Supporting Information

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SI Text

The following antibodies were used for Western blot analysis: rabbit anti-PKM1 (Sigma-Aldrich), rabbit anti-PKM2 (Cell Signaling Technology), rabbit anti-PKM1/2 (Cell Signaling Technology), rabbit anti-GAPDH (Cell Signaling Technology), rabbit anti-polypyrimidine tract binding protein (PTB) (Abcam), mouse anti-hnRNPA1 (Santa Cruz Biotechnology), mouse anti-hnRNP1/A2 (Abcam), rabbit anti-hnRNPH, mouse anti-CELF1 (Millipore), mouse anti-MBNL1 (LifeSpan BioSciences), mouse anti-SRSF3 (Invitrogen), mouse anti-MBNL2 (Santa Cruz Biotechnology), and the Glycolysis Sampler Antibody Kit (Cell Signaling Technology).

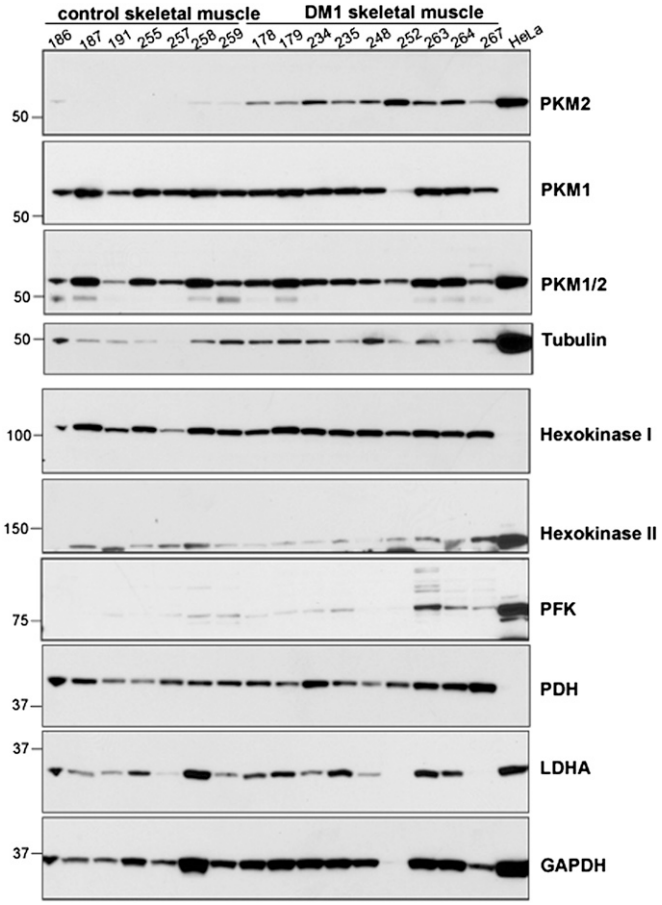

Fig. S1. No apparent change in glycolytic enzymes other than PKM2 in myotonic dystrophy type 1 (DM1). Shown are Western blots of enzymes in the glycolysis pathway in control and DM1 skeletal muscle samples using antibodies to hexokinase I, hexokinase II, phosphofructokinase (PFK), lactate dehydrogenase A (LDHA), pyruvate dehydrogenase (PDH), and GAPDH antibodies. The blots for PKM1, PKM2, PKM total, and tubulin are the same as in Fig. 2 and are shown for visual reference.

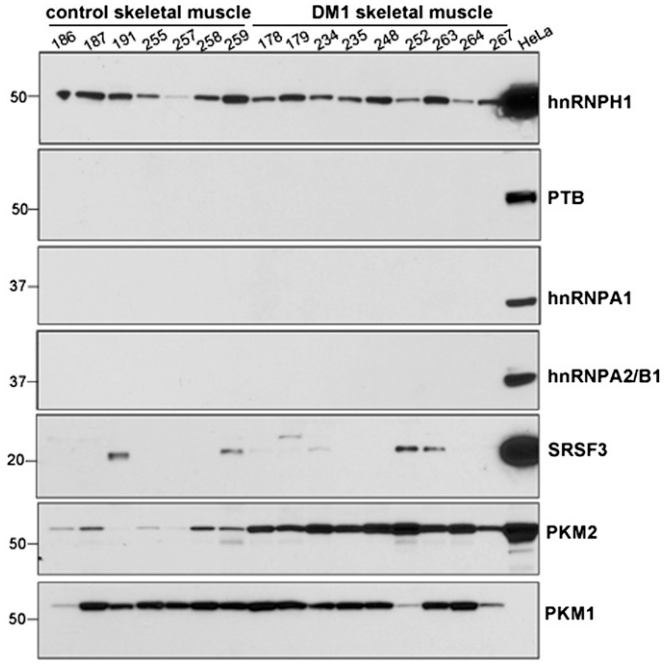

Fig. S2. No correlation between expression of known PKM splicing regulators and that of PKM2 in DM1 samples. Shown are Western blots of splicing factors and PKM isoforms in control and DM1 skeletal muscle samples using antibodies to PTB, hnRNPA1, hnRNPA2/B1, SRSF3, hnRNPH1, PKM2, and PKM1, as indicated.

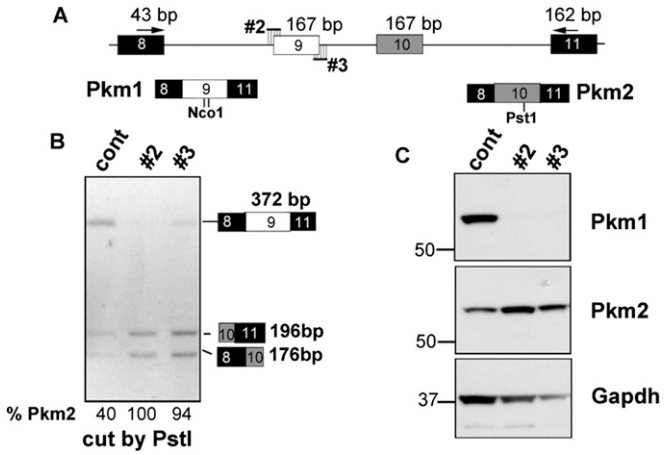

Fig. S3. Redirected Pkm splicing by morpholino oligonucleotides (MOs) in C2C12 myotubes. (*A*) Schematic of the Pkm genomic region containing exons 8–11. Primers used for PCR, two NcoI sites in exon 9 (6 nt apart), and a PstI site in exon 10 are indicated. MOs (#2 and #3) complementary to the 3′ and 5′ splice sites of exon 9 are shown. (*B*) RT-PCR analysis of Pkm transcripts in C2C12 myotubes at 6 d after differentiation in the presence of 10 μM control, #2 MOs, or #3 MOs. (*C*) Western blot analysis of proteins from C2C12 myotubes treated with MOs.

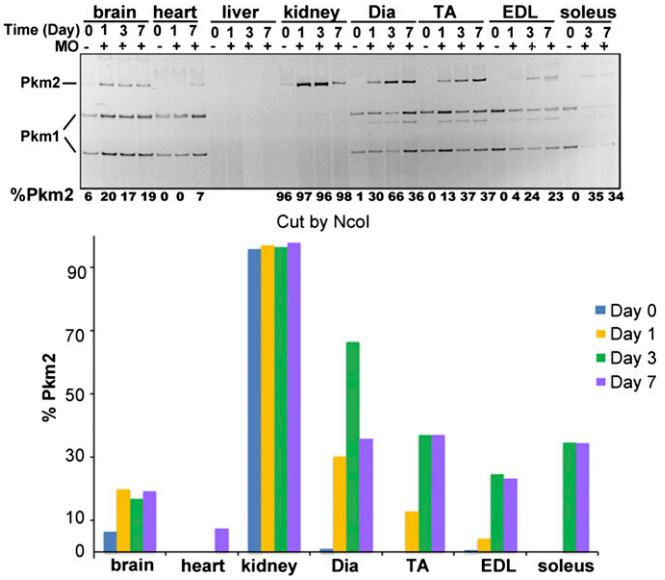

Fig. S4. Vivo-MOs shift Pkm splicing primarily in skeletal muscle. A single dose of 16 mg/kg of PKM vivo-MO #2 was delivered into 4-wk old mice by tail vein injection, followed by RT-PCR analysis/NcoI cut of Pkm in different tissues at the indicated time points. The apparent increased expression of Pkm2 in kidneys is not reproducible and likely reflects an issue with the time 0 RNA sample. Dia, diaphragm; EDL, extensor digitorum longus; TA, tibialis anterior.

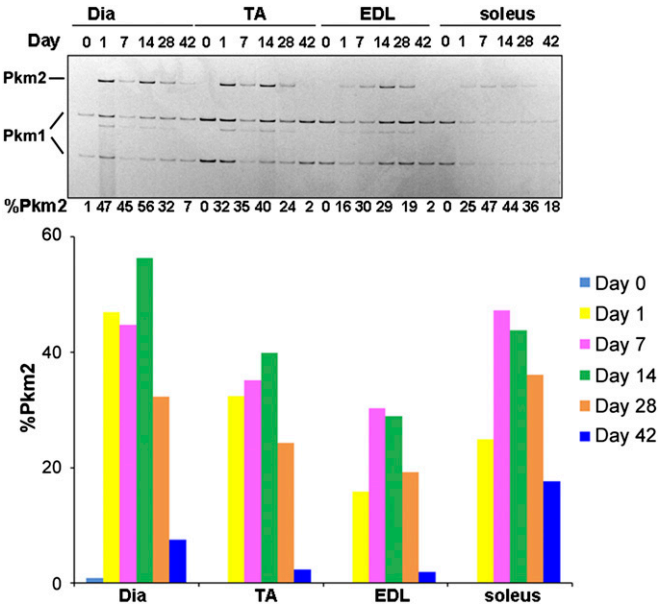

Fig. S5. Effect of Pkm vivo-MOs in different skeletal muscles over time. A single dose of 16 mg/kg Pkm vivo-MO #2 was delivered into 4-wk-old mice, followed by RT-PCR analysis/NcoI cut of Pkm in different skeletal muscle tissues at the indicated time points.

Table S1. Sequences of siRNAs and MOs

	Sense sequence	Antisense sequence/comments
siRNAs		
MBNL1-A5	ACGACGUCAUUAGCCAUAUUGUAUA	UAUACAAUAUGGCUAAUGACGUCGU
MBNL1-A7	CACAGCCAACCAGAUACCCAUAAUA	UAUUAUGGGUAUCUGGUUGGCUGUG
MBNL2-A1	GAGAUUAAUGGGAGGAACAAUUUGA	UCAAAUUGUUCCUCCCAUUAAUCUC
MBNL2-A3	GCGUUGCAUGAGGGAGAAAUGCAAA	UUUGCAUUUCUCCCUCAUGCAACGC
MOs		
PKM-#1	GCTATCTGTAAGGTTTAGGGTAGGA	Complementary to 3′ SS of PKM exon 9
PKM-#2	TCCCGAGCTATCTGTAAGGTTTAGG	Complementary to 3′ SS of PKM exon 9
PKM-#3	ACCTGCCCTTAGGTCCCTACCTGCC	Complementary to 5′ SS of PKM exon 9
Inverted control	GGATTTGGAATGTCTATCGAGCCCT	Inverted sequence of #2

SS, splice site.