Figure 1

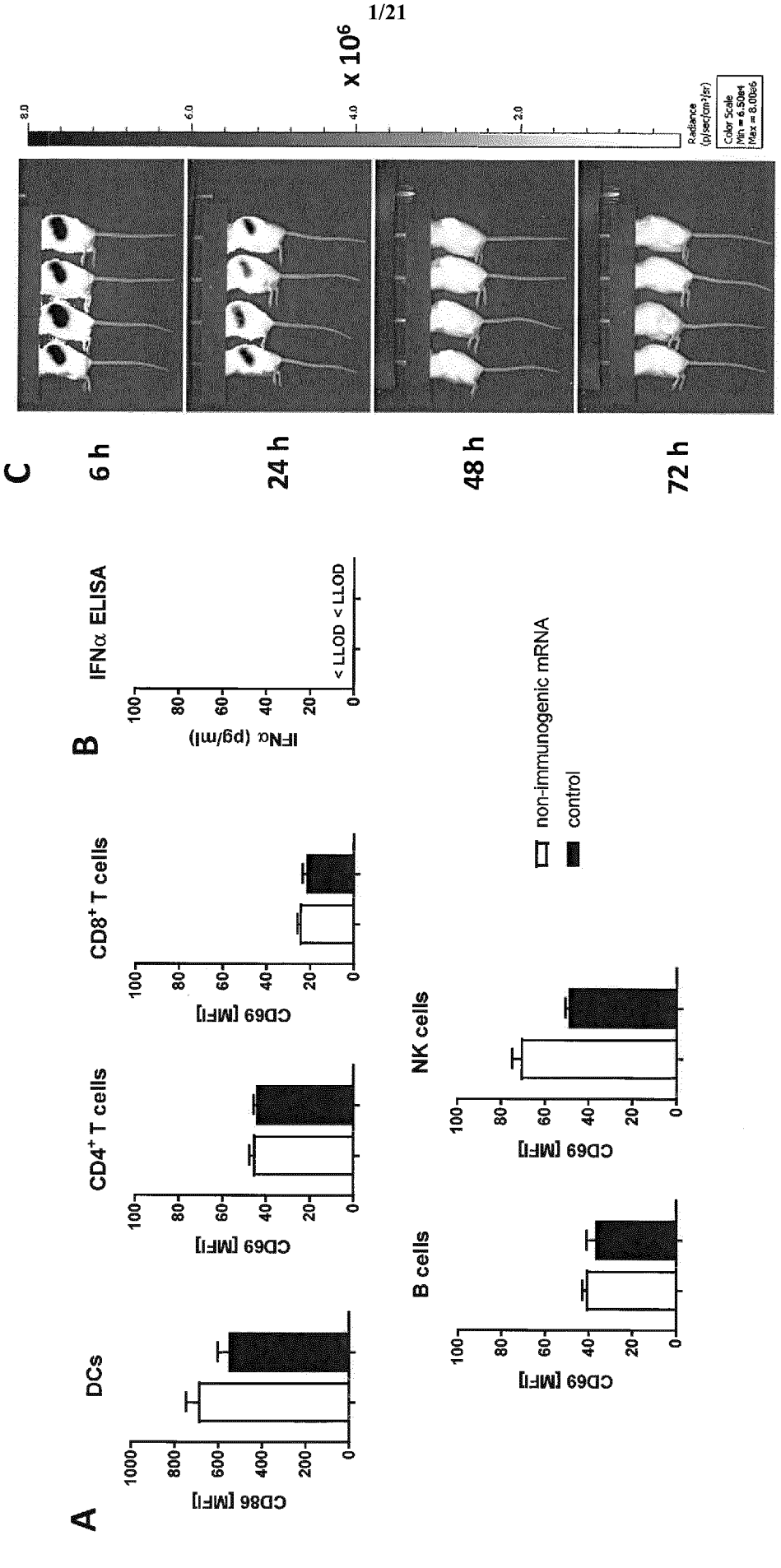
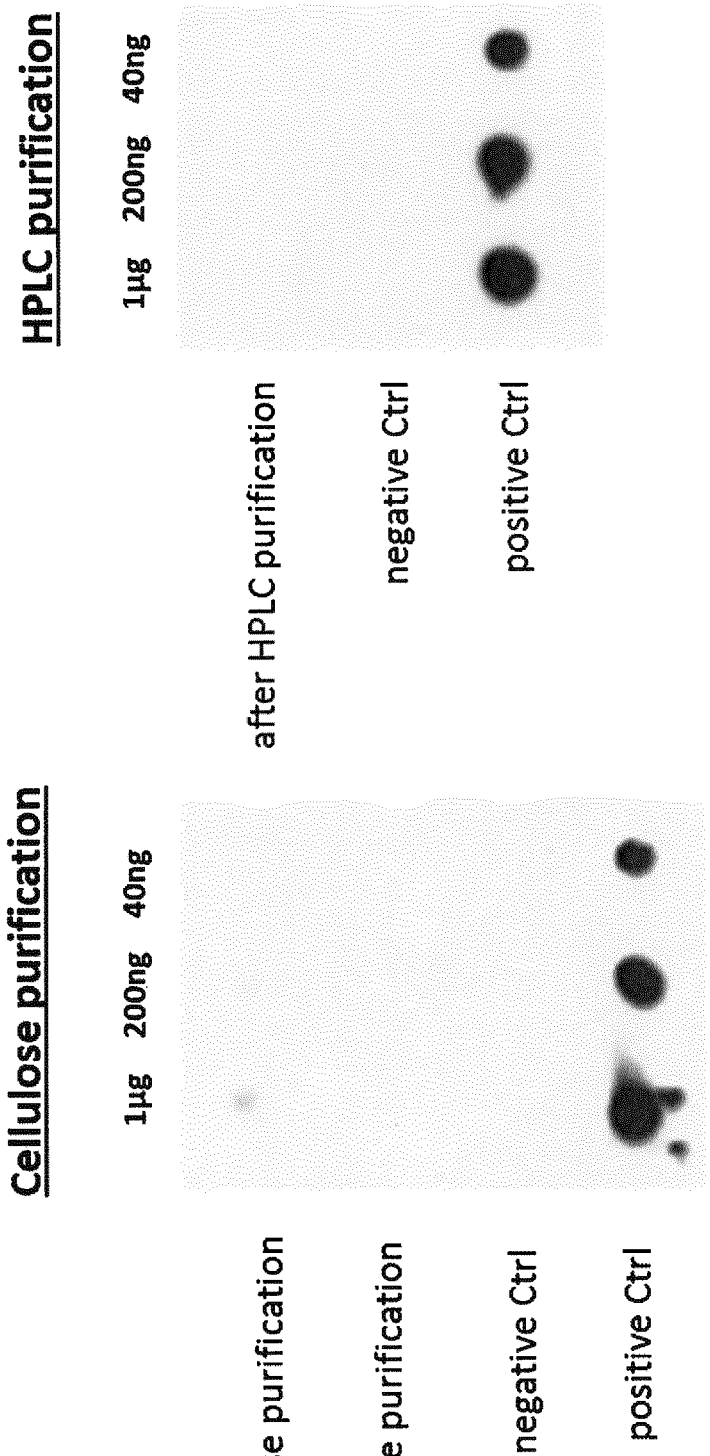

A
DCs
CD86 [MFI]
CD4+ T cells
CD8+ T cells
CD69 [MFI]
B
IFNα ELISA
IFNα (pg/ml)
< LLOD < LLOD
non-immunogenic mRNA
control
NK cells
B cells
C
6 h
24 h
48 h
72 h
x 10^6
Radiance (p/sec/cm²/sr)
Color Scale
Min = 6.50e4
Max = 8.00e6

Figure 2


HPLC purification
1µg 200ng 40ng
after HPLC purification
negative Ctrl
positive Ctrl
Cellulose purification
1µg 200ng 40ng
before cellulose purification
after cellulose purification
negative Ctrl
positive Ctrl

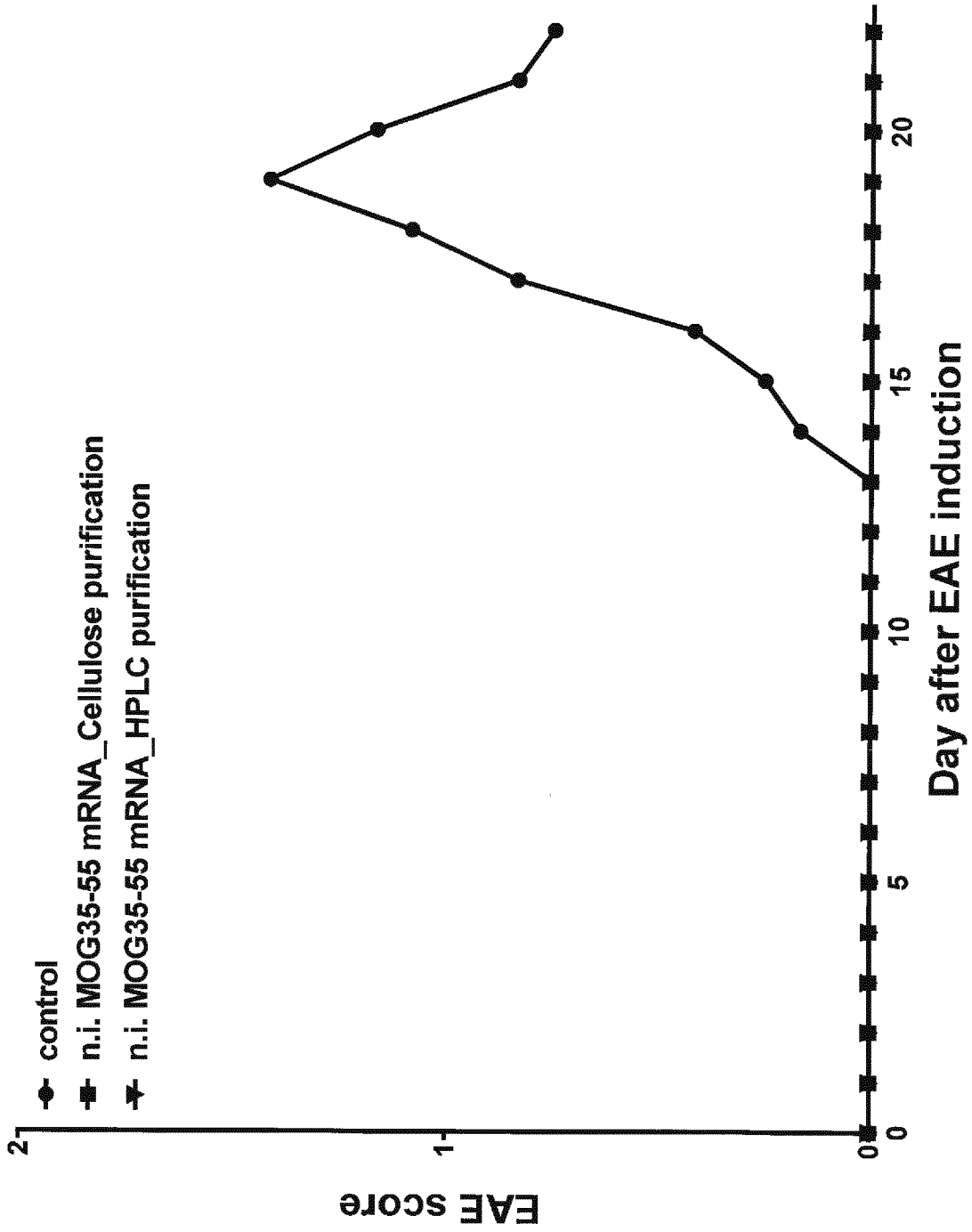

control
n.i. MOG35-55 mRNA_Cellulose purification
n.i. MOG35-55 mRNA_HPLC purification
EAE score
0
1
2
0
5
10
15
20
Day after EAE induction

Figure 3

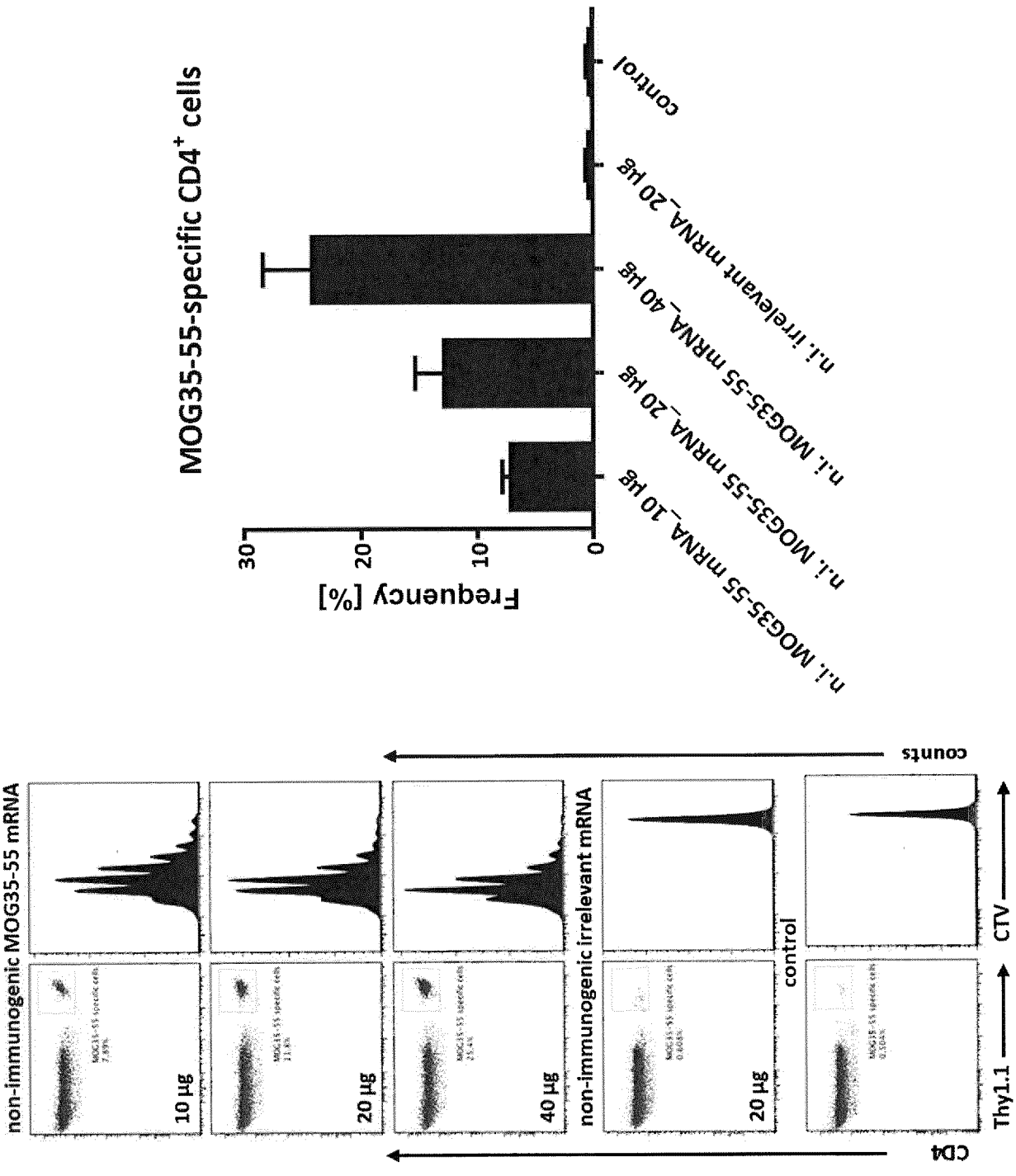

MOG35-55-specific CD4+ cells
Frequency [%]
0
10
20
30
n.i. MOG35-55 mRNA_10 µg
n.i. MOG35-55 mRNA_20 µg
n.i. MOG35-55 mRNA_40 µg
n.i. irrelevant mRNA_20 µg
control
non-immunogenic MOG35-55 mRNA
10 µg
20 µg
40 µg
non-immunogenic irrelevant mRNA
20 µg
control
CD4
Thy1.1
CTV
counts

Figure 4

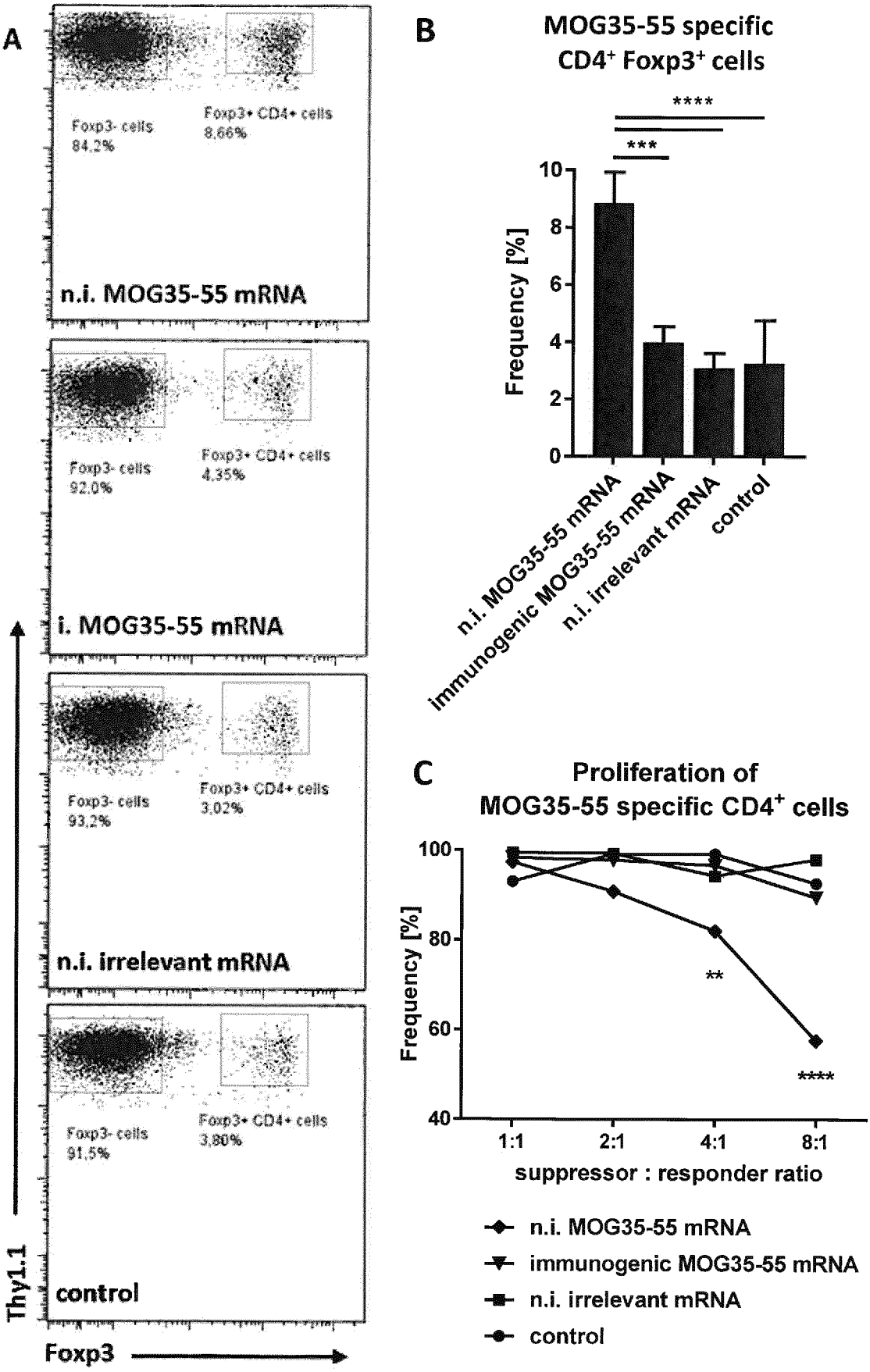
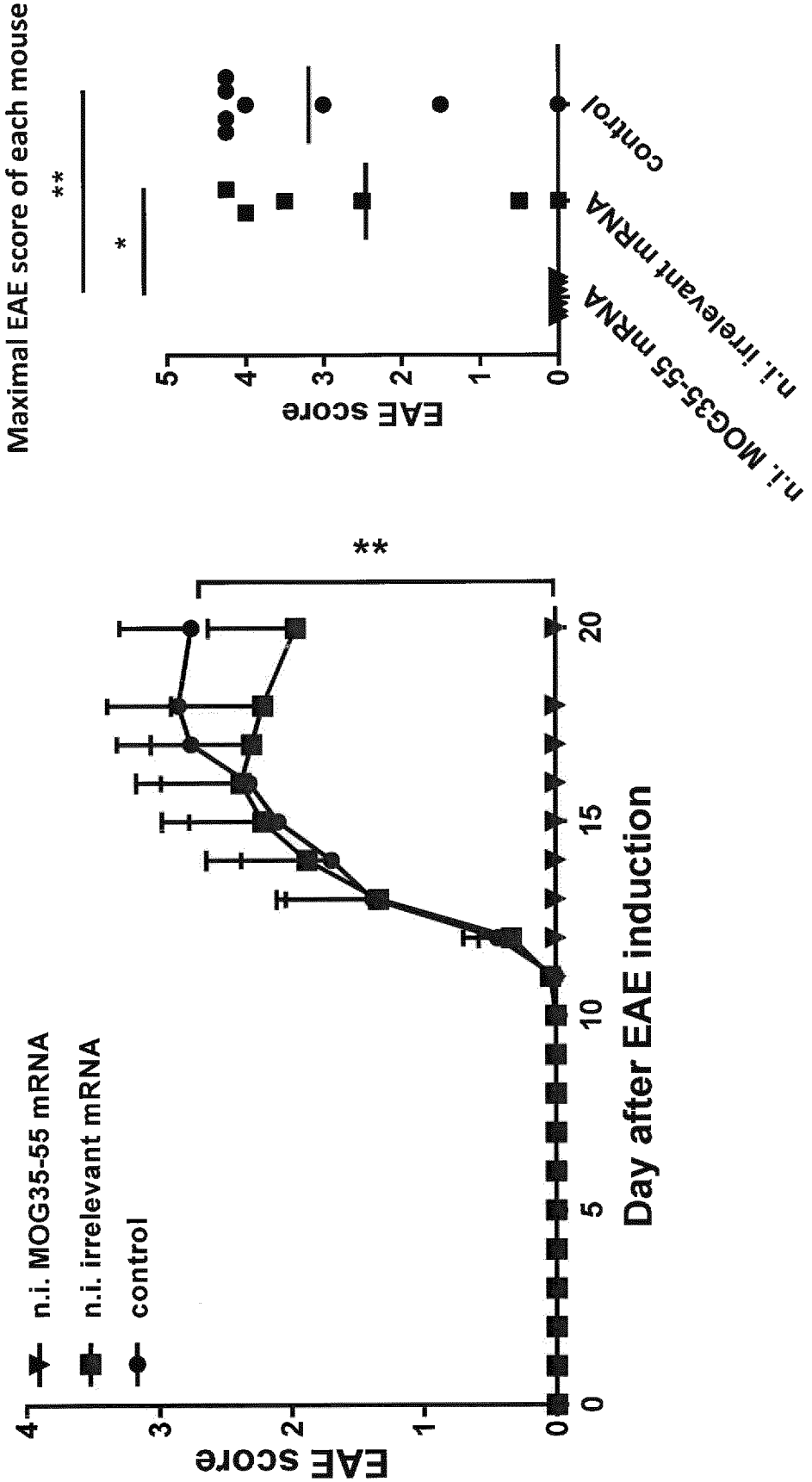

A
Foxp3- cells
84,2%
Foxp3+ CD4+ cells
8,66%
n.i. MOG35-55 mRNA
Foxp3- cells
92,0%
Foxp3+ CD4+ cells
4,35%
i. MOG35-55 mRNA
Foxp3- cells
93,2%
Foxp3+ CD4+ cells
3,02%
n.i. irrelevant mRNA
Foxp3- cells
91,5%
Foxp3+ CD4+ cells
3,80%
control
Thy1.1
Foxp3
B
MOG35-55 specific
CD4+ Foxp3+ cells
****
***
Frequency [%]
10
8
6
4
2
0
n.i. MOG35-55 mRNA
immunogenic MOG35-55 mRNA
n.i. irrelevant mRNA
control
C
Proliferation of
MOG35-55 specific CD4+ cells
Frequency [%]
100
80
60
40
**
****
1:1
2:1
4:1
8:1
suppressor : responder ratio
n.i. MOG35-55 mRNA
immunogenic MOG35-55 mRNA
n.i. irrelevant mRNA
control

Figure 5

Figure 6


n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
EAE score
Day after EAE induction
**
Maximal EAE score of each mouse
**
*
EAE score
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control

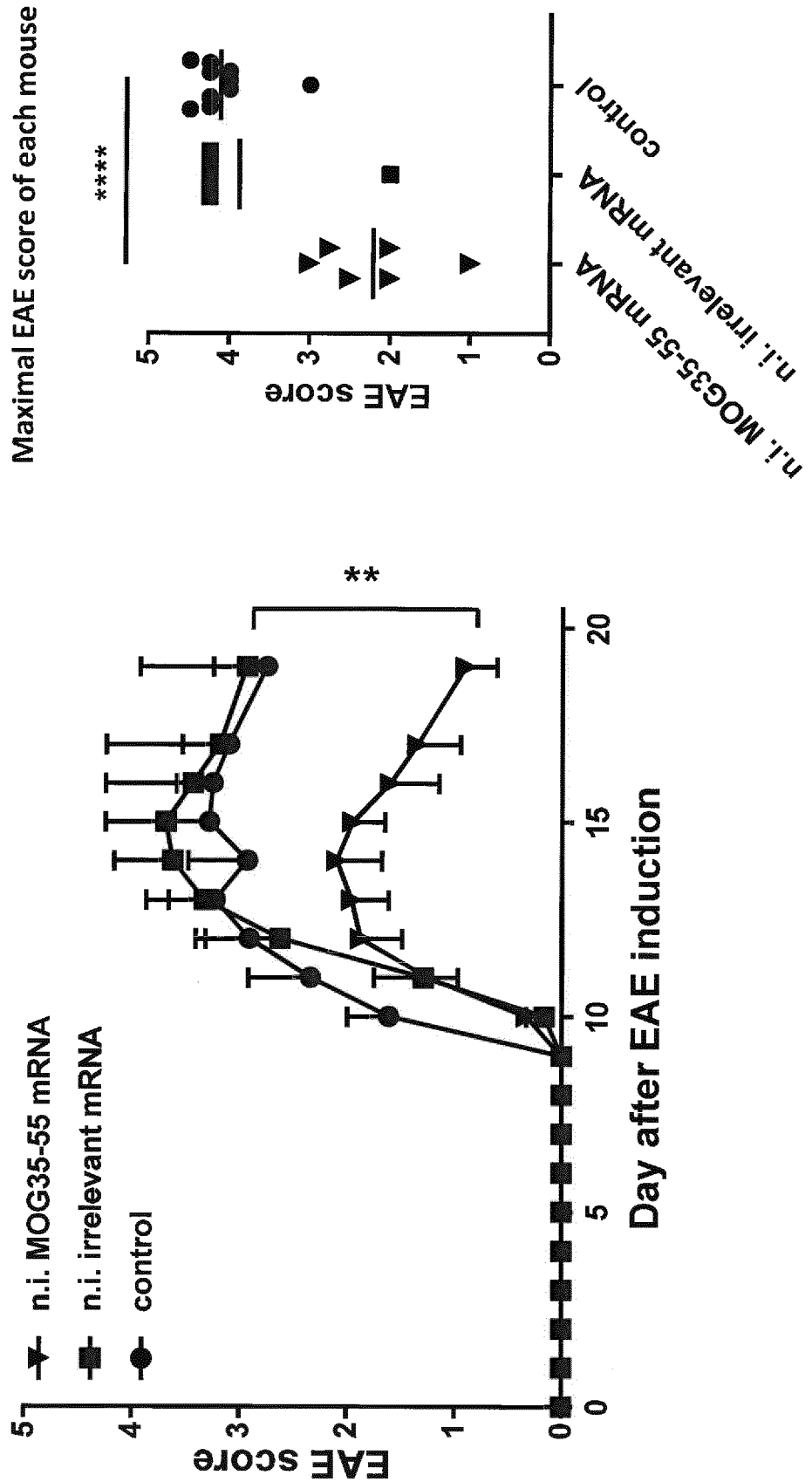

Maximal EAE score of each mouse
****
EAE score
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
**
EAE score
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
Day after EAE induction

Figure 7

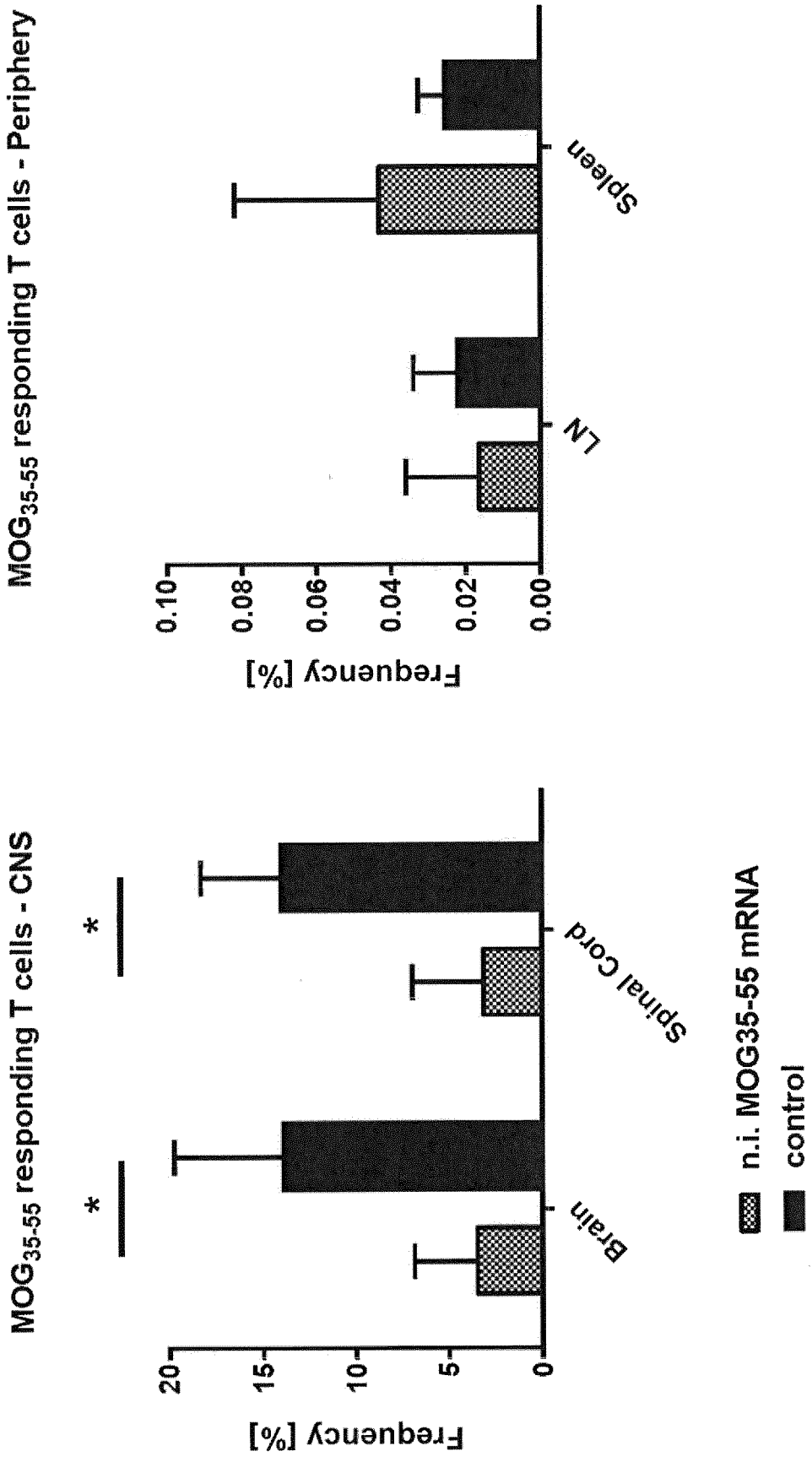

MOG35-55 responding T cells - Periphery
Frequency [%]
0.10
0.08
0.06
0.04
0.02
0.00
LN
Spleen
MOG35-55 responding T cells - CNS
Frequency [%]
20
15
10
5
0
*
*
Brain
Spinal Cord
n.i. MOG35-55 mRNA
control

Figure 8

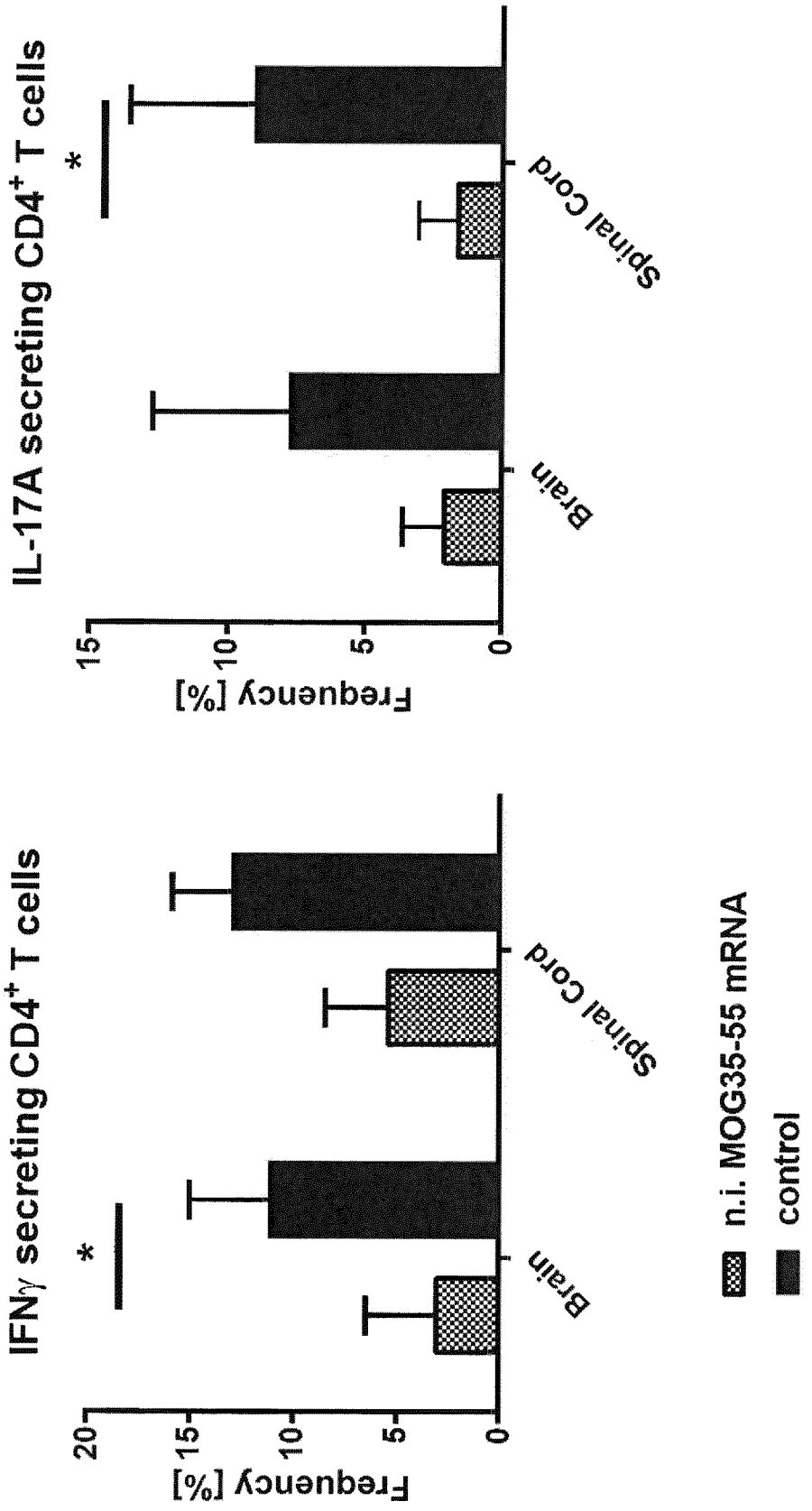

IL-17A secreting CD4+ T cells
*
Frequency [%]
0
5
10
15
Brain
Spinal Cord
IFNγ secreting CD4+ T cells
*
Frequency [%]
0
5
10
15
20
Brain
Spinal Cord
n.i. MOG35-55 mRNA
control

Figure 9

Figure 10

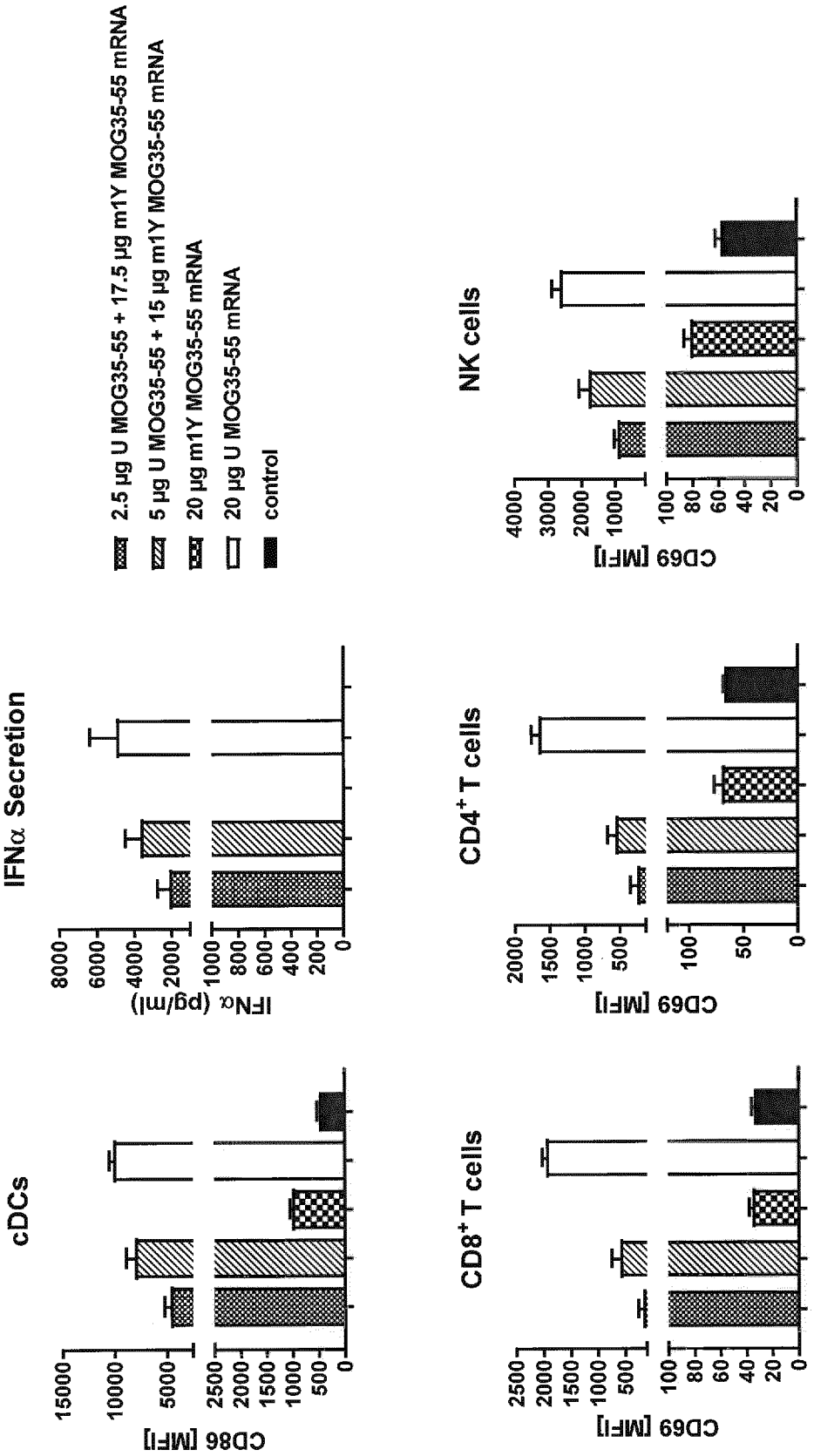

2.5 µg U MOG35-55 + 17.5 µg m1Y MOG35-55 mRNA
5 µg U MOG35-55 + 15 µg m1Y MOG35-55 mRNA
20 µg m1Y MOG35-55 mRNA
20 µg U MOG35-55 mRNA
control
cDCs
CD86 [MFI]
IFNα Secretion
IFNα (pg/ml)
NK cells
CD69 [MFI]
CD4+ T cells
CD69 [MFI]
CD8+ T cells
CD69 [MFI]

Figure 11

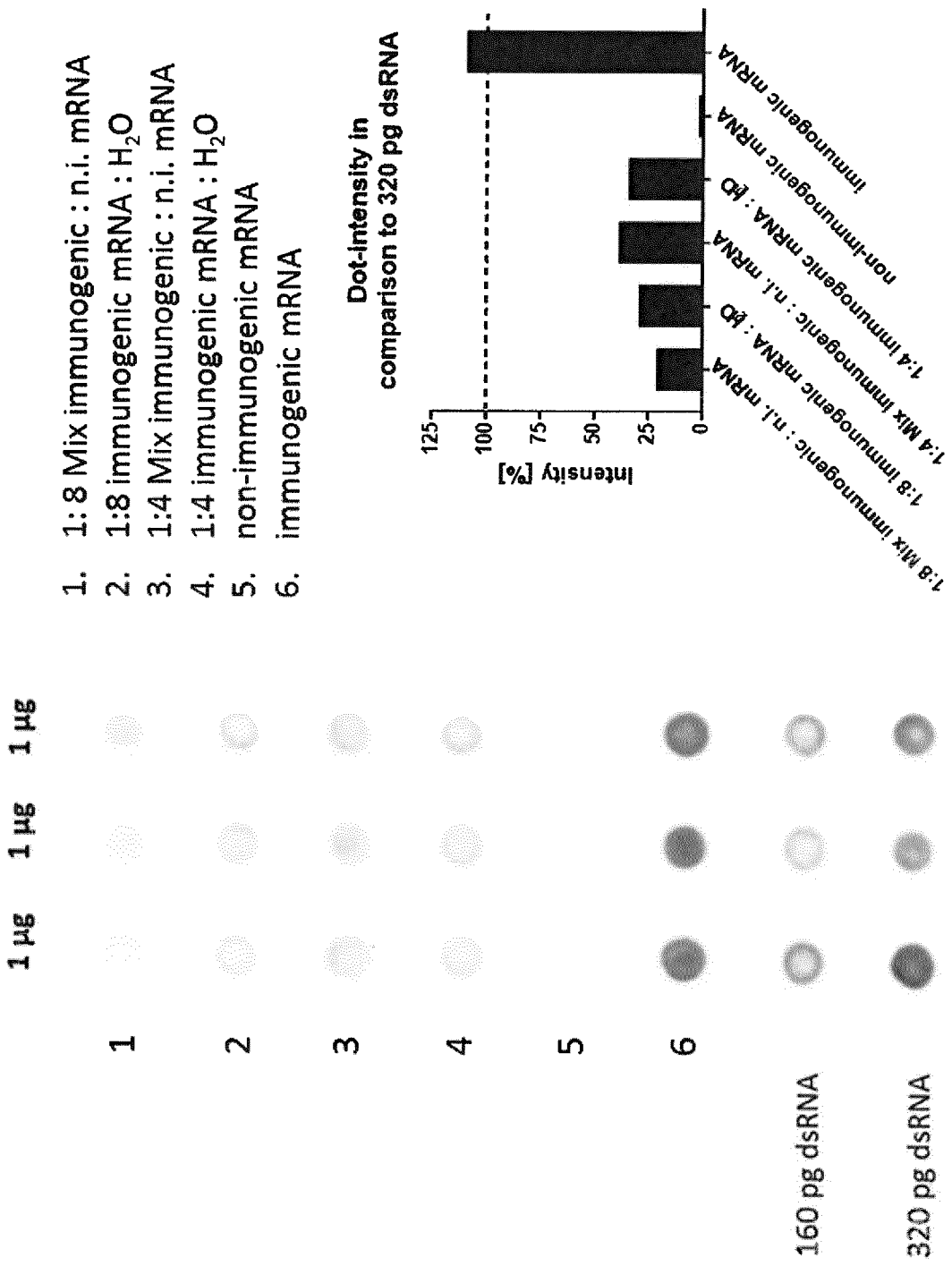

1 µg
1 µg
1 µg
1
2
3
4
5
6
160 pg dsRNA
320 pg dsRNA
1. 1: 8 Mix immunogenic : n.i. mRNA
2. 1:8 immunogenic mRNA : H2O
3. 1:4 Mix immunogenic : n.i. mRNA
4. 1:4 immunogenic mRNA : H2O
5. non-immunogenic mRNA
6. immunogenic mRNA
Dot-intensity in comparison to 320 pg dsRNA
Intensity [%]
125
100
75
50
25
0
1:8 Mix immunogenic : n.i. mRNA
1:8 immunogenic mRNA : H2O
1:4 Mix immunogenic : n.i. mRNA
1:4 immunogenic mRNA : H2O
non-immunogenic mRNA
immunogenic mRNA

Figure 12

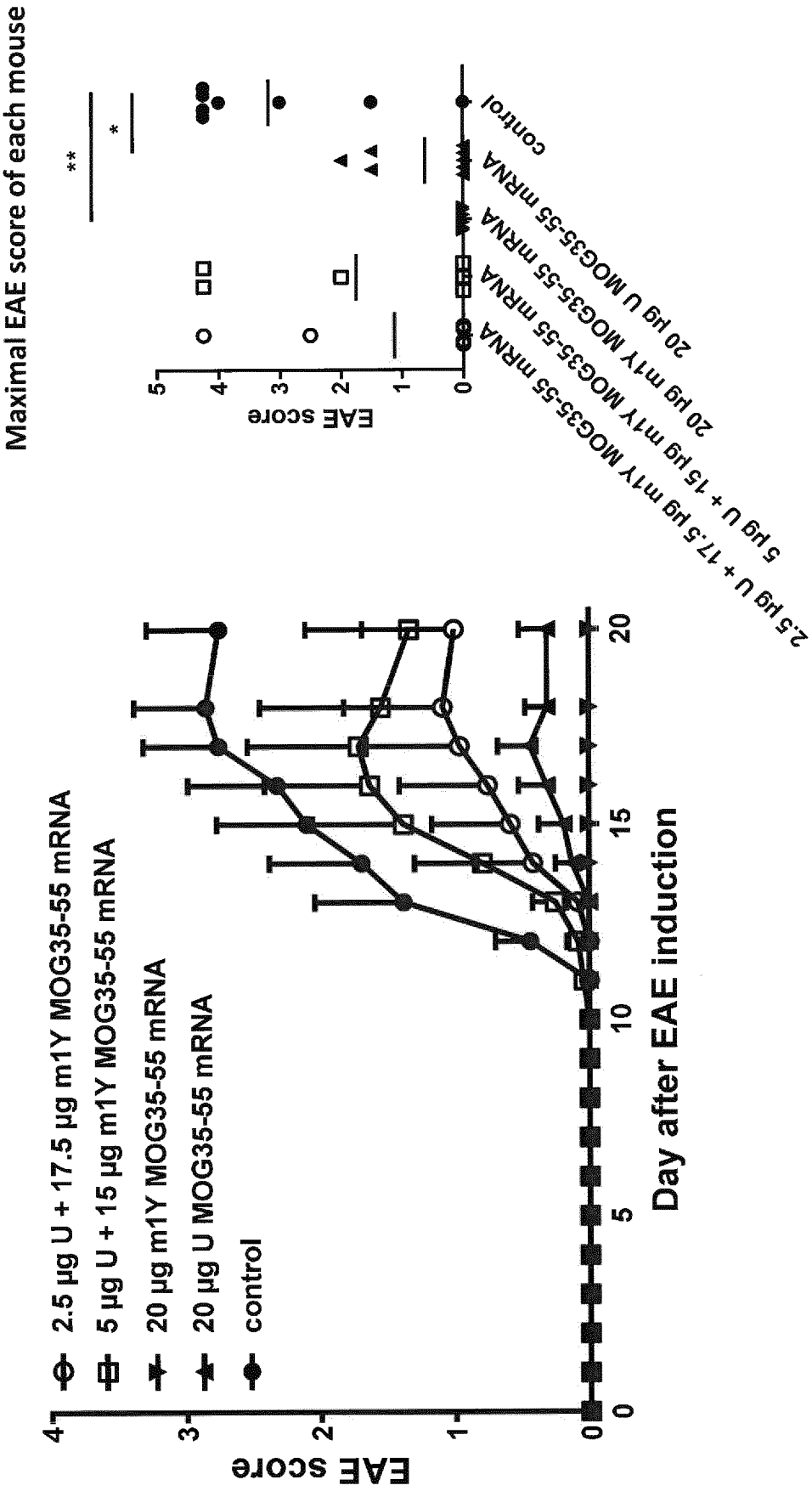

EAE score
Day after EAE induction
2.5 µg U + 17.5 µg m1Y MOG35-55 mRNA
5 µg U + 15 µg m1Y MOG35-55 mRNA
20 µg m1Y MOG35-55 mRNA
20 µg U MOG35-55 mRNA
control
Maximal EAE score of each mouse
**
*
2.5 µg U + 17.5 µg m1Y MOG35-55 mRNA
5 µg U + 15 µg m1Y MOG35-55 mRNA
20 µg m1Y MOG35-55 mRNA
20 µg U MOG35-55 mRNA
control

Figure 13

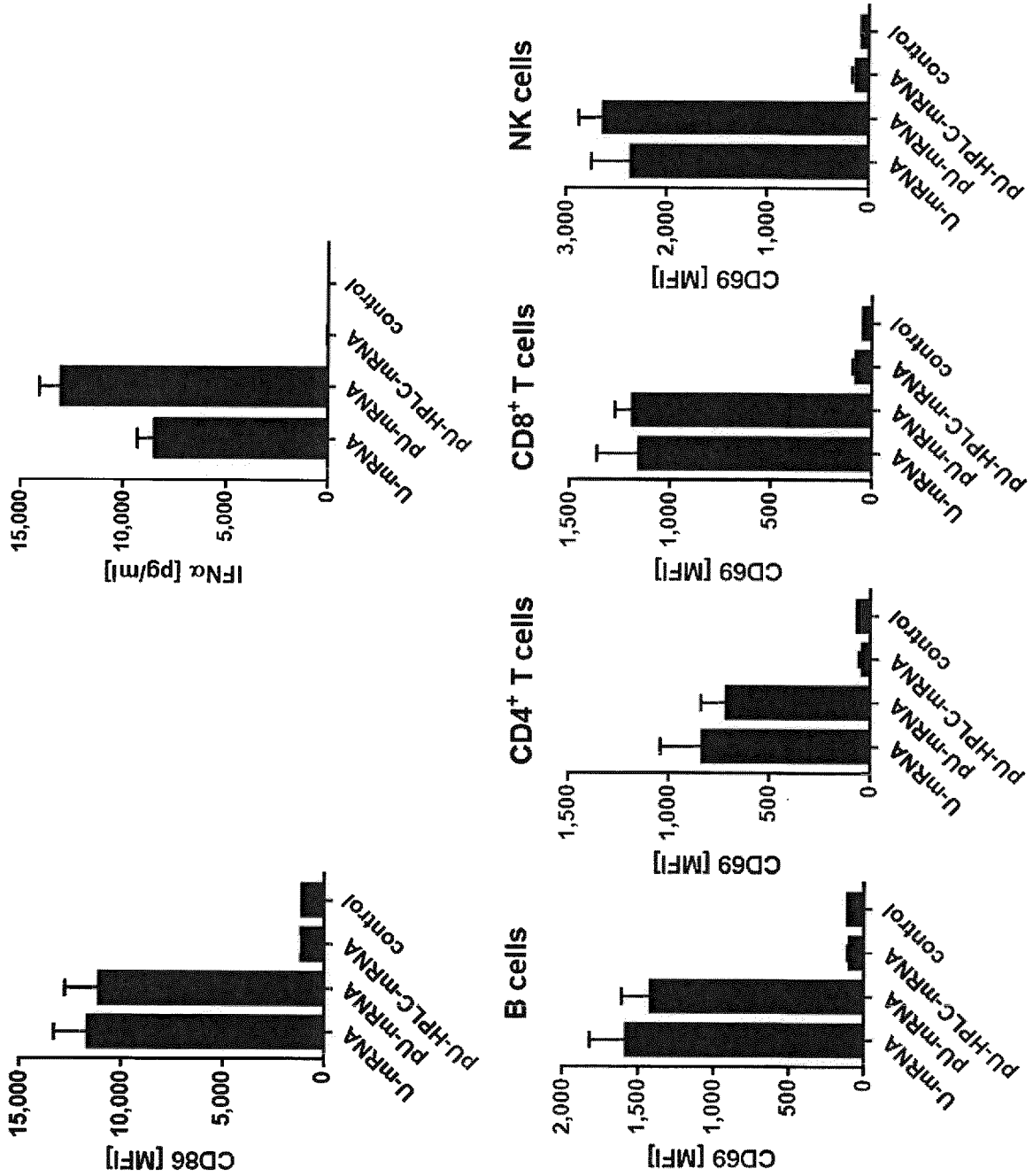
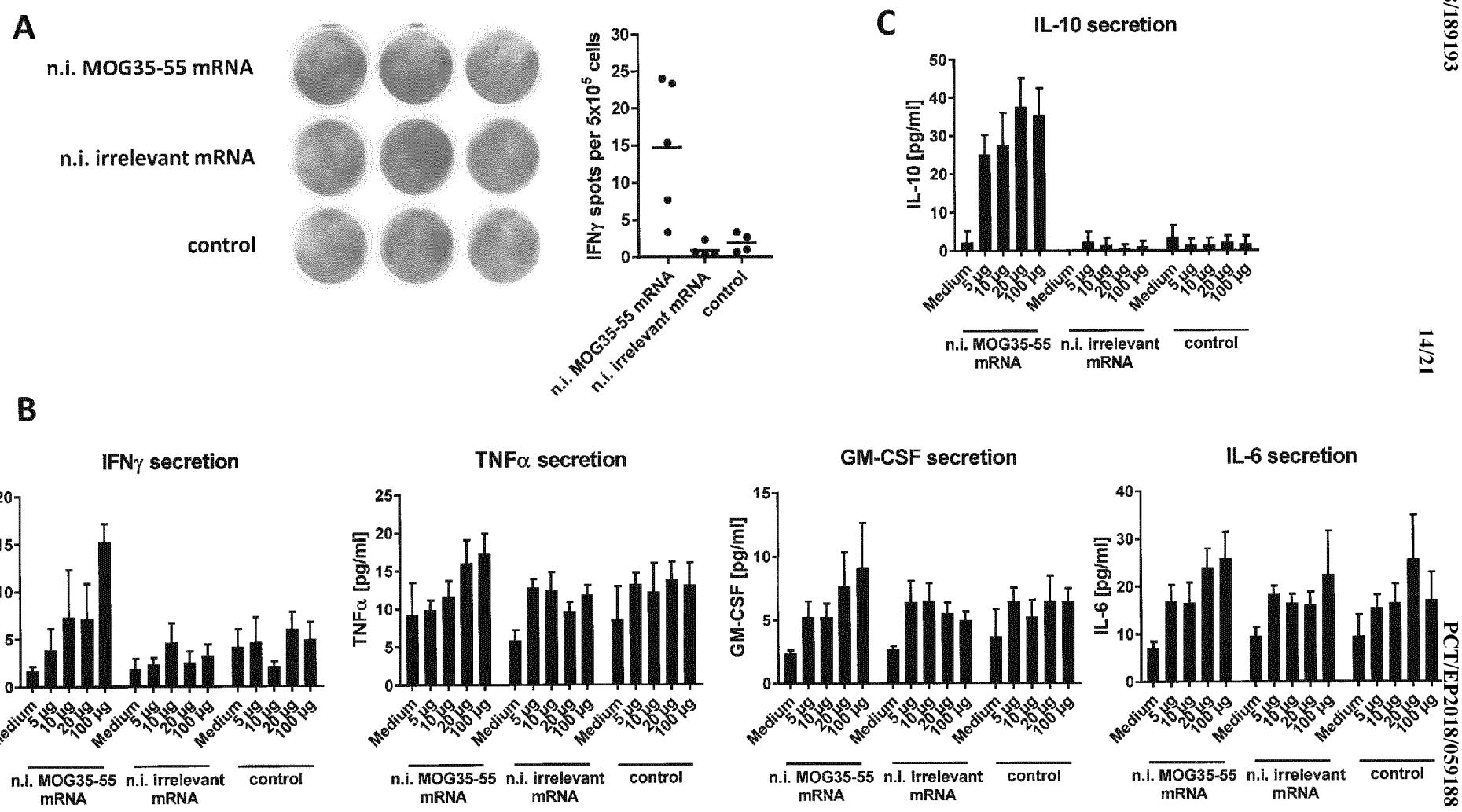

DCs
CD86 [MFI]
15,000
10,000
5,000
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control
IFNα secretion
IFNα [pg/ml]
15,000
10,000
5,000
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control
B cells
CD69 [MFI]
2,000
1,500
1,000
500
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control
CD4+ T cells
CD69 [MFI]
1,500
1,000
500
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control
CD8+ T cells
CD69 [MFI]
1,500
1,000
500
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control
NK cells
CD69 [MFI]
3,000
2,000
1,000
0
U-mRNA
PU-mRNA
PU-HPLC-mRNA
control

Figure 14


A
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
IFNγ spots per 5x10^5 cells
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
C
IL-10 secretion
IL-10 [pg/ml]
Medium
5 µg
10 µg
20 µg
100 µg
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
B
IFNγ secretion
IFNγ [pg/ml]
TNFα secretion
TNFα [pg/ml]
GM-CSF secretion
GM-CSF [pg/ml]
IL-6 secretion
IL-6 [pg/ml]

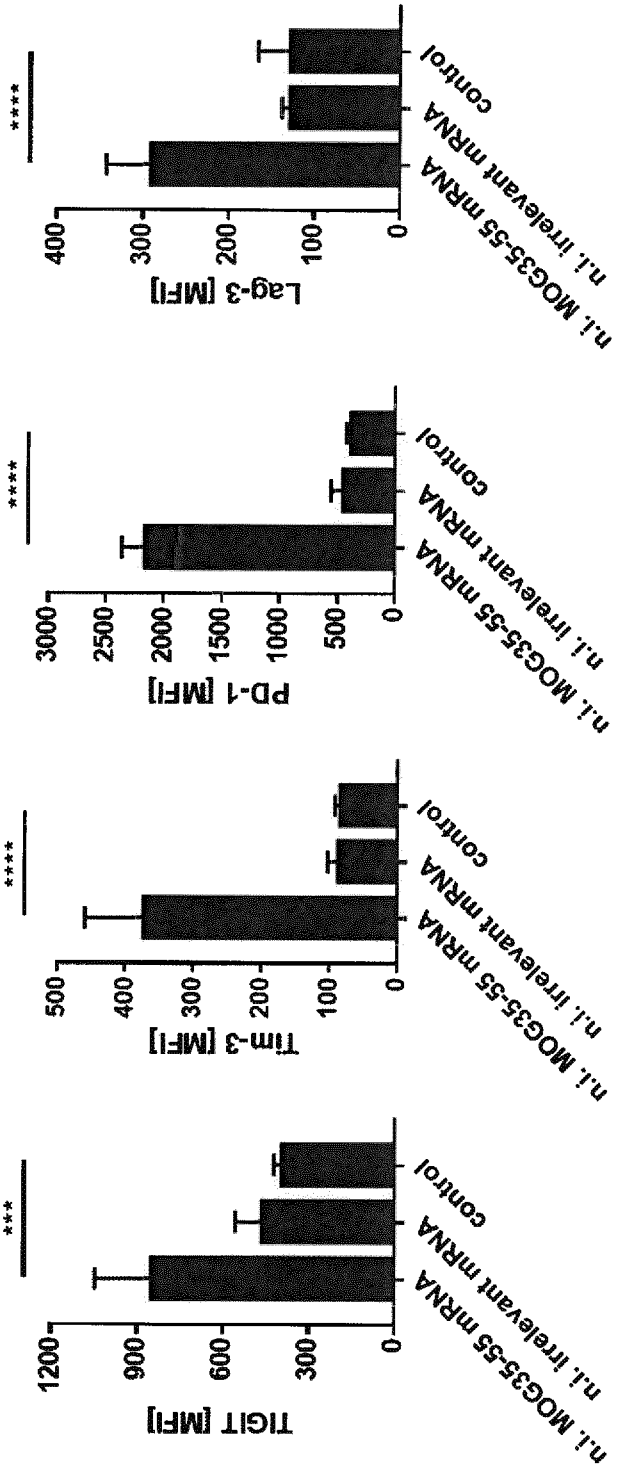

TIGIT [MFI]
Tim-3 [MFI]
PD-1 [MFI]
Lag-3 [MFI]
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
***
****

Figure 15

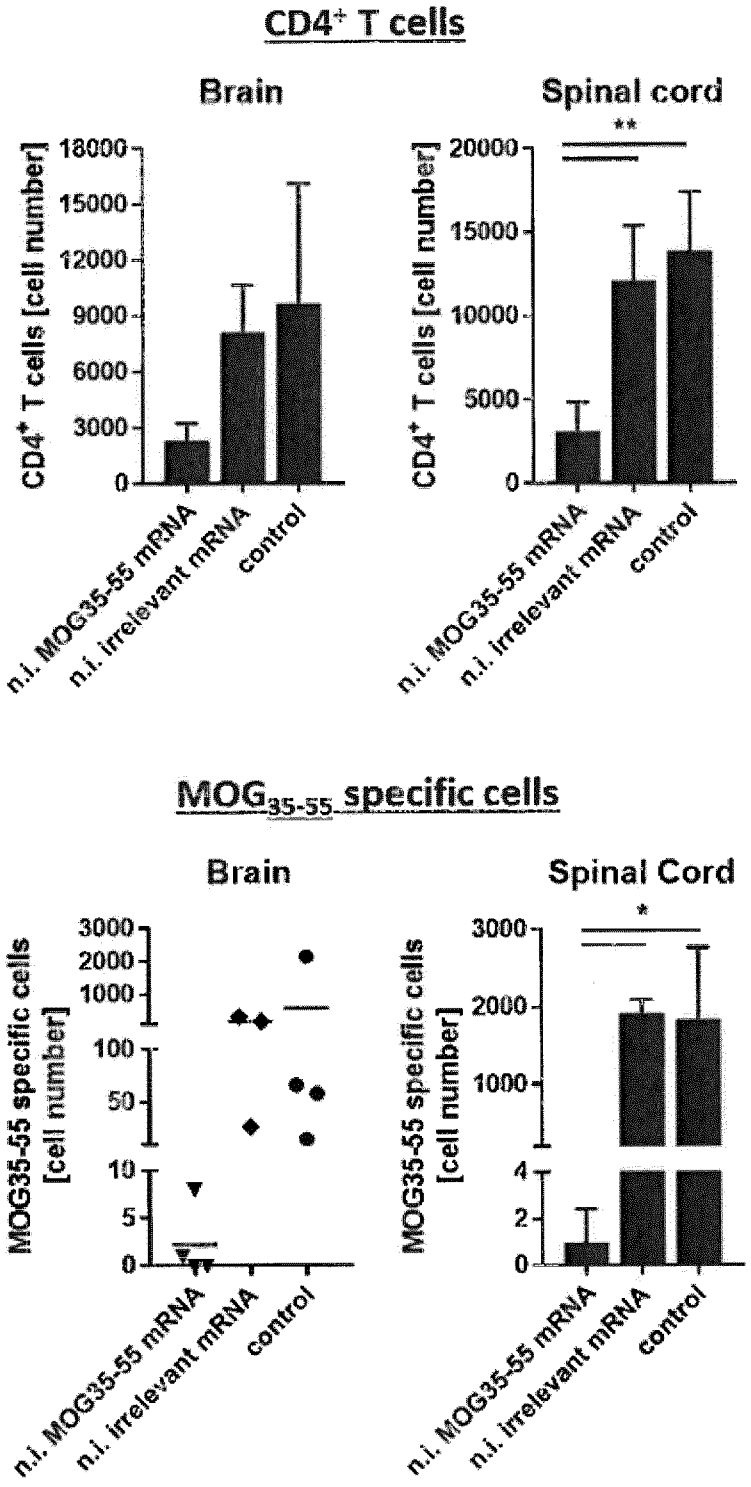

CD4+ T cells
Brain
Spinal cord
CD4+ T cells [cell number]
18000
15000
12000
9000
6000
3000
0
20000
10000
5000
**
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
MOG35-55 specific cells
Brain
Spinal Cord
MOG35-55 specific cells [cell number]
3000
2000
1000
100
50
10
5
4
2
*

Figure 16

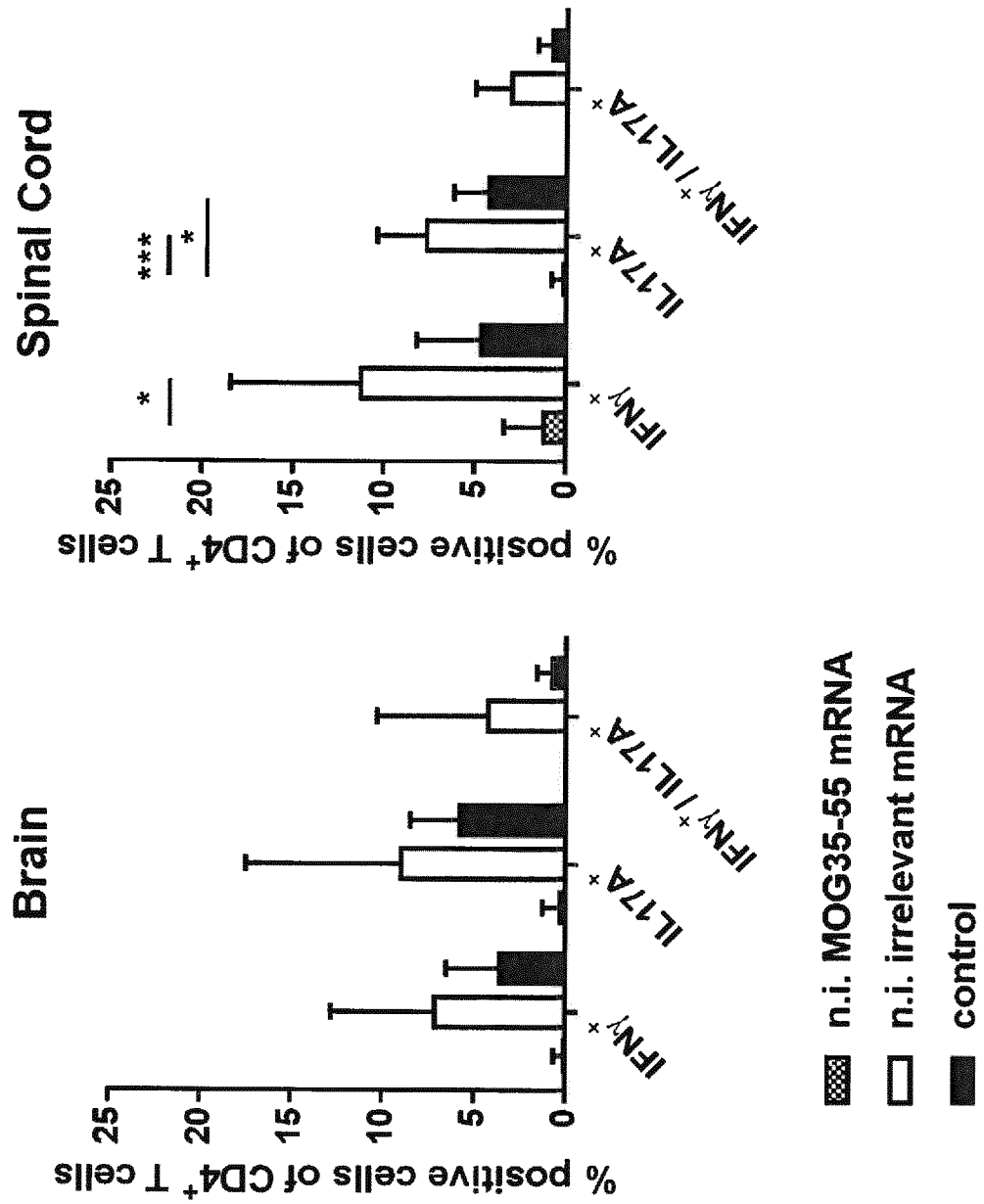

Spinal Cord
Brain
% positive cells of CD4+ T cells
0
5
10
15
20
25
IFNγ +
IL17A +
IFNγ / IL17A +
*
***
*
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control

Figure 17

Figure 18

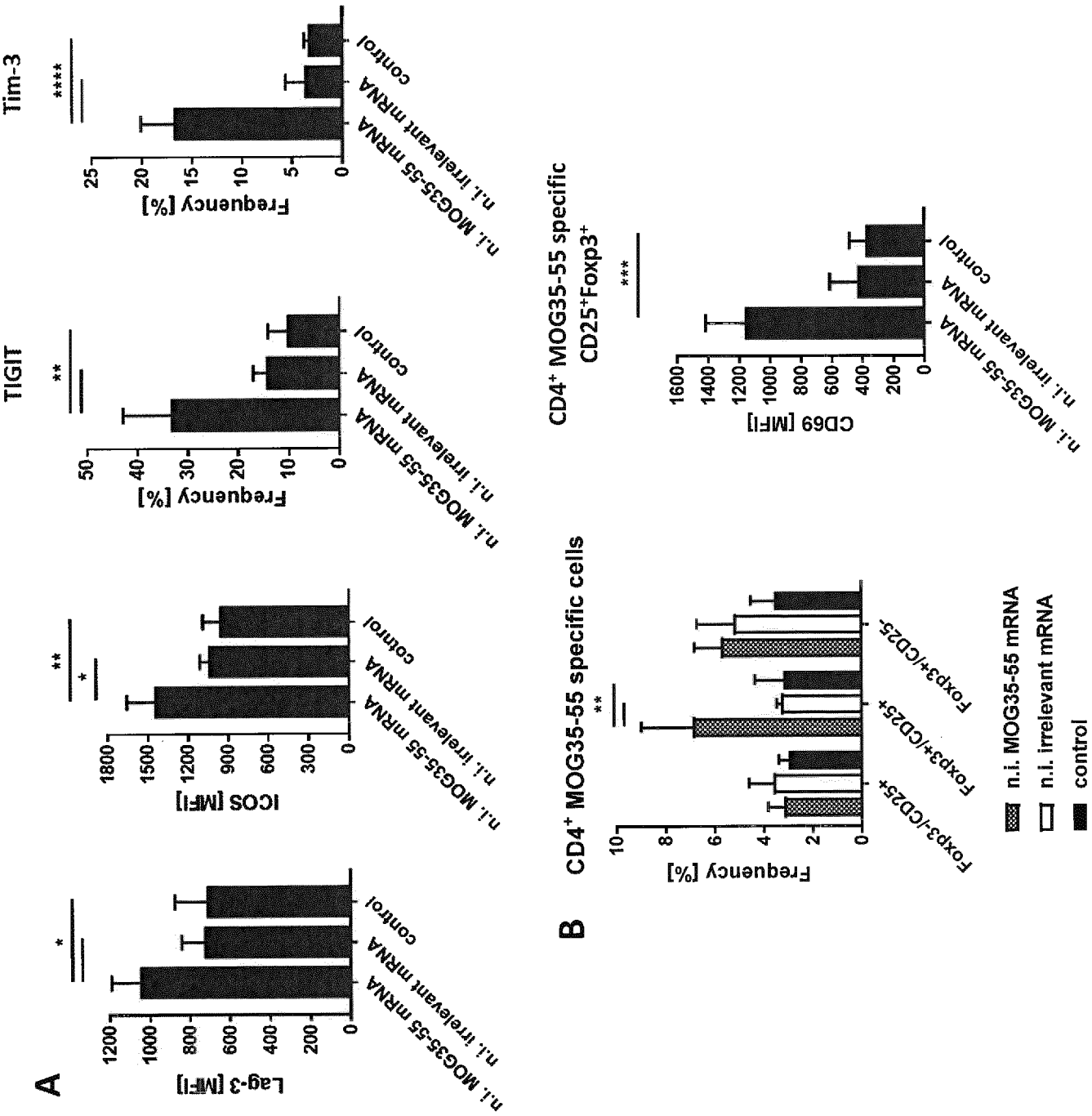

A
Lag-3 [MFI]
ICOS [MFI]
TIGIT
Frequency [%]
Tim-3
Frequency [%]
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
B
CD4+ MOG35-55 specific cells
Frequency [%]
Foxp3-/CD25+
Foxp3+/CD25+
Foxp3+/CD25-
n.i. MOG35-55 mRNA
n.i. irrelevant mRNA
control
CD4+ MOG35-55 specific CD25+Foxp3+
CD69 [MFI]

Figure 19

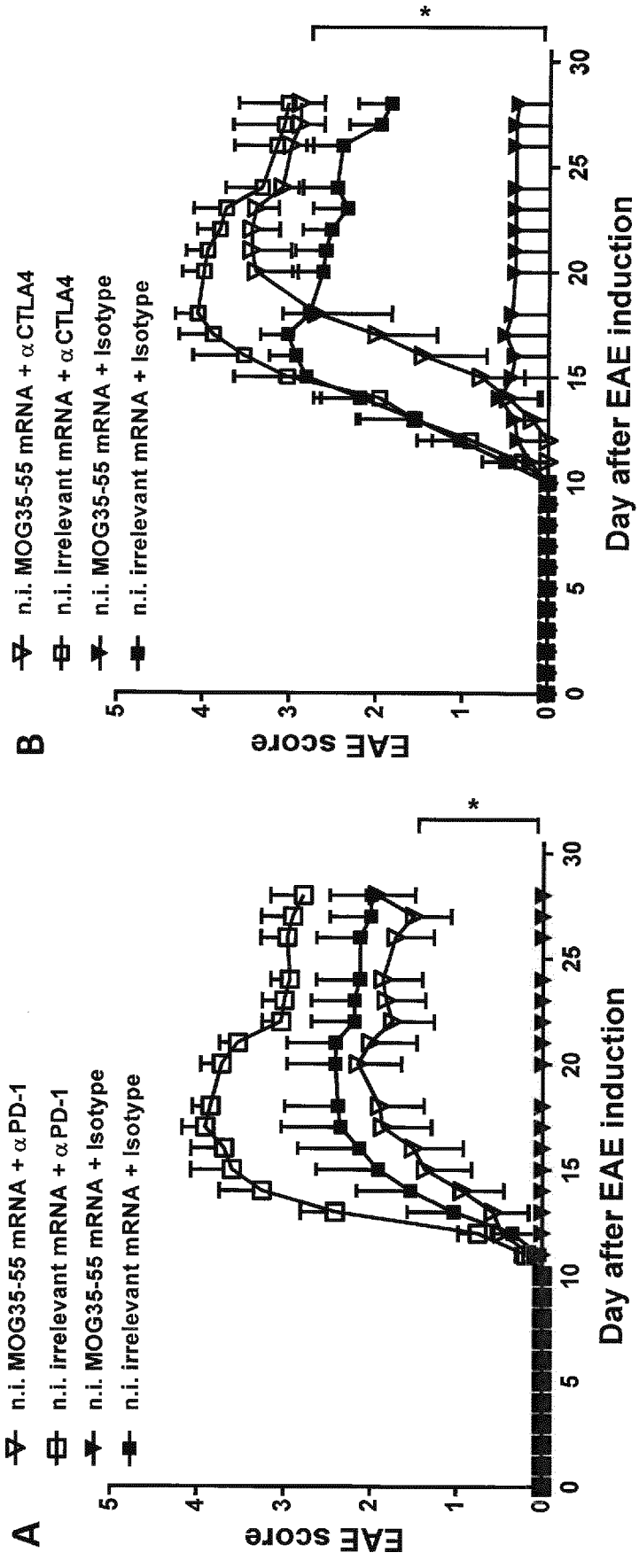

A
n.i. MOG35-55 mRNA + αPD-1
n.i. irrelevant mRNA + αPD-1
n.i. MOG35-55 mRNA + Isotype
n.i. irrelevant mRNA + Isotype
EAE score
Day after EAE induction
*
B
n.i. MOG35-55 mRNA + αCTLA4
n.i. irrelevant mRNA + αCTLA4
n.i. MOG35-55 mRNA + Isotype
n.i. irrelevant mRNA + Isotype
EAE score
Day after EAE induction
*

Figure 20

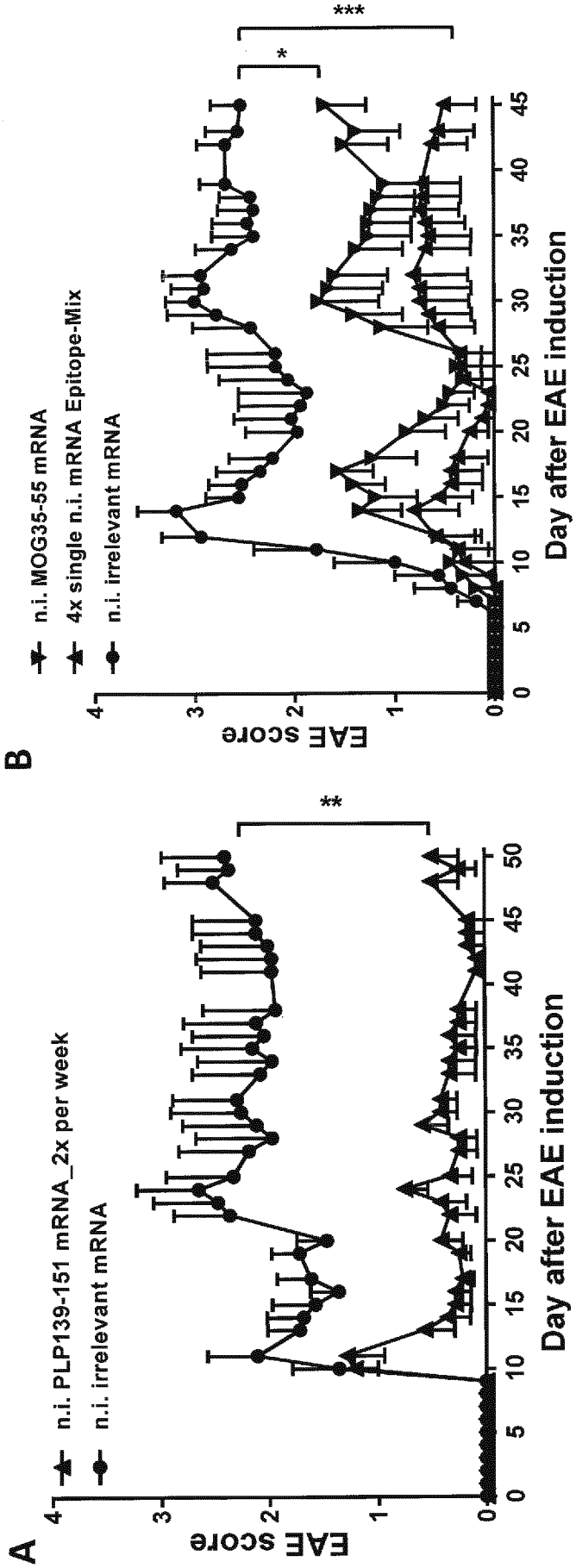
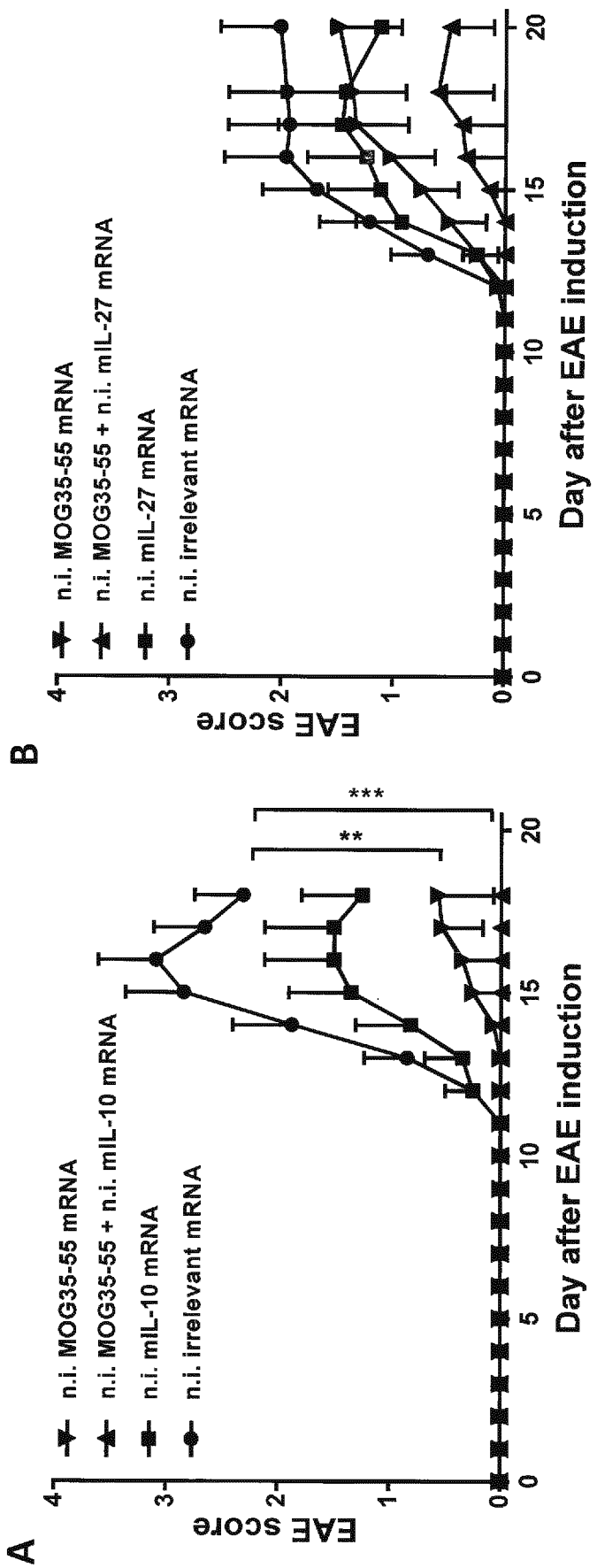

A
n.i. PLP139-151 mRNA_2x per week
n.i. irrelevant mRNA
EAE score
Day after EAE induction
**
B
n.i. MOG35-55 mRNA
4x single n.i. mRNA Epitope-Mix
n.i. irrelevant mRNA
EAE score
Day after EAE induction
*
***

Figure 21


A
B
EAE score
Day after EAE induction
n.i. MOG35-55 mRNA
n.i. MOG35-55 + n.i. mIL-10 mRNA
n.i. mIL-10 mRNA
n.i. irrelevant mRNA
n.i. MOG35-55 + n.i. mIL-27 mRNA
n.i. mIL-27 mRNA
**
***