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Supplemental information

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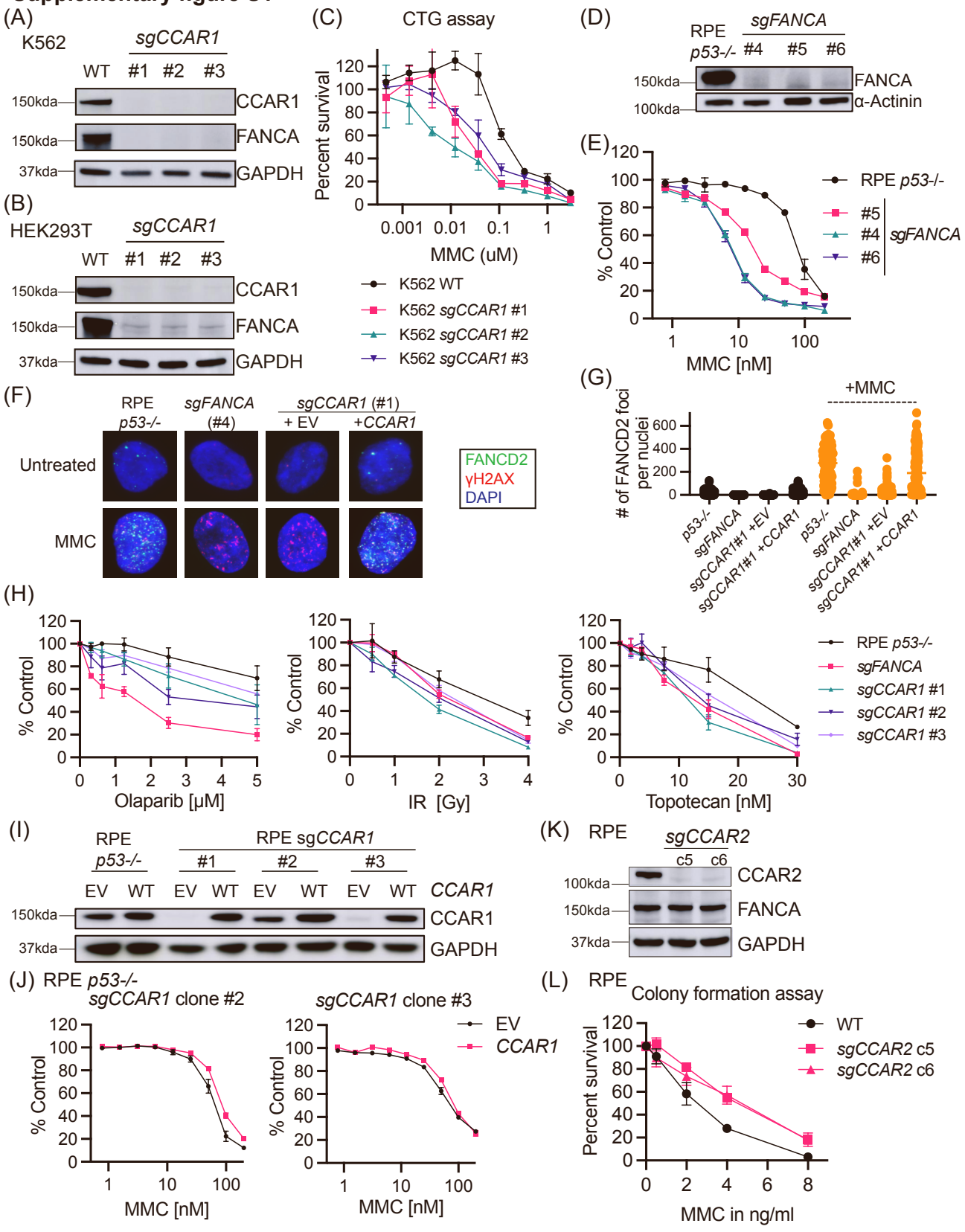
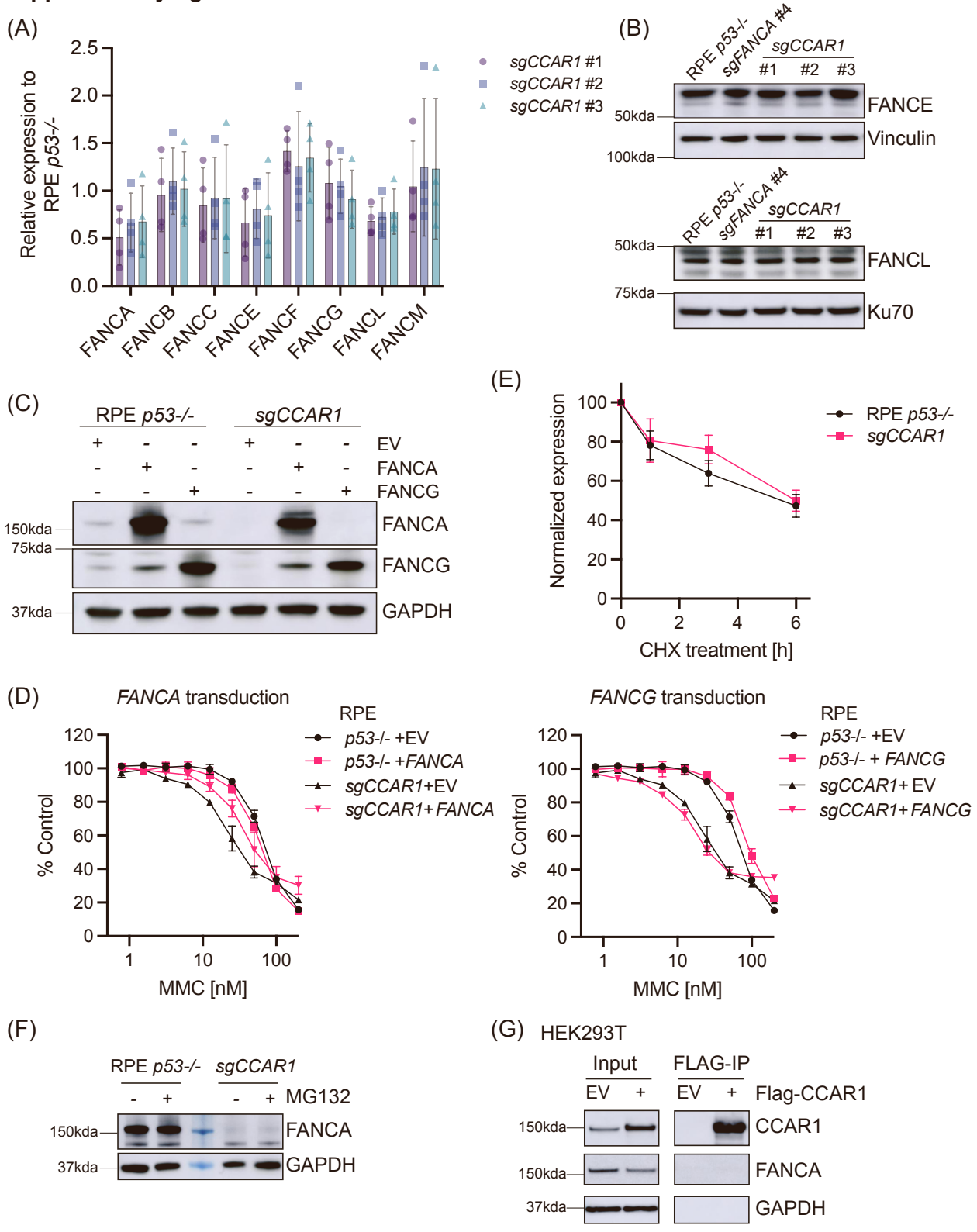
Supplementary figure S1



Supplementary Figure S1: *CCAR1* knock-out cells show FA-like phenotypes in multiple cell lines, related to Figure 1

(A-B) *CCAR1* knock-out clones in K562 (A) and HEK293T (B) cell lines show loss of FANCA similar to RPE data in Figure 1. (C) K562 *CCAR1* knockout clones are sensitive to MMC treatment as evaluated by CTG assay. Mean and SD from n=3 independent experiments are plotted. (D) WB of FANCA in *FANCA* knock-out clones. (E) MMC sensitivity of *FANCA* knock-out clones. Cells were treated with MMC for 6 days, and then cell viability was measured by CellTiter-Glo assay. Mean and SD from n=2 independent experiments are plotted. (F, G) Representative immunofluorescence images and quantification of the number of FANCD2 foci (n=2). (H) Colony formation assay data of RPE p53-/-*CCAR1* knock-out clones. Cells were treated with Olaparib, X-ray, or Topotecan. Mean and SEM from n=3 independent experiments are plotted. (I) WB of CCAR1 in *CCAR1* transduced RPE *p53-/- CCAR1* knock-out clones. RPE *p53*-/- and *p53-/-CCAR1* knock-out clones were transduced with pLV-Blast (EV) or WT *CCAR1* cDNA, and selected using 10 μg/mL Blasticidin S HCl for 7 days. (J) MMC resistance of WT *CCAR1* transduced RPE *p53-/- CCAR1* knock-out clones #2 and #3. The cells were treated with MMC for 6 days, and then cell viability was measured by CellTiter-Glo assay. Mean and SEM from n=3 independent experiments are plotted. (K) WB showing RPE *CCAR2* knock-out clones do not have a defect in FANCA protein expression. (L) *CCAR2* loss does not sensitize cells to MMC treatment. Colony formation assay of RPE *CCAR2* knock-out clones treated with MMC for 7 days. Mean and SD from n=3 independent experiments are plotted.

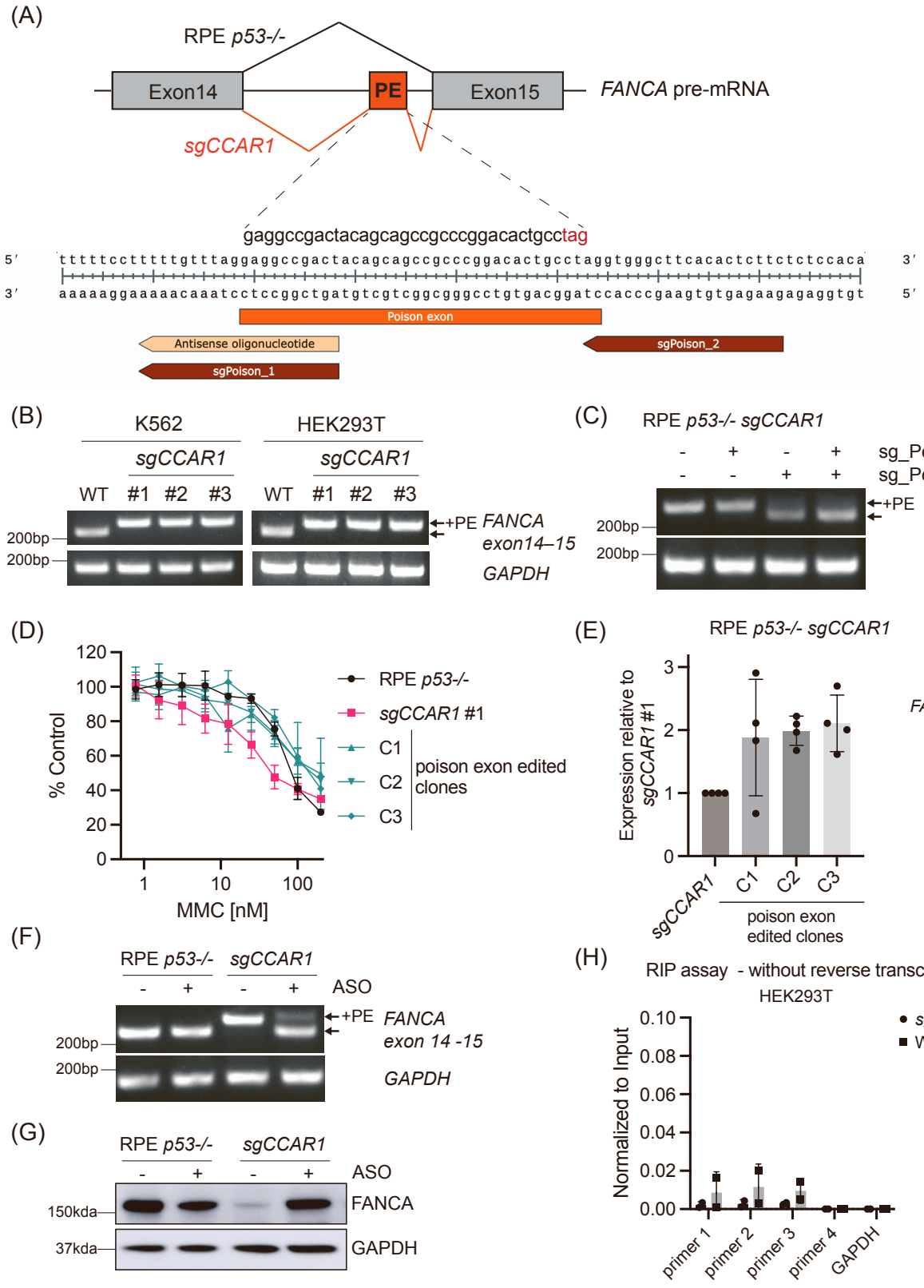
Supplementary figure S2



Supplementary Figure S2: CCAR1 loss does not affect other genes in the FA pathway, related to Figure 2

(A) mRNA expression levels of FA core-complex genes in RPE *p53-/-CCAR1* knock-out clones. Mean and SEM from n=4 independent experiments are plotted. (B) WB of FANCE and FANCL in RPE *p53-/-CCAR1* knock-out clones. (C) WB of FANCA and FANCG in RPE *p53*-/- and *p53-/-CCAR1* knock-out cells transduced with pMMP-puro (EV), *FANCA*, or *FANCG* cDNA, and selected using 12 μg/mL puromycin for 3 days (n=2). (D) Cell survival was evaluated for *FANCA* or *FANCG* cDNA transduced RPE *p53*-/- or *p53-/-CCAR1* knock-out cells treated with MMC using the CellTiter Glo assay. The cells were treated with MMC for 6 days. Mean and SEM from n=3 independent experiments are plotted. (E) Time-course of the degradation of FANCA protein in CHX-treated RPE *p53*-/- and *p53-/-CCAR1* knock-out cells transduced with *FANCA* cDNA. WB images were quantified using ImageJ. Mean and SD from n=3 independent experiments are plotted. (F) WB of FANCA in RPE *p53*-/- and *p53-/-CCAR1* knock-out cells treated with MG-132 (10 μM) for 6 h (n=2). (G) Co-immunoprecipitation assay of FLAG-CCAR1 and FANCA in HEK293T cells (n=2).

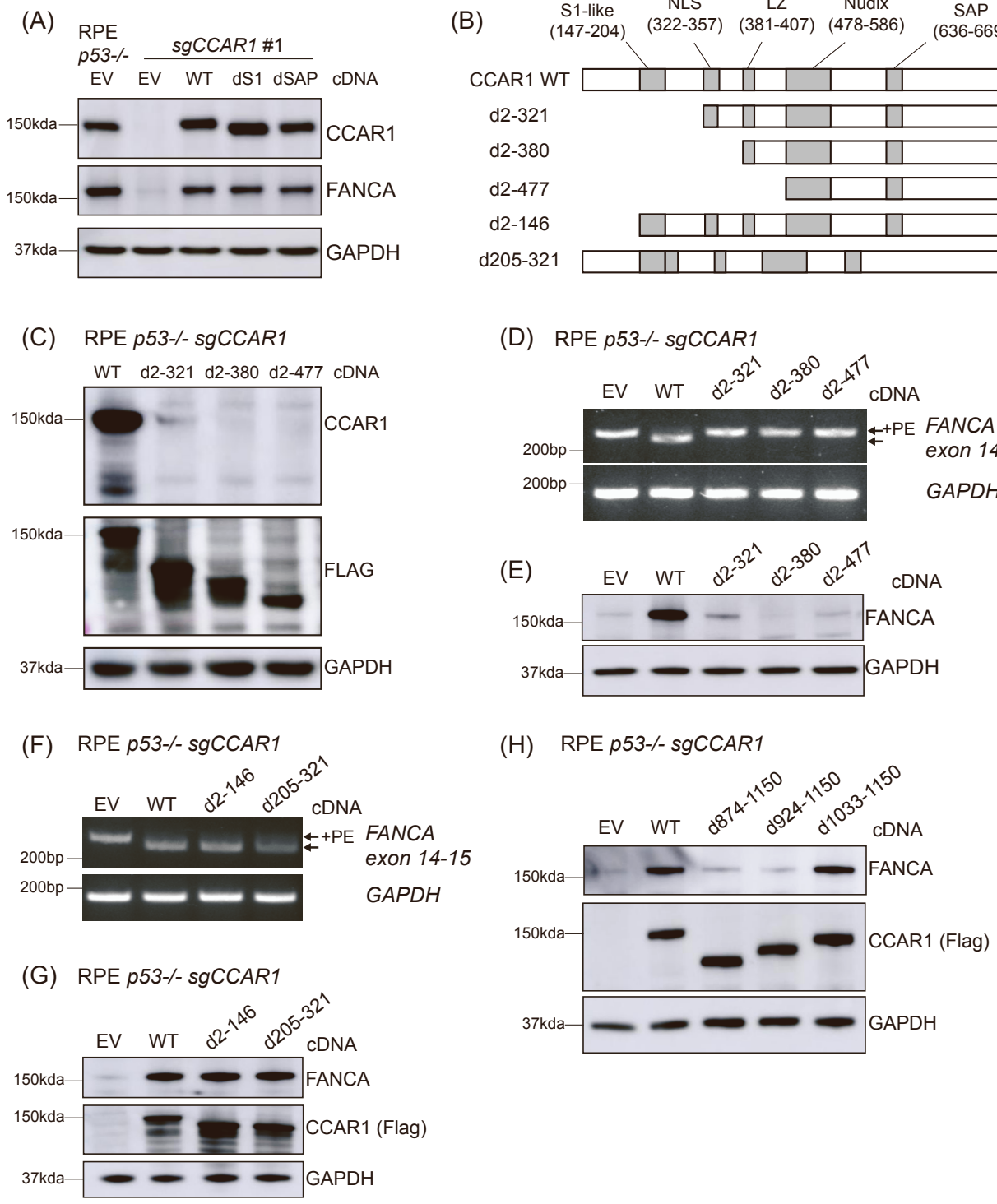
Supplementary figure S3



Supplementary Figure S3: CCAR1 regulates the exclusion of the poison exon from *FANCA* transcript in multiple cell lines, related to Figure 3

(A) Schematics of the *FANCA* poison exon (PE) between exon14 and exon15 and the design of antisense oligonucleotide (ASO) and sgRNAs targeting the poison exon. (B) RT-PCR evaluation of the *FANCA* poison exon (PE) inclusion in K562 and HEK293T *CCAR1* knockout clones. (C) RT-PCR evaluation of the *FANCA* poison exon (PE) inclusion in RPE *p53-/-CCAR1* knock-out cells after transfection with sgRNAs targeting the PE (sgPoison-1 and sgPoison-2). (D) Restoration of MMC resistance in *FANCA* poison exon-edited RPE *p53-/-CCAR1* knock-out cells. Cells were treated with MMC for 6 days, and then cell viability was measured by CellTiter-Glo assay. Mean and SEM from n=3 independent experiments are plotted. (E) *FANCA* transcript level in PE-edited RPE *p53-/-CCAR1* knock-out clones. Mean and SEM from n=4 independent experiments are plotted. *:$P<0.05$ (One-way ANOVA, Dunnett's multiple comparisons test). Statistical analysis was performed by comparing to *CCAR1* knock-out cells. (F) RT-PCR evaluation of the *FANCA* poison exon (PE) inclusion in RPE *p53-/-* and *p53-/-CCAR1* knock-out cells after transfection with antisense oligonucleotide (ASO) (n=2). (G) WB of FANCA in RPE *p53-/-CCAR1* knock-out cells transfected with ASO (n=2). (H) Control showing qPCR signal in the RNA immuno-precipitation (RIP) assay is derived from RNA converted to cDNA and not genomic DNA contamination. qPCR was set up with immuno-precipitated RNA converted to cDNA without adding the reverse transcriptase enzyme. The Ct values obtained were normalized to Ct values obtained from input RNA converted to cDNA with reverse transcriptase enzyme. Mean and SD of (n=2).

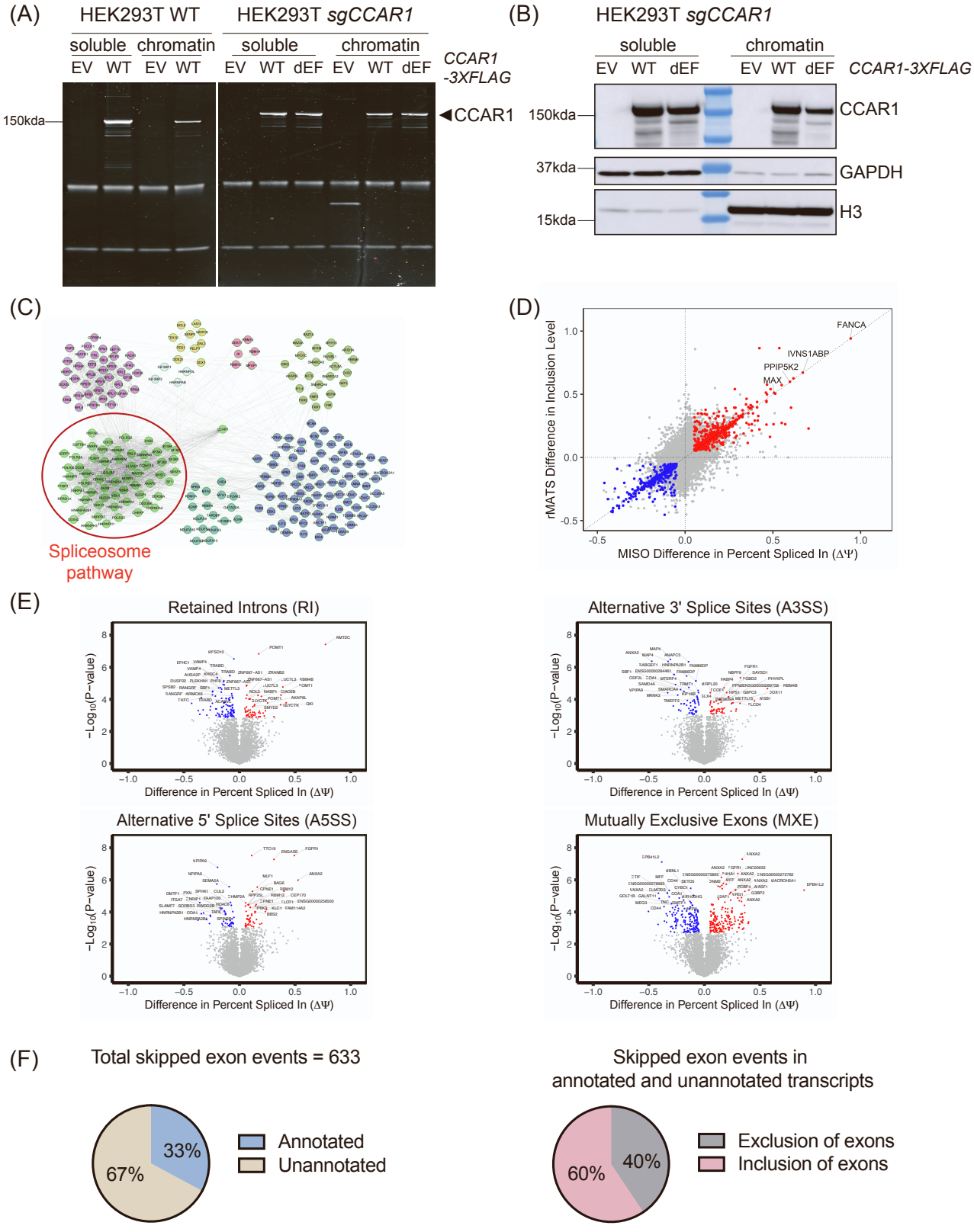
Supplementary figure S4



Supplementary Figure S4: Functional evaluation of various CCAR1 deletion mutants, related to Figure 4

(A) WB of CCAR1 and FANCA in RPE *p53-/-CCAR1* knock-out cells transduced with empty vector (EV), WT *CCAR1*, dS1 mutant, or dSAP mutants (n=2). (B) Schematics of d2–321, d2–380, d2–477, d2–145, and d205–321 mutants used in this study. (C) WB of CCAR1 in RPE *p53-/-CCAR1* knock-out cells transduced with d2–321, d2–380, or d2–477 mutants. Anti-CCAR1 and anti-FLAG antibodies were used to detect the CCAR1 mutant proteins (n=2). (D) RT-PCR showing *FANCA* poison exon (PE) inclusion in RPE *p53-/-CCAR1* knock-out cells transduced with empty vector (EV), WT *CCAR1*, d2–321, d2–380, or d2–477 mutants (n=2). (E) FANCA protein expression in RPE *p53-/-CCAR1* knock-out cells transduced with empty vector (EV), WT *CCAR1*, d2–321, d2–380, or d2–477 mutants (n=2). (F) RT-PCR showing inclusion of the *FANCA* poison exon in RPE *p53-/-CCAR*1 knock-out cells transduced with empty vector (EV), WT *CCAR1*, d2–146 or d205–321 mutants (n=2). (G) FANCA protein expression in RPE *p53-/-CCAR1* knock-out cells transduced with empty vector (EV), WT *CCAR1*, d2–146, or d205–321 mutants (n=2). (H) FANCA protein expression in RPE *p53-/-CCAR1* knock-out cells transduced with empty vector (EV), WT *CCAR1*, d874–1150, d924–1150, or d1033–1150 mutants (n=2).

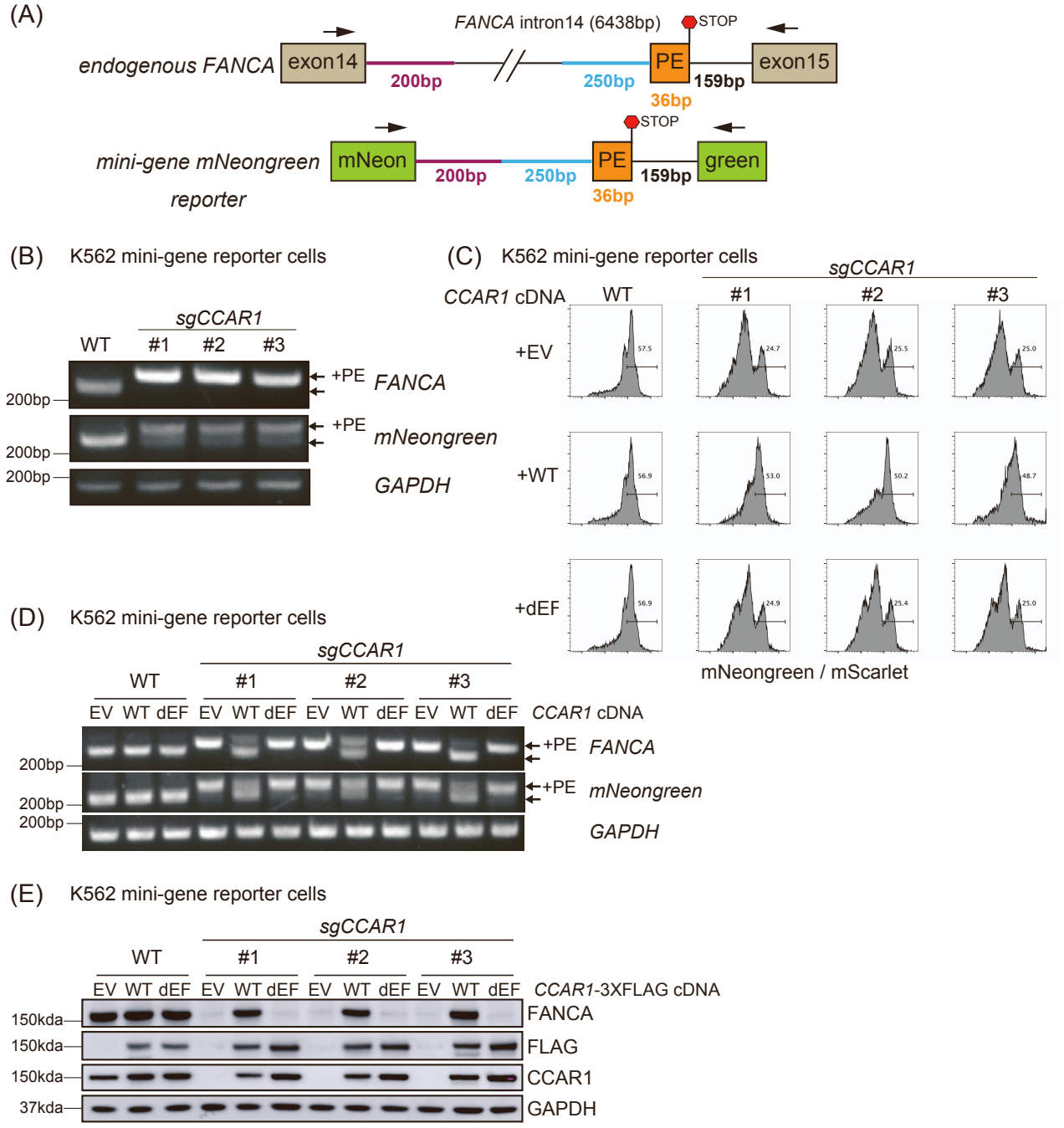
Supplementary figure S5



Supplementary Figure S5: CCAR1 interacts with the spliceosome and affects various alternative splicing event types, related to Figure 5

(A) Oriole stained SDS-PAGE gel image of 2% FLAG-CCAR1 IP samples from HEK293T WT and *CCAR1* knock-out cells transfected with EV, WT-*CCAR1*-3xFLAG, or dEF-*CCAR1*-3xFLAG. The chromatin fraction samples were used for mass spectrometry analysis (n=2). (B) WB showing fractionation of input samples used for FLAG IP in **Fig. 5B**. HEK293T *CCAR1* knock-out cells transfected with empty vector (EV), WT *CCAR1*-3xFLAG, or dEF mutant-*CCAR1*-3xFLAG (dEF) were fractionated and the chromatin fraction was used for FLAG-IP. (C) Reactome functional interaction analysis of the potential binding partners of CCAR1 identified by FLAG-CCAR1 IP mass spectrometry. The factors of the spliceosome pathway are highlighted by a red circle. (D) Scatterplot comparing the differences in levels of exon inclusion between MISO and rMATS, demonstrating high concordance between the two methods. Red and blue dots represent events whose differences in levels of exon inclusion were significantly increased (exons included), or decreased (exons excluded), respectively, in both algorithms. The *FANCA* PE inclusion is the top event by both MISO and rMATS algorithm analysis. (E) Volcano plots showing alternatively spliced retained intron (RI), alternative 3´splice site (A3SS), alternative 5´splice site (A5SS), and mutually exclusive exon (MXE) events in RPE *p53-/-CCAR1* knock-out cells compared to RPE *p53-/-*. (F) Skipped exon event analyzed using rMATS framework analysis with stringent a cut-off of FDR ($10^{\wedge-10}$) and dPSI (0.2). The pie chart on the left shows annotated and unannotated skipped exon events in RPE *p53-/-CCAR1* knock-out cells compared to RPE *p53*-/-. We also segregated skipped exon events based on exon inclusion or exclusion in RPE *p53-/-CCAR1* knock-out cells relative to RPE *p53*-/- (pie chart on the right).

Supplementary figure S6



Supplementary Figure S6: Validation of the fluorescence-based synthetic *FANCA* poison exon mini-gene reporter construct, related to Figure 6

(A) Schematic for the mini-gene reporter construct used in Figure 6. mNeongreen CDS was split into two exons and a synthetic intron derived from intron 14 of *FANCA* was inserted in-between. The *FANCA* intron 14 is >6kb, so a minimal region necessary for CCAR1-dependent splicing was inserted (200bp from the start of intron14 and 250bp upstream of the poison exon and 159bp downstream of the poison exon). The arrows show the position of the primers used for the RT-PCR assay in **Fig. S6B**. (B) RT-PCR showing inclusion of the poison exon in the endogenous *FANCA* and in the ectopic *mNeongreen* mRNA in K562 *CCAR1* knock-out clones. (C) Raw histogram data for **Fig. 6C**. K562 WT mini-gene reporter cells transduced with empty vector (EV) and *CCAR1* knock-out mini-gene reporter cells transduced with EV, wild-type *CCAR1* (WT), or dEF-*CCAR1* (dEF) were evaluated by FACS for mNeongreen /mScarlet ratio. (D) RT-PCR showing inclusion of the poison exon in the endogenous *FANCA* and in the ectopic *mNeongreen* mRNA in the cells shown in **Fig. S6C**. (E) WB analysis of FANCA, CCAR1, and FLAG in the cells shown in **Fig. S6C**.