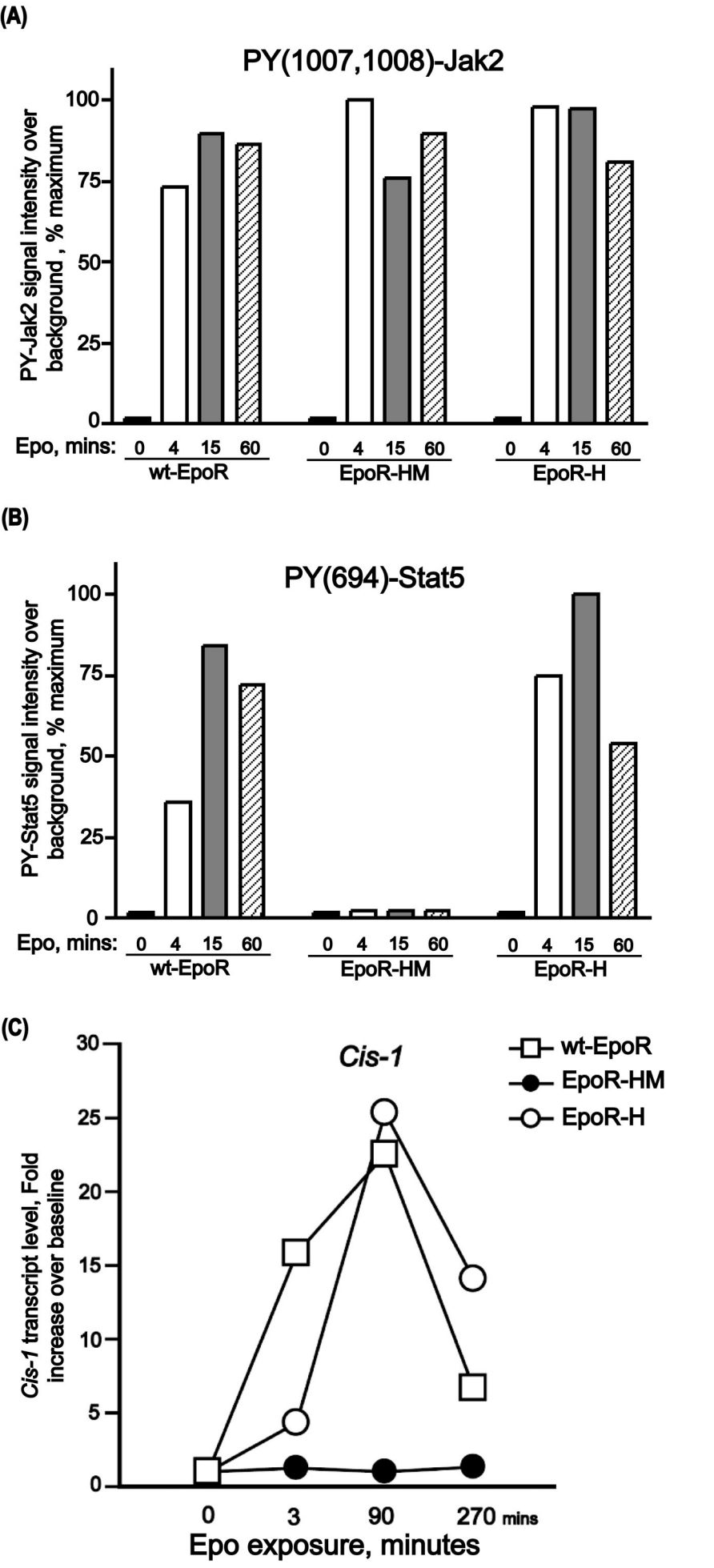
METHODS SUPPLEMENT

PCR primer pairs:

Epo, 5'-AGAATGGAGGTGGAAGAACAGG-3' and 5'-CTGGTGGCTGGGAGGAATTG-3';
Pim-1, 5'-TTCTGGACTGGTTCGAGAGG-3' and 5'-GCTCCTCGTTCGGTGATAAA-3';
Onco-M, 5'-AACTGAGCAAGCCTCACTTCC-3' and 5'-ATGCCGAGGATATTGTGCCG-3';
Bcl-x 5'-ACTGTGCGTGGAAAGCGTAGA-3' and 5'-TGCTGCATTGTTCCCGTAGAG-3';
SOCS3 5'-CCGCTTCGACTGTGTACTCAAG-3' and 5'-TCTTCTCGCCCCCAGAATAGAT-3'
Gas-6, 5'-GCCATCCAGCAGACAGTCAAG-3' and 5'-TGGTTTCCGTGCCGACATC-3';
Pim-2, 5'-CCAGAACCTCTGGTCCCTAA-3' and 5'-CTAAAGAGCTGCTGGGGATG-3';
Onco-MRβ, 5'-CCAGCCCTCAGCACAAACC-3' and 5'-AGGACCATCAAGGACTCAGGAG-3';
Actin, 5'-CGTGCGTGACATTAAAGAGAAG-3' and 5'-TGGATGCCACAGGATTCCATA-3';
Cis-1 - 5'-CCA-CTG-GCT-TTG-TCA-AGA-AGG-3' and 5'-AGG-CCA-CAT-AGT-GCT-GCA-CAA-3'.

Primary Antibodies – antibodies used were from Cell Signaling Technology (CS), Abcam Ltd. (AC), or Upstate Cell signaling Solutions (UPS) as designated: Pim-1, CS # 4722; phospho-Stat5, CS #9351; phospho-Stat3, CS #9131; phospho-Stat1, CS #9171, Stat-5, CS #9352; Stat-3, CS #9132; Stat-1, CS #9172; phospho-ERK, CS #9101; ERK, CS #9102; Jak2, UPS #06-255; phospho-Jak2, UPS #07-123; GAPDH, AC #ab9485.


(A)
PY(1007,1008)-Jak2
PY-Jak2 signal intensity over background , % maximum
100
75
50
25
0
Epo, mins: 0 4 15 60 0 4 15 60 0 4 15 60
wt-EpoR
EpoR-HM
EpoR-H
(B)
PY(694)-Stat5
PY-Stat5 signal intensity over background, % maximum
100
75
50
25
0
Epo, mins: 0 4 15 60 0 4 15 60 0 4 15 60
wt-EpoR
EpoR-HM
EpoR-H
(C)
Cis-1
wt-EpoR
EpoR-HM
EpoR-H
Cis-1 transcript level, Fold increase over baseline
30
25
20
15
10
5
0
0 3 90 270 mins
Epo exposure, minutes

SUPPLEMENTAL FIGURE LEGEND

Figure S-1. Jak2 and Stat5 activation profiles for wt-EpoR-, EpoR-H and EpoR-HM erythroblasts. A] Jak2 is activated with comparable kinetics via wt-EpoR, EpoR-H and EpoR-HM alleles: $Kit^{pos}CD71^{high}$ erythroblasts were isolated from expanded bone marrow preparations. Cytokines then were withdrawn for six hours, and erythroblasts were exposed to Epo at 2.5U/mL. At the indicated intervals, phospho-Jak2 levels then were determined by western blotting, and quantitative imaging (with standardization for GAPDH). **B]** Stat5 activation via wt-EpoR and EpoR-H, but not EpoR-HM alleles: In the above samples, levels of phospho-Stat5 also were quantitatively analyzed. Note the essential failure of EpoR-HM to detectably stimulate Stat5. **C**] $Kit^{pos}CD71^{high}$ erythroblasts were isolated from expanded wt-EpoR, EpoR-HM and EpoR-H marrow preparations, and cultured for six hours in 0.5% BSA, 10ng/mL insulin, 0.1mM 2-ME, IMDM. At 0, 30, 90 and 270 minutes of subsequent Epo-exposure (1U/mL), cells were lysed in Trizol reagent. Quantitative RT-PCR then was used to determine Epo-induced levels of *Cis-1*. *Actin* was used as a normalizing control.