```
Improved RecAP (LVL-RecAP) Sequence (SEQ ID NO: 1):
  1 VIPAEENPAFWNRQAAEALDAAKKLQPIQKVAKNLILFLGDGLGVPTVTATRILKGQKNGKLGPETPLAMDRFPYLALSKTYNVDRQVPDSAATATAYL
101 CGVKANFQTIGLSAAARFNQCNTTRGNEVISVMNRAKQAGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSDADMPASARQEGCQDIATQLISNMDIDVIL
201 GGGRKYMFPMGTPDPEYPADASQNGIRLDGKNLVQEWLAKHQGAWYVWNRTELMQASLDQSVTHLMGLFEPGDTKYEILRDPTLDPSLMEMTEAAILRLLS
301 RNPRGFYLFVEGGRIDHGHHECVAYQAVTEAVMFDDAIERAGQLTSEEDTLTLVTADHSHVFSFGGYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYV
401 LKDGARPDVTESESGSPEYRQQSAVPLDEETHGGEDVAVFARGPQAHLVHGVQEQSFVAHVMAFAACLEPYTACDLALPACTTD

RefSEQ hALPI (mature protein sequence of accession number: NM_001631.3) (SEQ ID NO:2):
  1 VIPAEENPAFWNRQAAEALDAAKKLQPIQKVAKNLILFLGDGLGVPTVTATRILKGQKNGKLGPETPLAMDRFPYLALSKTYNVDRQVPDSAATATAYL
101 CGVKANFQTIGLSAAARFNQCNTTRGNEVISVMNRAKQAGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSDADMPASARQEGCQDIATQLISNMDIDVIL
201 GGGRKYMFPMGTPDPEYPADASQNGIRLDGKNLVQEWLAKHQGAWYVWNRTELMQASLDQSVTHLMGLFEPGDTKYEIHRDPTLDPSLMEMTEAALRLLS
301 RNPRGFYLFVEGGRIDHGHHECVAYQALTEAVMFDDAIERAGQLTSEEDTLTLVTADHSHVFSFGGYPLRGSSIFGLAPGKAYTSILYGNGPGYV
401 FNSCVRPDVNESESGSPDYQQQAAVPLSSETHGGEDVAVFARGPQAHLVHGVQEQSFVAHVMAFAACLEPYTACDLAPPACTTD

RefSEQ hALPP (mature protein sequence of accession number: NM_001632.3) (SEQ ID NO: 3):
  1 IIPVEENPDFWNREAAEALGAAKKLQPAQTAAKNLIIFLGDGMGVSTVTAARILKGQKKDKLGPEIPLAMDRFPYVALSKTYNVDKHVPDSGATATAYL
101 CGVKGNFQTIGLSAAARFNQCNTTRGNEVISVMNRAKKAGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSDADVPASARQEGCQDIATQLISNMDIDVIL
201 GGGRKYMFRMGTPDPEYPDDYSQGGTRLDGKNLVQEWLAKRQGARYVWNRTELMQASLDPSVTHLMGLFEPGDMKYEIHRDSTLDPSLMEMTEAALRLLS
301 RNPRGFFLFVEGGRIDHGHHESRAYRALTETIMFDDAIERAGQLTSEEDTLSLVTADHSHVFSFGGYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYV
401 LKDGARPDVTESESGSPEYRQQSAVPLDEETHAGEDVAVFARGPQAHLVHGVQEQTFIAHVMAFAACLEPYTACDLAPPAGTTD

sALPI-ALPP-CD Sequence (SEQ ID NO: 4):
  1 VIPAEENPAFWNRQAAEALDAAKKLQPIQKVAKNLILFLGDGLGVPTVTATRILKGQKNGKLGPETPLAMDRFPYLALSKTYNVDRQVPDSAATATAYL
101 CGVKANFQTIGLSAAARFNQCNTTRGNEVISVMNRAKQAGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSDADMPASARQEGCQDIATQLISNMDIDVIL
201 GGGRKYMFPMGTPDPEYPADASQNGIRLDGKNLVQEWLAKHQGAWYVWNRTELMQASLDQSVTHLMGLFEPGDTKYEIHRDPTLDPSLMEMTEAALRLLS
301 RNPRGFYLFVEGGRIDHGHHESRAYRALTETIMFDDAIERAGQLTSEEDTLSLVTADHSHVFSFGGYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYV
401 LKDGARPDVTESESGSPEYRQQSAVPLDEETHGGEDVAVFARGPQAHLVHGVQEQSFVAHVMAFAACLEPYTACDLAPPACTTD

N-Terminal Sequence (1 - 365) of hALPI (SEQ ID NO:5):
  1 VIPAEENPAFWNRQAAEALDAAKKLQPIQKVAKNLILFLGDGLGVPTVTATRILKGQKNGKLGPETPLAMDRFPYLALSKTYNVDRQVPDSAATATAYL
101 CGVKANFQTIGLSAAARFNQCNTTRGNEVISVMNRAKQAGKSVGVVTTTRVQHASPAGTYAHTVNRNWYSDADMPASARQEGCQDIATQLISNMDIDVIL
201 GGGRKYMFPMGTPDPEYPADASQNGIRLDGKNLVQEWLAKHQGAWYVWNRTELMQASLDQSVTHLMGLFEPGDTKYEIHRDPTLDPSLMEMTEAALRLLS
301 RNPRGFYLFVEGGRIDHGHHECVAYQALTEAVMFDDAIERAGQLTSEEDTLTLVTADHSHVFSFG

Crown domain Sequence (366 - 430) of PLAP (SEQ ID NO: 6):
366 GYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYVLKDGARPDVTESESGSPEYRQQSAVPLDEE

C-Terminal Sequence (431 - 484) of hALPI (SEQ ID NO: 7):
431 THGGEDVAVFARGPQAHLVHGVQEQSFVAHVMAFAACLEPYTACDLAPPACTTD
```

FIG. 1

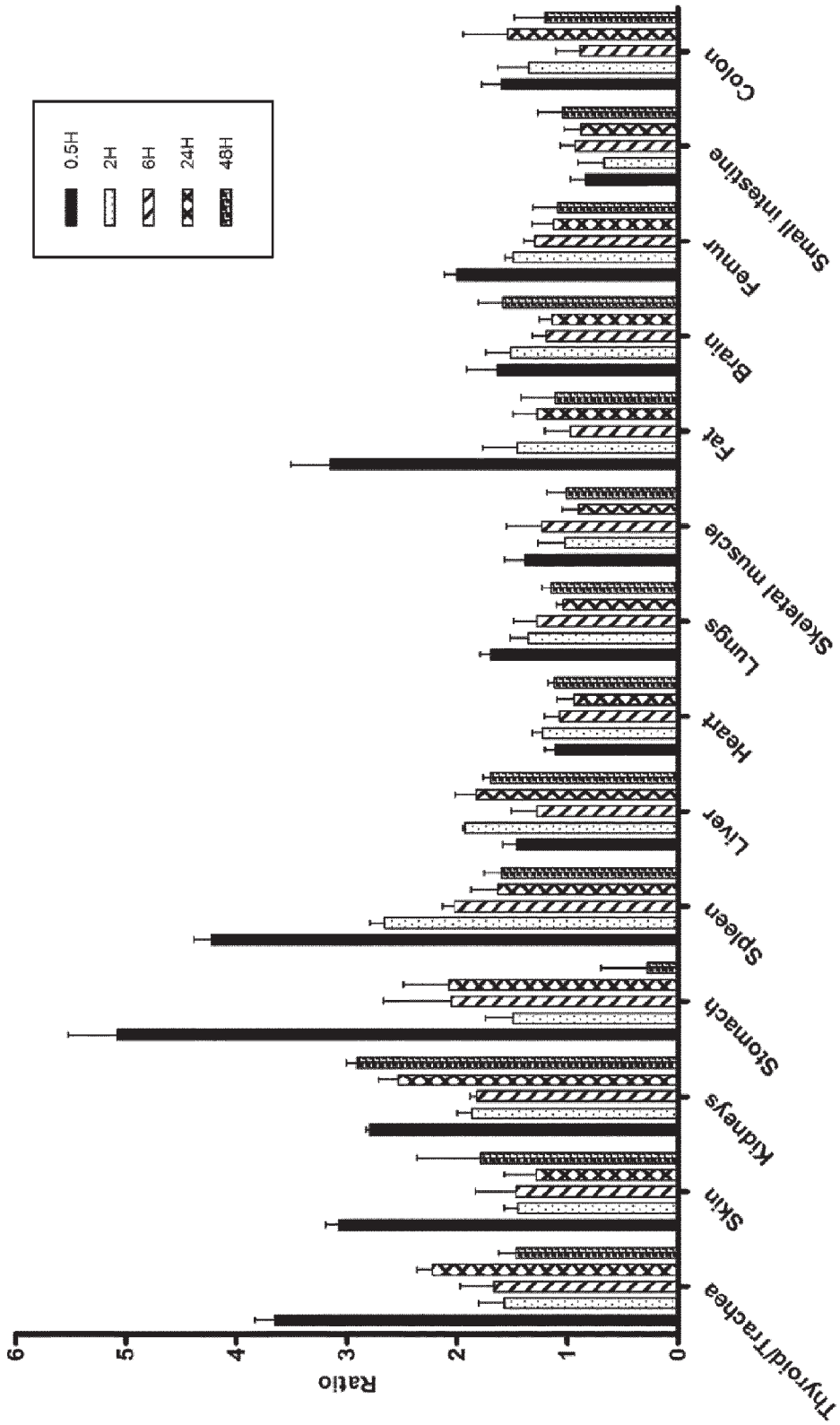

0.5H
2H
6H
24H
48H
Ratio
0
1
2
3
4
5
6
Thyroid/Trachea
Skin
Kidneys
Stomach
Spleen
Liver
Heart
Lungs
Skeletal muscle
Fat
Brain
Femur
Small intestine
Colon

FIG. 2

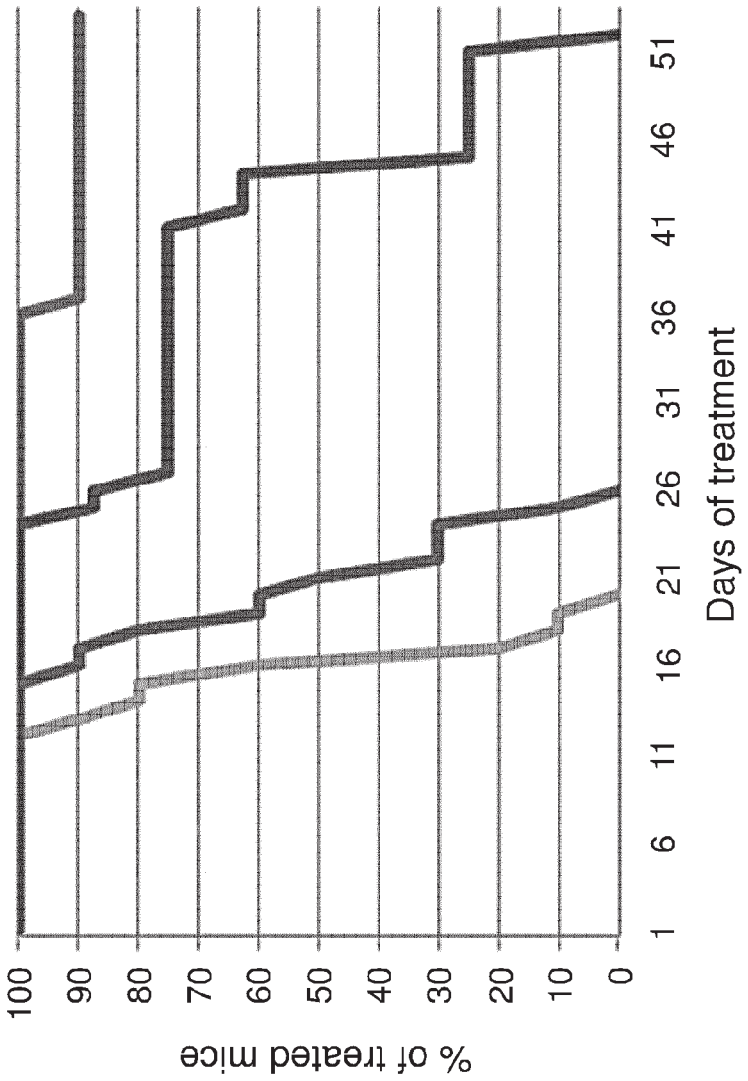

% of treated mice
100
90
80
70
60
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
Days of treatment

FIG. 3

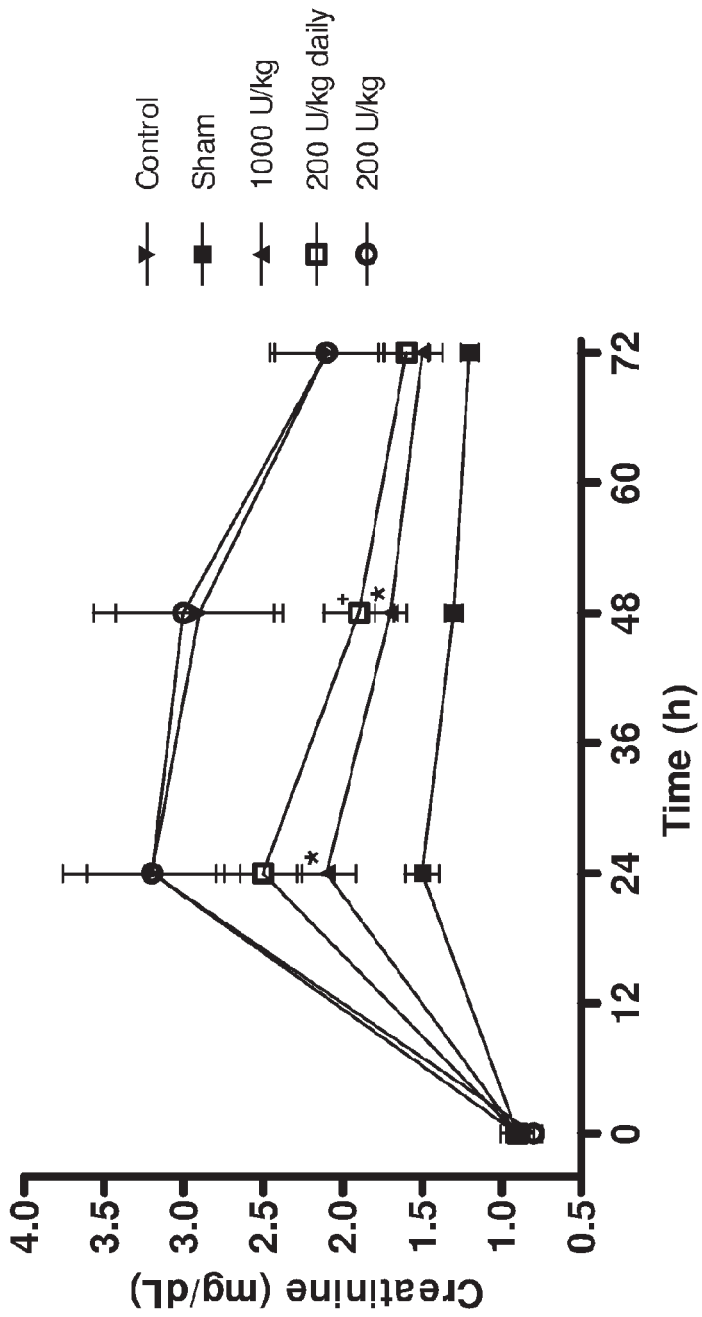

Control
Sham
1000 U/kg
200 U/kg daily
200 U/kg
Creatinine (mg/dL)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
12
24
36
48
60
72
Time (h)
*
*

FIG. 4

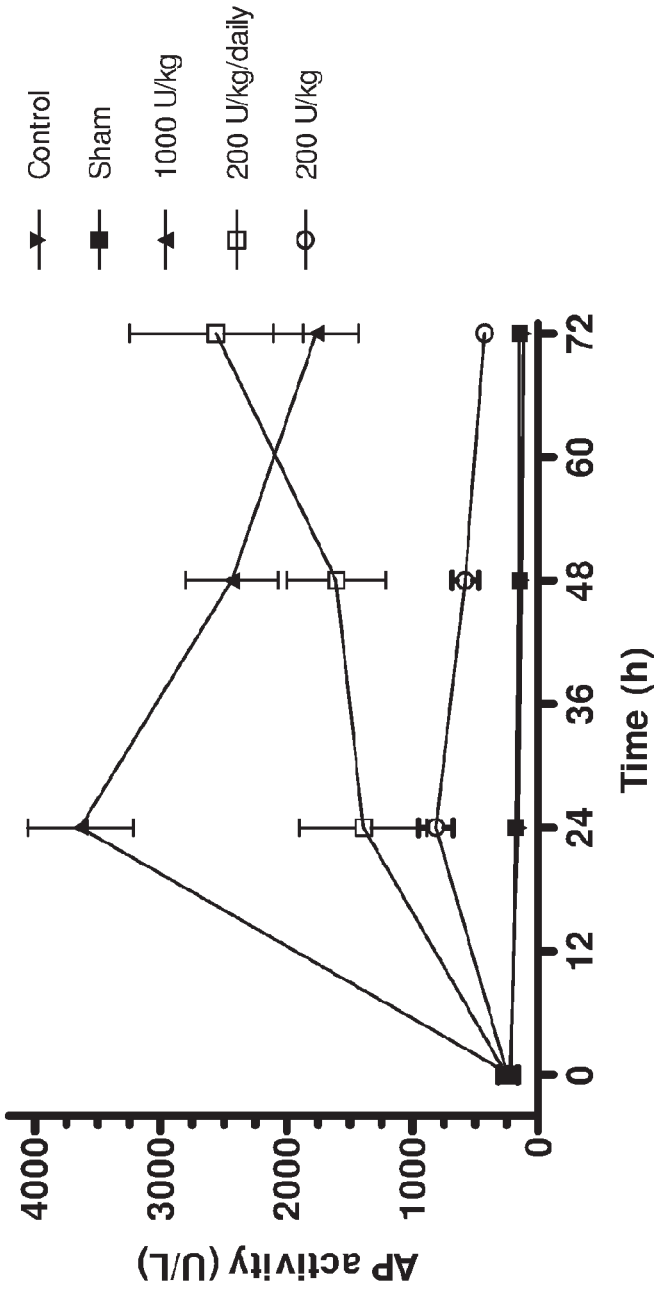

Control
Sham
1000 U/kg
200 U/kg/daily
200 U/kg
AP activity (U/L)
4000
3000
2000
1000
0
0
12
24
36
48
60
72
Time (h)

FIG. 5

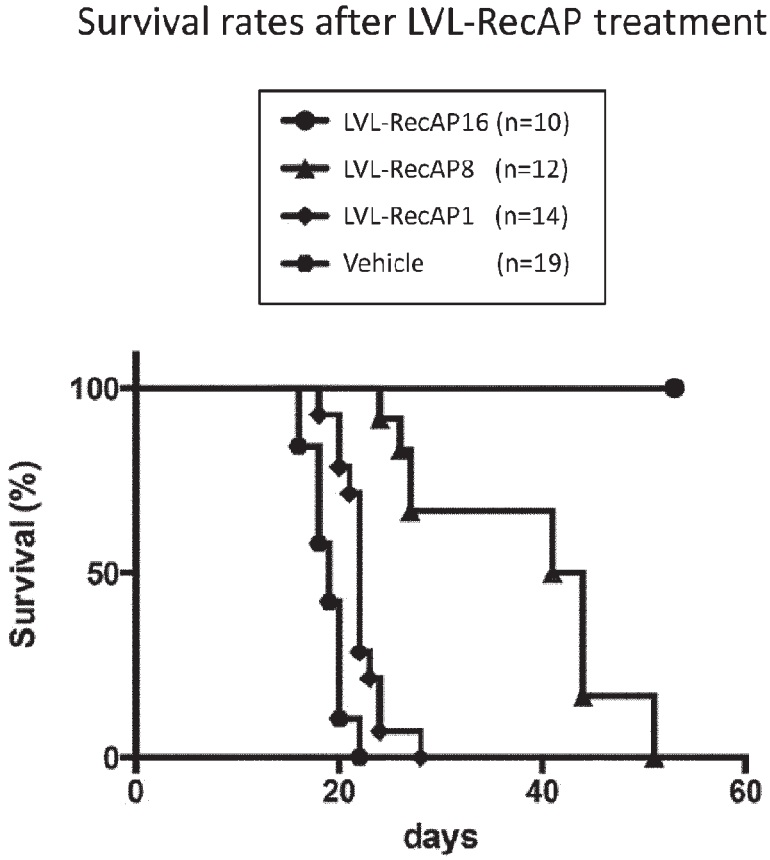

Survival rates after LVL-RecAP treatment
LVL-RecAP16 (n=10)
LVL-RecAP8 (n=12)
LVL-RecAP1 (n=14)
Vehicle (n=19)
Survival (%)
100
50
0
0
20
40
60
days

FIG. 6A

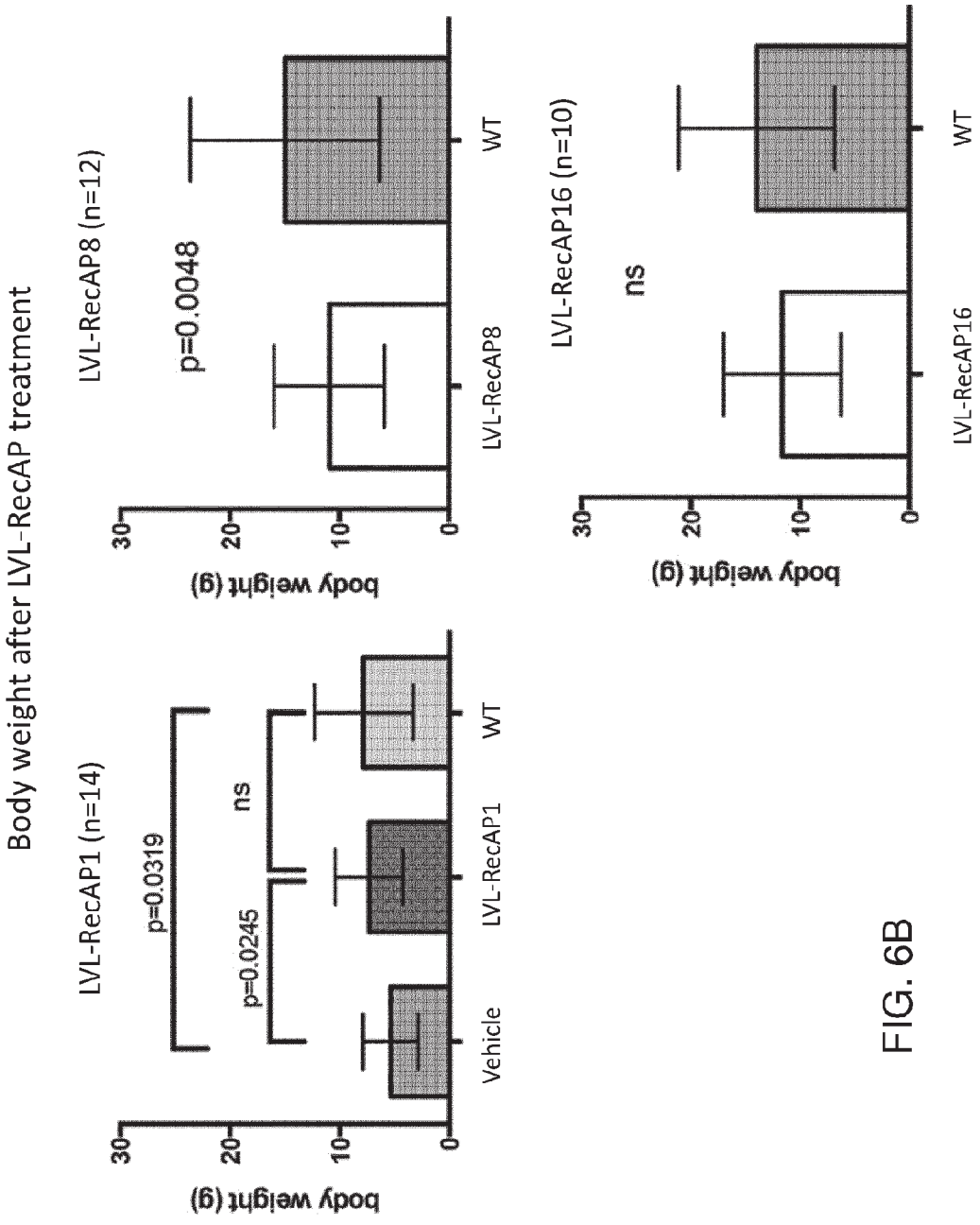

Body weight after LVL-RecAP treatment
LVL-RecAP1 (n=14)
body weight (g)
30
20
10
0
p=0.0319
p=0.0245
ns
Vehicle
LVL-RecAP1
WT
LVL-RecAP8 (n=12)
p=0.0048
LVL-RecAP8
WT
LVL-RecAP16 (n=10)
ns
LVL-RecAP16
WT

FIG. 6B

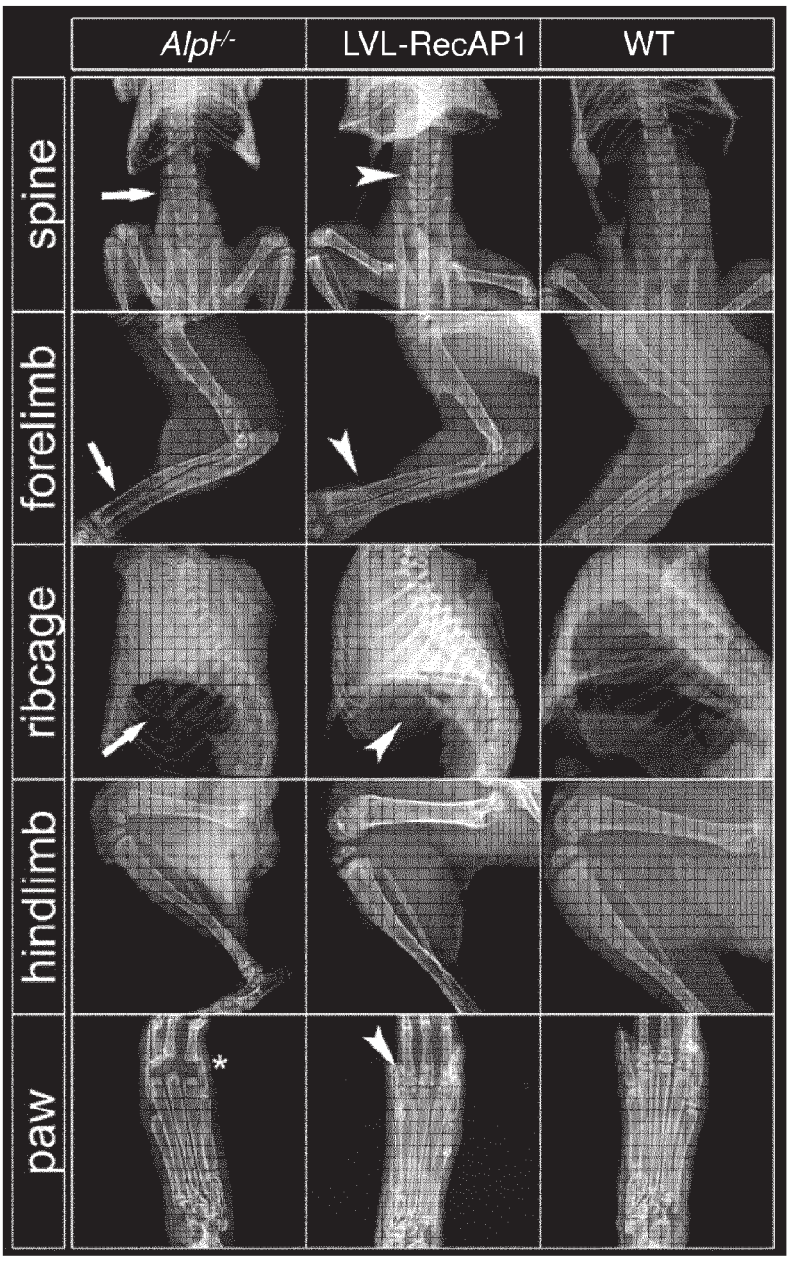


FIG. 7A

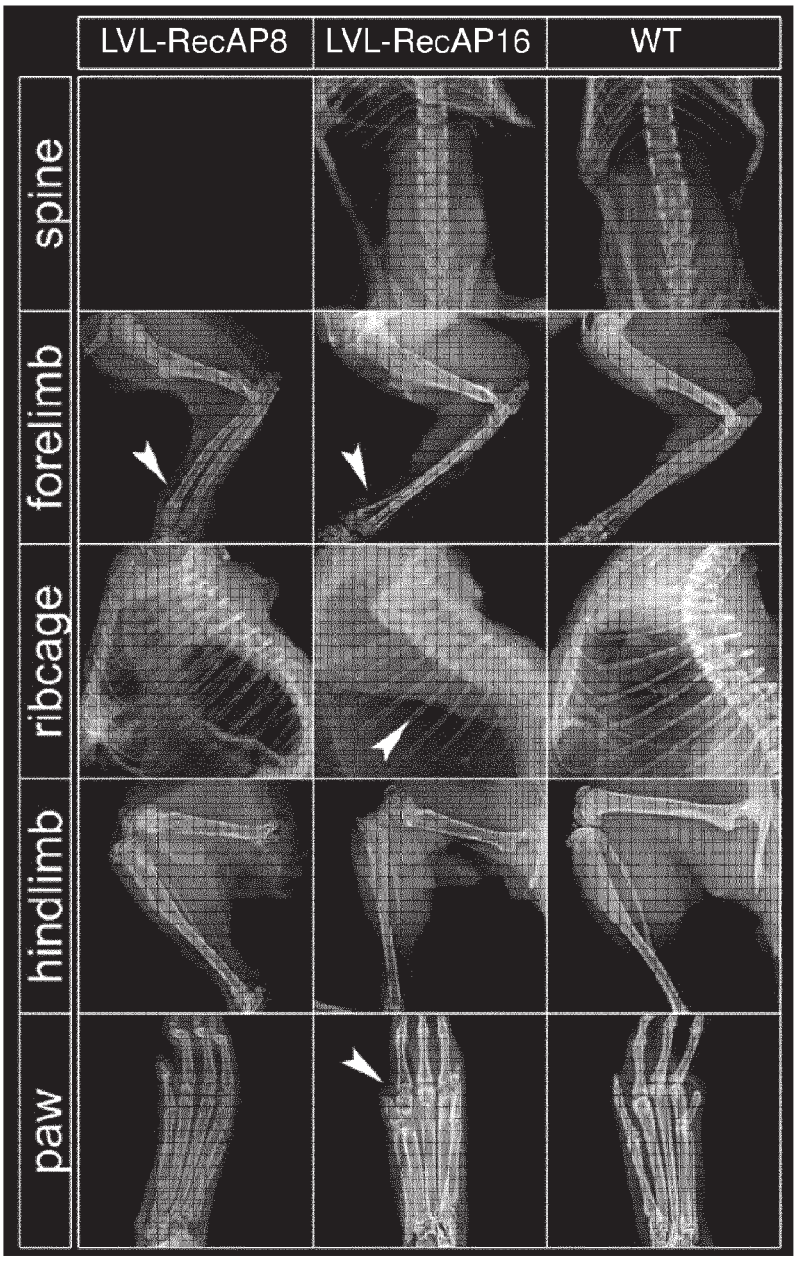

LVL-RecAP8
LVL-RecAP16
WT
spine
forelimb
ribcage
hindlimb
paw

FIG. 7B

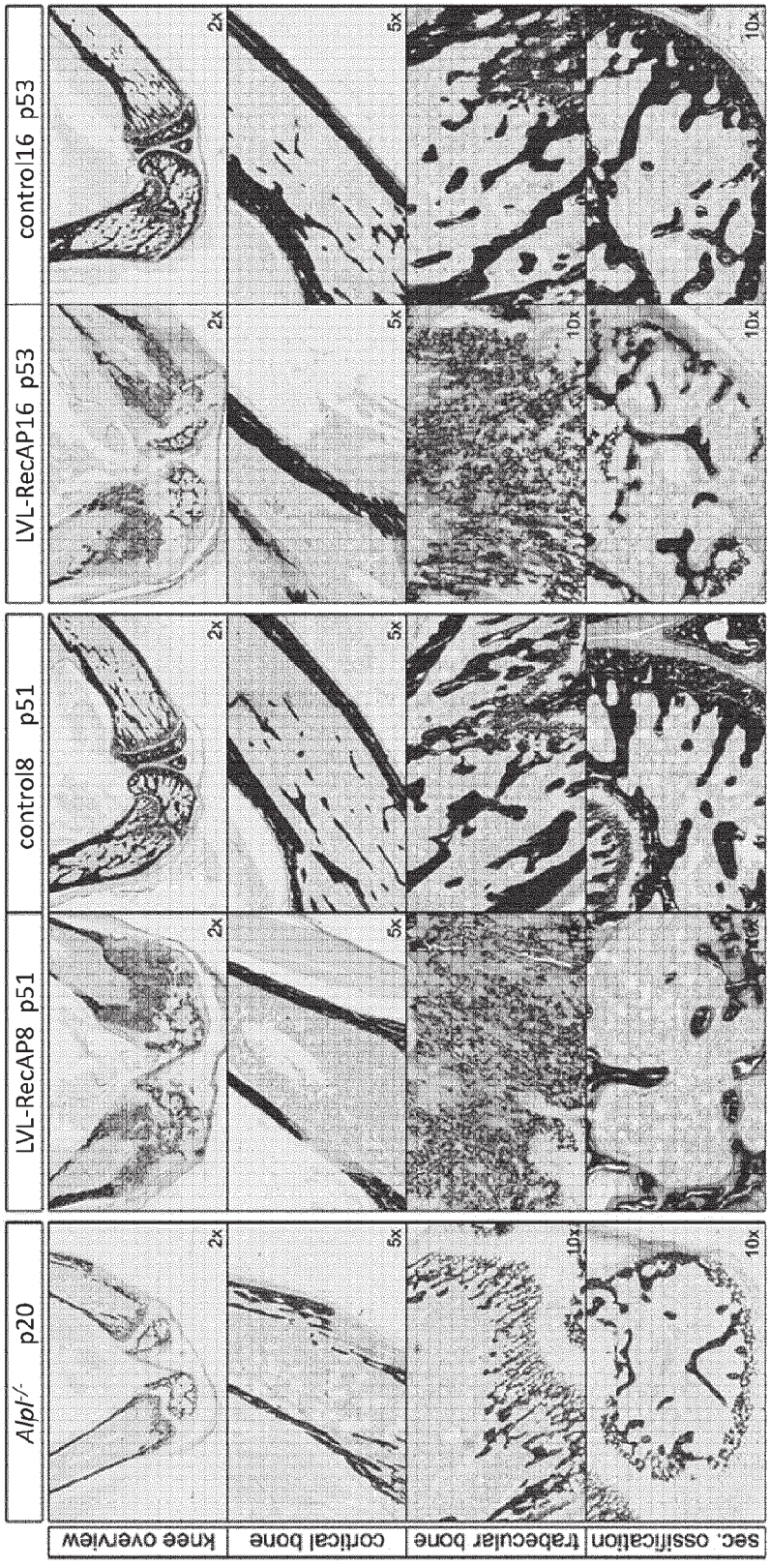

Alpl-/- p20
LVL-RecAP8 p51
control8 p51
LVL-RecAP16 p53
control16 p53
knee overview
cortical bone
trabecular bone
sec. ossification
2x
5x
10x

FIG. 8A

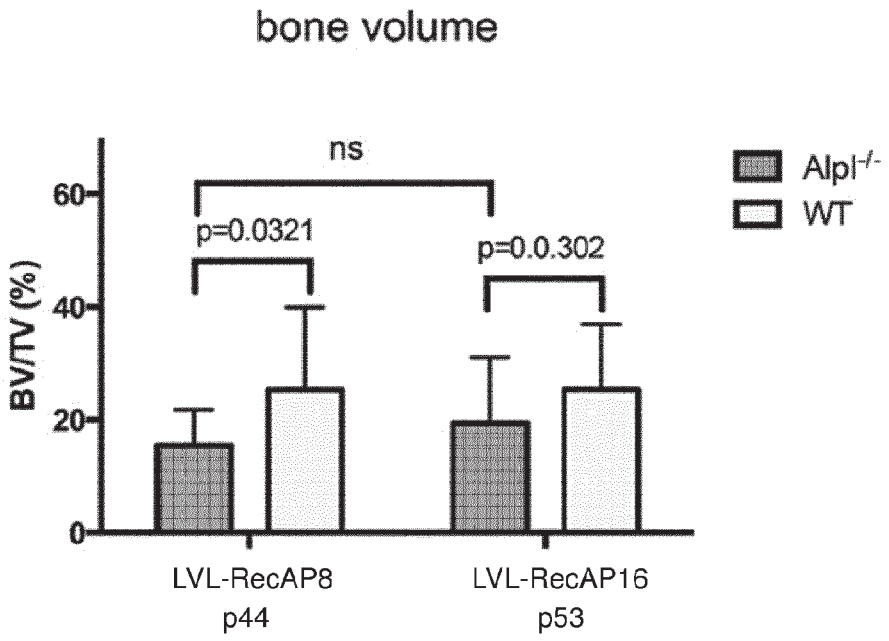

bone volume
ns
p=0.0321
p=0.0.302
BV/TV (%)
60
40
20
0
Alpl-/-
WT
LVL-RecAP8
p44
LVL-RecAP16
p53

FIG. 8B

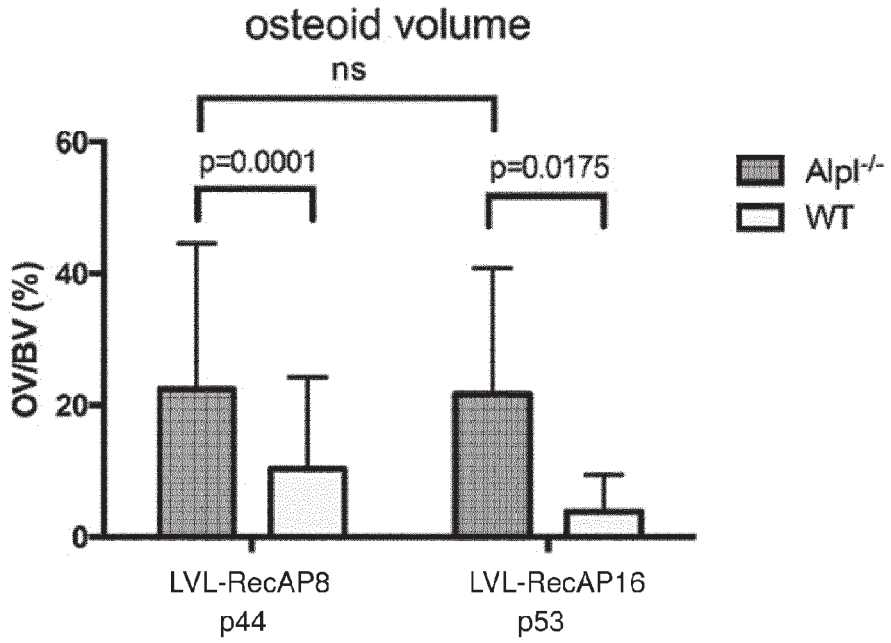

osteoid volume
ns
p=0.0001
p=0.0175
OV/BV (%)
60
40
20
0
Alpl-/-
WT
LVL-RecAP8
p44
LVL-RecAP16
p53

FIG. 8C

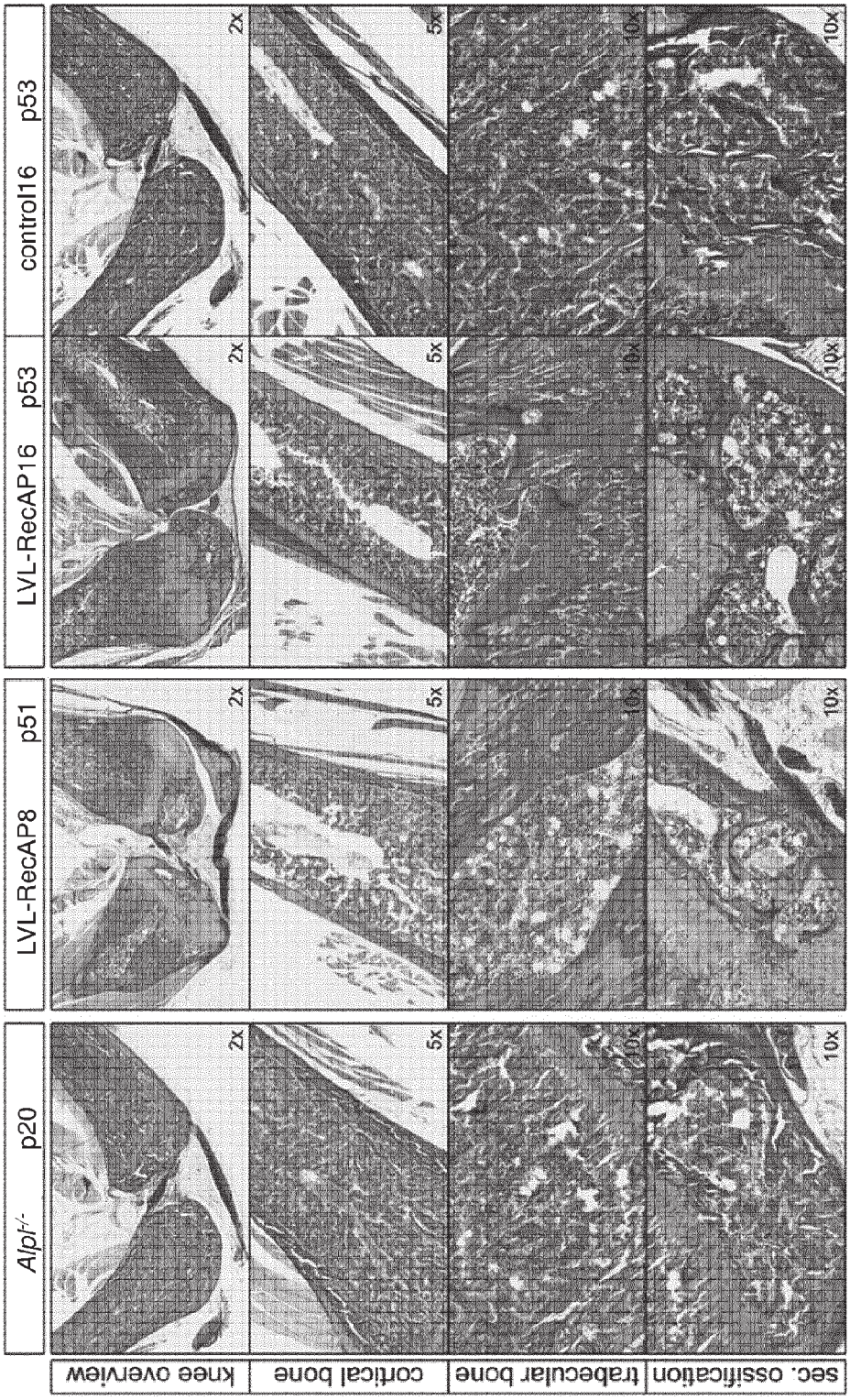

Alpl-/- p20
LVL-RecAP8 p51
LVL-RecAP16 p53
control16 p53
knee overview
cortical bone
trabecular bone
sec. ossification
2x
5x
10x

FIG. 9A

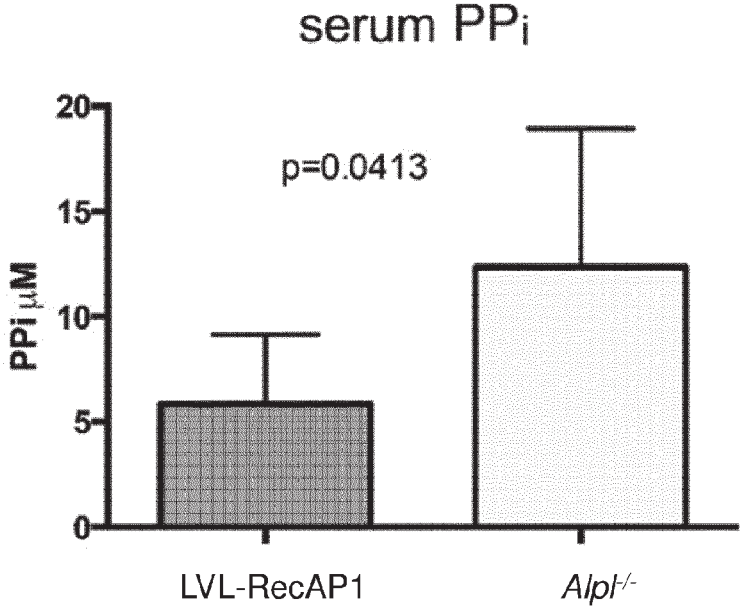

serum PPi
p=0.0413
PPi µM
20
15
10
5
0
LVL-RecAP1
Alpl-/-

FIG. 9B

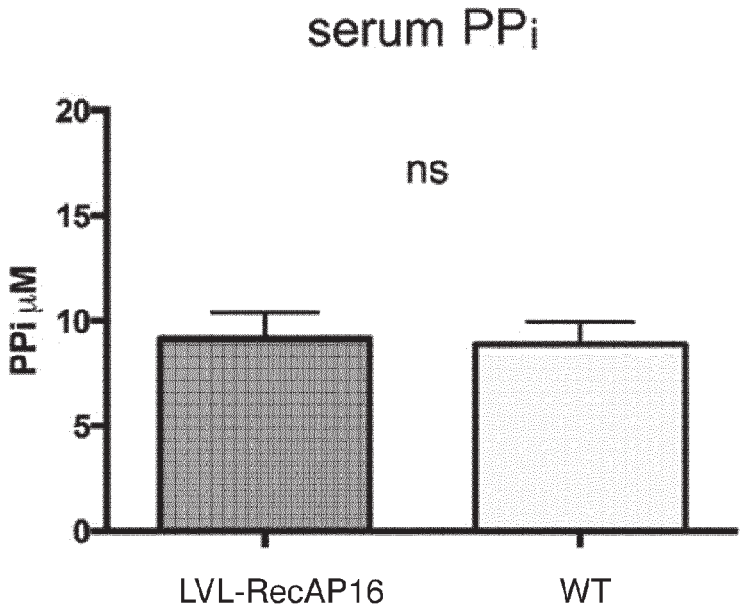

serum PPi
ns
PPi µM
20
15
10
5
0
LVL-RecAP16
WT

FIG. 9C

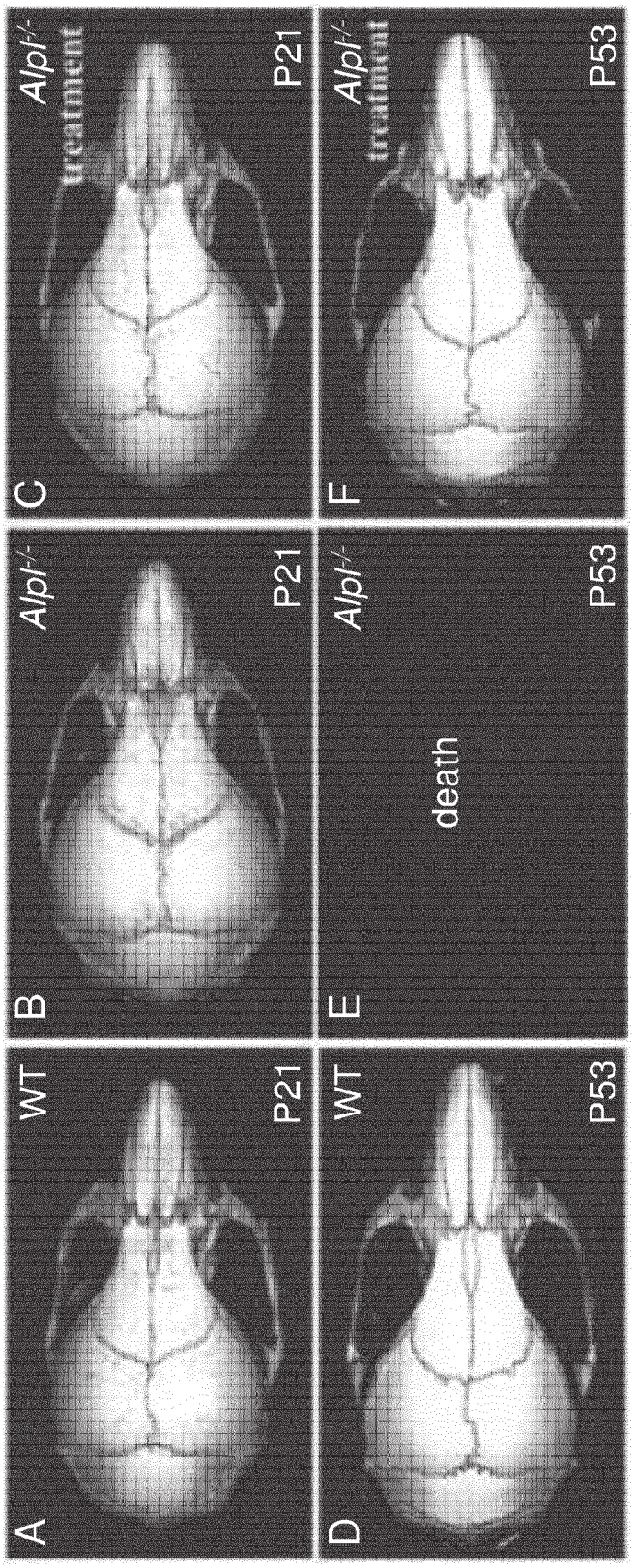

A
WT
P21
B
Alpl-/-
P21
C
Alpl-/-
treatment
P21
D
WT
P53
E
Alpl-/-
death
P53
F
Alpl-/-
treatment
P53

FIG. 10

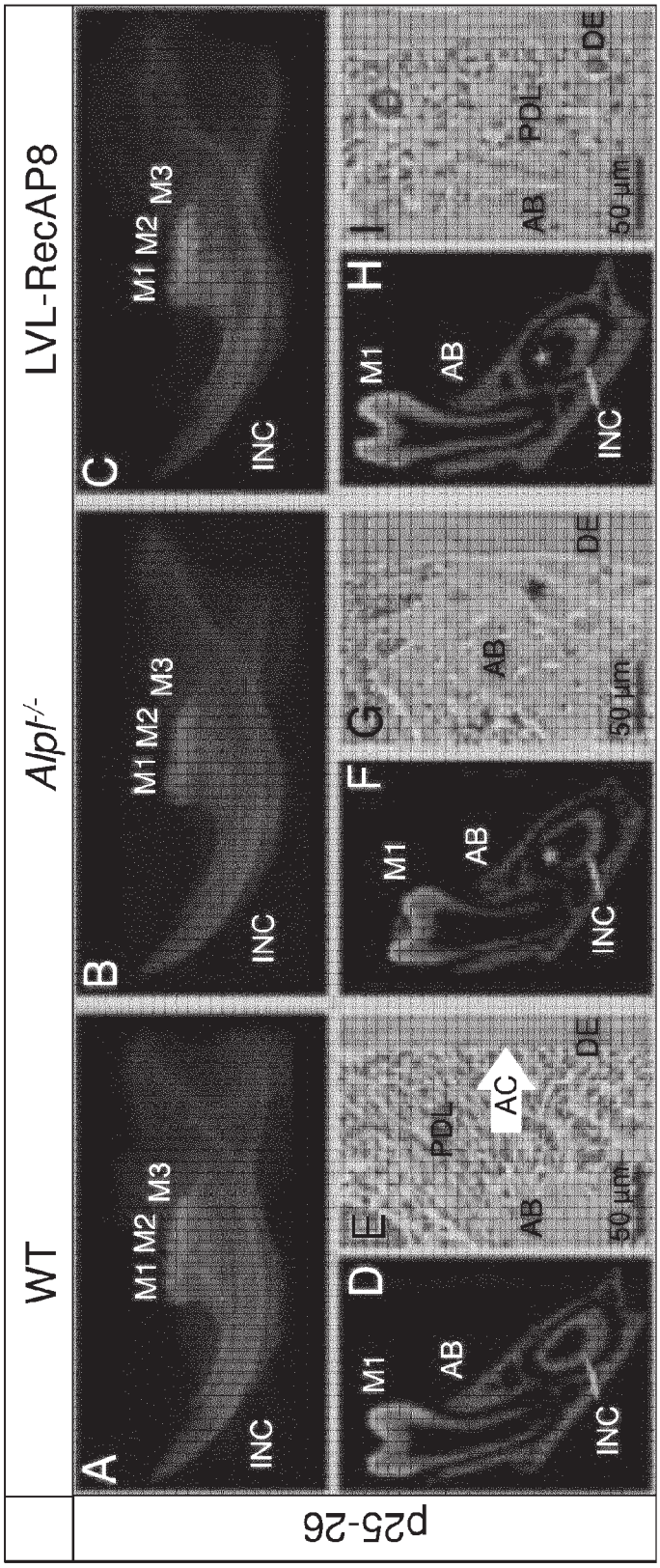

WT
Alpl-/-
LVL-RecAP8
p25-26
A
B
C
D
E
F
G
H
I
M1 M2 M3
INC
M1
AB
PDL
AC
DE
50 µm

FIG. 11

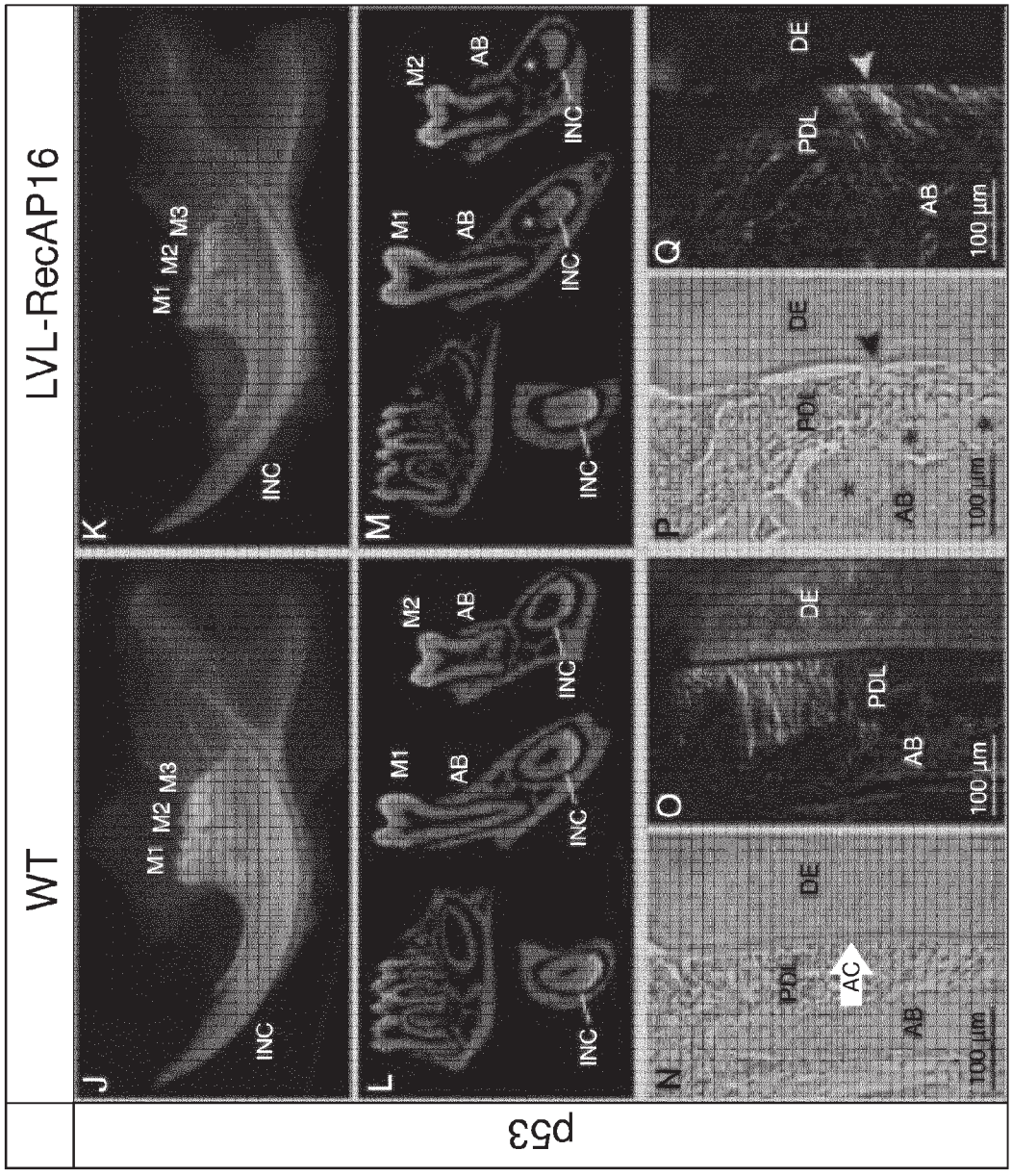


FIG. 11, Cont'd

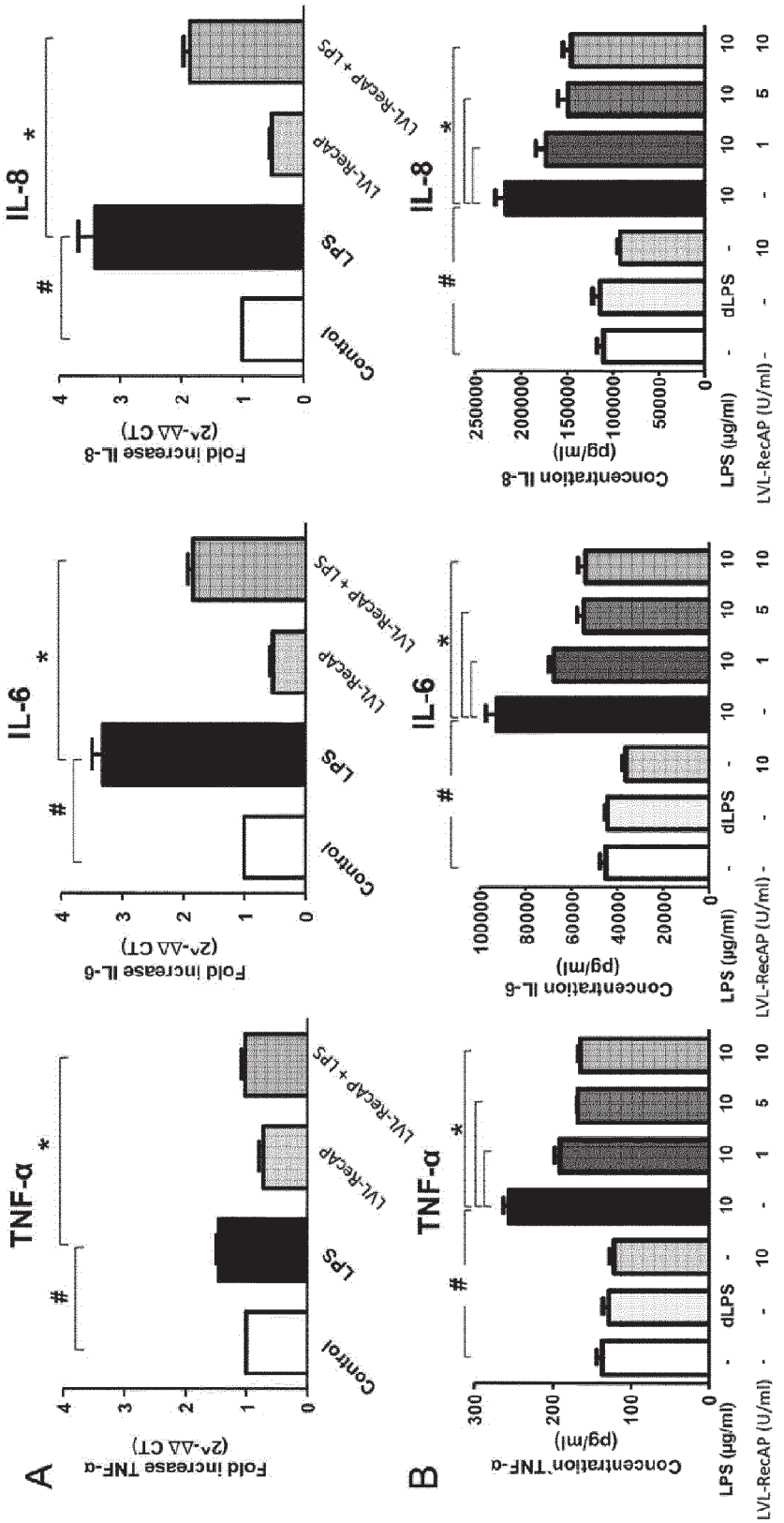

A
TNF-α
Fold increase TNF-α (2^-ΔΔ CT)
IL-6
Fold increase IL-6 (2^-ΔΔ CT)
IL-8
Fold increase IL-8 (2^-ΔΔ CT)
Control
LPS
LVL-RecAP
LVL-RecAP + LPS
B
Concentration TNF-α (pg/ml)
Concentration IL-6 (pg/ml)
Concentration IL-8 (pg/ml)
LPS (µg/ml) - dLPS - 10 - 1 5 10
LVL-RecAP (U/ml) - - 10 - 1 5 10

FIG. 12

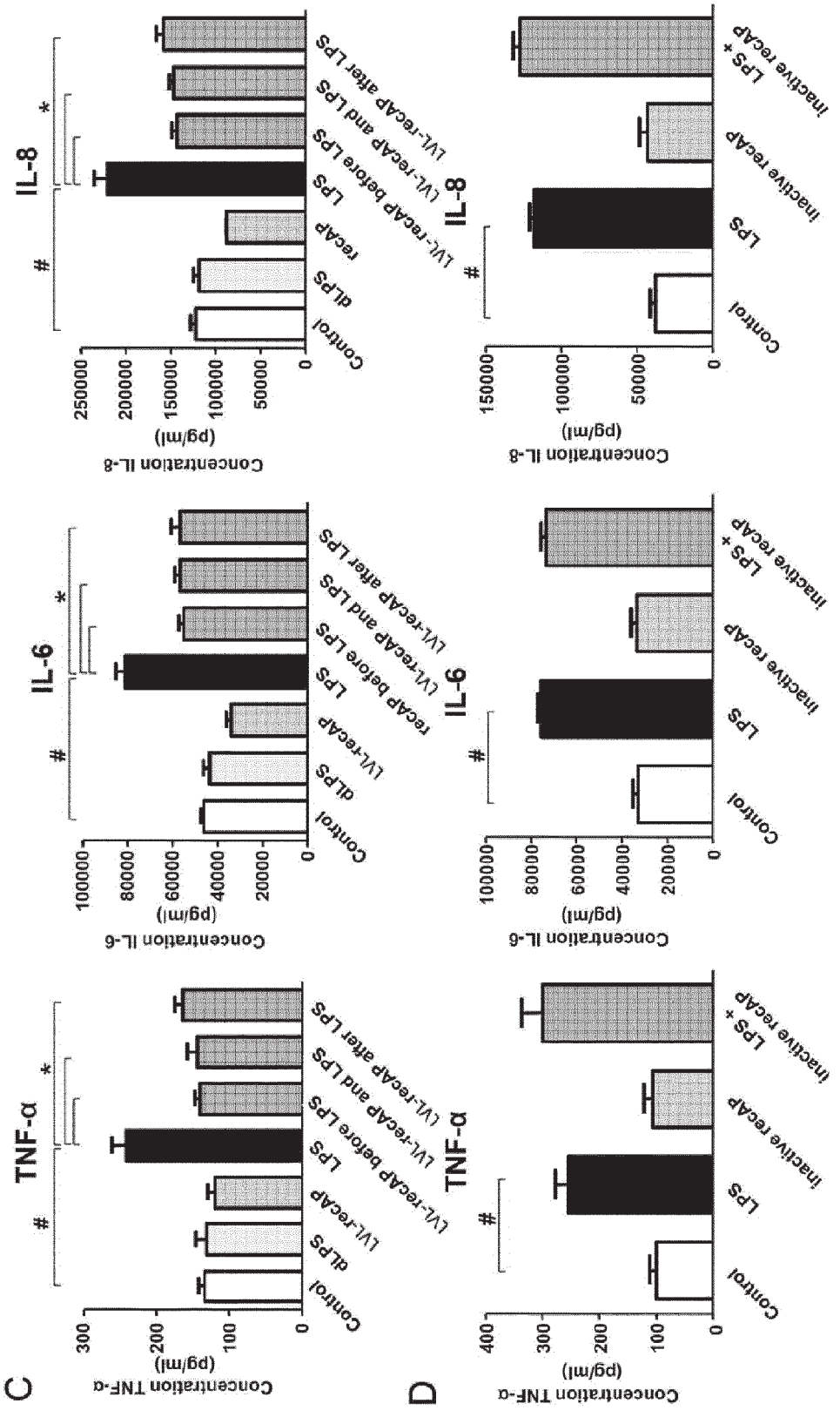

C
D
TNF-α
IL-6
IL-8
Concentration TNF-α (pg/ml)
Concentration IL-6 (pg/ml)
Concentration IL-8 (pg/ml)
Control
dLPS
LVL-recAP
recAP
LPS
LVL-recAP before LPS
LVL-recAP and LPS
LVL-recAP after LPS
Inactive recAP
LPS + Inactive recAP
#
*

FIG. 12, Cont'd

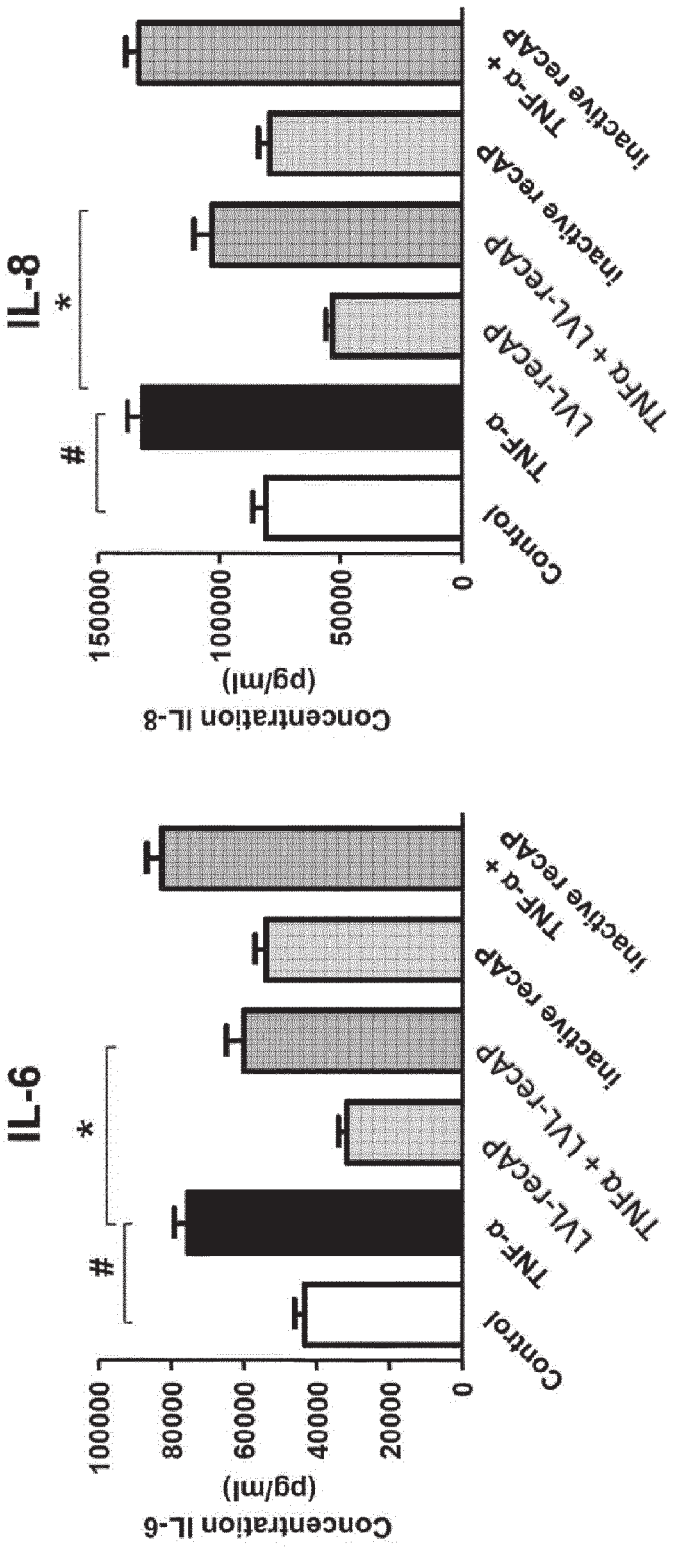

IL-8
Concentration IL-8 (pg/ml)
150000
100000
50000
0
#
*
Control
TNF-α
LVL-rECAP
TNFα + LVL-rECAP
inactive recAP
TNF-α + inactive recAP
IL-6
Concentration IL-6 (pg/ml)
100000
80000
60000
40000
20000
0
#
*
Control
TNF-α
LVL-rECAP
TNFα + LVL-rECAP
inactive recAP
TNF-α + inactive recAP

FIG. 13A

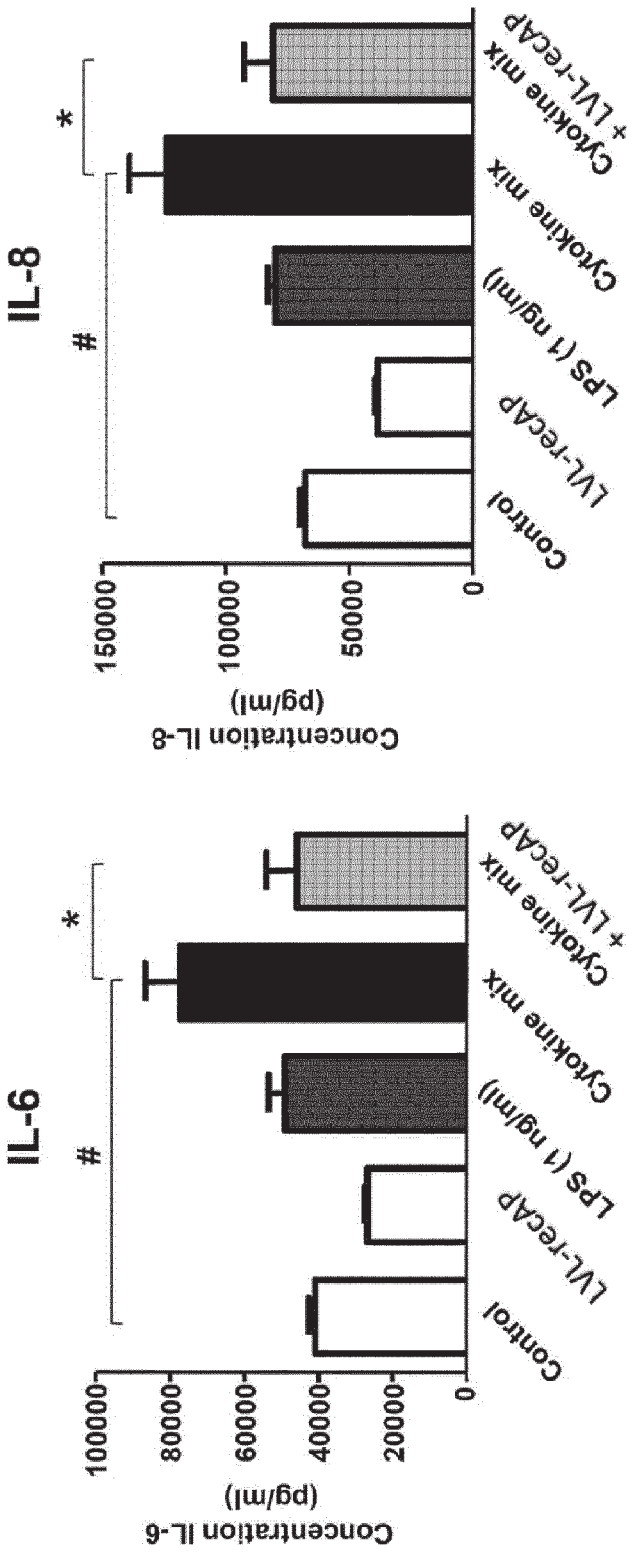

IL-8
Concentration IL-8 (pg/ml)
150000
100000
50000
0
Control
LVL-rECAP
LPS (1 ng/ml)
Cytokine mix
Cytokine mix + LVL-rECAP
#
*
IL-6
Concentration IL-6 (pg/ml)
100000
80000
60000
40000
20000
0
Control
LVL-rECAP
LPS (1 ng/ml)
Cytokine mix
Cytokine mix + LVL-rECAP
#
*

FIG. 13B

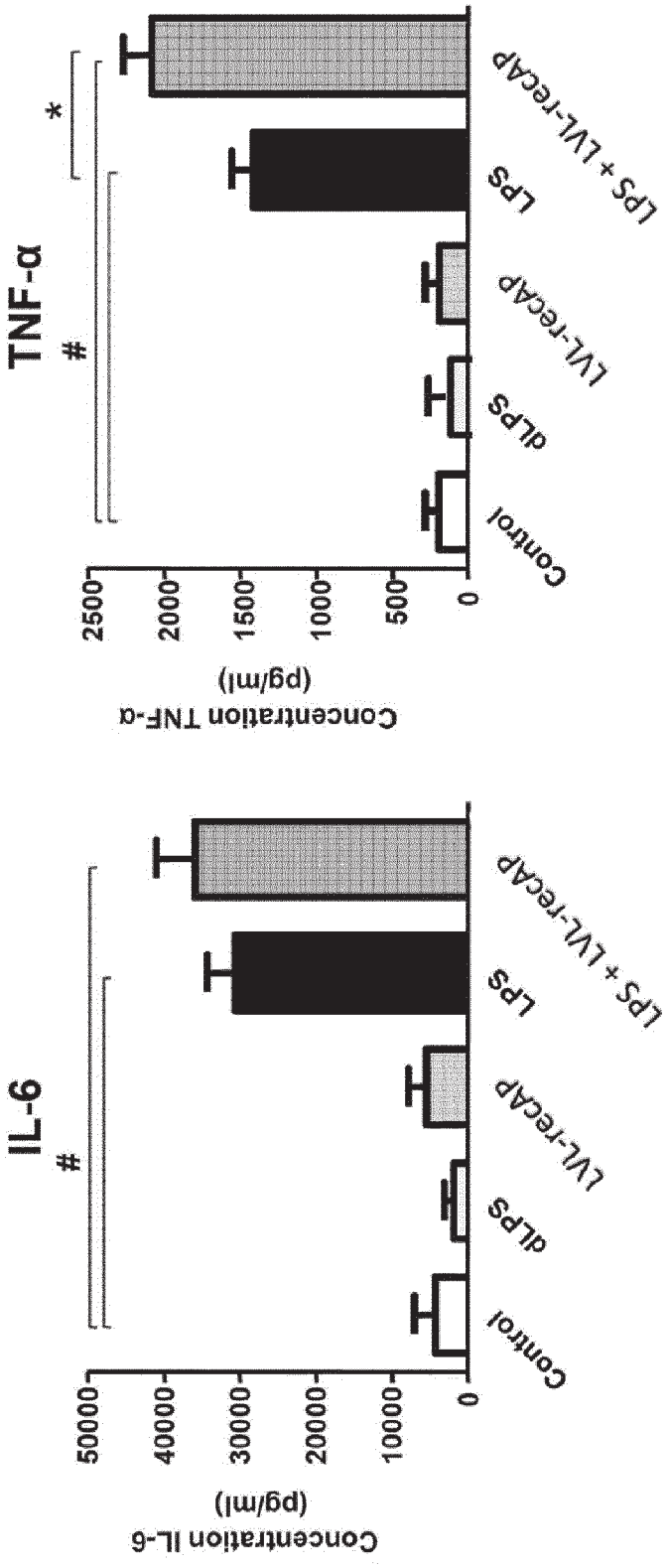

TNF-α
Concentration TNF-α (pg/ml)
2500
2000
1500
1000
500
0
Control
dLPS
LVL-reCAP
LPS
LPS + LVL-reCAP
#
*
IL-6
Concentration IL-6 (pg/ml)
50000
40000
30000
20000
10000
0
Control
dLPS
LVL-reCAP
LPS
LPS + LVL-reCAP
#

FIG. 13C

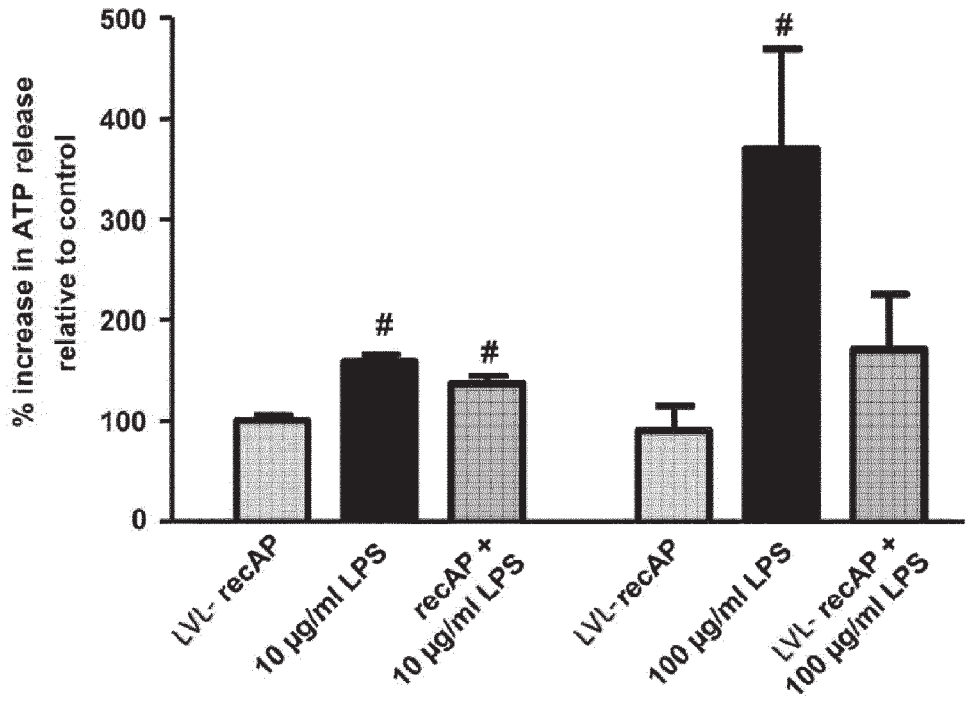

% increase in ATP release relative to control
500
400
300
200
100
0
#
#
#
LVL- recAP
10 µg/ml LPS
recAP + 10 µg/ml LPS
LVL-recAP
100 µg/ml LPS
LVL- recAP + 100 µg/ml LPS

FIG. 14

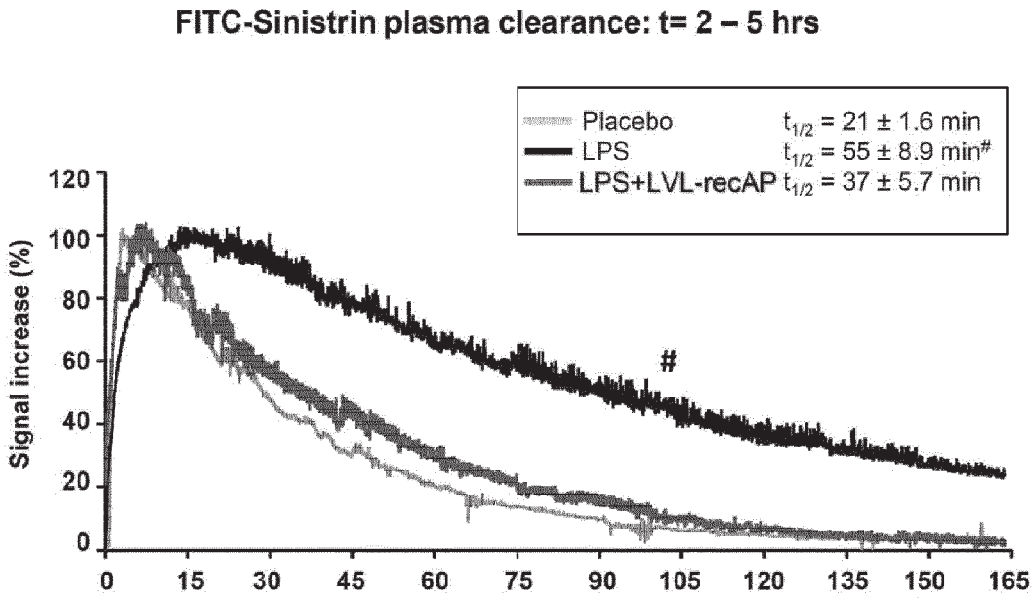


FIG. 15A

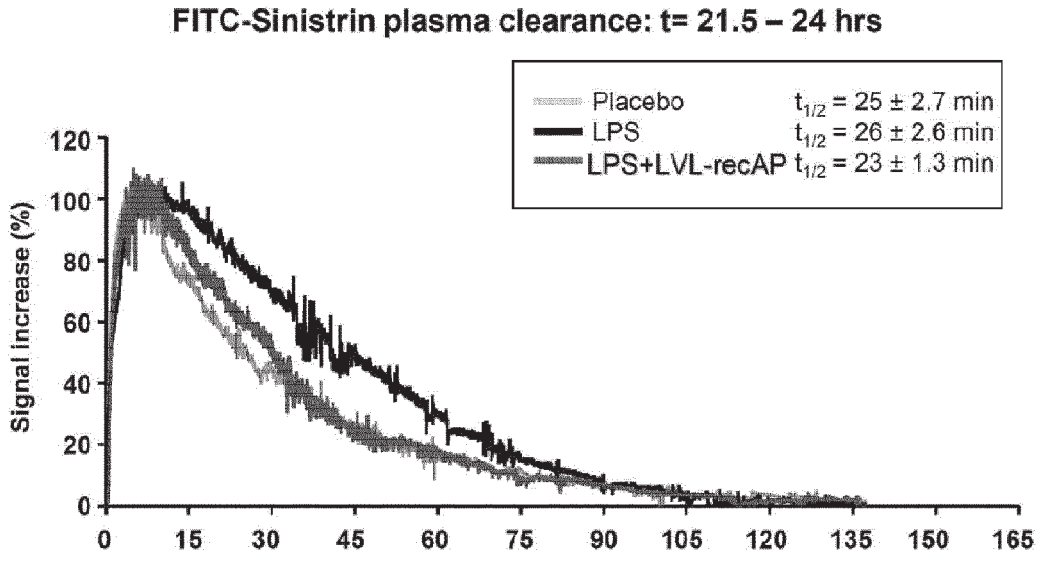


FIG. 15B

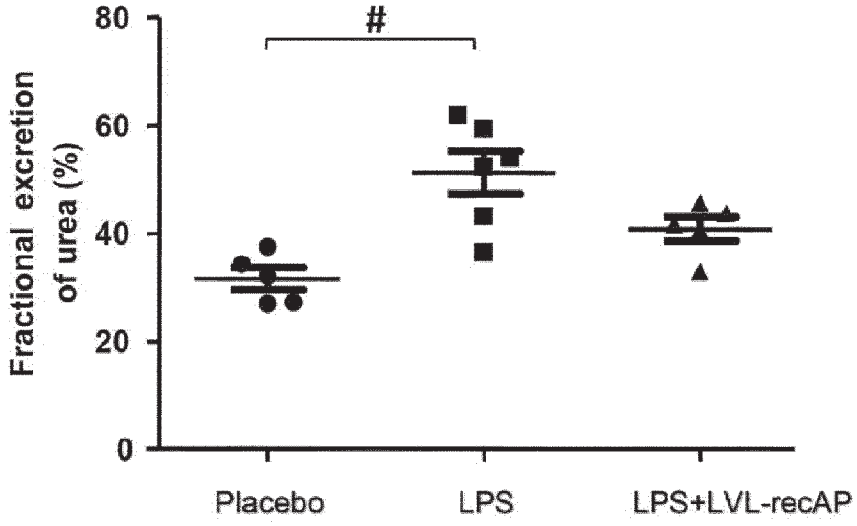


FIG. 15C

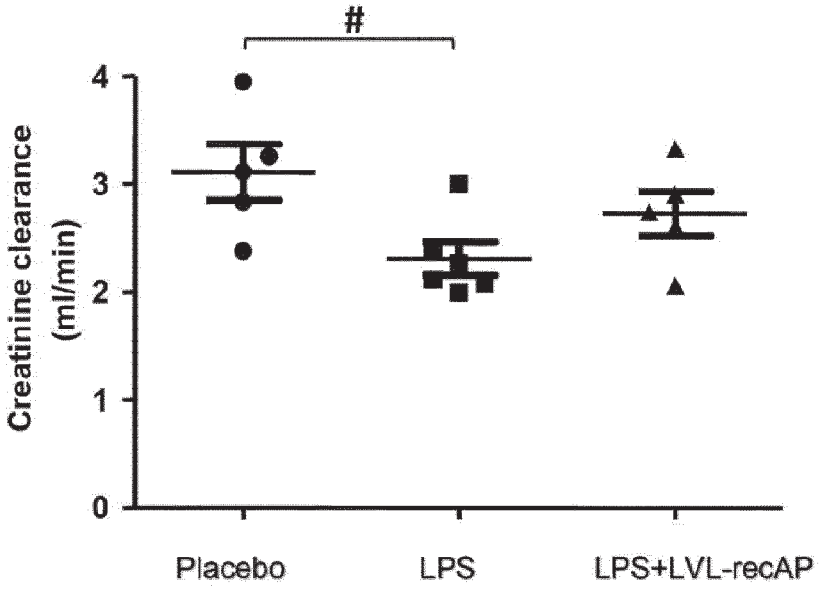


FIG. 15D

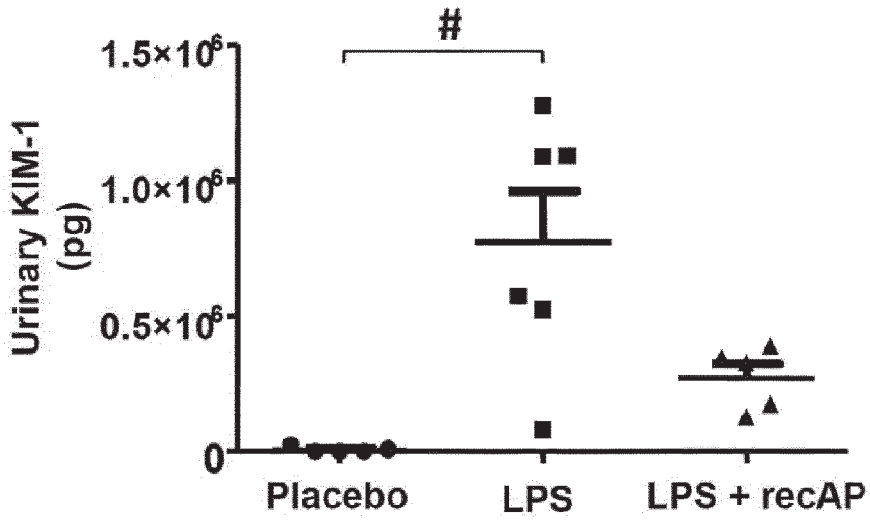


FIG. 16A

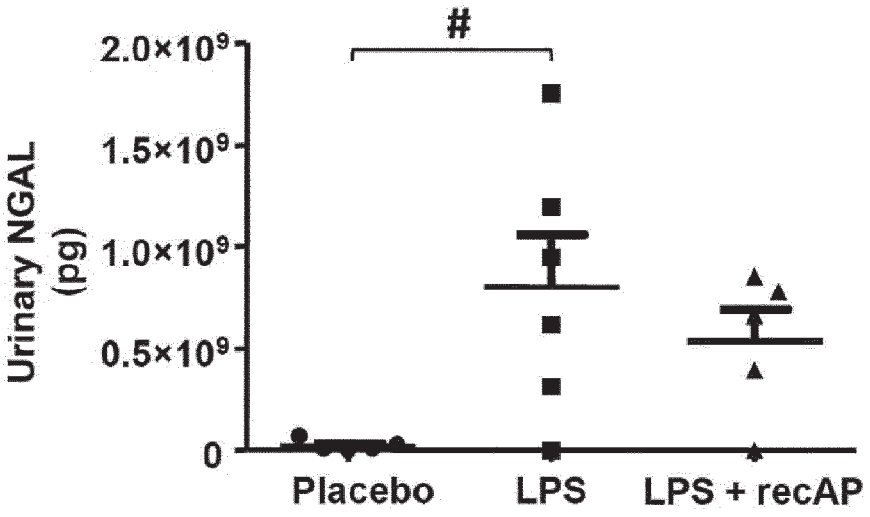


FIG. 16B

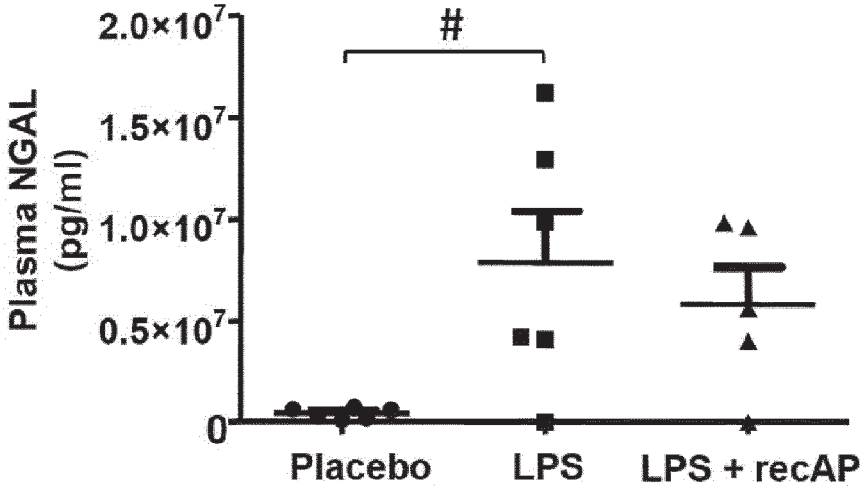


FIG. 16C

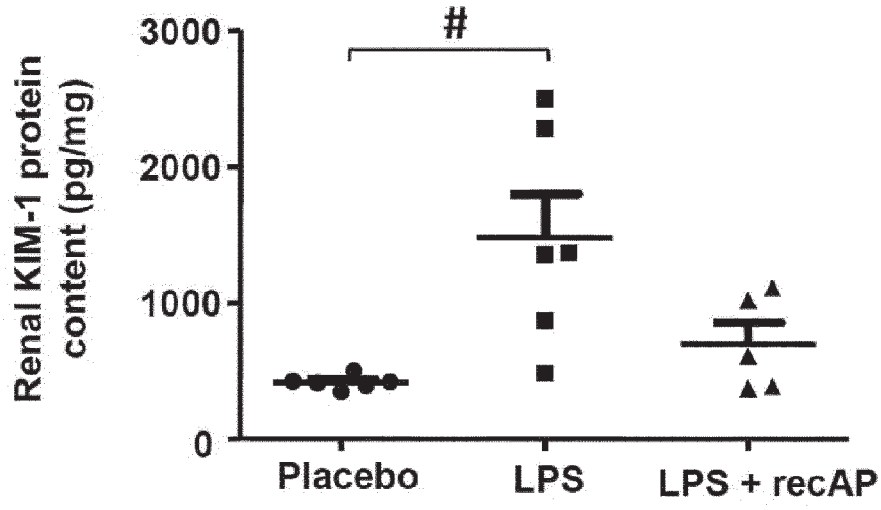


FIG. 16D

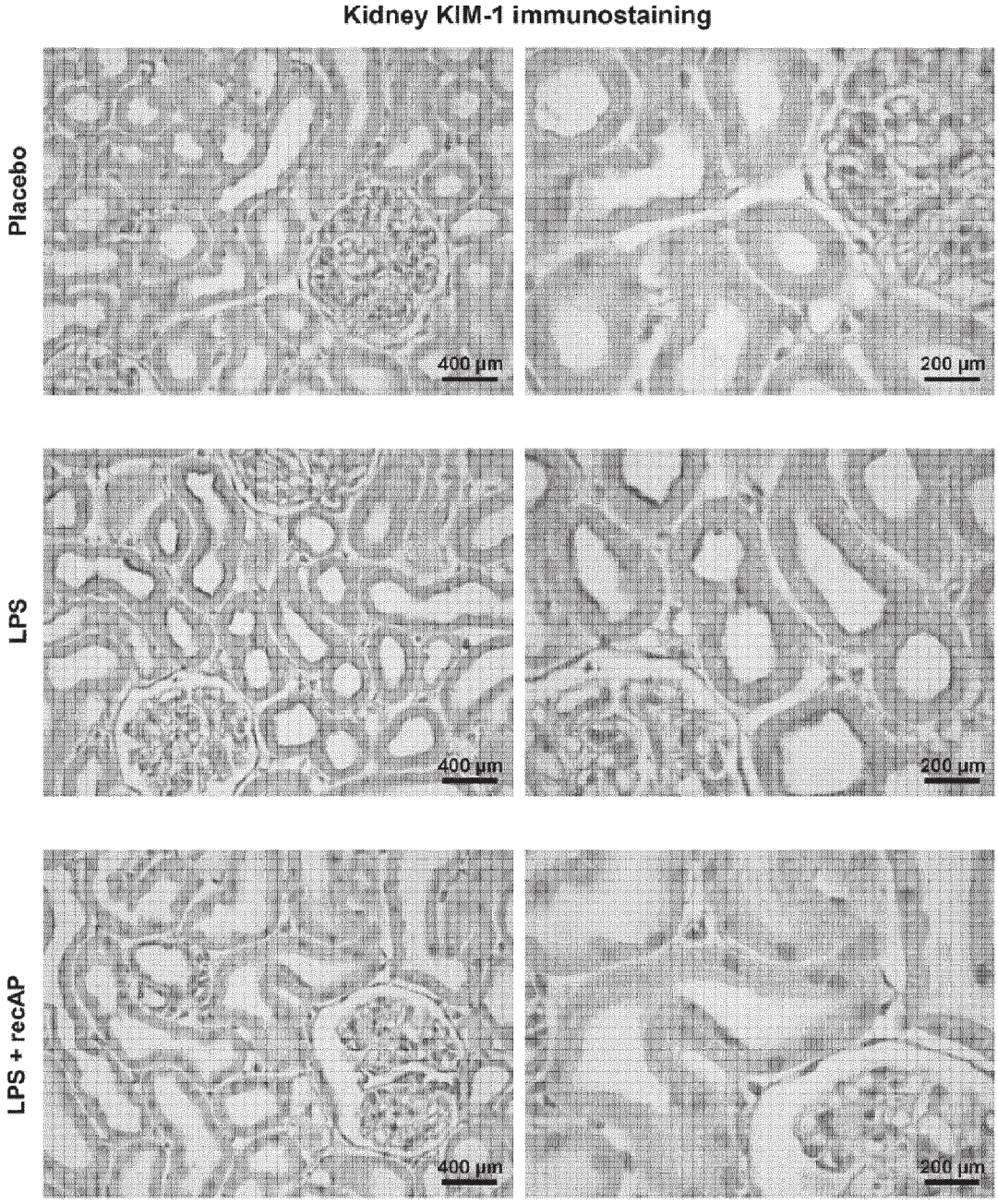

Kidney KIM-1 immunostaining
Placebo
LPS
LPS + recAP
400 µm
200 µm
400 µm
200 µm
400 µm
200 µm

FIG. 16E

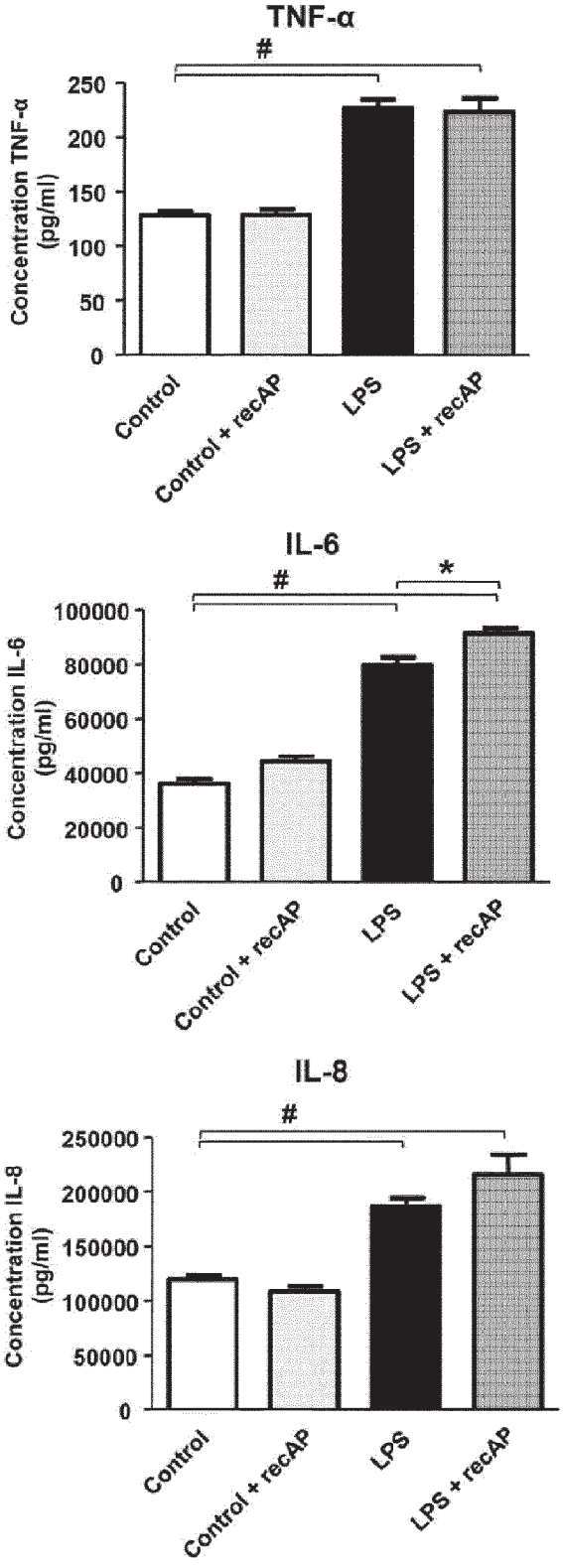

TNF-α
#
Concentration TNF-α (pg/ml)
250
200
150
100
50
0
Control
Control + recAP
LPS
LPS + recAP
IL-6
#
*
Concentration IL-6 (pg/ml)
100000
80000
60000
40000
20000
0
Control
Control + recAP
LPS
LPS + recAP
IL-8
#
Concentration IL-8 (pg/ml)
250000
200000
150000
100000
50000
0
Control
Control + recAP
LPS
LPS + recAP

FIG. 17

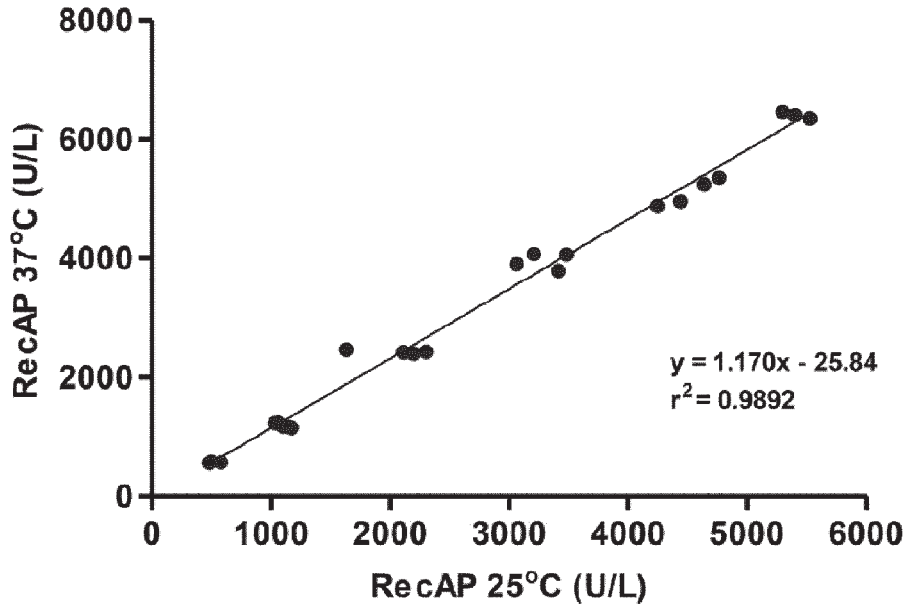

8000
6000
4000
2000
0
RecAP 37°C (U/L)
0
1000
2000
3000
4000
5000
6000
RecAP 25°C (U/L)
y = 1.170x - 25.84
r2 = 0.9892

FIG. 18

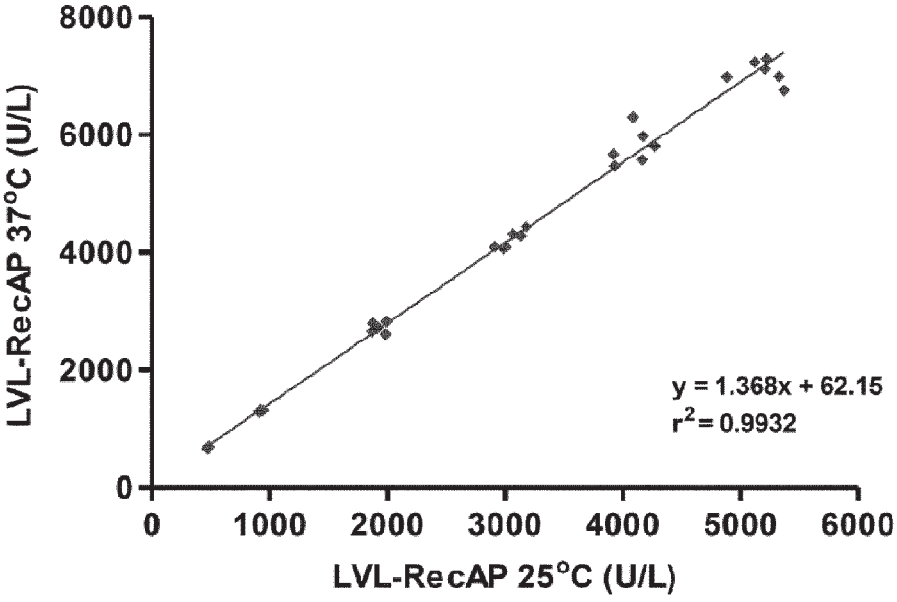

8000
6000
4000
2000
0
LVL-RecAP 37°C (U/L)
0
1000
2000
3000
4000
5000
6000
LVL-RecAP 25°C (U/L)
y = 1.368x + 62.15
r2 = 0.9932

FIG. 19

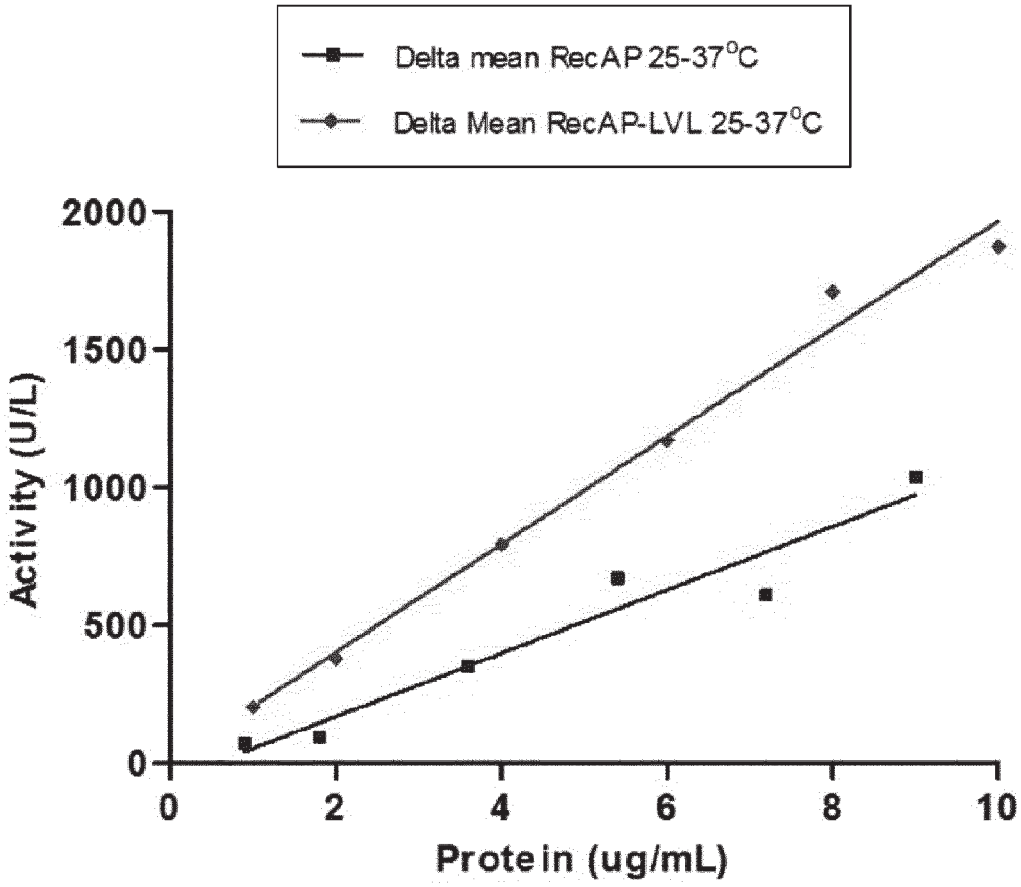

Delta mean RecAP 25-37°C
Delta Mean RecAP-LVL 25-37°C
Activity (U/L)
2000
1500
1000
500
0
0
2
4
6
8
10
Protein (ug/mL)

FIG. 20