Supplemental Materials

Table 1. Antibodies

Antigen	Fluorophore	Clone	Company
CD3	PE	17A2	BioLegend
CD3	PerCP-Cy5.5	145-2C11	eBioscience
B220	PE	RA3-6B2	BD Biosciences
Ly6C/Ly6G (Gr1)	PE	RB6-8C5	BD Biosciences
Ly6C/Ly6G (Gr1)	APC-Cy7	RB6-8C5	BD Biosciences
Ter119	PE	TER-119	BD Biosciences
Ter119	BV421	TER-119	BD Biosciences
CD11b	PE	M1/70	eBioscience
CD11b	BV605	M1/70	BioLegend
CD48	AF700	HM48-1	BioLegend
NK1.1	AF700	PK136	BioLegend
Sca1	PE-Cy7	D7	eBioscience
CD150	BV605	TC15-12F12.2	BioLegend
CD117 (c-Kit)	BV421	2B8	BD Biosciences
CD117 (c-Kit)	APC	2B8	BD Biosciences
CD31	BV605	390	BioLegend
CD41	eFluor450	MWReg30	eBioscience
CD45	AF700	30-F11	BioLegend
CD16/32	PE	93	eBioscience
CD4	AF700	GK1.5	eBioscience
CD8	PE-Cy5	53-6.7	BioLegend
CD19	BV650	6D5	BioLegend
CD45.1	APC	A20	BD Biosciences
CD45.2	PE	104	BD Biosciences

Table 2. Primers and g-RNAs

Genotyping	**Forward 5' – 3'**	**Reverse 5'- 3'**
VEC-Cre	CCCAGGCTGACC AAGCTGAG	GCCTGGCGATCC CTGAACATG
Vav-Cre	GGCGACAGTTAC AGTCACAGAAGAGG	GCCTGGCGATCC CTGAACATG
Gpr56$^{fl/fl}$	TGGTAGCTAACCTAC TCCAGGAGC	CACGAGACTAGTGA GACGTGCTAC
Gpr56^{WT}	TGGTAGCTAACCTAC TCCAGGAGC	GGTGACTTTGGTGT TCTGCACGAC
Cre recombination		
Gpr56	GTGAGGTCCAGGCA TACTCG	AGGAGCTCTGTGCA TTGGAG
qRT-PCR		
ß-actin	CACCACACCTTCTTA CAATGAG	GTCTCAAACATGAT CTGGGTC
Gpr56	TCTGCTCTGGCTTGT CTTC	AGGTTCATGTGGAC TTTGATG
Gpr97	CTGGGATATGGCTAA AGGAGAC	AAGGCGAAGAAGG TCAAGTG
gRNAs		
Gpr56 top	CACCGtctgttgggtctggtt ccgc	
Gpr56 bottom	AAACgcggaaccagaccc aacagaC	
Gpr97-ex2-top	CACCGgaatgtctgccgtc ggcttc	
Gpr97-ex2-bottom	AAACgaagccgacgg cag acattcC	
Gpr97-ex10-top	CACCgcggttctcctggtcgc gaa	
Gpr97-ex10-bottom	AAACttcgcgaccaggaga accgc	
Zebrafish mRNA generation		
gpr56 coding zf	ATGAACCAGAATCCA GCAAAG	TTAACACTTCTCGTT AGTTTGTA
Mouse mRNA generation		
gpr56 cDNA	TAGGAGTATAATGGC TGTCCA	CTTAGATGCGGCTG GAGGA
gpr97 cDNA	CTGATGGCGACAGC CAGGA	CTGCAGCCACCCAT CATCA
gpr114 cDNA	AATACTGGCGAGGAC ATGGA	GAGCTGGGTCAGT GTGTCAT
MO sequences		
gpr56 Spl E2 I2-3	TGTAATGCTCGTTTA CTTACCTTGA	

Supplemental Figures and Legends

Figure S1

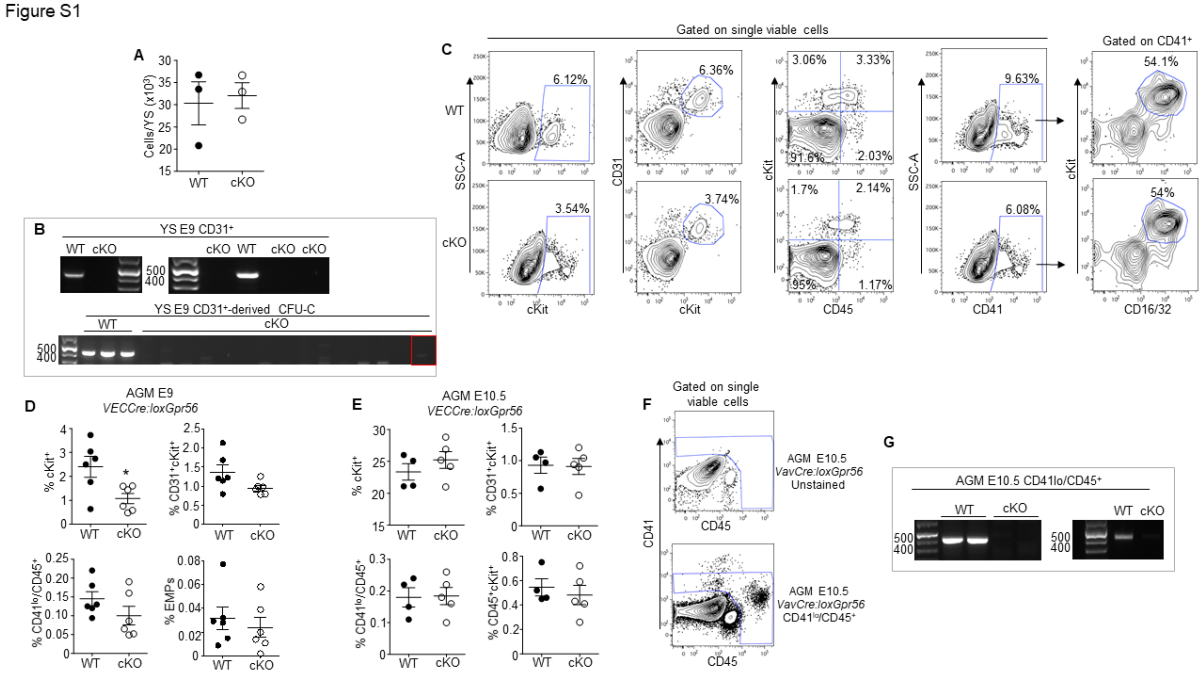

Figure S1. Gating strategy for progenitor analysis and *Gpr56* deletion in YS and AGM. **A)** Number of cells in WT and *VECCre:loxGpr56* cKO E9 YS. WT=black; cKO=white. **B)** DNA PCR showing the deletion of *Gpr56* in sorted CD31$^+$ WT control and *VECCre:loxGpr56* cKO E9 YS cells (top panel) and in WT and *VECCre:loxGpr56* E9 YS CD31-derived CFU-C (bottom panel). WT band=460bp. **C)** Contour plots showing the gating strategy for the progenitor analyses in Fig 1D and Fig1H. One WT and one E9 YS *VECCre:loxGpr56* are shown as representative examples. **D)** Percentages of cKit$^+$, CD31$^+$cKit$^+$, CD41^{lo}/CD45$^+$ and CD41$^+$cKit$^+$CD16/32$^+$ (EMP=erythromyeloid progenitor) cells in WT (black) and cKO (white) E9 AGM (n=6). **E)** Percentages of cKit$^+$, CD31$^+$cKit$^+$, CD41^{lo}/CD45$^+$ and CD45$^+$cKit$^+$ cells in WT (black) and cKO (white) E9 AGM (WT n=4, cKO n=5). **F)** Contour plots showing the gating strategy for the cell sorting of CD41^{lo}/CD45$^+$ from E10.5 *VavCre:loxGpr56* AGM. **G)** DNA PCR showing the deletion of *Gpr56* in sorted CD41^{lo}/CD45$^+$ WT control and *VavCre:loxGpr56* cKO cells from E10.5 *AGM.* WT band=460bp.

Figure S2

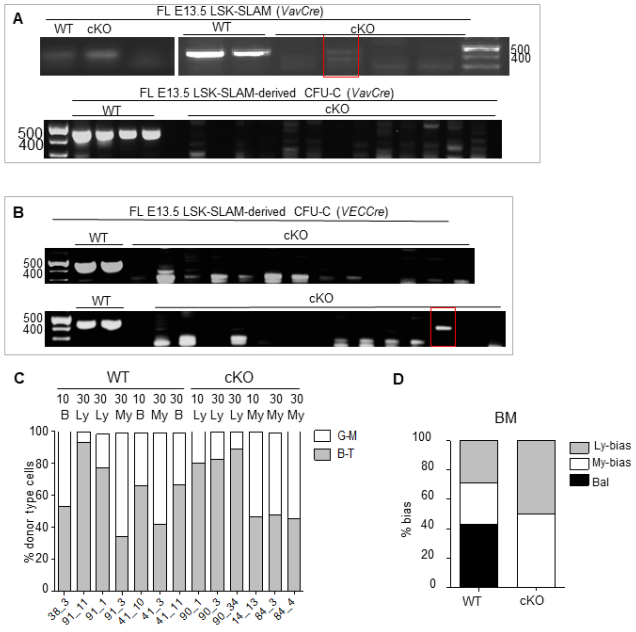

Figure S2. *Gpr56* is deleted upon Cre activation in E13.5 FL and influences HSC lineage bias. **A)** DNA PCR showing the deletion of *Gpr56* in sorted LSK-SLAM cells from WT and *VavCre:loxGpr56* cKO E13.5 FL (top panel) and in LSK SLAM *VavCre:loxGpr56 FL*-derived CFU-C (bottom panel). WT band=460bp. **B)** DNA PCR showing the deletion of *Gpr56* in sorted LSK-SLAM cells from E13.5 *VECCre:loxGpr56* FL-derived CFU-C. WT band=460bp. **C)** Percentage of lymphoid and myeloid cell contribution in BM of 13 individual adult irradiated recipient mice as measured by flow cytometry at 23 weeks post-injection of 10 and 30 LSK-SLAM WT control and *VavCre:loxGpr56* cKO E13.5 FL cells. **D)** Percentages of lymphoid-biased, balanced and myeloid-biased HSC engrafted recipients from panel C.

Figure S3

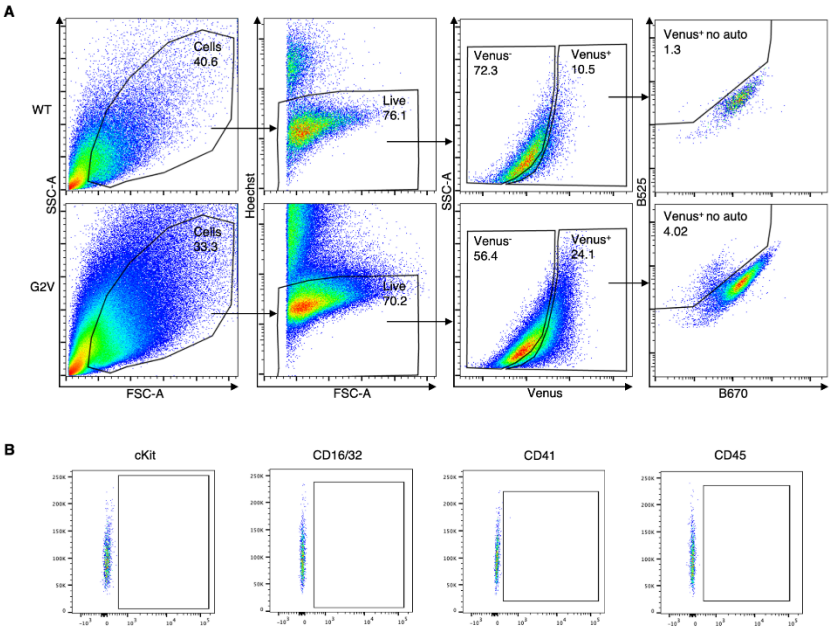

Figure S3. Gating strategy for flow cytometry of WT and *G2V* ESC differentiation cultures. Forward scatter (FSC), side scatter (SSC), Venus, and Venus minus autofluorescence plots are shown in upper panels. After gating on live cells, the Venus$^+$ cells are plotted against a dump neighbor channel (B525-Venus vs B670) to exclude auto-fluorescent cells. Only the cells positive in the B525 channel and not in the B670 channel are considered Venus$^+$. WT cells are used as control to set the gates. Fluorescent minus one (FMO) controls for cKit, CD16/32, CD41, CD45 antibodies in lower panels.

Figure S4

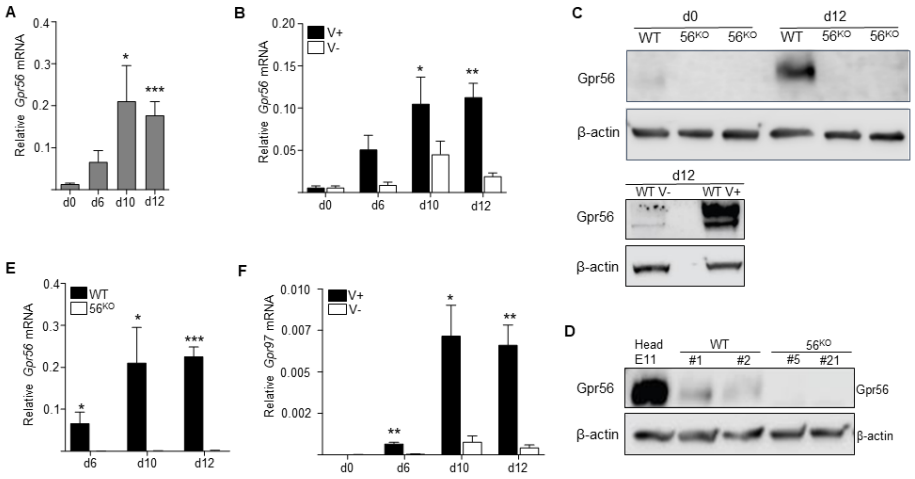

Figure S4. Gpr56 and Gpr97 are expressed in mouse G2V.WT differentiated cells.
Time course qRT-PCR analysis of relative *Gpr56* expression (normalized to *b-actin*) **A)** in unsorted and **B)** in Venus sorted *G2V ESC* hematopoietic differentiation cultures. **C)** Western blot analysis of Gpr56 protein expression in day 0 and day12 unsorted *G2V* (WT) and *G2V Gpr56* deleted (*Gpr56^{KO}*) ESC hematopoietic differentiation cultures (upper panel) and day 12 Venus sorted *G2V* ESC (WT V^{+} and WT V^{-}) hematopoietic differentiation cultures (lower panel), with β-actin as protein normalization control. **D)** Western blot analysis of Gpr56 protein expression in day 0 *G2V.WT* (clones #1 and #2) and *G2V. 56KO* (clones #5 and #21) ESCs, with β-actin as protein normalization control. Mouse embryonic head used as positive control. **E)** Time course qRT-PCR analysis of relative *Gpr56* expression (normalized to *β-actin*) in unsorted *G2V.WT* and *G2V.56KO* (clone #5) ESC hematopoietic differentiation cultures. **F)** Time course RT-qPCR analysis of relative Gpr97 expression (normalized to *β-actin*) in *G2V.WT* Venus sorted cells. d=day of culture harvest; *p<0.05: **p<0.01; ***p<0.001.

Figure S5

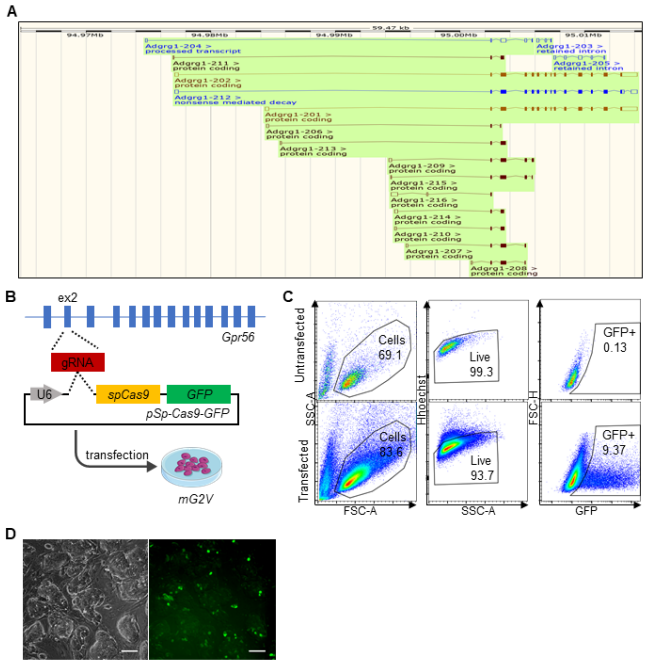

Figure S5. Generation of *Gpr56* deleted *G2V* ESCs.
A) *Gpr56* gene and splice isoforms as taken from Ensemble database. **B)** CRISPR/Cas9 strategy showing gRNA for *Gpr56* exon 2 and insertion in *pSp-Cas9-2A-GFP* vector used for transfection of G2V ESCs. **C)** Flow cytometric analysis of transfected and untransfected control ESCs for forward (FSC) and side scatter (SSC), viability and GFP expression. **D)** Fluorescence microscopic images of untransfected and transfected ESCs at 48 hours post-transfection. Size bar=100μm.

Figure S6

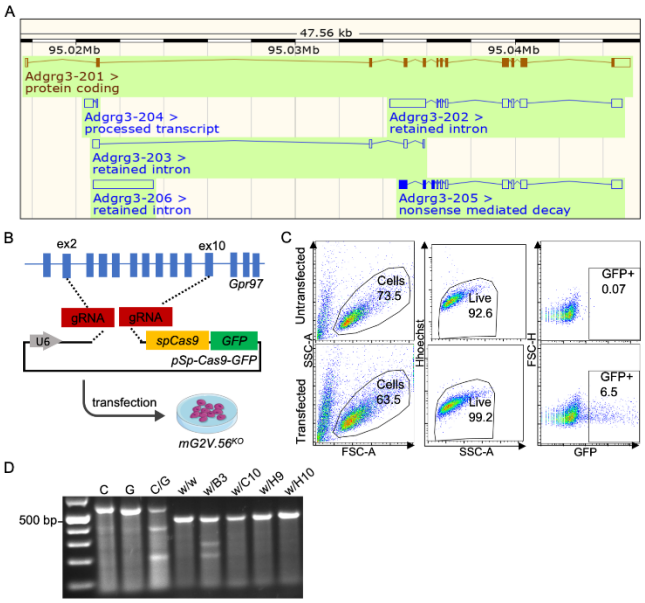

Figure S6. Generation of *Gpr56:Gpr97* double deleted *G2V* ESCs.
A) Mouse *Gpr97* gene and splice isoforms as taken from Ensemble database. **B)** CRISPR/Cas9 strategy showing gRNAs for *Gpr97* exons 2 and 10 and insertion in *pSp-Cas9-2A-GFP* vector used for transfection of *G2V.56*KO ESCs. Two rounds of transfection were performed, first with exon 2 gRNA, and after sorting for GFP+ cells a second transfection was performed with exon 10 gRNAs. **C)** Flow cytometric analysis of transfected and untransfected control ESCs for forward and side scatter, viability and GFP expression at 48 hours post-transfection. **D)** Surveyor assay on DNA from 4 CRISPR/Cas9 ESC clones (B3, C10, H9, H10) that were negative for *Gpr97* mRNA. Clone B3 shows a mismatch in *Gpr97* genomic sequence. C and G are negative and C/G is the positive control.