TRPM7: Ca2+ signaling, actomyosin remodeling and metastasis
Visser, Daan

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Ion channels represent a major class of transmembrane signal transducers that facilitate the passage of ions over the plasma membrane. Ions and ionic fluxes regulate numerous fundamental cell processes and are crucial to cellular signaling. Ions, for example, act as second messengers that propagate extracellular signals into the cytosol, which enables the cell to communicate with and adapt to its environment. TRPM7 is unique among ion channels, because it also comprises a functional α-kinase domain; a feature that is only shared by its closest family member TRPM6. As discussed in detail in Chapter 1, TRPM7 is an incredibly diverse protein when it comes to activation mechanisms, ion selectivity (divalent cations, particularly $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$), and downstream signaling and it could, therefore, potentially act as an integrator of multiple signaling pathways. In accordance with this notion, TRPM7 has been proposed to function in several fundamental physiological processes as well as to contribute to pathological conditions, such as cancer.

In this thesis, we provide the first evidence that TRPM7 (expression) contributes to metastasis in vivo [Chapters 2 and 3]. In addition, using a variety of biophysical and cell biological techniques we addressed several aspects of TRPM7 functioning and signaling, with a main focus on actomyosin remodeling, cell adhesion and $\text{Ca}^{2+}$ signaling [Chapters 2, 3 and 5], as well as the PLC/PIP$_2$ signaling axis [Chapter 4].

**TRPM7 in cancer progression**

*TRPM7 promotes metastasis formation.*

We used a multidisciplinary approach to investigate the potential contribution of TRPM7 to the metastatic potential of breast cancer and neuroblastoma cells. **TRPM7 knockdown** in highly metastatic breast cancer cells (MDA-MB-231) significantly impaired metastasis formation following intravenous injections into immunodeficient mice [Chapter 2]. In line with this observation, **TRPM7 overexpression** in mouse neuroblastoma cells (N1E-115) strongly increased their capacity to form metastases, as compared to empty-vector controls [Chapter 3]. We additionally found that high(er) TRPM7 mRNA levels correlate with poor disease outcome and metastasis formation in two independent breast cancer cohorts,
independently of other clinical parameters such as tumor size [Chapter 2]. Consistent with our findings, TRPM7 has been found upregulated in primary breast and pancreatic tumor samples using immunohistochemistry to score TRPM7 protein expression. Although those studies report a positive correlation between TRPM7 protein levels and tumor grade, conclusions on associations with pathological parameters, such as survival rates, are largely hampered by small sample sizes (Dhennin-Duthille et al., 2011; Guilbert et al., 2009; Guilbert et al., 2013; Rybarczyk et al., 2012) [see also Table 1 in Chapter 1]. We validated our results from a large microarray-based dataset (368 samples) by RT-qPCR analyses of primary breast cancer samples in an independent patient cohort (144 samples). We, however, did not observe a correlation of TRPM7 with cancer progression in all patient cohorts examined [Chapters 2 and 3; three breast cancer cohorts and a neuroblastoma cohort, respectively]. Discrepancies between microarray-based gene expression profiles are frequently observed and remain a serious challenge, often reflecting differences in patient populations, tissue handling and probe selection (Ahmed and Brenton, 2005; Ein-Dor et al., 2005; Subramanian and Simon, 2010). Taken together, our findings suggest that TRPM7 contributes to metastasis formation. Future studies will have to uncover to what extent TRPM7 expression levels have prognostic value in cancer types.

How, tell me how? Proliferation, cell adhesion and migration.

In addition to the general growth advantage of tumor cells compared to non-tumor cells, metastatic tumor cells have acquired a number of other traits that enable them to successfully complete the different steps of the metastatic cascade; most importantly, loss of cell-cell adhesion, migration or local invasion and anchorage-independence.

We found that cell proliferation of breast cancer cells (MDA-MB-231) and neuroblastoma cells (N1E-115) in cell culture conditions was not affected by TRPM7 knockdown and overexpression, respectively [Chapters 2 and 3]. In addition, the size of metastases in experimental metastasis assays for both tumor cell models was similar as for control cells. Furthermore, primary tumor size in breast cancer patients was not associated with TRPM7 expression levels and multivariate analysis showed that TRPM7 is an independent prognostic marker for disease progression in breast cancer cohorts. These findings illustrate that proliferation and cell viability are not affected by altered TRPM7 expression levels, neither in vitro nor in vivo [Chapters 2 and 3]. In support of our results, conditional and tissue-specific deletion of TRPM7 in mice has highlighted several cell types that proliferate independently of TRPM7 (Jin et al., 2012; Sah et al., 2013). In addition, Guilbert and colleagues (2013) demonstrated that siRNA-mediated TRPM7 knockdown also did not affect proliferation of
MDA-MB-231 and MDA-MB-435S breast cancer cells (Guilbert et al., 2013). In contrast, however, other studies have demonstrated that TRPM7 contributes to the proliferation and survival of several other cell types, including MCF-7 breast cancer cells (Du et al., 2010; Guilbert et al., 2009; Jin et al., 2008; Sahni et al., 2010; Sun et al., 2013; Yee et al., 2011). The requirement of TRPM7 for cell proliferation, therefore, appears to depend on cell type, developmental stage and different experimental strategies used, most notably transient overexpression and knockdown (by siRNA) versus stable transduction strategies [see also Table 1 and paragraph ‘Proliferation and survival’ in Chapter 1].

We showed that manipulating the expression levels of TRPM7 particularly affects cell-matrix adhesions in both breast cancer cells and neuroblastoma cells [Chapters 2 and 3]. TRPM7 downregulation in breast cancer cells results in a contractile phenotype and increased focal adhesion numbers, as demonstrated by increased myosin light chain phosphorylation and paxillin tyrosine phosphorylation, respectively [Chapter 2]. TRPM7 overexpression in neuroblastoma cells, on the other hand, is associated with increased cell flattening (a ‘relaxed morphology’) and cell-adhesive properties, accompanied by the formation of invadosomes [Chapter 3] (Clark et al., 2006). These findings are consistent with the general idea that focal adhesion formation is promoted by increased actomyosin-based tension (Chrzanowska-Wodnicka and Burridge, 1996; Geiger et al., 2009), while invadosome formation requires actomyosin relaxation (Alexander et al., 2008; Burgstaller and Gimona, 2004; Clark et al., 2006; van Helden et al., 2008). Indeed, inhibition of myosin II-based tension by Rho-kinase blockers was sufficient to revert the phenotype of TRPM7-knockdown MDA-MB-231 cells, reducing cellular tension and decreasing focal adhesion numbers towards the level observed in control cells [Chapter 2]. In addition, in Chapter 5 we demonstrated that TRPM7 inhibition by waixenicin-A reduced cellular tension in neuroblastoma cells, which coincided with invadosome disassembly and the subsequent formation of focal adhesions and stress fibers. The dynamic formation and turnover of cell adhesions (‘cell adhesion dynamics’) as well as actomyosin remodeling are essential requirements for cell migration (Ridley et al., 2003; Ridley, 2011). Accordingly, the profound effects of TRPM7 on cellular tension, actomyosin remodeling and cell adhesions were reflected by strongly altered migratory properties, whereby breast cancer cells and neuroblastoma cells with higher TRPM7 expression levels migrate significantly better [Chapters 2 and 3]. Combined with observations in other cell types (Baldoli et al., 2013; Baldoli and Maier, 2012; Chen et al., 2010; Gao et al., 2011; Guilbert et al., 2013; Liu et al., 2011; Rybarczyk et al., 2012), our findings suggest that TRPM7 acts as a common regulator of tumor cell migration [see also Table 1 and paragraph ‘Adhesion and migration’ in Chapter 1].
Regulation of adhesion and migration by TRPM7

TRPM7 as a channel: Local Ca\(^{2+}\) signaling.

We previously proposed that TRPM7, perhaps acting as a mechanosensor to detect stress and matrix rigidity, is a key regulator of localized Ca\(^{2+}\)-dependent actomyosin remodeling, cell adhesion and migration (Clark et al., 2007). This model held that Ca\(^{2+}\) influx through TRPM7 at cell adhesions controls MHC-II phosphorylation by its kinase-domain as well as the activation of Ca\(^{2+}\)-sensitive effector proteins [see Figure 6 in Chapter 1]. Ca\(^{2+}\) signals are well-known to regulate cell adhesion dynamics and migration and several Ca\(^{2+}\) channels and Ca\(^{2+}\) signaling molecules have been found to locate to cell adhesions, including TRPM7, TRPV2, Orai1, STIM1 and calmodulin. Orai1 and STIM1, the molecular components responsible for store-operated Ca\(^{2+}\)-entry (SOCE), were demonstrated to be involved in focal adhesion dynamics, by which they regulate migration and breast cancer metastasis, much like our results for TRPM7 (Schafer et al., 2012; Yang et al., 2013; Yang et al., 2009). In addition, Orai1 inhibition impaired invadosome formation in microglia, while TRPV2 is involved in invadosome disassembly in macrophages (Nagasawa and Kojima, 2012; Siddiqui et al., 2012). Although these findings suggest that cell adhesions are a hub for Ca\(^{2+}\) signaling, the contribution of localized Ca\(^{2+}\) influx at cell adhesions to cell adhesion dynamics has remained mainly ambiguous. TRPM7 was shown to ignite highly localized Ca\(^{2+}\) signals at the leading edge of migrating fibroblasts in response to mechanical force or growth factor signaling (Wei et al., 2009). The spatial overlap between Ca\(^{2+}\) sparks and focal adhesions was minimal, however, and the effect of TRPM7-mediated Ca\(^{2+}\) sparks on focal adhesion dynamics was not addressed. Our detailed investigation into this matter showed that TRPM7 can indeed mediate localized Ca\(^{2+}\) signals, but these did not correlate with invadosome regulation or location in N1E-115 cells [Chapter 5]. Furthermore, also perturbing cell-wide Ca\(^{2+}\) signals by removal of extracellular Ca\(^{2+}\) did not affect invadosome dynamics, nor did it impair cell migration. Since inhibition of TRPM7 by waixenicin-A induced cell contraction and invadosome dissolution, this suggests that TRPM7 regulates invadosomes and migration through actomyosin-based tension and independent of its Ca\(^{2+}\) permeability. While future experiments should determine whether this holds true for focal adhesion dynamics and other cell types too, TRPM7 was recently demonstrated to also contribute to migration of MDA-MB-231 and MDA-MB-435S breast cancer cells largely independent of mediating Ca\(^{2+}\) influx (Guilbert et al., 2013).

While some studies have proposed that TRPM7-mediated Ca\(^{2+}\) influx regulates cell migration (Chen et al., 2010; Kuras et al., 2012; Wei et al., 2009), other studies have challenged this view and argue for a prominent role of Mg\(^{2+}\) influx (Abed and Moreau, 2009; Callera et al., 2009; Rybarczyk et al., 2012; Su et al., 2011). The defective directional migration of Swiss 3T3 fibroblasts and the deficiency in their ability to form lamellipodia as a...
consequence of TRPM7-knockdown, for example, could be rescued by introducing the Mg\(^{2+}\)-transporter SLC41A2, whereas changing \([\text{Ca}^{2+}]_o\) had no effect (Su et al., 2011). Since TRPM7 knockdown lowered the levels of activated Rac and Cdc42, it was suggested that TRPM7’s Mg\(^{2+}\) conductance regulates directional cell migration by acting on these Rho-family GTPases (Su et al., 2011). Similar results were obtained for polarized cell movements in *Xenopus* embryos by the same groups (Liu et al., 2011). In N1E-115 cells, however, we did not find a role for Mg\(^{2+}\) in the control of TRPM7 over actomyosin remodeling, invadosome formation or migration [*Chapter 5*]. I have extensively discussed the controversy surrounding the potential contribution of Mg\(^{2+}\) influx through TRPM7 to several cell processes in subsection ‘The Mg\(^{2+}\) versus Ca\(^{2+}\) controversy’ in Chapter 1. Since Rho-family GTPases are common regulators of the actomyosin cytoskeleton (Burridge and Wennerberg, 2004), the potential contribution of TRPM7 to Rho and Rac signaling asks for further study. It would be particularly interesting to track the dynamics of Rho and Rac (de)activation, in response to TRPM7-mediated signaling, at high spatiotemporal resolution by genetically-engineered FRET-based sensors. We have shown that waixenicin-A is a powerful new tool for further studies into the function of TRPM7 in adhesion and migration.

**TRPM7 as a kinase or a scaffold: Interaction with a cytoskeletal complex.**

TRPM7’s functional α-kinase-domain can phosphorylate serine and threonine residues in annexin-A1 and MHC-II isoforms *in vitro* (Clark et al., 2006; Clark et al., 2008a; Clark et al., 2008b; Dorovkov et al., 2011; Dorovkov and Ryazanov, 2004). Clark and colleagues (2006) have previously shown that TRPM7 associates with myosin II in a kinase-dependent manner in N1E-115 cells, which coincided with a decrease in cellular tension and the formation of invadosomes (Clark et al., 2006). Since this effect could be mimicked by pharmacological inhibition of myosin II function, this supports a model whereby TRPM7-mediated MHC-II phosphorylation regulates cellular tension and cell adhesion dynamics. In contrast, two consecutive reports from the Habas – and Runnels labs argue for a less profound role of TRPM7’s kinase function in controlling cellular tension and actomyosin remodeling as expression of a kinase-inactive TRPM7 mutant (G1618D) was sufficient to rescue disruption of the actomyosin cytoskeleton and cell migration caused by reduced TRPM7 expression in different model systems (Liu et al., 2011; Su et al., 2011). Although MHC-II phosphorylation was decreased in TRPM7 knockdown MDA-MB-231 cells (Guilbert et al., 2013) and MHC-II phosphorylation and TRPM7 have independently been shown to regulate the motility of these cells [*Chapter 2*] (Dulyaninova et al., 2007; Middelbeek et al., 2012), there is to date no formal proof that TRPM7 phosphorylates MHC-II *in vivo*. The identification of substrates of the TRPM7 kinase-domain, especially *in vivo*, remains a challenge for future studies.

While TRPM7’s kinase-function is shared by TRPM6 and TRPM2 also contains an
enzymatic domain, this intriguing feature has not been observed in any other ion channel discovered to date! There is, however, commonly more to ion channels than just their capacity to permeate ions; they can serve as molecular scaffolds and organize and function in macromolecular signaling complexes. TRP channels, for example, appear to particularly associate with components of the actomyosin cytoskeleton (Clark et al., 2008c; Goel et al., 2005; Tsunoda et al., 2001; Venkatachalam and Montell, 2007), although only little is known about the molecular composition and regulatory function of such complexes. In Chapter 3 we described a proteomic analysis of TRPM7 immunoprecipitates in N1E-115 neuroblastoma cells, which revealed that TRPM7 associates with a largely cytoskeletal complex. In line with our other findings, the vast majority of components in the TRPM7 interactome are involved in actomyosin remodeling and cell adhesion formation. Our literature survey also highlighted that many TRPM7 interactome components are associated with cancer progression in various cancers, but their role in neuroblastoma was largely unknown [see Table 1 in Chapter 3]. In contrast to our results in breast cancer [Chapter 2], TRPM7 did not significantly correlate with clinical parameters in the NB-88 neuroblastoma patient cohort and the custom array of the Oberthuer-251 cohort did not contain probes for TRPM7 [Chapter 3]. However, we found that a substantial proportion of the TRPM7 interactome components did associate with neuroblastoma progression in the NB-88 patient cohort and we validated six of these in the independent Oberthuer-251 cohort. Moreover, of these six genes TRIM28, MYO5A, TAX1BP1 and TMOD2 strongly correlated with bone marrow metastasis formation and, therefore, might be of general significance for neuroblastoma pathogenesis. Mutations in neuritogenesis-regulating genes were recently shown to correlate with aggressive, high-stage neuroblastoma tumors (Molenaar et al., 2012). Since MYO5A and TMOD2 have been shown to function in neuritogenesis (Fath et al., 2011; Lise et al., 2009; Wang et al., 1996) this further implicates defects in neuritogenesis in neuroblastoma pathogenesis. We therefore propose that TRPM7 modulates cell migration and ultimately promotes metastasis formation by controlling cell adhesion dynamics, cell protrusion formation and actomyosin-based cellular tension as part of a cytoskeletal complex.

Our current efforts are focused on elucidating how TRPM7 and these interactors may modulate each other’s function and which interactors are (most) important to TRPM7 signaling, emphasizing on cell adhesion, migration and metastasis. We showed that local TRPM7-mediated Ca\(^{2+}\) sparks (and also Mg\(^{2+}\) influx) did not contribute to cell adhesion dynamics [Chapter 5]. However, we cannot fully exclude the possibility that Ca\(^{2+}\) signals ignited by TRPM7 affect more subtle events, such as association of TRPM7 with components of the TRPM7 interactome. Since TRPM7 phosphorylates MHC-II isoforms in vitro it is tempting to speculate that the TRPM7 interactome contains yet-unidentified substrates for its kinase domain. We should, however, also seriously consider the possibility that TRPM7
exerts regulatory control by acting as a molecular scaffold, independent from its channel and kinase function. In order to differentiate between these functionalities it will be essential to establish viable model systems with a variety of TRPM7 mutants; channel-inactive, kinase-inactive and mutations that impair its interaction with specific interactors; preferably on a background of minimal expression of TRPM7 wildtype channels, because TRPM7 assembles in tetramers. For short-term experiments, pharmacological inhibition of either the TRPM7 channel or its kinase-domain may provide further insight, although specificity and mode of inhibition remain serious challenges. In our opinion, waixenicin-A is a promising and currently the most specific inhibitor of TRPM7 ([Chapter 5] (Kim et al., 2013; Zierler et al., 2011)). The mechanism by which it blocks TRPM7 is, however, not clear. While its potency appears to depend on $[\text{Mg}^{2+}]$, and it was described to inhibit TRPM7 currents (Zierler et al., 2011), our results suggest that waixenicin-A may affect TRPM7 activity and signaling unrelated to just ion fluxes [Chapter 5]. Future experiments should resolve if waixenicin-A may perturb the association of TRPM7 with specific interactors or abrogates TRPM7’s potential scaffold-function altogether by inducing TRPM7 endocytosis.

**TRPM7 activation by PLC(s)**

In Chapter 4 we extended our previous observations that showed that TRPM7 opening and subsequent $\text{Ca}^{2+}$ influx correlates with PLC activation in intact cells (Langeslag et al., 2007). Activation of Gq/PLC-coupled receptors by Bradykinin (or other ligands, such as LPA and TRP) triggers PIP$_2$ hydrolysis, generating DAG, IP$_3$, and a drop in PIP$_2$. We demonstrated that IP$_3$-mediated $\text{Ca}^{2+}$ release was required for TRPM7 activation, while IP$_3$ did not directly act on TRPM7 [Chapter 4]. Importantly, also the drop in membrane PIP$_2$ levels was essential to activate TRPM7, since clamping PIP$_2$ levels by overexpression of PI(4)P 5-kinase 1α blocked TRPM7-mediated $\text{Ca}^{2+}$ influx. In addition, we showed for the first time that TRPM7-mediated $\text{Ca}^{2+}$ influx is a prerequisite to keep the channel open, because it facilitates further or prolonged PIP$_2$ hydrolysis by PLCδ$_1$, a $\text{Ca}^{2+}$-sensitive PLC. Although we showed that TRPM7 and PLCδ$_1$ are both located in invadosomes, we emphasize that this should not be interpreted as support for the local regulation of the actomyosin cytoskeleton by TRPM7, since we falsified this model in Chapter 5. We merely used the invadosome as a tool to demonstrate that PLCδ$_1$ may localize in close proximity to TRPM7, because it is the only structure in N1E-115 cells with a distinctive localization of TRPM7. We propose that the feedforward regulatory loop for TRPM7 activation does not locally control cell adhesion dynamics or actomyosin remodeling. The role of the TRPM7 feedforward regulatory network, other than sustaining TRPM7 activation, remains to be determined.

Our results indicate that PIP$_2$ regulates TRPM7 activation and that TRPM7 can modulate PIP$_2$ levels. The regulation by PIP$_2$ is not unique to TRPM7 nor to TRP channels.
and is commonly rather complex (Hilgemann et al., 2001; Rohacs, 2007). For example, PIP$_2$ regulates TRPV1 sensitization and tunes its gating depending on the strength of the stimulus (Lukacs et al., 2007; Lukacs et al., 2013). Ca$^{2+}$ influx through TRPV1 activates a Ca$^{2+}$-sensitive PLC (PLC$\delta_3$), similar to our results for PLC$\delta_1$ and TRPM7. Whereas we observed that PLC$\delta_1$ activation sustains TRPM7 channel opening, PLC$\delta_3$ activation was proposed to contribute to capsaicin-induced desensitization of TRPV1 (Lukacs et al., 2007) and similar results have been obtained for TRPM8 (Rohacs et al., 2005; Yudin et al., 2011).

**Concluding remarks and future directions**

1. TRPM7 contributes to metastasis formation *in vivo* and may represent a useful marker for cancer progression and disease outcome. Although our cell biological investigations *in vitro* suggest that TRPM7 controls cell-matrix adhesions and the migratory potential of tumor cells, but not proliferation and survival, future experiments should be aimed at resolving which aspect(s) of TRPM7 signaling and functioning is particularly involved in *in vivo* metastasis and during which steps of the metastatic cascade. Intravital imaging of disseminating tumor cells in living mice should allow to study this in more detail by tracking their faith in circulation, during extravasation and subsequent colonization of the distant organ.

2. TRPM7 acts as a regulator of cellular tension, cell adhesion dynamics and migration independent from mediating (localized) Ca$^{2+}$-signaling. Rather our results suggest that TRPM7 may control these processes by functioning as a scaffold.

3. TRPM7 activation regulates PIP$_2$ levels and future efforts should be aimed at elucidating the biologically relevant consequence of the resulting prolonged activation of TRPM7.

In sum,

‘TRPM7: a M$^1$agnificent, M$^2$ultifunctional, M$^3$agnesium-regulated cation channel-kinase that triggers localized Ca$^{2+}$ events, potentially in response to M$^4$echanical cues, but promotes M$^5$igration and M$^6$etastasis by controlling cell adhesion dynamics, actomyosin remodeling and M$^7$yosin II-based cellular tension, Ca$^{2+}$-independently, as part of a cytoskeletal complex.’

**References**


Baldoli, E and Maier, JA. (2012) Silencing TRPM7 mimics the effects of magnesium deficiency in human microvascular endothelial cells. Angiogenesis.; 15 (1); 47-57.


Callera, GE, He, Y, Yogi, A, Montezano, AC, Paravicini, T, Yao, G, and Touyz, RM. (2009) Regulation of the novel Mg2+ transporter transient receptor potential melastatin 7 (TRPM7) cation channel by bradykinin in vascular smooth muscle cells. J.Hypertens.; 27 (1); 155-166.


Clark, K, Middelbeek, J, Dorovkov, MV, Figdor, CG, Ryazanov, AG, Lasonder, E, and van Leeuwen, FN. (2008a) The alpha-kinases TRPM6 and TRPM7, but not eEF-2 kinase, phosphorylate the assembly domain of myosin IIA, IIB and IIC. FEBS Lett.; 582 (20); 2993-2997.


Dulyaninova, NG, House, RP, Betapudi, V, and Bresnick, AR. (2007) Myosin-IIA heavy-chain phosphorylation regulates the motility of MDA-MB-231 carcinoma cells. Mol.Biol.Cell; 18 (8); 3144-


Hilgemann, DW, Feng, S, and Nasuhooglu, C. (2001) The complex and intriguing lives of PIP2 with ion channels and transporters. Sci.STKE.; 2001 (111); re19-


Kuras, Z, Yun, YH, Chimote, AA, Neumeier, L, and Conforti, L. (2012) KCa3.1 and TRPM7 channels at the uropod regulate migration of activated human T cells. PLoS.One.; 7 (8); e43859-


Lukacs, V, Yudin, Y, Hammond, GR, Sharma, E, Fukami, K, and Rohacs, T. (2013) Distinctive changes in
plasma membrane phosphoinositides underlie differential regulation of TRPV1 in nociceptive neurons. 

*J.Neurosci.;* 33 (28); 11451-11463.


Ridley, AJ. (2011) Life at the leading edge. *Cell;* 145 (7); 1012-1022.


Schafer, C, Rymarczyk, G, Ding, I, Kirber, MT, and Bolotina, VM. (2012) Role of molecular determinants of store-operated Ca(2+)+ entry (Orai1, phospholipase A2 group 6, and STIM1) in focal adhesion formation and cell migration. *J.Biol.Chem.;* 287 (48); 40745-40757.


Yang, S, Zhang, JJ, and Huang, XY. (2009) Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell*; 15 (2); 124-134.

