To the editor:

Nuclear JAK2

We note with interest the recent letter published by Girodon et al[1] in *Blood* that disputes the nuclear localization of JAK2 that we and others have previously reported.[2-10] We would like to make 3 points related to this publication.

First, the majority of the data presented in this manuscript rely on tracking JAK2 tagged with a relatively large fluorescent epitope, which may interfere with its tertiary structure, interacting partners, or subcellular localization.[11] Nevertheless, it is worth noting that other groups have demonstrated a nuclear localization of JAK2 tagged with either EGFP[10] or smaller epitope tags.[6] Moreover, close inspection of the immunofluorescence (IF) images supplied by Girodon et al (Figure 1C)[1] demonstrates that the nuclei of HEL cells contain several prominent fluorescent speckles, which were not discussed in their letter. We agree with the authors' general sentiment that antibody based IF methodologies have limitations, and that several commercially available antibodies for JAK2 (but not including the ones we used) do detect other bands on Western blot and therefore need to be used with caution. Stringent controls are required to ensure that the antibody used is specific, reproducible and reliable. For these reasons, in our original study,[2] we used 2 separate antibodies raised in different species and performed several controls including demonstrating the absence of any signal by both IF and Western blotting in γ2A cells that are genetically null for JAK2. We also demonstrated that both antibodies detect a single band at the molecular weight of JAK2 in Western blotting assays and showed that these antibodies only detect JAK2 in γ2A cells transfected with JAK2 but not nontransfected cells within the same visual field. Importantly, these data are in keeping with the findings from multiple other independent groups that have also used IF to demonstrate nuclear JAK2 (reviewed in Qian et al[12] and Zouein et al[13]).

Second, in addition to IF studies there is an abundant literature demonstrating nuclear localization of janus kinases using stringent biochemical fractionation. These results benefit from the fact that separation of denatured proteins by SDS-PAGE allows rigorous assessment of both molecular weight and purity of the fractionation. The fractionation methodology used by the Girodon et al was not detailed in their manuscript, and so we are unable to comment on this. However, it is worth noting that several groups have used biochemical fractionation to demonstrate JAK2 in the nucleus of mammary cells,[7] kidney and neuroepithelial cells,[4] amniotic epithelium,[8] and adrenocortical cells.[9] In hematopoietic cells, the Nimer laboratory has used cell fractionation techniques to demonstrate that JAK2V617F is found in both the cytoplasm and the nucleus, and that nuclear JAK2V617F interacts with the chromatin modifier PRMT5 to regulate its histone methyltransferase activity.[5] Similarly, we now present new data from the Levine laboratory showing that JAK2V617F-EGFP fusion proteins expressed in Ba/F3 and TF-1 cells can also be reliably detected in the nucleus in fractionation experiments in a similar manner to untagged, endogenous JAK2 protein (Figure 1A). Furthermore, we show that endogenous JAK2V617F can be reliably detected in the nucleus in SET2, HEL, and UKE1 cells (Figure 1B). Taken together these data from multiple independent laboratories with expertise in hematopoiesis demonstrate that JAK2 can reliably and reproducibly be detected in the nucleus of hematopoietic cells.

Third, in our original study we also demonstrated that JAK2 directly and specifically phosphorylates histone H3Y41 which results in reduced HP1α binding and increased transcription of target genes,[2] findings which have now been confirmed in other cell types.[3,14] These data further support a nuclear role for JAK2.

Two recent reviews include over 20 publications that have reported JAK2 in the nucleus but only 2 that dispute this claim.[12,13] We believe that much of the debate is likely related to methodologic differences, which are not easily resolved, and it seems highly likely that the amount of nuclear JAK2 varies in different cellular contexts. However, the current weight of evidence strongly supports a nuclear role for JAK2, a view that is consistent with emerging nuclear roles for a large number of kinases previously thought to function solely in the cytoplasm.[15]

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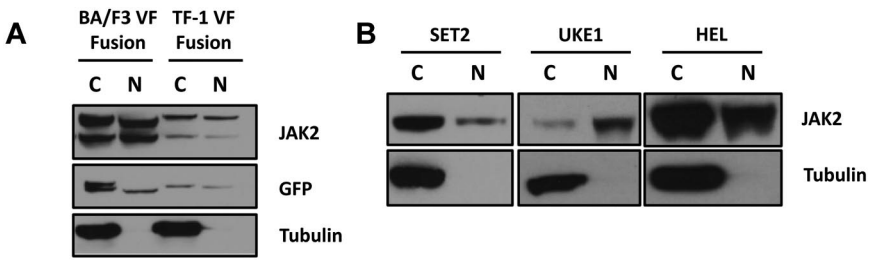

Figure 1. JAK2 is present in both the cytoplasm and the nucleoplasm. (A) Subcellular fractionation was performed in Ba/F3 EPO-R and TF-1 cell lines stably expressing a JAK2V617F EGFP fusion construct and subcellular distribution was assessed by immunoblotting. Endogenous JAK2 and JAK2 GFP Fusion protein were present in both the cytoplasm [C] and the nucleoplasm [N]. α-tubulin was used as a cytoplasmic marker. (B) JAK2 compartmentalizes to both the nuclear and cytoplasmic fractions in fractionation experiments in a panel of leukemic cell lines expressing endogenous JAK2V617F mutation (SET2, UKE1, and HEL).

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To the editor:

A novel splice donor mutation in the thrombopoietin gene leads to exon 2 skipping in a Filipino family with hereditary thrombocythemia

In contrast to the familial predisposition observed in somatically acquired myeloproliferative neoplasms (low penetrance, clonal hematopoiesis), the hereditary thrombocythemias (HT) are characterized by Mendelian inheritance, high penetrance, and polyclonal hematopoiesis, and appear to only affect the megakaryocytic lineage.[1] All the molecular alterations identified thus far in patients with HT have involved either *THPO* (thrombopoietin) or its receptor *MPL* (myeloproliferative leukemia virus oncogene) genes, with 4 and 3 distinct mutations reported, respectively. The HT-associated *THPO* mutations were either confirmed or expected to increase the translational efficiency of thrombopoietin without altering the sequence of the mature protein.[1] Thrombopoietin, the primary regulator of megakaryopoiesis and platelet production, is produced in the liver, kidney, spleen, and bone marrow.[2] Thrombopoietin binds to its receptor and activates the *JAK-STAT* signaling pathway.[3] The presence of multiple upstream AUG codons (uAUG) within the 5′-untranslated region (5′-UTR) precludes efficient translation and prevents harmful overproduction of this potent cytokine.[2]

We identified a novel point mutation at the splice donor site of *THPO* intron 2 (position +2) in a Filipino family with HT. Approval was obtained from the Stanford University institutional review board for these studies and informed consent was provided according to the Declaration of Helsinki. The proband and her 2 children manifested with moderate to severe elevations of the serum thrombopoietin levels and platelet counts, while those were normal in her biologic parents and husband (Figure 1A). The absence of the mutation in either parent suggests the de novo nature of the mutation. Sequencing of the 5′-UTR of *THPO* in all the family members revealed a heterozygous T > C transition at the splice donor of intron 2 (Figure 1B) in subjects II-1, III-1 and III-2, but not in I-1, I-2 and II-2, indicating the co-segregation of the mutation with the thrombocytosis phenotype. The patients were negative for all known HT-associated *MPL* mutations. Serum thrombopoietin levels (measured with the human TPO Quantikine kit, R&D Systems) in the affected family members were significantly higher than in the non-affected family members or healthy controls, while there was no statistically significant difference