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Supplemental Information

Polyploid Superficial Cells that Maintain the Urothelial Barrier Are Produced via Incomplete Cytokinesis and Endoreplication

Jia Wang, Ekatherina Batourina, Kerry Schneider, Spenser Souza, Theresa Swayne, Chang Liu, Christopher D. George, Tiffany Tate, Hanbin Dan, Gregory Wiessner, Yelena Zhuravlev, Julie C. Canman, Indira U. Mysorekar, and Cathy Lee Mendelsohn

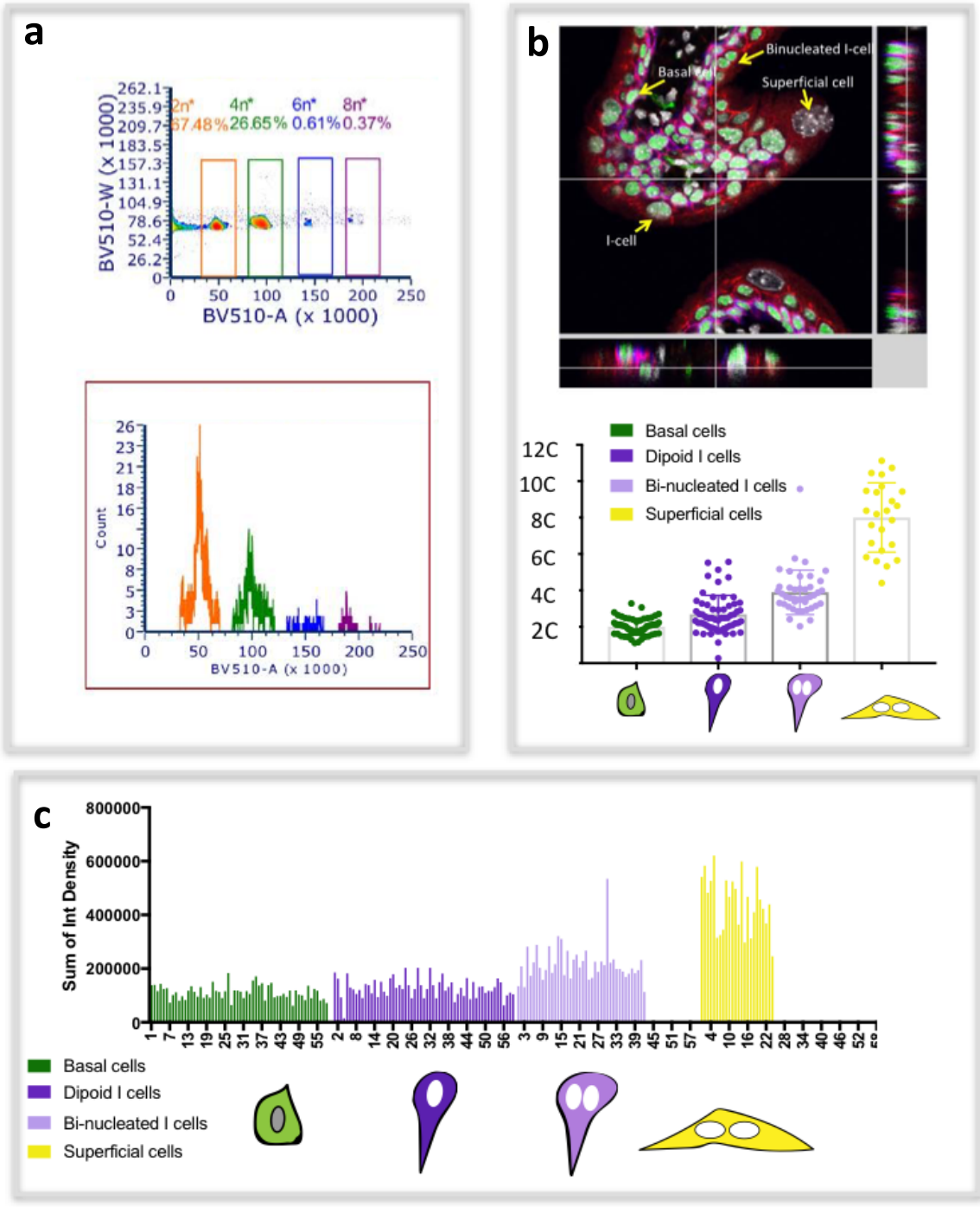

Figure S1. DNA content measurements, related to Figure 1.

(a) Relative DNA content obtained by flow cytometric analysis of propidium iodide-stained nuclei from the adult mouse urothelium. Basal cells which are 2*n*, served as internal reference standard. **(b)** A section of a urothelium from a UPEC infected mouse 25h post-infection stained for EdU and P63. EdU was administered 24h after infection and tissue was collected one hour later. The Dot Plot shows the estimated DNA content of urothelial sub-populations based on integrated fluorescence density of DAPI staining. For this experiment, we analyzed 59 basal cells, 58 mononucleated cells, 42 bi-nucleated I-cells and 24 S-cells. **(c)** The estimated DNA content of EdU+ cells was assessed by analysis of the Integrated Fluorescence density of DAPI stained sections The graph below shows the Sum of Estimated Density from analysis of EdU+ Basal cells, mononucleated I-cells, binucleated I-cells and S-cells at 25h and 72h post infection. For quantification, a minimum of three independent experiments were performed, and numbers are mean of percentages ± s.e.m.

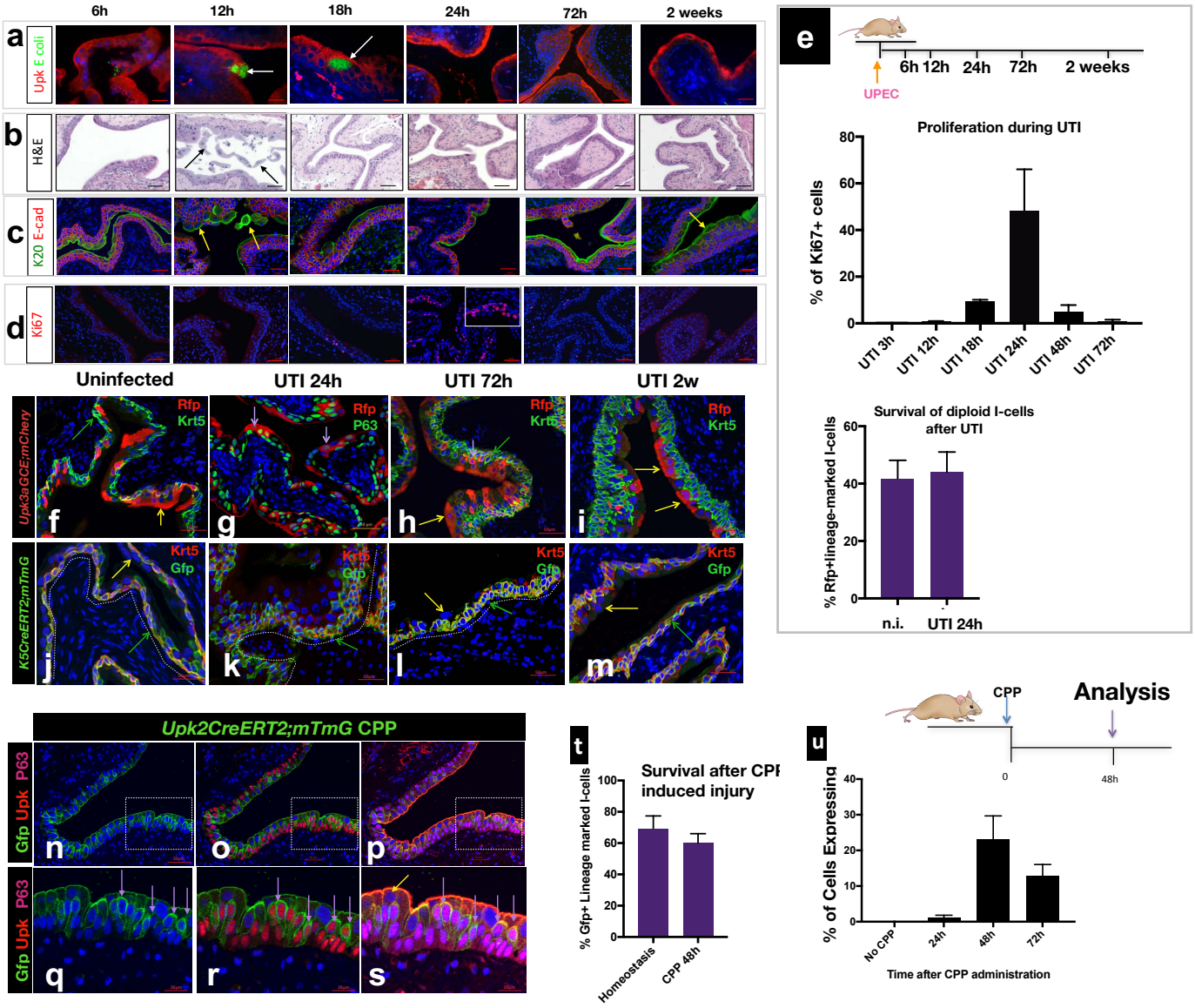

Figure S2. Models of injury and repair, related to Figure 2. (a) Animals were inoculated UPEC which invade S-cells initially produce intracellular bacterial communities (E.coli is stained green). **(b)** Invasion is followed by exfoliation (arrows). **(c)** The S-cell layer is replaced 72h after infection and the urothelial barrier is restored after 2 weeks. **(d)** Ki67 expression after UTI. **(e)** Graphs showing Ki67 expression at different times after UTI (left) and quantification of diploid I-cells in uninfected urothelium and in the urothelium 24h post-infection (right). **(f-i)** Lineage tracing I-cells in a UTI model of injury and repair. **(f)** A paraffin section from a Tm-induced adult *Upk3aGCE;mCherry* mouse that is uninfected, stained with P63 and Rfp. **(g)** A paraffin section from a *Upk3aGCE;mCherry* mouse 24h after infection, stained with P63 and Rfp. **(h)** A paraffin *Upk3aGCE;mCherry* mouse 72h p.i. stained for Krt5 and Rfp expression. **(i)** A paraffin section from a *Upk3aGCE;mCherry* mouse 2 weeks after infection, stained with Krt5 and Rfp. **(j)** A paraffin section from a *Krt5CreERT2;mTmG* mouse that is uninfected, stained with Krt5 and Gfp. **(k)** A paraffin section from a Tm-induced adult *Krt5CreERT2;mTmG* mouse 24h p.i., stained with Krt5 and Gfp. **(l)** A paraffin section from a Tm-induced adult *Krt5CreERT2;mTmG* mouse 72h p.i., stained with Krt5 and Gfp. **(m)** A paraffin section from *Krt5CreERT2;mTmG* mouse 2 weeks after infection, stained with Krt5 and Gfp. **(n-s)** Lineage tracing using the Upk2CreERT2;mTmG line in the CPP-induced model injury and regeneration. **(n)** Section from a *Upk2CreERT2;mTmG* mouse 48h after CPP stained for expression of Upk and Gfp. **(o)** Section from a *Upk2CreERT2;mTmG* mouse 48h after CPP stained for expression of Gfp and P63. **(p)** A section from a *Upk2CreERT2;mTmG* mouse 48h after CPP stained for expression of Gfp, Upk and P63. **(q)** Same sample as in **(n)** at a higher magnification. **(r)** Same sample as in **(o)** at a higher magnification. **(s)** Same section as in **(p)** at a higher magnification. **(t)** Bar graph showing the numbers of surviving I-cells after CPP. **(u)** Graph showing kinetics of proliferation after CPP. Scale bars: **(a,c)**: 25μm; **(b,d)** 50μm; **(f-m)**: 50μm; Scale bars **(n-p)** 50μm; **(q-s)** 25μm.

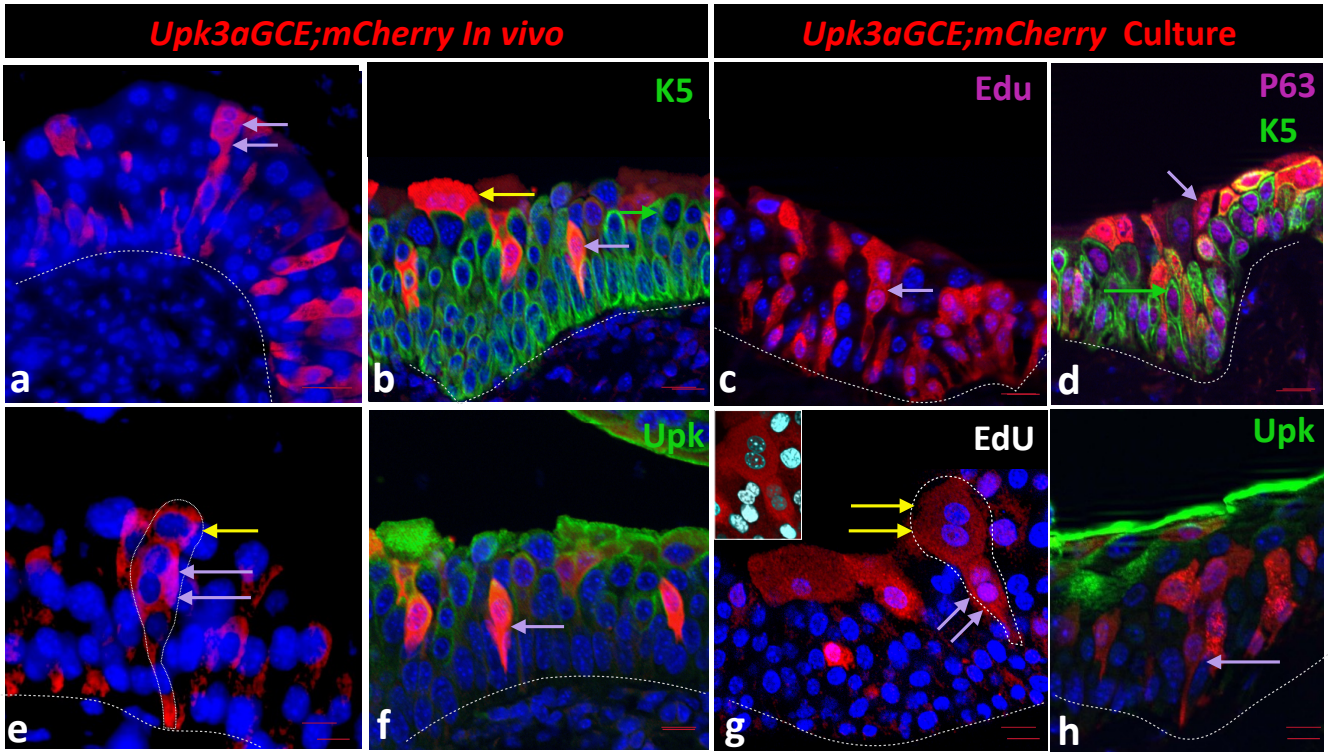

Figure S3. Comparison of in vivo regeneration and organotypic culture, related to Fig. 3. (**a)** Section from a *Upk3aGCE;mCherry* mouse 24h after UTI, stained for expression *mCherry.* Scale bar: 20mm. **(b)** A section from a *Upk3aGCE;mCherry* mouse 24h after UTI stained for expression of K5 and *mCherry*. **(c)** A section from an organotypic culture of urothelium and stroma isolated from a Tamoxifen-induced *Upk3aGCE;mCherry* mouse 4 days after plating, stained for expression of EdU and *mCherry*. Scale Bar: 20mm. **(d)** A section from an organotypic culture of urothelium and stroma isolated from a Tamoxifen-induced *Upk3aGCE;mCherry* mouse 4 days after plating, stained for expression of *mCherry,* P63 and K5. Scale Bar: 25mm **(e)** A Section from a *Upk3aGCE;mCherry* mouse bladder 24h after UTI, stained for *mCherry* expression. Scale bar: 20mm. **(f)** A Section from a *Upk3aGCE;mCherry* mouse bladder 24h after UTI, stained for *mCherry* and Upk expression. Scale bar: 20mm. **(g)** A section from an organotypic culture of urothelium and stroma isolated from a Tamoxifen-induced *Upk3aGCE;mCherry* mouse 4 days after plating, stained for expression of *mCherry* and P63. The inset shows EdU staining (white). Scale Bar: 20mm. **(h)** A section from an organotypic culture of urothelium and stroma isolated from a Tamoxifen-induced *Upk3aGCE;mCherry* mouse 4 days after plating, stained for expression of mCherry and Upk. Scale Bar:50 mm.