Supplemental Information

Self-assembled micelle RNAi for effective and safe targeting of dysregulated genes in pulmonary fibrosis

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I. Extended Chemical Synthesis : Detailed conjugation process in the generation of SAMiRNA

1) General information:

All the starting materials were obtained from commercial supplies and used as received. The reactions were monitored by thin-layer chromatography (TLC) analysis on silica gel glass plates (TLC Silicagel 60 F254, Merck). Column chromatography was performed on silica gel (240-400 mesh, Watchers). ^{1}H and ^{13}C NMR spectra were recorded with a Varian Mercury 300MHz)

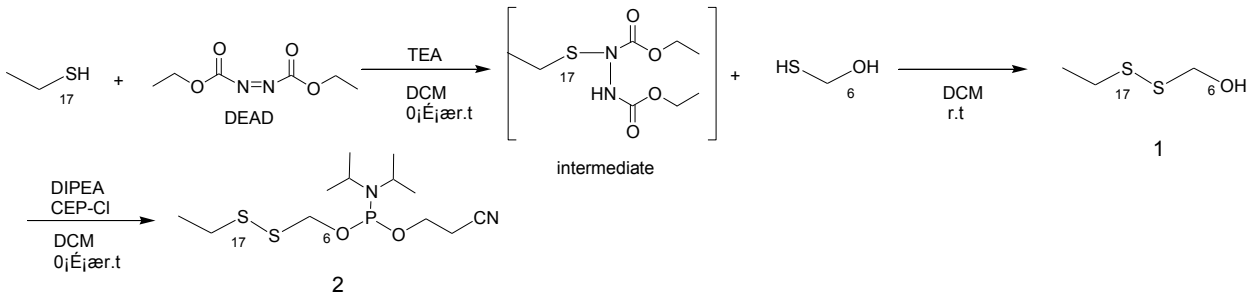
2) List of chemical abbreviations

CEP-Cl: 2-cyanoethyl diisopropylaminophosphorochloridite
DCM: Dichloromethane
DEAD: Diethyl azodicarboxylate
DIPEA: *N*,*N*-Diisopropylethylamine
distl. Water: distilled water
DMT-Cl: 4,4′-Dimethoxytrityl chloride
sat. $NaHCO_3$: **saturated** $NaHCO_3$
TEA: Triethylamine

3) Preparation of $C_{18\text{-}6}$ Disulfide Phosphoramidite

In order to bond $C_{18\text{-}6}$ Disulfide to a double-helix oligo RNA structure, $C_{18\text{-}6}$ Disulfide Phosphoramidite was prepared as shown in the following reaction scheme 1.

Reaction scheme 1



$C_{18\text{-}6}$Disulfide-OH (1)

1-octadecanthiol 15g(52.35mmol) was dissolved in dichloromethane(400 ml) and DEAD(40% in toluene,ca.2.2mol/L) 22.79g(23.74ml, 52.35mmol, 1eq)was slowly added to the reaction solution at 0 °C. triethylamine 2.65g(3.65ml, 26.17mmol, 0.5eq) was added to the reaction solution at 0 °C. Then, the reaction solution was stirred at room temperature for 15 minuten. 6-mercapto-1-hexanol 7.03g(7.16ml, 52.35mmol, 1eq) was added dropwise thereto and the reaction solution was stirred at room temperature for 2h. After completion of the reaction, the reaction solution was diluted with dichloromethane (500 ml) and extracted with distil. water (500 ml). The organic layer was collected, dried with anhydrous magnesium sulfate and filtered, evaporated to a syrup. The resulting residue was dissolved in 400 ml mixture solution(volume ratio 1:10:16 = n-hexane: distl. water: acetone), stired at 60 °C. Then stired the reaction solution at room temperature for 1h, filted. The filtercake washed

with n-hexane(100ml) to remove further impurities. A white solid was recovered(18.25g, 83%).
^{1}H-NMR($CDCl_3$)δ3.64(t, 2H, OCH_2), 2.71-2.65(m, 4H, SCH_2), 1.70-1.58(m, 6H, CH_2), 1.42-1.39(m, 3H, CH_2, OH), 1.32(s, 32H, CH_2), 0.87(t, 3H, CH_3)

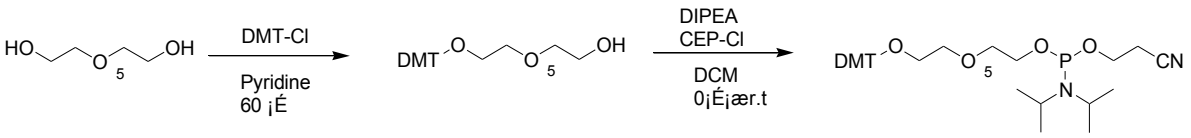
C_{18-6} Disulfide Phosphoramidite (2)

C_{18-6}Disulfide-OH(1) 18.25g(43.58mmol) was dissolved in dichloromethane (400ml), DIPEA 16.93g(22.82ml, 130.73mmol, 3eq) was added to the solution at 0 ℃ and then CEP-Cl 12.38g(11.66ml, 52.29mmol, 1.2eq) was added dropwise to reaction solution at 0 ℃. The reaction solution was stirred at room temperature for 2 h. After completion of the reaction, the reaction solution was diluted with dichloromethane (500ml) and extracted with distl. water (500mlX2). the organic layer was collected, dried with anhydrous $MgSO_4$, filtered, evaporated to a syrup. The resulting residue was purified by silica column (1:4 ethyl acetate /n-hexane), a clear oil was recovered (21.28g, 78.9%)
^{1}H-NMR($CDCl_3$)δ3.86-3.80(m, 2H, OCH_2), 3.67-3.56(m, 4H, $POCH_2$, NCH), 2.71-2.60 (m, 6H, $CNCH_2$, SCH_2), 1.69-1.54 (m, 6H, CH_2), 1.42-1.39(m, 6H, CH_2), 1.25(s, 28H, CH_2), 1.20-1.16(m, 12H, ipr-CH_3), 0.88(t, 3H, CH_3)
^{31}P-NMR(CDCl3);148-151(s)

4) Preparation of C_{18-6} Disulfide Phosphoramidite

In order to bond Atom 18 Spacer to a double-helix oligo RNA structure, Atom 18 Spacer phosphoramidite was prepared as shown in the following reaction scheme 2.

Reaction scheme 2



3 4

DMT-Hexa(ethylene glycol) (3)

Hexa(ethylene glycol) 30.14g (106.75mmol) was dissolved in pyridine(100ml), DMT-Cl 39.8g (111.43.mmol, 1.1eq) in pyridine(300ml) was added to the solution at 60 ℃ for 1h, stirred at 60 ℃ for 10h. After completion of the reaction, the reaction solution was concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate(500ml) and extracted with sat. $NaHCO_3$ (500mlX2). The organic layer was dried with anhydrous $MgSO_4$, filtered, evaporated to a syrup, and chromatographed by silica gel column chromatography using 0-4% gradient of methanol in ethyl acetate. Fractions containing the product were pooled and evaporated to a oil (31g, 49.6%)
^{1}H-NMR($CDCl_3$); δ7.47-7.18(m, 9H, DMT-H), 6.82(d, 4H, DMT-H), 3.78(s, 6H, DMT-OCH_3), 3.67-3.57(m,23H, CH_2CH_2O, OH), 3.20(t, 2H, CH_2O)

Atom 18 Spacer phosphoramidite (4)

DMT-Hexa(ethylene glycol)(3) 31g(53.02mmol) was dissolved in dichloromethane(400ml). DIPEA 13.71g(18.47ml, 106.04mmol,2eq) was added to the solution at 0 ℃ and then CEP-Cl 18.83g (17.74ml, 79.53mmol, 1.5eq) was added dropwise to reaction solution at 0 ℃. The

reaction solution was stirred at room temperature for 2 h. After completion of the reaction, the reaction solution was diluted with Methylene chloride(500ml) and extracted with distl. water (500mlX2). The organic layer was dried with anhydrous $MgSO_4$, filtered, evaporated to a syrup. The resulting residue was purified by silica column (1:1 ethyl acetate /n-hexane), a clear oil was recovered (30.14 g, 72%)

^{1}H-NMR($CDCl_3$);δ^{1}H-NMR($CDCl_3$); δ7.47-7.18(m, 9H, DMT-H), 6.82(d, 4H, DMT-H), 4.13-3.81(m, 8H, DMT-OCH_3, OCH_2), 3.67-3.57(m,24H, CH_2CH_2O), 3.22(t, 2H, NCH), 2.63(t, 2H, CH_2CN), 1.28-1.23(m, 12H, ipr-CH_3)

^{31}P-NMR(CDCl3);148-151(s)

II. Supplemental Figures

Supplemental Fig.1. Biochemical and hematologic evaluation on the effects in vivo SAMiRNA Treatment.

A. Biochemical and hematologic indicators measured 24 hr after i.t. injection of SAMiRNA (3mg/kg).

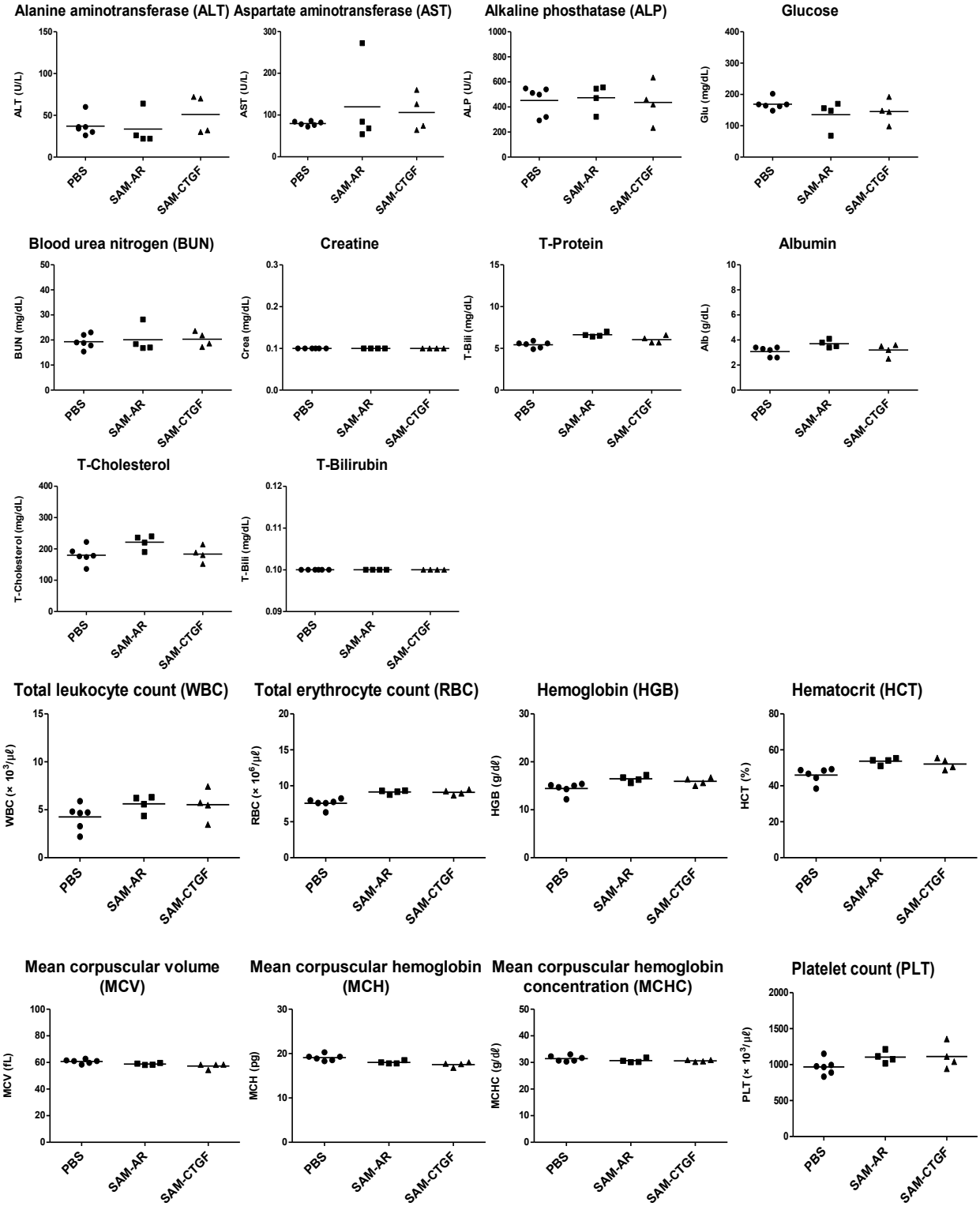
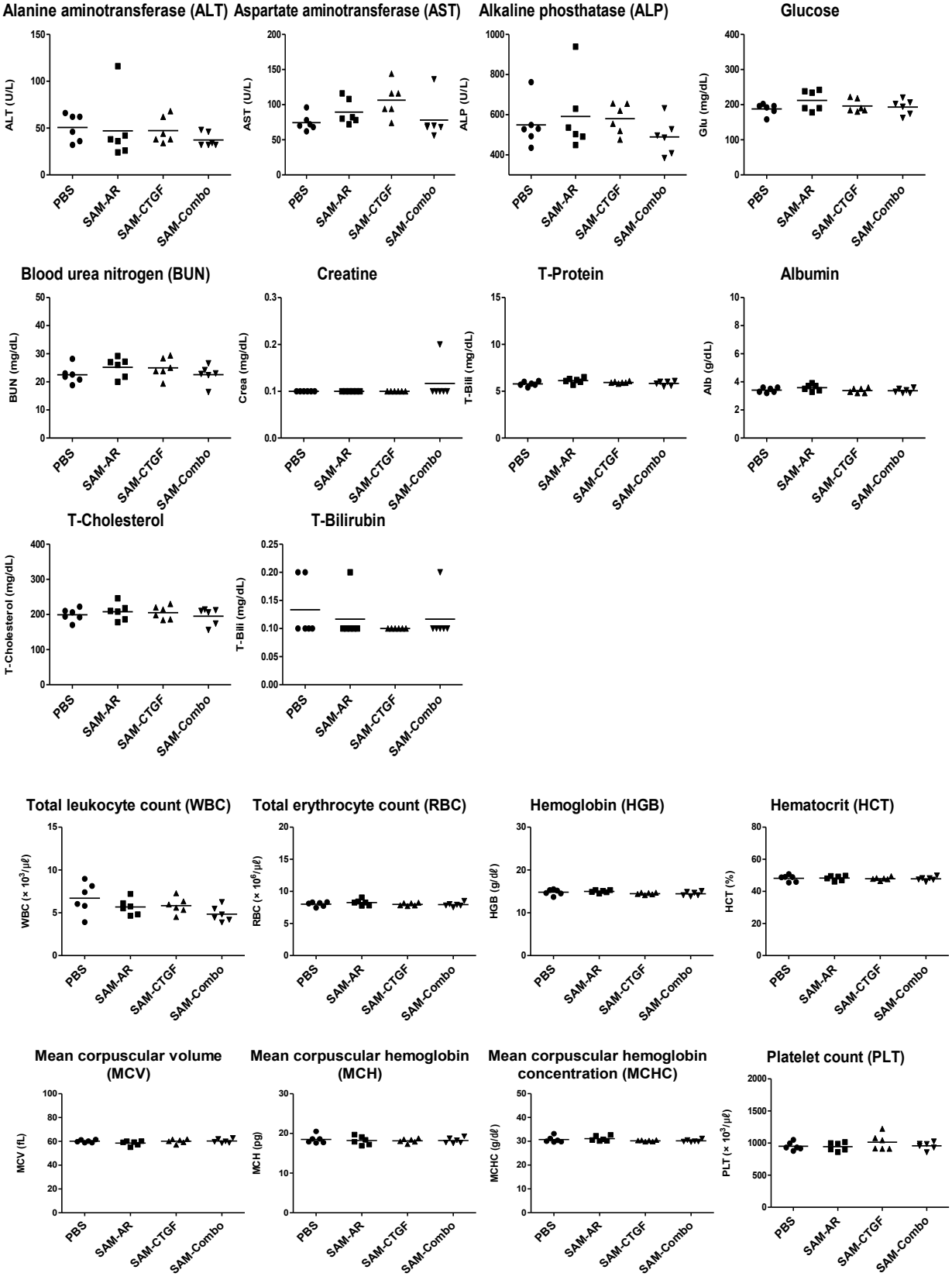
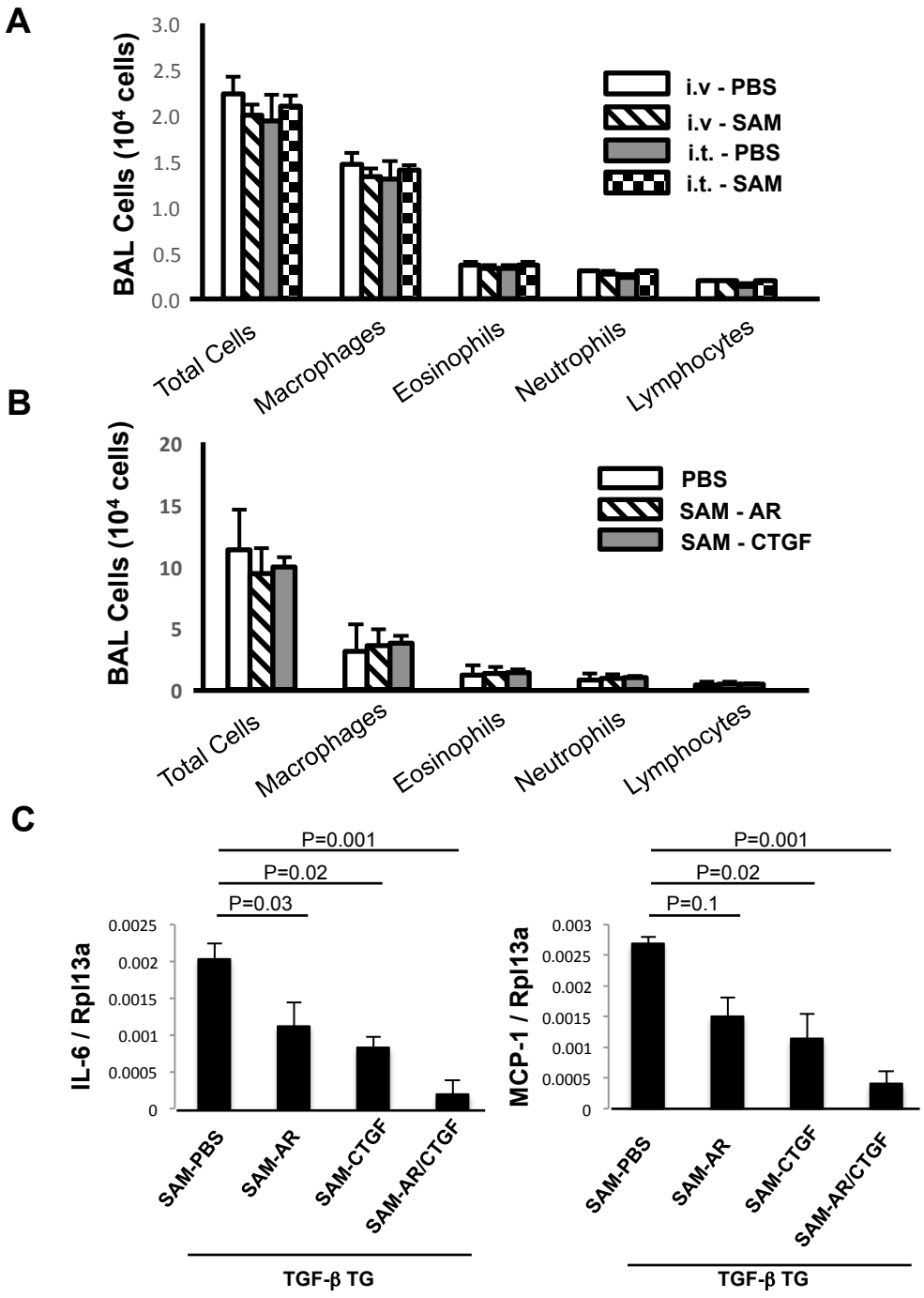


Fig.1 continued

B. Biochemical and hematologic indicators measured 24 hr after i.v. injection of SAMiRNA (5mg/kg).



Supplemental Fig. 2. Effects of SAMiRNAs on inflammation and innate cytokine expression in the BAL and lung.



A, Total and differential counts of BAL cells 24 hrs after administration of PBS or SAMiRNA on WT mice via i.v. (5mg/kg) or i.t. (3mg/kg). N=5 mice each. SAM, amphiregulin(AR) SAMiRNA. B, BAL cell recovery 24 hrs after administration of PBS or SAMiRNAs on TGF-β Tg mice. SAM-AR, amphiregulin SAMiRNA; SAM-CTGF, CTGF SAMiRNA. N=5 mice each. C. Real-time RT-PCR analysis on lungs from TGF-β Tg mice with PBS or SAMiRNA (5mg/kg) administration via i.v. injection, n=5 mice each.