Title: Specific and versatile monoclonal antibodies for hantavirus research

Running title: Novel anti-hantavirus antibodies

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Abstract

Rodent-borne hantaviruses pose a continual public health threat to humans through zoonotic transmission, with case fatality rates of up to 50% in some cases. Human infections can lead to hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardio-pulmonary syndrome (HCPS), depending on the viral species. Despite the morbidity and mortality associated with this family of viruses, no anti-viral therapeutics or vaccines are available to treat and prevent hantavirus disease. The relative shortage of commercially available reagents to study hantavirus infections *in vitro* and *in vivo* likely contributes to the challenges in developing viral countermeasures. This report describes the generation of a panel of mouse monoclonal antibodies that collectively recognize the four viral proteins of Seoul virus (*Orthohantavirus seoulense*), an Old World hantavirus with worldwide distribution and the causative agent of HFRS. We have validated the specificity and versatility of these antibodies against a subset of Old World and New World hantaviruses in assays relying on antigen recognition in denatured or native conformations. We present several antibodies that specifically recognize the Seoul virus nucleoprotein and polymerase protein in Western blotting and immunostaining assays. We also identified three novel antibodies directed against the glycoprotein complex that are capable of binding to the N-terminal glycoprotein of all hantaviruses tested. These antibodies are freely available to all hantavirus researchers to add to the small, but growing, collection of reliable and available reagents to be used to study hantavirus biology, identify novel antiviral compounds, and measure viral prevalence in the laboratory and the field.

Importance

Pathogenic hantaviruses cause severe hemorrhagic disease and pose a significant public health threat world-wide. Insufficient research into the biology of these viruses has slowed the development of effective direct-acting antivirals and vaccines. Here, we describe the generation and validation of novel, specific monoclonal antibodies for the detection of Seoul virus proteins

51 *in vitro*. These reagents can be used to fill in critical gaps in knowledge regarding hantavirus
52 entry, protein expression, and particle generation.
53
54 **Introduction**
55 Hantaviruses (genus *Orthohantavirus*, order *Elliovirales*, class *Bunyaviricetes*) are zoonotic
56 segmented, negative-sense RNA viruses that can cause severe hemorrhagic disease in
57 humans. Old World hantaviruses (OW) are distributed throughout Southeast Asia, Russia, and
58 Europe and cause a form of hantavirus disease termed Hemorrhagic Fever with Renal
59 Syndrome (HFRS). New World hantaviruses (NW) are found in rodent reservoir populations in
60 the Americas and are responsible for Hantavirus Cardio-Pulmonary Syndrome (HCPS).
61 Infections with OW hantaviruses are more common with high morbidity but lower case-fatality
62 rates (1-15%) compared to NW hantavirus infections (30-45%). Despite the public health
63 concern, there are no FDA approved vaccines or targeted therapeutics for hantavirus infections.
64
65 The type species, *Orthohantavirus hantanense* (Hantaan virus, HTNV), was first isolated in
66 1978, and NW hantaviruses were discovered in 1993 (1-4). Yet much of the basic biology of this
67 family of viruses remains underexplored. This can be explained, in part, due to a scarcity of
68 tractable tools and models to study hantavirus infections *in vitro* and *in vivo*, especially
69 compared to other segmented viruses, such as influenza. We report here the generation of
70 monoclonal antibodies targeting the OW *Orthohantavirus seoulense* (Seoul virus, SEOV)
71 nucleoprotein, glycoprotein complex, and polymerase and demonstrate their use for Western
72 blotting and immunostaining assays. We tested these antibodies for specificity against SEOV
73 and other NW and OW hantaviruses, finding cross-reactivity only in a subset of anti-glycoprotein
74 antibodies. Several of the antibodies generated can be used to detect denatured proteins in cell
75 lysates as well as native proteins in immunoassays. These tools will be made available for the
76 research community through public repositories and have widespread applicability to test

77 antiviral efficacy, study pathogenesis in culture models, and to explore virus-host interactions for
78 this understudied virus family.
79
80 **Results**
81 Antibody development
82 Through Genscript, we purchased custom-generated hybridomas from mice immunized against
83 the four SEOV proteins: nucleoprotein (N), N-terminal glycoprotein (G_n), C-terminal glycoprotein
84 (G_c), and polymerase (L). Mice were immunized with linear peptides from Gn
85 (WRKKANQESANQNSC), Gc (CGDPGDVMGPKDKPF), or L (LEKKVIPDHPSGKTC). For N,
86 the entire recombinant protein was synthesized as the target antigen for immunization. From the
87 immunization scheme, we received five hybridomas per protein target, selected for antigen
88 reactivity by peptide ELISA. Supernatants from the hybridomas were purified on protein A/G
89 columns, concentrated, and tested for reactivity and specificity by Western blot and
90 immunostaining assays, as described below.
91
92 Nucleoprotein
93 The hantavirus nucleoprotein coats the viral RNA, plays a critical role in viral transcription and
94 translation, and is abundantly expressed in infected cells. We tested antibodies from each of the
95 five hybridomas generated against the SEOV nucleoprotein for reactivity by Western blot (WB),
96 immunofluorescence (IFA), and focus-forming unit assay (FFU). To test for specificity against
97 SEOV N, we transfected HEK293T cells with plasmids expressing the N protein from the OW
98 hantaviruses SEOV and HTNV, as well as the NW hantaviruses *Orthohantavirus*
99 *sinnombreense* (Sin Nombre, SNV) and *Orthohantavirus andesense* (Andes virus, ANDV).
100 Although expression for HTNV N was low, all ectopically-expressed proteins were detected in
101 the lysates by their HA tag but only SEOV N was detected by the monoclonal antibodies (Fig
102 1a). Next, we tested the specificity of these antibodies in immunofluorescence assays (IFA).

103 HEK293T cells were transfected with the N expression plasmids used above for SEOV, HTNV,
104 SNV, and ANDV. Two days post-transfection, the cells were fixed, permeabilized, and probed
105 with the monoclonal antibodies followed by an anti-mouse IgG fluorescent secondary. We
106 observed that all transfected N proteins were detectable with the anti-HA primary antibody, but
107 only SEOV N was detected with the anti-N monoclonal antibodies (Fig 1b, c). Fig 1c shows the
108 absence of N staining for HTNV, SNV, and ANDV N using a representative anti-N, clone 17E11.
109 Importantly, very low background staining was detected in mock transfected cells, supporting
110 the utility of these antibodies for IFA (Supp Fig 1a).
111 We then asked whether these antibodies could detect N during infection. We infected Vero E6
112 cells with SEOV, HTNV, SNV, or ANDV for 12 days and performed WB on collected cell lysates
113 (Fig 2a). Our standard hantavirus propagation protocols call for 12-day growth in Vero E6 cells
114 as the peak of viral production. Therefore, this time point was chosen to be reasonably assured
115 of abundant viral protein in cell lysates for all hantaviruses tested. Again, we see that the SEOV
116 N antibodies are highly specific for SEOV N and can be used to assess SEOV infection status in
117 susceptible cells. Similar results were observed when probing hantavirus-infected Vero E6 cells
118 for IFA (Fig 2b). Again, we noted very low background staining for these antibodies on mock-
119 infected cells (Supp Fig 1b). Finally, we tested the five anti-N antibodies for their efficacy in FFU
120 assays. Hantaviruses are typically non-cytopathic in healthy cells and therefore do not form
121 plaques. Instead, virus titrations can be executed by immunostaining. Vero E6 cells were
122 infected with serial dilutions of SEOV for 1 hour, after which a methylcellulose overlay was
123 added, and cells were incubated for 7 days post-infection. Cells stained with each of the anti-N
124 antibodies showed well-defined foci of infection with very low background staining of uninfected
125 monolayers (Fig 2c). These monoclonal antibodies, therefore, are specific for the SEOV
126 nucleoprotein and are effective in denaturing WB conditions and IF assays.
127
128 <u>Glycoproteins</u>

129 The hantavirus M segment encodes a precursor glycoprotein that is co-translationally cleaved at
130 a conserved WAASA site into two mature glycoproteins (G_N and G_C) (5). These glycoproteins
131 form trimeric complexes in the ER consisting of a G_N dimer and a G_C monomer (known as
132 glycoprotein complex, GPC), and are trafficked to the Golgi (6-11). Co-expression of both
133 proteins is necessary for trafficking from the ER to the Golgi, as individual expression results in
134 retention of the proteins in the ER (12-17). We immunized mice with peptides from either G_N or
135 G_C, but found that none of the antibodies raised against G_C-immunization were able to detect
136 glycoproteins from any of the viruses tested either in transfected or virus-infected cells (data not
137 shown). For these experiments, we transfected HEK293T cells with plasmids expressing the M
138 segment sequence for both G_N and G_C (GPC) for SEOV, HTNV, and ANDV. These plasmids
139 encode for the M segment coding region with an HA tag at the C-terminus. Thus, upon
140 expression and cleavage of the polyprotein, only the 52-58kDa G_C protein will be detectable with
141 an anti-HA antibody. SNV GPC was omitted because this plasmid failed to express detectable
142 HA-tagged G_C. Curiously, we found that several anti-G_N antibodies detected a protein migrating
143 at the same size as our viral G_N (~72-74kDa) in transfected HEK293T cells (Fig 3a). This cross-
144 reactive protein was only detected in HEK293T and not in other commonly used cells for
145 hantavirus research (A549, Vero E6, HUVEC-C, and primary rat lung microvascular endothelial
146 cells) (Fig 3b). Surprisingly, we found that three of our tested anti-G_N antibodies (1B12, 11A5,
147 and 12D10) detected the G_N from HTNV, SNV, and ANDV-infected Vero E6 cell lysates by WB
148 (Fig 3c). Unfortunately, none were able to recognize native GPC by IFA (data not shown).
149
150 <u>Polymerase</u>
151 The RNA-dependent RNA polymerase protein of hantaviruses (L) is expressed at low, almost
152 undetectable levels in cultured cells due to its presumed toxicity (18, 19). Even overexpression
153 of plasmid derived L protein has proven difficult without mutations that inhibit its transcriptional
154 activity (18). We generated HA-tagged plasmids for ectopic expression of SEOV L and SNV L in

155 HEK293T cells to test the anti-L antibodies in WB and IF assays (Fig 4a, b). Two of the four
156 antibodies tested were able to detect SEOV L by both assays using transfected HEK293T cells,
157 but none were cross-reactive against SNV L. We also fund that clone 13G8 was able to detect
158 SEOV L from infected Vero E6 cell lysates, but not from cells infected with HTNV, SNV, or
159 ANDV (Fig 4c). Despite considerable effort, we were unable to detect L expression in SEOV-
160 infected cells by IFA (data not shown).
161
162 **Conclusions:**
163 We have characterized novel monoclonal antibodies for their specificity and versatility for
164 detection of hantavirus proteins in exogenous and endogenously expressed conditions. These
165 tools for SEOV detection and quantification should replace less specific and less reliable
166 commercial sources for hantavirus antibodies. We are actively working to make these reagents
167 available to the hantavirus research community though trusted biorepositories, such as BEI
168 Resources.
169
170 **Methods:**
171 Cell culture
172 Vero E6 cells (ATCC, CRL-1586), A549 (ATCC, CCL-185), and HEK293T (ATCC, CRL-3216)
173 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10%
174 heat-inactivated FBS, 1% pen/strep, 1% nonessential amino acids, 2.5% HEPES. Primary rat
175 microvascular endothelial cells (RLMVEC, VEC Technologies) were cultured in MCDB-131 base
176 medium (Corning) supplemented with EGMTM-2 Endothelial SingleQuots Bullet Kit (Lonza, CC-
177 4176) and 10% heat-inactivated FBS. Human umbilical endothelial cells (HUVEC-C; ATCC,
178 CRL-1730) were cultured in Lonza EGM-Plus (Lonza, CC-4542) supplemented with bullet kit
179 (Lonza, CC-5036) and 10% heat-inactivated FBS. All cells were cultured in tissue culture-

180 treated plastics and plate for endothelial cells were coated with rat-tail collagen (VWR, 47747-
181 218).
182
183 Viruses and *in vitro* infections
184 Seoul virus (strain SR11), Hantaan virus (strain 76-118) Sin Nombre virus (strain NMR11), and
185 Andes virus (CHI7913) were propagated on Vero E6 cells (ATCC, CRL-1586) for 12 days, with
186 a maximum of 3 passages. Infectious virus was isolated by harvesting supernatant and
187 centrifuging at 1000 rpm for 10 minutes to remove cellular debris. For virus infections, cells were
188 seeded in cell culture vessels 18-24 hours prior to infection at a target density of 70%. Virus
189 stock was diluted to the target MOI using serum-free Dulbecco's modified Eagle's medium
190 (DMEM) supplemented with 1x pen/strep, 1% nonessential amino acids, and 2.5% HEPES.
191 Virus was allowed to adsorb for 1 hour at 37ºC. Cells were washed twice with sterile PBS
192 solution (FisherScientific, SH30264FS), and appropriate culture medium was added for the
193 duration of the experiment.
194
195 Antibody preparation
196 Hybridomas were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with
197 10% heat-inactivated FBS, 1% pen/strep, 1% nonessential amino acids, 1% L-Glutamine, 2.5%
198 HEPES, and 1x hybridoma fusion and cloning supplement (HFCS; MilliporeSigma
199 11363735001) for the first passage. Hybridomas were then maintained in in Dulbecco's
200 modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS, 1% pen/strep,
201 1% nonessential amino acids, 1% L-Glutamine, and 2.5% HEPES. Supernatants were collected
202 every 2-3 days when cells were at 90% confluency. They were then centrifuged at 1,000xg for
203 10min to remove cell debris and stored at 4C until purified. To purify antibody, a 2mL Pierce
204 protein A/G agarose column (Thermo Scientific, 20422, 29922) was prepared according to
205 manufacturer instructions. Supernatants from the same hybridoma culture were pooled together

206 and 1/3 volume of Pierce protein A/G IgG binding buffer (Thermo Scientific, 54200) was added
207 to the supernatants. Supernatant and binding buffer were run through the column according to
208 manufacturer instructions. Antibody was eluted from the column using 8mL of Pierce IgG elution
209 buffer (Thermo Scientific, 21004) and neutralized with 400uL 1M Tris pH 7.5. Amicon Ultra-15
210 centrifugal filters, 30kDa MW (MilliporeSigma, UFC903024), were used to concentrate the
211 purified antibody down to 200uL. Antibody concentration was determined via Pierce BCA
212 Protein Assay Kit (ThermoFisher Scientific, 23225) and stocks stored at 1ug/mL.
213
214 Plasmids
215 To validate the detection of hantavirus proteins by each monoclonal antibody, we subcloned
216 each of the described proteins from SEOV, HTNV, SNV, and ANDV into our previously
217 described pCAGGS expression vector (20, 21). This vector contains the coding sequence of
218 each viral protein followed by a C-terminal hemagglutinin (HA) epitope tag (YPYDVPDYA). Viral
219 protein coding sequences were PCR amplified from viral cDNA and subcloned into the vector
220 using Gateway Technology (Invitrogen) following manufacturer's instructions. The nucleotide
221 sequence of each plasmid was verified by DNA sequencing (Plasmidsaurus). Viral protein
222 sequences were subcloned from the following virus strains: SEOV strain SR-11, HTNV strain
223 76-118, SNV strain NMR11, and ANDV strain CHI-7913. For the SEOV L protein plasmid, the
224 previously reported K44A mutation was made to increase expression of L (18).
225
226 Plasmid transfections
227 HEK 293T cells were seeded in 10cm dishes at 2x10^5 cells/mL. Transfections were
228 accomplished 24hrs later using jetPRIME (VWR, 89129-924) according to manufacturer's
229 specifications. Briefly, 5ug (GPC and L) or 10ug (N) of the indicated expression plasmid were
230 diluted in 500uL jetPRIME buffer and 10uL of jetPRIME reagent was added. The mixture was
231 incubated at room temperature for 10min and was then added dropwise to the 10cm dishes of

232 cells. Lysates were harvested 48hrs post-transfection in RIPA buffer (50mM Tris HCl pH 7.4,
233 150mM NaCl, 1% (v/v) NP-40, 0.5% (w/v) Na-deoxycholate) and clarified through 25,000rcf
234 centrifugation for 15 minutes at 4ºC. Protein was quantified via Pierce BCA Protein Assay Kit
235 (ThermoFisher Scientific, 23225). 30ug of protein was loaded per sample into an 8% (L) or 10%
236 (N and GPC) polyacrylamide gel and, after denaturing electrophoresis, transferred to a 0.45µm
237 nitrocellulose membrane (VWR, 10120-006). Membranes were blocked at room temperature in
238 10% FCS in PBS-T. Primary antibodies (1ug/mL) were incubated at 1:500 at 4º overnight. HRP-
239 conjugated secondary antibodies against primary antibody species were incubated 1:10,000 for
240 1 hour at room temperature. Blots were imaged on BioRad Chemidoc MP Imaging System
241 using chemiluminescence Pierce Substrate for Western Blotting (VWR, PI80196).
242
243 Generation of infected-cell lysates
244 Cell lysates to be interrogated for protein expression by immunoblot analysis were harvested in
245 protein lysis buffer 12 days post-infection and prepared as previously described (22). Briefly,
246 protein was harvested in RIPA buffer (50mM Tris HCl pH 7.4, 150mM NaCl, 1% (v/v) NP-40,
247 0.5% (w/v) Na-deoxycholate) and clarified through 25,000rcf centrifugation for 15 minutes at
248 4ºC. Protein was quantified via Pierce BCA Protein Assay Kit (ThermoFisher Scientific, 23225).
249 30ug of protein was loaded per sample into an 8% (L) or 10% (N and GPC) polyacrylamide gel
250 and, after denaturing electrophoresis, transferred to a 0.45µm nitrocellulose membrane (VWR,
251 10120-006). Membranes were blocked at room temperature in 10% FCS in PBS-T. Primary
252 antibodies (1ug/mL) were incubated at 1:500 at 4º overnight. HRP-conjugated secondary
253 antibodies against primary antibody species were incubated 1:10,000 for 1 hour at room
254 temperature. Blots were imaged on BioRad Chemidoc MP Imaging System using
255 chemiluminescence Pierce Substrate for Western Blotting (VWR, PI80196).
256
257 Immunofluorescence assay

258 Vero E6 cells were seeded in 48 well plates at 2.5x10^3 cells/well. When cells had reached 70-
259 80% confluency, they were infected with either SEOV, HTNV, ANDV, or SNV at an MOI of 0.1.
260 Virus was allowed to adsorb for 1hr at 37° C, then cells were washed 2x with 1x PBS and
261 complete DMEM was added. At 4 days post-infection, supernatants were removed and cells
262 were washed 1x with 1x PBS. Cells were fixed with 95% ethanol:5% acetic acid for 10min at -
263 20° C. Cells were permeabilized with 0.01% Triton X-100 in PBS for 15min at room temperature
264 and then blocked with 3% FBS in PBS for at least 30min. Primary antibodies (1ug/mL) were
265 incubated at 1:500 at 4°C overnight. Goat α mouse 647 fluorescent secondary antibodies
266 (Invitrogen A21235) were incubated at 1:400 for at least 2hrs at room temperature. Nuclei were
267 stained with DAPI at 1:1000 for 5min at room temperature. Stained cells were visualized using
268 the 20x objective on the EVOS Cell Imaging System (ThermoScientific). Microscope settings for
269 fluorescence intensity were set on mock-transfected/infected cells to remove background and
270 remained constant for all imaging of transfected/infected cells.
271
272 Focus Forming Unit Assay
273 Infectious virus was quantified using immunostaining as previously described (23). Vero E6 cells
274 were infected with 100μL of culture supernatants and incubated for 2 hours at 37ºC. After
275 incubation, a 2% carboxymethylcellulose overlay containing supplemented DMEM (2%
276 pen/strep, 2% nonessential amino acids, 2% HEPES, 4% heat-inactivated FBS). Cells were
277 incubated for 7 days at 37ºC. After incubation, cells were fixed with 95% EtOH:5% Acetic Acid
278 for 10 minutes at -20ºC, blocked with 3% FBS in PBS, and probed for SEOV N (α SEOV
279 nucleocapsid, 17E11 or others presented here) or HTNV N (anti-HTNV nucleocapsid 76–118,
280 BEI resources NR-12152, cross-reacts with HTNV, SNV, and ANDV) at 4º for 24 hours. HRP
281 conjugated secondary (donkey α rabbit for α HTNV, Jackson Immunoresearch 711-035-152; or
282 goat α mouse for α SEOV, Jackson Immunoresearch 115-035-003) for 2 hours at room

temperature. Foci were then stained using Vector VIP Substrate Kit (Vector Laboratories, SK-4600) and counted under a light microscope to calculate titer.

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377

378 **Figure legends:**

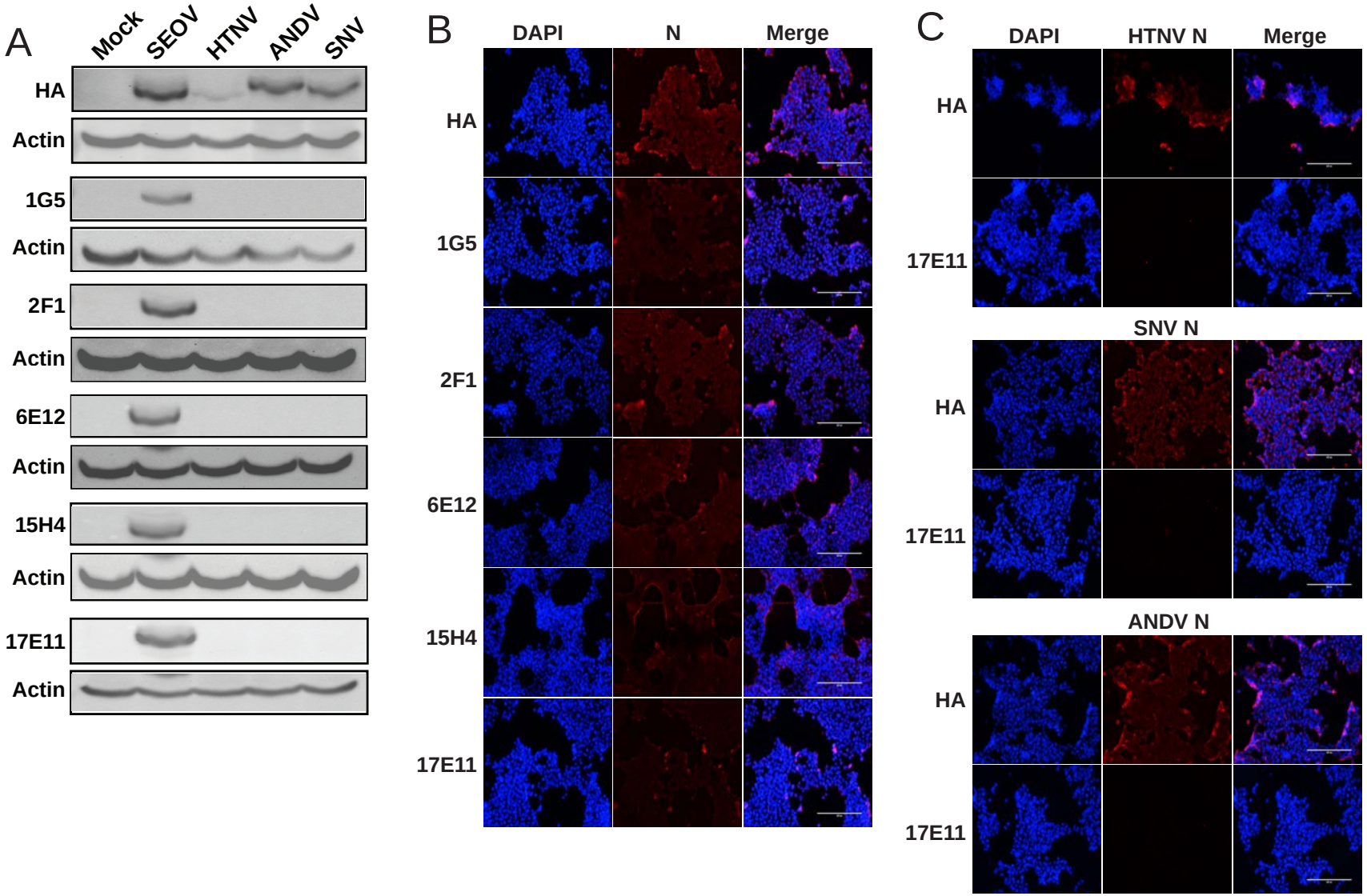
379 **Figure 1. Antibodies specifically recognize SEOV N in transfected cells.** HEK293T cells
380 expressing HA-tagged N protein from SEOV, HTNV, SNV, and ANDV were interrogated by
381 Western blotting and IFA. A) 30ug of cell lysates were loaded into 10% polyacrylamide gels in
382 denaturing conditions and probed for N expression using anti-HA and newly generated anti-
383 SEOV N antibodies. Actin served as a loading control. B) HEK293T cells transfected with SEOV
384 N plasmid, probed using DAPI (nuclei, blue) and anti-HA or antibodies against SEOV N (red). C)
385 HEK293T cells transfected with N plasmids from the indicated virus and probed with anti-HA
386 antibodies or SEOV N antibody 17E11 (red) and DAPI (nuclei, blue). Cells visualized on the
387 EVOS Cell Imaging System.

388

389 **Figure 2. Antibodies specifically recognize SEOV N in hantavirus infected cells.** Vero E6
390 cells were infected with SEOV, HTNV, ANDV or SNV and interrogated by Western blotting (A),
391 IFA (B), and FFU assay (C). A) 30ug cell lysates were loaded into 10% polyacrylamide gels in
392 denaturing conditions and probed for N expression using antibody clones against N. Actin

393 serves as loading control. B) SEOV-infected Vero E6 cells probed with anti-N antibodies (red)
394 and DAPI (nuclei, blue). Cells visualized on the EVOS Cell Imaging System. C) Focus forming
395 unit assay using Vero E6 cells infected with SEOV and incubated with a 2% methylcellulose
396 overlay for 7 days. Cells were probed with anti-N antibody clones, HRP-conjugated secondary
397 antibody, and visualized for foci of stained cells, representing viral infectious units. Mock-
398 infected cells represent non-specific background antibody interactions.
399
400 **Figure 3. Several anti-G_N antibodies recognize G_N from OW and NW hantaviruses.** A)
401 HEK293T cells were transfected with plasmids expressing the indicated hantavirus GPCs from
402 SEOV, HTNV, or ANDV. Each GPC encodes a C-terminal HA tag which, upon cleavage allows
403 for the detection of expressed G_C only (52-58 kDa). Cell lysates were interrogated by Western
404 blotting for recognition by anti-G_N antibodies or the anti-HA antibody. B) Un-transfected cell
405 lysates from several cell types commonly used in hantavirus research were interrogated by
406 Western blotting for cross-reactivity with anti-G_N antibodies. C) Vero E6 cells infected with
407 SEOV, HTNV, ANDV, SNV, or mock infected were analyzed by Western blot for detection of G_N
408 by anti-G_N monoclonal antibodies. Actin served as a loading control.
409
410 **Figure 4. Monoclonal antibodies specifically recognize SEOV L protein.** A) HEK293T cells
411 transfected with plasmids expressing HA-tagged SEOV L or SNV L were analyzed by Western
412 blotting using anti-L monoclonal antibodies. B) SEOV L plasmid transfected HEK293T cells
413 were probed with anti-HA antibody or clones 13G8 or 17F3 (red) and DAPI (nuclei, blue). Cells
414 visualized on the EVOS Cell Imaging System. C) Vero E6 cells infected with SEOV, HTNV,
415 ANDV, SNV, or mock-infected were analyzed by Western blot for detection by anti-L
416 monoclonal antibodies. Actin served as a loading control.
417

418 **Supplementary Figure 1. Anti-N antibodies exhibit low background staining in mock**
419 **transfected or mock infected cells.** Mock-transfected HEK293T cells (A) and mock-infected
420 Vero E6 cells (B) probed with anti-HA and anti-N monoclonal antibodies (red) and DAPI (nuclei,
421 blue). Cells visualized on the EVOS Cell Imaging System.
422
423 **Supplementary Figure 2. Anti-L antibodies exhibit low background staining in mock**
424 **transfected cells.** Mock-transfected HEK293T cells probed with anti-HA and anti-L monoclonal
425 antibodies (red) and DAPI (nuclei, blue). Cells visualized on the EVOS Cell Imaging System.
426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ANDV
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17E11
Actin
B
DAPI
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Merge
HA
1G5
2F1
6E12
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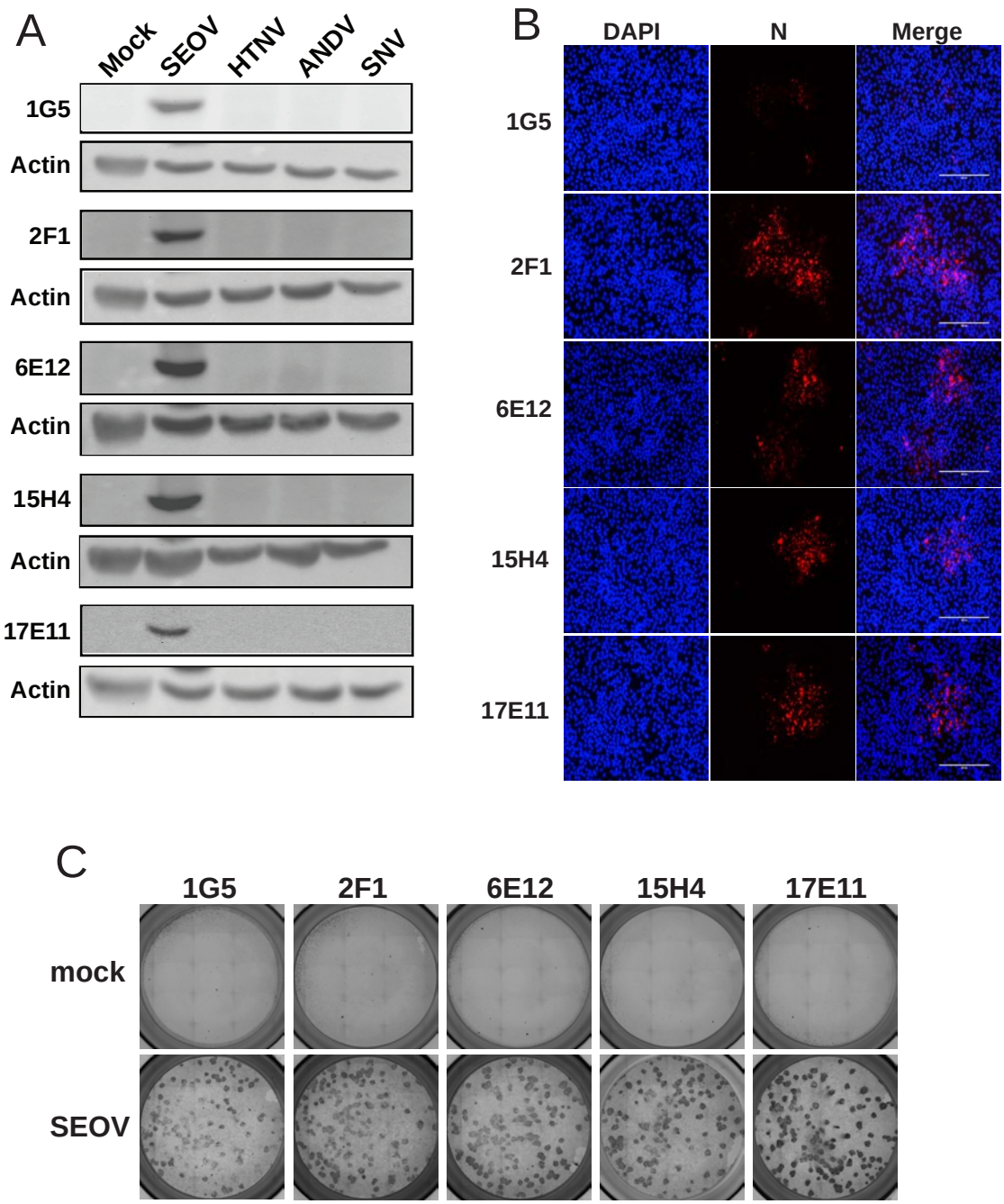
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mock
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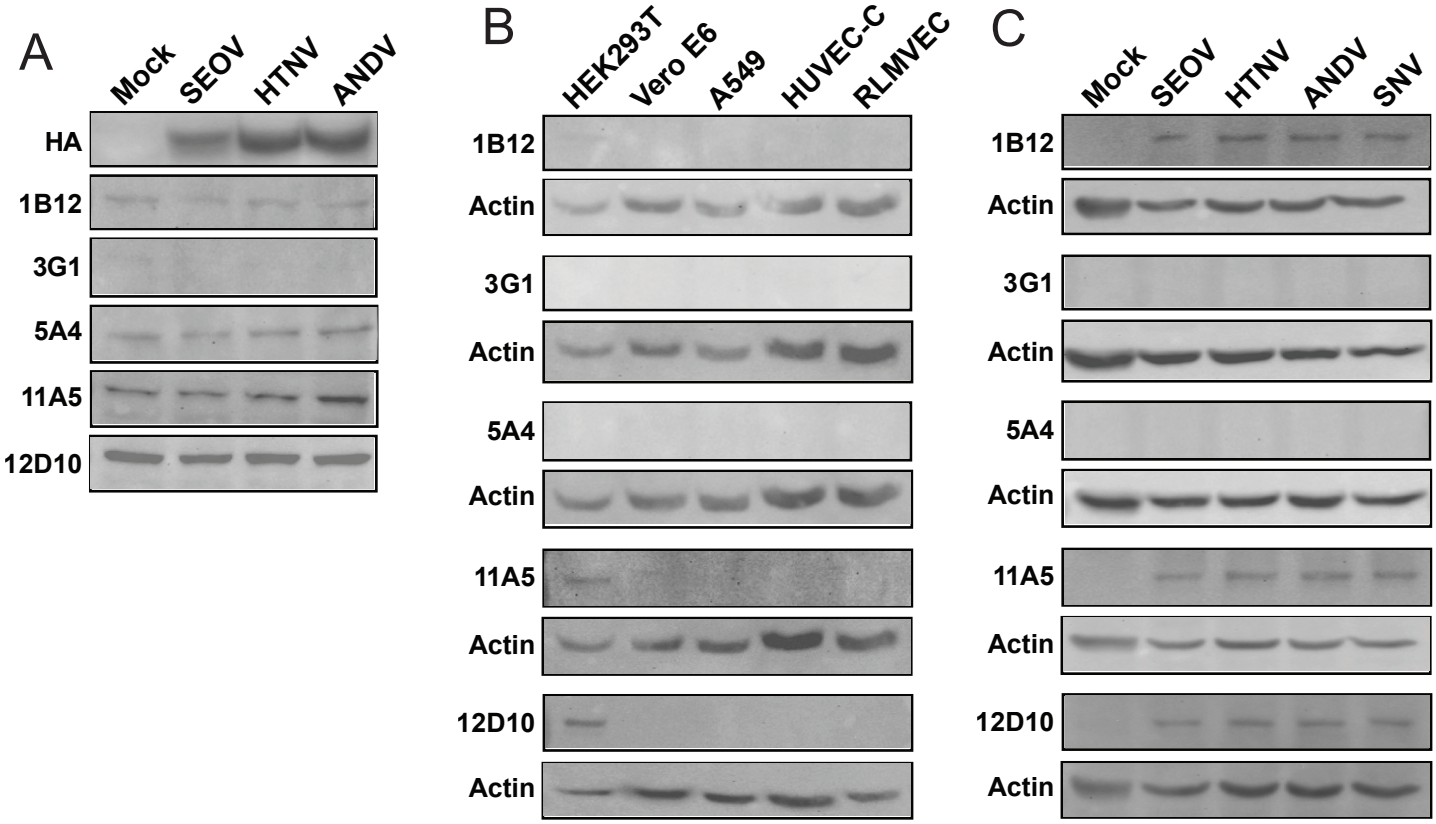
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HUVEC-C
RLMVEC
1B12
Actin
3G1
Actin
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Actin
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12D10
Actin
C
Mock
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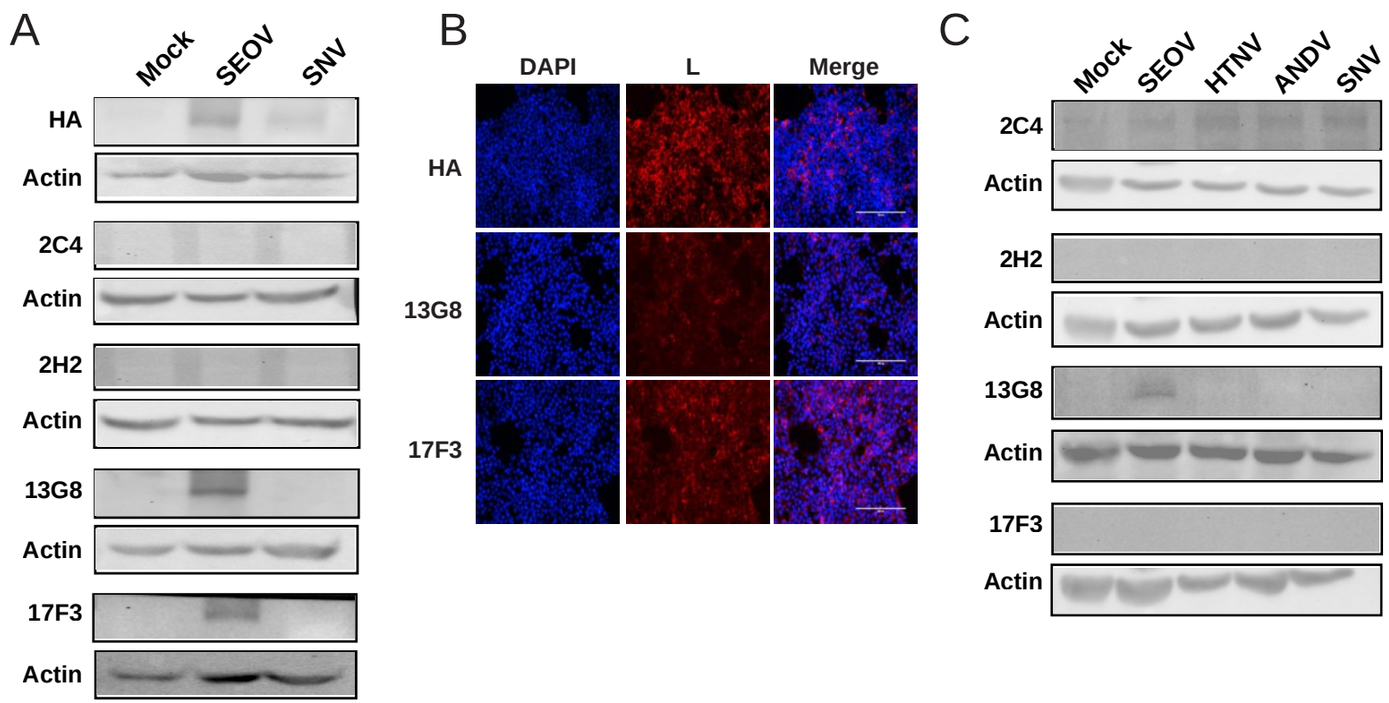
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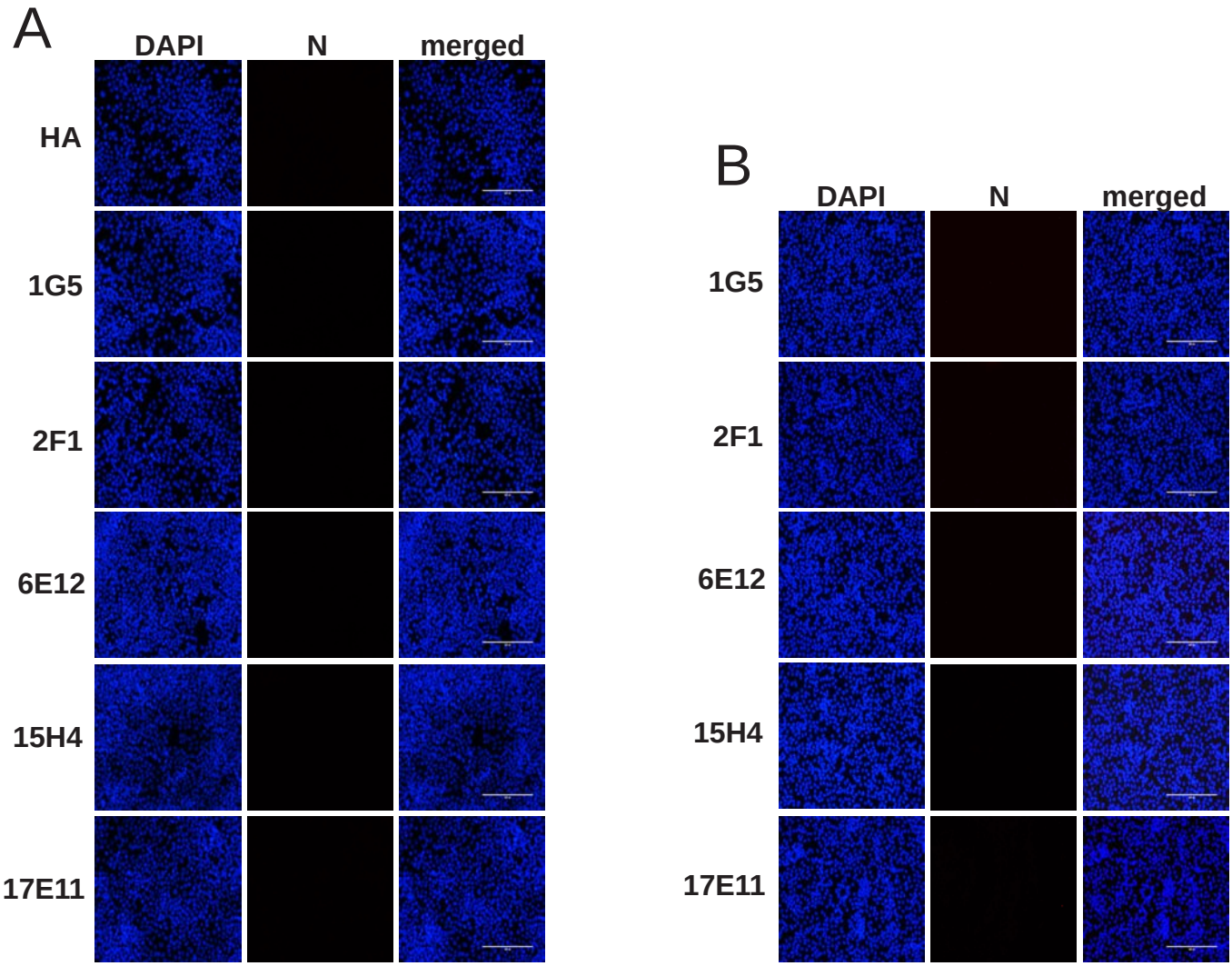
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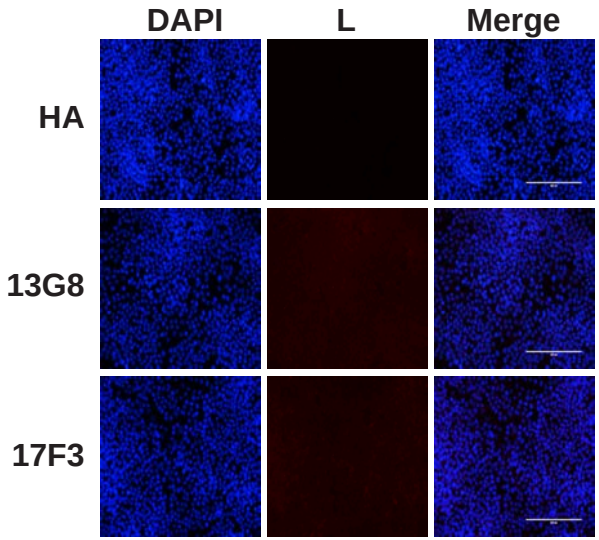
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