Supplemental Material

Poc1A and Poc1B act together in human cells to ensure centriole integrity

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Validation of Poc1A and Poc1B antibody specificity

A. Isoform specificity of Poc1A and Poc1B antibodies was tested by Western blot of extracts from mock, GFP-Poc1B and MYC-Poc1A expressing HeLa cells. In parallel, Western blots were performed with anti-GFP and anti-MYC. α-tubulin antibodies served as a loading control. M. wts (kDa) are indicated on the left. **B, C.** Immunofluorescence microscopy of methanol fixed HeLa cells following mock depletion or depletion with Poc1A and/or Poc1B siRNAs and staining with Poc1A (B) or Poc1B (C) antibodies, as indicated (green), and γ-tubulin (red) antibodies. Merge panels include DNA stained with Hoechst 33258 (blue). Magnified views of centrosomes are shown. Scale bars, 10 μm. **D, E.** HeLa cells were fixed and stained with γ-tubulin (red) antibodies, and Poc1A (green, D) or Poc1B (green, E) antibodies before (top panels) or after (bottom panels) pre-incubation with MBP-Poc1A or MBP-Poc1B, respectively. Merge panels include DNA stained with Hoechst 33258 (blue); magnified views of centrosomes are shown. Scale bars, 6 μm.

Figure S2. A fraction of Poc1 protein is resistant to depletion

A-D. HeLa cells were transiently transfected with GFP-Poc1A (A, B) or GFP-Poc1B (C, D) plasmids for 24 hrs and then with siRNAs against siGL2 (A, C), siPoc1A (B) or siPoc1B (D) for 48 hrs. Cells were then stained with GFP (green) and Poc1A (red) or Poc1B (red) antibodies. Untransfected (upper panels) and transfected (lower panels) cells are shown. Merge panels include DNA stained with Hoechst 33258 (blue); magnified views of centrosomes are showed. Scale bars, 6 μm. **E, F**. Western blots

were performed on extracts prepared from cells treated as in A-D (and as indicated on the panels) using GFP antibodies again showing incomplete depletion when following expression of recombinant protein. M. wts (kDa) are indicated on the left.

Figure S3. Association of recombinant Poc1A and Poc1B in cells

Stable TAP-Poc1A expressing cells were transfected with GFP, GFP-Poc1A or GFP-Poc1B, and 24 hours later precipitations were carried out with IgG beads to test for interactions between the differently tagged proteins. Western blots were carried out using Protein A (Pr A) antibodies to detect the TAP-tag, and GFP antibodies to detect GFP-fusion proteins (arrowheads). The GFP antibodies also detected the TAP-Poc1A (*) through reactivity with the Protein A epitope. M. wts (kDa) are indicated on the left.

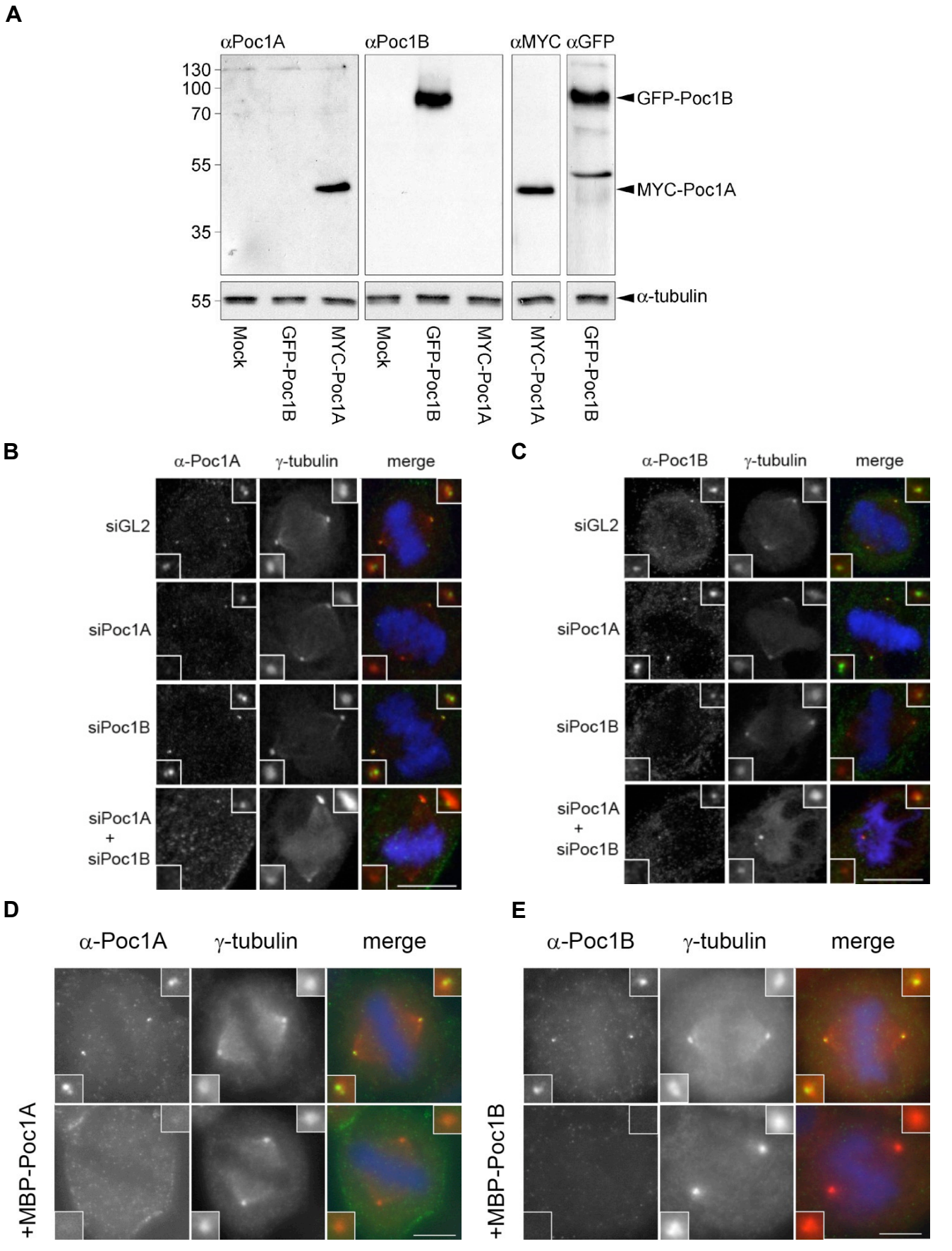
Figure S4. Mitotic phosphorylation of Poc1B

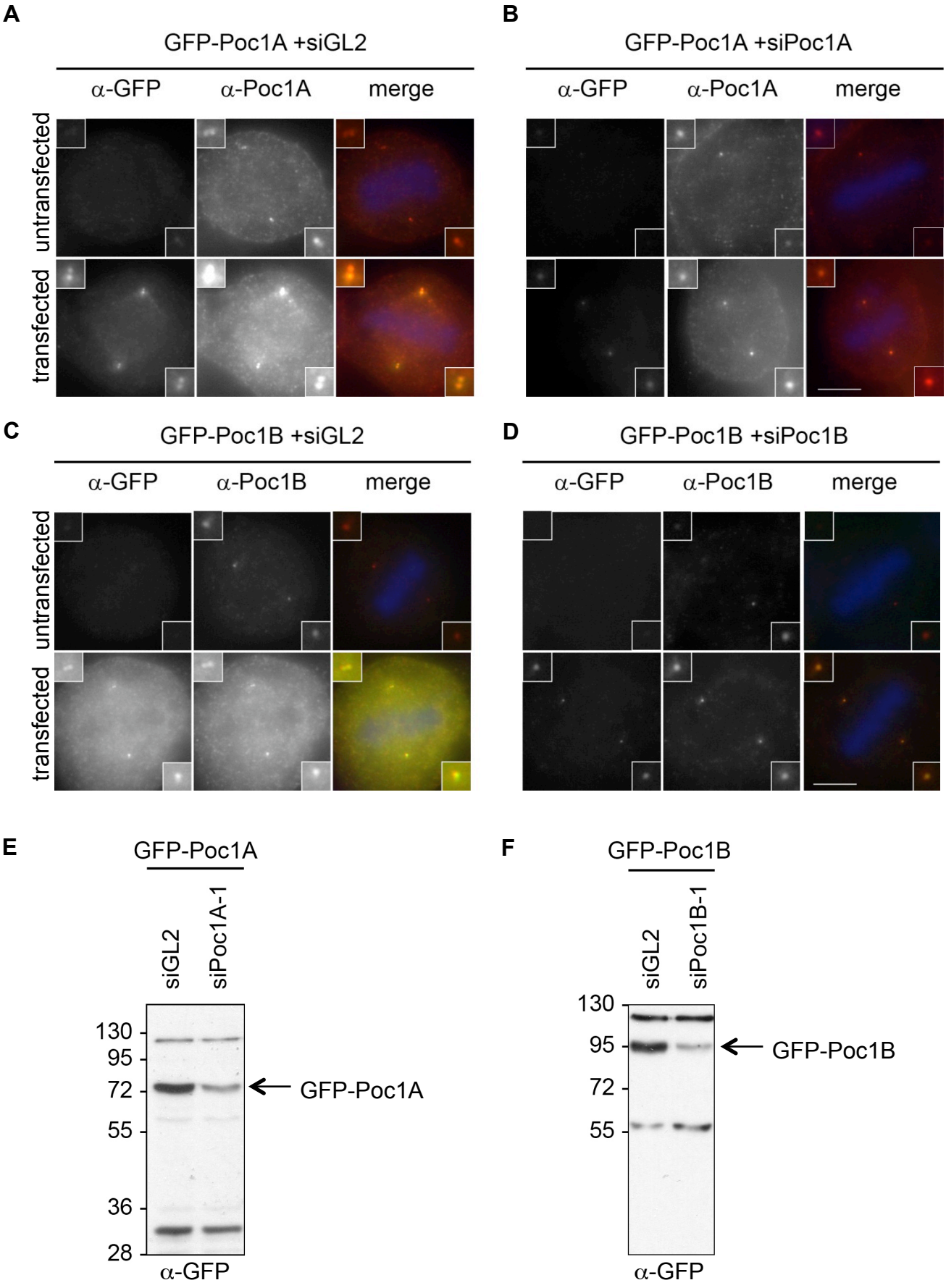
A. Extracts were prepared from HEK293 cells stably expressing TAP-Poc1B that were either growing asynchronously (A), or arrested in S-phase with hydroxyurea (S), or M-phase (M) with either nocodazole or BI-2536. In addition, cells were treated for 4 hours with or without nocodazole, as indicated, prior to harvesting. Samples were analysed by Western blot with antibodies against Poc1B and α-tubulin. **B.** Histone H1, maltose-binding protein (MBP) or MBP-tagged Poc1B full-length (FL) were used as substrates in assays with the kinases indicated for 30 min at 30°C. Samples were analysed by SDS-PAGE, Coomassie Blue staining (CB) and autoradiography (^{32}P). M. wts (kDa) are indicated on the left.

Figure S5. Depletion of Poc1B but not Poc1A blocks cell proliferation

A. Growth curves of HeLa cells transfected with siRNAs against luciferase (siGL2) or two different siRNAs Poc1A and/or Poc1B are shown; data represent means from two separate experiments. **B-D**. hTERT-RPE1 cells treated with siRNAs for 55 hrs

were treated with 0.5 μg/ml nocodazole or DMSO for 17 hrs, fixed, and analyzed by flow cytometry. FACS profiles are shown in B; histograms representing the percentage of cells in G2/M phase and variation in G1 phase relative to siGL2 control are shown in C and D, respectively.


A
αPoc1A
αPoc1B
αMYC
αGFP
130
100
70
55
35
55
GFP-Poc1B
MYC-Poc1A
α-tubulin
Mock
GFP-Poc1B
MYC-Poc1A
Mock
GFP-Poc1B
MYC-Poc1A
MYC-Poc1A
GFP-Poc1B
B
α-Poc1A
γ-tubulin
merge
siGL2
siPoc1A
siPoc1B
siPoc1A + siPoc1B
C
α-Poc1B
γ-tubulin
merge
siGL2
siPoc1A
siPoc1B
siPoc1A + siPoc1B
D
α-Poc1A
γ-tubulin
merge
+MBP-Poc1A
E
α-Poc1B
γ-tubulin
merge
+MBP-Poc1B


A
GFP-Poc1A +siGL2
α-GFP
α-Poc1A
merge
untransfected
transfected
B
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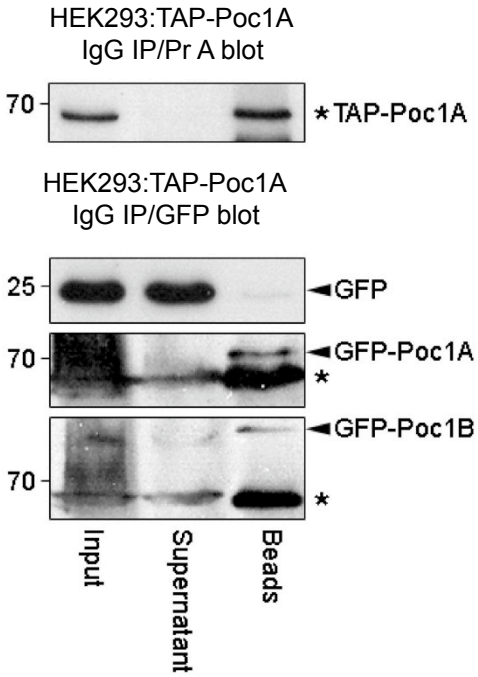
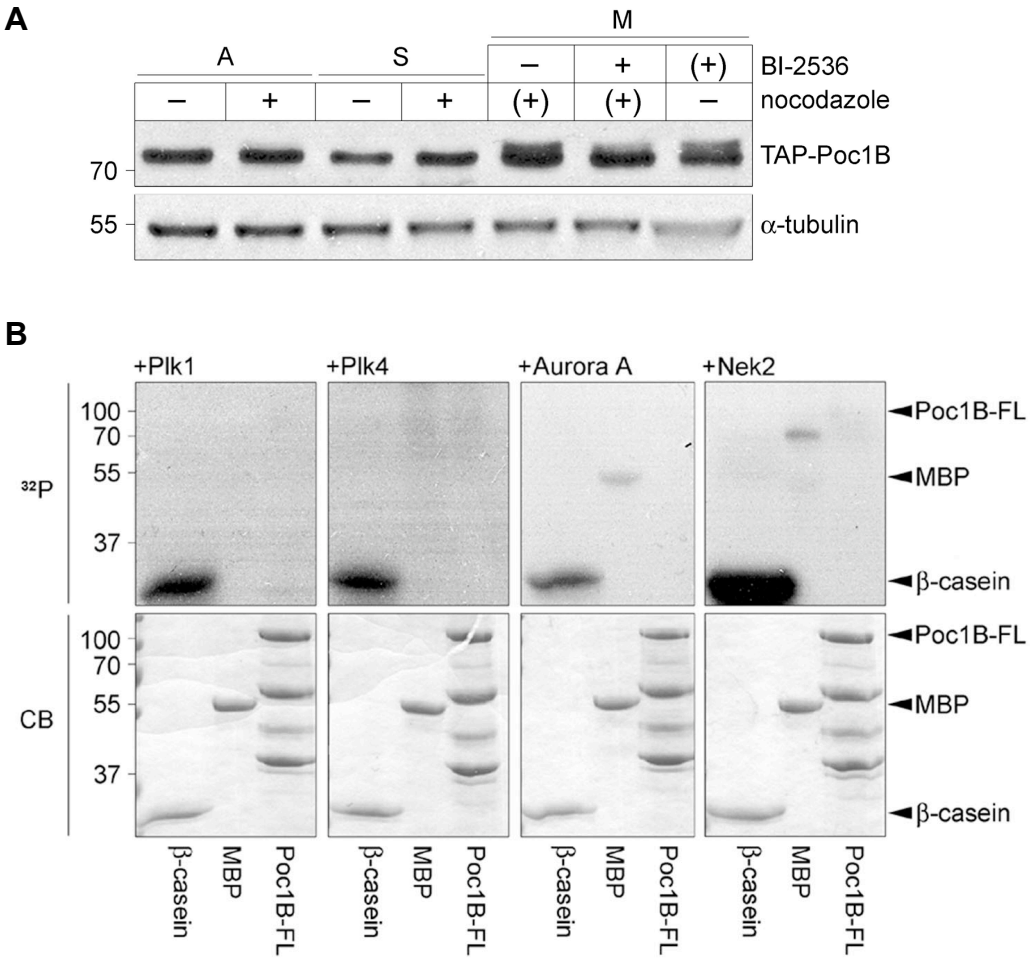
α-GFP
α-Poc1A
merge
C
GFP-Poc1B +siGL2
α-GFP
α-Poc1B
merge
untransfected
transfected
D
GFP-Poc1B +siPoc1B
α-GFP
α-Poc1B
merge
E
GFP-Poc1A
siGL2
siPoc1A-1
130
95
72
55
36
28
GFP-Poc1A
α-GFP
F
GFP-Poc1B
siGL2
siPoc1B-1
130
95
72
55
GFP-Poc1B
α-GFP


HEK293:TAP-Poc1A
IgG IP/Pr A blot
70
* TAP-Poc1A
HEK293:TAP-Poc1A
IgG IP/GFP blot
25
GFP
70
GFP-Poc1A
*
GFP-Poc1B
70
*
Input
Supernatant
Beads


A
M
A
S
–
+
(+)
BI-2536
–
+
–
+
(+)
(+)
–
nocodazole
70
TAP-Poc1B
55
α-tubulin
B
+Plk1
+Plk4
+Aurora A
+Nek2
32P
100
70
55
37
Poc1B-FL
MBP
β-casein
CB
100
70
55
37
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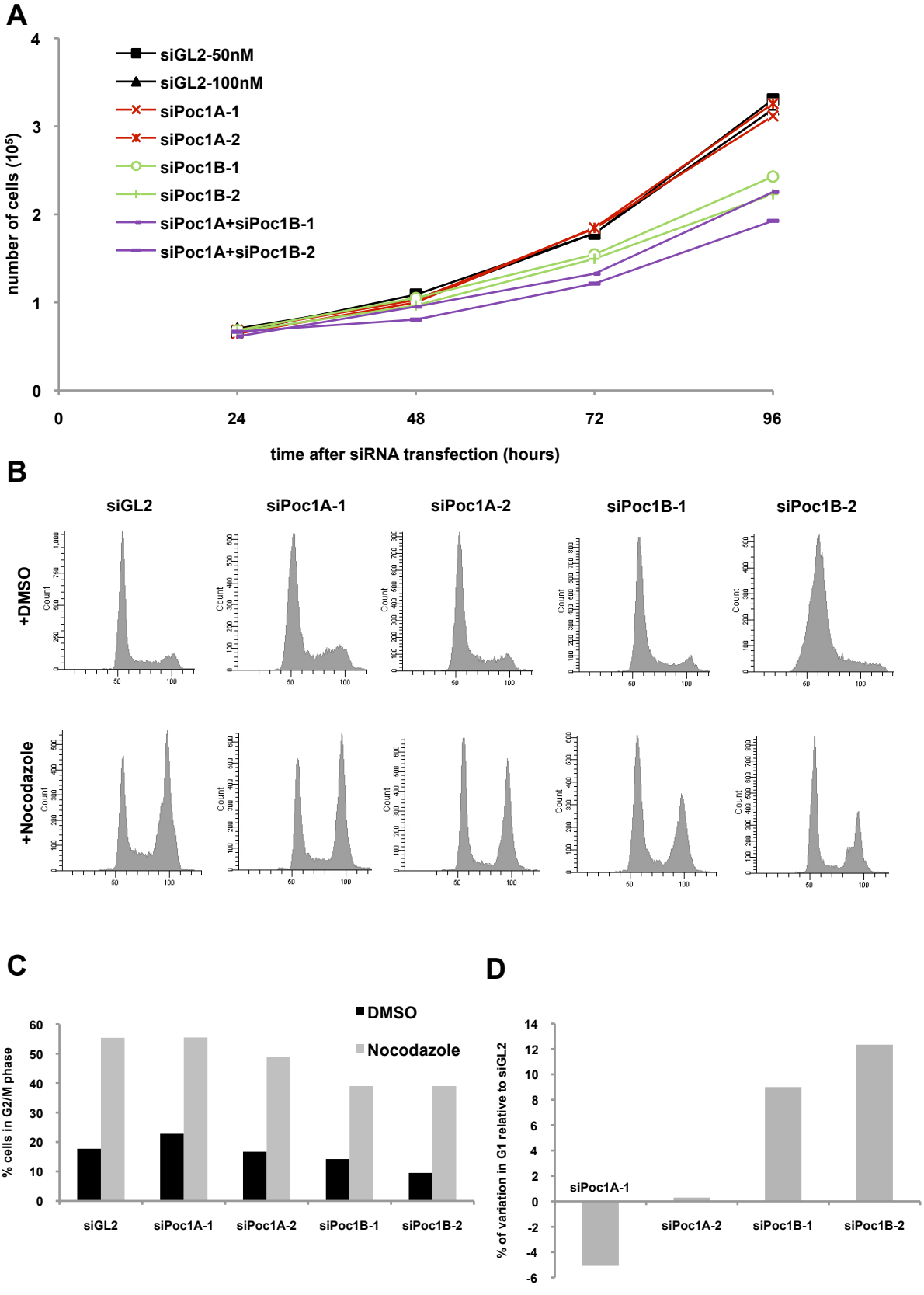
Poc1B-FL


A
number of cells (10⁵)
time after siRNA transfection (hours)
siGL2-50nM
siGL2-100nM
siPoc1A-1
siPoc1A-2
siPoc1B-1
siPoc1B-2
siPoc1A+siPoc1B-1
siPoc1A+siPoc1B-2
B
siGL2
siPoc1A-1
siPoc1A-2
siPoc1B-1
siPoc1B-2
+DMSO
+Nocodazole
Count
C
% cells in G2/M phase
DMSO
Nocodazole
D
% of variation in G1 relative to siGL2