SUPPLEMENTARY APPENDIX

Implementation of erythroid lineage analysis by flow cytometry in diagnostic models for myelodysplastic syndromes

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Supplementary

Table S1 The applied 4-color (panel A) and 8-color (panel B) markers. Per antibody CD number, (clone), and manufacturer are depicted. DAKO: DakoCytomation, Glostrup, Denmark; BD: BD Biosciences, San Jose, CA, USA. BL: BioLegend, San Diego, Ca, USA. Sanquin, Amsterdam, The Netherlands. BC: Beckman Coulter, Miami, FL, USA. IS: Immunostep, Salamanca, Spain.

Table S1A

	FITC	**PE**	**PerCP**	**APC**
1			**CD45** (2D1) BD	
2	**CD16** (DJ130c) DAKO	**CD13** (L138) BD	**CD45**	**CD11b** (D12) BD
3	**CD34** (8G12) BD	**CD11b** (D12) BD	**CD45**	**HLA-DR** (L243) BD
4	**CD36** (CLB-IVC7) Sanquin	**CD33** (P67.6) BD	**CD45**	**CD14** (MoP9) BD
5	**CD36**	**CD64** (10.1) DAKO	**CD45**	**CD14**
6	**CD15** (MMA) BD	**CD10** (SS2/36) DAKO	**CD45**	**CD34** (8G12) BD
7	**CD34**	**CD117** (104D2) BD	**CD45**	**CD13** (WM15) BD **CD33** (P67.6) BD
8			**CD45**	**CD34**
9	**CD5** (DK23) DAKO	**CD19** (SJ25C1) BD	**CD45**	**CD34**
10	**CD2** (MT910) DAKO	**CD56** (My31) BD	**CD45**	**CD34**
11	**CD13** (WM-47) DAKO	**CD7** (M-T701) BD	**CD45**	**CD34**
12	**CD13**	**CD25** (ACT-1) DAKO	**CD45**	**CD34**
13	**CD71** (Ber-T9) BD	**CD235a** (JC159) DAKO	**CD45**	**CD117** (104D2) Dako

Table S1B

	FITC	PE	PerCP-Cy5.5	PC7	APC	APC-H7	V450	KO
1			**CD34** (8G12) BD	**CD117** (104D2D1) BC			**HLA-DR** (L243) BD	**CD45** (J.33) BC
2	**CD16** (DJ130c) DAKO	**CD13** (L138) BD	**CD34**	**CD117**	**CD11b** (D12) BD	**CD10** (HI10A) BD	**HLA-DR**	**CD45**
3	**CD2** (MT910) DAKO	**CD64** (10.1) DAKO	**CD34**	**CD117**	**IREM2** (UP-H2) IS	**CD14** (MoP9) BD	**HLA-DR**	**CD45**
4	**CD36** (CLB-IVC7) Sanquin	**CD105** (43A3) BL	**CD34**	**CD117**	**CD33** (P67.6) BD	**CD71** (M-A712) BD	**HLA-DR**	**CD45**
5	**CD5** (DK23) DAKO	**CD56** (My31) BD	**CD34**	**CD117**	**CD7** (M-T701) BD	**CD19** (SJ25C1) BD	**HLA-DR**	**CD45**
6	**CD15** (MMA) BD	**CD25** (ACT-1) DAKO	**CD34**	**CD117**	**CD123** (9F5) BD	**CD38** (HB7) BD	**HLA-DR**	**CD45**
7	**CD7** (M-T701) BD	**CD235a** (JC159) DAKO	**CD34**	**CD117**	**CD13** (WM15) BD	**CD71** (M-A712) BD	**HLA-DR**	**CD45**

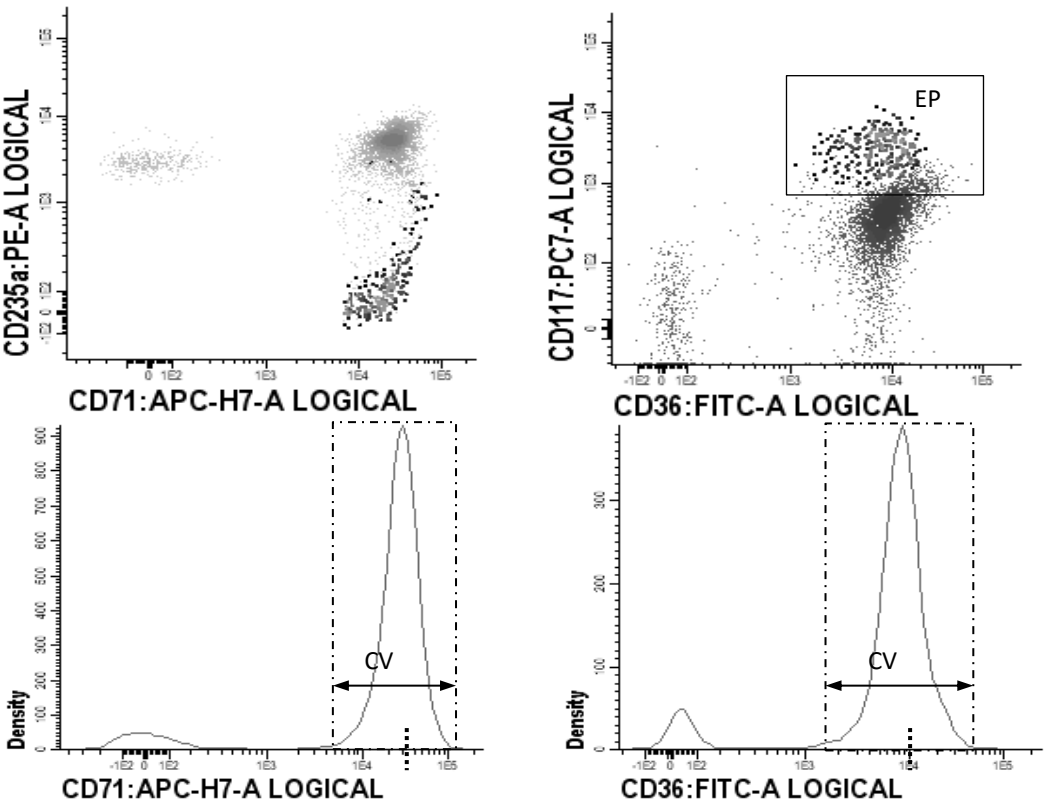
Table 2S Upgraded patients per WHO-Classification after addition of the erythroid evaluation. This table provides the absolute patient counts belonging to Figure 2.

WHO-classification	Upgrade A > B 'No MDS'	Upgrade B > C 'MDS'	C 'MDS' after addition erythroid evaluation (group total)
RA	1	0	2 (4)
RARS	2	2	16 (20)
RCMD	1	5	17 (23)
RCMD-RS	1	4	23 (27)
RAEB-1	0	0	13 (14)
Del(5q)	0	1	9 (12)
MDS-U	0	0	1 (2)
CMML	0	0	4 (4)

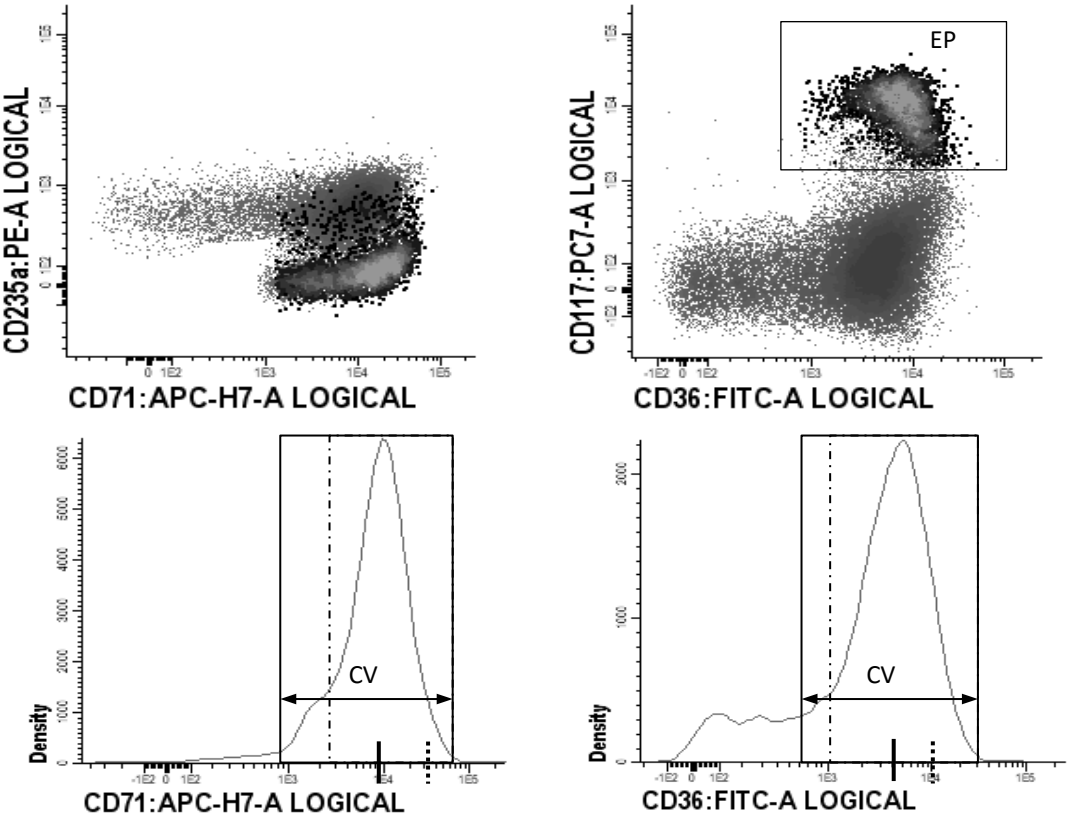
Figure S1 Example of a normal bone marrow (A) and a MDS patient (B)

Figure A provides an example of a healthy control. Figure B provides an example of a MDS patient (RCMD-RS) which has an increased percentage of erythroid progenitors (EP). Furthermore, CD71 expression is longer retained during maturation. This is reflected in the increased CV of CD71 and CD36. Finally, the MDS patient shows a decreased MFI of CD71. This patient scores 4 out of 4 points, revealing clear MDS specific dyserythropoiesis as assessed by FC. The dotted lines plotted in figure B are the normal reference subtracted from figure A.

A


CD235a:PE-A LOGICAL
CD71:APC-H7-A LOGICAL
CD117:PC7-A LOGICAL
CD36:FITC-A LOGICAL
EP
CV
CV
Density
Density
CD71:APC-H7-A LOGICAL
CD36:FITC-A LOGICAL

B


CD235a:PE-A LOGICAL
CD71:APC-H7-A LOGICAL
CD117:PC7-A LOGICAL
CD36:FITC-A LOGICAL
EP
CV
CV
Density
Density
CD71:APC-H7-A LOGICAL
CD36:FITC-A LOGICAL