Esophageal cancer as initial presentation of Fanconi anemia in patients with a hypomorphic *FANCA* variant.

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Supplemental material:

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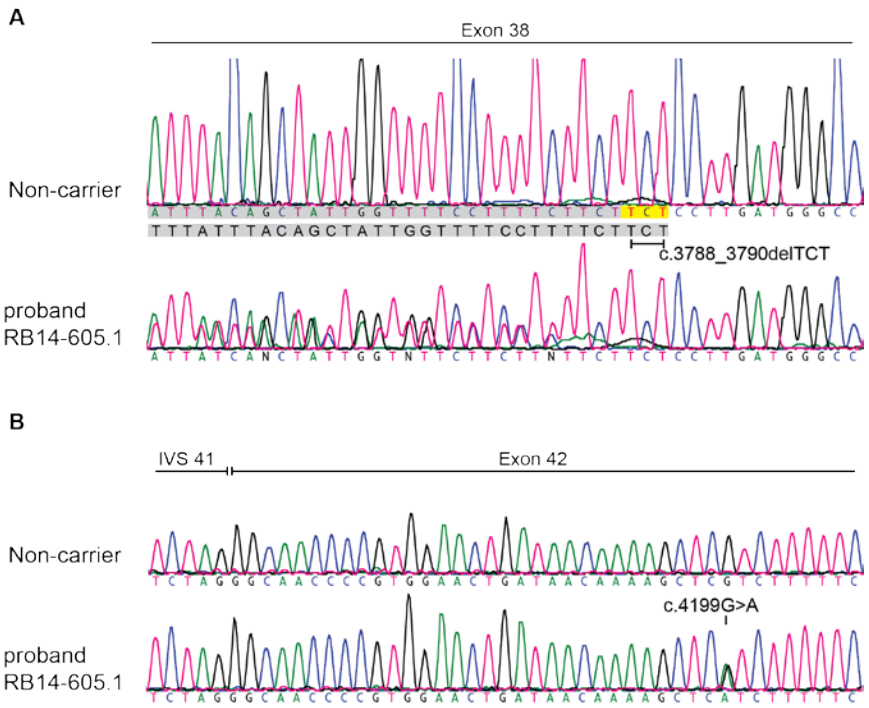
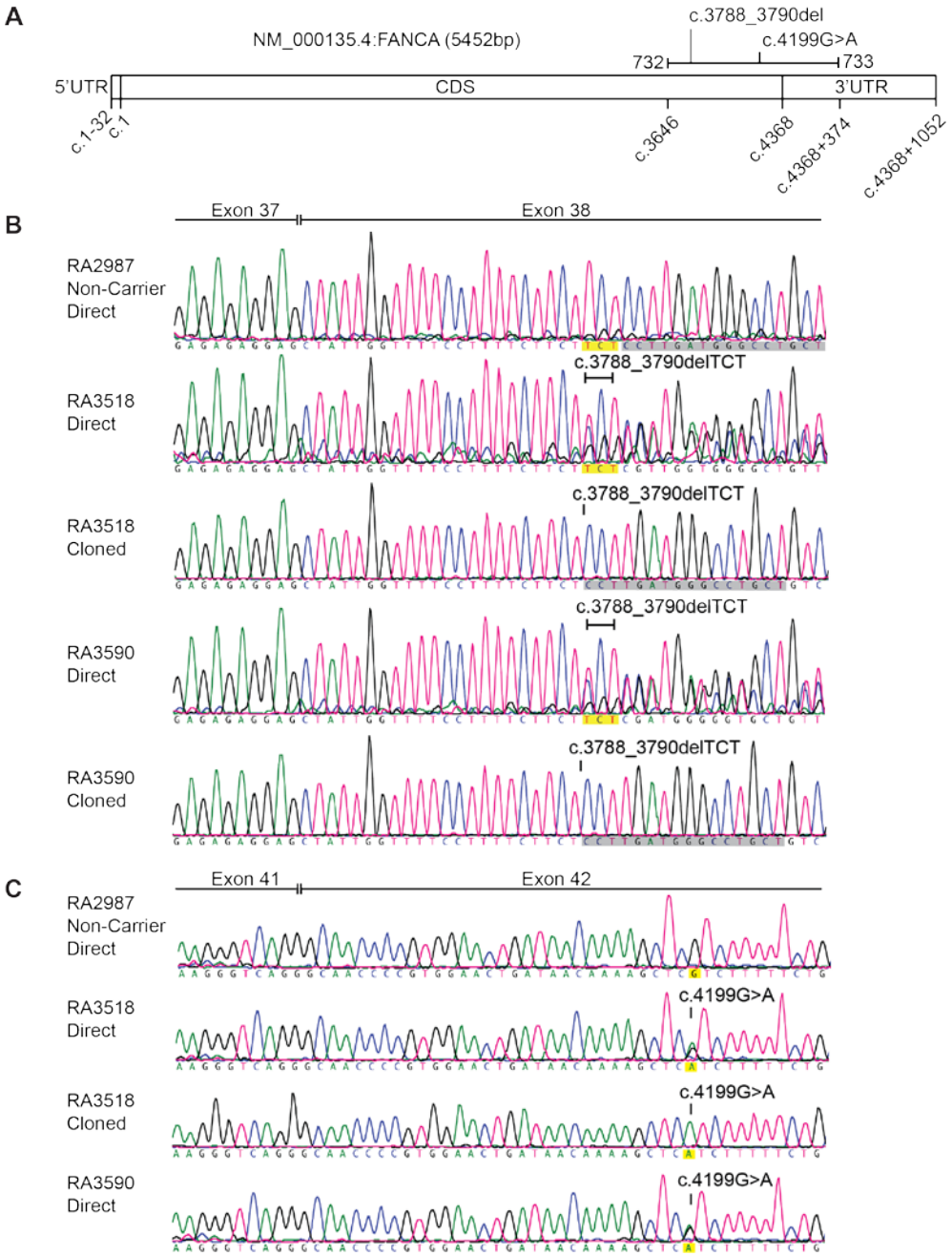
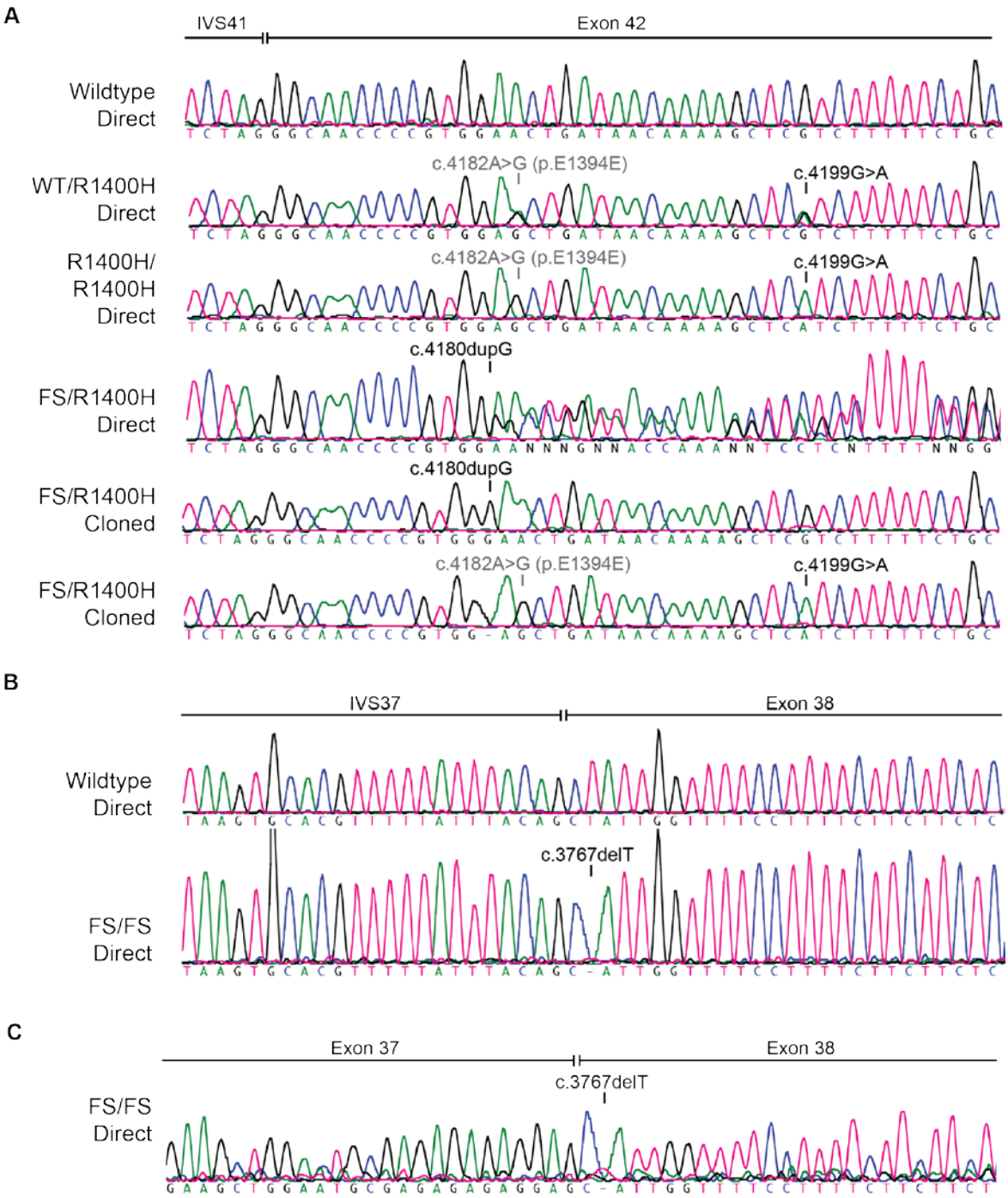


Supplemental Figure 1. *FANCA* Genomic Sequencing of proband's blood **A.** Genomic *FANCA* exon 38 sequencing confirming the heterozygous c.3788_3790delTCT variant in the proband's peripheral blood. **B.** Genomic *FANCA* exon 42 sequencing confirming the heterozygous c.4199G>A variant in the proband's peripheral blood.



Supplemental Figure 2. cDNA Sequencing (direct and cloned) from RA3518 and RA3590 showing no aberrant splicing. The variants are confirmed to be *in-trans.* **A.** Mapping of partial RT-PCR product 732-733 against *FANCA* transcript (NM_000135.4). The causal variants c.3788_3790delTCT (exon 38) and c.4199G>A (exon 42) are nested within. The amplicon was sequenced with the end primers 732 and 733. **B.** Direct sequencing of RT-PCR and TOPO cloned (pBlunt) RT-PCR products covering the exon 37-38 splice junction and the causal c.3788_3790delTCT variant in Exon 38 in both sibling fibroblasts RA3518 and RA3590. **C.** Direct sequencing of RT-PCR and TOPO cloned (pBlunt) RT-PCR products covering the exon 41-42 splice junction and the causal c.4199G>A variant in exon 42.



Supplemental Figure 3. Sequencing of the CRISPR-Cas9 mediated knock-in of FANCA variants. **A.** Genomic *FANCA* exon 42 sequencing illustrating successful knock-in of heterozygous, homozygous, and compound heterozygous variants in BJ E6E7/hTERT fibroblasts (direct sequencing). TOPO cloning was used to segregate the compound heterozygous alleles (Cloned). Note the silent c.4182A>G (p.E1394E) variant introduced *in-cis* with the causal c.4199G>A since the PAM site could not be mutated in this design**. B.** Genomic FANCA exon 38 sequencing illustrating a frameshift-causing mutation. **C.** Direct cDNA sequencing illustrating normal splicing of FANCA exon 37 and exon 38.

Table S1. Genes in the CancerNext-Expanded panel from Ambry Genetics.

APC	*ATM*	*BARD1*	*BRCA1*	*BRCA2*	*BRIP1*	*BMPR1A*	*CDH1*	*CDK4*
CDKN2A	*CHEK2*	*EPCAM*	*FH*	*FLCN*	*MAX*	*MET*	*MITF*	*MLH1*
MRE11	*MSH2*	*MSH6*	*MUTYH*	*NBN*	*NF1*	*PALB2*	*PMS2*	*PTEN*
RAD50	*RAD51C*	*RAD51D*	*RET*	*SDHA*	*SDHAF2*	*SDHB*	*SDHC*	*SDHD*
SMAD4	*STK11*	*TMEM127*	*TP53*	*TSC1*	*TSC2*	*VHL*		

Table S2. Primers

Primer name	Purpose	Sequence
FANCAex38F	gDNA PCR and SEQ	AGGATTTATGGCCTAGATGTAAAAA
FANCAex38R	gDNA PCR and SEQ	CTGGTGCCCCTGCCTGG
FANCAex38F_2	gDNA PCR and SEQ	AGTGTGAAATGACCCAGCCA
FANCAex38F_2	gDNA PCR and SEQ	ACACTTCCGCAAACACAAGG
FANCAex42F	gDNA PCR and SEQ	GACTATGGGGGACACACCAG
FANCAex42R	gDNA PCR and SEQ	GGCTCTGGCAGAAATAGTCG
FANCAex42F_2	gDNA PCR and SEQ	GCTCTTCGTGGCTGGGGATACAA
FANCAex42R_2	gDNA PCR and SEQ	TCGGGGCCGGACAGTTCATAAATA
FANCA nt3678_3696 Fwd	cDNA PCR and SEQ	GCCTCCCCAGCACCCAACC
FANCA nt4774_4754 Rvs	cDNA PCR and SEQ	TGAAGCCCGAACCCACCTGAG
FANCA MUT G4199A Fwd	Mutagenesis	TATTAACTGCAGCAGAAAAAGATGAGCTTTTGTTATCAGTTCCAC
FANCA MUT G4199A Rvs	Mutagenesis	GTGGAACTGATAACAAAAGCTCATCTTTTTCTGCTGCAGTTAATA
FANCA MUT 3788_3790del Fwd	Mutagenesis	GGTTTTCCTTTTCTTCTCCTTGATGGGCCTGCTGTCG
FANCA MUT 3788_3790del Rvs	Mutagenesis	CGACAGCAGGCCCATCAAGGAGAAGAAAAGGAAAACC
FANCA c.4199G>A KI DONOR ssDN	CRISPR	T*G*A*GAAGCTCTTTTTCGGGCACCGAGGTATTAACTGCAGCAGAAAAAGATGAGCTTTTGTTATCAGCTCCACGGGGTTGCCCTAGAGAGAAAACAGG*C*A*A

SG1 FANCAex38 GUIDE RNA	CRISPR	ACGTTTTTATTTACAGCTAT
SG2 FANCAex42 GUIDE RNA	CRISPR	GCTTTTGTTATCAGCTCCAC