Figure S1

A

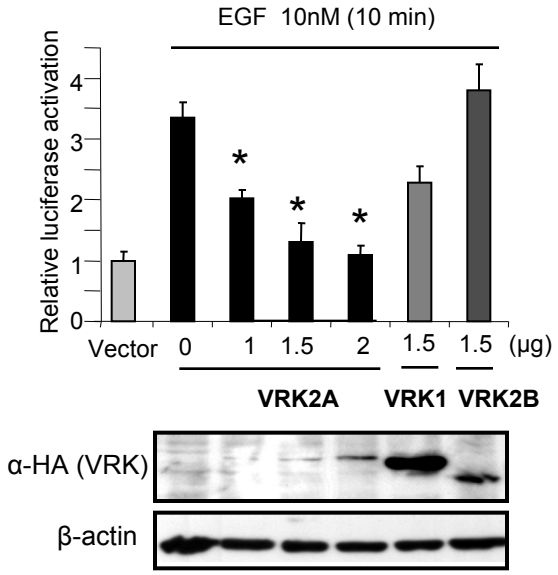


**Figure S1A.** Effect of VRK2 overexpression on transcriptional response mediated by SRE in HeLa cells stimulated with EGF. HeLa cells were transfected with plasmid pCEFL-HA-VRK2A, pCEFL-HA-VRK1 or pCEFL-HA-VRK2B. Forty-eight hours after transfection 10 nM EGF was added to the culture for 10 minutes. Luciferase was determined using a dual-luciferase reporter system. Mean of three independent experiments. $P<0.05$. At the bottom is shown a western blot to detect the transfected proteins and the loading control. The three VRK proteins were detected with an anti-HA antibody, the common tag epitope in the three VRK proteins.

B

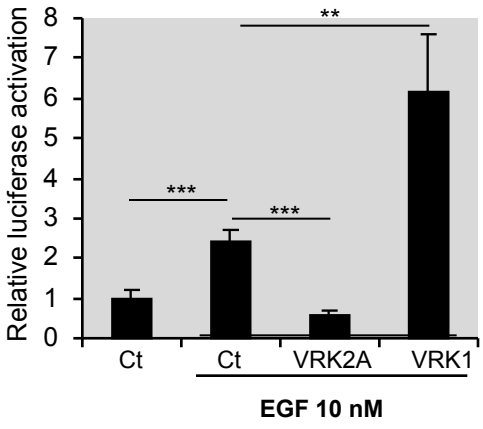


**Figure S1B**. Effect of VRK proteins overexpression on the transcriptional response to EGF of cyclin D1 promoter in MCF7 cells. MCF7 cells were transfected with 1 µg of plasmid pA·cyclin D1 (-1720)-Luc construct, Cells were serum starved for 12 hours before stimulation with 10 nM EGF for 6 hours. Ct: control with empty vector plasmid. ** ($P<0.001$). *** ($P< 0.0005$).

Figure S2

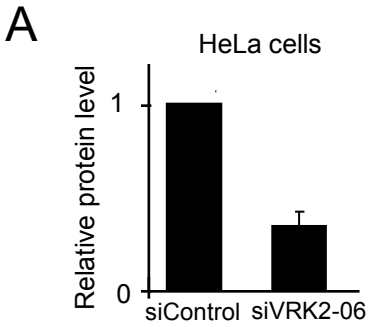


**Figure S2A.** Quantification of VRK2A protein level in HeLa cells that were transfected with siVRK2-06 or siControl (Fig. 2). Data are presented as the mean of three independent experiments.

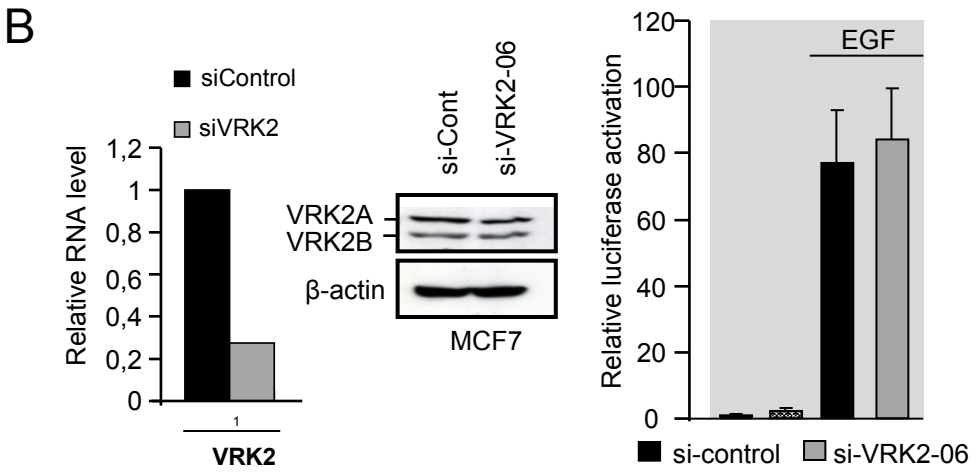


**Figure S2B**.Quantification by semiquantitative RT-PCR of the VRK2 mRNA in MCF7 cells transfected with si-VRK2-06 or si-control (left) and effect on VRK2 protein level (middle) showing it has no effect on protein despite the effective knockdown of its RNA. Therefore this lack of VRK2 protein reduction predicts that in this cell line on EGF stimulation of transcription should not be affected as shown using the pSRE-Luc reporter plasmid (right).

Figure S3

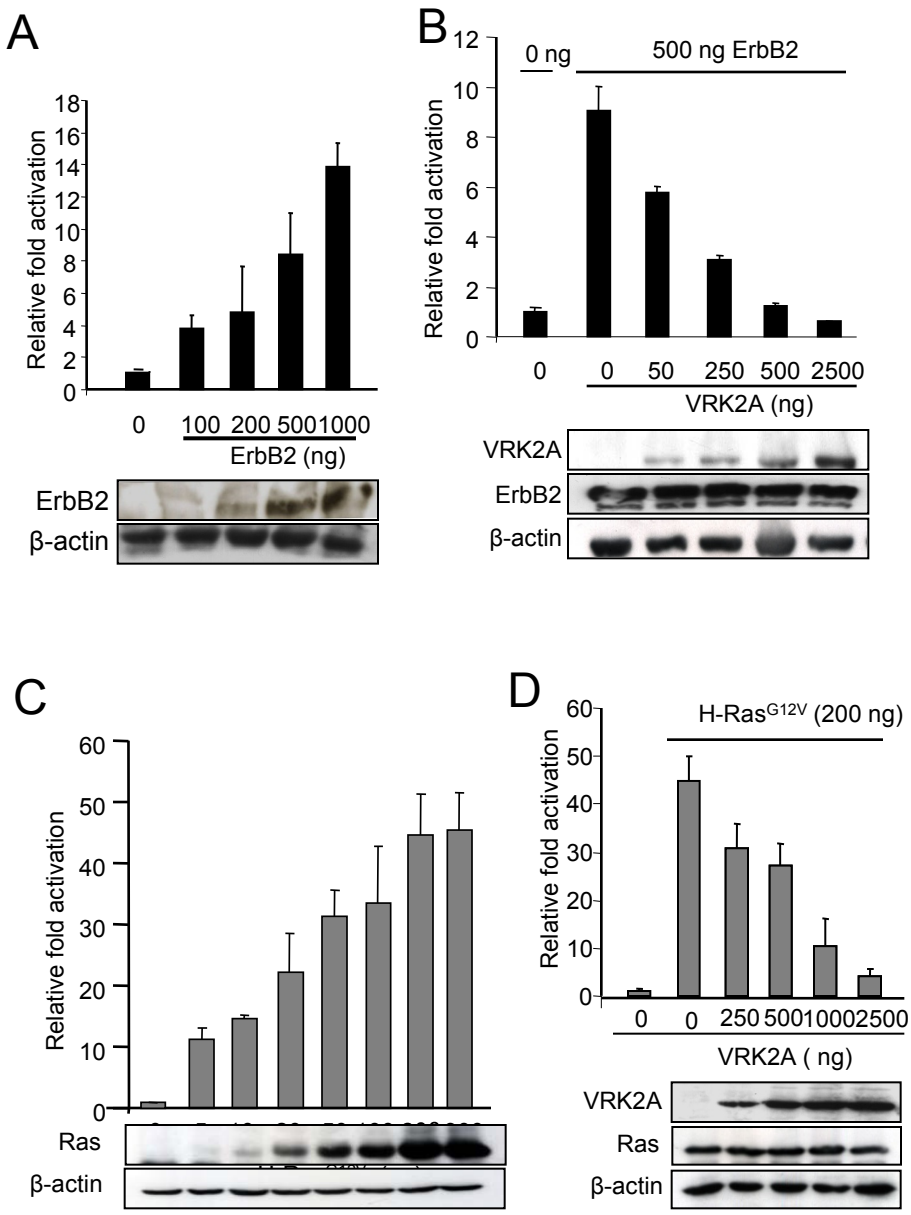


**Figure S3. A.** Dose–dependent activation of transcription induced by ErbB2 in HEK293T cells. **B**. Inhibition of ErbB2 (500 ng) activation of transcription by VRK2 in a dose-dependent manner. **C.** Dose–dependent activation of transcription induced by K-Ras$^{G12V}$. **D.** Inhibition of H-Ras$^{G12V}$ (200 ng) activation of transcription by VRK2 in a dose-dependent manner. These experiments were performed in HEK293T cells. Luciferase activity was measured with an SRE-Luc reporter plasmid. In control points (0) the maximaun amount of empty vector was used, and the specific points it was used to reach the same amount of DNA in transfections.

Figure S4

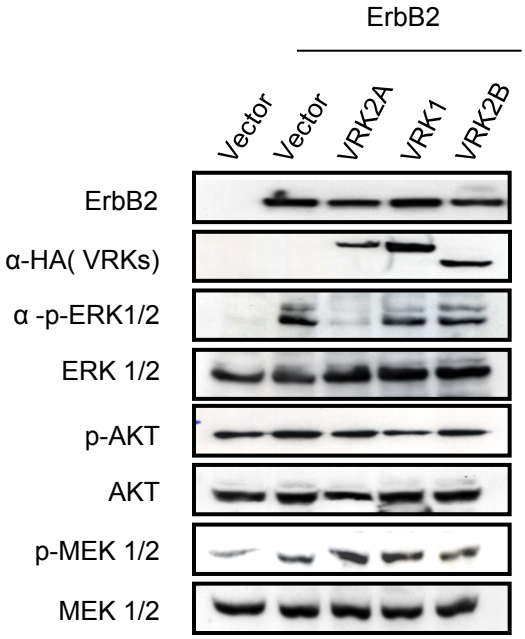


**Figure S4.** VRK2A overexpression leads to reduced, ERK1/2 phosphorylation in response to ErbB2 overexpression, without affecting AKT or MEK1/2 phosphorylation, in HEK293T cell line. VRK1 and VRKK2B overexpression does not affect ERK1/2 phosphorylation. HEK293T cells were transfected with the correponding plasmids and forty-eight hours later the lysates were used for immunoblot analysis with specific antibodies indicated in the Methods section.

Figure S5

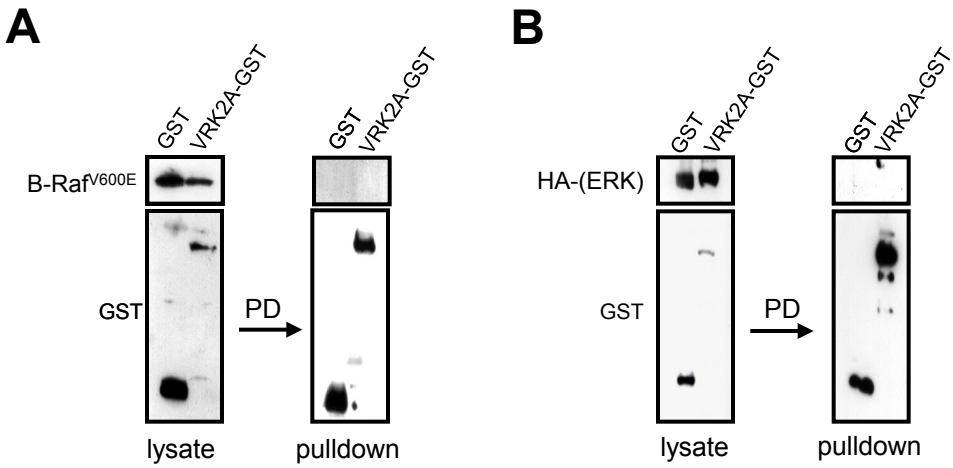


**Figure S5**. Lack of interaction between VRK2A and B-RafV600E (left) or ERK-HA (right).**A** 293T cells were transfected with plasmid pCEFL-GST-VRK2A or pCEFL-GST as control in combination with plasmids pG12B-Raf$^{V600E}$ or empty vector. After forty-eight hours cell lysates (left) were used for pull-down (PD), and the proteins in it (right) detected by western blot with the corresponding antibodies. **B**. 293T cells were transfected with plasmids pCEFL-GST-VRK2A or pCEFL-GST as control in combination with pCEFL-HA-ERK1 or empty vector.

Figure S6

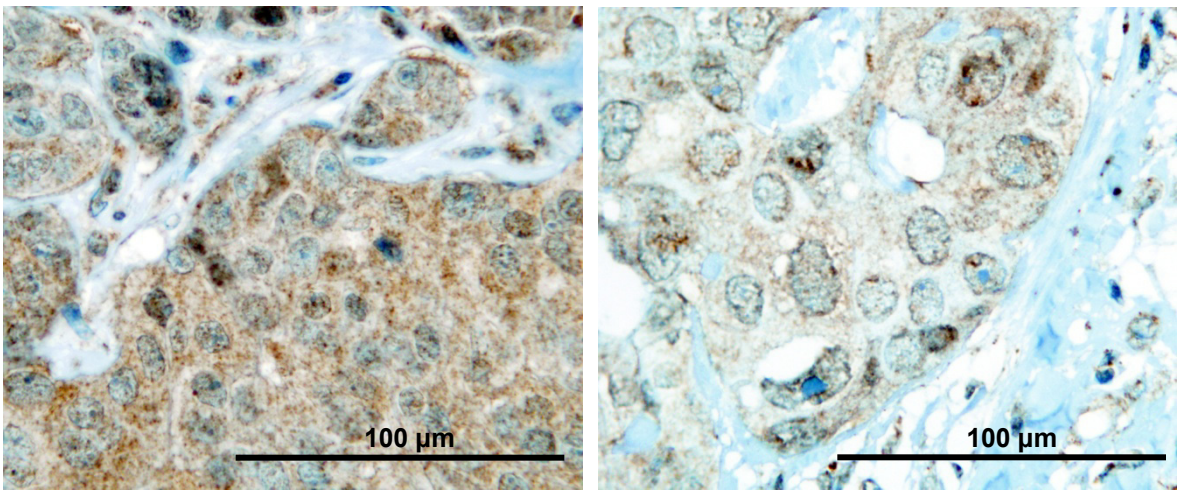


**Figure S6**. Intracellular distribution of VRK2 in two breast cancers, ErbB2- (left) and ErbB2+ (right) cases. In both cases VRK2 is mostly granular and cytosolic as expected from its binding to intracellular membranes. The ErbB2- (left) case has high levels of VRK2 and the ErbB2+ case has low levels of VRK2.

Figure S7

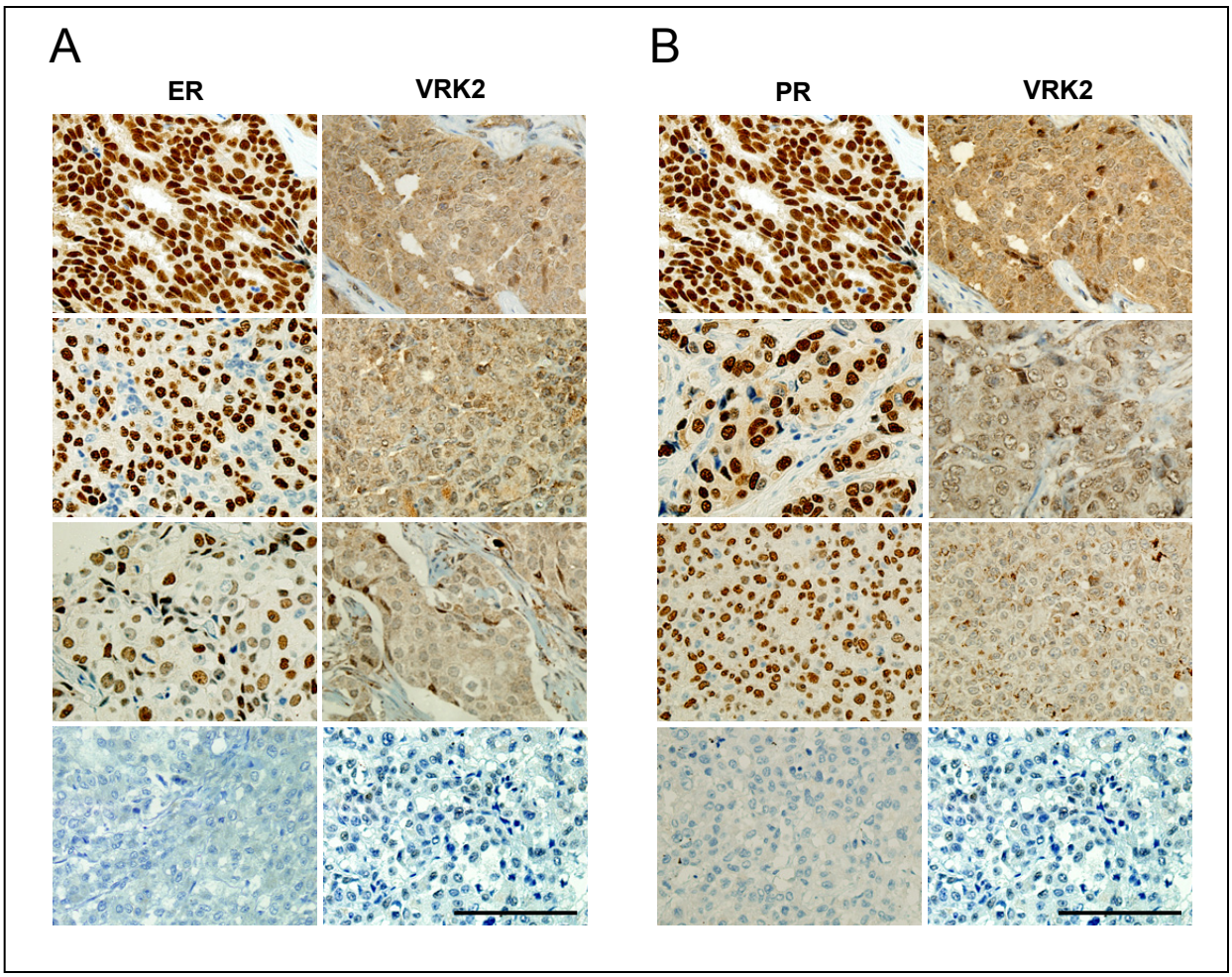


**Figure S7.** Immunohistochemical detection of estrogen (ER) and progesterone (PR) receptors, and VRK2 protein in human breast carcinomas. The presence of ER and PR was determined in the same series of cases. Four cases with different levels of ER or PR expression are shown. A. Detection of ER and VRK2. B. Detection of PR and VRK2. The bar represents 200 µM. .