**Supplemental figure legends**

**Supplementary Figure S1. Antiviral effect of exogenous HS and heparin, and enzymatic removal of HS on the infectivity of RABV CVS in A549 cells.**

(A) Surface expression levels of NCAM on A549 cells were examined by flow cytometry. (B and C) RABV CVS was mixed with the indicated concentrations of HS (B) and heparin (C), and then inoculated to A549 cells. (D and E) A549 cells were incubated with HS (D) or heparin (E) and substantially washed with PBS, prior to the infection with RABV CVS. (F) A549 cells were pretreated with heparinase III and then infected with RABV CVS. RABV-infected cells were stained with anti-RABV N antibody and Hoechst 33342, and counted by imaging cytometry. The values in the graphs are shown as means ± S.D. of triplicates from a representative experiment. Statistical analyses were performed using one-way ANOVA with Dunnett’s test. \* *p* < 0.01, significantly different from the control.

**Supplementary Figure S2. Competitive inhibition of RABV CVS entry with recombinant G protein.**

RABV CVS were inoculated to SK-N-SH cells in the presence of recombinant soluble CVS-G (A) or bovine serum albumin (B) as a negative control. RABV-infected cells were stained with anti-RABV N antibody and Hoechst 33342, and counted by imaging cytometry. The values in the graphs are shown as means ± S.D. of triplicates from a representative experiment. Statistical analyses were performed using one-way ANOVA with Dunnett’s test. \* *p* < 0.01, significantly different from the control.

**Supplementary Figure S3. Endogenous expression levels of HS sulfotransferases on SK-N-SH cells.**

The mRNA expression profiles of sulfotransferases on SK-N-SH cells were analyzed by RT-qPCR. The expression levels of the indicated genes were normalized to the mRNA levels of *β-actin*. The values in the graphs are shown as means ± S.D. of triplicates.

**Supplementary Figure S4. Cell viability measurement after gene knockdown.**

SK-N-SH cells were transfected with siRNAs targeting the indicated genes. Three siRNAs were assayed for each gene knockdown. Cell viability of individual siRNA transfected cells were measured by CellTiter-Glo luminescent cell viability assay. The luminescence values in the graphs are shown as means ± S.D. of triplicates. RLU, relative light units.

**Supplementary Figure S5. Effect of the treatment with exogenous HS, heparin and heparinase on the infectivity of RABV HEP strain.**

(A and B) RABV HEP was mixed with the indicated concentrations of HS (A) or heparin (B), and then inoculated to SK-N-SH cells. (C) SK-N-SH cells were pretreated with the indicated concentrations of heparinase III and then infected with RABV HEP. RABV-infected cells were identified by indirect immunofluorescence with anti-RABV N antibody and Hoechst 33342, and counted by imaging cytometry. The values in the graphs are shown as means ± S.D. of triplicates from a representative experiment.

**Supplementary Figure S6. Results of positional scanning with RABV G mutants to identify amino acid residues involved in heparin-neutralization.**

VSVΔG\* bearing the indicated G mutant proteins of RABV were pretreated with 100 μg/ml of heparin and then inoculated onto SK-N-SH cells. Infection with pseudotyped VSVs was identified by GFP reporter expression. The 50% reduction of infectivity by heparin treatment was judged as neutralization.

**Supplementary Figure S7. Effect of heparin treatment on the infectivity of VSVΔG\* pseudotyped with G protein mutants.**

VSVΔG\* pseudotyped with mutant G proteins of CVS (A) or HEP (B and C) were pretreated with heparin and then inoculated to SK-N-SH cells. Infection with pseudotyped VSVΔG\* was identified by GFP reporter expression. Cellular nuclei were stained with Hoechst 33342. The number of GFP-positive cells and total cells were counted by imaging cytometry. Relative infectivity was calculated as a percentage of untreated conditions. The values in the graphs are shown as means ± S.D. of triplicates from a representative experiment.

**Supplementary Figure S8. Comparison of the heparin sensitivity and multiple amino acid sequence alignment with G proteins of RABV fixed and street strains.**

(A) VSVΔG\* pseudotyped with G proteins of RABV HEP, CVS, ERA, Nishigahara and 1088 strains were pretreated with heparin and then inoculated to SK-N-SH cells. Relative infectivity was calculated as described in the legend to Figure S7. The values in the graphs are shown as means ± S.D. of triplicates from a representative experiment. (B) Multiple amino acid sequence alignment based on G proteins of RABV fixed strains: CVS, HEP-Flurry, ERA, Nishigahara and street strains: 1088, 9147FRA, 8764THA. GenBank accession numbers for each gene is shown in parentheses. The three amino acid residues of the CVS G protein identified in this study and the corresponding residues in G proteins of other RABV strains are boxed in blue.