

## **Defective CFTR leads to aberrant $\beta$ -catenin activation and kidney fibrosis**

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### **Supplementary information:**

#### **Supplementary Figure legends**

**Supplementary Figure 1. Suppression of CFTR during EMT is not related to TGF- $\beta$ /Smads signaling. (a)** Immunofluorescent staining shows that CFTR protein is expressed at cell-cell adhesion and cytoplasm in tubular epithelium in normal human kidney, whereas significantly decreased in fibrotic kidney tissue (n=2), scale bar=50 $\mu$ m.

**(b)** MDCK cells were treated with 2ng/ml TGF- $\beta_1$  for 3 days. Western blot analysis shows that while TGF- $\beta_1$  significantly induces EMT markers, it does not have any effect on CFTR expression in MDCK cells (Full-length blot is shown in supplementary figure S7m); **(c)** Immunofluorescent staining shows that the expression of CFTR is decreased, whereas the expression of HIF-1 $\alpha$  is significantly induced in UUO kidney in both wild type and Smad3 knockout (*smad3 KO*) mice; **(d)** Western blot analysis shows that CFTR inhibitors have no effect on the expression of phosphorylated-Smad2 in MDCK cells (Full-length blot is shown in supplementary figure S7n).

**Supplementary Figure 2. Dysregulation of CFTR induces EMT in kidney epithelial cells.**

**(a)** Phase-contrast photomicrographs of MDCK cells treated with 10 $\mu$ M CFTRinh-172 (inh172) or 5 $\mu$ M GlyH101 for 48 hours; **(b)** Western blot analysis showing increased Vimentin expression and decreased Occludin expression in MDCK cells treated with different concentration of GlyH101 for 48hours; quantification analysis is shown in the lower panel, \* $p < 0.05$ , \*\* $p < 0.01$ . (Full-length blot is shown in supplementary figure S7o.); **(c)** Quantification of western blot showing significantly reduced CFTR expression in MDCK cells 48 hours post transfection with CFTR miRNA, \* $p < 0.05$ ; **(d)** Real time-PCR showing significantly reduced CFTR expression by ribozyme (rib) –mediated CFTR knockdown in HK-2 cells; **(e)** Western blot showing decreased E-cadherin expression and increased SMA expression in CFTR knockdown HK2 cells compared to control cells; quantification analysis is shown in the lower panel, \* $p < 0.05$ . (Full-length blot is shown in supplementary figure S7p); **(f)** MTS assay shows no significant changes in cell proliferation in CFTR knockdown HK-2 cells compared to control cells; **(g)** Wound-healing migration assay showing enhanced cell migration in CFTR knockdown HK-2 cells compared to control cells.

**Supplementary Figure 3. Focused PCR analysis showing upregulated mRNA expression of various EMT and migration-associated genes in CFTR knockdown HK-2 cells.** A human fibrosis-focused PCR array was used to analyze the expression of a panel of genes involved in fibrosis. Expression levels of 19 genes show more than 2-folds change in CFTR knockdown HK-2 cells compared to control cells. ( $p < 0.05$ )

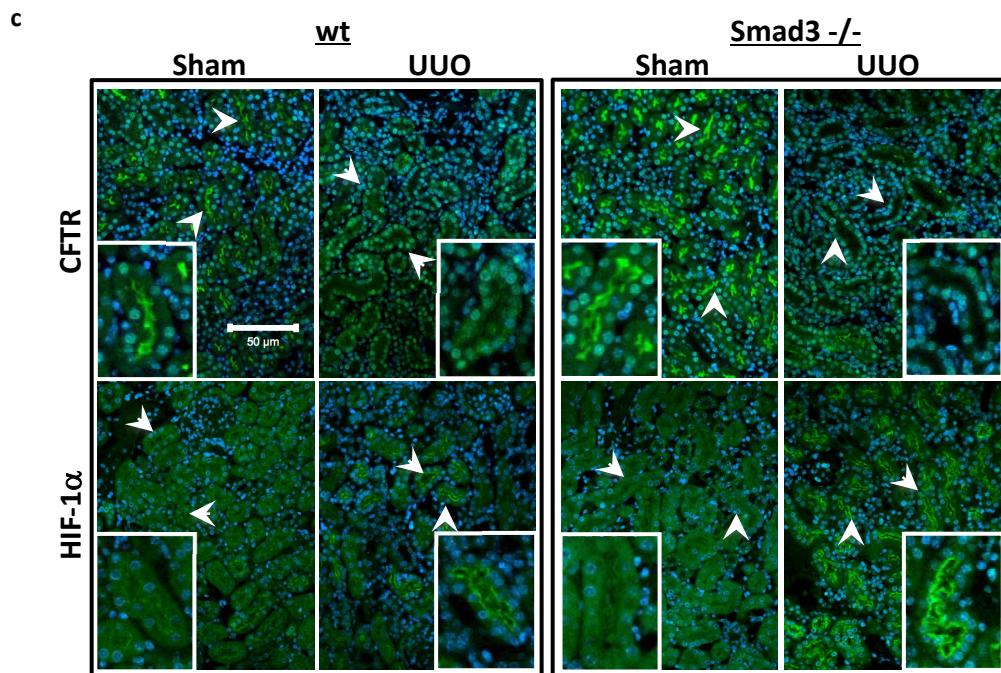
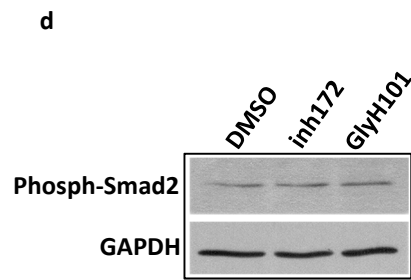
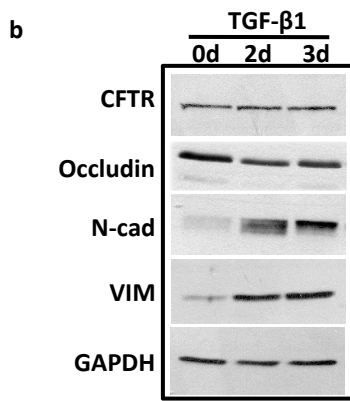
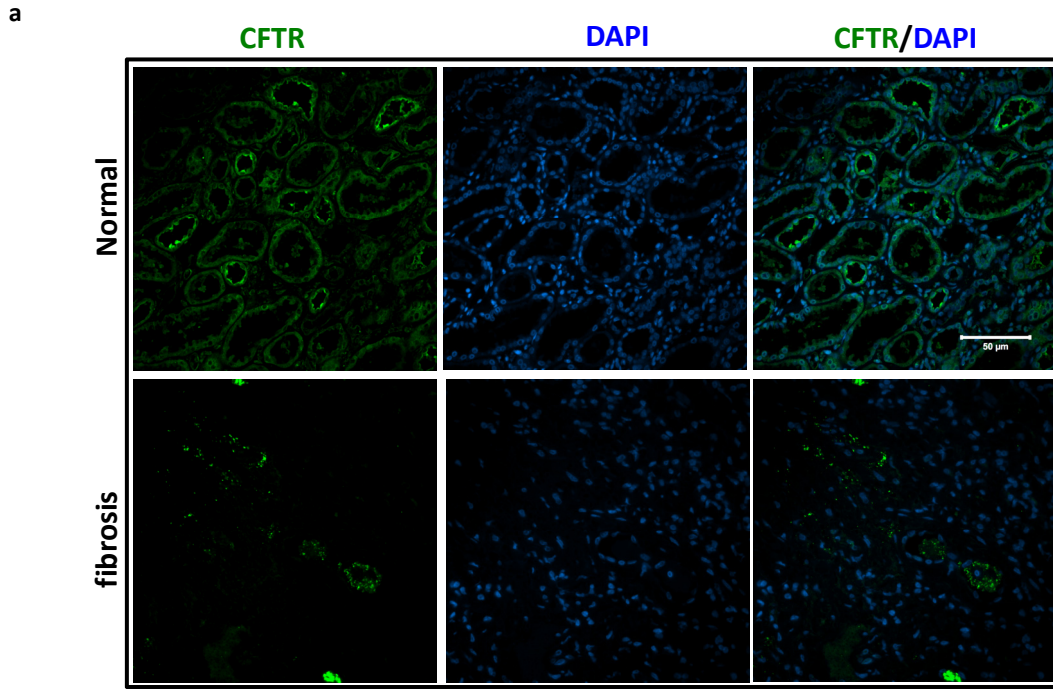
**Supplementary Figure 4. CFTR regulates  $\beta$ -catenin signaling.** (a) Immunofluorescent staining showing the nuclear translocation of  $\beta$ -catenin in CFTR inhibitor-treated MDCK cells; (b) Western blot showing overexpression of CFTR in HK-2 cells (Full-length blot is shown in supplementary figure S7q); (c) Western blot showing knockdown of CFTR in HK-2 cells does not affect the expression of  $\beta$ -catenin destruction complex proteins (Full-length blot is shown in supplementary figure S7r); (d) HK-2 cells were treated with 10 $\mu$ M  $\beta$ -catenin activator, CHIR 99021, for 48 hours. Western blot showing activation of  $\beta$ -catenin promotes EMT (Full-length blot is shown in supplementary figure S7s); (e) Western blot using primary antibody against GFP, showing the expression level of wild type CFTR (CFTR<sub>wt</sub>) and CFTR with deletion of the PDZ binding domain (CFTR delPDZ) (Full-length blot is shown in supplementary figure S7t).

**Supplementary Figure 5. Defective CFTR aggravates the development of renal fibrosis.** (a) Western blot showing the expression levels of CFTR in WT and  $\Delta$ F508 mice (Full-length blot is shown in supplementary figure S7u); (b) PCR result showing the genotype of WT,  $\Delta$ F508 hetero and  $\Delta$ F508 homo mice. (c) H&E staining showing more inflammatory cell invasion in  $\Delta$ F508 CFTR mice after UUO procedure compare

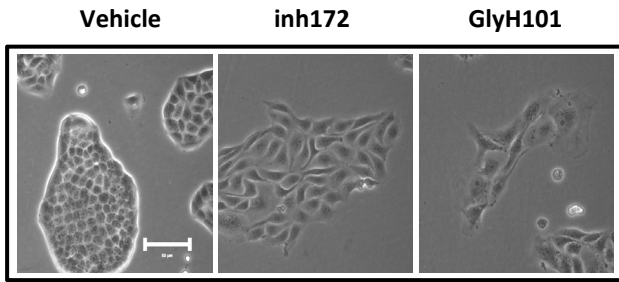
to wild type mice. Note the accumulation of inflammatory cells (arrow) in the UUO kidneys at 7 days after ligation; **(c)** Masson's trichrome stain showing more interstitial collagen expression in  $\Delta F508$  CFTR mice after UUO procedure compared to wild type mice.

**Supplementary Figure 6. Forced expression of CFTR downregulates  $\beta$ -catenin target in UUO model.** **(a)** Real time-PCR analysis showing the significantly overexpression of CFTR in peGFP-CFTR overexpression kidneys compared to peGFP-C3 control kidneys. \* $p < 0.05$ , quantification analysis represents data from 9 control and 10 CFTR overexpressed UUO kidneys; **(b)** Representative western blot showing reduced Axin2 expression in CFTR overexpressed UUO kidneys compared to control kidneys. (Full-length blot is shown in supplementary figure S7v.) Quantification data is shown in the right panel (n=6-7).

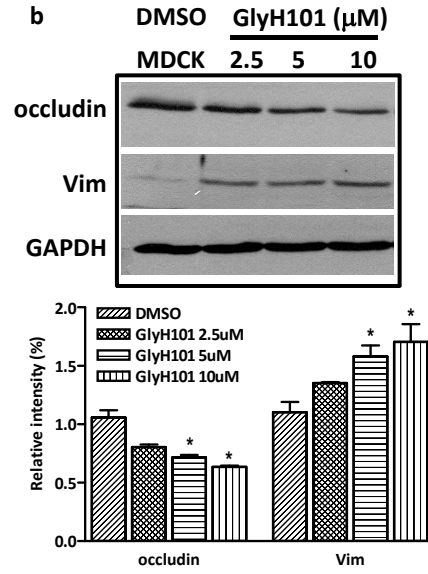
**Supplementary Figure 7. Full-length gel images of western blots.**



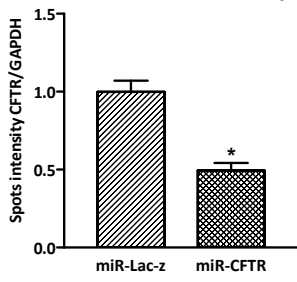
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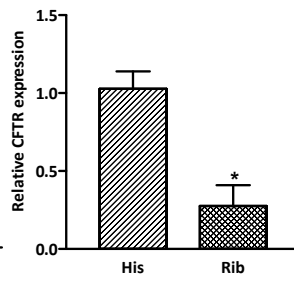
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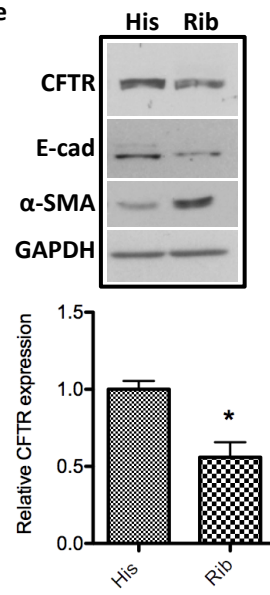
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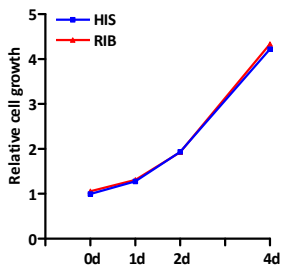
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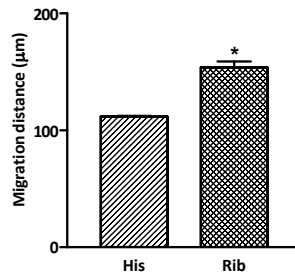
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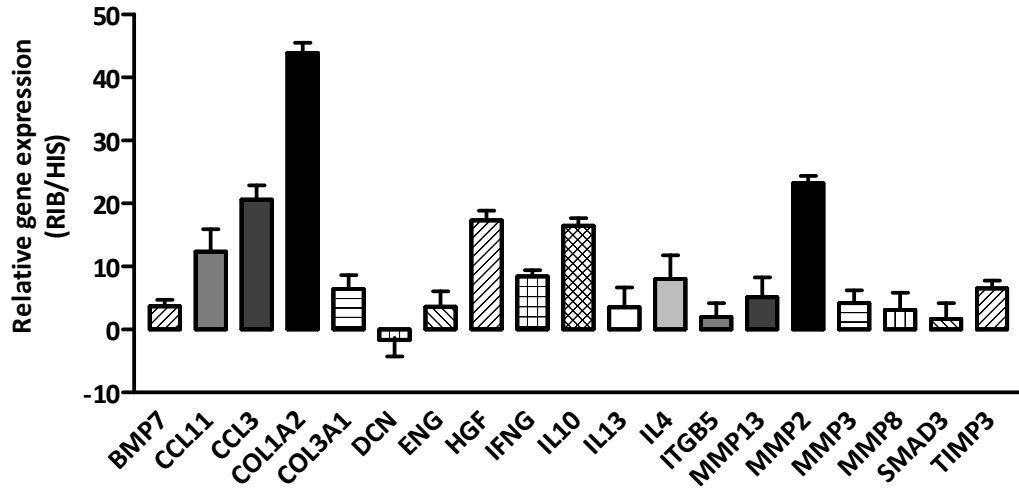


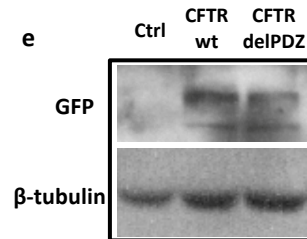
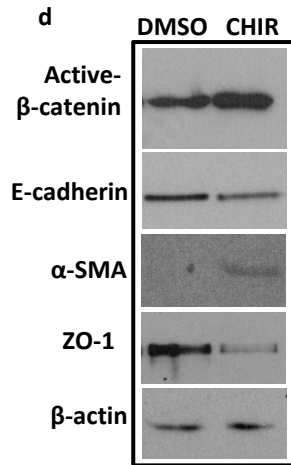
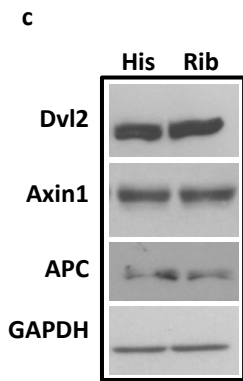
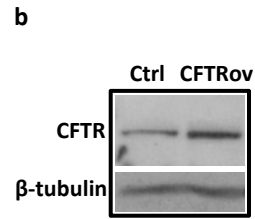
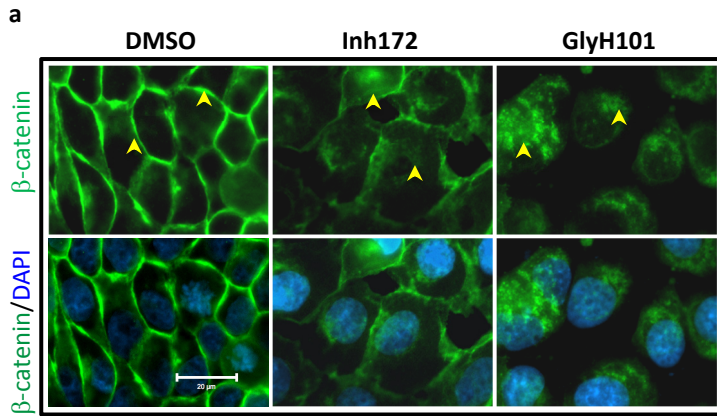
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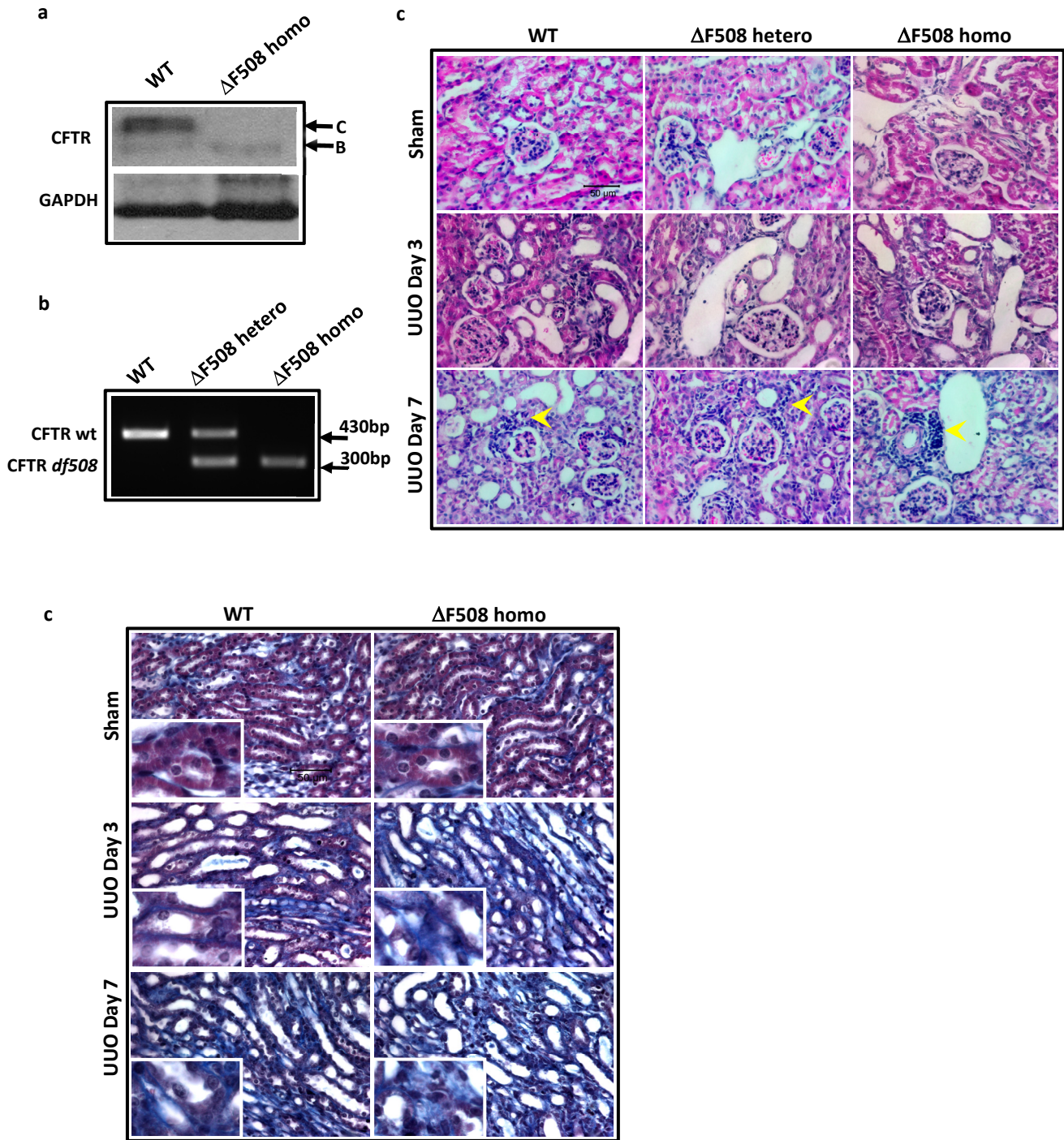
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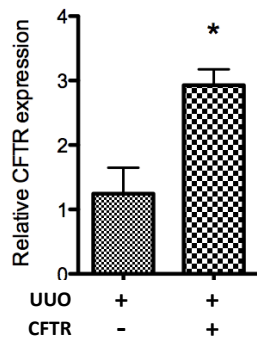




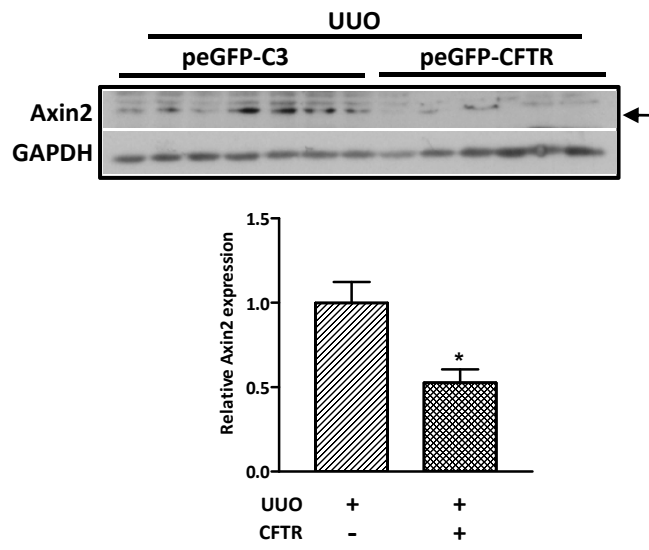


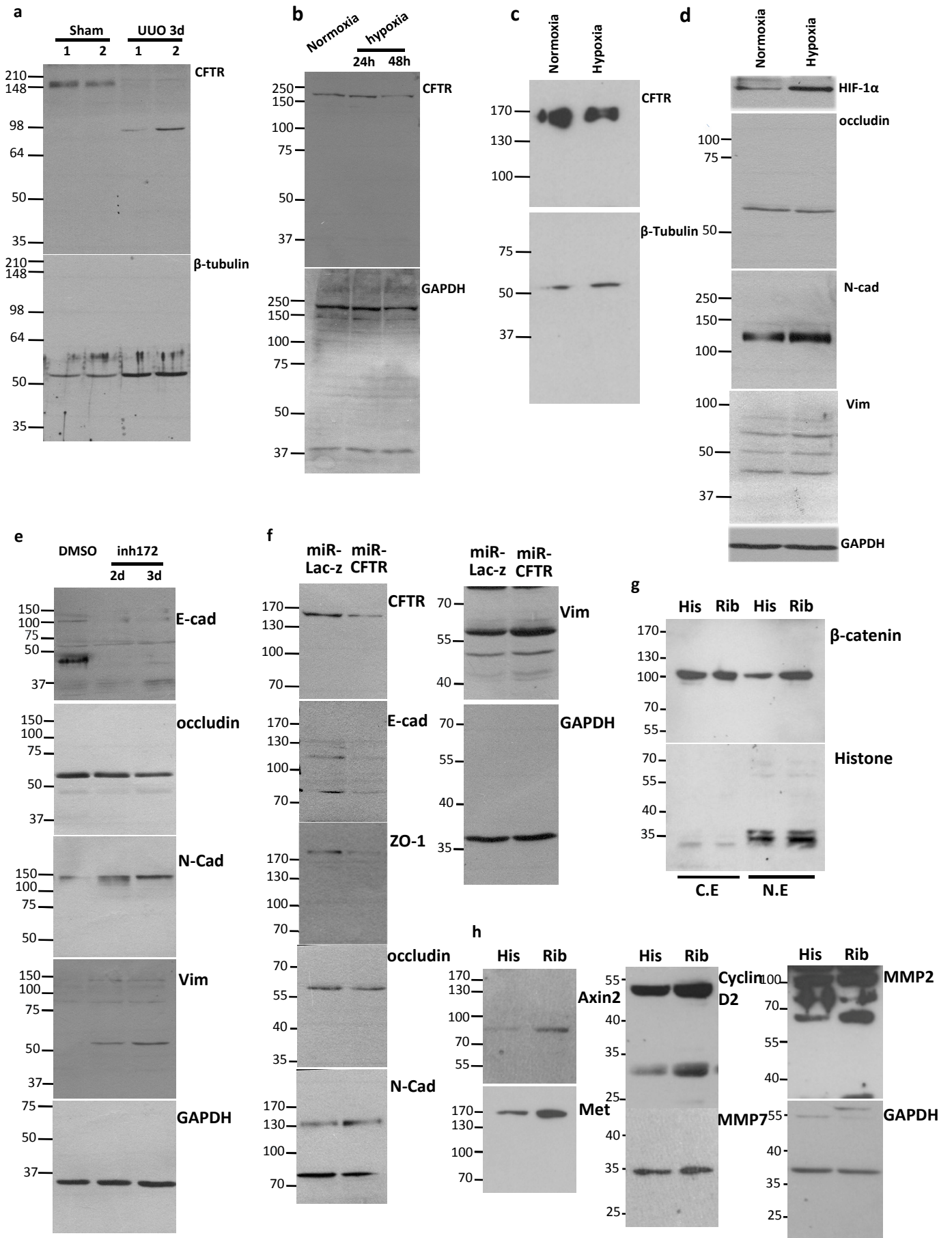


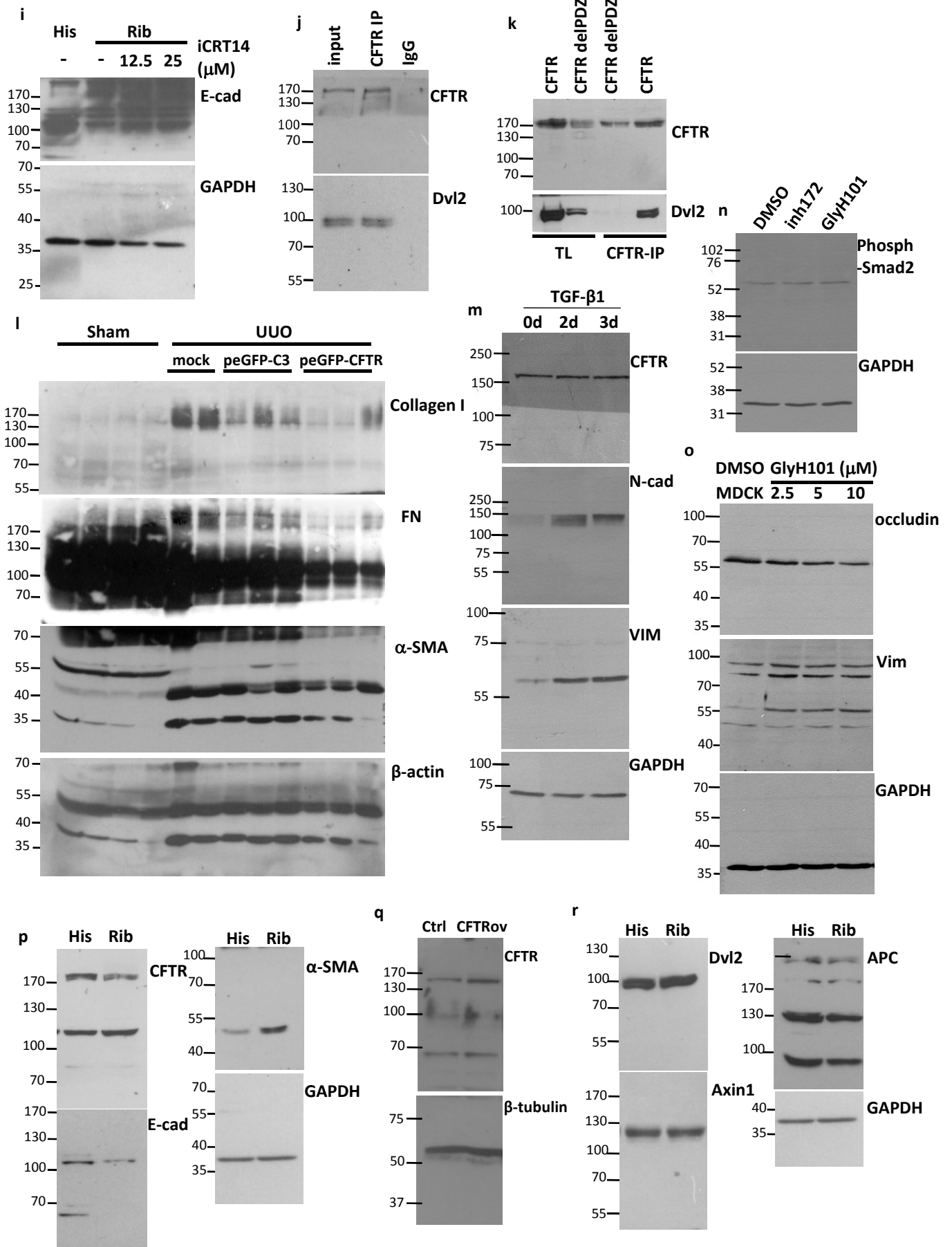
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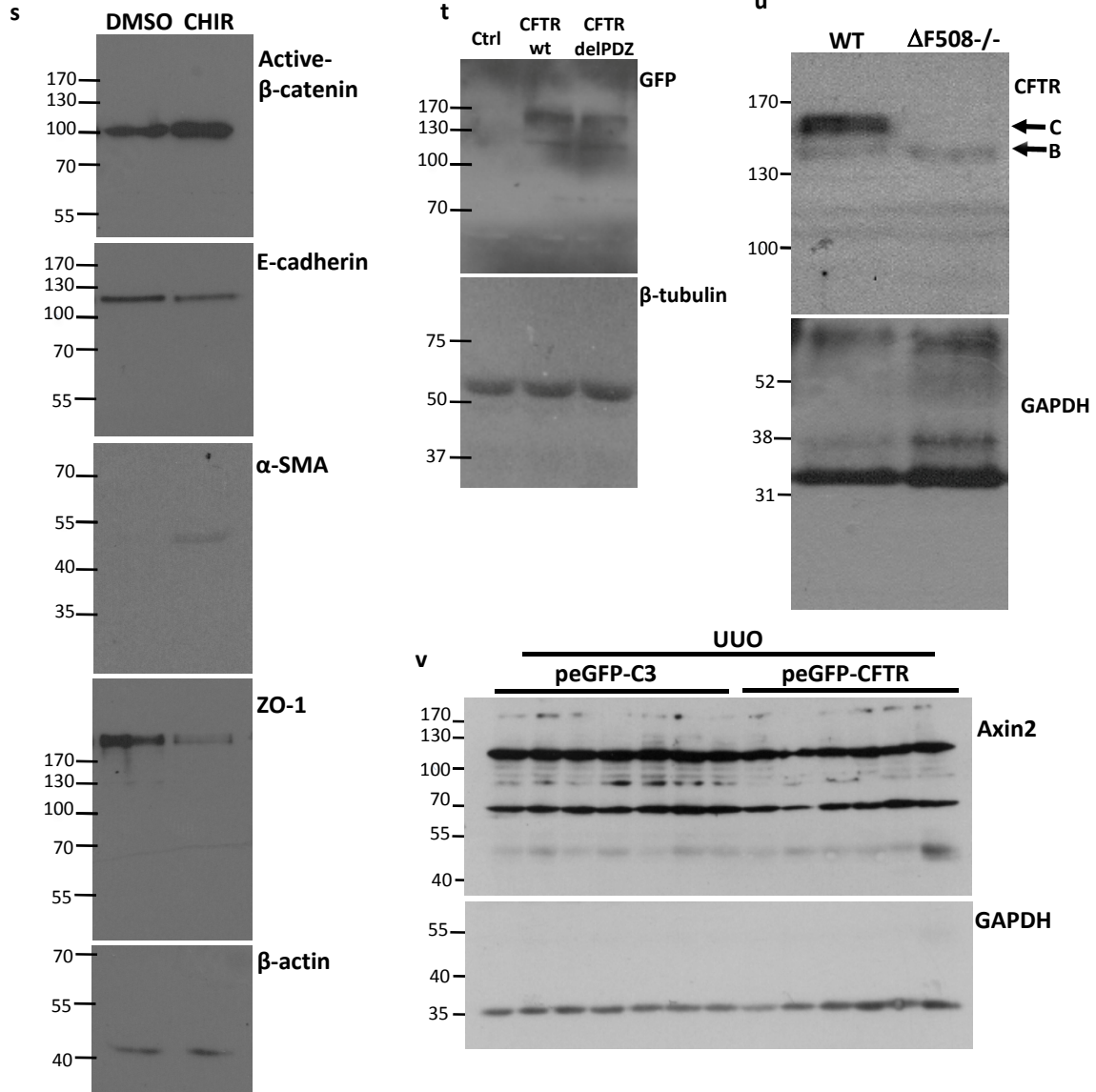


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**Supplementary Table1 Primer information (Human)**

Gene		Primer sequence (5'-3')	size	Tm
CFTR	forward	GTGTGATTCCACCTTCTCCAA	149bp	60°C
	reverse	GCCTGGCACCATTAAAGAAA		
Axin2	forward	CTGGTGCAAAGACATAGCCA	103bp	60°C
	reverse	AGTGTGAGGTCCACGGAAAC		
C-Met	forward	TGTTCGATATTCATCACGGC	94bp	60°C
	reverse	GCATTTTTACGGACCCAATC		
MMP7	forward	GCATCTCCTTGAGTTTGGCT	103bp	60°C
	reverse	GAGCTACAGTGGGAACAGGC		
GAPDH	forward	AGGGTCATCATCTCTGCC	245bp	60°C
	reverse	CCATCACGCCACAGTTTC		

**Supplementary Table2 Primer information (Mouse)**

Gene		Primer sequence (5'-3')	size	Tm
Axin2	forward	ACTGACCGACGATTCCATGT	96bp	60°C
	reverse	TGCATCTCTCTCTGGAGCTG		
c-jun	forward	GGGACACAGCTTTCACCCTA	104bp	60°C
	reverse	GAAAAGTAGCCCCAACCTC		
MMP7	forward	GCATTCCTTGAGGTTGTCC	102bp	60°C
	reverse	CACATCAGTGGGAACAGGC		
a-SMA	forward	GGGATCCTGACGCTGAAGTA	147bp	60°C
	reverse	G TTCAGTGGTGCCTCTGTCA		
Col1A1	forward	ACATGTTTACGCTTTGTGGACC	110bp	60°C
	reverse	TAGGCCATTGTGTATGCAGC		
FN	forward	ACCTCTGCAGACCTACCCAG	120bp	60°C
	reverse	TTGGTGATGTGTGAAGGCTC		
GAPDH	forward	AACGACCCCTTCATTGAC	190bp	60°C
	reverse	TCCACGACATACTCAGCAC		
CFTR (Genotype)		TTCAAGCCCAAGCTTTCGCGAG	WT:430 bp	
		CTCCCTTCTTCTAGTCACAACCG	Hetero:430+300 bp	
		CATCTTGATAGAGCCACGGTGC	Homo:300 bp	