Supplementary information

Further Characterization of the Antiviral Transmembrane Protein MARCH8

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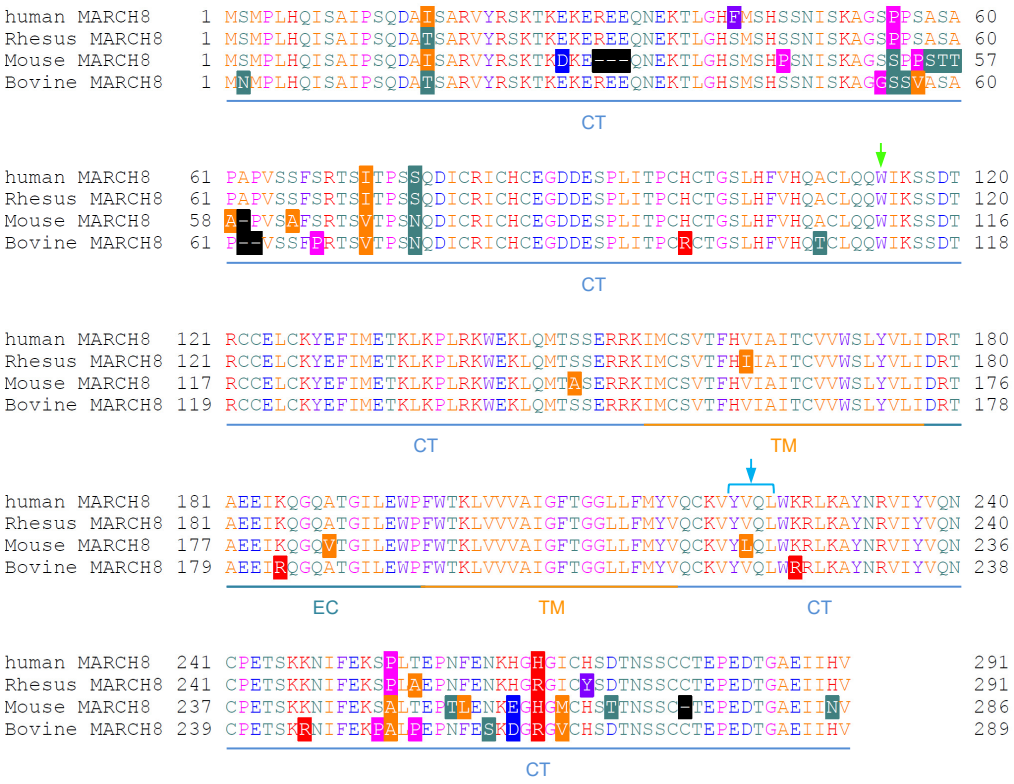

Figure S1. Amino acid sequence alignments of the human, rhesus macaque, mouse, and bovine MARCH8 proteins. Amino acid differences are boxed in several colors (polar neutral in green, aliphatic/hydrophobic in orange, aromatic/hydrophobic in purple, basic in red, acidic in blue, unique in pink, and absence in black). EC, extracellular domain; TM, transmembrane domain; CT, cytoplasmic tail. A well-conserved tryptophan residue, which is essential for ubiquitin ligase activity, indicated by a light green arrow; A well-conserved Yxxϕ motif, indicated by a light blue arrow.

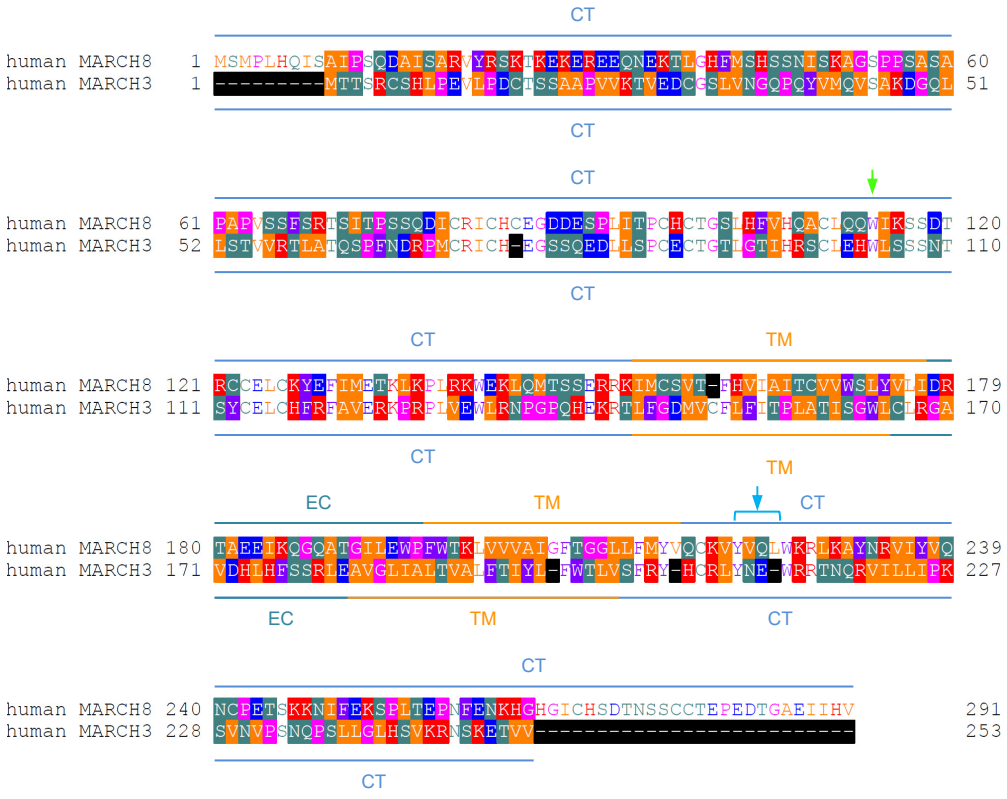

Figure S2. Amino acid sequence alignments of the human MARCH8 and MARCH3 proteins. Amino acid differences are boxed as depicted in Figure S1. EC, extracellular domain; TM, transmembrane domain; CT, cytoplasmic tail. A well-conserved tryptophan residue among mammalian MARCH proteins, which is indicated by a light green arrow, is also conserved in the human MARCH3 protein. MARCH8's Yxxϕ motif in the C-terminal CT, indicated by a light blue arrow, is not seen in MARCH3.

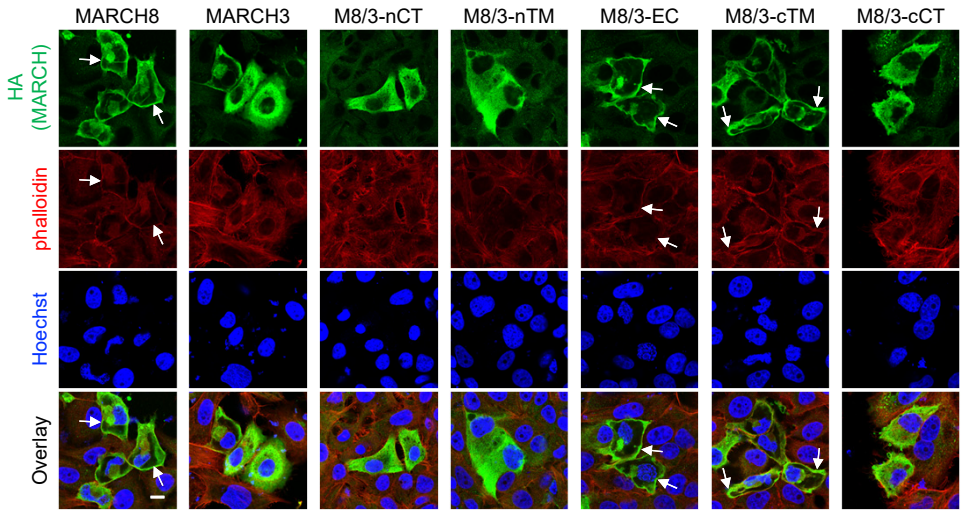

Figure S3. Subcellular localization of MARCH8, MARCH3, and their chimeric proteins. HeLa cells transfected with HA-tagged MARCH protein plasmids were analyzed using immunofluorescence microscopy. Anti-HA monoclonal antibody (primary) and Alexa 488–conjugated anti-mouse IgG (secondary) were used for detecting MARCH proteins (green). Hoechst 33342 (blue) and Phalloidin-TRITC (red) were used to stain the nucleus, and to provide cell morphology, respectively. MARCH8, M8/3-EC, and M8/3-cTM were detected at the plasma membrane (arrows), whereas MARCH3, M8/3-cCT, M8/3-nTM, and M8/3-cTM were exclusively cytoplasmic. Bar, 10 μm.

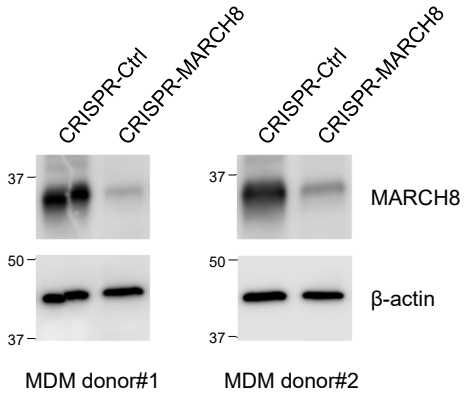

Figure S4. Lentiviral CRISPR-Cas9–mediated knockout (right) of MARCH8 expression in MDMs. Immunoblot images showing the expression of MARCH8 and β-actin in MDMs obtained from two donors. Lentiviral CRISPR-Cas9 technology was employed to knock out MARCH8 expression in MDMs.