Supplementary information

The semi-quantitative analysis was conducted on the histological images as shown in Figure 7 in the manuscript. The columns represented the tumor sections from mouse treated with U, UA, UG, UAG respectively. The rows labeled with "AFP", "GPC3" and "Prussian blue" were further analyzed by quantifying the ratio of DAB- or Prussian-blue-stained pixel among the pixels involved in the tumor region. The DAB-stained pixel ratio could reflect the AFP or GPC3 expression level and Prussian-blue-stained pixel ratio could reflect the targeting effects of iron-contained USPIO probes. The analysis steps were as follows.

Analysis for DAB-stained pixel ratio

Step1: Open the targeted AFP or GPC3 stained image in the Image J (Wayne Rasband and contributors, National Institutes of Health, USA. http://imagej.nih.gov/ij).

Step2: Subtract the image background with rolling ball radius set as 50 pixels.

Step3: Crop the image to only involve tumor region as much as possible.

Step4: Use IHC Profiler plugin to split the DAB-stained regions by choosing "Cytoplasmic Stained Image" mode. [*Varghese F, Bukhari AB, Malhotra R, De A (2014) IHC Profiler: An Open Source Plugin for the Quantitative Evaluation and Automated Scoring of Immunohistochemistry Images of Human Tissue Samples. PLoS ONE 9(5): e96801. doi:10.1371/journal.pone.0096801*] The DAB-stained ratio could be reported automatically.

Analysis for Prussian-blue-stained pixel ratio

Step1: Open the targeted Prussian-blue-stained image in the Image J (Wayne Rasband and contributors, National Institutes of Health, USA. http://imagej.nih.gov/ij).

Step2: Subtract the image background with rolling ball radius set as 50 pixels.

Step3: Split color channels into Red, Green and Blue. Choose the image in the Red channel and select the region automatically based on the threshold set as 210. Select the wrongly segmented region and fill them as background. Convert the retained segmented region into stained ROIs.

Step4: Delineate the tumor region and calculated the total pixel numbers Ntotal. Then calculate the pixel numbers located in the overlayed stained ROIs Nstained. The stained ratio is calculated as Ntotal/Nstained.

Supplementary Table 1 The targeted stained pixel ratio for AFP, GPC3 and iron in the U-, UA-, UG- and UAG-treated mice

	U	**UA**	**UG**	**UAG**
AFP-stained ratio	25.96%	43.01%	25.49%	27.22%
GPC3-stained ratio	29.10%	38.19%	38.55%	39.72%
Prussian-blue-stained ratio	0.0040 (270328 px)	0.0051 (357189 px)	0.0124 (446022 px)	0.3848 (735700 px)

Note: px-pixels, which represents the approximated pixels number for the tumor tissues in each analyzed image.

Supplementary Table 1 summarized the results derived from the semi-quantitative methods above. Although it existed many aspects to improve the IHC sample staining and quantitative analysis in the current study, the semi-quantitative results might indicate that UAG-treated mice could have higher iron concentrated and targeting specificity in the tumor.