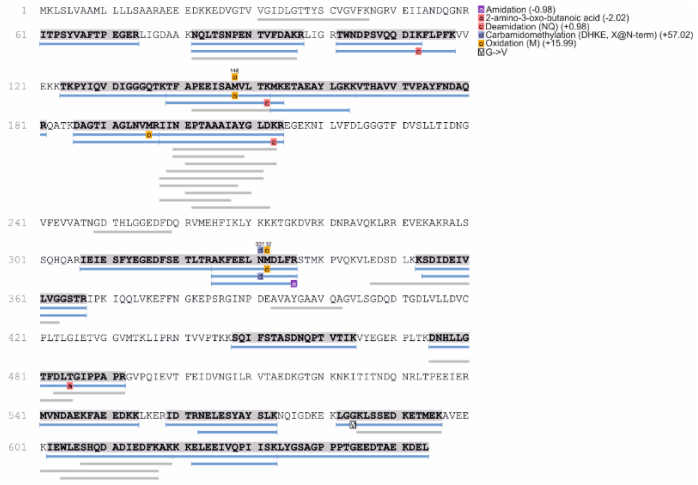
### a



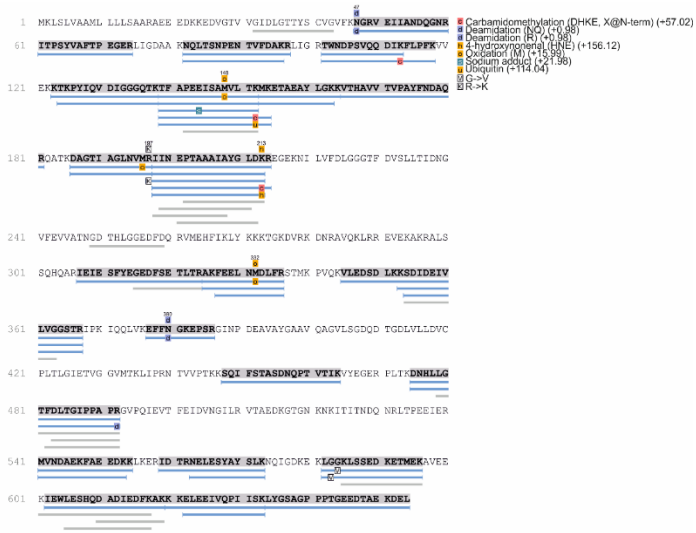

### b



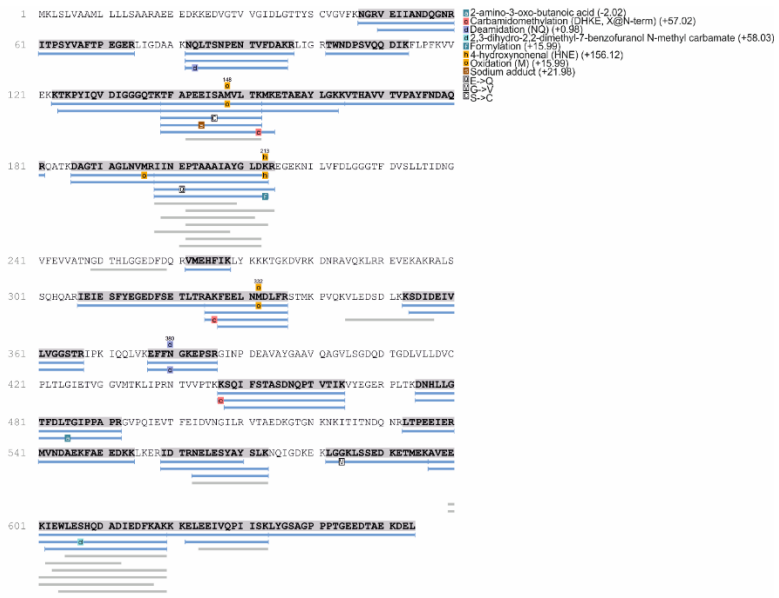

### c



**d**

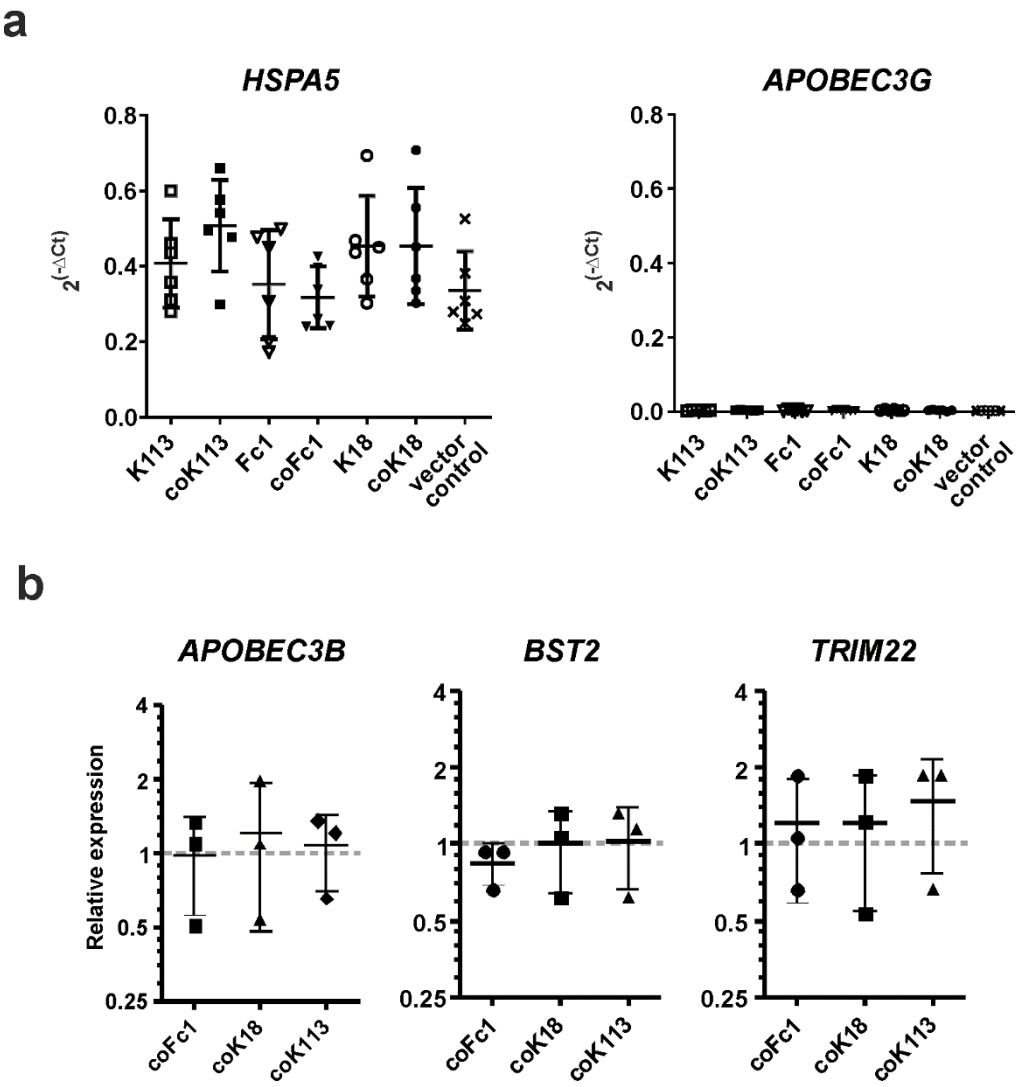



**e**



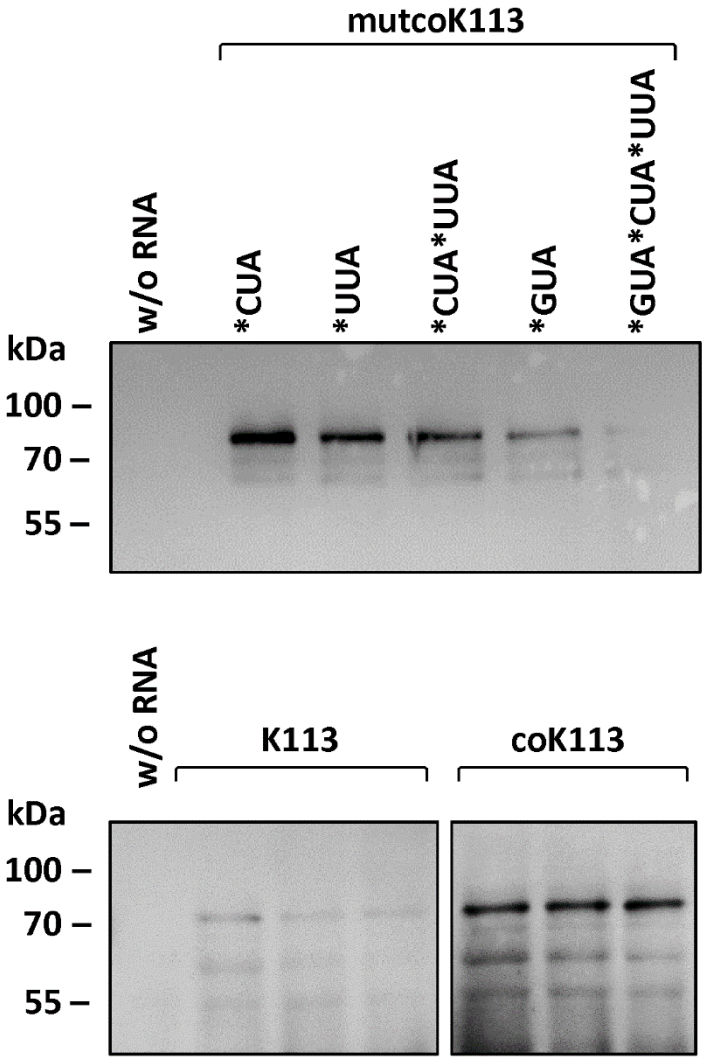

**Supplementary Figure S8.** Sequence coverage for the endoplasmic reticulum chaperone BiP (UniProt: P11021) identified in (a) the empty vector (pcDNA3.1)-expressing HEK293 cells, (b) K113-expressing HEK293 cells, (c) coK113-expressing HEK293 cells, (d) Fc1-expressing HEK293 cells and (e) coFc1-expressing HEK293 cells.

**Supplementary Figure S9**



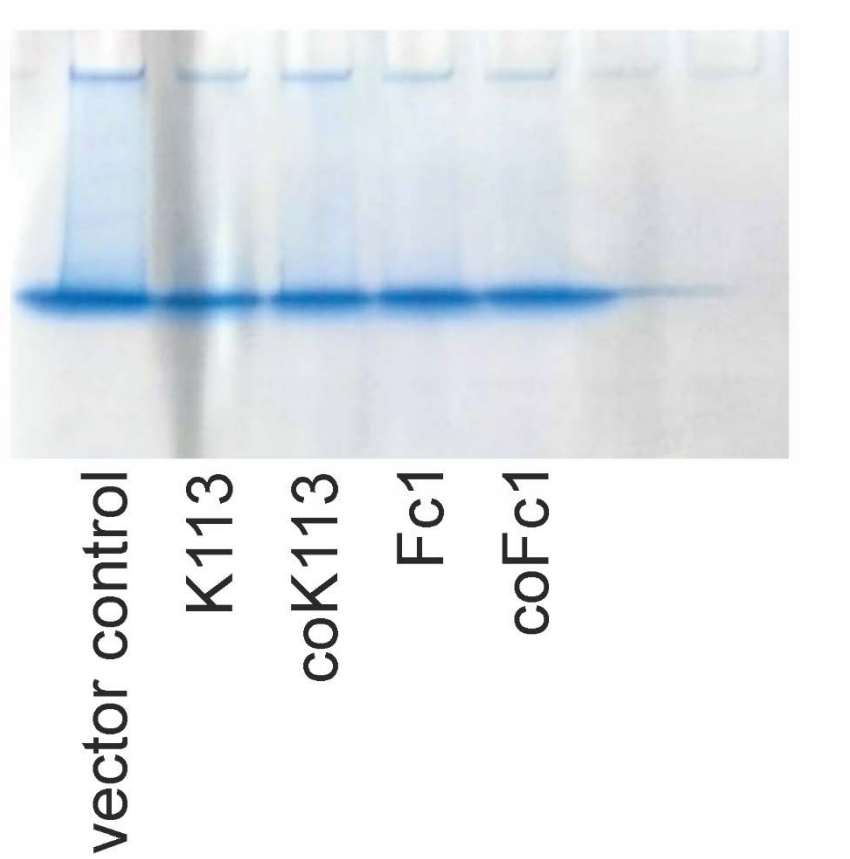

**Supplementary Figure S9.** (a) Comparison of $2^{(-\Delta Ct)}$ values for *HSPA5* and *APOBEC3G* in HEK293 cells. Expression of *HSPA5* and *APOBEC3G* was analyzed in transfected HEK293 cells by quantitative PCR. The expression was normalized to *GAPDH*. (b) Relative gene expression of additional genes involved in viral defense mechanisms after transfection of codon-optimized HERV envelope protein sequences studied in COS-7 cells (*APOBEC3B*) or HEK293 cells (*BST2*, *TRIM22*). The expression was normalized to *GAPDH* and relative to empty vector-transfected cells (dashed grey line)

**Supplementary Figure S10**




**Supplementary Figure S10.** Cell-free expression of HERV-K113 envelope variants. 4 µg of transcribed mRNA of K113 envelope sequences were translated into protein using reticulocyte lysate from New Zealand white rabbits including mixture of all components necessary for translation. Three independent experiments of wildtype and codon-optimized and one experiment of mutant variants of codon-optimized K113 envelope protein are shown. A translation reaction without RNA served as negative control. An equal aliquot of all reactions was loaded for Western blotting. The non-glycosylated precursor of K113 ENV was observed at 80 kDa using anti-HERV-K-TM (HERM1811-5) antibody. Densitometric analysis of expression level is shown in Figure 7 of the manuscript.

**Supplementary Figure S11**




**Supplementary Figure S11.** 6 % PAA stacking gel used to focus cellular lysates that were subsequently applied to tryptic digestion and LC-MS/MS analysis.