Supplementary Materials and Methods

Western Blotting

All human breast tumor cell lines were purchased directly from the American Type Culture Collection and grown in RPMI-1640 supplemented with 10% FBS, 100 units/mL penicillin, 100 µg/mL streptomycin sulfate, and 2.5 µg/mL amphotericin-B. Cells were lysed using Mammalian Protein Extraction Reagent (Thermo Fisher Scientific) with protease and phosphatase inhibitors (cat. nos. 539134 and 78420, EMD Chemicals, and Thermo Fisher Scientific, respectively). Lysates were resolved on 4-12% Bis-Tris gels and transferred to PVDF membranes (NuPage system, Life Technologies). Membranes were blocked for 1 hour at room temperature (for JAK2) or overnight at 4°C (for TUBULIN) with 5% non-fat dry milk in PBS, 0.15% Tween-20 (PBS-T). Primary antibodies were rabbit JAK2 (D2E12, Cell Signaling Technology) at 1:1,000 in 5% BSA, PBS-T (overnight, 4°C), and mouse TUBULIN (T7816, Sigma) at 1:20,000 in 2.5% non-fat dry milk, PBS-T (2 hours, room temperature). Anti-rabbit and anti-mouse HRP-conjugated secondary antibodies (cat. nos. 111-035-045 or 115-035-174, Jackson ImmunoResearch) were used at 1:75,000 (1 hour, room temperature) in 2.5% non-fat dry milk, PBS-T. Blots were developed and exposed using enhanced chemiluminescence and Hyperfilm (GE Healthcare).

Immunohistochemistry

Primary breast tumor specimens were randomly selected for staining from the highest and lowest quartiles of *JAK2* mRNA expression (n=10 from each quartile). Staining and washing steps were performed using the Bond Automated Immunostainer (Leica). Primary breast tumor sections (4um) were deparaffinized in Bond Dewax Solution (Leica) and rehydrated through a graded ethanol series. Following antigen retrieval using citrate buffer, pH 6 (Bond Epitope Retrieval Solution 1, Leica) at 100°C for 20 minutes, endogenous peroxidase blocking using 3% hydrogen peroxide for 5 minutes, and blocking with 10% normal goat serum in TBS for 10 minutes, the sections were incubated at 1:400 with rabbit monoclonal JAK2 antibody (clone D2E12, Cell Signaling Technology) in Bond Primary Antibody Diluent (Leica) for 30 minutes at room temperature. Sections were then incubated with Goat Polymer Anti-Rabbit Poly-HRP-IgG (Leica) for 8 minutes followed by Bond Mixed Refine DAB Substrate (Leica) for 10 minutes at room temperature. After washing with distilled water, the sections were counter-stained with hematoxylin solution (Leica), dehydrated through a graded ethanol series, cleared in xylene, and mounted. Negative and positive controls for JAK2 were *JAK2*-deficient γ2A cells (a gift of Dr. George Stark and Dr. Peter Sayeski) and γ2A cells transiently-transfected with a human *JAK2* expression vector (a gift of Dr. Juan Li). JAK2 staining was quantified using the Nanozoomer Digital Pathology system (Hamamatsu) and image analysis software (Visiopharm) and also scored manually by a pathologist blinded to *JAK2* mRNA levels.

T cell activation

Murine splenocytes were collected under a protocol approved by the University of Washington Institutional Animal Care and Use Committee and plated at $1x10^6$ cells/mL in RPMI, 10% FBS, 50 µM beta-mercaptoethanol, 100 units/mL penicillin, 100 µg/mL

streptomycin sulfate, and 2.5 μg/mL amphotericin-B. Cultures were treated with 25 ng/mL anti-CD3 (from the UCSF monoclonal antibody core) plus the indicated concentrations of ruxolitinib (Selleck Chemicals). After 48 hours, interferon-gamma (IFN-γ) levels in culture supernatants were measured using the Ready-Set-Go ELISA System (ebioscience).