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Supplementary Materials

Towards a physiological scale of vocal fold agent-based models of surgical injury and repair: sensitivity analysis, calibration and verification

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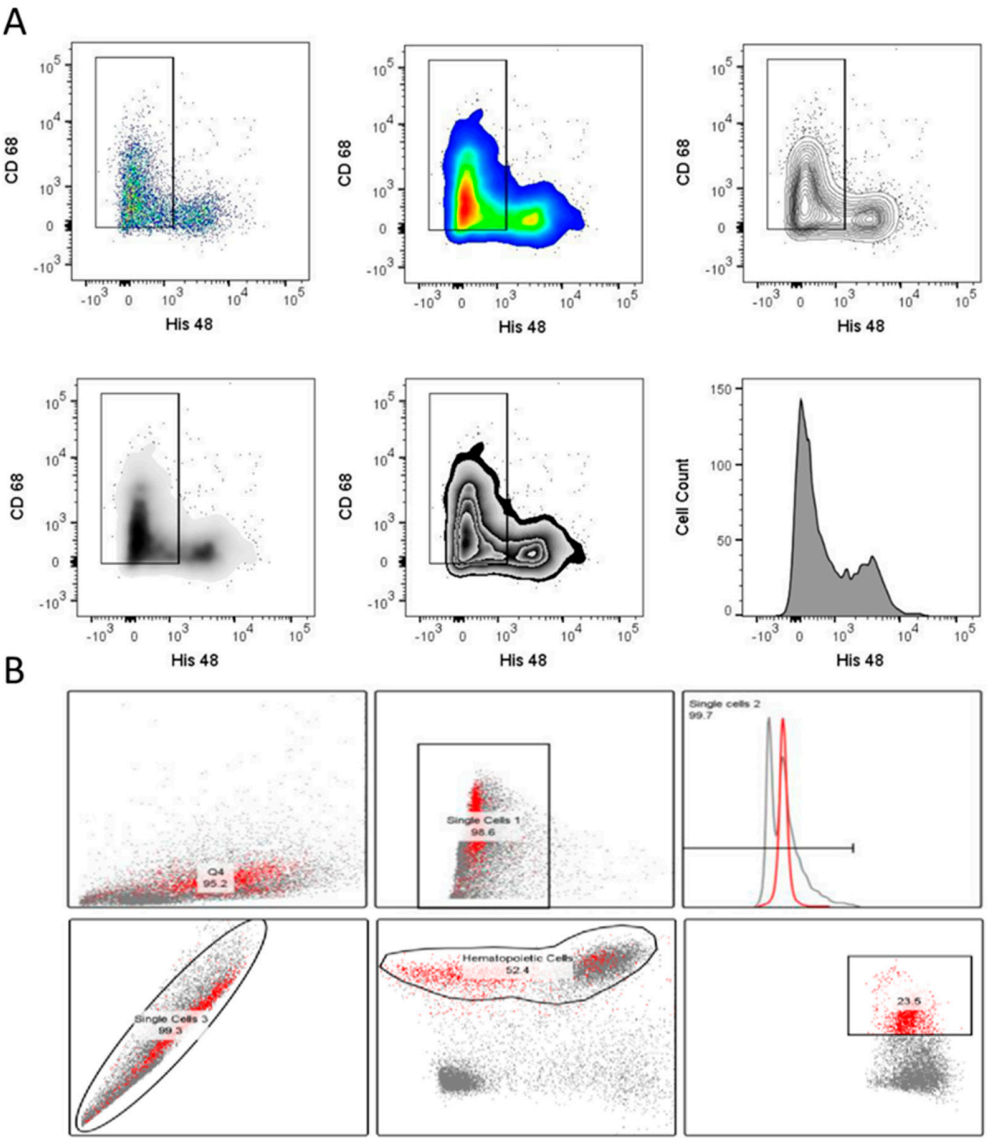

Figure S1: Verification of Gating Strategy. (A) Various plots were used for verification: (i) Original plot, (ii) Smoothing curve, (iii) Contour plot, (iv) Density plot, (v) Zebra Plot and (vi) Histogram. (B) Backgating for neutrophils. The final gated population is overlayed on each gating step as red dots on the dot plot.

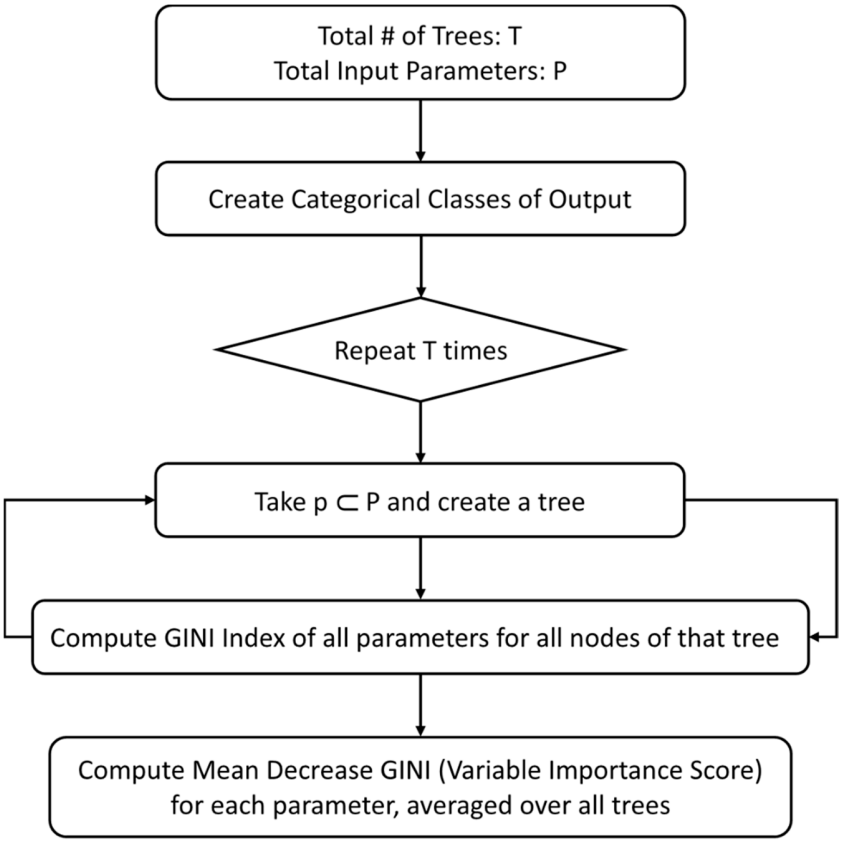

Figure S2: Workflow of Random Forests. Three factors were taken into account for sensitivity analysis namely, *T* trees, *P* input parameters and one output parameter. The algorithm produced categorical classes of output. It produced *T* trees by repeating the following procedure *T* times. It took *p* number of random parameters and then created and optimized a tree. GINI Index of all *p* parameters was computed for all the nodes in this tree. It was used to decide the further splitting of the node. After creating all *T* trees, mean decrease GINI was estimated for each parameter by aggregating the weighted GINI Index for all nodes in those trees where that parameter was used.

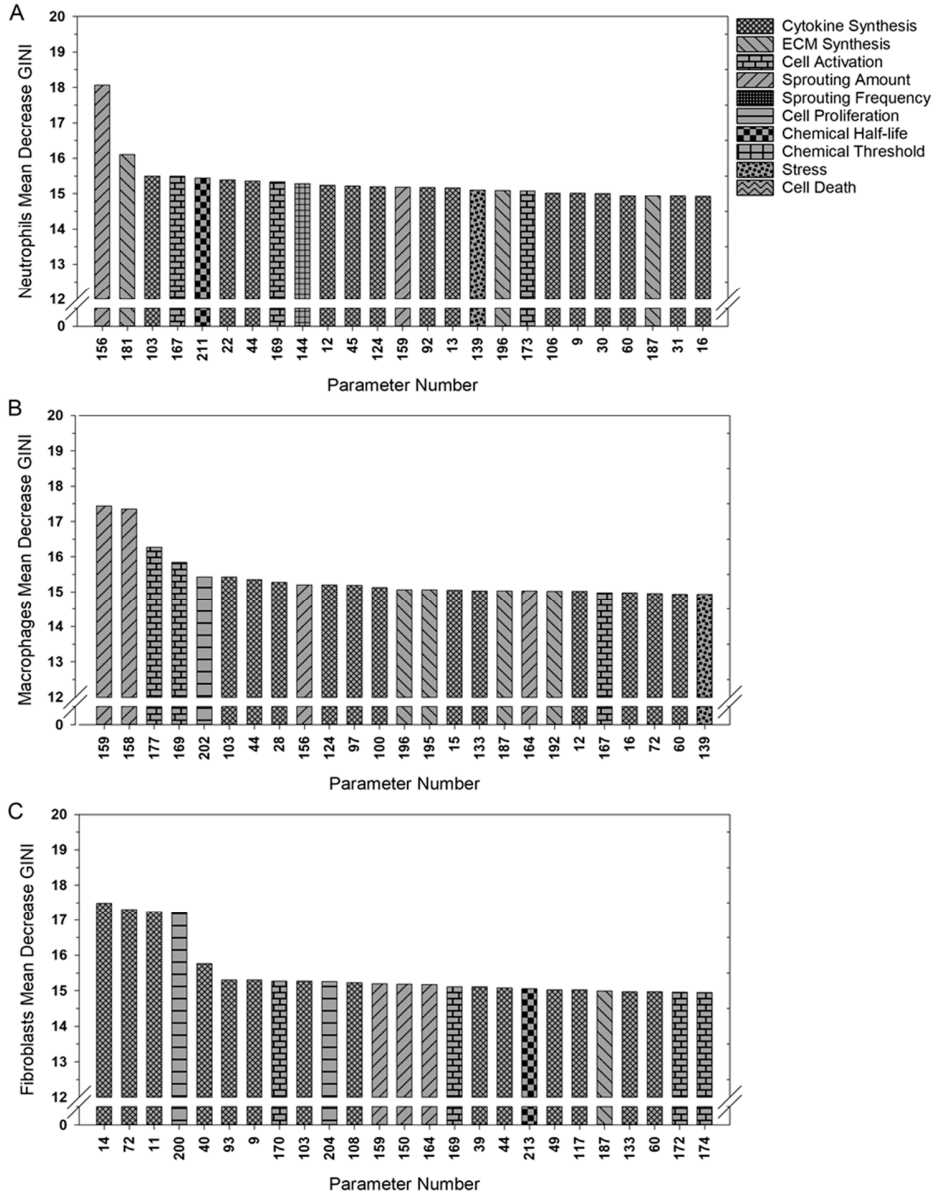

Figure S3: Top 25 Parameters obtained by Sensitivity Analysis for Day 2. (A) Neutrophils. (B) Macrophages. (C) Fibroblasts. The biological function of each parameter is coded by the shade of the bar.

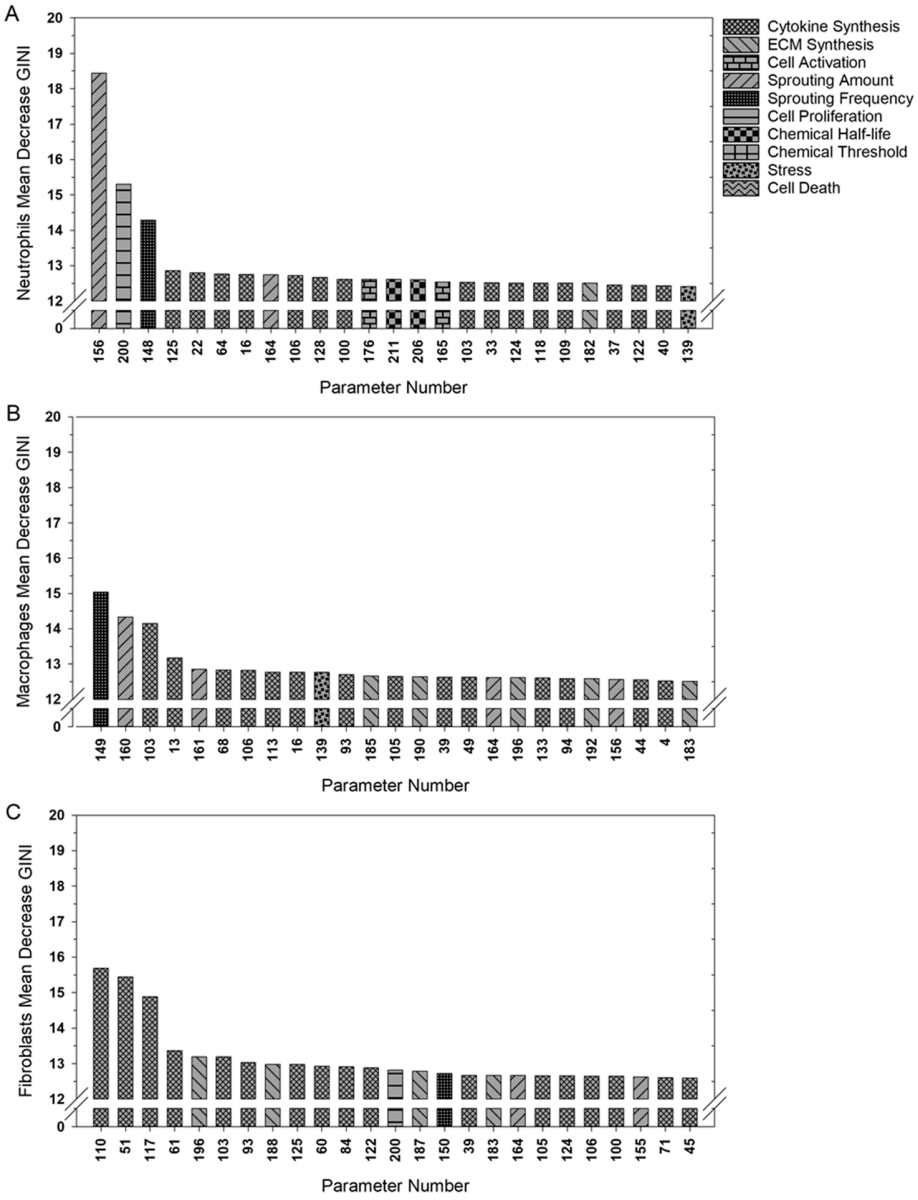

Figure S4: Top 25 parameters obtained by sensitivity analysis for Day 3. (A) Neutrophils. (B) Macrophages. (C) Fibroblasts. The biological function of each parameter is coded by the shade of the bar.

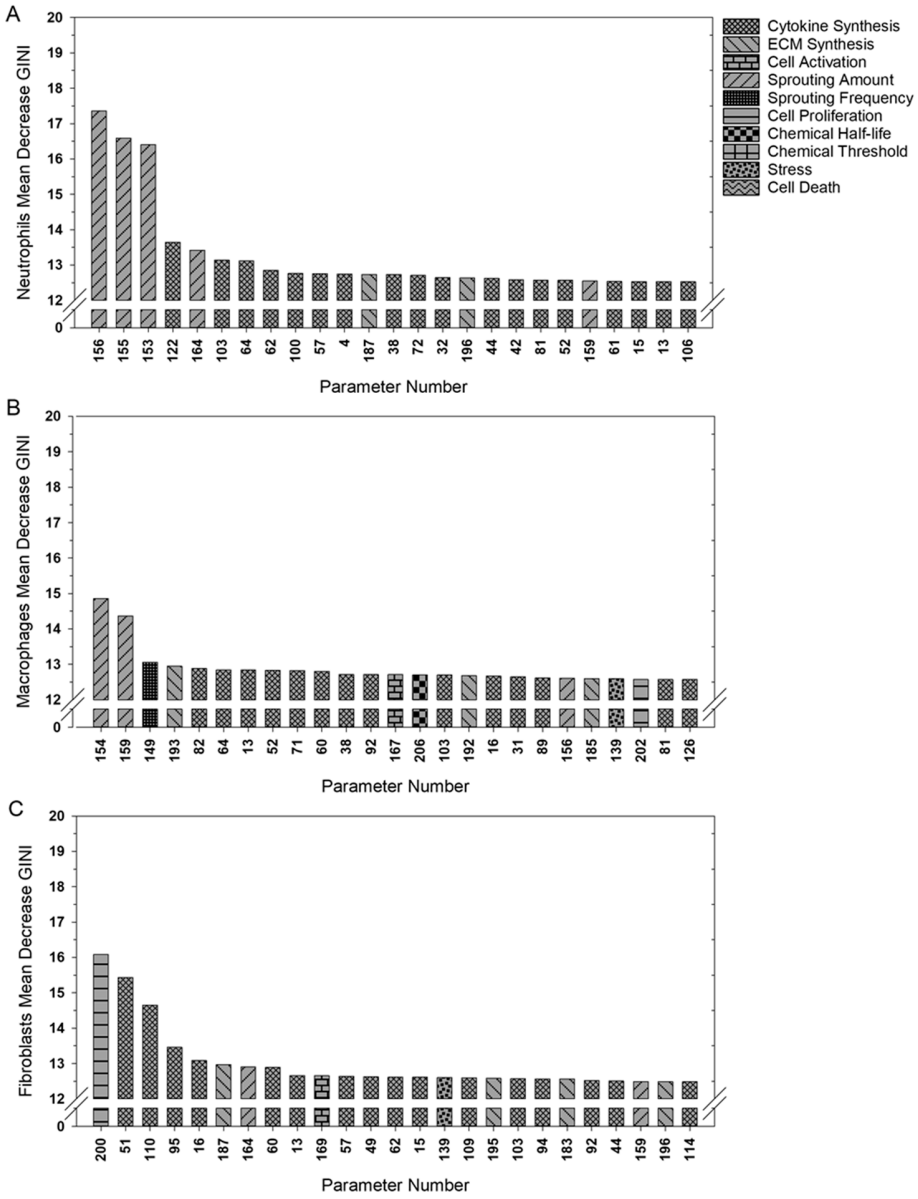

Figure S5: Top 25 parameters obtained by sensitivity analysis for Day 5. (A) Neutrophils. (B) Macrophages. (C) Fibroblasts. The biological function of each parameter is coded by the shade of the bar.

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Table S1: Cell Surface Markers used in Panel A and Their Corresponding Biological Functions.

	Markers	Significance
1	CD11b/c	CD11b/c is involved in adhesion activities of leukocytes including granulocytes, monocytes and macrophages. CD11b/c plays an important role in chemotaxis and apoptosis. CD11b/c can also be used as a marker to discriminate macrophage subtypes in the study of functional heterogeneity of macrophages.
2	CD29	CD29 is a member of the integrin family that plays an important role in cell-cell or cell-matrix interaction. CD29 binds to extracellular matrix proteins including collagen, laminin, fibronectin and vitronectin. During inflammation, CD29 helps neutrophils migrate to the wound site. Other functions of CD29 include cell adhesion, signal transduction and cell differentiation.
3	CD44H	CD44H acts as cell adhesion receptors. Hyaluronate has the ligand of CD44H. CD44H also acts as regulators of cell migration, cell-to-cell and cell-to-substrate interactions.
4	CD45	CD45 is the primary surface marker to distinguish between hematopoietic and non-hematopoietic cells. CD45 helps in controlling the signals originating from cytokine and integrin receptors. CD45 also plays an important role to regulate B-cell and T-cell antigen receptor signaling, cell growth and cell differentiation.
5	CD68	CD68 mediates the process of phagocytosis for macrophages. CD68 helps in both intracellular and extracellular activities like lysosomal metabolism, cell-to-cell and cell-to-pathogen interactions. The expression of CD68 on macrophages and monocytes is mostly cytoplasmic.
6	CD105	CD105 acts as a regulator of angiogenesis and neovascularization, and facilitates the binding of endothelial cells to integrins. The expression of CD105 results in the cytoskeletal reorganization that affects cell morphology and migration. During the process of inflammation and healing, the expression of CD105 is enhanced on activated endothelium in tissues that undergo angiogenesis.
7	CD106	CD106 mediates cell adhesion of leukocytes such as lymphocytes and monocytes to activated endothelium and functions in leukocyte-endothelial cell signal transduction. The expression of CD106 is enhanced in endothelial cells under the stimulation of inflammatory cytokines.
8	His48	His48 has been shown to react with monocytes and granulocytes via an antigen molecule which is expressed on their surface. His48 is mainly expressed by neutrophils and used as a marker to distinguish neutrophils from other cell populations. His48 is also commonly used in combination with other surface markers (such as CD11, CD45 and CD68) to identify granulocytes.
9	Cell Viability	To distinguish between live and dead cells.
10	FSC	Forward-scattered light (FSC) reflects the cell-surface area or size.
11	SSC	Side-scattered light (SSC) reflects the cell granularity or intracellular complexity.

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Table S2: Cell Surface Markers used in Panel B and Their Corresponding Biological Functions.

	Markers	Significance
1	CD31	CD31 is also known as platelet-endothelial cell adhesion molecule-1 (PECAM-1). CD31 plays a major role in cell-cell and cell-matrix interactions and signal transduction. CD31 mediates in both homotypic and heterotypic cell adhesion by binding to either itself or the leukocyte integrin $\alpha v\beta 3$. CD31 also plays a role in neutrophils recruitment, transendothelial migration of leukocytes, vasculogenesis, angiogenesis, integrin activation as well as in cardiovascular development.
2	CD45	CD45 is the primary surface marker to distinguish between hematopoietic and non-hematopoietic cells. CD45 helps in controlling the signals originating from cytokine and integrin receptors. CD45 also plays an important role to regulate B-cell and T-cell antigen receptor signaling, cell growth and cell differentiation.
3	CD90	CD90 interacts with CD45 and regulates the vascular permeability during the process of inflammation. Most peripheral T cells, fibroblasts, thymocytes and endothelial cells express CD90 on their cell surface. Other functions include differentiation of hematopoietic stem cells, proliferation and activation of lymphocytes, and adhesion of thymocytes.
4	CD163	CD163 acts as a scavenger receptor for both hemoglobin and hemoglobin-haptoglobin complex. CD163 is exclusively expressed by most of the subtypes of macrophages and mediates the activation of macrophages during inflammation.
5	His48	His48 has been shown to react with monocytes and granulocytes via an antigen molecule which is expressed on their surface. His48 is mainly expressed by neutrophils and used as a marker to distinguish neutrophils from other cell populations. His48 is also commonly used in combination with other surface markers (such as CD11, CD45, CD68 etc.) to identify granulocytes.
6	Cell Viability	To distinguish between live and dead cells.
7	FSC	Forward-scattered light (FSC) reflects the cell-surface area or size.
8	SSC	Side-scattered light (SSC) reflects the cell granularity or internal complexity.

Table S3: Laser Configuration of FACSAria II and Preconjugated Primary Antibody-Fluorochrome for Panel A.

Marker	Fluoro-chrome	Bandpass Filter (nm/nm)	Excitation (nm)	Fluorescence Emission Color	Description	Company / Catalog Number
CD11b/c	FITC	530/30	488	Green	Mouse Anti-CD11b/c equivalent antibody [MRC OX-42]	Abcam/ ab112170
CD29	PE-Cy7	780/60	488	Infrared	Anti-mouse/Rat CD29 (Integrin beta 1)	eBiociences/ 25-0291
CD44H	APC-Cy7	780/60	633	Infrared	Anti-Rat CD44H APC-eFluor 780 [OX49]	eBiosciences/ Custom order
CD45	PerCP-Cy5.5	695/40	488	Far Red	Mouse Anti-rat CD45 Antibody [OX-1]	Biolegend/ 202220
CD68	PE-Texas Red	610/20	488	Orange	Mouse Anti Rat CD68 RPE-Texas Red [ED1]	AbD Serotec/ Custom order
CD105	PE	575/26	488	Yellow	Rabbit Anti-CD105/Endoglin polyclonal antibody	BIOSS/ bs-4609R-PE
CD106	Brilliant Violet 421	450/50	405	Blue	Mouse Anti-rat CD106 [MR106]	BD/ Custom order
His48	APC	660/20	633	Red	Anti-Rat Granulocyte Marker [HIS48]	eBiosciences/ Custom Order
Cell Viability Dye	AmCyan	525/20	405	Green	Fixable Viability Dye eFluor® 506	eBiosciences/ 65-0866-14

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Table S4: Laser Configuration of FACSAria II and Preconjugated Primary Antibody-Fluorochrome for Panel B.

Marker	Fluoro-chrome	Bandpass Filter (nm/nm)	Excitation (nm)	Fluorescence Emission Color	Description	Company / Catalog Number
CD31	APC	660/20	633	Red	Anti-Rat CD31 (PECAM-1) [TLD-3A12] eFluor 660 / APC	eBiosciences / 50-0310
CD45	PerCP-Cy5.5	695/40	488	Far Red	Mouse Anti-rat CD45 Antibody [OX-1]	Biolegend/ 202220
CD90	FITC	530/30	488	Green	Mouse Anti- Rat THY1 / CD90 [HIS51]	LSBio/ LS-C105942
CD163	PE-Cy7	780/60	488	Infrared	Mouse Anti Rat CD163 [ED2]	AbD Serotec/ Custom order
His48	PE	575/26	488	Yellow	Anti-Granulocytes antibody [HIS48]	eBioscience/ 12-0570
Cell Viability Dye	AmCyan	525/20	405	Green	Fixable Viability Dye eFluor® 506	eBiosciences / 65-0866-14