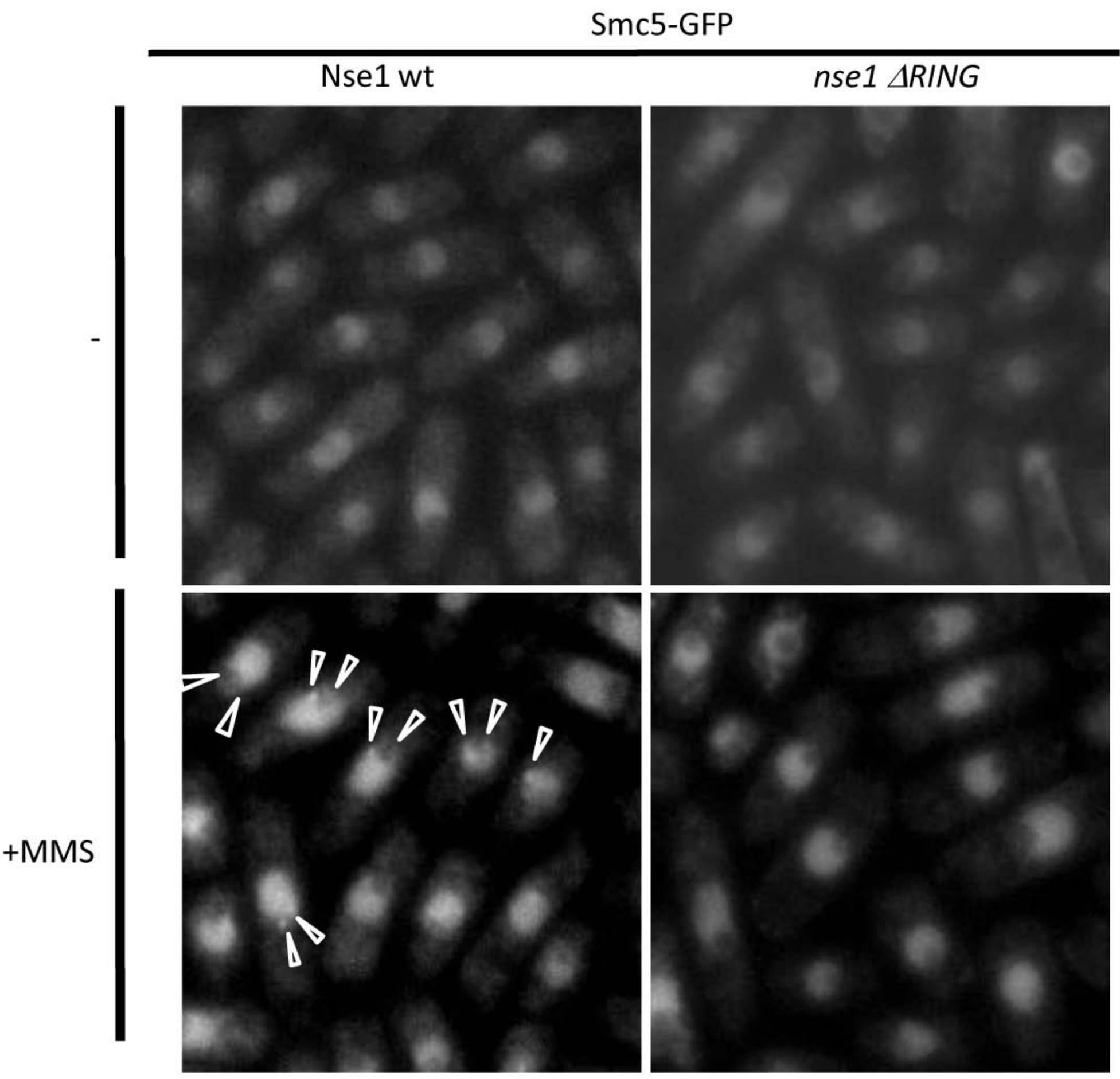
\E08-02-0226 Boddy

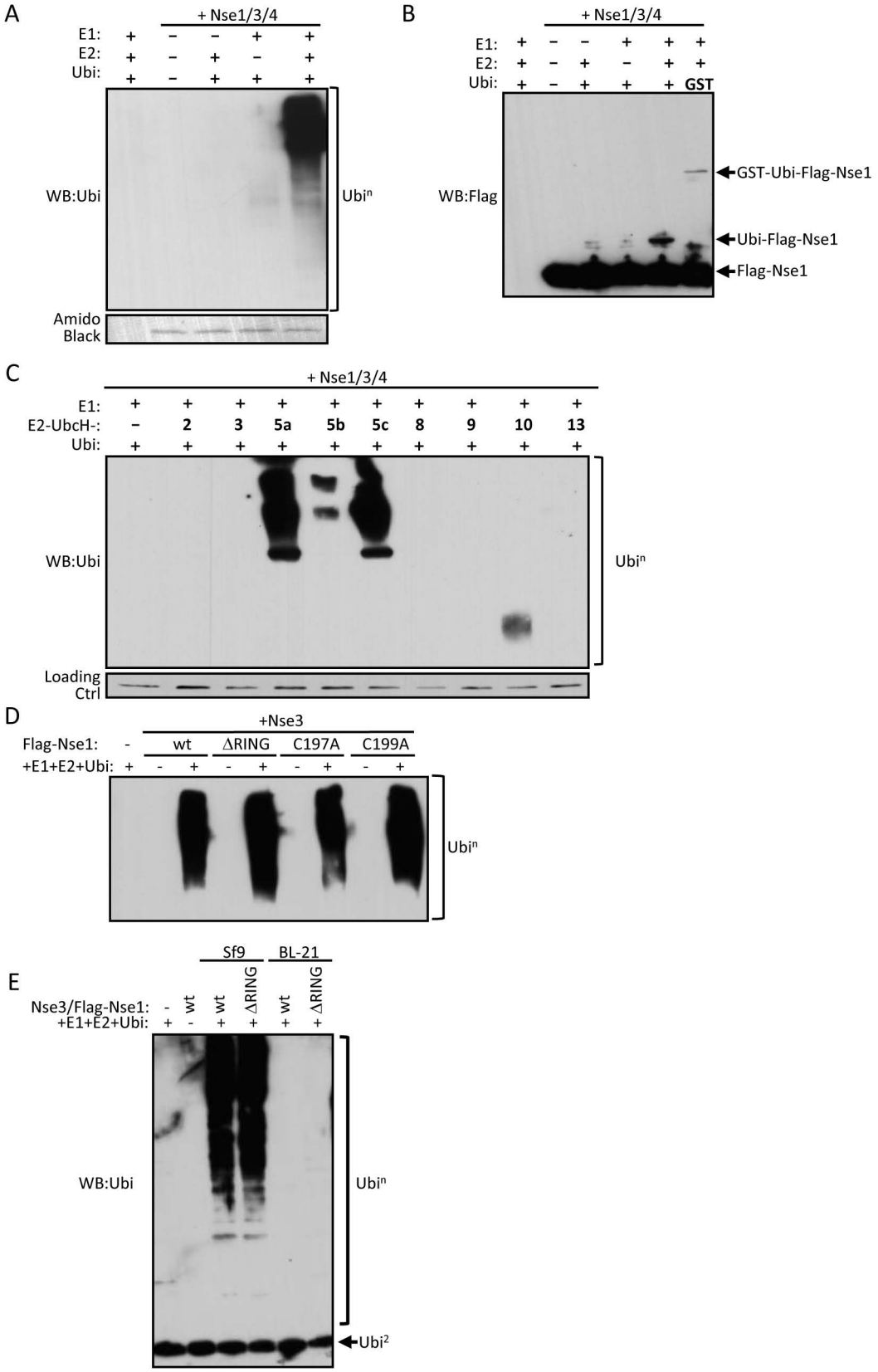
Supplemental Figure Legends

Supplemental Figure 1. Smc5-GFP localization upon DNA damage is also dependent on Nse1 RING motif. Wild-type (wt) or *nse1ΔRING* mutant cells carrying an endogenously tagged Smc5-GFP allele were exposed to MMS and tested for Smc5-GFP focus formation. Arrows point at the multiple but discrete Smc5 foci forming in MMS-treated wild-type cells, which do not form in cells lacking a functional Nse1 NH-RING domain.

Supplemental Figure 2. Nse1-Nse3 dimer expressed in insect cells displays weak ubiquitin E3 ligase activity. (A) Recombinant Nse1-Nse3-Nse4 trimer from Sf9 insect cells weakly catalyzes poly-ubiquitin chain formation *in vitro*. Ubiquitination reactions were performed using the indicated sets of proteins, and a recombinant trimer produced in Sf9 cells and containing Flag-Nse1, HA-Nse3 and Nse4 cleaved from its GST tag by PreScission protease cleavage. Reactions were immunoblotted with anti-ubiquitin antibody. Amido black-stained Nse3 was used as loading control. (B) Nse1 is mono-ubiquitinated within the Nse1-Nse3-Nse4 trimer *in vitro*. Ubiquitination reactions were performed using the indicated protein combinations, and the same recombinant trimer as in A. Where indicated, untagged ubiquitin was replaced by GST-tagged ubiquitin. Flag-tagged Nse1 was detected by anti-Flag immunoblotting. (C) The Nse1-Nse3-Nse4 trimer preferentially utilizes UbcH5A/C E2 enzymes to stimulate *in vitro* ubiquitination. Ubiquitination reactions were performed using E1, free ubiquitin, the same recombinant trimer as in (A), and various human recombinant E2 enzymes (UbcH-2,3,5a,5b,5c,8,9,10, and UbcH13/Uev1a dimer). Reactions were immunoblotted using anti-ubiquitin antibody. (D) Nse1-Nse3 dimer purified from insect cells catalyzes poly-ubiquitin chain formation *in vitro*, independently of the Nse1 NH-RING domain. Ubiquitination reactions were performed as in Figure 4, using the indicated combinations

of recombinant proteins. (E) The Nse1-Nse3 dimer produced in and purified from bacteria does not support ubiquitin E3 ligase activity. *In vitro* ubiquitination reactions were performed as in Figure 4, using equal amounts of recombinant Nse1-Nse3 complexes co-expressed in and purified from Sf9 insect or BL21 bacterial cells.


Smc5-GFP
Nse1 wt
nse1 ΔRING
-
+MMS


A
+ Nse1/3/4
E1:
E2:
Ubi:
WB:Ubi
Ubi^n
Amido Black
B
+ Nse1/3/4
E1:
E2:
Ubi:
GST
WB:Flag
GST-Ubi-Flag-Nse1
Ubi-Flag-Nse1
Flag-Nse1
C
+ Nse1/3/4
E1:
E2-UbcH-:
2
3
5a
5b
5c
8
9
10
13
Ubi:
WB:Ubi
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Loading Ctrl
D
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Flag-Nse1:
wt
ΔRING
C197A
C199A
+E1+E2+Ubi:
Ubi^n
E
Sf9
BL-21
Nse3/Flag-Nse1:
wt
wt
ΔRING
wt
ΔRING
+E1+E2+Ubi:
WB:Ubi
Ubi^n
Ubi^2