

Supplementary Information

Supplementary Table 1

Target mRNA	Primer identity	Sequence	T_A (°C)	Expected product size (nt)
Nav1.1	hNav1-1F	5'-ATG ACA ATG ATG AAC CCT CC-3'	58	568
Nav1.1	hNav1-1R	5'-GTGCAT CTA AAA AAC CCT CC-3'	58	
Nav1.2	2HB1	5'-TGT GCA CGA TTC TTA CCA AC-3'	58	513
Nav1.2	2HB3	5'-AAG TAG TAC CAT TCC CAT CC-3'	58	
Nav1.3	H3A	5'-TTG AGC AAC CCT CCT GAC TG-3'	60	405
Nav1.3	H3C	5'-GGG CCA CTG CAA ACA TTT AT-3'	60	
Nav1.4	HSK1A	5'-GGG ATC TAC ACC TTT GAG TC-3'	60	428
Nav1.4	HSK1C	5'-CCA TAC CAT GTG TCA TTG CC-3'	60	
Nav1.5	hH1	5'-CAT CCT CAC CAA CTG CGT GT-3'	58	509
Nav1.5	hH2	5'-CAC TGA GGT AAA GGT CCA GG-3'	58	
Nav1.6	H6D	5'-AGA CCA TCC GCA CCA TCC TG-3'	60	517 (adult) 464 (neonatal)
Nav1.6	H6C	5'-TGT CAA AGT TGA TCT TCA CG-3'	60	
Nav1.7	HNE1	5'-TAT GAC CAT GAA TAA CCC GC-3'	59	389
Nav1.7	HNE3	5'-TCA GGT TTC CCA TGA ACA GC-3'	59	
Nav1.8	scn10a-P1	5'-TGG AAC TGG CTG GAT TTT AG-3'	58	434
Nav1.8	scn10a-P2	5'-TCC GGG TTG TCA GAA GTT TT-3'	58	
Nav1.9	scn11a-P1	5'-ATT ATC GGC ACC GTT ATC AT-3'	56	448
Nav1.9	scn11a-P2	5'-TGC ATT TCA GGT TCA GAC TT-3'	56	
NaX	hNaxF	5'-TGC GGC TTC CAT CTT GTG TA-3'	60	562
NaX	hNaxR	5'-GTG CAG GGT TTC ATT TTC ATT-3'	60	
VGSCβ1	h1bF	5'- AGAAGGGCACTGAGGAGTTT-3'	60	379
VGSCβ1	h1bR	5'- GCAGCGATCTTCTTGTAGCA-3'	60	
VGSCβ2	h2bF	5'-GAGATGTTCCCTCCAGTTCCG-3'	62	310
VGSCβ2	h2bR	5'-TGACCACCATCAGCACCAAG-3'	62	
VGSCβ3	h3bF	5'-CTGGCTTCTCTCGTGCTTAT-3'	60	353
VGSCβ3	h3bR	5'-TCAAACCTCCCGGACACATT-3'	60	
VGSCβ4	h4bF	5'-TAACCCTGTCGCTGGAGGTG-3'	64	459
VGSCβ4	h4bR	5'-TGAGGATGAGGAGCCCGATG-3'	64	
B-actin	hb-actinF	5'-CAC TGA GGT AAA GGT CCA GG-3'	59	
B-actin	hb-actinR	5'-TGT CAA AGT TGA TCT TCA CG-3'	59	

Supplementary Figures

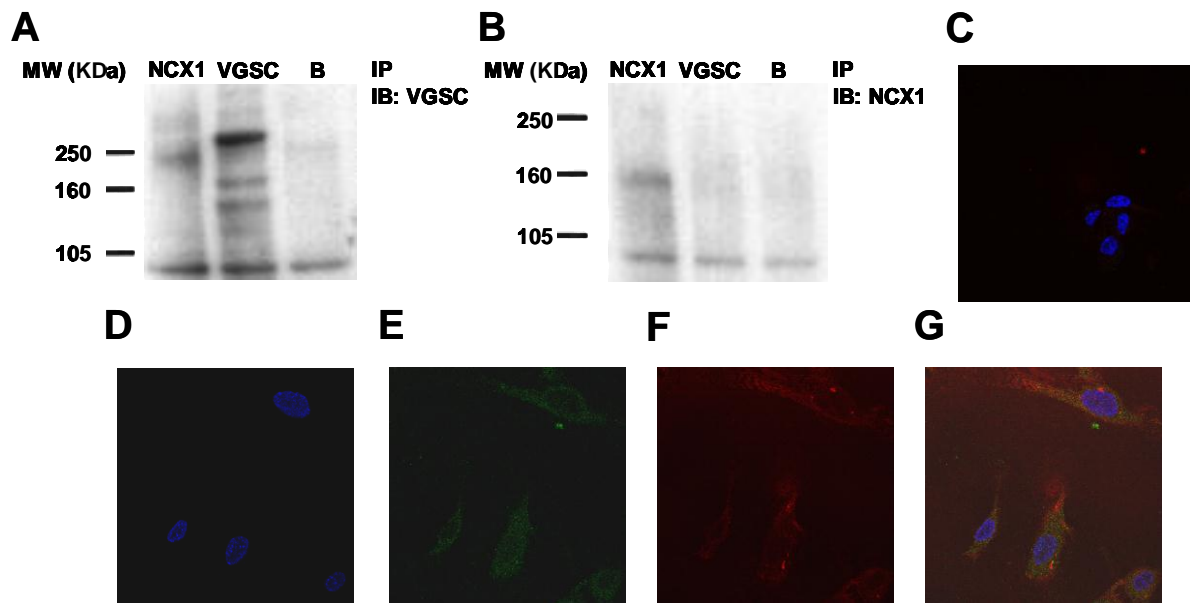
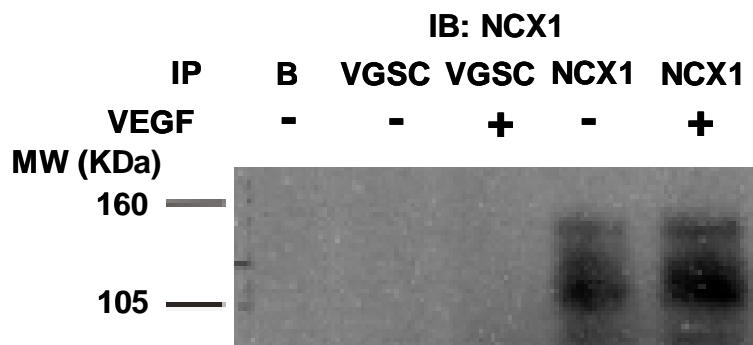


Figure S.1. VGSC and NCX1 are not physically associated in HUVECs. Proteins extracted from HUVECs were solubilized and immunoprecipitated (IP) with antibodies to NCX1, VGSC or protein-A agarose beads only, B. Immunoblots were probed (IB) for NCX1 (A) or VGSC (B). (N=3). Confocal fluorescence images of HUVECs stained with anti-NCX1 (green, E), PAN anti-VGSC (red, F). Nuclei were stained with tropo-3 (blue, D); merged image G. Controls with primary antibodies omitted is shown in C. Images were acquired with a Leica SP1 scanning confocal microscope at 60X magnification.

A



B

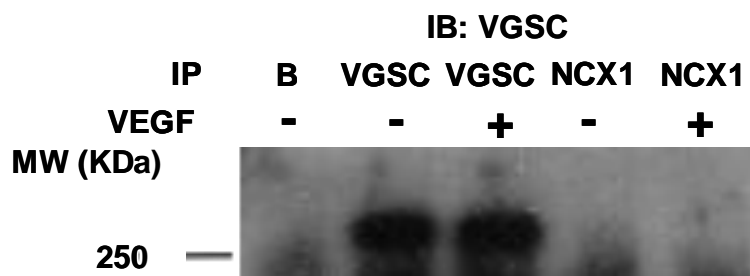


Figure S.2. Serum-starved HUVECs were exposed to VEGF (50ng/ml) for 10min. Subsequently, proteins were solubilised and subjected to immunoprecipitation (IP) with the following antibodies absorbed on protein-A coated agarose beads NCX1: NCX1 antibody, VGSC: PAN anti-VGSC antibody, B: protein-A agarose beads only. Immunoblots were probed (IB) with an anti NCX1 antibody (A) or a PAN anti-VGSC antibody (B). Representative immunoblots from N=2 identical repeats is shown.

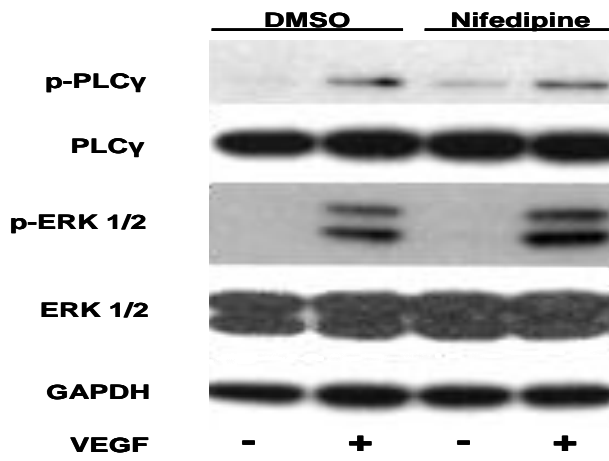


Figure S.3. HUVECs were preincubated for 30min with 1 μ M nifedipine, or vehicle and then stimulated with 50ng/ml VEGF for 10min. ERK 1/2 and PLC γ activation in cell lysates was determined by western blot analysis as described in the legend of Figure 5A. Representative immunoblots are shown (N=3).