Supplementary Information

Experimental Section

Chemicals and Materials

Methacryloxytrimethylsilane (SIMA, 97 %) and N-methacryloyloxysuccinimide (MAOS) were purchased from ABCR GmbH. 2-aminoethylmethacrylate (AEM, 90 %) was supplied by Aldrich. 2,2′-azo-bis-(2,4-dimethylvaleronitrile) (ABDV) was obtained from Wako and recrystallized from cold ethanol before use. Rhodamine 123 (R123, > 90 %) was obtained from Sigma-Aldrich and Rhodamine 6G (R6G, 99 %) and rhodamine B (RB, + 99 %) were purchased from Acros. HPLC-grade methanol and acetonitrile were purchased from SDS. HPLC-grade acetone and diethyl ether were from Panreac. Dimethylformamide (DMF) was obtained from Carlo Erba and absolute ethanol was from BDH Prolabo Chemicals. Triethylamine (TEA) and trifluoroacetic acid (TFA) (peptide synthesis) were from Fisher Scientific and Fluorochem, respectively. Water was purified with a Milli-Q system.

^{1}H NMR spectra (UCM Central Instrumentation Facilities) were acquired on a Bruker Avance DPX 300MHz-BACS60 spectrometer. NMR chemical shifts are expressed relative to the signals of the non-deuterated traces of the solvent (DMSO-d_6 at 2.54 ppm).

Weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity (M_w/M_n) were determined by gel permeation chromatography (GPC) using a Perkin-Elmer chromatograph with an isocratic pump (250 series) connected to a differential refractometric detector (200 series). Three resin columns (PL-gel, 500, 104 and 105 nm pore size) (Polymer Laboratories) were connected in series to elute the samples (concentration 1 mg mL^{-1}). DMF containing 0.1 % (*v/v*) of LiBr was used as the mobile phase at a flow rate of 0.3 mL min^{-1}. Monodispersive poly(methyl

methacrylate) standards in the range of 10.3 – 480 kDa (Polymer Laboratories) were used for GPC calibration. The measurements were carried out at 70 ºC.

Synthesis of poly((trimethylsilylmethacrylate-co-2-aminoethylmethacrylate) [P(SIMA-co-AEM)]

The copolymer was prepared by standard free radical polymerization. In a typical synthesis, SIMA (4.5 mL, 25.2 mmol), AEM (525 mg, 3.2 mmol) and ABDV (0.2 mmol) were dissolved in methanol (20 mL) in a 100 mL round bottom flask sealed with a rubber septa (SIMA:AEM, mole ratio 9:1). The mixture was purged with argon for 20 min, and stirred at 60 °C for 24 h. The resulting copolymer was precipitated in acetone and the solid was collected by centrifugation and washed with acetone (3 x 60 mL) and diethyl ether (3 x 60 mL), centrifuged and dried under vacuum to give the purified copolymer (yield: 4.10 g, 91 %).

^{1}H NMR (300 MHz, DMSO-d_6, δ): 5.70 and 5.36 (s, C=CH_2), 3.91 (s, br, NH_2-CH_2-), 1.25, 1.17 and 0.94 (s, br, C-CH_3).

Synthesis of poly(methacrylic acid-co-2-methacrylamidoethylmethacrylate) [P(MAA-co-MAAEMA)]

P(SIMA-*co*-AEM) (4.01 g) was dissolved in DMF (40 mL) at room temperature and then 543 mg of MAOS (3.0 mmol) were added. The mixture was purged with argon for 20 min prior to the addition of 1 mL of TEA dropwise. The reaction mixture was stirred at room temperature for 12 h and, afterwards, it was added dropwise to a cooled solution of 10% TFA (*v/v*) to precipitate the hydrolyzed copolymer P(MAA-*co*-MAAEMA). The solid was centrifuged, dissolved in methanol and precipitated into

acetone. The resulting solid was washed in acetone (3 x 60 mL) and dried under vacuum to give the final purified copolymer (yield: 1.28 g, 28 %).

A solution of 20 g/L of P(MAA-*co*-MAAEMA) in methanol was stored at 4 ºC. The reaction scheme is shown in Figure S1. The ^{1}H NMR spectrum of P(MAA-*co*-MAAEMA) (Figure S2) showed that the ratio of the areas from any of the proton signals of the double bound and carboxylic acid was 1:6.5. Therefore, considering the initial mole ratio of the monomers (1:9, AEM:SIMA), we concluded that 28% of trimethylsilyl ester groups were not hydrolyzed. This fact could explain the ease of this linear copolymer to adhere to silicon wafers via a silanization reaction.

^{1}H NMR (300 MHz, DMSO-d_6, δ): 12.39 (s, br, HOOC-), 5.99 and 5.63 (s, C=CH_2), 4.10 (s, br, CO-NH-), 1.85 (s, =C(CH_3)-), 1.23, 1.04 and 0.95 (s, br, CH_3-C). GPC (THF): M_w = 20 kDa. Polydispersity index: 1.6.

Photo- and EBL-film structuring and characterization

Two prepolymerization solutions containing 3 and 30 μg of R123 (template molecule) in 1 mL of a solution of 20 g/L of P(MAA-*co*-MAAEMA) in methanol were prepared for EBL and UV photolithography, respectively. A non-imprinted polymer was prepared in a similar manner except that the imprinting solution did not contain the template molecule. Prepolymerization solutions were spun on (100) Si substrates, previously cleaned using piranha solution (H_2SO_4 96 % + H_2O_2 30 % 3:1 v/v at 130 ºC for 10 minutes) at 5000 rpm. The resulting films had thicknesses of approximately 100 nm and were patterned by both, EBL and photolithography as follows.

EBL lithography was performed with a Crestec CABL-9000C system at an acceleration voltage of 50 keV and beam currents of 10 pA and 1 nA. 40x60 μm^2 and 20x50 μm^2 rectangular features were written with electron doses ranging from 0.05 $\mu C/cm^2$ to 20

mC/cm^2. After the EBL process, the films were immediately developed in THF for 1 minute at room temperature and dried with N_2. The resulting patterns (remaining film on substrates) were thermally cross-linked on a hot plate at 170 ºC for 30 minutes. Photolithography-defined micropatterns were achieved with a SUSS MicroTec MJB4 mask aligner equipped with a Xe-Hg 255 nm (DUV) lamp at an output power of approximately 650 W. A Cr/quartz lithography mask was placed in close proximity (non-contact mode) to the film and DUV exposure was carried out for 30 minutes. The exposed film material was removed by developing the samples in THF for 1 minute and drying them with N_2. The obtained polymeric film features were then cross-linked by heating the samples at 170 ºC for 30 minutes on a hot plate.

The morphology of the fabricated structures was characterized by optical microscopy (Leica Leitz DMRX optical microscope), atomic force microscopy (AFM) with a Digital Instruments MultiMode Scanning Probe AFM (Model MMAFM-2) equipped with Veeco probes made of 0.01–0.025 Ωcm antimony (n) doped Si and using tapping mode and scanning electron microcopy (JEOL JSM 7600 F, at the National Center for Electron Microscopy, Complutense University).

MicroRaman spectra were recorded in a Renishaw inVia Raman microscope (Renishaw Iberica SAU, Barcelona, Spain), using a 50X magnification objective. The excitation line, 532 nm, was provided by Nd:YAG laser. The output laser power was 100 mW. The acquisition time of each spectrum was 5 min

Analysis with the nanostructured MIP based sensors

The polymeric patterns were washed thoroughly for 1 h with a mixture of ethanol:TFA (10% v/v). Uptake experiments were carried out incubating the MIP-based sensors, or the corresponding NIPs, for 3 h, under gentle agitation and protected from light, in 5

mL of R123 solutions in acetonitrile. For the cross-reactivity studies R123 was replaced by R6G or RB. The sensors were rinsed with 10 mL of acetonitrile and dried with Ar, for 3 min, prior to fluorescence microscopy measurements.

Fluorescence images were acquired at room temperature with a fluorescence microscope (Olympus BX51) equipped with a CCD camera (Infinity 3, Lumera Corporation). A 488 nm interference filter (FF01-488/6-25, Semrock Inc) was placed in the excitation path and a 600 nm dichroic mirror (T600LPXR, Chroma) was placed in the filter cube. A 590 nm cut-off filter (CG-OG-590-1.00-3, CVI Technical Optics, UK) was used to minimize the detection of scattered light.

The patterns were imaged at 10x magnification with a 3 s exposure time. Other fluorescence microscope parameters (detector gain, pinhole, iris diaphragm) were kept constant throughout the measurements with the MIP/NIP sensors. All images were analysed using ImageJ (v. 1.44p, Wayne Rasband, National Institutes of Health, USA). The average fluorescence intensity signal in the patterned region (n = 6, 50 x 50 pixel) was background corrected by subtracting the corresponding average fluorescence intensity signal of the substrate (n = 4, 50 x 50 pixel).

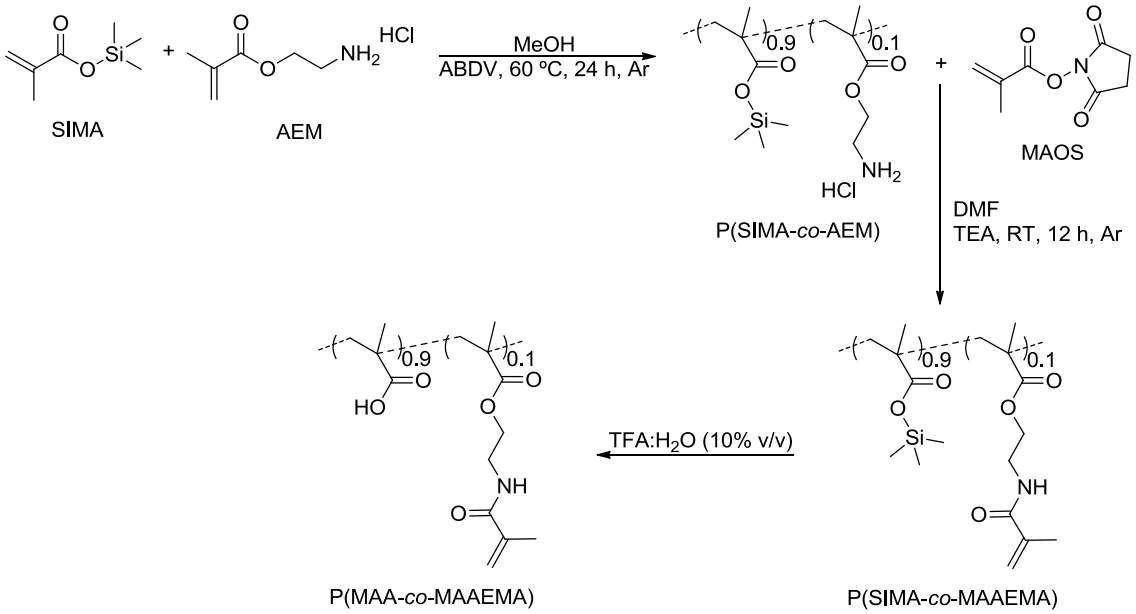

Figure S1. Scheme of the synthesis of P(MAA-*co*-MAAEMA).

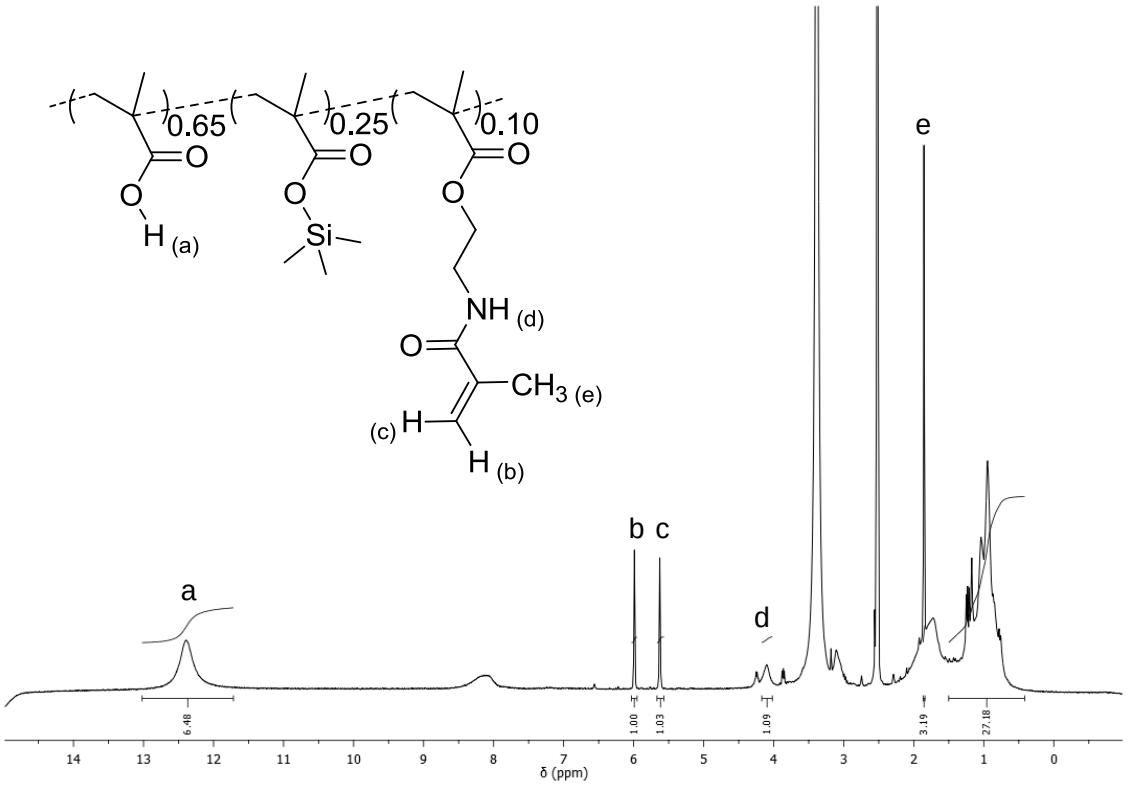

Figure S2. ^{1}H NMR spectra of P(MAA-*co*-MAAEMA). Inset: co-polymer composition estimated based on the proton signal ratios.

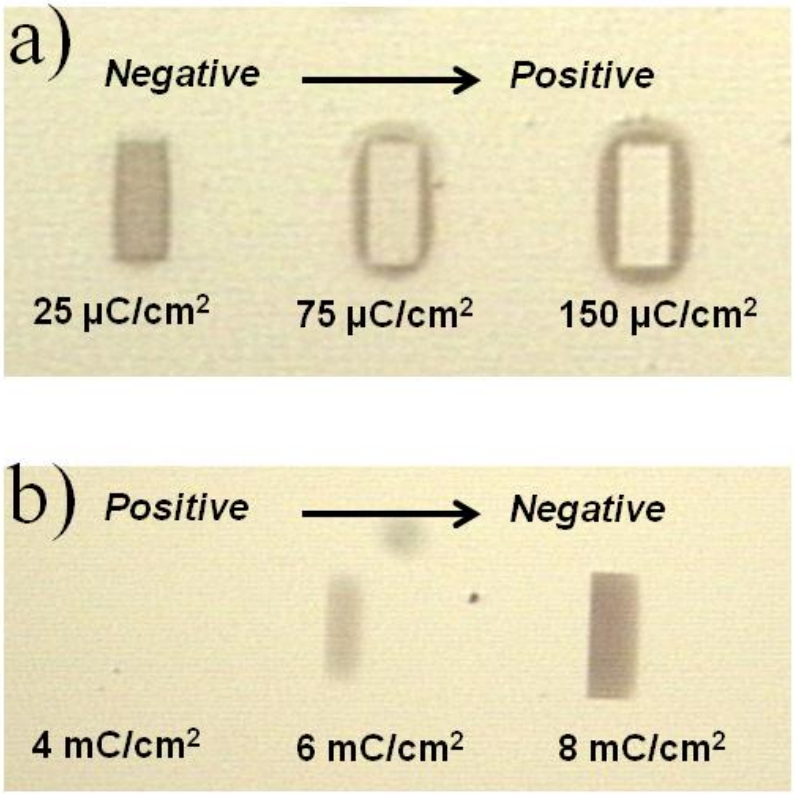

Figure S3. Optical microscope images of 20×50 μm^2 P(MAA-*co*-MAAEMA) rectangles written by EBL at different doses. (a) First tone reversal observed at approximately 100 $\mu C/cm^2$. (b) Second tone reversal observed at approximately 8 mC/cm^2. Acceleration voltage: 50 keV. Electron beam current: (a) 10 pA, (b) 1 nA.

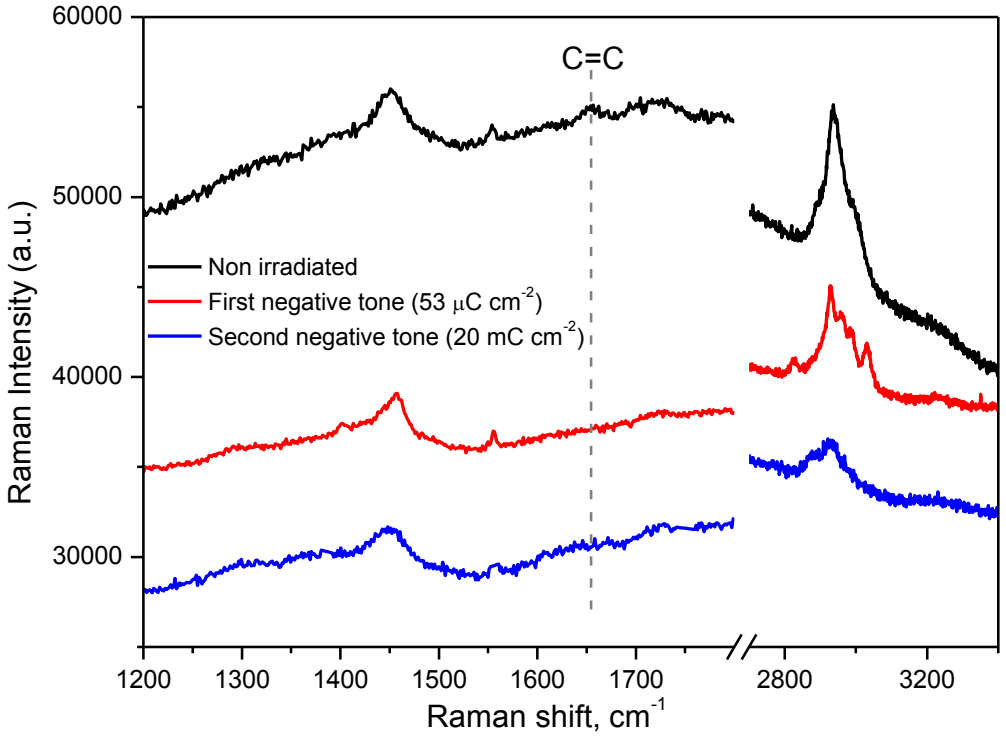

Figure S4. Micro-Raman spectra corresponding to a non irradiated P(MAA-co-MAAEMA) spin coated on a silica wafer (—); a nanostructure fabricated by e-beam direct writing at an electron radiation dose of 53 μC/cm^2 (—); a nanostructure fabricated by e-beam direct writing at an electron radiation dose of 20 mC/cm^2 (—).

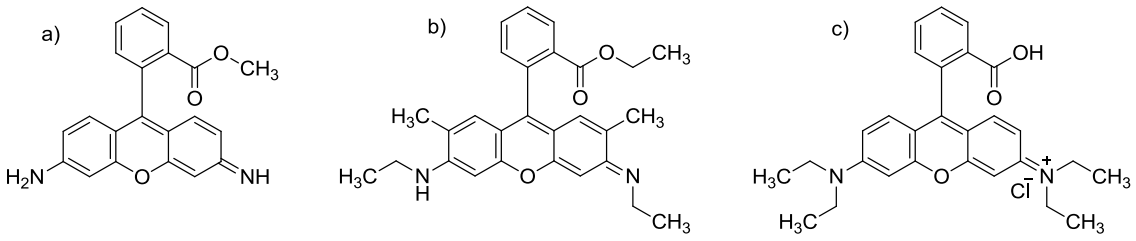

Figure S5. Chemical structure of: a) rhodamine 123 (R123); b) rhodamine 6G (R6G) and, c) rhodamine B (RB).