Landes Bioscience

www.landesbioscience.com

Supplemental Material to:

Pauline C. Conroy, Chiara Saladino, Tiago J. Dantas, Pierce Lalor, Peter Dockery and Ciaran G. Morrison

C-NAP1 and rootletin restrain DNA damage-induced centriole splitting and facilitate ciliogenesis

2012; 11(20)

http://dx.doi.org/10.4161/cc.21986

http://www.landesbioscience.com/journals/cc/article/21986

Supplemental Figure Legends

Supplemental figure 1. Composition of split centrioles following IR

Immunofluorescence microscopy analysis of untreated and IR-split centrioles. hTERT-RPE1 cells were fixed 48 hours after 5 Gy IR, where indicated, then stained with the indicated antibodies (green) and γ-tubulin (red). Cells were counterstained with DAPI to visualise the DNA. Inserts show blow-ups of centrioles. Scale bar, 10 μm.

Supplemental figure 2. Comparison of different siRNA and culture conditions

A. Immunoblot analysis of RNAi efficiency. Cells were treated with 50nM siRNA for the indicated times. α-tubulin was used as a loading control.

B. Ciliogenesis in RPE1 cells that had been siRNA treated for 48 hours before being serum starved in medium containing 0.2% NCS for 48 hours, then fixed and stained for acetylated tubulin and γ-tubulin. Data show the mean ± s.e.m of 3 separate experiments in which at least 100 cells were counted. ***P ≤0.001 compared with scrambled siRNA control.

C. Ciliogenesis in RPE1 cells that had been siRNA treated for 48 hours before being serum starved in serum-free medium (SFM) for 48 hours, then fixed and stained for acetylated tubulin and γ-tubulin. Data show the mean ± s.e.m of 3 separate experiments in which at least 100 cells were counted. * P ≤0.05 compared with scrambled siRNA control.

Supplemental Tables

Supplemental Table 1: Dharmacon On-target smart pool siRNA sequences used in this study

NEK2
CGAUCUGGCUAGUGUAAUU
GCAGACAGAUCCUGGGCAU
GGCAAUACUUAGAUGAAGA
GCUAGAAUAUUAAACCAUG

CEP250
GAGCAGAGCUACAGCGAAU
GGACCUCGCUGAACAACUA
AAGCUGACGUGGUGAAUAA
GAGAAUAUGAUCCAAGAGA

CROCC
AGGCAGAGAGCGAGCGCAU
GCGAGCAGGUGCAGACGUU
GGGAGAUUGUCACCCGCAA
CAGCGACUCCAGCGUGAAA

KIZUNA
UGUCCAAGCUCAUGUCGUA
GAGAAAUACUGACACGGGA
CCAAGAAUAUUUAAAGCGA
GAAUAUUGGCAGCGGUGCA

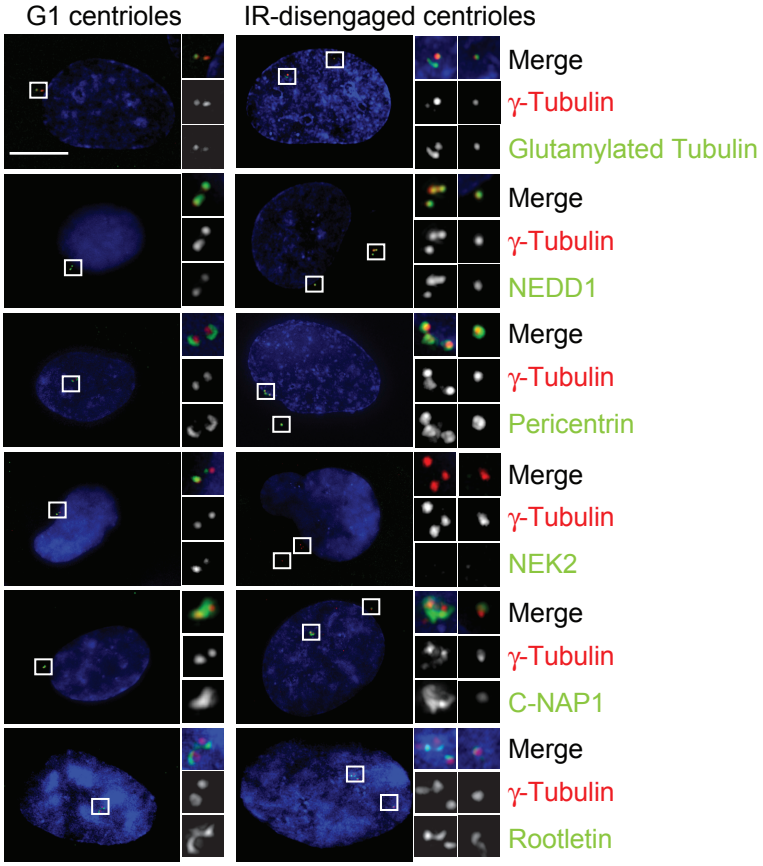
CHK1
CAAGAUGUGUGGUACUUUA
GAGAAGGCAAUAUCCAAUA
CCACAUGUCCUGAUCAUAU
GAAGUUGGGCUAUCAAUGG

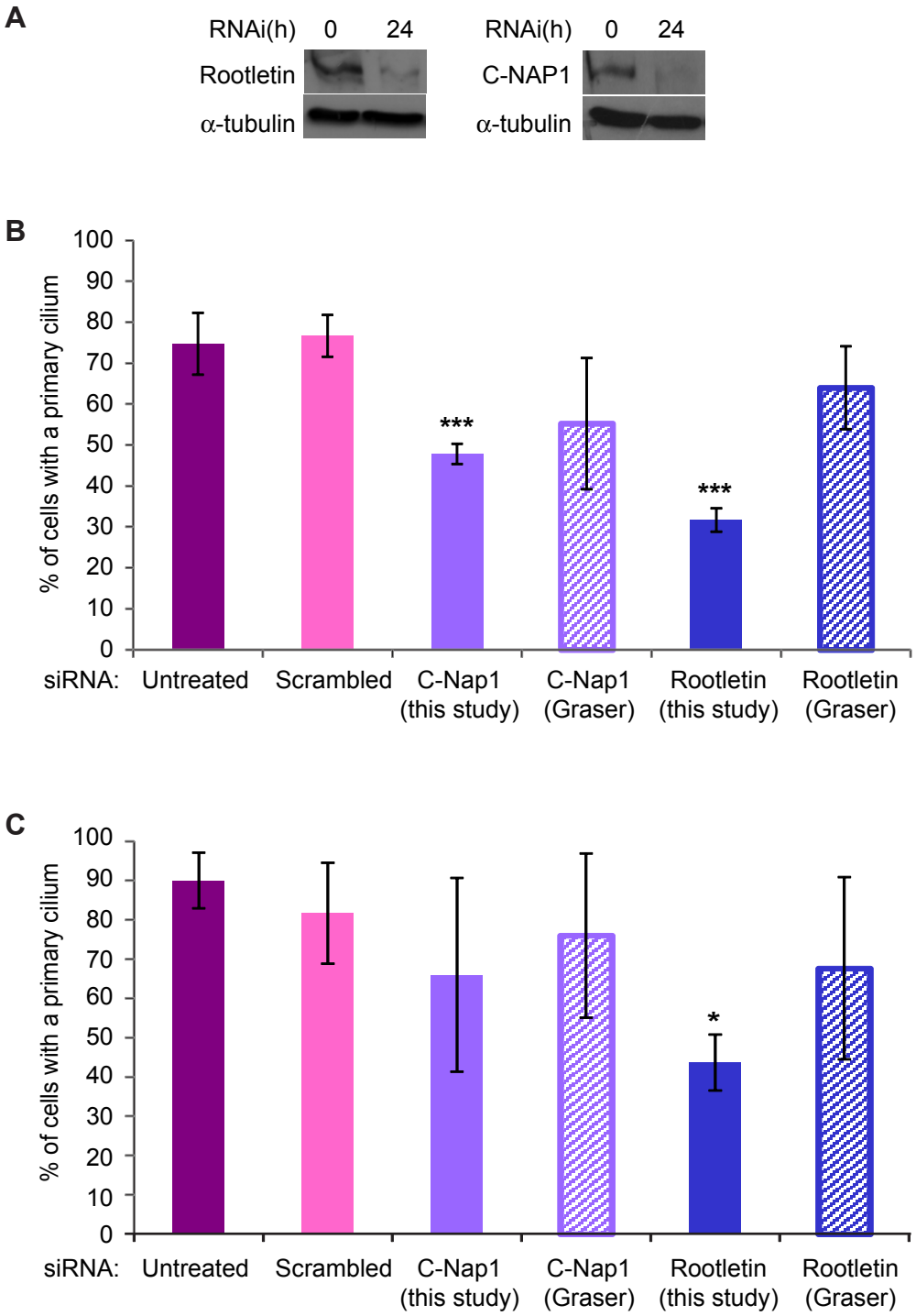
Supplemental Table 2: Qiagen custom siRNA target sequences used in this study

CEP250
CUGGAAGAGCGUCUAACUGAU

CROCC
(AA)AAGCCAGUCUAGACAAGGA

CEP164
CAGGUGACAUUUACUAUUUCA


G1 centrioles
IR-disengaged centrioles
Merge
γ-Tubulin
Glutamylated Tubulin
Merge
γ-Tubulin
NEDD1
Merge
γ-Tubulin
Pericentrin
Merge
γ-Tubulin
NEK2
Merge
γ-Tubulin
C-NAP1
Merge
γ-Tubulin
Rootletin


A
RNAi(h) 0 24
Rootletin
α-tubulin
RNAi(h) 0 24
C-NAP1
α-tubulin
B
% of cells with a primary cilium
100
90
80
70
60
50
40
30
20
10
0
***
***
siRNA:
Untreated
Scrambled
C-Nap1 (this study)
C-Nap1 (Graser)
Rootletin (this study)
Rootletin (Graser)
C
% of cells with a primary cilium
100
90
80
70
60
50
40
30
20
10
0
*
siRNA:
Untreated
Scrambled
C-Nap1 (this study)
C-Nap1 (Graser)
Rootletin (this study)
Rootletin (Graser)