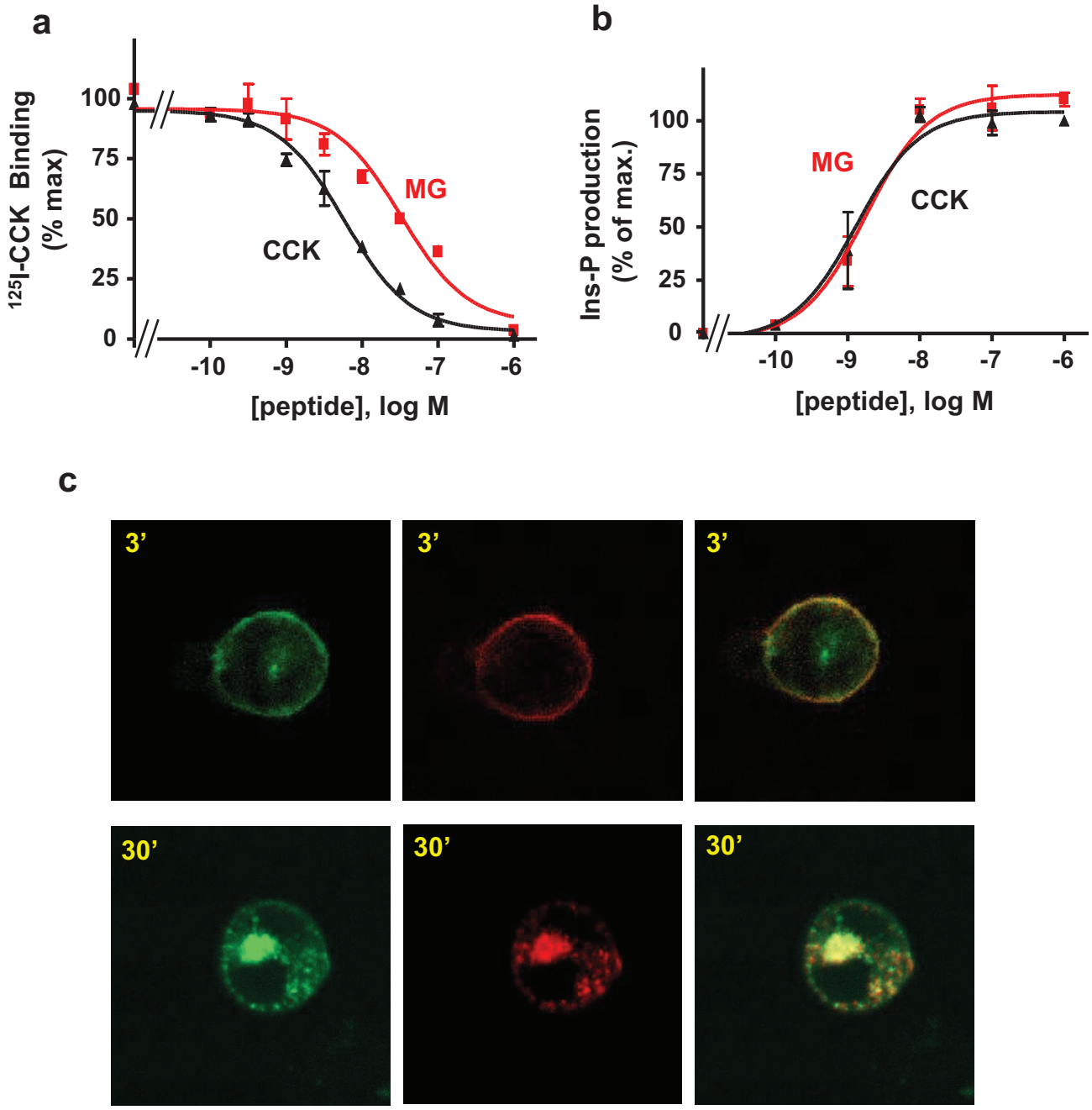
Targeting a G-Protein Coupled Receptor Overexpressed in Endocrine Tumors by Magnetic Nanoparticles to Induce Cell Death

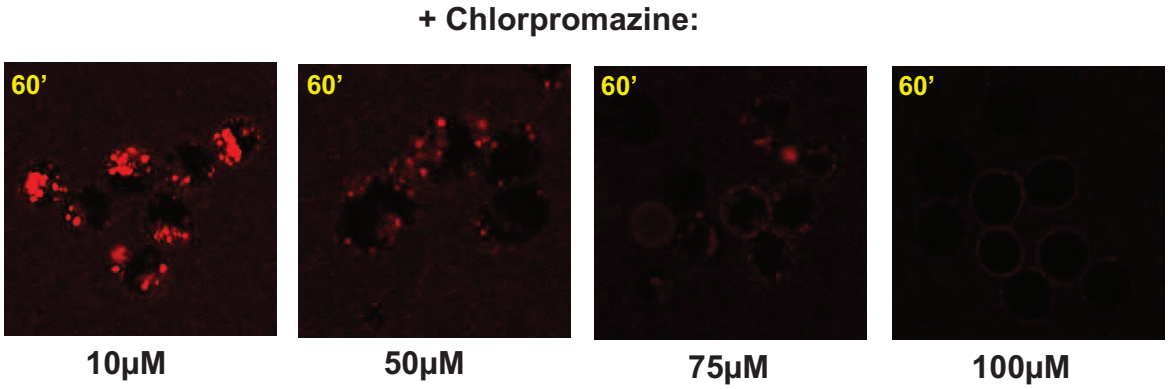
Claire Sanchez, Darine El Hajj Diab, Vincent Connord, Pascal Clerc, Etienne Meunier, Bernard Pipy, Bruno Payré, Reasmey P. Tan, Michel Gougeon, Julian Carrey, Véronique Gigoux and Daniel Fourmy

NIMT FeOdot PEG-Amine (Produc numbers N1-FA1-020, N1-FA1-050)	
Iron concentration	1.5 $mg \cdot mL^{-1}$
Iron oxide (Fe_3O_4) concentration	2.0 $mg \cdot mL^{-1}$
Number of particles in 1 mL of solution	~ $5 \cdot 10^{14}$
Molar concentration of particles	~ 0.75 µM
Number of Amino groups / particle	~ 700
Core diameter (TEM)	11 ± 1 nm
Particle diameter including coating (TEM)	18 ± 2 nm
Hydrodynamic particle diameter including coating (DLS)	30 nm
Magnetic Susceptibility	80 $emu \cdot g^{-1}$
r2 relaxivity	~ 300 $mM^{-1} \cdot S^{-1}$

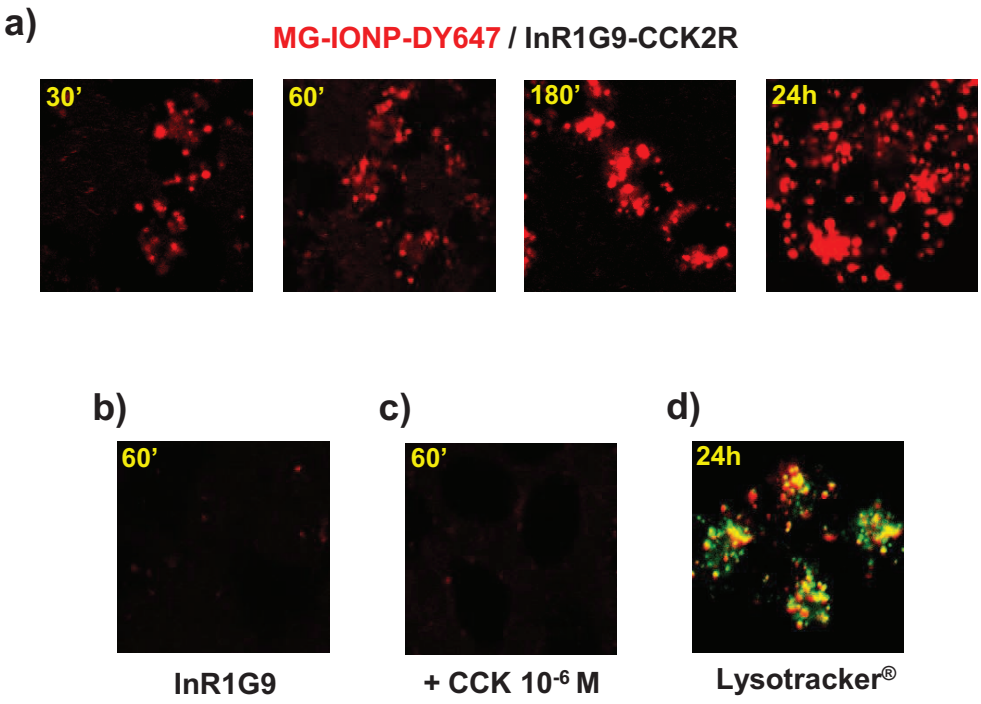
Supplementary table 1



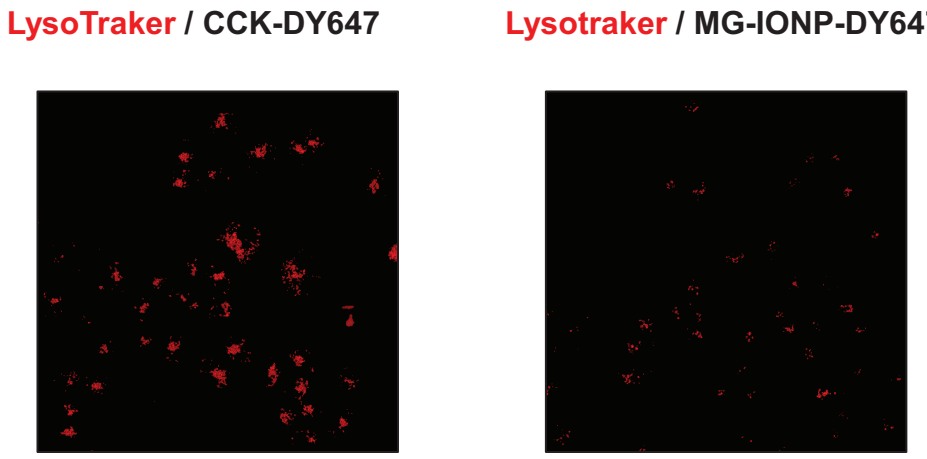
Supplementary information, Fig. 1: MG is a high affinity full agonist of the CCK2R which stimulates CCK2R internalization. **a**: **Competitive binding of MG to CCK2R.** Inhibition binding was carried out by incubating radio-iodinated CCK (^{125}I-CCK) with HEK293 cells transiently expressing CCK2R in the presence of increasing concentrations of competitor as described in experimental procedure section. Calculated IC_{50} was 6.0 ± 1.4 nM for CCK and 44.0 ± 1.0 nM for MG. Results are the mean ± SEM of 3 distinct experiments. **b**: **MG-induced inositol phosphate production.** Inositol phosphate production (Ins-P) was measured on HEK293 cells transiently expressing CCK2R cell following 1h of stimulation with CCK or MG. Concentrations giving half-maximal production (EC_{50}) for CCK was 1.4 ± 0.5 nM and for MG was 1.8 ± 0.3 nM. Results are the mean ± SEM of 3 experiments. **c**: **MG-induced CCK2R internalization.** HEK293 cells transfected with CCK2R-GFP were incubated at 37°C with MG-DY647 (50 nM). The images illustrate initial binding of MG-DY647 at the cell surface and internalization of CCK2R-GFP following stimulation with MG-DY647 after 30 min.



Supplementary information, Fig. 2: Dose-response effect of chlorpromazine on Flp-In^{TM}CCK2R-293 cells incubated with MG-IONP-DY647. Flp-In^{TM}CCK2R-293 cells were incubated at 37°C for 60 min with MG-IONP-DY467 (8 µM/mL) in the presence of the clathrin coated-pits inhibitor, chlorpromazine, at indicated concentrations.



Supplementary information, Fig.3: Endocytosis of MG-IONP-DY647 in InR1G9-CCK2R.
a: Time-course of internalization. InR1G9-CCK2R cells were incubated with MG-IONP-DY647 (8µg Fe/ml) at 37°C for indicated times. **b: Absence of significant MG-IONP-DY647 uptake in InR1G9 lacking CCK2R**. InR1G9 cells were incubated with MG-IONP-DY647 at 37°C. **c: Specificity of internalization**. InR1G9-CCK2R cells were incubated with MG-IONP-DY647 at 37°C in the presence of CCK 1µM. **d: Intracellular trafficking to lysosomes.** InR1G9-CCK2R cells were incubated with MG-IONP-DY647 for 24h at 37°C. Cells were pre-loaded with LysoTracker® (75 µM) for 30 min before observation with confocal microscope.



Supplementary information, Fig. 4: Application of an alternating magnetic field to tumoral cells having internalized MG-IONP-DY647 affects lysosome integrity. InR1G9-CCK2R were incubated with CCK-DY647 (0.1 µM) or MG-IONP-DY647 (16 µg Fe/ml) for 24h at 37°C. Cells were washed and incubated in presence of Lysotraker (75 nM) and exposed for 2h to an alternating magnetic field (275kHz, 40 mT at 37°C). At the end of magnetic field exposure, cells were washed and lysosome staining was observed by confocal microscopy. Staining was quantified using Morpho ExpertTM Software.