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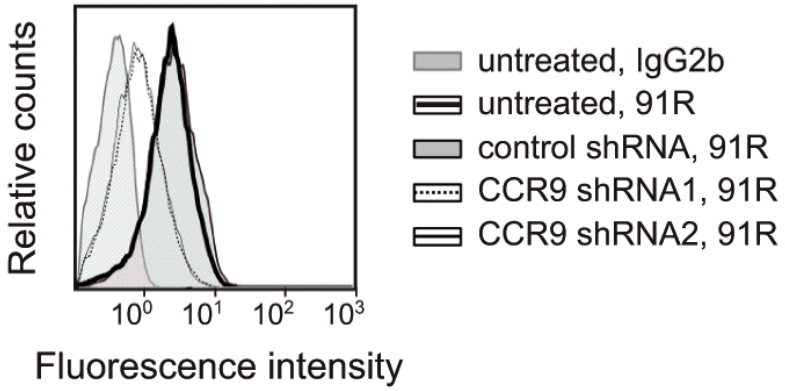
Supplemental Material to:

Sonia Chamorro, Maria Vela, Ana Franco,
Laura Carramolino, Julio Gutiérrez, Lucio Gómez,
María Lozano, Beatriz Salvador, Mónica García-Gallo,
Carlos Martínez-A, and Leonor Kremer

Antitumor effects of a monoclonal antibody to human CCR9 in leukemia cell xenografts

mAbs 2014; 6(4)
http://dx.doi.org/10.4161/mabs.29063

http://www.landesbioscience.com/journals/mabs/article/29063/



Supplementary Figure S1:

91R binding to CCR9-silenced MOLT-4 cells

Untreated, control shRNA-, hCCR9 shRNA1- or hCCR9 shRNA2-infected MOLT-4 cells were analyzed by flow cytometry using 91R or isotype control antibody. One representative experiment is shown of three.

Supplementary Materials and Methods:

Lentivirus-mediated shRNA silencing

shRNAi lentiviral particles were prepared using the lentivirus-compatible vectors pCMV-dR8.91 and pMD2.G (Addgene); and non-target-shRNA control or CCR9-shRNA (Mission SHCLNG_10051011MN,Sigma-Aldrich). Each shRNA vector was cotransfected with pCMV-dR8.91 and pMD2.G in the HEK-293T cell line (using Opti-MEM, Invitrogen). At 48 h post-transfection, medium containing viruses was collected and filtered through a 0.45 µm filter (*Tiscornia et al. 2006*). Viral titer was determined by standard procedures.

MOLT-4 cells (2 x 10^5) were plated in 1 ml complete medium 24 h before infection with 350 plaque-forming units (pfu) of the indicated pseudovirus (48 h). Cells were cultured for an additional 7 days in complete medium supplemented with puromycin (2 µg/ml) to select lentivirus-infected cells prior to flow cytometry analysis of CCR9 expression using 91R antibody.

- Tiscornia G, Singer O, Verma IM. Production and purification of lentiviral vectors. Nature Protocols. 2006. 1:241-245.