

# Suramin interacts with the positively charged region surrounding the 5-fold axis of the EV-A71 capsid and inhibits multiple enterovirus A

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**Supplementary Table 1.** Sequence information

| Group  | Name                | Strain/Isolate                    | GenBank accession No.             |
|--------|---------------------|-----------------------------------|-----------------------------------|
| HEV-A  | EV-A71              | Fuyang 573                        | <a href="#"><u>ADI40103.1</u></a> |
|        |                     | SH12-036                          | <a href="#"><u>AGK07541.1</u></a> |
|        |                     | SH12-276                          | <a href="#"><u>AGK07542.1</u></a> |
|        |                     | BrCr                              | <a href="#"><u>Q66478.3</u></a>   |
|        |                     | FY23                              | <a href="#"><u>ACF21980.1</u></a> |
|        |                     | FY0805                            | <a href="#"><u>AEK98534.1</u></a> |
|        |                     | Nagoya                            | <a href="#"><u>BAB69808.1</u></a> |
|        | CV-A2               | Fleetwood                         | <a href="#"><u>AAR38840.1</u></a> |
|        | CV-A3               | Olson                             | <a href="#"><u>AAD17698.1</u></a> |
|        | CV-A4               | JR                                | <a href="#"><u>BAH24182.1</u></a> |
|        | CV-A5               | CA5/25-1010/osaka.JPN/2013        | <a href="#"><u>BAP27871.1</u></a> |
|        | CV-A6               | Gdula                             | <a href="#"><u>AAD17701.1</u></a> |
|        | CV-A7               | Parker                            | <a href="#"><u>AEL16676.1</u></a> |
|        | CV-A8               | Donovan                           | <a href="#"><u>AAD17703.1</u></a> |
|        | CV-A10              | Kowalik                           | <a href="#"><u>AAD17704.1</u></a> |
|        | CV-A12              | Texas-12                          | <a href="#"><u>AAD17706.1</u></a> |
| CV-A14 | G-14                | <a href="#"><u>AAD17708.1</u></a> |                                   |
| CV-A16 | shzh05-1            | <a href="#"><u>ABX55895.1</u></a> |                                   |
| EV-A76 | 40961_EV76_TJK_2011 | <a href="#"><u>AHJ38831.1</u></a> |                                   |
| EV-A89 | N289                | <a href="#"><u>AEX15160.1</u></a> |                                   |
| EV-A90 | 21364               | <a href="#"><u>AHJ38775.1</u></a> |                                   |
| HEV-B  | CV-A9               | RO-609/4/80                       | <a href="#"><u>CAC08134.1</u></a> |
|        | CV-B3               | Nancy                             | <a href="#"><u>AAA74400.1</u></a> |

|       |                    |                   |                          |
|-------|--------------------|-------------------|--------------------------|
|       | CV-B4              | J.V.B. Benschoten | <b><u>P08292.4</u></b>   |
|       | Echo1              | Farouk            | <b><u>AAD17718.1</u></b> |
|       | Echo2              | Cornelis          | <b><u>AAD17719.1</u></b> |
|       | Echo3              | Morrissey         | <b><u>AAD17720.1</u></b> |
|       | Echo5              | Noyce             | <b><u>AAD17724.1</u></b> |
|       | Echo8              | Bryson            | <b><u>AAD17729.1</u></b> |
|       | Echo9              | Hill              | <b><u>AAK13385.1</u></b> |
|       | Echo11             | Gregory           | <b><u>AAK13386.1</u></b> |
|       | Echo13             | Del Carmen        | <b><u>AAD17731.1</u></b> |
|       | Echo14             | Tow               | <b><u>AAD17732.1</u></b> |
|       | Echo15             | CH96-51           | <b><u>AAD17733.1</u></b> |
|       | Echo18             | Metcalf           | <b><u>AAD17735.1</u></b> |
|       | Echo19             | Burke             | <b><u>AAD17736.1</u></b> |
|       | Echo20             | JV-1              | <b><u>CAB53942.1</u></b> |
|       | Echo24             | DeCamp            | <b><u>CAB53945.1</u></b> |
|       | Echo25             | JV-4              | <b><u>AAD17740.1</u></b> |
|       | Echo26             | Coronel           | <b><u>AAD17741.1</u></b> |
|       | Echo27             | Bacon             | <b><u>AAD17742.1</u></b> |
|       | Echo29             | JV-10             | <b><u>CAB53947.1</u></b> |
|       | Echo30             | Bastianni         | <b><u>AAD17744.1</u></b> |
|       | Echo31             | Caldwell          | <b><u>AAD17748.1</u></b> |
|       | Echo32             | PR-10             | <b><u>AAD17749.1</u></b> |
|       | EV-B69             | Toluca            | <b><u>AAD17753.1</u></b> |
|       | Human poliovirus 1 | Sabin-1           | <b><u>AHB86319.1</u></b> |
|       | Human poliovirus 1 | Mahoney           | <b><u>CAA24461.1</u></b> |
| HEV-C | Human poliovirus 2 | Sabin-2           | <b><u>AAN85443.1</u></b> |
|       | Human poliovirus 3 | Sabin-3           | <b><u>AAN85444.1</u></b> |
|       | Human poliovirus 3 | P3/Leon/37        | <b><u>AAA46914.1</u></b> |
| HEV-D | EV-D68             | Fermon            | <b><u>AAD17752.1</u></b> |

**Supplementary Table 2.** Plasma Concentration of Suramin ( $\mu\text{g/mL}$ ) after Single Intramuscular Injection at 46.5 mg/kg

| <b>Time (h)</b>  | <b>P101#</b> | <b>P102</b> | <b>P103</b> | <b>Mean</b> |
|--|--------------|-------------|-------------|-------------|
| <b>0.00</b>  | BQL          | BQL         | BQL         | n.d.        |
| <b>0.25</b>  | 273          | 250         | 292         | 272         |
| <b>0.5</b>   | 309          | 454         | 512         | 425         |
| <b>1</b>   | 407          | 593         | 594         | 531         |
| <b>3</b>   | 368          | 414         | 549         | 444         |
| <b>6</b>   | 291          | 356         | 387         | 345         |
| <b>9</b>   | 211          | 273         | 305         | 263         |
| <b>24</b>  | 165          | 201         | 163         | 176         |
| <b>48</b>  | 100          | 122         | 118         | 113         |
| <b>72</b>  | 69.5         | 90.8        | 89.1        | 83.1        |
| <b>96</b>  | 53.8         | 64.1        | 74.8        | 64.2        |
| <b>120</b>   | 54.3         | 75.2        | 67.8        | 65.8        |
| <b>144</b>   | 45.9         | 62.4        | 57.4        | 55.2        |
| <b>168</b>   | 50.0         | 58.1        | 45.4        | 51.2        |
| <b>C<sub>max</sub> (<math>\mu\text{g/mL}</math>)</b>                   | 407          | 593         | 594         | 531         |
| <b>t<sub>max</sub> (h)</b>   | 1.00         | 1.00        | 1.00        | 1.00        |
| <b>t<sub>1/2</sub> (h)</b>   | 64.33        | 128.96      | 82.96       | 92.08       |
| <b>AUC<sub>0-last</sub> (<math>\mu\text{g}\cdot\text{h/mL}</math>)</b> | 15800        | 19900       | 19600       | 18433       |
| <b>AUC<sub>0-inf</sub> (<math>\mu\text{g}\cdot\text{h/mL}</math>)</b>  | 20500        | 30700       | 25000       | 25400       |
| <b>MRT<sub>0-last</sub> (h)</b>  | 55.92        | 56.74       | 54.70       | 55.79       |
| <b>MRT<sub>0-inf</sub> (h)</b>   | 102.33       | 161.30      | 105.29      | 122.97      |

NA = not available

n.d. = not determined

BQL= Below the lower limit of quantitation (LLOQ)

**Supplementary Table 3. Primers**

| <b>Target gene</b>          | <b>Primer/probe</b> | <b>Sequence (5'~3')</b>                 |
|-----------------------------|---------------------|---|
| Enterovirus 5'UTR(122)      | EV-5UTR-F           | CCCTGAATGCGGCTAATC                      |
|                             | EV-5UTR-R           | ATTGTCACCATAAGCAGCCA                    |
|                             | EV-5UTR-P           | FAM-AACCGACTACTTTGGGTGTCC GTGTTTC-TAMRA |
| Enterovirus 71<br>VP1(123)  | EV71-VP1-F          | GTTCACCTACATGCGCTTTGA                   |
|                             | EV71-VP1-R          | TGGAGCAATTGTGGGACAA                     |
|                             | EV71-VP1-P          | HEX-TCTTGCGTGCACACCCACCG-TAMRA          |
| EV-A71 VP-1 145<br>deletion | 145 delta-F         | GTGCACACCCACTGGGGTTGTCCCAC              |
|                             | 145 delta-F         | CCCAGTGGGTGTGCACGCAACAAAAG              |
| EV-A71 VP-1 145 E           | 145E-F              | GTGCACACCCACTGGGGAAGTTGTCCCAC           |
|                             | 145E-R              | CCCCAGTGGGTGTGCACGCAACAAAAG             |
| EV-A71 VP-1 145 Q           | 145Q-F              | GTGCACACCCACTGGGCAGGTTGTCCCAC           |
|                             | 145Q-R              | CTGCCAGTGGGTGTGCACGCAACAAAAG            |
| EV-A71 VP-1 145 G           | 145G-F              | GTGCACACCCACTGGGGGGTTGTCCCAC            |
|                             | 145G-R              | GAAAACAACGCACGTGTGGGTGACCCCCC           |
| EV-A71 VP-1 145 K           | 145K-F              | GTGCACACCCACTGGGAAAGTTGTCCCAC           |
|                             | 145K-R              | TCCCAGTGGGTGTGCACGCAACAAAAG             |