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Supplemental Information

Improved Genome Editing through

Inhibition of FANCM and Members

of the BTR Dissolvase Complex

Gustavo de Alencastro, Francesco Puzzo, Mara Pavel-Dinu, Feijie Zhang, Sirika Pillay, Karim Majzoub, Matthew Tiffany, Hagoon Jang, Adam Sheikali, M. Kyle Cromer, Ruhikanta Meetei, Jan E. Carette, Matthew H. Porteus, Katja Pekrun, and Mark A. Kay

Supplementary figures:



Supplementary Figure 1. Characterization of the AAV-GAPDH(Hap1)-2A-BLAS targeting vector. (A) Scheme of the PCR strategy used to detect TI events in the *GAPDH* locus after transduction with AAV-GAPDH(Hap1)-2A-BLAS. The forward primer was designed to anneal outside the *GAPDH* homology arm sequence (i.e. outside of the part that is also included in the rAAV vector) and the reverse primer was designed to anneal within the blasticidin resistance gene coding sequence. Upon TI, a 2.1 kb fragment is generated. (B) Detection of TI events using amplification of the *GAPDH* locus. Individual HAP1 cell clones were picked and expanded after transducing HAP1 cells with AAV-GAPDH(Hap1)-2A-BLAS and selecting with three different blasticidin drug concentrations (5, 10 and 20 μ g/ml). Untransduced HAP1 cells were used as negative controls. (C) Southern blot analysis of DNA obtained from HAP1 cells after transduction with AAV-GAPDH(Hap1)-2A-BLAS and selection (5 and 20 μ g/ml). The expected size for TI is approximately 5.9 kb. The lower band (~4.3 kb) corresponds to episomal

AAV. Expected sizes for control plasmids are approximately 7.0 kb and 4.0 kb for BLAScontaining plasmid.

Α

HAP1-FANCM K	/O clo	ne B1	2 (32 bj	o deleti	on)							
Consensus Coverage	GCCTAAT	90 GAGCGGACC	100 GGCAAAGAAC	GCTTTTTCA	120 GACGTINNINN	130 NNNNNNNNNN	140 INNININININININI	150 NINNINN CCGG	160 IGTTGCAGCTC	170 CGGAACTGAG	180 GGACCTCAG	190 AGCCCTGGC
D* FANCM wt seq	GCCTAAT	90 GAGCGGAC	100 GGCAAAGAAC	110 GCTTTTTCA								
De FANCM-WT;FANCM_seq_F.ab1										CGGAACTGAG		
De FANCM-B12;FANCM_seq_F.ab1	GCCTAAT	GAGCGGAC	GCAAAGAAC	GCTTTTTCA	ACGT				GTTGCAGCTC	CGGAACTGAG	CGACCTCAG	AGCCCTGGC

HAP1-FANCM K/O clone C1 (77bp insertion)

Consensus	120 ACGTGGGGCTC	130 AAGTATCTCC	140 200 CGATC	210 CMCBGNNNNN	220 	230 NINNINNNNNNN	240 มากการเกิดการการการการการการการการการการการการการก	250 NINNNNNNNN	260 11 11 11 11 11 11 11 11 11 11 11 11 11	270 NININININGGTT	280 GCAGCTCC	290 GGAACTGAGCGACC
Coverage 0												
D• FANCM wt seq	120 द्रद्रतन्तन्तन्तन्त			154 ACTCCC								
De FANCM-WT:FANCM seg F.ab1										GGTT		
C+ FANCM-C1;FANCM_seq_F.ab1	ACGTGGGGCTC			MMM		MMMM	www	MMM	MMM	MMM GGTT	MMM GCAGCTCC	

В



Supplementary Figure 2. Characterization of FANCM knockout clones and the AAV-GAPDH(Hap1)-2A-GFP targeting vector. (A) Nucleotide analysis by Sanger sequencing of the FANCM knockout #B12 and FANCM knockout #C1 HAP1 clones compared to the wild-type sequence. (B) Scheme of the PCR strategy used to detect TI events in the *GAPDH* locus after transduction with AAV-GAPDH(Hap1)-2A-GFP. The forward primer was designed to anneal outside the *GAPDH* homology arm sequence (i.e. outside of the part that is also included in the rAAV vector) and the reverse primer was designed to anneal within the GFP coding sequence. Upon TI, a 2.4 kb fragment is generated. (C) FACs plots depicting GFP expressing cells sorted for Southern blot analysis. (D) Southern blot analysis of DNA obtained from sorted HAP1 cells after transduction with AAV-GAPDH(Hap1)-2A-GFP. Untransduced HAP1 cells were used as control. Expected size for TI is approximately 6.2 kb. The lower band (~4.3 kb) corresponds to episomal AAV. Expected sizes for control plasmids are approximately 7.3 kb and 4.3 kb for GFP-containing plasmid.



Supplementary Figure 3. Flow cytometry analysis of FANCM knockout HAP1 cells after AAV-HR treatment. Representative flow cytometry plots illustrating the typical gating strategy used for untransduced, wild-type, FANCM knockout #B12, and FANCM knockout #C1 HAP1 cells transduced with AAV-GAPDH(Hap1)-2A-GFP (MOI:16,000).





Supplementary Figure 4. Analysis of FANCM knockout and knockdown experiments in HeLa cells. (A-B) The graphs show the indel analysis of HeLa cells treated with CRISPR/Cas9 and two sgRNAs targeting, respectively, the exon 1 and exon 4 of the FANCM gene. Indels were analyzed using the TIDE software. (C) Representative flow cytometry plots illustrating the typical

gating strategy used for HeLa cells treated with CRISPR/Cas9 in combination with a scramble sgRNA or FANCM-targeted sgRNAs followed by transduction with AAV-GAPDH(HeLa)-2A-GFP (MOI:50,000). (**D**) Representative flow cytometry plots illustrating the typical gating strategy used for HeLa cells treated with a scramble siRNA and FANCM-targeted siRNA followed by transduction with AAV-GAPDH(HeLa)-2A-GFP (MOI:50,000).



HAP1-RMI1 K/O clone D10 (2 bp deletion)

Consensus Coverage	2 0]	640 GAATATGCCCA	650 AGGAAAAAGTA	660 ACTTGCAAGA	670 TTAATAGGGGA	680 AACCTGAT	690 NTGTAGTTTC	700 CAGTCATACC	710 AAACAATTCTA	720 ACGAAAACAT	730 TCCCAGAGT	740 TACAGATGI
🖙 RMI1 WT seq		640			670		688				728	738 TACAGATGI
Ce REV RMI1-WT;RMI-R.ab1				MACTTGCAAGA								
De REV D10(wt+RMI1);RMI-R	.a					MACCTGAT-						

HAP1-RMI1 K/O clone E5 (61 bp insertion)



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Supplementary Figure 5. FANCM protein interactions and analysis of RMI1 knockout cells.

(A) Scheme of FANCM protein interactions adapted from⁴³. (B) Nucleotide analysis by Sanger sequencing of the RMI1 D10 and RMI1 E5 knockout HAP1 cells compared to the wild-type sequence. (C) Representative flow cytometry plots illustrating the typical gating strategy used for the untransduced, wild-type, RMI1 knockout #D10, and RMI1 knockout #E5 HAP1 cells, respectively, transduced with AAV-GAPDH(Hap1)-2A-GFP (MOI:16,000).



Supplementary Figure 6. Analysis of RMI1 knockout experiments in HeLa cells. (A-B) The graphs show indels analysis of HeLa cells treated with CRISPR/Cas9 and two different sgRNAs

targeting the exon 1 of RMI1 gene. Indels were analyzed using the TIDE software. **(C)** Representative flow cytometry plots illustrating the typical gating strategy used for HeLa cells treated with a scramble sgRNA and RMI1-targeted sgRNA followed by transduction with AAV-GAPDH(HeLa)-2A-GFP (MOI:50,000).



Supplementary Figure 7. Flow cytometry analysis of knockdown and knockout experiments in Hela and HAP1 cells after AAV-HR transduction. (A) Representative flow cytometry plots illustrating the typical gating strategy used for HeLa cells treated with a scramble siRNA,

FANCM-targeted, RMI1-targeted, and BLM-targeted, FANCM+BLM-targeted, FANCM+RMI1targeted, RMI1+BLM-targeted and FANCM+BLM+RMI1 targeted siRNAs, followed by transduction with AAV-GAPDH(HeLa)-2A-GFP (MOI:50,000). **(B)** Representative flow cytometry plots illustrating the typical gating strategy used for the untransduced, wild-type, FANCM knockout #B12, and RMI1 knockout #D10 HAP1 cells, respectively, treated with CRISPR/Cas9 targeting GAPDH locus followed by transduction with AAV-GAPDH(Hap1)-2A-GFP (MOI:800).



Supplementary Figure 8. Analysis of TP53BP1 knockout cells. (A) Nucleotide analysis by Sanger sequencing of the TP53BP1 knockout HAP1 cells compared to the wild-type sequence. (B) Representative flow cytometry plots illustrating the typical gating strategy used for the untransduced, wild-type, FANCM knockout #B12, and TP53BP1 knockout HAP1 cells, respectively, transduced with AAV-GAPDH(Hap1)-2A-GFP (MOI:16,000). (C) Representative flow cytometry plots illustrating the typical gating strategy used for the untransduced, wild-type, FANCM knockout #B12, and TP53BP1 knockout HAP1 cells, respectively plots illustrating the typical gating strategy used for the untransduced, wild-type, FANCM knockout #B12, and TP53BP1 knockout HAP1 cells, respectively treated with CRISPR/Cas9 targeting GAPDH locus followed by transduction with AAV-GAPDH(Hap1)-2A-GFP (MOI:800).