

Abstract

The bladder urothelium forms a highly specialized watertight barrier to urinary wastes. The urothelium offers an unusual example of tissue regeneration: although urothelial cells do not rapidly turn over under physiological conditions, they have an impressive capacity to regenerate tissue upon injury. Even more remarkable, depending on the modality of injury (sterile, infectious) there appear to be two distinctive modes of urothelial regeneration. We have previously shown that in response to a urinary tract infection (UTI), the urothelial stem cell niche becomes activated and induces rapid restoration of the urothelium, whereas, regeneration following sterile injury does not involve stem cell activity. However, the key driver(s) of mode of regeneration choice has yet to be elucidated. To better understand the regulatory pathways important for tissue regenerative response, we performed large unbiased RNA-Seq and proteomics analyses. We identified interferon-related developmental regulator 1 (IFRD1), a transcriptional co-regulator, as a gene that is rapidly activated upon the induction of a UTI. Ifrd1 has been shown recently to be important for paligenosis, a process differentiated cells use to reenter the cell cycle to regenerate lost tissue. Interestingly, we observe that even in the absence of injury, loss of Ifrd1 results in gross urothelial defects: excess vesicular congestion in terminally differentiated cells including aberrant accumulation of mitochondria and abnormal endoplasmic reticulum (ER). Furthermore, we show that Ifrd1 affects localization and trafficking of uroplakins, tetraspanin proteins that constitute organized urothelial plaques, and which dimerize in the ER and assemble into heterotetramer in the Golgi and trans-Golgi network (TGN), where they undergo chain-specific glycosylation and proteolytic processing and eventual degradation. Loss of Ifrd1 results in dysfunctional uroplakin ER-> Golgi translocation and aberrant accumulation in ER. Proteomic analyses revealed a significant increase in the unfolded protein response (UPR) and stress. In agreement with this, we note an increase in the ER chaperone, Bip and increased phosphorylation of eIF2 α , the critical translation initiation factor that quenches global, cellular mRNA translation and can ultimately trigger apoptosis. Indeed, induction of injury in the Ifrd1^{-/-} mouse results in massive epithelial exfoliation into the urine and dysregulated recruitment of progenitor cells for regeneration. Ongoing work is elucidating the molecular underpinnings of this response. In sum, we suggest IFRD1 plays a role in the decision-making matrix of urothelial regeneration and that IFRD1 plays a role in urothelial plasticity.

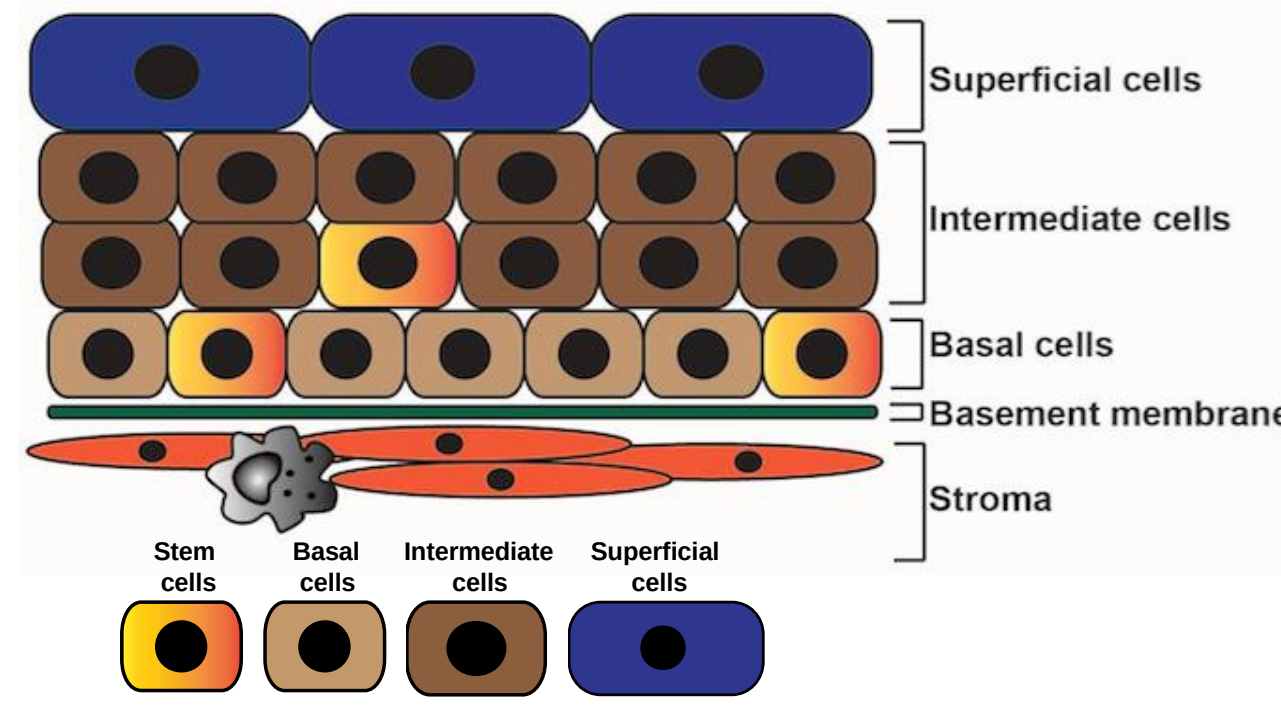


Fig. 1 The urinary bladder is lined by a transitional epithelium that forms a watertight barrier to urinary wastes

The multilayered bladder epithelium (urothelium) consists of 3 main cellular layers: UP11+ superficial cells, KRT7+ intermediate cells, and KRT5+ basal cells. Within the KRT5+ cells layer, a subset of KRT14+ stem cells have been identified. These cells have been shown to have long-term repopulating capabilities in vivo. Under homeostatic conditions, the urothelium is normally quiescent. The urothelium has an extremely slow turnover rate of 3-6 months.

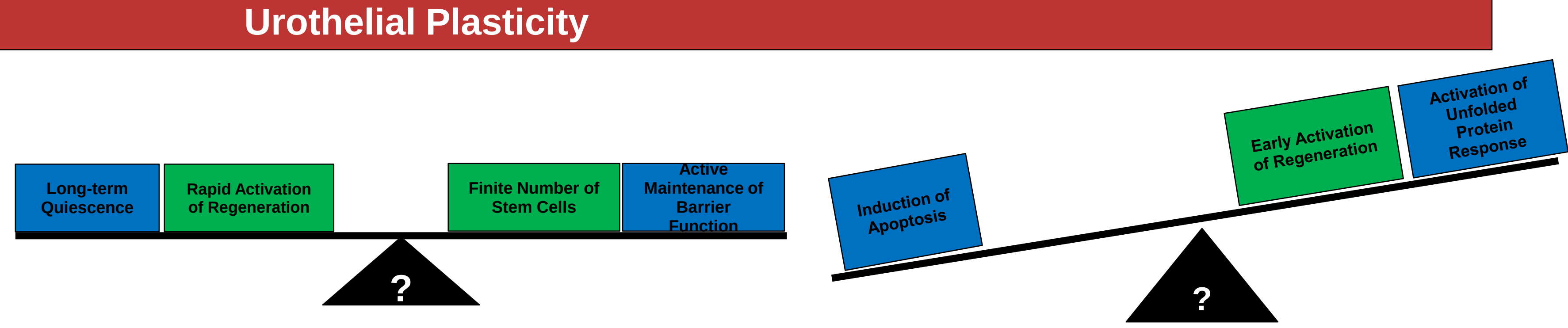


Fig. 2 Under homeostatic conditions, the urothelium maintains “active” long-term quiescence. While traditionally thought of as dormant, the urothelium, and in particular the superficial cells, are actively regulating a number of important processes. How is this tissue able to remain “dormant” when they are constantly in contact toxic hypertonic urine and are mechanically active nonstop? The underlying molecular pathways that control this, are not well understood.

Fig. 3 Upon injury, a decision has to be quickly made whether to regenerate and respond to injury, or undergo apoptosis.

Both protamine sulfate injury and injury due to urinary tract infection, quickly activate regeneration within hours of insult. In the murine model, we see the complete restoration of the urothelium within 72 hours. the urothelium is able regenerate

Here, we propose IFRD1 sits at the interface of these two states:

1. Actively maintaining homeostatic quiescence and
2. Modulating the response to insult in the forms of injury and/or environmental assault (i.e urine)

IFRD1 in Urothelial Maintenance

Loss of IFRD1 results in vesicular congestion, dysfunctional intracellular cargo recycling, and accumulation of dysfunctional mitochondria and intravesicular endoplasmic reticulum

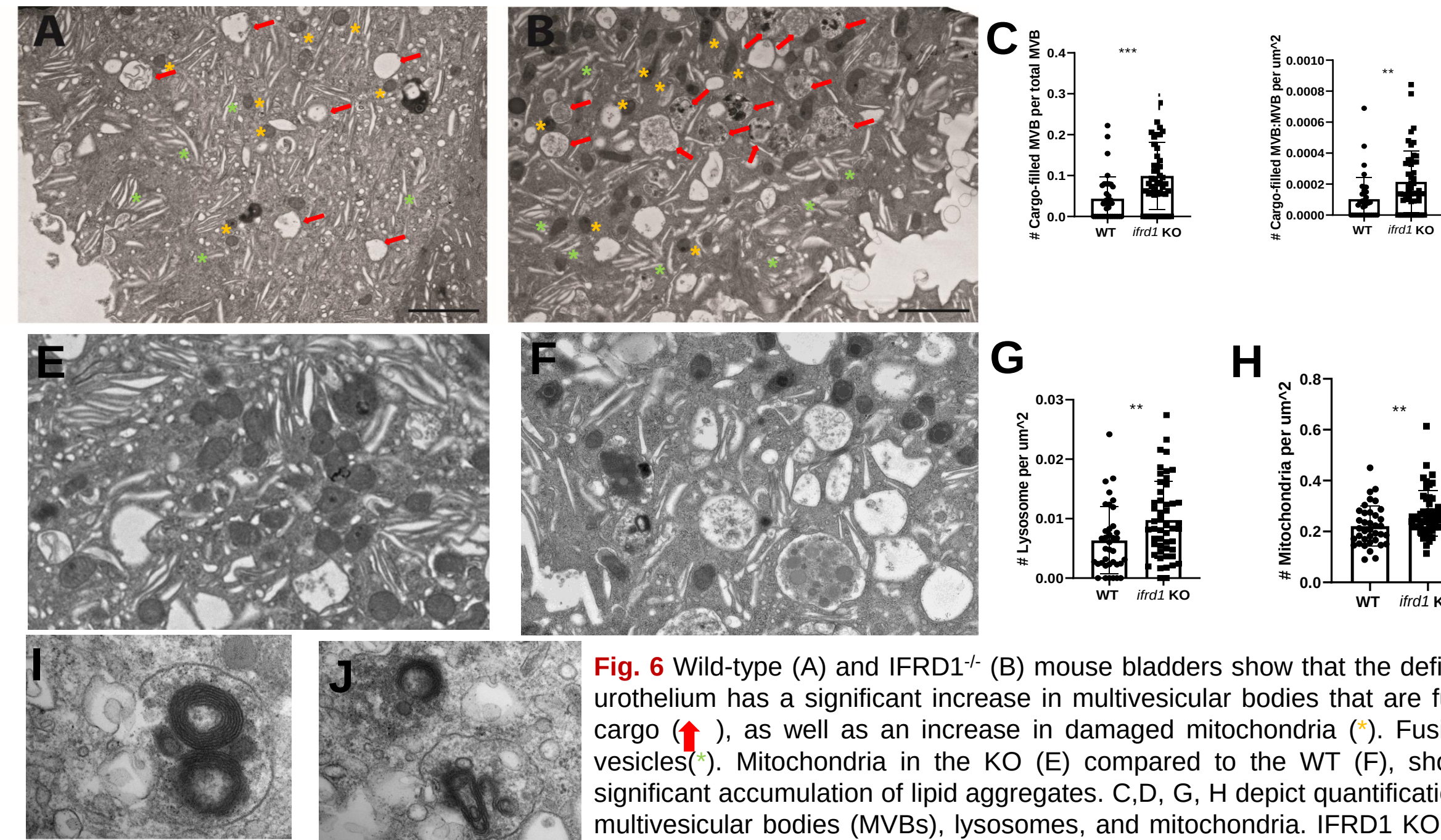


Fig. 6 Wild-type (A) and IFRD1^{-/-} (B) mouse bladders show that the deficient urothelium has a significant increase in multivesicular bodies that are full of cargo (●), as well as an increase in damaged mitochondria (*). Fusiform vesicles(*). Mitochondria in the KO (E) compared to the WT (F), show a significant accumulation of lipid aggregates. C,D, G, H depict quantification of multivesicular bodies (MVBs), lysosomes, and mitochondria. IFRD1 KO also exhibited significant accumulation of intravesicular endoplasmic reticulum (I,J). WT bladders did not depict any ER accumulation. Transmission electron microscopy images were taken at 5000x. Scale bar = 2 μ m

IFRD1 is associated with urothelial ultrastructure defects and decreased expression of terminal differentiation markers

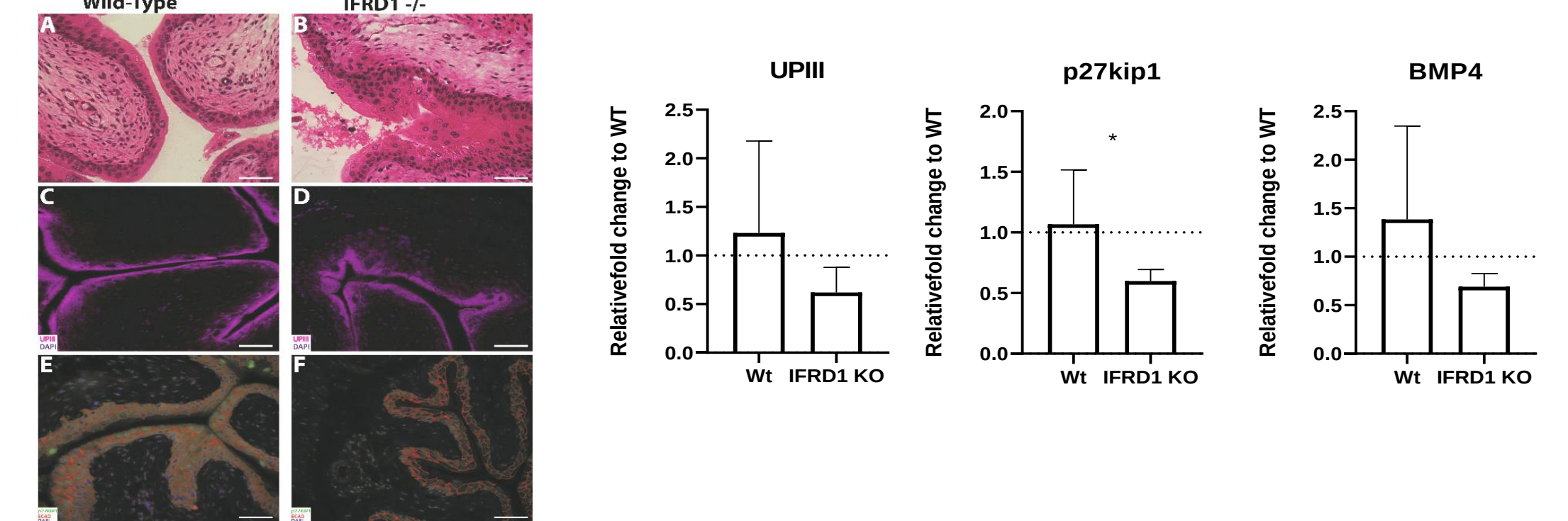


Fig. 9 Uninjured urothelium from male wild-type (A, C, E) and male IFRD1^{-/-} (B, D, E) mice. IFRD1^{-/-} shows enlarged superficial cells with an increase in vesicles (B). These mice also have a decrease in expression of superficial cell marker Uroplakin III (UPIII) (D), and marker of terminal differentiation p27KIP1 (F). Quantification of UPIII, p27KIP1, and Bmp4 (upstream regulator of p27kip1) expression in the half bladders using qRT-PCR (G). Images taken at 40x, scale bar = 50 μ m

Loss of IFRD1 is associated with the disruption of the urothelial stem cell niche and increased urothelial cell apoptosis and oxidative stress

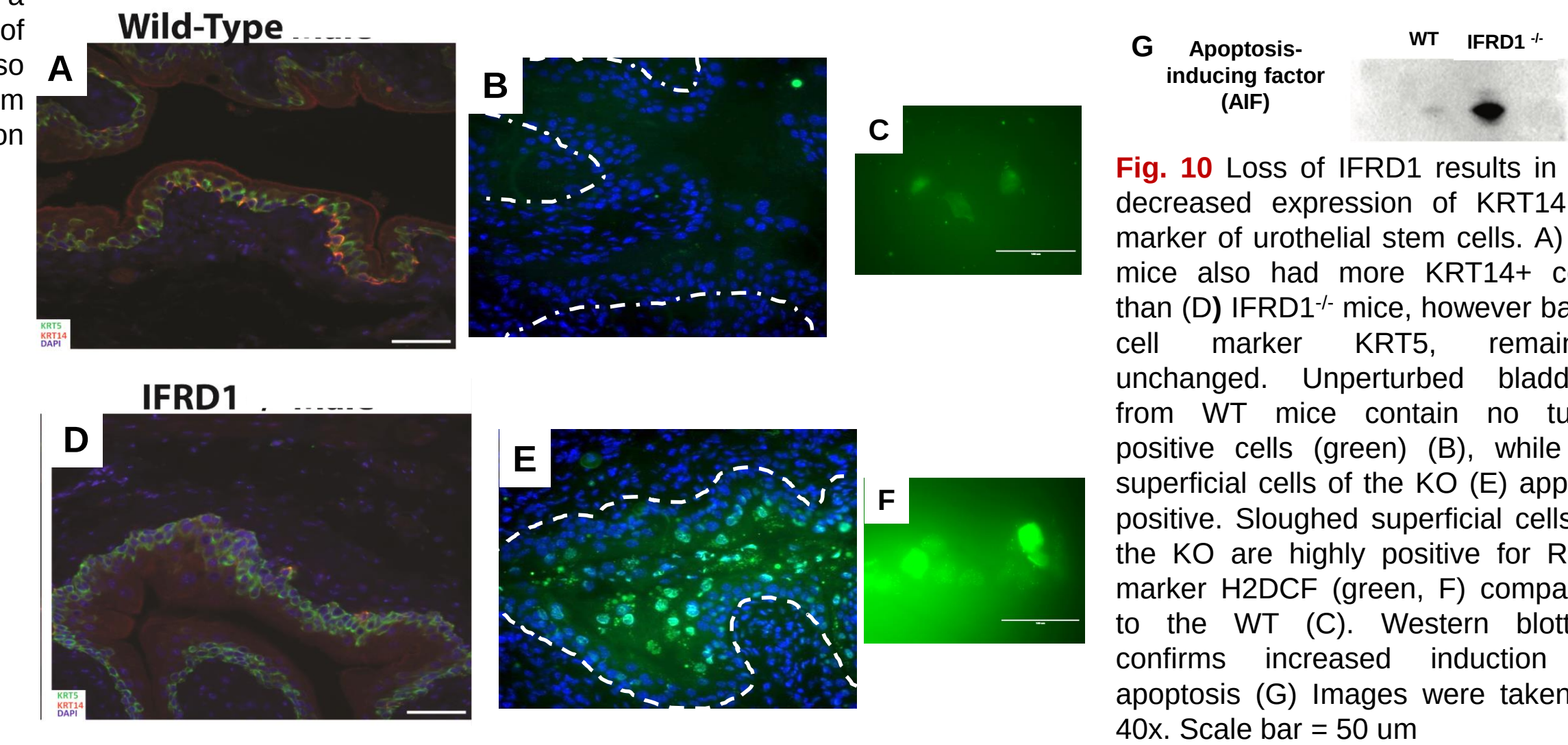


Fig. 10 Loss of IFRD1 results in the decreased expression of KRT14, a marker of urothelial stem cells. A) Wt mice also had more KRT14+ cells than (D) IFRD1^{-/-} mice, however basal cell marker KRT5, remained unchanged. Unperturbed bladders from WT mice contain no tunnel positive cells (green) (B), while all superficial cells of the KO (E) appear positive. Sloughed superficial cells of the KO are highly positive for ROS marker H2DCF (green, F) compared to the WT (C). Western blotting confirms increased induction of apoptosis (G) Images were taken at 40x. Scale bar = 50 μ m

Role of interferon-related developmental regulator 1 (IFRD1) in tissue regeneration and gene regulation

- Stimulates muscle regeneration and differentiation (Vetor, 2002)
- Can act as both transcriptional co-repressor and activator (Vadivelu, 2004)
- Expressed as early as E12 in the developing bladder (Mendelsohn, unpublished)
- Promotes re-entry into the cell cycle following injury in the liver and gut Phase 3 of paligenosis (Willet et al, 2018; Miao et al, 2020)
- Through a large unbiased RNA-sequencing analysis, we found that IFRD1 is activated early upon early upon UPEC infection, reaching peak expression 6 hours post infection (Fig. 4)

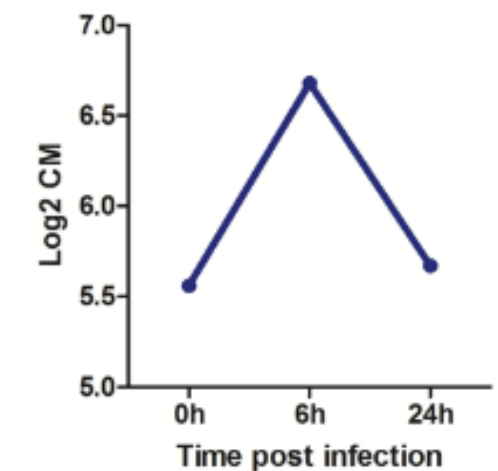


Fig. 4 Unbiased RNA-sequencing analysis of WT bladders following UPEC infection. Log10 Counts per Million

OBJECTIVE

TO BETTER UNDERSTAND THE MOLECULAR REGULATION OF UROTHelial REGENERATIVE RESPONSE

Hypothesis
INTERFERON-RELATED DEVELOPMENTAL REGULATOR 1 (IFRD1) PLAYS A KEY ROLE IN THE DECISION-MAKING MATRIX OF UROTHelial REGENERATION

Loss of IFRD1 is associated with increased urothelial exfoliation

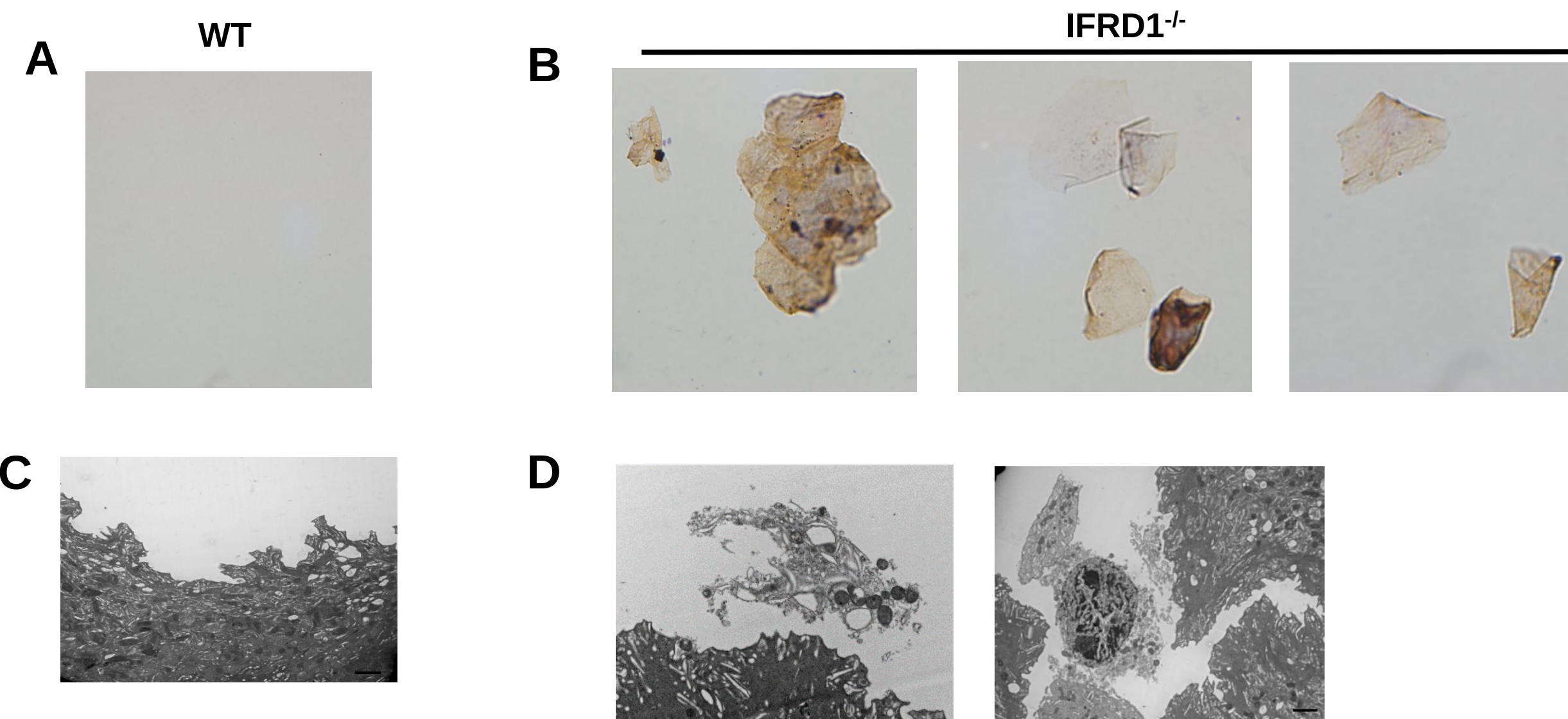


Fig. 5 Loss of IFRD1 results in increased sloughing of superficial cells. Wt mice have few sloughed superficial cells that are captured in both urine (A), and in the lumen of bladders shown via transmission electron microscopy(TEM) (C). However, KO mice have a significant amount sloughed superficial cells shown in urine (B) as well as TEM (D). Images were taken at 40x. Transmission electron microscopy images were taken at 5000x. Scale bar = 2 μ m

Proteomic analysis and western blotting, reveals IFRD1 is significantly associated with unfolded protein response pathway gene regulation

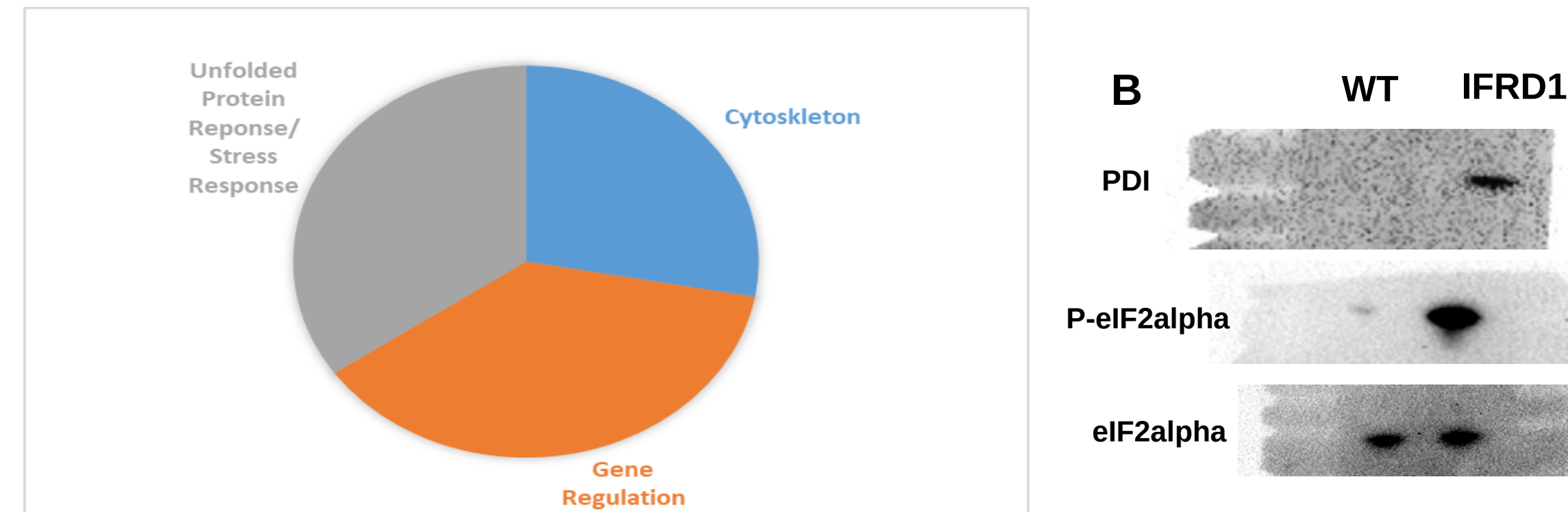


Fig. 7 (A) Co-immunoprecipitation followed by mass spectrometry reveals that the top 30 proteins found to bind to IFRD1, are associated with the unfolded protein response, gene regulation, and the cytoskeleton. The human cancer cell line 5637 cells were used for this analysis. Preliminary western blot analysis reveals the accumulation of protein disulfide-isomerase and phospho-eIF2alpha in IFRD1 KO, compared to the WT (B).

Loss of IFRD1 results in dysfunctional uroplakin endoplasmic reticulum to Golgi translocation

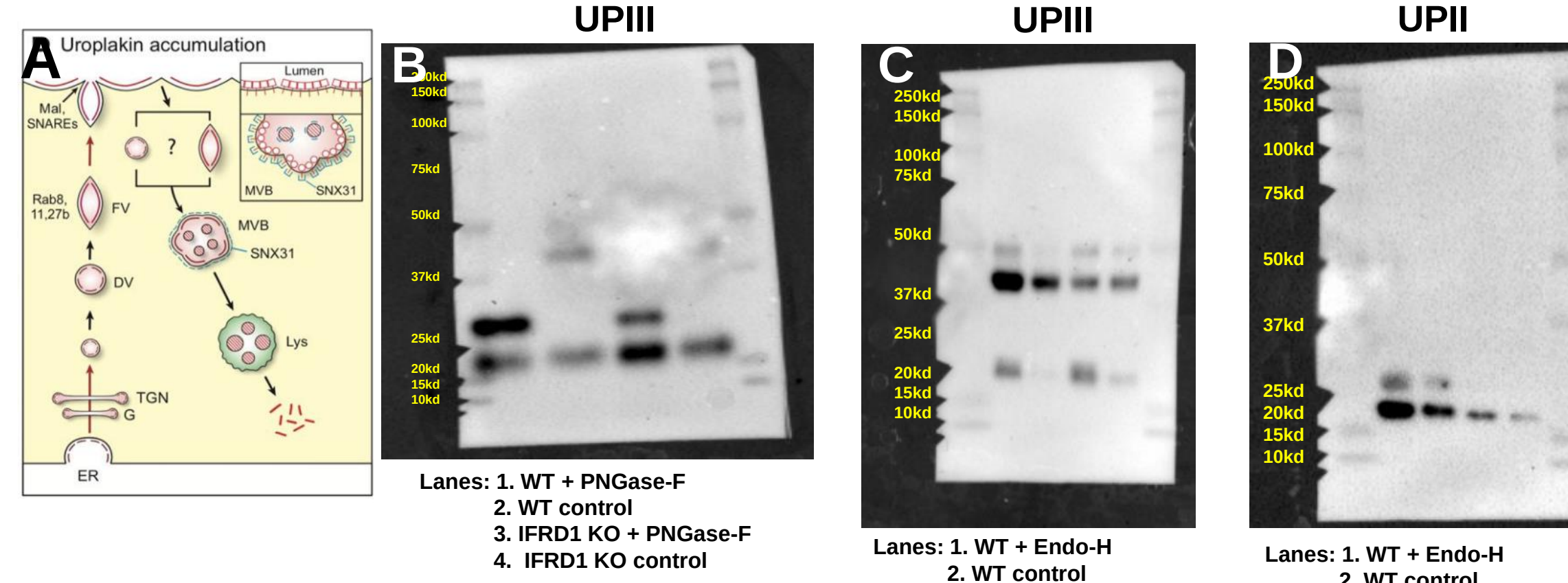
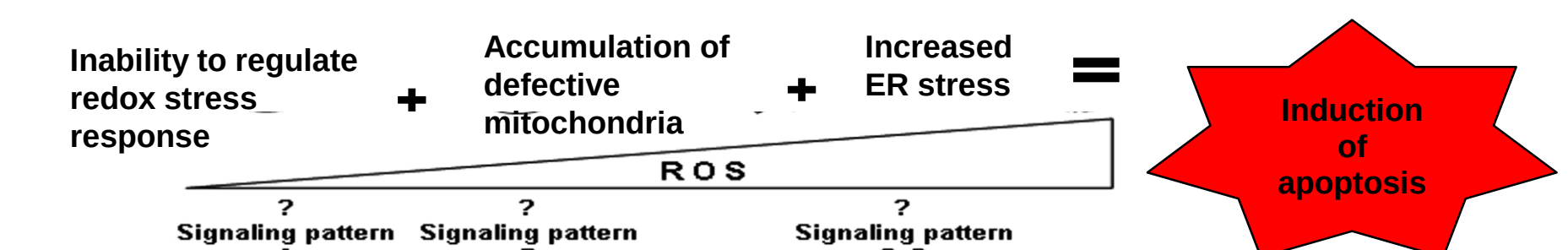


Fig. 8 Uroplakin are transmembrane proteins that are a part of the tetrapanin family. In the bladder uroplakin are trafficked from the ER, through the golgi and trans-golgi network, and are eventually expressed upon the apical surface superficial cells (A; *Liao 2019, Mol Biol Cell*). Protein extracts from WT and IFRD1^{-/-} were treated with endoglycosidases, and then blotted for uroplakin III and uroplakin II, to track their locations along the transportation pathway. Following treatment with PNGase-F (B), we see the accumulation of the cleaved UPIII glycan (lane 3) in the KO, which suggest an increased amount of UPIII is remaining in the ER. After incubation with Endo-H (C), we see the crosslinkage of the mature band remains in the KO, which suggest UPII and UPIB have not dimerized and are stuck in the ER. UPII appears to be stuck in the ER following Endo-H cleavage (D) in the KO.

Conclusions

- IFRD1 plays a role in urothelial cell vesicle trafficking
- IFRD1 KOs have a defect in the degradation and/or recycling of cellular components in the urothelium
- IFRD1 may regulate long-term homeostasis of the urothelium by mediating integrated stress response in urothelial cells
- Loss of ifrd1 leads to urothelial cell decision to die versus regenerate



Next Steps...

Examine the molecular underpinnings of IFRD1 mediated unfolded protein response and how this mediates homeostatic and injury responses in the urothelium

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