Single nuclei RNAseq reveals cell-type specific responses to disease and enalapril in the gddY mouse model of IgAN

CHINOOK THERAPEUTICS

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Background

IgA Nephropathy (IgAN) is the leading cause of primary glomerulonephritis worldwide, with limited treatment options

IgAN Is defined by the deposition of IgA-containing immune complexes in the mesangium that induce inflammatory and profibrotic responses resulting in proteinuria and tubular injury via cellular crosstalk.

The Grouped ddy (gddY) mouse model is a spontaneous model of early-onset IgAN

The gddY mouse model is characterized by IgA immune complex deposition in the mesangium of the kidney, characterized by significant proteinuria, glomerular hypercellularity, mesangioproliferative glomerular lesions, glomerulosclerosis and reduced kidney function, all hallmarks of human IgAN[1].

Deep and high-resolution single-cell datasets to investigate the complex pathogenesis of IgAN are limited

This dataset was then utilized to gain insights into specific kidney cell responses and gene programs of interest, in addition to responses to pharmacological interventions with the angiotensin converting enzyme inhibitor (ACEi) enalapril or the potent and selective endothelin A (ETA) receptor antagonist atrasentan*.

Study Aims:
The gddY mouse was utilized as a model to create a high-resolution dataset to characterize the cell-type specific transcriptional responses in IgAN

Methods/Study Design

Mouse IgAN model:

Generation and characterization of gddY mice with early onset IgAN have been previously described[1] BALB/c mice were used as control mice. gddY mice treated with the ACEi enalapril or the potent and selective ETA receptor antagonist atrasentan.

Group	Strain	n/group	Treatment	Duration*
Group 1	BALB/c (control)	n=6	Vehicle (control)	8 weeks
Group 2	gddY	n=8	Vehicle (control)	8 weeks
Group 3	gddY	n=8	ACEi† (15 mg/kg/day)	8 weeks
Group 4	gddY	n=8	Atrasentan (30 mg/kg/day)	8 weeks

*Treated from 4-12 weeks of age; [1]ACEi (angiotensin converting enzyme inhibitor, enalapril)
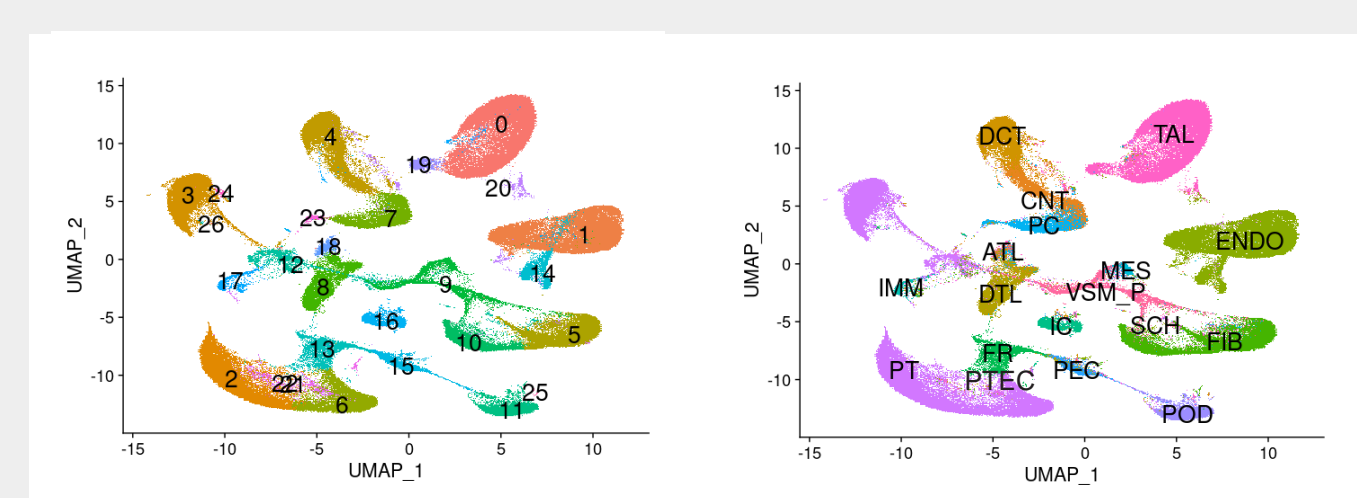
RNA sequencing and data processing:

Nuclei were isolated from snap-frozen kidney cortex, sequenced using the 10x Genomics Platform and analyzed using Seurat 4.0. Number of nuclei per sample ranged from 4,000-10,000. Feature cutoffs used in QC included min.cells = 10, min.features = 500, percent.mt < 1, percent.hb < 1, percent.ribo > 0.5. Additionally, nuclei with top 5%, bottom 1% of nuclei by counts and features were removed. DoubletFinder was used to remove 8% of the nuclei. Nuclei were integrated by mouse using the Seurat IntegrateData function. The top 20 principal components were used for downstream analyses.

Data analysis:

Azimuth was used to annotate cell clusters at the L1 level of annotation[2, 3]. Failed repair proximal tubular cells were annotated using a failed repair proximal tubule signature[4] and TNF-α [5] signature. Mesangial cells were annotated from Azimuth L3 annotation. Genes were tested for differential expression (llog2FC > 0.25|, adjusted p < 0.05) between treatment groups using the FindMarkers function in Seurat by cell type. Enrichment of Hallmark gene sets in differentially expressed genes was assessed using the enrichR library in R. The Connectome R toolkit v1.0.0 was used to infer cell-cell interaction networks from single-cell transcriptome data. Only receptors and ligands expressed in more than 10% of the cells in their respective cell type were considered to construct cell type-specific interactions.

Cellular Composition

A Seurat Clusters

B Enhanced Azimuth L1 annotation

C

	Control	gddY			
	Vehicle	Vehicle	Atrasentan	ACEi	Total
ATL	313	160	126	165	764
CNT	1,615	2,291	2,158	2,472	8,536
DCT	2,963	3,170	3,753	3,480	13,366
DTL	2,539	2,269	2,205	2,681	9,694
ENDO	5,267	7,874	8,408	9,744	31,293
FIB	3,291	5,780	4,950	6,915	20,936
FR PTEC	278	1,564	971	1,648	4,461
IC	771	886	899	983	3,539
IMM	42	650	380	490	1,562
MES	195	378	366	721	1,660
PC	2,432	1,698	1,418	1,929	7,477
PEC	509	765	737	1,005	3,016
POD	2,248	1,334	1,656	2,310	7,548
PT	8,308	10,768	15,287	15,356	49,719
SCH	74	189	132	322	717
TAL	10,120	7,785	7,830	9,550	35,285
VSM_P	667	1,435	1,278	2,649	6,029
Total	41,622	48,996	52,554	62,420	205,592

A Seurat clusters at resolution 0.3 generated 26 clusters

B Cell types were annotated using azimuth level 1 annotation which was supplemented with mesangial cells from azimuth level 3 annotation. Additionally, failed repair proximal tubular epithelial cells (FR PTEC) were annotated using the approach described in Figure 2.

C Number of nuclei for each cell group by treatment. The majority of kidney cell types have well over 100 nuclei per treatment group, **providing good coverage of the tubulointerstitial and glomerular compartments.**

ATL – Ascending thin limb; CNT – connecting tubule; DCT – distal convoluted tubule; DTL – descending thin limb; ENDO – endothelial; FIB – fibroblast; FR PTEC– failed repair proximal tubular epithelial cells; IC – intercalated; IMM – immune; MES – mesangial; PC – principal; PEC – parietal epithelial; POD–podocyte; PT – proximal tubule; SCH – schwann; TAL – thick ascending limb; VSM_P – vascular smooth muscle / pericyte

Results

1 Identification of Failed Repair Proximal Tubular Epithelial cells (FR PTEC) in the gddY IgAN model
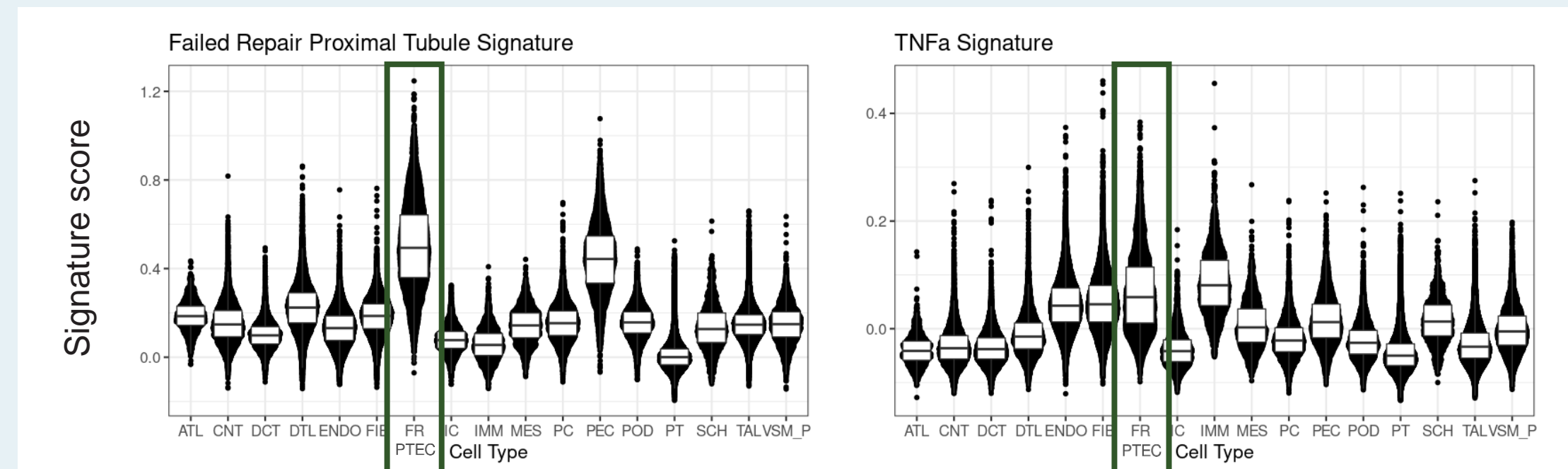
- A cluster of cells with the highest score for the FR PTEC signature[4] and a high score for a TNF-α signature[5] was identified and labeled as FR PTEC
- Analysis of patient samples has also shown that FR PTEC are a hallmark of human CKD and that these cells are associates with eGFR decline[6].
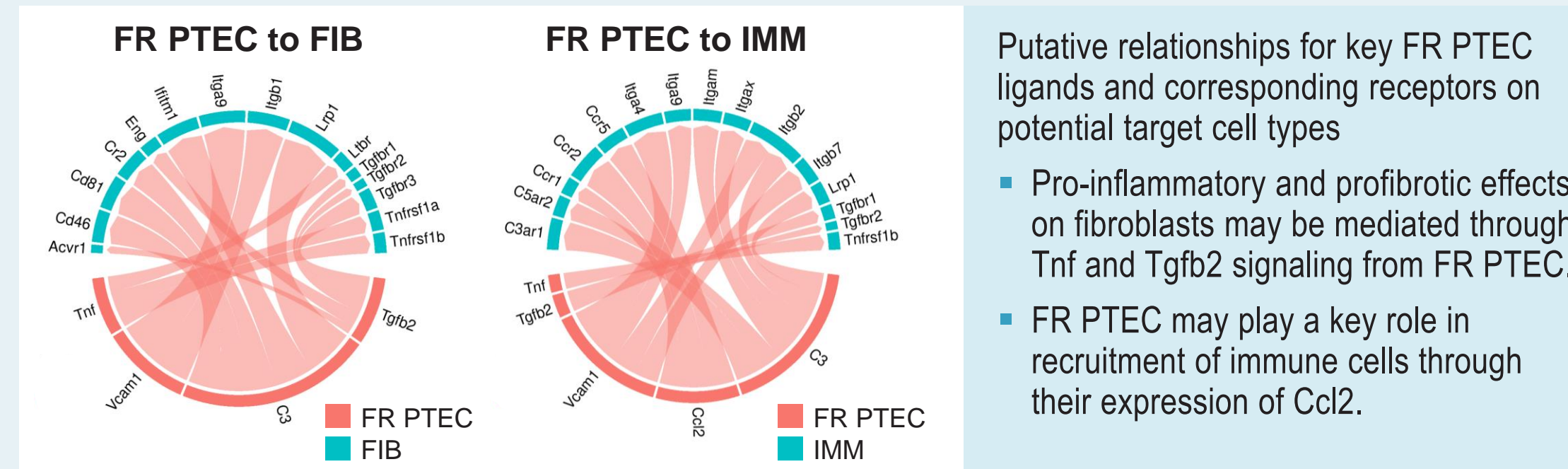
2 FR PTEC are the most expanded kidney cell type in gddY

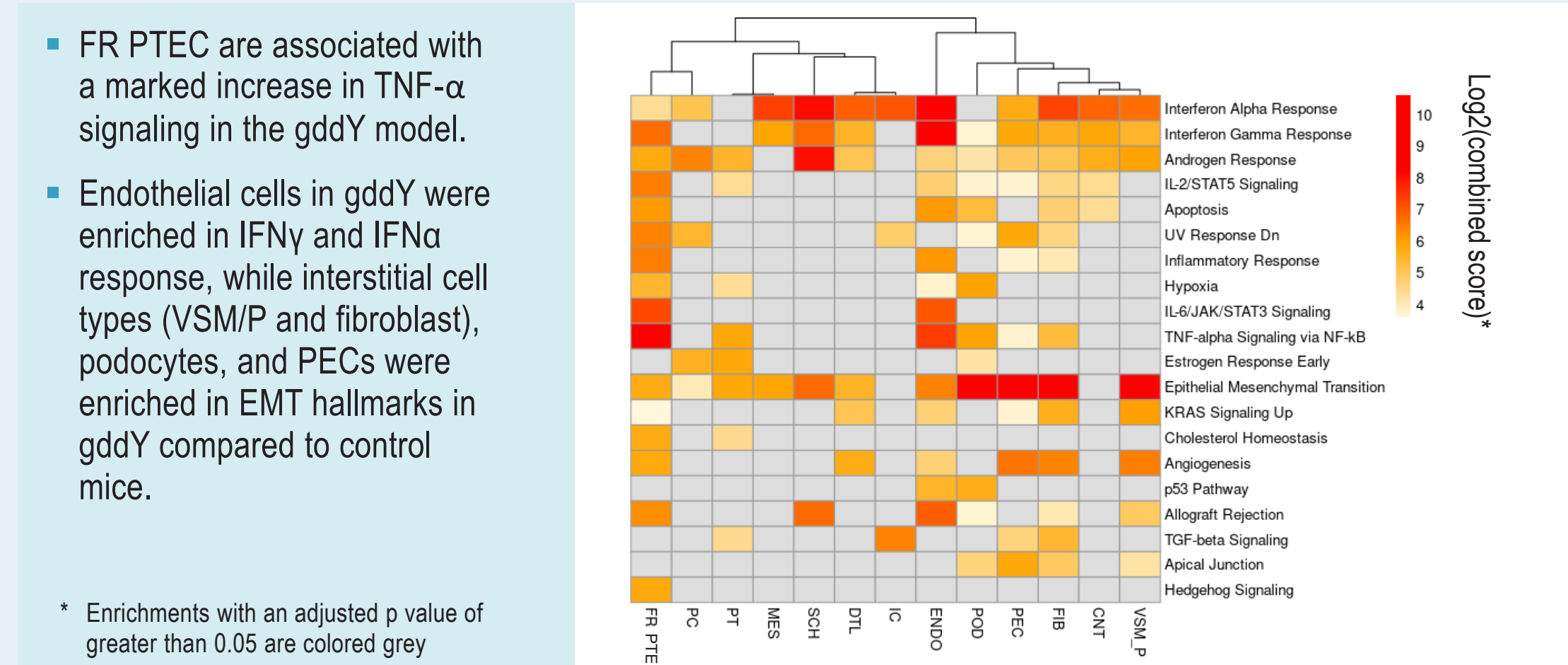**A Cell proportion**

B Differentially expressed genes
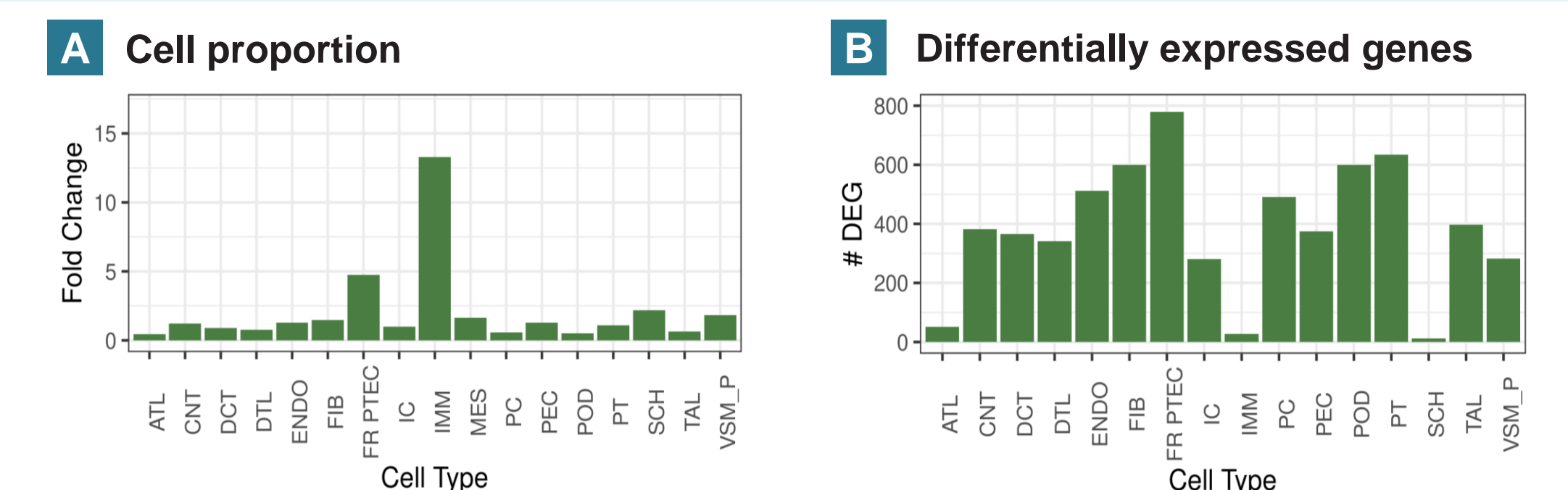
A FR PTEC are the most expanded kidney cell type in gddY compared to control, with ~4.7x the proportion of nuclei in gddY compared with control.

B FR PTEC have the largest number of differentially expressed genes (DEG), with 779 genes differentially expressed in gddY compared to control.

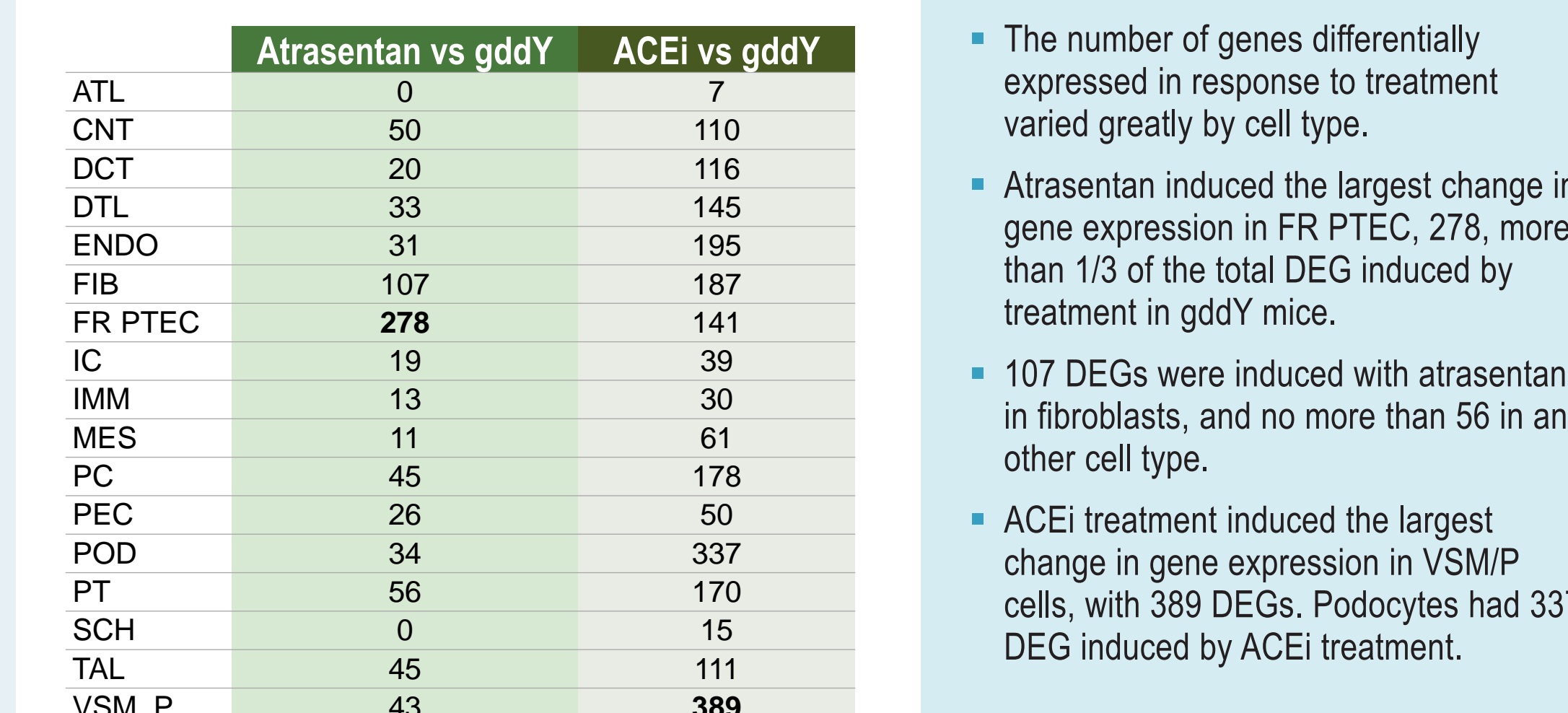## 3 FR PTEC are a source of chemokines and cytokines for immune cells and fibroblasts

FR PTEC to FIB   **FR PTEC to IMM**

Putative relationships for key FR PTEC ligands and corresponding receptors on potential target cell types

- Pro-inflammatory and profibrotic effects on fibroblasts may be mediated through Tnf and Tgfb2 signaling from FR PTEC.
- FR PTEC may play a key role in recruitment of immune cells through their expression of Ccl2.

4 TNF-α signaling is enriched in FR PTEC

- FR PTEC are associated with a marked increase in TNF-α signaling in the gddY model.
- Endothelial cells in gddY were enriched in IFNγ and IFNα response, while interstitial cell types (VSM/P and fibroblast), podocytes, and PECs were enriched in EMT hallmarks in gddY compared to control mice.
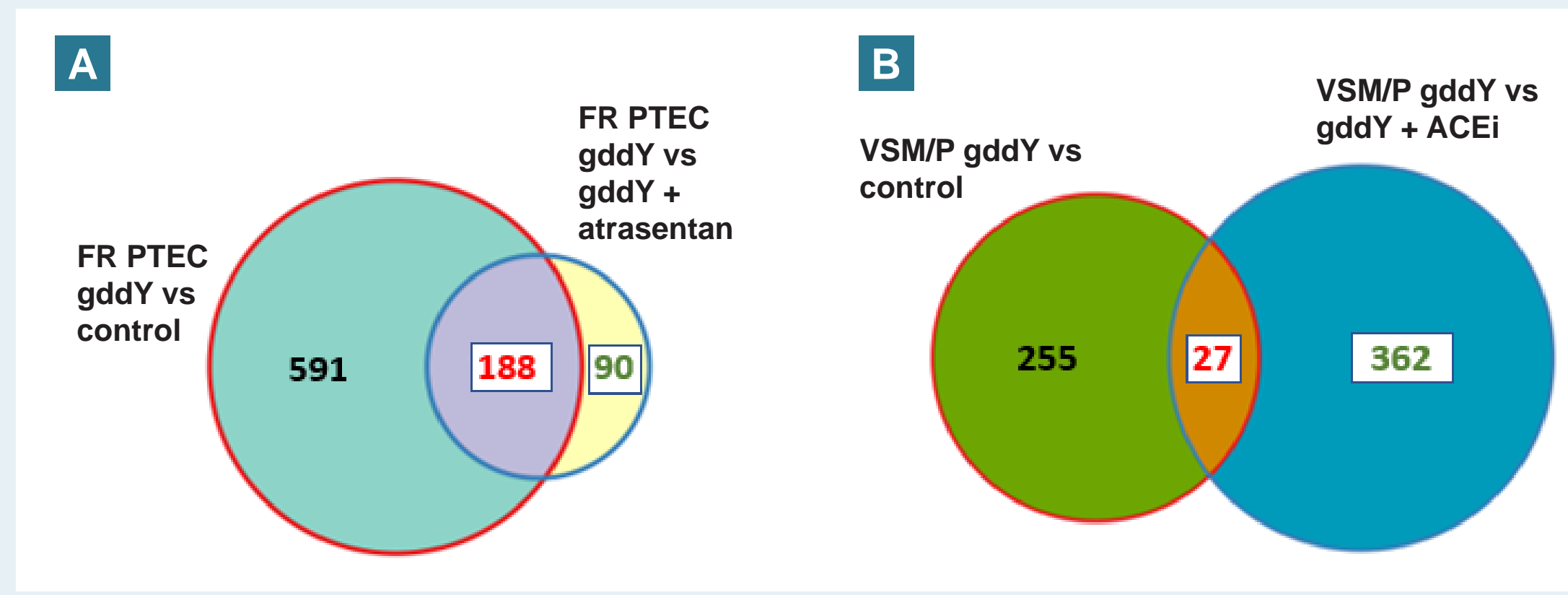
* Enrichments with an adjusted p value of greater than 0.05 are colored grey

5 Treatments have differing effects on gene expression

	Atrasentan vs gddY	ACEi vs gddY
ATL	0	7
CNT	50	110
DCT	20	116
DTL	33	145
ENDO	31	195
FIB	107	187
FR PTEC	278	141
IC	19	39
IMM	13	30
MES	11	61
PC	45	178
PEC	26	50
POD	34	337
PT	56	170
SCH	0	15
TAL	45	111
VSM_P	43	389

- The number of genes differentially expressed in response to treatment varied greatly by cell type.
- Atrasentan induced the largest change in gene expression in FR PTEC, 278, more than 1/3 of the total DEG induced by treatment in gddY mice.
- 107 DEGs were induced with atrasentan in fibroblasts, and no more than 56 in any other cell type.
- ACEi treatment induced the largest change in gene expression in VSM/P cells, with 389 DEGs. Podocytes had 337 DEG induced by ACEi treatment.

6 Treatments have differing effects on gene expression

A

FR PTEC gddY vs control

FR PTEC gddY vs gddY + atrasentan

591 188 90

B

VSM/P gddY vs control

VSM/P gddY vs gddY + ACEi

255 27 362

A In FR PTEC, 188 genes are reversed by atrasentan treatment in FR PTEC (red text, center), while 90 genes were induced by atrasentan, but not in gddY alone (green text, right).

B In VSM/P cells, ACEi treatment reversed expression of only 27 genes (red text, center), while 362 genes were induced by ACEi, but not gddY alone (green text, right).

Atrasentan treatment in FR PTEC reverses gene expression changes induced in the gddY model, while ACEi treatment in VSM/P induces new gene expression.

7 Comparison of pathway enrichments for atrasentan and ACEi treatment

A   Enriched Hallmarks in FR PTEC

B   Enriched Hallmarks in VSM/P

Increased in gddY
Decreased by atrasentan
Decreased by ACEi

A In FR PTEC, gddY-induced DEGs were highly enriched for TNF-α signaling. Atrasentan treatment of gddY mice led to downregulation of genes that are enriched in the TNF-α hallmark.

B In VSM/P cells, EMT was the primary hallmark increased in gddY. ACEi treatment had a modest effect on the increased expression of these genes.

8 Characterization of the gddY FR PTEC atrasentan response gene signature in the anti-Thy1.1 model of Mesangio-proliferative Glomerulonephritis

A

B   Decreased   Increased

Anti-Thy1.1 vs Control
Atrasentan + Anti-Thy1.1 vs Anti-Thy1.1
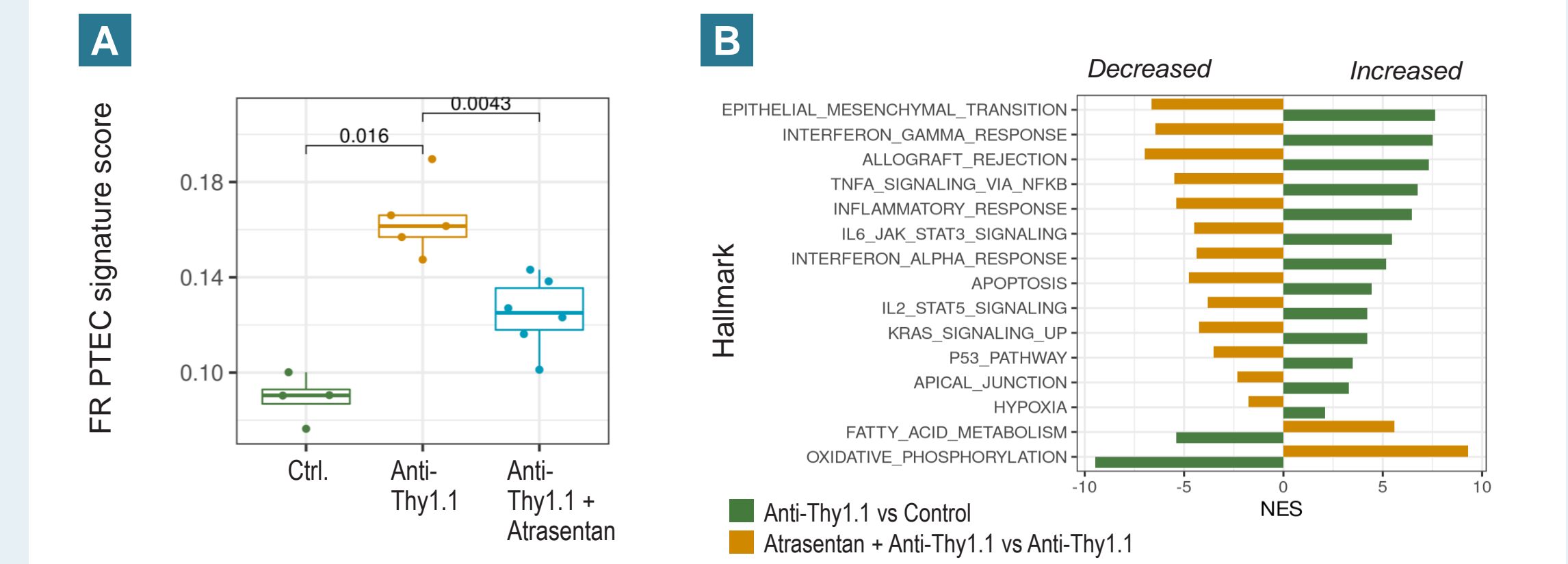
A The top 108 genes that were increased in the FR PTEC in gddY and decreased by atrasentan were used as an atrasentan response signature. Expression of this signature is elevated in anti-Thy1.1 rats and atrasentan significantly reduced the expression of the FR PTEC atrasentan signature.

B Functional enrichments of Hallmark genesets for DEGs show that TNF-α signaling, as well as EMT, IFNg and other genesets are enriched in anti-Thy1.1 rats. Atrasentan treatment reversed these enrichments.

These findings suggest that the gene expression changes we identified in atrasentan treated FR PTEC in the gddY model are not unique to that model.

Conclusions

Failed repair proximal tubular epithelial cells (FR PTEC) are a prominent feature of the gddY mouse model of IgAN

- This study identified a cluster of cells that scored highly for a FR PTEC signature and a TNF signature and were the most highly expanded kidney cell type in gddY.
- We propose that these cells represent FR PTEC, and that the expansion of these cells is a major characteristic of the gddY model and may play a major role in tubulointerstitial inflammation and fibrosis and progressive kidney function loss.
- We have also found that FR PTEC are a key characteristic of CKD progression in human disease[6].

Atrasentan and ACEi treatment resulted in different effects on gene expression

- Atrasentan induces the most gene expression changes in FR PTEC and these gene expression changes reverse pathogenic changes that are induced in the gddY disease model.
- ACEi treatment tends to induce new gene expression changes, most prominently in VSM/P.

Gene expression changes observed in atrasentan treated FR PTEC were also observed in atrasentan treated anti-Thy1.1 rats

- An FR PTEC- associated atrasentan response signature derived from the gddY dataset was applied to the anti-Thy1.1 rat model of mesangio-proliferative glomerulonephritis.
- This signature was increased by anti-Thy1.1 treatment and that atrasentan treatment reduced this increase.
- Atrasentan treatment reversed inflammation and fibrosis associated gene expression in anti-Thy1.1 rats .

Ongoing efforts to characterize gene expression associated with atrasentan response

- This atrasentan response signature is currently being evaluated in IgAN patient kidney biopsies and matched urine and serum samples are being screened for non-invasive surrogate biomarkers.

References

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*Atrasentan is an investigational drug that has not been approved by regulatory authorities. Efficacy and safety have not been established. There is no guarantee that it will become commercially available for the use(s) under investigation.