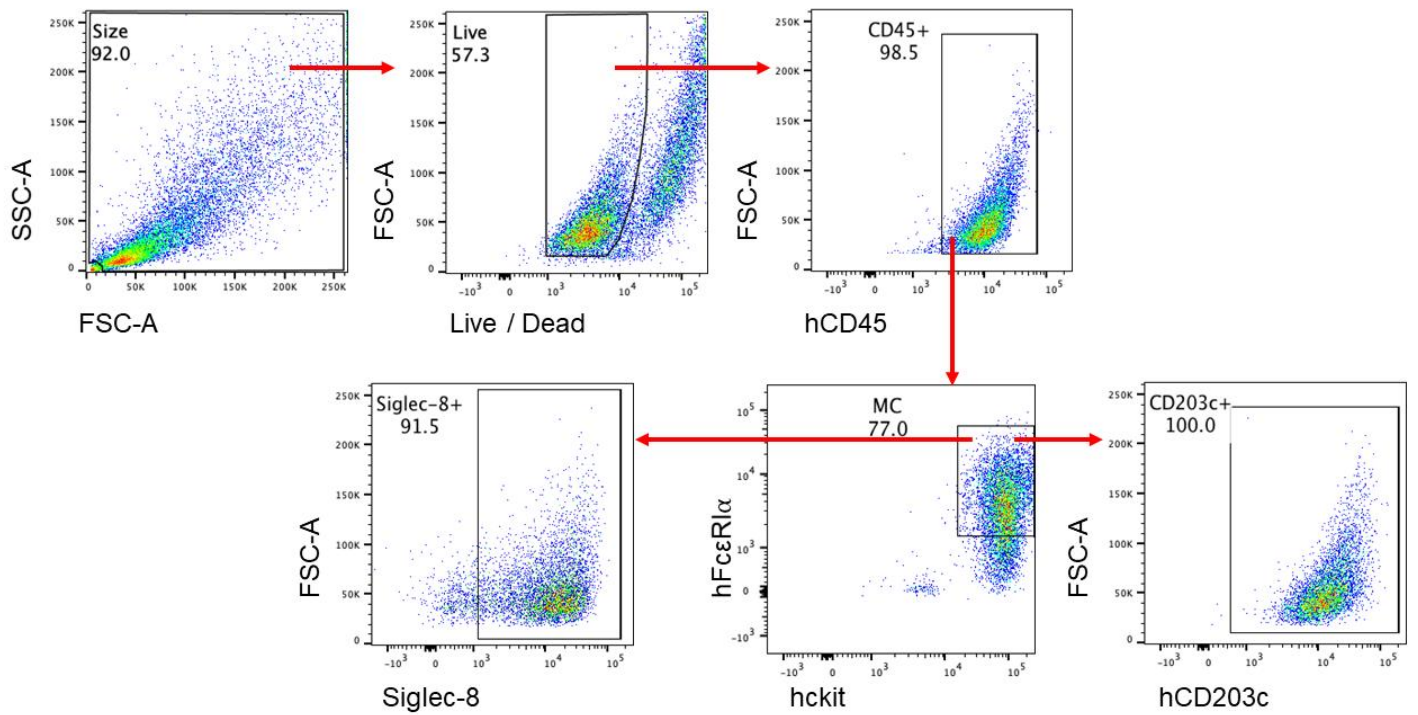


Supplementary Tables and Figures

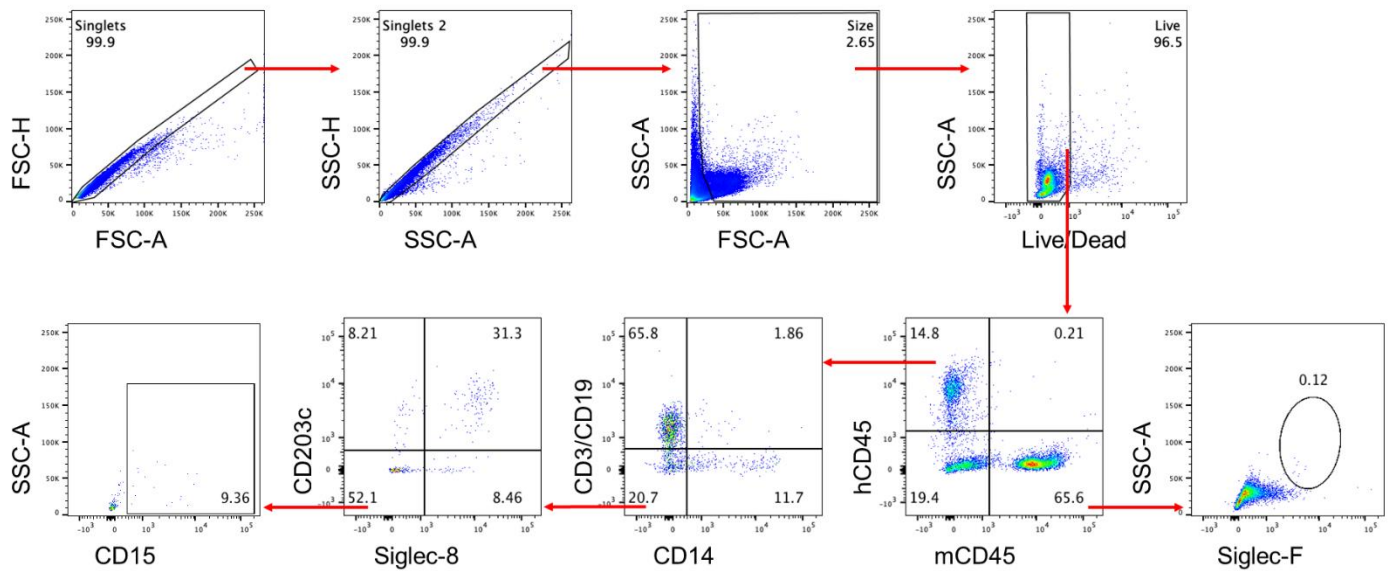
Supplemental Table 1: Antibodies used for flow cytometry

	Clone	Fluorophore Conjugation	Source
Antibodies for SDMC surface marker upregulation			
Anti-human CD117 (c-Kit)	YB5.B8	APC	BD Biosciences
Anti-human CD203c	NP4D6	PE-Cy7	Biolegend
Anti-human CD107a (LAMP1)	H4A3	PE	Invitrogen
Antibodies for NSG-SGM3 humanized mouse engraftment analysis			
Anti-mouse CD45	30-F11	AF700	Biolegend
Anti-human CD45	2D-1	FITC	Biolegend
Anti-human CD3	OKT3	PerCP-Cy5.5	Biolegend
Anti-human CD19	HIB19	PerCP-Cy5.5	Biolegend
Anti-human CD14	63D3	APC-Cy7	Biolegend
Anti-human CD203c	NP4D6	PE-Cy7	Biolegend
Anti-mouse Siglec F	W6D3	PE	Becton Dickinson
Anti-Siglec-8	2C4	AF647	Conjugated in-house (1)
Antibodies for NSG-SGM3 humanized mouse dispersed tissue-resident mast cell analysis			
Anti-mouse CD117 (c-Kit)	2B8	FITC	eBioscience
Anti-mouse CD45	30F16	PE-Cy7	Biolegend
Anti-mouse FcεRI	MAR-1	BV421	eBioscience
Anti-human CD45	HI30	AF700	Biolegend
Anti-human FcεRI	AER-37	APC-Cy7	Biolegend
Anti-human CD117 (c-Kit)	A3C6E2	PerCP-Cy5.5	Biolegend
Anti-Siglec-8	2C4	AF647	Conjugated in-house (1)

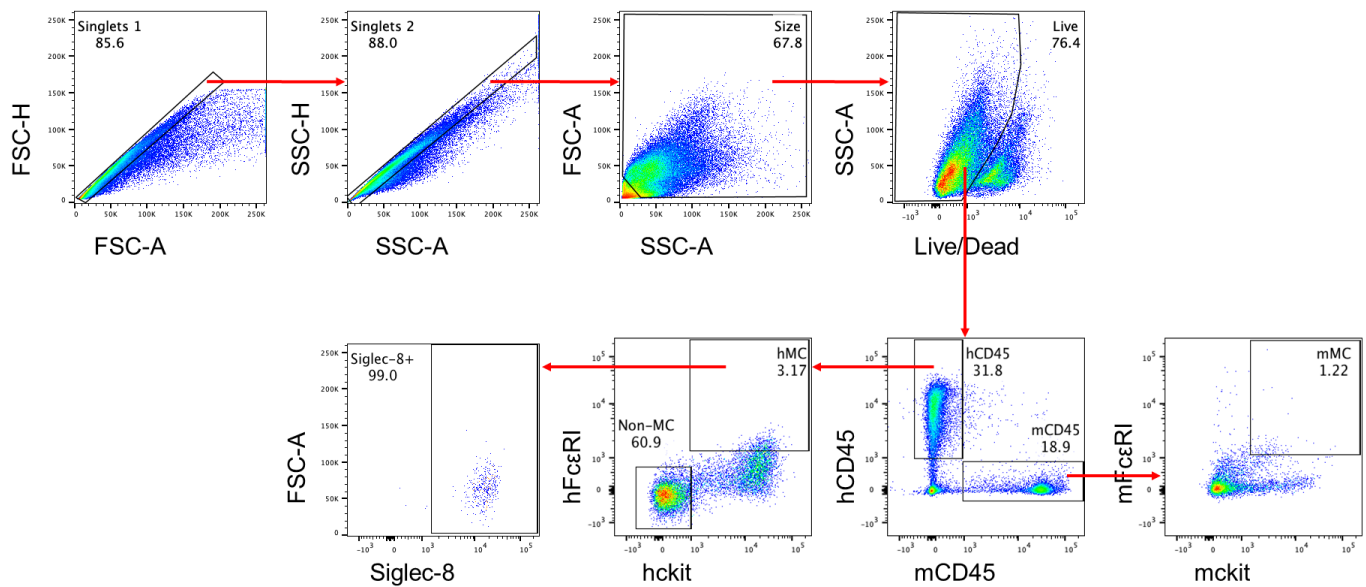
Supplemental Figure 1: Human skin-derived mast cell (SDMC) cultures. Human SDMCs were isolated from normal skin fragments as described. After 4 weeks in culture, SDMCs from each donor consistently showed 70-90% purity as assessed by human CD45 (hCD45), cKit (hcKit) and FcεRI (hFcεRI) expression by flow cytometry. CD203c (hCD203c) and Siglec-8 expression were also analyzed. Plots are representative of three separate experiments.



Supplemental Figure 2: Gating strategy for engraftment analysis of humanized mice. Whole blood from engrafted NSG-SGM3 mice was obtained at specific time points after HSC injection, labeled with fluorescently-conjugated antibodies for both murine and human markers, and analyzed by flow cytometry. Cells were first gated on forward and side scatter parameters to isolate single cells, then Aqua Fluorescent Reactive Dye (Invitrogen) was used to gate on live cells. Cells were then further gated on human CD45 (hCD45) or murine CD45 (mCD45) expression. Human mast cell and basophil progenitors were analyzed by gating on hCD45+ CD3- CD19- CD14- CD15- Siglec-8+ CD203c+ cells.



Supplemental Figure 3: Gating strategy for analysis of tissue-resident mast cells in humanized mice after HSC engraftment. Engrafted NSG-SGM3 mice were sacrificed 16 weeks after HSC injection. Indicated tissues were harvested, processed as described to generate single cell suspensions, and then labeled with fluorescently-conjugated antibodies for various murine and human markers for analysis by flow cytometry. Cells were first gated on forward and side scatter parameters to isolate single cells, then Aqua Fluorescent Reactive Dye (Invitrogen) was used to gate on live cells. Cells were then further gated on human CD45 (hCD45) or murine CD45 (mCD45) expression. Human mast cells were analyzed by gating on hCD45+ hKit+ hFcεRI+ Siglec-8+ cells.



References:

1. Kikly KK, Bochner BS, Freeman SD, Tan KB, Gallagher KT, D'Alessio K J, et al. Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells, and basophils. *J Allergy Clin Immunol.* 2000;105(6 Pt 1):1093-100.