Supplementary data

Fig. S1. Exemplary FACS analysis of a HBMC plus HMVEC plus HDF co-culture grown on TCPS. The bulk of the total cell mix was gated in range 1 (R1) (A) excluding most of the debris. In the FL1-FL4 diagram (B) the ranges R2, R3, and R4 were defined in a way that in single cell type cultures more than 96 % of HDF cells were located in R2, more than 90% of HBMC were located in R3 and more than 92% of the HMVEC were located in R4 (some exchange of CFSE/PKH2 of the HBMC towards HMVEC was observed). The percentage of cells in the other regions were determined and used to correct cell counts for the different cell types in co-culture situations. In the FL1-FL2 diagram (C) R5 was defined in a way that 99% of the HBMC control treated cells (absence of first antibody) were located in this region. The bALP stained HBMC outside R5 were defined as being bALP^{+}. R6 represents the region of the bALP+ HBMC which strongly express bALP. The region between R5 and R6 represents the faintly bALP expressing HBMC.

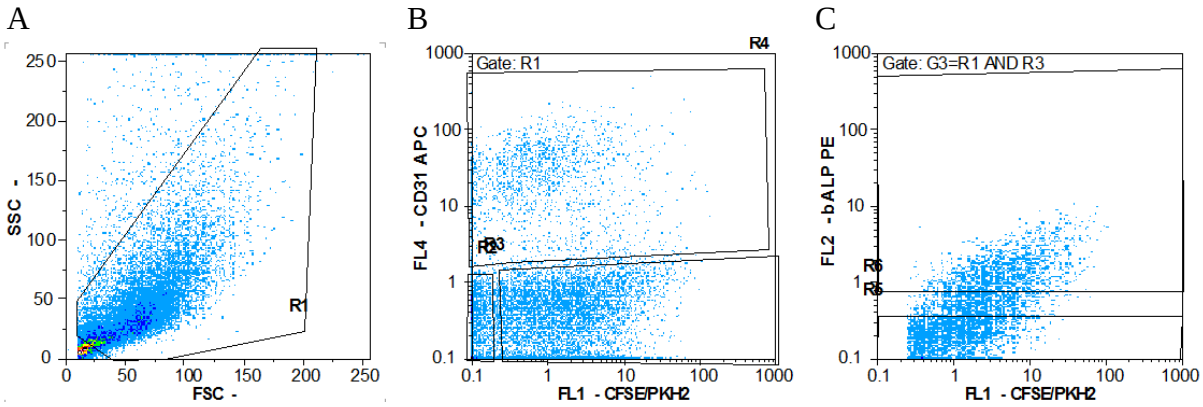
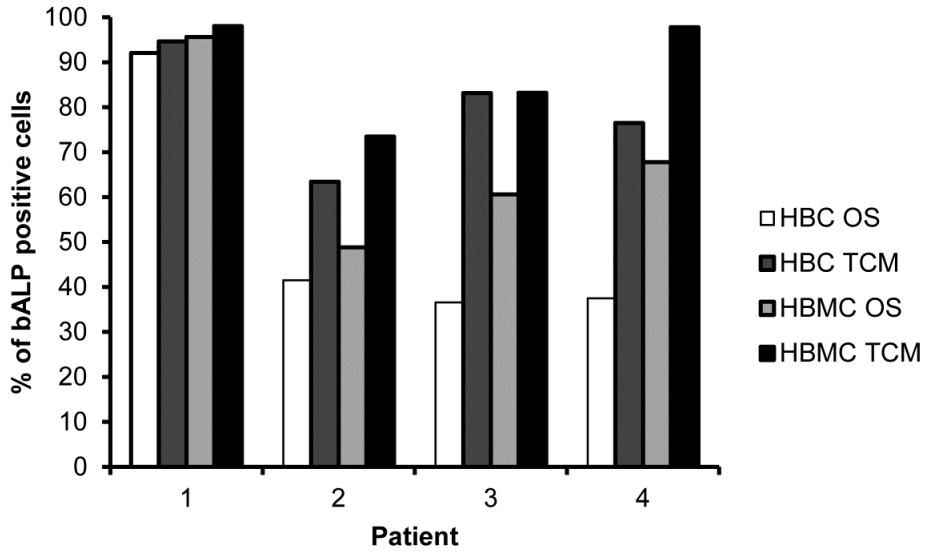


Fig S2. Effect of OS and TCM on expression of bALP. The data of 4 patients are separately shown. A. The percentage of HBMC and HBC being bALP positive. B. Discrimination between faintly and strongly fluorescent bALP positive cells based on cell cultures kept in OS. The range of faintly and strongly fluorescent bALP positive cells is defined by the threshold level where 50% of the cells are located in the faintly and 50% in the strongly fluorescent bALP positive cell range.

A



B

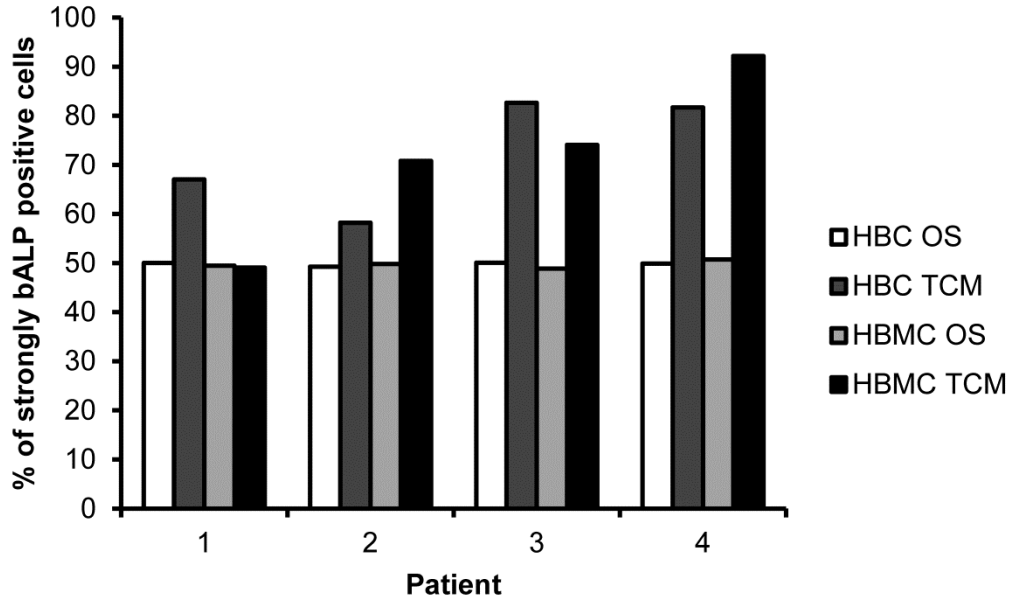
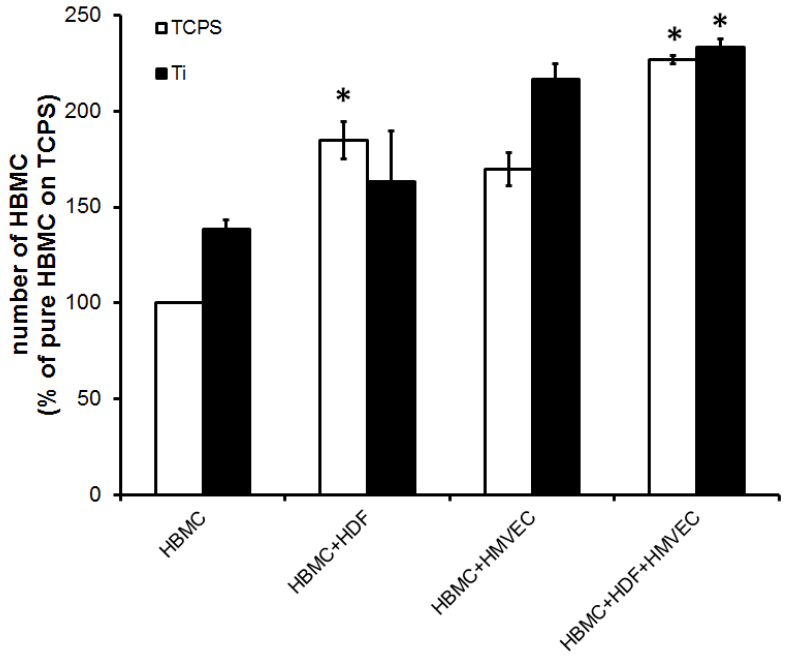
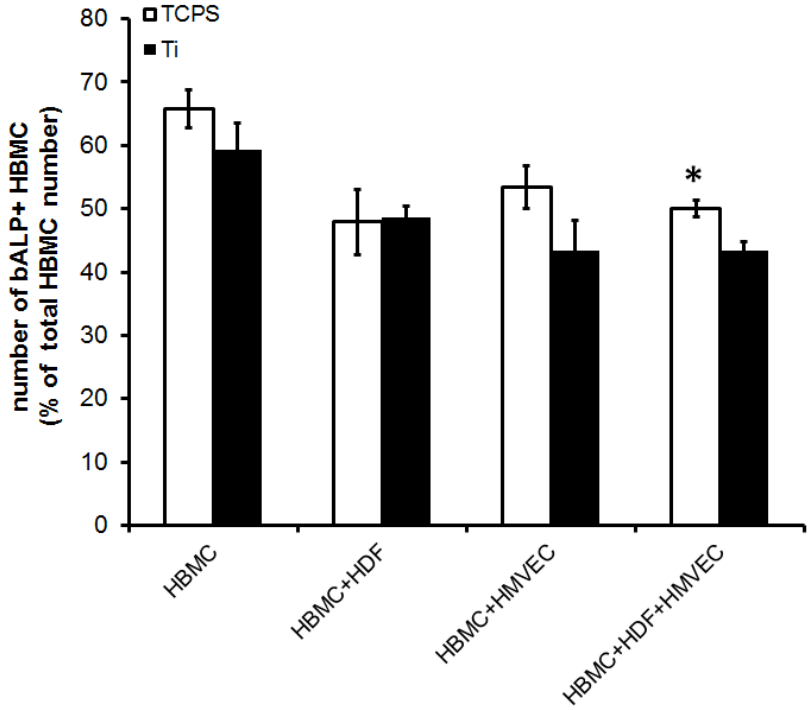


Fig. S3. Effects of substratum and co-culturing on percentage of the total amount of HBMC and HBMC being bALP positive relative to pure HBMC cultures. A: Total number of HBMC in the co-cultures relative to the pure HBMC cultures on TCPS and Ti coated TCPS. B: Percentage of HBMC being bALP+ in pure HBMC and in HBMC-HDF, HBMC-HMVEC and HBMC-HDF-HMVEC co-cultures. Bars represent mean over three independent experiments ± S.E.M. *: significant different from single cell type cultures ($P < 0.05$; two-sided t test; n=3)



A



B

Fig. S4. Flow cytometry analysis of single, double and triple cell type cultures. The number of each cell type was set relative to values of the single cell type cultures (single) kept on Ti. *: Significant effects relative to single cell type culture controls. #: significant different from the single cell type cell type in corresponding triple cell type culture ($P < 0.05$; two-sided t test; n=3).