

Supplementary Table

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 reference 33.

Legends to Supplementary Figures

Supplementary Figure 1. Characterization of DPI-treated Src-3T3 cells.

Src-3T3 cells were treated with DMSO or 20 μ M DPI as indicated, then stained for Tks5 (Panel A), cortactin (panel B) or MT1-MMP (panel C). In each case, the phalloidin stain is in red, and the antibody stain in green.

Supplementary Figure 2. Knockdown of p22 in Src-3T3 cells.

Panel A. RT-PCR analysis of p22 and actin levels for the experiment shown in Figure 3A and B.

Panel 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 the F-actin.

Panel C. qPCR analysis of p22 levels (normalized to cyclophilin levels) following transfection of pooled or individual p22 siRNAs, as shown in panel B.

Panel D. Quantitation of percentage of cells containing rosettes for the transfected cells in panel B.

Supplementary Figure 3. Knockdown of Nox4 in Src-3T3 cells.

Panel A. RT-PCR analysis of Nox4 and actin levels for the experiment shown in Figure 3D.

Panel B. RT-PCR analysis of Nox4 and actin levels following transfection of scrambled or Nox4#1 siRNA.

Panel C. Representative pictures (top) and quantitation (bottom) of rosette formation of the transfected cells analyzed for mRNA levels in panel B.

Supplementary Figure 4. Knockdown of Nox components in human cancer cells.

Panel A. RT-PCR analysis of p22 and actin levels for the experiment shown in Figure 5A (invadopodia assay).

Panel B. RT-PCR analysis of p22 and actin levels for the experiment shown in Figure 5A (gelatin assay).

Panel C. RT-PCR analysis of p22 and actin levels following transfection of SCC61 cells scrambled, p22 pooled, siRNA#1 and siRNA#2.

Panel D. Representative images of SCC61 cells transfected with scrambled, siRNA#1 and siRNA#2, as analyzed in panel C.

Panel E. qPCR analysis of p22 mRNA levels, normalized to cyclophilin levels, for the experiment shown in Figure 6B.

Panel F. Representative images of C8161.9 cells transfected with scrambled and p22^{phox} siRNA pool, stained with phalloidin.

Panel G. RT-PCR analysis of p22 and actin mRNA levels, for the experiment shown in panel F.

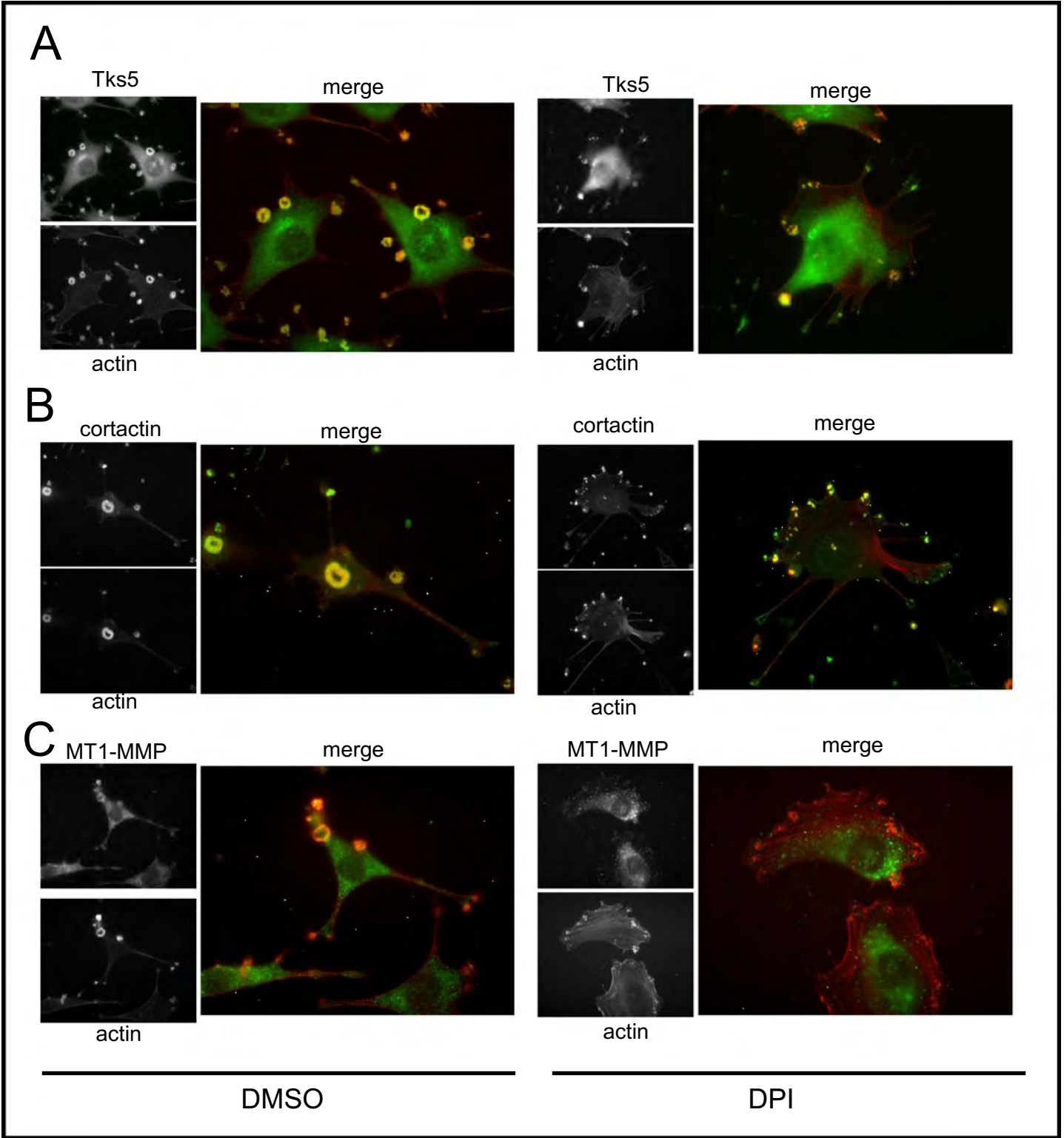
Panel H. RT-PCR analysis of p22 and actin mRNA levels, for the experiment shown in Figure 5B.

Panel I. RT-PCR analysis of Nox4 and actin levels, corresponding to the experiment shown in Figure 5C.

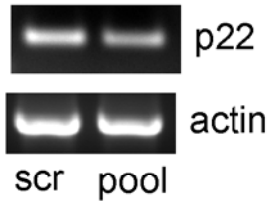
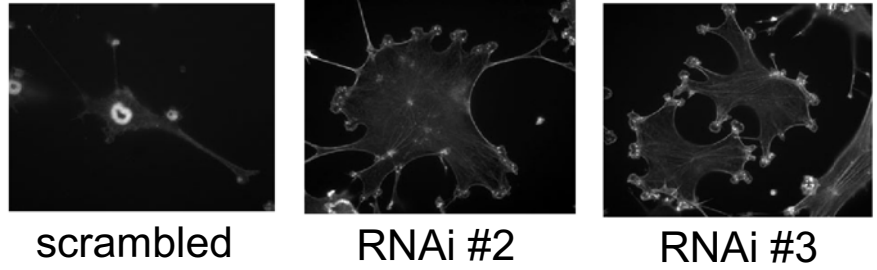
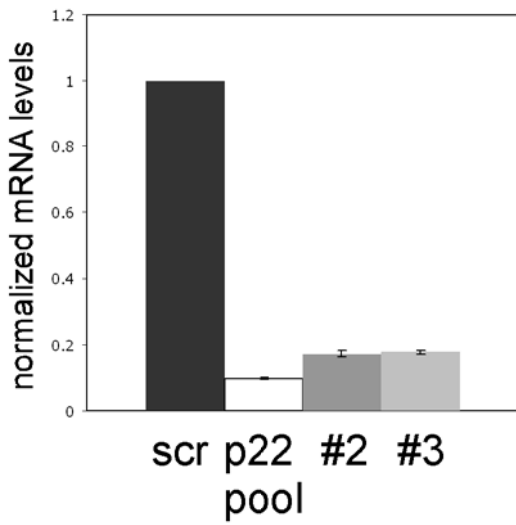
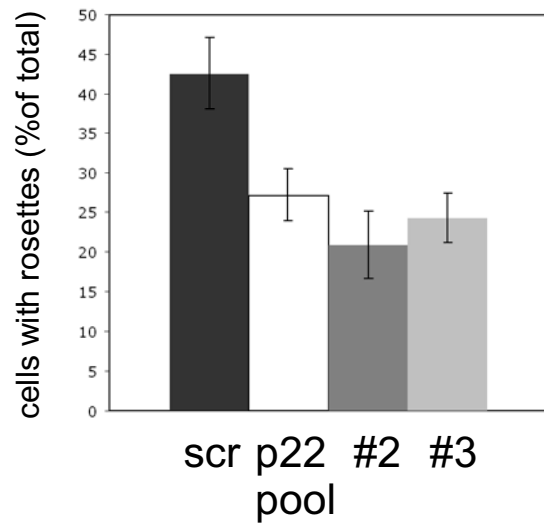
Supplementary Figure 5. Knockdown of Tks5 and ROS production.

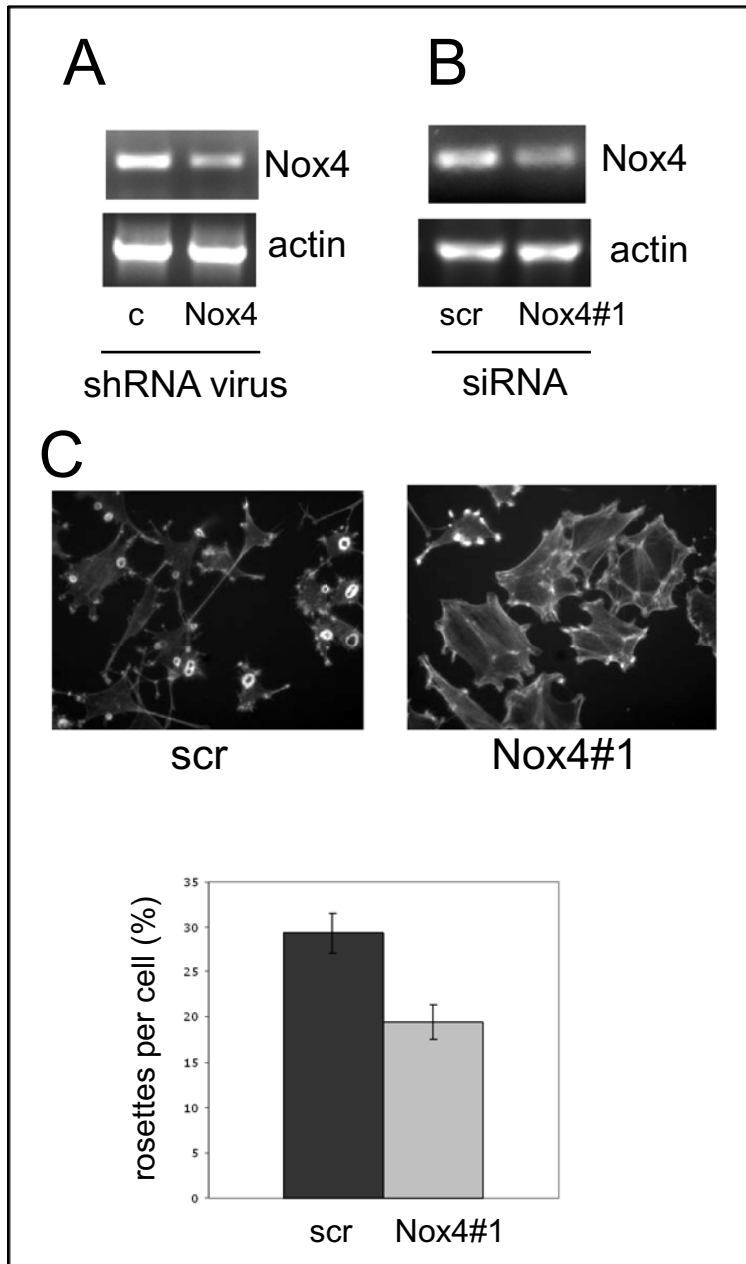
Tks5 levels were determined by immunoblotting for the experiments shown in Figure 6A (Panel A), 6B (Panel B) and 6C (Panel C). B16-F10 melanoma cells from the experiment shown in Figure 6D were probed by immunoblot for Tks5 and p22^{phox} levels (Panel D, left) and Nox4 and actin mRNA levels by RT-PCR (Panel D, right).

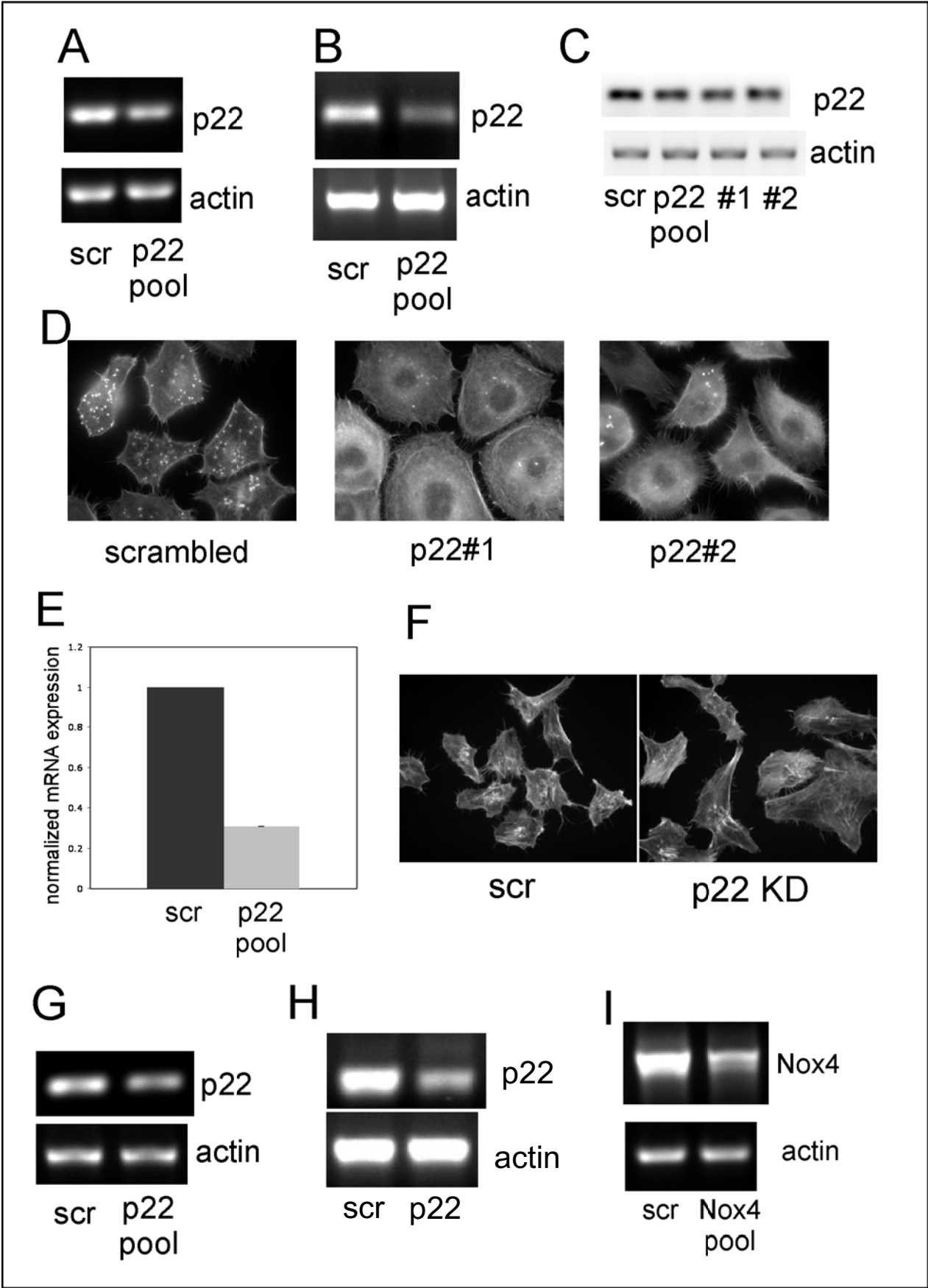
Supplementary Figure 6. Relative phosphotyrosine levels of the Src substrates shown in Figure 8A.



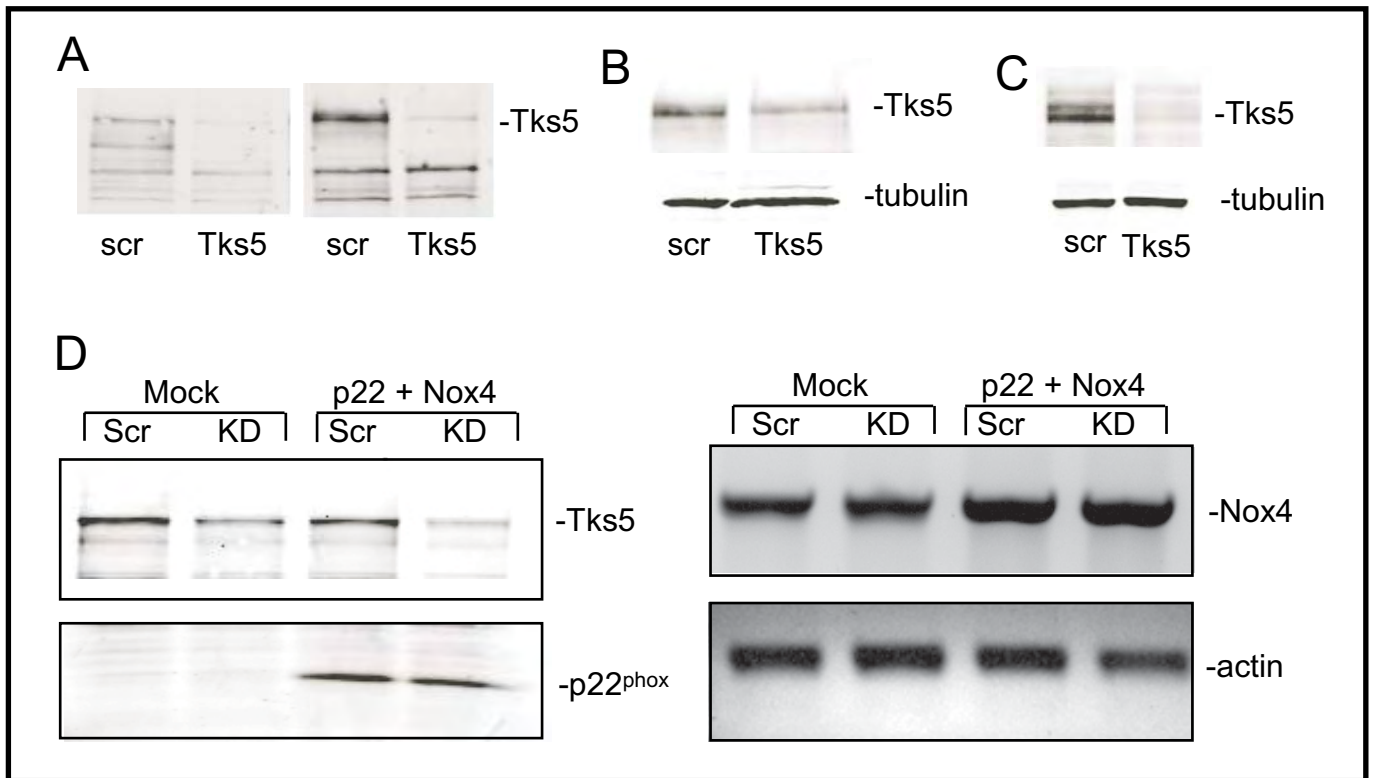
Diaz et al Supplementary Figure 1

A**B****C****D**

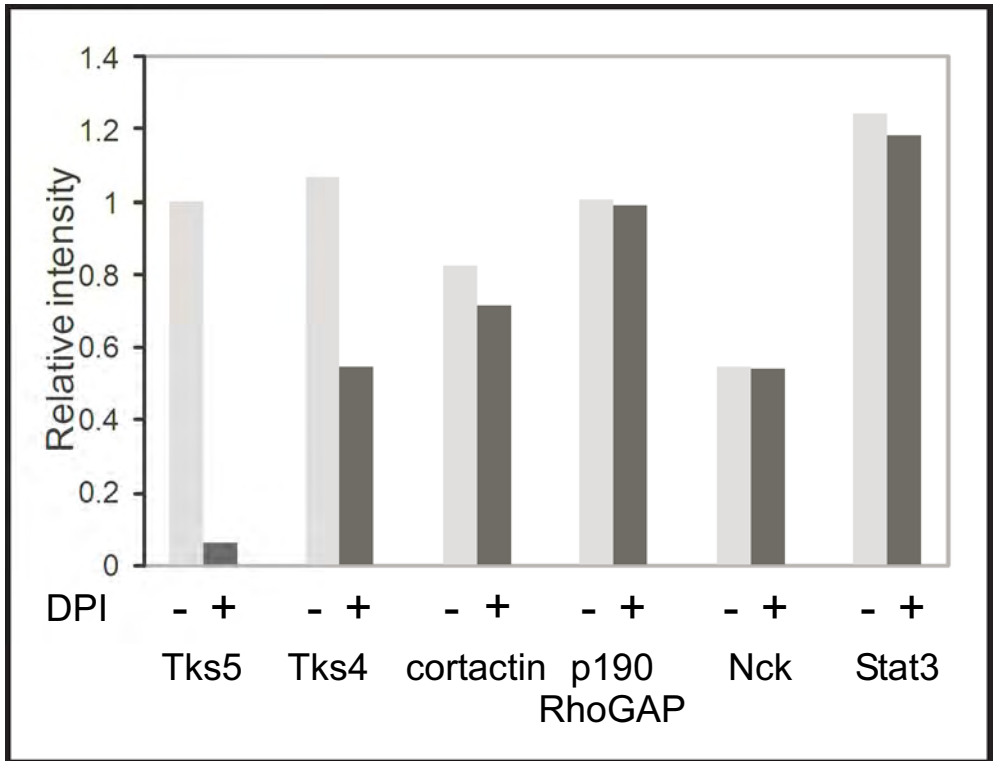




Diaz et al, Supplementary Figure 4



Diaz et al Supplementary Figure 5



Diaz et al Supplementary Figure 6