Expanded View Figures

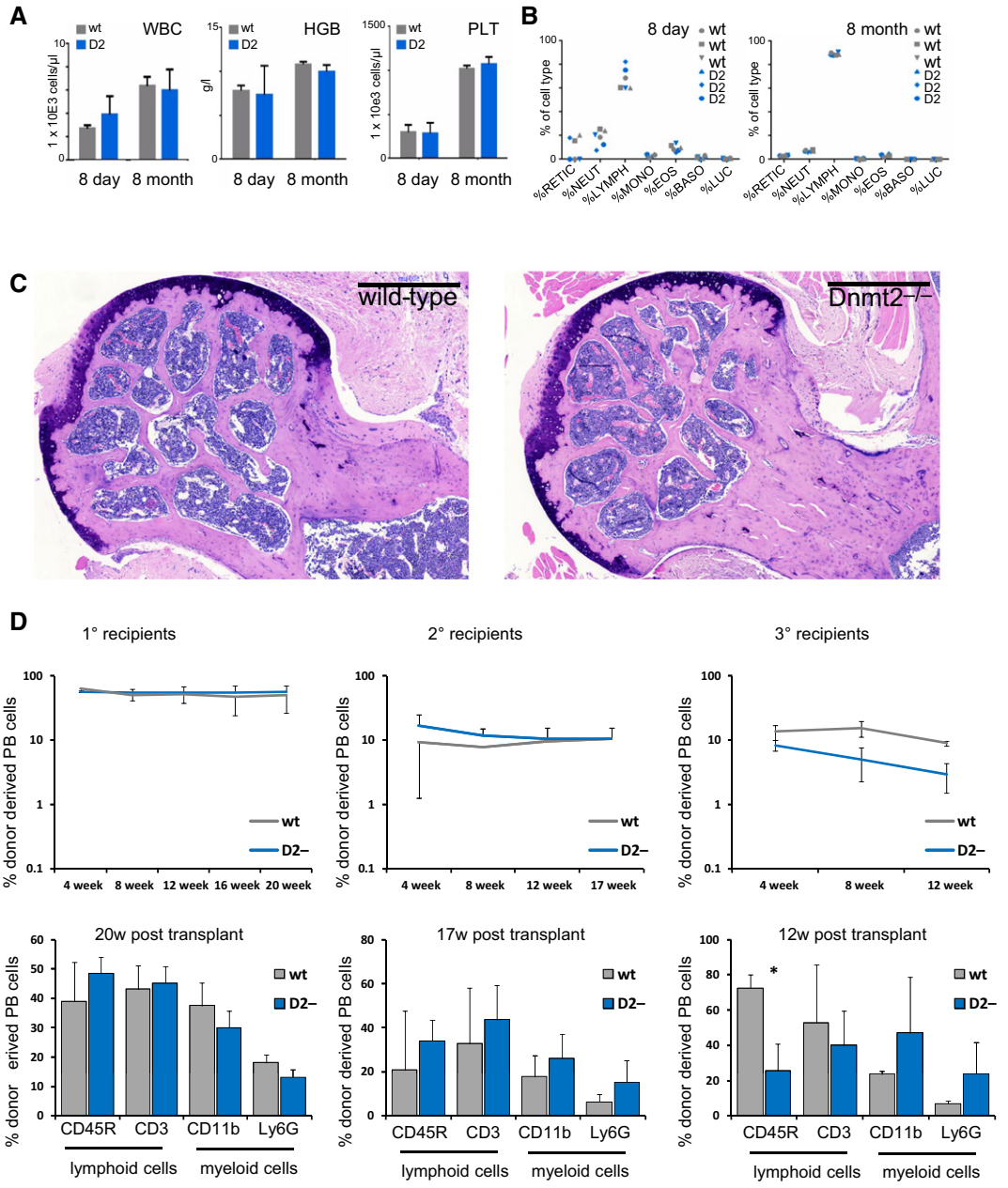

Figure EV1. Peripheral blood cell counts.

A Total blood cell counts of wild-type and *Dnmt2*$^{-/-}$ mice showing no significant differences. Total white blood cell counts, hemoglobin concentration, and platelet cell counts of wild-type and *Dnmt2*$^{-/-}$ mice (n = 3 mice per group). WBC: white blood cell counts; HGB: hemoglobin; PLT: platelets.

B Lineage distribution of peripheral blood analyzed by the ADVIA hematology analyzer system. RETIC: reticulocytes; NEUT: neutrophiles; LYMPH: lymphocytes; MONO: monocytes; EOS: eosinophiles; BASO: basophiles; LUC: large unstained cells.

C Giemsa staining of a representative femoral section from 4-month-old mice showing restoration of ossification defects. Scale bar: 500 µm.

D Stable engraftment (upper panels) and multi-lineage reconstitution (lower panels) in wild-type and *Dnmt2*$^{-/-}$ recipient mice. Donor chimerism is indicated by CD45.1 expression of peripheral blood (PB) cells using flow cytometry. Peripheral blood was collected monthly and analyzed for engraftment and lineage distribution (CD11b: macrophages; Ly6G: granulocytes; CD45R: B cells; CD3: T cells).

Data information: Data are presented as mean ± SD. Asterisks indicate statistically significant ($P < 0.05$, t-test) differences.

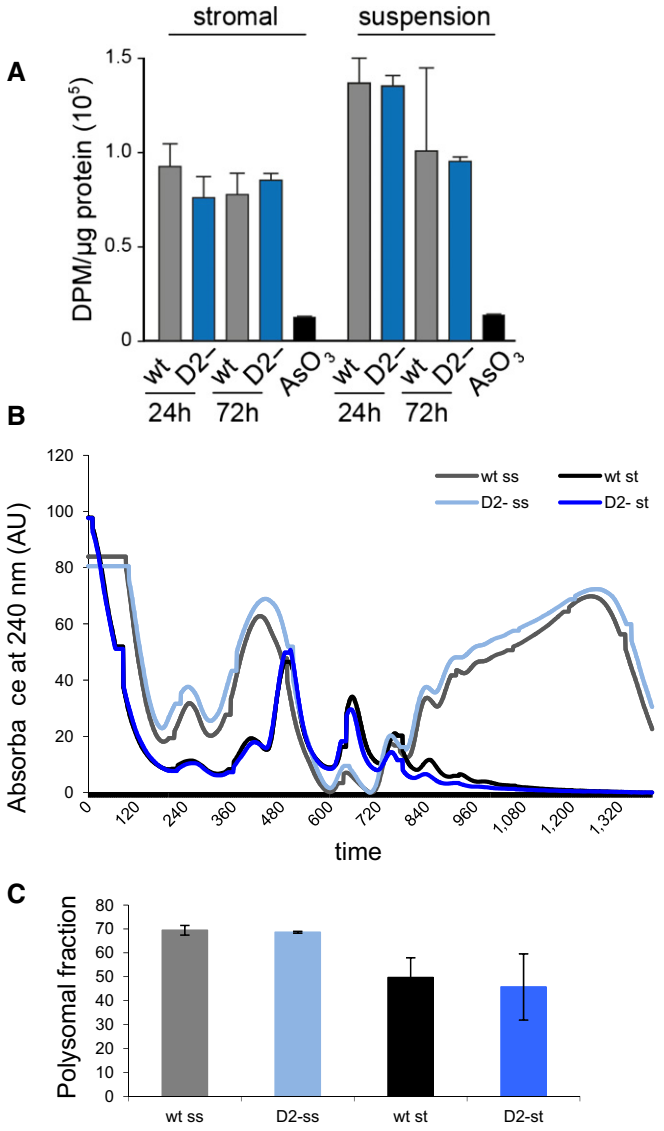

Figure EV2. Analysis of protein synthesis rates.

A Analysis of total protein synthesis in primary bone marrow stromal and haematopoietic cells by pulse-labeling with ^{3}H-Gly. As a negative control, protein synthesis was blocked in wild-type cells by treatment with 250 µM sodium arsenite (AsO_3). Results were obtained from three independent experiments, and error bars represent standard deviations.

B Representative polysome profiles from *Dnmt2*$^{-/-}$ and control primary bone marrow culture. Haematopoietic cells growing in suspension (ss) and attached stromal (st) cells were independently harvested and showed a higher proteins synthesis rate.

C As a measure for the global rate of translation, the fraction of polysomal ribosomes was quantified from polysome profiles. Results are shown for two independent biological replicates, and error bars represent standard deviations.

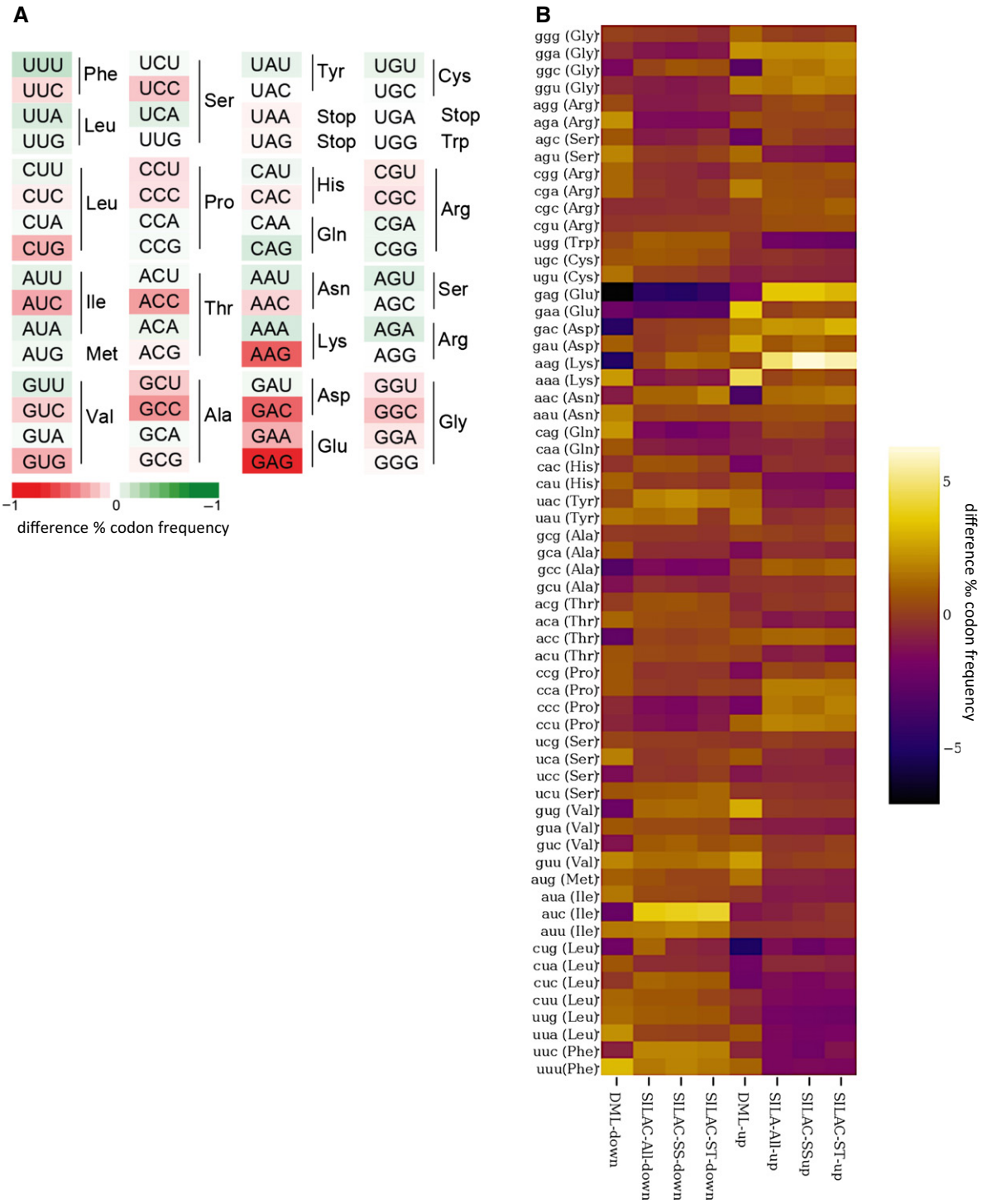

Figure EV3. Regulation of codon fidelity.

A Heatmaps showing the significant codon biases in Dnmt2 down-regulated proteins in the DML dataset. An increase of a codon frequency relative to the average frequency of that codon in all identified proteins is displayed in red, whereas a reduction is depicted in green. Codons showing no deviation from average values are displayed in white according to the scale provided in the figure.

B Heatmaps showing codon biases in all protein datasets. Changes of codon frequencies relative to the average frequency of the indicated codon are displayed according to the scale provided in the figure.

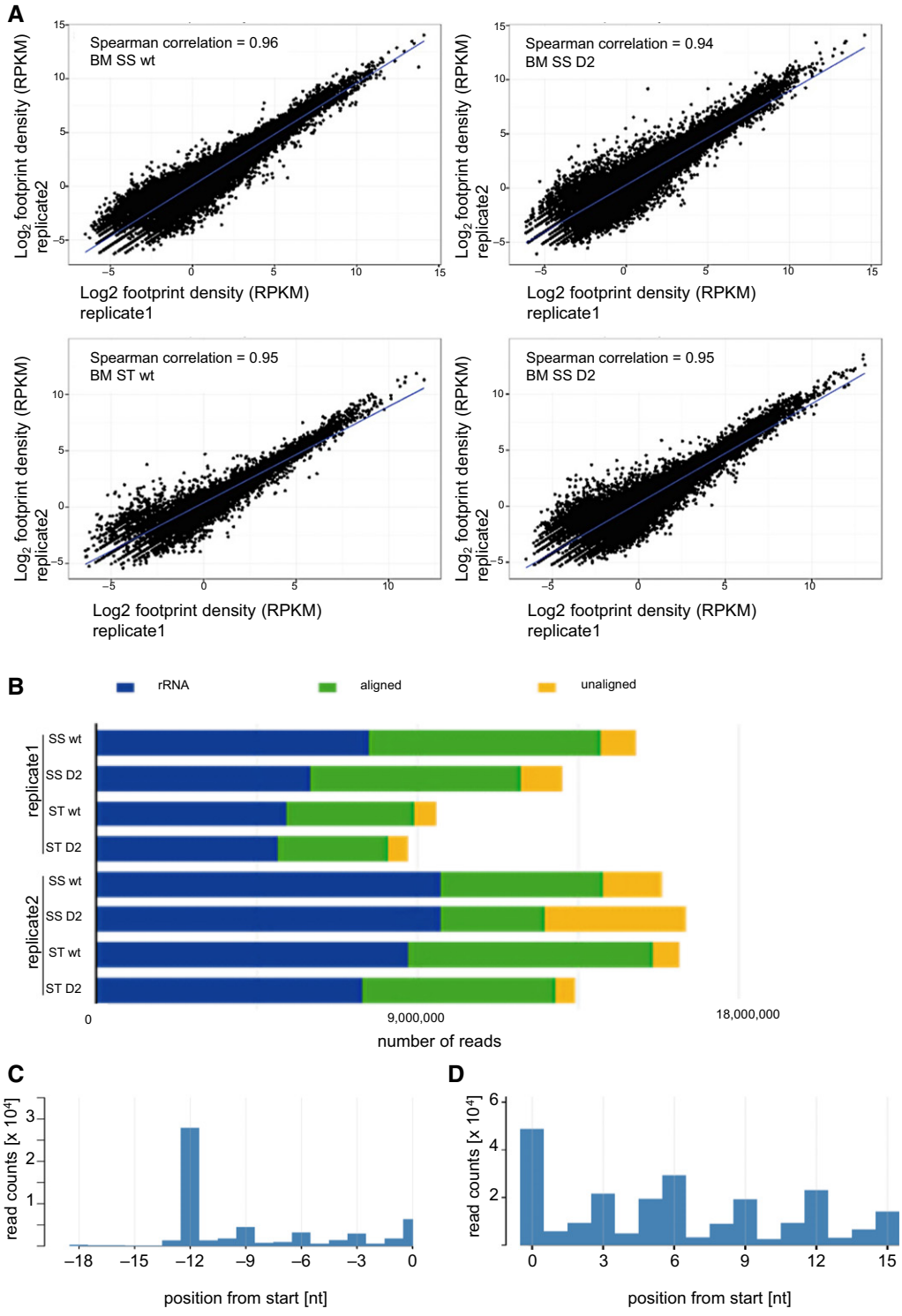

Figure EV4. Reproducibility and quality control of ribosome profiling.

A Correlation between ribosome profiling dataset replicates. Haematopoietic cells growing in suspension (ss) and attached stromal cells (st) were independently harvested.

B Mapping results from the two independent ribosome profiling replicates.

C Representative metaplot of wild-type haematopoietic (ss) cells. 28-mer ribosome footprint reads were summed across all start codons.

D Representative in-frame 28-mer ribosome footprint abundance in wild-type haematopoietic (ss) cells showing the correct periodicity.

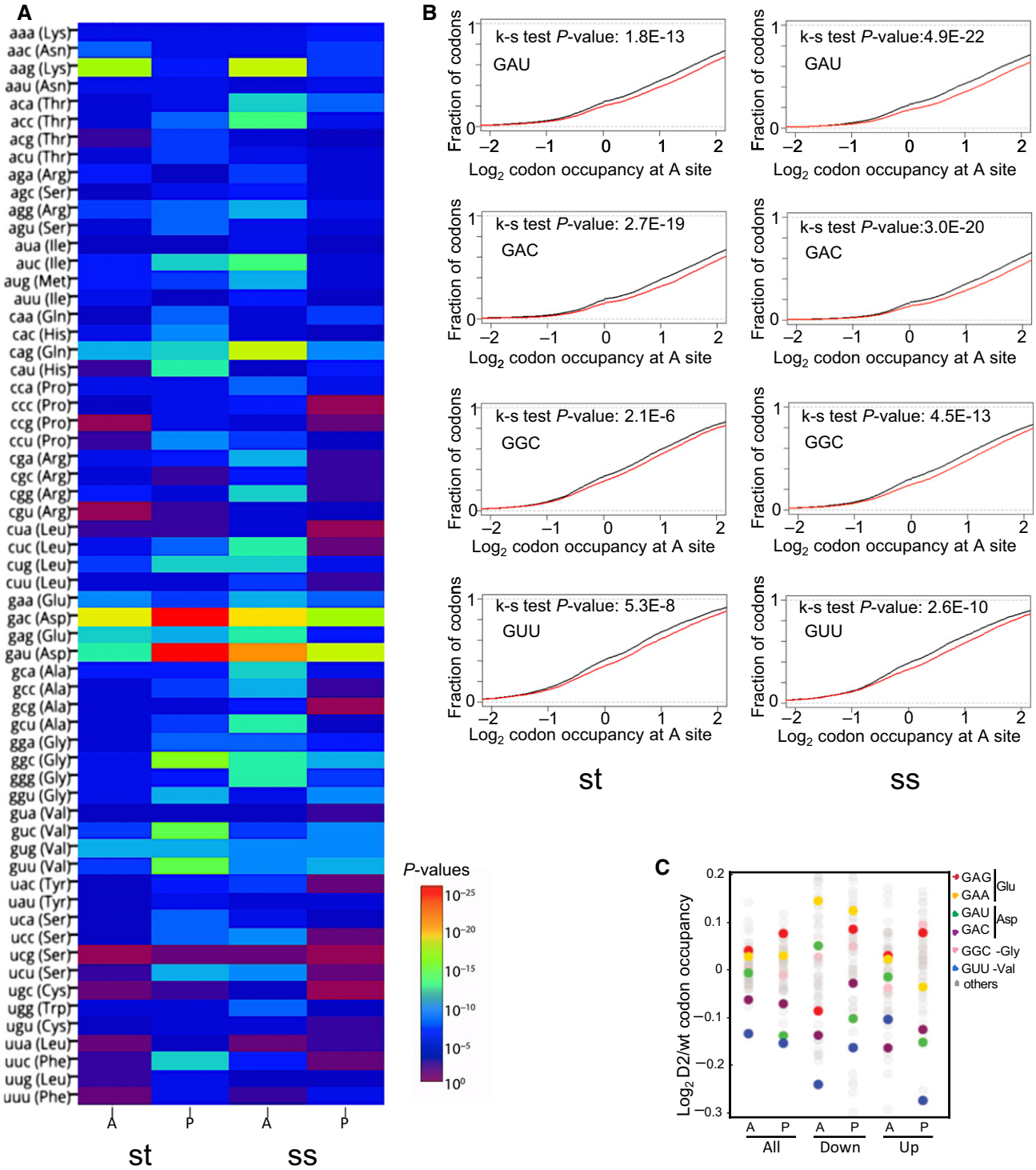

Figure EV5. Single-codon occupancy metric shows that differences in ribosome footprint occupancy at Dnmt2 target codons are statistically significant.

A Heatmap of *P*-values (Kolmogorov–Smirnov test) for all sense codons in wild-type and *Dnmt2*$^{-/-}$ ribosome A and P sites. The two biological replicates of each cell type were pooled. A: ribosomal A-site; P: ribosomal P-site; st: stromal cells, ss: suspension cells.

B Cumulative distributions of single-codon occupancy for select codons in wild-type and *Dnmt2*$^{-/-}$ stromal (st) and suspension (ss) cells.

C Changes in bulk codon occupancy in *Dnmt2*$^{-/-}$ bone marrow suspension cells versus wild-type cells. Dnmt2 target codons and Glu codons are highlighted with the indicated colors. The two biological replicates of each cell type were pooled. A: ribosomal A-site; P: ribosomal P-site.