Supplemental Data

The p21-activated Kinase PAK3 Forms Heterodimers with PAK1 in Brain Implementing Trans-regulation of PAK3 Activity

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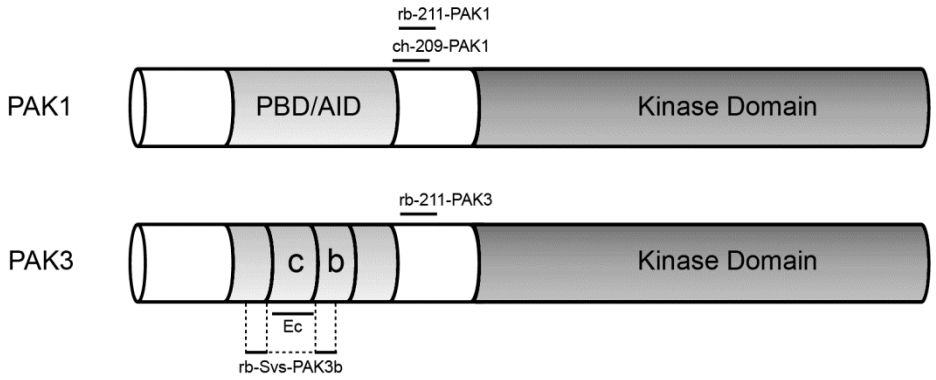
A

	ANTIBODY NAME	SPECIES	POLY/MONOCLONAL	origin	EPITOPE	WB	IP	IF
PAK1	ch-209-PAK1	chicken	polyclonal	Barnier's lab	LTVTPTRDVAT	+	-	ND
	rb-211-PAK1	rabbit	polyclonal	Barnier's lab	VTPTRDVATSPISPTEN	+	+	+
	N20-PAK1	rabbit	polyclonal	Santa Cruz	N20	+	+	ND
PAK3	rb-211-PAK3	rabbit	polyclonal	Barnier's lab	ASPAAPNKEDIPPSAENA	+	+	ND
	3A12-PAK3	mouse	monoclonal	Sigma	Nter-90-AA	+	ND	+
	N19-PAK3	goat	polyclonal	Santa Cruz	N19	+	+	ND
	rb-Svs-PAK3b	rabbit	polyclonal	Barnier's lab	GEFTPDLY	+	+	ND
	Ec	rabbit	polyclonal	Barnier's lab*	PFQTSRPVTVASSQSEGKM	+	+	ND

*Kreis et al., 2008

B



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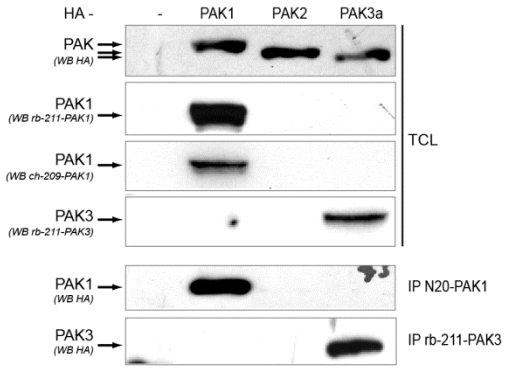

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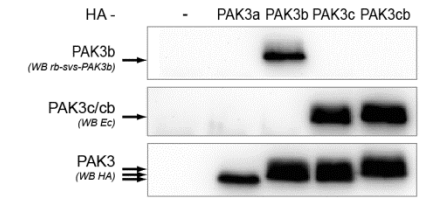


Supplemental Fig. S1: Characterization of PAK specific sera. To analyze co-localization, co-purification and co-immunoprecipitation of endogenous PAK proteins, we developed PAK1 and PAK3 isoform-specific antibodies and PAK3 splice variant-specific antibodies (SD 1*A*) that would be appropriate for immunofluorescence, for Western blotting, and for immunoprecipitation assays. Location of antigens is indicated on PAK structure (SD 1*B*). We produced them in two host species (chicken and rabbit) to permit simultaneous use with mouse monoclonal or goat polyclonal antibodies. The specificity of each affinity-purified antibody was tested on HeLa cell lysates previously transfected with HA-tagged PAK1, PAK2, or PAK3a plasmids (SD 1*C*). We produced PAK1 antibodies directed against a unique sequence (209-219 amino-acids) in chicken and named them ch-209-PAK1, and also a rabbit polyclonal antiserum against a 211-227 peptide, named rb-211-PAK1. Both sera are specific to PAK1 in Western blotting, and the rabbit serum is well suited for immunofluorescence assay. The commercial N20-PAK1 is specific to PAK1 protein in immunoprecipitation assay. For PAK3 proteins, the mouse monoclonal 3A12 gave a specific fluorescent labeling on hippocampal neurons and no signal on *pak3*- neurons (data not shown). For PAK3 immunoprecipitation, we used a rabbit polyclonal serum generated against a unique PAK3 sequence located between amino acids 211-228 shared by all PAK3 splice variants: this new antibody named rb-211-PAK3 was able to specifically immunoprecipitate PAK3a and not PAK1 nor PAK2 (SD 1*C*). For analyzing

PAK3 splice variants, we also produced rabbit antibodies (SD 1*D*) directed against a peptide restricted to the PAK3b splice variant by designing a 8-mer peptide whose sequence encompasses the last four amino-acids of the coding exon 2 and the first four amino-acids encoded by the exon b. We carefully showed that this serum named rb-svs-PAK3b recognizes the PAK3b protein and does not recognize the PAK3cb splice variant, since the epitope is interrupted by the sequence encoded by the exon c. The rabbit antibody directed against a peptide issued from an internal sequence of the exon c, named Ec was previously described (17). Thus, we developed very specific antibodies, able to discriminate between the different PAK proteins.

A, table summary of the characteristics and properties of the PAK sera used in this study. *B*, location of antigens in the PAK structures. *C*, characterization of PAK1 and PAK3 specific antibodies. HA-tagged PAK1, PAK2, PAK3a plasmids were transfected in HeLa cells and their expression was checked by HA immunoblotting (first panel). Western blot specificity of the rabbit rb-211-PAK1 (second panel), ch-209-PAK1 (third panel), and rb-211-PAK3 (fourth) sera were analyzed on TCL of transfected cells. The specificity of the sera N20-PAK1 (fifth panel) and rb-211-PAK3 (sixth panel) in immunoprecipitation assays were tested on transfected cells and immunoprecipitates were analyzed by Western blot using the HA antibody. *D*, characterization of the PAK3 variant antibodies. Antibodies specific to the PAK3b variant (rb-svs-PAK3b, first panel) or of the insert c (Ec, second panel) were tested on lysates of HeLa cells transfected with HA-PAK3a, b c or cb plasmids by Western blotting using HA antibodies as control (third panel).

set 1
F: 5'CTGAGCAATGGGCACGACTATTCCAAACCTCCAACATAAC3'
R: 5'GTTATGTTGGAGGTTTGGAATAGTCGTGCCCATTGCTCAG3'

set 2
F: 5'GGATGGCACCTGAAGTGGTAACTGAAGATGCATATGGTCCAAAAGTTGATATCT3'
R: 5' CCAGATATCAACTTTTGGACCATATGCATCTTCAGTTACCACTTCAGGTGCCAT3'.

set 3
F: 5'ATGCGGATCCACTTTGTACAGGAATACAGAATCG3'
R: 5'GTCGACCTACTGTTCTTAATTGCTTCC3'

set 4
R: 5'GCCAGGATCCGCTTTGACCCGGAATACTG3'
F: 5'GGCTCTAGATCAGTGATTGTTCTTGGTTGC3'

set 5
R: 5'ATGCGGATCCACTTTGTACAGGAATACAGAATCG3'
F: 5'GGCTCTAGACTAACGGCTACTGTTCTTAATTGC 3'

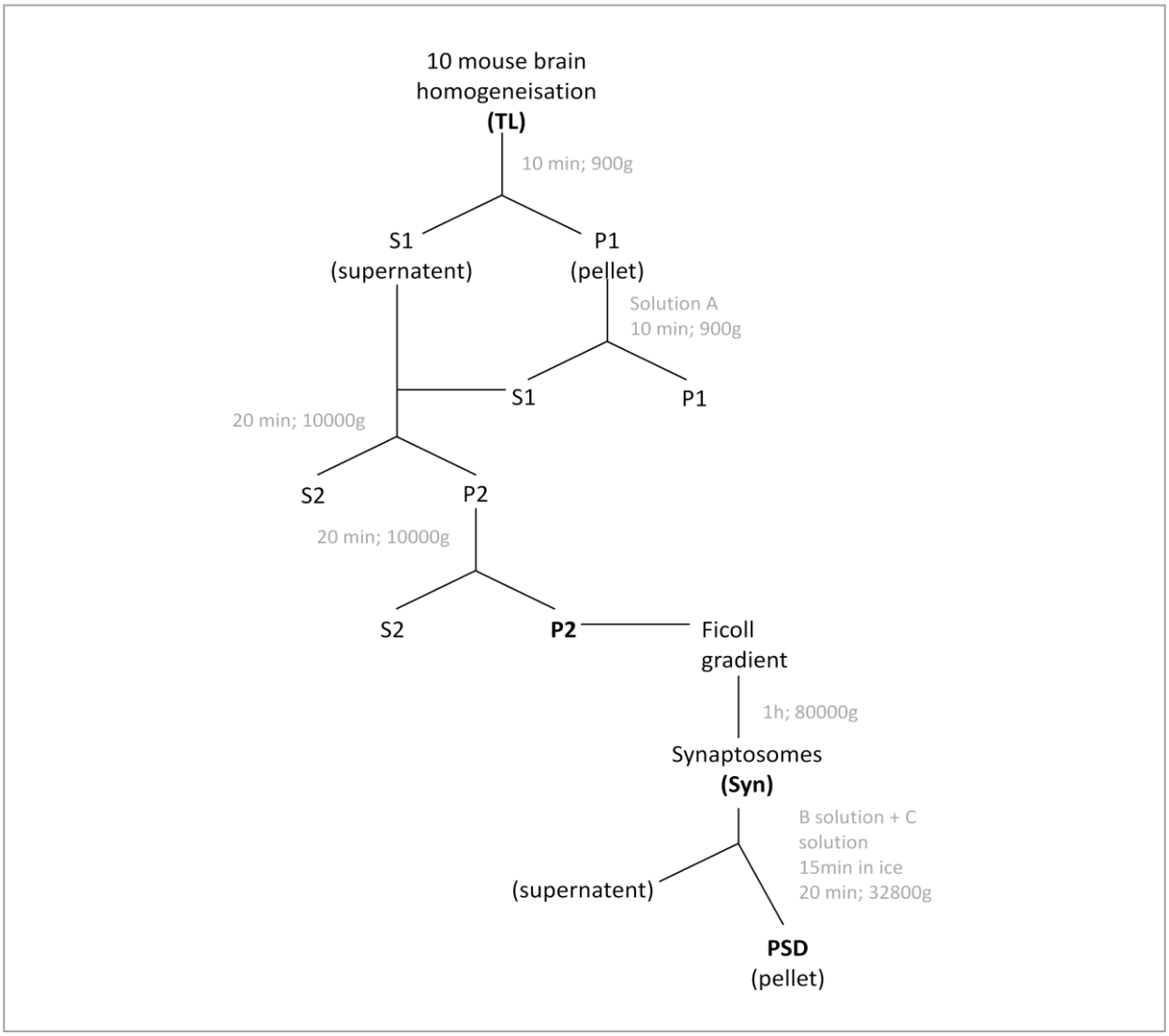
set 6
R: 5'GGGAATTCGCTCAAATAACGGCGTAGACATCC-3'
F: 5' CCGCTCGAGTTACTACGGTGTGTACTTCTTCTTGGGG3'

set 7
F: 5'CAGGATCCTCTGACAGCTTGGATAACG3'
R: 5'GCGTCGACCTATCTCGTATATTTCTTCTTTGGGTC3'

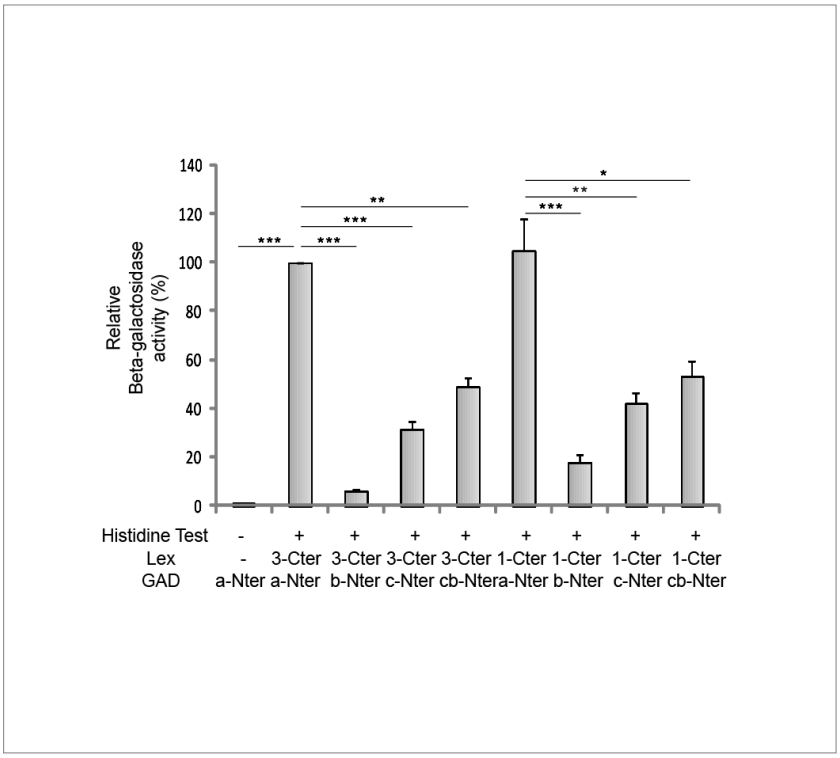
set PAK1
F: 5'CCCCTCCGATGAGAAACACC3'
R: 5'CTGGCATCCCCGTAAACTCC3'

set actin
F: 5'CTAAGGCCAACCGTGAAAAGATG3'
R: 5'AGATGGGCACAGTGTGGGTGACC3'.

Supplemental Table S2: List of primers used for plasmid construction or RT-PCR. Sets 1 to 7 were used to amplify PAK sequences by PCR in order to construct plasmids. PAK1 set and actin set were used to amplify the PAK1 and actin cDNAs, respectively, to detect their expression in cytoplasm of single neurons.



Supplemental Fig. S3: Schematic representation of the method used to prepare synaptosomes and postsynaptic fraction from mice brain. Along the preparation, protein samples of the different fractions were conserved: the Total Lysate (TL) corresponds to the total brain homogenate from adult male mice; the second pellet (P2) corresponds to synaptosomes with mitochondria, synaptic vesicles and plasma membrane; the synaptosomal (Syn) fraction was obtained after purification from a 7.5% and 14% Ficoll gradient and the postsynaptic density (PSD) fraction was obtained after detergent extraction of the synaptosomes.



Supplemental Fig. S4: Two-hybrid analysis of the PAK3 splice variant interactions. N-terminal portion of the different splice variants of PAK3 fused to GAD bait were co-expressed in yeast with the C-terminal moieties of PAK1 and PAK3 fused to Lex. Growth on histidine-medium indicates an interaction which was measured by the β-galactosidase assay. Quantifications were done relatively to the PAK3a-Nter/PAK3-Cter interaction. The presence of the alternatively spliced exons c and b induces a strong decrease of the interactions between the C-terminal moieties of PAK1 and PAK3, suggesting that the inserts modify structures involved in dimer formation. However this decrease is less pronounced toward PAK1 than PAK3, reinforcing the idea that the splice variants heterodimerize with PAK1 *in vivo*. Yeast growth on histidine minus media (Histidine test) indicates a protein-protein interaction (+). β-galactosidase activity was expressed relative to the PAK3a-Nter/PAK3a-Cter interaction. Comparison with Student t test: *, $p<0.05$, ***, $p<0.001$, n=3.