Supplementary Materials for

Helminth egg derivatives as pro-regenerative immunotherapies

David R. Maestas Jr.[1], Liam Chung[1,2], Jin Han[1], Xiaokun Wang[1], Sven D. Sommerfeld[1], Sean H. Kelly[1], Erika Moore[1,3], Helen Hieu Nguyen[1], Joscelyn C. Mejías[1], Alexis N. Peña[1], Hong Zhang[1], Joshua S. T. Hooks[1], Alexander F. Chin[1], James I. Andorko[1,2], Cindy Berlicke[1], Kavita Krishnan[1], Younghwan Choi[1], Amy E. Anderson[1], Ronak Mahatme[1], Christopher Mejia[1], Marie Eric[1], JiWon Woo[1], Sudipto Ganguly[2,4], Donald J. Zack[5], Liang Zhao[2,4], Edward J. Pearce[2,4,6], Franck Housseau[2,4], Drew M. Pardoll[2,4], and Jennifer H. Elisseeff[1,2,4*]

[1]Translational Tissue Engineering Center, Johns Hopkins University, Baltimore, MD 21287, USA

[2]Bloomberg~Kimmel Institute for Cancer Immunotherapy and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

[3]Materials Science and Engineering, University of Florida, Gainesville, FL, USA

[4]Department of Oncology and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[5]Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

[6]Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21287, USA.

***** Corresponding author. Email: jhe@jhu.edu

This PDF file includes:

Materials and Methods

Mice

Mice were housed and maintained in the Johns Hopkins Cancer Research Building animal facility in compliance with ethical guidelines outlined by the Animal Care and Use Committee (ACUC). All procedures performed on animals were approved by Johns Hopkins ACUC. Investigators involved with the studies were blinded whenever possible. All mice used in these studies were maintained as Helicobacter negative.

Mouse Strain	Alternative Name	Background	Company	Stock #
C57BL/6J	-	-	The Jackson Laboratory	000664
BALB/cJ	-	-	The Jackson Laboratory	000651
4Get	C.129-Il4^{tm1Lky}/J	BALB/cJ	The Jackson Laboratory	004190
ΔdblGATA	C.129S1(B6)-Gata1^{tm6Sho}/J	BALB/cJ	The Jackson Laboratory	005653

Volumetric Muscle Loss (VML) Model

The VML injury was performed in female mice as a bilateral surgical removal of the quadriceps femoris as previously described. A unilateral longitudinal incision measuring approximately 1.5 cm in length was made in the epidermis, dermis, and the underlying fascia above the muscle. Using sterilized microdissection scissors, a 3 mm x 4 mm x 4 mm full thickness segment of skeletal muscle was resected from each hindlimb. The remaining defect space was filled with 50 µL to 75 µL of vehicle (1X DPBS or treatment). Immediately after treatment the epidermis and dermis were closed using a wound clipper with 7 mm sterile wound clips (Roboz, USA).

Anterior Cruciate Ligament Transection (ACLT) Model and Joint Evaluation

Post-traumatic osteoarthritis (PTOA) was induced in male mice by utilizing an anterior cruciate ACLT injury model in 10-week-old male C57BL/6j mice. SEA, rSEA, and various components were administered to the joint space of the operated knee via a 30-gauge needle or intraperitoneally. The joint cavity was opened in the sham group, but the ACL was not transected. Weight-bearing in mice was measured in the un-operated control animals and compared to ACLT animals receiving PBS control or rSEA therapy using an incapacitance tester (Columbus Instruments). The percentage if weight distributed on the ACLT limb was used as an index of joint discomfort in OA (*55*). The mice were positioned to stand on their hind paws in an angled box placed above the incapacitance tester so that each hind paw rested on a separate force plate. The force (g) exerted by each limb was measured. Three consecutive 3-sec readings were taken and averaged to obtain the mean score (*64*). To determine pain response times in post-injury and treated animals, mice were placed on an enclosed hotplate set to 55 ºC. The latency period for hind limb response (marked as jumping or paw-licking) was recorded as the response time before surgery and 4 weeks after surgery in all animal groups (*55*). At least three readings were taken per mouse and averaged to obtain the mean response time for each time point. After 4 weeks, animals were sacrificed, and mouse knees were fixed in 10 % neutral-buffered formalin, decalcified for approximately 2 weeks in 10% EDTA at 4°C, step-wise dehydrated in EtOH, cleared in xylenes, and embedded in paraffin. 7 µm sections were taken throughout the joint, dried, and stained for proteoglycans with Safranin-O and Fast Green (Applied biosciences) per manufacturer's instructions. Evaluation of the cartilage damage was performed according to the Osteoarthritis Research Society International (OARSI) scoring system and was performed by blinded histological assessment the medial plateau of the tibia (*65*). Osteophytes on the tibial plateau were scored from 0 to 3, with 0 indicating no osteophytes or an osteophyte up to 100 µm

in diameter; a score of 1 indicating an osteophyte measuring 100 μm to 200 μm in diameter; a score of 2 indicating an osteophyte of 200 μm to 300 μm in diameter; and a score of 3 indicating an osteophyte measuring more than 300 μm in diameter (*64, 65*).

Corneal debridement surgery and scar quantification

All surgical procedures were performed under the guideline of the Johns Hopkins University Animal Care and Use Committee (ACUC). Male adult (8-12 weeks old) BALB/c mice, GATA1 KO mice, and IL4-IRES-eGFP (4get) mice were purchased from Jackson Labs. The corneal debridement wound was adapted with minor modifications from Stepp et al., 2014(*56*). Mice were weighed and anesthetized with 90 mg/kg ketamine HCl (VetOne) with10 mg/kg Xylazine HCl (VetOne) by injection. Proparacaine hydrochloride ophthalmic eye drops (Sandoz) were applied after the mice were sedated. The center area of the cornea was marked by a 1.5 mm biopsy punch, and the epithelium layer was removed within the area by a 1.5 mm flat blade (Fine Science Tools). After epithelium removal, a volume of 50 μL PBS solution with or without rSEA, were injected to the subconjunctival space of the wounded eye. After injection, drops of sterile PBS solution were applied to both wounded and unwounded eyes to keep the eyes moist until the mice were recovered from anesthesia. At 14 days post-surgery, the mice were euthanized, and the eye globes were collected. The picture of each globe was taken under surgical microscope (Nikon), and the scar areas and cornea areas were determined with ImageJ. The ratio was quantified as: scar ratio = A_s (scar area)/ A_c (whole corneal area).

S. mansoni egg collection and isolation of SEA

S. mansoni (from infected NMRI mice) reagents were provided by the NIAID Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD) through NIH-NIAID Contract HHSN272201700014I, supplied frozen at -80°C. Standard SEA was prepared according to standard operating procedures utilized by the center and based on Boros *et. al* (*28*). After thawing on ice in the dark, eggs were re-suspended in 4°C 1X DPBS at a concentration of 100,000 eggs/mL and were homogenized on ice using a motorized pestle, or with a 2 mL dounce homogenizer (Kimble, USA). 95% to 100% of the eggs were disrupted, verified by visualization with a phase contrast microscope. The crude mixture was then centrifuged at 4°C at 200 x g for 45 minutes. The supernatant was retrieved and ultracentrifuged for 90 min at 100,000 x g at 4°C. The entirety of the final supernatant was passed through a 0.22 μm sterile filter and stored at -80°C. Concentrations were determined using standard Bradford assays and the Qubit™ Protein Assay Kit (Invitrogen).

Isolation of rSEA formulations

S. mansoni eggs were homogenized to isolate SEA as stated above with several modifications in the extraction process to generate rSEA. Initially the eggs are homogenized to 95% to 100%, verified by phase contrast microscopy. Centrifuged at 21,000 x g for 45 minutes, then ultra-centrifuged at 100,000 x g at 4°C for 90 min. After ultra-centrifugation, an insoluble mixture that forms at the top layer is harvested and stored in sterile low-protein binding 1.5 mL Eppendorf tubes. The top half of the resulting soluble antigen volume is carefully removed by pipet, and sterile filtered using a low-protein binding 0.2 μm filter into a low-protein binding 1.5 mL tube. 900 μL of the soluble fraction is then combined with 100 μL of the lipid fraction that was sterile filtered using lipid 1.2 μm medical Supor disc filters (B Braun Medical, USA). The final mixture is then stored at -80°C. Concentrations were determined using standard Coomassie Bradford assays (ThermoFisher) and the Qubit Protein Assay Kit (Invitrogen).

Lipid analysis

Protein concentration of SEA and rSEA samples were measured prior to lipid analysis using a Qubit Protein Assay Kit (Invitrogen, Q33211). SEA or rSEA solutions at 2 mg/mL protein concentration were mixed 1:1 with chloroform, vortexed, and centrifuged at 15,000 x g for 1 minute at 4 °C. The organic phase of the resulting mixture was removed, dried with nitrogen gas, and resuspended in a 2:1:1 mixture of isopropyl alcohol/acetonitrile/water for LC-MS. Reversed-phase HPLC was performed with a C18 column (Phenomenex, 00D-4726-AN), MS was performed with a Bruker timsTOF Pro instrument, and post-run analysis was performed with Bruker MetaboScape software.

Preparation of decellularized extracellular matrix from porcine small intestines

Decellularized extracellular matrix (ECM) was produced from porcine small intestinal sub-mucosa (SIS) following procedures developed under Stephen Badylak and described in Keane, et al., with minor modifications (*66*). Fresh porcine small intestines were obtained from Wagner Meats (Maryland, USA), harvested from a five-year old animal. The tissues were thoroughly washed to remove debris and mechanically processed to remove mucosal, serosal, and muscular layers by scraping with sterile pyrogen-free plastics. The resulting tissue identified as SIS include the submucosa and basilar layers of the tunica mucosa, was treated using 0.1 % peracetic acid (Sigma Aldrich) and 4 % EtOH prepared type-1 sterile water in pyrogen-free plastics for 2 hrs while stirring. The ECM was then returned to neutral pH using serial washes of quality-1 water and sterile culture grade 1X DPBS. Upon return to neutral pH the samples were lash frozen in liquid nitrogen and lyophilized. All tissues were then cryo-milled in liquid nitrogen to particle mesh sizes approximately < 400 μm and stored at -20°C until use.

Preparation of vitrified extracellular matrix hydrogels (Vitrigels)

To enable delivery of rSEA, a vitrified ECM was utilized to combine the benefits of ECM biomaterials with rSEA for enhancing pro-regenerative outcomes. SIS-ECM vitrigels were verified to enable a measurable release of biological payloads while acting as an immunomodulator for immune type 2 responses. SIS-ECM is digested with 1 mg of pepsin (Sigma Aldrich) and 10 mg of ECM with 0.01 HCl in type-1 water, covered, and stirred for 48 hrs at room temperature. The working solution is then cooled on ice and neutralized with 1 mL of a 60 mM HEPES and 0.05 mM NaOH solution, and therapeutics like rSEA are added immediately after the neutralization is confirmed by a pH of 7. Gelation is then allowed to occur at 37°C for 2 hrs and then placed into a 40 °C vitrification chamber for 7 days. Just before implantation, the vitrified gels are hydrated with 100 μL to 200 μL and trimmed into 2 mm x 2mm pieces.

Gene expression tissue processing and qRT-PCR

Harvested tissues were immediately placed into RNALater for at least 24 hrs at 4°C, transferred into TRIzol reagent (Thermo Fisher Scientific), flash frozen, and stored in a -80˚C freezer. For mRNA isolation, samples were homogenized using a Bead Ruptor 12 (OMNI International) using the highest speed setting for 3 rounds of 15 secs with 2.8 mm ceramic beads (OMNI International). RNA was isolated from whole tissue using TRIzol reagent and chloroform extraction. RNA was purified using Qiagen's RNeasy PLUS kits (mini-kit and micro-kit), with gDNA eliminator columns. All qRT-PCR was performed using TaqMan Gene Expression Master Mix (Applied Biosystems) and TaqMan probes according to manufacturer's instructions. Briefly, 2.5 μg of mRNA was used to synthesize cDNA using Superscript IV VILO Master Mix

(ThermoFisher Scientific) utilizing manufacturer guidelines with a C1000 Touch Thermocycler (Bio-Rad). The cDNA concentration was set to 100 ng/well (in a total volume of 20 µL qRT-PCR reaction). The qRT-PCR reactions were performed on the StepOne Plus Real-Time PCR System and software (Applied Biosystems, ThermoFisher Scientific), as TaqMan single-plex FAM-MGB assays, TaqMan Gene Expression Master Mix, using manufacturer recommended settings for quantitative and relative expression. All qRT-PCR reactions were performed in 96-well MicroAmp Fast Optical Plates (Life Sciences). For tissue samples, *B2m*, *Rer1*, *Hprt*, and *Ppia* were used as endogenous controls (reference housekeeping genes), with samples normalized to the most stable endogenous control. Samples were normalized to vehicle treated (saline) controls, unless otherwise stated. All qRT-PCR data was analyzed using the Livak Method, wherein ΔΔCt values are calculated and reported as relative quantification values (RQ), established by the result of the $2^{-\Delta\Delta Ct}$ calculation (*67*). These results were further verified by analysis using the appliedbiosystems relative quantification online software (Thermo Fisher Scientific, ver. 2020.2.1-Q2-20-build4). RQ, same as fold-change (FC), values are represented by the geometric means with error bars representing the geometric standard deviation or by Log_2(FC) wherein the data are displayed linearly as means with the error bars representing standard deviation. All qRT-PCR assays were completed within the laboratory at Johns Hopkins by the authors involved with the study.

Murine TaqMan gene expression assay probes

Probe:	Assay ID:	Probe:	Assay ID:	Probe:	Assay ID:
B2m	Mm00437762_m1	***Gata3***	Mm00484683_m1	***Lyve1***	Mm00475058_m1
Acan	Mm00545794_m1	***Hprt***	Mm03024075_m1	***Myf5***	Mm00435125_m1
Acta2	Mm00725412_s1	***Igf1***	Mm00439560_m1	***Mymk***	Mm00481256_m1
Areg	Mm00437583_m1	***Ifng***	Mm01168134_m1	***Myod1***	Mm00440387_m1
Cd31	Mm01242584_m1	***Il1b***	Mm00434228_m1	***Myog***	Mm00446194_m1
Cd36	Mm00432403_m1	***Il4***	Mm00445259_m1	***Pax7***	Mm01354484_m1
Col1a1	Mm00801666_g1	***Il5***	Mm00439646_m1	***Ppia***	Mm02342430_g1
Col2a1	Mm01309565_m1	***Il6***	Mm00446190_m1	***Prg4***	Mm01284582_m1
Col3a1	Mm00802300_m1	***Il13***	Mm00434204_m1	***Ptgs2***	Mm00478374_m1
Col4a2	Mm00802386_m1	***Il17a***	Mm00439618_m1	***Rer1***	Mm00471276_m1
Col5a1	Mm01332580_m1	***Il17f***	Mm00521423_m1	***Tgfb2***	Mm00436955_m1
Col6a5	Mm01231908_m1	***Il23a***	Mm00518984_m1	***Vegfc***	Mm00437310_m1

Tissue preparation and flow cytometry

Tissue samples were obtained by cutting the quadriceps femoris muscle from the hip to the knee. Tissues were finely diced and digested for 45 min at 37ºC with 1.67 Wünsch U/mL (5 mg/mL) of Liberase TL (Roche Diagnostics, Sigma Aldrich) and 0.2 mg/mL DNase I (Roche Diagnostics, Sigma Aldrich) in RPMI-1640 medium supplemented with L-Glutamine and 15 mM HEPES (Gibco). The digested tissues were ground through 70 µm cell strainers (ThermoFisher Scientific) with excess RPMI-1640 (supplemented as before), and then washed twice with 1X DPBS. A discontinuous Percoll (GE Healthcare) density gradient centrifugation was used to enrich the leukocyte fraction (80%, 40%, and 20% layers) and to remove blood and debris from the muscle samples, centrifuged at 2,100 xg for 30 min with the lowest acceleration, no brake, at room temperature. For intracellular staining, cells were stimulated for 4 hrs with Cell Stimulation Cocktail Plus Protein Transport Inhibitors (eBioscience) diluted in complete culture media (RPMI-1640 supplemented with 10% FBS, 15 mM HEPES, and 5 mM Sodium pyruvate). Cells were washed and surface stained, followed by fixation/permeabilization (Cytofix/Cytoperm, BD), and then stained for intracellular markers. Flow cytometry was performed using Attune NxT Flow Cytometer (ThermoFisher Scientific). Gating schemes are

provided in **fig. S18**. The enriched cells were washed and stained with the antibody panels as shown below.

Mouse Pan-Immune Surface

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	Ly6G	PacBlue	1-250	1A8	Biolegend
VL2	Ly6C	BV510	1-250	HK1.4	Biolegend
VL3	CD45	BV605	1-150	30-F11	Biolegend
VL4	CD8a	BV711	1-300	53-6.7	Biolegend
BL1	CD4	FITC	1-250	GK1.5	Biolegend
BL3	CD19	PerCP-Cy5.5	1-250	6D5	Biolegend
GL1	CD3	PE	1-300	17A2	Biolegend
GL2	SiglecF	PE-CF594	1-350	E50-2440	BD
GL4	F4/80	PE-Cy7	1-400	BM8	Biolegend
RL1	CD11c	APC	1-250	N418	Biolegend
RL2	CD11b	AF700	1-400	M1/70	Biolegend
RL3	Viability	eFlour780	1-1000	NA	ThermoFisher

Mouse Intracellular Cytokine Staining

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	Foxp3	BV421	1-500	MF-14	Biolegend
VL2	CD45	V500	1-150	30-F11	BD
VL3	NK1.1	BV605	1-300	PK136	Biolegend
VL4	CD8a	BV711	1-300	53-6.7	Biolegend
BL1	CD3	AF488	1-200	GK1.5	Biolegend
BL3	CD19	PerCpCy5.5	1-200	6D5	Biolegend
GL1	IL-4	PE	1-200	11B11	Biolegend
GL2	γδ	PE-594	1-300	GL3	Biolegend
GL4	CD4	PE-Cy7	1-300	GK1.5	Biolegend
RL1	IFNγ	APC	1-200	XMG1.2	Biolegend
RL2	IL-17a	AF700	1-200	TC11-18H10.1	Biolegend
RL3	Viability	eFlour780	1-1000	N/A	Thermo

Mouse 4get IL4:GFP Pan-Immune

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	SiglecF	BV421	1-200	E50-2440	Biolegend
VL2	Ly6c	BV510	1-150	HK1.4	Biolegend
VL3	CD45	BV605	1-150	30-F11	Biolegend
VL4	CD8	BV711	1-200	53-6.7	Biolegend
BL1	IL-4	GFP	N/A	N/A	Biolegend
BL2	CD19	BB700	1-250	1D3	Biolegend
GL1	CD4	PE	1-300	11B11	Biolegend
GL2	MHCII	PE-594	1-250	M5/114.15.2	Biolegend
GL4	F4/80	PE-Cy7	1-200	EMR1	Biolegend
RL1	CD206	APC	1-250	C068C2	Biolegend
RL2	CD11b	AF700	1-400	M1/70	Biolegend
RL3	Viability	Fixable Yellow	1000	N/A	Thermo

Mouse Myeloid Panel

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	CD86	BV421	1-300	GL1	Biolegend
VL2	Ly6c	BV510	1-300	HK1.4	Biolegend
VL3	CD45	BV605	1-150	30-F11	Biolegend
VL4	CD11b	BV711	1-400	M1/70	Biolegend
BL1	CD9	FITC	1-200	MZ3	Biolegend
BL3	-	-	-	-	-
GL1	CD301b	PE	1-250	URA-1	Biolegend
GL2	MHCII	PE-594	1-250	M5/114.15.2	BD
GL4	F4/80	PE-Cy7	1-250	EMR1	Biolegend
RL1	CD206	APC	1-250	C068C2	Biolegend
RL2	CD11c	AF700	1-200	M1/70	Biolegend
RL3	Viability	eFlour780	1-1000	NA	ThermoFisher

Fluorescence activated cell sorting (FACS)

T cells (Live $CD45^{+}CD11b^{-}CD3^{+}$Singlets) and macrophages (Live $CD45^{+}CD11b^{+}CD3^{-}F4/80^{Hi}$ Singlets) were sorted from quadriceps femoris muscles 1-week post-injury. Tissue processing is the same as described above for flow cytometry, but without Percoll isolation. Only viability and surface staining were performed for FACS, and these experiments were performed using a BD FACSAria Fusion SORP. The cell sort gating scheme is provided in **fig. S17.** Antibody clones and dilutions utilized for the sort are listed in the table below:

Marker	Fluorophore	Dilution	Clone	Source
CD31 Lin^{-}	BV421	1-100	390	Biolegend
CD19 Lin^{-}	BV421	1-400	1A9	BioLegend
Viability	AQUA	1-1000	HK1.4	ThermoFisher
CD45	BV605	1-150	30-F11	Biolegend
CD11b	BV711	1-300	53-6.7	Biolegend
CD3	AF488	1-200	GK1.5	Biolegend
CD34	PerCP-Cy5.5	1-100	6D5	Biolegend
F4/80	PE-Cy7	1-250	BM8	Biolegend
CD11b	AF700	1-400	M1/70	Biolegend
CD29	APC-Cy7	1-100	HMβ1-1	Biolegend

Immunofluorescence staining and imaging

Dystrophin (rabbit anti-mouse monoclonal antibody, clone EPR21189, Abcam, dilution: 1:1000) was stained using tyramide signal amplification (TSA) method with Opal-650. Briefly, after blocking with bovine serum albumin, the first primary antibody was incubated at room temperature for 30 mins, followed by 30 mins of incubation with HRP polymer conjugated secondary antibody, and 10 mins of Opal-650. Slides were then counterstained with DAPI for 5 mins before being mounted using DAKO mounting medium. Imaging of the histological samples was performed on a Zeiss AxioObserver.Z2 and images were stitched on Zen Blue software.

Cornea tissue processing and flow cytometry

Wounded corneas were collected from each experimental group (Saline vs. rSEA), 4-5 corneas were pooled for one "flow cytometry sample". Cornea samples were processed similar to what is described in Ogawa, et al., with minor modification(*68*). Briefly, corneas in each group were digested in RPMI-1640 media containing 0.5 mg/mL Liberase TL (Sigma Aldrich) + 0.2 mg/mL DNase I (Roche) for 45 min while gently rocking. Digested tissues were ground through 70 µm cell strainers and digestion stopped with FBS supplemented RPMI-1640. Cell suspensions were centrifuged, washed, and each cell pellet was resuspended in 200 µL 1X DPBS for staining and blocked with anti-mouse CD16/32 TruStain FcX (BioLegend) per manufacturer recommendations. The antibodies used were listed below:

Mouse Cornea 4get IL4:GFP Pan-Immune

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	Ly6g	Pacific Blue	1-250	1A8	Biolegend
VL2	Ly6c	BV510	1-250	HK1.4	Biolegend
VL3	CD45	BV605	1-100	30-F11	Biolegend
VL4	CD8	BV711	1-200	53-6.7	Biolegend
BL1	IL4-GFP	N/A	N/A	N/A	N/A
BL3	CD11c	PerCP-Cy5.5	1-250	N418	Biolegend
GL1	CD3	PE	1-150	17A2	Biolegend
GL2	Siglec F	PE-594	1-200	E50-2440	BD
GL4	F4/80	PE-Cy7	1-150	EMR1	Biolegend
RL1	CD4	APC	1-250	GK1.5	Biolegend
RL2	CD11b	AF700	1-250	M1/70	Biolegend
RL3	Viability	eFluor780	1-1000	N/A	Thermo

Cornea Model Immune profile in draining lymph nodes

Draining lymph nodes (submandibular lymph nodes) were collected and grinded through a 70 µm filter. Cells were collected after centrifugation and washing with 1X DPBS. Lymphocytes were stimulated for 4 hrs with Cell Stimulation Cocktail, plus protein transport inhibitors (eBioscience), followed by staining of surface markers. After permeabilization and fixation of cells, cytokines IL-17A, and IFN-γ were stained for 4get mice and IL-17A, IL-4, and IFN-γ were stained for wild-type (WT) and GATA1 KO mice.

The antibodies used were listed below:

Mouse Cornea iLN ICS Panel

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	Thy1.2	PacBlue	1-500	53-2.1	Biolegend
VL2	CD45	V500	1-100	30-F11	BD
VL3	NK1.1	BV605	1-300	PK136	Biolegend
VL4	CD8a	BV711	1-300	53-6.7	Biolegend
BL1	CD3	AF488	1-200	GK1.5	Biolegend
BL3	CD19	PerCP-Cy5.5	1-200	6D5	Biolegend
GL1	IL-4	PE	1-200	11B11	Biolegend
GL2	γδ	PE-594	1-300	GL3	Biolegend
GL4	CD4	PE-Cy7	1-300	GK1.5	Biolegend
RL1	IFNγ	APC	1-200	XMG1.2	Biolegend
RL2	IL-17a	AF700	1-200	TC11-18H10.1	Biolegend
RL3	Viability	eFlour780	1-1000	N/A	Thermo

Mouse Cornea iLN 4get IL4:GFP Modified ICS

Filter	Marker	Fluorophore	Dilution	Clone	Source
VL1	Thy1.2	PacBlue	1-500	53-2.1	Biolegend
VL2	CD45	V500	1-100	30-F11	BD
VL3	NK1.1	BV605	1-300	PK136	Biolegend
VL4	CD8a	BV711	1-300	53-6.7	Biolegend
BL1	IL4:GFP	N/A	N/A	N/A	N/A
BL3	CD19	PerCP-Cy5.5	1-200	6D5	Biolegend
GL1	CD3	PE	1-200	11B11	Biolegend
GL2	γδ	PE-594	1-300	GL3	Biolegend
GL4	CD4	PE-Cy7	1-300	GK1.5	Biolegend
RL1	IFNγ	APC	1-200	XMG1.2	Biolegend
RL2	IL-17a	AF700	1-200	TC11-18H10.1	Biolegend
RL3	Viability	eFlour780	1-1000	N/A	Thermo

Cornea Immunostaining

Dissected corneas were fixed in 100% methanol at -20ºC for 30 min, and permeabilized with PBS containing 0.25% Triton-X (PBST). The cornea samples were blocked with 1% goat serum + 1% BSA in PBST for 30 mins and stained with rabbit anti-mouse αSMA (Abcam) overnight at 4 ºC. Following washing with PBST, corneas were stained with goat anti-rabbit 633 for 2 hrs at

room temperature, and mounted flat in SlowFade Diamond Antifade Mountant (Thermo Fisher Scientific). Zeiss Apotome microscope was used for fluorescent imaging.

Statistics

Data points for all in vivo experiments are biological replicates and were not randomly assigned. Investigators were not formally blinded to separation during experiments and outcome assessment, except for histological assessment for scoring. All experiments were independently replicated with similar results and trends at least twice, except sorted T cell and macrophages NanoString results in Fig. 1h and 1m as previously noted, which were each performed once. No data were excluded from the study. No formal statistical methods were used to determine sample size and differences of intra-group variances; however, sample sizes were determined by previous experiences with injury models and immunotherapy treatments and their respective previous power analyses in previous publications within the laboratory (*4, 57, 69*). All statistical differences were determined using GraphPad Prism (version 9.2.0 for Windows, GraphPad Software), excluding NanoString Codeset results, which were analyzed using nSolver Advanced Analysis Software. All other data was analyzed using two-way ANOVA with Sidak's multiple comparisons for experiments with two or more independent factors (KO mouse models vs. WT, and gene expression of multiple genes) wherein experimental group conditions were arranged by column and mouse strain or gene of interest were listed as row factors. Ordinary one-way ANOVA with Tukey's multiple comparisons was used for experiments with three or more experimental groups, comparing one factor each. In all other cases, unpaired, two-tailed Student's t-tests were used for single factor conditions wherein saline treatment controls were compared only to rSEA treated groups. NanoString differential expression results for sorted $CD3^+$ T cells and $F4/80^{Hi+}$ macrophages were analyzed using the manufacturer supplied nSolver Advanced Analysis Software (version 4.0.70, NanoString Technologies, inc.) according to manufacturer guidelines and recommendations. NanoString differential gene expression analysis was performed using the Advanced Analysis with an analysis threshold of 20 counts (minimum) per gene probe was used and the automated software selected the top 16 reference genes for analysis of the sorted macrophages and the top 10 reference genes were selected by the software for the sorted T cell analysis. For NanoString analysis, False Discovery rate-adjusted P-values were determined for each gene by applying the Benjamini-Yekutieli method.

Flow Cytometry Analysis

All flow cytometry data was visualized, analyzed, and gated using FlowJo (version 10.7.1 for Windows, BD Life Sciences). Gating for positive populations utilized fluorescence-minus-one controls, and all reported populations for these studies are from events on-scale, singlets (using the diagonal gating of FSC-Height vs. FSC-Area cells, live (negative for amine-reactive dye stain), CD45-positive events, with negative/positive gating as shown in **fig. S17.** To ensure the integrity of the reported results, populations were backgated and screened and quality controlled for unusual characteristics (*e.g.,* $CD3^+$ sub-populations expressing CD19 were omitted from evaluation).

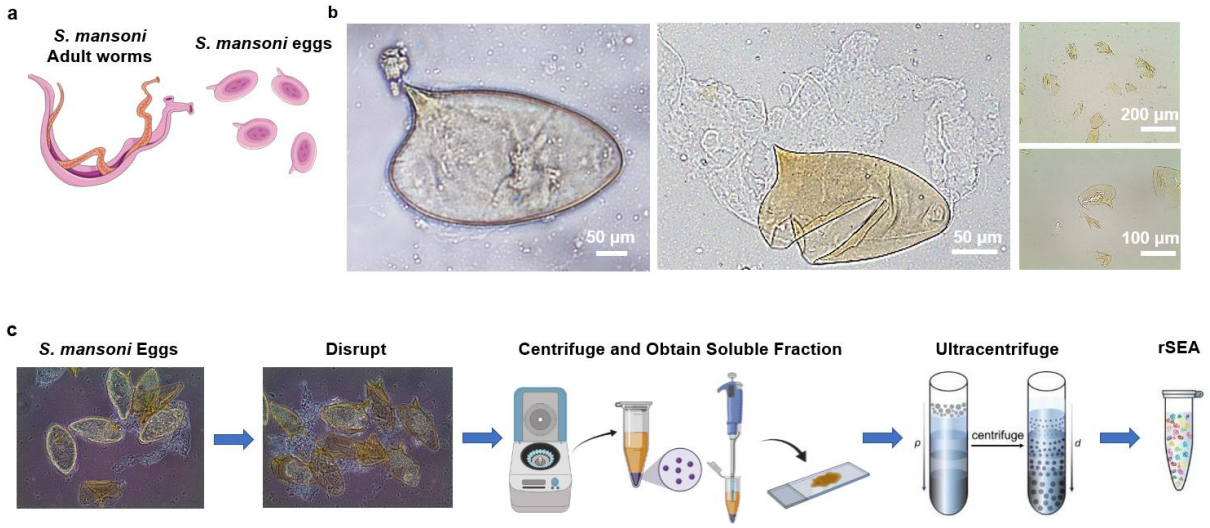

Fig. S1. SEA Isolation Process from *S. mansoni* eggs

a, Graphical images of *S. mansoni* adult worms and eggs, and brightfield images of the egg disruption process. **b,** Images of the *S. mansoni* parasite eggs at various stages of disruption. **c**, a graphical schematic of SEA isolation and removal of eggshell debris.

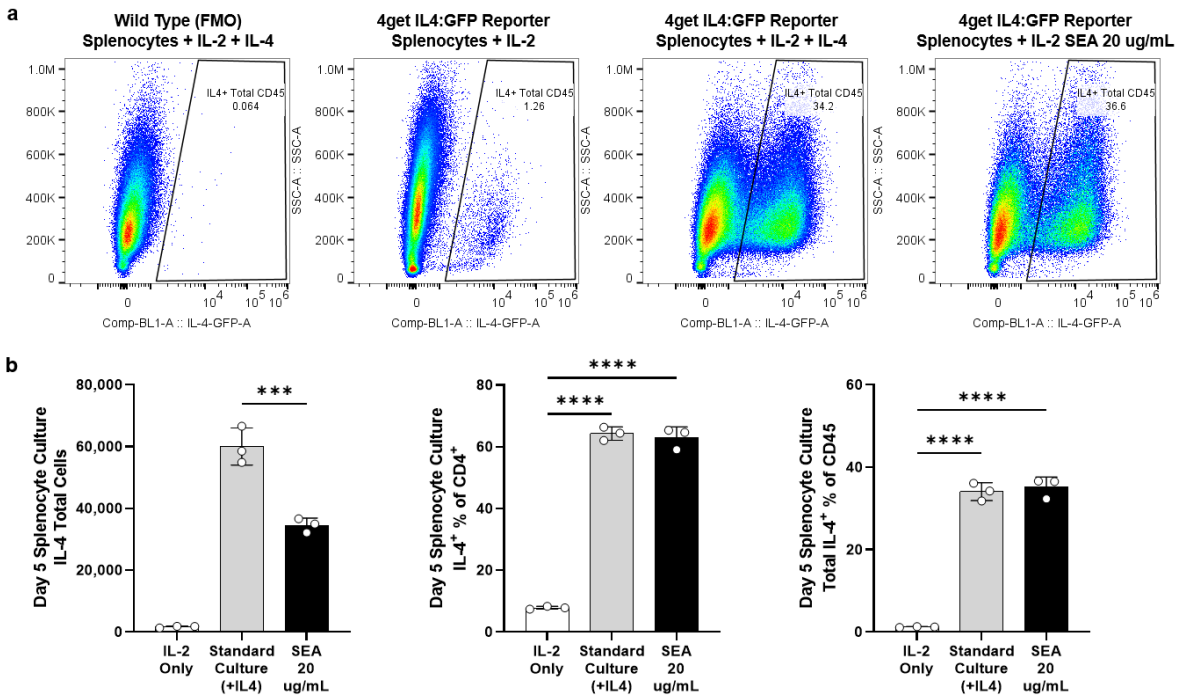

Fig. S2. SEA *In vitro* screening CD4$^+$IL4$^+$

a, Representative flow cytometry plots of the IL-4 expression obtained in 4get splenocyte cultures. **b,** Flow cytometry populations for IL4$^+$CD4$^+$ cells as percentages of CD4 and CD45 live with SEA treatment versus controls. **c,** Flow cytometry population counts from treatment with IL-4 and SEA. Statistical tests represent all *in vitro* replicates, and all experiments were replicated at least twice. Graphs show mean ± s.d. (b), n = 3. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by one-way ANOVA with Tukey's multiple comparisons (b).

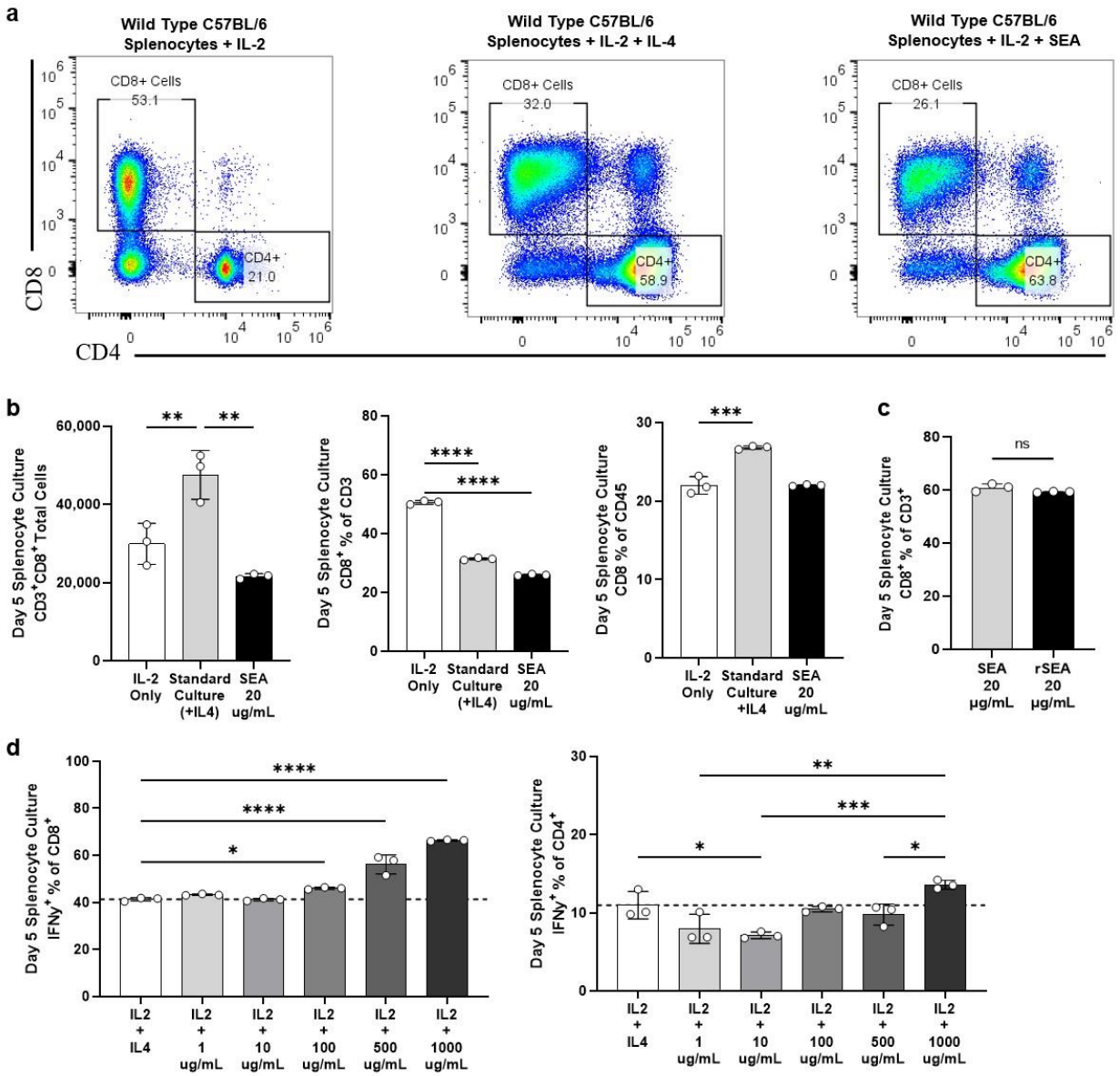

Fig. S3. SEA *In vitro* Screening CD8$^+$ T Cells

a, Representative flow cytometry plots of the CD4$^+$ and CD8$^+$ populations in day 5 splenocyte cultures. **b,** Flow cytometry populations for CD4$^+$ and CD8$^+$ cells of different dosage schemes of SEA. **c,** Flow cytometry populations for CD8$^+$ cells in day 5 splenocyte cultures from an independent experiment comparing rSEA and SEA. **d,** Flow cytometry population counts from treatment with standard SEA and an alternative SEA formulation. Statistical tests represent all *in vitro* replicates, and all experiments were replicated at least twice. Graphs show mean ± s.d. (b, c), n = 3. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by one-way ANOVA with Tukey's multiple comparisons (b, d) or unpaired, two-tailed T-test (c)

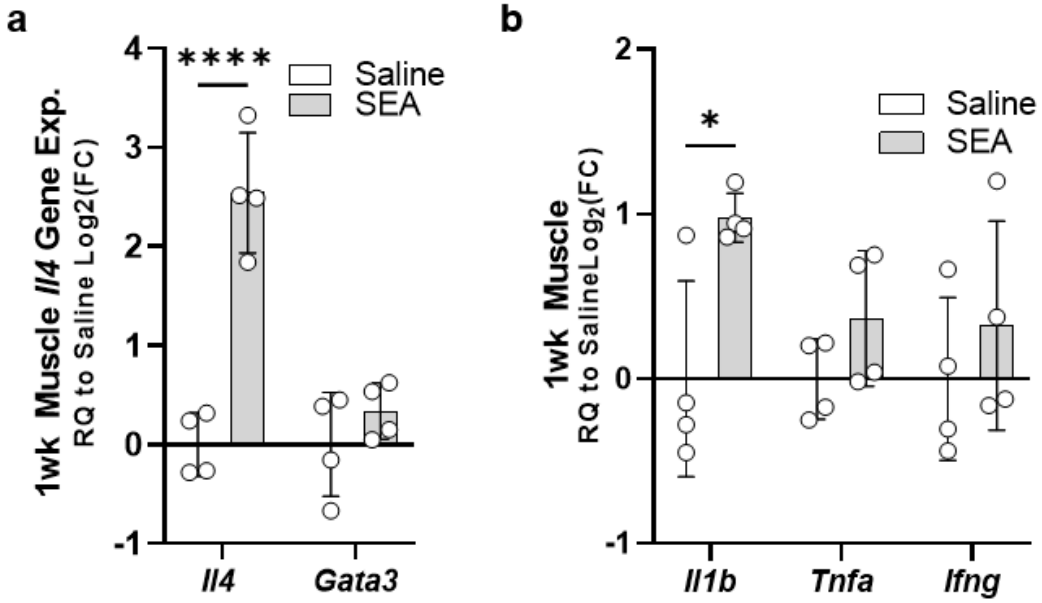

Fig. S4. Treatment with SEA increases *Il4* gene expression with a small rise in pro-inflammatory genes in local muscle injury tissue

a, Gene expression of *Il4* and *Gata3* in muscles treated with SEA, assessed 1-week post-injury. **b,** Gene expression of *Il1b*, *Tnfa*, and *Ifng* in muscles treated with SEA, assessed 1-week post injury. Statistical tests represent all biological replicates, and all experiments were replicated at least twice. Graphs show mean ± s.d. (a, b), n = 4. *$P < 0.05$, ****$P < 0.0001$ by two-way ANOVA with Sidak's multiple comparisons.

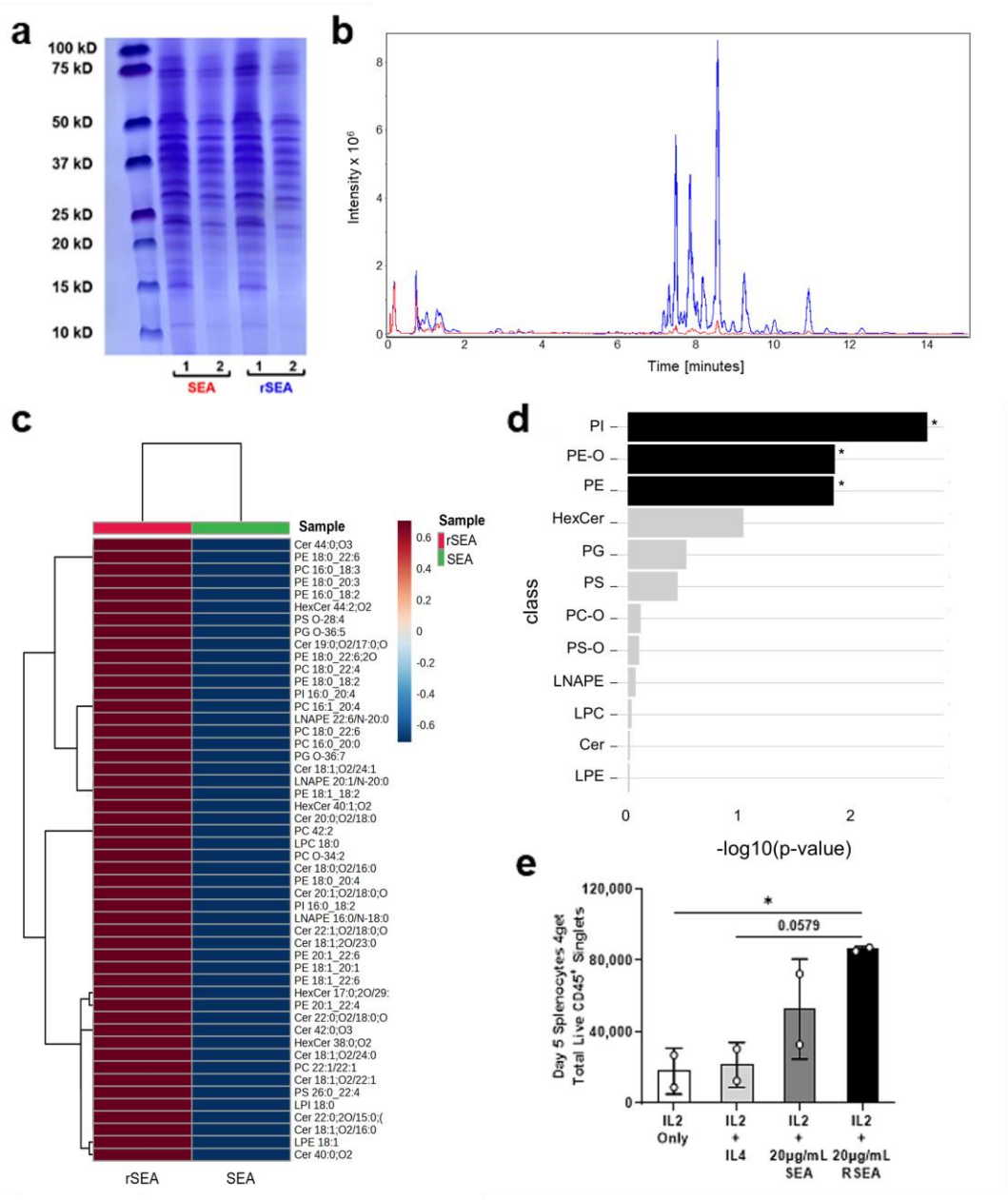

Fig. S5. SEA vs rSEA compositional comparison and impact on cell proliferation
a, SDS-PAGE gel to visualize changes to the SEA proteins when alternate isolation techniques are performed, using 5µg of protein/lane. Columns 1 and 2 represent SEA and rSEA before (1) and after (2) sterile filtration. **b,** Mass chromatogram from LC-MS run showing increased lipid levels in rSEA versus SEA. **c,** List of the 50 most highly upregulated lipid compounds in rSEA vs SEA. **d,** Comparison of lipid class levels; black bars indicate classes that are significantly upregulated in rSEA vs SEA. **e,** Primary 4get splenocyte culture day 5 results on total live $CD45^+$ cell number increases with SEA and rSEA at the same dosage of 20 µg in 200 µL of complete culture media. Statistical tests (b) represent all *in vitro* replicates (n = 2), and all experiments were replicated at least twice. Graphs show mean ± s.d. (b). *P < 0.05, by one-way ANOVA with Tukey's multiple comparisons. Abbreviations: PI: phosphatidylinositol; PE-O: akylphosphatidylethanolamine; PE: phosphatidylethanolamine; HexCer: hexosylceramide; PG: phosphatidylglycerol; PS: phosphatidylserine; PC-O: alkylphosphatidylcholine; PS-O: alkylphosphatidylserine; LNAPE: lyso-*N*-acyl-phosphatidylethanolamine; LPC: lysophosphatidylcholine; Cer: ceramide; LPE: lysophosphatidylethanolamine.

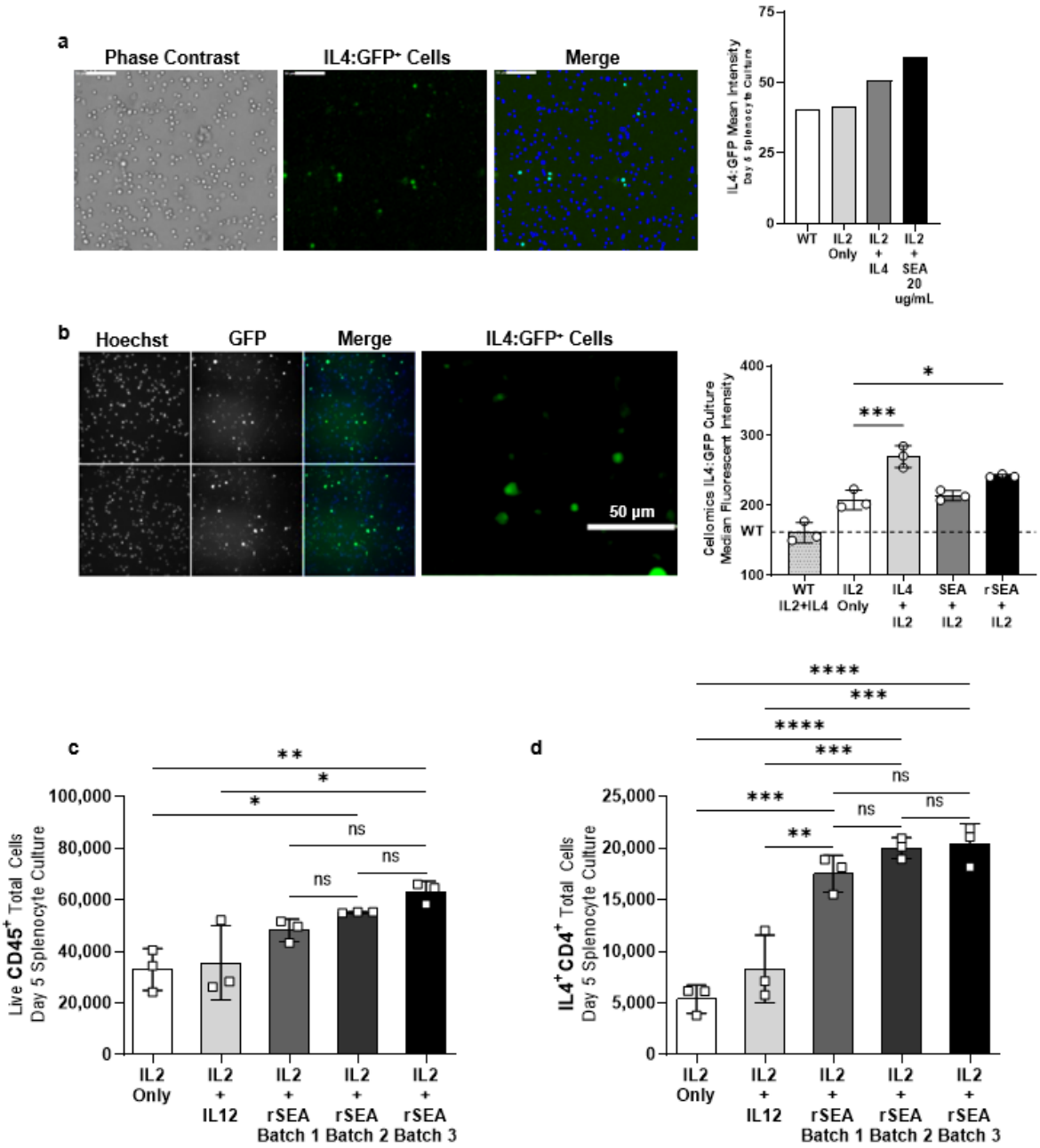

Fig. S6. *In vitro* Screening

a, Representative images of the IL4:GFP expression obtained from 4get splenocyte cultures at day 5 using the Celigo plate imaging system. **b,** Representative images of the IL4:GFP expression obtained from 4get splenocyte cultures at day 5 using the Cellomics HTS. **c**, Comparison of rSEA batches in a splenocyte culture system to expand $CD45^+$ cells. **d**, Reproducibility between batches of rSEA to stimulate $IL4{:}GFP^+CD4^+$ cells *in vitro*. Statistical tests represent all *in vitro* replicates, and all experiments were replicated at least twice, excluding (a). Graphs show mean ± s.d. (b-d), n = 3. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ by one-way ANOVA with Tukey's multiple comparisons (b-d).

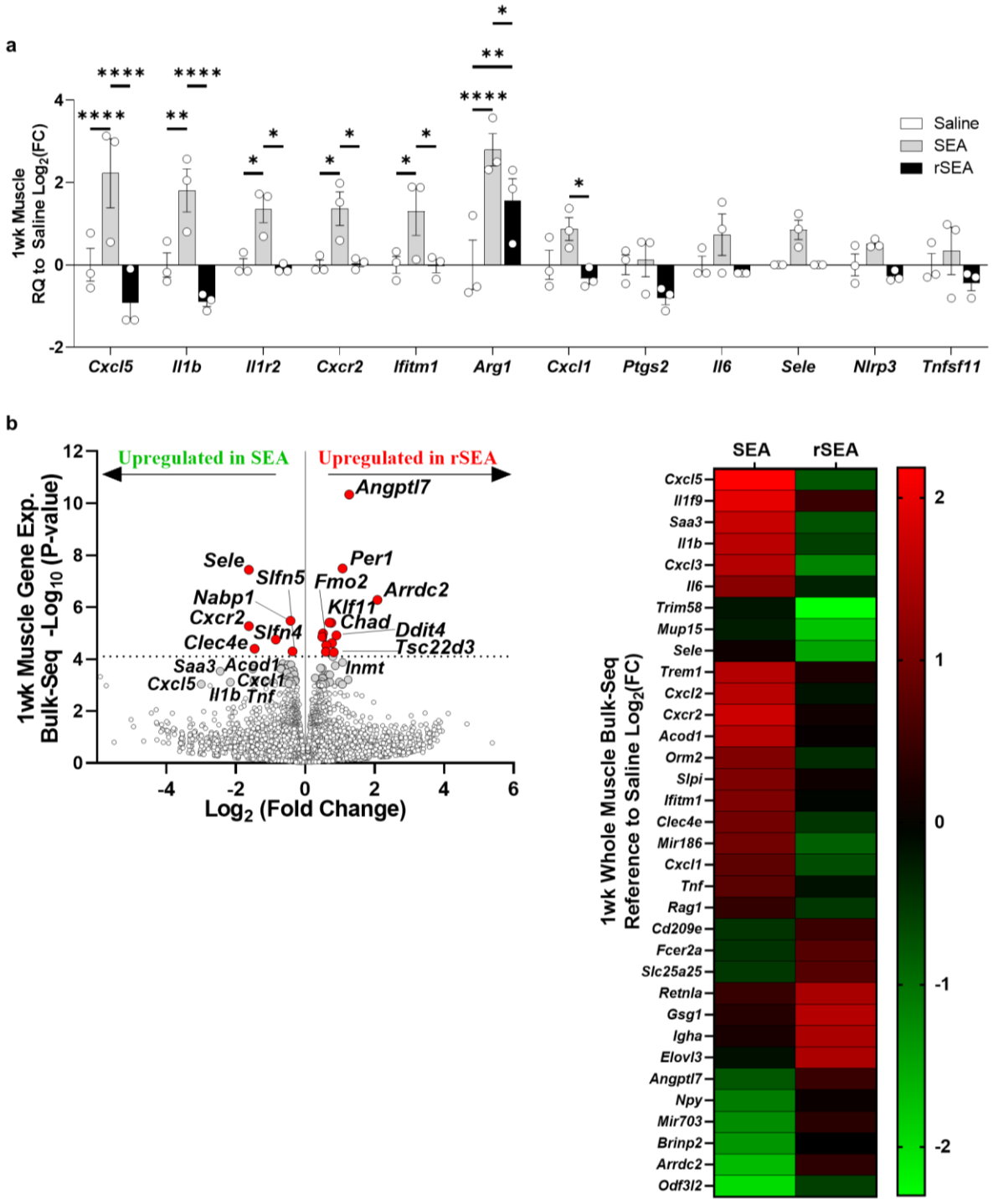

Fig. S7. Comparison of SEA vs rSEA treated muscles
a, Gene expression of pro-inflammatory associated genes of 1-week post-VML muscles comparing SEA or rSEA, normalized to saline treated controls. **b,** Bulk RNASeq comparing SEA and rSEA. Statistical tests represent all biological replicates. Graphs show mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ by two-way ANOVA with Sidak's multiple comparisons.

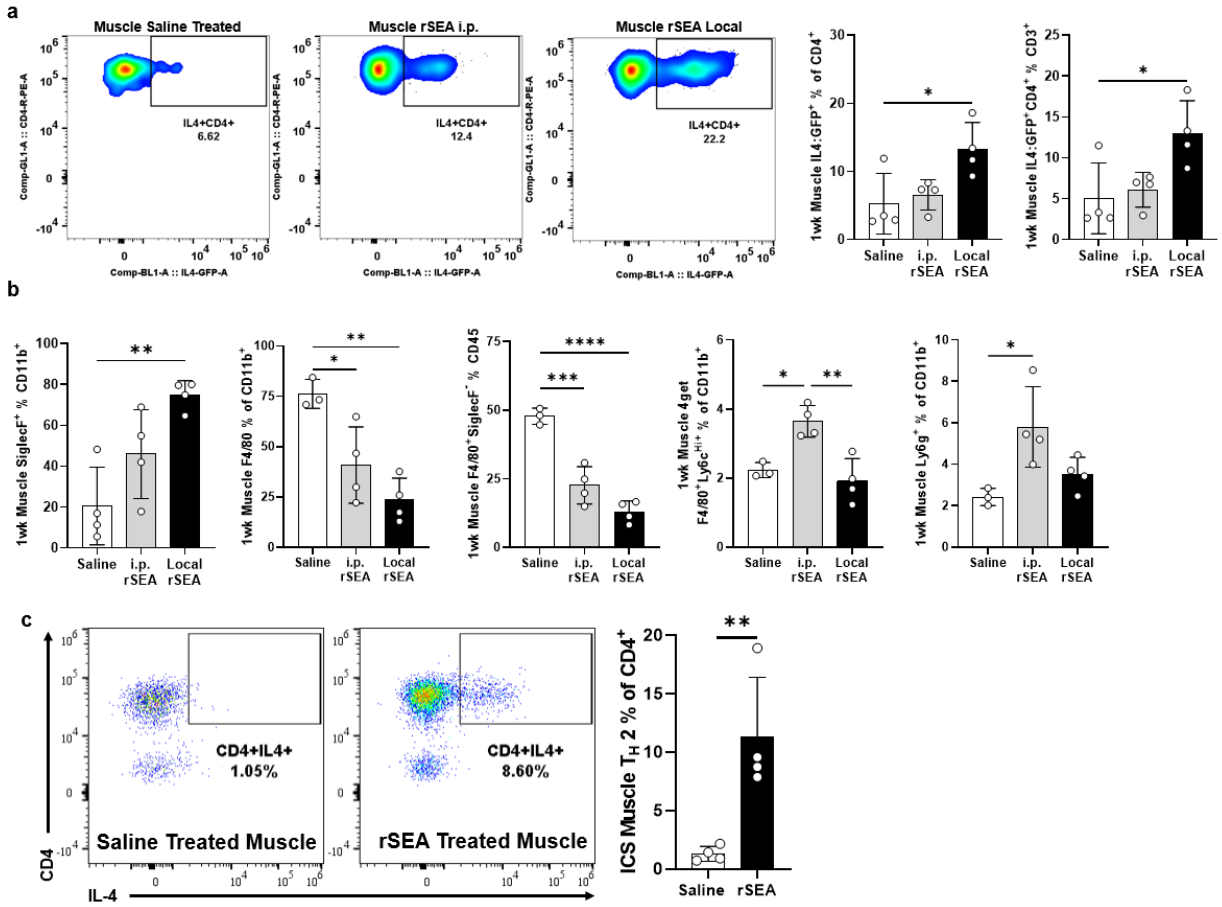

Fig. S8. Local vs. systemic administration of rSEA

a, Representative flow cytometry plots of IL4:GFP^{+}CD4^{+} of local vs. systemic rSEA administration in muscle 1-week post-VML and respective percentages in 4get IL4:GFP reporter mice. **b,** Flow cytometry populations for myeloid populations in the muscle post-injury and treatment. **c,** 1-week muscle injury ICS IL-4 cytokine staining in C57BL/6 mice. Statistical tests represent all biological replicates, except when otherwise noted. Graphs show mean ± s.d.. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by one-way ANOVA with Tukey's multiple comparisons.

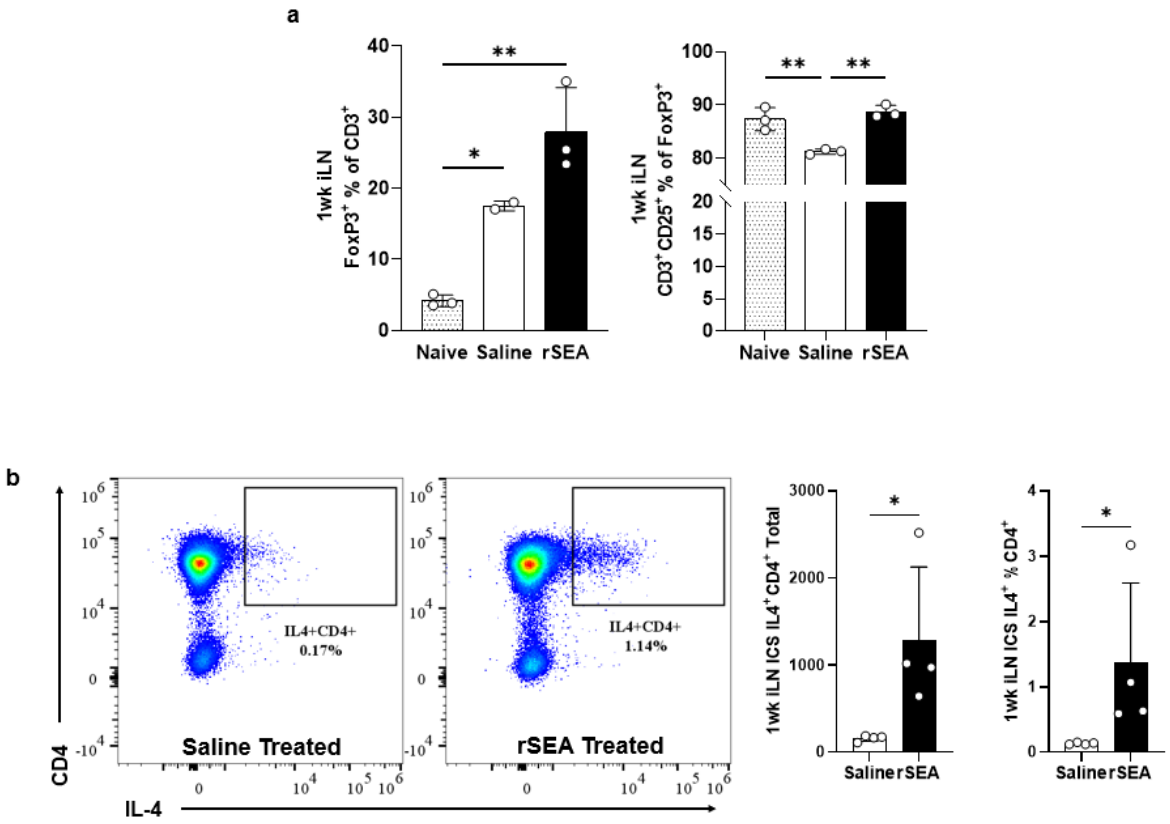

Fig. S9. Treg Populations in the iLNs after rSEA treatment of muscle injuries
a, FoxP3$^+$ populations in 1-week post-VML in the draining inguinal lymph nodes. **b,** Flow cytometry intracellular staining of cytokines (ICS) in iLNs taken from C57BL/6 mice treated with rSEA, harvested 1-week after injury and treatment. Data are means ± s.d., n = 3-4. Statistical tests represent all biological replicates, except when otherwise noted. Graphs show mean ± s.d.. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by one-way ANOVA with Tukey's multiple comparisons (a), and unpaired two-tailed Student's t-test (b).

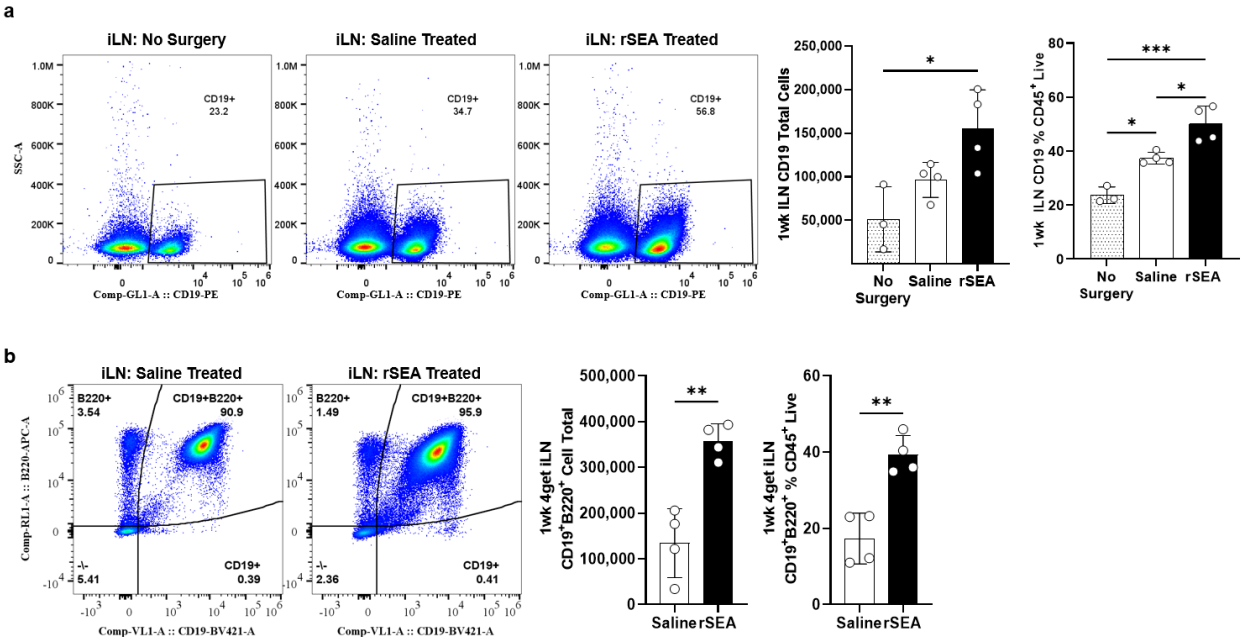

Fig. S10. iLN and B Cell Responses to rSEA Treatment

a, Flow cytometry counts and % population of $CD19^+$ cells in the iLNs at 1-week post-injury and rSEA treatment. **b,** Flow cytometry % population of $B220^+CD19^+$ cells in 4get mouse 1-week post-injury and treatment with rSEA. Statistical tests represent all biological replicates. Graphs show mean ± s.d., n = 3-4. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by one-way ANOVA with Tukey's multiple comparisons (a), and unpaired two-tailed Student's t-test (b).

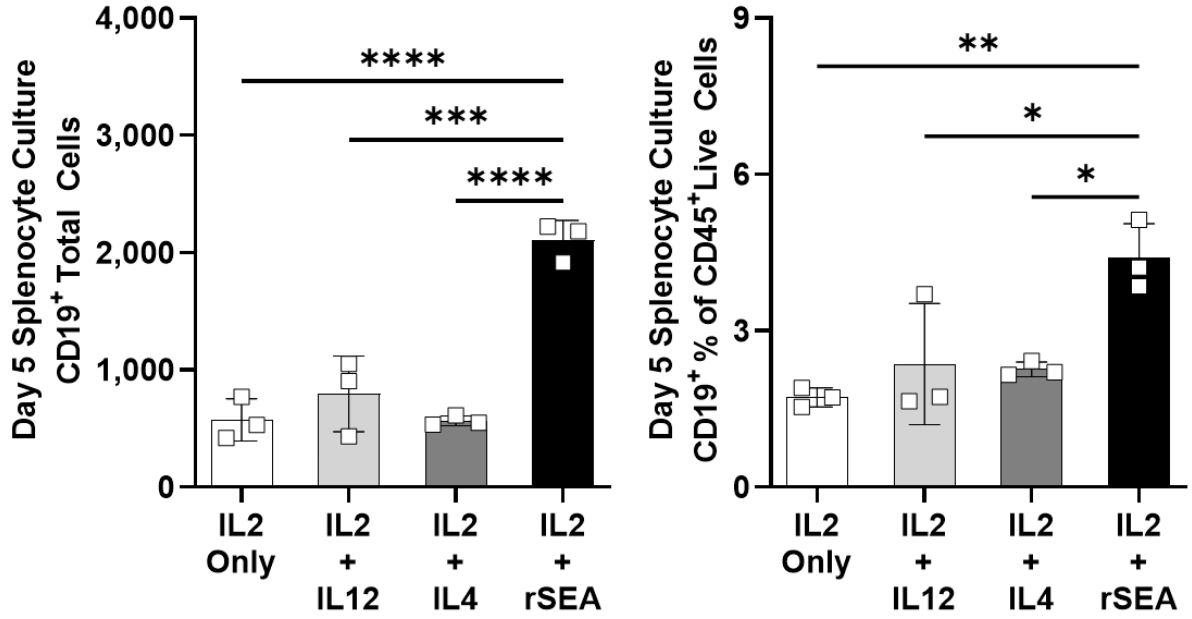

Fig. S11. *In vitro* B Cell Responses to rSEA Treatment
Flow cytometry total counts and % populations of $CD19^+$ cells from *in vitro* splenocyte cultures taken from live $CD45^+$ cells. Statistical tests represent all technical replicates. Graphs show mean ± s.d., n = 3. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by one-way ANOVA with Tukey's multiple comparisons.

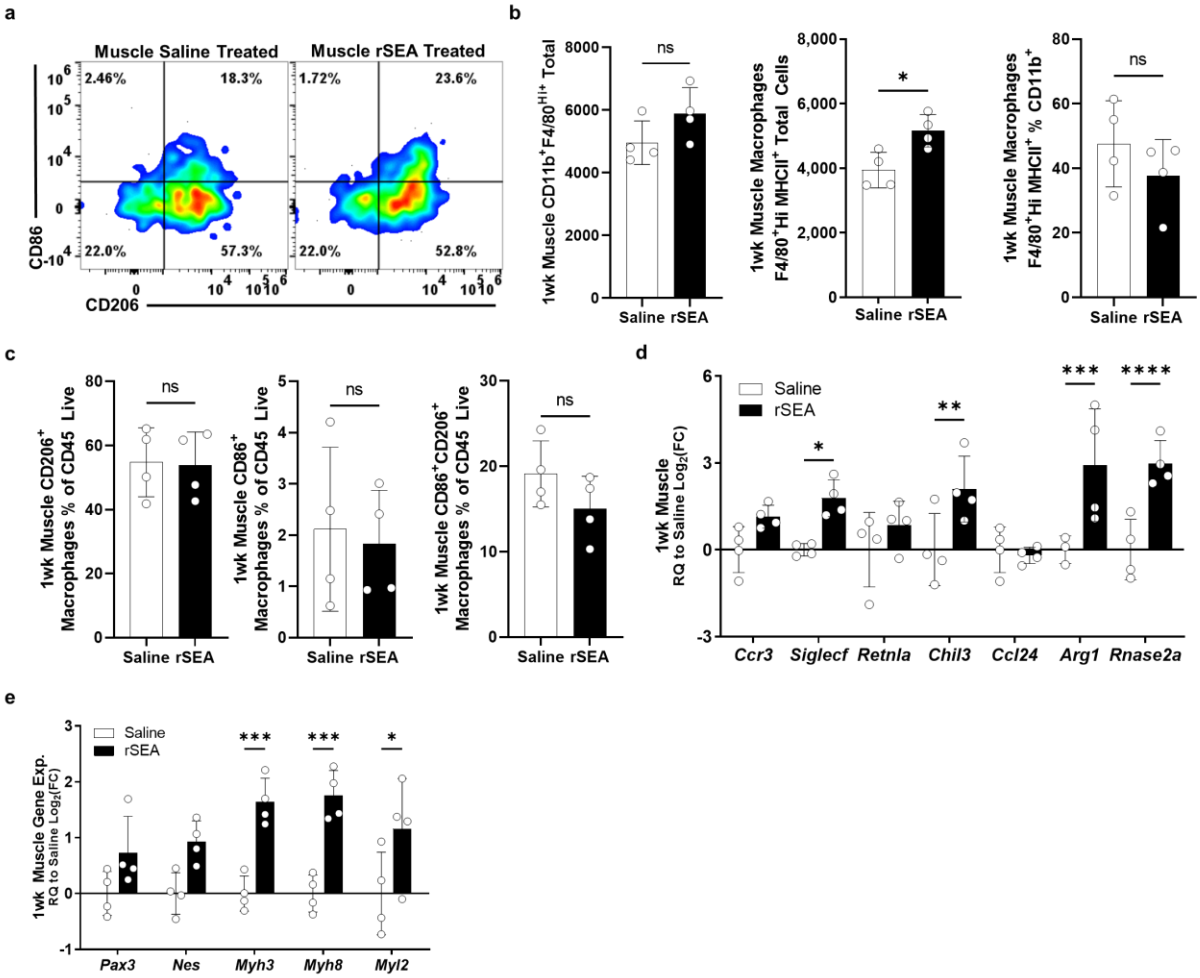

Fig. S12. Macrophage populations in 1-week muscle post-rSEA treatment

a, Representative flow cytometry plots of the $CD206^{+}$ and $CD86^{+}$ populations in muscle 1-week post-VML and rSEA treatment. **b,** Flow cytometry population cell counts of $F4/80^{Hi+}MHCII^{+}$ cells as percentages of $CD11b^{+}$ cells. **c,** Flow cytometry population cell counts of $F4/80^{Hi+}SiglecF^{Neg}CD206^{+}$ or $CD86^{+}$ cells and their percent of $CD45^{+}$ live. **d,** 1-week post-injury muscle gene expression of macrophage and eosinophil associated genes after rSEA treatment. **e,** 1-week gene expression of injured muscle for skeletal muscle regeneration associated genes with rSEA treatment. Statistical tests represent all biological replicates. Graphs show mean ± s.d., n = 4. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ by unpaired two-tailed Student's t-test (b, c), and two-way ANOVA with Sidak's multiple comparisons (d).

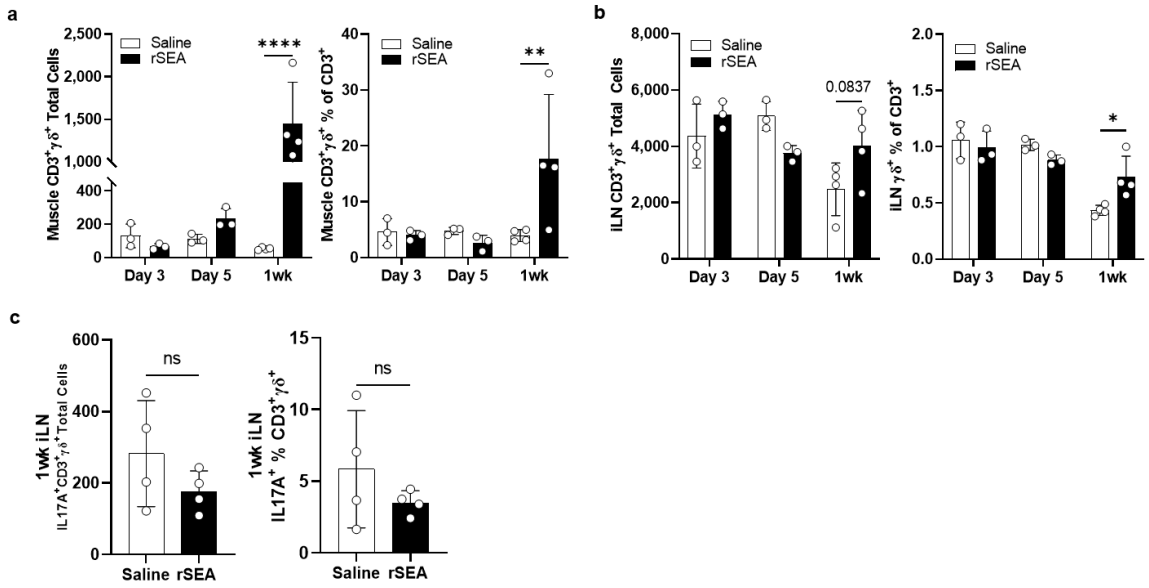

Fig. S13. Gamma Delta T cell responses in muscle and iLN with rSEA treatment
a, Post-injury muscle γδ T cell total cells and percentages kinetics. **b,** iLN γδ T cell counts and percentages after muscle injury and treatment. **c,** iLN IL17A$^+$ γδ counts and percentages 1-week post-injury of the muscle and treatment. Statistical tests represent all biological replicates. Graphs show mean ± s.d., n = 3-4. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by two-way ANOVA with Sidak's multiple comparisons (a, b), and unpaired two-tailed Student's t-test (c).

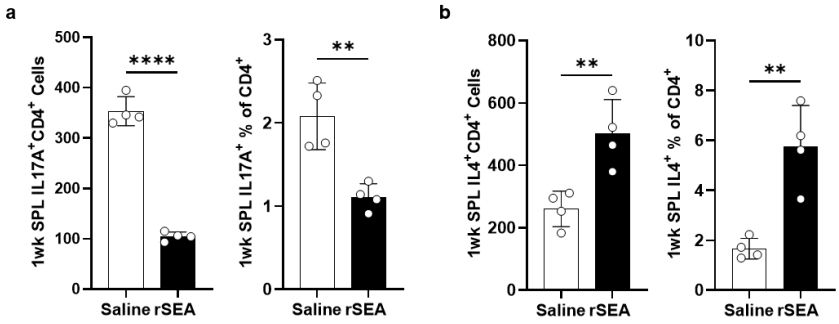

Fig. S14. T helper type 17 responses in the spleen with rSEA treatment
a, $IL17A^+CD4^+$ total cells and % of CD4 in spleen 1-week post-injury and treatment. **b,** $IL4^+CD4^+$ total cells and % of CD4 in spleen 1-week post-injury and treatment. Statistical tests represent all biological replicates. Graphs show mean ± s.d., n = 4. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by unpaired two-tailed Student's t-test (a, b).

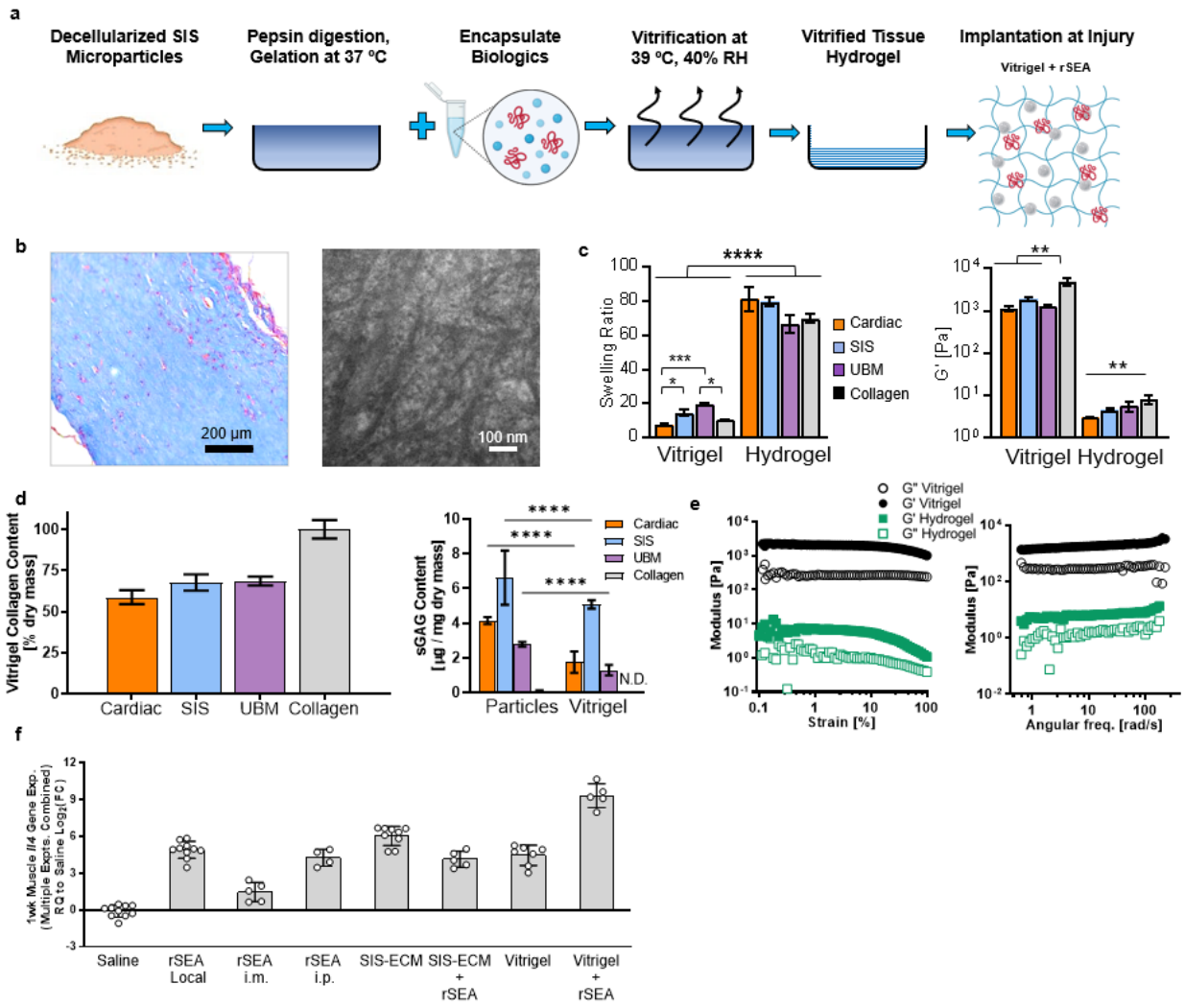

Fig. S15. Vitrified gel development and delivery for rSEA

a, Graphical synopsis of vitrified gel and packaging of rSEA. **b,** Representative images of SIS-vitrigel characterization in H&E (gross) and TEM (micro-architecture). **c,** (left) Swelling ratio comparison of various vitrified gels and hydrogel sources, the (right) assessment of storage modulus (G') in Pascals, and values for vitrified gels (SIS, vit) and hydrogels (SIS, H). **d,** Characterization of collagen content (% of dry mass) in vitrigels (left) and the sGAG content of the source material (particle form) compared to post-vitrification. **e,** Biomechanical assessment of SIS-vitrigels in comparison to hydrogel forms for storage modulus, loss modulus, and viscosity in reference to % strain and angular frequency, respectively. **f,** 1-week post-VML muscle gene expression of *Il4* with the indicated treatment and normalized to saline treated control muscles (graph represents multiple independent experiments combined). Graphs show mean ± s.d., n = 3. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by two-way ANOVA with Sidak's multiple comparisons (c, d-right). Scale bars: 200 µm (b, left), and 100 nm (b, right).

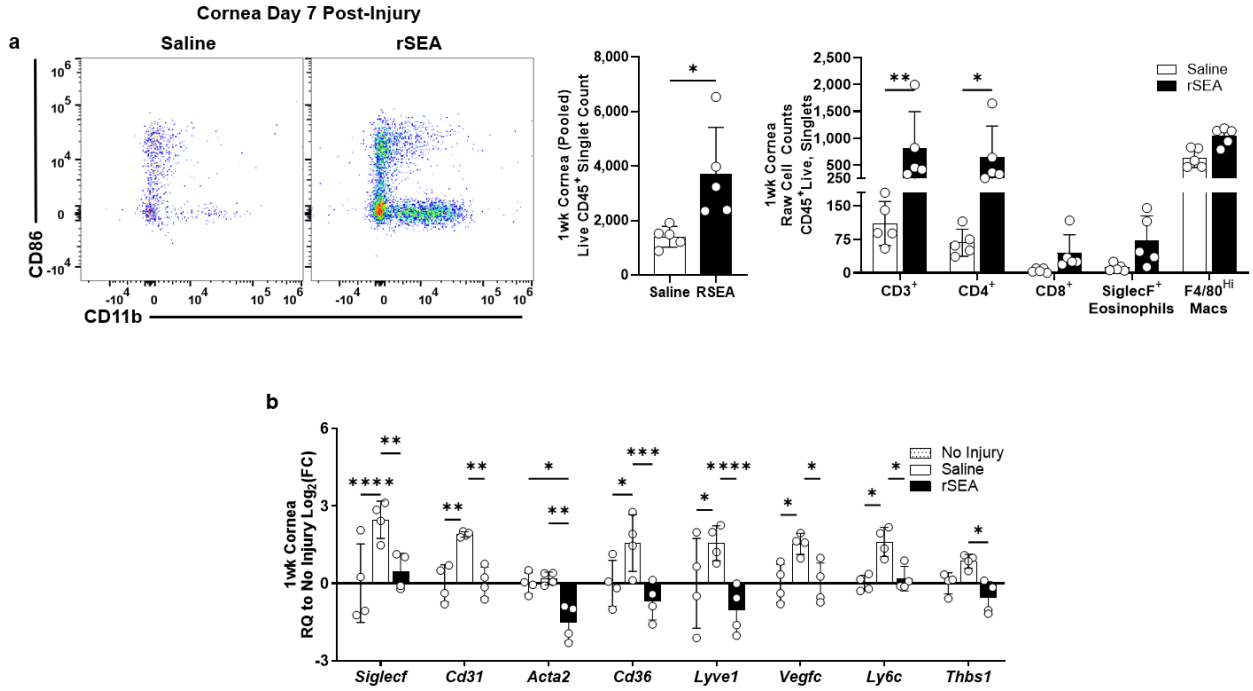

Fig. S16. Injured Cornea immune populations with rSEA treatment

a, Representative flow cytometry plots of immune cell populations in injured cornea 1-week post-injury and % populations of CD45^{+}Live cells. Flow cytometry populations for T helper cell populations in the spleen 1-week post-injury and treatment. Flow Data generated by pooling 6 corneas to represent 1 sample (n = 5). **b,** Gene expression of cornea samples 1-week post-injury and treatment with rSEA or saline (n = 4). Graphs show mean ± s.d.. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by Student's t-test (**a**), and two-way ANOVA with Sidak's multiple comparisons (**b**).

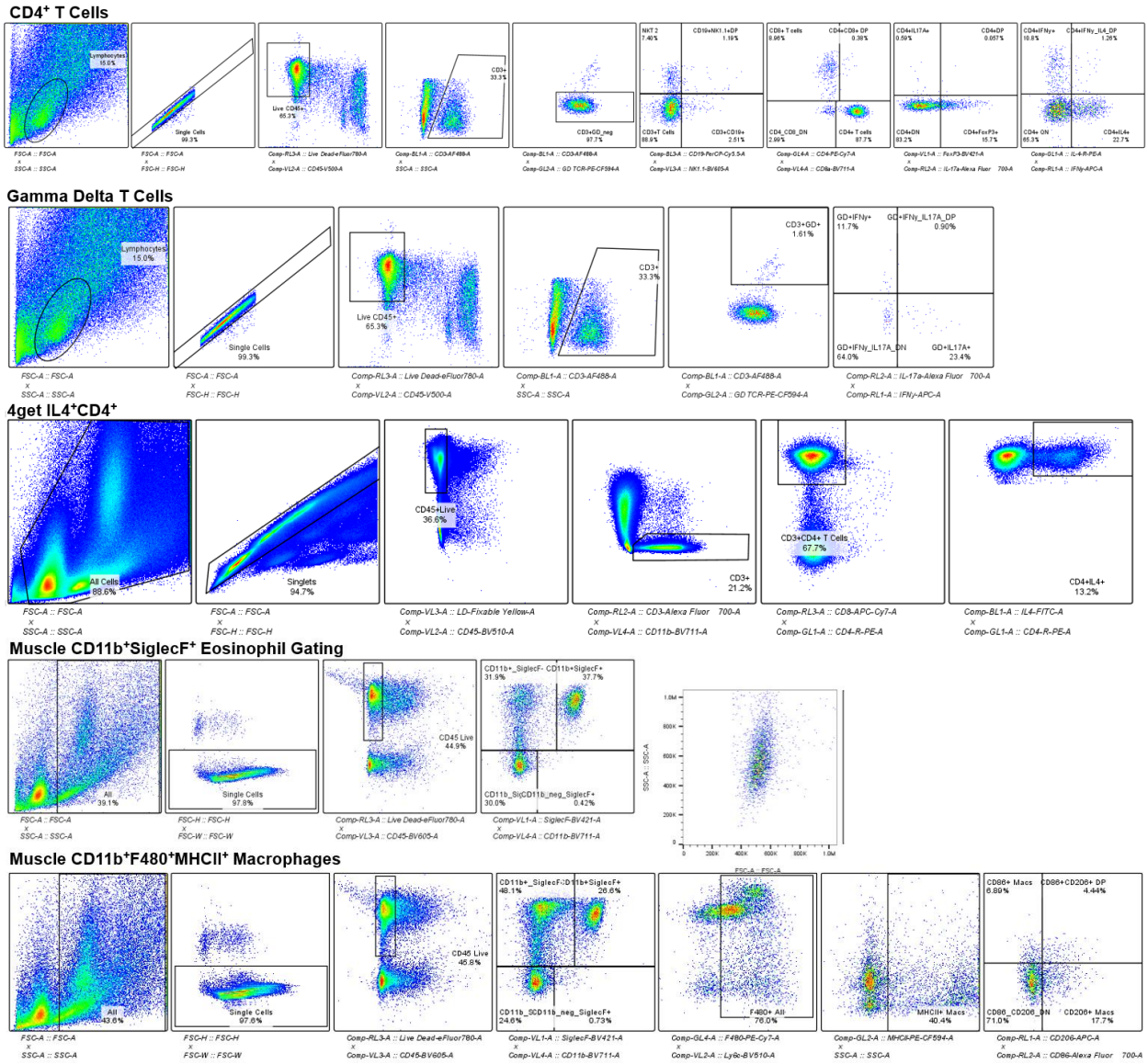

Fig. S17. Gating Schemes. General gating scheme

Table S1. RNA Bulk-sequencing of VML Muscle Tissues 1-week Post-Injury & rSEA

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Gimap3	1.774081225	0.001014808
Atp1b4	0.989509205	0.001035301
Retnlg	2.027560396	0.001324974
Gatm	0.964943966	0.001616492
Icos	1.879668492	0.001831082
Cd3e	2.629457564	0.001831082
Iqsec3	0.86606271	0.001831082
Tm6sf1	0.639572302	0.001831082
Cd6	2.281516917	0.001831082
Ms4a4b	1.939024737	0.001900998
Clec7a	1.336884384	0.001900998
Cxcr6	2.181408089	0.003257059
Vash2	0.631238466	0.003297846
Lck	1.544819208	0.003340315
Traf3ip3	0.851254836	0.004170501
Card11	1.149250752	0.004170501
Zdbf2	0.757353056	0.004170501
Cd3g	2.287459767	0.004170501
Il2rb	1.371027256	0.004170501
Cd5	2.006777738	0.004170501
Themis	2.963198744	0.004170501
Stra6l	1.425483694	0.004170501
Il7r	1.187737935	0.004170501
Fmo1	0.638195278	0.004170501
Myf5	0.973625834	0.00568638
Ppfia4	0.746441167	0.00568638
Cd83	0.82843717	0.00568638
Rasal3	1.016682102	0.005856345
Mycl	0.798236513	0.006919635
Ppp1r27	-0.932630095	0.007167036
Slamf7	0.880797602	0.007978069
Tmem37	0.65915643	0.007978069
Cyp4f18	1.316153996	0.008469018
Aif1l	0.761876136	0.008469018
Klra13-ps	2.313017578	0.008862624
Ltb	1.407502929	0.008862624
Izumo1r	4.042164981	0.008862624
Mrgprg	4.722399407	0.008892888
Cd3d	1.912507389	0.00907744
Irf8	0.74119657	0.00945388

Gene Name	RSEA LOG2(FC)	EDGE R FDR
H2-T23	0.610937864	0.00945388
Acap1	1.224430208	0.009713101
Gstm2	-0.586071007	0.009713101
Ciita	0.810464103	0.00995403
Itk	1.481613368	0.010086007
Shisal1	0.823129696	0.010086007
Mdfi	0.879013473	0.010086007
H2-Q5	0.9845686	0.010086007
Spon2	-1.277600493	0.010086007
Trbc1	1.820173232	0.010479107
AW112010	0.81225642	0.010479107
Sla2	2.004608338	0.010892934
Apobec1	0.845130829	0.010892934
Rab44	1.279718226	0.010892934
Zap70	0.966377907	0.010892934
Skap1	1.83346263	0.010892934
Slamf8	1.126284884	0.010892934
Myl4	0.601768015	0.011040333
Mpeg1	0.770643045	0.011160855
Rgs4	-0.711968522	0.011610116
Ikzf3	1.407876452	0.011664359
Mroh2a	1.989042933	0.011664359
Cysltr2	2.373203581	0.011683291
Mymx	0.757737528	0.011683291
Pglyrp2	1.775772842	0.01177976
Pcbd1	0.950335204	0.011895076
Cd300c2	0.635262072	0.012520707
Mymk	0.762518825	0.012520707
Itgal	0.938463557	0.012702262
Lair1	0.826924818	0.012702262
Napsa	0.876640816	0.013824615
Flnb	-0.532382412	0.013824615
Sell	1.199061719	0.013917042
Arhgap25	0.724774454	0.013984898
Gimap7	2.079902981	0.014144678
Cxcr3	1.194714561	0.014220686
H2-Q4	0.700165392	0.014220686
Pax7	0.619742274	0.014220686
Lat2	0.719892138	0.014220686
Galnt5	0.515951376	0.014249948

Table S1 Continued

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Tnfrsf18	1.47349947	0.01449763
Ptprc	0.730409667	0.014942951
Cd40lg	6.394679562	0.014942951
Tceal3	0.902042352	0.014942951
Pnmal2	0.816945012	0.014942951
Tcf7	0.790346612	0.015447083
AU020206	0.728493508	0.015456081
Adamts3	-0.852167887	0.015924902
Ninl	0.833606865	0.015924902
Rhoh	0.835490551	0.015947606
Gzmb	1.632963097	0.01647679
F5	1.479467736	0.01647679
Ms4a7	0.659934363	0.01647679
Gpr65	0.868091118	0.01647679
Fgfrl1	0.687827093	0.01647679
Kcnj2	-0.677460472	0.01647679
Slc15a3	0.696268435	0.016531819
Neurl3	0.890350267	0.016531819
Chil3	1.729950182	0.016531819
Hexb	0.478583455	0.016531819
Ubash3a	1.667418719	0.016839485
Trac	1.613445515	0.016839485
Siglech	1.11860435	0.016839485
Septin1	0.756597008	0.016839485
Gm11243	1.800647165	0.016839485
Ccl5	1.002775988	0.016839485
Rtkn2	0.813259595	0.016839485
Bcl11b	1.520176893	0.016839485
Fgfr4	0.791767117	0.016839485
Cdk5r1	0.683872331	0.016901209
Loxl2	-0.534472869	0.016972871
Irf7	0.614664325	0.016972871
Nkg7	1.089445228	0.016972871
Ctss	0.894274205	0.016972871
Itgb7	0.866355192	0.016972871
Jpt1	0.440059004	0.016972871
Klrc1	2.407648291	0.017084327
Erbb3	0.426132693	0.017731346
Slfn2	0.695843798	0.017761234
Cracd	-0.932450089	0.01782567
Myod1	0.586957045	0.01782567

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Cdkn1c	0.499689081	0.01782567
Cd300a	0.747003552	0.01782567
Lgals3	0.809897439	0.018295341
Trim12c	0.545426162	0.018337938
Gm3830	0.916942956	0.018482818
Gbp3	0.645697324	0.018482818
Mapk8ip1	0.517150874	0.018482818
P2ry14	0.986061032	0.020522595
Pde3a	-0.462021559	0.020522595
Gm14635	1.27829582	0.020864486
2310043L19Rik	1.532807489	0.020914086
Ly86	0.701639678	0.021886266
Cd2	1.116445519	0.021886266
Ccr3	2.175071664	0.021886266
Otog	1.038769913	0.021886266
Adap1	0.774366267	0.022281262
Pgm5	0.443422664	0.022307191
Slfn1	1.102949585	0.022482858
Ifit1	0.83221217	0.022482858
Sytl2	0.42735373	0.022482858
Slc9a3r2	-0.451757442	0.022482858
A630023P12Rik	4.15812957	0.022482858
Nuak1	-0.408553432	0.022482858
Trbc2	1.811767895	0.022482858
Patj	0.703552208	0.022482858
Ccr4	3.352362265	0.022482858
Vcam1	0.654383692	0.022482858
Cytip	0.819007246	0.022482858
Sele	-1.49680031	0.022482858
H2-Aa	0.872031818	0.022746521
Zfp185	-0.699363114	0.02277294
Gdf15	1.395769212	0.02277294
Cd52	0.863732181	0.023517717
Prune2	0.601806389	0.023553097
Foxp3	1.552171236	0.023991236
Plekhh1	0.749905754	0.023991236
Rcan1	-0.445639927	0.023993592
Olfr1372-ps1	1.413258489	0.024037961
Sh2d2a	1.343267424	0.024037961
Ipcef1	1.086447627	0.024260598
Id2	0.779833034	0.024260598

Table S1 Continued

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Lpcat2	0.841645789	0.024260598
Sap30	0.672006761	0.024260598
Slamf6	1.279177857	0.024260598
Gbp8	1.198103852	0.024390679
Alox15	2.456865703	0.024390679
H2-M2	2.74241885	0.024390679
Fcgr4	0.841491825	0.024390679
Mmel1	1.080338641	0.024469362
Fgd5	-0.469197654	0.024679312
Siglecg	1.081475208	0.024778719
Cebpe	1.609893133	0.024922707
Resf1	0.520722269	0.02535228
Enho	0.637747054	0.02535228
Mamstr	0.497002253	0.025395155
H2-Eb1	0.846753911	0.025395155
Itgax	0.933921865	0.025395155
R3hdml	1.075418321	0.025967085
Ctla4	2.057700843	0.026686783
Coro1a	0.626072201	0.027030939
Tnnt2	0.559893519	0.027063831
Klrk1	1.106386517	0.027106533
Npnt	0.423652395	0.027193933
Cd22	0.901981731	0.027452852
Plcb2	0.683624615	0.027470048
Galnt16	-0.527205659	0.027470048
Tlr12	1.261613232	0.027470048
Gm49654	1.182247455	0.027561142
Pdzrn3	-0.450999491	0.027612909
Ptprcap	0.995839479	0.027784426
Gm47992	2.624835237	0.027784426
Il18rap	0.872373966	0.027938763
Myog	0.581467392	0.028020689
Bcat1	-0.719509862	0.028644926
Gm8369	1.532397581	0.028944016
Tbc1d8	0.395174299	0.029412281
Lat	1.781419333	0.030519306
Palm3	0.737096113	0.030519306
Ddx60	0.639162327	0.030601259
Cd300lf	1.031116269	0.030601259
Klrg1	1.871663116	0.030601259
Hspa12b	-0.384728096	0.03078527

Gene Name	RSEA LOG2(FC)	EDGE R FDR
P2ry10	0.963506571	0.030819143
Parm1	-0.479015964	0.030819143
Wnt2b	-0.84172015	0.031402135
Eln	-0.954410711	0.031544896
Atp6v0d2	1.074245627	0.031704488
Ccdc141	0.584170954	0.031711884
Htr2a	-0.706011003	0.031812597
Ikzf1	0.642086495	0.031943659
Tmem86a	0.55022246	0.031943659
H2-DMb1	0.695076314	0.031943659
Fbn2	-0.828907818	0.031943659
H2-Ab1	0.841452475	0.031943659
Cyria	0.376624396	0.031998056
Gzma	1.213832426	0.032659141
Unc13d	0.791582052	0.032659141
Jaml	0.944407219	0.032659141
Sla	0.946216256	0.032659141
Ccdc134	0.467083519	0.032659141
H2-DMa	0.713480165	0.032659141
Mfsd12	0.628531101	0.032659141
Gpr55	1.627874478	0.032659141
Pcdh12	-0.530553348	0.032659141
Iigp1	0.673710706	0.032725333
Pcp4l1	-0.509201304	0.032785894
Kcnma1	-0.639873641	0.033025646
Atf3	0.54743947	0.033459286
Stmn4	-0.924092492	0.033552969
Fndc3c1	0.709033093	0.033841773
Tlr1	0.687738382	0.033917164
Ptgds	1.339613098	0.033917164
Gm45867	1.081883115	0.033917164
Gm4951	0.759214824	0.033917164
Flt3	0.848262906	0.033917164
Tubb4b	-0.364577636	0.033917164
Adamts4	-0.589364541	0.033917164
Cxcr4	0.550796114	0.033917164
Arntl	-0.55020371	0.033947996
Hk3	0.580138919	0.034098072
Rab11fip4	0.885421817	0.034098072
Treml2	1.132409903	0.034312196
Rsad2	0.730982347	0.034365381

Table S1 Continued

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Epsti1	0.705526268	0.034468208
Lax1	0.844662067	0.034468208
Cpe	-0.676864761	0.034468208
Lacc1	0.549664346	0.034468208
Gpr174	1.328695861	0.034468208
Slc44a4	1.244062098	0.034468208
Tbx4	0.495827782	0.034468208
Tap1	0.621941062	0.034468208
Myo1f	0.585952359	0.034686363
Il18r1	1.195470761	0.034838793
Ctsw	1.244757716	0.035012634
Arhgap42	-0.344640414	0.035012634
Rassf7	0.804895674	0.035012634
Gstm1	-0.380854367	0.035212128
St8sia1	1.437598983	0.035212128
Gamt	0.400409236	0.035212128
Slc2a9	0.833426321	0.035212128
Nlrp1b	0.790719529	0.035864933
Rasgrp1	0.739165507	0.035864933
Eomes	1.455063296	0.035864933
Il1a	1.886532679	0.035864933
A430093F15Rik	1.876909536	0.036035689
Mirt1	1.528082956	0.036035689
St3gal2	-0.36949867	0.036035689
Tapbpl	0.450579667	0.036035689
Cybb	0.634826894	0.036035689
Tmem71	0.889817308	0.036035689
Rnase2a	5.059515946	0.036035689
Fndc3b	-0.546850651	0.036244419
Gbp7	0.473441494	0.036426396
AB124611	0.68282944	0.036623256
Has3	-0.834611495	0.036773036
Dym	-0.409421834	0.036834063
Trit1	0.409532285	0.036834063
Dll1	0.549245597	0.036834063
Slc8a1	-0.364811577	0.036834063
Piezo2	-0.476712587	0.036834063
Togaram2	-0.639179347	0.036879592
Smim6	1.717852717	0.036910898
Tap2	0.408039862	0.036910898
H2-T24	0.461962001	0.037095935

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Coro2a	0.835879418	0.037095935
Abcg1	0.707529309	0.037095935
Samsn1	0.871335494	0.037095935
Nr1d1	-0.685606911	0.037095935
Blnk	0.731914358	0.037095935
Ms4a14	0.553406618	0.037178737
Fzd9	-0.568860747	0.037178737
F2rl1	-0.904869668	0.037178737
Marchf1	0.524305446	0.037178737
Sat1	0.503616149	0.037178737
Tifab	0.620463748	0.037178737
Cd84	0.750274677	0.037353396
Tnc	-0.831608758	0.037805842
Psmb9	0.636961799	0.037842163
2410006H16Rik	0.446597775	0.037842163
Trim25	0.314681693	0.037882711
Aph1c	0.800620959	0.038104412
Hvcn1	0.597481125	0.038124734
P4ha2	-0.435577498	0.03834952
Celsr3	1.143381456	0.03834952
Sdsl	1.011682242	0.03834952
Aldh1l2	-0.62840189	0.03834952
Grin2a	1.509818526	0.03861339
Ifi208	0.886325243	0.038737167
Klrd1	1.3672707	0.038737167
Arhgap45	0.534820686	0.038737167
Sit1	2.292654702	0.038737167
Il10ra	0.530499534	0.038737167
Igfbp2	1.051632314	0.038737167
Efr3b	-0.639582132	0.038737167
Cd200r4	0.823100788	0.038894303
Rasgef1b	0.533532356	0.039067339
Pycr1	-0.478254643	0.039205288
Vstm2a	1.602975248	0.040011216
Parvg	0.795249187	0.040421056
Crim1	-0.39210798	0.040885426
Xcr1	1.137449203	0.041515018
Ifi209	0.632553477	0.041559611
Pamr1	-0.391606223	0.041559611
Igsf6	0.714111159	0.041559611
Dna2	0.523513198	0.041559611

Table S1 Continued

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Coro2a	0.835879418	0.037095935
Abcg1	0.707529309	0.037095935
Samsn1	0.871335494	0.037095935
Nr1d1	-0.685606911	0.037095935
Blnk	0.731914358	0.037095935
Ms4a14	0.553406618	0.037178737
Fzd9	-0.568860747	0.037178737
F2rl1	-0.904869668	0.037178737
Marchf1	0.524305446	0.037178737
Sat1	0.503616149	0.037178737
Tifab	0.620463748	0.037178737
Cd84	0.750274677	0.037353396
Tnc	-0.831608758	0.037805842
Psmb9	0.636961799	0.037842163
2410006H16Rik	0.446597775	0.037842163
Trim25	0.314681693	0.037882711
Aph1c	0.800620959	0.038104412
Hvcn1	0.597481125	0.038124734
P4ha2	-0.435577498	0.03834952
Celsr3	1.143381456	0.03834952
Sdsl	1.011682242	0.03834952
Aldh1l2	-0.62840189	0.03834952
Grin2a	1.509818526	0.03861339
Ifi208	0.886325243	0.038737167
Klrd1	1.3672707	0.038737167
Arhgap45	0.534820686	0.038737167
Sit1	2.292654702	0.038737167
Il10ra	0.530499534	0.038737167
Igfbp2	1.051632314	0.038737167
Efr3b	-0.639582132	0.038737167
Cd200r4	0.823100788	0.038894303
Rasgef1b	0.533532356	0.039067339
Pycr1	-0.478254643	0.039205288
Vstm2a	1.602975248	0.040011216
Parvg	0.795249187	0.040421056
Crim1	-0.39210798	0.040885426
Xcr1	1.137449203	0.041515018
Ifi209	0.632553477	0.041559611
Pamr1	-0.391606223	0.041559611
Igsf6	0.714111159	0.041559611
Dna2	0.523513198	0.041559611

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Arhgap9	0.712503675	0.041559611
Creb3l1	-0.865616922	0.041559611
Col26a1	0.510755934	0.041559611
Ogdhl	0.669315353	0.041559611
Ap1s3	1.433898703	0.041559611
Fcho1	1.028392561	0.041559611
Stk17b	0.410569622	0.041687058
Jcad	-0.485486249	0.042172923
Gm45512	0.735982419	0.042418902
Ifit2	0.842423464	0.042777243
Lrrc17	-0.467419675	0.042777243
Krt8	0.905314267	0.042777243
Slc38a1	0.570037105	0.042777243
Mypop	-0.526028724	0.042777243
Kdm3a	0.306834967	0.042777243
Ly9	0.679601423	0.042777243
H2-Q7	0.906950083	0.042777243
Cd82	0.427879879	0.042777243
Pglyrp1	0.862597139	0.042777243
Trim30a	0.505194863	0.043036209
Rgs2	0.493291454	0.043347892
Oas3	0.850472612	0.043347892
Ttc39a	0.855496121	0.043347892
Hsh2d	1.119041471	0.043347892
Gal	2.633418974	0.043436345
Gm8113	2.425659458	0.043436345
Manf	-0.443449248	0.043701864
Parp10	0.369145034	0.043701864
Kcnh2	0.720406589	0.043846894
Map7d3	0.997715956	0.044218604
B3gnt5	0.884014731	0.044218604
Siglecf	1.842229775	0.044218604
H2-K1	0.626893736	0.044218604
Sh2d1b1	0.921322008	0.044218604
Cmah	-0.627998276	0.044218604
Jam3	0.307453607	0.044218604
Arhgef15	-0.471622801	0.044218604
Al504432	0.649653117	0.044328397
Tm4sf1	-0.339333547	0.044328397
Serpine2	-0.366135538	0.044460176
Lrrn1	0.433060515	0.044460176

Table S1 Continued

Gene Name	RSEA LOG2(FC)	EDGE R FDR
Lrrc15	-0.8413941	0.044485355
Clec2i	0.817470563	0.044500889
Stk40	-0.423188904	0.044584118
Klra7	1.311161815	0.044641596
Wnt16	-1.105478639	0.044859237
Kcnk13	0.56761148	0.044859237
Fhad1	1.522754044	0.045286847
Svbp	0.362532279	0.045902211
Srgap1	-0.545932139	0.04597027
Ifi213	0.889138759	0.045995667
Morc3	0.32036656	0.045995667
Cd180	0.831454606	0.045995667
Trim13	0.441029275	0.045995667
Pianp	0.930559587	0.045995667
Gm36161	0.818110366	0.045995667
Adamts16	-0.82521823	0.045995667
Pcdh18	-0.494062581	0.046190835
Hlf	0.459349187	0.046418932
Amz1	0.680259234	0.04678159
Bcorl1	-0.334571079	0.047634494
Arhgap15	0.783272879	0.047641588
Gucy1b2	-1.428563918	0.047939781
Tmem132a	0.323782877	0.047939781
Bex1	0.426685795	0.04799333
Cdc42se2	0.325897344	0.04799333
Smc4	0.501636764	0.048225116
Abcg3	0.716376154	0.048461459
Mir142hg	1.124262812	0.048486991
Ifit1bl1	1.226810418	0.048811929
Dusp18	-0.439769454	0.048811929
Barx2	-0.505824196	0.048811929
Sp110	0.561438334	0.049084886
Plat	-0.452959728	0.049084886
Vcan	-0.567021246	0.049084886
Evi2a	0.519206368	0.049237412
Bcar3	0.435503585	0.049855187

Table S2. NanoString Top Differential Expression of 1-week Muscle $CD3^{+}SSC^{Low}$ Sorted Cells

1-week post-injury: $CD3^{+}SSC^{Low}$ sorted cells from skeletal muscle								
Gene	**Log2 FC**	**Std Error**	**Lower Conf. Limit**	**Upper Conf. Limit**	**P-value**	**BY.p.value**	**Analysis Method**	**nCounter Probe.ID**
Il10	**3.49**	0.778	1.97	5.02	0.00203	0.44	lm.nb	NM_010548.1:985
Il10ra	**3.03**	0.527	2	4.07	0.00043	0.33	loglinear	NM_008348.2:75
Tnfrsf18	**2.99**	0.425	2.15	3.82	0.00011	0.18	loglinear	NM_009400.2:840
Ccr4	**2.15**	0.498	1.18	3.13	0.00253	0.47	lm.nb	NM_009916.2:394
Il13	**1.92**	0.352	1.23	2.6	0.00061	0.33	loglinear	NM_008355.2:425
Isg20	**1.9**	0.428	1.06	2.74	0.00215	0.44	lm.nb	NM_020583.5:552
Nfatc3	**1.86**	0.383	1.11	2.61	0.00125	0.38	loglinear	NM_010901.2:2260
Tigit	**1.65**	0.391	0.885	2.42	0.00291	0.49	lm.nb	NM_001146325.1:730
Cxcr6	**1.48**	0.252	0.987	1.98	0.00037	0.33	lm.nb	NM_030712.4:650
Cd5	**1.46**	0.296	0.883	2.04	0.00113	0.38	loglinear	NM_007650.3:1395
Ly9	**1.45**	0.337	0.788	2.11	0.00261	0.47	loglinear	NM_008534.2:1190
Cd4	**1.37**	0.188	1	1.74	8.63E-05	0.18	loglinear	NM_013488.2:950
Ccr2	**1.17**	0.213	0.751	1.59	0.00059	0.33	lm.nb	NM_009915.2:2965
Gata3	**1.17**	0.247	0.686	1.65	0.00147	0.38	loglinear	NM_008091.3:1943
Cd3e	**1.1**	0.226	0.653	1.54	0.00128	0.38	lm.nb	NM_007648.4:380
Il2rg	**1.09**	0.263	0.578	1.61	0.00318	0.49	loglinear	NM_013563.3:1226
H2-Aa	**-0.916**	0.189	-1.29	-0.546	0.00126	0.38	lm.nb	NM_010378.2:450
Cd81	**-1.09**	0.26	-1.6	-0.582	0.003	0.49	lm.nb	NM_133655.2:575
Cxcl12	**-1.27**	0.312	-1.88	-0.66	0.00354	0.51	lm.nb	NM_021704.3:259
Cd36	**-1.38**	0.293	-1.95	-0.806	0.00152	0.38	lm.nb	NM_007643.3:1520

Table S3. NanoString Top Differential Expression of 1-week Muscle CD11b^{+}F4/80^{Hi+} Sorted Cells

1-week post-injury: F4/80^{Hi+} sorted cells from skeletal muscle								
Gene	**Log2 FC**	**Std Error**	**Lower Conf. Limit**	**Upper Conf. Limit**	**P-value**	**BY.p.value**	**Analysis Method**	**nCounter Probe.ID**
Rnase2a	**4.43**	0.763	2.94	5.93	0.000403	0.0974	lm.nb	NM_053113.2:332
Chil4	**4.42**	0.382	3.67	5.17	2.80E-06	0.00258	lm.nb	NM_145126.2:545
Chil3	**4.27**	0.288	3.7	4.83	4.28E-07	0.00118	lm.nb	NM_009892.2:823
Arg1	**2.39**	0.415	1.58	3.21	0.000424	0.0974	lm.nb	NM_007482.3:626
Csf2ra	**2.37**	0.426	1.53	3.2	0.000536	0.114	lm.nb	NM_009970.2:296
Lgals3	**1.92**	0.236	1.46	2.38	3.84E-05	0.0265	loglinear	NM_001145953.1:1005
Siglec1	**1.79**	0.255	1.29	2.29	0.000111	0.051	lm.nb	NM_011426.3:4550
Bcl2	**1.76**	0.589	0.607	2.91	0.0202	0.705	Wald	NM_009741.3:1844
Ikbke	**1.64**	0.322	1.01	2.27	0.000921	0.159	lm.nb	NM_019777.3:618
Tlr4	**-0.82**	0.165	-1.14	-0.497	0.00109	0.176	loglinear	NM_021297.2:2510
Prdx1	**-0.835**	0.318	-1.46	-0.212	0.034	0.977	Wald	NM_011034.4:1131
Tlr2	**-0.84**	0.273	-1.37	-0.305	0.0151	0.602	lm.nb	NM_011905.2:255
S100a11	**-0.853**	0.0681	-0.987	-0.72	1.55E-06	0.00213	loglinear	NM_016740.3:304
Fcgr3	**-0.863**	0.181	-1.22	-0.508	0.00143	0.18	lm.nb	NM_010188.5:1175
Cd151	**-0.896**	0.279	-1.44	-0.348	0.0149	0.602	Wald	NM_009842.3:610
Pros1	**-0.921**	0.335	-1.58	-0.264	0.0252	0.827	loglinear	NM_011173.2:2720
Itgam	**-0.94**	0.139	-1.21	-0.667	0.000143	0.055	loglinear	NM_001082960.1:3025
Prdx3	**-0.958**	0.144	-1.24	-0.676	0.00016	0.055	loglinear	NM_007452.2:496
Itgal	**-1.1**	0.234	-1.56	-0.642	0.00219	0.217	Wald	NM_008400.2:950
Cysltr1	**-1.24**	0.307	-1.85	-0.64	0.00372	0.244	loglinear	NM_021476.4:1190