Multiple oligomerization domains of KANK1-PDGFRβ are required for JAK2-independent hematopoietic cell proliferation and signaling via STAT5 and ERK

Sandrine Medves,[1] Laura A. Noël,[1] Carmen P. Montano-Almendras,[1] Roxana I. Albu,[1,2] Hélène Schoemans,[3,4] Stefan N. Constantinescu,[1,2] and Jean-Baptiste Demoulin[1]

[1]de Duve Institute, Université Catholique de Louvain, Brussels; [2]Ludwig Institute for Cancer Research, Brussels Branch; [3]Hematology Department, University Hospitals Leuven, Leuven; and [4]Leuvense Navelstrengbloed Bank, Leuven, Belgium

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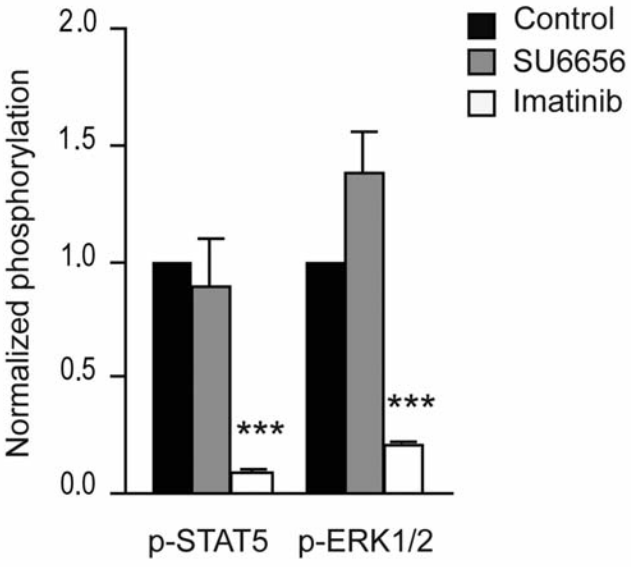
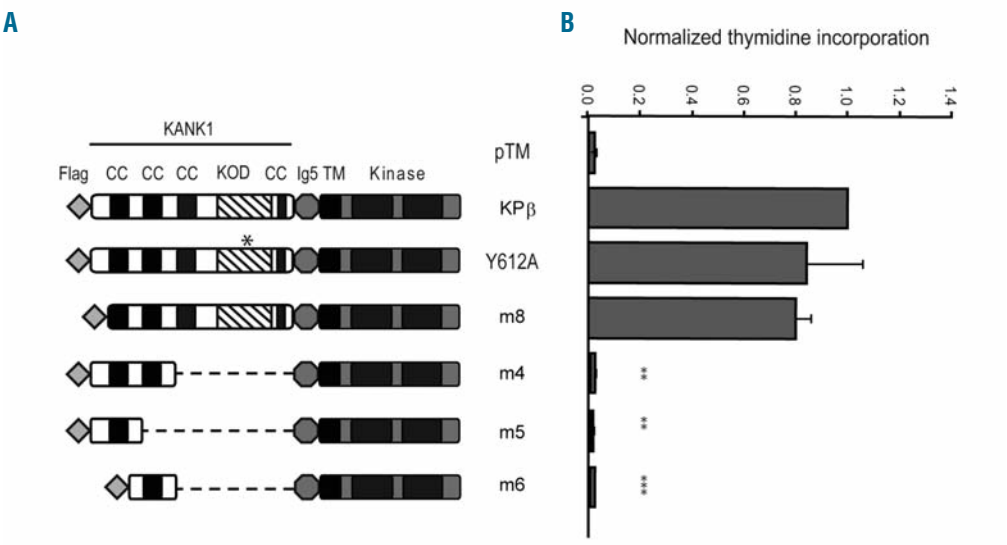
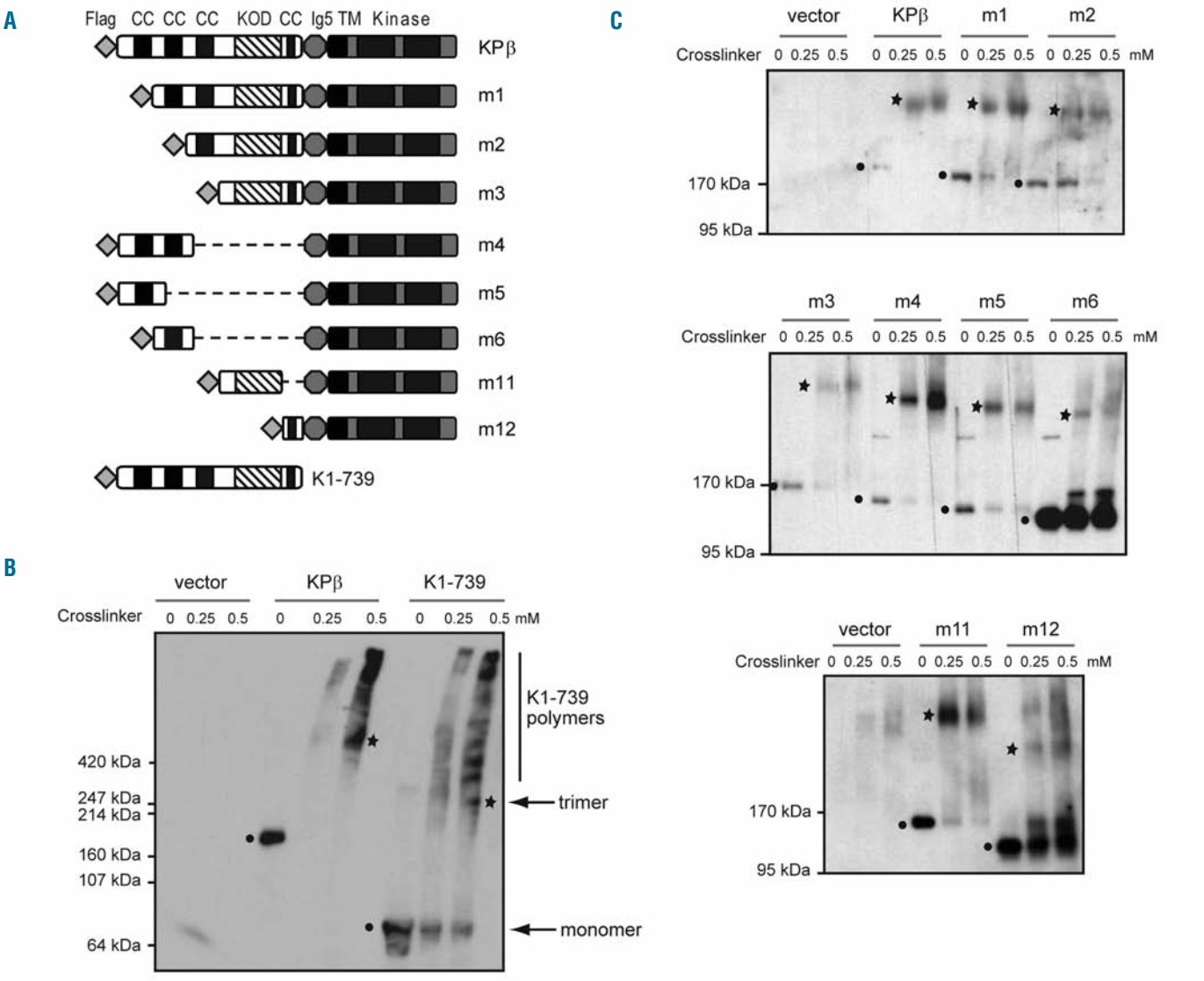


Online Supplementary Figure S1. Role of SRC in KPβ-induced signaling and proliferation. Ba/F3 cells were transduced with KPβ and grown in the absence of cytokines. Cells were treated with imatinib, SU6656 (both at 1 μM) or vehicle for 4 h. The phosphorylation of STAT5 and ERK was monitored by flow cytometry. The average of three experiments is shown with SEM. ***$P<0.001$ compared to control (Student's t-test).



Online Supplementary Figure S2. Identification of the KANK1 domains required for Ba/F3 cell transformation by KPβ. Additional deletion mutants of KPβ were generated as described in the *Design and Methods* section: m8: residues 100 to 739 of KANK1; m4: residues 2 to 287; m5, residues 2 to 202 and m6, residues 159 to 287 (according to SWISS-PROT accession number #Q6PIB3). The KPβ-Y612A mutant contains a point mutation corresponding to the tyrosine 612 to alanine substitution. Mutagenesis was performed using the QuickChange XL-II kit (Stratagene) according to the manufacturer's instructions. All mutants were checked by sequencing. (A) A schematic representation of KPβ and mutants. CC: coiled-coil domain; KOD, KANK1 oligomerization domain; Ig5 (octagon): Ig-like domain 5 of PDGFRβ; TM: transmembrane domain; Kinase: split tyrosine kinase domain. (B) Ba/F3 cells were transduced with KPβ, mutants or the pTM-898-neo empty vector as a control. Cells were grown for 72 h in the absence of IL3 and proliferation was estimated by thymidine incorporation. All cell lines proliferated to a similar extent in the presence of IL3 (*data not shown*). The average of multiple independent experiments is shown with SEM. $^{**}P<0.01$ compared to KPβ (Student's t-test).

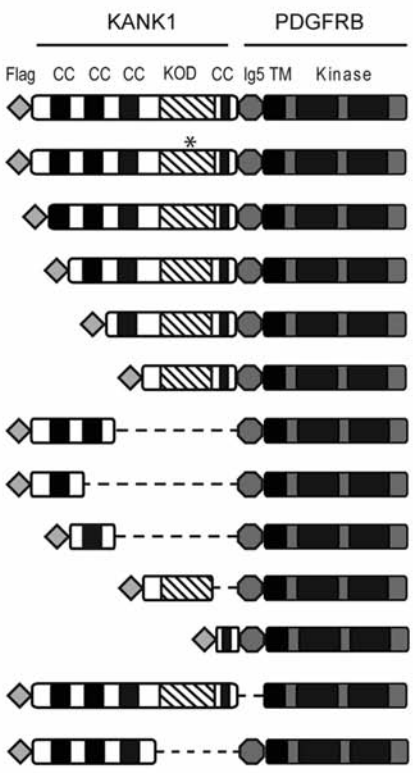


Online Supplementary Figure S3. Analysis of KPβ oligomerization by protein cross-linking. Cross-linking assays were performed in the presence of bis(sulfosuccinimidyl)-suberate (BS3, Pierce) as described elsewhere.[1] Briefly, 2×10^5 cells were washed once with ice-cold PBS and then lysed in 200 μL of 50 mM HEPES pH 7.5, 150 mM NaCl, glycerol 10% w/v, Triton 1% w/v, EDTA 1 mM, 1 mM Pefabloc (Roche), 1 μg/mL aprotinin and 1 mM Na_3VO_4. After clearing by centrifugation, lysates were incubated with 0.25 or 0.5 mM BS3 for 1.5 h at 4°C. Reactions were stopped by addition of 50 mM Tris-HCl for 15 min at room temperature. Sample proteins were separated on a gradient gel (4-12%, Invitrogen) and analyzed by western blot with anti-FLAG antibodies (Sigma) or with the anti-PDGFR antiserum CED.[2] High molecular weight protein standards (Invitrogen) were used to evaluate the molecular weight of the KPβ complexes. (A) A schematic representation of KPβ and mutants. CC: coiled-coil domain; KOD, KANK1 oligomerization domain; Ig5 (octagon): Ig-like domain 5 of PDGFRβ; TM: transmembrane domain; Kinase: kinase domain. (B-C) Lysates of Ba/F3 cells expressing the indicated mutant were treated with BS3 (0.25 or 0.5 mM) to cross-link protein complexes. Lysates were run on a gradient denaturing polyacrylamide gel and subjected to western blotting with anti-FLAG (B) or anti-PDGFRβ antibodies (C). Balls and stars indicate monomers and trimeric complexes, respectively. Disappearance of the monomeric forms (m1 to m5 and m11) is indicative of efficient oligomerization.

References

1. Toffalini F, Hellberg C, Demoulin JB. Critical role of the platelet-derived growth factor receptor (PDGFR)-beta transmembrane domain in the TEL-PDGFRbeta cytosolic oncoprotein. J Biol Chem. 2010;285(16):12268-78.
2. Demoulin JB, Seo JK, Ekman S, Grapengiesser E, Hellman U, Ronnstrand L, et al. Ligand-induced recruitment of Na+/H+-exchanger regulatory factor to the PDGF (plateletderived growth factor) receptor regulates actin cytoskeleton reorganization by PDGF. Biochem J. 2003;376(Pt 2):505-10.

Online Supplementary Figure S4. Summary of the results. CC, coiled coil; TM, transmembrane domain; nd, not determined.



	Oligomers	Proliferation	STAT5	ERK1/2	Phosphorylation
KPβ	+	+++	++	++	+
Y612A	nd	+++	++	nd	+
m8	+	+++	++	nd	+
m1	+	++	++	+	+
m2	+	++	+	+	+
m3	+	+	+	+	+
m4	+	-	-	nd	+
m5	+	-	-	nd	+
m6	-	-	-	nd	-
m11	+	+	+	+	+
m12	-	-	-	-	-
m13	+	+++	++	nd	+
m14	+	++	++	-	+