Supplementary figure S6

1D and 2D plots and concentration graphs from Quantasoft for ddPCR on 12 patients

ddPCR; digital droplet PCR, pre; diagnostic plasma sample, post; plasma sample from after surgery, NK; negative control (wild type), NTC; no template control, pos ctrl; positive control (from tissue DNA)

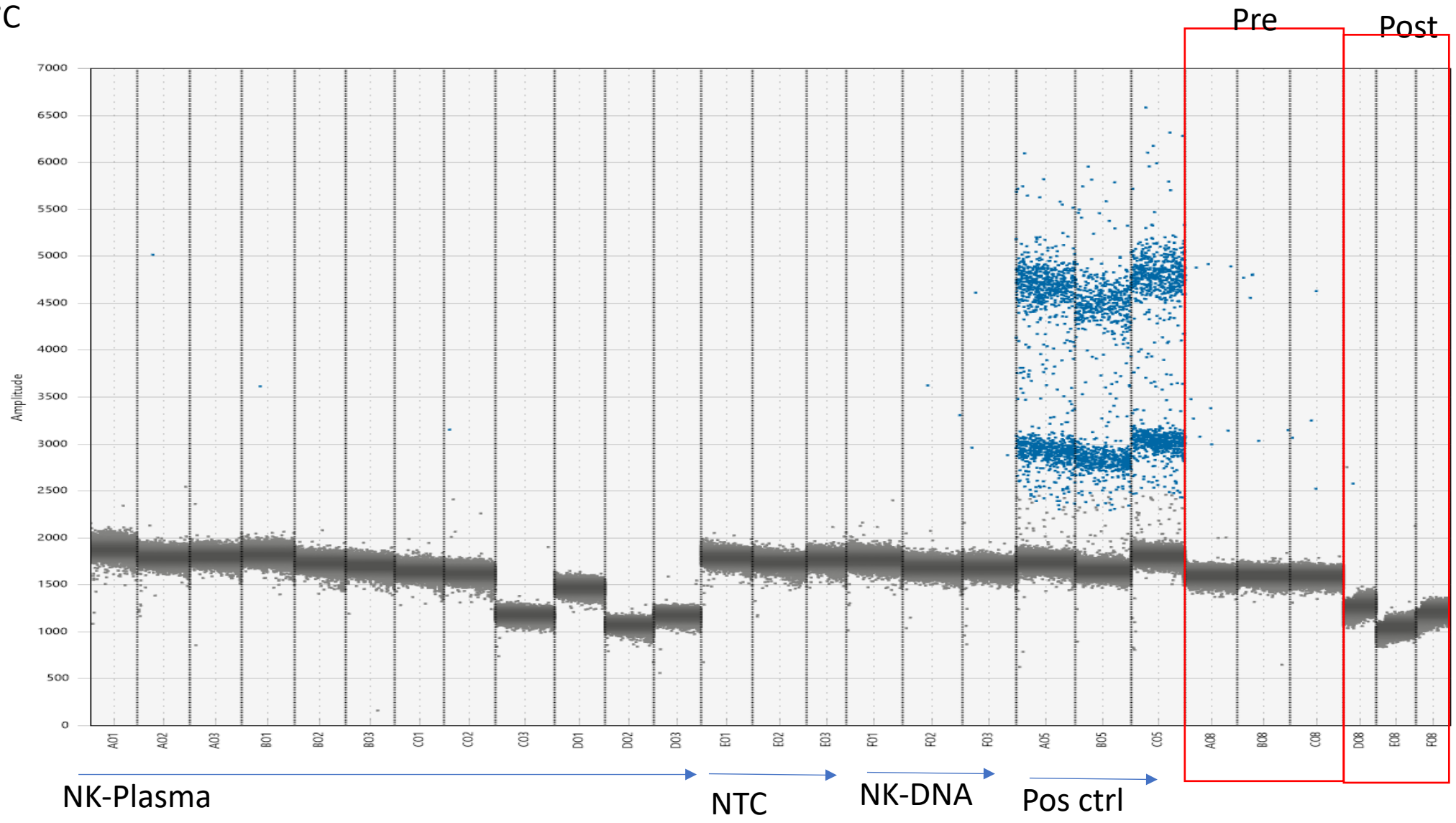
P28 Final- pre and post

Gene targets : TP53,p.R273H, c.818G>A and KRAS p.G13D, c.38G>A

Protocol : Exp 12

Assays: 0.7X TP53,p.R273H mut (FAM) and 1X dark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and 1X KRAS WT p.G13D (HEX)

Annealing temp: 60°C



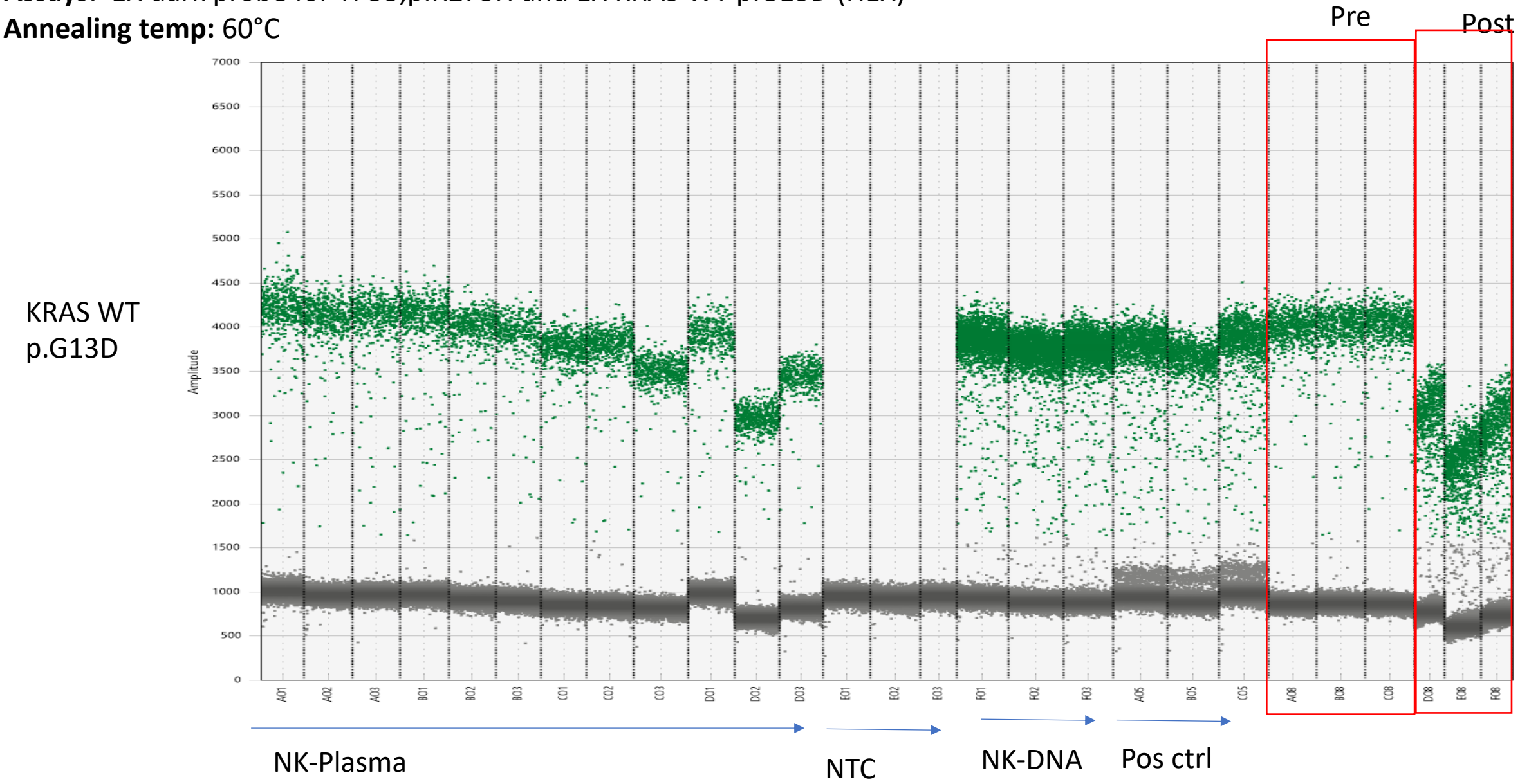
P28 Final- pre and post

Gene targets : TP53,p.R273H, c.818G>A and KRAS p.G13D, c.38G>A

Protocol : Exp 12

Assays: 1X dark probe for TP53,p.R273H and 1X KRAS WT p.G13D (HEX)

Annealing temp: 60°C



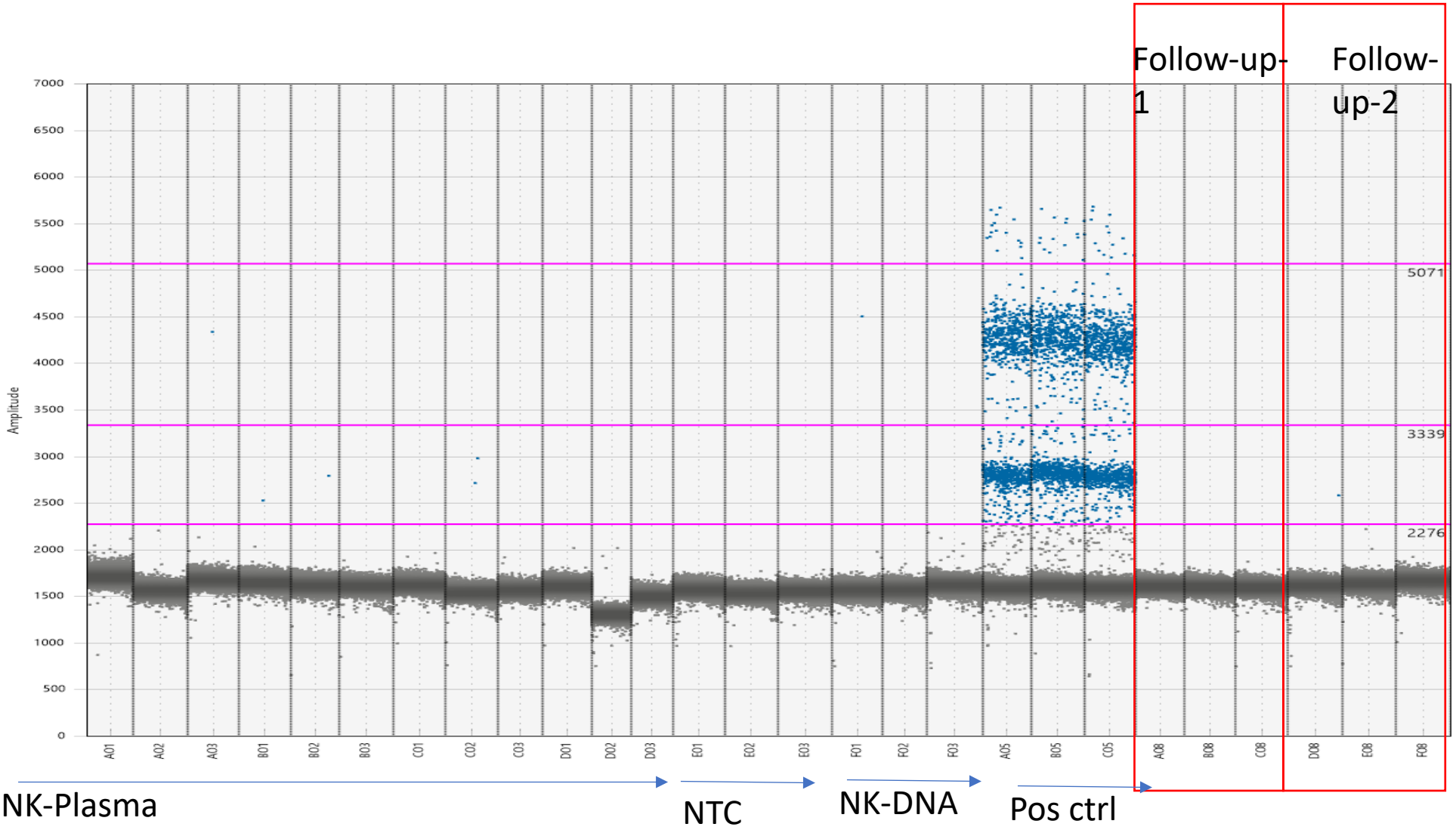
P28 Final- fp1 and fp2

Gene targets : TP53,p.R273H, c.818G>A and KRAS p.G13D, c.38G>A

Assays: 0.7X TP53,p.R273H mut (FAM) and 1X dark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and 1X KRAS WT p.G13D (HEX)

Protocol : Exp 12

Annealing temp: 60°C



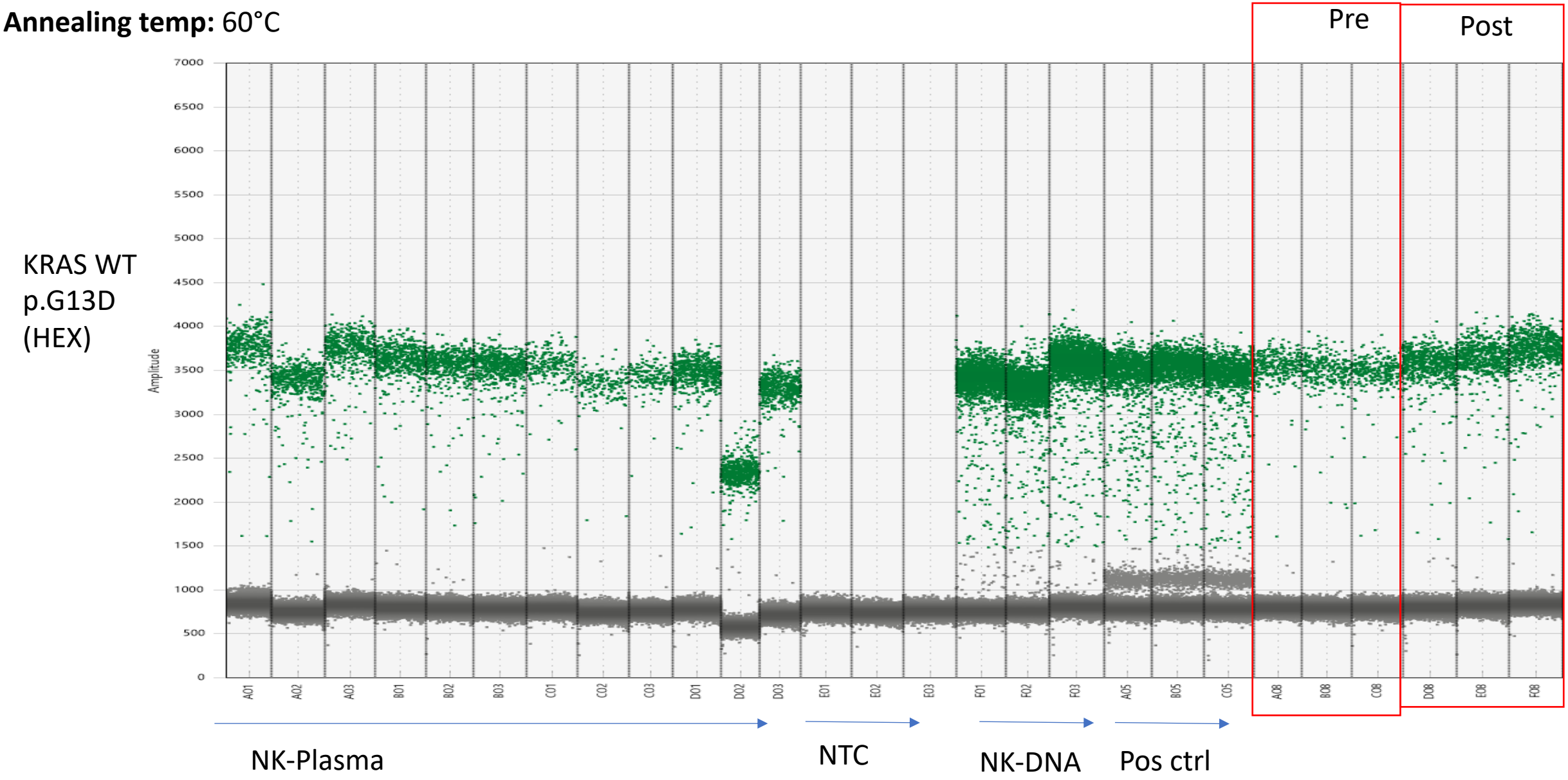
P28 Final- fp1 and fp2- WT

Gene targets : TP53,p.R273H, c.818G>A and KRAS p.G13D, c.38G>A

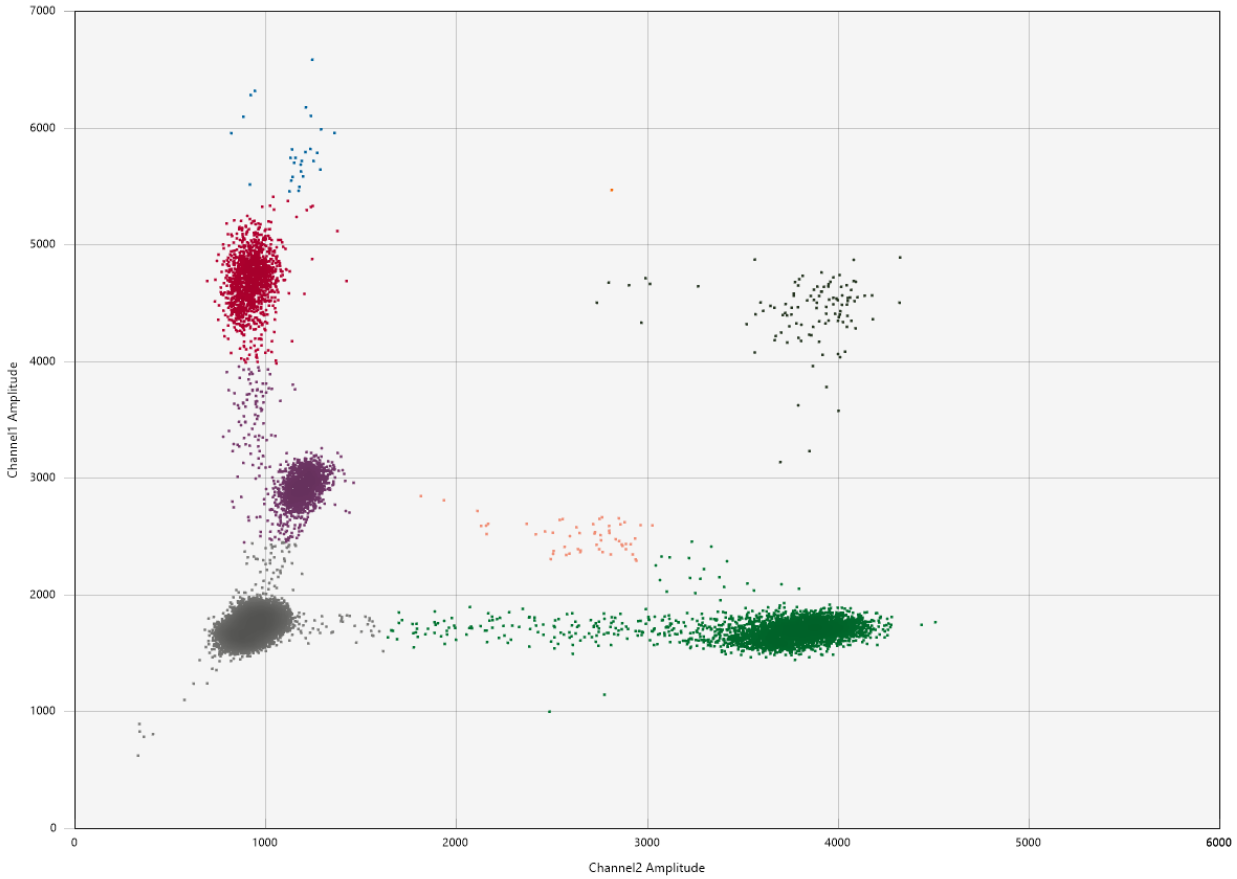
Assays: 1X dark probe for TP53,p.R273H and 1X KRAS WT p.G13D (HEX)

Protocol : Exp 12

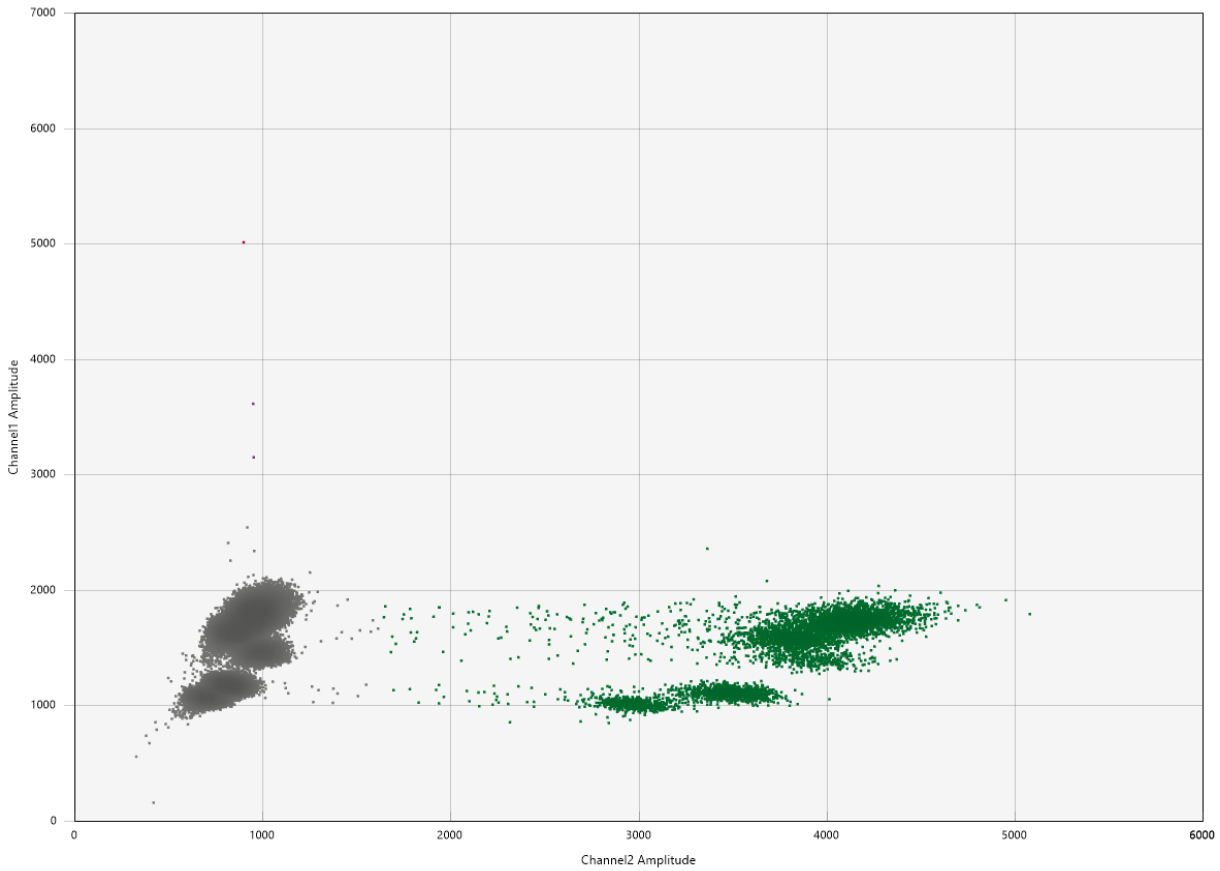
Annealing temp: 60°C



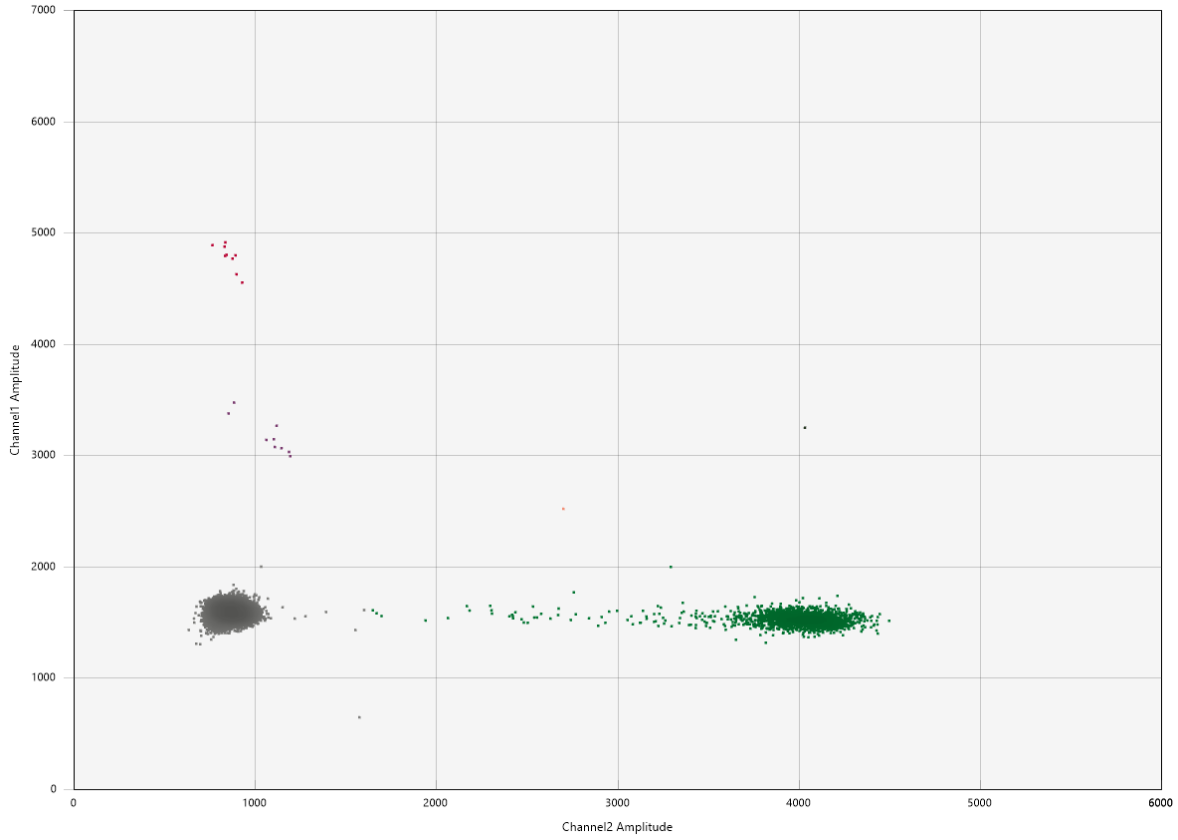
Positive – control



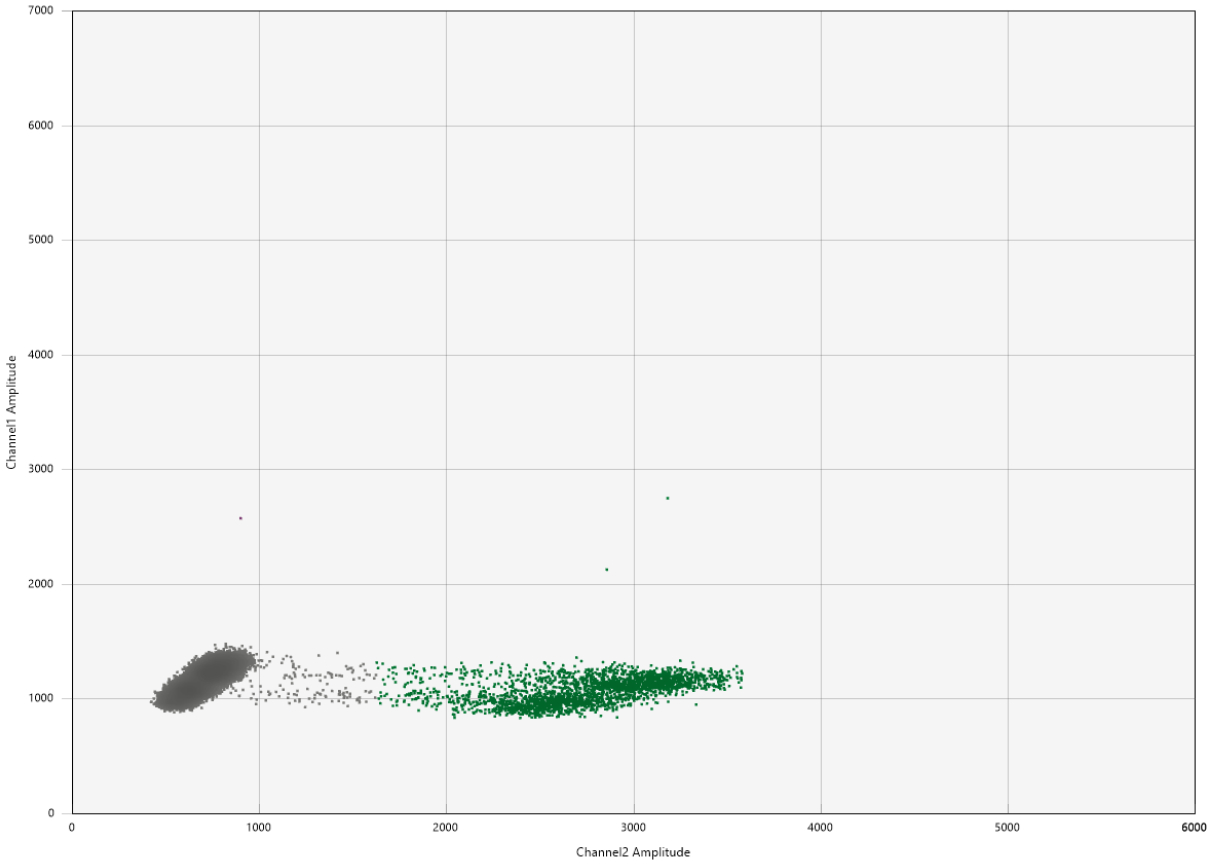
NK-Plasma



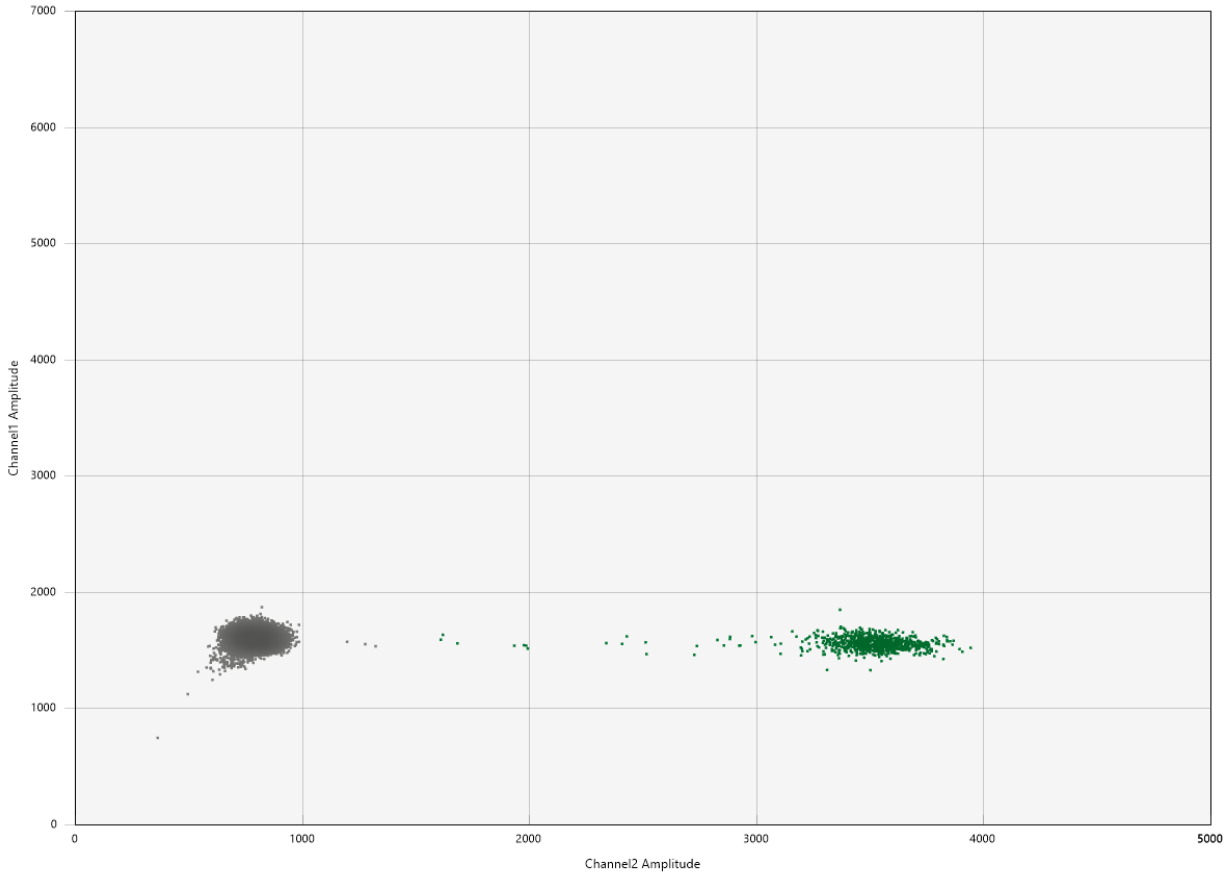
Pre-surgery



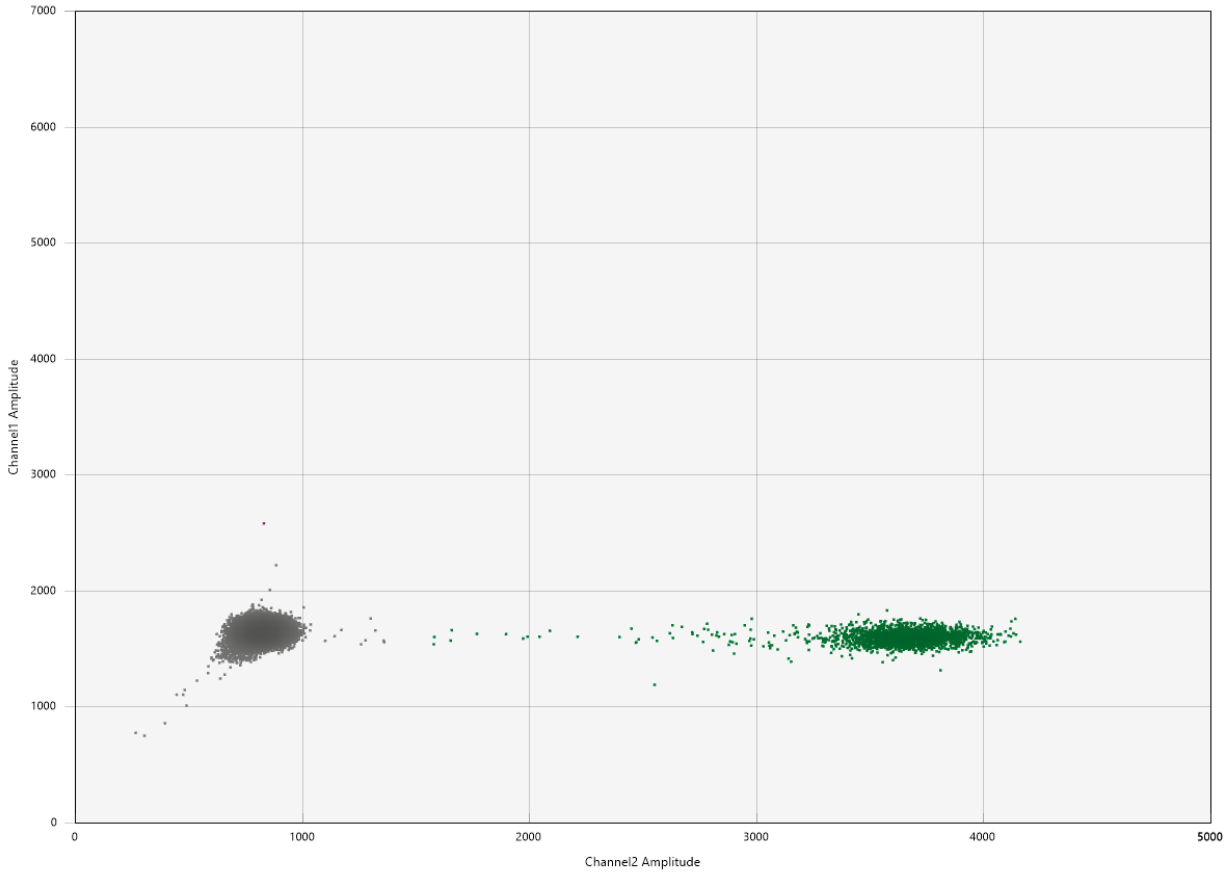
Post-surgery



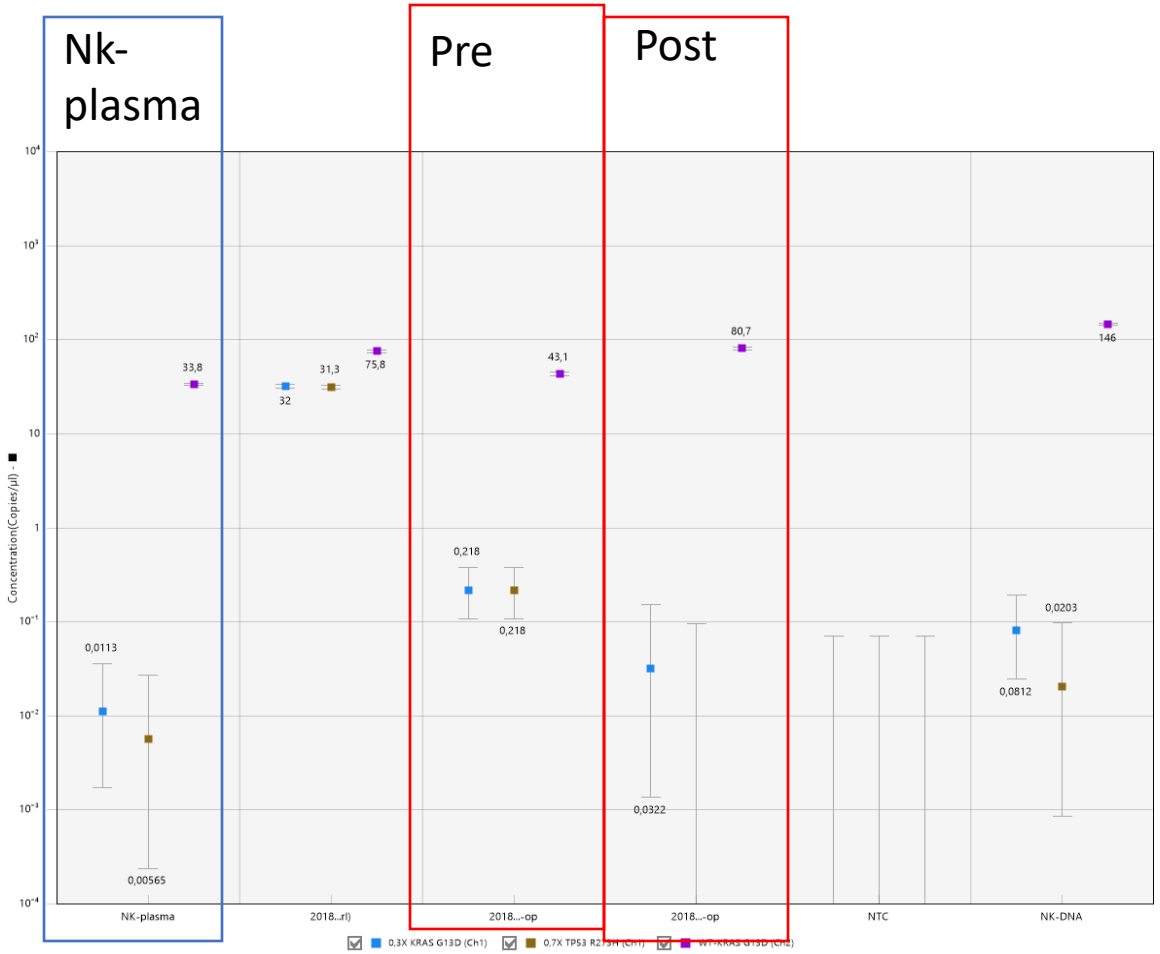
Follow-up 1

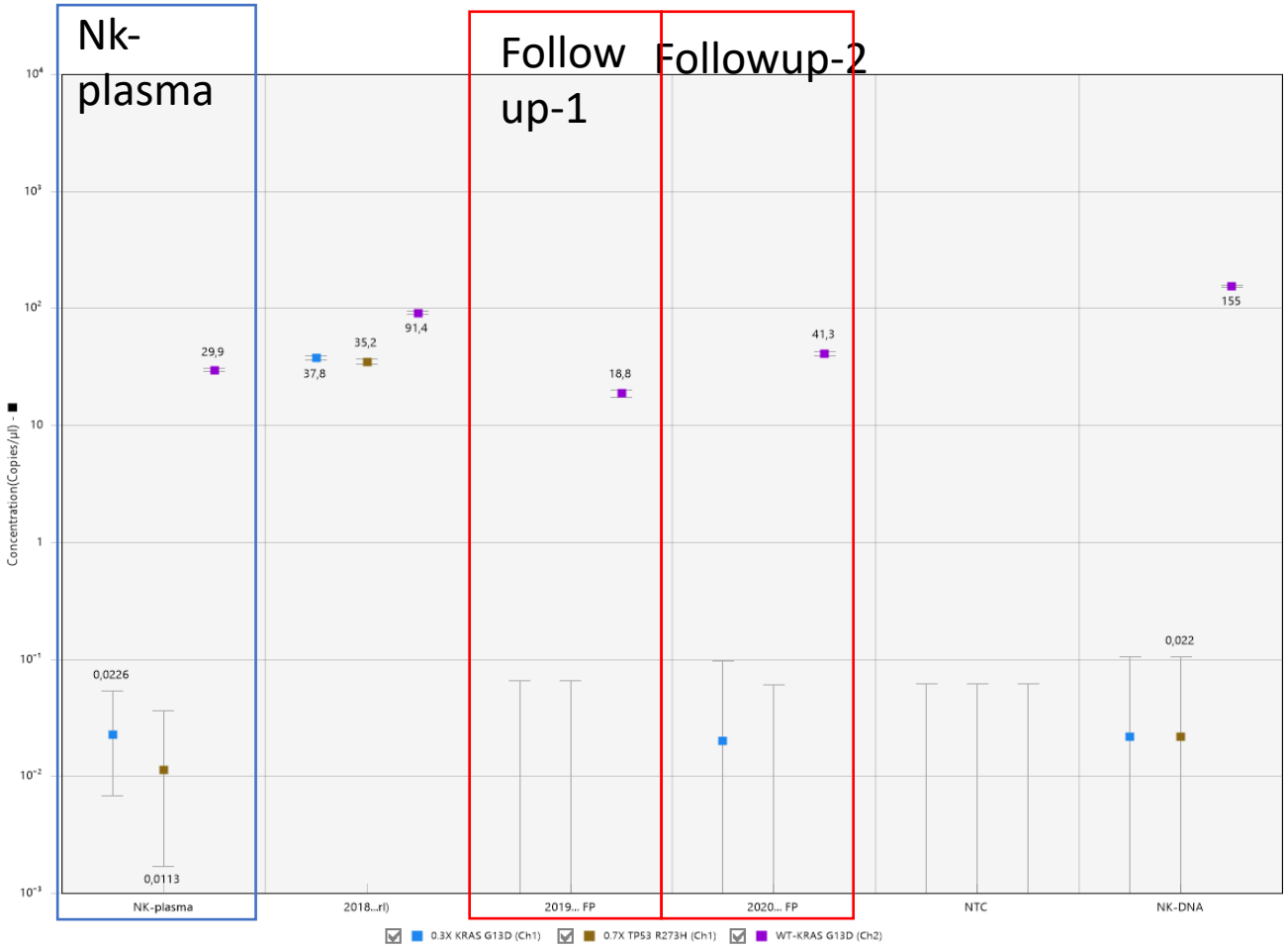


Follow-up 2



Concentration plots





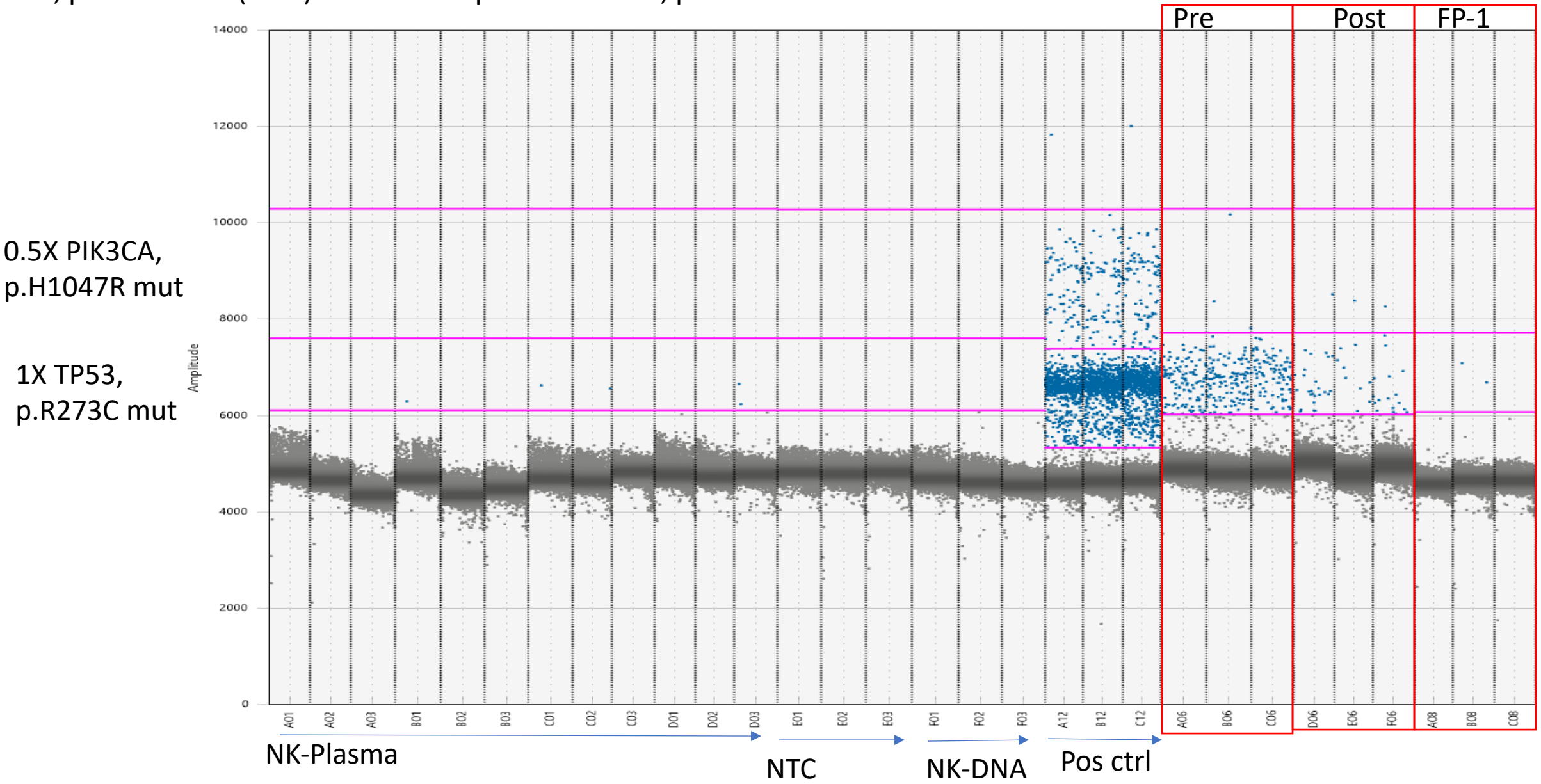
P27 - Final

Gene targets : PIK3CA, p.H1047R,c.3140A>G and TP53, p.R273C, c.817C>T

Annealing temp: 55°C

Protocol: Exp 21

Assays: 0.5X PIK3CA, p.H1047R mut (FAM) and 1X PIK3CA WT, p.H1047R (HEX)
1X TP53, p.R273C mut (FAM) and 1X dark probe for TP53, p.R273C



P27 - Final -WT

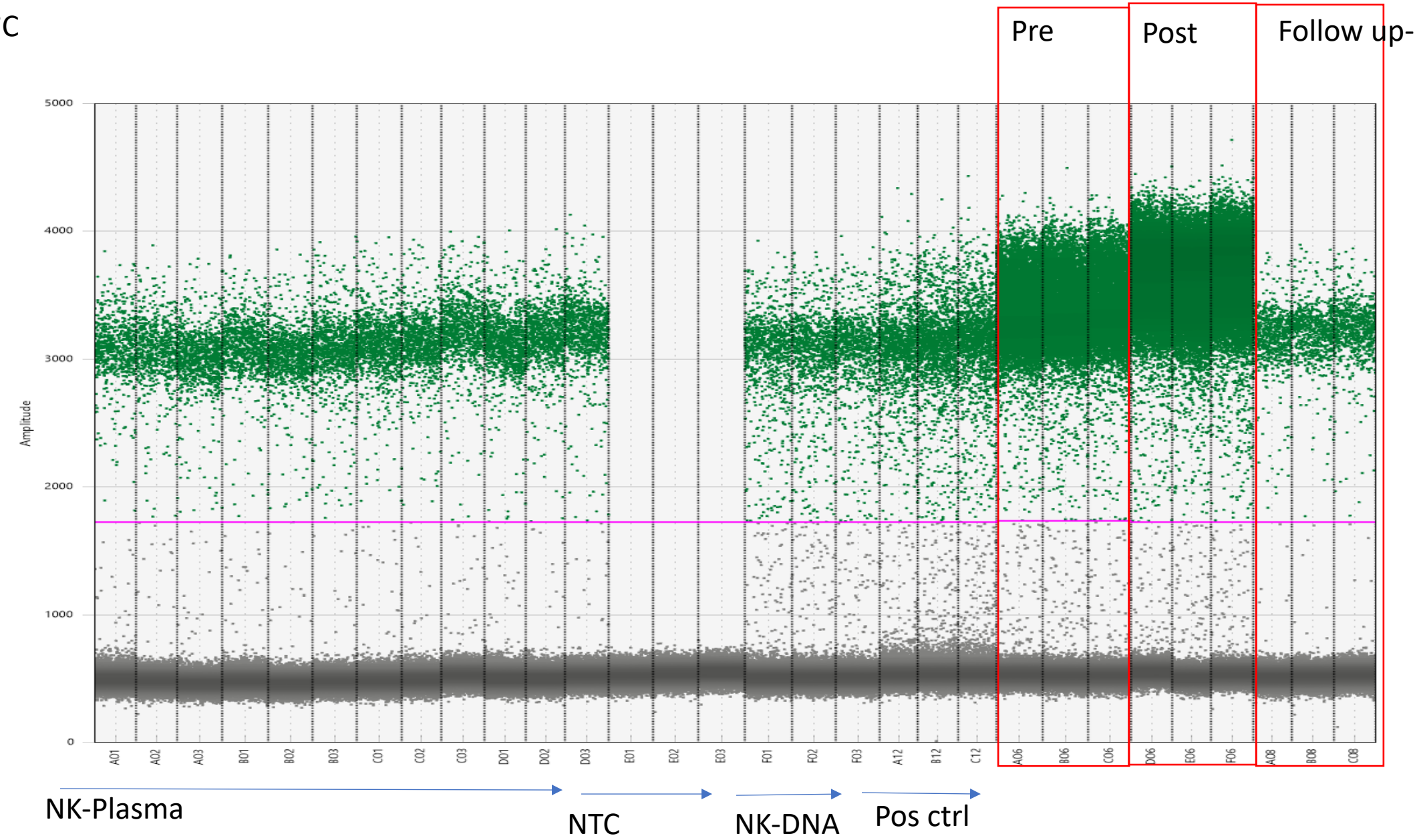
Gene targets : PIK3CA, p.H1047R,c.3140A>G and TP53, p.R273C, c.817C>T

Assays: 1X PIK3CA WT, p.H1047R (HEX) and 1X dark probe for TP53, p.R273C

Annealing temp: 55°C

Protocol: Exp 21

0.5X PIK3CA WT, p.H1047R

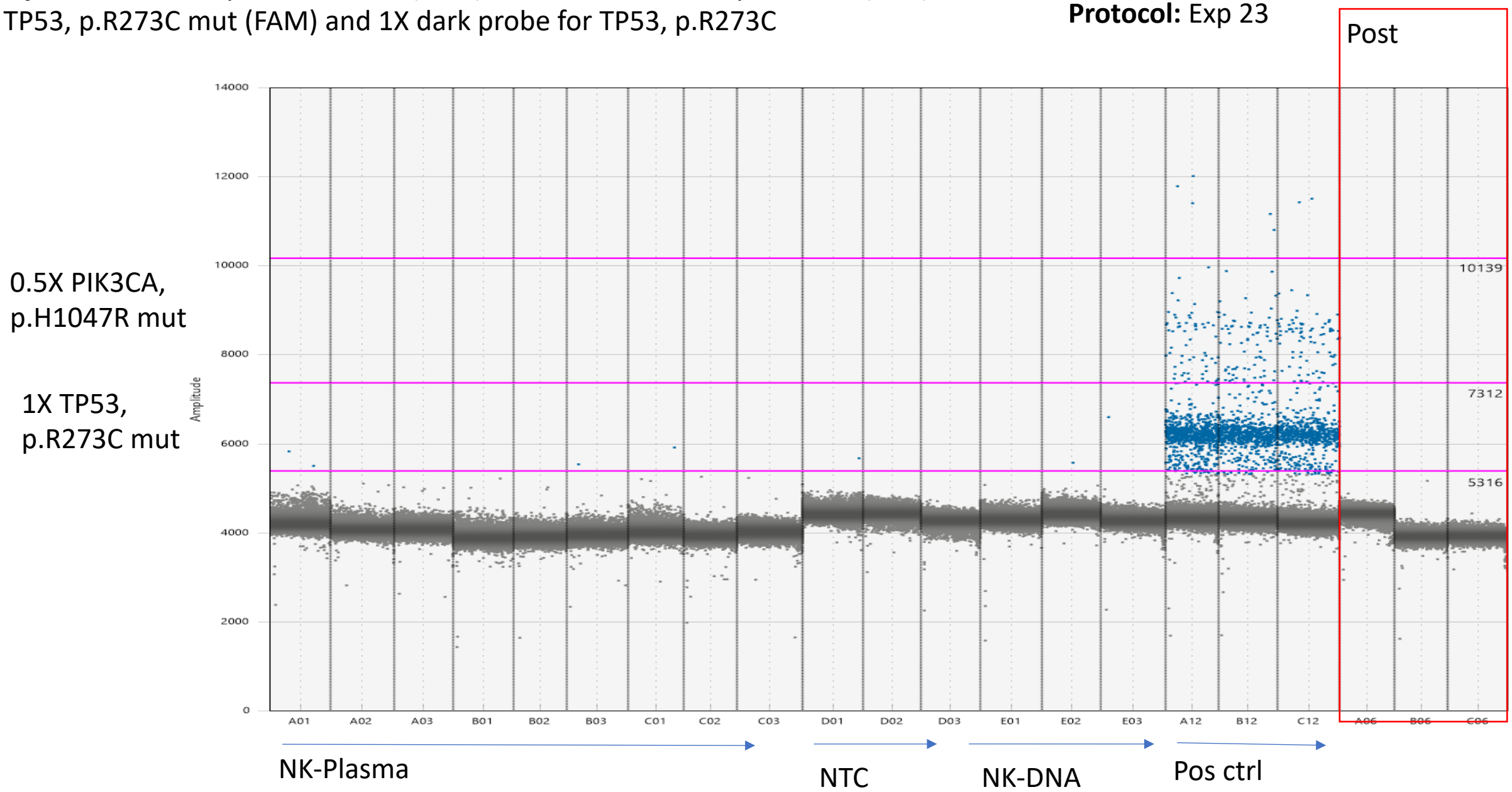


P27 - Final diluted

Gene targets : PIK3CA, p.H1047R,c.3140A>G and TP53, p.R273C, c.817C>T

Assays: 0.5X PIK3CA, p.H1047R mut (FAM) and 1X PIK3CA WT, p.H1047R (HEX)
1X TP53, p.R273C mut (FAM) and 1X dark probe for TP53, p.R273C

Annealing temp: 55°C
Protocol: Exp 23



P27 - Final diluted -WT

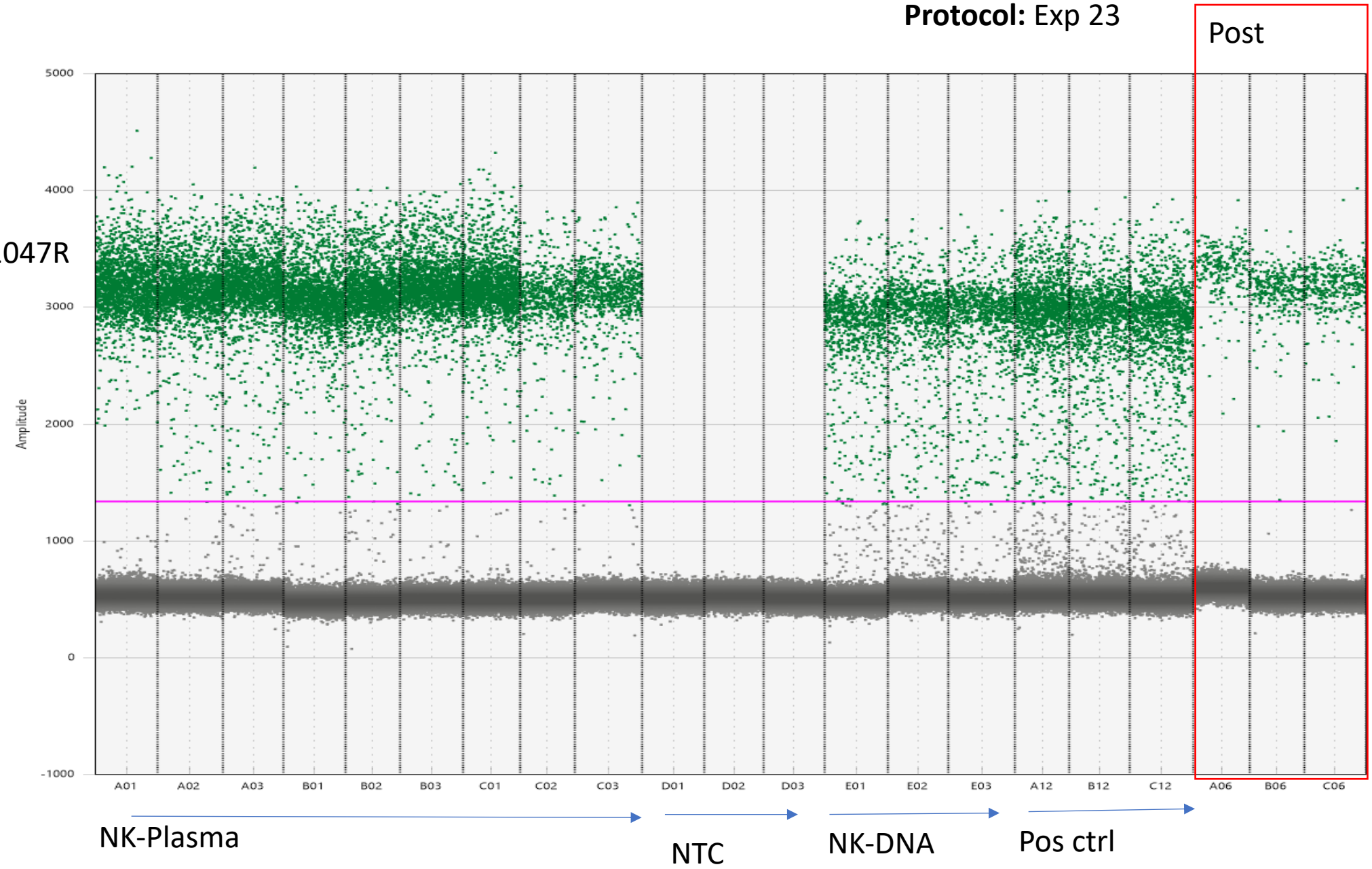
Gene targets : PIK3CA, p.H1047R,c.3140A>G and TP53, p.R273C, c.817C>T

Assays: 1X PIK3CA WT, p.H1047R (HEX) and 1X dark probe for TP53, p.R273C

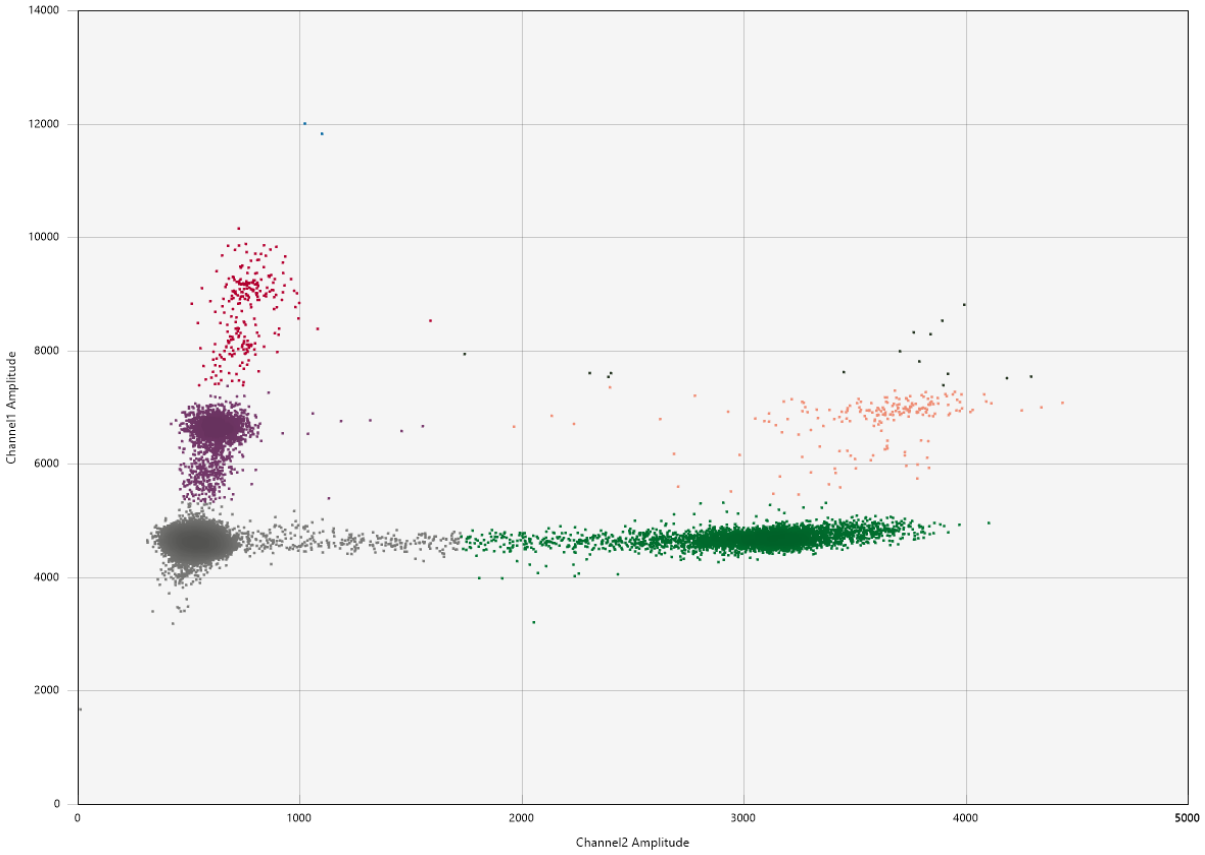
Annealing temp: 55°C

Protocol: Exp 23

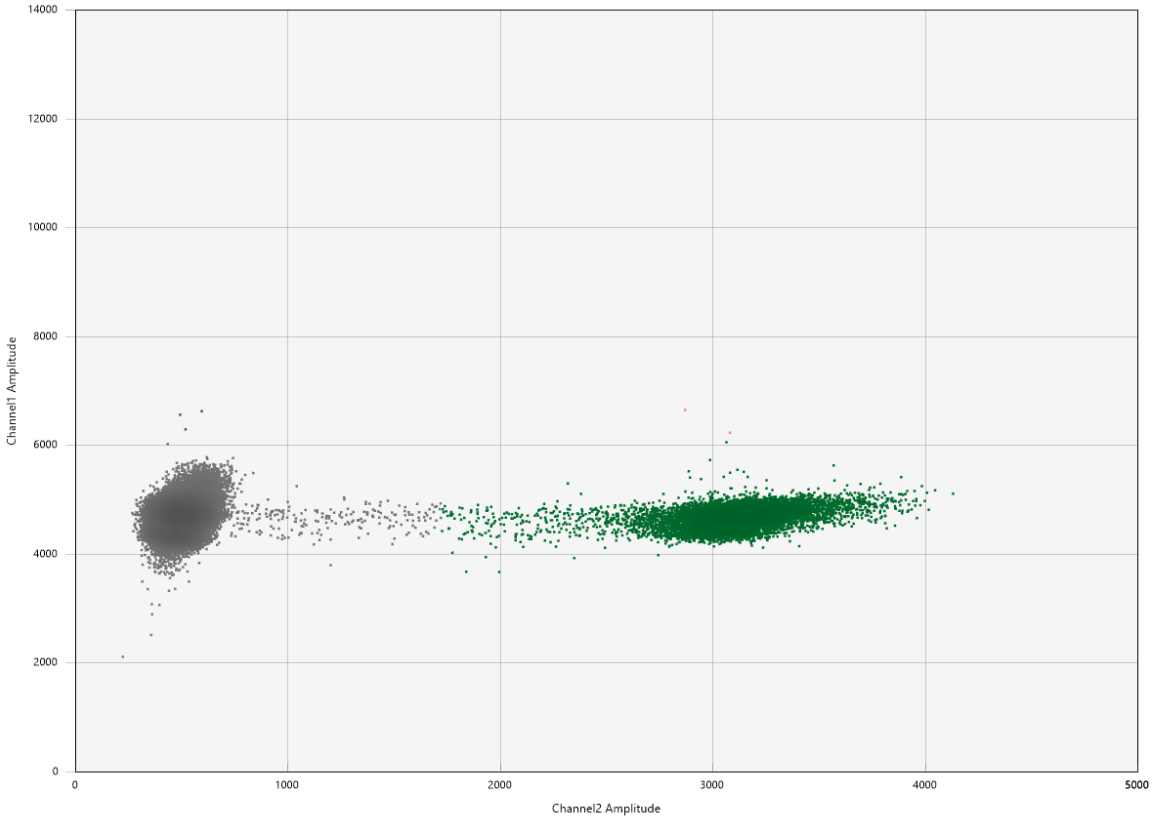
0.5X PIK3CA WT, p.H1047R



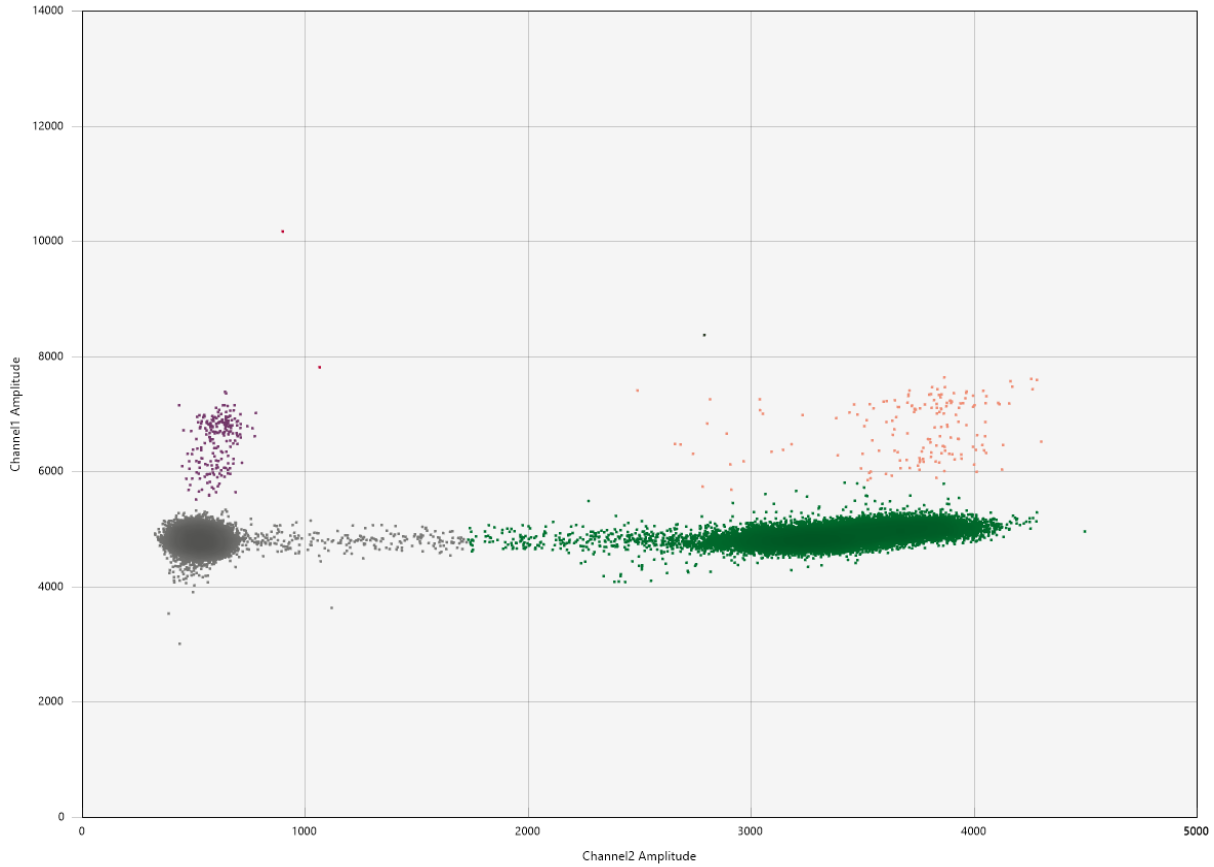
Positive – control



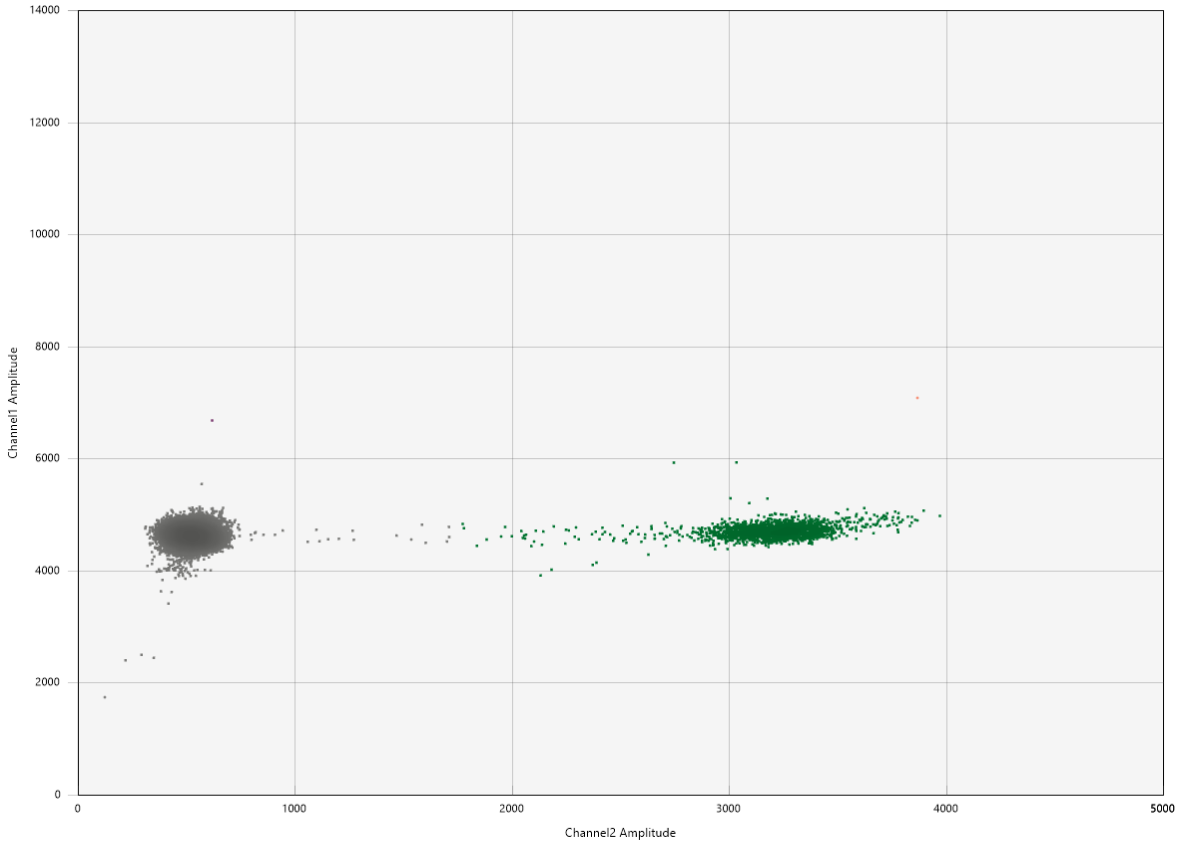
NK-Plasma



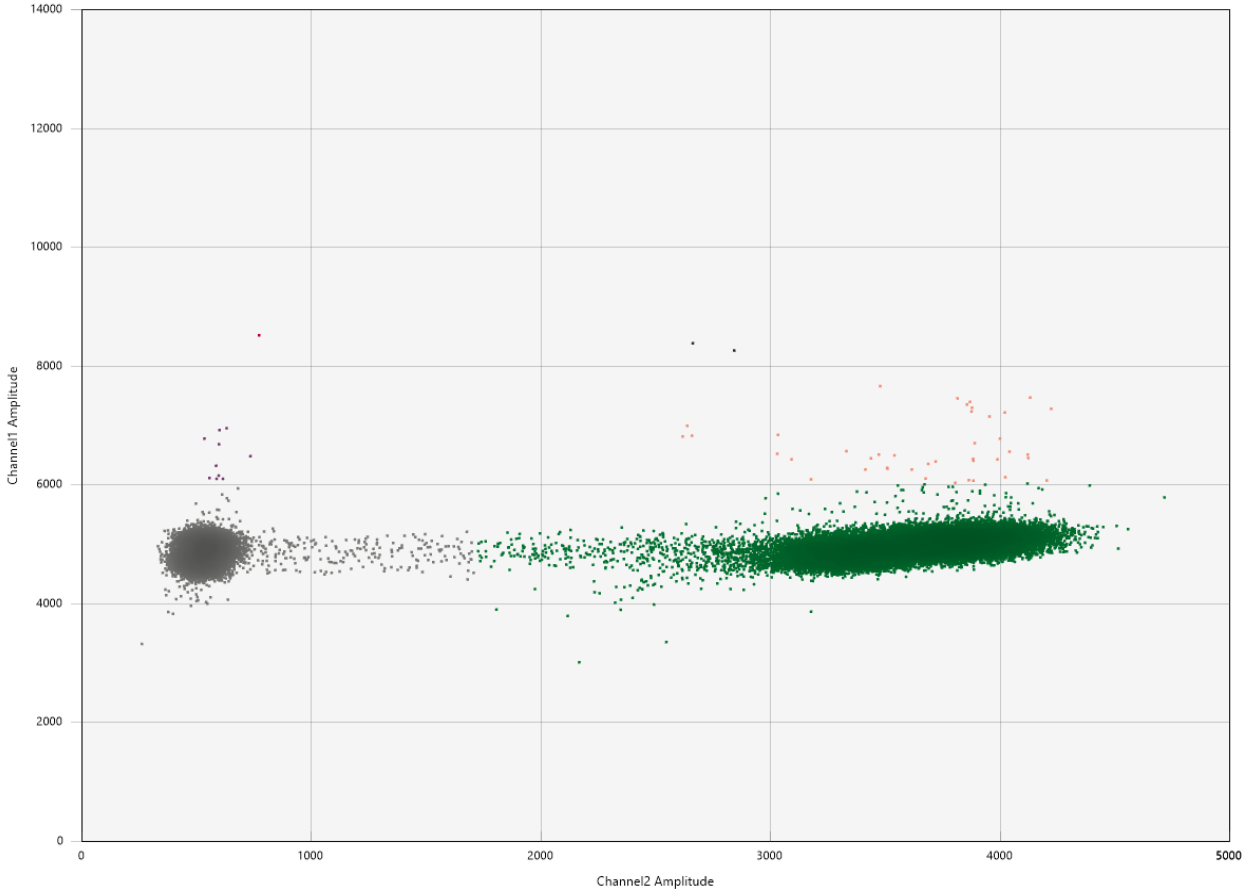
Pre-surgery



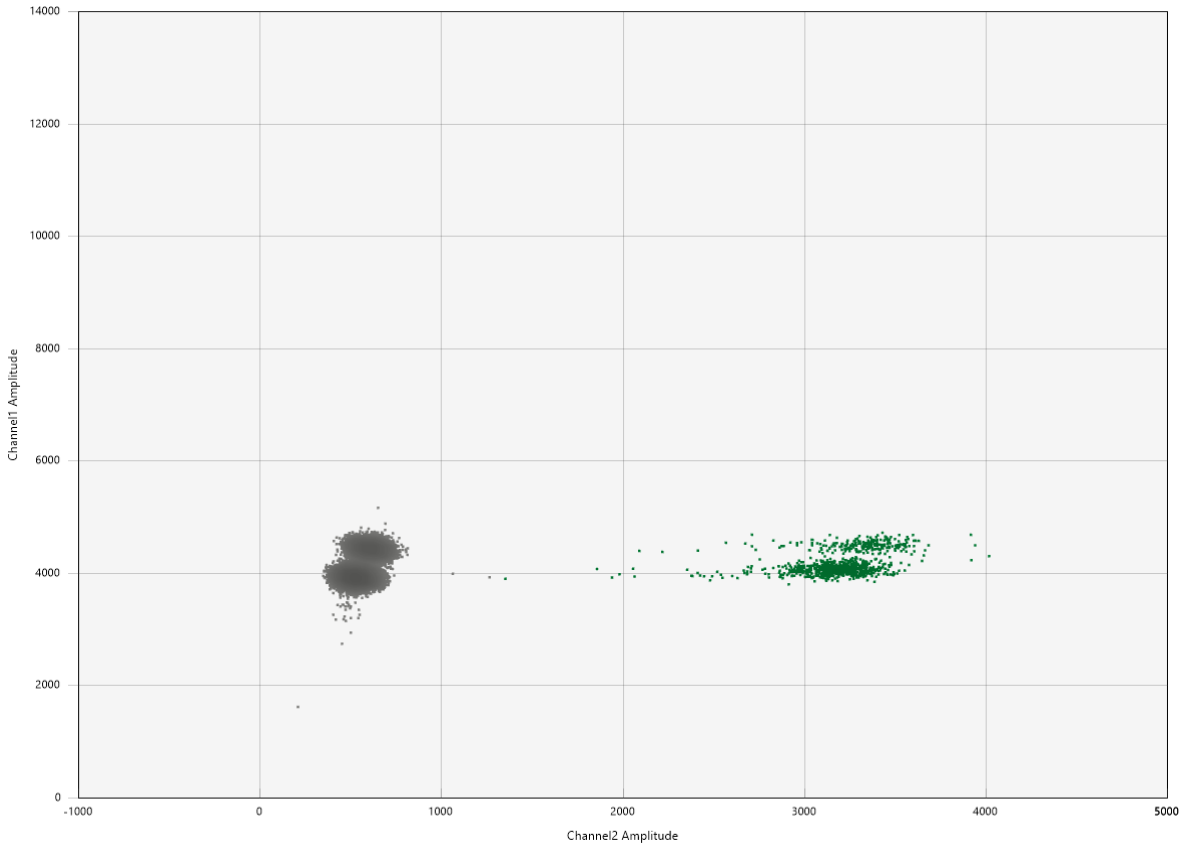
Follow-up 1



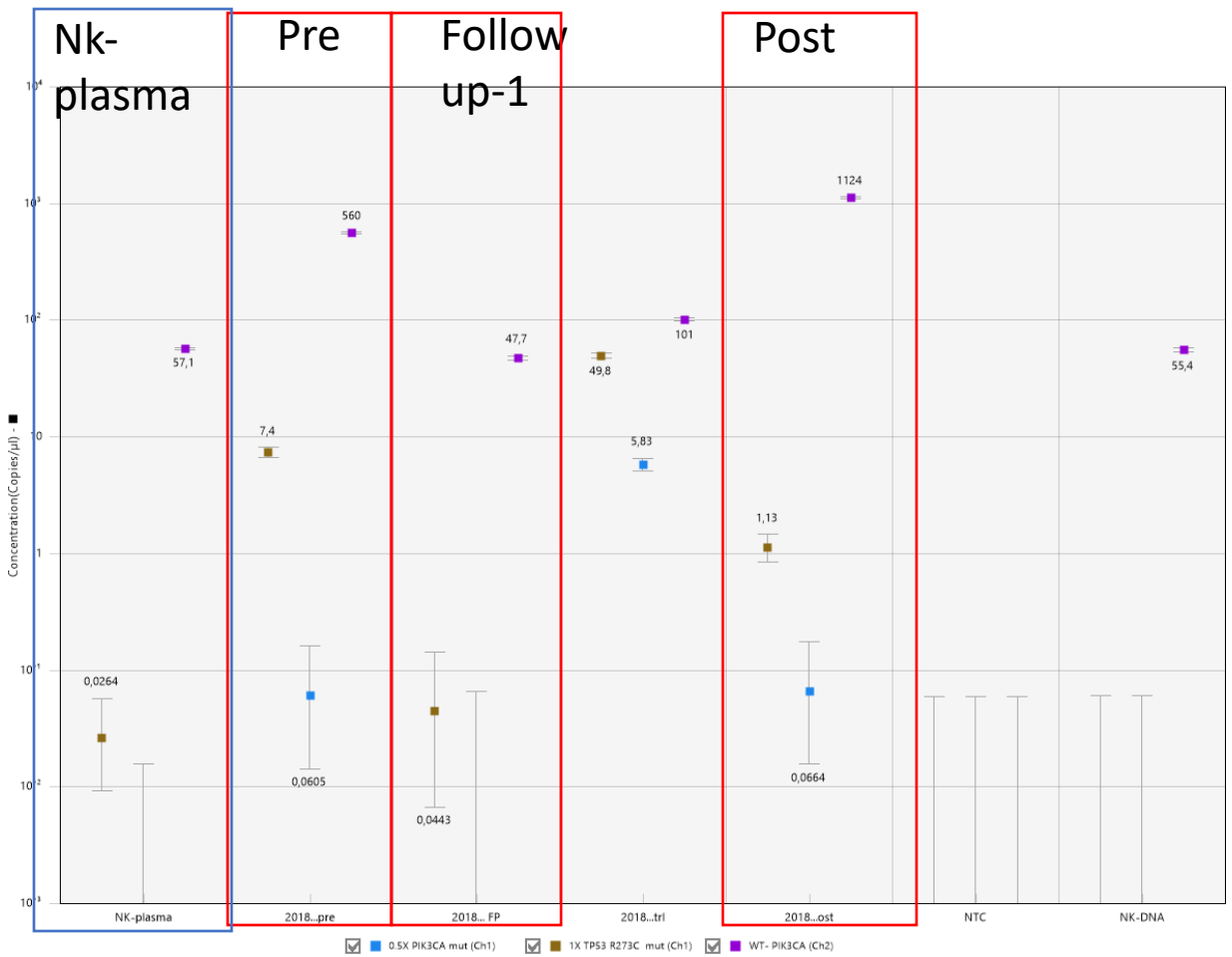
Post-surgery



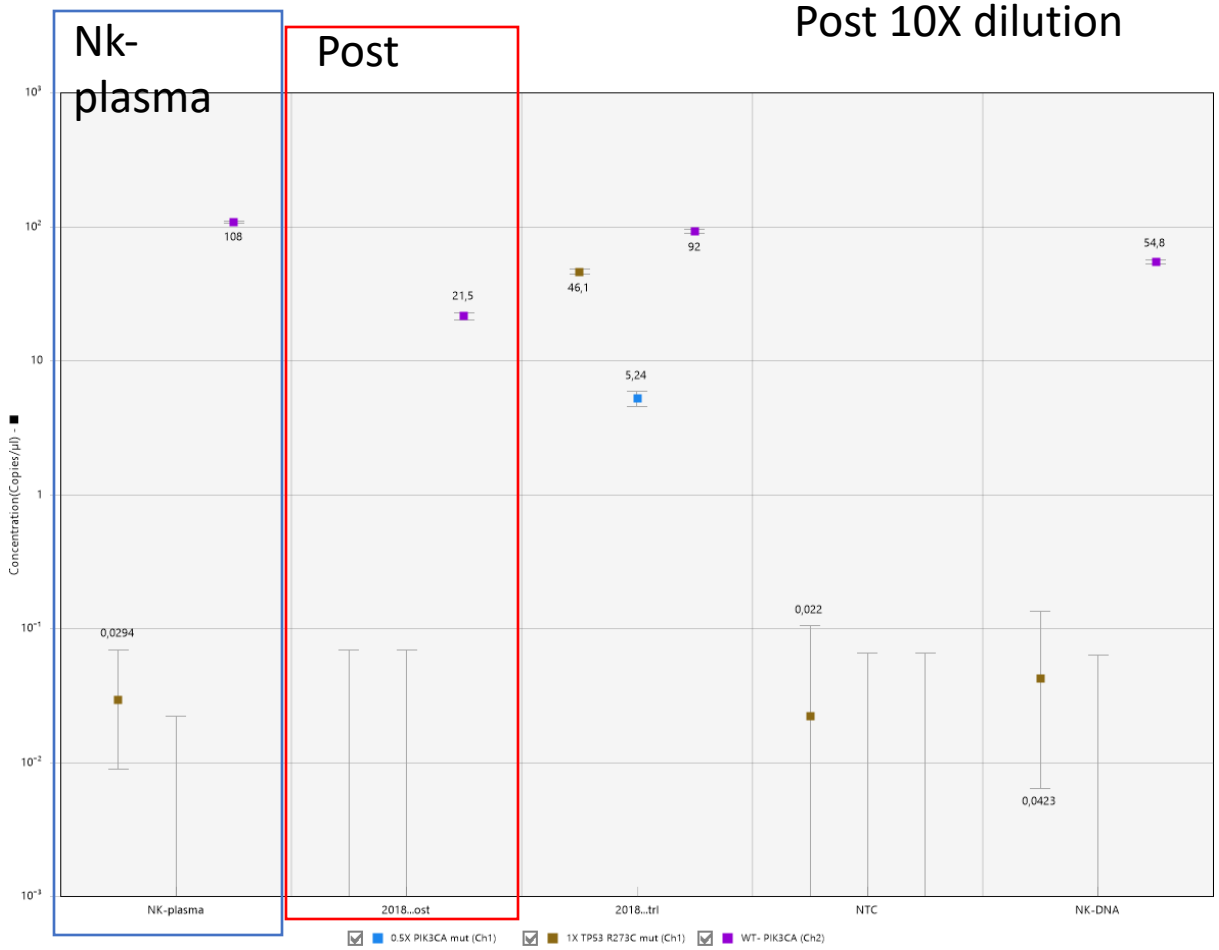
Post-surgery diluted



Concentration plots Pre, post and follow-up



Post 10X dilution



Calling – Pre-op- positive for –PIK3CA and TP53 R273C
Post-op- positive for PIK3CA and TP53 R273C
Follow-up- negative for PIK3CA and TP53 R273C

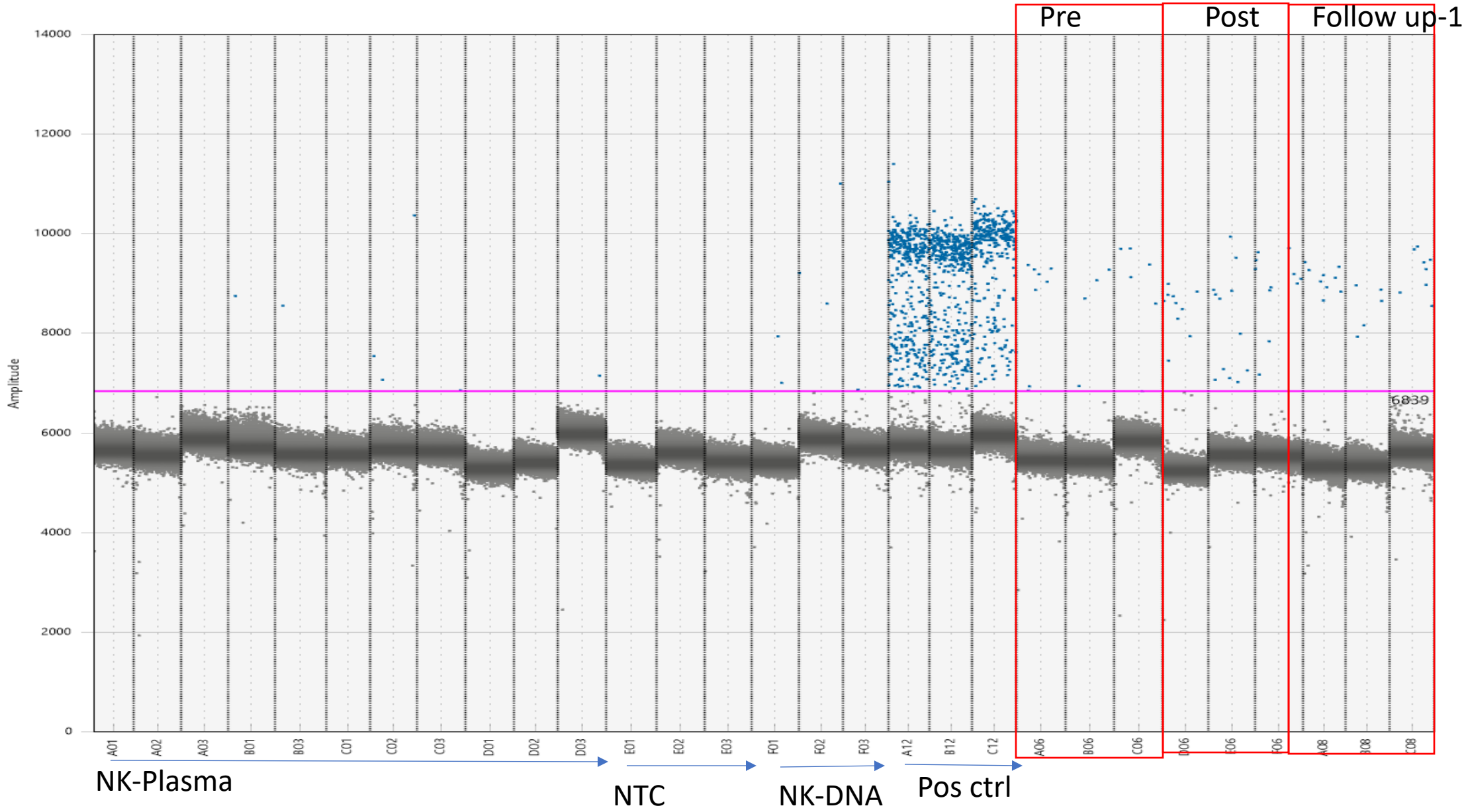
Calling – After dilution
Post-op- negative for PIK3CA and TP53 R273C

P43 - Final

Gene target : KRAS, p.G12D, c.35G>A

Assays: 1X KRAS, p.G12D mut (FAM) and 1X KRAS WT, p.G12D (HEX)

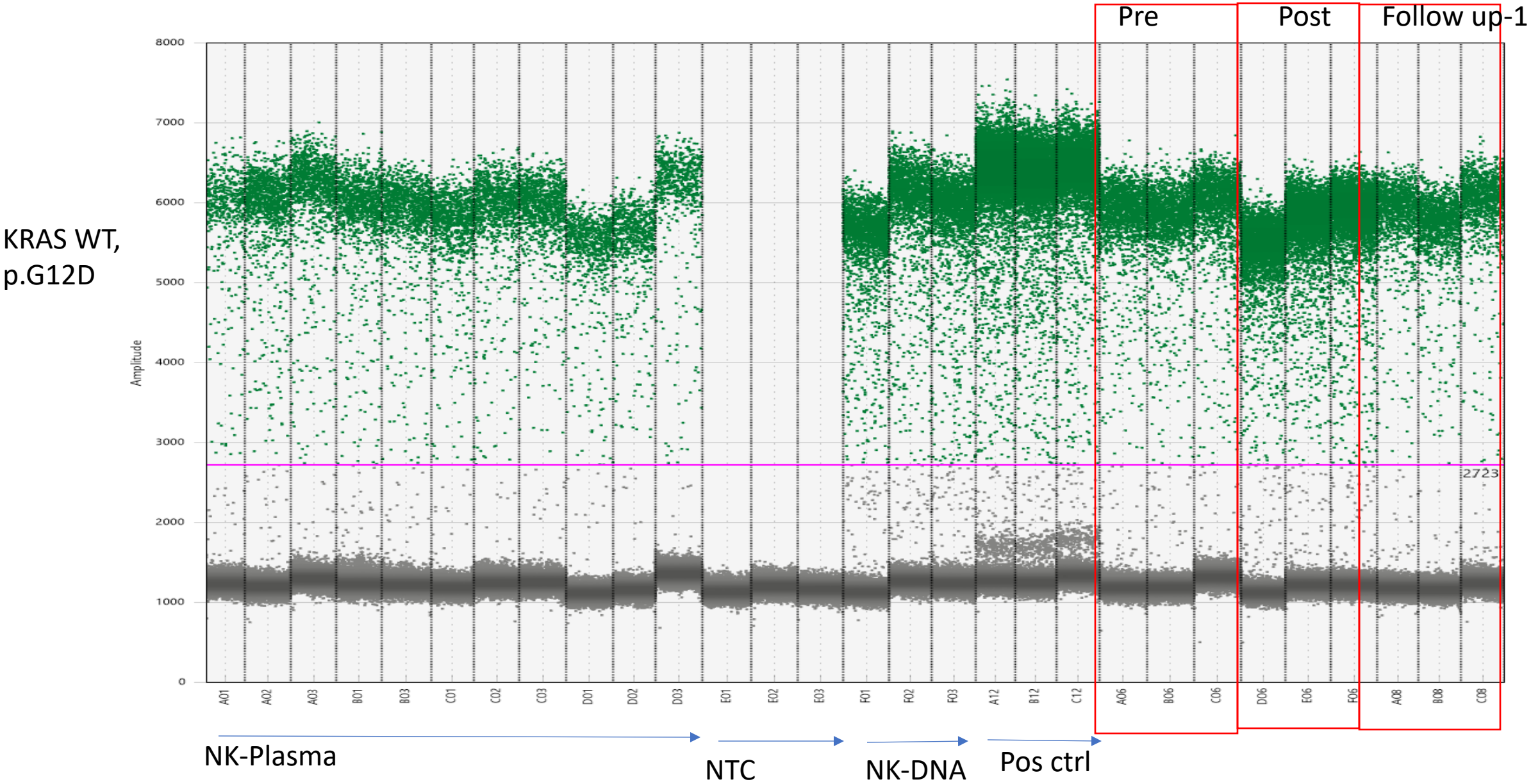
KRAS, p.G12D mut



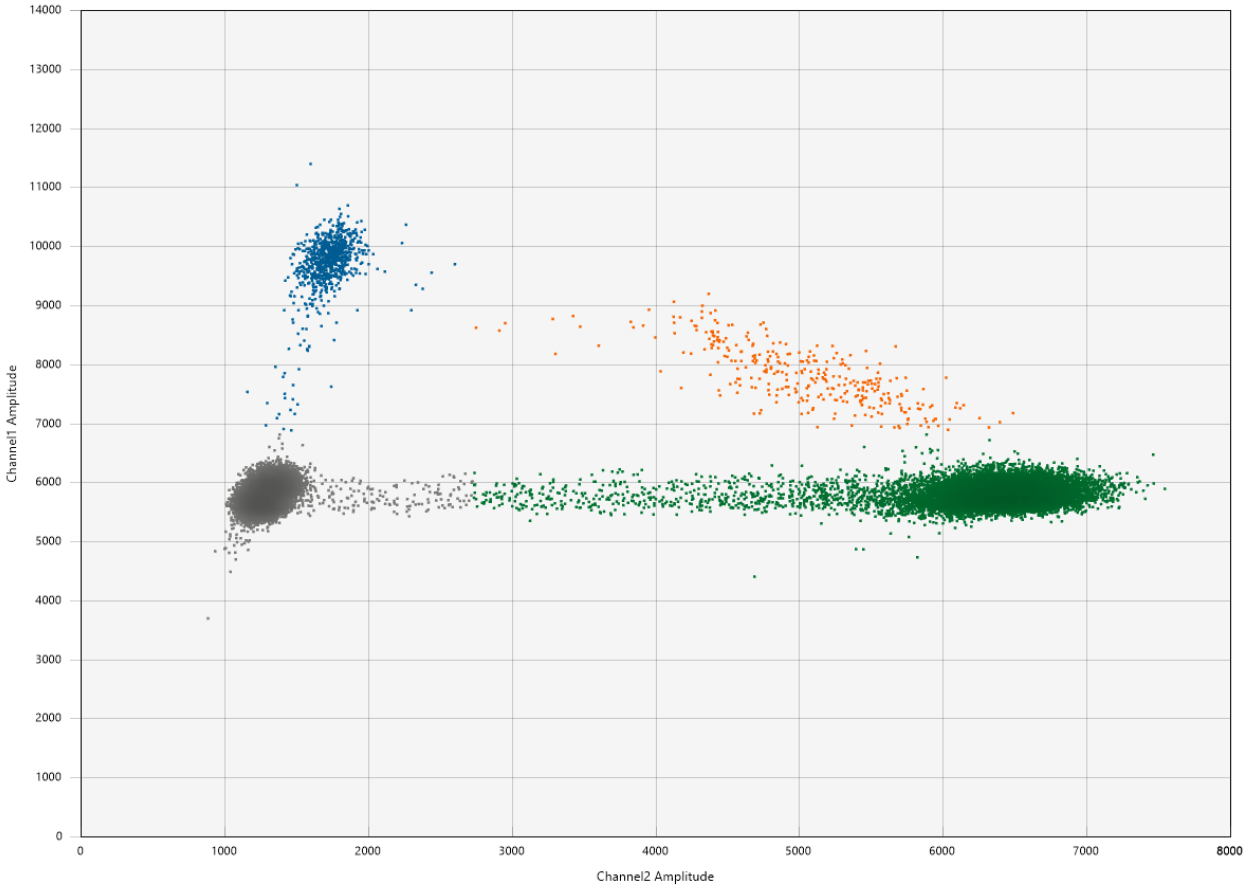
P43 - Final-WT

Gene target : KRAS, p.G12D, c.35G>A

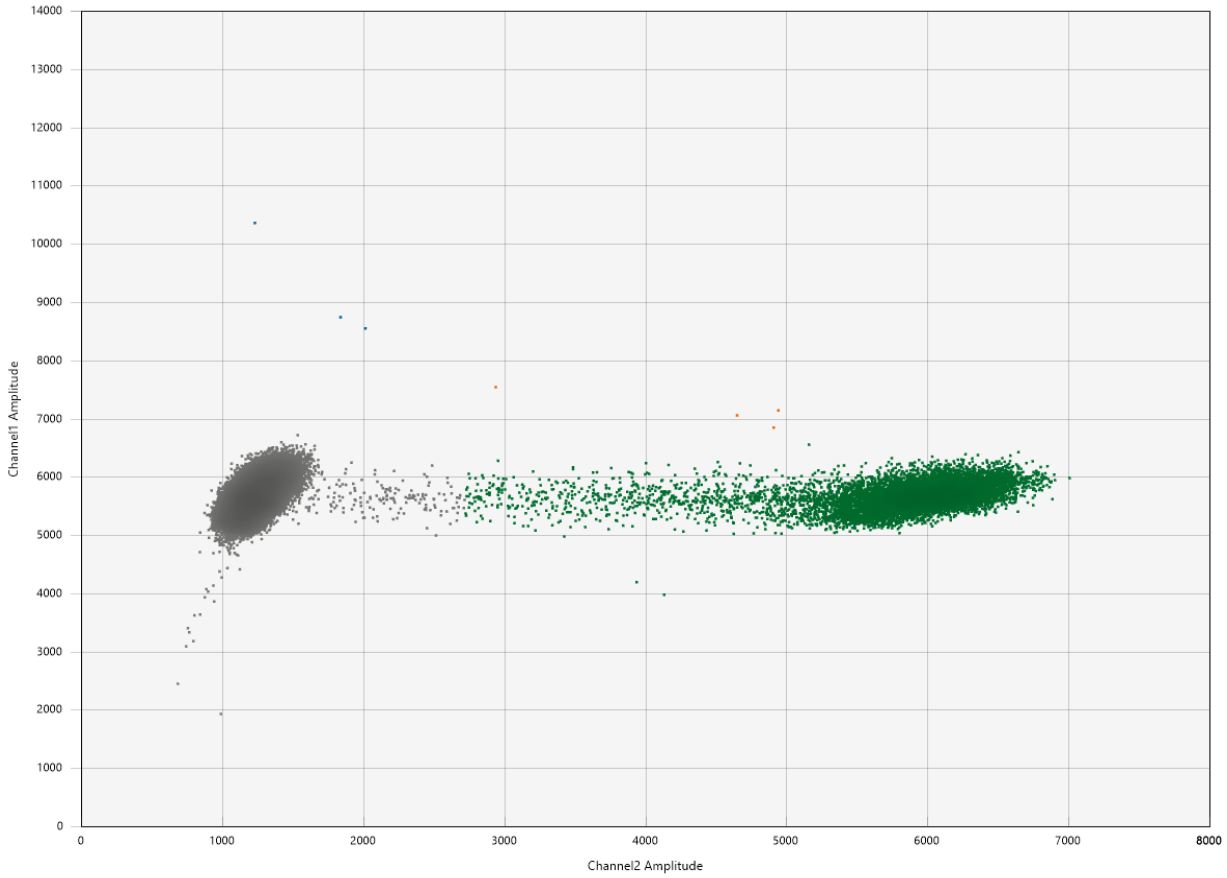
Assay: 1X KRAS WT, p.G12D (HEX)



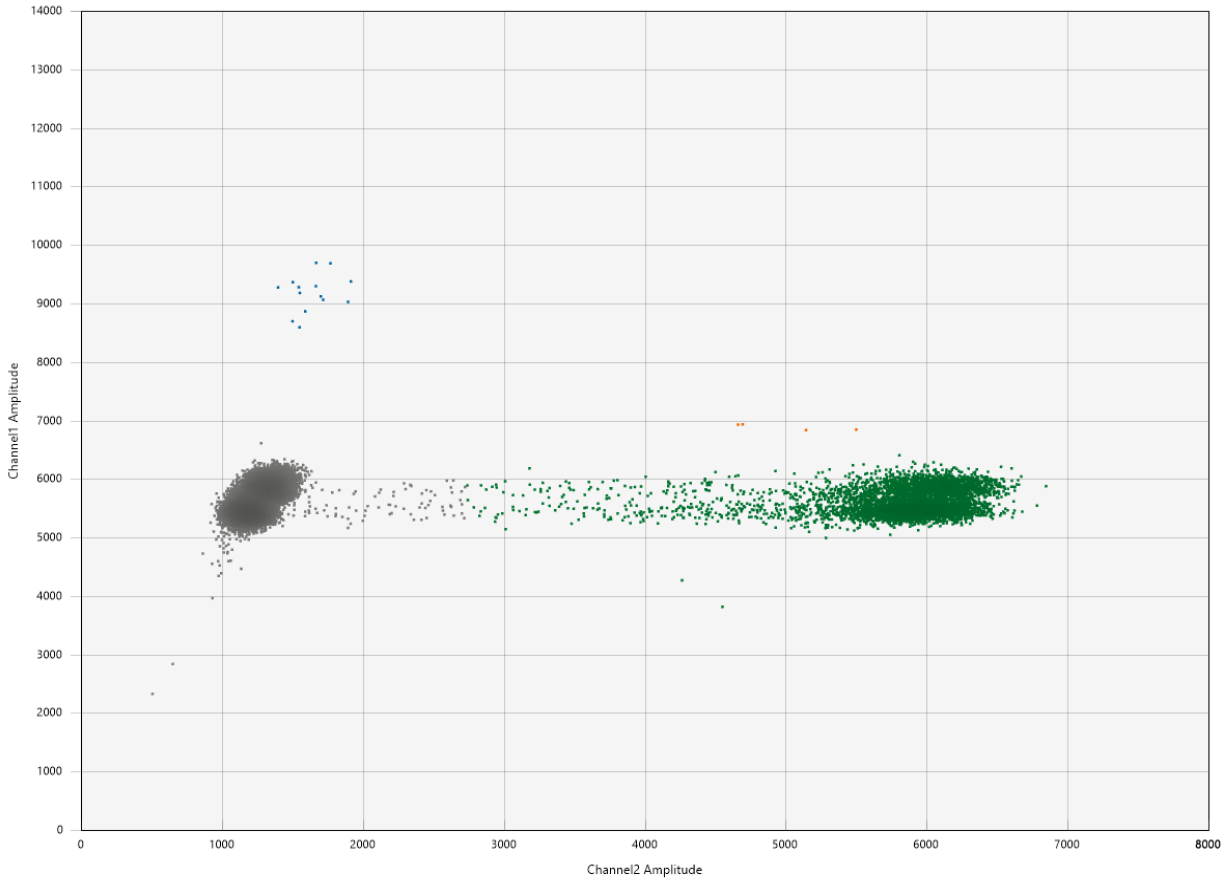
Positive – control



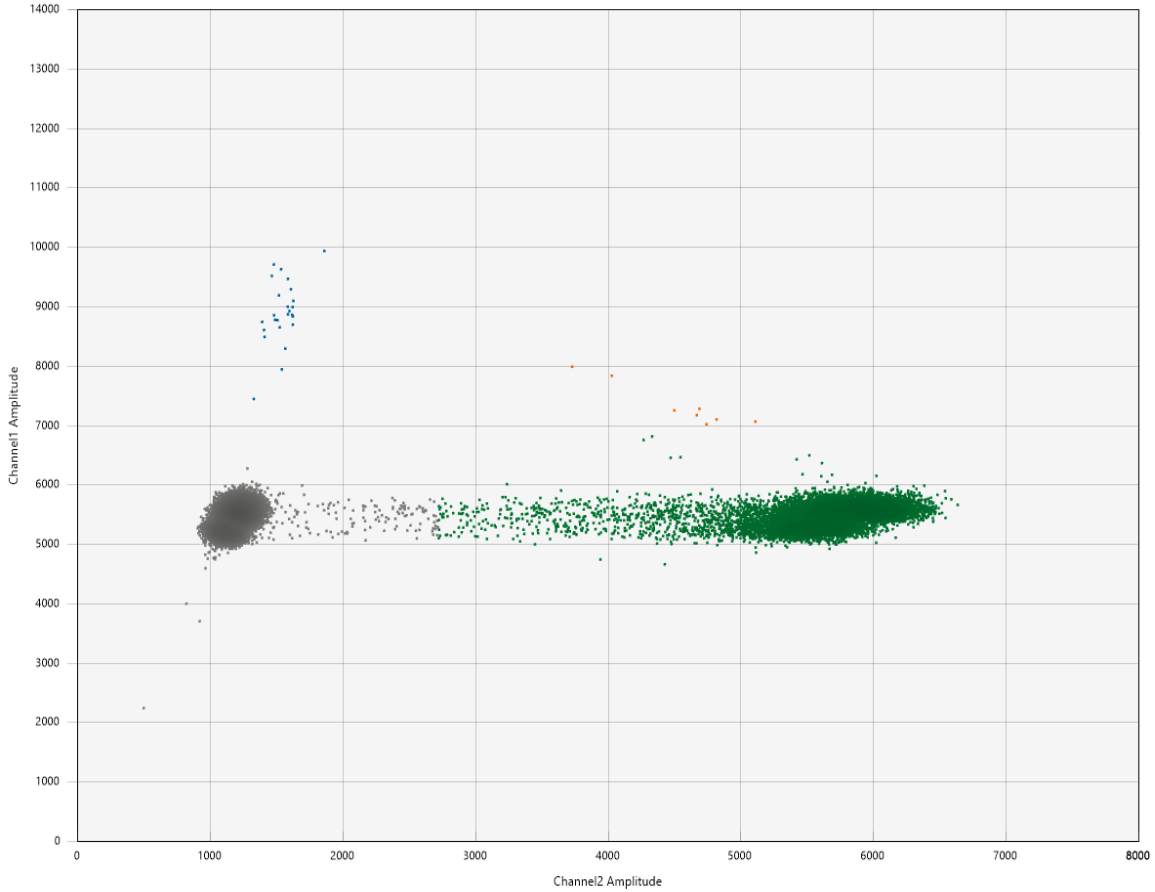
NK-Plasma



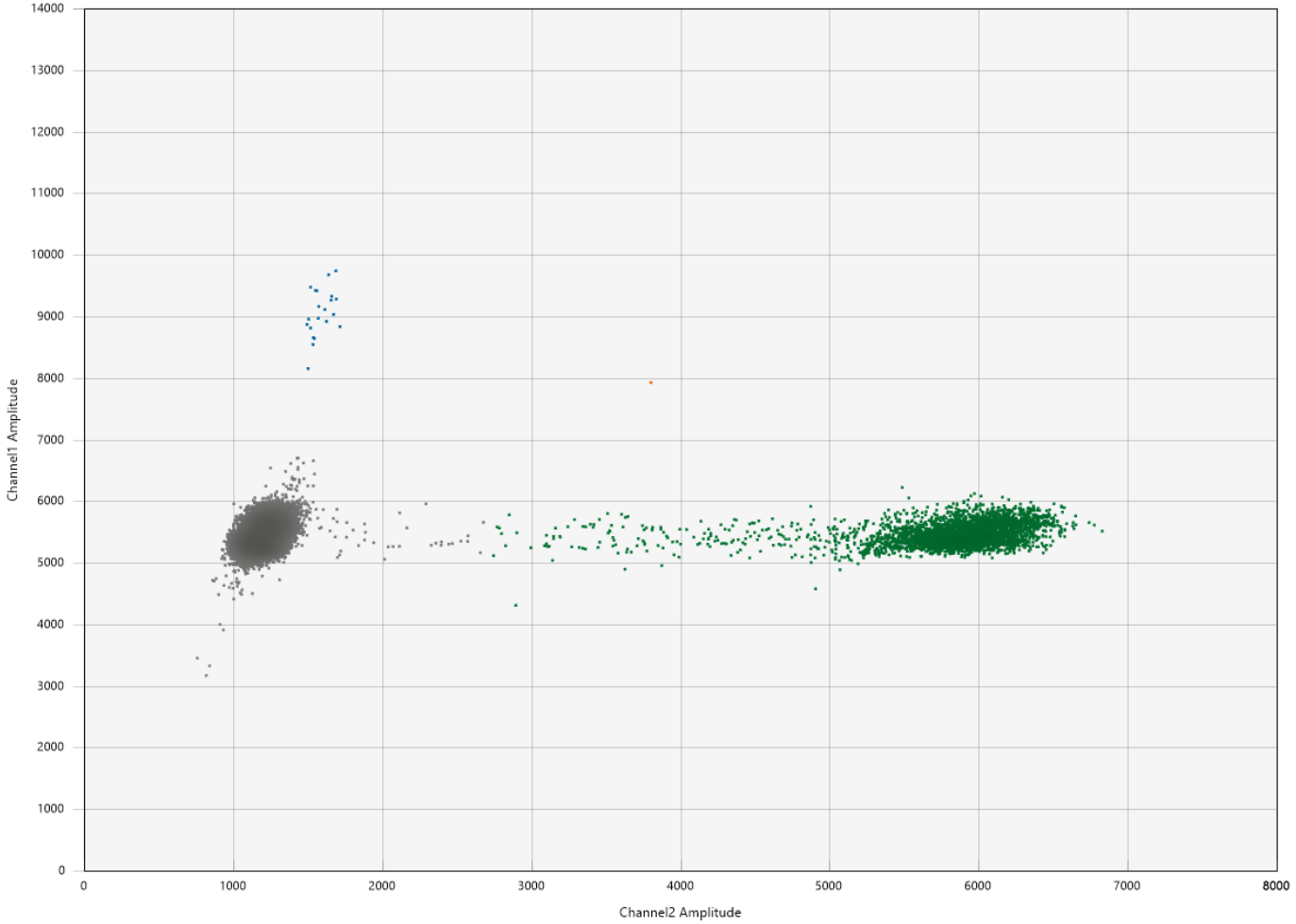
Pre-surgery



Post-surgery

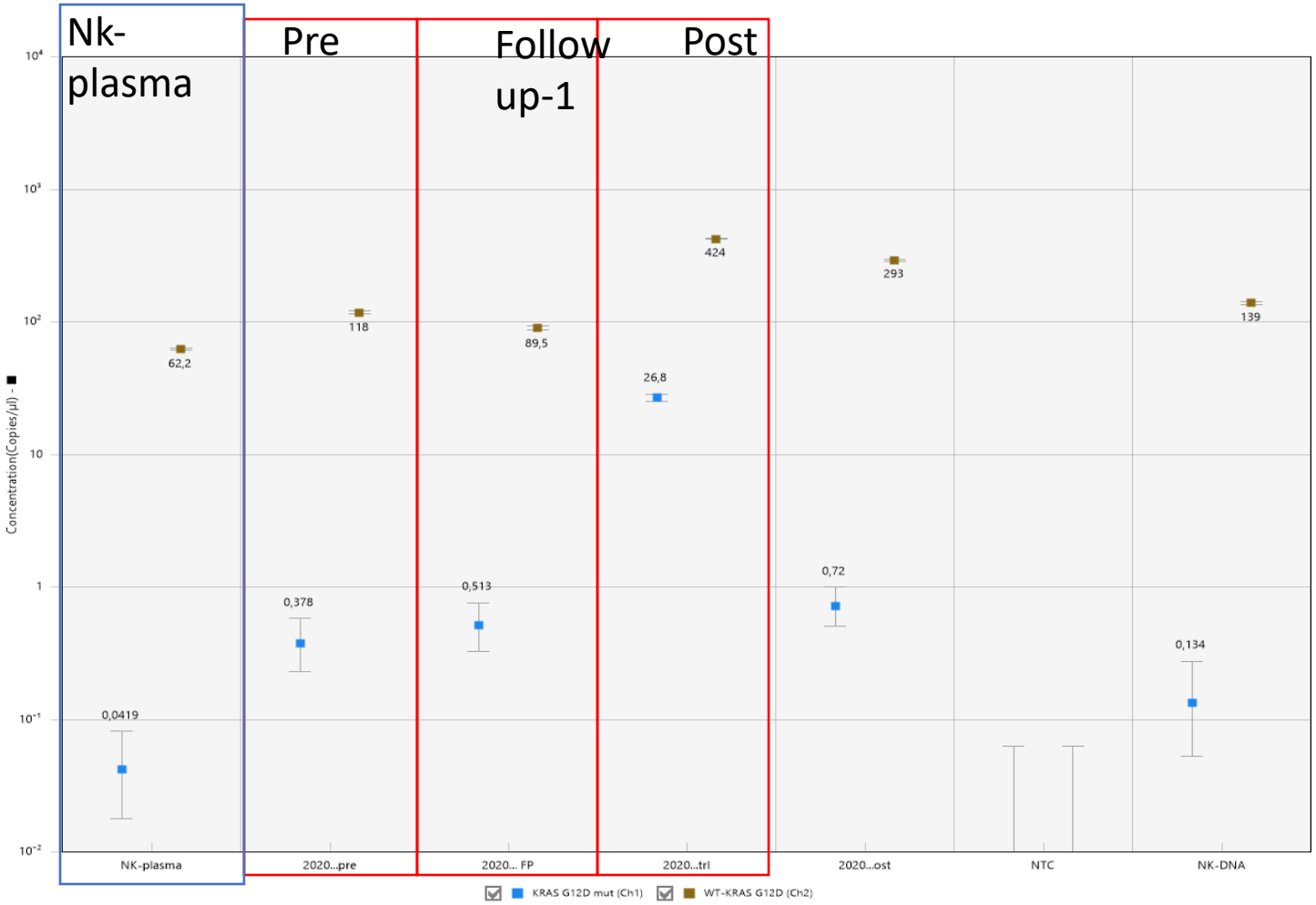


Follow-up 1


14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Channel1 Amplitude
0
1000
2000
3000
4000
5000
6000
7000
8000
Channel2 Amplitude

Concentration plots

Pre, post and follow-up



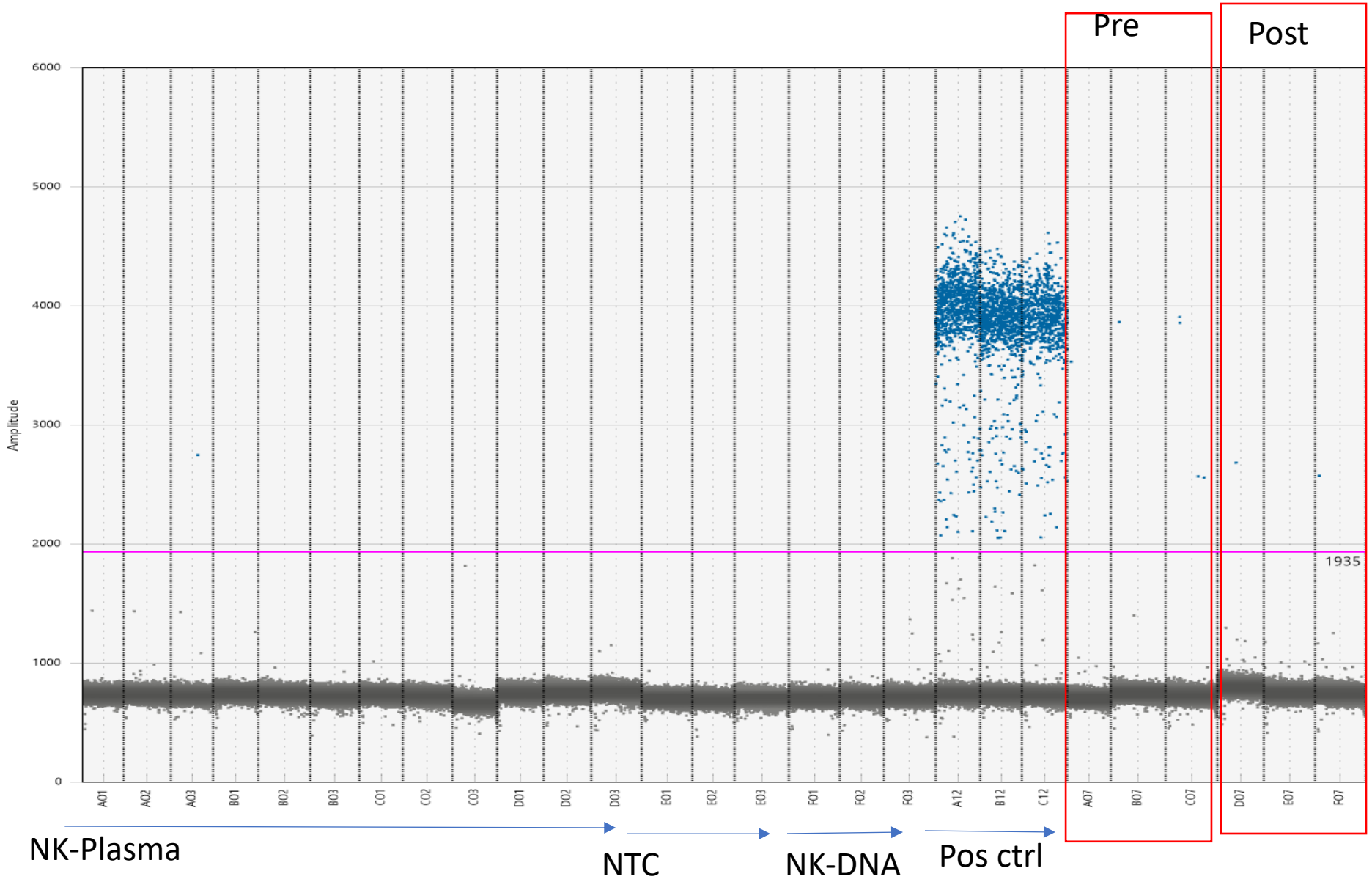
Calling – Pre-op- positive for –KRAS G12D
Post-op- positive for KRAS G12D
Follow-up- positive for KRAS G12D

P38 - Final

Gene target :TP53, p.R248W, c.742C>T

Assays: 1X TP53, p.R248W mut (FAM) and 1X TP53 WT, p.R248W (HEX)

1X TP53,
p.R248W mut

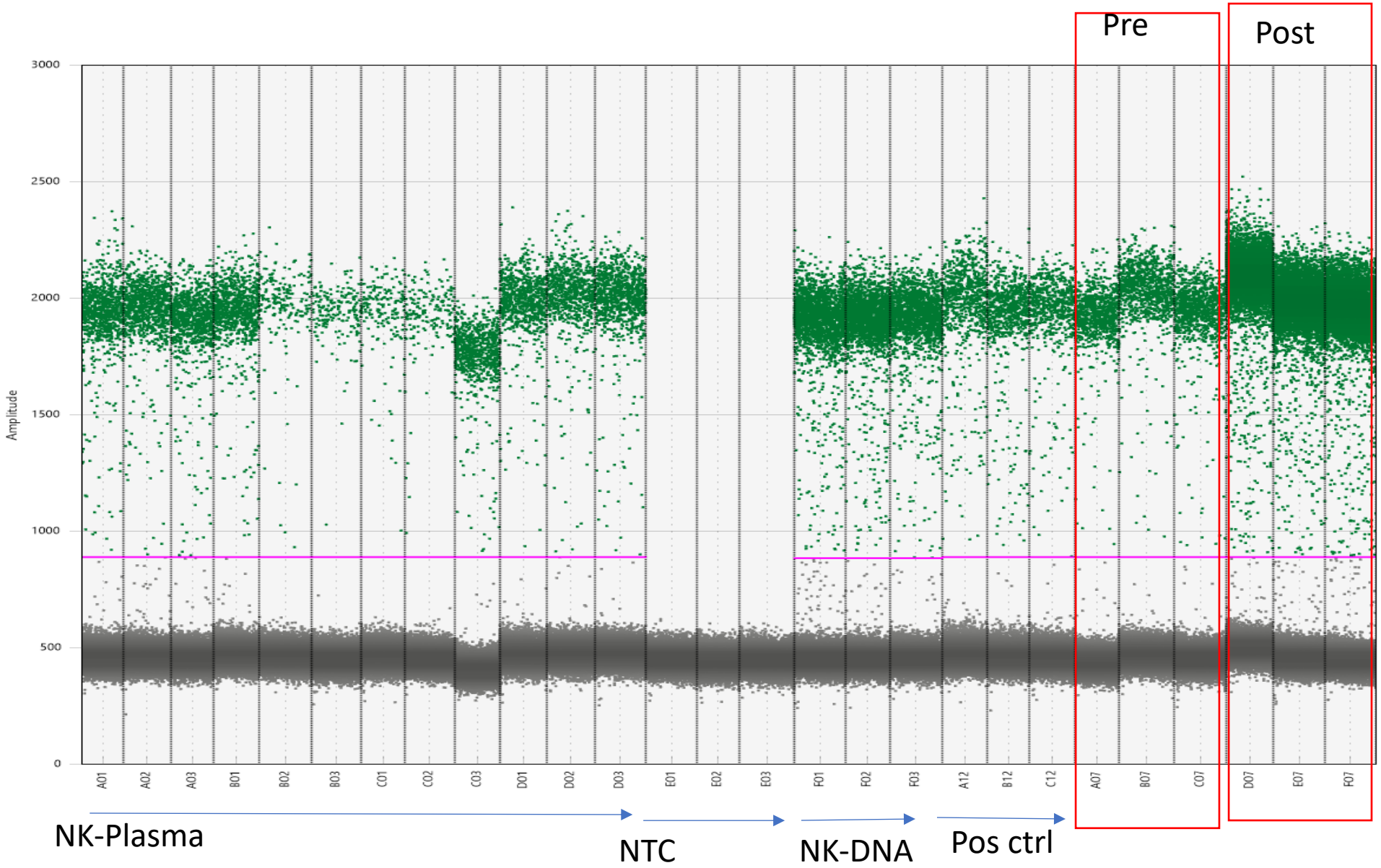


P38 -Final -WT

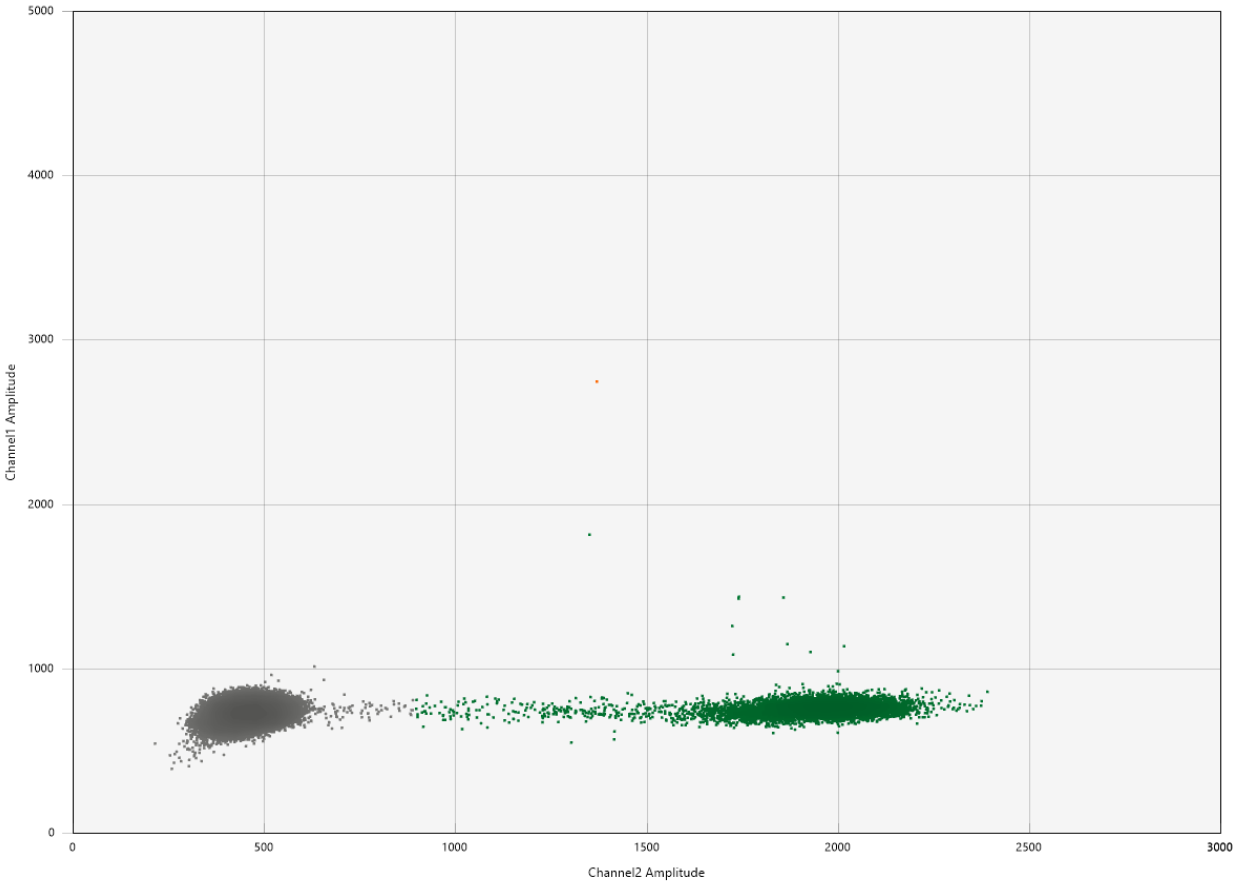
Gene target :TP53, p.R248W, c.742C>T

Assays: 1X TP53 WT, p.R248W (HEX)

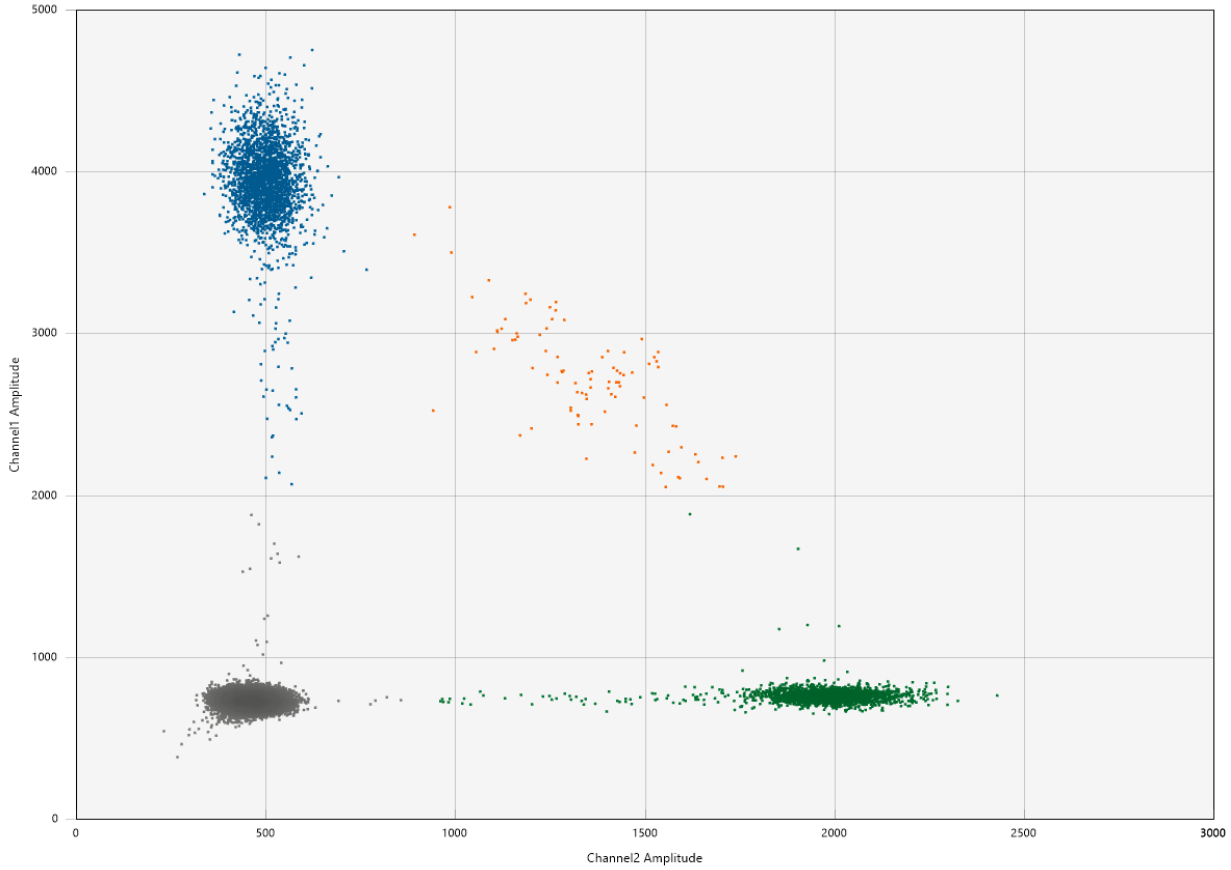
1X TP53 WT, p.R248W



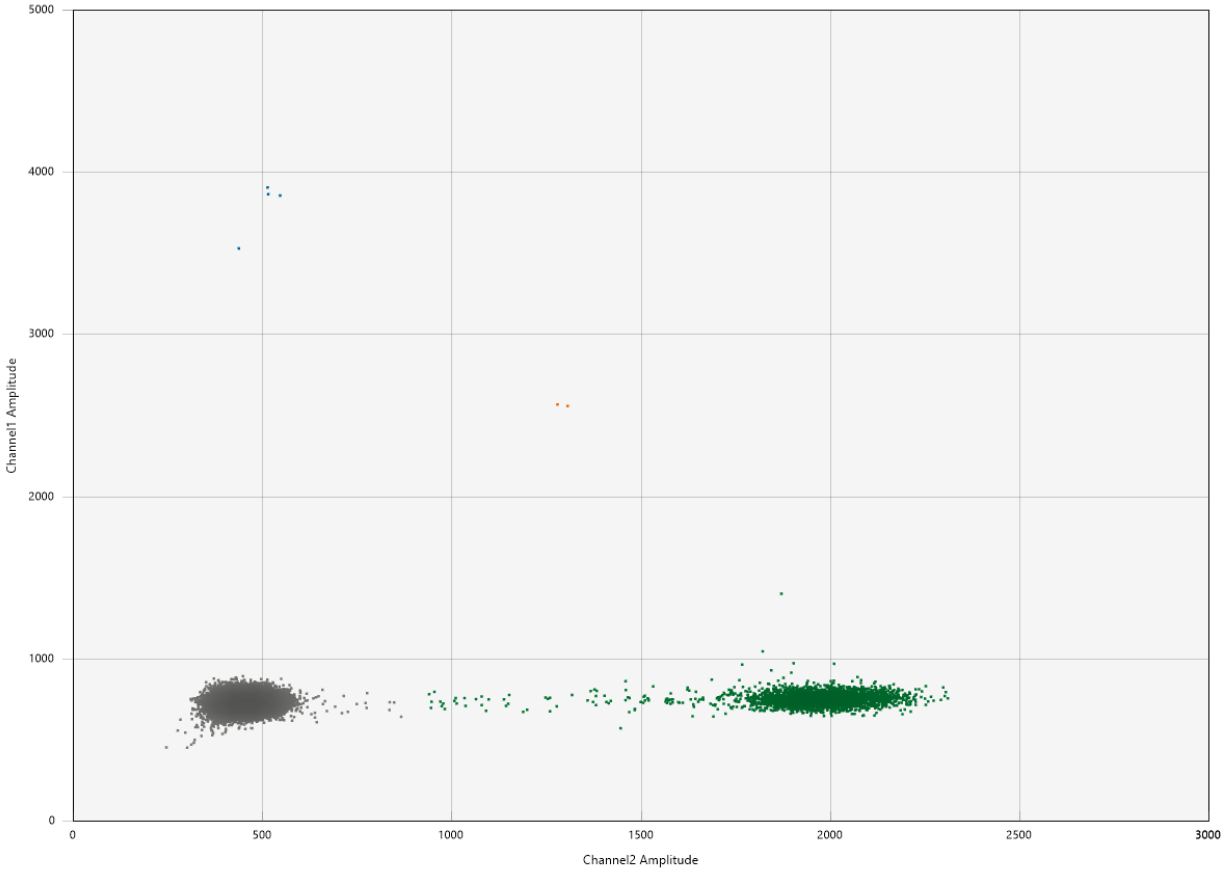
NK-plasma DNA



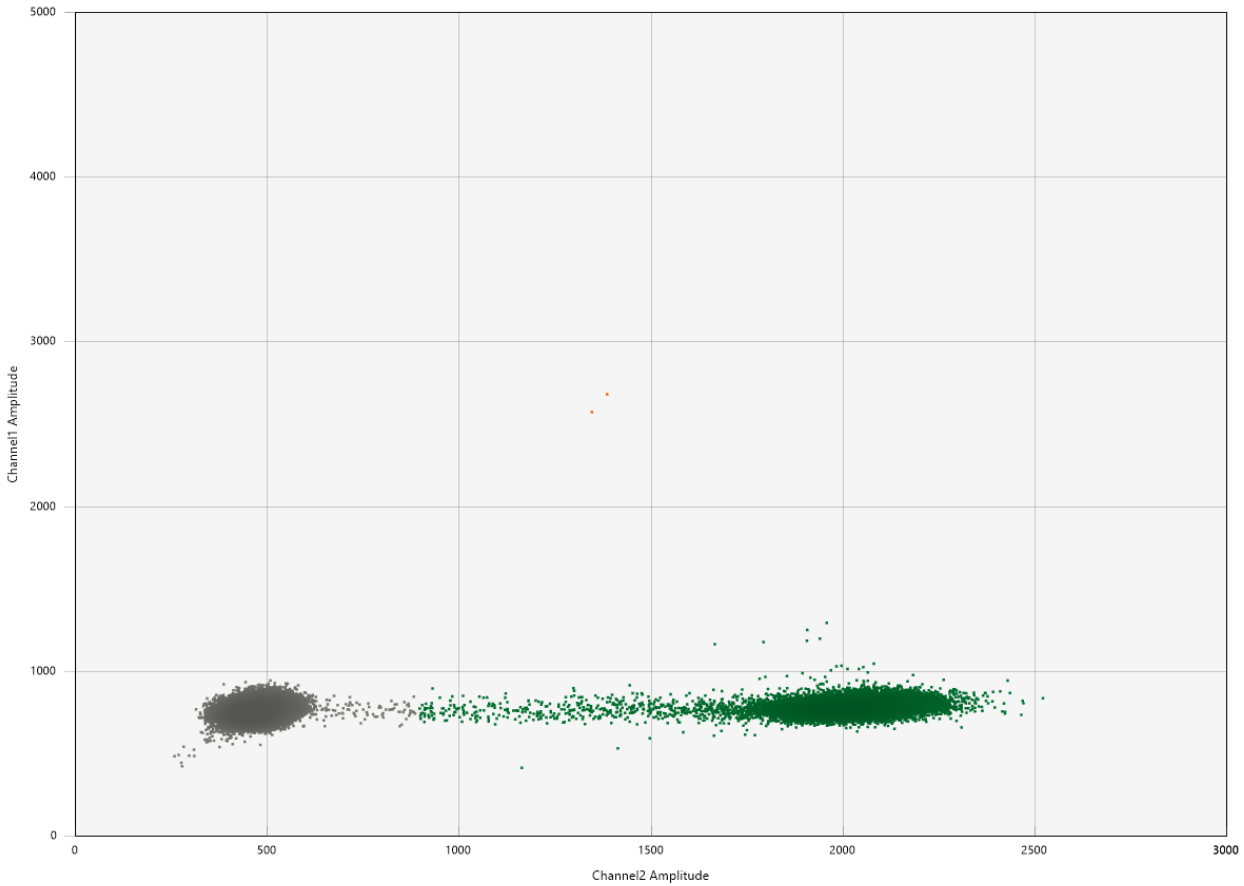
Positive control



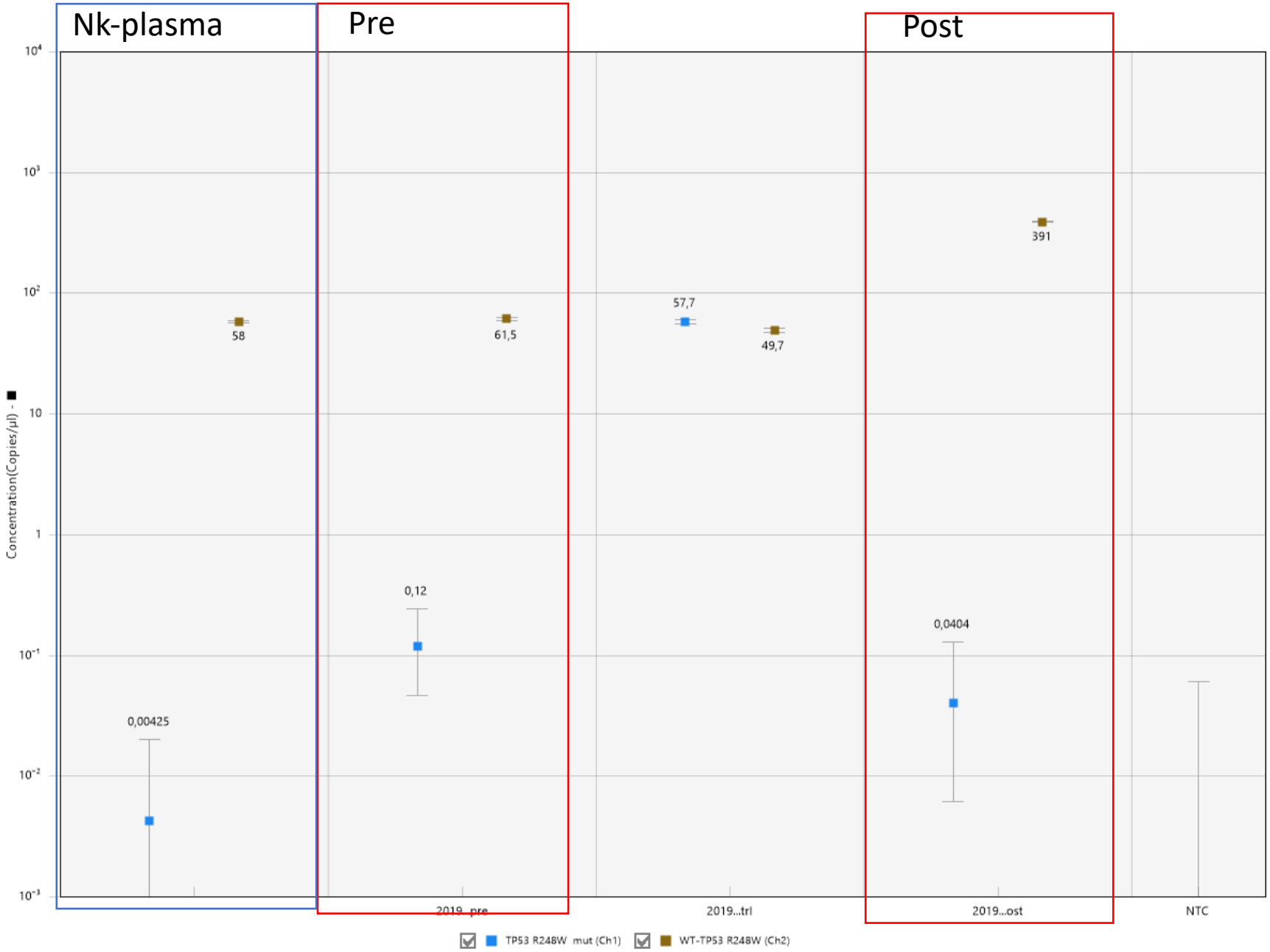
Pre-surgery



Post-surgery



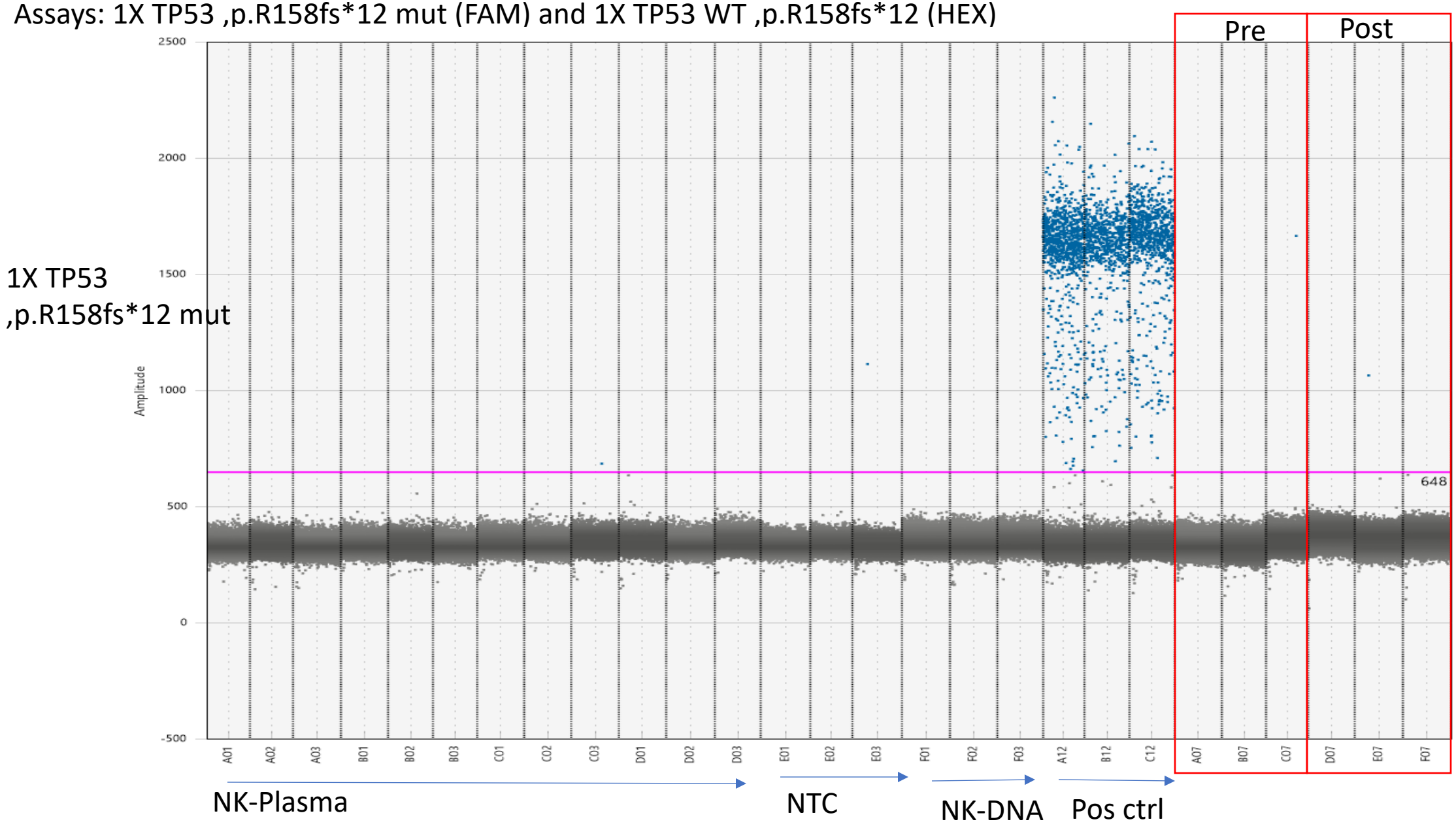
Concentration plots



P42 - Final

Gene target : TP53,p.R158fs*12 , c.472del

Assays: 1X TP53 ,p.R158fs*12 mut (FAM) and 1X TP53 WT ,p.R158fs*12 (HEX)

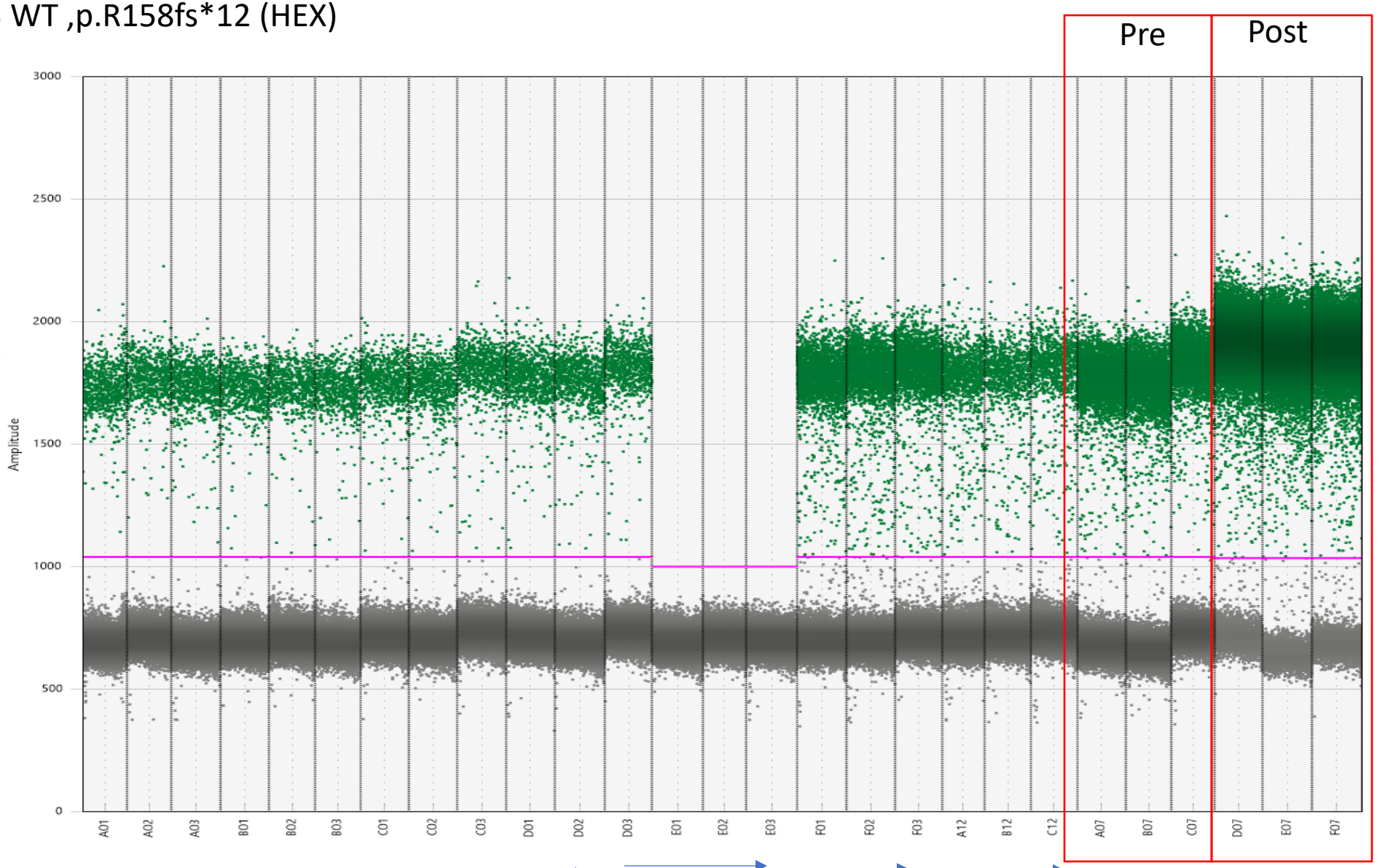


P42 - Final -WT

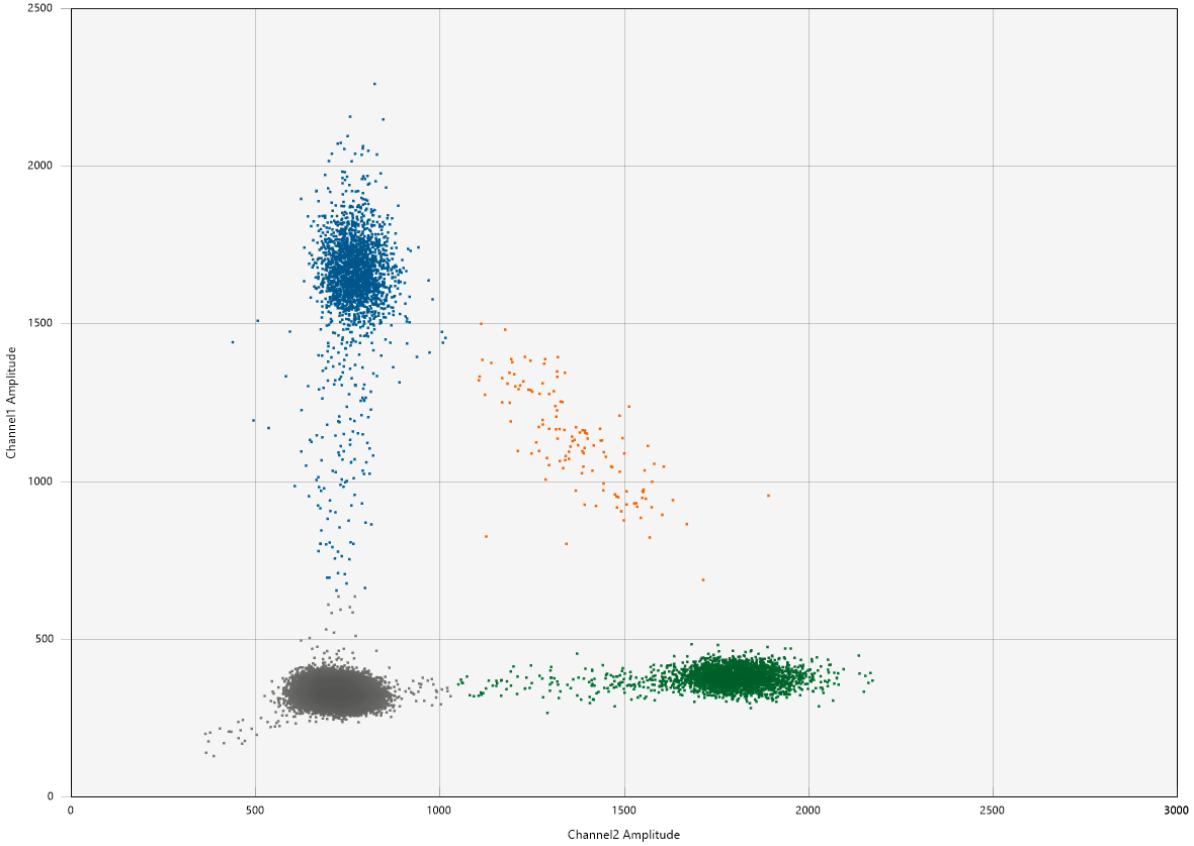
Gene target : TP53,p.R158fs*12 , c.472del

Assays: 1X TP53 WT ,p.R158fs*12 (HEX)

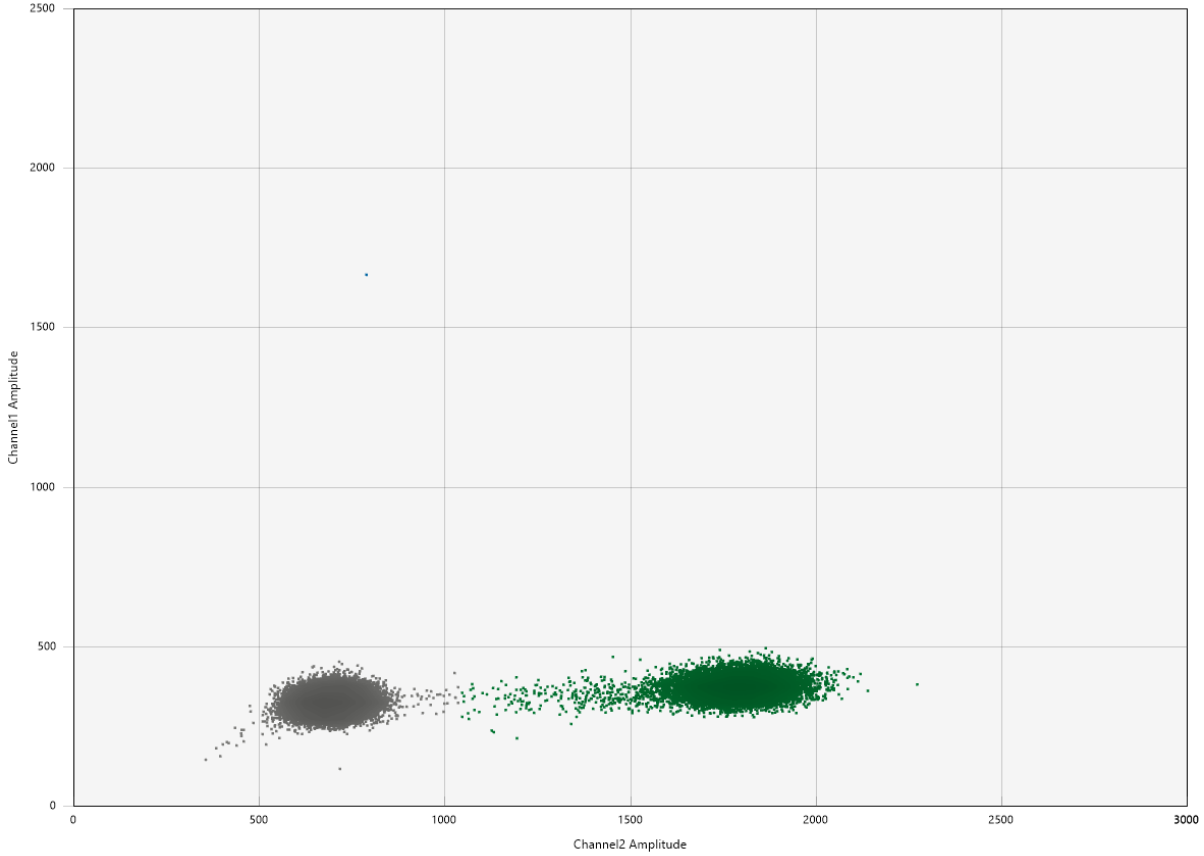
1X TP53
WT ,p.R158fs*12



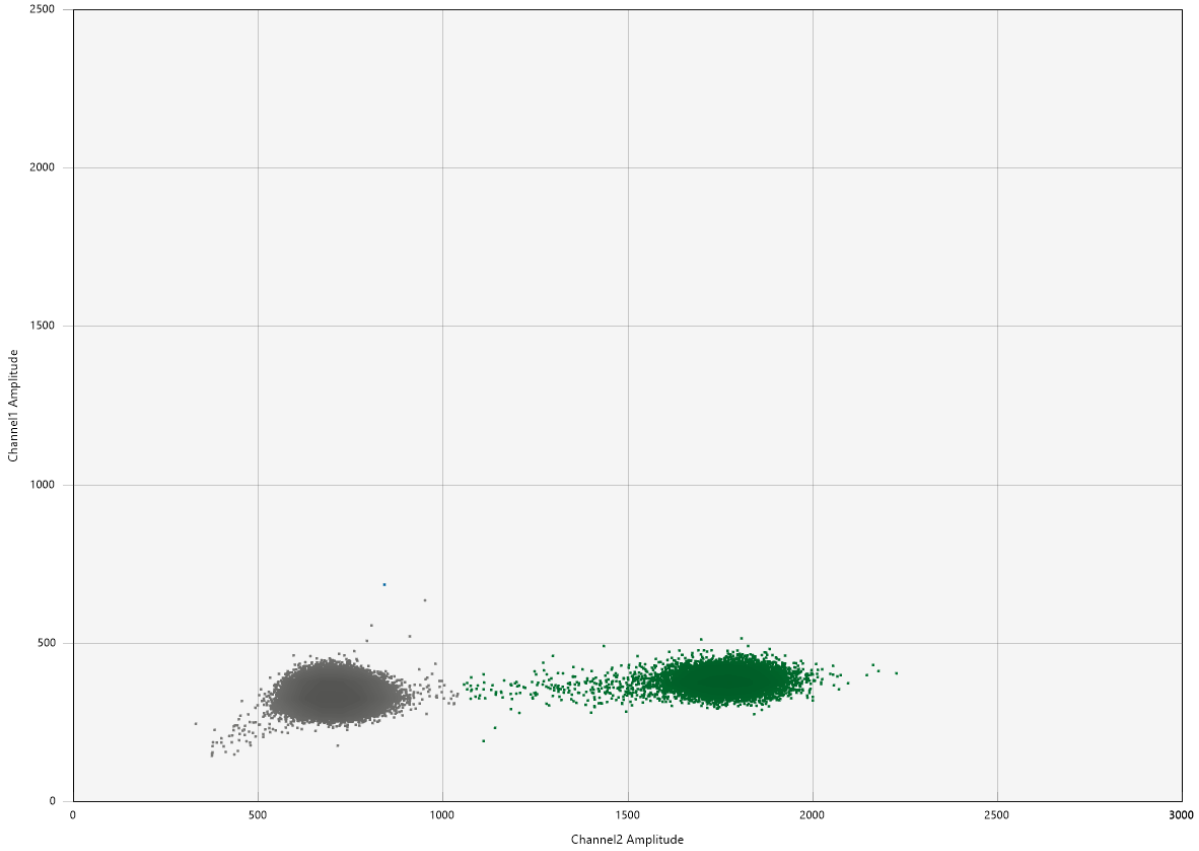
Positive – control



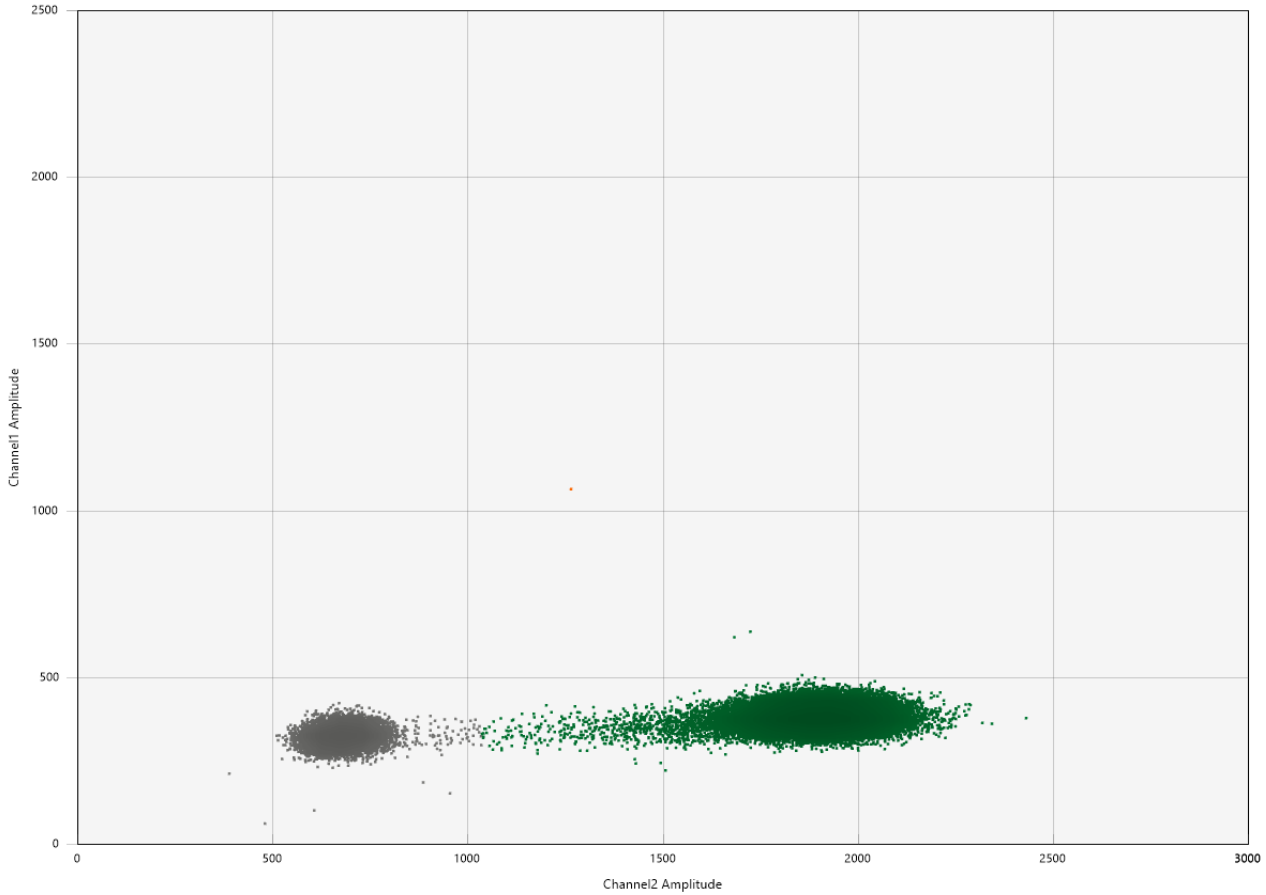
Pre-surgery



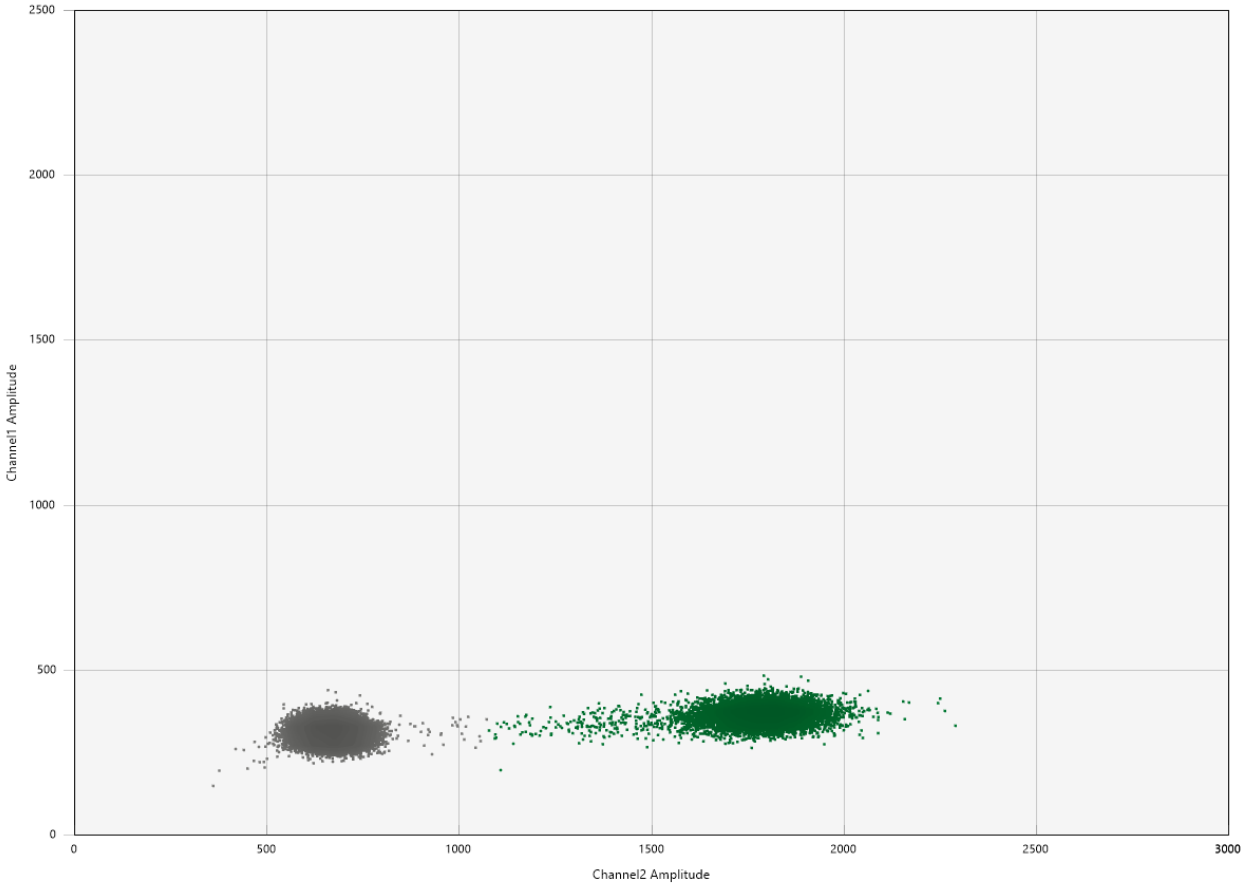
NK-Plasma


2500
2000
1500
1000
500
0
Channel1 Amplitude
0
500
1000
1500
2000
2500
3000
Channel2 Amplitude

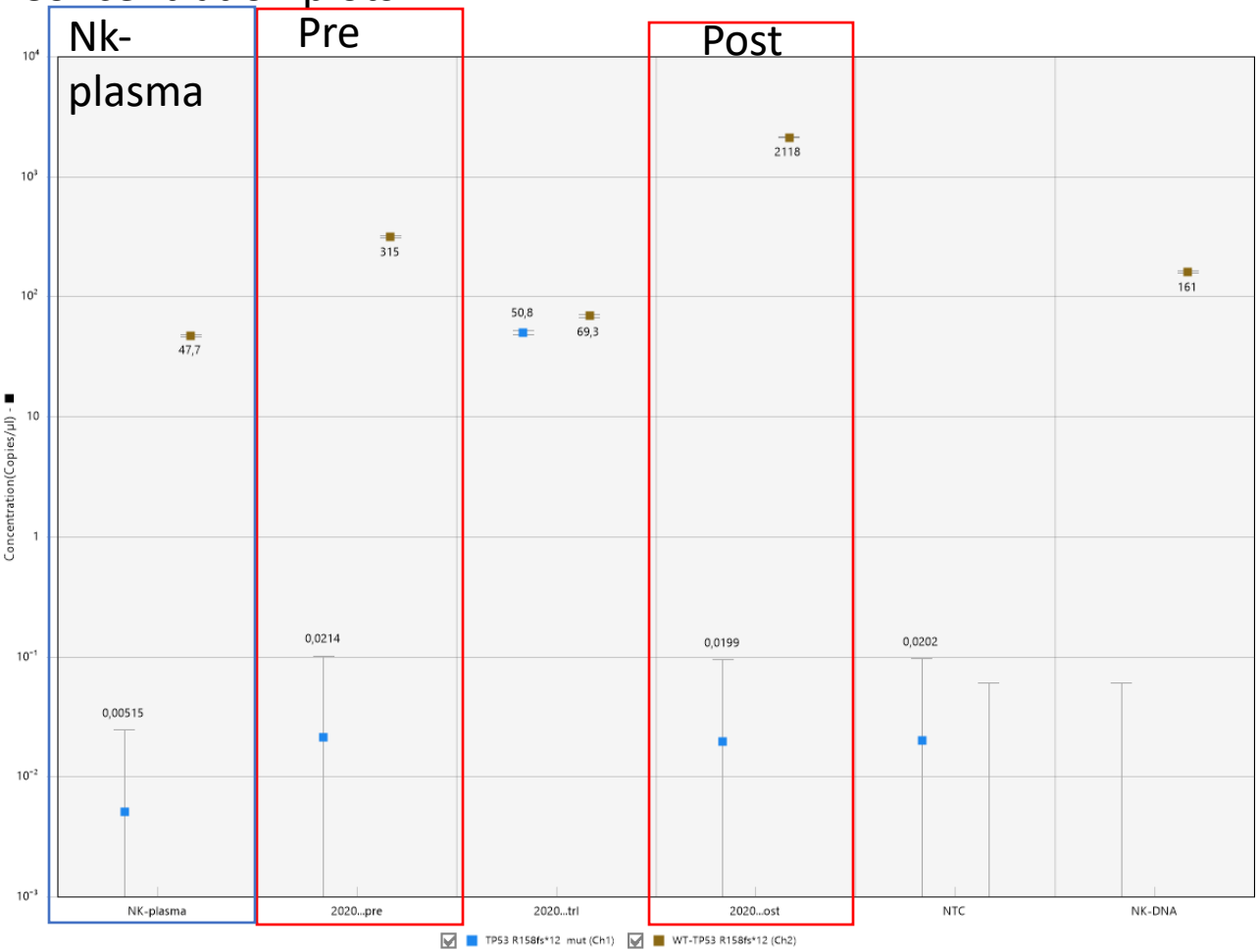
Post-surgery



Post-surgery diluted

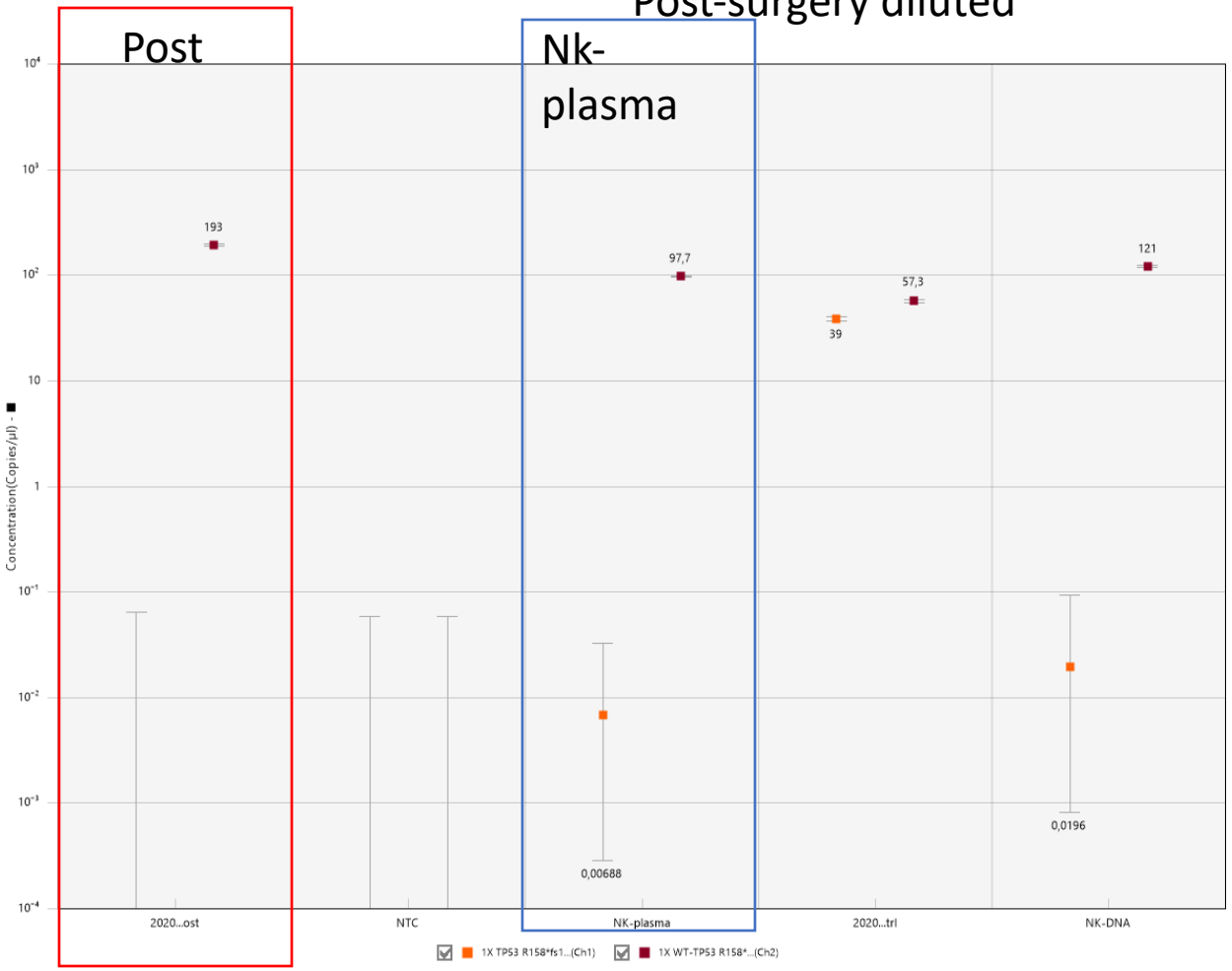


Concentration plots



Pre, post

Post-surgery diluted



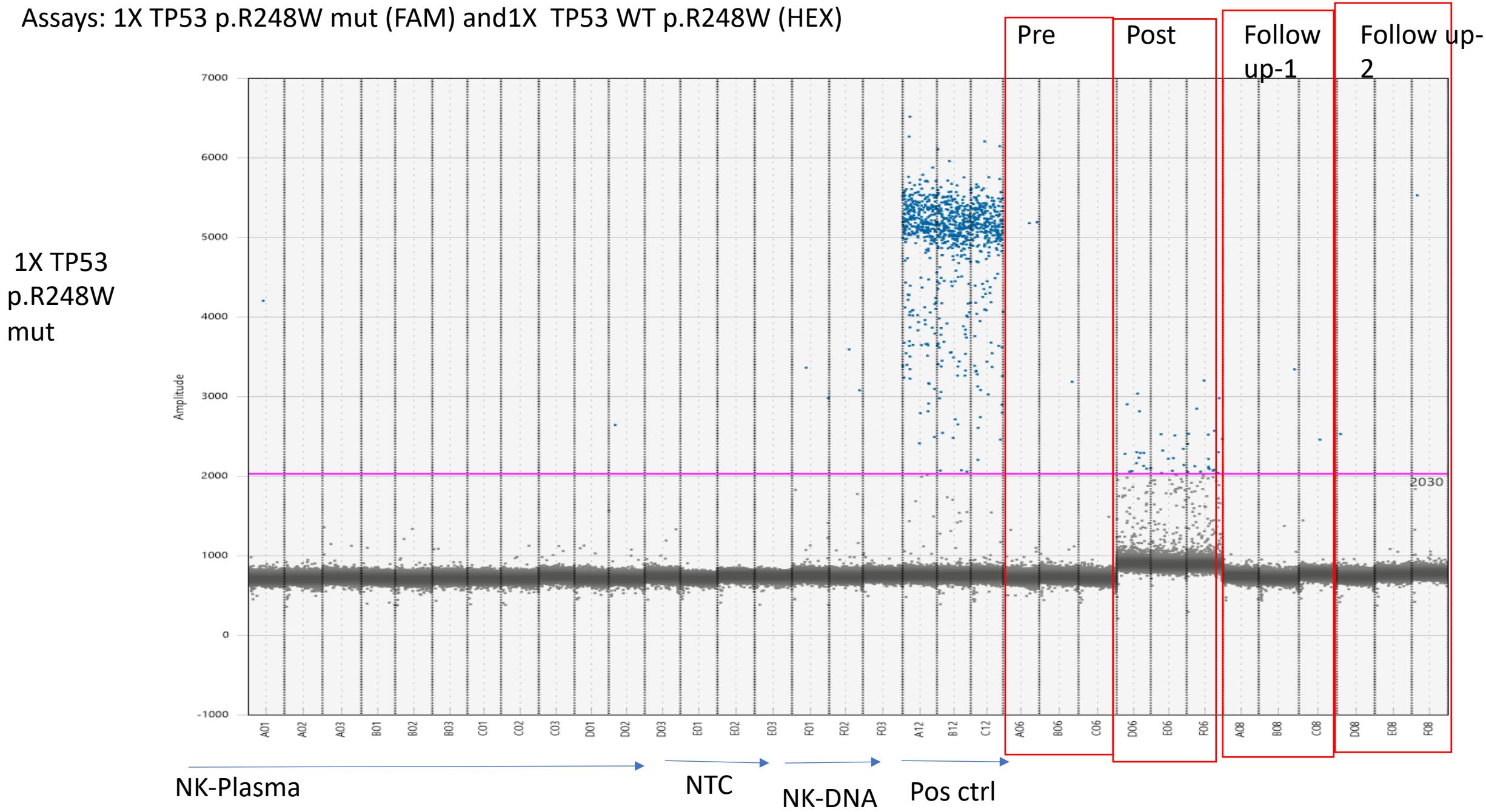
Calling – Pre-op- negative for –TP53 R158fs*- did not met droplet criteria and overlap

Post-op and diluted- negative for TP53 R158fs*

P32 -Final

Gene target : TP53 p.R248W, c.742C>T and TP53,p.?, c.673-2A>G

Assays: 1X TP53 p.R248W mut (FAM) and1X TP53 WT p.R248W (HEX)

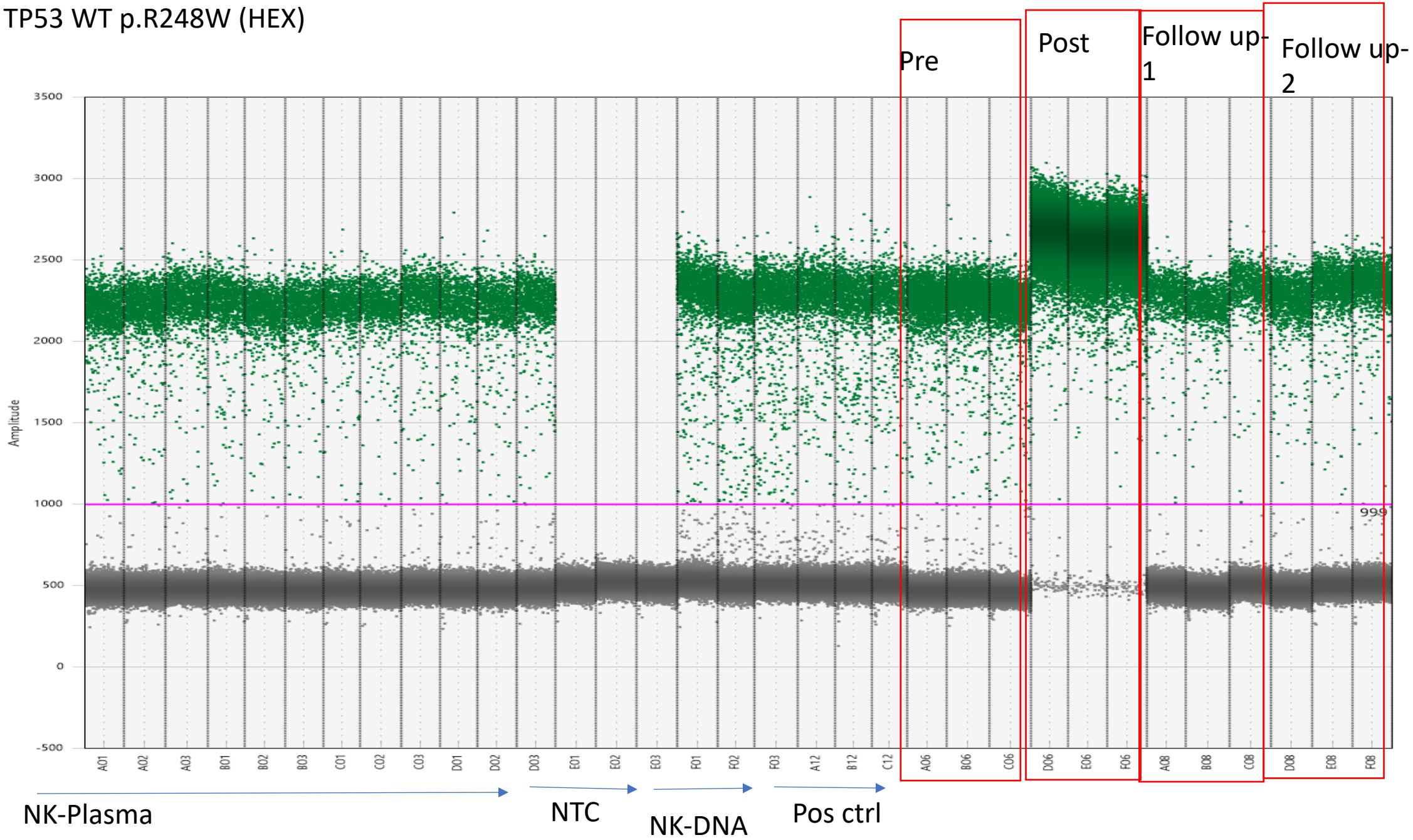


P32 - Final-WT

Assays: 1X TP53 WT p.R248W (HEX)

Gene target : TP53 p.R248W, c.742C>T and TP53,p.?, c.673-2A>G

1X TP53 p.R248W mut

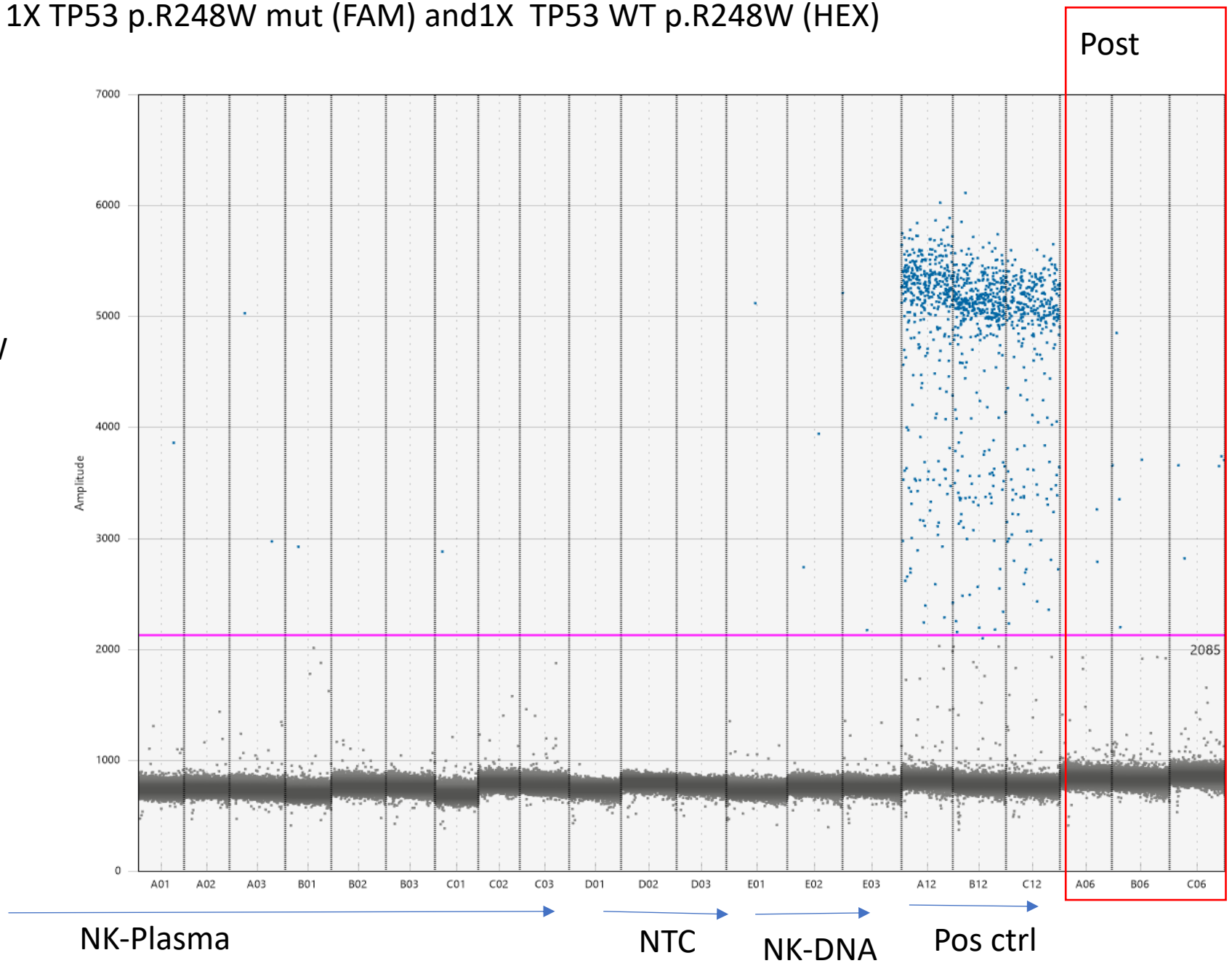


P32 -Final diluted

Gene target : TP53 p.R248W, c.742C>T and TP53,p.?, c.673-2A>G

Assays: 1X TP53 p.R248W mut (FAM) and1X TP53 WT p.R248W (HEX)

1X TP53 p.R248W mut

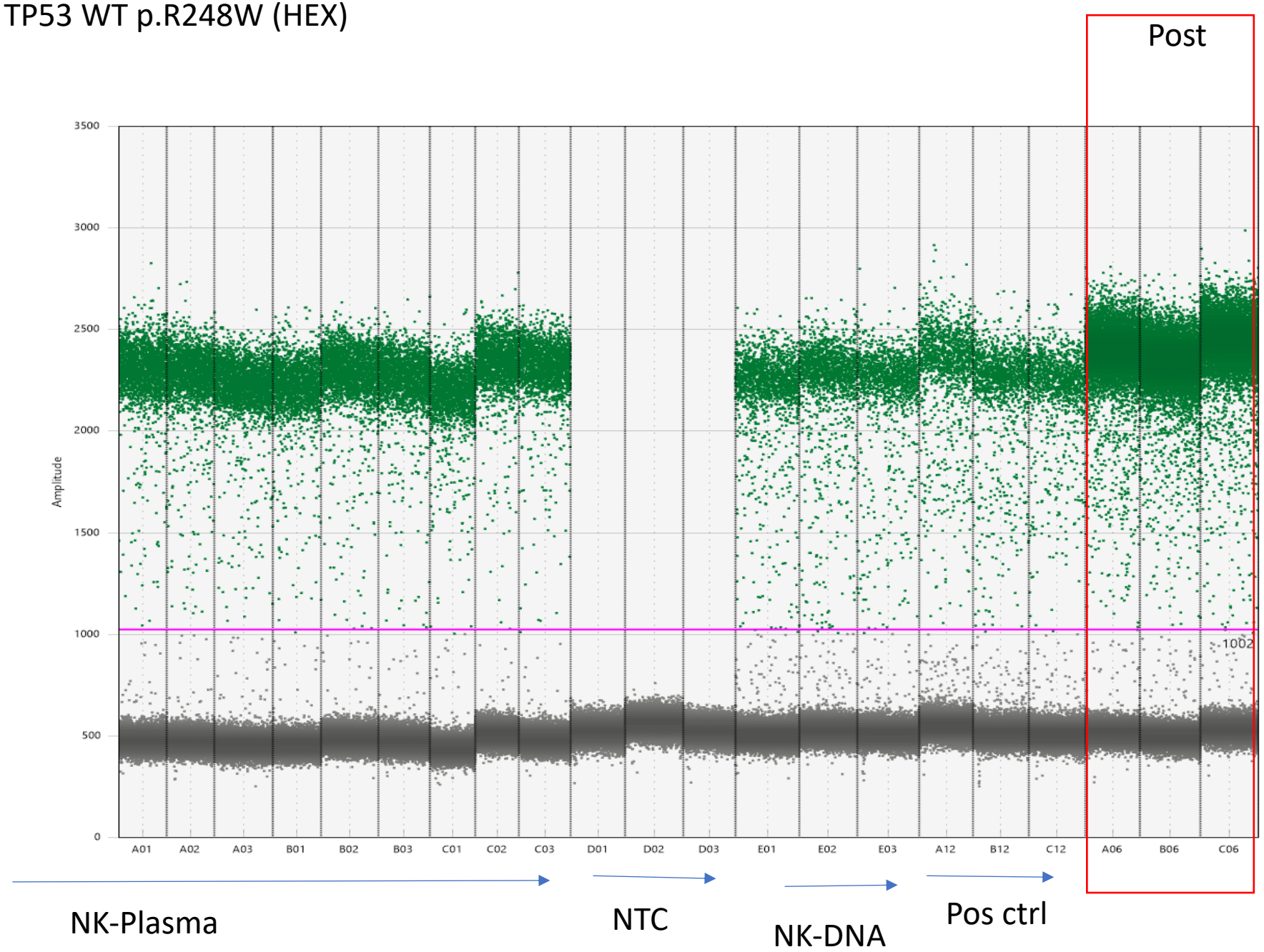


P32 -Final-WT

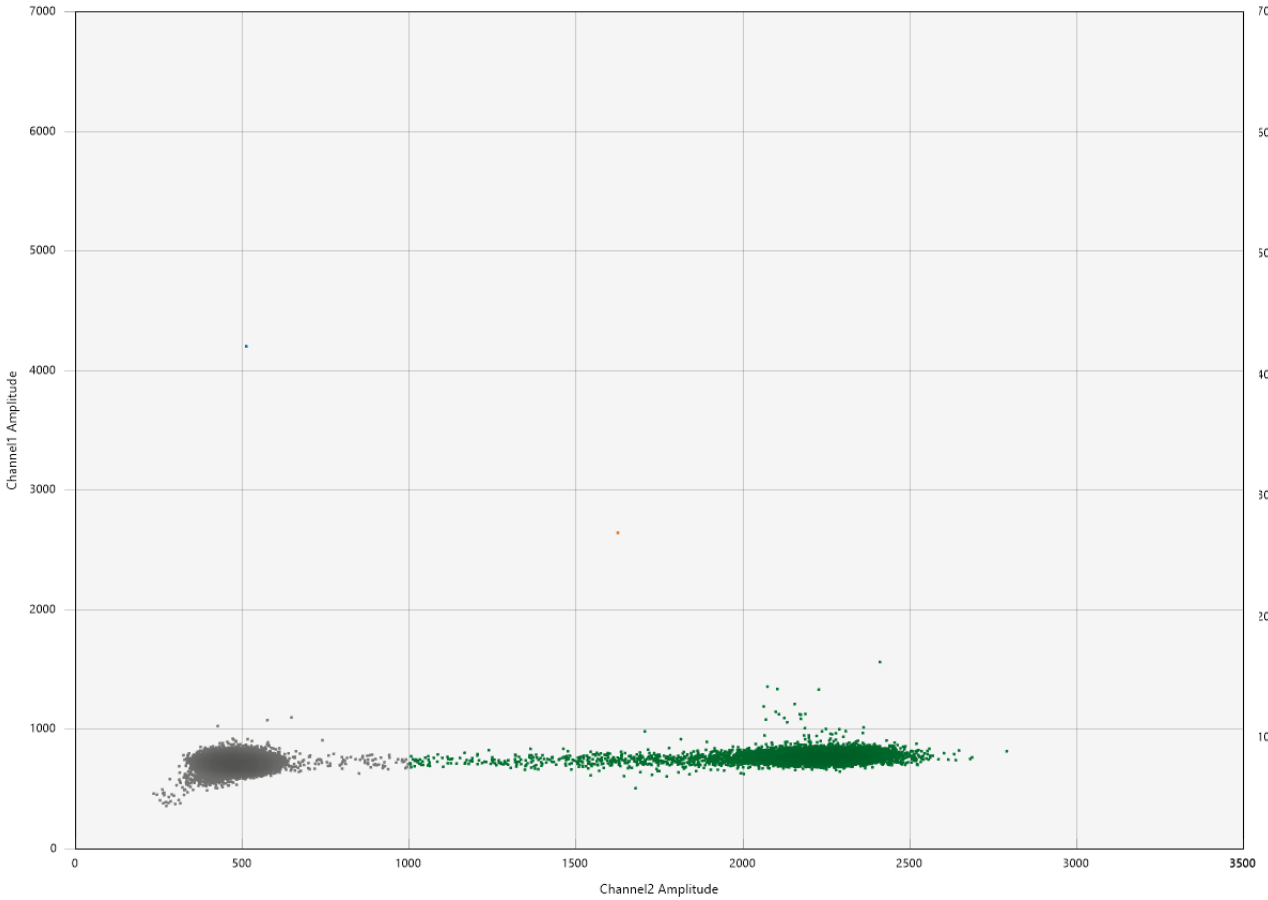
Assays: 1X TP53 WT p.R248W (HEX)

Gene target : TP53 p.R248W, c.742C>T and TP53,p.?, c.673-2A>G

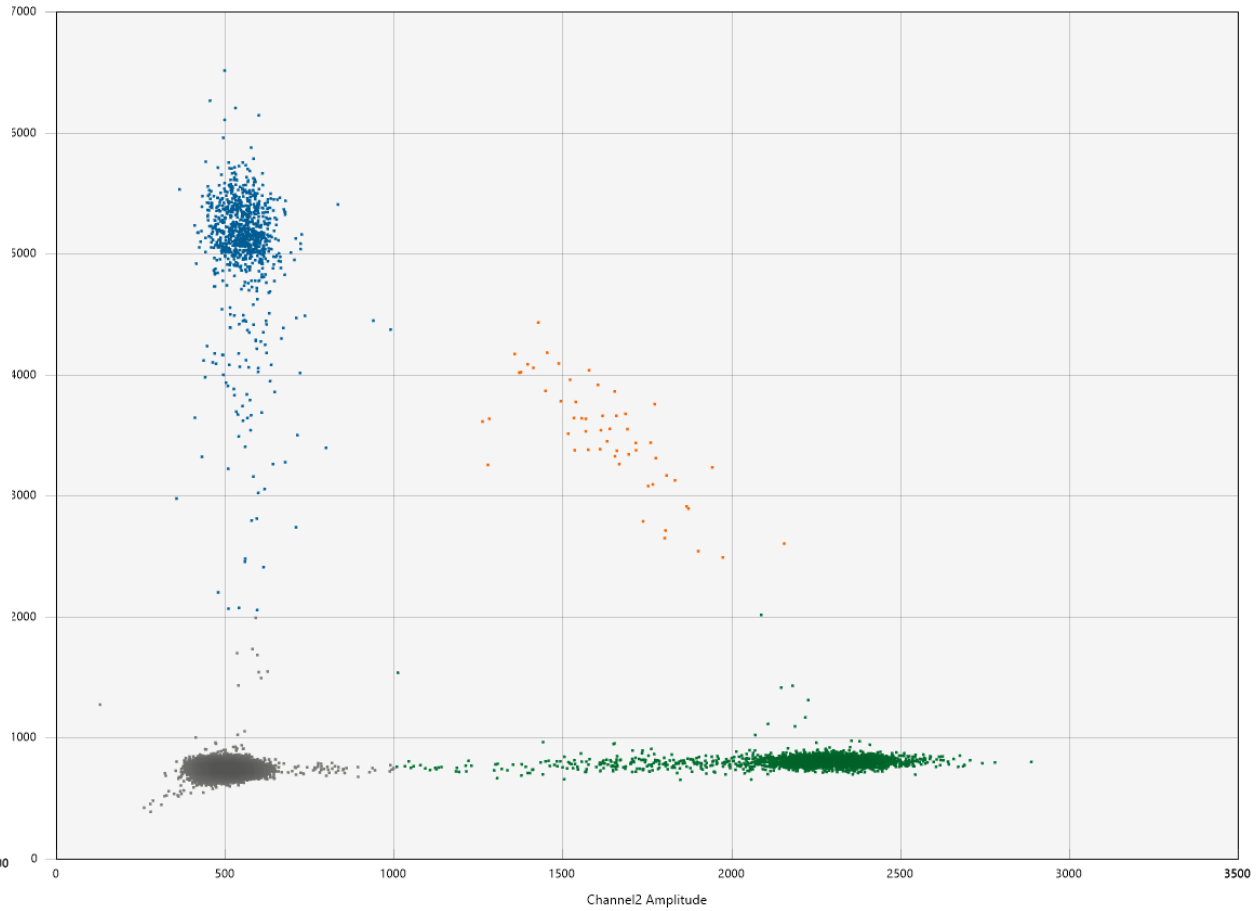
1X TP53 WT p.R248 W



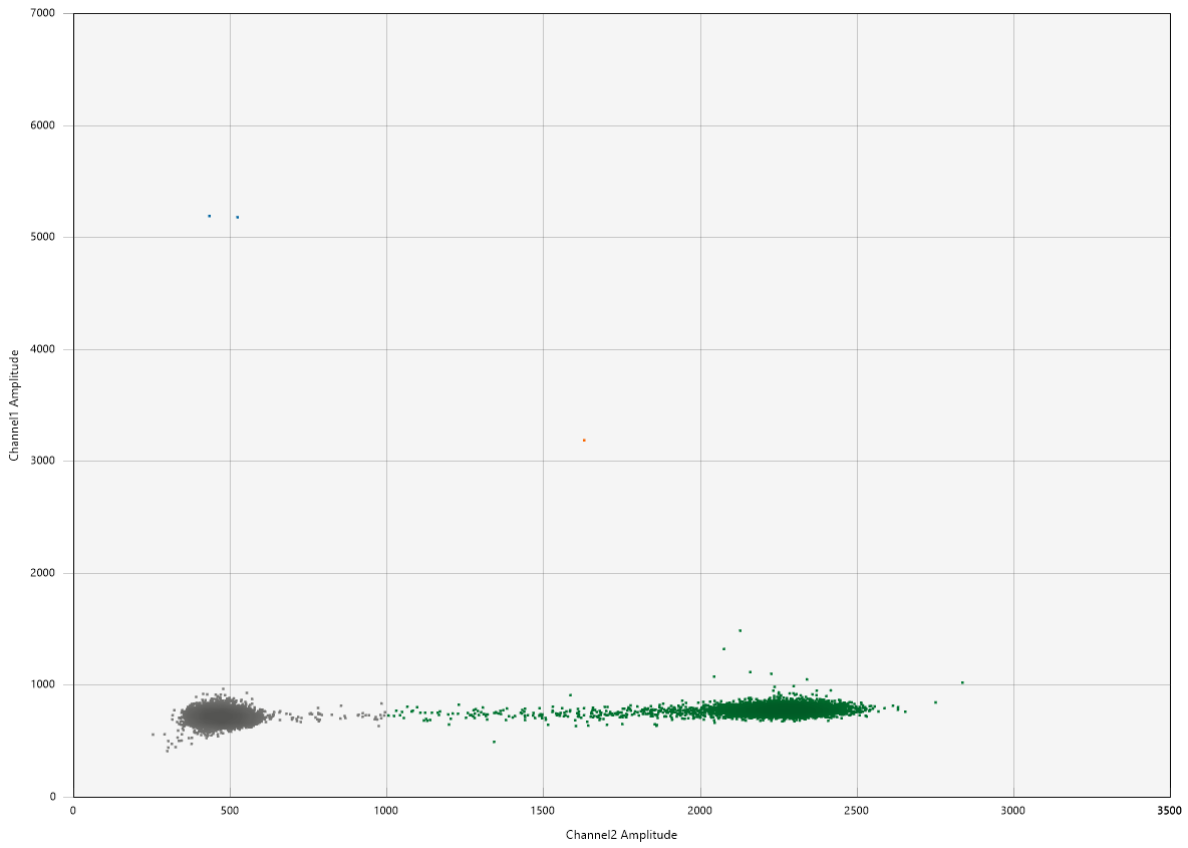
NK-plasma DNA



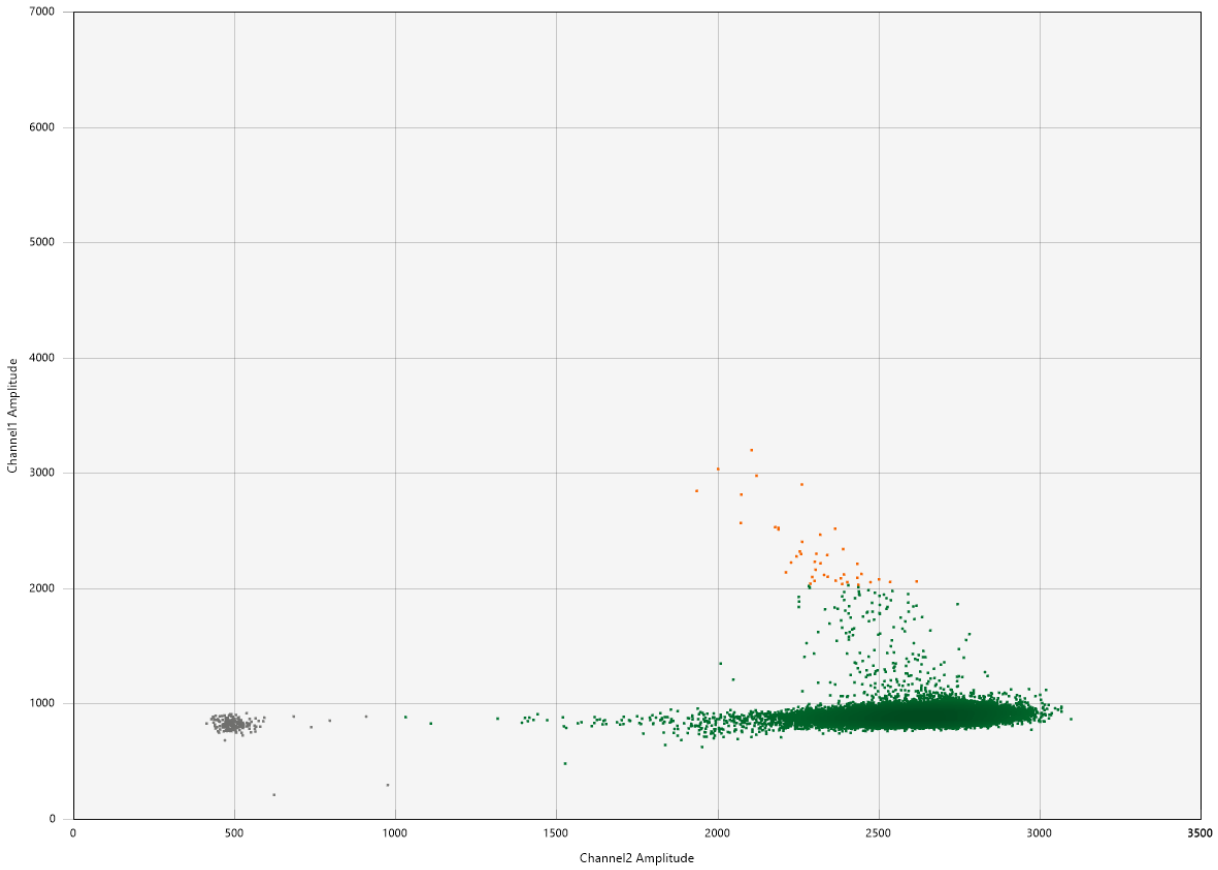
Positive control



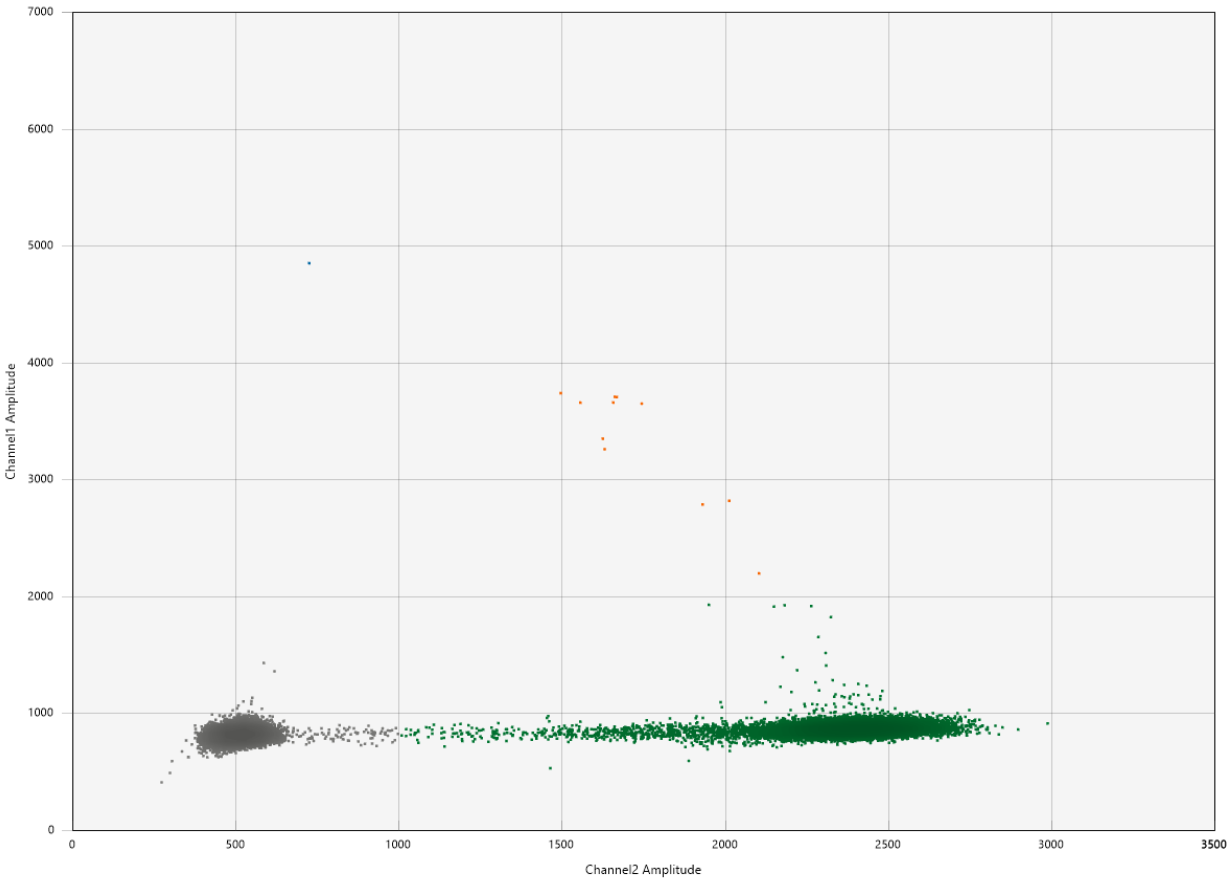
Pre-surgery



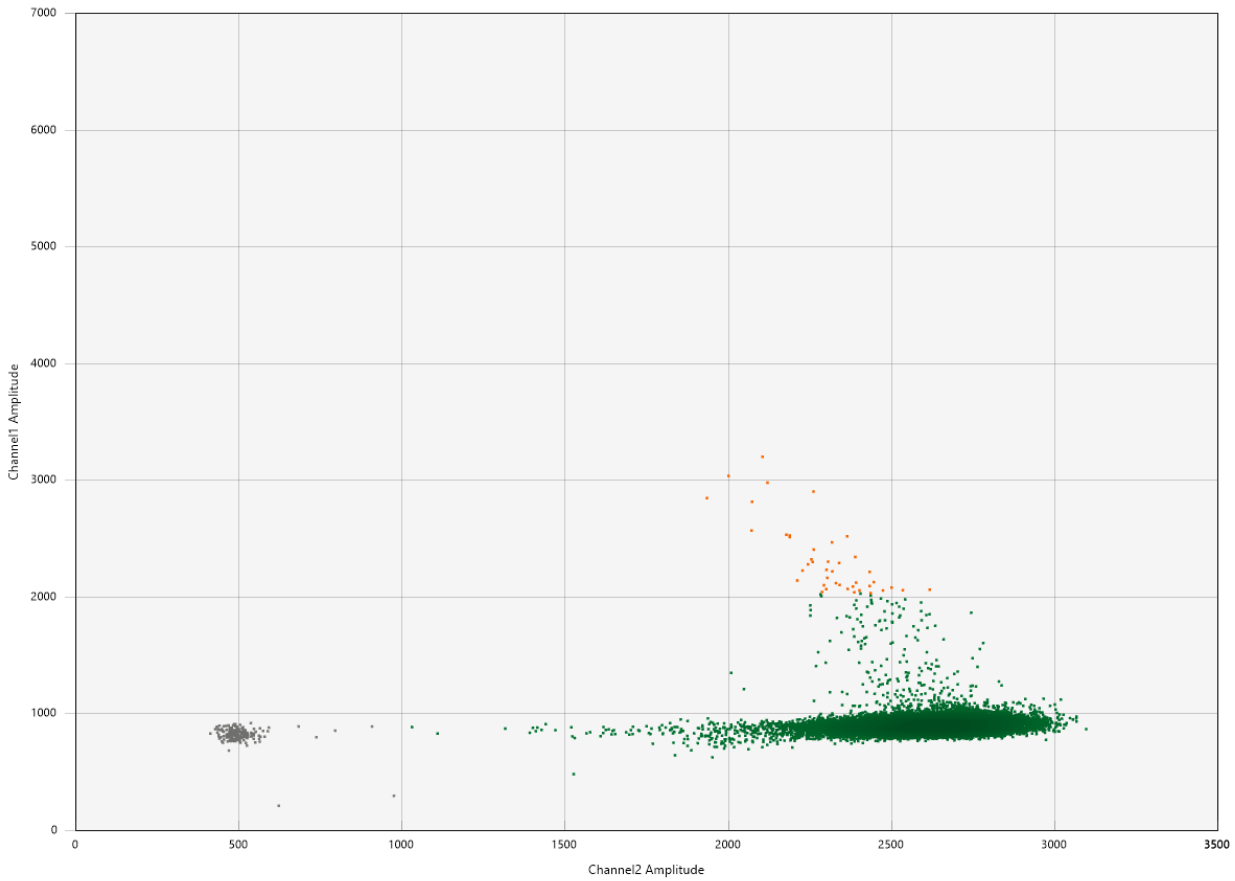
Post-surgery



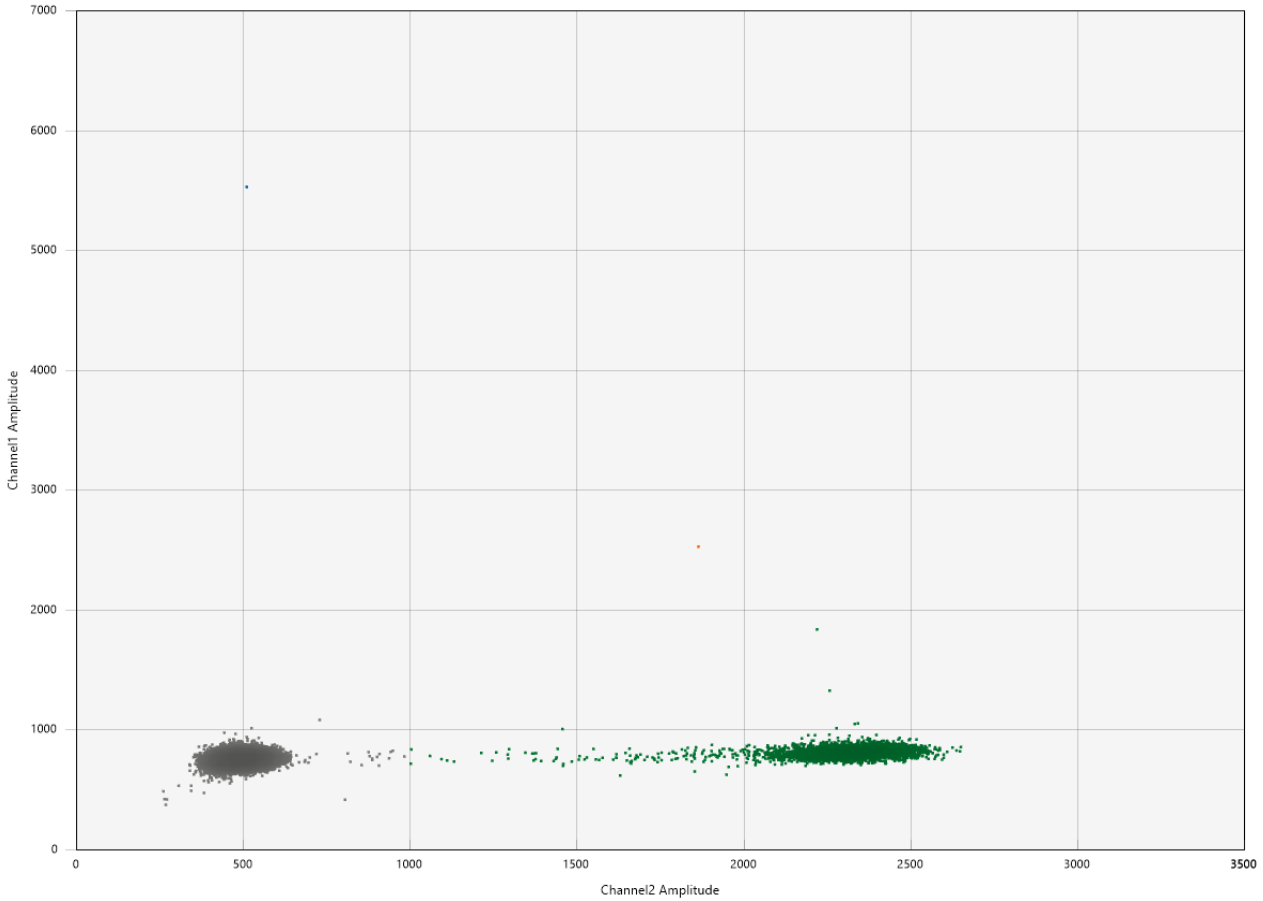
Post-surgery diluted



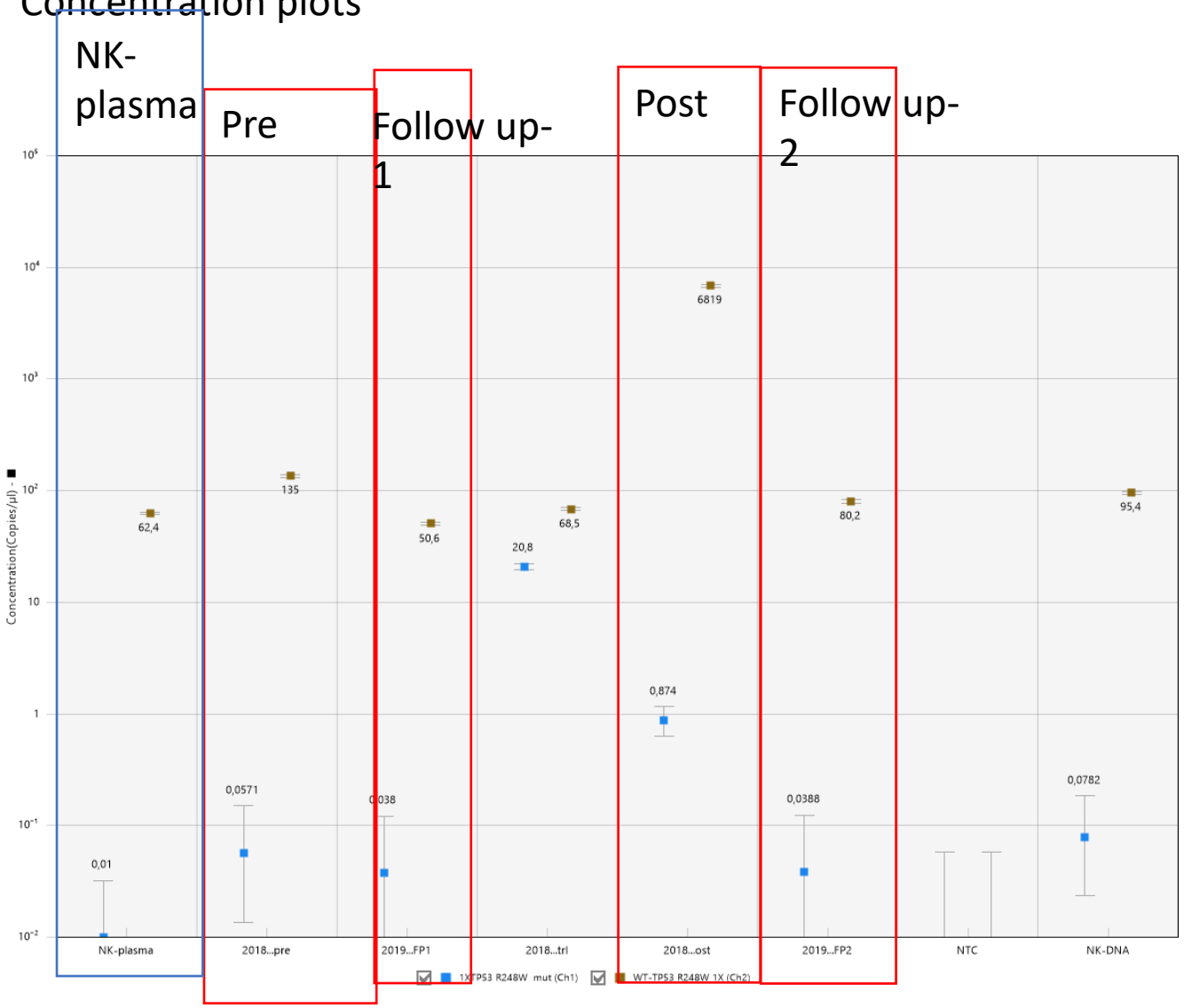
Follow-up 1



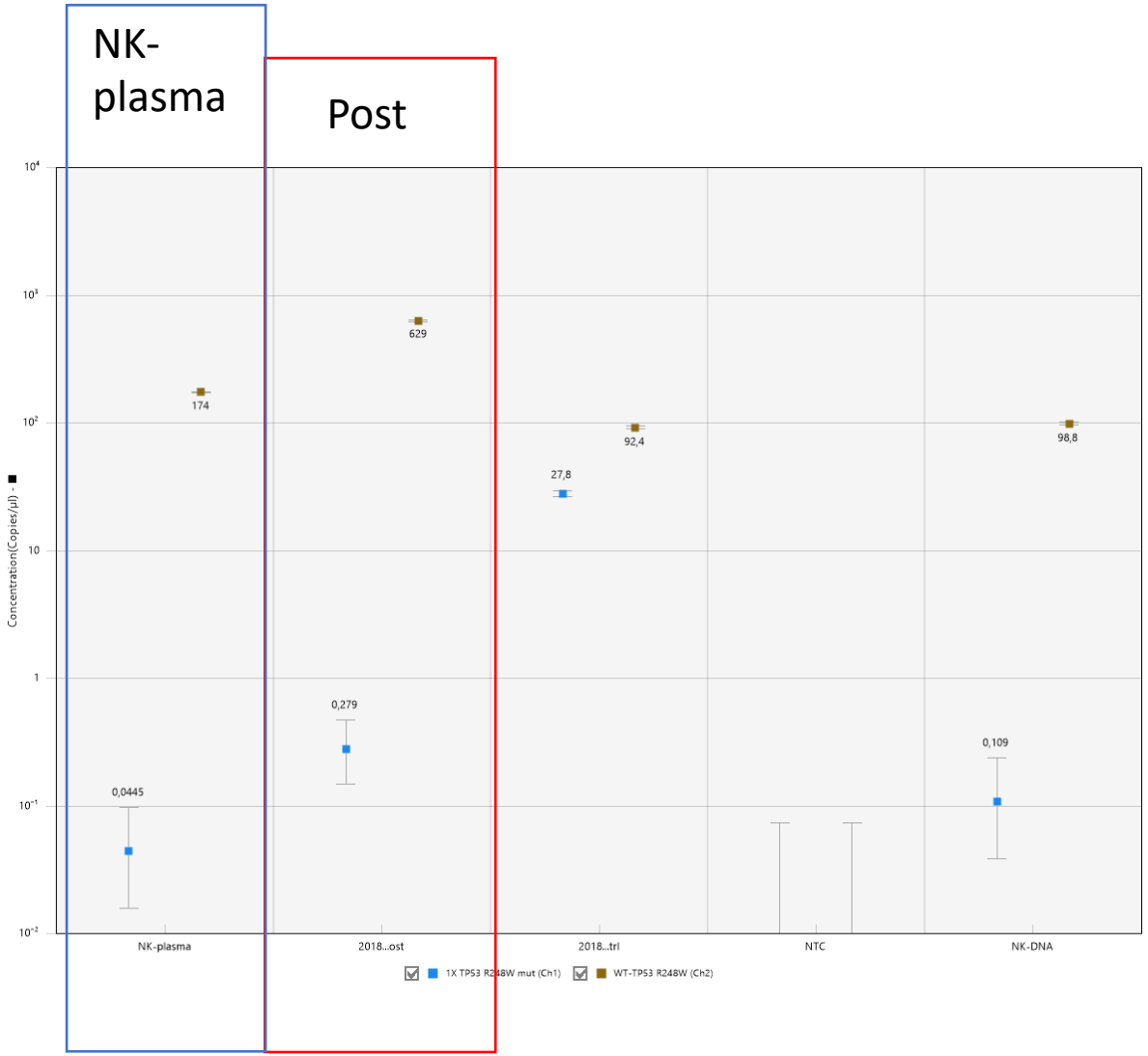
Follow-up 2



Concentration plots



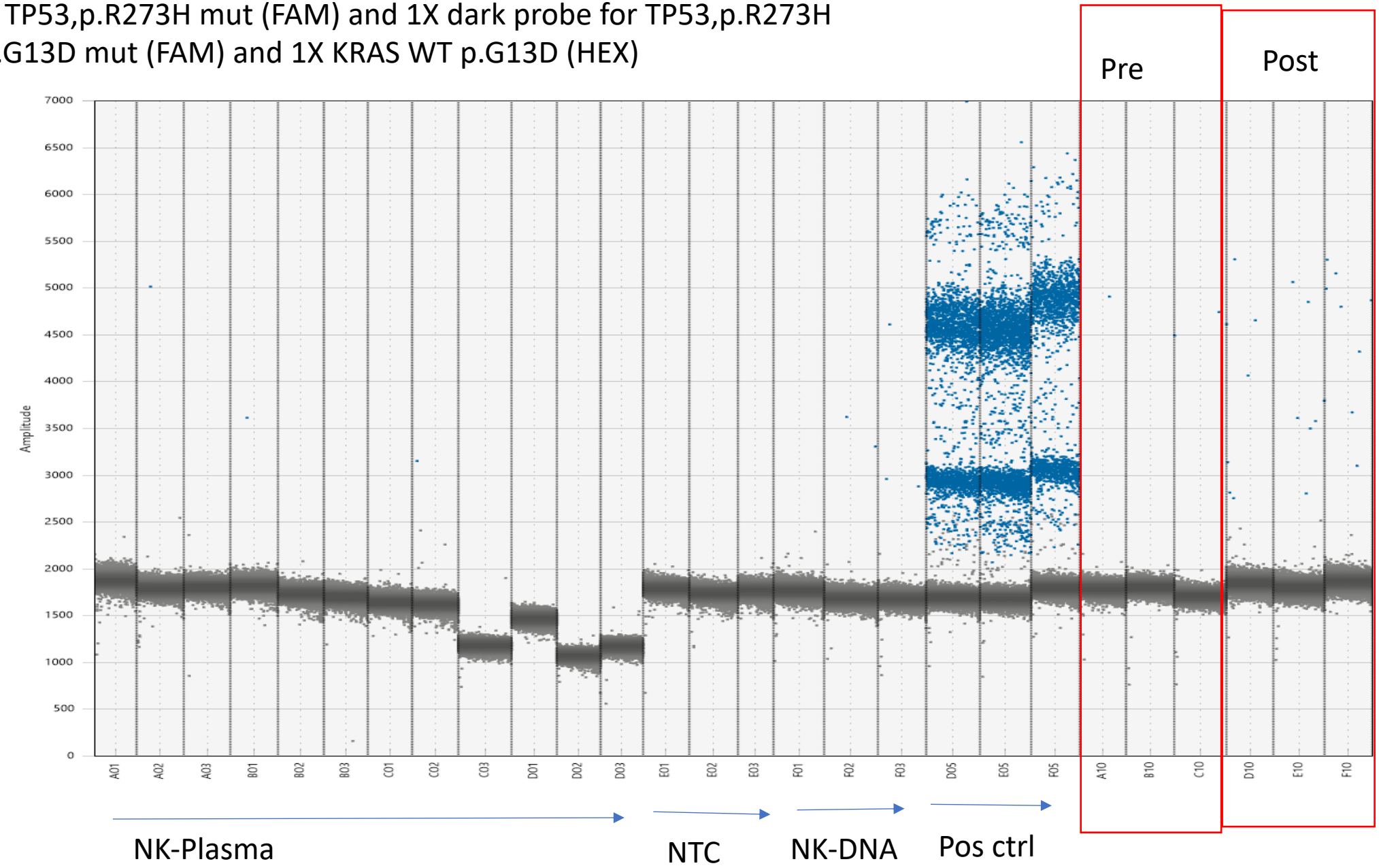
Diluted



P44 -Final

Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

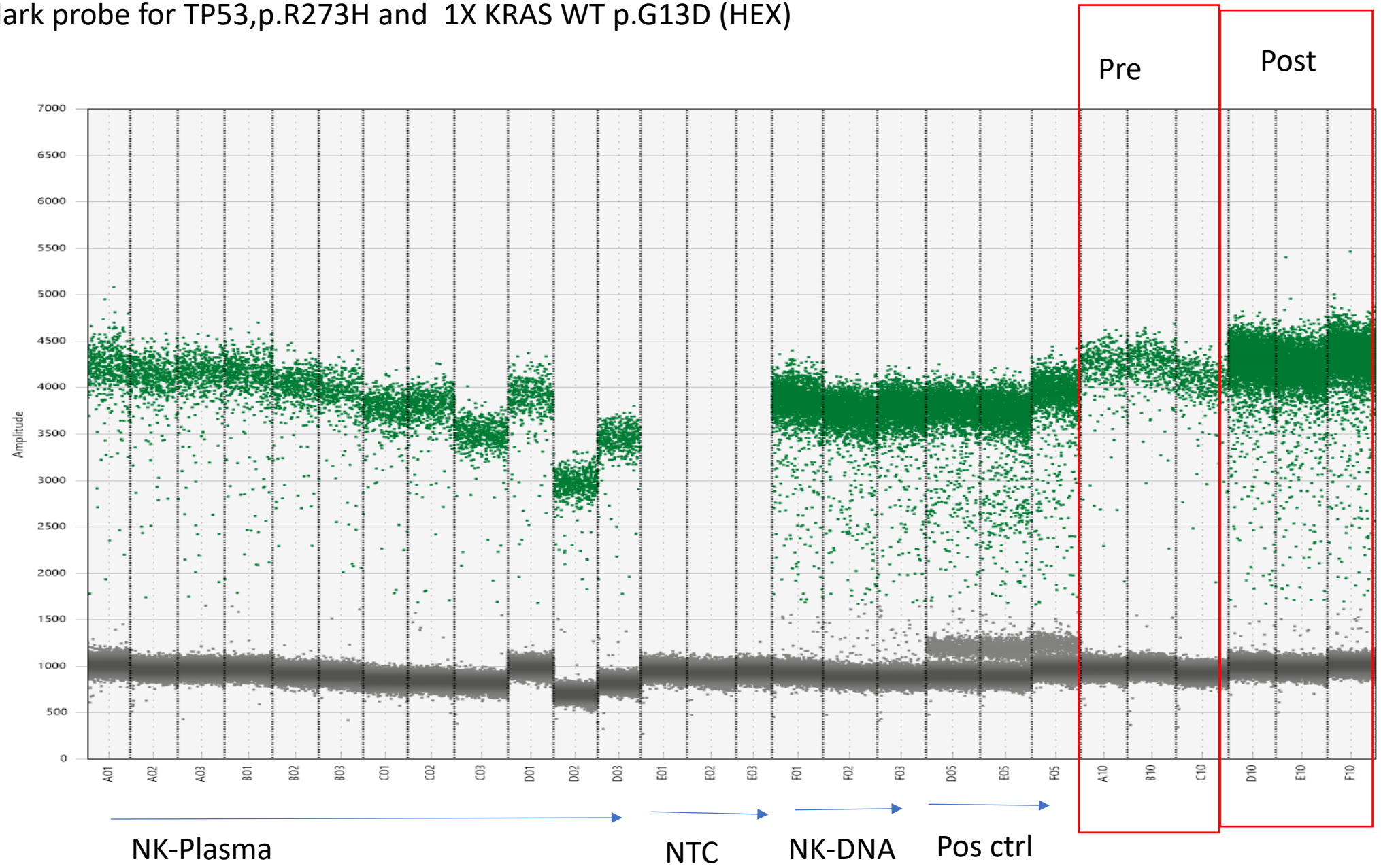
Assays: 0.7X TP53,p.R273H mut (FAM) and 1X dark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and 1X KRAS WT p.G13D (HEX)



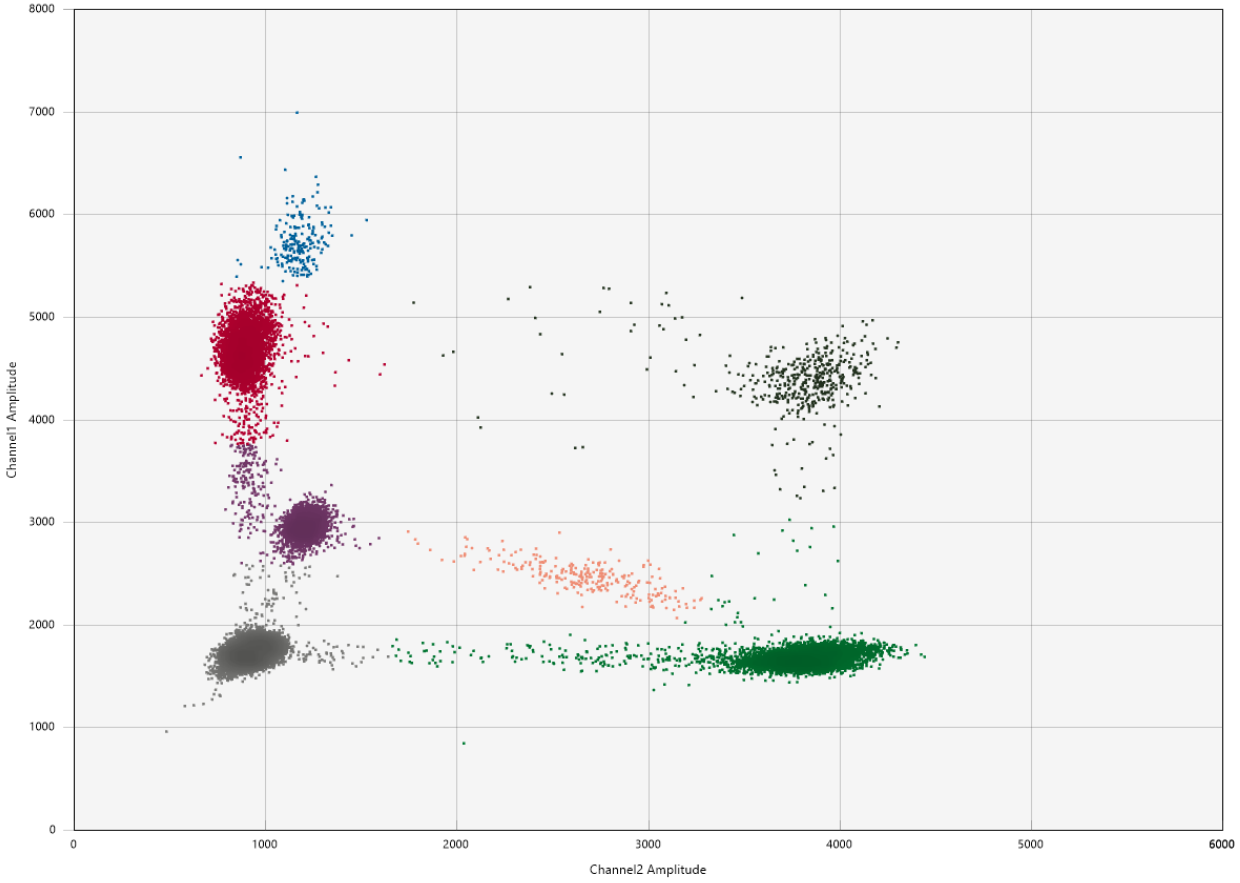
P44 -Final -WT

Assays: 1X dark probe for TP53,p.R273H and 1X KRAS WT p.G13D (HEX)

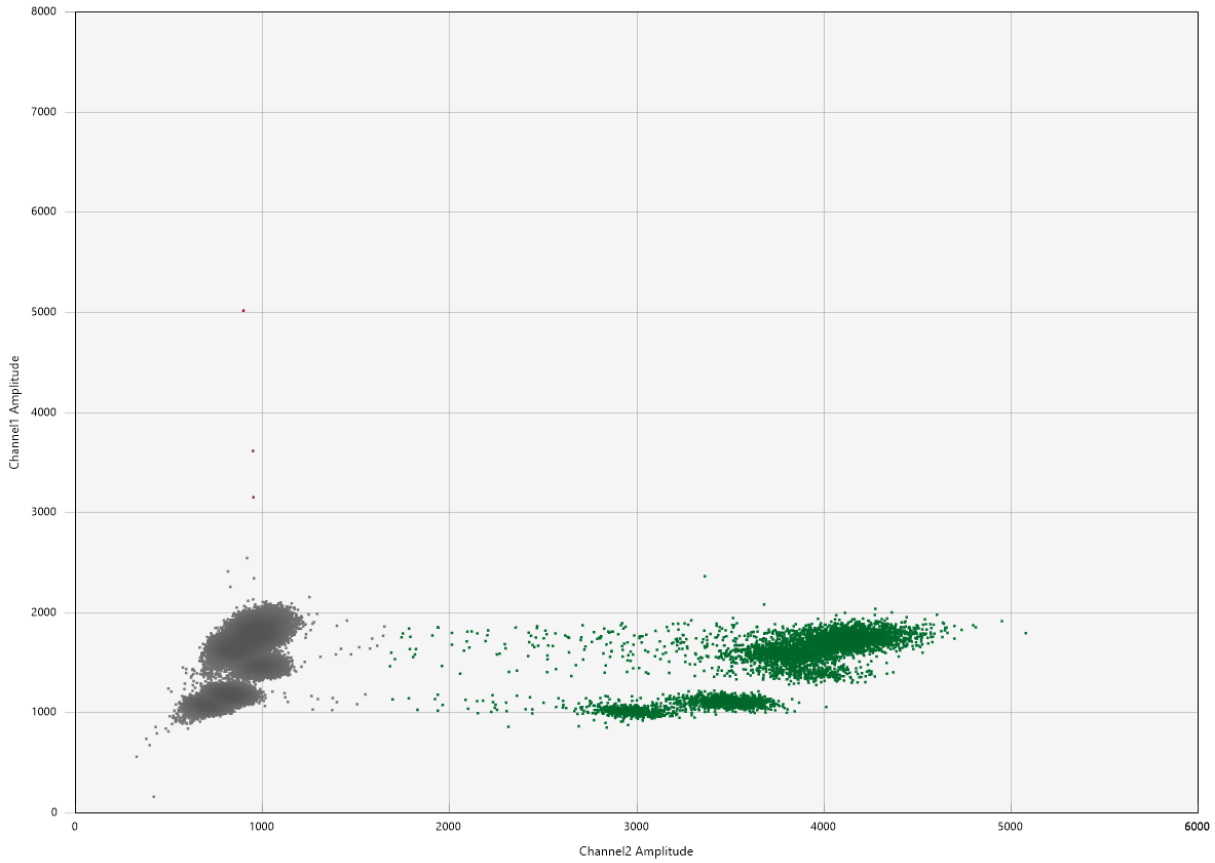
0.3X KRAS WT p.G13D



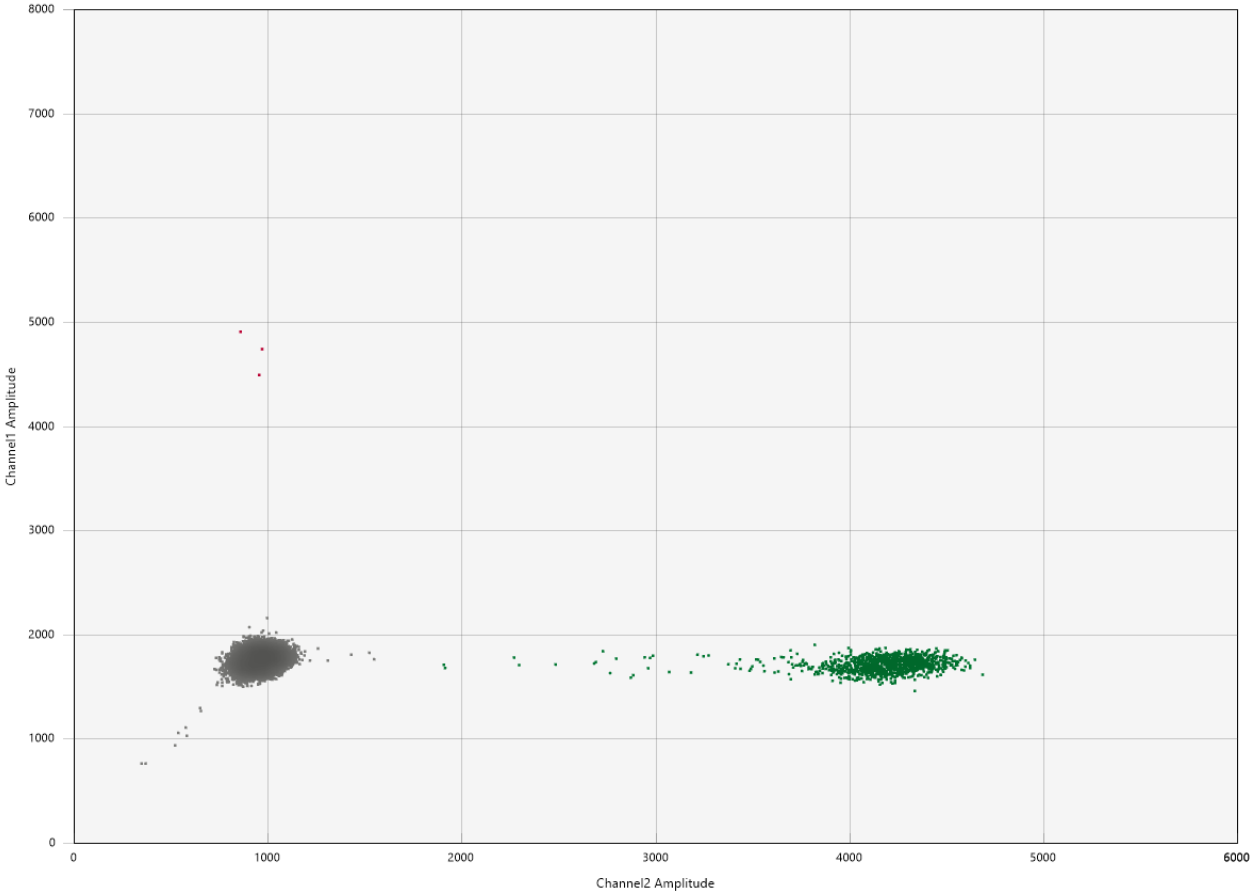
Positive control



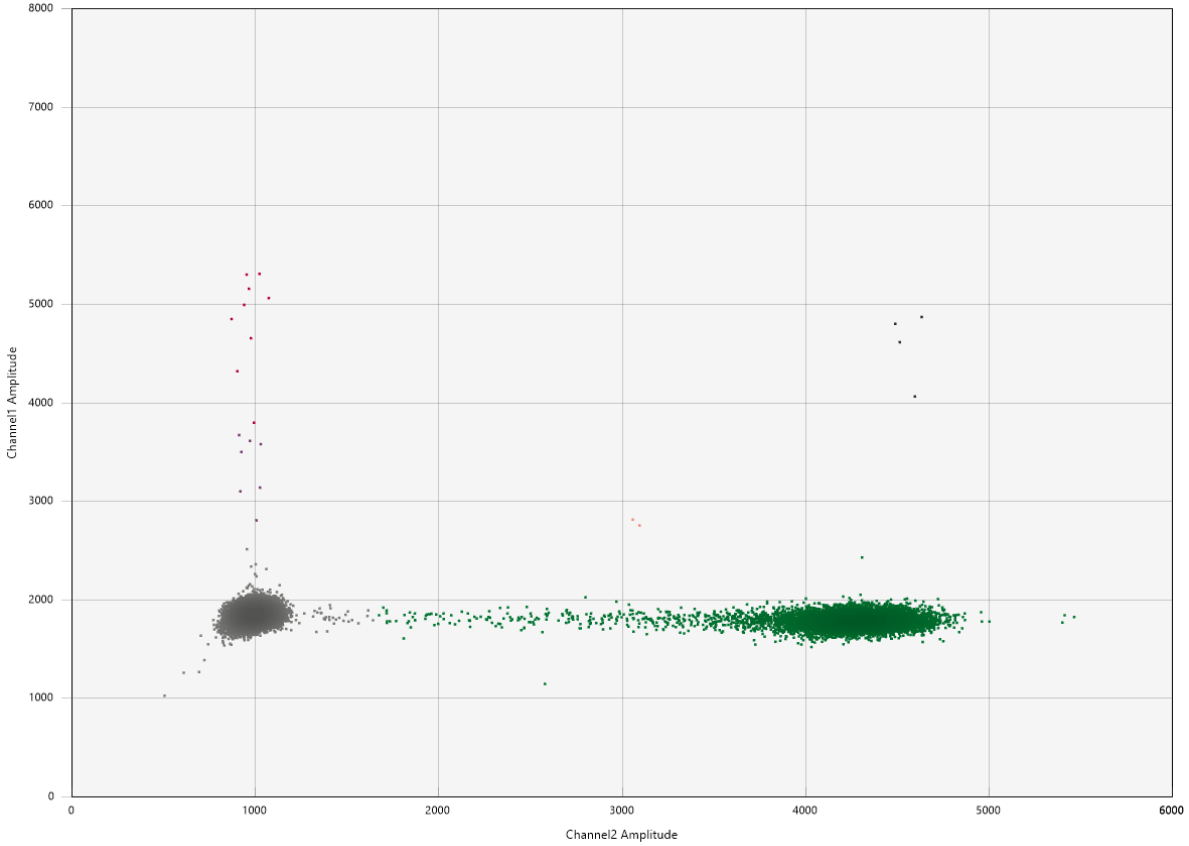
NK-plasma DNA



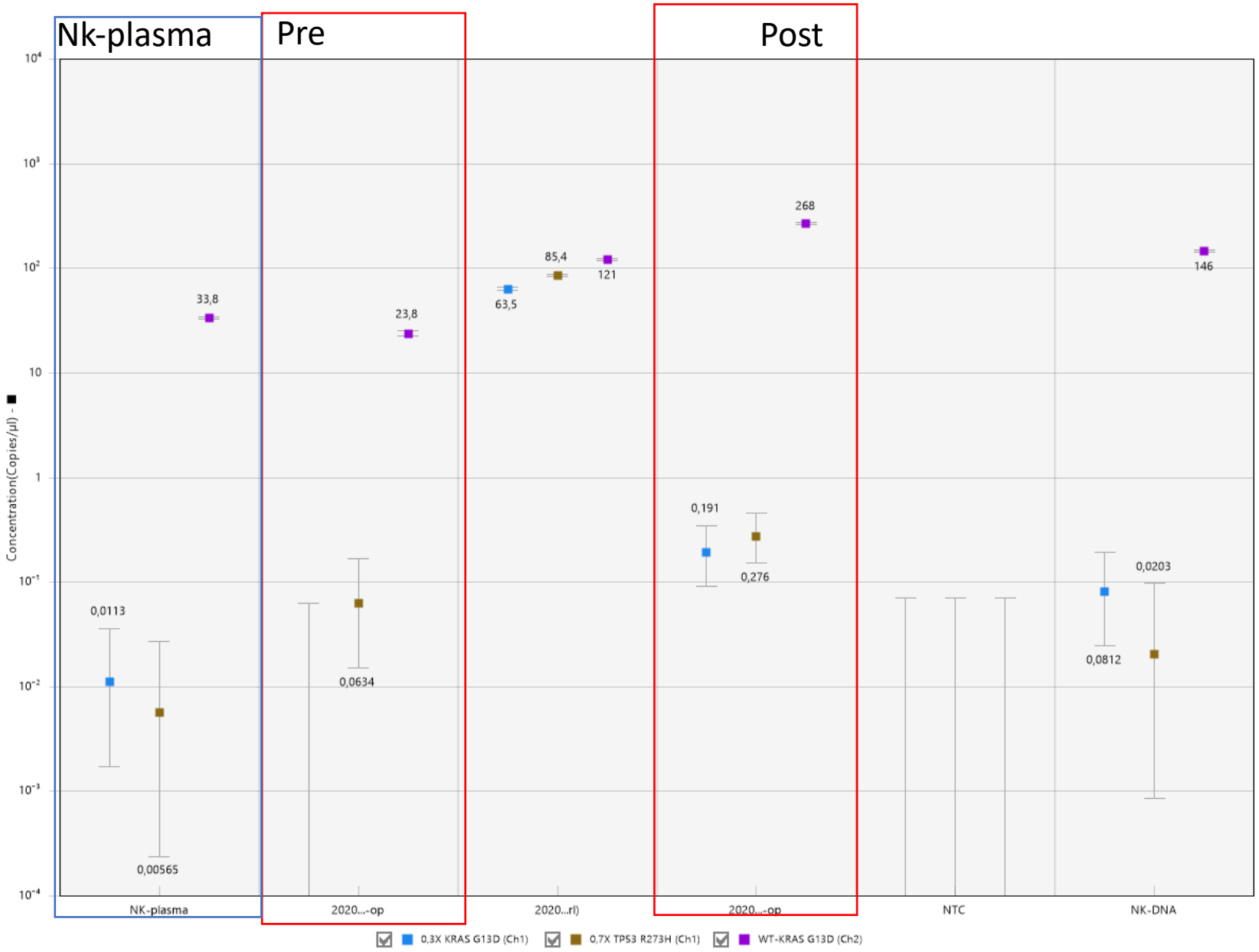
Pre-surgery



Post-surgery



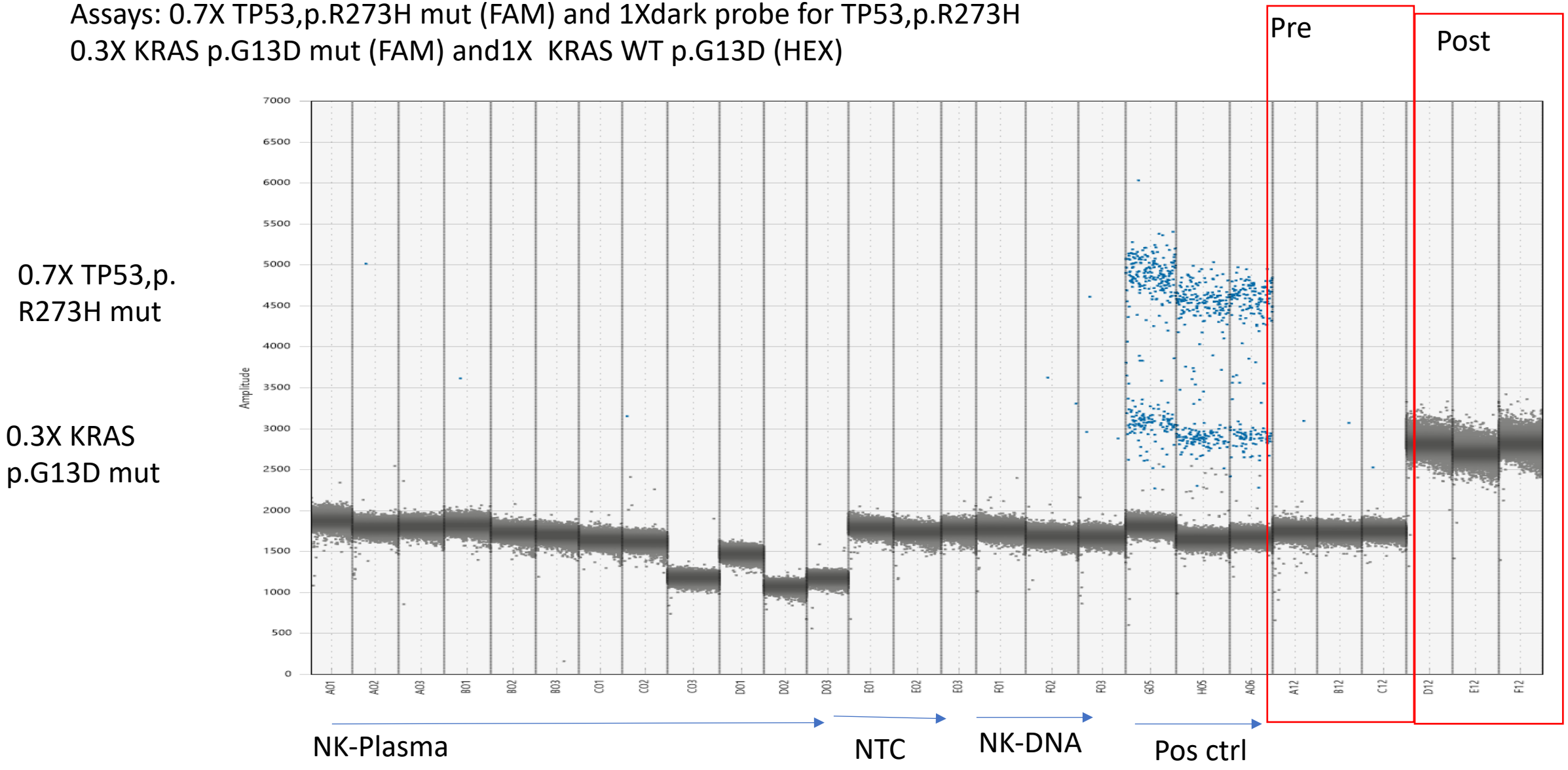
Concentration plots



P23 -Final

Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

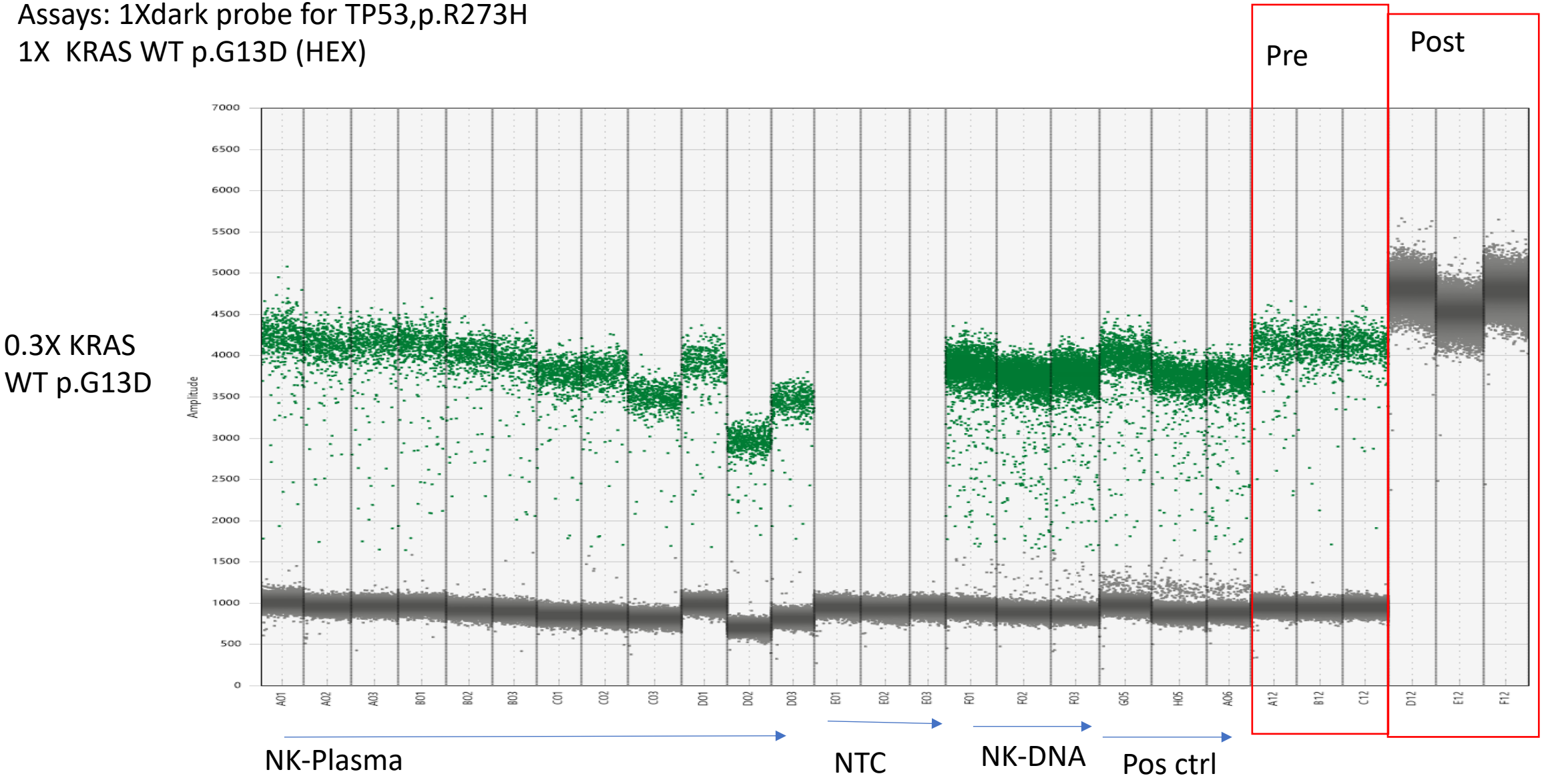
Assays: 0.7X TP53,p.R273H mut (FAM) and 1Xdark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and1X KRAS WT p.G13D (HEX)



P23 -Final -WT

Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

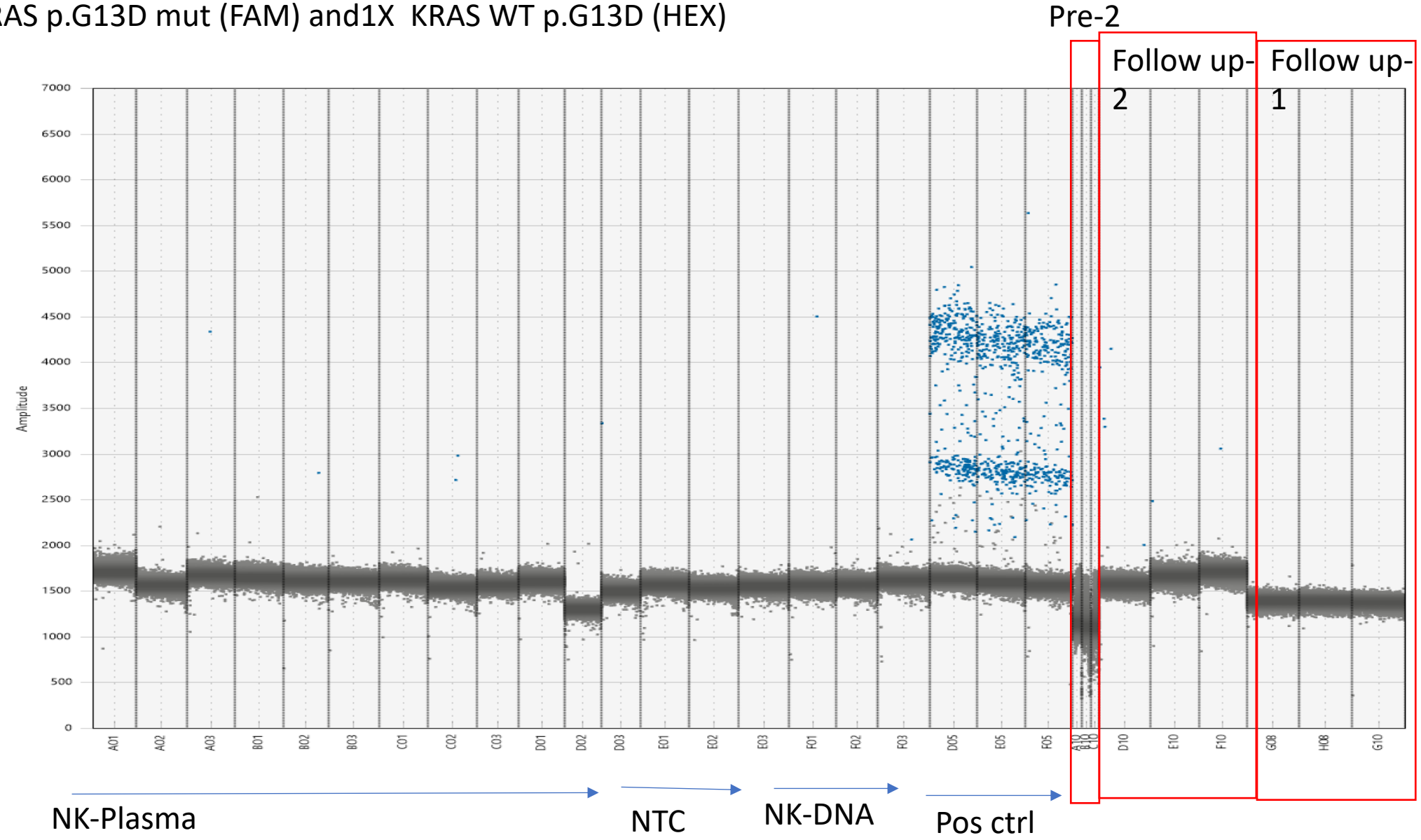
Assays: 1Xdark probe for TP53,p.R273H
1X KRAS WT p.G13D (HEX)



P23 - Final

Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

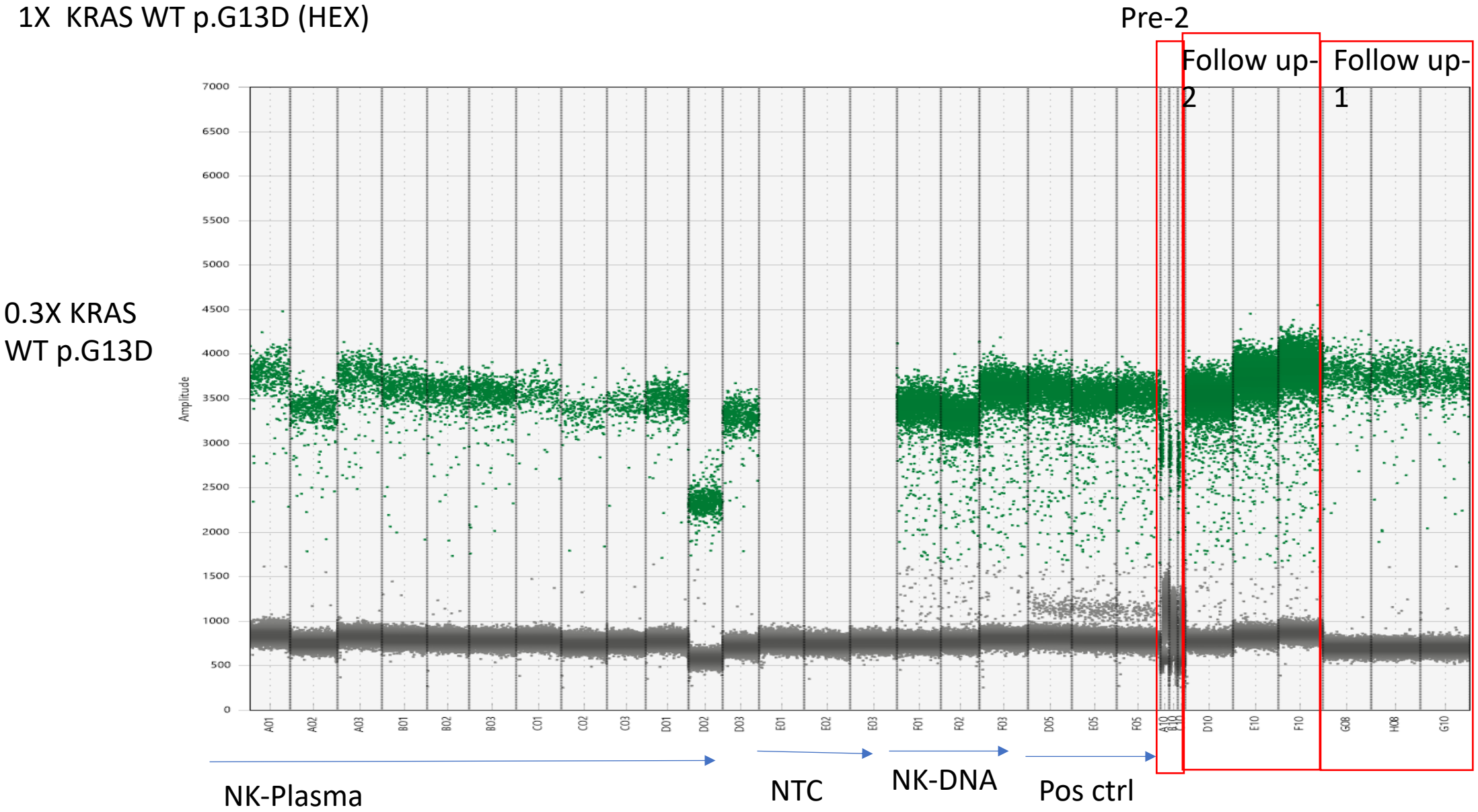
Assays: 0.7X TP53,p.R273H mut (FAM) and 1Xdark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and1X KRAS WT p.G13D (HEX)



P23 -Final-WT

Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

Assays: 1Xdark probe for TP53,p.R273H
1X KRAS WT p.G13D (HEX)



P23 -diluted

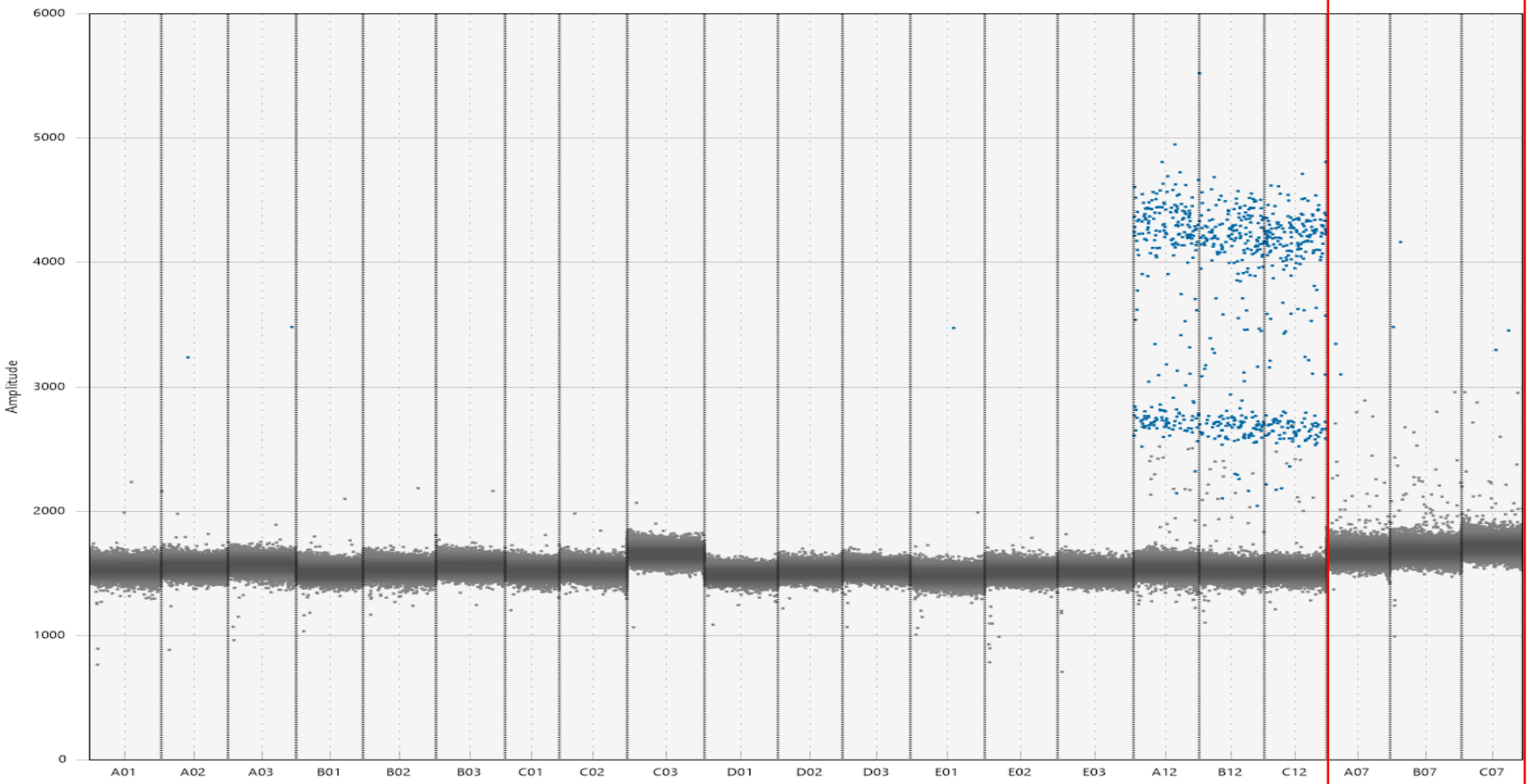
Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

Assays: 0.7X TP53,p.R273H mut (FAM) and 1Xdark probe for TP53,p.R273H
0.3X KRAS p.G13D mut (FAM) and1X KRAS WT p.G13D (HEX)

Post

0.7X TP53,p. R273H mut

0.3X KRAS p.G13D mut



NK-Plasma

NTC

NK-DNA

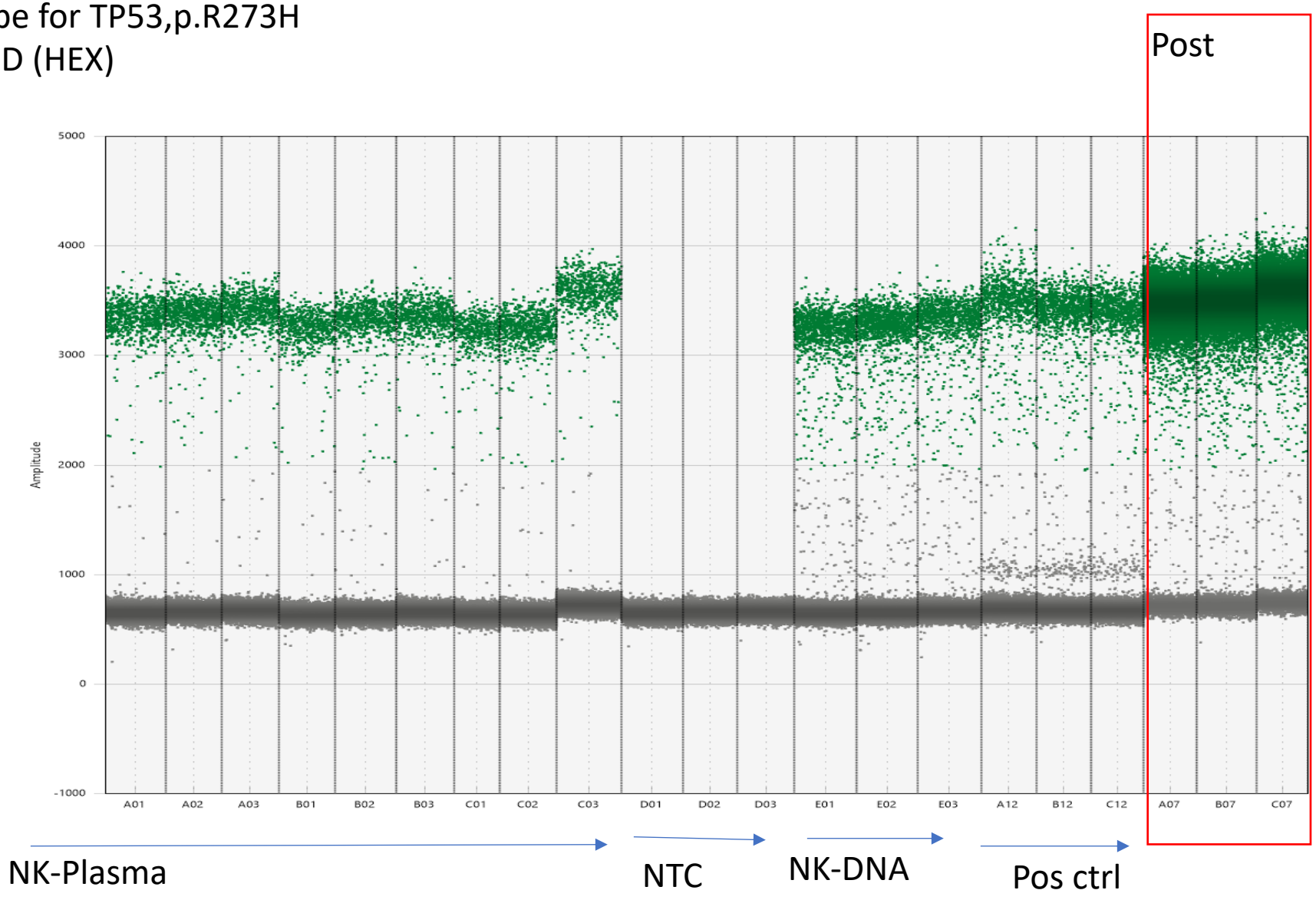
Pos ctrl

P23 -Final

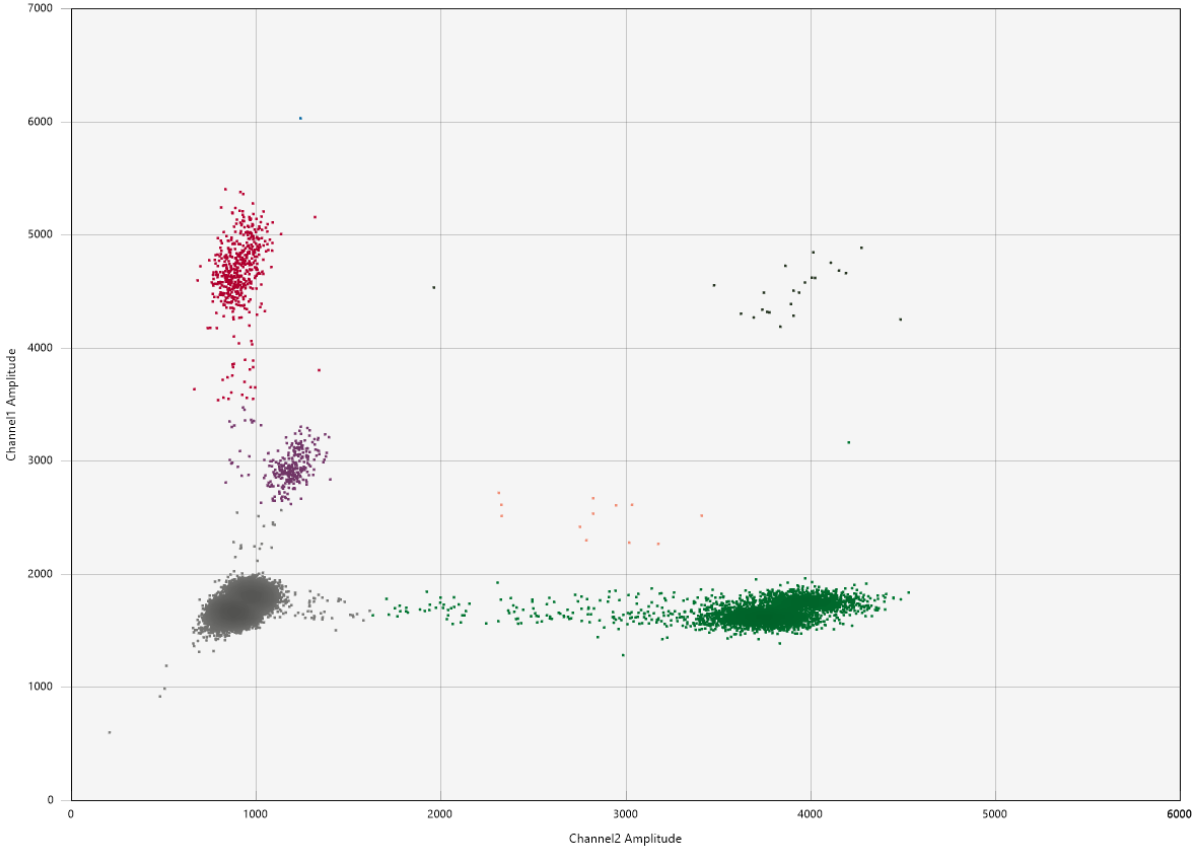
Gene target : TP53,p.R273H, c.818G>A and KRAS,p.G13D, c.38G>A

Assays: 1Xdark probe for TP53,p.R273H
1X KRAS WT p.G13D (HEX)

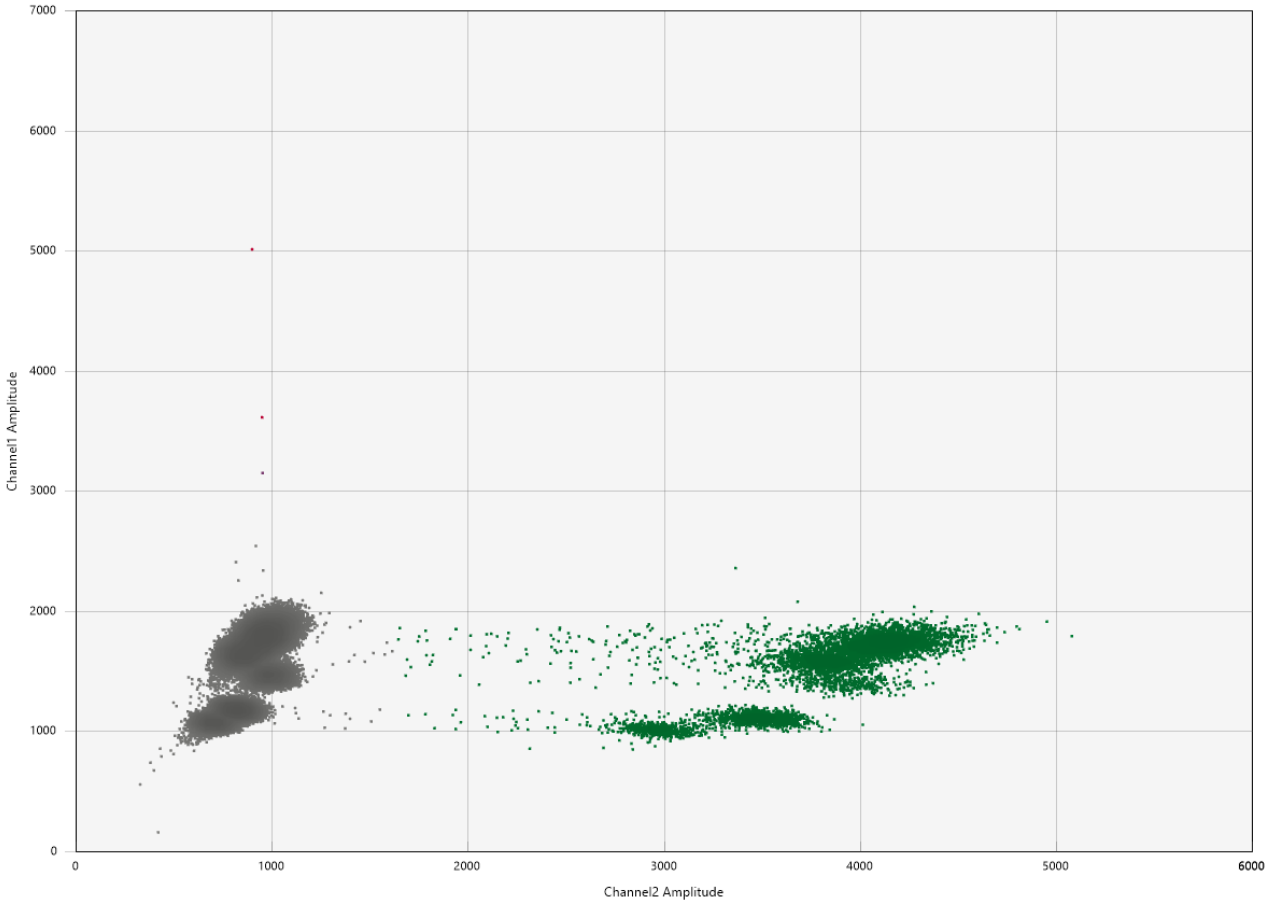
0.3X KRAS
WT p.G13D



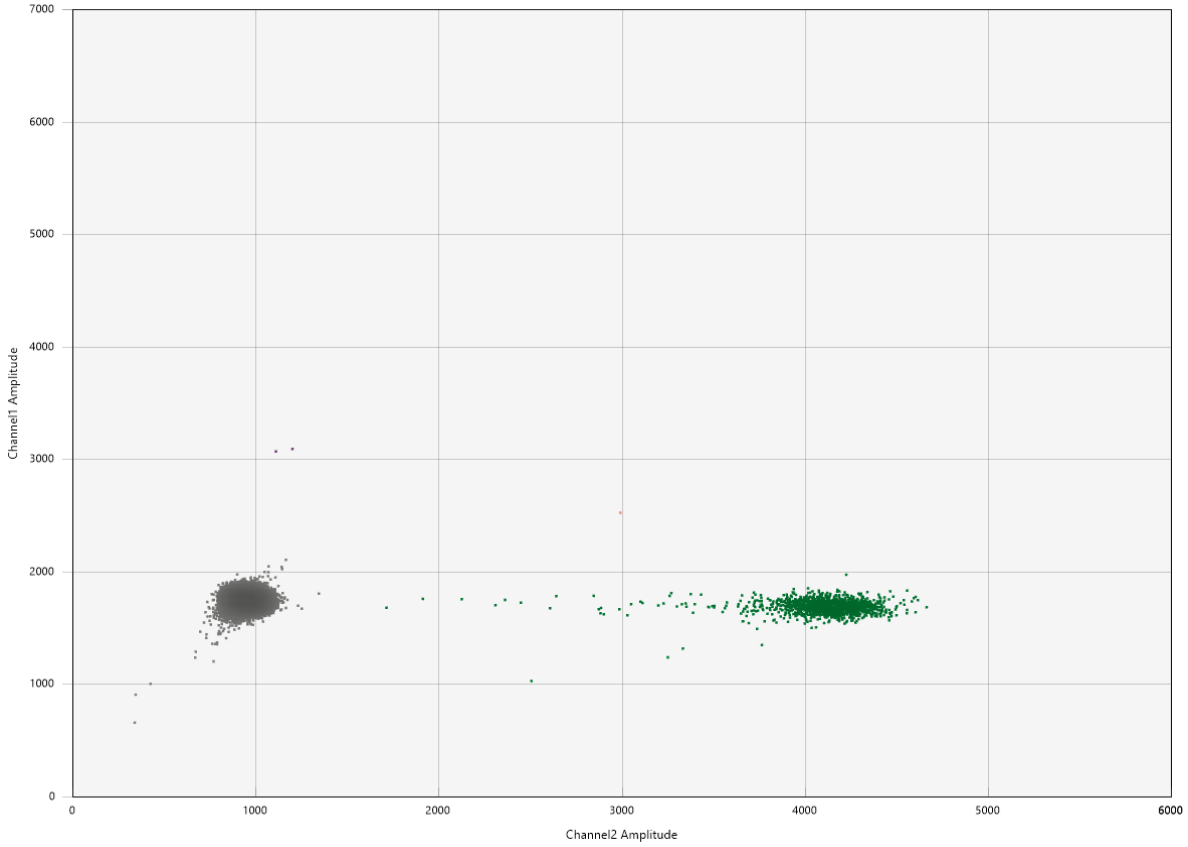
Positive control



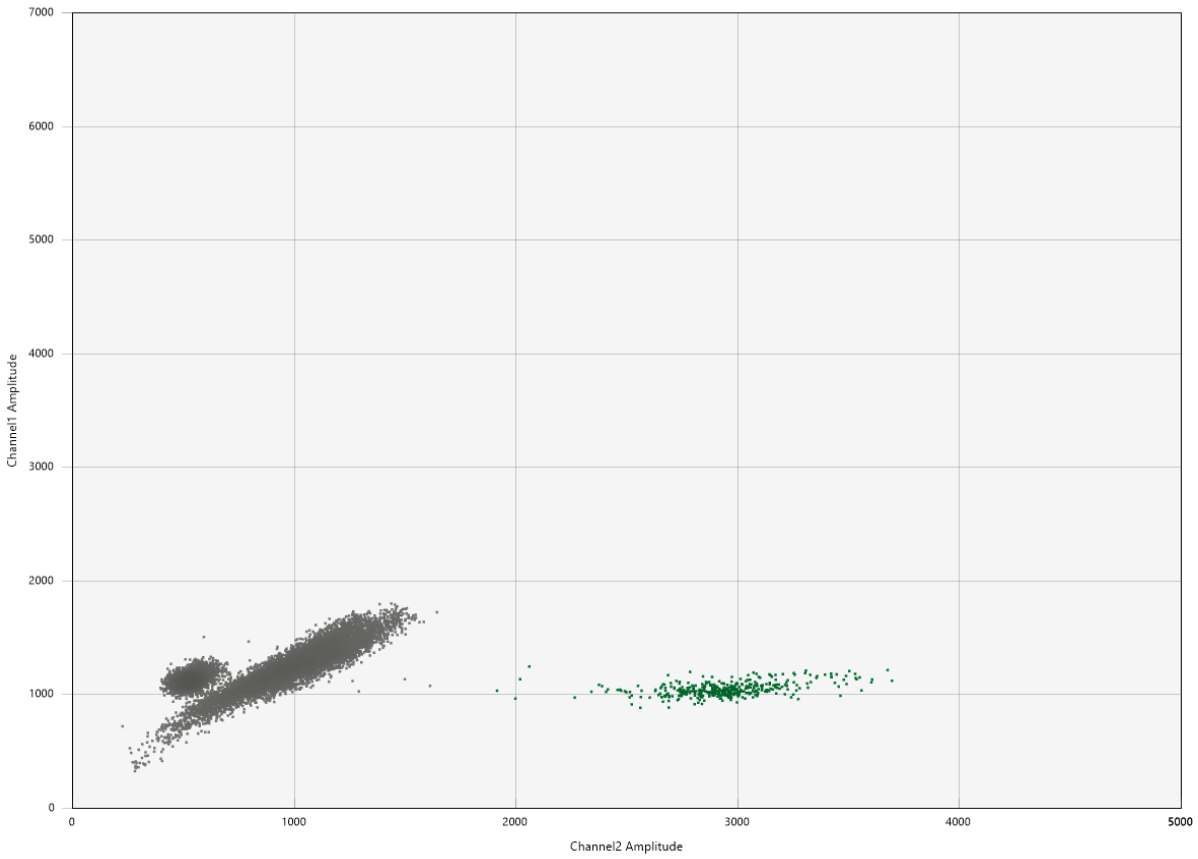
Nk-Plasma



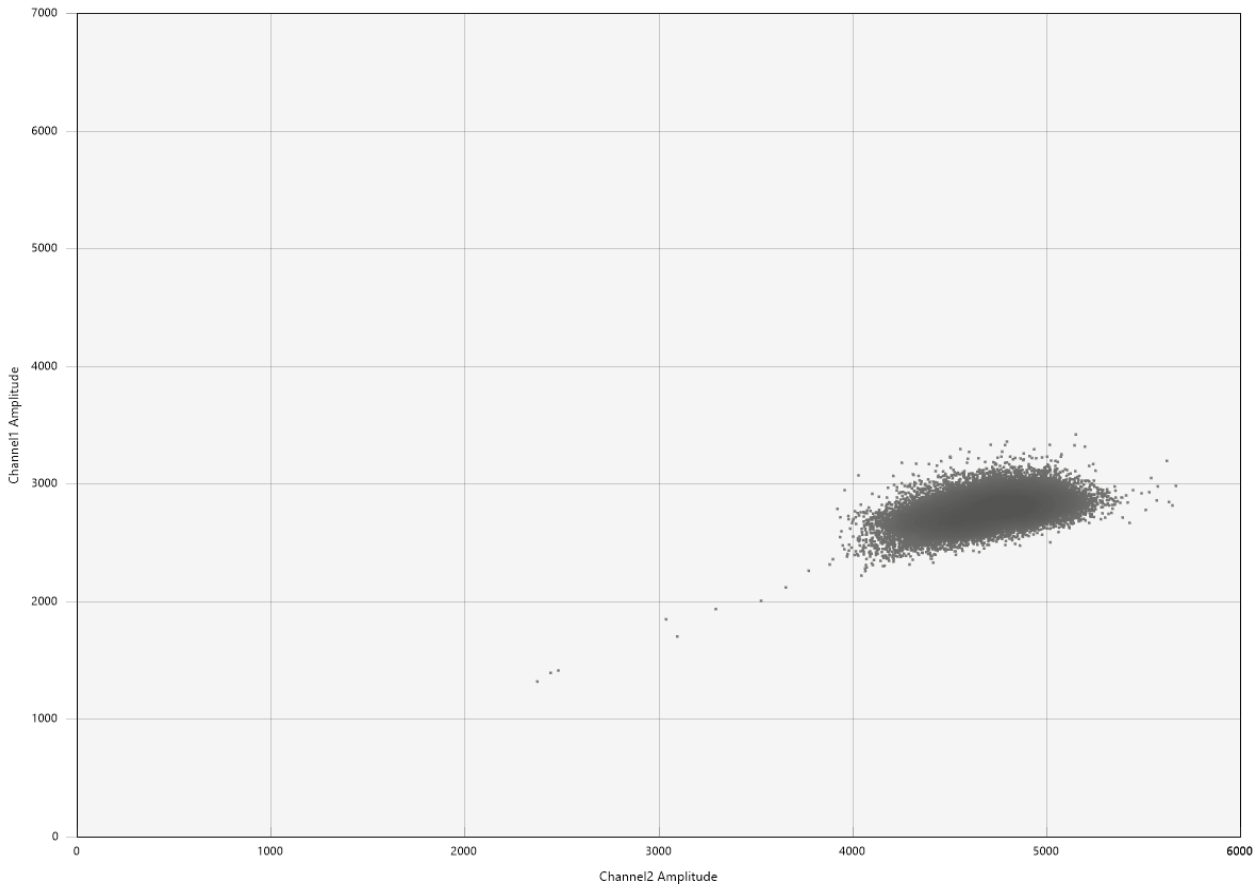
Pre-surgery



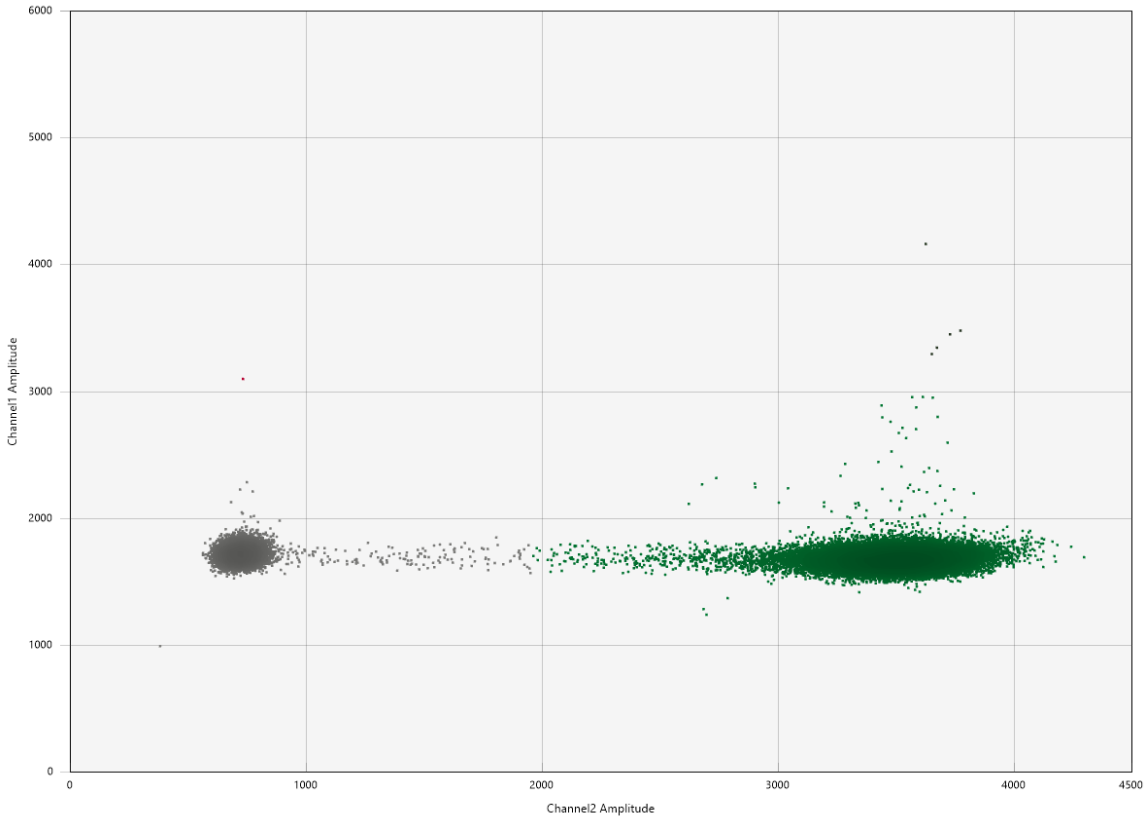
Pre-surgery-2



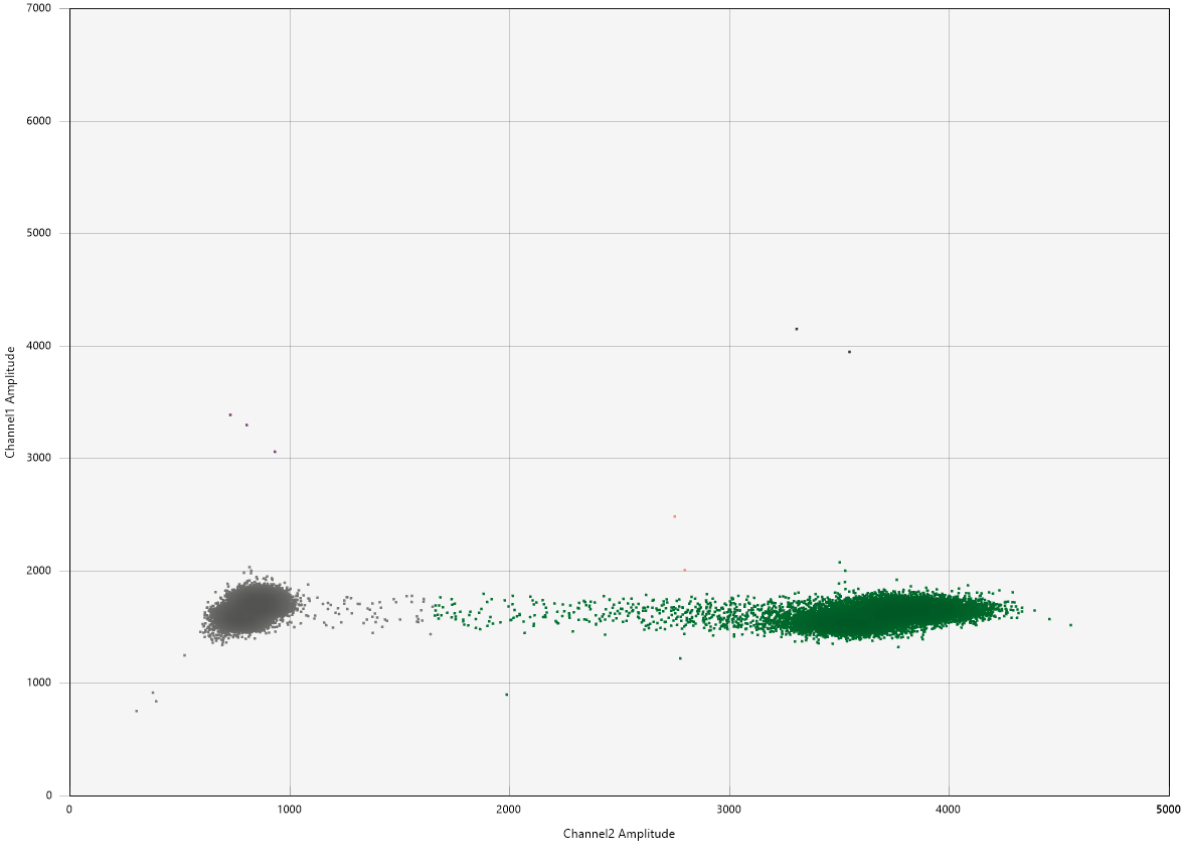
Post-surgery*



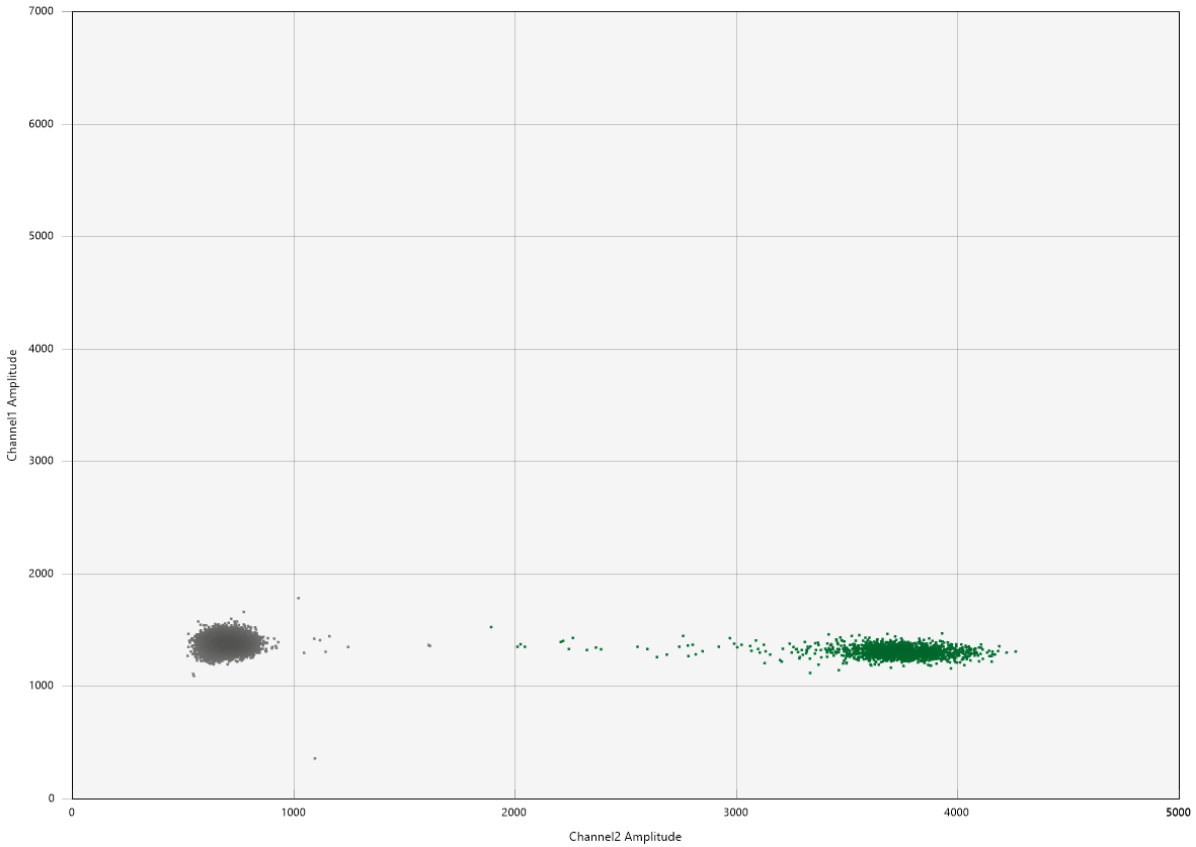
Post-surgery*- diluted



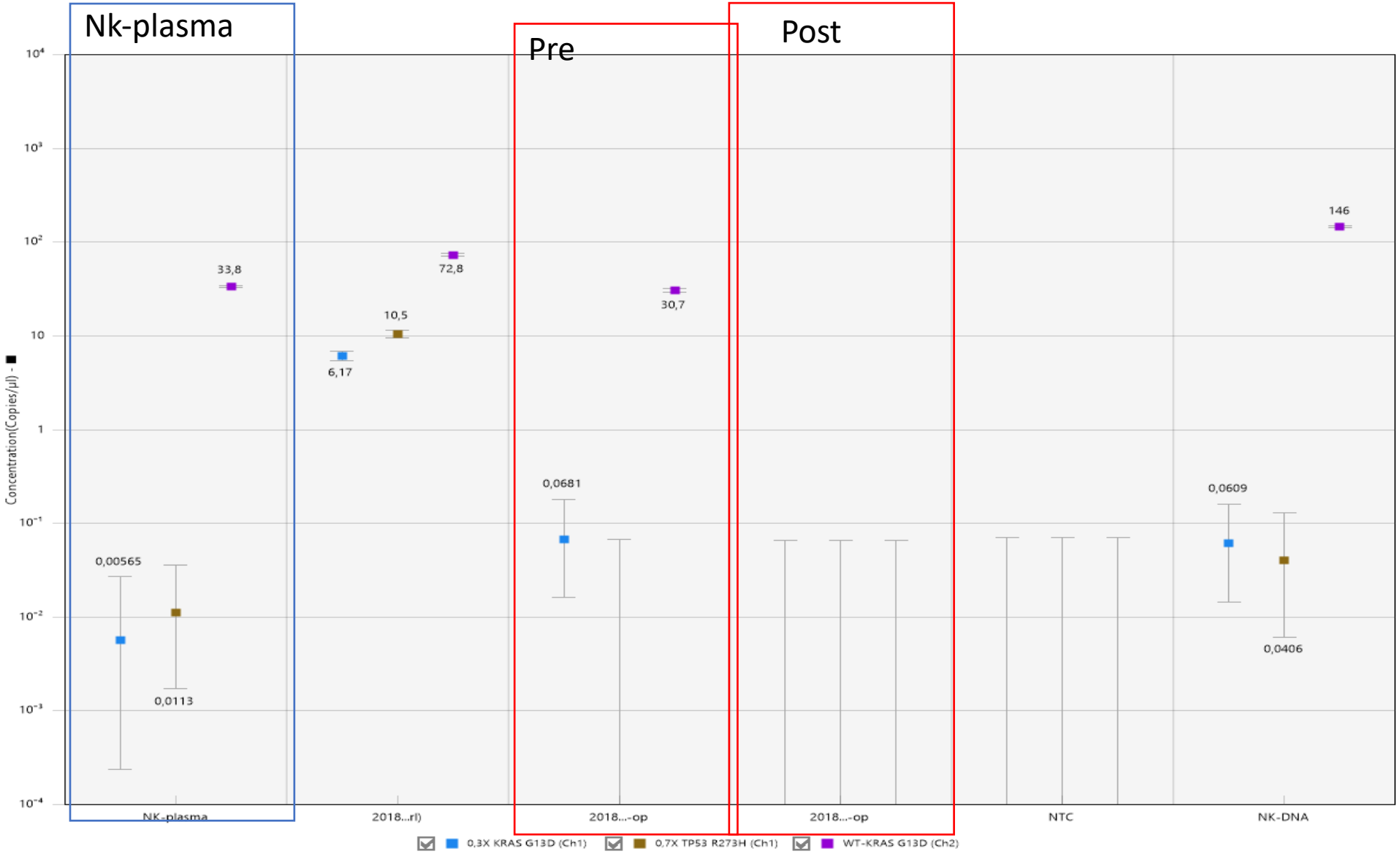
Follow- up-1



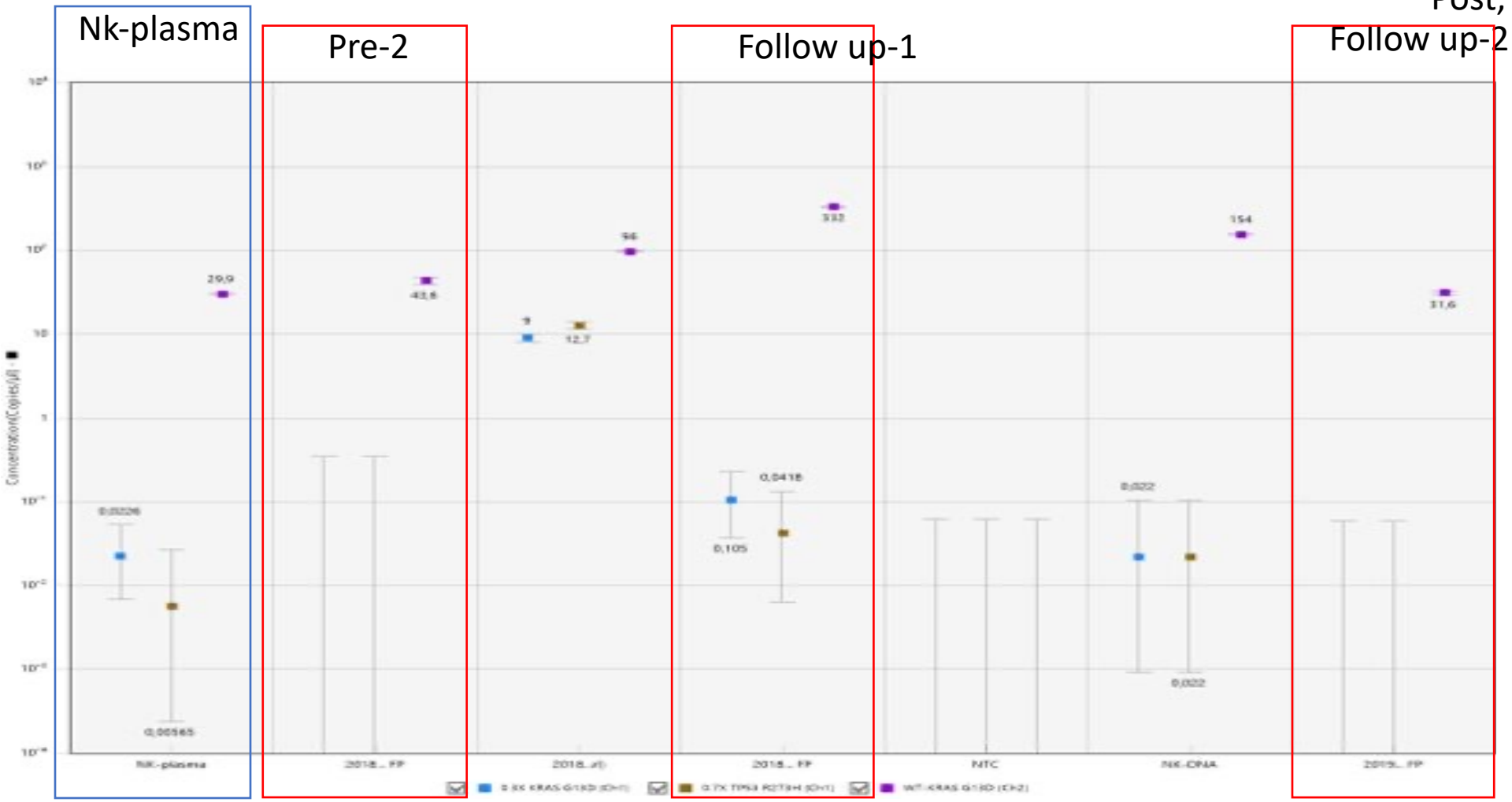
Follow-up-2



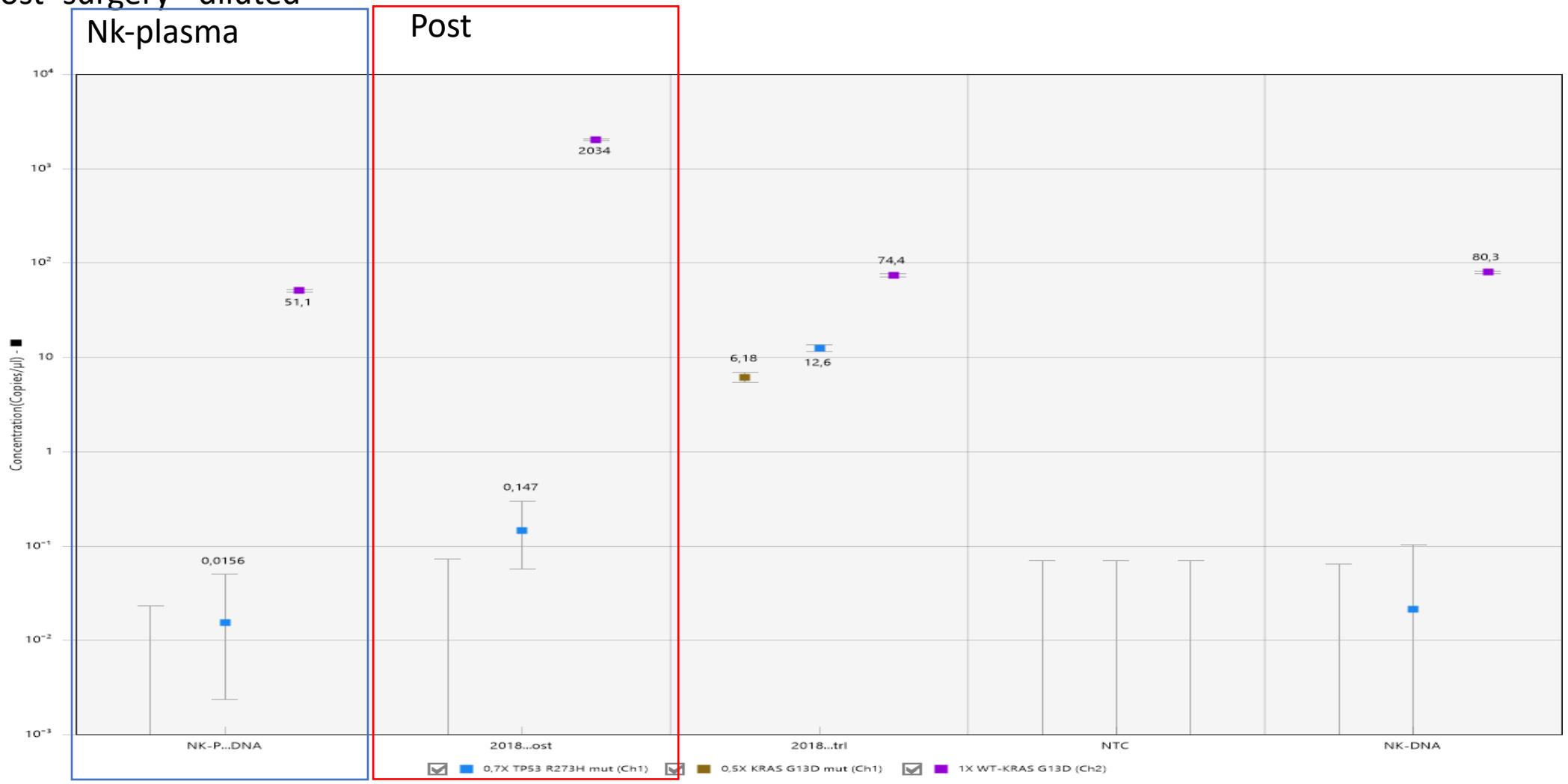
Concentration plots



Post, Follow-up 1 and 2



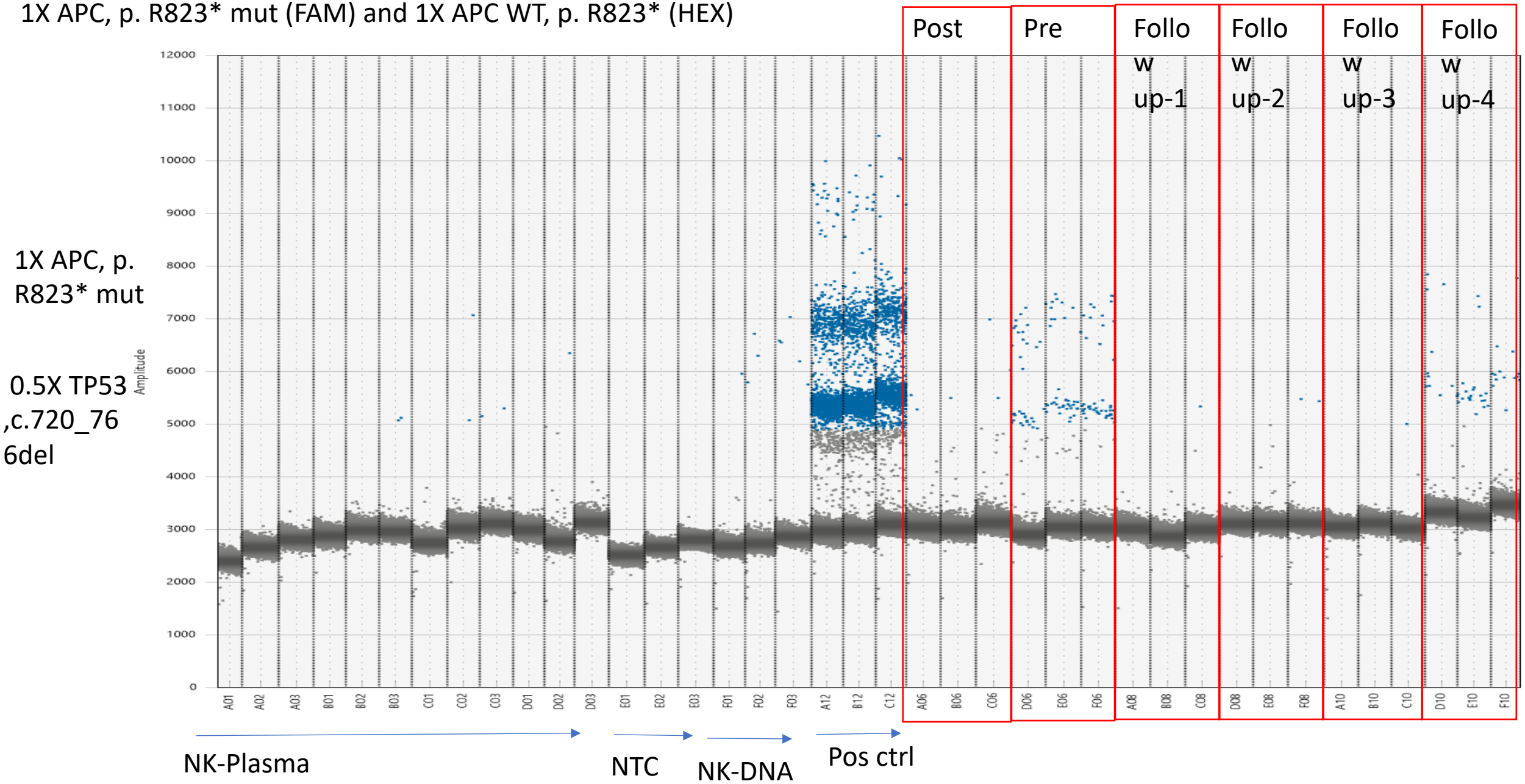
Post- surgery - diluted



P12 - Final

Gene target : APC, p. R823*, c.2467C>T and TP53,c.720_766del

Assays: 0.5X TP53,c.720_766del mut (FAM)
1X APC, p. R823* mut (FAM) and 1X APC WT, p. R823* (HEX)

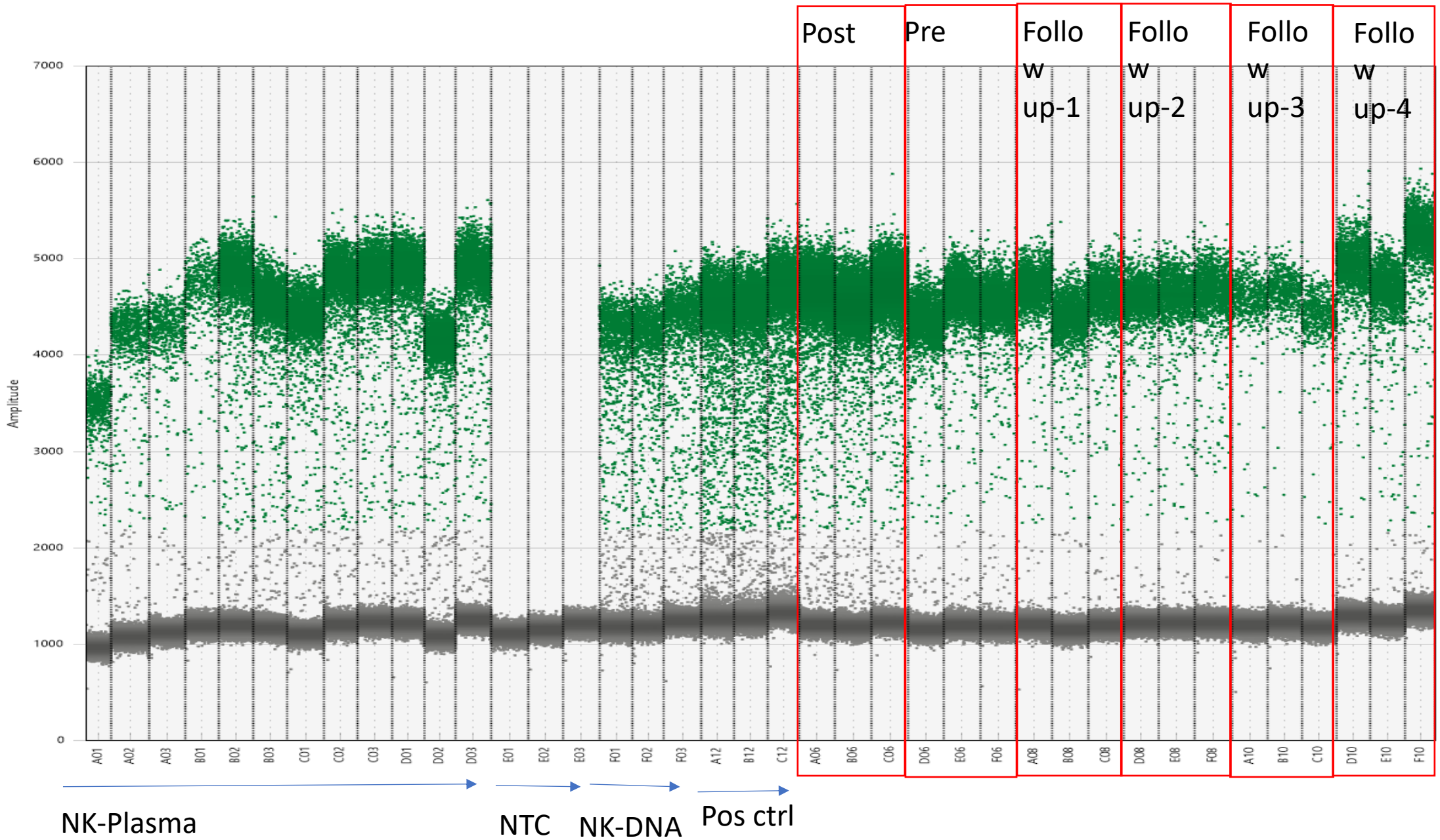


P12 – Final WT

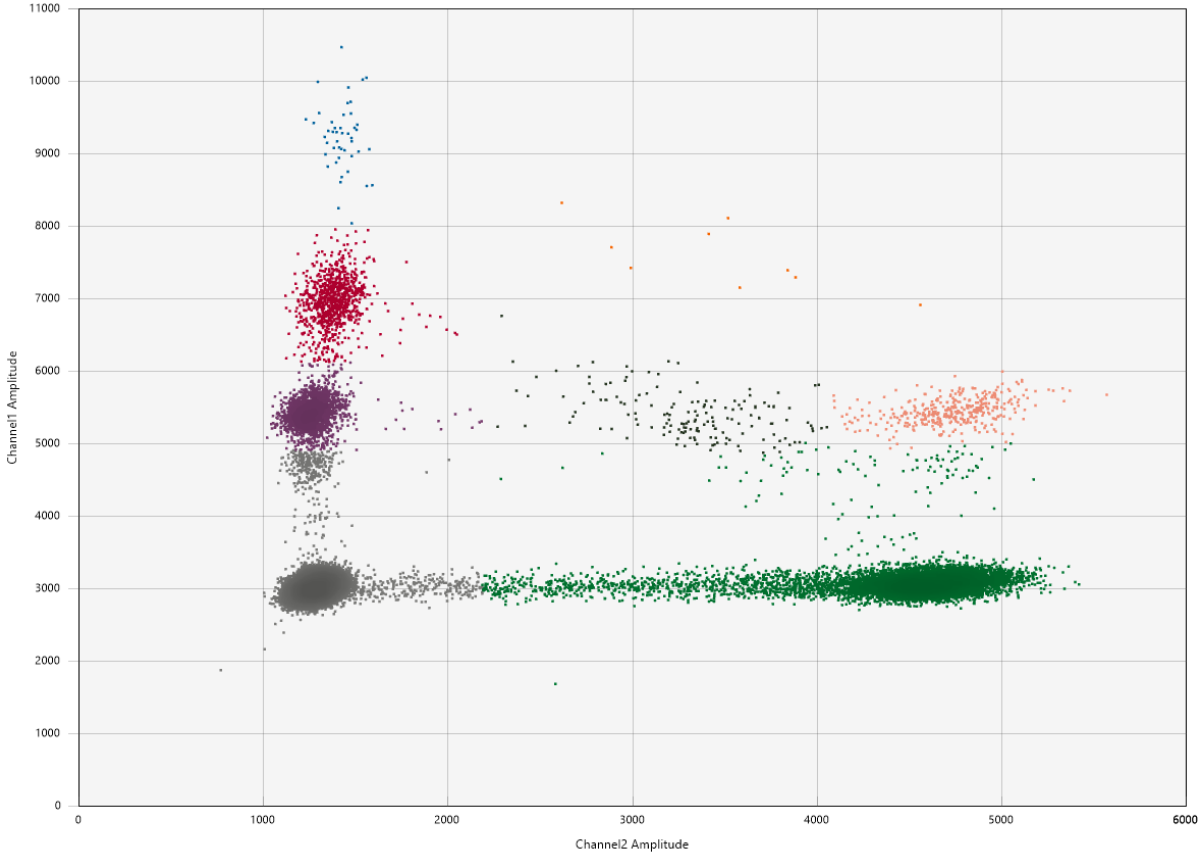
Gene target : APC, p. R823*, c.2467C>T and TP53,c.720_766del

Assays: 1X APC WT, p. R823* (HEX)

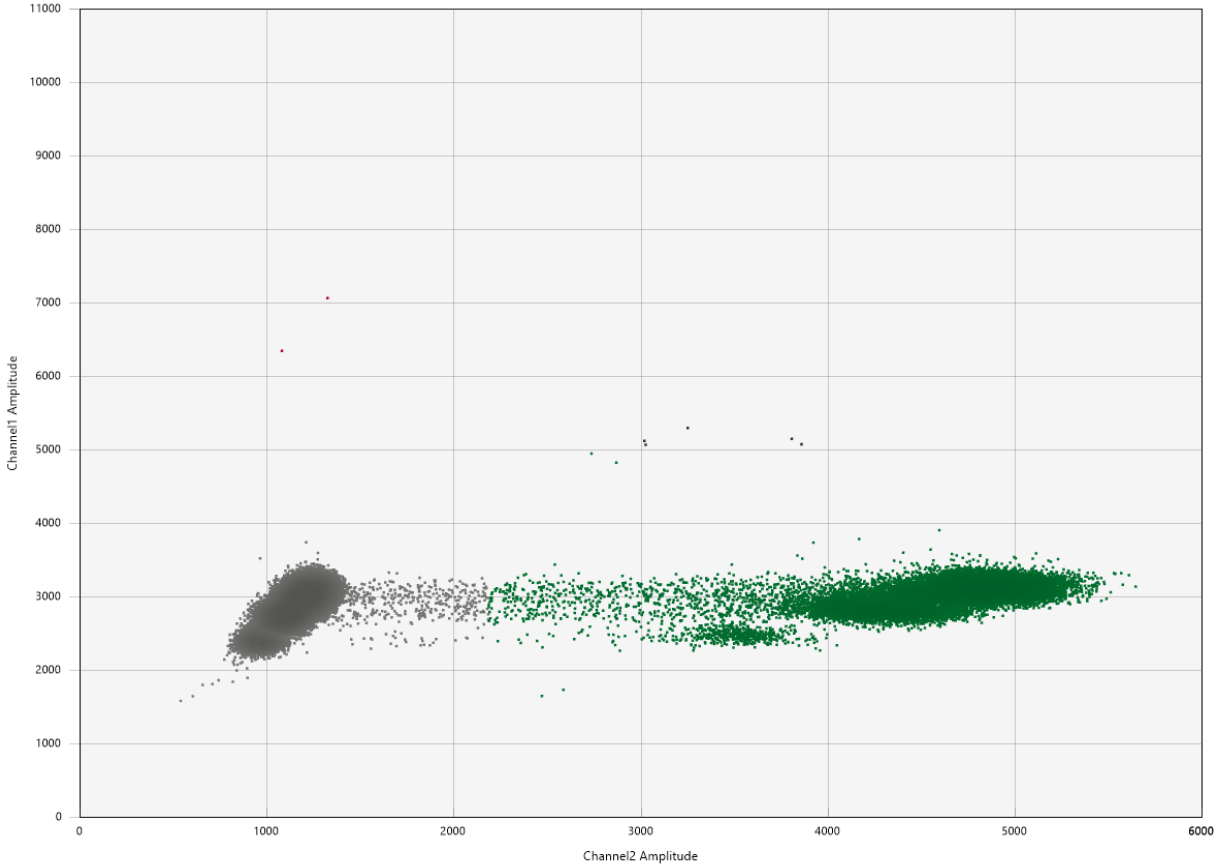
1X APC WT, p. R823*



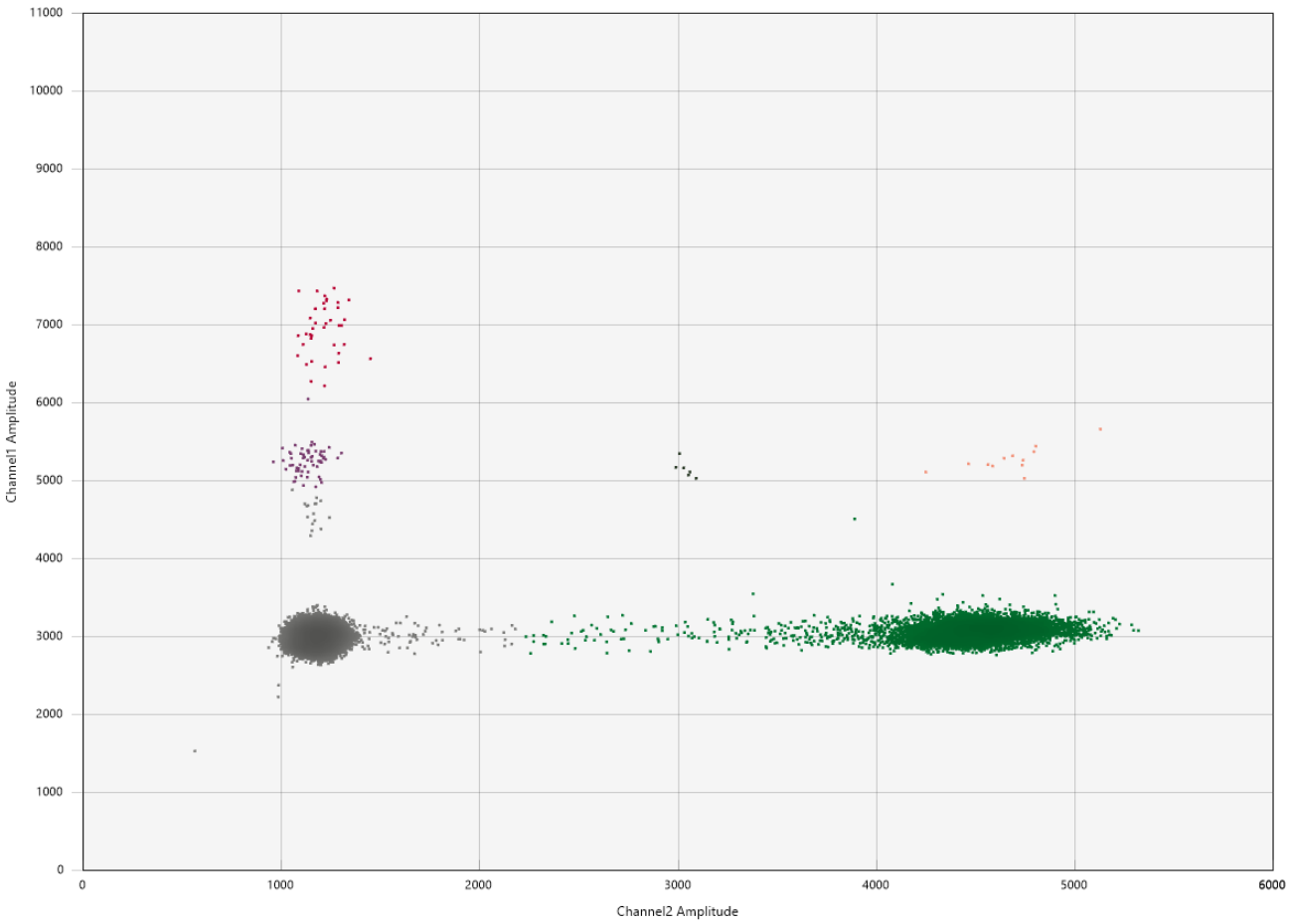
Positive control



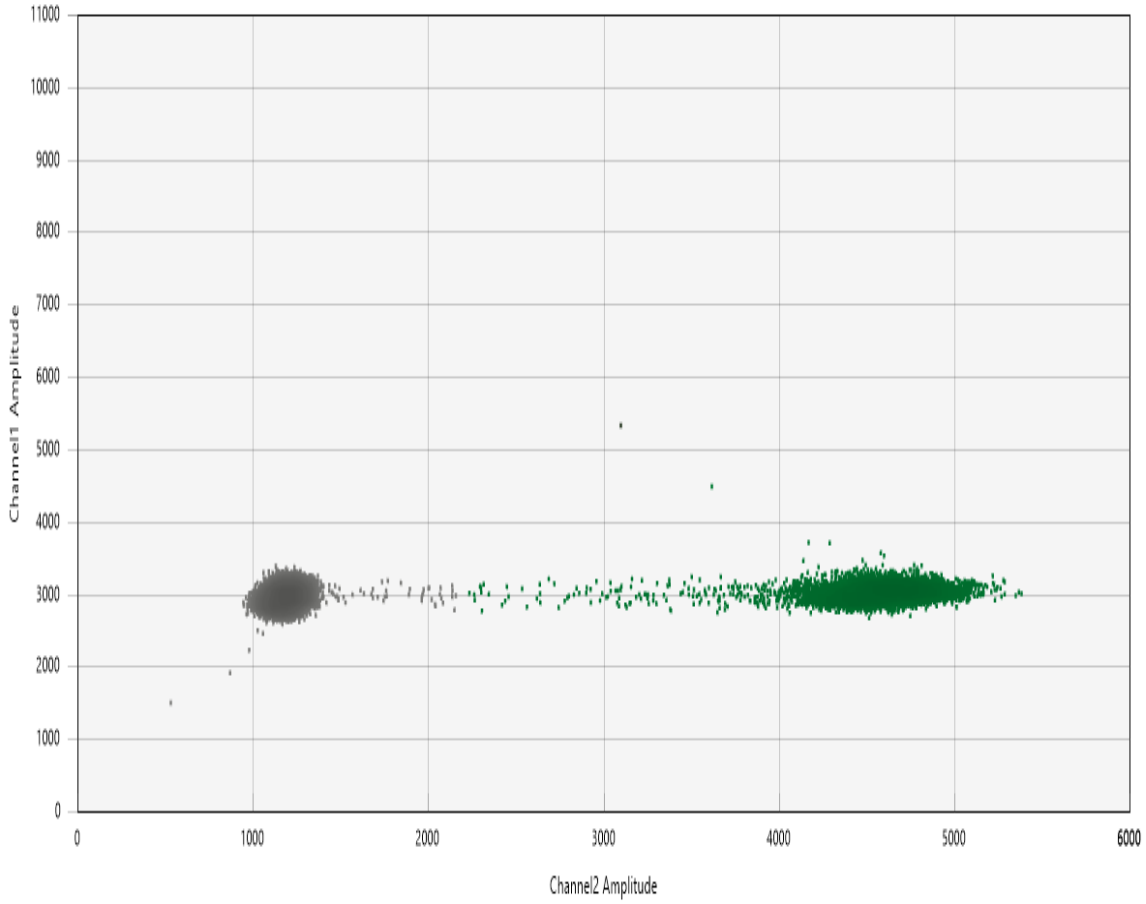
NK-Plasma



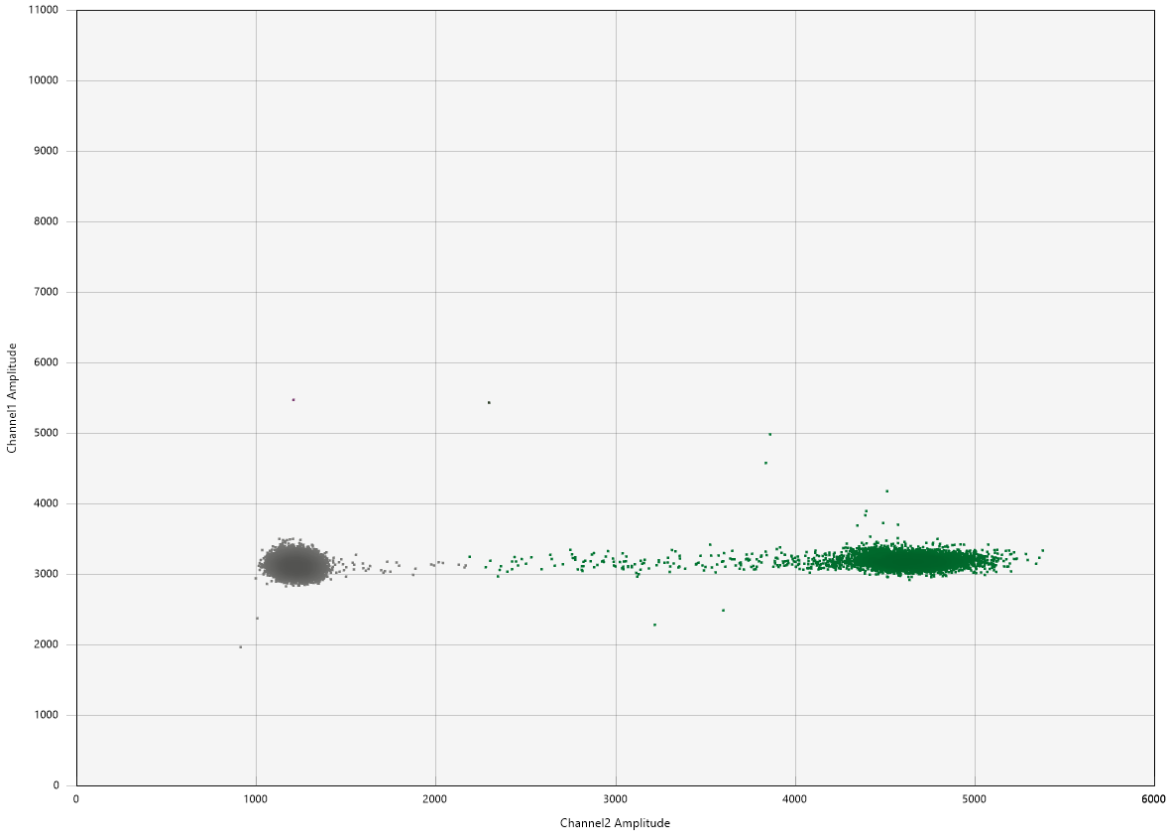
Pre-surgery



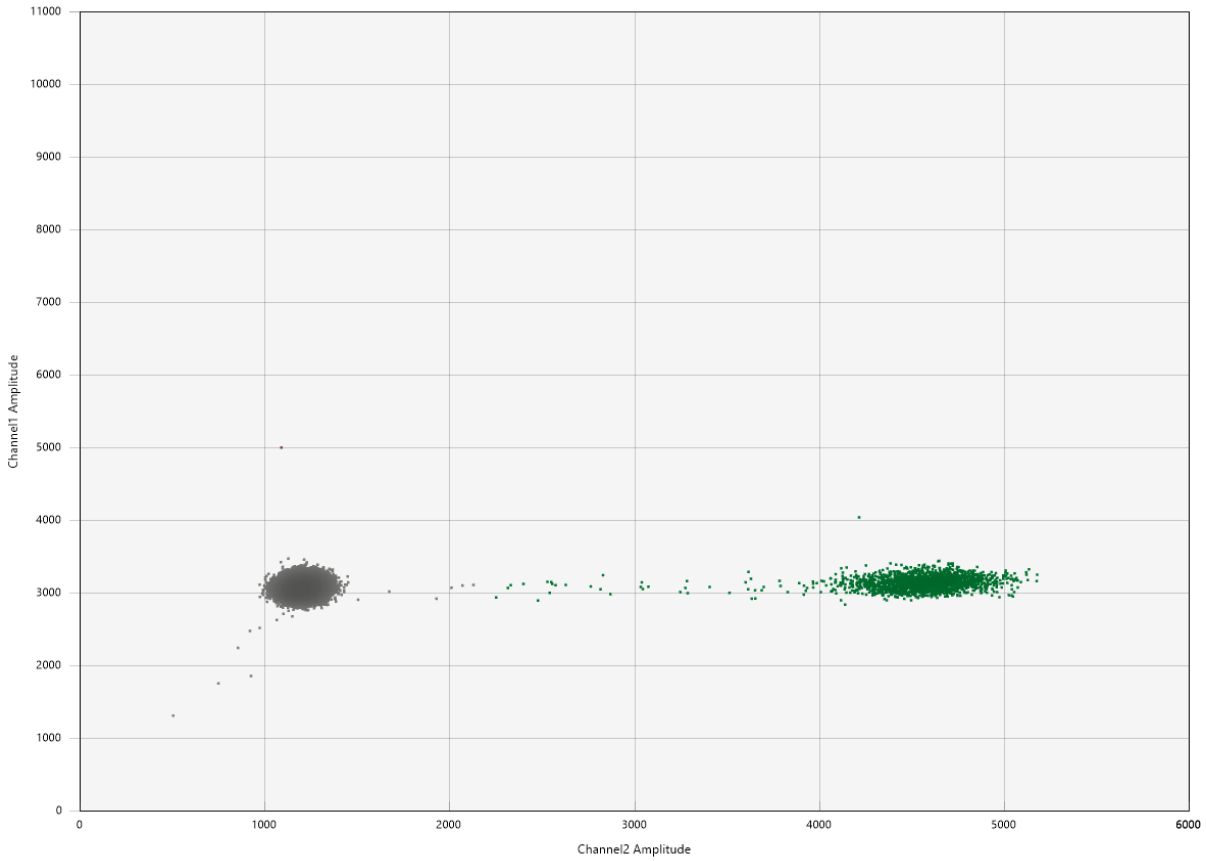
Follow up during chemo-1



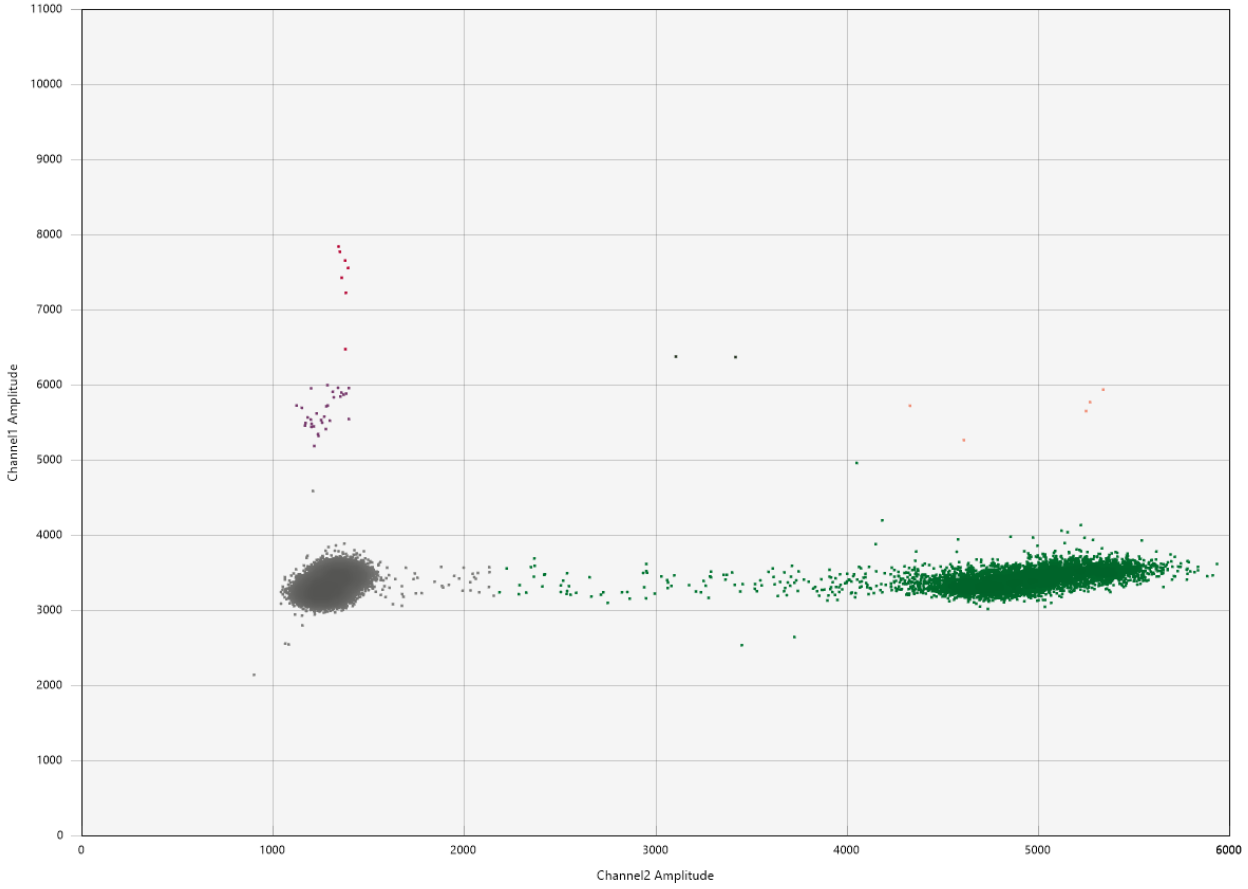
Follow up during chemo-2



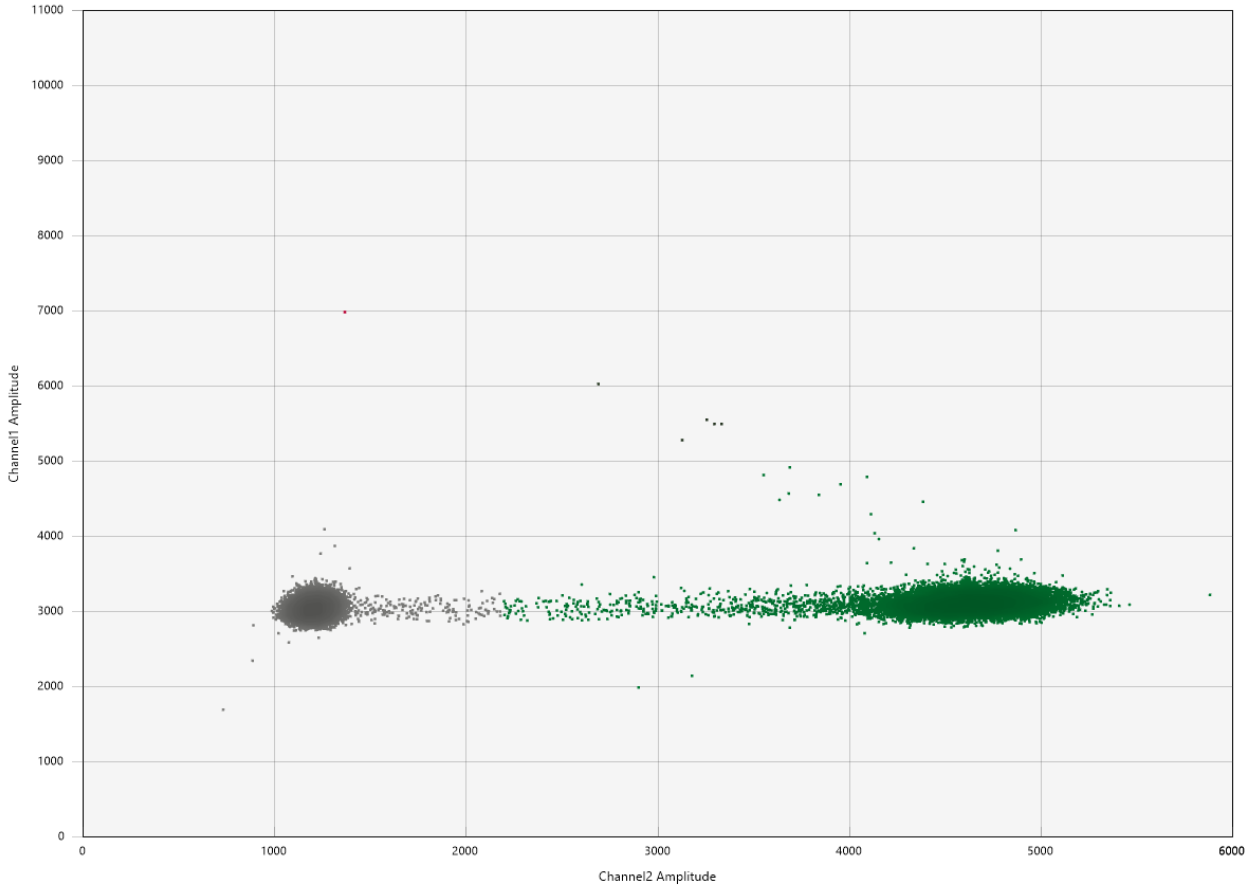
Follow up during chemo-3



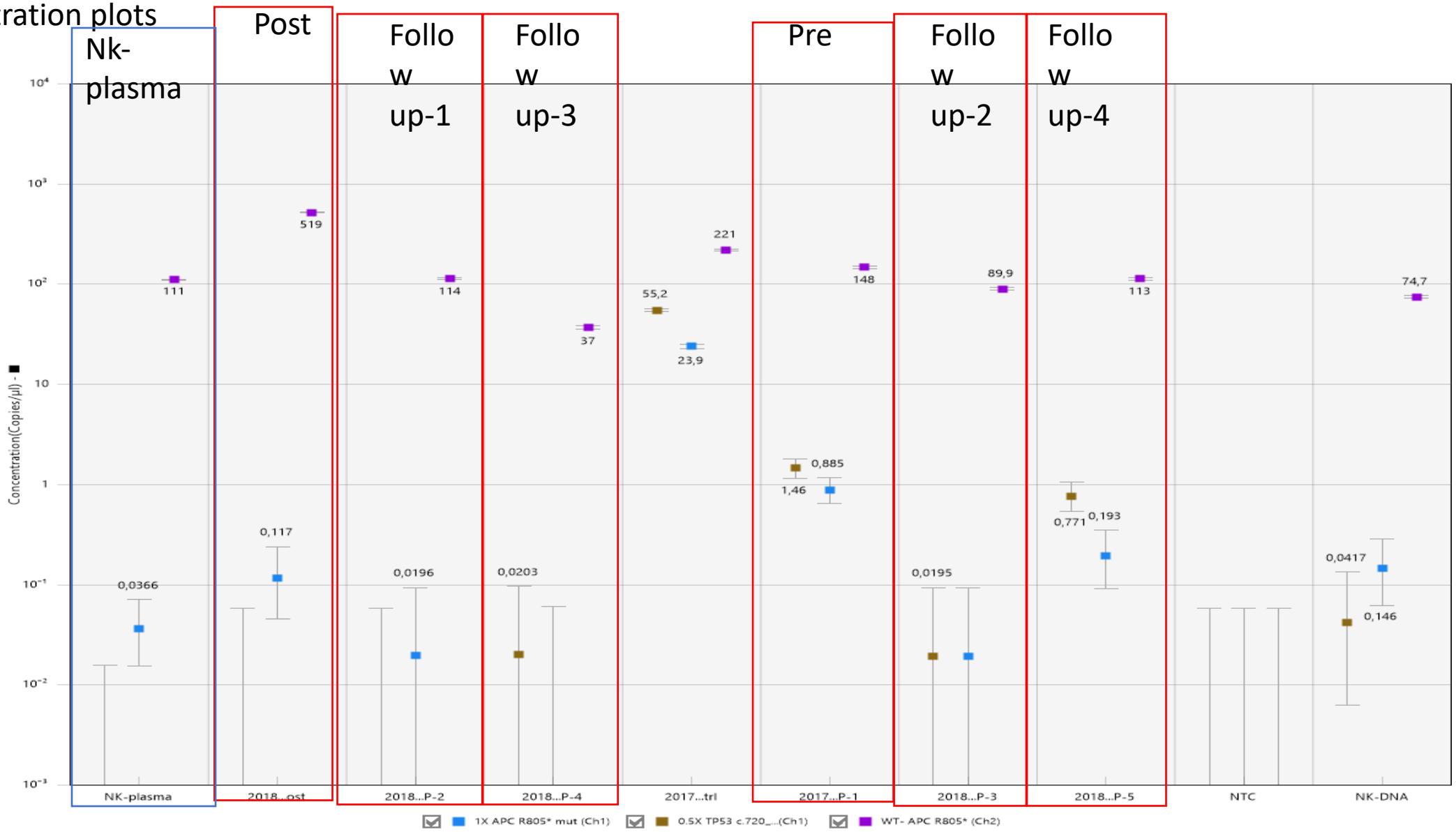
Follow up during chemo-4



Post-surgery



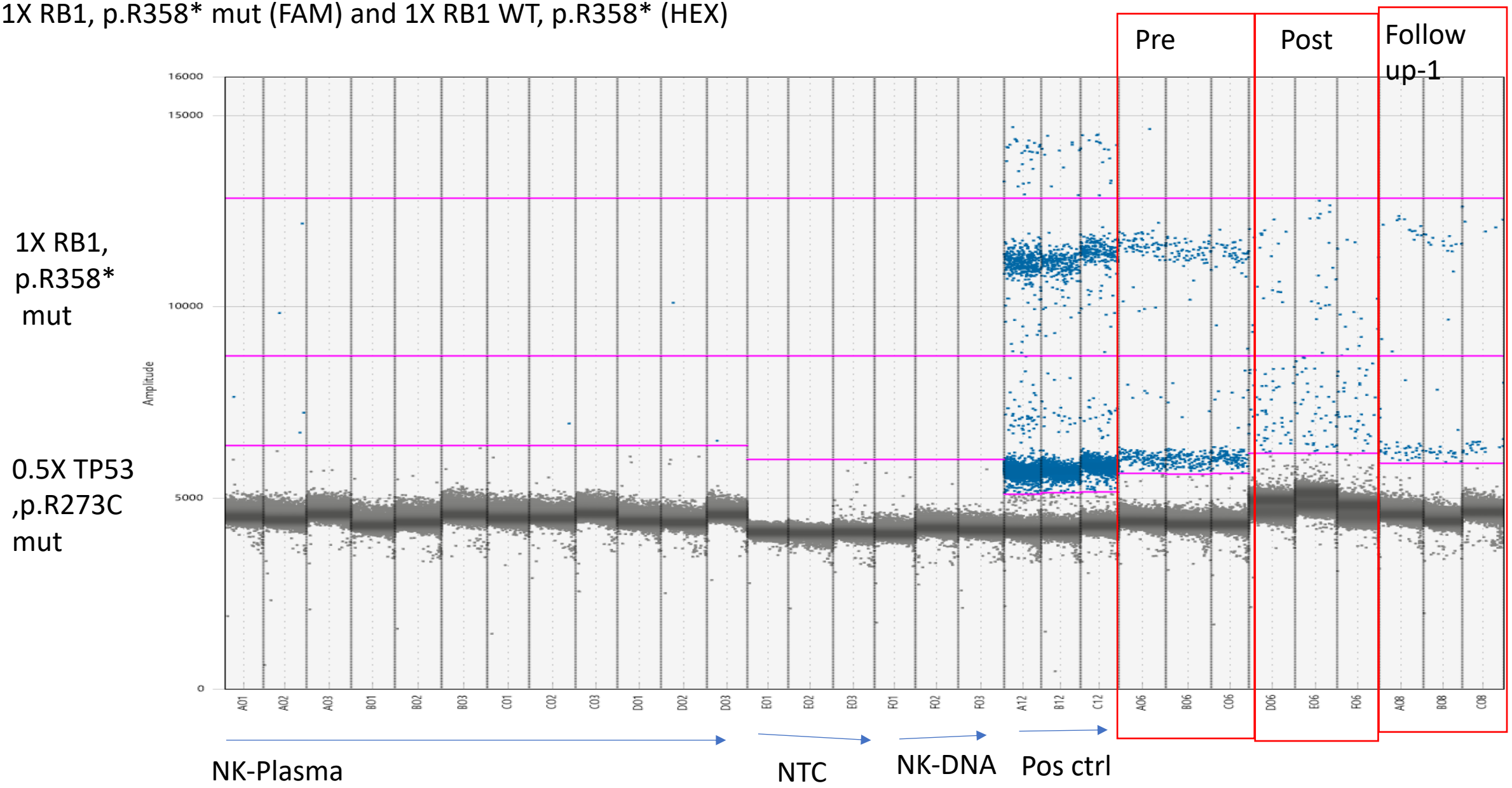
Concentration plots



P41 - Final

Gene targets : RB1, p.R358*, c.1072C>T and TP53,p.R273C, c.817C>T

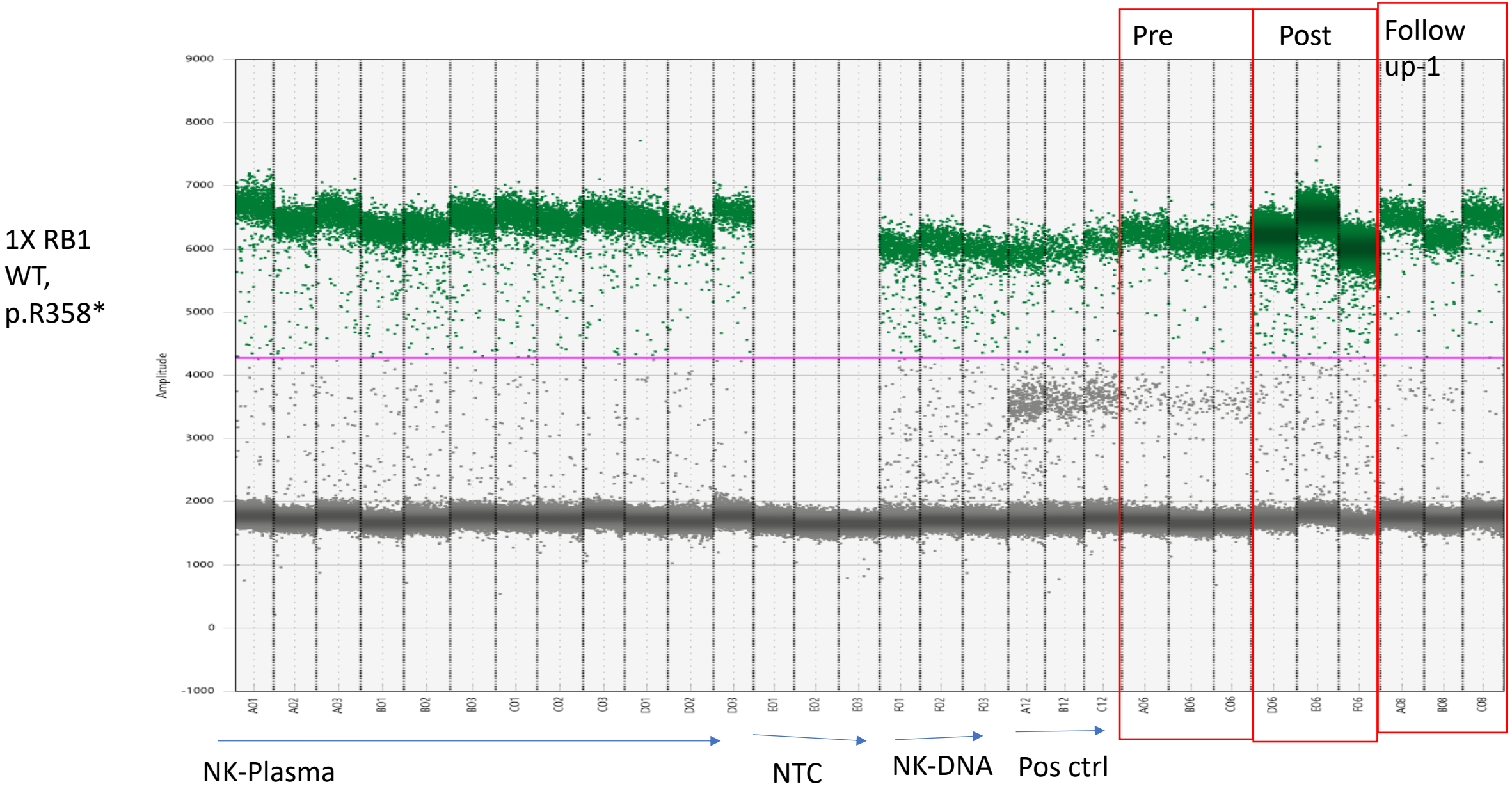
Assays: 0.5X TP53,p.R273C mut (FAM) and 1X dark probe for TP53,p.R273C
1X RB1, p.R358* mut (FAM) and 1X RB1 WT, p.R358* (HEX)



P41 - Final WT

Gene targets : RB1, p.R358*, c.1072C>T and TP53,p.R273C, c.817C>T

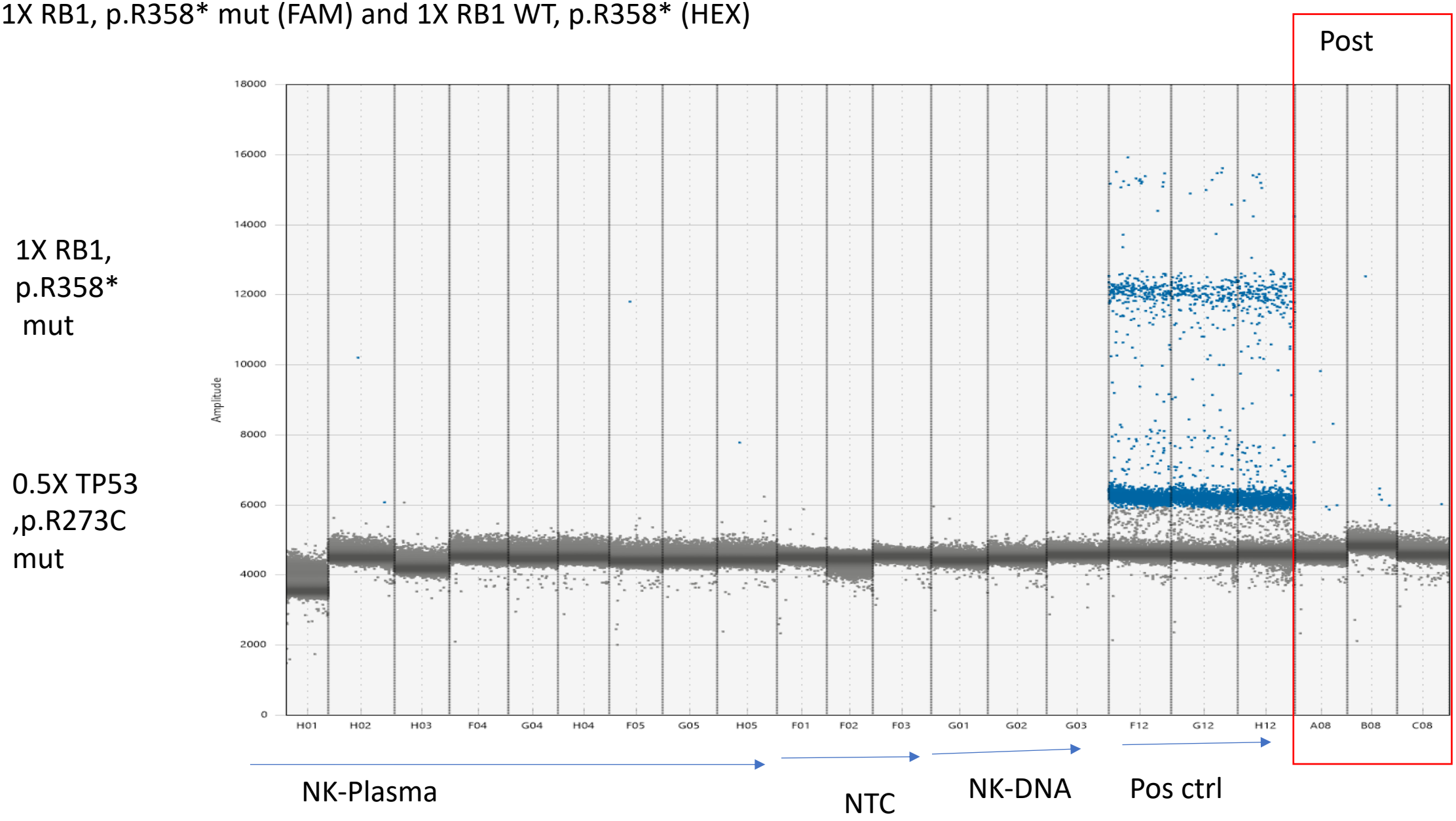
Assays: 1X dark probe for TP53,p.R273C and 1X RB1 WT, p.R358* (HEX)



P41 - Final dil

Gene targets : RB1, p.R358*, c.1072C>T and TP53,p.R273C, c.817C>T

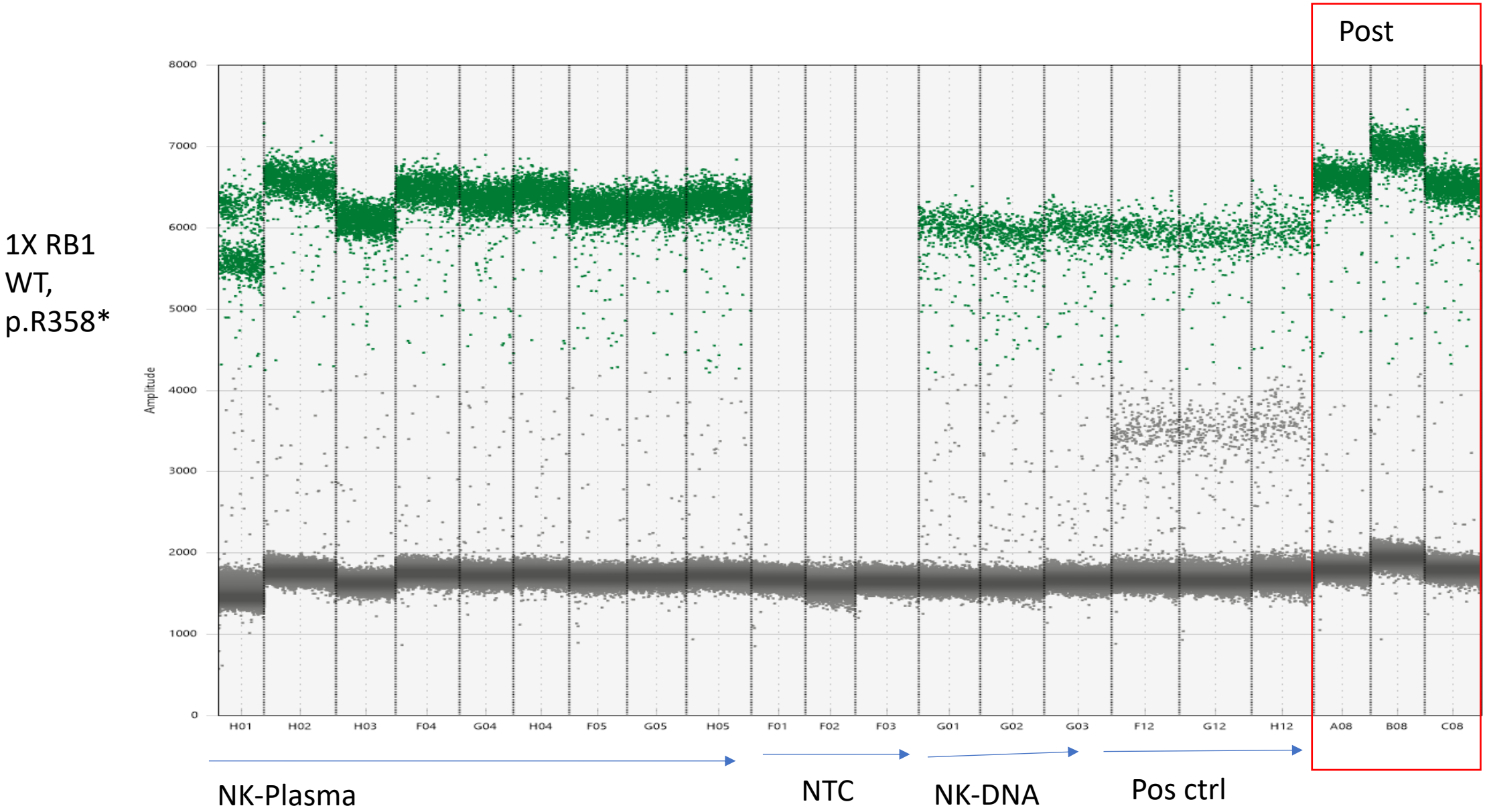
Assays: 0.5X TP53,p.R273C mut (FAM) and 1X dark probe for TP53,p.R273C
1X RB1, p.R358* mut (FAM) and 1X RB1 WT, p.R358* (HEX)



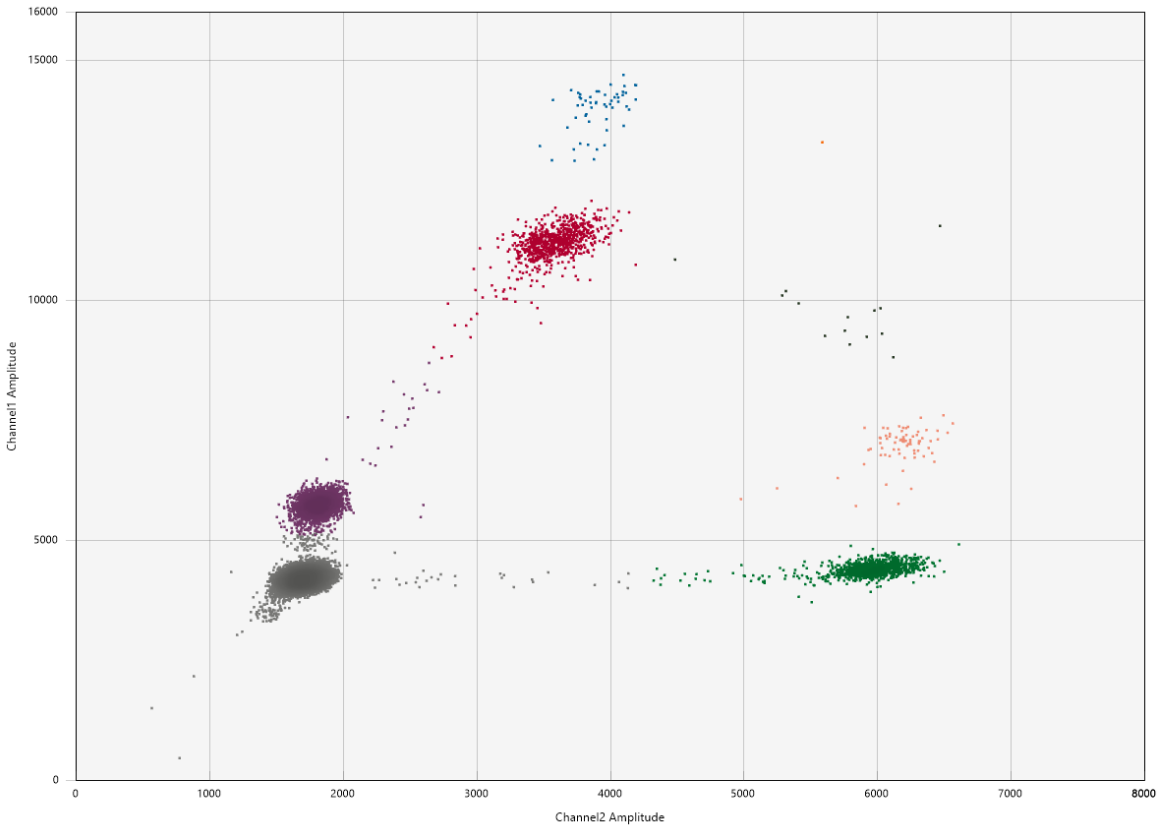
P41 - Final dil -WT

Gene targets : RB1, p.R358*, c.1072C>T and TP53,p.R273C, c.817C>T

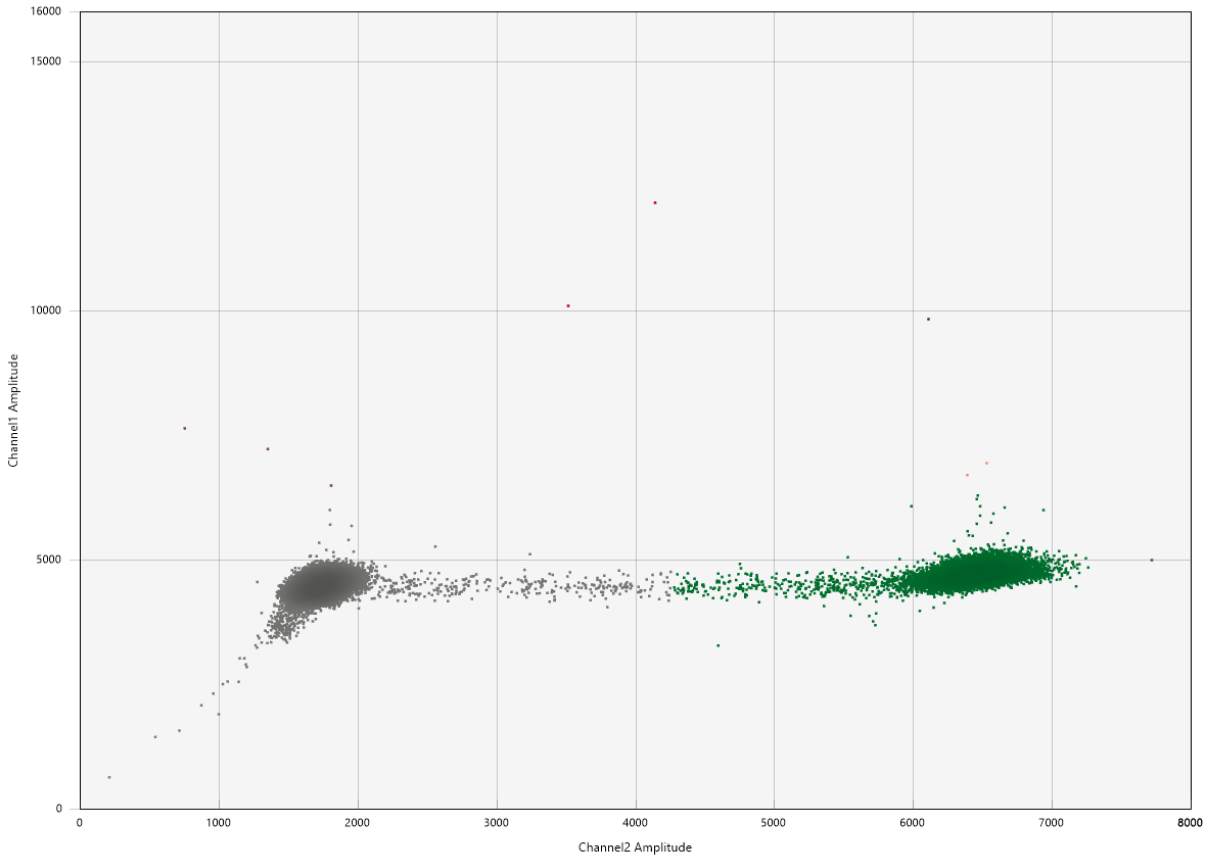
Assays: 1X dark probe for TP53,p.R273C and 1X RB1 WT, p.R358* (HEX)



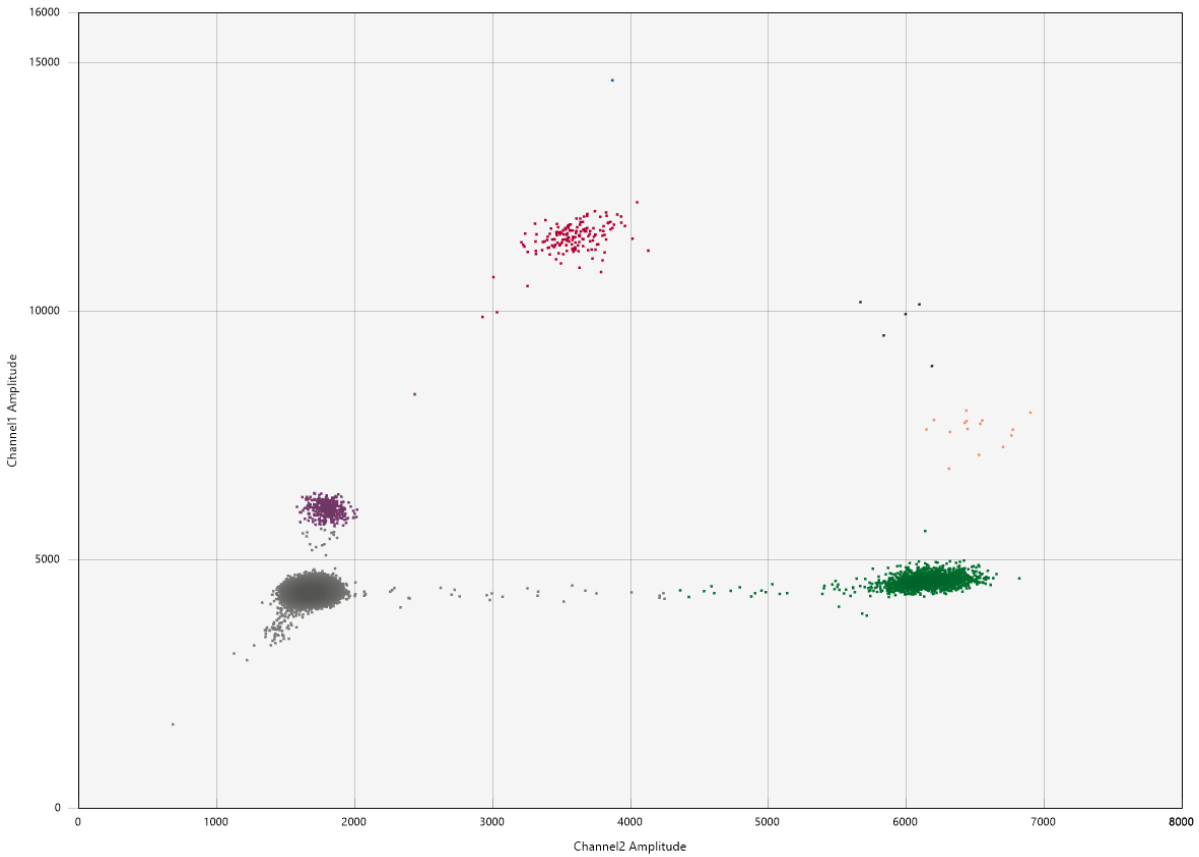
Positive control



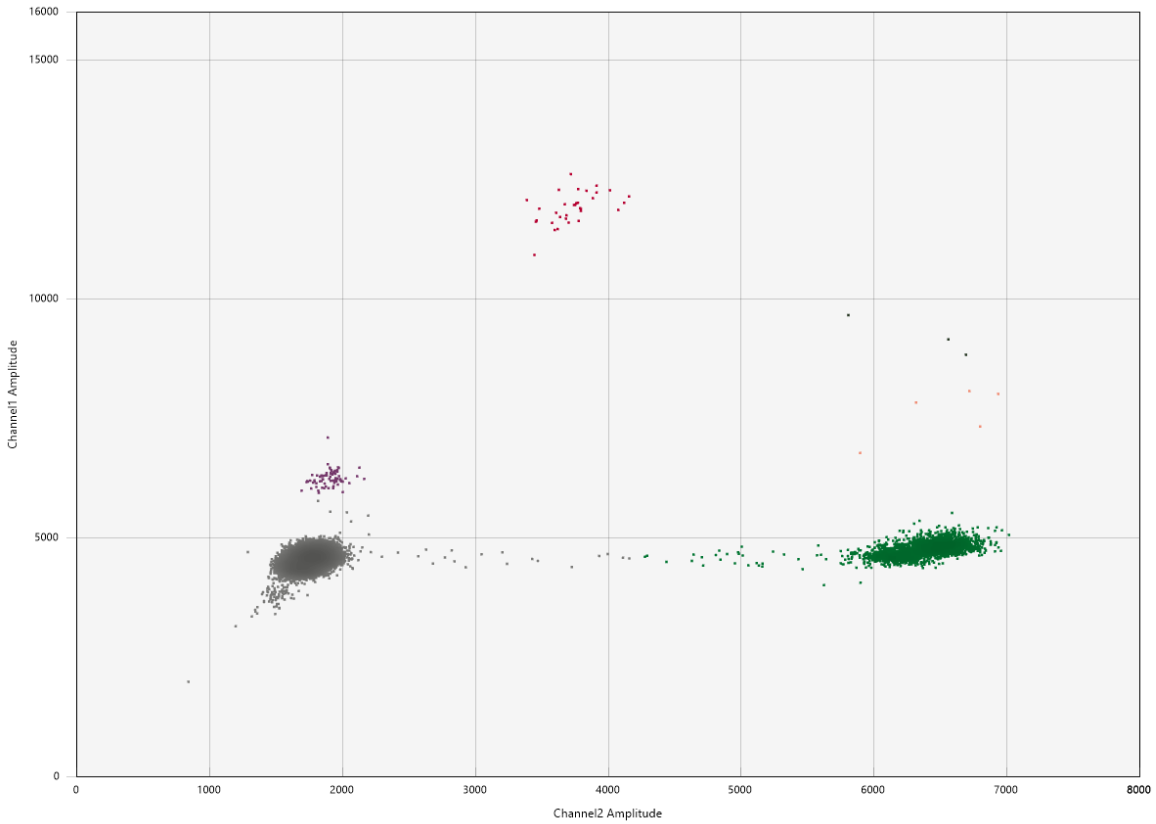
NK-Plasma



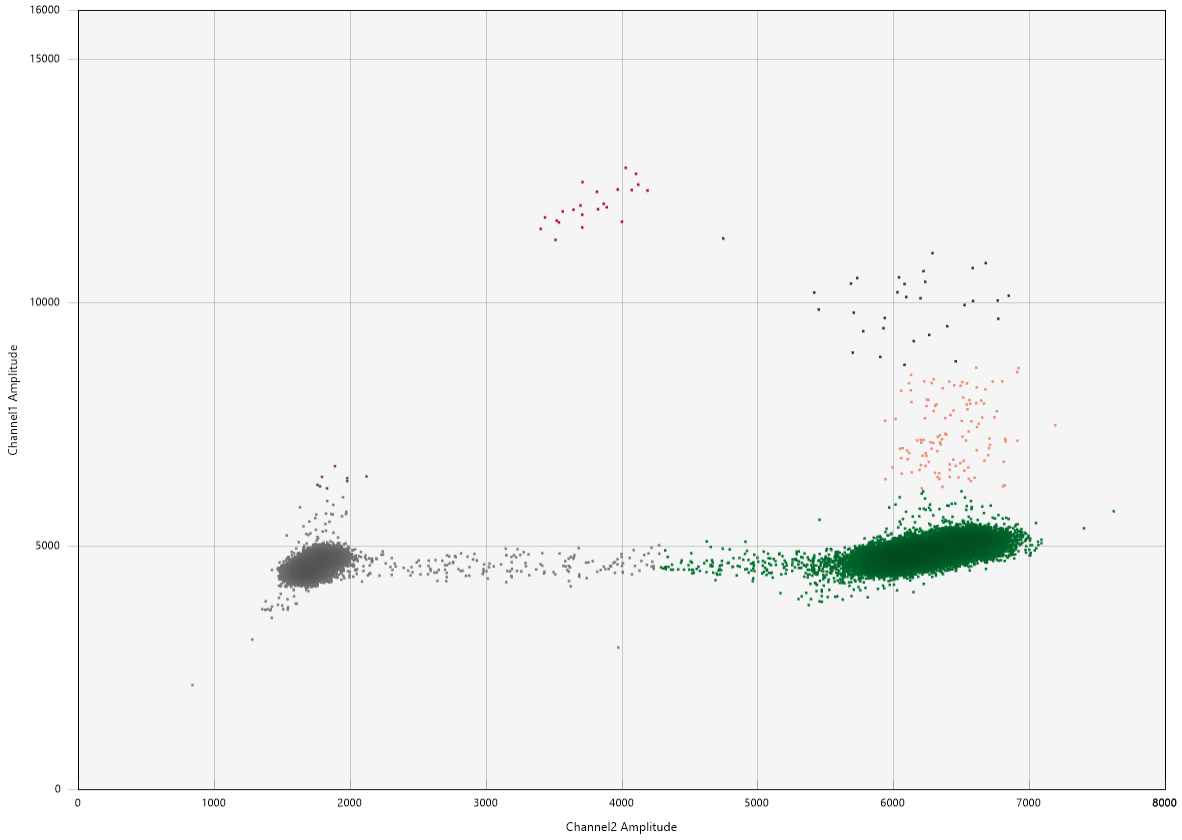
Pre-surgery



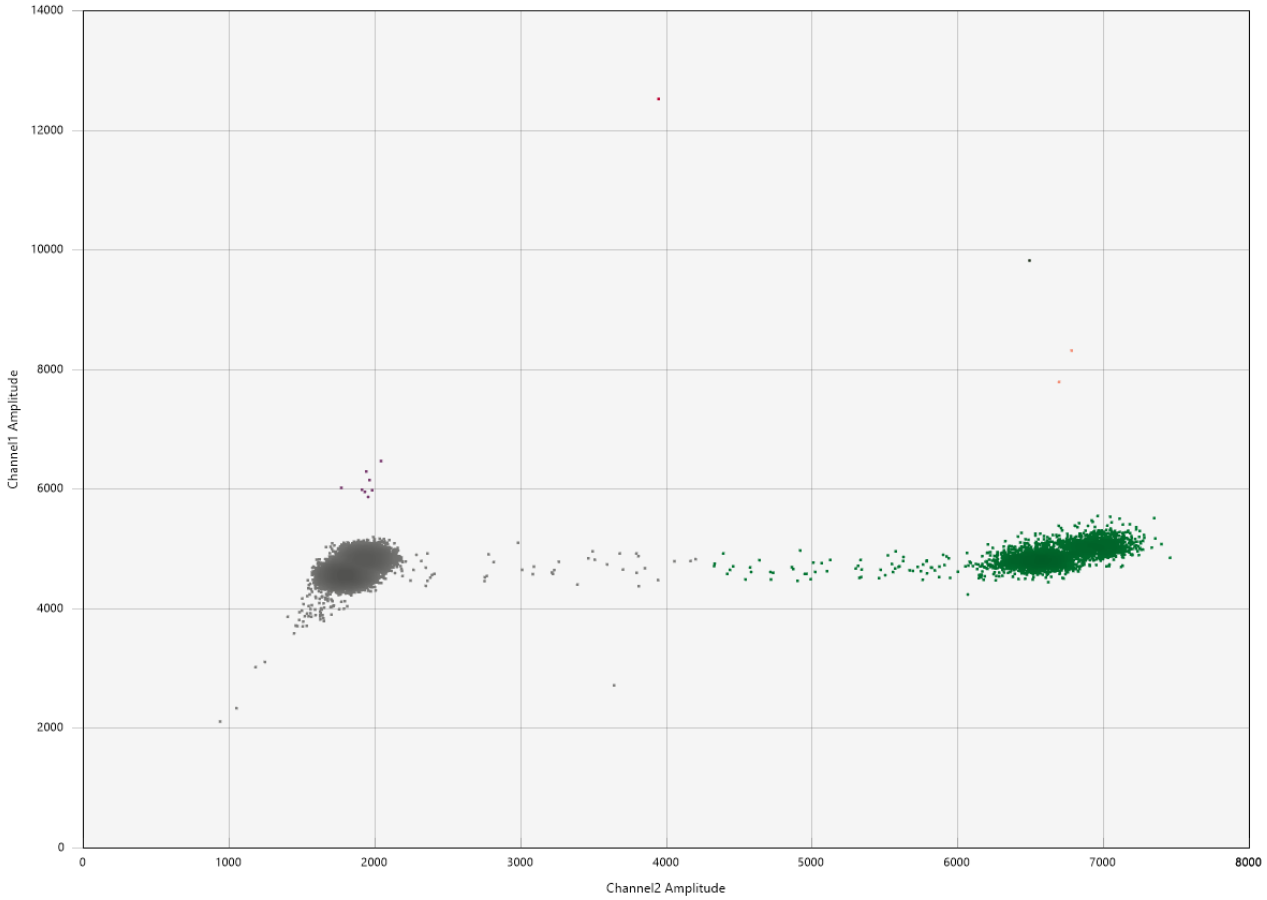
Follow-up 1



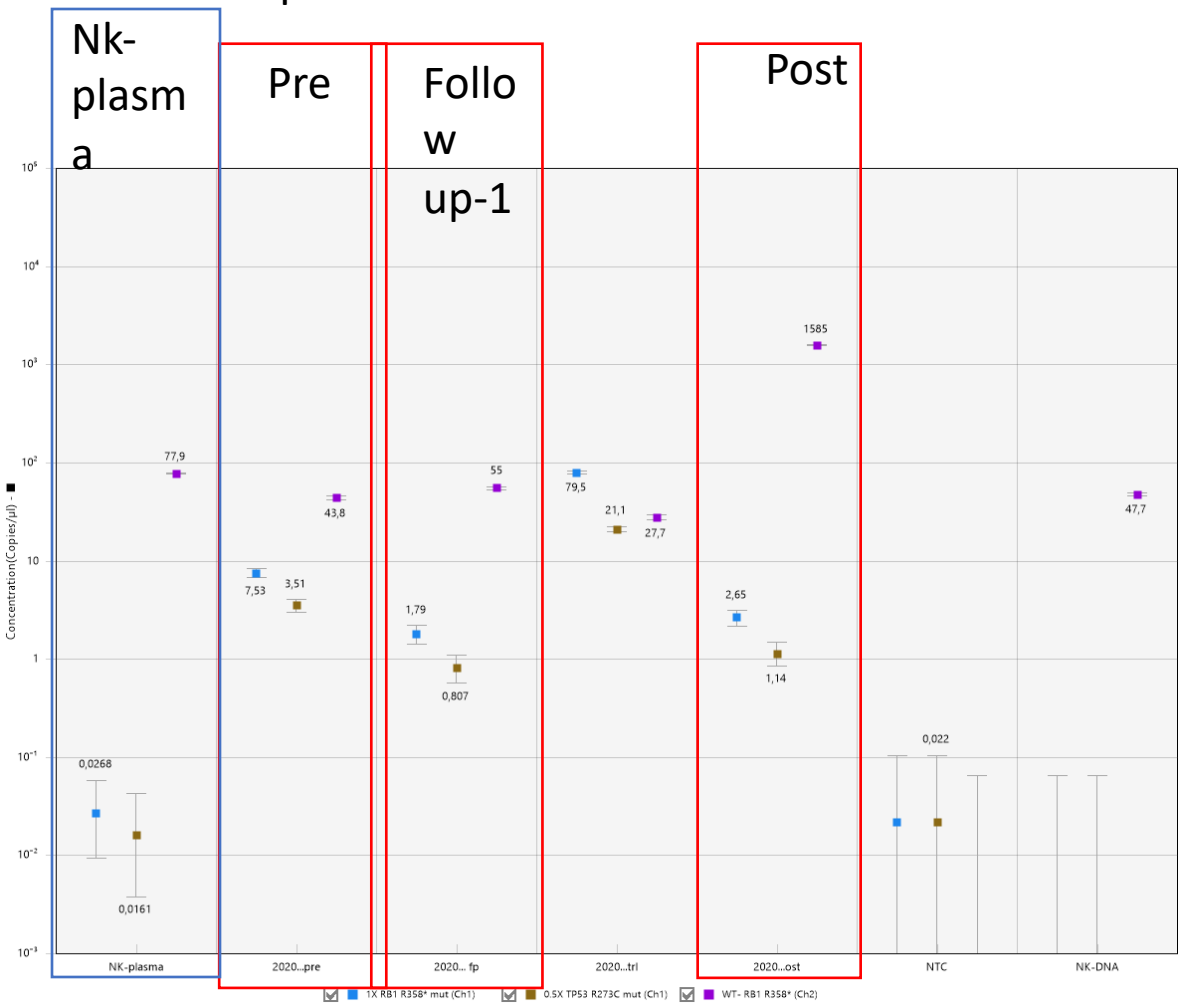
Post-surgery



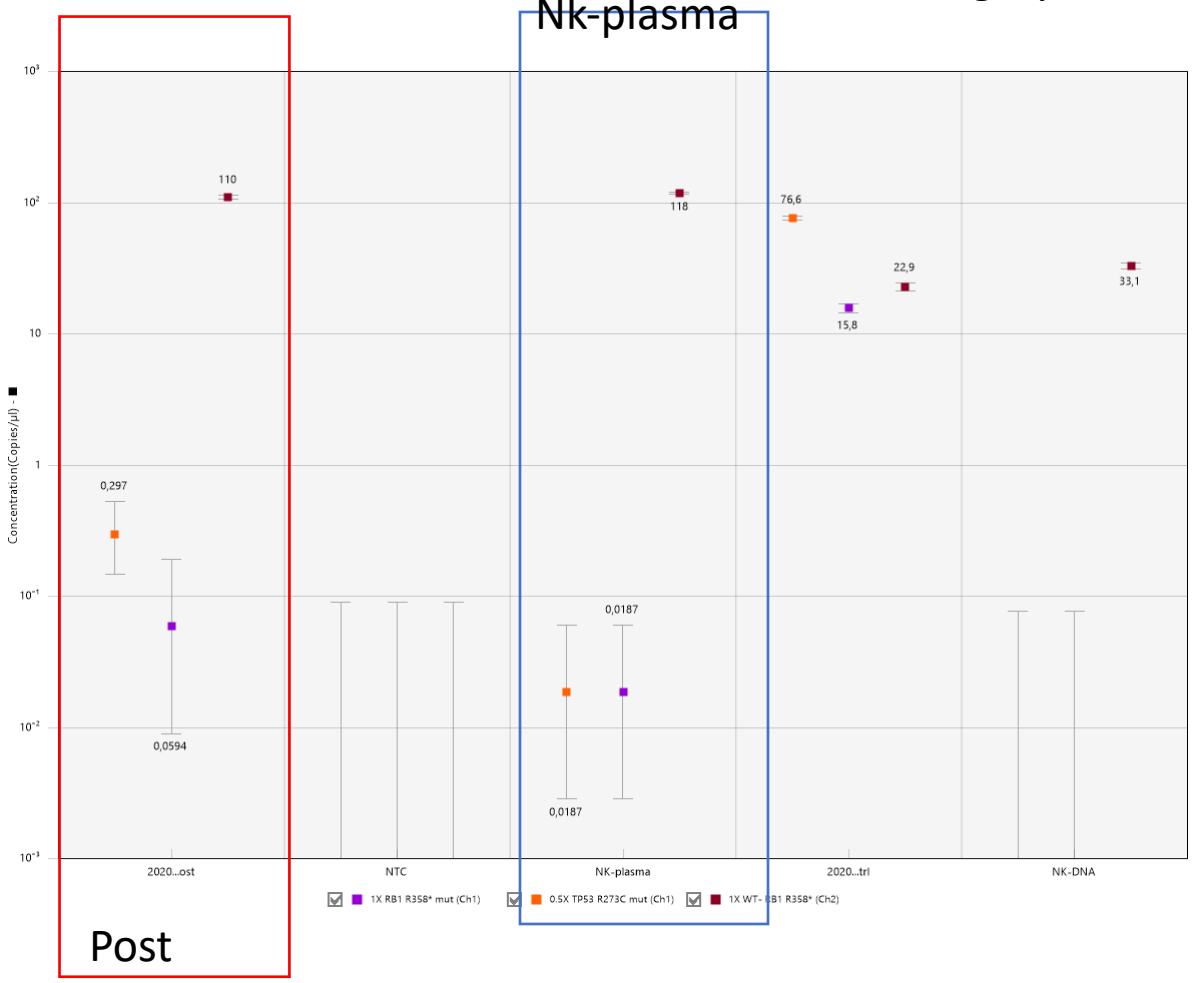
Post-surgery diluted



Concentration plots



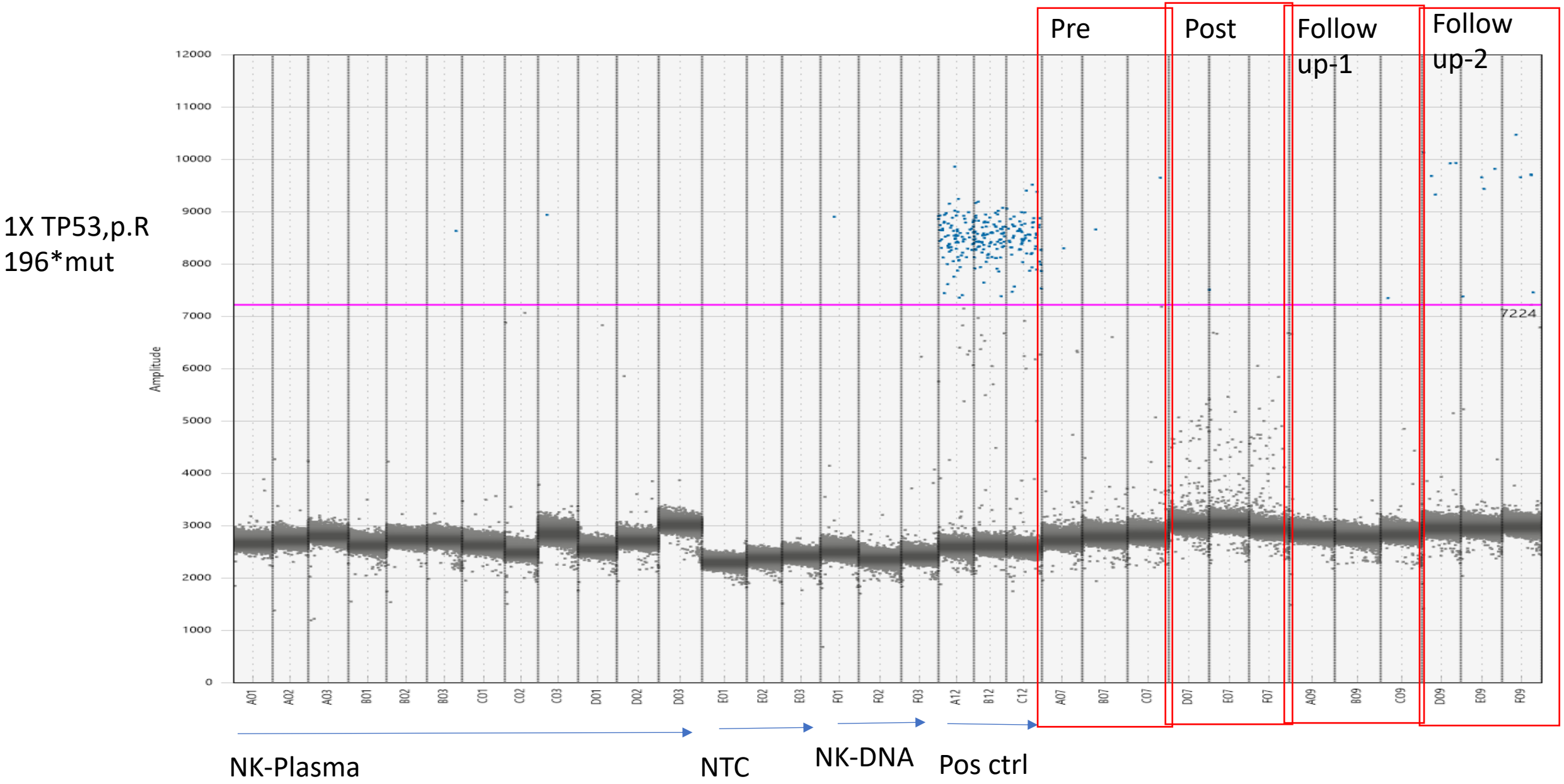
Post-surgery diluted



P25 - Final

Assays: 1X TP53,p.R196*mut (FAM) and 1X TP53 WT,p.R196* (HEX)

Gene target :TP53,p.R196*,c.586C>T

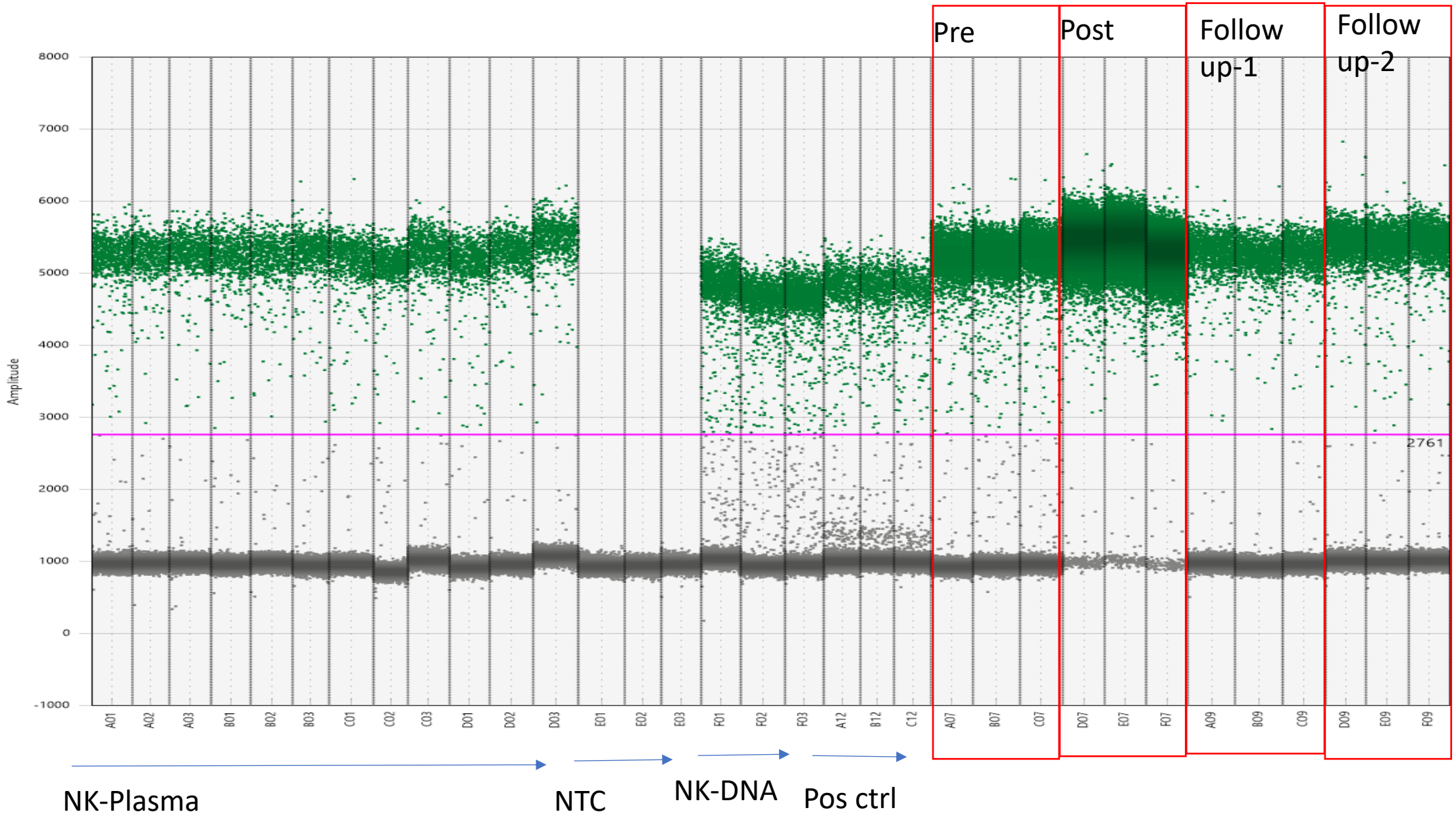


P25 - Final-WT

Assays: 1X TP53 WT,p.R196* (HEX)

Gene target :TP53,p.R196*,c.586C>T

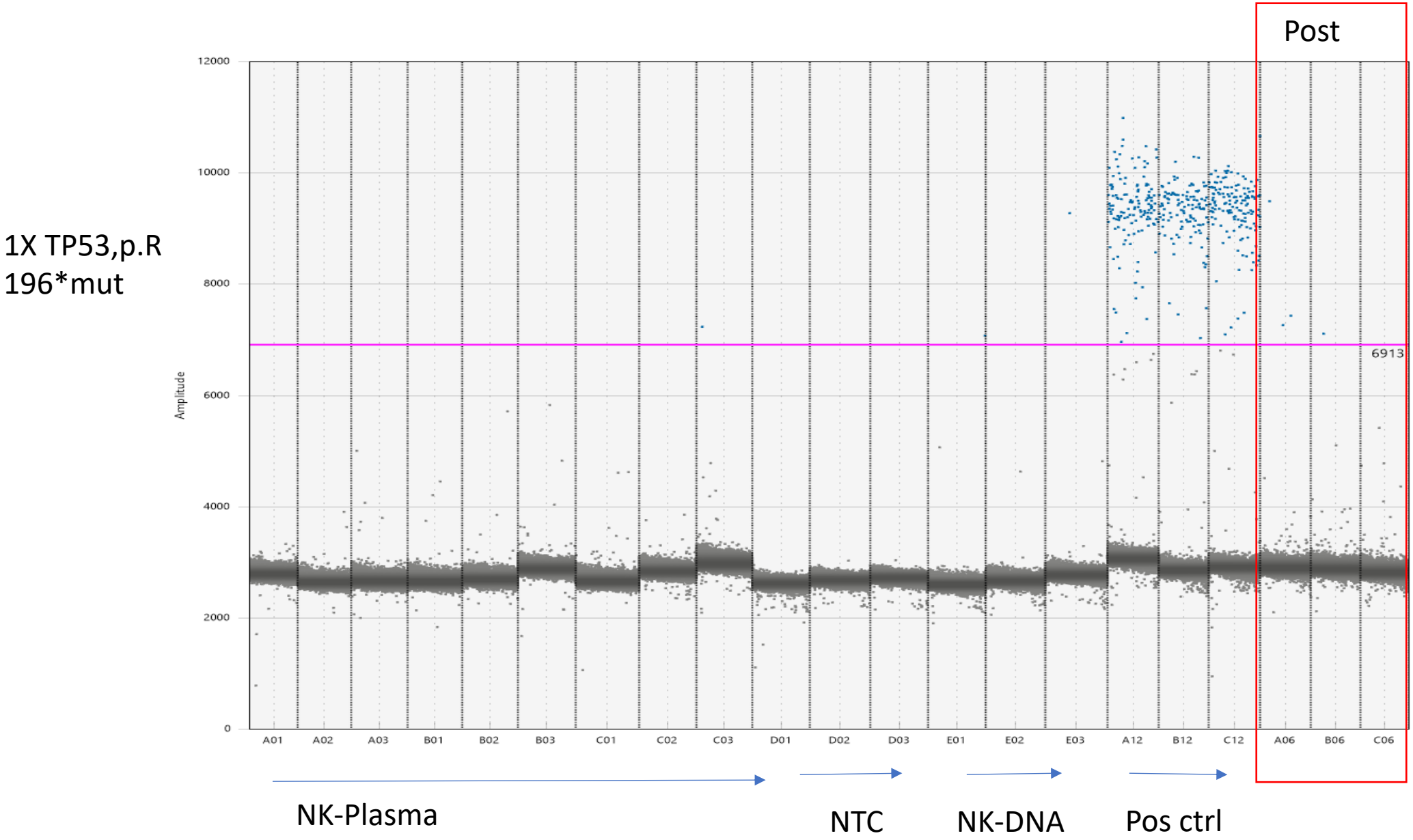
1X TP53 WT ,p.R196*



P25 - Final- diluted

Assays: 1X TP53,p.R196*mut (FAM) and 1X TP53 WT,p.R196* (HEX)

Gene target :TP53,p.R196*,c.586C>T

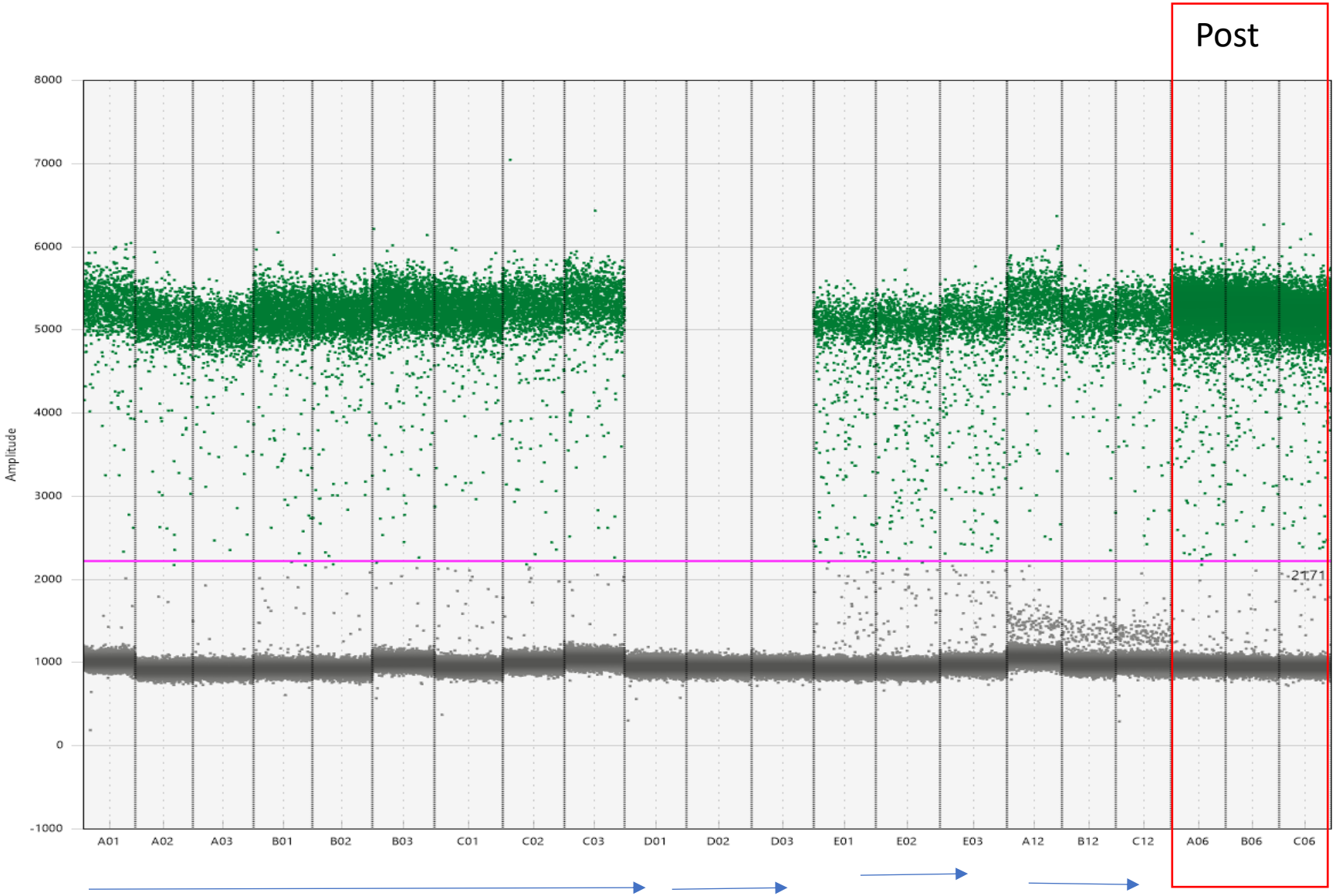


P25 - Final diluted

Assays: 1X TP53 WT,p.R196* (HEX)

Gene target :TP53,p.R196*,c.586C>T

1X TP53 WT,p.R196*



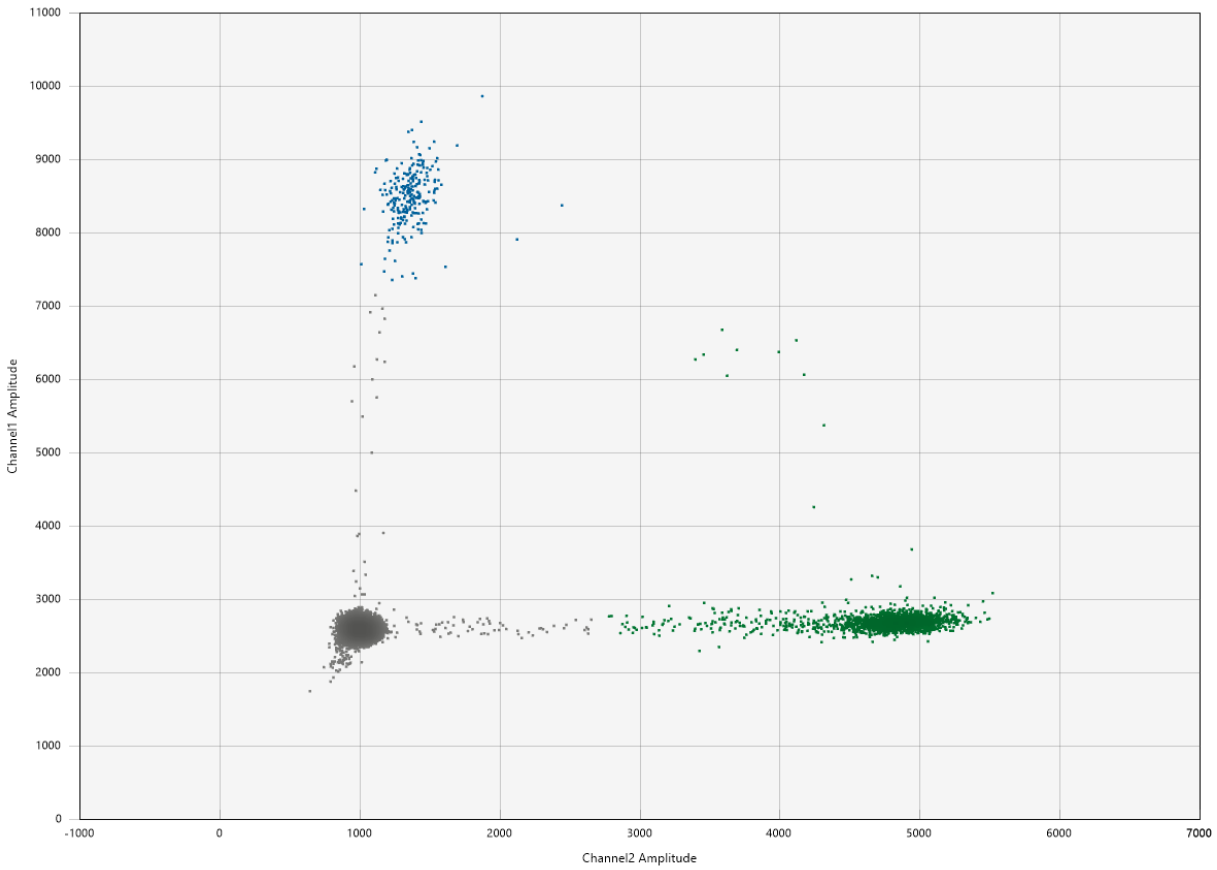
NK-Plasma

NTC

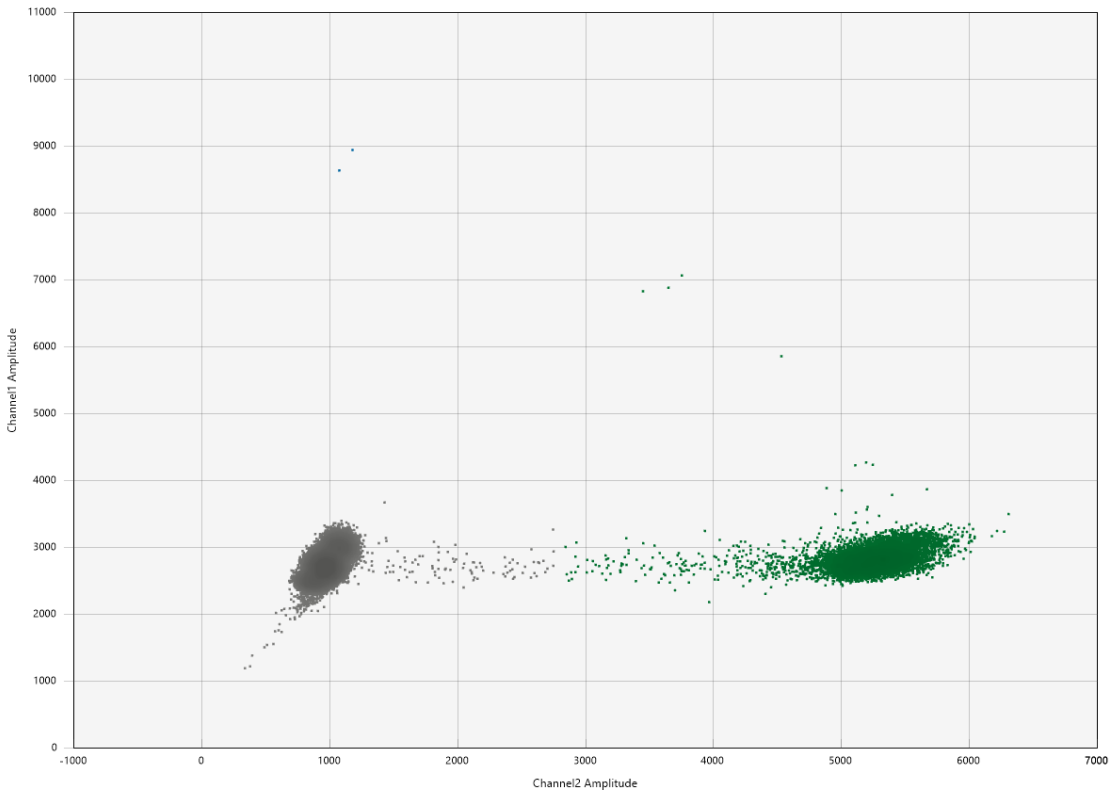
NK-DNA

Pos ctrl

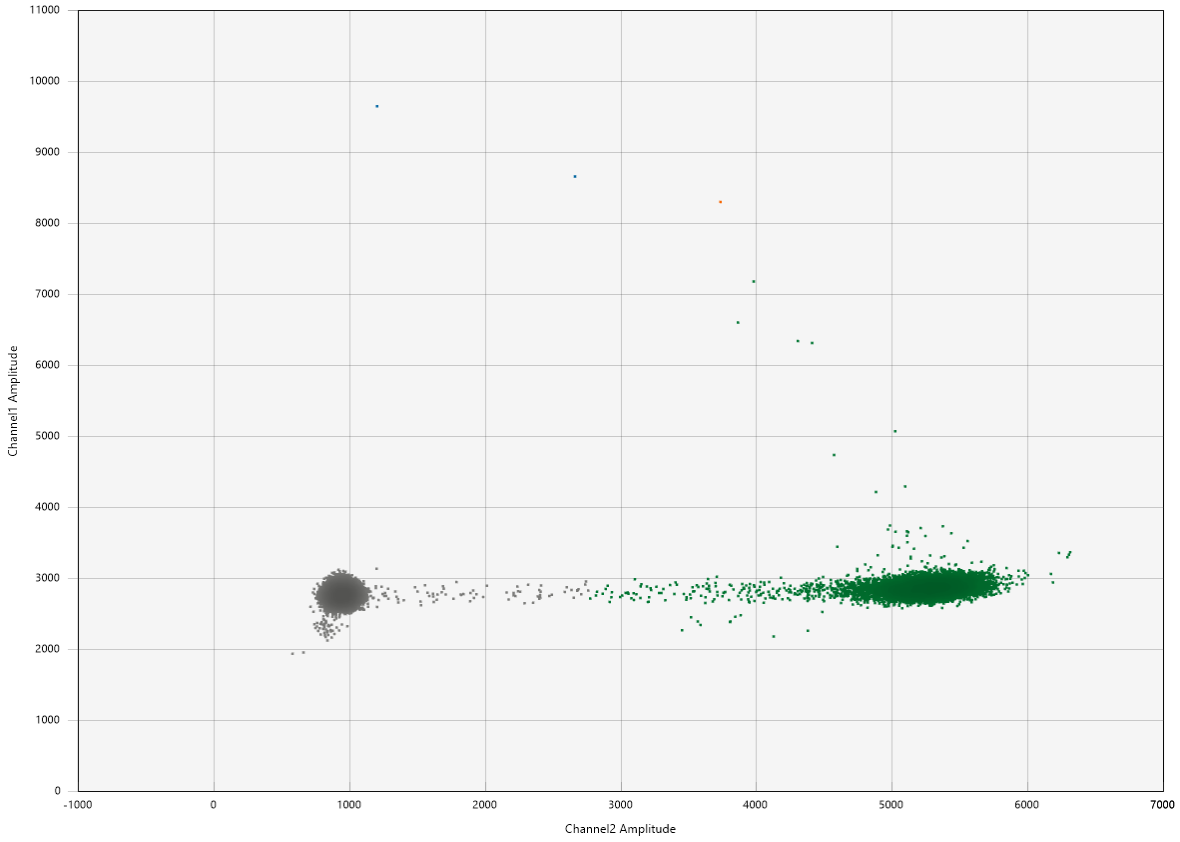
Positive control



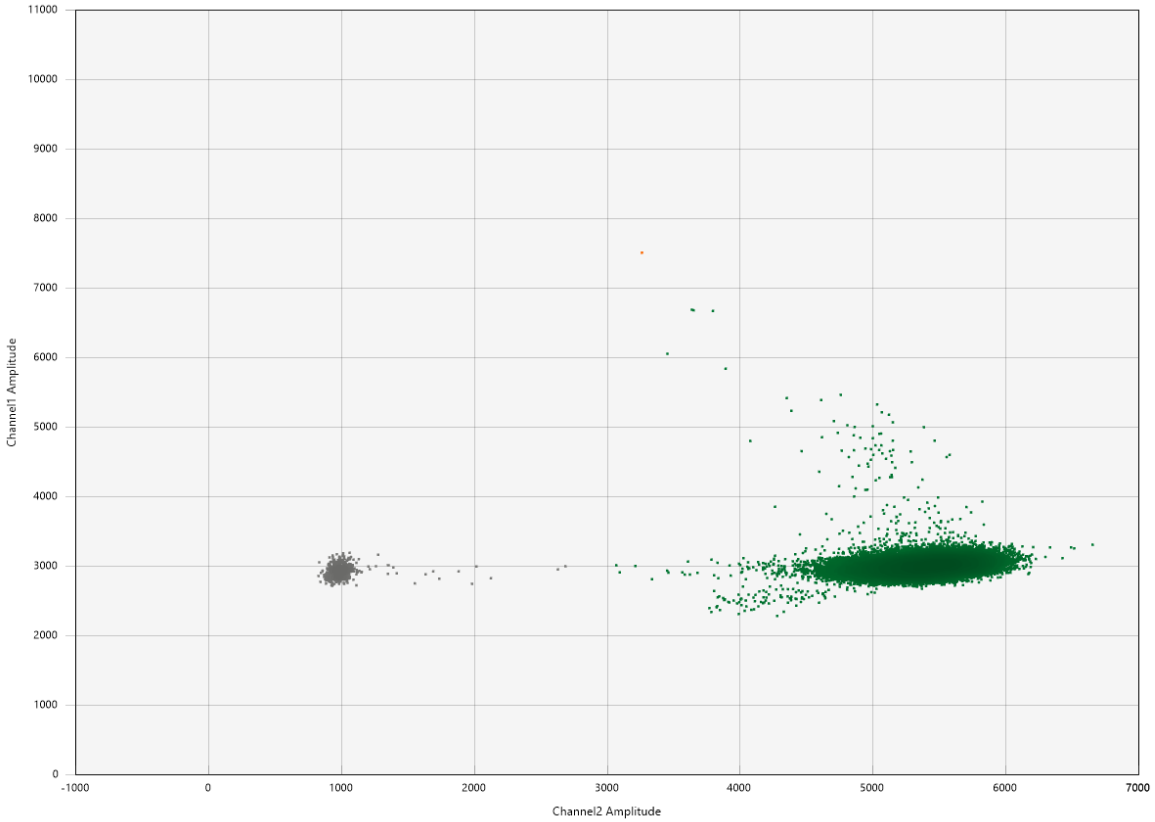
NK-plasma cfDNA



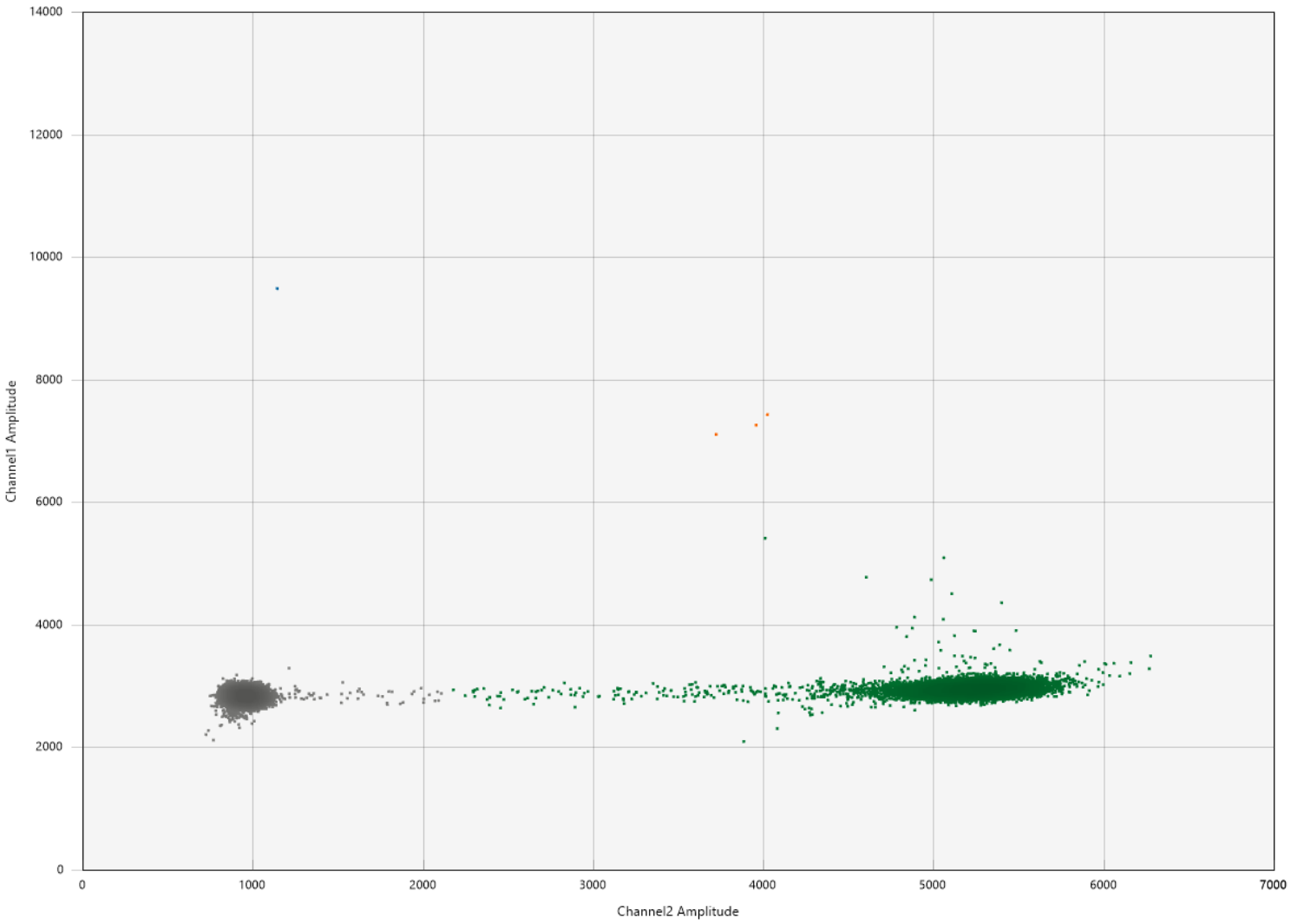
Pre-surgery



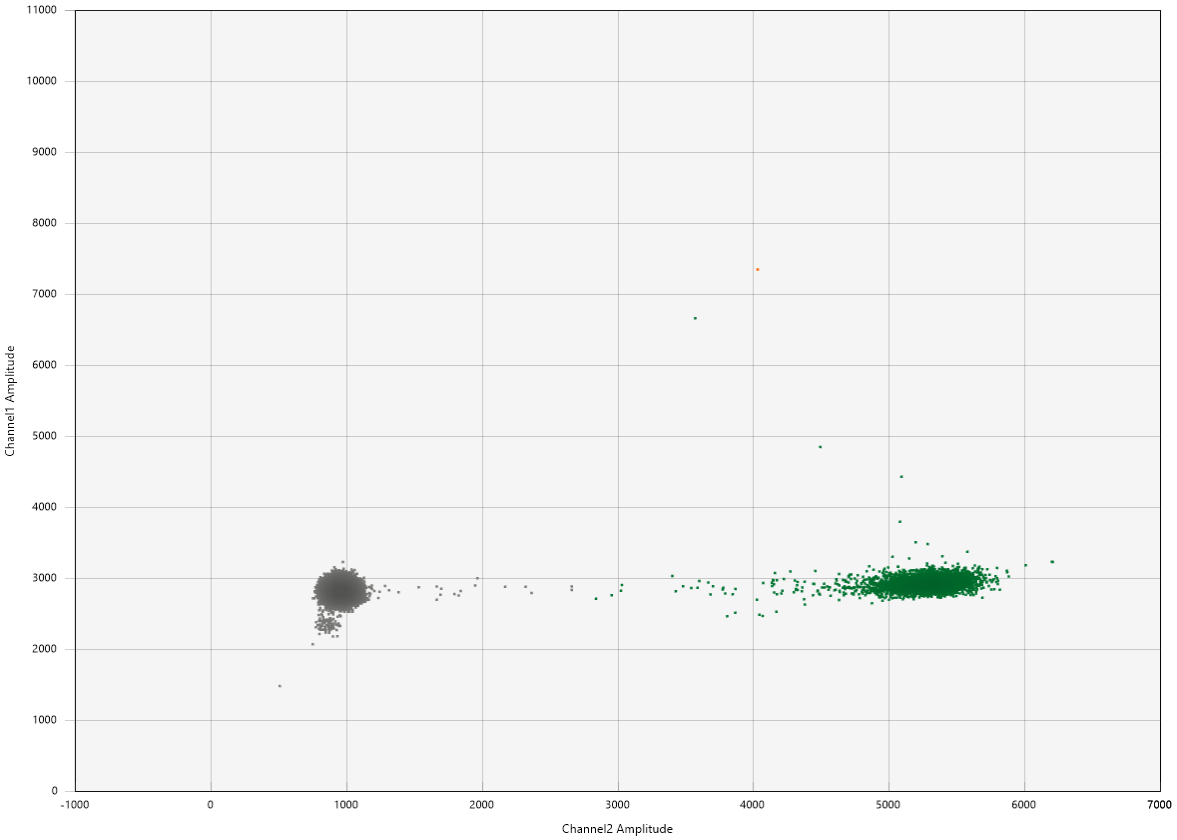
Post-surgery



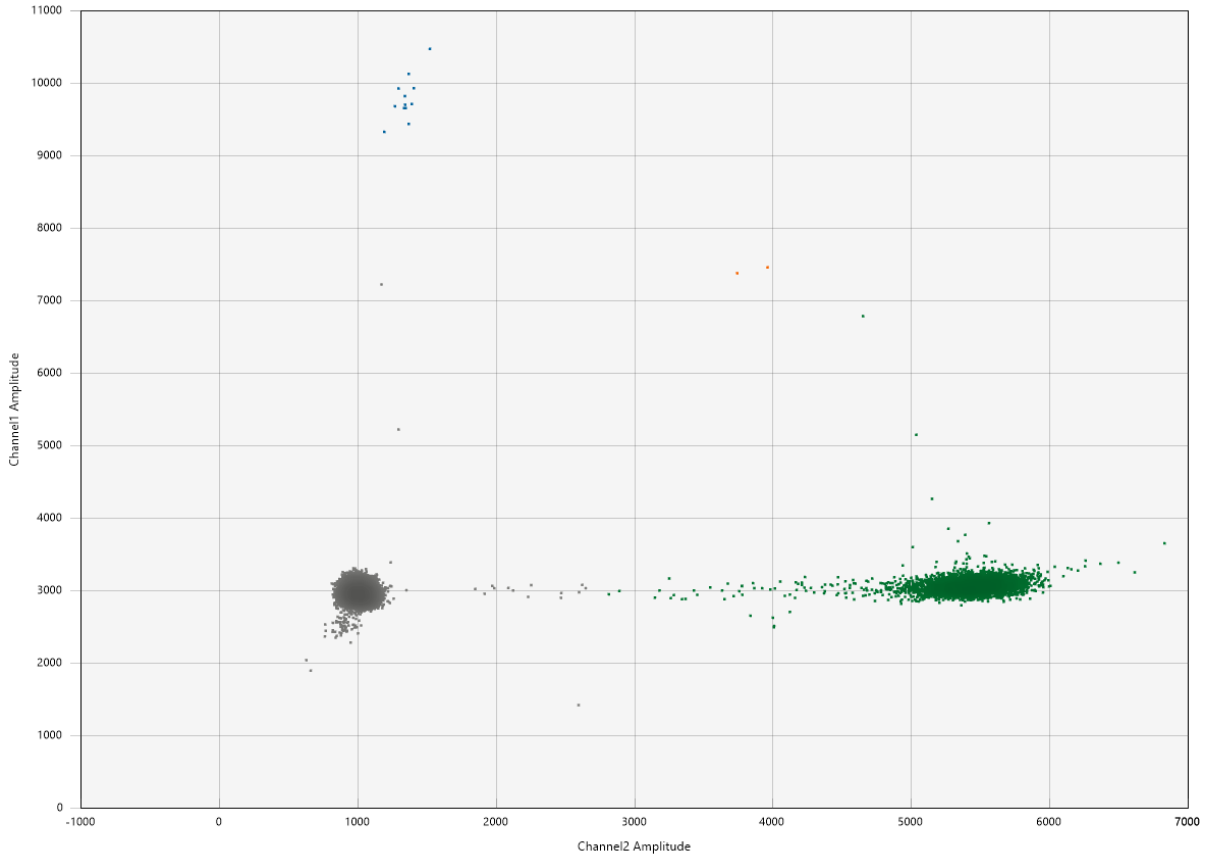
Post-surgery - diluted



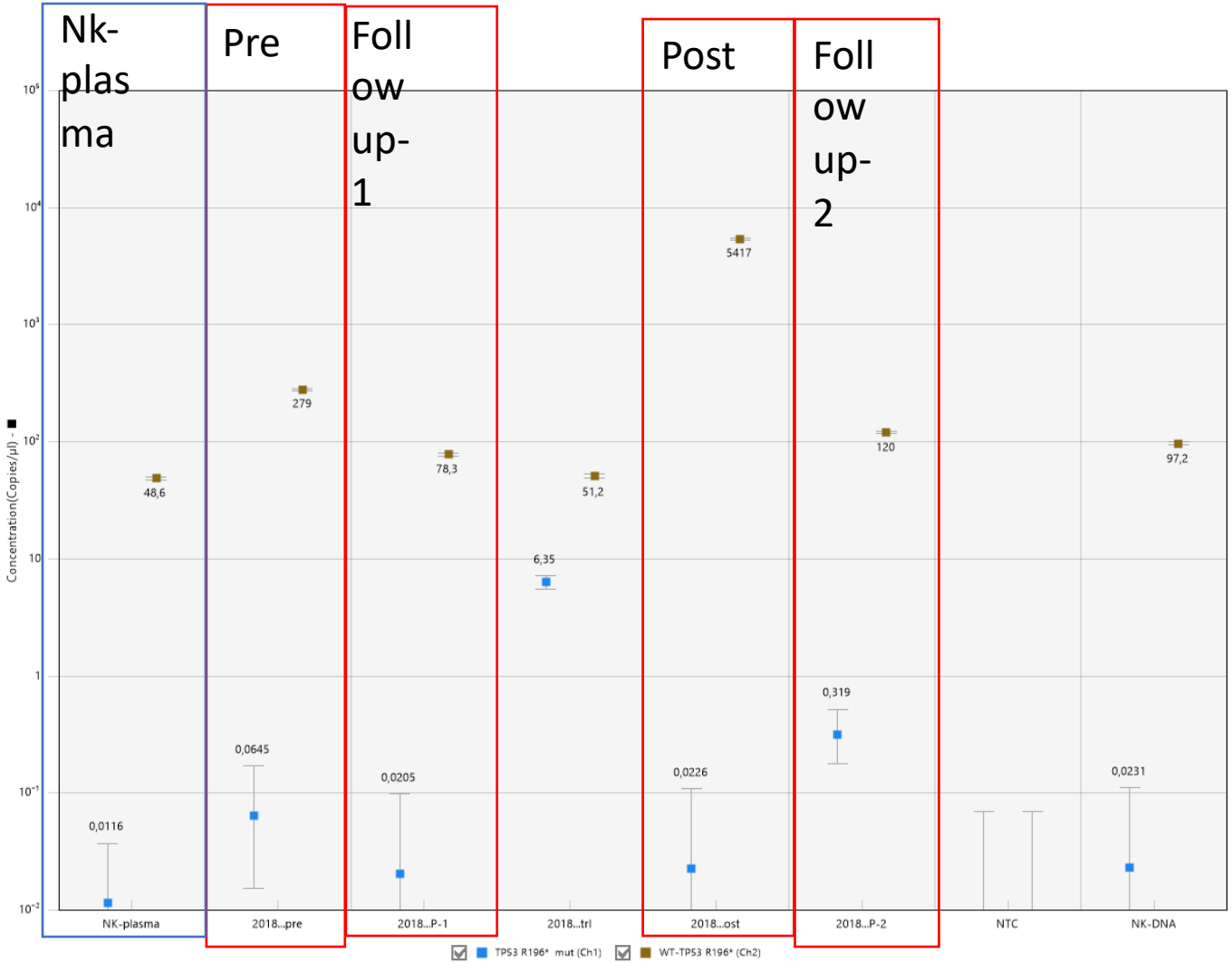
Follow up 1



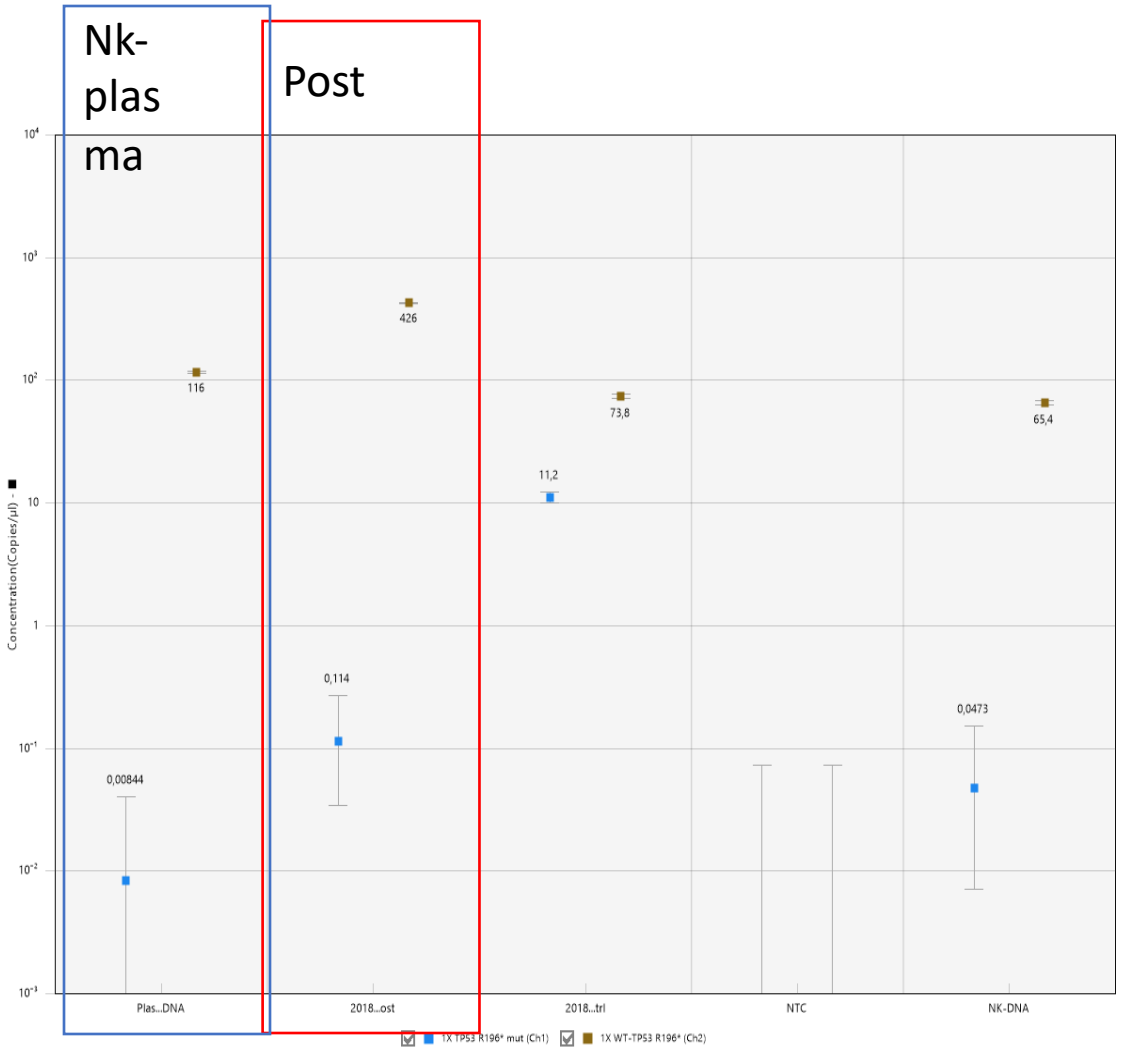
Follow up 2



Concentration plots Pre ,Post-surgery and Follow-up1 and 2



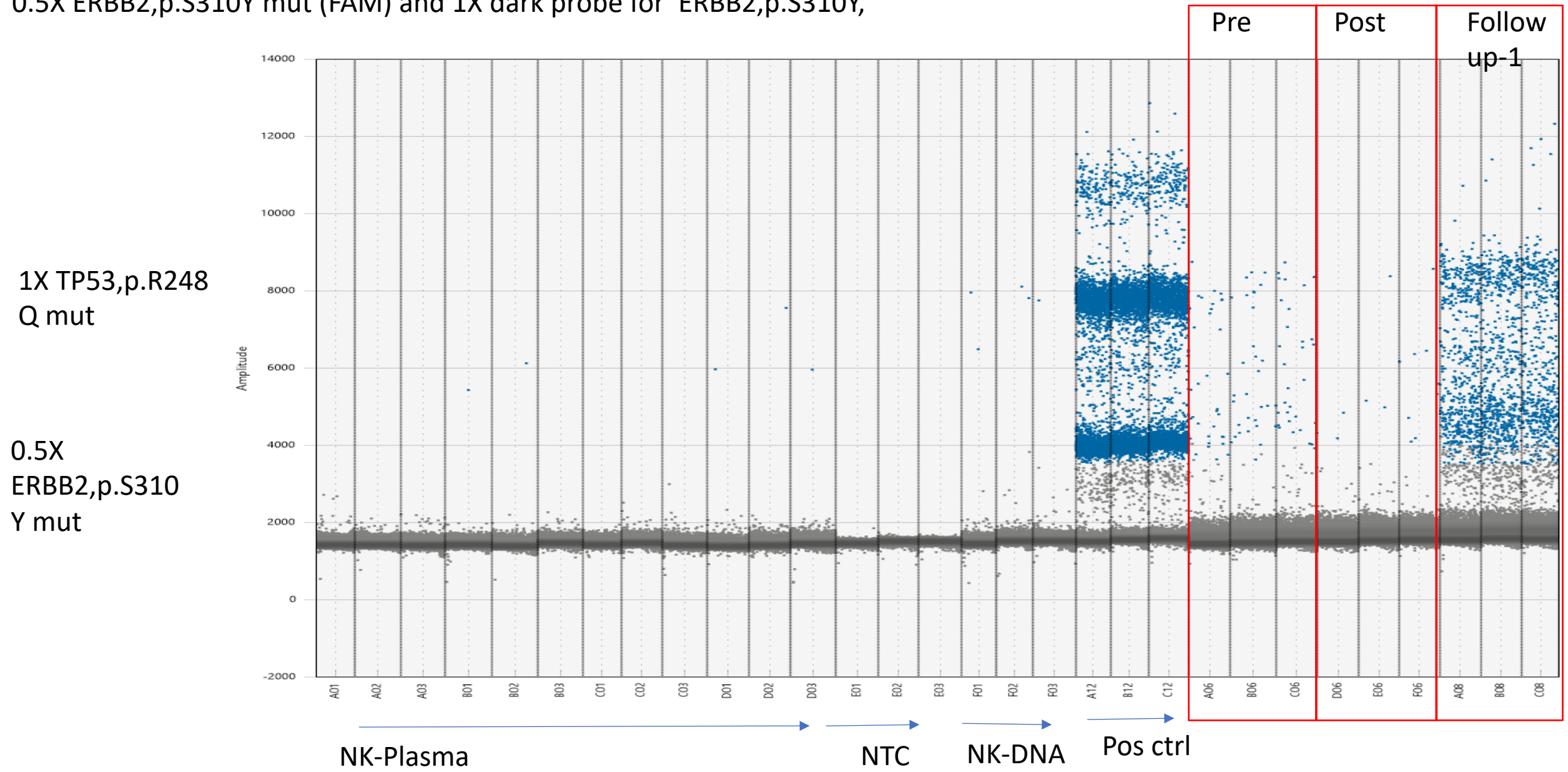
Post-dilution



P15 - Final

Gene targets : TP53,p.R248Q, c.743G>A and ERBB2,p.S310Y, c.929C>A

Assays: 1X TP53,p.R248Q mut (FAM) and 1X TP53 WT,p.R248Q (HEX)
0.5X ERBB2,p.S310Y mut (FAM) and 1X dark probe for ERBB2,p.S310Y,

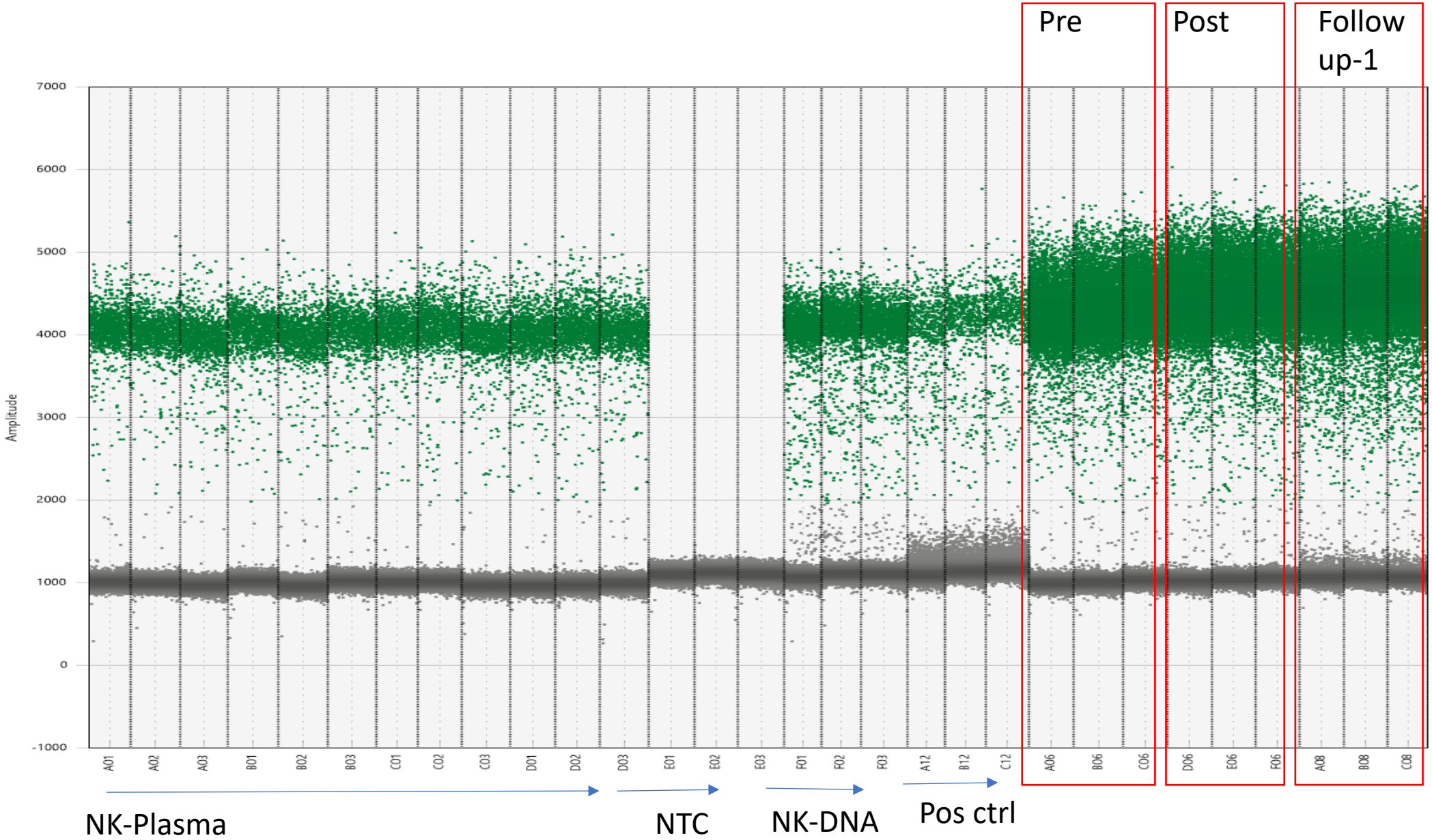


P15 - Final-WT

Gene targets : TP53,p.R248Q, c.743G>A and ERBB2,p.S310Y, c.929C>A

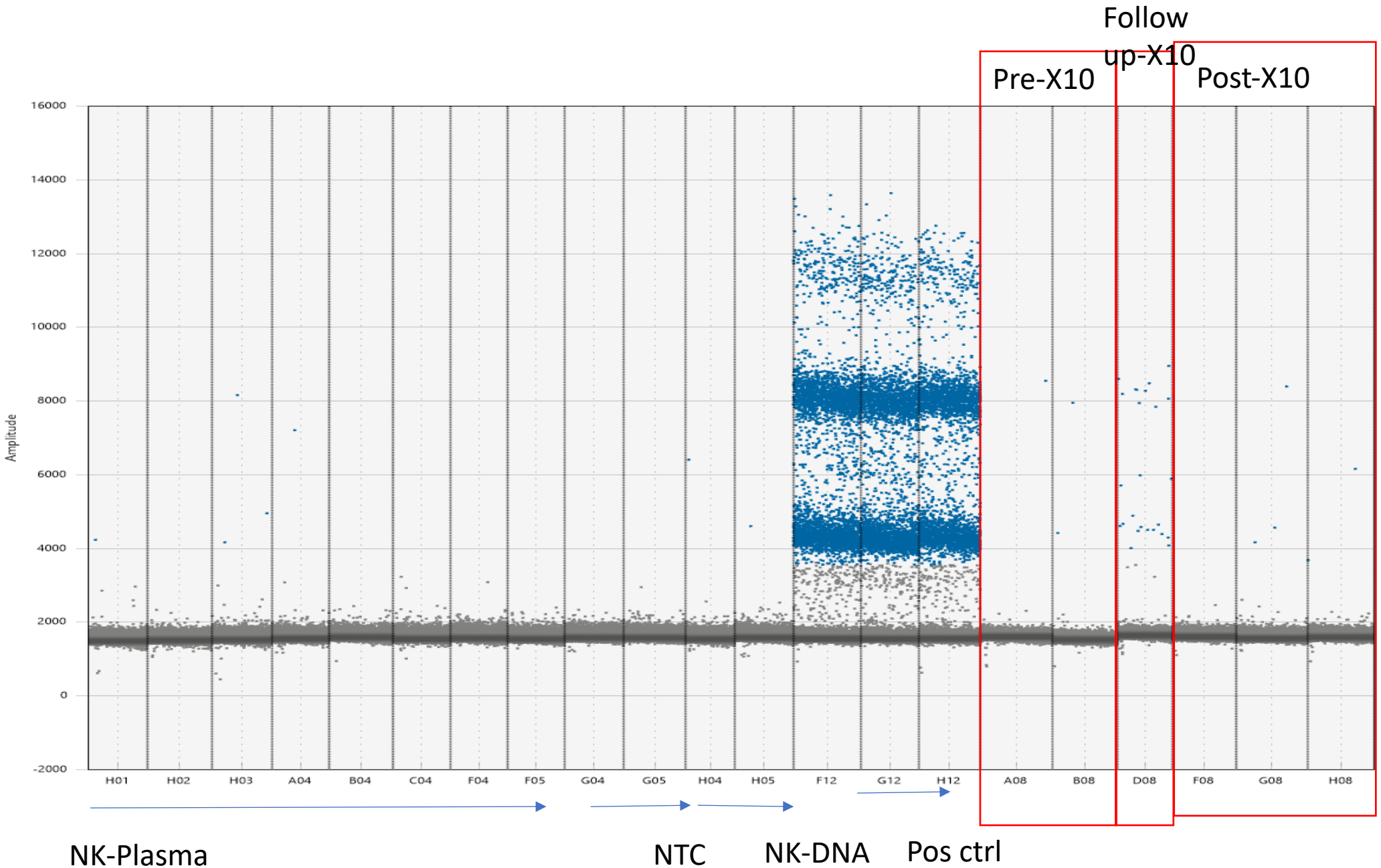
Assays:1X TP53 WT,p.R248Q (HEX) and 1X dark probe for ERBB2,p.S310Y,

1X TP53 WT,p.R248Q mu t



P15 Diluted

Gene targets : TP53,p.R248Q, c.743G>A and ERBB2,p.S310Y, c.929C>A

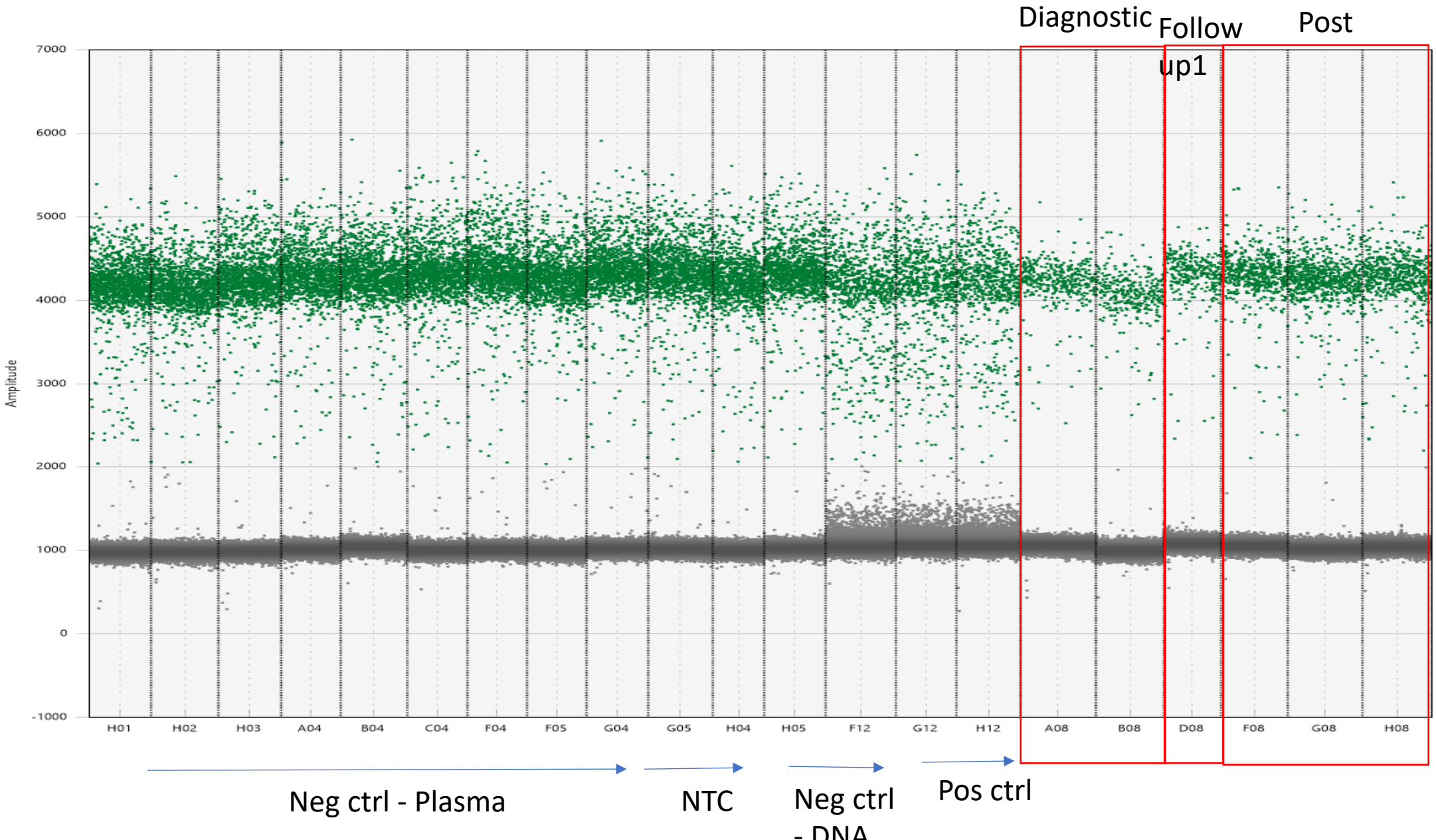


P15 - Final diluted

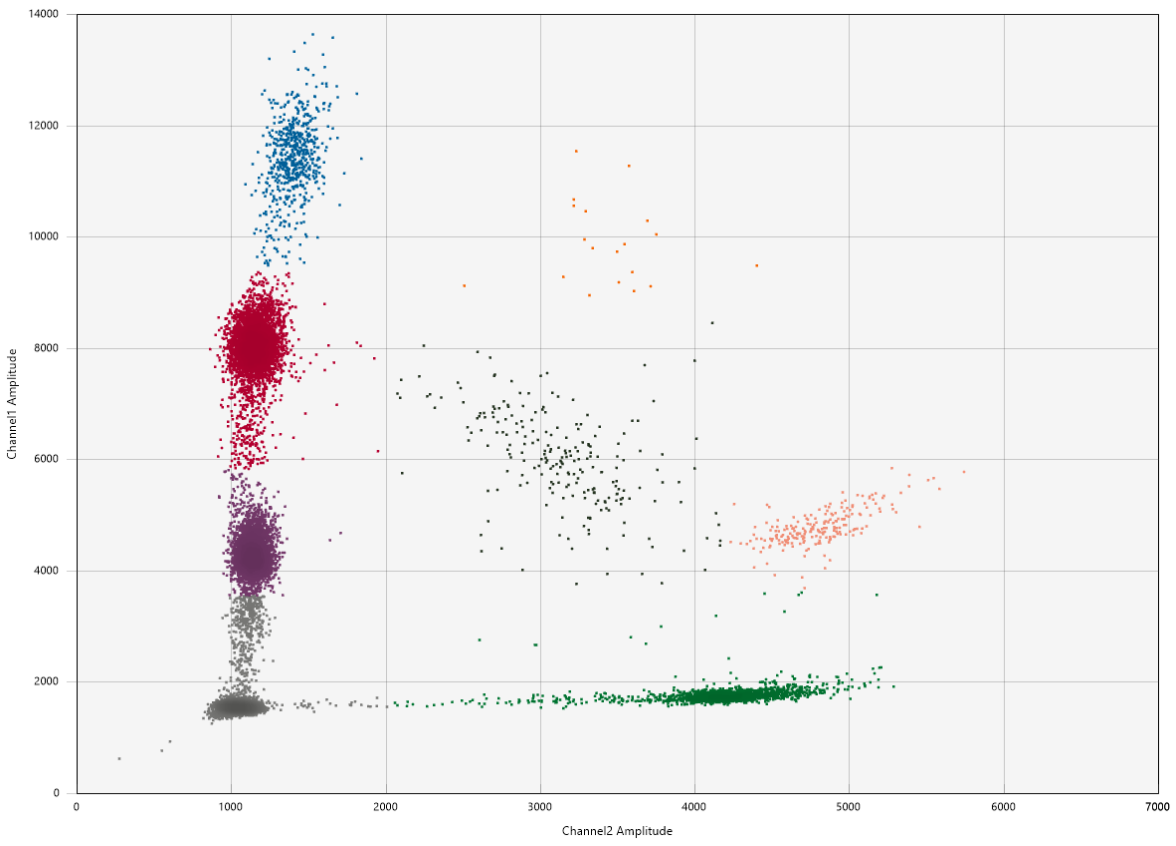
Gene targets : TP53,p.R248Q, c.743G>A and ERBB2,p.S310Y, c.929C>A

Assays: 1X TP53 WT,p.R248Q (HEX) and 1X dark probe for ERBB2,p.S310Y

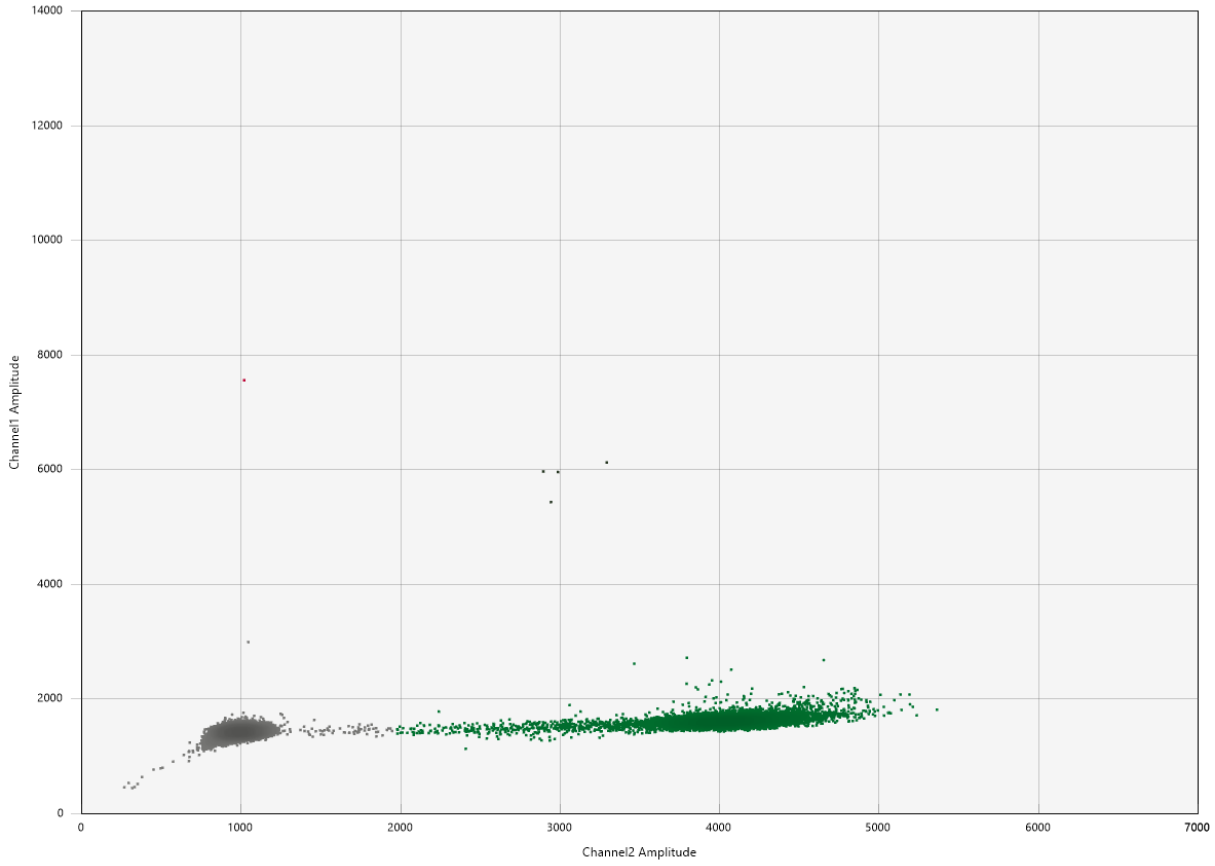
1X TP53 WT,p.R248Q



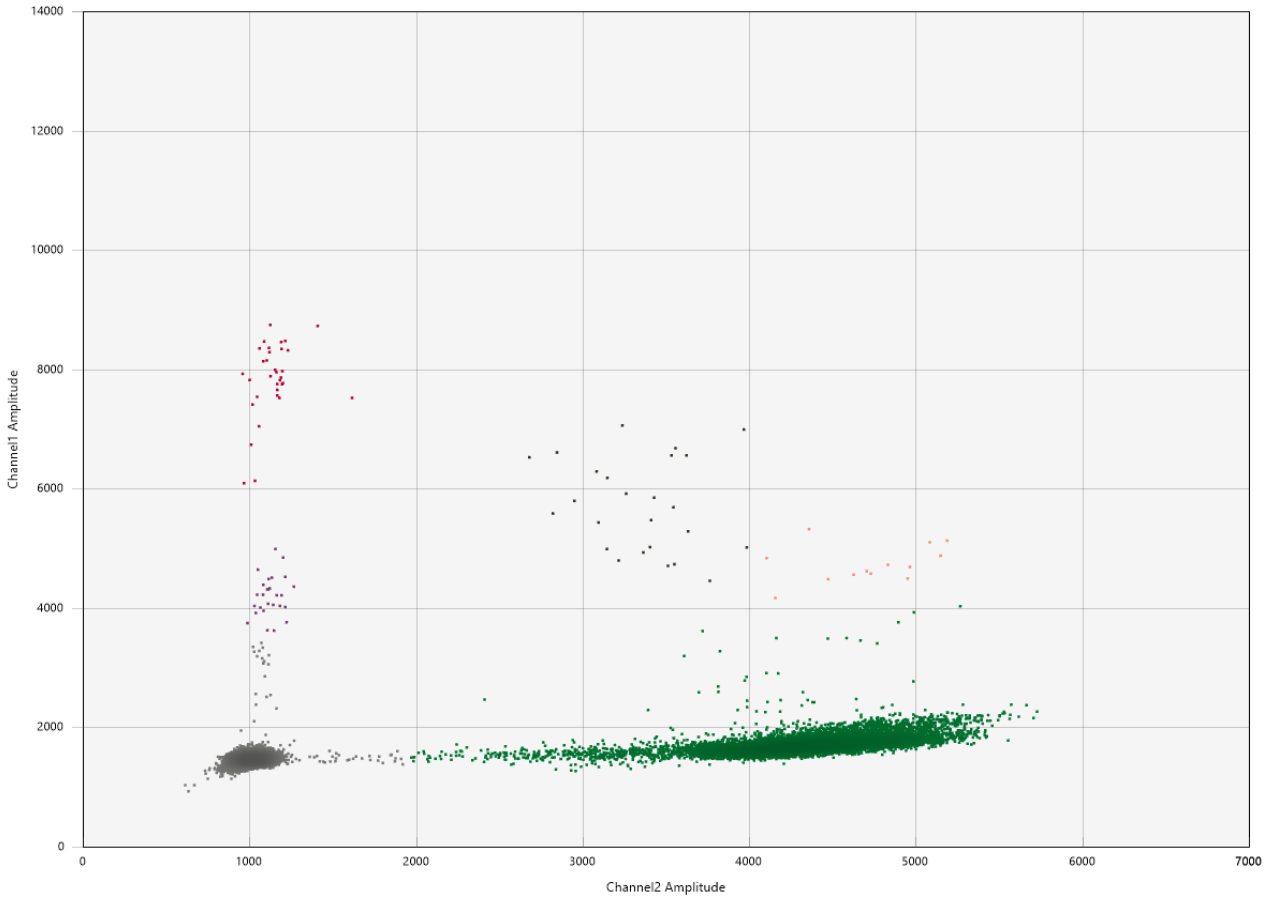
Positive control



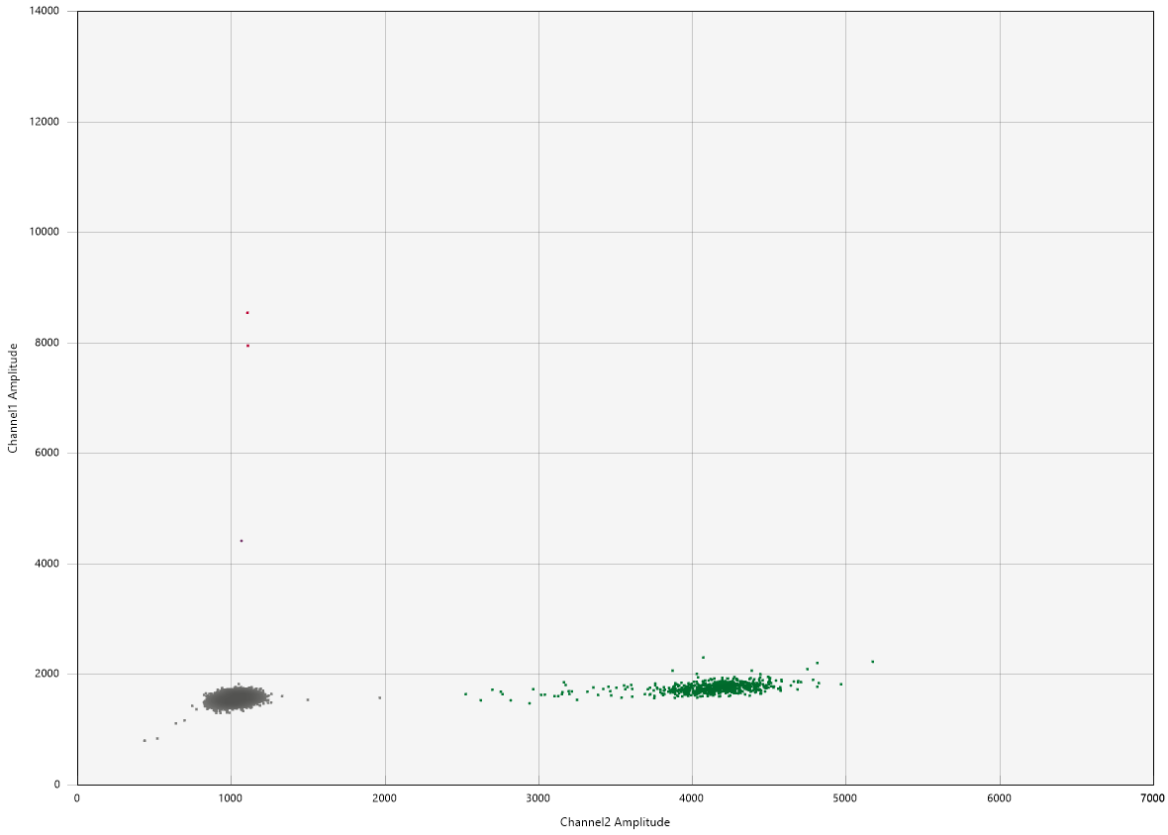
NK-plasma



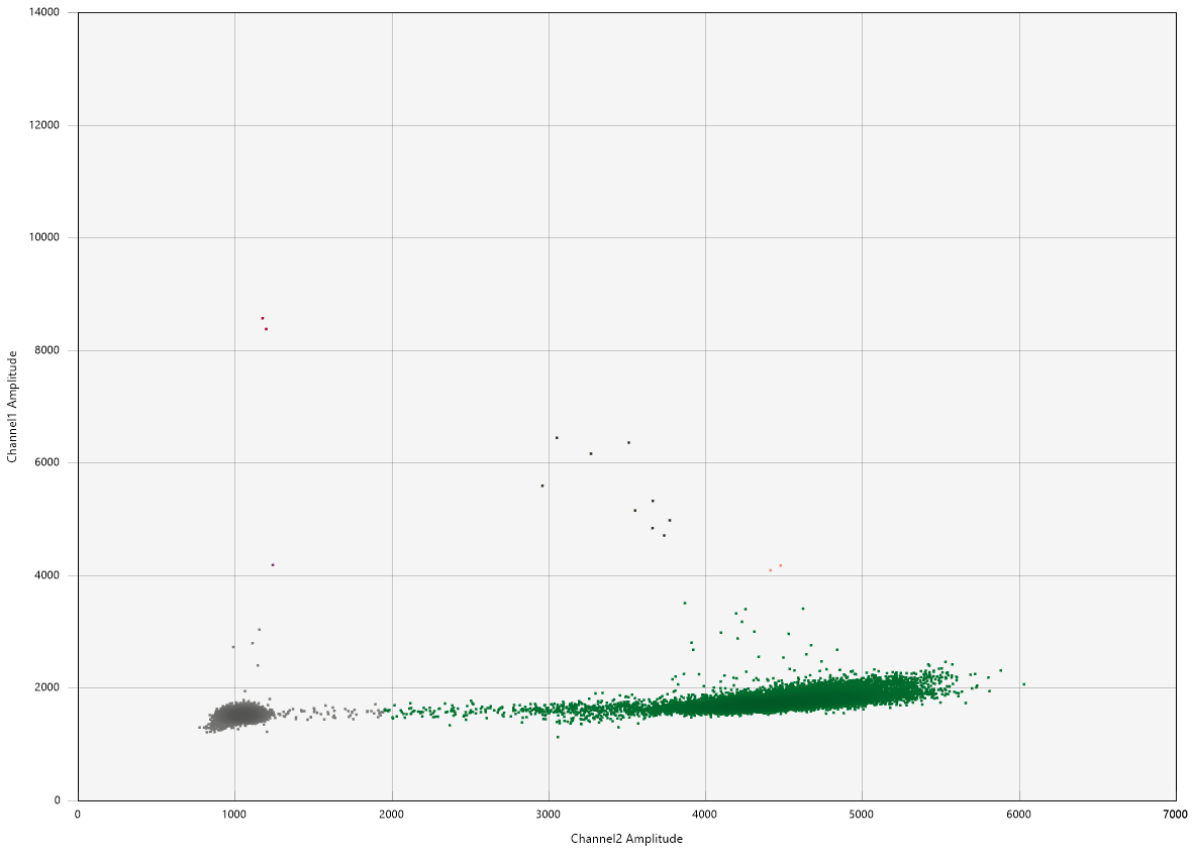
Pre-surgery



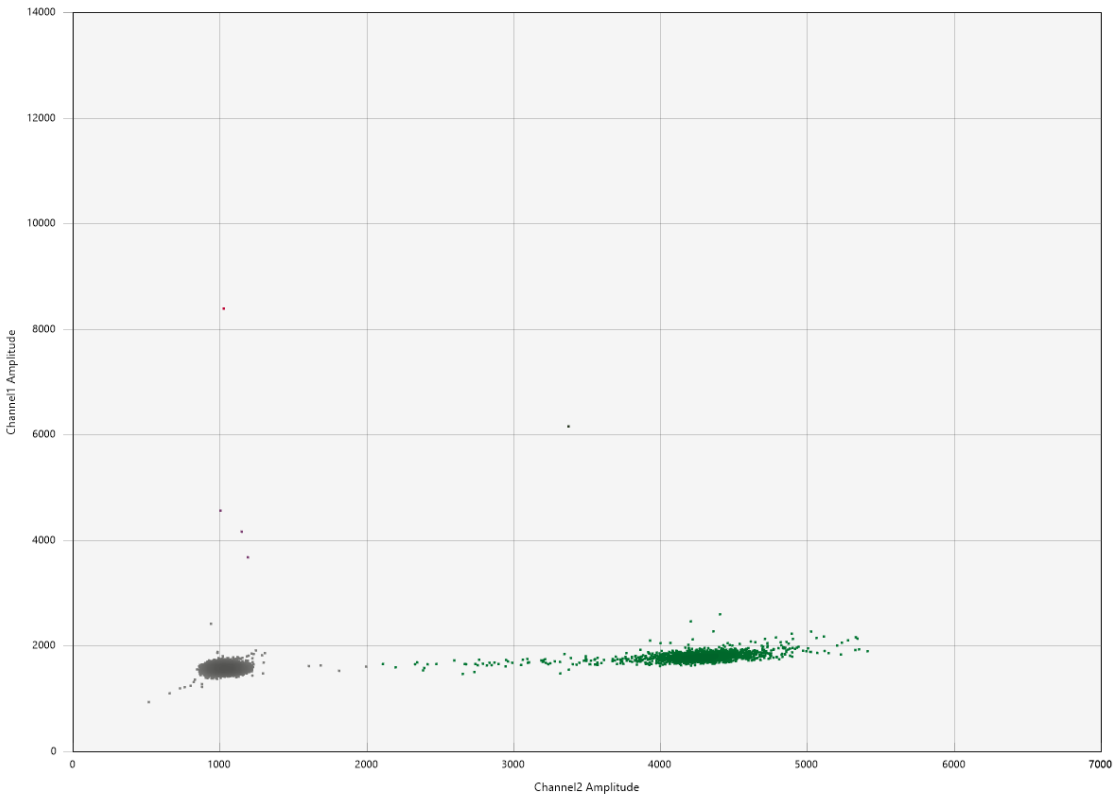
Pre-surgery- 10X dilution



Post-surgery

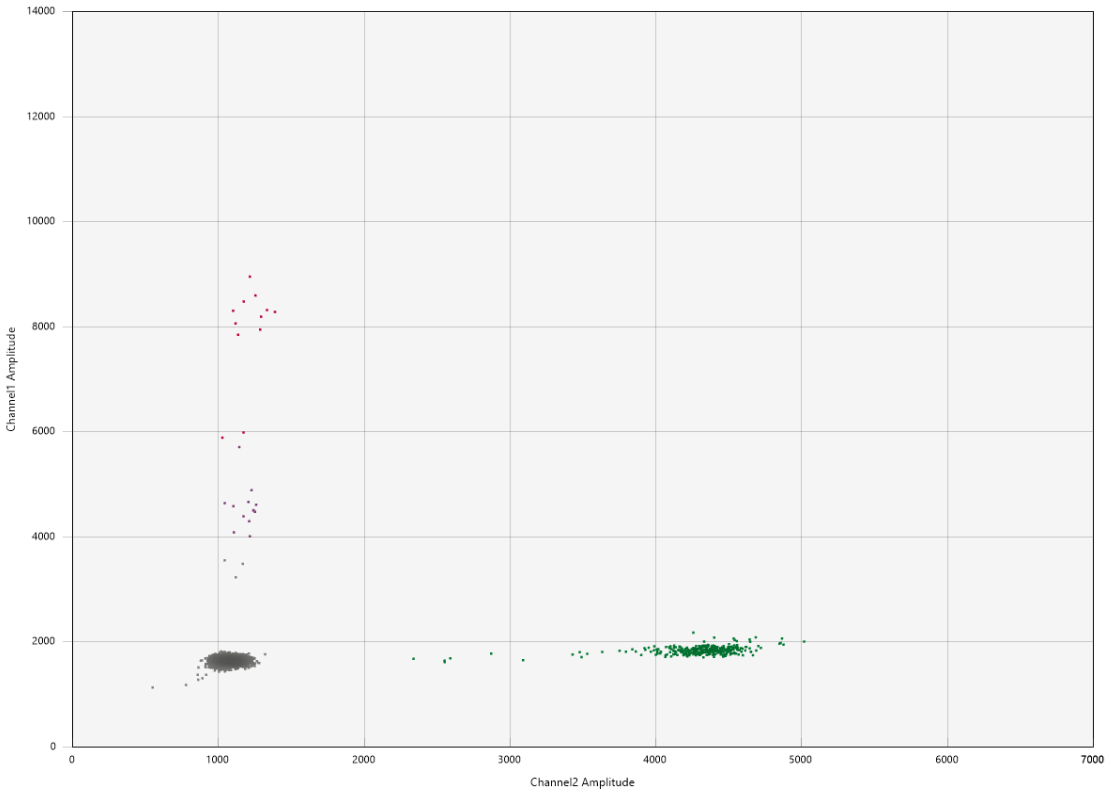


Post-surgery-10X dilution

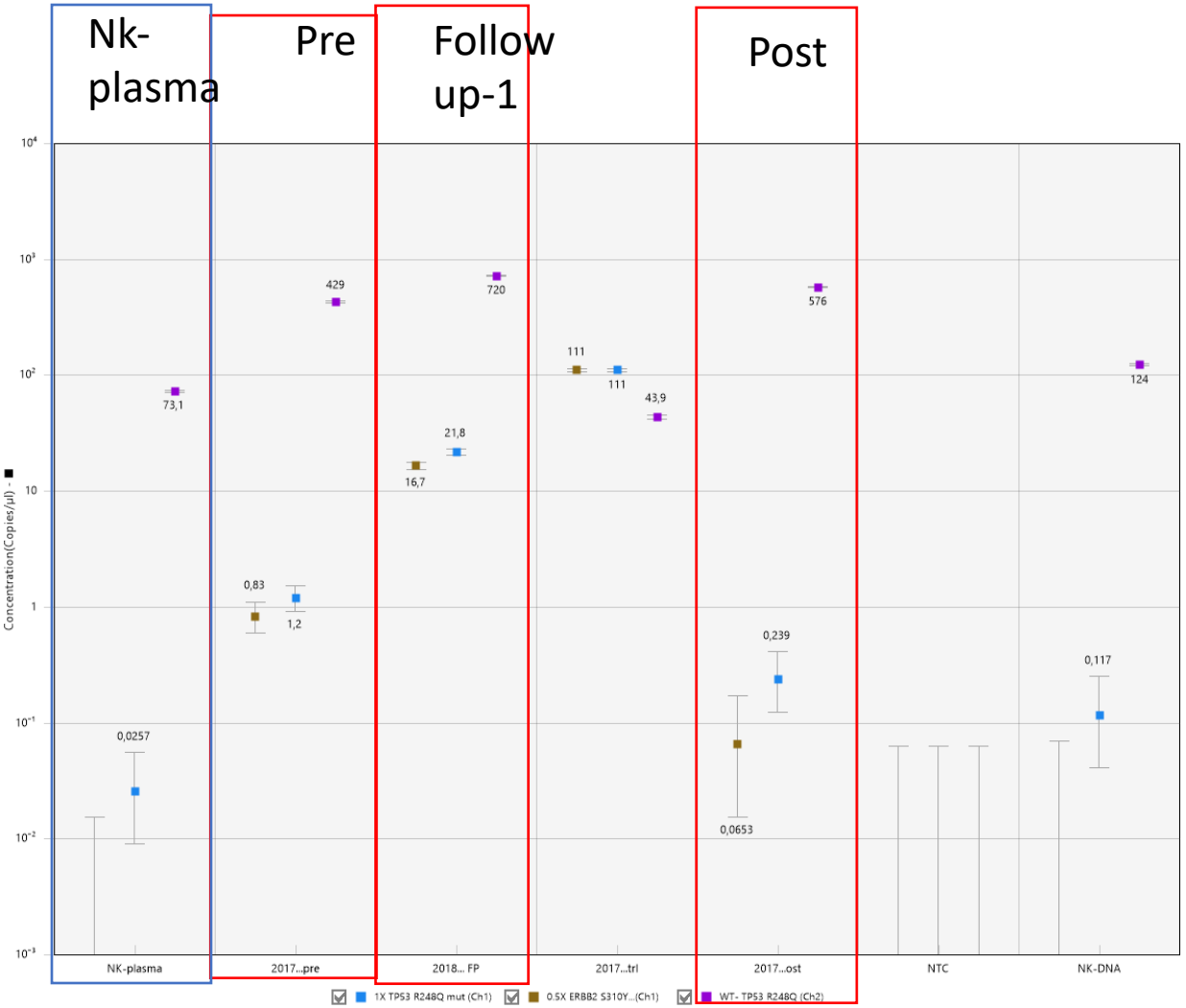
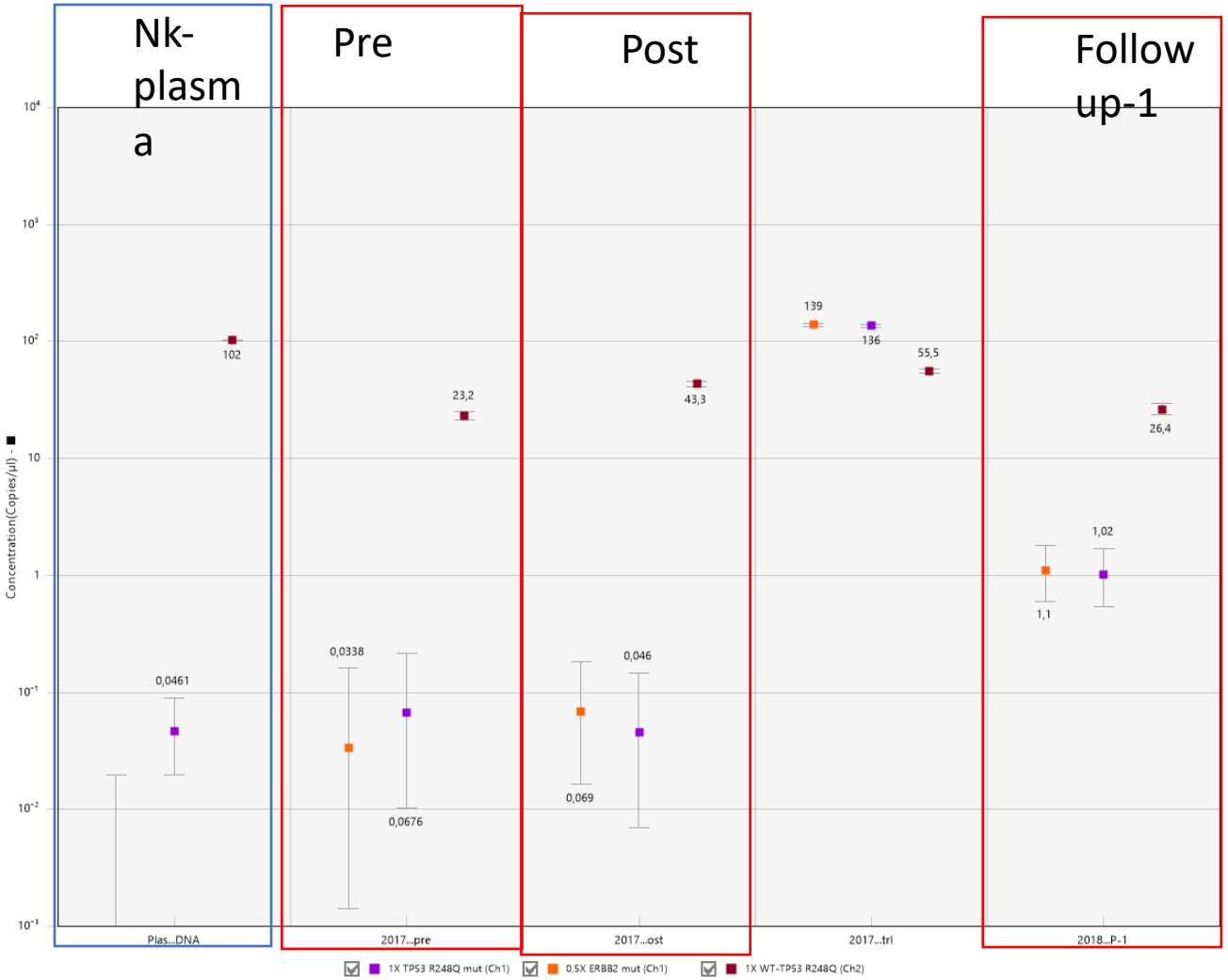


follow-up 1

follow-up 1- 10 X dilution



Concentration plots Pre ,Post-surgery and Follow-up1



Diluted-Pre ,Post-surgery and Follow-up1