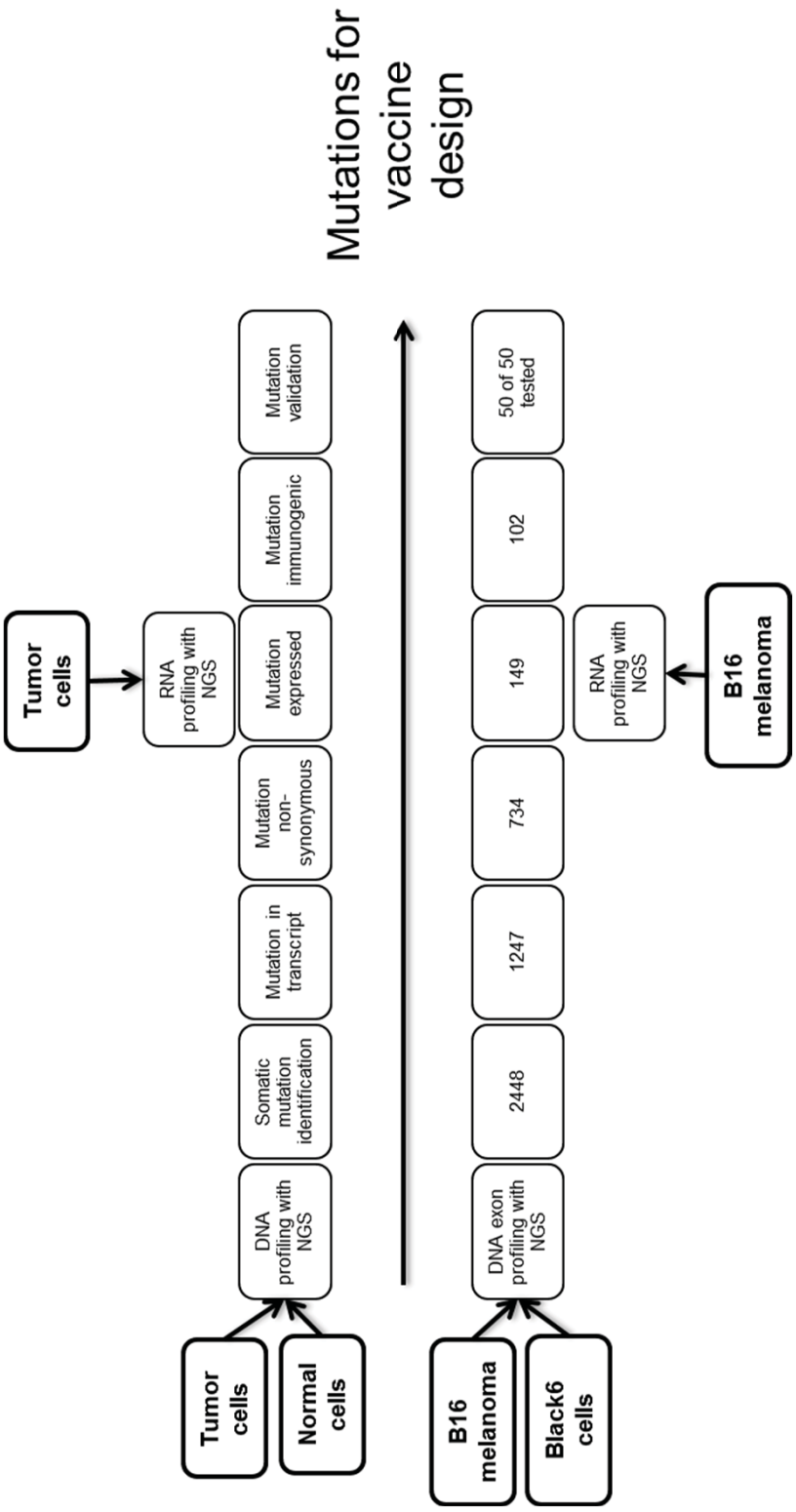

Mutations for vaccine design
Tumor cells
Normal cells
DNA profiling with NGS
Somatic mutation identification
Mutation in transcript
Mutation non-synonymous
Tumor cells
RNA profiling with NGS
Mutation expressed
Mutation immunogenic
Mutation validation
B16 melanoma
Black6 cells
DNA exon profiling with NGS
2448
1247
734
149
102
50 of 50 tested
B16 melanoma
RNA profiling with NGS

Fig. 1

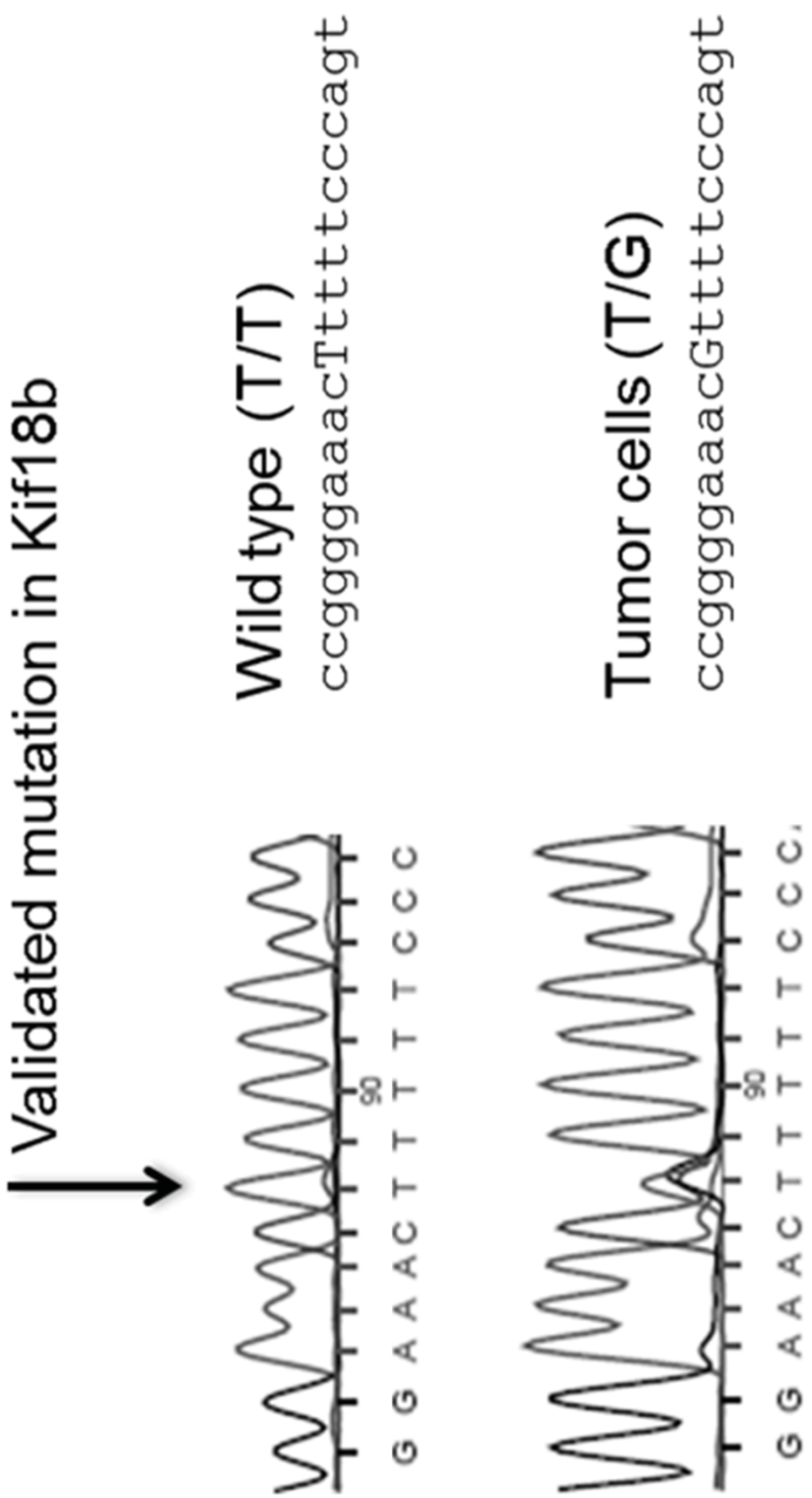

Validated mutation in Kif18b
Wild type (T/T)
ccggggaaacTttttcccagt
Tumor cells (T/G)
ccggggaaacGttttcccagt
G G A A A C T T T T T C C C
90
G G A A A C T T T T T C C C
90

Fig. 2

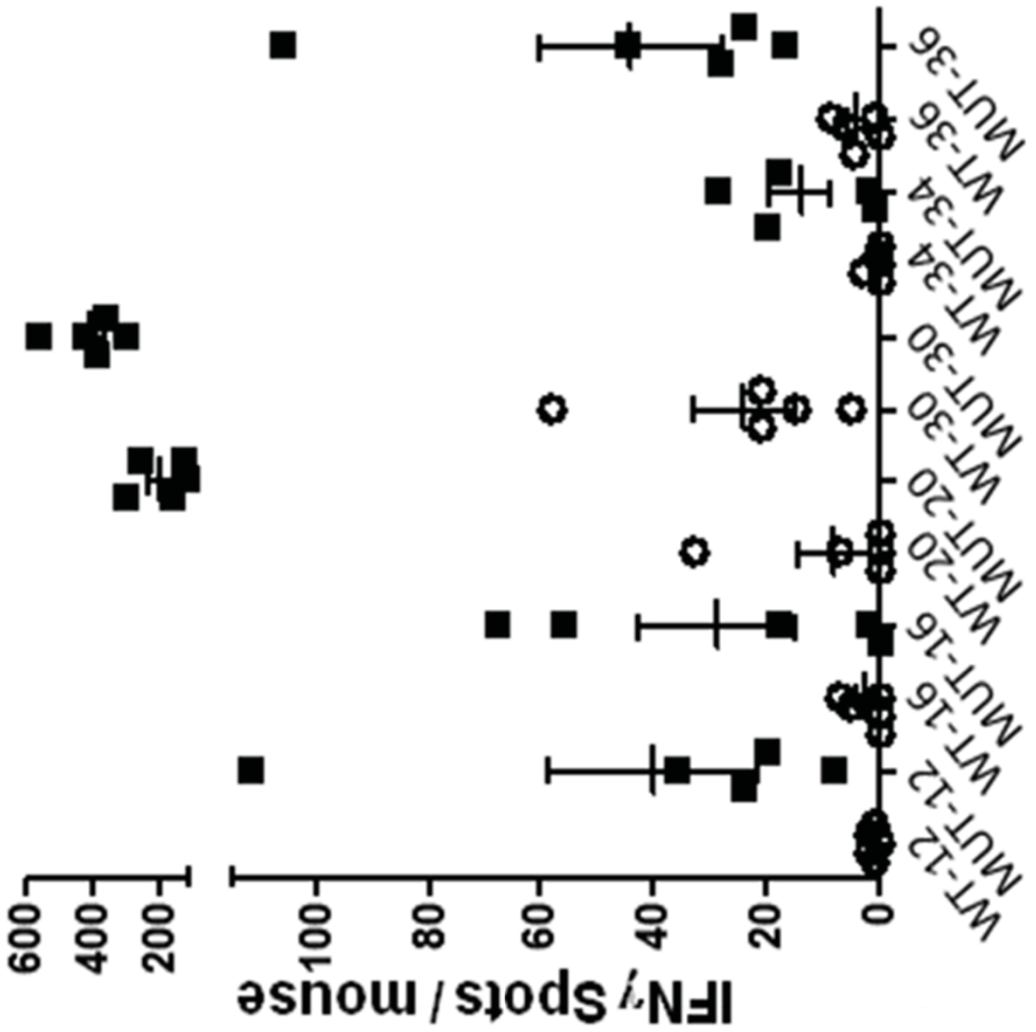

IFNγ Spots / mouse
600
400
200
100
80
60
40
20
0
WT-12
MUT-12
WT-16
MUT-16
WT-20
MUT-20
WT-30
MUT-30
WT-34
MUT-34
WT-36
MUT-36

Fig. 3

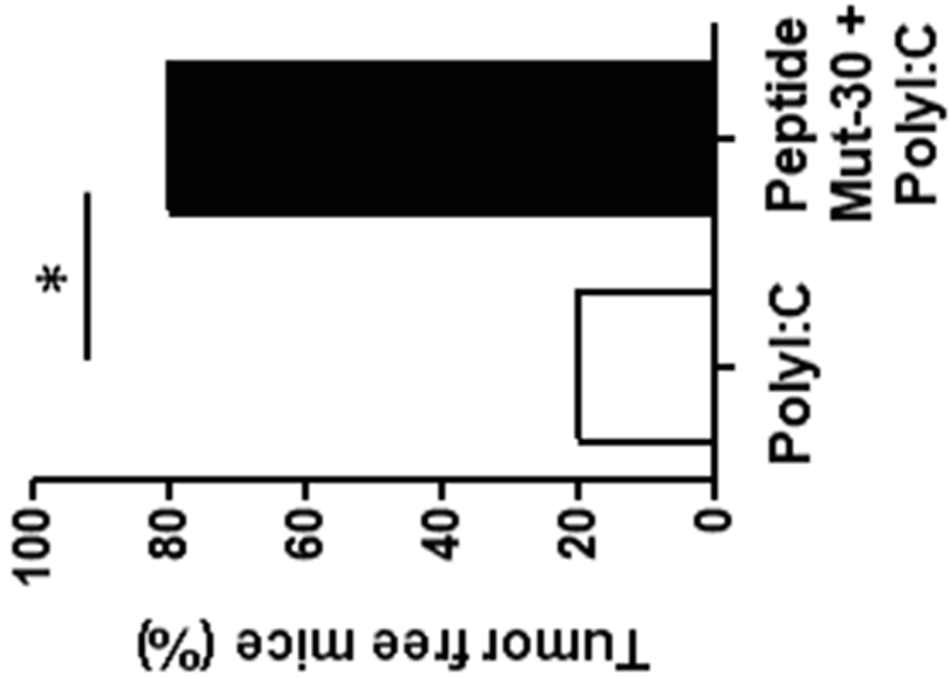


Fig. 4

Fig. 5

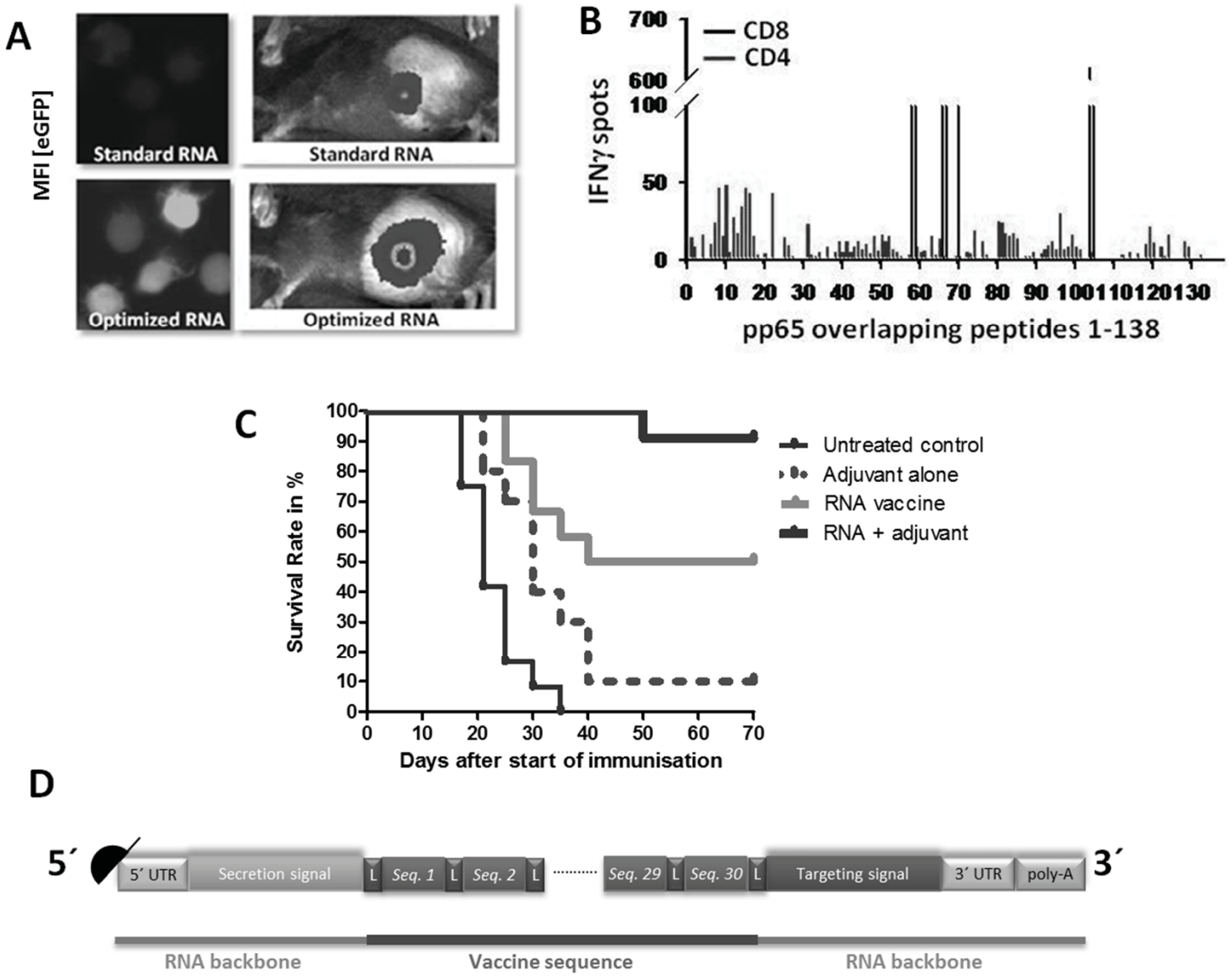
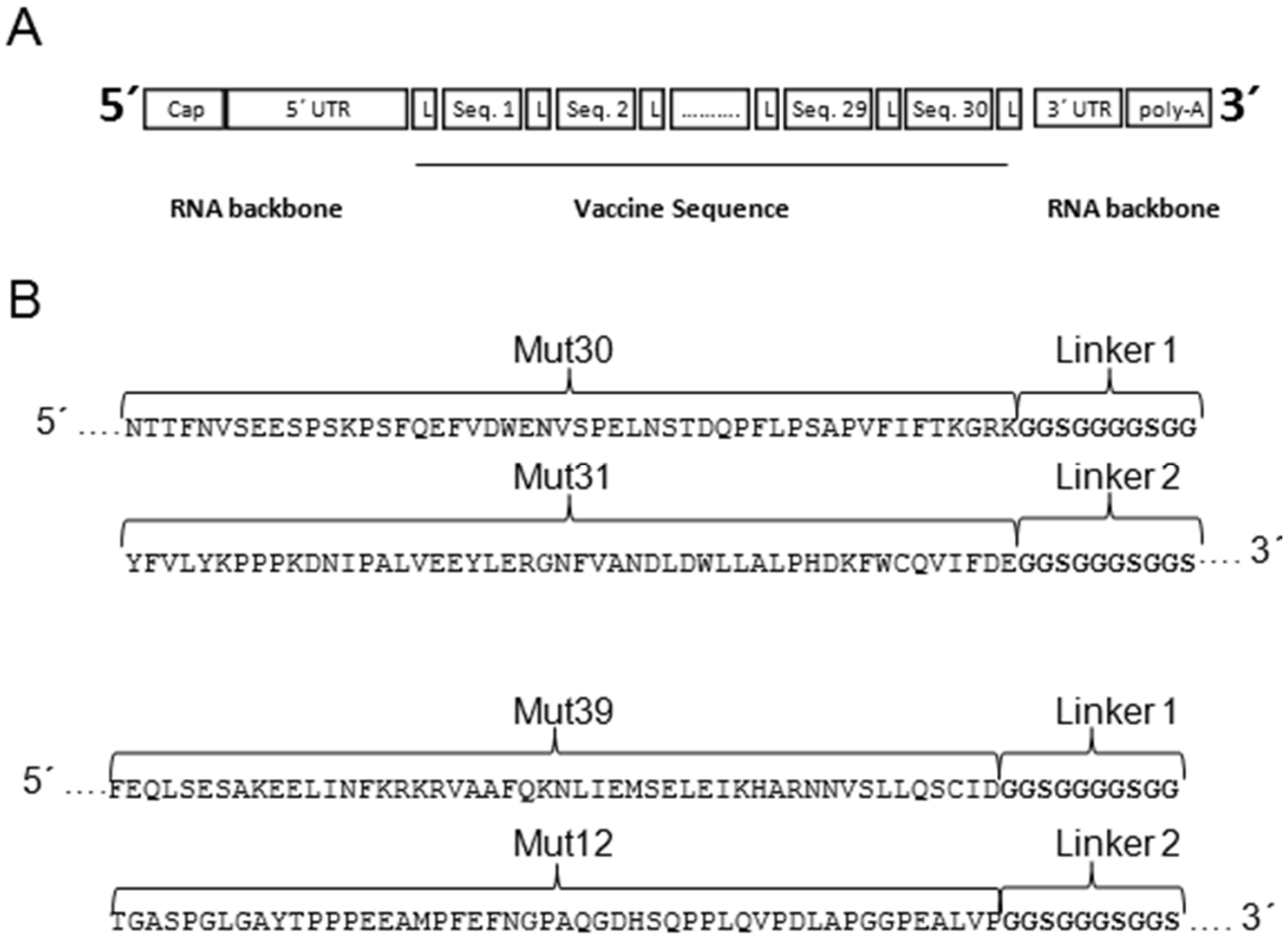

A
MFI [eGFP]
Standard RNA
Standard RNA
Optimized RNA
Optimized RNA
B
IFNγ spots
CD8
CD4
700
600
100
50
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130
pp65 overlapping peptides 1-138
C
Survival Rate in %
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70
Days after start of immunisation
Untreated control
Adjuvant alone
RNA vaccine
RNA + adjuvant
D
5´
5´ UTR
Secretion signal
L
Seq. 1
L
Seq. 2
L
Seq. 29
L
Seq. 30
L
Targeting signal
3´ UTR
poly-A
3´
RNA backbone
Vaccine sequence
RNA backbone

Fig. 6


A
5´
Cap
5´ UTR
L
Seq. 1
L
Seq. 2
L
.....
L
Seq. 29
L
Seq. 30
L
3´ UTR
poly-A
3´
RNA backbone
Vaccine Sequence
RNA backbone
B
Mut30
Linker 1
5´ ....NTTFNVSEESPSKPSFQEFVDWENVSPELNSTDQPFLPSAPVFIFTKGRKGGSGGGGSGG
Mut31
Linker 2
YFVLYKPPPKDNIPALVEEYLERGNFVANDLDWLLALPHDKFWCQVIFDEGGSGGGSGGS.... 3´
Mut39
Linker 1
5´ ....FEQLSESAKEELINFKRKRVAAFQKNLIEMSELEIKHARNNVSLLQSCIDGGSGGGGSGG
Mut12
Linker 2
TGASPGLGAYTPPPEEAMPFEFNGPAQGDHSQPPLQVPDLAPGGPEALVPGGSGGGSGGS.... 3´

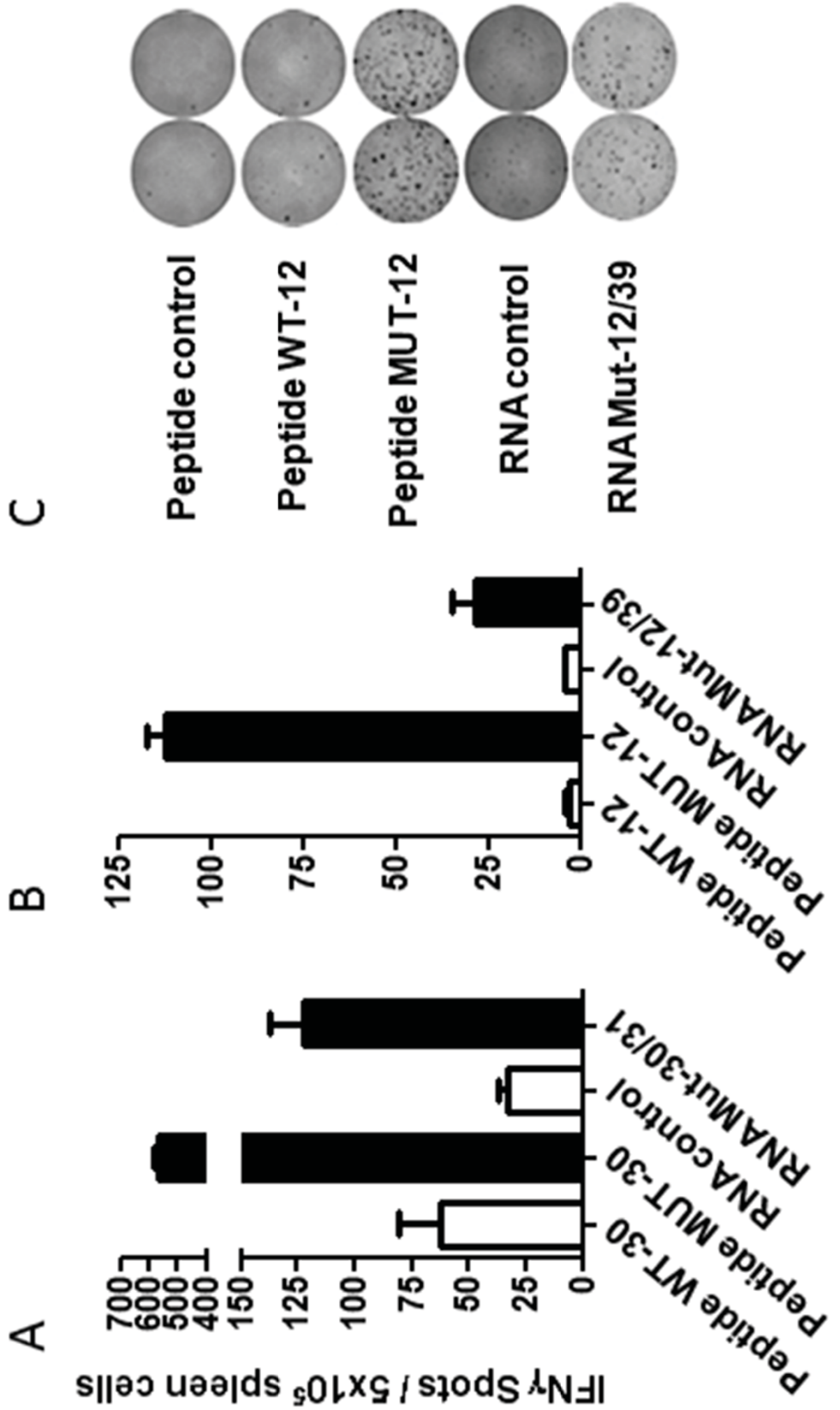

A
IFNγ Spots / 5x10⁵ spleen cells
700
600
500
400
150
125
100
75
50
25
0
Peptide WT-30
Peptide MUT-30
RNA control
RNA Mut-30/31
B
125
100
75
50
25
0
Peptide WT-12
Peptide MUT-12
RNA control
RNA Mut-12/39
C
Peptide control
Peptide WT-12
Peptide MUT-12
RNA control
RNA Mut-12/39

Fig. 7

Fig. 8

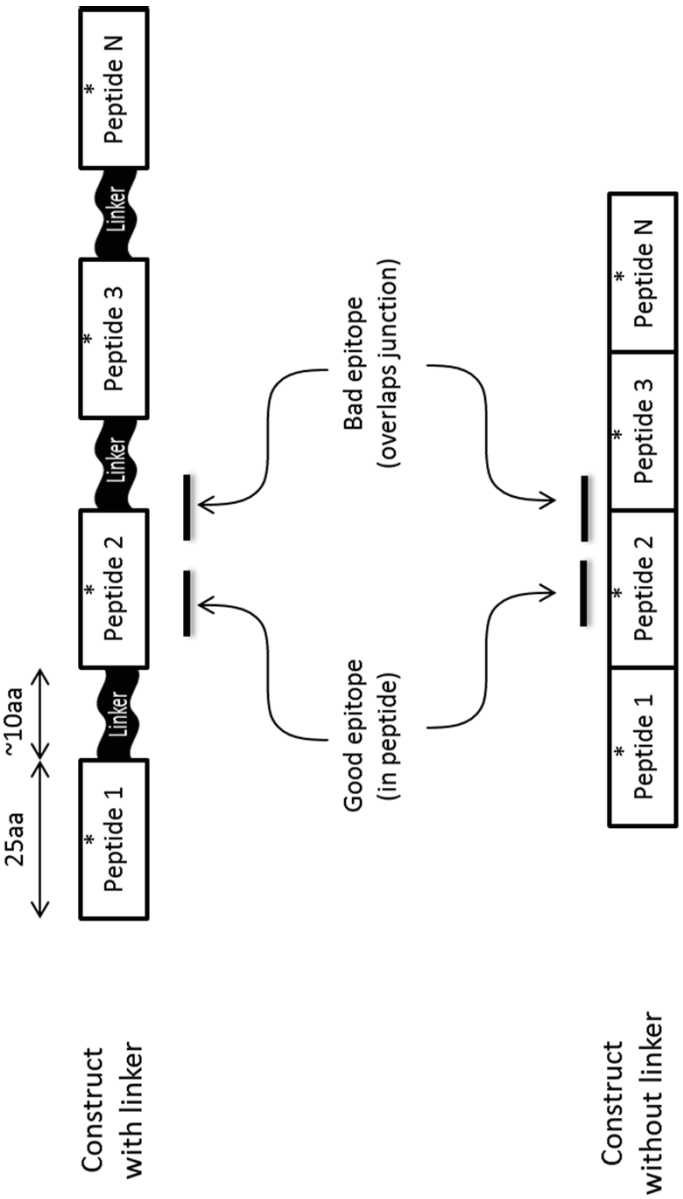
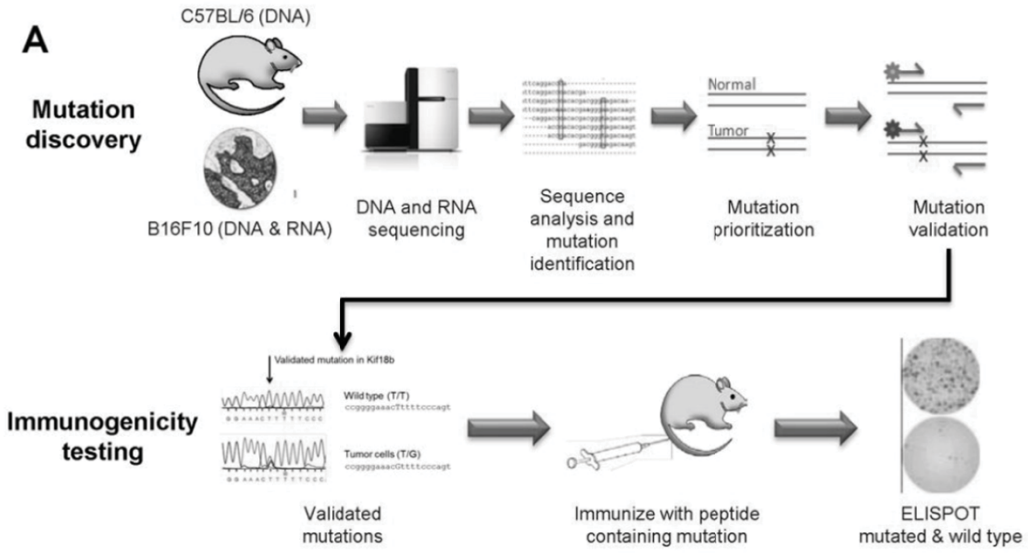

Construct with linker
25aa
~10aa
Peptide 1
Linker
Peptide 2
Linker
Peptide 3
Linker
Peptide N
Good epitope (in peptide)
Bad epitope (overlaps junction)
Construct without linker
Peptide 1
Peptide 2
Peptide 3
Peptide N

Fig. 9


A
Mutation discovery
C57BL/6 (DNA)
B16F10 (DNA & RNA)
DNA and RNA sequencing
Sequence analysis and mutation identification
Normal
Tumor
Mutation prioritization
Mutation validation
Immunogenicity testing
Validated mutation in Kif18b
Wild type (T/T)
Tumor cells (T/G)
Validated mutations
Immunize with peptide containing mutation
ELISPOT mutated & wild type

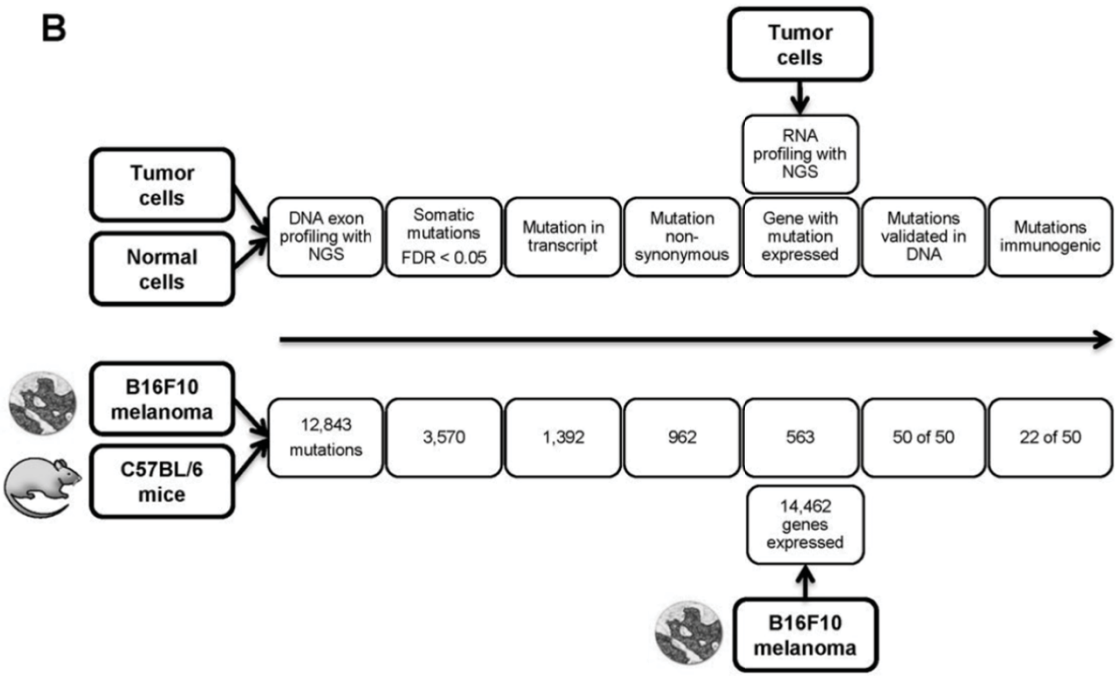

B
Tumor cells
RNA profiling with NGS
Tumor cells
Normal cells
DNA exon profiling with NGS
Somatic mutations FDR < 0.05
Mutation in transcript
Mutation non-synonymous
Gene with mutation expressed
Mutations validated in DNA
Mutations immunogenic
B16F10 melanoma
C57BL/6 mice
12,843 mutations
3,570
1,392
962
563
50 of 50
22 of 50
14,462 genes expressed
B16F10 melanoma

Fig. 9

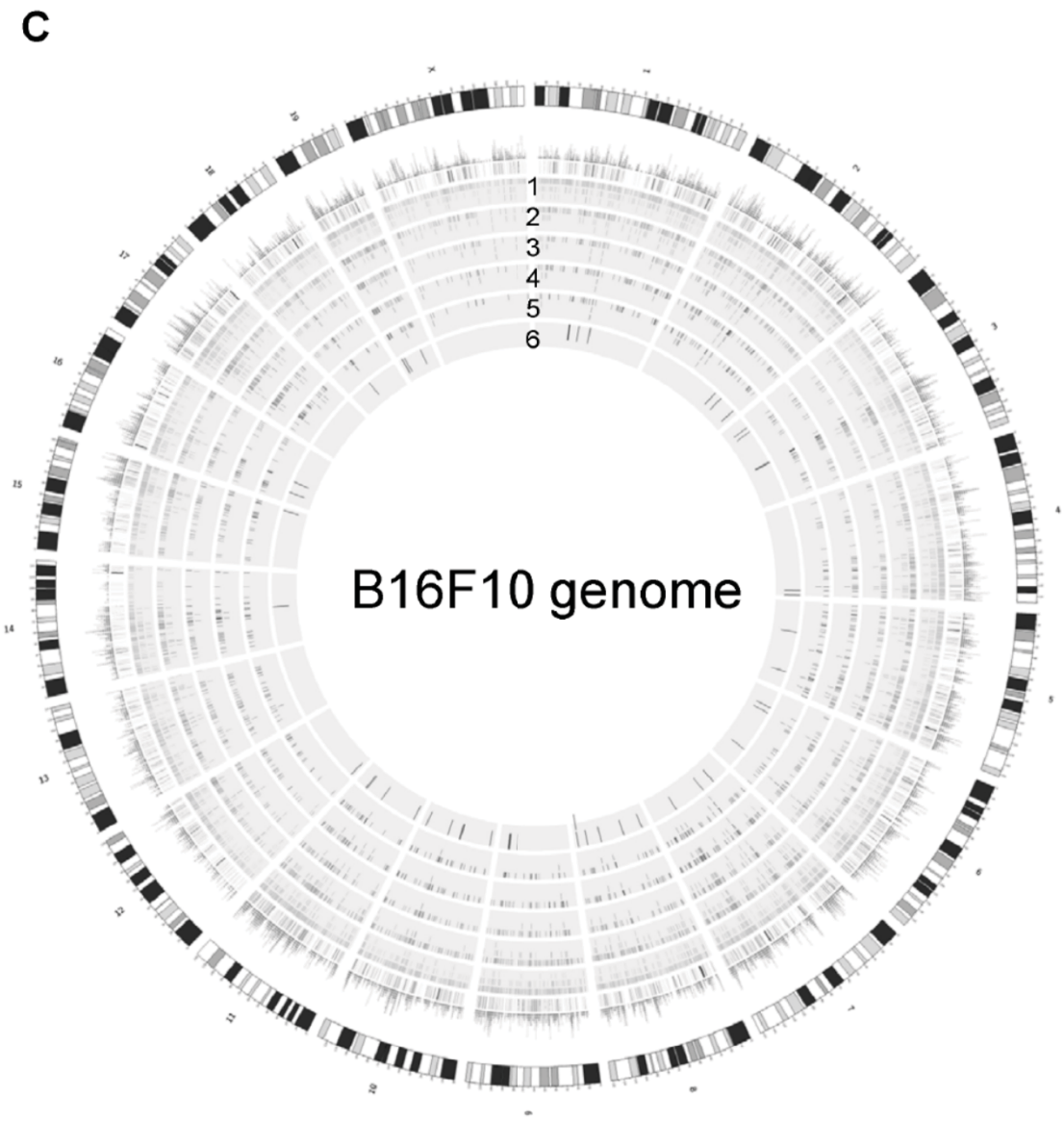


Fig. 10

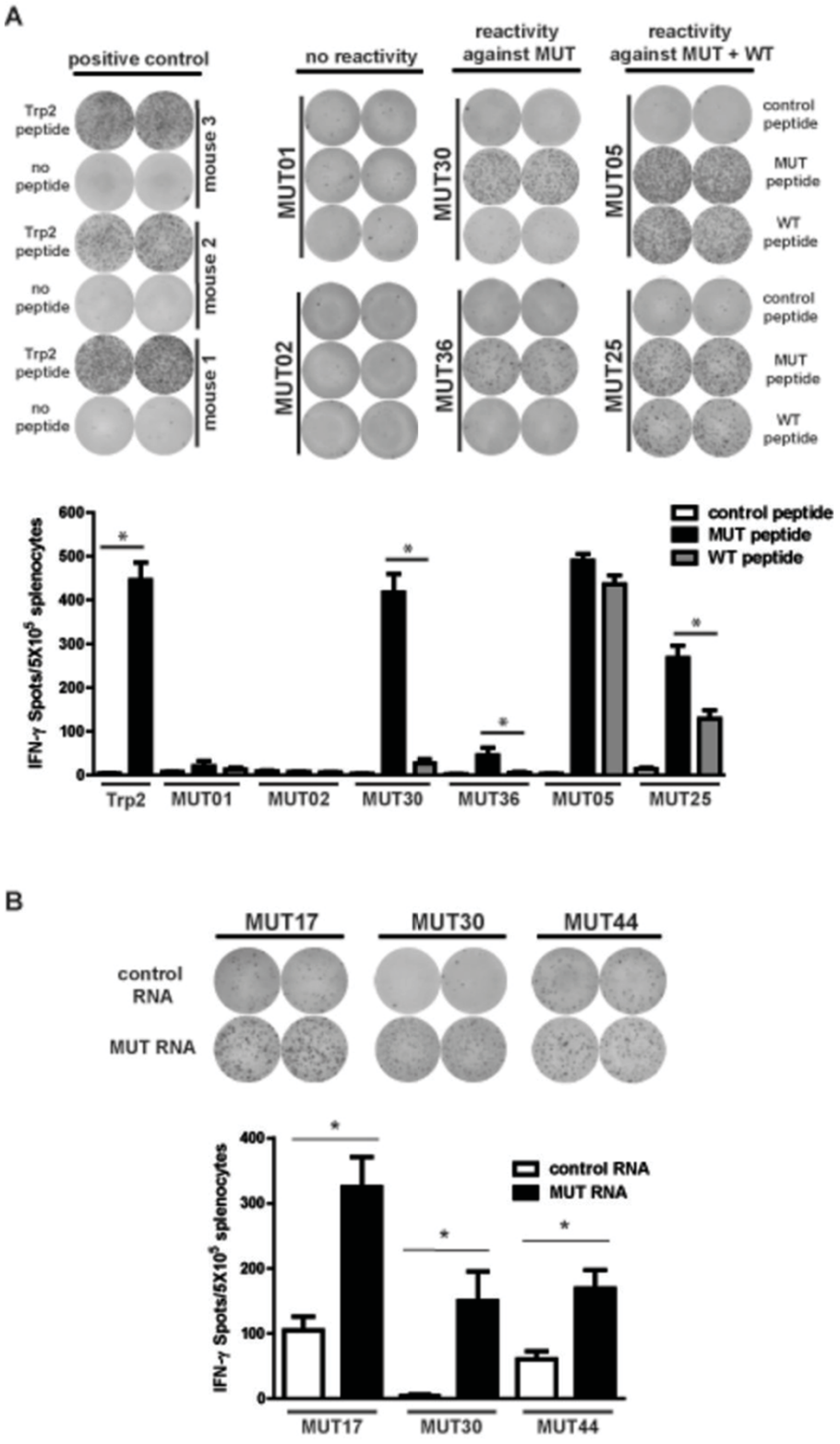

A
positive control
no reactivity
reactivity against MUT
reactivity against MUT + WT
Trp2 peptide
no peptide
mouse 3
mouse 2
mouse 1
MUT01
MUT30
MUT05
MUT02
MUT36
MUT25
control peptide
MUT peptide
WT peptide
IFN-γ Spots/5X10^5 splenocytes
Trp2
MUT01
MUT02
MUT30
MUT36
MUT05
MUT25
B
MUT17
MUT30
MUT44
control RNA
MUT RNA

Fig. 10

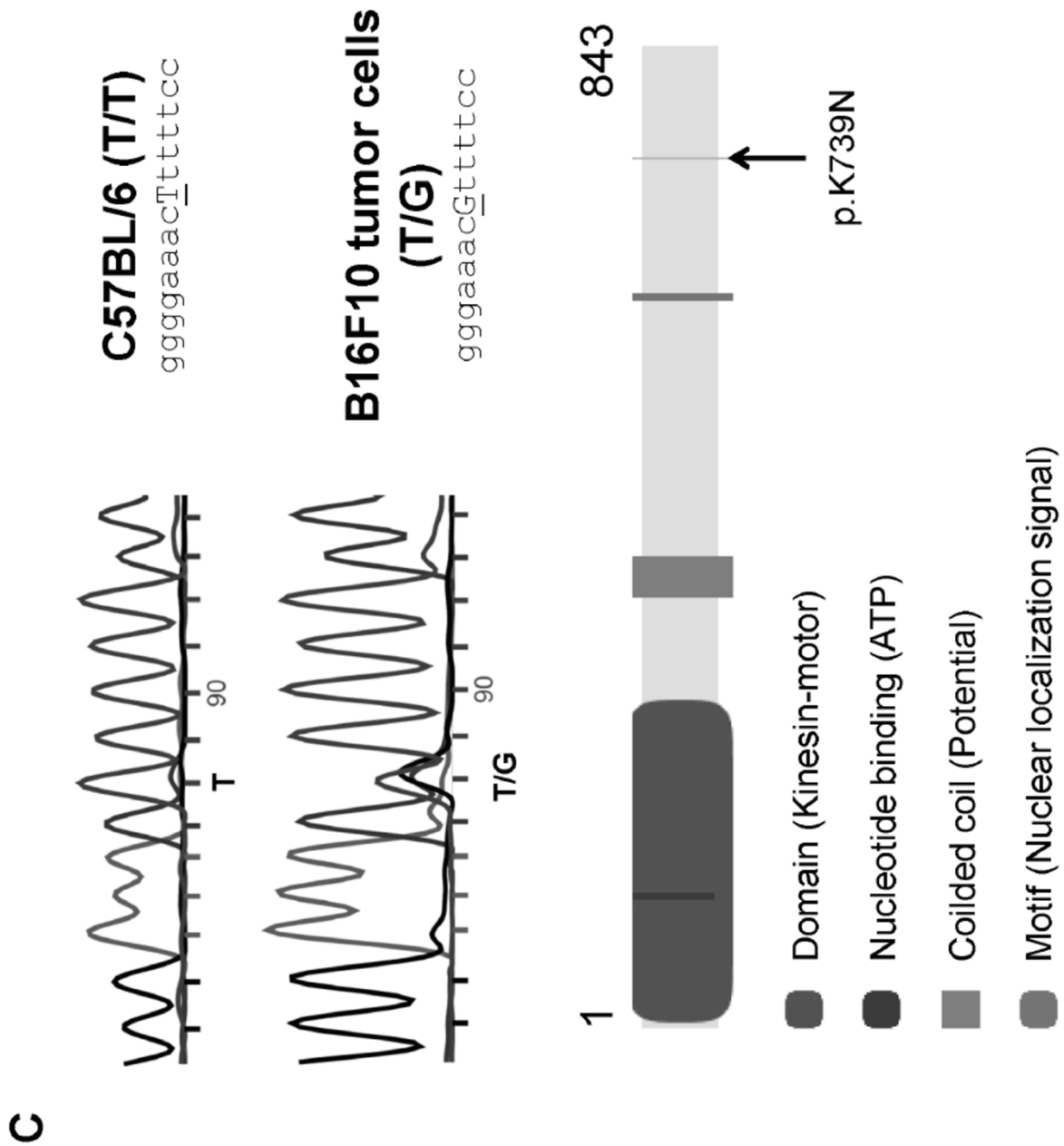

C
C57BL/6 (T/T)
ggggaaacTttttcc
T
90
B16F10 tumor cells
(T/G)
gggaaacGttttcc
T/G
90
1
843
p.K739N
Domain (Kinesin-motor)
Nucleotide binding (ATP)
Coilded coil (Potential)
Motif (Nuclear localization signal)

Fig. 11

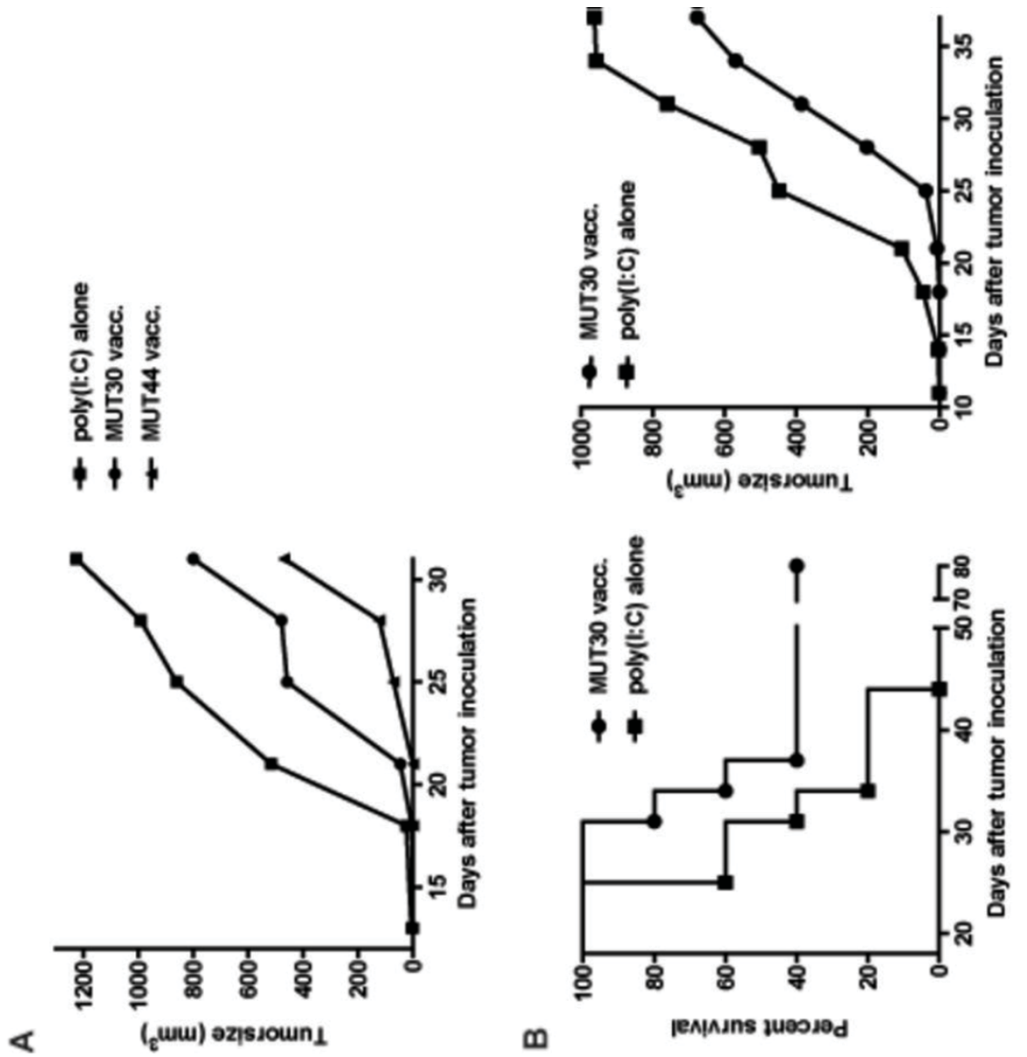
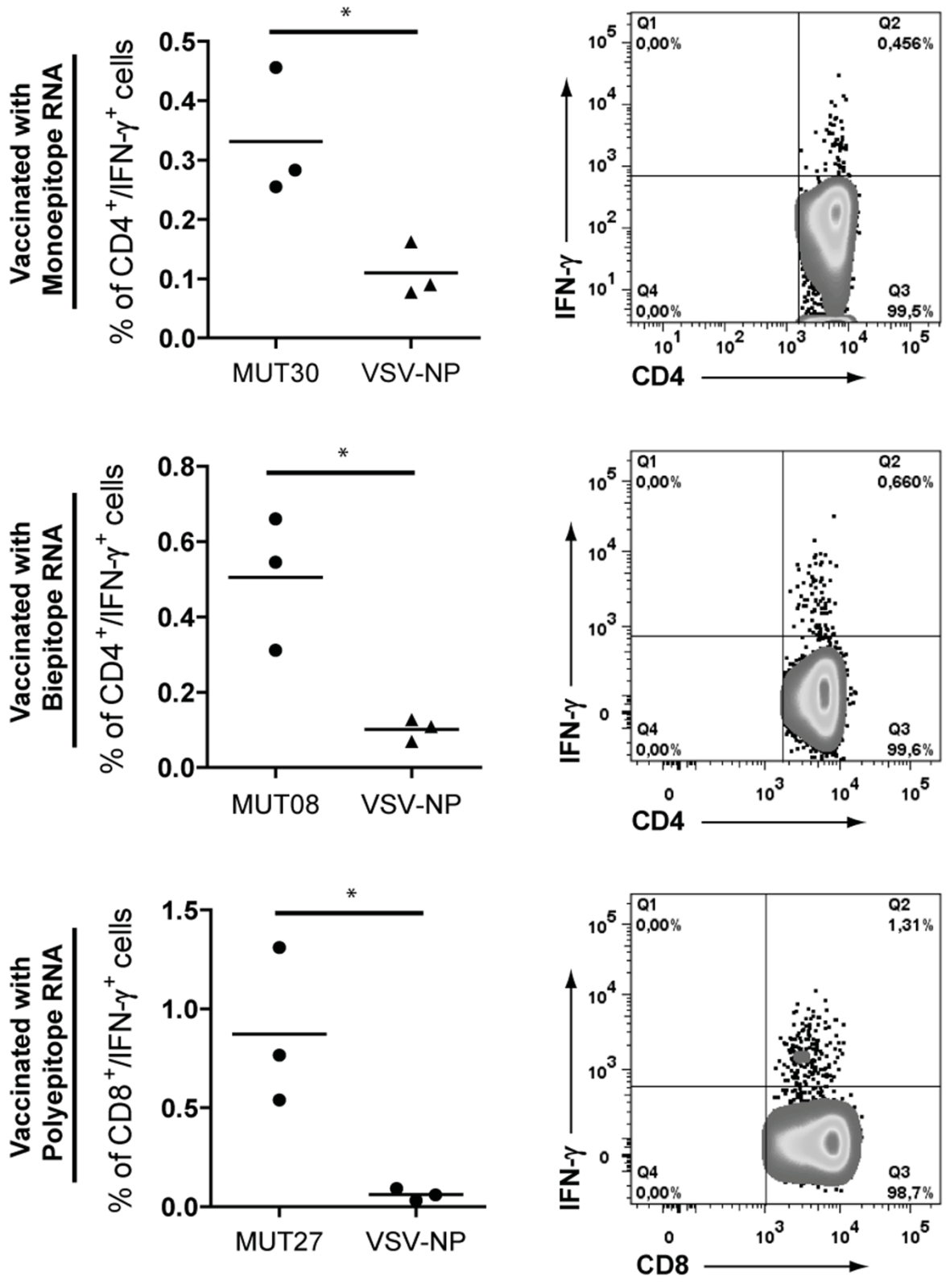

A
poly(I:C) alone
MUT30 vacc.
MUT44 vacc.
Tumorsize (mm³)
1200
1000
800
600
400
200
0
15
20
25
30
Days after tumor inoculation
B
MUT30 vacc.
poly(I:C) alone
Percent survival
100
80
60
40
20
0
20
30
40
50 70 80
Days after tumor inoculation
MUT30 vacc.
poly(I:C) alone
Tumorsize (mm³)
1000
800
600
400
200
0
10
15
20
25
30
35
Days after tumor inoculation

Fig. 12


Vaccinated with Monoepitope RNA
% of CD4+/IFN-γ+ cells
0.5
0.4
0.3
0.2
0.1
0.0
*
MUT30
VSV-NP
Q1 0,00%
Q2 0,456%
Q4 0,00%
Q3 99,5%
IFN-γ
CD4
Vaccinated with Biepitope RNA
% of CD4+/IFN-γ+ cells
0.8
0.6
0.4
0.2
0.0
*
MUT08
VSV-NP
Q1 0,00%
Q2 0,660%
Q4 0,00%
Q3 99,6%
IFN-γ
CD4
Vaccinated with Polyepitope RNA
% of CD8+/IFN-γ+ cells
1.5
1.0
0.5
0.0
*
MUT27
VSV-NP
Q1 0,00%
Q2 1,31%
Q4 0,00%
Q3 98,7%
IFN-γ
CD8

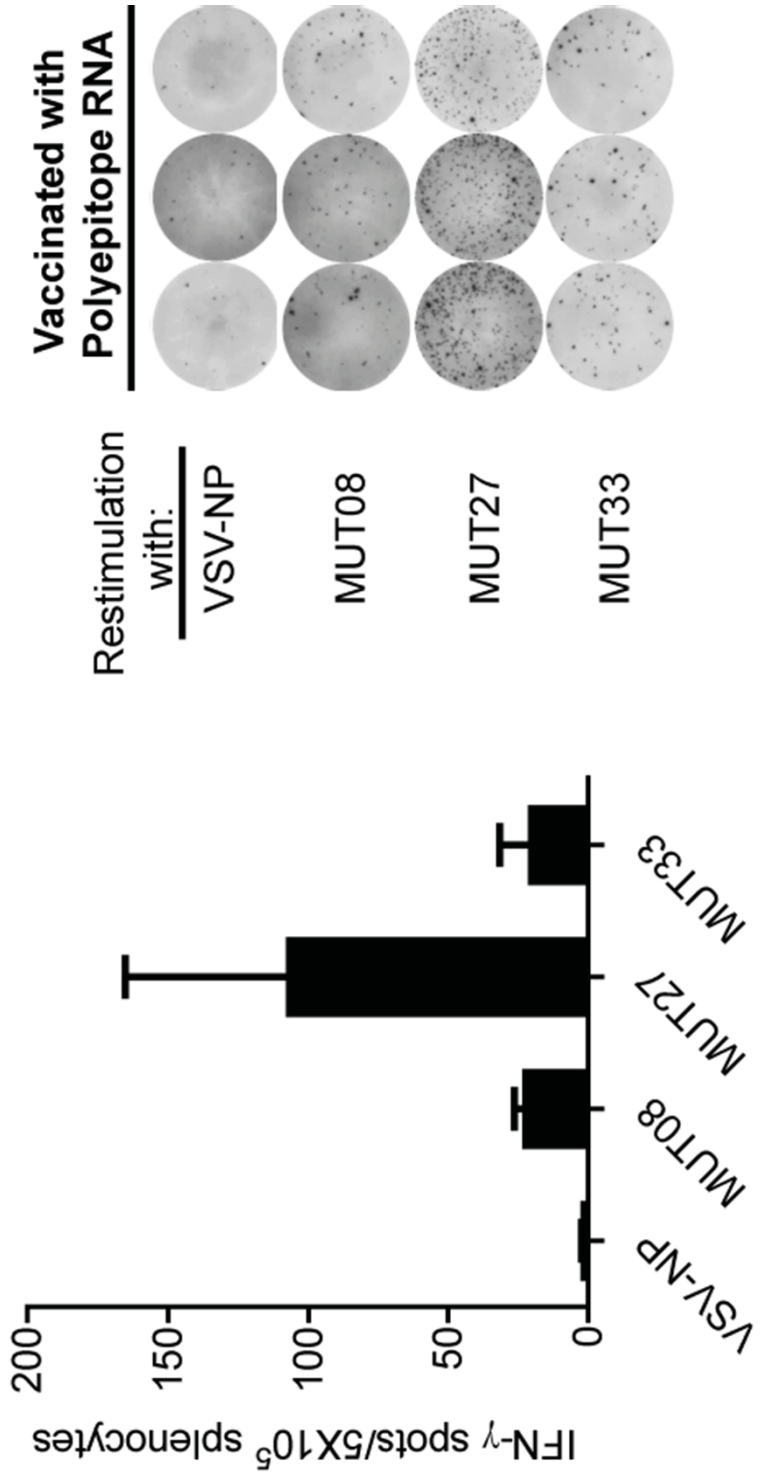

Vaccinated with Polyepitope RNA
Restimulation with:
VSV-NP
MUT08
MUT27
MUT33
IFN-γ spots/5X10^5 splenocytes
200
150
100
50
0
VSV-NP
MUT08
MUT27
MUT33

Fig. 13

Fig. 14

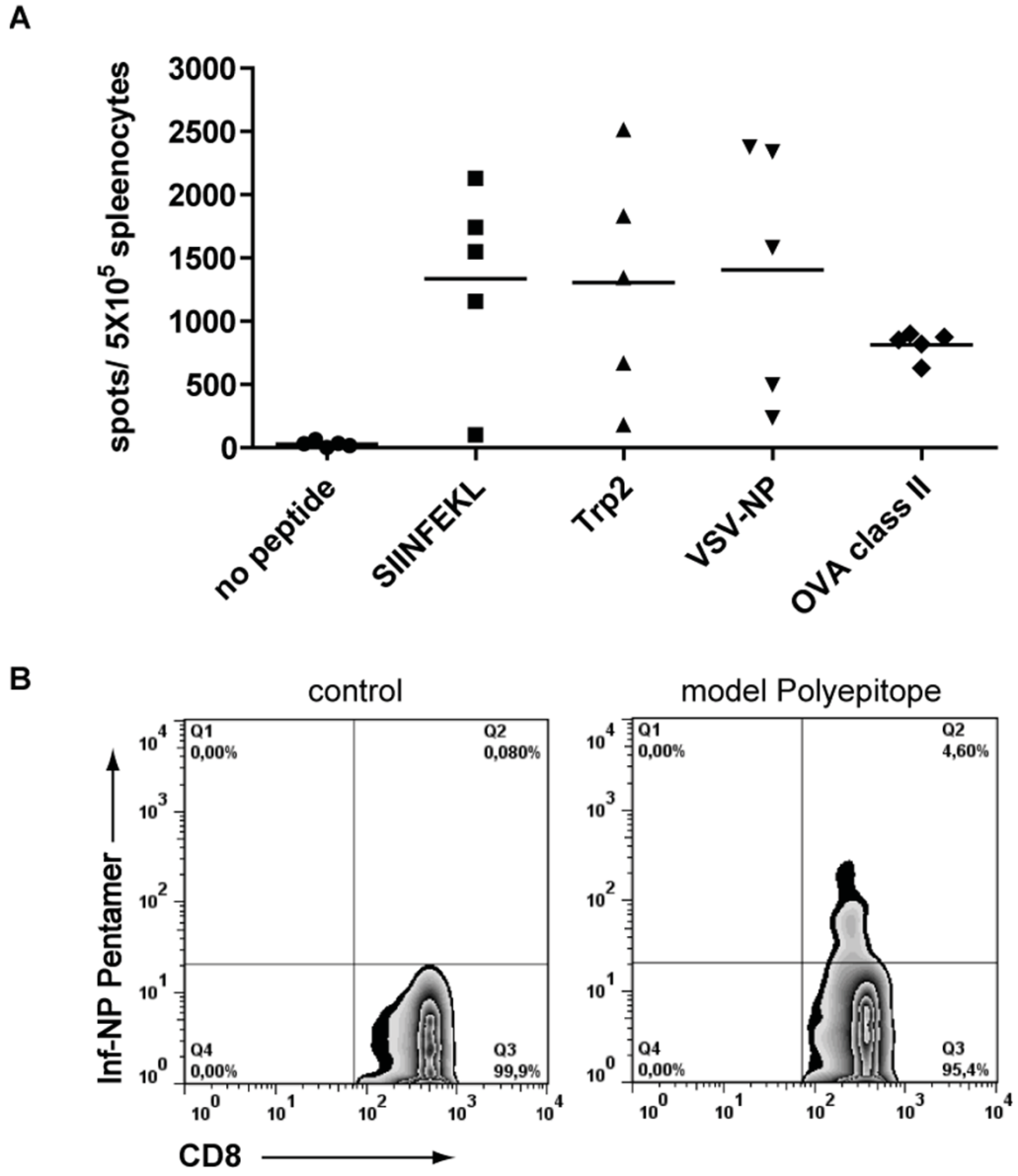
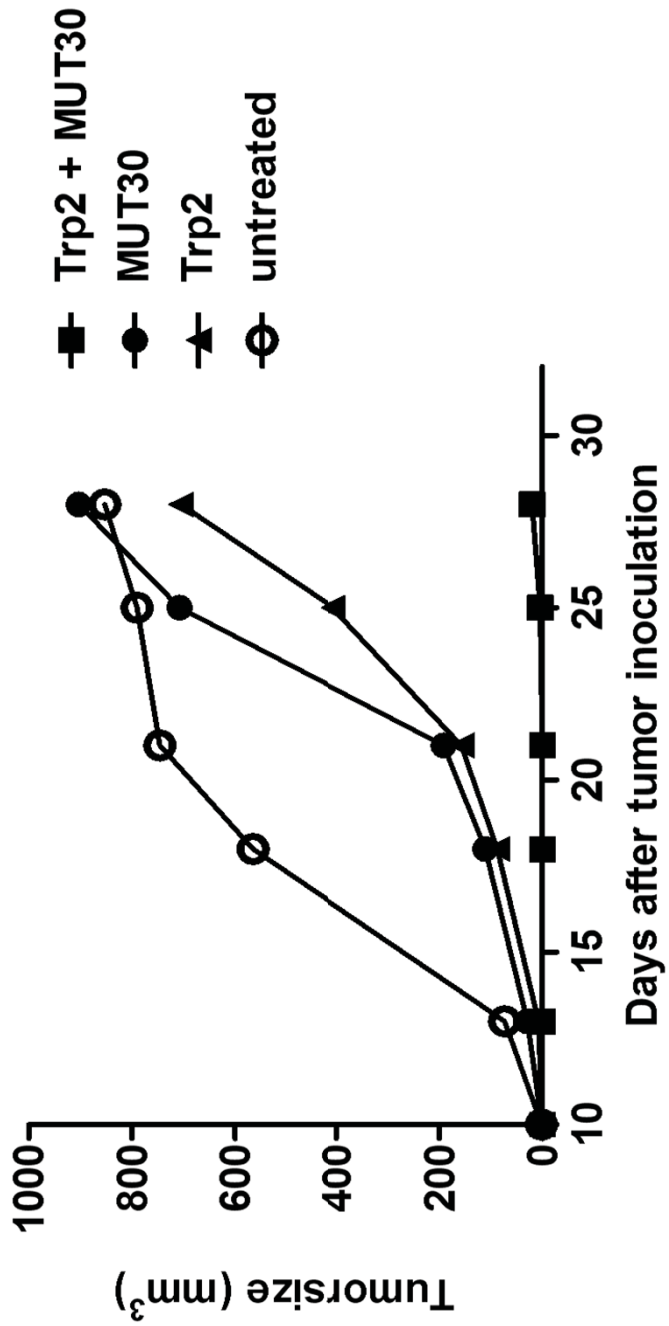

A
spots/ 5X10^5 spleenocytes
3000
2500
2000
1500
1000
500
0
no peptide
SIINFEKL
Trp2
VSV-NP
OVA class II
B
control
model Polyepitope
Inf-NP Pentamer
CD8
Q1 0,00%
Q2 0,080%
Q4 0,00%
Q3 99,9%
Q1 0,00%
Q2 4,60%
Q4 0,00%
Q3 95,4%

Fig. 15 A


Trp2 + MUT30
MUT30
Trp2
untreated
Tumorsize (mm³)
1000
800
600
400
200
0
10
15
20
25
30
Days after tumor inoculation

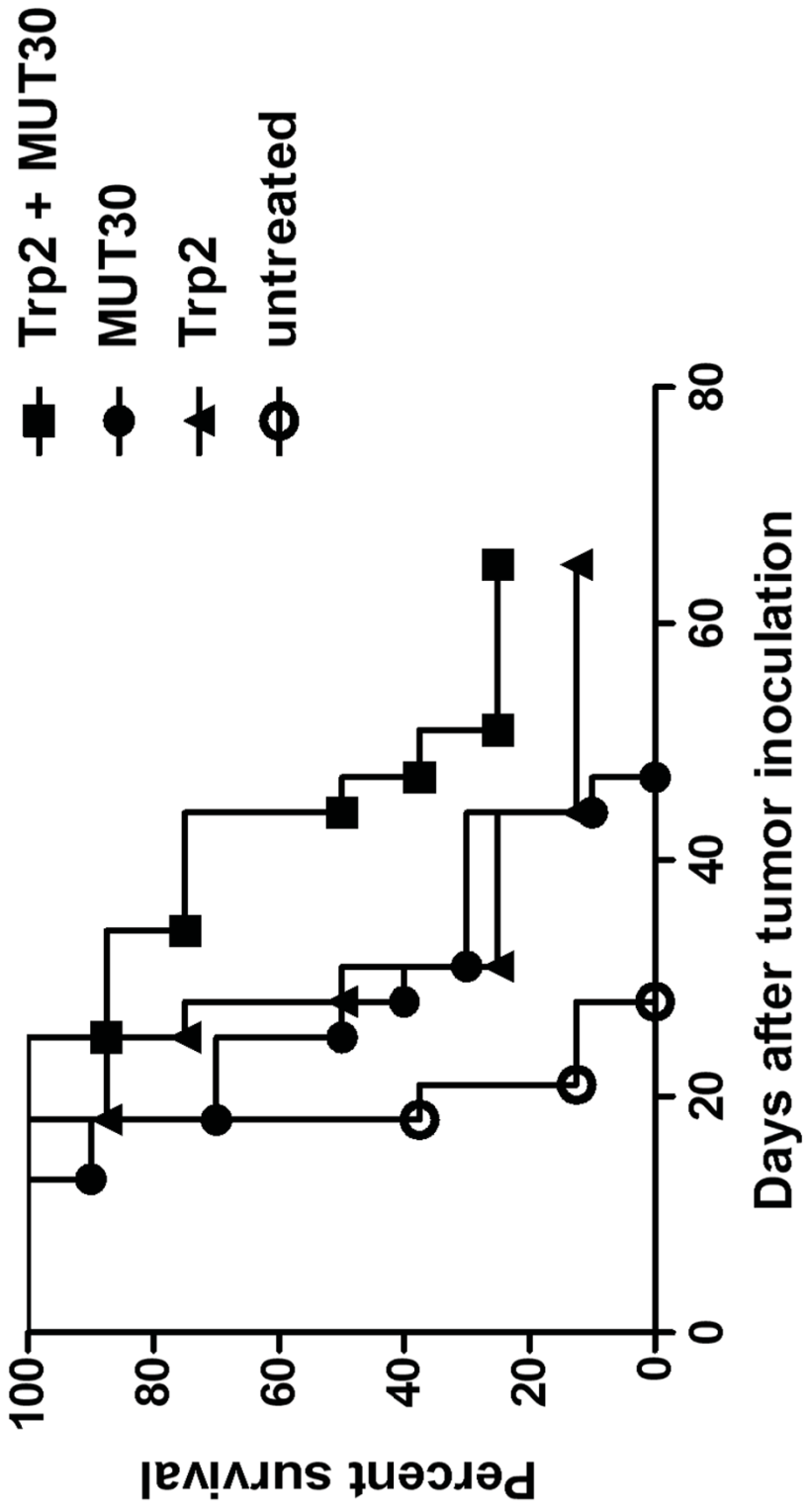

Trp2 + MUT30
MUT30
Trp2
untreated
Percent survival
100
80
60
40
20
0
0
20
40
60
80
Days after tumor inoculation

Fig. 15 B

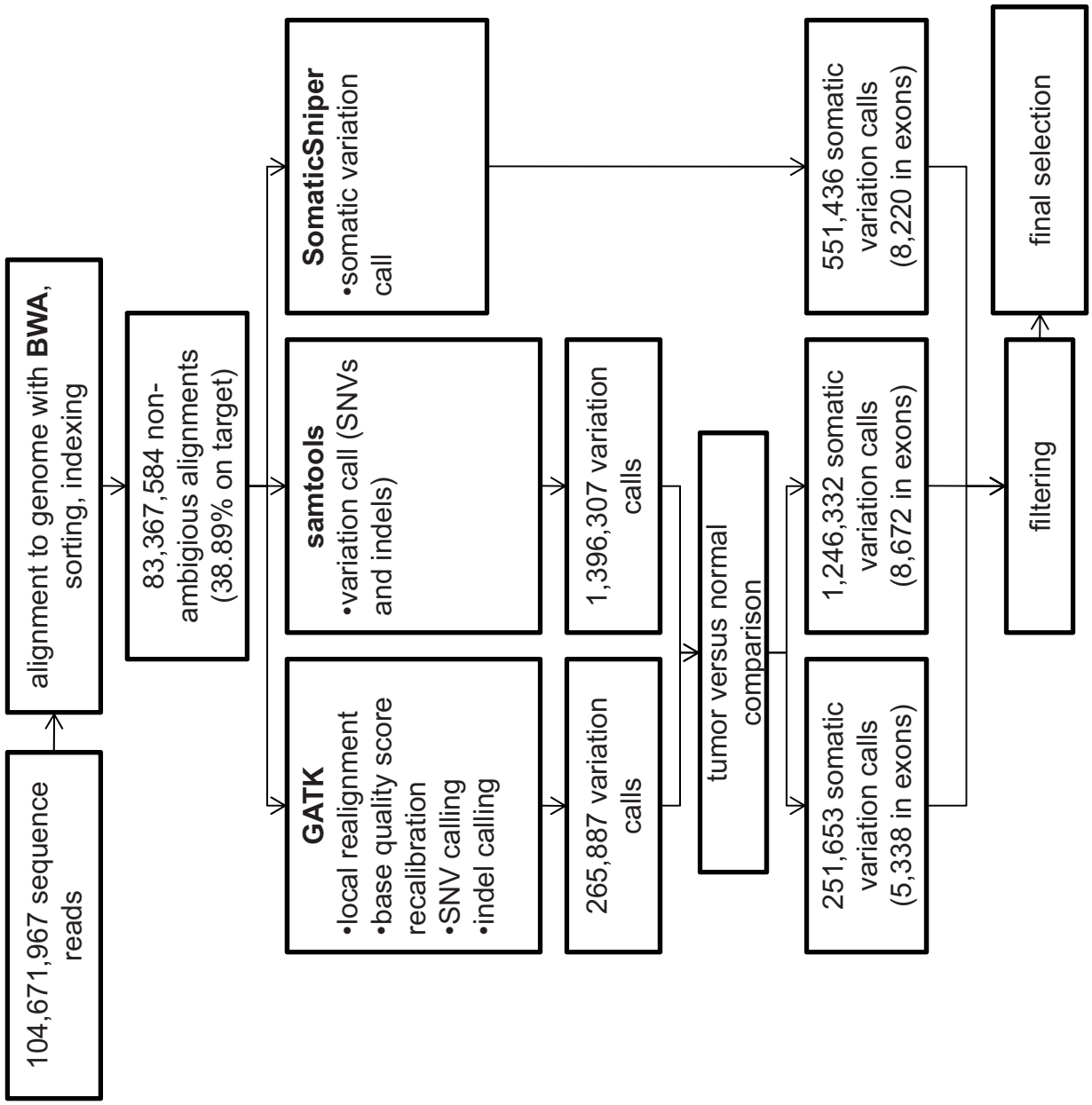

104,671,967 sequence reads
alignment to genome with **BWA**, sorting, indexing
83,367,584 non-ambigious alignments (38.89% on target)
GATK
•local realignment
•base quality score recalibration
•SNV calling
•indel calling
265,887 variation calls
samtools
•variation call (SNVs and indels)
1,396,307 variation calls
SomaticSniper
•somatic variation call
551,436 somatic variation calls (8,220 in exons)
tumor versus normal comparison
1,246,332 somatic variation calls (8,672 in exons)
251,653 somatic variation calls (5,338 in exons)
filtering
final selection

Fig. 16

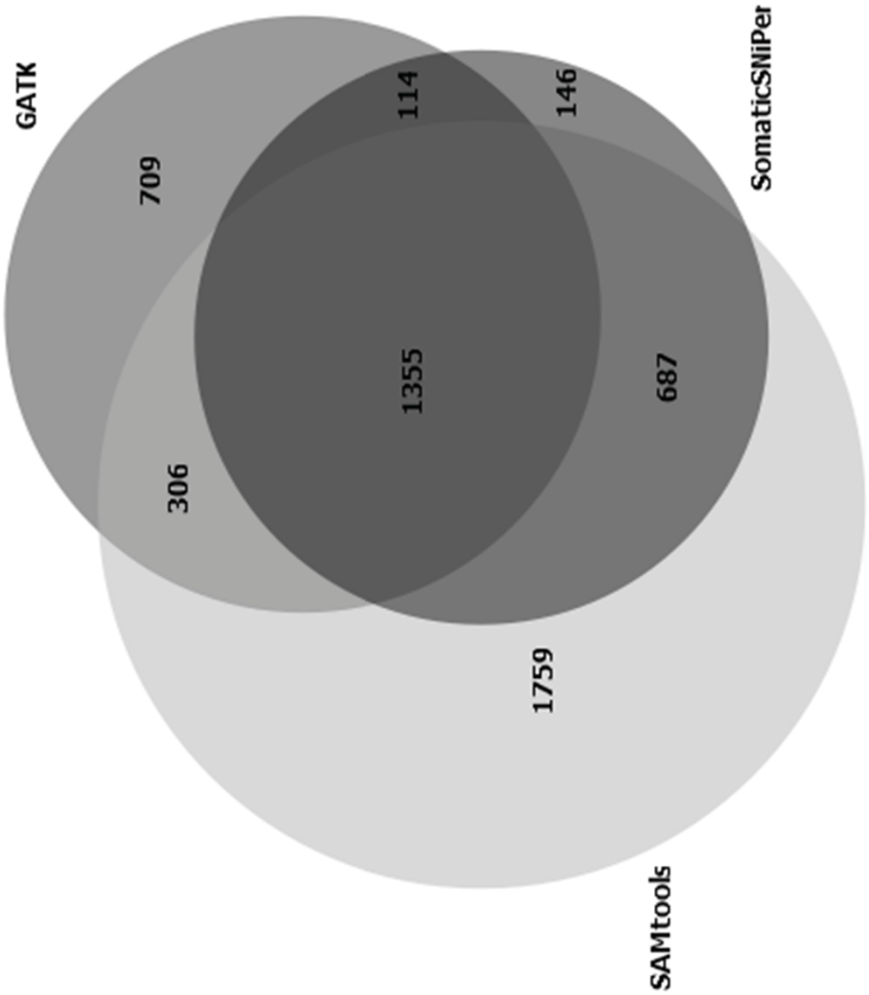
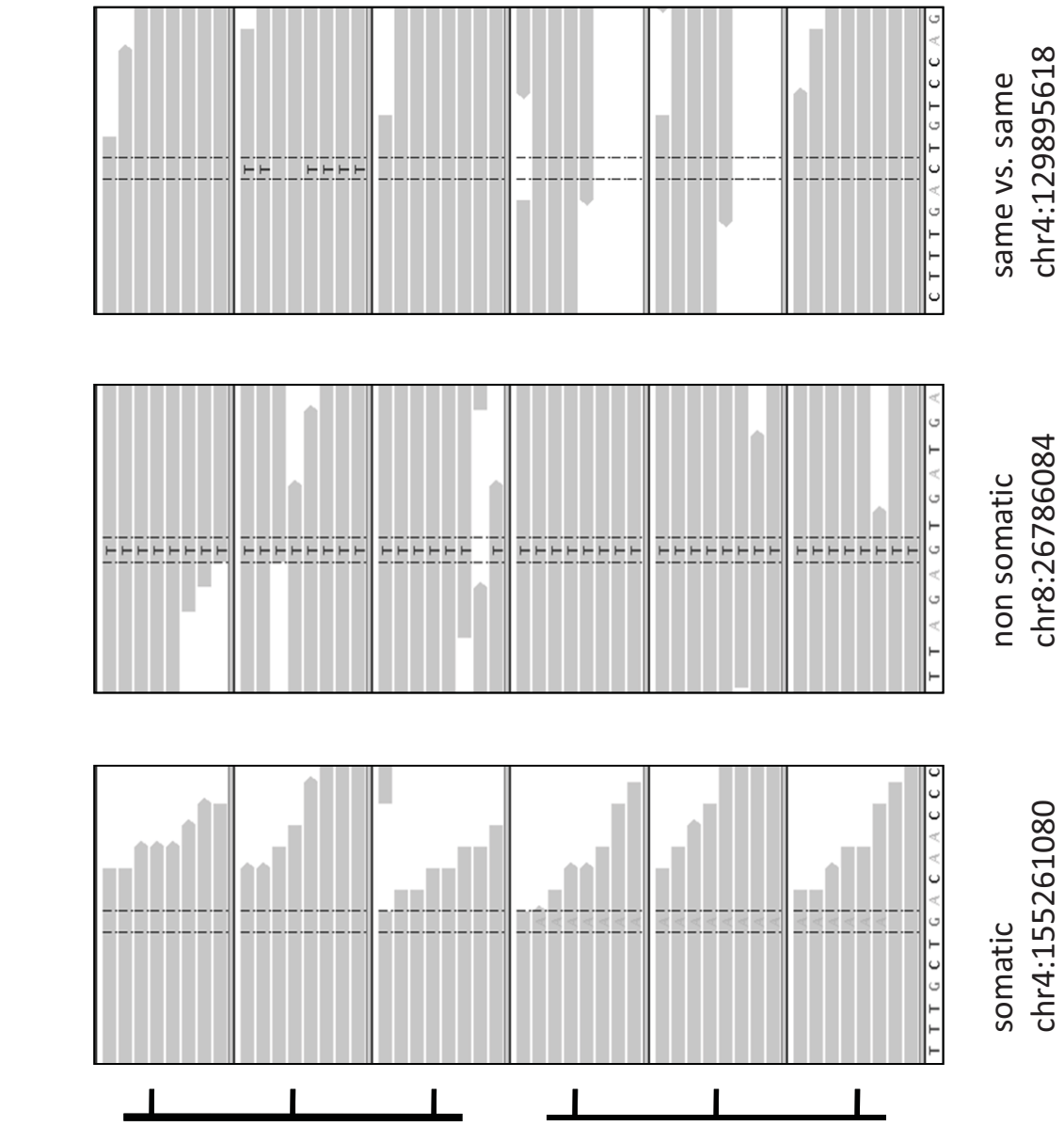


Fig. 17

Fig. 18


A
black 6
b16
somatic
chr4:155261080
non somatic
chr8:26786084
same vs. same
chr4:129895618

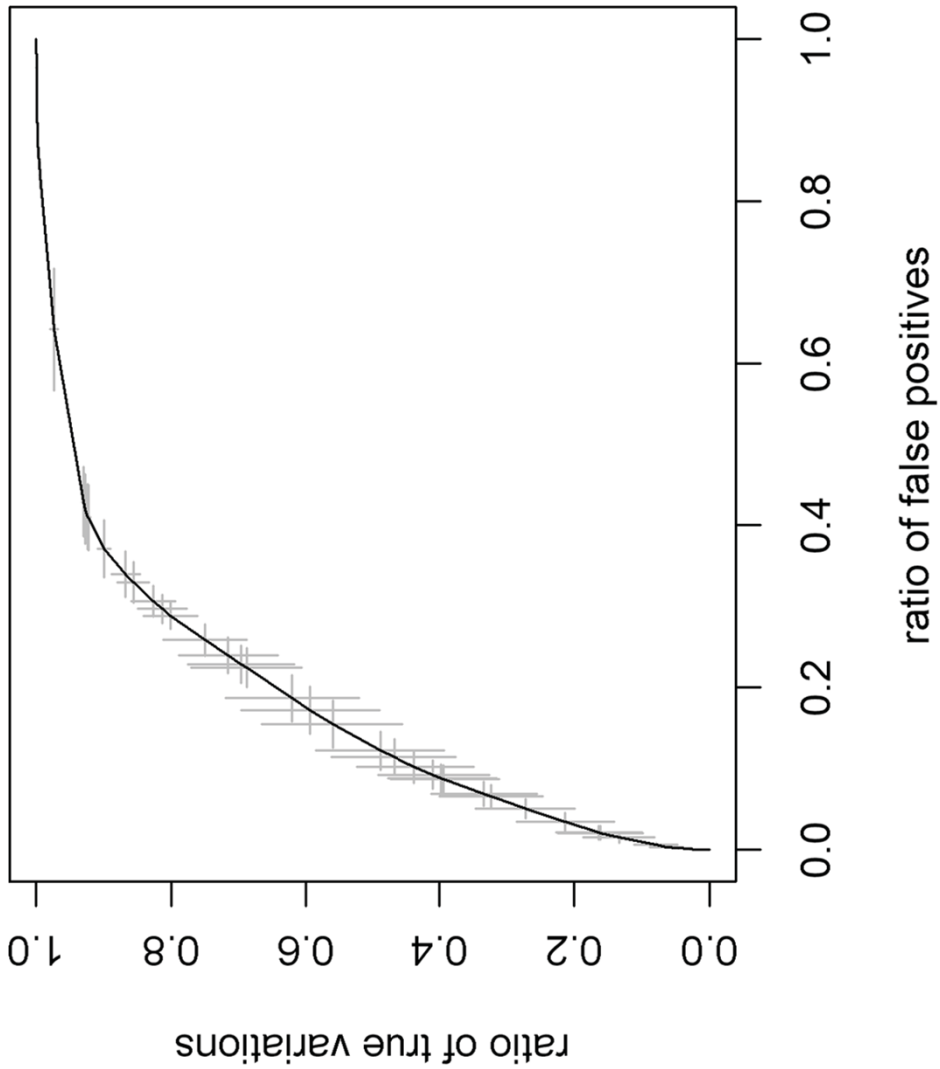

B
ratio of true variations
0.0
0.2
0.4
0.6
0.8
1.0
ratio of false positives
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 18

Fig. 19

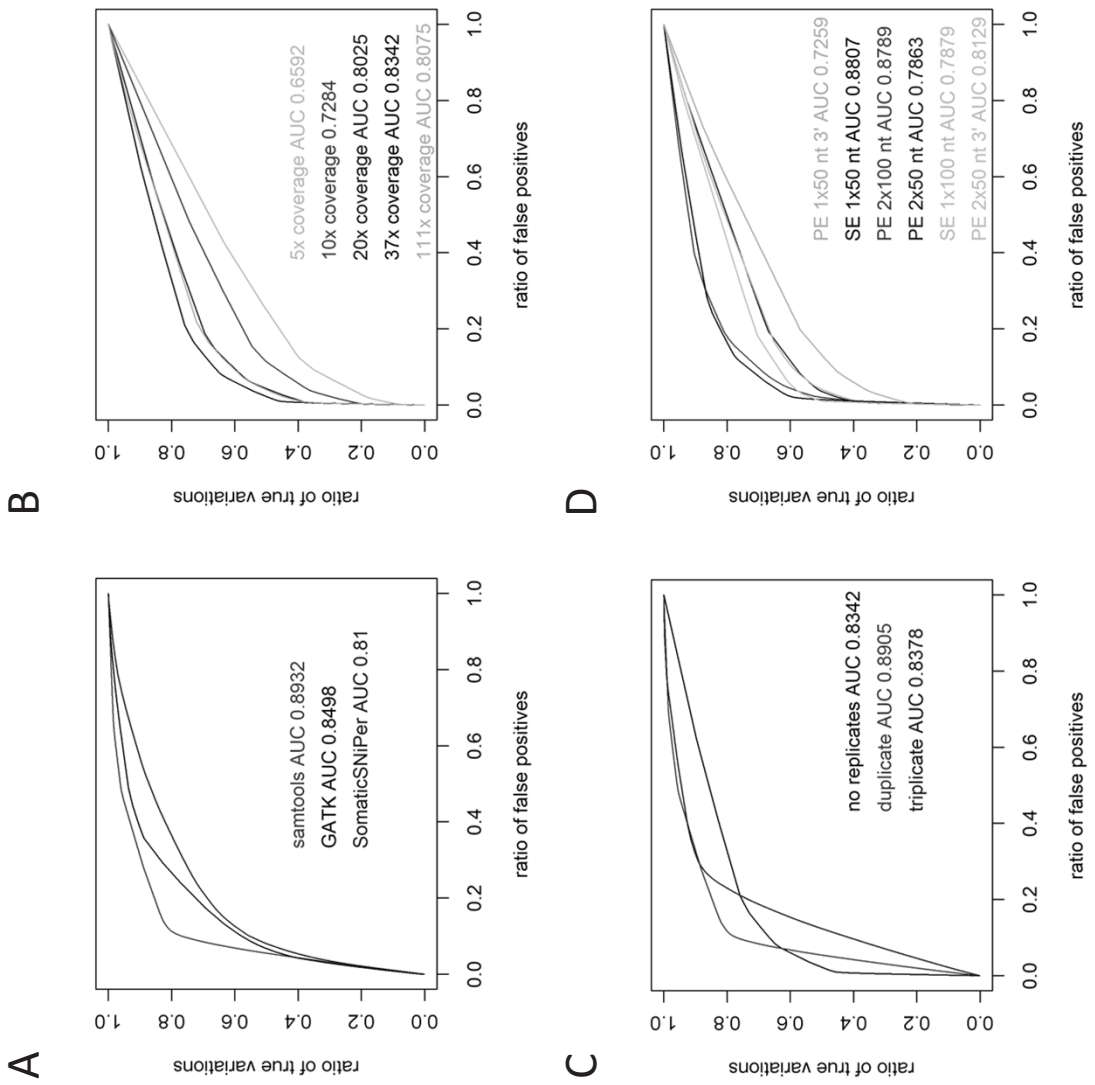
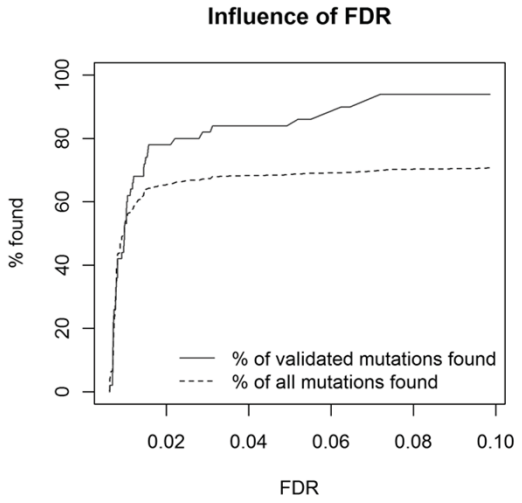

A
B
C
D
ratio of true variations
ratio of false positives
0.0
0.2
0.4
0.6
0.8
1.0
samtools AUC 0.8932
GATK AUC 0.8498
SomaticSNiPer AUC 0.81
5x coverage AUC 0.6592
10x coverage 0.7284
20x coverage AUC 0.8025
37x coverage AUC 0.8342
111x coverage AUC 0.8075
no replicates AUC 0.8342
duplicate AUC 0.8905
triplicate AUC 0.8378
PE 1x50 nt 3' AUC 0.7259
SE 1x50 nt AUC 0.8807
PE 2x100 nt AUC 0.8789
PE 2x50 nt AUC 0.7863
SE 1x100 nt AUC 0.7879
PE 2x50 nt 3' AUC 0.8129

Fig. 20

A

Chromosome	Position	Reference allele	Sample allele(s)	relative rank [%]	FDR [%]
8	110078987	G	R	3,2	0,6
1	59540714	G	S	7,6	0,7
5	124854313	G	K	7,8	0,7
10	59352802	C	M	8,3	0,7
16	36919828	A	M	9,8	0,7
2	144078227	C	Y	10,1	0,7
8	12834637	G	S	10,9	0,7
19	6121411	T	Y	11,4	0,7
1	58533360	A	M	12,1	0,7
15	98478052	A	R	13,6	0,7

B



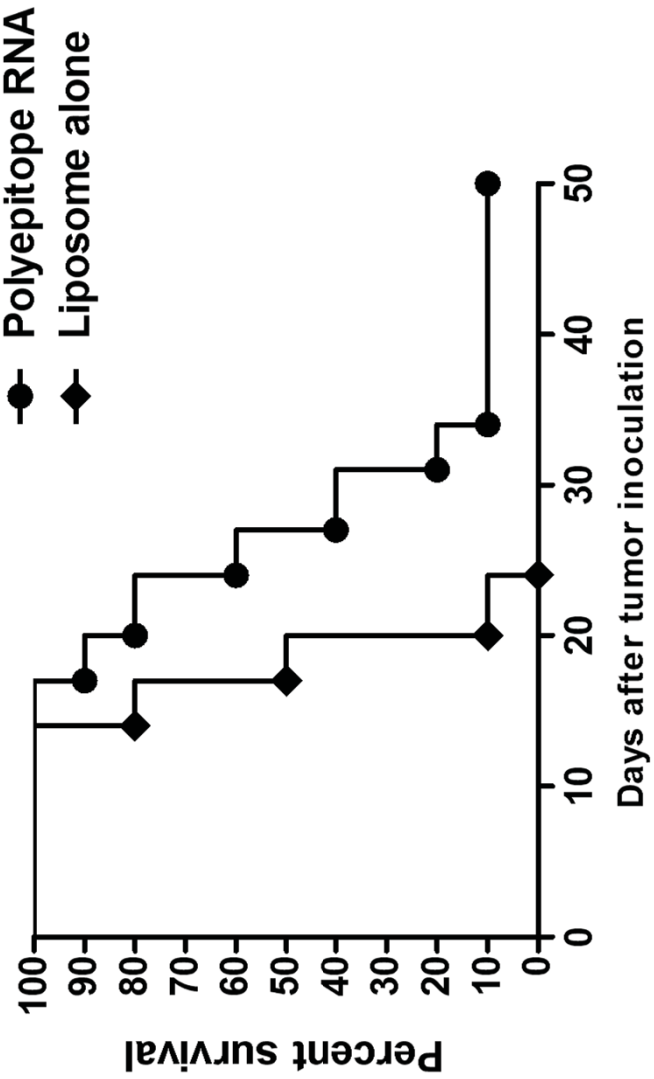

Polyepitope RNA
Liposome alone
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Days after tumor inoculation

Fig. 21

SEQUENCE LISTING

<110> Biontech AG;
TRON -Translationale Onkologie an der Universitätsmedizin
der Johannes Gutenberg-Universität Mainz gemeinnützige GmbH

<120> INDIVIDUALIZED VACCINES FOR CANCER

<130> SG4096A

<150> PCT/EP2011/002576
<151> 2011-05-24

<150> PCT/EP2012/000006
<151> 2012-01-02

<160> 39

<170> PatentIn version 3.5

<210> 1
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<220>
<221> REPEAT
<222> (1)..(3)
<223> Portion of sequence repeated a times, wherein a is independently a number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20

<220>
<221> REPEAT
<222> (4)..(6)
<223> Portion of sequence repeated b times, wherein b is independently a number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20

<220>
<221> REPEAT
<222> (7)..(9)
<223> Portion of sequence repeated c times, wherein c is independently a number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20

<220>
<221> REPEAT
<222> (10)..(12)
<223> Portion of sequence repeated d times, wherein d is independently a number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20

<220>
<221> REPEAT
<222> (13)..(15)
<223> Portion of sequence repeated e times, wherein e is independently a number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20

<220>
<221> MISC_FEATURE
<222> (1)..(15)

<223> a + b + c + d + e are different from 0 and preferably are 2 or more, 3 or more, 4 or more or 5 or more

<400> 1

Gly Gly Ser Gly Ser Ser Gly Gly Gly Ser Ser Gly Gly Ser Gly
1 5 10 15

<210> 2
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 2

Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5

<210> 3
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Epitope sequence

<400> 3

Ser Ile Ile Asn Phe Glu Lys Leu
1 5

<210> 4
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutated peptide sequence

<400> 4

Ser Pro Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val
1 5 10 15

Ser Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Pro Ser
20 25 30

<210> 5
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Wildtype peptide sequence

<400> 5

Ser Pro Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Lys Val
1 5 10 15

Ser Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu Pro Ser
20 25 30

<210> 6
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutated peptide sequence

<400> 6

Thr Pro Pro Pro Glu Glu Ala Met Pro Phe Glu Phe Asn Gly Pro Ala
1 5 10 15

Gln Gly Asp His Ser Gln Pro Pro Leu Gln Val
20 25

<210> 7
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Wildtype peptide sequence

<400> 7

Thr Pro Pro Pro Glu Glu Ala Met Pro Phe Glu Phe Asn Glu Pro Ala
1 5 10 15

Gln Gly Asp His Ser Gln Pro Pro Leu Gln Val
20 25

<210> 8
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutated peptide sequence

<400> 8

Arg Val Thr Cys Asn Arg Ala Gly Glu Lys His Cys Phe Ser Ser Asn
1 5 10 15

Glu Ala Ala Arg Asp Phe Gly Gly Ala Ile Gln
20 25

<210> 9
<211> 27
<212> PRT
<213> Wildtype peptide sequence

<400> 9

Arg Val Thr Cys Asn Arg Ala Gly Glu Lys His Cys Phe Thr Ser Asn
1 5 10 15

Glu Ala Ala Arg Asp Phe Gly Gly Ala Ile Gln
20 25

<210> 10
<211> 27
<212> PRT
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<220>
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<400> 10

Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Ala Met Asp
1 5 10 15

Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met
20 25

<210> 11
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Wildtype peptide sequence

<400> 11

Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Gly Met Asp
1 5 10 15

Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met
20 25

<210> 12
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutated peptide sequence

<400> 12

Pro Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Asn Val Ser
1 5 10 15

Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu
20 25

<210> 13
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Wildtype peptide sequence

<400> 13

Pro Ser Lys Pro Ser Phe Gln Glu Phe Val Asp Trp Glu Lys Val Ser
1 5 10 15

Pro Glu Leu Asn Ser Thr Asp Gln Pro Phe Leu
20 25

<210> 14
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<220>
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<400> 14

His Leu Thr Gln Gln Leu Asp Thr Tyr Ile Leu Lys Asn Val Val Ala
1 5 10 15

Phe Ser Arg Thr Asp Lys Tyr Arg Gln Leu Pro
20 25

<210> 15
<211> 27
<212> PRT
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<220>
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<400> 15

His Leu Thr Gln Gln Leu Asp Thr Tyr Ile Leu Lys Asn Phe Val Ala
1 5 10 15

Phe Ser Arg Thr Asp Lys Tyr Arg Gln Leu Pro
20 25

<210> 16
<211> 27
<212> PRT
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<220>
<223> Mutated peptide sequence

<400> 16

Cys Gly Thr Ala Phe Phe Ile Asn Phe Ile Ala Ile Tyr His His Ala
1 5 10 15

Ser Arg Ala Ile Pro Phe Gly Thr Met Val Ala
20 25

<210> 17
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Wildtype peptide sequence

<400> 17

Cys Gly Thr Ala Phe Phe Ile Asn Phe Ile Ala Ile Tyr Tyr His Ala
1 5 10 15

Ser Arg Ala Ile Pro Phe Gly Thr Met Val Ala
20 25

<210> 18
<211> 7
<212> PRT
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<220>
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<400> 18

Gly Gly Ser Gly Gly Gly Gly
1 5

<210> 19
<211> 10
<212> PRT
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<220>
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<400> 19

Thr Ser Leu Asn Ala Leu Leu Asn Ala His
1 5 10

<210> 20
<211> 10
<212> PRT
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<220>
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<400> 20

Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser
1 5 10

<210> 21
<211> 10
<212> PRT
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<220>
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<400> 21

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
1 5 10

<210> 22
<211> 10
<212> PRT
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<400> 22

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
1 5 10

<210> 23
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<400> 23

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
1 5 10

<210> 24
<211> 9
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<220>
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<400> 24

Thr Ser Leu Asn Ala Leu Leu Asn Ala
1 5

<210> 25
<211> 9
<212> PRT
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<220>
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<400> 25

Ser Ser Ser Ser Ser Ser Ser Ser Ser
1 5

<210> 26
<211> 9
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<220>
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<400> 26

Gly Gly Gly Gly Gly Gly Gly Gly Gly
1 5

<210> 27
<211> 9
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<220>
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<400> 27

Gly Gly Ser Gly Gly Gly Ser Gly Gly
1 5

<210> 28
<211> 5
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<220>
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<400> 28

Gly Gly Ser Gly Gly
1 5

<210> 29
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<400> 29

Gly Gly Ser Gly Gly Gly
1 5

<210> 30
<211> 7
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<220>
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<400> 30

Gly Gly Ser Gly Gly Gly Gly
1 5

<210> 31
<211> 8
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<220>
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<400> 31

Gly Gly Ser Gly Gly Gly Gly Ser
1 5

<210> 32
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
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<400> 32

Gly Gly Ser Gly Gly Gly Ser Gly Gly Ser
1 5 10

<210> 33
<211> 14
<212> DNA
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<220>
<223> Oligonucleotide

<400> 33
ggaaactttt tccc 14

<210> 34
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 34
ccggggaaac tttttcccag t 21

<210> 35
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide

<400> 35
ccggggaaac gttttcccag t 21

<210> 36
<211> 60
<212> PRT
<213> Artificial Sequence

<220>
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<400> 36

Asn Thr Thr Phe Asn Val Ser Glu Glu Ser Pro Ser Lys Pro Ser Phe
1 5 10 15

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Gln Glu Phe Val Asp Trp Glu Asn Val Ser Pro Glu Leu Asn Ser Thr
            20                  25                  30

Asp Gln Pro Phe Leu Pro Ser Ala Pro Val Phe Ile Phe Thr Lys Gly
        35                  40                  45

Arg Lys Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    50                  55                  60
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<210> 37
<211> 60
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial construct

<400> 37

```
Tyr Phe Val Leu Tyr Lys Pro Pro Pro Lys Asp Asn Ile Pro Ala Leu
1               5                   10                  15

Val Glu Glu Tyr Leu Glu Arg Gly Asn Phe Val Ala Asn Asp Leu Asp
            20                  25                  30

Trp Leu Leu Ala Leu Pro His Asp Lys Phe Trp Cys Gln Val Ile Phe
        35                  40                  45

Asp Glu Gly Gly Ser Gly Gly Gly Ser Gly Gly Ser
    50                  55                  60
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<210> 38
<211> 60
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial construct

<400> 38

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Phe Glu Gln Leu Ser Glu Ser Ala Lys Glu Glu Leu Ile Asn Phe Lys
1               5                   10                  15

Arg Lys Arg Val Ala Ala Phe Gln Lys Asn Leu Ile Glu Met Ser Glu
            20                  25                  30

Leu Glu Ile Lys His Ala Arg Asn Asn Val Ser Leu Leu Gln Ser Cys
        35                  40                  45

Ile Asp Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    50                  55                  60
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<210> 39
<211> 60

<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial construct

<400> 39

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Thr Gly Ala Ser Pro Gly Leu Gly Ala Tyr Thr Pro Pro Pro Glu Glu
1               5                   10                  15


Ala Met Pro Phe Glu Phe Asn Gly Pro Ala Gln Gly Asp His Ser Gln
            20                  25                  30


Pro Pro Leu Gln Val Pro Asp Leu Ala Pro Gly Gly Pro Glu Ala Leu
        35                  40                  45


Val Pro Gly Gly Ser Gly Gly Gly Ser Gly Gly Ser
    50                  55                  60
```