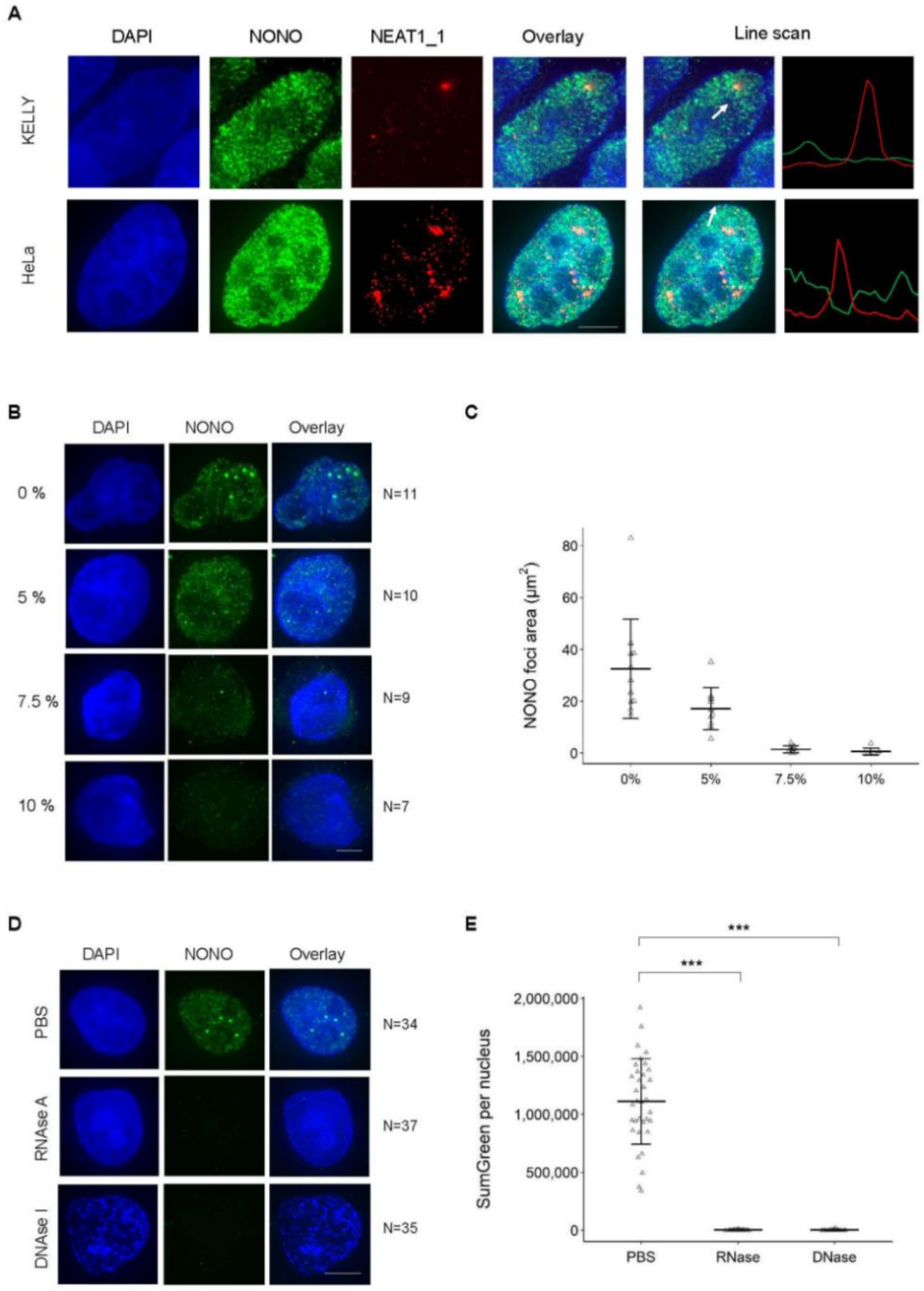
Appendix information

Zhang *et al.* NONO enhances mRNA processing of super enhancer-associated GATA2 and HAND2 genes in neuroblastoma

Appendix Table S1. A list of primers

Primer	Direction	5' - 3'
U6	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT
RPLP0	Forward	AGCCCAGAACACTGGTCTC
	Reverse	ACTCAGGATTTCAATGGTGCC
β-actin	Forward	GCCAACACAGTGCTGTCTGG
	Reverse	TACTCCTGCTTGCTGATCCA
Total NEAT1	Forward	GTGGCTGTTGGAGTCGGTAT
	Reverse	TAACAAACCACGGTCCATGA
NEAT1_2	Forward	GTCTTTCCATCCACTCACGTCTATTT
	Reverse	GTACTCTGTGATGGGGTAGTCAGTCAG
MYCN	Forward	CGACCACAAGGCCCTCAGTA
	Reverse	CAGCCTTGGTGTTGGAGGAG
pre_MYCN	Forward	CTGCCTGGACAGAAACCTGTTAG
	Reverse	TGCACAGCCCTTGAATCTTCTC
DAZAP1	Forward	CAGACCGCACACGCTAGATG
	Reverse	GTTATCGCTCCTGGGTCCTTTC
pre_DAZAP1	Forward	TGGAACTGGAGAGAGAGGTTTATGG
	Reverse	CCTCCCTCTGTGACTTTCCTACAA
HAND2	Forward	AAACAGGGCCGCTAACATTTC
	Reverse	TAGAGGACGGAAGTGCACAAA
pre_HAND2	Forward	AACTGGCTTCGGTAGGGTAGAG
	Reverse	GGTCTGAGGGCTAATGGAGGTTA
GATA2	Forward	CTGACGACAACCACCACCTTAT
	Reverse	CTTCATGGTCAGTGGCCTGTTA
pre_GATA2	Forward	AGCGCCAGCATTTCCAACTATAC
	Reverse	AGGCCTGGTGAGAGCAGATTTA
MALAT1	Forward	GAC GGA GGT TGA GAT GAA GC
	Reverse	ATT CGG GGC TCT GTA GTC CT
KCNQ2	Forward	CCCTCATCGGTGTCTCCTTCTT
	Reverse	TTCTCAAAGTGCTTCTGCCTGTG
pre_KCNQ2	Forward	TCATCCACTGTTGCTCCTCTGT

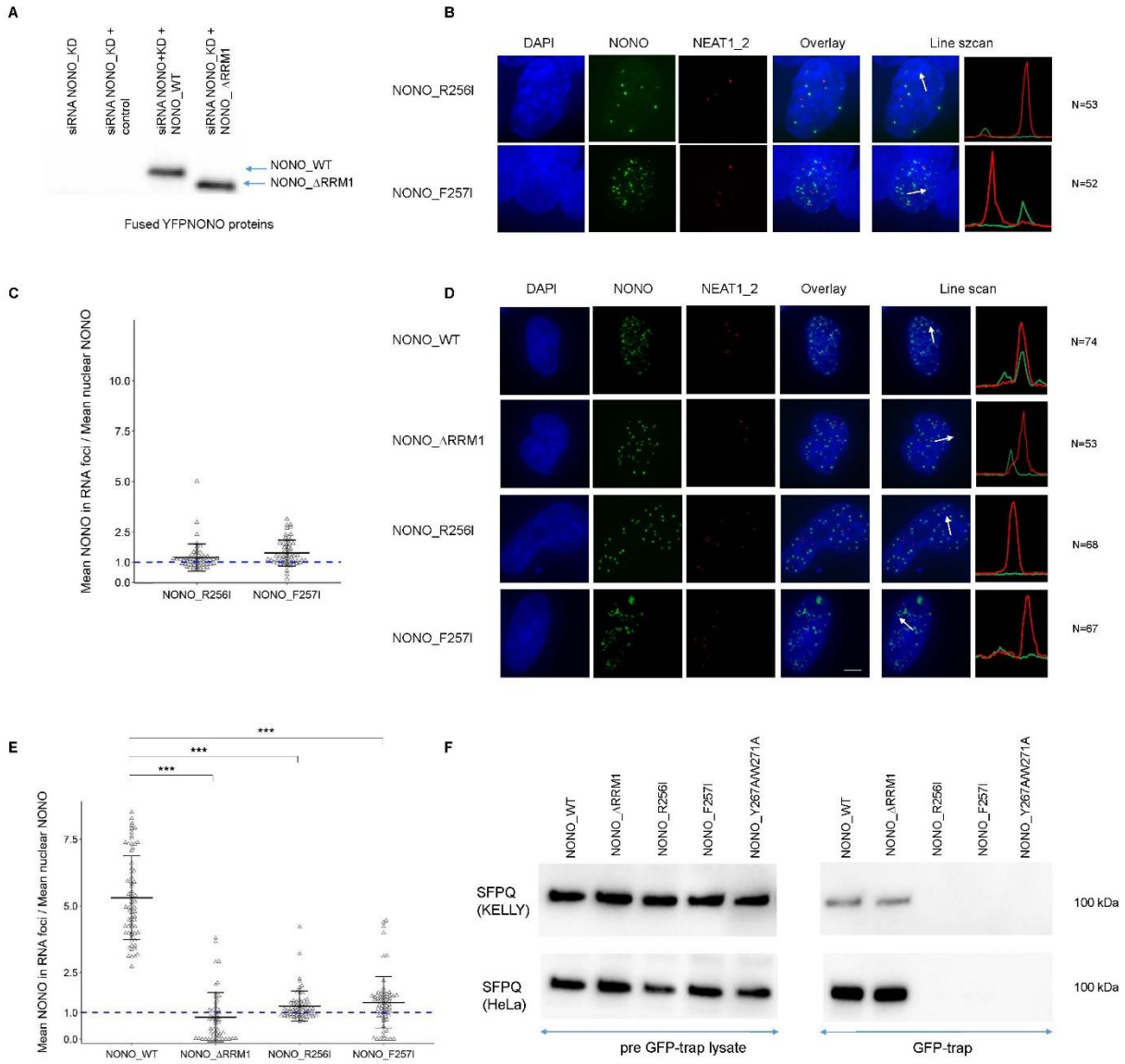
	Reverse	CAGGTCTGACGCCCTTCTAACT
RPLP2	Forward	ATGGATGCAGGAAGTGAGCC
	Reverse	AAGCCTGAGGAGTGATTGCC
CEP55	Forward	ATTCGCTCAATCACTGTGGTTCT
	Reverse	TGAGAGTGATTCTTTGGTTGGTATCT
GATA2_2	Forward	CTGCTCCCAGCTCTACTCCAG
	Reverse	AGGGAAGGTGGAAGTGGAAGAA
GATA2_3	Forward	GAAGTGTCTCCTGACCCTAGCA
	Reverse	GGGACTGCCACTTTCCATCTTC
HAND2_2	Forward	GAGGAAGAAGGAGCTGGTCAGTA
	Reverse	CGCAGCCAAAGAACACGAGAT
HAND2_3	Forward	AGACCGACGTGAAAGAGGAGAA
	Reverse	TTTCTTGTCGTTGCTGCTCACT
GATA2_4	Forward	AACGTGTCCCGAGCTTAGATTC
	Reverse	GGTCACTACATCAGCACAATCCT
GATA2_S	Forward	GGCTTACAGGGTAGGAGCTG
	Reverse	TTCATGTCTGTGCAGGAGTCG
GATA2_M	Forward	GAACAGCAGGAGCCGAGAG
	Reverse	TCCACTGGGTCAAGCACAG
GATA2_L	Forward	TGGAGTAGAGCTGGGAGCA
	Reverse	CCCACCAGGCGGACAAA



Appendix Figure S1: NONO distribution is not co-localising with microspeckles, and both RNA and DNA are required for NONO distribution in HeLa cells

(A) Fluorescence micrograph images of representative cells stained for NONO and NEAT1_1 in KELLY and HeLa cells showing NONO puncta and microspeckles (as marked by NEAT1_1). DAPI (blue) stain indicates cell nuclei, NONO immunofluorescence (green) and NEAT1_1 RNA FISH (red). Scale bar: 5 µm. (B) Fluorescence micrograph images of representative cells treated with 0, 5, 7.5 or 10% 1,6 hexanediol. (C) Dot plot of NONO foci area (µm2) per nucleus at different concentrations of 1,6 hexanediol in (B). Bars are SD. (D)

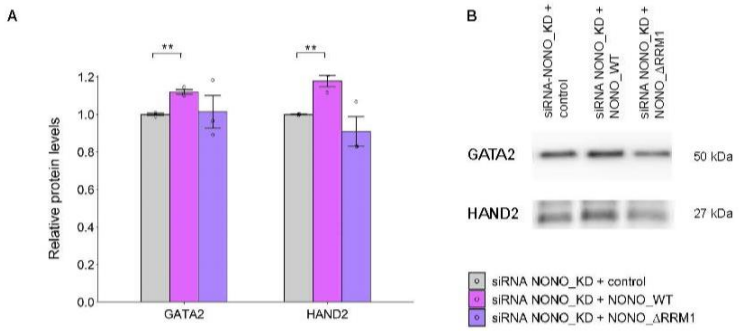
Fluorescence micrograph images of representative cells treated with PBS, RNase A or DNase I and stained for NONO. (E) Dot plot of SumGreen per nucleus in (D). Bars are SD. ***P<0.001.



Appendix Figure S2: Two single mutants of NONO play roles in dimerization

(A) Representative Western blot images of YFP fused NONO_WT and NONO_ΔRRM1 proteins in KELLY cells. (B) Fluorescence micrograph images of representative KELLY cells stained for NONO and NEAT1_2 after transfection with YFP fused NONO_R256I and NONO_F257I exogenous protein plasmids. DAPI (blue) stain indicates cell nuclei, YFP fused NONO (green) and NEAT1_2 RNA FISH (red). Scale bar: 5μm. (C) The enrichment of mean NONO fluorescence detected within RNA FISH foci is quantitatively determined as a ratio relative to mean nuclear NONO fluorescence in (B). Bars are SD. (D) Fluorescence micrograph images of representative HeLa cells stained for NONO and NEAT1_2 after transfection with YFP fused NONO_WT, NONO_ΔRRM1, NONO_R256I and NONO_F257I exogenous protein plasmids. DAPI (blue) stain indicates cell nuclei, YFP

fused NONO (green) and NEAT1_2 RNA FISH (red). Scale bar: 5μm. (E) The enrichment of mean NONO fluorescence detected within RNA FISH foci is quantitatively determined as a ratio relative to mean nuclear NONO fluorescence in (D). Bars are SD. ***$p<0.001$. (F) Representative Western blot images of SFPQ protein in pre-GFP-trap lysate samples and GFP-trapped samples transfected with YFP fused NONO_WT, NONO_ΔRRM1, NONO_R256I, NONO_F257I and NONO_Y267A/W271A exogenous protein plasmids in KELLY and HeLa cells.



Appendix Figure S3: Overexpression of NONO_WT after endogenous NONO knockdown, leads to increased GATA2 and HAND2 protein levels.

(A) Western blot quantitation analysis of GATA2 and HAND2 protein levels in KELLY cells transfected sequentially with NONO KD siRNA and then siRNA-resistant control (YFP only), YFP fused NONO_WT or NONO_ΔRRM1 plasmids. Bars are SEM. n≥3. (B) Representative Western blot images for GATA2 and HAND2 proteins in (A).