Supporting Information

Chloroquine-containing HPMA Copolymers as Polymeric Inhibitors of Cancer Cell Migration Mediated by the CXCR4/SDF-1 Chemokine Axis

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Experimental section

Chemicals

Hydroxychloroquine sulfate and methacryloyl chloride were obtained from ACROS Organics (Fair Lawn, NJ). Triethylamine was purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis of methacryloyl chloroquine (MA-CQ)

Hydroxychloroquine sulfate (3.0 g, 6.9 mmol) was dissolved in water (20 mL), to which the ammonium hydroxide (30 % aq., 2.5 mL, 21 mmol) was added dropwise while vigorously stirring for 30 min. Dichloromethane (DCM) (20 mL) was added and the mixture was continuously stirred for 10 min to dissolve HCQ. The mixture was then transferred to a separatory funnel. DCM layer was drained and another 10 mL of DCM was added to extract the HCQ. The DCM layers were combined and washed with brine (20 mL). After drying with anhydrous sodium sulfate, the DCM was evaporated under reduced pressure and viscous colorless paste was obtained (1.38 g, 59 %). ^{1}H NMR (Bruker-AVIII-500 MHz, Chloroform-*d*) δ 8.48 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 2.1 Hz, 1H), 7.76 (d, *J* = 8.9 Hz, 1H), 7.31 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.38 (d, *J* = 5.4 Hz, 1H), 5.19 (d, *J* = 7.7 Hz, 1H), 3.73 – 3.66 (m, 1H), 3.63 (s, 1H),

3.57 (td, J = 5.6, 1.2 Hz, 2H), 2.63 – 2.54 (m, 6H), 1.76 – 1.69 (m, 1H), 1.65 – 1.54 (m, 3H), 1.30 (d, J = 6.4 Hz, 3H), 1.01 (t, J = 7.1 Hz, 3H).

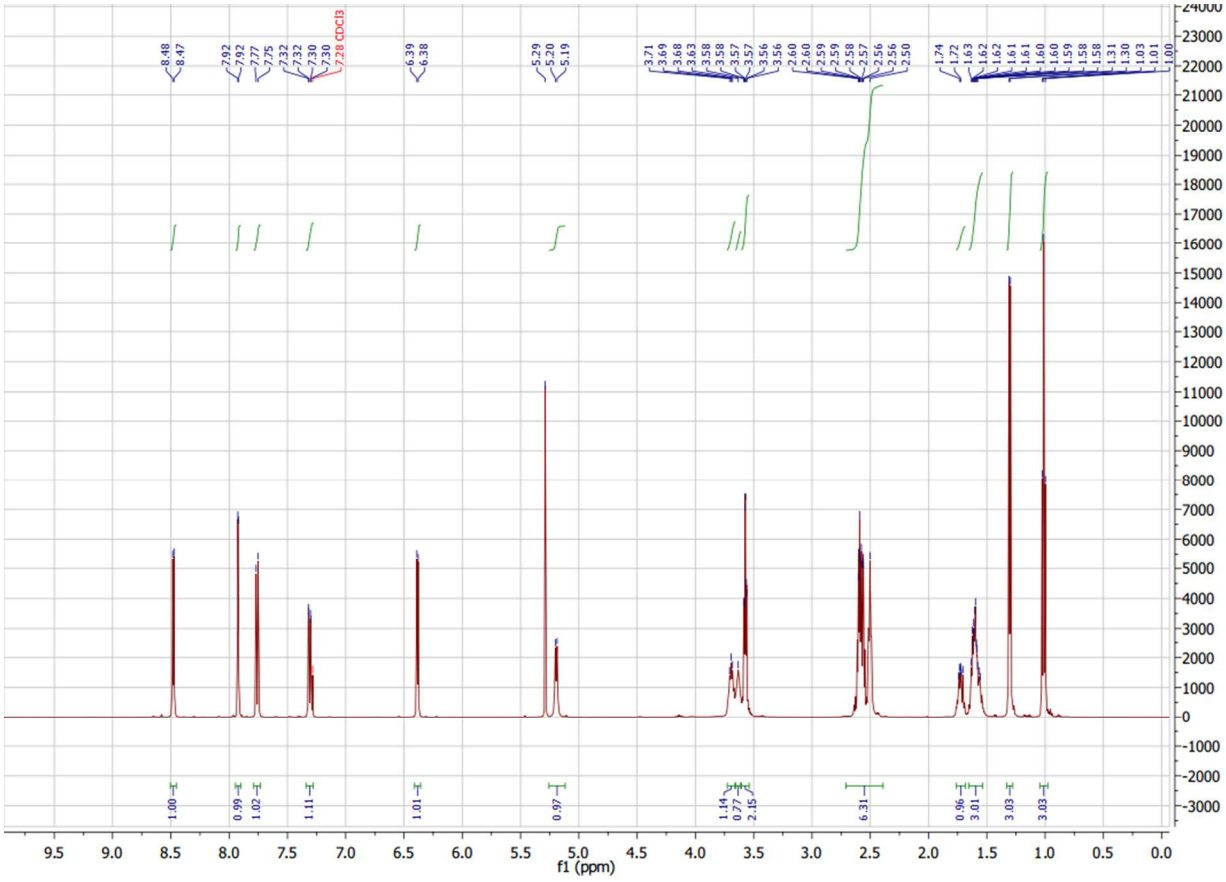

Figure S1. ^{1}H-NMR of hydroxychloroquine (HCQ).

HCQ (2.0 g, 5.95 mmol) and triethylamine (1.92 g, 2.4 mL, 19 mmol) were dissolved in chloroform (100 mL) and cooled down in the ice bath. Methacryloyl chloride (1.99 g, 1.84 mL, 19 mmol) was dissolved in anhydrous chloroform (100 mL) and added to the HCQ dropwise with vigorously stirring at 0 °C. The mixture was stirred overnight, followed by washing with saturated sodium carbonate (2 × 50 mL) and brine (50 mL). The resulted organic layer was concentrated and purified by silica gel chromatography (10 : 1 dichloromethane : methanol) to give the MA-CQ (0.96 g, 40 %) as a light yellow paste. ^{1}H NMR (499 MHz, Chloroform-*d*) δ 8.46 (d, J = 5.5 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.33 (dd, J = 8.9, 2.2 Hz, 1H), 6.41 (d, J = 5.6 Hz, 1H), 6.07 (s, 1H), 5.51 (s, 1H), 5.43 (d, J = 7.4 Hz, 1H), 4.21 (td, J = 6.1, 2.1 Hz, 2H), 3.71 (m, 1H), 3.41 (s, 1H), 2.75 (t, J = 6.2 Hz, 2H), 2.64 – 2.40 (m, 4H), 1.90 (s, 3H), 1.74 – 1.51 (m, 4H), 1.31 (d, J = 6.3 Hz, 3H), 1.01 (t, J = 7.1 Hz, 3H).

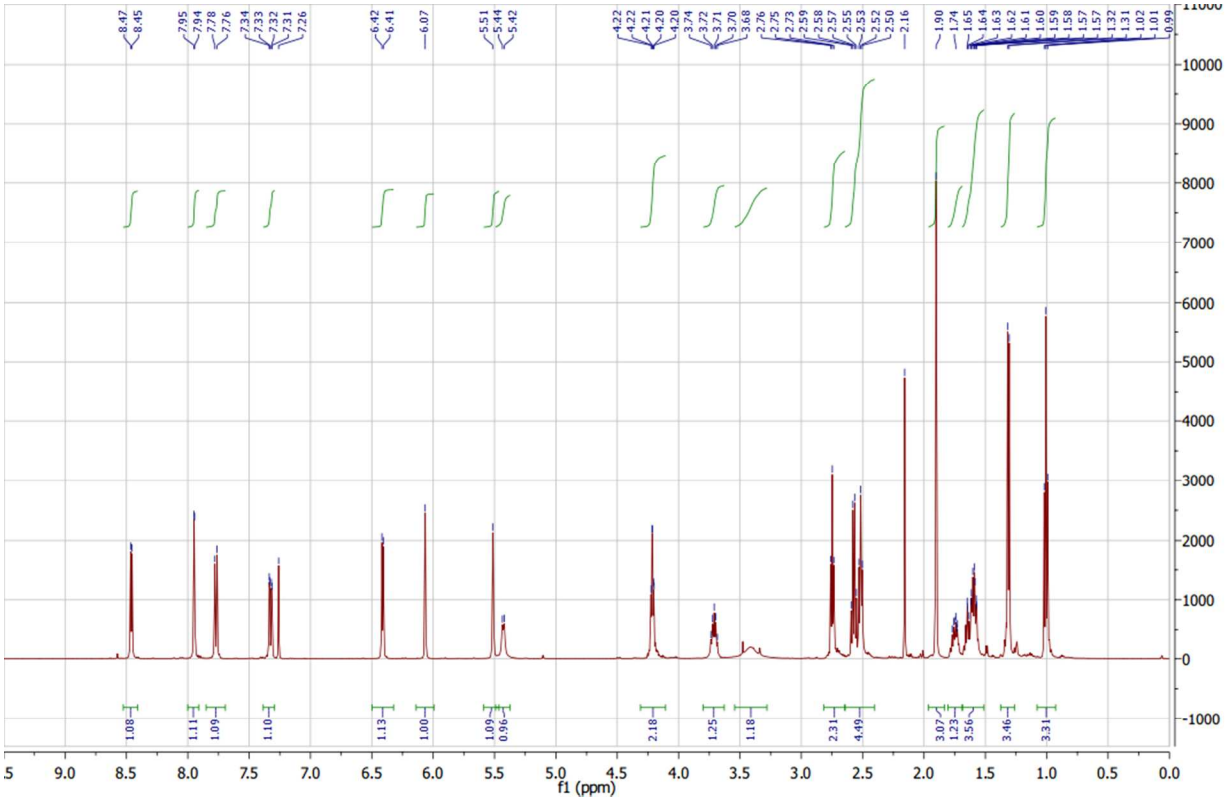

Figure S2. ^{1}H-NMR of MA-CQ.

Synthesis of pCQ

The typical polymerization procedure is shown below. MA-CQ (31 mg, 0.0767 mmol), HPMA (110 mg, 0.767 mmol) and AIBN (7 mg, 0.042 mmol) were dissolved in methanol (1 mL) under N_2 and stirred at 55 °C overnight. The polymer was precipitated out by adding the mixture to cold diethyl ether under vigorous stirring. The precipitate was centrifuged and re-dissolved in methanol, followed by precipitation in diethyl ether. The white solid was collected and dialyzed against water (membrane cut-off MW: 8000) for 2 days. White solid (pCQ-10.0, 79 mg, 56 %) was obtained after freeze-drying. The content of HCQ was calculated by the integration of protons on HCQ (δ 8.38, 7.77, 6.53) and HPMA (δ 4.70, 3.68, 2.91):

mol% of HCQ of pCQ-10.0=[(1.87+1+1.1)/4]/ [(1.87+1+1.1)/4+(8.91+9.77+16.74)/4]=10.0%

The pCQ-16.7 (62 mg, 61 %) was synthesized using MA-CQ (40 mg, 0.099 mmol), HPMA (57 mg, 0.4 mmol) and AIBN (4 mg, 0.025 mmol) following the procedure above.

mol% of HCQ of pCQ-16.7=[(1.66+1+0.99)/4]/ [(1.66+1+0.99)/4+(4.59+5.26+8.50)/4]=16.7%

The molecular weight of pCQ was analyzed by gel permeation chromatography (GPC) operated in 0.1 M sodium acetate buffer (pH 5.0) using Agilent 1260 Infinity LC system equipped with a miniDAWN TREOS multi-angle light scattering (MALS) detector and a Optilab T-rEX refractive index detector from Wyatt Technology (Santa Barbara, CA). The column TSKgel G3000PWXL-CP (Part No. 0021873, Tosoh Bioscience LLC, King of Prussia, PA) was used at a

flow rate of 0.5 mL/min. Results were analyzed using Astra 6.1 software from Wyatt Technology. The degree of polymerization was calculated based on GPC and ^{1}H NMR.

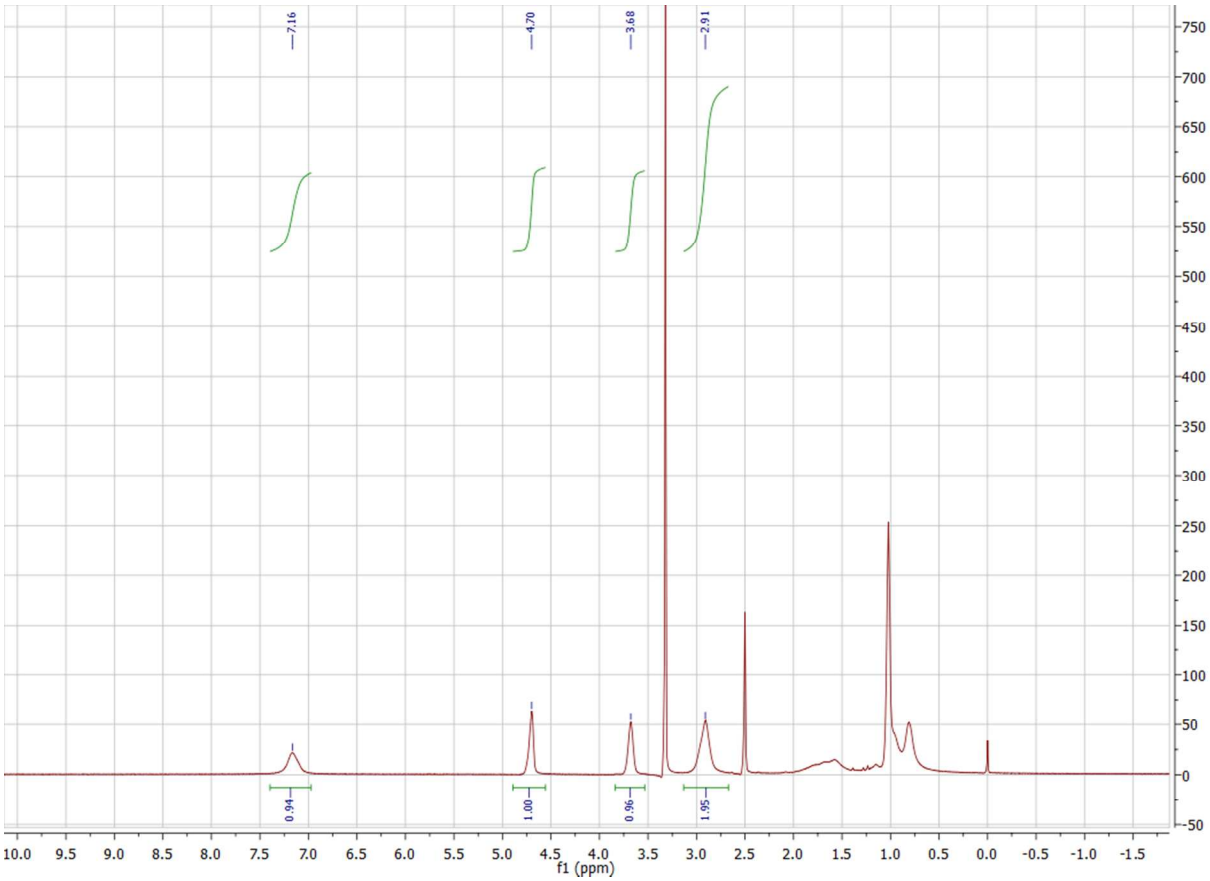

Figure S3. ^{1}H-NMR of pHPMA.

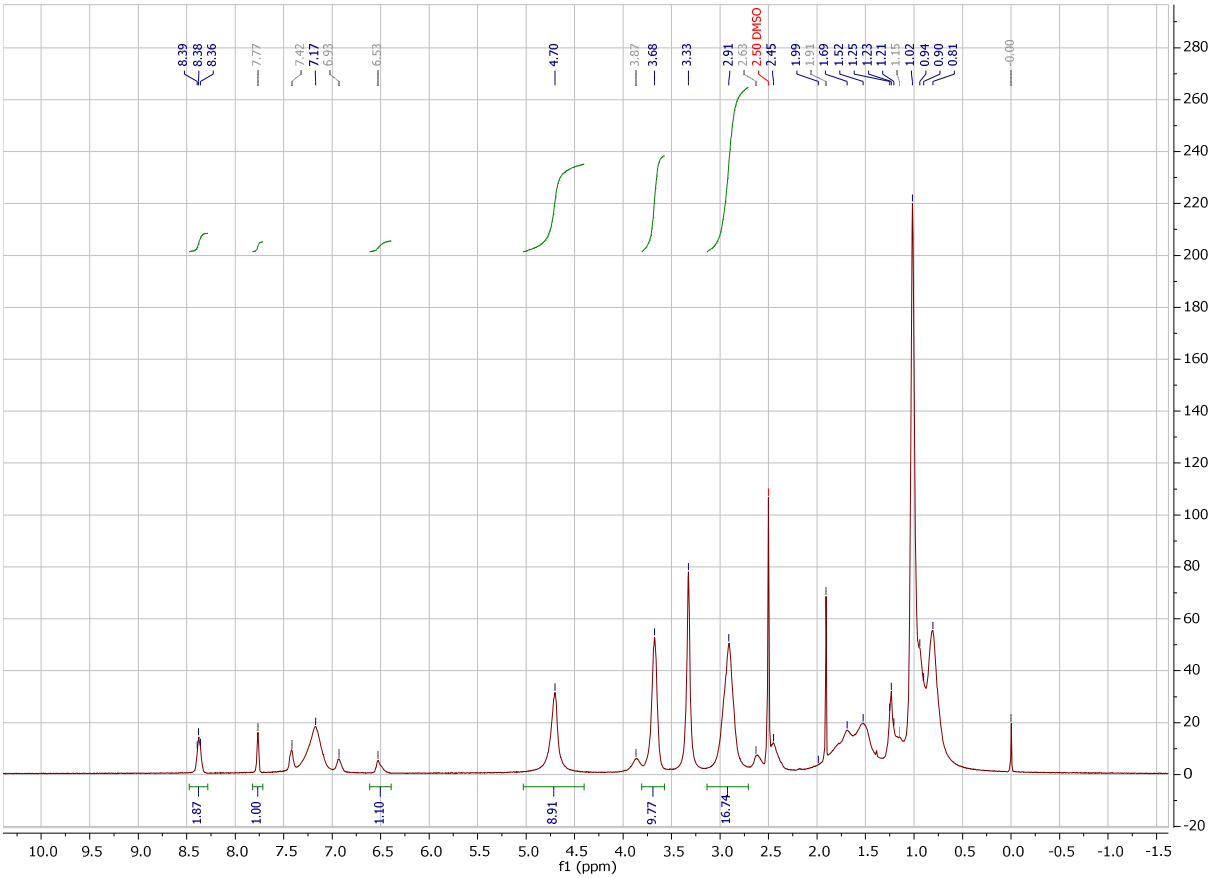

Figure S4. ^{1}H-NMR of pCQ-10.0.

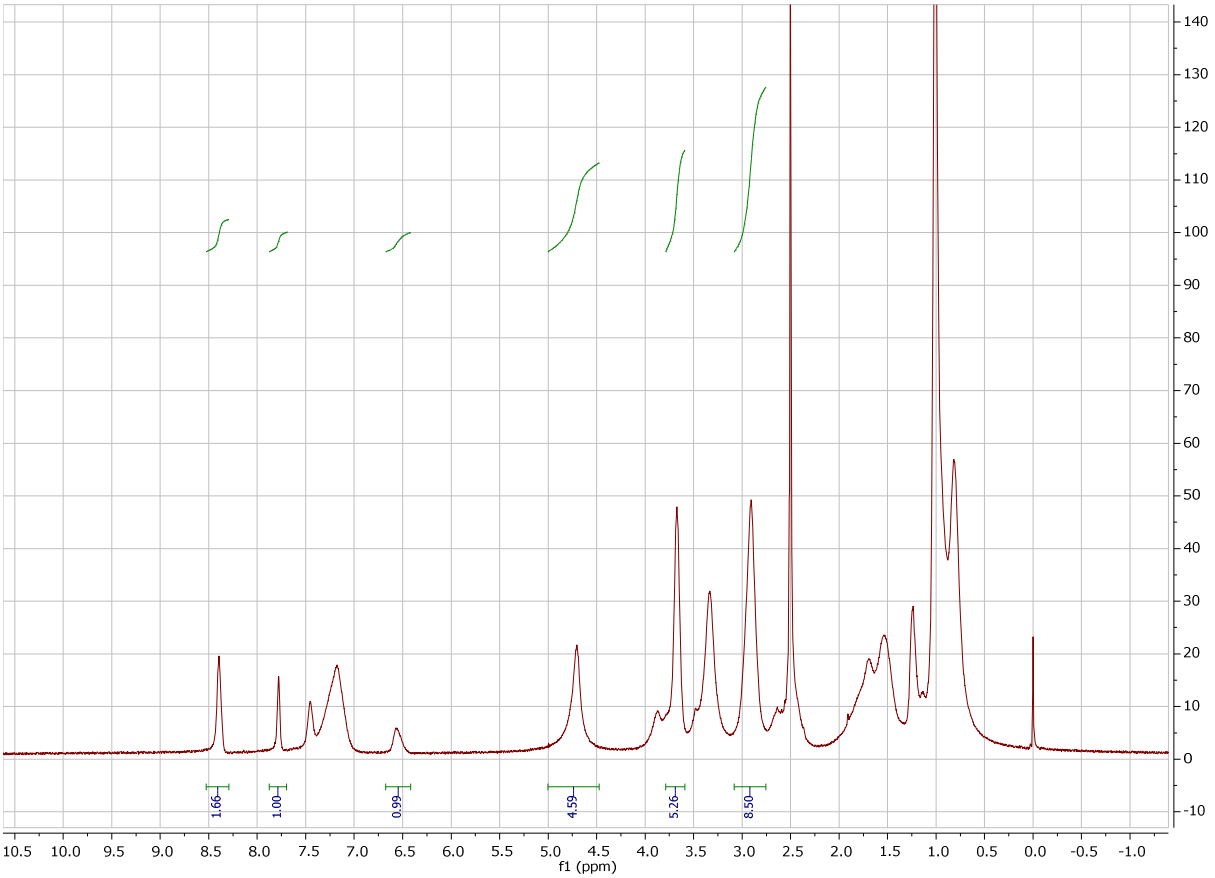

Figure S5. ^{1}H-NMR of pCQ-16.7.

Cell culture

Jurkat and Raji cells were from Dr. Michael Green. The cells were cultured in RPMI-1640 (Thermo Scientific, Waltham, MA) supplemented with 10% FBS (Atlanta Bio., Flowery Branch, GA). The cells were maintained at 37°C with 5% CO2 in a humidified incubator.

Cytotoxicity

Cytotoxicity of pCQ was evaluated by Celltiter-Blue cell viability assay (Promega) in Jurkat and Raji cells. The cells were seeded in 96-well microplates at a density of 10,000 cells per well with 200 µL of serial dilutions of drugs in serum-supplemented medium. After 24 h incubation, 40 µL of Celltiter-Blue reagent was added to each well and incubated for 1 h. The fluorescence intensity [I] was measured using SpectraMaxM5e Multi-Mode microplate Reader (Molecular Devices, CA) at $579_{Ex}/584_{Em}$. The relative cell viability (%) was calculated as $[I]_{sample}/[I]_{treated} \times 100$ %.

Flow cytometry

Jurkat and Raji cells (2×10^{6} mL^{-1}) were treated with drugs in serum-free media for 24 h before sorting. Cells were stained with APC Mouse B Anti-Human CD184 and APC Mouse IgG2a, κ

Isotype Control (BD Biosciences) according to the suggested protocol. Samples were analyzed using FACSCalibur, and data were processed using FlowJo software.

Western blot

Jurkat cells (2×10^6 mL^{-1}, 5 mL) were treated with or without drugs in serum-free media for 4 h in incubator. After that, the cells were centrifuged and treated with SDF-1 (10 nM, 5 mL) for 10 min in incubator. Cells were lysed by RIPA buffer with EDTA and Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, Waltham, MA), and total protein was extracted by lysis with Laemmli buffer (Bio-rad, Hercules, CA) according to the suggested protocol. Protein (30 μg) was separated by SDS/PAGE and transferred to nitrocellulose membranes that were probed with pERK (Cell Signaling, Beverly, MA) and incubated with anti-rabbit IgG HRP-linked antibody (Cell Signaling, Beverly, MA). The membranes were visualized by enhanced chemiluminescence. The results were quantified by ImageJ.