Electronic Supplementary Material (ESI) for Nanoscale.

Quantum dot multiplexing for the profiling of cellular receptors

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Supporting information:

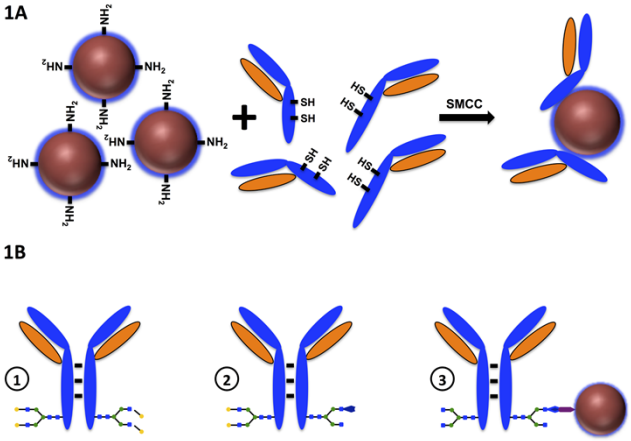

Figure S1. Schematic of two different Qdot antibody conjugation strategies. (S-1A) This conjugation reaction is based on the coupling of thiols that are present in reduced antibodies to reactive maleimide groups present on the PEG coating of the Qdots after SMCC activation. (S-1B) Copper-free click chemistry consists of the initial modification of the n-terminal carbohydrate with an azide group; after this modification occurs the DIBO groups present on the surface of the Qdots reacts with the azide to form an azide–alkyne bond, as described in the manual of the SiteClick technology (available: https://tools.lifetechnologies.com/content/sfs/manuals/mp10469.pdf)

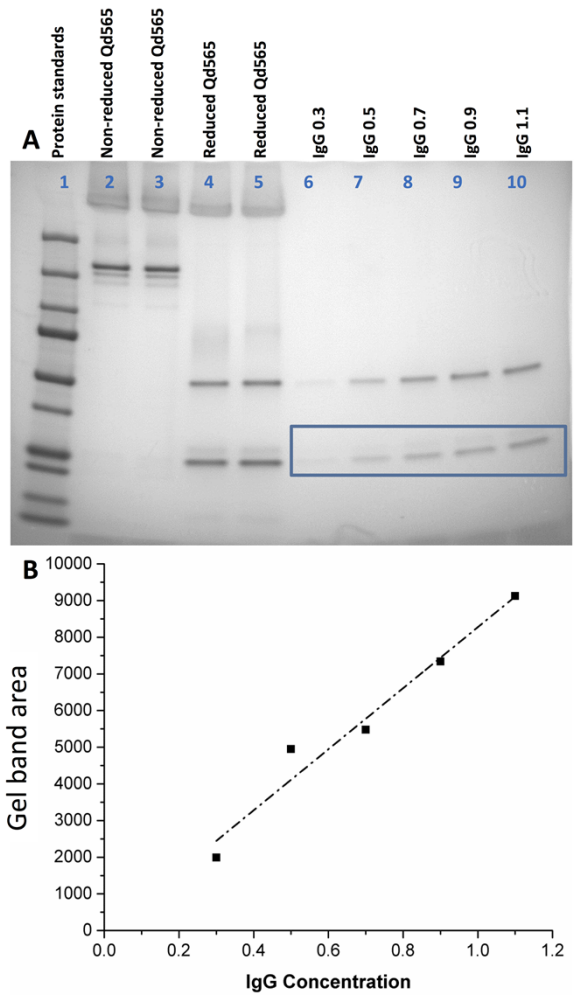

Figure S2. (A) SDS-PAGE used to make the calibration curve for Qdots 565 conjugated with IgG antibodies. Lane numbers correspond to: 1) Biorad precision plus dual color protein size marker; 2 and 3) Qdots 565 conjugated with antibodies in a non-reduced form travel relatively slowly in the gel indicating that the antibodies were unfractionated prior to denaturation; 4 and 5) Qdots 565 conjugated with antibodies reduced to separate the heavy chain and the receptor-bearing light chains of the IgG; 6 to 10) Antibodies of known concentration reduced to separate the heavy chain and the receptor bearing light chains of the IgG loading 0.3 µl, 0.5 µl, 0.7 µl, 0.9 µl and 1.1 µl of 1 mg/ml sample per well, respectively. (B) Calibration curve of the light chain fraction of the IgG indicating a

linear relationship used to calculate the concentration of experimental samples of antibodies bound to Qdots. The intensity of the gel bands (arbitrary units) was calculated using Fiji densitometry gel analysis software, which measures the area under the peak of a plot of each gel band.

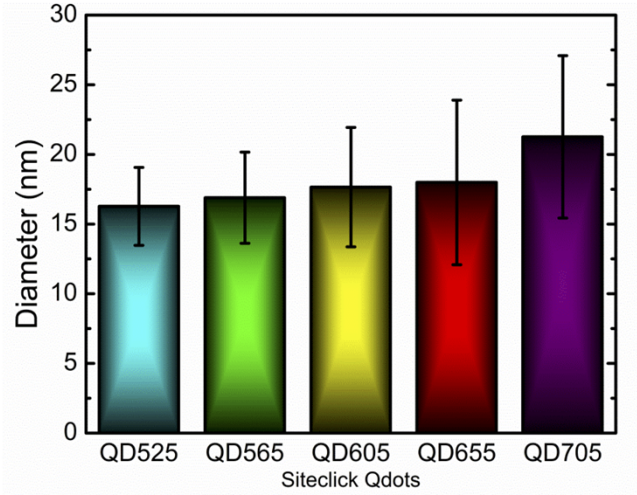

Figure S3. Hydrodynamic diameter of Copper-free Click Qdots conjugated to IgG, calculated using a dynamic light scattering analysis. The mean size ranged from 16.27 nm to 21.26 nm, larger than when unbound to the antibody. Error bars indicate standard deviation. The error was larger for the rod-shaped Qdots.

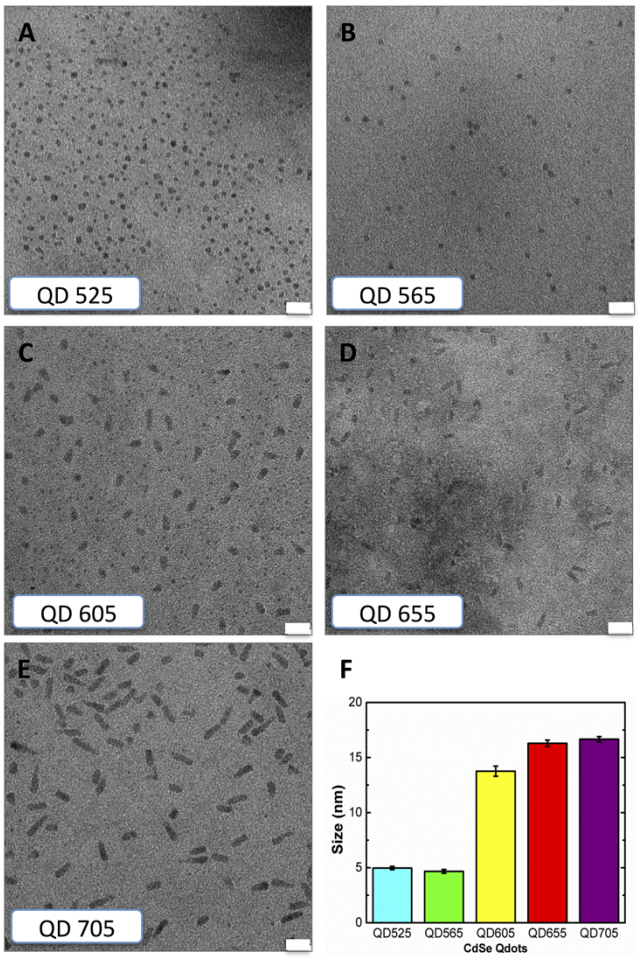

Figure S4. Transmission electron microscopy images of the inorganic CdSe core/ZnS shell of Qdots 525, 565, 605, 655 and 705 nm, showing that the Qdots 525 and 565 nm have a spherical structure and the Qdots 605, 655 and 705 nm have a rod shape (A-E). (F) shows how the size of the Qdots ranged from 5.05 to 16.56 nm and the size increased with increased Qdot emission spectra. Scale bars represent 20 nm in length and error bars indicate standard error.

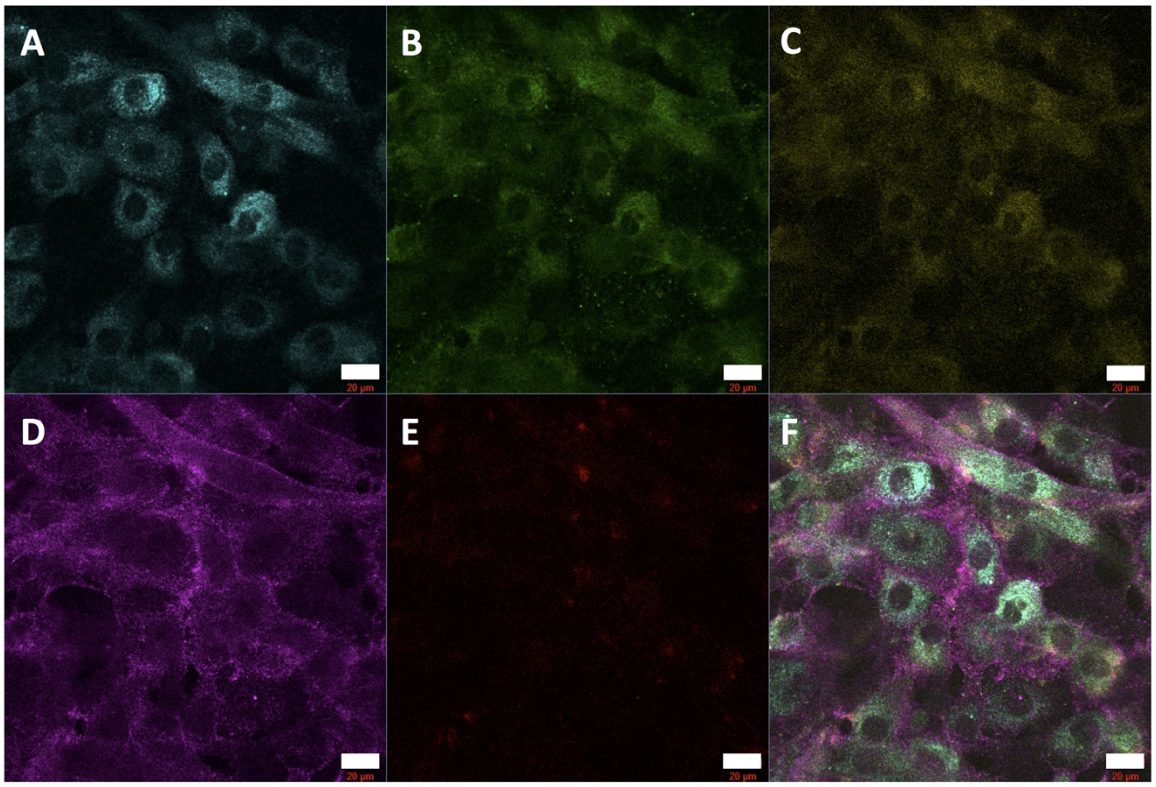

Figure S5. Confocal image of human live fibroblasts labeled in a single-step procedure with five different Qdots-Ab probes targeting the five different angiogenic receptors A) VEGFR1, B) VEGFR2, C) VEGFR3, D) NRP1, E) NRP2, and F) merged image with all five receptor types.

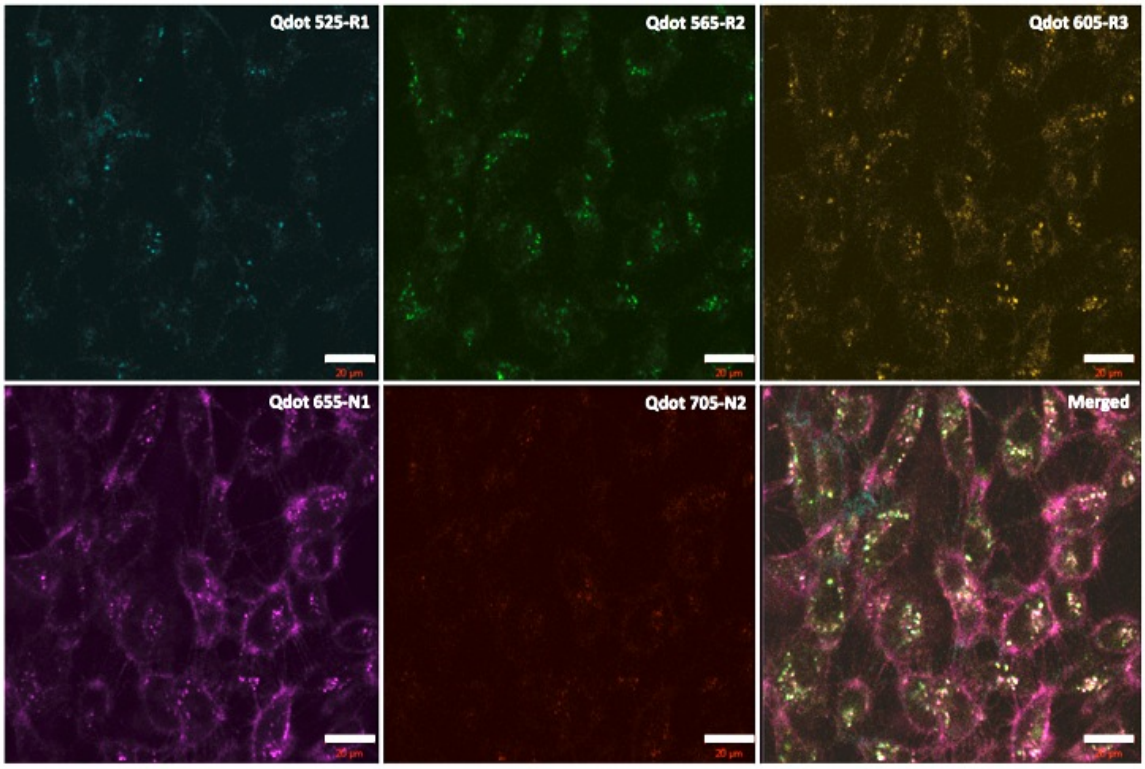

Figure S6. Confocal image of live HUVECs

Receptor combination	Pearson Coefficient (no threshold)	Conclusion	Pearson Coefficient (Costes threshold)	Conclusion
R1R2	0.722	Colocalization	0.637	Colocalization
R1R3	0.625	Colocalization	0.532	Colocalization
R1N1	0.526	Colocalization	0.385	No Colocalization
R1N2	0.502	Colocalization	0.451	No Colocalization
R2R3	0.749	Colocalization	0.657	Colocalization
R2N1	0.639	Colocalization	0.480	No Colocalization
R2N2	0.597	Colocalization	0.540	Colocalization
R3N1	0.642	Colocalization	0.538	Colocalization
R3N2	0.396	No Colocalization	0.300	No Colocalization
N1N2	0.547	Colocalization	0.508	Colocalization

Table S7. Colocalization results of a Z-stack HUVEC image labeled with Qdots.

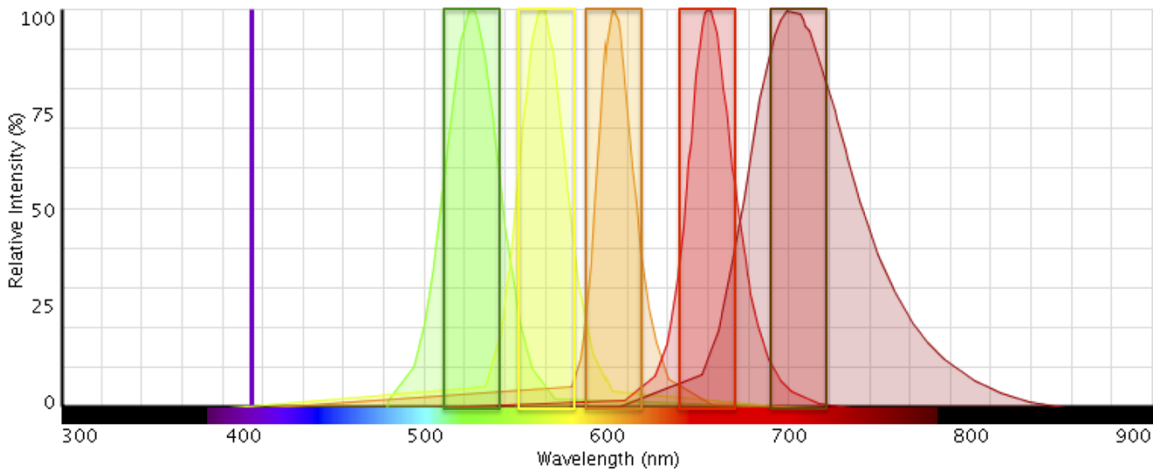

Figure S8. Quantum dots emission spectra using a 405 nm excitation laser source. Rectangular boxes represent the emission filters used on each of the five channels.

Fluorophore / Filter	Qdot 525/30	Qdot 565/30	Qdots 605/30	Qdot 655/30	Qdot 705/30
Qdot 525	68.00%	6.40%	0.10%	0.00%	0.00%
Qdot 565	2.80%	73.70%	4.60%	0.00%	0.00%
Qdot 605	0.00%	1.40%	81.70%	1.30%	0.00%
Qdot 655	0.00%	0.00%	1.50%	69.30%	3.30%
Qdot 705	0.10%	0.10%	0.20%	6.10%	37.80%

Table S9. Percentages of spillover of the emission signal of non-target (neighboring) Qdots detected in the filtered channel of the target wavelength range.