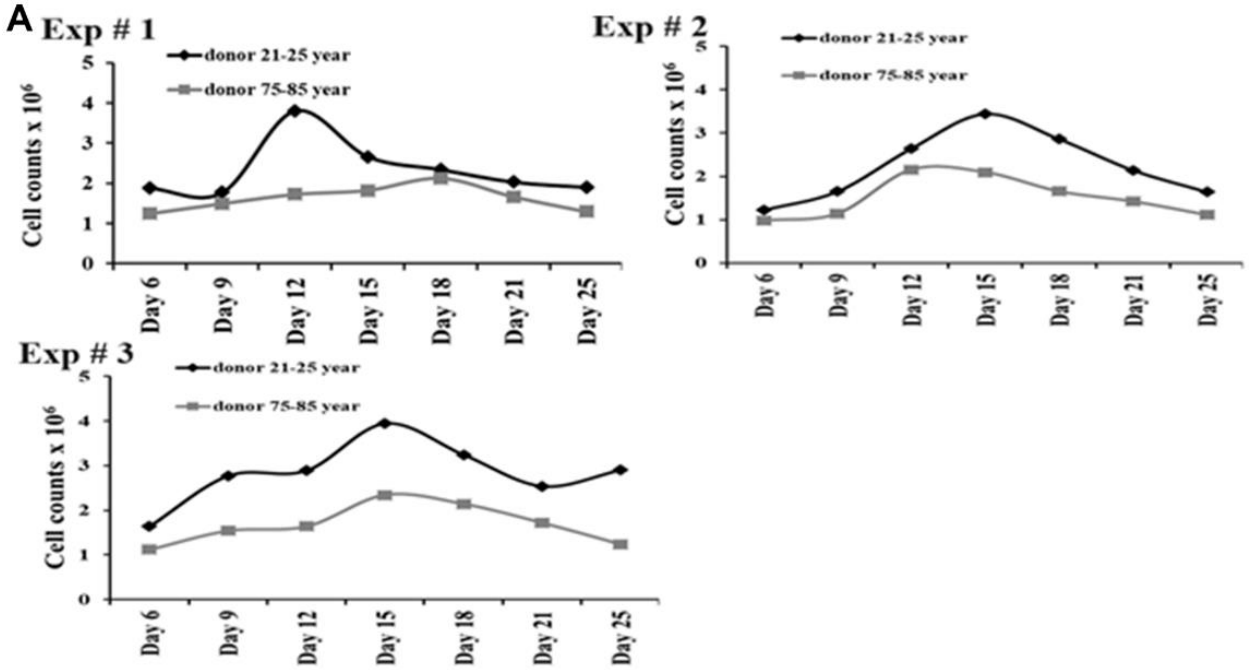
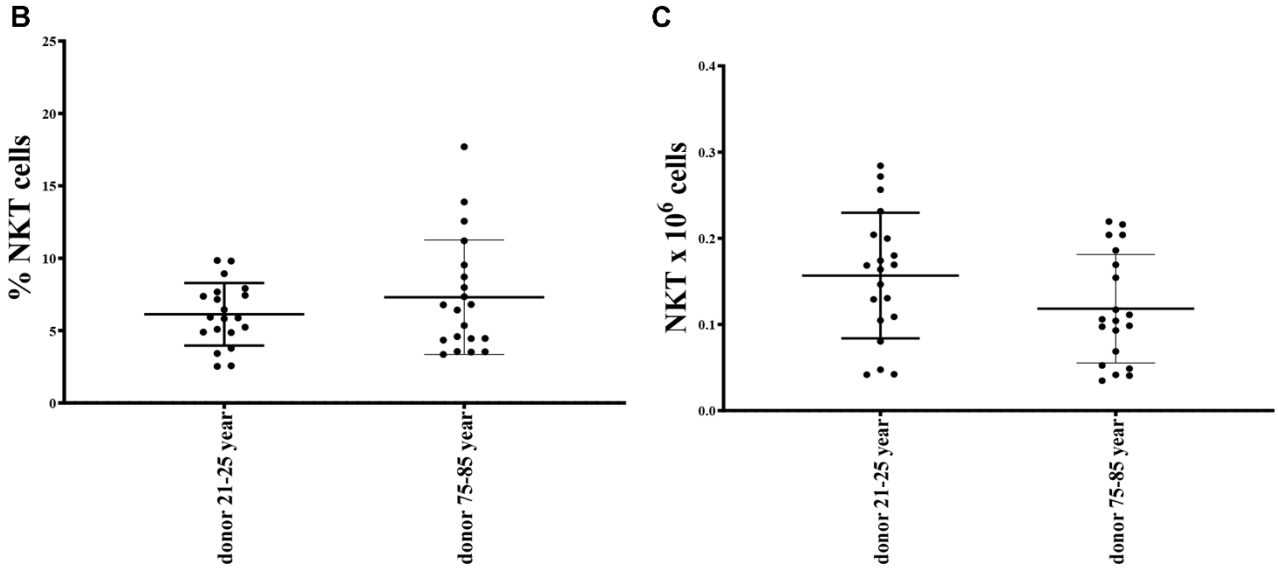
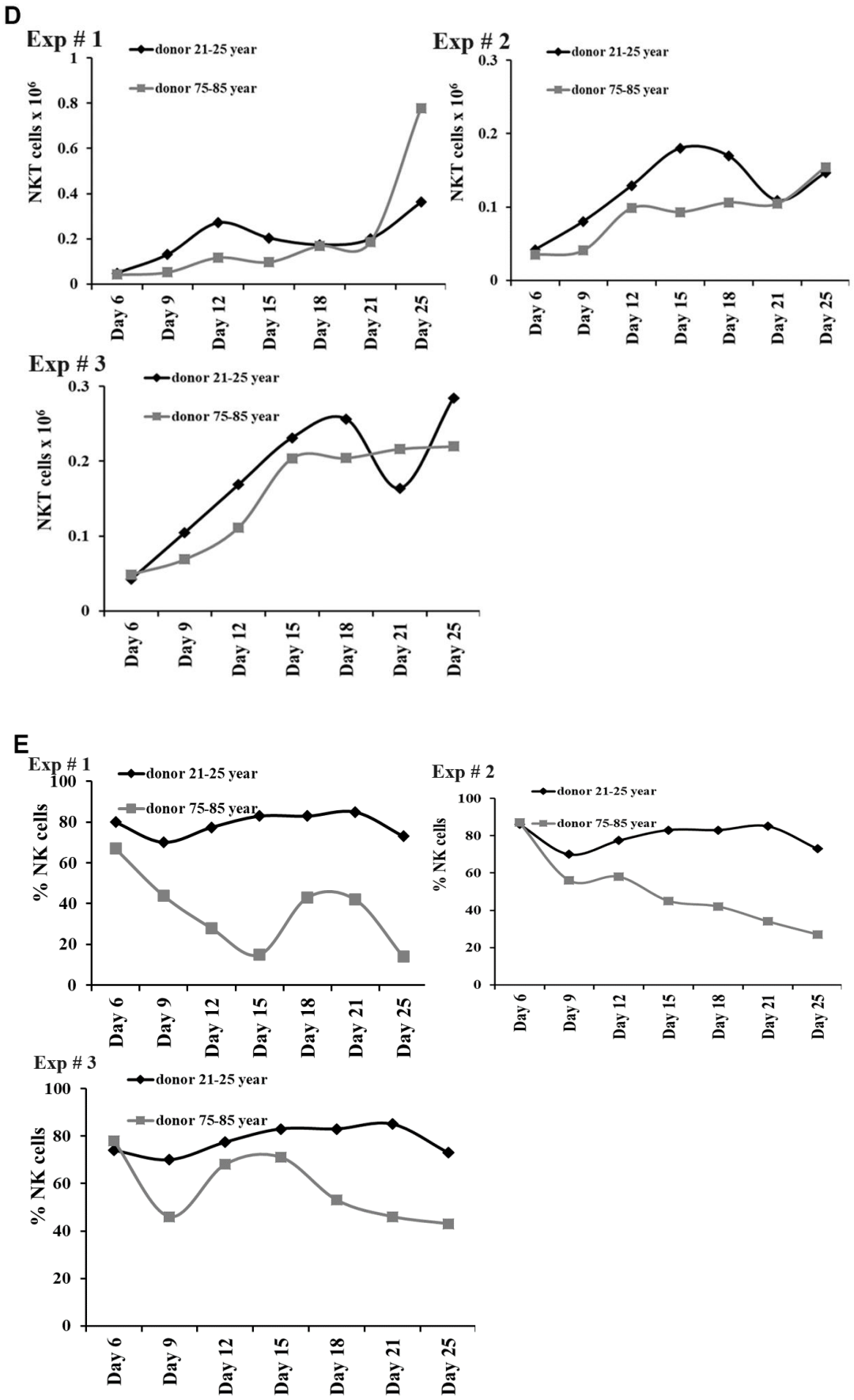
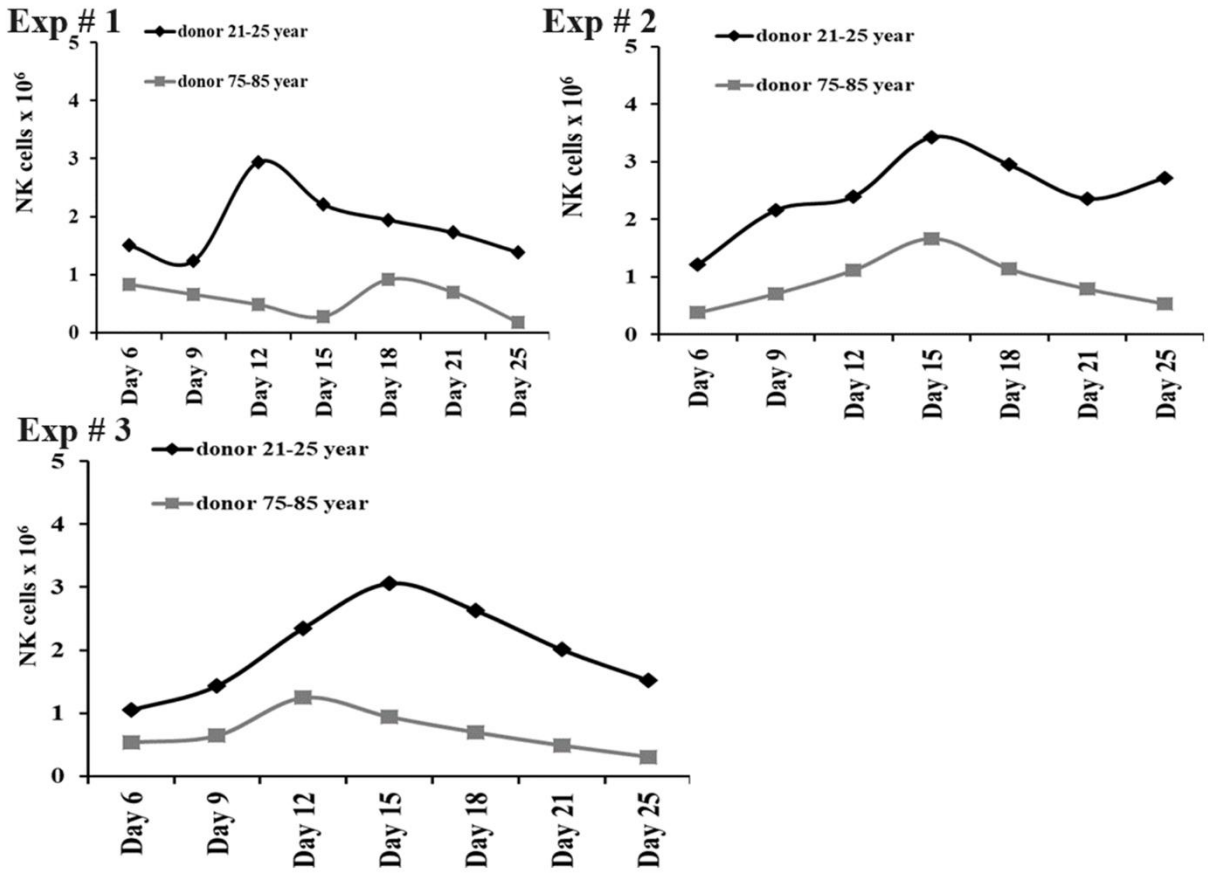
SUPPLEMENTARY FIGURES



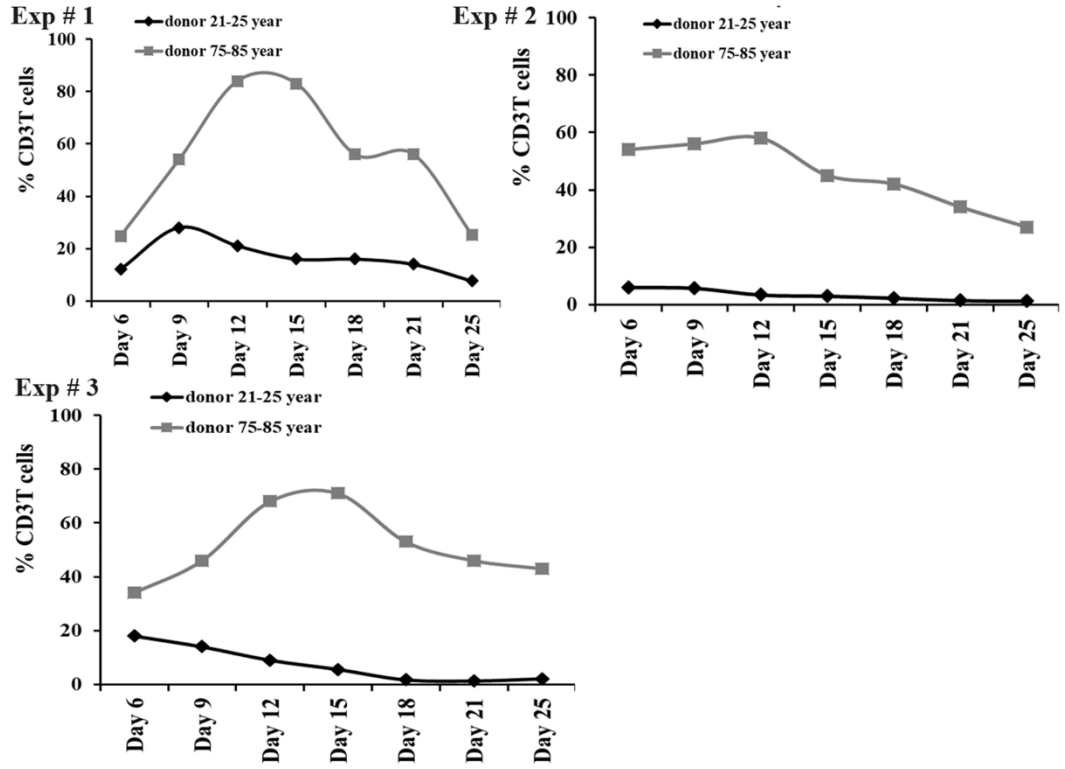



D
Exp # 1
donor 21-25 year
donor 75-85 year
NKT cells x 10⁶
Exp # 2
Exp # 3
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25
E
Exp # 1
% NK cells
Exp # 2
Exp # 3

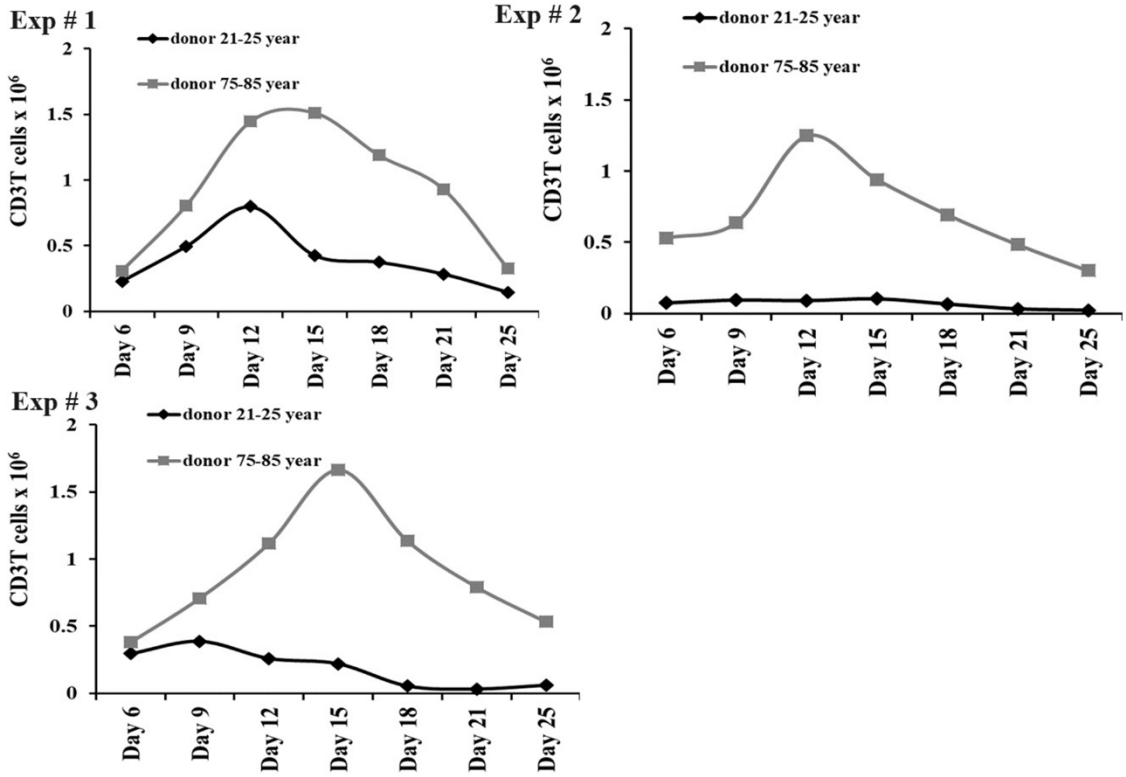
F


Exp # 1
donor 21-25 year
donor 75-85 year
NK cells x 10⁶
0
1
2
3
4
5
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25
Exp # 2
donor 21-25 year
donor 75-85 year
NK cells x 10⁶
Exp # 3
donor 21-25 year
donor 75-85 year
NK cells x 10⁶

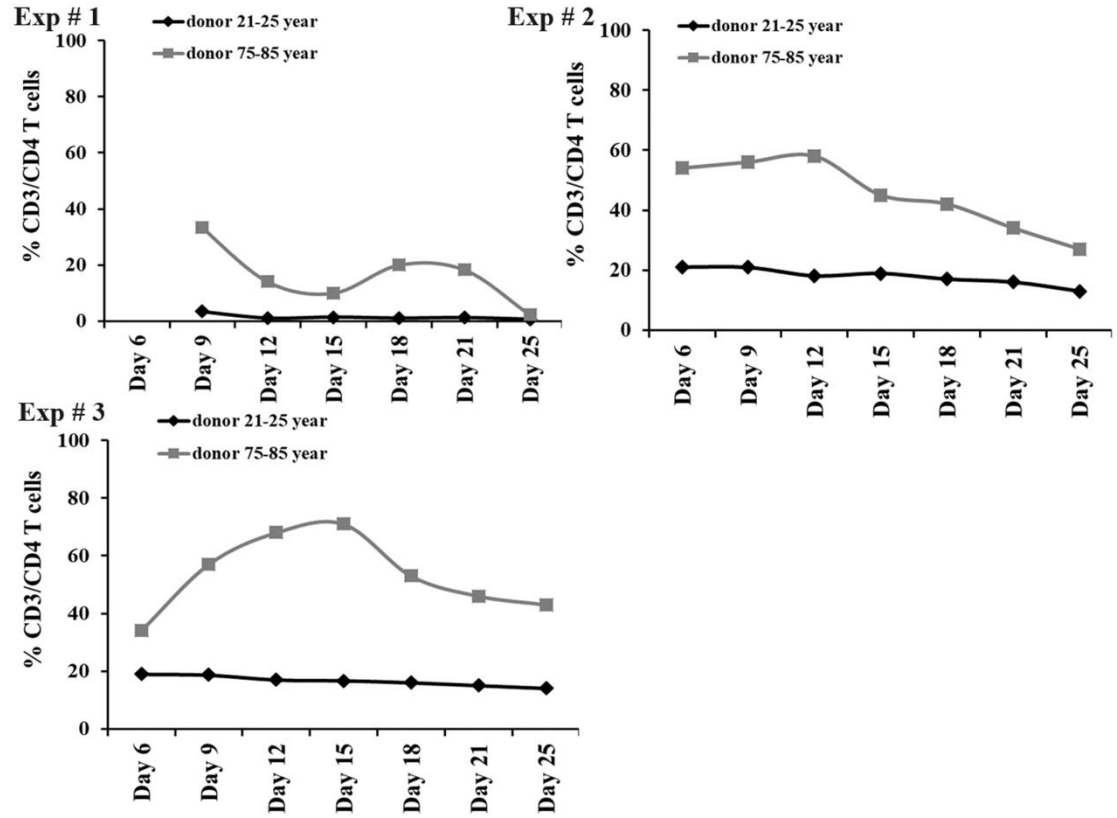
G

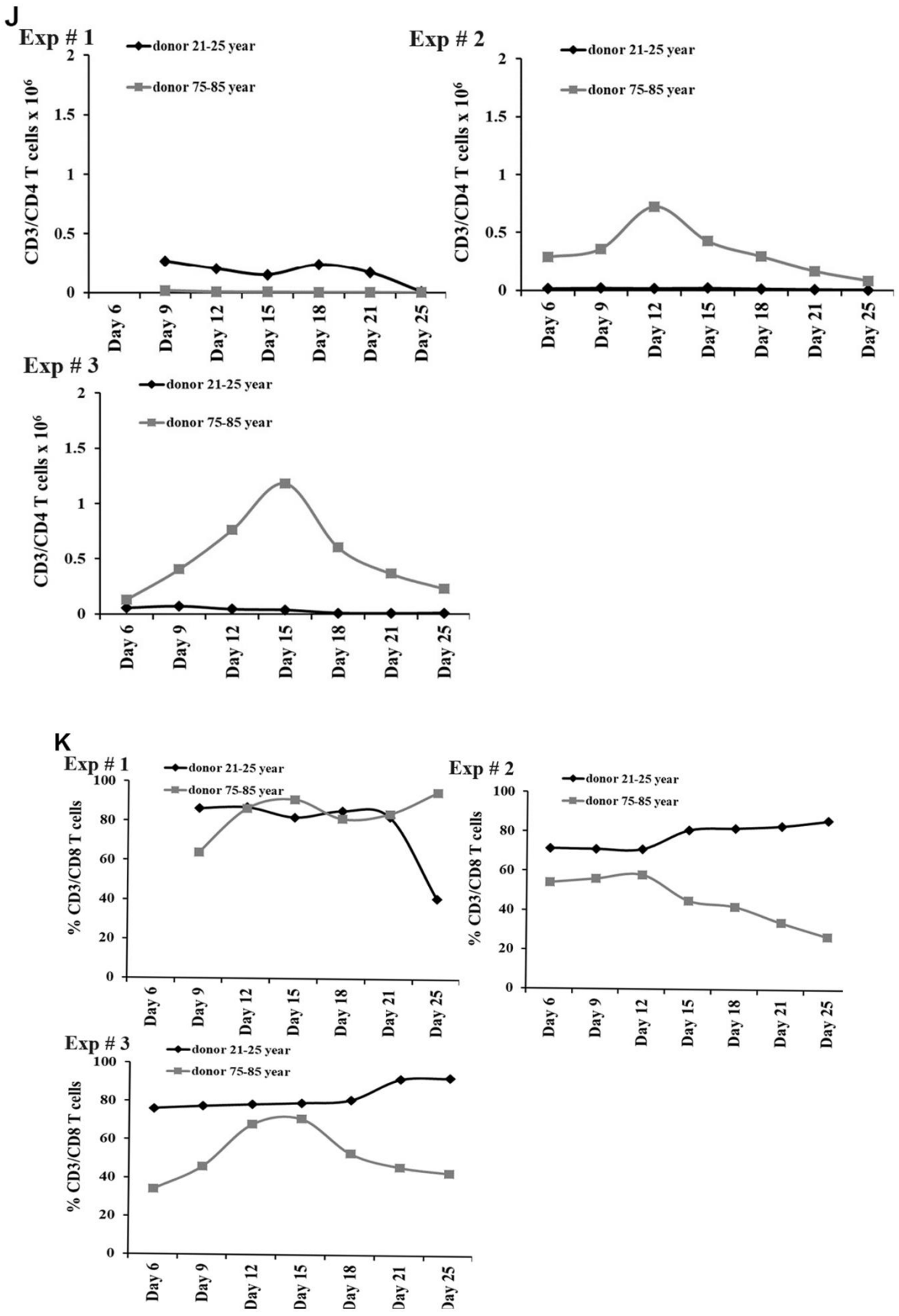

Exp # 1
donor 21-25 year
donor 75-85 year
% CD3T cells
0
20
40
60
80
100
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25
Exp # 2
donor 21-25 year
donor 75-85 year
% CD3T cells
Exp # 3
donor 21-25 year
donor 75-85 year
% CD3T cells

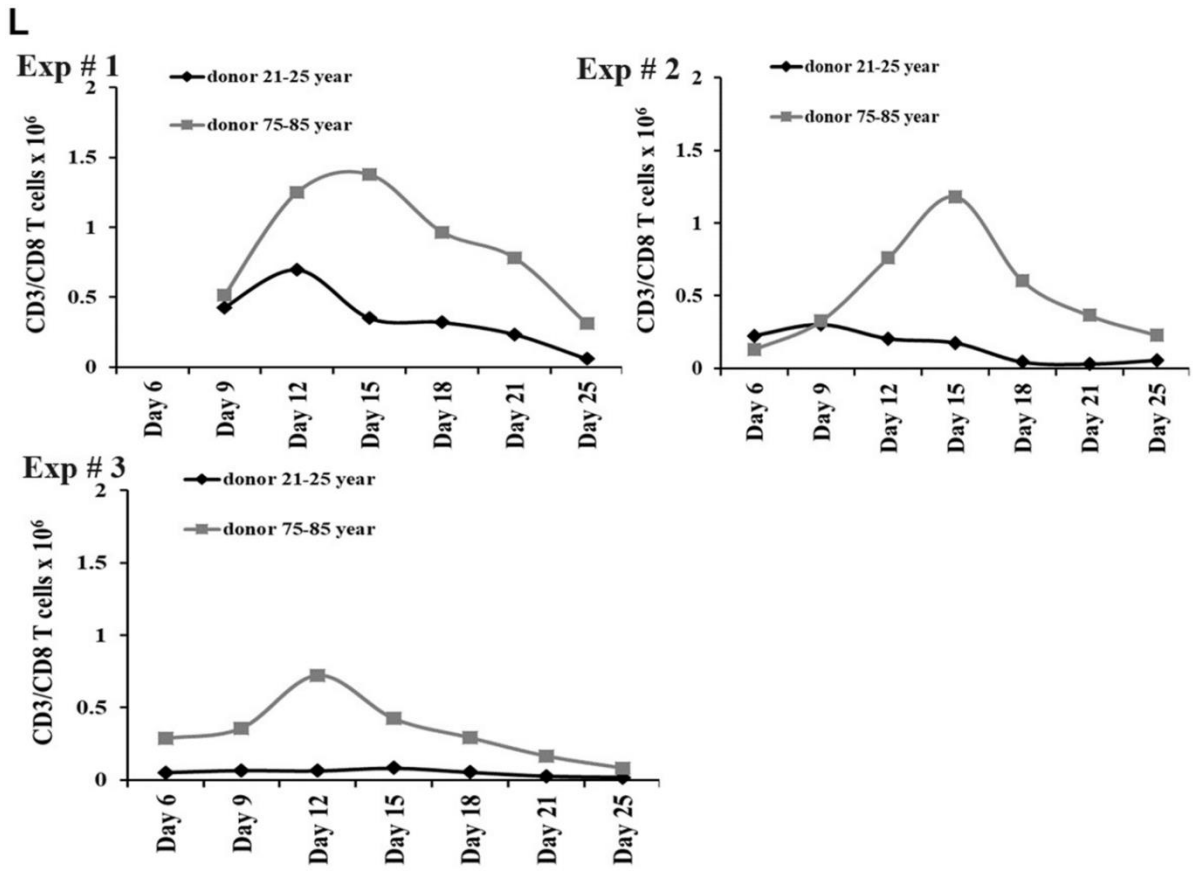
H


Exp # 1
donor 21-25 year
donor 75-85 year
CD3T cells x 10^6
Exp # 2
donor 21-25 year
donor 75-85 year
CD3T cells x 10^6
Exp # 3
donor 21-25 year
donor 75-85 year
CD3T cells x 10^6
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25

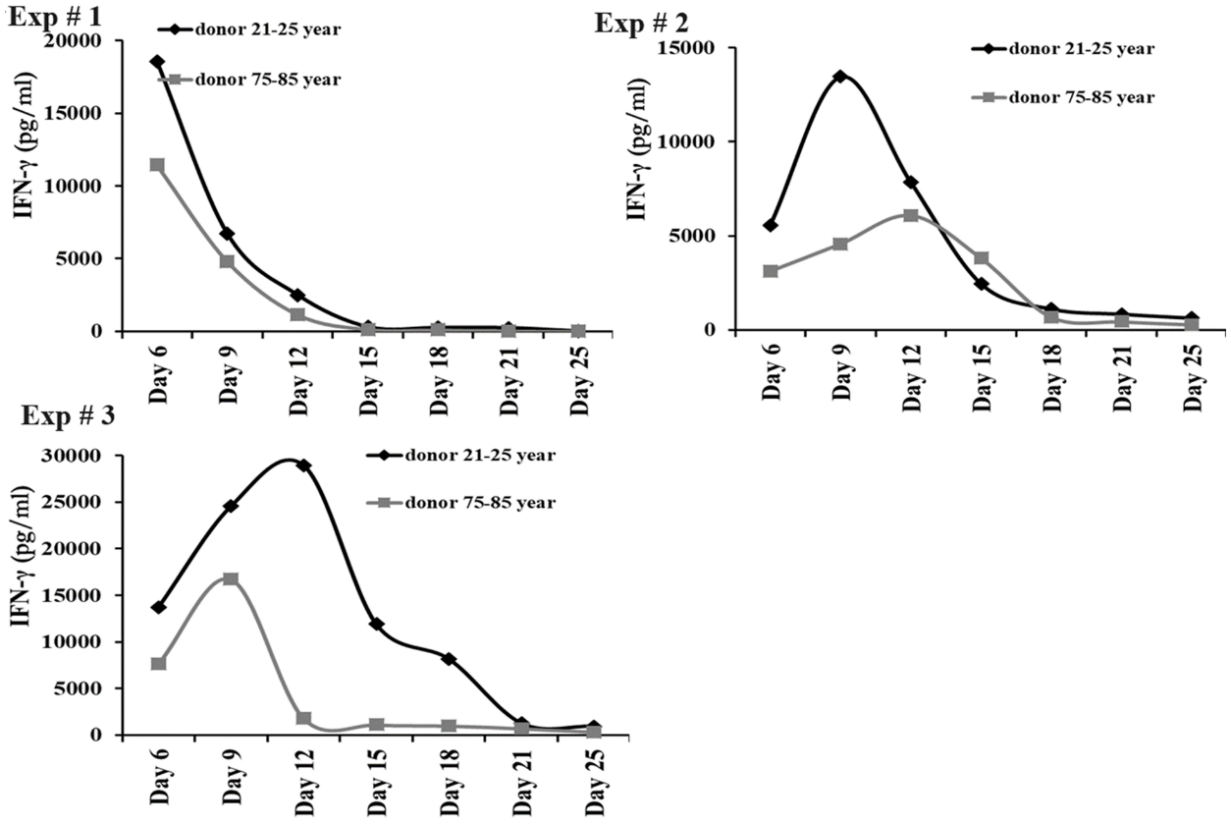
I


Exp # 1
donor 21-25 year
donor 75-85 year
% CD3/CD4 T cells
Exp # 2
donor 21-25 year
donor 75-85 year
% CD3/CD4 T cells
Exp # 3
donor 21-25 year
donor 75-85 year
% CD3/CD4 T cells
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25

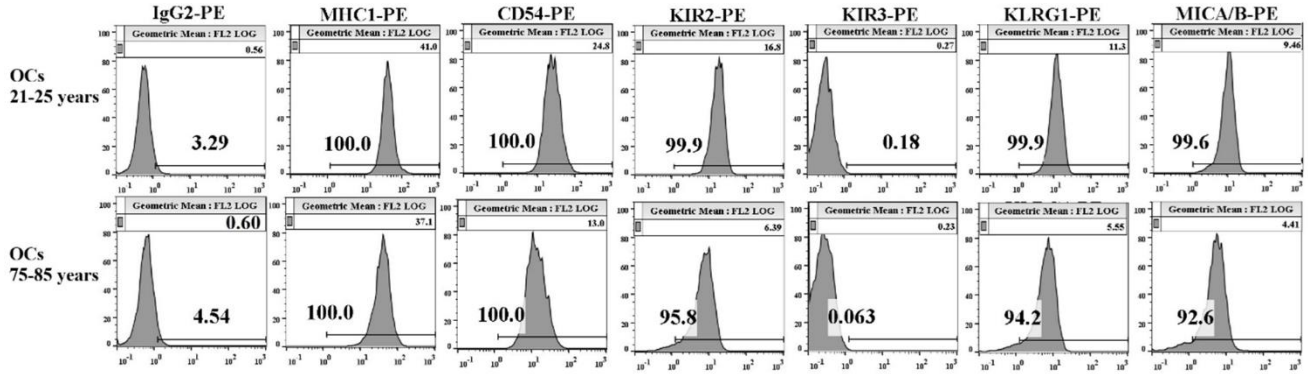

J
Exp # 1
donor 21-25 year
donor 75-85 year
CD3/CD4 T cells x 10⁶
Day 6
Day 9
Day 12
Day 15
Day 18
Day 21
Day 25
Exp # 2
Exp # 3
K
% CD3/CD8 T cells



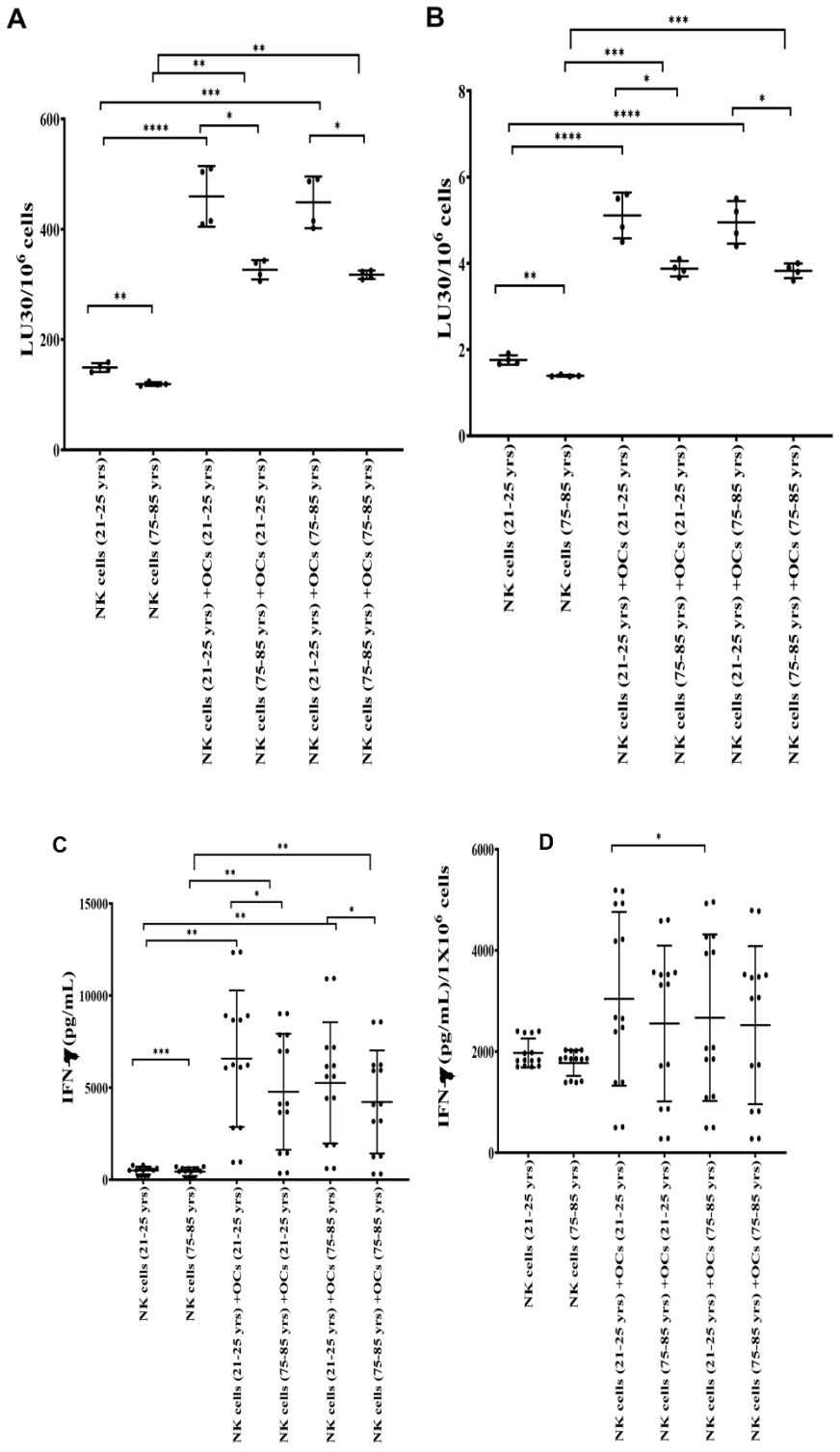
Supplementary Figure 1. OCs induced lower levels of cell expansion in old-age donor NK cells. Osteoclasts (OCs) were generated as described in the Materials and Methods section. NK cells ($0.5x10^6$ cells/ 2ml) were a combination of IL-2 (1000 U/ml) and anti-CD16mAb (3µg/ml) for 18 hours before they were co-cultured with OCs and sAJ2 (1:2:4: OCs:NK:sAJ2). NK cells were counted on days 6, 9, 12, 15, 18, 21, and 25, day 0 counts were $0.5x10^6$ cells/2 ml, and $0.5x10^6$ cells/2 ml were cultured every 3 days (**A**). CD3+CD16+CD56+ NKT cells, and the number of NKT cells (**B**–**D**), CD16+CD56+ NK cells and the number of NK cells (**E**, **F**), CD3+ T cells and the number of T cells (**G**, **H**), CD3+CD4+ T cells and the number of CD4+ T cells (**I**, **J**), CD3+CD8+ T cells and the number of CD8+ T cells (**K**, **L**), were determined counted on days 6, 9, 12, 15, 18, 21, and 25 in expanding cells.



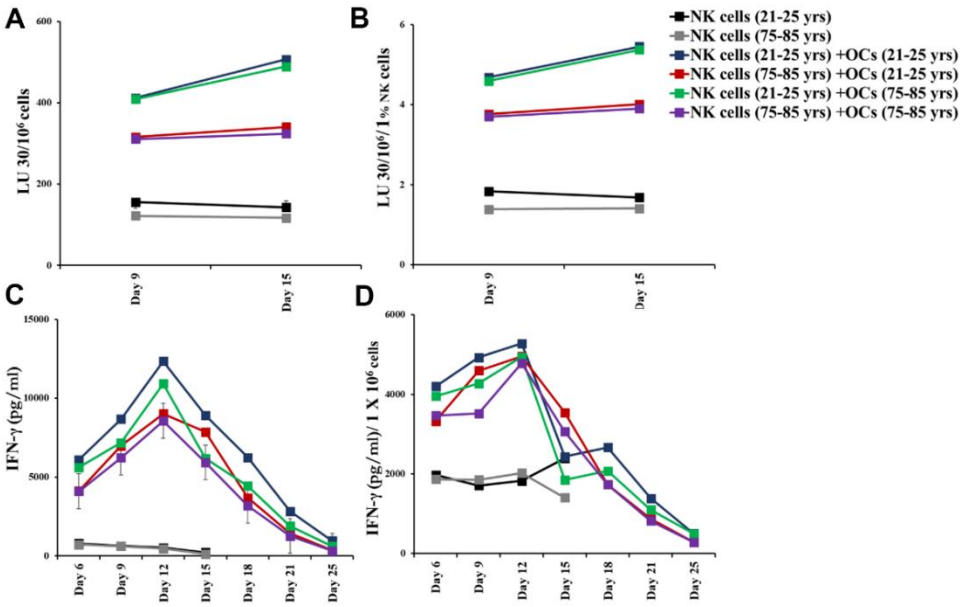
Supplementary Figure 2. OCs induced lower levels of increased IFN-γ secretion in old-age donor NK cells. Osteoclasts (OCs) were generated as described in the Materials and Methods section. NK cells and OCs co-culture was performed as described in Supplementary Figure 1. The supernatants were harvested from the cultures on days 6, 9, 12, 15, 18, 21, and 25 to determine IFN-γ secretion using single ELISA.



Supplementary Figure 3. Reduced surface receptor expressions on old age Ocs. Osteoclasts (OCs) were generated as described in the Materials and Methods section. The surface markers MHC-class I, CD54, KIR2, KIR3, KLRG1, and MICA/B were determined on OCs using flow cytometric analysis (one of two representative experiments is shown in the figure).



Supplementary Figure 4. Old-age donor-derived OCs induced lower levels of activation in NK cells. Osteoclasts (OCs) were generated as described in the Materials and Methods section. NK cells ($0.5x10^6$ cells/ 2ml) were a combination of IL-2 (1000 U/ml) and anti-CD16mAb (3μg/ml) for 18 hours before they were co-cultured with criss-cross OCs and sAJ2 (1:2:4: OCs:NK:sAJ2). NK cell-mediated cytotoxicity against OSCSCs was determined on days 9 and 15 using a standard 4-hour ^{51}Cr release assay. The lytic units $30/10^6$ cells were determined using the inverse number of NK cells required to lyse 30% of OSCSCs x 100 (**A**). Lytic units per 1 % NK cells were determined based on the percentages of CD16+/CD56+ NK cells in the cultures obtained by flow cytometric analysis (**B**). The supernatants were harvested from the cultures on days 6, 9, 12, 15, 18, 21, and 25 to determine IFN-γ secretion using single ELISA (**C**), and the levels were adjusted based on per million of cells (**D**). ****(p-value <0.0001), ***(*p*-value 0.0001-0.001).



P-values of Supplementary Figure 5

A

P values	Day 9	Day 15
NK (21-25 y) vs. NK(75-85 y)	*	*
NK (21-25 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*
NK (21-25 y) vs. NK(75-85 y)+OCs(21-25 y)	*	****
NK (21-25 y) vs. NK(21-25 y)+OCs(75-85 y)	*	****
NK (21-25 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*
NK (75-85 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*
NK (75-85 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*
NK (75-85 y) vs. NK(21-25 y)+OCs(75-85 y)	*	*
NK (75-85 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(21-25 y)	*	*
NK(21-25 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	ns	ns
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*
NK(75-85 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	*	****
NK(75-85 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	ns	ns
NK(21-25 y)+OCs(75-85 y) vs. NK (75-85 y)+OCs(75-85 y)	ns	*

B

P values	Day 9	Day 15
NK (21-25 y) vs. NK(75-85 y)	ns	*
NK (21-25 y) vs. NK(21-25 y)+OCs(21-25 y)	ns	*
NK (21-25 y) vs. NK(75-85 y)+OCs(21-25 y)	*	ns
NK (21-25 y) vs. NK(21-25 y)+OCs(75-85 y)	ns	ns
NK (21-25 y) vs. NK(75-85 y)+OCs(75-85 y)	ns	ns
NK (75-85 y) vs. NK(21-25 y)+OCs(21-25 y)	ns	*
NK (75-85 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*
NK (75-85 y) vs. NK(21-25 y)+OCs(75-85 y)	ns	*
NK (75-85 y) vs. NK(75-85 y)+OCs(75-85 y)	ns	ns
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(21-25 y)	ns	*
NK(21-25 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	ns	ns
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	*	ns
NK(75-85 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	ns	*
NK(75-85 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	ns	ns
NK(21-25 y)+OCs(75-85 y) vs. NK (75-85 y)+OCs(75-85 y)	****	ns

C

P values	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 25
NK (21-25 y) vs. NK(75-85 y)	*	ns	ns	ns			
NK (21-25 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*	*	*			
NK (21-25 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*	*	*			
NK (21-25 y) vs. NK(21-25 y)+OCs(75-85 y)	*	*	*	*			
NK (21-25 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*	*	*			
NK (75-85 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*	*	*			
NK (75-85 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*	*	*			
NK (75-85 y) vs. NK(21-25 y)+OCs(75-85 y)	*	*	*	*			
NK (75-85 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*	*	*			
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(21-25 y)	*	*	*	*	*	*	*
NK(21-25 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	*	*	*	*	*	*	*
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*	*	*	*	*	*
NK(75-85 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	*	*	*	*	*	ns	ns
NK(75-85 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	ns	*	*	*	*	ns	ns
NK(21-25 y)+OCs(75-85 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*	*	ns	*	ns	*

D

P values	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 25
NK (21-25 y) vs. NK(75-85 y)	ns	*	****	*			
NK (21-25 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*	*	ns			
NK (21-25 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*	*	*			
NK (21-25 y) vs. NK(21-25 y)+OCs(75-85 y)	*	*	*	*			
NK (21-25 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*	*	*			
NK (75-85 y) vs. NK(21-25 y)+OCs(21-25 y)	*	*	*	*			
NK (75-85 y) vs. NK(75-85 y)+OCs(21-25 y)	*	*	*	*			
NK (75-85 y) vs. NK(21-25 y)+OCs(75-85 y)	****	*	*	*			
NK (75-85 y) vs. NK(75-85 y)+OCs(75-85 y)	*	*	*	*			
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(21-25 y)	*	*	*	*	*	*	*
NK(21-25 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	*	*	*	ns	*	*	ns
NK(21-25 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*	*	ns	*	****	*
NK(75-85 y)+OCs(21-25 y) vs. NK (21-25 y)+OCs(75-85 y)	*	*	*	*	*	*	*
NK(75-85 y)+OCs(21-25 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*	*	*	ns	*	ns
NK(21-25 y)+OCs(75-85 y) vs. NK (75-85 y)+OCs(75-85 y)	*	*	ns	*	*	*	*

Supplementary Figure 5. Old-age donor-derived OCs induced lower levels of activation in NK cells. Osteoclasts (OCs) were generated as described in the Materials and Methods section. NK cells and OCs co-culture was performed as described in Fig. S4. NK cell-mediated cytotoxicity against OSCSCs was determined on days 9 and 15 using a standard 4-hour ^{51}Cr release assay. The lytic units 30/10^6 cells were determined using the inverse number of NK cells required to lyse 30% of OSCSCs x 100 (**A**). Lytic units per 1 % NK cells were determined based on the percentages of CD16+CD56+ NK cells in the cultures obtained by flow cytometric analysis (**B**). The supernatants were harvested from the cultures on days 6, 9, 12, 15, 18, 21, and 25 to determine IFN-γ secretion using single ELISA (**C**), and the levels IFN-γ pg/ml were adjusted based on per million of cells (**D**). ****(p-value <0.0001), ***(*p*-value 0.0001-0.001).