E06-12-1140 Jabrane-Ferrat

Figure S1. (A) Immunofluorescence of COS-7 and DLD-1 cells, control or treated for 16h with proteasome inhibitor PS-341. Cells were stained for gamma-tubulin (red), and DNA (blue). Images were deconvolved using DeltaVision software. Bar, 15 μm. (B) Western blot analysis of centrosome-enriched fractions prepared from control and PS-341 treated Raji cells on a sucrose step gradient, probed with antibody against gamma-tubulin.

Figure S2. Gamma-tubulin accumulation at the centrosome increases specifically after proteasome inhibition. (A) Cell cycle analysis by flow cytometry of propidium iodide-stained HeLa cells, treated with proteasome inhibitor PS-341, or control cells (Ctrl). Top: asynchronous cells, bottom: cells that were synchronized in the S phase by a 5mM thymidine block for 24h, followed by 4 h release in fresh media, followed by 16h control treatment (Ctrl), or treatment with PS-341. The table summarizes the cell cycle analysis using DIVA software. (B) Immunofluorescence of HeLa cells, treated for 16h with proteasome inhibitor PS-341, or with a set of chemotherapeutic drugs, including Bleomycin (Bleo), Adriamycin (Adr), Etoposide (VP16), Camptothecin (CAM), 5-Fluorouracil (5-FU), Cisplatin (Cis), and Vinorelbine (VNB). Cells were stained for gamma-tubulin (green) and DNA (blue). Bar, 10 μm.

Figure S3. Proteasome components co-sediment with centrosome proteins. (A) Western blot analysis of centrosome-enriched fractions (1 through 16) prepared from HeLa cells on a step gradient of 70, 50, and 40% sucrose, probed with antibodies against gamma-tubulin, centrin and against the beta7-subunit of the proteasome. (B) Centrosomes were purified as in A except that larger volumes were collected per fraction, resulting in seven fractions only. Proteasome peptidase activities were determined from 20 μl of fractions 1 to 7. 100 μM of Z-

LLE-AMC or Suc-LLVY-AMC were used as substrate for caspase- or chymotrypsine-like (CTL) activities. 50 mM of lactacystin (+Lact) was included as a specific proteasome inhibitor. (C) 20 μl of each centrosome fraction from B were resolved on a 4.5% native gel overlaid with 2% stacking gel. The gel was subsequently developed in the presence of ATP and Suc-LLVY-AMC substrate peptide. The 26S (5 μg) and 20S (5 μg) proteasomal complexes were included for reference (lanes a and b), to depict the relative migration of proteasomal sub-complexes.

Figure S4. Immunofluorescence of HeLa cells, control or treated for 16h with proteasome inhibitor PS-341. (A) Cells were stained for gamma-tubulin (red) and NEDD1 (green), (B) gamma-tubulin (red) and GCP4 (green) or (C) gamma tubulin and PCM1 (green). Enlarged views of the centrosomes are shown on the left side. Scale bar, 10 μm.

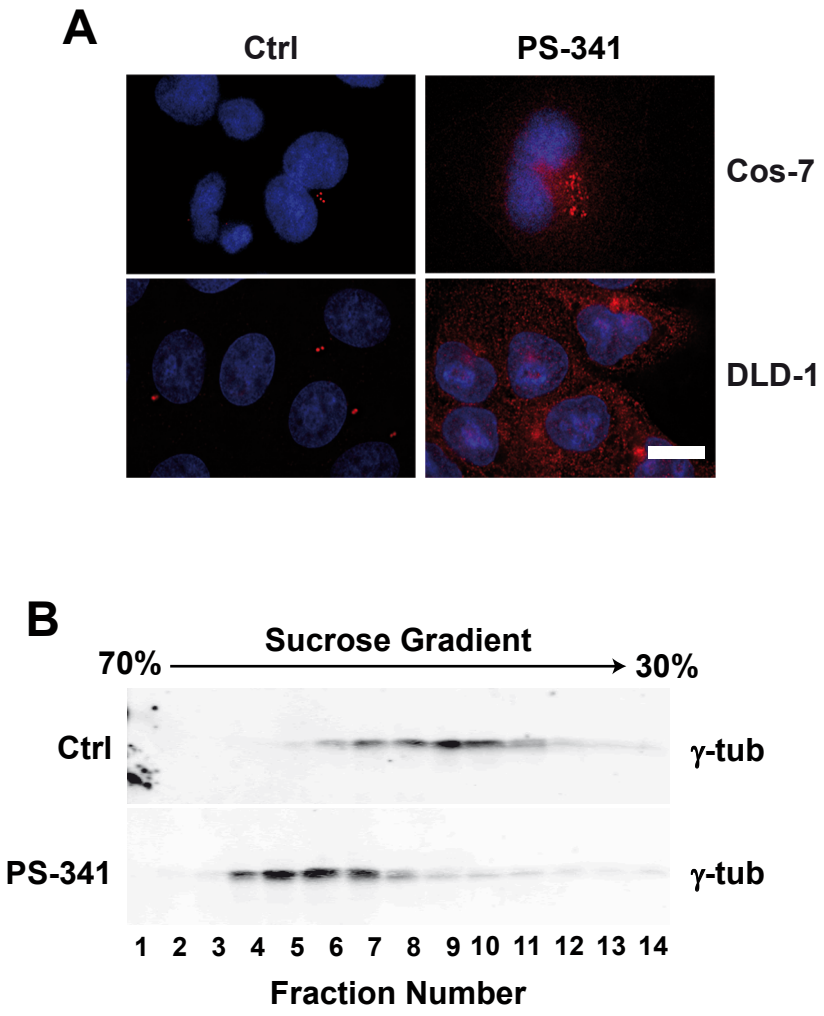
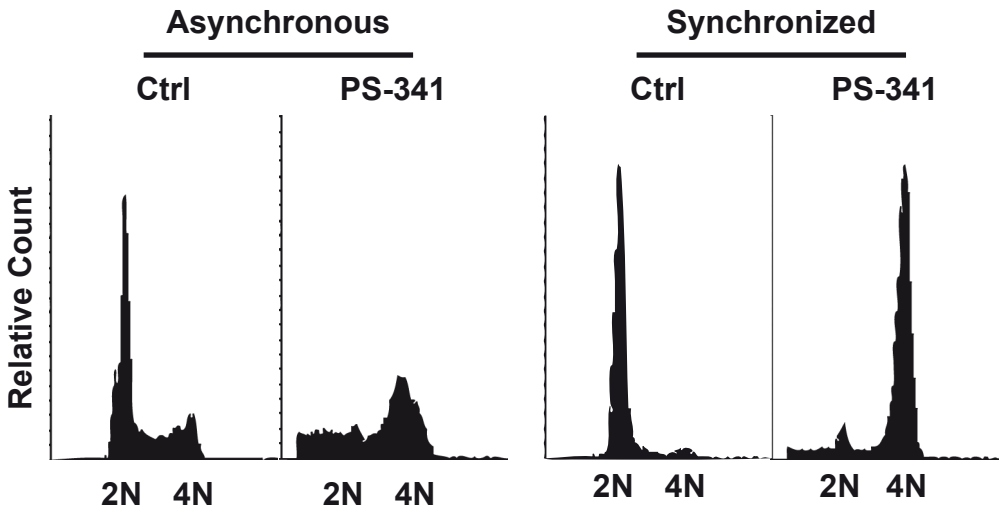

A
Ctrl
PS-341
Cos-7
DLD-1
B
Sucrose Gradient
70%
30%
Ctrl
γ-tub
PS-341
γ-tub
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Fraction Number

Fig. S1

A



	Asynchronous		Synchronized	
%	Ctrl	PS-341	Ctrl	PS-341
G1	58.3	12.5	88.9	9.3
G2/M	22	55.5	4.4	72.1
S	19.7	9.4	3.6	11.3

B

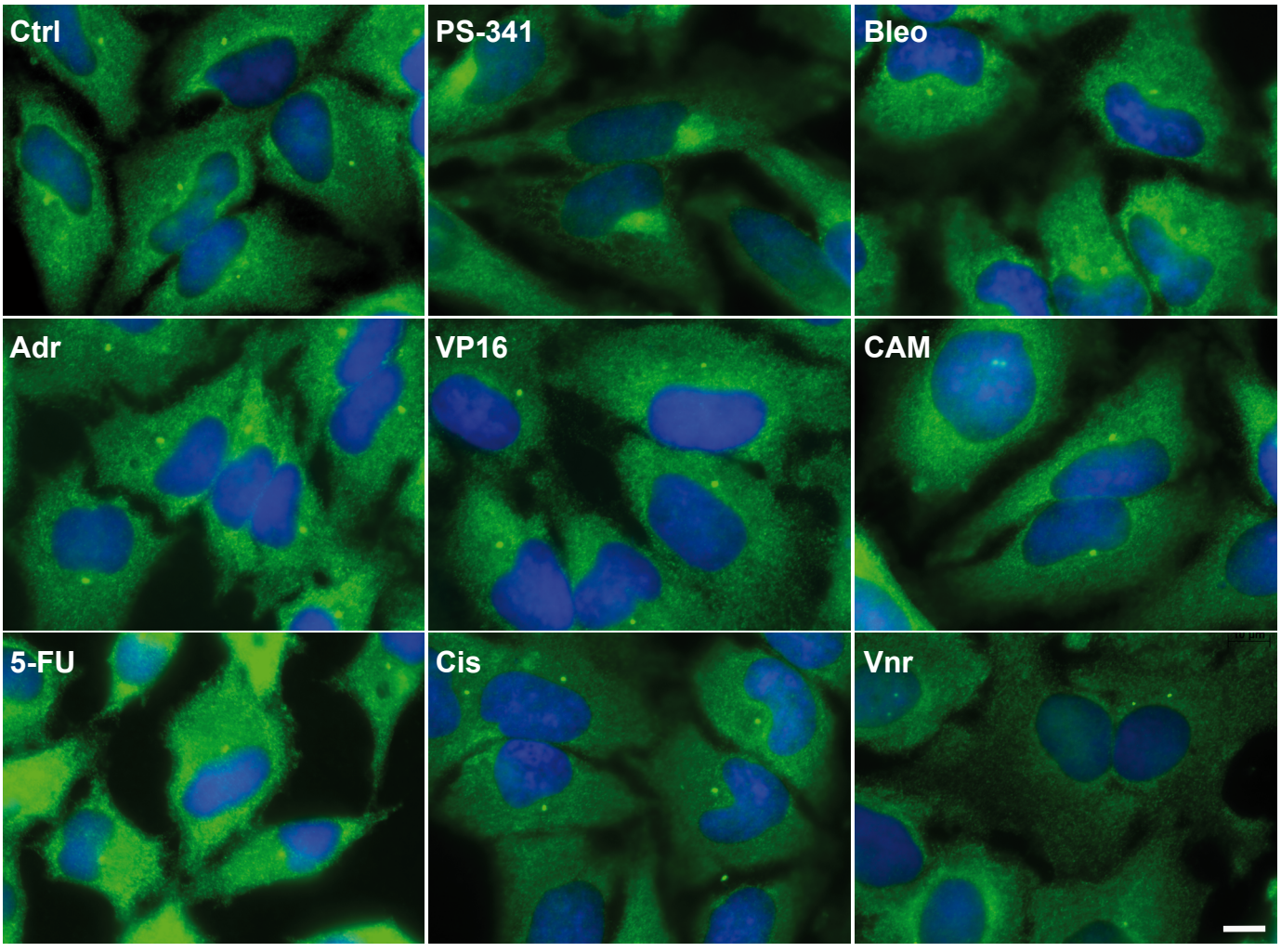


Fig. S2

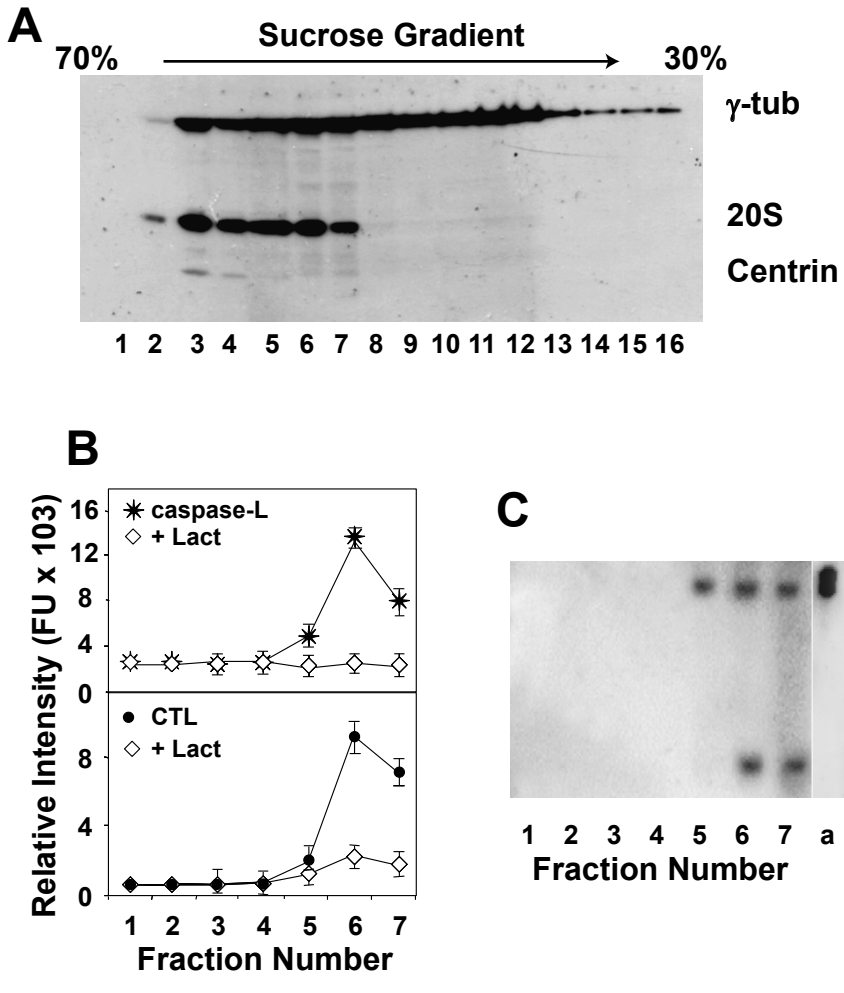

A
Sucrose Gradient
70%
30%
γ-tub
20S
Centrin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
B
caspase-L
+ Lact
CTL
+ Lact
Relative Intensity (FU x 103)
16
12
8
4
0
8
4
0
1 2 3 4 5 6 7
Fraction Number
C
26S
20S
1 2 3 4 5 6 7 a b
Fraction Number

Fig. S3

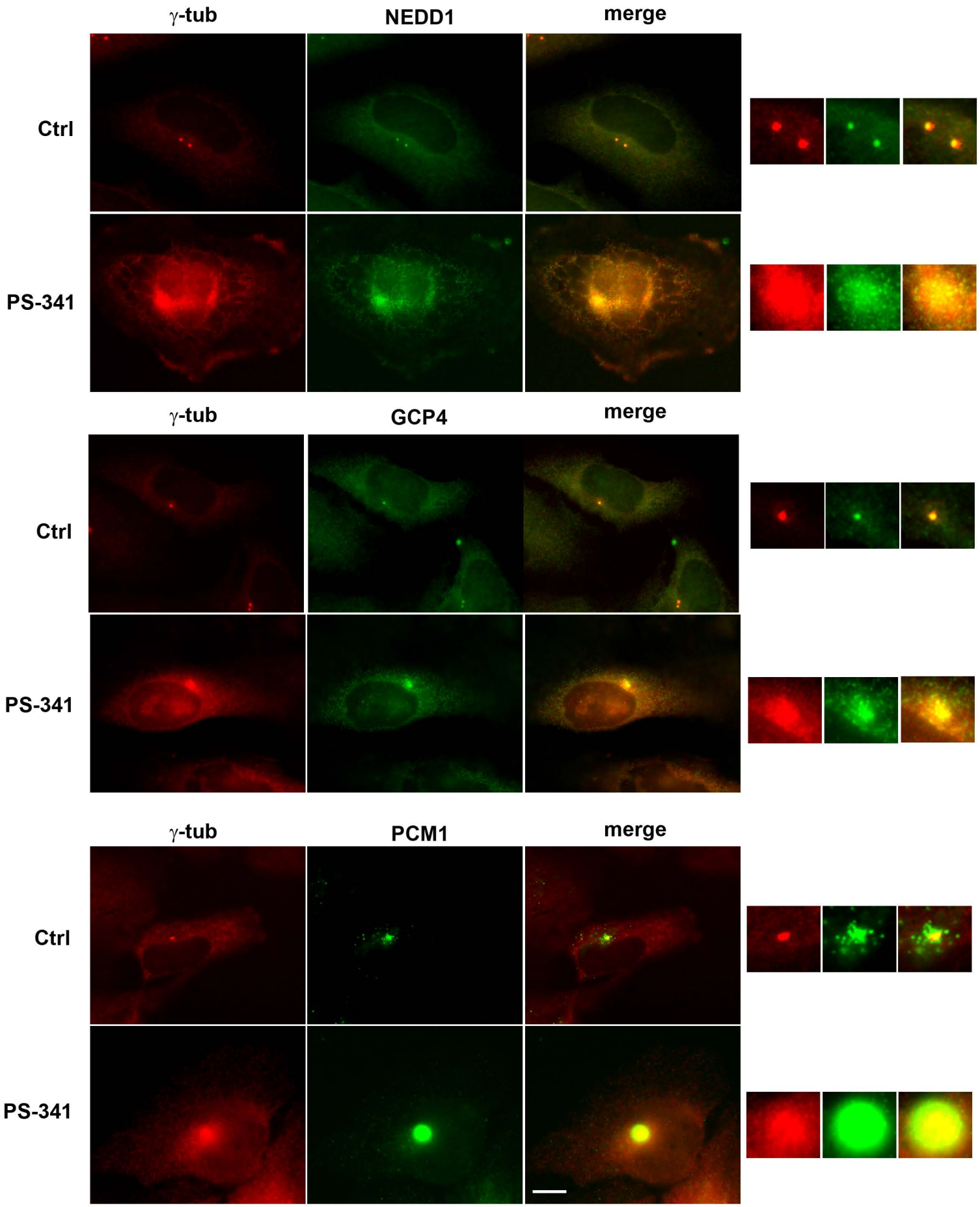

γ-tub
NEDD1
merge
Ctrl
PS-341
γ-tub
GCP4
merge
Ctrl
PS-341
γ-tub
PCM1
merge
Ctrl
PS-341

Fig. S4