

YMTHE, Volume 26

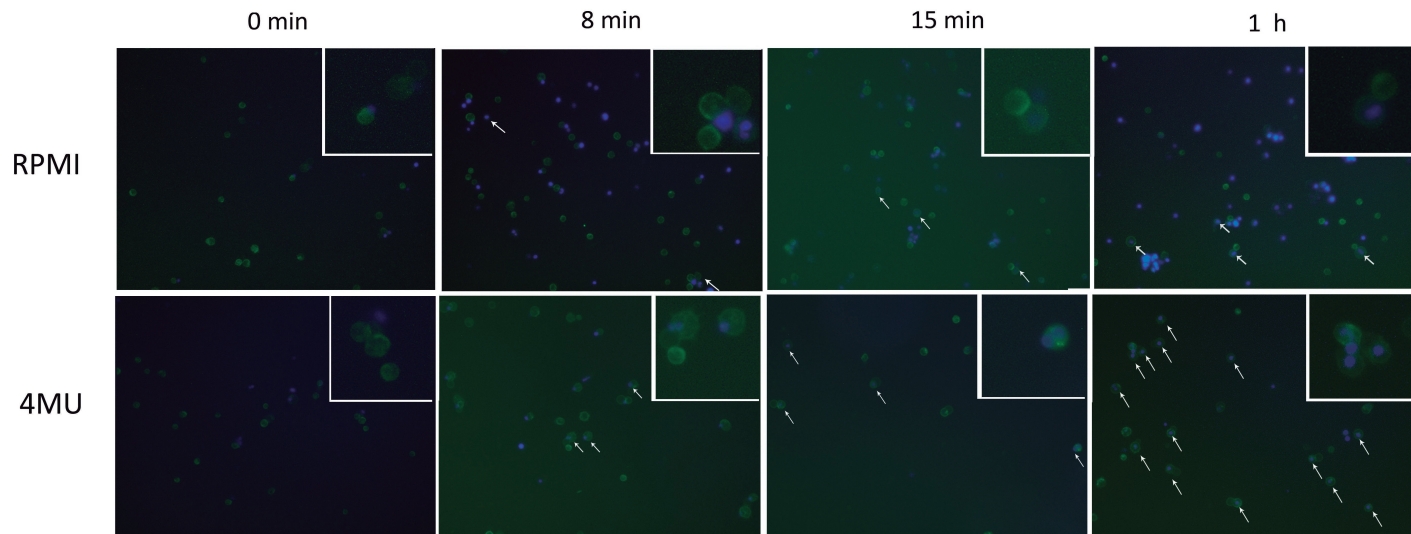
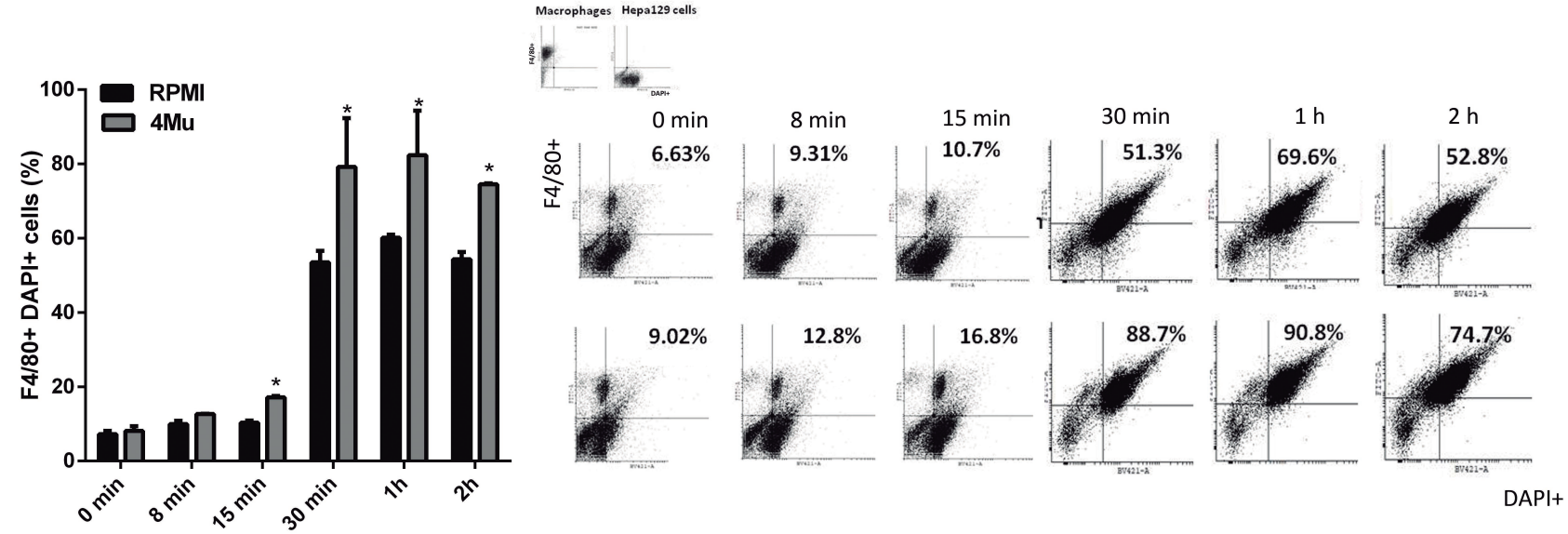
Supplemental Information

4Mu Decreases CD47 Expression on Hepatic Cancer

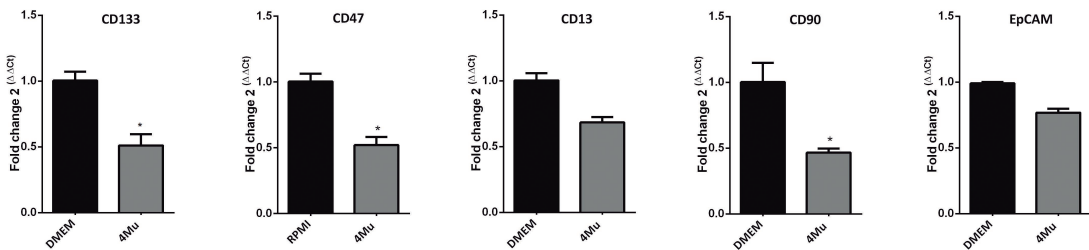
Stem Cells and Primes a Potent Antitumor T Cell

Response Induced by Interleukin-12

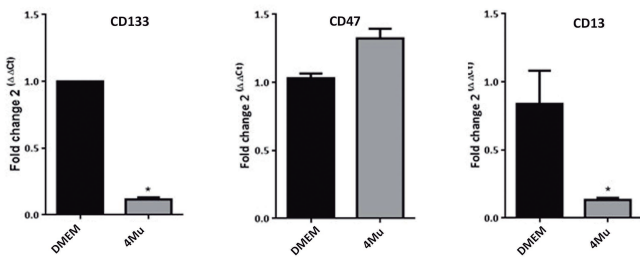
Marcelo M. Rodríguez, Esteban Fiore, Juan Bayo, Catalina Atorrasagasti, Mariana García, Agostina Onorato, Luciana Domínguez, Mariana Malvicini, and Guillermo Mazzolini



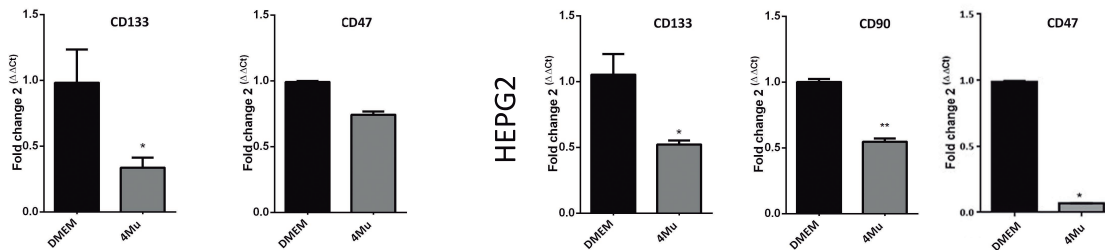
Hepa 1.6



BNL



HuH7



HEPG2

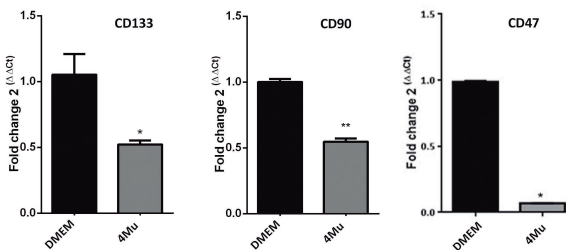


Figure S2

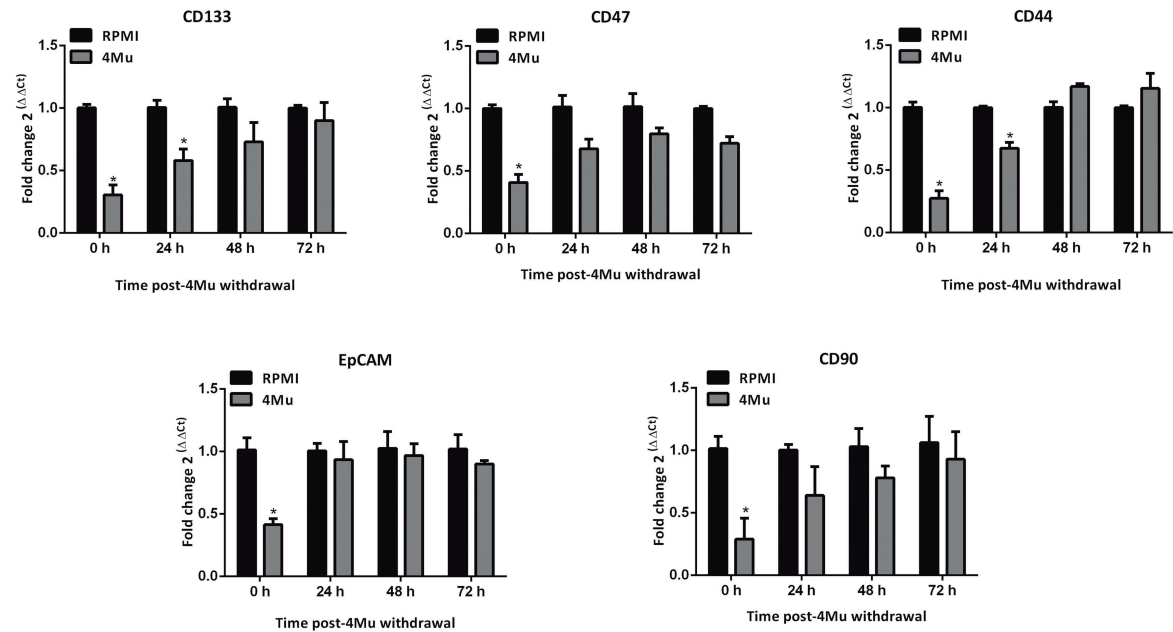


Figure S3

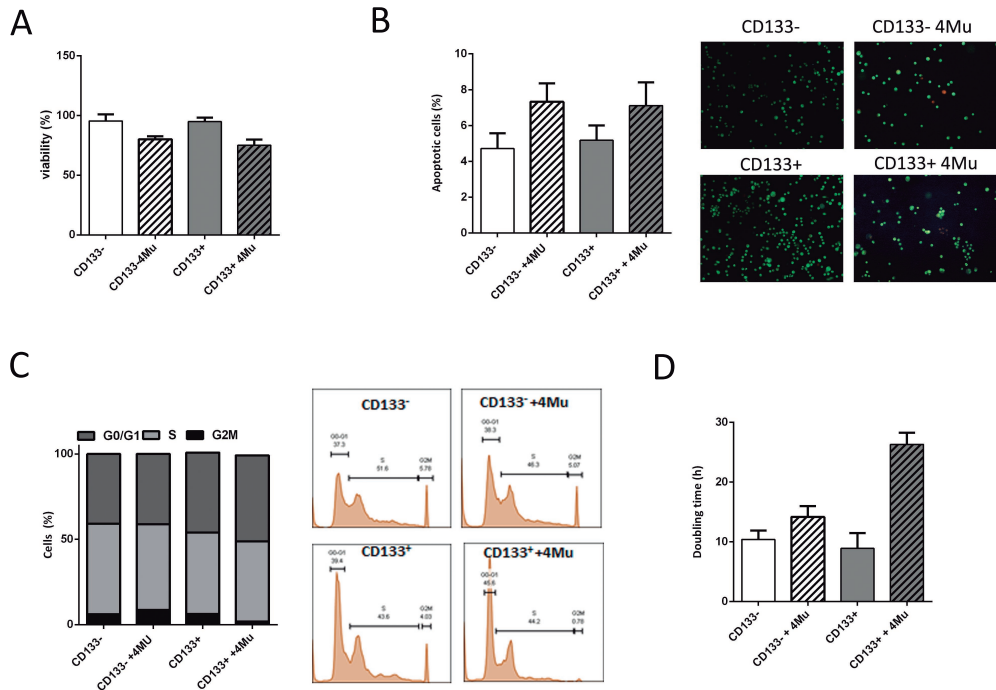


Figure S4

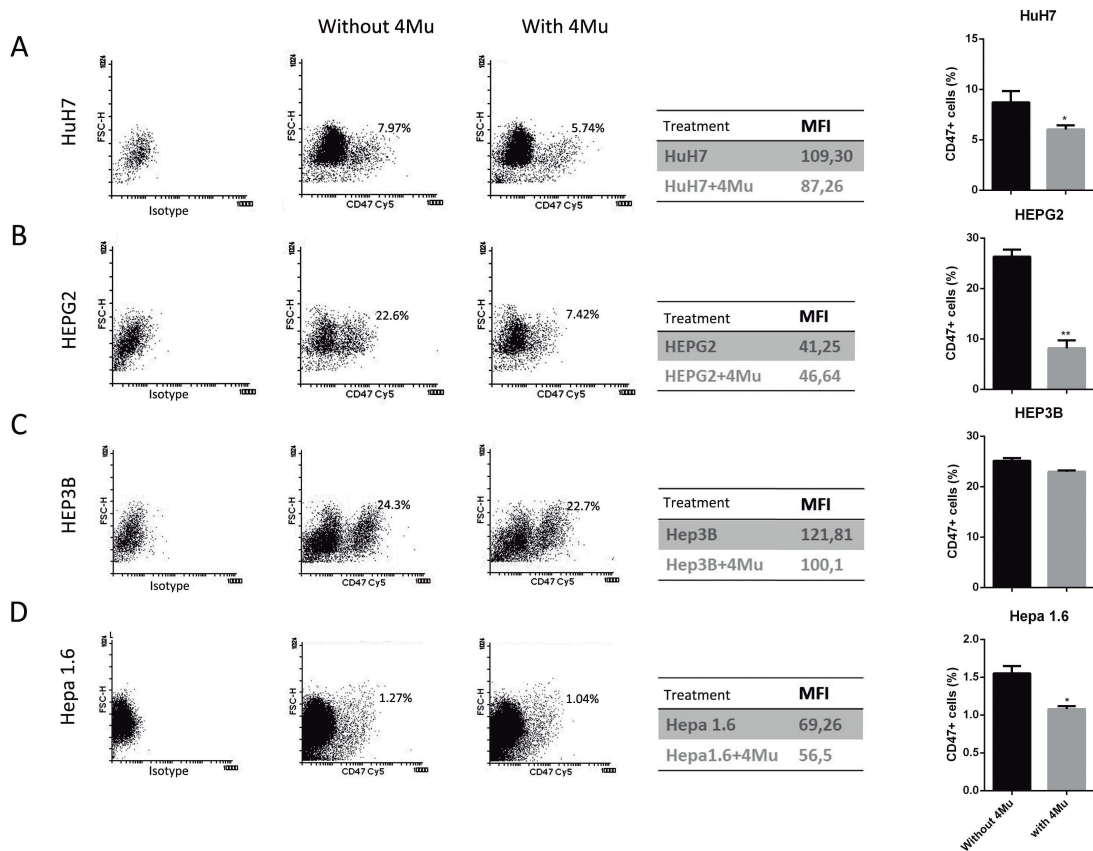


Figure S5

Supplementary Figure 1. Kinetic of HCC cells phagocytosis.

A) Hepa 129 cells untreated or treated with 0.5mM 4Mu were incubated with macrophages and analyzed by flow cytometry (F4/80+DAPI+) at different time points. * $p < 0.05$ 4Mu vs. RPMI at 15min, 30 min, 1h and 2h after co-culture of Macrophages with Hepa129 cells. Two-way ANOVA, Sidak's multiple comparisons B) Dot plots for each assayed time. Small dot plot corresponds to control Hepa 129 cells (DAPI) or macrophages alone (F4/80+) test. C) Fluorescence microscopy analysis showed phagocytosis at early times.

Supplementary Figure 2. Decreased mRNA levels of CSCs markers on murine and human HCC lines induced by treatment 4Mu *in vitro*. CSC markers profile was determined in murine Hepa1.6 and BNL cells as well as in human HepG2 and HuH7 HCC cell lines. Real-time qPCR showed reduced mRNA levels of CD133, CD13, CD90, EpCAM and CD47 when HCC cells were exposed to 0.5mM 4Mu for 72h.

Supplementary Figure 3. mRNA levels of CSCs markers on Hepa129 cells are restored after 4Mu withdrawn *in vitro*. CSC markers profile was determined on murine Hepa129 cells by Real-time qPCR after 4Mu withdrawn. mRNA levels of CD133, CD44, CD90, EpCAM and CD47 were analyzed at 24, 48 and 72h post 4Mu withdrawal. CD133: * $p < 0.05$ RPMI vs. 4Mu at 0h and 24h; CD44: * $p < 0.05$ RPMI vs. 4Mu at 0 and 24h; CD90: * $p < 0.05$ RPMI vs. 4Mu at 0h and; EpCAM $p < 0.05$ RPMI vs. 4Mu at 0h; CD47: $p < 0.05$ RPMI vs. 4Mu at 0h and

Supplementary Figure 4. 4Mu effects on Hepa 129 cells. A) Hepa129 cell viability after 72 h of culture in presence of 0.5mM 4Mu determined by MTT assay. B) Cell cycle was assessed by flow cytometry on CD133+ and CD133- Hepa 129 cells at 72h: 4Mu halted CD133+ Hepa 129 cells at G0/G1, (* $p < 0.05$ Kruskal-Wallis test). C) *In vitro* CD133+ and CD133- Hepa 129 cells apoptosis was assessed using acridin orange/ethidium bromide staining. D) CD133+Hepa 129 cells plus 4Mu showed a one and a half -fold increase in duplication time compared with CD133+/CD133- and CD133- plus 4Mu cells, statistically non significant (Kruskal-Wallis test).

Supplementary Figure 5. CD47 expression levels in human and murine HCC cell lines. A) HuH7; B) HepG2 and C) HEP3B human HCC cell lines or D) murine Hepa1.6

cells were cultured alone or with 0.5mM 4Mu for 72h; CD47 expression was measured by FACS. Representative data showing percentages of CD47+ in HCC cells and mean fluorescence intensity (MFI) was represented. HuH7 and HepG2 *p<0.05; HEP3B **p<0.01 and Hepa1.6 *p<0.05 vs 4Mu (Mann-Whitney test)