Supplemental Data

Methods

Mice. Srsf2 conditional knockout mice (B6;129S4-*Srsf2*$^{tm1Xdfu/J}$; *SC35*^{fl}) with *loxP* sites flanking exons 1–2 (*Srsf2*^{fl}) and albumin-*Cre* (Alb-*Cre*) transgenic mice (B6.Cg-Tg(Alb-cre)21Mgn/J) were obtained from The Jackson Laboratory (Bar Harbor, Me). The *Srsf2*^{fl} allele was genotyped with forward primer 5′–GTTATTTGGCCAAGAATCACA–3′ and reverse primer 5′–AACCTTGTTCGTTGACCGAT–3′; this reaction yields a 321-bp product for the wild-type allele and a 410-bp product for the *Srsf2*^{fl} allele. *Cre*-mediated recombination excises exons 1–2 and inactivates *Srsf2* (42). The *Srsf2* knockout allele was detected by PCR with forward primer 5′–GTTATTTGGCCAAGAATCACA–3′ and reverse primer 5′–ACTCTCCACACCTAGTATTGTAAA–3′; this reaction yields a 518-bp product. The Alb-*Cre* transgenic allele (Alb-*Cre*$^{+}$) was genotyped with forward primer 5′–GCATTACCGGTCGATGCAACGAGTGATGAG–3′ and reverse primer 5′–GAGTGAACGAACCTGGTCGAAATCAGTGCG–3′, which yields a 408-bp product. The *Lmna*^{G609G} allele was created with a sequence-replacement vector as described (21), except that a C>T mutation in codon 609 was introduced into the 5′ arm of the *Lmna* targeting vector by site-directed mutagenesis (QuikChange kit, Stratagene). After electroporating the vector into 129/OlaHsd embryonic stem (ES) cells, targeted ES cell clones were identified by long-range PCR (TaKaRa LA Taq polymerase, Clontech). Targeted ES cells were injected into C57BL/6 blastocysts, and the resulting chimeras were bred with C57BL/6 females to generate heterozygous knock-in mice, which were then intercrossed to generate homozygotes. All mice were fed a chow diet and housed in a virus-free barrier facility with a 12-h light/dark cycle.

Hepatocyte isolation. Srsf2$^{fl/fl}$ mice were bred with Alb-*Cre*$^{+}$*Srsf2*$^{fl/+}$ mice to generate *Srsf2*$^{fl/fl}$ and *Srsf2*$^{fl/fl}$Alb-*Cre* mice. At age P23, the mice were anesthetized and the inferior vena cava cannulated with a 22-gauge catheter. The portal vein was cut, and the liver was perfused with warm PBS, followed by liver digest medium (17703-034, Invitrogen) containing 0.015%

collagenase IV (Gibco). The liver was removed, dispersed, and filtered through a 70-μm basket. Cells were suspended in William's E medium (Gibco) and fractionated on a Percoll gradient (P1644, Sigma-Aldrich). Hepatocytes were isolated, and extracts were prepared for protein and RNA analyses.

LMNA reporter construct. A *LMNA* fragment spanning exons 8–12 (including the 3′ UTR) was amplified from human genomic DNA with forward primer 5′–GAGATGATCCCTTGCTGACTTACC–3′ and reverse primer 5′–CCAAAGTGCTCTGATCTCTAATTGT–3′. The fragment was purified and subcloned into pGEM-T (A3600, Promega; Madison, WI), and the sequence was verified by sequencing. Three potential SRSF2 sites (site-1, site-2, and site-3) in exon 11 were mutated individually (or in combination) by site-directed mutagenesis with the QuickChange kit (Agilent Technologies; Santa Clara, CA). SRSF2 site 1, located between nucleotides 24–31 of exon 11, was mutated with forward primer 5′–GTACTCAGCGGGTTCGCCCGAGCTGCTG–3′ and reverse primer 5′–CAGCAGCTCGGGCGAACCCGCTGAGTAC–3′. SRSF2 site 2, located between bases 46–53 of exon 11, was mutated with forward primer 5′–GCTGAGTACAACCTGAGATCTCGCACCGTGCTGTGC–3′ and reverse primer 5′–GCACAGCACGGTGCGAGATCTCAGGTTGTACTCAGC–3′. SRSF2 site 3, located between bases 54–60, was mutated with forward primer 5′–CCGCACAGCACTGTGCGCGAGCG–3′ and reverse primer 5′–CGCTCGCGCACAGTGCTGTGCGG–3′. The SRSF6 site, located between bases 64–69 of exon 11, was mutated with forward primer 5′–GGGACCCCGCCGAGTTCAACCTGCGCT–3′ and reverse primer 5′–AGCGCAGGTTGAACTCGGCGGGTCCCC–3′. The cytosine in codon 608 (c.1824 or base 126 of exon 11) was changed to a thymidine with forward primer 5′–AGCCCAGGTGGGTGGACCCATCTCC–3′ and reverse primer 5′–GGAGATGGGTCCACCCACCTGGGCT–3′. All nucleotide changes were confirmed by DNA sequencing. A DNA fragment from exon 10 to exon 12 (including the 3′ UTR) was amplified

from each plasmid and subcloned into the β-globin reporter RHCglo (a gift from Dr. Thomas A. Cooper, Baylor College of Medicine; Houston, TX) with the In-Fusion HD kit (Clontech Laboratories; Mountain View, CA). Each fragment was amplified with exon 10 primer 5′–ACCTCCAAGCTCCGGAGAAGTGGCCATGCGCAAGCTG–3′ and exon 12 primer 5′–ACCGCGGTGGCGGCCGCGCCAGGGGTAGAAACAACTAG–3′ and subcloned with restriction enzymes *Bsp*EI and *Not*I. All constructs were verified by DNA sequencing.

Table 1. Sequence of ASOs and primers.

Sequence	Length	Mouse ASO	ISIS #
GCAGGTTGTACTCAGC	16	E11-31	641439
CAGGTTGTACTCAGCGGG	18	E11-28	641412
GCAGGTTGTACTCAGCGG	18	E11-29	641413
CGCAGGTTGTACTCAGCG	18	E11-30	641414
GCGCAGGTTGTACTCAGC	18	E11-31	641415
AGCGCAGGTTGTACTCAG	18	E11-32	641416
GAGCGCAGGTTGTACTCA	18	E11-33	641417
TGAGCGCAGGTTGTACTC	18	E11-34	641418
GTGAGCGCAGGTTGTACT	18	E11-35	641419
CGTGAGCGCAGGTTGTAC	18	E11-36	641420
GCGTGAGCGCAGGTTGTA	18	E11-37	641421
TGCGTGAGCGCAGGTTGT	18	E11-38	641422
GTGCGTGAGCGCAGGTTG	18	E11-39	641423
GGTGCGTGAGCGCAGGTT	18	E11-40	641424
CGGTGCGTGAGCGCAGGT	18	E11-41	641425
CAGCTTGCGCATGGCCACTT	20	E10-2	549468
CGCACCAGCTTGCGCATGGC	20	E10-7	549469
GTGAGCGCACCAGCTTGCGC	20	E10-12	549470
GGTCAGTGAGCGCACCAGCT	20	E10-17	549471
ACCATGGTCAGTGAGCGCAC	20	E10-22	549472
CCTCAACCATGGTCAGTGAG	20	E10-27	549473
ATTGTCCTCAACCATGGTCA	20	E10-32	549474
TCCTCATTGTCCTCAACCAT	20	E10-37	549475
CGTCATCCTCATTGTCCTCA	20	E10-42	549476
CTCGTCGTCATCCTCATTGT	20	E10-47	549477
CCATCCTCGTCGTCATCCTC	20	E10-52	549478
CTTCTCCATCCTCGTCGTCA	20	E10-57	549479
GAGCTCTTCTCCATCCTCGT	20	E10-62	549480
TGGAGGAGCTCTTCTCCATC	20	E10-67	549481
GGTGATGGAGGAGCTCTTCT	20	E10-72	549482
ACGGTGGTGATGGAGGAGCT	20	E10-77	549483
CTCACACGGTGGTGATGGAG	20	E10-82	549484
TGCCACTCACACGGTGGTGA	20	E10-87	549485
GCGGCTGCCACTCACACGGT	20	E10-92	549486
CAGCGGCGGCTGCCACTCAC	20	I10-1	549487
GGCCTCAGCGGCGGCTGCCA	20	I10-6	549488
GGCTGGGCCTCAGCGGCGGC	20	I10-11	549489
TTGTGGGCTGGGCCTCAGCG	20	I10-16	549490
CTAGGCTGGCAGGGCTACCC	20	I10-36	549494

CTGCCCTAGGCTGGCAGGGC	20	I10-41	549495
GAGAGCTGCCCTAGGCTGGC	20	I10-46	549496
GGTGGGAGAGCTGCCCTAGG	20	I10-51	549497
ATGGAGGTGGGAGAGCTGCC	20	I10-56	549498
TTGGCATGGAGGTGGGAGAG	20	I10-61	549499
AGACTTTGGCATGGAGGTGG	20	I10-66	549500
TGAAAAGACTTTGGCATGGA	20	I10-71	549501
TTTAATGAAAAGACTTTGGC	20	I10-76	549502
CATTCTTTAATGAAAAGACT	20	I10-81	549503
CAAAACATTCTTTAATGAAA	20	I10-86	549504
CATTCCAAAACATTCTTTAA	20	I10-91	549505
AGTGGCATTCCAAAACATTC	20	I10-96	549506
CAGCAAGTGGCATTCCAAAA	20	I10-101	549507
CAGGGCAGCAAGTGGCATTC	20	I10-106	549508
AAGGCCAGGGCAGCAAGTGG	20	I10-111	549509
GAAGAAAGGCCAGGGCAGCA	20	I10-116	549510
AGAGAGAAGAAAGGCCAGGG	20	I10-121	549511
GCTCTTGGAGCTTCCTGGCC	20	I10-126	549512
TGTGGGCTCTTGGAGCTTCC	20	I10-131	549513
GTTTGGGACTGACTTCTTAG	20	i10-651	549520
AGCGAGTTTGGGACTGACTT	20	I10-656	549521
GGGACAGCGAGTTTGGGACT	20	I10-661	549522
CAGGAGGGACAGCGAGTTTG	20	I10-666	549523
AGGCTCAGGAGGGACAGCGA	20	I10-671	549524
AGACAAGGCTCAGGAGGGAC	20	I10-676	549525
AAGGGAGACAAGGCTCAGGA	20	I10-681	549526
CTGGGAAGGGAGACAAGGCT	20	I10-686	549527
GAGCCGCTGCAGTGGGAACC	20	E11-1	549531
CCCCCGAGCCGCTGCAGTGG	20	E11-6	549532
GGGGTCCCCCGAGCCGCTGC	20	E11-11	549533
TCAGCGGGGTCCCCCGAGCC	20	E11-16	549534
TGTACTCAGCGGGGTCCCCC	20	E11-21	386363
CAGGTTGTACTCAGCGGGGT	20	E11-26	549535
GAGCGCAGGTTGTACTCAGC	20	E11-31	549536
TGCGTGAGCGCAGGTTGTAC	20	E11-36	549537
CACGGTGCGTGAGCGCAGGT	20	E11-41	549538
CACAGCACGGTGCGTGAGCG	20	E11-46	549539
TCCCGCACAGCACGGTGCGT	20	E11-51	549540
Sequence	**Length**	**Human ASO**	**ISIS #**
CGCAGGTTGTACTCAGCGGG	20	E11-28	386364

GAGCGCAGGTTGTACTCAGC	20	E11-31	549536
GCGAGCGCAGGTTGTACTCA	20	E11-33	573298
TGCGCGAGCGCAGGTTGTAC	20	E11-36	573299
GGTGCGCGAGCGCAGGTTGT	20	E11-38	573300
CACGGTGCGCGAGCGCAGGT	20	E11-41	573301

Sequence	**Length**	**Name**	**ISIS #**
TGGTGCACGGTCTACGAGAC	20	Control ASO	376024
ACTCCAGGCCTATGAGGGTG	20	Control ASO	463309
GTCACTTGCCAGGGTCAGGA	20	Control ASO	556311
GCTCATTTAGTCTGCCTGAT	20	Control ASO	389629

qPCR primers

Human transcript

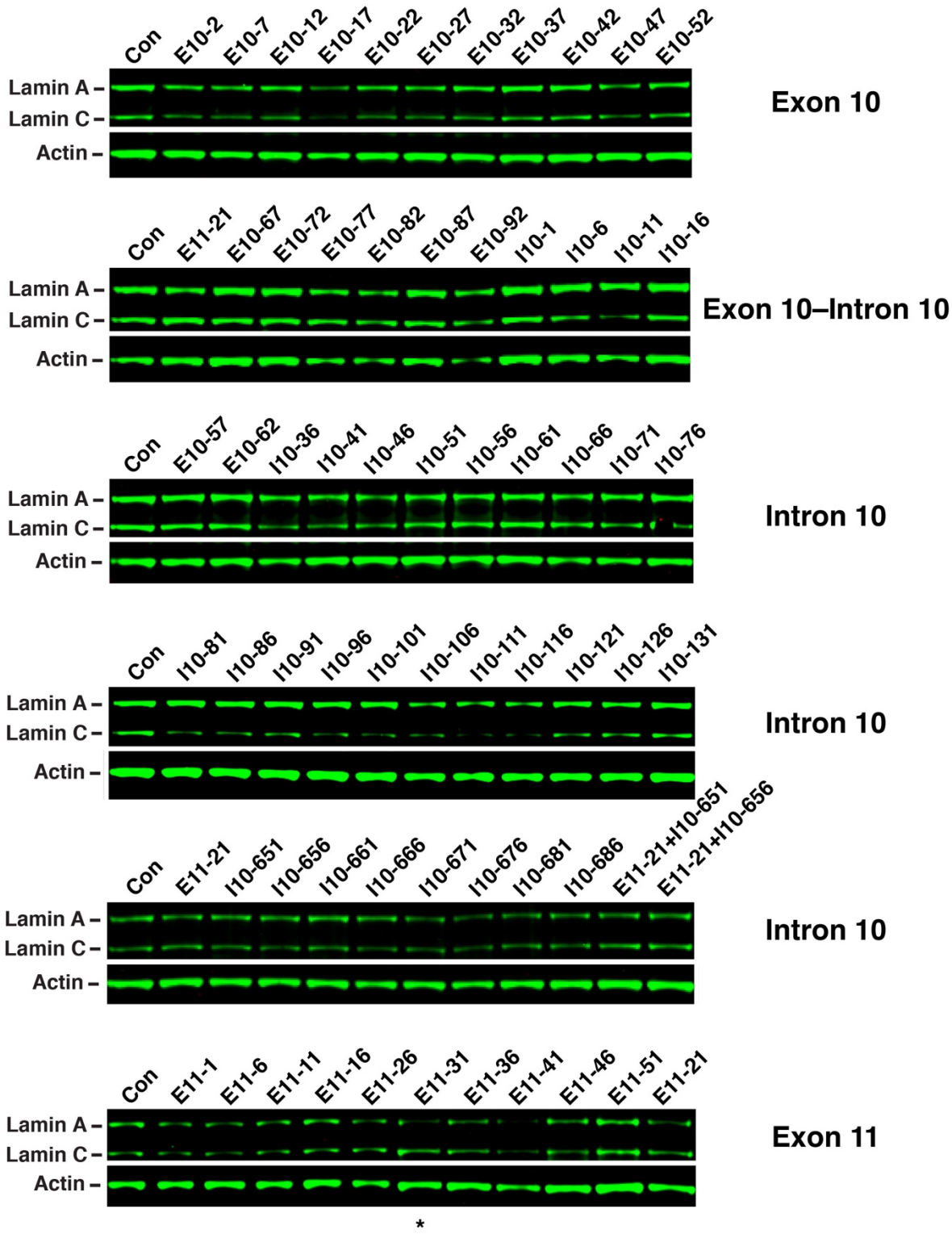
Prelamin A	atgaggatggagatgacctgc	aggcagaagagccagaggaga
Lamin C	tggtgtggaaggcacagaaca	agcggcggctaccactca
LMNA	agcaaagtgcgtgaggagtt	tcaggtcaccctccttcttg
Progerin	gctcaggagcccagagc	gacgcaggaagcctccac
SRSF1	tgcctacatccgggttaaag	ctgctgttgcttctgctacg
SRSF2	ccttacctttcttcaccttggtt	caaaggctaccatcagcatgta
SRSF5	agacctcgaaatgatagacgaaa	tgtctcatgaaatctttgagatcc
SRSF6	aaatacggaccacctgttcg	cttcacctgcttgtcgcata
LMNB1	gctgctcctcaactatgctaaga	gaattcagtgctgcttcatattctc

Mouse transcript

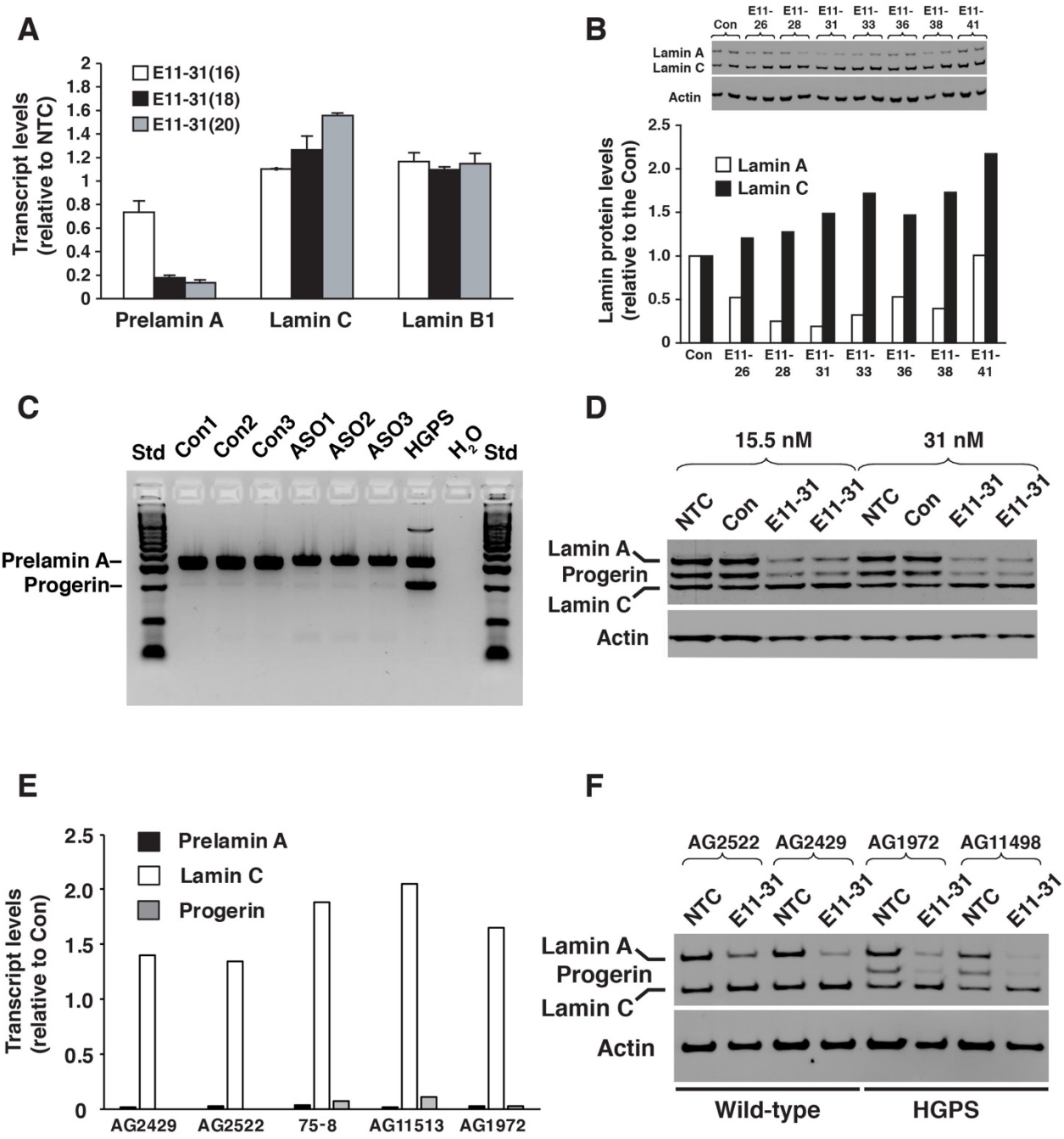
Prelamin A	ggttgaggacaatgaggatga	tgagcgcaggttgtactcag
Lamin C	gacaatgaggatgacgacgag	ttaatgaaaagactttggcatgg
Lmna	cctatcgaaagctgctggag	cctgagactgggatgagtgg
Lmnb1	caactgacctcatctggaagaac	tgaagactgtgcttctctgagc
Ppia	tgagcactggagagaaagga	ccattatggcgtgtaaagtca
Sfsr2	gagcccacccaagtctcc	cgcttgccgattcatcat
CD31	aaccgtatctccaaagccagt	ccagacgactggaggagaact

RNA sequences

Wild-type	GGGACCCCGCUGAGUACAACCUGCGCUCGCGCACCGUGCUG
ΔSRSF-1	GCGAACCCGCUGAGUACAACCUGCGCUCGCGCACCGUGCUG
ΔSRSF-2	GGGACCCCGCUGAGUACAACCUGAGAUCUCGCACCGUGCUG
ΔSRSF-3	GGGACCCCGCUGAGUACAACCUGCGCUCGCGCACAGUGCUG
ΔSRSF-2/3	GGGACCCCGCUGAGUACAACCUGAGAUCUCGCACAGUGCUG
Scrambled	CAUCAACCUGUAUGGGAACUUUCUAUAUGGUUCUUCGACGG

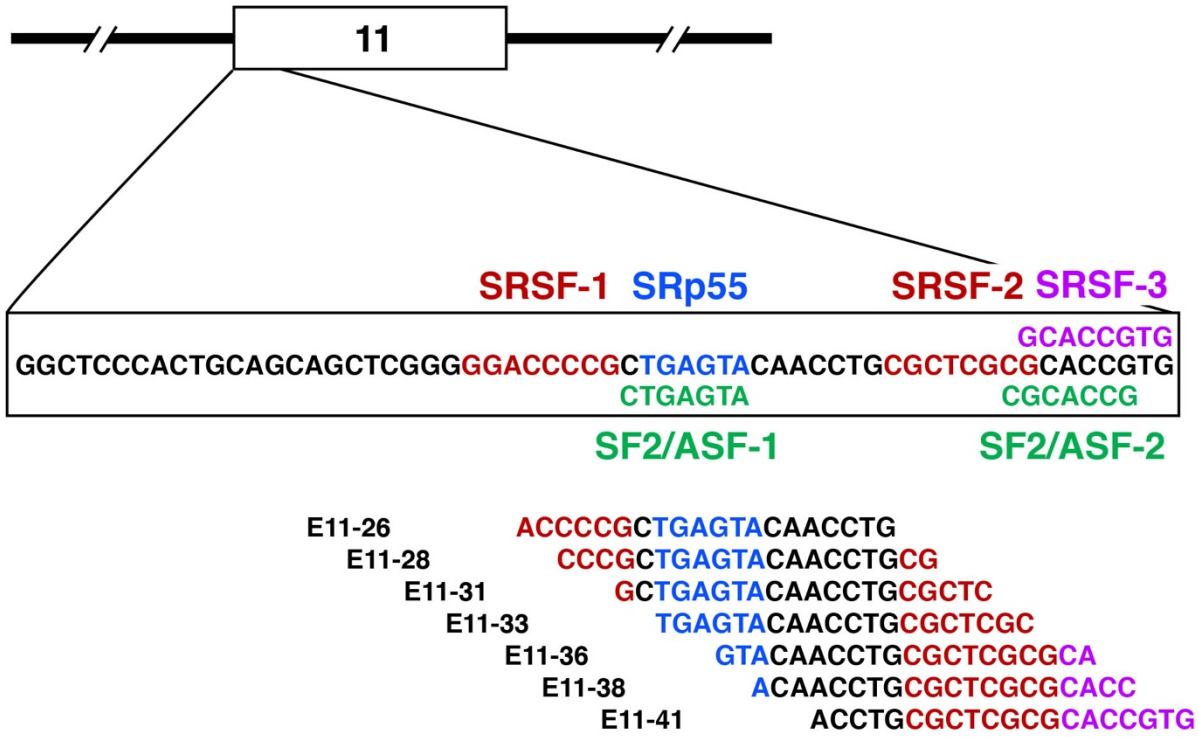


Supplemental Figure 1. Screening of *Lmna* antisense oligonucleotides that affect lamin C/prelamin A mRNA splicing. Wild-type mouse embryonic fibroblasts were transfected with ASOs corresponding to sequences in exon 10, intron 10, and exon 11 of *Lmna*. After 2 days, extracts were prepared and analyzed by western blotting with antibodies against lamins A/C and actin (as a loading control). Cells treated with ASO E11-31 are marked with an asterisk.

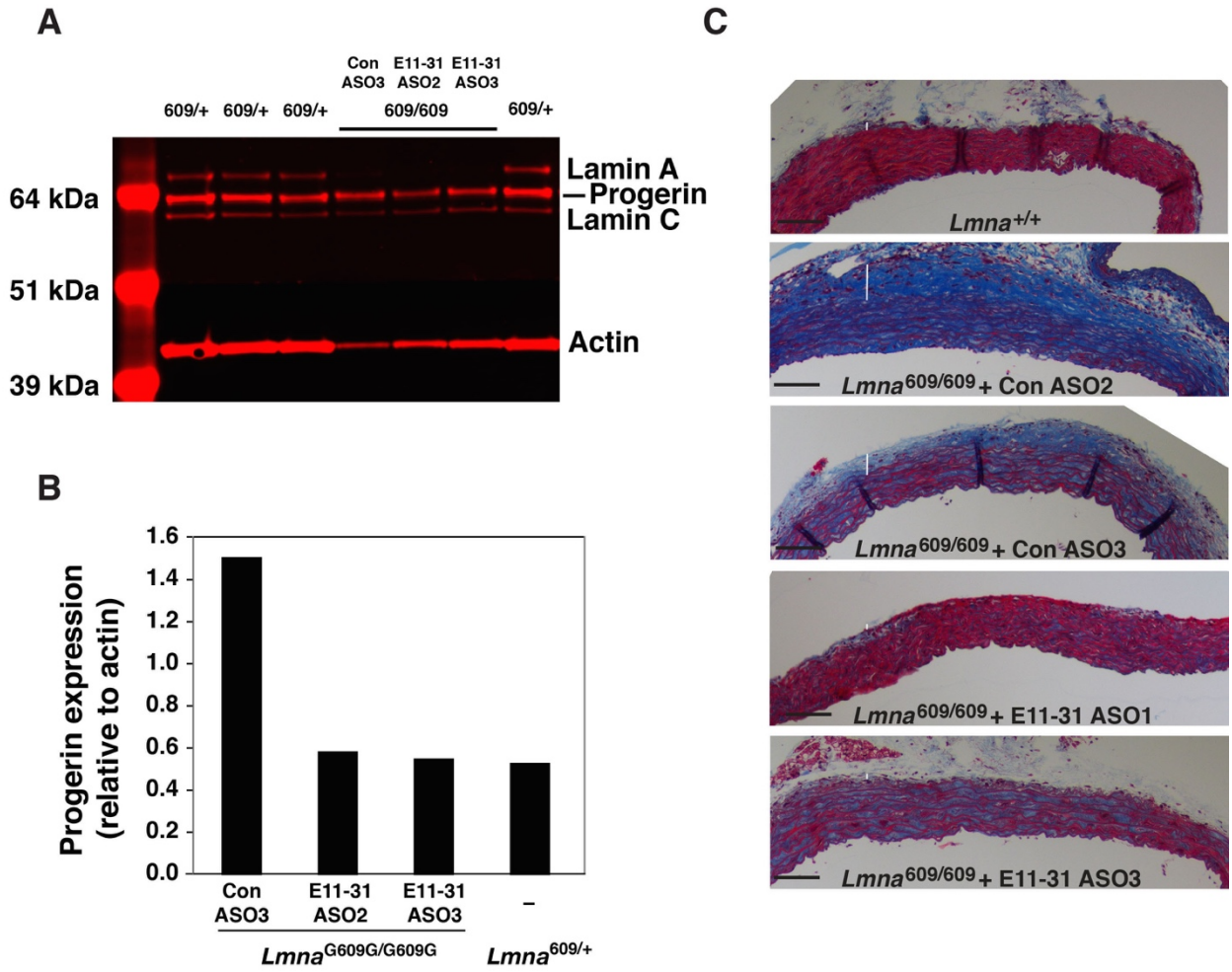


Supplemental Figure 2. Modulation of *LMNA* alternative splicing with exon 11 ASOs. (A) Longer ASOs are more effective in promoting lamin C splicing. Wild-type cells were transfected with E11-31 ASOs of different lengths (16 nt, 18 nt, 20 nt). After 2 days, transcript levels were measured by qRT-PCR. (B) Multiple ASOs near E11-31 promote lamin C splicing. Human fibroblasts (AG2429) in duplicate were transfected with ASOs. After 3 days, lamin A and lamin C protein levels were measured by western blotting. The bar graph shows lamin protein expression relative to cells treated with a scrambled ASO (set at a value of 1.0). (C) RT-PCR showing that ASO E11-31 does not increase usage of the HGPS donor splice site. Triplicate wells of human fibroblasts (AG2522) were transfected with ASO E11-31 or a scrambled control ASO. After 2 days, prelamin A transcripts were amplified by RT-PCR. RNA from

nontransfected HGPS cells (AG11513) was included as a control (HGPS). Only trace amounts of progerin transcripts were detected in the ASO-treated cells. (D) Western blot analysis showing that the effects of ASO E11-31 on lamin A and progerin levels in HGPS cells are dose dependent. (E) ASO E11-31 reduces prelamin A and progerin transcript levels in multiple HGPS cell lines. Wild-type cells (AG2429 and AG2522) and HGPS cells (hTERT immortalized 75-8, AG11513, and AG1972) were transfected twice with ASO E11-31 or transfection reagent alone (NTC). One day after the last transfection, transcript levels were measured by qRT-PCR and expressed relative to the NTC (set at a value of 1.0). (F) Western blot analysis showing that ASO E11-31 reduces lamin A and progerin protein levels in multiple HGPS cell lines.



Supplemental Figure 3. Location of potential exonic splice enhancer (ESE) binding sites within exon 11 of *LMNA*. Exon 11 *LMNA* sequences were analyzed with the program ESE Finder. Six potential ESE binding sites were identified: 3 SRSF2 sites (red and purple), one SRp55 site (blue), and 2 SF2/ASF sites (green). The sequences of several ASOs used in this study are shown at the bottom of the schematic.



Supplemental Figure 4. ASO E11-31 treatment lowers progerin levels in the aortas of *Lmna*$^{G609G/G609G}$ mice and improves the arterial disease phenotype. (A) Western blot showing ASO E11-31 lowers progerin levels in the aorta of *Lmna*$^{G609G/G609G}$ mice. Lamin A, progerin, and lamin C levels in four untreated *Lmna*$^{G609G/+}$ mice (609/+) and three *Lmna*$^{G609G/G609G}$ mice (609/609) treated with ASO E11-31 (ASO) or a scrambled ASO (Con) are shown. Actin levels were measured as a loading control. The results are shown for two *Lmna*$^{G609G/G609G}$ mice treated with ASO E11-31 (E11-31 ASO2 & E11-31 ASO3), and a *Lmna*$^{G609G/G609G}$ mouse treated with the control ASO (Con ASO3; not reported in Figures 6C–6D). (B) ASO E11-31 lowers progerin levels in the aorta. Progerin levels shown in panel A were measured by laser scanning and normalized to actin levels. Levels reported for the *Lmna*$^{G609G/+}$ mice are the average of four animals. (C) Histological images showing less disease in the aortas from *Lmna*$^{G609G/G609G}$ mice treated with ASO E11-31. Images (10× magnification) of Masson's trichrome-stained cross sections through the ascending aorta are shown for one wild-type, two *Lmna*$^{G609G/G609G}$ mice treated with a control ASO (Con ASO2 and Con ASO3), and two *Lmna*$^{G609G/G609G}$ mice treated with ASO E11-31 (E11-31 ASO1 and E11-31 ASO3). White colored bars identify the adventitia. Scale bars, 100 μm.