**Supplemental Digital Content**

**SDC, Materials and Methods**

*Cloning of LeGO vectors and production of lentiviral particles*

Cloning of LeGO vector was performed as previously described[1](#_ENREF_1) (for detailed protocols and vector maps refer to <http://www.lentigo-vectors.de>). For overexpression of human *AXL*, the full-length cDNA was cloned from pDONR223 AXL (Addgene Plasmid #23945) by PCR (forward primer: 5’-atatGAATTCcgccaccATGGCGTGGCGGTGCC and reverse primer: 5’-atatGCGGCCGCCTAGGCACCATCCTCCTGCC) into the lentiviral vector LeGO-iG2-Puro+ using EcoRI and NotI restriction enzymes (New England Biolabs) according to standard protocols. All PCR amplified sequences were verified by sanger sequencing. Overexpression of *AXL* was confirmed by qPCR. Percentage of transduced cells was determined using flow cytometry. Only cells with a minimum of 80-fold overexpression as measured by qPCR compared to controls were used for experiments.

*Co-Immunoprecipitation*

For co-immmunoprecipitation, proteins were crosslinked by incubation with 1mM DSP (Pierce) for 30 minutes followed by cell lysis using NP-40 buffer (both ThermoFisher). 1mg of protein was incubated with anti-JAK2 antibody or the same amount of anti-IgG isotype (both purchased from Cell Signaling) and Protein G Sepharose (Invitrogen) overnight. After centrifugation and washing at 4°C, protein-bead complexes were boiled and subjected to immunoblot analysis.

*Measurement of transaminases and creatinine*

Alanine transaminase (ALT), aspartate transaminase (AST) (both IFCC method) and creatinine levels (Jaffé method) were determined by spectrophotometric analysis using a Siemens Atelica CH 930.

**SDC, References**

1. Weber K, Bartsch U, Stocking C, et al. A multicolor panel of novel lentiviral "gene ontology" (LeGO) vectors for functional gene analysis. *Mol Ther* 2008; 16: 698-706. 2008/03/26. DOI: 10.1038/mt.2008.6.

**SDC, Tables**

**SDC, Table 1. Patient characteristics.** This table summarizes characteristics of patients of which PB and BM samples were used in this study. All patients were untreated at time point of sample taking.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient #** | **Disease** | **Genetics** | **Sex** | **Age** | **Source** | **Material** | **Experiment** |
| 1 | PV | *JAK2V617F* | male | 58 | BM | protein, plasma | WB, ELISA |
| PB | MNC, plasma | WB, ELISA |
| 2 | PV | *JAK2V617F* | female | 74 | BM | protein, plasma | WB, ELISA |
| 3 | PV | *JAK2V617F* | male | 54 | BM | protein, plasma | WB, ELISA |
| 4 | ET | *JAK2V617F* | male | 38 | BM | protein, plasma | WB, ELISA |
| 5 | PV | *JAK2V617F* | male | 56 | BM | protein, plasma | WB, ELISA |
| 6 | MPN | *JAK2V617F* | female | 40 | BM | protein, plasma | WB, ELISA |
| PB | MNC, plasma | CFU, ELISA |
| 7 | PV | *JAK2V617F* | Male | 46 | BM | plasma | ELISA |
| 8 | ET | *CALR* | Male | 41 | BM | plasma | ELISA |
| 9 | ET | *CALR* | female | 61 | BM | plasma | ELISA |
| 10 | MPN | *JAK2V617F* | Male | 70 | BM | plasma | ELISA |
| 11 | ET | *JAK2V617F* | female | 79 | BM | plasma | ELISA |
| 12 | ET | n.a. | female | 36 | PB | MNC | CFU |
| 13 | PV | *JAK2V617F* | female | 45 | PB | plasma, MNC | ELISA, CFU |
| 14 | MF | *JAK2V617F* | female | 75 | PB | plasma, MNC | ELISA, CFU |
| 15 | ET | *CALR* | male | 77 | PB | plasma | ELISA |
| 16 | PV | *JAK2V617F* | male | 42 | PB | plasma | ELISA |
| 17 | PMF | *JAK2V617F* | male | 75 | PB | plasma | ELISA |
| 18 | ET | *JAK2V617F* | female | 71 | PB | plasma | ELISA |
| 19 | ET | WT | female | 37 | PB | plasma | ELISA |
| 20 | PV | *JAK2V617F* | female | 77 | PB | plasma | ELISA |
| 21 | PV | *JAK2V617F* | female | 69 | PB | plasma | ELISA |
| 22 | PV | *JAK2V617F* | male | 66 | PB | plasma | ELISA |
| 23 | ET-MF | *JAK2V617F* | male | 70 | PB | plasma | ELISA |
| 24 | MF | n.a. | female | n.a. | PB | plasma | ELISA |
| 25 | MF | n.a. | male | n.a. | PB | plasma | ELISA |
| 26 | MF | n.a. | female | 76 | PB | plasma | ELISA |
| 27 | MF | n.a. | n.a. | n.a. | PB | plasma | ELISA |
| 28 | MF | n.a. | n.a. | n.a. | PB | plasma | ELISA |
| 29 | MF | n.a. | male | 81 | PB | plasma | ELISA |
| 30 | MF | n.a. | female | 82 | PB | plasma | ELISA |
| 31 | MF | WT | male | 45 | PB | plasma | ELISA |
| 32 | MF | n.a. | male | 40 | PB | plasma | ELISA |
| 33 | ET | n.a. | female | n.a. | PB | plasma | ELISA |
| 34 | PV | *JAK2V617F* | male | 51 | PB | plasma | ELISA |
| 35 | PV | *JAK2V617F* | male | 51 | PB | plasma | ELISA |
| 36 | MF | *JAK2V617F* | male | 70 | PB | plasma | ELISA |
| 37 | MPN | *JAK2V617F* | female | 63 | PB | plasma | ELISA |
| 38 | PV | *JAK2V617F* | male | 40 | PB | plasma | ELISA |
| 39 | PV | *JAK2V617F* | female | 64 | PB | plasma | ELISA |
| 40 | ET | *JAK2V617F* | female | 73 | PB | plasma | ELISA |
| **BM** Bone Marrow; **PB** Peripheral Blood; **ET** Essential Thrombocythemia; **MF** Myelofibrosis; **PMF** Primary Myelofibrosis; **PV** Polycythemia Vera; **MNC** Mononuclear cells; **WB** Western blot; **CFU** Colony Formation Assay; **n.a.** data not available. | | | | | | | |

**SDC, Table 2. Western blot antibodies.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Company** | **Catalogue No.** | **Source** | **Dilution** |
| pAXL (Y779) | R&D | AF2228 | rabbit | 1:333 |
| AXL | gift from Björn Dahlbäck | n.a. | rabbit | 1:1000 |
| pAKT (Ser473) (193H12) | Cell Signaling | 4058 | rabbit | 1:1000 |
| AKT | Cell Signaling | 9272 | rabbit | 1:1000 |
| p-p44/42 MAPK (pERK1/2) | Cell Signaling | 4370 | rabbit | 1:2000 |
| p44/42 MAPK (ERK1/2) (3A7) | Cell Signaling | 9107 | mouse | 1:1000 |
| pSTAT5 (Tyr694) | Cell Signaling | 9351 | rabbit | 1:1000 |
| STAT5 (D2O6Y) | Cell Signaling | 94205 | rabbit | 1:1000 |
| STAT5A (4H1) | Cell Signaling | 4807 | mouse | 1:1000 |
| STAT5B | Cell Signaling | 34662 | rabbit | 1:1000 |
| pSTAT3 (Tyr705) | Cell Signaling | 9131 | rabbit | 1:1000 |
| STAT3 | Cell Signaling | 9139 | mouse | 1:1000 |
| BCL-2 (D17C4) | Cell Signaling | 3498 | rabbit | 1:1000 |
| Cleaved Caspase 3 (Asp175) | Cell Signaling | 9664 | rabbit | 1:1000 |
| XIAP (3B6) | Cell Signaling | 2045 | rabbit | 1:1000 |
| β-Actin (C4) | Santa Cruz | sc-47778 | mouse | 1:1000 |
| β-Tubulin (D-10) | Santa Cruz | sc5274 | mouse | 1:500 |
| JAK2 (D2E12) | Cell Signaling | 3230 | rabbit | 1:50 (IP) |
| α-Tubulin (B-5-1-2) | Sigma Aldrich | T5168 | mouse | 1:8000 |
| Lamin B1 (D4Q4Z) | Cell Signaling | 12586 | rabbit | 1:1000 |
| Rabbit IgG Isotype (DA1E) | Cell Signaling | 3900 | rabbit | 50 µg/ml (IP) |

**SDC, Figures**

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**SDC, Figure 1.** **AXL is bound to JAK2.** SET-2 cells were used for co-immunoprecipitation. Immunoprecipitation was performed using anti-IgG Isotype antibody (used as negative control) or an anti-JAK2 antibody. Western blot analysis was performed to detect AXL.

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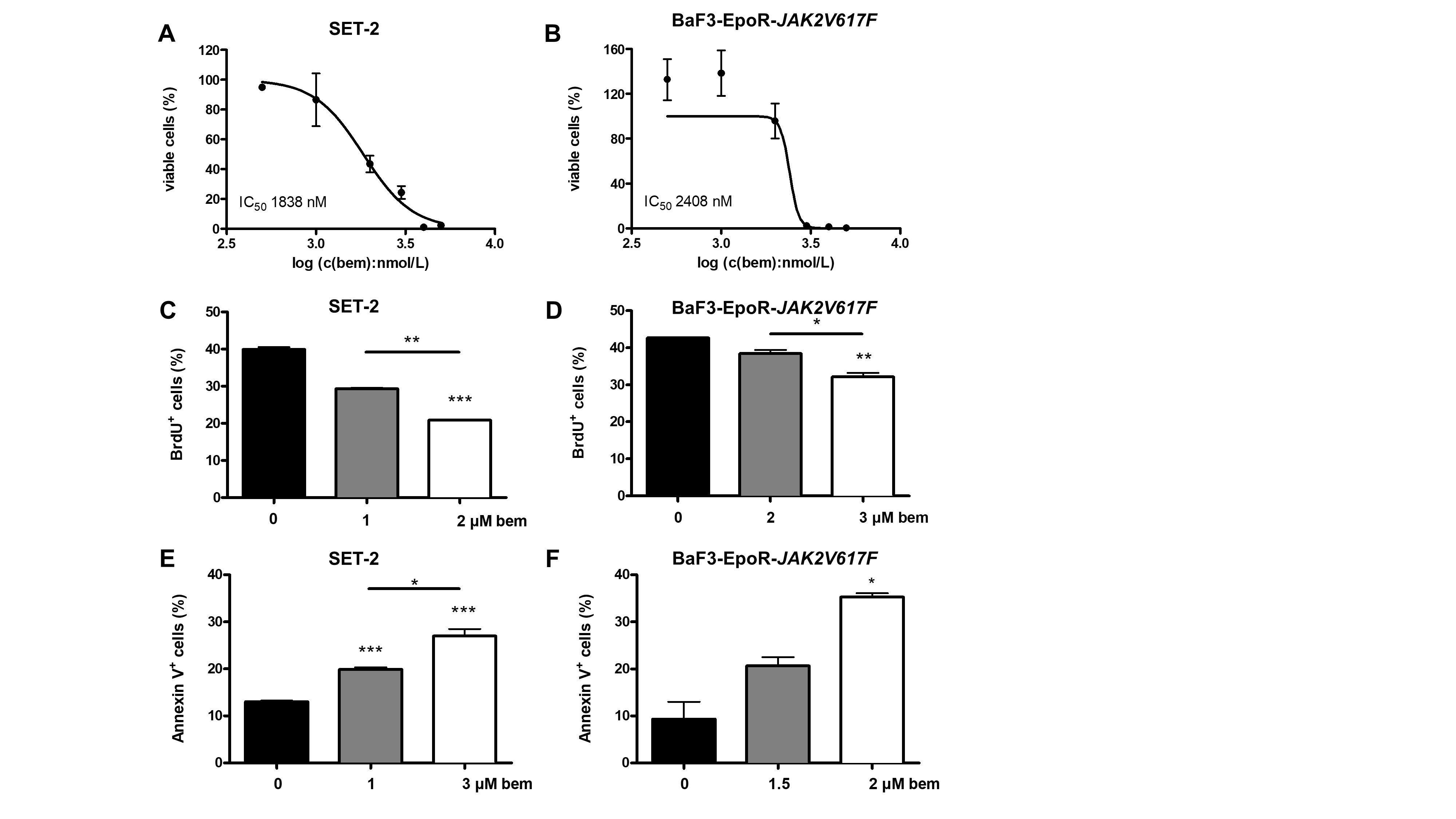
Automatisch generierte Beschreibung**

**SDC, Figure 2. Bemcentinib reduces viability in AXL- MPN cell lines.** Assessment of half maximal inhibitory concentration (IC50) for bemcentinib (bem). Treatments were performed with increasing concentrations (0, 0.5, 1, 2, 3, 4, 5 µmol/L) of bem (log10 scale) for 48h with human HEL (A) and UKE-1 (B) cells in serum deprived conditions. WST-1 cell viability assays were performed (n=3). Percentage of viable cells was normalized to control-treated cells.

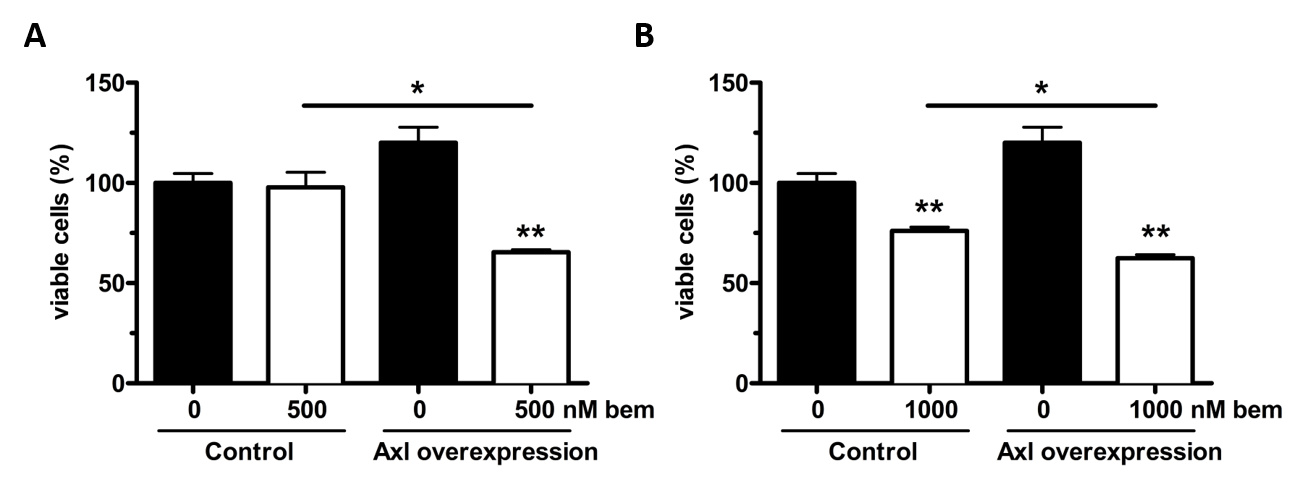
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Automatisch generierte Beschreibung

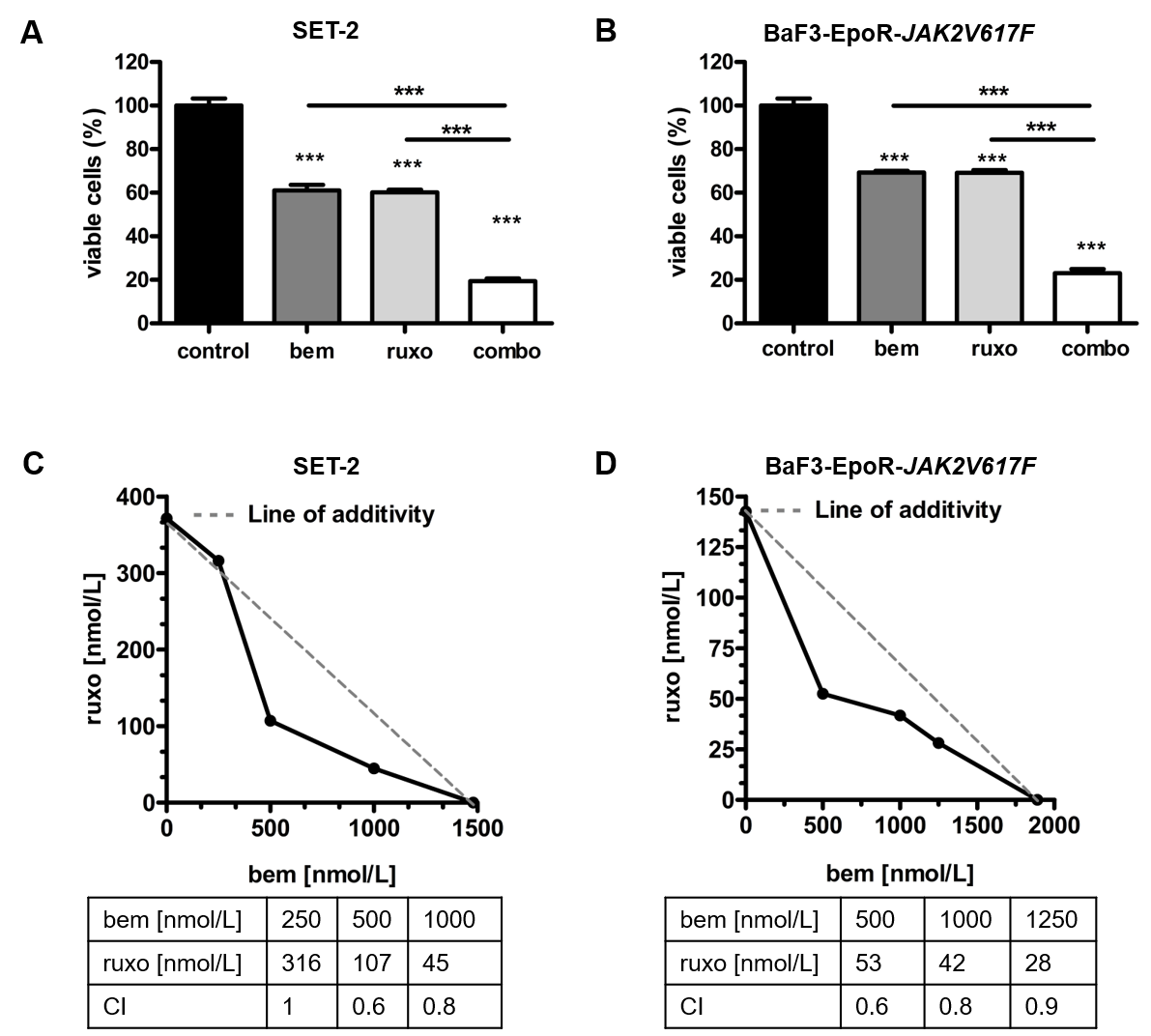
**SDC, Figure 3. GAS6 increases viability of AXL+ but not AXL- MPN cell.** SET-2, BaF3-EpoR-*JAK2V617F*, HEL, and UKE-1 cells were treated with increasing concentrations (0, 3.5, 7, 10, and 30 ng/ml) of recombinant human or murine GAS6, respectively, in serum deprived conditions. After 48h WST-1 cell viability assays were performed. Percentage of viable cells was normalized to control-treated cells (n=3, \*p<0.05).



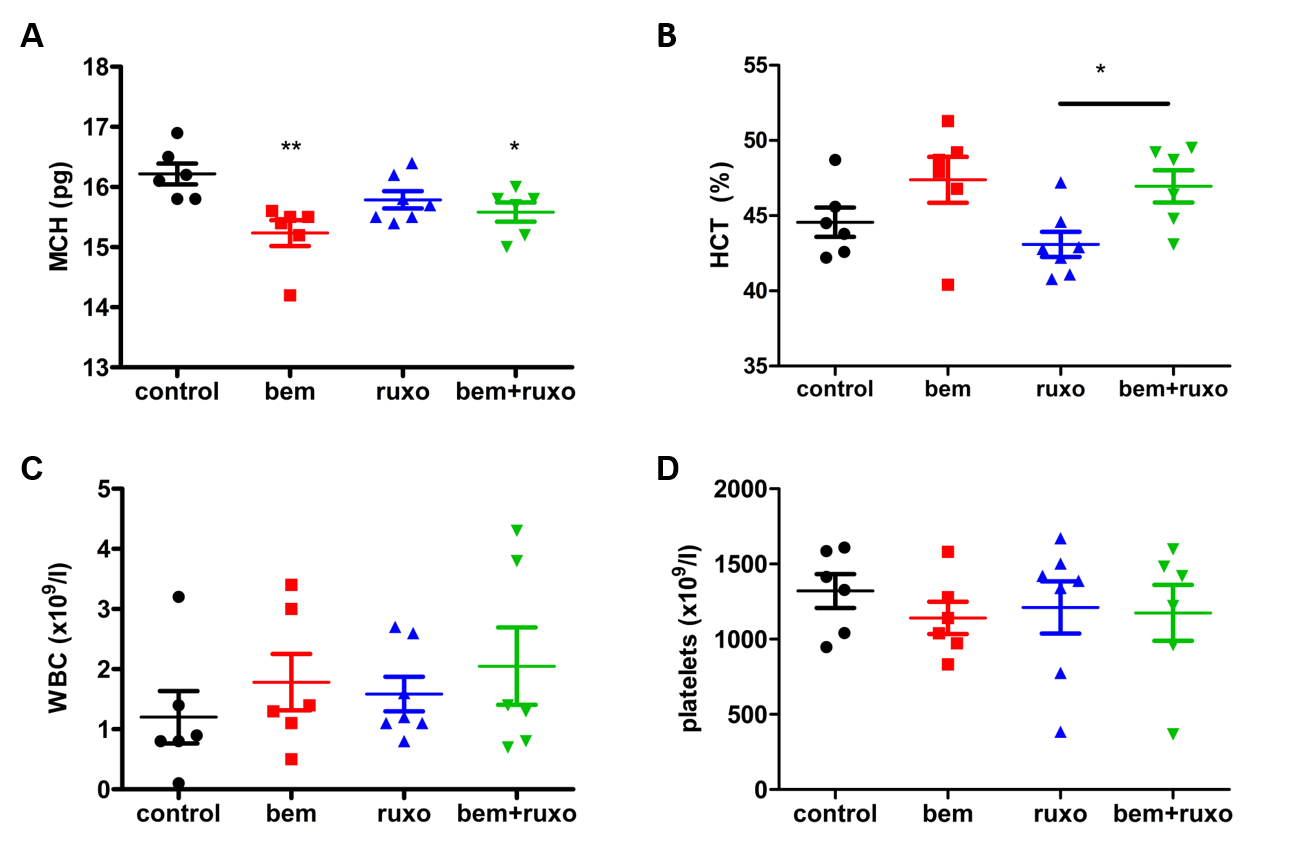
**SDC, Figure 4. Bemcentinib inhibits proliferation and induces apoptosis in MPN cell lines.** Assessment of half maximal inhibitory concentration (IC50) for bemcentinib (bem). Treatments were performed in normal medium (10% FBS) with increasing concentrations (0, 0.5, 1, 2, 3, 4, 5 µmol/L) of bem (log10 scale) for 48h with human SET-2 and murine BaF3-EpoR-*JAK2V617F* cells, WST-1 cell viability assays were performed. Percentage of viable cells was normalized to control-treated cells (A-B). FACS quantification of proliferation (BrdUstaining) showed a dose dependent decrease in proliferation rates after incubation of SET-2 and BaF3-EpoR-*JAK2V617F* cells with bem for 24h in serum containing medium (n=2) (C-D). FACS quantification of apoptosis (Annexin V staining) showed increased apoptosis of SET-2 and BaF3-EpoR-*JAK2V617F* cells after treatment with bem in normal medium conditions (n=2, \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001) (E-F).

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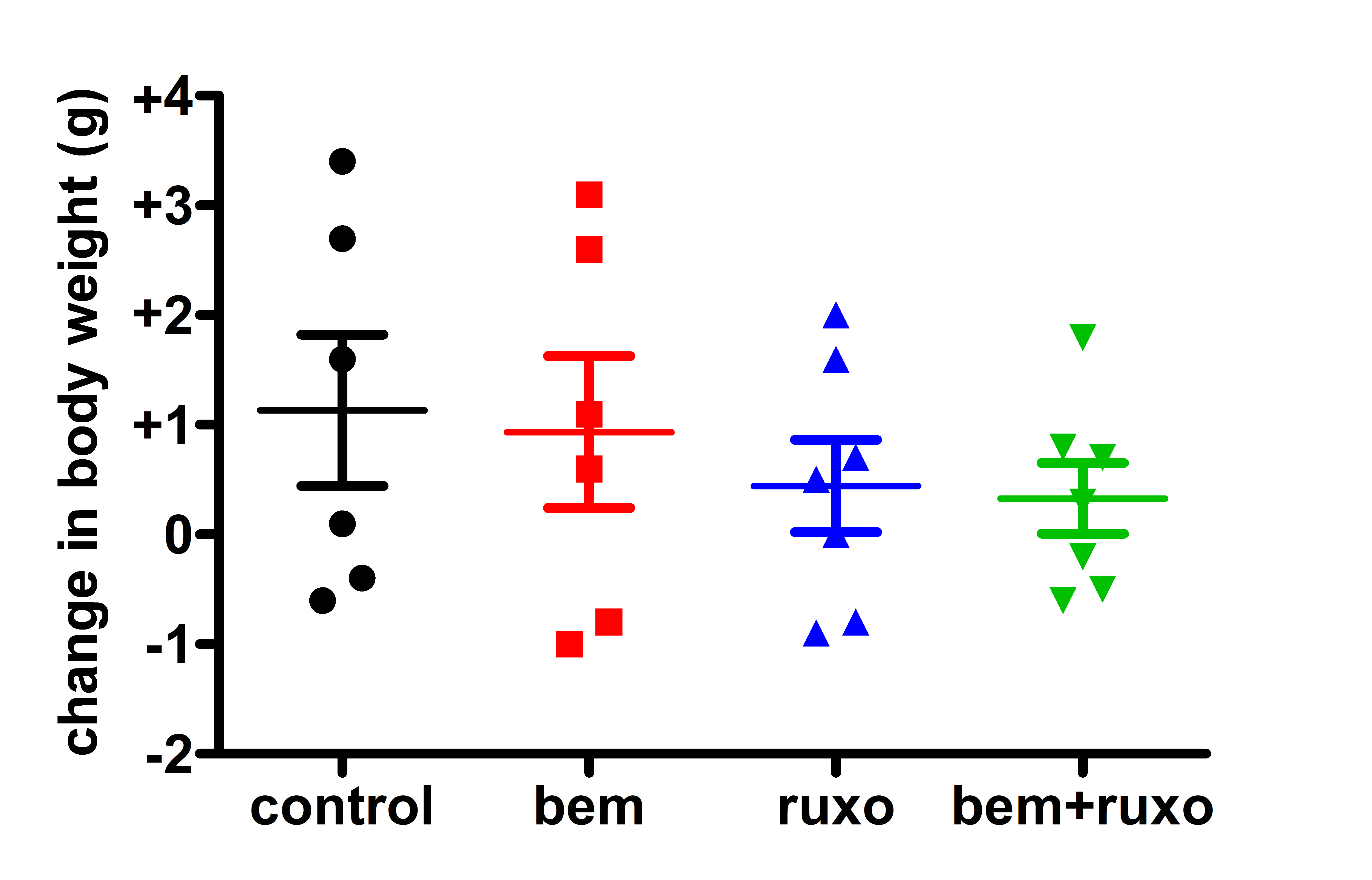
**SDC, Figure 5. Overexpression of Axl increases sensitivity to bemcentinib.** Overexpression of AXL was induced by lentiviral gene transfer. AXL overexpressing cells and control-transduced cells were incubated with 500nM (A) and 1000nM (B) bemcentinib (bem) for 48h in normal medium conditions followed by WST-1 cell viability assay. Percentage of viable cells was normalized to control-transduced untreated cells (n=3, \*p<0.05, \*\*p<0.01).



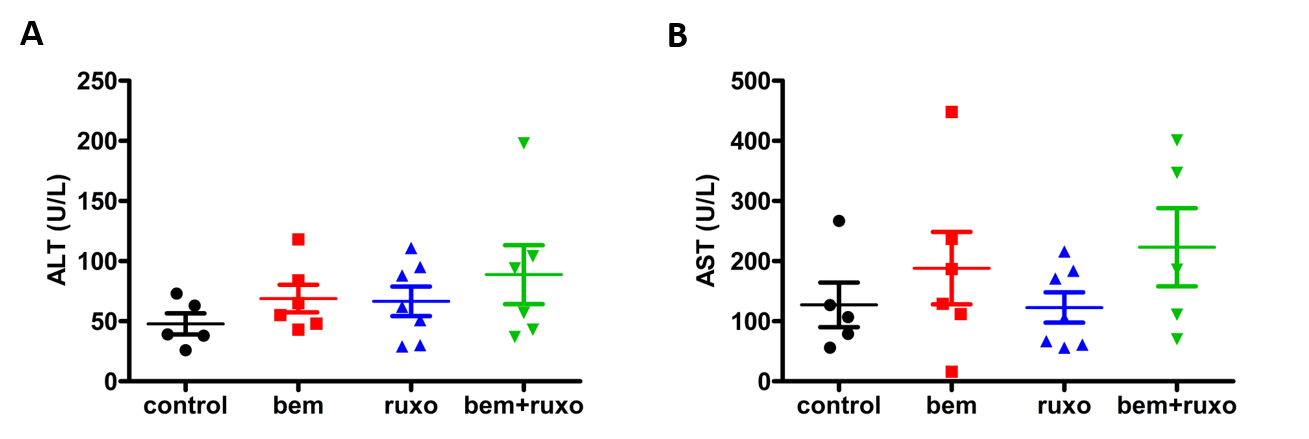
**SDC, Figure 6. Bemcentinib and ruxolitinib exert additive inhibitory effects *in vitro.*** SET-2 and BaF3-EpoR-*JAK2V617F* cells were treated with different concentrations of bemcentinib (bem) and ruxolitinib (ruxo) as single treatment or in combination for 48 hours in normal medium conditions, and cell viability was measured by WST-1 assay (n=3). Percentage of viable cells was normalized to control-treated cells (\*\*\*p<0.001). For SET-2 cells combination of 1000 nM bem and 200 nM ruxo is shown (A), for BaF3-EpoR-*JAK2V617F* cells combination of 1000 nM bem and 100 nM ruxo is shown (B). Isobologram analysis was performed to test for combination effects of bem and ruxo, combination indices (CI) were calculated based on *Loewe Additivity*. CI <0.8 indicate synergism, CI 0.8-1.2 indicate additivity (C-D).



**SDC, Figure 7. Blood count analysis shows good tolerability of combined bemcentinib and ruxolitinib treatment *in vivo*.** In a systemic model of *JAK2V617F*-driven disease, mice were treated with vehicle, bem (50mg/kg), ruxo (50mg/kg) or bem and ruxo (50mg/kg each) twice daily. Mice were sacrificed on day 18 (n=6/6/7/7) and blood counts were analyzed. Mean cellular hemoglobin (MCH) of erythrocytes (A), hematocrit (B), white blood cell count (WBC) (C) and platelet count (D) are presented. Significant outliers were identified using Grubb’s test and excluded from analysis. T-test was performed for analysis of statistical difference (\*p<0.05, \*\*p<0.01).



**SDC, Figure 8. Combined bemcentinib and ruxolitinib treatment showed no effect on body weight *in vivo*.** In a systemic model of *JAK2V617F*-driven disease, mice were treated with vehicle, bemcentinib (bem, 50mg/kg), ruxolitinib (ruxo, 50mg/kg) or bem and ruxo (50mg/kg each) twice daily and body weight was monitored (n=6/6/7/7). Change in body weight after 18 days of treatment in relation to body weight pre-treatment is presented.



**SDC, Figure 9. Combined bemcentinib and ruxolitinib treatment shows no hepatotoxicity *in vivo*.** In a systemic model of *JAK2V617F*-driven disease, mice were treated with vehicle, bem (50mg/kg), ruxo (50mg/kg) or bem and ruxo (50mg/kg each) twice daily. On day 18, alanine transaminase (ALT) (A) and aspartate transaminase (AST) (B) levels were measured by spectrophometric analysis.