Supplemental Figure 1

**Uptake with variable substrate concentrations:** T3 and T4 were titrated in uptake buffer and incubated on MDCK1 cells (MOCK (■) vs MCT8 (●)) for 5 or 10 min. For both substrates, MCT8-mediated uptake was detectable starting from ~1µM. In higher concentrations, T4 produced an elevated background signal on MOCK cells, probably due to unspecific adhesion to the cells or plastic surface. This effect was not seen for T3.
Linear range of measured TH; Dilution series of differently iodinated TH were prepared to define the total amount of imported substrate in relationship to the total amount within the uptake buffer (usually 10 µM in a total volume of 100 µl). Samples underwent APS-digestion and were quantified after appropriate dilution (1:4) in ddH2O. We found, that the linear range depends to some degree upon the substrate compound. Reduced dOD values in the higher concentration range of T4 result from the destaining even before capturing the first data points.
Rational Design and Setup of the Non-Radioactive Substrate Uptake Assay.

(A) Schematic Work-Flow of the non-radioactive uptake assay. After cell seeding into 96-well microtiter plates, the assay continued with the (co-)incubation of substrates (e.g. Iodothyronines) and testing compounds on cells at 80-100% confluency. After a specified incubation time period, the plates were washed with PBS supplemented with 0.1% BSA and ice-cold ddH2O. After oxidative digest with 0.68 M APS at 90°C for 1h, the quantification of free iodide is achieved by adding the ceric and arsenic solutions. The absorption was measured at 415 nm every 60 s with 30 iterations. The difference between the absorption after 1 min and 21 min was recorded as the assay read-out.

(B) The destaining reaction of cerium (Ce$^{4+}$(yellow) $\rightarrow$ Ce$^{3+}$(colorless)) occurs very fast in presence of iodide which acts as the catalyst in the reaction between cerium and arsenic moieties (Sandell-Kolthoff Reaction (15)). Here, these changing absorption rates are easy to assess in a non-radioactive fashion, reliably reflecting the iodide concentrations and thereby allowing a precise and fast signal read-out.