

Supplementary Materials for

DNA Deamination Is Required for Human APOBEC3A-Driven Hepatocellular Carcinoma *In Vivo*

Running title: Human APOBEC3A as a Master Driver of Cancer

Jordan A Naumann^{1,2}, Prokopios P Argyris^{1,2,3,4}, Michael A Carpenter^{5,6}, Harshita B Gupta⁵, Yanjun Chen⁵, Nuri A Temiz^{2,7}, Yufan Zhou⁵, Cameron Durfee⁵, Joshua Proehl⁵, Brenda L Koniar², Silvestro Conticello⁸, David A Largaespada^{2,9}, William L. Brown^{1,2}, Hideki Aihara^{1,2}, Rachel I Vogel^{2,10}, Reuben S Harris^{5,6,*}

¹ Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA

² Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA

³ Howard Hughes Medical Institute, University of Minnesota, Minneapolis, MN 55455, USA

⁴ Division of Oral and Maxillofacial Pathology, College of Dentistry, Ohio State University, Columbus, OH 43210, USA

⁵ Department of Biochemistry and Structural Biology, University of Texas Health San Antonio, San Antonio, TX 78229, USA

⁶ Howard Hughes Medical Institute, University of Texas Health San Antonio, San Antonio, TX 78229, USA

⁷ Institute for Health Informatics, University of Minnesota, Minneapolis, MN 55455, USA

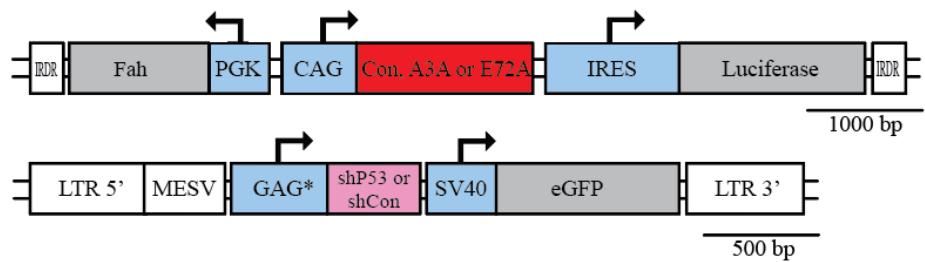
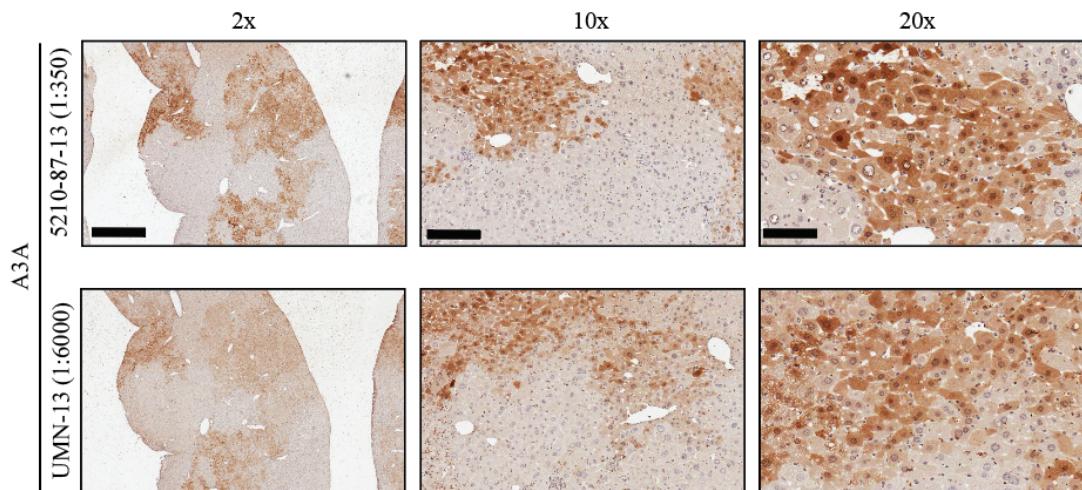
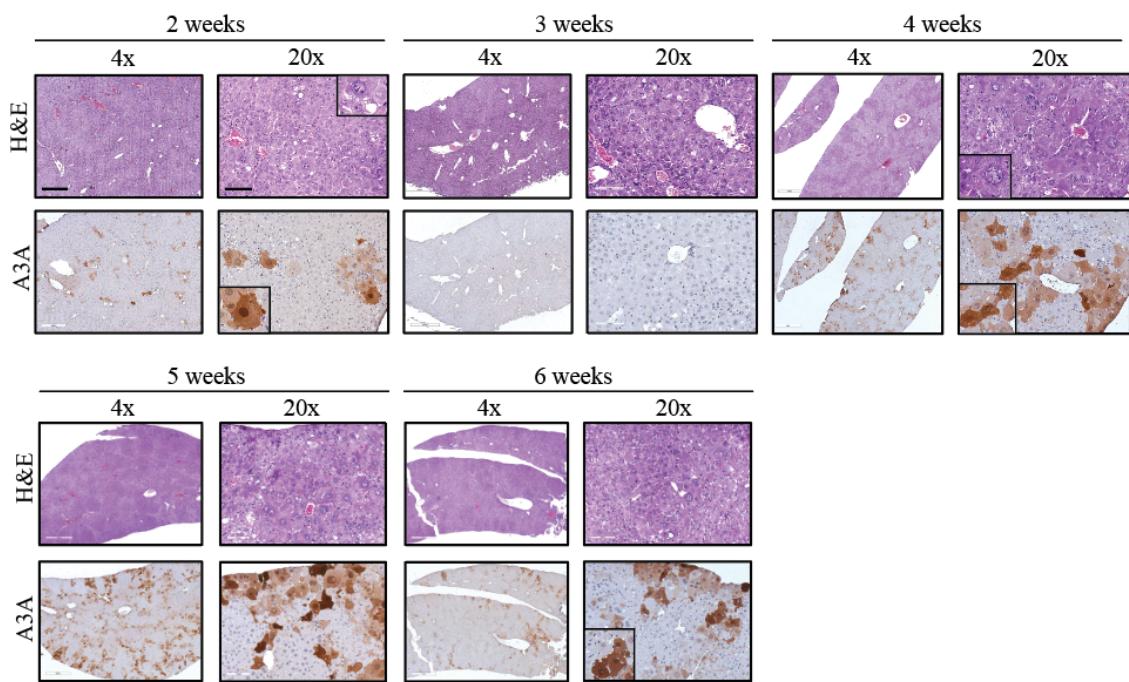
⁸ Core Research Laboratory, ISPRO, Florence, Italy

⁹ Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA

¹⁰ Department of Obstetrics, Gynecology, and Women's Health, University of Minnesota, Minneapolis, Minnesota, USA

* Correspondence to Reuben S. Harris, email: rsh@uthscsa.edu

Supplementary Information: Supplementary Figures S1-S3

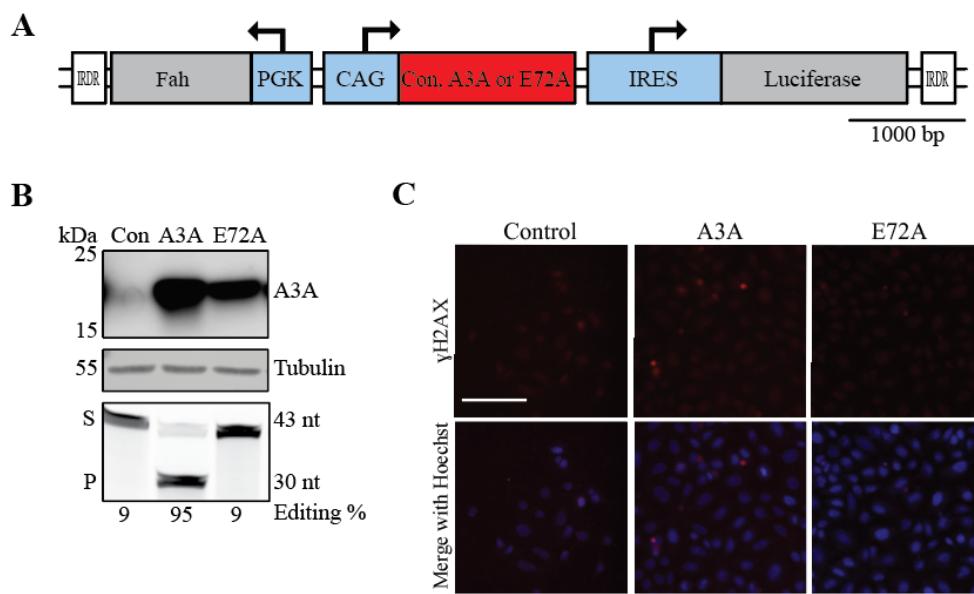
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Supplementary Figure S1. Human A3A expression in murine hepatocytes.

(a) Schematic representation of Fah-restorative and shRNA constructs used for hydrodynamic injections in Figure 1. Scale bar, 1000 bp and 500 bp, respectively.

(b) Comparative IHC analysis of consecutive sections of A3A expressing livers stained with α -A3A monoclonal antibodies, 5210-87-13 or UMN-13, at 1:350 or 1:6,000 dilutions, respectively. Scale bar, 1mm, 200 μ m, and 100 μ m for 2x, 10x, and 20x magnification, respectively.

(c) Representative H&E and A3A IHC (UMN-13) photomicrographs of A3A livers harvested 2-6 weeks p.i. Scale bar, 1 mm and 100 μ m for 4x and 20x magnification, respectively, and inset images are magnified 4-fold.

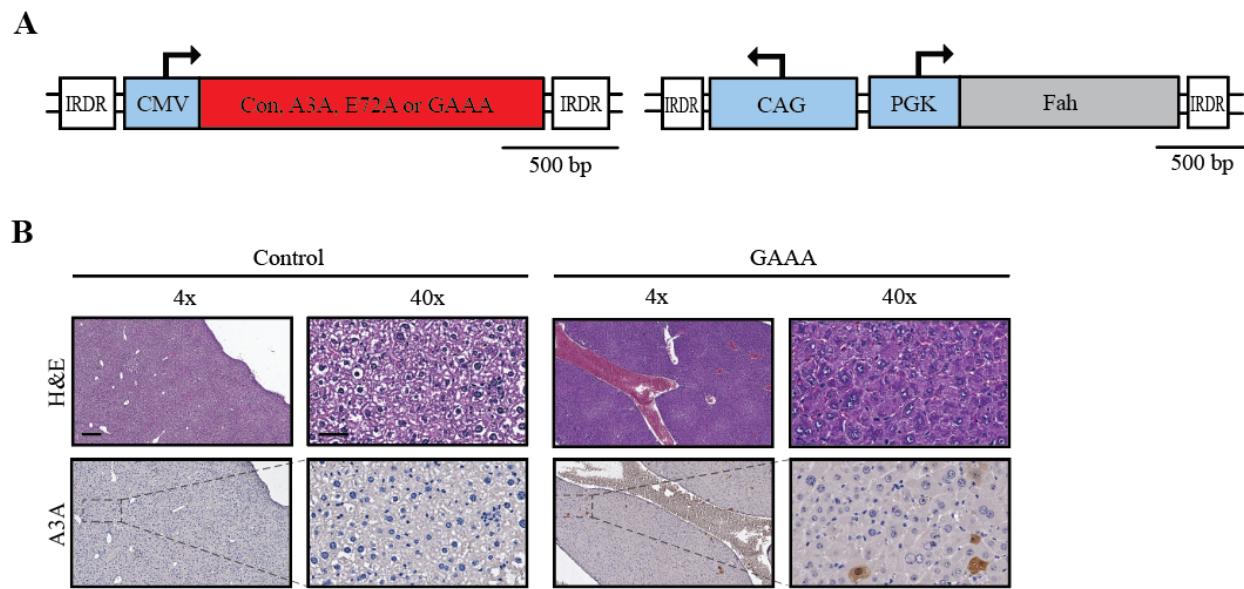


Supplementary Figure S2. The catalytic activity of A3A induces genomic damage.

(a) Schematic representation of E72A construct used for hydrodynamic injections. Scale bar, 1000 basepairs.

(b) Immunoblot (upper panels; 5210-87-13) and DNA deaminase activity assay (lower panel) of whole cell lysates of HeLa cells transfected with constructs containing A3A, E72A or control. Control (Con); substrate (S); product (P); nucleotides (nt). DNA deaminase activity quantification of band volume intensities (ratio of substrate to product) by densitometry reported below as Editing %.

(c) Immunofluorescence analysis of transfected HeLa cells stained for γ -H2AX and counterstained for DNA with Hoechst. Scale bar, 100 μ m.



Supplementary Figure S3. A3A-GAAA mutant expression in hepatocytes.

(a) Schematic representations of constructs used for all experiments in Figure 4. Scale bars, 500 bp.

(b) H&E and IHC (UMN-13) of representative livers from the Control and GAAA cohorts harvested 72 hours p.i. Scale bar, 500 μ m or 50 μ m for 4x and 40x magnification, respectively.