Figure Legends

Figure 1: Representative FACS profiles of quiescent and activated MuSCs. The digested tissue preparations, as described in this protocol, from uninjured or injured limb muscles were stained with PI, APC anti-mouse CD31, APC anti-mouse CD45, Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. Cells in the P4 gate are MuSCs. (A) Profiles of the unstained quiescent MuSCs. (B) Profiles of quiescent MuSCs after antibody staining. The population hierarchy is shown under the plots. (C) Profiles of the unstained activated MuSCs and their progeny. (D) Profiles of activated MuSCs and their progeny after antibody staining. The population hierarchy is shown under the plots.

Figure 2: Confirmation of the myogenicity of isolated MuSCs. (A) Immediately following FACS isolation, 5,000 quiescent MuSCs were plated in a well of an 8-well chamber slide that had been coated with Poly-D lysine and ECM. Cells were fixed with 4% paraformaldehyde 6 hours (6 hr), 3 days (3 d) and 5 days (5 d) following plating, and stained with antibodies against Pax7, MyoD1 and Myogenin, respectively. DAPI stains all nuclei. Note the changes in cell size and morphology. Scale bars represent 70 μm in the images and 10 μm in the inserts. (B) Immediately following FACS isolation, 5,000 activated MuSCs or their progeny from limb muscles that had been injured three days earlier were plated in a well of an 8-well chamber slide that had been coated with Poly-D lysine and ECM. Cells were fixed with 4% paraformaldehyde 6 hours later and stained with an anti-MyoD1 antibody. DAPI stains all nuclei. Scale bars represent 70 μm in the image and 10 μm in the insert.

Supplemental Figure 1: Validation of the VCAM1^{+}Sca1^{-}CD31^{-}CD45^{-} population from muscle as MuSCs using a genetic lineage tracer. Pax7$^{CreER/+}$; ROSA26$^{eYFP/+}$ mice were injected with tamoxifen at 8-10 weeks of age. Mononucleated cells were isolated from limb muscles of these mice at different ages (indicated in parentheses) in the absence of injury or at different times following the injury, as indicated. Cells were then stained with APC anti-mouse CD31, APC anti-mouse CD45, Pacific Blue anti-mouse Sca1 and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. Cells positive for CD31 and CD45 (CD31^{+}/CD45^{+}), cells positive for Sca1 but negative for CD31/CD45 (CD31^{-}/CD45^{-}, Sca1^{+}), and cells positive for

VCAM1 but negative for CD31/CD45/Sca1 ($CD31^-/CD45^-/Sca1^-$, $VCAM1^+$) were analyzed for YFP-expressing cells. The FITC channel (for the detection of YFP) is shown on the Y axis and FSC-S is shown on the X axis of all plots. qMuSCs: quiescent MuSCs; aMuSCs: activated MuSCs and their progeny; mo: months; DPI: days post-injury; MPI: months post-injury.

Supplemental Figure 2: Representative FACS profiles of quiescent and activated MuSCs without PI staining. The digested tissue preparations, as described in this protocol, from uninjured or injured limb muscles were stained with APC anti-mouse CD31, APC anti-mouse CD45, Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. Cells in the P4 gate are MuSCs. (A) Profiles of the unstained quiescent MuSCs. (B) Profiles of quiescent MuSCs after antibody staining. The population hierarchy is shown under the plots. (C) $VCAM1^+/CD31^-/CD45^-/Sca1^-$ SCs were isolated from a 2-month-old C57BL/6 mouse according to the protocol described. Cells were then incubated with 0.3 μg/ml PI and analyzed by FACS. (D) Profiles of the unstained activated MuSCs and their progeny. (E) Profiles of activated MuSCs and their progeny after antibody staining. The population hierarchy is shown under the plots. (F) $VCAM1^+/CD31^-/CD45^-/Sca1^-$ SCs were isolated from a 2-month-old C57BL/6 mouse injured by $BaCl_2$ injection according to the protocol described. Cells were then incubated with 0.3 μg/ml PI and analyzed by FACS for the percentage of PI-positive cells.

Supplemental Figure 3: Identification and isolation of different cell-types in skeletal muscle. (A) Distinct separation of mononucleated cells from myofiber debris following isolation from uninjured skeletal muscle. 2,000 events are shown in the FSC-A and SSC plot. The region of the plot containing cellular debris is highlighted in red. (B) Representative FACS profiles to isolate highly pure quiescent MuSCs (P6) and muscle-derived mesenchymal stem cells (P5) simultaneously. The suspension of resident mononucleated cells from uninjured limb muscles was stained with APC anti-mouse CD31, APC anti-mouse CD45, Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. 20,000 events were recorded and are shown in the plots. The population hierarchy is shown under the plots. Note the size difference of the P1 gate in comparison to that in Figure 1A and 1B. Mesenchymal stem cells are much larger than MuSCs and therefore a larger gate is needed to

acquire these cells. Therefore we recommend using the FSC-W parameter to further select for small cells within the $CD31^-/CD45^-/Sca1^-/VCAM1^+$ population (P4). (C) Typical FACS profiles to identify and isolate highly pure quiescent MuSCs (P8) and muscle-derived mesenchymal stem cells (P5), as well as populations enriched for hematopoietic (P6) and endothelial (P7) cells simultaneously. The suspension of resident mononucleated cells from uninjured limb muscles was stained with APC anti-mouse CD31, FITC anti-mouse CD45, Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. 20,000 events were recorded and are shown in the plots. The population hierarchy is shown under the plots. The FSC-W parameter further selected for small cells within the $CD31^-/CD45^-/Sca1^-/VCAM1^+$ population (P4) in order to maximize the purity of MuSCs.

Supplemental Figure 4: Typical FACS profiles for single channel staining controls. The suspension of resident mononucleated cells from uninjured limb muscles of a 2-month-old C57BL/6 mouse was stained with (A) APC anti-mouse CD31 and APC anti-mouse CD45, (B) Pacific Blue anti-mouse Ly-6A/E (Sca1), and (C) Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin. The population hierarchy is shown under the plots.

Supplemental Figure 5: Typical FACS profiles for Fluorescence Minus One (FMO) staining controls. The suspension of resident mononucleated cells from uninjured limb muscles of a 2-month-old C57BL/6 mouse was stained with (A) Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin, (B) APC anti-mouse CD31, APC anti-mouse CD45 and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin, and (C) APC anti-CD31, APC anti-mouse CD45 and Pacific Blue anti-mouse Ly-6A/E (Sca1). The population hierarchy is shown under the plots.

Supplemental Figure 6: Typical FACS profiles for staining using isotype control antibodies. The suspension of resident mononucleated cells from uninjured limb muscles of a 2-month-old C57BL/6 mouse was stained with (A) APC IgG2a, APC IgG2b, Pacific Blue anti-mouse Ly-6A/E (Sca1) and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin, (B) APC anti-mouse CD31, APC anti-mouse CD45, Pacific Blue IgG2a, and Biotin anti-mouse CD106 (VCAM1) followed by PE/Cy7 Streptavidin, and (C) APC anti-CD31, APC anti-mouse

CD45, Pacific Blue anti-mouse Ly-6A/E (Sca1), and Biotin IgG2a followed by PE/Cy7 Streptavidin. The population hierarchy is shown under the plots.

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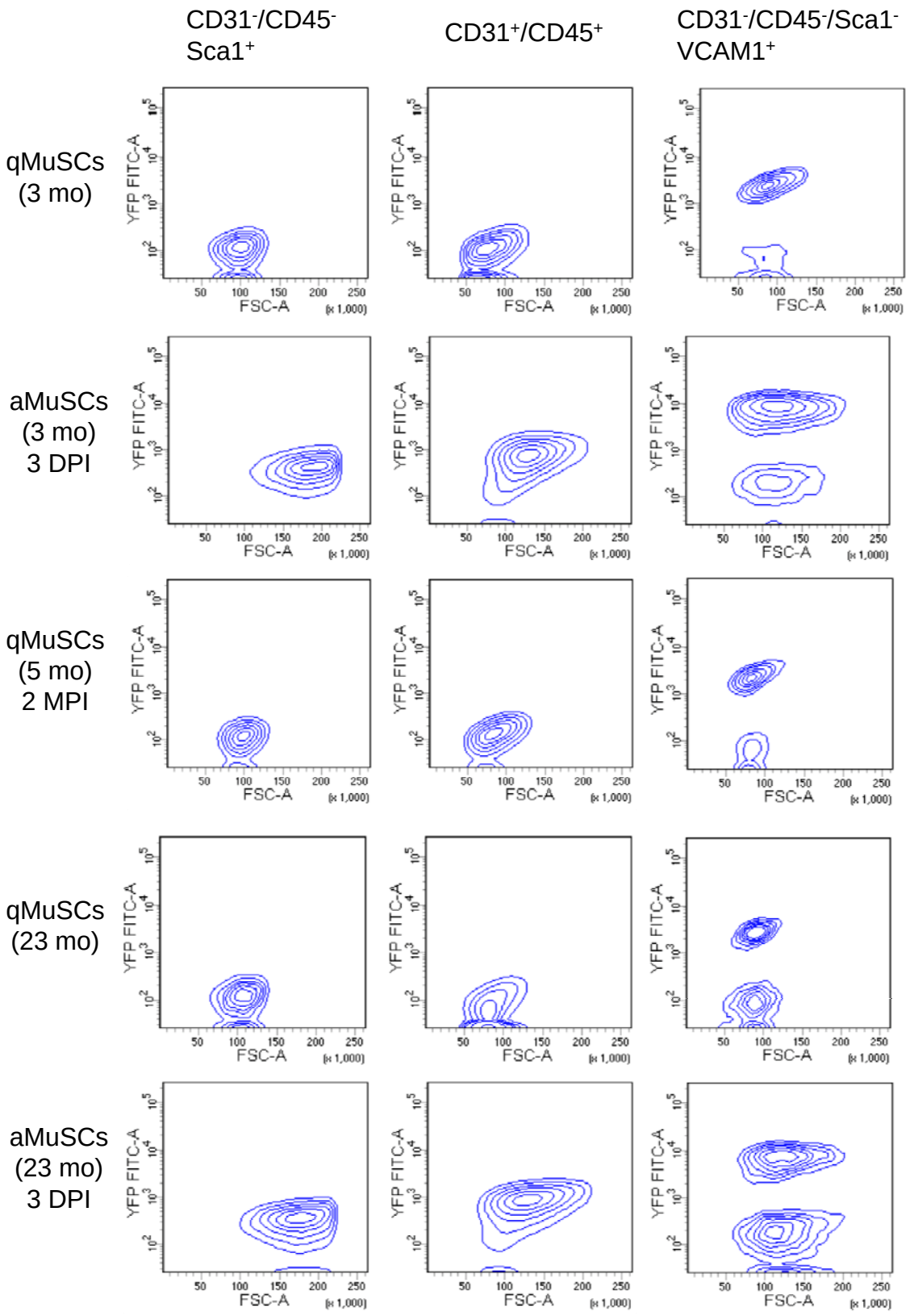
Author Contribution

All authors contribute to the design and interpretation of the experiments; L.L., T.H.C. and G.W.C. conducted the experiments; L.L. and T.A.R. wrote the manuscript.

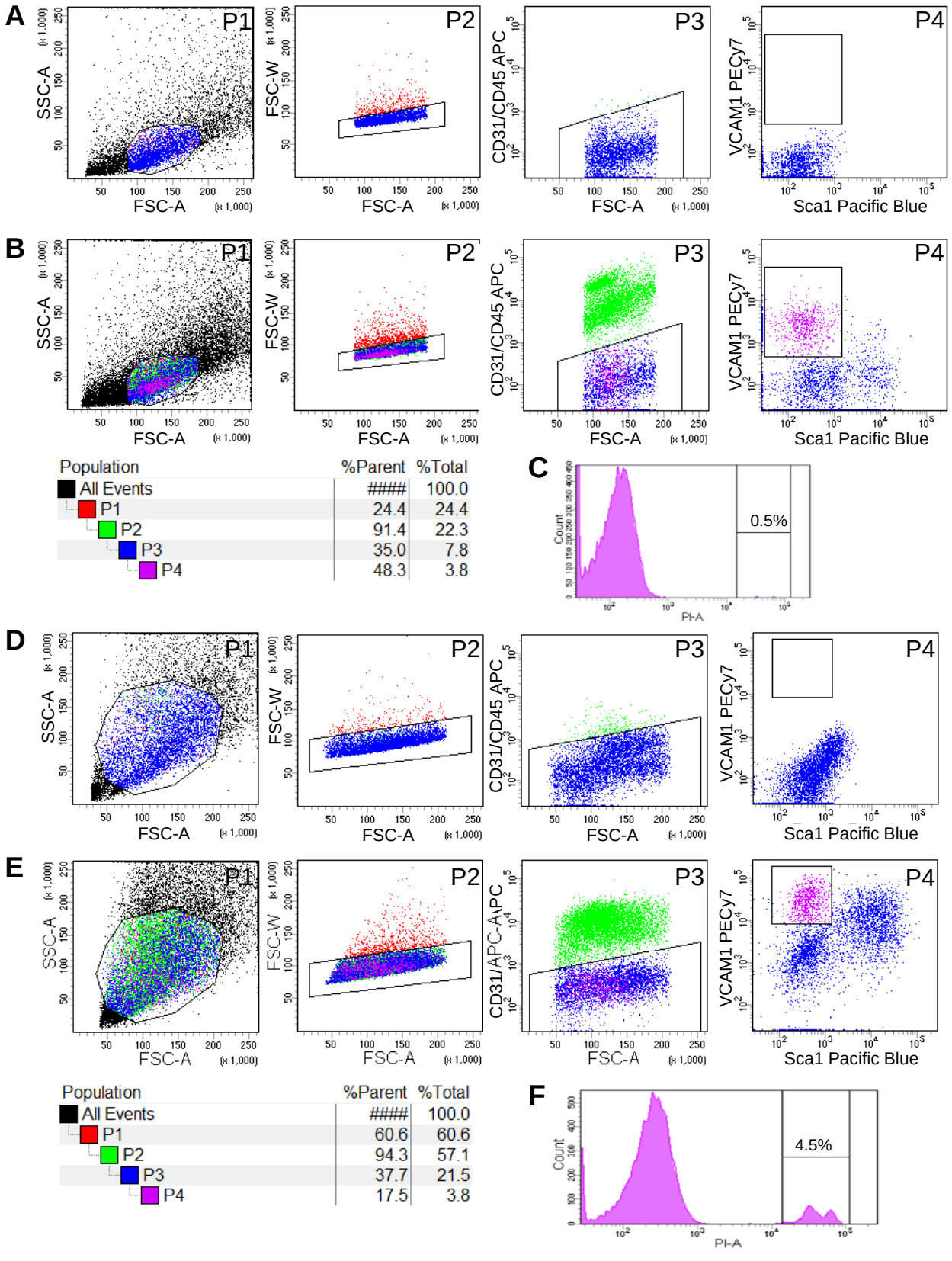
Competing Financial Interest

The authors declare no competing financial interests.

Supplemental Figure 1

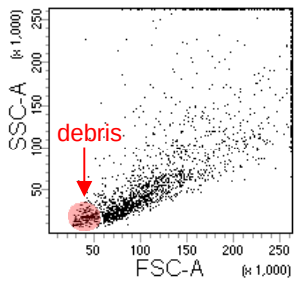


Supplemental Figure 2



Supplemental Figure 3

A



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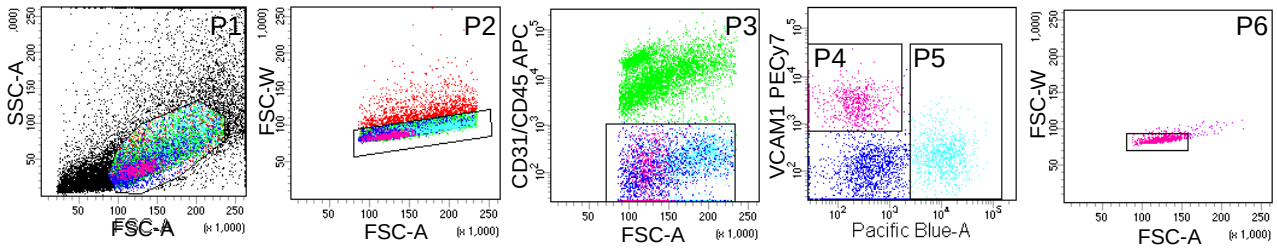
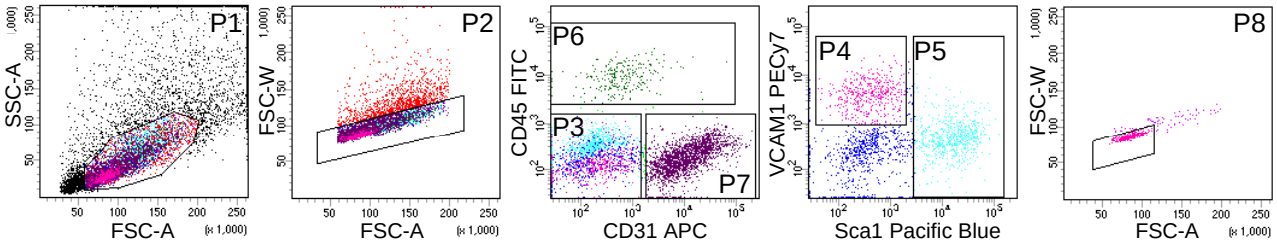


Population	%Parent	%Total
All Events	####	100.0
P1	58.5	58.5
P2	93.6	54.8
P3	39.2	21.5
P4	19.9	4.3
P6	85.3	3.6
P5	26.0	5.6

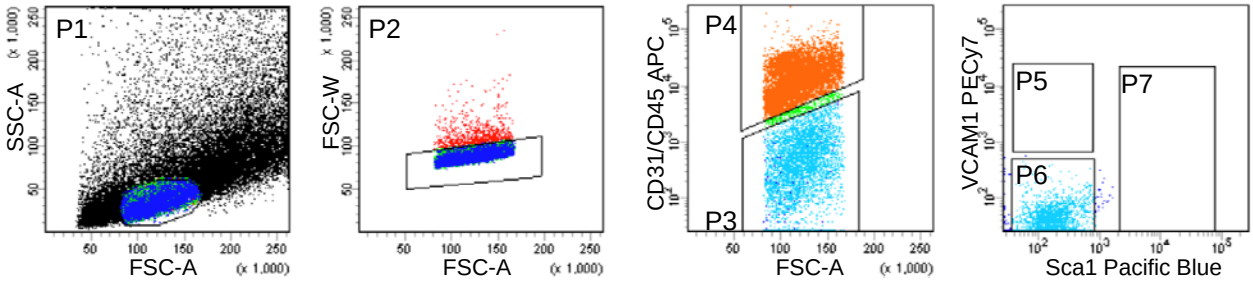
C



Population	%Parent	%Total
All Events	####	100.0
P1	54.1	54.1
P2	80.3	43.4
P3	43.2	18.8
P4	23.7	4.4
P8	84.3	3.8
P5	36.3	6.8
P6	5.8	2.5
P7	50.3	21.9

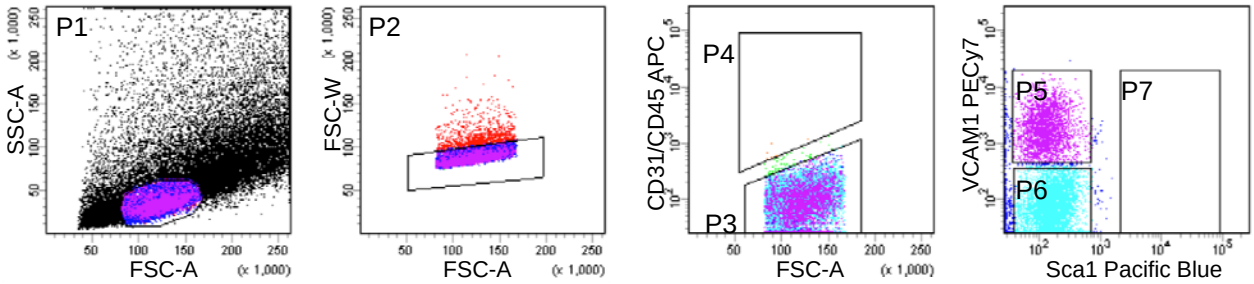
Supplemental Figure 4

A



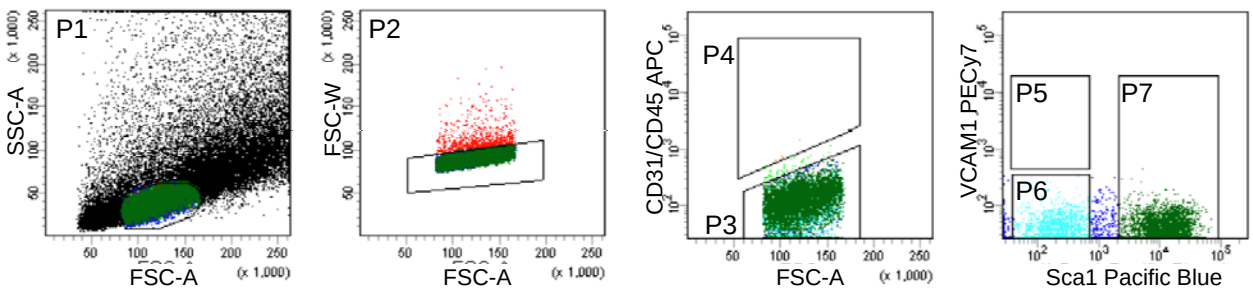
Population	%Parent	%Total
All Events	####	100.0
P1	25.5	25.5
P2	93.8	23.9
P3	32.4	7.7
P5	0.0	0.0
P6	95.1	7.4
P7	0.0	0.0
P4	65.0	15.6

B



Population	%Parent	%Total
All Events	####	100.0
P1	24.1	24.1
P2	92.7	22.3
P3	99.4	22.2
P5	21.4	4.8
P6	66.4	14.7
P7	0.0	0.0
P4	0.0	0.0

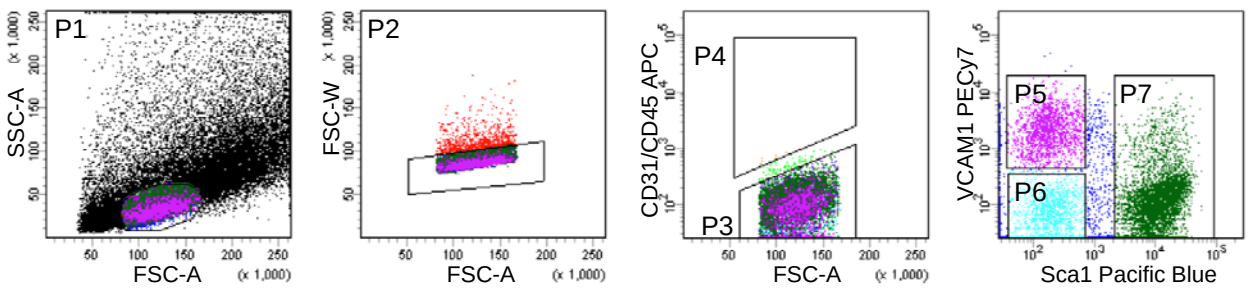
C



Population	%Parent	%Total
All Events	####	100.0
P1	24.0	24.0
P2	93.2	22.3
P3	99.6	22.2
P5	0.0	0.0
P6	34.9	7.8
P7	57.1	12.7
P4	0.0	0.0

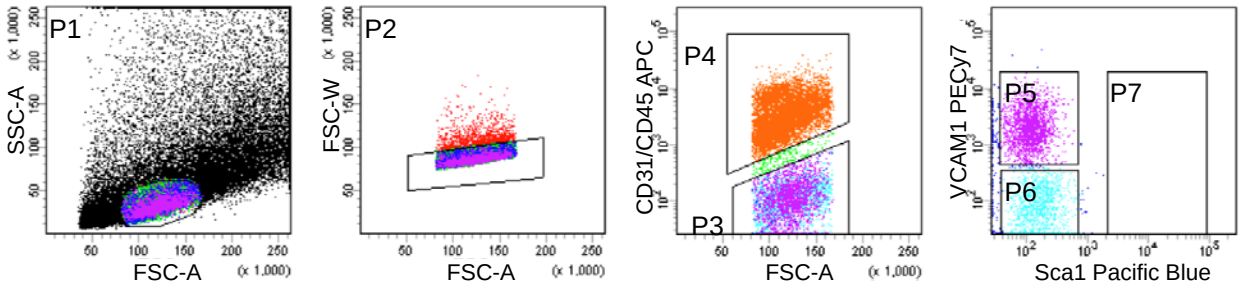
Supplemental Figure 5

A



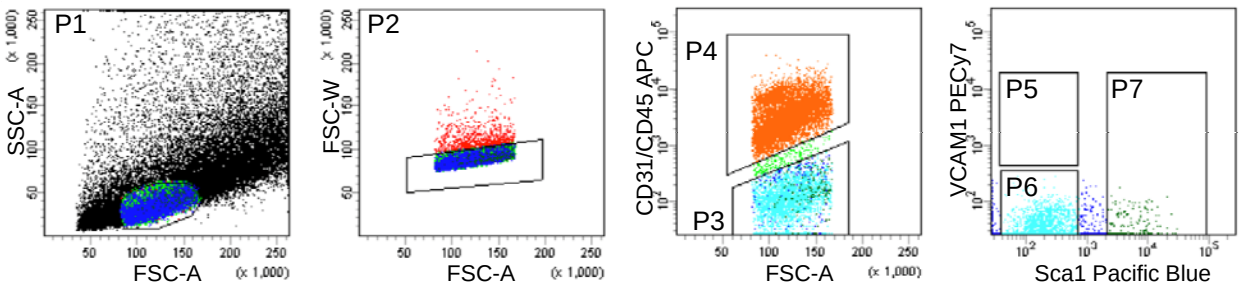
Population	%Parent	%Total
All Events	####	100.0
P1	23.7	23.7
P2	93.0	22.1
P3	99.3	21.9
P5	15.1	3.3
P6	22.4	4.9
P7	54.0	11.9
P4	0.0	0.0

B



Population	%Parent	%Total
All Events	####	100.0
P1	24.3	24.3
P2	91.4	22.2
P3	34.4	7.6
P5	46.5	3.6
P6	47.2	3.6
P7	0.0	0.0
P4	63.8	14.2

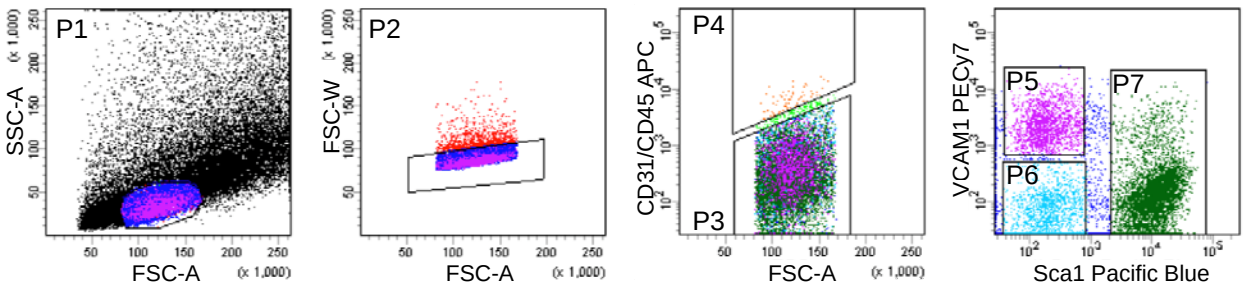
C



Population	%Parent	%Total
All Events	####	100.0
P1	23.3	23.3
P2	93.1	21.7
P3	33.9	7.4
P5	0.0	0.0
P6	83.0	6.1
P7	4.3	0.3
P4	64.2	13.9

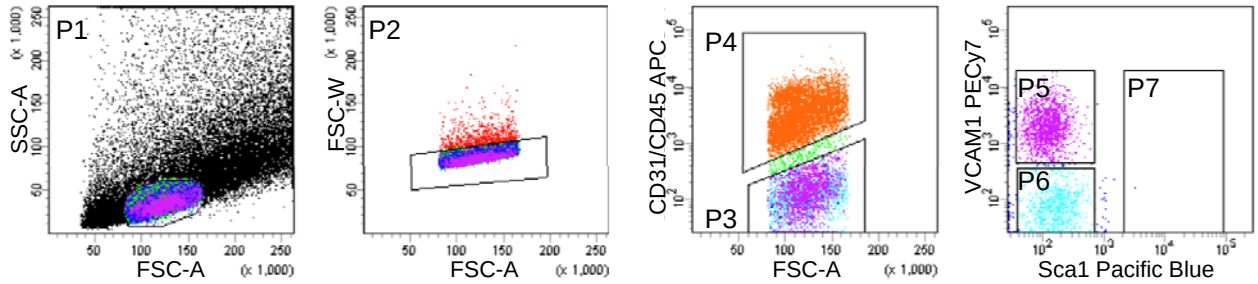
Supplemental Figure 6

A



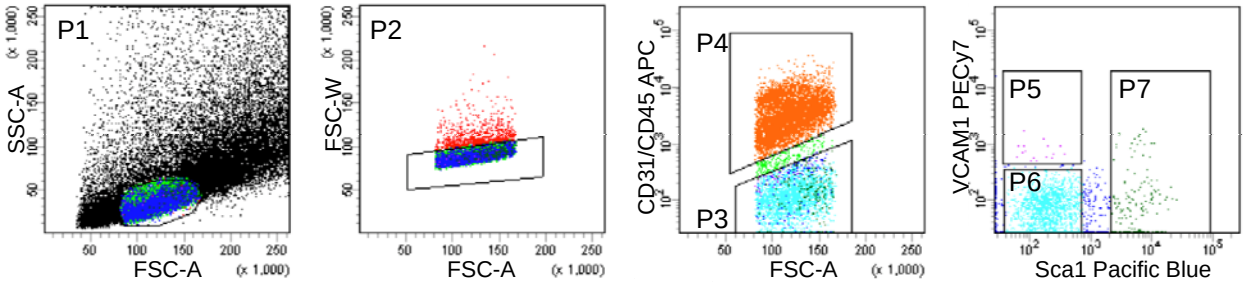
Population	%Parent	%Total
All Events	####	100.0
P1	22.3	22.3
P2	91.8	20.5
P3	98.0	20.1
P5	14.3	2.9
P6	20.6	4.1
P7	57.1	11.5
P4	0.8	0.2

B



Population	%Parent	%Total
All Events	####	100.0
P1	22.7	22.7
P2	92.4	21.0
P3	32.6	6.8
P5	44.8	3.1
P6	49.3	3.4
P7	0.0	0.0
P4	65.1	13.7

C



Population	%Parent	%Total
All Events	####	100.0
P1	20.4	20.4
P2	91.7	18.7
P3	32.4	6.1
P5	0.8	0.0
P6	82.6	5.0
P7	4.8	0.3
P4	65.4	12.3