



Supplemental Material to:

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and Reuben S. Harris**

**Subcellular localization of the APOBEC3 proteins during
mitosis and implications for genomic DNA deamination**

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Supplemental online materials for

Subcellular localization of the APOBEC3 proteins during mitosis and implications for genomic DNA deamination

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The authors declare that there are no conflicts of interest.

This section contains 10 figures (S1-S10) and 4 movies (Movies S1-4).

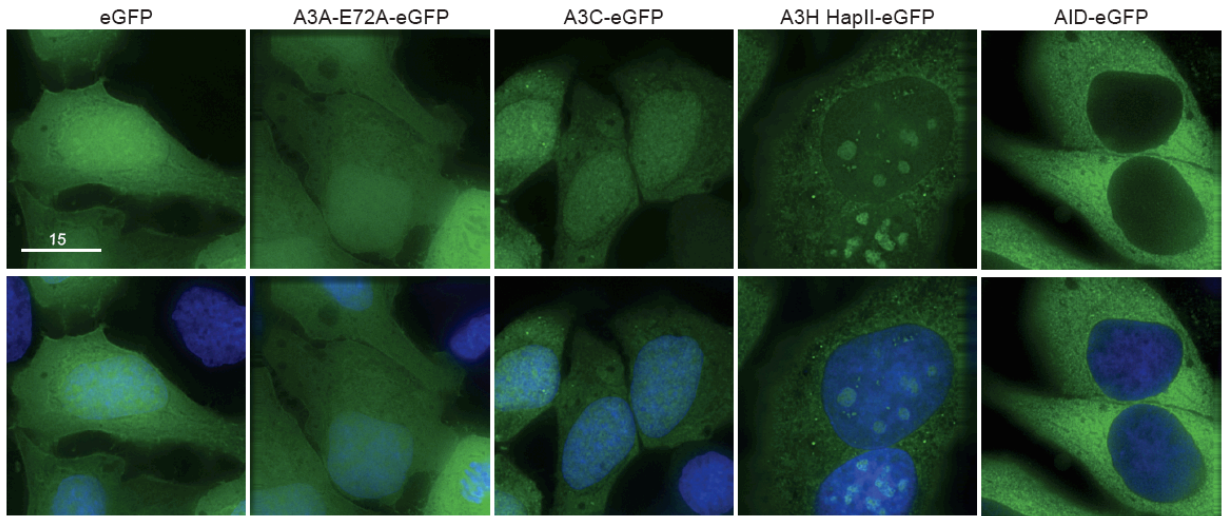


Figure S1. Interphase localization of the single-domain APOBEC3 proteins and AID.

HeLa cells were transfected with the indicated eGFP-tagged constructs and imaged by fluorescent microscopy (top). The DNA is indicated by Hoechst staining (merge, bottom).

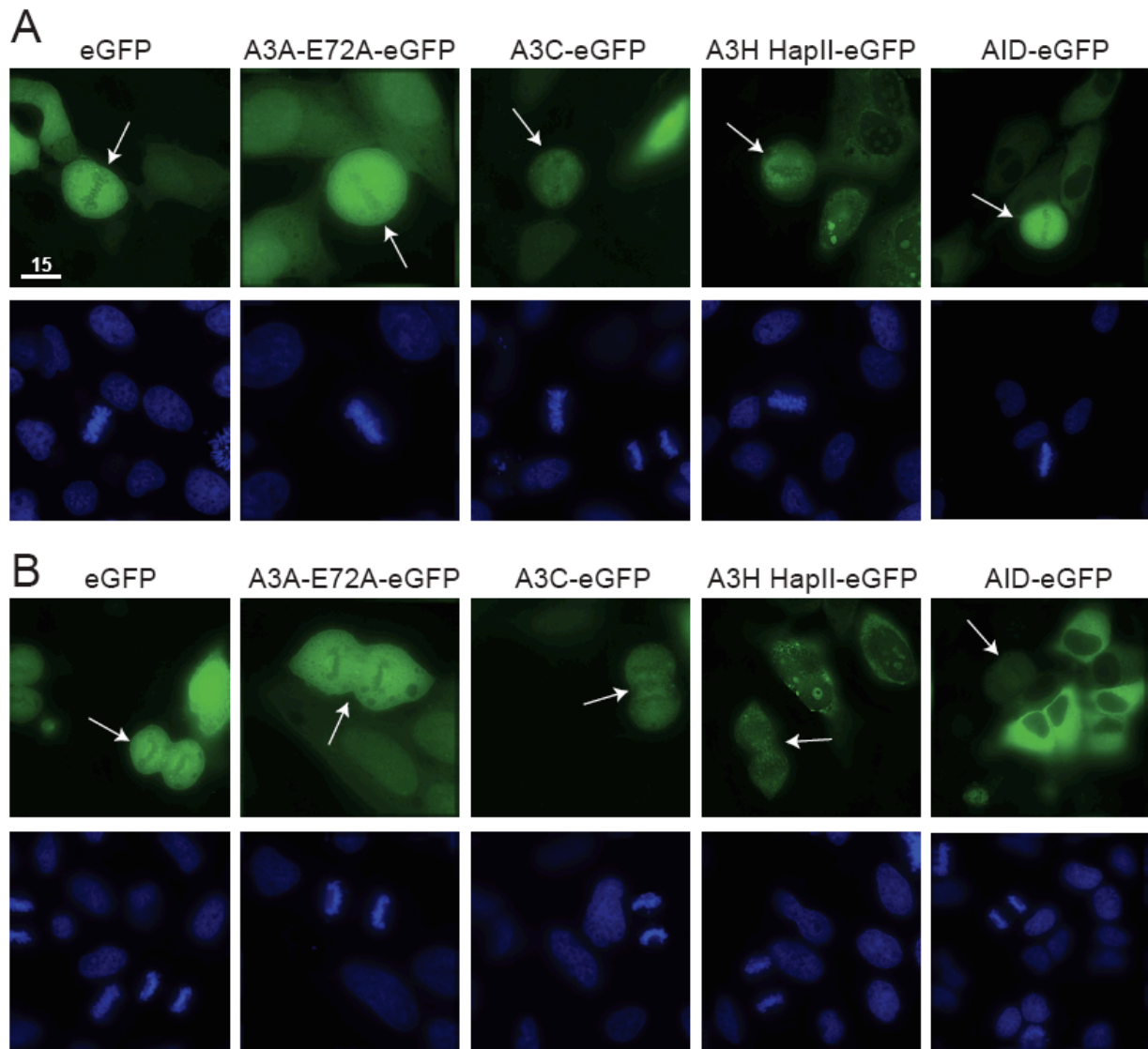


Figure S2. A3A-E72A, A3C, and A3H are excluded during anaphase and metaphase.

(A) HeLa cells in metaphase expressing the indicated eGFP-tagged constructs (top). Cells were stained with Hoechst dye to identify the nuclei (bottom). (B) HeLa cells in anaphase expressing eGFP tagged APOBEC3s (top) and nuclear stain (bottom). Arrows indicate mitotic cells. All images are representative of at least three mitotic cells.

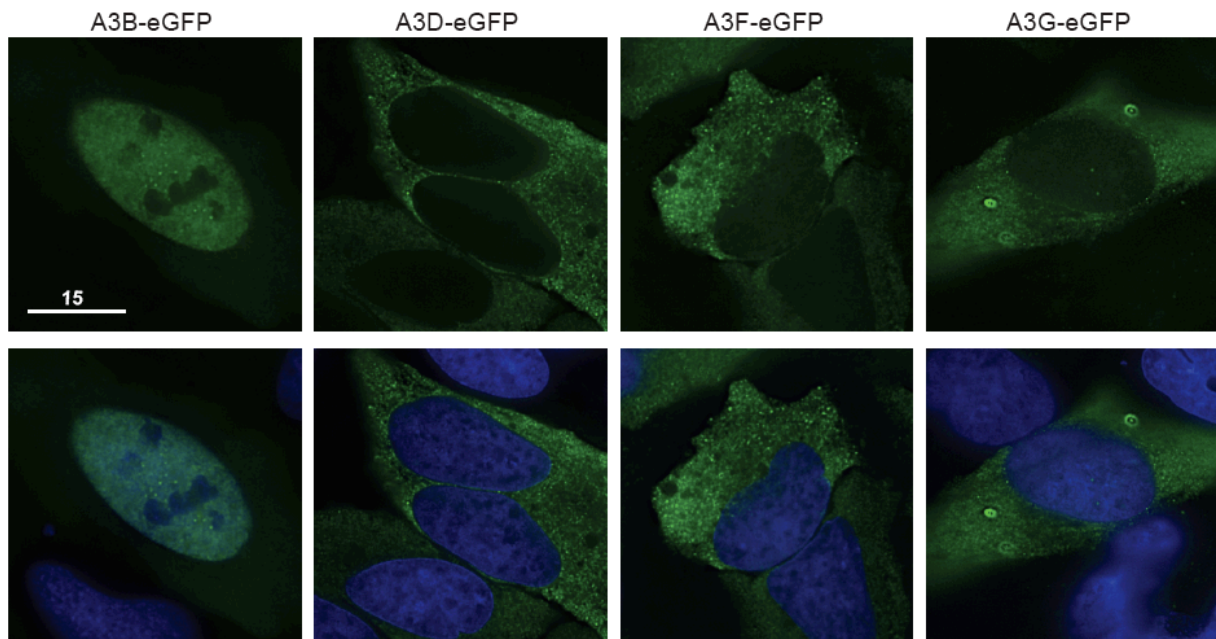


Figure S3. Interphase localization of the double-domain APOBEC3-eGFP proteins.

HeLa cells were transfected with the indicated APOBEC3-eGFP tagged constructs (top) and imaged by fluorescent microscopy. DNA is indicated by Hoechst staining (bottom).

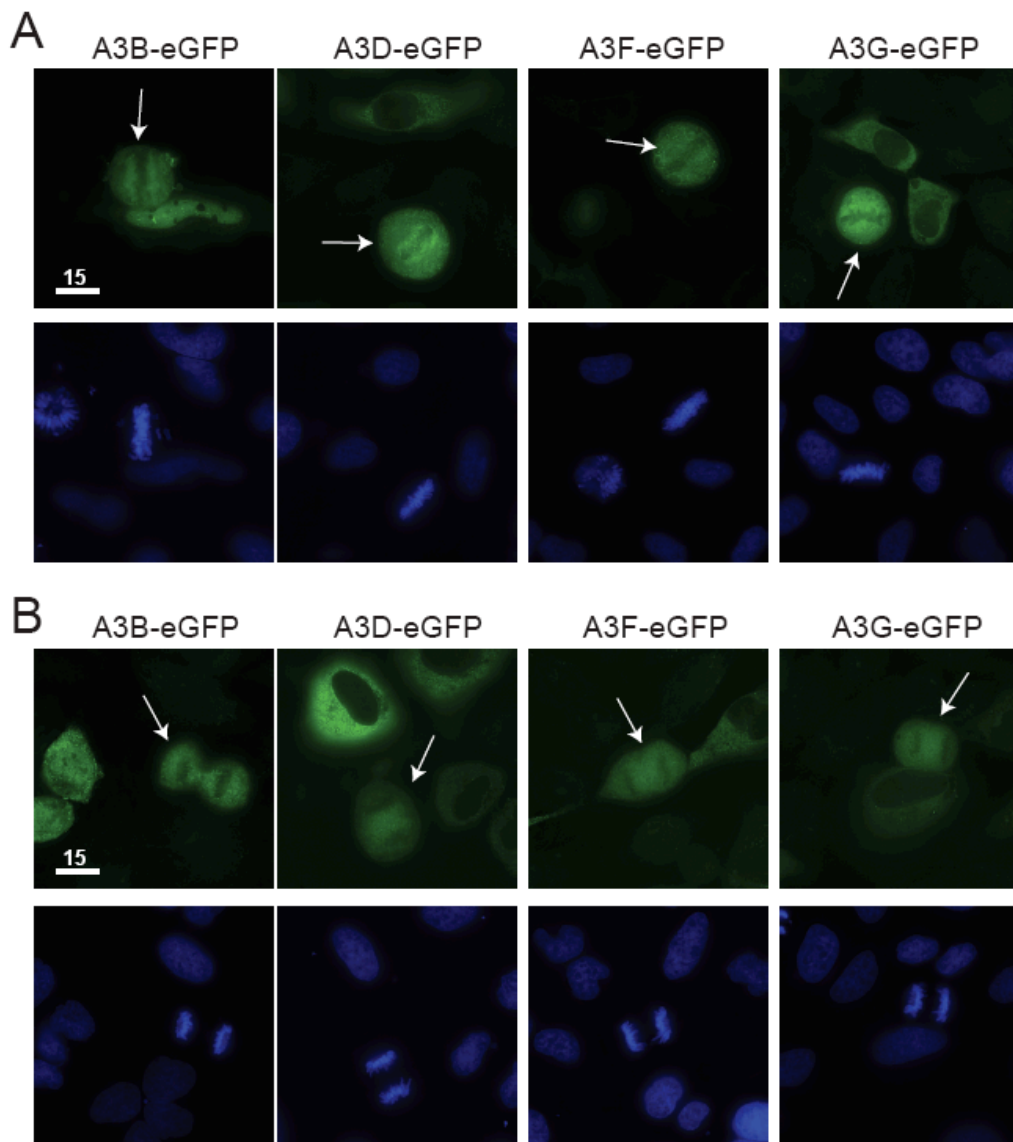


Figure S4. A3B, A3D, A3F, and A3G are excluded from DNA during metaphase and anaphase mitosis. (A) HeLa cells in metaphase expressing the indicated eGFP-tagged constructs (top). Cells were stained with Hoechst dye to identify the nuclei (bottom). (B) HeLa cells in anaphase expressing eGFP tagged APOBEC3s (top) and nuclear stain (bottom). Arrows indicate mitotic cells. All images are representative of at least three mitotic cells.

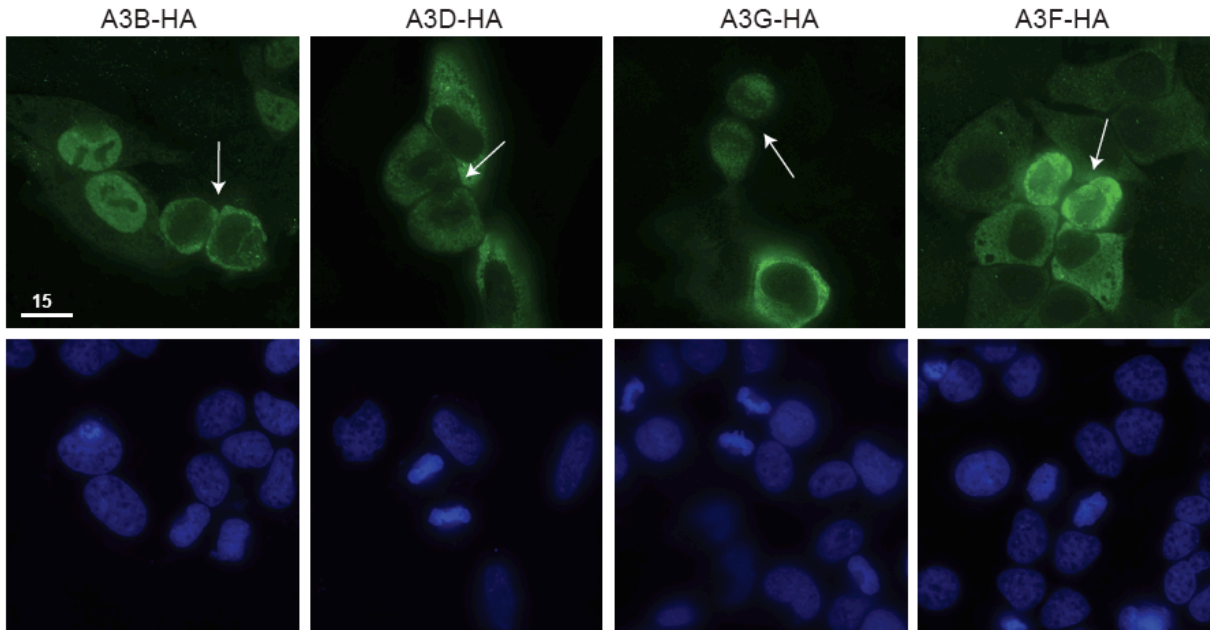


Figure S5. Double domain HA tagged APOBEC3 proteins are excluded from telophase DNA. Telophase images of APOBEC3B-HA expressing HeLa cells fixed and stained with anti-HA and anti-mouse FITC antibodies (top). DNA was stained with Hoechst dye (bottom). Representative images are shown from several telophase cells.

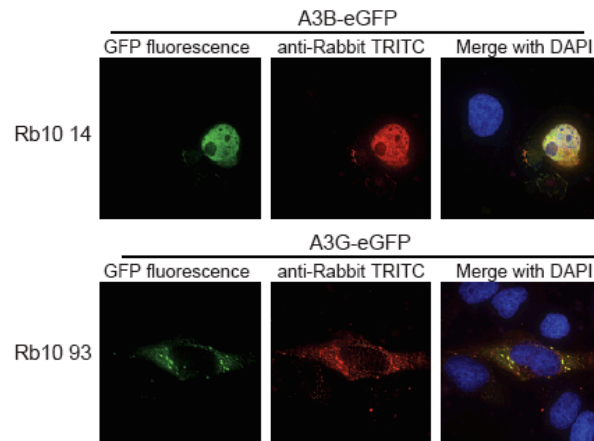


Figure S6. Recognition of A3B and A3G by rabbit polyclonal antibodies. A3B- or A3G-eGFP were transiently transfected into HeLa cells and the cells were fixed and screened as described in the Methods. Rb10 14 strongly recognized A3B, while Rb10 93 recognized A3G. These two polyclonal sera were used for subsequent experiments as anti-A3B and anti-A3G respectively.

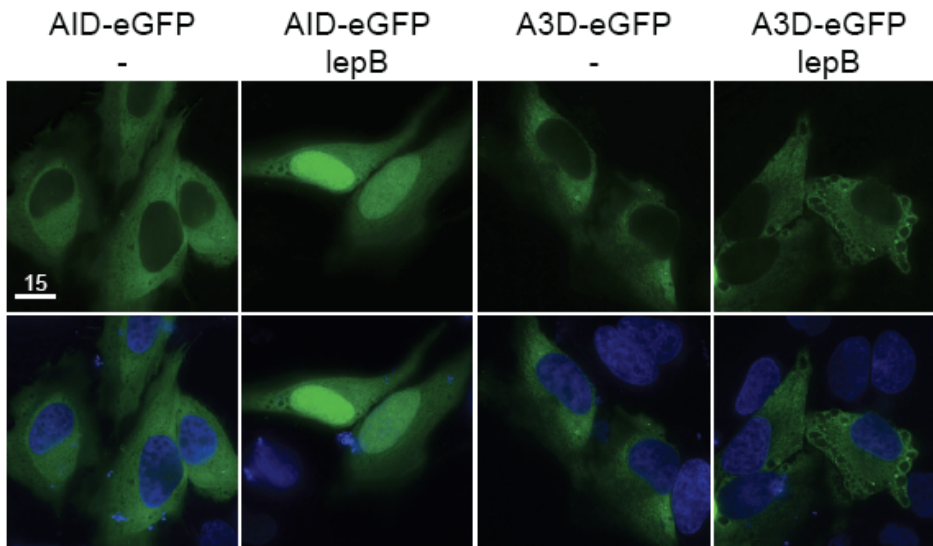


Figure S7. APOBEC3D does not shuttle through the CRM1 export pathway. HeLa cells expressing the indicated constructs were treated with either ethanol or leptomycin B in ethanol for three hours before imaging (top). Nuclei are indicated by Hoechst staining (merge, bottom). Images are representative of at least ten cells.

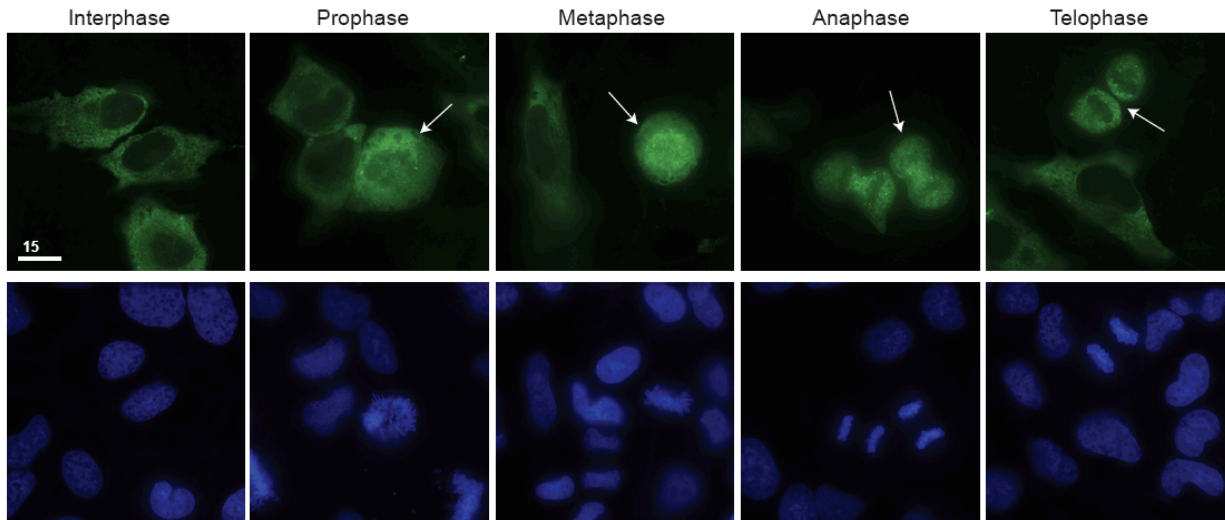


Figure S8. Localization of A3C-A3C-eGFP. Interphase and mitotic stages were imaged for A3C-A3C-eGFP expressed in HeLa cells (top) with DNA stain indicated (bottom). Arrows specify mitotic cells. All images are representative of at least three cells.

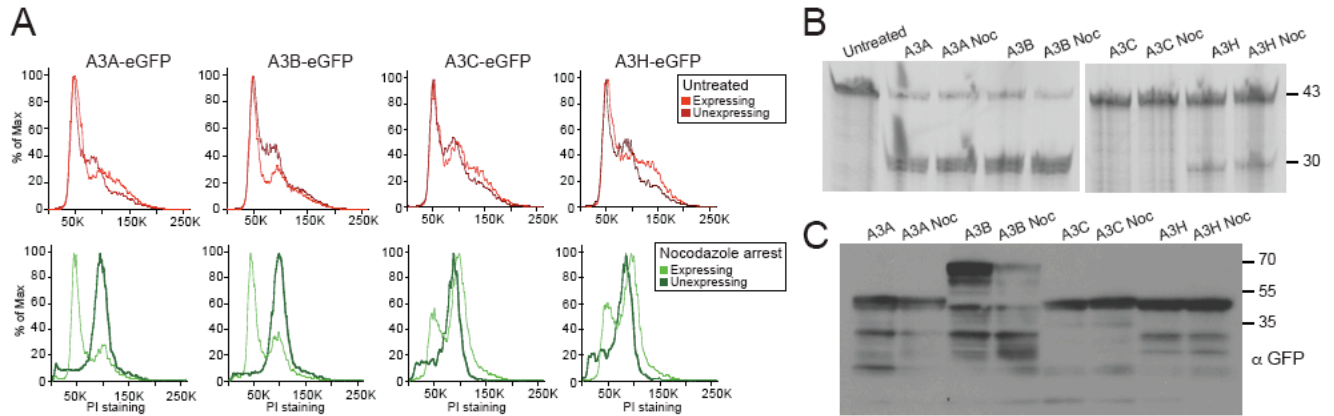


Figure S9. DNA deaminase activity in extracts from transfected cell lysates. (A) Cell cycle profiles of HEK293T cells transiently transfected with the indicated APOBEC3-eGFP and stained with propidium iodide (PI) to measure the DNA content of the cell. The cells were untreated or treated with 120 ng/mL of nocodazole for 16 hours to arrest the cells in mitosis. Non-APOBEC3 expressing cells are represented by darker shaded lines. (B) Deaminase activity on a cytosine containing fluorescent deoxyoligonucleotide. (C) Immunoblot of the APOBEC3 proteins using an anti-GFP antibody.

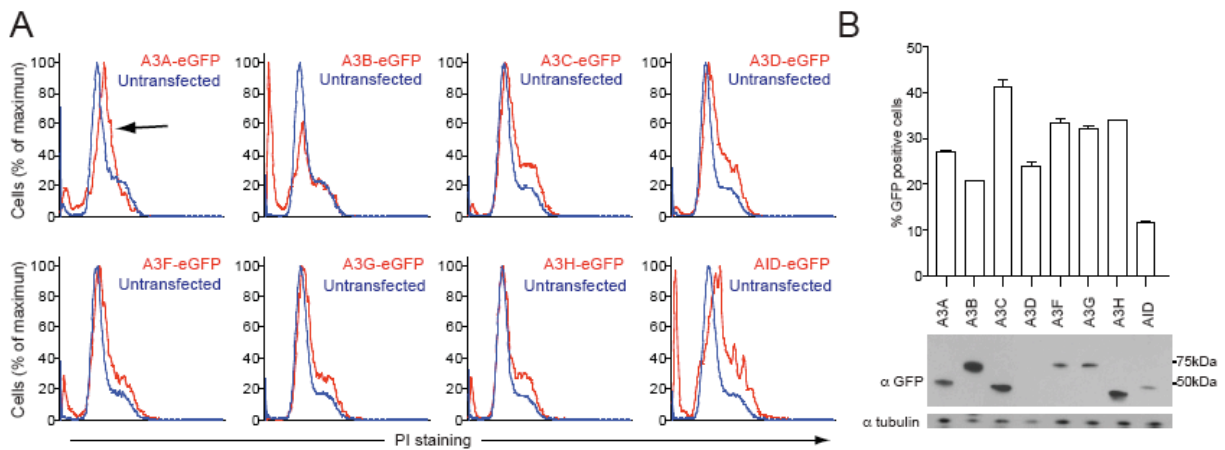


Figure S10. APOBEC3 effects on cell cycle progression in HeLa cells. (A) HeLa cells transiently expressing APOBEC3-eGFP proteins were monitored over several days by flow cytometry. Representative profiles for 96 hours are shown. (B) Expression of the indicated APOBEC3-eGFP proteins in HeLa cells as shown by flow cytometry (top) and immunoblotting (bottom).

Supplemental movie legends

Movie S1. A3A-E72A-mCherry is excluded during metaphase and anaphase and re-enters during telophase. A3A-E72A-mCherry localization in a HeLa cell starting in metaphase over a 105 minute period with images taken every 3 minutes. Exclusion is indicated by the shadow of the condensed chromosomes and is visible in metaphase and anaphase. Autofocus changes can obscure this effect in some frames. Immediately upon the separation of the two cells A3A-E72A-mCherry is fully cell-wide (57 minute time-point).

Movie S2. A3A-E72A-mCherry and H2B-eGFP during mitosis. A3A-E72A-mCherry (as in Movie S1) co-expressed with H2B-eGFP in a dividing HeLa cell. The chromosomes can be seen as the cell begins in metaphase followed by their separation in anaphase (39-42 minute time-points). Once the cells separate (57 minute time-point) their chromosomes begin to relax and they re-enter G1.

Movie S3. A3F-mCherry is excluded during metaphase, anaphase and telophase. A3F-mCherry localization in a dividing HeLa cell over a 3 hour period with images taken every 3 minutes. This cell begins in prophase and A3F changes from cytoplasmic to more cell-wide (18 minute time-point). The exclusion of A3F from the condensed chromosomes in metaphase and anaphase is visible as a shadow. Once the cells separate (1h 48 minute time-point), A3F remains excluded from the DNA, even when the cell is re-entering G1. Over time the fluorescence fades as the cell bleaches. This movie is representative of other A3F-mCherry and other double domain APOBEC3-mCherry movies.

Movie S4. A3F-mCherry and H2B-eGFP during mitosis. A3F-mCherry (as in Movie S3) co-expressed with H2B-eGFP in a dividing HeLa cell. The chromosomes can be seen condensing and forming the metaphase plate (such as at the 45 minute time-point) followed by their separation in anaphase (1h 36-1h 39 minute time-points). Once the cells separate (1h 48 minute time-point) their chromosomes begin to relax and they re-enter G1.