Supporting Information (Final version)

Overcoming Endocytosis Deficiency by Cubosome Nanocarriers

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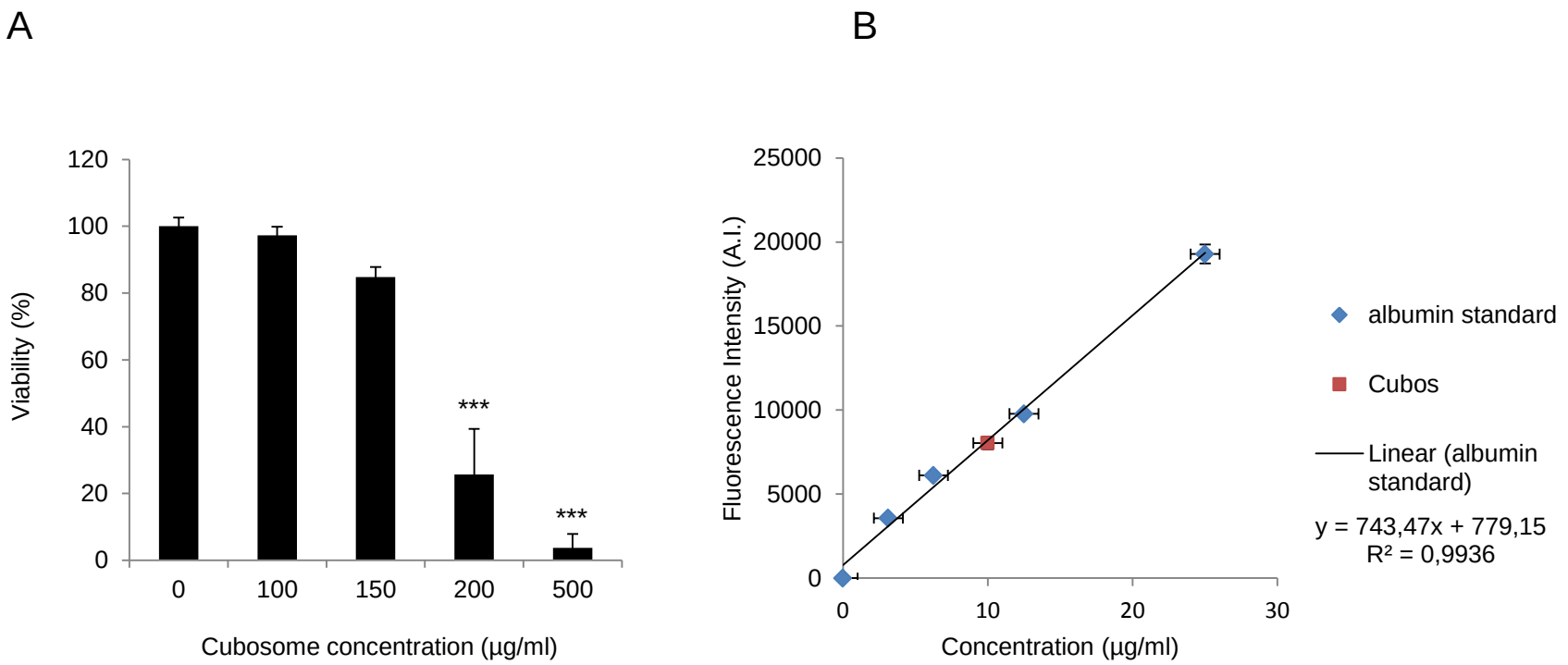
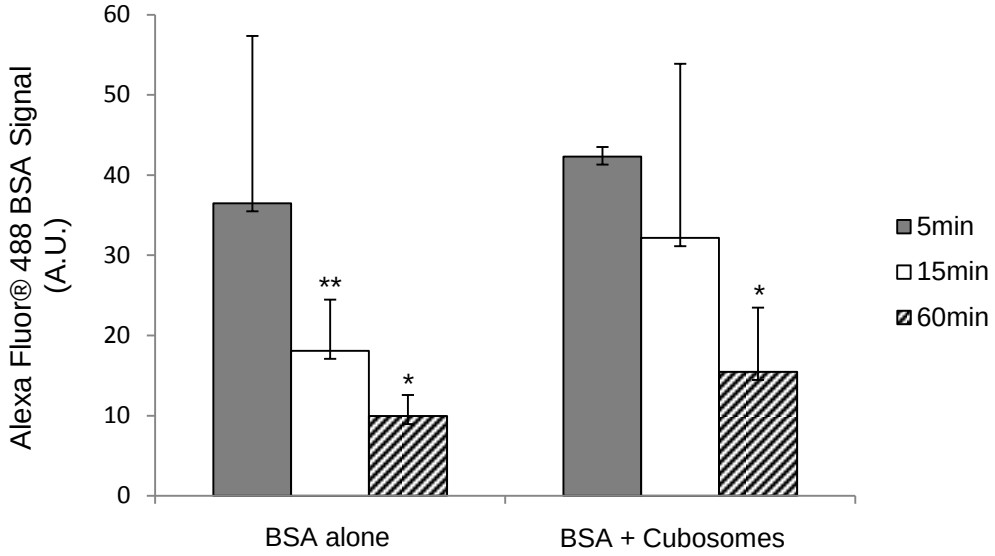
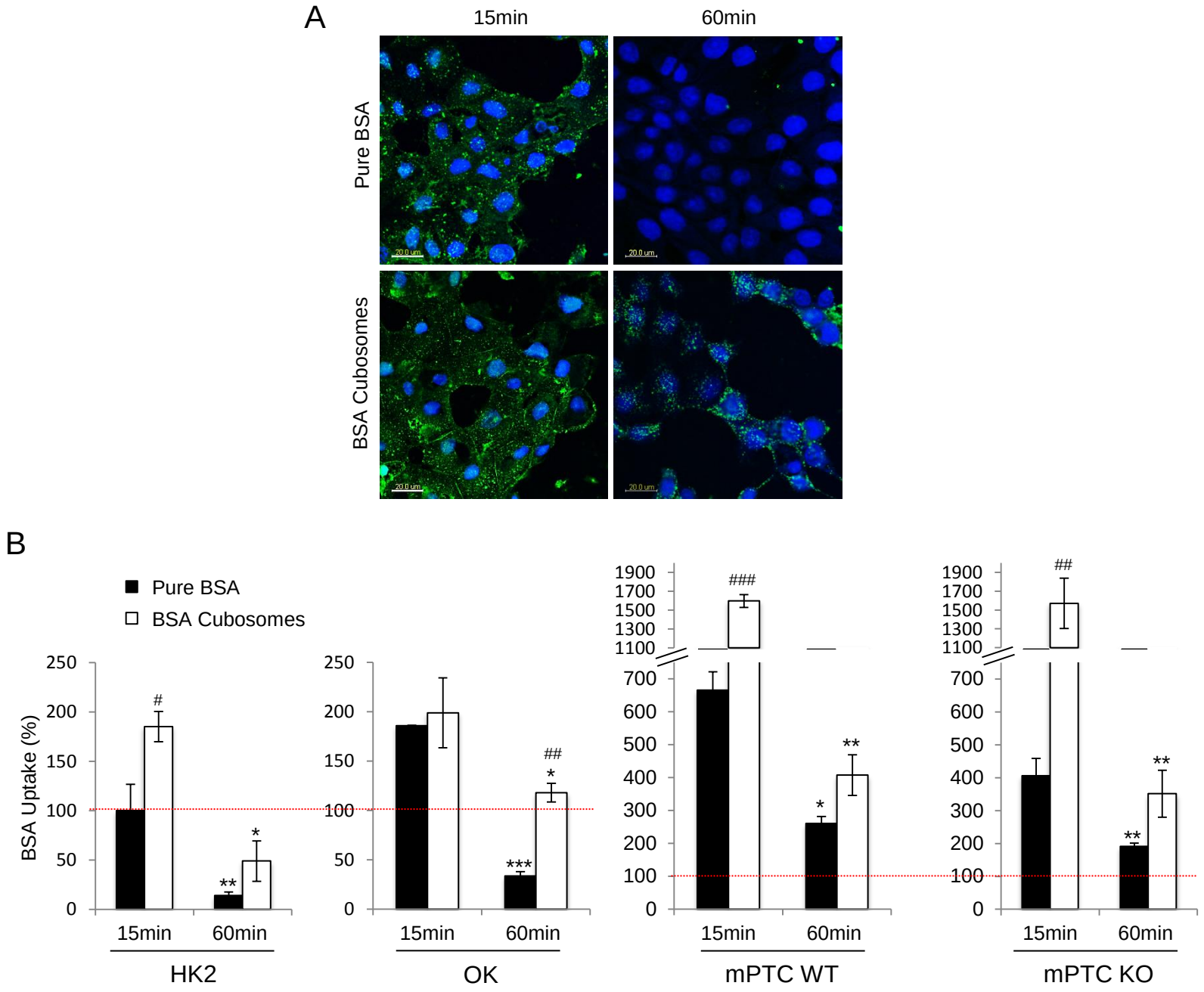
Supplementary Figures 1-4
Supplementary Video



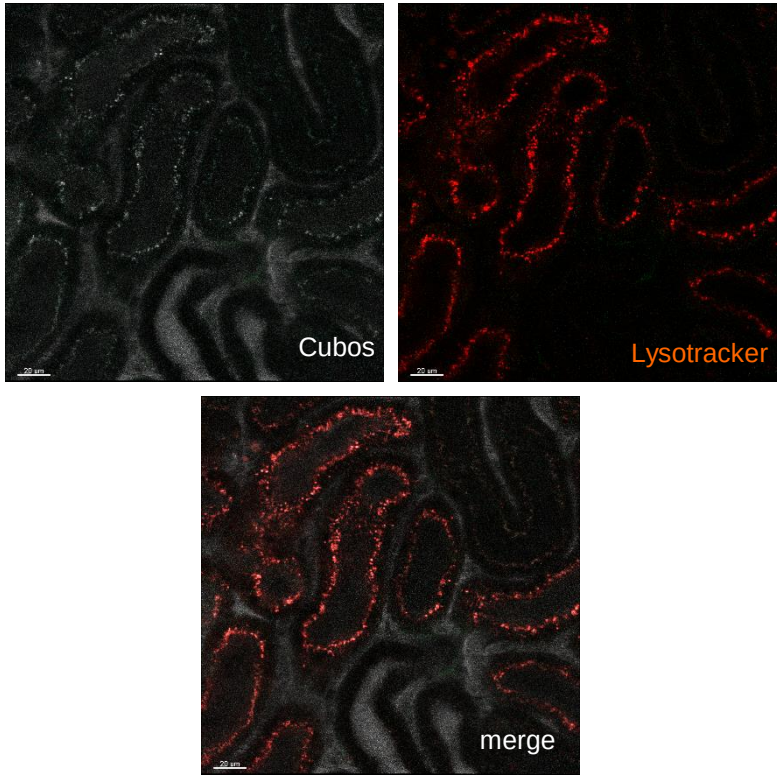
Suppl. Figure 1. Assessment of cubosome toxicity and optimal BSA concentration.
(A) HK2 cells were incubated with indicated concentrations of cubosomes for 1h and viability was tested using a standard MTT assay. The viability of HK2 cells was not significantly affected at cubosome concentrations of up to150µg/ml. (B) A solution of 150µg/ml of cubosome containing 100µg/ml Alexa Fluor® BSA. After dialysis, the effective concentration of Alexa Fluor® BSA of a 1:10 dilution of the cubosome solution was verified using a standard BCA protein quantification. *** $p<0.001$, compared to control incubation.



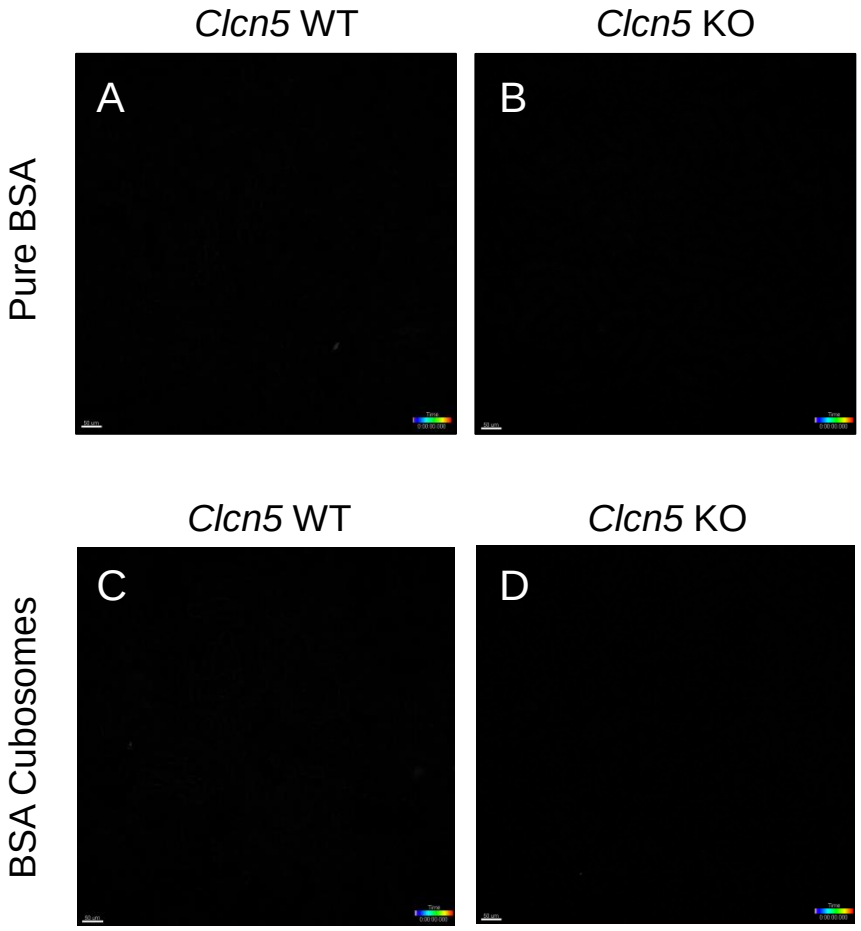
Suppl. Figure 2. Impact of empty cubosomes on endocytosis in HK2 cells.
HK2 cells were cultured on chamber slides, incubated with 100μg/ml Alexa Fluor® 488 BSA alone (BSA alone) or with 150μg/ml empty cubosomes (BSA+Cubosomes) and BSA degradation was monitored for the indicated time points. The signal of Alexa Fluor® BSA significantly decreased over time in both conditions, with no influence of the co-incubation with empty cubosomes. ** $p<0.01$, *$p<0.05$ compared to 5min.


A
15min
60min
Pure BSA
BSA Cubosomes
B
Pure BSA
BSA Cubosomes
BSA Uptake (%)
HK2
OK
mPTC WT
mPTC KO
15min
60min

Suppl. Figure 3. Albumin (BSA) uptake and degradation in cells with different endocytic activity.
(A) Opossum kidney (OK) cells were cultured on chamber slides, incubated with 100μg/ml Alexa Fluor® BSA, either pure or loaded in 150μg/ml cubosomes, and degradation was monitored for the indicated time points. The fluorescence signal in cells exposed to pure BSA is cleared after 60min whereas the signal in cells exposed to BSA loaded in cubosomes remains strong.
(B) Uptake (15 min) and processing (60 min) of BSA in various cells lines (HK2, human kidney; OK, opossum kidney; mPTC, primary cultures of proximal tubule cells obtained from *Clcn5* wild-type (WT) and knock-out (KO) mice) exposed to 100μg/ml Alexa Fluor® BSA, either pure or loaded in 150μg/ml cubosomes. Values are normalized to the uptake in HK2 after 15min time point (dotted red line, 100%). See text for details.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to 15min, # $p<0.05$, ## $p< 0.01$, ### $p< 0.001$ compared to pure BSA.



Suppl. Figure 4. *In vivo* uptake and co-localization of Alexa Fluor® BSA with lysotracker in mouse kidney.
250µl of 100µg/ml Alexa Fluor® BSA alone or 150µg/ml cubosome solution (containing 100µg/ml Alexa Fluor® BSA) was injected via a cannulated internal jugular vein into WT and KO *Clcn5* mice. The left kidney was externalized and mounted for better visualization. Co-injection with lysotracker shows co-localization of BSA-loaded cubosomes with lysosomes.



Video S5 *In vivo* uptake and processing of cubosomes in *Clcn5* mice.
250µl of 100µg/ml Alexa Fluor® BSA alone (upper panels) or 150µg/ml cubosome solution containing 100µg/ml Alexa Fluor® BSA (lower panels) were injected into WT (A, C) or KO (B, D) *Clcn5* mouse kidneys respectively. BSA delivered in cubosome solution was taken up faster and in higher amounts in both, WT and KO kidneys.