Figure S7

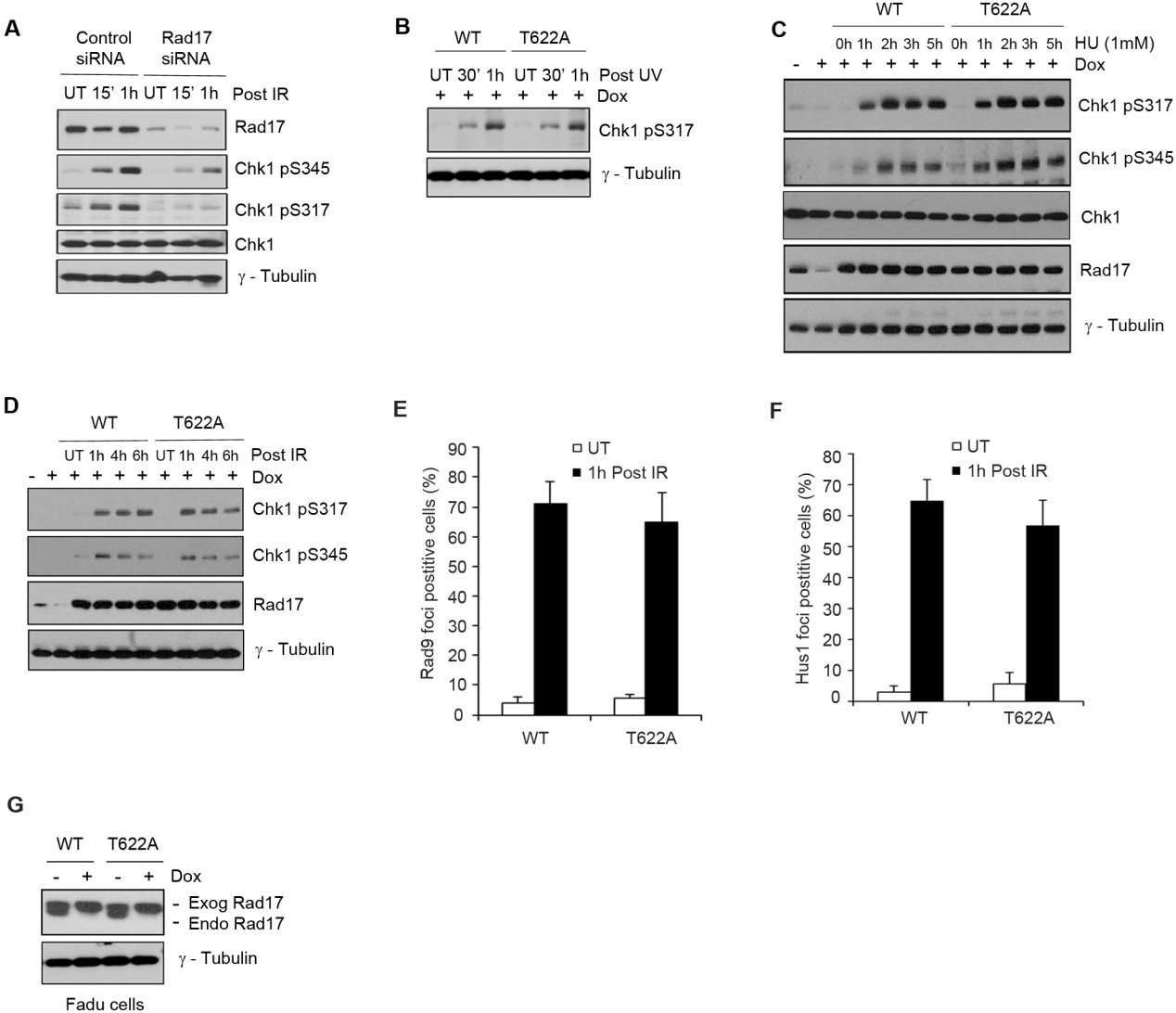
A
Control siRNA
Rad17 siRNA
UT 15' 1h UT 15' 1h Post IR
Rad17
Chk1 pS345
Chk1 pS317
Chk1
γ - Tubulin
B
WT
T622A
UT 30' 1h UT 30' 1h Post UV
+ + + + + + Dox
Chk1 pS317
γ - Tubulin
C
WT
T622A
0h 1h 2h 3h 5h 0h 1h 2h 3h 5h HU (1mM)
- + + + + + + + + + + Dox
Chk1 pS317
Chk1 pS345
Chk1
Rad17
γ - Tubulin
D
WT
T622A
UT 1h 4h 6h UT 1h 4h 6h Post IR
- + + + + + + + + + Dox
Chk1 pS317
Chk1 pS345
Rad17
γ - Tubulin
E
Rad9 foci postitive cells (%)
UT
1h Post IR
WT
T622A
F
Hus1 foci postitive cells (%)
UT
1h Post IR
WT
T622A
G
WT
T622A
- + - + Dox
- Exog Rad17
- Endo Rad17
γ - Tubulin
Fadu cells

Figure S7 Rad17 Thr622 phosphorylation has no significant effect on Chk1 activation and Rad9/Hus1 recruitment to DSBs. (**A**) Rad17 is required for Chk1 activation after IR. U2OS cells transfected with control siRNA or Rad17 siRNA were either left untreated or exposed to 5 Gy of IR. Cell lysates prepared at the indicated times were blotted as indicated. (**B**-**D**) Rad17 Thr622 phosphorylation is dispensable for Chk1 activation following UV, HU but has moderate effect on Chk1 phosphorylation at Ser317 after IR treatment. Inducible Rad17 knockdown cells (U2OS) stably expressing shRNA-resistant Rad17 WT or Rad17 T622A mutant treated with Dox for two days were either left untreated or exposed to UV (50 J/M^2) (**B**), HU (1 mM) (**C**) or IR (5 Gy) (**D**). Cell lysates were prepared at the indicated times. Western blot was performed as indicated. (**E** and **F**) Inducible Rad17 knockdown cells (U2OS) stably expressing shRNA-resistant Rad17 WT or Rad17 T622A mutant treated with Dox for two days were either left untreated or exposed to 5Gy of IR. Cells were fixed and stained with anti-Rad17 and anti-Rad9 (**E**) or anti-Hus1 (**F**). The percentage of Rad9 or Hus1 foci positive cells was plotted (mean ± SD, n=3). (**G**) Inducible Rad17 knockdown cells (Fadu) stably expressing shRNA-resistant Rad17 WT or Rad17 T622A mutant were treated with or without Dox for two days. Western blot was performed as indicated. Dox induced down-regulation of endogenous Rad17 in Fadu cells.