

siRNA sequences used for knockdown.

mouseRalA : 5'-AAGGCAGGTTCTGTAGAA-3' [1,2],
mouseRalB: 5'-GGTGGTTCTCGACGGAGAA-3' [2,3],
mouseRalBP1: 5'-ACATCATGGTCCTCTAC-3' [4],
sh-mouseArf6:5'-AGCTGCACCGCATTATCAA-3' [5,6],
humanRalA: 5'-CAGAGCTGAGCAGTCCAAT-3' [7],
humanRalB: 5'-GGTGATCATGGTTGGCAGC-3' [7],
humanRalBP1: 5'-GTAGAGAGGACCATGATG-3' [8],
human RalBP1#2 : 5'-ACATCATGGTCCTCTAC-3' [4],
humanArf6#1: 5'-TCCTCATCTTCGCCAACAA-3' [9] and
humanArf6#2: 5'-GCACCGCATTATCAATGACCG-3' [9]
humanCyth3#1: GTCGCCAGTTCCCTTATA [10]
humanCyth3#2: CAGCAGAGATCCCTTCTAT [10]
humanCYTH1: AACGACCTCCTGAAGAACACT [11]
humanARNO#2 (Cyth2) : AAGATGGCAATGGGCAGGAAG [11]

siRNA SMARTPOOL SEQUENCES

mouse Sec5 (L-042601)

mSec5_SM1 AGAAACTATTAGTCGGAAA
mSec5_SM2 TCAACGTACTTCAGCGATT
mSec5_SM3 CAGCAGAGATTACACGTCA
mSec5_SM4 GTGAGTGGCTTGCGCAGTA

Mouse ARNO ((L-059077)

mARNO_SM1 TAAGTGAAGCTATGAGCGA
mARNO_SM2 CAGAAAATTGATCGAATGA
mARNO_SM3 AATTAAGATTCCAGAACGT
mARNO_SM4 GCAAGAAAGAAGCGAATT

Human ARNO #1 (L-011925)

hARNO_SM1 TGGCAGTGCTCCATGCTTT
hARNO_SM2 AAACCGAACTGCTTTGAAC
hARNO_SM3 GTAAGACCTTGCAACGGAA
hARNO_SM4 GAACACACCCGAGGAGATC

RTPCR primers

mARNO_F: 5'-CCTTGCCCAGAGATACTGC-3'
mARNO_R: 5'-GGATTGTGAAGGCTGGTGT-3'
hCYTH1_F: 5'-GTTGCCAGCGATATTGTCAG-3'
hCYTH1_R: 5'-GTGCAGACTGGTGTCAACATG-3'
hARNO_F: 5'-TGGTGGAGAATGAAGTGC-3'
hARNO_R: 5'-TCATGCAGATCCACAAAAGC-3'
hCYTH3_F: 5'-TGCTACAGAGTTCCCCAGAA-3'
hCYTH3_R: 5'-TTCATCCCTTCACCCAGGTAG-3'
mActin_F: 5'-GCTACAGCTTCACCACACA-3'
mActin_R: 5'-TCTCCAGGGAGGAAGAGGAT-3'
hActin_F: 5'-GATTCCATGTGGCGAC-3'
hActin_R: 5'-GGTAGTCAGTCAGGTCCCG-3'

Site Directed mutagenesis primers

R79L_A: 5'-GGCAGGAGGACTATGCTGCAATTCTAGACAACTACTTCCGA-3'

R79L_B: 5'-TCGGAAGTAGTTGTCTAGAATTGCAGCATAGTCCTCTGCC-3'

RalBP1*A: 5'-TTGGAATTCCCTTGGCTGATGCAGTCGAACGAACATGATGTATGGCATTGGCTGC -3'

RalBP1*B: 5'-GCAGCCGAATGCCATCATACATCATAGTCGTTGACTGCATCAGCCAAAGGAATTCCAA -3'

REFERENCES

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- [1] N. Vitale, J. Mawet, J. Camonis, R. Regazzi, M.-F. Bader, S. Chasserot-Golaz, The Small GTPase RalA controls exocytosis of large dense core secretory granules by interacting with ARF6-dependent phospholipase D1, *J. Biol. Chem.* 280 (2005) 29921–29928. doi:10.1074/jbc.M413748200.
 - [2] N. Balasubramanian, J.A. Meier, D.W. Scott, A. Norambuena, M.A. White, M.A. Schwartz, RalA-exocyst complex regulates integrin-dependent membrane raft exocytosis and growth signaling, *Curr. Biol.* 20 (2010) 75–79. doi:10.1016/j.cub.2009.11.016.
 - [3] G. Li, L. Han, T.-C. Chou, Y. Fujita, L. Arunachalam, A. Xu, et al., RalA and RalB function as the critical GTP sensors for GTP-dependent exocytosis, *Journal of Neuroscience*. 27 (2007) 190–202. doi:10.1523/JNEUROSCI.2537-06.2007.
 - [4] L.E. Goldfinger, C. Ptak, E.D. Jeffery, J. Shabanowitz, D.F. Hunt, M.H. Ginsberg, RLIP76 (RalBP1) is an R-Ras effector that mediates adhesion-dependent Rac activation and cell migration, *J. Cell Biol.* 174 (2006) 877–888. doi:10.1083/jcb.200603111.
 - [5] S. Choi, J. Ko, J.-R. Lee, H.W. Lee, K. Kim, H.S. Chung, et al., ARF6 and EFA6A regulate the development and maintenance of dendritic spines, *Journal of Neuroscience*. 26 (2006) 4811–4819. doi:10.1523/JNEUROSCI.4182-05.2006.
 - [6] N. Balasubramanian, D.W. Scott, J.E. Casanova, M.A. Schwartz, Arf6 and microtubules in adhesion-dependent trafficking of lipid rafts, *Nat. Cell Biol.* 9 (2007) 1381–1391. doi:10.1038/ncb1657.
 - [7] G. Oxford, C.R. Owens, B.J. Titus, T.L. Foreman, M.C. Herlevsen, S.C. Smith, et al., RalA and RalB: antagonistic relatives in cancer cell migration, *Cancer Res.* 65 (2005) 7111–7120. doi:10.1158/0008-5472.CAN-04-1957.
 - [8] K.-H. Lim, D.C. Brady, D.F. Kashatus, B.B. Ancrile, C.J. Der, A.D. Cox, et al., Aurora-A phosphorylates, activates, and relocates the small GTPase RalA, *Mol. Cell. Biol.* 30 (2010) 508–523. doi:10.1128/MCB.00916-08.
 - [9] A. Béglé, P. Tryoen-Tóth, J. de Barry, M.-F. Bader, N. Vitale, ARF6 regulates the synthesis of fusogenic lipids for calcium-regulated exocytosis in neuroendocrine cells, *J. Biol. Chem.* 284 (2009) 4836–4845. doi:10.1074/jbc.M806894200.
 - [10] Y. Fu, J. Li, M.-X. Feng, X.-M. Yang, Y.-H. Wang, Y.-L. Zhang, et al., Cytohesin-3 is upregulated in hepatocellular carcinoma and contributes to tumor growth and vascular invasion, *Int J Clin Exp Pathol.* 7 (2014) 2123–2132.
 - [11] M. Morishige, S. Hashimoto, E. Ogawa, Y. Toda, H. Kotani, M. Hirose, et al., GEP100 links epidermal growth factor receptor signalling to Arf6 activation to induce breast cancer invasion, *Nat. Cell Biol.* 10 (2008) 85–92. doi:10.1038/ncb1672.