

Supplemental Figure 1: HASMC response to LIF, IL-6, IL-31 and IL-11.HASMC cultures were prepared and stimulated as in Fig 1 with 10ng/ml of LIF, IL-6, IL-31 or IL-11 and concentrations of IL-17A up to 10 ng/ml. 24- hour supernatants were collected and cytokine concentrations were quantified by ELISA for MCP-1/CCL-2 (upper panel) or IL-6 (lower panel).



Supplemental Figure 2: Steady state mRNA levels of Eotaxin-3.HASMC were plated and prepared for RNA analysis as in methods after 6 hours(top) and 18 Hours (Bottom) of stimulation with the indicated cytokines at 5ng/ml (OSM at 1 ng/ml). RNA was prepared and analyzed by qRT-PCR using probes for eotaxin-3 as indicated. Levels are expressed as fold change relative to control using b-actin as a reference control gene.



Supplemental Figure 3: IL-8 protein regulation by OSM/IL-17A from HASMC. HASMC cultures were prepared and stimulated as in Fig 1 with 5 ng/ml of IL-4, IL-13 or IL-17A and concentrations of OSM at 0.5, 1 and 5 ng/ml. 18- hour supernatants were collected and cytokine concentrations were quantified by ELISA for IL-8/ CXCL8. ***=P<0.0001 using One-Way ANOVA comparing treatment to unstimulated control (C). *** (above bars) indicate P<0.0001 comparing treatment to OSM alone at the indicated concentration.



Supplemental Figure 4: OSM synergistic in serum free conditions. Panel A-B: HASMC cultures were prepared and stimulated as in Fig 2A with 0-10 ng/ml IL-17A and concentrations of OSM at 0.1, 1 and 10 ng/ml in serum free RPMI containing 1% BSA. 24- hour supernatants were collected and cytokine concentrations were quantified by ELISA for(A) MCP-1 and (B) IL-6. Panel C: HASMC cultures were prepared and stimulated as described previously with 10 ng/ml of IL-4 or IL-17A and/or OSM in 2%FBS/RPMI or serum free RPMI 24-Hour supernatants were assessed by ELISA for Eotaxin-1/CCL11. *=P<0.05; ***=P<0.0001; # =p<0.05 comparing treatment to either cytokine alone using two way ANOVA with Tukey post-test



C OSM LIF IL-31 IL-11 IL-6

Cell line 2



Supplemental Figure 5: HASMC cell signaling in response to OSM and gp130 cytokines in serum-free conditions. HASMC cultures from two different subjects were prepared as previously described and stimulated with 10 ng/ml of OSM, LIF, IL-31, IL-11 or IL-6 in serum-free RPMI medium containing 1% BSA for 20 minutes and lysed. Total cell lysates were probed for indicated proteins using standard immunoblot procedures



Supplemental Figure 6: Time course of HASMC responses to OSM in combination with IL-4, IL-13 or IL-17A in serum free and serum containing conditions. HASMC cultures were prepared as described previously and stimulated with 10 ng/ml of IL-4, IL-13 or IL-17A with or without 10 ng/ml of OSM in 2% FBS RPMI(Left panel) or serum free RPMI containing 1% BSA (Right panel) for 12 (white bars), 24 (grey bars) and 48 hours (black bars). Cell supernatants for each time point were assessed for protein levels of MCP-1 (A), IL-6 (B) and Eotaxin-1/CCL11 (C) by ELISA.