## SUPPLEMENTARY APPENDIX

## Abnormal Hedgehog pathway in myelodysplastic syndrome and its impact on patients' outcome

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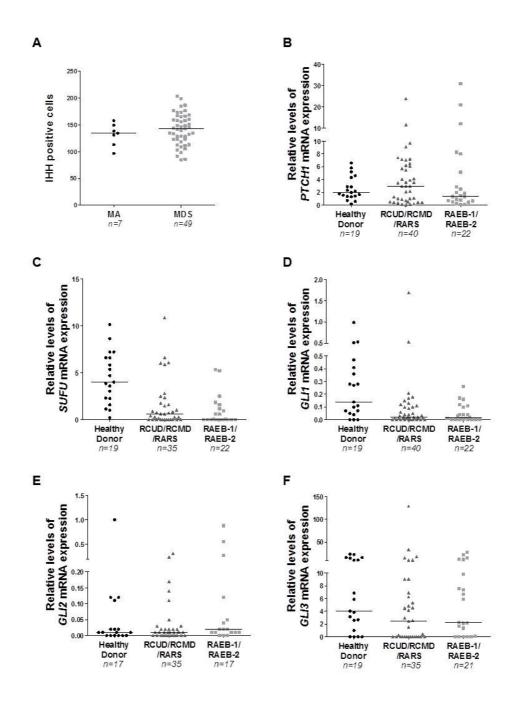
## **SUPLEMENTAL DATA MANUSCRIPT 2015/124040**

## Supplemental Table 1. Multivariate analyses of survival outcomes.

Factor	Event-free survival		Overall survival		AML evolution	
	Hazard Ratio* (95% CI)	P	Hazard Ratio* (95% CI)	P	Hazard Ratio* (95% CI)	P
WHO 2008 classification RAEB-1/RAEB-2 vs RCUD/RCMD/RARS IPSS risk group	3.08 (1.27-7.48)	0.01	3.38 (1.34-8.55)	0.01	7.75 (2.33-25.75)	0.001
Int-2 / High <i>vs</i> Int-1 /Low	2.85 (1.10-7.40)	0.03	3.11 (1.18-8.13)	0.02	-	-

Abbreviations: MDS: myelodysplastic sydromes; AML: acute myeloid leukemia; WHO: World Health Organization; RCUD: Refractory Cytopenia with Unilineage Dysplasia; RCMD: Refractory Cytopenia with Multilineage Dysplasia; RARS: Refractory Anemia with Ringed sideroblasts; RAEB-1: Refractory Cytopenia with Excess Blasts – 1; RAEB-2: Refractory Cytopenia with Excess Blasts – 2 IPSS: International Prognostic Scoring System; Int: intermediate.

<sup>\*</sup> Hazard ratio >1 indicates that the first feature has the poorest outcome.



**Supplemental Figure 1. Hedgehog pathway element expressions in MDS bone marrow.** (A) Immunohistochemical quantification of IHH in bone marrow biopsies from MDS, and from megaloblastic anemia (MA) patients. Graph shows quantitative analysis of IHQ, where random four high-powered fields from stained slides were captured at 20x objective magnification and visualized

for manual scoring for positive cells, using ImageJ (http://imagej.nih.gov/ij/). Horizontal lines indicate medians of positive cells for IHH. Quantitative PCR analysis of (B) PTCH1, (C) SUFU, (D) GL11, (E) GL12 and (F) GL13 mRNA expression in total bone marrow cells from healthy donors and from patients with MDS stratified according to the WHO 2008 classification. Expression of HPRT transcripts was used as an endogenous control. The relative gene expression was calculated using the equation  $2^{-\Delta\Delta CT}$  <sup>17</sup>. Horizontal lines indicate medians. The numbers of individuals are indicated in the graph. ANCOVA followed by post-hoc Tukey. After adjustment for age, P values were >0.05 for all gene expressions shown.