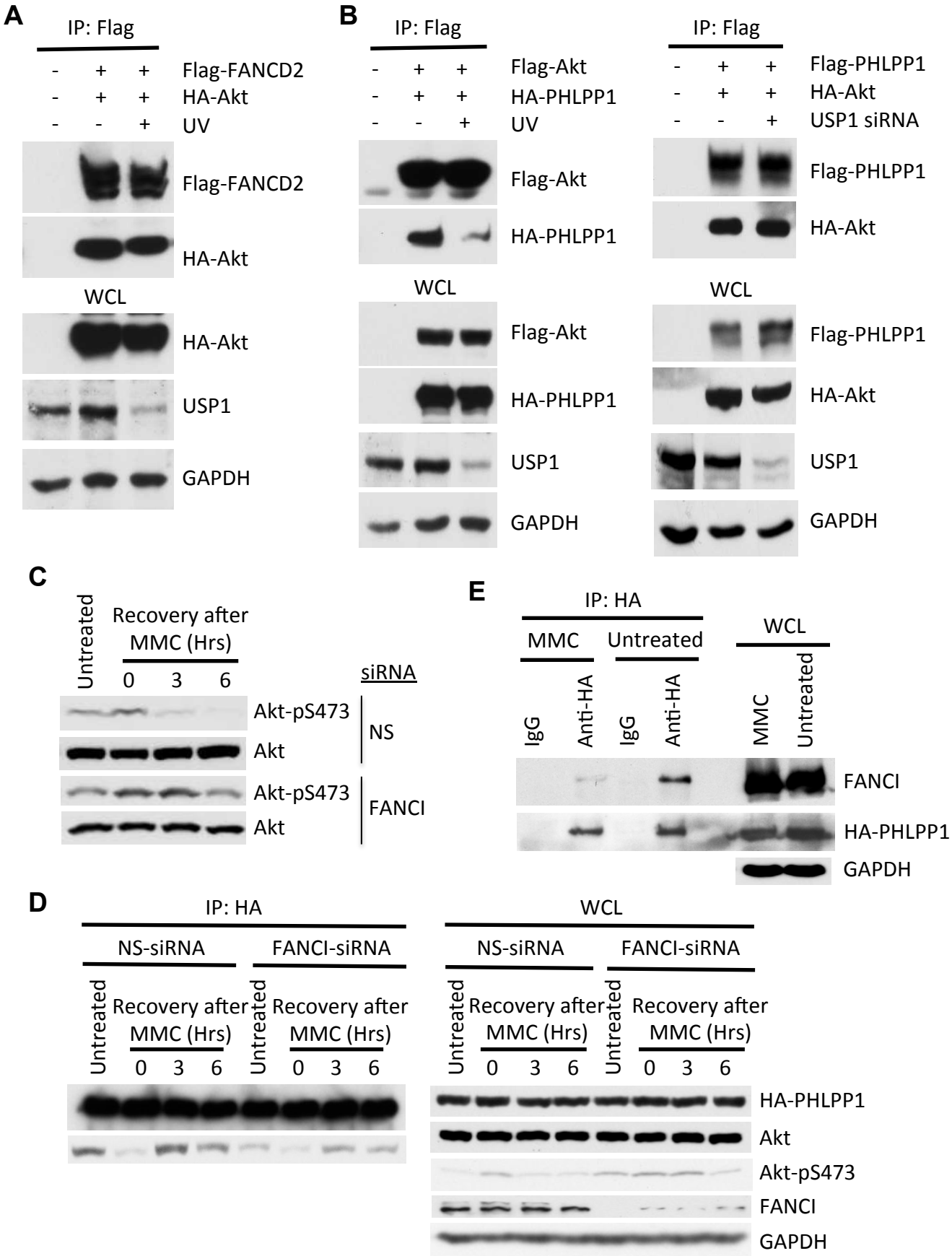
A
IP: Flag
- + + Flag-FANCD2
- + + HA-Akt
- - + UV
Flag-FANCD2
HA-Akt
WCL
HA-Akt
USP1
GAPDH
B
IP: Flag
- + + Flag-Akt
- + + HA-PHLPP1
- - + UV
Flag-Akt
HA-PHLPP1
WCL
Flag-Akt
HA-PHLPP1
USP1
GAPDH
IP: Flag
- + + Flag-PHLPP1
- + + HA-Akt
- - + USP1 siRNA
Flag-PHLPP1
HA-Akt
WCL
Flag-PHLPP1
HA-Akt
USP1
GAPDH
C
Untreated
Recovery after MMC (Hrs)
0 3 6
siRNA
Akt-pS473
Akt
NS
Akt-pS473
Akt
FANCI
E
IP: HA
MMC
Untreated
WCL
IgG
Anti-HA
IgG
Anti-HA
MMC
Untreated
FANCI
HA-PHLPP1
GAPDH
D
IP: HA
NS-siRNA
FANCI-siRNA
Untreated
Recovery after MMC (Hrs)
0 3 6
Untreated
Recovery after MMC (Hrs)
0 3 6
WCL
NS-siRNA
FANCI-siRNA
Untreated
Recovery after MMC (Hrs)
0 3 6
Untreated
Recovery after MMC (Hrs)
0 3 6
HA-PHLPP1
Akt
Akt-pS473
FANCI
GAPDH

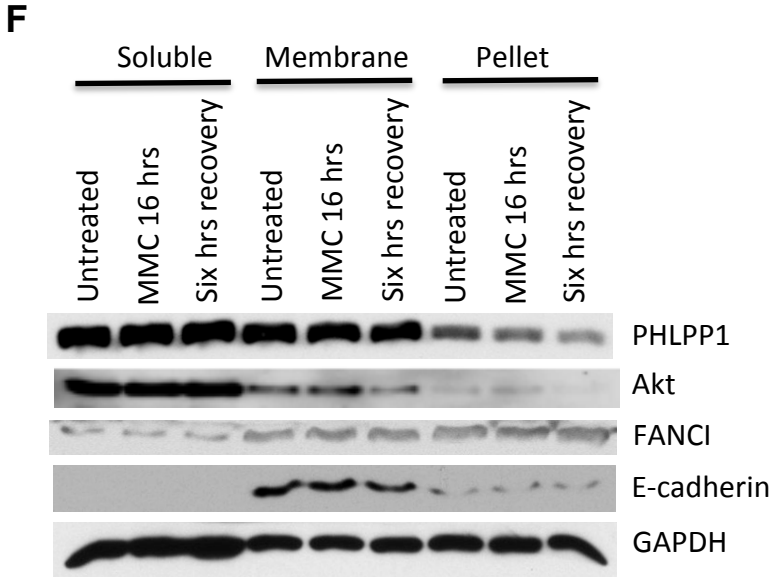

Figure S5. The effects of DNA damage on interactions among components of the PAF complex. WCL and IPs were derived from HEK293 cells transfected with the indicated Flag or HA tagged constructs. (**A**) Immunoblots of WCL and co-IPs indicating that UV irradiation did not affect the interaction between FANCD2 and Akt. GAPDH indicates a loading control. (**B**) Immunoblots of WCL and co-IPs indicating that UV irradiation, but not depletion of USP1 by siRNA, decreased the interaction between PHLPP1 and Akt. (**C**) Immunoblots of WCL showing that depletion of FANCI by siRNA resulted in enhanced phosphorylation of Akt during recovery from MMC treatment. Cells were incubated with MMC for 16 hrs and then changed to normal media for the indicated lengths of time. (**D**) Immunoblots of WCL and co-IPs indicating that during recovery from MMC treatment the interaction between PHLPP1 and Akt was reduced by depletion of FANCI. (**E**) Immunoblots of WCL and co-IPs showing that MMC treatment reduced the interaction between FANCI and PHLPP1. (**F**) Localization of PAF complex proteins in response to DNA damage. HEK293 cells were either untreated or treated as indicated and lysates were subsequently separated into soluble, membrane or pellet fractions.