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Supplementary Materials for

Tks5-Dependent, Nox-Mediated Generation of Reactive Oxygen Species Is Necessary for Invadopodia Formation

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Table S1. The expression of Nox family members in cancer cells.

Gene	**Src-3T3**	**SCC61**	**C8161.9**	**RPMI-7951**	**Bt549**
Nox1	+	+/-	-	-	-
Nox2	-	+/-	-	+/-	+/-
Nox3	+/-	-	-	-	-
Nox4	+	+	+	+	+

The expression of Nox 1-4 was determined by RT-PCR using primer pairs as described in *D. Gianni, B. Bohl, S. A. Courtneidge, G. M. Bokoch, The involvement of the tyrosine kinase c-Src in the regulation of reactive oxygen species generation mediated by NADPH oxidase-1. Mol Biol Cell **19**, 2984 (2008).*

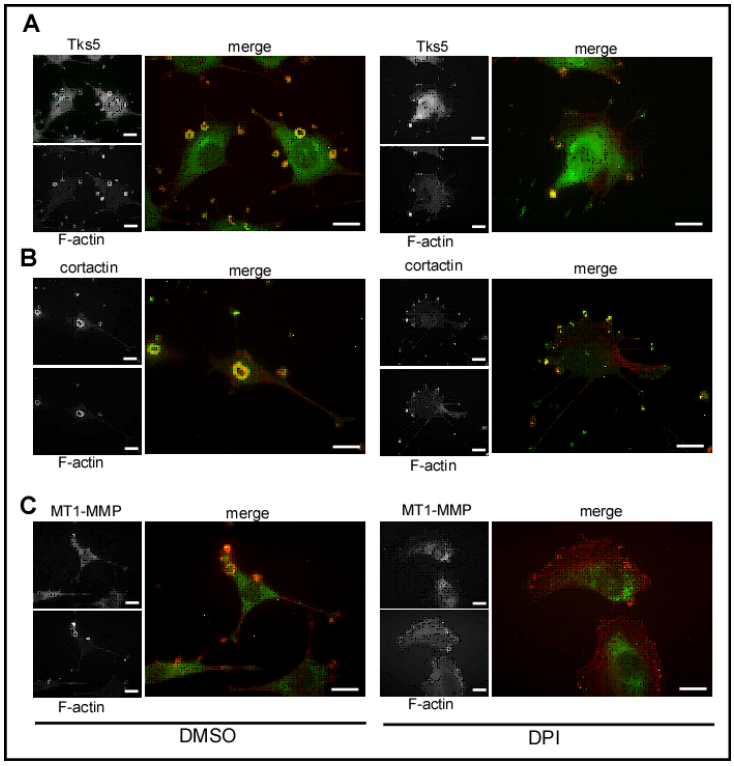

Fig. S1. Characterization of DPI-treated Src-3T3 cells.
Src-3T3 cells were treated with DMSO or 20μM DPI as indicated, then stained for Tks5 (**A**), cortactin (**B**), or MT1-MMP (**C**). In each case, the phalloidin stain is in red, and the antibody stain in green. Bars, 4μm.

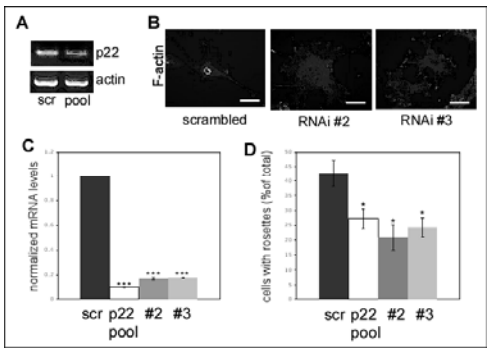

Fig. S2. p22 knockdown in Src-3T3 cells.
A. RT-PCR analysis of p22 and actin for the experiment shown in Fig. 3A and B. **B.** Representative pictures of the effect of scrambled (scr) and two siRNAs specific for p22 (RNAi#2 and RNAi#3) on invadopodia formation, as judged by phalloidin staining of F-actin. Bar, 4μm. **C.** qPCR analysis of p22 abundance (normalized to cyclophilin) following transfection of pooled or individual p22 siRNAs, as shown in panel B. (***) $p<0.0001$ for the indicated samples with respect to control (Student's t-test). **D.** Quantitation of percentage of cells containing rosettes for the transfected cells in panel B. (*) $p<0.05$ for the indicated samples with respect to control (Student's t-test).

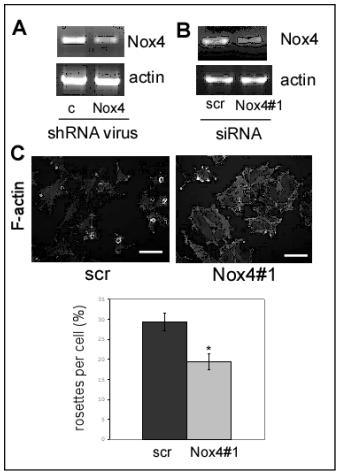

Fig. S3. Nox4 knockdown in Src-3T3 cells.
A. RT-PCR analysis of Nox4 and actin abundance for the experiment shown in Figure 3D. **B.** RT-PCR analysis of Nox4 and actin abundance following transfection of scrambled or Nox4#1 siRNA. **C.** Representative pictures (top) and quantitation (bottom) of rosette formation of the transfected cells analyzed for mRNA levels in panel B. Bar, 20μm. (*) $p<0.05$ (Student's t-test).

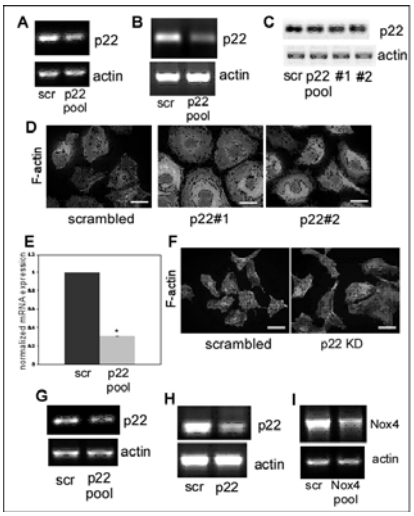

Fig. S4. Knockdown of Nox components in human cancer cells.
A. RT-PCR analysis of p22 and actin abundance for the experiment shown in Figure 5A (invadopodia assay). **B.** RT-PCR analysis of p22 and actin abundance for the experiment shown in Figure 5A (gelatin assay). **C.** RT-PCR analysis of p22 and actin abundance following transfection of SCC61 cells scrambled, p22 pooled, siRNA#1 and siRNA#2. **D.** Representative images of SCC61 cells transfected with scrambled, siRNA#1 and siRNA#2, analyzed in panel C. Bar, 4μm. **E**. qPCR analysis of p22 mRNA abundance, normalized to cyclophilin, for the experiment shown in Figure 6B. (*) $p<0.0005$ (Student's t-test). **F.** Representative images of C8161.9 cells transfected with scrambled and $p22^{phox}$ siRNA pool, stained with phalloidin. Bar, 20μm. **G.** RT-PCR analysis of p22 and actin mRNA abundance, for the experiment shown in panel F. **H.** RT-PCR analysis of p22 and actin mRNA levels, for the experiment shown in Figure 5B. **I.** RT-PCR analysis of Nox4 and actin abundance, corresponding to the experiment shown in Figure 5C.

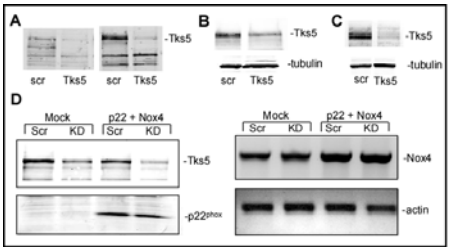

Fig S5. Knockdown of Tks5 and ROS production.
Tks5 abundance determined by immunoblotting for the experiments shown in Figure 6A (**A**), 6B (**B**) and 6C (**C**). B16-F10 melanoma cells from the experiment shown in Figure 6D were probed by immunoblot for Tks5 and $p22^{phox}$ (**D**, left) and Nox4 and actin mRNA abundance by RT-PCR (**D**, right).

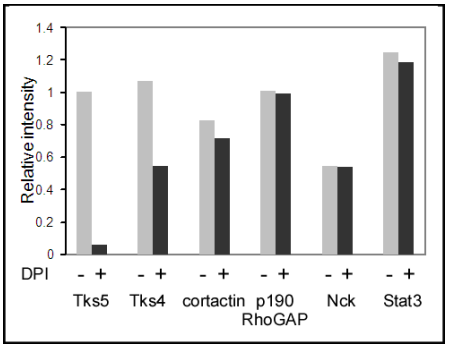

Fig. S6. Relative phosphotyrosine levels of Src substrates.
Relative intensity (pixel density) of the phosphotyrosine bands with respect to total protein bands in the immunoprecipitation experiment in Fig. 8 calculated using Adobe Photoshop Software.