Online Supplementary Materials

Supplementary Methods

Genotyping--Conditional *Sulf1* and *Sulf2* mutant alleles were generated by inserting loxP sites that flank the second coding exon of the *Sulf* genes (Ai, et al., 2007). Primers were designed to distinguish between the wildtype ($Sulf^{+}$), the floxed ($Sulf^{f}$) and the mutant ($Sulf^{-}$) *Sulf* alleles. Their relative locations to the targeted exon 2 were shown in Supplementary Figures 1&2. Sequences of these primers follow:

Sulf1_F1: 5' GCATAGAGTCAGTGGGTCAAAGTTG 3';

Sulf1_R1: 5' GCCTCCTGACAAGGTTACTAGG 3';

Sulf1_F2: 5' GACACTGGAATCTCCCTCATATC 3';

Sulf2_F1: 5'ATCTCAACAGCACAGGCTACCG 3';

Sulf2_R1: 5' ATGGCAACCCCTTCGTCATC 3';

Sulf2_F2: 5' GCAGGTTTGTACCCAACGC 3'

Sulf2_R2: 5' GGTTACTCCCACAATAAACTGGTG 3'

Annealing temperature in all PCR reactions was 58^{o}C. After PCR, different *Sulf* alleles generate DNA fragments of following sizes.

Primer pair Sulf1_F1 & R1:

156 bp from the $Sulf1^{+}$allele, 204 bp from the $Sulf1^{f}$ allele, no product from the $Sulf1^{-}$ allele

Primer pair Sulf1_F2 & R1:

871 bp from the $Sulf1^{+}$allele, 291 bp from the $Sulf1^{-}$ allele

Primer pair Sulf2_F1 & R1:

492 bp from the $Sulf2^{+}$allele, 540 bp from the $Sulf2^{f}$ allele, no product from the $Sulf2^{-}$ allele

Primer pair Sulf2_F2 & R2:

861 bp from the $Sulf2^{+}$allele, 910 bp from the $Sulf2^{f}$ allele, 480 bp from the $Sulf2^{-}$ allele

Quantitative RT-PCR—Total RNA was extracted from primary satellite cell cultures established from the hindlimb skeletal muscles of control and *Sulf*$^{\delta K}$-DN mice using RNeasy Mini Kit (Qiagen). To quantify *Sulf1* and *Sulf2* mRNA levels, total RNA (1 μg) was reverse transcribed using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and StepOnePlus Real-Time PCRSystem. The relative level of *Sulf* mRNAexpression was calculated by normalizing to β-actinmRNA using ΔCt (cycle threshold difference).

Primers used in PCR follow:

Sulf1_forward: 5'-TGTTTGTCGCAACGGCATC-3';

Sulf1_reverse: 5'-GGACCACGAATGAAGAAAGGC-3';

Sulf2_forward: 5'-ACACCAATGTGCTGTCCGTCTC-3';

Sulf2_reverse: 5'-CGTGAGGTAATCCGTGGAGTAGTC-3'

β-actin_forward: 5'-GCAGCTCCTTCGTTGCCGGT-3';

β-actin_reverse: 5'-TACAGCCCGGGGAGCATCGT-3'.

Supplementary Figure Legends

Supplementary Figure 1. Generation and validation of conditional *Sulf1* mutant mice. (**A**) Schematic representation of the wildtype (*Sulf1*$^{+}$) allele, the floxed allele (*Sulf1*^{f}) and the recombined null (*Sulf1*$^{-}$) allele. Two loxP sites were inserted to flank the second *Sulf1* coding exon. PCR primers for genotyping *Sulf1*^{f} allele (F1 and R1) and *Sulf1*$^{-}$ allele (F2 and R1) are shown. (**B**) PCR genotyping of *Sulf1*$^{+}$ and *Sulf1*^{f} alleles were performed using primers F1 and R1 and genomic DNA from tails. *Sulf1*$^{+}$ allele generated a 156-bp DNA fragment, while *Sulf1*^{f} allele generated a 204-bp DNA fragment. Cre-mediated excision of the floxed *Sulf1* exon led to a 291-bp fragment by PCR using primers F2 and R1 and genomic DNA from the tibialis anterior (TA) muscle of *Myf5-Cre*$^{+}$*;Sulf1*$^{f/f}$ mice, consistent with the Cre expression pattern. PCR using primers F2 and R1 generated a ~900 bp DNA fragment from *Sulf1*$^{+}$ allele.

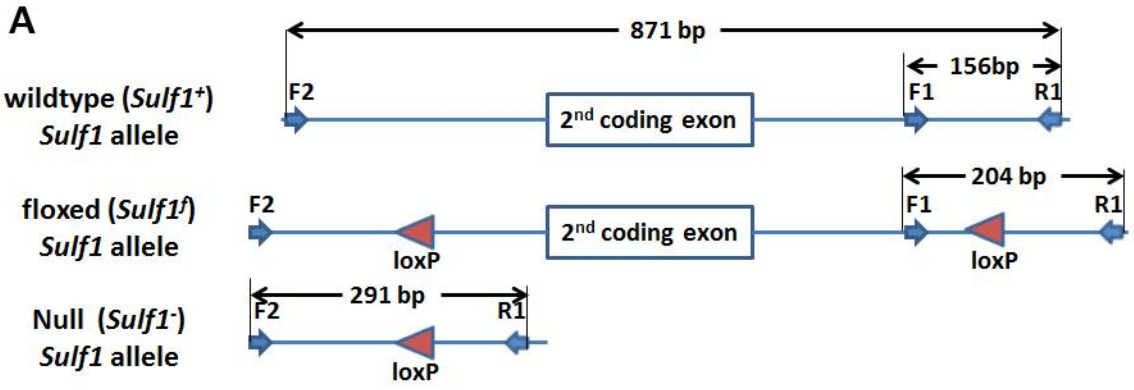
Supplementary Figure 2. Generation and validation of conditional *Sulf2* mutant mice. (**A**) Schematic representation of the wildtype (*Sulf2*$^{+}$) allele, the floxed allele (*Sulf2*^{f}) and the recombined null (*Sulf2*$^{-}$) allele. Two loxP sites were inserted to flank the second *Sulf2* coding exon. PCR primers for genotyping *Sulf2*^{f} allele (F1 and R1) and *Sulf2*$^{-}$ allele (F2 and R2) are shown. (**B**) PCR genotyping of *Sulf2*$^{+}$ and *Sulf2*^{f} alleles were performed using primers F1 and R1 and genomic DNA from tails. *Sulf2*$^{+}$ allele generated a 492-bp DNA fragment, while *Sulf2*^{f} allele generated a 540-bp DNA fragment. Cre-mediated excision of the floxed *Sulf2* exon leads to a 480-bp fragment by PCR using primers F2 and R2 and genomic DNA from the tibialis anterior (TA) muscle of *Myf5-Cre*$^{+}$*;Sulf2*$^{f/+}$ and *Myf5-Cre*$^{+}$*;Sulf2*$^{f/f}$ and mice, consistent with the Cre expression pattern. PCR using primers F2 and R2 generate an 861 bp DNA fragment from *Sulf2*$^{+}$ allele.

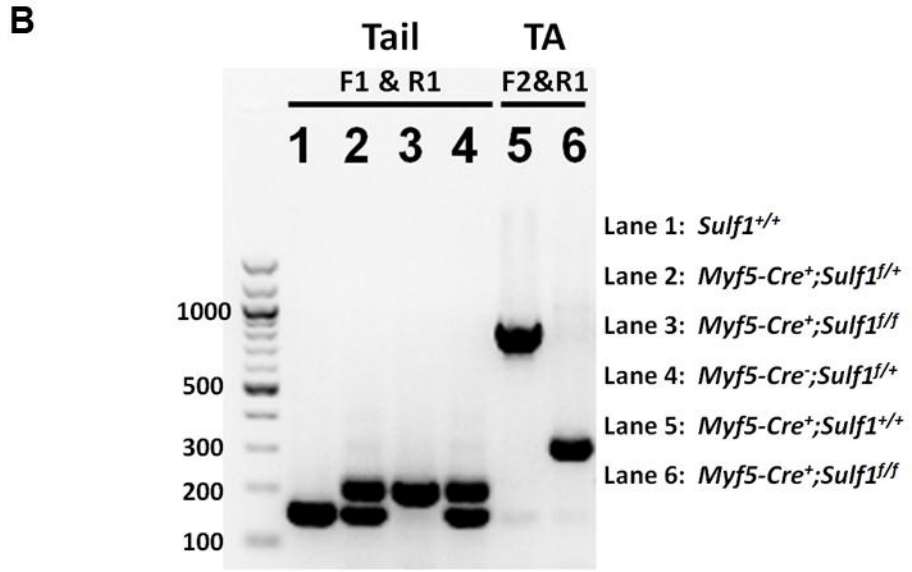
Supplementary Figure 3: Validation of loss of Sulf expression in primary myoblasts isolated from *Sulf^{SK}*-DN mice. Primary myoblast cultures were established from *Myf5-Cre^{-};Sulf1$^{f/f}$;Sulf2$^{f/f}$* control *mice and Myf5-Cre^{+};Sulf1$^{f/f}$;Sulf2$^{f/f}$* (*Sulf^{SK}*-DN) mice. Total RNA was extracted from the culture and then subjected to quantitative RT-PCR to measure *Sulf1* and *Sulf2* mRNA expression levels. *β-actin* mRNA expression was internal control. Relative levels of *Sulf1* (in **A**) and *Sulf2* (in **B**) mRNA expression in *Sulf^{SK}*-DN myoblasts were normalized to control myoblasts. **p<0.01.

Supplemenetary Figure 4: Sulf deficiency has no effects on the efficacy of skeletal muscle regeneration at day 14 post-injury. TA muscles of WT (**a**) and *Sulf^{SK}*-DN (**b**) mice were injured with cardiotoxin (100 μl of 10 μM) and collected on day 14 post injury. Immuno-histochemical analyses using mid-belly muscle cross sections were performed to identify Pax7^{+} SCs and laminin^{+} basement membrane. Nuclei were labeled by Hoechst dye. Relative abundance of Pax7^{+} SCs (in **c**), new myofibers with increasing sizes (μm^2) (in **d**) and number of myonuclei (in **e**) were quantified. (**f**) The average size and number of new myofibers within a regenerating area of 1 mm^2 of WT and *Sulf^{SK}*-DN muscles were quantified. Data presented were mean and standard deviation of more than 1000 new myofibers from 3 independent experiments. Scale bars,

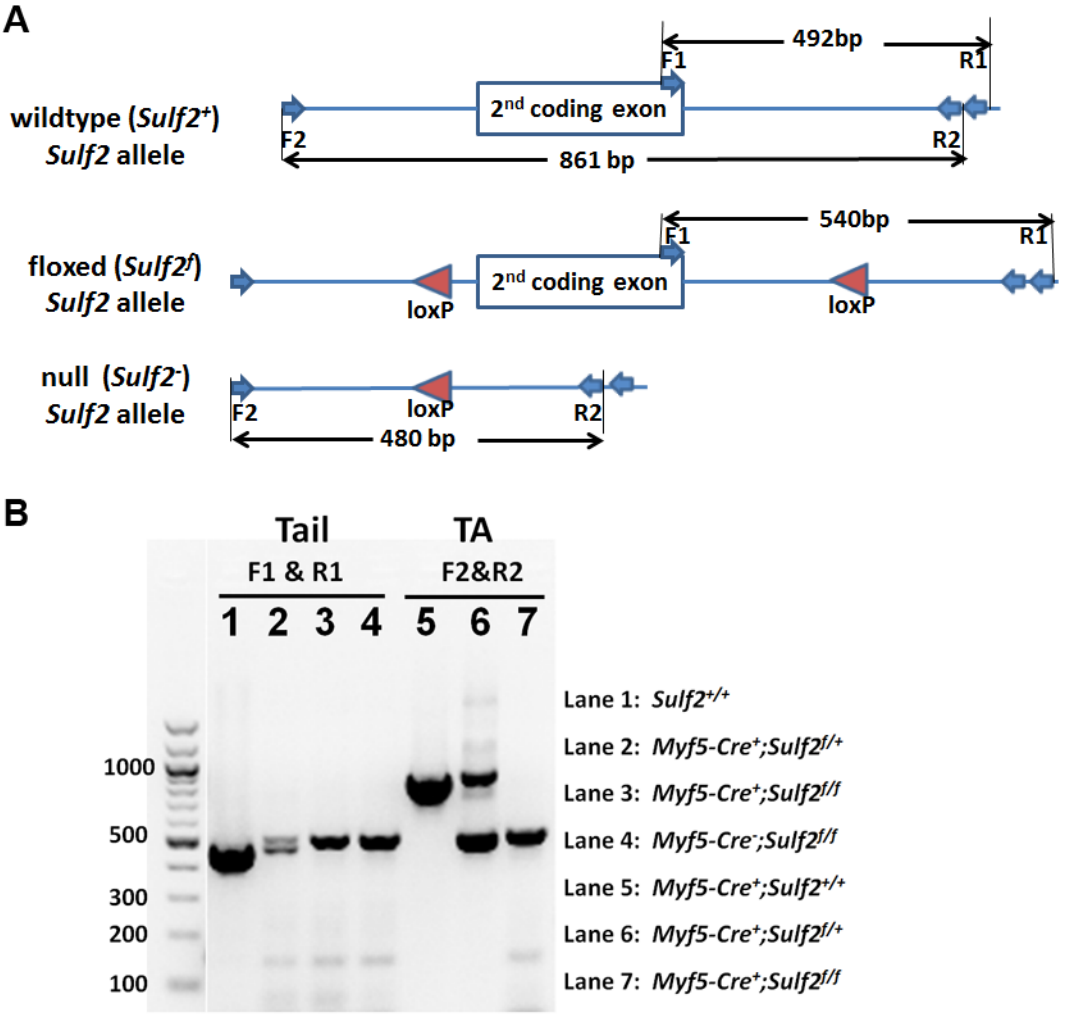
Supplementary Figure 5. No effects on pFAK clustering by Wnt7a or Sulf-deficiency during early myoblast differentiation. Primary culture of satellite cells from hindlimb muscles of WT (**A-B**) and *Sulf^{SK}-DN* (**C**) mice were treated with or without Wnt7a in Differentiating Media for 24 hours, fixed, and stained for Myf5, pFAK, and Hoechst. No difference in cell surface clustering of pFAK was observed. Images shown represented one of two independent experiments.

Supplementary Figure 1. Tran et al.

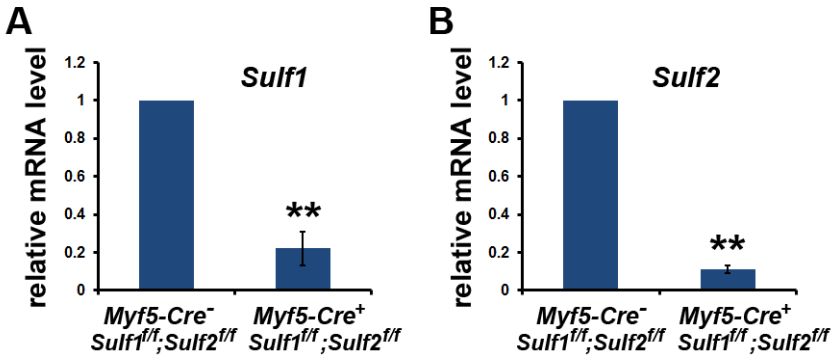




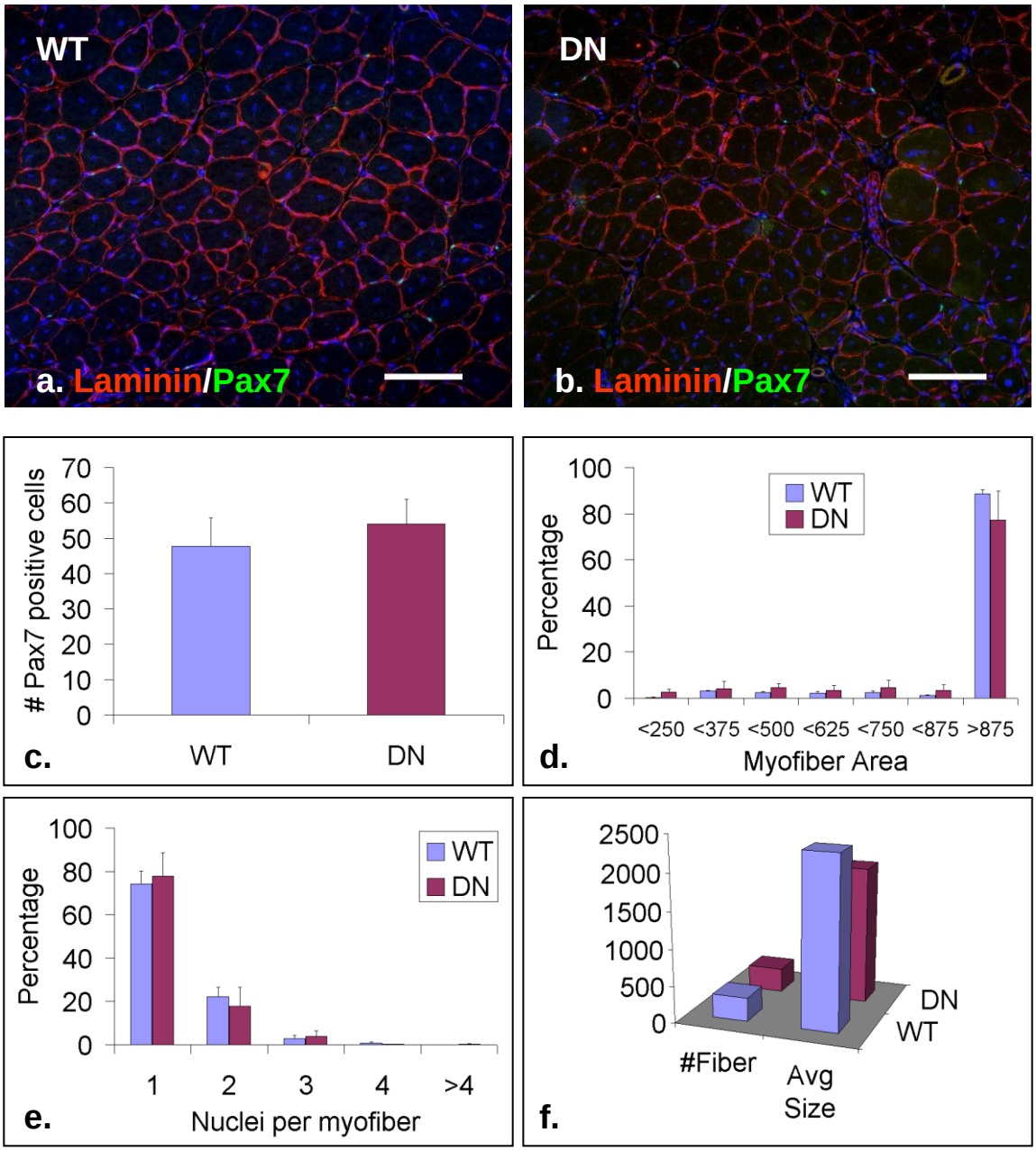
Supplementary Figure 2. Tran et al.



Supplementary Figure 3. Tran et al



Supplementary Figure 4. Tran et al.



Supplementary Figure 5. Tran et al.