## SUPPLEMENTARY INFORMATION

## SUPPLEMENTAL MATERIALS AND METHODS

**MSC generation:** MSC were obtained from BM aspirates as previously described (1). Briefly, BM was separated by density gradient (Biocoll, Biochrom AG, Berlin, Germany) and mononuclear cells were cultured on plastic flaks and after 48 hours non adherent cells were washed; plastic adherent cells were lifted with Trypsin (Trypsin-EDTA, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) once they reached confluence. Generated cells were expanded to obtain a homogeneous MSC population. MSC were frozen until the start of experiments.

**HSPC generation:** healthy volunteers were treated with Colony Stimulating Factor Granulocytes 24 hours previous to peripheral blood apheresis. Inmunomagnetic sorting with the CD34+ antibody conjugated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) was used to obtain the cells.

**Media:** Media used for experiments were DMEM-Glutamax® and Alpha-MEM-Glutamax® (Gibco Life Technologies) supplemented with 10 % fetal calf serum (Biochrom AG, Berlin, Germany) containing penicillin-streptomycin (Gibco Life Technologies). RPMI 1640 medium (Biochrom) supplemented with 2 mM L-glutamine, 10 mM sodium pyruvate, 1% nonessential amino acids, 100 µg/ml penicillin, 100 µg/ml streptomycin (all from Biochrom), and 10% fetal calf serum.

**Lenalidomide (LEN) treatment:** two concentrations of the drug were used: 0.1 µM and 1 µM. They were based on several other prior investigations (2,3,4) and were mainly selected to represent the range of plasmatic concentration reached by the drug in patients treated with oral doses of 5 to 30 mg of Lenalidomide and with different degrees of renal function (5).

**MSC clonality assays:** MSC were plated in T25 flasks ($1x10^3$ cells per flask) or on 96 well plates at 0.2 cells per well to show ability of the cells to form single cell derived (SCD) colonies. They were cultured in Alpha-MEM media for 14 days. Colonies in T25 flasks were stained with Giemsa and counted under a microscope (Axiovert 25 Zeiss, Jena, Germany) or trypsinized and counted using Trypan Blue (to determine viability) and a hemocytocounter.

**Flow cytometry:** expression of cell surface markers and adhesion molecules was determined using antibodies against CD73, CD29, CD166, CD44, and CD54 (BD bioscience), CD105 (Abd Serotec, Düsseldorf, Germany), CD90, CD146, CD34, CD45, and CD49e (Miltenyi Biotec). The antibodies were conjugated with the fluorophores fluorescein isothiocyanate, phycoerythrine and allophycocyanin. Appropriate mouse antibodies were used as isotype controls (Miltenyi biotec). All flow measurement was made using FACScalibur (BD Biosciences, Heidelberg, Germany) using BD CellQuest Pro software**.**

**Differentiation assays:** MSC were seeded in 24 well plates ($2x10^4$ cells per well). After reaching subconfluence, media was changed to osteo inductive media or adipo inductive media with or without LEN. All reagents for differentiation induction were purchased from Sigma, recipes are described extensively (1). Cells were

differentiated for 21 days with media change twice per week. Afterward, the layers were stained with von Kossa and their alkaline phosphatase activity was measured as described elsewhere (6) for evaluating osteogenic differentiation, and Oil red staining and expression of fatty acid binding protein 4 gene (FABP4) determined by real time polymerase chain reaction (RT-PCR) (using the primers sense 5′-tac tgg gcc agg aat ttg ac-3′ and reverse 5′-tgg ttg att ttc cat ccc at-3′) were used to evaluate adipogenic differentiation.

**Vitality, growth, and proliferation of MSC:** cells were seeded in 6 well plates ($5x10^4$ cells per well) and in some cases LEN was added after 2 days of culture. Layers were observed daily on an inverted microscope and evaluated for confluency and morphology. Cells were harvested at different time points. Cells from day 14 were stained with Annexin V kit, PI (eBioscience), and measured for apoptosis by flow cytometry. Ethynil-deoxi-Uridyl (EdU, Click IT Invitrogen) incorporation after 96 hours pulse and 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE, Cell Trace Invitrogen) dilution after 14 days was studied with flow cytometry. MSC were cultured on 96 well plates ($5x10^3$ cells per well) for 24 hours and afterward LEN was added and sustained for 5 days. At this time point a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) proliferation assay (Roche Diagnostics GmbH, Mannheim, Germany) was performed according to manufacturer´s directions.

**Senescence studies:** $1x10^3$ MSC were seeded in 24 well plates and after 72 h culture, stained with β-Galactosidase (β-Gal) kit. Number of β-Gal positive cells was counted under an inverted microscope.

**Cell cycle:** MSC were transfected with the Premo Fucci cell cycle sensor (80 particles per cell, 16 h, Invitrogen). *Nuclei* were stained with Hoechst 33342 (Invitrogen) for quantification of DNA content. 10 cells per replicate were seeded on 18 well slides (ibidi, Martinsried, Germany) and imaged live using a Leica TCS SP5 confocal microscope at different time points to determine cell cycle phase.

**Cytokine concentration measurement:** supernatant from MSC was obtained after 7-days culture with or without LEN (SN1) and after washing the cells and further culturing without drug for 3-days (SN2). The fallowing cytokines were measured with Enzyme Linked Immunoabsorbant Assay: Stromal Derived Factor-1α (SDF-1α, R&D systems kit), Stem Cell Factor (SCF Peprotech kit), and Angiopoietin 1 (ANG1 RayBiotech kit), according to the manufacturer's instructions.

**HSPC transwell migration assay:** purification of $CD34^+$ HSPC and transwell migration toward the conditioned medium MSC with or without LEN treatment were performed as described previously (1). Briefly, $1 \times 10^5$ HSPC were added to the upper chamber in 0.1 mL medium and allowed to migrate for 4 hours at 37°C toward 600 µL conditioned medium (SN2) through a 5-µm polycarbonate membrane. Afterward, cells in the lower chamber were counted. The CXCR4 antagonist AMD3100 (Sigma) was used at a concentration of 10 µM.

**MSC-HSPC cocultures and colony assays, proliferation and apoptosis of HSPC and KG1-α with stroma coculture:** a 4 week cobblestone area forming assay (CAF-C) was performed by plating MSC in 24 well plates. After reaching subconfluent stage, MSC were treated with LEN for additional 7 days. Layers were washed and $1x10^3$ magnetically isolated CD43+ cells were cocultured using LTC-IC media. (Stem

Cell Technologies, Cologne, Germany) supplemented with 1x10-6 M Hydrocortisone (Sigma). CFSE was used to track proliferation of HSPC or the cell line KG1-α during 72 hours coculture with MSC. In other experiments, these cells were cocultured with stroma for 24 hours and then stained with Annexin V to measure apoptosis. The experiments were performed with or without addition of 100 nM recombinant Tumor Necrosis Factor alpha (TNF-α, Peprotech) in RPMI media. Apoptosis and proliferation of HSPC, KG1-α cell line in coculture with MSC was studied using flow cytometry. For a Colony Forming Unit Granulocyte-Erythrocyte-Monocyte/Macrophage assay (CFU-GEMM), $1x10^4$ MSC were plated in 35 mm dishes and treated with LEN for 7 days. At this time, the MSC layers were washed to remove residual LEN, and $1x10^3$ CD34+ cells were seeded with MyeloCult media (Stem Cell Technologies). After 14d, colonies were counted and classified under a microscope.

**RNA extraction, complementary DNA synthesis and reverse transcriptase polymerase chain reaction (RT-PCR):** mRNA was isolated from MSC cultures using Trizol reagent (Invitrogen) and reverse transcribed into complementary DNA with the RevertAid First Strand C-DNA Synthesis kit (Fermentas). Besides the aforementioned primers for FABP4, primers for SDF-1α (sense 5´- GGT CCG TCC TGT CTT GAT GT-3´ and reverse 5´- ACT GGG TGT ACC ACC TGC TC-3´), for ANG1 (sense 5´-gaa ggg aac cga gcc tat tc-3´ and reverse 5´-gct ctg ttt tcc tgc tgt cc-3´), and for Stem Cell Factor (SCF, sense 5´-ctc cag taa gtg gcc ttt gc-3´ and reverse 5´-tat ctg agg gcc tga aca cc-3´) were used for RT-PCR with SYBR Green mastermix (Fermentas) in a Taqman 7000 Fast cycler (Applied Biosystems, Darmstadt, Germany). Abelson murine leukemia viral oncogene homolog 1 (ABL-1) sense 5´-tgg tag ggg aga acc act tg-3´ and reverse 5´-ata ctc caa atg ccc aga cg-3´ was used to normalize values of relative gene expression.

**Inmunomodulatory activity of MSC:** healthy and MDS-MSC were cultured with and without LEN for 7 days and then irradiated (30 Gy) and seeded on 96 well plates. On each well, 1 $x10^5$ stimulator peripheral blood mononuclear cells (PBMC, polled cells form 5 donors and then irradiated with 30 Gy) and responder PBMC (1 x $10^5$ / well) were added and cocultured for 7 days; 1 µCi of [3H]thymidine (Hartmann Analytic, Braunschweig, Germany) was given to each well for the last 18 h of culture. Cells were harvested and [3H]thymidine incorporation was determined in a beta counter (Wallac, Freiburg, Germany).

## SUPPLEMENTAL RESULTS

### MSC from MDS patients suppress T-cell proliferation regardless of disease stage

Proliferation of PBMC induced by allogenic stimulation is clearly reduced by MSC (Supplementary Figure S7). This suppressive effect was seen with stromal cells from healthy donors and all MDS subtypes without differences. LEN priming of MSC was not accompanied by an alteration of this capacity (data not shown).

## Supplementary References


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3. Verhelle D, Corral LG, Wong K, Mueller JH, Moutouh-de Parseval L, Jensen-Pergakes K, et al. Lenalidomide and CC-4047 inhibit the proliferation of malignant B cells while expanding normal CD34+ progenitor cells. Cancer Res. 2007;67(2):746-55.
4. Wei S, Chen X, Rocha K, Epling-Burnette PK, Djeu JY, Liu Q, et al. A critical role for phosphatase haplodeficiency in the selective suppression of deletion 5q MDS by lenalidomide. Proc Natl Acad Sci U S A. 2009;106(31):12974-9.
5. Chen N, Lau H, Kong L, Kumar G, Zeldis JB, Knight R, Laskin OL. Pharmacokinetics of lenalidomide in subjects with various degrees of renal impairment and in subjects on hemodialysis. J Clin Pharmacol. 2007 Dec;47(12):1466-75.
6. Hempel U, Hefti T, Kalbacova M, Wolf-Brandstetter C, Dieter P, Schlottig F. Response of osteoblast-like SAOS-2 cells to zirconia ceramics with different surface topographies. Clin Oral Implants Res. 2010;21(2):174-81.

## SUPPLEMENTARY TABLE

### Supplementary Table 1. Characteristics of patients

| Age | Gender | WHO type | BM blasts % | Karyotype | Group |
|---|---|---|---|---|---|
| 84 | m | MDS-del(5q) | <5% | 46,XY,del(5q) | LR-5q |
| 75 | m | MDS-del(5q) | <5% | 46,XY,del(5q) | LR-5q |
| 68 | f | MDS-del(5q) | <5% | 46,XX,del(5q) | LR-5q |
| 41 | f | MDS-del(5q) | <5% | 46,XX,del(5q) | LR-5q |
| 85 | f | MDS-del(5q) | <5% | 46,XX,del(5q) | LR-5q |
| 73 | f | MDS-del(5q) | <5% | 46,XX,del(5q) | LR-5q |
| 55 | f | MDS-del(5q) | <5% | 46,XX,del(5q) | LR-5q |
| 59 | m | MDS-del(5q) | <5% | 46,XY,del(5q) | LR-5q |
| 32 | m | MDS-del(5q) | <5% | 46,XY,del(5q) | LR-5q |
| 64 | m | RCMD | <5% | 47,XY,+8 | LR |
| 54 | m | RCUD | <5% | 46, XY | LR |
| 68 | f | RCMD | <5% | 46, XX | LR |
| 70 | m | RCMD | <5% | 46, XY | LR |
| 66 | m | RCMD | <5% | 45, X, -Y | LR |
| 72 | m | RCMD | <5% | 46, XY | LR |
| 70 | f | RAEB-1 | 10,00% | Complex | HR |
| 71 | f | RAEB-1 | 7,00% | 46, XX | HR |
| 60 | f | RAEB-1 | 15,00% | Complex | HR |
| 62 | f | RAEB-1 | 10,00% | 46, XX | HR |
| 74 | m | RAEB-1 | 5,00% | 45, X, -Y | HR |

## SUPPLEMENTARY FIGURES LEGENDS

### Supplementary Figure S1

Exemplary histograms showing the flow cytometry characterization of healthy MSC donors

### Supplementary Figure S2

Related to Figure 1. Characteristics of MSC. **(A)** From all studied positive MSC surface markers, CD105 expression was reduced in MDS-HR stroma. **(B,C)** MSC derived by plastic adherence and by SDC plating present distinct degrees of osteogenic potential, quantified by assigning points from 1 to 10 to the degree of differentiation seen with von Kossa staining (B); this was confirmed quantitatively by ALP activity determination (C). **(D,E)** Adipogenic differentiation is also possessed by MSC as demonstrated by Oil red staining (D, index is equal to % of cells with lipid vacuoles) and quantified by RT-PCR for FABP-4 (E). Ω indicates significance between the MDS patients and the healthy group. Results are expressed as mean ± SEM of independent cases, for A n were Healthy=6, LR5q=9, LR=6 and HR=5; for B and D n were Healthy=6, LR-5q=6, LR=6 and HR=5; for C n =4 for all groups; for E n=3 for all groups. Significance was set as * p≤0.05; **p≤0.005; Ω Ω Ω /*** p≤0.001.

### Supplementary Figure S3

Related to Figure 2. Proliferation defects of MDS-MSC. **(A)** Proliferation of MSC was determined after seeding $5x10^4$ cells for 14 days and counting cells at the end of culture; proliferation indexes were calculated (final cell count minus number of seeded cells). **(B,C)** EdU pulse of 96h (B) and CFSE labeling (C) were used to track short term and proliferation of MSC during 14 days of culture. **(D,E)** Cells were transfected with a baculovirus vector containing Germinin-GFP (expressed in S and G2-M phases of cell cycle), and Cdt1-RFP (expressed in G1) and stained with Hoechst 33342 for DNA content quantification and imaged every 24 h for 2 days to determinate cell cycle phases, the pictures show representative examples of cell *nuclei* expressing fluorescent proteins depending on cell cycle phase (20X, scale bar=50 μm) and quantification of results in the graph. Results are expressed as mean ± SEM of independent cases, for A-C n were Healthy=6, LR-5q=6, LR=6 and HR=5; for D all groups n=3 for all groups. Significance was set as */† p≤0.05; **/††/‡‡ p≤0.005; *** p≤0.001 comparing MDS patients with healthy donors.

### Supplementary figure S4

Treatment with LEN of MSC does not affect their proliferation and viability. **(A)** MSC were culture for 2 days (5000 cells per well, 96 well plate) and then treated for 5 days with LEN; afterwards, a MTT assay was performed. **(B)** MSC were cultured for 28 d with LEN treatment (recharged every media change, twice a week), after culture, cells were harvested and stained with Annexin V and PI as counterstain and analyzed with FACS. Healthy n=6, LR-5q n=7, LR and HR n=4 each.

**Supplementary Figure S5**

Expression of adhesion molecules on MDS-MSC is altered. MSC in culture were trypsined and stained with antibodies for adhesion molecules and measured by flow cytometry. The figure shows mean fluorescence intensity (MFI) for each molecule. Results are expressed as mean ± SEM of independent cases, n were Healthy=6, LR-5q=9, LR=6 and HR=5 for CD166, CD29 and CD146, and n=4 for all groups for CD49e, CD44 and CD54. Significance was set as * p≤0.05; ** p≤0.005; *** p≤0.001.

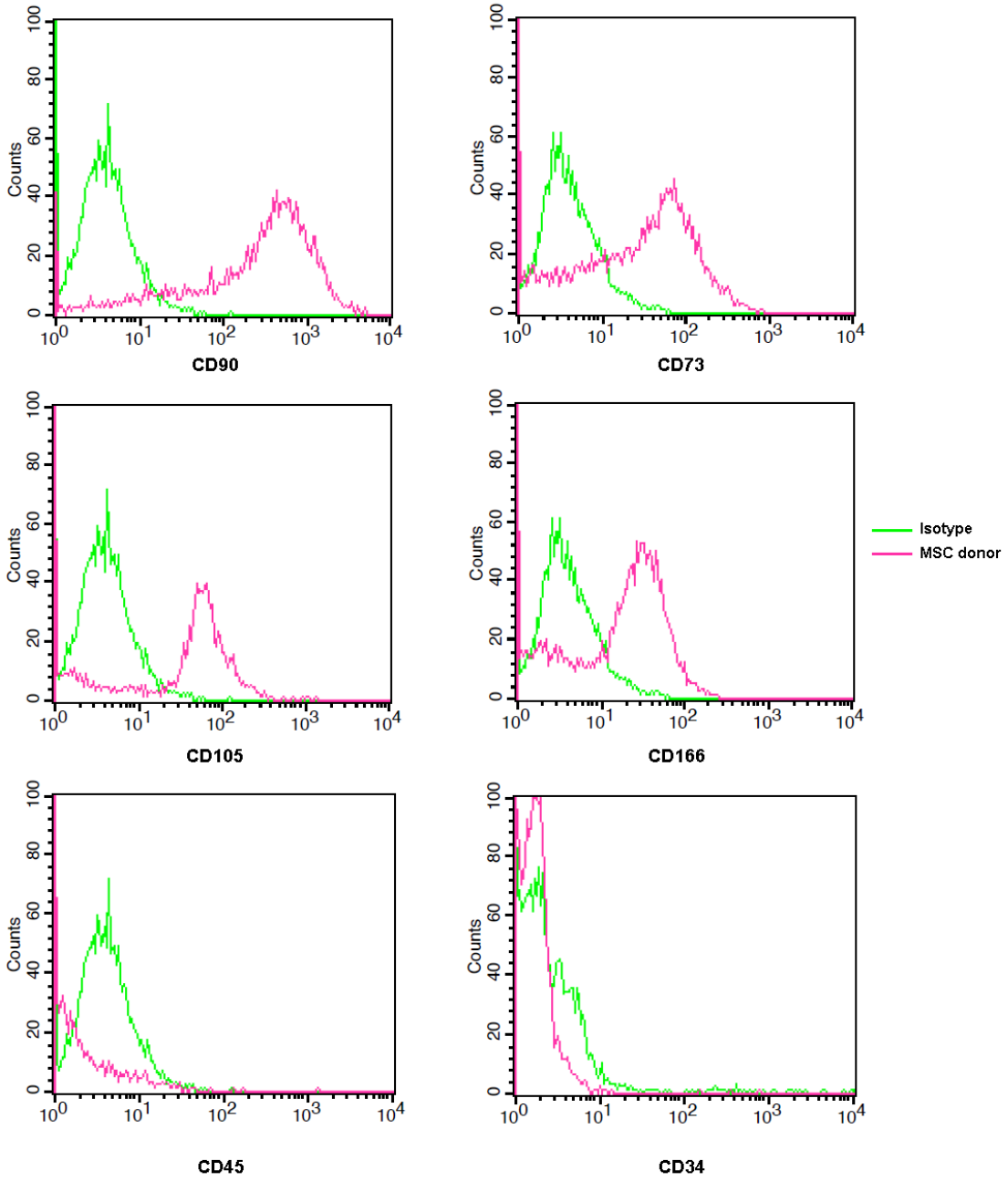
**Supplementary Figure S6**

Support of expansion of healthy clonogenic progenitors by MDS-MSC is defective and modified by LEN. MSC were seeded on 35 mm dishes with or without LEN treatment, then washed and used for coculture with $1x10^3$ healthy CD34+ cells using semi-solid media. 14 days after coculture, hematopoietic colony output was determined under an inverted microscope; The graphs show the effect of LEN pre-treatment of stroma as the fold change of the colony number compared to HSPC alone. Results are expressed as mean ± SEM of independent cases, n were HSPC alone n=3, Healthy n=5, LR-5q n=6, LR n=5 and HR n=5. Significance was set as * p≤0.05; **p≤0.005; *** p≤0.001.

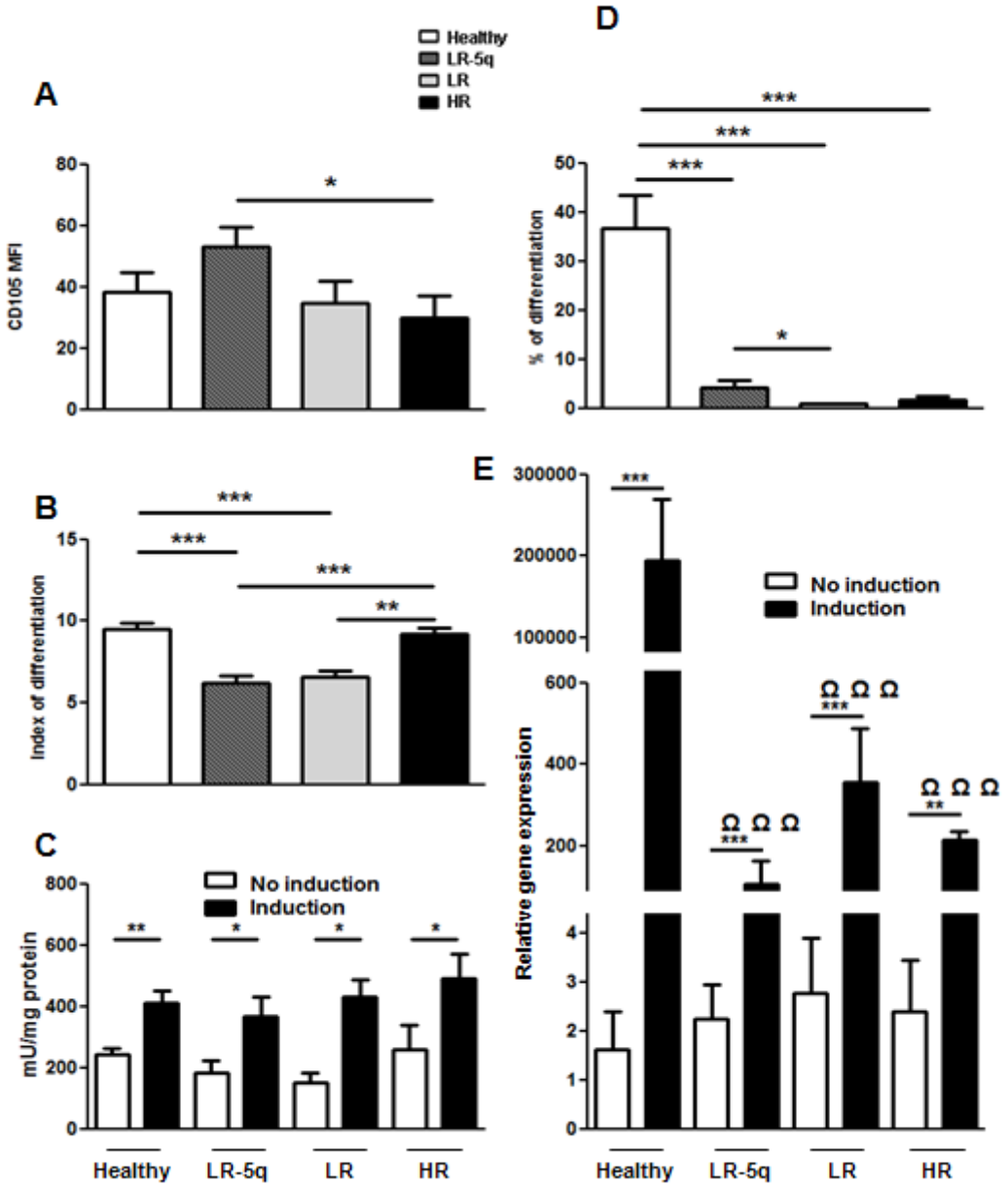
**Supplementary Figure S7**

MDS-MSC are able to suppress T-cell proliferation. PBMNC were allowed to react to $3^{rd}$ party polled irradiated PBMNC and their incorporation of [3H]thymidine was measured with and without present of MSC layers. % change reduction of incorporation of [3H]thymidine was similar in the MDS groups to that of healthy donors regardless of disease group. n=3 for all groups.

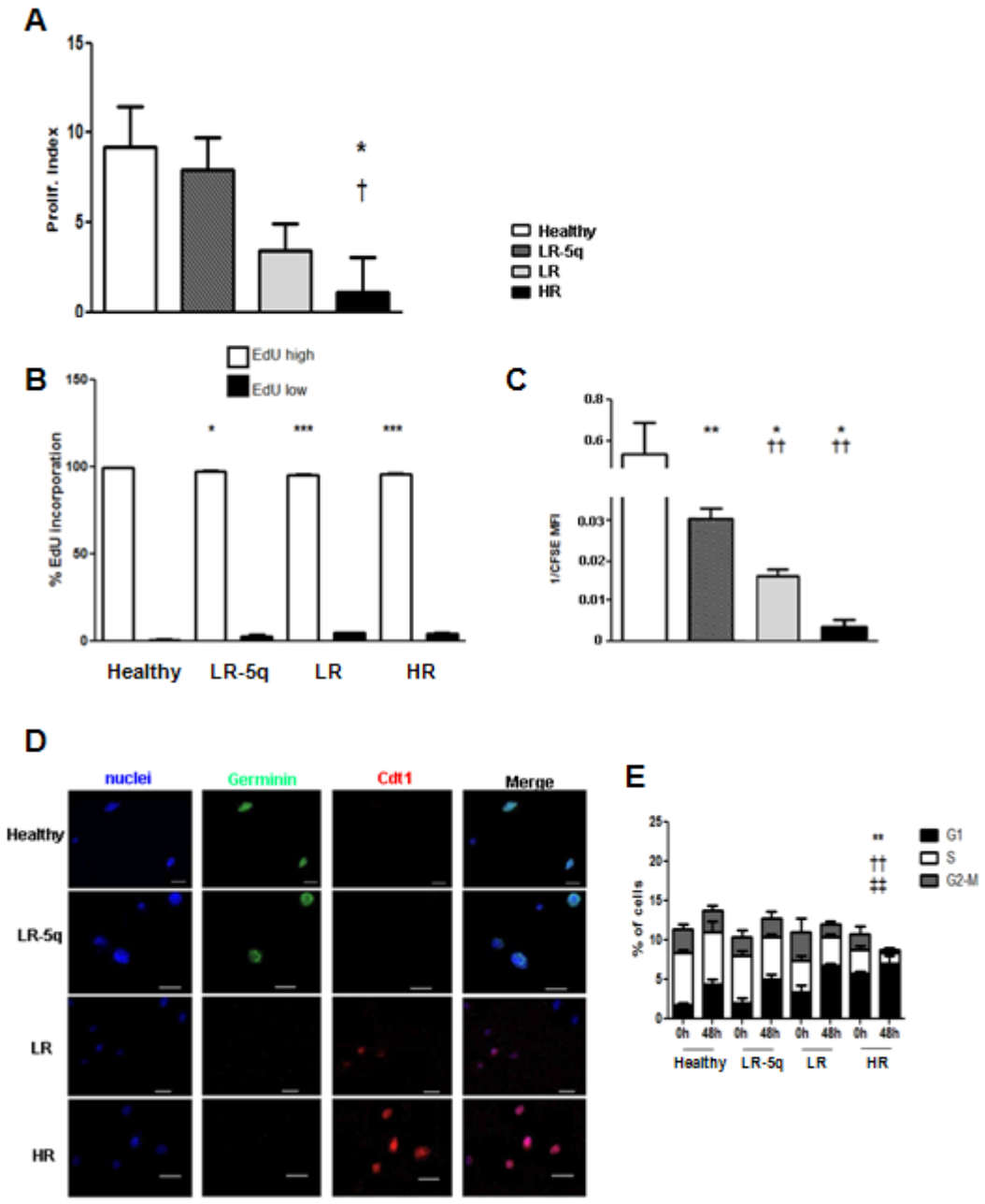
## Supplementary Figure S1



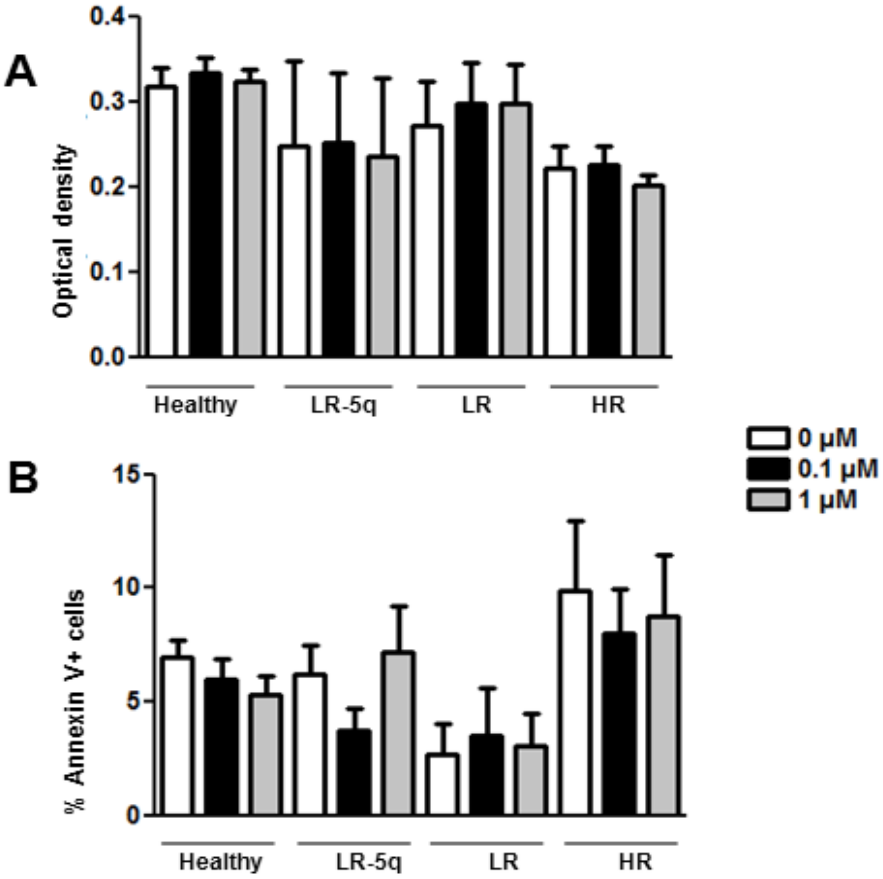
**Supplementary Figure S2**



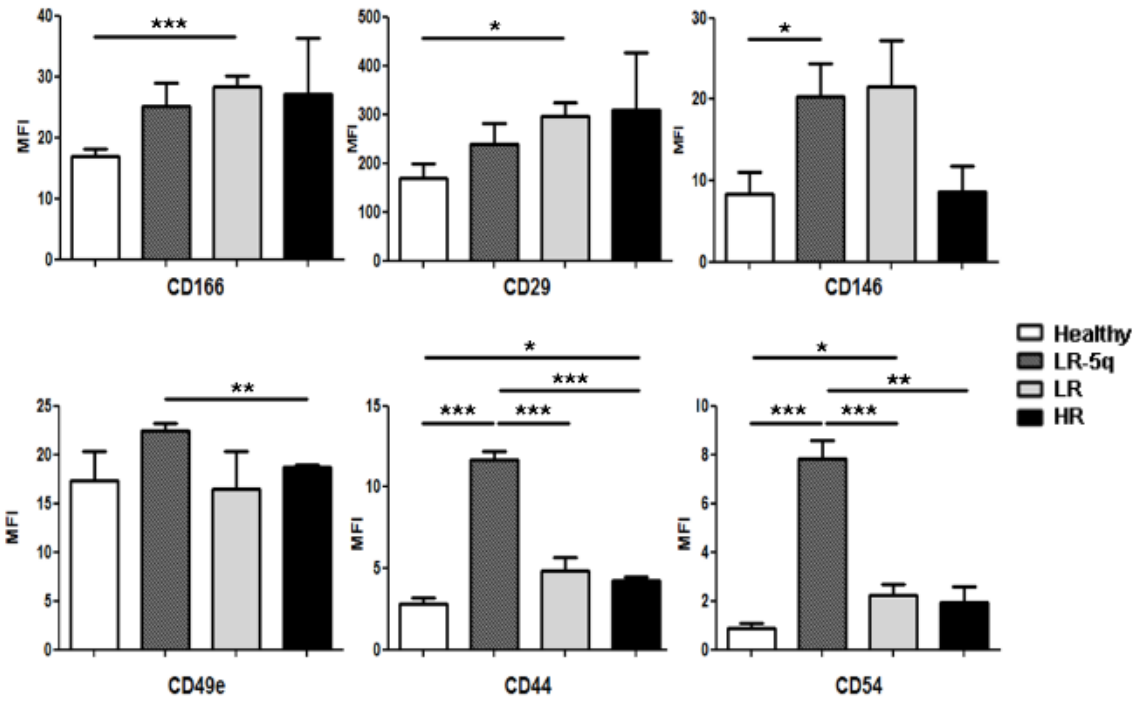
## Supplementary Figure S3



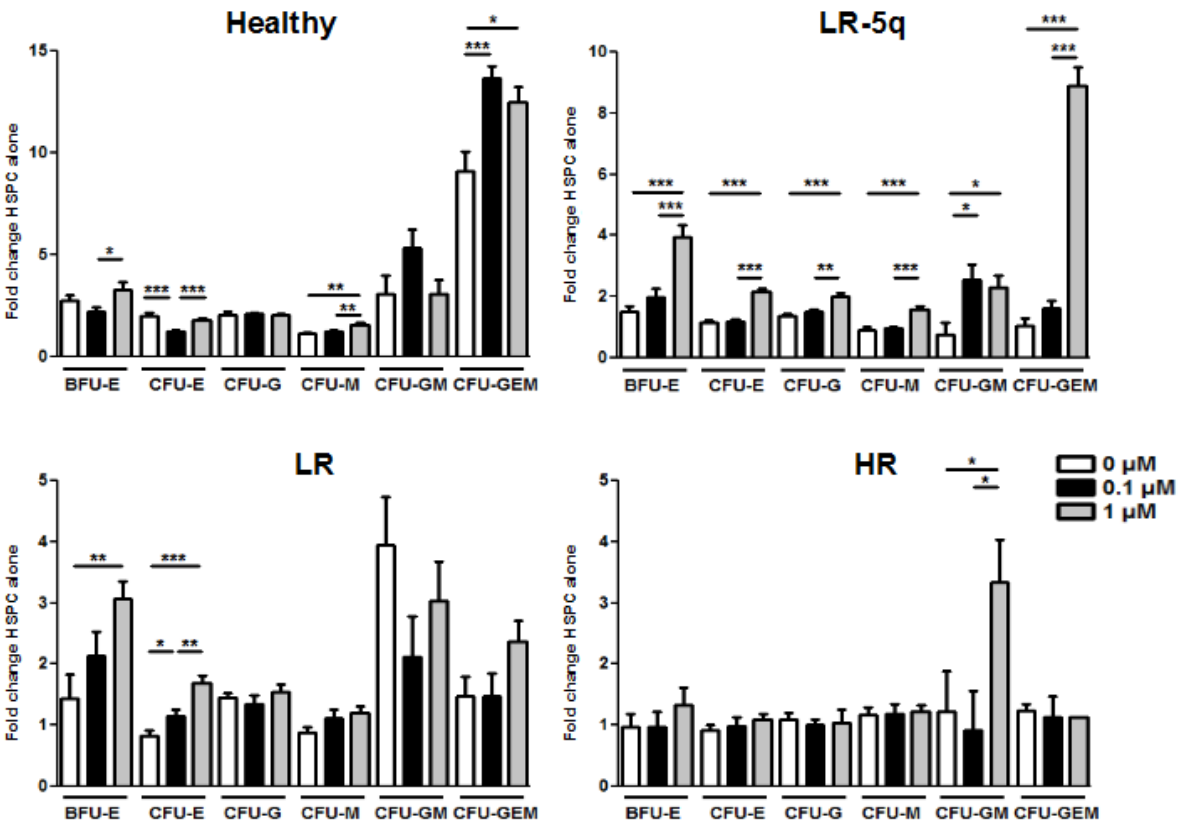
**Supplementary Figure S4**




**Supplementary Figure S5**



## Supplementary Figure S6




## Supplementary Figure S7