

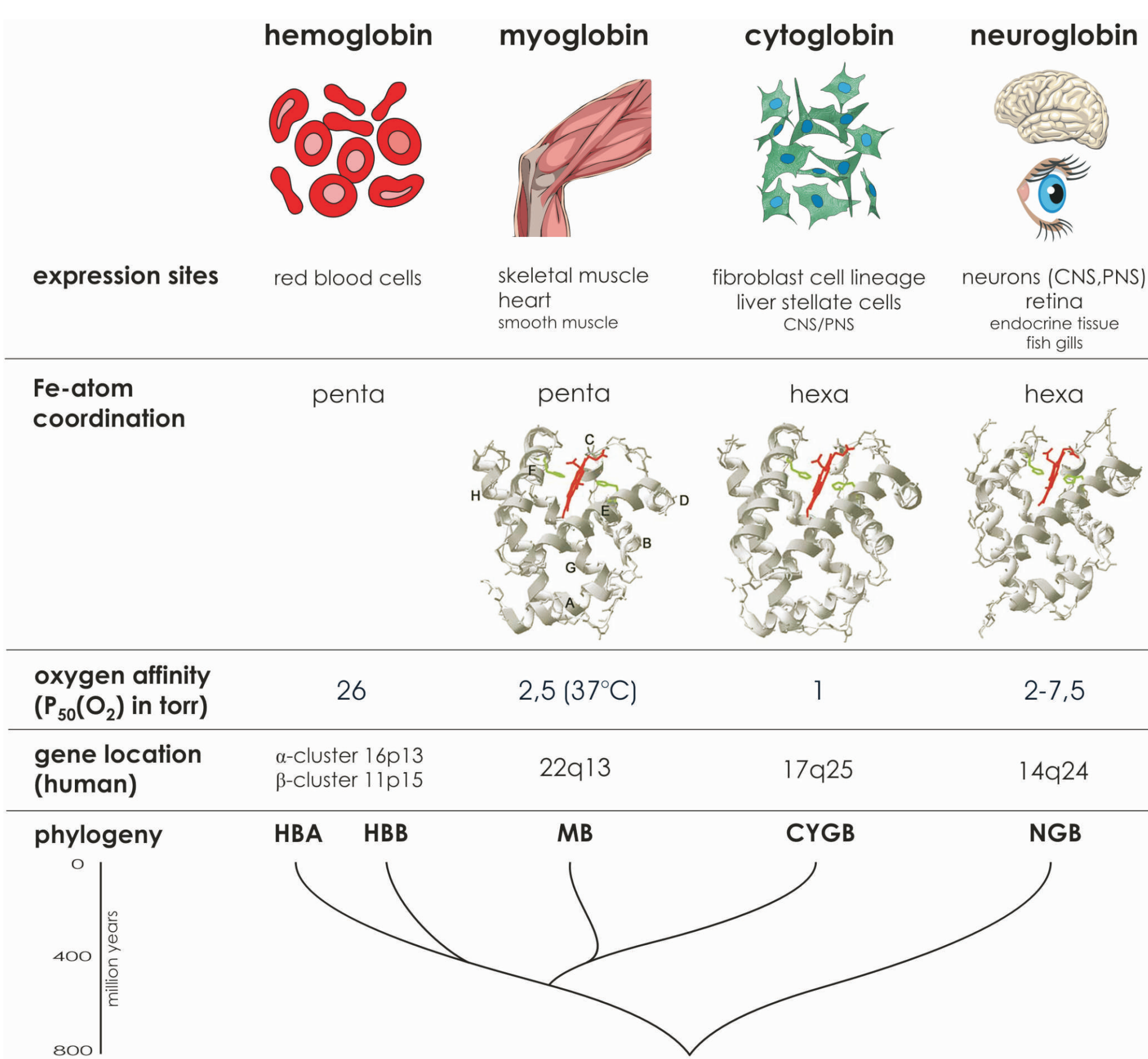


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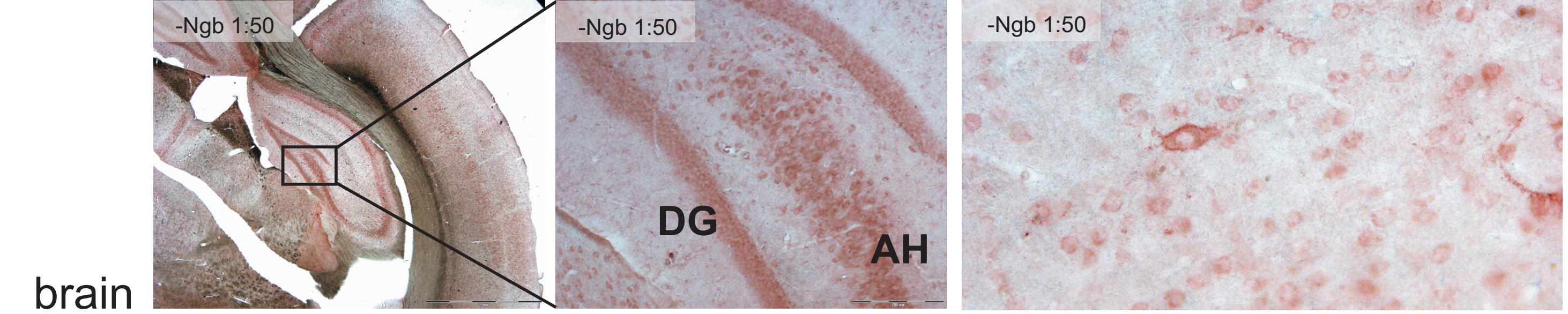
Introduction



Neuroglobin (Ngb) and Cytoglobin (Cygb) are two recently discovered O₂ binding proteins [1,2]. Ngb is primarily expressed in neurons of the CNS and PNS. Cygb is found in fibroblasts and related cells and in distinct neurons. The physiological function of both globins is discussed in terms of O₂ supply, ROS scavenging, NO detoxification and other mechanisms [3]. Here we investigate the expression of Ngb, Cygb and Myoglobin (Mb) in the blind mole rat *Spalax ehrenbergi* and rat. We compare the expression levels at normoxia and under different hypoxic conditions. Among mammals, *Spalax* is a unique model of hypoxia tolerance [4,5]. It lives in underground burrows and survives prolonged times of severe hypoxia (11h of 3% oxygen) without neurological damage. In Israel, there are four different chromosomal species, which differ slightly in their hypoxia tolerance. *S. galili* (2n=52), which lives in flooding areas, is the most tolerant species, whereas *S. judaei* (2n=60) is slightly more sensitive towards oxygen deprivation. In contrast, rats are near death after 4h of 3% oxygen [6], representing the majority of non-hypoxia-adapted mammals.

Results

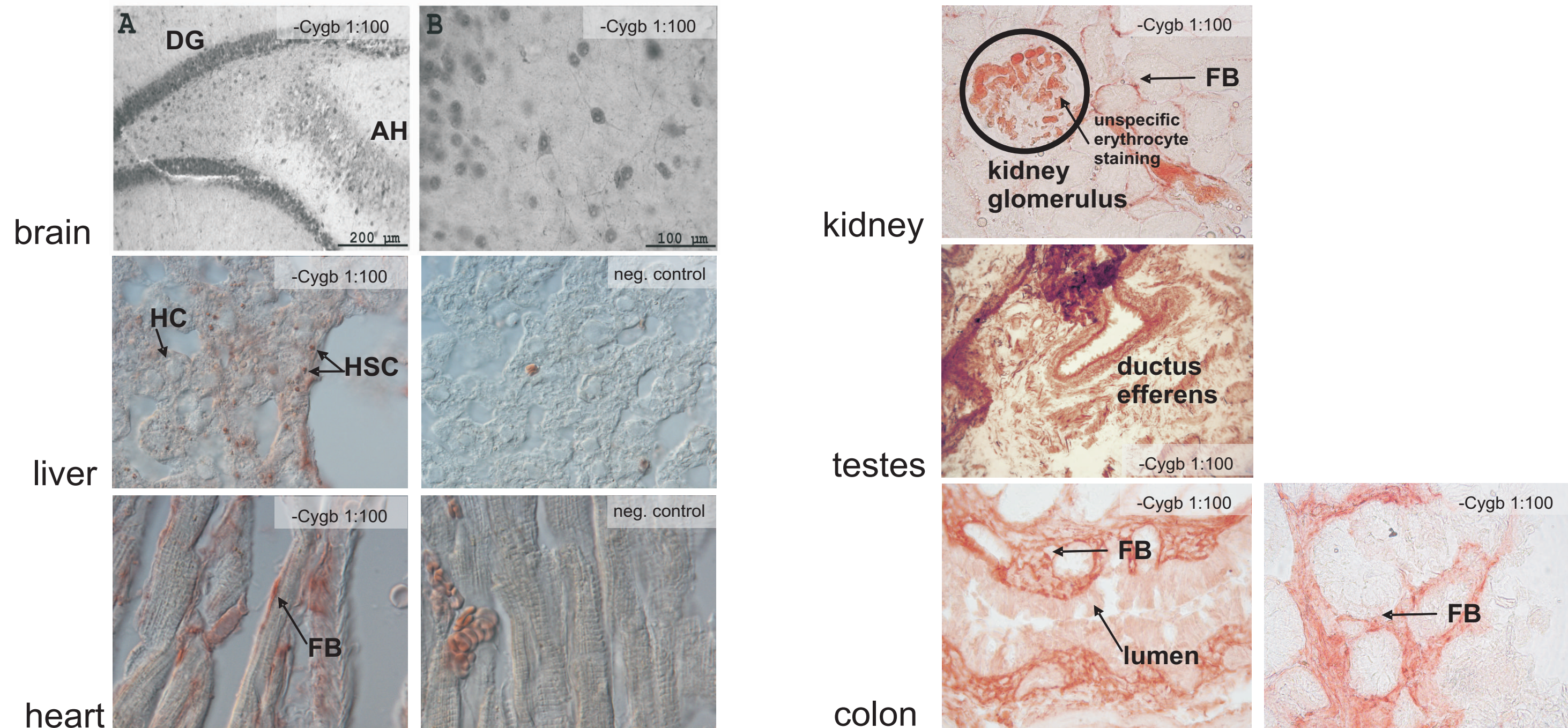
Expression analysis of Ngb protein in *Spalax* (2n=54) brain tissue



Ngb expression is restricted to neuronal cells, e.g. in the hippocampal area including Ammon's horn (AH) and dentate gyrus (DG) (see right). We observe a typical cytoplasmic immunostaining signal including cellular processes. No differences between *Spalax* and *Rattus* were observed in Ngb protein or mRNA expression patterns.

Expression analysis of Cygb in different tissues

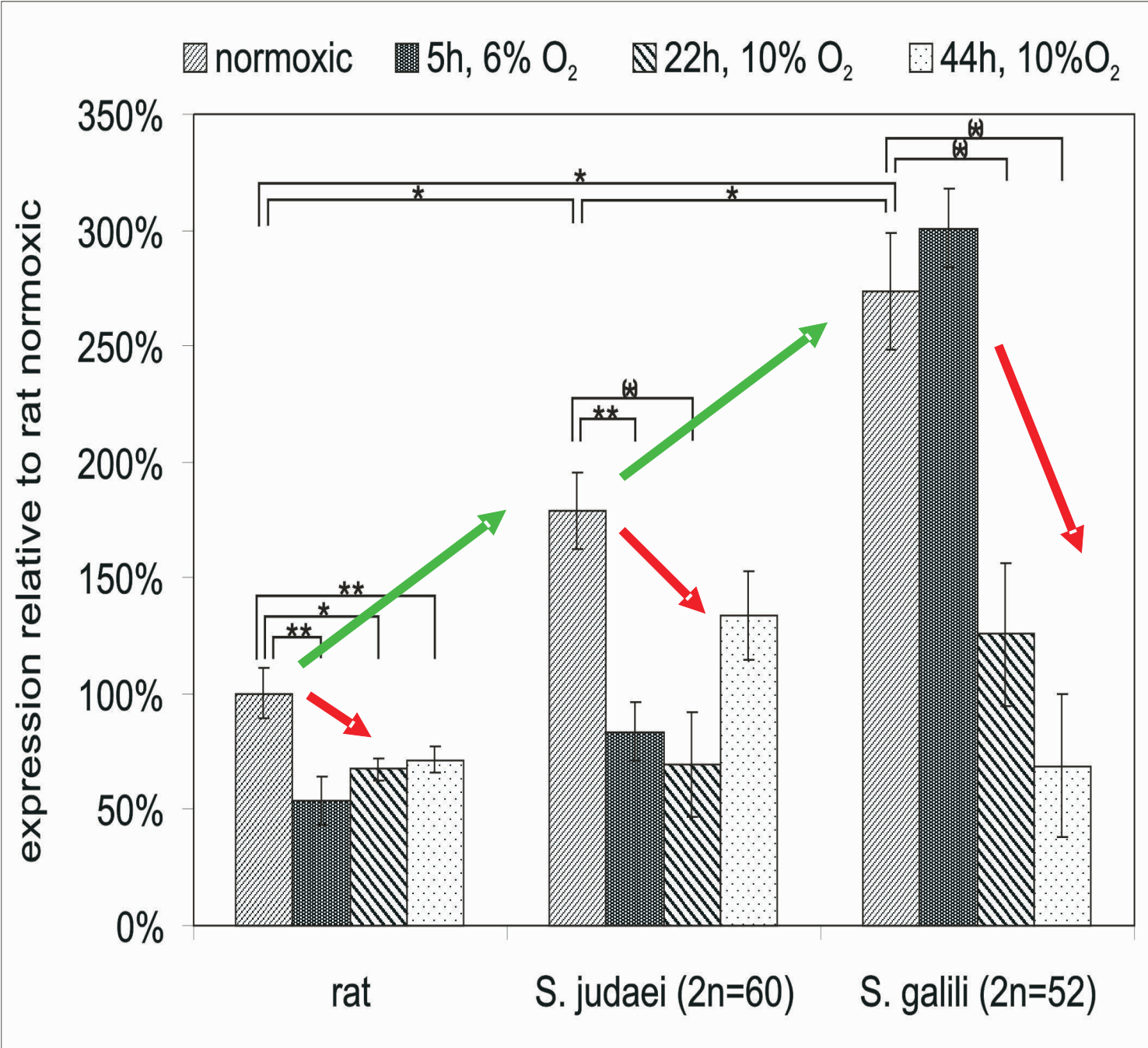
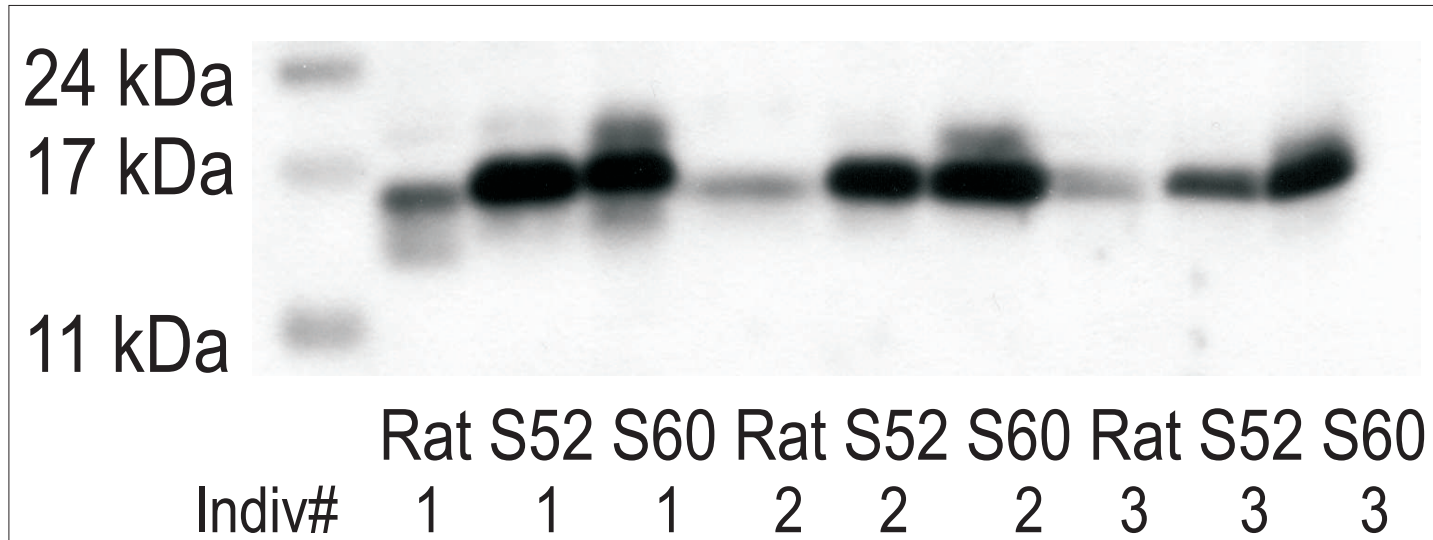
Cygb is present in fibroblasts (FB) of the heart, kidney and colon. We also find Cygb staining in neuronal cells of the brain (AH and DG) and hepatic stellate cells of the liver. This cellular expression pattern is the same in *Spalax* and other mammals.



Expression analysis of Ngb mRNA and Protein

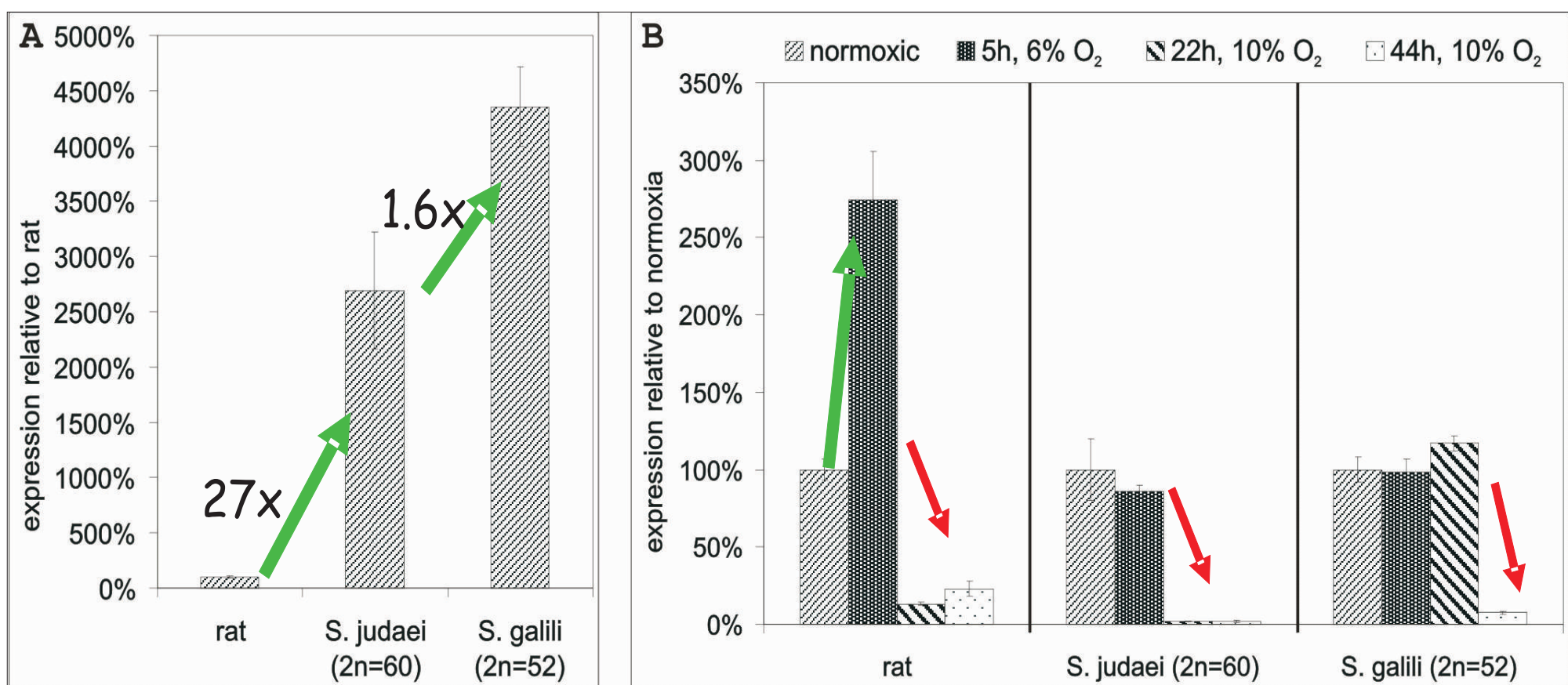
Spalax constitutively expresses 1.8-2.7 fold more Ngb mRNA (realtime RT-PCR; *p<0.05) than rat under normoxic conditions and several fold more Ngb protein (Western Blot).

Under hypoxia, Ngb mRNA is down-regulated by approximately 2-fold (realtime RT-PCR). Significant down-regulation occurs in all species ((*)p<0.1; *p<0.05; p<0.01). The most hypoxia resistant species *S. galili* (2n=52) does not yet react by down-regulation after 5h of 6% oxygen.



Expression analysis of Mb mRNA

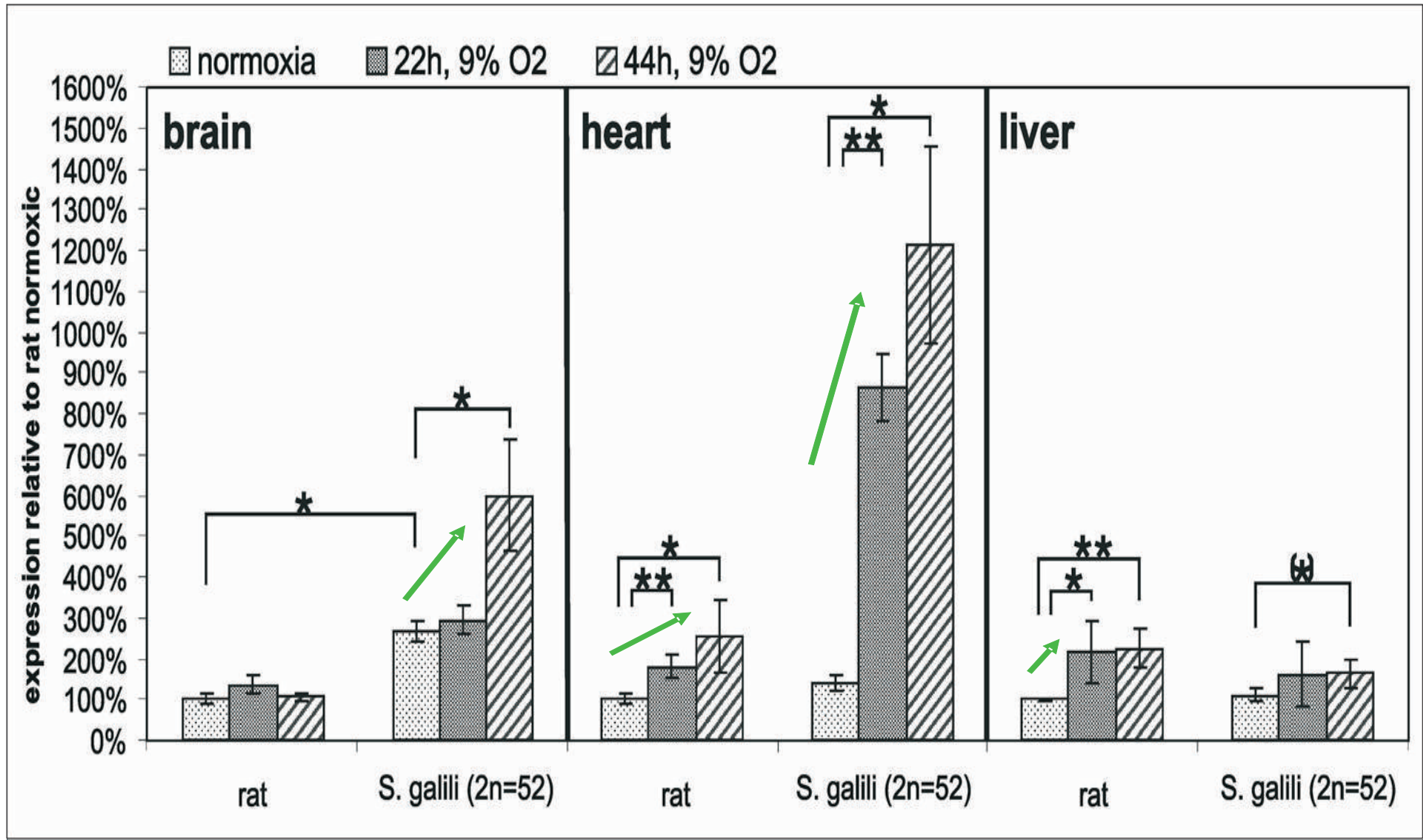
Mb is constitutively more highly expressed (27-43 fold) in neck muscle of both *Spalax* species as compared to rat. Under short, severe hypoxia (5h, 6% oxygen) the expression rises 2.7-fold in rat. Mb expression does not change in *Spalax* under these conditions. Mild, long-time hypoxia leads to a down-regulation of Mb mRNA.



Expression analysis of Cygb mRNA

While Cygb expression levels are similar in rat and *Spalax* heart and liver, the brain of *Spalax* harbors near 3 times more Cygb mRNA than the rat brain.

In *Spalax* but not rat, we observe a higher Cygb level in brain after 44h of 9% oxygen. Cygb is also up-regulated in heart of rat and *Spalax*, but in *Spalax* to a much higher extent.



Discussion

The data suggest that Ngb and Mb contribute to a pre-adaptation of *Spalax* towards a lack of O₂ by sharing several fold stronger expression under normoxia. After extended periods of hypoxia, both globins are down-regulated at the mRNA level in *Spalax* and rat, showing that an increase in O₂ binding capacity is possibly not advantageous in these mammals. The largely parallel pattern of gene regulation of Ngb and Mb suggests similar cellular functions, possibly in O₂ supply and/or the detoxification of NO.

Cygb shows an augmented normoxic expression in *Spalax* vs. rat only in brain tissue, but not in heart and liver. Hypoxia specifically induces Cygb in heart and liver of both mammals, with the most pronounced response (12x up) in *Spalax* heart. The data indicate that Cygb possibly fulfils different roles in fibroblast-like cells than in neurons.

Literature

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