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Supplemental Information

Rapid Mast Cell Generation from *Gata2* Reporter Pluripotent Stem Cells

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Supplemental Figures and Legends

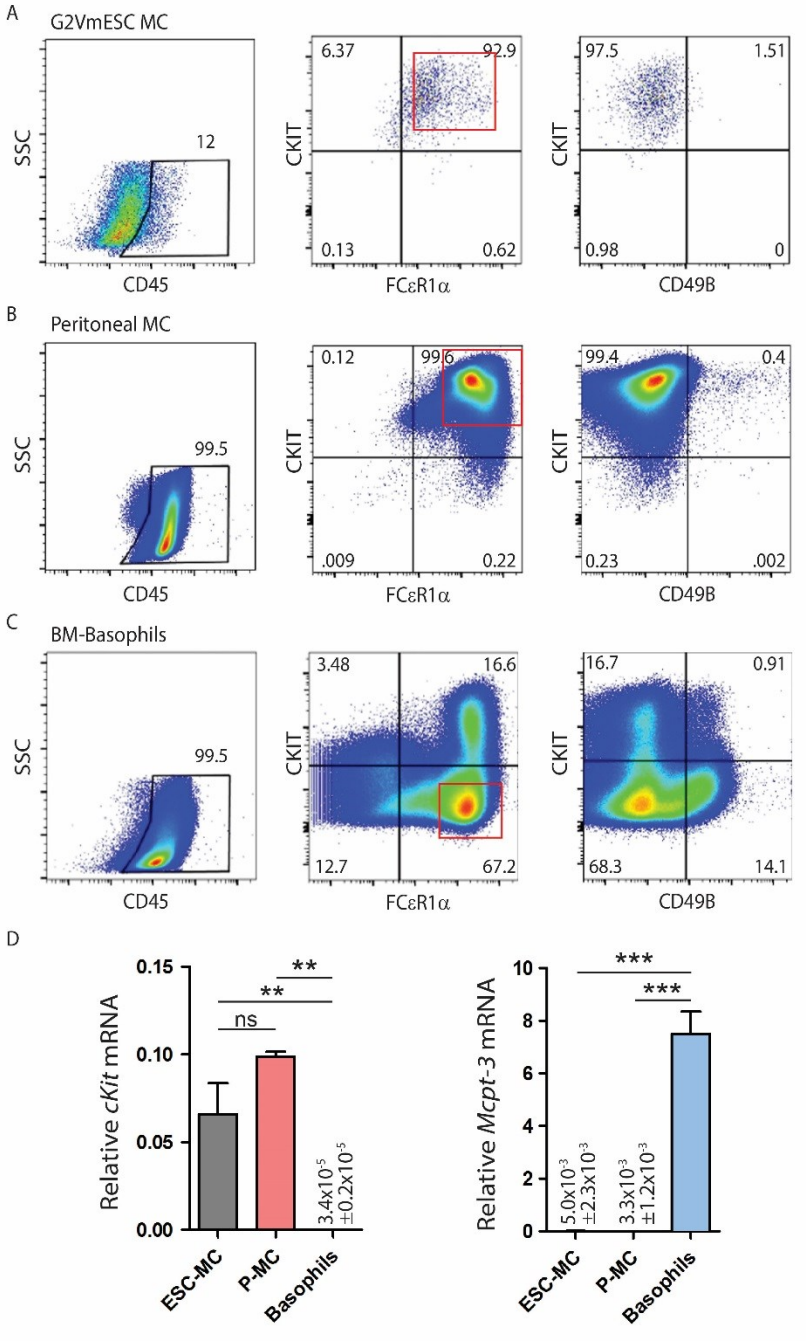

Figure S1. G2V mESC differentiation cultures specifically enrich for mast cell and not for basophil generation. Related to figures 1 and 2. FACS analysis of **(A)** ESC-MC isolated after stage 3, **(B)** Peritoneal MC, and **(C)** bone marrow (BM) cultures containing basophils for CD45, CKIT, FCƐR1α and CD49B expression. Dot plots show staining for mast cell markers (CKIT+, FcƐR1α+ CD49b-) and staining for basophil markers (CKIT-, FCƐR1α+, CD49+). Percentages of cells in each quadrant are shown. Quadrants are set according to FMOs. Red boxes indicate the populations sorted for qRT-PCR analysis. **(D)** Gene expression analysis of mast cell specific marker *cKit* and basophil specific marker *Mcpt-8* in ESC-MC, Peritoneal (P)-MC and Basophils. Relative expression levels were normalized to *β-actin* expression. Mean±SEM, **p<0.01, ***p<0.001, number of independent experiments=3.

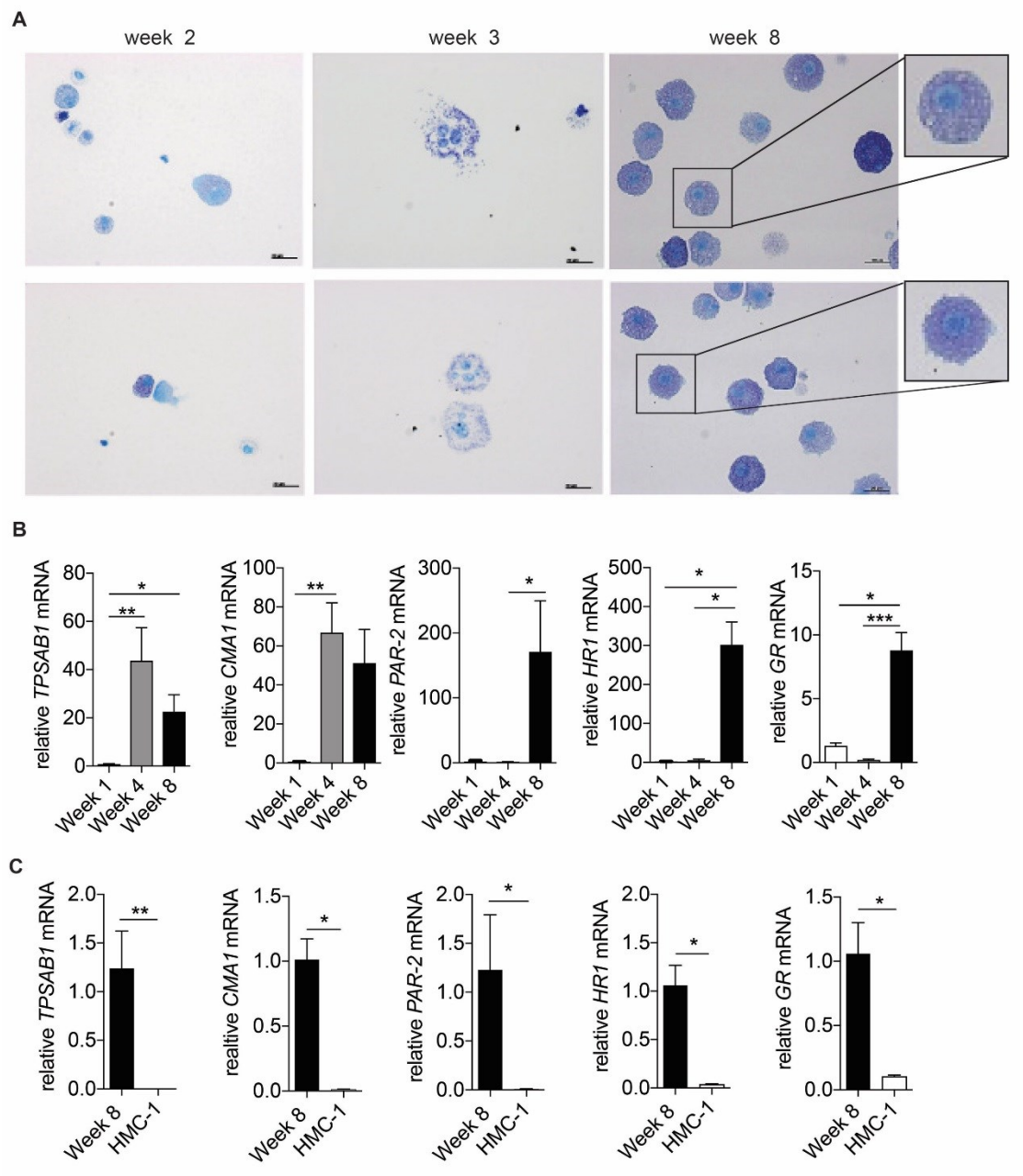

Figure S2. Mast cell differentiation of human peripheral blood progenitor cells. Related to Figure 3. **(A)** Human peripheral blood CD34⁺ cells were isolated and mast cell differentiation performed during 8 weeks of culture. Images of toluidine blue stained cells from week 2, 3 and week 8 (first appearance of mature mast cells) cultures. Number of experiments (n)=3 for each differentiation week, scale bar=20 µm. **(B)** Gene expression analysis of mast cell proteases: *tryptase* (*TPSAB1*), *chymase* (*CMA1*), surface receptors: *protease activated receptor 2*(*PAR-2*), *histamine receptor 1*(*HR1*) and inflammatory marker: *glucocorticoid* receptor(GR) in week 1, 4 and 8 differentiated peripheral blood CD34⁺progenitors. Expression levels were normalized to *GAPDH* and gene expression set as 1 in the undifferentiated cell sample. Week 1, n=7-10; week 4, n=6; week 8, n=2-8. Mean±SEM, *p<0.05, **p<0.01, ***p<0.001. **(C)**Mast cell specific gene expression in mast cells derived via 8-week peripheral blood CD34⁺ progenitor differentiation and human mast cell line (HMC-1). Gene expression in primary mast cell samples was normalized to *GAPDH* and to *cyclophillin D1* in HMC-1 samples. Week 8 n=3-4, HMC-1 n=3-5. Mean±SEM, *p<0.05, **p<0.01.

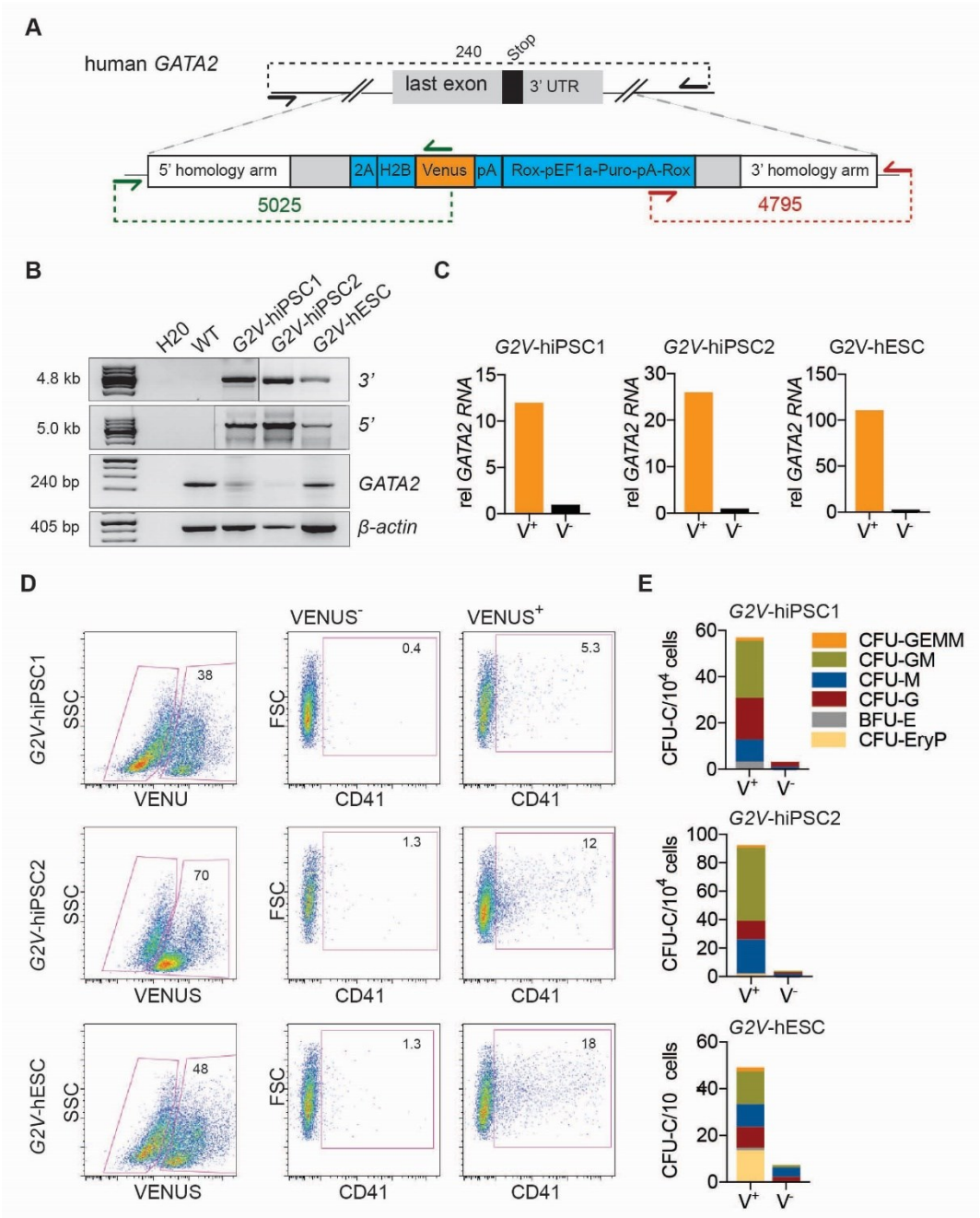

Figure S3. Generation and hematopoietic differentiation of *G2V* reporter human pluripotent stem cells. Related to Figure 3. **(A)** Schematic of the *2A-H2B-Venus* reporter and *Rox* site flanked puromycin selection cassette insertion into the 3' untranslated region (UTR) of the human *GATA2* locus. Primers used for detection of wild type (WT) and recombined alleles are indicated (black=WT; green=5'-junction; red=3'-junction). **(B)** DNA PCR analysis of the genomic locus of *GATA2* and the recombined *GATA2* locus (*5'* and *3'*) in untargeted (WT) and targeted human ESC (*G2V*-hESC) and human iPSC clones (*G2V*-hiPSC-1 and -2). **(C)** Bar graphs showing relative *GATA2* gene expression in VENUS$^+$ (V^+) and VENUS$^-$ (V^-) FACS sorted cells from day 6 differentiated *G2V*-hESCs and *G2V*-iPSCs. *HPRT1* expression was used for normalization and *Gata2* expression was set as 1 in the V^- fraction. **(D)** FACS analysis of hematopoietic marker CD41 expression on V^- and V^+ populations of day 12 differentiated *G2V*-hiPSC1 and *G2V*-hESC. **(E)** Hematopoietic progenitor potential of V^+ and V^- cells isolated from day 12 differentiated *G2V*-hiPSC1 (number of independent experiments(n)=2), *G2V*-hiPSC2 (n=1) and *G2V*-hESC (n=1) lines. Number of colony forming unit cells-culture (CFU-C) per 10^4 FACS sorted cells is shown. CFU types designated by colored bars are granulocyte, erythroid, macrophage, megakaryocyte (GEMM); granulocyte, macrophage (GM); macrophage (M); granulocyte (G); Burst forming unit-erythroid (BFU-E) and primitive erythroid (E). Representative data are shown.

Supplemental Table 1. Primer sequences.

Murine RT-PCR	forward 5'-3'	reverse 5'-3'
cKit	CCATCCATCCATCCAGCACA	CTGTTGCTGCACGTGTATGT
FcεR1α	ACCGTTCAAGACAGTGGAAA	AGACGGGGCTCTCATAACTG
mMCP-6	TAATGACGAGCCTCTCCCAC	CAGCCAGGTACCCTTCACTT
mMCP-5	TCATCTGCTGCTCCTTCTCC	ATAGACCTTCCCGCACAGTG
Gata2	ATGGCAGCAGTCTCTTCCAT	CACAGGCATTGCACAGGTAG
MMCP-1	CCACACTCCCGTCCTTACAT	ACATCATGAGCTCCAAGGGT
CPA-3	ACACCAACAAACCATGCCTC	TGGTGGTTAGGAGGCAGTTT
β-actin	CACCACACCTTCTTACAATGAG	GTCTCAAACA TGATCTGGGTC
Murine TaqMan® qRT-PCR		
cKit	GATCTGCTCTGCGTCCTGTT	CTTGCAGATGGCTGAGACG
FcεR1α	CCATGGATCCTTTGACATCAG	GATCACCTTGCGGACATTC
FcεR1γ	CTTACCCTACTCTACTGTCGACTCAA	AGGCCCGTGTAGACAGCAT
Gata2	TGGCACCACAGTTGACACA	TGGCACCACAGTTGACACA
mMCP-5	ATCTGCTGCTCCTTCTCCTG	ACTCCGTGCCTCCAATGA
mMCP-6	TGCTGTGTGCTGGAAATACC	CCCTTCACTTTGCAGACCA
CPA-3	GCTATTAATTCCTTATGGCTACACATT	GTGGCAATCCTTGCAACTTT
mMCPT8	CCACTCCCGGCCCTATATG	TGCTGTCATTACGATGTCTCTTG
β-actin	CACCACACCTTCTTACAATGAG	GTCTCAAACA TGATCTGGGTC
Human TaqMan® qRT-PCR		
TPSAB1	CCTGCCTCAGAGACCTTCC	ACCTGCTTCAGAGGAAATGG
CMA1	TTCACCCGAATCTCCCATTA	TCAGGATCCAGGATTAATTTGC
PAR-2	GAGCCATGTCTATGCCCTGT	CGATGCAGCTGTTAAGGGTAG
HR1	AGAATCAGACCTGGGTGGAA	AATGAGTCTGAGGCTCCCATAG
GR	TCCCTGGTCGAACAGTTTTT	GCTGGATGGAGGAGAGCTTA
human qRT-PCR		
GATA2	CAGCAAGGCTCGTTCCTGTT	GGCTTGATGAGTGGTCGGT
HPRT-1	CCTGGCGTCGTGATTAGTGAT	AGACGTTCAGTCCTGTCCATAA
human PCS genotyping		
β-ACTIN	AGCTGTCACATCCAGGGTCC-	CCTCGGCCACATTGTGAACT
5'-junction	AGTGCTTCCAGTGTACCCCCA	TGGTCGAGCTGGACGGCGA
3'-junction	GAAGGACCGCGCACCTGGT	GATTCTGAGGTCTGGGCTCTGG
wt GATA2	ACCTCCCGCCCTTCAGCC	GAGGGGGTGCTGGGCCGAG
human CRISPR/Cas9 engineering		
GATA2 Out-5	*ttgcggccgc GCAAAATTCCCAGGACCTGCTC*	*ccaagctt TGCAAAACAAACAGGAGAAAGG ACC*
GATA2 Out-3	*ccaagcttggatcc TCCTACCTGATGCATAGTGGC*	*ccctcgag GAGTTCTGGGGCCTAGAGCTAT GG*
GATA2 In-5	*cggaattc CTGGCTTCCTGGGACCCTCAG*	*gggctagcgtcgac GCCCATGGCGGTCACCATG*
GATA2 In-3	*ccctcgagggcgcgcc GGAACAGATGGACGTCGAGGACC*	*aggcggccgc AGGACTTGGGACAGCTCAGACC AC*

Supplemental experimental procedures

Generation of human GATA2-Venus hESCs and iPSCs

G2V hPSCs were generated by *CRISPR/Cas9* engineering. *GATA2-T2A-H2B-Venus-pA-EF1α-Puro-pA* donor vector was generated from a BAC (CTD-3248G10; Life Technologies). pSIM18Hygro (gift from P. Liu, Wellcome Trust Sanger Institute) transformed bacteria (heat-inducible recombinase expression) were used. Mini-homology arms (*5'-out, 3'-out, 5'-in* and *3'-in*) were generated by PCR amplification of BAC DNA using primers (Suppl Table 1).

5'-out arm was digested with NotI/HindIII and *3'-out* arm with XhoI/HindIII and ligated to XhoI/NotI digested, gel purified pBlueScript to generate outarm-pBlueScript. *5'-in* arm was digested with EcoRI/NheI and *3'-in* arm with XhoI/NotI and ligated with a NheI/AscI digested *ZeoR* (gift from P.Liu) and EcoRI/NotI digested pBlueScript to generate *5'in-arm-ZeoR-3'in-arm-pBlueScript*. The *out-arm-pBlueScript* backbone was PCR amplified for recombineering. Competent BAC/pSIM18Hygro bacteria were transformed with PCR amplified *out-arm-pBlueScript* to generate the full homology region within *pBlueScript*. Full homology region-*pBluescrip* and a EcoRV/NotI linearised *5'in-arm-ZeoR 3'in-arm* fragment were transformed into competent SIM18 E.coli (strain with stable phage incorporated SIM18 recombinase to replace the *GATA2* stop codon with *ZeoR*). *ZeoR* was digested from the homology arms using SalI/AscI and homology arms ligated with a SpeI/AscI digested *T2A-Venus-pA-EF1a-PuroR-pA* DNA fragment (provided by W. Wang, Wellcome Trust Sanger Institute) and sequencing verified.

GATA2-stop codon-targeting gRNA was ligated to *pSpCas9(BB)-2AGFP(PX458)* (Addgene) vector. $8x10^5$ hPSCs were electroporated with Human Stem Cell Nucleofector Kit 2 (A-023), AMAXA Nucleofector (Lonza). 3µg of *GATA2-T2A-H2B-Venus-pA-EF1α-Puro-pA-pBlueScript* and 2µg of *gRNA-pSpCas9(BB)-2A-GFP* were used for transfection and cells seeded on puromycin-resistant MEFs (DR4, ATCC). 48 hours later puromycin selection was initiated (1µg/ml, 1 week). 33 iPSC and 16 ESC prospective *G2V* clones were established and PCR genotyped (primers spanning 3'(4795bp) and 5'(5025bp) junctions of the construct and *GATA2* genomic sequence (Fig3A). *GATA2* WT allele primers(240bp) assessed the homo-/heterozygosity of the insertion. Correctly integrated clones were karyotyped. 1 clone of *G2V*-hESC and 2 clones of *G2V*-hiPSC with normal karyotypes were used.

hPB $CD34^+$ cell isolation and differentiation

Peripheral blood (PB; 40-80ml, venipuncture) was collected under the LREC08/S1103/38 (MRC-CIR) in 4ml of sodium citrate (3.8%, Sigma Aldrich) from healthy female donors (age 24-47, number of independent experiments=20). 10ml PB in 25ml of complete StemPro serum-free medium (Gibco), 2 mM L-glutamine, 100 IU/ml penicillin, and 50µg/ml streptomycin (LifeTechnologies) was layered onto 14 ml of Histapaque (Sigma Aldrich), centrifuged (675g, 20min, RT) and mononuclear cell layer harvested, washed twice and resuspended in PBS, 0.5% bovine serum albumin (Sigma Aldrich) and 2mM ethylene diamine tetra-acetic acid (EDTA, Gibco). $CD34^+$ enrichment was by MicroBead Kit (MACS Miltenyi Biotec), Magnetic separation and mini MACS separator (Miltenyi Biotec). Cells were counted on a haemocytometer (Immune Systems).

Mast cell differentiation was performed by $CD34^+$ cell culture in Stem-Pro34® SFM (Gibco) with rhSCF, rhIL-6, and rhIL-3 (only week 1) (Kirshenbaum and Metcalfe, 2006). Hemi-depletion/feeding was performed weekly. Recovered cells were washed and resuspended in 25ml StemPro media with rhIL-6 and rhSCF for further differentiation. Complete differentiation (95-100%) was achieved over 8–10-weeks.

Control HMC-1 cells were cultured at $4x10^5$ cells/ml (37°C, 5%CO_2) in IMDM, 25mM HEPES, 3.024g/l sodium bicarbonate, 4mM L-glutamine (ThermoFisher Scientific), 10% iron-supplemented calf serum (Hyclone, USA), 1.2mM α-thioglicerol (Sigma Aldrich) and 10ml/l P/S (Sigma Aldrich).