Supplemental Data

Pentraxin-2 suppresses c-Jun/AP-1 signaling to inhibit progressive fibrotic disease

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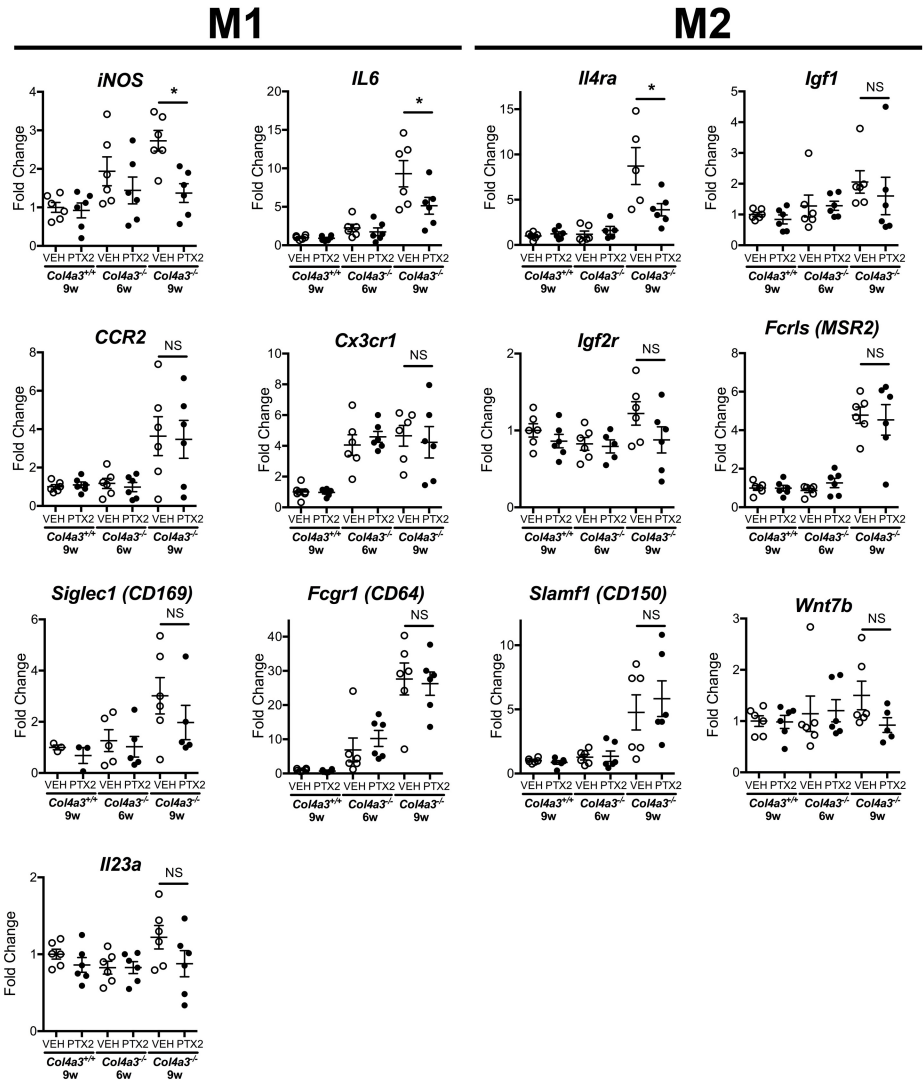
1. Supplemental Figures
2. Supplemental Tables

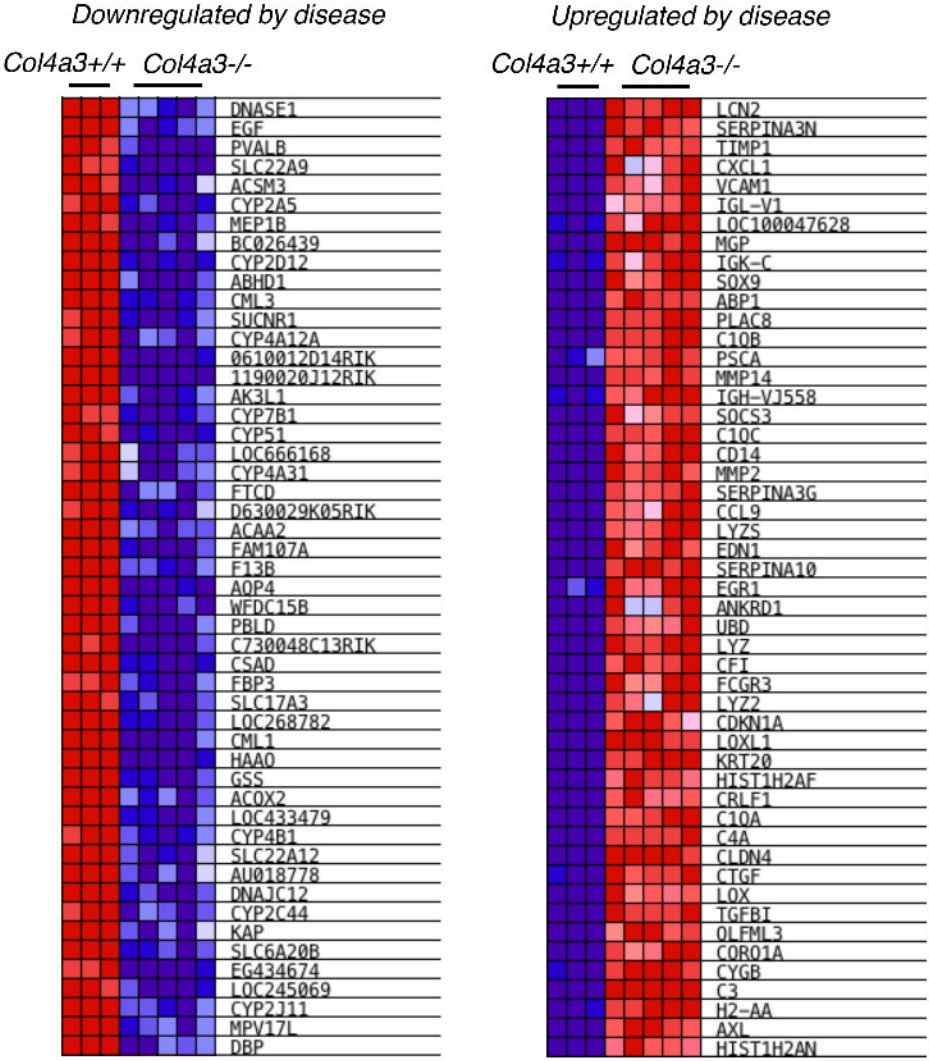
Figure S1



Supplemental Figure 1. rhPTX-2 decreases some M1-type but not M2-type markers of macrophage activation.

Q-PCR analysis of markers associated with M1-type or M2-type macrophage activation in whole kidney tissue at 9wk. N = 6-12/group. * $P < 0.05$; NS, not significant (ANOVA with post hoc testing for multiple comparisons, or Mann-Whitney test).

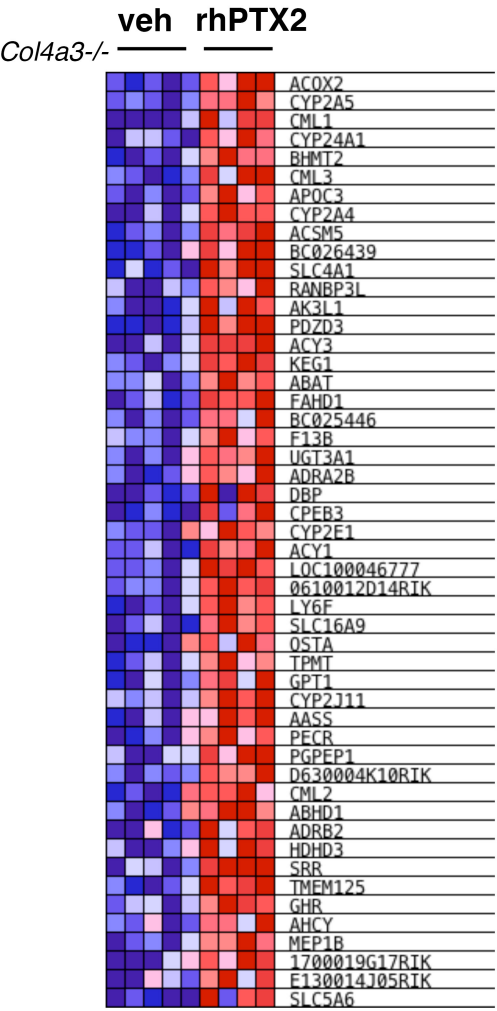
Figure S2



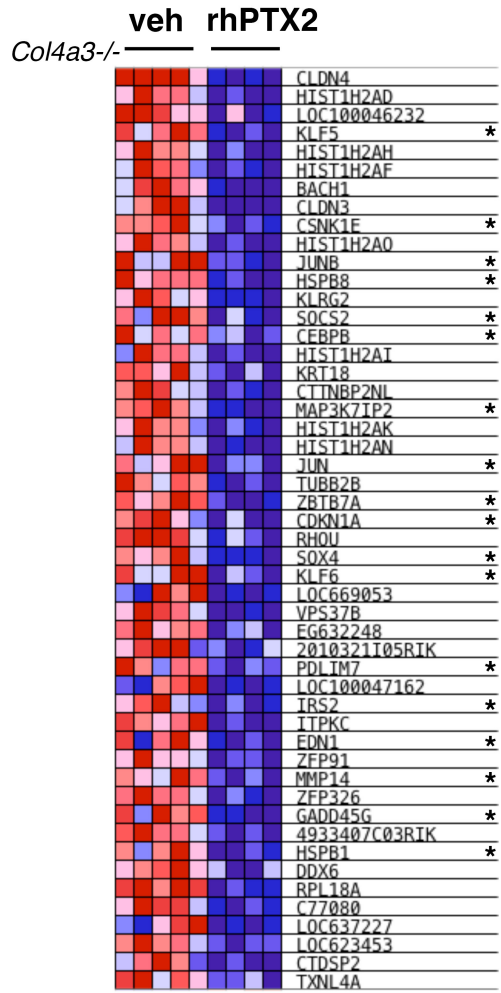
Supplemental Figure 2. The most differentially expressed kidney genes in healthy controls versus Alport nephropathy. Heat map of the top 50 upreglated and downregulated kidney genes in Alport neuropathy comparing healthy WT control (N = 3) to Col4a3-/- (N = 5) mice at 9wk by RNA microarray. Higher (red) and lower (blue) expression of individual mice relative to mean WT levels are shown.

Figure S3

Upregulated by rhPTX2



Downregulated by rhPTX2



Supplemental Figure 3. The most differentially expressed kidney genes in untreated Alport neuropathy versus rhPTX-2 treatment. Heat map of the top 50 upreglated and downregulated kidney genes with rhPTX-2 treatment of Alport neuropathy comparing vehicle control (N = 5) to rhPTX-2 treated (N = 4) Col4a3-/- mice at 9wk by RNA microarray. Higher (red) and lower (blue) expression of individual mice relative to mean WT levels are shown. Asterisks indicate downregulated genes predicted to interact with one another by STRING analysis (Figure 6C).

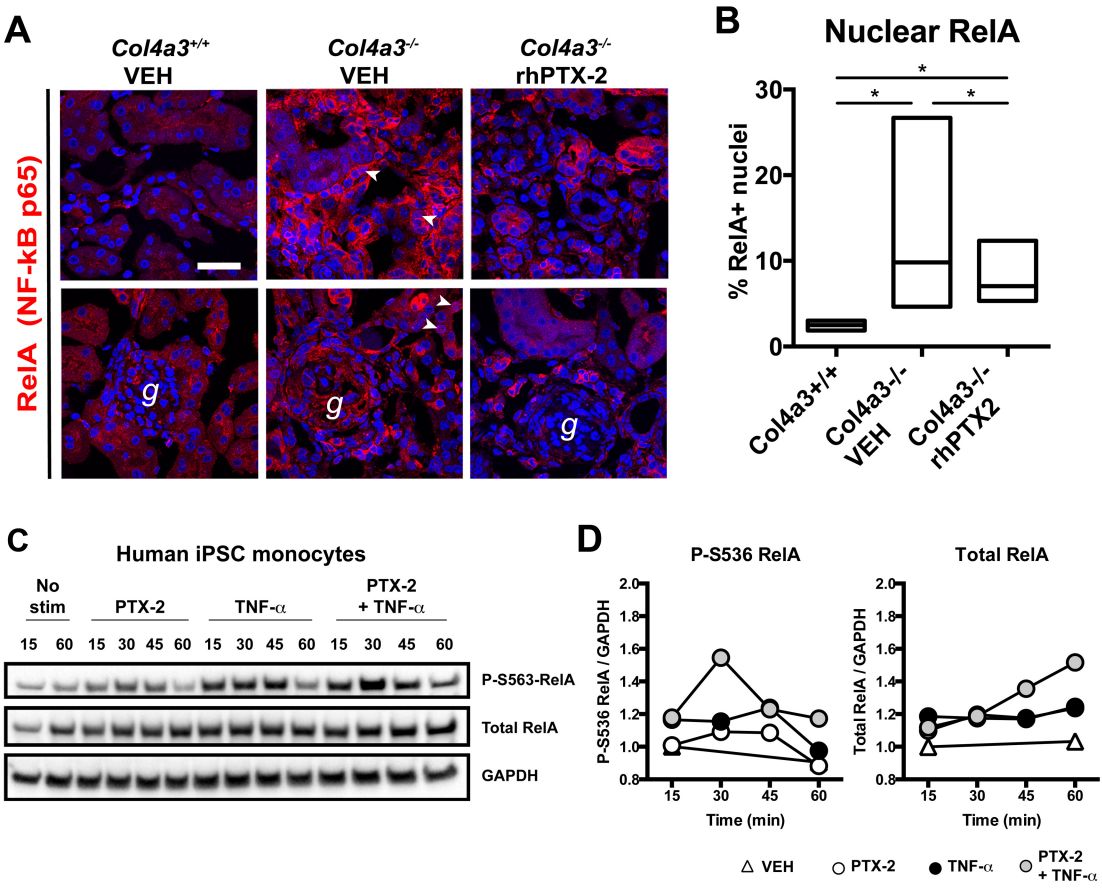
Figure S4

Rank	Pathway map	Network objects: c-Jun and AP-1	MAPK	NF-κB	JAK/STAT	TRAF6	MyD88
1	Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling	■	■			■	■
2	Immune response_HSP60 and HSP70/ TLR signaling pathway	■	■	■		■	■
3	Immune response_MIF in innate immunity response	■	■			■	■
4	Transcription_P53 signaling pathway		■	■			
5	Immune response_IL-1 signaling pathway	■	■			■	■
6	Cell adhesion_Chemokines and adhesion	■		■			
7	Development_Regulation of epithelial-to-mesenchymal transition (EMT)	■		■			
8	Immune response_Bacterial infections in normal airways		■	■	■	■	■
9	Development_Thrombopoetin signaling via JAK-STAT pathway				■		
10	Immune response_IL-18 signaling	■	■	■		■	■
11	Development_WNT signaling pathway. Part 2	■					
12	Immune response_Oncostatin M signaling via JAK-Stat in mouse cells				■		
13	Signal transduction_PTMs in IL-17-induced CIKS-dependent MAPK signaling pathways	■	■			■	
14	Immune response_TLR2 and TLR4 signaling pathways	■	■	■		■	■
15	Development_PEDF signaling	■		■			
16	Development_NOTCH1-mediated pathway for NF-KB activity modulation		■	■		■	
17	Immune response_Oncostatin M signaling via JAK-Stat in human cells				■		
18	IGF family signaling in colorectal cancer	■		■			
19	IL-6 signaling in multiple myeloma	■			■		
20	Signal transduction_AKT signaling			■			

■ Present in pathway map
□ Absent from map

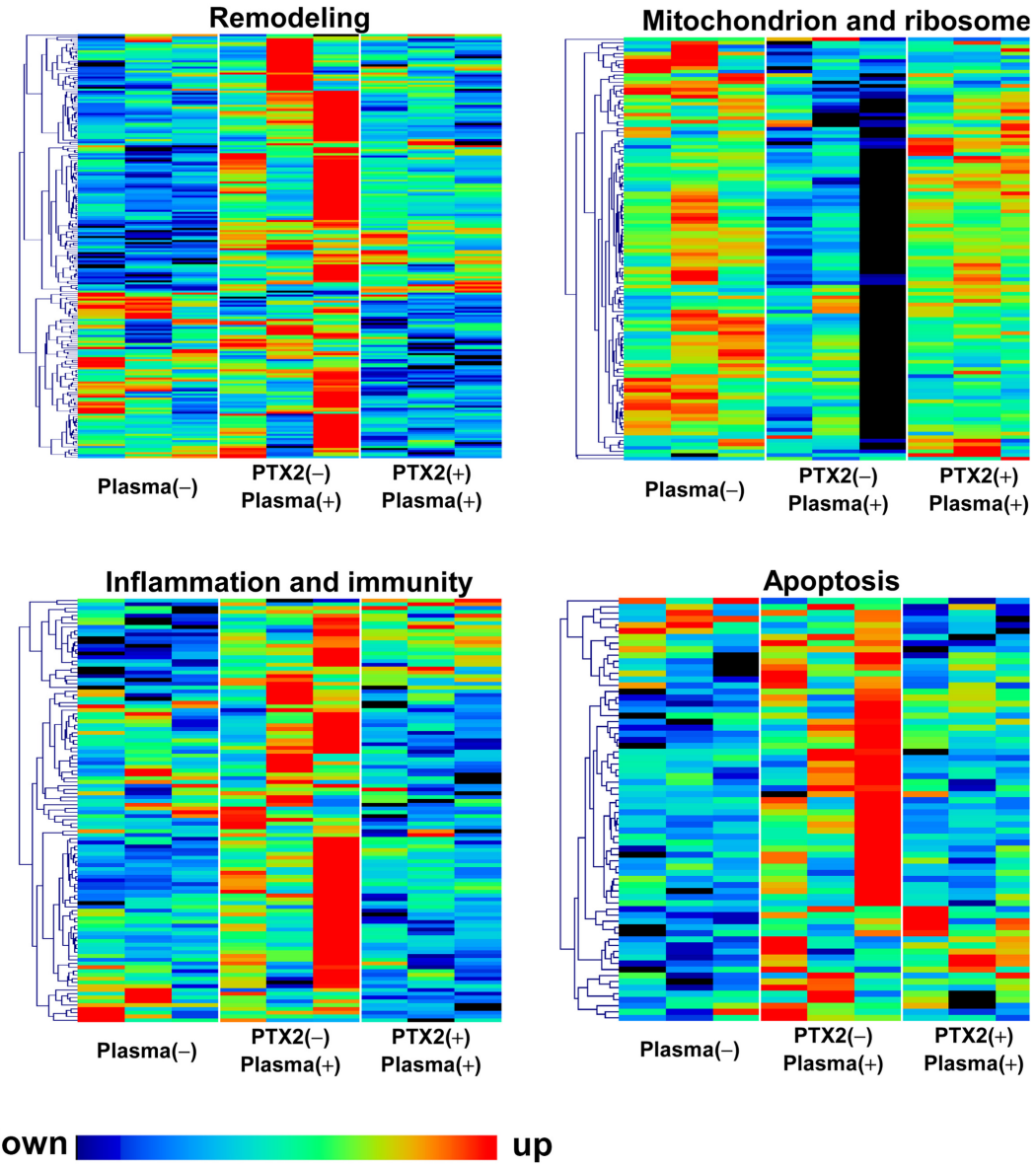
Supplemental Figure 4. Gene pathway enrichment analysis of genes downregulated by rhPTX-2 widely involves AP-1, MAPK, and NF-κB components. Genes downregulated by rhPTX-2 treatment versus vehicle control were uploaded to the GeneGo knowledge base and analyzed for pathway enrichment. The top 20 affected pathways were ranked and scored for including key signaling components as network objects.

Figure S5

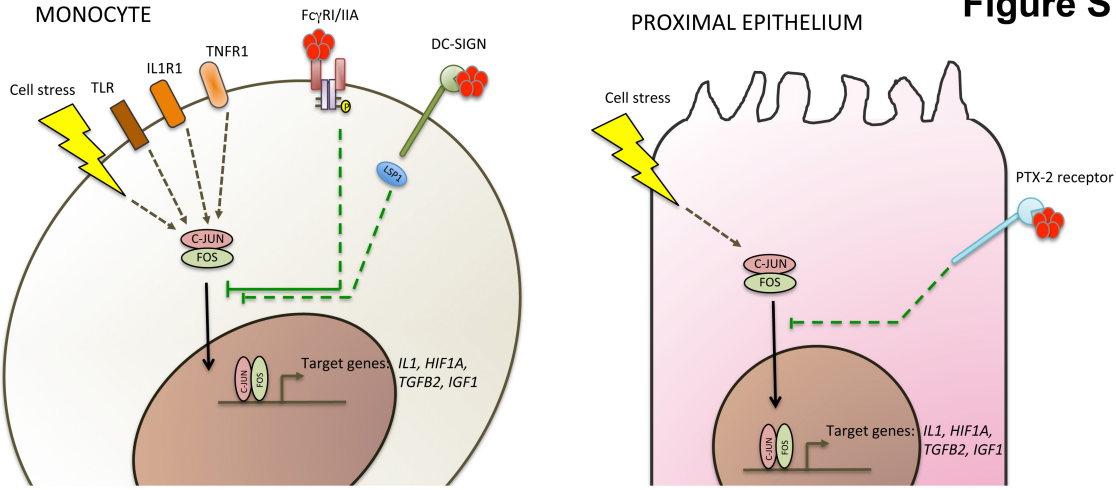


Supplemental Figure 5. Col4a3-/- kidneys show overall decreased RelA activation in response to rhPTX-2 but rhPTX-2 does not suppress active RelA in human monocytes in vitro. (**A**) Representative images of kidney tissue sections from 9wk Col4a3+/+ and vehicle or rhPTX-2 treated Col4a3-/- mice showing RelA (NF-κB p65) intensity and localization. Examples of RelA co-localized with nuclear staining (DAPI, blue) indicated by arrowheads. g = glomerulus. (**B**) Box plots (Maximum, median, and minimum percentages) showing % cells in kidney sections with nuclear localization of RelA. ~1300 nuclei across 10 kidney cortex section images from each mouse were scored. Note nuclear RelA increases with disease and is inhibited by rhPTX-2 in vivo. (**C-D**) Time course western blots of RelA phosphorylation and total RelA from 15-60 min after rhPTX-2 and/or TNF-α stimulation in iPSC-derived monocytes, normalized to GAPDH. Note PTX-2 does not directly suppress active RelA in monocytes. Bar = 50μm. N = 3-5/group. * $P < 0.05$ by one-tailed Mann-Whitney test.

Figure S6



Supplemental Figure 6. Human primary proximal kidney tubule epithelial cells resist the effects of plasma shock across major pathways after rhPTX-2 treatment. Human PTECs were cultured 24hr with no treatment, human plasma, or rhPTX-2 treatment for 1hr followed by plasma. Gene expression profiles were determined by microarray and analyzed by GSEA. Plasma shock primarily activated pathways for tissue remodeling, inflammation and immunity, and apoptosis while suppressing mitochondrion and ribosome functions. rhPTX-2 treatment reduced the response to plasma in each of these broad categories. Enriched gene sets are depicted as heat maps of individual leading edge genes (rows) and replicate cultures (columns) (N = 3/group from 3 different donors).



Supplemental Figure 7. Schema showing the effect of PTX-2 on inflammatory or stress pathway signaling in monocytes and proximal tubule epithelial cells. PTX-2 can bind to activating FcγRs or DC-SIGN on monocytes. It triggers partial phosphorylation of FcRγ chain. Such partial receptor activation imparts an inhibitory signal, which counteracts activating signals from other receptors including TNF receptor 1. The inhibitory effect is predominantly by blocking AP-1 complex translocation to the nucleus and activation of AP1-dependent genes. In epithelium, evidence suggests an unknown receptor can interact with PTX-2 to transduce an inhibitory signal which similarly reduces AP-1 signaling in response to cell stress or cytokines such as TGFβ.

Table S1: Top GSEA pathways activated in kidney by Alport nephropathy

Rank	Upregulated gene set in Col4a3-/- versus control mice at 9wk
1	DEFENSE_RESPONSE
2	IMMUNE_SYSTEM_PROCESS
3	CELL_ACTIVATION
4	PROTEINACEOUS_EXTRACELLULAR_MATRIX
5	EXTRACELLULAR_REGION_PART
6	EXTRACELLULAR_MATRIX
7	KEGG_ECM_RECEPTOR_INTERACTION
8	PID_INTEGRIN1_PATHWAY
9	KEGG_CHEMOKINE_SIGNALING_PATHWAY
10	REACTOME_INTERFERON_GAMMA_SIGNALING
11	REACTOME_INTERFERON_SIGNALING
12	KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION
13	PID_IL6_7PATHWAY
14	REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM
15	REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
16	REACTOME_INTERFERON_ALPHA_BETA_SIGNALING
17	KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY
18	KEGG_LEISHMANIA_INFECTION
19	PID_IL23_PATHWAY
20	PID_TCR_PATHWAY
21	PID_PDGFRB_PATHWAY
22	KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS
23	REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
24	EXTRACELLULAR_MATRIX_STRUCTURAL_CONSTITUENT
25	PID_INTEGRIN3_PATHWAY

Table S2: Top genes identified by leading edge analysis of GSEA

Gene name	Enrichment Score
CLDN4	1.49058
HIST1H2AD	1.32975
LOC100046232	1.26426
KLF5	1.26420
HIST1H2AH	1.20503
HIST1H2AF	1.19361
BACH1	1.18299
CLDN3	1.17773
CSNK1E	1.17255
HIST1H2AO	1.15799
JUNB	1.15649
HSPB8	1.15450
KLRG2	1.15306
SOCS2	1.13105
CEBPB	1.12654
HIST1H2AI	1.11656
KRT18	1.10294
CTTNBP2NL	1.09788
MAP3K7IP2	1.09747
HIST1H2AK	1.08938
HIST1H2AN	1.08856
JUN	1.08497
TUBB2B	1.08370
ZBTB7A	1.08127
CDKN1A	1.07322
RHOU	1.06619
SOX4	1.06390
KLF6	1.01302
LOC669053	1.00752
VPS37B	1.00402
EG632248	0.99455
2010321I05RIK	0.97755
PDLIM7	0.97753
LOC100047162	0.96719
IRS2	0.96220
ITPKC	0.95866
EDN1	0.95767
ZFP91	0.95502
MMP14	0.94396
ZFP326	0.94312

GADD45G	0.92965
4933407C03RIK	0.92220
HSPB1	0.91852
DDX6	0.91571
RPL18A	0.90159
C77080	0.89511
LOC637227	0.89290
LOC623453	0.88459
CTDSP2	0.88432
TXNL4A	0.87974
2410006H16RIK	0.87189
PHF13	0.86722
SLC10A6	0.86533
SPPL3	0.86155
IGH-4	0.85895
NGFRAP1	0.85412
ANKRD11	0.85267
ASXL1	0.85176
SMOX	0.84992
BCL2L11	0.84895
PRR8	0.84841
6720427H10RIK	0.84524
SUPT5H	0.84364
SLPI	0.84194
GADD45A	0.84191
IGF2BP2	0.84113
AXUD1	0.83853
NFKBIA	0.83677
5530400B01RIK	0.83584
SERPINB6A	0.83500
5730494J16RIK	0.83341
FOSL2	0.82916
CREBBP	0.82661
IFNGR1	0.82481
NIPBL	0.82376
RAB11FIP5	0.81662
CHKA	0.81576
CHD1	0.81396
D2ERTD391E	0.81360
MAP3K3	0.81193
BMP1	0.81149
AXIN1	0.80972
LOC676222	0.80918

LOC100047093	0.80734
CHD2	0.80718
SESN2	0.80634
PLP2	0.80571
SULF1	0.80546
SERPINE2	0.80371
ERDR1	0.80031
SCL0001118.1_0	0.80028
SAMD4B	0.79537
STAT3	0.79405
YBX3	0.79306
RELL1	0.79151
RASL11A	0.79114
BBC3	0.78901
ACSL1	0.78898
BC003236	0.78875
CRLF1	0.78437
EG626367	0.78329
DDX3Y	0.78185
FZD5	0.78081
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SYF2	0.77799
ORMDL3	0.77488
NCAPH2	0.77445
NUAK1	0.77408
RPL18	0.77368
ITGB4	0.77322
PI16	0.77222
ZFP664	0.76939
DUSP8	0.76938
EG237361	0.76853
RNF4	0.76822
SPHK1	0.76714
GSK3B	0.76676
GLIPR2	0.76576
KCTD10	0.76241
PABPN1	0.76104
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HOXD9	0.75761
SOCS3	0.75663
TNIP1	0.75516
IGK-C	0.75063
COL15A1	0.75035

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PNPLA2	0.74878
MAF1	0.74778
RGS16	0.74734
MFAP5	0.74588
EG382843	0.74486
ARNTL	0.74328
WDR45L	0.74310
A530089A20RIK	0.74143
CSNK1D	0.73900
SYNE2	0.73737
SERINC2	0.73621
LOC626152	0.73601
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RORA	0.73413
GATS	0.73066
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KLF7	0.72937
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LOC100047628	0.72566
AI450540	0.72377
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MMP2	0.71497
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WIPI1	0.71106
1700023B02RIK	0.71018

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IER3	0.70439
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NEURL	0.70250
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CHK	0.70112
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BCAM	0.69837
IGH-VJ558	0.69765
CD14	0.69739
ERCC2	0.69720
ZYX	0.69336
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IFNGR2	0.68656
COL4A2	0.68602
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CBR3	0.68326
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COL12A1	0.55261
LOC100044221	0.55254
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SPSB1	0.55118
MYH9	0.55088
KLF4	0.55070
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STK24	0.54974
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LOC672474	0.54905

RFXAP	0.54893
E030034J16RIK	0.54771
BIN1	0.54753
MYD88	0.54728
RPS7	0.54645
2310007G05RIK	0.54608
BC017647	0.54585
DUSP1	0.54566
BAG3	0.54549
PTGDS	0.54485
ATG16L1	0.54442
CLTA	0.54438
DDAH2	0.54343
HIST1H1C	0.54341
0610009J05RIK	0.54336
NR1D1	0.54300
CHIC2	0.54279
ERGIC3	0.54264
BC039210	0.54237
HMGA1	0.54172
CSRP1	0.54129
MGP	0.54129
AW228700	0.54119
TRP53INP1	0.54116
ID3	0.54114
BRD4	0.54106
ZBTB39	0.54099
HN1	0.54080
LOC230253	0.54063
FOXO3	0.54044
MBD1	0.53967
9430080K19RIK	0.53950
LGALS4	0.53929
5730559C18RIK	0.53928
RPS15	0.53870
TRABD	0.53867
LOC547380	0.53708
LOC381215	0.53618
BTBD6	0.53584
TUBB5	0.53551
SERPINF1	0.53514
LOC100046552	0.53472
PDGFA	0.53472

BC014795	0.53449
RXRB	0.53447
FKHL18	0.53442
TRPM6	0.53441
TBC1D20	0.53424
RPL29	0.53412
LOC634327	0.53394
TMPRSS2	0.53390
IGHG	0.53381
NCOA5	0.53347
CTSK	0.53329
MYST2	0.53296
LOC636944	0.53295
H2AFV	0.53252
ZBTB2	0.53232
SIAH1A	0.53199
2310005L22RIK	0.53100
CSDA	0.53093
ITPK1	0.53077
SCARA3	0.53075
LOC100045644	0.53075
DAXX	0.53038
FAM110B	0.53004
1110008P14RIK	0.52937
RNF125	0.52921
5430406J06RIK	0.52829
C030014F05RIK	0.52786
MCRS1	0.52763
IPMK	0.52760
SFTPD	0.52754
LOC100047427	0.52741
PLA2G12B	0.52731
WDR43	0.52670
NRM	0.52663
PER	0.52633
MTAP1S	0.52577
KLF2	0.52523
ACBD6	0.52508
8430427H17RIK	0.52462
PHKG2	0.52454
PDZRN3	0.52410
RSC1A1	0.52366
FMNL3	0.52363

WNK4	0.52327
APP	0.52311
YPEL3	0.52285
2600005C20RIK	0.52250
2310044H10RIK	0.52199
2700094K13RIK	0.52100
TMEM63B	0.52073
CDCA3	0.52062
LAMA5	0.52022
NISCH	0.51951
DEFB36	0.51929
LMBR1L	0.51845
GRASP	0.51844
2610028A01RIK	0.51802
XBP1	0.51760
CAPN2	0.51736
2810454F19RIK	0.51699
PIP5K1A	0.51687
LY6E	0.51656
GATAD2B	0.51580
UBA1	0.51572
8430408G22RIK	0.51565
BC004044	0.51562
SLC4A4	0.51562
BSPRY	0.51532
MAPKAPK3	0.51514
RTN2	0.51496
FN1	0.51489
MDM2	0.51481
ICAM1	0.51466
PARD3B	0.51454
PKP4	0.51448
MFF	0.51426
PHF21B	0.51405
IRX2	0.51396
KRT17	0.51374
EHMT2	0.51352
EIF3I	0.51339
ITPRIP	0.51327
CDCA8	0.51264
C630024B01RIK	0.51241
CHD7	0.51235
LOC100043192	0.51234

DYNC1H1	0.51161
VPS37C	0.51160
MOBK1B	0.51137
BCAR1	0.51096
MAST2	0.51087
PDK4	0.51040
4930546H06RIK	0.51029
POM121	0.50960
MFHAS1	0.50940
LOC100044165	0.50936
SETD1A	0.50935
PDLIM1	0.50896
OCIAD2	0.50857
1300014I06RIK	0.50719
RPS9	0.50692
GSTT2	0.50659
ABTB1	0.50563
TUBB6	0.50559
PIP5K1B	0.50502
TSPAN17	0.50482
DSCR1	0.50472
NFATC4	0.50457
JMJD1A	0.50444
IRF2BP2	0.50425
TULIP1-PENDING	0.50414
DUSP16	0.50376
TNFRSF12A	0.50366
TBC1D15	0.50351
BRMS1	0.50336
LOC100046853	0.50327
A130010C12RIK	0.50288
SNHG10	0.50285
DDIT3	0.50268
AL022832	0.50238
CSRNP2	0.50211
SLC5A3	0.50198
FRAT2	0.50188
ANKRD56	0.50146
AW146242	0.50119
LOC665235	0.50116
TATDN2	0.50059
CLASP1	0.50025
WFIKKN1	0.50004

Table S2: GeneGo pathway enrichment for genes downregulated by rhPTX-2 in Col4a3-/- mice at 9wk

Enrichment by Pathway Maps

					DEGs_sym_genelist			
#	Maps	Total	pValue	Min FDR	p-value	FDR	In Data	Network Objects from Active Data
1	Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling	111	3.253E-08	1.293E-05	3.253E-08	1.293E-05	22	Talin, p130CAS, VEGF-A, Actin cytoskeletal, Cyclin D1, GSK3 beta, Caveolin-1, Collagen IV, p21, Fibronectin, MYLK1, WNT, Actin, p38 MAPK, Axin, MLCK, Frizzled, FOXO3A, Paxillin, c-Jun, TAB1, MDM2
2	Immune response_HSP60 and HSP70/ TLR signaling pathway	54	4.662E-08	1.293E-05	4.662E-08	1.293E-05	15	IL-1 beta, I-kB, TIRAP (Mal), AP-1, UBE1, NF-kB, TAB2, CD14, HSP60, ICAM1, MyD88, p38 MAPK, TRAF6, c-Jun, TAB1
3	Immune response_MIF in innate immunity response	40	4.667E-08	1.293E-05	4.667E-08	1.293E-05	13	IL-1 beta, I-kB, TAB2, CD14, SITPEC (ECSIT), MyD88, LBP, MEKK1(MAP3K1), C/EBPbeta, p38 MAPK, TRAF6, c-Jun, TAB1
4	Transcription_P53 signaling pathway	39	3.058E-07	6.353E-05	3.058E-07	6.353E-05	12	MKP-7, CBP, VEGF-A, XPA, NF-kB, p21, MEKK1(MAP3K1), HSP27, APEX, MKP-1, MDM2, MMP-2
5	Immune response_IL-1 signaling pathway	44	1.305E-06	2.168E-04	1.305E-06	2.168E-04	12	IL-1 beta, I-kB, AP-1, TAB2, SITPEC (ECSIT), Endothelin-1, MyD88, MEKK1(MAP3K1), c-Jun/c-Jun, TRAF6, c-Jun, TAB1
6	Cell adhesion_Chemokines and adhesion	100	2.554E-06	3.537E-04	2.554E-06	3.537E-04	18	Talin, p130CAS, VEGF-A, Actin cytoskeletal, GSK3 beta, NF-kB, Caveolin-1, Collagen IV, CD44, SERPINE2, Fibronectin, Actin, Zyxin, Rap1GAP1, CD47, Paxillin, c-Jun, MMP-2
7	Development_Regulation of epithelial-to-mesenchymal transition (EMT)	64	3.105E-06	3.686E-04	3.105E-06	3.686E-04	14	PDGF-A, IL-1 beta, EGF, TGIF, RelA (p65 NF-kB subunit), NOTCH1 receptor, Endothelin-1, Fibronectin, WNT, SRF, Frizzled, ACTB, c-Jun, MMP-2
8	Immune response_Bacterial infections in normal airways	49	4.517E-06	4.692E-04	4.517E-06	4.692E-04	12	IL-1 beta, I-kB, TIRAP (Mal), JAK2, IFN-gamma receptor, NF-kB, TAB2, CD14, MyD88, LBP, TRAF6, TAB1
9	Development_Thrombopoetin signaling via JAK-STAT pathway	22	7.435E-06	6.503E-04	7.435E-06	6.503E-04	8	STAT3, Bcl-XL, JAK2, Cyclin D1, CISH, p21, SERPINA3 (ACT), SOCS3
10	Immune response_IL-18 signaling	60	7.825E-06	6.503E-04	7.825E-06	6.503E-04	13	IL-1 beta, I-kB, AP-1, NF-kB, TAB2, ICAM1, MyD88, Fibronectin, p38 MAPK, TRAF6, c-Jun, Bcl-XS, TAB1
11	Development_WNT signaling pathway. Part 2	53	1.083E-05	8.185E-04	1.083E-05	8.185E-04	12	Casein kinase I epsilon, CBP, VEGF-A, Cyclin D1, GSK3 beta, CD44, DAB2, WNT, Axin, Frizzled, c-Jun, TAB1
12	Immune response_Oncostatin M signaling via JAK-Stat in mouse cells	18	1.695E-05	1.174E-03	1.695E-05	1.174E-03	7	STAT3, JAK2, VEGF-A, Cyclin D1, SERPINA3 (ACT), SOCS3, TIMP1
13	Signal transduction_PTMs in IL-17-induced CIKS-dependent MAPK signaling pathways	32	2.155E-05	1.377E-03	2.155E-05	1.377E-03	9	AP-1, UBE1, ABIN-2, TAB2, MAP3K3, C/EBPbeta, p38 MAPK, TRAF6, TAB1
14	Immune response_TLR2 and TLR4 signaling pathways	57	2.383E-05	1.415E-03	2.383E-05	1.415E-03	12	IL-1 beta, I-kB, TIRAP (Mal), AP-1, NF-kB, TAB2, CD14, MyD88, p38 MAPK, TRAF6, c-Jun, TAB1
15	Development_PEDF signaling	49	2.753E-05	1.525E-03	2.753E-05	1.525E-03	11	IL-1 beta, Bcl-XL, VEGF-A, PEDF (serpinF1), PEDF-R (iPLA2-zeta), RelA (p65 NF-kB subunit), NF-kB, JunB, SOD2, Fra-2, NFKBIA
16	Development_NOTCH1-mediated pathway for NF-KB activity modulation	34	3.677E-05	1.794E-03	3.677E-05	1.794E-03	9	NOTCH1 (NICD), I-kB, N-CoR, NF-kB, NOTCH1 receptor, MEKK1(MAP3K1), NOTCH1 (NEXT), TRAF6, NFKBIA
17	Immune response_Oncostatin M signaling via JAK-Stat in human cells	20	3.776E-05	1.794E-03	3.776E-05	1.794E-03	7	STAT3, JAK2, VEGF-A, Cyclin D1, SERPINA3 (ACT), SOCS3, TIMP1
18	IGF family signaling in colorectal cancer	60	4.100E-05	1.794E-03	4.100E-05	1.794E-03	12	GSK3 alpha/beta, Bcl-XL, I-kB, VEGF-A, Cyclin D1, GSK3 beta, RelA (p65 NF-kB subunit), NF-kB, IRS-2, IBP, MAT2A, c-Jun
19	IL-6 signaling in multiple myeloma	51	4.101E-05	1.794E-03	4.101E-05	1.794E-03	11	STAT3, SPHK1, Cyclin D2, Bcl-XL, JAK2, VEGF-A, Cyclin D1, GSK3 beta, p21, c-Jun, MDM2
20	Signal transduction_AKT signaling	43	4.614E-05	1.917E-03	4.614E-05	1.917E-03	10	GSK3 alpha/beta, Bcl-XL, I-kB, NF-kB, HSP90, p21, Bim, FOXO3A, Cyclin D, MDM2
21	Cell adhesion_ECM remodeling	52	4.966E-05	1.965E-03	4.966E-05	1.965E-03	11	MMP-12, HB-EGF, TIMP3, Collagen IV, CD44, SERPINE2, Fibronectin, MMP-15, TIMP1, MMP-14, MMP-2
22	Cytoskeleton remodeling_Cytoskeleton remodeling	102	5.440E-05	2.055E-03	5.440E-05	2.055E-03	16	Talin, p130CAS, VEGF-A, Actin cytoskeletal, GSK3 beta, Caveolin-1, Collagen IV, p21, Fibronectin, MyHC, MYLK1, p38 MAPK, Zyxin, MLCK, Paxillin, c-Jun
23	Immune response_HMGB1/TLR signaling pathway	36	6.023E-05	2.176E-03	6.023E-05	2.176E-03	9	IL-1 beta, I-kB, TIRAP (Mal), UBE1, RelA (p65 NF-kB subunit), TAB2, MyD88, TRAF6, TAB1
24	Immune response_CCL2 signaling	54	7.173E-05	2.415E-03	7.173E-05	2.415E-03	11	IL-1 beta, VEGF-A, Actin cytoskeletal, AP-1, NF-kB, Claudin-5, Caveolin-1, ICAM1, c-Jun, MMP-14, MMP-2
25	Immune response_CD137 signaling in immune cell	29	7.267E-05	2.415E-03	7.267E-05	2.415E-03	8	Cyclin D2, Bcl-XL, NF-kB, MEKK1(MAP3K1), p38 MAPK, Bim, NFKBIA, c-Jun
26	Signal transduction_PTMs in IL-17-induced CIKS-independent signaling pathways	46	8.545E-05	2.731E-03	8.545E-05	2.731E-03	10	STAT3, JAK2, AP-1, GSK3 beta, MAPKAPK2, NF-kB, C/EBPbeta, p38 MAPK, c-Jun, MKP-1
27	Development_YAP/TAZ-mediated co-regulation of transcription	56	1.017E-04	3.099E-03	1.017E-04	3.099E-03	11	Bcl-XL, ID3, VEGF-A, TAZ, Cyclin D1, Catalase, Endothelin-1, SOD2, KLF5, PUMA, SOX9
28	Immune response_IL-12 signaling pathway	23	1.044E-04	3.099E-03	1.044E-04	3.099E-03	7	STAT3, JAK2, G6NT, PDLIM2, Perforin, SOCS3, c-Jun
29	Immune response_Role of PKR in stress-induced antiviral cell response	57	1.203E-04	3.248E-03	1.203E-04	3.248E-03	11	IL-1 beta, I-kB, TIRAP (Mal), RelA (p65 NF-kB subunit), IFN-gamma receptor, NF-kB, TAB2, MyD88, TRAF6, NFKBIA, c-Jun
30	IL-1 beta-dependent CFTR expression	31	1.220E-04	3.248E-03	1.220E-04	3.248E-03	8	IL-1 beta, I-kB, TAB2, SITPEC (ECSIT), MyD88, MEKK1(MAP3K1), TRAF6, TAB1
31	Immune response_TLR5, TLR7, TLR8 and TLR9 signaling pathways	48	1.251E-04	3.248E-03	1.251E-04	3.248E-03	10	IL-1 beta, I-kB, AP-1, NF-kB, TAB2, MyD88, p38 MAPK, TRAF6, c-Jun, TAB1
32	Cell adhesion_Integrin-mediated cell adhesion and migration	48	1.251E-04	3.248E-03	1.251E-04	3.248E-03	10	Talin, p130CAS, Actin cytoskeletal, Collagen IV, Fibronectin, MyHC, MYLK1, Zyxin, MLCK, Paxillin
33	Translation_Non-genomic (rapid) action of Androgen Receptor	40	1.457E-04	3.670E-03	1.457E-04	3.670E-03	9	HB-EGF, EGF, GSK3 beta, NF-kB, Caveolin-1, WNT, Frizzled, FOXO3A, MDM2
34	Signal transduction_NF-kB activation pathways	51	2.129E-04	5.054E-03	2.129E-04	5.054E-03	10	IL-1 beta, I-kB, TIRAP (Mal), RelA (p65 NF-kB subunit), NF-kB, TAB2, MyD88, ZFP91, TRAF6, TAB1
35	Some pathways of EMT in cancer cells	51	2.129E-04	5.054E-03	2.129E-04	5.054E-03	10	STAT3, PDGF-A, I-kB, JAK2, EGF, GSK3 beta, RelA (p65 NF-kB subunit), Endothelin-1, Axin, TAB1
36	Development_EGFR signaling pathway	71	2.248E-04	5.190E-03	2.248E-04	5.190E-03	12	STAT3, HB-EGF, I-kB, JAK2, EGF, Amphiregulin, GSK3 beta, NF-kB, p38 MAPK, c-Jun, MKP-1, MMP-2
37	Development_WNT signaling pathway. Part 1. Degradation of beta-catenin in the absence	19	2.648E-04	5.947E-03	2.648E-04	5.947E-03	6	Casein kinase I epsilon, Casein kinase I delta, GSK3 beta, DAB2, SKP1, Axin
38	Development_Growth hormone signaling via STATs and PLC/IP3	35	3.039E-04	6.646E-03	3.039E-04	6.646E-03	8	STAT3, p130CAS, JAK2, SOCS2, SOCS3, Paxillin, c-Jun, GHR
39	Th17 cells in CF	54	3.474E-04	7.269E-03	3.474E-04	7.269E-03	10	STAT3, IL-1 beta, JAK2, NF-kB, CD14, ROR-alpha, ICAM1, MyD88, LBP, p38 MAPK

40	Cell adhesion_Tight junctions	36	3.735E-04	7.269E-03	3.735E-04	7.269E-03	8	CSDA, WNK4, Claudin-5, Caveolin-1, AF-6, Claudin-3, Actin, Claudin-4
41	Immune response_IL-12-induced IFN-gamma production	36	3.735E-04	7.269E-03	3.735E-04	7.269E-03	8	STAT3, I-kB, JAK2, GADD45 gamma, NF-kB, TRAF6, c-Jun, GADD45 beta
42	Immune response_TNF-R2 signaling pathways	45	3.762E-04	7.269E-03	3.762E-04	7.269E-03	9	Bcl-XL, I-kB, AP-1, RelA (p65 NF-kB subunit), NF-kB, ZFP91, p38 MAPK, Bim, c-Jun
43	Transcription_Androgen Receptor nuclear signaling	45	3.762E-04	7.269E-03	3.762E-04	7.269E-03	9	STAT3, EGF, N-CoR, Cyclin D1, GSK3 beta, p21, WNT, Frizzled, MMP-2
44	Immune response_CD40 signaling	65	4.034E-04	7.489E-03	4.034E-04	7.489E-03	11	STAT3, Cyclin D2, Bcl-XL, I-kB, JAK2, NF-kB, ICAM1, MEKK1(MAP3K1), p38 MAPK, TRAF6, c-Jun
45	Immune response_Platelet activating factor/ PTAFR pathway signaling	55	4.056E-04	7.489E-03	4.056E-04	7.489E-03	10	STAT3, HB-EGF, IL-1 beta, JAK2, Actin cytoskeletal, MAPKAPK2, NF-kB, p38 MAPK, NFKBIA, NF-AT
46	Immune response_MIF-induced cell adhesion, migration and angiogenesis	46	4.469E-04	7.888E-03	4.469E-04	7.888E-03	9	VEGF-A, AP-1, NF-kB, ICAM1, CD44, MEKK1(MAP3K1), p38 MAPK, c-Jun, MKP-1
47	Role of red blood cell adhesion to endothelium in vaso-occlusion in Sickle cell disease	37	4.556E-04	7.888E-03	4.556E-04	7.888E-03	8	TfR1, Beta-2 adrenergic receptor, cAMP-GEFI, CD44, Fibronectin, LAMA5, BCAM, CD47
48	Cooperative action of IFN-gamma and TNF-alpha on astrocytes in multiple sclerosis	37	4.556E-04	7.888E-03	4.556E-04	7.888E-03	8	I-kB, JAK2, IFN-gamma receptor, Beta-2 adrenergic receptor, NF-kB, ICAM1, C/EBPbeta, NFKBIA
49	Immune response_CD28 signaling	56	4.716E-04	7.998E-03	4.716E-04	7.998E-03	10	Bcl-XL, I-kB, NF-AT3(NFATC4), GSK3 beta, NF-kB, MEKK1(MAP3K1), PIP5KI, p38 MAPK, NF-AT, c-Jun
50	Role of ZNF202 in regulation of expression of genes involved in atherosclerosis	21	4.852E-04	8.065E-03	4.852E-04	8.065E-03	6	VEGF-A, APOA4, SDP1, Beta-adrenergic receptor, APOC3, HDL proteins

Table S4: TGF-b-induced epithelial secretome

Fold change = TGF-b / untreated
Ranked by %increase

				Promoter binding sites	
Rank	**Secreted factor**	**P value**	**Fold change**	**c-Jun**	**AP-1**
1	Fractalkine	0.014	4.07		
2	Activin A	0.005	3.37		
3	IGFBP-2	0.027	2.79		
4	PDGF-AA	0.000	2.64		
5	IGFBP-3	0.011	2.58		
6	Decorin	0.021	2.57		
7	MMP-10	0.018	2.33		
8	PDGF-BB	0.011	2.15		
9	TGF-beta 2	0.021	1.69		
10	ICAM-3 (CD50)	0.030	1.68		
11	Thrombospondin-1	0.047	1.64		
12	IL-17	0.019	1.56		
13	Follistatin-like 1	0.045	1.54		
14	sFRP-4	0.038	1.47		
15	HB-EGF	0.013	1.47		
16	TROY / TNFRSF19	0.020	1.44		
17	Inhibin A	0.003	1.44		
18	LBP	0.006	1.43		
19	Angiogenin	0.021	1.43		
20	TWEAK R / TNFRSF12	0.002	1.43		
21	Growth Hormone (GH)	0.046	1.36		
22	EGF R / ErbB1	0.040	1.36		
23	EDA-A2	0.037	1.35		
24	IL-9	0.043	1.32		
25	Siglec-9	0.047	1.31		
26	NT-4	0.017	1.30		
27	Progranulin	0.016	1.30		
28	MCP-2	0.006	1.29		
29	PDGF-AB	0.004	1.29		
30	IP-10	0.036	1.28		
31	IL-1 sRI	0.046	1.26		
32	IL-17RD	0.023	1.26		
33	Insulin R	0.003	1.26		
34	uPAR	0.010	1.26		
35	HGFR	0.015	1.25		
36	M-CSF R	0.035	1.25		
37	HCR / CRAM-A/B	0.020	1.24		
38	NT-3	0.007	1.24		

39	TNF-alpha	0.017	1.23		
40	GREMLIN	0.005	1.22		
41	TMEFF2	0.014	1.22		
42	IL-15	0.007	1.22		
43	Leptin (OB)	0.026	1.22		
44	IL-13	0.000	1.21		
45	IL-10	0.012	1.21		
46	ErbB3	0.030	1.20		
47	IL-1 R8	0.035	1.20		
48	VEGF-D	0.037	1.19		
49	PlGF	0.047	1.18		
50	TCCR / WSX-1	0.035	1.18		
51	TNF RII / TNFRSF1B	0.009	1.17		
52	MMP-8	0.035	1.16		
53	IL-18 BPa	0.035	1.15		
54	CNTF	0.033	1.14		
55	Vasorin	0.045	1.14		
56	IL-7	0.007	1.14		
57	NCAM-1 / CD56	0.009	1.12		
58	BCMA / TNFRSF17	0.035	0.49		
59	BMP-8	0.005	0.40		

Table S5: rhPTX-2 regulation of epithelial secretome

Fold change = PTX-2 + TGF-b / TGF-b
Ranked by %inhibition by rhPTX-2

				Promoter binding sites	
Rank	Secreted factor	P value	Fold change	c-Jun	AP-1
1	MDC	0.048	0.465		
2	Fractalkine	0.041	0.491		
3	TGF-beta RI / ALK-5	0.031	0.728		
4	TGF-beta 2	0.032	0.731		
5	NT-4	0.007	0.803		
6	IL-17RD	0.014	0.806		
7	LBP	0.035	0.810		
8	VEGF-D	0.029	0.815		
9	NT-3	0.007	0.819		
10	M-CSF R	0.041	0.826		
11	TNF RI / TNFRSF1A	0.052	0.846		
12	MMP-8	0.001	0.849		
13	IL-1 sRI	0.023	0.856		
14	MMP-3	0.024	0.864		
15	OSM	0.018	0.874		
16	IL-18 BPa	0.043	0.897		
17	TIMP-3	0.033	1.511		

Table S6: Quantitative RT-PCR primer sequences

Gene	Forward	Reverse
Acta2	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA
Arg1	GAATGGAAGAGTCAGTGTGGT	AGTGTTGATGTCAGTGTGAGC
Ccr2	GAGCCATACCTGTAAATGCCA	ACTGAGGTAACATATTATTGTCTTCCA
CD206	TATCTCTGTCATCCCTGTCTCT	CAAGTTGCCGTCTGAACTGA
CD86	CAGACTCCTGTAGACGTGTTC	AACAGCATCTGAGATCAGCA
Col1a1	CTGGTGAACAGGGTGTTCCT	AGAACCA TCAGCACCTTTGG
Csf1	CAGCAGTTGATCGACAGTCA	TCTTTAGGTAGCAAACAGGATCA
Cx3cr1	TCCCTTCCCATCTGCTCA	CACAATGTCGCCCAAATAACAG
Fcgr1	TGGCTTCTAACAACTCTGCTAC	GCCCCTCACACCATAAAGTG
Fcrls	CAGTGTCTCAAAGCAGAGTCA	TCCCCTTCAACGACCAGT
Gapdh	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG
Igf1	ATGCTCTTCAGTTCGTGTGT	AGTACATCTCCAGTCTCCTCAG
Igf2r	GGAAGGCCAGGAACTGTC	AGTGTAAGAGAGCTGTATGTGTC
Il10	TCAGCCAGGTGAAGACTTTC	GGCATCACTTCTACCAGGTAA
Il23a	ACCAGCGGGACATATGAATC	GATCCTTTGCAAGCAGAACTG
Il4ra	CTGACCTGGAATAACCTGTACC	GATGTTGATCGGGAAGCTCA
IL6	GGTGACAACCACGGCCTTCCC	AAGCCTCCGACTTGTGAAGTGGT
iNOS	AGCCTTGCATCCTCATTGGGCCTGG	ATGCGGCCTCCTTTGAGCCCTTTG
Silec1	ACGTCCAGCCTAGACTTCTATG	CAGGAGAAGCGAGTGTCAG
Slamf1	CAGCATCAAGACAGCATCTACA	CCCCCAGTGGTACAAGAGTA
Tnfa	CGCTCTTCTGTCTACTGAACTT	GATGAGAGGGAGGCCATT
Trem1	CTGTGCGTGTTCTTTGTCTC	ATGTGGACTTCACTGGGTCT
Wnt7b	GACTTTTCTCGTCGCTTTGTG	CACTTACATTCCAGCTTCATGC