Expanded View Figures

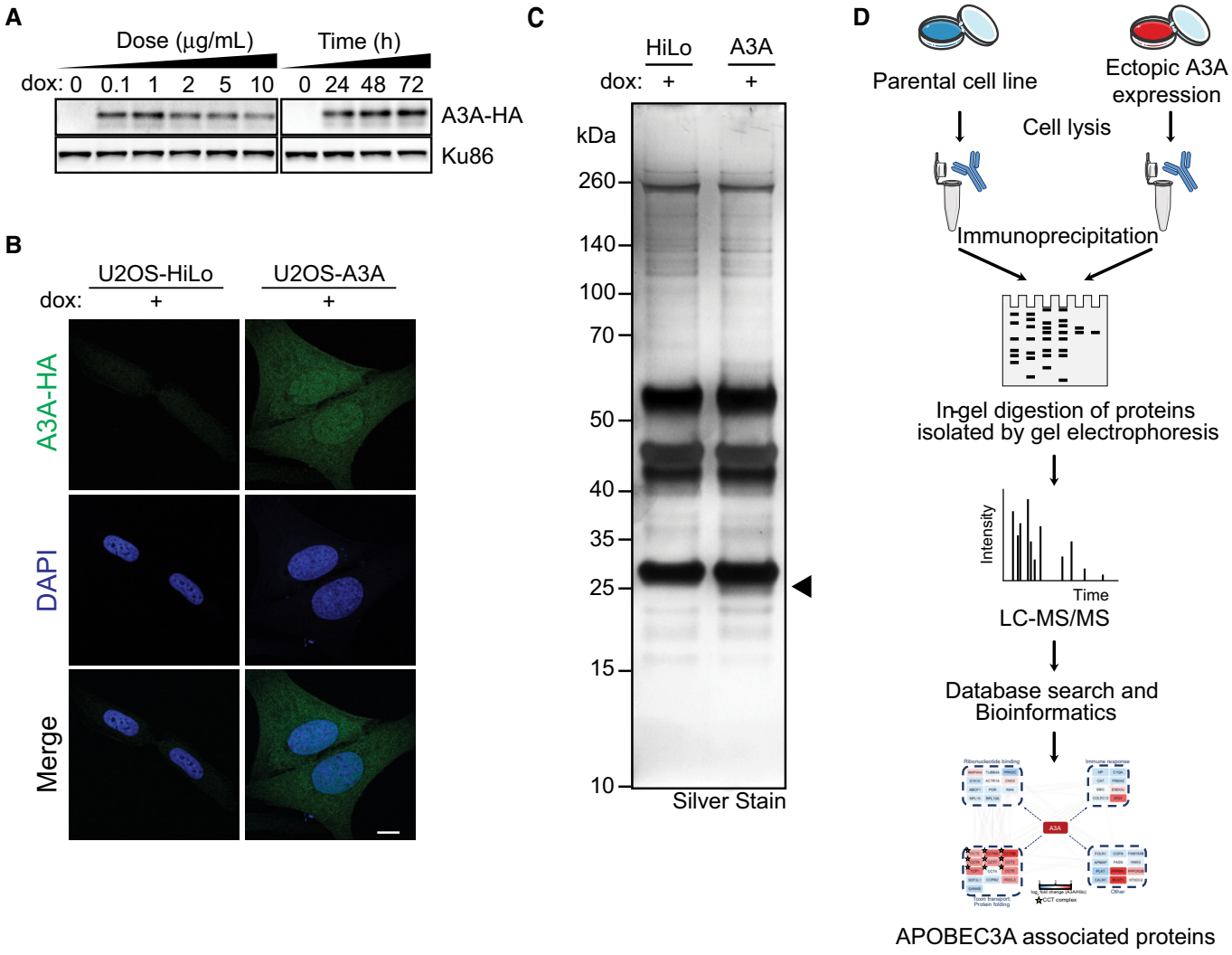

Figure EV1. Approach for discovery of APOBEC3A protein interactors.

A Ectopically expressed HA-tagged A3A was induced by treatment with dox at varying concentrations and duration in U2OS-HiLo cells. Lysates were analyzed by immunoblot using an HA antibody. Ku86 was used as a loading control. Based on these results, proteomics experiments were performed on both the U2OS-A3A and the parental U2OS-HiLo cells treated with 1 µg/ml dox for 72 h.

B A3A expression is pan-cellular in U2OS cells. Parental U2OS-HiLo cells and U2OS-A3A cells were treated with dox, stained with HA antibody, and analyzed by confocal microscopy. Nuclei were stained with DAPI. Scale bar is 10 µm.

C Silver stain detection of A3A-HA immunoprecipitation in U2OS-HiLo cells. U2OS-HiLo cells and U2OS-A3A cells were immunoprecipitated with HA antibody after treatment with 1 µg/ml dox for 72 h. Lysates were separated by PAGE and analyzed by silver stain. APOBEC3A appears under the immunoglobulin light chain at ~ 24 kDa (indicated by arrow).

D A quantitative proteomics approach was employed to identify protein interactors of A3A. Schematic of the approach for discovery of protein interactors of A3A in a U2OS cell line by immunoprecipitation followed by quantitative proteomics.

Source data are available online for this figure.

Figure EV2. Comparison of APOBEC mutational signature burden in CCT-deficient and control tumors.

A All cancer genome sequences in TCGA were evaluated for mutation burden and specific base substitutions. Cancers with deleterious mutations in one or more CCT complex genes were compared to a control group of primary cancers that harbor deleterious mutations in a random gene set. The random gene set was constructed from 9 genes of similar size to those that comprise CCT members, and with similarly frequent mutational rate among the 9 genes relative to mutation rate among CCT complex genes. Wilcoxon rank sum test was used for statistical comparison. For each column on the *x*-axis, a comparison between tumors without CCT mutations and those with CCT mutations is significant ($P < 0.01$). Similarly, for each x-axis column a comparison between tumors without CCT mutations and those with random gene mutations is significant ($P < 0.01$). For each *x*-axis column, a comparison between tumors with CCT gene mutations and tumors with random gene mutations is not statistically significant.

B The burden of mutations attributed to APOBEC3 activity (SBS2 and SBS13) was evaluated in CCT-mutated, random gene set-mutated, and all other cancer genome sequences. Depicted is the quantity of mutations within mutational signatures attributable to APOBEC3 activity (SBS2 and SBS13) in comparison with all other SBS mutational patterns (other). Wilcoxon rank sum test was used for statistical comparison. $^{**}P < 0.01$ and ns non-significant.

C Quantification of APOBEC3 mutational signatures among breast, bladder, and cervical cancer samples from the TCGA database was divided into CCT-mutated, random gene set-mutated, and non-mutated genomes, as above. The number of mutations contributing to SBS2 and SBS13 in each tumor type is quantified and displayed as an average. Wilcoxon rank sum test was used for statistical comparison. $^{**}P < 0.01$, $^{*}P < 0.05$, and ns non-significant.

D The contribution of SBS2, SBS13, and all other SBS signatures was evaluated in all tumors and independently in breast, bladder, and cervical cancer genomes within the TCGA database. A comparison of mutational signature contributions between CCT-mutated, random gene set-mutated (Ran.), and non-mutated tumors is shown. A z-test of proportions was used to compare whether the percentage of "other" signatures is significantly different between CCT-mutated and non-mutated tumors. $^{**}P < 0.01$, $^{*}P < 0.05$, and ns non-significant.

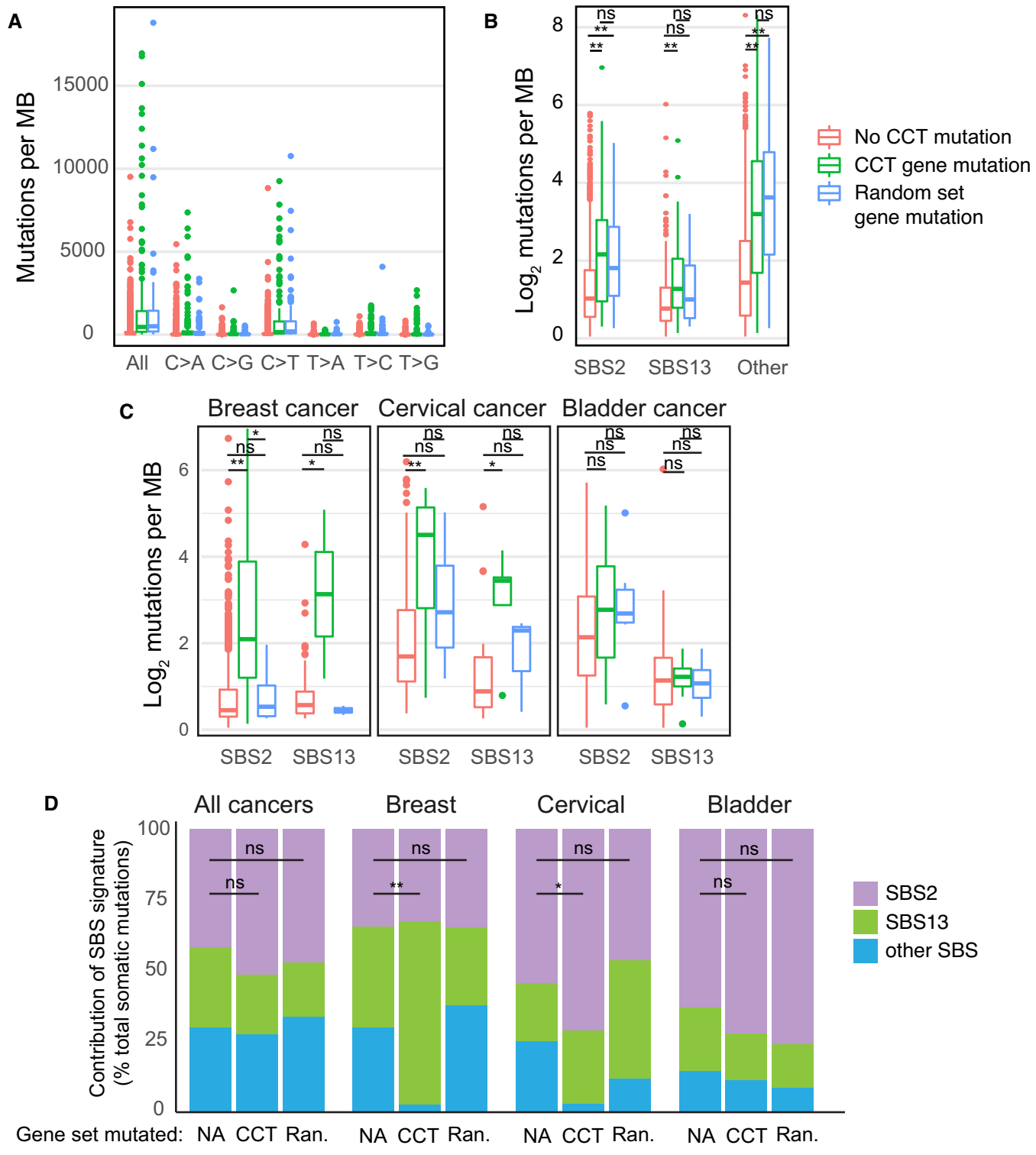
A
Mutations per MB
15000
10000
5000
0
All C>A C>G C>T T>A T>C T>G
B
Log2 mutations per MB
8
6
4
2
0
SBS2 SBS13 Other
No CCT mutation
CCT gene mutation
Random set gene mutation
C
Breast cancer
Cervical cancer
Bladder cancer
Log2 mutations per MB
SBS2 SBS13 SBS2 SBS13 SBS2 SBS13
D
All cancers
Breast
Cervical
Bladder
Contribution of SBS signature (% total somatic mutations)
100
75
50
25
0
Gene set mutated: NA CCT Ran. NA CCT Ran. NA CCT Ran. NA CCT Ran.
SBS2
SBS13
other SBS

Figure EV2.