Eosinophil-derived IL-13 Promotes Emphysema

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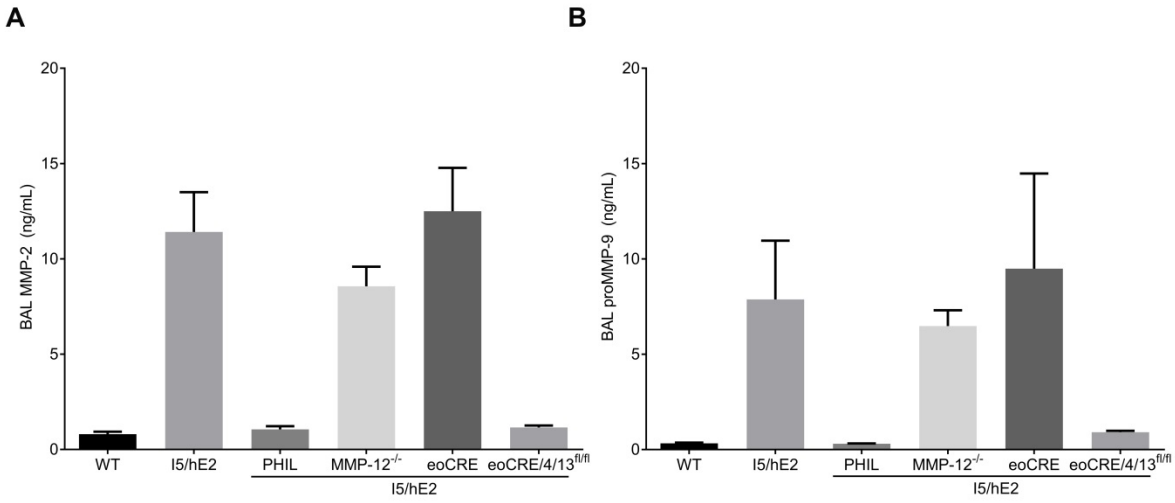
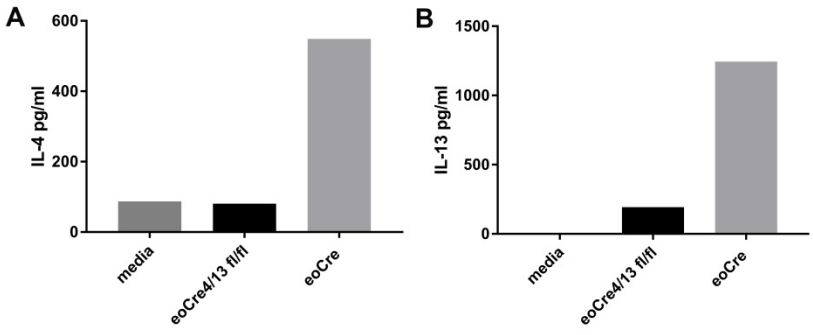
ONLINE SUPPLEMENTARY MATERIAL

Supplementary Table 1. Multivariate Regression analysis showing MMP-12 as the predictor for presence of emphysema

Model Summary						
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Independent variables	Method
1	0.361	0.13	0.107	0.4709	IL-13, Eosinophilia, EPX, MMP-12	Stepwise
Analysis of Variance						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	**Regression**	**1.231**	**1**	**1.231**	**5.553**	**0.024**
	Residual	8.205	37	0.222		
	Total	9.436	38			
Coefficient						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.215	0.112		1.924	0.062
	MMP-12	**0**	**0**	**0.361**	**2.356**	**0.024**
Excluded Variables						
Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	IL-13	.126b	0.806	0.426	0.133	0.967
	Eosinophil	-.225b	-1.411	0.167	-0.229	0.903
	EPX	-.004b	-0.026	0.98	-0.004	0.842
a Dependent Variable: Emphysema						
b Predictors in the Model: (Constant), MMP-12						



Supplementary Figure 1. Eosinophil-derived IL-4/13 induces MMP-2 and MMP-9 production in *I5/hE2* mice. (A) ELISA measurement of MMP-2 in BAL showed MMP-2 in *I5/hE2* mice is dependent on eosinophil-derived IL-4/13. (B) ELISA measurement of proMMP-9 in BAL showed proMMP-9 in *I5/hE2* mice is dependent on eosinophil-derived IL-4/13. n≥3 mice.



Supplementary Figure 2. eoCre4/13$^{fl/fl}$ mice are significantly reduced in their expression of IL-4 and IL-13. Eosinophils from NJ.1638 or NJ.1638/eoCre4/13$^{fl/fl}$ were purified and then cultured at 1 million/ml for 24 hours with IL-33 (30ng/ml) and GM-CSF (10ng/ml). Supernatants were tested by Multiplex array for cytokine expression.

Supplementary Methods

Cell isolation and culture

Eosinophils were isolated as described previously (1) from ***NJ.1638*** or ***IL-13$^{-/-}$/NJ.1638*** (2, 3) mice and resuspended at 5x10^6/mL in RPMI Glutamax (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Invitrogen), 50μM beta-mercaptoethanol (Sigma), 10μg/mL penicillin, 10μg/mL streptomycin, 2mM L-glutamine (Life Technologies, Carlsbad, CA), 1mM sodium pyruvate, and 1x MEM non-essential amino acids (Gibco,Thermo Fisher) (RPMI supp.). IL-5 (Peprotech, Rocky Hill, NJ) was added for eosinophil survival to 5ng/mL and IL-33 (Peprotech) was added to activate the eosinophils to 50ng/mL (IL-33 was omitted from the resting eosinophil experimental group). Cells were placed at 37°C, 5%CO_2 and 24 hours later were washed three times to remove added cytokines and resuspended to 1.5x10^6/mL in RPMI supp. + 5ng/mL IL-5. Macrophages were isolated from C57BL/6J or MMP-12$^{-/-}$ mice as described by Lasbury et al (4). Briefly, mice were euthanized then BAL was performed with 1ml of sterile saline and placed on ice. The BAL was repeated 10 times per mouse. Collected BAL was pooled and counted for macrophage numbers by morphology on a hemocytometer. Cells were then centrifuged at 300g for 10 min. at 4°C and resuspended in RPMI supp. to 300,000 cells per mL. Cells were then plated at 500μl (150,000 cells) per well in 24 well culture plates (Corning, Corning, NY) and incubated at 37°C, 5%CO_2 for 2 hours. Plates were then washed three times with PBS to remove non-adherent cells. Adherent cells (macrophages) remained in the wells and 500μl RPMI supp. was added to each well. Cells were then cultured for 48 hours with the addition of either eosinophils (750,000 cells) in 500μl RPMI supp. or media alone. To

assess contact dependency the above was performed in transwell plates (0.4μm pore) (Corning).

Supplementary References

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