Hormonal Profile

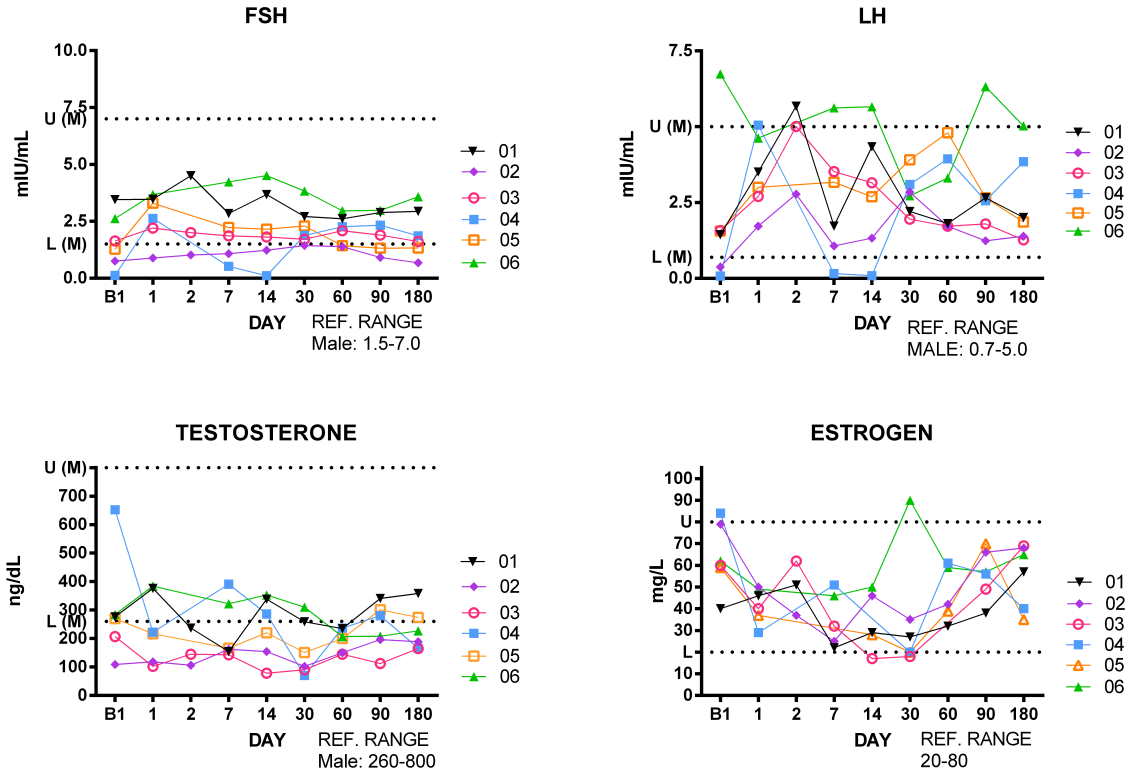


Figure S1. Hormonal profile for follistatin-treated patients. Follicle stimulating hormone (FSH), Luteinizing Hormone (LH), Testosterone, and Estrogen levels are shown for each of the patients in the trial over 180 days.

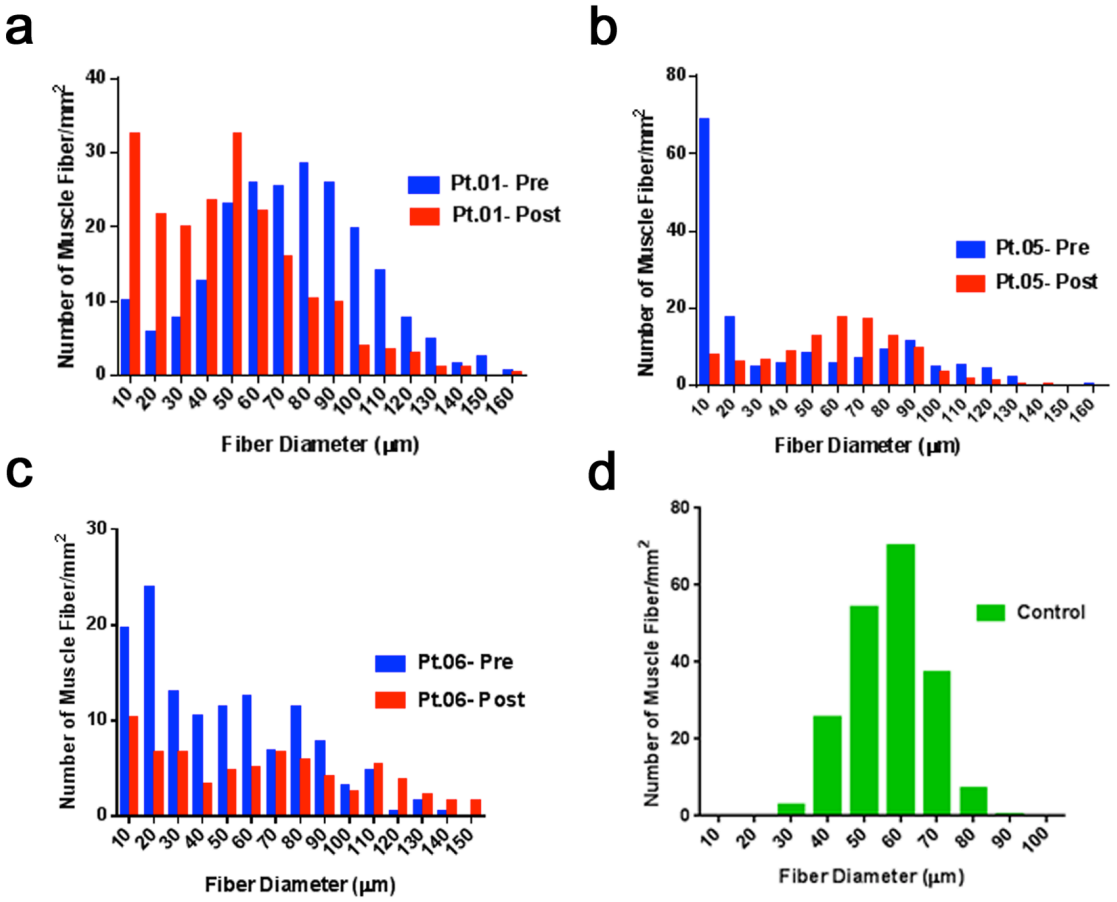


Figure S2. **Muscle fiber size distribution histograms from pre and post treatment biopsies.** (a) Post treatment histogram from Pt.01 shows an increase in the number of fibers with diameters between 10 and 50 µm per mm^2 area. (b) The pre treatment biopsy from Pt.05 shows a marked increase of severely atrophic round fibers (also see Fig. 6a) and abnormally hypertrophic fibers. (c) A more normalized Poisson-like fiber size distribution with a shift to larger fibers with 40 to 80 µm range is seen with treatment. Decease in abnormally small fibers with treatment is seen in Pt.06 (d) For comparison, normal fiber size distribution from a normal control biopsy.

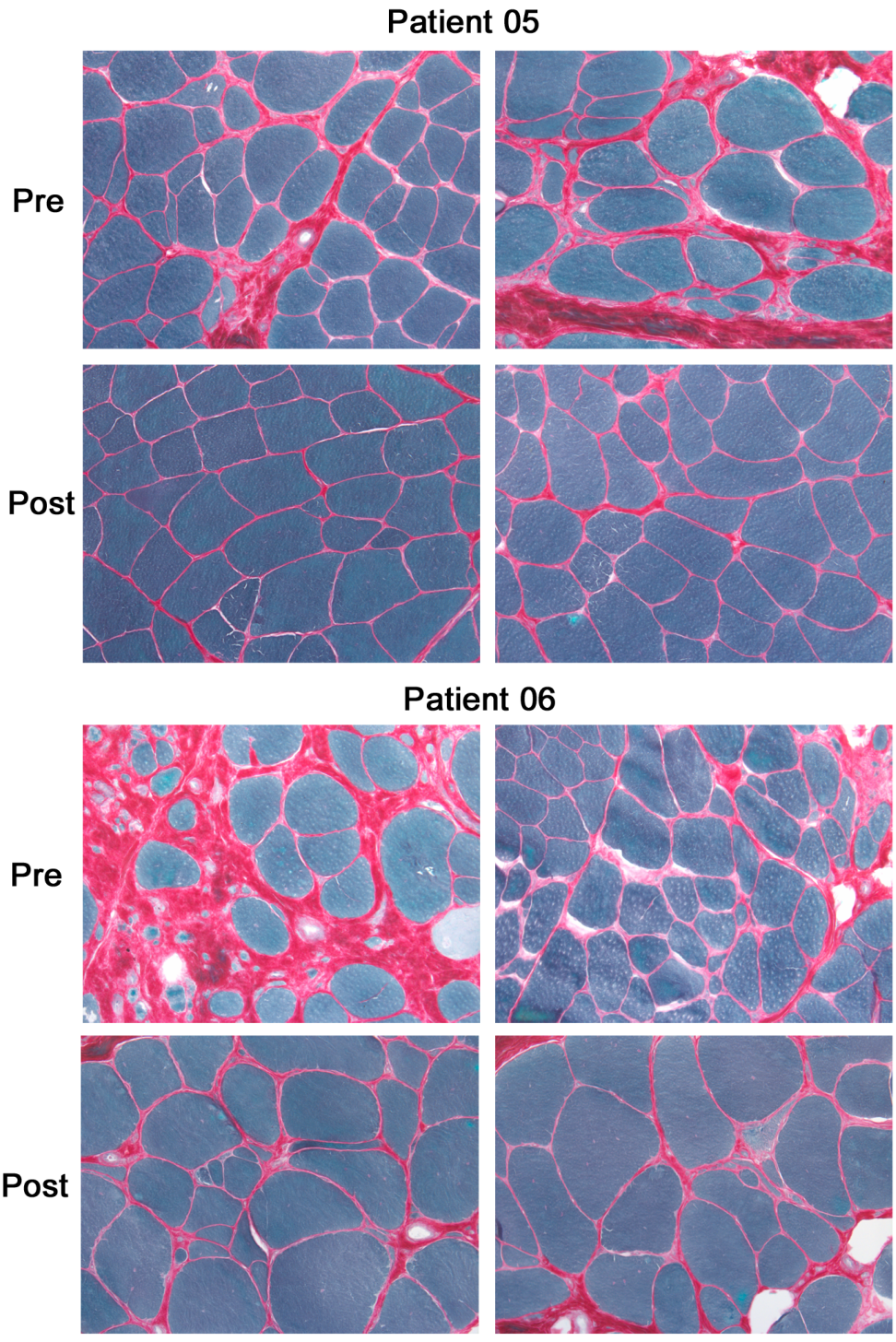


Figure S3. Picrosirius red collagen staining of muscle pre- and post-follistatin treatment. In post-treatment muscle biopsies, collagen deposition was reduced by FS344 gene therapy (quantified in Fig 7).

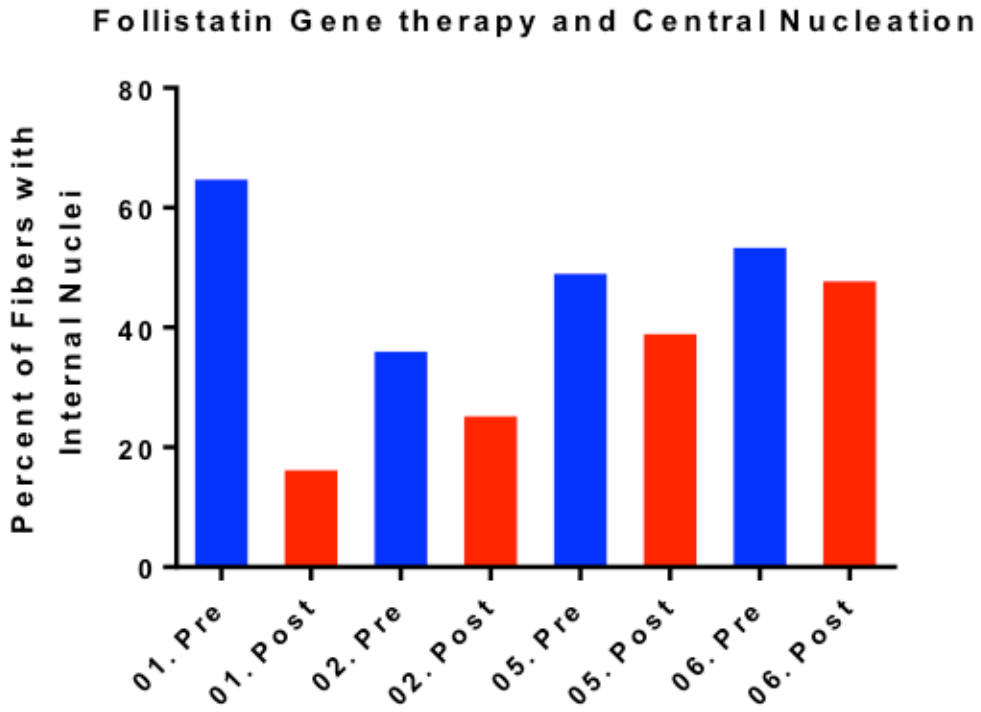


Figure S4. Follistatin gene therapy and central nucleation. Pre- and post-treatment comparisons show percent of fibers with central nuclei was reduced following FS344 gene delivery.

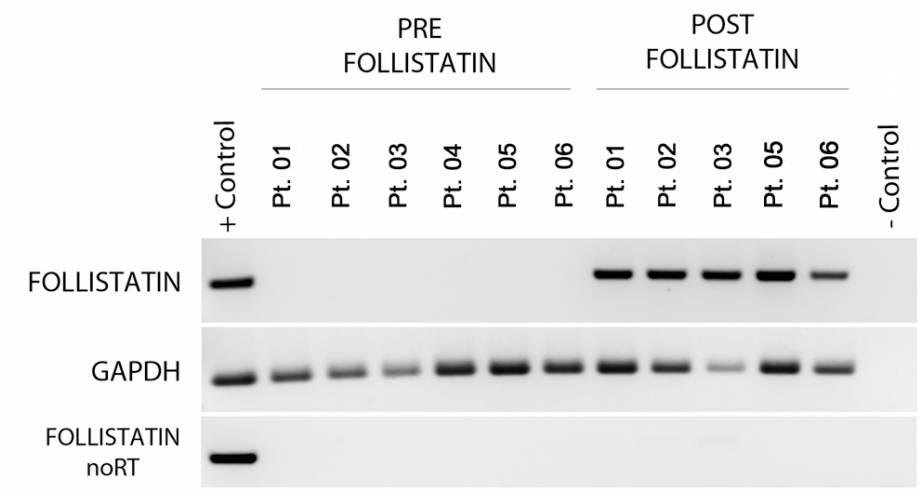


Figure S5. Pre-and post-treatment RT-PCR on muscle biopsies. There is well-defined follistatin expression specific for FS344 isoform in post treatment muscle biopsies that is not present pre-treatment. Patient 04 had only a pre-treatment muscle biopsy.

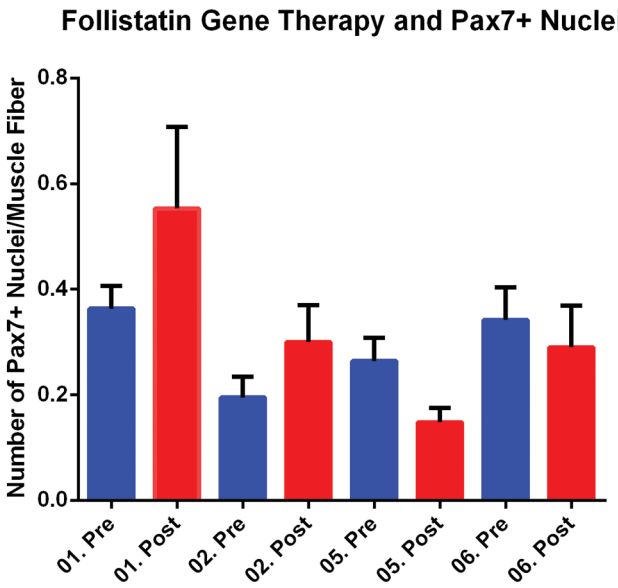


Figure S6. Pax7 positive nuclei per muscle fiber in pre- and post-treatment biopsies for Patients 01, 02, 05 and 06. The findings refute concerns that follistatin expression reduces the number of Pax7 + satellite cell nuclei.

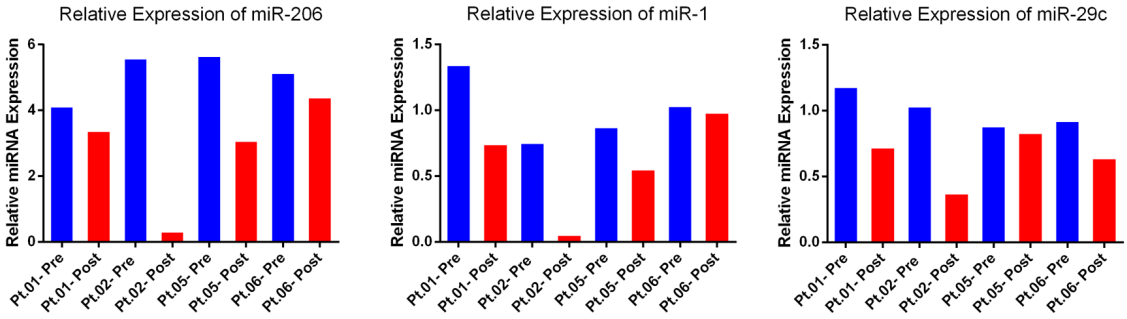
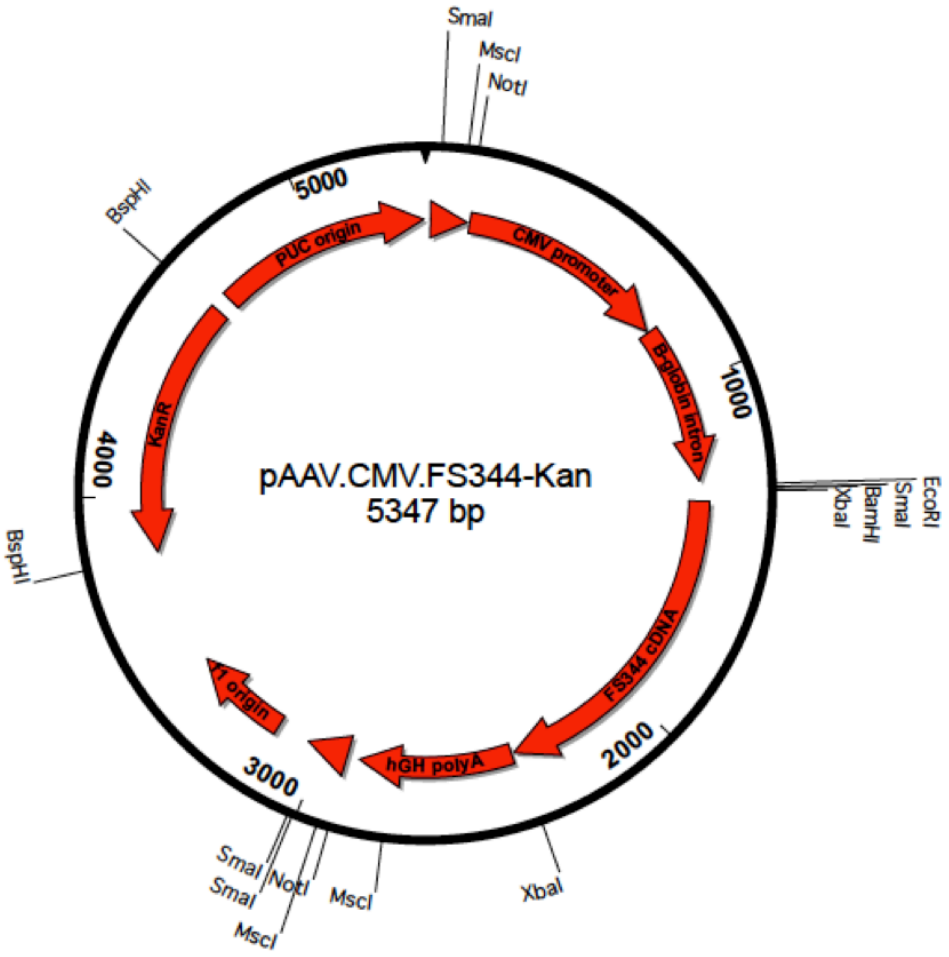


Figure S7. miR-206, miR-1, and miR29c levels in pre- and post-treatment muscle biopsies for Patients 01, 02, 05 and 06. The down-regulation of miRs was observed following gene transfer serving as additional biomarker confirming follistatin expression in skeletal muscle.



Type	Start	End	Description
Region	1	138	Inverted terminal repeat
REGION	139	804	CMV enhancer/promoter (CMV)
REGION	805	1298	β-globin intron
GENE	1362	2396	Human follistatin isoform 344 cDNA
REGION	2403	2881	Human growth hormone polyA signal
REGION	2921	3050	Inverted terminal repeat
GENE	3828	4625	Kanamycin resistance gene
REGION	3153	3459	Plasmid origin of replication (ori)

Figure S8. AAV.CMV.FS344-Kan plasmid used for vector production. The orientation and placement sites are shown in relation to restriction sites. The human follistatin gene expression cassette FS344 cDNA is flanked by AAV2 inverted terminal repeats (ITRs). The CMV immediate early promoter/enhancer used the β-globin intron. The plasmid contains the kanamycin resistance gene.