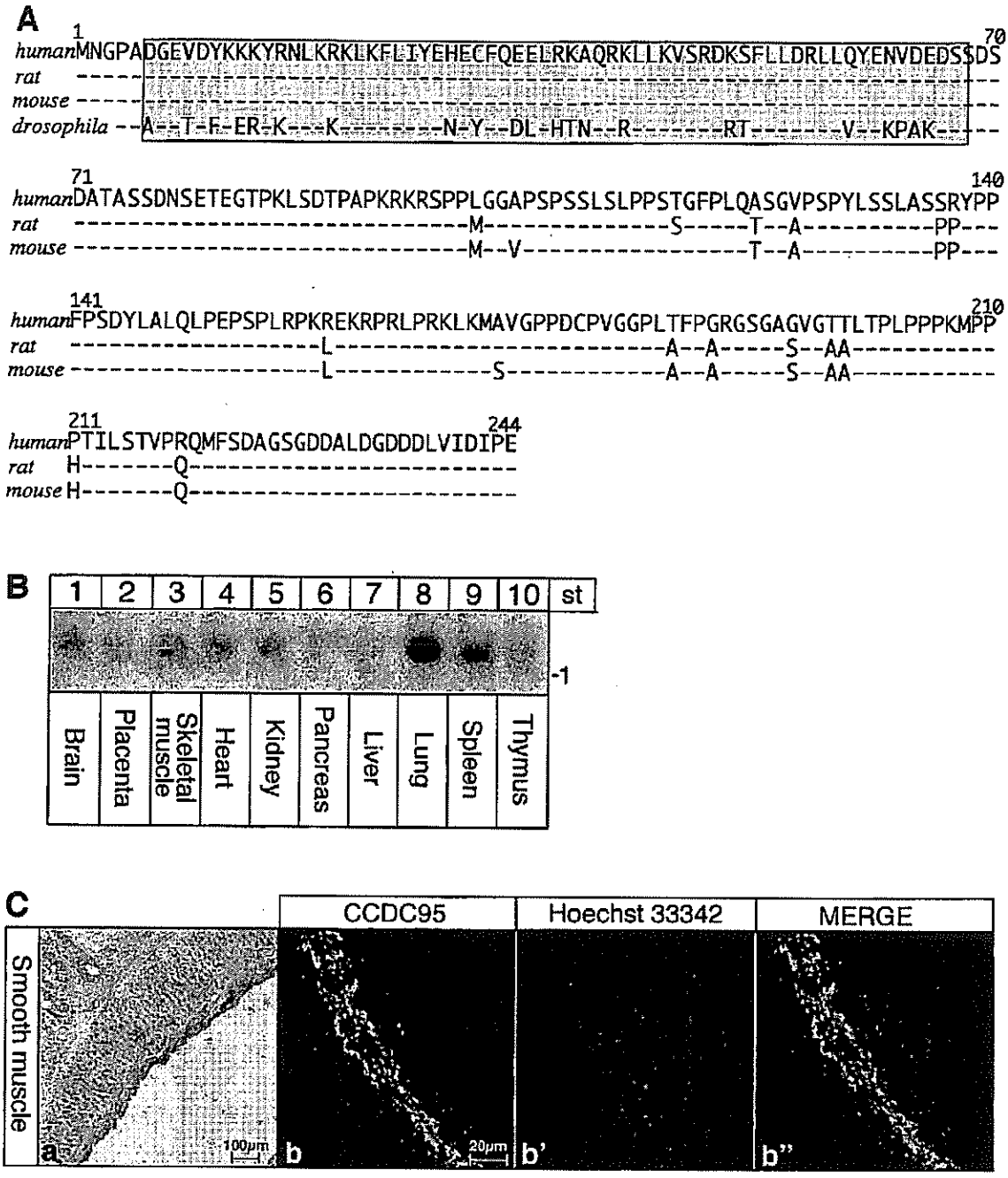
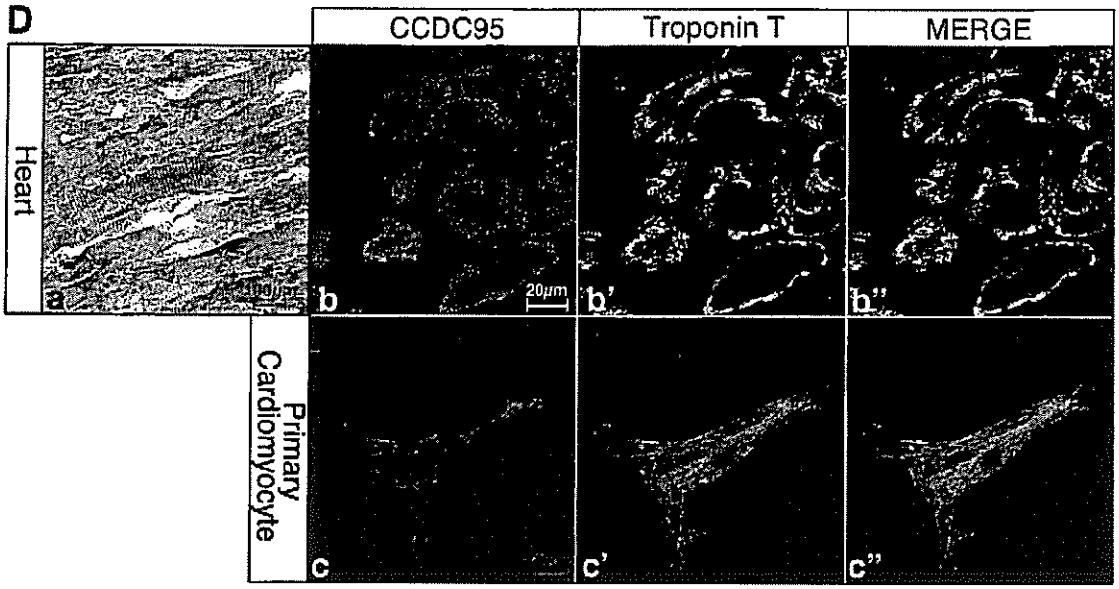

A
1
70
human MNGPADGEVDYKKKYRNLKRKLKFLIYEHECFQEELRKAQRKLLKVSRDKSFLLDRLLQYENVDEDSSDS
rat
mouse
drosophila --A--T-F-ER-K---K---------N-Y--DL-HTN--R-------RT--------V--KPAK-----
71
140
human DATASSDNSETEGTPKLSDTPAPKRKRSPPLGGAPSPSSLSLPPSTGFPLQASGVPSPYLSSLASSRYPP
rat ----------M----------S-----T--A----------PP---
mouse ----------M--V----------T--A----------PP---
141
210
human FPSDYLALQLPEPSPLRPKREKRPRLPRKLKMAVGPPDCPVGGPLTFPGRGSGAGVGTTLTPLPPPKMPP
rat ----------L----------A--A-----S--AA----------
mouse ----------L----------S----------A--A-----S--AA----------
211
244
human PTILSTVPRQMFSDAGSGDDALDGDDDLVIDIPE
rat H-------Q----------
mouse H-------Q----------
B
1 2 3 4 5 6 7 8 9 10 st
-1
Brain
Placenta
Skeletal muscle
Heart
Kidney
Pancreas
Liver
Lung
Spleen
Thymus
C
CCDC95
Hoechst 33342
MERGE
Smooth muscle
a
100µm
b
20µm
b'
b''

Fig. 1


D
CCDC95
Troponin T
MERGE
Heart
a
b
20μm
b'
b''
Primary Cardiomyocyte
c
c'
c''

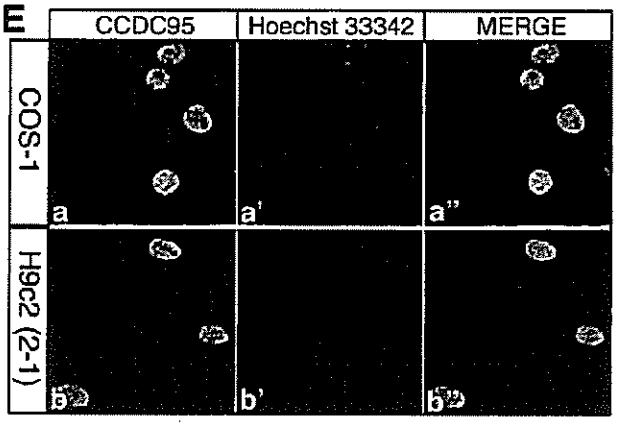
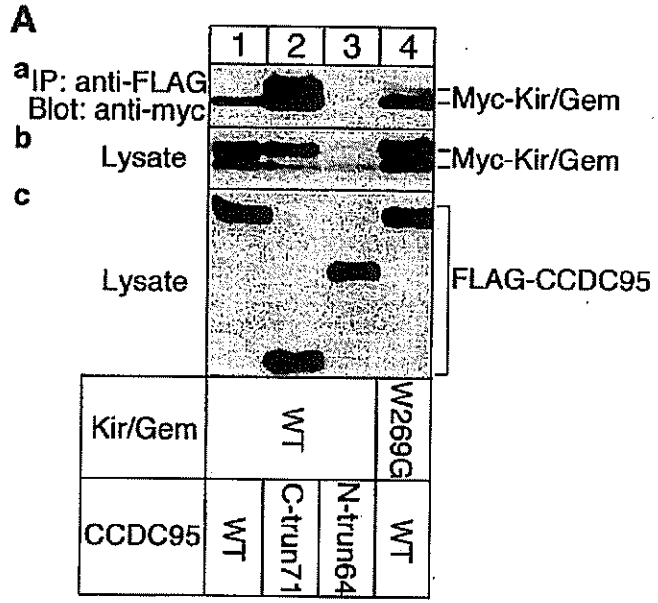

E
CCDC95
Hoechst 33342
MERGE
COS-1
a
a'
a''
H9c2 (2-1)
b
b'
b''

Fig. 1





CCDC95 mutants	β-galactosidase activity
Y11A	–
K14A	–/+
Y15A	–
R16A	–/+
L18A	–
K19A	–
K21A	–
L22A	–
F24A	–/+
F32A	–
K50A	–
L54A	–/+
L58A	–
Y60A	–
E61A	–

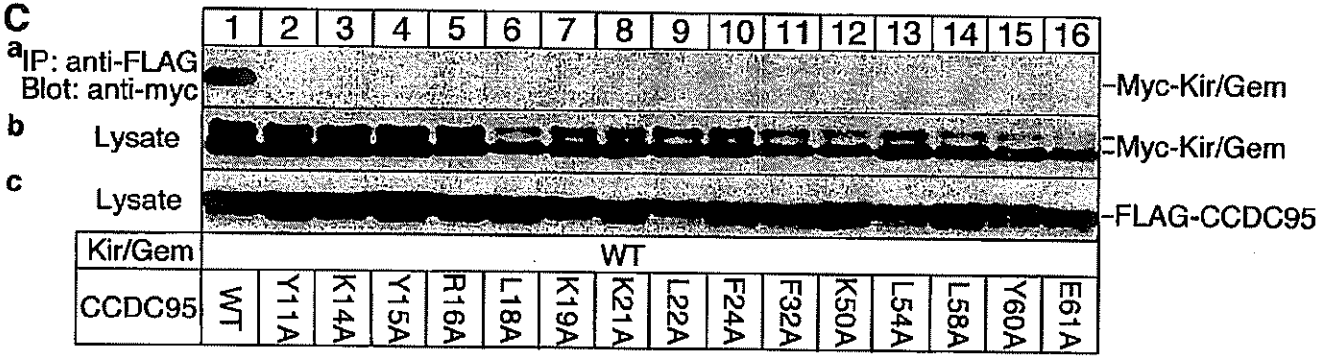


Fig. 2

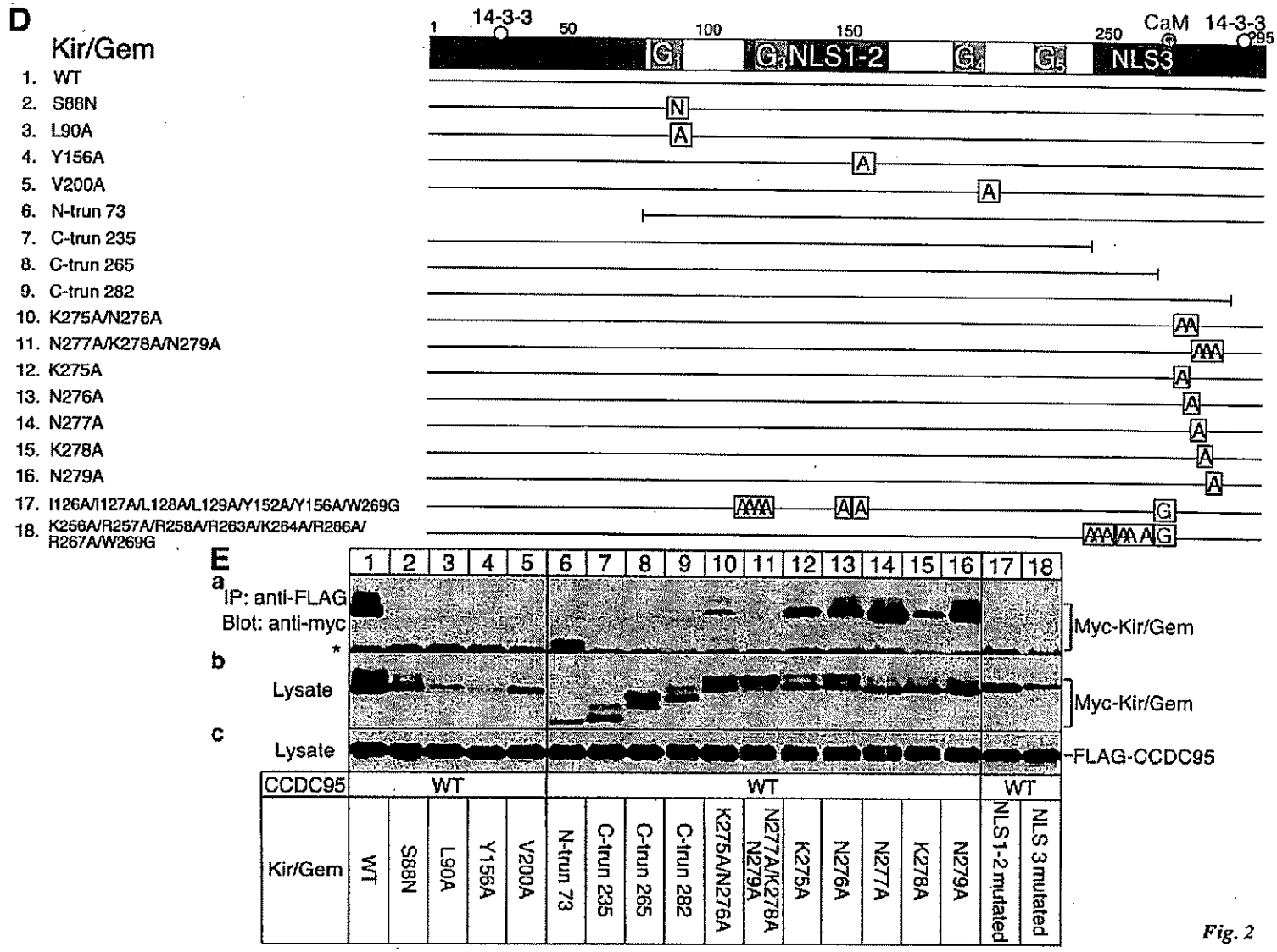
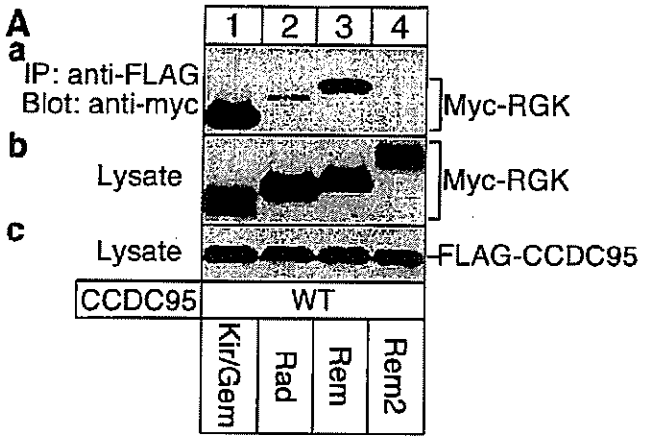

D
Kir/Gem
1. WT
2. S88N
3. L90A
4. Y156A
5. V200A
6. N-trun 73
7. C-trun 235
8. C-trun 265
9. C-trun 282
10. K275A/N276A
11. N277A/K278A/N279A
12. K275A
13. N276A
14. N277A
15. K278A
16. N279A
17. I126A/I127A/L128A/L129A/Y152A/Y156A/W269G
18. K256A/R257A/R258A/R263A/K264A/R266A/R267A/W269G
1
14-3-3
50
100
150
250
CaM
14-3-3
295
G1
G3
NLS1-2
G4
G5
NLS3
E
a
IP: anti-FLAG
Blot: anti-myc
b
Lysate
c
Lysate
Myc-Kir/Gem
Myc-Kir/Gem
FLAG-CCDC95
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
CCDC95
WT
WT
WT
Kir/Gem
WT
S88N
L90A
Y156A
V200A
N-trun 73
C-trun 235
C-trun 265
C-trun 282
K275A/N276A
N277A/K278A/N279A
K275A
N276A
N277A
K278A
N279A
NLS1-2 mutated
NLS 3 mutated

Fig. 2

4/21


A
a
IP: anti-FLAG
Blot: anti-myc
1
2
3
4
Myc-RGK
b
Lysate
Myc-RGK
c
Lysate
FLAG-CCDC95
CCDC95
WT
Kir/Gem
Rad
Rem
Rem2

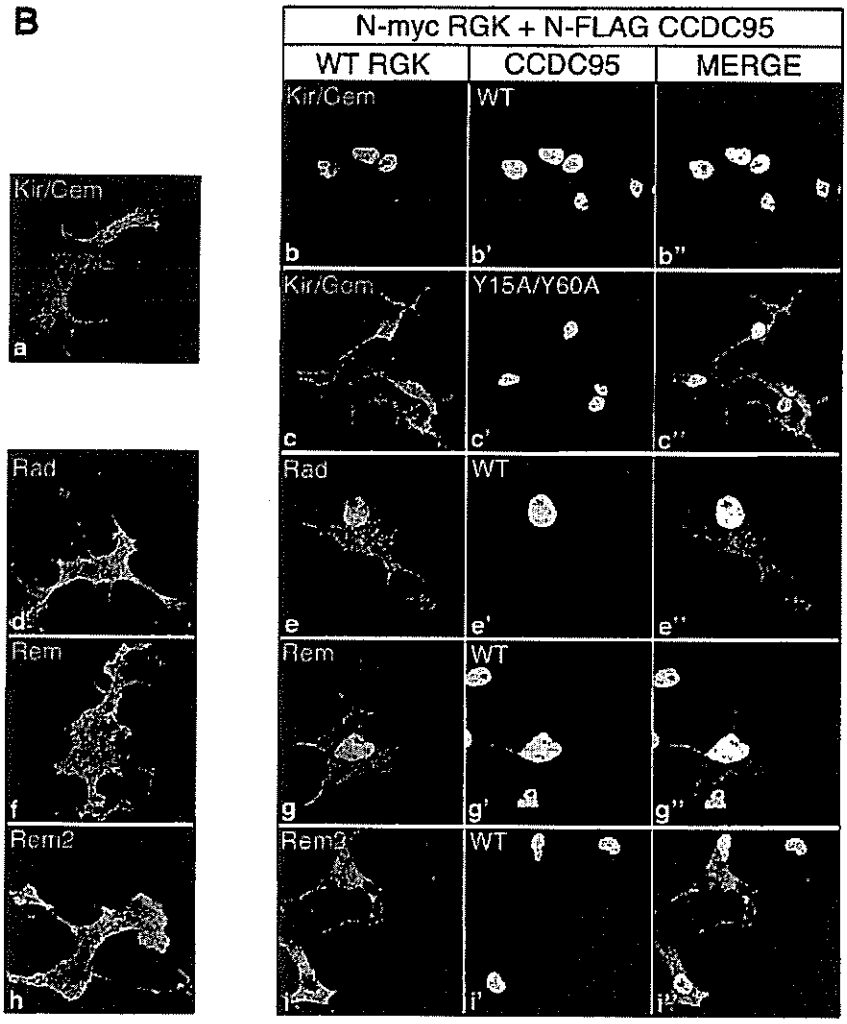
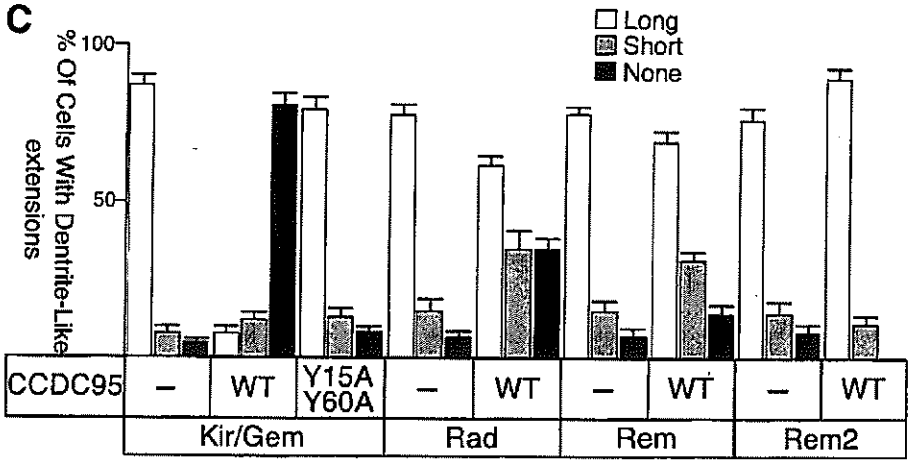

B
N-myc RGK + N-FLAG CCDC95
WT RGK
CCDC95
MERGE
Kir/Gem
WT
Kir/Gem
Y15A/Y60A
Rad
WT
Rem
WT
Rem2
WT
Kir/Gem
Rad
Rem
Rem2

Fig. 3


C
% Of Cells With Dentrite-Like extensions
100
50
Long
Short
None
CCDC95
–
WT
Y15A Y60A
–
WT
–
WT
–
WT
Kir/Gem
Rad
Rem
Rem2

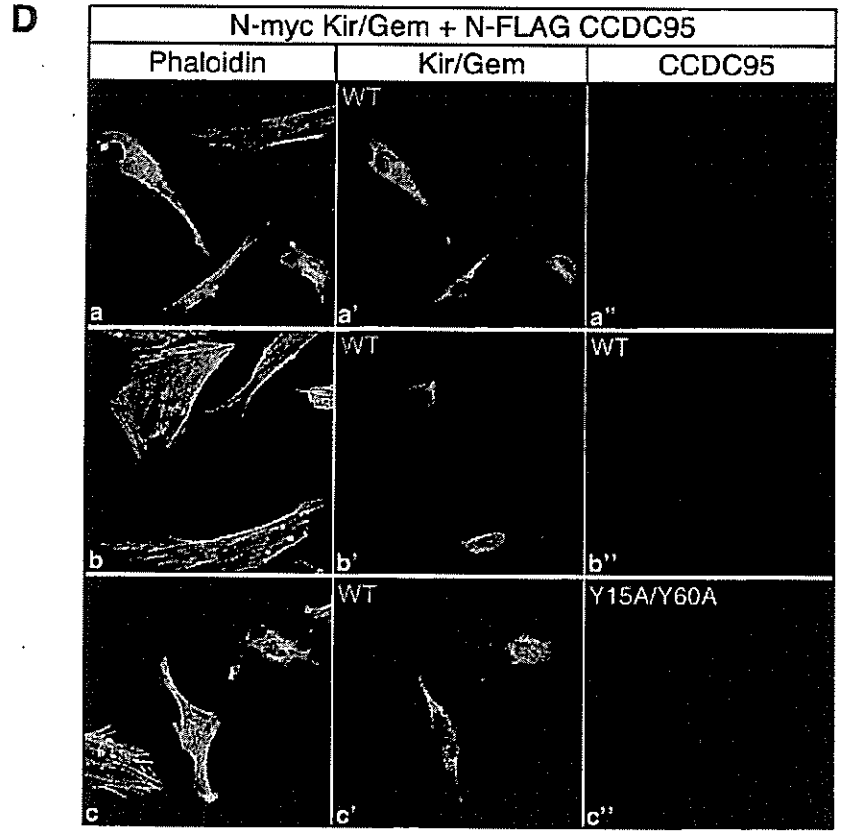

D
N-myc Kir/Gem + N-FLAG CCDC95
Phaloidin
Kir/Gem
CCDC95
WT
a
a'
a''
WT
WT
b
b'
b''
WT
Y15A/Y60A
c
c'
c''

Fig. 3

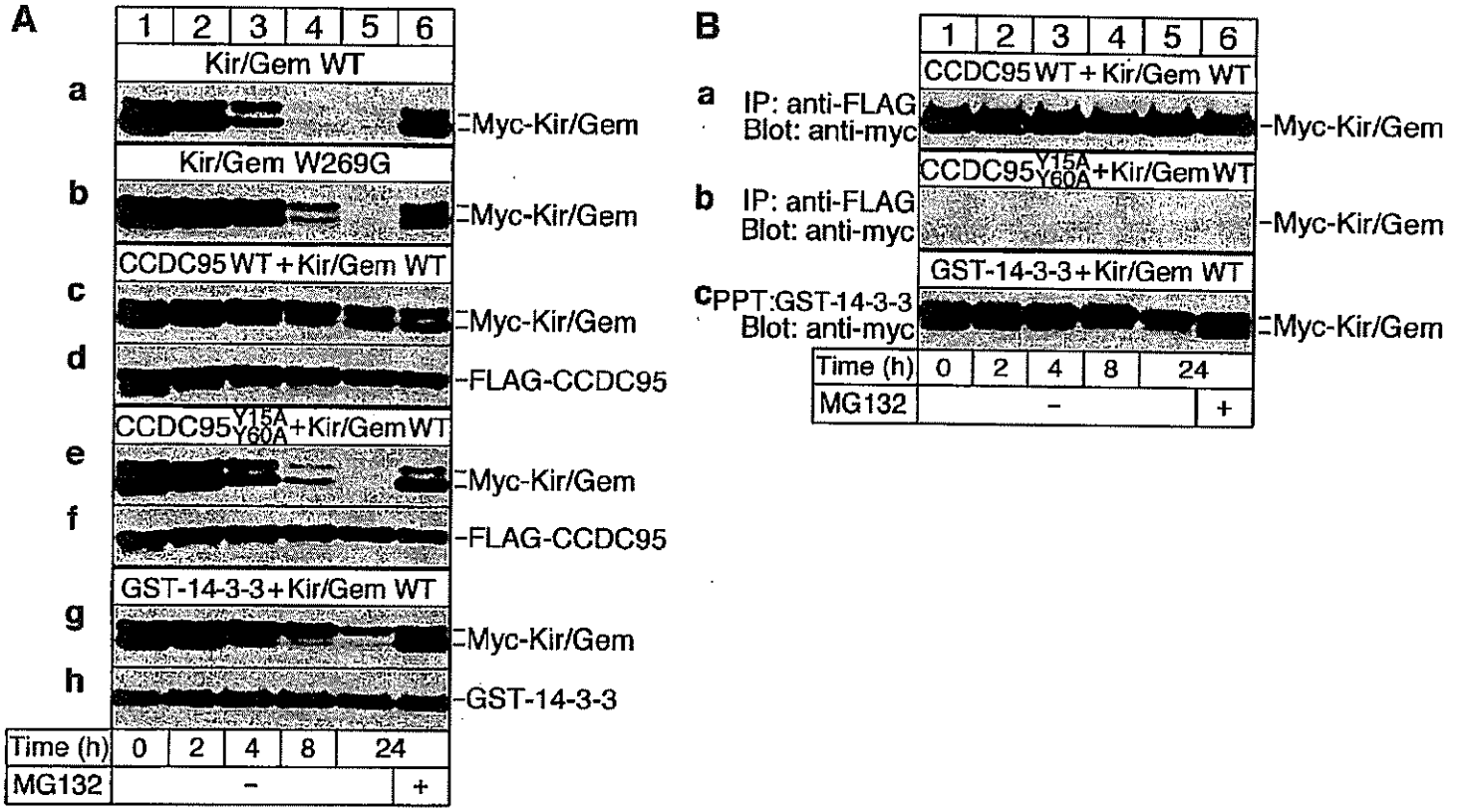

A
1 2 3 4 5 6
Kir/Gem WT
a
Myc-Kir/Gem
Kir/Gem W269G
b
Myc-Kir/Gem
CCDC95 WT + Kir/Gem WT
c
Myc-Kir/Gem
d
FLAG-CCDC95
CCDC95 Y15A Y60A + Kir/Gem WT
e
Myc-Kir/Gem
f
FLAG-CCDC95
GST-14-3-3 + Kir/Gem WT
g
Myc-Kir/Gem
h
GST-14-3-3
Time (h) 0 2 4 8 24
MG132 - +
B
1 2 3 4 5 6
CCDC95 WT + Kir/Gem WT
a IP: anti-FLAG
Blot: anti-myc
Myc-Kir/Gem
CCDC95 Y15A Y60A + Kir/Gem WT
b IP: anti-FLAG
Blot: anti-myc
Myc-Kir/Gem
GST-14-3-3 + Kir/Gem WT
c PPT:GST-14-3-3
Blot: anti-myc
Myc-Kir/Gem
Time (h) 0 2 4 8 24
MG132 - +

Fig. 4

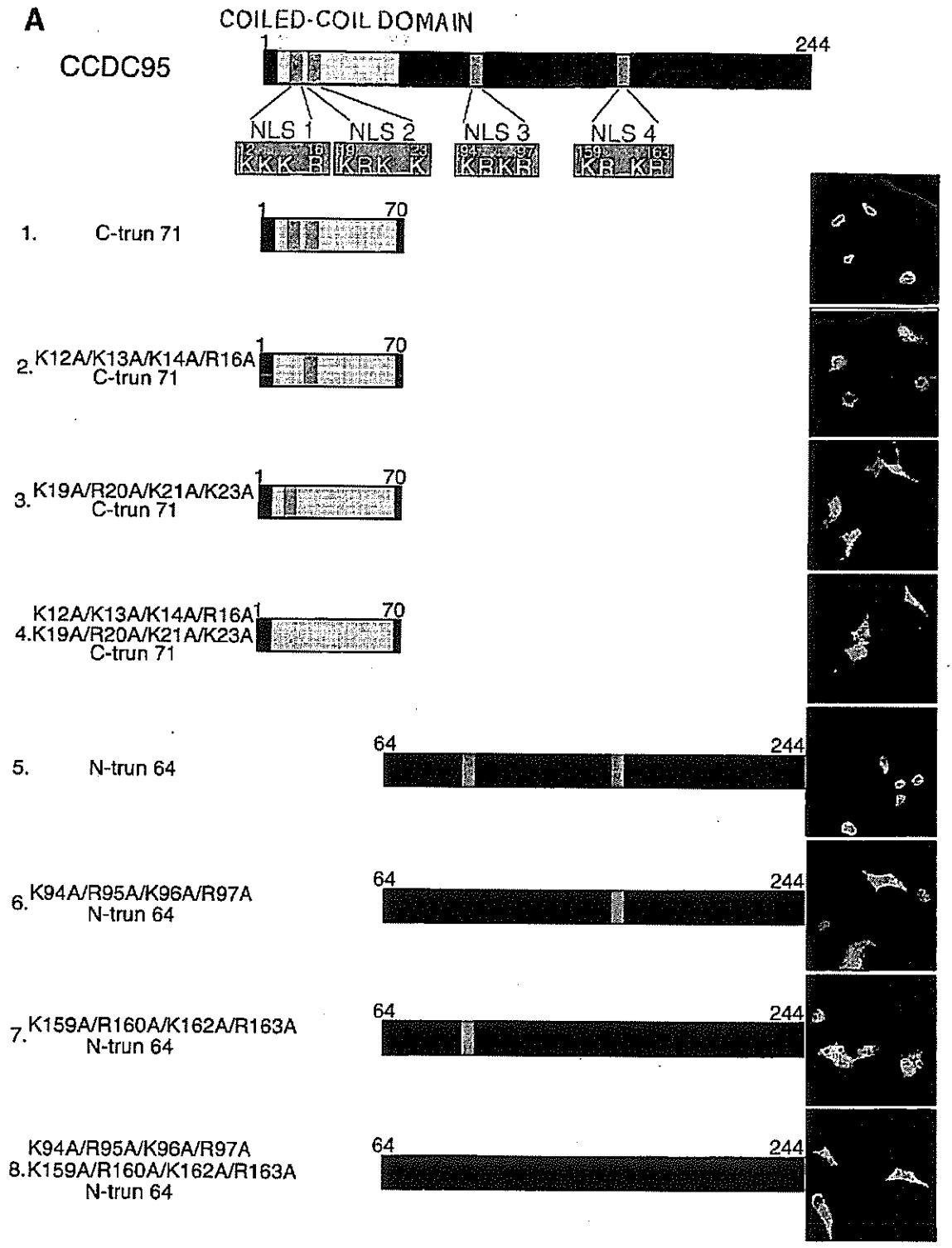
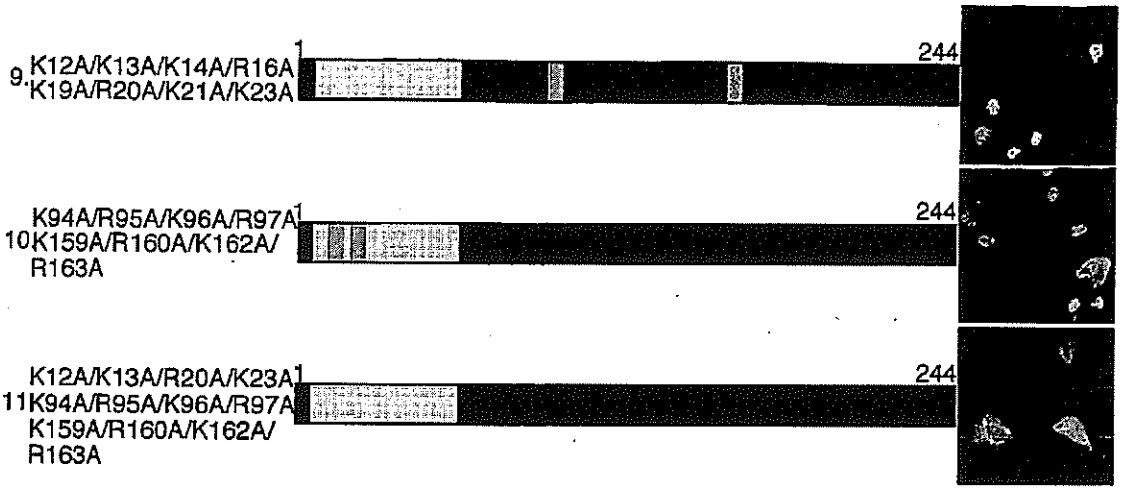
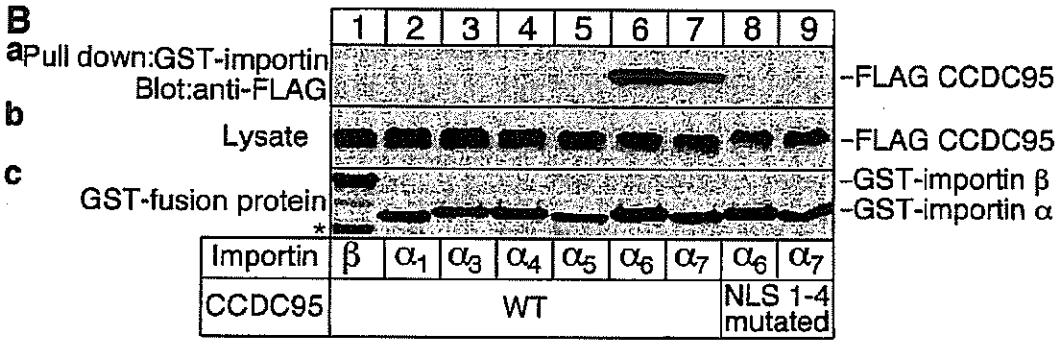

A
COILED-COIL DOMAIN
CCDC95
1
244
NLS 1
NLS 2
NLS 3
NLS 4
1. C-trun 71
2. K12A/K13A/K14A/R16A C-trun 71
3. K19A/R20A/K21A/K23A C-trun 71
4. K12A/K13A/K14A/R16A K19A/R20A/K21A/K23A C-trun 71
5. N-trun 64
6. K94A/R95A/K96A/R97A N-trun 64
7. K159A/R160A/K162A/R163A N-trun 64
8. K94A/R95A/K96A/R97A K159A/R160A/K162A/R163A N-trun 64

Fig. 5


9. K12A/K13A/K14A/R16A
K19A/R20A/K21A/K23A
1
244
10 K94A/R95A/K96A/R97A
K159A/R160A/K162A/
R163A
1
244
11 K12A/K13A/R20A/K23A
K94A/R95A/K96A/R97A
K159A/R160A/K162A/
R163A
1
244


B
a Pull down:GST-importin
Blot:anti-FLAG
b Lysate
c GST-fusion protein
1 2 3 4 5 6 7 8 9
-FLAG CCDC95
-FLAG CCDC95
-GST-importin β
-GST-importin α
*
Importin β α1 α3 α4 α5 α6 α7 α6 α7
CCDC95 WT NLS 1-4 mutated

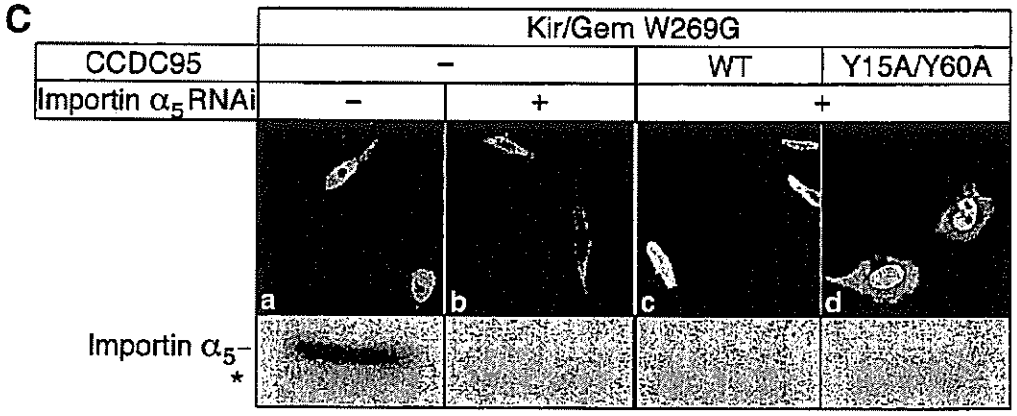

C
Kir/Gem W269G
CCDC95 – WT Y15A/Y60A
Importin α5 RNAi – + +
a
b
c
d
Importin α5–
*

Fig. 5

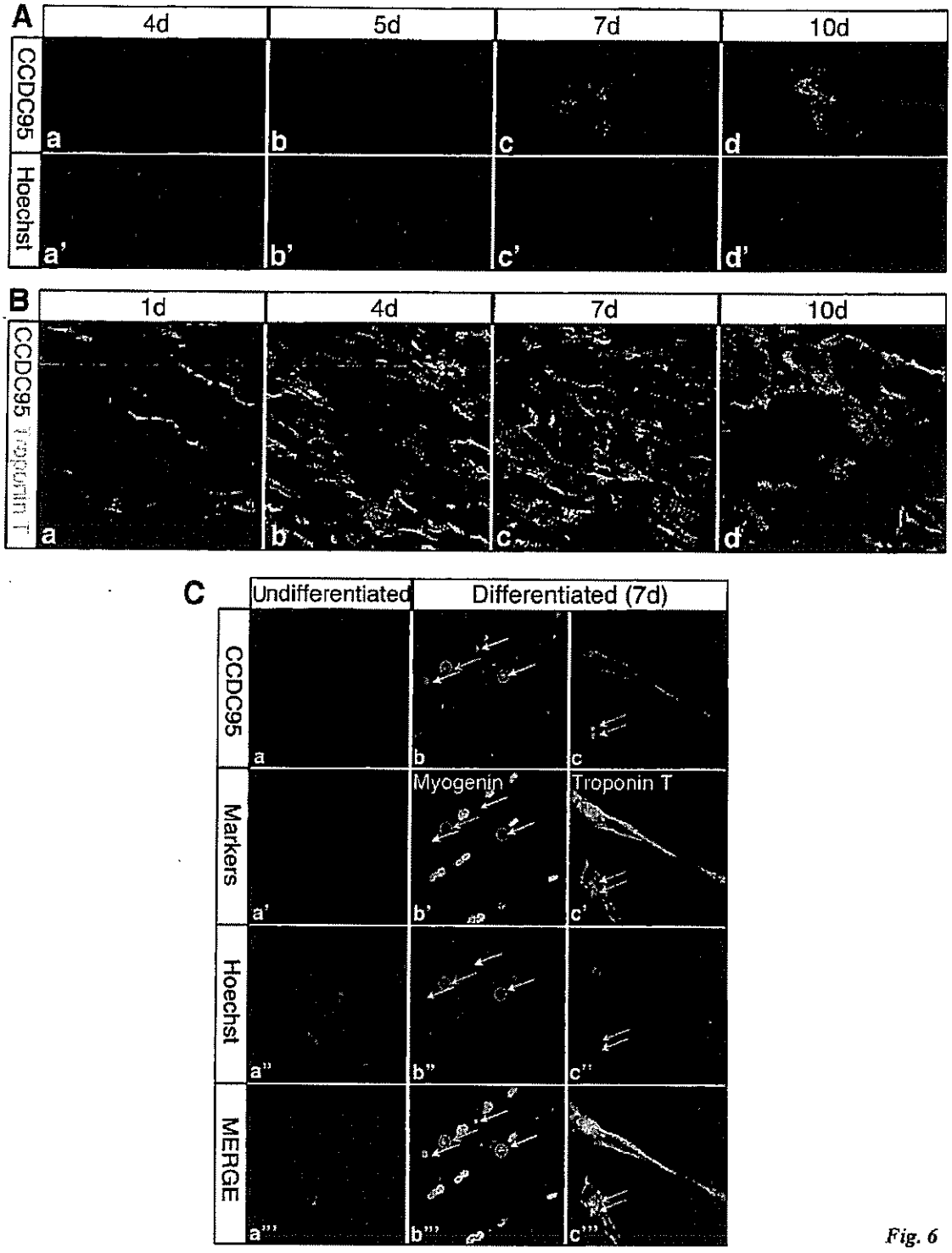
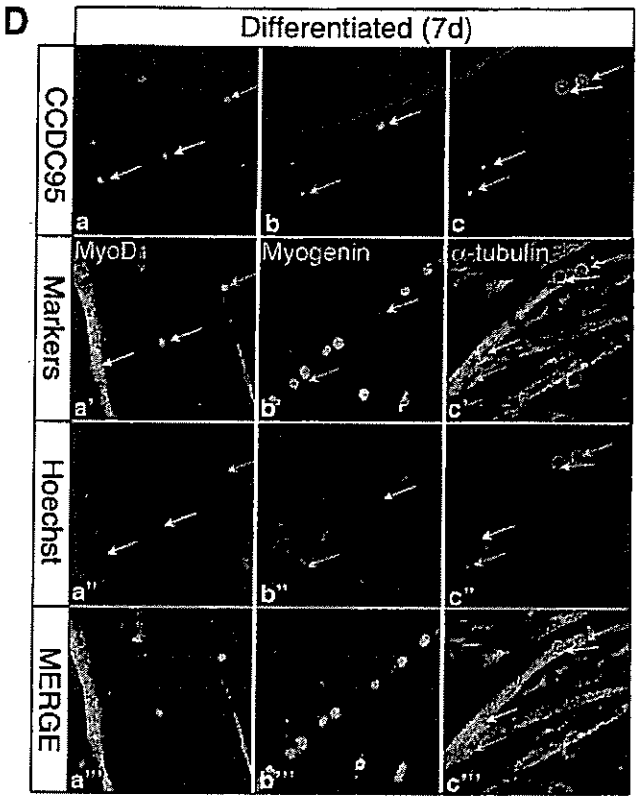

A
4d
5d
7d
10d
CCDC95
Hoechst
a
b
c
d
a'
b'
c'
d'
B
1d
4d
7d
10d
CCDC95
Troponin T
a
b
c
d
C
Undifferentiated
Differentiated (7d)
CCDC95
Markers
Hoechst
MERGE
Myogenin
Troponin T
a
b
c
a'
b'
c'
a''
b''
c''
a'''
b'''
c'''

Fig. 6


D
Differentiated (7d)
CCDC95
Markers
Hoechst
MERGE
MyoD1
Myogenin
α-tubulin
a
b
c
a'
b'
c'
a''
b''
c''
a'''
b'''
c'''

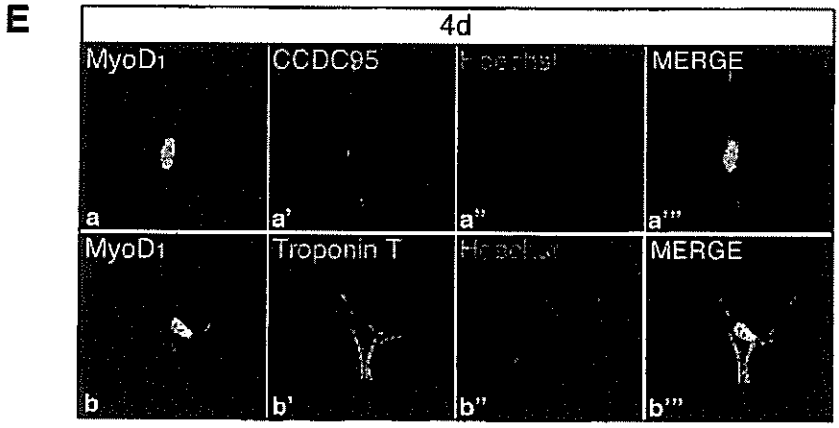

E
4d
MyoD1
CCDC95
MERGE
MyoD1
Troponin T
MERGE
a
a'
a''
a'''
b
b'
b''
b'''

Fig. 6

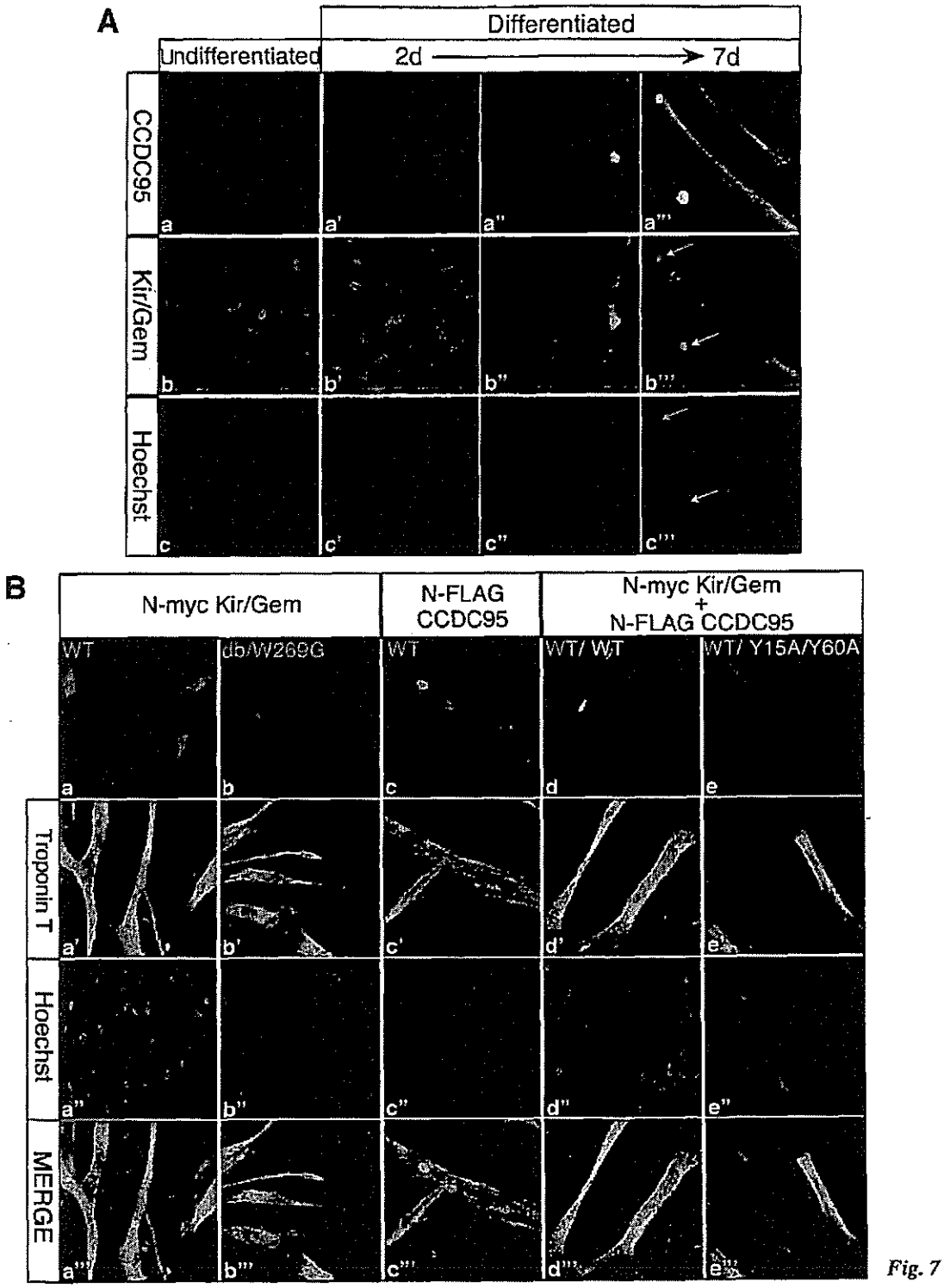
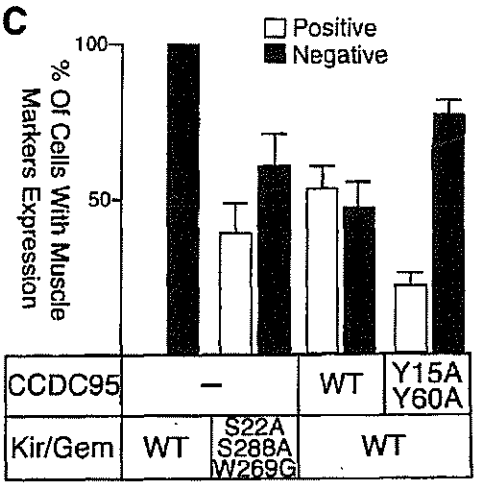

A
Differentiated
Undifferentiated
2d
7d
CCDC95
Kir/Gem
Hoechst
a
a'
a''
a'''
b
b'
b''
b'''
c
c'
c''
c'''
B
N-myc Kir/Gem
N-FLAG CCDC95
N-myc Kir/Gem + N-FLAG CCDC95
WT
db/W269G
WT
WT/ WT
WT/ Y15A/Y60A
Troponin T
Hoechst
MERGE
a
b
c
d
e
a'
b'
c'
d'
e'
a''
b''
c''
d''
e''
a'''
b'''
c'''
d'''
e'''

Fig. 7


C
Positive
Negative
100
50
% Of Cells With Muscle Markers Expression
CCDC95
–
WT
Y15A Y60A
Kir/Gem
WT
S22A S288A W269G
WT

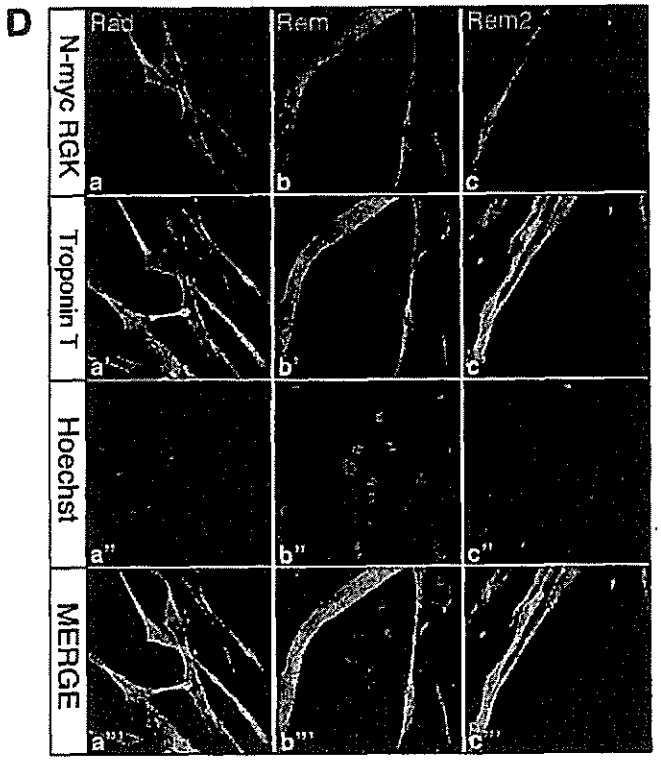
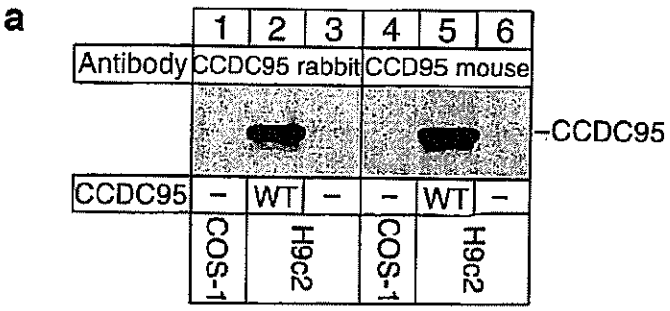

D
Rad
Rem
Rem2
N-myc RGK
Troponin T
Hoechst
MERGE
a
b
c
a'
b'
c'
a''
b''
c''
a'''
b'''
c'''

Fig.7


a
1
2
3
4
5
6
Antibody
CCDC95 rabbit
CCD95 mouse
CCDC95
CCDC95
–
WT
–
–
WT
–
COS-1
H9c2
COS-1
H9c2

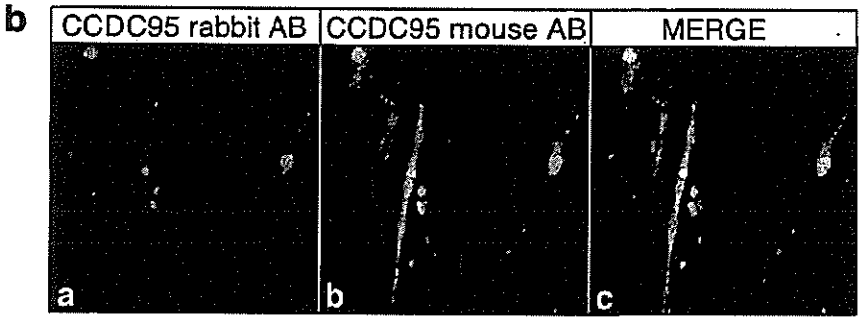
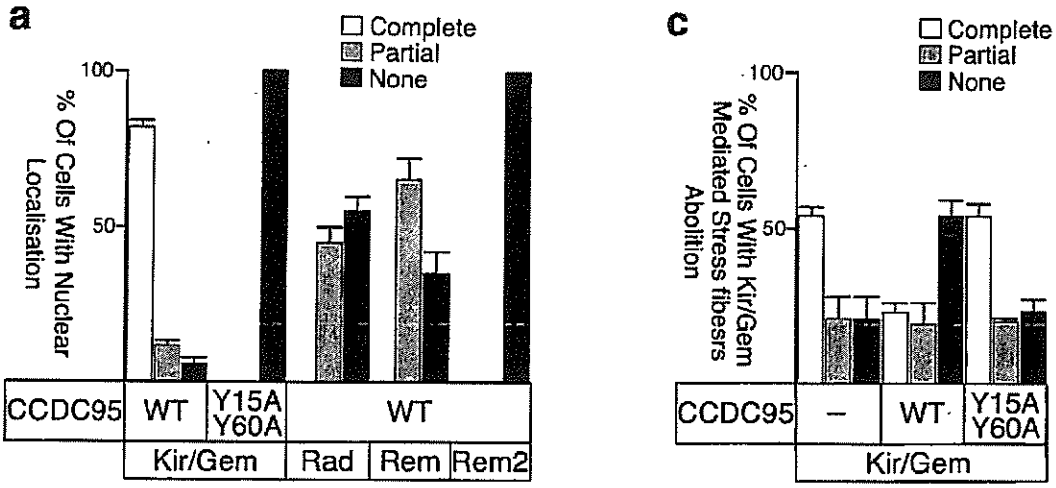

b
CCDC95 rabbit AB
CCDC95 mouse AB
MERGE
a
b
c

Figure 8


a
Complete
Partial
None
100
50
% Of Cells With Nuclear Localisation
CCDC95
WT
Y15A Y60A
WT
Kir/Gem
Rad
Rem
Rem2
c
Complete
Partial
None
100
50
% Of Cells With Kir/Gem Mediated Stress fibesrs Abolition
CCDC95
–
WT
Y15A Y60A
Kir/Gem

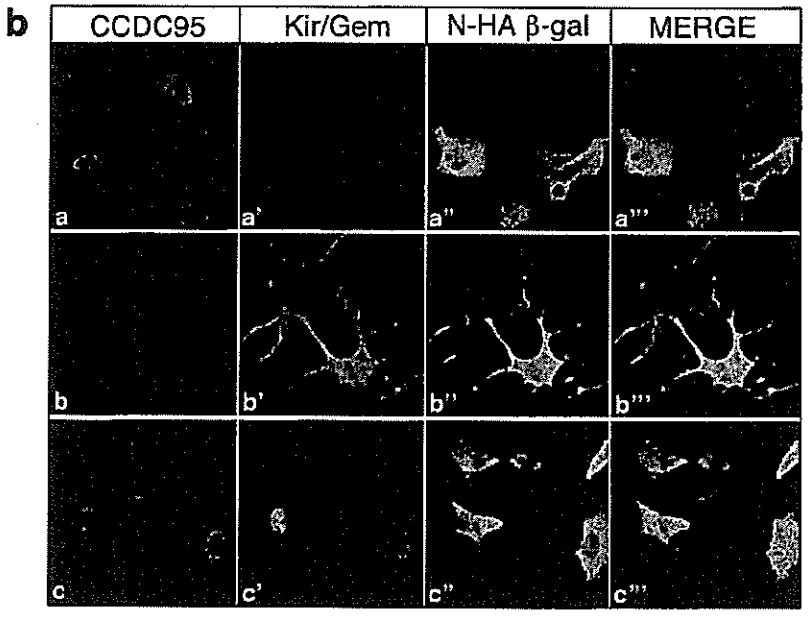

b
CCDC95
Kir/Gem
N-HA β-gal
MERGE
a
a'
a''
a'''
b
b'
b''
b'''
c
c'
c''
c'''

Figure 9

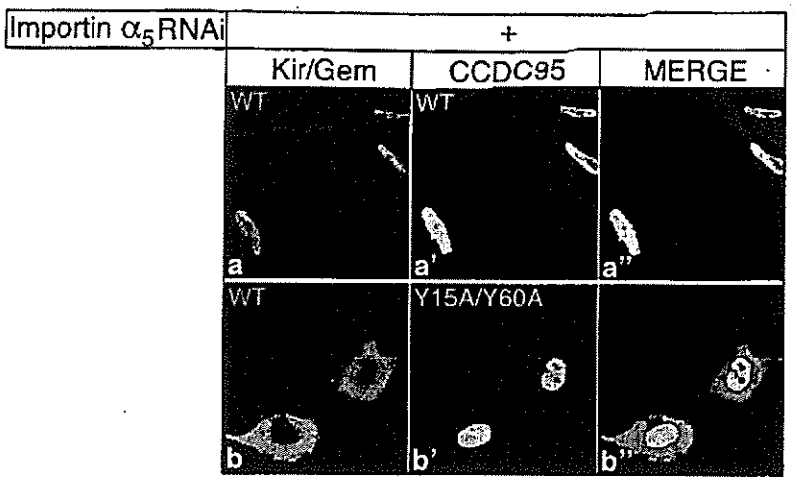
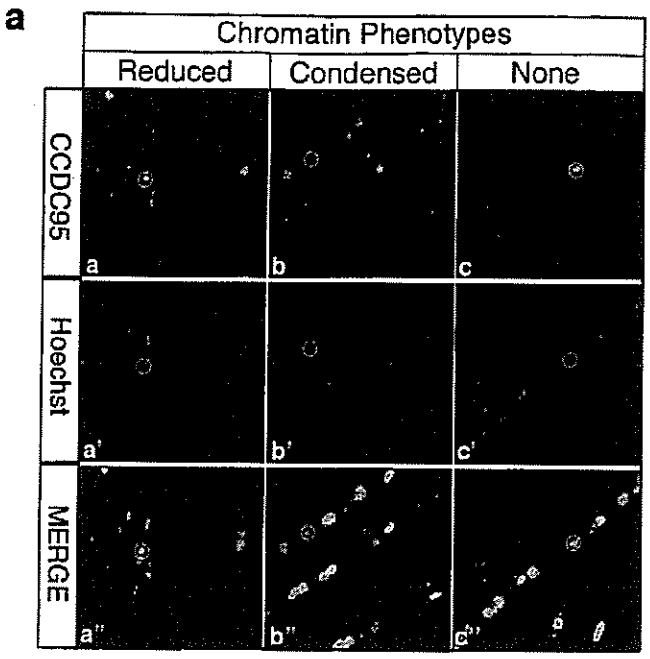

Importin α_5RNAi
+
Kir/Gem
CCDC95
MERGE
WT
WT
a
a'
a''
WT
Y15A/Y60A
b
b'
b''

Figure 10


a
Chromatin Phenotypes
Reduced
Condensed
None
CCDC95
Hoechst
MERGE
a
b
c
a'
b'
c'
a''
b''
c''

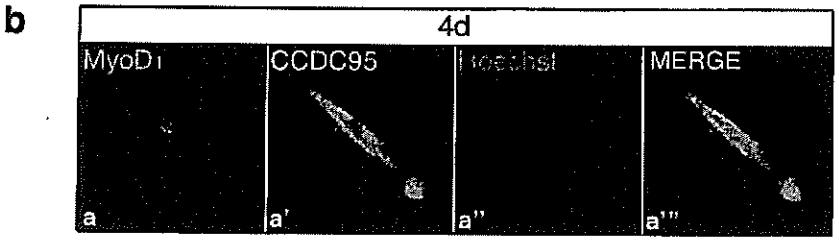

b
4d
MyoD1
CCDC95
Hoechst
MERGE
a
a'
a''
a'''

Figure 11

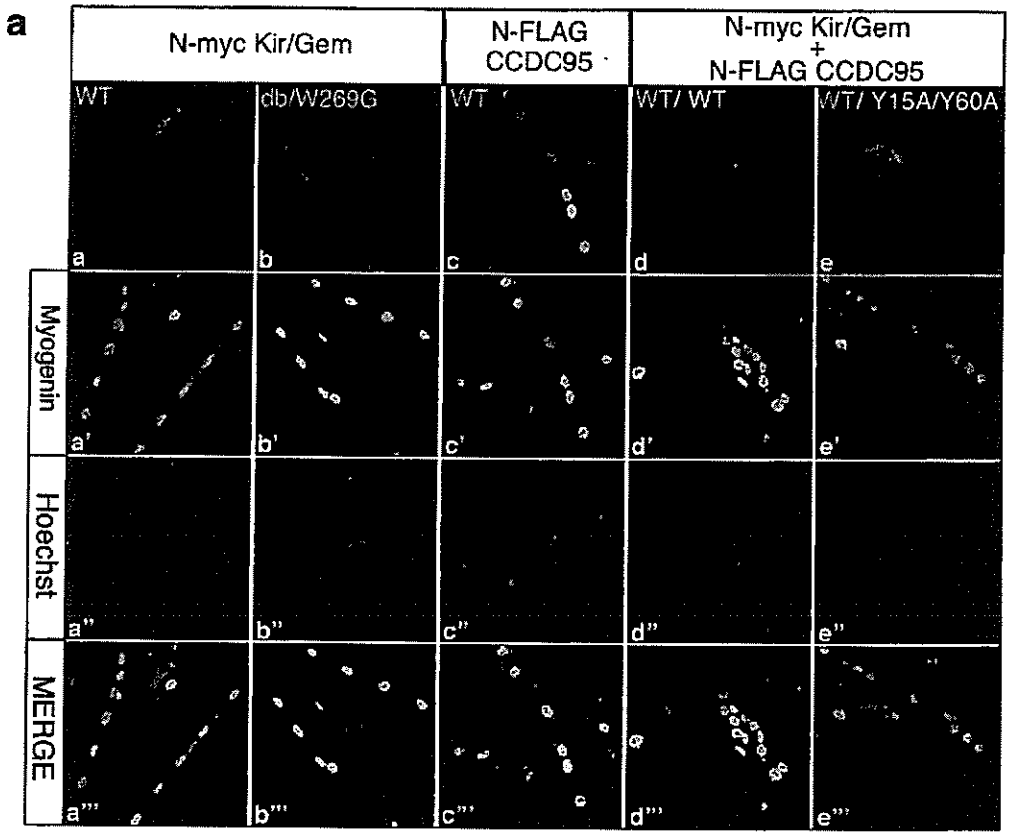

a
N-myc Kir/Gem
N-FLAG CCDC95
N-myc Kir/Gem + N-FLAG CCDC95
WT
db/W269G
WT
WT/ WT
WT/ Y15A/Y60A
Myogenin
Hoechst
MERGE
a
b
c
d
e
a'
b'
c'
d'
e'
a''
b''
c''
d''
e''
a'''
b'''
c'''
d'''
e'''

Figure 12

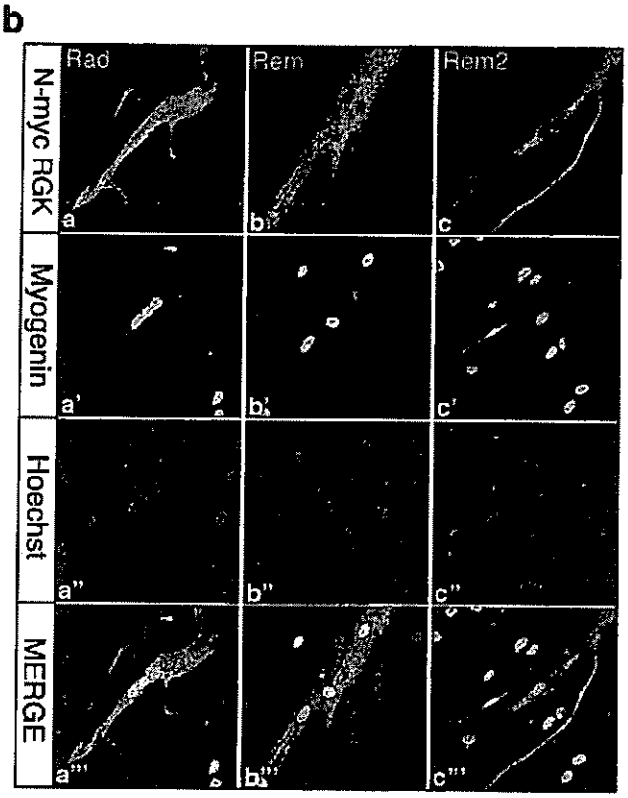
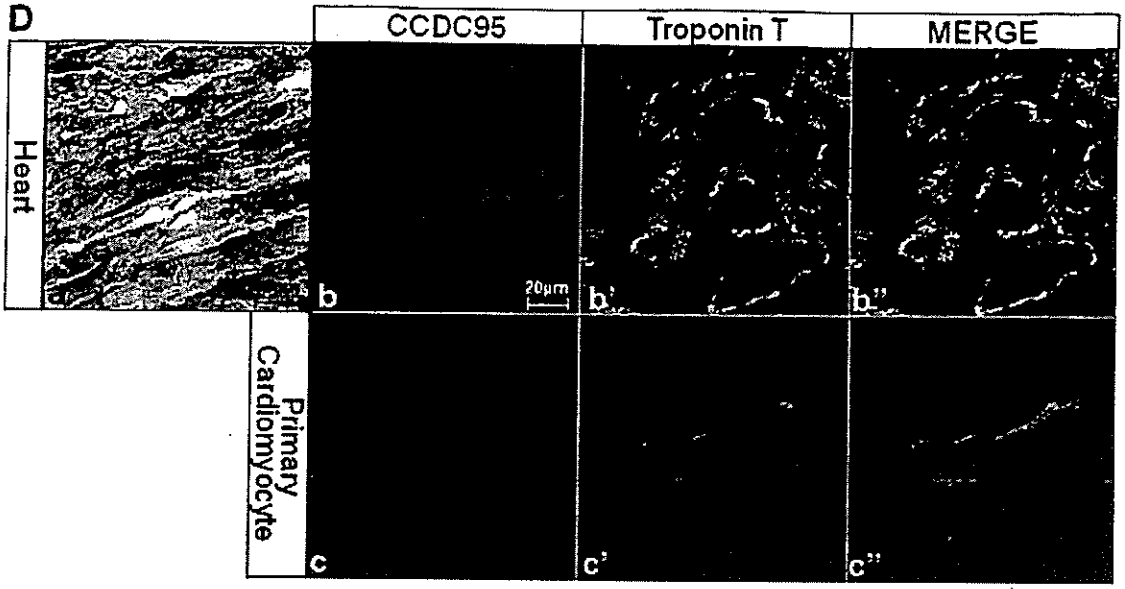

b
Rad
Rem
Rem2
N-myc RGK
Myogenin
Hoechst
MERGE
a
b
c
a'
b'
c'
a''
b''
c''
a'''
b'''
c'''

Figure 12


D
CCDC95
Troponin T
MERGE
Heart
Primary Cardiomyocyte
20µm
a
b
b'
b''
c
c'
c''

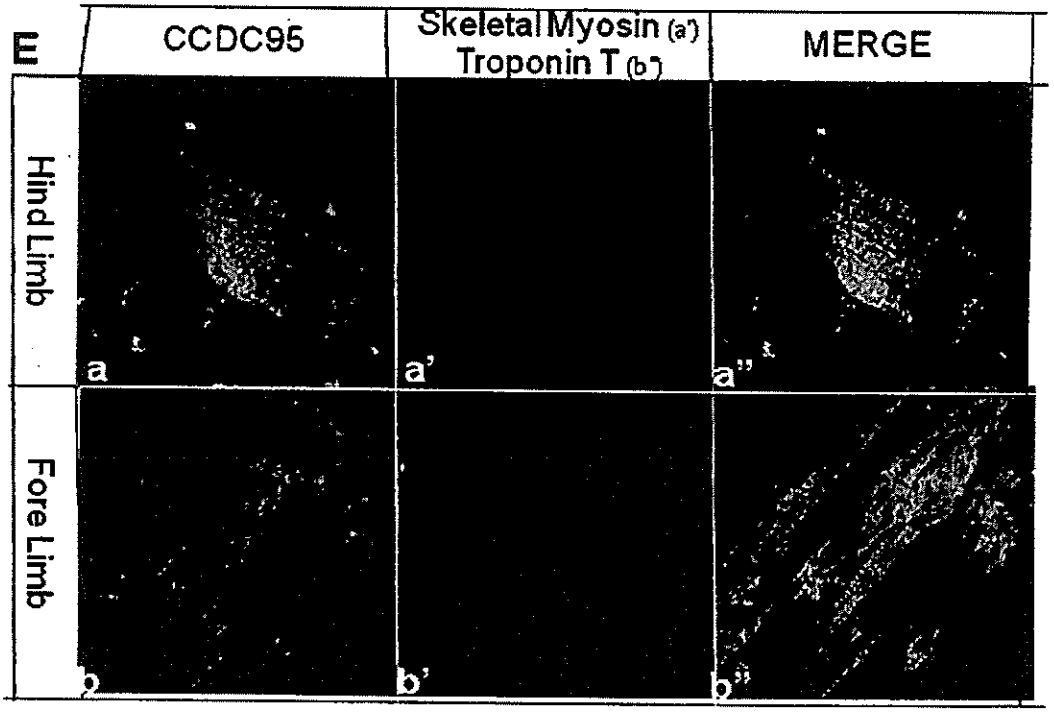

E
CCDC95
Skeletal Myosin (a')
Troponin T (b')
MERGE
Hind Limb
Fore Limb
a
a'
a''
b
b'
b''

Figure 13

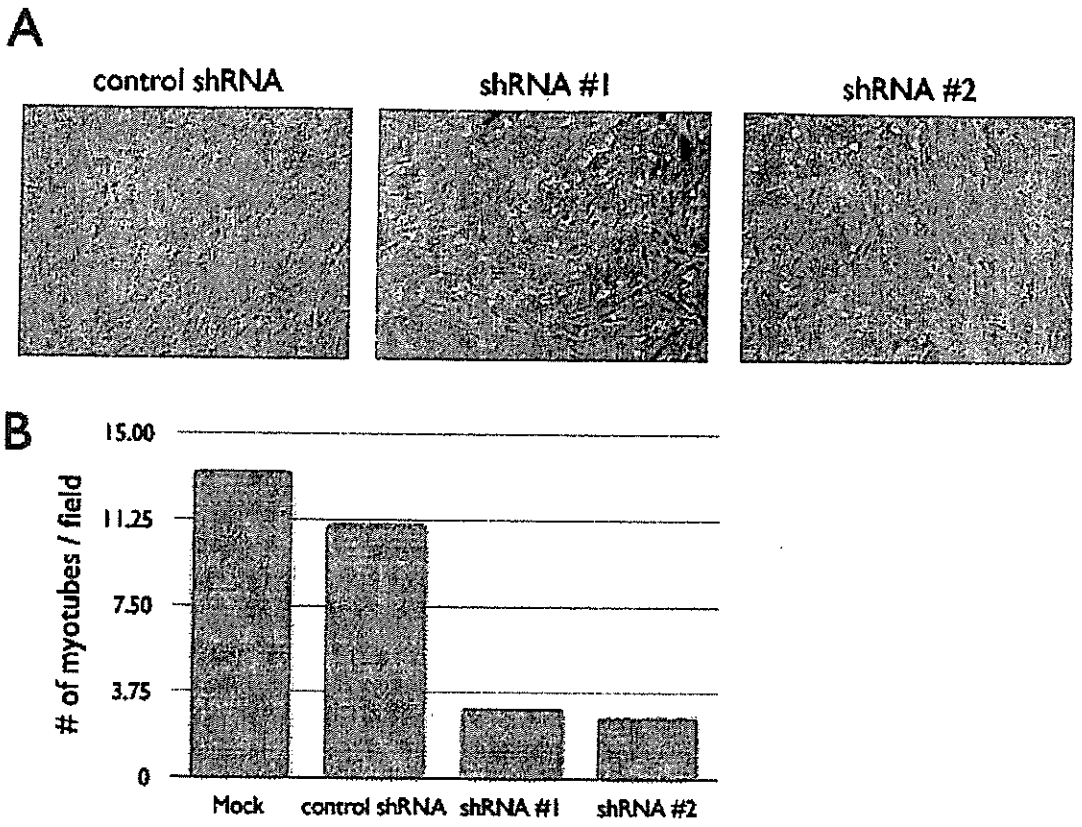

A
control shRNA
shRNA #1
shRNA #2
B
15.00
11.25
7.50
3.75
0
# of myotubes / field
Mock
control shRNA
shRNA #1
shRNA #2

Figure 14