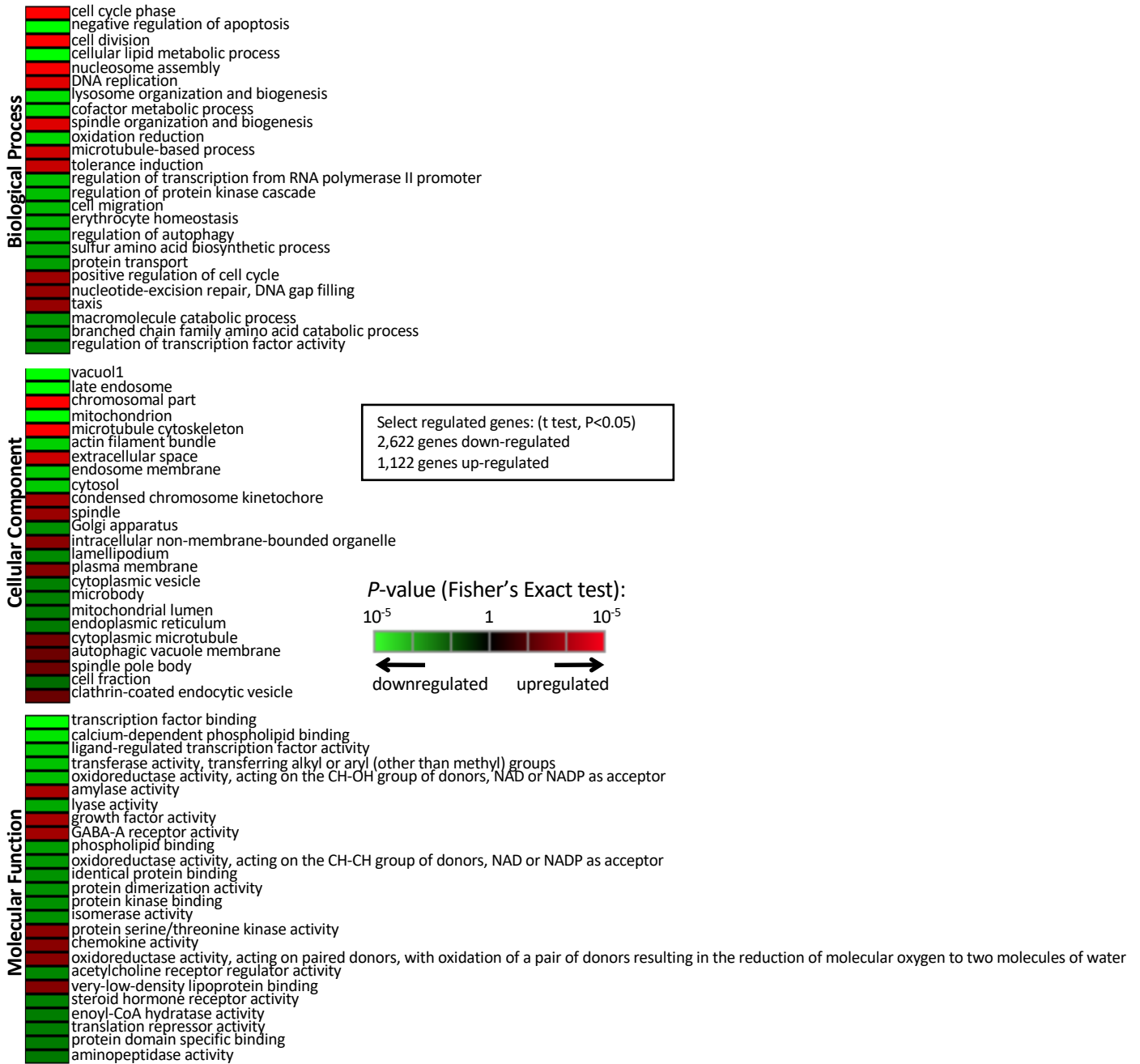


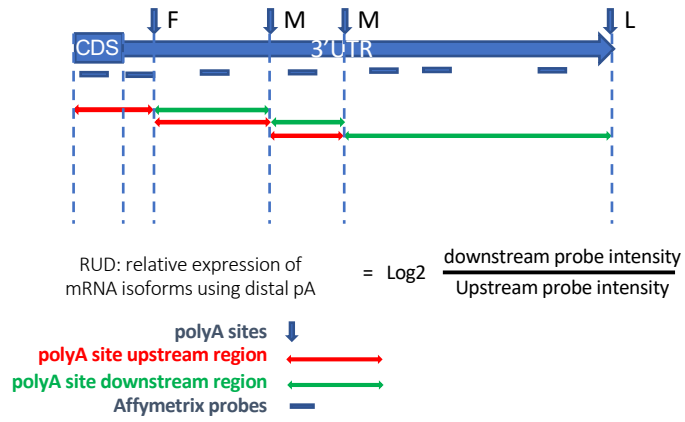
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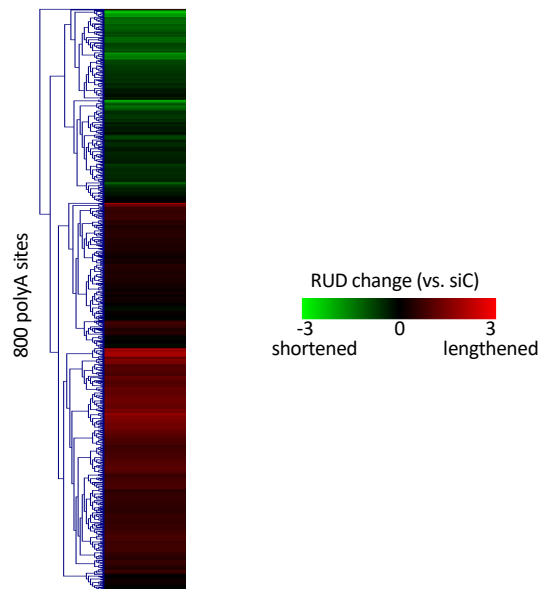
B

Gene symbol	log2 expression change	Gene description
ATP6V1C1	-1.49	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1
ATP6V0C	-0.18	ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c
ATP6V1H	-1.16	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H
ATP6V1E1	-0.93	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1
ATP6V0D2	-4.03	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2
ATP6V1G1	-0.61	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G1
ATP6V0E1	-0.91	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1
ATP6V0D1	-0.43	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1
ATP6V1B2	-1.15	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2

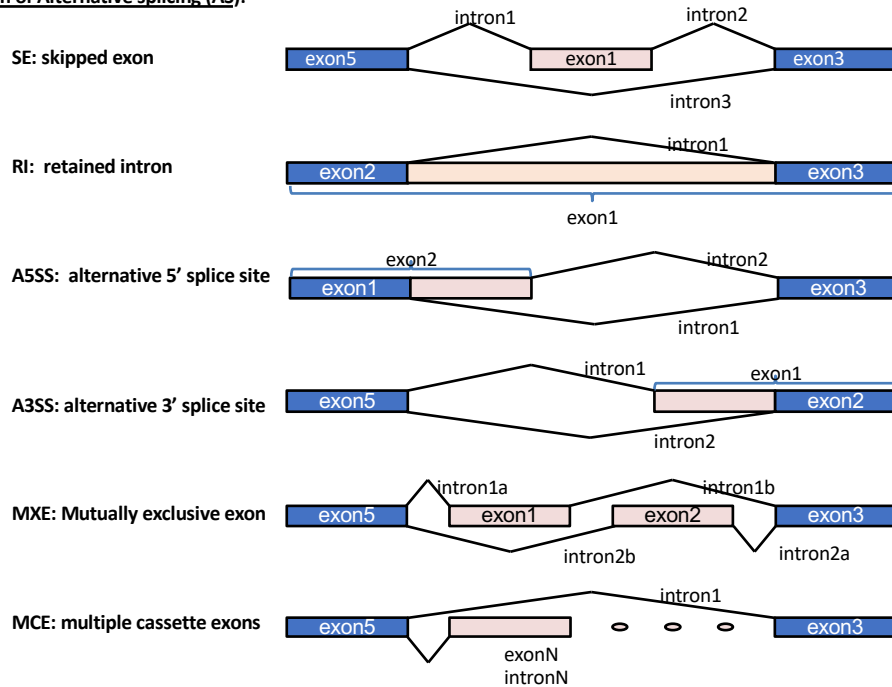
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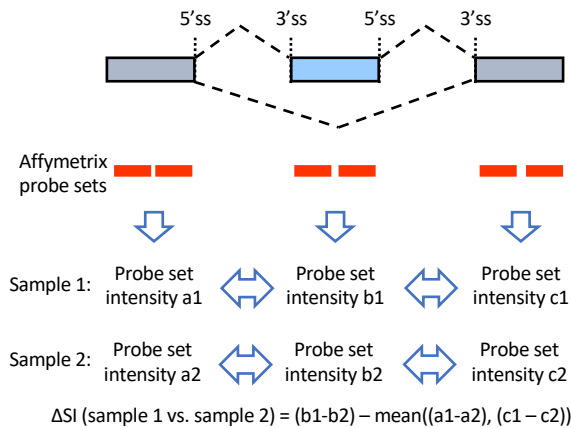
B



A Definition of Alternative splicing (AS):



B Splicing index calculating using microarray



C AS regulation

	se	a5ss	a3ss	mxe	ri	mce
ex	869	103	134	14	78	788
in	1035	114	239	40	192	1443
nc	21981	2616	3670	642	2755	18130

Comments:

alternative splicing regulation (t-test, $P < 0.05$):

ex, exon more excluded

in, exon more included

nc, no change

gene expression regulation:

dn, gene down-regulated

up, gene up-regulated

nc, gene expression no significant change

D

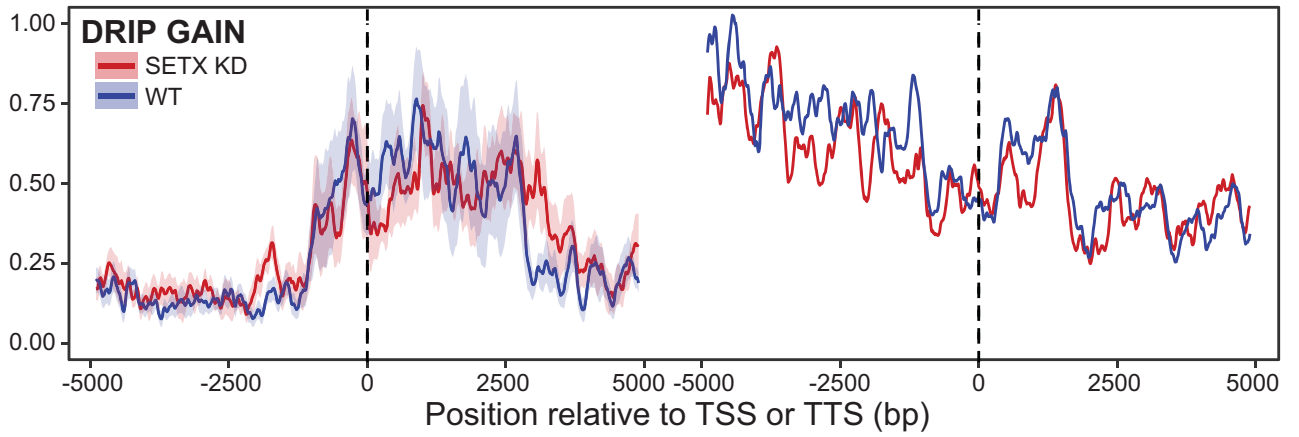
Correlation between AS regulation and gene expression regulation

Alternative splicing type	Alternative splicing regulation	Gene expression regulation			Total number of gene	Fisher's exact test P value	Spearman correlation
		dn	nc	up			
se	ex	129	306	45	7172	2.6E-04	p= 1.3e-29 rho= -0.133
	in	216	337	29			
	nc	759	5057	294			
a5ss	ex	18	47	9	1732	6.5E-03	p= 1.4e-20 rho= -0.221
	in	43	29	3			
	nc	245	1263	75			
a3ss	ex	21	51	9	2218	2.1E-02	p= 7.4e-16 rho= -0.17
	in	73	74	9			
	nc	313	1556	112			
mxe	ex	0	12	1	493	1.3E-01	p= 1.1e-20 rho= -0.403
	in	14	13	1			
	nc	62	374	16			
ri	ex	11	36	10	1554	6.8E-06	p= 1.1e-49 rho= -0.363
	in	71	54	3			
	nc	226	1070	73			
mce	ex	81	265	59	6403	1.3E-15	p= 8.9e-106 rho= -0.268
	in	255	336	23			
	nc	670	4471	243			

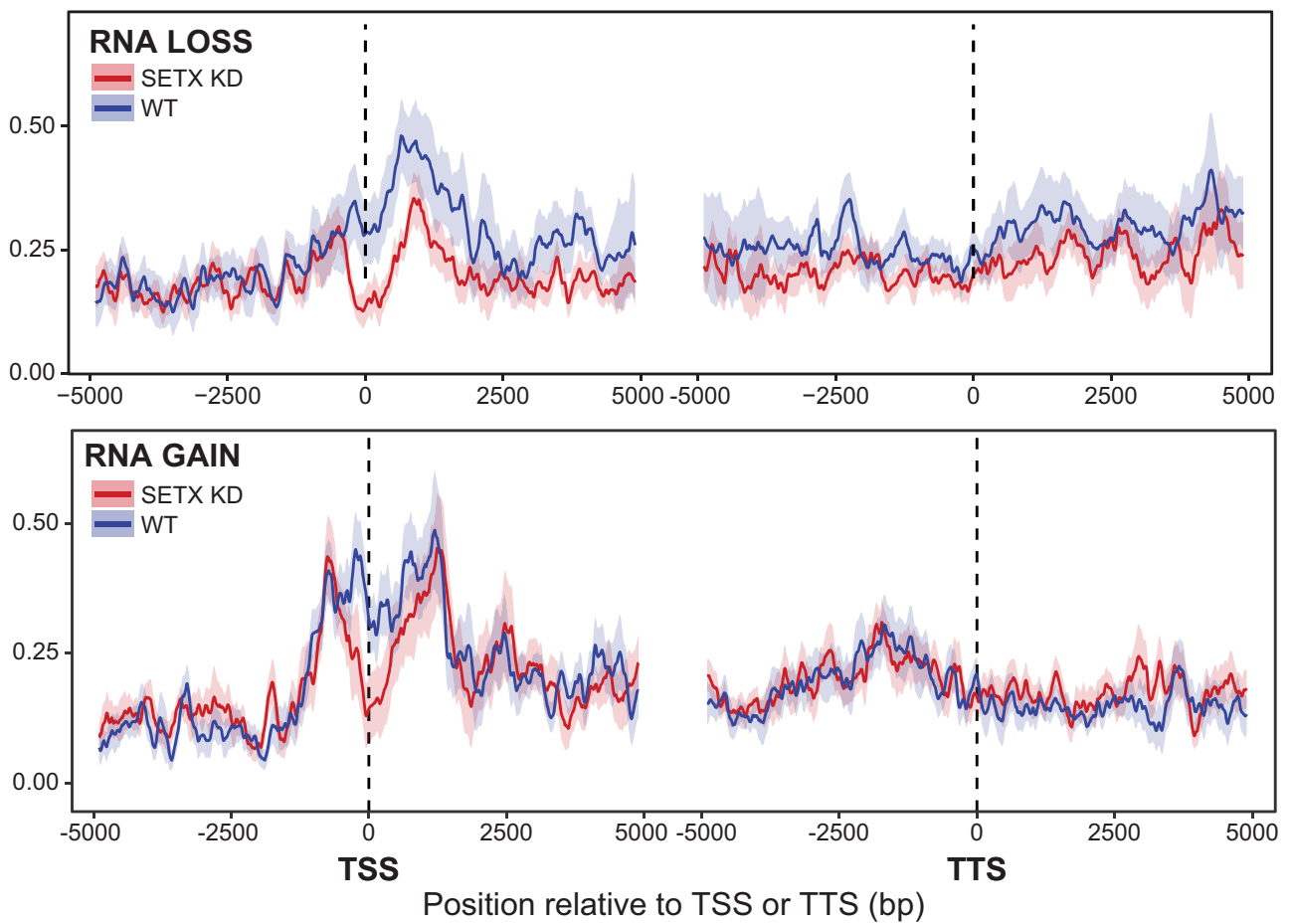
Note: for one gene only select most regulated AS events.

Supplementary Figure S4

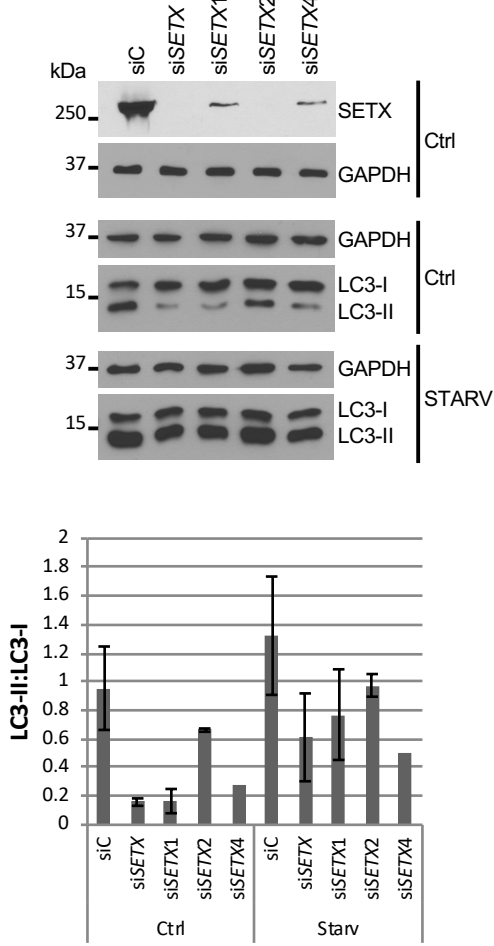
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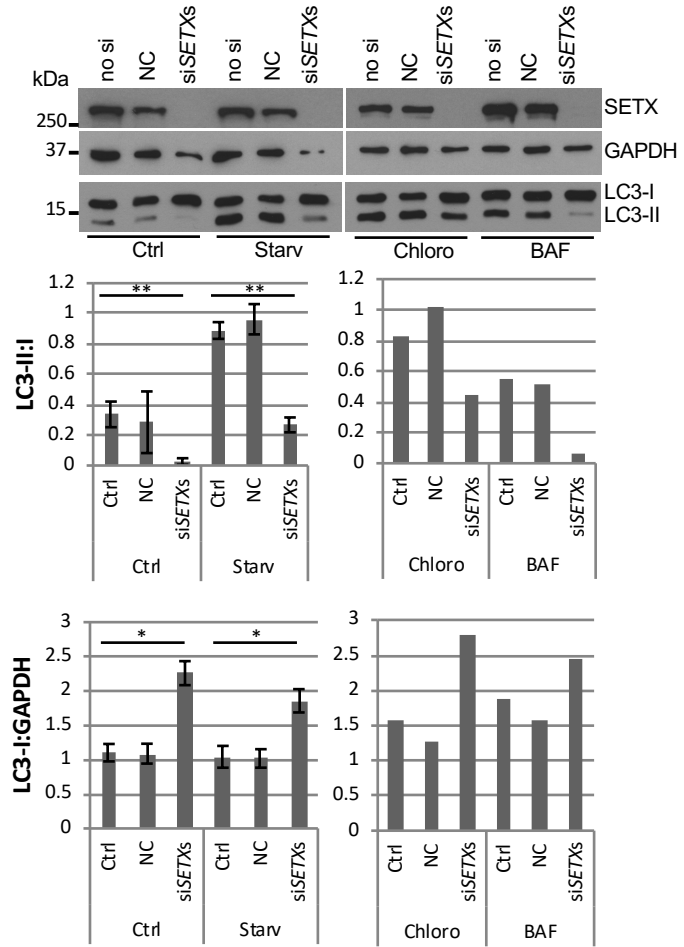
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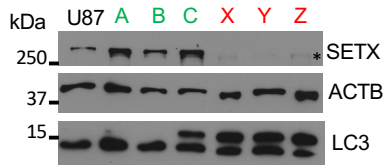
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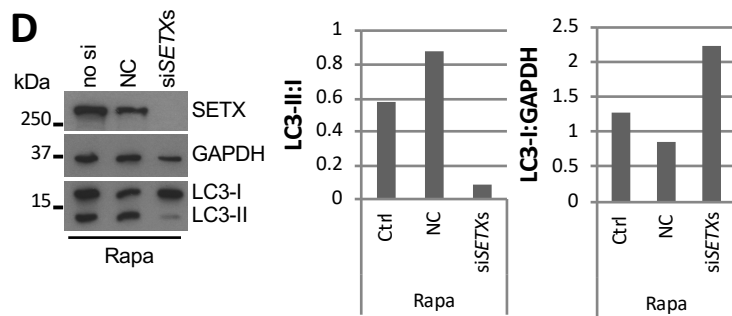
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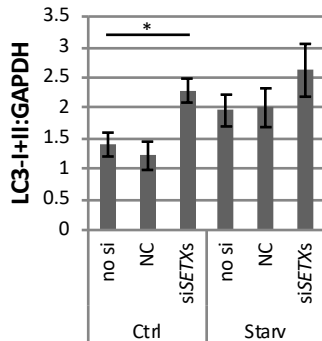
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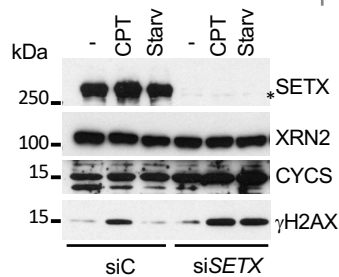
D



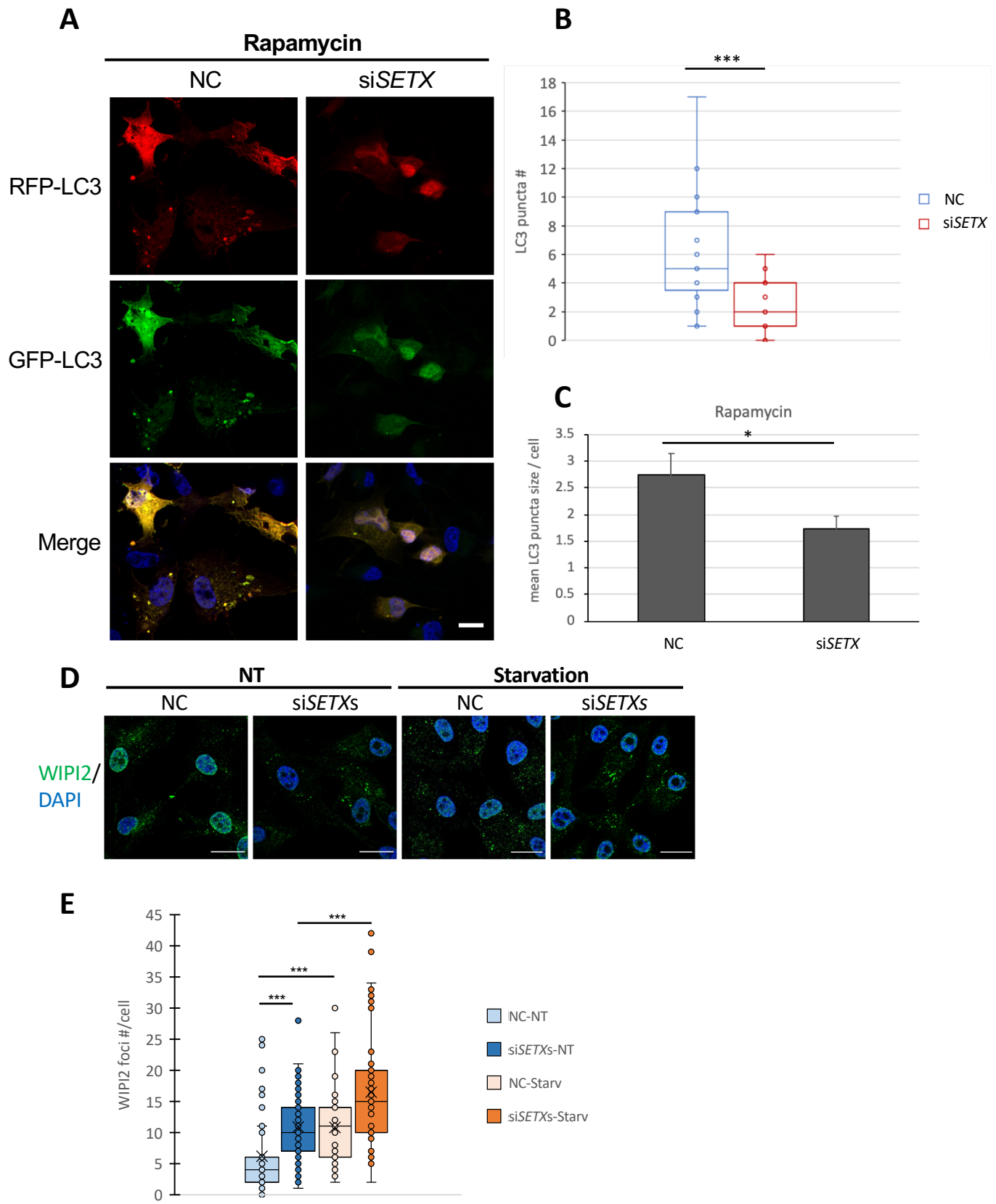
E



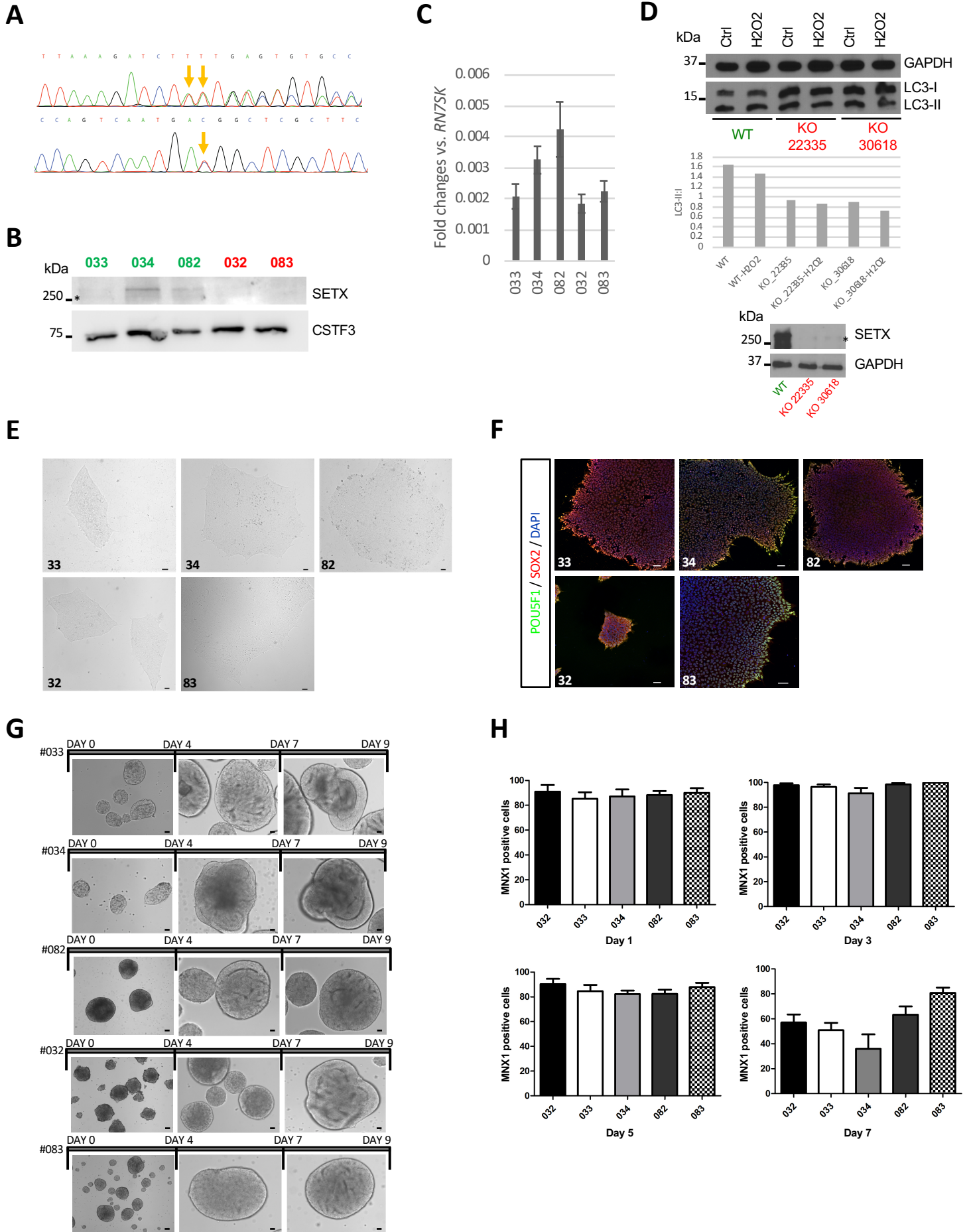
F



Supplementary Figure S6



Supplementary Figure S7



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Gene regulation after SETX KD affects genes involved in various processes including autophagy and lysosomal degradation. **(A)** GO analysis of genes regulated by SETX KD obtained from the microarray data. **(B)** List of V-ATPase genes downregulated by SETX KD.

Figure S2. SETX KD impairs APA. **(A)** method for APA analysis from the microarray data. F, M and L denote the first, middle and last polyA sites in 3'-most exon. **(B)** Heatmap showing RUD changes in SETX KD.

Figure S3. AS analysis from the microarray data. **(A)** Definition of AS events analyzed in the study. In each AS type, two isoforms were compared, with the pink- and blue-colored regions indicating alternative and common regions, respectively. **(B)** Explanation of splicing index (SI) calculation. Probes mapped to different regions of exons were used to calculate a splicing index (SI). The difference of probe signal between alternative region (pink-colored in A) and common region is defined as SI, except for MXE, in which the difference between exon1 and exon2 is used. The Δ SI is the difference of SI between two samples (e.g., knockdown vs. control, denoted as sample 1 vs. sample 2 here). All raw probe set signals are log 2 based. An increase of SI indicates an increased signal of the alternative region in sample 1. Here we call significant increase of SI "inclusion" (in) and decrease of SI "exclusion" (ex). **(C)** and **(D)** correlation between splicing event and gene expression after SETX KD in U87 cells.

Figure S4. SETX depletion does not increase R-loop signal. **(A)** Metagene plots showing DRIP-seq signal increase over promoters and terminal regions in SETX-depleted cells. The lines represent the average signal surrounded by the standard error (shaded) for

control and SETX-depleted cells, respectively. **(B)** Metagene plots showing DRIP-seq signal over promoter and terminal regions of genes showing loss of gene expression (RNA loss [top]) and gain of gene expression (RNA gain [bottom]) in SETX-depleted cells.

Figure S5. SETX regulates the autophagic flux. **(A)** U87 transfection with control (siC) and 4 different siRNAs targeting *SETX* at 20 nM for 72 h. Protein extracts were analyzed by WB in normal conditions (Ctrl) and after starvation for 24 h (STARV) in medium containing 0.1% FBS. Graph shows LC3-II:LC3-I quantification. $n = 2$, SD shown. **(B)** Steady-state levels of LC3 expression in U87 cells. Cells were cultured in DMEM with (Ctrl) or without FBS for 6 h (Starv), treated with chloroquine (Chloro; 20 μ M) or Bafilomycin (BAF; 100 nM) for 6 h after transfection without siRNA (no si), with a negative control siRNA (NC) and a pool of 4 siRNAs targeting *SETX* (si*SETX*s) at 2 nM each for 72 h. Cell extracts were analyzed by WB. The upper graph shows LC3-II:LC3-I quantification. The lower graph shows LC3-I ratio to GAPDH. For Ctrl and Starv, $n = 4$, SE shown. Significance was analyzed by a student's t-test, * $p < 0.05$, ** $p < 0.01$. **(C)** Steady-state levels of SETX and LC3 in WT (green) and *SETX* KO (red) U87 CRISPR cells. ACTB is used as a loading control. * indicates non-specific band. **(D)** WB as in B. Extracts were prepared after rapamycin treatment at 100 nM for 6 h. **(E)** Quantification of total LC3 levels after SETX KD (I + II) as shown in WB **Fig. S5B**. $n = 3$, SE shown. Significance was analyzed by a student's t-test, * $p < 0.05$. **(F)** Proteins extract and analysis of SETX, CYCS (cytochrome c) and γ H2AX levels after siC and si*SETX* transfection for 72 h in U87 cells after no treatment (-), CPT treatment and starvation for 6 h in medium without FBS (Starv). XRN2 is used as loading control. * indicates non-specific band.

Figure S6. SETX KD compromises autophagosome and autolysosome formation. **(A)** mRFP-GFP-LC3 puncta formation assay in rapamycin-treated (10 nM for 6 h) U87 cells transfected with NC or siSETX for 48 h prior *mRFP-GFP-LC3* transfections for 24 h. Merge signal (yellow) indicate the formation of autophagosomes. Scale bar: 15 μ m. **(B)** Quantification of autophagosomes and autolysosomes number per cell in control and SETX KD cells. Total cells analyzed: n = 44; Unpaired t-test ***p < 0.001. **(C)** Quantification of mean LC3 puncta size per cell in control and SETX KD cells. Total number of 91 LC3 puncta from 20 cells were analyzed. Data is displayed in mean \pm s.e.m. Unpaired t-test *p < 0.05. **(D)** U87 cells were transfected with a siRNA control (NC) or a pool of siRNAs (siSETXs) for 72 h. Cells were untreated (NT) or starved for 6 h and stained for endogenous WIPI2 (green). Scale bar: 15 μ m. **(E)** Quantification of WIPI2 foci per cell as shown in D. Significance was analyzed by a student's t-test, ***p < 0.001.

Figure S7. Characterization of control and AOA2 patients' fibroblasts and the corresponding iPSCs and EBs. **(A)** Chromatogram of sequencing covering *SETX* exons 10 (top) and 21 (bottom) of AOA2 patient #032 that carry new mutations. **(B)** SETX protein level in control (green) and AOA2 (red) fibroblasts. CSTF3 (cleavage stimulation factor subunit 3) is used as loading control. * indicates non-specific band. **(C)** *SETX* mRNA quantification by RT-qPCR in control (033, 034, 082) and AOA2 fibroblasts (032 and 083), n = 10, SE shown. **(D)** Steady-state levels of LC3 in WT (green) and AOA2 (red) lymphoblastic cells in normal condition (Ctrl) and after H₂O₂ treatment at 0.5 mM for 30 min. The graph shows LC3-II:LC3-I quantification and the lower WB shows SETX protein level in each cell line. * indicates non-specific band. **(E)** Representative brightfield pictures of iPSCs colonies obtained from the 3 asymptomatic individuals (033, 034, 082) and 2 AOA2 patients (032 and 083) from the same family. All five iPSC cell lines presented a normal morphology, and they were also positive for specific stem cell markers. Scale bar:

50 μm . **(F)** Characterization of the iPSC colonies with specific stem cell markers, POU5F1 (POU class 5 homeobox 1) in green, SOX2 (SRY-box transcription factor 2) in red and nuclear staining in blue (DAPI). Scale bar: 50 μm . **(G)** Brightfield representative images of EBs during the differentiation process. Scale bar: 50 μm (DAY 0 to DAY 4); 25 μm (DAY 4 to DAY 9). **(H)** MNs count at day 1, 3, 5 and 7 after plating.