SUPPLEMENTARY APPENDIX

# New pathogenic mechanisms induced by germline erythropoietin receptor mutations in primary erythrocytosis

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# New pathogenic mechanisms induced by germline erythropoietin receptor mutations in primary erythrocytosis

**Running title:** Erythropoietin receptor mutations in PFCP

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**Supplemental figures**

Figure S1

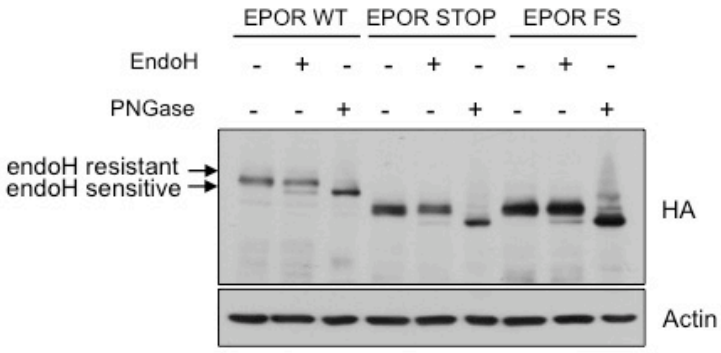
The glycosylation state of the HA EPOR WT, HA-EPOR STOP, HA-EPOR FS was investigated in Ba/F3 cells. Cell lysates were treated with either Endo H or PGNase glycosidase overnight at 37°C and then were analyzed by Western blot. Endo H-resistant HA-EPOR and Endo H-sensitive HA-EPOR are indicated by arrows.

Figure S2

(**a**) UT7 cells were transduced with pMX-HA-huEPOR-IRES-GFP retrovirus to stably express the wild-type receptor (EPOR WT), a truncated mutant at position 444 (p.Gln444*, EPOR STOP) or the frameshift mutant *EPOR* c.1300dup (p.Gln434Profs*11, EPOR FS). Proliferation was assessed 72 hours after culturing UT7-EPOR cells in absence or in presence of increasing doses of EPO (0.01, 0.02, 0.05, 0.1, 0.3, and 1 U/mL) by proliferation assay. Dose-response curves are means expressed in percentages of maximum growth value ± SD (n = 2). (**b**) Effect of increasing EPO concentration on EPOR signaling. UT7 cells expressing different EPOR constructs were examined by western blotting for the presence and phosphorylation status of various signaling molecules. Cells were serum- and cytokine-starved for 5 hours prior to 15 minute stimulation with increasing doses of EPO (0, 0.01, 0.1 and 1 U/mL). Expression of β-actin was used as loading control. One out of two independent experiments is presented.

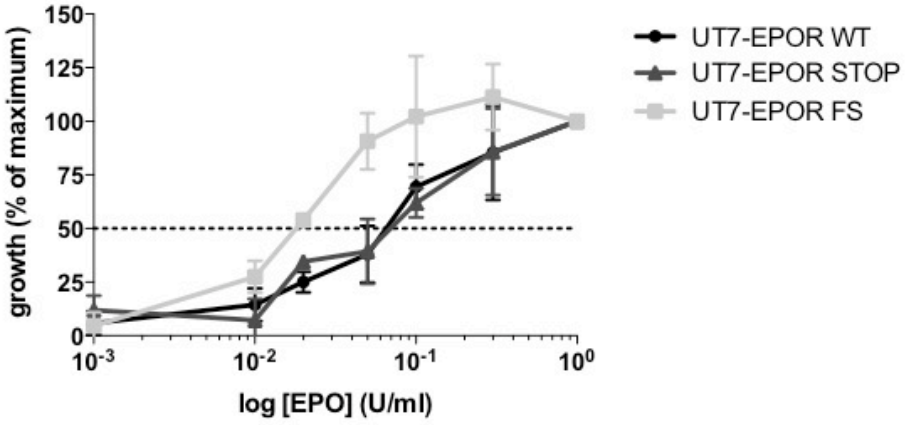
Figure S3

Cell-surface expression of the different EPORs was assessed by flow cytometry using PE fluorescent labeling of the extracellular HA-tag. Representative histograms are shown in (**a**) Ba/F3 and (**b**) UT7 cell lines.

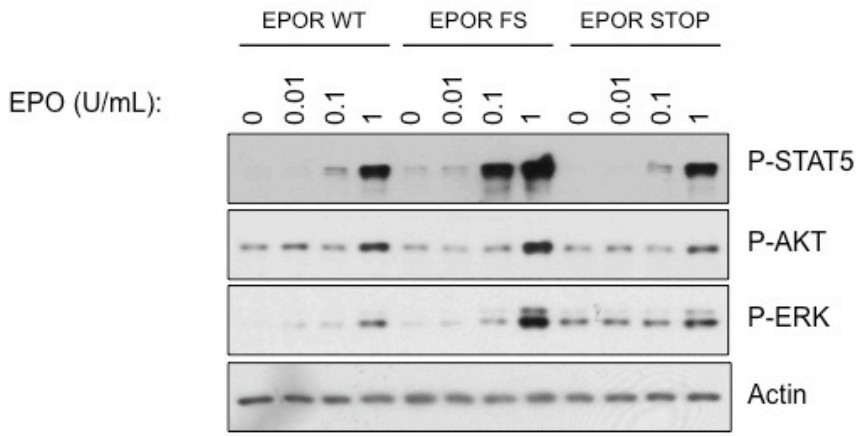



**Pasquier *et al*. Figure S1**

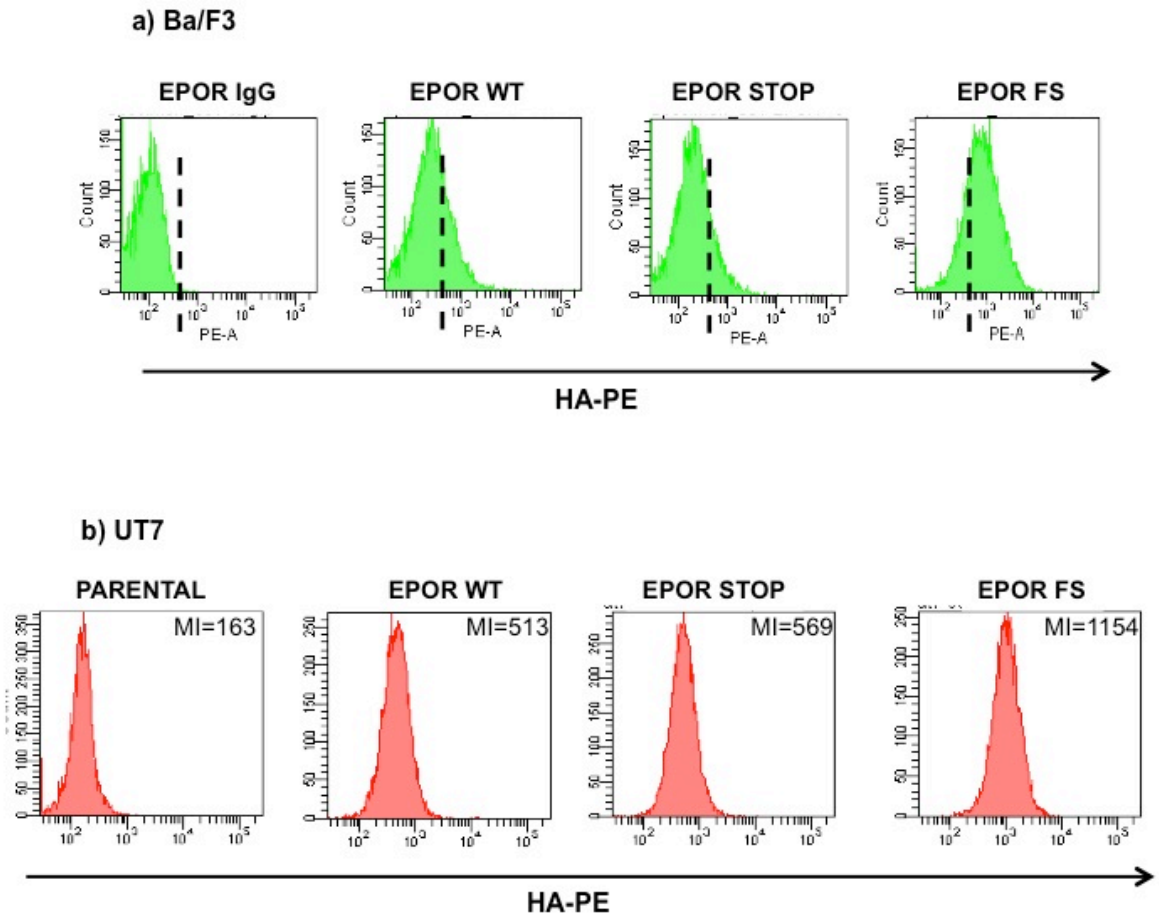
a




b




**Pasquier *et al*. Figure S2**




**Pasquier *et al*. Figure S3**