How To Apply Gate and Analysis Procedures

Fig. 1

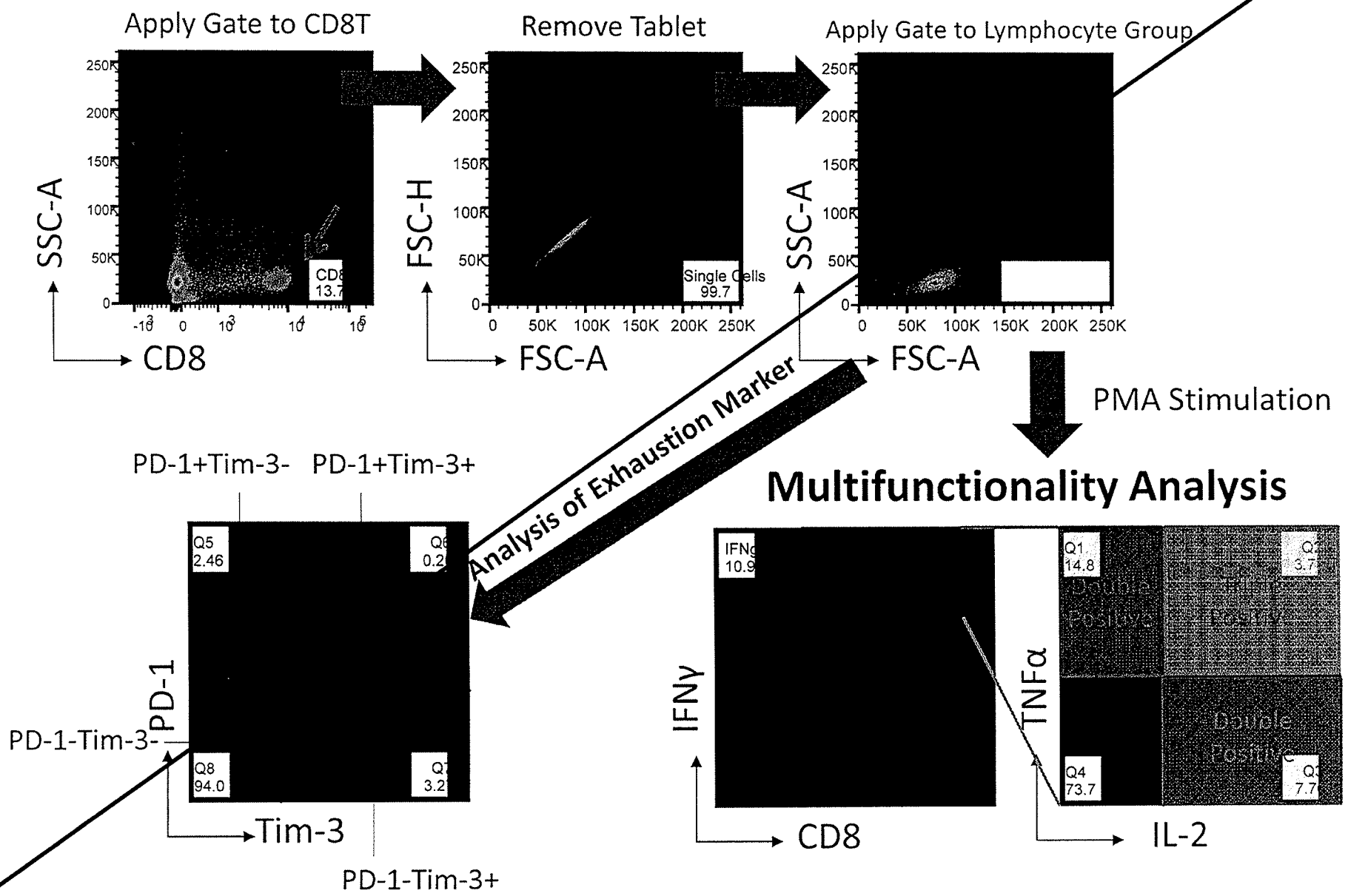


Fig. 2

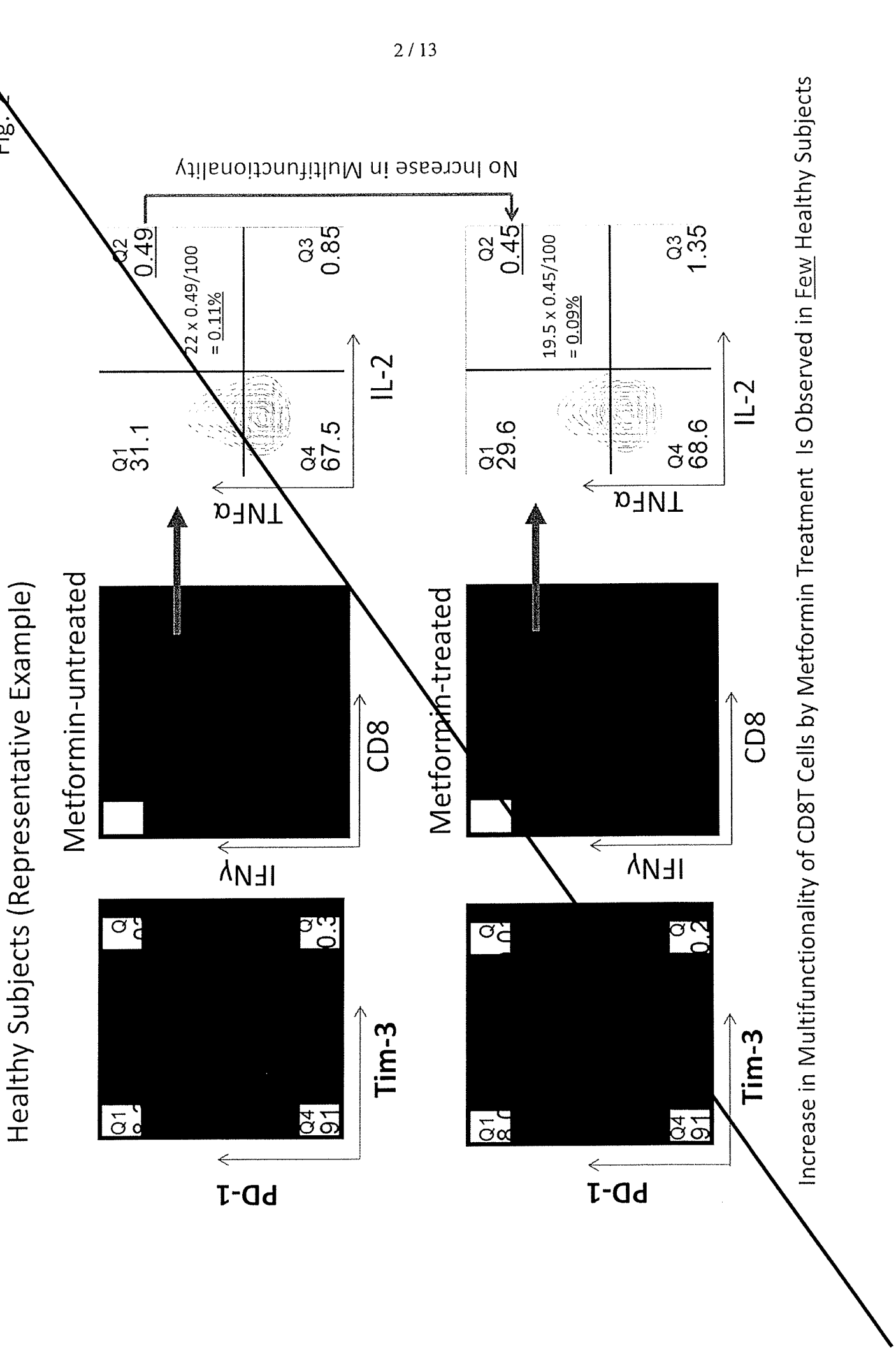

Healthy Subjects (Representative Example)
Metformin-untreated
Metformin-treated
PD-1
Tim-3
IFNγ
CD8
TNFα
IL-2
Q1 31.1
Q2 0.49
Q3 0.85
Q4 67.5
22 x 0.49/100 = 0.11%
Q1 29.6
Q2 0.45
Q3 1.35
Q4 68.6
19.5 x 0.45/100 = 0.09%
No Increase in Multifunctionality
Increase in Multifunctionality of CD8T Cells by Metformin Treatment Is Observed in Few Healthy Subjects

Fig. 3

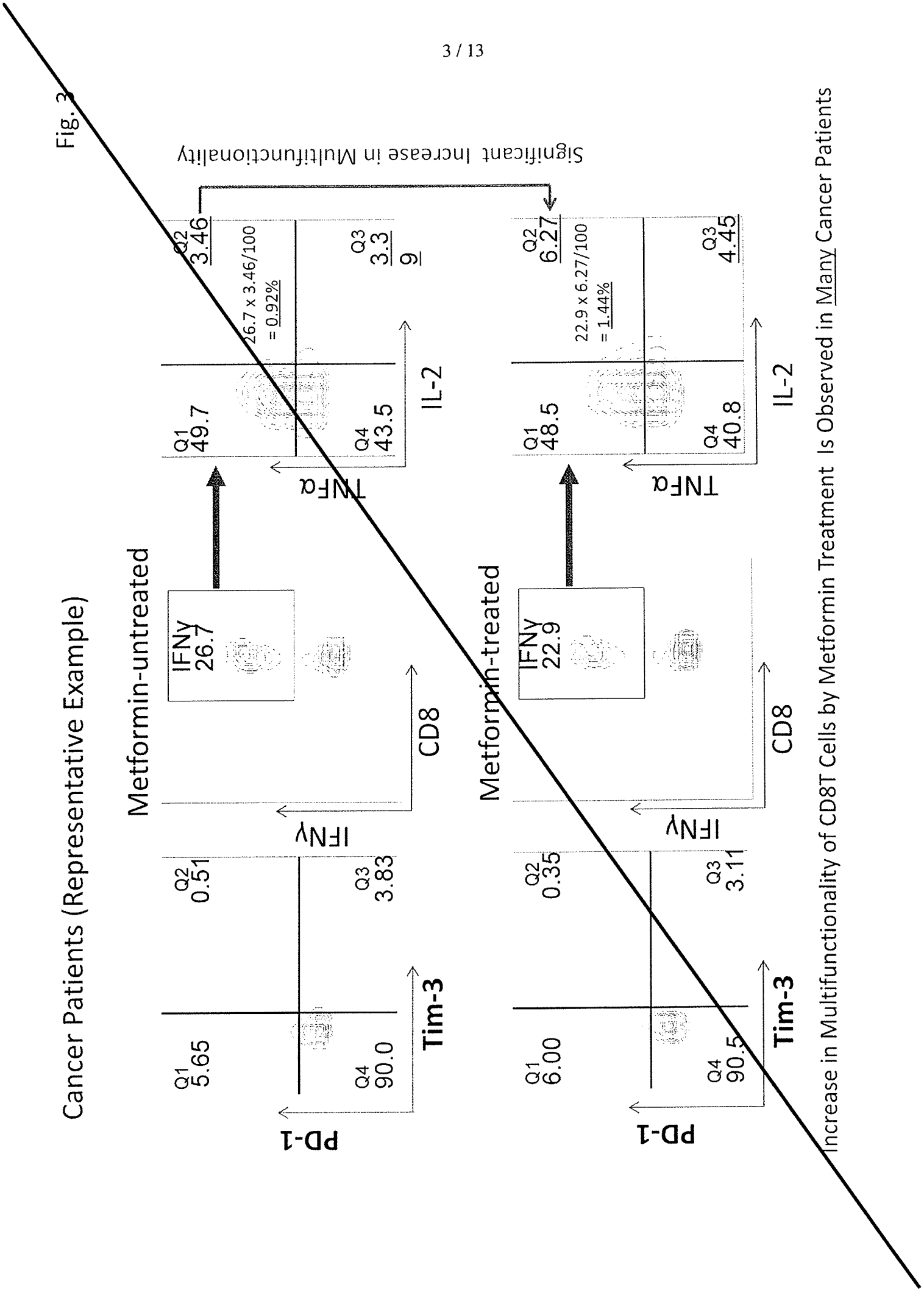

Cancer Patients (Representative Example)
Increase in Multifunctionality of CD8T Cells by Metformin Treatment Is Observed in Many Cancer Patients
Metformin-untreated
Metformin-treated
Significant Increase in Multifunctionality
PD-1
Tim-3
IFNγ
CD8
TNFα
IL-2
IFNγ 26.7
IFNγ 22.9
Q1 5.65
Q2 0.51
Q3 3.83
Q4 90.0
Q1 6.00
Q2 0.35
Q3 3.11
Q4 90.5
Q1 49.7
Q2 3.46
Q3 3.3
Q4 43.5
Q1 48.5
Q2 6.27
Q3 4.45
Q4 40.8
26.7 x 3.46/100 = 0.92%
22.9 x 6.27/100 = 1.44%

Fig. 6

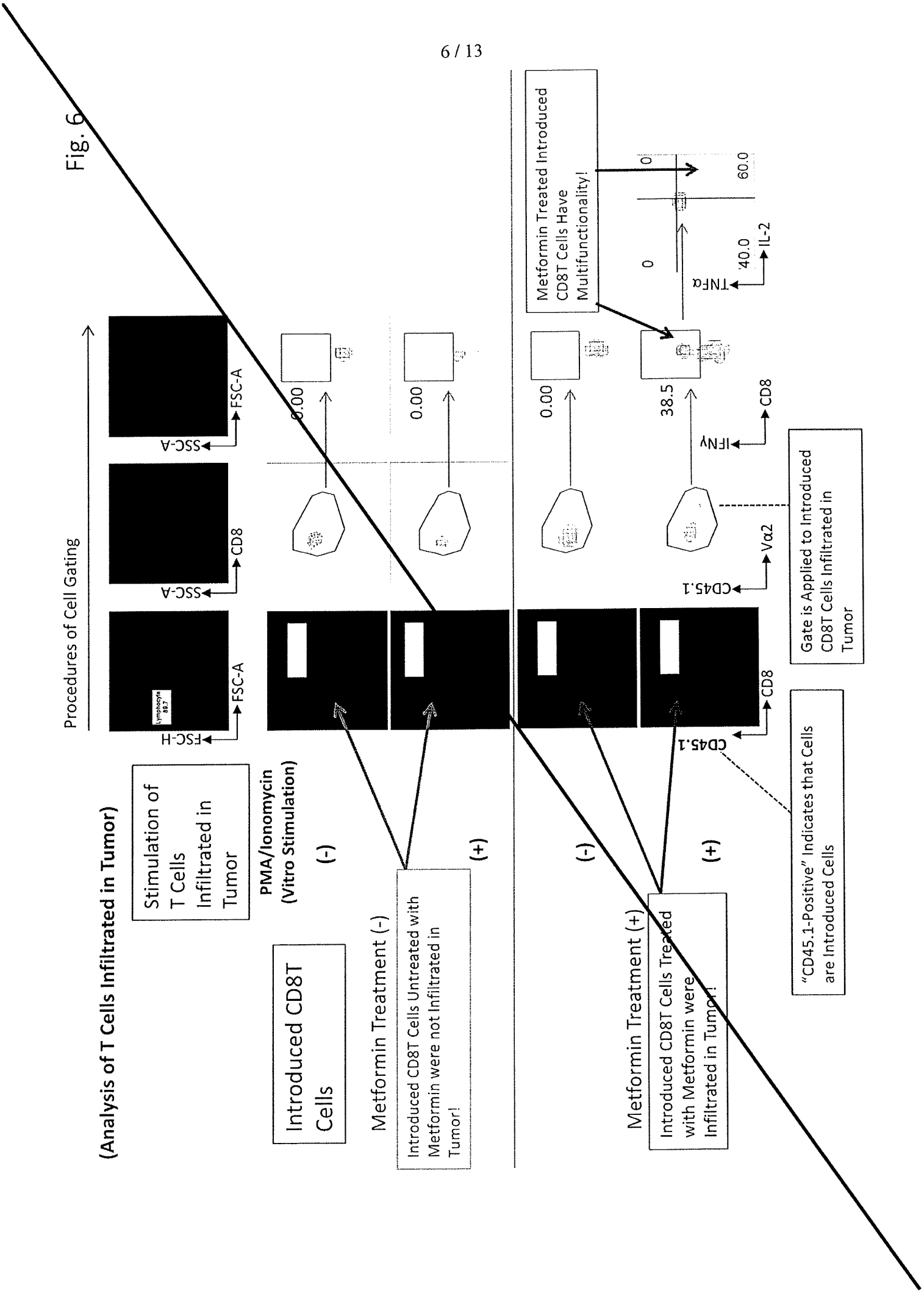
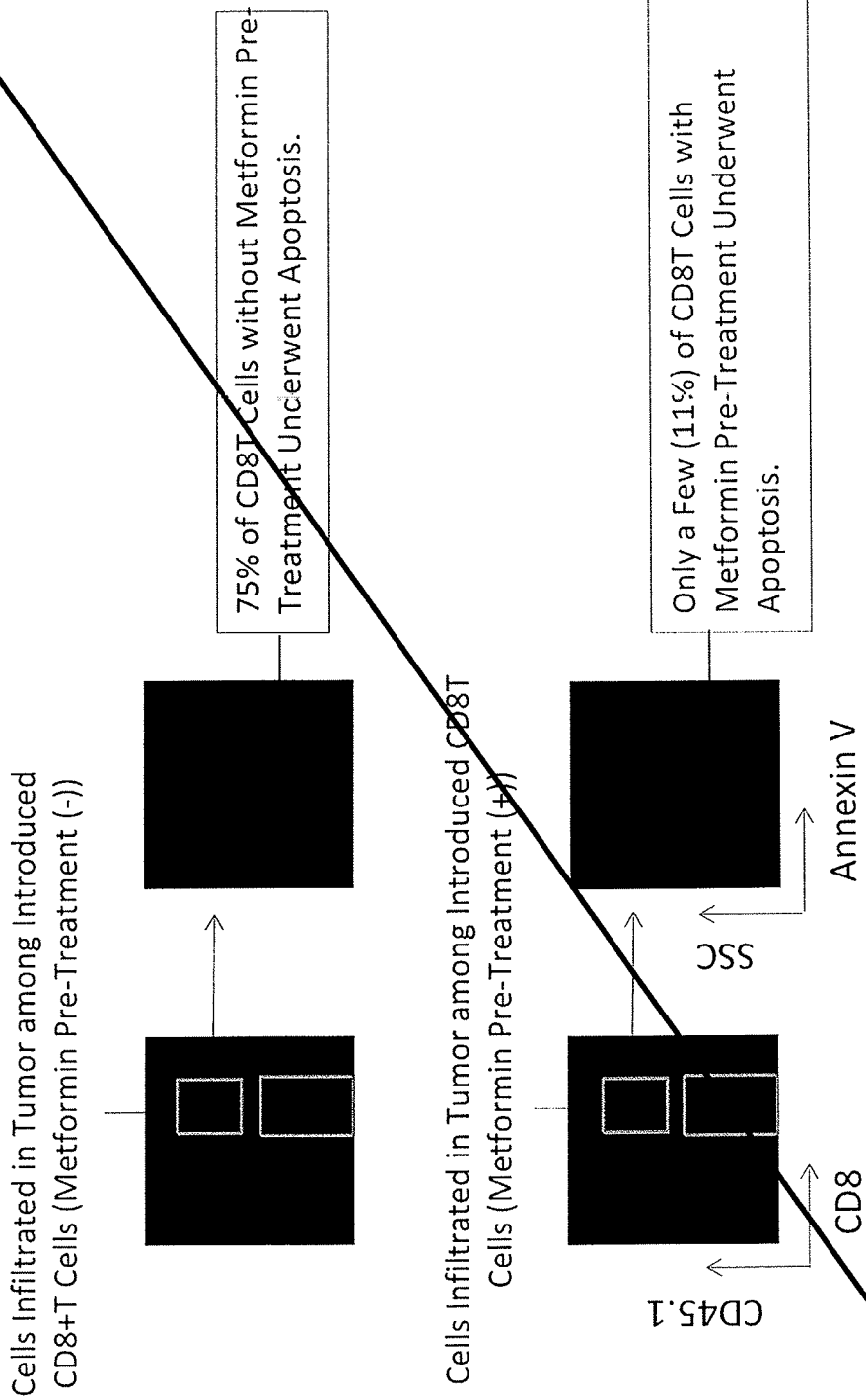

(Analysis of T Cells Infiltrated in Tumor)
Procedures of Cell Gating
Stimulation of T Cells Infiltrated in Tumor
PMA/Ionomycin (Vitro Stimulation)
Introduced CD8T Cells
Metformin Treatment (-)
Introduced CD8T Cells Untreated with Metformin were not Infiltrated in Tumor!
Metformin Treatment (+)
Introduced CD8T Cells Treated with Metformin were Infiltrated in Tumor!
Metformin Treated Introduced CD8T Cells Have Multifunctionality!
Gate is Applied to Introduced CD8T Cells Infiltrated in Tumor
"CD45.1-Positive" Indicates that Cells are Introduced Cells
FSC-H
FSC-A
SSC-A
CD8
CD45.1
Vα2
IFNγ
TNFα
IL-2
0.00
38.5
40.0
60.0

Fig. 7


Cells Infiltrated in Tumor among Introduced CD8+T Cells (Metformin Pre-Treatment (-))
75% of CD8T Cells without Metformin Pre-Treatment Uncerwent Apoptosis.
CD45.1
CD8
Cells Infiltrated in Tumor among Introduced CD8T Cells (Metformin Pre-Treatment (+))
Only a Few (11%) of CD8T Cells with Metformin Pre-Treatment Underwent Apoptosis.
SSC
Annexin V

How To Apply Gate and Analysis Procedures

Fig. 1

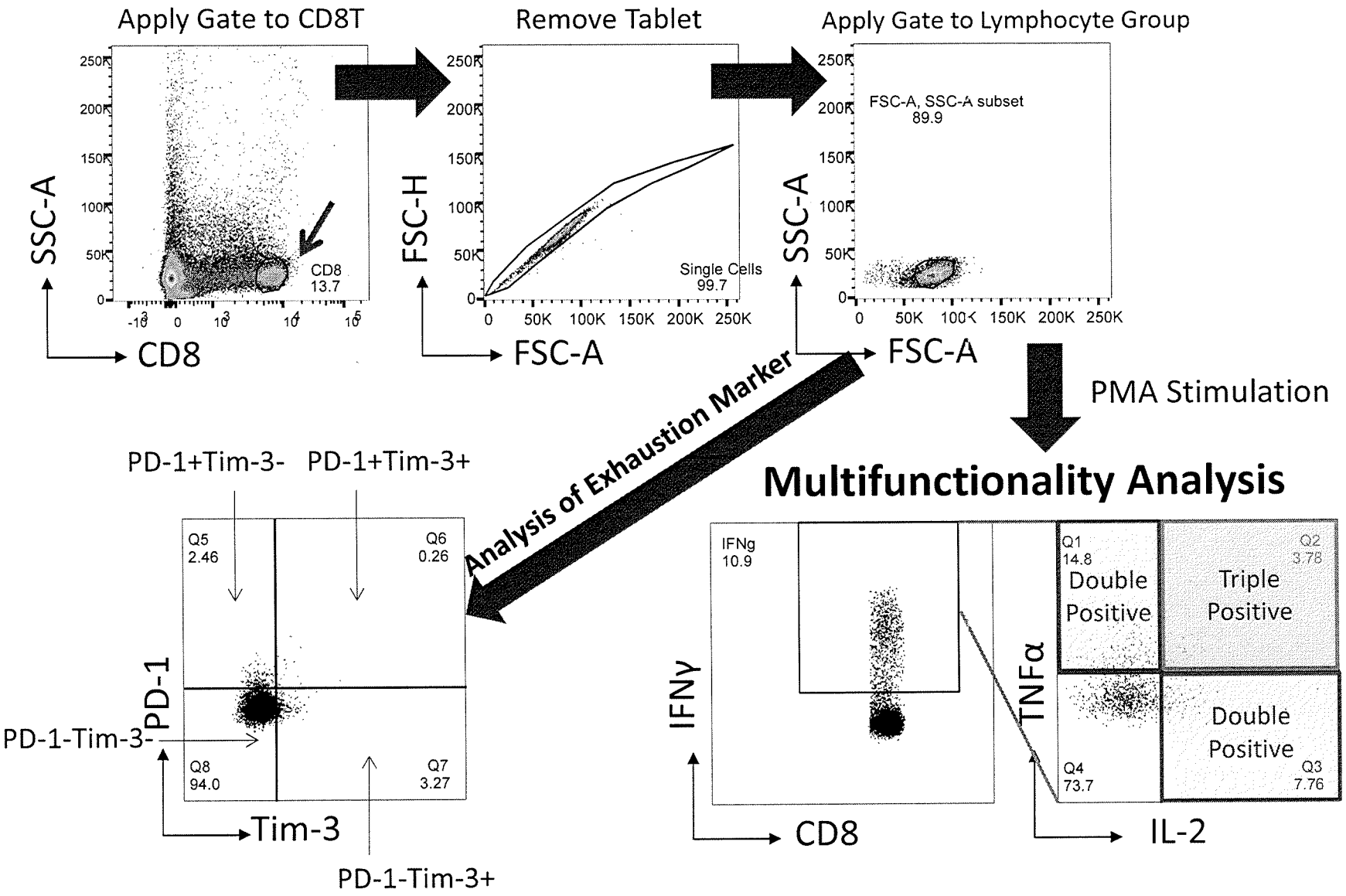
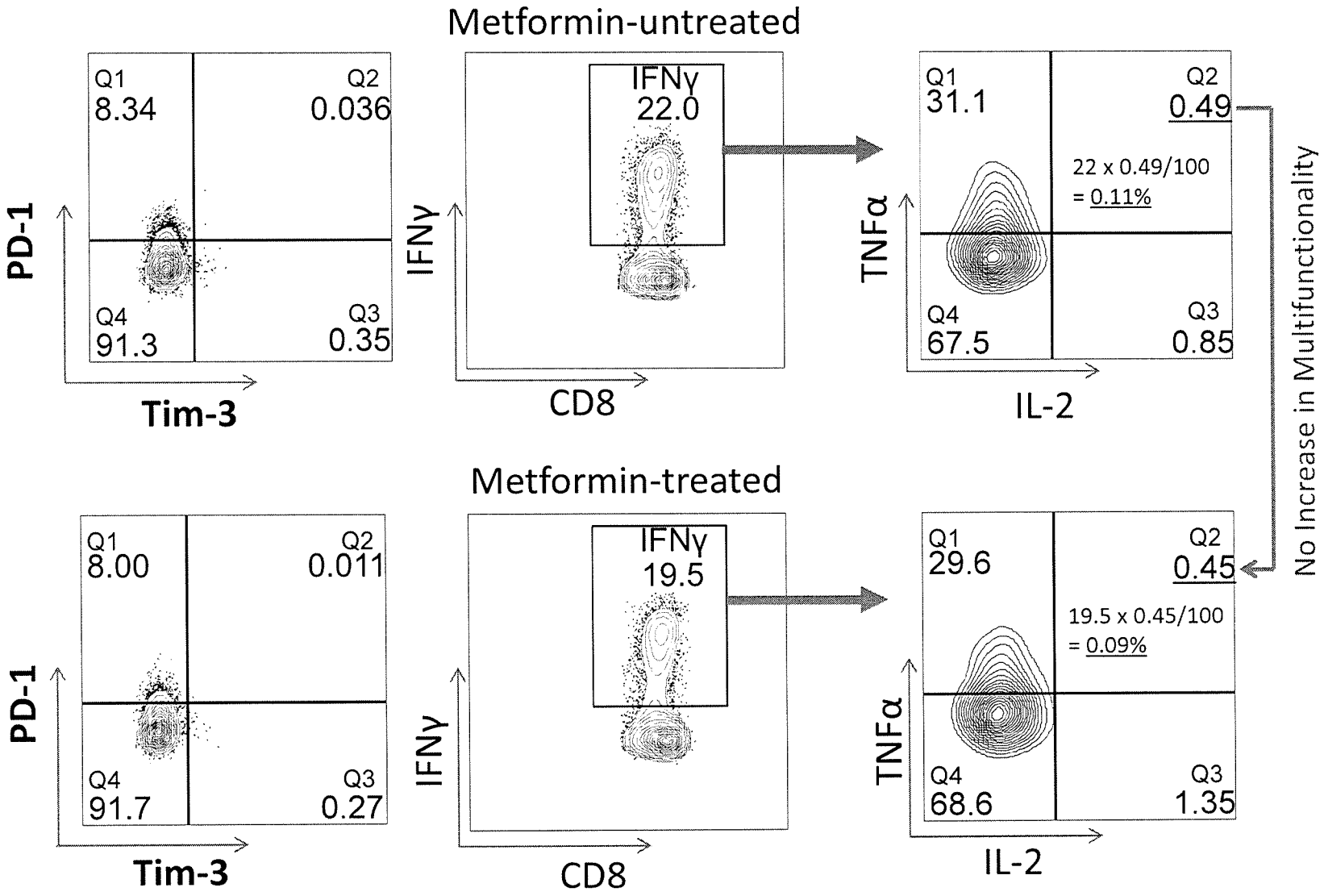
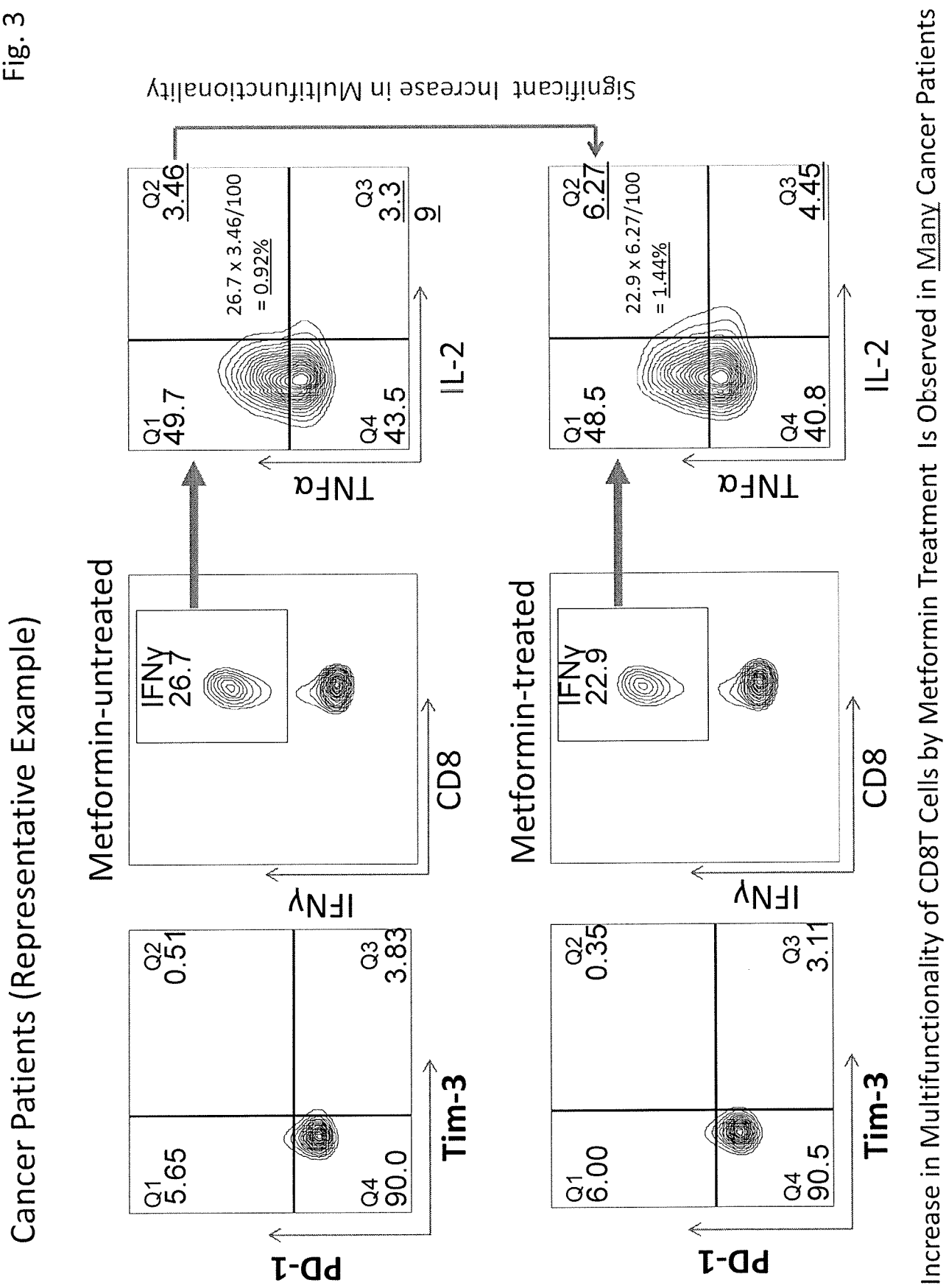


Fig. 2



Increase in Multifunctionality of CD8T Cells by Metformin Treatment Is Observed in Few Healthy Subjects

Fig. 3



Increase in Multifunctionality of CD8T Cells by Metformin Treatment Is Observed in Many Cancer Patients

Fig. 6

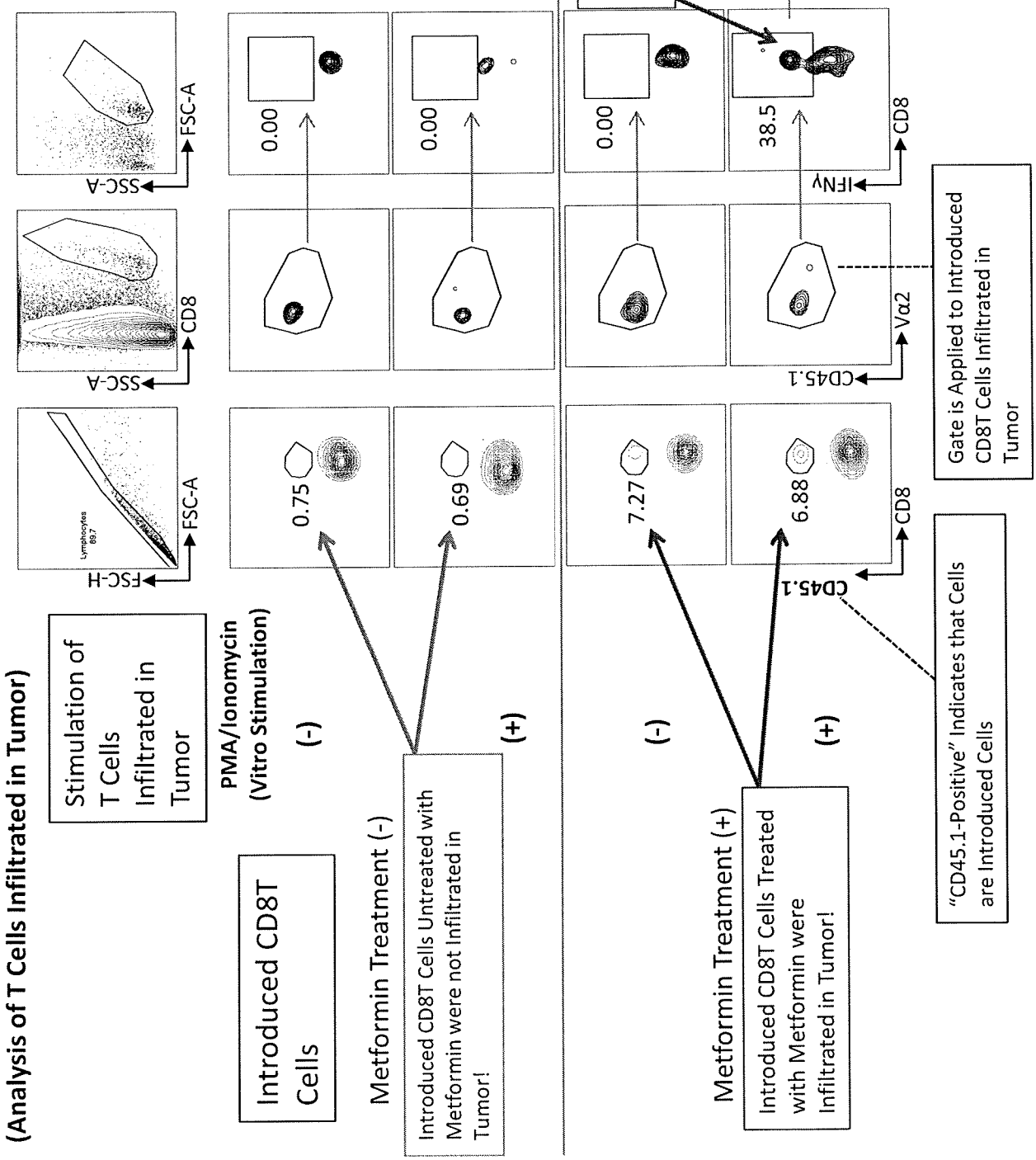
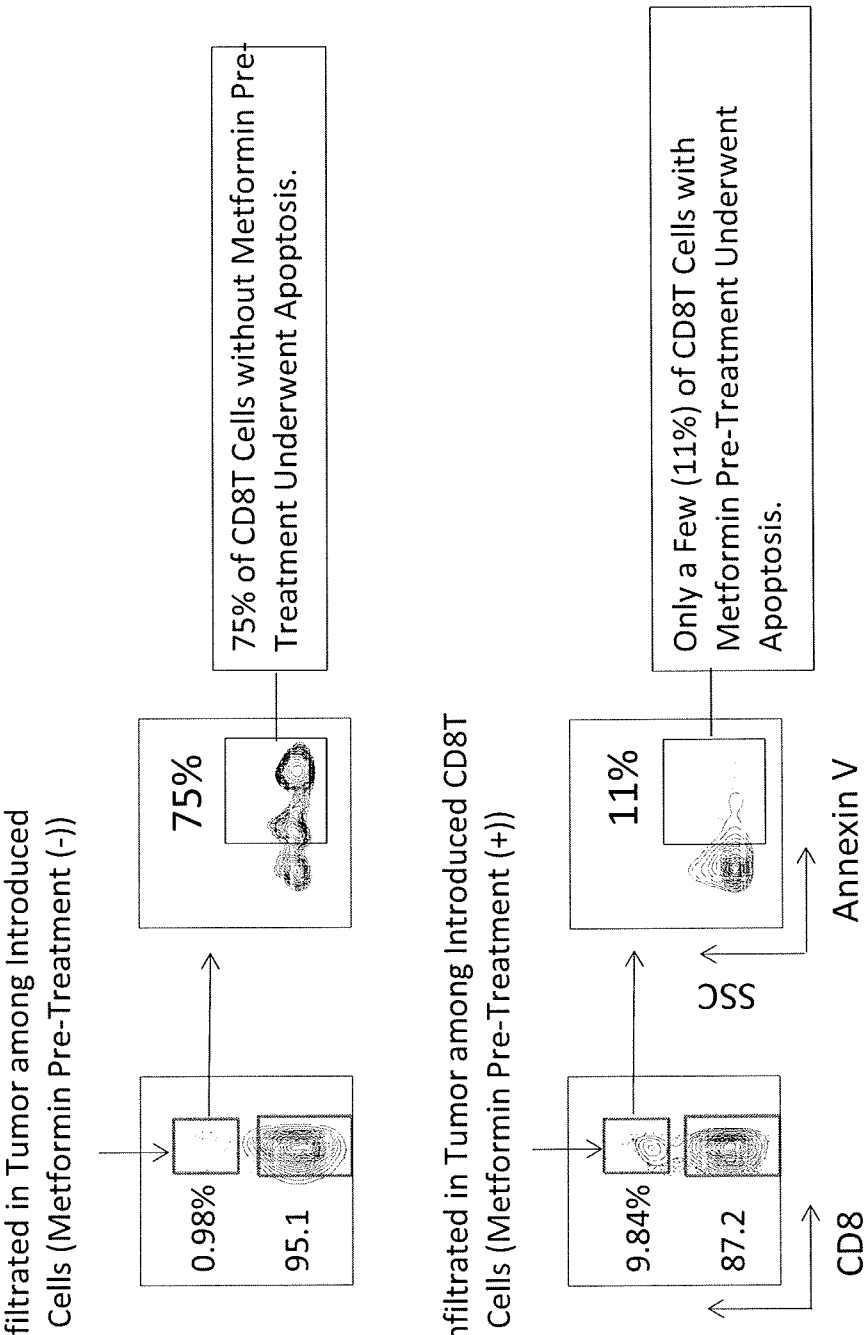

(Analysis of T Cells Infiltrated in Tumor)
Procedures of Cell Gating
Stimulation of T Cells Infiltrated in Tumor
FSC-H
FSC-A
SSC-A
CD8
FSC-A
SSC-A
Introduced CD8T Cells
PMA/Ionomycin (Vitro Stimulation)
(-)
(+)
Metformin Treatment (-)
Introduced CD8T Cells Untreated with Metformin were not Infiltrated in Tumor!
0.75
0.69
0.00
0.00
Metformin Treatment (+)
Introduced CD8T Cells Treated with Metformin were Infiltrated in Tumor!
7.27
6.88
CD45.1
CD8
CD45.1
Vα2
IFNγ
CD8
0.00
38.5
Metformin Treated Introduced CD8T Cells Have Multifunctionality!
TNFα
IL-2
0
0
0
40.0
60.0
"CD45.1-Positive" Indicates that Cells are Introduced Cells
Gate is Applied to Introduced CD8T Cells Infiltrated in Tumor

Fig. 7


Cells Infiltrated in Tumor among Introduced CD8+T Cells (Metformin Pre-Treatment (-))
0.98%
95.1
75%
75% of CD8T Cells without Metformin Pre-Treatment Underwent Apoptosis.
Cells Infiltrated in Tumor among Introduced CD8T Cells (Metformin Pre-Treatment (+))
9.84%
87.2
11%
Only a Few (11%) of CD8T Cells with Metformin Pre-Treatment Underwent Apoptosis.
CD45.1
CD8
SSC
Annexin V