Fig. 1

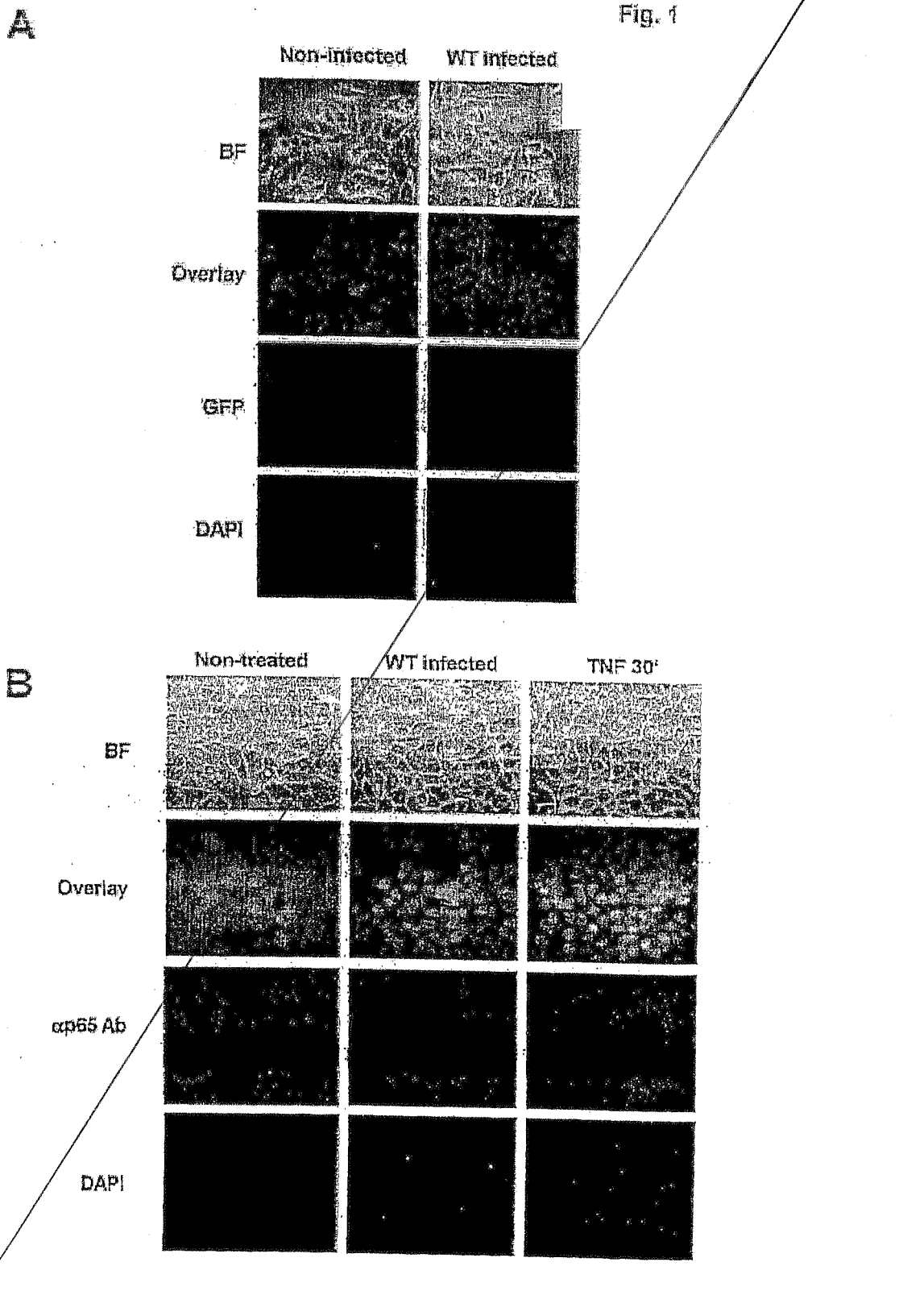

A
Non-infected
WT infected
BF
Overlay
GFP
DAPI
B
Non-treated
WT infected
TNF 30'
BF
Overlay
αp65 Ab
DAPI

Fig. 2

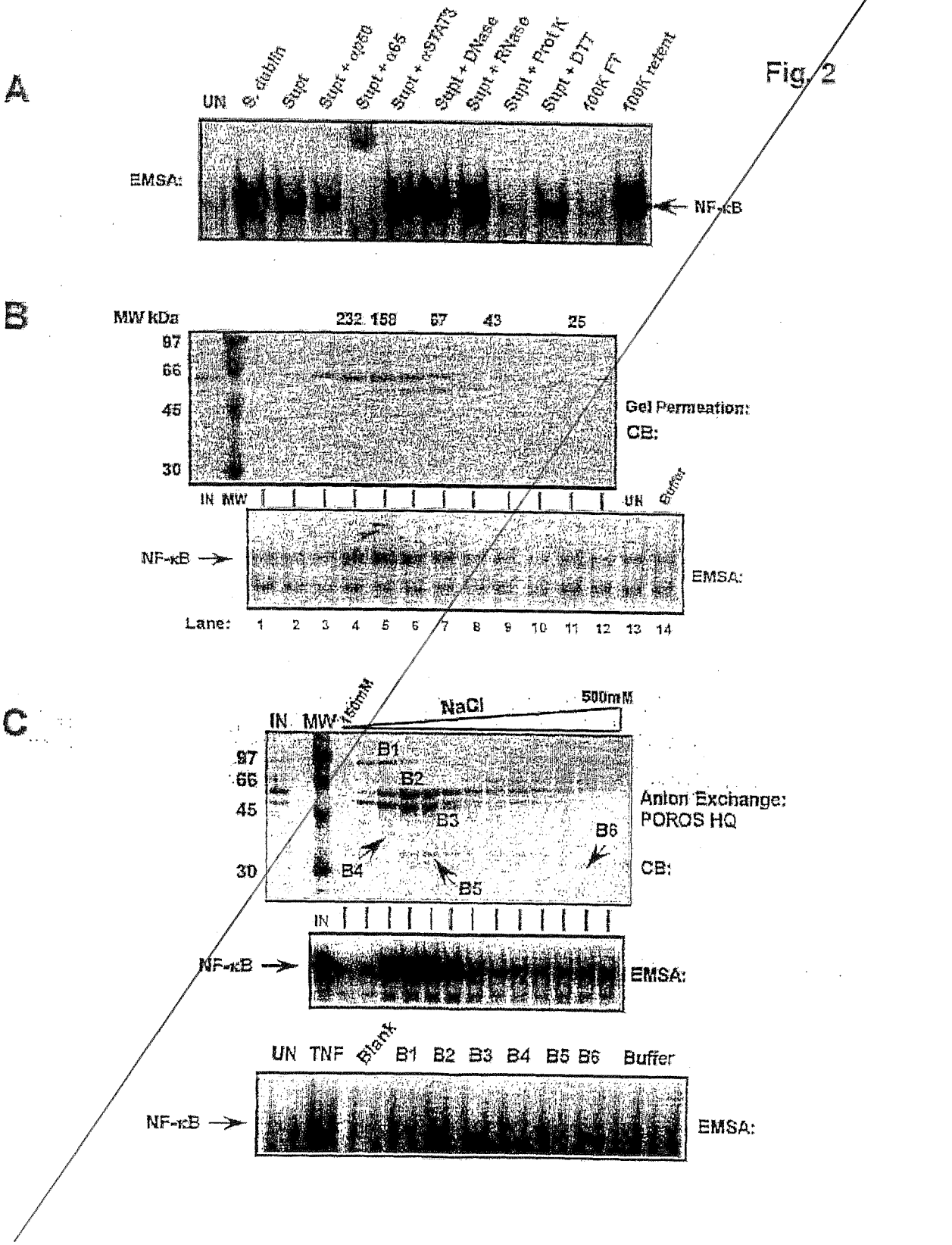

A
UN
S. diablin
Supt
Supt + αp65
Supt + αSTAT3
Supt + DNase
Supt + RNase
Supt + Prot K
Supt + DTT
100K FT
100K retent
EMSA:
NF-κB
B
MW kDa
232 158 67 43 25
97
66
45
30
Gel Permeation:
CB:
IN MW
UN
Buffer
NF-κB
EMSA:
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14
C
IN MW
150mM
NaCl
500mM
B1
B2
B3
B4
B5
B6
Anion Exchange:
POROS HQ
CB:
IN
NF-κB
EMSA:
UN TNF Blank B1 B2 B3 B4 B5 B6 Buffer
NF-κB
EMSA:

Fig. 3

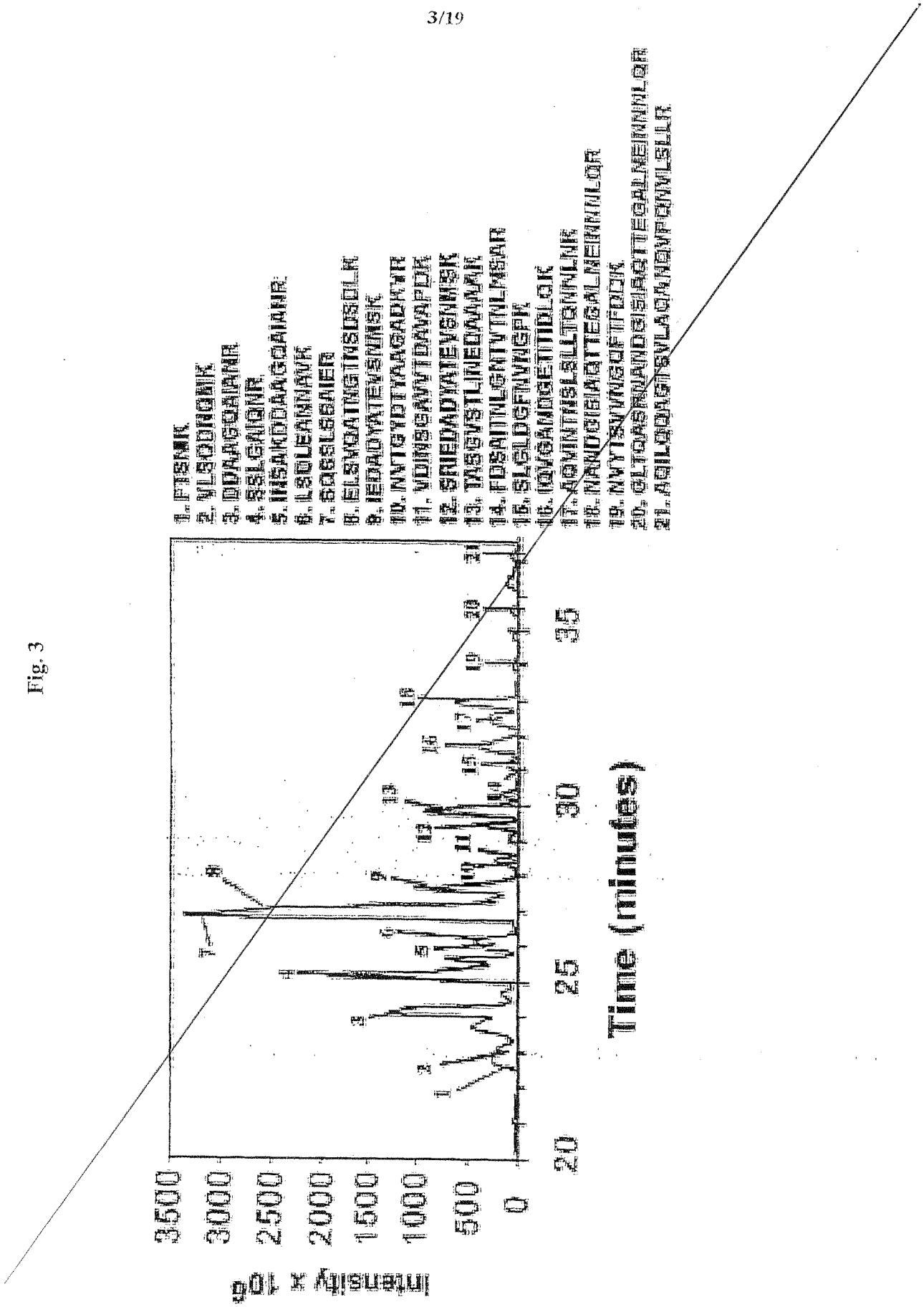

1. FTSMK
2. VLSDDNGMK
3. DDAAGQAIANR
4. SSLGAIQNR
5. INSAKDDAAGQAIANR
6. LSDLEANNAVK
7. SDESLSEAIER
8. ELSNVATNGTVSDSDLK
9. IEDADYATEVSNMSK
10. NVTGYDTYAAGADTVR
11. VDNSEAVTDAVAPLDK
12. SRIEDADYATEVSNMSK
13. TASGVSTLNEDAAAAK
14. FDSAITNLGNTVTNLMSAR
15. SLSLDGFNVNSPK
16. IQVGANDGETITDLDK
17. ADVINTNSLLTDNNLNR
18. NANDGEGAQTTEGALNEINNLDR
19. NVYTSVVNGDFTFDDK
20. GLTQASRNANDGSGAQTTEGALNEINNLDR
21. AGLQDAGTSVLAQLNQVPDNVLSLLR
Intensity x 10⁶
3500
3000
2500
2000
1500
1000
500
0
20
25
30
35
Time (minutes)

Fig. 4

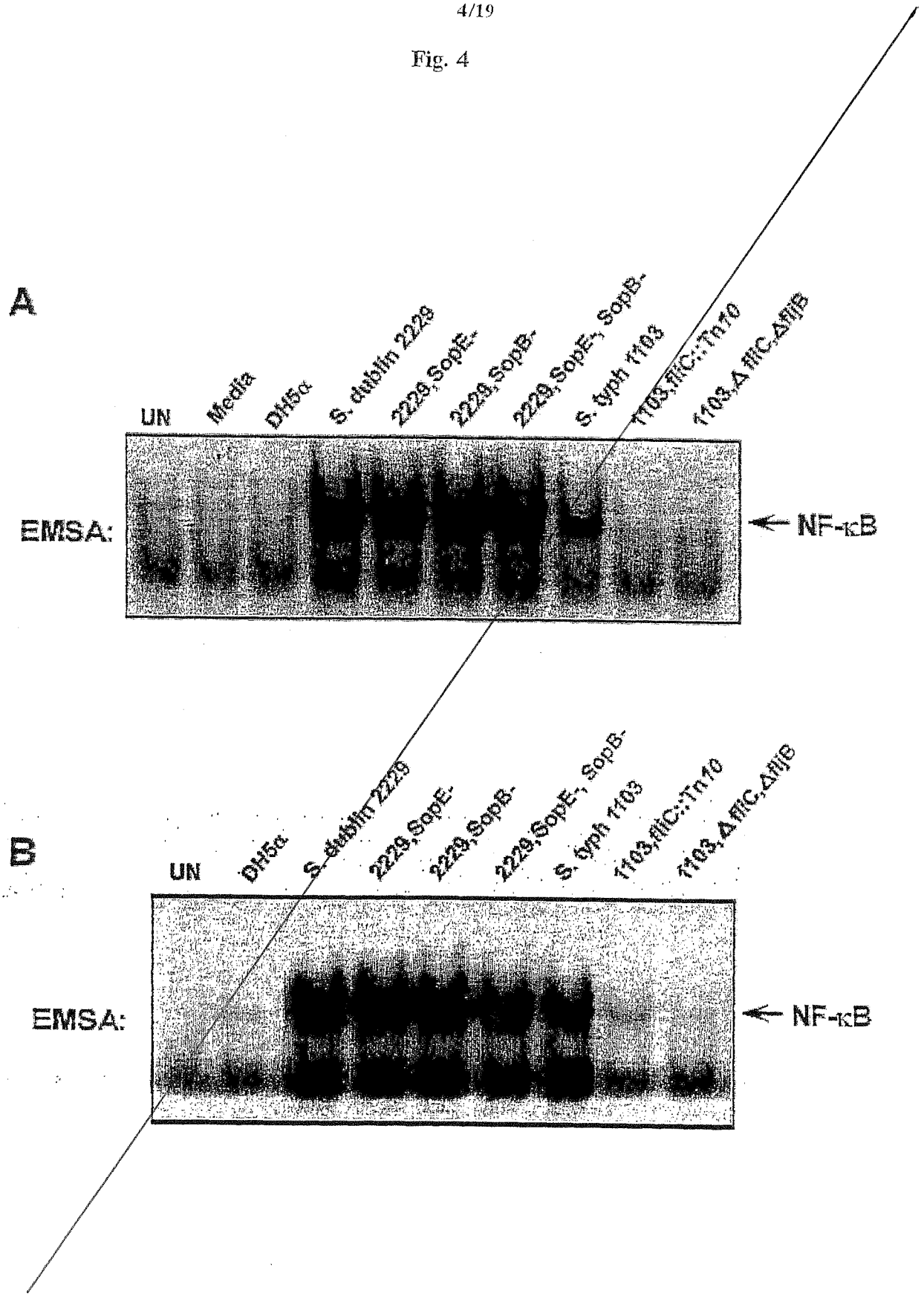

A
UN
Media
DH5α
S. dublin 2229
2229,SopE-
2229,SopB-
2229,SopE-, SopB-
S. typh 1103
1103,fliC::Tn10
1103,Δ fliC,ΔfljB
EMSA:
NF-κB
B
UN
DH5α
S. dublin 2229
2229,SopE-
2229,SopB-
2229,SopE-, SopB-
S. typh 1103
1103,fliC::Tn10
1103,Δ fliC,ΔfljB
EMSA:
NF-κB

Fig. 5

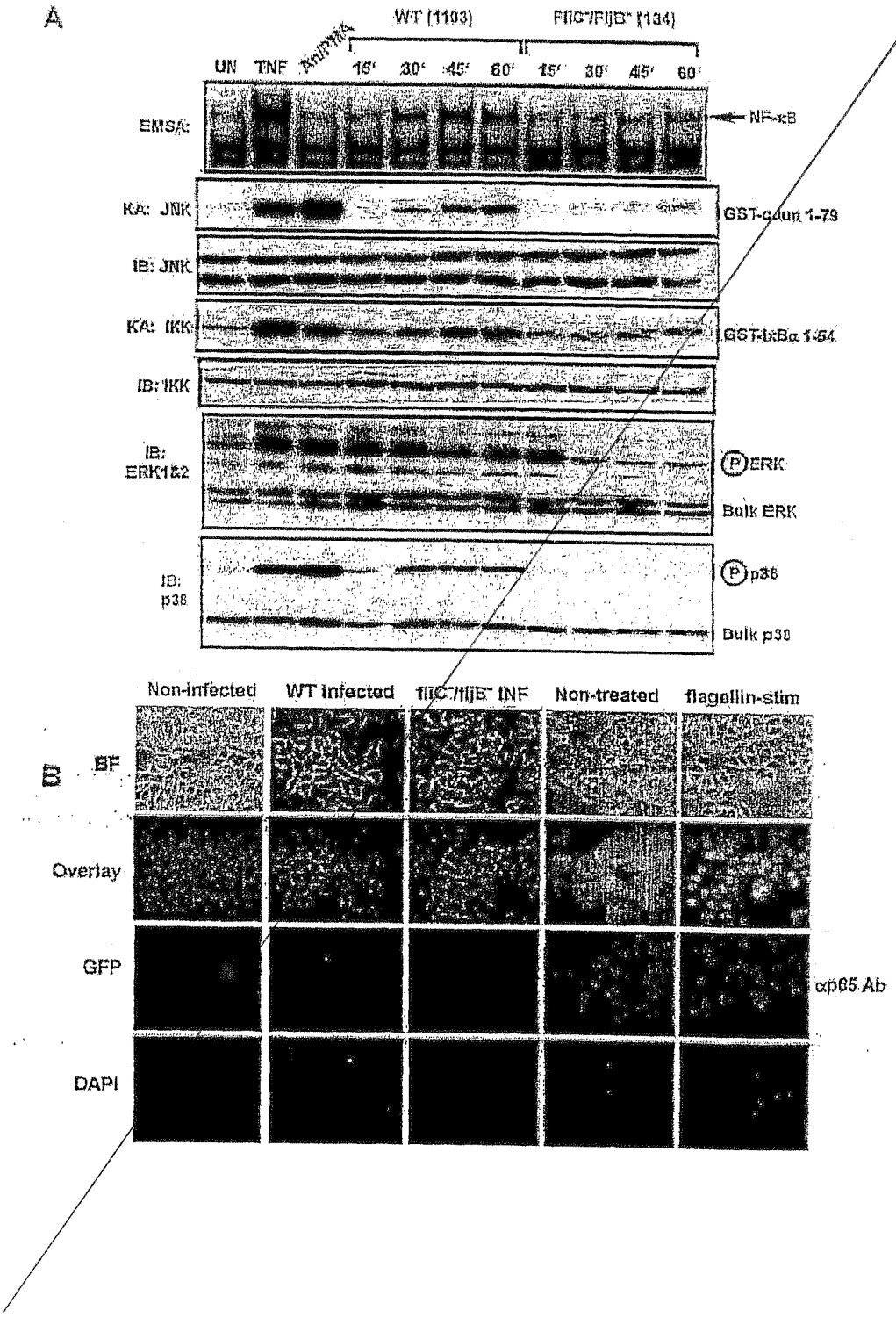
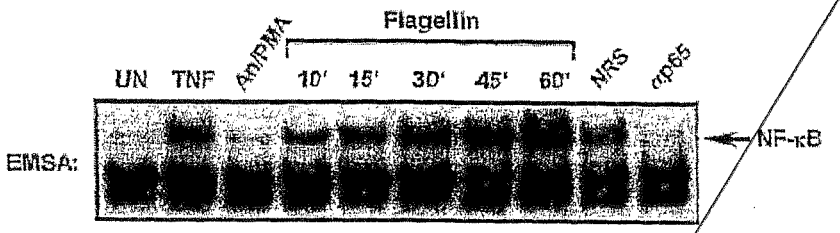
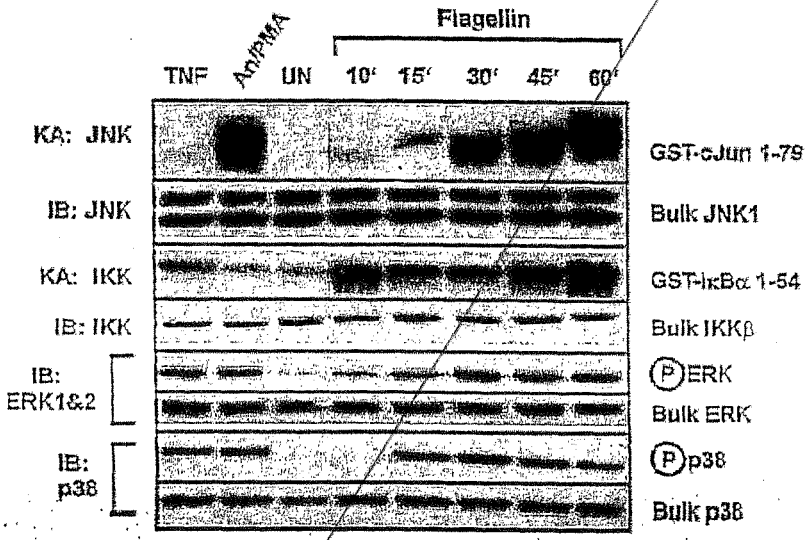

A
WT (1103)
FliC⁻/FljB⁻ (134)
UN
TNF
Ad/βMA
15'
30'
45'
60'
15'
30'
45'
60'
EMSA:
NF-κB
KA: JNK
GST-c-Jun 1-79
IB: JNK
KA: IKK
GST-IκBα 1-54
IB: IKK
IB: ERK1&2
PERK
Bulk ERK
IB: p38
Pp38
Bulk p38
Non-infected
WT infected
fliC⁻/fljB⁻ INF
Non-treated
flagellin-stim
B
BF
Overlay
GFP
αp65 Ab
DAPI

Fig. 6

A


Flagellin
UN
TNF
Ab/PMA
10'
15'
30'
45'
60'
NRS
αp65
EMSA:
NF-κB

B


Flagellin
TNF
Ab/PMA
UN
10'
15'
30'
45'
60'
KA: JNK
GST-cJun 1-79
IB: JNK
Bulk JNK1
KA: IKK
GST-IκBα 1-54
IB: IKK
Bulk IKKβ
IB: ERK1&2
ERK
Bulk ERK
IB: p38
p38
Bulk p38

C

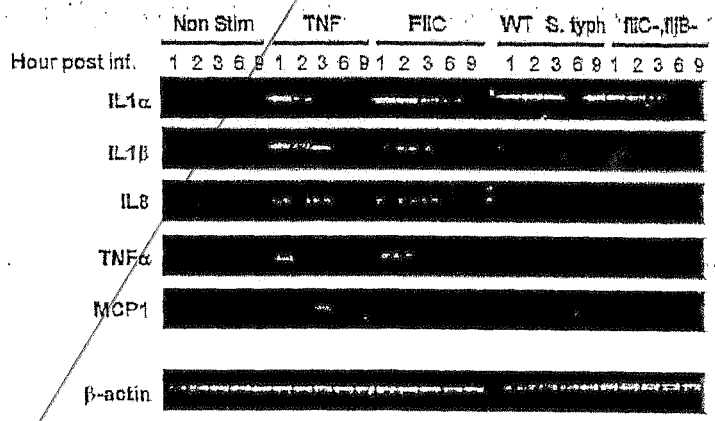

Non Stim
TNF
FliC
WT S. typh
fliC-,fljB-
Hour post inf.
1 2 3 6 9 1 2 3 6 9 1 2 3 6 9 1 2 3 6 9 1 2 3 6 9
IL1α
IL1β
IL8
TNFα
MCP1
β-actin

Fig. 7

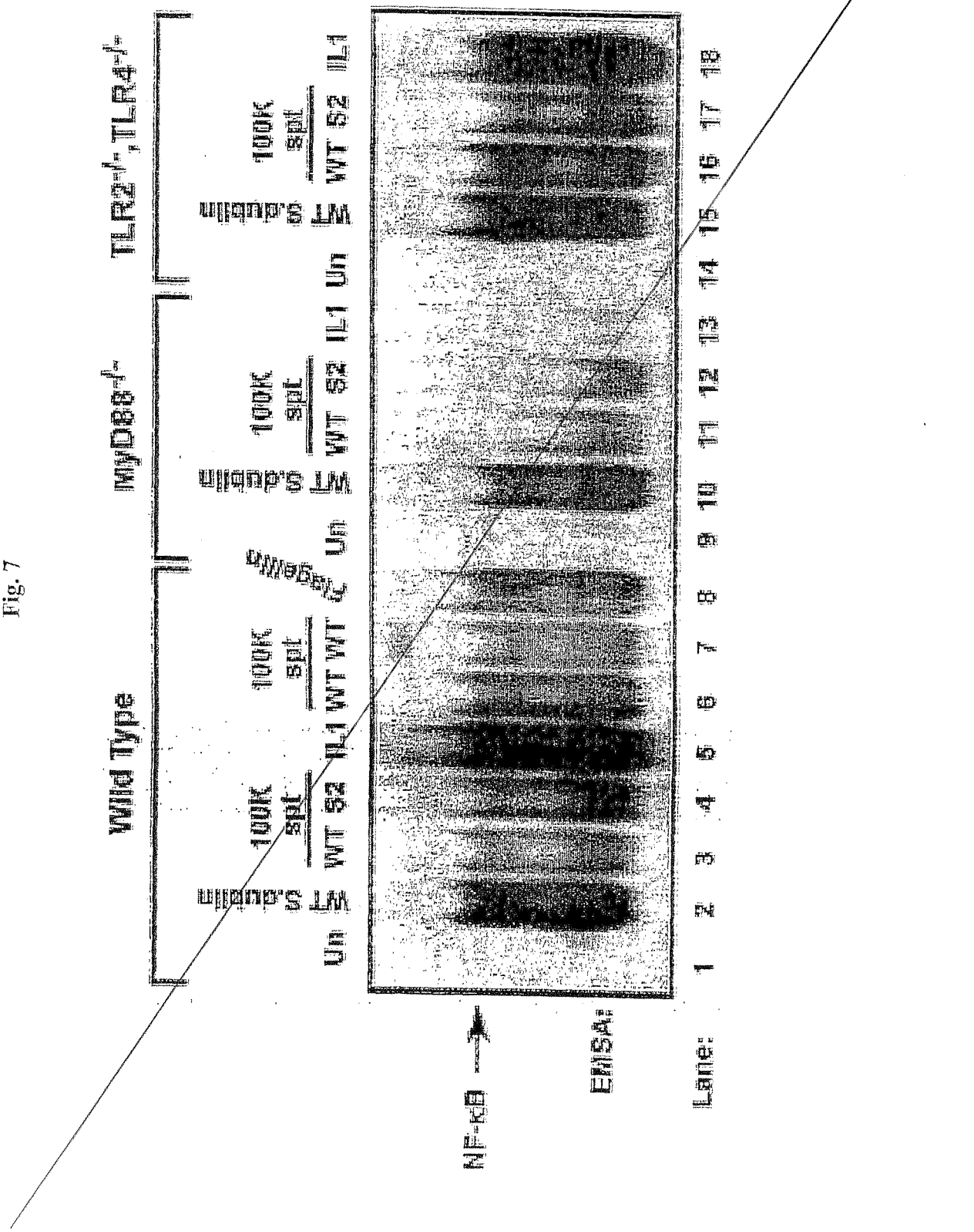

Wild Type
MyD88-/-
TLR2-/-, TLR4-/-
Un
WT S.aureus
100K spl
WT S2
IL1
Un
WT S.aureus
100K spl
WT S2
IL1
Un
WT S.aureus
100K spl
WT S2
IL1
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
NF-κB
EMSA

Fig. 8

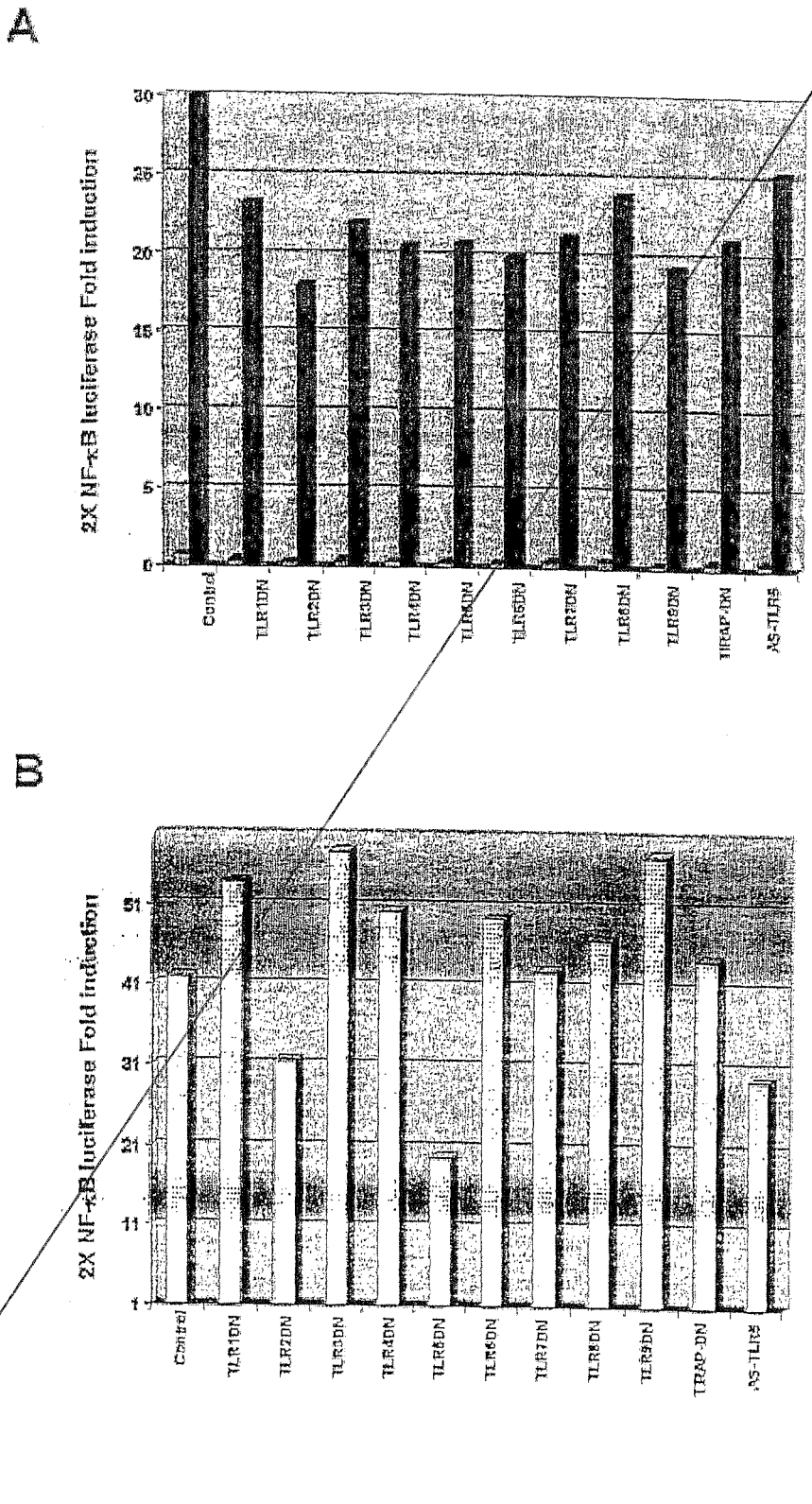

A
2X NF-κB luciferase Fold induction
30
25
20
15
10
5
0
Control
TLR1DN
TLR2DN
TLR3DN
TLR4DN
TLR5DN
TLR6DN
TLR7DN
TLR8DN
TLR9DN
TRAP-DN
A5-TLR5
B
2X NF-κB luciferase Fold induction
51
41
31
21
11
1
Control
TLR1DN
TLR2DN
TLR3DN
TLR4DN
TLR5DN
TLR6DN
TLR7DN
TLR8DN
TLR9DN
TRAP-DN
A5-TLR5

Fig. 9

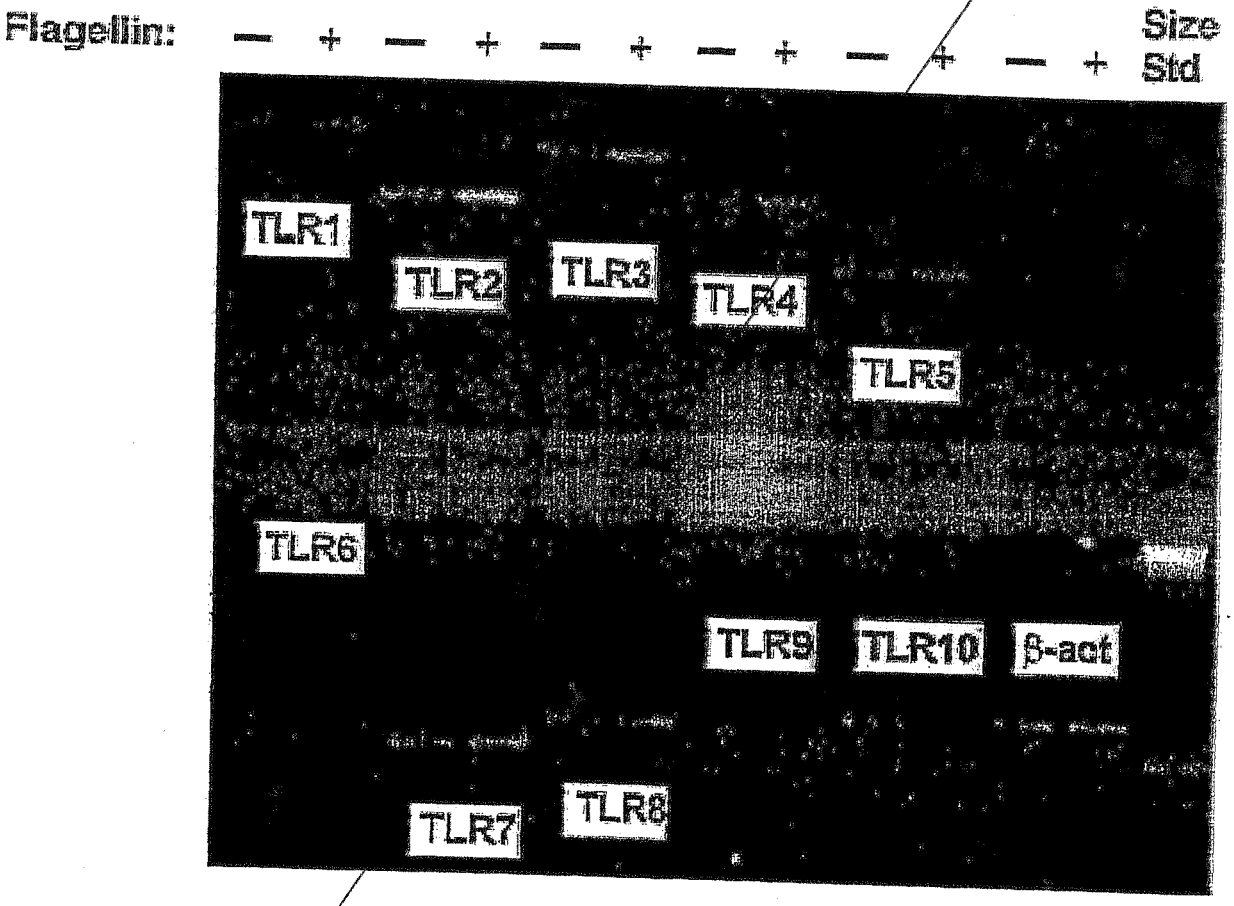

Flagellin:
— + — + — + — + — + — +
Size Std
TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR9
TLR10
β-act
TLR7
TLR8

Fig. 10

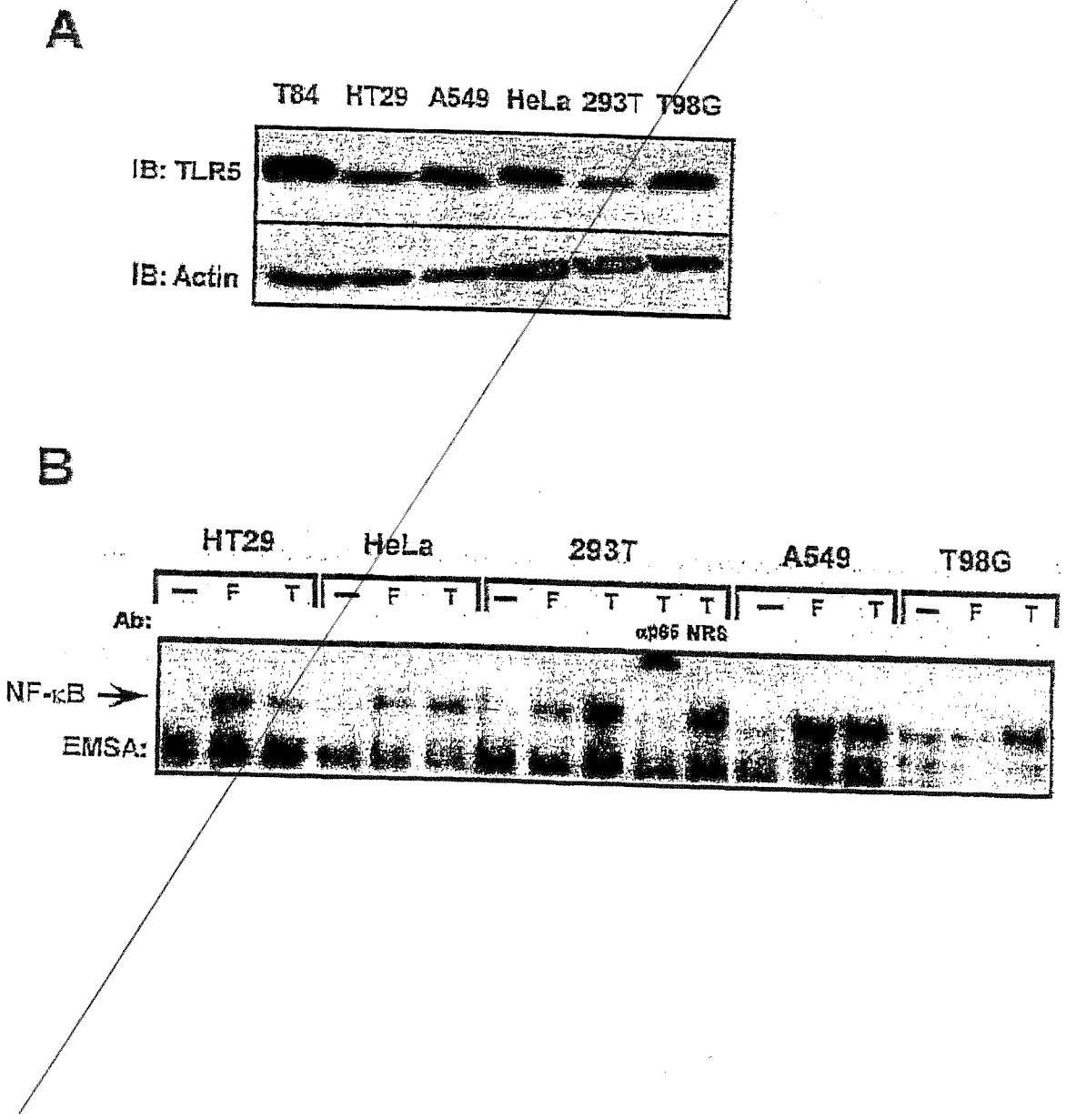

A
T84 HT29 A549 HeLa 293T T98G
IB: TLR5
IB: Actin
B
HT29 HeLa 293T A549 T98G
— F T — F T — F T T T — F T — F T
Ab:
αp65 NRS
NF-κB
EMSA:

Fig. 11

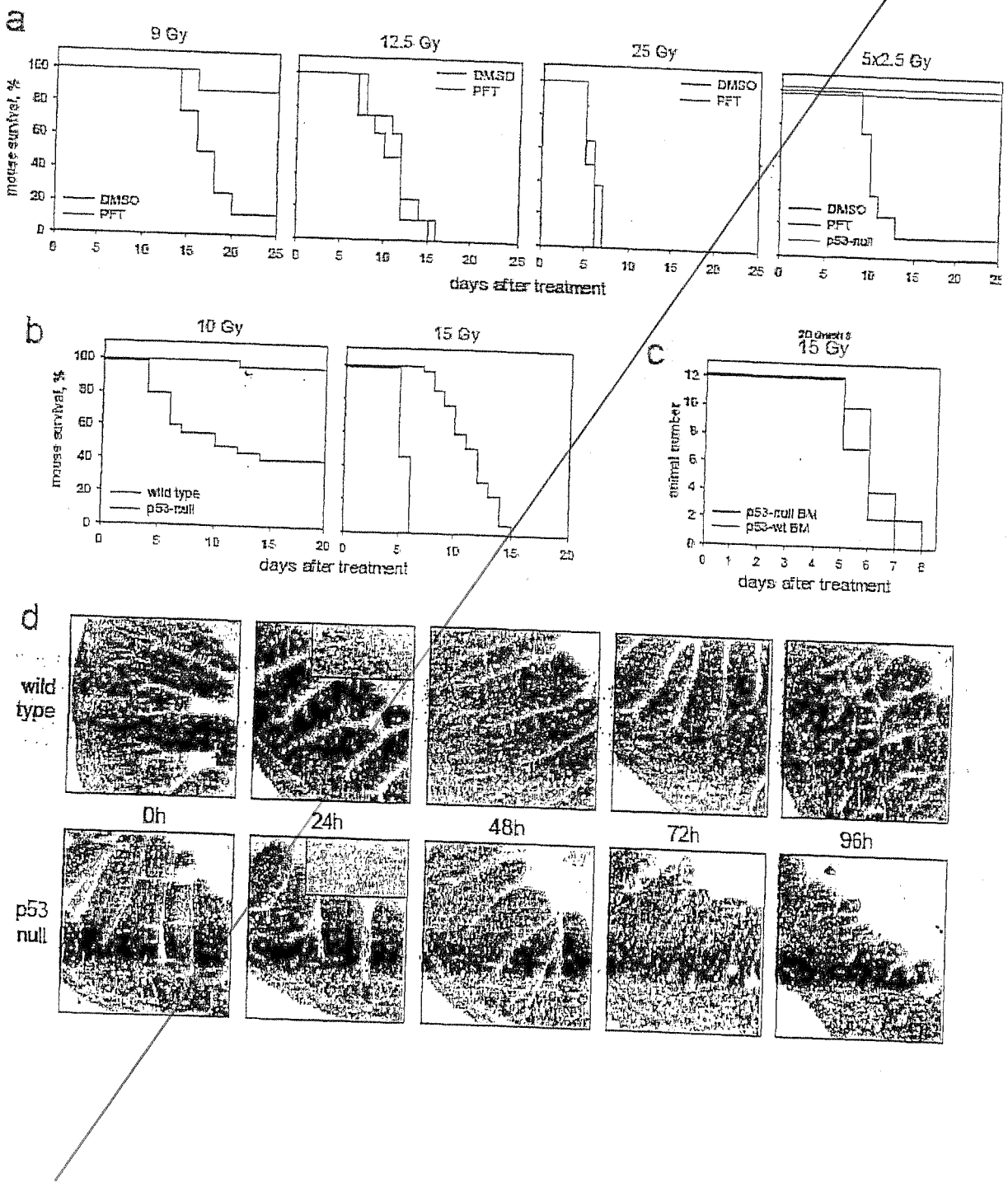

a
9 Gy
12.5 Gy
25 Gy
5x2.5 Gy
mouse survival, %
DMSO
PFT
p53-null
days after treatment
b
10 Gy
15 Gy
wild type
p53-null
c
15 Gy
animal number
p53-null BM
p53-wt BM
d
wild type
p53 null
0h
24h
48h
72h
96h

Fig. 12

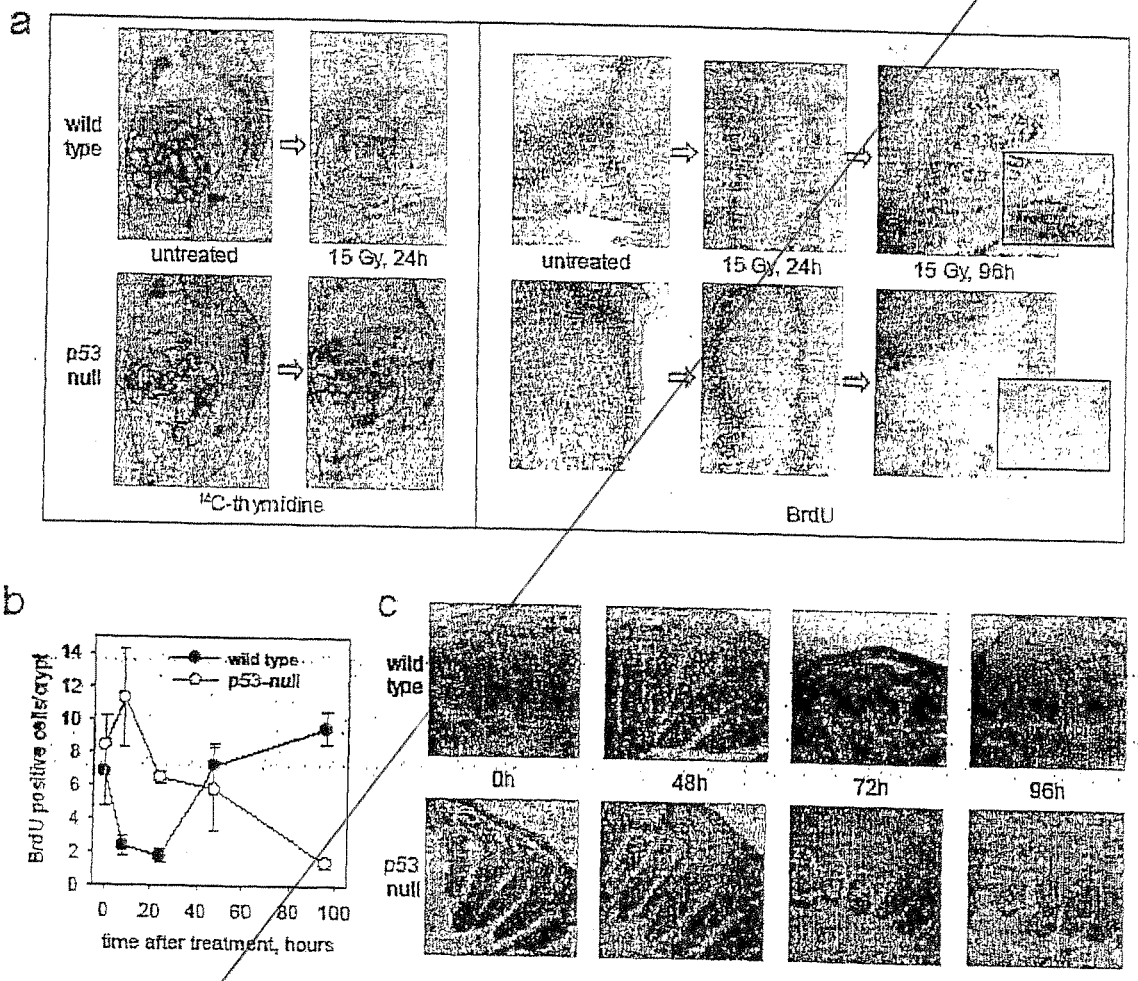

a
wild type
p53 null
untreated
15 Gy, 24h
^{14}C-thymidine
untreated
15 Gy, 24h
15 Gy, 96h
BrdU
b
BrdU positive cells/crypt
wild type
p53-null
time after treatment, hours
c
wild type
p53 null
0h
48h
72h
96h

Fig. 13

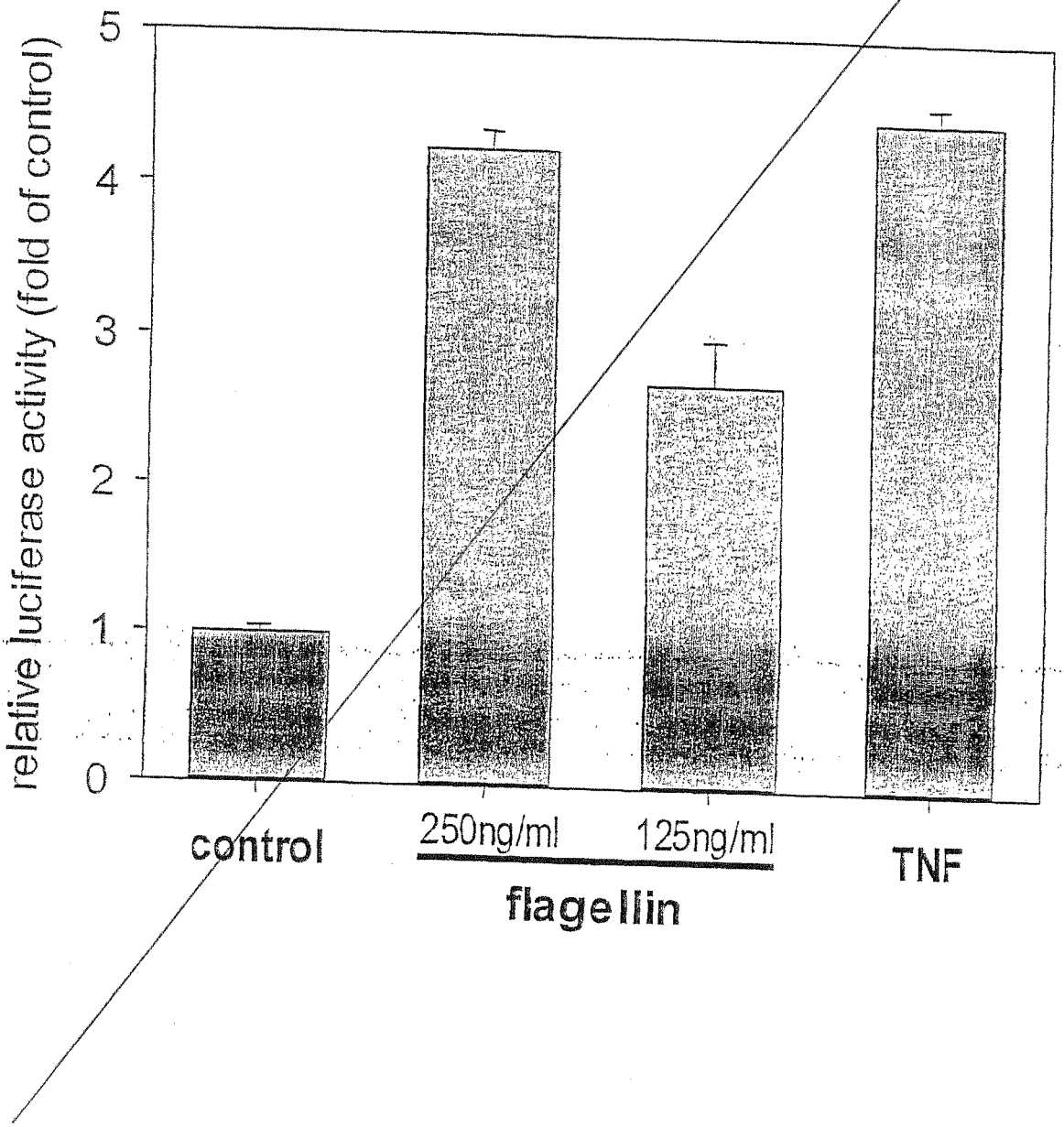

relative luciferase activity (fold of control)
0
1
2
3
4
5
control
250ng/ml
125ng/ml
flagellin
TNF

Fig. 14

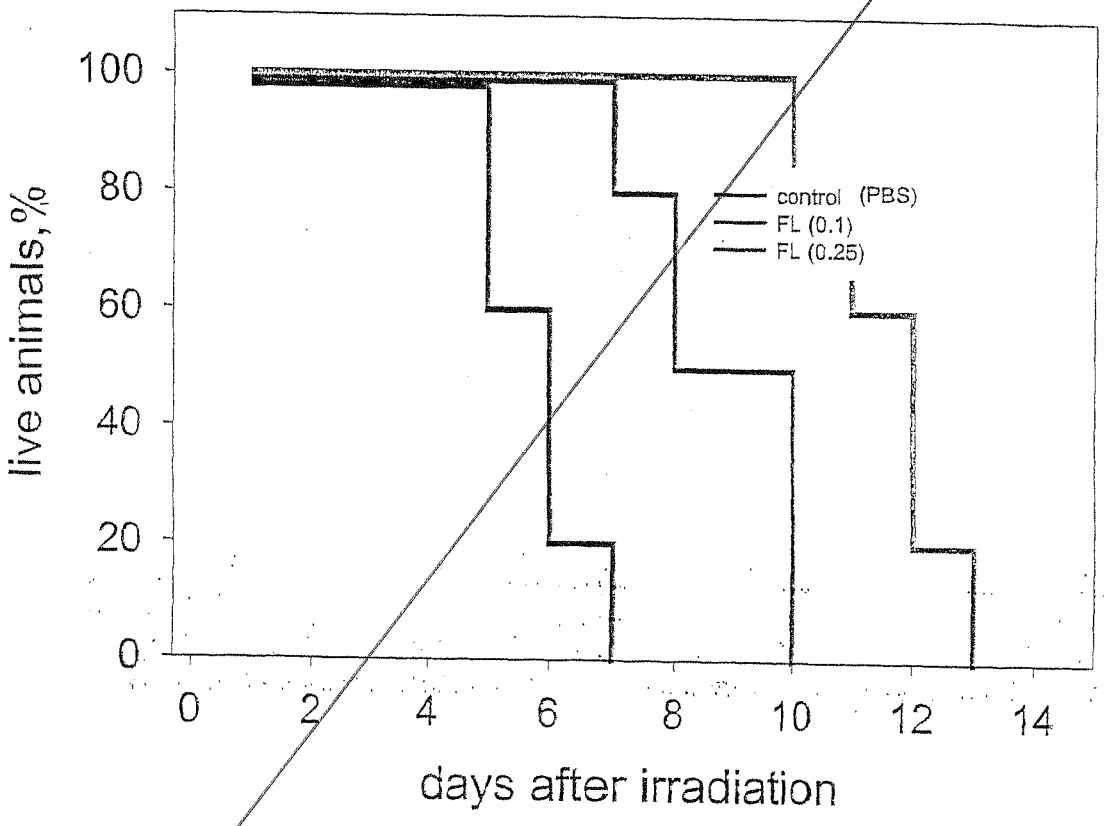

100
80
60
40
20
0
live animals,%
0
2
4
6
8
10
12
14
days after irradiation
control (PBS)
FL (0.1)
FL (0.25)

Fig. 15

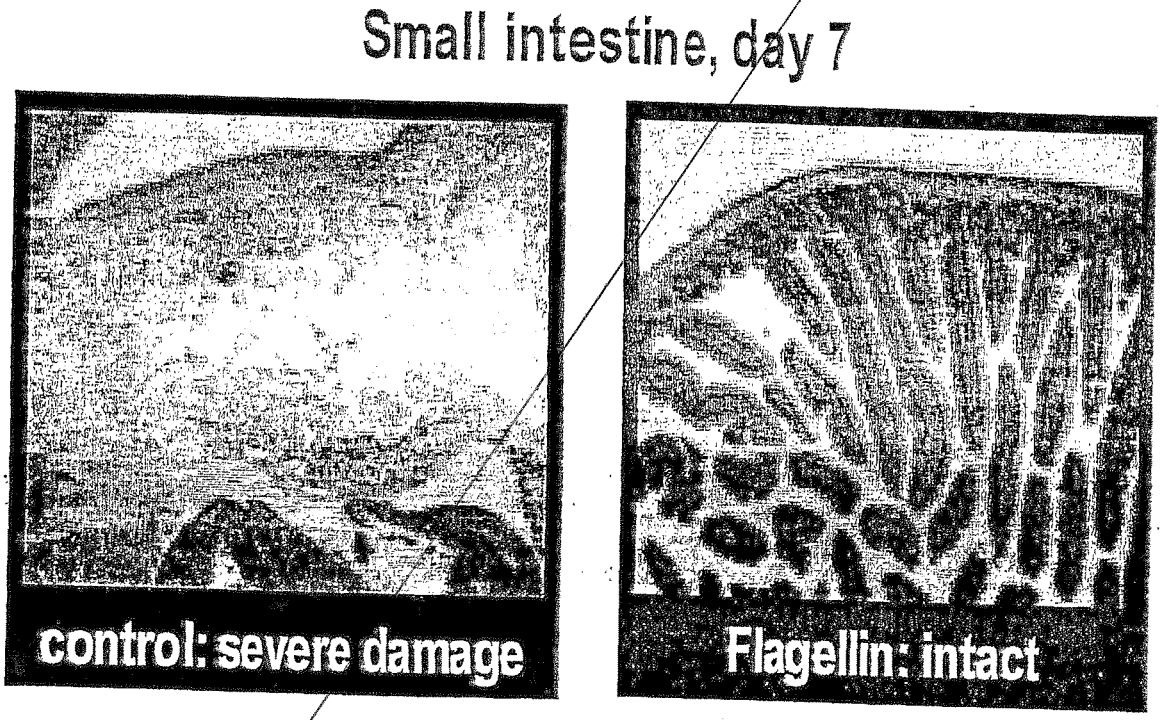

Small intestine, day 7
control: severe damage
Flagellin: intact

Fig. 16

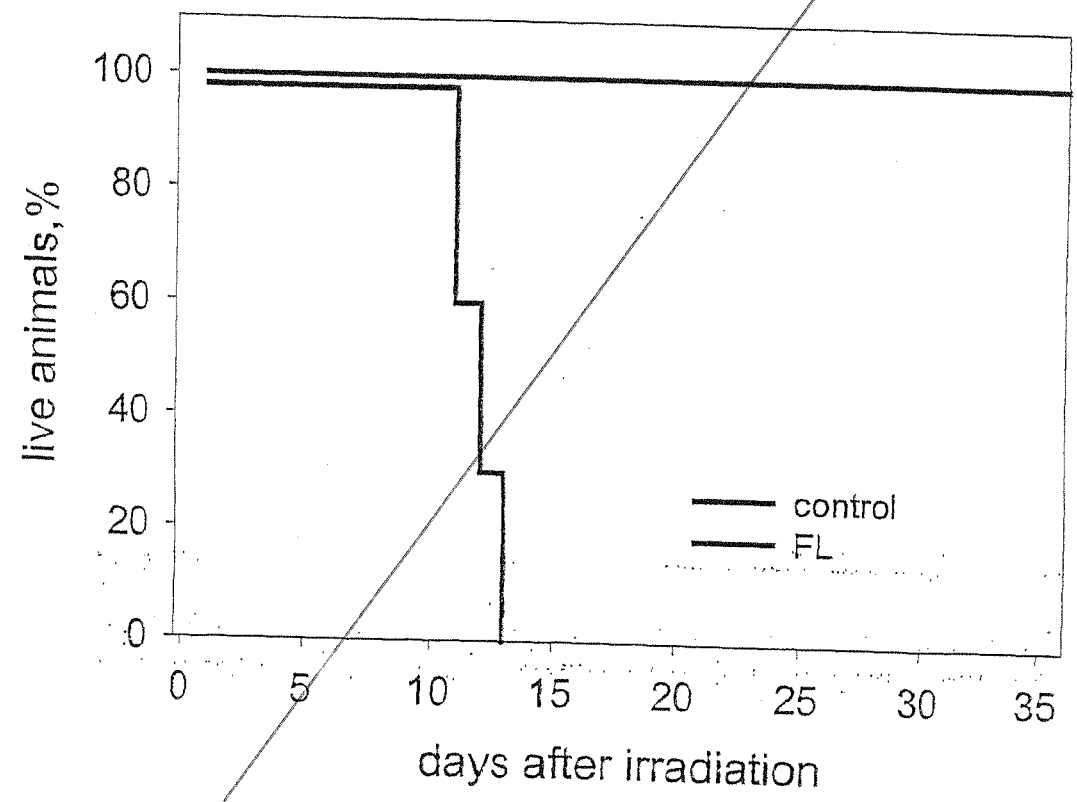

100
80
60
40
20
0
live animals, %
0
5
10
15
20
25
30
35
days after irradiation
control
FL

Fig. 17

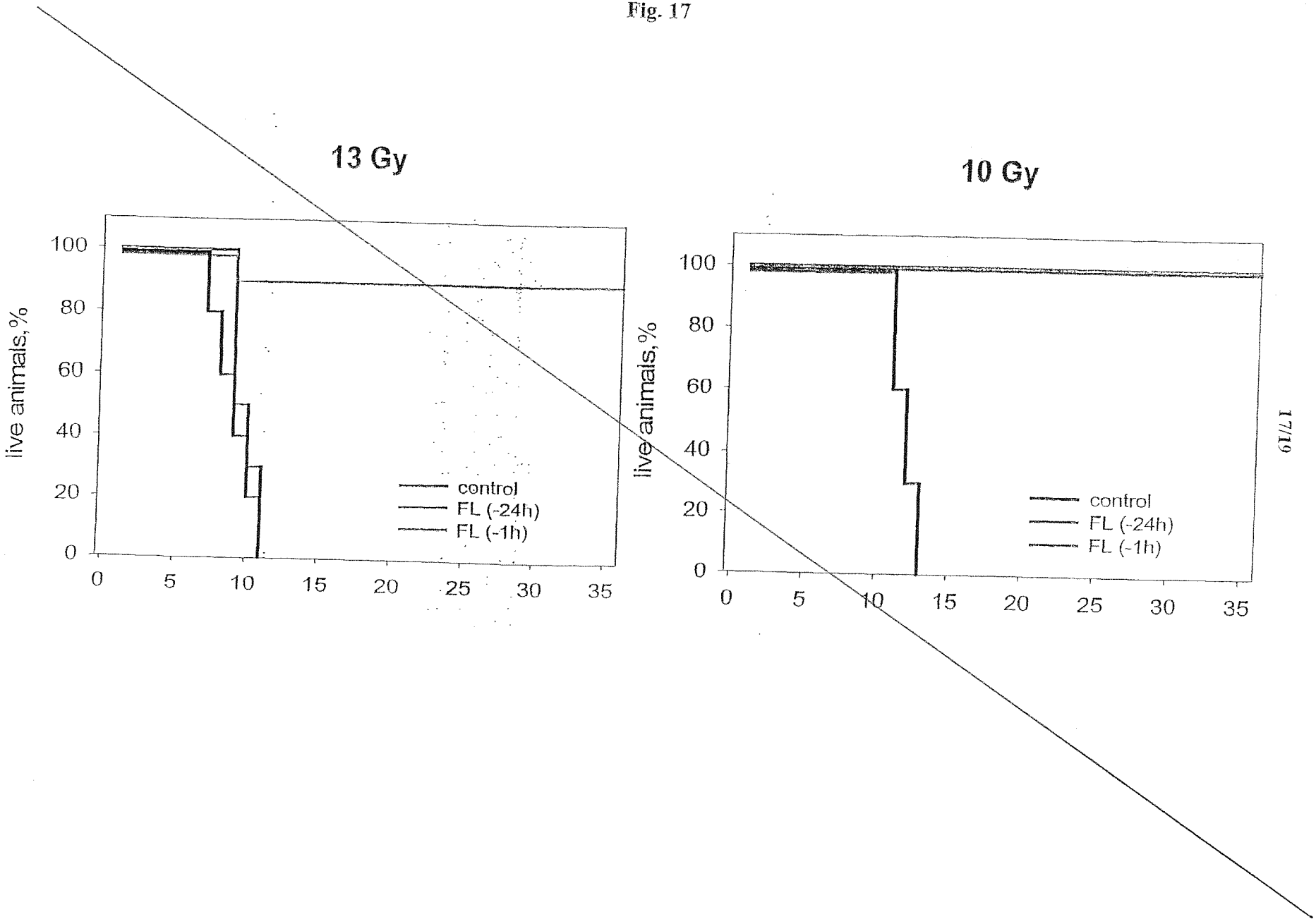

13 Gy
10 Gy
live animals, %
100
80
60
40
20
0
0
5
10
15
20
25
30
35
control
FL (-24h)
FL (-1h)

Fig. 18

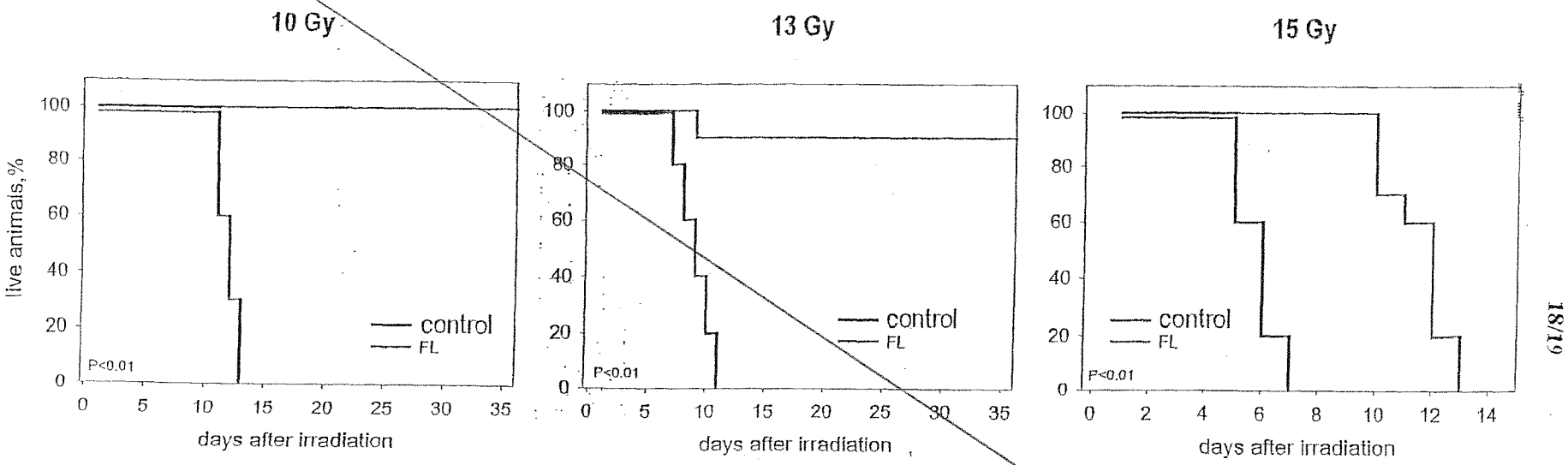

10 Gy
13 Gy
15 Gy
live animals, %
control
FL
P<0.01
days after irradiation

Fig. 19

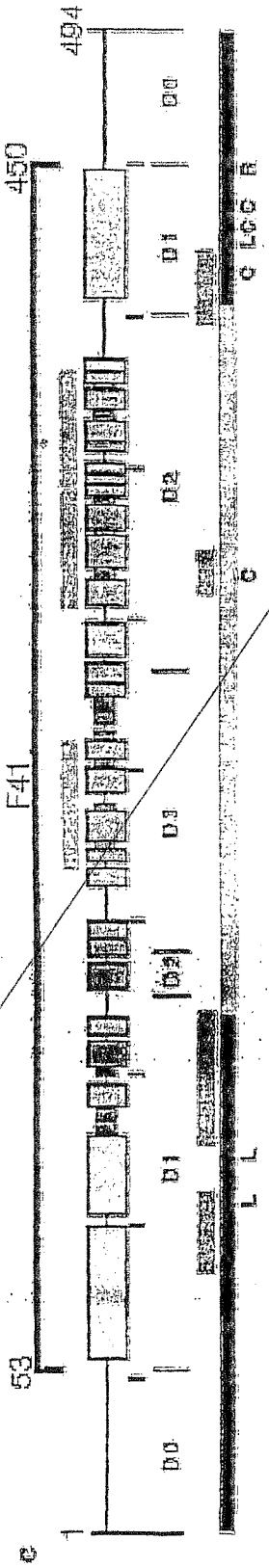


Fig. 1

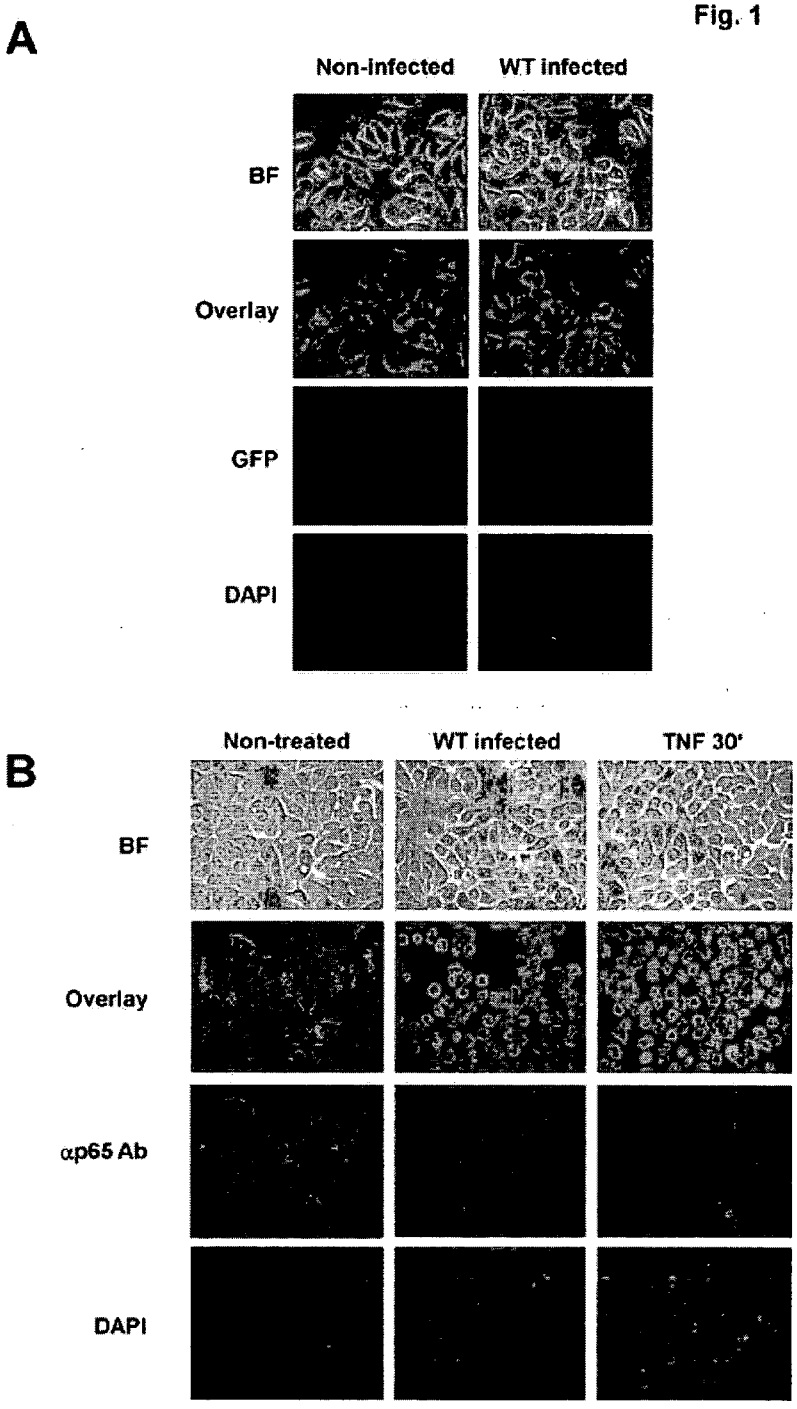

A
Non-infected
WT infected
BF
Overlay
GFP
DAPI
B
Non-treated
WT infected
TNF 30'
BF
Overlay
αp65 Ab
DAPI

Fig. 2

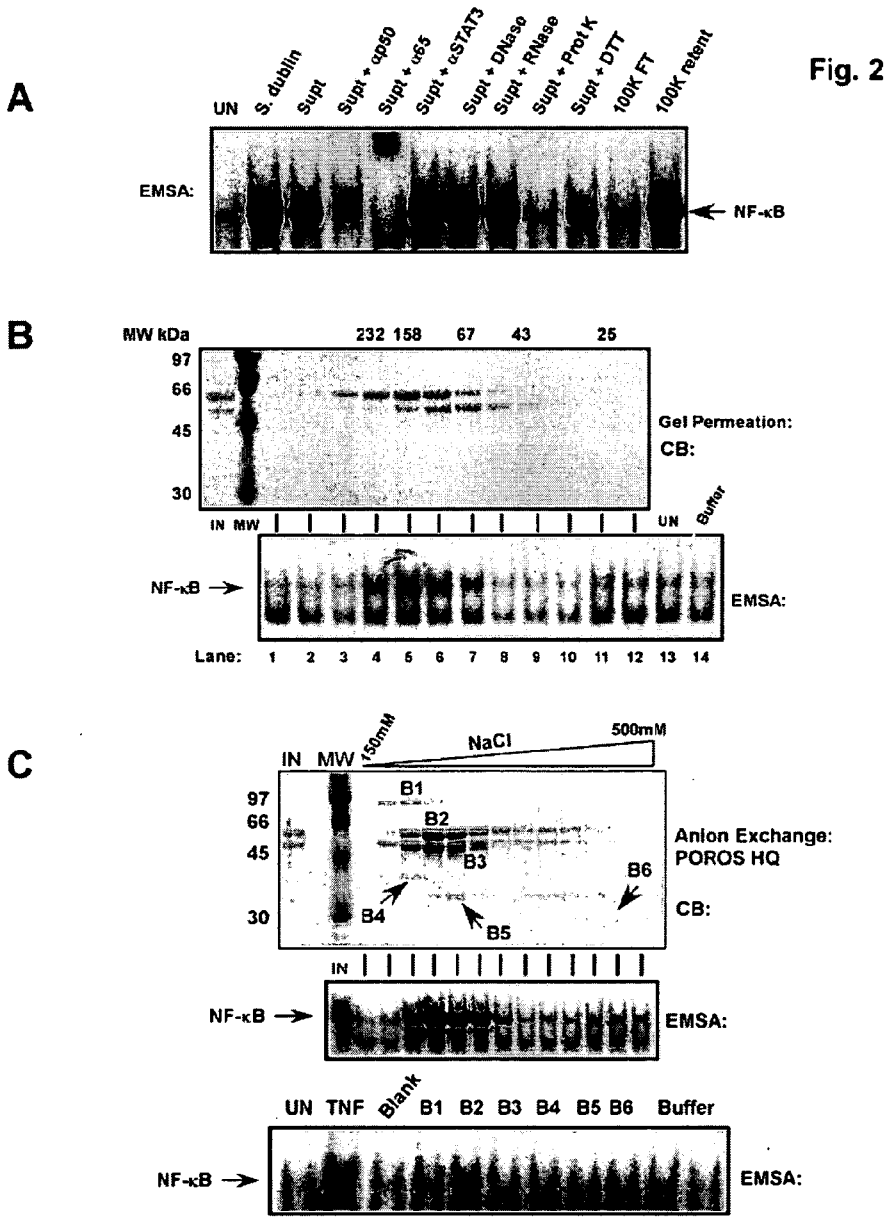
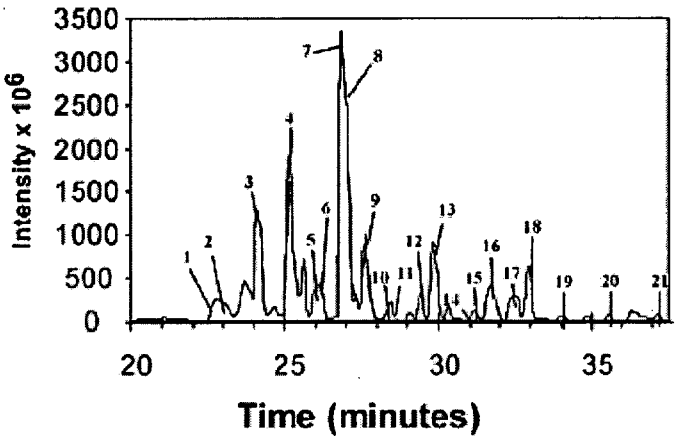
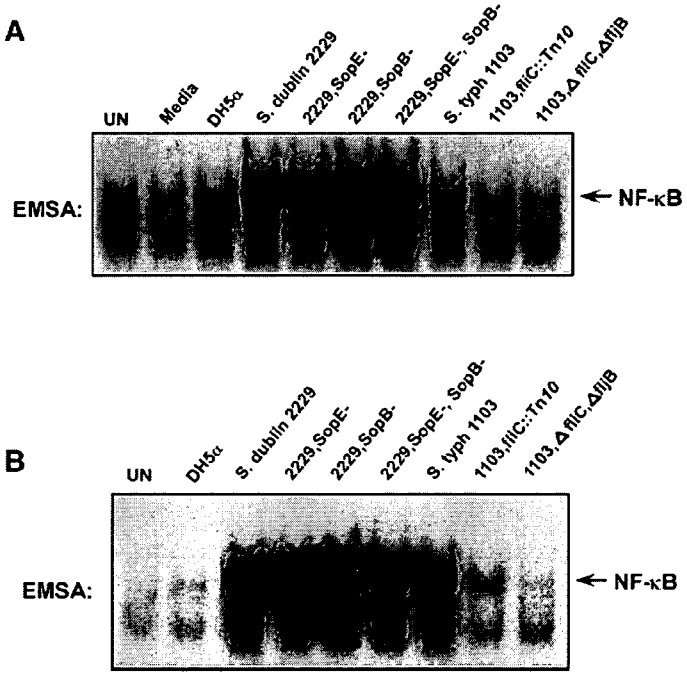

A
UN
S. dublin
Supt
Supt + αp50
Supt + α65
Supt + αSTAT3
Supt + DNase
Supt + RNase
Supt + Prot K
Supt + DTT
100K FT
100K retent
EMSA:
NF-κB
B
MW kDa
97
66
45
30
232 158 67 43 25
Gel Permeation:
CB:
IN MW
UN
Buffer
NF-κB
EMSA:
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14
C
IN MW
150mM
NaCl
500mM
B1
B2
B3
B4
B5
B6
Anion Exchange:
POROS HQ
CB:
IN
NF-κB
EMSA:
UN TNF Blank B1 B2 B3 B4 B5 B6 Buffer
NF-κB
EMSA:

Fig. 3



1. FTSNIK
2. VLSQDNQMK
3. DDAAGQAIANR
4. SSLGAIQNR
5. INSAKDDAAGQAIANR
6. LSDLEANNAVK
7. SQSSLSSAIER
8. ELSVQATNGTNSDSDLK
9. IEDADYATEVSNMSK
10. NVTGYDTYAAGADKYR
11. VDINSGAVVTDAVAPDK
12. SRIEDADYATEVSNMSK
13. TASGVSTLINEDAAAAK
14. FDSAITNLGNTVTNLNSAR
15. SLGLDGFNVNGPK
16. IQVGANDGETITIDLQK
17. AQVINTNSLSLLTQNNLNK
18. NANDGISIAQTTEGALNEINNNLQR
19. NVYTSVVNGQFTFDDK
20. GLTQASRNANDGISIAQTTEGALNEINNNLQR
21. AQILQQAGTSVLAQANQVPQNVLSLLR

Fig. 4


A
UN
Media
DH5α
S. dublin 2229
2229,SopE-
2229,SopB-
2229,SopE-, SopB-
S. typh 1103
1103,fliC::Tn10
1103,Δ fliC,ΔfljB
EMSA:
NF-κB
B
UN
DH5α
S. dublin 2229
2229,SopE-
2229,SopB-
2229,SopE-, SopB-
S. typh 1103
1103,fliC::Tn10
1103,Δ fliC,ΔfljB
EMSA:
NF-κB

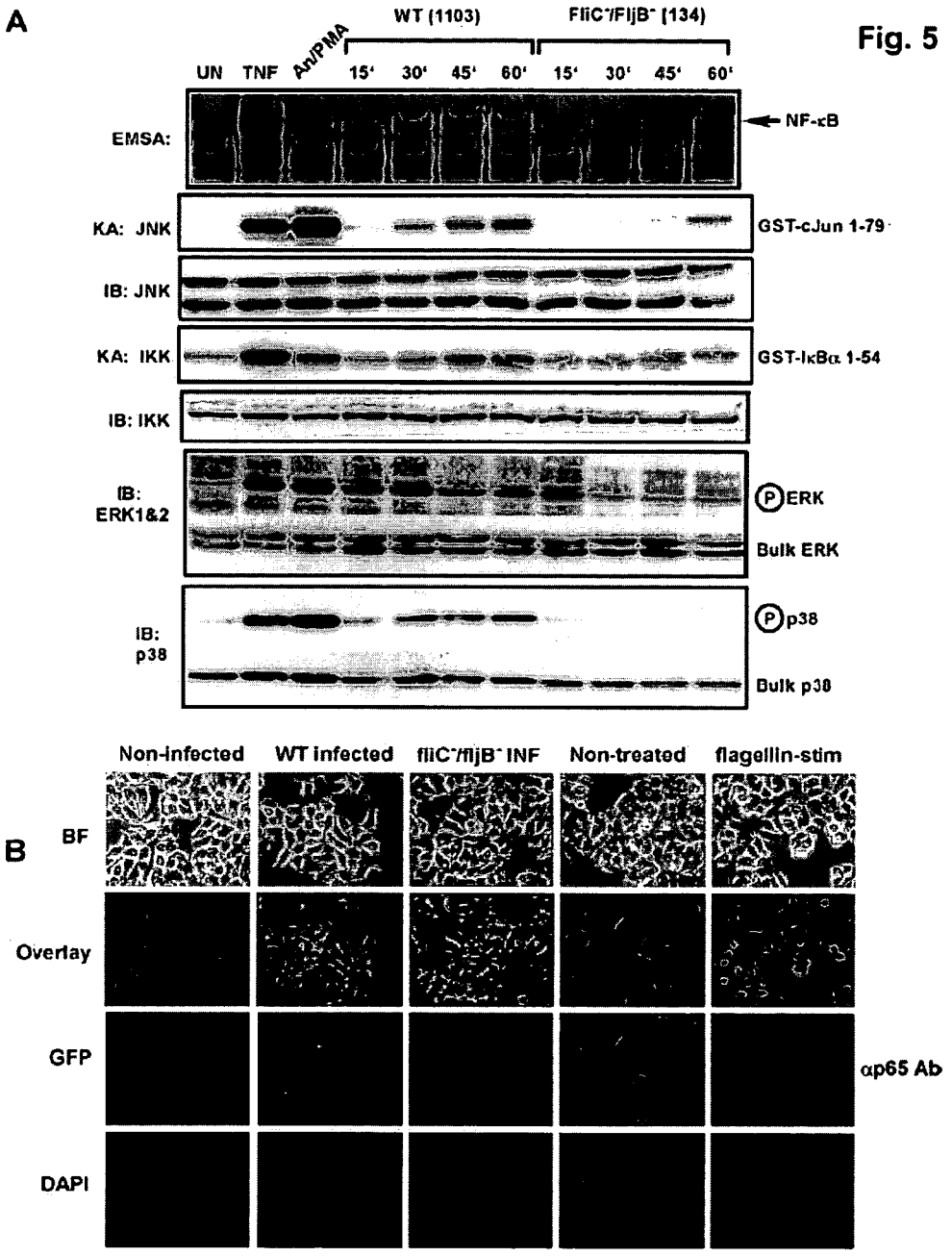
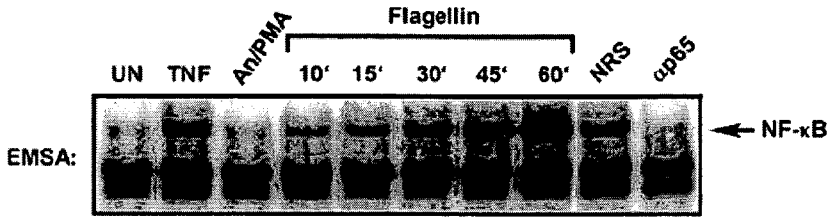

A
WT (1103)
FliC⁻/FljB⁻ (134)
UN
TNF
Anv/PMA
15'
30'
45'
60'
15'
30'
45'
60'
EMSA:
NF-κB
KA: JNK
GST-cJun 1-79
IB: JNK
KA: IKK
GST-IκBα 1-54
IB: IKK
IB: ERK1&2
PERK
Bulk ERK
IB: p38
Pp38
Bulk p38
B
Non-infected
WT infected
fliC⁻/fljB⁻ INF
Non-treated
flagellin-stim
BF
Overlay
GFP
DAPI
αp65 Ab

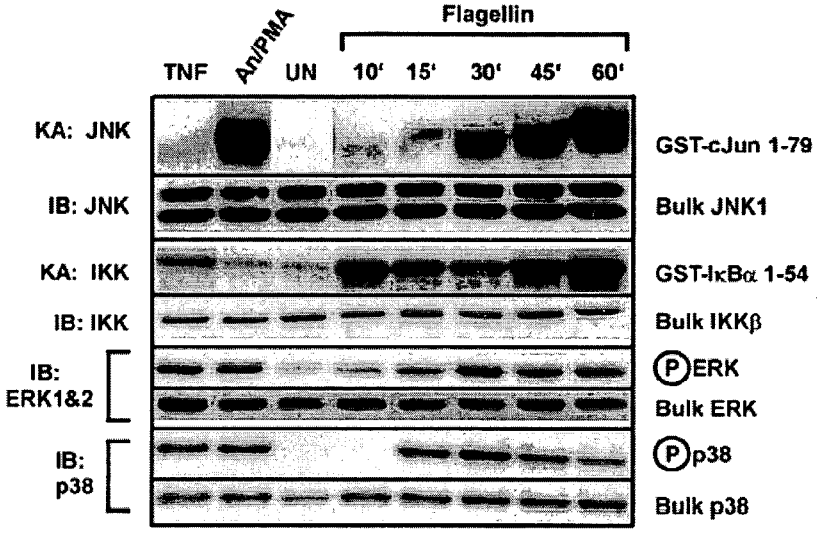
Fig. 5

Fig. 6

A


Flagellin
UN
TNF
An/PMA
10'
15'
30'
45'
60'
NRS
αp65
EMSA:
NF-κB

B


Flagellin
TNF
An/PMA
UN
10'
15'
30'
45'
60'
KA: JNK
GST-cJun 1-79
IB: JNK
Bulk JNK1
KA: IKK
GST-IκBα 1-54
IB: IKK
Bulk IKKβ
IB: ERK1&2
PERK
Bulk ERK
IB: p38
Pp38
Bulk p38

C

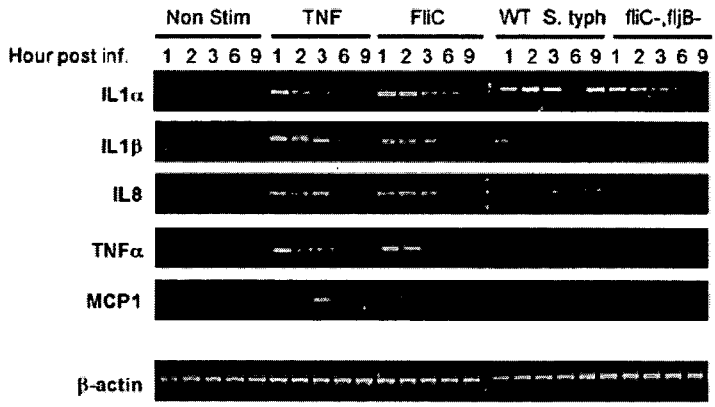

Non Stim
TNF
FliC
WT S. typh
fliC-,fljB-
Hour post inf.
1 2 3 6 9 1 2 3 6 9 1 2 3 6 9 1 2 3 6 9 1 2 3 6 9
IL1α
IL1β
IL8
TNFα
MCP1
β-actin

Fig. 7

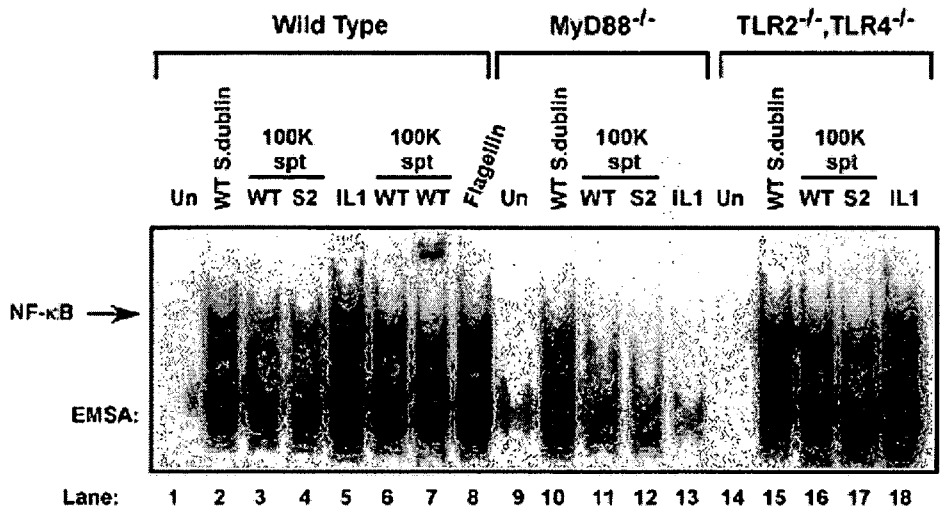
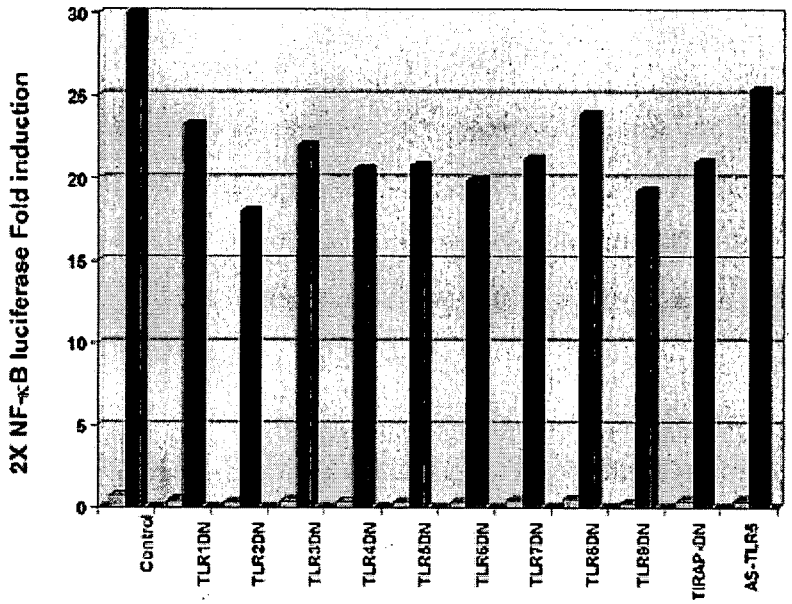

Wild Type
MyD88-/-
TLR2-/-,TLR4-/-
Un
WT S.dublin
100K spt
WT S2
IL1
WT WT
Flagellin
NF-κB
EMSA:
Lane:
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 8

A


2X NF-κB luciferase Fold induction
30
25
20
15
10
5
0
Control
TLR1DN
TLR2DN
TLR3DN
TLR4DN
TLR5DN
TLR6DN
TLR7DN
TLR8DN
TLR9DN
TIRAP-DN
AS-TLR5

B

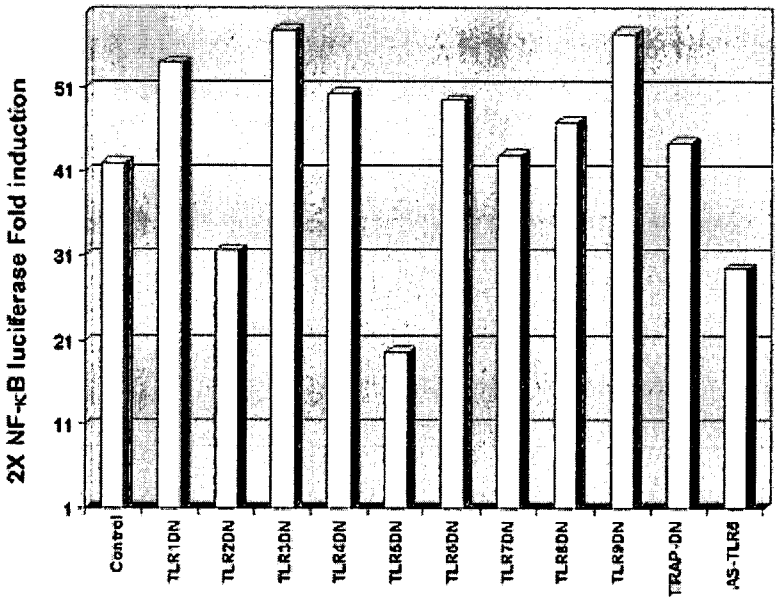

2X NF-κB luciferase Fold induction
51
41
31
21
11
1
Control
TLR1DN
TLR2DN
TLR3DN
TLR4DN
TLR5DN
TLR6DN
TLR7DN
TLR8DN
TLR9DN
TRAP-DN
AS-TLR5

Fig. 9

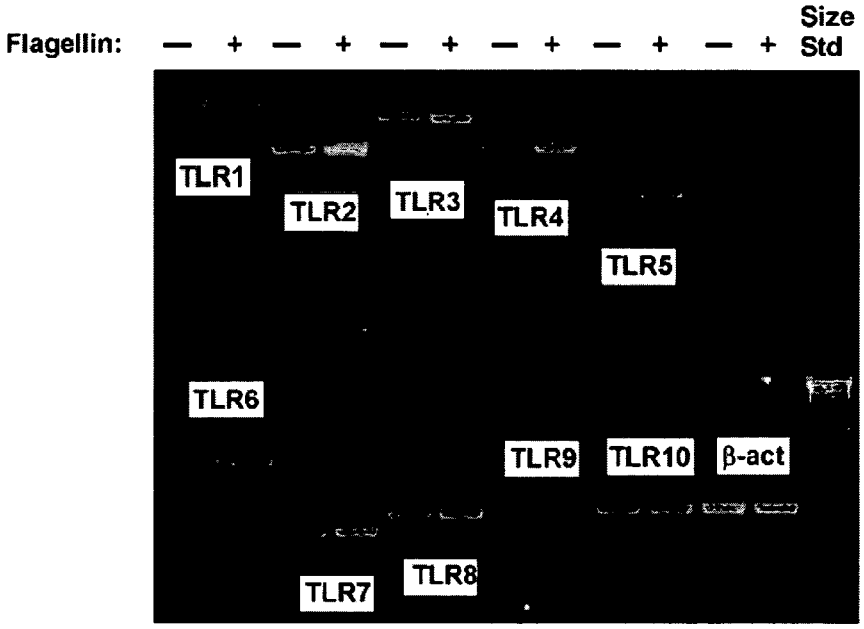

Flagellin: — + — + — + — + — + — + Size Std
TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR9
TLR10
β-act
TLR7
TLR8

Fig. 10

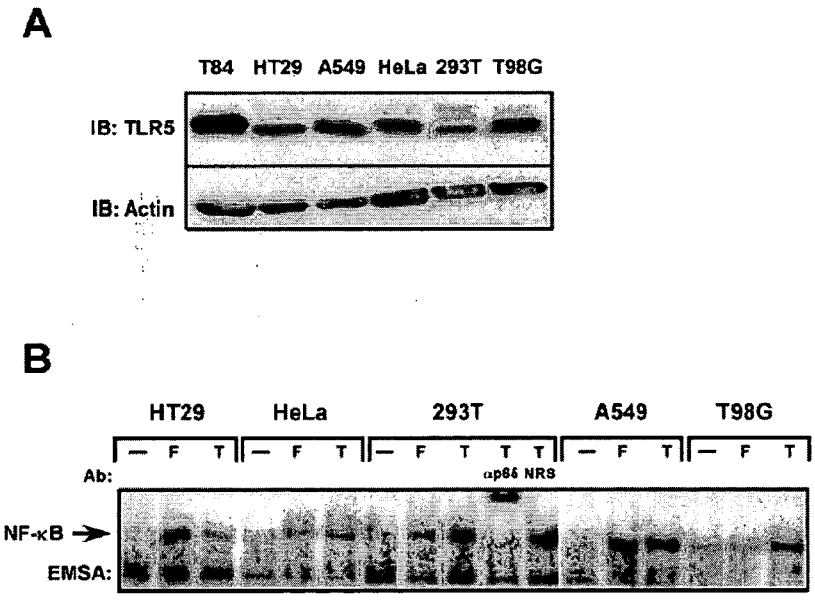

A
T84 HT29 A549 HeLa 293T T98G
IB: TLR5
IB: Actin
B
HT29 HeLa 293T A549 T98G
— F T — F T — F T T T — F T — F T
Ab:
αp65 NRS
NF-κB
EMSA:

Fig. 11

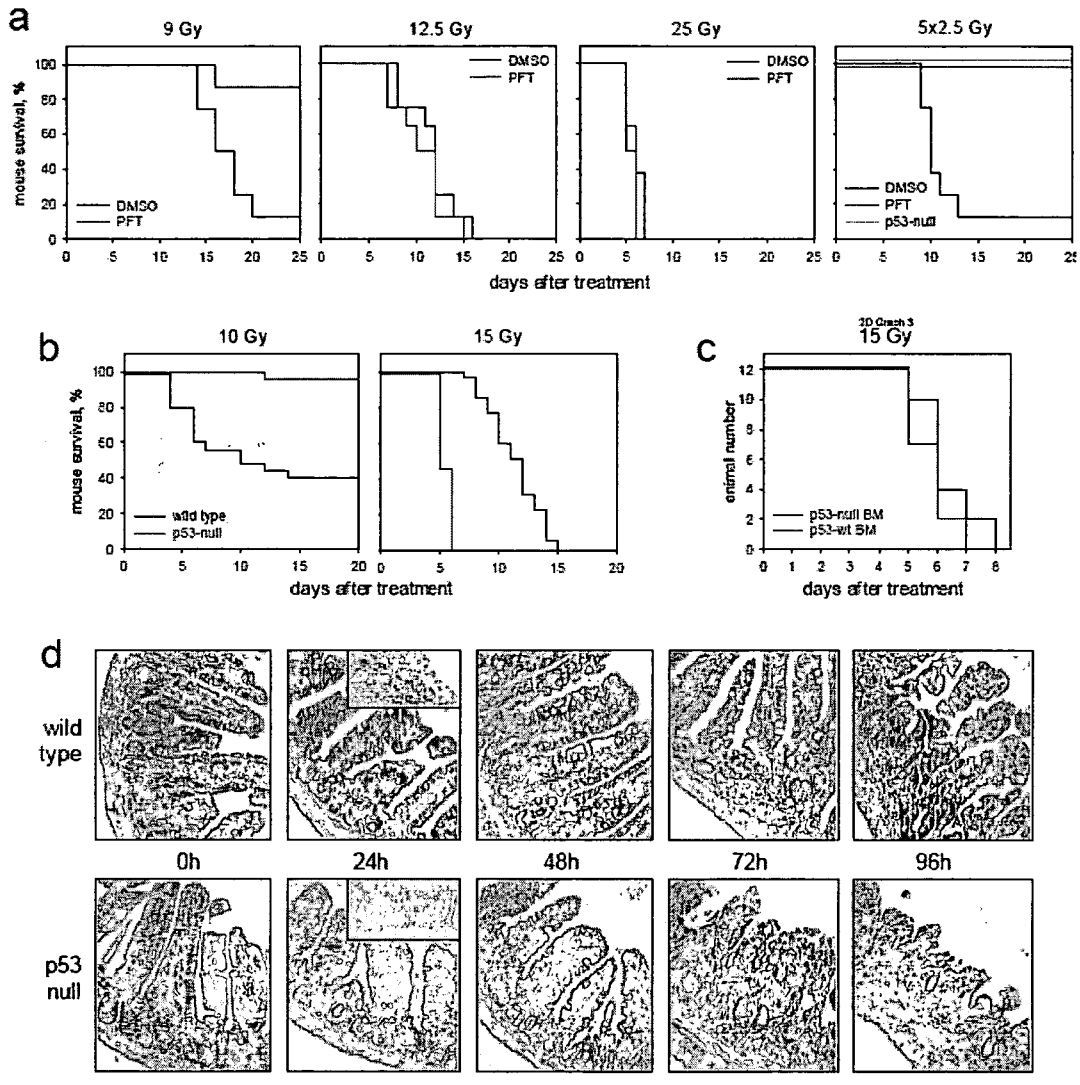

a
9 Gy
12.5 Gy
25 Gy
5x2.5 Gy
mouse survival, %
DMSO
PFT
p53-null
days after treatment
b
10 Gy
15 Gy
wild type
p53-null
c
15 Gy
animal number
p53-null BM
p53-wt BM
d
wild type
p53 null
0h
24h
48h
72h
96h

Fig. 12

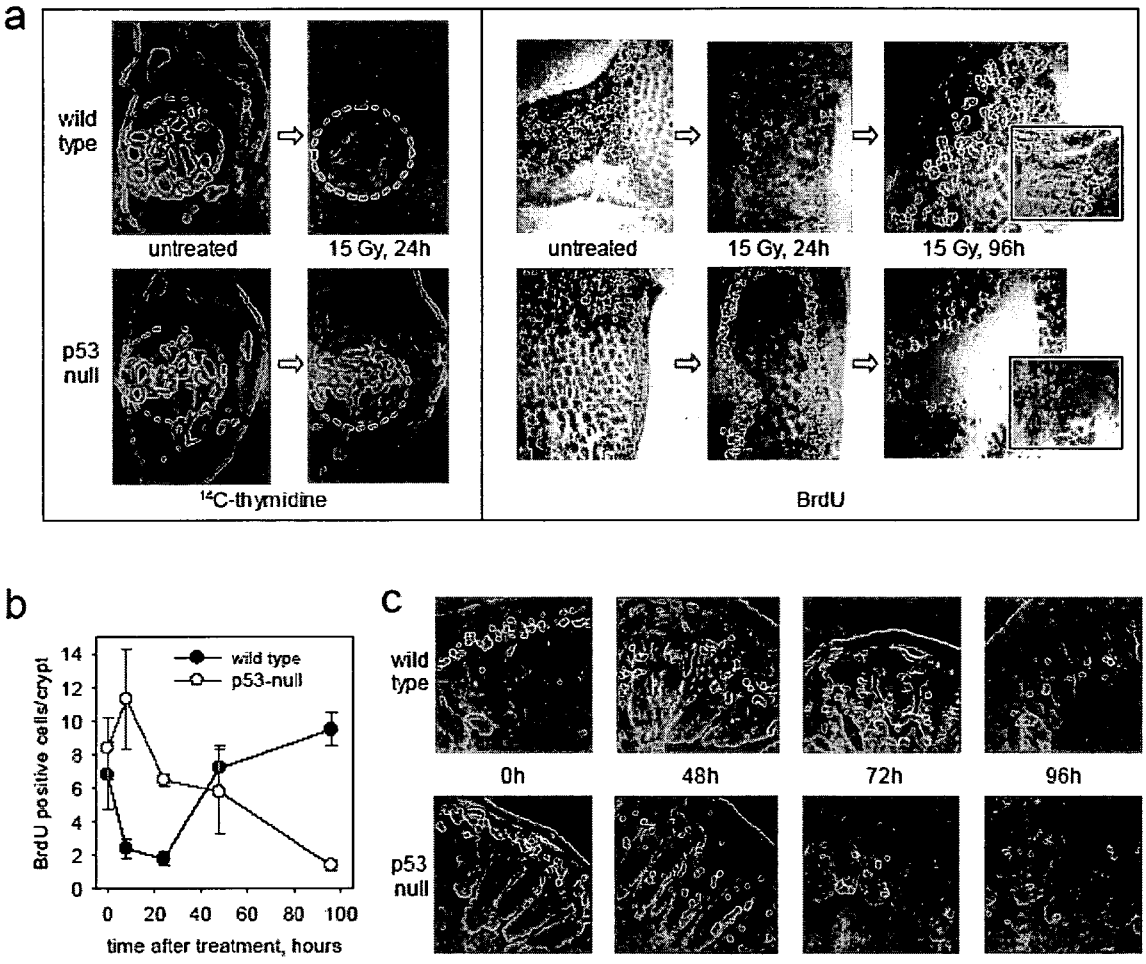

a
wild type
p53 null
untreated
15 Gy, 24h
14C-thymidine
untreated
15 Gy, 24h
15 Gy, 96h
BrdU
b
BrdU positive cells/crypt
wild type
p53-null
time after treatment, hours
c
wild type
p53 null
0h
48h
72h
96h

Fig. 13

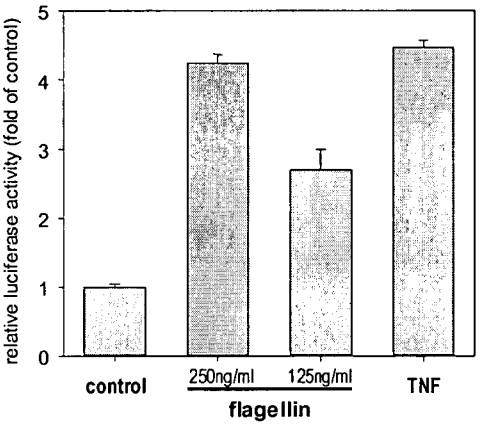

relative luciferase activity (fold of control)
0
1
2
3
4
5
control
250ng/ml
125ng/ml
TNF
flagellin

Fig. 14

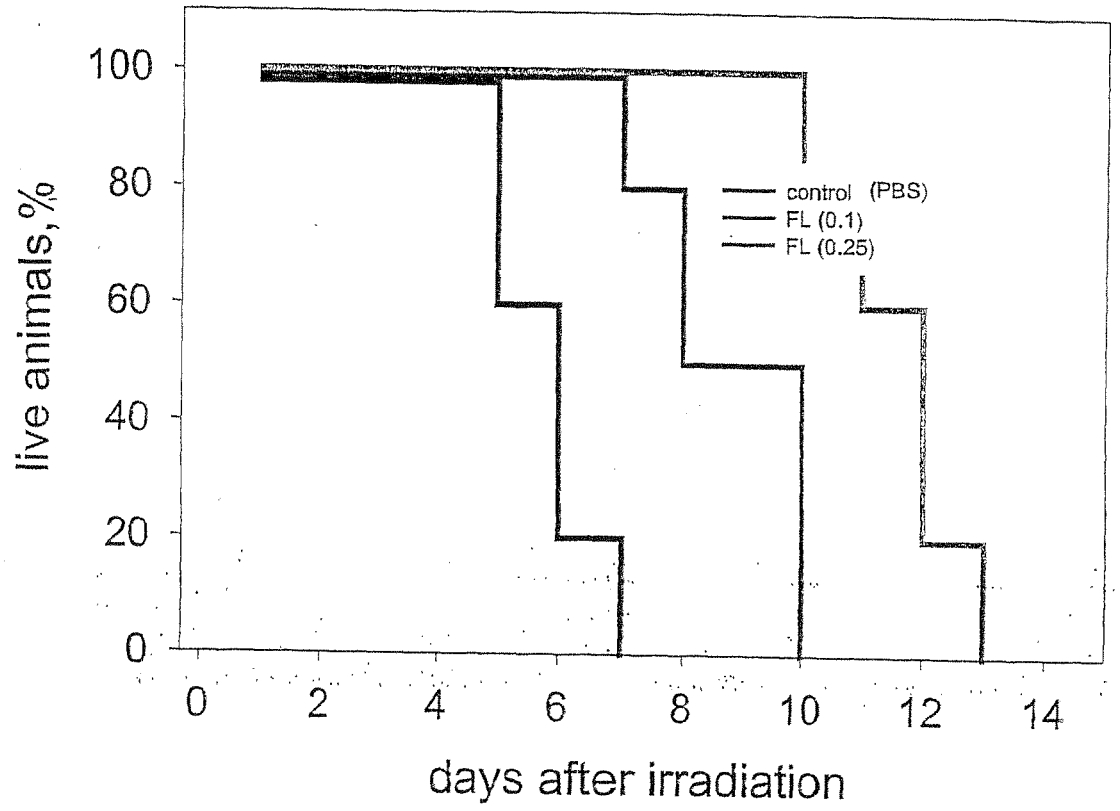

100
80
60
40
20
0
live animals,%
0
2
4
6
8
10
12
14
days after irradiation
control (PBS)
FL (0.1)
FL (0.25)

Fig. 15

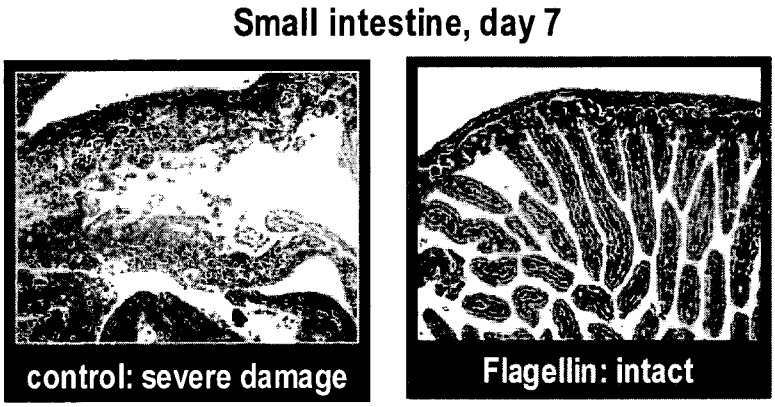
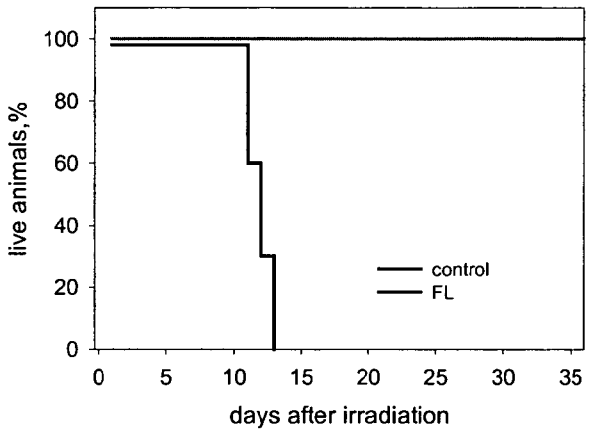

Small intestine, day 7
control: severe damage
Flagellin: intact

Fig. 16


100
80
60
40
20
0
live animals,%
0
5
10
15
20
25
30
35
days after irradiation
control
FL

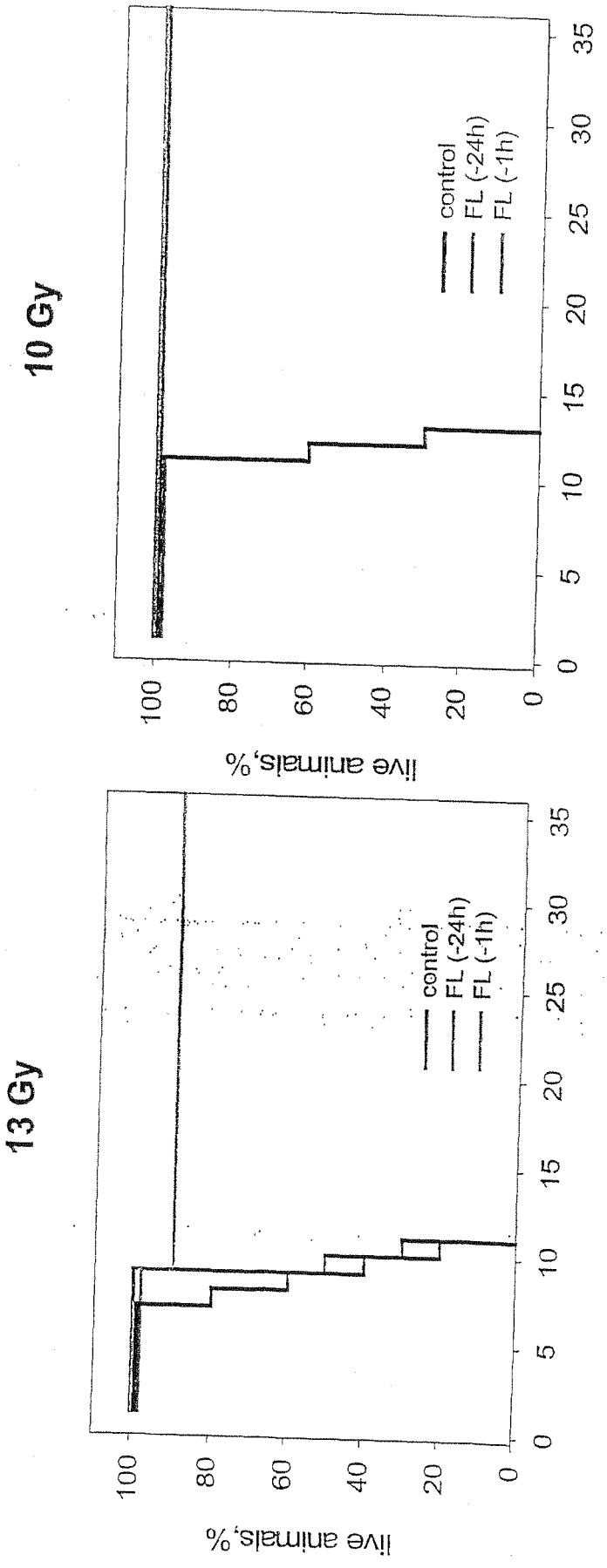

13 Gy
10 Gy
live animals, %
live animals, %
control
FL (-24h)
FL (-1h)
control
FL (-24h)
FL (-1h)
0
20
40
60
80
100
0
5
10
15
20
25
30
35

Fig. 17

Fig. 18

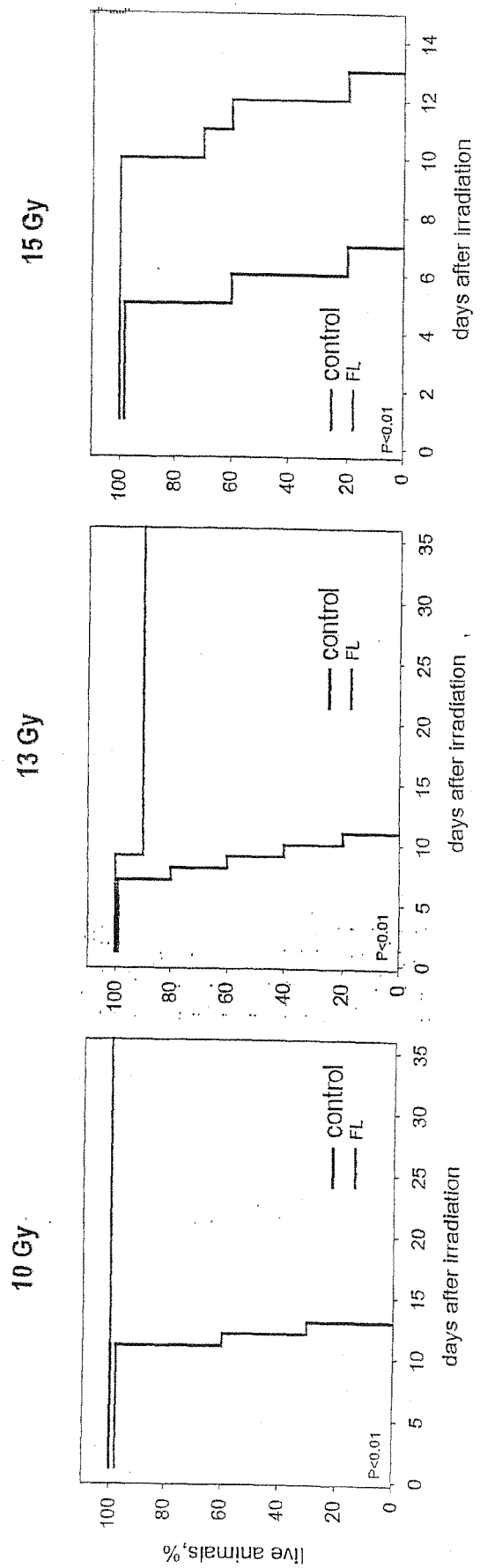

10 Gy
13 Gy
15 Gy
live animals, %
days after irradiation
control
FL
P<0.01

Fig. 19

