802

803 Supplemental Table Legends

804 **Sup Table 1. Lysine-methylated peptides in WT and *SETD2*-KO human kidney cell lines.**
805 "Position" corresponds to location of methylated lysine residue within the full-length protein.
806 "MeK" indicates whether the lysine residue was mono-, di-, or tri-methylated. "Methyl K
807 Probabilities" indicates the calculated relative probability that the indicated lysine residue was
808 modified. A-D indicates the four biological mass spectrometry replicates and the ratio of the wild
809 type (WT) and *SETD2*-knock out (KO) ratios (i.e., WT/KO ratio). If the lysine predicted to be
810 modified corresponds to a canonical *SETD2* methylation motif (e.g., KxP or KxxG), it was
811 indicated with "x". "K motif" corresponds to the modified lysine and the four amino acids that
812 follow (N-terminal to C-terminal).
813
814 **Sup Table 2. Differentially expressed proteins in WT and *SETD2*-KO human kidney cell**
815 **lines.** Proteins quantified in only one sample or proteins with inconsistent relative quantification
816 change direction (knockout vs wild type) were discarded. Differentially expressed proteins with
817 fold change larger than 1.5 in at least one sample were included in pathway analysis.
818
819 **Sup Table 3. Differentially expressed genes in WT and *SETD2*-KO human kidney cell lines.**
820 Differential gene expression is expressed as log2 fold change of WT/KO cell lines (three
821 replicates for each genotype).

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825 # Supplemental Figure Legends

826 **Sup Fig 1. Immunoblots validating loss of SETD2 protein and catalytic activity. a)** *SETD2*
827 was knocked out of HKC cells using TALEN. Successful knock out was confirmed through
828 immunoblot analysis. Functional loss of SETD2 was further validated by H3K36me3
829 immunoblot. Both SETD2 and H3K36me3 were decreased relative to loading control in *SETD2*-
830 KO cell line (relative quantifications shown). **b)** Validation of rescue with the truncated SETD2
831 variants by SETD2 and H3K36me3 immunoblot (relative quantifications shown). **c)** Uncut films
832 of SETD2-WT, KO, tSETD2, SET-mt, and SRI-mt cell lines. The rescue vectors included a flag
833 tag, showing successful construct expression.
834
835 **Sup Fig 2. Distribution of heavy isotope-labeled (H) and unlabeled (L) peptides in each**
836 **replicate.** Shown are heavy:light ratios of proteins in **a)** raw and **b)** normalized data. Normalized
837 ratios were used for all subsequent analyses.
838
839 **Sup Fig 3. eEF1A1 lysine methylated peptides detected in SILAC-labeled replicates.** Ratios
840 represent WT/KO.
841
842 **Sup Fig 4. Extracted ion chromatograms for K165-containing peptide from the eEF1A1**
843 **protein.** Shown are the extracted ion chromatograms for K165me0-3 (C replicate from Fig. 2e,
844 no lysine methylated peptide immunoprecipitation).
845

846 **Sup Fig 5. Low expression of EEF1AKMT2 in ccRCC correlates with poorer survival.**

847 Figure obtained from UALCAN (42).

848

849 **Sup Fig 6. Total Transcriptome Analysis Demonstrates Changes in Protein Translation in**

850 ***SETD2*-KO Cells. a)** Individual genes are up- and down-regulated in WT versus *SETD2*-KO

851 HKC cell lines. WT/KO protein ratios are ranked and evaluated for Gene Set Enrichment

852 Analysis (GSEA) using the Gene Ontology datasets. Gene sets with an FDR q-value <0.25 were

853 considered to be significant ($-\log_{10} = 0.60$). Displayed are the ten most significant gene sets that

854 are **b)** increased and **c)** decreased in *SETD2*-KO cells relative to wild type cells. **d)** The Gene

855 Ontology signature is GO_Translational_Initiation was highly significant and up-regulated in

856 *SETD2*-KO cells, just as the it was in the total protein dataset (Fig 4b). Increased levels of

857 several genes involved in protein translation were observed in the *SETD2*-KO cell lines. **e)** The

858 Gene Ontology signature with the lowest FDR q-value that is decreased in *SETD2*-KO cells is

859 GO_SWI_SNF_Superfamily_Type_Complex though it does not reach the significance threshold.

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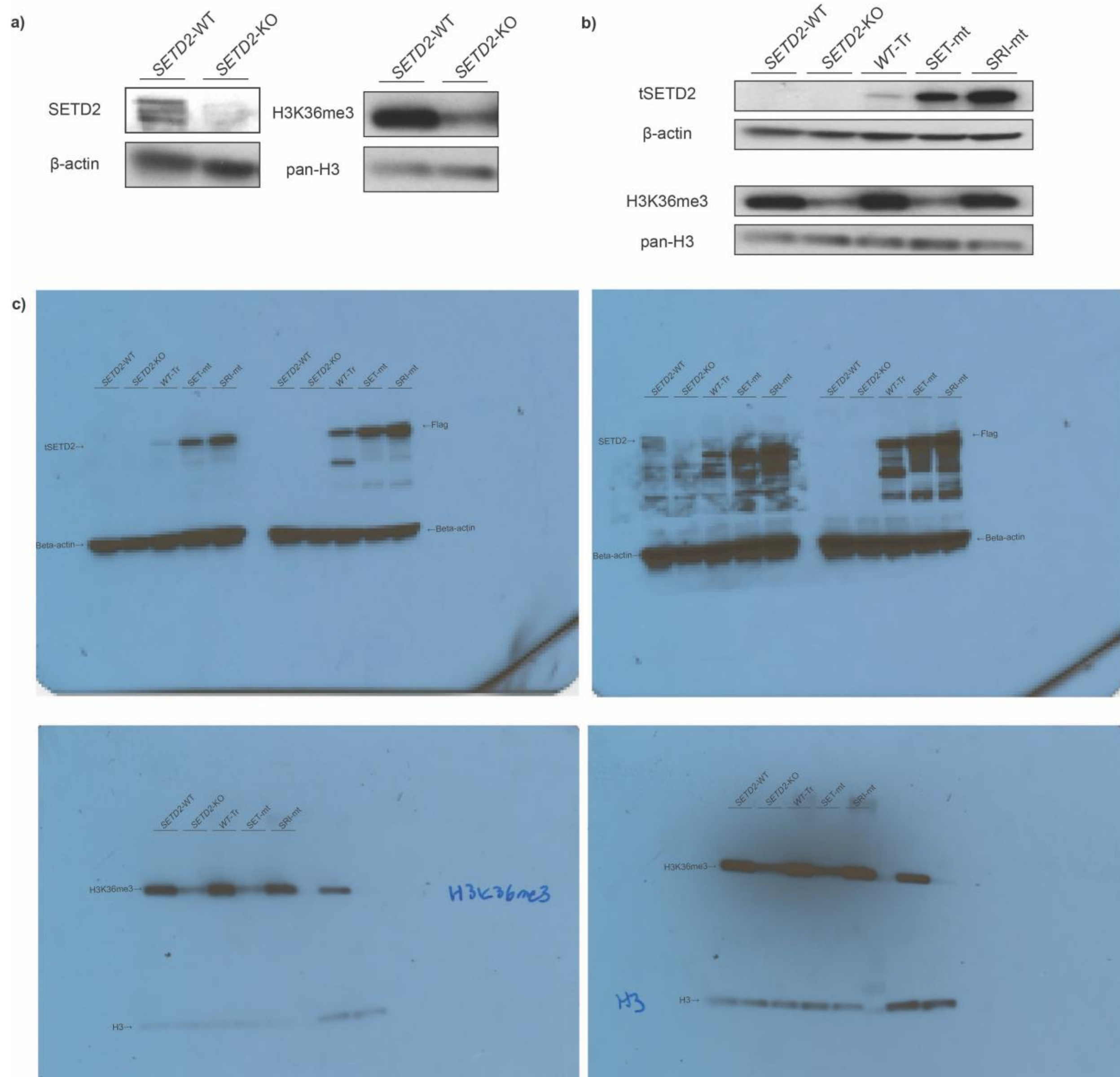
871 # Supplemental Figures

872 **Sup Fig 1.**



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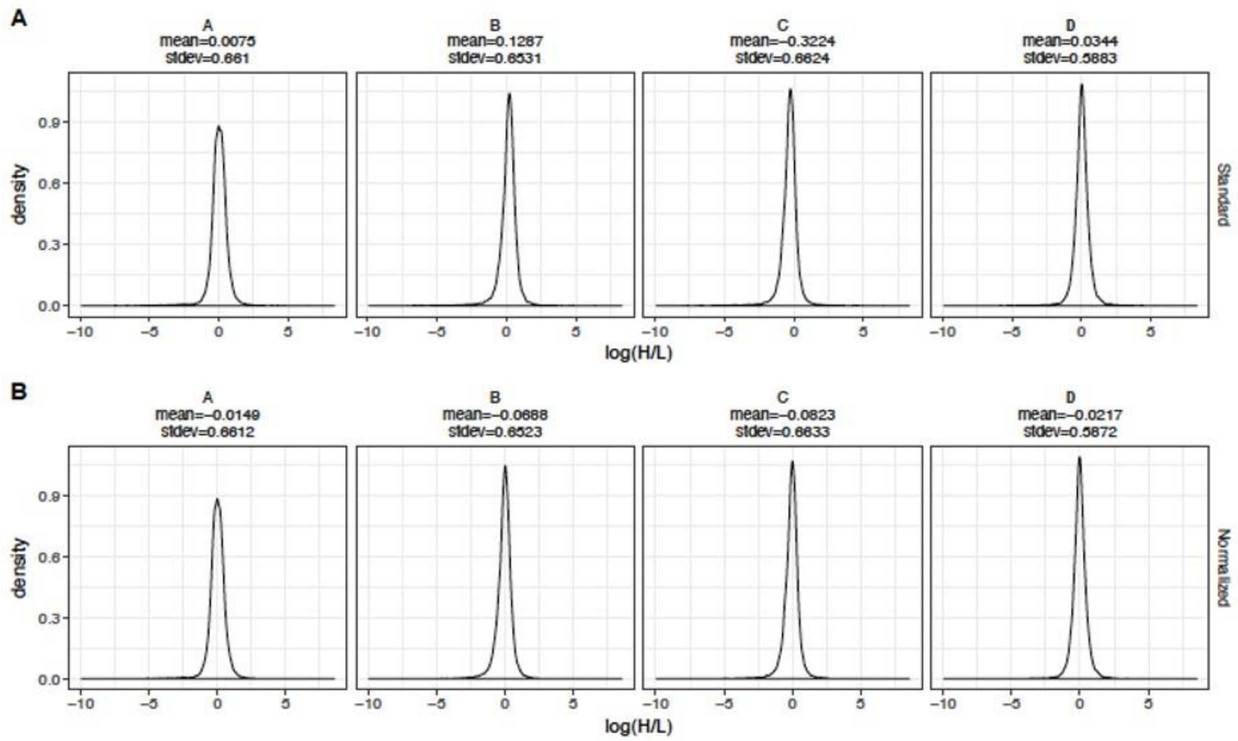
878 **Sup Fig 2.**



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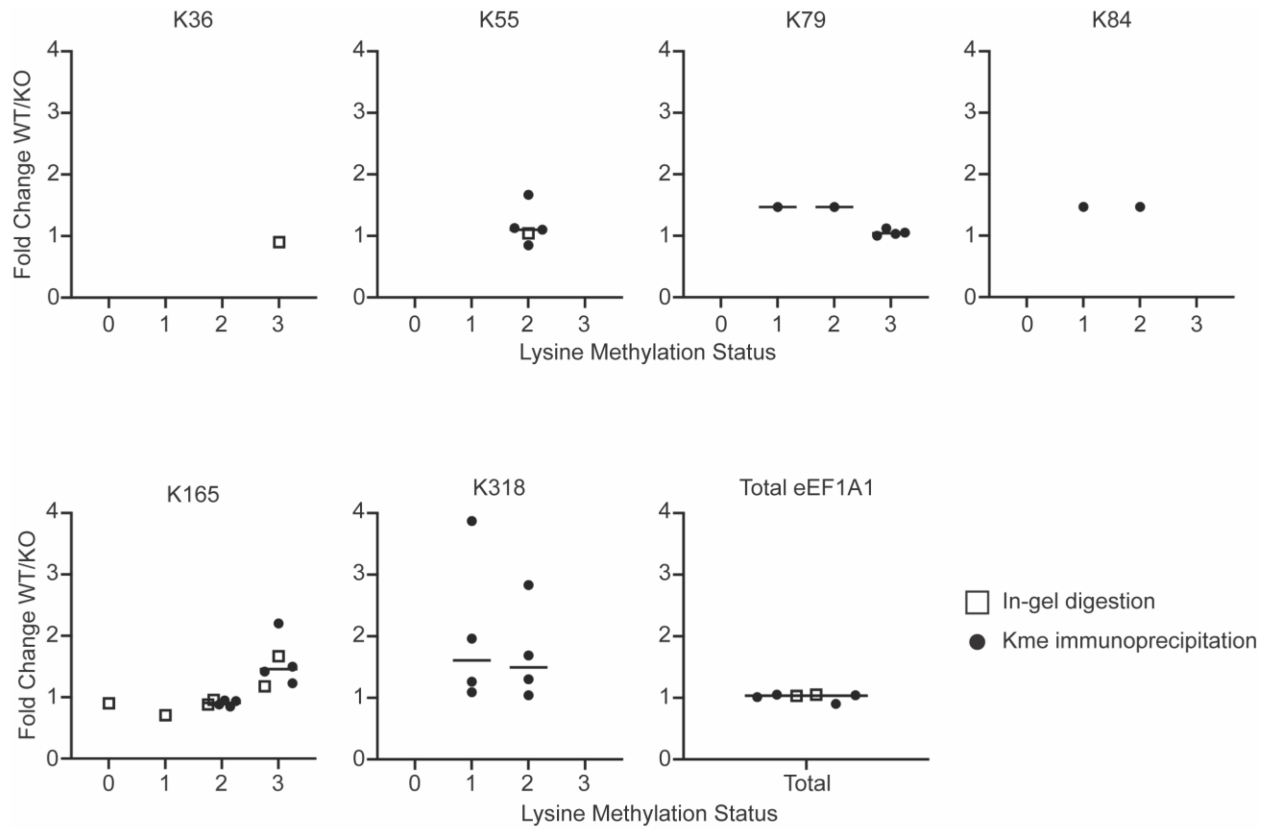
892 **Sup Fig 3.**



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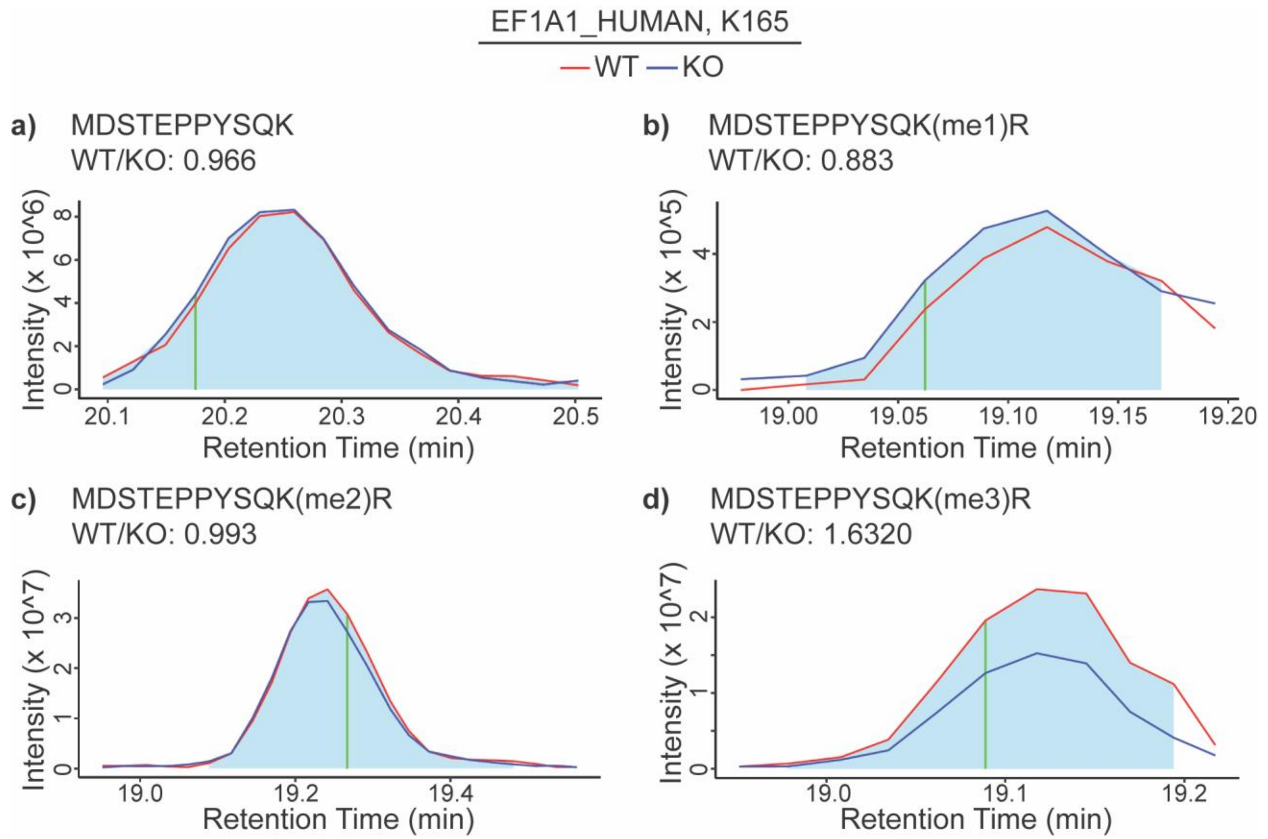
904 **Sup Fig 4.**



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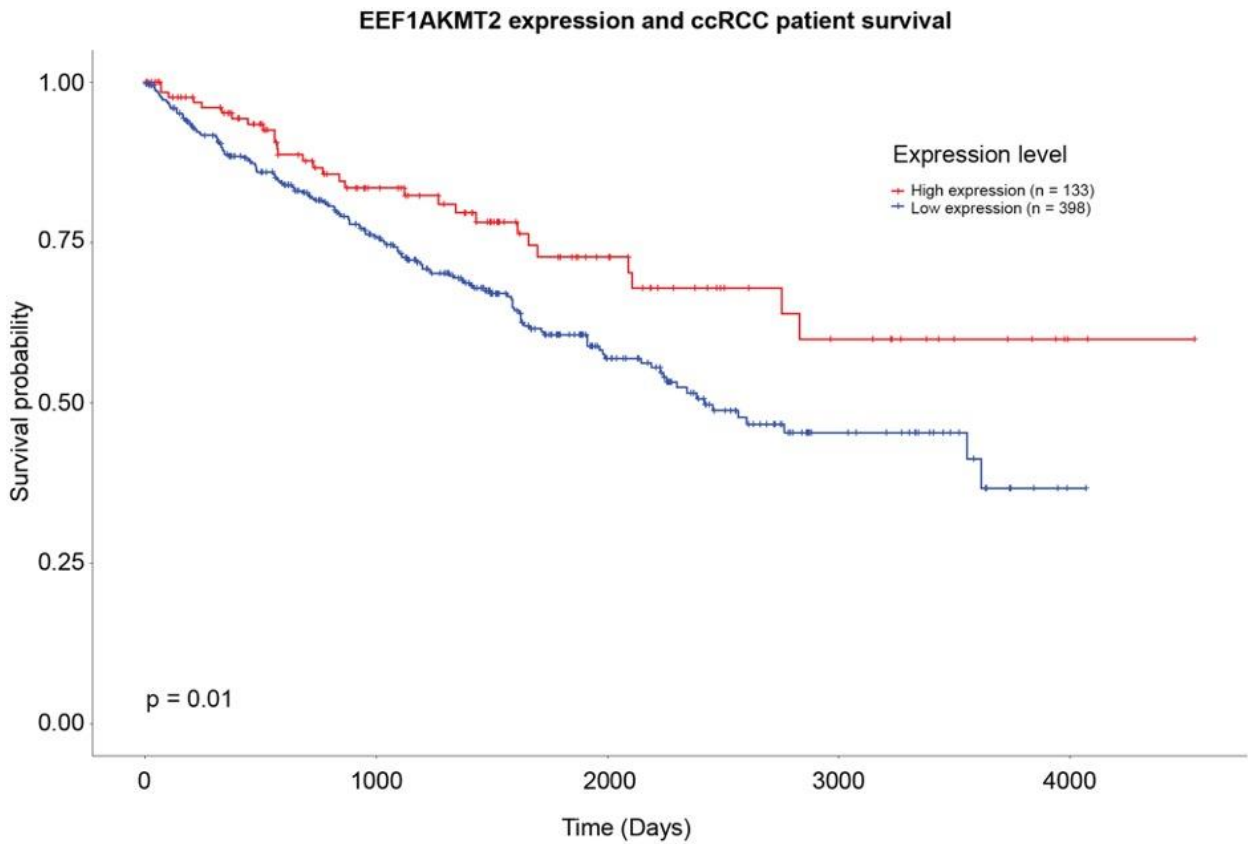
917 **Sup Fig 5.**



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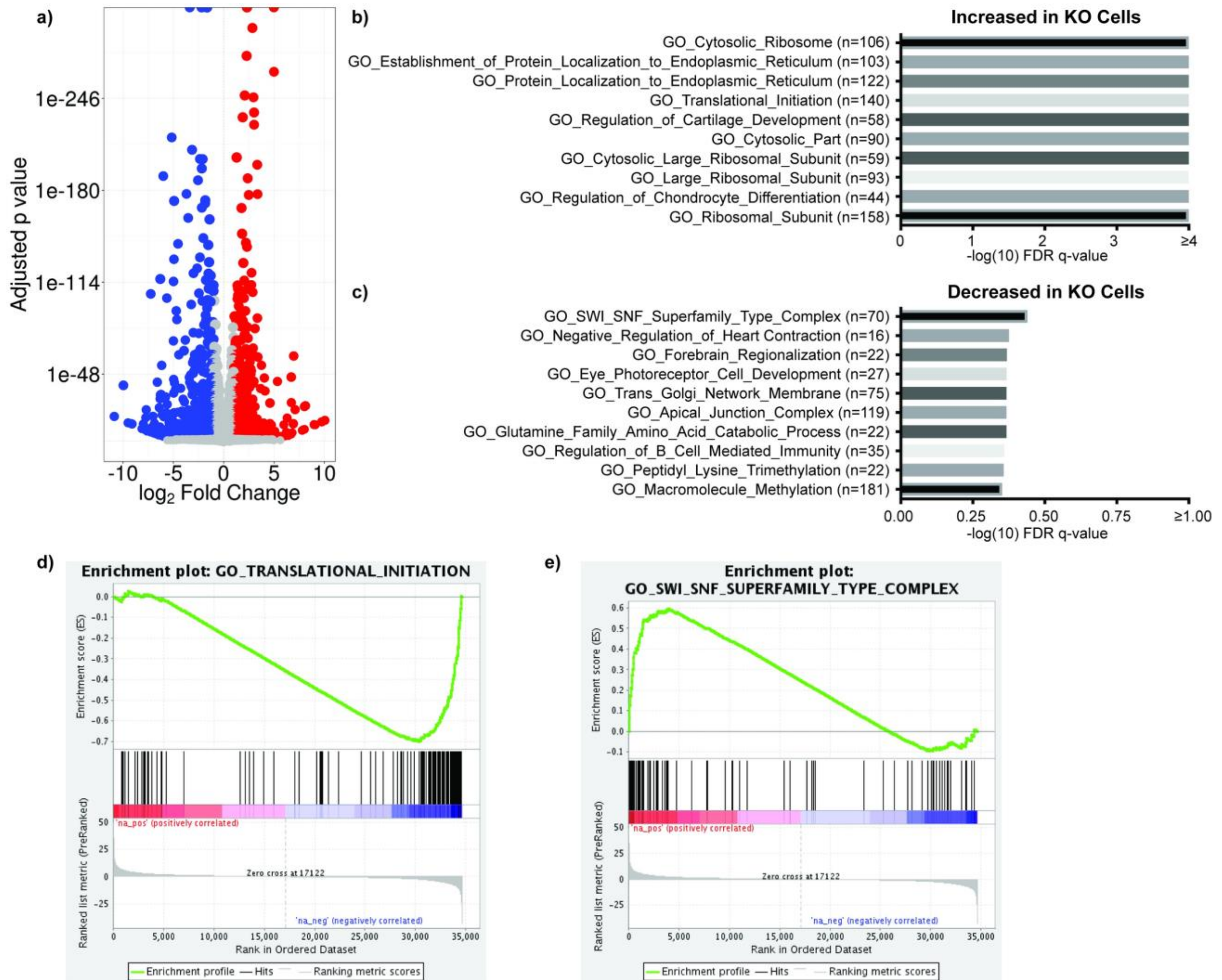
930 **Sup Fig 6.**



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940 # Supplemental Methods

941 ## Peptide Preparation

942 To prepare tryptic peptides for affinity purification, lysates (20 mg) were reduced with 4.5 mM
943 DTT for 30 minutes (min) and alkylated with 100 mM iodoacetamide for 30 min in the dark at
944 room temperature. Samples were then diluted 4-fold with 20mM Hepes, and proteins were digested
945 overnight at 37°C with 400 µg of proteomics-grade trypsin (1:50 enzyme to protein ratio).

946

947 The resulting peptides were then desalted by solid-phase extraction (Sep-pak C18 cartridges,
948 Waters Corporation). Digested samples were first acidified with TFA, diluted 2-fold with 0.1%
949 TFA, and loaded onto the Sep-pak SPE material. After sample loading, the cartridges were washed
950 with 0.1% TFA, and eluted with acetonitrile containing 0.1% TFA (80% acetonitrile with 0.1%
951 TFA). Eluates were dried using vacuum centrifugation.

952

953 ## Lysine Methylated Peptide Enrichment

954 Dried eluates were then reconstituted in 0.1% formic acid and analyzed by LC-coupled
955 tandem mass spectrometry (LC-MS/MS). An analytical column was packed with 22 cm of C18
956 reverse phase material (Jupiter, 3 µm beads, 300Å, Phenomenox) directly into a laser-pulled
957 emitter tip. Peptides were loaded on the capillary reverse phase analytical column (360 µm O.D.
958 x 100 µm I.D.) using a Dionex Ultimate 3000 nanoLC and autosampler. The mobile phase
959 solvents consisted of 0.1% formic acid, 99.9% water (solvent A) and 0.1% formic acid, 99.9%

960 acetonitrile (solvent B). Peptides were gradient-eluted at a flow rate of 350 nL/min, using a 180-
961 min gradient. The gradient consisted of the following: 1-165 min, 2-40 % B; 165-167 min, 40-
962 90 % B; 167-169 min, 90 % B; 169-172 min, 90-2% B; 172-180 min, 2% B. Peptides were
963 analyzed on a Q Exactive HF mass spectrometer (Thermo Scientific), equipped with a
964 nanoelectrospray ionization source, and a data-dependent method was used. Data dependent
965 acquisition is a traditional mass spectrometry-based proteomics approach (48). Proteomic
966 samples are digested into peptides, ionized, and analyzed by mass spectrometry. Peptides that
967 rise above a critical signal-to-noise level in the full scan mass spectrum are selected
968 fragmentation, producing tandem mass spectra (MS/MS) that can be matched to spectra in a
969 database for peptide identification. The instrument method included an AGC target value of 3e6
970 for MS1, followed by up to 15 MS/MS scans of the most abundant ions detected in the preceding
971 MS scan with an MS2 AGC target 1e5. HCD collision energy was set to 27 nce, and peptide
972 match and isotope exclusion were enabled. Each of the four SILAC samples were prepared and
973 analyzed as described above for four biological replicates.
974

975 # Total Proteome Analysis

976 For whole proteome analysis, aliquots of the mixed SILAC-labeled lysates containing 20 μg of
977 protein were precipitated with ice-cold acetone overnight at -20°C. Following precipitation,
978 samples were centrifuged at 18,000x*g* at 4°C, precipitates were washed with cold acetone, and
979 pellets were allowed to air dry. A solution of 50mM Tris buffer, pH 8, containing 50%
980 trifluoroethanol was used for reconstitution of the precipitated protein. Proteins were next reduced
981 with TCEP for 1 hour, alkylated with iodoacetamide for 30 min, and diluted 5-fold with 100 mM
982 Tris to obtain a final 10% TFE solution prior to proteolytic digestion. Proteins were digested

983 overnight at 37°C with 0.5 µg of trypsin. LC-MS/MS analysis of the peptides was performed using
984 a Q Exactive mass spectrometer (Thermo Scientific) equipped with a nanospray source and a
985 Dionex Ultimate 3000 nanoLC and autosampler. The peptides were loaded onto a self-packed
986 biphasic C18/SCX MudPIT column using a Helium-pressurized cell (pressure bomb). The
987 MudPIT column consisted of 360 µm x 150 µm i.d. fused silica, fritted with a filter-end fitting
988 (IDEX Health & Science), and packed with 5 cm of Luna SCX material (5 µm bead, Phenomenex)
989 and 4 cm of Jupiter C18 material (5 µm bead, Phenomenex). After sample loading, the MudPIT
990 column was connected using an M-520 microfilter union (IDEX Health & Science) to an analytical
991 column (360 µm x 100 µm i.d.), equipped with a laser-pulled emitter tip. The analytical column
992 was packed with 20 cm Jupiter C18 material (3 µm bead, Phenomenex). LC-MS/MS was
993 performed with an 11-step salt pulse gradient (25 mM, 50 mM, 75 mM, 100 mM, 150 mM, 200
994 mM, 250 mM, 300 mM, 500 mM, 750 mM, and 1 M ammonium acetate). Peptides were eluted
995 from the analytical column after each salt step with a 90 min reverse gradient (2-50% acetonitrile,
996 0.1% formic), followed by a 10-min equilibration a 2% B, for the first 10 salt pulses. For the final
997 salt step, a gradient consisting of 2-98 % acetonitrile was used. Data were collected using a data-
998 dependent method. The instrument method included an AGC target value of 1e6 for MS1, followed
999 by 20 MS/MS scans of the most abundant ions detected in the preceding MS scan with an intensity
1000 threshold of 5e4.
1001
1002 MS/MS spectra were searched against a human subset database created from the UniprotKB
1003 protein database (www.uniprot.org). Variable modifications included carbamidomethylation of
1004 cysteines (+57.0214) and oxidation of methionines (+15.9949). Precursor mass tolerance was set
1005 to 10 ppm, enzyme specificity was set to Trypsin/P, and a maximum of 2 missed cleavages were

1006 allowed. The target-decoy false discovery rate (FDR) for peptide and protein identification was
1007 set to 1% for peptides and proteins. For SILAC protein ratios, a minimum of 2 unique peptides
1008 and a minimum ratio count of 2 were required, and the requantify option was enabled. All reported
1009 protein groups were identified with two or more distinct peptides and were quantified with two or
1010 more ratio counts.

1011

1012 **Size Enrichment of eEF1A1 Proteins**

1013 SILAC-labeled protein lysates from SETD2-WT and -KO cells were mixed 1:1, and 50 μg of the
1014 mixed lysates (25 μg of WT lysate and 25 μg KO lysate) were loaded onto a NuPAGE 10% Bis-
1015 Tris gel. The gel was stained with Novex colloidal Coomassie stain, and the region
1016 corresponding to approximately 48 – 52 kDa was excised for in-gel digestion. Gel regions were
1017 diced into $1mm^3$ cubes, proteins were treated for 30 min with 45 mM DTT, and Cys residues
1018 were carbamidomethylated with 100 mM iodoacetamide for 45 min. Gel pieces were destained
1019 with 50% MeCN in 25 mM ammonium bicarbonate, and proteins were digested with trypsin (10
1020 ng/μL) in 25 mM ammonium bicarbonate overnight at 37°C. Peptides were extracted by gel
1021 dehydration with 60% MeCN, 0.1% TFA, the extracts were dried by speed vac centrifugation,
1022 and peptides were reconstituted in 0.1% formic acid and analyzed by LC-coupled tandem mass
1023 spectrometry (LC-MS/MS). Similar to previously described methods for affinity purified lysates,
1024 peptides were loaded on a capillary reverse phase analytical column using a Dionex Ultimate
1025 3000 nanoLC and autosampler. Peptides were gradient-eluted at a flow rate of 350 nL/min,
1026 using a 90-min gradient. The gradient consisted of the following: 2-45% B in 75 min; 45-90% B
1027 in 4 min; 90% B for 1 min; 90-2% B in 1 min; 2 %B for 9 min (column re-equilibration). A Q
1028 Exactive Plus mass spectrometer (Thermo Scientific), equipped with a nanoelectrospray

1029 ionization source, was used to mass analyze the eluting peptides using a data-dependent method,
1030 with an inclusion list of specific m/z values corresponding to various forms of the eEF1A1
1031 peptide MDSTEPPYSWKR. The inclusion list included methylated forms of the peptide in both
1032 light and heavy labeled conditions. The instrument method consisted of MS1 using an MS AGC
1033 target value of 1e6, followed by up to 15 MS/MS scans of the most abundant ions detected in the
1034 preceding MS scan. The MS2 AGC target was set to 5e4, dynamic exclusion was set to 15s,
1035 HCD collision energy was set to 27 nce, and peptide match and isotope exclusion were enabled.
1036 For identification of peptides, LC-MS/MS raw data were searched with Maxquant as described
1037 previously. Variable modification included oxidation of Met, carbamidomethylation of Cys, and
1038 methylation, dimethylation, and trimethylation of lysine. Comparison of the heavy and light
1039 forms of eEF1A1 MDSTEPPYSQK(me3)R (eEF1A1_K318) peptide were conducted in WT and
1040 KO cells in two replicates independent of lysine methyl-peptide enrichment.
1041

1042 **Synthetic Heavy-Labeled Peptide-Aided Peptide Quantification**

1043 A synthetic peptide labeled with heavy arginine (13C, 15N) was formulated for the
1044 MDSTEPPYSQK(me3)R (eEF1A1_K318) peptide (HeavyPeptide AQUA custom synthesis
1045 service, Life Technologies). Synthetic peptides were spiked into the WT and *SETD2*-KO lysates
1046 from the HKC and 786-O cells, allowing for relative quantification of the endogenous
1047 methylated peptide in non-SILAC-labeled cells. First, HKC and 786-O lysates (50 μg) were
1048 separated on gel as described previously, and the eEF1A1 gel regions were excised and in-gel
1049 digested with trypsin. Lysates from WT and KO lysates were prepared in triplicate for LC-
1050 MS/MS analysis of eEF1A1. Following in-gel digestion, the peptides were reconstituted in 20
1051 μL of 0.1% formic acid. Aliquots (8 μL) of each digest from the triplicate WT and KO lysates

1052 were then spiked with the synthetic peptide to make a solution of 12 μL containing 50 fmol/μL
1053 of the synthetic peptide. For LC-MS/MS, 2.5 μL of the spiked in-gel digests were analyzed on a
1054 Q Exactive Plus mass spectrometer. The method consisted of both data-dependent and targeted
1055 PRM scan events. First, MS1 were acquired using an AGC target value of 3e6, followed by 4
1056 MS/MS data-dependent using an MS2 AGC target of 5e4. Dynamic exclusion was set to 15s,
1057 HCD collision energy was set to 27 nce, and peptide match and isotope exclusion were enabled.
1058 Following data-dependent MS2 scan events, the method included targeted PRM scans of m/z
1059 values corresponding to the light and heavy eEF1A1 peptide MDSTEPPYSWK(me3)R.
1060 Targeted m/z values included oxidized and unoxidized forms of the peptide in both light and
1061 heavy SILAC states. PRM data were imported into Skyline, product ions were evaluated, and
1062 integrated areas were calculated in Skyline for y-type ions, y6 - y11, for each peptide precursor.
1063 Areas were summed for the light precursors and heavy precursors separately, and then ratios of
1064 the summed areas for WT and KO samples were calculated and used to determine the difference
1065 in the relative amount of the peptide MDSTEPPYSWK(me3)R peptide.