Supplemental Methods

Immunoblot

$8x10^6$ cells were harvested and re-suspended in 2-4 ml lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1 % SDS, 0.2 % Triton X-100, Protease Inhibitor). Then 100 µl of this cell suspension was mixed with 20 µl of 6xLaemmli buffer (375 mM Tris-HCl, 9 % SDS, 50 % Glycerol, 9 % β-mercaptoethanol, 0.03 % Bromophenol blue) and heated for 5 min at 95 °C. Then sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 10 % SDS separation gels. Afterwards the gel was blotted onto a membrane using a semi-dry blotting technique. This membrane was then blocked via incubation in blocking buffer (Odyssey® Blocking Buffer (TBS), Li-Cor) for 2 h before it was incubated with our primary anti-LCN2R antibody (Anti-Slc22A17 rabbit polyclonal, Merck #ABC846) diluted 1:800 in blocking buffer (Odyssey® Blocking Buffer (TBS), Li-Cor) and with addition of 1:500 diluted Tween 20 overnight at 4 °C. The next day, the membrane was washed two times with TBST for 5 min and once for 10 min and then incubated with the secondary anti-rabbit antibody (IRDye® 800CW Goat anti-Rabbit, Lot#D00825-14, Li-Cor) 1:5000 diluted in blocking buffer (Odyssey® Blocking Buffer (TBS), Li-Cor) and with addition of 1:500 diluted Tween 20 for 1-2 h at room temperature (RT). Next, two 5 min and one 15 min washing steps in TBST followed by a 3 min washing step in TBS were conducted. Then the membrane was dried for 15-30 min at 4 °C and finally analyzed using the Odyssey DLx imaging system.

Crystal violet staining

$1x10^4$ cells were seeded in a 96-well plate. After incubation for 24 h in a cell culture incubator the cells were infected with 1000 vpc and then incubated for another 24 h in a cell culture incubator. Subsequently to the CCK-8 assay, cells could still be used for the following procedure as well. The medium was aspirated from the wells, 200 µl of 2 % formalin were added to each well and incubated for 20 min at RT, to fix the cells and inactivate the virus. After formalin was aspirated and 50 µl of 0,5 % crystal violet staining solution were immediately added to each well, the plate was incubated for 20 min at RT on a bench rocker (frequency = 20 osc/min). Afterwards the plate was carefully washed using a water bath at least four times. Then the plates were air-dried until all wells were completely dry (usually around 24 h). Finally, 200 µl methanol were added to each well, the plates were incubated with their lid on for 20 min at RT on a bench rocker (frequency = 20 osc/min) and then the optical density of each well at 570 nm was measured with a TECAN infinite f plex plate reader.

Supplemental Figures

Figure S1, Schellhorn et al.

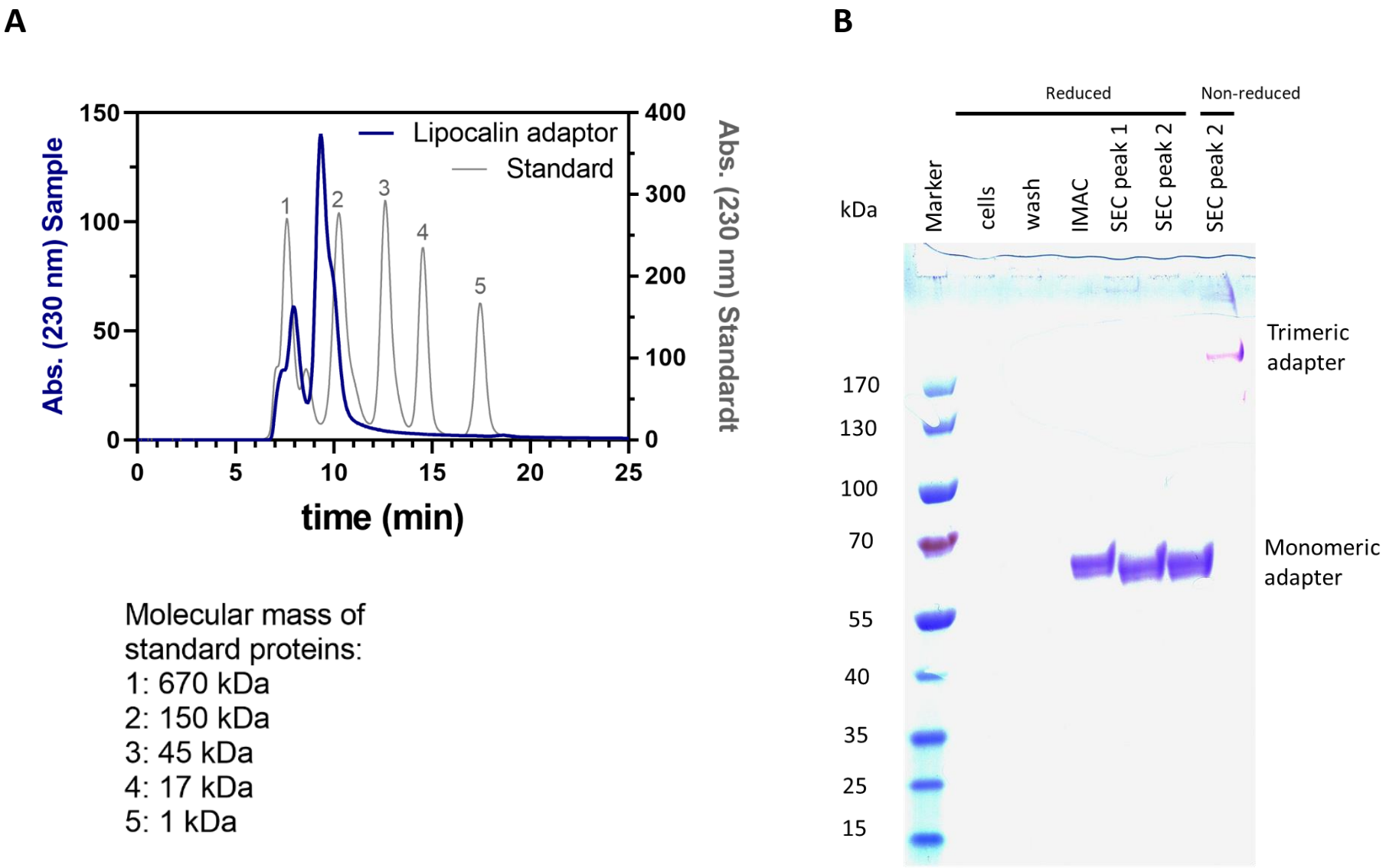

Figure S1: Analytical size exclusion chromatography (SEC) analysis and SDS gel electrophoresis of the produced Lipocalin adapter (LA) molecule. (A) The SEC shows that the majority of the lipocalin adapter molecules form stable trimers (right main peak more than 150 kDa) with some detectable aggregates (left peak close to 670 kDa). Aggregates form at high concentrations and can be resolved at lower concentrations which are usually used for retargeting. (B) The predicted molecular weight of the monomer is 49.4928 kDa resulting in 148.484 kDa for the trimer. The SDS gel shows a molecular weight of around 65 kDa for the produced monomer and around 200 kDa for the trimeric adapter.

Figure S2, Schellhorn et al.

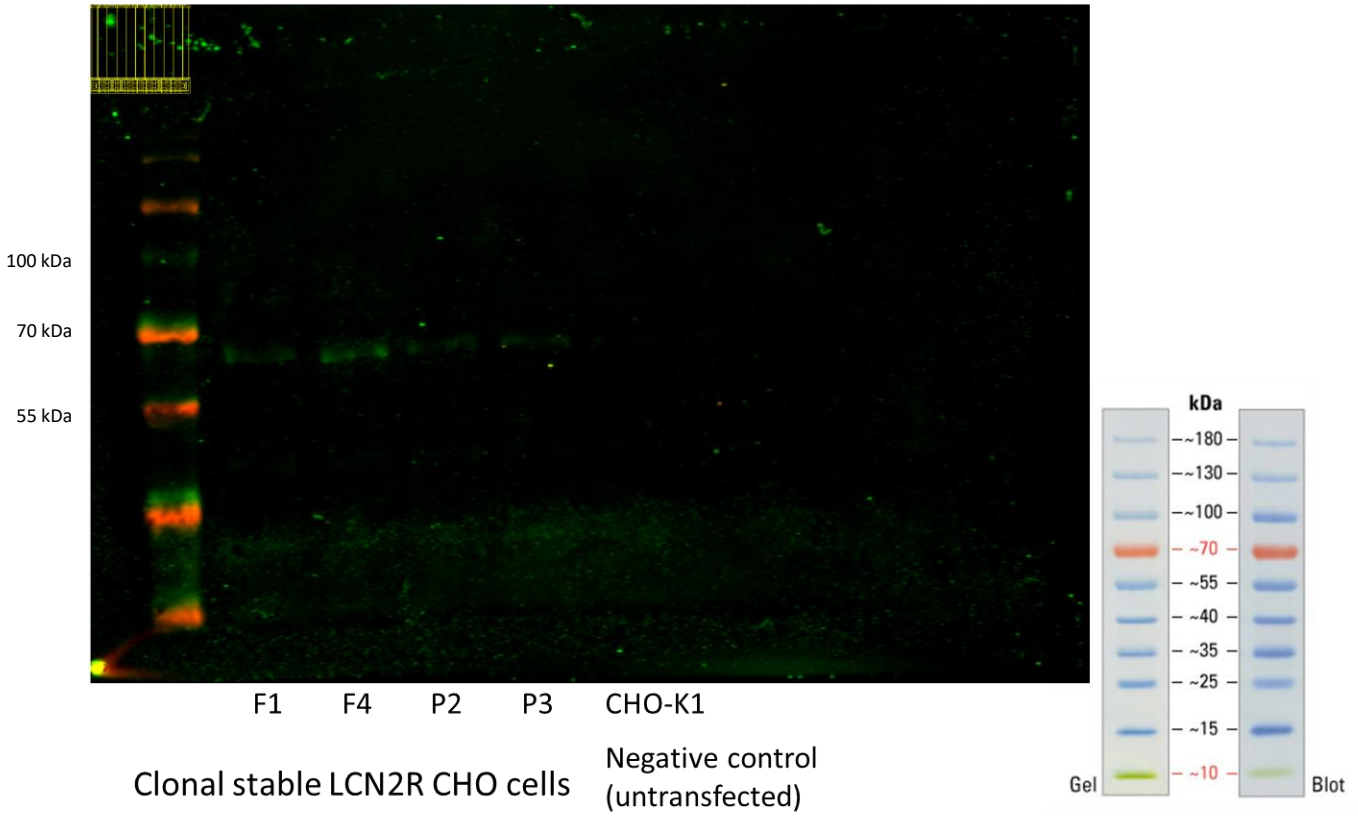

Figure S2: Immunoblot shows LCN2R expression in the CHO model cell lines. Four stable LCN2R expressing cell clones (F1, F4, P2 and P3) were tested, as well as the CHO K1 negative control cell line. The predicted size of the LCN2R is around 60 kDa.

Figure S3, Schellhorn et al.

```
// GFP Quantification of spheroid pictures after infection: “Flu-QoSI”
// Sebastian Schellhorn
// 11.03.2021

// GFP quantification from i pictures of the spheroids

//Save pictures in a folder and select the folder in the following:


/*
 * Macro template to process multiple images in a folder
 */

#@ String(value="Take pictures - save pictures in folder - select folder (input is enough)", visibility="MESSAGE") hint;
#@ File (label = "Input directory", style = "directory") input
#@ File (label = "Output directory", style = "directory") output
#@ String (label = "File suffix", value = ".tif") suffix


// Define global variables

var x = 0;

// Main command

processFolder(input);


// Define functions
// function to scan folders/subfolders/files to find files with correct suffix

function processFolder(input) {
	list = getFileList(input);
	list = Array.sort(list);
	x = list.length;
	//print(x);

	for (i = 0; i < list.length; i++) {
		if(File.isDirectory(input + File.separator + list[i]))
			processFolder(input + File.separator + list[i]);
		if(endsWith(list[i], suffix))
			processFile(input, output, list[i]);
			if(i == list.length-1) {

				print(x + " pictures were analyzed");

				selectWindow("Results");
				close("Results");
			}
	}
}
```

```
function processFile(input, output, file) {
	// Do the processing here by adding your own code.
	// my BatchProcessingMacro GFPquantification

	open(input + File.separator + file);
	name = getTitle();
	foldername = File.getNameWithoutExtension(input);

	run("Split Channels");
	selectWindow(name + " (blue)");
	close();
	selectWindow(name + " (red)");
	close();
	selectWindow(name + " (green)");

	//run("Set Scale...", "distance=72.000 known=1 pixel=1.000 unit=inch");

	run("Duplicate...", "title=[maskblueprint_" + name + " (green)]");
	selectWindow("maskblueprint_" + name + " (green)");

	//choose fitting background substraction for your pictures!
	//run("Auto Threshold", "method=Li white"); //only for high fluorescence possible
	//run("Auto Threshold", "method=MaxEntropy white"); //choose fitting thresholdingmethod carefully
	run("Auto Threshold", "method=Triangle white");


	run("Create Mask");

	run("Set Measurements...", "area mean standard integrated redirect=[" + name + " (green)] decimal=0");
	run("Measure");
	saveAs("Results", input + "/" + foldername + "_Results.csv");
	selectWindow(name + " (green)");
	close();
	selectWindow("maskblueprint_"+ name + " (green)");
	close();
	selectWindow("mask");
	close();

}
```

Figure S3: ImageJ macro Flu-QoSI for GFP-Quantification of spheroid infection.

Figure S4, Schellhorn et al.

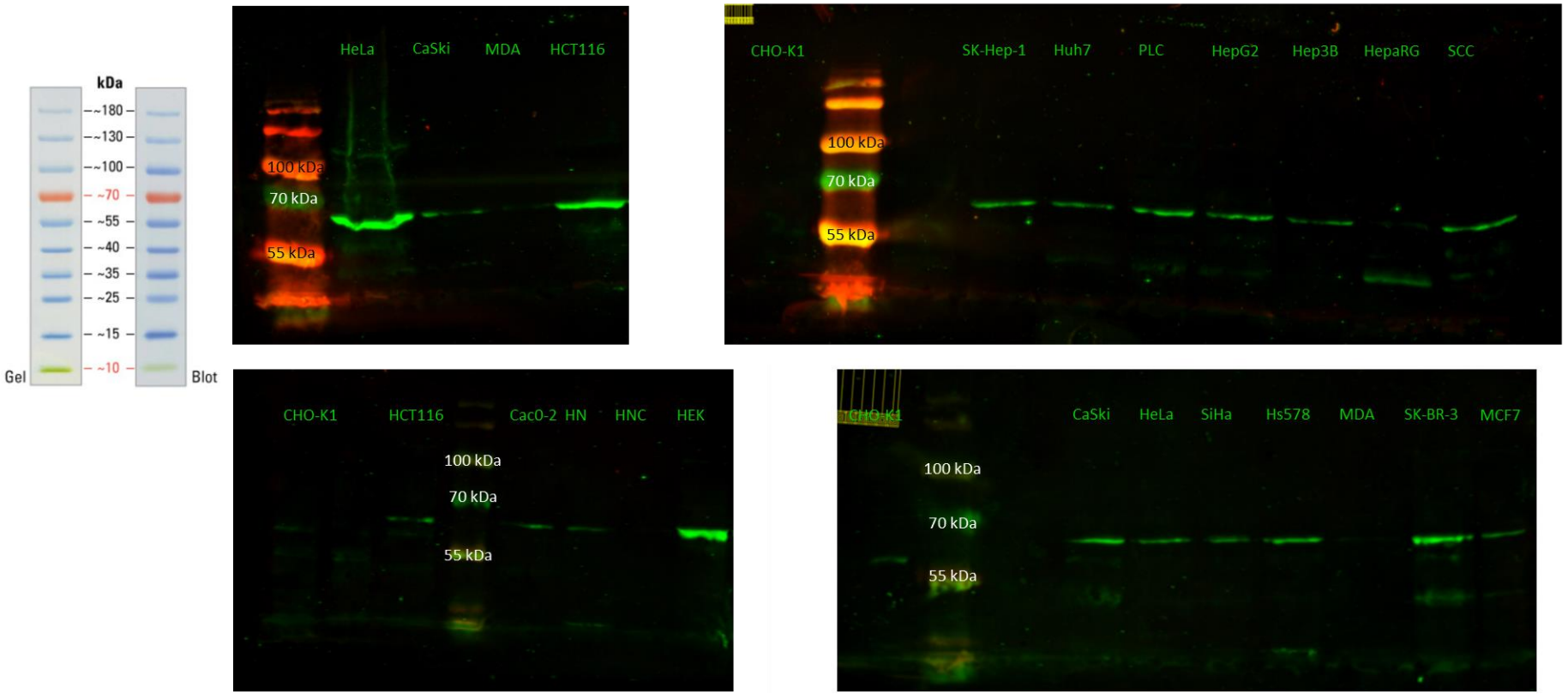

Figure S4: Immunoblots show LCN2R expression in cancer cell lines (CCLs). The predicted size of the LCN2R is around 60 kDa. A smaller isoform of around 30 kDa could be detected as well. Analyzed cancer cell lines include: HeLa, CaSki, MDA-MB-231, HCT-116, SK-Hep-1, Huh-7, PLC-PRF-5, HepG2, Hep3B, HepaRG, SCC, CaCo-2, HN, HNC, HEK-293, CaSki, HeLa, SiHa, Hs578T, SK-BR- and MCF-7.

Figure S5, Schellhorn et al.

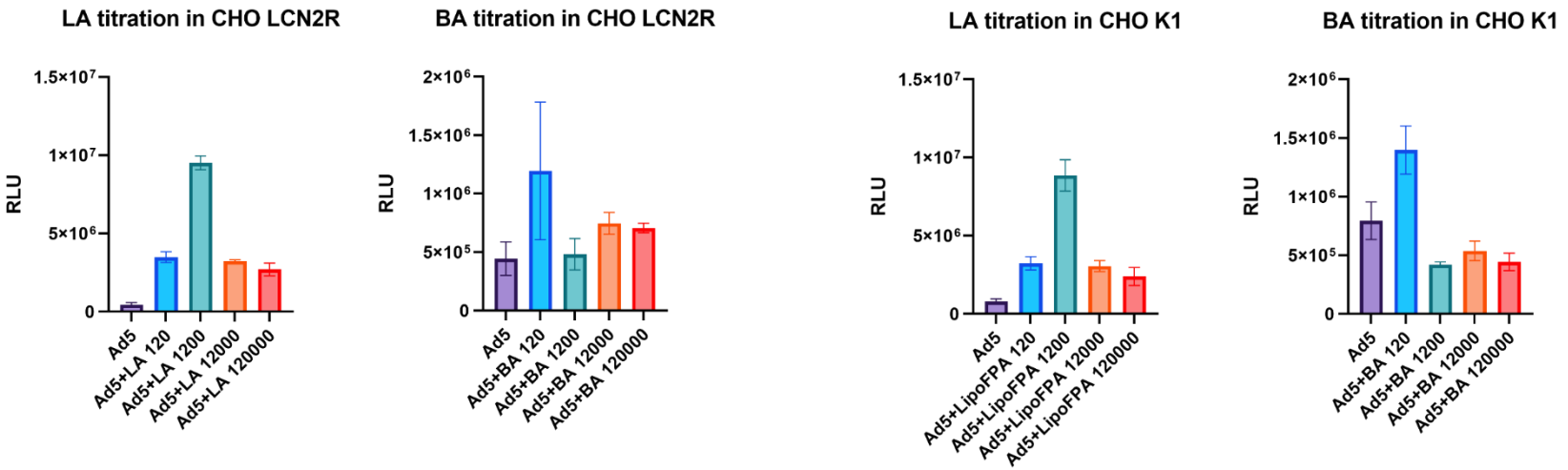

Figure S5: The ratio of virus to LCN2 adapter (LA) that shows the highest viral uptake increase is 1:1200. Luciferase assay performed on CHO K1 and CHO LCN2R cell lines. Cells were infected with different virus to adapter ratios and incubated beforehand for 1-2 h at RT (ratios: 120 = 1 viral particle (12 knobs) + 120 LCN2 adapter (LA) / blocking adapter (BA) molecules; 1 200 = 1 viral particle (12 knobs) + 1 200 LA/BA molecules; 12 000 = 1 viral particle (12 knobs) + 12 000 LA/BA molecules; 120 000 = 1 viral particle (12 knobs) + 120 000 LA/BA molecules). The mean value and standard deviation of three technical replicates are shown here. (N = 1; 3 technical replicates)

Figure S6, Schellhorn et al.

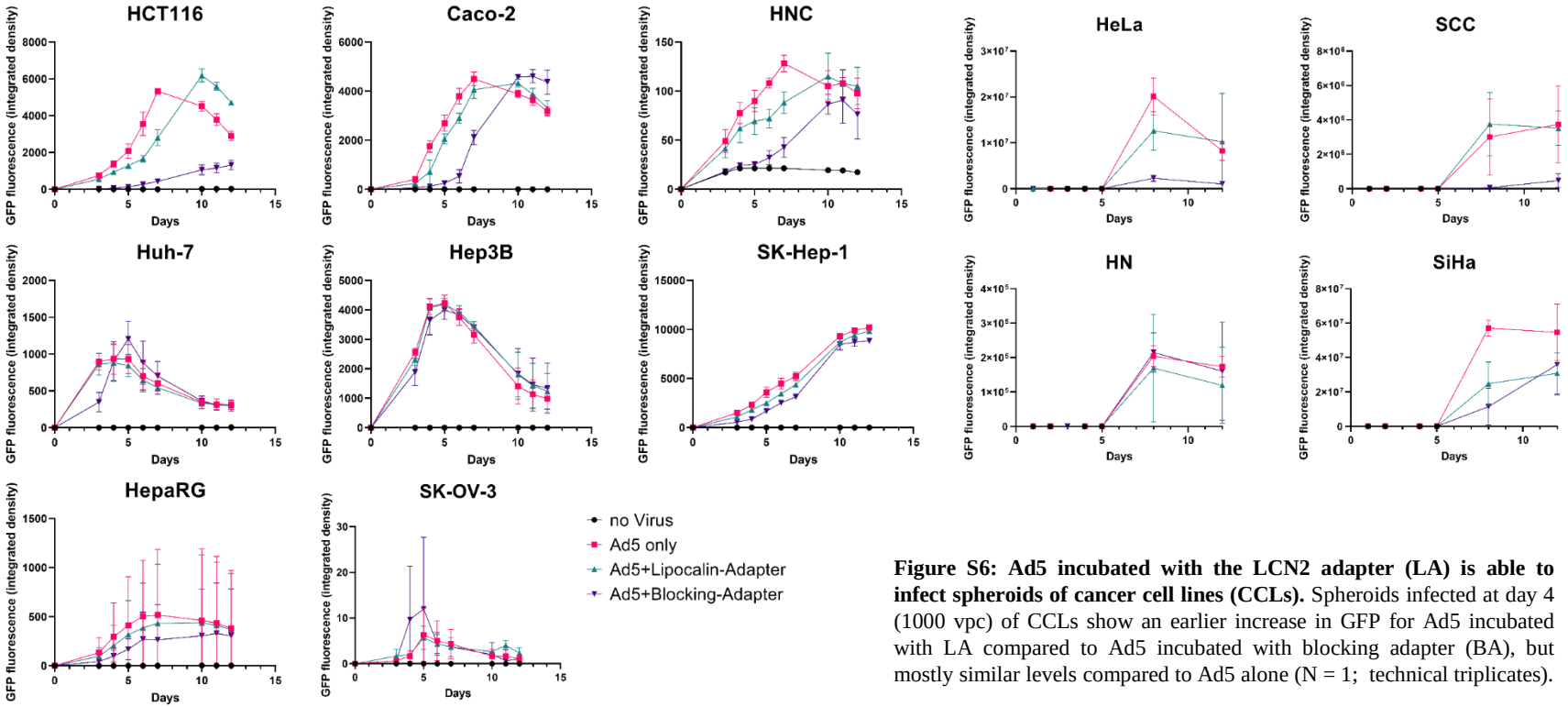

Figure S6: Ad5 incubated with the LCN2 adapter (LA) is able to infect spheroids of cancer cell lines (CCLs). Spheroids infected at day 4 (1000 vpc) of CCLs show an earlier increase in GFP for Ad5 incubated with LA compared to Ad5 incubated with blocking adapter (BA), but mostly similar levels compared to Ad5 alone (N = 1; technical triplicates).

Figure S7, Schellhorn et al.

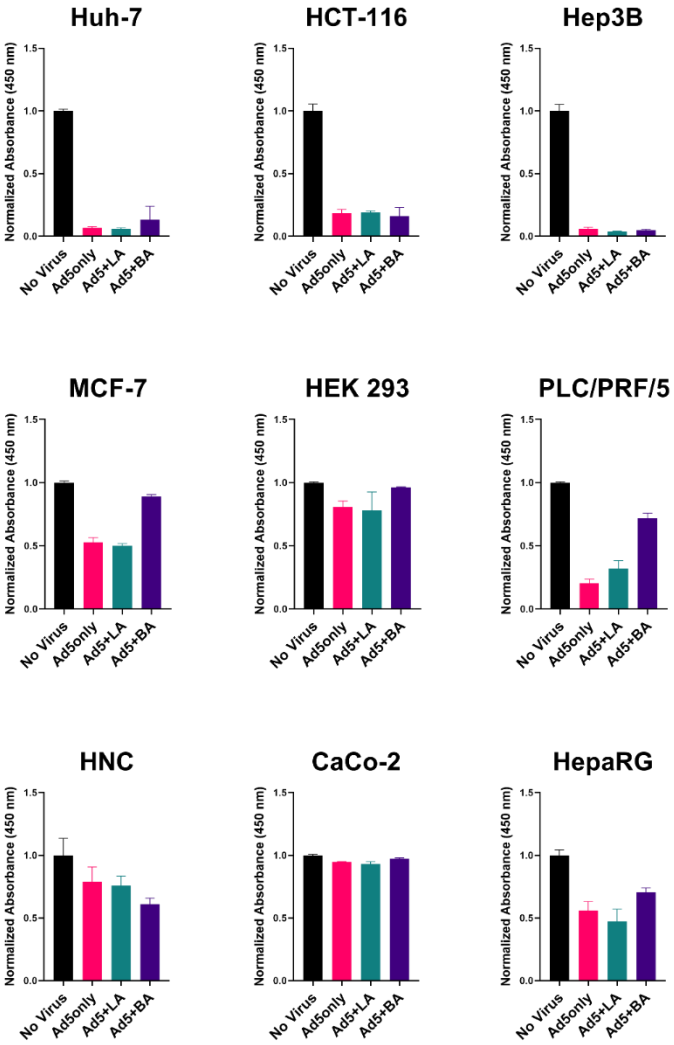

Figure S7: Ad5 incubated with the LCN2 adapter (LA) is able to kill cancer cells. The Crystal Violet cell viability assay was performed to analyze cancer cell lines (CCLs) killing efficiency. Various CCLs showed lower viability 5 days post-infection for Ad5 incubated with LA compared to Ad5 incubated with the blocking adapter (BA), but a similar viability as Ad5 alone (N=1; technical triplicates).

Figure S8, Schellhorn et al.

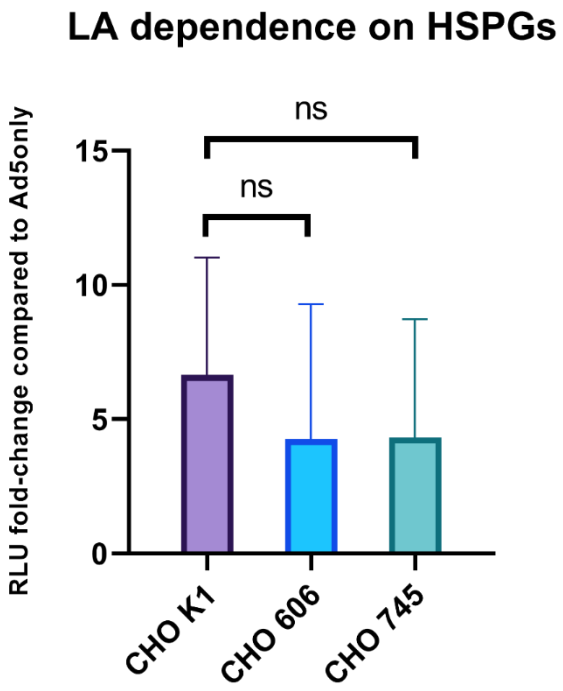

Figure S8: LCN2 adapter (LA) effect does not depend on Heparan Sulfate Proteoglycan (HSPG) expression. Luciferase assays on CHO K1 and HSPG deficient CHO 606 and CHO 745 cell lines showed that the viral uptake increasing effect of Ad5 incubated with LA is not abolished when HSPG deficient cell lines are infected. (Mann-Whitney U test; N=5-7; *= p-value<0,0332; **= p-value<0,0021; ***= p-value<0,0002; ****= p-value<0,0001).

Videos S1-S4, Schellhorn et al.

Videos S1-S4: Ad5 incubated with LCN2 adapter (LA) infection of HeLa spheroids. Video of HeLa cell spheroids generated from fotos taken at time of infection until 14 days later every 12 hours. Video S1 shows uninfected HeLa cells. Video S2 shows cells infected with Ad5 only. Video S3 shows results of adapter LA-bound Ad5 infection. Video S4 shows results of blocking adapter (BA)-bound Ad5 virus.