# SUPPLEMENTARY FIGURE LEGENDS

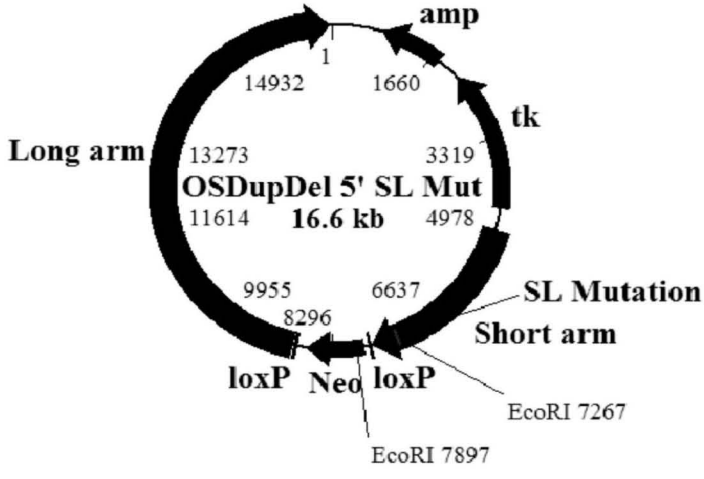
Supplementary Fig. 1. Schematic of targeting vector and homologous recombination. *A*, Diagram of targeting plasmid. Restriction fragments of 2.8 kb ("*Short arm*") and 7.7 kb ("*Long arm*") of mouse α1(I) collagen DNA were cloned into the OSDupDel vector. The short arm contains the mutated 5′ stem-loop sequence ("*SL Mutation*"). The arms were cloned on either side of a neomycin resistance gene ("*Neo*"), which is flanked by loxP sites. The plasmid also contains ampicillin ("*amp*") and thymidine kinase ("*tk*") selection cassettes; *Eco* RI restriction sites are located within the SL mutation sequence and the neo cassette. *B*, Representation of targeted recombination. *Top*, mouse α1(I) collagen genomic DNA; only the first 3 exon regions are indicated. *Middle*, targeting vector sequence, including the SL mutation in exon 1 and thymidine kinase and neomycin resistance cassettes. *Bottom*, the product of a homologous recombination event. Hatched lines represent homologous sequence between genomic and construct DNA. *E*, *Eco* RI restriction endonuclease sites.

Supplementary Fig. 2. Introduction of the SL mutant construct did not affect splicing of α1(I) collagen mRNA. *A*, Schematic of PCR strategy. Following reverse transcription of the RNA, the resulting cDNA was PCR-amplified using a sense primer which bound the first exon of the mouse α1(I) collagen message; the antisense primer bound a region of the second exon. Inefficient or non-splicing of the message would result in a 3020-bp product, while proper splicing would generate a 290-bp product. *B*, Results of RT-PCR. 293 cells were transfected with SL mutant plasmid (*Tr)* or empty vector (*Un)*. RNA was harvested 48 hours post-transfection and reverse-transcribed followed by PCR using the described α1(I) collagen primers (*C*). 18S primers were used as positive control for reverse transcription. -, water template; *3T3*, RNA from NIH3T3 cells used as positive control for spliced collagen expression; *SL*, OSDupDel SL mutant DNA used as positive control for unspliced collagen expression; *M*, 100-bp (left) and 1-kb (right) DNA ladders; sizes are indicated to left of markers. In the case of multiple bands in the same lane, products are delineated on the gel as either collagen (*C*) or 18S.

Supplementary Fig. 3. Southern blot analysis for SL mutant. Schematic of Southern blot approach (Figure 1). Proper homologous recombination results in the introduction of two new *Eco* RI restriction sites. Digestion of mutant genomic DNA with *Eco* RI followed by hybridization with a probe for homologous recombination ("*Recomb*") generates a 4.1-kb fragment (Figure 1); hybridization with a probe for the neomycin cassette ("*Neo*") results in a 10.3-kb band (Figure1). Hybridization of digested wild-type DNA with *Recomb* produces a 13.9-kb fragment; no band is present with *Neo*. Thicker lines represent endogenous DNA; thinner lines represent recombined mutant construct DNA. *C*, Representative Southern blot. DNA from isolated embryonic stem cell clones was digested with *Eco* RI; the resulting fragments were separated by gel electrophoresis followed by transfer to nylon membrane. The membrane was hybridized with the *Recomb* probe (*top*), stripped, and re-probed with *Neo* (*bottom*). Lanes 7 and 8 represent two positive heterozygous clones.

Supplementary Fig. 4. Mutation of the 5′ SL did not affect α2(I) collagen mRNA levels in MEFs. A, Representative RPA using α2(I) collagen probe. RNA from wild-type (+/+) and mutant (-/-) MEFs was incubated with α2(I) collagen riboprobe; each lane represents a separate cell isolation. *L*, DNA ladder (fragment sizes indicated to left of gel); *C,* undigested mouse α2(I) collagen riboprobe; *G*, undigested mouse GAPDH riboprobe; *t*, yeast tRNA; *3T3*, NIH3T3 RNA. Arrows: *G*, protected GAPDH; *C*, protected mouse α2(I) collagen riboprobe. B, Result of 4 independent RPAs. α2(I) collagen-specific bands were quantitated and normalized to GAPDH values. Data is expressed as relative amount of message vs. wild-type MEFs (N=12). For all graphs, error bars represent S.E.M.

A

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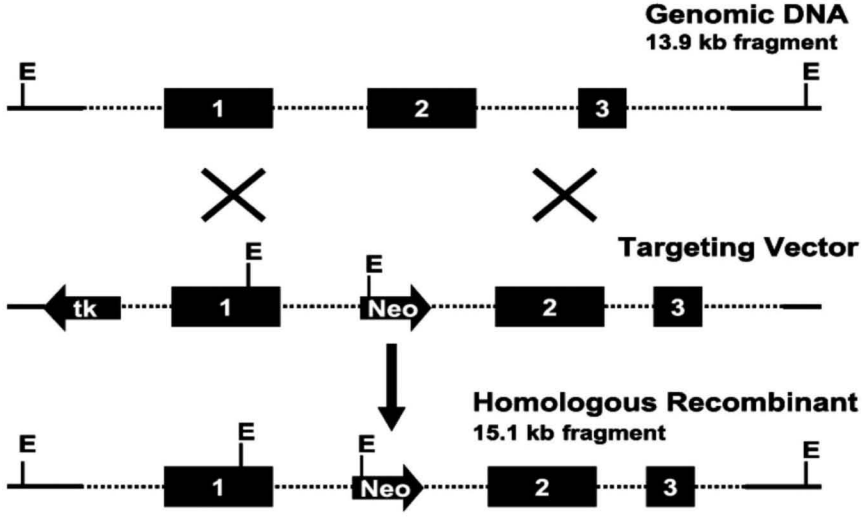
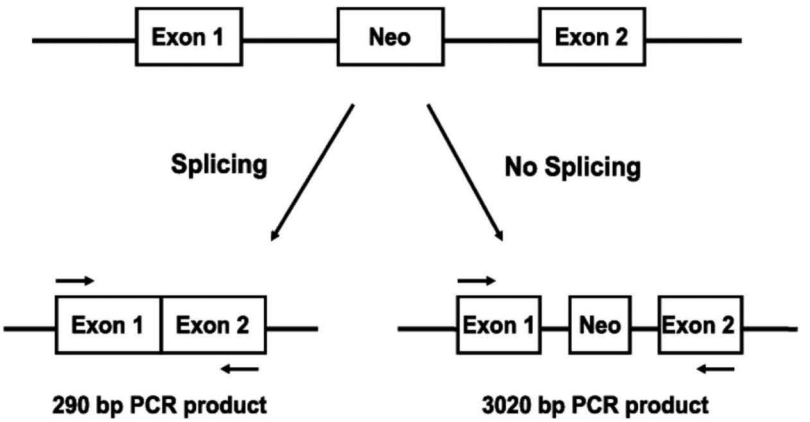



**Supplementary Figure 1 Parsons et al.**

A

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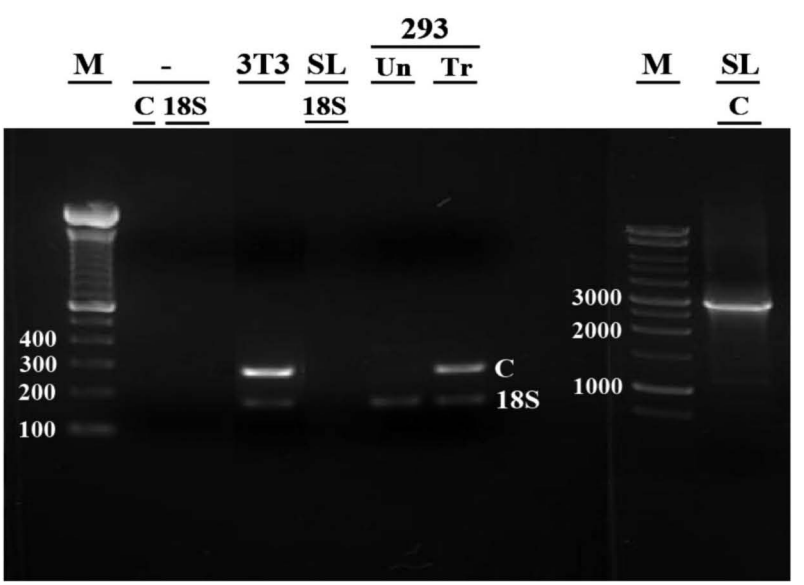
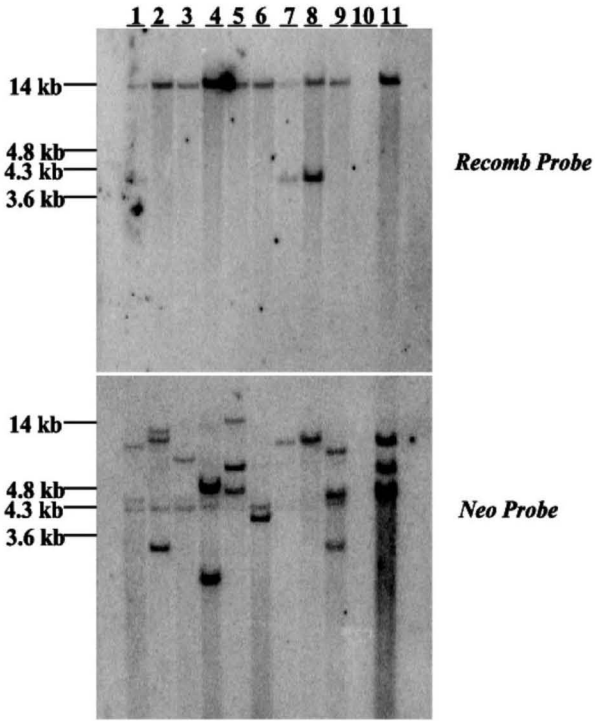
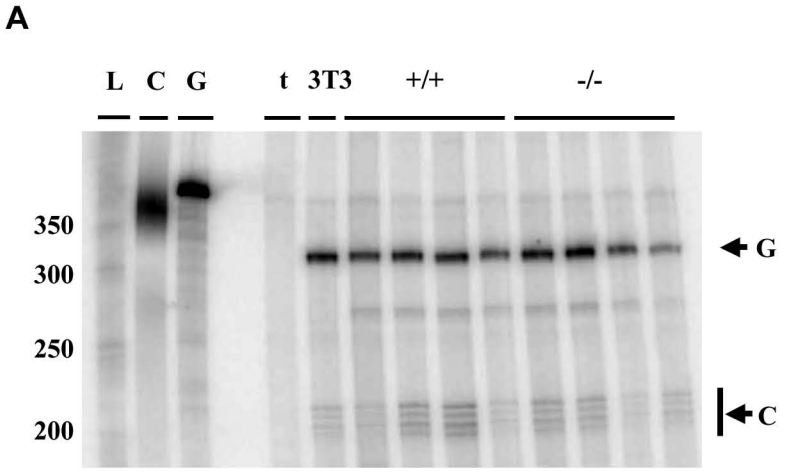
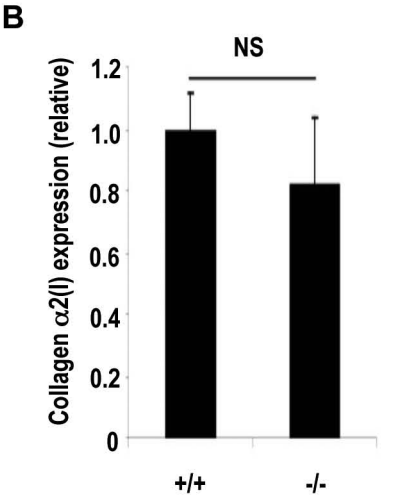



**Supplementary Figure 2 Parsons et al.**




**Supplementary Figure 3 Parsons et al.**




**Supplementary Figure 4 Parsons et al.**