Supplemental Figures

Fig. S1**.** Effect of a 15 min preincubation with S1P on the action of endothelin-I. Upper panel: representative tracings of intracellular free-calcium concentration ($[Ca^{2+}]_i$) of cells preincubated in the absence (left panel) or presence of 1 μM S1P. Where indicated (arrow), 100 nM endothelin-1 was added. Lower panel: increases of free-calcium concentration ($[Ca^{2+}]_i$) induced by 100 nM endothelin-1 in cells preincubated in the absence (solid bar) or presence of 1 μM S1P (open bar). The means are plotted with vertical lines representing the S.E.M of 4-5 determinations using different cell preparations.

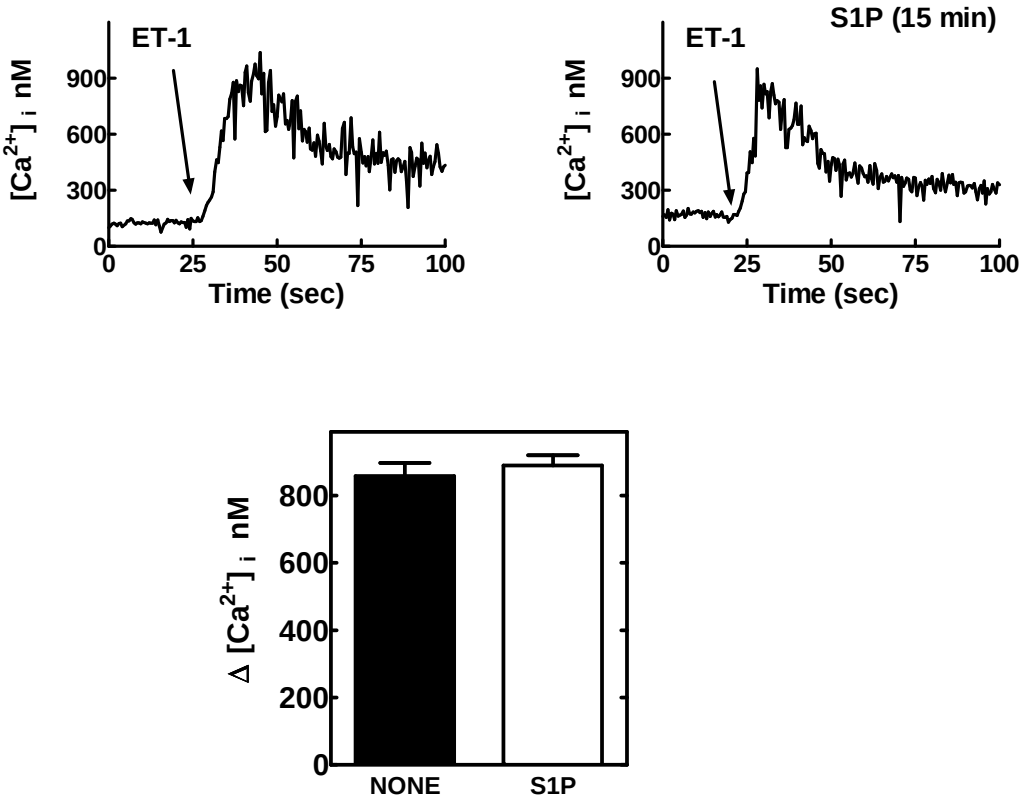


Fig. S2. *Plasma membrane associated fluorescence of cells expressing α_{1B}-adrenergic receptor-enhanced green fluorescent protein construction.* Cells were incubated in the absence of any agent (BASAL, time 0; Vehicle, 15 min) or presence of 1 μM S1P or 10 μM noradrenaline (NA). Data correspond to experiments shown in Fig 10. * $p < 0.001$ vs Basal or Vehicle.

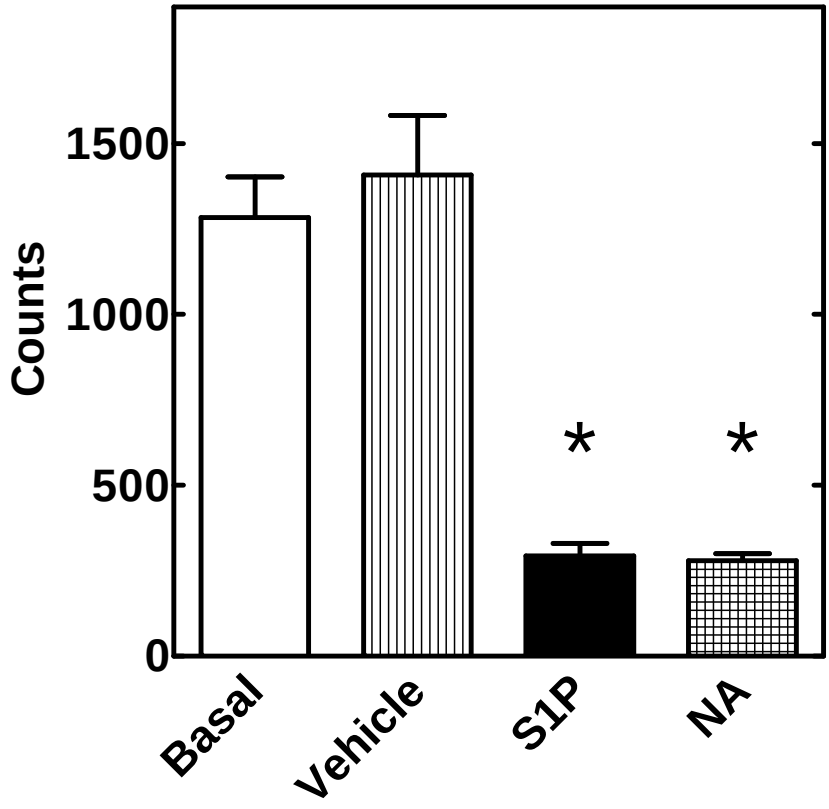
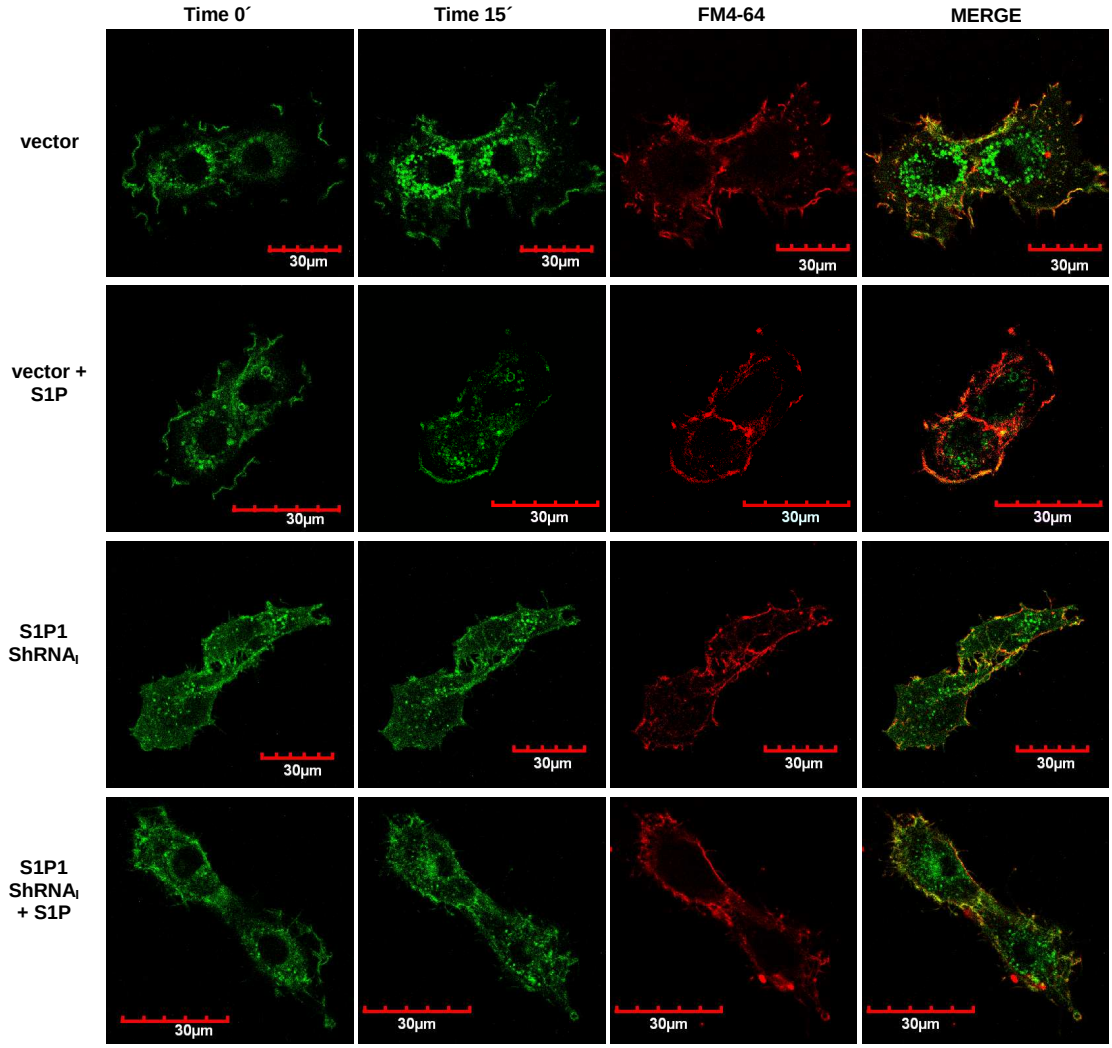


Fig. S3. *Confocal microscopy images of cells expressing α_{1B}-adrenergic receptor-enhanced green fluorescent protein construction; effect of $S1P_1$ receptor knock down.* Cells transfected with the empty vector or the $S1P_1$ shRNA were used and incubated in the absence or presence of 1 μM S1P. Images were taken at the beginning of incubation (Time 0') and at the end of the incubation with the agents (Time 15'); immediately thereafter, the membrane marker FM4-64 (red) was added and images were taken. Merge images (end of encubation -FM4-64) are presented for colocalization (yellow). Quantitative analysis of plasma membrane-associated fluorescence is shown below the images.* $p< 0.001$ vs. vector basal; ** $p< 0.01$ vs. vector basal.



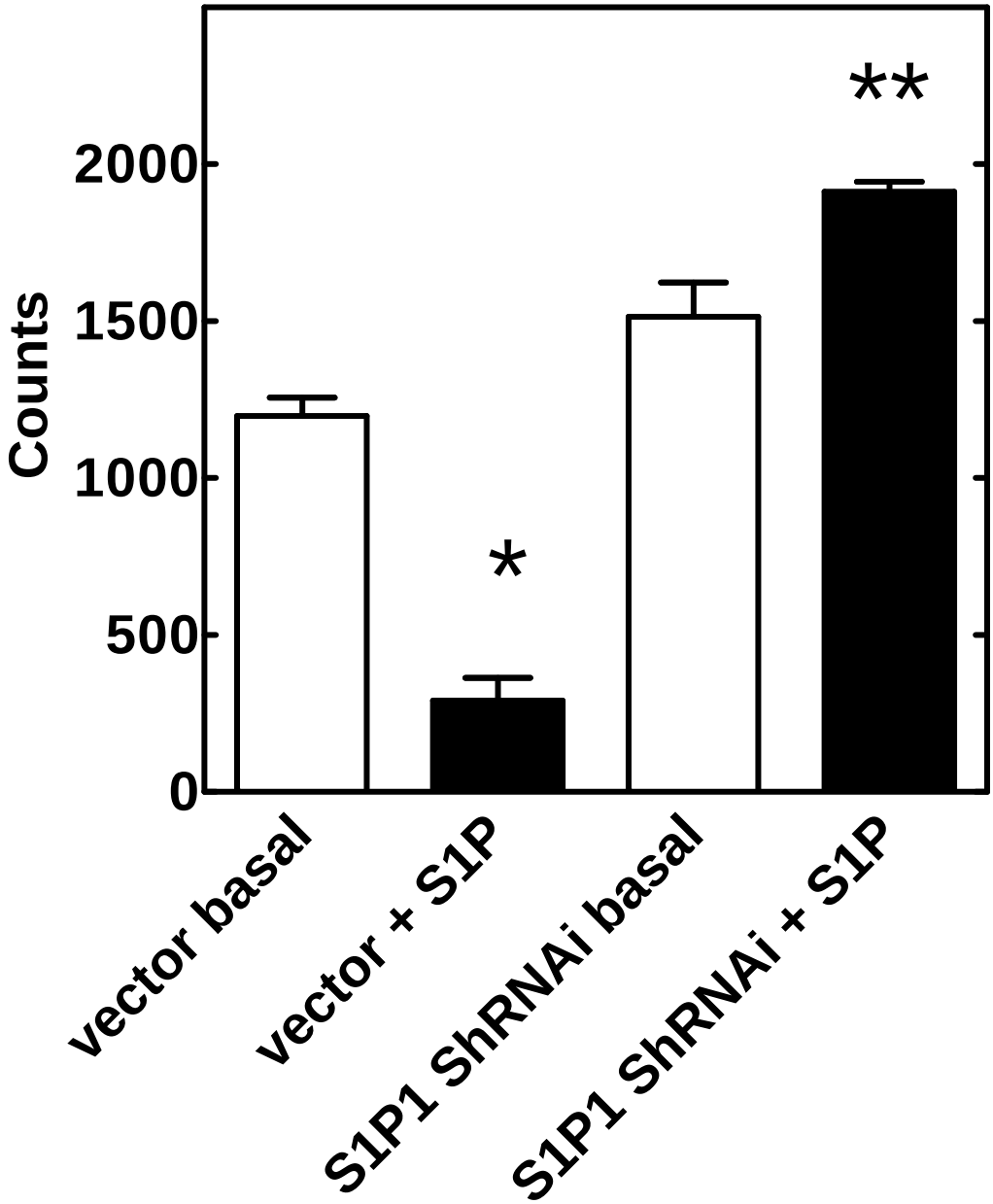
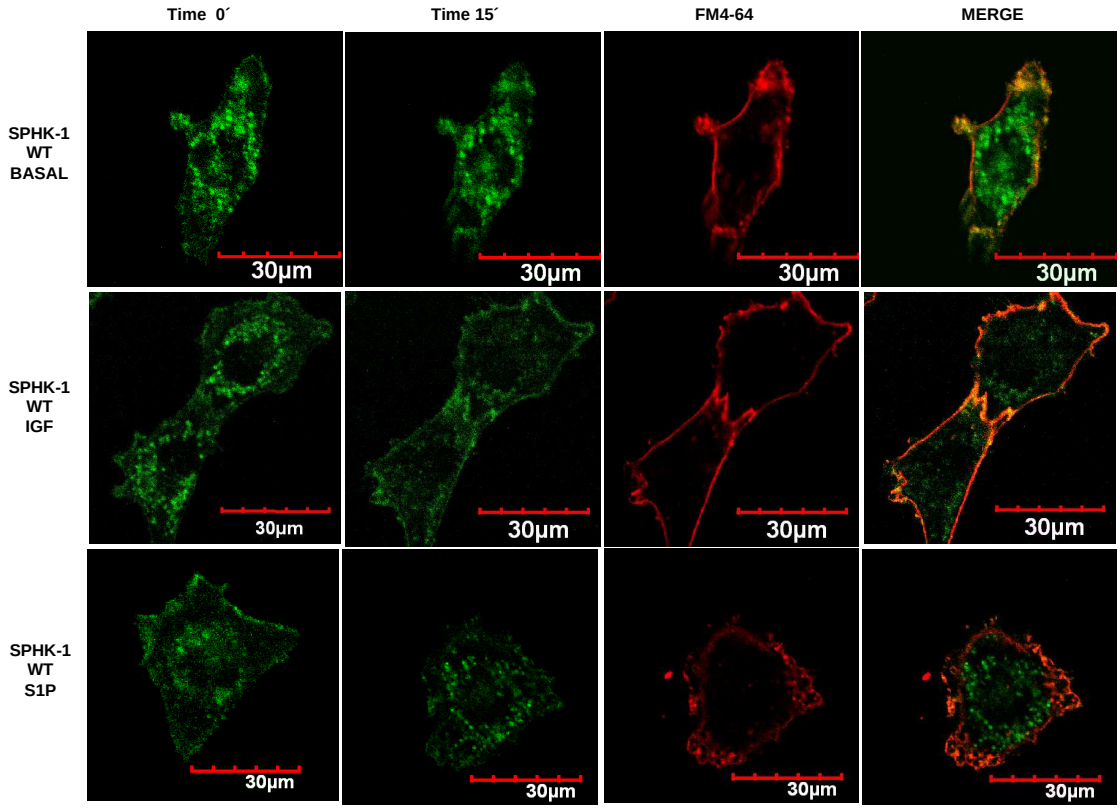

Counts
2000
1500
1000
500
0
*
**
vector basal
vector + S1P
S1P1 ShRNAi basal
S1P1 ShRNAi + S1P

Fig. S4. Confocal microscopy images of cells expressing α_{1B}-adrenergic receptor-enhanced green fluorescent protein construction; effect of wild type sphingosine kinase-1 expression. Cells transfected with wild type sphingosine kinase-1 were used and incubated in the absence or presence of 100 ng/ml IGF-I or 1 μM S1P. Images were taken at the beginning of incubation (Time 0') and at the end of the incubation with the agents (Time 15'); immediately the membrane marker FM4-64 (red) was added and images were taken. Merge images (end of incubation-FM4-64) are presented for colocalization (yellow). Quantitative analysis of plasma membrane-associated fluorescence is shown below the images. * $p < 0.001$ vs. SPHK-1 wild-type.



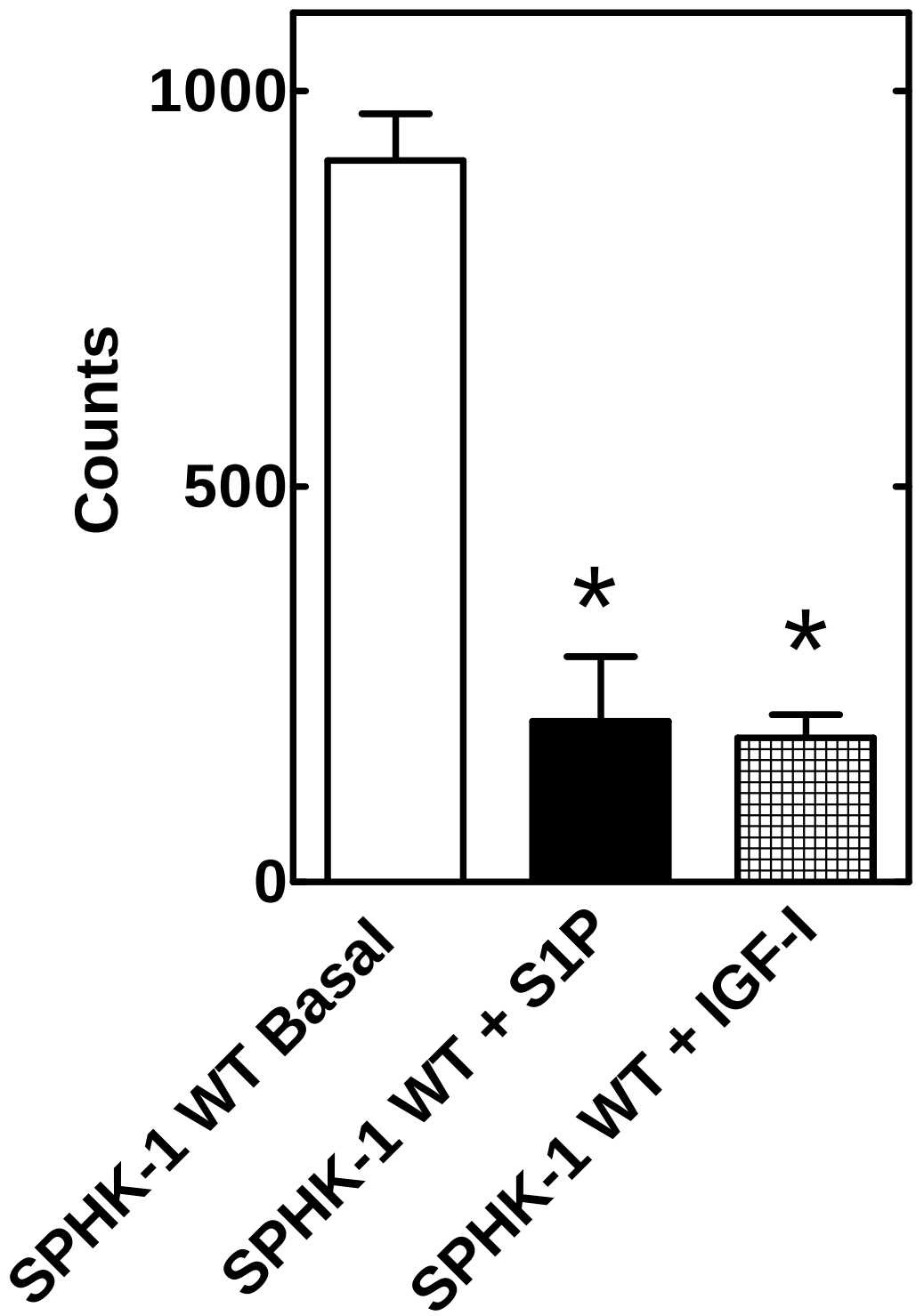
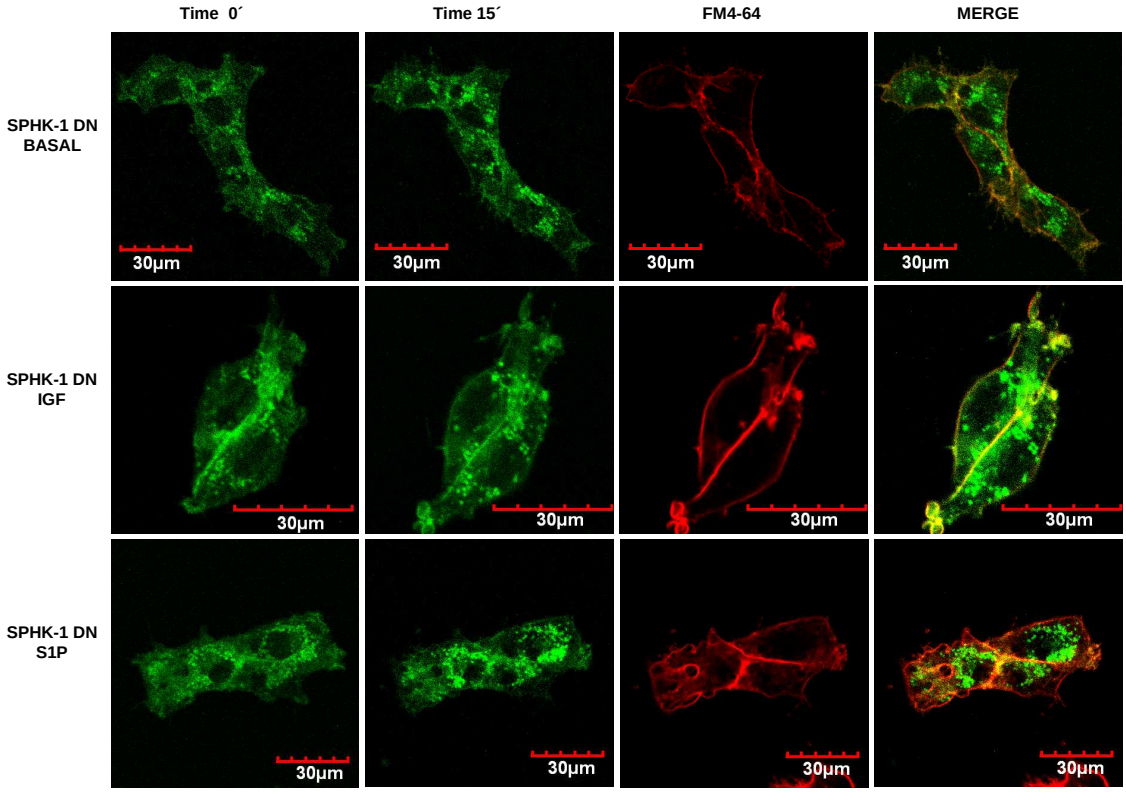
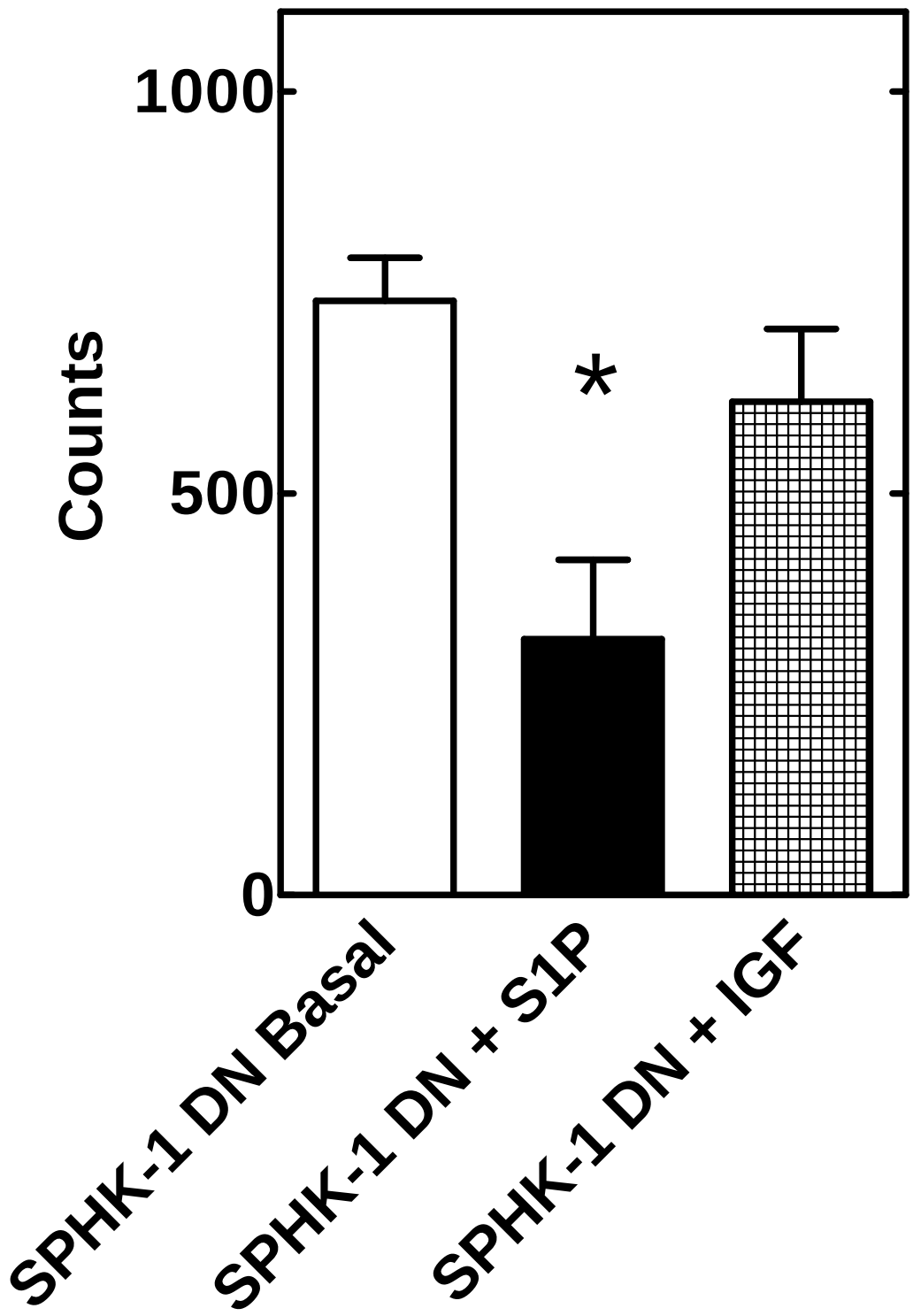

1000
500
0
Counts
*
*
SPHK-1 WT Basal
SPHK-1 WT + S1P
SPHK-1 WT + IGF-I

Fig. S5. Confocal microscopy images of cells expressing α_{1B}-adrenergic receptor-enhanced green fluorescent protein construction; effect of expression of a dominant-negative mutant of sphingosine kinase-1 (SPHK-1 DN). Cells transfected with the dominant negative mutant of sphingosine kinase-1 were used and incubated in the absence or presence of 100 ng/ml IGF-I or 1 μM S1P. Images were taken at the beginning of incubation (Time 0') and at the end of incubation with the agents (Time 15'); immediately thereafter, the membrane marker FM4-64 (red) was added and images were taken. Merge images (end of incubation-FM4-64) are presented for colocalization (yellow). Quantitative analysis of plasma membrane-associated fluorescence is shown below the images. * $p < 0.01$ vs. SPHK-1 DN Basal.




1000
500
0
Counts
*
SPHK-1 DN Basal
SPHK-1 DN + S1P
SPHK-1 DN + IGF