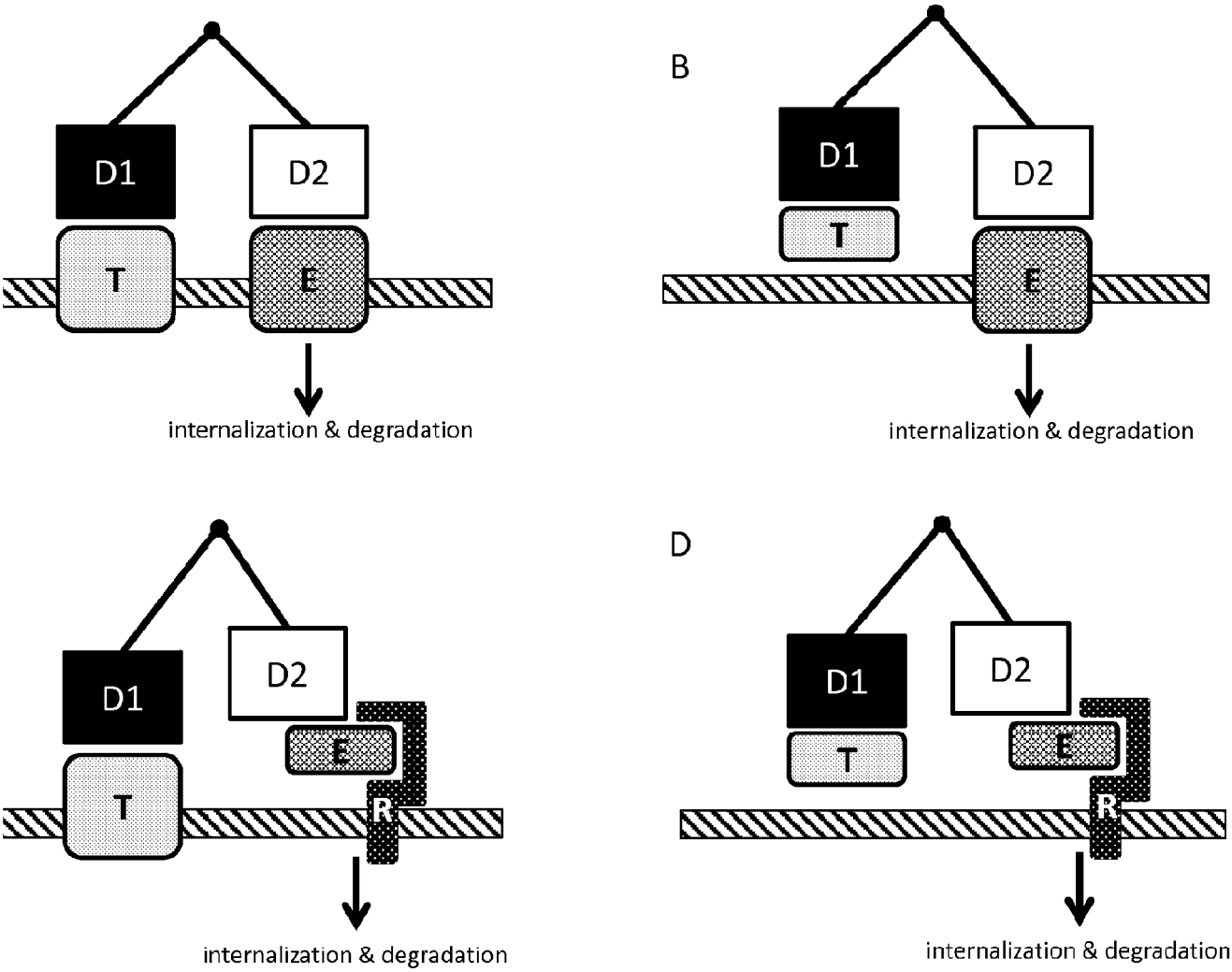

D1
D2
T
E
internalization & degradation
B
D1
D2
T
E
internalization & degradation
D1
D2
T
E
R
internalization & degradation
D
D1
D2
T
E
R
internalization & degradation

Figure 1

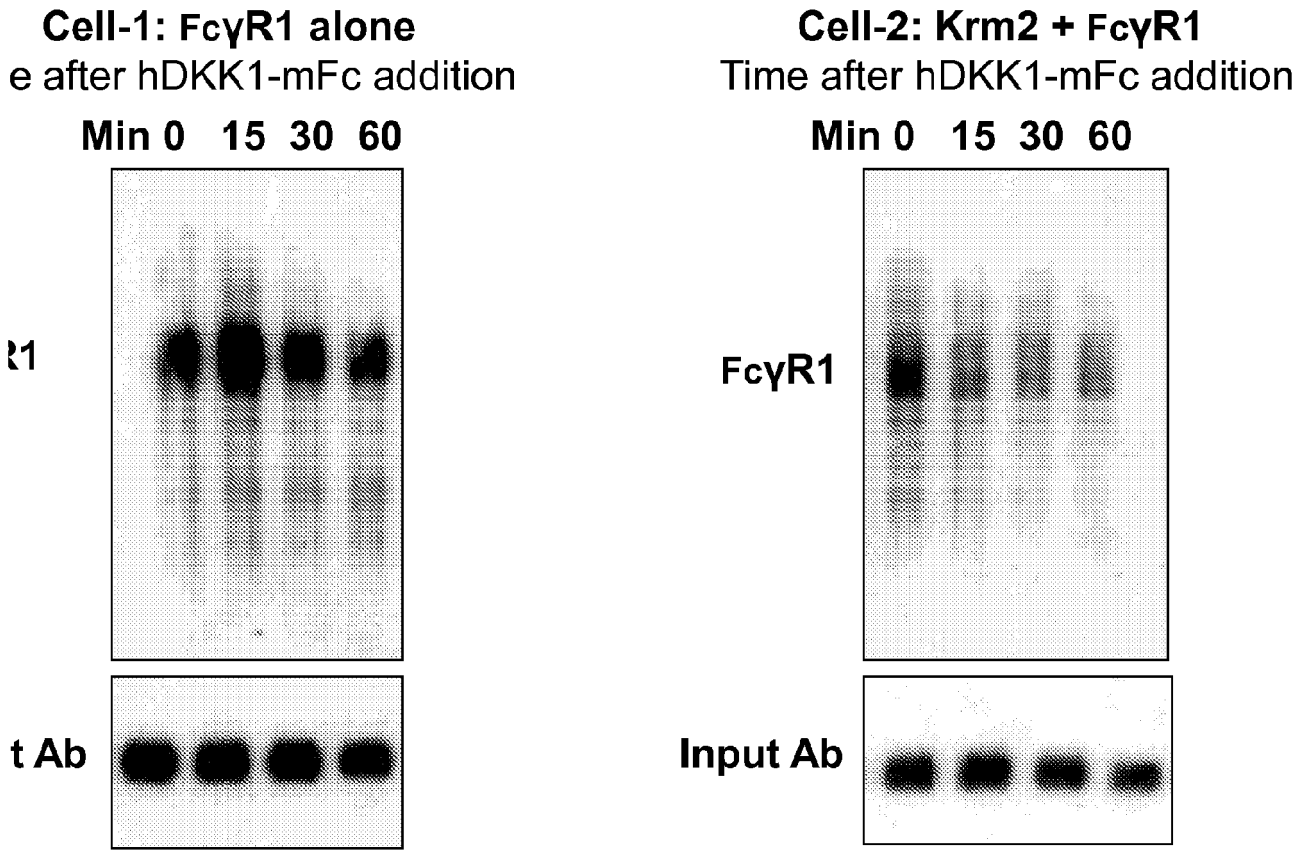

Cell-1: FcγR1 alone
e after hDKK1-mFc addition
Min 0 15 30 60
R1
t Ab
Cell-2: Krm2 + FcγR1
Time after hDKK1-mFc addition
Min 0 15 30 60
FcγR1
Input Ab

Figure 2

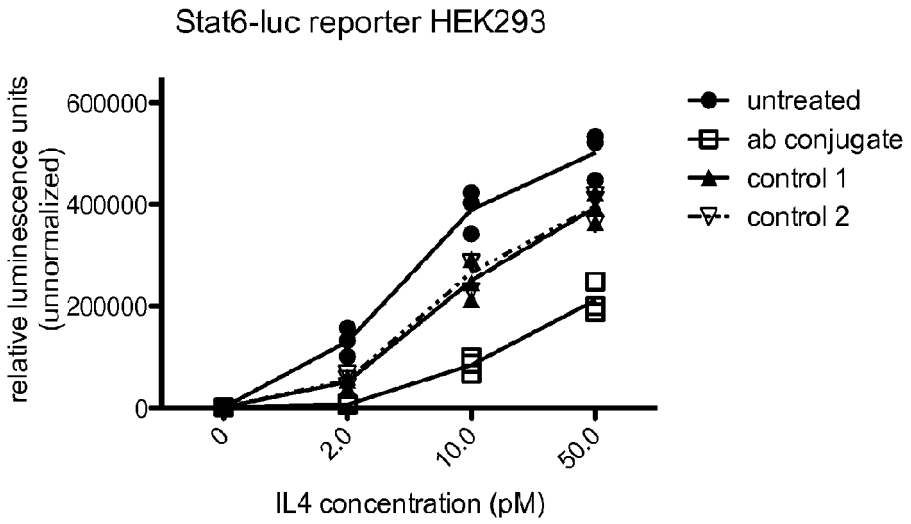

Stat6-luc reporter HEK293
relative luminescence units (unnormalized)
600000
400000
200000
0
0
2.0
10.0
50.0
IL4 concentration (pM)
untreated
ab conjugate
control 1
control 2

Figure 3

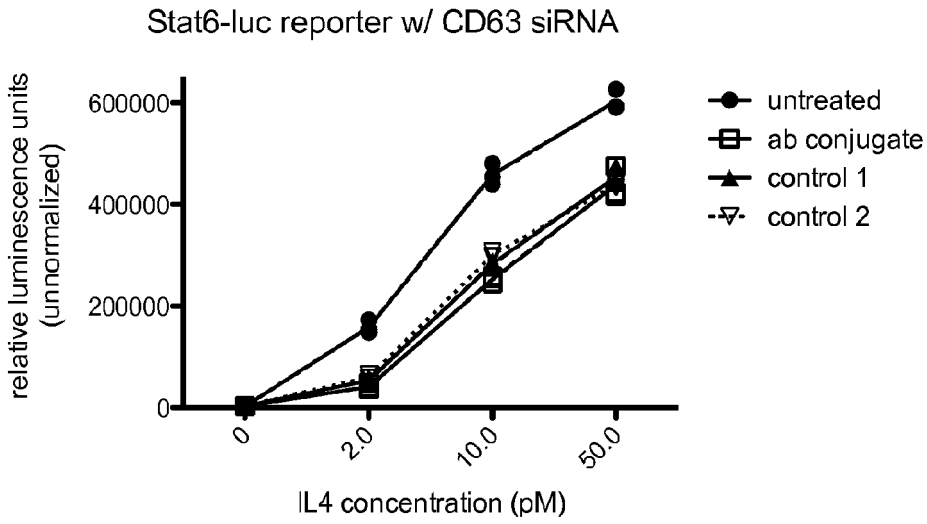

Stat6-luc reporter w/ CD63 siRNA
relative luminescence units (unnormalized)
600000
400000
200000
0
0
2.0
10.0
50.0
IL4 concentration (pM)
untreated
ab conjugate
control 1
control 2

Figure 4

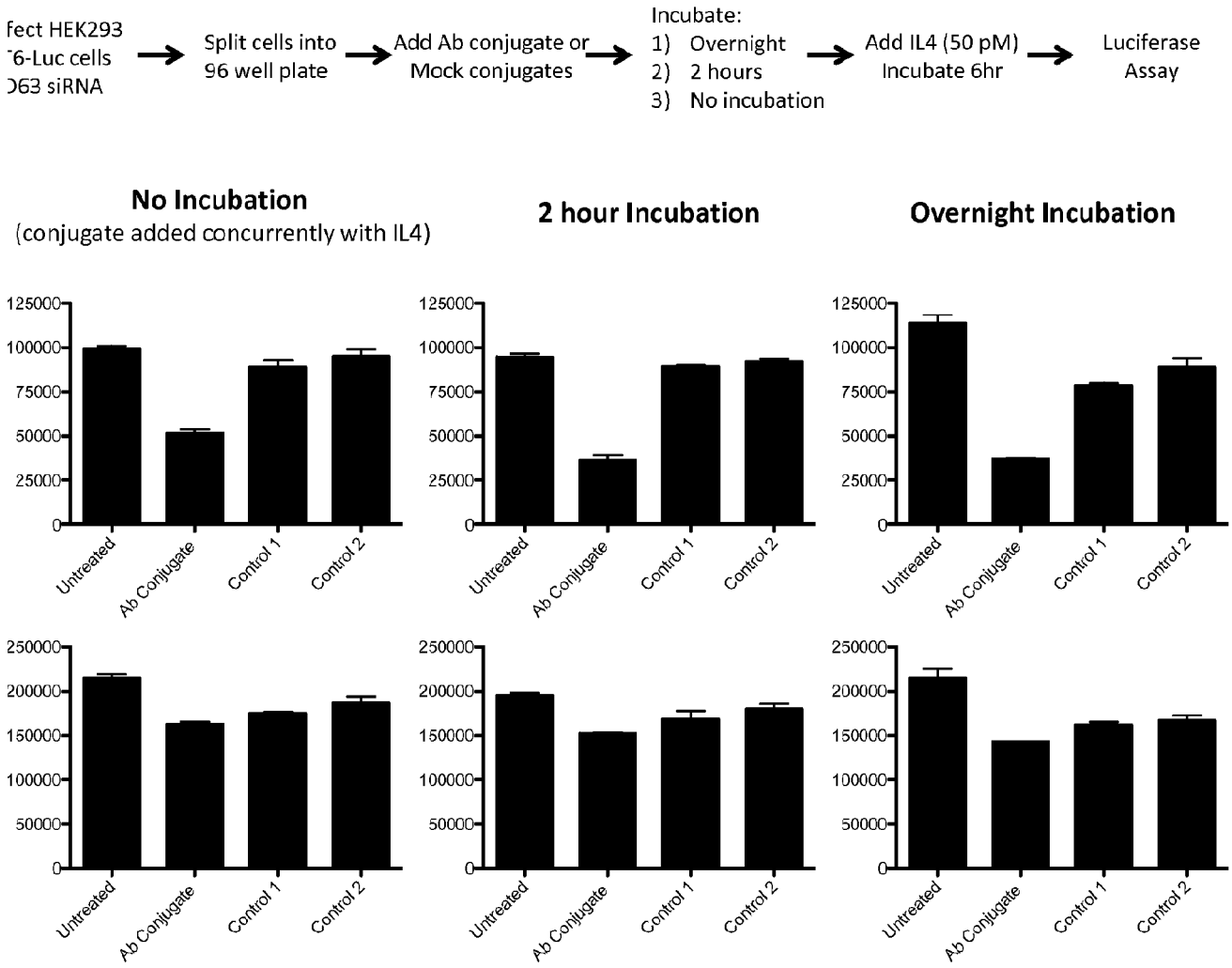
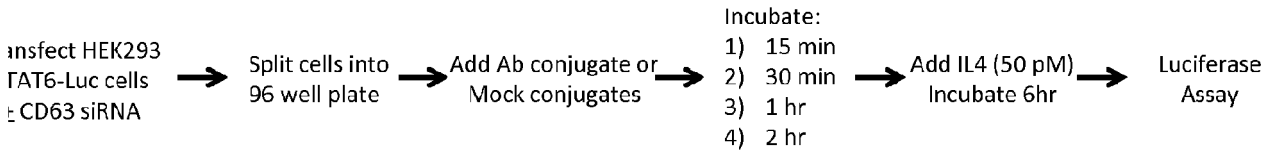

fect HEK293
6-Luc cells
063 siRNA
Split cells into
96 well plate
Add Ab conjugate or
Mock conjugates
Incubate:
1) Overnight
2) 2 hours
3) No incubation
Add IL4 (50 pM)
Incubate 6hr
Luciferase
Assay
No Incubation
(conjugate added concurrently with IL4)
2 hour Incubation
Overnight Incubation
125000
100000
75000
50000
25000
0
250000
200000
150000
100000
50000
0
Untreated
Ab Conjugate
Control 1
Control 2

Figure 5


ınsfect HEK293
ΓAT6-Luc cells
ᵵ CD63 siRNA
Split cells into
96 well plate
Add Ab conjugate or
Mock conjugates
Incubate:
1) 15 min
2) 30 min
3) 1 hr
4) 2 hr
Add IL4 (50 pM)
Incubate 6hr
Luciferase
Assay

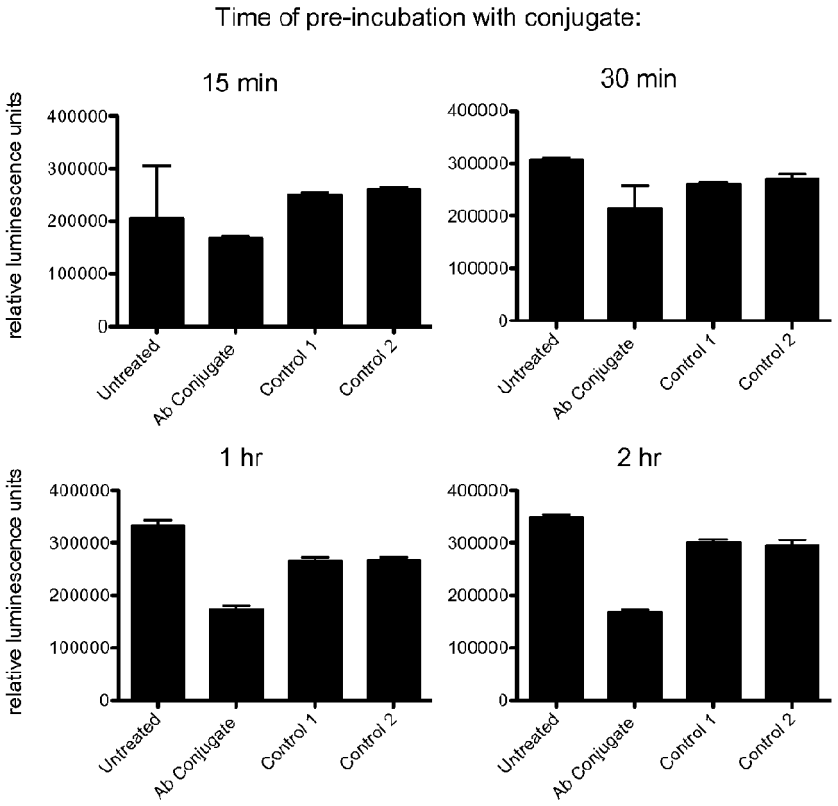

Time of pre-incubation with conjugate:
15 min
30 min
1 hr
2 hr
relative luminescence units
400000
300000
200000
100000
0
Untreated
Ab Conjugate
Control 1
Control 2

Figure 6

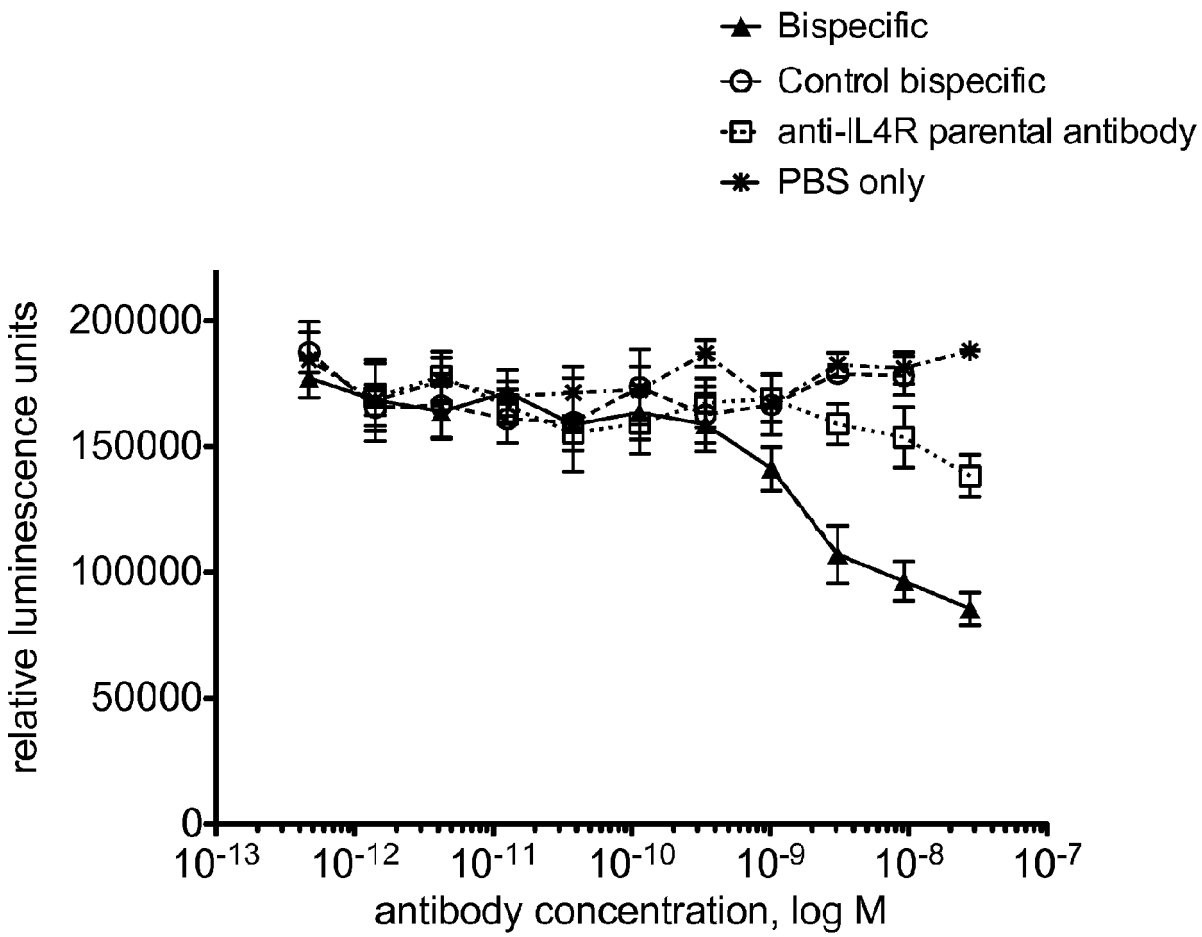

Bispecific
Control bispecific
anti-IL4R parental antibody
PBS only
relative luminescence units
200000
150000
100000
50000
0
10-13
10-12
10-11
10-10
10-9
10-8
10-7
antibody concentration, log M

Figure 7

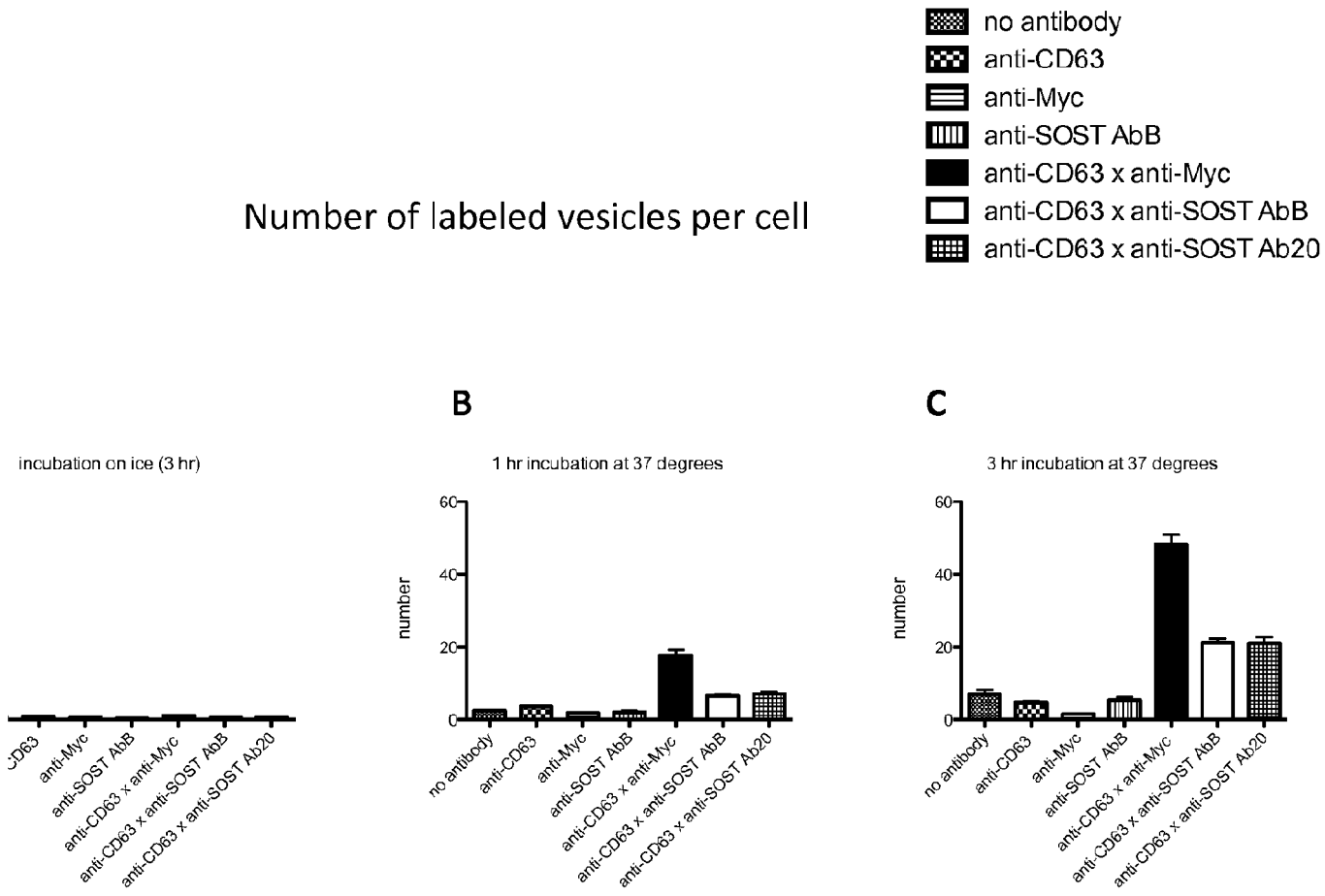

Number of labeled vesicles per cell
no antibody
anti-CD63
anti-Myc
anti-SOST AbB
anti-CD63 x anti-Myc
anti-CD63 x anti-SOST AbB
anti-CD63 x anti-SOST Ab20
B
C
incubation on ice (3 hr)
1 hr incubation at 37 degrees
3 hr incubation at 37 degrees
number
60
40
20
0
anti-Myc
anti-SOST AbB
anti-CD63 x anti-Myc
anti-CD63 x anti-SOST AbB
anti-CD63 x anti-SOST Ab20
no antibody
anti-CD63

Figure 8

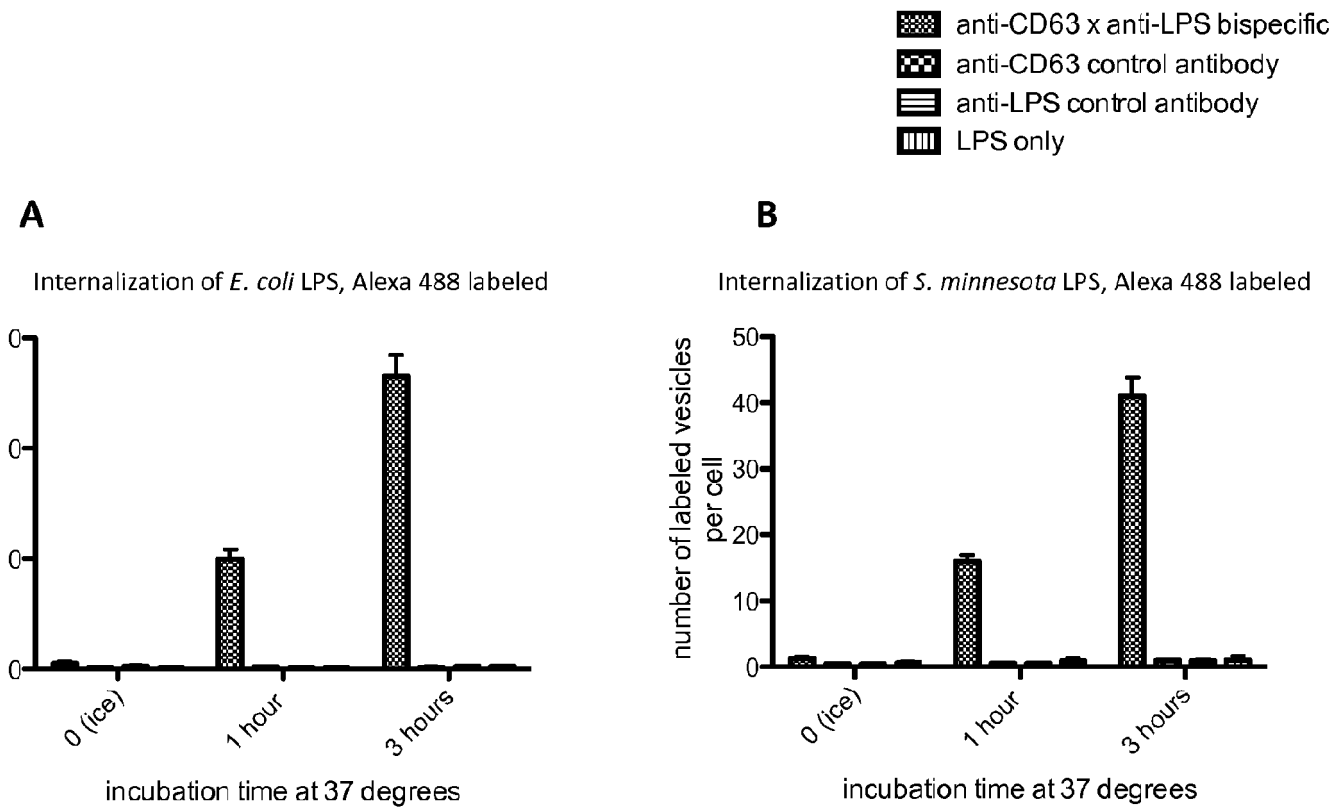

anti-CD63 x anti-LPS bispecific
anti-CD63 control antibody
anti-LPS control antibody
LPS only
A
Internalization of E. coli LPS, Alexa 488 labeled
0
0
0
0
0 (ice)
1 hour
3 hours
incubation time at 37 degrees
B
Internalization of S. minnesota LPS, Alexa 488 labeled
number of labeled vesicles per cell
50
40
30
20
10
0
0 (ice)
1 hour
3 hours
incubation time at 37 degrees

Figure 9