Supplemental Table 1. Key materials

Reagent or resource	Source	Identifier
Stem cell isolation, culture, transduction and electroporation		
Cas9 nuclease	Integrated DNA Tech	Cat#: 1081059
Ficoll-Paque Plus	GE Healthcare	Cat#: 17-1440-02
Indirect CD34 Microbead Kit	Miltenyi Biotec	Cat#: 130-046-701
Retronectin	Takara Bio Inc	Cat#: T100B
Stemline II HSC Expansion Medium	Sigma-Aldrich	Cat#: S0192
Cytokines, complements and inhibitors		
AMD3100	Sigma-Aldrich	Cat#: A5602
C-C motif chemokine ligand 1 (CCL1)	R&D Systems	Cat#: 272-I/CF
Complement component 3a (C3a)	R&D Systems	Cat#: 3677-C3
Complement component 5a (C5a)	R&D Systems	Cat#: 2037-C5
Fms-related tyrosine kinase 3 ligand (Flt3-L)	Miltenyi Biotec	Cat#: 130-096-479
Pertussis toxin (PTX)	Sigma-Aldrich	Cat#: P7208
Stem cell factor (SCF)	Miltenyi Biotec	Cat#: 130-093-991
Stromal cell-derived factor-1 (SDF-1)	R&D Systems	Cat#: 350-NS/CF
Thrombopoietin (TPO)	Miltenyi Biotec	Cat#: 130-094-011
Tumor necrosis factor (TNF-α)	PeproTech	Cat#: 300-01A
Quantitative PCR		
High Capacity cDNA RT Kit	Applied Biosystems	Cat#: 4368814
RNeasy Micro Kit	Qiagen	Cat#: 74004
Taqman Gene Expression Master Mix	Applied Biosystems	Cat#: 4369016
TRIzol reagent	Invitrogen	Cat#: 15596026
Taqman assays		
C-C motif chemokine ligand 1	Applied Biosystems	CCL1; Hs00171072_m1
Coagulation factor II thrombin receptor like 2	Applied Biosystems	F2RL2; Hs00187982_m1
Complement component 3a receptor 1	Applied Biosystems	C3AR1; Hs00377780_m1
Complement component 5a receptor 1	Applied Biosystems	C5AR1; Hs00383718_m1
Complement component 5a receptor 2	Applied Biosystems	C5AR2; Hs00218495_m1
Cyclin A1	Applied Biosystems	CCNA1; Hs00171105_m1
Glyceraldehyde-3-phosphate dehydrogenase	Applied Biosystems	GAPDH; Hs99999905_m1
Heparanase	Applied Biosystems	HPSE; Hs00935036_m1
Interleukin 1 receptor like 1	Applied Biosystems	IL1RL1; Hs00249384_m1
Lysophosphatidic acid receptor 5	Applied Biosystems	LPAR5; Hs01051307_m1
Matrix metallopeptidase 14	Applied Biosystems	MMP14; Hs00237119_m1
Protein S, alpha	Applied Biosystems	PROS1; Hs00165590_m1
Regulator of G-protein signaling 1	Applied Biosystems	RGS1; Hs00175260_m1
Regulator of G-protein signaling 2	Applied Biosystems	RGS2; Hs00180054_m1
Regulator of G-protein signaling 3	Applied Biosystems	RGS3; Hs00367777_m1
Regulator of G-protein signaling 4	Applied Biosystems	RGS4; Hs00194501_m1

Regulator of G-protein signaling 5	Applied Biosystems	RGS5; Hs00186212_m1
Regulator of G-protein signaling 8	Applied Biosystems	RGS8; Hs00364277_m1
Regulator of G-protein signaling 13	Applied Biosystems	RGS13: Hs00243182_m1
Regulator of G-protein signaling 16	Applied Biosystems	RGS16; Hs00892674_m1
Regulator of G-protein signaling 18	Applied Biosystems	RGS18; Hs00329468_m1
Regulator of G-protein signaling 21	Applied Biosystems	RGS21; Hs00982207_m1
Secreted phosphoprotein 1	Applied Biosystems	SPP1; Hs00959010_m1
Thrombospondin 1	Applied Biosystems	THBS1; Hs00962908_m1
TIMP metallopeptidase inhibitor 3	Applied Biosystems	TIMP3; Hs00165949_m1
Single-guide RNA		
AAVS1	Synthego	GGGGCCACUAGGGACAGGAU
RGS1	Synthego	AAUGAAAUCUUCCAAGUCCA
RGS13	Synthego	CGAGAAUAUUCAAUUCUGGA
RGS16	Synthego	CAGAGCUGGGCUGCGAUACU
Western blotting		
Anti-GAPDH (rabbit monoclonal/14C10)	Cell Signaling Tech	Cat#: 2118S; 1:1000
Anti-RGS1 (rabbit polyclonal)	Invitrogen	Cat#: PA5-29579; 1:1000
Anti-RGS2 (rabbit polyclonal)	Invitrogen	Cat#: PA5-28162; 1:1000
Anti-RGS13 (rabbit polyclonal)	Invitrogen	Cat#: PA5-22291; 1:1000
Anti-RGS16 (rabbit polyclonal)	ABclonal	Cat#: A4078; 1:1000
Goat-anti-rabbit HRP	Cell Signaling Tech	Cat#: 7074S; 1:5000
Protease inhibitor cocktail	Roche Diagnostics	Cat#: 11836170001
RIPA buffer	Sigma-Aldrich	Cat#: R0278
SignalFire Elite ECL Reagent	Cell Signaling Tech	Cat#: 12757S
Functional assays		
7-AAD	BD Biosciences	Cat#: 555816
Annexin V-APC	BD Biosciences	Cat#: 550475
Annexin V binding buffer	BD Biosciences	Cat#: 51-66121E
Bovine serum albumin	Sigma-Aldrich	Cat#: A3059
CellTracker Deep Red	Molecular Probes	Cat#: C34565
CellTracker Orange CMRA	Molecular Probes	Cat#: C34551
Fetal bovine serum	Gibco	Cat#: 10473028
Fibronectin	Gibco	Cat#: 33016015
Fixation/Permeabilization Solution Kit	BD Biosciences	Cat#: 554714
Fluo-3AM	Molecular Probes	Cat#: F14218
Hank's Balanced Salt Solution	Gibco	Cat#: 14175095
HEPES	Gibco	Cat#: 15630080
Iscove's Modified Dulbecco's Medium	Gibco	Cat#: 12440053
Matrigel	BD Biosciences	Cat#: 354234
MethoCult H4434 Classic	Stem Cell Tech	Cat#: 04444
Phalloidin-AF647	Molecular Probes	Cat#: A22287

Pluronic acid	Molecular Probes	Cat#: P3000MP
VLA4 blocking antibody	BD Biosciences	Cat#: 555501; clone 9F10
Flow cytometry antibodies		
FcR Blocking Reagent, Human	Miltenyi Biotec	Cat#: 130-059-901
Human AKT (pS473)-AF647	BD Biosciences	Cat#: 560343; clone M89-61
Human C3AR1-APC	BioLegend	Cat#: 345805; clone hC3aRZ8
Human C5AR1-APC	BioLegend	Cat#: 344309; clone S5/1
Human CD19-PE	BD Biosciences	Cat#: 555413; clone HIB19
Human CD33-PE	BD Biosciences	Cat#: 555450; clone WM53
Human CD34-FITC	BD Biosciences	Cat#: 348053; clone 8G12
Human CD34-PE	BD Biosciences	Cat#: 560941; clone 581
Human CD45-APC	Beckman Coulter	Cat#: IM2473U; clone J.33
Human CD71-PE	BD Bioscience	Cat#: 555537; clone M-A712
Human CXCR4-APC	BD Biosciences	Cat#: 555976; clone 12G5
Human CXCR7-APC	BioLegend	Cat#: 391405; clone 10D1-J16
Human ERK1/2 (pT202/pY204)-AF647	BD Biosciences	Cat#: 612593; clone 20A
Human MMP14-APC	R&D Systems	Cat#: FAB9181A; clone 128527
Human STAT3 (pS727)-AF647	BD Biosciences	Cat#: 558099; clone 49/pStat3
Mouse CD16/CD32	BD Biosciences	Cat#: 553140; clone 2.4G2
Special culturewares		
High-binding 96-well plates	Corning	Cat#: 9018
Non-TC treated 6-well plates	Falcon	Cat#: 351146
Transwells 6.5-mm diameter; 5-μm pore	Corning	Cat#: 3421
Softwares		
FlowJo	TreeStar	v10.4
GeneChip Operating Software	Affymetrix	https://www.affymetrix.com/
ICE CRISPR Analysis Tool	Synthego	https://synthego.com/
Image J	NIH	https://imagej.nih.gov/ij
MetaCore	Clarivate Analytics	https://portal.genego.com/
Partek Genomics Suite	Partek	v6.5
SPSS	IBM Corporation	v21.0

Supplemental Table 2. GO analyses of genes exclusively altered by specific RGS members

RGS1			
#	**Processes**	***P* value**	**FDR**
1	Regulation of interleukin-5 production	1.48E-07	7.77E-05
2	Immune system process	1.56E-05	4.09E-03
3	Inflammatory response	7.66E-05	1.33E-02
4	Defense response	1.97E-04	1.81E-02
5	Cellular response to cytokine stimulus	2.62E-04	1.81E-02
6	Positive regulation of microglial cell migration	3.46E-04	1.81E-02
7	Positive regulation of response to macrophage colony-stimulating factor	3.46E-04	1.81E-02
8	Positive regulation of macrophage colony-stimulating factor signaling pathway	3.46E-04	1.81E-02
9	Mammary gland fat development	3.46E-04	1.81E-02
10	Positive regulation of cellular response to macrophage colony-stimulating factor stimulus	3.46E-04	1.81E-02
RGS13			
#	**Processes**	***P* value**	**FDR**
1	Oxygen transport	5.96E-09	1.31E-05
2	Gas transport	4.04E-08	4.46E-05
3	Nucleosome assembly	8.06E-08	5.92E-05
4	Protein heterooligomerization	1.29E-07	7.11E-05
5	Chromatin assembly	2.07E-07	9.16E-05
6	Nucleosome organization	4.25E-07	1.56E-04
7	Chromatin assembly or disassembly	7.02E-07	2.21E-04
8	Defense response to bacterium	1.09E-06	3.00E-04
9	DNA packaging	2.17E-06	5.32E-04
10	Protein-DNA complex assembly	3.96E-06	8.21E-04
RGS16			
#	**Processes**	***P* value**	**FDR**
1	Postsynaptic neurotransmitter receptor internalization	2.05E-06	1.54E-03
2	Dynamin family protein polymerization involved in mitochondrial fission	1.22E-05	1.85E-03
3	Synaptic vesicle budding from presynaptic endocytic zone membrane	1.22E-05	1.85E-03
4	Dynamin family protein polymerization involved in membrane fission	1.22E-05	1.85E-03
5	Sperm ejaculation	1.22E-05	1.85E-03
6	Negative regulation of dendritic spine morphogenesis	1.87E-05	2.36E-03
7	Synaptic vesicle budding	2.24E-05	2.42E-03
8	Membrane fission	2.65E-05	2.50E-03
9	Neurotransmitter receptor internalization	3.57E-05	2.99E-03
10	Insemination	7.84E-05	5.89E-03

Supplemental Table 3. Array data of 36 differentially expressed transcripts commonly regulated by RGS1/13/16

Column ID	Gene Assignment	Gene Symbol	Fold change (RGS1 *vs.* CTRL)	*P* value (RGS1 *vs.* CTRL)	Fold change (RGS13 *vs.* CTRL)	*P* value (RGS13 *vs.* CTRL)	Fold change (RGS16 *vs.* CTRL)	*P* value (RGS16 *vs.* CTRL)
16980245	NM_198682 // GYPE // glycophorin E (MNS blood group) // 4q31.1 // 2996 /// ENST00000358	GYPE	1.712	0.008	1.700	0.008	1.606	0.015
16760516	NM_001142961 // LPAR5 // lysophosphatidic acid receptor 5 // 12p13.31 // 57121 /// NM_0	LPAR5	-1.525	0.000	-1.513	0.000	-1.583	0.000
16782054	ENST00000390502 // TRAJ35 // T cell receptor alpha joining 35 (non-functional) // --- /	TRAJ35	-1.591	0.042	-1.720	0.021	-1.561	0.050
16690881	NR_034126 // LINC01160 // long intergenic non-protein coding RNA 1160 // 1p13.2 // 1001	LINC01160	-1.627	0.005	-1.697	0.003	-1.650	0.005
16722081	NM_001282663 // MICAL2 // microtubule associated monooxygenase, calponin and LIM domain	MICAL2	-1.634	0.021	-1.852	0.006	-1.616	0.023
16679717	NM_001281834 // GCSAML // germinal center-associated, signaling and motility-like // 1q	GCSAML	-1.655	0.000	-1.719	0.000	-1.547	0.001
16855355	OTTHUMT00000448305 // RP11-839G9.1 // novel transcript, antisense to RAB27B // --- // -	RP11-839G9.1	-1.667	0.004	-1.580	0.008	-1.679	0.004
16929442	NM_000362 // TIMP3 // TIMP metallopeptidase inhibitor 3 // 22q12.3 // 7078 /// uc003anb	TIMP3	-1.677	0.004	-1.739	0.003	-1.797	0.002
16968680	NM_001251830 // SPP1 // secreted phosphoprotein 1 // 4q22.1 // 6696 /// ENST00000509659	SPP1	-1.693	0.000	-1.929	0.000	-1.858	0.000
16852463	NM_004163 // RAB27B // RAB27B, member RAS oncogene family // 18q21.2 // 5874 /// ENST00	RAB27B	-1.745	0.004	-1.615	0.010	-1.651	0.008
16883690	NM_003856 // IL1RL1 // interleukin 1 receptor-like 1 // 2q12 // 9173 /// NM_016232 // I	IL1RL1	-1.752	0.000	-1.509	0.002	-1.691	0.000

16977537	NM_001098540 // HPSE // heparanase // 4q21.3 // 10855 /// NM_001199830 // HPSE // hepar	HPSE	-1.763	0.000	-1.513	0.000	-1.561	0.000
17012598	NM_005021 // ENPP3 // ectonucleotide pyrophosphatase/phosphodiesterase 3 // 6q22 // 516	ENPP3	-1.793	0.007	-1.758	0.008	-1.879	0.004
16760889	NM_004054 // C3AR1 // complement component 3a receptor 1 // 12p13.31 // 719 /// ENST000	C3AR1	-1.795	0.000	-1.735	0.000	-1.760	0.000
16863593	NM_001271749 // C5AR2 // complement component 5a receptor 2 // 19q13.33 // 27202 /// NM	C5AR2	-1.797	0.000	-1.991	0.000	-1.723	0.000
16697370	NM_000963 // PTGS2 // prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase	PTGS2	-1.813	0.004	-1.692	0.009	-1.736	0.007
16872248	NM_001828 // CLC // Charcot-Leyden crystal galectin // 19q13.1 // 1178 /// ENST00000221	CLC	-1.837	0.005	-1.991	0.002	-1.956	0.003
16901593	NM_144710 // SEPT10 // septin 10 // 2q13 // 151011 /// NR_047585 // SEPT10 // septin 10	SEPT10	-1.845	0.001	-1.500	0.019	-1.673	0.005
17010760	NM_001204813 // NT5E // 5-nucleotidase, ecto (CD73) // 6q14-q21 // 4907 /// NM_002526 /	NT5E	-1.914	0.000	-1.702	0.001	-1.906	0.000
16782187	ENST00000548162 // MMP14 // matrix metallopeptidase 14 (membrane-inserted) // 14q11.2 /	MMP14	-1.923	0.000	-1.822	0.000	-1.718	0.000
16984010	NM_002185 // IL7R // interleukin 7 receptor // 5p13 // 3575 /// ENST00000303115 // IL7R	IL7R	-1.925	0.010	-1.634	0.039	-1.823	0.015
16774053	NM_001111045 // CCNA1 // cyclin A1 // 13q12.3-q13 // 8900 /// NM_003914 // CCNA1 // cyc	CCNA1	-1.944	0.001	-1.787	0.003	-1.880	0.002
16956569	NM_000313 // PROS1 // protein S (alpha) // 3q11.2 // 5627 /// ENST00000394236 // PROS1	PROS1	-1.993	0.000	-1.853	0.000	-1.996	0.000
16997383	NM_004101 // F2RL2 // coagulation factor II (thrombin) receptor-like 2 // 5q13 // 2151	F2RL2	-2.079	0.009	-2.043	0.011	-2.100	0.008

16738803	NM_001062 // TCN1 // transcobalamin I (vitamin B12 binding protein, R binder family) //	TCN1	-2.107	0.003	-1.679	0.024	-2.318	0.001
16709245	NM_000681 // ADRA2A // adrenoceptor alpha 2A // 10q25.2 // 150 /// ENST00000280155 // A	ADRA2A	-2.148	0.001	-2.150	0.001	-2.109	0.001
16694764	NM_001161441 // SH2D2A // SH2 domain containing 2A // 1q21 // 9047 /// NM_001161442 //	SH2D2A	-2.266	0.000	-1.896	0.000	-1.923	0.000
16799315	NM_003246 // THBS1 // thrombospondin 1 // 15q15 // 7057 /// ENST00000260356 // THBS1 //	THBS1	-2.391	0.024	-2.405	0.023	-2.510	0.018
16996234	NM_003711 // PPAP2A // phosphatidic acid phosphatase type 2A // 5q11 // 8611 /// NR_103	PPAP2A	-2.440	0.000	-1.971	0.000	-2.055	0.000
16888106	NM_001201480 // OSBPL6 // oxysterol binding protein-like 6 // 2q32.1 // 114880 /// NM_0	OSBPL6	-3.155	0.001	-3.000	0.002	-3.135	0.002
16782122	AK026255 // YME1L1 // YME1-like 1 ATPase // 10p14 // 10730 /// BC020840 // YME1L1 // YM	YME1L1	-3.386	0.000	-3.561	0.000	-3.432	0.000
16753630	OTTHUMT00000401554 // RP11-221N13.3 // novel transcript // --- // --- /// OTTHUMT000004	RP11-221N13.3	-3.412	0.000	-2.105	0.003	-2.454	0.001
16754895	ENST00000550879 // NTS // neurotensin // 12q21 // 4922 /// OTTHUMT00000406112 // NTS //	NTS	-3.788	0.008	-3.447	0.012	-3.342	0.014
16863589	NM_001736 // C5AR1 // complement component 5a receptor 1 // 19q13.3-q13.4 // 728 /// EN	C5AR1	-3.991	0.000	-3.125	0.000	-3.522	0.000
16843309	NM_002981 // CCL1 // chemokine (C-C motif) ligand 1 // 17q12 // 6346 /// ENST0000022584	CCL1	-6.169	0.000	-5.511	0.000	-5.611	0.000
16659637	NM_015849 // CELA2B // chymotrypsin-like elastase family, member 2B // 1p36.21 // 51032	CELA2B	-9.528	0.000	-6.691	0.000	-10.214	0.000

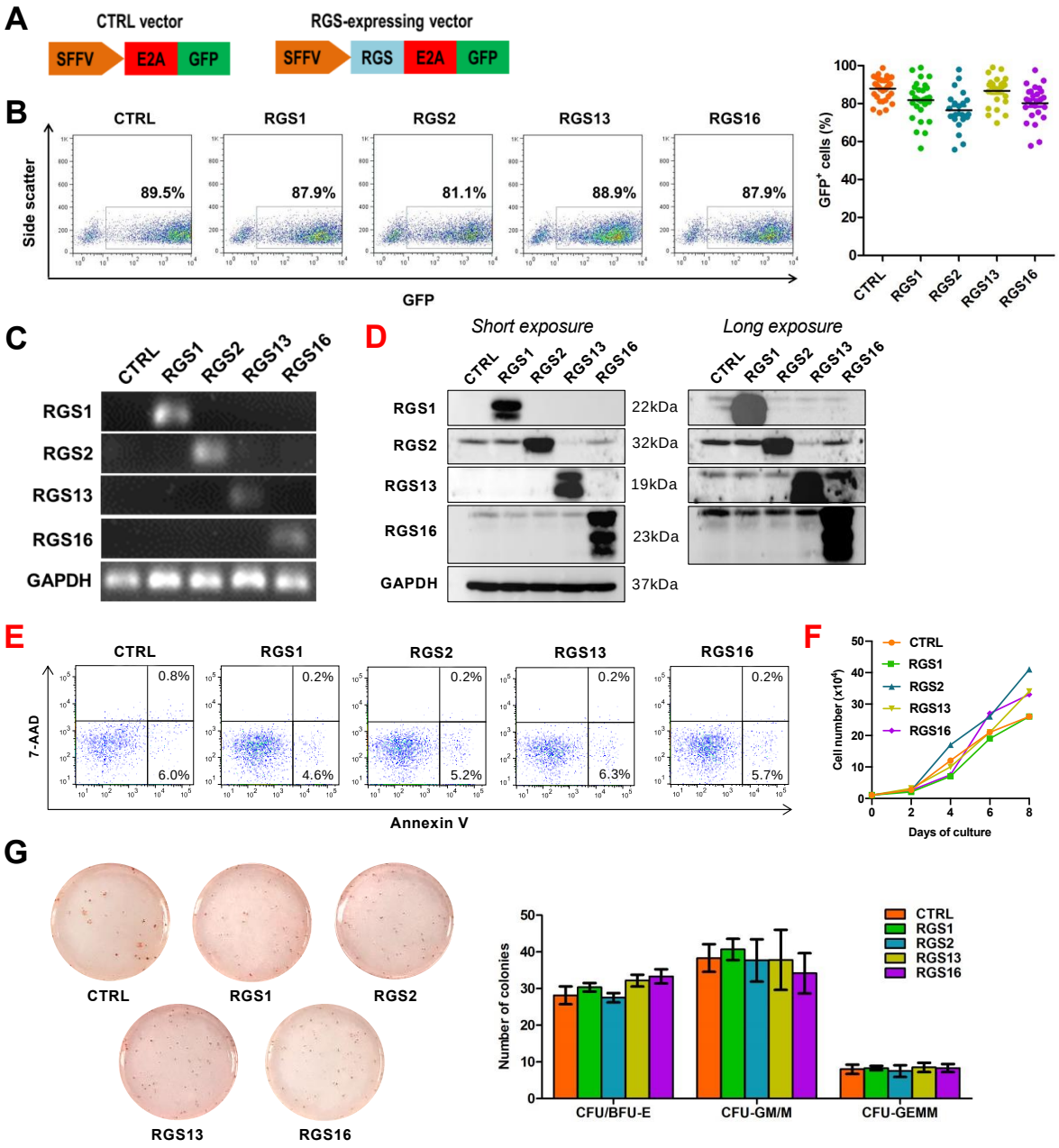
Supplemental Table 4. Selected genes commonly regulated by RGS1/13/16 and their reported functions in hematopoietic cells

Gene Symbol	Description	Roles in cell trafficking or hematopoiesis	References
C3AR1	Complement C3a Receptor 1	• C3a potentiates SDF-1-directed chemotaxis, primes trans-Matrigel migration, stimulates MMP-9 secretion and promotes VLA-4-mediated adhesion to VCAM-1 in human $CD34^+$ cells • C3a priming enhances engraftment kinetics of murine Sca-1^+ cells	Reca *et al.*, 2003[1]
		• G-CSF-induced HSPC mobilization is enhanced in $C3aR^{-/-}$ mice • C3aR antagonist SB 290157 accelerates G-CSF-induced HSPC mobilization	Ratajczak *et al.*, 2004[2]
		• HSPCs from $C3aR^{-/-}$ mice exhibit defective homing and engraftment • Pharmacologic inhibition of C3aR impairs homing of human $CD34^+$ cells	Wysoczynski *et al.*, 2009[3]
C5AR1 *C5AR2*	Complement C5a Receptor 1 Complement C5a Receptor 2	• $C5^{-/-}$ mice exhibits impaired G-CSF-induced HSPC mobilization • C5a stimulates $C5aR^+$ granulocytes to produce MMP-9 and secretes cationic peptides that enhances responsiveness of murine HSPCs to plasma SDF-1 gradient	Lee *et al.*, 2009[4]
		• Plasma C5a level is higher in good mobilizers and correlates with the number of mobilized $CD34^+$ cells • Stimulation of human granulocytes and monocytes with C5a increases MMP-9 secretion and MT1-MMP expression that may facilitate HSPC egress	Jalili *et al.*, 2010[5]
CCL1	C-C Motif Chemokine Ligand 1	• CCL1 is required for pulmonary recruitment of eosinophils in a murine model of allergic airway disease	Bishop and Lloyd, 2003[6]

		• CCL1/CCR8 axis regulates skin homing of human T cells for immune surveillance	Schaerli *et al.*, 2004[7]
		• $CD4^+$ T cells produces CCL1 that recruits and activates murine macrophages in type I diabetes	Cantor and Haskins, 2007[8]
F2RL2	Coagulation Factor II Thrombin Receptor Like 2	• LPS-matured human dendritic cells expressed F2RL2 (PAR3) that mediates ERK1/2- and Rho kinase 1-dependent chemotaxis	Li *et al.*, 2008[9]
PROS1	Protein S	• Exogenous Protein S inhibits VEGF-A-induced migration and activation of ERK1/2 and Akt in human endothelial cells	Fraineau *et al.*, 2012[10]
THBS1	Thrombospondin 1	• Endogenous THBS1 acts as a ligand of LRP1 and CRT for regulation of T cell adhesion	Li *et al.*, 2006[11]
		• Monocytic cells from $THBS1^{-/-}$ mice display reduced migration to MCP-1 and adhesion to fibronectin	Liu *et al.*, 2015[12]
MMP14	Matrix Metallopeptidase 14	• MMP14 (MT1-MMP) expression positively correlates with the level of human $CD34^+$ cells in G-CSF-mobilized peripheral blood • Antibody or siRNA targeting MMP14 decreases trans-Matrigel migration and homing of $CD34^+$ cells in NOD/SCID mice • HSPCs from $MMP14^{-/-}$ mice exhibit defective engraftment	Vagima *et al.*, 2009[13]
		• $MMP14^{-/-}$ mice display severe pancytopenia and reduced secretion of SCF, SDF-1 and IL-7 in the BM niche	Nishida *et al.*, 2012[14]
TIMP3	Tissue Inhibitor of Metalloproteinases 3	• TIMP-3 enhances murine HSPC proliferation by stimulating cell-cycle entry which is independent of its metalloproteinase inhibitory activity • BM recovery from myelosuppression is impaired in $TIMP3^{-/-}$ mice	Nakajima *et al.* 2010[15]

		• Overexpression of TIMP-3 increases murine HSPC proliferation *in vivo* and enhances colony-forming cell trafficking to blood and spleen	Shen *et al.*, 2010[16]
CCNA1	Cyclin A1	• Conditional deletion of Cyclin A1/A2 induces severe anemia and reduces the reconstitution potential of murine HSPCs	Kalaszczynska *et al.*, 2009[17]
		• Loss of cyclin A1 increases murine HSPC migration and homing to the BM niche	Miftakhova *et al.*, 2015[18]
HSPE	Heparanase	• Transgenic mice overexpressing heparanase exhibits increased retention of HSPCs in the BM through enhancement of SDF-1 turnover and reduction of protease activity	Spiegel *et al.*, 2008[19]
IL1RL1	Interleukin 1 Receptor Like 1	• IL-33 primes migration of human $CD34^+$ cells to SDF-1 and homing to the lungs in patients with allergic asthma, which can be abolished by antibodies against IL1RL1 (IL33R, ST2)	Smith *et al.*, 2015[20]
LPAR5	Lysophosphatidic Acid Receptor 5	• Lysophosphatidic acid enhances survival of cord blood $CD34^+$ cells in hypoxic and serum-deprived cultures through activation of ERK and Akt pathways	Kostic *et al.*, 2015[21]
SPP1	Secreted Phosphoprotein 1	• HSPCs from Osteopontin (SPP1) knockout mice exhibit enhanced cycling and aberrant distribution in the BM niche after transplantation	Nilsson *et al.*, 2005[22]
		• Thrombin-cleaved osteopontin is chemotactic for human $CD34^+$ cells and is essential for homing of murine HSPCs • Osteopontin knockout mice exhibit elevated HSPC mobilization	Grassinger *et al.* 2009[23]

Supplemental Figure 1



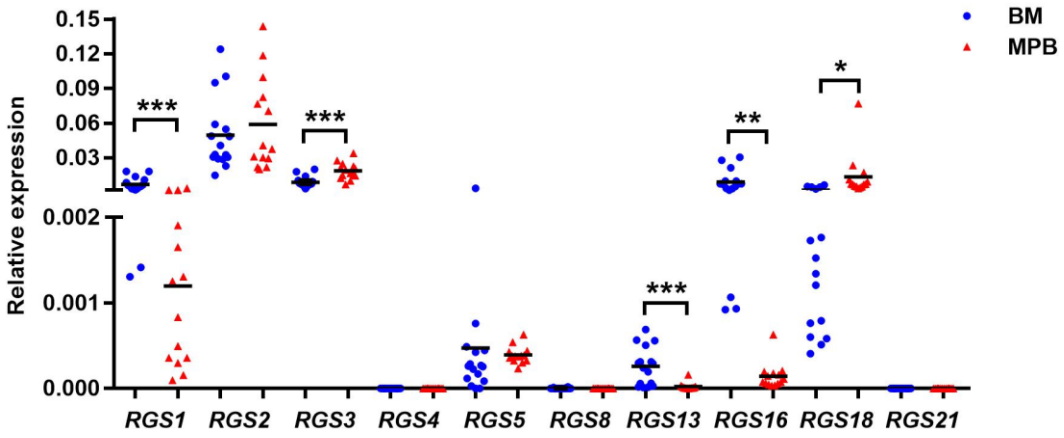
Supplemental Figure 1. Lentiviral transduction of human CD34$^+$ cells. (A) Schematic diagram showing the lentiviral vector backbone for RGS overexpression. SFFV, spleen focus-forming virus U3 promoter; E2A, a self-cleavage site derived from equine rhinitis A virus; GFP, green fluoresence protein. (B) Transduction efficiencies represented by the percentages of GFP$^+$

populations (n=21-35). (C) RT-PCR confirmation of specific mRNA expression of RGS members in transduced $CD34^+$ cells. (D) Protein expression of RGS members in transduced $CD34^+$ cells revealed by Western blotting. (Left) Images with short exposure showing specific overexpression of RGS members. (Right) Images with long exposure showing their basal expression. (E) Representative flow cytometry plots showing the apoptosis level of transduced $CD34^+$ cells (n=3). (F) Proliferation of transduced $CD34^+$ cells was measured by Trypan Blue exclusion over time. (G) Colony forming ability of transduced $CD34^+$ cells (n=3). Statistics: two-tailed, paired Student's *t*-test. None of the comparisons achieved statistical significance.

Supplemental Figure 2

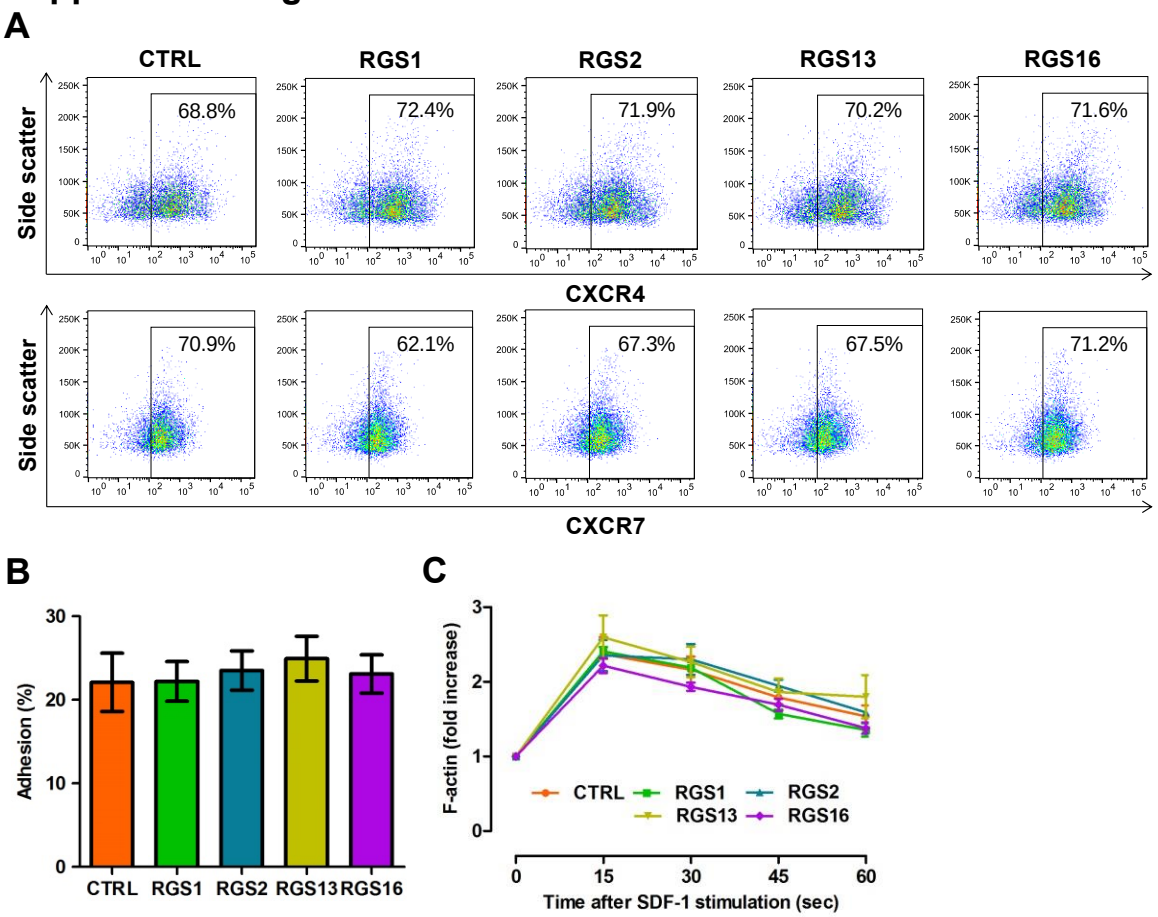
Supplemental Figure 2. RGS knockout in human CD34^{+} cells. Representative ICE analysis reports on *RGS1*-, *RGS13*-, and *RGS16*-edited HSPCs. The wild type DNA sequences are indicated as "+". The black vertical dotted lines represent the cut sites. Indel (insertion or deletion) percentages depict editing efficiencies. KO scores are defined as the inferred proportion of cells likely with a functional knockout.

Supplemental Figure 3



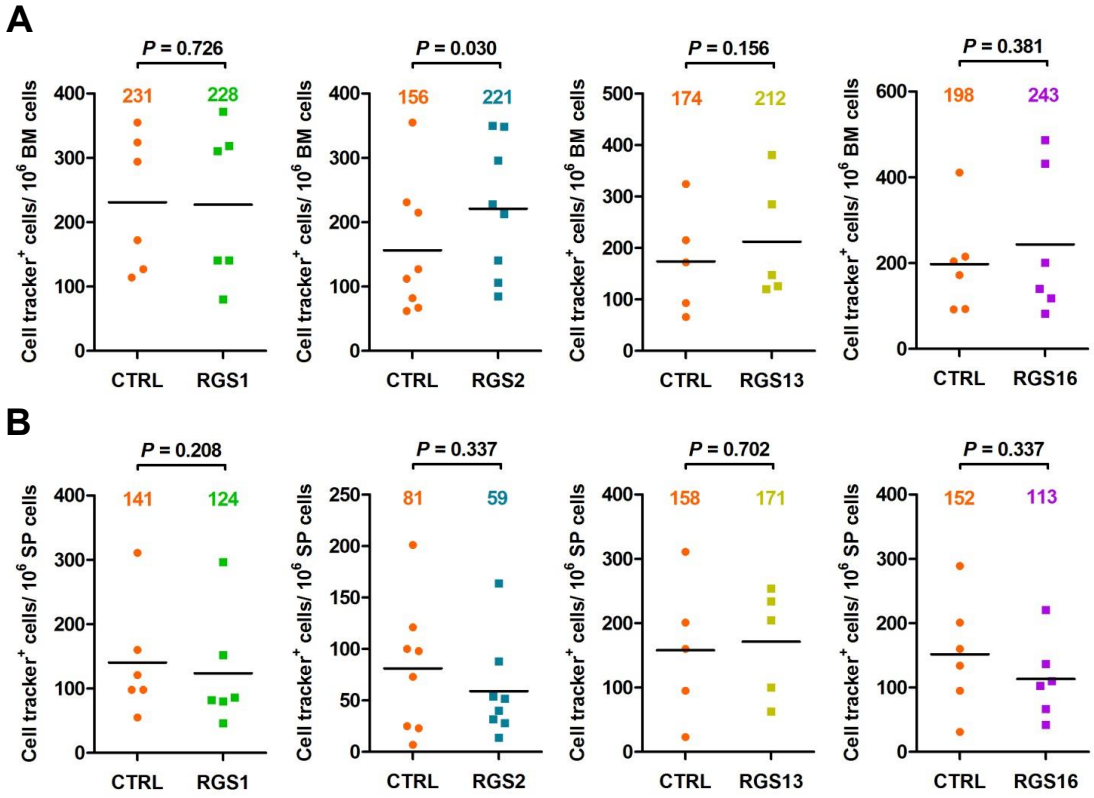
Supplemental Figure 3. Expression of R4 RGS in BM and MPB CD34$^+$ cells. Basal mRNA expression of R4 RGS in CD34$^+$ cells derived from normal BM (n=16) and G-CSF-mobilized MPB (n=14). Reported values are RGS expression relative to GAPDH. Statistics: two-tailed, unpaired Student's *t*-test. ***P*<0.05, ****P*<0.01, *****P*<0.001.

Supplemental Figure 4



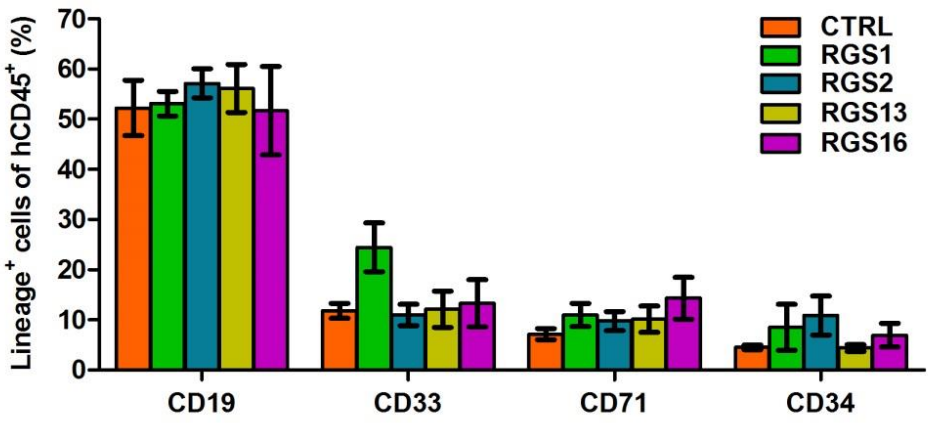
Supplemental Figure 4. R4 RGS does not affect SDF-1 receptor expression, adhesion and actin polymerization. (A) Representative flow cytometry plots showing the cell surface CXCR4 and CXCR7 expression on transduced $CD34^+$ cells. (B) Adhesion of transduced $CD34^+$ cells to fibronectin in the presence of SDF-1 (n=4). (C) Levels of F-actin in transduced $CD34^+$ cells following SDF-1 stimulation (n=3). SDF-1 at 100 ng/mL was applied for these functional assays. Statistics: two-tailed, paired Student's *t*-test. None of the comparisons achieved statistical significance.

Supplemental Figure 5



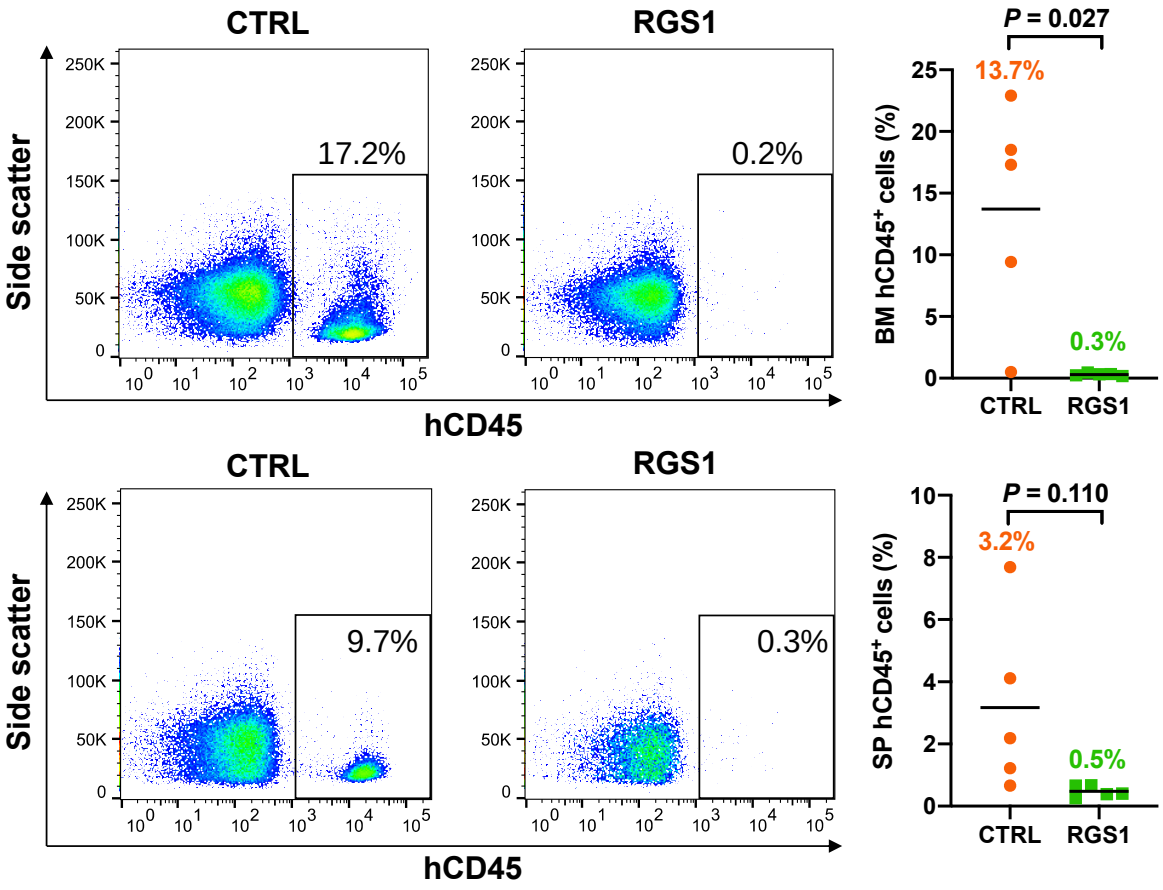
Supplemental Figure 5. R4 RGS does not influence HSPC homing. Transduced CD34$^+$ cells were labeled with CellTracker Deep Red Dye, and infused into sublethally irradiated NOD/SCID mice. Homing of CD34$^+$ cells to the recipent (A) BM or (B) spleens was enumerated by flow cytometry at 20h post-transplantation. Each data point represents the average homing level of 2 animals in a single experiment, with 5-8 independent experiments performed (*i.e.* 10-16 mice/group). Statistics: two-tailed, paired Student's *t*-test. *P* values are indicated.

Supplemental Figure 6



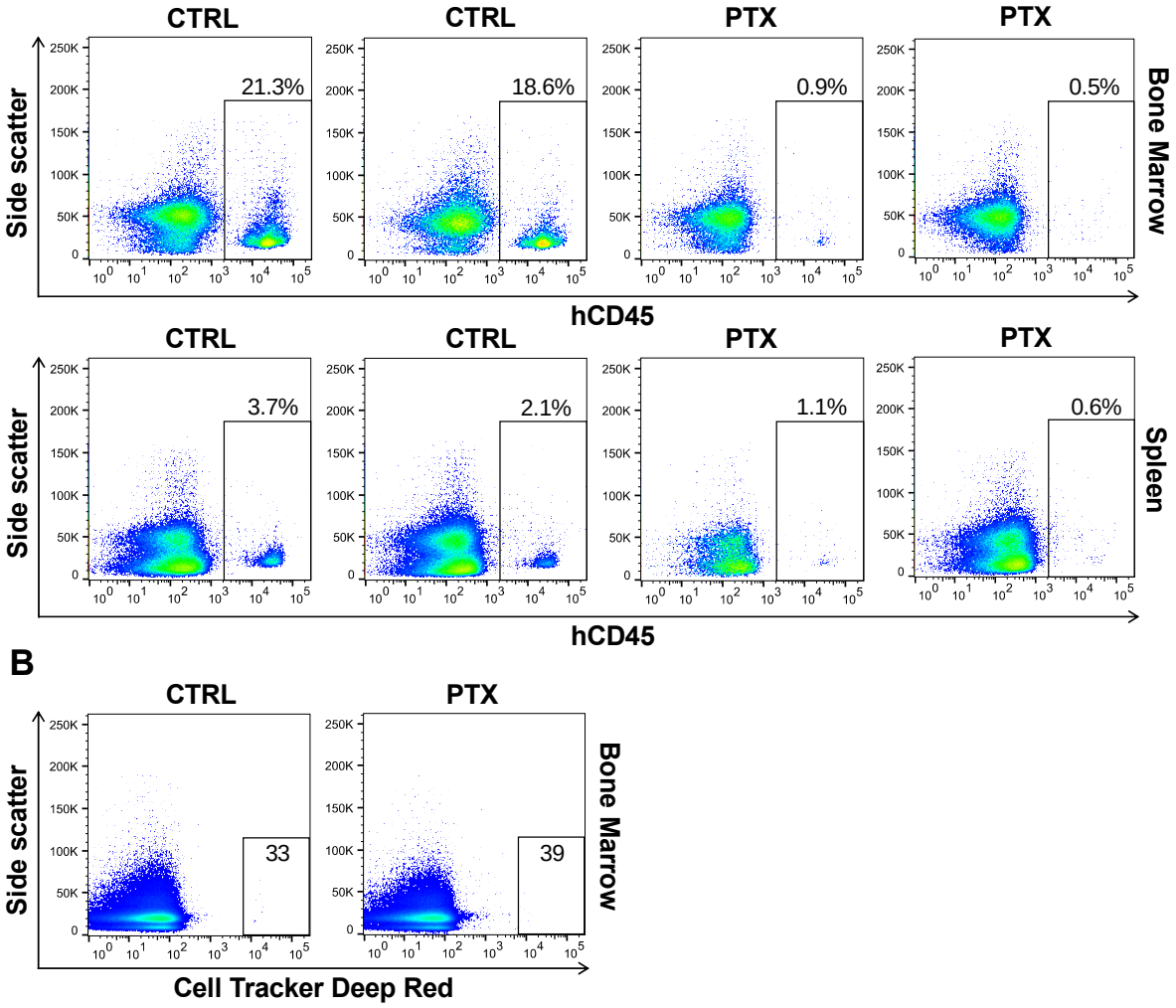
Supplemental Figure 6. R4 RGS does not bias lineage output. Control or RGS-overexpressing $CD34^+$ cells were intravenously infused into sublethally irradiated NOD/SCID mice. In animals with ≥1% engraftment in the BM, human $CD45^+$ cells were analyzed for lineage compositions (4-5 mice/group). Statistics: two-tailed, paired Student's *t*-test. None of the comparisons achieved statistical significance.

Supplemental Figure 7



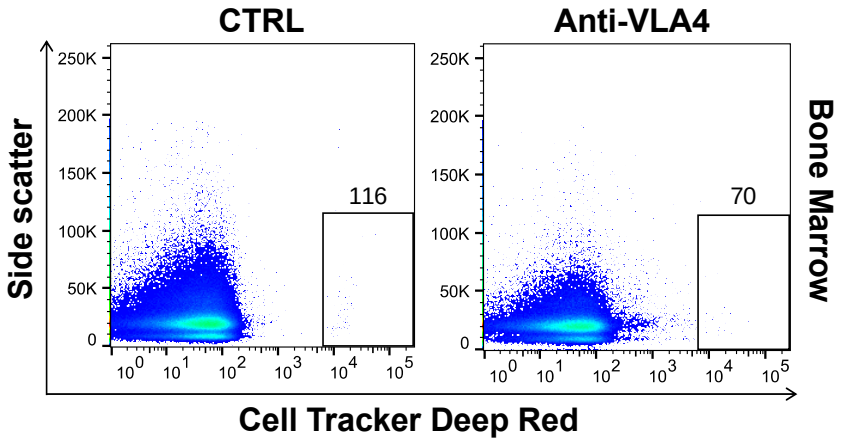
Supplemental Figure 7. Overexpression of *RGS1* inhibits longer-term HSPC engraftment. Control or *RGS1*-overexpressing $CD34^+$ cells were intravenously infused into sublethally irradiated NOD/SCID mice (5 animals/group). Human $CD45^+$ cells in the recipient BM and spleens were enumerated by flow cytometry at 16 weeks post-transplantation. Statistics: two-tailed, paired Student's *t*-test. *P* values are indicated.

Supplemental Figure 8



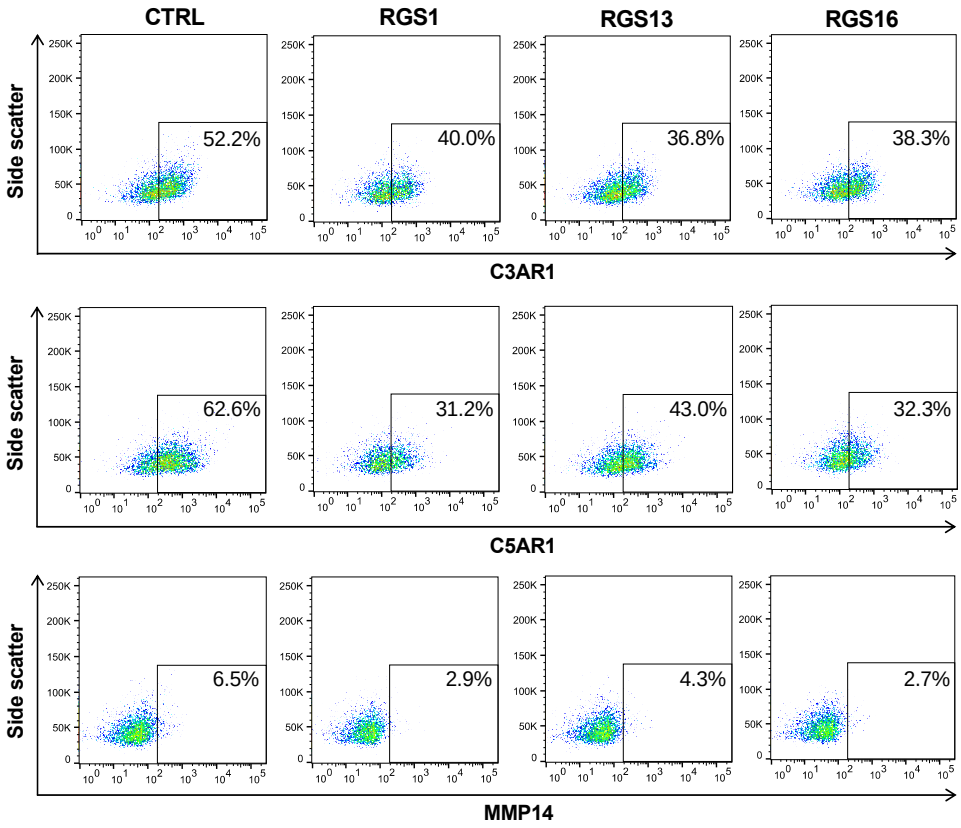
Supplemental Figure 8. Pertussis toxin inhibits HSPC engraftment but not homing. (A) $CD34^+$ cells were treated with medium or PTX (1 μg/mL, 1h), and intravenously infused into sublethally irradiated NOD/SCID mice (2 animals/group). Human $CD45^+$ cells in the recipient BM and spleens were measured by flow cytometry at 8 weeks post-transplantation. (B) Homing of PTX-treated $CD34^+$ cells at 20h post-transplantation. Reported values are Cell Tracker Deep Red^+ cells/10^6 BM cells.

Supplemental Figure 9



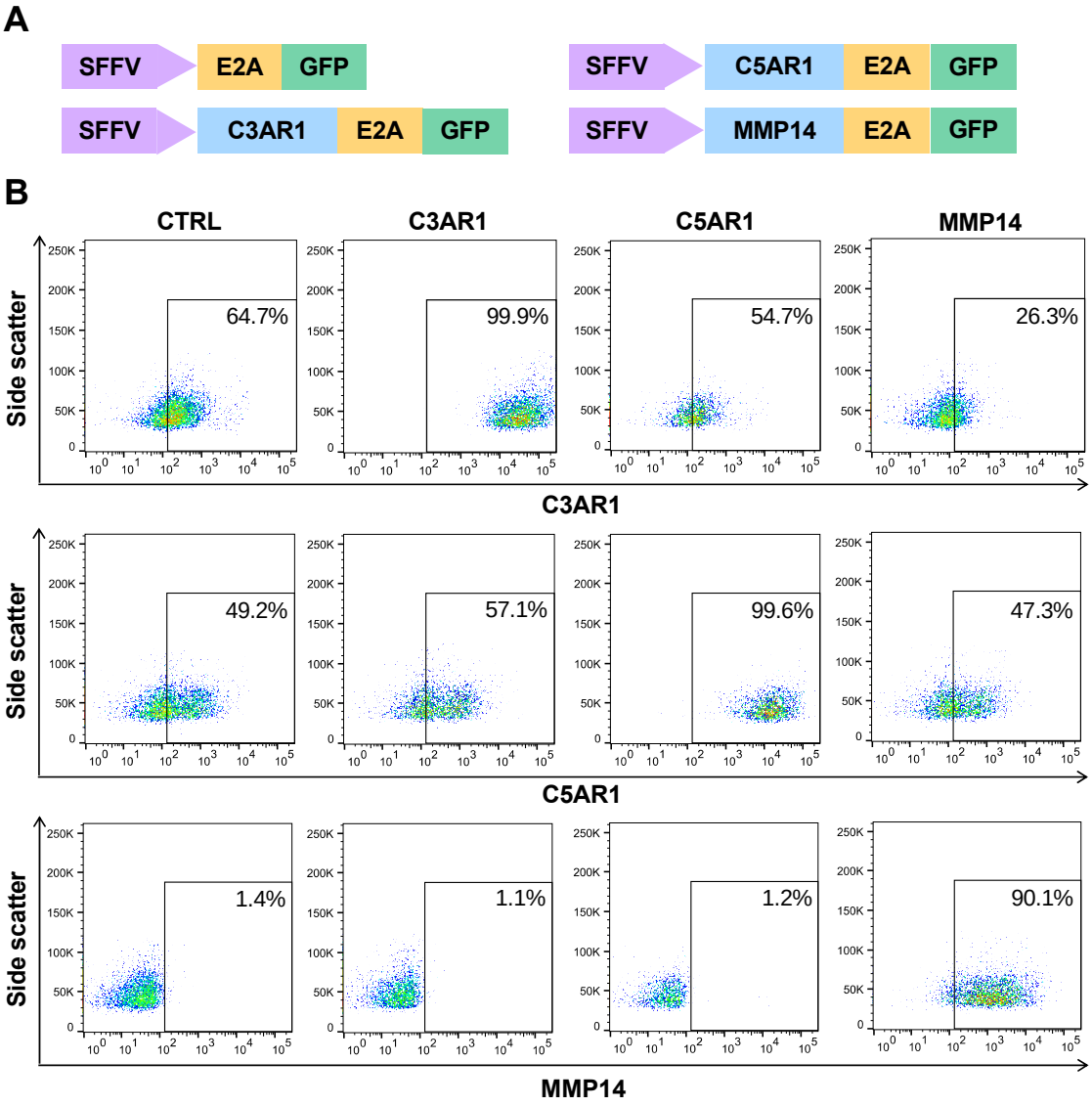
Supplemental Figure 9. Integrin blockade inhibits HSPC homing. $CD34^+$ cells were labeled with Cell Tracker Deep Red, and treated with IgG_1 control or anti-VLA4 (20 μg/mL, 1h) before infusion into sublethally irradiated NOD/SCID mice. Homed cells were detected in the recipient BM at 20h post-transplantation. Reported values are Cell $Tracker^+$ cells/10^6 BM cells.

Supplemental Figure 10



Supplemental Figure 10. R4 RGS decreases the protein expression of downstream effectors. Representative flow cytometry plots showing the cell surface expression of C3AR1, C5AR1 and MMP14 on RGS-transduced $CD34^{+}$ cells.

Supplemental Figure 11



Supplemental Figure 11. Lentiviral transduction of RGS downstream effectors in CD34$^+$ cells. (A) Schematic diagrams showing the lentiviral vector backbone for C3AR1, C5AR1 and MMP14 overexpression. (B) Representative flow cytometry plots showing the specific overexpression of target proteins on transduced CD34$^+$ cells.

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