Supplemental data

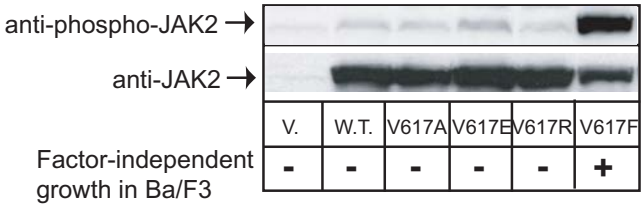
Supplemental Fig. 1. Replacing Val^{617} with Ala, Glu or Arg does not activate JAK2. Full-length JAK2 and JAK2 mutants at Val^{617} were expressed in γ2A cells. Kinase activity was examined using anti-phosphoJAK2 antibodies. Ba/F3 cells stably expressing these mutants were examined for factor-independent growth. V: vector, WT: wild-type.

Supplemental Fig. 2. JAK2 mutants confer factor-independent growth in Ba/F3 cells. Ba/F3 cells stably expressing wild-type or mutant JAK2 and sorted to similar expression levels were cultured in the absence of IL-3. Mitogenic activity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT*)* assay. Cells expressing JAK2 mutants with higher mitogenic activities (such as N622I and V617F) proliferate until confluent and subsequently die from lack of nutrients, and their growth curves between Days 0 to 5 are shown in panel A. The mitogenic activities of weaker JAK2 mutants measured on Day 4 are shown in panel B.

Supplemental Fig. 3. Kinase-dead JAK2 with mutations L611S, R683S, F694L or F694S interacted with the EpoR to promote its surface expression. Kinase-dead JAK2 with or without mutations were examined for their ability to promote HA-EpoR surface expression in γ2A cells. The surface expression of HA-EpoR was measured by median APC fluorescence shown in the upper right corner of each histogram. Surface expression normalized against JAK2(KD) was shown in parenthesis.

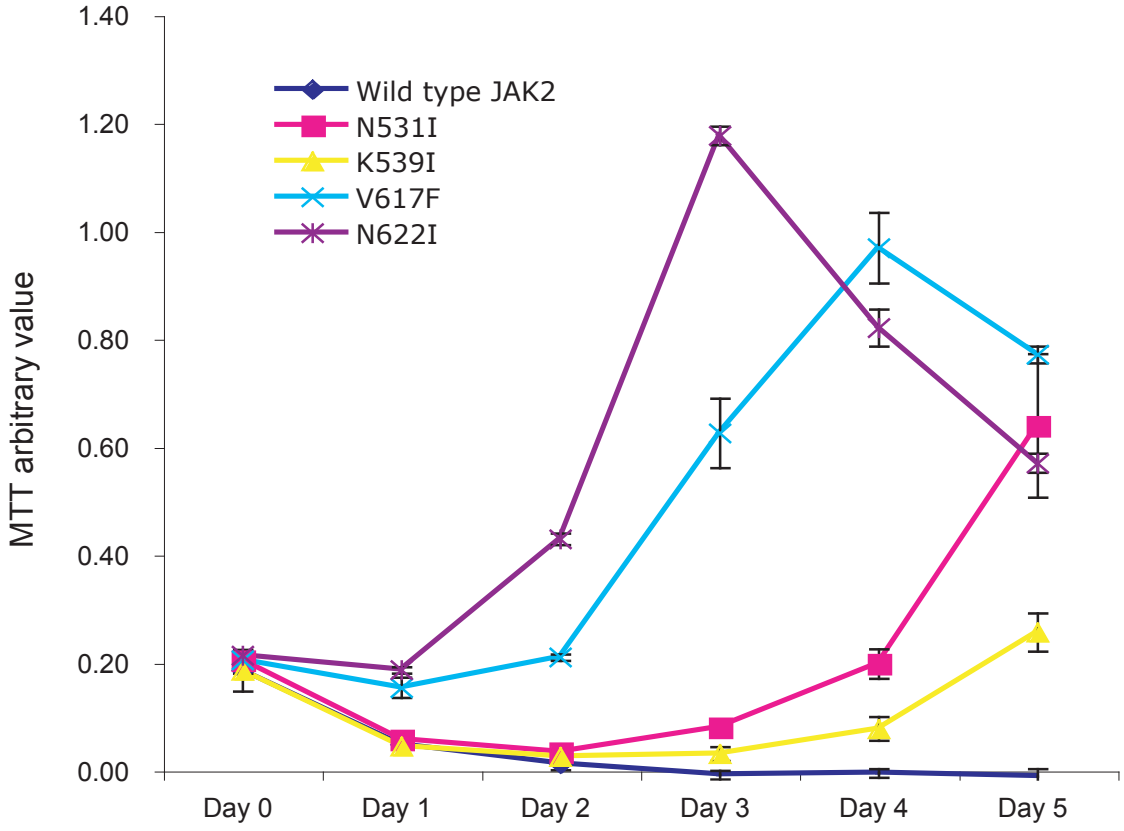
Supplemental Fig. 4. Residues in the SH2-pseudokinase linker are essential for interaction with EpoR. Surface expression of HA-EpoR in cells co-expressing GST-JAK2(N) or serial truncations in the SH2-pseudokinase linker was is measured by median APC fluorescence shown in the upper right corner of each histogram. Surface expression normalized against GST-JAK2(1-544) was shown in parenthesis.

Supplemental Figure 1



Supplemental Fig. 2

A



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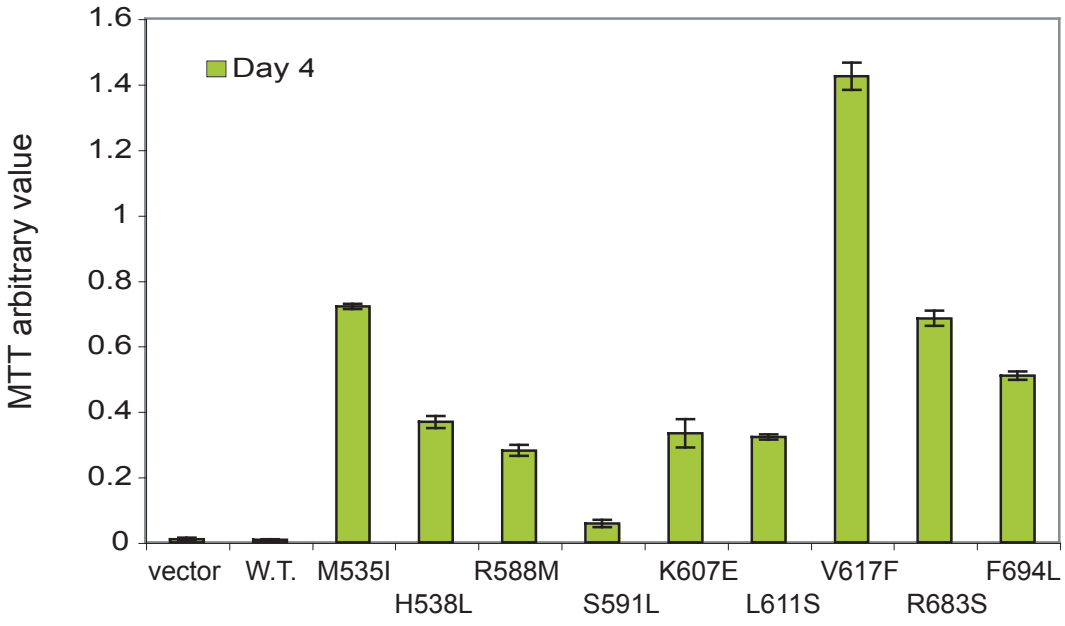
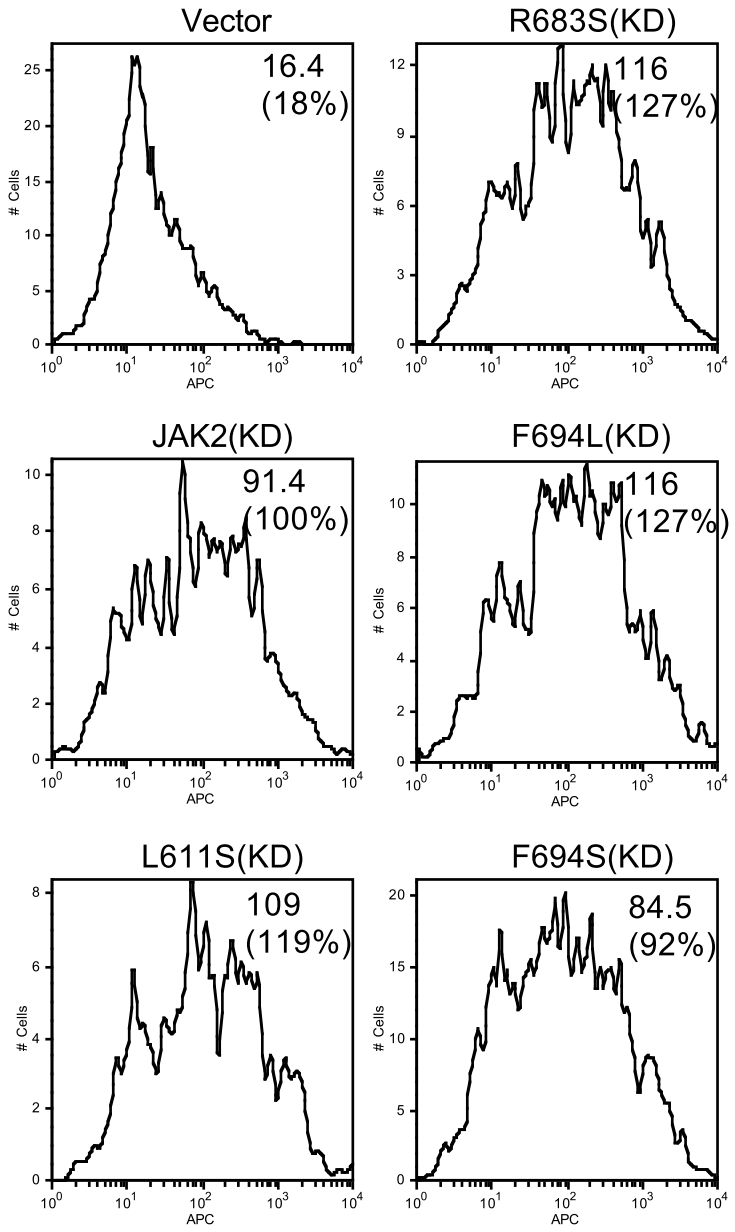


Supplemental Fig. 3



Supplemental Fig. 4