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**Supplemental information**

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blood cell deformability**

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Supplementary Information for

## $\alpha$ I-spectrin represents evolutionary optimization of spectrin for red blood cell deformability.

### **Authors**

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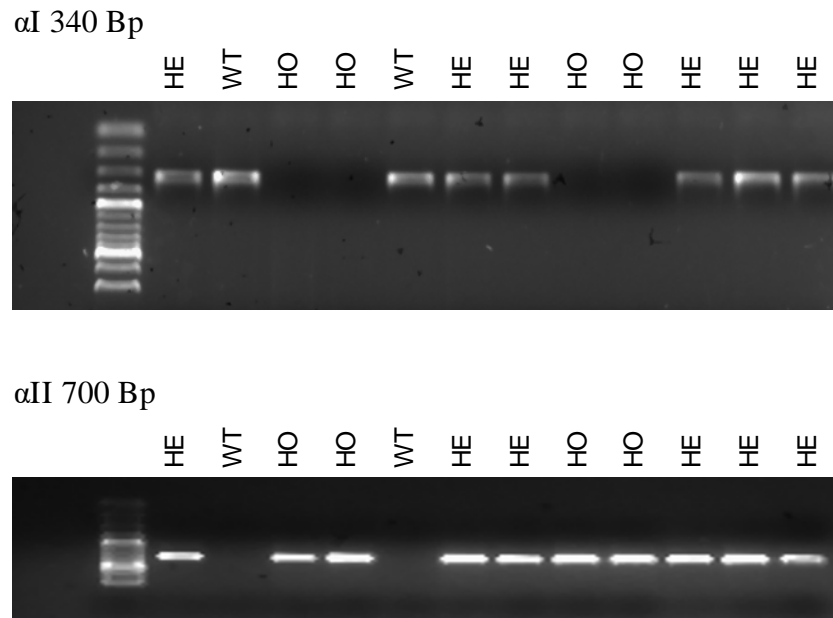
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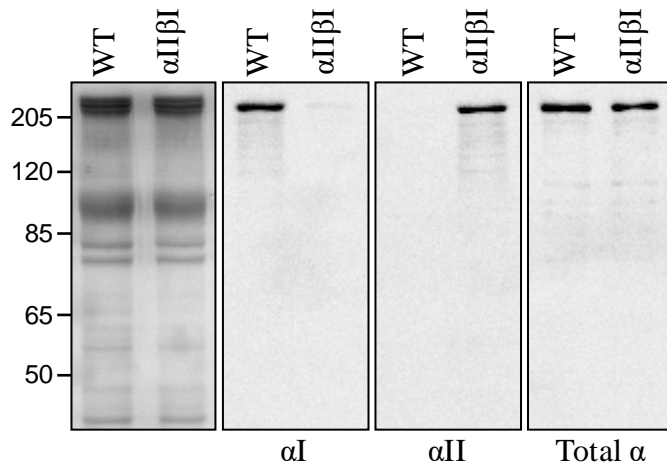
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**Figure S1.** Genotyping by PCR of litter mates: (WT) wild-type mice, (HE) heterozygous and (HO) homozygous knock-in mice. 340-bp indicates presence of  $\alpha$ I-spectrin and 700-bp indicates presence  $\alpha$ II-spectrin.



**Figure S2.** SDS gel electrophoresis and immunoblot analysis of WT and knock-in red cell membranes. Equal protein loadings of ghosts from either WT or  $\alpha II\beta I$  were separated by SDS gel electrophoresis and either stained with Coomassie brilliant blue (left panel) or transferred to PVDF and probed with antibodies specific for  $\alpha I$ ,  $\alpha II$  or total  $\alpha$ -spectrin as indicated. The positions of standard marker proteins (kDa) are indicated on the left edge.

	WT (n=9)	$\alpha$ II $\beta$ I (n=7)
WBC (x10 <sup>3</sup> / $\mu$ l)	7.76 $\pm$ 1.57	7.07 $\pm$ 3.45
RBC (x 10 <sup>6</sup> / $\mu$ l)	9.93 $\pm$ 0.36	8.69 $\pm$ 0.21 ***
HGB (g/dl)	14.28 $\pm$ 0.49	12.9 $\pm$ 0.41 ***
HCT (%)	44.92 $\pm$ 1.81	40 $\pm$ 0.96 ***
MCV (fl)	45.32 $\pm$ 0.73	46 $\pm$ 0.43 *
MCH (pg)	14.38 $\pm$ 0.17	14.84 $\pm$ 0.16 ***
MCHC (g/dl)	31.77 $\pm$ 0.5	32.26 $\pm$ 0.5
CHCM (g/dl)	30.81 $\pm$ 0.32	31.27 $\pm$ 0.71
CH (pg)	13.93 $\pm$ 0.15	14.39 $\pm$ 0.23 **
RDW (%)	13.46 $\pm$ 0.99	13.03 $\pm$ 0.31
HDW (%)	1.81 $\pm$ 0.13	1.94 $\pm$ 0.05 *
PLT (x 10 <sup>3</sup> / $\mu$ l)	1217.56 $\pm$ 216.16	1233.57 $\pm$ 273.23
MPV (fl)	7.08 $\pm$ 0.73	7.03 $\pm$ 0.96
Retic (%)	3.17 $\pm$ 0.36	4.29 $\pm$ 0.58 **

**Table S1.** The hematological parameters of WT (n=9 mice) and  $\alpha$ II $\beta$ I (n=7 mice) red blood cell samples as measured by AVIDA 120. WBC indicates white blood cell count; RBC red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; CHCM, corpuscular Hemoglobin concentration mean; CH, corpuscular hemoglobin content; RDW, red cell distribution width; HDW, hemoglobin distribution width; PLT, platelet count; MPV, mean platelet volume; Retic, reticulocyte count. Data is expressed as the mean  $\pm$  SD (standard deviation), P  $\leq$  0.05, \*\* P  $\leq$  0.01, \*\*\* P  $\leq$  0.001).

allbl-FIMD.avi

**Movie S1 (separate file).** Fluorescence imaged micro-deformation: Video of the captured image sequence of the micropipette aspirated  $\alpha$ II $\beta$ I RBC as the buffer is exchanged adjusting the RBC volume and thereby changing the aspiration tongue length.