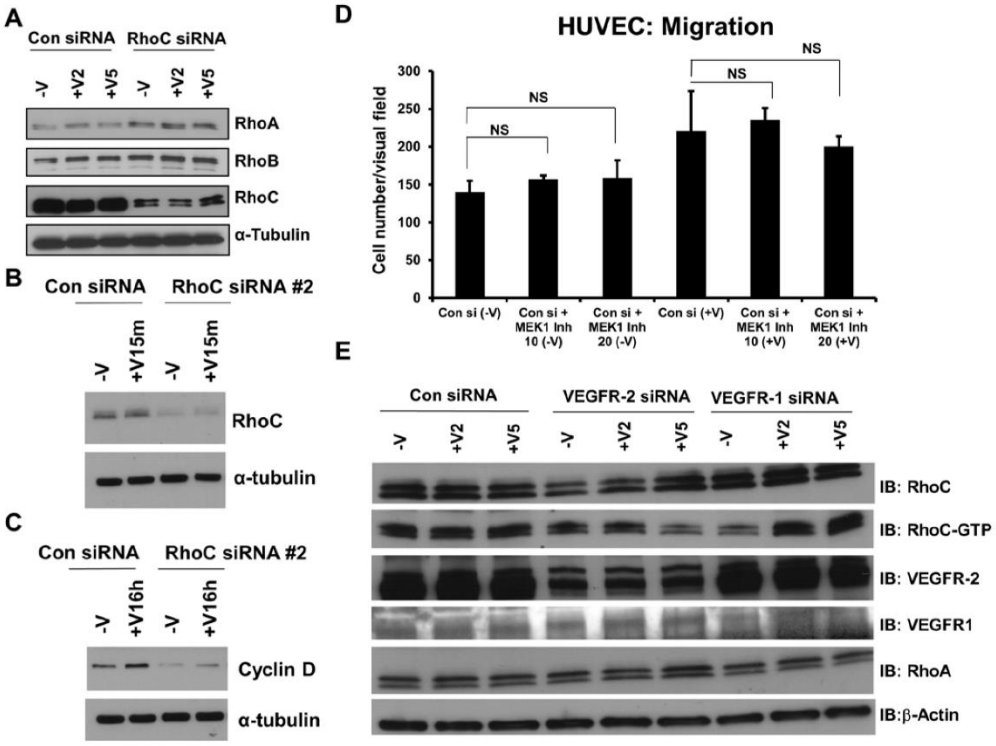
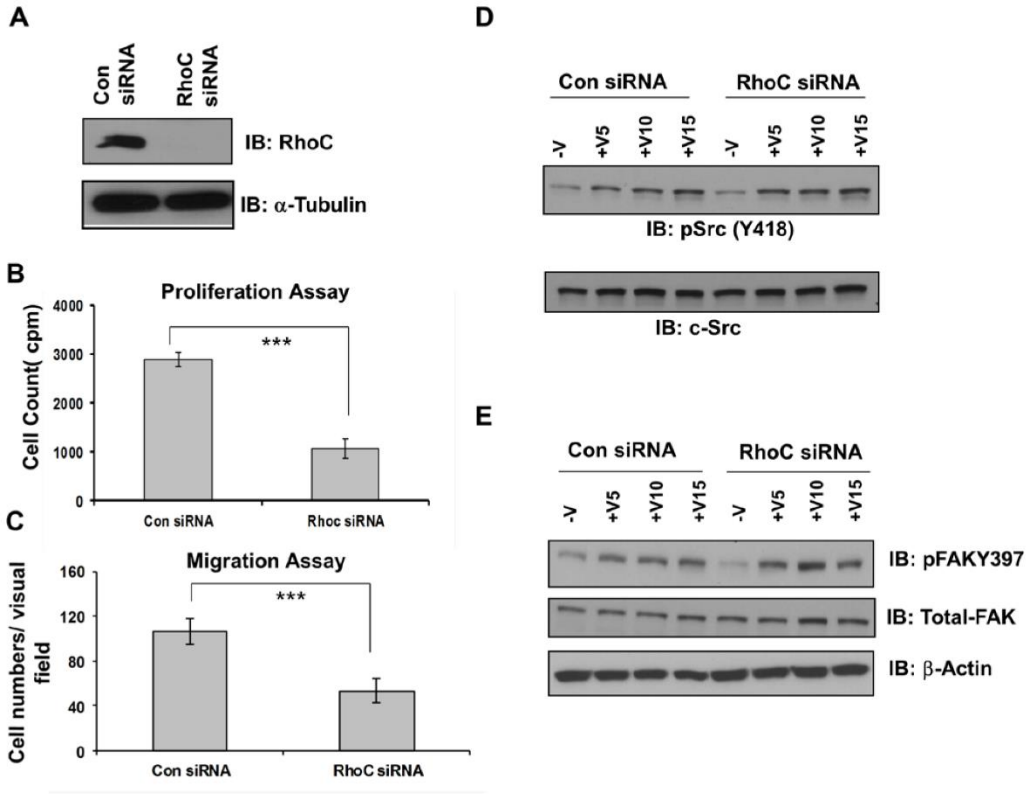
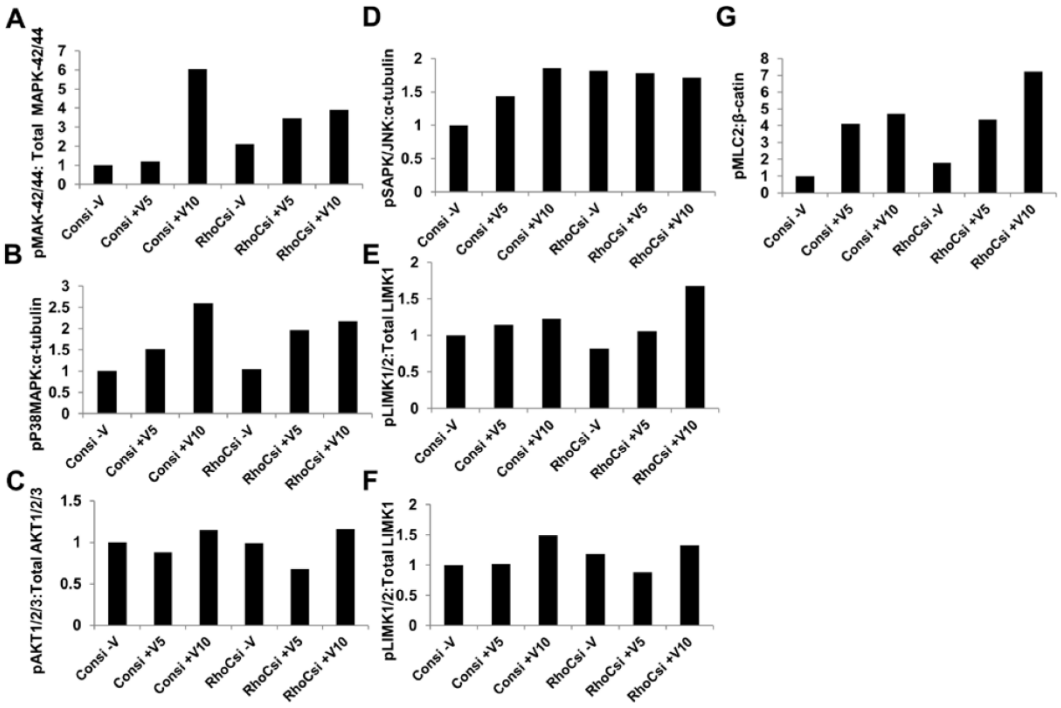
Supplementary Figures:



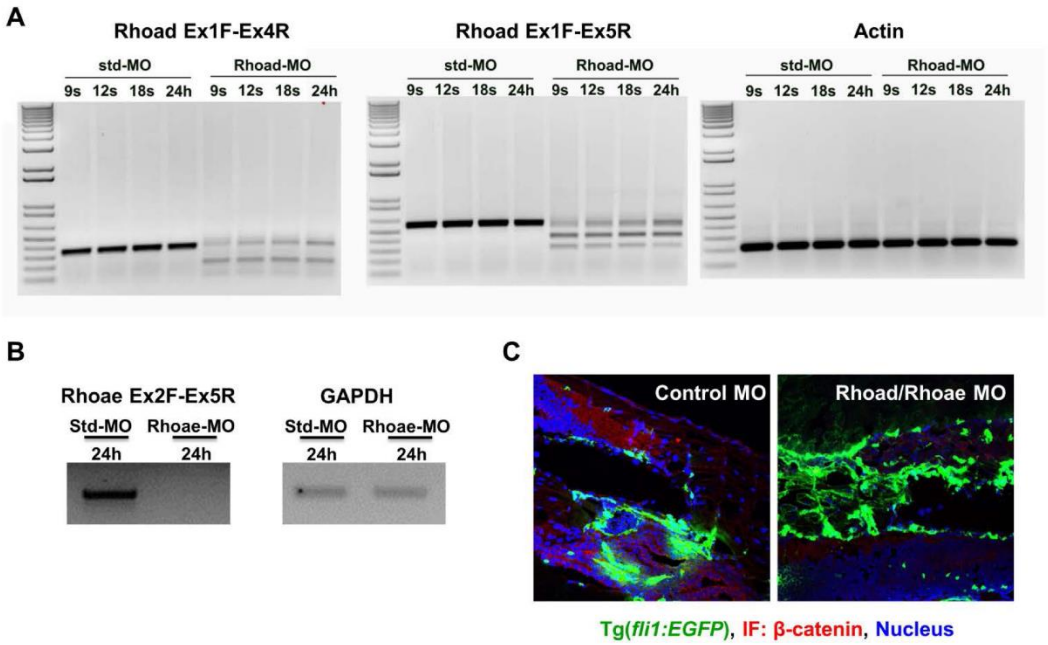
Supplemental Figure 1: VEGF activates RhoC through VEGFR2 (not VEGFR1) and does not affect protein expression of Rho isoforms, a second independent RhoC siRNA #2 decreases Cyclin D expression, and MEK1 inhibition does not affect migration in control siRNA-treated HUVEC. (**A**) HUVEC were treated with control or RhoC siRNA for 48 h and stimulated with VEGF for 2 or 5 min. Cells were lysed and immunoblotted using antibody against RhoA, RhoB, and RhoC. α-Tubulin was used as a loading control. Experiments were repeated at least three times. (**B**) HUVEC were transfected with control or RhoC siRNA #2 (second, independent RhoC siRNA with different target sequence than primary RhoC siRNA used for all other experiments), serum starved overnight, and treated with 10 ng/ml VEGF for 15 minutes. (**C**) HUVEC were transfected with control or RhoC siRNA #2 for 48 h, serum-starved overnight, and treated with VEGF for 16 h. Cyclin D1 and α-tubulin (loading control) were detected by western blot of cell lysates. (**D**) $5x10^4$ cells were seeded into collagen coated transwell chambers overnight and were then inserted into 24-well plates containing serum-starved EGM. In the serum starvation conditions, cells were treated with 10 or 20 μM of MEK1 inhibitor for 1 h and VEGF (10ng/ml) was added in the lower chamber and transwell migration assay was performed for 4 h. Experiments were repeated at least three times in triplicates. NS: Not significant (paired t test, 2 tailed) $p>0.05$. (**E**) HUVEC were transfected with control, VEGFR-1, or VEGFR-2 siRNA, serum starved overnight, and treated with 10 ng/ml VEGF for the indicated times with the indicated antibodies.



Supplemental Figure 2: RhoC promotes proliferation and invasion of MDA-MB-231 breast cancer cells. In HUVEC, RhoC does not affect phosphorylation of c-Src or FAK. (**A-C**) MDA-MB-231 cells were transfected with control or RhoC siRNA for 48 h. Experiments were repeated at least three times. (**A**) Cell lysates were collected, and immunoblotting was performed using RhoC and α-tubulin (loading control) antibodies. (**B**) After 48 h, cells were trypsinized and 4 x 10^4 cells were plated in a 24-well plate and next day thymidine incorporation assay was performed. (**C**) After 48 h, cells were trypsinized and 2 x 10^5 cells/ml were used for invasion assay as described in Methods. (**D-E**) HUVEC were transfected with control or RhoC siRNA for 48 h, serum-starved for overnight, and treated with 10 ng/ml VEGF for 5, 10, or 15 min. Cell lysates were collected and western blotted with antibodies against phospho-c-Src, total c-Src, or β-actin (loading control) (**D**) or antibodies against phospho-FAK, total-FAK and β-actin (**E**). Experiments were repeated at least three times.



Supplemental Figure 3: RhoC ablation in HUVEC results in earlier or increased expression of pMAPK-42/44, pP38MAPK, pSAPK/JNK, and pMLC2 in the presence of VEGF. (**A-G**) HUVEC were transfected with control or RhoC siRNA for 48 h, serum-starved for overnight, and treated with 10 ng/ml VEGF for indicated times. Cell lysates were collected and western blotted with the indicated antibodies. Densitometry was analyzed using ImageJ from the immunoblots depicted in Fig. 3A,C.



Supplemental Figure 4: MO-mediated RhoC ablation decreases β-catenin in the vessels of zebrafish. (**A-B**) RhoC (Rhoad/Rhoae) or control (std) MOs were injected into 1-2 cell stage *Tg(Fli:GFP)* zebrafish embryos. Embryos were collected at the indicated times, RNA was collected using an RNeasy Plus Mini Kit (Qiagen) and converted to cDNA, PCR was performed using the indicated oligonucleotide pairs, and visualized after running on an agarose gel. (**A**) Oligonucleotides were used to amplify Rhoad Exon 1-4 (**A**, left), Rhoad Exon 1-5 (**A**, middle) and loading control β-actin (**A**, right). (**B**) Oligonucleotides were used to amplify Rhoae Exon 2-5 (**B**, left) and loading control GAPDH (**B**, right). (**C**) RhoC (Rhoad/Rhoae) or control MOs were injected into 1-2 cell stage *Tg(Fli:GFP)* zebrafish embryos. At 3 dpf, the zebrafish were formalin-fixed, placed in OCT, frozen, sectioned with a microtome onto slide, stained with a primary β-catenin antibody followed by a corresponding secondary Alexa-Fluor 568 (red) antibody, and visualized using a Zeiss LSM 780 confocal microscope. Green: *Tg(Fli:GFP)*; Red: β-catenin; Blue: DAPI-stained nuclei.