In Vivo Recognition of Human Vascular Endothelial Growth Factor by Molecularly Imprinted Polymers

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Table S1. Summary of dissociation constants (K_D) of QD-MIPs imprinted in PBS against the epitope of hVEGF tested for either the epitope itself or non-target vancomycin or EGFR epitope**.**

Template for imprinting	K_D [nM]	Chi^2	Analyte	Analyte concentration [nM]
hVEGF epitope	1.78 1.39	0.48 0.76	hVEGF epitope	0.097 - 100
hVEGF epitope	/ /	>1 >1	Vancomycin	0.097 - 100
hVEGF epitope	/ /	>1 >1	Vancomycin	10 - 1000
hVEGF epitope	/	>1	EGFR epitope	0.097 - 100

Table S2. Results obtained with ICP-MS on the content of Cd ions after the injection of zebrafish embryos with QD-MIPs incubated at different time points.

Sample	Number of embryos per group	Cd content per embryo (a.u.)	Normalized content of Cd
Control (no injection)	10	1.26	0
Overnight incubation	13	95.98	0.36
2.5-hour incubation	20	151.15	0.57
0-hour incubation	10	264.18	1

Table S3. Z potential and diameter of functionalized nanoparticles and non-functionalized MIPs.

	Z potential (mV)	NTA Hydrodynamic diameter (nm)
QD-MIP (attached)	-11.9 ± 0.8	140.0 ± 6.8
QD-MIP (embedded)	-27.5 ± 4.8	123.2 ± 11.2
MIPs	-26.9 ± 2.7	120.6 ± 10.5
Free QDs	-3.76 ± 2.0	N/A

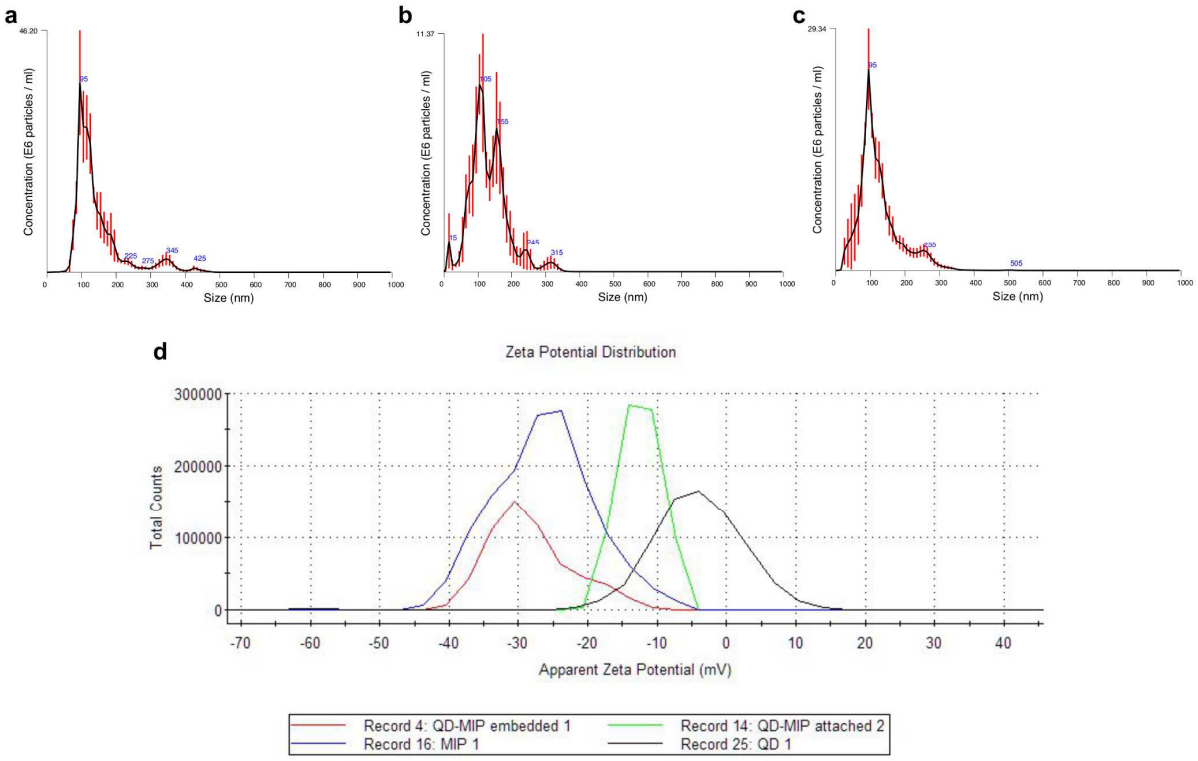

Figure S1. NTA measurements and Z potential curves on hybrid nanoparticles and non-functionalized MIPs. QD-MIPs (attached) have diameter of 140.0 ± 6.8 nm (a), QD-MIPs (embedded) 123.2 ± 11.2 nm (b) and non-functionalized MIPs 120.6 ± 10.5 nm (c), with n=5 measurements for each sample. In (d) are reported the curves of z potential measurements. Particularly, MIPs and QD-MIPs (embedded) show similar values, whereas QD-MIPs (attached) show signal shifted towards those values obtained for free-QDs.

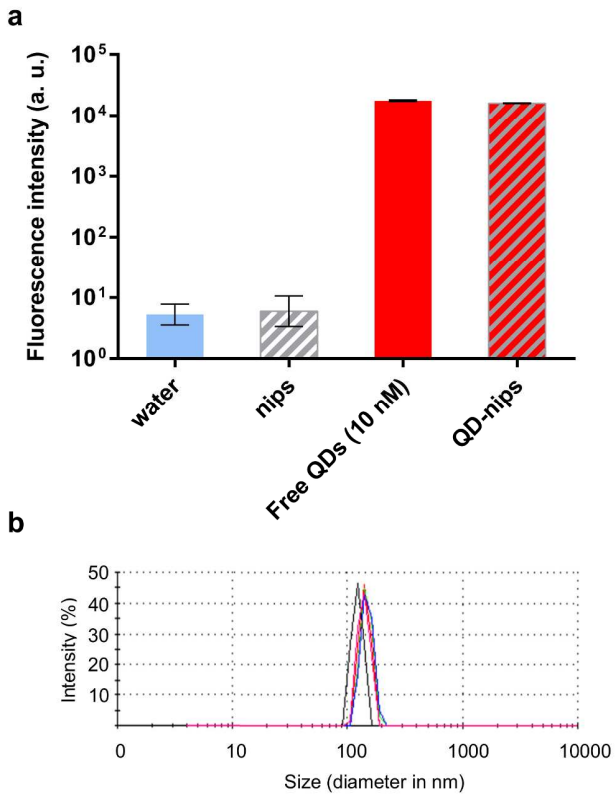

Figure S2. Characterization of control hybrid nanoparticles QD-nips in terms of fluorescence signal and hydrodynamic diameter. QD-nips show fluorescence intensity similar to that of a 10-nM solution of free QDs. On the contrary, as expected deionized water and non-functionalized nips, used as controls, did not show fluorescence. For this graph, logarithmic scale was exploited (a). In (b) are reported the size distribution curves of QD-nips obtained with DLS. QD-nips have hydrodynamic diameter of 140.2 ± 16 nm (n=6).

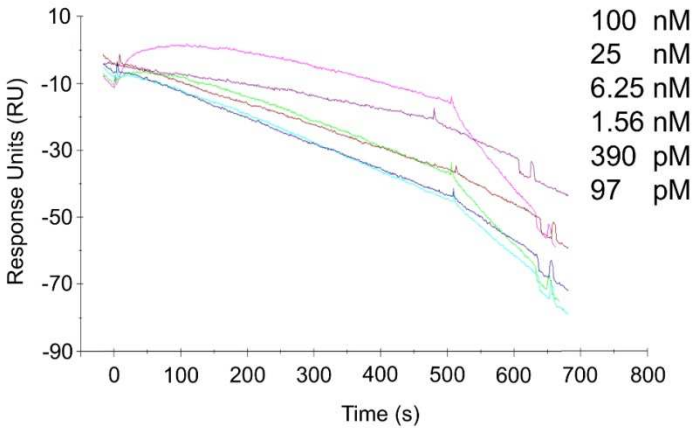

Figure S3. SPR measurements to assess the ability of QD-MIPs imprinted against hVEGF epitope to bind EGFR epitope. As can be observed from the graphs, the injection of concentrations of EGFR epitope ranging from 97 pM to 100 nM did not allow acquiring curves which could be fitted using the fit model exploited to obtain K_D for the hVEGF epitope. Hence, no K_D was calculated for EGFR epitope. QD-MIPs did not bind the epitope of EGFR.

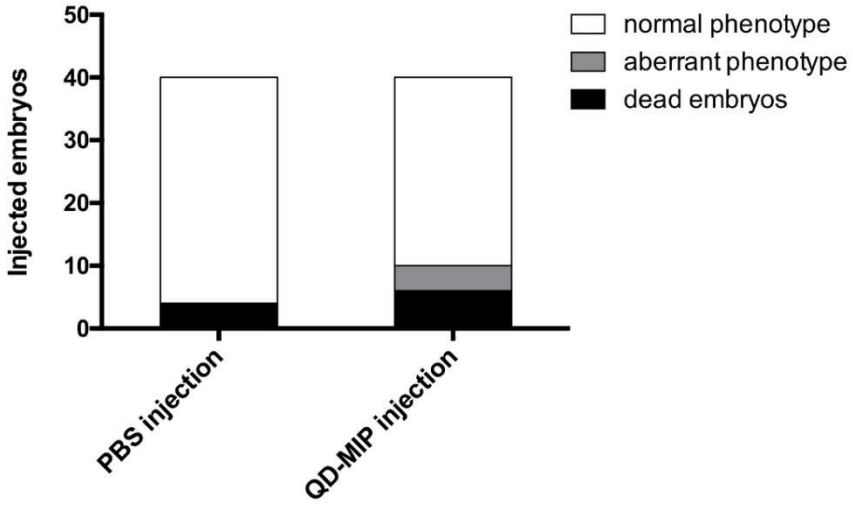

Figure S4. Graphical representation of toxic effect data of nanoparticles injected in zebrafish embryos. Forty embryos were injected either with PBS 1 X buffer (control) or QD-MIPs and the number of normal phenotypes, aberrant phenotypes and dead embryos were statistically analyzed (Chi2 test).

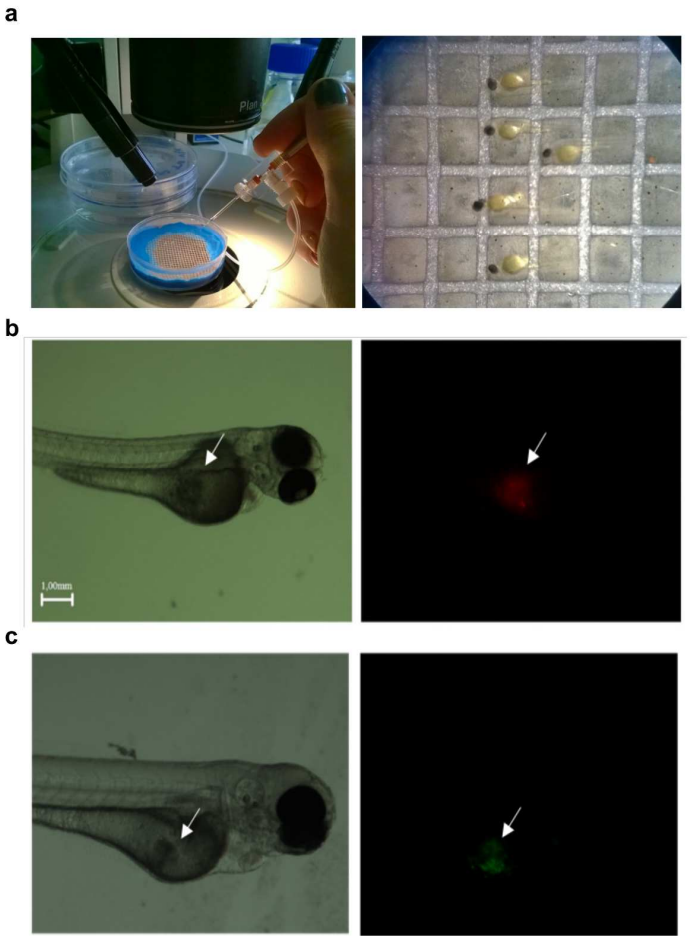

Figure S5. Panel illustrating the set up for the injection of zebrafish embryos and images of 72 hpf zebrafish embryos previously injected with melanoma cells. (a) Set up for the injection of 48 hpf and 72 hpf zebrafish embryos with either human melanoma cells WM-266, A-375 or nanoprobes. (b) Bright field and epifluorescence images of 72 hpf embryo 24 hours after the

injection of WM-266 hVEGF (+) human melanoma cell line, labelled with DiI (scale bar 1 mm). (c) Bright field and epifluorescence images of 72 hpf embryo 24 hours after the injection of A375 hVEGF (-) human melanoma cell line, stably transfected with GFP (scale bar 1 mm).

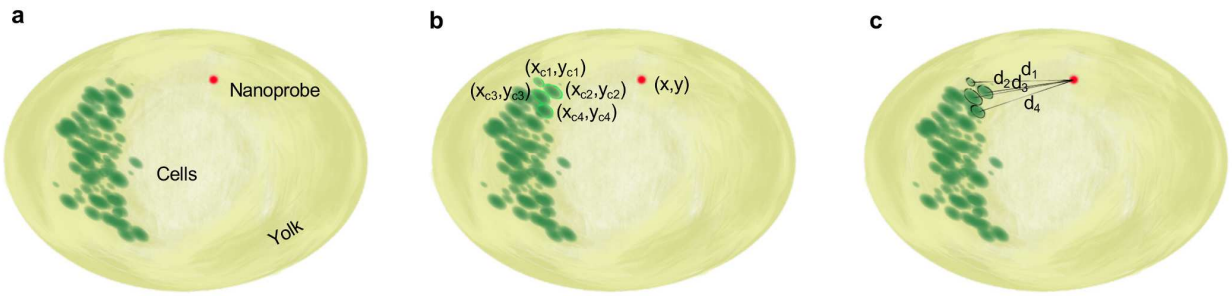

Figure S6. Scheme of the strategy applied to standardize the measurement of the distances nanoprobe-cell. (a) Cartoon of an example of localization scenario of the nanoprobes. (b) The FIJI plugin 'measure' was exploited to obtain the coordinates (x, y) of the centers of mass of nanoprobes and human melanoma cells. (c) Measurement of the single distances nanoprobe-cell by using the Pythagoras's equation $d = \sqrt{(x - x_c)^2 + (y - y_c)^2}$. The analysis was performed on embryos in which several 'red spots'≥10 was observed. The standardized protocol was applied to n≥7 embryos for each group.