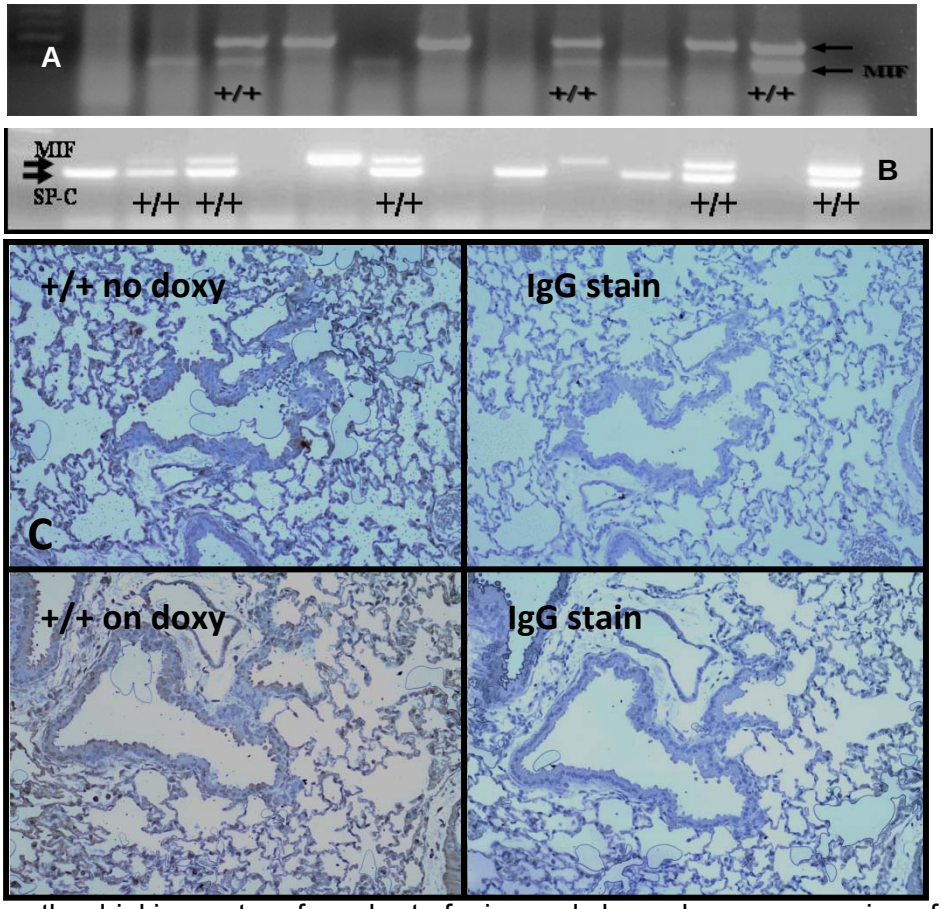
Macrophage migration inhibitory factor (MIF) promotes tumor growth in the context of lung injury and repair.

Douglas Arenberg, Tracy R. Luckhardt, Shannon Carskadon, Liujian Zhao, Mohammad A. Amin, and Alisa E. Koch

ONLINE DATA SUPPLEMENT



Supplemental data: We generated transgenic mice bearing the mMIF cDNA controlled by a Tet-O promoter, and crossed them with mice expressing the reverse tetracycline transactivator (rtTA) on lung specific promoters- either CCSP (CCSP-rtTA), or surfactant protein C (SPC-rtTA). Figures A and B show transgene identification by PCR in a litter showing single, double (++), and non-transgenic mice (A; CCSP-rtTA driver. B; SP-c-rtTA driver).

We added doxycycline to the drinking water of a cohort of mice and showed over-expression of MIF in the lung. Figure C (Top and bottom left panels) shows staining of double transgenic (CCSP-rtTA/tet(O)-MIF) mice with (bottom) or without (top) doxycycline. Control stain of the adjacent sections (IgG; top and bottom right panels). Top left MIF stain shows alveolar macrophages expressing MIF, but minimal to no MIF in bronchiolar epithelial cells showing no significant inflammation. Bottom left panel shows MIF upregulated in bronchiolar epithelium in the presence of doxycycline.

.

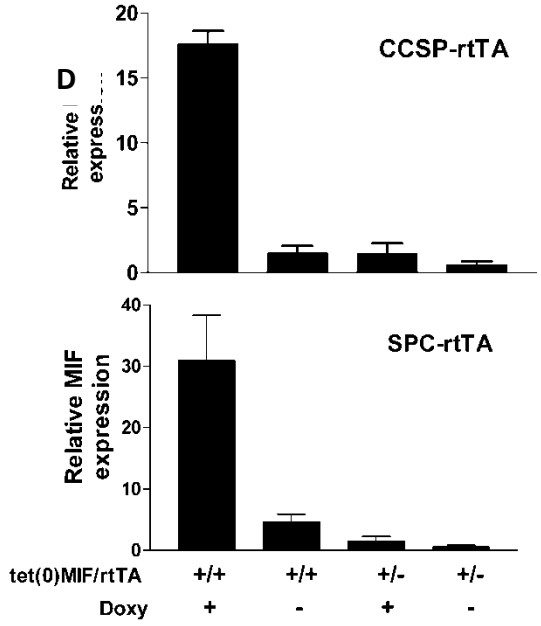
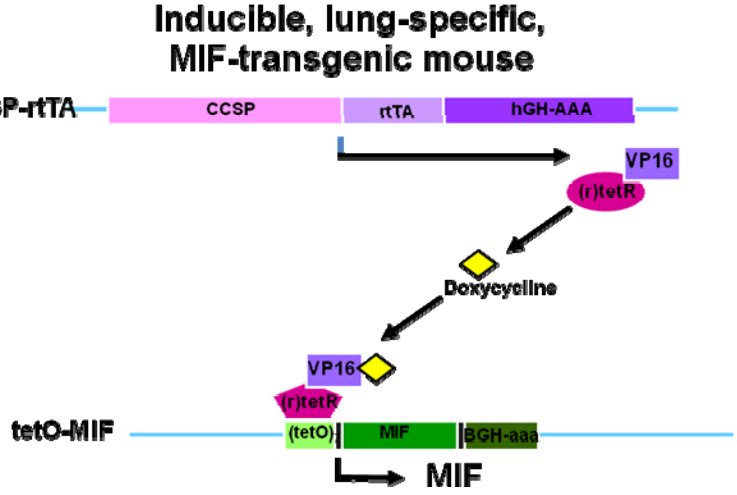
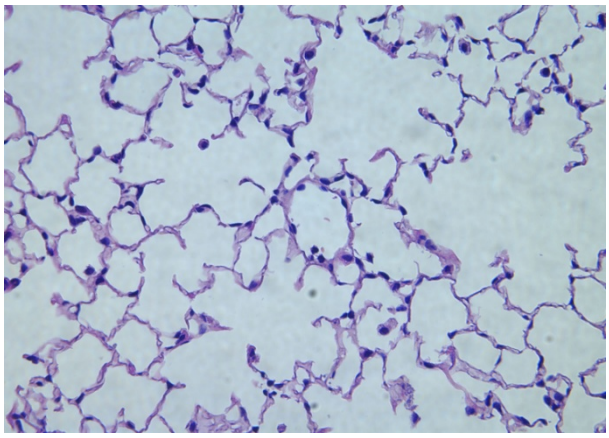


Figure D (left): PCR confirmation of MIF over-expression in double transgenic mice (using either the SP-C, or the CCSP promoter) on doxycycline.





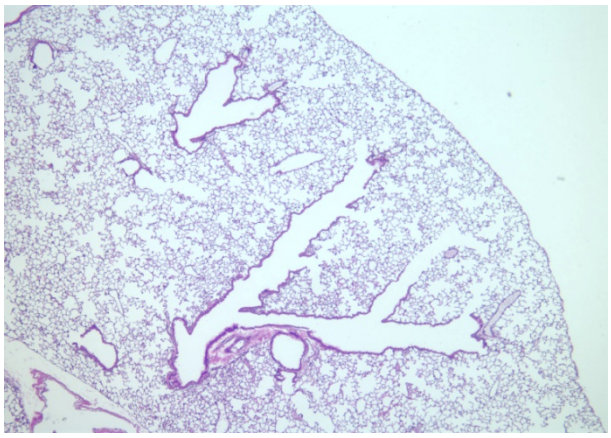
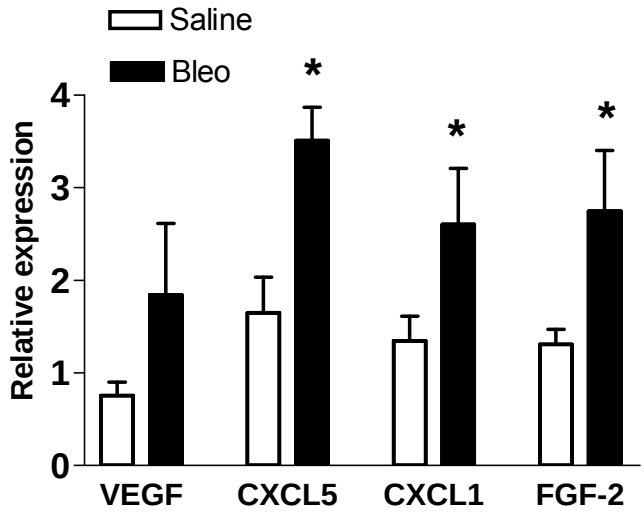


Figure E. Histology of lungs from double transgenic mice (CCSP-rtTA/tet(O)MIF) after receiving two months of doxycycline in the drinking water showing that MIF over-expression by itself induces no histological evidence of lung inflammation or lung injury when the animals are maintained in a pathogen free colony.

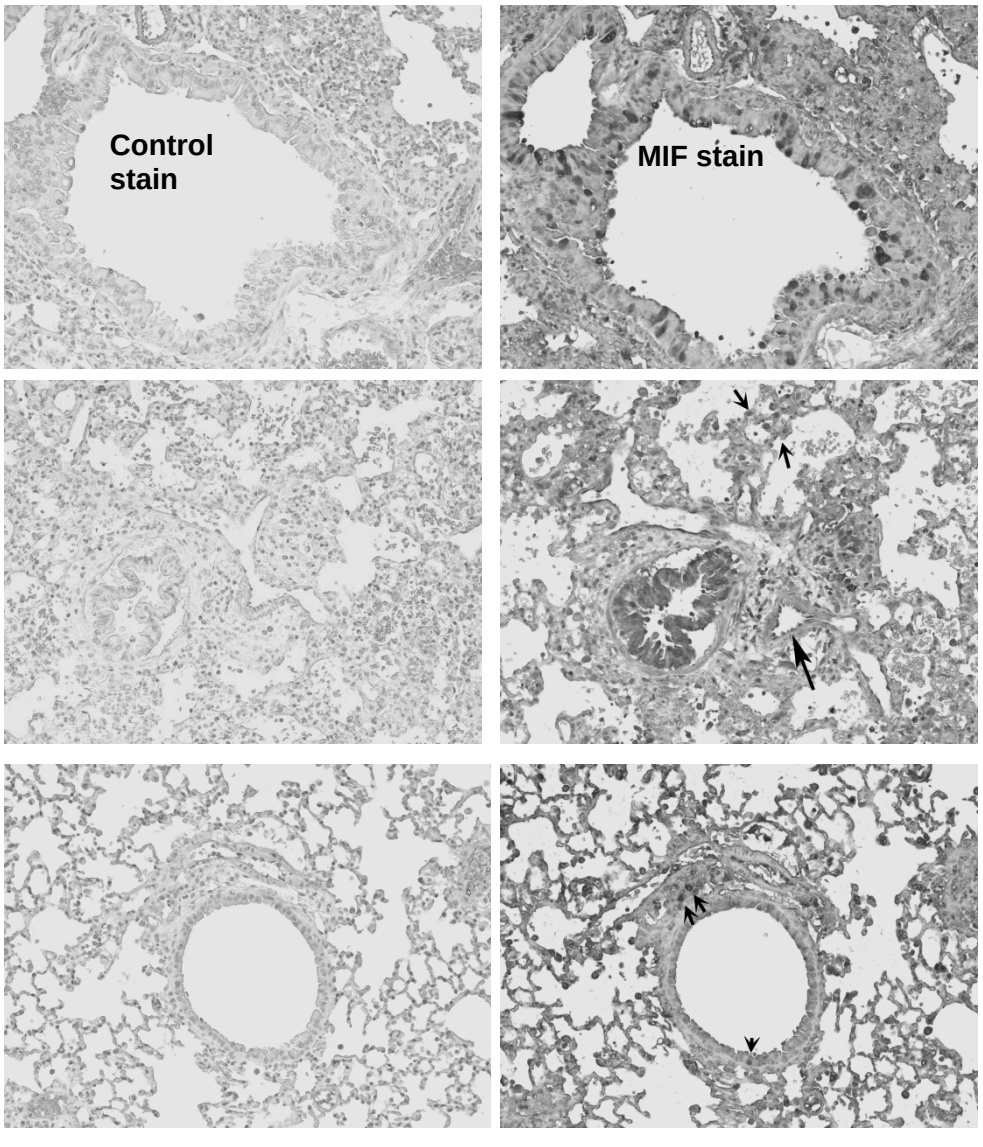


Supplemental figure F: Real-time semi quantitative RT-PCR was used to quantify the expression of angiogenic cytokines in tumors removed from the lungs of mice recovering from bleomycin or shame (saline) induced lung injury. * p <0.05

RNA processing and semi-quantitative real time polymerase chain reaction (RT-PCR). Lung tumors were dissected away from surrounding tissue under a dissecting microscope. We isolated RNA from lung tumors with Trizol reagent (Invitrogen, Carlsbad, CA) using the manufacturer instructions. We reversed transcribed with poly-adenosine and random primers to make cDNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA) following manufacturer's protocols. Semi-quantitative real-time PCR was performed on an ABI PRISM 7000 Sequence Detection System apparatus (Applied Biosystems) using primers probes from Applied Biosystems (Foster City,

CA); Master Mix, Assay-On-Demand primers for murine CXCL1 , CXCL5, VEGF, and basic fibroblast growth factor (FGF2). We use beta-actin to normalize expression levels as described in our previous work (24). The PCR reactions were performed in 25-µL volume containing 1 µL of cDNA per reaction. Data was analyzed with the sequence detection system software. Relative gene expression was calculated using the comparative cycle-threshold (C_T) method. The reference standard for all reactions (assigned a value of 1) is the age matched lung tissue from a genotype matched normal (non-experimental) mouse.

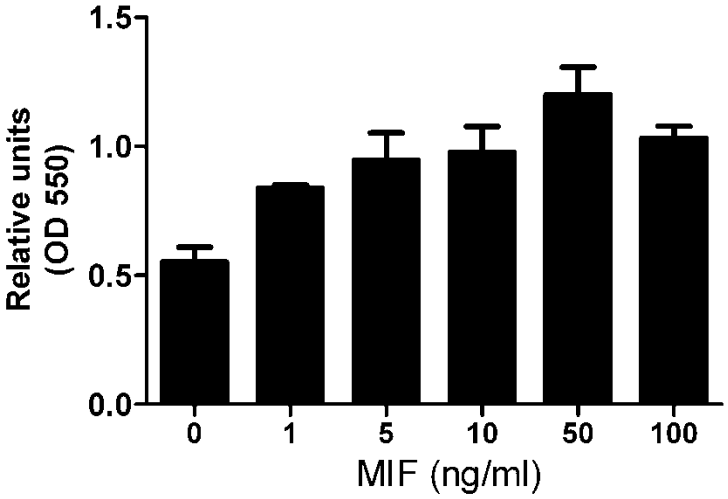
Below: supplemental figure G. Immunolocalization of MIF expressing cells after lung injury. Left column; control IgY. Right column, stain with Chicken anti-human MIF as described in McClelland M,. *Am J Pathol* 2009;174(2), All photographed at 250X magnification.

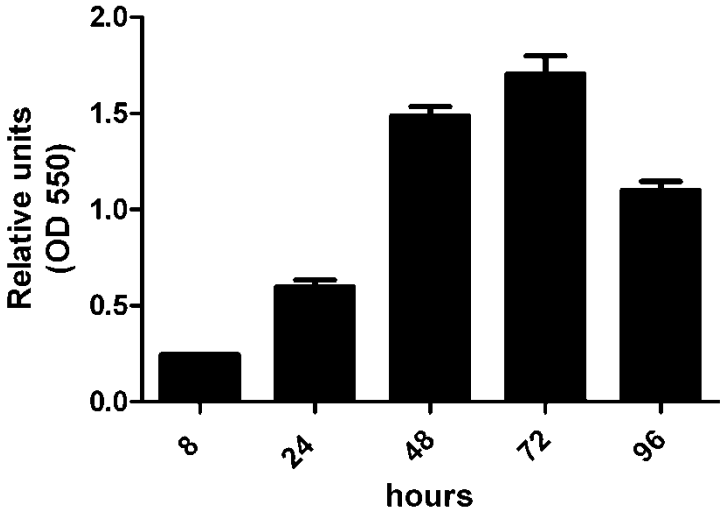


Naphthalene day 7, showing MIF staining in bronchial epithelial cells

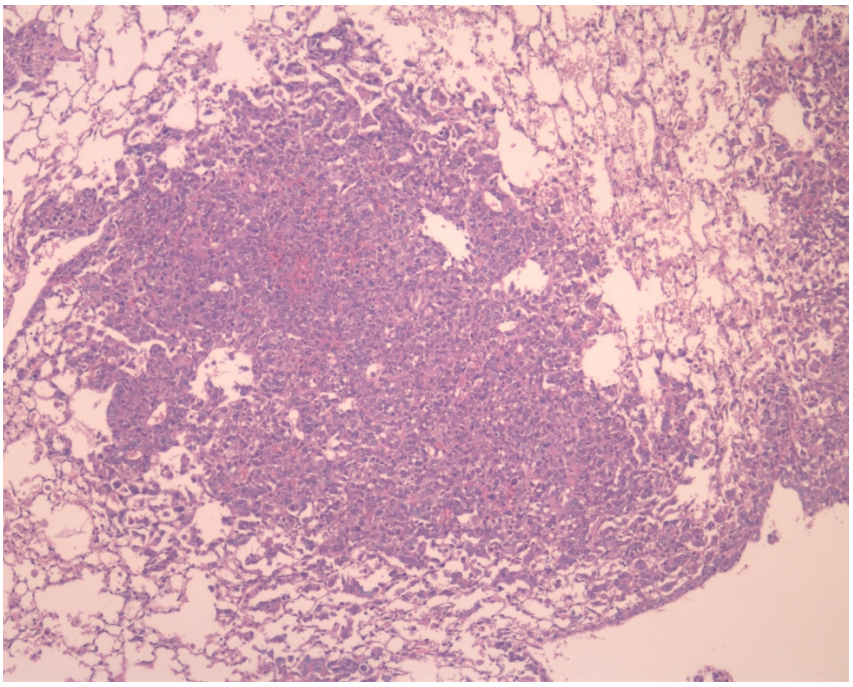
Bleomycin, day 3 showing bronchial, endothelial (long arrow), and alveolar epithelial (small arrow) localization

Bleomycin, day 14 showing MIF expression in bronchial epithelium (single arrow) and adjacent alveoli (double arrow)





Supplemental figure H:. Dose (top) and time course (bottom) responses of LLC cell prolikferation in the presence of MIF.



Supplemental figure I: Below: Low power magnification of 28 day tumors from naphthalene (A and B), bleomycin (C), corn oil (D), or saline (E-F) treated mice.

High power magnification (left) shows the cellularity of the tumors from a naphthalene treated mice.