EMBO reportsMinghua Nie et al

Expanded View Figures

75 - endo. FAM111A

EV1

Figure EV1. Endogenous FAM111A levels in various cell lines.

Western blot analysis of FAM111A levels in the indicated cell lines. Total protein from an equal number of cells was loaded in each lane. The blots were probed with FAM111A and Tubulin antibodies.

 Minghua Nie et al EMBO reports

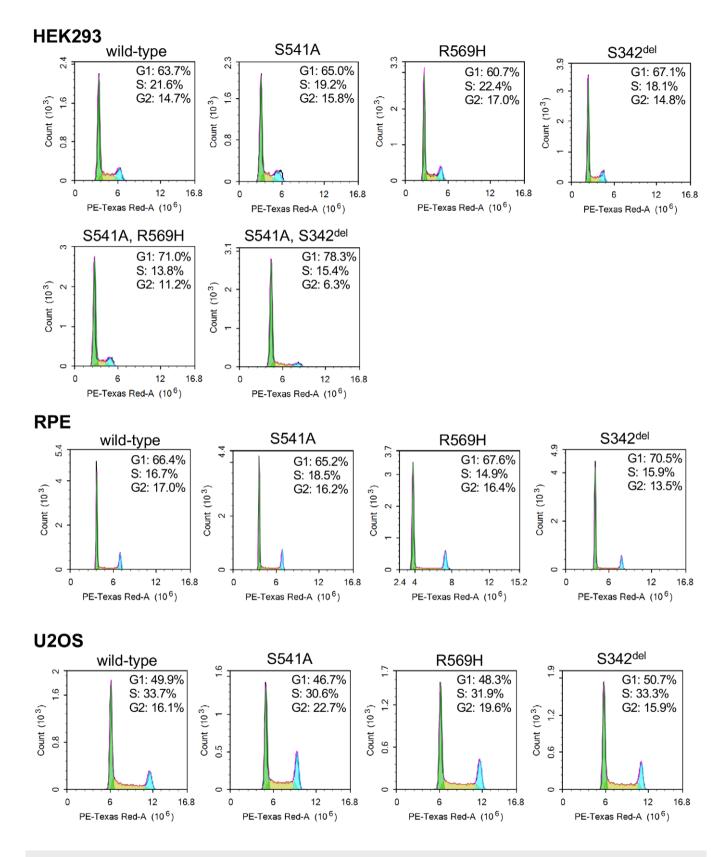


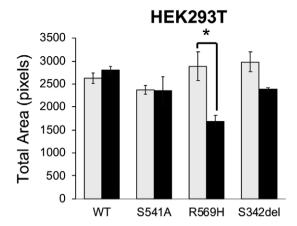
Figure EV2. FACS analysis of cell cycle in control conditions (- Dox).

Flow cytometry analysis of the cell cycle profiles of HEK293, RPE, and U2OS FAM111A cell lines without doxycycline induction was performed as described in Fig 2B.

© 2021 The Authors 22: e50803 | 2021 **EV2**

EMBO reports

Minghua Nie et al



EV3

Figure EV3. Cytotoxicity of FAM111A overexpression in HEK293T cells.

HEK293T/MycBioID-FAM111A cell lines were induced with doxycycline to express for 24 h. The colonies were fixed then stained with crystal violet. Cell growth was quantitated as total area on ImageJ. Values are mean \pm s.d. of independent experiments (n=3). *P<0.05 (two-tailed unpaired t-test).

EMBO reports 22: e50803 | 2021 The Authors

Minghua Nie et al EMBO reports

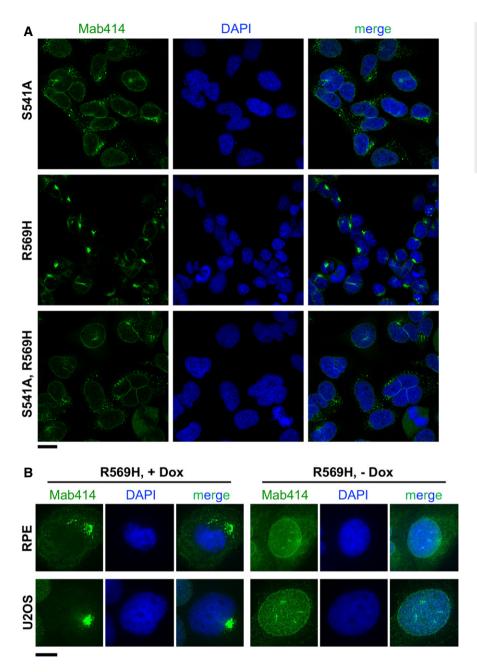
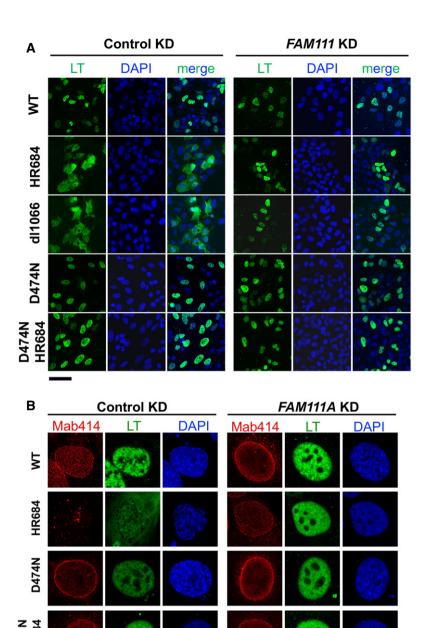


Figure EV4. Immunofluorescence of NPCs in cells expressing FAM111A mutants.

- A Cells captured through a 63×/1.4 objective of an LSM880 confocal microscope (a selection of individual cells is shown in Fig 4C). Scale bar: 20 μm .
- B Representative immunofluorescence images of RPE or U2OS/MycBioID-FAM111A-R569H stained with Mab414 and DAPI. Images were captured on a Zeiss Axio Imager through a 60x objective. Scale bar: 10 μ m.

EMBO reports

Minghua Nie et al



EV5

Figure EV5. Representative immunofluorescence images of SV40 LT and NPC.

- A SV40 LT (green) and DAPI (blue) staining in control or FAM111A shRNA infected U2OS cells 72 h after transfection with SV40 plasmids. Images were taken by Axio Imager 20×/0.6 objective. Scale bar: 100 $\,\mu m$.
- B SV40 LT and NPC (Mab414) localization 72 h after transfection into U2OS cells infected with control or FAM111A shRNA. Images were captured through a $63\times/1.4$ objective of LSM880 confocal microscope. Scale bar: 10 μ m.