163 determined carefully by showing several lines of evidence such as acquisition of
164 susceptibility by expressing a putative receptor in nonsusceptible cells, loss of
165 susceptibility by knocking down of the receptor in susceptible cells, and direct binding
166 of the virus to the receptor, etc.
167
168 L-PSGL-1.1 cells did not support PSGL-1-dependent replication of the HEV-B and
169 HEV-C strains (Figure 1B); however, the prototype EV70 strain (HEV-D) replicated in
170 L-PSGL-1.1 cells more efficiently than in L-bsd cells. Although EV70 replication was
171 not affected by KPL1 (Figure 1B), we cannot exclude the possibility that EV70 utilizes
172 α2,3-linked sialic acid, which could be a receptor for EV70 (Nokhbeh et al., 2005).
173
174 Recently we reported that that four out of five EV71-PB strains replicated poorly in
175 L-PSGL-1.1 cells (Miyamura et al., 2011). We found that EV71 variants, which were
176 propagated once in L-PSGL-1.1 cells, have several possible adaptive mutations,
177 including a putative amino acid determinant of the adaptive phenotype in L-PSGL-1.1
178 cells at VP2-149 (Miyamura et al., 2011). The results suggest that adaptive mutations,
179 along with a PB phenotype, may facilitate efficient PSGL-1-dependent replication of
180 the EV71 variants in L-PSGL-1.1 cells. It is possible that HEV-A strains other than
181 EV71 also require adaptive mutations for efficient replication in L-PSGL-1.1 cells.
182
183 **3. SCARB2**
184 Yamayoshi et al. (2009) identified SCARB2 (also known as lysosomal integral
185 membrane protein II, or CD36b like-2) as an EV71 receptor on RD cells, widely used
186 for isolation of EV71 from clinical specimens. They transfected EV71-nonsusceptible
187 L929 cells with the genomic DNA of RD cells and selected two cell clones that were
188 susceptible for EV71 infection. By a transcriptome analysis, SCARB2 was identified as
189 an EV71 receptor on RD cells.

SCARB2 is a heavily *N*-glycosylated type III transmembrane protein consists from 478 amino acids and belongs to the CD36 family of scavenger receptor proteins (Fujita et al., 1992;Calvo et al., 1995). SCARB2 has a N-terminal transmembrane domain, a ~400 amino acid lumeral domain, C-terminal transmembrane domain, and a C-terminal cytoplasmic tail of ~20 amino acids (Fujita et al., 1992). SCARB2 involves in an enlargement of early endosomes and late endosomes/lysosomes and an impairment of endocytic membrane out of the enlarged compartments (Kuronita et al., 2002). SCARB2 deficiency caused ureteric pelvic junction obstruction, deafness, and peripheral neuropathuy in mice (Gamp et al., 2003). SCARB2 is expressed ubiquitously in human tissues (Eskelinen et al., 2003); therefore, it might be involved in systemic EV71 infections (Yamayoshi et al., 2009).

Human SCARB2 has 10 potential *N*-glycosylation sites (Fujita et al., 1992). But the carbohydrate chains of human SCARB2 are not essential for the interaction between EV71 and human SCARB2 (Yamayoshi and Koike, 2011). Experiments using a series of chimeric proteins between human and mouse SCARB2 identified that the amino acids 142 to 204 of human SCARB2 (encoded by human *SCARB2* exon 4) are responsible for EV71 binding and infection (Yamayoshi and Koike, 2011).

L929 cells expressing human SCARB2 in the presence of puromycin (L-SCARB2 cells) permitted the replication of all EV71 strains tested, including the non-PB strains (Yamayoshi et al., 2009). CVA16 induced CPE in L-SCARB2 cells, whereas CVA2, CVA3, CVA4, CVA5, CVA6, CVA8 and CVA12 did not. CVA16 grew efficiently in L-SCARB2, whereas CVA2, CVA3, CVA4, CVA5, CVA6, CVA8 and CVA12 did not (Table 1). Yamayoshi et al. (2009) concluded that CVA16 also infect L-SCARB2 cells in a SCARB2-dependent manner and that infection with most other HEV-A is not

217 dependent upon SCARB2. CVA7, CVA10 and CVA14 induced CPE in both L-Empty
218 cells and L-SCARB2 cells (Yamayoshi et al., 2009). They could not to determine
219 whether the CPE induced by these viruses were due to hSCARB2–mediated infection.
220
221 **4. Annexin II**
222 Yang et al. (2011) identified annexin II as an EV71 VP1-binding protein on RD cells.
223 Using a recombinant VP1 protein of EV71 fused with a calmodulin-binding peptide
224 (VP1-CBP), they tried to identify VP1-binding proteins from the total cellular proteins
225 of RD cells. A virus-overlay protein-binding assay followed by a mass spectrometory
226 analysis identified annexin II as a VP1-binding protein.
227
228 Annexin II is a member of the annexin family – the multifunctional
229 phospholipid-binding proteins. Annexin II on the surface of endothelial cells acts as a
230 profibrinolytic coreceptor for both plasminogen and tissue plasminogen activator
231 facilitating the generation of plasmin (Kim and Hajjar, 2002). The interaction to annexin
232 II was specific to EV71; CVA16 did not bind to annexin II in the virus-overlay
233 protein-binding assay (Yang et al., 2011).
234
235 **5. Sialic acid**
236 Sialic acid (SA) is usually found as terminal monosaccharides on the glycan chains of
237 glycolipids and glycoproteins (Varki and Varki, 2007). Coxsackievirus A24 variant
238 (CVA24v) uses SA-containing glycoconjugates as attachment receptors on corneal cells
239 (Nilsson et al., 2008). Yang et al. (2009) hypothesized that SA would be important for
240 EV71 infection, as the transmission route of EV71 and CVA24v is fecal-oral and/or
241 droplet-aerosol route. EV71 infection to DLD-1 intestinal cells was inhibited by an
242 *O*-glycan synthesis inhibitor, but not by an *N*-glycan synthesis inhibitor. Sialidase
243 treatment decreased EV71 replication in DLD-1 cells. Furthermore, DLD-1 cells

co-cultured with SA-linked galactose significantly reduced the EV71 infection. Thus Yang et al. (2009) concluded that SA-linked glycans are EV71 receptors on DLD-1 cells. Recently, Neu5Acα2,3Gal disaccharides on PSGL-1 was reported as a candidate receptor of CVA24v (Mistry et al., 2011). It is unknown whether other enteroviruses, including HEV-A, recognize SA-containing glycans as the entry receptors.

6. DC-SIGN

DCs play crucial roles in antiviral immunity by functioning as professional antigen-presenting cells to prime T cells and by secreting cytokines to modulate immune responses. In a mouse model of EV71 infection, DCs from the brains of EV71-infected, but not of uninfected, mice expressed viral antigen and primed T cells efficiently (Lin et al., 2009a). Lin et al. (2009b) reported that EV71 infection enhances mouse DCs to elicit protective immune response and also found that EV71 infects human immature DCs and that viral entry is partially inhibited by anti-DC-SIGN antibody. However, the direct interaction between EV71 and DC-SIGN is still unclear. It is essential to characterize the role of DC-SIGN and other receptors for EV71 in DCs for understanding the host immunological responses and immunopathogenesis of HEV-A including EV71.

7. Conclusion

Identification of PSGL-1 and SCARB2 as the cellular receptors for EV71 and CVA16 has advanced our understanding of the early stages of HEV-A infections at the molecular level. However, further experiments using clinical HEV-A isolates are necessary to clarify the general role of PSGL-1 and SCARB2 in HEV-A infection and their pathogenesis. Most of the prototype (laboratory-adapted) HEV-A strains other than EV71 and CVA16 may use unidentified receptor(s) to infect susceptible human cells such as RD cells. Characterization of the identified and unidentified HEV-A receptors is

271 essential to understand the mechanism of HEV-A infection and development of a
272 diverse array of the clinical outcomes of HEV-A-associated diseases.
273
274 **8. Acknowledgements**
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280

281 **References**

282

283 Alexander, J.P., Jr., Baden, L., Pallansch, M.A., and Anderson, L.J. (1994). Enterovirus
284 71 infections and neurologic disease--United States, 1977-1991. *J. Infect. Dis.*
285 169, 905-908.
286 Calvo, D., Dopazo, J., and Vega, M.A. (1995). The CD36, CLA-1 (CD36L1), and
287 LIMPII (CD36L2) gene family: cellular distribution, chromosomal location, and
288 genetic evolution. *Genomics* 25, 100-106.
289 Eskelinen, E.L., Tanaka, Y., and Saftig, P. (2003). At the acidic edge: emerging
290 functions for lysosomal membrane proteins. *Trends Cell. Biol.* 13, 137-145.
291 Fujimoto, T., Iizuka, S., Enomoto, M., Abe, K., Yamashita, K., Hanaoka, N., Okabe, N.,
292 Yoshida, H., Yasui, Y., Kobayashi, M., Fujii, Y., Tanaka, H., Yamamoto, M., and
293 Shimizu, H. (in press). Hand, foot, and mouth disease caused by coxsackievirus
294 A6, Japan, 2011. *Emerg. Infect. Dis.*
295 Fujita, H., Takata, Y., Kono, A., Tanaka, Y., Takahashi, T., Himeno, M., and Kato, K.
296 (1992). Isolation and sequencing of a cDNA clone encoding the 85 kDa human
297 lysosomal sialoglycoprotein (hLGP85) in human metastatic pancreas islet tumor
298 cells. *Biochem. Biophys. Res. Commun.* 184, 604-611.
299 Gamp, A.C., Tanaka, Y., Lüllmann-Rauch, R., Wittke, D., D'hooge, R., De Deyn, P.P.,
300 Moser, T., Maier, H., Hartmann, D., Reiss, K., Illert, A.L., von Figura, K., and
301 Saftig, P. (2003). LIMP-2/LGP85 deficiency causes ureteric pelvic junction
302 obstruction, deafness and peripheral neuropathy in mice. *Hum. Mol. Genet.* 12,
303 631-646.
304 Hirata, T., Furukawa, Y., Yang, B.G., Hieshima, K., Fukuda, M., Kannagi, R., Yoshie, O.,
305 and Miyasaka, M. (2004). Human P-selectin glycoprotein ligand-1 (PSGL-1)
306 interacts with the skin-associated chemokine CCL27 via sulfated tyrosines at the
307 PSGL-1 amino terminus. *J. Biol. Chem.* 279, 51775-51782.

308 Kim, J., and Hajjar, K.A. (2002). Annexin II: a plasminogen-plasminogen activator
309 co-receptor. *Front. Biosci.* 7, d341-348.
310 Kuronita, T., Eskelinen, E.L., Fujita, H., Saftig, P., Himeno, M., and Tanaka, Y. (2002).
311 A role for the lysosomal membrane protein LGP85 in the biogenesis and
312 maintenance of endosomal and lysosomal morphology. *J. Cell. Sci.* 115,
313 4117-4131.
314 Laszik, Z., Jansen, P.J., Cummings, R.D., Tedder, T.F., McEver, R.P., and Moore, K.L.
315 (1996). P-selectin glycoprotein ligand-1 is broadly expressed in cells of myeloid,
316 lymphoid, and dendritic lineage and in some nonhematopoietic cells. *Blood* 88,
317 3010-3021.
318 Lin, T.Y., Hsia, S.H., Huang, Y.C., Wu, C.T., and Chang, L.Y. (2003). Proinflammatory
319 cytokine reactions in enterovirus 71 infections of the central nervous system.
320 *Clin. Infect. Dis.* 36, 269-274.
321 Lin, Y.W., Chang, K.C., Kao, C.M., Chang, S.P., Tung, Y.Y., and Chen, S.H. (2009a).
322 Lymphocyte and antibody responses reduce enterovirus 71 lethality in mice by
323 decreasing tissue viral loads. *J. Virol.* 83, 6477-6483.
324 Lin, Y.W., Wang, S.W., Tung, Y.Y., and Chen, S.H. (2009b). Enterovirus 71 infection of
325 human dendritic cells. *Exp. Biol. Med. (Maywood)* 234, 1166-1173.
326 Liu, W.J., Ramachandran, V., Kang, J., Kishimoto, T.K., Cummings, R.D., and McEver,
327 R.P. (1998). Identification of N-terminal residues on P-selectin glycoprotein
328 ligand-1 required for binding to P-selectin. *J. Biol. Chem.* 273, 7078-7087.
329 McMinn, P.C. (2002). An overview of the evolution of enterovirus 71 and its clinical
330 and public health significance. *FEMS Microbiol. Rev.* 26, 91-107.
331 Mistry, N., Inoue, H., Jamshidi, F., Storm, R.J., Oberste, M.S., and Arnberg, N. (2011).
332 Coxsackievirus A24 variant uses sialic acid-containing *O*-linked
333 glycoconjugates as cellular receptors on human ocular cells. *J. Virol.* 85,
334 11283-11290.

335 Miyamura, K., Nishimura, Y., Abo, M., Wakita, T., and Shimizu, H. (2011). Adaptive
336 mutations in the genomes of enterovirus 71 strains following infection of mouse
337 cells expressing human P-selectin glycoprotein ligand-1. *J. Gen. Virol.* 92,
338 287-291.
339 Modlin, J.F. (2007). Enterovirus déjà vu. *N. Engl. J. Med.* 356, 1204-1205.
340 Nadkarni, S.S., and Deshpande, J.M. (2003). Recombinant murine L20B cell line
341 supports multiplication of group A coxsackieviruses. *J. Med. Virol.* 70, 81-85.
342 Nilsson, E.C., Jamshidi, F., Johansson, S.M.C., Oberste, M.S., and Arnberg, N. (2008).
343 Sialic acid is a cellular receptor for coxsackievirus A24 variant, an emerging
344 virus with pandemic potential. *J. Virol.* 82, 3061-3068.
345 Nishimura, Y., Shimojima, M., Tano, Y., Miyamura, T., Wakita, T., and Shimizu, H.
346 (2009). Human P-selectin glycoprotein ligand-1 is a functional receptor for
347 enterovirus 71. *Nat. Med.* 15, 794-797.
348 Nishimura, Y., Wakita, T., and Shimizu, H. (2010). Tyrosine sulfation of the amino
349 terminus of PSGL-1 is critical for enterovirus 71 infection. *PLoS Pathog.* 6,
350 e1001174.
351 Nokhbeh, M.R., Hazra, S., Alexander, D.A., Khan, A., McAllister, M., Suuronen, E.J.,
352 Griffith, M., and Dimock, K. (2005). Enterovirus 70 binds to different
353 glycoconjugates containing α2,3-linked sialic acid on different cell lines. *J. Virol.*
354 79, 7087-7094.
355 Oberste, M.S., Jiang, X., Maher, K., Nix, W.A., and Jiang, B. (2008). The complete
356 genome sequences for three simian enteroviruses isolated from captive primates.
357 *Arch. Virol.* 153, 2117-2122.
358 Oberste, M.S., Maher, K., Michele, S.M., Belliot, G., Uddin, M., and Pallansch, M.A.
359 (2005). Enteroviruses 76, 89, 90 and 91 represent a novel group within the
360 species *Human enterovirus A*. *J. Gen. Virol.* 86, 445-451.
361 Oberste, M.S., Maher, K., and Pallansch, M.A. (2007). Complete genome sequences for

362 nine simian enteroviruses. *J. Gen. Virol.* 88, 3360-3372.
363 Oberste, M.S., Peñaranda, S., Maher, K., and Pallansch, M.A. (2004). Complete
364 genome sequences of all members of the species *Human enterovirus A. J. Gen.*
365 *Virol.* 85, 1597-1607.
366 Pallansch, M., and Roos, R. (2007). "Enteroviruses: polioviruses, coxsackieviruses,
367 echoviruses, and newer enteroviruses," in *Fields Virology 5th edition,* eds. D.M.
368 Knipe & P.M. Howley (Philadelphia, PA: Lippincott Williams & Wilkins),
369 839-893.
370 Patel, K.P., and Bergelson, J.M. (2009). Receptors identified for hand, foot and mouth
371 virus. *Nat. Med.* 15, 728-729.
372 Pouyani, T., and Seed, B. (1995). PSGL-1 recognition of P-selectin is controlled by a
373 tyrosine sulfation consensus at the PSGL-1 amino terminus. *Cell* 83, 333-343.
374 Sako, D., Chang, X.J., Barone, K.M., Vachino, G., White, H.M., Shaw, G., Veldman,
375 G.M., Bean, K.M., Ahern, T.J., Furie, B., Cumming, D.A., and Larsen, G.R.
376 (1993). Expression cloning of a functional glycoprotein ligand for P-selectin.
377 *Cell* 75, 1179-1186.
378 Sako, D., Comess, K.M., Barone, K.M., Camphausen, R.T., Cumming, D.A., and Shaw,
379 G.D. (1995). A sulfated peptide segment at the amino terminus of PSGL-1 is
380 critical for P-selectin binding. *Cell* 83, 323-331.
381 Somers, W.S., Tang, J., Shaw, G.D., and Camphausen, R.T. (2000). Insights into the
382 molecular basis of leukocyte tethering and rolling revealed by structures of P-
383 and E-selectin bound to SLe^X and PSGL-1. *Cell* 103, 467-479.
384 Varki, N.M., and Varki, A. (2007). Diversity in cell surface sialic acid presentations:
385 implications for biology and disease. *Lab. Invest.* 87, 851-857.
386 Wang, S.M., Lei, H.Y., Huang, K.J., Wu, J.M., Wang, J.R., Yu, C.K., Su, I.J., and Liu,
387 C.C. (2003). Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric
388 patients: roles of cytokines and cellular immune activation in patients with

389 pulmonary edema. *J. Infect. Dis.* 188, 564-570.
390 Wilkins, P.P., Moore, K.L., McEver, R.P., and Cummings, R.D. (1995). Tyrosine
391 sulfation of P-selectin glycoprotein ligand-1 is required for high affinity binding
392 to P-selectin. *J. Biol. Chem.* 270, 22677-22680.
393 Yamayoshi, S., and Koike, S. (2011). Identification of a human SCARB2 region that is
394 important for enterovirus 71 binding and infection. *J. Virol.* 85, 4937-4946.
395 Yamayoshi, S., Yamashita, Y., Li, J., Hanagata, N., Minowa, T., Takemura, T., and
396 Koike, S. (2009). Scavenger receptor B2 is a cellular receptor for enterovirus 71.
397 *Nat. Med.* 15, 798-801.
398 Yang, B., Chuang, H., and Yang, K.D. (2009). Sialylated glycans as receptor and
399 inhibitor of enterovirus 71 infection to DLD-1 intestinal cells. *Virol. J.* 6, 141.
400 Yang, S.L., Chou, Y.T., Wu, C.N., and Ho, M.S. (2011). Annexin II binds to capsid
401 protein VP1 of enterovirus 71 and enhances viral infectivity. *J. Virol.* 85,
402 11809-11820.
403

404 **Table 1**. Induction of CPE by the HEV-A strains.

Serotype	Strain	Accession No.	L-bsd[1)]	L-PSGL-1.1	L-Empty[2),3)]	L-SCARB2[3)]
CVA2	Fleetwood	AY421760	-	-	-	-
CVA3	Olson	AY421761	-	-	-	-
CVA4	JR[3)]	AB457644	-	-	-	-
CVA5	Swartz	AY421763	-	-	-	-
CVA6	Gdula	AY421764	-	-	-	-
CVA7	Parker	AY421765	-	+	+	+
CVA8	Donovan	AY421766	-	-	-	-
CVA10	Kowalik	AY421767	+	+	+	+
CVA12	Texas-12	AY421768	-	-	-	-
CVA14	G-14	AY421769	-	+	+	+
CVA16	G-10	U05876	-	+	-	+

405

406

407 1) Blasticidin-resistant L929 cells (a negative control for L-PSGL-1.1 cells)

408 2) Puromycin-resistant L929 cells (a negative control for L-SCARB2 cells)

409 3) Yamayoshi et al., 2009.

410 4) Prototype CVA4 strain (High Point) is unavailable from ATCC, therefore we used an

411 in-house reference strain of CVA4, the JR strain.

412

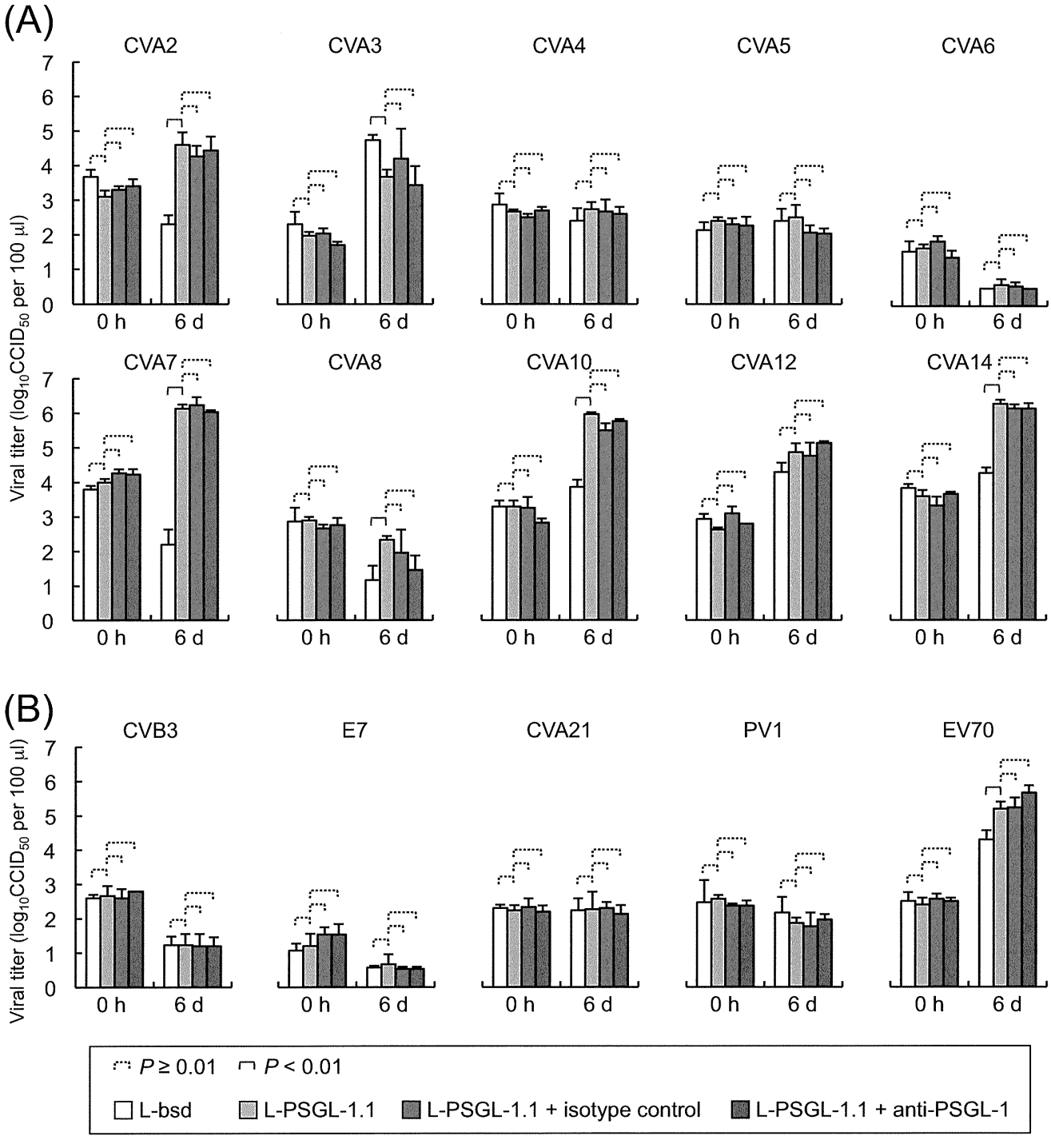
413 **Figure legend**

414

415 **Figure 1. HEV replication in L-PSGL-1.1 cells**

416 (A) Replication of the HEV-A strains (Table 1) in L-PSGL-1.1 cells in the presence or
417 absence of anti-PSGL-1 mAb (KPL1) or an isotype control. Cells were inoculated with
418 viruses at 10 $CCID_{50}$/cell for 1 h, washed, and incubated in the medium, as described
419 previously (Nishimura et al., 2009). Cell were incubated at 34°C. For mAb inhibition,
420 the cells were pretreated with 10 μg/ml mAb for 1 h, washed, and maintained in the
421 medium with 10 μg/ml mAb. At the indicated time (just after infection (0 h) and six
422 days postinfection (6 d)), the infected cells and supernatants were freeze-thawed and
423 viral titers were determined by $CCID_{50}$ titration using RD cells. The titers are expressed
424 as the mean and error bars indicate SD of triplicate analyses. The mean viral titers were
425 compared using Student's *t*-test. *P* values < 0.01 were considered statistically
426 significant.
427 (B) Viral replication of HEV-B, C, and D in L-PSGL-1.1 cells. Replication of two
428 HEV-B (CVB3-Nancy and echovirus 7(E7)-Wallace) and two HEV-C strains
429 (CVA21-Coe and poliovirus 1 (PV1)-Sabin 1), and one HEV-D (EV70-J670/71) strains
430 in L-PSGL-1.1 cells in the presence or absence of KPL1 or an isotype control. The titers
431 are expressed as the mean and error bars indicate SD of triplicate analyses. The mean
432 viral titers were compared using Student's *t*-test. *P* values < 0.01 were considered
433 statistically significant.

Figure 1


(A)
CVA2
CVA3
CVA4
CVA5
CVA6
CVA7
CVA8
CVA10
CVA12
CVA14
Viral titer ($log_{10}CCID_{50}$ per 100 µl)
0 h
6 d
(B)
CVB3
E7
CVA21
PV1
EV70
$P \geq 0.01$
$P < 0.01$
L-bsd
L-PSGL-1.1
L-PSGL-1.1 + isotype control
L-PSGL-1.1 + anti-PSGL-1

Analysis of amino acid determinants of enterovirus 71 responsible for the PSGL-1-binding phenotype

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15 September, 2011 IUMS 2011 Sapporo

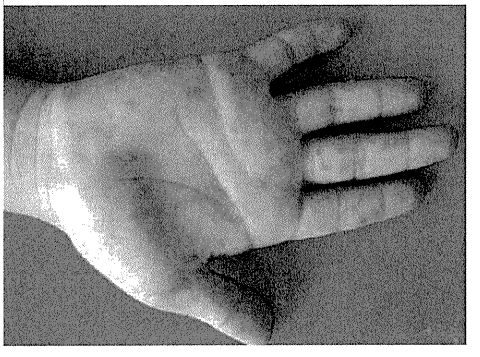
Erratum in the Abstract

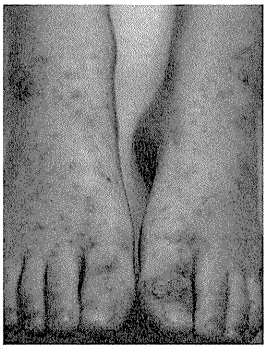
Analysis of amino acid determinants of enterovirus 71 (EV71) responsible for the PSGL-1-binding phenotype

1. Background information
   EV71 and PSGL-1
2. Methods
3. PSGL-1-binding assay
4. PSGL-1-dependent replication

Enterovirus 71 (EV71)

- Hand, foot, and mouth disease
- Neurological diseases
  Aseptic meningitis Acute encephalitis
  Polio-like paralysis
- Large outbreaks in the Asia-Pacific region

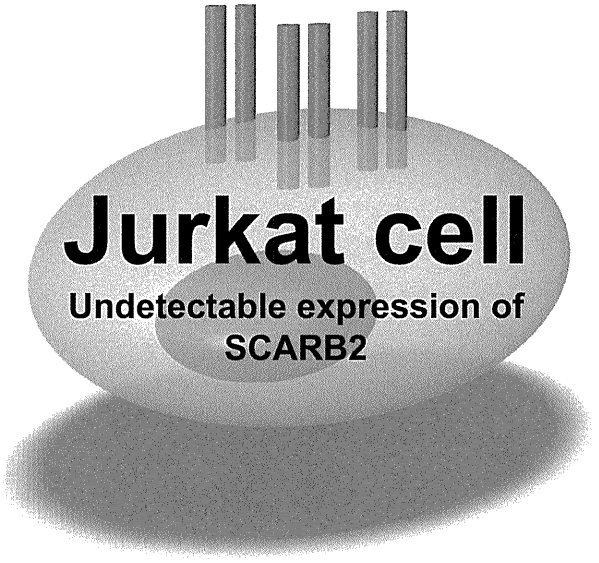




Photos by Dr. Ryo Uejima

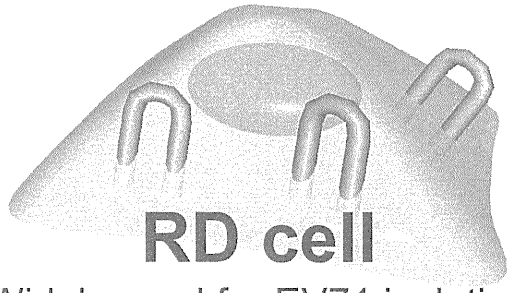
EV71 receptors

PSGL-1



Nishimura *et al. Nat. Med.* 2009

SCARB2

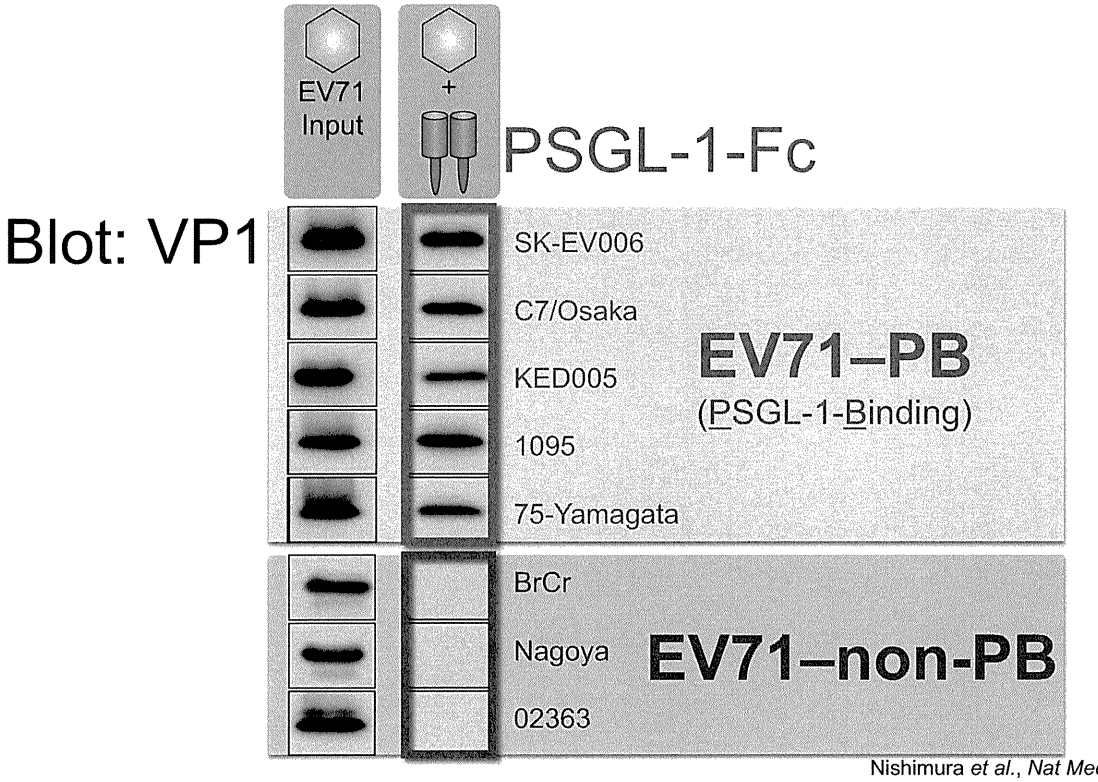


Widely used for EV71 isolation from clinical specimens

Yamayoshi *et al. Nat. Med.* 2009

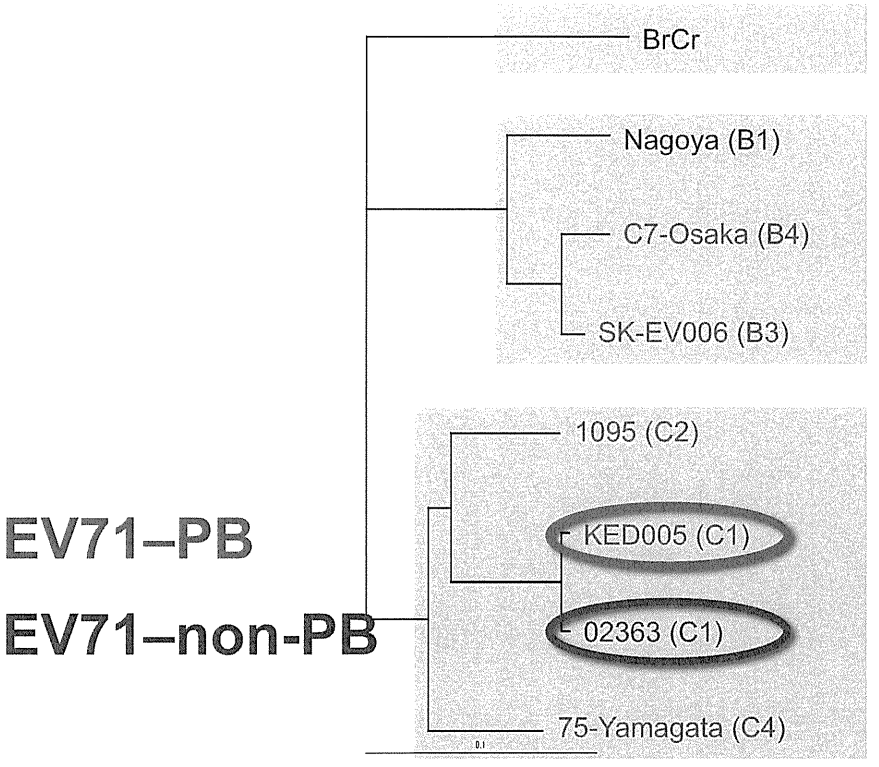
The PSGL-1-binding phenotypes of EV71

Co-precipitation of EV71 with soluble PSGL-1-Fc



Nishimura *et al.*, *Nat Med.*, 2009

EV71 genogroups (VP1)



Nishimura *et al.*, *Nat Med.*, 2009

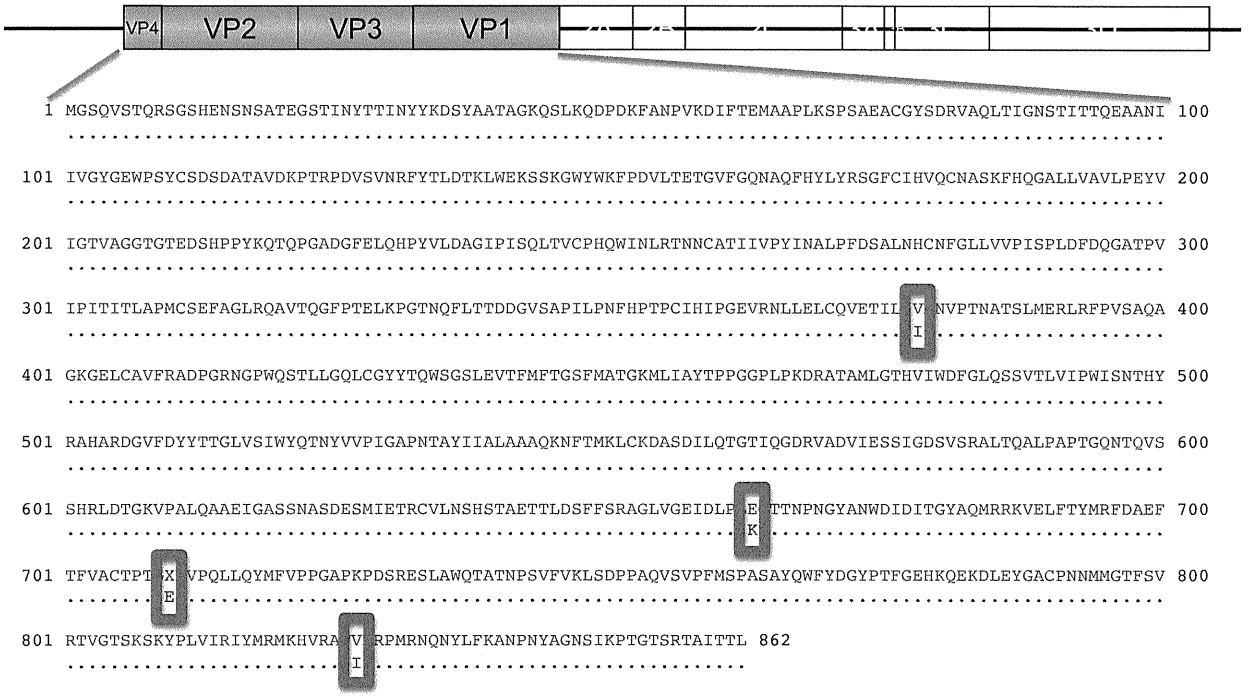
Methods

1. Direct sequencing of the EV71 genomes
2. Construction of cDNA-derived EV71
3. Propagation of EV71 mutants in RD cells
4. PSGL-1-binding capability
5. PSGL-1-dependent replication (Jurkat cells)

Comparison of the capsid region

EV71–PB (KED005, genogroup C1)

EV71–non-PB (02363, genogroup C1)



Comparison of the capsid region

Strain	PSGL-1 -binding	VP3-55	VP1-98	VP1-145	VP1-262
KED005	PB	V	E	X*	V
02363	Non-PB	I	K	E	I

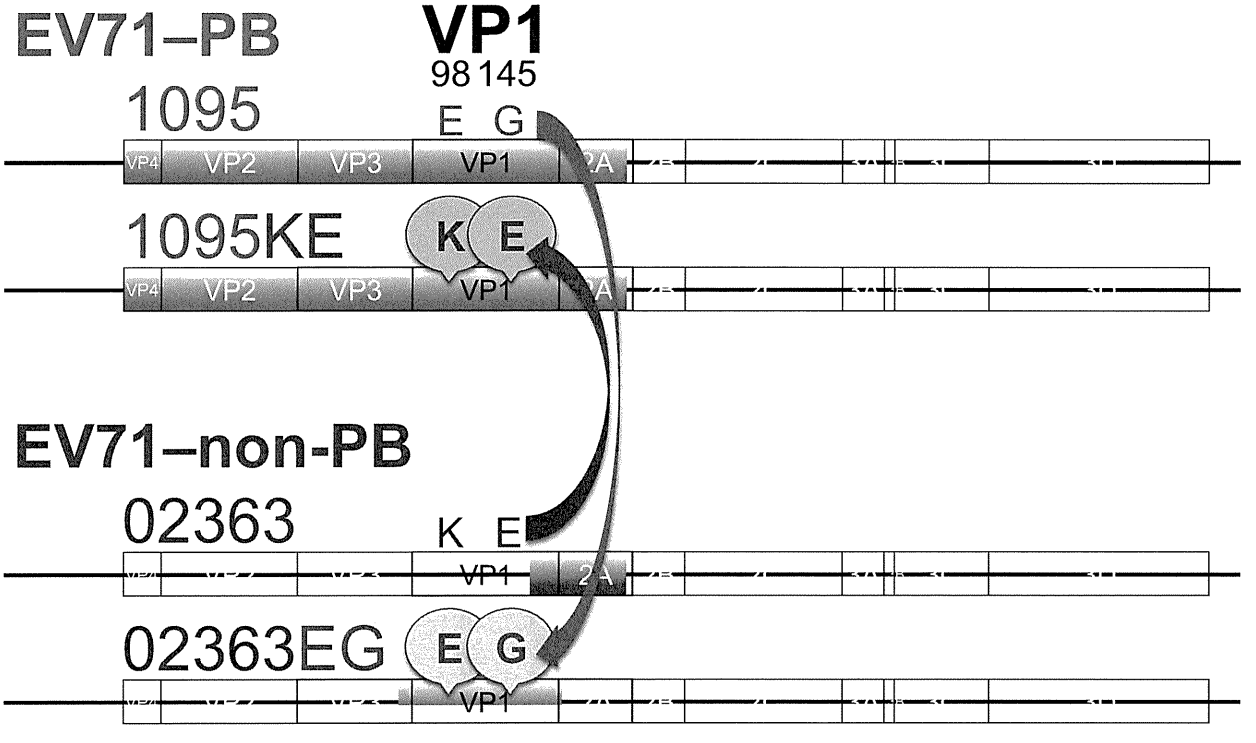
X*; The codon contained mixed nucleotides.

Comparison of the capsid region

Strain	PSGL-1 -binding	VP3-55	VP1-98	VP1-145	VP1-262
Osaka	PB	V	E	G	I
SK-EV006	PB	V	E	G	I
1095	PB	V	E	G	I
KED005	PB	V	E	X*	V
02363	Non-PB	I	K	E	I
BrCr	Non-PB	V	K	E	I

X*; The codon contained mixed nucleotides.

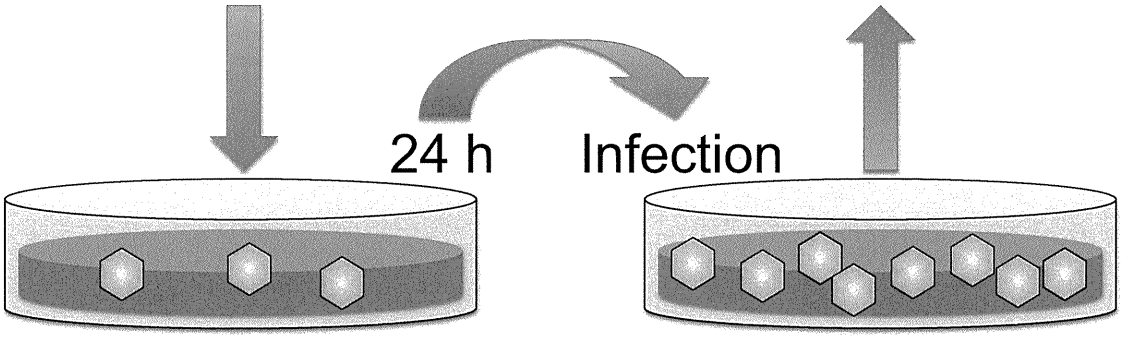
Structure of the cDNA-derived EV71



Propagation of the cDNA-derived EV71

Transfection of EV71 genome
(*in vitro*-transcribed RNA)

• Ultracentrifugation
(EV71-binding assay)
• Infection (Jurkat cells)



RD cells

RD cells

Co-precipitation of EV71 with soluble PSGL-1-Fc