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Supplemental information

Nuclear respiratory factor-1 negatively regulates TGF-β1 and attenuates pulmonary fibrosis

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Supplement Data:

Table S1. List of primer sequences used in ChIP assay to quantify genes promoter by quantitative real-time PCR polymerase chain reaction, related to STAR method.

Mouse lung	Gene	Position	Primer FW 5' --3'	Primer RV 5' --3'
	CCN5	-44	TCCTGAGCCCTGAAGCTCAA	GCCAGAAACCACAGGCTGG
		-55	CTGACCCACGGTGTCCTGA	CCACAGGCTGGGTCTGGG
		-79	GTTTCTGGCAGGGAGACTGG	TTGGTTGCCAAGAGCAGACA
		-144	CACCCAGAGGTGGGTGTGAC	GATAGGGAGGCTCTTGGTTGC
		-350	GCCTAAGCAGACTGAGCCTA	ACCCCTCAGAGCTGGTAGAAT
		-550	GCTAACAAGGCTCTGCGGTA	CTGTACTGAGTATGCCAGGCT
	Smad7	-80	CTCCGCCCCCTTCCCTCC	GTGGAACCAGCCGAGCAGAG
		-169	CTACCCCTCCCTCCCCCG	CTAGACGGCCACGTGACGA
		-208	AATGAGCCGGTTAGTGGCCC	TCCGTCCTGGCCGGTGTAAA
		-400	TCTAGAGAGGGGTACCCACGG	CCCCCAGCTCTTCCGATTTC
		-502	GAGTTTGGAGCGGAATGAGC	TCCTCCCCTTTCCAAGGGAT
		-599	TTGGAAAGGGGAGGAGATCG	TCTAGCGCACACTTCCCAG
		-806	CTGGAGGGAGGAGGGGTG	TGGCTGGGCAAGGTTAGC
Human MRC5	*CCN5*	-44	CTGTCCGAACCCCAGCG	TGCAAAGGGGTTGTTTACTGA
		-55	GTCCGAACCCCAGCGG	CTGCAAAGGGGTTGTTTACTGA
		-79	GTCTCCTAAGCTCCGTGGC	CCAGAAACCGCAGGCTGG
		-144	GCGGTTTCTGGCAGGCA	GTTTTGCTCTGGGTCACACC
	SMAD7	-169	ACGAAAGGCGTTCGGC	CTTAGCAAGGGGGAAAGAGGC
		-208	TTAATTTGGGGGTGGGGAGAAG	CGGAATTCGAGGAGATCCGGT
		-806	TGACAGCGCGGGGGA	CCCCTGCACTAGCAAGCAG

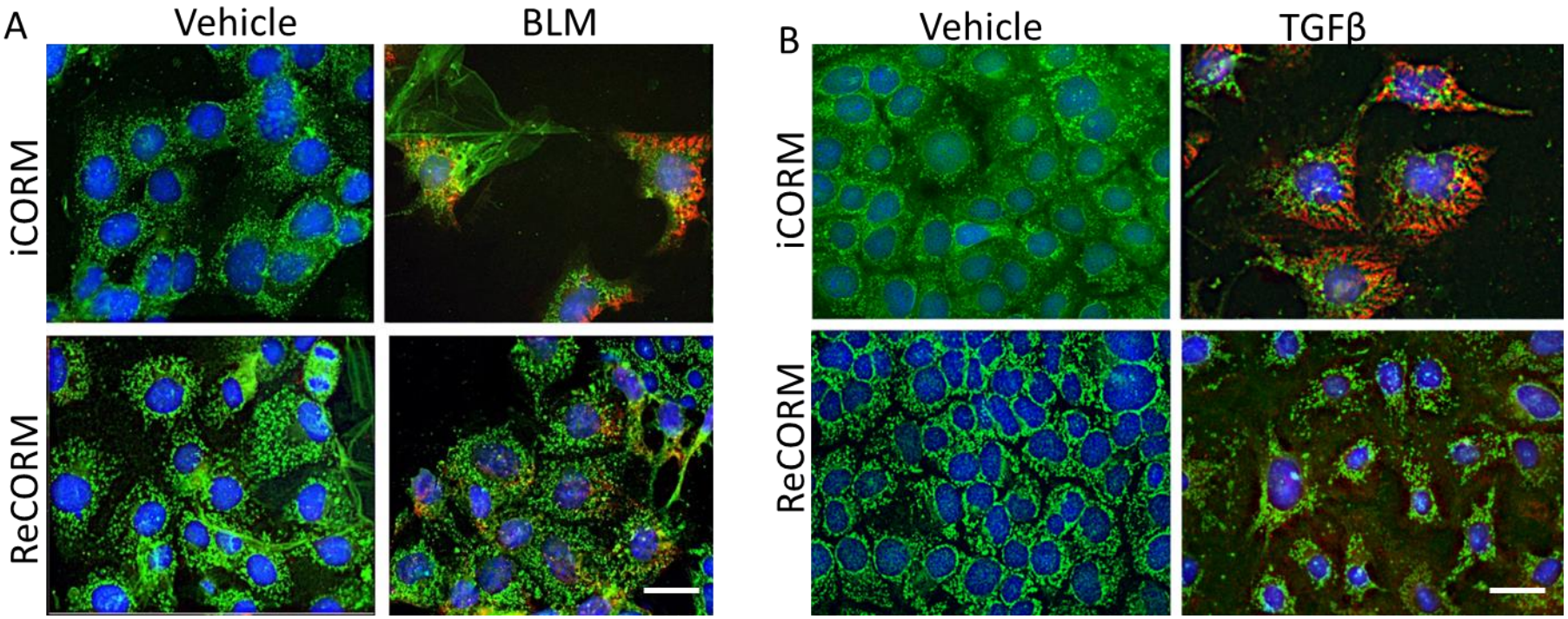

Figure S1. ReCORM protect mouse alveolar epithelial cells (MLE12) from BLM/ TGFβ1 induced-expression of fibrotic markers, related to Figure 5. (A) MLE12 cells cultured in uni-well glass slides were exposed to 5μg/mL BLM with or without ReCORM (50μM) for 96 hours. Cells were stained with MitoTracker green for mitochondrial mass and αSMA present as red wrinkle fluorescence (arrow). Nuclei stained with DAPI (blue). (B) MLE12 cells treated with TGFβ1 (10ng/ml) and stained with MitoTracker green and αSMA shown as red wrinkle fluorescence (arrow). Images of fluorescence were analyzed by confocal microscopy. All experiments were performed three times independently, and representative data are shown. Scale Bar=20μm.

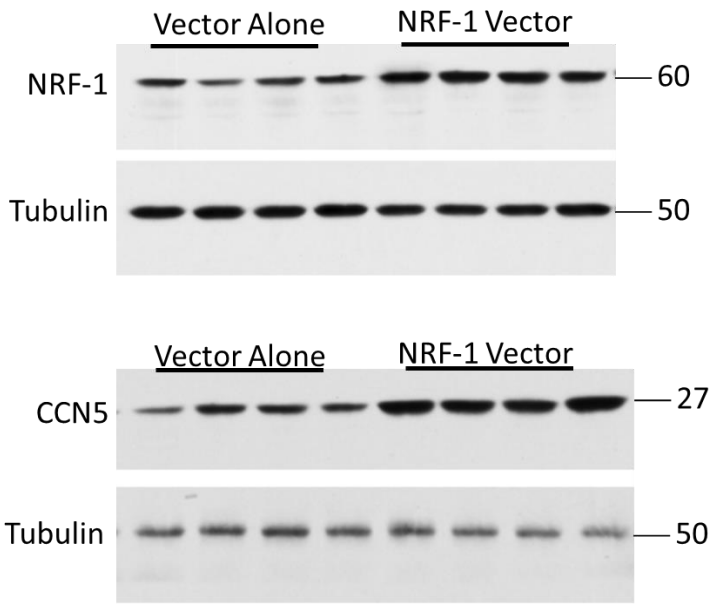

Figure S2. Immunoblot of NRF-1 or NCC5 transfection efficiency in MRC5, related to Figure 9.. MRC5 transfected with NRF-1 vector or empty vector. (**A**). MRC5 transfected with of CCN5 vector or empty vector (**B**).

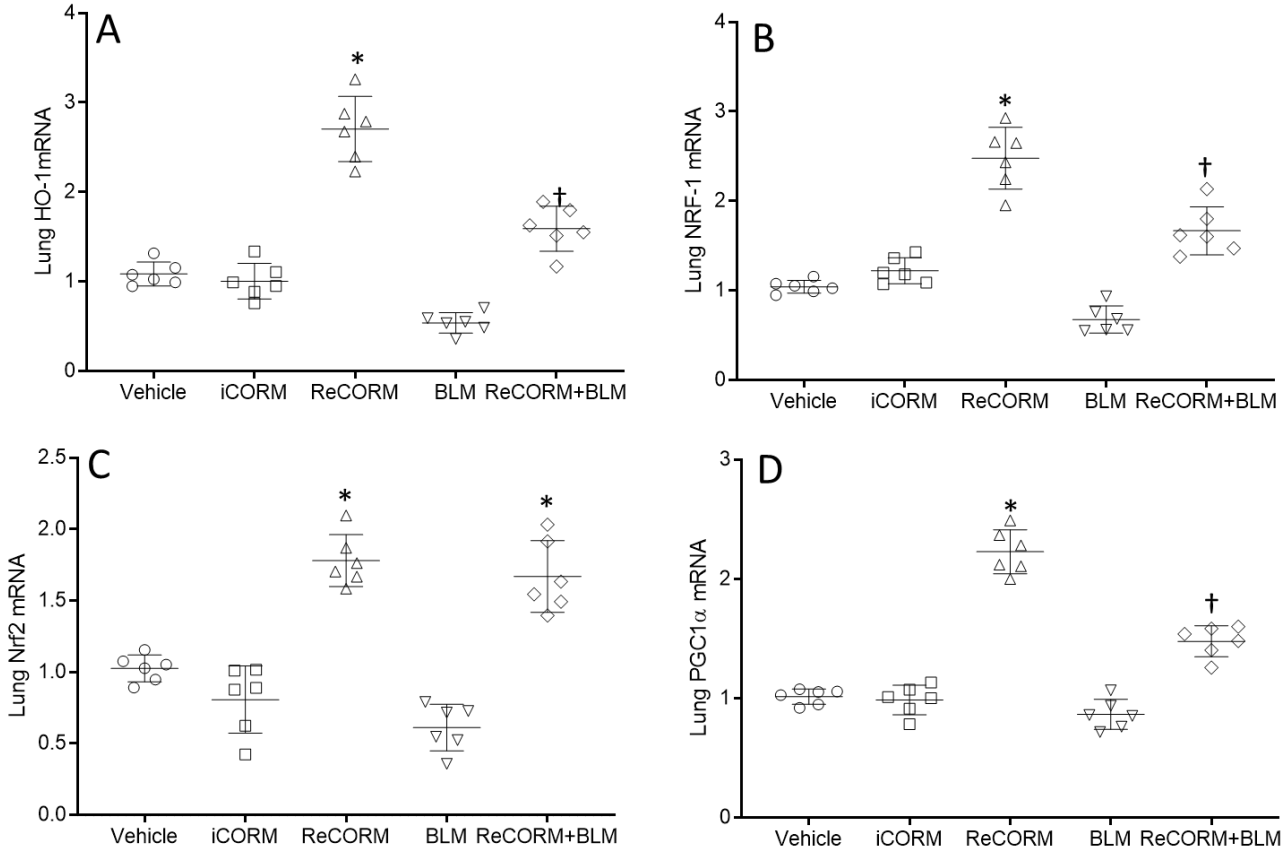

Figure S3. CORM2 increases HO-1 and mitochondrial transcriptional genetic program mRNA, releated to Figure 9. C57BL/6 mice were injected with 3 consecutive doses of 1mg/kg CORM2 or iCORM. Seven days post-BLM with or without CORM2, lungs were excised and analyzed for mRNA for HO-1, NRF-1, Nrf2 and PGC-1α were measured by qRT-PCR, with 18s rRNA as internal standard (**A-D**).

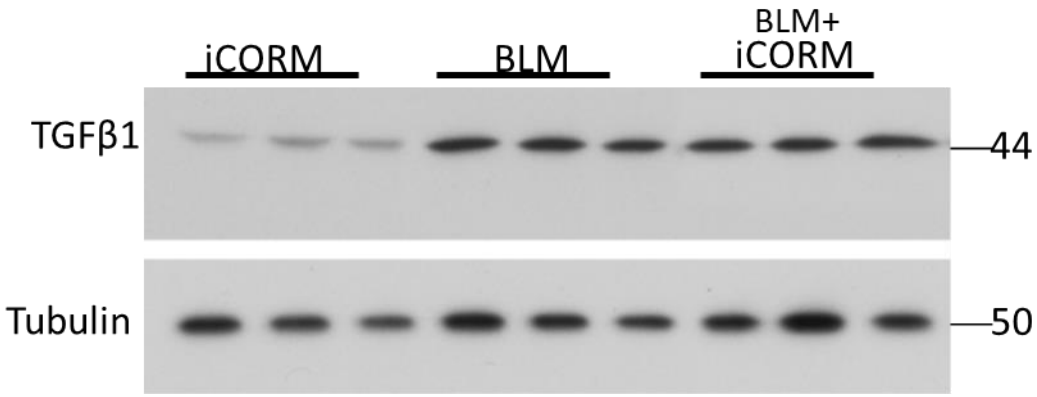

Figure S4. The effect of iCORM alone and with BLM (Figure S4) on TGFβ1 expression by immunoblotting, **related to STAR method.** The iCORM +BLM did not differ from BLM alone for induction of TGFβ1.