YMTHE, Volume *25*

Supplemental Information

Adoptive Transfer of mRNA-Transfected T Cells Redirected against Diabetogenic CD8 T Cells Can Prevent Diabetes

Sigal Fishman, Mark D. Lewis, L. Khai Siew, Evy De Leenheer, Dimitri Kakabadse, Joanne Davies, Doron Ziv, Alon Margalit, Nathan Karin, Gideon Gross, and F. Susan Wong

Legend for supplementary figure S1.

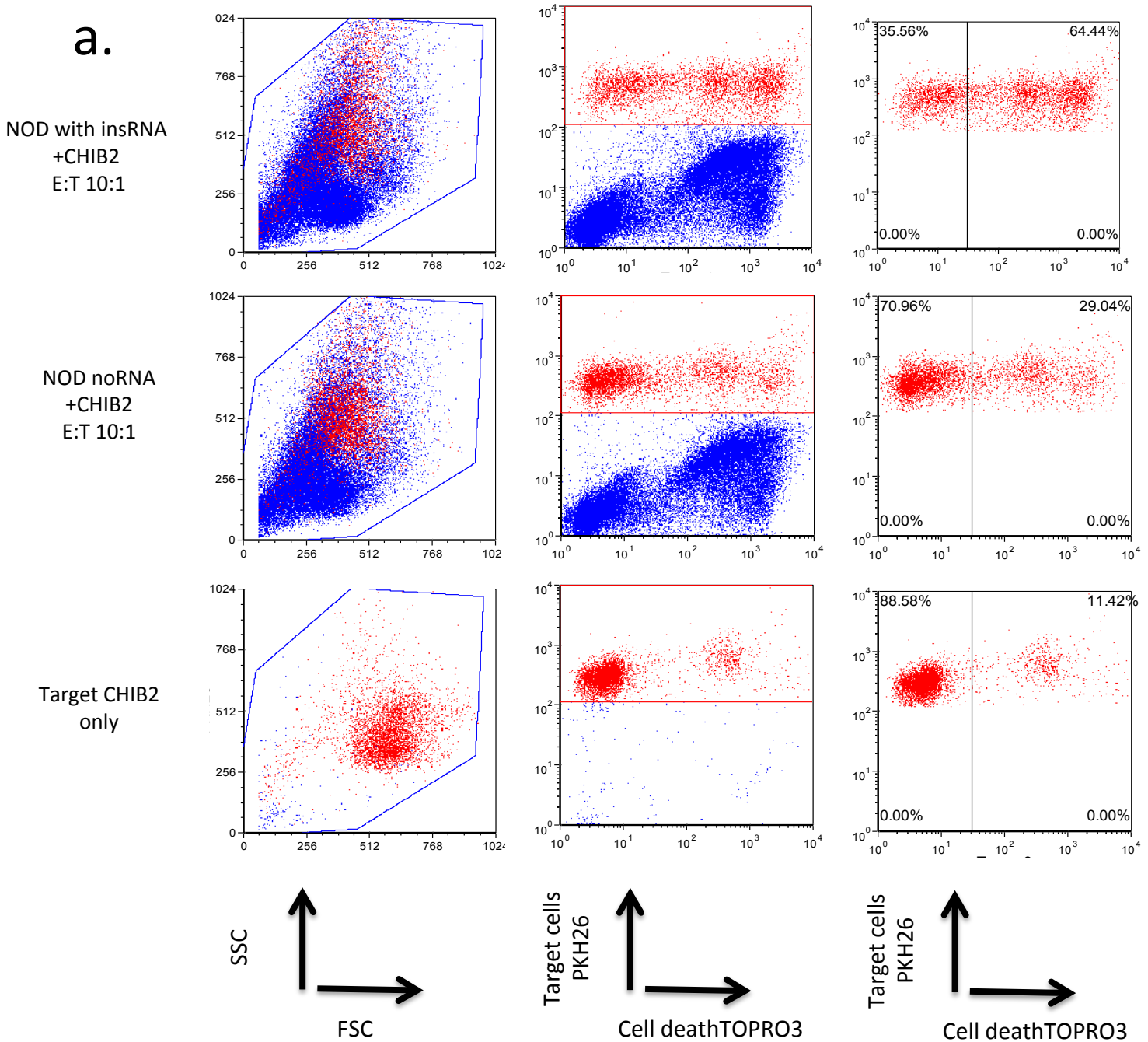
S1 Gating strategy for cytotoxicity assay

a. NOD T cells were electorporated with $InsB_{15\text{-}23}/\beta_2m$/CD3-ζ mRNA (top row) or electroporated without RNA (second row) and were incubated with PKH26-labeled target CHIB2 hybridoma cells, at an effector:target E:T ratio of 10:1 for 24 hours. PKH26-labeled target cells alone are shown in the bottom row. The analysis was performed by flow cytometry, detecting cells damaged by cytotoxicity, with TOPRO-3 added just prior to analysis. The gating strategy is shown. Total cells were gated and then after gating for single cells, the populations that were labeled with PKH26 and those stained by TOPRO-3 are shown in the middle panel. The % killed cells are shown in the right panels with cells stained with TOPRO3 calculated as a percentage of total PKH26-labeled target cells.

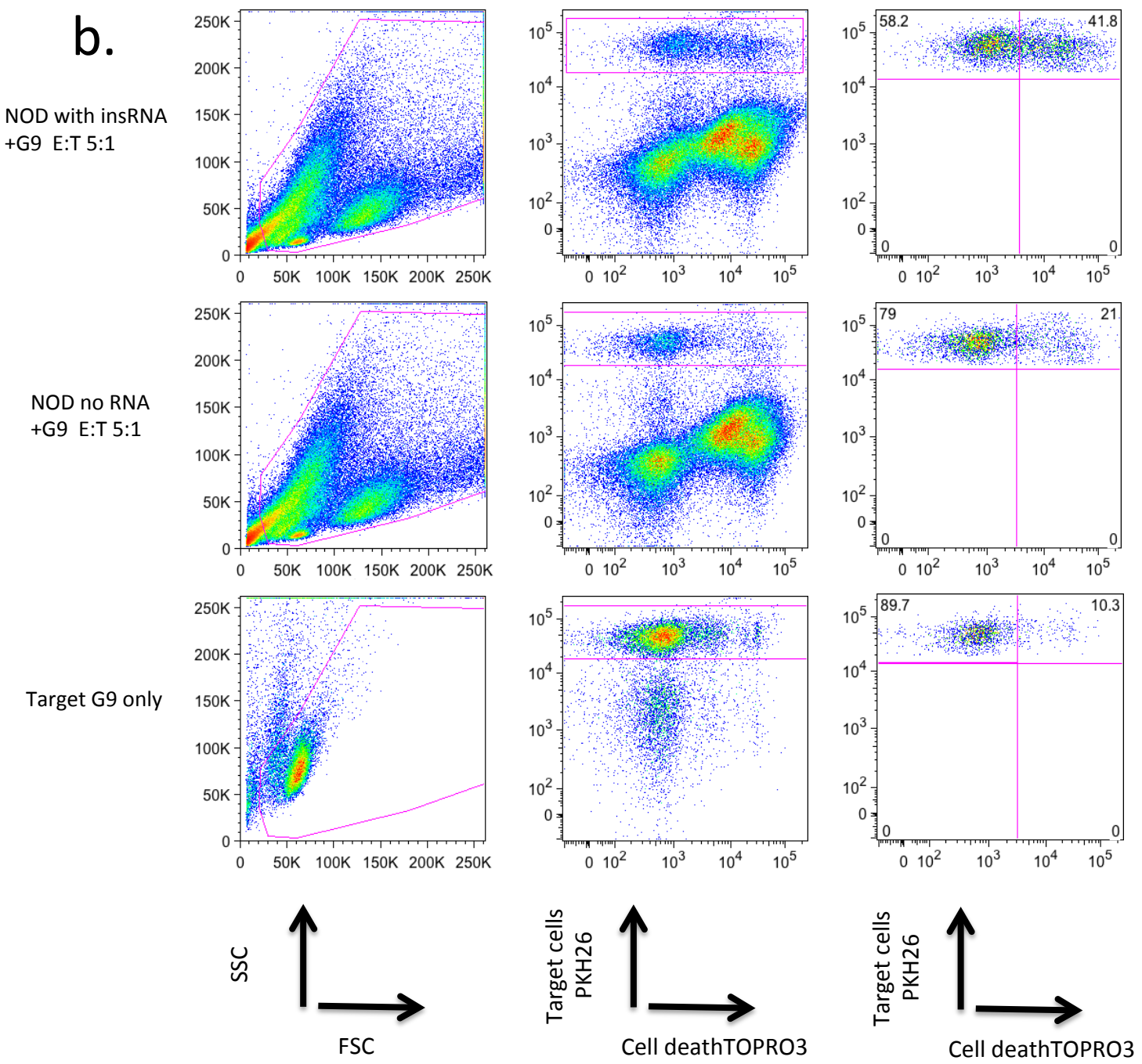
b. NOD T cells were electorporated with $InsB_{15\text{-}23}/\beta_2m$/CD3-ζ mRNA (top row) or electroporated without RNA (second row) and were incubated with PKH26-labeled target G9 insulin-reactive CD8 T cells at an effector:target E:T ratio of 5:1 for 24 hours. PKH26-labeled target cells alone are shown in the bottom row. The analysis was performed by flow cytometry, detecting cells damaged by cytotoxicity, with TOPRO-3 added just prior to analysis. The gating strategy is shown. Total cells were gated and then, after gating for single cells, the populations that were labeled with PKH26 and those stained by TOPRO-3 are shown in the middle panel. The % killed cells are shown in the right panels with cells stained with TOPRO3 calculated as a percentage of total PKH26-labeled target cells.

S1

a.


NOD with insRNA
+CHIB2
E:T 10:1
NOD noRNA
+CHIB2
E:T 10:1
Target CHIB2
only
35.56%
64.44%
0.00%
0.00%
70.96%
29.04%
0.00%
0.00%
88.58%
11.42%
0.00%
0.00%
SSC
FSC
Target cells
PKH26
Cell deathTOPRO3
Target cells
PKH26
Cell deathTOPRO3

S1


b.
NOD with insRNA
+G9 E:T 5:1
NOD no RNA
+G9 E:T 5:1
Target G9 only
58.2
41.8
79
21
89.7
10.3
SSC
FSC
Target cells PKH26
Cell deathTOPRO3
Target cells PKH26
Cell deathTOPRO3