

## Supplemental Figure Legends

**Supplemental Figure 1.** Validation of global AM response to cigarette smoking by independent data set. Expression of 123 significant smoking-responsive probe sets detected in a study of Woodruff et al. (17) in which AM were isolated by flow cytometry was compared to that in our study. **A.** Log<sub>2</sub>-transformed fold-change value for each significant smoking-responsive probe set detected in a study of Woodruff et al. (17) (blue columns) is compared to that detected in our study (red columns); indicated overlap of significantly up- and down-regulated genes is expressed as a percentage of smoking-responsive genes found in the study of Woodruff et al. (17). **B.** Correlation of fold-change values for smoking-responsive probe sets identified in the study of Woodruff et al. (17) with those in the present study based on the gene list of Woodruff et al (17); Spearman nonparametric correlation analysis; p value is indicated.

**Supplemental Figure 2.** TaqMan real-time PCR confirmation of the differential expression of examples of M1- and M2-related polarization genes in alveolar macrophages of healthy nonsmokers (n=5) and healthy smokers (n=5). Data are shown as fold-changes (healthy smokers vs healthy nonsmokers) of gene expression assessed by TaqMan PCR (white bars) in parallel with microarray data (black bars). See Tables II, III for gene names. \*p<0.05; \*\*p<0.01; \*\*\*p<0.005.

**Supplemental Figure 3.** Examples of M1- and M2-related protein expression assessed by FACS analysis. **A.** Representative histograms of expression of M1-related surface molecules; and **B.** M2-related surface molecules. Shown are comparative data from AM of healthy nonsmokers (green) and healthy smokers (orange). For the M1-related genes, shown is CD80 and TNFSF10; for the M2-related genes, shown is CCR5 and CD36. Isotype controls are shown as dashed line. **C.** Quantitative analysis displayed as mean fluorescence intensity index expressed as a percentage increase of mean fluorescence for a given antibody over isotype control for healthy non-

smokers (NS; green; n=3) and healthy smokers (S; orange; n=3). Error bars indicate standard deviation. See Tables II, III for gene names. \*p<0.05; \*\*p<0.01.

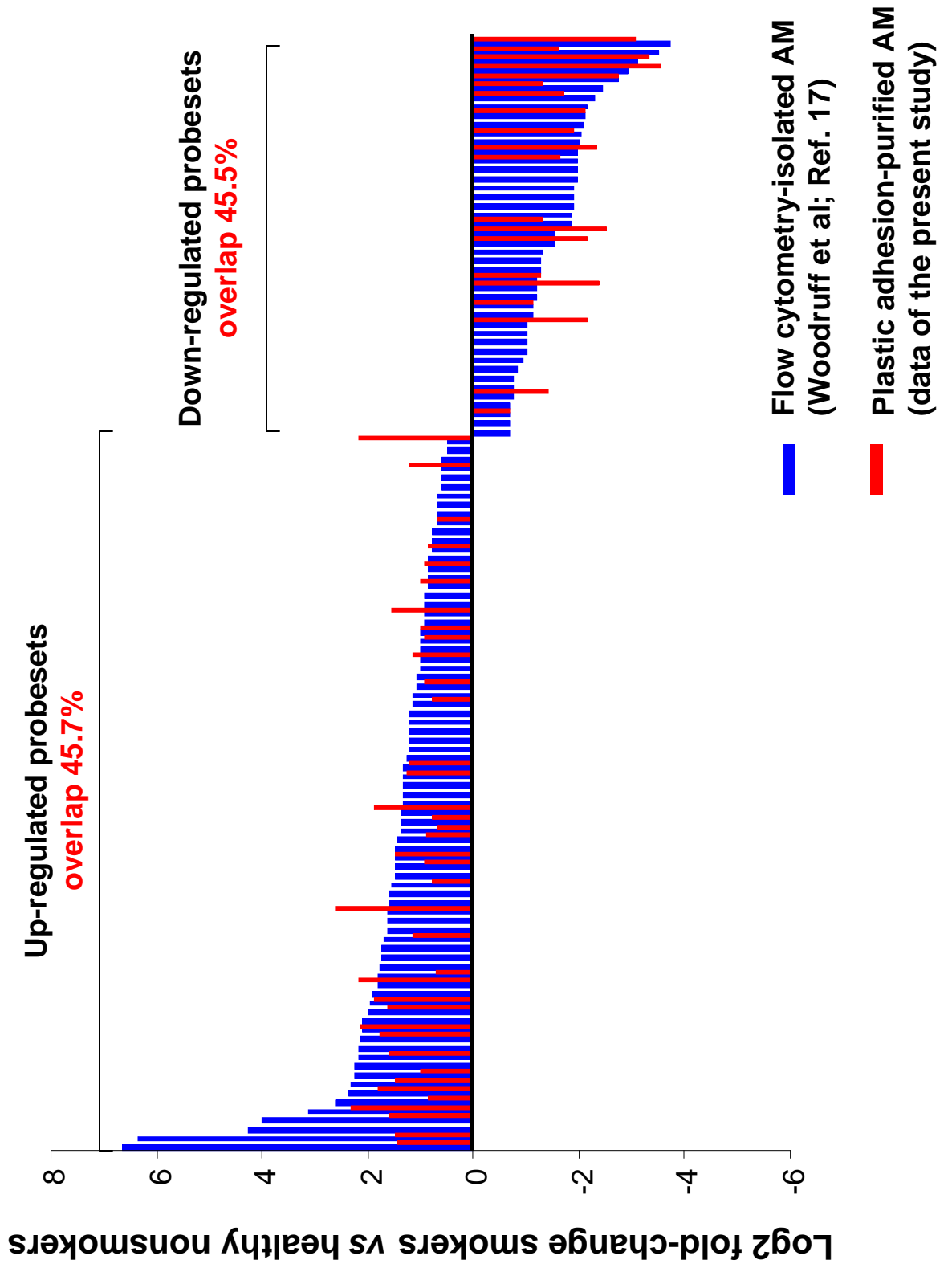
**Supplemental Figure 4.** Progressive alteration of polarization-related gene expression in alveolar macrophage with the development of COPD. **A.** Expression of examples of M1-related genes. **B.** Expression of examples of M2-related genes. For both panels, the analysis was by TaqMan PCR in AM of healthy nonsmokers (n=5; green bars), healthy smokers (n=5; orange bars) and COPD smokers (n=5; blue bars). Data are shown as mean normalized relative to expression of  $\beta$ -actin. Error bars indicate standard deviation. N.S., non-significant. See Tables II, III for gene names. p values are indicated.

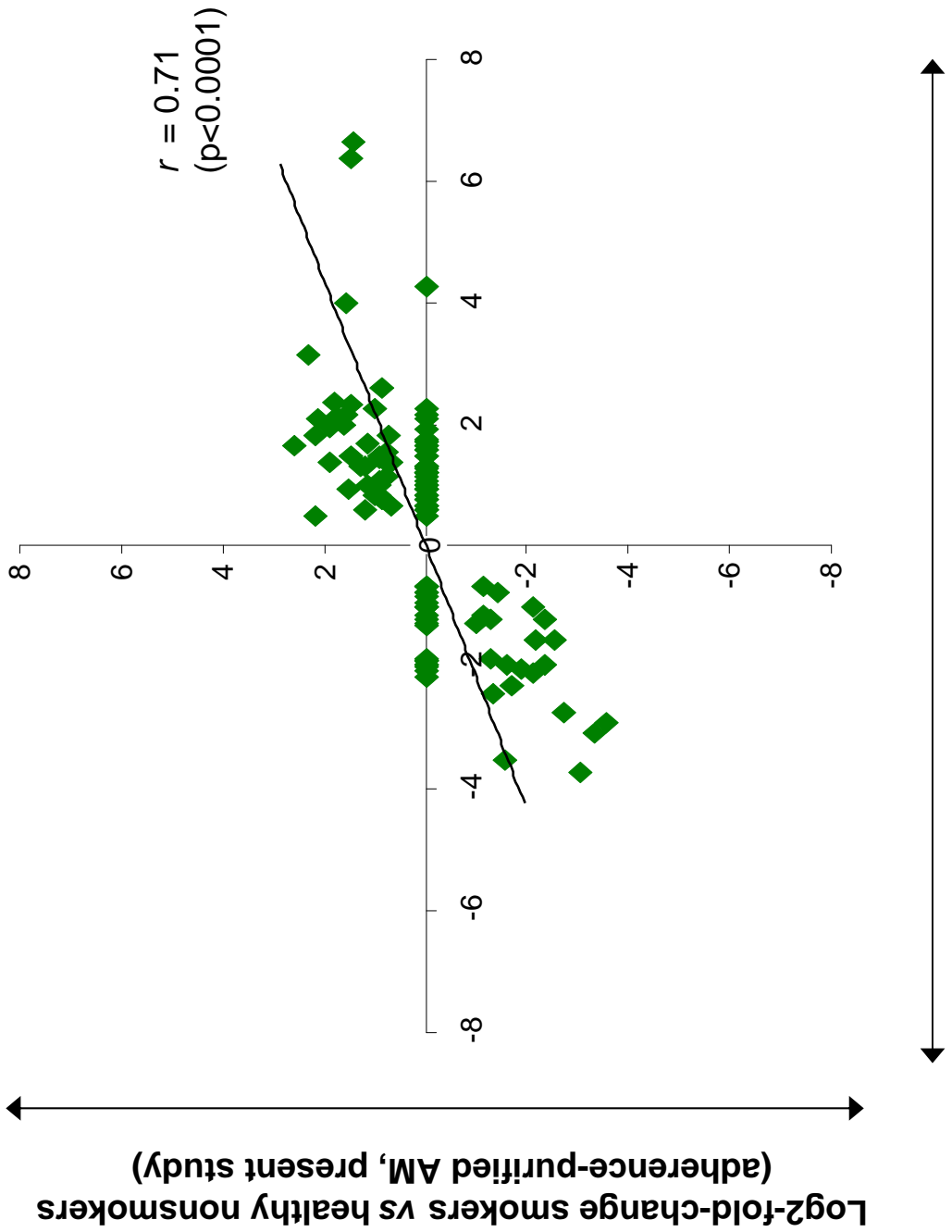
**Supplemental Figure 5.** Correlation of COPD-relevant M1-related gene probe sets with age in **A.** healthy nonsmokers (n=24) and **B.** in COPD smokers (n=12) based on Spearman nonparametric correlation analysis. Significantly correlating probe sets are highlighted in red (positive correlation with age) or blue (negative correlation). The names of significantly correlating genes are given. Non-significantly correlating probe sets are highlighted in grey.

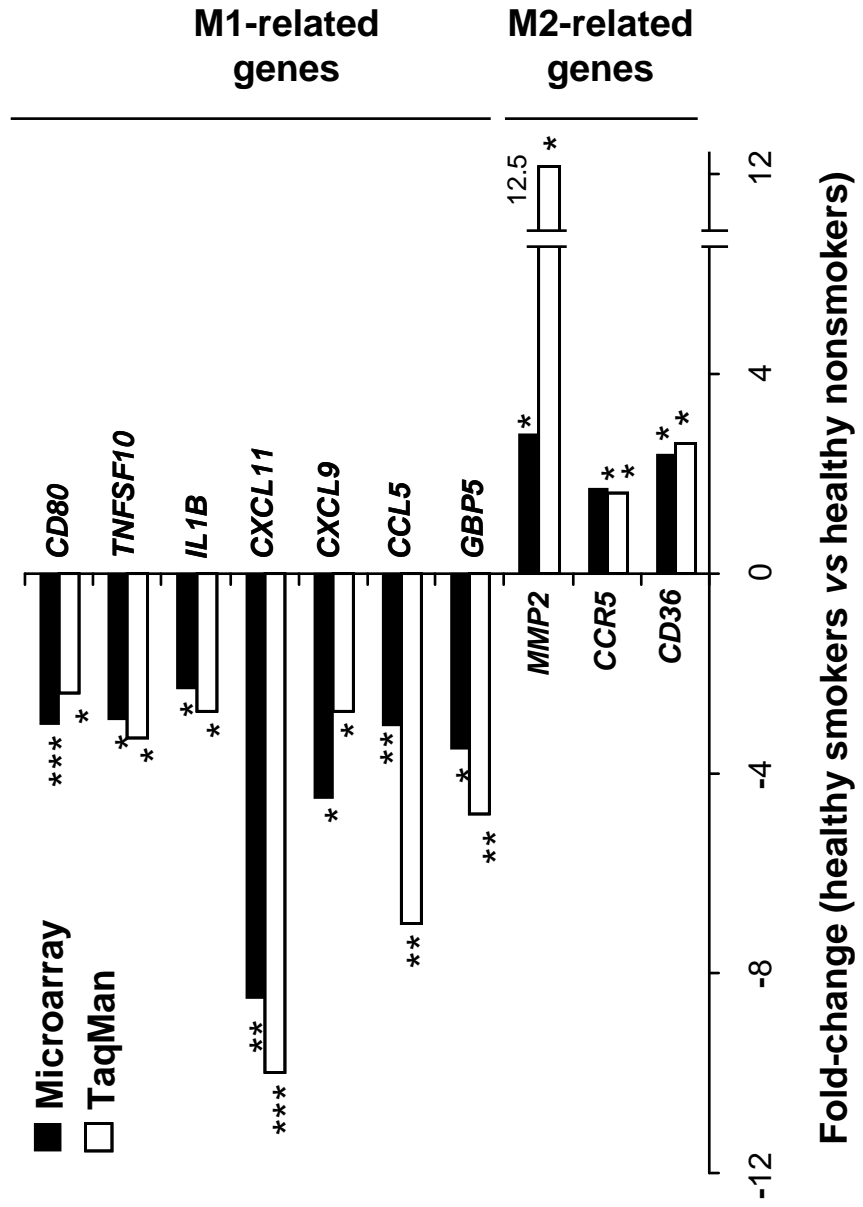
**Supplemental Figure 6.** Correlation of COPD-relevant M2-related gene probe sets with age in **A.** healthy nonsmokers (n=24) and **B.** in COPD smokers (n=12) based on Spearman nonparametric correlation analysis. Significantly correlating probe sets are highlighted in red (positive correlation with age) or blue (negative correlation). The names of significantly correlating genes are given. Non-significantly correlating probe sets are highlighted in grey.

**Supplemental Figure 7.** Correlation of COPD-relevant **A.** M1-related gene probe sets. **B.** M2-related probe sets with pack-years in COPD smokers (n=12) based on Spearman nonparametric correlation analysis. Significantly correlating probe sets are highlighted in red (positive correlation with age) or blue (negative correlation). The names of significantly correlating genes are

given. Non-significantly correlating probe sets are highlighted in grey.

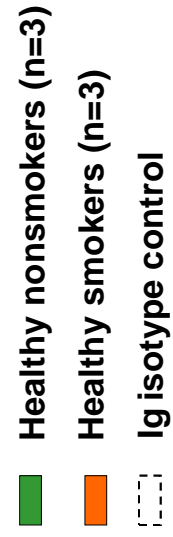
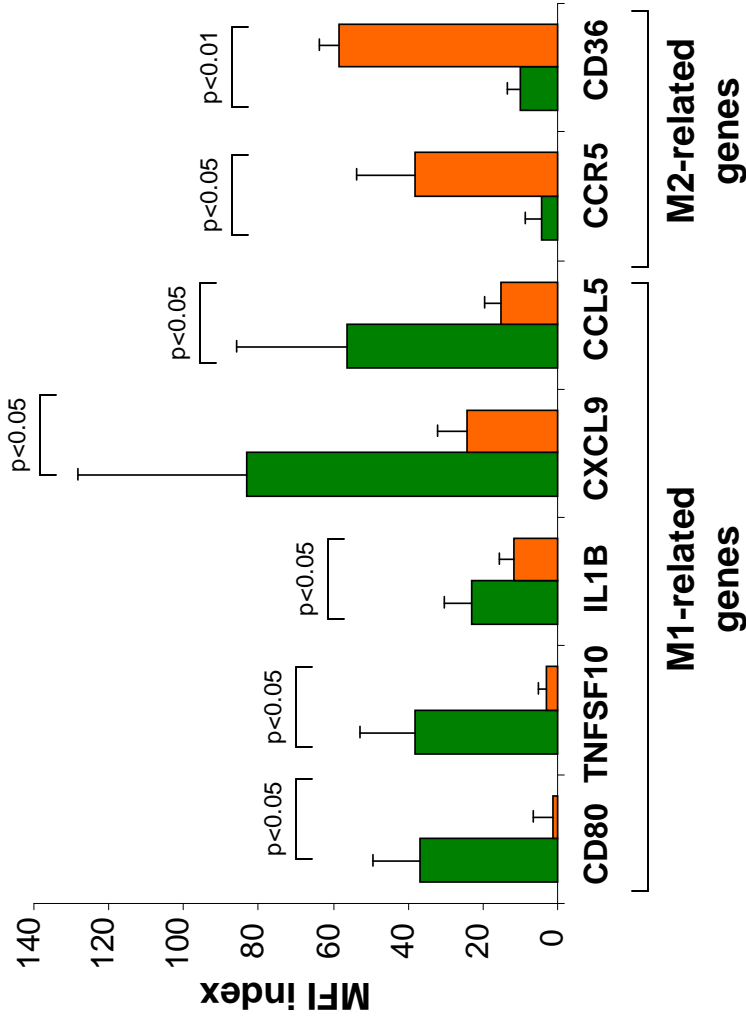




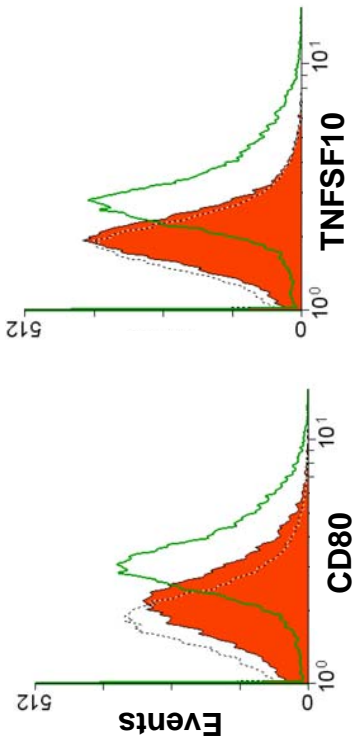


Fold-change (healthy smokers vs healthy nonsmokers)

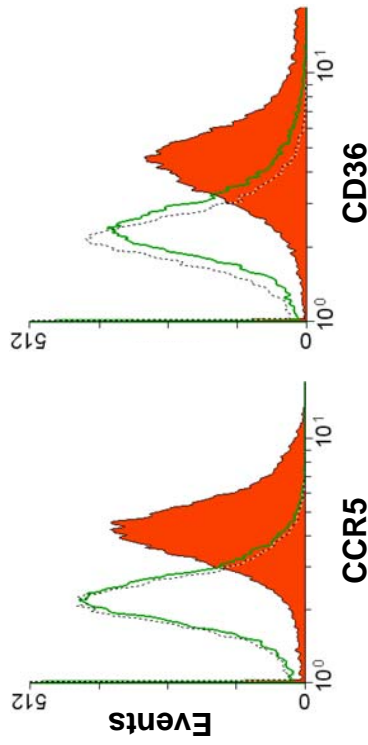
C.



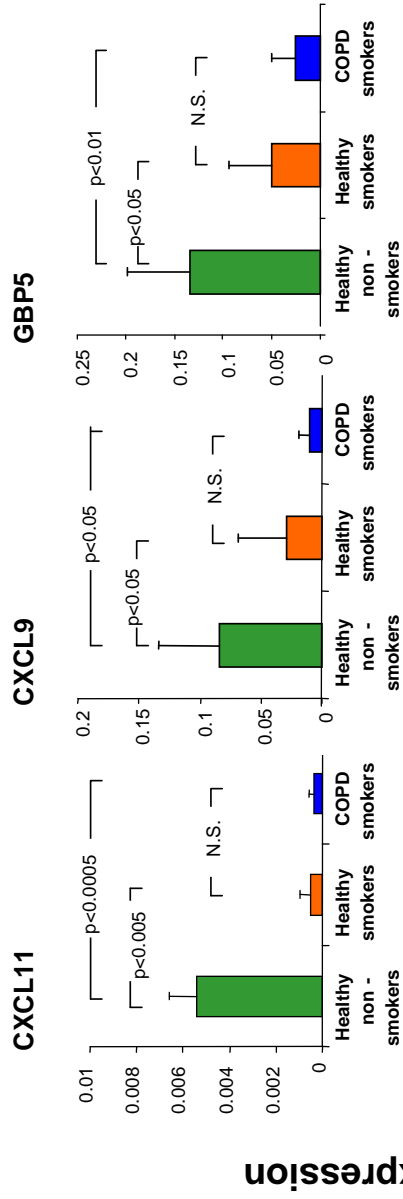
A. M1-related genes



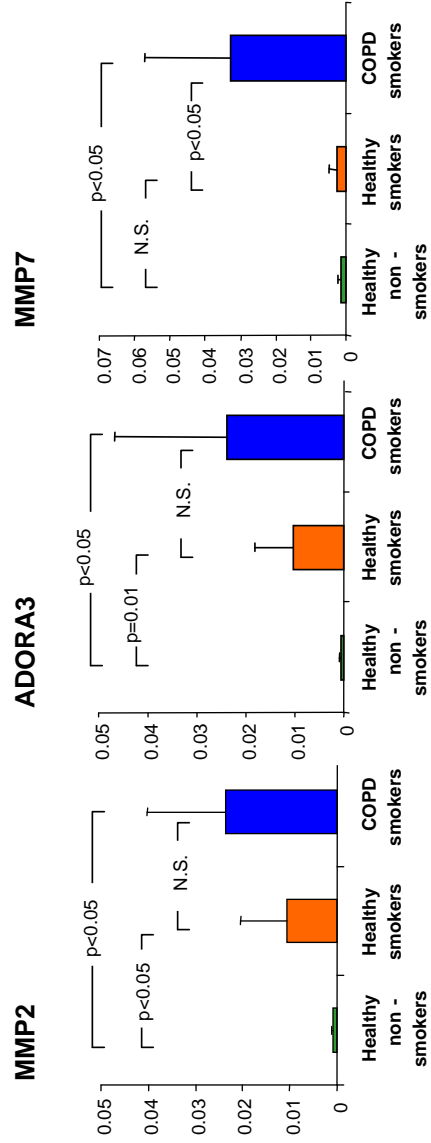
B. M2-related genes



**A. M1-related genes**



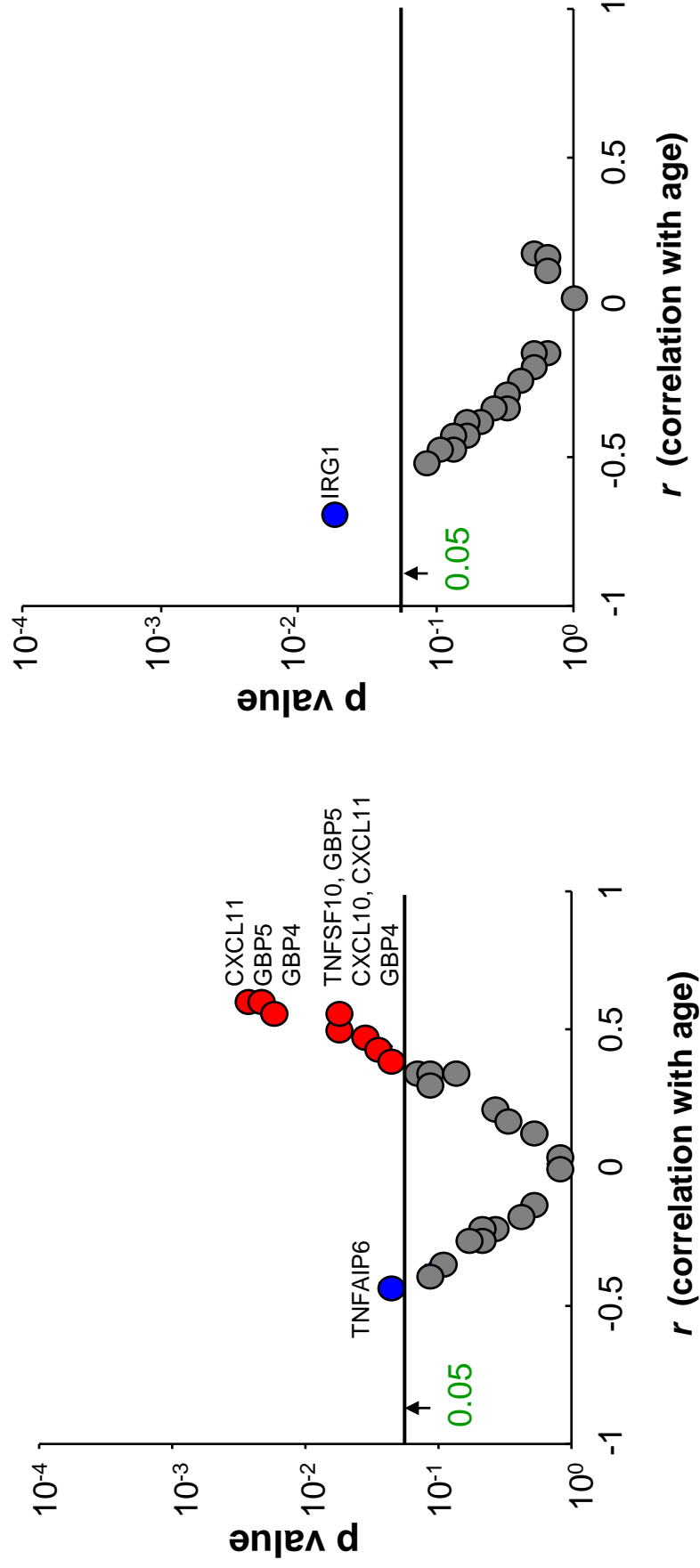
**B. M2-related genes**



Normalized expression

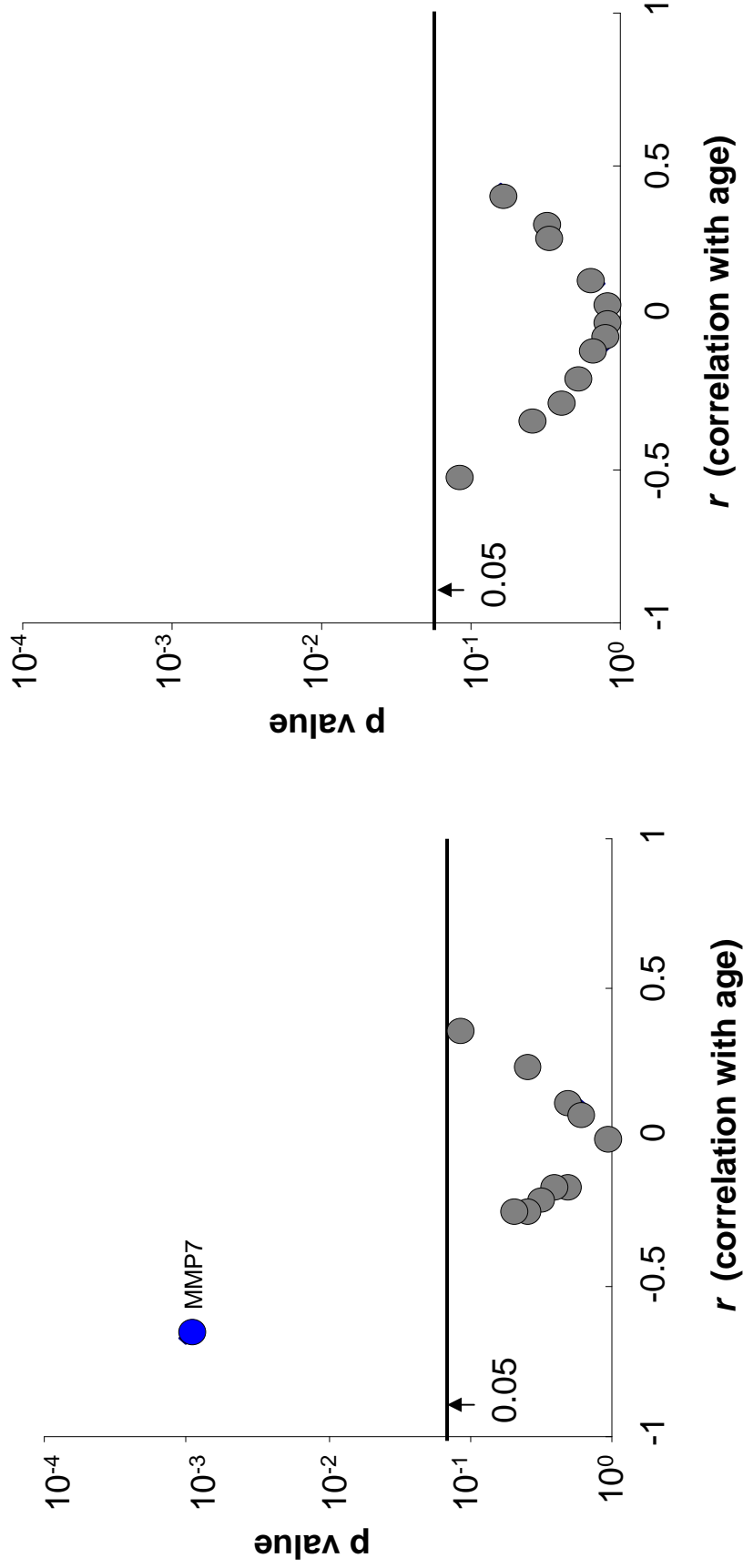


**A. Healthy nonsmokers COPD-relevant M1-related gene probe sets**      **B. COPD smokers COPD-relevant M1-related gene probe sets**

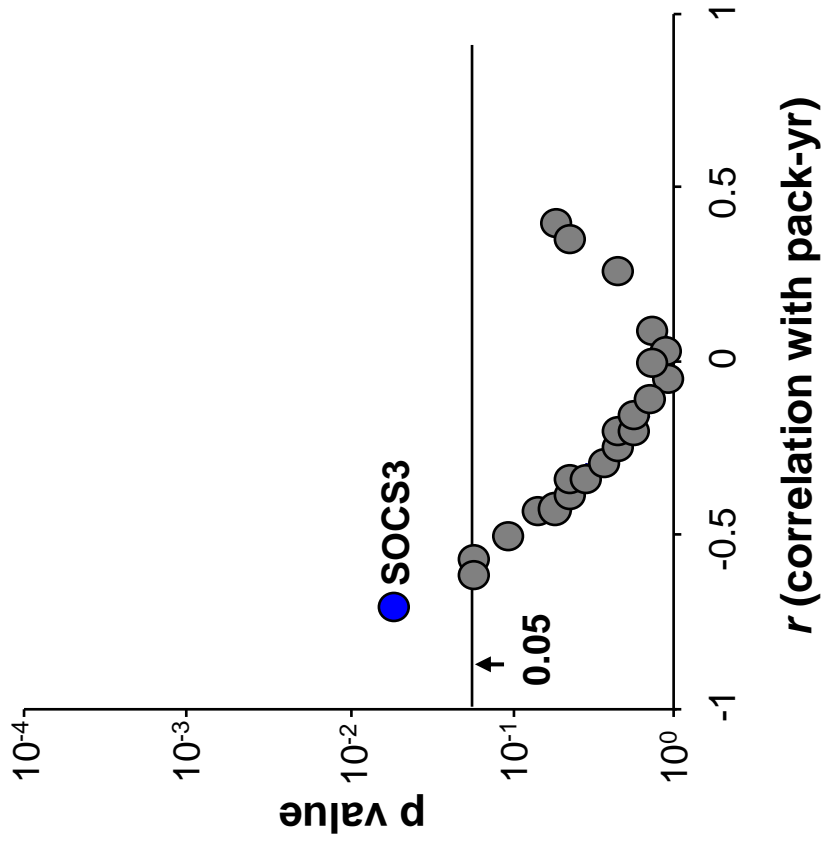


- Significant positive correlation
- Significant negative correlation
- No significant correlation

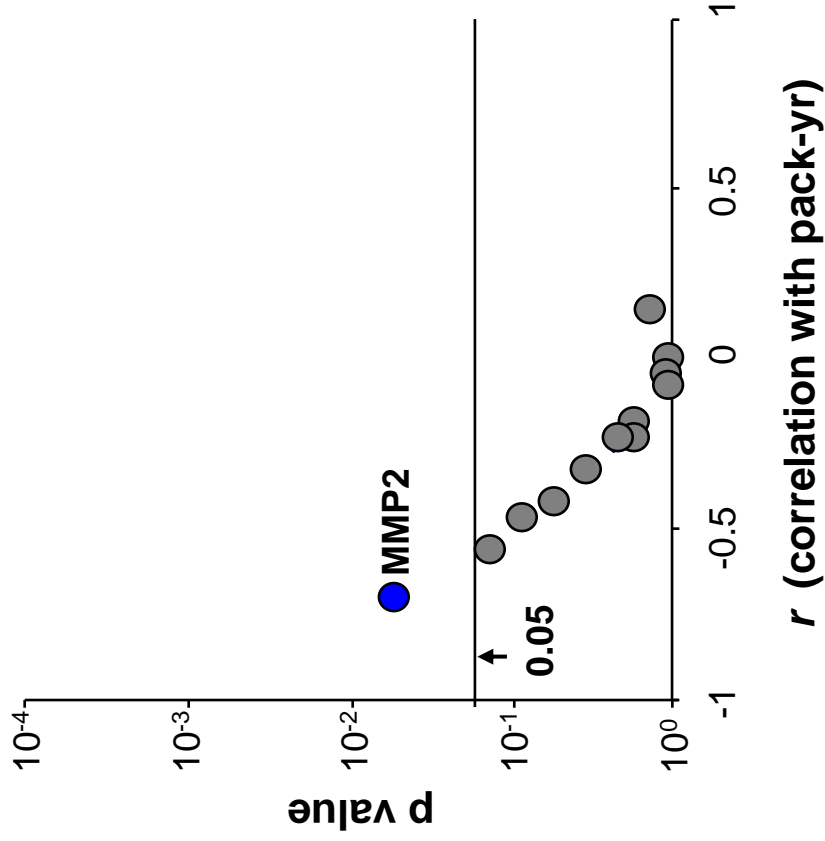
**A. Healthy nonsmokers COPD-relevant M2-related gene probe sets**      **B. COPD smokers COPD-relevant M2-related gene probe sets**



**A. COPD smokers COPD-relevant  
M1-related gene probesets**



**B. COPD smokers COPD-relevant  
M2-related gene probesets**



- Significant positive correlation
- Significant negative correlation
- No significant correlation