Online Supplemental Material for

Takeda et al. http://www.jem.org/cgi/doi/10.1084/jem.20031457

Spontaneous Metastasis of 4T1. The spontaneous 4T1 metastasis model was performed essentially as previously described (Cretney, E., K. Takeda, H. Yagita, M. Glaccum, J.J. Peschon, and M.J. Smyth. 2002. *J. Immunol.* 168:1356–1361 and Pulaski, B.A., and S. Ostrand-Rosenberg. 1998. *Cancer Res.* 58:1486–1493). In brief, wild-type or gene-targeted female BALB/c mice were inoculated with 5×10^4 4T1 tumor cells in the abdominal mammary gland. The primary tumor mass was resected on day 28 and then 200 μg MD5-1 or control Ig was administered i.p. on days 28, 32, and 36. The mice were then killed on day 39, and metastatic nodules on the lungs and liver were counted on a per organ basis. Data are represented as the mean $\pm$ SE of five to seven mice in each group. Some treated cohorts ($n = 5$–7 in each group) were not killed on day 39 and were monitored for survival.

Experimental Metastasis of R331-mock and R331-FLIP. BALB/c mice were inoculated with 10^5 R331-mock or R331-FLIP cells intrasplenically or i.v. for liver or lung metastasis, respectively (Seki, N., Y. Hayakawa, A.D. Brooks, J. Wine, R.H. Wiltrout, H. Yagita, J.E. Tanner, M.J. Smyth, and T.J. Sayers. 2003. *Cancer Res.* 63:207–213). The mice were then administered i.p. with 200 μg MD5-1 or control Ig on days 0, 4, and 8. The mice were killed on day 14, and metastatic nodules on the lungs and liver were counted under a dissecting microscope. Data are represented as the mean $\pm$ SE of five to seven mice in each group.

J. Exp. Med. © The Rockefeller University Press • 0022-1007
Volume 199 February 16, 2004
http://www.jem.org/cgi/doi/10.1084/jem.20031457