Time: 2024.05.21-2024.05.28

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1. **Experiment:** NK cells co-cultured with tumor cells
2. **Time:** 2024.05.21-2024.05.28
3. **Member:** Xiaoyuan Chen, Xinyu Zhu, Yaqi Gao
4. **Material:** NK cell media (Gibco, supplemented with 5% FBS and 500 IU/mL IL-2), IL-2 (Gibco), 10 μg/mL Mitomycin C, 5% hAB serum (Gibco), Trypan Blue
5. **Method:**
   (1) Cell Preparation and Initial Co-culture:
   ①Prepare NK92 cell library and Mitomycin-treated K562 cells. Treat K562 cells with 10 μg/mL Mitomycin C for 2 hrs, then wash thoroughly three times to remove the residual drug.
   ②Co-cultivate NK92 cells and Mitomycin-treated K562 cells in three 15 cm culture dishes (labeled A, B, and C), with each dish containing 5×10^6 NK92 cells and 5×10^6 K562 cells. Add 20 mL RPMI-1640 medium (supplemented with 10% FBS, 1% Penicillin-Streptomycin, and 100 IU/mL IL-2) and incubate at 37°C with 5% CO_2.
   (2) Co-culture Conditions and Cell Addition:
   ①Dish A: After 36 hrs of incubation, sort and collect EGFP+ NK92 cells using flow cytometry.
   ②Dish B: After 36 hrs of incubation, add an additional 5×10^6 Mitomycin-treated K562 cells to Dish B. Continue incubation for another 36 hrs (total 72 hrs), then sort and collect EGFP+ NK92 cells.
   ③Dish C: After 36 hrs of incubation, add 5×10^6 Mitomycin-treated K562 cells to Dish C, and add another 5×10^6 K562 cells at 72 hrs. Continue incubation for a total of 108 hrs, then sort and collect EGFP+ NK92 cells.
   (3) Distribution and Fluorescence Detection:
   ①After sorting, distribute the NK-92 cells from dishes A, B, and C into three groups of ten 96-well plates each (30 plates total). Ensure that cells are evenly distributed among the wells.
   ②Incubate the plates for 24 hrs at 37°C with 5% CO_2.
   ③Perform fluorescence detection by photographing the wells in a dark box equipped with a fluorescence filter. Capture images of each well to assess EGFP expression levels based on fluorescence intensity.
6. **Result:**
   The images show the varying levels of EGFP expression across different conditions, reflecting the proliferation status of the 3 dishes of NK92 cells.

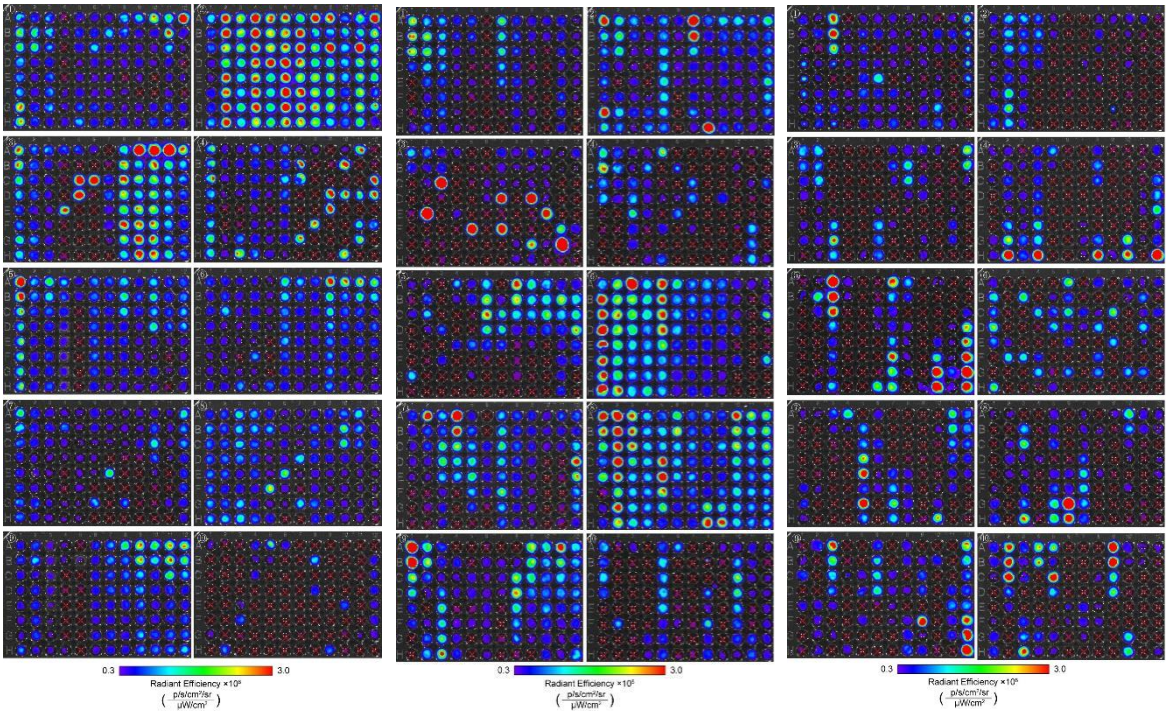

Fig.1 EGFP Fluorescence of 3 dishes NK92 cells