# Supplemental Information

# Thrombopoietin induces production of nucleated thrombocytes from liver cells in *Xenopus laevis*

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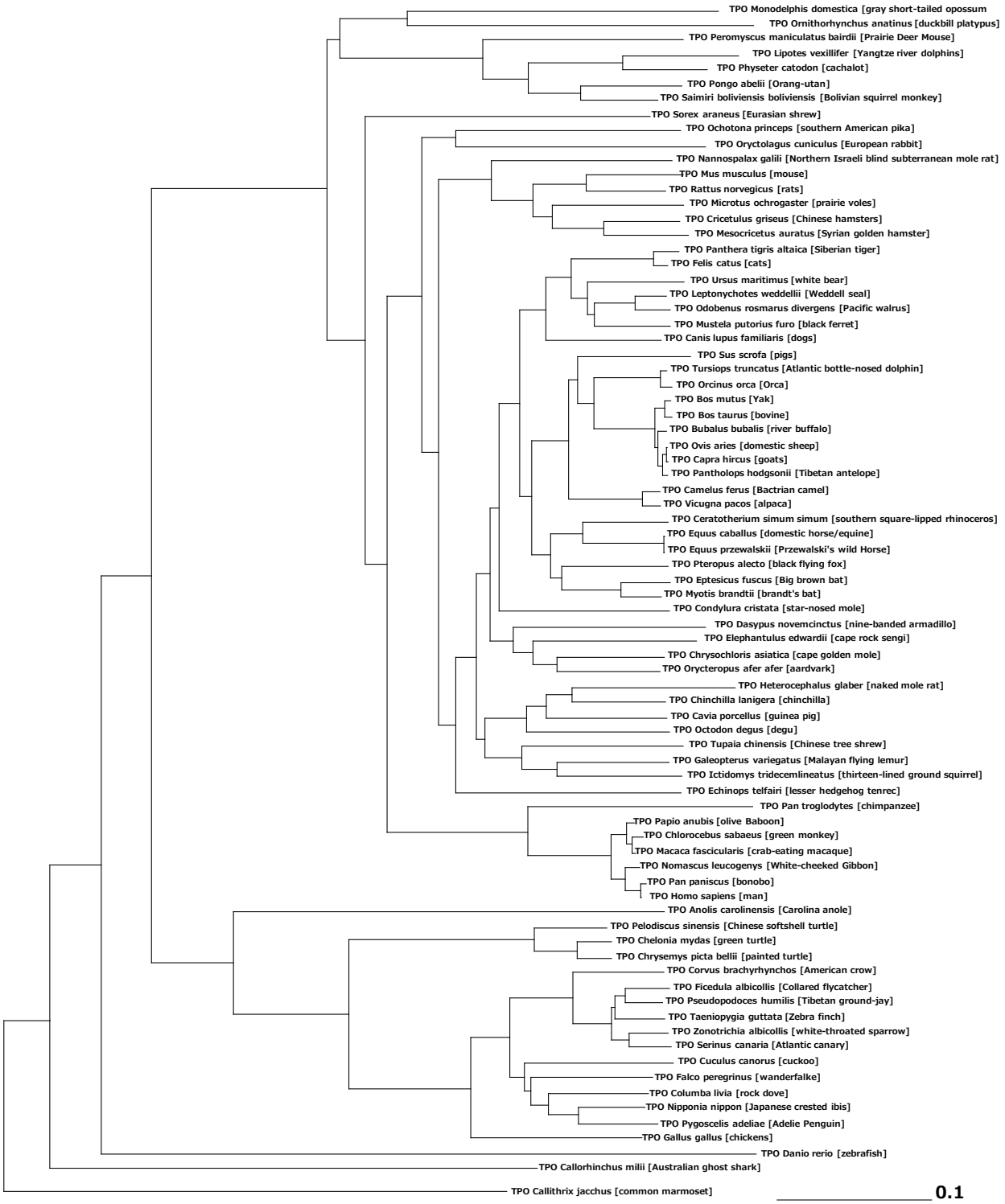
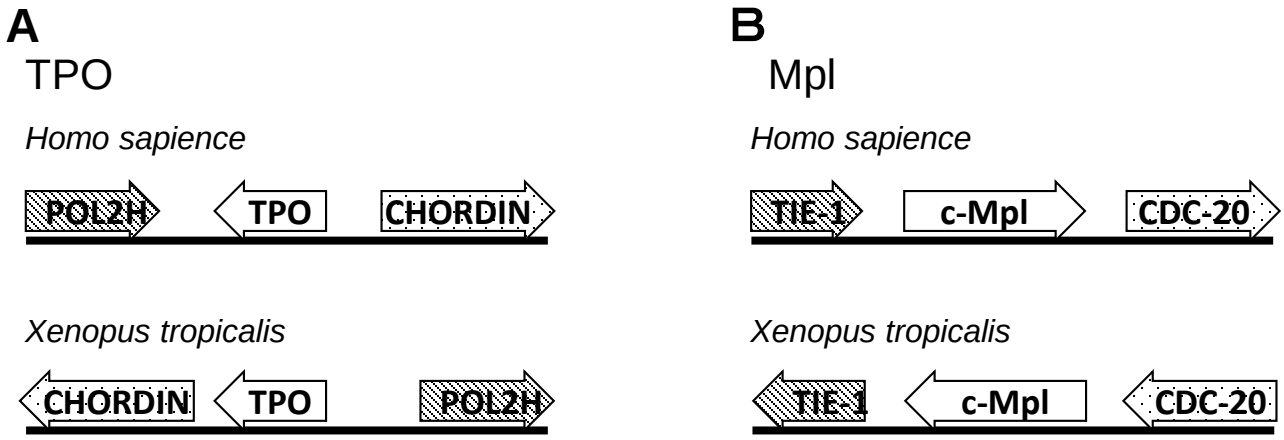
# Supplementary Figure 1




**Supplementary Figure 1. Phylogeny of putative thrombopoietin.** Phylogeny analyses were performed by use of Clustal Omega and Clustal Phylogeny for proteins, provide through the analysis tool web services from the EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/clustalo/). The deduced sequences of TPO were aligned with the program Clustal Omega and a tree was calculated using TreeViewX software[1-3].

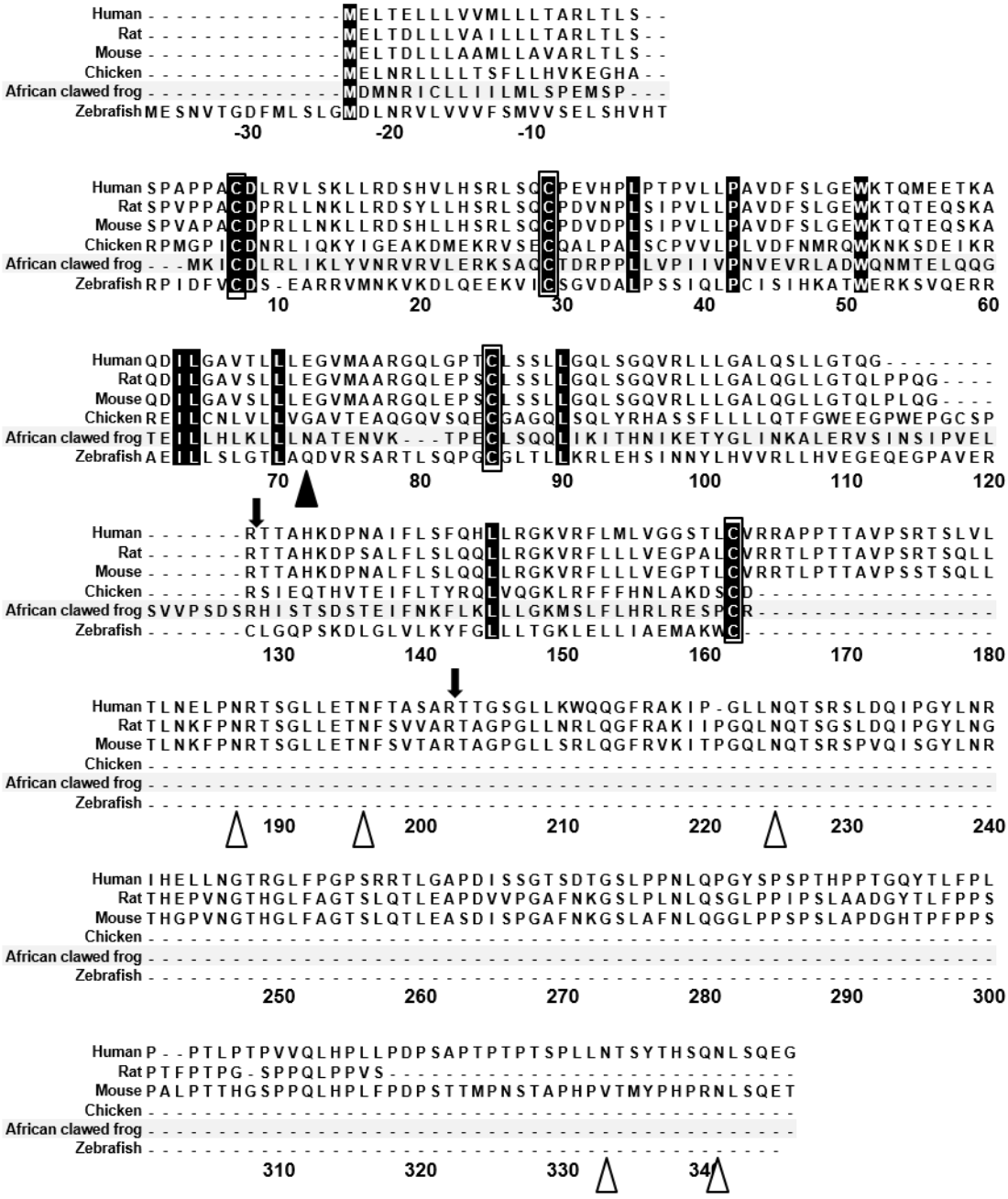

1. Sievers, F. *et al.* Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology* **7,** (2011).
2. Goujon, M. *et al.* A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res.* **38,** (2010).
3. McWilliam, H. *et al.* Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res.* **41,** (2013).

# Supplementary Figure 2



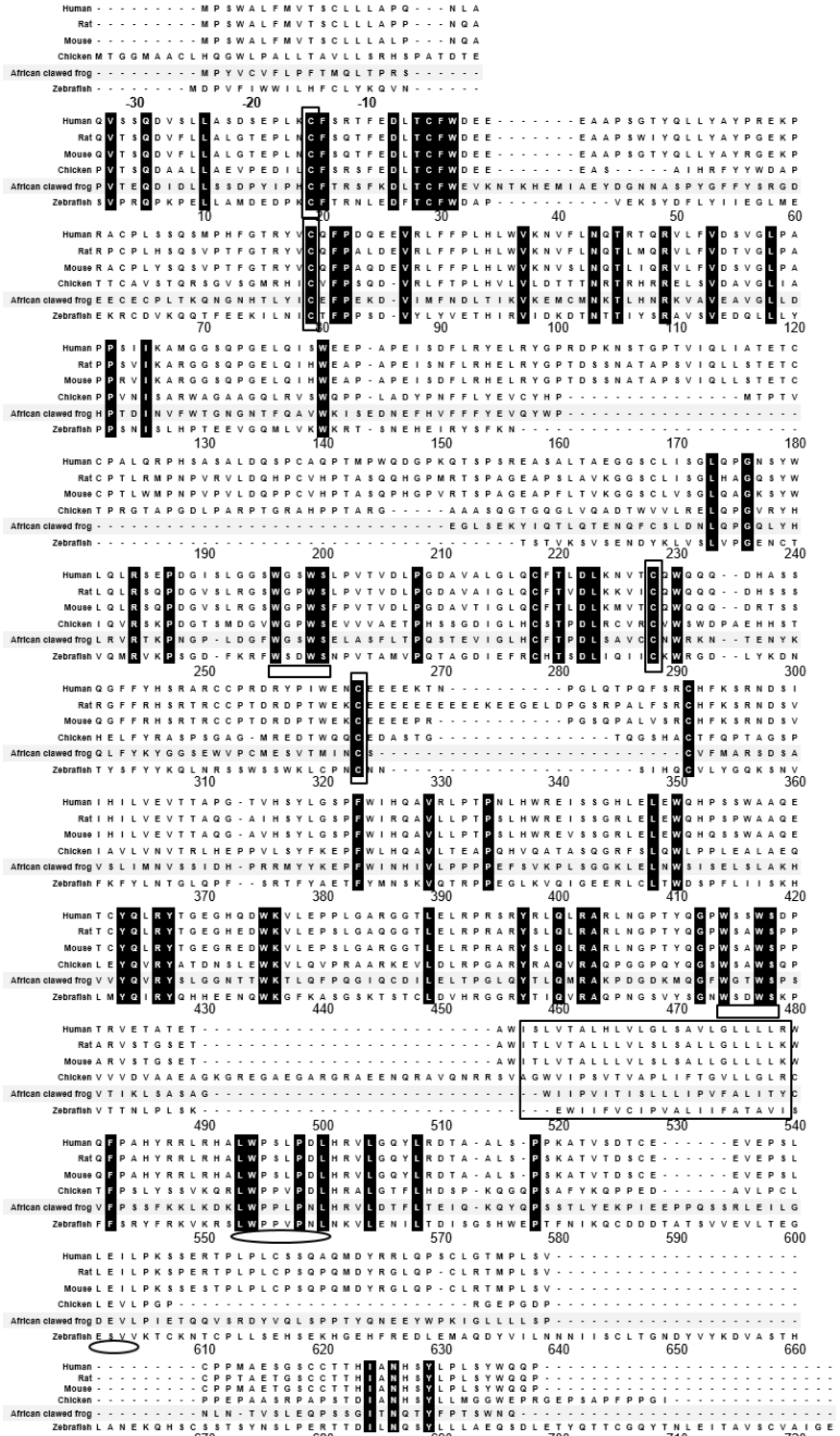

**Supplementary Figure 2. Conserved synteny and homology between the *X. tropicalis* and human TPO and Mpl loci.** (A) RNA polymerase II H (POL2H) and Chordin, mapped in the hTPO locus, were identified by NCBI Homologene or BLAST search in the *X. tropicalis* genome database. The synteny of POL2H, CHORDIN, and TPO at the human, mouse, chicken, and *X. tropicalis* TPO loci was conserved, although their order and direction of transcription has been shuffled (B) TIE-1 and CDC-20 in *xl*Mpl were identified by NCBI Homologene or BLAST in the *X. tropicalis* genome database. The synteny of TIE-1, CDC-20, and Mpl at the human, mouse, chicken, and *X. tropicalis* Mpl loci was also conserved, although their order and direction of transcription has been shuffled.

# Supplementary Figure 3



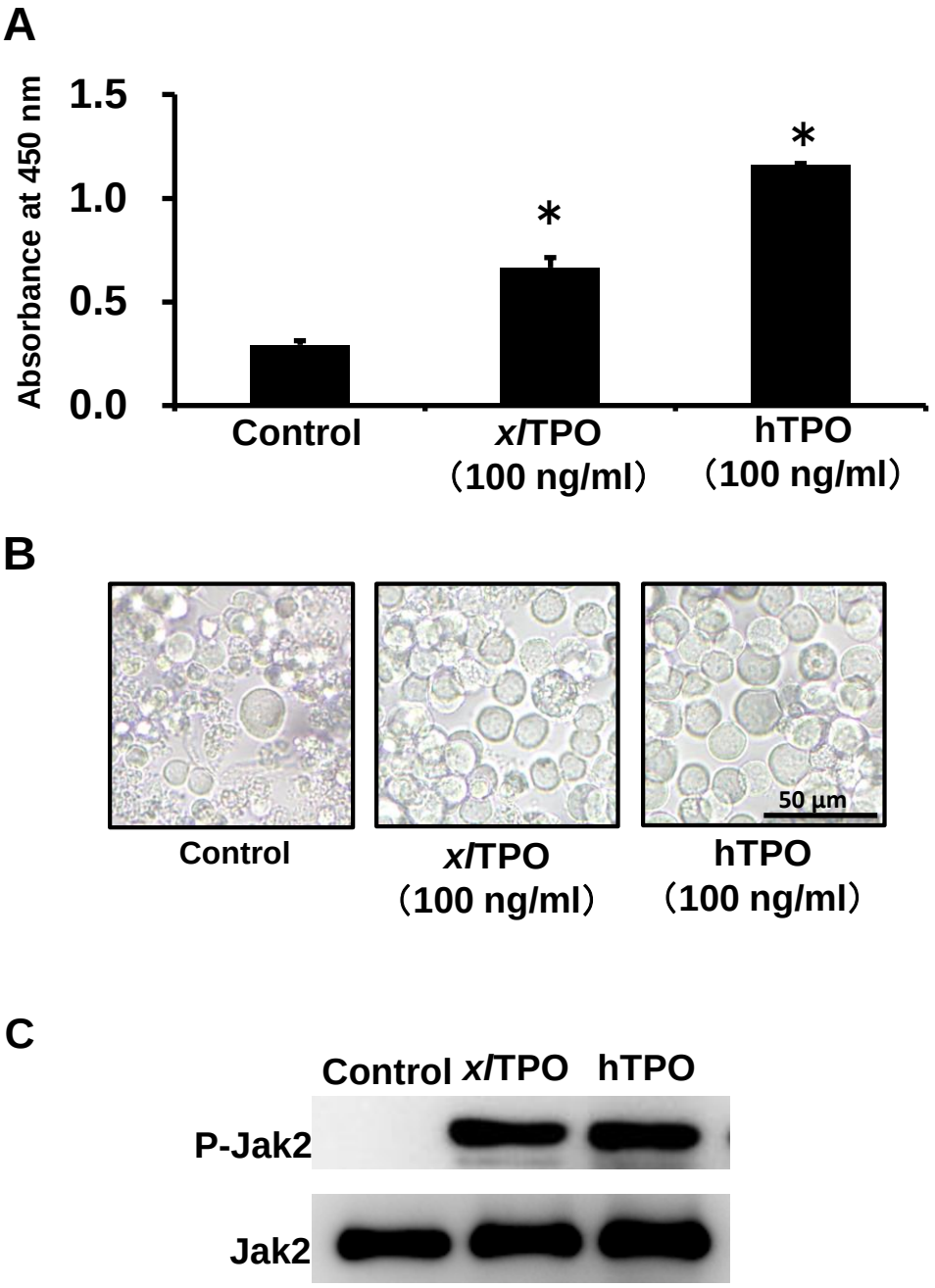

**Supplementary Figure 3. Homology of TPOs from various vertebrate species.** Amino acid sequence homology of mature TPOs from human, rat, mouse, chicken, *X. laevis*, and zebrafish. Numbers represent positions from the N-terminal end of mature *xl*TPO. The predicted signal peptide is shown at the top and indicated in lowercase letters. Identical amino acid residues are shaded black. Cysteine residues that form possible disulfide bridges in *xl*TPO are boxed. Positions of potential *N*-linked carbohydrate on *xl*TPO and hTPO are indicated by solid and open arrowheads, respectively. Potential sites for *N*-glycosylation are conserved in human, rat, and murine TPOs, whereas zebrafish, chicken, and *X. laevis* TPOs lack a C-terminal domain. The thrombin cleavage site in hTPO is indicated by an arrow. The tertiary structure of human TPO has been solved as an antiparallel four-helix bundle fold. TPO sequences were obtained from GenBank (accession numbers: human, AAI30323; rat, BAA06906; mouse, AAA40436; chicken, AAT45554; zebrafish, NP_001137225).

# Supplementary Figure 4



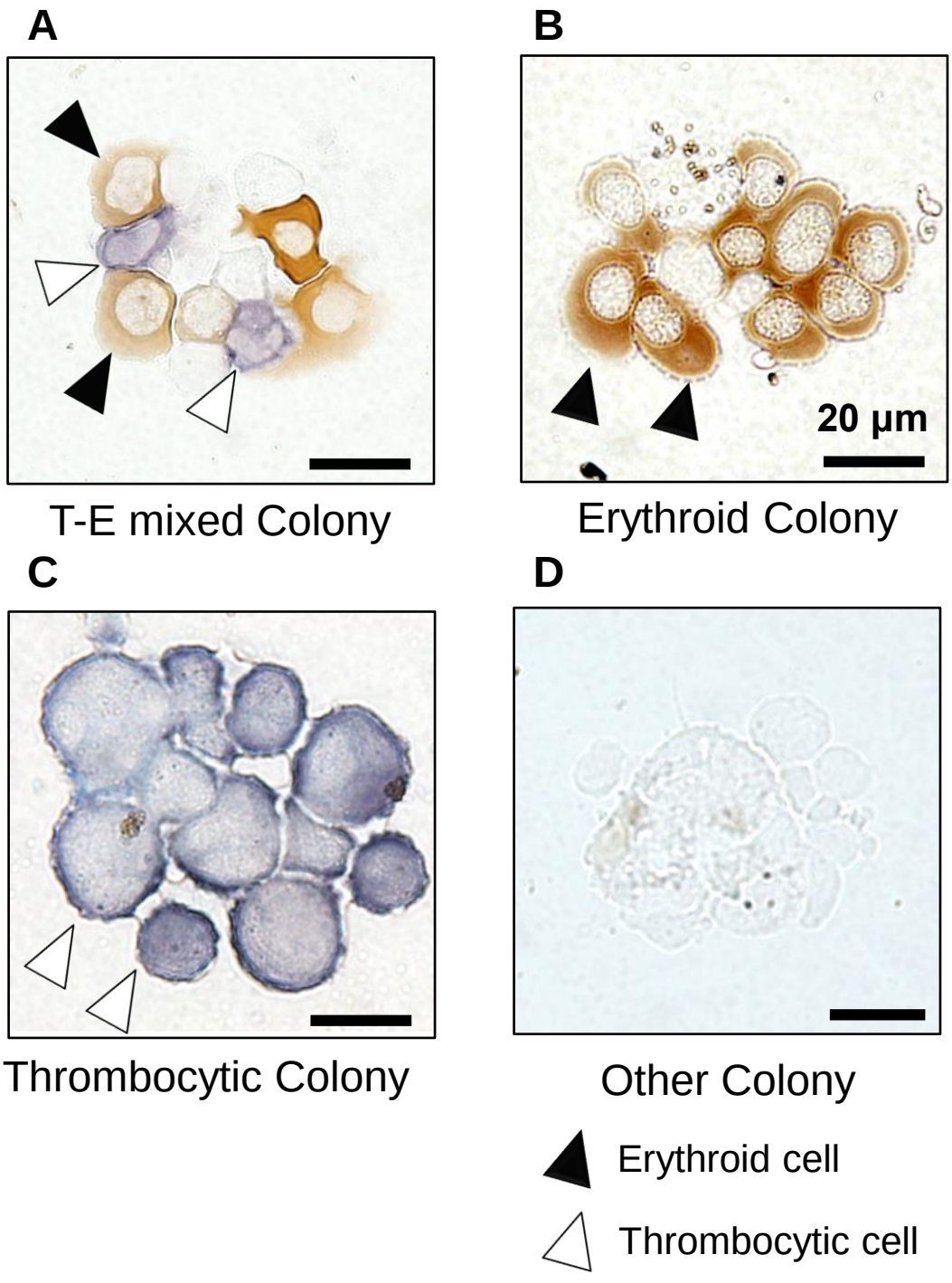

**Supplementary Figure 4. Homology of Mpls from various vertebrate species.** Amino acid sequence homology of mature Mpls from human, rat, mouse, chicken, zebrafish, and *X. laevis*. Numbers indicate positions from the N terminus of the mature *xl*TPO. The predicted signal peptide is shown at the top and indicated by lowercase letters. Identical amino acid residues are shaded black. Cysteine residues that form possible disulfide bridges in *xl*Mpl are boxed. Putative transmembrane domains are underlined. White boxes indicate tryptophan-serine-x-tryptophan-serine (WSXWS) motifs and the Box1 and Box2 regions, thought to be important for mitogenic signaling. Mpl sequences were obtained from GenBank (accession numbers: human, NP_005364; mouse, NP_001116421; chicken, NP_001001782; zebrafish, NP_001003858). The *xl*TPO and *xl*Mpl sequences have been submitted to DDBJ/EMBL/GenBank with accession numbers AB256538 and AB272077.

# Supplementary Figure 5



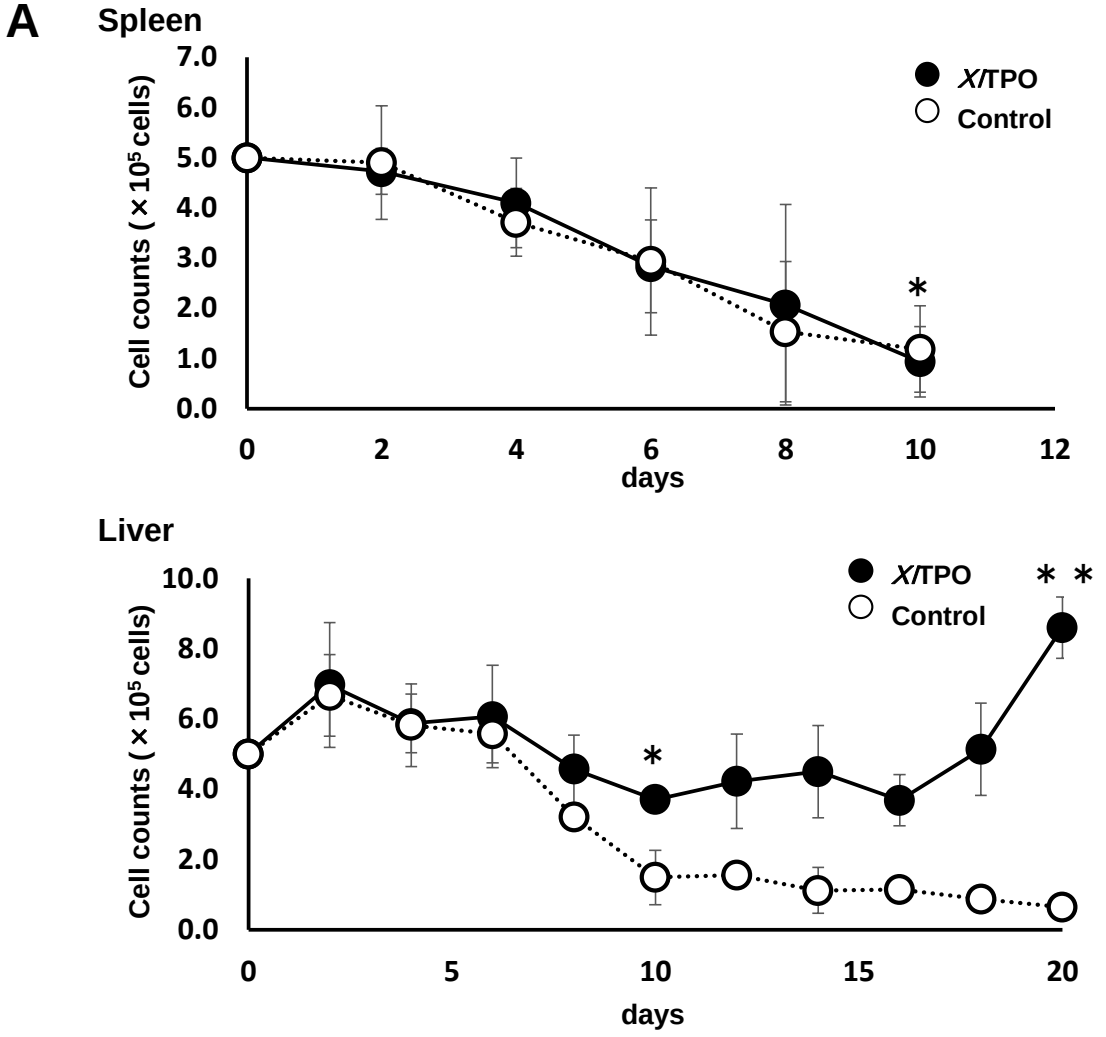

**Supplementary Figure 5. Proliferation of UT-7/TPO cells after *xl*TPO stimulation.** (A) MTS assay of UT-7/TPO cells. UT-7/TPO cells were stimulated with *xl*TPO or hTPO for 72 h in 96-well plates and analyzed by MTS assay. Controls were the UT-7/TPO cells cultured in the absence of TPO for 72 h. *P < 0.05 vs. the control. (B) Cell morphology of UT-7/TPO cells. UT-7/TPO cells were stimulated with *xl*TPO or hTPO for 72 h. (C) JAK2 phosphorylation. UT-7/TPO cells were cultured overnight in the absence of serum and growth factor in IMDM. Cells were stimulated with *xl*TPO or hTPO for 5 min and phosphorylated JAK2 was measured by western blotting.

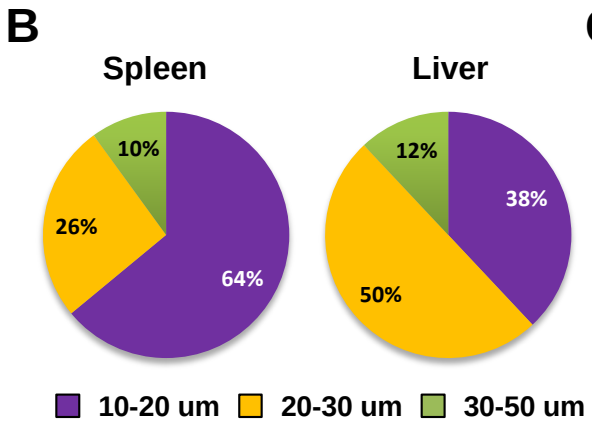
# Supplementary Figure 6

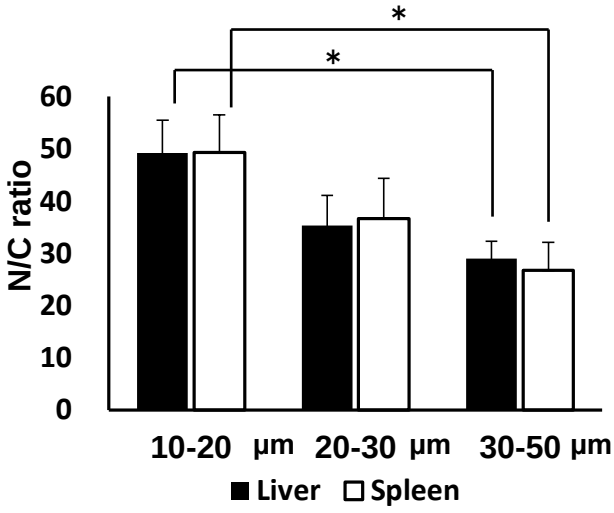



**Supplementary Figure 6. Colonies derived from hepatic cells in the presence of anemic serum.** Hepatic cells from anemic frogs were cultured in semi-solid medium in the presence of anemic serum. After four days, the cell were collected and cytocentrifuged smears were prepared using a cytospin cytocentrifuge. Colonies were immunostained for T12 and counterstained with *o*-dianisidine. (A) Colony of T12- and *o*-dianisidine positive cells. (B and C) Colony composed of only *o*-dianisidine or T12-positive cells. (D) Colony composed of non-hemoglobinized cells.

# Supplementary Figure 7

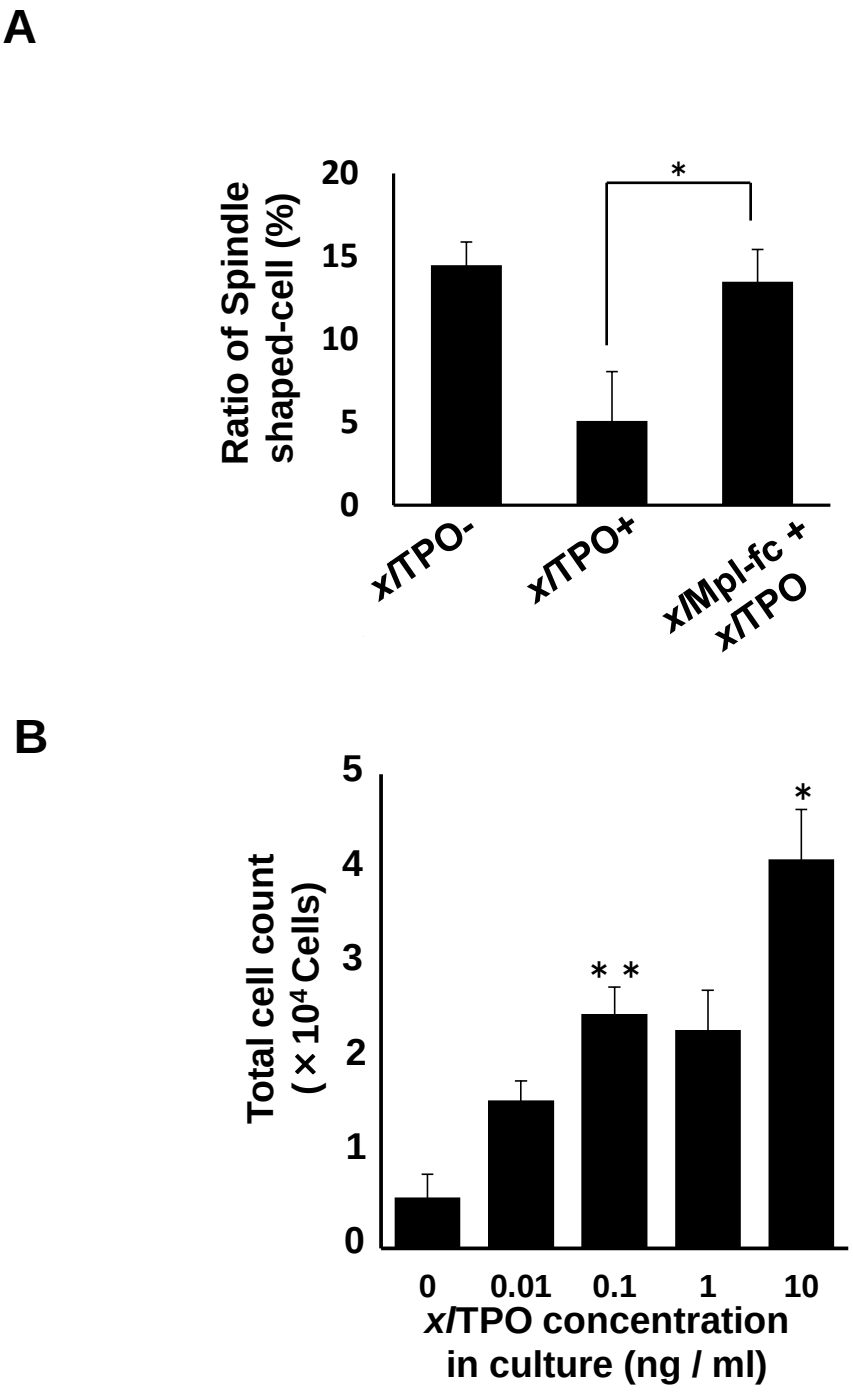



**Supplementary Figure 7. Viability of splenic or hepatic cells after *x*/TPO stimulation.** (A) Whole cells were counted during liquid culture of spleen or liver cells in the presence of *x*/TPO. Open squares represent the control. Black squares represent *x*/TPO-stimulated cells. *P < 0.05 vs. day 0; ** P < 0.05 vs. day 10. The data represent mean ± SD (N = 3). (B)Cellular distribution was investigated on cytospin preparations from hepatic and splenic cells after culturing with *x*/TPO for 8 days. (C) Morphological features of hepatic and splenic cells at 8 days of culture such as N/C ratio was calculated using images of cells by image-J software. Black bars indicates hepatic *x*/TPO-stimulated cells. White bars indicates splenic *x*/TPO-stimulated cells. Analysis was performed three times (N=3) . Results are presented as mean SD. *P < 0.05

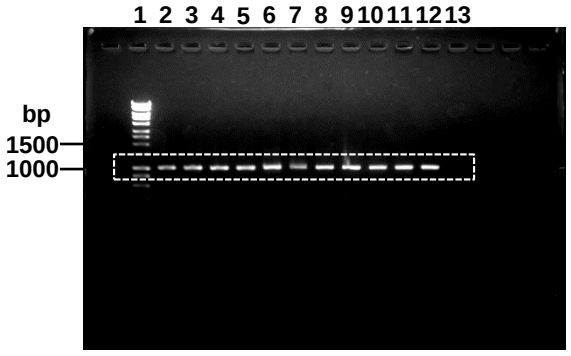
# Supplementary Figure 8




**Supplementary Figure 7. *xl*TPO activity in the MKs and peripheral thrombocytes**. (A) MKs were enriched by density gradient method and cultured with *xl*TPO in the presence or absence of *xl*Mpl-Fc fusion protein for 4 days. Graphs represent means + SD, n = 3. *P < 0.05 (B) Peripheral thrombocytes were enriched by density gradient method and cultured in the presence of in the presence of increasing concentrations of *xl*TPO for 8 days. Analysis was performed three times (N=3) . Results are presented as mean SD. *P < 0.05 vs 0.1 ng/mL *xl*TPO ; ** P < 0.05 vs 0 ng/mL *xl*TPO .

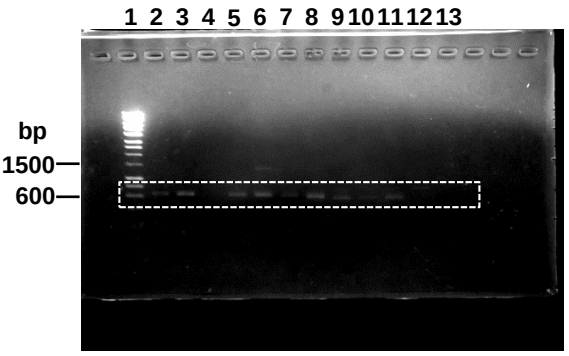
# Supplementary Figure 9

## A Full uncropped gel image from Fig. 1E

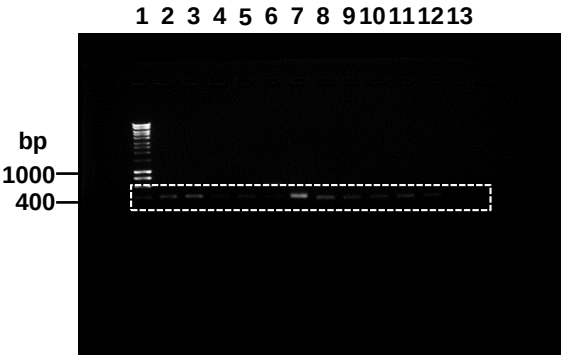



1: Marker
2: Brain
3: Lung
4: Muscle
5: Heart
6:PB
7 :Liver
8 :Spleen
9 :Kidney
10:Stomach
11:Intestine
12:Testis
13:Water

GAPDH

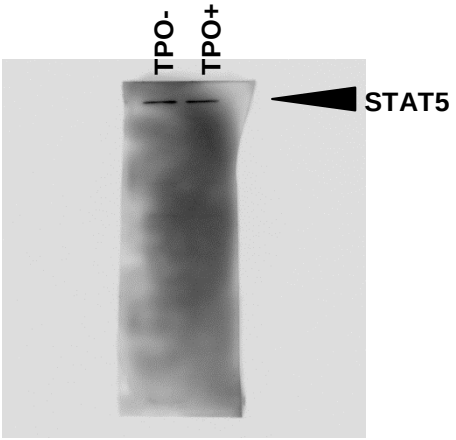



Mpl

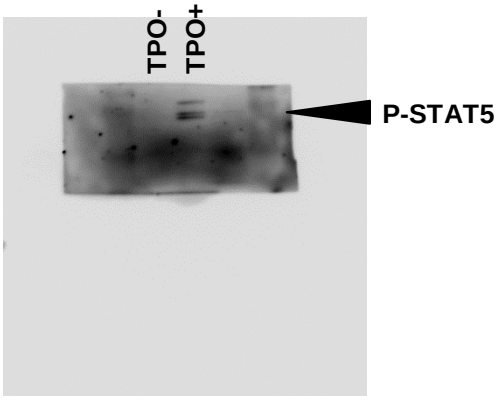



TPO

## B Full uncropped gel image from Fig. 6D




STAT5




P-STAT5