Supplementary data

Modelling and prevention of acute kidney injury through ischemia and reperfusion in a combined human renal proximal tubule/blood vessel-on-a-chip

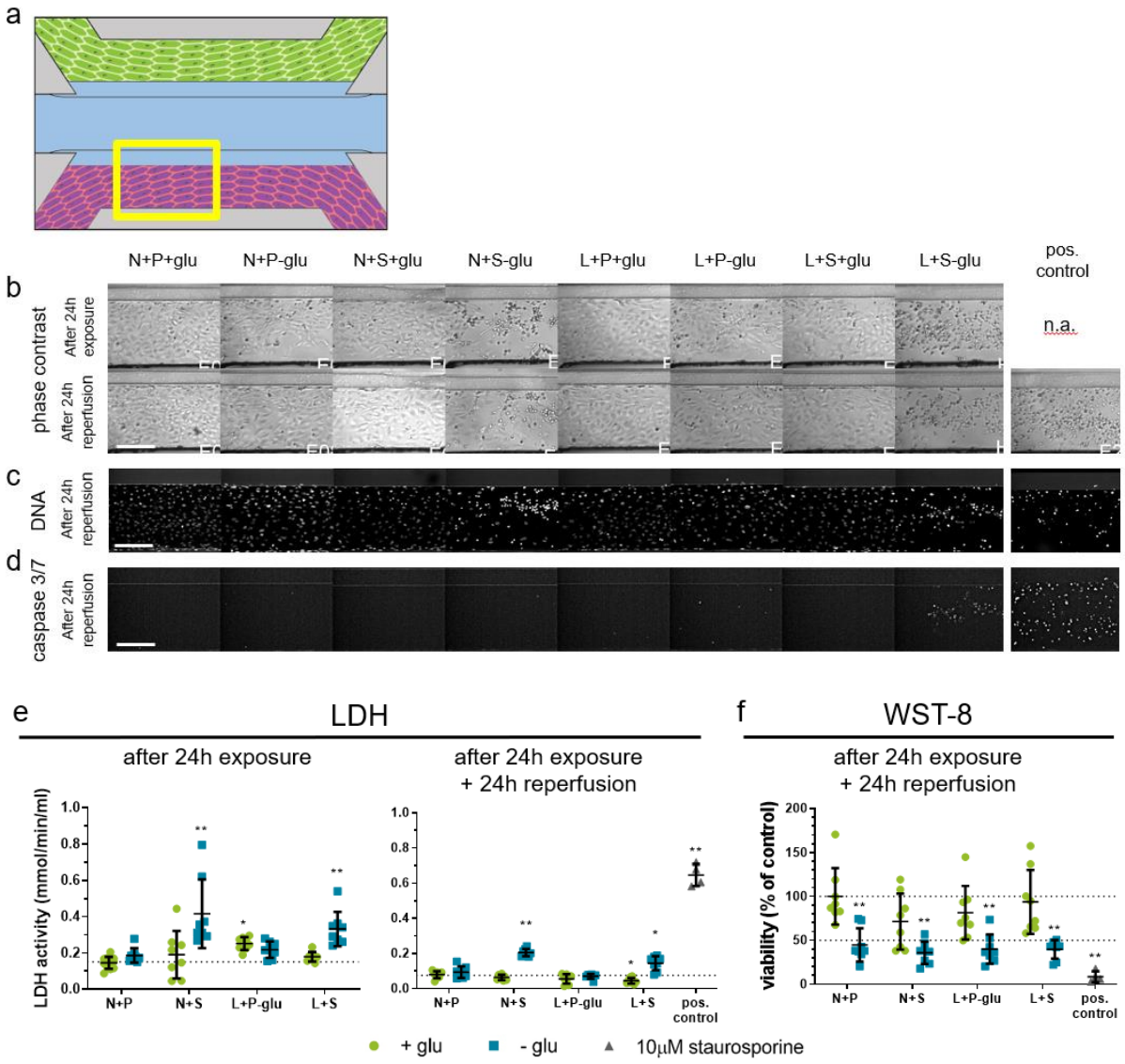


Figure S1: ***Modelling AKI upon ischemic parameter exposure showing results of HUVEC.*** *Ischemia was modelled on the OrganoPlate by exposing the coculture to a combination of low oxygen (L), static incubation (S), and glucose and nutrient poor medium (-glu) for 24-hours, followed by a 24h reperfusion in normoxia (N), perfusion on the rocker (P), and in glucose and nutrient rich medium (+glu).* ***a*** *Region of the HUVEC vessel (yellow square) that is used for the images shown in b-d.* ***b*** *Representative phase-contrast images after 24-hour exposure (top) and subsequent 24-hour reperfusion (bottom). Different ischemia inducing conditions were tested (columns) and compared to the normal condition N+P+glu. N.a.= not available.* ***c*** *DNA staining after 24h reperfusion.* ***d*** *Caspase 3-7 staining after 24h reperfusion. Scalebar = 200µm.* ***e*** *LDH release in the medium was measured after 24h exposure (left) and 24h exposure plus 24h reperfusion (right) respectively.* ***f*** *WST-8 viability relative to the normal condition N+P+glu was assessed after 24h reperfusion. 10µM staurosporine was included as a positive control. Error bars represent standard deviation. One-way ANOVA compares the conditions to the N+P+glu control condition, ** p<0.01 n=8-16 chips per condition.*

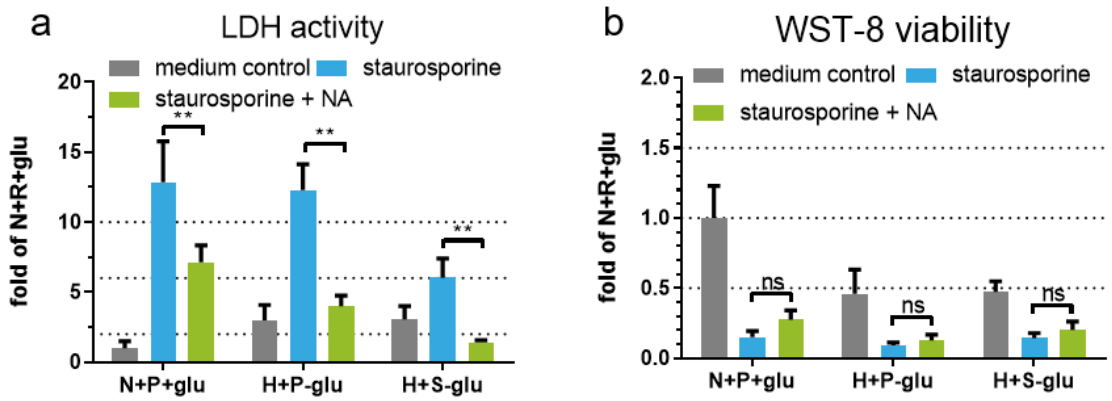
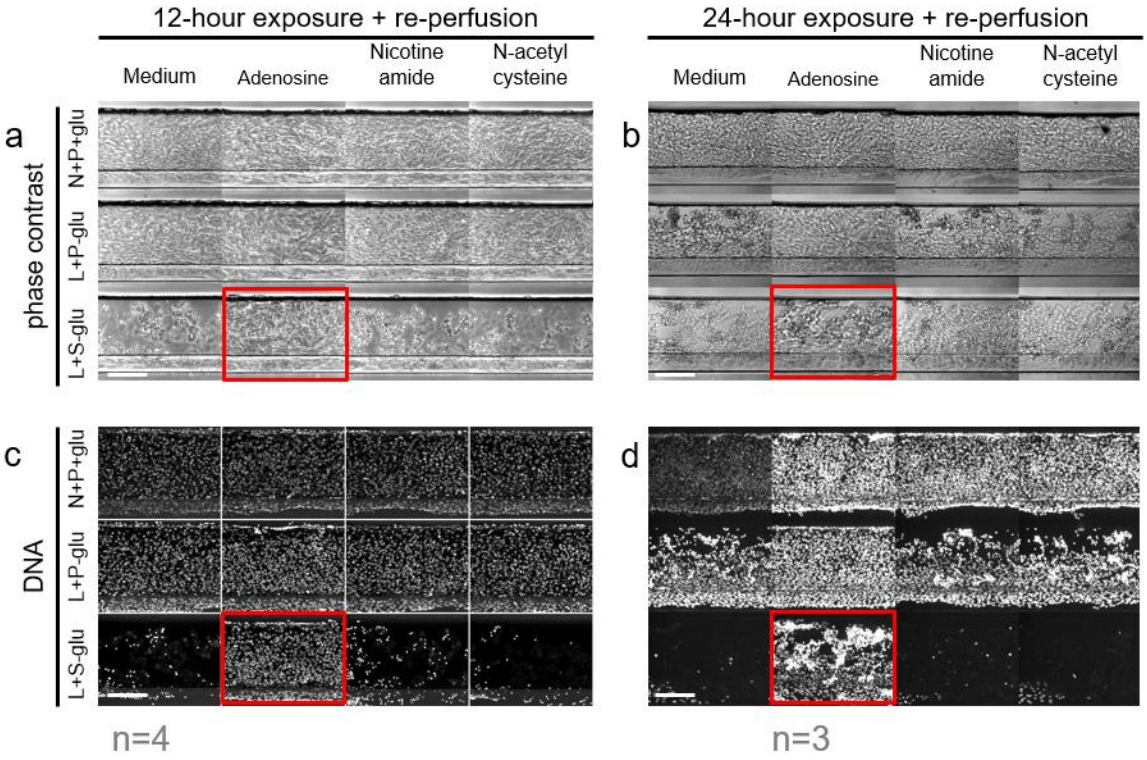


Figure S2: ***LDH activity and WST-viability measured on cocultures exposed to staurosporine with and without co-incubation of nicotinamide (NA).*** ***a*** *LDH activity was significant lower when cocultures exposed to staurosporine were co-incubated with NA.* ***b*** *WST-8 viability was not significant higher when cocultures exposed to staurosporine were co-incubated with NA, indicating no protective effect of NA. ** p<0.01. ns: not significant. Error bars represent the standard deviation. n=4-8 chips per condition.*



Representative images. Scalebar = 200 µm.
N=normoxia, H=5% O2, R=rocker (perfusion), S=static (no perfusion), glu=glucose.

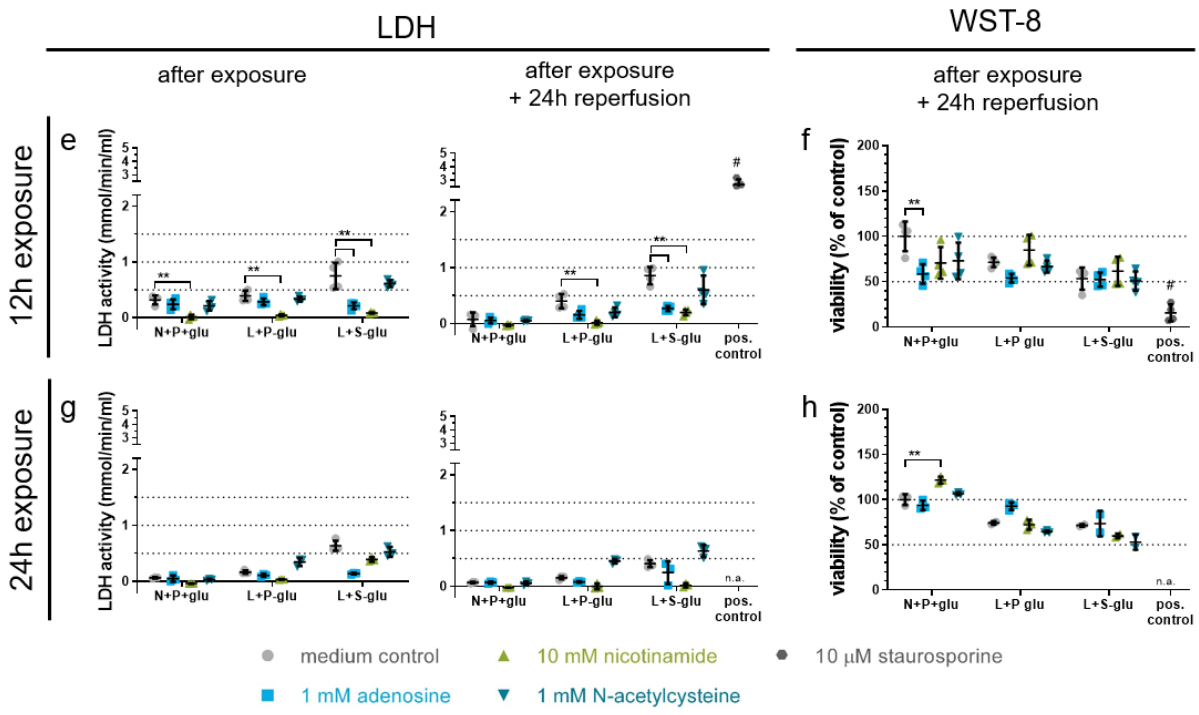


Figure S3: ***Repetition of experimental data presented in figure 5 of the main text. Cultures were exposed to the selected ischemic conditions L+P-glu and L+S-glu for either 12 or 24 hours, followed by a 24 hour reperfusion, in the presence of adenosine, nicotinamide or N-acetylcysteine. N+P+glu medium only is the normoxic control condition. a-d*** *A zoom of the RPTEC tubule (see Fig. 4b) was imaged after 12-hour exposure and reperfusion (**a, c**) or after 24-hour exposure and reperfusion (**b, d**). Red squares indicate a protective effect of adenosine compared to the medium control of the same ischemic condition in phase contrast imaging and DNA staining. Scalebar = 200µm.* ***e-h*** *After the ischemic exposure of either 12 hours (**e, f**) or 24 hours (**g, h**) and a reperfusion of 24 hours for both, medium from the RPTEC channel was sampled and analyzed for LDH activity (**e, g**) and WST-8 viability relative to the N+P+glu medium control (**f, h**) was determined. One-way ANOVA compares the co-incubations to the medium control of the same ischemic condition, * p < 0.05, ** p<0.01. # indicates the positive control differs significantly with all medium controls (p<0.01). Error bars represent the standard deviation. 10 µM staurosporine was included as a positive control. n=3-4 chips per condition.*