

# Supplemental Materials

*Molecular Biology of the Cell*

Shin et al.

## Supplemental Figure Legends

**FIGURE S1:** (A) LC-MS/MS analysis leading to validation of phosphorylation of S223 of PAK1. GFP-PAK1 expressed in 293T cells with (CK2 $\alpha$ -shRNA) or without (CK2 $\alpha$ -NT, a non-targeting shRNA) CK2a knockdown were processed for mass spectrometric analysis by LC-MS/MS. Fragmentation tables for  $^{215}\text{RDVATSPISPTENNTTPPDAL}^{235}$  (left) and  $^{215}\text{RDVATSPISPTENNTTPPDAL}^{235}$  (right).  $m/z$  values highlighted in orange and blue indicate the presence of standard b- and y-type fragment ions, respectively, whereas those highlighted in green indicate special gains and losses from the b and y ions. (B) Sequence comparison between PAK1 sequences (215-235) in human, mouse and rat. (C) Sequence comparison between the sequences of PAK1, PAK2 and PAK3 in human.

**FIGURE S2:** MTT proliferation assays of NIH 3T3 (A) and Rat1 (B) cells. Cell viability over time was expressed as fold increase over untreated control at day 1. Error bars represent mean  $\pm$  S.D (n=5). The mouse embryo fibroblast cell line NIH 3T3 and the rat fibroblast *cell line* Rat1 cells were cultured in RPMI 1640 or DMEM containing 10% FBS and penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Western blot analysis of expression of GFP-PAK1 (WT), GFP-PAK1<sup>S223A</sup> (S223A), and empty vector (V) in corresponding cells (inserts).

**FIGURE S3:** (A) Interaction between GFP-Rac1 and Myc-PAK1. Myc-PAK1 (WT) or Myc-PAK1<sup>S223A</sup> was coexpressed with GFP-Rac1 in 293T cells. Cell extracts were immunoprecipitated with anti-Myc antibody (IP) and then immunoblotted with anti-GFP or anti-Myc antibody. (B) Rac1 activity is not regulated by CK2. Rac1 activity was determined by its ability to interact with the PBD domain of Pak1 in vitro, as described previously (Benard and Bokoch, 2002). Top: PC3 cell lysates (500 $\mu$ g) were treated with GTP $\gamma$ S (positive control) to activate Rac1 or treated with GDP (negative control) to inactivate Rac1. Activated Rac1 was enriched by the pull-down assay and detected by Western blot (GTP $\gamma$ S). Levels of active Rac1 in PC3 cells with (sh1 and sh2) or without (NT, non-targeting) CK2a knockdown were determined by GST pull-down (PD) assays. GTP $\gamma$ S and GDP were purchased from Pierce. Bottom: Active Rac1-GTP and total Rac1 in the extracts were assessed by Western blot using anti-Rac1 antibody (WB).

**FIGURE S4:** Tumor growth of RWPE-1 cells infected with lentivirus expressing the vector, GFP-PAK1, and GFP-PAK1<sup>S223A</sup>. Subcutaneous growth of RWPE-1 cells expressing the vector plasmid, GFP-PAK1, GFP-PAK1<sup>S223A</sup>. RWPE-1 ( $4 \times 10^6$ ) cells were injected subcutaneously into female nude mice at the age of 5 weeks (n=8).

A

CK2 $\alpha$ -NT

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	157.1	79.1	140.1		R	2,276.0	1,138.5	2,259.0	2,258.0	21
2	272.1	136.6	255.1	254.1	D	2,119.9		2,102.9	2,101.9	20
3	371.2	186.1	354.2	353.2	V	2,004.9		1,987.9	1,986.9	19
4	442.2	221.6	425.2	424.2	A	1,905.8		1,888.8	1,887.8	18
5	543.3	272.1	526.3	525.3	T	1,834.8		1,817.8	1,816.8	17
6	630.3	315.7	613.3	612.3	S	1,733.8		1,716.7	1,715.8	16
7	727.4	364.2	710.3	709.4	P	1,646.7		1,629.7	1,628.7	15
8	840.5	420.7	823.4	822.4	I	1,549.7		1,532.7	1,531.7	14
9	1,007.5	504.2	990.4	989.4	S+80	1,436.6		1,419.6	1,418.6	13
10	1,104.5	552.8	1,087.5	1,086.5	P	1,269.6		1,252.6	1,251.6	12
11	1,205.6	603.3	1,188.5	1,187.5	T	1,172.5		1,155.5	1,154.5	11
12	1,334.6	667.8	1,317.6	1,316.6	E	1,071.5		1,054.5	1,053.5	10
13	1,448.6	724.8	1,431.6	1,430.6	N	942.5		925.4	924.4	9
14	1,562.7	781.8	1,545.7	1,544.7	N	828.4		811.4	810.4	8
15	1,663.7	832.4	1,646.7	1,645.7	T	714.4			696.4	7
16	1,764.8	882.9	1,747.8	1,746.8	T	613.3			595.3	6
17	1,861.8	931.4	1,844.8	1,843.8	P	512.3			494.3	5
18	1,958.9	979.9	1,941.9	1,940.9	P	415.2			397.2	4
19	2,073.9	1,037.5	2,056.9	2,055.9	D	318.2			300.2	3
20	2,144.9	1,073.0	2,127.9	2,126.9	A	203.1				2
21	2,276.0	1,138.5	2,259.0	2,258.0	L	132.1				1

CK2 $\alpha$ -shRNA

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	157.1	79.1	140.1		R	2,196.1	1,098.5	2,179.1	2,178.1	21
2	272.1	136.6	255.1	254.1	D	2,040.0		2,023.0	2,022.0	20
3	371.2	186.1	354.2	353.2	V	1,925.0		1,907.9	1,906.9	19
4	442.2	221.6	425.2	424.2	A	1,825.9		1,808.9	1,807.9	18
5	543.3	272.1	526.3	525.3	T	1,754.8		1,737.8	1,736.8	17
6	630.3	315.7	613.3	612.3	S	1,653.8		1,636.8	1,635.8	16
7	727.4	364.2	710.3	709.4	P	1,566.8		1,549.7	1,548.8	15
8	840.5	420.7	823.4	822.4	I	1,469.7		1,452.7	1,451.7	14
9	927.5	464.2	910.5	909.5	S	1,356.6		1,339.6	1,338.6	13
10	1,024.5	512.8	1,007.5	1,006.5	P	1,269.6		1,252.6	1,251.6	12
11	1,125.6	563.3	1,108.6	1,107.6	T	1,172.5		1,155.5	1,154.5	11
12	1,254.6	627.8	1,237.6	1,236.6	E	1,071.5		1,054.5	1,053.5	10
13	1,368.7	684.8	1,351.6	1,350.7	N	942.5		925.4	924.4	9
14	1,482.7	741.9	1,465.7	1,464.7	N	828.4		811.4	810.4	8
15	1,583.8	792.4	1,566.7	1,565.8	T	714.4			696.4	7
16	1,684.8	842.9	1,667.8	1,666.8	T	613.3			595.3	6
17	1,781.9	891.4	1,764.8	1,763.9	P	512.3			494.3	5
18	1,878.9	940.0	1,861.9	1,860.9	P	415.2			397.2	4
19	1,993.9	997.5	1,976.9	1,975.9	D	318.2			300.2	3
20	2,065.0	1,033.0	2,048.0	2,047.0	A	203.1				2
21	2,196.1	1,098.5	2,179.1	2,178.1	L	132.1				1

B

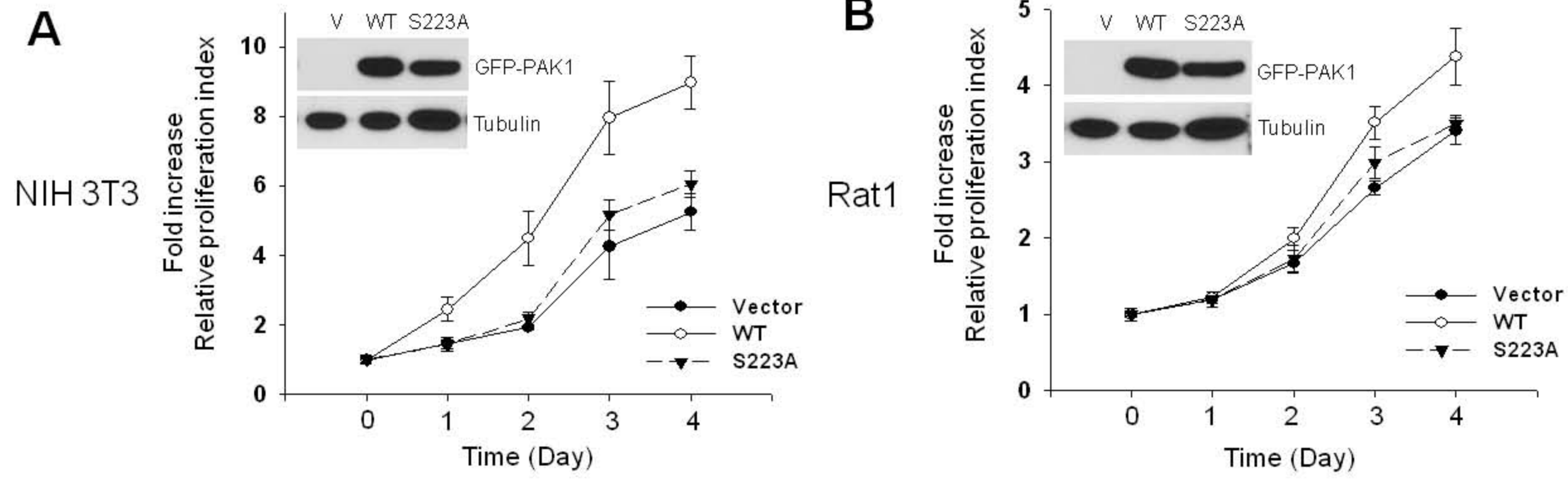
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Homo sapiens      215-RDVATSPISPTENNTTPPDAL-234  
Mus musculus      215-RDVATSPISPTENNTTPPDAL-234  
Rattus norvegicus 215-RDVATSPISPTENNTTPPDAL-234

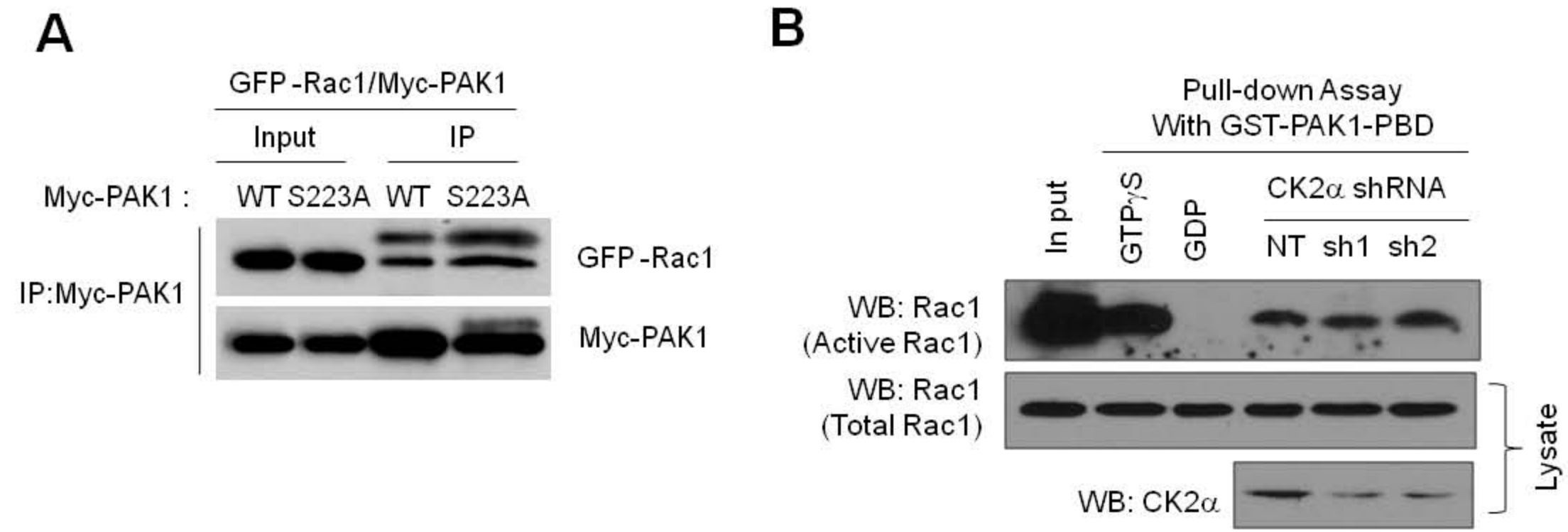
C

Human PAK1    200 VYTRSVIEPL--PVTPTRDVATSPISPTENNTTPPDALTRNTE    240  
Human PAK2    193 IYTRSVIDP--VP-APVGD~~SHV~~-----DGAAKSLD    219  
Human PAK3    201 IYTRSVVESIASPAVPNKEV-TPPSAENANSST----LYRNTD    238

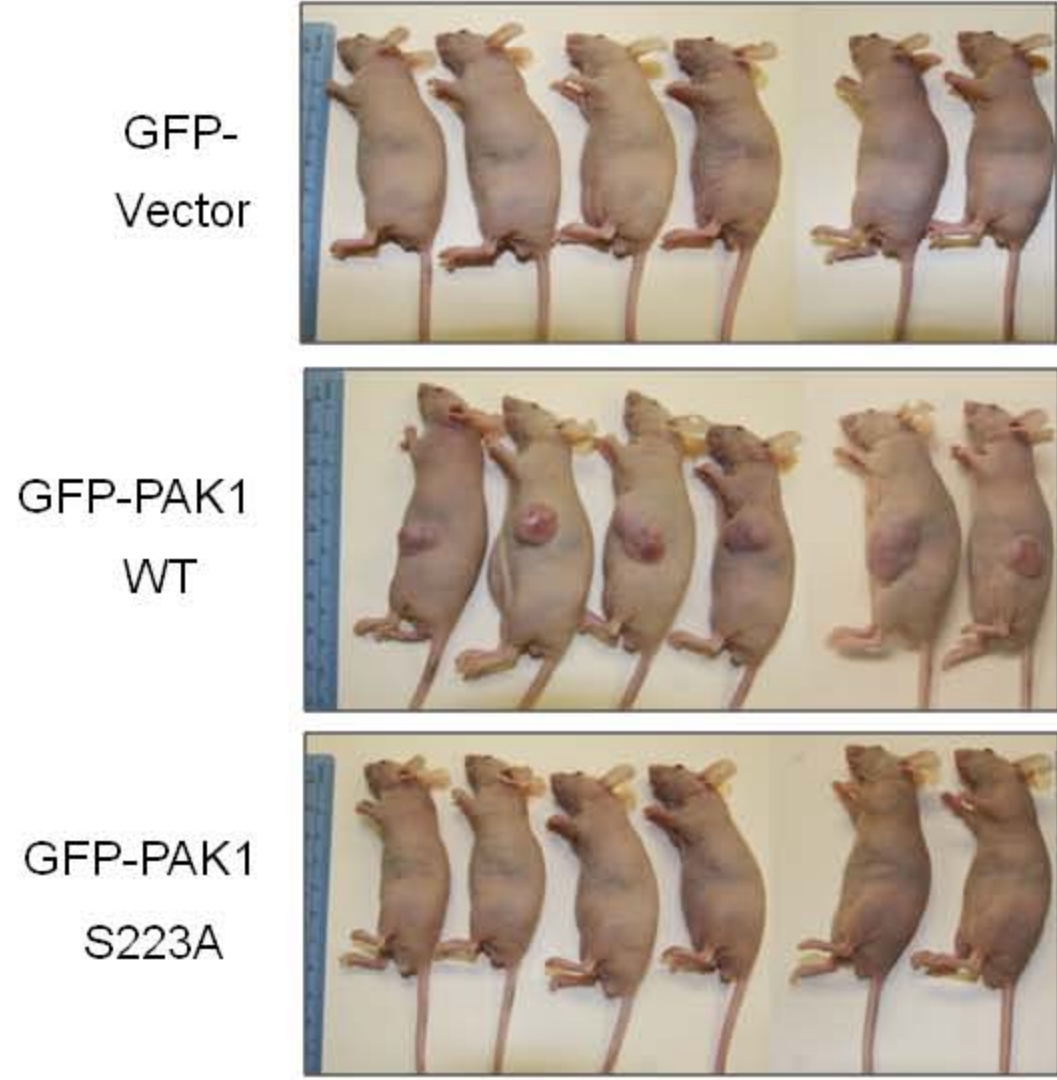
Figure S1



**Figure S2**



**Figure S3**



**Figure S4**