Title

The role of HERC2 and RNF8 ubiquitin E3 ligases in the promotion of translesion DNA synthesis in the chicken DT40 cell line

Supplementary Data

Fig.S1 Molecular mechanisms for Ig gene diversification, yielding single base substitutions at dC/dG pairs and Ig gene conversion in DT40 cells. AID-mediated deamination of deoxycytidine (C) generates U, which is excised by Uracil DNA glycosylase (UNG) leading to the generation of an abasic site (AP). Abasic sites cause replication blockage. Bottom right: release of replication blockage by TLS leads to point mutations at C/G pairs. Bottom left: alternatively, HR-dependent template switching from the functional V segment to one of the pseudo-V (ΨV) genes induces Ig gene conversion. Gray boxes indicate ΨV genes. This figure is based on previously described results [1,2].

Fig.S2 No detectable sensitivity of *RNF8*$^{-/-}$, *HERC2*$^{-/-}$, *RNF8*$^{-/-}$/*HERC2*$^{-/-}$ and *RNF168*$^{-/-}$ cells to cisplatin, MMS, and UV. The x-axis represents the dose of the DNA-damaging agents in a linear scale, and the y-axis represents the % of survival relative to non-treated cells.

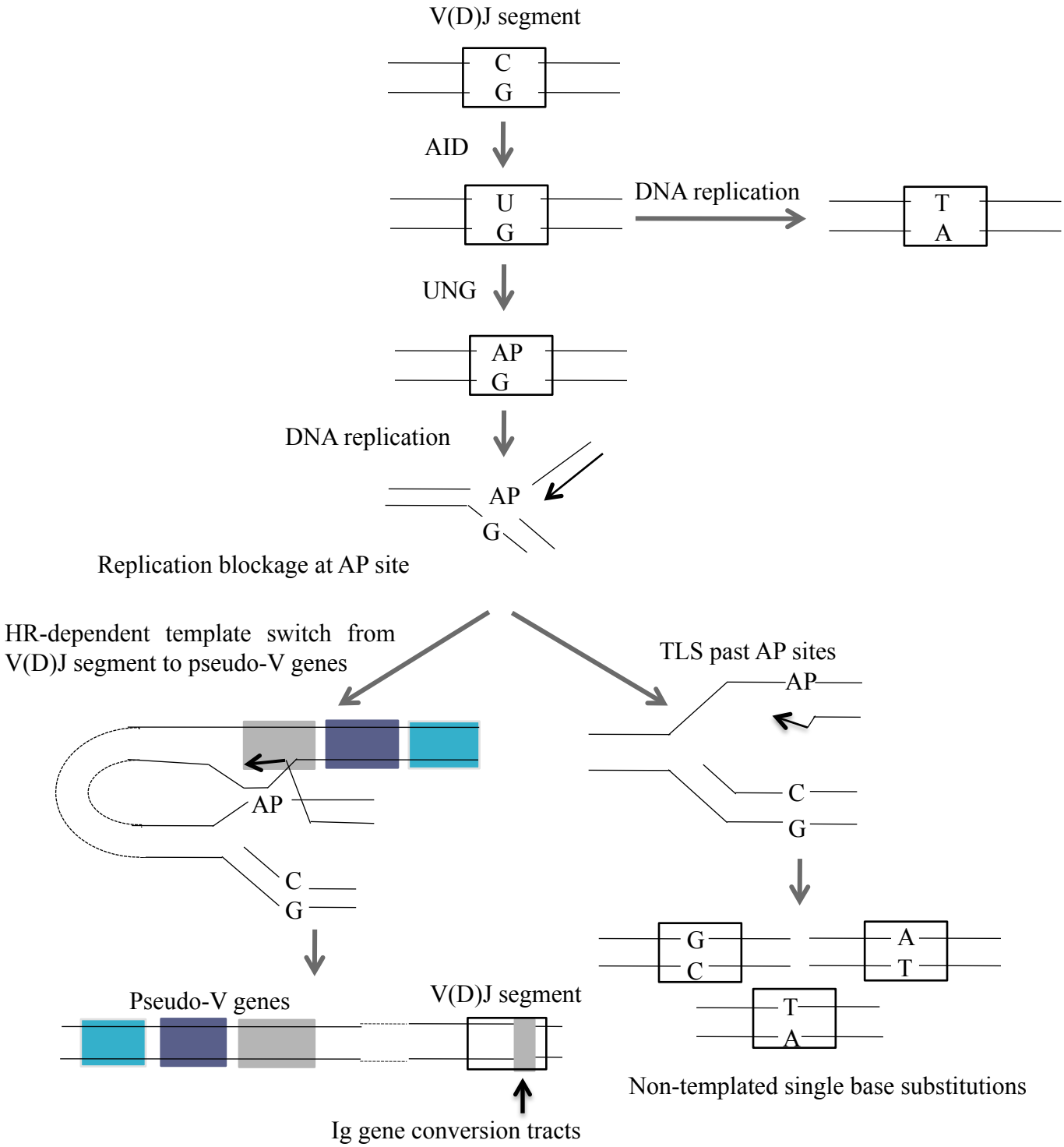
Fig.S3 The important role of HERC2 and RNF8 in TLS past abasic sites during Ig V_λ hypermutation. Clones over-expressing AID were expanded for two weeks before isolating genomic DNA and sequencing the V_λ segment. Each dashed line represents the V_λ segment region (450 bp), with single base substitution (lollipop shapes), gene conversion (horizontal bars), mutations of ambiguous origin (vertical bars), single-base deletion (triangle), oligonucleotides deletion (filled boxes) and duplication (vertical bars with rectangle) are indicated.

Fig.S4 Analysis of ubiquitination of PCNA as in fig.3. Whole western blot images used for quantification of mono-ubiquitinated PCNA in *HERC2*$^{-/-}$ and *RNF8*$^{-/-}$ mutants. Neither HERC2 nor RNF8 is required for the mono-ubiquitination of PCNA in UV-irradiated cells**.**

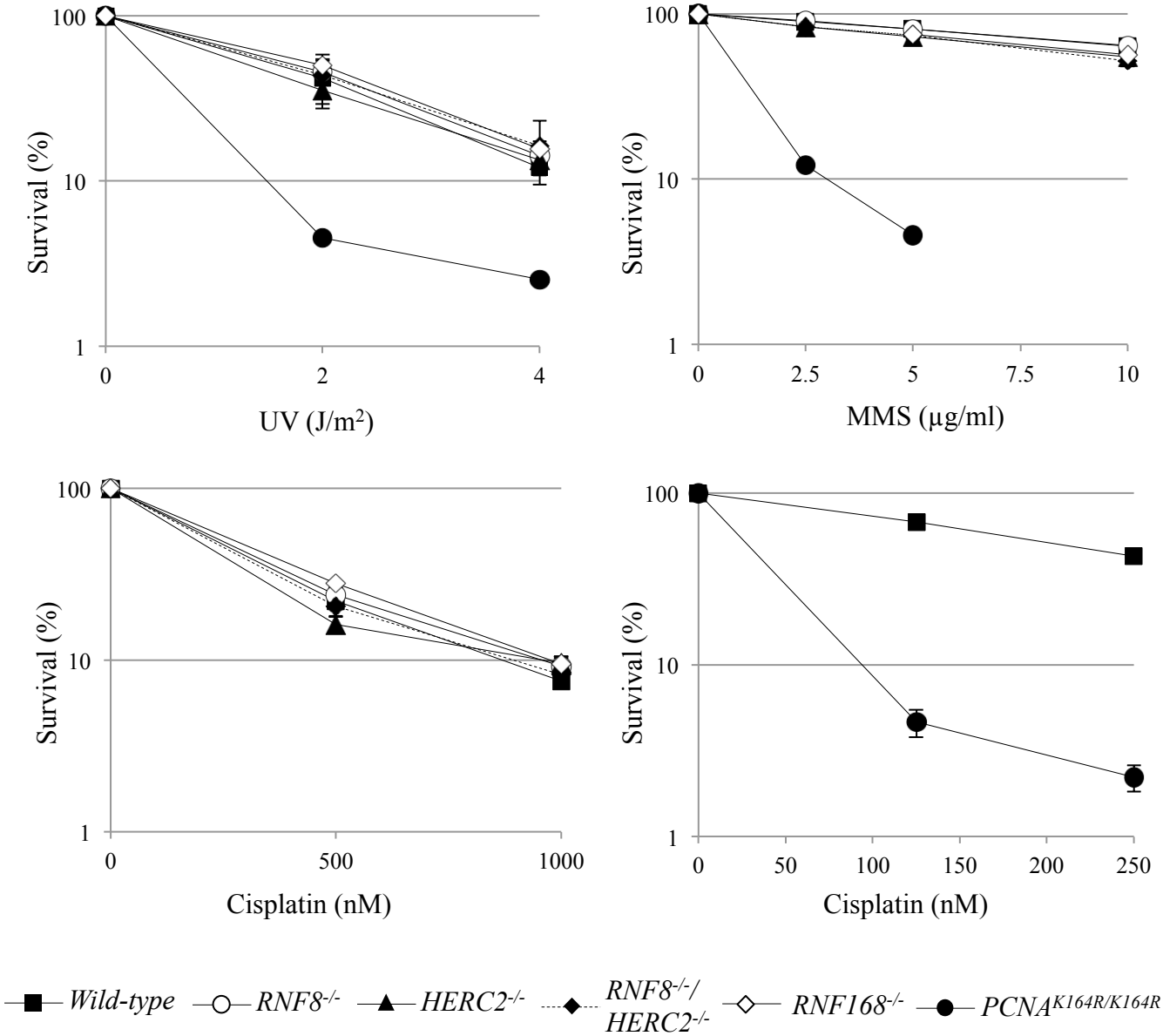
References

[1] J.E. Sale, D.M. Calandrini, M. Takata, S. Takeda, M.S. Neuberger, Ablation of XRCC2/3 transforms immunoglobulin V gene conversion into somatic hypermutation., Nature. 412 (2001) 921-926.

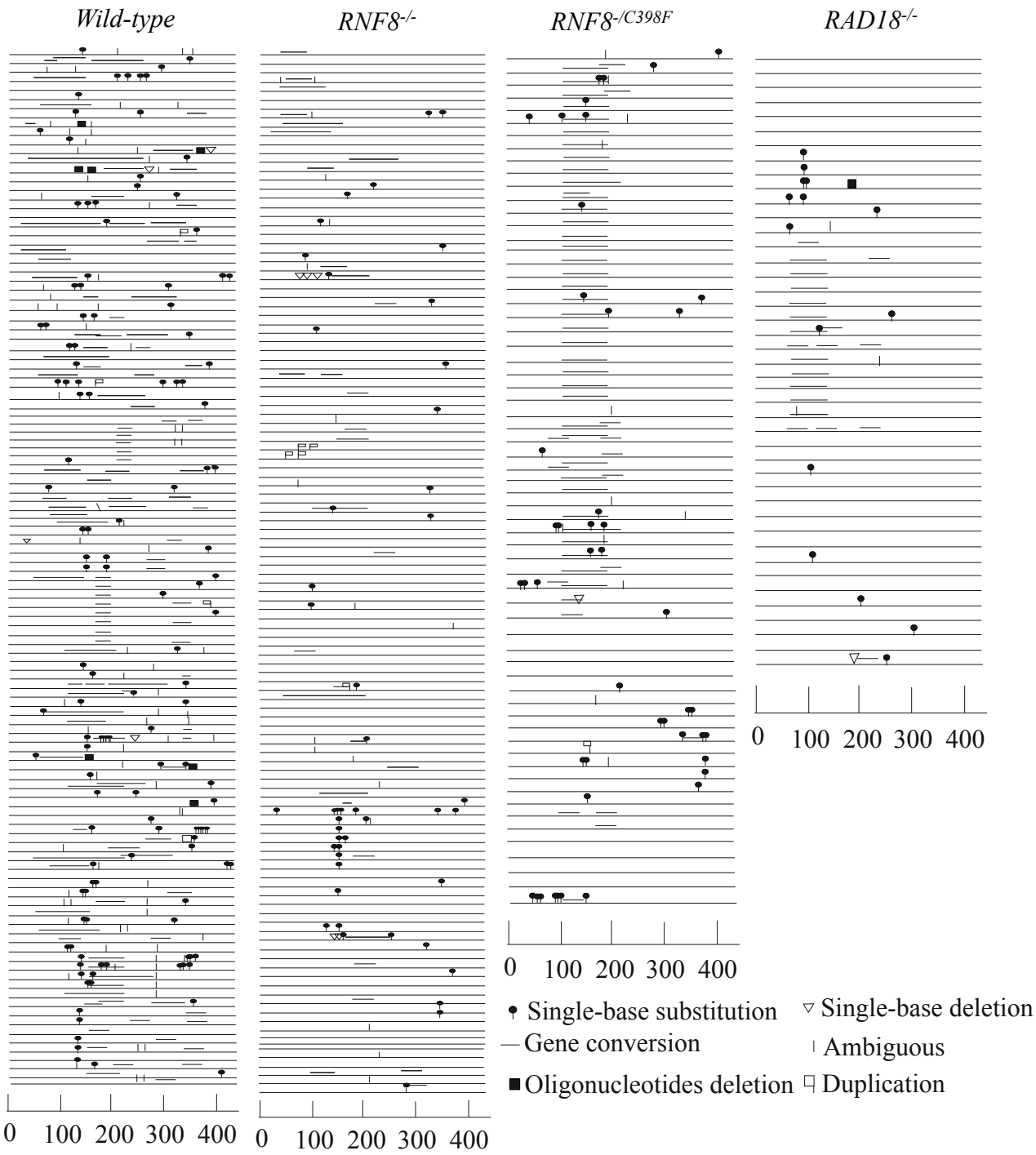
[2] H. Arakawa, J. Hauschild, J.-M. Buerstedde, Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion., Science. 295 (2002) 1301-1306.

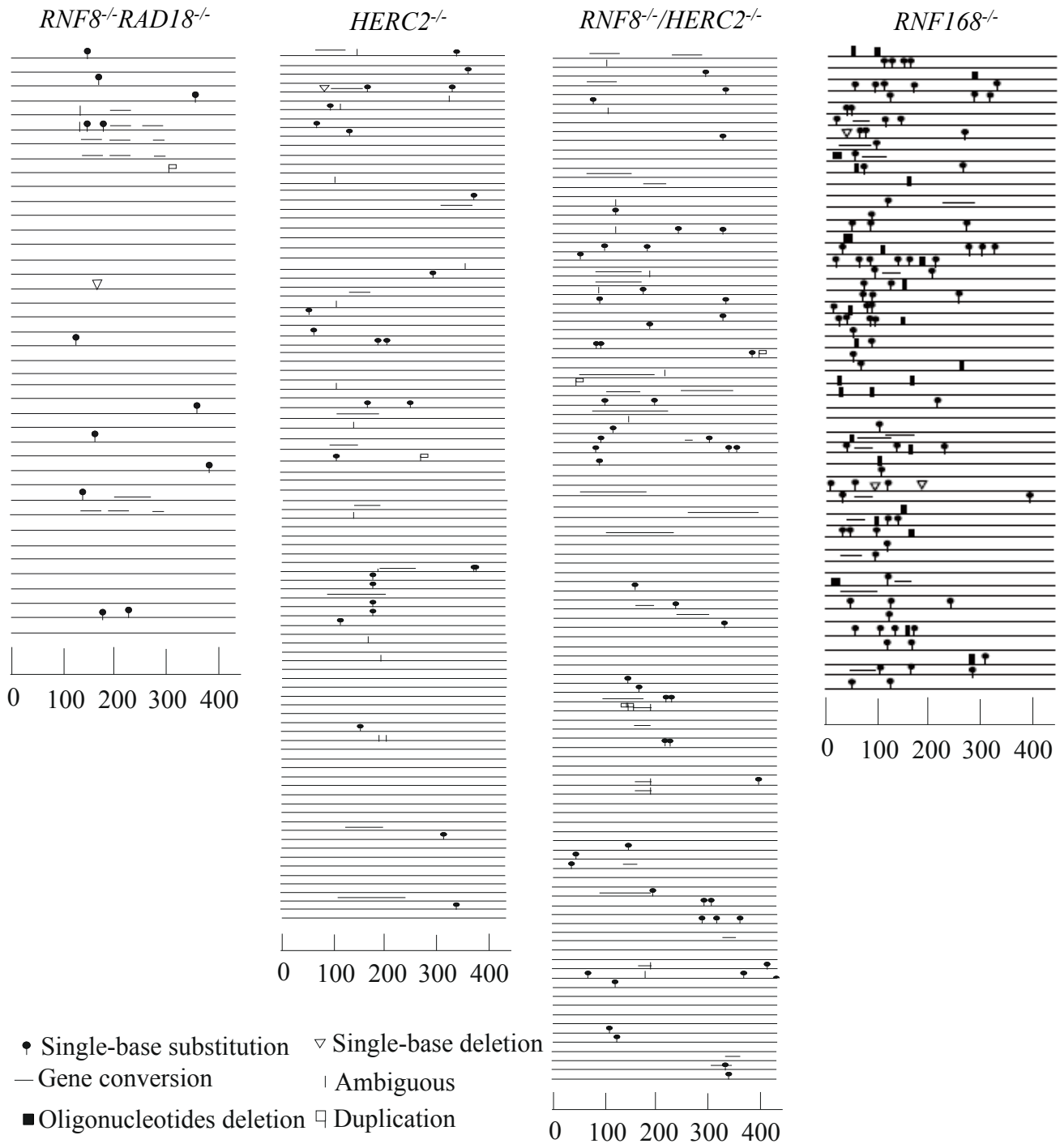


Supplement Fig.1

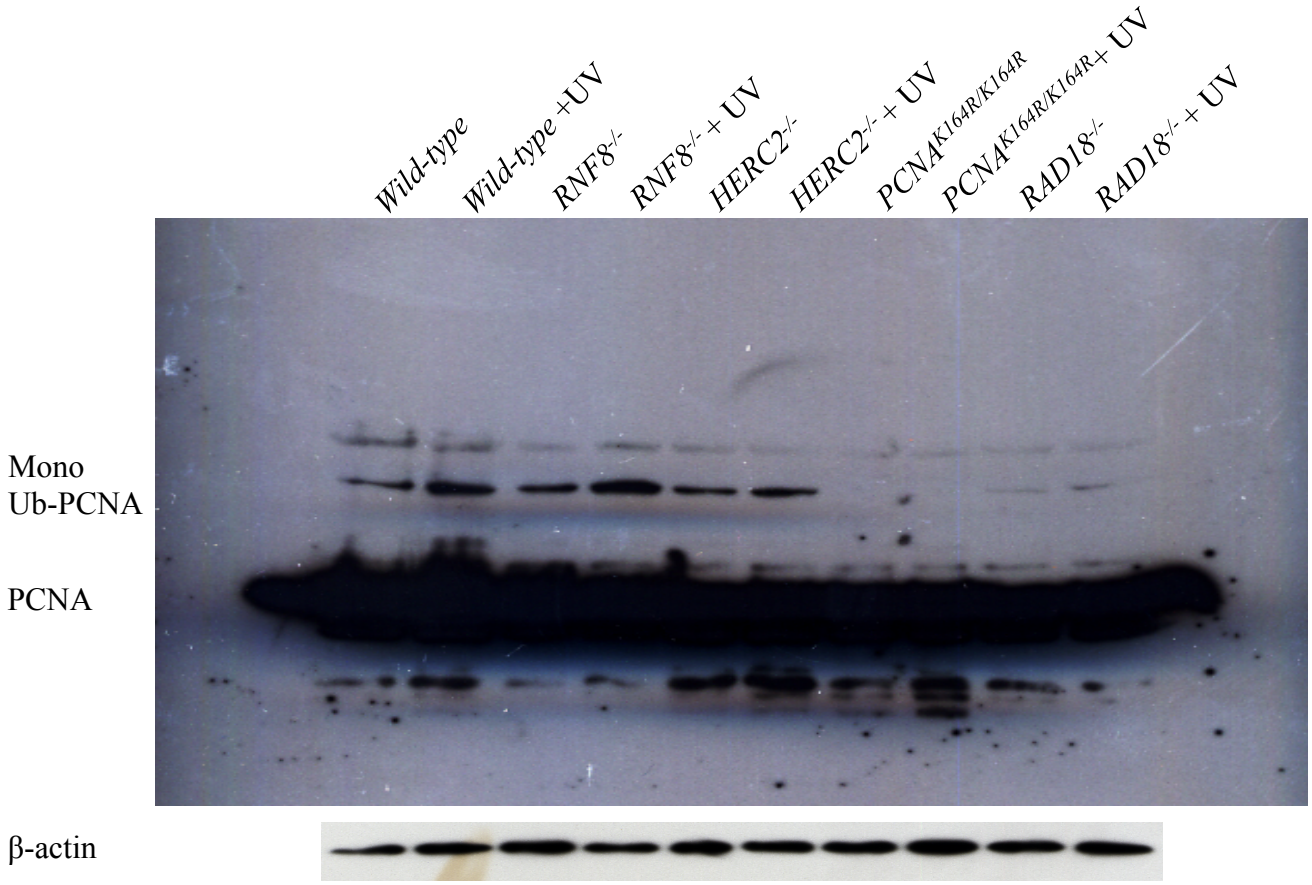


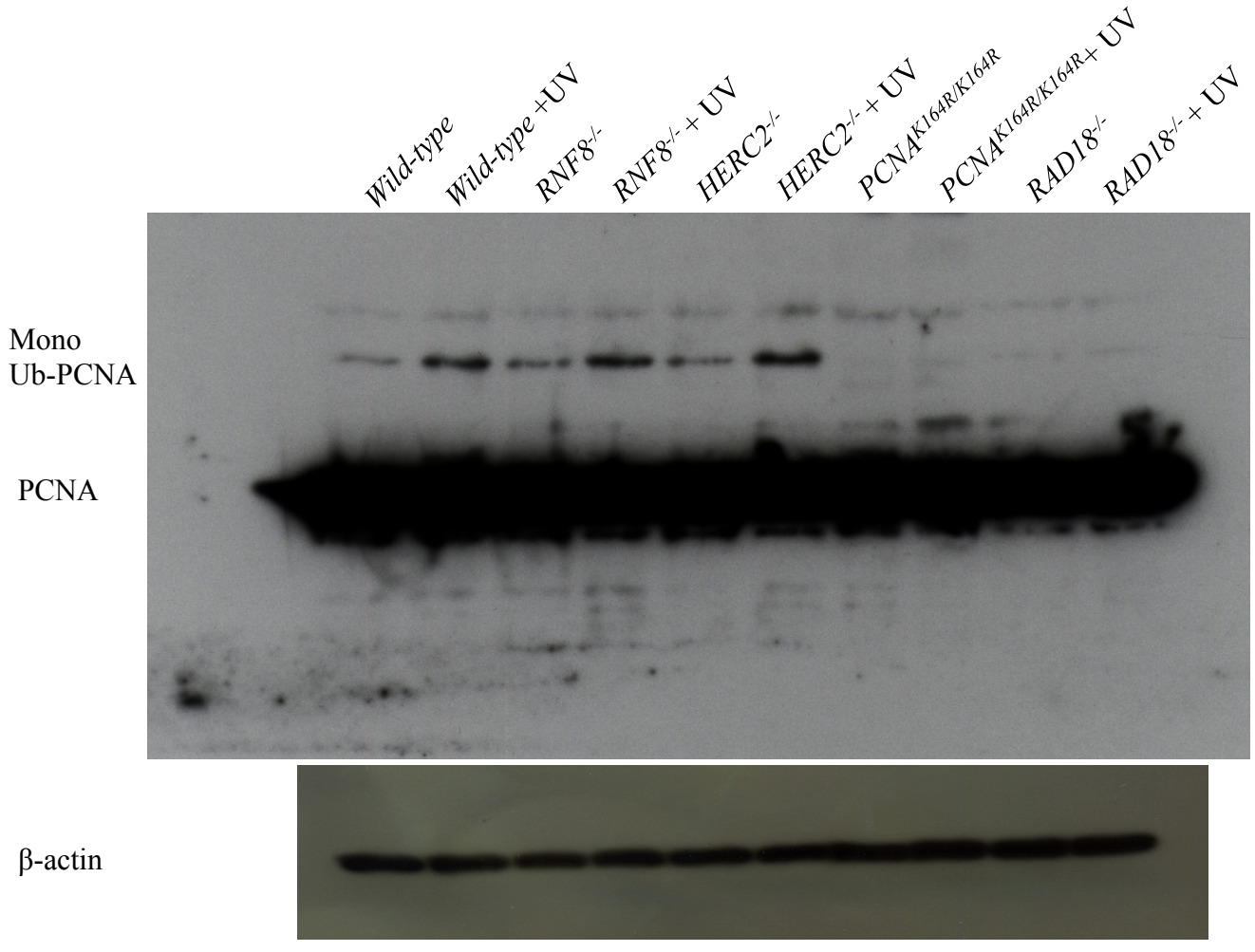
Supplement Fig.2


Wild-type
RNF8-/-
RNF8-/C398F
RAD18-/-
0 100 200 300 400
0 100 200 300 400
0 100 200 300 400
0 100 200 300 400
Single-base substitution
Gene conversion
Oligonucleotides deletion
Single-base deletion
Ambiguous
Duplication



Supplement Fig.3





Supplement Fig.4