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Supplemental Information

Plasmid-Mediated Gene Therapy in Mouse Models of Limb Girdle Muscular Dystrophy

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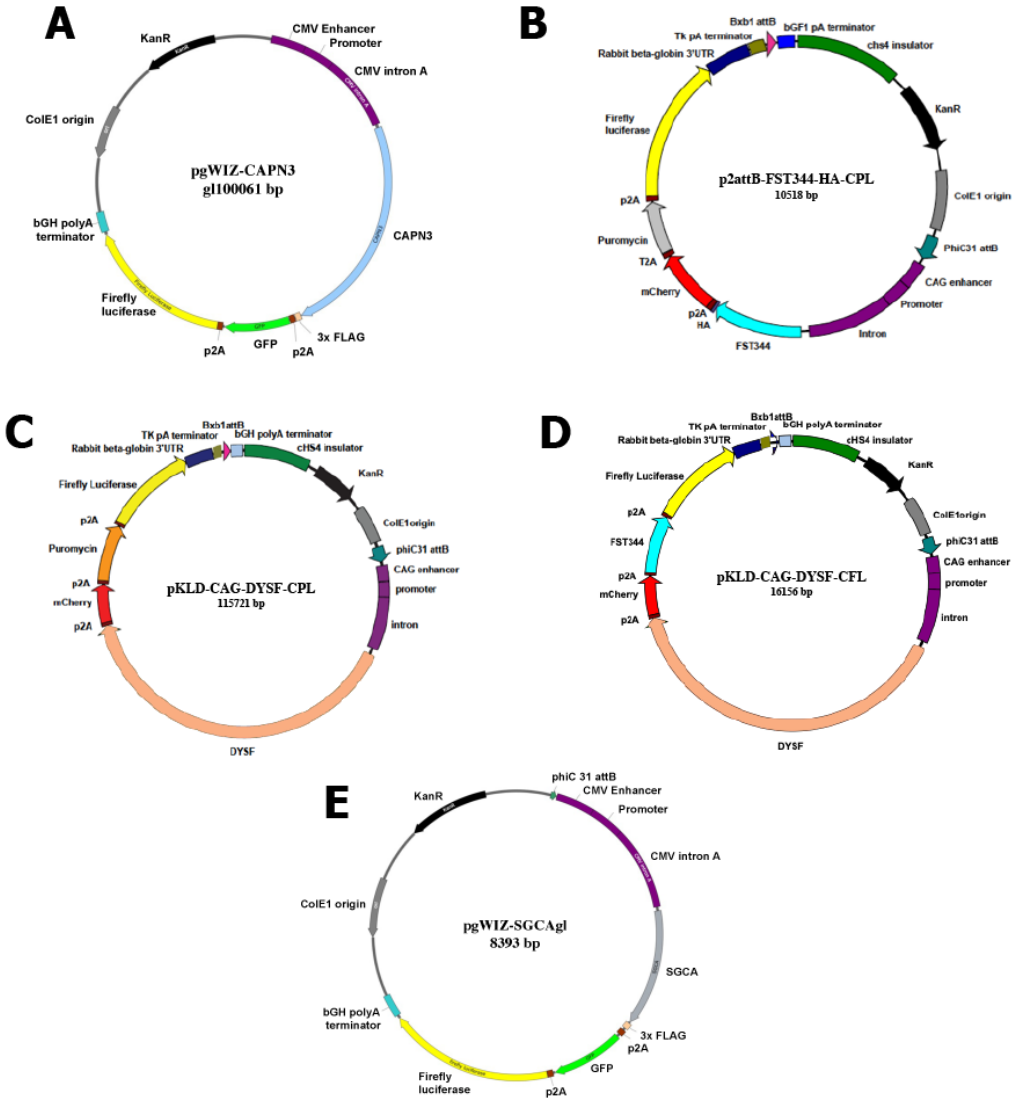
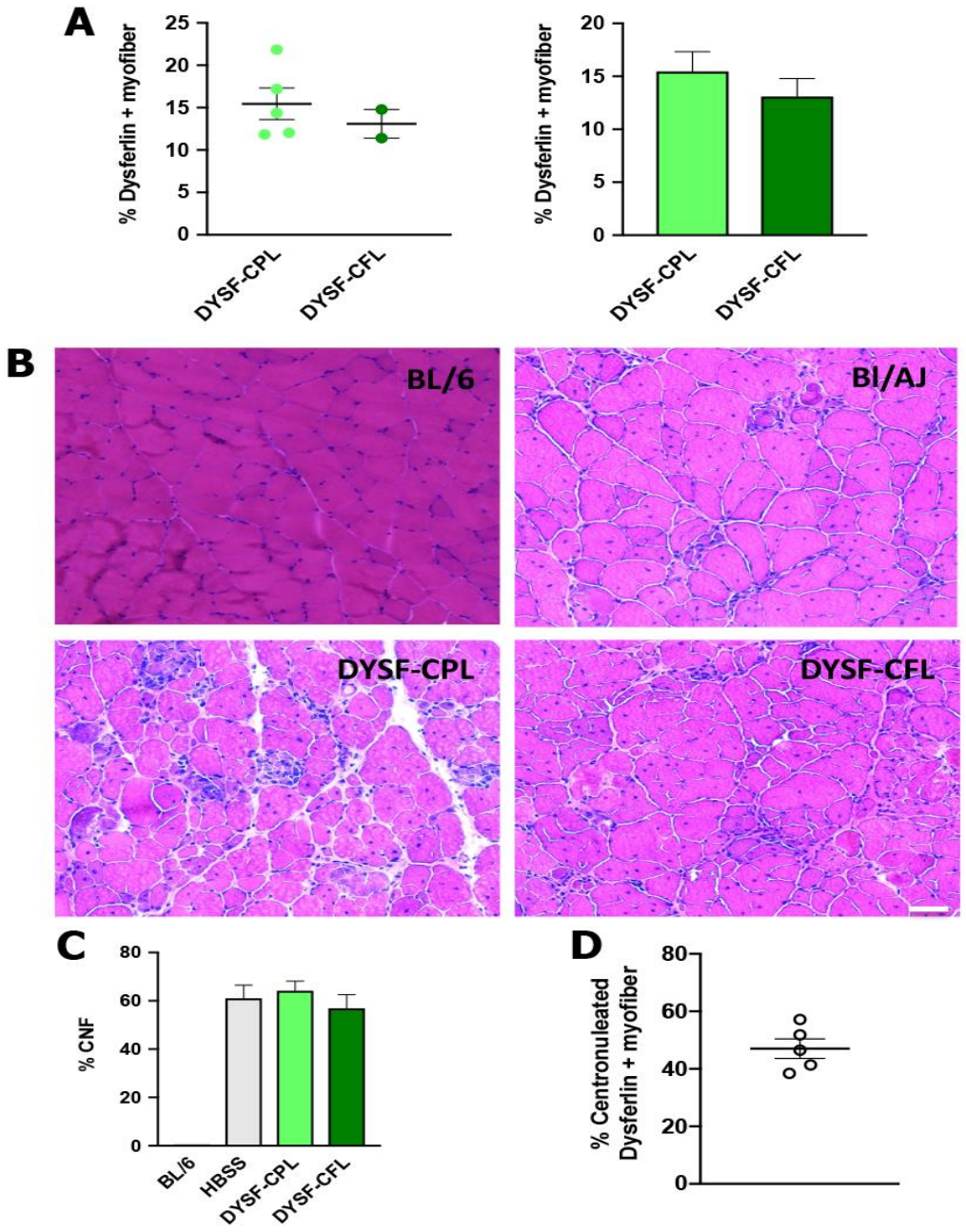
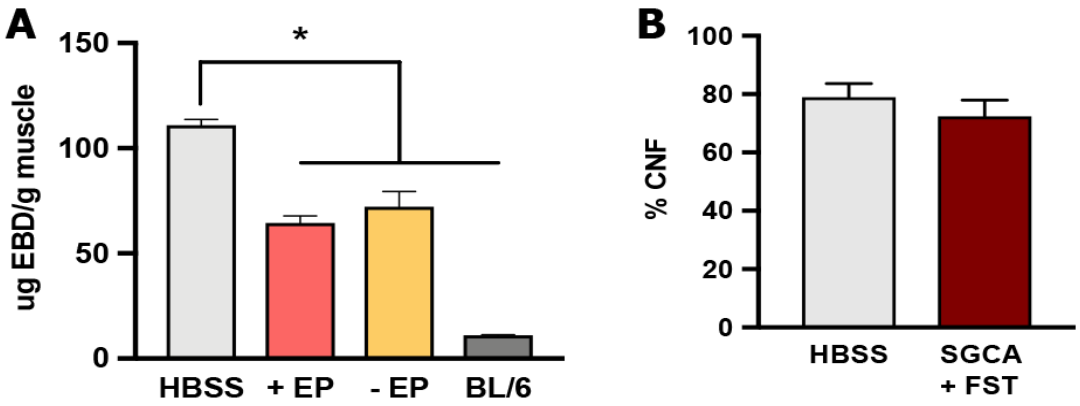


Supplementary Figure 1. Plasmids (A) pgWIZ-CAPN3gl. This plasmid uses the CMV enhancer-promoter–intron of the gWIZ vector to express human calpain3 (CAPN3), including a 3xFLAG tag to facilitate detection. The firefly luciferase gene is also in the expression unit, to enable live imaging of DNA delivery. (B) p2attB-FST344-HA-CPL. The plasmid carries the 344-amino acid version of human follistatin (FST). The follistatin gene, as well as firefly luciferase, are expressed from a CAG enhancer-promoter-intron unit. (C) pKLD-CAG-DYSF-CPL. This plasmid, abbreviated DYSF-CPL, expresses human dysferlin (DYSF) and firefly luciferase from a CAG enhancer-promoter-intron unit. (D) pKLD-CAG-DYSF-CFL. This plasmid, abbreviated DYSF-CFL, expresses human dysferlin (DYSF), the 344-

amino acid version of human follistatin (FST), and firefly luciferase from a CAG enhancer-promoter-intron unit. (E) pgWIZ –SGCAgl. This plasmid expresses human alpha-sarcoglycan (SGCA), including a 3xFLAG tag to facilitate detection, from CMV enhancer-promoter-intron expression sequences from the gWIZ vector, and also expresses firefly luciferase for live monitoring of DNA delivery.



Supplementary Figure 2. Additional data from Bl/AJ study (A) Percentage of dysferlin-positive fibers. The numbers of dysferlin-positive fibers were counted on several sections of muscles treated with pDYSF-CPL or pDYSF-CFL in the one-month study, 1 month after injection. Some of the images were shown in Fig. 2C. Each dot represents the count from one section. Two representations of the same data are shown. Data are mean +/- SEM with n=2-5. (B) Hematoxylin and eosin staining of muscle sections used for determining centronucleation frequency. Representative sections are from quadriceps muscles of wild-type C57Bl/6, untreated Bl/AJ, and Bl/AJ injected with pDYSF-CPL or pDYSF-CFL, harvested three months after injection. Bar = 50 um. (C) Centronucleation frequency in muscle sections from control and treated Bl/AJ mice from the 3-month experiment. Centronucleated fibers were counted for 2 mice per group, 2 fields per mouse, without regard to whether the fibers were positive for dysferlin. ~60% were centronucleated in each case. Data are mean +/- SEM, with n=4. (D) Centronucleation frequency in dysferlin-positive fibers. When only DYSF-positive myofibers were analyzed, a lower fraction, ~47%, of dysferlin-positive myofibers were centronucleated. Data are mean +/- SEM with n=5



Supplementary Figure 3. Additional data from *Sgca-null* studies (A) Independent 1-month study in *Sgca-null* mice. Gastrocnemius muscles were injected with plasmids encoding SGCA or SGCA + FST, with or without electroporation, versus untreated mice. n=6 legs per group. Data are mean +/-

SEM, with n=4-8 and * $p<0.05$. (B) Centronucleation study in young mice. Downward trend one month after injection in young *Sgca-null* mice. Data are mean +/- SEM with n=4.