**Supplementary Figure 1.** Sequential PRR stimulation with poly(I:C) and R848 enhances activation of immune cells. BMDC were stimulated with combinations of poly(I:C) and R848 at a 24-h interval. (A) Surface expression of activation markers was measured. Mean fluorescence intensities (MFI) + SEM of quadruples are shown. (B) CCL2 chemokine levels in supernatants measured by ELISA 18 h after the second stimulation and (C) surface expression of chemokine receptors are shown. Mean + SEM of triplicates are shown. Data are representative of at least 2 independent experiments.

**Supplementary Figure 2.** Sequential injections of TLR3/MDA5 and TLR7 ligands or single agent injections are unable to delay growth of large non-immunogenic tumors. Growth of B16-F10 tumors in mice treated with combinations of poly(I:C) and R848 on 2 consecutive days, repeated twice (arrows) was measured. Mean + SEM of tumor size is shown (n=5).
**A**

- **Graphs**
  - **CD80 (MFI)**
  - **CD86 (MFI)**
  - **MHC-II (MFI)**

- **Legend**
  - 1st stimulation:
    - Ø
    - Ø
    - piC
    - Ø
    - R8
    - piC
  - 2nd stimulation:
    - Ø
    - plC
    - piC
    - R8
    - R8
    - piC
    - +R8

**B**

- **Graph**
  - **CCL2 (pg/ml)**

- **Legend**
  - 1st stimulation:
    - Ø
    - Ø
    - piC
    - Ø
    - piC
  - 2nd stimulation:
    - Ø
    - piC
    - piC
    - R8
    - R8

**C**

- **Graph**
  - **CD80 (MFI)**
  - **CD86 (MFI)**
  - **MHC-II (MFI)**

- **Legend**
  - 1st stimulation:
    - Ø
    - Ø
    - piC
    - piC
    - Ø
    - piC
  - 2nd stimulation:
    - Ø
    - piC
    - piC
    - R8
    - R8
    - piC
    - +R8

**Additional Text**

- **1st stimulation**
- **2nd stimulation**
- Ø
- piC
- R8
- +R8

- **CXCR4**
- **CCR7**

**Notes**

- Bars with error bars indicate standard deviation.
- Graphs compare MFI values for different conditions.
Supplementary Figure 2

![Graph showing tumor size (mm²) over days after tumor induction. The x-axis represents days after tumor induction ranging from 0 to 20, while the y-axis represents tumor size from 0 to 300 mm². Four different lines are plotted: open squares for Untreated, open circles for R8 / R8, filled triangles for plC / plC, and filled squares for plC / R8. Arrows indicate specific points on the graph.](image-url)