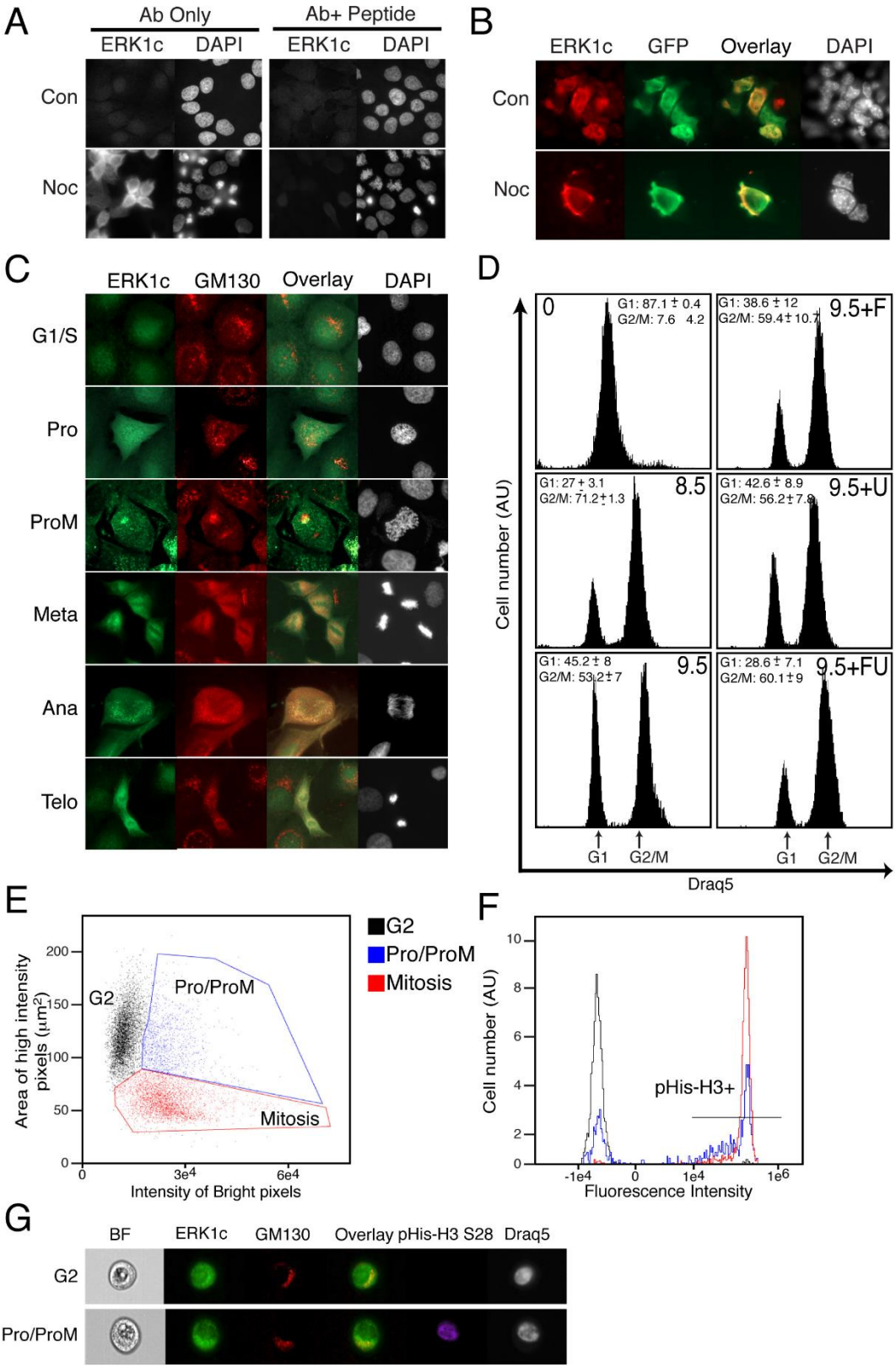
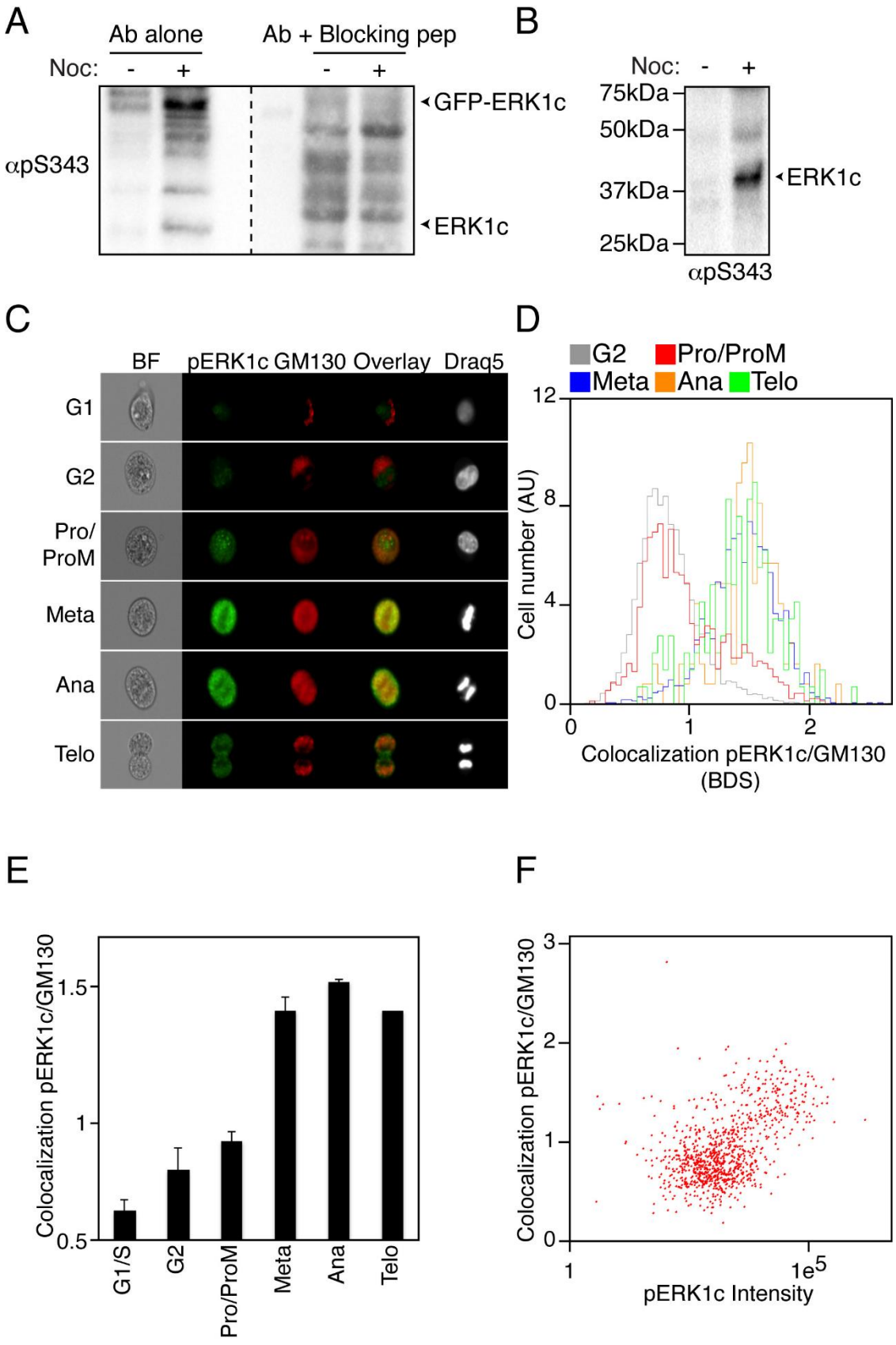
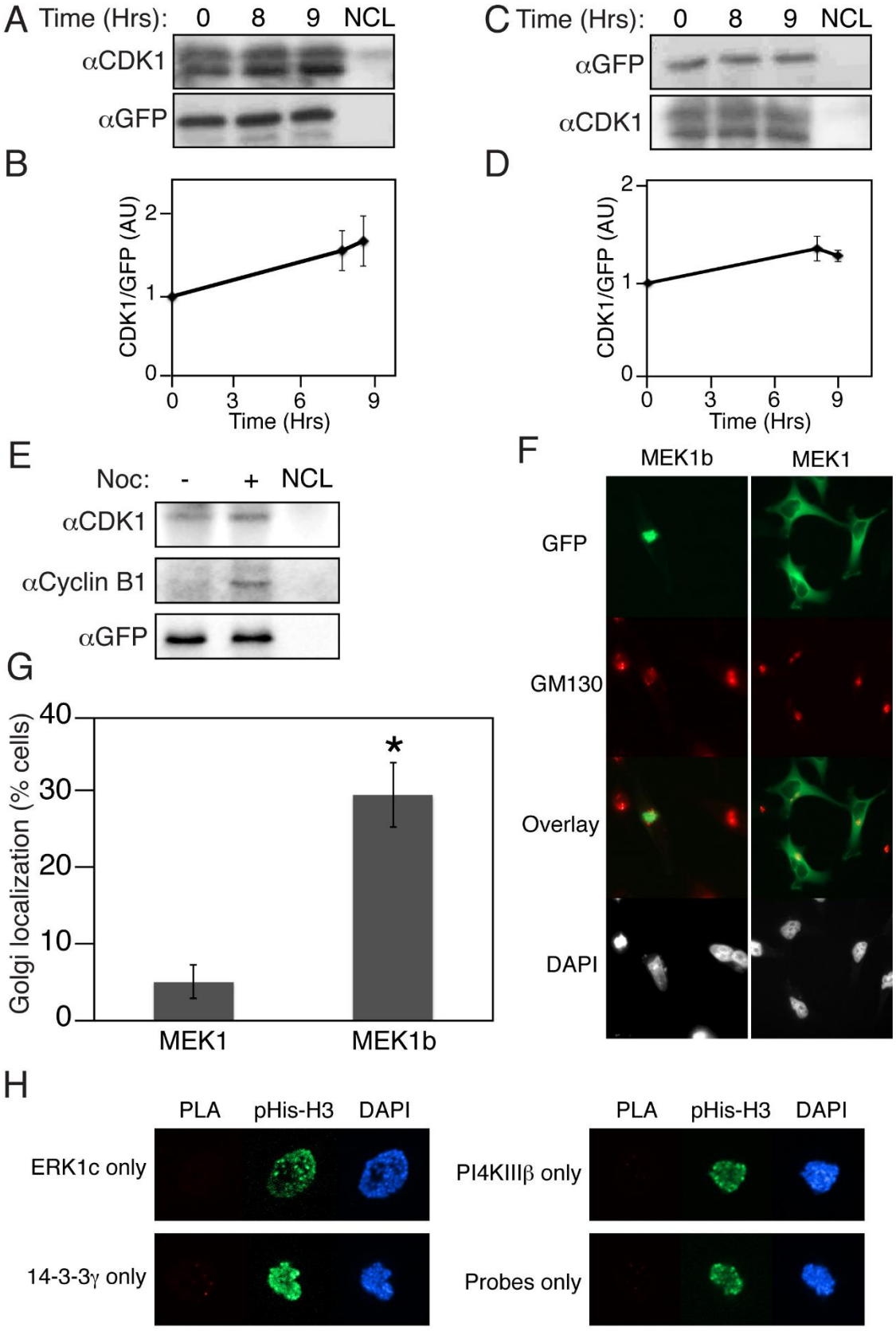

A
Ab Only
Ab+ Peptide
ERK1c
DAPI
Con
Noc
B
ERK1c
GFP
Overlay
DAPI
C
GM130
G1/S
Pro
ProM
Meta
Ana
Telo
D
Cell number (AU)
Draq5
G1
G2/M
9.5+F
8.5
9.5+U
9.5
9.5+FU
E
G2
Pro/ProM
Mitosis
Area of high intensity pixels (µm²)
Intensity of Bright pixels
F
pHis-H3+
Fluorescence Intensity
G
BF
Overlay pHis-H3 S28

Figure S2: ImageStream analysis of cell cycle and Golgi localization. (A) HeLa cells were synchronized as described in A, stained for GRASP55, CCDC86, GM130 and DRAQ5, and analyzed by ImageStream. **(B)** The co-localization of GRASP55 or CCDC86 with GM130 was quantified using the bright detail similarity feature. **(C)** Synchronized HeLa cells were fixed at peak mitosis, and then stained with ERK1c and GM130 Abs, and DRAQ5 followed by ImageStream analysis. Cells from prophase, prometaphase, metaphase, anaphase and telophase were selected by their DNA structure according to their DRAQ5 staining. **(D)** The co-localization of ERK1c and the GM130 was quantified using the bright detail similarity feature, and was measured for G2 and mitotic cells. **(E)** HeLa cells were synchronized for 9 h, and either left untreated (NT), or treated with 20μM roscovitine (Ros), 2 μM PD184352 (), or both for 1 h. Cells were fixed and stained with ERK1c and GM130 Abs, as well as DRAQ5 followed by ImageStream analysis. **(F)** The co-localization of ERK1c and he GM130 was quantified using the bright detail similarity feature, and was measured for G2, Pro/ProM, and mitotic (metaphase-telophase) for each treatment.


A
Ab alone
Ab + Blocking pep
Noc: - + - +
αpS343
GFP-ERK1c
ERK1c
B
Noc: - +
75kDa
50kDa
37kDa
25kDa
ERK1c
αpS343
C
BF
pERK1c
GM130
Overlay
Draq5
G1
G2
Pro/ ProM
Meta
Ana
Telo
D
G2
Pro/ProM
Meta
Ana
Telo
Cell number (AU)
Colocalization pERK1c/GM130 (BDS)
E
Colocalization pERK1c/GM130
G1/S
G2
Pro/ProM
Meta
Ana
Telo
F
Colocalization pERK1c/GM130
pERK1c Intensity


A
Time (Hrs): 0 8 9 NCL
αCDK1
αGFP
B
CDK1/GFP (AU)
Time (Hrs)
C
Time (Hrs): 0 8 9 NCL
αGFP
αCDK1
D
CDK1/GFP (AU)
Time (Hrs)
E
Noc: - + NCL
αCDK1
αCyclin B1
αGFP
F
MEK1b
MEK1
GFP
GM130
Overlay
DAPI
G
Golgi localization (% cells)
MEK1
MEK1b
H
PLA
pHis-H3
DAPI
ERK1c only
14-3-3γ only
PI4KIIIβ only
Probes only