EV-A71 induced IL-1β production in THP-1 macrophages is dependent on NLRP3, RIG-I, and TLR3

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**Figure 1A**

|   |   |
|---|---|
| 1 | PMA_THP-1_Mock |
| 2 | PMA_THP-1_EV-A71_12 hr |
| 3 | PMA_THP-1_EV-A71_24 hr |
| 4 | PMA_THP-1_EV-A71_48 hr |

1 2 3 4

Casp-1 (p20)

Pro-Casp-1

beta-actin

VP0
**Figure 1C**

|   |   |
|---|---|
| 1 | PMA_THP-1_Mock |
| 2 | PMA_THP-1_EV-A71_6 hr |
| 3 | PMA_THP-1_EV-A71_12 hr |
| 4 | PMA_THP-1_EV-A71_24 hr |

- **Pro-Casp-8**
- **VP0**
- **beta-actin**
- **Casp-6 (p18)**
### Figure 2A

|   | Description                                    |
|---|------------------------------------------------|
| 1 | PMA_THP-1_Scrambled siRNA_Mock                 |
| 2 | PMA_THP-1_Scrambled siRNA_EV-A71_12 hr         |
| 3 | PMA_THP-1_Scrambled siRNA_EV-A71_24 hr         |
| 4 | PMA_THP-1_siNLRP3_Mock                         |
| 5 | PMA_THP-1_siNLRP3_EV-A71_12 hr                 |
| 6 | PMA_THP-1_siNLRP3_EV-A71_24 hr                 |

![Western Blot](image1.png)

- **NLRP3**
- **beta-actin**
Figure 2C

|   | Description                                           |
|---|-------------------------------------------------------|
| 1 | PMA_THP-1_Scrambled siRNA_Mock                        |
| 2 | PMA_THP-1_Scrambled siRNA_EV-A71_12 hr                |
| 3 | PMA_THP-1_Scrambled siRNA_EV-A71_24 hr                |
| 4 | PMA_THP-1_siRIG-I_Mock                                |
| 5 | PMA_THP-1_siRIG-I_EV-A71_12 hr                        |
| 6 | PMA_THP-1_siRIG-I_EV-A71_24 hr                        |
**Figure 2E**

|   | Sample Description                  |
|---|-------------------------------------|
| 1 | PMA_THP-1_Scrambled siRNA_Mock      |
| 2 | PMA_THP-1_Scrambled siRNA_EV-A71_12 hr |
| 3 | PMA_THP-1_Scrambled siRNA_EV-A71_24 hr |
| 4 | PMA_THP-1_siASC_Mock                |
| 5 | PMA_THP-1_siASC_EV-A71_12 hr        |
| 6 | PMA_THP-1_siASC_EV-A71_24 hr        |

![Image showing gel electrophoresis with ASC and VP0 bands](image-url)
**Figure 3A**

|   | Description                                      |
|---|--------------------------------------------------|
| 1 | PMA_WT THP-1_Mock                               |
| 2 | PMA_NLRP3 KD THP-1_Scrambled siRNA_Mock_12 hr    |
| 3 | PMA_NLRP3 KD THP-1_Scrambled siRNA_EV-A71_12 hr  |
| 4 | PMA_NLRP3 KD THP-1_siRIG-I_Mock_12 hr            |
| 5 | PMA_NLRP3 KD THP-1_siRIG-I_EV-A71_12 hr          |

![Image of gel electrophoresis](image_url)

**Top Left:** RIG-I

**Top Right:** VP0

**Bottom Left:** NLRP3

**Bottom Right:** beta-actin
Figure 3C

|   |   |
|---|---|
| 1 | PMA_THP-1_Scrambled siRNA_Mock |
| 2 | PMA_THP-1_siRIG-I_Mock         |
| 3 | PMA_THP-1_siNLRP3_Mock         |
| 4 | PMA_THP-1_siRIG-I + siNLRP3_Mock |
| 5 | PMA_THP-1_Scrambled siRNA_EV-A71 |
| 6 | PMA_THP-1_siRIG-I_EV-A71       |
| 7 | PMA_THP-1_siNLRP3_EV-A71       |
| 8 | PMA_THP-1_siRIG-I + siNLRP3_EV-A71 |
Figure 3D

|   | Sample Description               |
|---|----------------------------------|
| 1 | PMA_THP-1_Mock                   |
| 2 | PMA_THP-1_EV-A71                 |
| 3 | IP: PMA_THP-1_IgG control        |
| 4 | IP: PMA_THP-1_Mock               |
| 5 | IP: PMA_THP-1_EV-A71             |

[Image of gel electrophoresis showing bands for RIG-I, ASC, NLRP3, beta-actin, and VP0.]
**Figure 4A**

|   | Description         |
|---|---------------------|
| 1 | PMA_THP-1_scrambled siRNA |
| 2 | PMA_THP-1_siRIG-I |

**Figure 4B**

|   | Description         |
|---|---------------------|
| 1 | PMA_WT THP-1       |
| 2 | PMA_NLRP3 KD THP-1 |

beta-actin | RIG-I

beta-actin | NLRP3
**Figure 5A**

1. PMA_THP-1_scrambled siRNA_Mock
2. PMA_THP-1_scrambled siRNA_EV-A71_12 hr
3. PMA_THP-1_scrambled siRNA_EV-A71_24 hr
4. PMA_THP-1_siTLR3 MOCK
5. PMA_THP-1_siTLR3_12 hr
6. PMA_THP-1_siTLR3_24 hr

**Figure 5C**

1. PMA_THP-1_scrambled siRNA_Mock
2. PMA_THP-1_siTLR8 Mock
3. PMA_THP-1_scrambled siRNA_EV-A71
4. PMA_THP-1_siTLR8_EV-A71
**Figure 5E**

|   |   |
|---|---|
| 1 | PMA_WT THP-1_scrambled siRNA_EV-A71 |
| 2 | PMA_WT THP-1_siTLR3_EV-A71 |
| 3 | PMA_NLRP3 KD THP-1_scrambled siRNA_EV-A71 |
| 4 | PMA_NLRP3 KD THP-1_siTLR3_EV-A71 |
**Figure 6F**

|    |                                |
|----|--------------------------------|
| 1  | PMA_THP-1_scrambled siRNA      |
| 2  | PMA_THP-1_siRIG-I              |

![Image of gel showing bands for beta-actin and RIG-I with lanes 1 and 2 highlighted.](image-url)
### Figure 7C

|   | Sample Description              |
|---|---------------------------------|
| 1 | PMA_THP-1_untreated_Mock        |
| 2 | PMA_THP-1_untreated_EV-A71      |
| 3 | PMA_THP-1_CHX_EV-A71           |

![Image showing bands on a gel for beta-actin and 3D](image)

### Figure 7E

|   | Sample Description              |
|---|---------------------------------|
| 1 | PMA_THP-1_untreated             |
| 2 | PMA_THP-1_LF2K                  |
| 3 | PMA_THP-1_LF2K_RD RNA           |
| 4 | PMA_THP-1_LF2K_0.5ug EV-A71 vRNA|
| 5 | PMA_THP-1_LF2K_1ug EV-A71 vRNA  |
| 6 | PMA_THP-1_LF2K_2ug EV-A71 vRNA  |

![Image showing bands on a gel for Pro-Casp-8, Casp-8 (p18), and beta-actin](image)
### Supplementary Figure 1B

|   |   |
|---|---|
| 1 | THP-1_Mock |
| 2 | THP-1_EV-A71_6 hr |
| 3 | THP-1_EV-A71_12 hr |
| 4 | THP-1_EV-A71_24 hr |
| 5 | THP-1_EV-A71_36 hr |
| 6 | THP-1_EV-A71_48 hr |
| 7 | PMA_THP-1_Mock |
| 8 | PMA_THP-1_EV-A71_6 hr |
| 9 | PMA_THP-1_EV-A71_12 hr |
| 10 | PMA_THP-1_EV-A71_24 hr |
| 11 | PMA_THP-1_EV-A71_36 hr |
| 12 | PMA_THP-1_EV-A71_48 hr |
Supplementary Figure 3

|   | THP-1_untreated |
|---|----------------|
| 1 |                |
| 2 | THP-1_LF2K    |
| 3 | THP-1_LF2K_poly(A:U) |