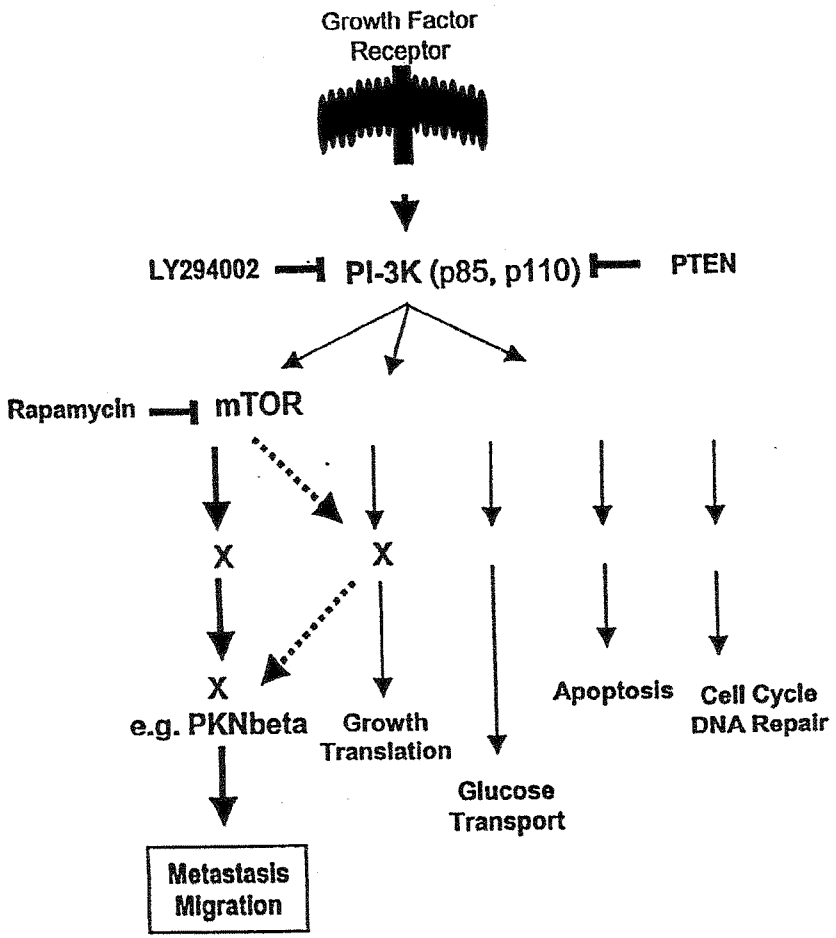

Growth Factor
Receptor
LY294002
PI-3K (p85, p110)
PTEN
Rapamycin
mTOR
X
X
X
e.g. PKNbeta
Growth
Translation
Glucose
Transport
Apoptosis
Cell Cycle
DNA Repair
Metastasis
Migration

Fig. 1

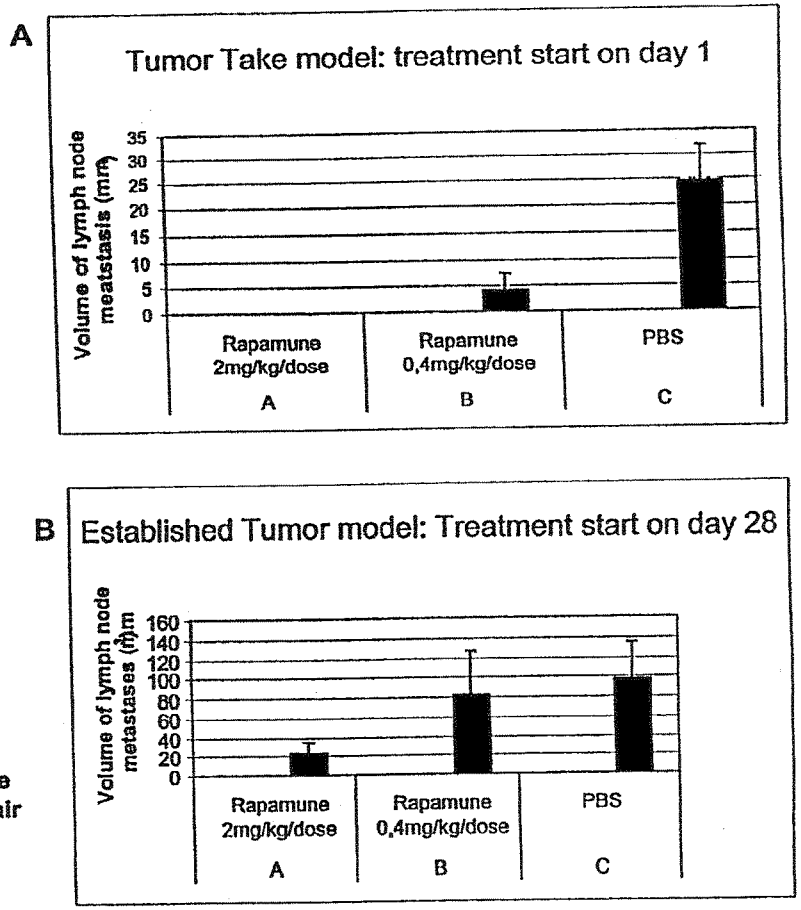

A
Tumor Take model: treatment start on day 1
Volume of lymph node meatstasis (mm)
35
30
25
20
15
10
5
0
Rapamune 2mg/kg/dose
A
Rapamune 0,4mg/kg/dose
B
PBS
C
B
Established Tumor model: Treatment start on day 28
Volume of lymph node metastases (mm)
160
140
120
100
80
60
40
20
0
Rapamune 2mg/kg/dose
A
Rapamune 0,4mg/kg/dose
B
PBS
C

Fig. 2

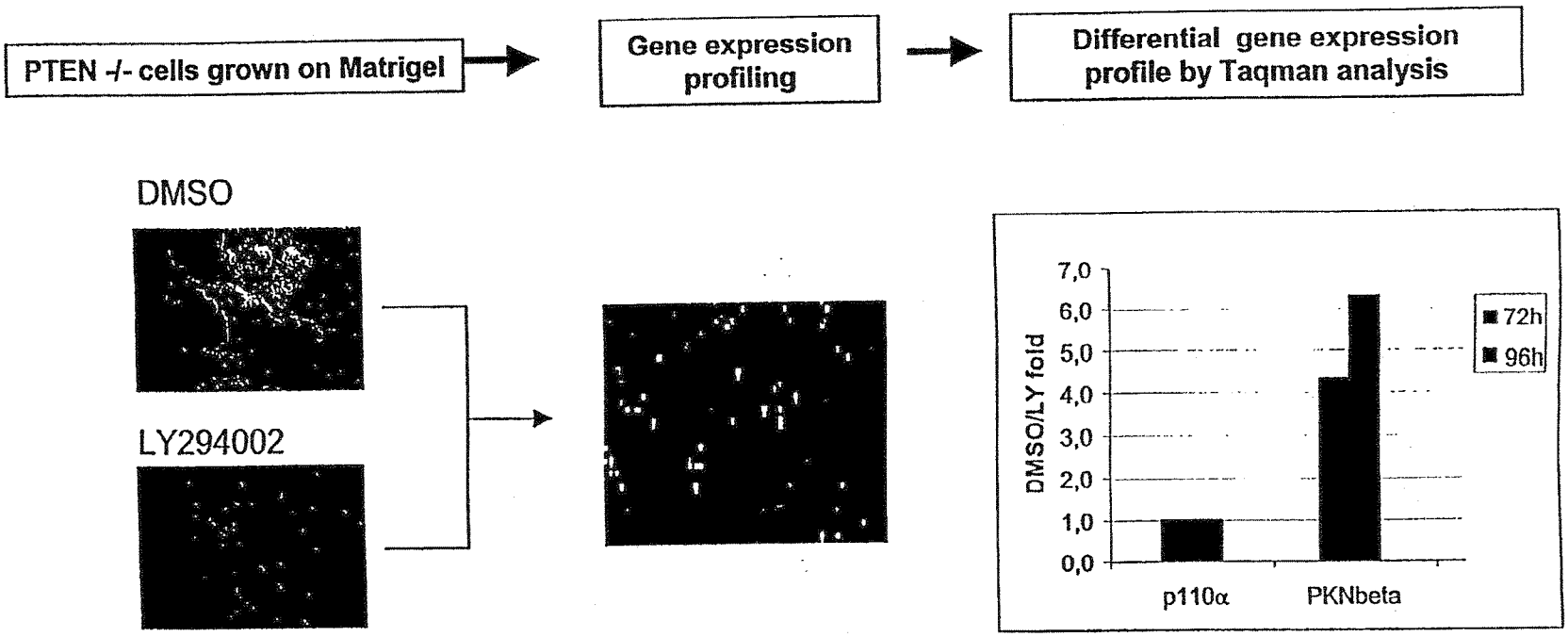

PTEN -/- cells grown on Matrigel
Gene expression profiling
Differential gene expression profile by Taqman analysis
DMSO
LY294002
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
DMSO/LY fold
72h
96h
p110α
PKNbeta
RNA isolated from differentially treated PC3 cells
Analyzed on Affymetrix chips
Verification of differential expression using Real-Time PCR

Fig. 3

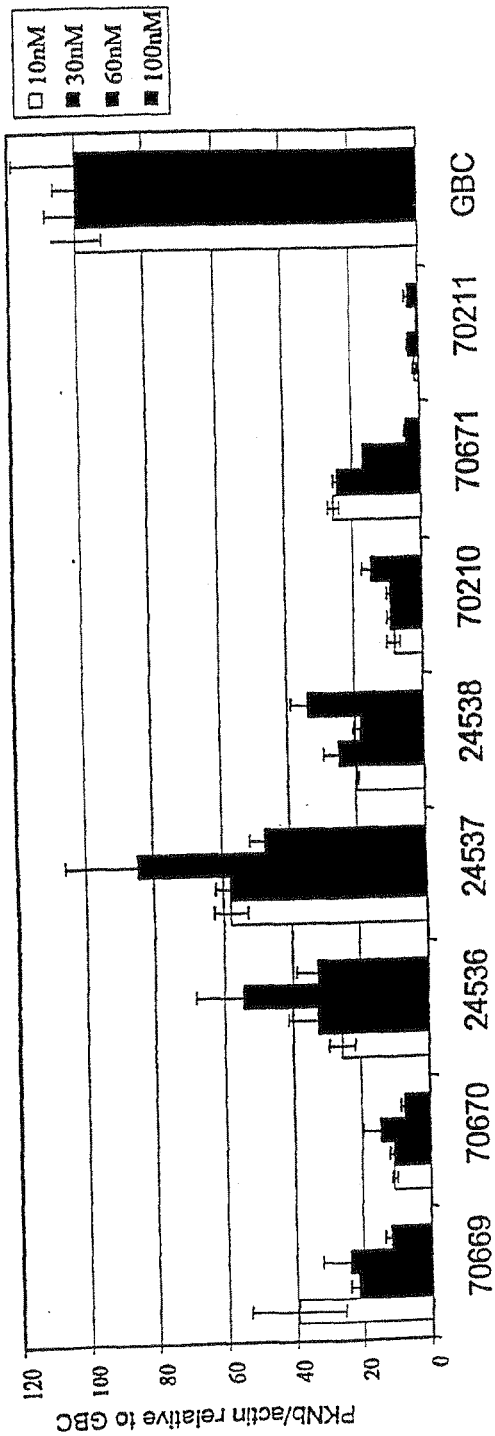
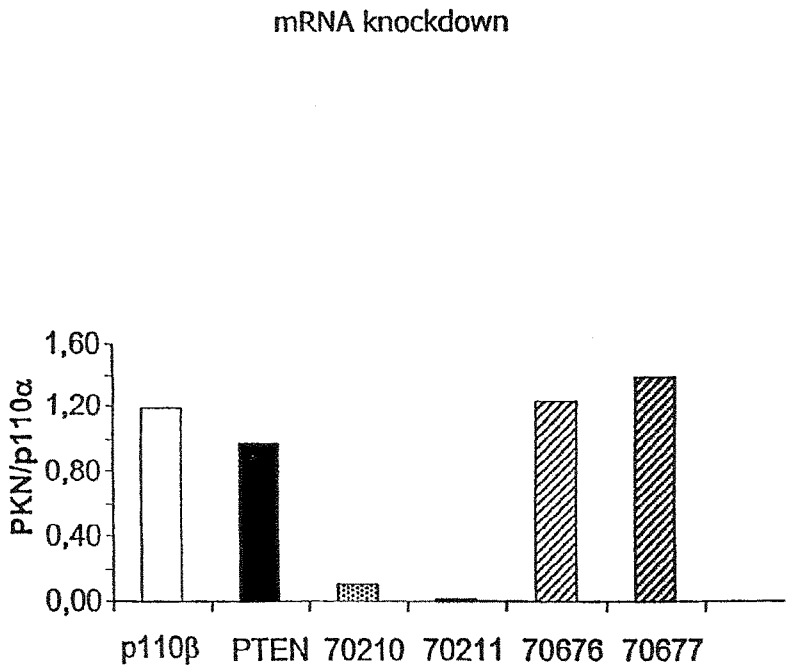

10nM
30nM
60nM
100nM
PKNb/actin relative to GBC
120
100
80
60
40
20
0
70669
70670
24536
24537
24538
70210
70671
70211
GBC

Fig. 4


mRNA knockdown
PKN/p110α
1,60
1,20
0,80
0,40
0,00
p110β
PTEN
70210
70211
70676
70677

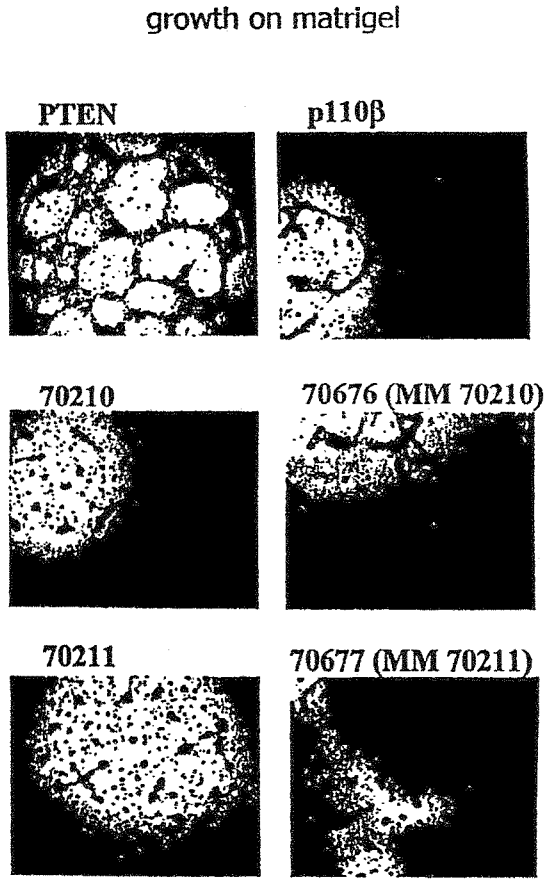

growth on matrigel
PTEN
p110β
70210
70676 (MM 70210)
70211
70677 (MM 70211)

Fig. 5

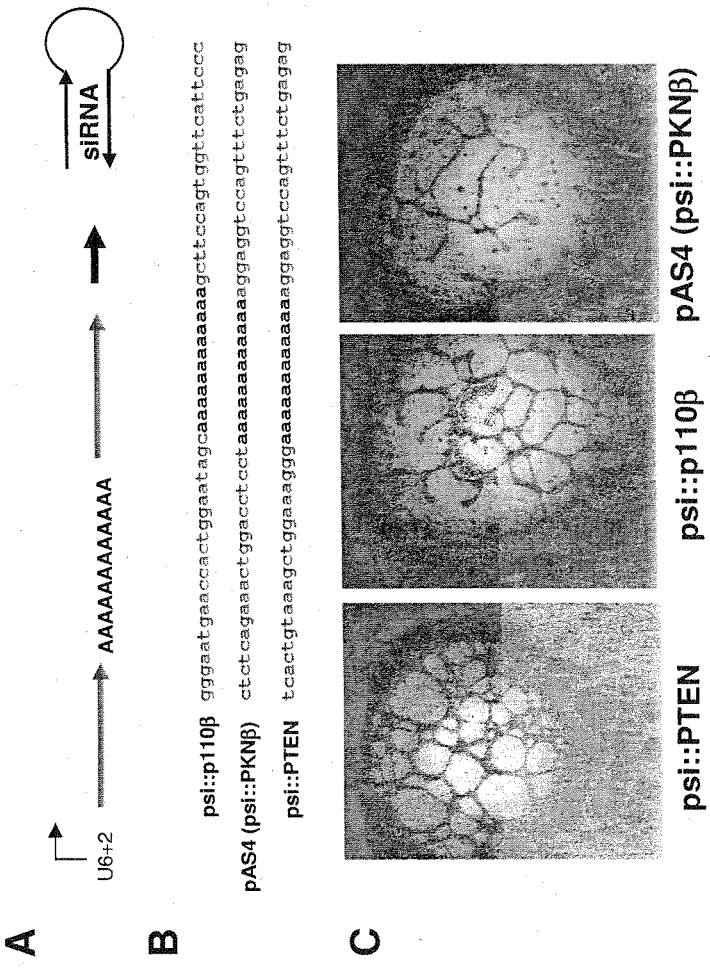

A
U6+2
AAAAAAAAAA
siRNA
B
psi::p110β gggaatgaaccactggaatagcaaaaaaaaaaaaagcttccagtggttcattccc
pAS4 (psi::PKNβ) ctctcagaaactggacctcctaaaaaaaaaaaaaaggaggtccagtttctgagag
psi::PTEN tcactgtaaagctggaaaggaaaaaaaaaaaaaggaggtccagtttctgagag
C
psi::PTEN
psi::p110β
pAS4 (psi::PKNβ)

Fig.6

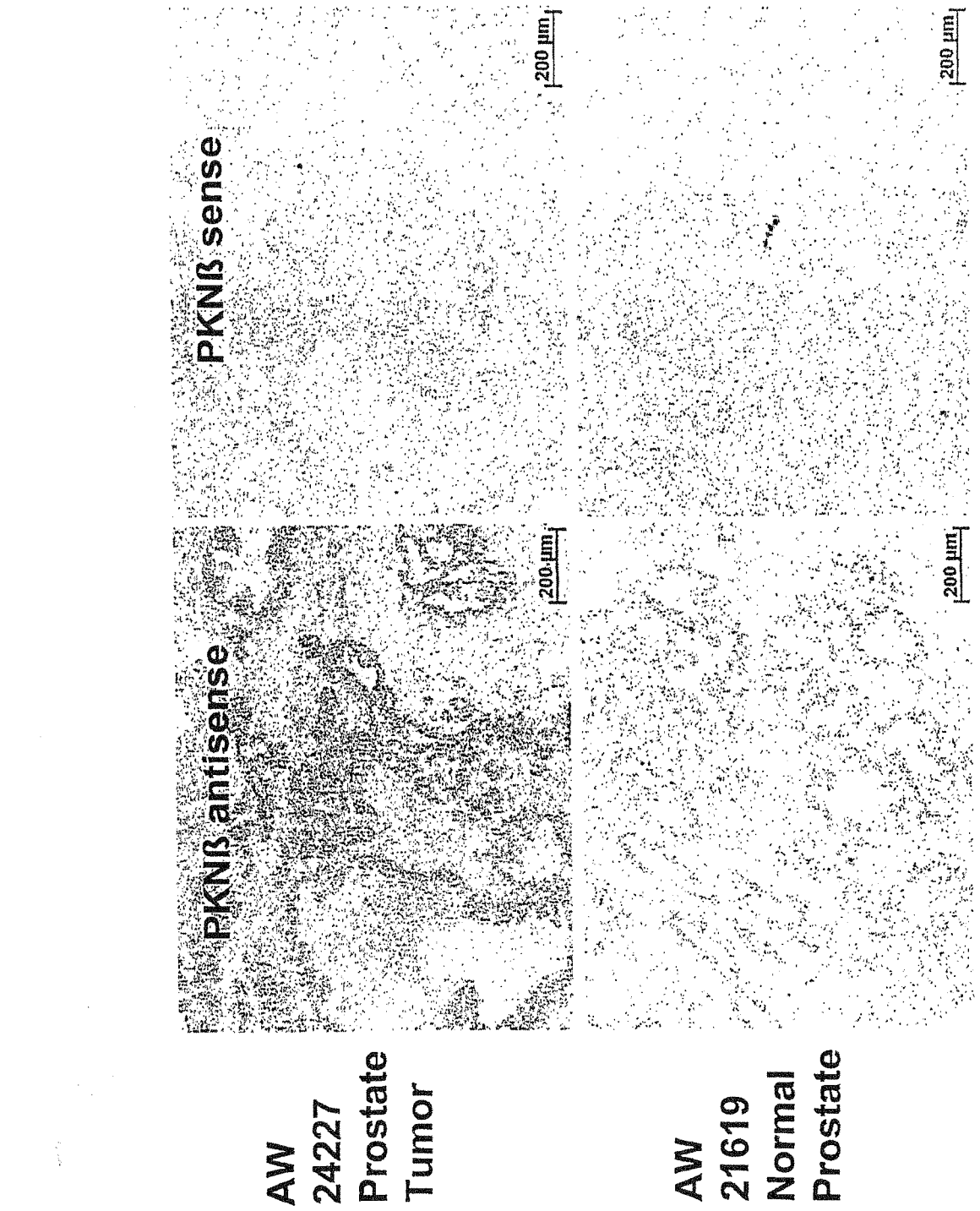
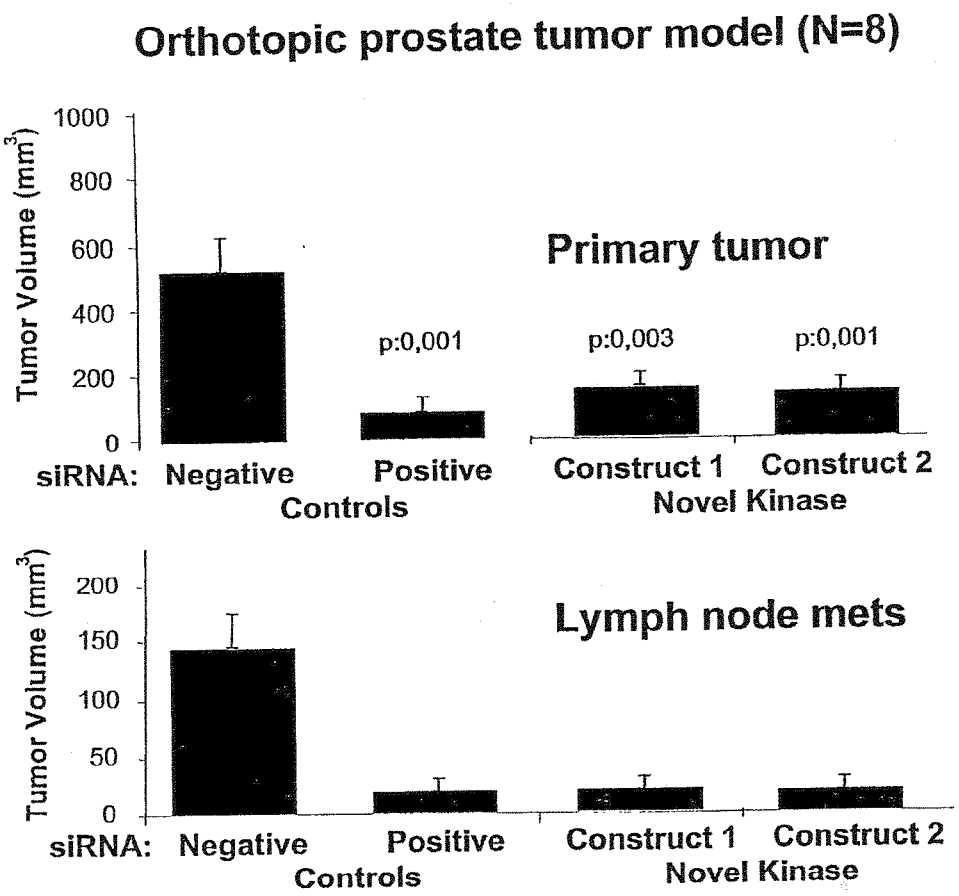

PKNß antisense
PKNß sense
AW 24227 Prostate Tumor
AW 21619 Normal Prostate
200 µm
200 µm
200 µm
200 µm

Fig. 7


Orthotopic prostate tumor model (N=8)
A
Tumor Volume (mm3)
1000
800
600
400
200
0
Primary tumor
p:0,001
p:0,003
p:0,001
siRNA: Negative
Positive
Construct 1
Construct 2
Controls
Novel Kinase
B
Tumor Volume (mm3)
200
150
100
50
0
Lymph node mets
siRNA: Negative
Positive
Construct 1
Construct 2
Controls
Novel Kinase

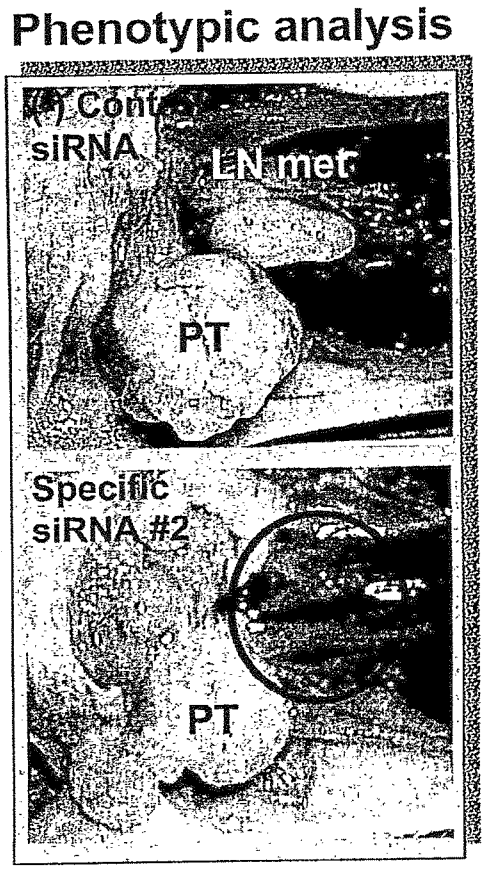

Phenotypic analysis
(-) Cont
siRNA
LN met
PT
C1
Specific
siRNA #2
PT
C2

Fig. 8

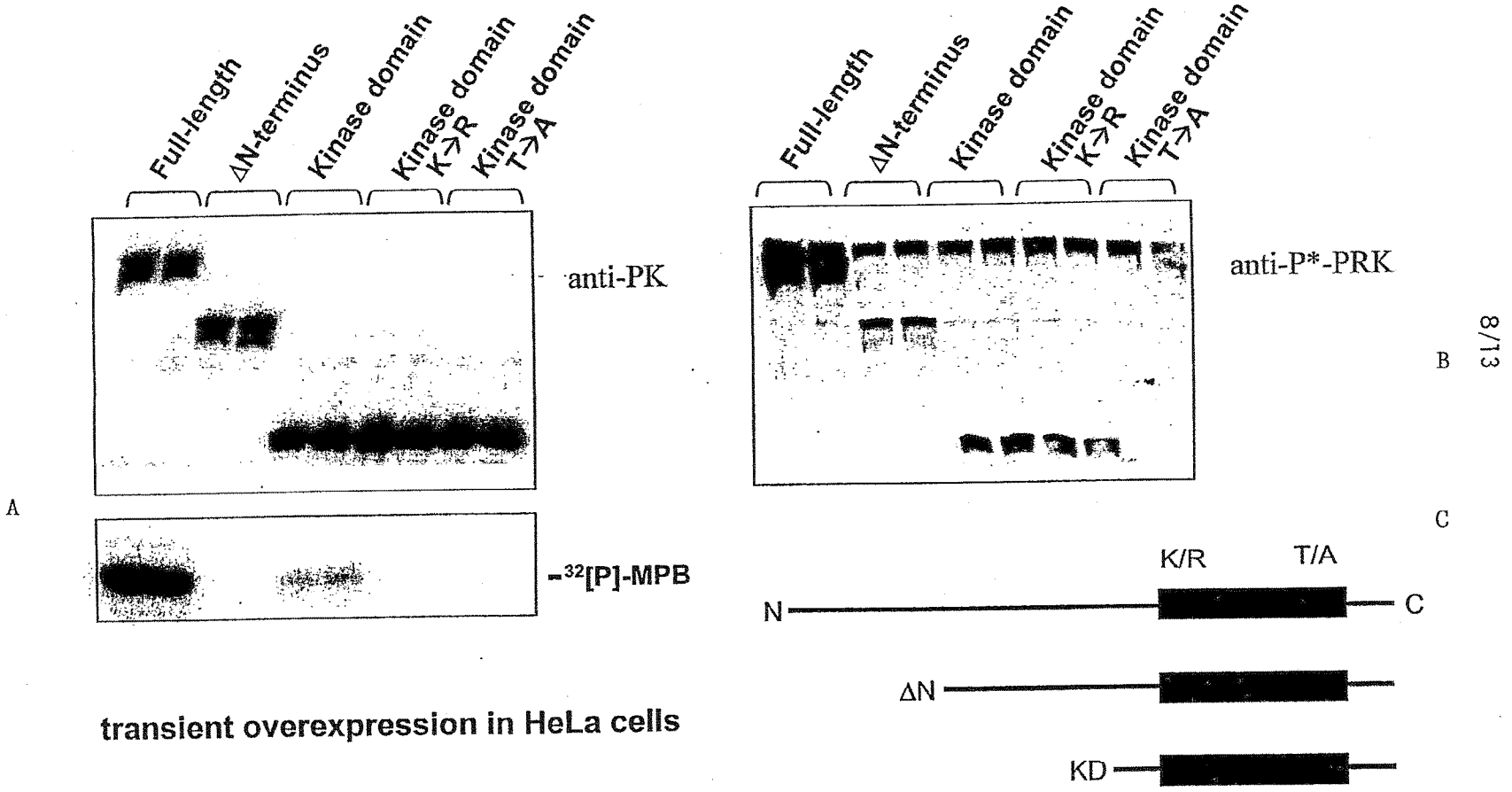
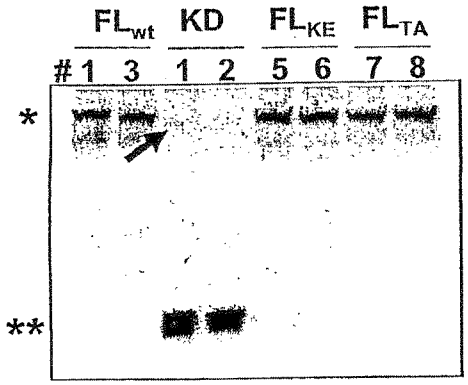
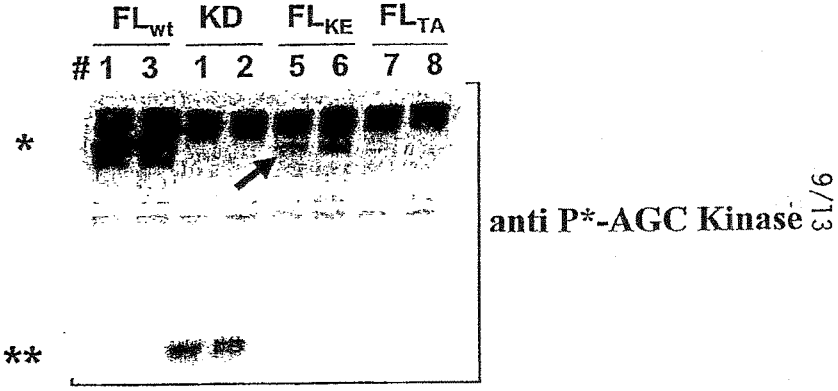

A
Full-length
ΔN-terminus
Kinase domain
Kinase domain K->R
Kinase domain T->A
anti-PK
-32[P]-MPB
transient overexpression in HeLa cells
B
Full-length
ΔN-terminus
Kinase domain
Kinase domain K->R
Kinase domain T->A
anti-P*-PRK
C
K/R
T/A
N
C
ΔN
KD

Fig. 9

A. Expression of recombinant "KINASE"
Western blot



B. In Vivo Phosphorylation of
recombinant "KINASE"



FL, Full length "KINASE"
KD, "KINASE" domain
FL_{KE}, mutation Lys→Glu in "KINASE" domain
FL_{TA}, mutation Thr→Ala in activation loop

Homology to AGC-Family of
Kinase Molecules

Fig. 10

Anti-Myc tag Immunoprecipitation/In Vitro Kinase Assay:

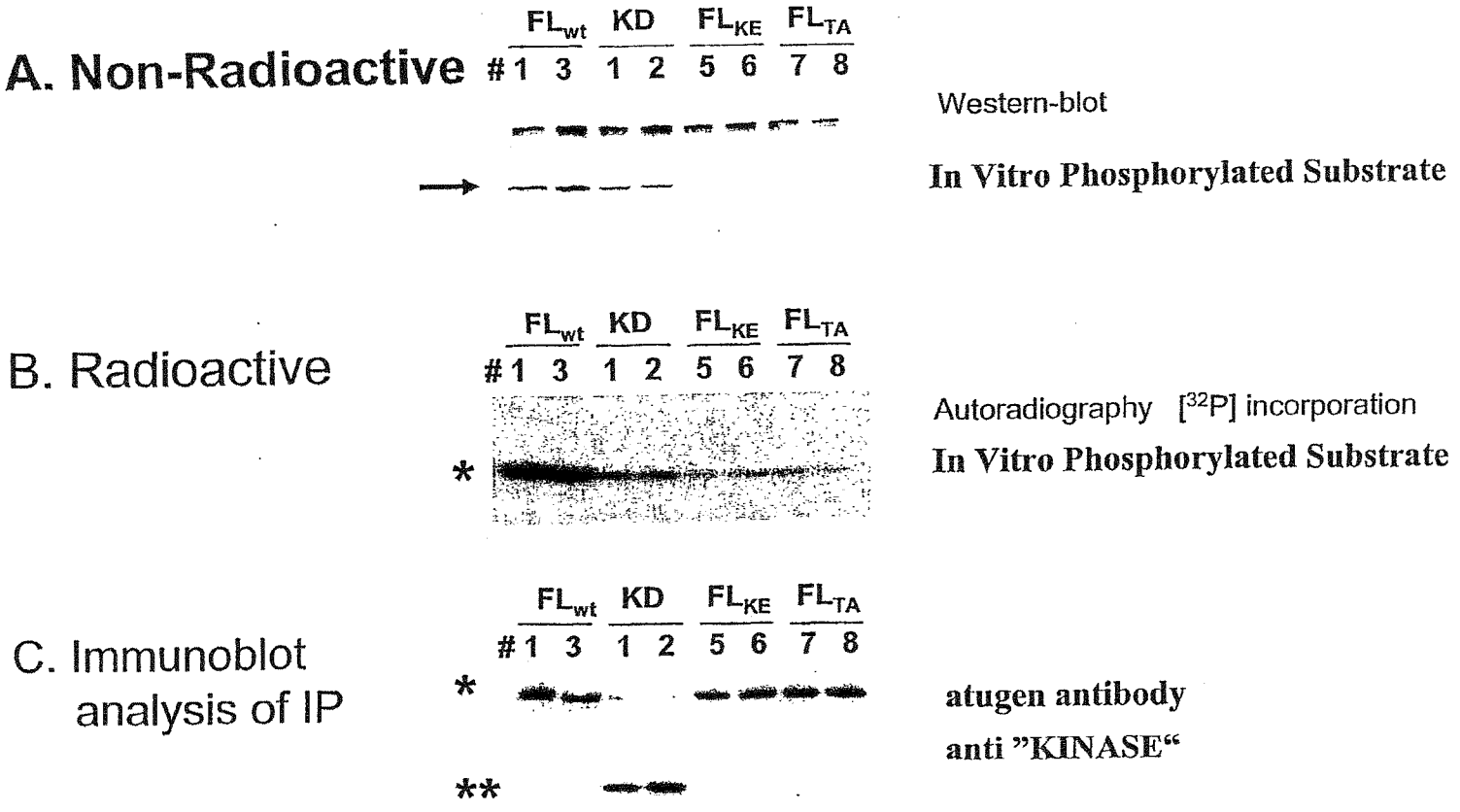


Fig. 11

PI 3-kinase-Dependent PK Expression



Fig. 12

PI 3-kinase Is Required for PK Activity

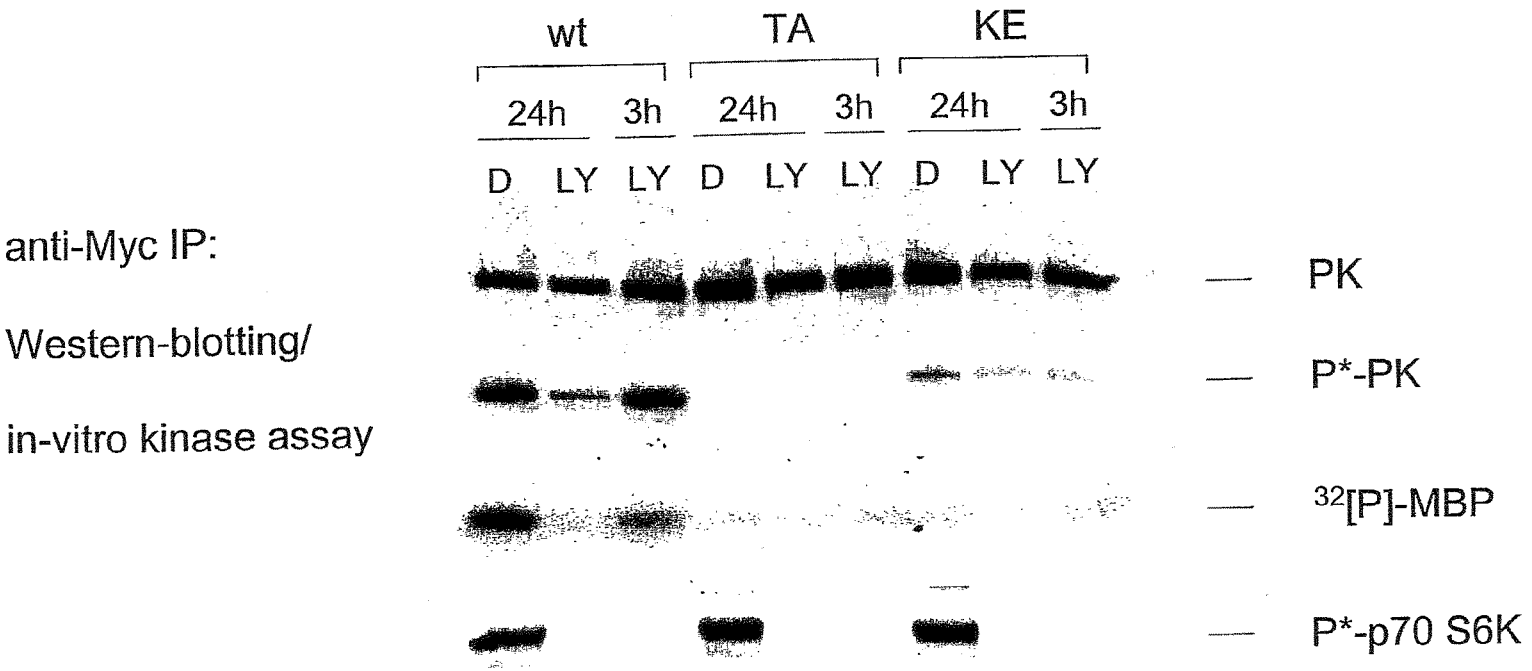


Fig. 13

Nuclear Localization of PK is Dependent on Kinase Activity and N-terminus

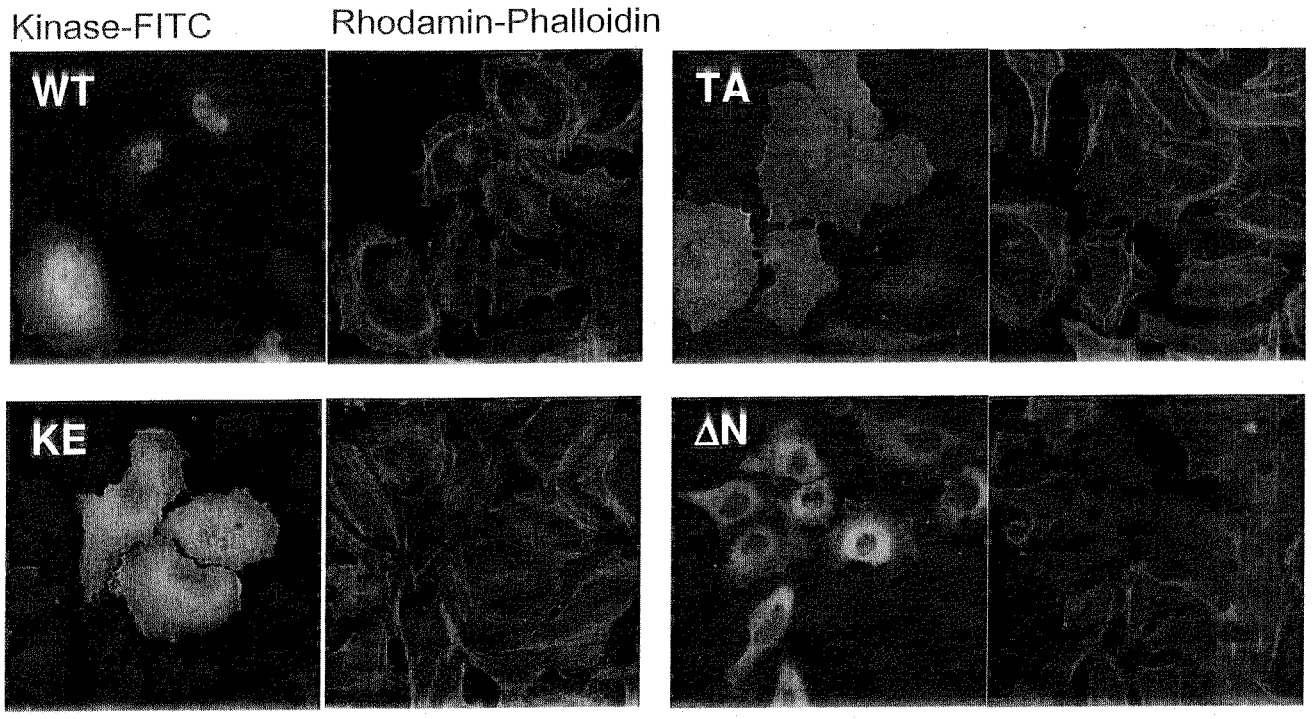


Fig. 14

SEQUENCE LISTING

<110> atugen AG

<120> Further use of protein kinase N beta

<130> SP6674A

<160> 12

<170> PatentIn version 3.1

<210> 1
<211> 889
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (1)..(889)
<223> protein kinase N beta (PKN beta)

<400> 1

Met Glu Glu Gly Ala Pro Arg Gln Pro Gly Pro Ser Gln Trp Pro Pro
1 5 10 15

Glu Asp Glu Lys Glu Val Ile Arg Arg Ala Ile Gln Lys Glu Leu Lys
20 25 30

Ile Lys Glu Gly Val Glu Asn Leu Arg Arg Val Ala Thr Asp Arg Arg
35 40 45

His Leu Gly His Val Gln Gln Leu Leu Arg Ser Ser Asn Arg Arg Leu
50 55 60

Glu Gln Leu His Gly Glu Leu Arg Glu Leu His Ala Arg Ile Leu Leu
65 70 75 80

Pro Gly Pro Gly Pro Gly Pro Ala Glu Pro Val Ala Ser Gly Pro Arg
85 90 95

Pro Trp Ala Glu Gln Leu Arg Ala Arg His Leu Glu Ala Leu Arg Arg
100 105 110

Gln Leu His Val Glu Leu Lys Val Lys Gln Gly Ala Glu Asn Met Thr
115 120 125

His Thr Cys Ala Ser Gly Thr Pro Lys Glu Arg Lys Leu Leu Ala Ala
130 135 140

Ala Gln Gln Met Leu Arg Asp Ser Gln Leu Lys Val Ala Leu Leu Arg
145 150 155 160

Met Lys Ile Ser Ser Leu Glu Ala Ser Gly Ser Pro Glu Pro Gly Pro
165 170 175

Glu Leu Leu Ala Glu Glu Leu Gln His Arg Leu His Val Glu Ala Ala
180 185 190

Val Ala Glu Gly Ala Lys Asn Val Val Lys Leu Leu Ser Ser Arg Arg
195 200 205

Thr Gln Asp Arg Lys Ala Leu Ala Glu Ala Gln Ala Gln Leu Gln Glu
210 215 220

Ser Ser Gln Lys Leu Asp Leu Leu Arg Leu Ala Leu Glu Gln Leu Leu
225 230 235 240

Glu Gln Leu Pro Pro Ala His Pro Leu Arg Ser Arg Val Thr Arg Glu
245 250 255

Leu Arg Ala Ala Val Pro Gly Tyr Pro Gln Pro Ser Gly Thr Pro Val
260 265 270

Lys Pro Thr Ala Leu Thr Gly Thr Leu Gln Val Arg Leu Leu Gly Cys
275 280 285

Glu Gln Leu Leu Thr Ala Val Pro Gly Arg Ser Pro Ala Ala Ala Leu
290 295 300

Ala Ser Ser Pro Ser Glu Gly Trp Leu Arg Thr Lys Ala Lys His Gln
305 310 315 320

Arg Gly Arg Gly Glu Leu Ala Ser Glu Val Leu Ala Val Leu Lys Val
325 330 335

Asp Asn Arg Val Val Gly Gln Thr Gly Trp Gly Gln Val Ala Glu Gln
340 345 350

Ser Trp Asp Gln Thr Phe Val Ile Pro Leu Glu Arg Ala Arg Glu Leu
355 360 365

Glu Ile Gly Val His Trp Arg Asp Trp Arg Gln Leu Cys Gly Val Ala
370 375 380

Phe Leu Arg Leu Glu Asp Phe Leu Asp Asn Ala Cys His Gln Leu Ser
385 390 395 400

Leu Ser Leu Val Pro Gln Gly Leu Leu Phe Ala Gln Val Thr Phe Cys
405 410 415

Asp Pro Val Ile Glu Arg Arg Pro Arg Leu Gln Arg Gln Glu Arg Ile
420 425 430

Phe Ser Lys Arg Arg Gly Gln Asp Phe Leu Arg Arg Ser Gln Met Asn
435 440 445

Leu Gly Met Ala Ala Trp Gly Arg Leu Val Met Asn Leu Leu Pro Pro
450 455 460

Cys Ser Ser Pro Ser Thr Ile Ser Pro Pro Lys Gly Cys Pro Arg Thr
465 470 475 480

Pro Thr Thr Leu Arg Glu Ala Ser Asp Pro Ala Thr Pro Ser Asn Phe
485 490 495

Leu Pro Lys Lys Thr Pro Leu Gly Glu Glu Met Thr Pro Pro Pro Lys
500 505 510

Pro Pro Arg Leu Tyr Leu Pro Gln Glu Pro Thr Ser Glu Glu Thr Pro
515 520 525

Arg Thr Lys Arg Pro His Met Glu Pro Arg Thr Arg Arg Gly Pro Ser
530 535 540

Pro Pro Ala Ser Pro Thr Arg Lys Pro Pro Arg Leu Gln Asp Phe Arg
545 550 555 560

Cys Leu Ala Val Leu Gly Arg Gly His Phe Gly Lys Val Leu Leu Val
565 570 575

Gln Phe Lys Gly Thr Gly Lys Tyr Tyr Ala Ile Lys Ala Leu Lys Lys
580 585 590

Gln Glu Val Leu Ser Arg Asp Glu Ile Glu Ser Leu Tyr Cys Glu Lys
595 600 605

Arg Ile Leu Glu Ala Val Gly Cys Thr Gly His Pro Phe Leu Leu Ser
610 615 620

Leu Leu Val Cys Phe Gln Thr Ser Ser His Ala Arg Phe Val Thr Glu
625 630 635 640

Phe Val Pro Gly Gly Asp Leu Met Met Gln Ile His Glu Asp Val Phe
645 650 655

Pro Glu Pro Gln Ala Arg Phe Tyr Val Ala Cys Val Val Leu Gly Leu
660 665 670

```
Gln Phe Leu His Glu Lys Lys Ile Ile Tyr Arg Asp Leu Lys Leu Asp
        675                 680                 685

Asn Leu Leu Leu Asp Ala Gln Gly Phe Leu Lys Ile Ala Asp Phe Gly
    690                 695                 700

Leu Cys Lys Glu Gly Ile Gly Phe Gly Asp Arg Thr Ser Thr Phe Cys
705                 710                 715                 720

Gly Thr Pro Glu Phe Leu Ala Pro Glu Val Leu Thr Gln Glu Ala Tyr
                725                 730                 735

Thr Gln Ala Val Asp Trp Trp Ala Leu Gly Val Leu Leu Tyr Glu Met
            740                 745                 750

Leu Val Gly Glu Cys Pro Phe Pro Gly Asp Thr Glu Glu Glu Val Phe
        755                 760                 765

Asp Cys Ile Val Asn Met Asp Ala Pro Tyr Pro Gly Phe Leu Ser Val
    770                 775                 780

Gln Gly Leu Glu Phe Ile Gln Lys Leu Leu Gln Lys Cys Pro Glu Lys
785                 790                 795                 800

Arg Leu Gly Ala Gly Glu Gln Asp Ala Glu Glu Ile Lys Val Gln Pro
                805                 810                 815

Phe Phe Arg Thr Thr Asn Trp Gln Ala Leu Leu Ala Arg Thr Ile Gln
            820                 825                 830

Pro Pro Phe Val Pro Thr Leu Cys Gly Pro Ala Asp Leu Arg Tyr Phe
        835                 840                 845

Glu Gly Glu Phe Thr Gly Leu Pro Pro Ala Leu Thr Pro Pro Ala Pro
    850                 855                 860

His Ser Leu Leu Thr Ala Arg Gln Gln Ala Ala Phe Arg Asp Phe Asp
865                 870                 875                 880

Phe Val Ser Glu Arg Phe Leu Glu Pro
                885
```

<210> 2
<211> 2670
<212> DNA
<213> Homo sapiens

<220>
<221> mRNA

<222> (1)..(2670)
<223> mRNA / cDNA of PKN beta

<400> 2
atggaggagg gggcgccgcg gcagcctggg ccgagccagt ggcccccaga ggatgagaag 60

gaggtgatcc gccgggccat ccagaaagag ctgaagatca aggagggggt ggagaacctg 120

cggcgcgtgg ccacagaccg ccgccacttg ggccatgtgc agcagctgct gcggtcctcc 180

aaccgccgcc tggagcagct gcatggcgag ctgcgggagc tgcacgcccg aatcctgctg 240

cccggccctg ggcctggccc agctgagcct gtggcctcag gaccccggcc gtgggcagag 300

cagctcaggg ctcggcacct agaggctctc cggaggcagc tgcatgtgga gctgaaggtg 360

aaacaggggg ctgagaacat gacccacacg tgcgccagtg gcaccccaa ggagaggaag 420

ctccttgcag ctgcccagca gatgctgcgg gacagccagc tgaaggtggc cctgctgcgg 480

atgaagatca gcagcctgga ggccagtggg tccccggagc cagggcctga gctactggcg 540

gaggagctac agcatcgact gcacgttgag gcagcggtgg ctgagggcgc caagaacgtg 600

gtgaaactgc ttagtagccg gagaacacag gaccgcaagg cactggctga ggcccaggcc 660

cagctacagg agtcctctca gaaactggac ctcctgcgcc tggccttgga gcagctgctg 720

gagcaactgc ctcctgccca ccctttgcgc agcagagtga cccgagagtt gcgggctgcg 780

gtgcctggat acccccagcc ttcagggaca cctgtgaagc ccaccgcccc aacagggaca 840

ctgcaggtcc gcctcctggg ctgtgaacag ttgctgacag ccgtgcctgg gcgctcccca 900

gcggccgcac tggccagcag cccctccgag ggctggcttc ggaccaaggc caagcaccag 960

cgtggccgag gcgagcttgc cagtgaggtg ctggctgtgc taaaggtgga caaccgtgtt 1020

gtggggcaga cgggctgggg gcaggtggcc gaacagtcct gggaccagac ctttgtcatc 1080

ccactggagc gagcccgtga gctggagatt ggggtacact ggcgggactg gcggcagcta 1140

tgtggcgtgg ccttcctgag acttgaagac ttcctggaca atgcctgtca ccaactgtcc 1200

ctcagcctgg taccgcaggg actgcttttt gcccaggtga ccttctgcga tcctgtcatt 1260

gagaggcggc cccggctgca gaggcaggaa cgcatcttct ctaaacgcag aggccaggac 1320

ttcctgaggc gttcgcagat gaacctcggc atggcggcct gggggcgcct cgtcatgaac 1380

ctgctgcccc cctgcagctc cccgagcaca atcagccccc ctaaaggatg ccctcggacc 1440

ccaacaacac tgcgagaggc ctctgaccct gccactccca gtaatttcct gcccaagaag 1500

acccccttgg gtgaagagat gacacccccа cccaagcccc cacgcctcta cctcccccag 1560

gagccaacat ccgaggagac tccgcgcacc aaacgtcccc atatggagcc taggactcga 1620

cgtgggccat ctccaccagc ctccccccacc aggaaacccc ctcggcttca ggacttccgc 1680

tgcttagctg tgctgggccg gggacacttt gggaaggtcc tcctggtcca gttcaagggg 1740

acagggaaat actacgccat caaagcactg aagaagcagg aggtgctcag ccgggacgag 1800

atagagagcc tgtactgcga gaagcggatc ctggaggctg tgggctgcac agggcaccct 1860

ttcctgctct ccctccttgt ctgcttccag acctccagcc atgcccgctt tgtgactgag 1920

tttgtgcctg gtggtgacct catgatgcag atccacgagg atgtcttccc cgagccccag 1980

gcccgcttct acgtggcttg tgttgtcctg gggctgcagt tcttacacga gaagaagatc 2040

atttacaggg acctgaagtt ggataacctt ctgctggatg cccagggatt cctgaagatc 2100

gcagactttg gactctgcaa ggaagggatc ggcttcgggg accggactag caccttctgt 2160

ggcaccccgg agttcctggc tcccgaggtg ctgacccagg aggcatacac acaggccgtc 2220

gactggtggg cgctgggtgt gctgctctac gagatgctgg tgggtgagtg cccgttccca 2280

ggggacacag aggaagaggt gtttgactgc atcgtcaaca tggacgcccc ctaccccggc 2340

tttctgtcgg tgcaagggct tgagttcatt cagaagctcc tccagaagtg cccggagaag 2400

cgcctcgggg caggtgagca ggatgccgag gagatcaagg tccagccatt cttcaggacc 2460

accaactggc aagccctgct cgcccgcacc atccagcccc ccttcgtgcc taccctgtgt 2520

ggccctgcgg acctgcgcta ctttgagggc gagttcacag ggctgccgcc tgccctgacc 2580

ccacctgcac cccacagcct cctcactgcc cgccaacagg ccgccttccg ggacttcgac 2640

tttgtgtcag agcgattcct ggaaccctga 2670

<210> 3
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 3
ggagguccag tttctgagag g 21

<210> 4
<211> 21

<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 4
uguuucacct tcagcuccac a 21

<210> 5
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(7)
<223> RNA

<220>
<221> misc_feature
<222> (8)..(16)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (17)..(23)
<223> RNA

<400> 5
aggacaacac aagccacgua gaa 23

<210> 6
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(7)
<223> RNA

<220>
<221> misc_feature
<222> (8)..(16)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (17)..(23)
<223> RNA

<400> 6
gcucugacac aaagtcgaag ucc 23

<210> 7
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(7)
<223> RNA

<220>
<221> misc_feature
<222> (8)..(16)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (17)..(23)
<223> RNA

<400> 7
gcagucaaac acctctuccu cug 23

<210> 8
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature

<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> DNA linked through phosphorothioate linkages

<400> 8
caacacggtt gtccaccuuu a 21

<210> 9
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 9
ucagugcttt gatggcguag u 21

<210> 10
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 10
cuucucgcag tacaggcucu c 21

<210> 11
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 11
caagacgctt gtgcacguuu a 21

<210> 12
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> antisense oligonucleotide

<220>
<221> misc_feature
<222> (1)..(6)
<223> RNA

<220>
<221> misc_feature
<222> (7)..(15)
<223> DNA linked through phosphorothioate linkages

<220>
<221> misc_feature
<222> (16)..(21)
<223> RNA

<400> 12
ucagagctta gttggcguug u 21