

ONLINE DATA SUPPLEMENT

A CRITICAL ROLE FOR MACROPHAGES IN PROMOTION OF URETHANE-INDUCED LUNG CARCINOGENESIS.

Rinat Zaynagetdinov*, Taylor P. Sherrill*, Vasiliy V. Polosukhin*, Wei Han*, Jamie A. Ausborn†, Allyson G. McLoed†, Frank B. McMahon*, Linda A. Gleaves*, Amber L. Degryse*, Georgios T. Stathopoulos*, Fiona E. Yull†, Timothy S. Blackwell*, †, ‡, §

*Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine,

†Department of Cancer Biology, ‡Department of Cell and Developmental Biology, Vanderbilt

University School of Medicine, Nashville, TN, and §Department of Veterans Affairs Medical

Center, Nashville, TN, 37232, USA

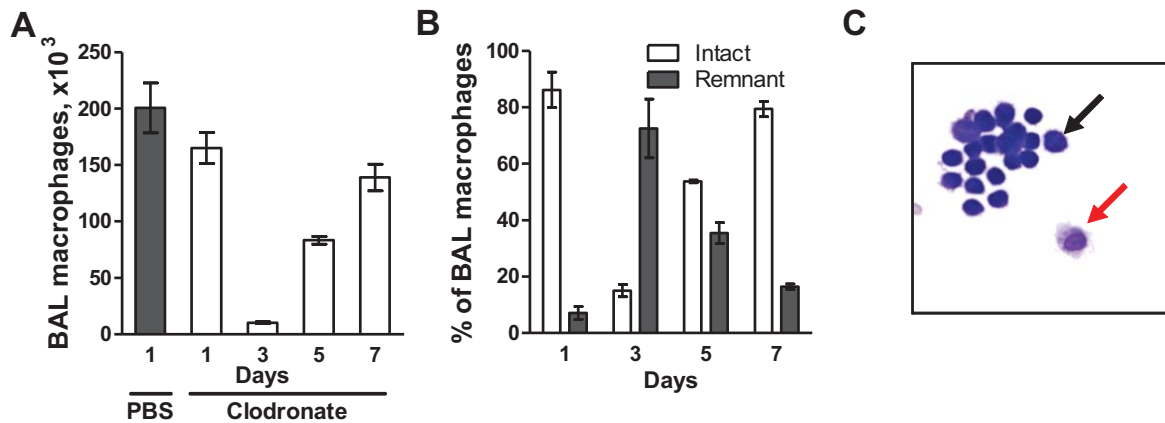


Figure S1. Depletion of alveolar macrophages by clodronate. A) The number of BAL macrophages in mice on days 1-7 after single intratracheal injection of PBS containing liposomes (black bar) or clodronate (open bar) liposomes. B) Identification of intact (open bars) and remnant (black bars) macrophages after single treatment with clodronate liposomes. Data are presented as mean \pm SEM of 3 mice per group. C) Representative microphotograph of normal (red arrow) and remnant (black arrow) macrophages in BAL.

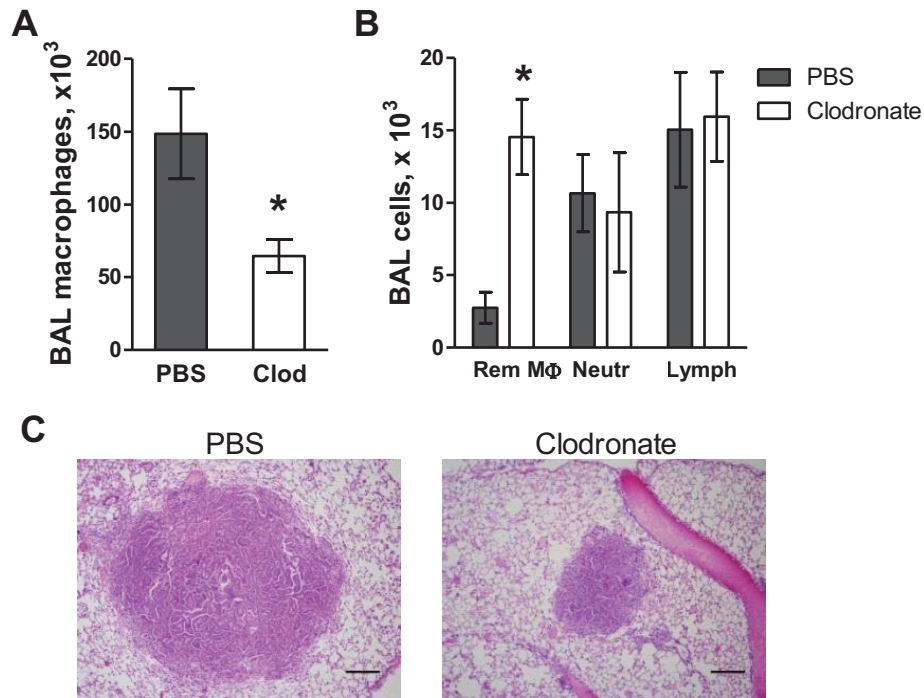


Figure S2. Weekly treatment with liposomal clodronate reduces the number of BAL macrophages at 4 months after injection of urethane. A) The number of intact macrophages and B) remnant macrophages (Rem MΦ), neutrophils (Neutr) and lymphocytes (Lymph) in BAL from mice injected with urethane and treated with weekly injection of PBS (black bar) or clodronate (open bar) liposomes for 4 months. Mice were euthanized 6 days after the last liposome injection. Data are presented as mean \pm SEM of 7 mice for the PBS group and 10 mice for the clodronate group, $*=p<0.05$ compared to the PBS group. C) Photomicrographs of H&E stained lung sections from tumor bearing mice treated with weekly IT injections of PBS liposomes (left panels) or clodronate liposomes (right panels) for 4 months. Bars: 100 μ M.

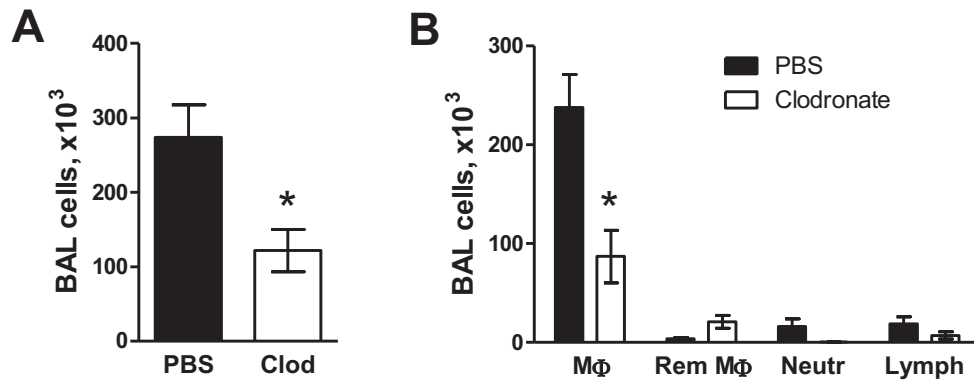


Figure S3. Treatment with clodronate after urethane injection significantly reduces the number of total inflammatory cells in the lungs and lung M1 macrophages. A) Total BAL cells, B) differential cell counts in BAL assessed at day 14 after urethane injection in mice treated with PBS or clodronate liposomes. Animals were given PBS or clodronate liposomes on days 0 and 7 after injection of urethane, n=5 per group, *=p<0.05 compared to PBS group.