

Supplemental Material

Supplemental Figure S1: FAM120A is localized to the cytoplasm of mESCs.

Supplemental Figure S2: iCLIP analysis of FLAG-HA-tagged FAM120A in mESCs.

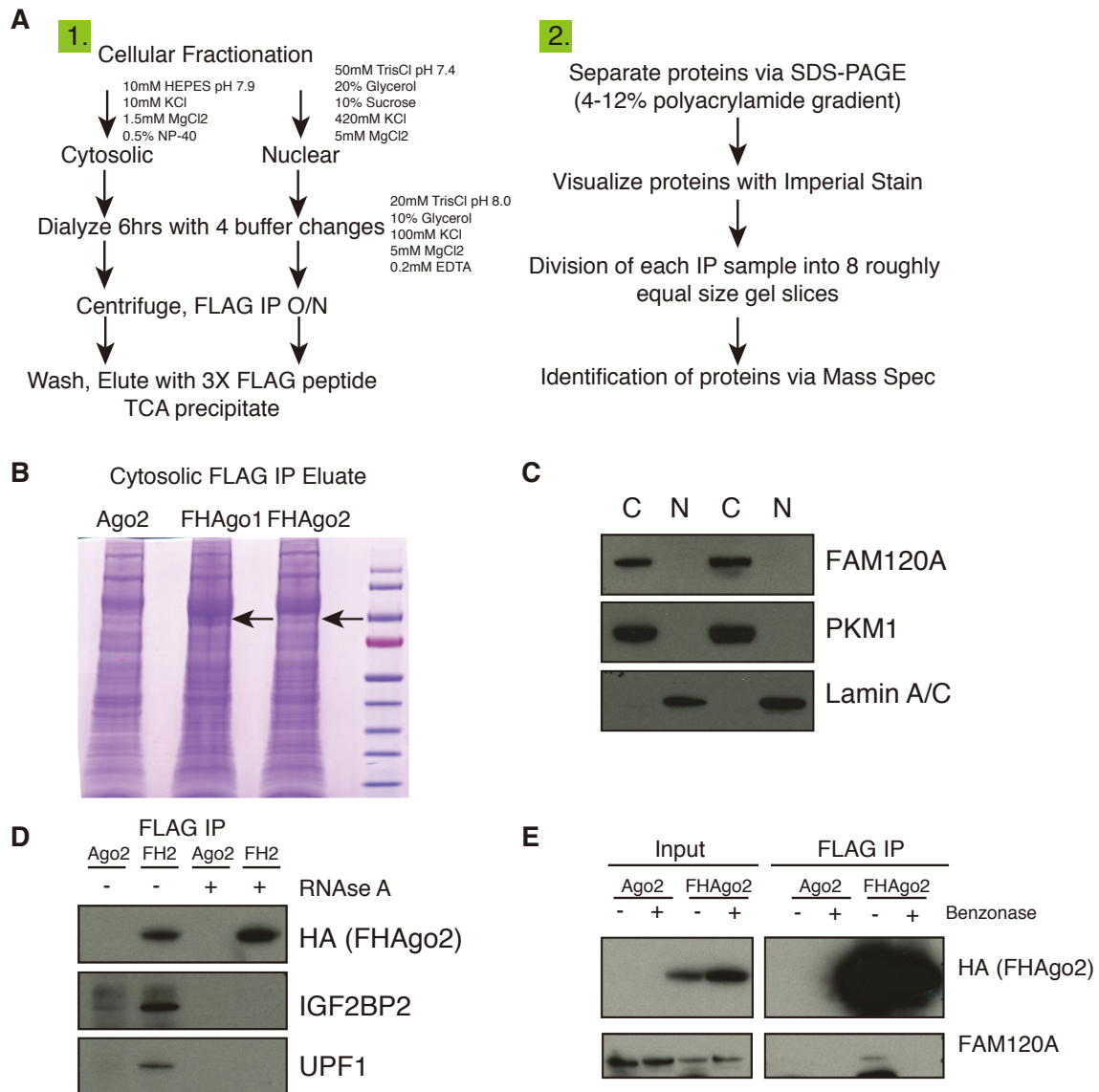
Supplemental Figure S3: FAM120A binds to highly expressed genes.

Supplemental Table S1. List of Ago2, FHago1, and FHago2 cytoplasmic protein binding partners.

>> Excel Spreadsheet

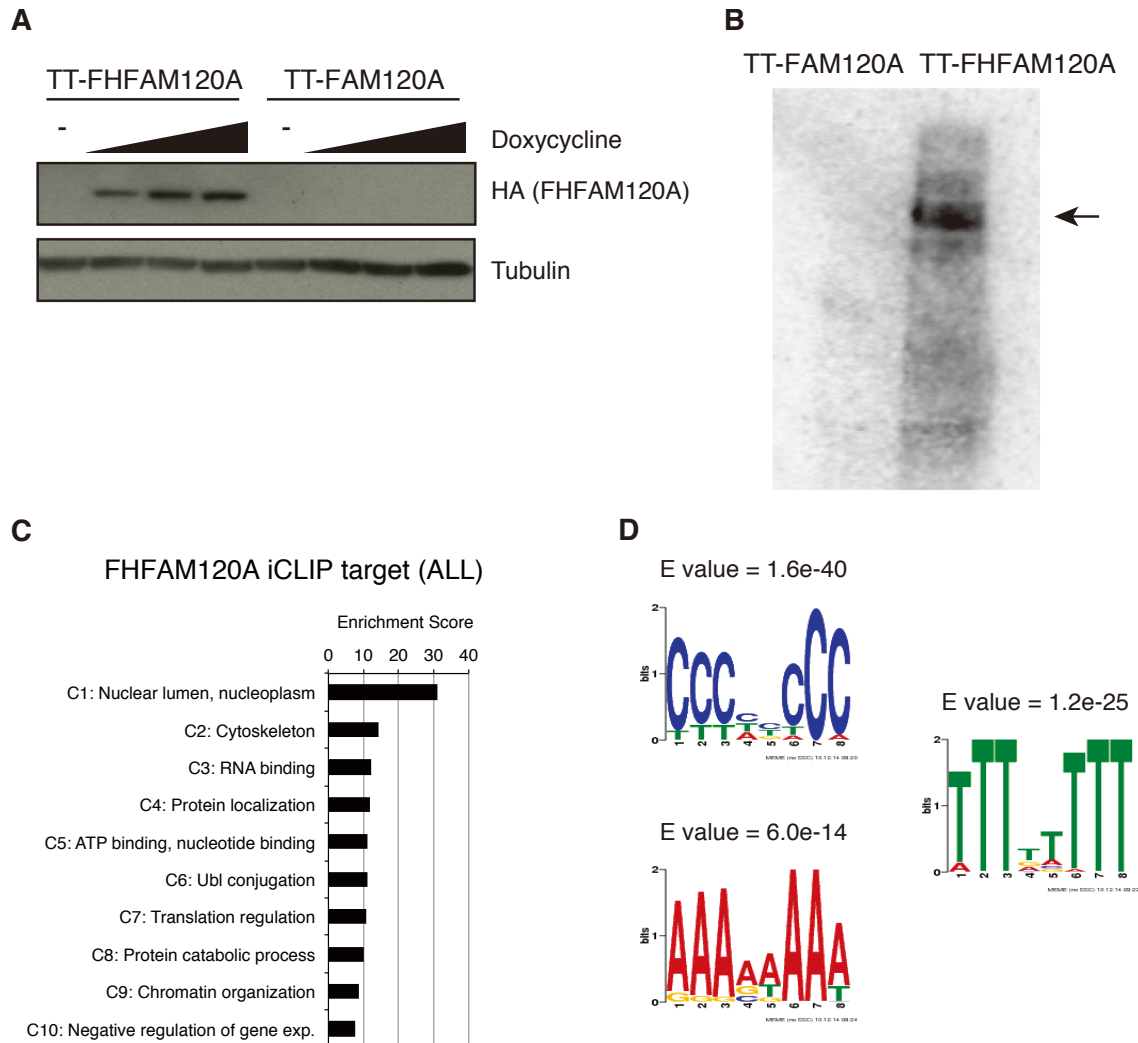
Supplemental Table S2. Primers used in this study.

>> Excel Spreadsheet



Supplemental Figure S1: FAM120A is localized to the cytoplasm of mESCs.

- (A) Workflow of Ago IP and identification of Ago binding proteins by mass spectrometry.
- (B) Visualization of proteins with Imperial staining after FLAG IP from Ago2, FHAgO1 or FHAgO2 expressing mESCs.
- (C) Western blot of FAM120A in cytoplasmic or nuclear extracts from mESCs.
- (D) RNase A sensitivity of Ago2-IGF2BP2 and Ago2-UPF1 interactions in mESCs. FH2: FHAgO2.
- (E) Ago2-FAM120A interaction is disrupted with benzonase treatment.



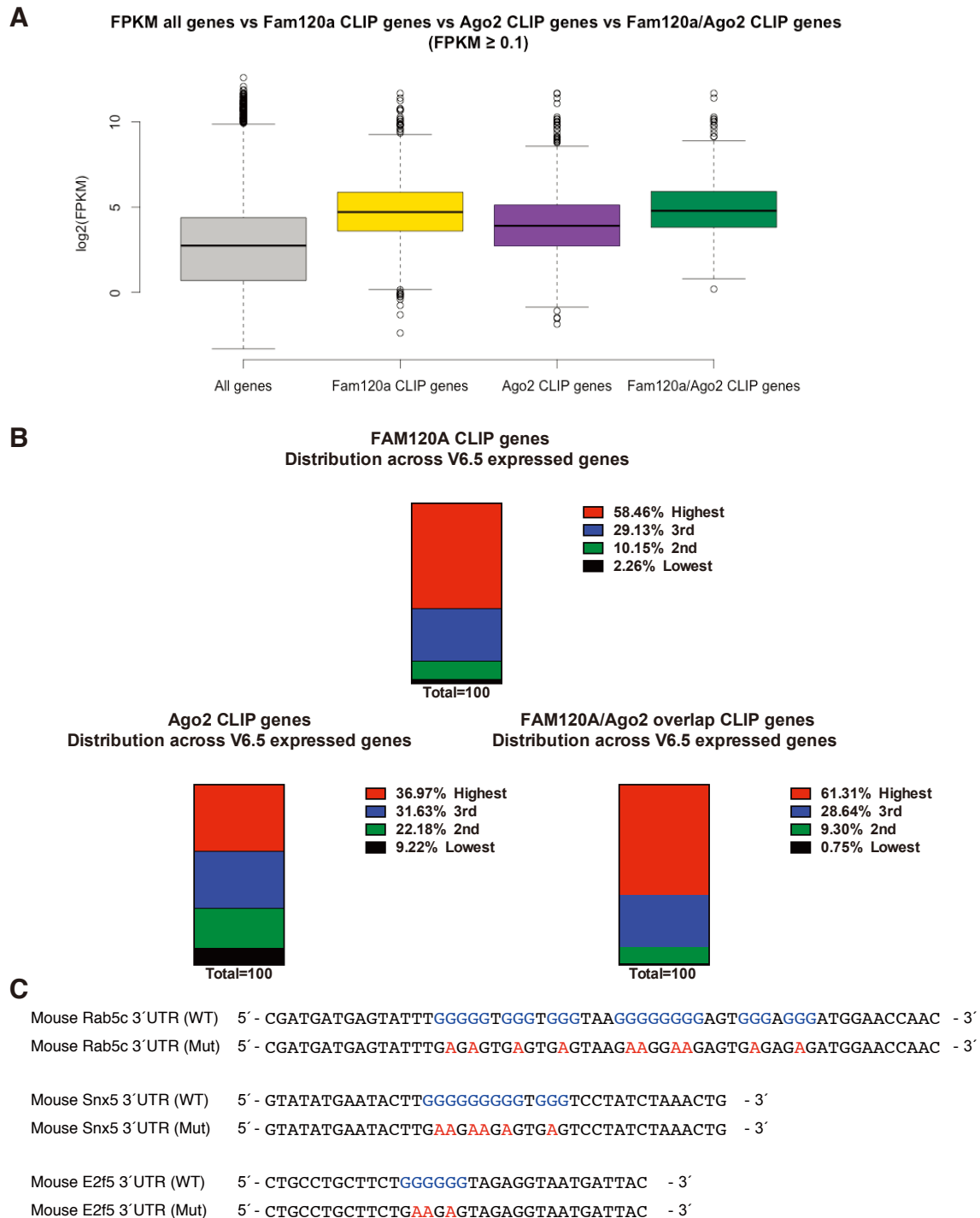
Supplemental Figure S2: iCLIP analysis of FLAG-HA-tagged FAM120A in mESCs.

(A) Establishment of mESCs expressing FLAH-HA-tagged and untagged FAM120A in a Dox-inducible manner.

(B) Autoradiography of FAM120A bound 5' end labeled RNA after UV cross-linking, tandem FLAG-HA immunoprecipitation of FHFAM120A, limited RNase I digestion, and separation via SDS-PAGE. Arrow denotes approximate location of FAM120A protein migration.

(C) GO analysis of FHFAM120A bound mRNAs.

(D) Enrichment of poly(C), poly(U), and poly(A) sequences identified in FHFAM120A iCLIP.



Supplemental Figure S3: FAM120A binds to highly expressed genes.

(A) Expression levels of FHFAM120A, FHAgO2, and FHFAM120A/FHAgO2 bound mRNAs compared to all genes expressed in mESCs.

(B) Quartile expression analysis of FHFAM120A, FHAgO2, and FHFAM120A/FHAgO2 bound genes.

(C) Sequences of wild-type and mutated G-rich motifs in FHFAM120A-bound genes used in 3' UTR reporter assay shown in Fig. 4C.