

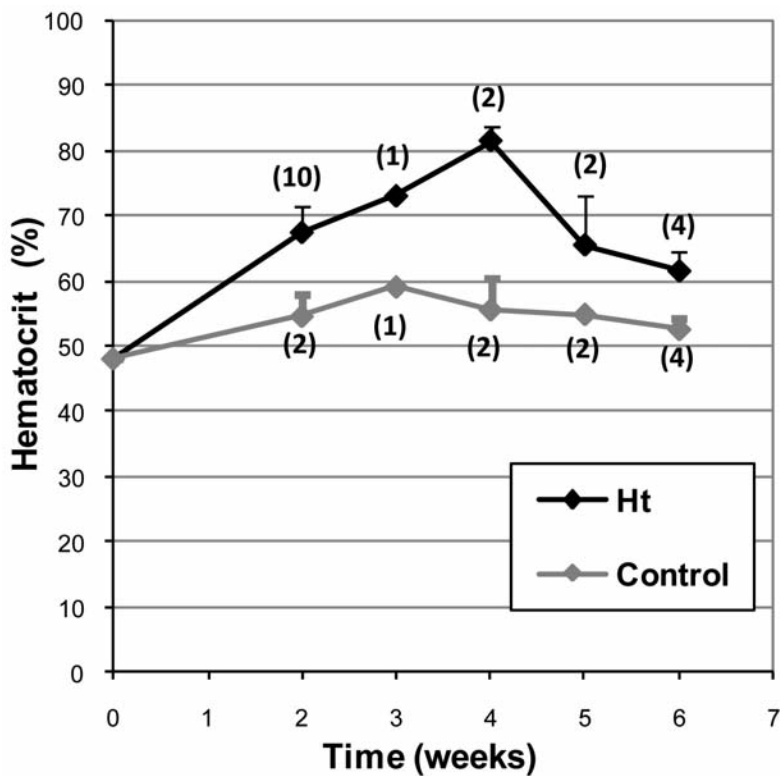
## Physiologically aged red blood cells undergo erythrophagocytosis *in vivo* but not *in vitro*

Yehonatan Gottlieb,<sup>1\*</sup> Orit Topaz,<sup>1\*</sup> Lyora A. Cohen,<sup>1</sup> Liat David Yakov,<sup>1</sup> Tom Haber,<sup>2</sup> Abigail Morgenstern,<sup>1</sup> Avital Weiss,<sup>1</sup> Karen Chait Berman,<sup>1</sup> Eitan Fibach,<sup>3</sup> and Esther G. Meyron-Holtz<sup>1</sup>

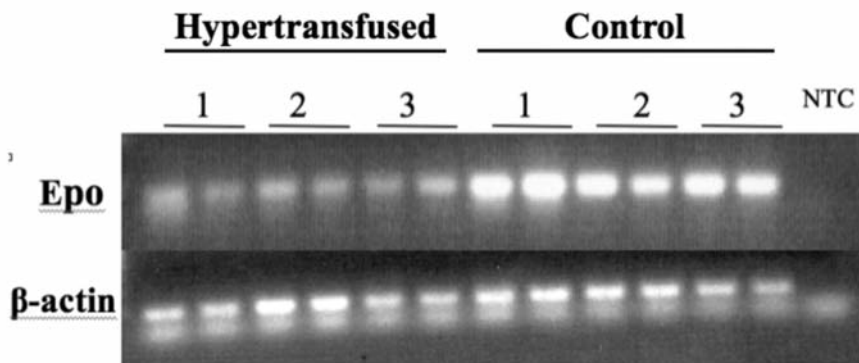
<sup>1</sup>Laboratory for Molecular Nutrition, Faculty of Biotechnology and Food Engineering, Technion, Technion City, Haifa, Israel; <sup>2</sup>Laboratory For Cancer Drug Delivery & Mammalian Cell Technology, Faculty of Biotechnology and Food Engineering, Technion, Technion City, Haifa, Israel, and <sup>3</sup>Hematology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Citation: Gottlieb Y, Topaz O, Cohen LA, Yakov LD, Haber T, Morgenstern A, Weiss A, Chait Berman K, Fibach E, and Meyron-Holtz EG. Physiologically aged red blood cells undergo erythrophagocytosis *in vivo* but not *in vitro*. *Haematologica* 2012;97(7):994-1002. doi:10.3324/haematol.2011.057620

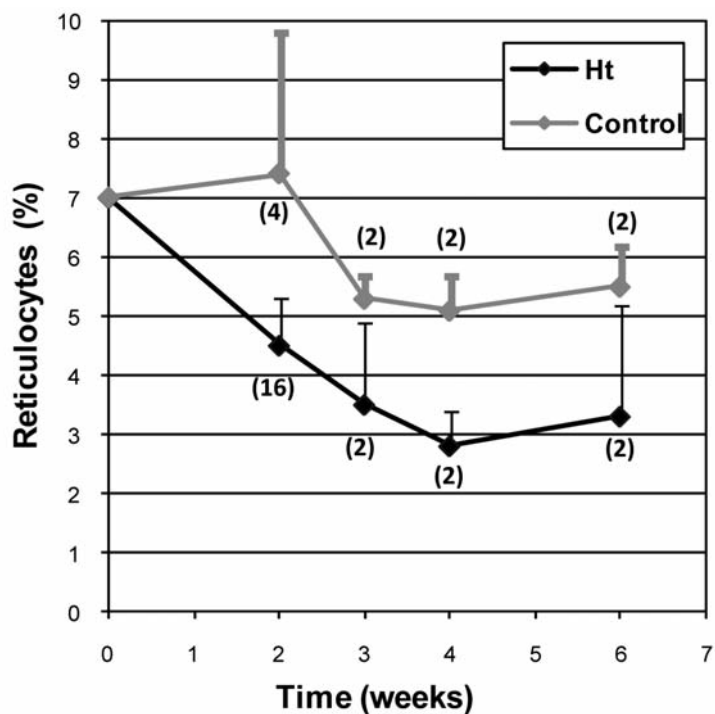
\* YG and OT contributed equally to this manuscript



Online Supplementary Figure S1. Hematocrit is elevated by hypertransfusion. Blood was drawn at the indicated times and hematocrit determined in untreated (control) and hypertransfused (ht)-mice.



**Online Supplementary Figure S2.** Down-regulation of erythropoietin (Epo) expression in hypertransfused mice 2 weeks after hypertransfusion. Two kidneys each of three mice hypertransfused with 3.2 mL blood and of three untreated mice were analyzed for epo mRNA levels by semi-quantitative polymerase chain reaction. For epo, 35 cycles were found to be in the linear amplification range and for  $\beta$ -actin 22 cycles were used. EtBr-stained, 2% agarose gels are shown. Epo in the upper gel and  $\beta$ -actin in the lower gel. No template control (NTC) was examined in the last well as a negative control.



**Online Supplementary Figure S3.** Reticulocyte count is decreased by hypertransfusion. Blood was drawn at indicated times and reticulocytes determined in untreated (control) and hypertransfused (ht)-mice. Six weeks after initial hypertransfusion, the reticulocyte count was  $3.3\% \pm 1.9$  in ht-mice and  $5.5\% \pm 0.7$  in control-mice (n=2).