Supplementary Figures

Figure S1. The HMEC-1 cell line also induced cytoprotection to CLL cells and manifested similar shear-induced changes in endothelial cell markers. (A) CLL cell viability in circulating and static co-culture with HMEC-1 cells. There was no significant difference between CLL viability in circulating culture and in static co-culture with HUVEC cells. All culture conditions were supplemented with IL-4 (5ng/ml). Expression of endothelial cell markers was measured by flow cytometry on HMEC-1 cells grown in both non-circulating static tissue culture flasks and on cells recovered from the hollow fibers after alignment under shear force. Expression of (B) ICAM-1, (C) VCAM-1, (D) PECAM-1 and (E) VEGFR2 were reduced on HMEC-1 cells under shear force.
Figure S2. Increased expression of cell surface markers on CLL cells circulated over endothelial cells is not a specific feature of the HUVEC endothelial cell line.

The alternative endothelial cell line, HMEC-1, was used to line the hollow fibers in the circulating system. CLL cells circulated for 48h over the HMEC cells showed increased expression of (A) CD62L, (B) CXCR4, (C) CD5, (D) CD49d and (E) CD69. Cell surface marker expression was measured by flow cytometry on doublet discriminated, CD19+ lymphocytes and compared with uncirculated CLL cells. Error bars indicate ±1S.D. around the mean fluorescence intensity. N = 6 for all experiments, * P<0.05.
Figure S3. CLL cell migration into the extravascular space displayed rapid kinetics. CLL cells were recovered from the extravascular space after 1h of being introduced into the circulation system. This was further enhanced at 2h and 48h.
Figure S4. CLL cells migrated across HMEC-lined hollow fibers and showed increased expression of CD49d, CD80, and CD38.

The expression of cell surface markers on CLL cells circulating in a bioreactor with hollow fibers lined with HMEC cells and those that had migrated through the hollow fibers into the extravascular space was determined by flow cytometry after 48h of circulation; (A) CD49d, (B) CD80, (C) CD38. Error bars indicate ±1S.D. around the mean fluorescence intensity on a population of doublet discriminated, CD19+ lymphocytes. N = 6 for all experiments; * P<0.05, ** P<0.0001.
Figure S5. Correlations between CLL cell migration and molecules induced by shear force

CLL cells were circulated in a closed system containing hollow fibers lined with HUVEC cells were recovered from both the circulating compartment and the extravascular space. The expression of (A) CD38 (B) CD69 (C) MMP-9 (D) CD80 and (E) CXCR4 was then correlated with the percentage of migration of CLL cells into the extravascular space. (F) CLL cells pre-treated with the anti-CD49d monoclonal antibody and then introduced into the circulating system showed a significant reduction in their capacity to migrate into the extravascular space at 48h but CD49d expression was still correlated with the percentage of migration of CLL cells into the extravascular space.