SUPPLEMENTARY INFORMATION

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SYBR-green RT-PCR

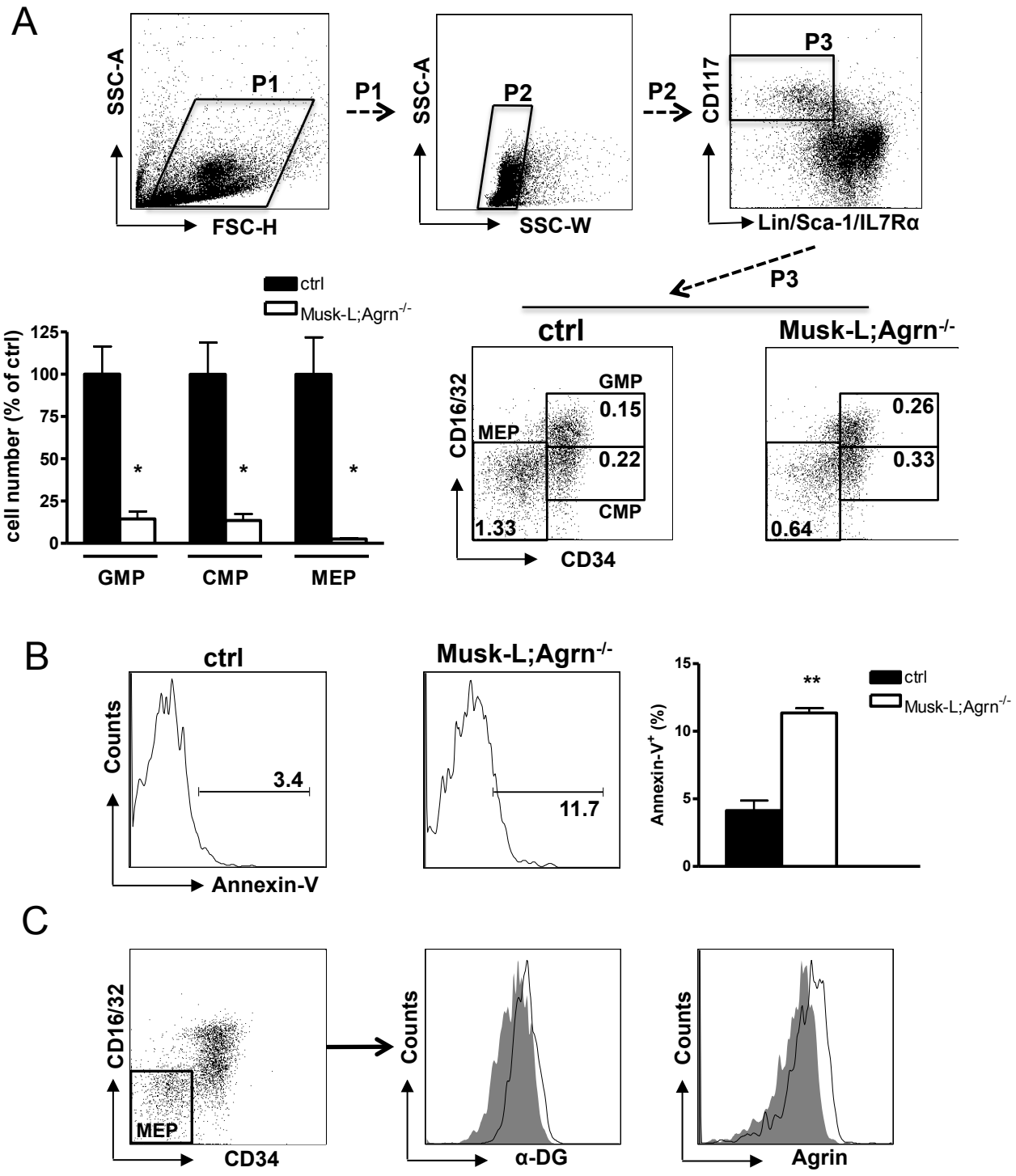
RNA was isolated using the RNeasy Mini Kit (QIAGEN) and digested with TURBO DNA-free™ Kit (Ambion). Complementary DNA was then made from the RNA by using the SuperScript™ III Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions. Primers to detect the expression of Agrin 31/34 (1), GAPDH (1), as well as of α-DG (2) have already described. To quantify the expression of HPGRT, the RTPrimerDB ID:50 primer set from RTPrimerDB was used. Real-time PCR was performed using an ABI 7900HT Fast Real-time PCR system (AB Applied Biosystem) with the SYBR® Green PCR Master Mix system (Applied Biosystems). The comparative threshold cycle method for relative quantification of mRNA expression was performed according to the manufacturer's recommendations.

Supplementary References

1. Hausser HJ, Ruegg MA, Brenner RE, Ksiazek I. Agrin is highly expressed by chondrocytes and is required for normal growth. Histochem Cell Biol 2007, 127:363-374.
2. Zhang J, Wang Y, Chu Y, Su L, Gong Y, Zhang R, et al.. Agrin is involved in lymphocytes activation that is mediated by α-dystroglycan. FASEB J. 2006;20:50–58.
3. Chen K, Liu J, Heck S, Chasis JA, An X, Mohandas N. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. Proc Natl Acad Sci U S A. 2009 Oct 13;106(41):17413-8.
4. Porpiglia E, Hidalgo D, Koulnis M, Tzafriri AR, Socolovsky M. Stat5 signaling specifies basal versus stress erythropoietic responses through distinct binary and graded dynamic modalities. PLoS Biol. 2012 Aug;10(8):e1001383.
5. Hu J, Liu J, Xue F, Halverson G, Reid M, Guo A, et al. Isolation and functional characterization of human erythroblasts at distinct stages: implications for understanding of normal and disordered erythropoiesis in vivo. Blood. 2013 Apr 18;121(16):3246-53.

Supplementary Figures

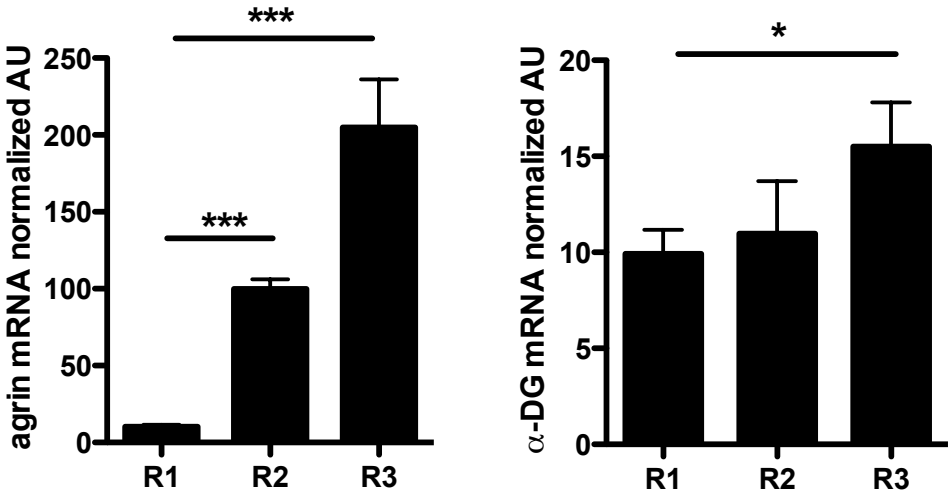
Supplementary Figure 1



Supplementary Figure 1

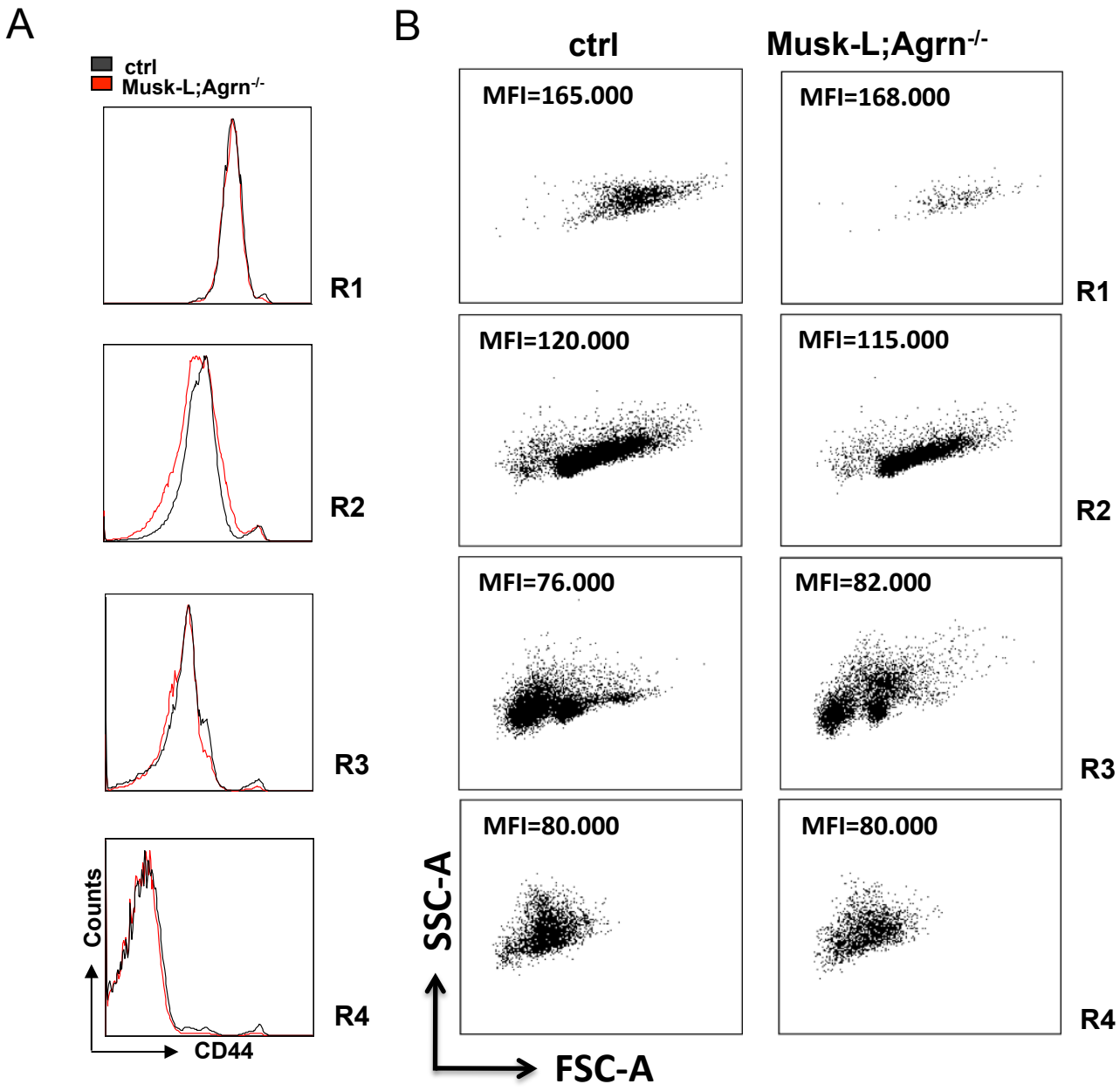
(A) Representative flow cytometric analysis of the bone marrow of control and agrin-deficient mice. After morphological gating (P1) and duplets discrimination (P2), $Lin^-/Sca\text{-}1^-/IL7R^-/CD117^+$ cells were gated (P3). Finally (right) $CD34^{+/low}/CD16/32^{int}$ (CMP), $CD34^+/CD16/32^+$ (GMP) and $CD34^-/CD16/32^{low}$ (MEP) subpopulations were gated. Frequency value of each subpopulation is referred to total bone marrow cells. Left: mean numbers relative to controls are shown (ctrl, n=15; Musk-L;$Agrn^{-/-}$, n=8) (B) Annexin-V analysis of MEP from the bone marrow of control and agrin-deficient mice (n=3). (C) Representative flow cytometry analysis of agrin and α-DG surface expression on MEP progenitors from wild-type bone marrows (the grey solid curve represents the Fluorescence Minus One plus isotype control). In all panels, error bars represent s.e.m and *P ≤0,05, **P ≤0,01

Supplementary Figure 2



Supplementary Figure 2
Real time PCR analysis of agrin 31/34 (alternative splicing of exons 32 and 33, z-site), and α-DG receptor in sorted R1-R3 subsets from control P5 spleen (n=3). Data are shown as Arbitrary Units (AU). The expression was normalized to the gene encoding glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and hypoxanthine-guanine phosphoribosyltransferase (HPGRT). In all panels, error bars represent s.e.m.

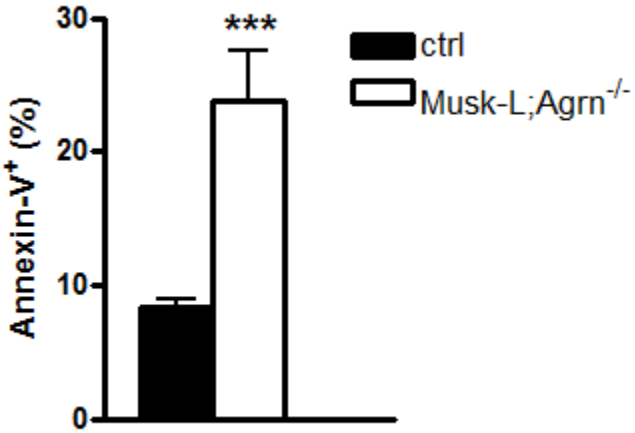
Supplementary Figure 3



Supplementary Figure 3

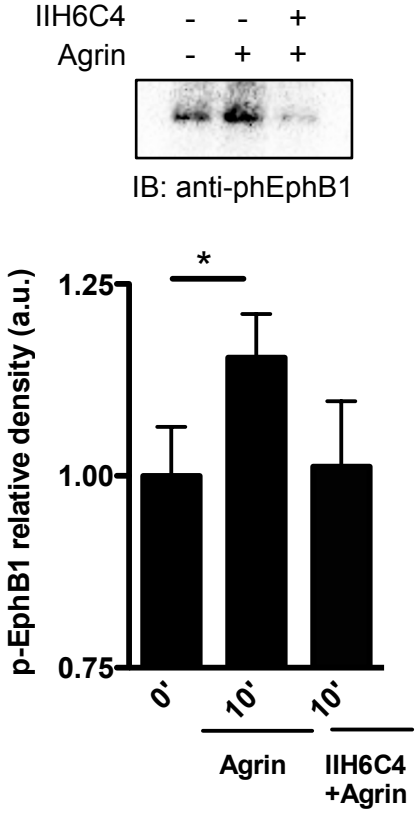
(**A**) Flow cytometric analysis of the CD44 transmembrane protein, as marker for discrimination of erythroblasts at different stages of maturation (3), showed similar expression levels in agrin-deficient and control erythroid subsets (R1-R4). (**B**) Flow cytometric analysis of MFI value of forward-scatter parameter (FCS-A) (4,5) in the R1-R4 erythroid subsets; no relevant morphological alterations both in earlier (R1 and R2) larger cells and in the more differentiated (R3 and R4) ones were detected in agrin-deficient mice, suggesting, together with the results obtained through the analysis of the erythroid markers, that the altered differentiation process in agrin-deficient mice is not accompanied by major phenotypic or morphological alterations.

Supplementary Figure 4



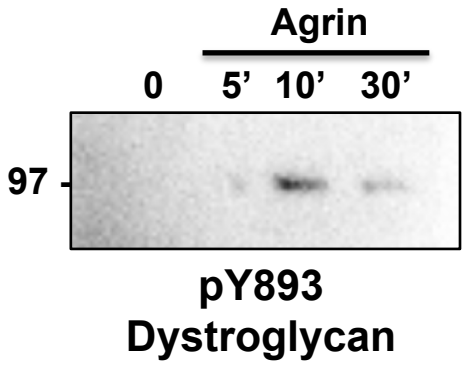
Supplementary Figure 4
Sorted R2 erythroid cells were cultured with control F4/80^{+}cells. Percentage of annexin V^{+} cells was measured by flow cytometry 2 days later. Data are shown as means (± s.e.m.); n=3; ***P<0.001.

Supplementary Figure 5



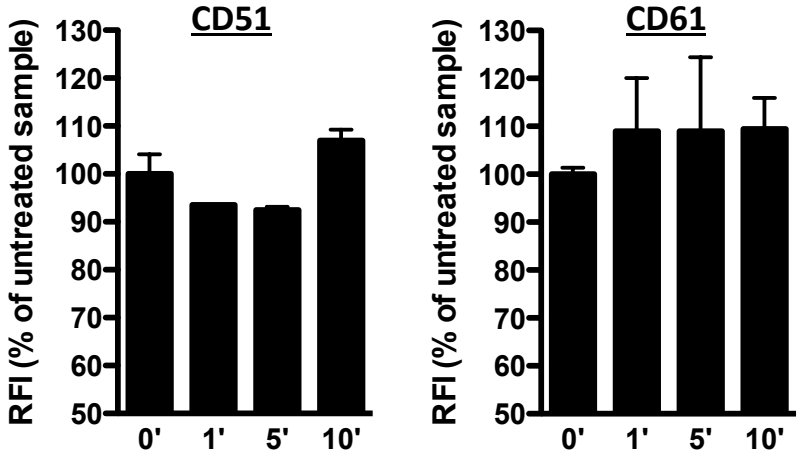
Supplementary Figure 5
Representative western blot analysis of phosphorylated Eph-B1 expression in primary sorted R2 cells pre-incubated with IIH6C4 (10µg/ml) and agrin (10µg/ml, 10 minutes).

Supplementary Figure 6



Supplementary Figure 6
Representative western blot analysis of the total expression of phosphorylated β-DG in K562 human erythromyeloblastoid leukemia cells treated with agrin (10μg/ml, 5-10-30 minutes).

Supplementary Figure 7



Supplementary Figure 7
Flow cytometry analysis of CD51 and CD61 membrane expression on the R2 sorted primary erythroid subset from the spleen of P5 ctrl mice stimulated for the indicated time points with agrin (10 µg/ml). (n=3 for CD51; n=6 for CD61). Results are reported as fold increase of the MFI corresponding to untreated samples. In all panels, error bars represent s.e.m.