Expanded View Figures

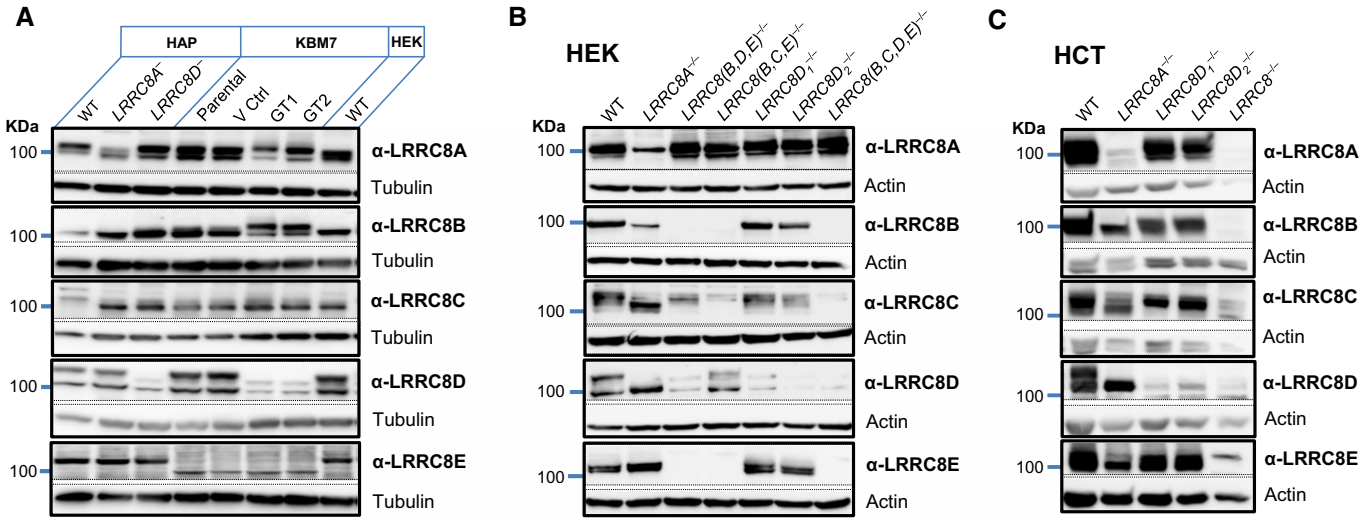


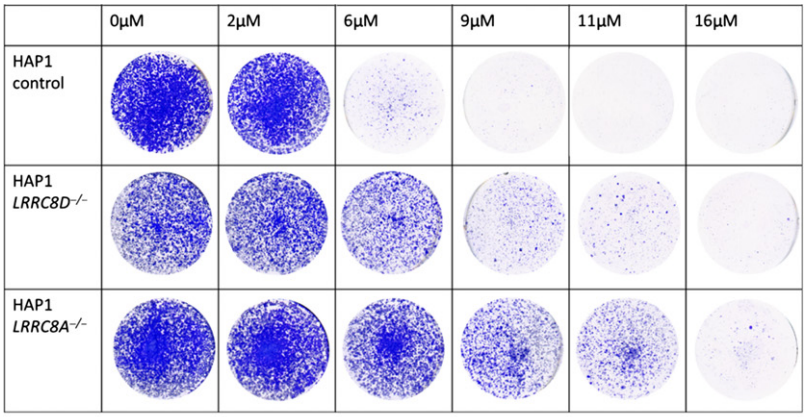
Figure EV1. LRRC8 subunit expression in different cell lines.

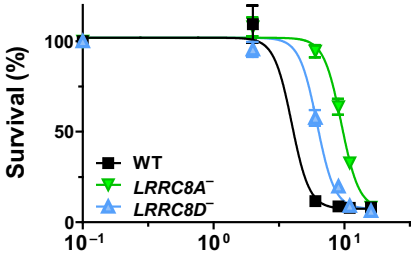
A–C Western blot showing the expression of all LRRC8 subunits in HAP1 and KBM7 (A), HEK (B), and HCT116 (C) cell lines, including knockout cell lines. Tubulin or actin was used as loading control. Note that KBM7 cells virtually lack LRRC8E, explaining the lack of inactivation of their $I_{Cl,vol}$ at clamped voltages (Fig 3B). Notice that disruption of LRRC8A changes the apparent sizes of the other LRRC8 subunits (prominently seen for LRRC8D in HCT cells) because LRRC8B through E need LRRC8A to leave the ER (Voss *et al*, 2014) and are therefore not fully glycosylated in its absence. *LRRC8D1*$^{-/-}$ and *LRRC8D2*$^{-/-}$ denote two independent HEK and HCT116 knockout clones.

Figure EV2. Increased resistance of *LRRC8A*$^{-/-}$ and *LRRC8D*$^{-/-}$ HAP1 cells to carboplatin and cisplatin, but not to oxaliplatin.

A–C Clonogenic growth of *LRRC8A*$^{-}$ and *LRRC8D*$^{-}$ or WT HAP1 cells treated with carboplatin, cisplatin, or oxaliplatin. Cells were exposed to the indicated concentrations of carboplatin (A), cisplatin (B), or oxaliplatin (C) for 7 days. Surviving colonies were formalin-fixed and stained with crystal violet. The optical absorption was determined at 590 nm after extracting the dye with 10% acetic acid. Data are presented as mean ± SEM (n = 6). CI, confidence interval.

A Carboplatin

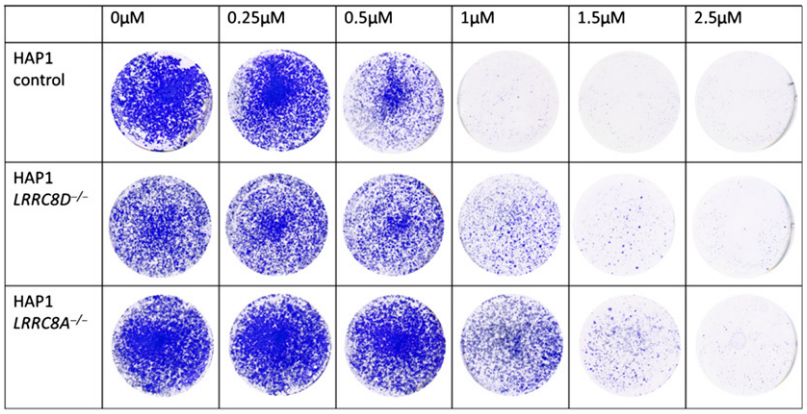


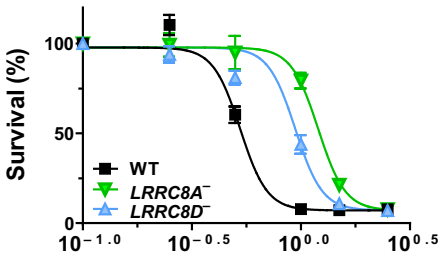


Carboplatin (μM)

	WT	LRRC8A−/−	LRRC8D−/−
IC50 (μM)	4.6	9.1	6.3
CI (95%)	4.1 – 5.1	8.9 – 9.3	6.2 – 6.5

B Cisplatin

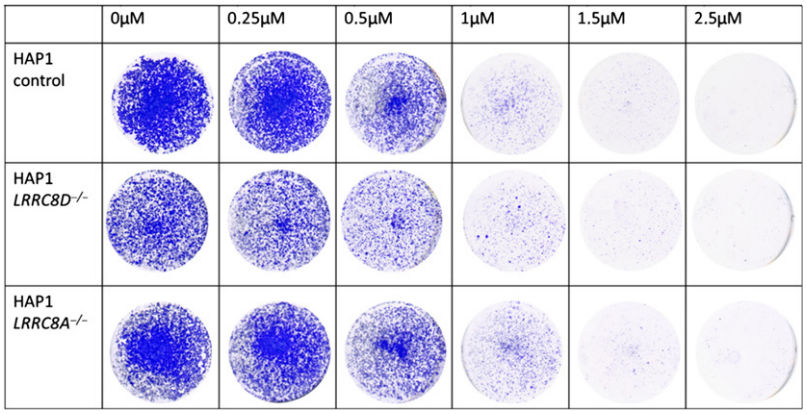


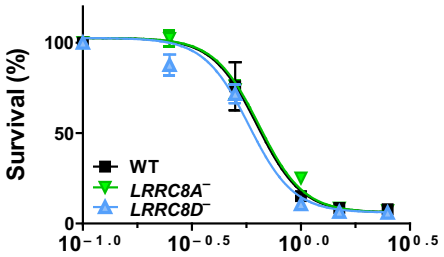


Cisplatin (μM)

	WT	LRRC8A−/−	LRRC8D−/−
IC50 (μM)	0.28	1.20	0.74
CI (95%)	–0.03 – 0.59	1.04 – 1.35	0.63 – 0.85

C Oxaliplatin





Oxaliplatin (μM)

	WT	LRRC8A−/−	LRRC8D−/−
IC50 (μM)	0.45	0.50	0.36
CI (95%)	0.16 – 0.75	0.34 – 0.66	0.16 – 0.57

Figure EV2.

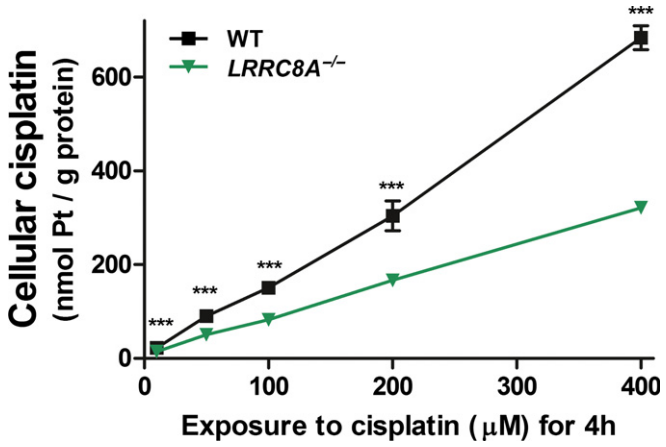


Figure EV3. Pt uptake after 4 h exposure to different concentrations of cisplatin in WT and *LRRC8A*$^{-/-}$ HEK cells.
Cells were exposed to the indicated drug concentrations in isotonic cell culture medium, and accumulated cisplatin was determined by Pt measurements. Data are presented as mean ± SEM (n = 4). ***P < 0.001.

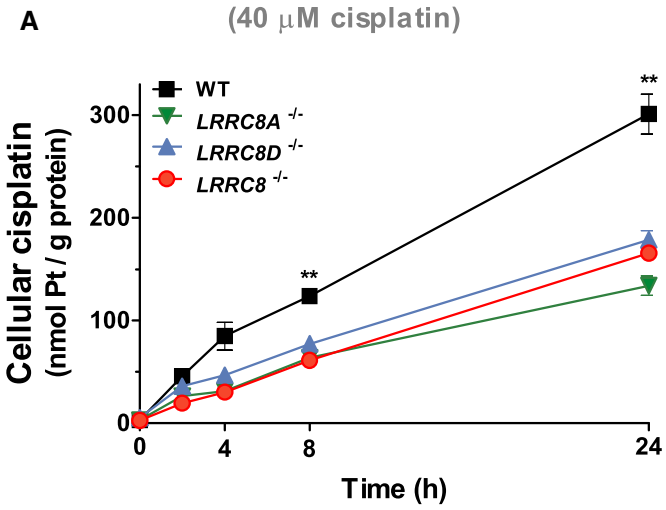
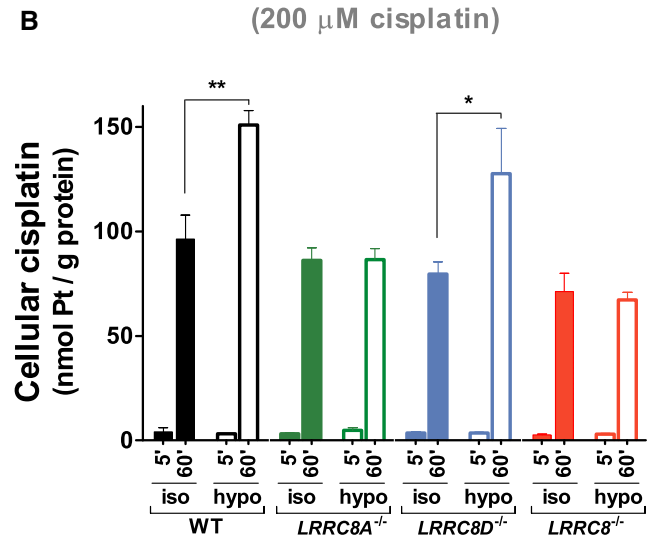


Figure EV4. LRRC8 subunit- and osmolarity-dependent cisplatin uptake in HCT116 cells.
A Long-term cisplatin uptake into WT, *LRRC8A*$^{-/-}$, *LRRC8D*$^{-/-}$, and *LRRC8*$^{-/-}$ HCT116 cells from isotonic culture medium containing 40 μM cisplatin.
B Comparison between short-term uptake from isotonic and hypotonic saline containing 200 μM cisplatin into WT, *LRRC8A*$^{-/-}$, *LRRC8D*$^{-/-}$, and *LRRC8*$^{-/-}$ HCT116 cells.

Data information: Results from two different *LRRC8A*$^{-/-}$ and *LRRC8D*$^{-/-}$ clones each are shown averaged as in Fig 4. Data are presented as mean ± SEM (n = 3). *P < 0.05; and **P < 0.01 compared to *LRRC8A*$^{-/-}$ cells.



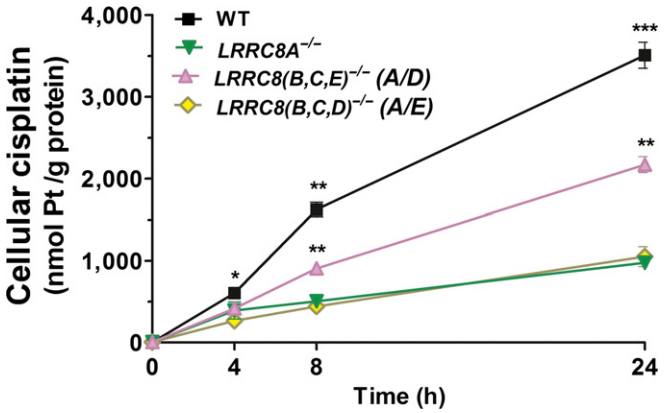


Figure EV5. LRRC8 subunit-dependent uptake of cisplatin into HEK cells of various genotypes.

Cisplatin uptake under isotonic conditions (200 μM cisplatin in culture medium) into HEK WT, *LRRC8A*$^{-/-}$, *LRRC8(B,C,E)*$^{-/-}$ (expressing only A and D subunits), and *LRRC8(B,C,D)*$^{-/-}$ (expressing only A and E) cells during the indicated times. Data are presented as mean ± SEM ($n = 3$). *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ compared to *LRRC8A*$^{-/-}$ cells.