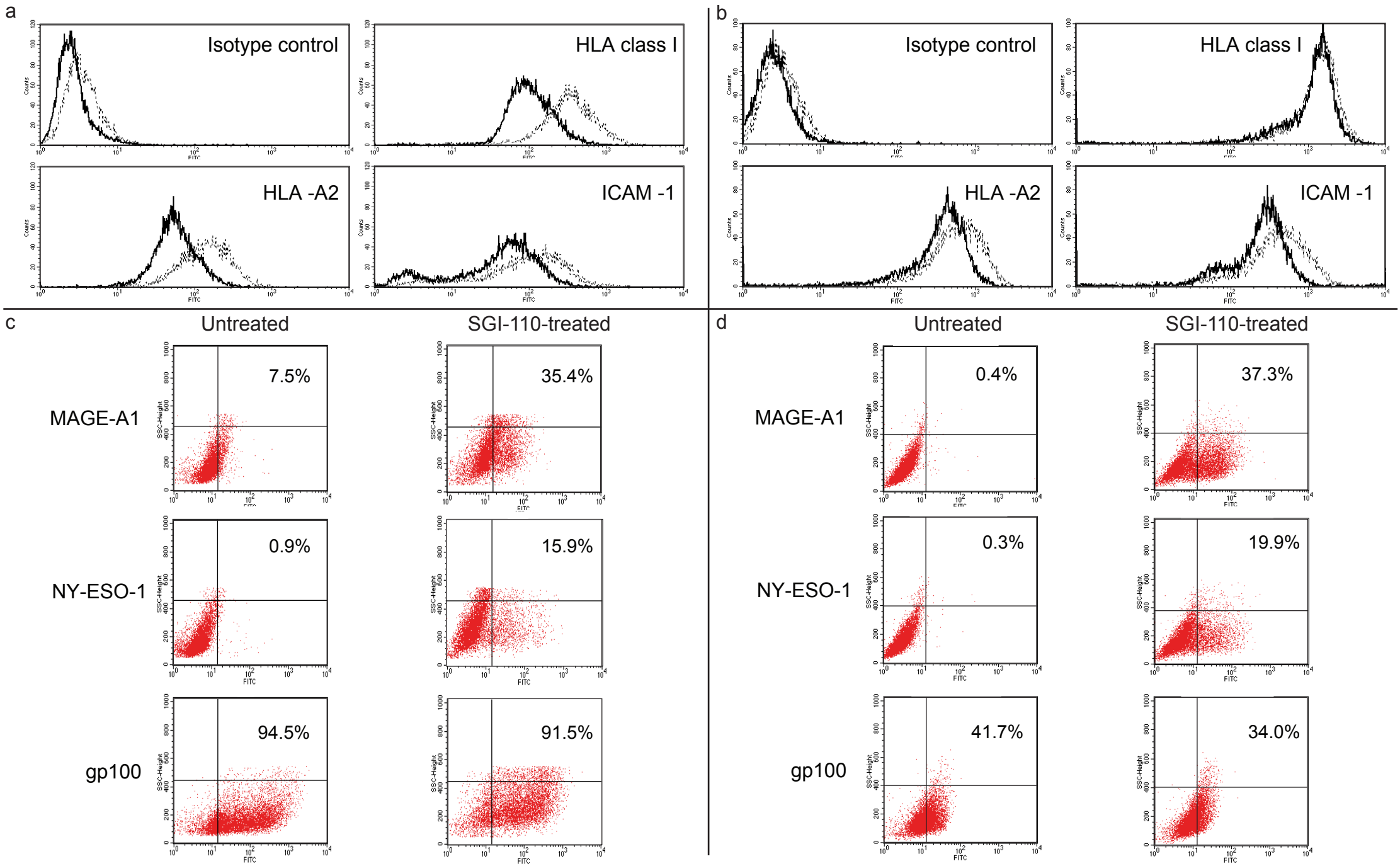
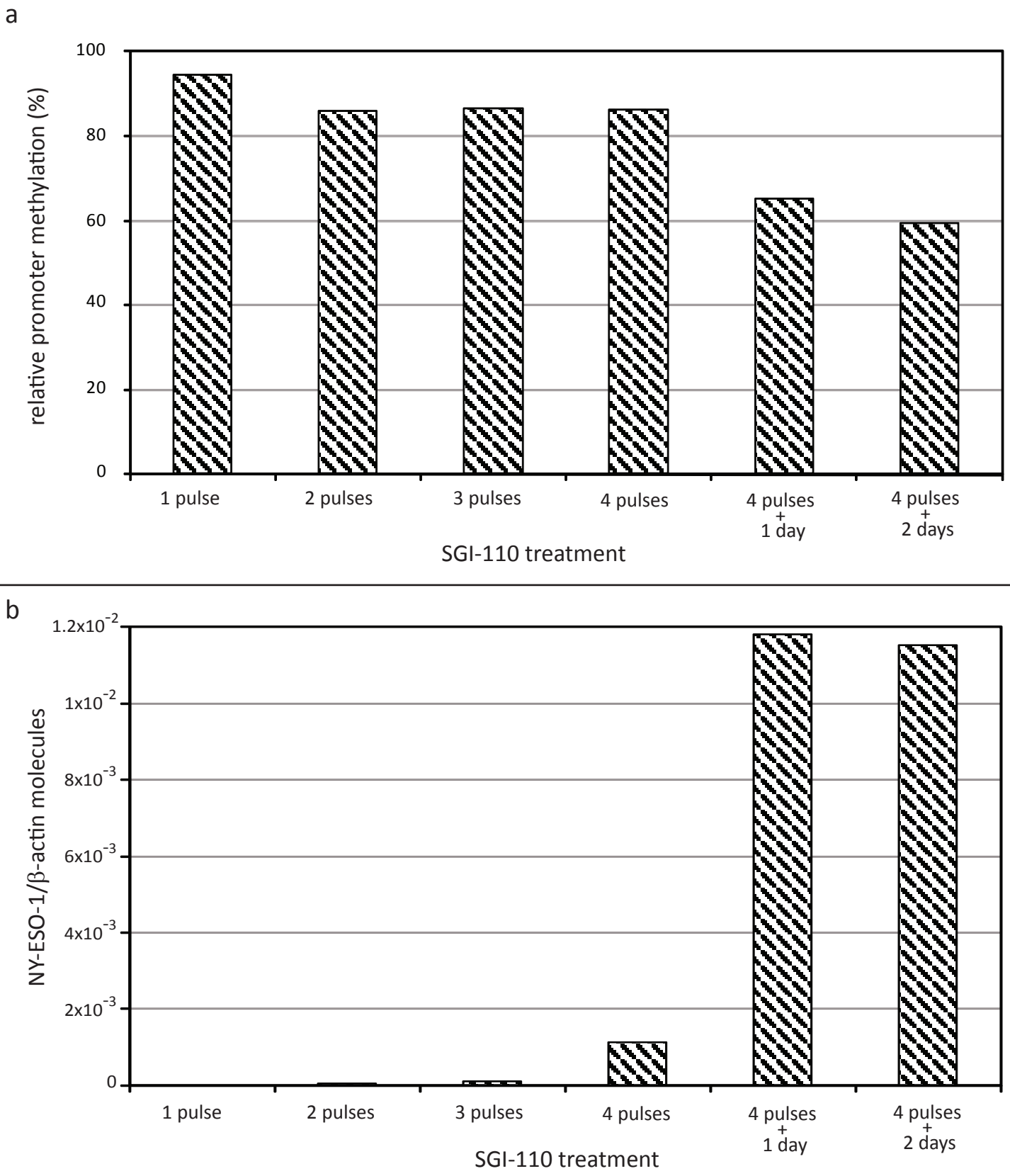


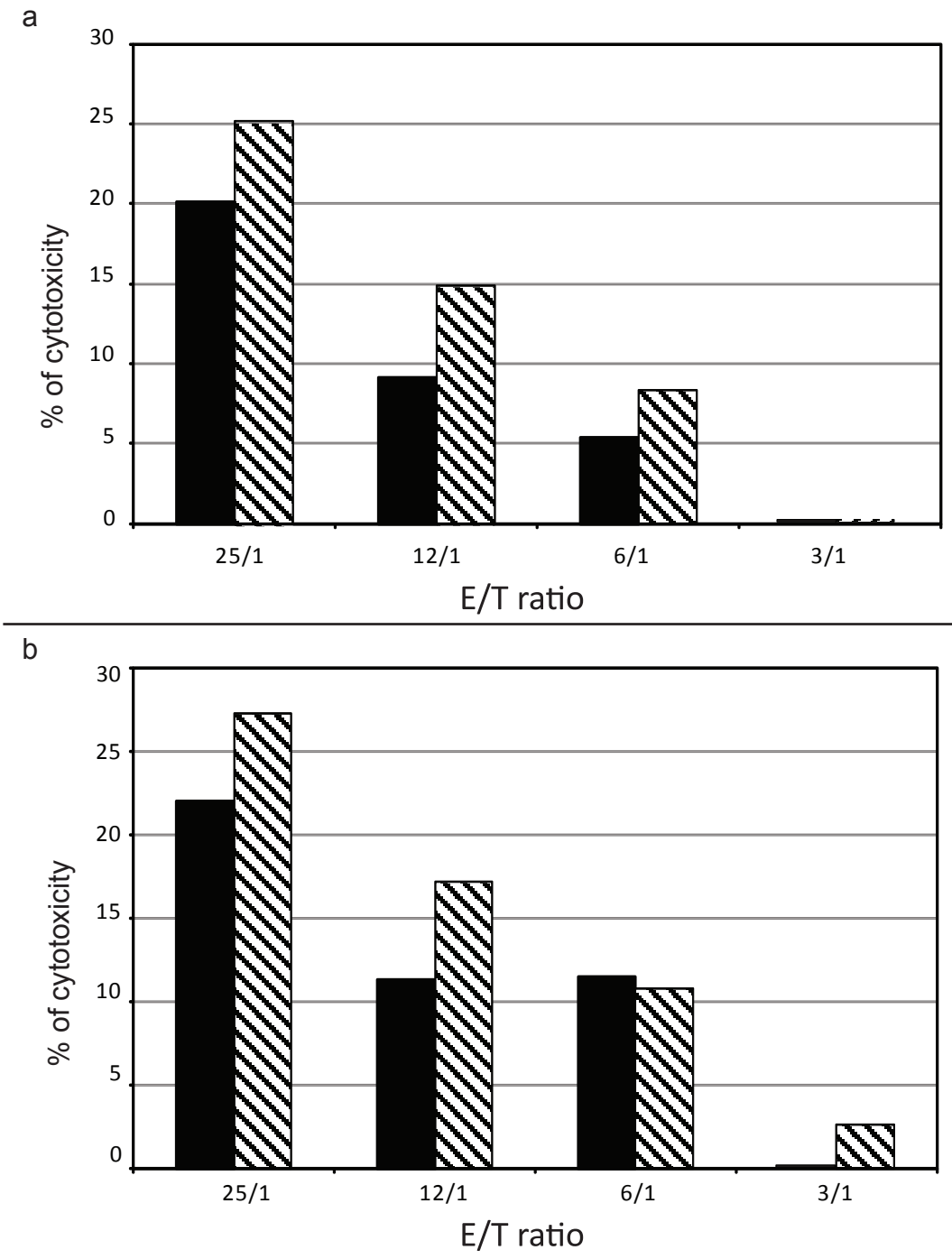
Supplementary Figure 1S. Indirect immunofluorescence analysis of the antigenic profile of SGI-110-treated cancer cells.
To further evaluate the immunomodulatory activity of SGI-110 in cancer cells, Mel 531 (a) and MZ-1257 (b) cells untreated (solid line) and treated with 1 μM SGI-110 (dashed line) were sequentially incubated with the isotype-matched mouse Ig, the anti-HLA class I mAb W6/32, the anti-HLA-A2 mAb BB7.2 or the anti-ICAM-1 mAb 84H10, and with FITC-conjugated F(ab')2 fragments of rabbit anti-mouse Ig. Cells were then analyzed by flow cytometry. Paraformaldehyde-fixed and permeabilized Mel 531 (c) and MZ-1257 (d) cells, either untreated or treated with 1 μM SGI-110, were sequentially incubated with the anti-MAGE-A1 mAb MA454, the anti-NY-ESO-1 mAb D8.38, or the anti-gp100 mAb HMB-45 and with FITC-conjugated F(ab')2 fragments of rabbit anti-mouse Ig, followed by flow cytometry. The reported numbers represent the percentage of TAA-positive cells in upper and lower right quadrants.



Supplementary Figure 2S. Kinetic study of CTA promoter hypomethylation and CTA expression induced in cancer cells throughout SGI-110 treatment

a) Genomic DNA was extracted from Mel 195 cells untreated and treated with 1μM SGI-110, at different time-points during the treatment. Real-time qMSP analysis of NY-ESO-1 promoter was performed on bisulfite-modified genomic DNA using methylated- or unmethylated-specific primer pairs. The percentage of promoter methylation was defined as the ratio between methylated molecules and the sum of methylated and unmethylated molecules. Data are reported as relative percentage of promoter methylation in SGI-110-treated cells vs. untreated ones, at different investigated time-points.

b) Total RNA was extracted from Mel 195 cells untreated and treated with 1μM SGI-110, at different time-points during the treatment. Quantitative RT-PCR reactions were performed on retrotranscribed total RNA, utilizing NY-ESO-1- and β-actin-specific primers. CTA expression was normalized to the expression of the β-actin gene. Values are reported as CTA molecules/β-actin molecules.



Supplementary Figure 3S. Susceptibility of SGI-110-treated melanoma cells to lysis by HLA-A2-restricted gp-100-specific CTL.

To further evaluate whether exposure to SGI-110 was effective in increasing immune recognition of cancer cells, cytotoxicity of HLA-A2-restricted gp100-specific CTL was tested by LDH-release assay against HLA-A2- and gp100-positive Mel 531 (a) and Mel 603 (b) melanoma cells, untreated (black columns) or treated (striped columns) with 1μM SGI-110, at effector/target (E/T) ratios of 25/1, 12/1, 6/1 and 3/1.