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Electronic Supplementary Material

Delayed Hemoglobin Switching and Perinatal Neocytolysis in Mice with Gain-of-Function Erythropoietin Receptor.

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Supplementary Material

Real-time PCR analysis of globin genes

PB was washed with cold PBS once and then lysed by Trizol (Molecular Research Center Inc; Cincinnati, OH). Total RNA was isolated and cDNA was synthesized using SuperScript II kit (Invitrogen; Carlsbad, CA). Expression of embryonic and adult globin genes was measured by real-time PCR (qRT-PCR) using SYBR green dye. Specific primer sets for each globin gene and for control 18S rRNA gene were as follows:

εy 5'-CTC TAG CTG TCC AGC AAT CCT G-3' and 5'-GCT TTC AAG GAA CAG TCC AGT ATT C-3';

βH1 5'-AGT TTG GAA ACC TCT CTT CTG CCC TG-3' and 5'-TGT TCT TAA CCC CCA AGC CCA AG-3';

β1 5'-GCT CTT GCC TGT GAA CAA TG -3' and 5'-GTC AGA AGA CAG ATT TTC AAA TG-3';

β2 5'-GCC CCT TTT CTG CTA TTG TCT A-3' and 5'-GAT AAA AGC TAG ATG CCC AAA GG-3';

α1/α2 5'-TGC TCT CTG GGG AAG ACA AAA G-3' and 5'-GGC TTC AGC TCC ATA TTC AGC AC-3';

ζ 5'-CCG CCA CGA CCC CCA TGA CC-3' and 5'-AAA GAC CTG AGG GAG GGT TCA AT-3';

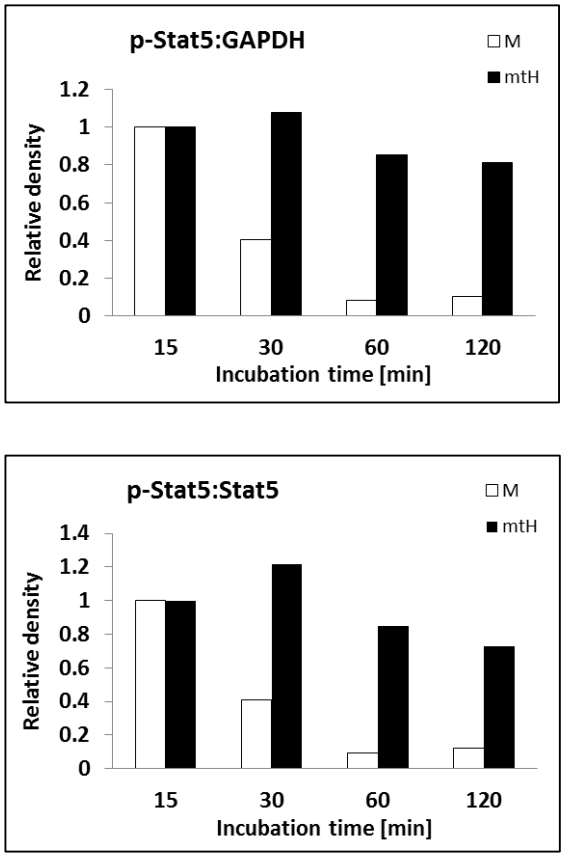
18S 5'-TTG ACG GAA GGG CAC CAC CAG-3' and 5'-GCA CCA CCA CCC ACG GAA TCG-3'.

Real-time PCR analysis of Epo transcripts

Total RNA from liver and kidney was isolated using RNeasy Mini Kit (QIAGEN, Valencia, CA) and reverse-transcribed by SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen), according to manufacturer's instructions. Transcript levels of Epo were measured by qRT-

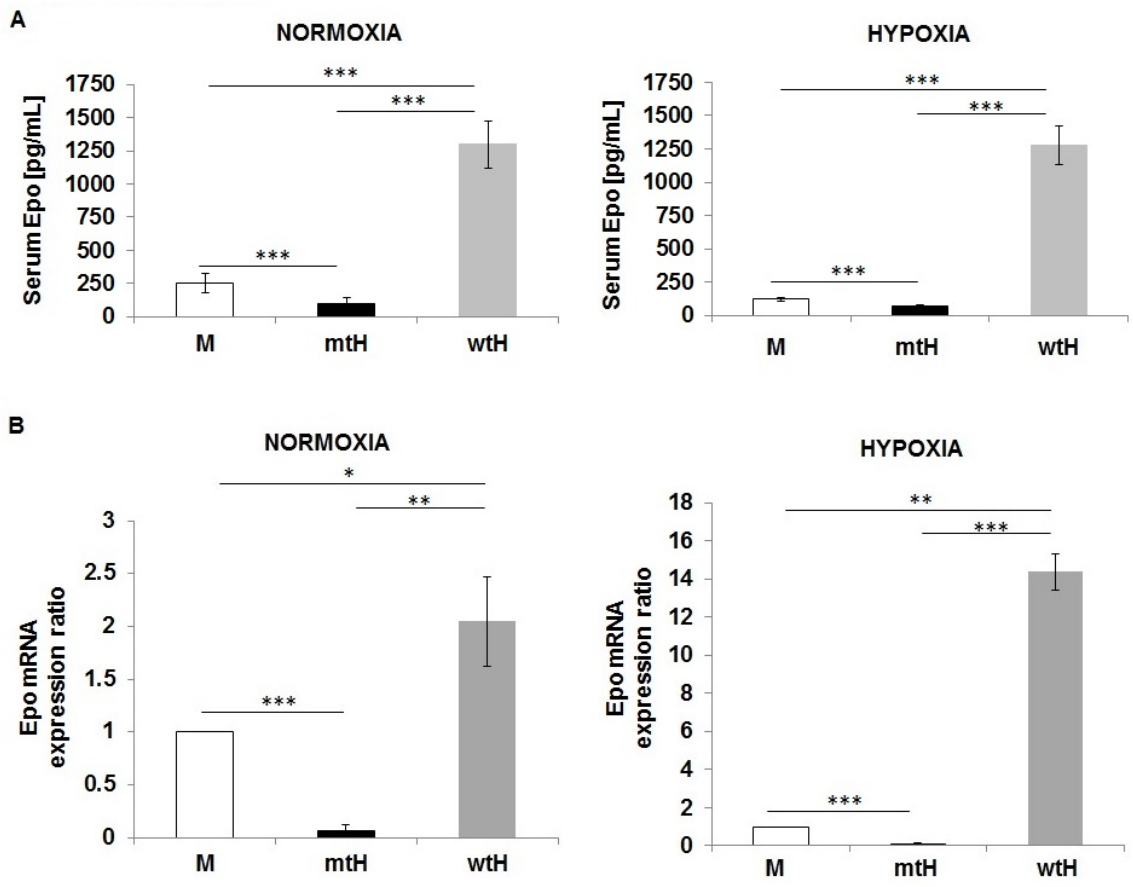
PCR using Epo specific Taqman probe, Mm01202755_m1. The data were normalized to the expression of beta-actin (Actb; 4352341E) and to mRNA levels of wild-type mouse control (mEpoR).

Supplementary Figure 1.



Supplementary Figure 1. Relative quantification of Stat5 phosphorylation for mtH*EPOR* (mtH) and m*EpoR* (M) FLCs. The relative quantification of gel bands from immunoblot analyses shown on Figure 4B was performed by ImageJ software (http://imagej.nih.gov/ij/). Each bar represents the ratio of the density of phosphorylated Stat5 (p-Stat5) to the density of loading control (GAPDH, top graph or total Stat5, bottom graph) and is presented as fold change against the 15 min time-point.

Supplementary Figure 2.



Supplementary Figure 2. The trends in the differences in the Epo levels between individual genotypes are consistent in normoxia and hypoxia. A) Serum Epo levels are highest in wtH*EPOR* (wtH) mice and lowest in mtH*EPOR* (mtH) mice in both normoxic and hypoxic conditions. Epo level increase in wtH*EPOR* mice compared to control m*EpoR* mice (M) is higher in hypoxic conditions (10x) than in normoxia (5x). On the other hand the reduction in serum Epo in mt*HEPOR* mice compared to m*EpoR* mice (M) is greater in normoxia (2.6-times) than in hypoxia (1.7-times). **B)** The differences in serum Epo levels were paralleled by differences in *Epo* mRNA expression in the kidney; the highest in wtH mice and the lowest in mtH mice in both normoxic and hypoxic conditions. The results were normalized to the expression of beta-actin and to mRNA levels of wild-type mouse control (M) using REST© 2009 software. M mice, n = 6 for normoxia and hypoxia; mtH mice, n = 3 for normoxia and n = 7 for hypoxia; wtH mice, n = 4 for normoxia and n = 6 for hypoxia. ***P<0.001, **P<0.01, *P<0.05.

Note: the analyses were separately performed using mice housed in two different institutions

- Olomouc, Czech Republic (the “normoxia” measurements, left panels of the figure) and in SLC, USA (the “hypoxia” measurements, right panels). Because the measured parameters could be influenced by the differences in the altitude of the two laboratories (Olomouc 230 m versus SLC 1300 m elevation above sea level), the graphs compare serum Epo levels and *Epo* mRNA expression between individual genotypes in normoxia and hypoxia separately.