

**E08-02-0138 Bokoch**

**Supplementary Figure S1: Nox1 is the only member of the Nox family expressed in HT29 cells and is responsible for the majority of ROS produced in these cells.**

RT-PCR was performed as described in *Materials and Methods* to detect the expression of other members of Nox family in HT29 cells. Expression plasmids for Nox2, Nox3, Nox4 and Nox5 were used as positive controls for primer sets. Expression of Nox1 is shown in Figure 1.

**Supplementary Figure S2: p-Src levels correlate with ROS production in some colon cancer cell lines.**

$2 \times 10^5$  HT29, DLD1, HCT116 or CCD481 were counted and resuspended in HBSS. ROS generation was measured by CL-assay as described in *Materials and Methods*. One representative experiment from three separate experiments is shown and results are given as mean of triplicates  $\pm$  S.D.

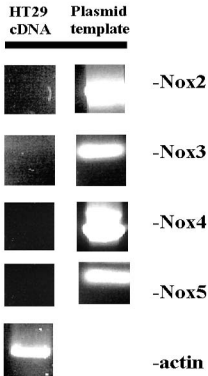
**Supplementary Figure S3: The inhibition of ROS generation caused by Vav2 siRNA#2 in HT29 cells is rescued by the overexpression of mVav.**

HT29 cells were transfected with control siRNA or Vav2 siRNA#2 (20nM) as described in *Materials and Methods*. After 48hrs, Vav2 siRNA-transfected cells were transfected again with expression plasmid for mVav or with empty vector by Lipofectamine 2000. 24hrs later ROS generation was measured. One representative experiment from three separate experiments is shown and results are given as mean of triplicates  $\pm$  S.D.

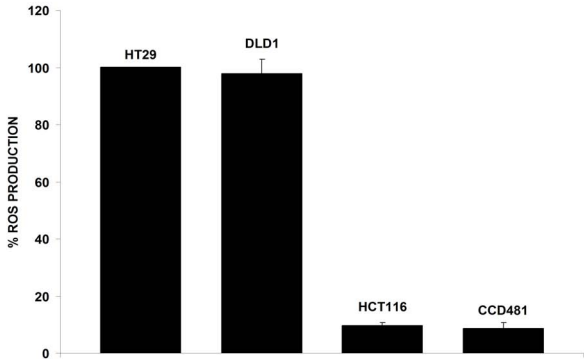
**Supplementary Figure S4: The ability to induce Nox1-dependent ROS generation is restricted to the Rac-GEF Vav2, and the Rho-GEF family member GEF-H1 cannot substitute for it.**

HEK293 cells were transfected with empty vector or with NoxO1, NoxA1 and Nox1 and, where indicated, with RacQL, Vav2-CA or with the constitutive active GEF-H1 (GEF-H1-CA) (upper panel) and after 24 hrs the ROS generation was measured. The lower panel shows similar amount of expression for all the transfected proteins. One representative experiment from three separate experiments is shown, and results are given as mean of triplicates +/- S.D.

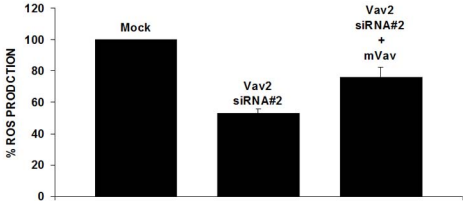
# Figure S1



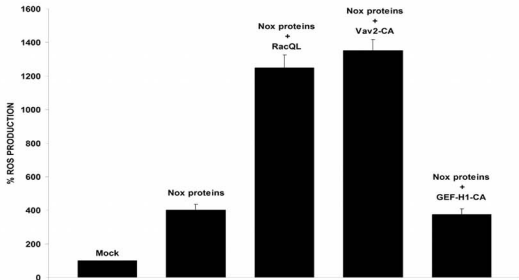
**Figure S2**



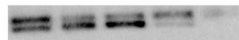
**Figure S3**



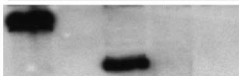
# Figure S4



-	-	-	-	+	<b>pRK5m</b>
+	+	+	+	-	<b>NoxO1-Myc + NoxA1-Myc+Nox1 wt</b>
+	-	-	-	-	<b>GEF-H1-CA-EGFP</b>
-	+	-	-	-	<b>Vav2-CA</b>
-	-	+	-	-	<b>RacQL-EGFP</b>



**IB: Nox1**



**-79Kd <--GEF-H1-CA**

**IB: GFP**

**-47Kd <--RacQL**



**IB: Vav2**



**IB: Myc**



**IB: actin**