Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/3/5/e1602505/DC1

Supplementary Materials for

The long noncoding RNA *SPRIGHTLY* acts as an intranuclear organizing hub for pre-mRNA molecules

Bongyong Lee, Anupama Sahoo, John Marchica, Erwin Holzhauser, Xiaoli Chen, Jian-Liang Li, Tatsuya Seki, Subramaniam Shyamala Govindarajan, Fatu Badiane Markey, Mona Batish, Sonali J. Lokhande, Shaojie Zhang, Animesh Ray, Ranjan J. Perera

Published 3 May 2017, *Sci. Adv.* **3**, e1602505 (2017)
DOI: 10.1126/sciadv.1602505

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/5/e1602505/DC1)

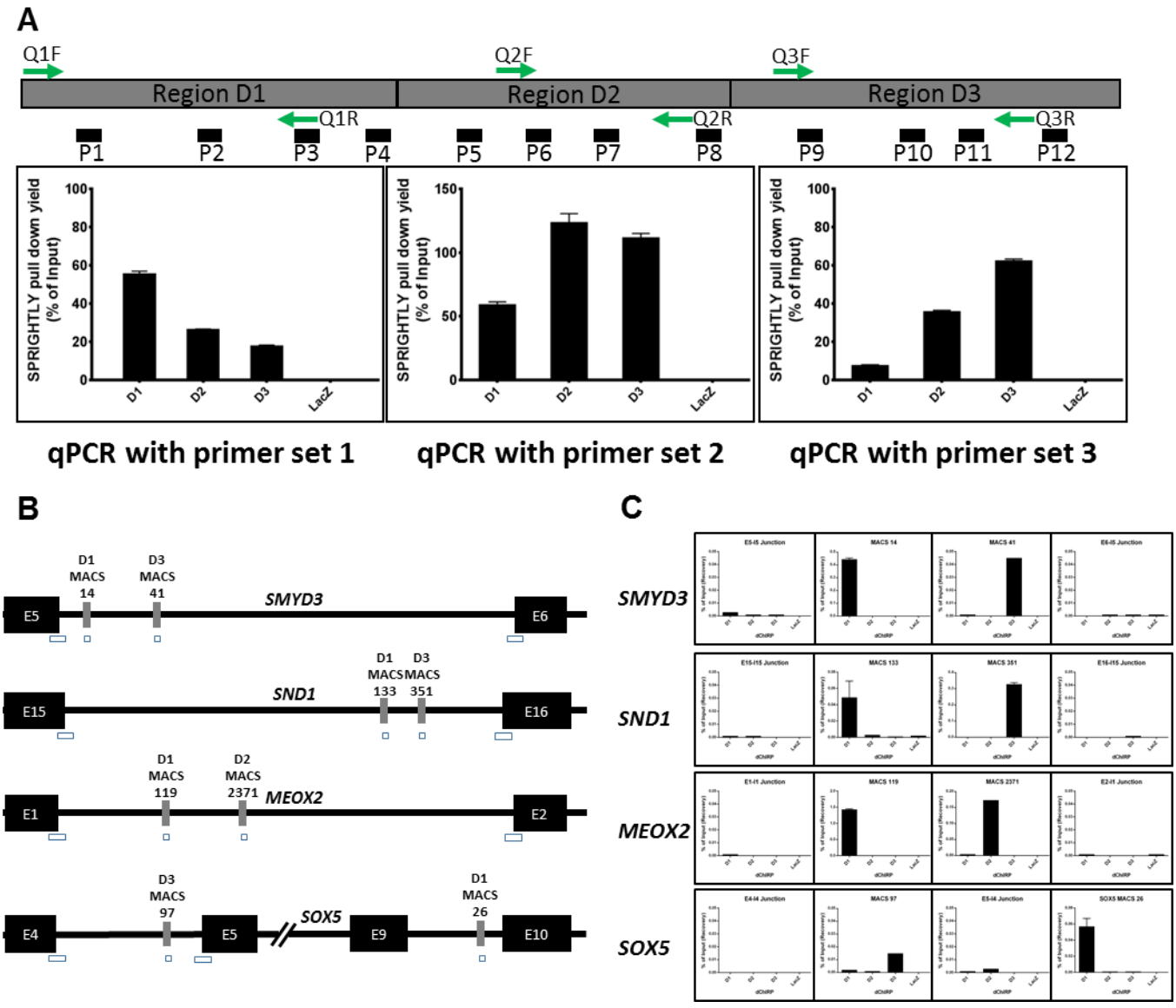
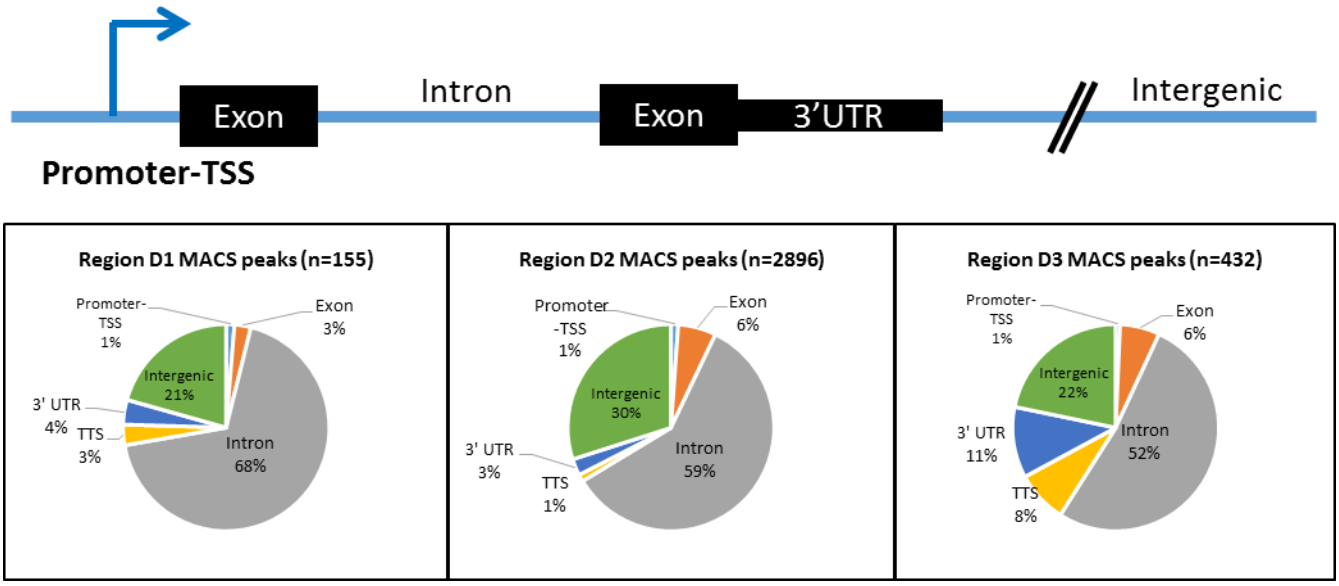


fig. S1 A-C. The pulldown efficiency of *SPRIGHTLY* and validation of copurification of RNA binding partners by qPCR. (**A**) Region-specific probes enrich corresponding *SPRIGHTLY* region. With region specific antisense oligo pools, *SPRIGHTLY* was pulldowned, and the recovery of *SPRIGHTLY* in each dChIRP was determined by qPCR using region specific qPCR primers. Within each sample, *SPRIGHTLY* domain recovery was quantified against input by qPCR (percent *SPRIGHTLY* RNA recovery). As expected, each antisense oligonucleotide pool best enriches for the target region. Black rectangles represent relative positions of antisense oligo binding sites and green arrows indicate the qPCR primer binding sites. Twelve probes are indicated as P1to P12. Primer set 1, 2, or 3 detects region 1, 2, or 3, respectively. (**B-C**) *SPRIGHTLY* dChIRP samples were analyzed by qPCR using primers for each MACS peak. (B) the relative position of MACS peak and its preferential binding region of *SPRIGHTLY*. e.g. D1

MACS 14: MACS 14 was found in region 1 dChIRP-Seq. (C) MACS peak recovery was quantified against input by qPCR (percent recovery). As expected, each MACS peak was recovered more from corresponding dChIRP samples. Black square represents exon and black line represents intron. Gray rectangle represent MACS peak and empty rectangle represents the qPCR amplicon positon.

D



E

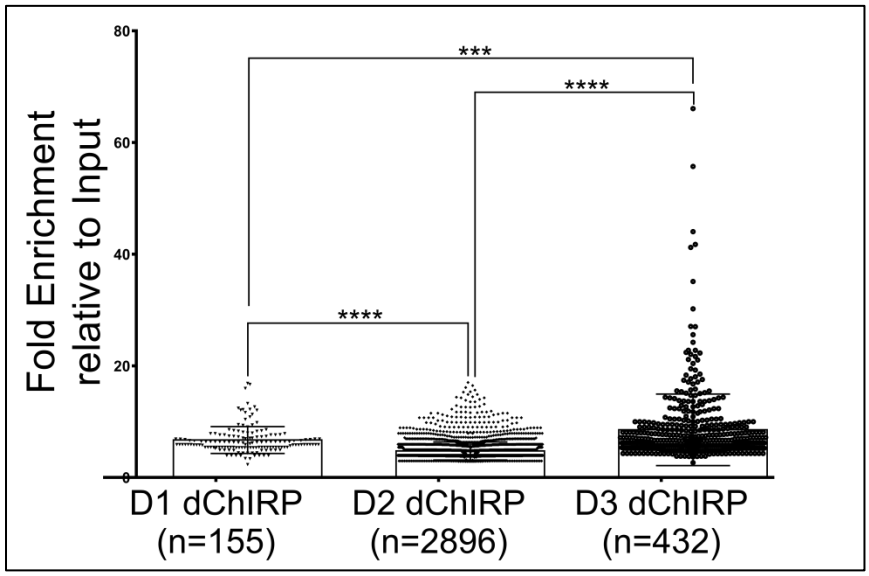


fig. S1 D-E. The pulldown efficiency of *SPRIGHTLY* and validation of copurification of RNA binding partners by qPCR. (**D**) The distribution of MACS peaks. The total MACS peaks from each dChIRP were categorized by exonic region including promoter-TSS, exon, 3' UTR, TTS, intronic region, and intergenic region. (**E**) Each MACS peak's fold enrichment compared to input was calculated and plotted. The overall fold enrichment of each dChIRP was significantly different. The average enrichment of MACS peaks from region three dChIRP was highest followed by region 2 and region 1. A middle line represents the median. *** $P \leq 0.001$, **** $P \leq 0.0001$, Student's *t*-test.

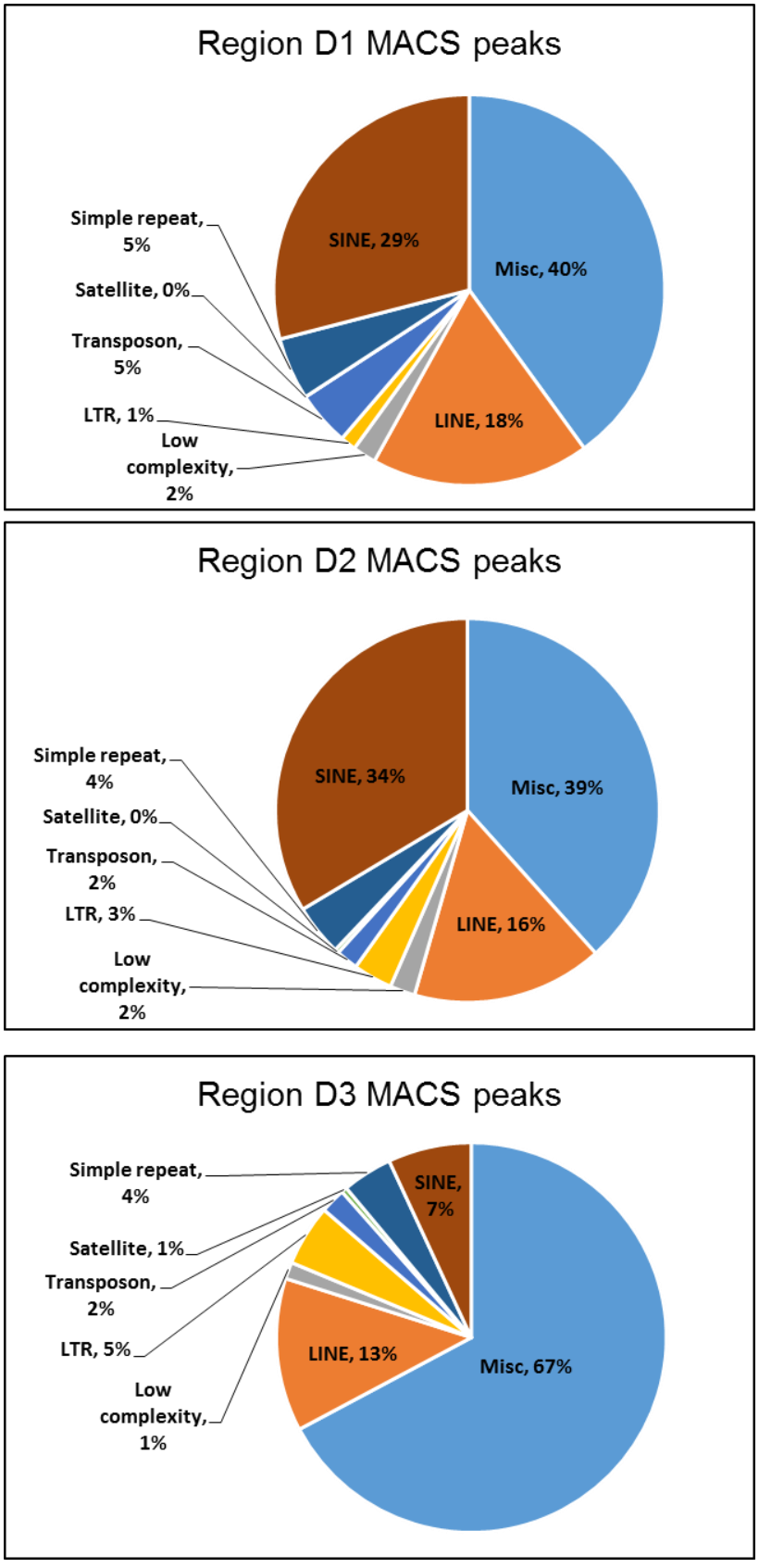


fig. S2. Many MACS peaks are composed of repetitive sequences. Many MACS peaks were found in repetitive regions. All MACS peaks from each dChIRP were categorized by their

sequence character, repetitive sequences such as SINE, LINE, simple repeat, satellite, transposon, and LTR or MISC including non-repetitive sequence found in 3'UTR, exon, intergenic, intron, non-coding, promoter-TSS, and TTS regions.

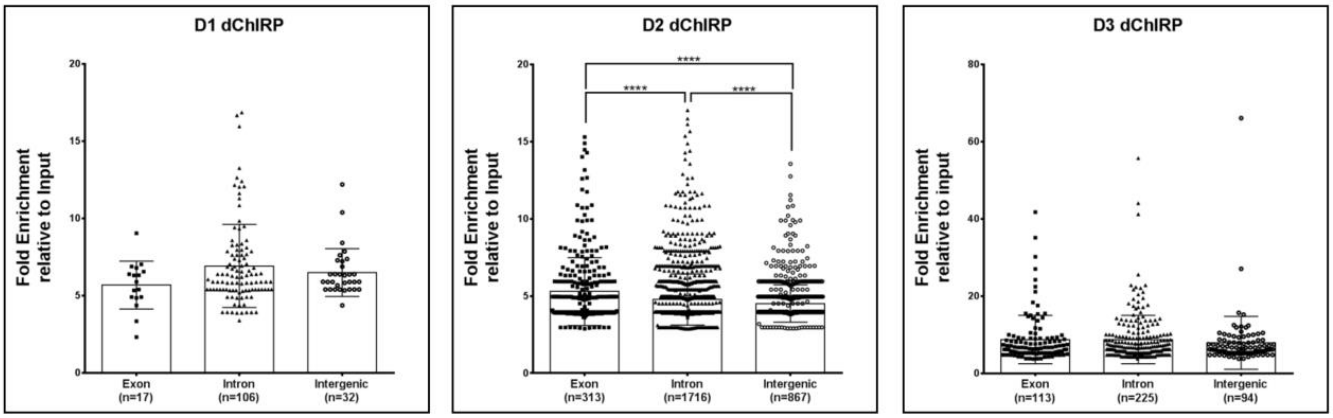


fig. S3. The fold enrichment of dChIRP MACS peaks. The fold enrichment of each MACS peak was plotted according to its category (Exon, intron, and intergenic regions). There were significant differences in fold enrichments of MACS peaks only from region 2 dChIRP (D2 dChIRP). A middle line represents median. **** $P \leq 0.0001$, Student's *t*-test.

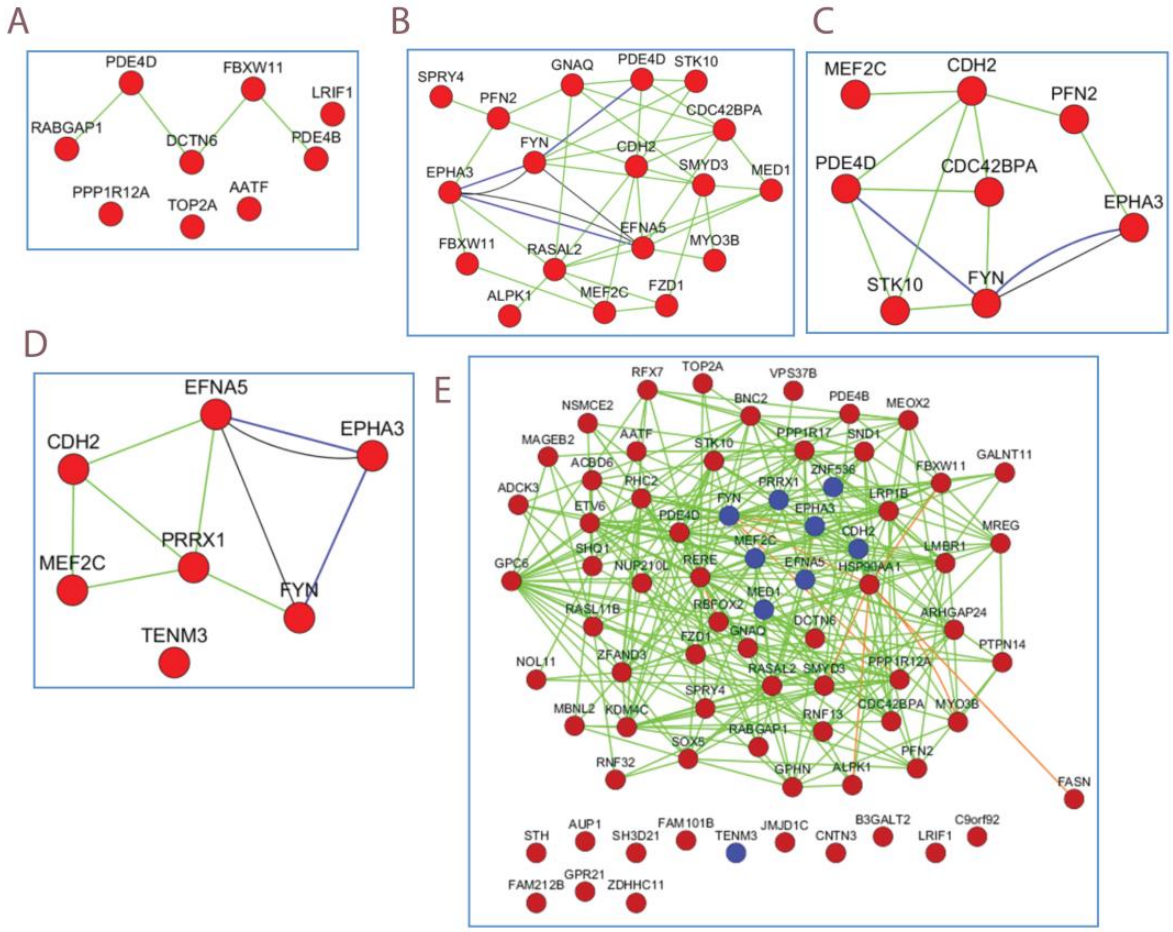


fig. S4. Gene interaction networks of *SPRIGHTLY*-interacting RNA molecules. The total of 115 *SPRIGHTLY* binding partners were subjected to GO enrichment analysis and the subsets of genes that exhibited GO enrichment were used to build gene interaction networks. Blue edges are physical interaction; green, genetic interaction; orange, miRNA-target interaction. Related networks shown in A-D are: (**A**) D2-D3 Gene subset network enriched for the GO term, microtubule-organizing center (P=1.42 x 10^{-13}; 9 genes); (**B**) D2-D3 gene subset network enriched for the GO term, protein phosphorylation (P=1.03 x 10^{-18}; 18 genes); (**C**) D2-D3 gene subset network enriched for the GO term, cell migration (P=1.29 x 10^{-5}; 8 genes); (**D**) D2-D3 gene subset network enriched for the GO term, regulation of neuron projection development (P=2.84 x 10^{-14}; 9 genes). (**E**) The interaction network of 74 *SPRIGHTLY* binding-partner RNAs.

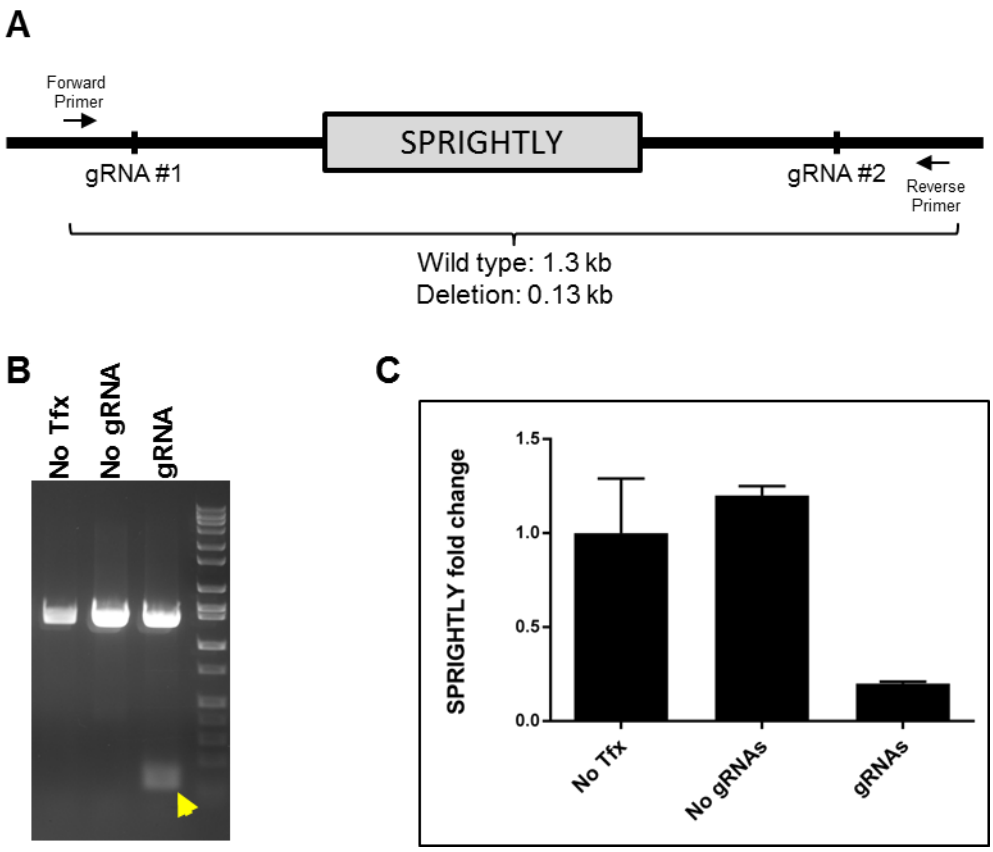


fig. S5. *SPRIGHTLY* knockout using a CRISPR system. (**A**) Schematic diagram showing the position of guide RNA binding sites and primer binding sites for genomic DNA PCR. Two guide RNAs named gRNA #1 and gRNA #2 bind upstream -319 region and downstream +176 region of *SPRIGHTLY*. (**B**) Genomic DNA PCR showing the deletion allele and wild-type allele. Yellow arrowhead indicates the deletion allele. (**C**) qPCR result indicating the reduction of *SPRIGHTLY* expression in *SPRIGHTLY* hemizygous knockout clone, SC2-17.

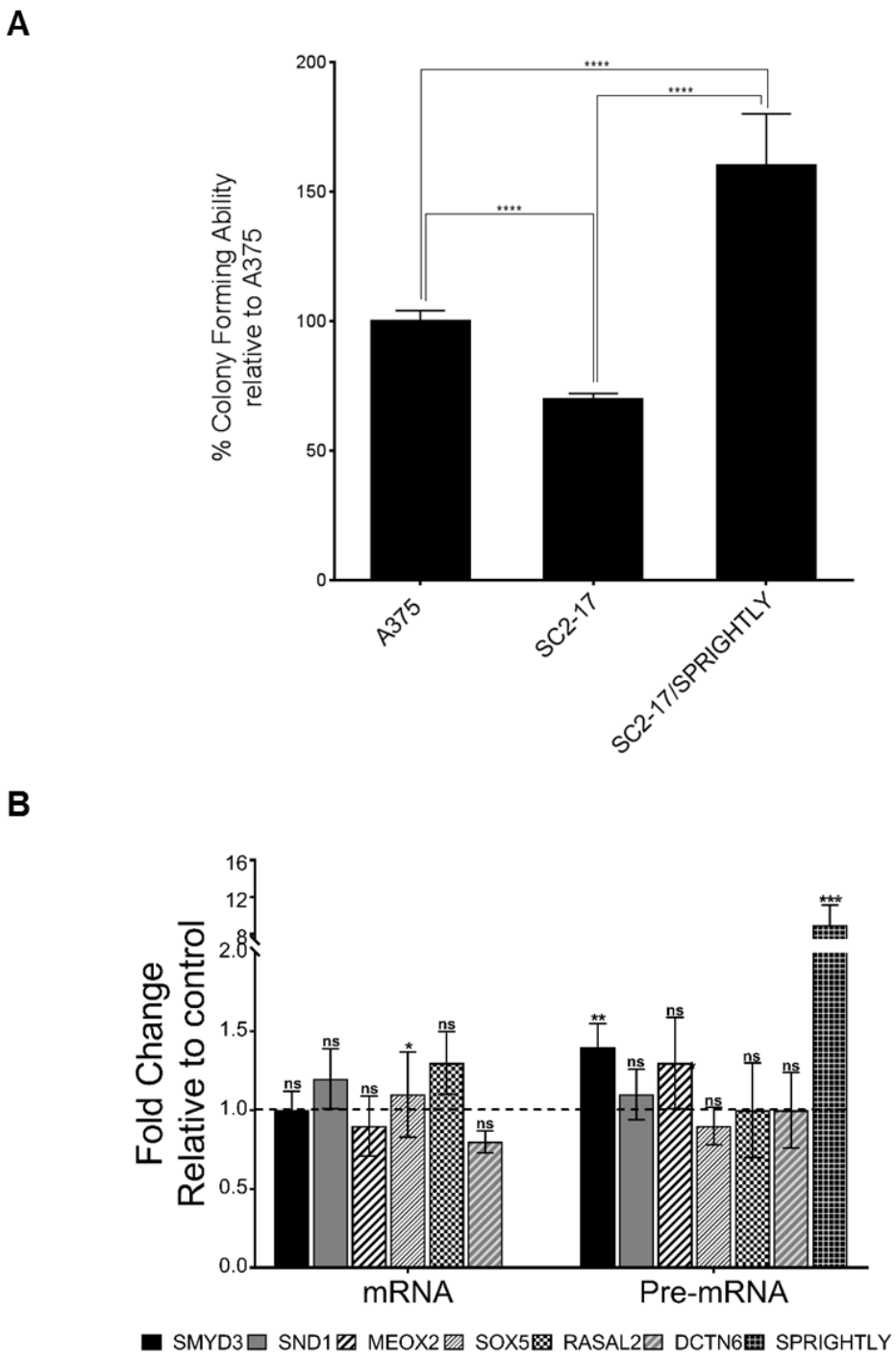


fig. S6. *SPRIGHTLY* overexpression recovered the loss of anchorage-independent growth of SC2-17 cells. (**A**) Stable SC2-17 cells ectopically expressing *SPRIGHTLY* were seeded at 10,000 cells per well and cultured for 7 days. MTT assay determined cell concentration. (**B**) The ectopic expression of *SPRIGHTLY* in SC2-17 cells recovered the expression levels of its RNA binding partners. Results in (A) are expressed as means ± SD from two independent experiments. ns, not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, Student's *t*-test. qPCR results are from a triplicate experiment.