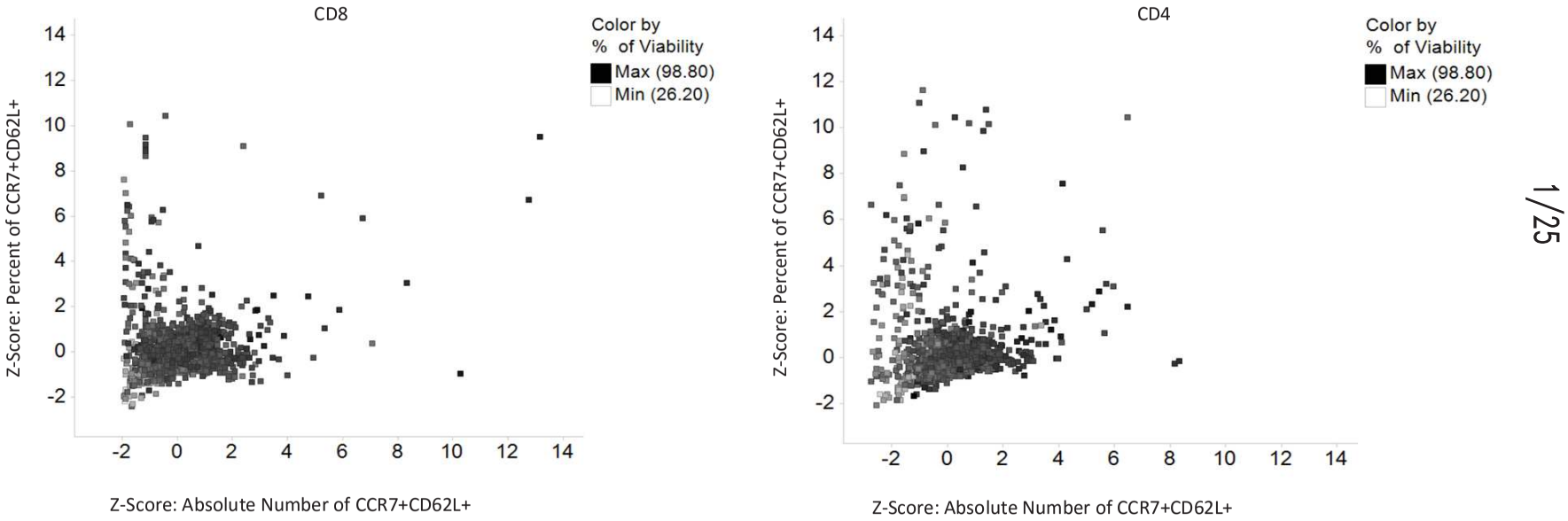

CD8
Color by
% of Viability
Max (98.80)
Min (26.20)
Z-Score: Percent of CCR7+CD62L+
Z-Score: Absolute Number of CCR7+CD62L+
CD4
Color by
% of Viability
Max (98.80)
Min (26.20)
Z-Score: Percent of CCR7+CD62L+
Z-Score: Absolute Number of CCR7+CD62L+

Figure 1

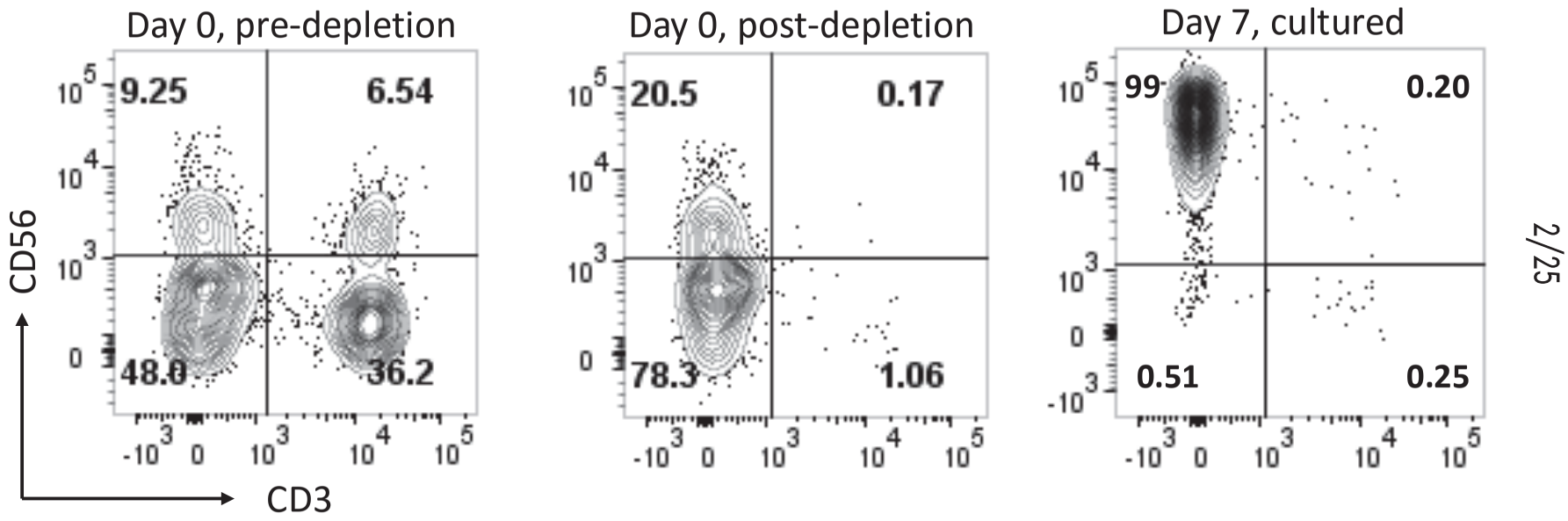

Day 0, pre-depletion
Day 0, post-depletion
Day 7, cultured
CD56
CD3
9.25
6.54
48.0
36.2
20.5
0.17
78.3
1.06
99
0.20
0.51
0.25

Figure 2

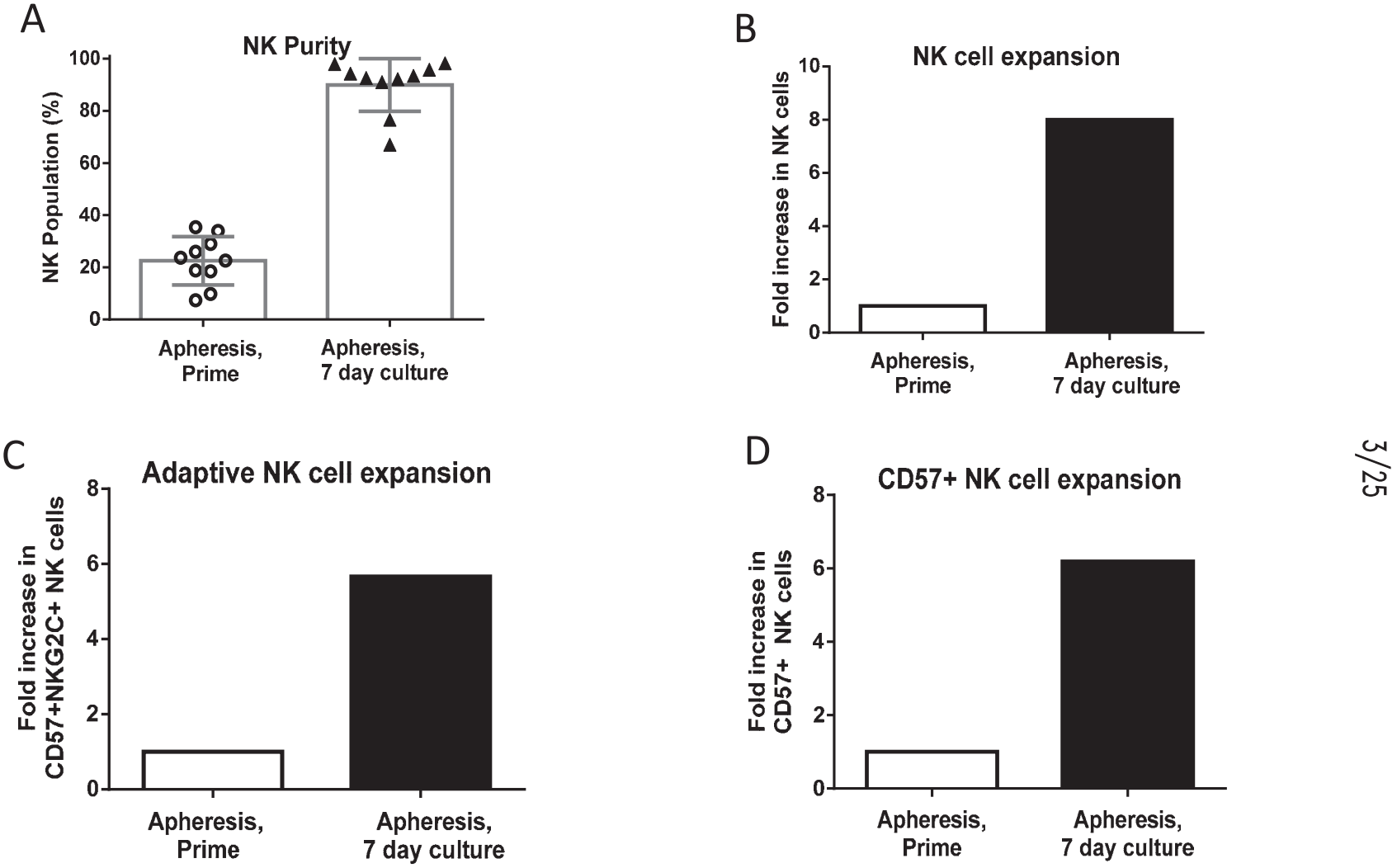
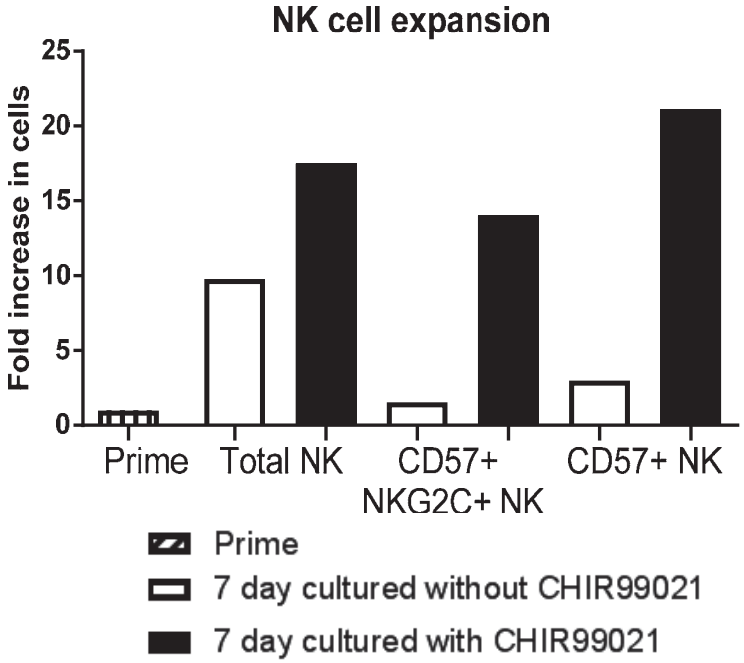

A
NK Purity
NK Population (%)
100
80
60
40
20
0
Apheresis, Prime
Apheresis, 7 day culture
B
NK cell expansion
Fold increase in NK cells
10
8
6
4
2
0
Apheresis, Prime
Apheresis, 7 day culture
C
Adaptive NK cell expansion
Fold increase in CD57+NKG2C+ NK cells
8
6
4
2
0
Apheresis, Prime
Apheresis, 7 day culture
D
CD57+ NK cell expansion
Fold increase in CD57+ NK cells
8
6
4
2
0
Apheresis, Prime
Apheresis, 7 day culture

Figure 3

A


NK cell expansion
Fold increase in cells
25
20
15
10
5
0
Prime
Total NK
CD57+ NKG2C+ NK
CD57+ NK
Prime
7 day cultured without CHIR99021
7 day cultured with CHIR99021

B

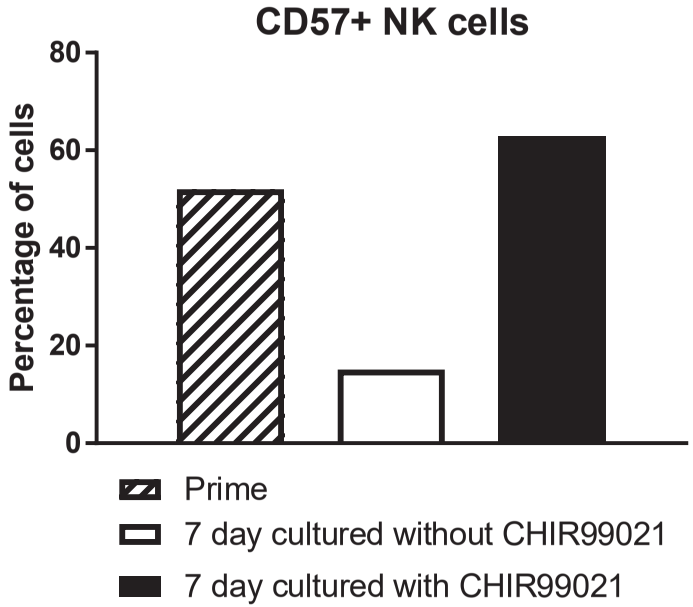

CD57+ NK cells
Percentage of cells
80
60
40
20
0
Prime
7 day cultured without CHIR99021
7 day cultured with CHIR99021

Figure 4

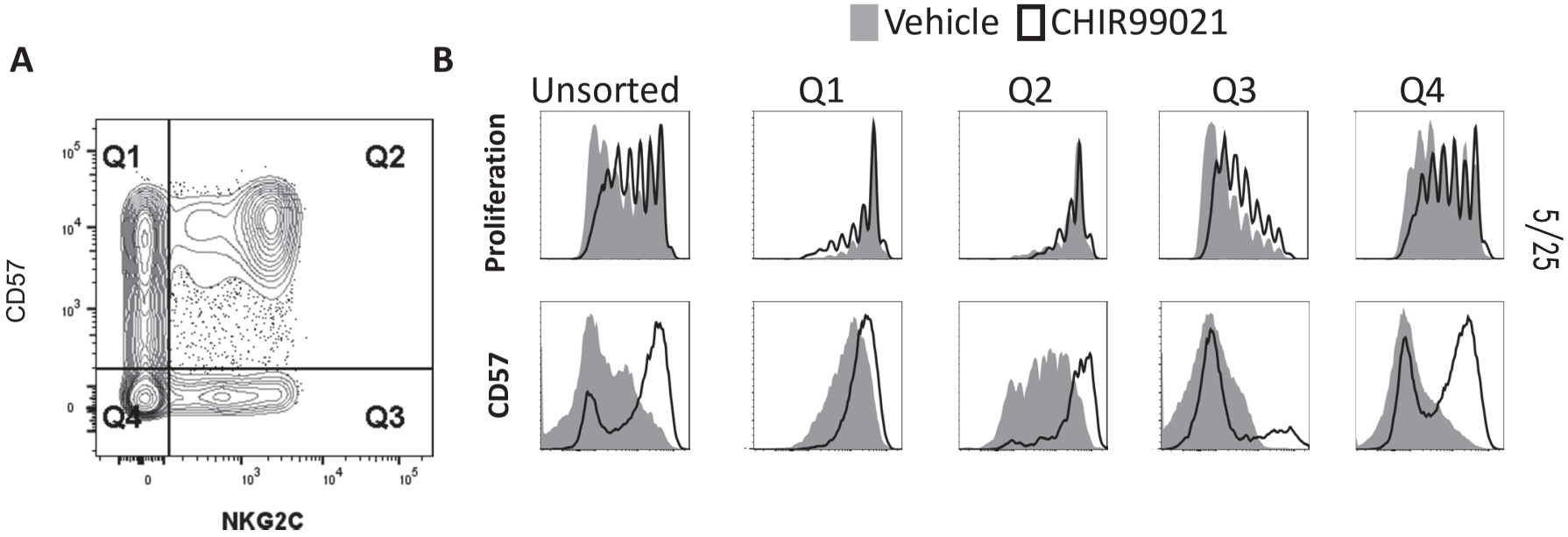

A
B
Vehicle
CHIR99021
Unsorted
Q1
Q2
Q3
Q4
Proliferation
CD57
CD57
NKG2C
0
10^3
10^4
10^5

Figure 5

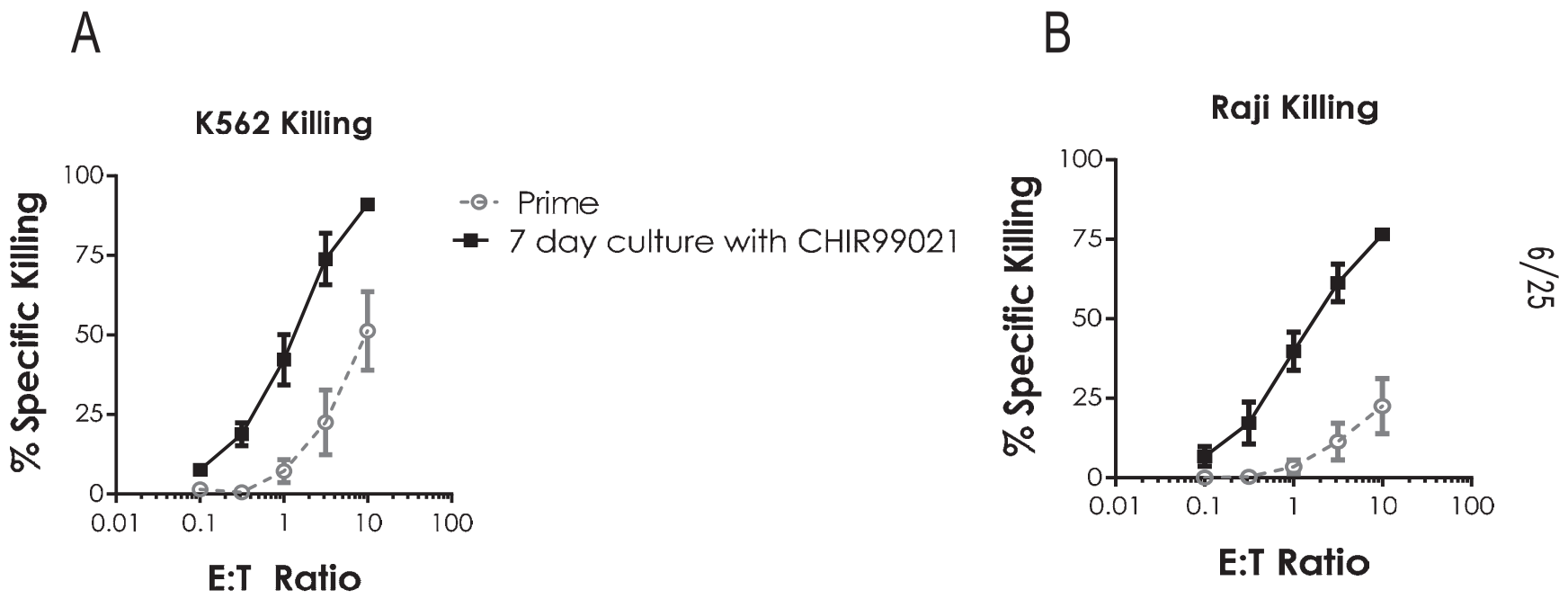

A
K562 Killing
% Specific Killing
100
75
50
25
0
0.01
0.1
1
10
100
E:T Ratio
Prime
7 day culture with CHIR99021
B
Raji Killing
% Specific Killing
100
75
50
25
0
0.01
0.1
1
10
100
E:T Ratio

Figure 6

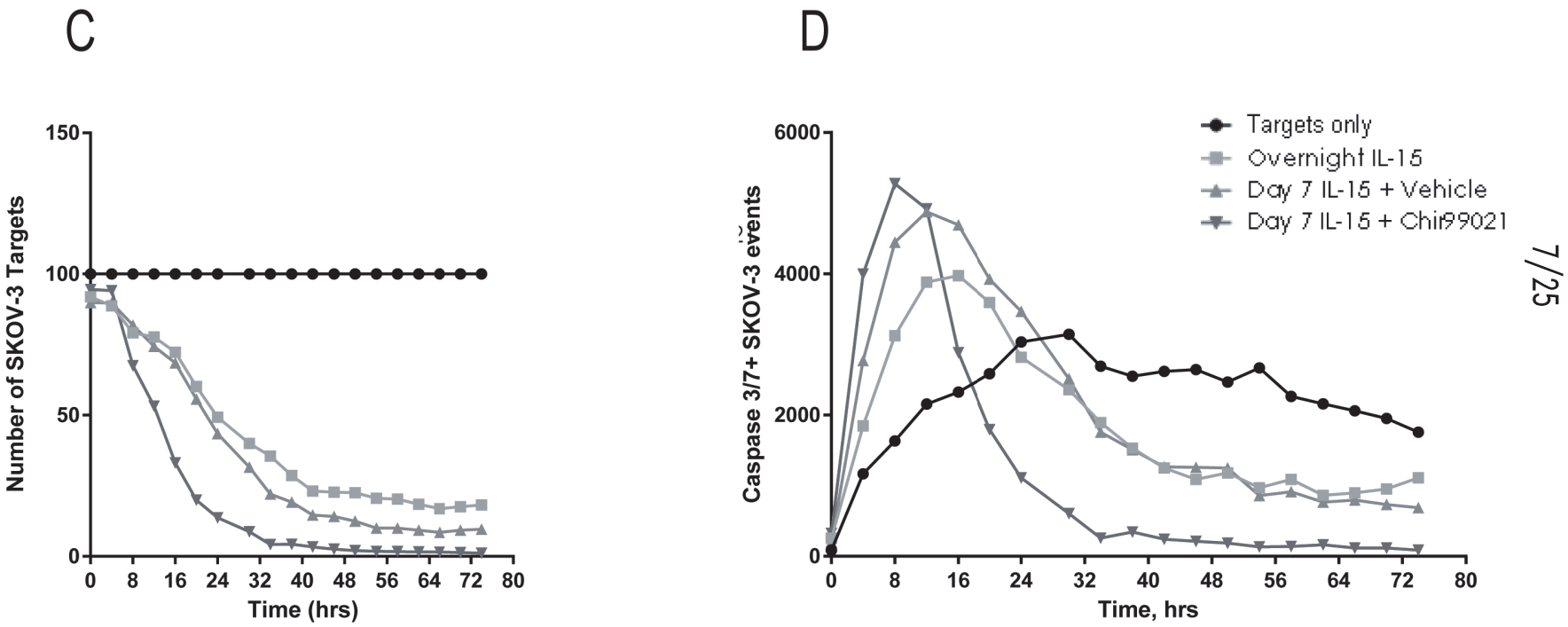

C
D
Number of SKOV-3 Targets
Time (hrs)
Caspase 3/7+ SKOV-3 events
Time, hrs
Targets only
Overnight IL-15
Day 7 IL-15 + Vehicle
Day 7 IL-15 + Chir99021

Figure 6

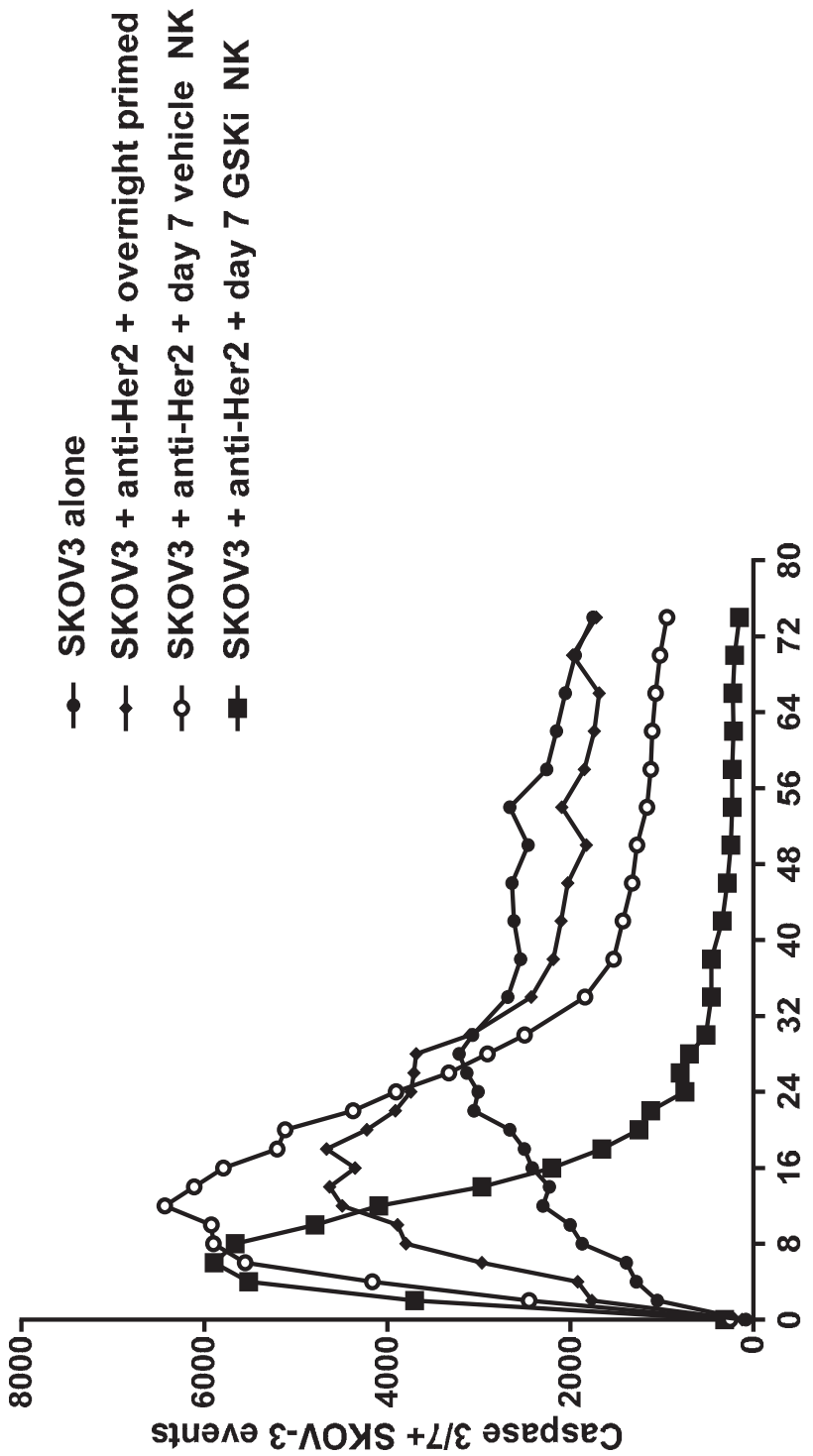

10:1 E:T Ratio
SKOV3 alone
SKOV3 + anti-Her2 + overnight primed
SKOV3 + anti-Her2 + day 7 vehicle NK
SKOV3 + anti-Her2 + day 7 GSKi NK
Caspase 3/7+ SKOV-3 events
8000
6000
4000
2000
0
0
8
16
24
32
40
48
56
64
72
80
Time, hrs

Figure 6E

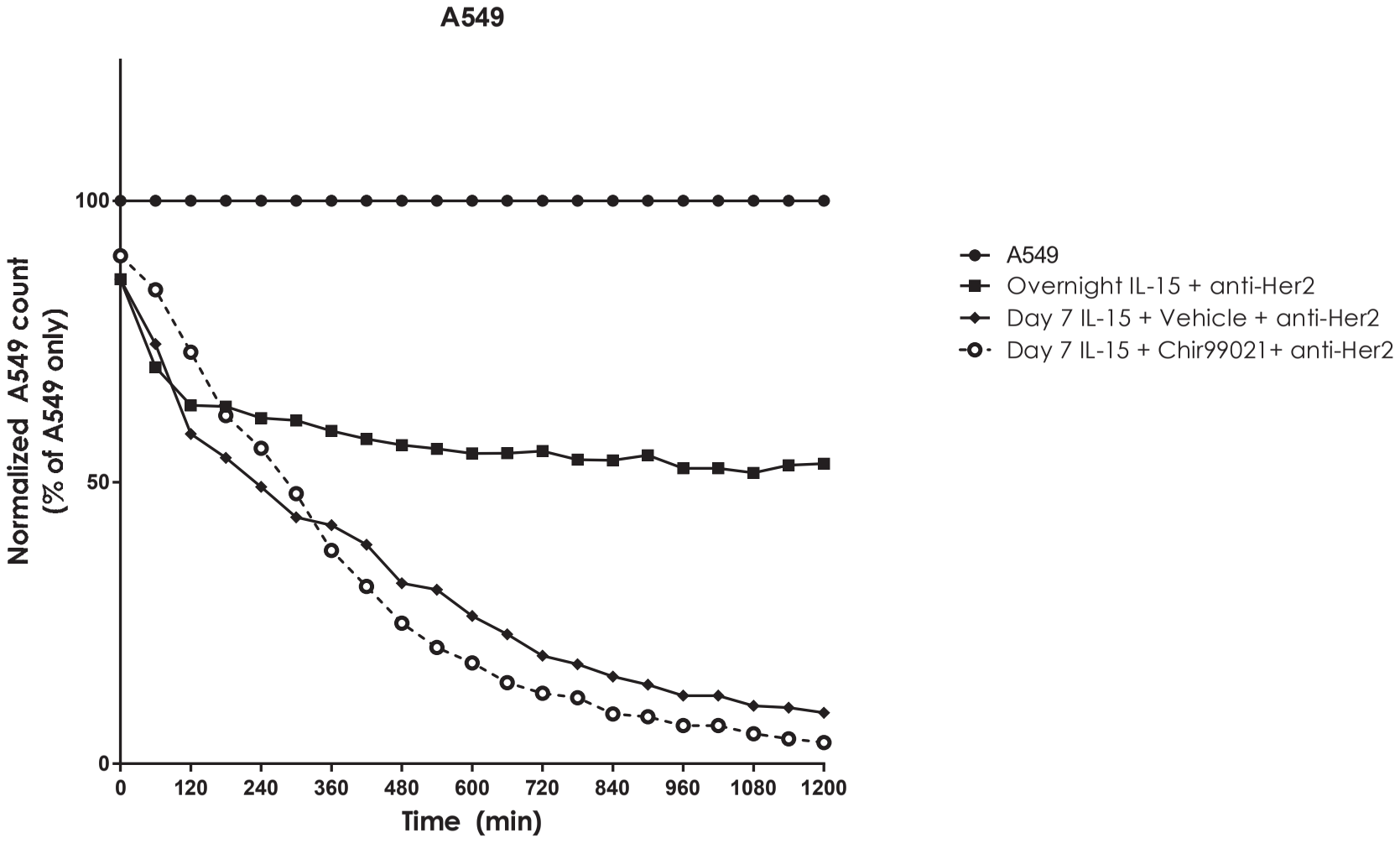

A549
Normalized A549 count
(% of A549 only)
100
50
0
0
120
240
360
480
600
720
840
960
1080
1200
Time (min)
A549
Overnight IL-15 + anti-Her2
Day 7 IL-15 + Vehicle + anti-Her2
Day 7 IL-15 + Chir99021+ anti-Her2

Figure 6F

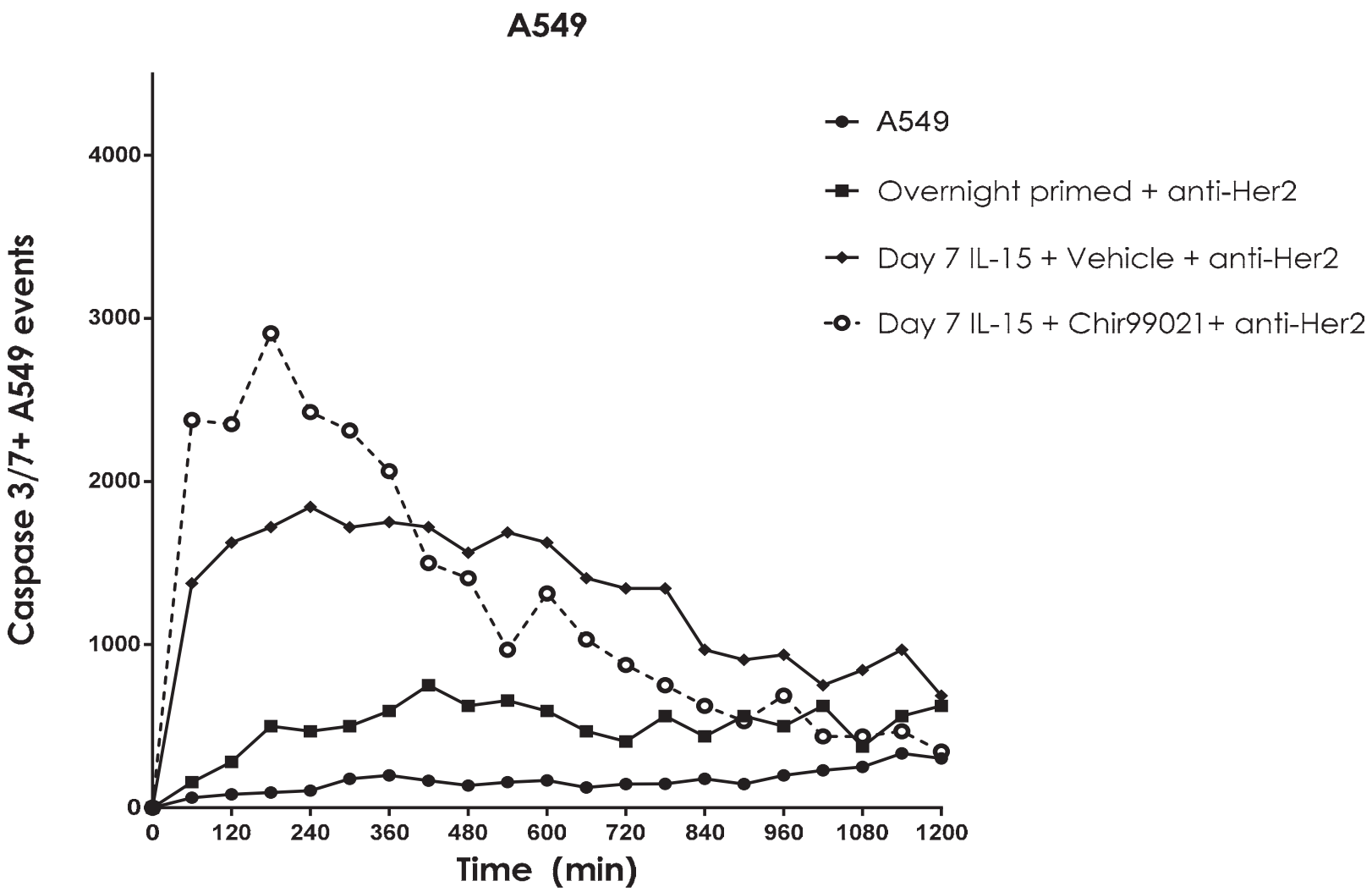

A549
Caspase 3/7+ A549 events
4000
3000
2000
1000
0
0
120
240
360
480
600
720
840
960
1080
1200
Time (min)
A549
Overnight primed + anti-Her2
Day 7 IL-15 + Vehicle + anti-Her2
Day 7 IL-15 + Chir99021+ anti-Her2

Figure 6G

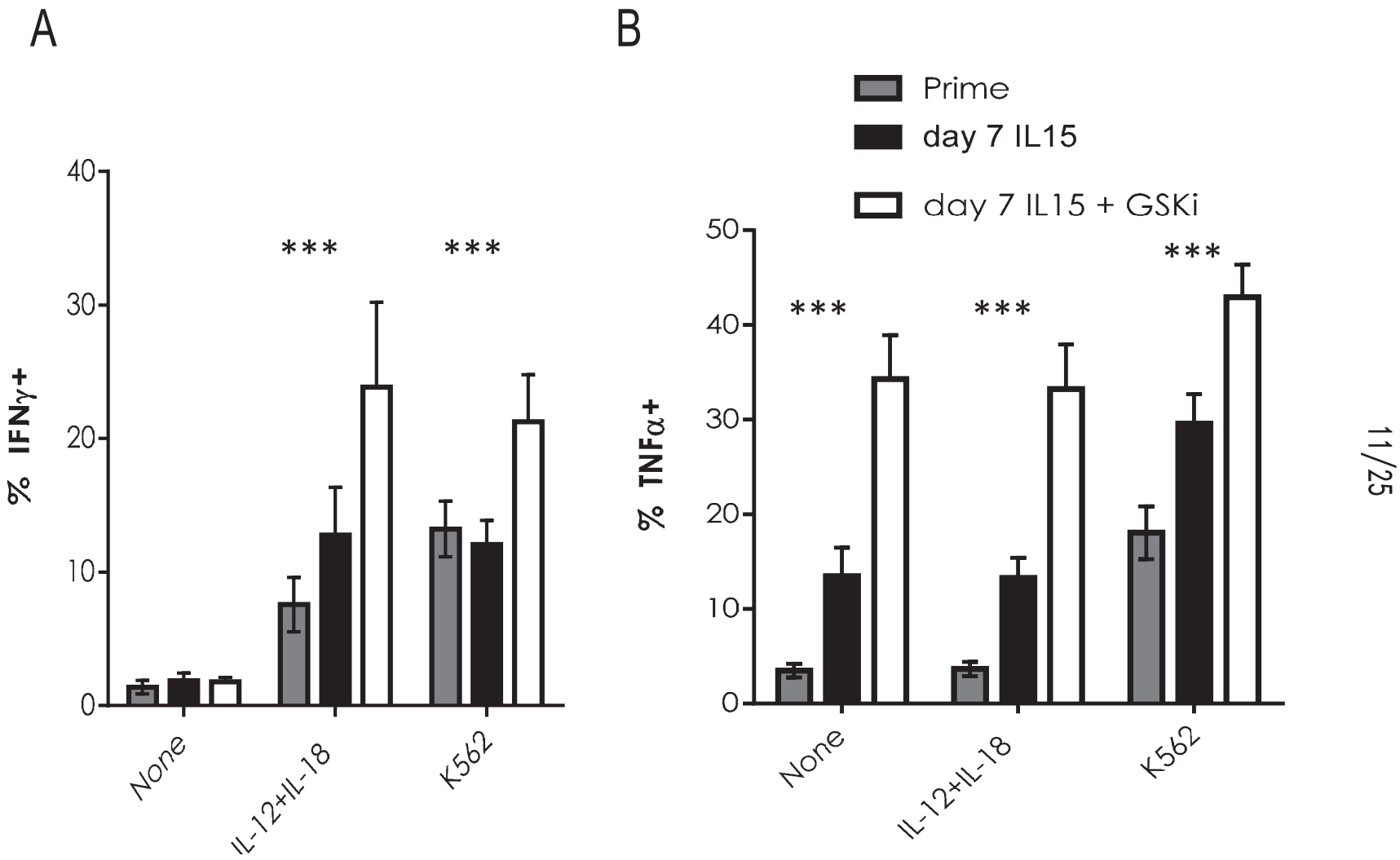
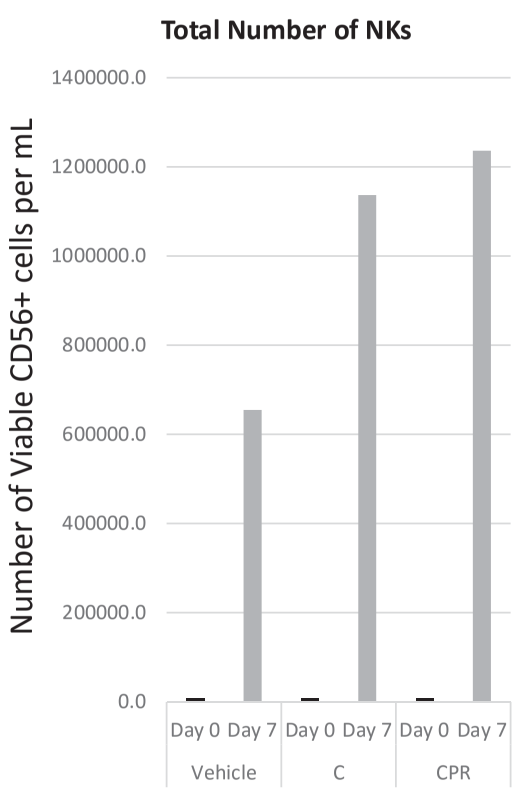
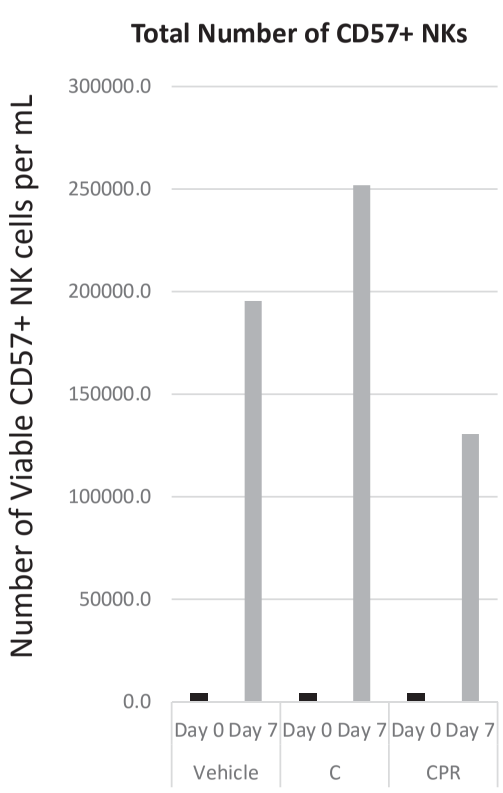

A
B
Prime
day 7 IL15
day 7 IL15 + GSKi
% IFNγ+
% TNFα+
***
***
***
***
None
IL-12+IL-18
K562
None
IL-12+IL-18
K562

Figure 7

A


Total Number of NKs
Number of Viable CD56+ cells per mL
1400000.0
1200000.0
1000000.0
800000.0
600000.0
400000.0
200000.0
0.0
Day 0 Day 7 Day 0 Day 7 Day 0 Day 7
Vehicle
C
CPR

B


Total Number of CD57+ NKs
Number of Viable CD57+ NK cells per mL
300000.0
250000.0
200000.0
150000.0
100000.0
50000.0
0.0
Day 0 Day 7 Day 0 Day 7 Day 0 Day 7
Vehicle
C
CPR

C

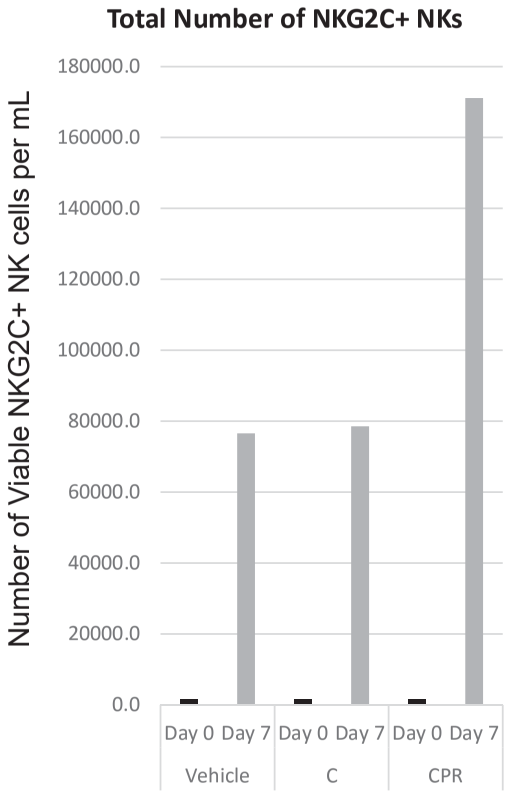
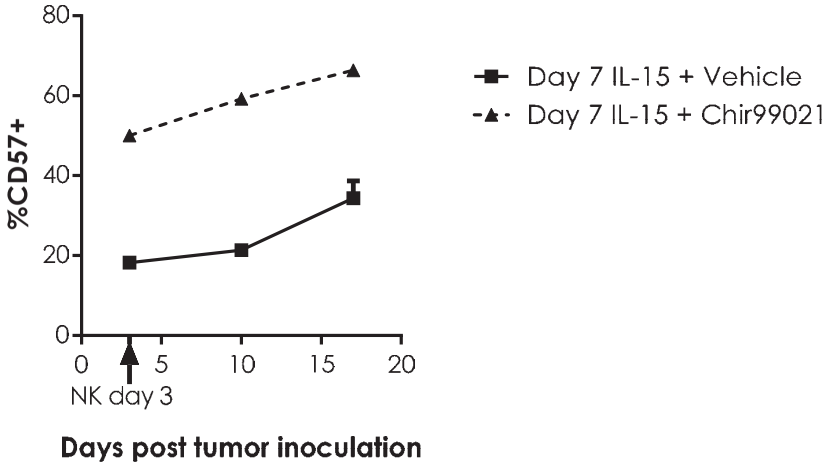

Total Number of NKG2C+ NKs
Number of Viable NKG2C+ NK cells per mL
180000.0
160000.0
140000.0
120000.0
100000.0
80000.0
60000.0
40000.0
20000.0
0.0
Day 0 Day 7 Day 0 Day 7 Day 0 Day 7
Vehicle
C
CPR

Figure 8

A


80
60
40
20
0
%CD57+
0
5
10
15
20
NK day 3
Days post tumor inoculation
Day 7 IL-15 + Vehicle
Day 7 IL-15 + Chir99021

B

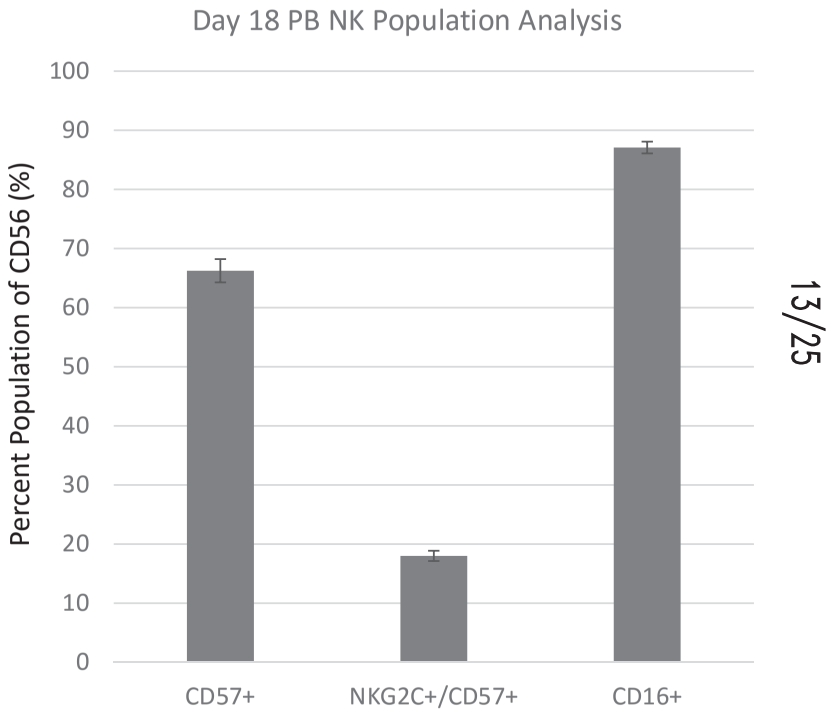

Day 18 PB NK Population Analysis
Percent Population of CD56 (%)
100
90
80
70
60
50
40
30
20
10
0
CD57+
NKG2C+/CD57+
CD16+

Figure 9

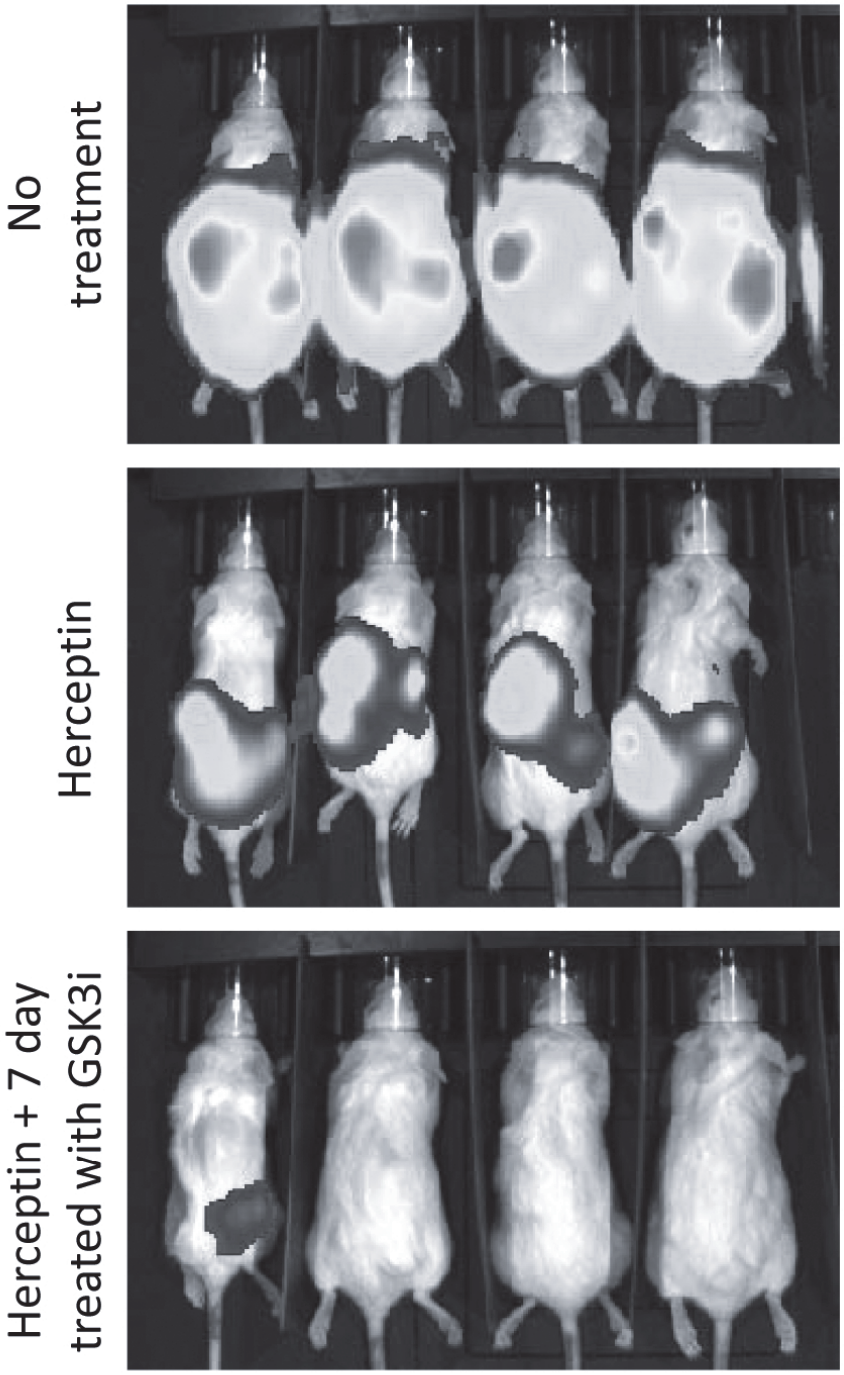


Figure 10

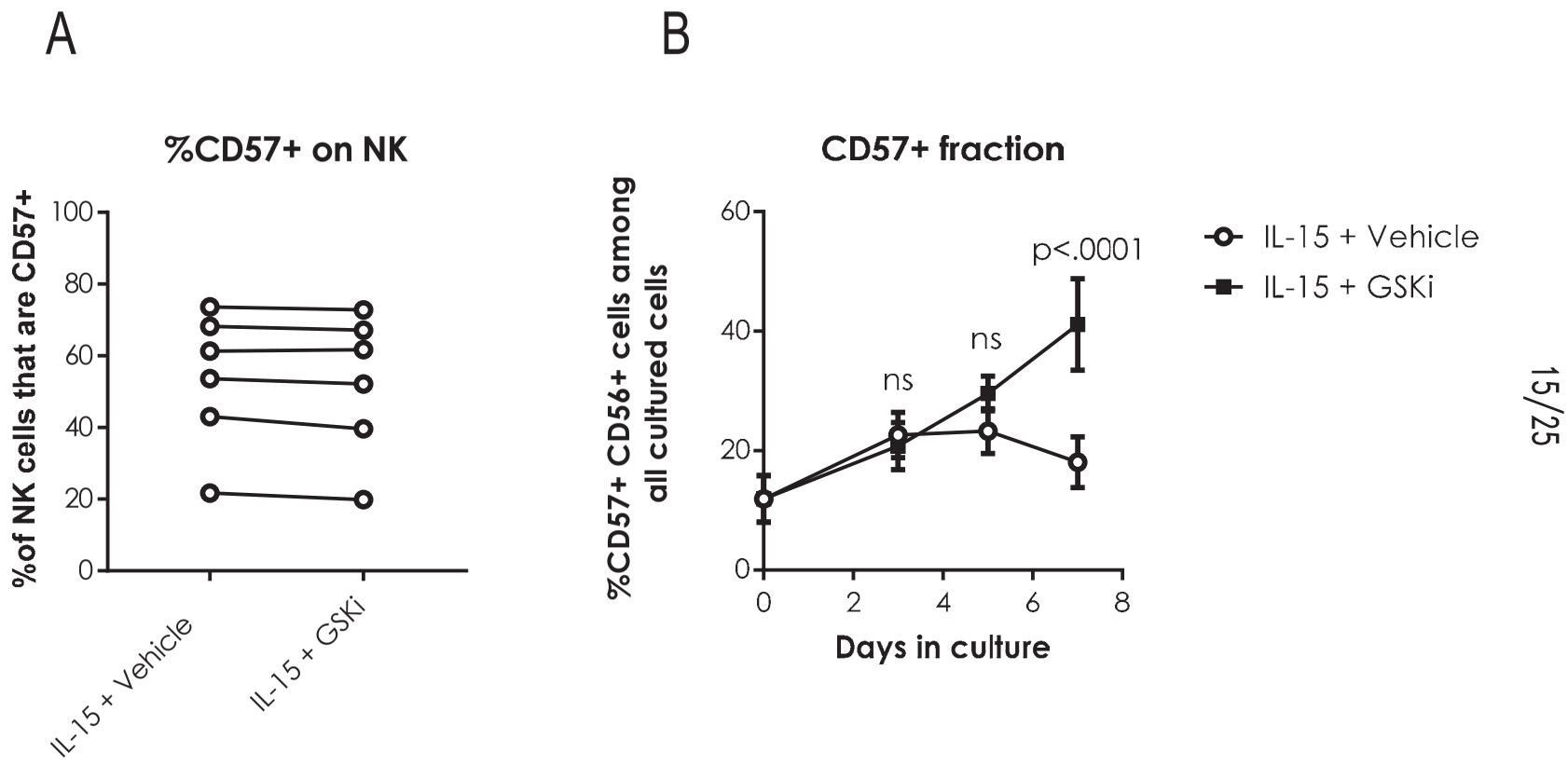

A
%CD57+ on NK
%of NK cells that are CD57+
100
80
60
40
20
0
IL-15 + Vehicle
IL-15 + GSKi
B
CD57+ fraction
%CD57+ CD56+ cells among all cultured cells
60
40
20
0
0
2
4
6
8
Days in culture
p<.0001
ns
ns
IL-15 + Vehicle
IL-15 + GSKi

Figure 11

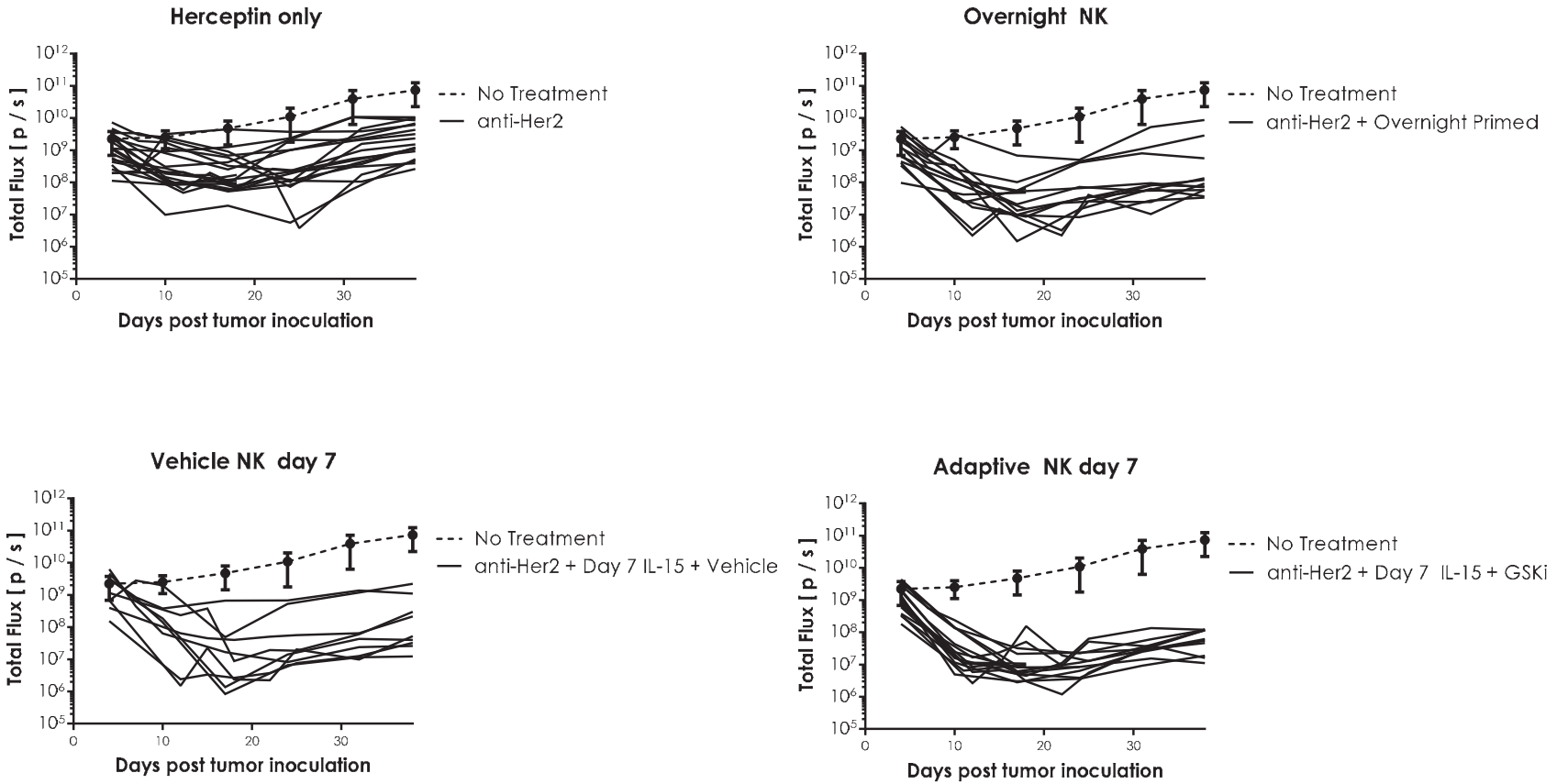

Herceptin only
Total Flux [p / s]
Days post tumor inoculation
No Treatment
anti-Her2
Overnight NK
Total Flux [p / s]
Days post tumor inoculation
No Treatment
anti-Her2 + Overnight Primed
Vehicle NK day 7
Total Flux [p / s]
Days post tumor inoculation
No Treatment
anti-Her2 + Day 7 IL-15 + Vehicle
Adaptive NK day 7
Total Flux [p / s]
Days post tumor inoculation
No Treatment
anti-Her2 + Day 7 IL-15 + GSKi

Figure 12A

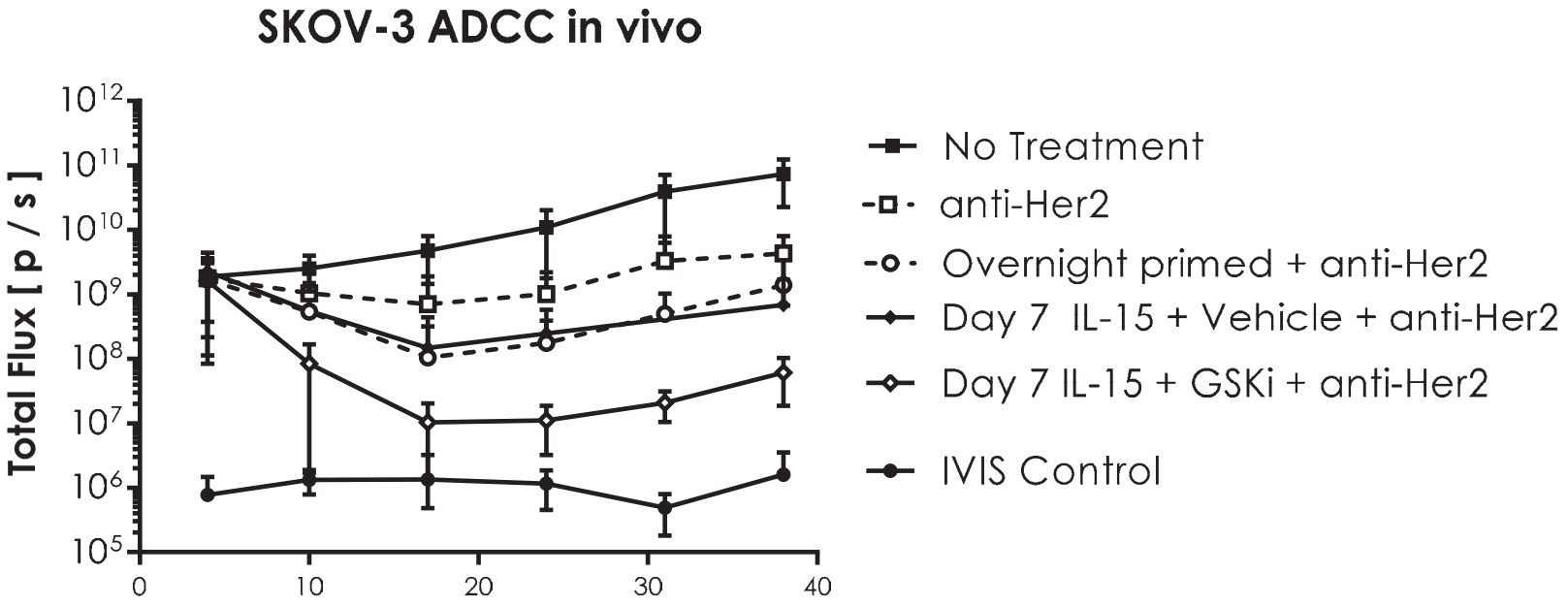

SKOV-3 ADCC in vivo
Total Flux [p / s]
10^12
10^11
10^10
10^9
10^8
10^7
10^6
10^5
0
10
20
30
40
No Treatment
anti-Her2
Overnight primed + anti-Her2
Day 7 IL-15 + Vehicle + anti-Her2
Day 7 IL-15 + GSKi + anti-Her2
IVIS Control

Figure 12B

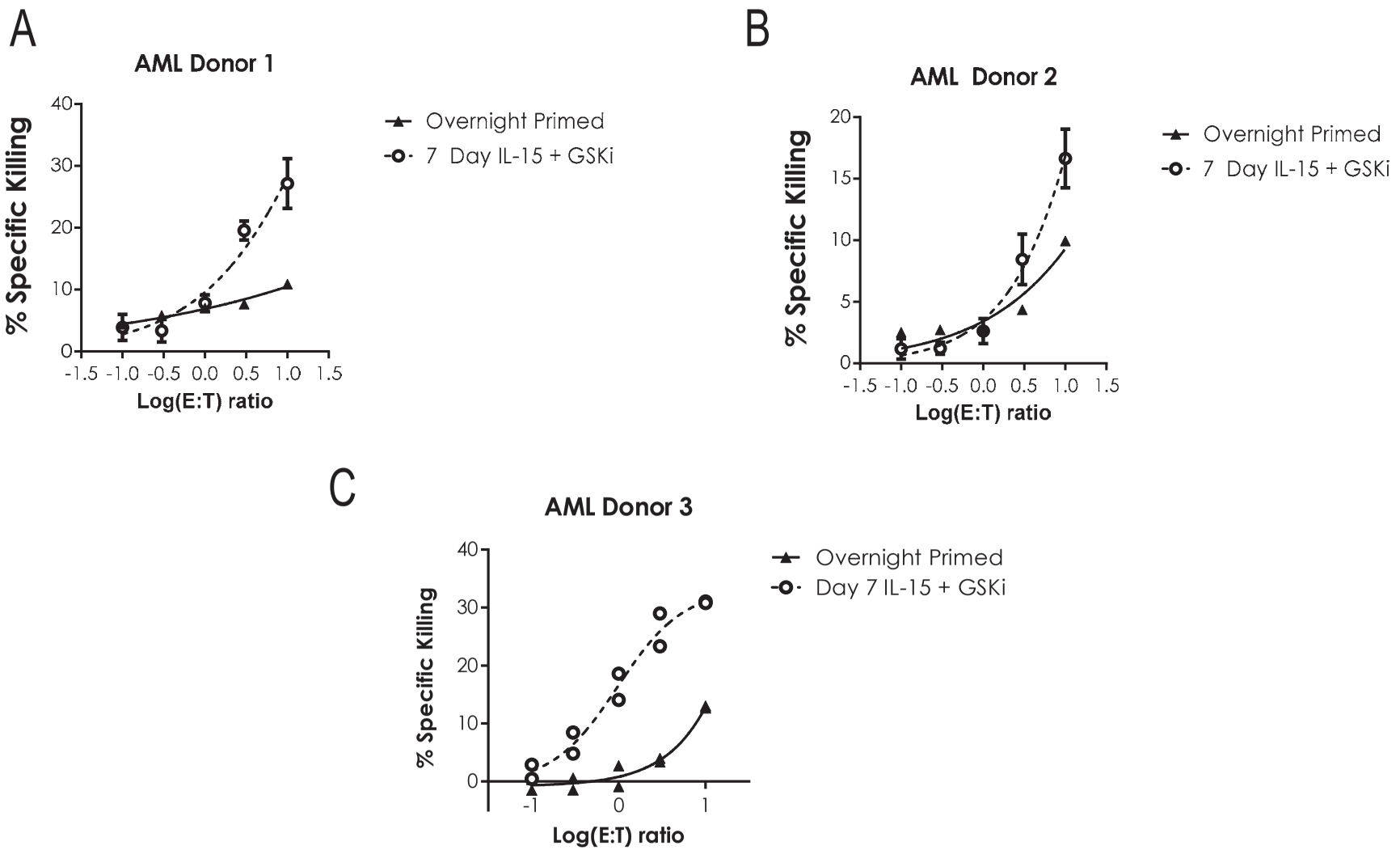

A
AML Donor 1
% Specific Killing
40
30
20
10
0
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
Log(E:T) ratio
Overnight Primed
7 Day IL-15 + GSKi
B
AML Donor 2
% Specific Killing
20
15
10
5
0
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
Log(E:T) ratio
Overnight Primed
7 Day IL-15 + GSKi
C
AML Donor 3
% Specific Killing
40
30
20
10
0
-1
0
1
Log(E:T) ratio
Overnight Primed
Day 7 IL-15 + GSKi

Figure 13

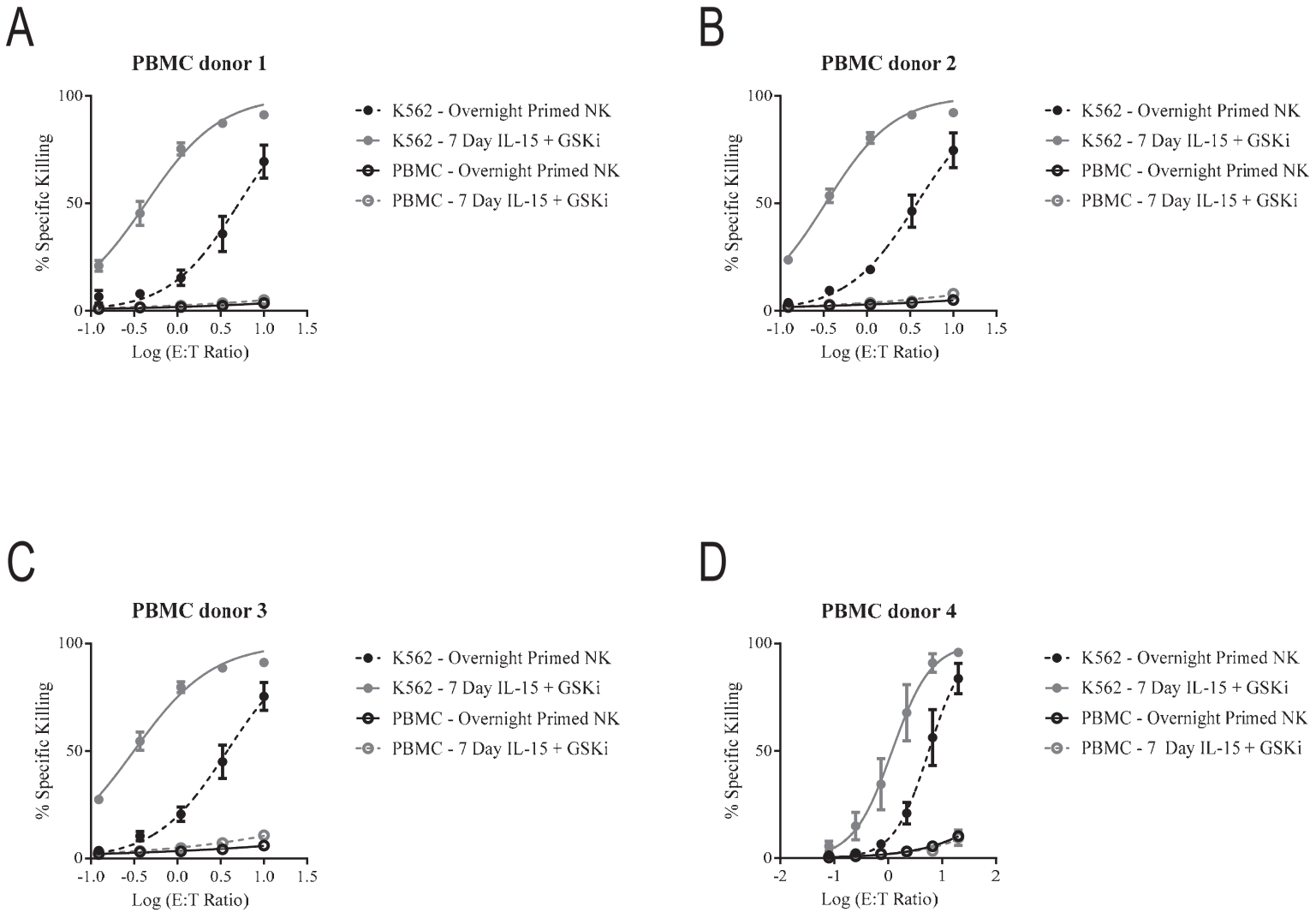
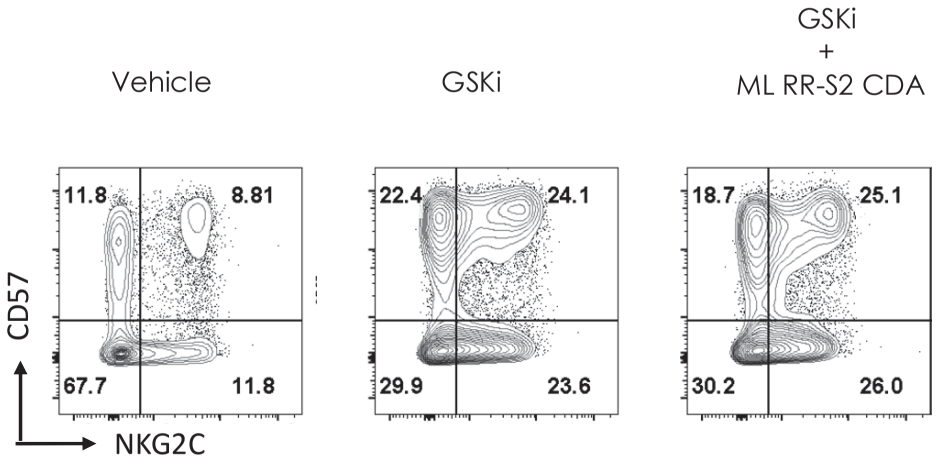

A
PBMC donor 1
% Specific Killing
Log (E:T Ratio)
K562 - Overnight Primed NK
K562 - 7 Day IL-15 + GSKi
PBMC - Overnight Primed NK
PBMC - 7 Day IL-15 + GSKi
B
PBMC donor 2
% Specific Killing
Log (E:T Ratio)
K562 - Overnight Primed NK
K562 - 7 Day IL-15 + GSKi
PBMC - Overnight Primed NK
PBMC - 7 Day IL-15 + GSKi
C
PBMC donor 3
% Specific Killing
Log (E:T Ratio)
K562 - Overnight Primed NK
K562 - 7 Day IL-15 + GSKi
PBMC - Overnight Primed NK
PBMC - 7 Day IL-15 + GSKi
D
PBMC donor 4
% Specific Killing
Log (E:T Ratio)
K562 - Overnight Primed NK
K562 - 7 Day IL-15 + GSKi
PBMC - Overnight Primed NK
PBMC - 7 Day IL-15 + GSKi

Figure 14

A


Vehicle
GSKi
GSKi
+
ML RR-S2 CDA
11.8
8.81
67.7
11.8
22.4
24.1
29.9
23.6
18.7
25.1
30.2
26.0
CD57
NKG2C

B

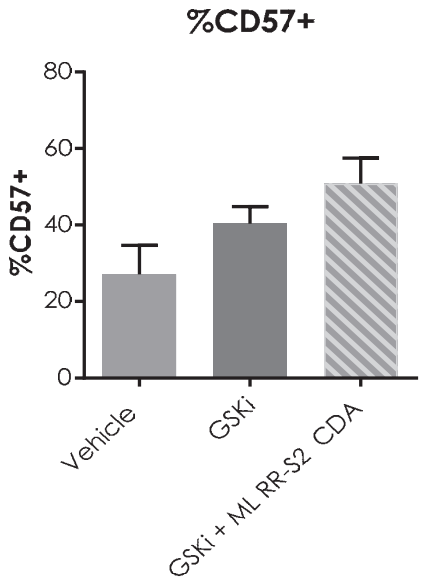

%CD57+
%CD57+
80
60
40
20
0
Vehicle
GSKi
GSKi + ML RR-S2 CDA

Figure 15

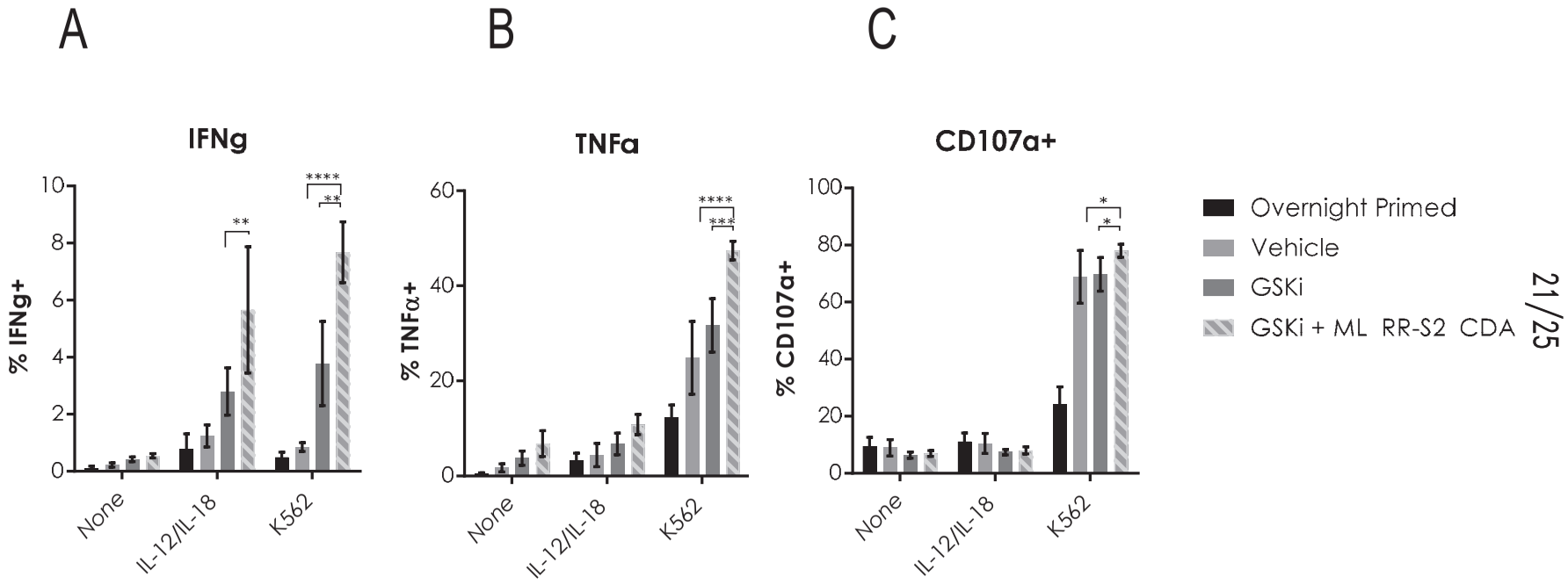

A
B
C
IFNg
TNFa
CD107a+
% IFNg+
% TNFα+
% CD107a+
None
IL-12/IL-18
K562
Overnight Primed
Vehicle
GSKi
GSKi + ML RR-S2 CDA

Figure 16

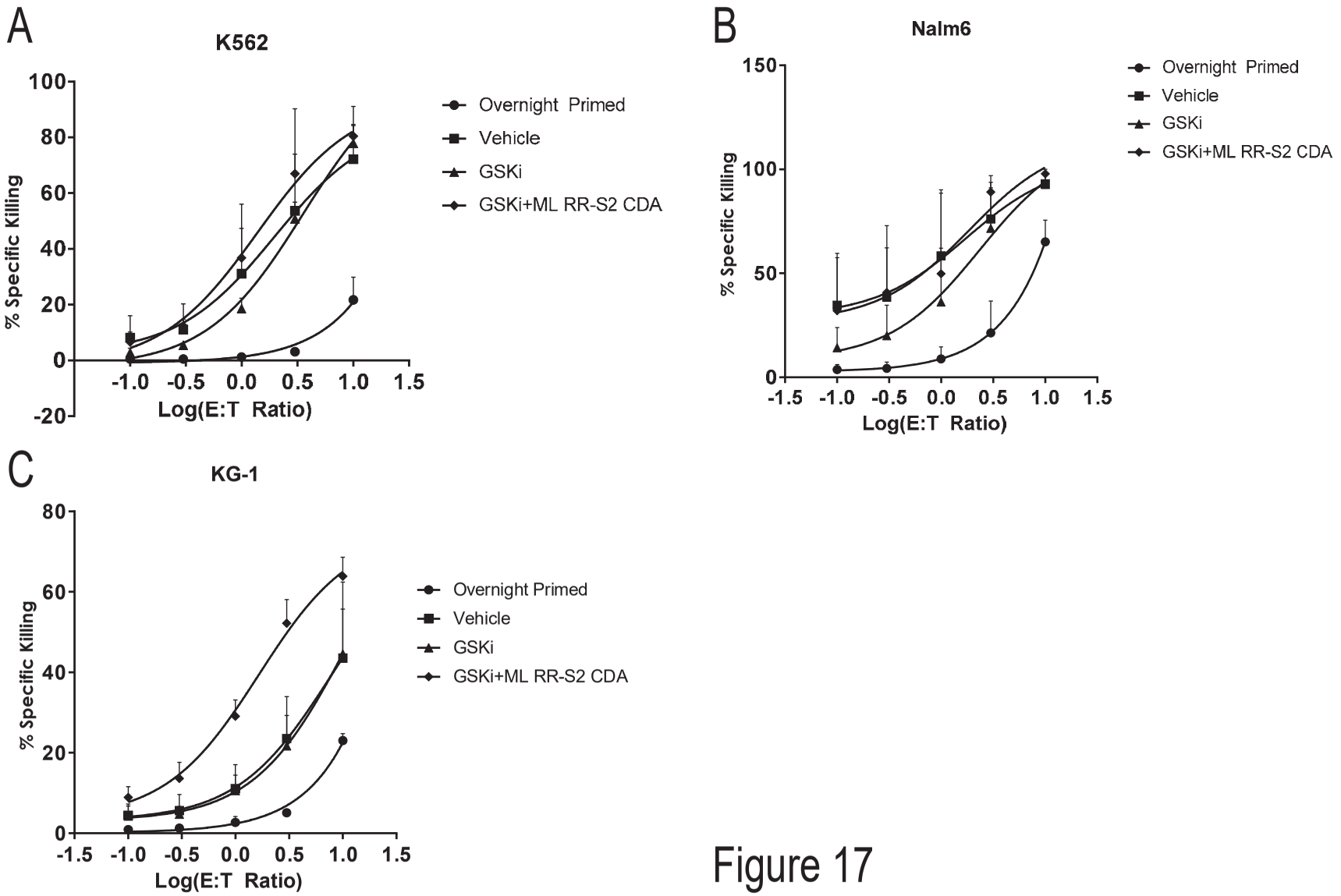

A
K562
% Specific Killing
Log(E:T Ratio)
Overnight Primed
Vehicle
GSKi
GSKi+ML RR-S2 CDA
B
Nalm6
% Specific Killing
Log(E:T Ratio)
Overnight Primed
Vehicle
GSKi
GSKi+ML RR-S2 CDA
C
KG-1
% Specific Killing
Log(E:T Ratio)
Overnight Primed
Vehicle
GSKi
GSKi+ML RR-S2 CDA

Figure 17

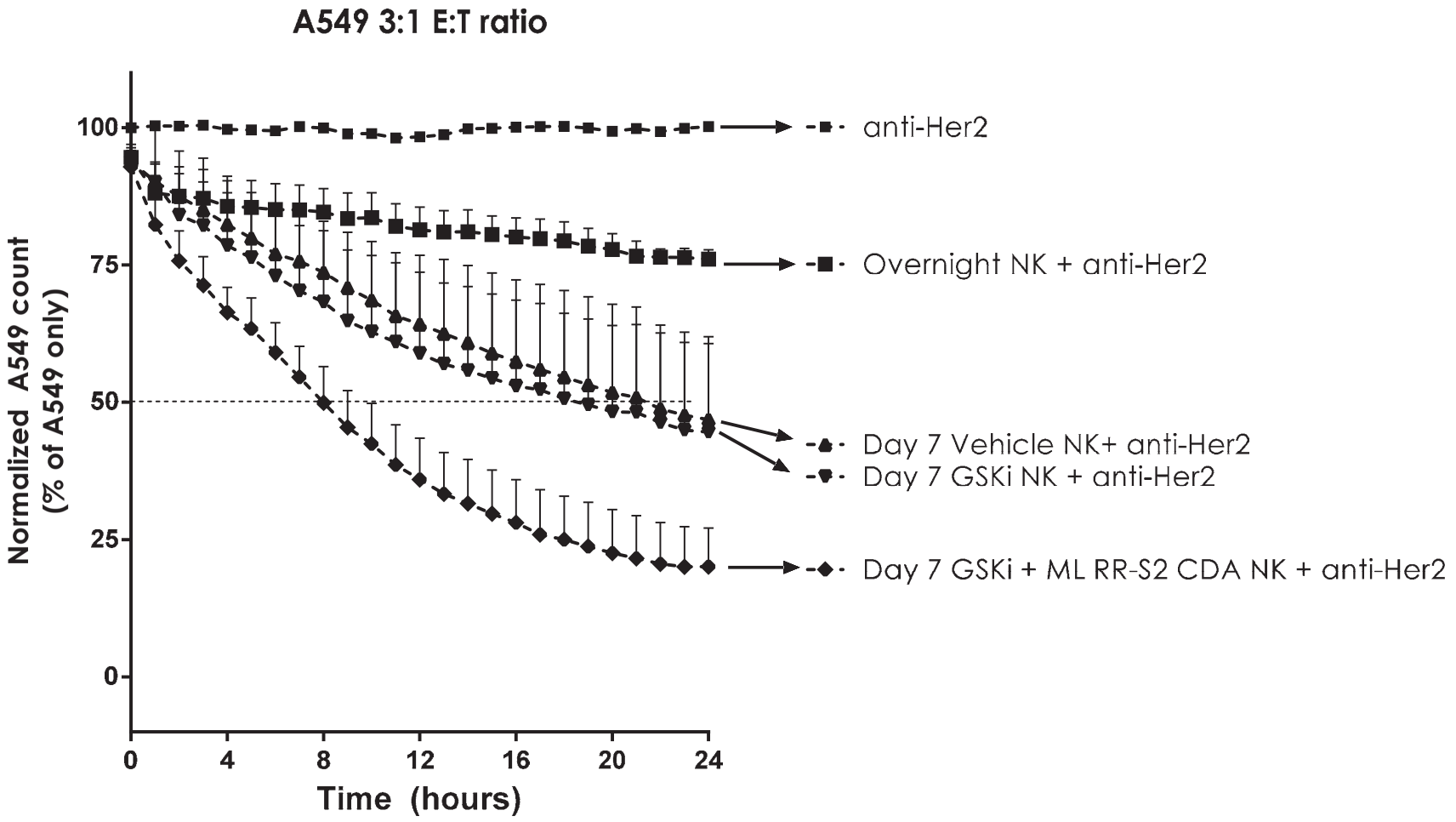

A549 3:1 E:T ratio
Normalized A549 count
(% of A549 only)
100
75
50
25
0
0
4
8
12
16
20
24
Time (hours)
anti-Her2
Overnight NK + anti-Her2
Day 7 Vehicle NK+ anti-Her2
Day 7 GSKi NK + anti-Her2
Day 7 GSKi + ML RR-S2 CDA NK + anti-Her2

Figure 18A

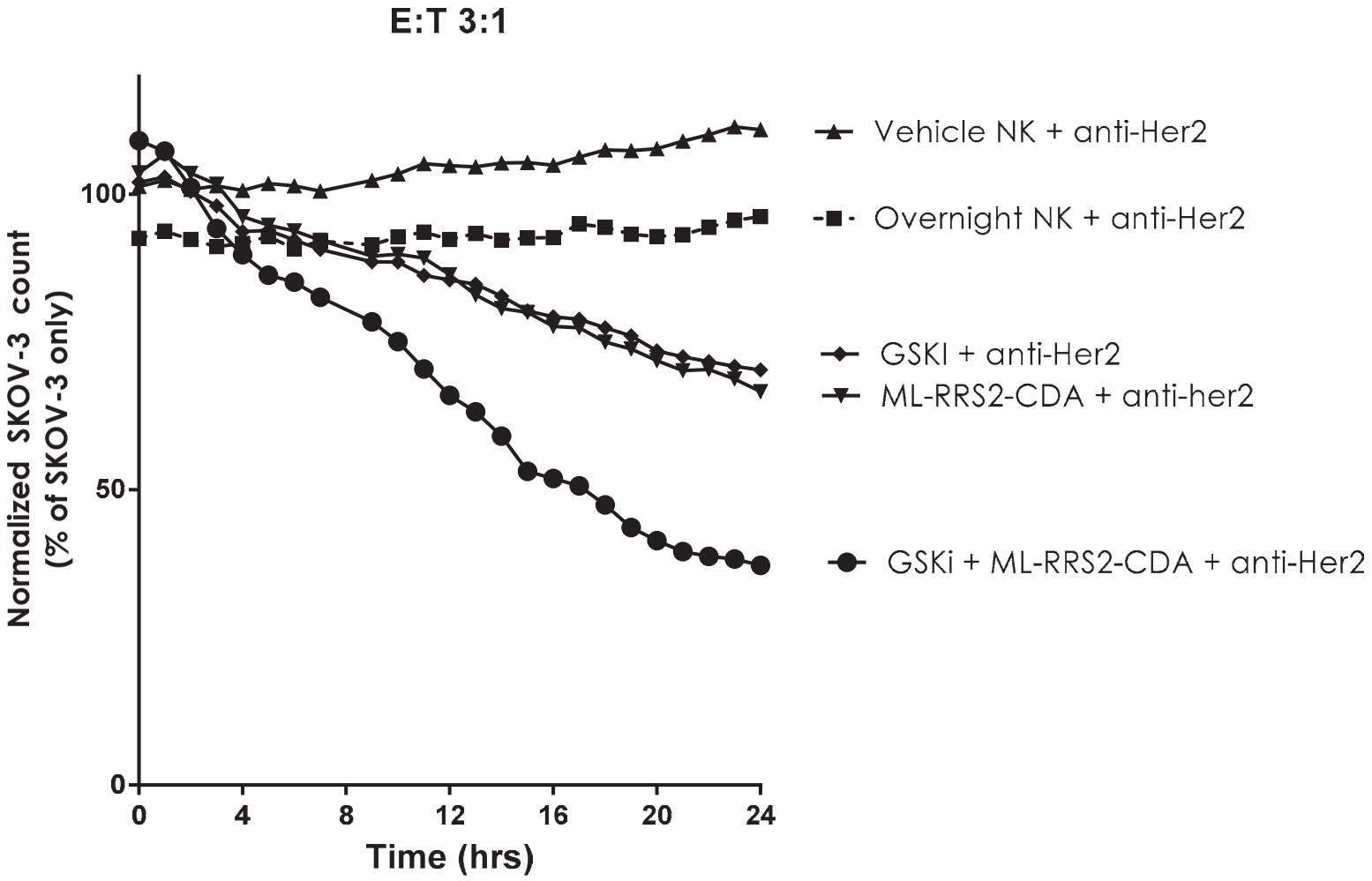

E:T 3:1
Normalized SKOV-3 count
(% of SKOV-3 only)
100
50
0
0
4
8
12
16
20
24
Time (hrs)
Vehicle NK + anti-Her2
Overnight NK + anti-Her2
GSKI + anti-Her2
ML-RRS2-CDA + anti-her2
GSKi + ML-RRS2-CDA + anti-Her2

Figure 18B

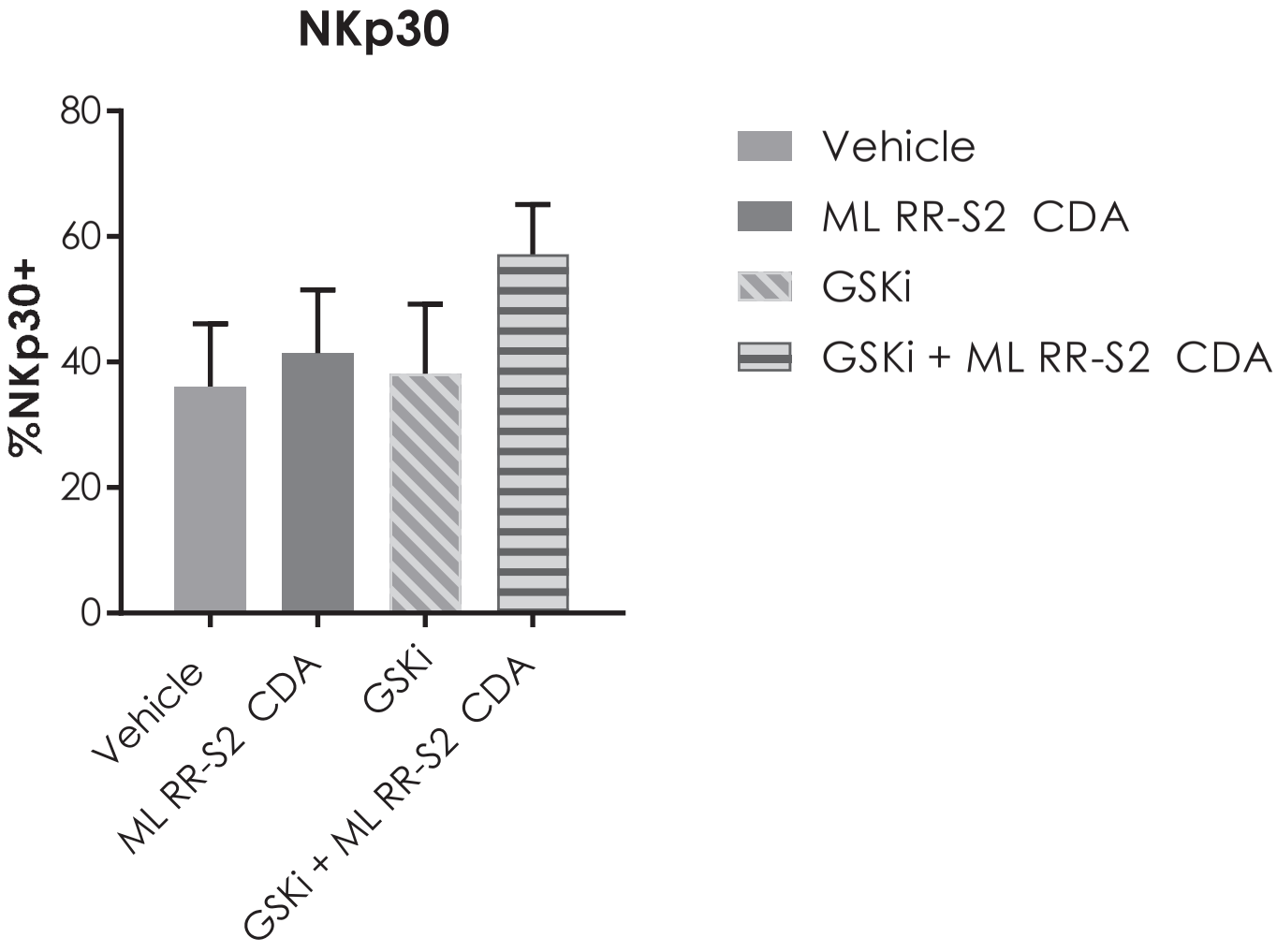

NKp30
%NKp30+
80
60
40
20
0
Vehicle
ML RR-S2 CDA
GSKi
GSKi + ML RR-S2 CDA
Vehicle
ML RR-S2 CDA
GSKi
GSKi + ML RR-S2 CDA

Figure 19