Supplemental Methods

Helper-dependent adenoviral vectors

The HDAd used for gene targeting at the ASL locus was constructed as follows: A 14,449 bp fragment from the ASL locus was PCR amplified from genomic DNA extract from a non-ASL human iPSC. PCR was performed with primers ASL F2 and ASL R3 using PrimeSTAR GXL DNA polymerase (Takara/Clontech, Mountain View, CA) with final concentrations of 0.2 mM dinucleotide triphosphate (dNTP) and 0.2 μM of each primer. Thermocycling conditions were as follows: 1 min at 94ºC, followed by 30 cycles of 98ºC for 10 seconds (sec) and 72ºC for 10 minutes (min), and a final extension of 10 min at 72ºC. The sequence of the PCR fragment was verified and used to replace the 18,745 SpeI-NotI fragment in plasmid p23.8-CFTRm-PuroTk-DTA (1). Next, the PACTk expression cassette, flanked by PB ITRs, was inserted into the first 5' TTAA 3' PiggyBac insertion site downstream of exon 9. The HDAd was produced in 116 cells (2) using the helper virus AdNG163 (3) as described elsewhere (4). HDAd titers were determined by absorbance at 260 nm as described elsewhere (2, 4).

Transduction and analysis of iPSCs

ASLD iPSCs was maintained in mTeSR 1 (STEMCELL Technologies) on Matrigel (Corning, Tewksbury, MA) coated plates. The iPSCs were transduced with HDAd as described previously (5). Briefly, $2x10^6$ cells were resuspended in 1 ml mTeSR 1 supplemented with Y27632 (Reagents Direct) to 10 μM in a 1.5 ml microfuge tube and transduced with HDAd at an MOI of 350 vp/cell for 1 hour at 37ºC with gentle rocking. Following transduction, cells were washed twice with 1 ml mTeSR 1 supplemented with Y27632 to 10 μM and plated into 11 Matrigel coated wells of 6 well plates in mTeSR 1 supplemented with Y27632 to 10 μM. Puromycin was added to the media to a final concentration of 0.5 μg/ml 48 hours post-transduction. Well

isolated colonies were picked and DNA was extracted from colonies for PCR. Transduction of iPSCs to achieve PB excision was performed as described previously (5). Briefly, $2x10^6$ cells were resuspended in 1 ml mTeSR 1 supplemented with Y27632 (Reagents Direct, Encinitas, CA) to 10 μM in a 1.5 ml microfuge tube and infected with HDAd-CAG-hyPB-VAI (6) at an MOI of 350 vp/cell for 1 hour at 37ºC with gentle rocking. Following infection, cells were washed twice with 1 ml mTeSR1 supplemented with Y27632 to 10 μM and plated onto 6 well plates at a density of $2x10^5$ and $1x10^5$ cells/well in nonselective media. 48 hours later, the media was replaced with media supplemented with gancyclovir to a final concentration of 2 μM. Well isolated colonies were picked and DNA was extracted from colonies for PCR. Parental and isogenic control iPSCs from the individual with ASLD were analyzed for pluripotency markers via immunocytochemistry of OCT4, SOX2, and TRA-1-81. In addition, karyotypic analysis showed both parental and isogenic controls showed the same cytogenetic makeup including chromosome 20 monosomy.

DNA analysis

Genomic DNA from iPSCs was extracted from a single confluent well of a 24 or 12 well plate as follows: Cells were washed once with 0.5 or 1 ml PBS, and 0.4 ml lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA pH 8.0, 0.5% SDS, 500 μg/ml pronase (Roche, Indianapolis IN)) was added to the well. The lysate was then transferred into a microfuge tube and incubated overnight at 37ºC. The next day, the DNA was precipitated by the addition of 1 ml 95% ethanol, washed once with 1 ml 70% ethanol and resuspended in 10 mM Tris-HCL pH 8.0.

Primer pair F1 and asL4 and primer pair sR6 and R4 were used to amplify the unique 5' and 3', respectively, vector/chromosome junction in correctly targeted clones using PrimeSTAR GXL DNA polymerase (Takara/Clontech, Mountain View, CA) with final concentrations of 0.2

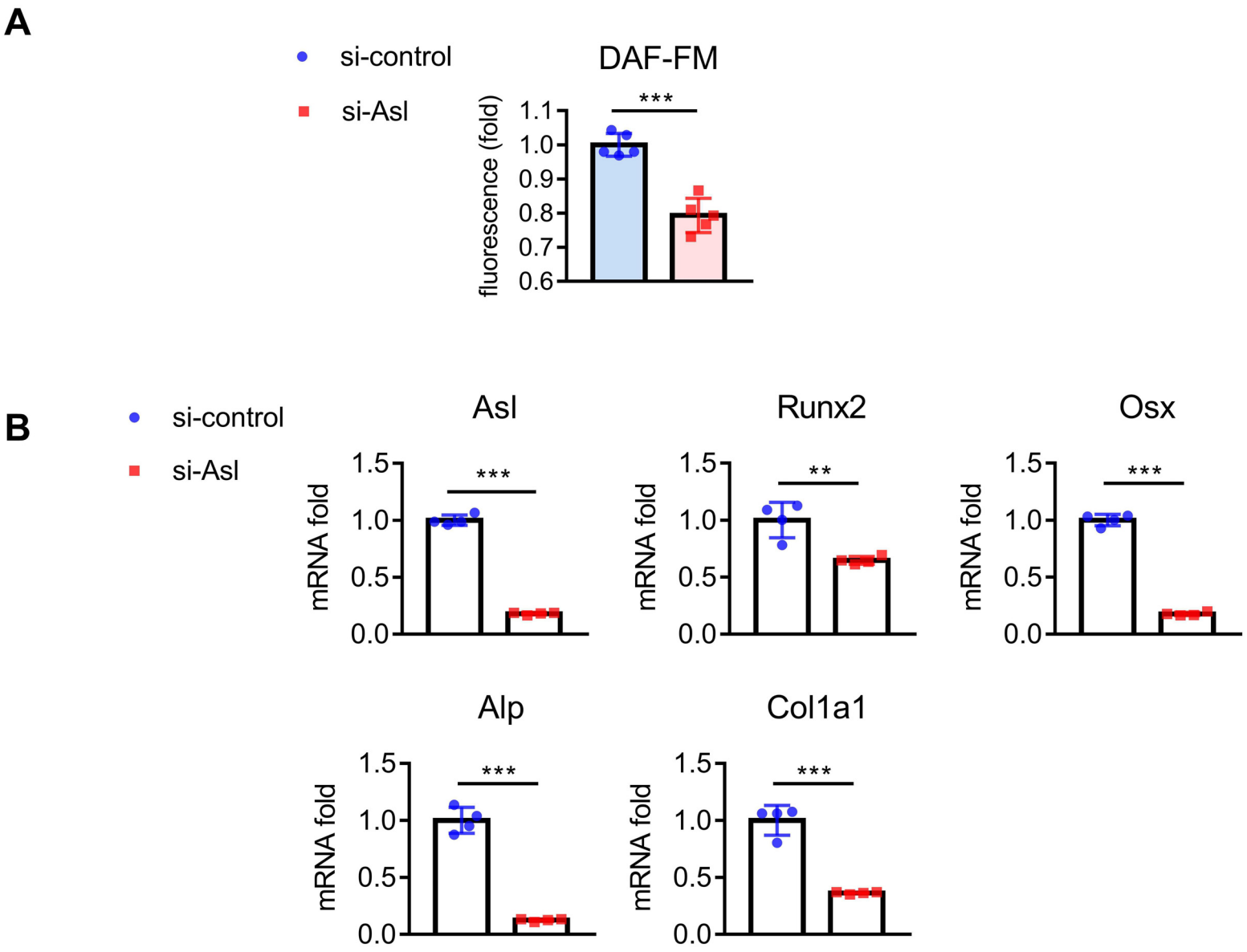
mM dinucleotide triphosphate (dNTP) and 0.2 μM of each primer. Thermocycling conditions were as follows: 1 min at 94ºC, followed by 30 cycles of 98ºC for 10 sec and 72ºC for 8 min, and a final extension of 10 min at 72ºC. The primer pair F3 and R4 is used to amplify a 7,447 bp and 10,442 bp amplicon from the non-targeted and correctly targeted ASL alleles using the same PCR reagents as described above and with thermocycling conditions were as follows: 1 min at 94ºC, followed by 35 cycles of 98ºC for 10 sec and 72ºC for 10 min, and a final extension of 10 min at 72ºC.

Primers ASL F6a and ASL R8a were used to amplify the unmodified ASL allele(s) encompassing exon 7 and exon 11 and the site of the selectable marker insertion from iPSC genomic DNA PrimeSTAR GXL DNA polymerase (Takara/Clontech, Mountain View, CA) with final concentrations of 0.2 mM dinucleotide triphosphate (dNTP) and 0.2 μM of each primer. Thermocycling conditions were as follows: 1 min at 94ºC, followed by 40 cycles of 98ºC for 10 sec and 70ºC for 3 min, and a final extension of 10 min at 70ºC. The primer ASL F6a was used to sequence the PCR product to determine the sequence of exon 7, the primer ASL R8a was used to sequence the PCR product to determine the sequence of exon 11, and the primer ASL F3 was used to sequence the PCR product to verify footprintless PB excision of the selectable marker.

References for Supplemental Methods

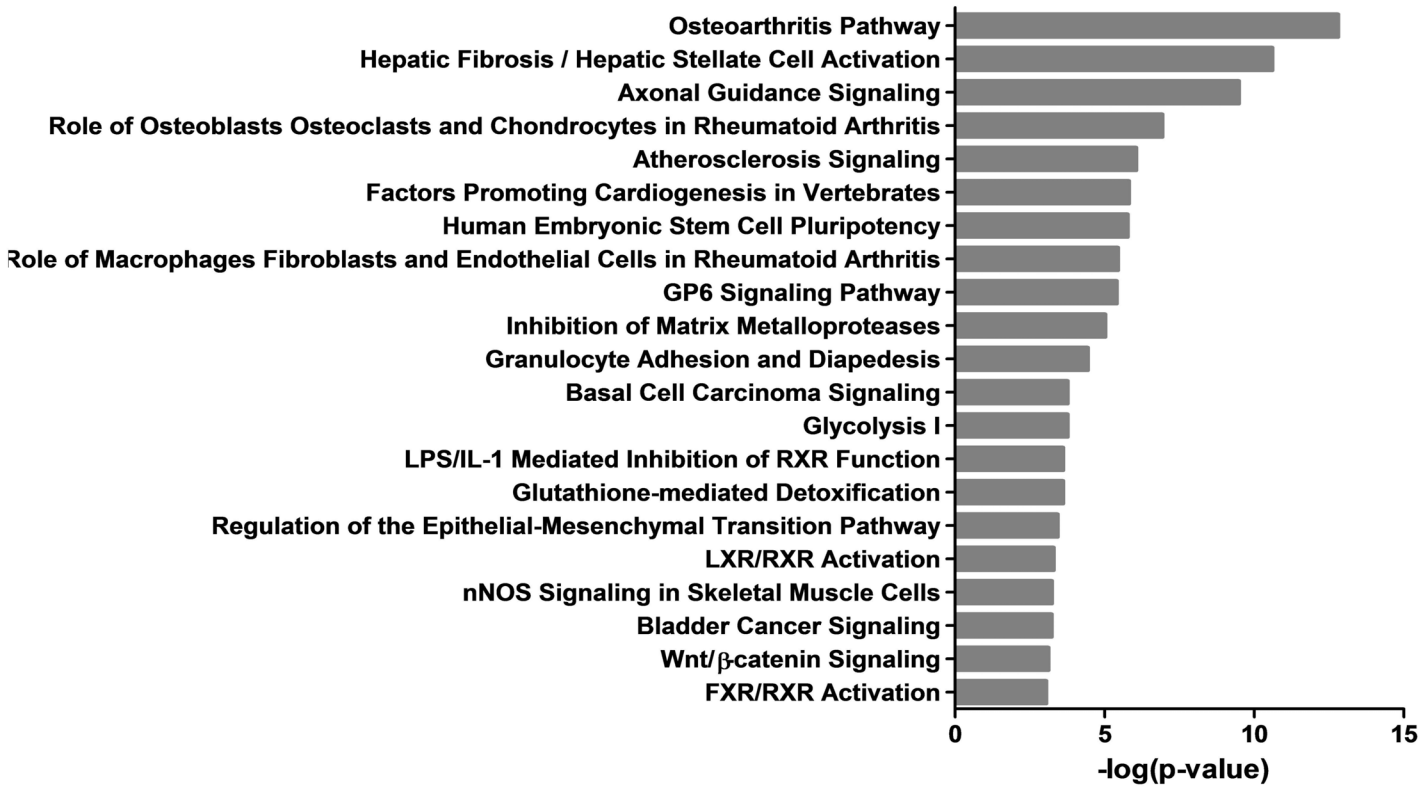
1. Palmer DJ, Grove NC, Turner DL, and Ng P. Gene Editing with Helper-Dependent Adenovirus Can Efficiently Introduce Multiple Changes Simultaneously over a Large Genomic Region. *Mol Ther Nucleic Acids.* 2017;8:101-10.
2. Palmer D, and Ng P. Improved system for helper-dependent adenoviral vector production. *Mol Ther.* 2003;8(5):846-52.

3. Palmer DJ, and Ng P. Physical and infectious titers of helper-dependent adenoviral vectors: a method of direct comparison to the adenovirus reference material. *Mol Ther.* 2004;10(4):792-8.
4. Palmer DJ, and Ng P. Methods for the production of helper-dependent adenoviral vectors. *Methods in molecular biology.* 2008;433:33-53.
5. Palmer DJ, et al. Homology Requirements for Efficient, Footprintless Gene Editing at the CFTR Locus in Human iPSCs with Helper-dependent Adenoviral Vectors. *Mol Ther Nucleic Acids.* 2016;5(10):e372.
6. Palmer DJ, Grove NC, and Ng P. Helper virus-mediated downregulation of transgene expression permits production of recalcitrant helper-dependent adenoviral vector. *Mol Ther Methods Clin Dev.* 2016;3:16039.

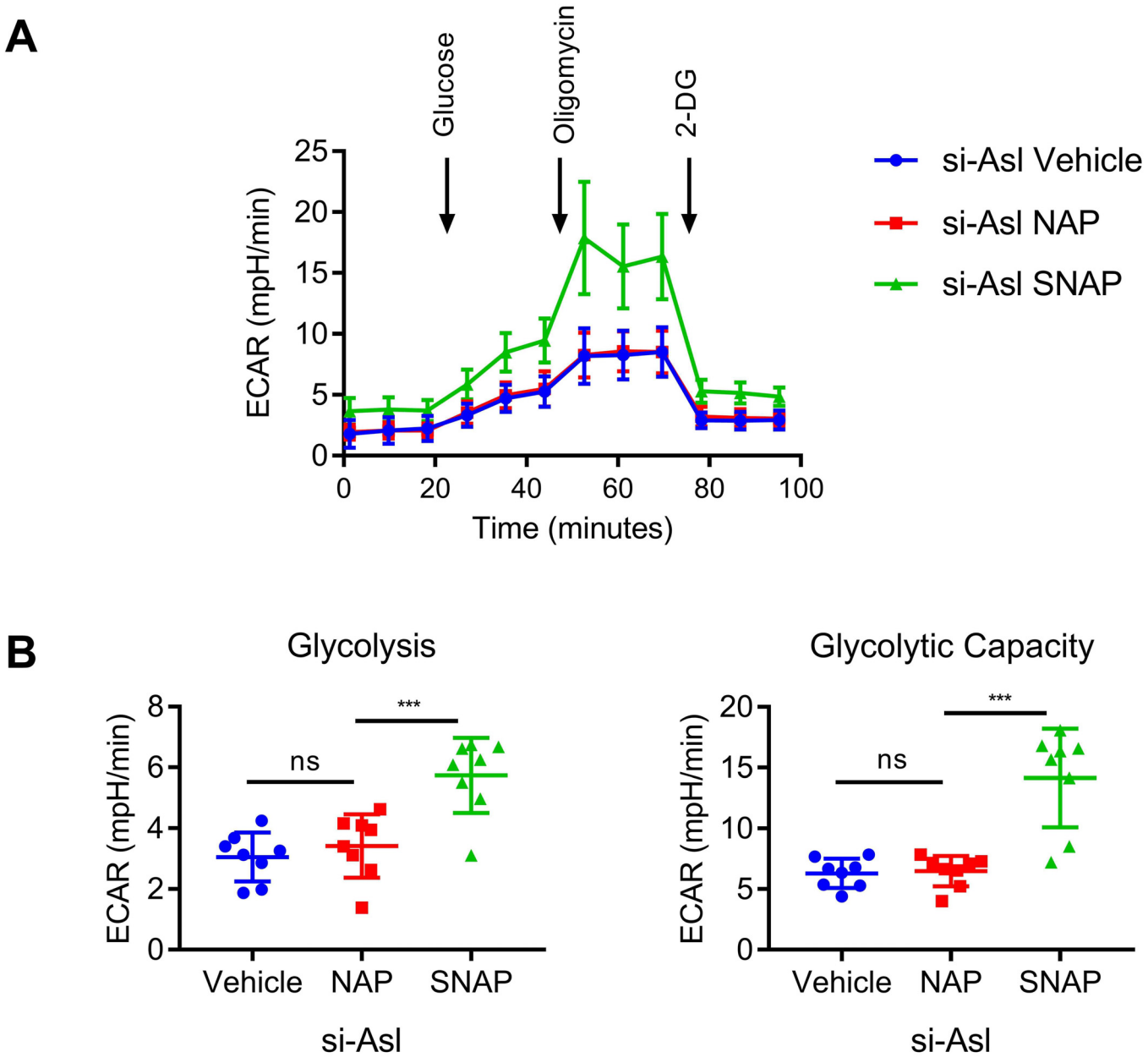


Supplemental Figure 1. Knockdown of *Asl* in MC3T3 cells leads to decreased NO production and decreased expression of osteoblast marker genes

(**A**) NO level was measured by DAF-FM fluorescence intensity in si-Asl knockdown MC3T3 cells. n=5.
(**B**) mRNA levels of *Asl, Runx2, Osx, Alp,* and *Col1a1* by qPCR in si-Asl knockdown MC3T3 cells. n=4.
Data are represented as mean ± SD. Student's t test. ** P<0.01; ***P<0.005.

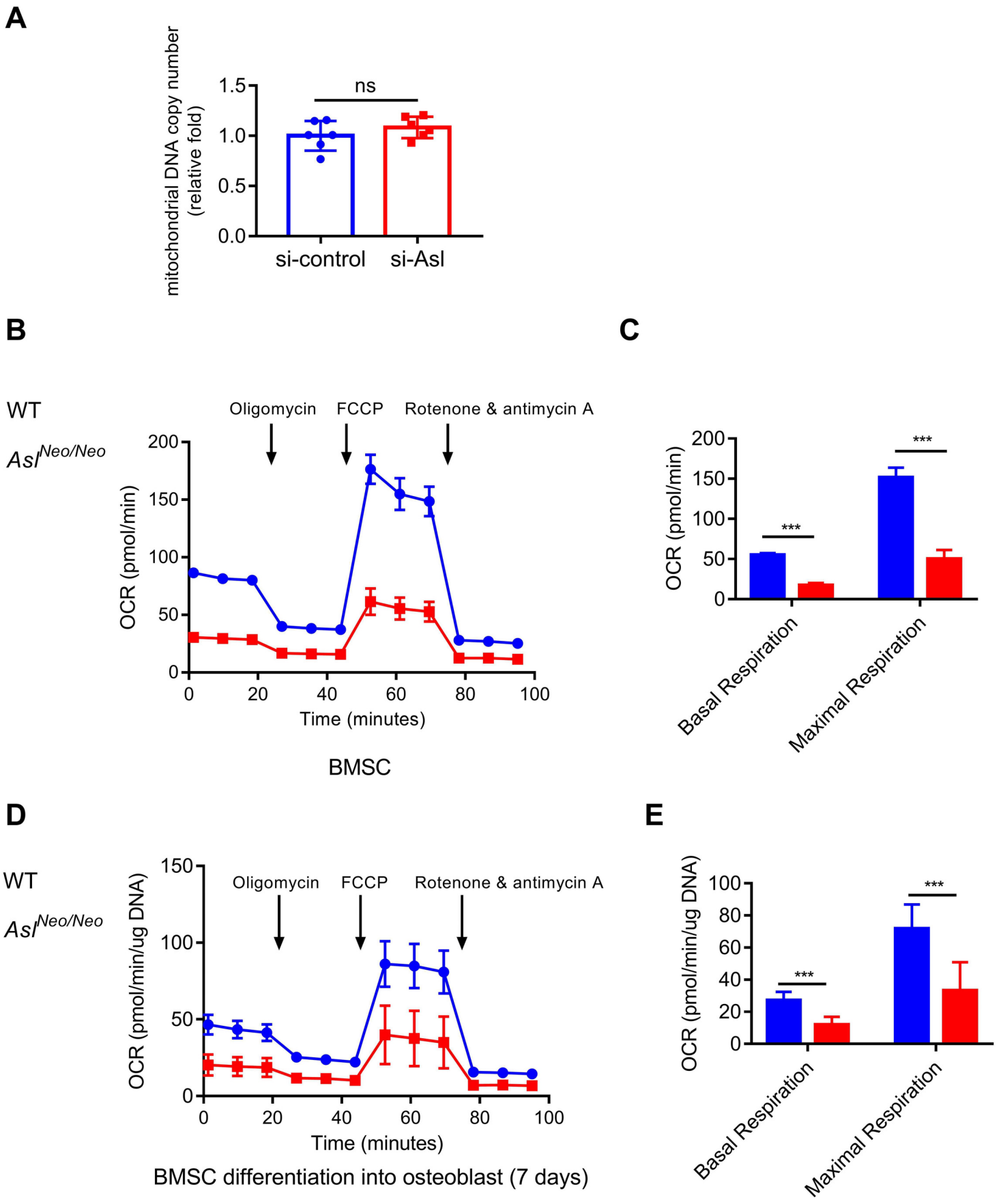


Supplemental Figure 2. Ingenuity Pathway Analysis (IPA) analysis of top canonical pathways from RNA-seq results



Supplemental Figure 3. SNAP treatment increases glycolysis rate in *Asl* knockdown ST2 cells as shown in the Seahorse assay

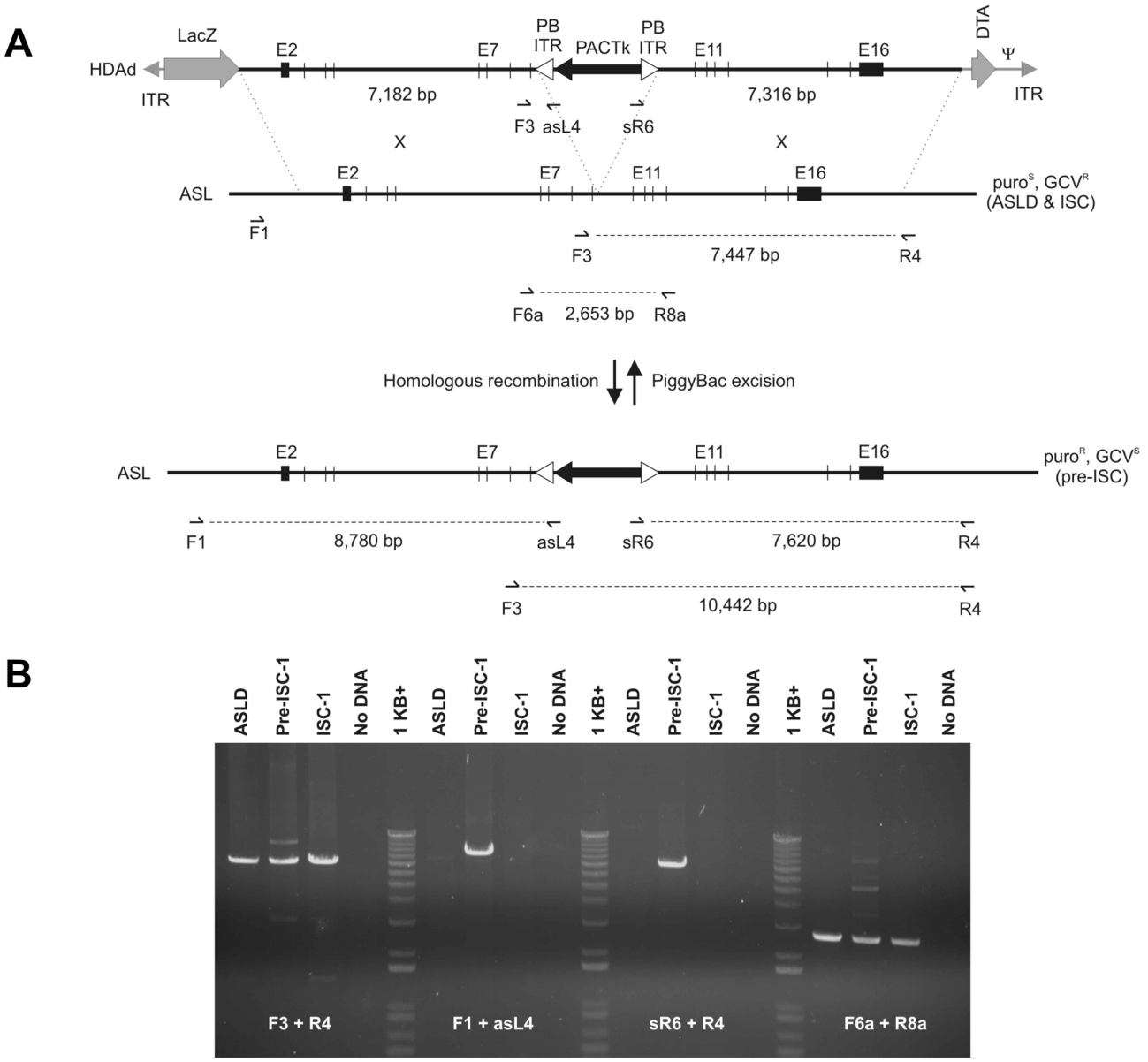
(**A**) Seahorse assay of glycolysis activity (ECAR) in si-RNA mediated *Asl* knockdown in ST2 cells. Vehicle: 5μM DMSO. NAP: 5μM N-acetylpenicillamine. SNAP: 5μM S-nitroso-N-acetylpenicillamine. 18 hours treatment. (n=8, data are representative of three independent experiments). (**B**) Quantification of ECAR in glycolysis rate and glycolytic capacity. Data are represented as mean ± SD. One-way ANOVA followed by Tukey's multiple comparisons test. ***P<0.005; ns: not significant P>0.05.



Supplemental Figure 4. Mitochondrial DNA copy number, and OCR levels

(**A**) Relative fold changes of mitochondrial DNA copy number in ST2 cells with si-RNA mediated knockdown of *Asl*. The amount of mitochondrial DNA was normalized by genomic DNA. n=6.

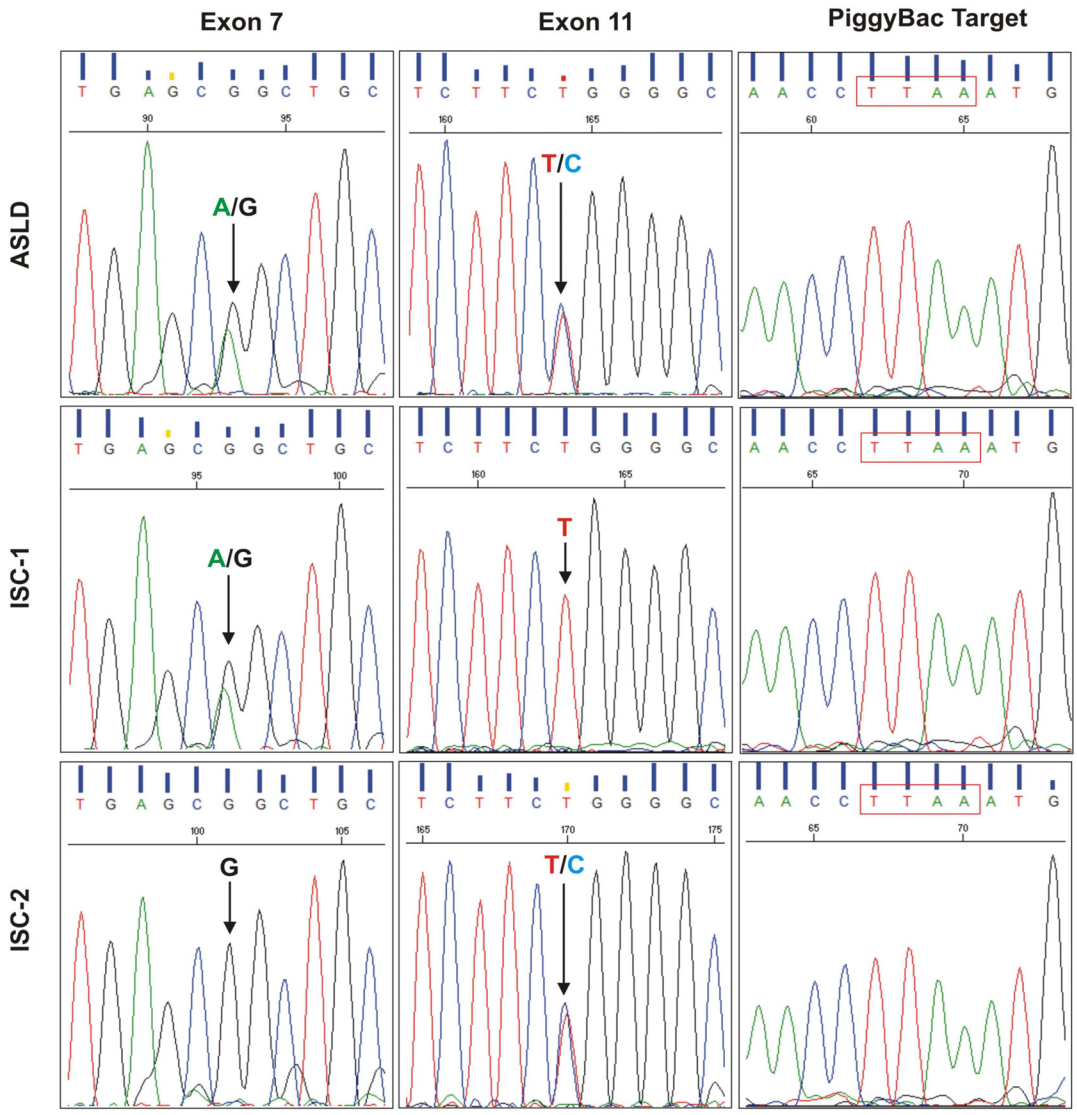
(**B-E**) OCR levels of mitochondrial respiration in BMSCs (**B, C**) and BMSCs differentiated osteoblasts (7 days; **D**, **E**) from WT and *Asl*$^{Neo/Neo}$ mice. Data are representative of three biological repeats. Data are represented as mean ± SD. Student's t test. ***P<0.005. ns: not significant P>0.05.



Supplemental Figure 5. Gene targeting at the *ASL* locus by HDAd

(A) The HDAd bears a wildtype exon 7 and exon 11. A single reciprocal crossover in the right and left homology arms results in integration of the PACTk marker into the *ASL* gene rendering clones puro^R. PB ITRs flank the PACTk cassette to permit its footprintless excision in the presence of PB transposase. The locations of PCR primers and the lengths of the PCR products are shown. The poly(A)-less diphtheria toxin A-fragment gene (DTA) in the HDAd permits negative selection against clones bearing random HDAd integrations. The position of the LacZ expression cassette, adenoviral packaging signal (ψ) and adenoviral inverted terminal repeats (Ad ITR) are shown.

(B) Representative agarose gel showing PCR amplifications from genomic DNA extracted from iPSCs confirming targeted integration of PACTk into pre-ISC-1 and excision of PACTk in ISC-1. In this gene targeting approach, PCR was used to identify targeted recombinants as follows: DNA was extracted from puromycin resistant clones and subjected to PCR with primer pair F1 and asL4 and primer pair sR6 and R4. All primers sequences are shown in Supplemental Table 1. As shown in Supplemental Figure 5, correct gene targeting juxtaposes primers F1 and asL4 to permit amplification of a unique 8,780 bp amplicon spanning the 5' vector/chromosome junction. Likewise, primers sR6 and R4 are juxtaposed only following correct gene targeting to permit amplification of a unique 7,447 bp amplicon spanning the 3' vector/chromosome junction. Additionally, correct gene targeting converts the 7,447 bp amplicon obtained with primers F3 and R4, into a 10,442 bp amplicon. Following gene targeting, the PACTk was excised by PiggyBac transposase.



Supplemental Figure 6. Sequence analyses of iPSCs

Sequence analyses confirmed that the ASLD iPSC line is compound heterozygous at exon 7 and exon 11, correction of exon 7 in ISC-1, correction of exon 11 in ISC-2, and that PiggyBac-mediated excision of PACTk in ISC-1 and ISC-2 is footprintless. To verify correction of the mutation in exon 7 or exon 11, PCR was used to amplify a 2,653 bp amplicon using primers F6a and R8a (Supplemental Figure 5). This amplicon was sequenced with primer F6a to determine if exon 7 had been corrected, or sequenced with primer R8a to determine if exon 11 was corrected (Supplemental Figure 6). Additionally, this amplicon was sequenced with primer F3 to verify that PiggyBac transposase-mediated excision of PACTk was footprintless as shown in Supplemental Figure 6.