Supplementary Figure S1: The interaction between NoxA1 and Tks5 is dependent on Src activity

An alternative immunoprecipitation approach is performed to prove that the interaction between NoxA1 and Tks5 is dependent on Src activity. HEK293 cells were transfected as indicated with Flag-tagged Tks5 and with Myc-tagged NoxA1 in presence of constitutive active or dominant negative Src (SrcYF or SrcKM respectively). After 24 h, cells were lysed and immunoprecipitation (IP) was carried out using Flag antibody. Lys indicates lysate before IP was performed. The interaction between NoxA1 and Tks5 and comparable levels of transfected NoxA1 was tested by immunoblot (IB) using NoxA1 antibody (upper section). Similar expression levels of transfected Flag-tagged Tks5 in cell lysates and immunoprecipitation efficiency was assessed by re-blotting the membrane with Tks5 specific antibody (lower section). One representative experiment from three separate experiments is shown.

Supplementary Figure S2: The abolishment of phosphorylation of Tyr508 of Tks4 blocks the interaction between NoxA1 and Tks4.

The GST-pulldown analysis reveals that the integrity of Tyr508 of Tks4 is important for its binding to NoxA1. HEK293 cells were transfected as indicated with empty vector or with wild type Tks4, unphosphorylable Tks4 Y508F and phosphomimetic Tks4 Y508E. After 24 h, cells were lysed and cell lysates were used as the source of different Tks4 protein and incubated as indicated with equal amounts of GST alone or GST-fusion NoxA1 protein, which were pre-bound to glutathione-Sepharose beads. GST-pulldown was performed and the interaction between GST-fusion NoxA1 and different Tks4 proteins and similar levels of transfected Tks4 proteins in cell lysates was tested using the Tks4 antibody (upper panel). The re-blot using GST-

specific antibody in the lower panel indicates that GST-fusion protein were present at comparable levels in the GST-pulldown analysis. One representative experiment from three separate experiments is shown.

Supplementary Figure S3: The contemporary presence of Tks4 and NoxA1 unphosphorylable mutants blocks their SrcYF-induced interaction and ROS generation

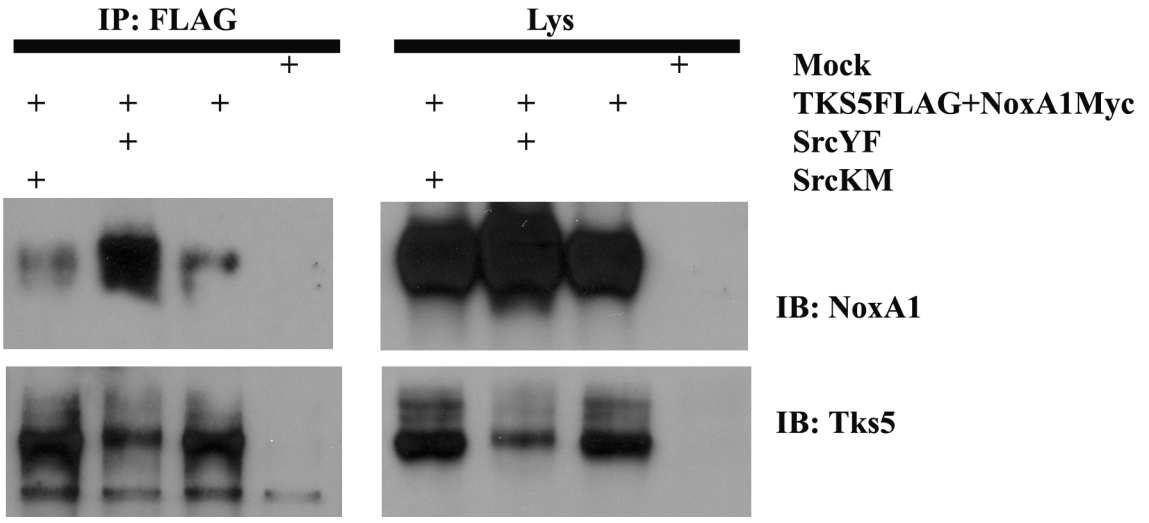
(A) Tks4 and NoxA1 unphosphorylable mutants block their SrcYF-induced interaction. Immunoprecipitation analysis was performed in HEK293 cells transfected as indicated. After 24 h, cells were lysed and immunoprecipitation (IP) was carried out using NoxA1 antibody. The interaction between NoxA1 and Tks4 (lower section) and comparable expression levels of different transfected Tks4 proteins (upper section) was tested by immunoblot (IB) using Tks4 antibody. One representative experiment from three separate experiments is shown.

(B) Only the contemporary presence of Tks4 and NoxA1 unphosphorylable mutants block SrcYF-induced, Nox1-dependent ROS generation. ROS generation was monitored in HEK293 cells transfected as indicated using CL assay. One representative experiment from three separate experiments is shown, and data are given as mean of triplicates +/- S.D. * $p<0.01$

Supplementary Figure S4: DLD1 cells shown in Figure 6 were transfected with GFP

DLD1 cells shown in Figure 6 were transfected with GFP and therefore also expressing the indicated expression vectors as they were transfected with GFP:indicated plasmid ratio of 1:5. We adopted this strategy to avoid scoring non-transfected cells in the analysis of this experiment (and the other experiments quantified in Figure 6B), which could hide the effect of the unphosphorylable mutants due to the endogenous NoxA1 and Tks4. Scale bars, 5 μm.

Figure S1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Lys
Mock
TKS5FLAG+NoxA1Myc
SrcYF
SrcKM
IB: NoxA1
IB: Tks5

Figure S2

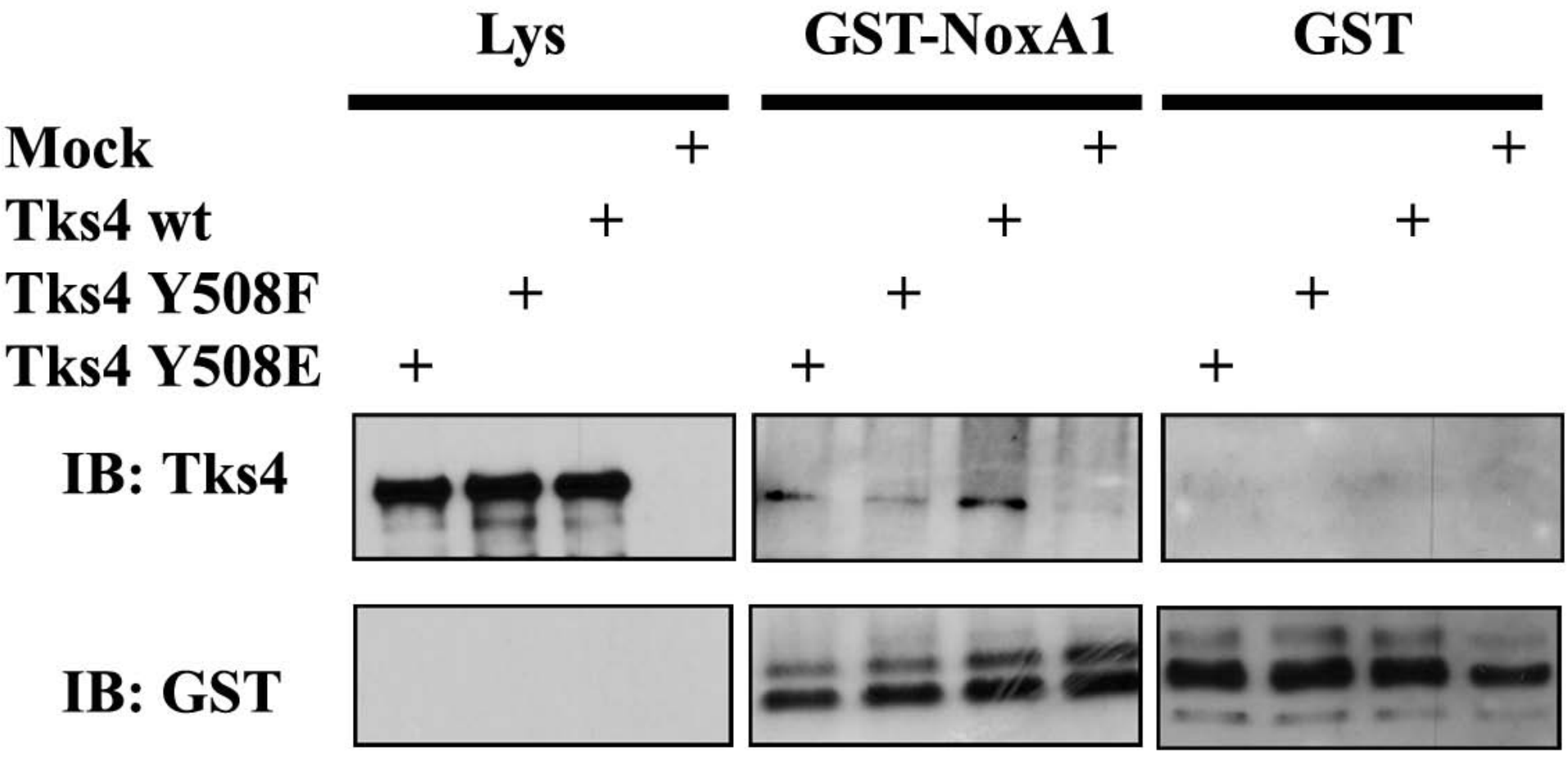
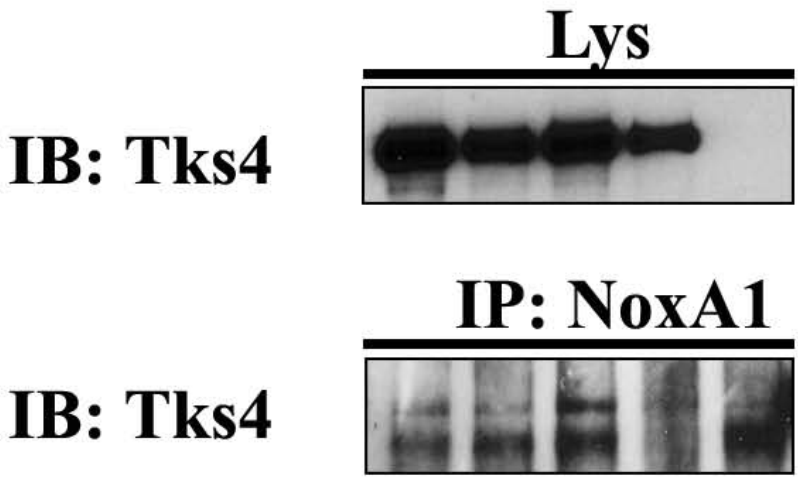

Lys
GST-NoxA1
GST
Mock
Tks4 wt
Tks4 Y508F
Tks4 Y508E
IB: Tks4
IB: GST

Figure S3

A

Mock					+
SrcYF	+	+	+	+	
NoxA1 wt	+		+		
NoxA1 Y110A		+		+	
Tks4 wt		+	+		
Tks4 Y508F	+			+	



B

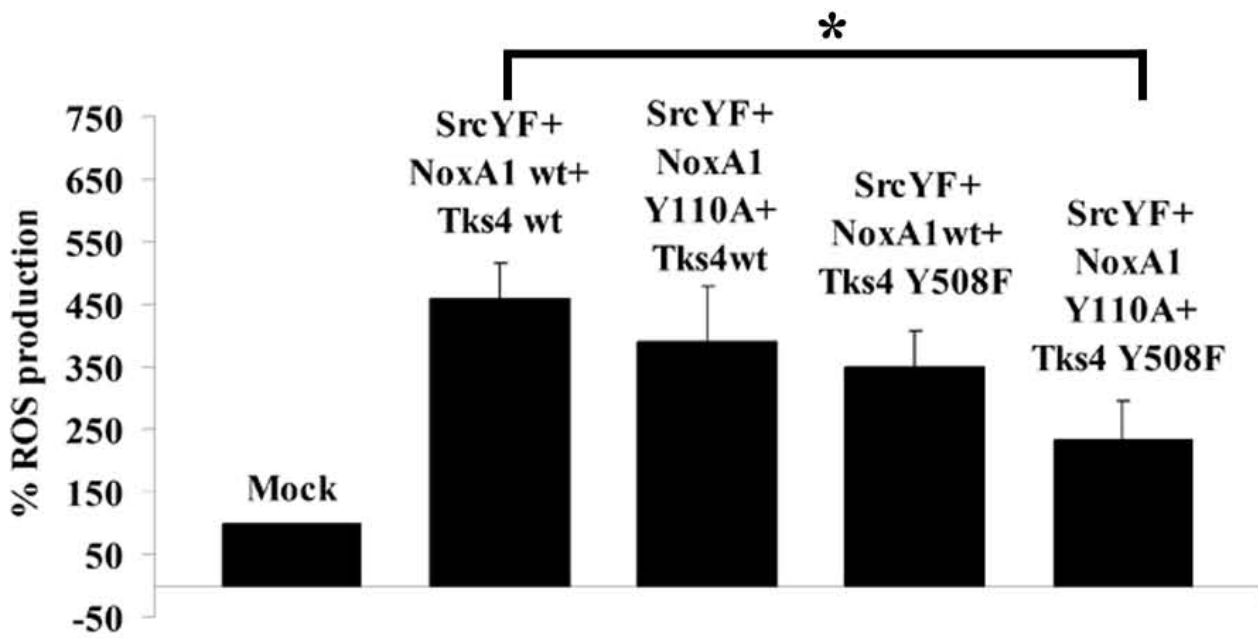
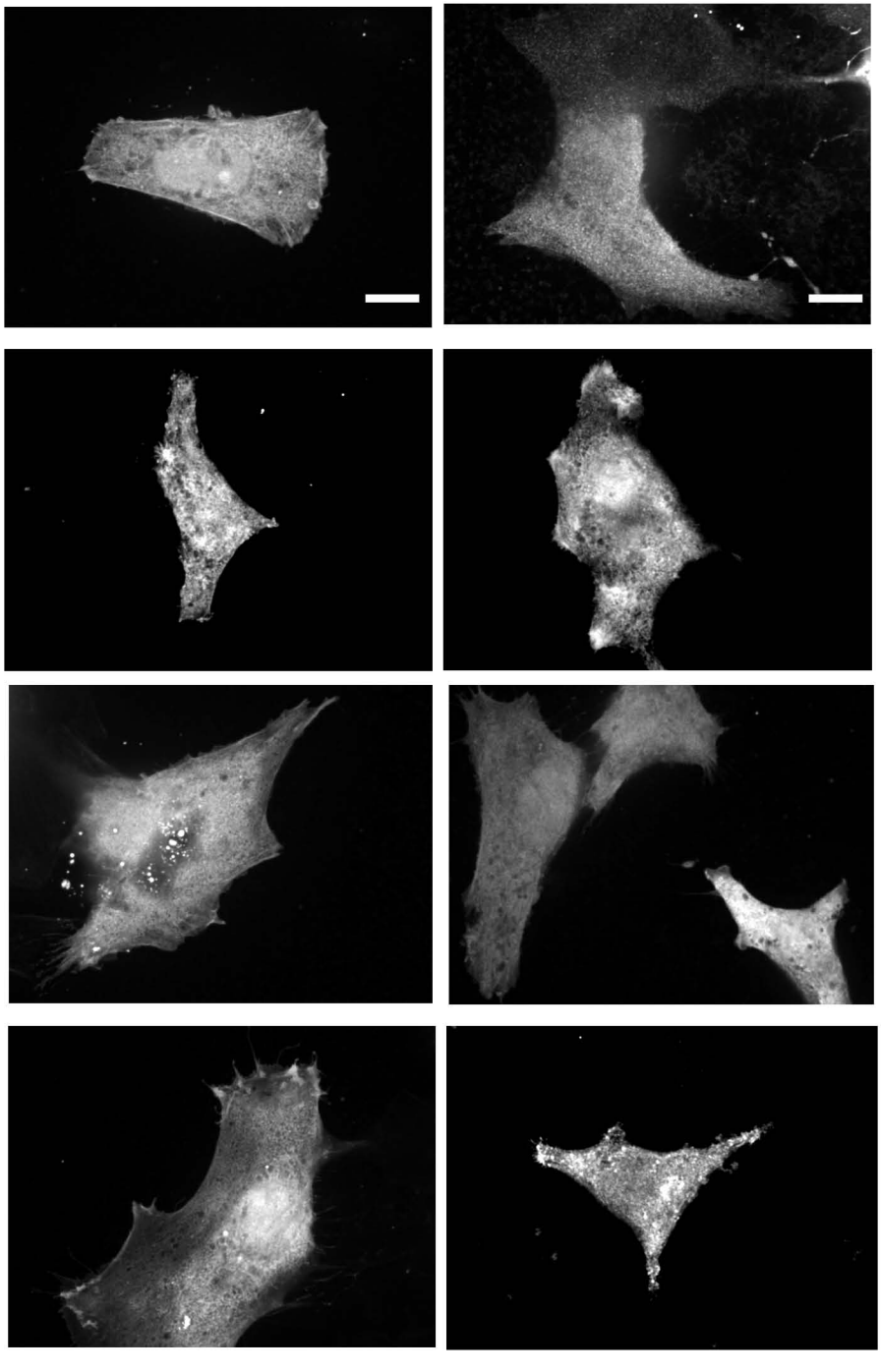


Figure S4


Mock
+SrcYF
+SrcYF
+Tks4 Y508F
+NoxA1 Y110A
+Tks4 Y508E
+NoxA1 Y110E