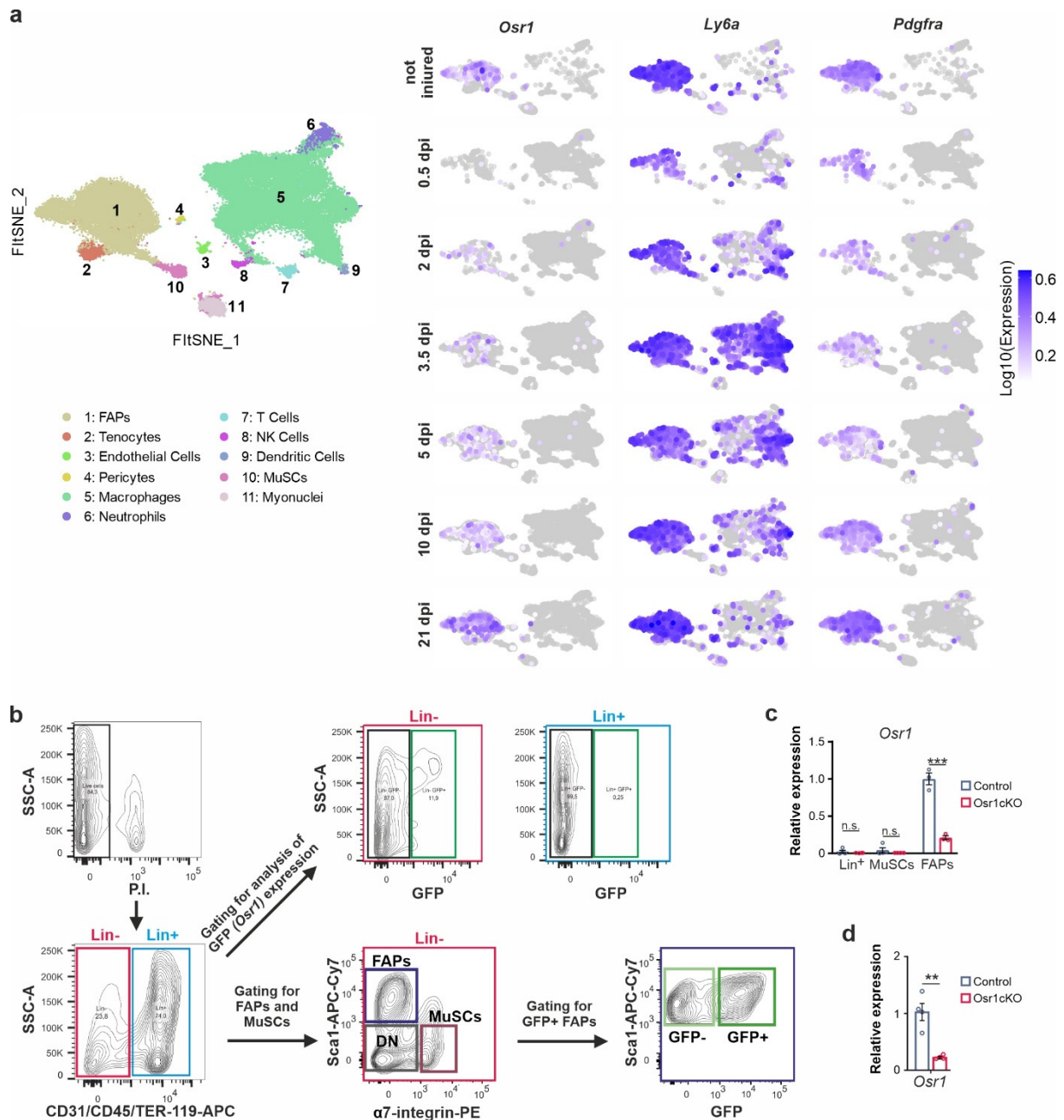


Odd skipped-related 1 controls the pro-regenerative response of Fibro-Adipogenic Progenitors

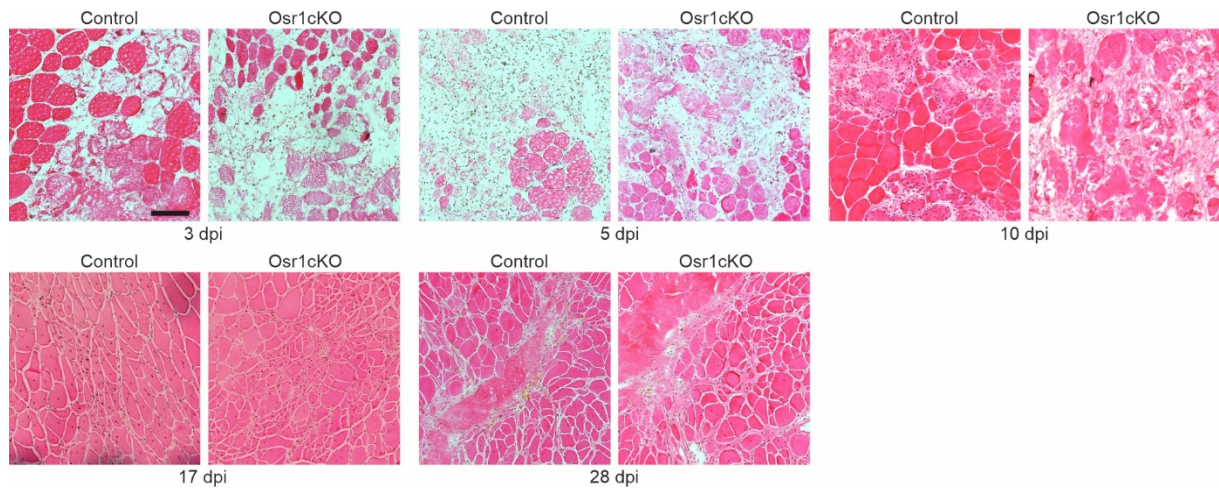
Georgios Kotsaris, Taimoor H. Qazi, Christian H. Bucher, Hafsa Zahid, Sophie Pöhle-Kronawitter, Vladimir Ugorets, William Jarassier, Stefan Börno, Bernd Timmermann, Claudia Giesecke-Thiel, Aris N. Economides, Fabien Le Grand, Pedro Vallecillo-García, Petra Knaus, Sven Geissler and Sigmar Stricker

Supplementary Figures and Tables



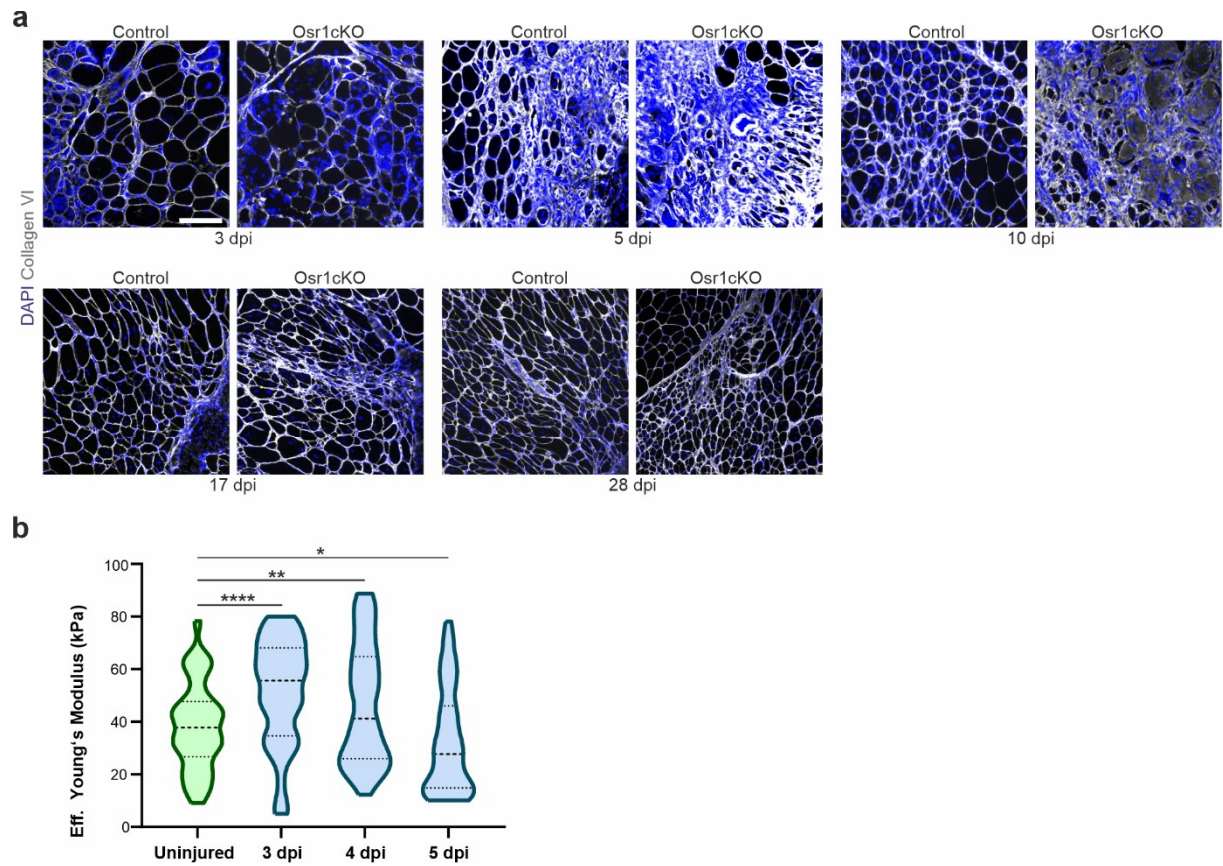
Supplementary Figure 1 Efficacy of *Osr1* conditional inactivation and specificity of *Osr1* expression.

a Analysis of *Osr1* expression in comparison to *Pdgfra* and *Ly6a* (Sca-1) in single cell data from Oprescu et al. of uninjured muscle and of indicated time points post injury. Cluster annotation shown left. **b** FACS sorting strategy for isolating propidium iodide (P.I.)-low live cells, followed by gating for Lin+ (CD31+ CD45+ TER-119+) and Lin- (CD31- CD45- TER-119-) cells. Top right panel: Analysis of GFP expression from the recombined *Osr1* locus in Lin- and Lin+ cells from 3 dpi muscle. Note no GFP+ cells were detected in Lin+ hematopoietic and endothelial cells. Bottom right panel: FACS sorting strategy for FAPs and MuSCs as used for Fig. 3b, c, d; Fig. 5b-h; Fig. 6b, d; Fig. 7g, h. Right: isolation of GFP+ FAPs as used for RNA Sequencing. **c** RT-qPCR analysis of *Osr1* mRNA expression in FACS-isolated FAPs, MuSCs and Lin+ cells from control and *Osr1*cKO mice at 3 dpi (n=3). **d** RT-qPCR analysis of *Osr1* mRNA expression in whole muscle tissue from control and *Osr1*cKO mice at 3 dpi (n=4). Data are mean $\pm$ SEM; P-value calculated by two-sided unpaired t-test; **p < 0.01. N-numbers indicate biological replicates (mice per genotype).



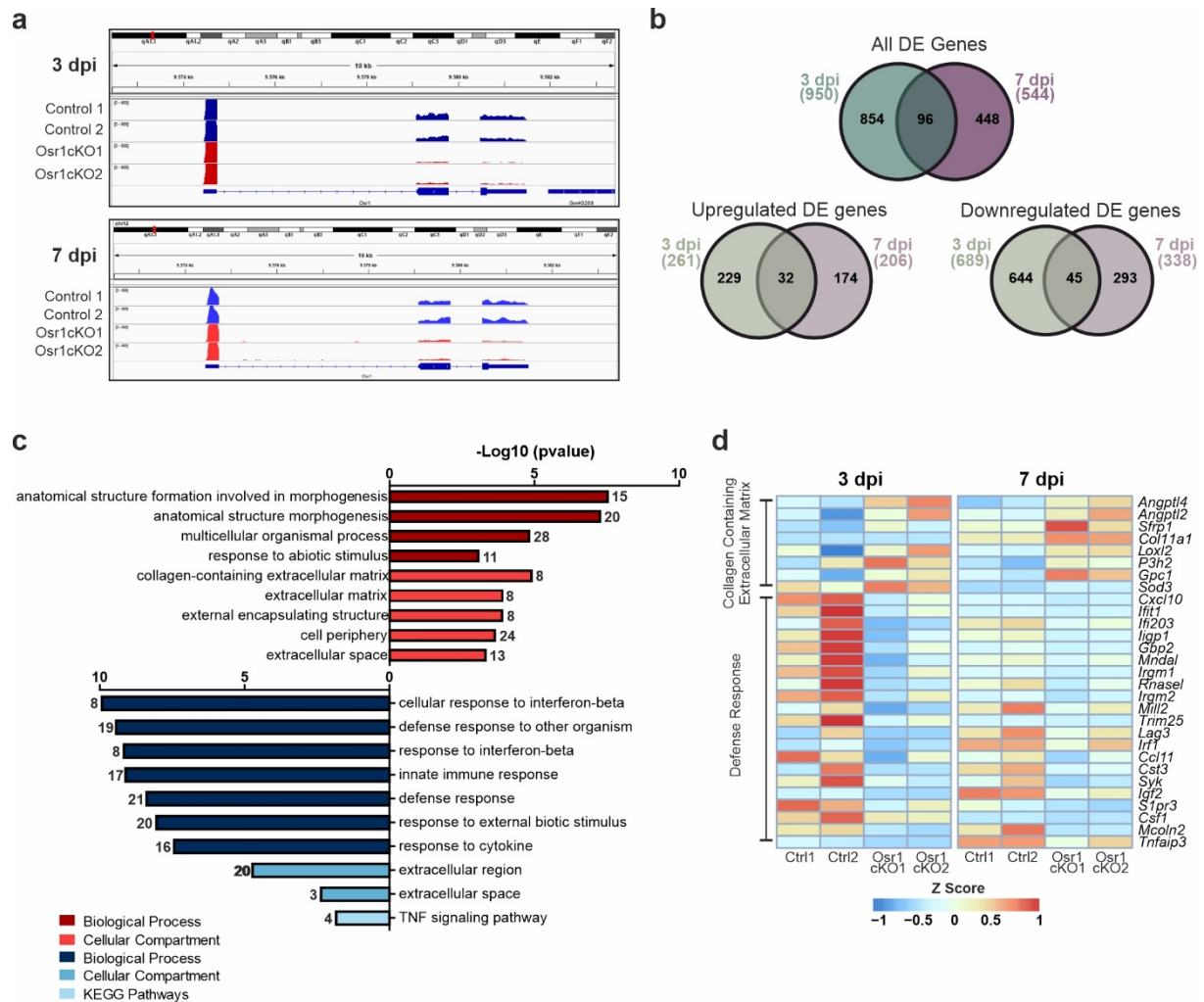
Supplementary Figure 2 Delayed muscle regeneration and fibrotic appearance of Osr1cKO regenerating muscle.

Hematoxylin and eosin staining of control and Osr1cKO muscle sections at indicated days post injury (dpi). Scale bar: 100 μm .



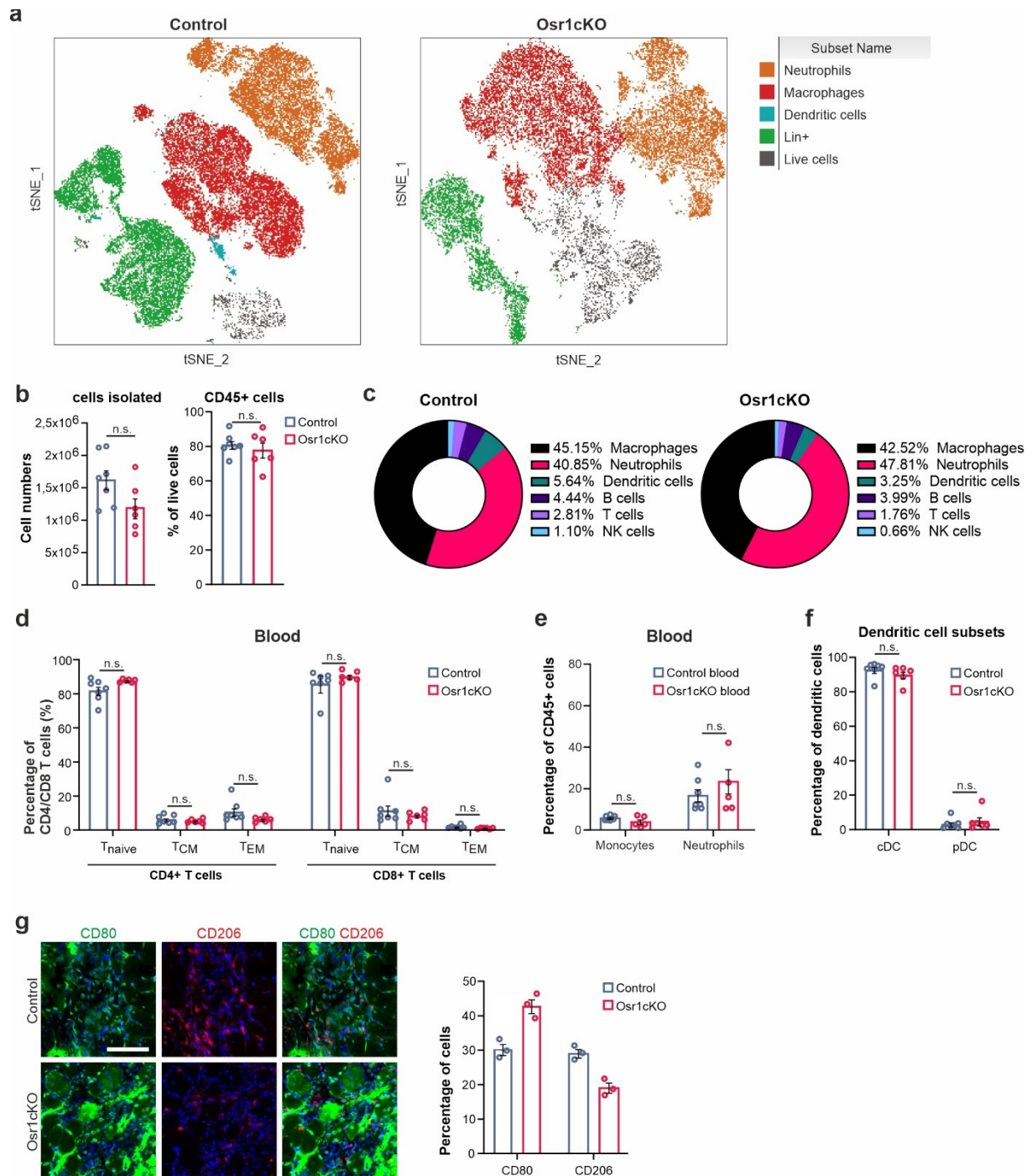
Supplementary Figure 3 Fibrotic appearance of *Osr1cKO* regenerating muscle.

a Immunolabeling for Collagen VI on control and *Osr1cKO* muscle sections at indicated dpi. **b** Tissue stiffness measurement assessed by nanoindentation of wild type regenerating muscle tissue sections at indicated dpi (n=3). Data are mean $\pm$ SEM; P-value calculated by two-sided unpaired t-test; * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. N-numbers indicate biological replicates (mice per genotype). Scale bar: 100 μm .



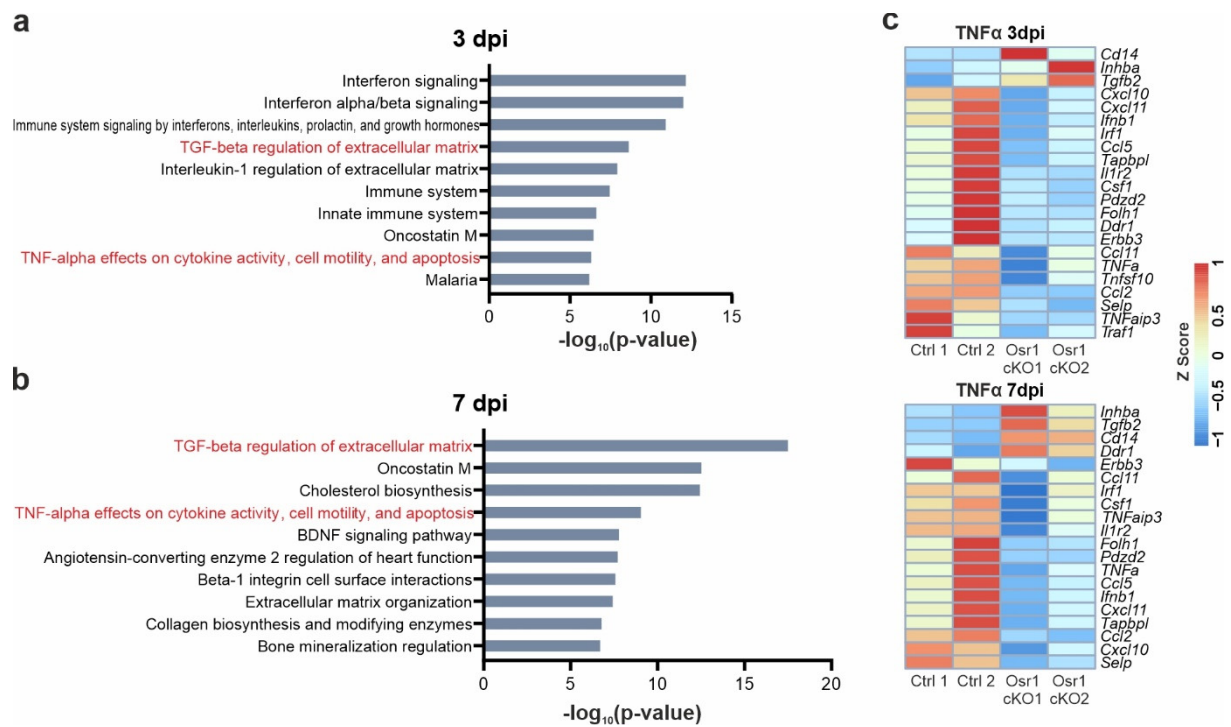
Supplementary Figure 4 Supplementary transcriptome analysis of *Osr1*cKO FAPs.

a Genome browser view of the *Osr1* locus with RNA seq data from 3 and 7 dpi. Note efficient recombination indicated by strongly reduced exon 2 and 3 reads. **b** Venn diagram depicting common regulated genes between the 3 and the 7 dpi *Osr1*cKO FAPs. **c** GO term analysis of genes commonly upregulated (top) and downregulated (bottom) in *Osr1*cKO FAPs at 3 and 7 dpi. **d** Heat maps showing genes belonging to the GO terms “Collagen-containing extracellular matrix” and “Defense response” at 3 and 7 dpi showing continuous deregulation.



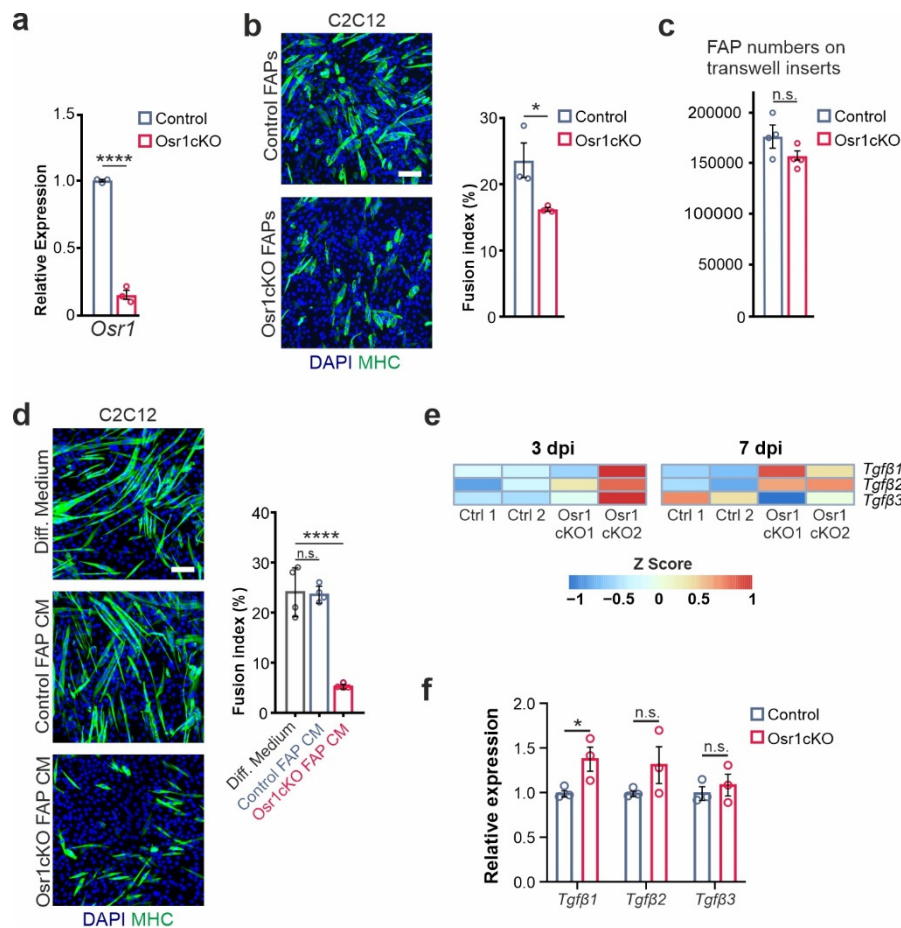
Supplementary Figure 5 Supplementary data immune cell profiling at 3 dpi.

a tSNE plot depiction of immune cell populations identified in 3 dpi control or Osr1cKO muscle. Lin+ is defined CD3+, CD19+, CD335+. **b** Flow cytometry quantification of all live cells analyzed and percentage of CD45+ cells in 3 dpi control or Osr1cKO muscle. **c** Percentages of immune cell populations identified in control and Osr1cKO muscle. **d** Flow cytometry quantification of T-cell cell subsets in blood of 3 dpi control or Osr1cKO mice. **e** Flow cytometry quantification of macrophages and neutrophils in blood of 3 dpi control and Osr1cKO mice. **f** Flow cytometry quantification of Dendritic cell subsets in 3 dpi control or Osr1cKO muscle. **g** Immunolabeling for CD80 and CD206 on tissue sections of 3 dpi control and Osr1cKO animals. Relative quantification of CD80+ vs. CD206+ cells is shown right. In b - f n=7 for control and n=6 for Osr1 cKO. In g n=3 for both groups. Data are mean $\pm$ SEM; P-value calculated by Mann-Whitney test; N-numbers indicate biological replicates (mice per genotype). Scale bar: 100 μ m.



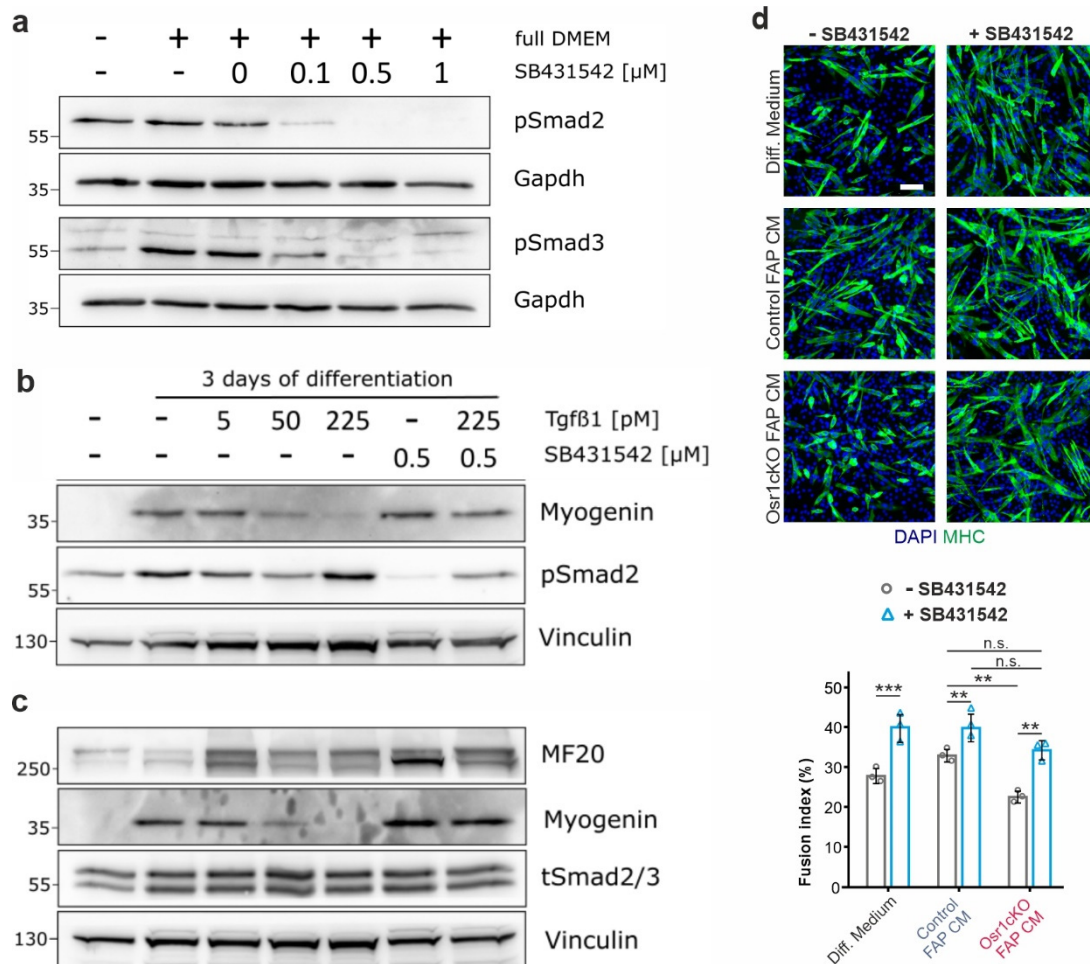
Supplementary Figure 6 TNFα pathway analysis in transcriptome data of Osr1cKO FAPs

a, b GO term analysis of DE genes in Osr1cKO FAPs relative to control FAPs at 3 dpi (top) and 7 dpi (bottom). **c** Heat maps showing downregulation of TNFα pathway genes belonging to the highlighted terms in Osr1cKO FAPs.



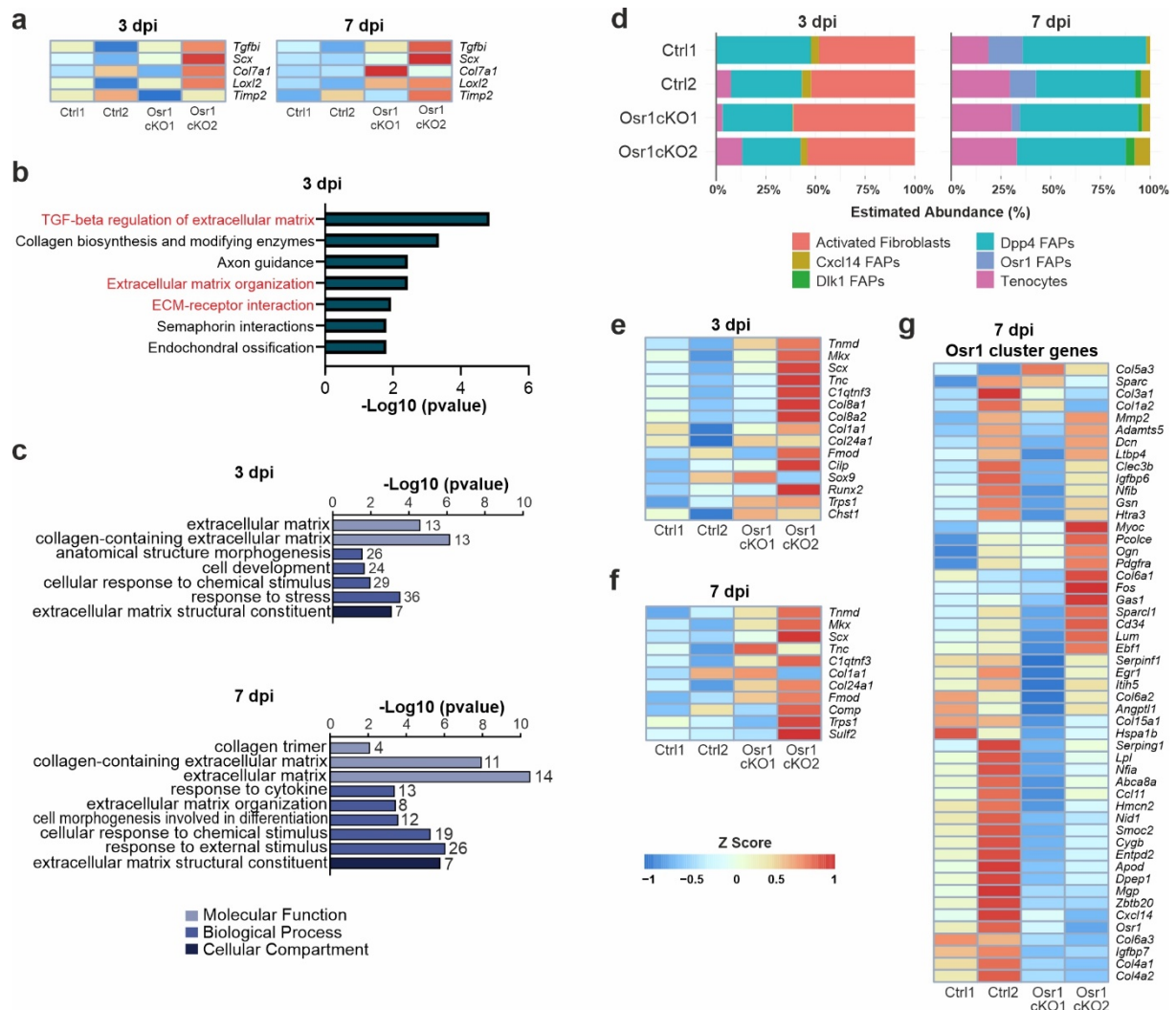
Supplementary Figure 7 Effects of FAP coculture and FAP conditioned medium on C2C12 cells, and upregulation of *Tgfb* genes in *Osr1*cKO FAPs.

a RT-qPCR analysis of *Osr1* mRNA expression in FAPs isolated at 7 dpi from contralateral muscle of injured control and *Osr1*cKO mice (n=3). **b** Immunolabeling for MHC to detect myotube formation from C2C12 cells co-cultured with control or *Osr1*cKO FAPs; quantification of fusion index is shown right (n=3). **c** Quantification of FAP numbers in transwell plates at the day of analysis. **d** Immunolabeling for MHC to detect myotube formation from C2C12 cells in differentiation medium or differentiation medium supplemented with control or *Osr1*cKO CM; quantification of fusion index is shown right (n=4). **e** Heat maps showing *Tgfb1,2* and 3 gene expression in control or *Osr1*cKO FAPs at 3 and 7 dpi. **f** RT-qPCR analysis of *Tgfb1,2* and 3 gene expression in *in vitro* recombined *Osr1*cKO FAPs (n=3). Data are mean $\pm$ SEM; P-value calculated by two-sided unpaired t-test; * $p < 0.05$, **** $p < 0.0001$. N-numbers indicate biological replicates (mice per genotype). Scale bars: 100 μ m.



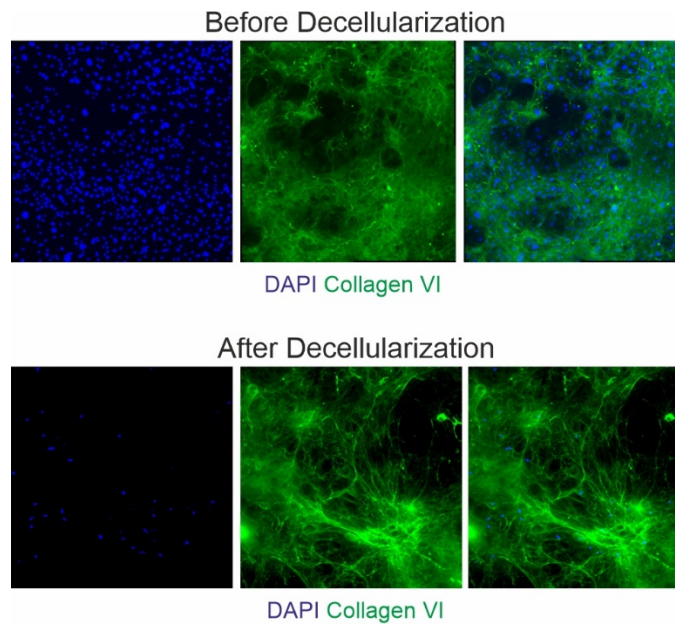
Supplementary Figure 8 Efficacy of TGF β pathway inhibition and effects of SB431542 in C2C12 cells.

a Western blot analysis of TGF β pathway activity in C2C12 cells cultured in DMEM and treated with increasing concentrations of SB431542 assessed by detection of phospho-Smad2 phospho-Smad3. **b, c** Western blot analysis of phospho-Smad2, Myogenin and myosin heavy chain (MF20) expression in C2C12 cells treated with recombinant TGF β 1 and SB431542. All blots of each panel are derived from the same experiment, **d** Immunolabeling for MHC to detect myotube formation from C2C12 cells in differentiation medium or differentiation medium supplemented with control or Osr1cKO CM, with or without TGF β pathway inhibitor SB431542; quantification of fusion index is shown right (n=3). Data are mean $\pm$ SEM; P-value calculated by ANOVA; ** p < 0.01, *** p < 0.001. N-numbers indicate biological replicates (mice per genotype). Scale bar: 100 μ m.



Supplementary Figure 9 Transcriptional fibrogenic shift of Osr1cKO FAPs.

a Heat maps showing TGFβ target gene expression in control or Osr1cKO FAPs at 3 and 7 dpi. **b** Bio planet 2019 pathway analysis of genes upregulated in 7 dpi Osr1cKO FAPs relative to controls. **c** GO terms analysis of the common deregulated genes between mdx FAPs and the 3 or 7 dpi Osr1cKO FAPs. **d** Deconvolution analysis of control and Osr1cKO FAP bulk transcriptome data on single cell sequencing data from Oprescu et al. **e, f** Heat maps showing upregulation of tendon- and cartilage-associated genes in Osr1cKO FAPs relative to controls. **g** Heat map depiction of signature genes characterizing the “Osr1” cluster in Oprescu et al.



Supplementary Figure 10 Efficiency of in vitro ECM deposition and the decellularization.

Supplementary Tables

Supplementary Table 1 Primers for RT-qPCR

	Forward	Reverse
<i>Pax7</i>	CGATTAGCCGAGTGCTCAGAA	CCAGACGGTTCCTTTGTCG
<i>Myod1</i>	CGCCACTCCGGGACATAG	GAAGTCGTCTGCTGTCTCAAAGG
<i>Myf5</i>	CAGCCCCACCTCCAAGT	GGGACCAGACAGGGCTGTTA
<i>Myog</i>	TCCAGTACATTGAGCGCCTAC	GCTGTGGGAGTTGCATTAC
<i>Osr1</i>	CCTGTATGGTTTCAGCGCTC	TGGCTTAGGGTGAATGACGT
<i>Gapdh</i>	CTGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTCT
<i>TGFβ1</i>	GACCCCCACTGATACGCCTG	GCGCTGAATCGAAAGCCCTG
<i>TGFβ2</i>	CCGGAGGTGATTTCCATCTA	GCGGACGATTCTGAAGTAGG
<i>TGFβ3</i>	GATGAGCACATAGCCAAGCA	ATTGGGCTGAAAGGTGTGAC
<i>CD68</i>	GGCGGTGGAATACAATGTGTCC	AGCAGGTCAAGGTGAACAGCTG
<i>CD86</i>	ACGTATTGGAAGGAGATTACAGCT	TCTGTCAGCGTTACTATCCCGC
<i>CD163</i>	GGCTAGACGAAGTCATCTGCAC	CTTCGTTGGTCAGCCTCAGAGA
<i>CD206</i>	GTTACCTGGAGTGATGGTTCTC	AGGACATGCCAGGGTCACCTTT
<i>Arg1</i>	AACACGGCAGTGGCTTTAACC	GGTTTTTCATGTGGCGCATTC
<i>Albumin</i>	CTGCAATCCTGAACCGTGT	TTCCACCAGGGATCCACTAC

Supplementary Table 2 Primary antibodies

Antibody	Clone	Conjugate	Concentration / Dilution	Source
Mouse anti-MHC3 (eMHC)	Monoclonal	Unconjugated	1:50	DSHB
Mouse anti-MF20 (MYH1)	Monoclonal	Unconjugated	1:100	DSHB
Mouse anti-Pax7 (supernatant)	Monoclonal	Unconjugated	1:20	DSHB
Guinea pig anti-Pax7	Polyclonal	Unconjugated	1:100	C. Birchmeier
Mouse anti-Ki67	Monoclonal	B56	1:100	BD Biosciences
Rabbit anti-Ki67	Polyclonal	Unconjugated	1:1000	Abcam
Rabbit anti-laminin	Polyclonal	Unconjugated	5 µg ml ⁻¹	Sigma-Aldrich
Goat anti-Collagen VI	Polyclonal	Unconjugated	1:200	Southern Biotechnology Associates
Mouse anti-MyoD	5.8A	Unconjugated	1:100	BD Biosciences
Mouse anti-fibronectin	Polyclonal	Unconjugated	1:500	Merck
Phalloidin		591/608	1:250	Thermo Fischer Scientific
Rabbit anti-tSmad2/3	D7G7	Unconjugated	1:1000	Cell Signaling
Rabbit anti-pSmad2	Ser465/467	Unconjugated	1:1000	Cell Signaling
Rabbit anti-pSmad3	Ser423/425	Unconjugated	1:1000	Cell Signaling
Rabbit anti-GAPDH	14C10	Unconjugated	1:2000	Cell Signaling
Mouse anti-myogenin	sc-12732	Unconjugated	1:100	Santa Cruz
Mouse anti-vinculin	V9131	Unconjugated	1:4000	Merck
Rabbit-anti-CD80	Polyclonal	Unconjugated	1:150	Invitrogen
Rat-anti-CD206	MR5D3	Unconjugated	1:150	Bio-Rad
Rabbit-anti-Perilipin A/B	Polyclonal	Unconjugated	1:300	Sigma-Aldrich
Goat-anti-PDGFRα	Polyclonal	Unconjugated	1:200	R & D systems

Supplementary Table 3 Secondary antibodies

Antibody	Conjugate(s)	Source
Donkey anti-mouse	Alexa Fluor 488, 568 and 680	Invitrogen
Donkey anti-rabbit	Alexa Fluor 488, 568 and 681	Invitrogen
Donkey anti-goat	Alexa Fluor 488, 568 and 682	Invitrogen
Goat anti-guinea pig	Alexa Fluor 682	Invitrogen

Supplementary Table 4 FACS antibodies

Antibody	Clone	Conjugate	Concentration/dilution	Source
Anti mouse CD31	390	APC conjugated	3 µg ml ⁻¹	Invitrogen
Anti mouse CD45	30-F11	APC conjugated	6 µg ml ⁻¹	Invitrogen
Anti mouse TER119	TER-119	APC conjugated	6 µg ml ⁻¹	Invitrogen
Anti rat α7-integrin	R2F2	PE conjugated	1.5 µg ml ⁻¹	Ablab
Anti mouse Ly-6A/E (Sca-1)	D7	APC-Cy7 conjugated	1.5 µg ml ⁻¹	Biolegend
CD45	30-F11	AF488	0.25 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD3e	145-2C11	PE-Cy7	0.4 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD19	6D5	PE-Cy7	0.2 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD335	29A1.4	PE-Cy7	0.6 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD11c	N418	PerCP-Cy5.5	0.4 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD11b/Mac-1	M1/70	BV510	0.4 µg ml ⁻¹	BioLegend, San Diego, CA, USA
Ly6-G	1A8	APC-Fire750	0.6 µg ml ⁻¹	BioLegend, San Diego, CA, USA
MHC class II	M5/114.15.2	BV785	0.05 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD80	16-10A1	BV650	0.2 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD86	GL-1	BV421	0.2 µg ml ⁻¹	BioLegend, San Diego, CA, USA
CD163	TNKUPJ	PE	0.1 µg ml ⁻¹	ThermoFisher, Waltham, MA, USA
VEGF	VG1	AF647	0.225 µg ml ⁻¹	Novus Biologicals, Littleton, CO, USA
CD206	C068C2	PE-Dazzle594	0.2 µg ml ⁻¹	BioLegend, San Diego, CA, USA