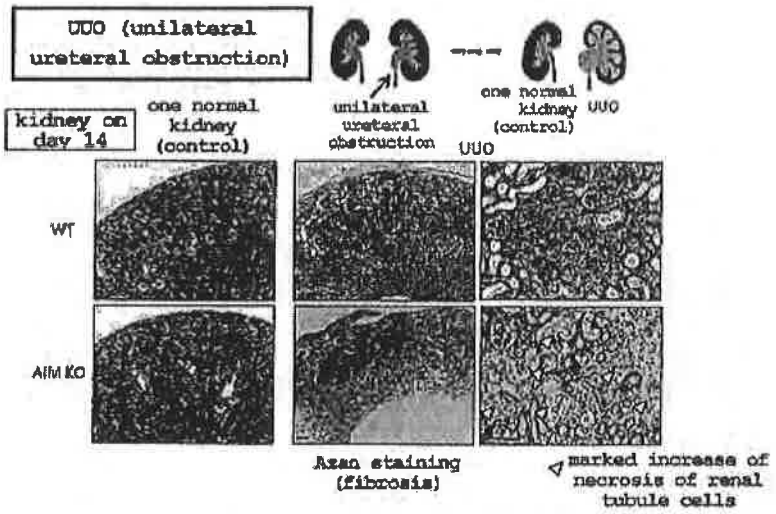
Fig. 1

A


UUO (unilateral ureteral obstruction)
kidney on day 14
one normal kidney (control)
unilateral ureteral obstruction
one normal kidney (control)
UUO
UUO
WT
AIM KO
Azan staining (fibrosis)
marked increase of necrosis of renal tubule cells

B

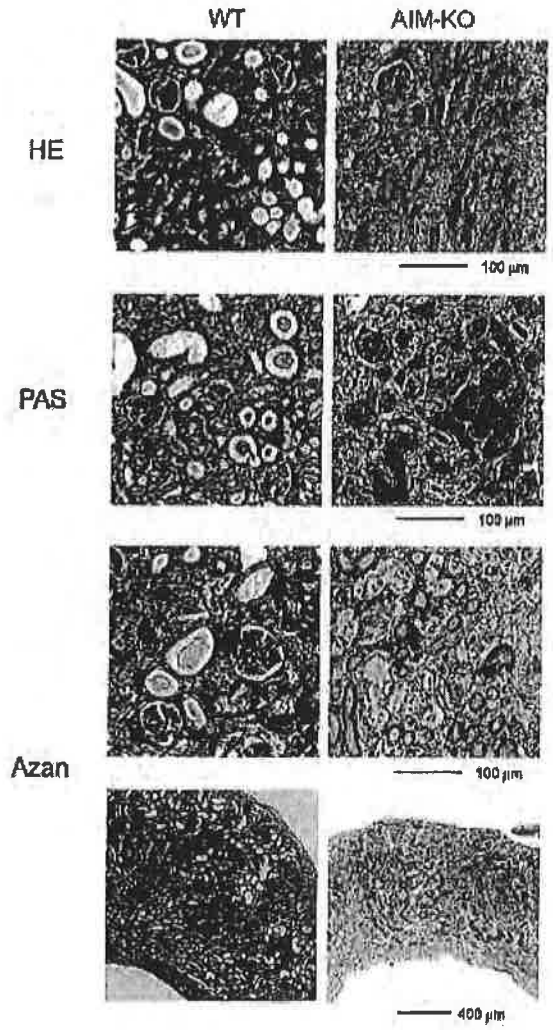

WT
AIM-KO
HE
PAS
Azan
100 μm
100 μm
100 μm
400 μm

Fig. 2

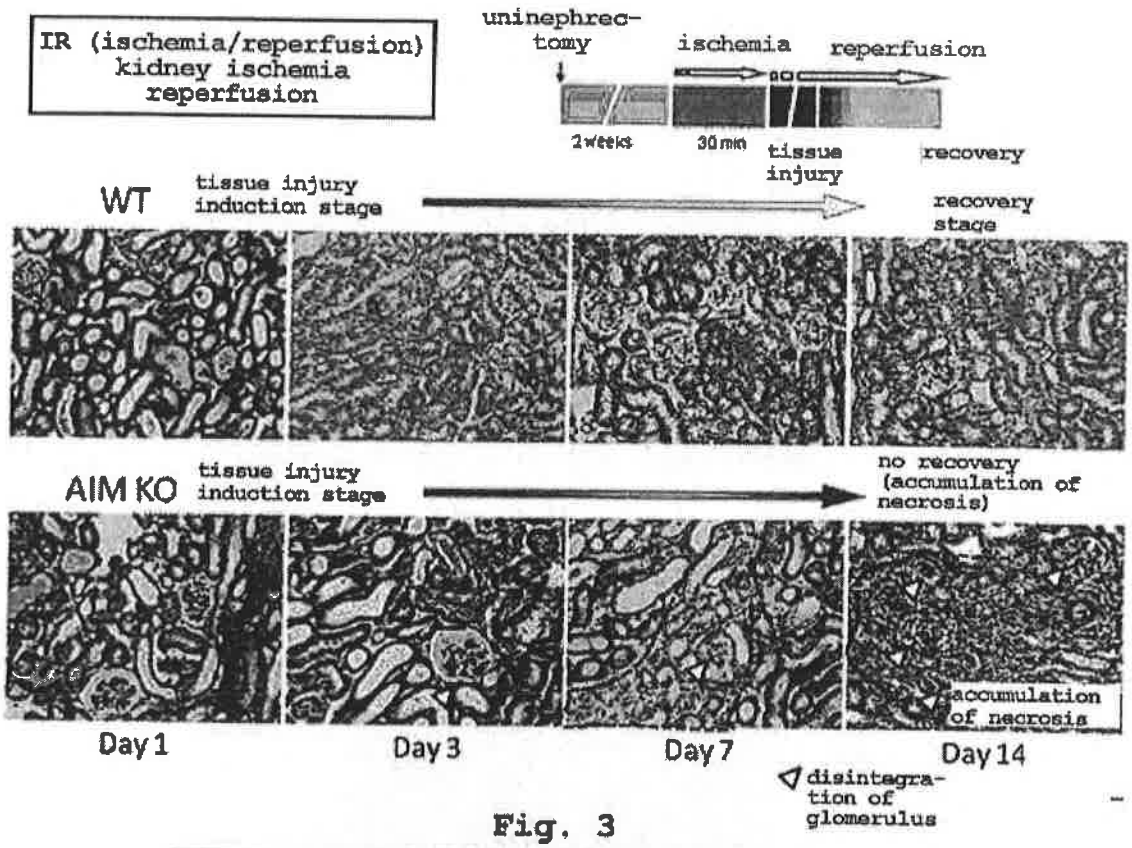
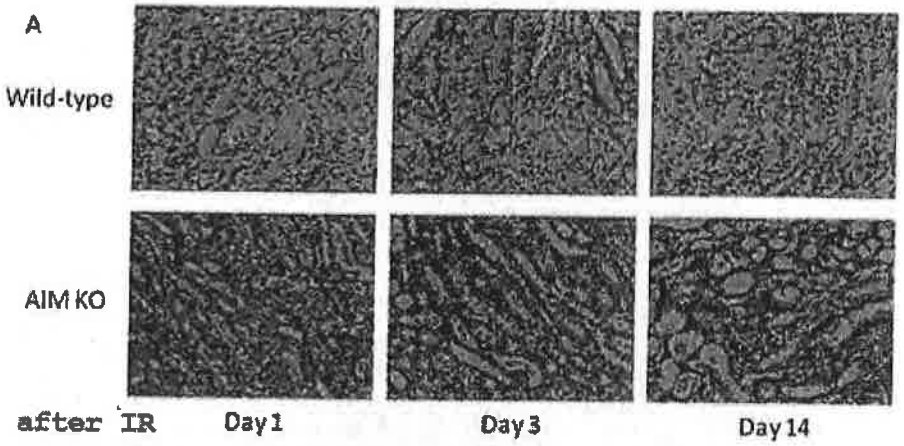

IR (ischemia/reperfusion)
kidney ischemia
reperfusion
uninephrec-
tomy
ischemia
reperfusion
2weeks
30mm
tissue
injury
recovery
WT
tissue injury
induction stage
recovery
stage
AIM KO
tissue injury
induction stage
no recovery
(accumulation of
necrosis)
accumulation
of necrosis
Day 1
Day 3
Day 7
Day 14
disintegra-
tion of
glomerulus

Fig. 3


A
Wild-type
AIM KO
after IR
Day 1
Day 3
Day 14

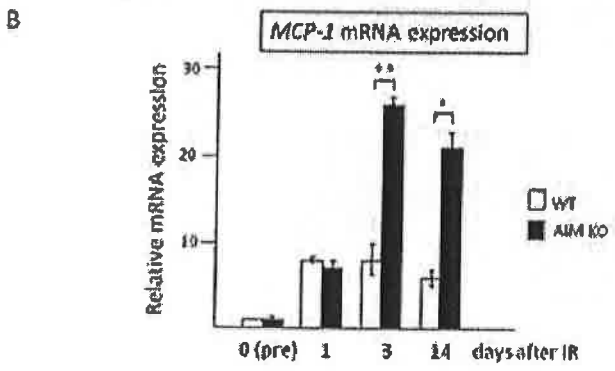
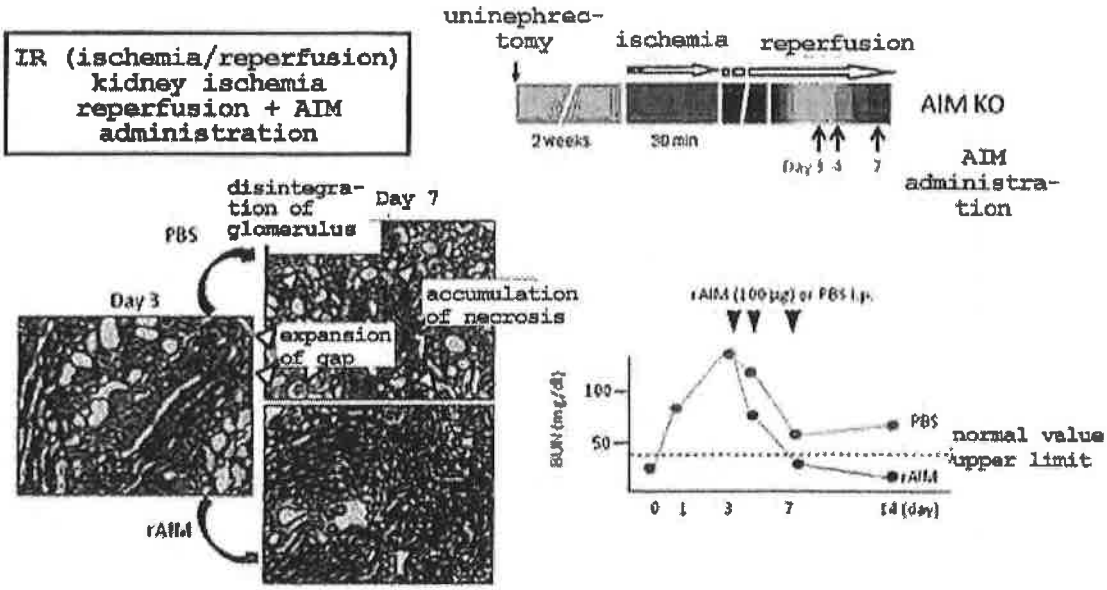

B
MCP-1 mRNA expression
Relative mRNA expression
30
20
10
0 (pre)
1
3
14
days after IR
WT
AIM KO

Fig. 4



kidney of AIM KO mouse subjected to IR

Fig. 5

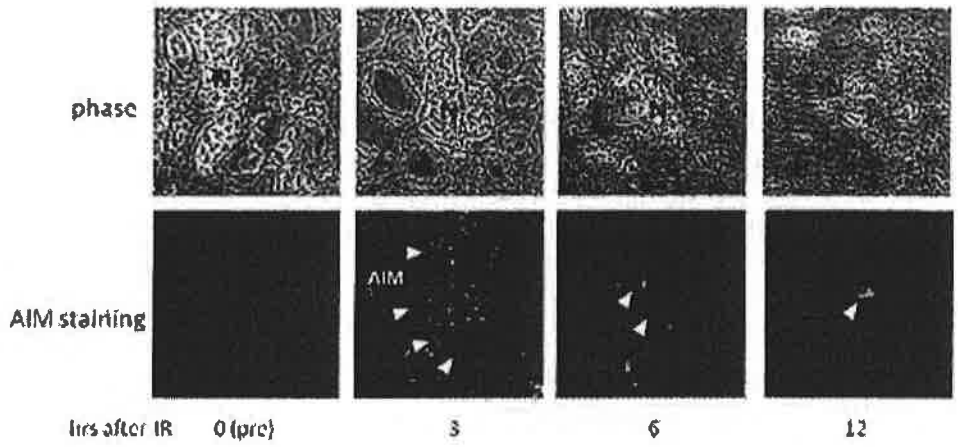


Fig. 6

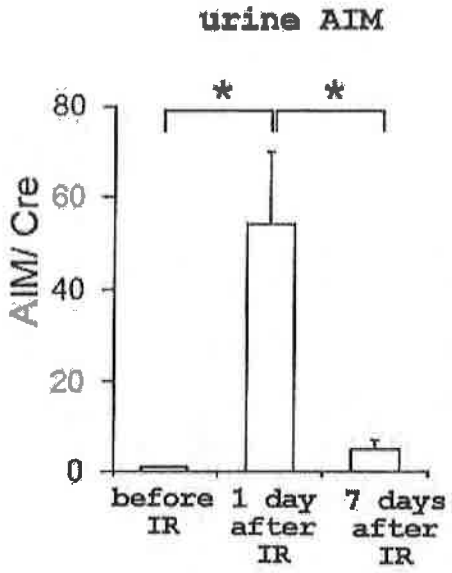

urine AIM
AIM/ Cre
80
60
40
20
0
*
*
before IR
1 day after IR
7 days after IR

Fig. 7

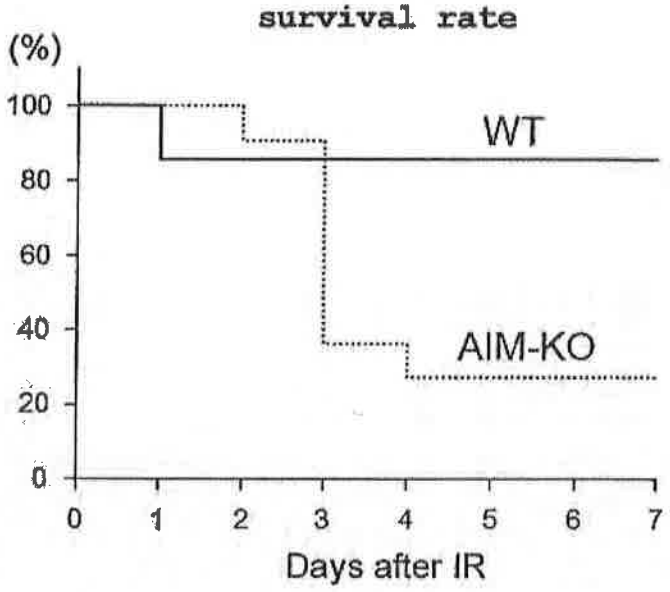

survival rate
(%)
100
80
60
40
20
0
WT
AIM-KO
0
1
2
3
4
5
6
7
Days after IR

Fig. 8

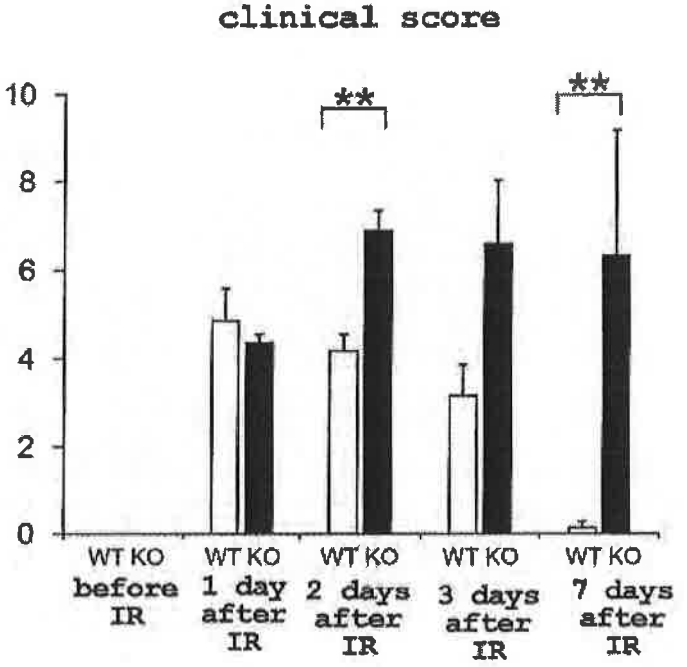

clinical score
10
8
6
4
2
0
**
**
WT KO
before IR
WT KO
1 day after IR
WT KO
2 days after IR
WT KO
3 days after IR
WT KO
7 days after IR

Fig. 9

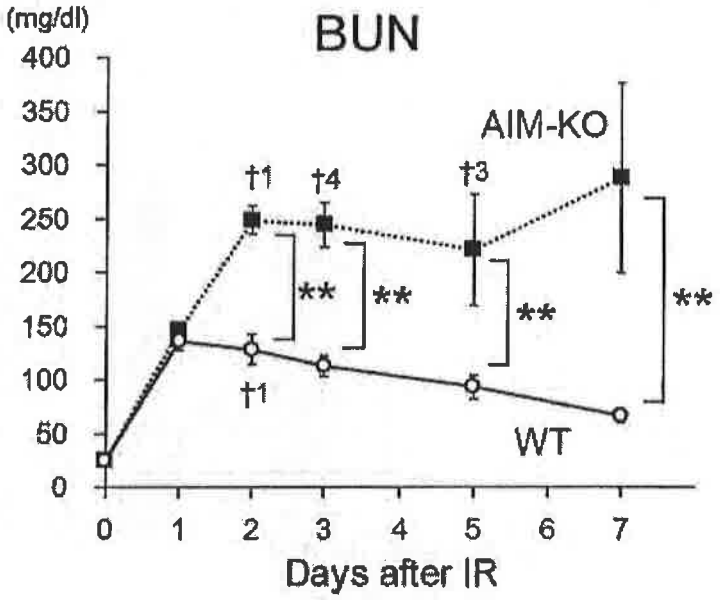

BUN
(mg/dl)
400
350
300
250
200
150
100
50
0
AIM-KO
WT
†1
†4
†3
†1
**
**
**
**
0
1
2
3
4
5
6
7
Days after IR

Fig. 10

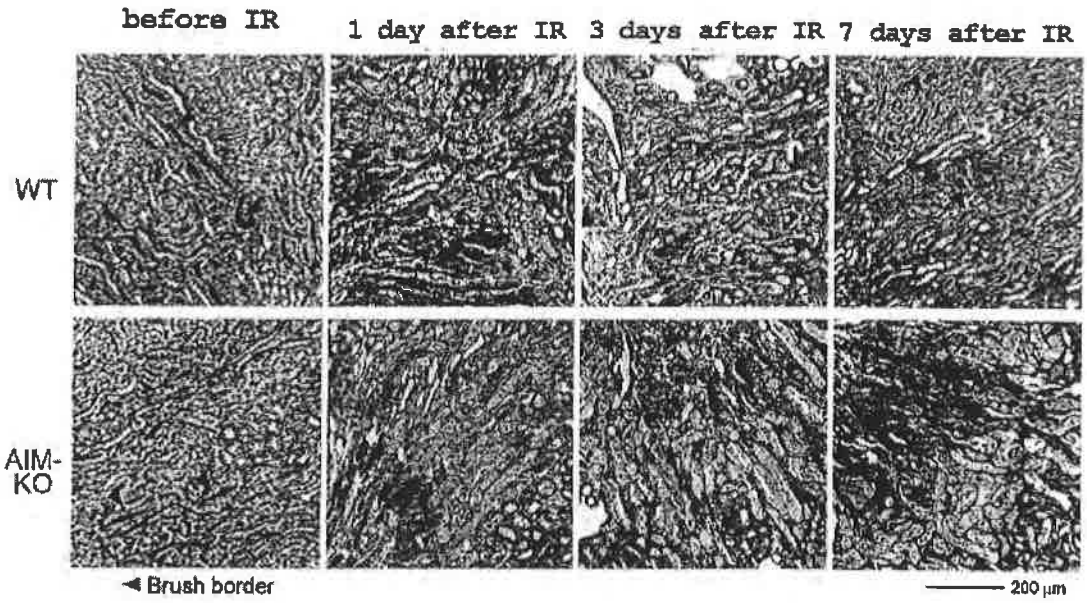

before IR
1 day after IR
3 days after IR
7 days after IR
WT
AIM-KO
Brush border
200 μm

Fig. 11

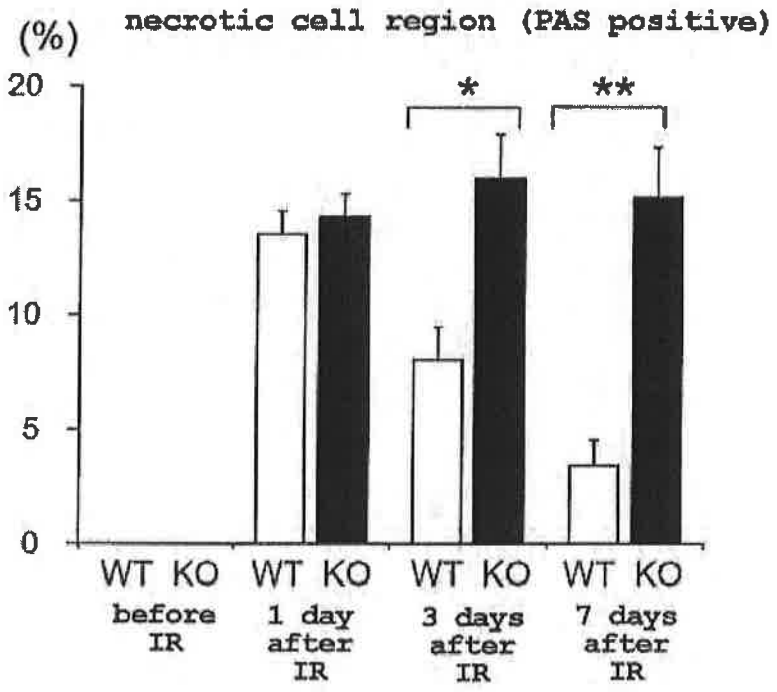

necrotic cell region (PAS positive)
(%)
20
15
10
5
0
*
**
WT KO
before IR
1 day after IR
3 days after IR
7 days after IR

Fig. 12

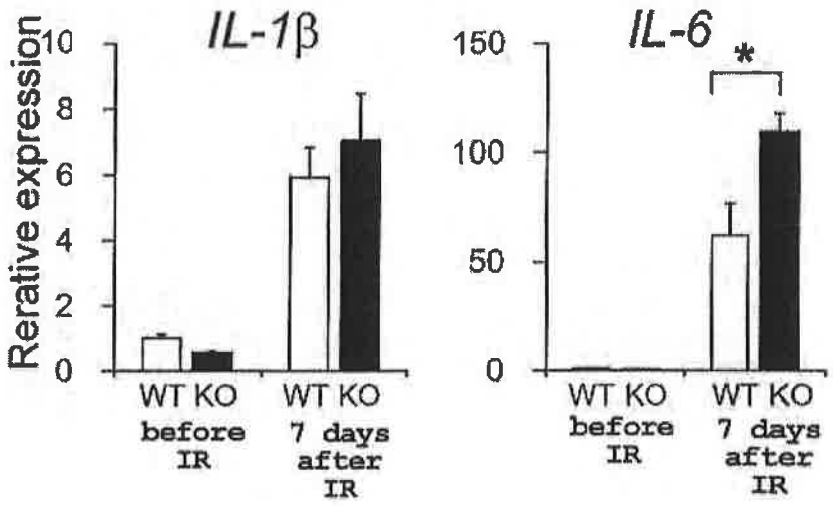

IL-1β
IL-6
Rerative expression
10
8
6
4
2
0
150
100
50
0
*
WT KO
before IR
WT KO
7 days after IR
WT KO
before IR
WT KO
7 days after IR

Fig. 13

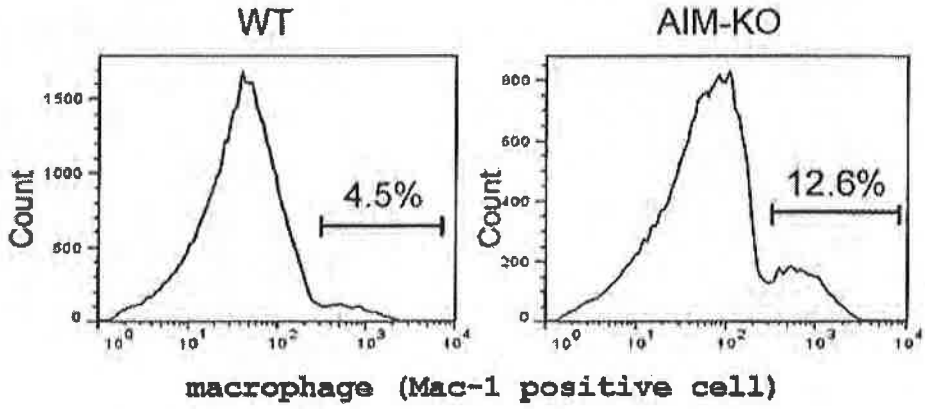
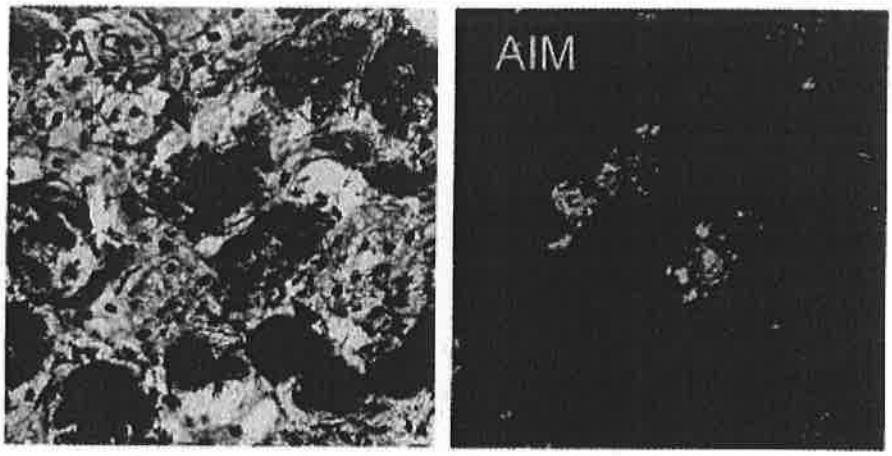

WT
AIM-KO
Count
1500
1000
500
0
4.5%
800
600
400
200
0
12.6%
macrophage (Mac-1 positive cell)

Fig. 14



◀ dead cell mass clogged in proximal renal tubule (intraluminal debris)

Fig. 15

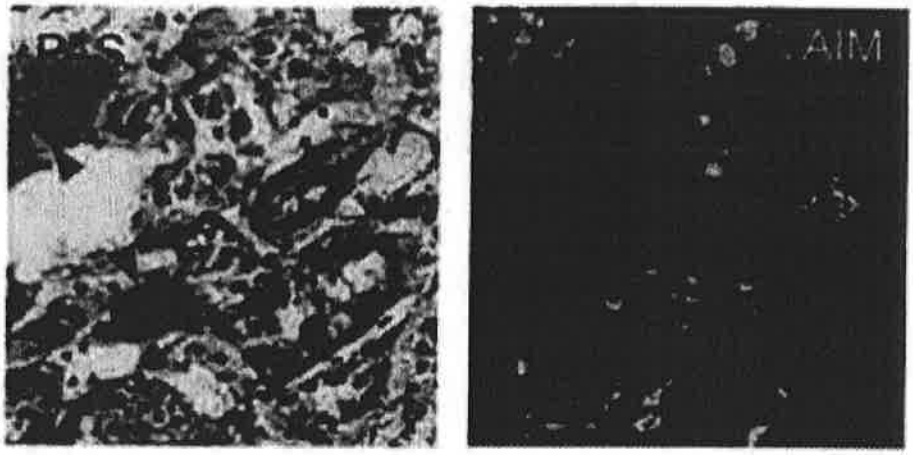


Fig. 16

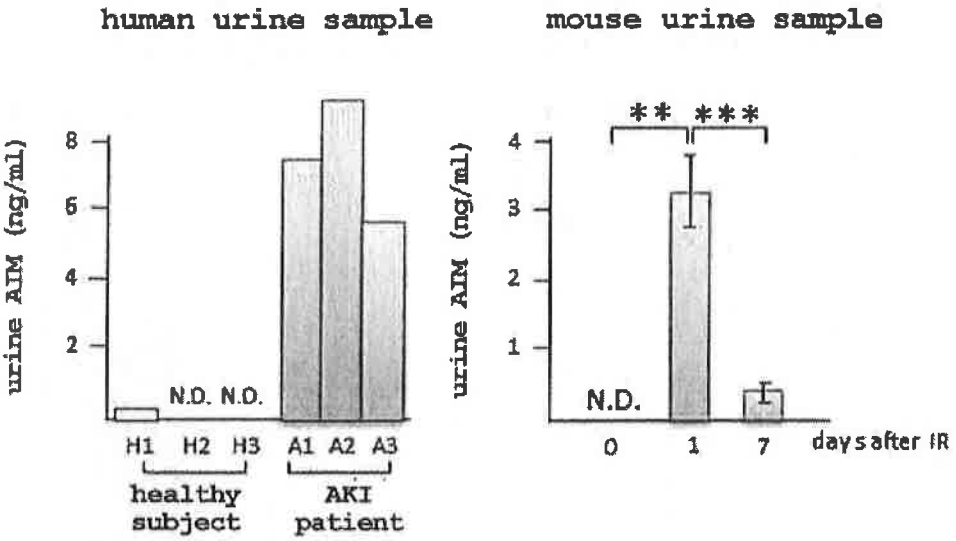

human urine sample
mouse urine sample
urine AIM (ng/ml)
8
6
4
2
N.D. N.D.
H1 H2 H3 A1 A2 A3
healthy subject
AKI patient
urine AIM (ng/ml)
4
3
2
1
** ***
N.D.
0 1 7
days after IR

Fig. 17

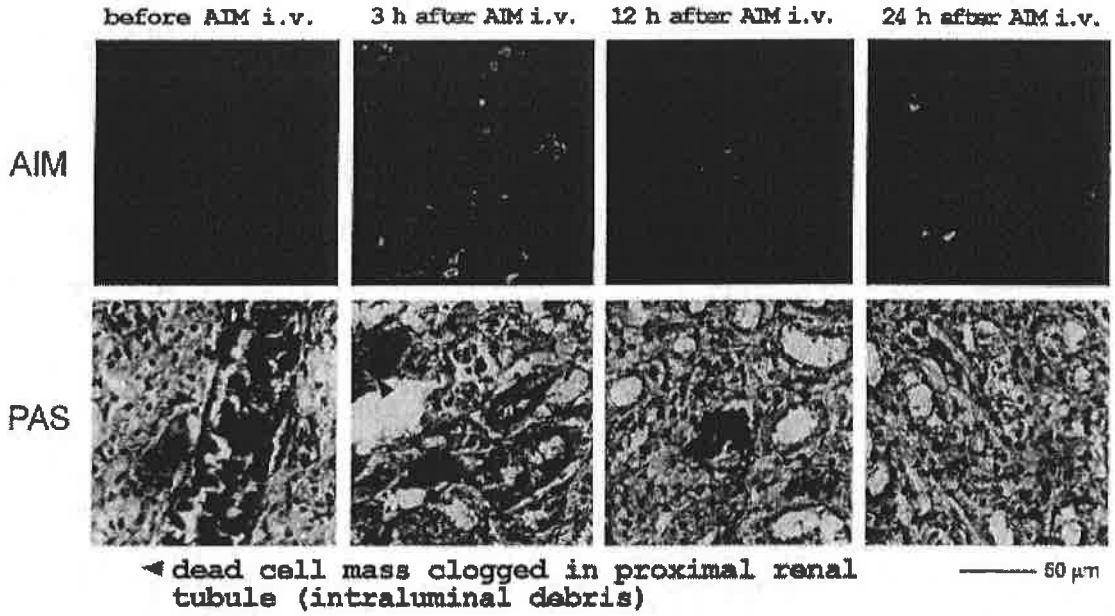

before AIM i.v.
3 h after AIM i.v.
12 h after AIM i.v.
24 h after AIM i.v.
AIM
PAS
dead cell mass clogged in proximal renal tubule (intraluminal debris)
50 μm

Fig. 18

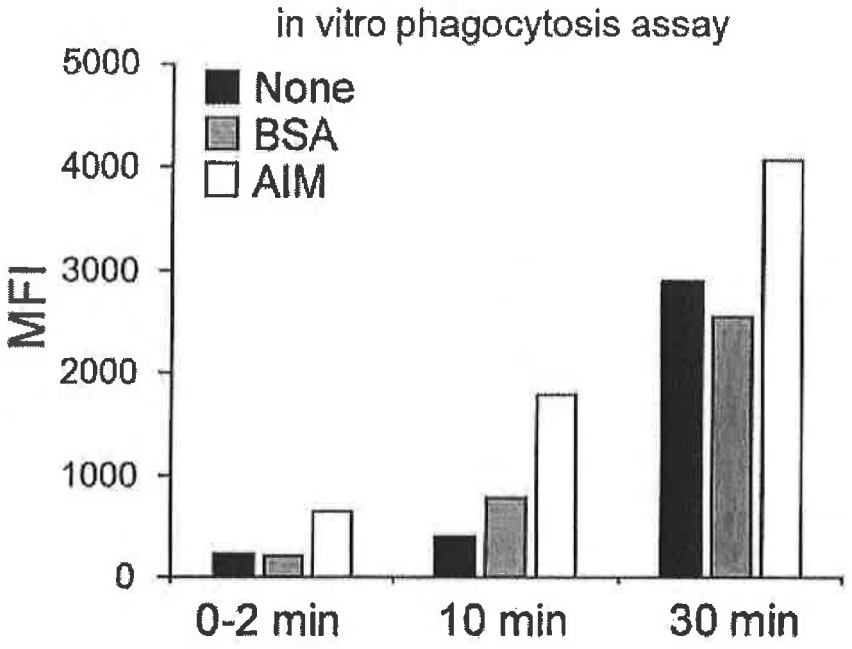

in vitro phagocytosis assay
5000
4000
3000
2000
1000
0
MFI
None
BSA
AIM
0-2 min
10 min
30 min

Fig. 19

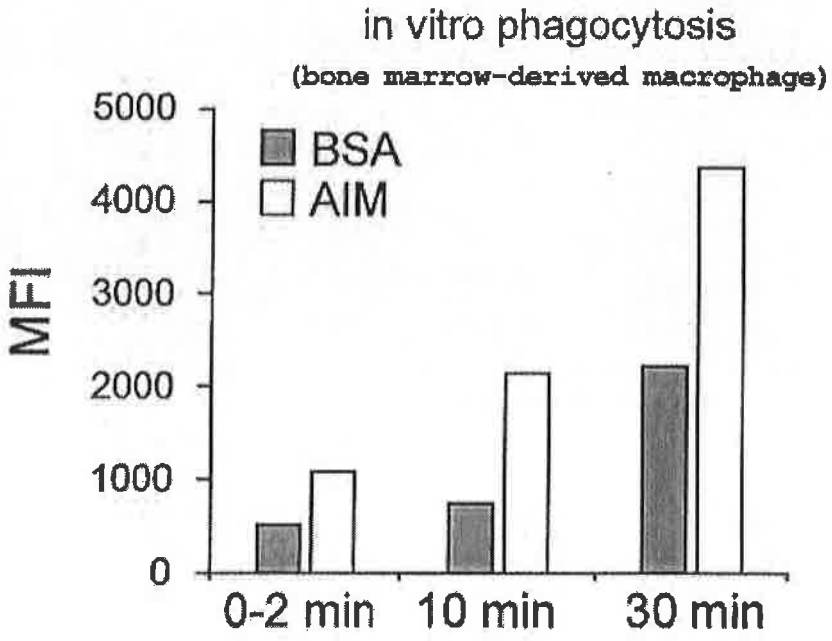

in vitro phagocytosis
(bone marrow-derived macrophage)
5000
4000
3000
2000
1000
0
MFI
BSA
AIM
0-2 min
10 min
30 min

Fig. 20

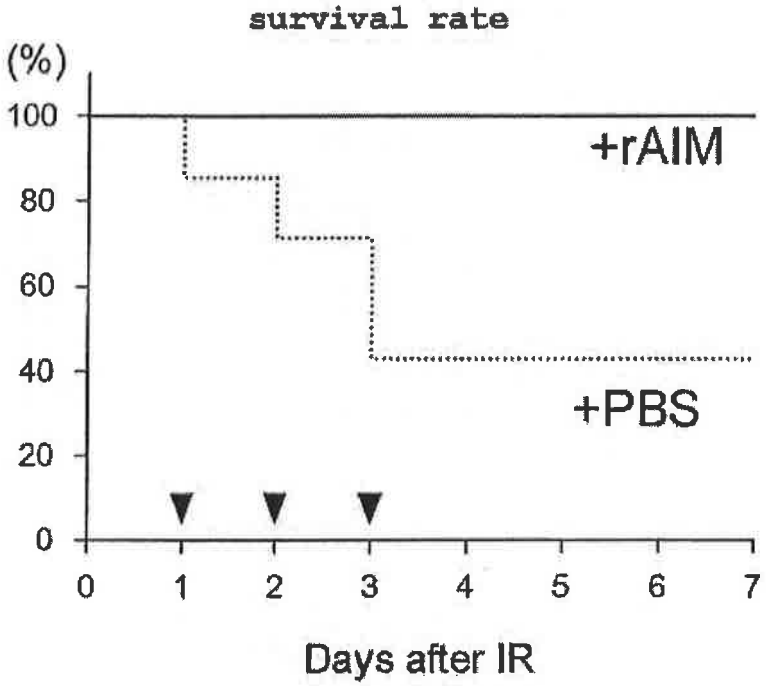

survival rate
(%)
100
80
60
40
20
0
+rAIM
+PBS
0
1
2
3
4
5
6
7
Days after IR

Fig. 21

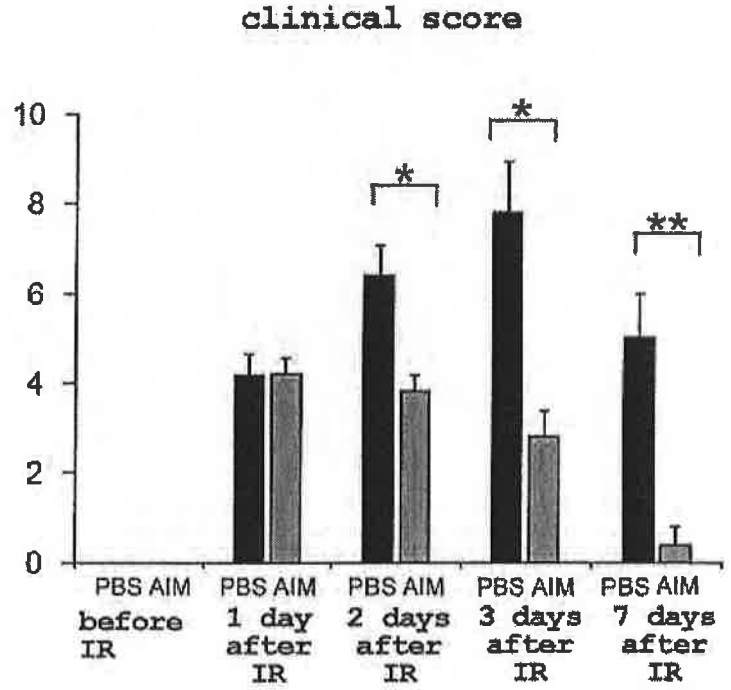

clinical score
10
8
6
4
2
0
*
*
**
PBS AIM
before IR
PBS AIM
1 day after IR
PBS AIM
2 days after IR
PBS AIM
3 days after IR
PBS AIM
7 days after IR

Fig. 22

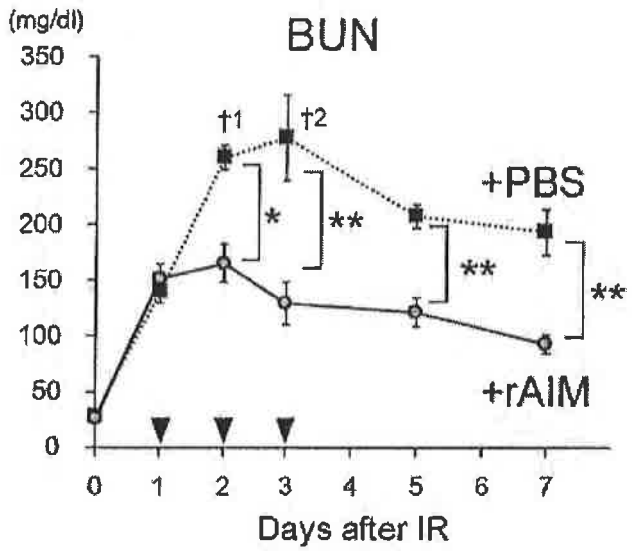

(mg/dl)
BUN
350
300
250
200
150
100
50
0
†1
†2
+PBS
+rAIM
*
**
**
**
0 1 2 3 4 5 6 7
Days after IR

Fig. 23

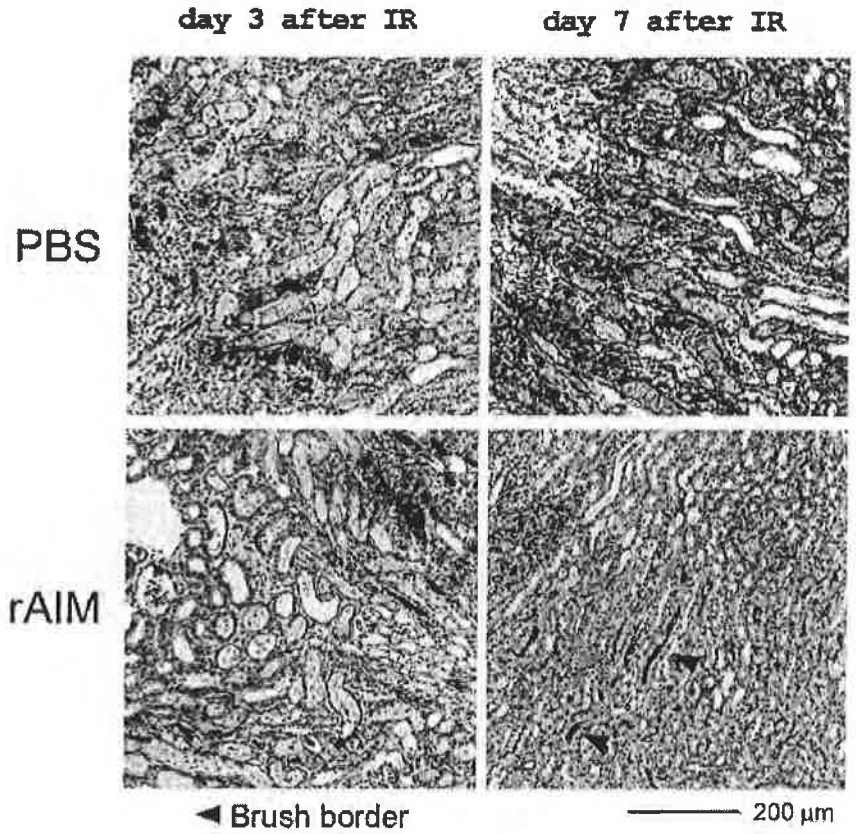

day 3 after IR
day 7 after IR
PBS
rAIM
◄ Brush border
200 μm

Fig. 24

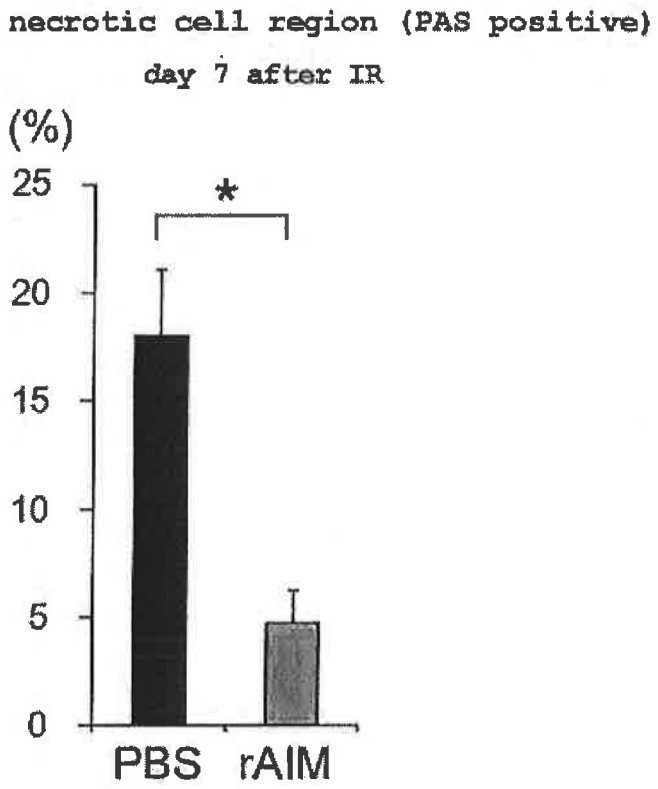

necrotic cell region (PAS positive)
day 7 after IR
(%)
25
20
15
10
5
0
*
PBS
rAIM

Fig. 25

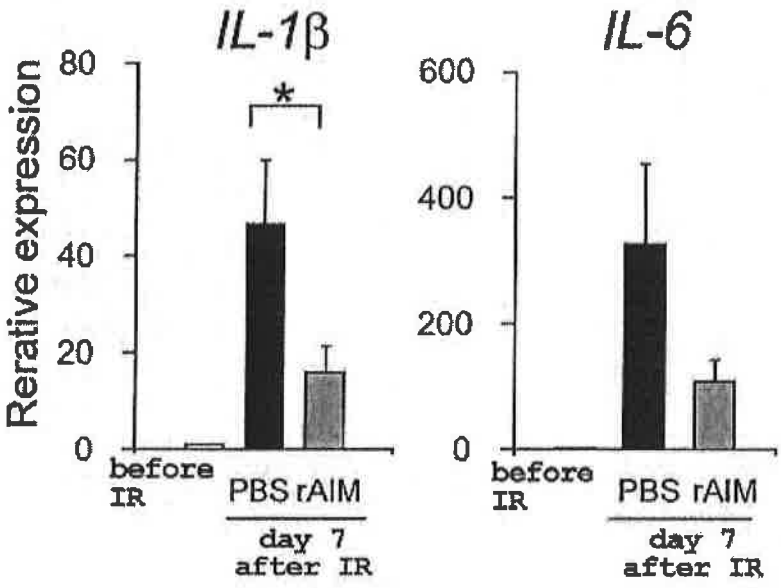

IL-1β
IL-6
Rerative expression
80
60
40
20
0
600
400
200
0
*
before IR
PBS
rAIM
day 7 after IR
before IR
PBS
rAIM
day 7 after IR

Fig. 26

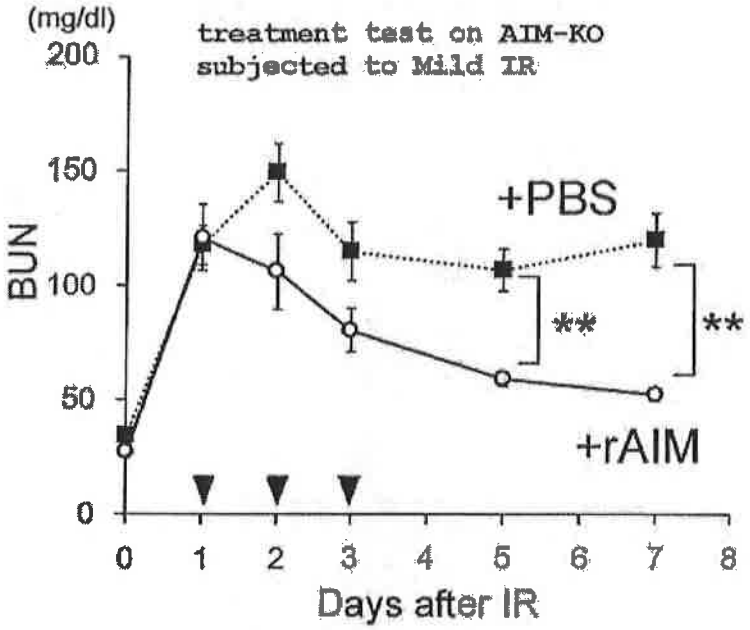

(mg/dl)
treatment test on AIM-KO
subjected to Mild IR
200
150
100
50
0
BUN
+PBS
+rAIM
**
**
0 1 2 3 4 5 6 7 8
Days after IR

Fig. 27

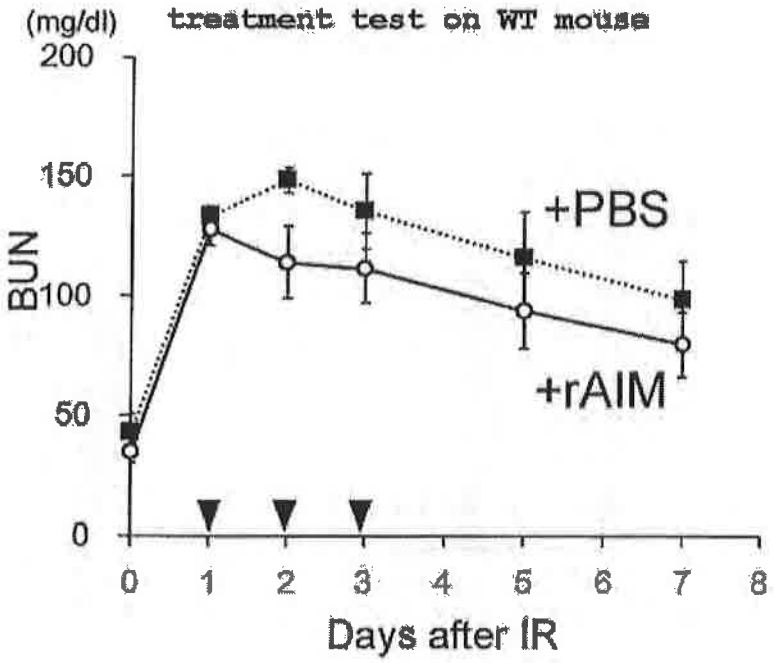

(mg/dl)
treatment test on WT mouse
200
150
100
50
0
BUN
+PBS
+rAIM
0 1 2 3 4 5 6 7 8
Days after IR

Fig. 28

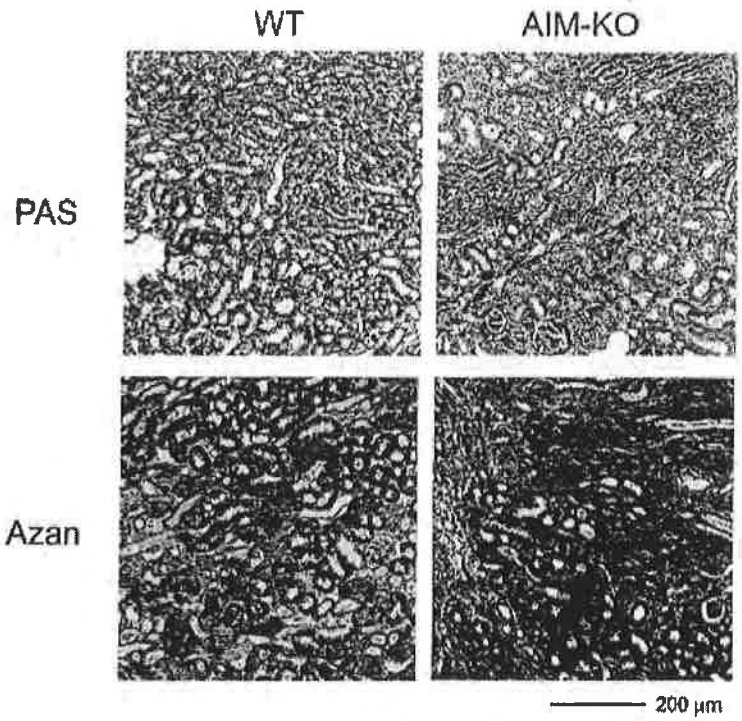

WT
AIM-KO
PAS
Azan
200 μm

Fig. 29

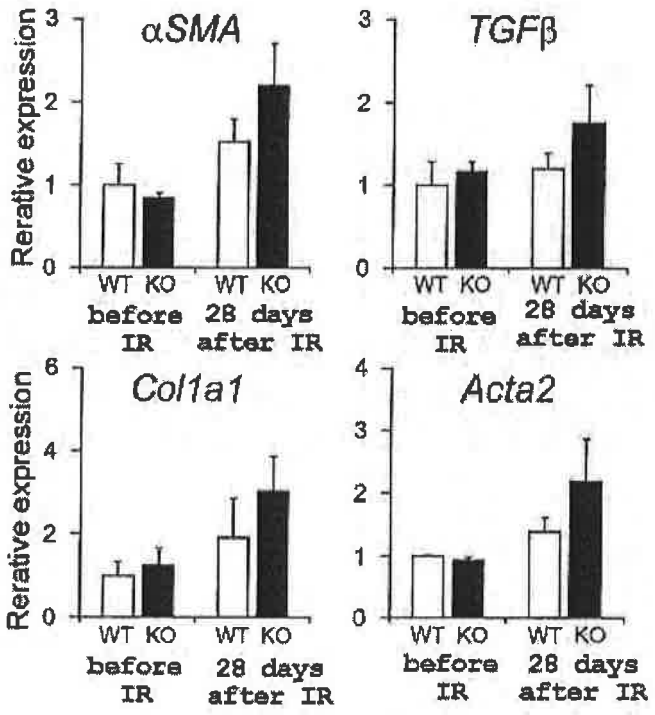

αSMA
TGFβ
Col1a1
Acta2
Rerative expression
WT KO
before IR
28 days after IR

Fig. 30

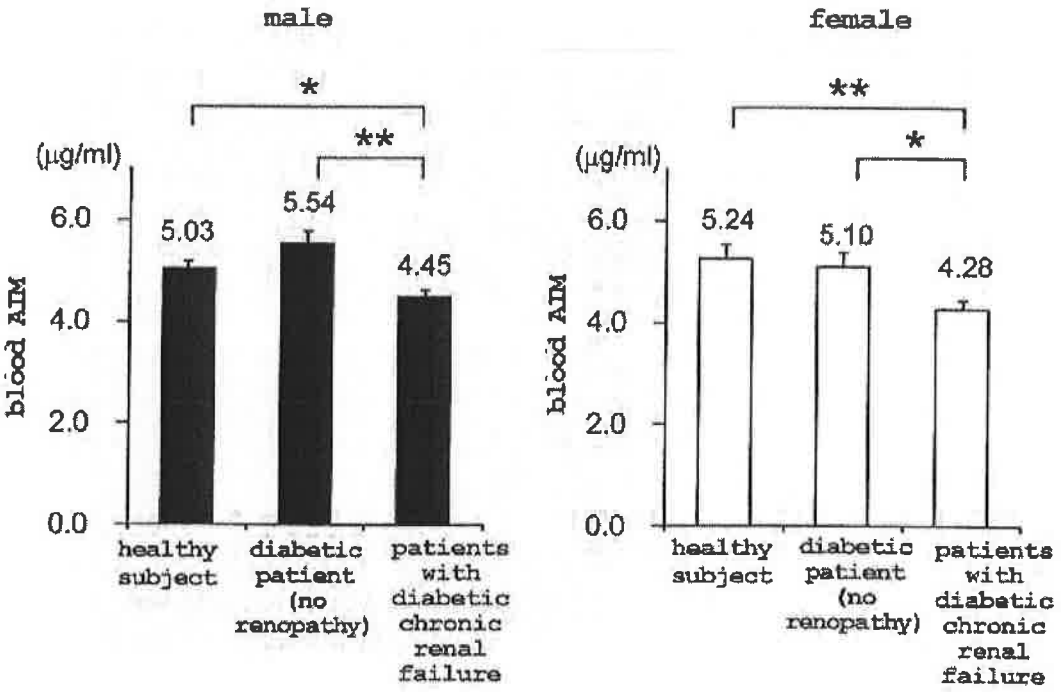

male
female
*
**
**
*
(μg/ml)
(μg/ml)
blood AIM
blood AIM
6.0
4.0
2.0
0.0
5.03
5.54
4.45
5.24
5.10
4.28
healthy subject
diabetic patient (no renopathy)
patients with diabetic chronic renal failure
healthy subject
diabetic patient (no renopathy)
patients with diabetic chronic renal failure

Fig. 31

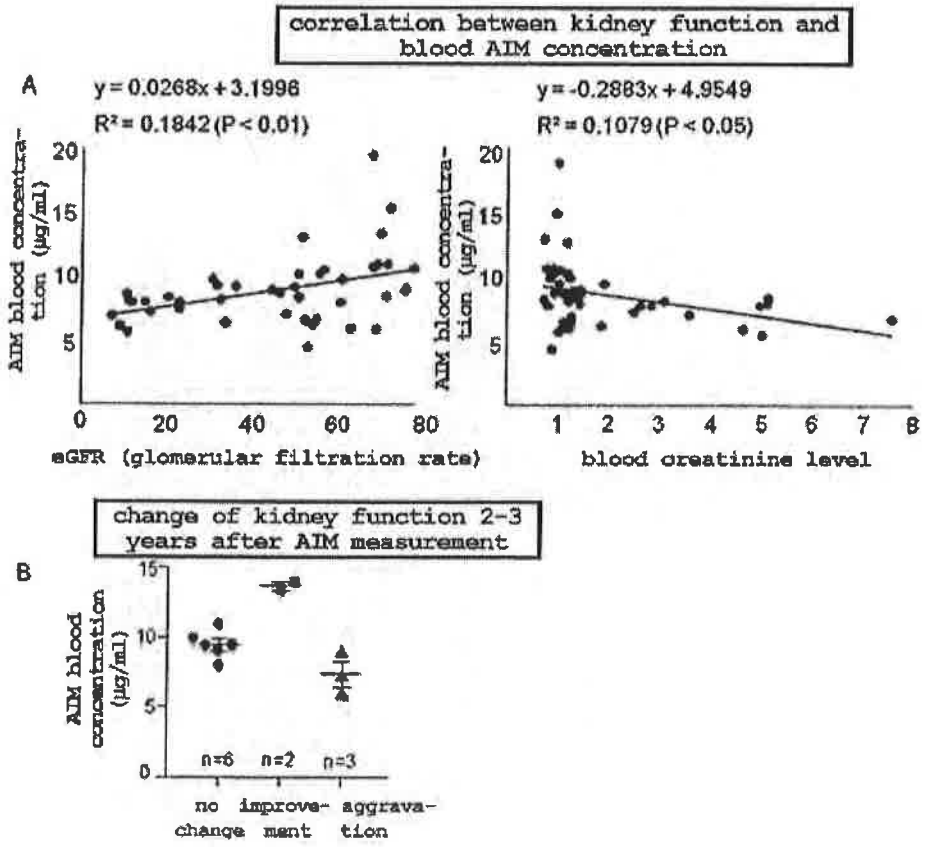

correlation between kidney function and blood AIM concentration
A
y = 0.0268x + 3.1996
R² = 0.1842 (P < 0.01)
y = -0.2883x + 4.9549
R² = 0.1079 (P < 0.05)
AIM blood concentration (μg/ml)
AIM blood concentration (μg/ml)
20
15
10
5
0
20
40
60
80
1
2
3
4
5
6
7
8
eGFR (glomerular filtration rate)
blood creatinine level
change of kidney function 2-3 years after AIM measurement
B
AIM blood concentration (μg/ml)
15
10
5
0
n=6
n=2
n=3
no change
improvement
aggravation

Fig. 32

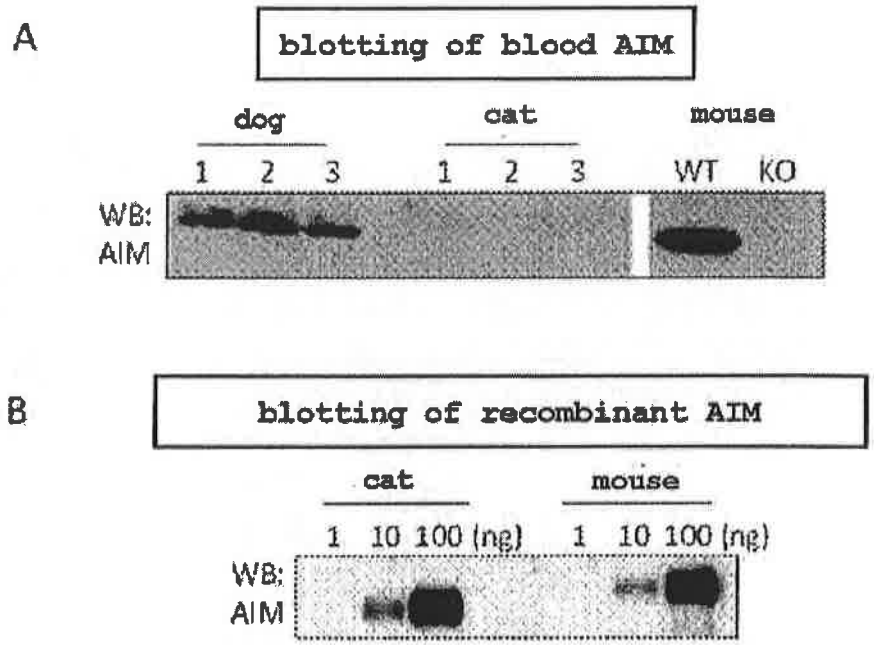
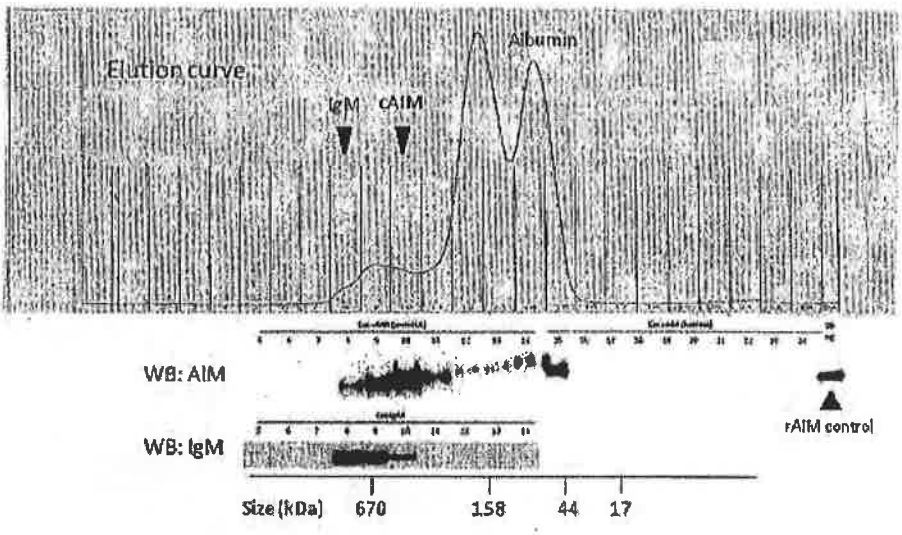

A
blotting of blood AIM
dog
cat
mouse
1 2 3 1 2 3 WT KO
WB:
AIM
B
blotting of recombinant AIM
cat
mouse
1 10 100 (ng) 1 10 100 (ng)
WB:
AIM

Fig. 33

A


Elution curve
IgM
cAIM
Albumin
WB: AIM
WB: IgM
rAIM control
Size (kDa)
670
158
44
17

B

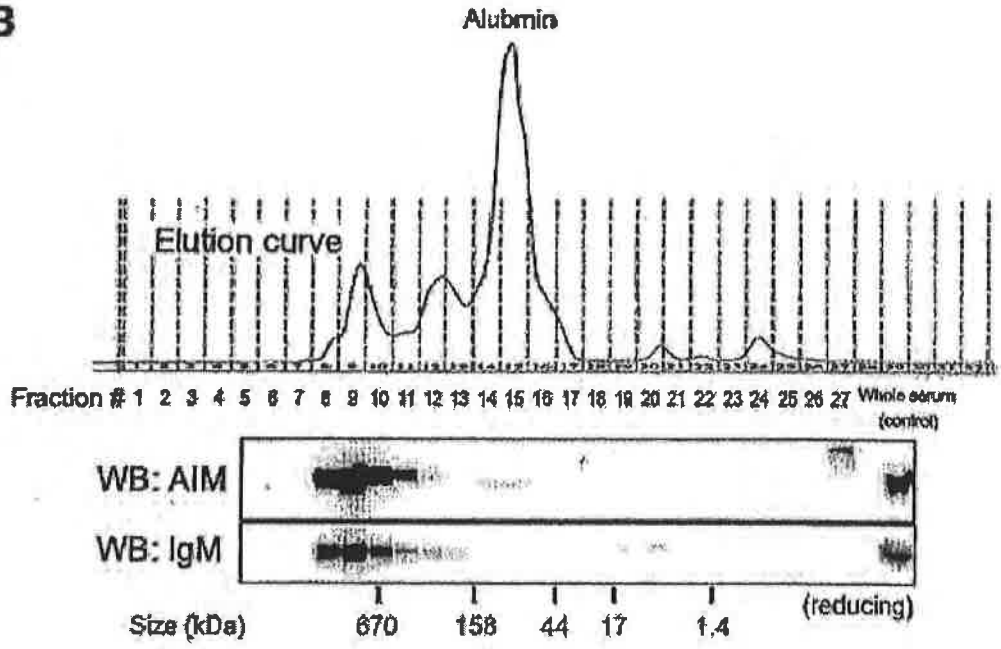

Albumin
Elution curve
Fraction # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Whole serum (control)
WB: AIM
WB: IgM
(reducing)
Size (kDa)
670
158
44
17
1.4

Fig. 34

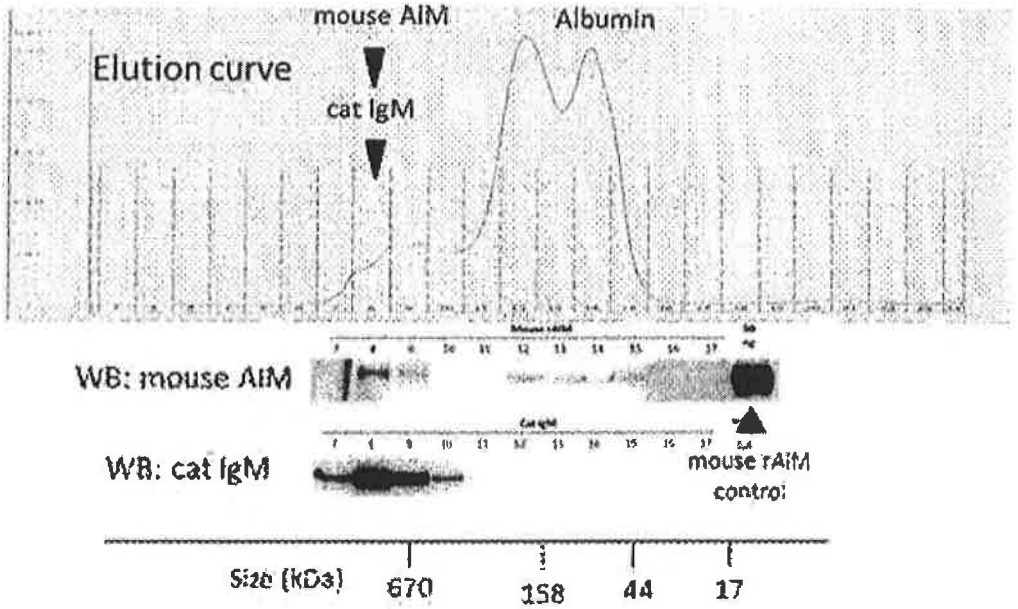
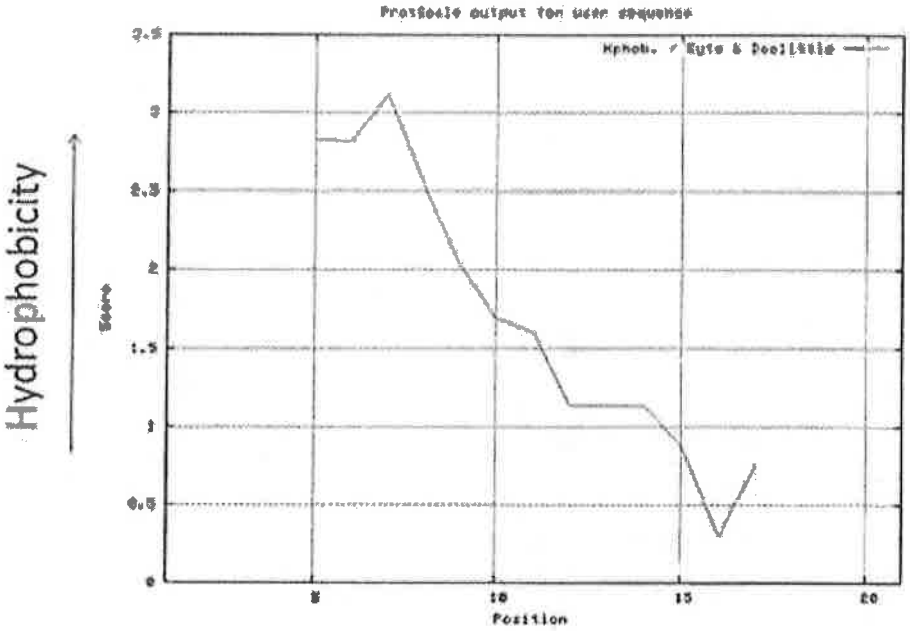


Fig. 35

cat AIM cDNA sequence

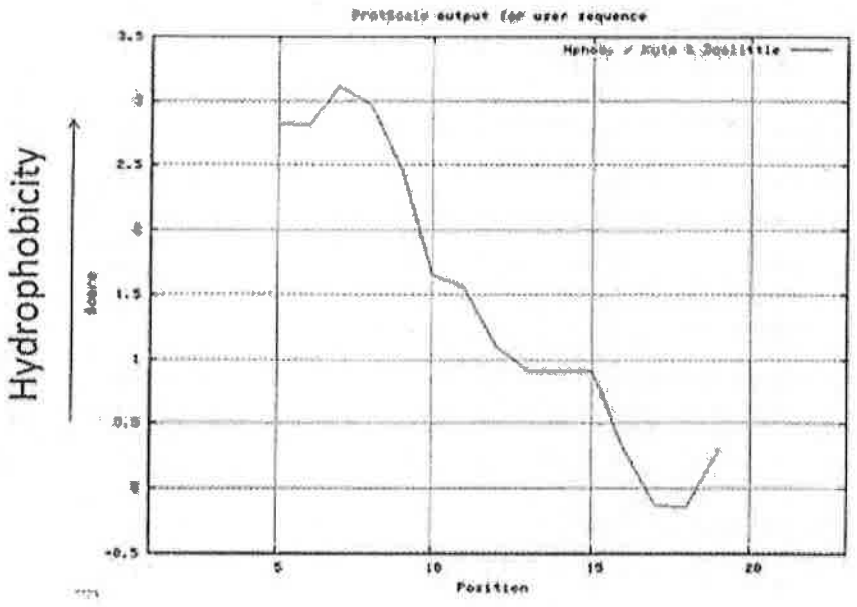
agaactctcc gttgctgccc tggggcctcc tcgcggcctc ggattccagc
tcagcctctc ccgtcgcctg gctcatggcg ctactcttct ccctaatcct
cgccatttac actggacctg gcattttagg gtctttttcc agagtgcggc
tagtgggagg cgaccaccgc tgtgaaggtc gtgtggagtt gcagcaggat
gacgagtggg tcaccgtgtg tgatgactac tggaacatgg actctgtggc
cgtgctgtgc cgggagctgg gctgtggggc ggccaggaag accatgagtg
gcaccgtgta tggaccagtg acaccaaagg accaaaaagt cttcatccac
ctgttcagat gcaatgggat cgaagaaagc ctgtctcagt gcgagaggga
agatgcaatc ggatgctccc atgttgagga tgcgggagcc gtgtgcgagc
ccatttacac tggacctggc attttagggc cggagagtgt gaggctggcc
gatggccccg ggcgctgcca gggccgagtg gaggtgaagt tccgagggga
gtggagctct gtgtgccaag caggctggag ctttgcagcc gccaaggtgg
tgtgccggca gctggggtgt ggacgggcca ccctgacccg gagaggctgc
aacaaagcga cccagggcca aggggccatc tggcagagaa aggcgtcatg
ctcaggacaa gaagtgagcc ttcaagattg cctttctgaa gtttgggaac
acaactgtac ccacaatgag gacgtgtggg tcgaatgtga agatccctttt
gccttgaagc tggtaggagg acgcagccac tgtgagggga ggctggaggt
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tgcacaggtc ctggggctac cacaactgta accacagaga ggatgtggct
gtggtctgtg aagaacagca gtctggccta cctgatgctt gacccacagg
ccccaagcgc tcttacttct cctgggccct gatcggcccg gcctga

Fig. 36



cat

Fig. 37



human

Fig. 38

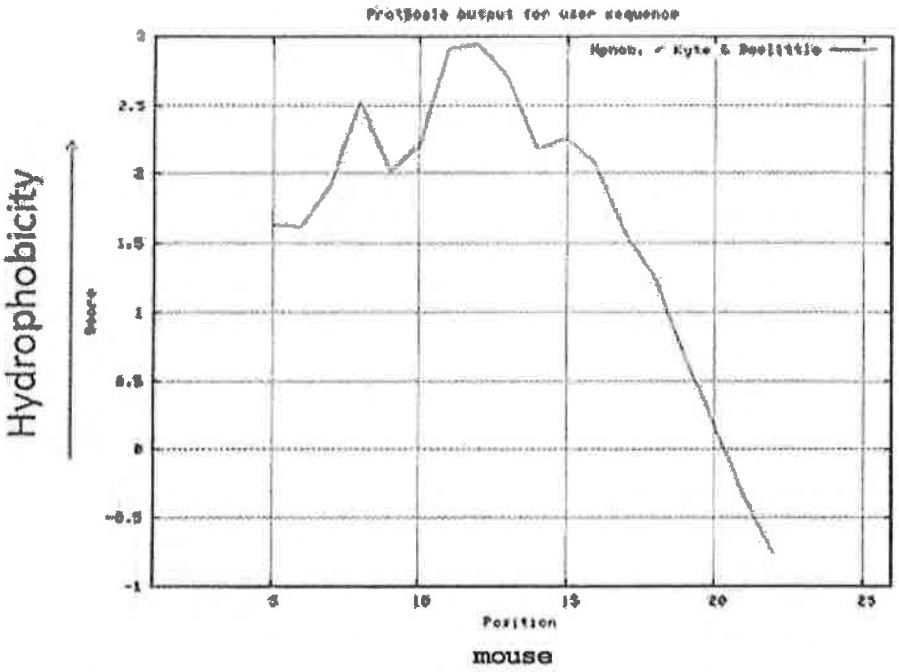

ProtScale output for user sequence
Hphob. / Kyte & Doolittle
Hydrophobicity
Score
Position
mouse

Fig. 39

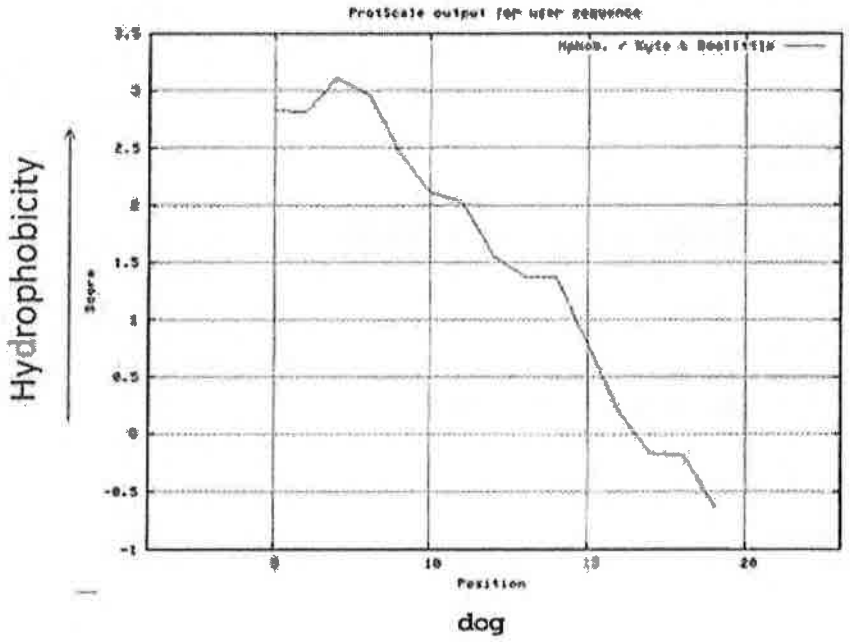
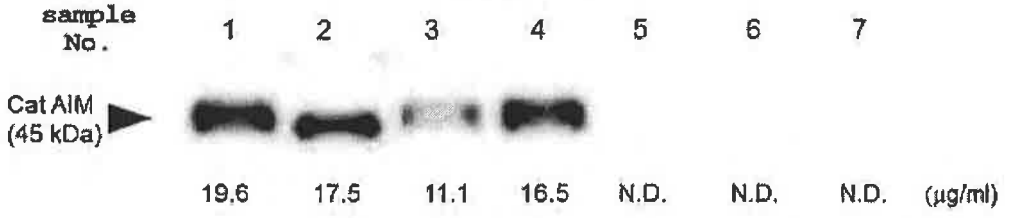

ProtScale output for user sequence
Hphob. / Kyte & Doolittle
Hydrophobicity
Score
Position
dog

Fig. 40



Fig. 41



mean serum AIM concentration in AIM+(total 48 samples): 16.8 μg/m
(mean of mouse・human AIM: 5 μg/m)

Fig. 42

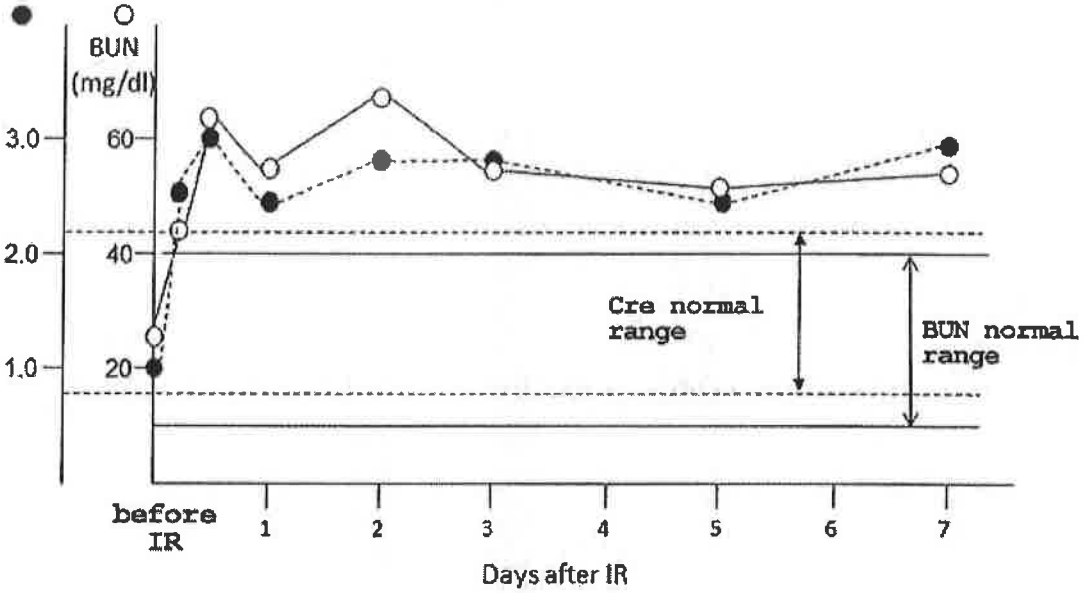


Fig. 43

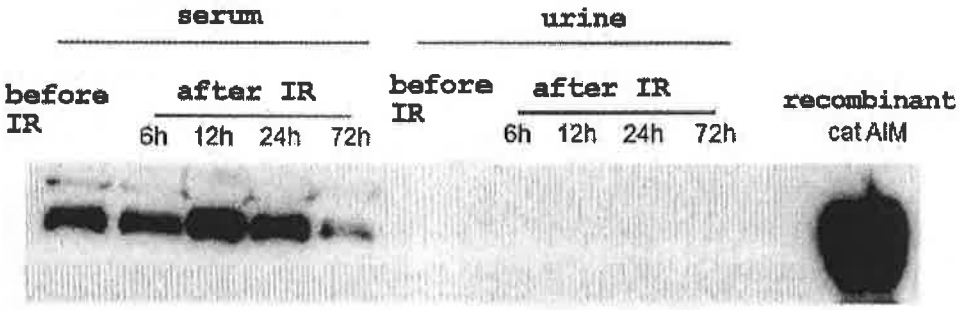
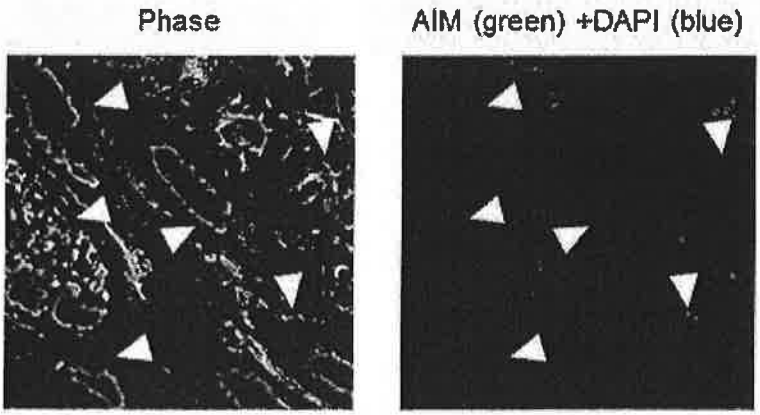


Fig. 44



arrowhead: dead cell mass in proximal renal tubule (intraluminal debris)

Fig. 45

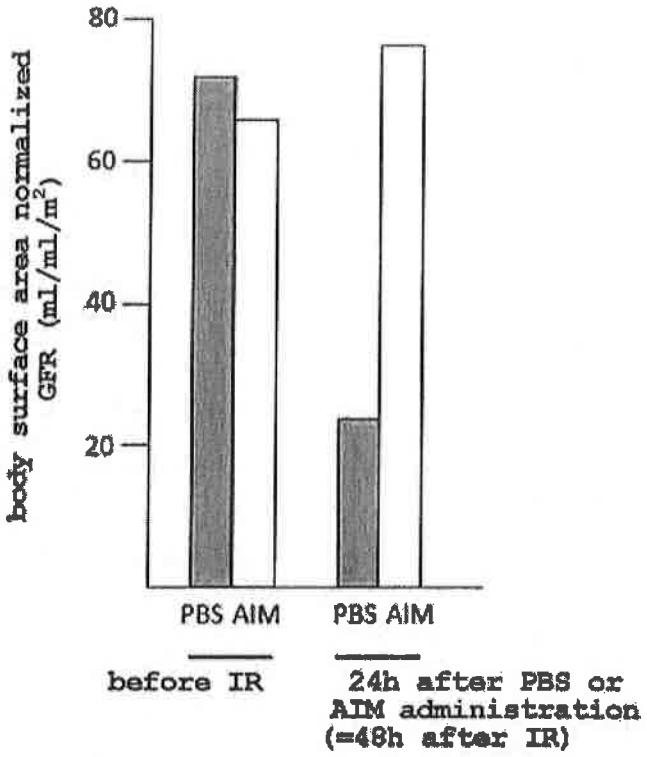
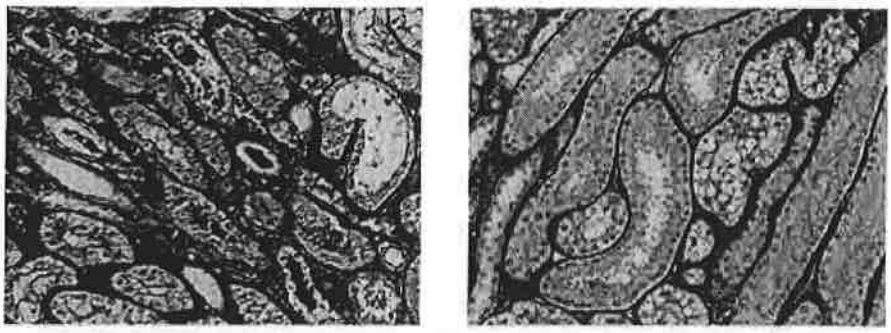


Fig. 46



PBS-administered cat kidney

rAIM-administered cat kidney