SUPPLEMENTAL DATA

Supplemental figures :

Supplemental figure 1. Expression of COX enzymes in stromal cells.

COX1 and COX-2 protein expressions were evaluated by western blotting in stromal cells, including HD-RESTO (n=4), FL-RESTO (n=4), FL-MSC (n=5) and HD-MSC (n=4). β-actin was used as a loading control.

Supplemental figure 2. Celecoxib potentiates TRAIL-induced apoptosis, even in the presence of bone marrow stromal cells.

HD-MSC were cultured at $0.5x10^5$ cells/cm². After 3 days, 10^5 cells of NHL-B cells lines were seeded on these stromal cells and stimulated or not with 30µM celecoxib and/or 50ng/ml of TRAIL. B cell apoptosis was analyzed by flow cytometry gating on $CD19^{pos}CD20^{pos}CD105^{neg}$ active capsase-3^{pos} cells. Apoptosis level of B cell lines without stromal cells (figure 2) was reported with dotted lines Mean±SD, n=2.

Supplemental figure 3. Soluble factors effects on B cell survival after TRAIL treatment

(A) SUDHL4 and BL2 cell lines (10^5 cells) were cultured on transwell (black bars) or co-cultured with HD-RESTO or HD-RESTO+B cells under transwell (white and grey bars, respectively). After TRAIL treatment (100ng/ml), indirect survival effect of soluble factors produced by stromal cells were evaluated, compared to co-culture without transwell (shaded bars), Mean±SD, n=5. (B) BL2 and SUDHL4 were cultured with supernatants from HD-RESTO co-cultured or not with B cell lines (white and grey bars respectively) for 24h and treated with or without 100ng/ml TRAIL. These results were compared with B cells co-cultured with HD-RESTO (shaded bars). Mean±SD, n=4. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$.

Supplemental figure 4. Inefficiency of PGE2 as a survival factor.

(A) Expression of the 4 EP receptors (PGE2 receptors) in BL2 and SUDHL4 was evaluated by western blotting. (B) and (C) BL2 and SUDHL4 were cultured with X-VIVO medium and stimulated with PGE2 for 24h. (B) B cell proliferation was evaluated with tritiated thymidine (^{3}H-TdR) incorporation. Results from one of three representative experiments. Mean ± SD. (C) After PGE2 stimulation for 1h, BL2 and SUDHL4 were treated or not with 100ng/ml TRAIL for 24h. Apoptotic B cells were analysed by flow cytometry using $CD19^{pos}$, $CD20^{pos}$ and active caspase-3 staining.

Supplemental material and methods

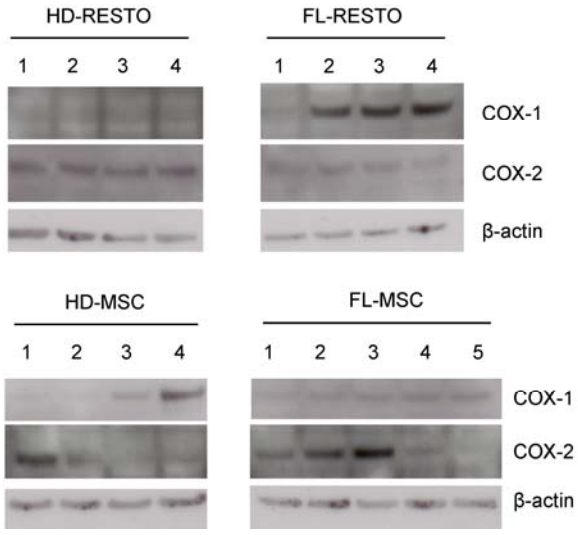
B cell growth assay

B cell growth was assessed by tritiated thymidine (^{3}HTdR, Perkin Elmer) incorporation. After serum deprivation, BL2 and SUDHL4 cell lines were cultured for 24h at 10^{5} cells/ml in X-VIVO 20 medium, notably deprived in prostaglandin (Lonza, Basel, Switzerland). Cells were treated with different concentrations of PGE2 and pulsed with 1µCi/well ^{3}HTdR during the last 16h of treatment.

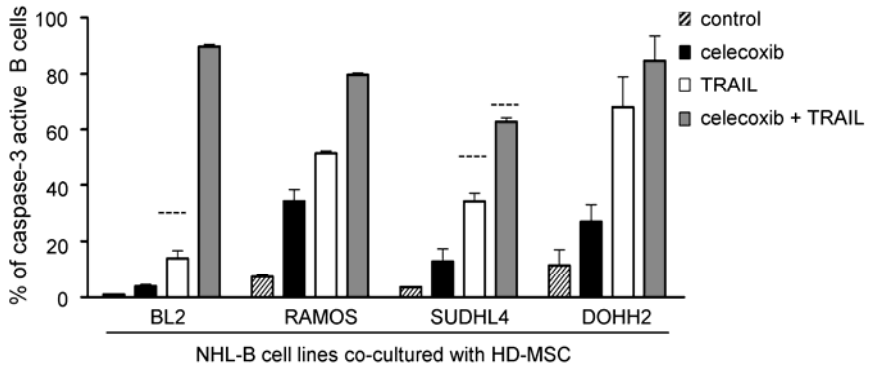
Chemical and antibodies

PGE2 was purchased from Sigma-Aldrich. The anti-COX1, anti-COX-2, EP1, EP2, EP3 and EP4 antibodies were obtained from Cayman chemical (Ann Arbor, Michigan, USA).

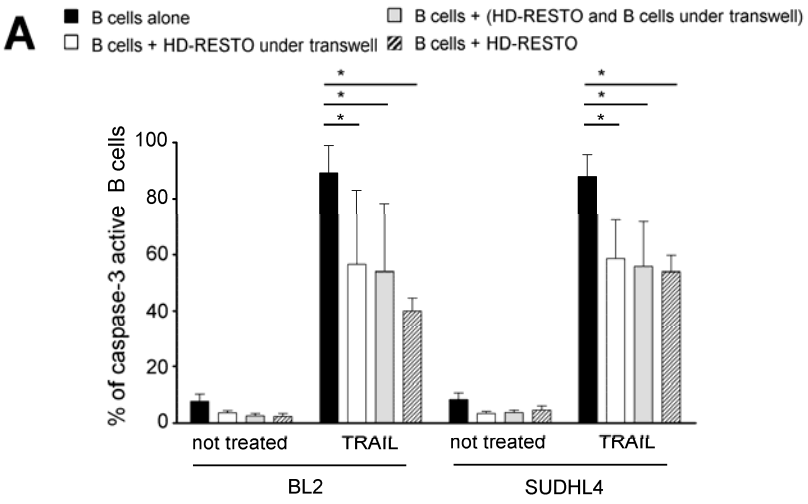
Supplemental figure 1

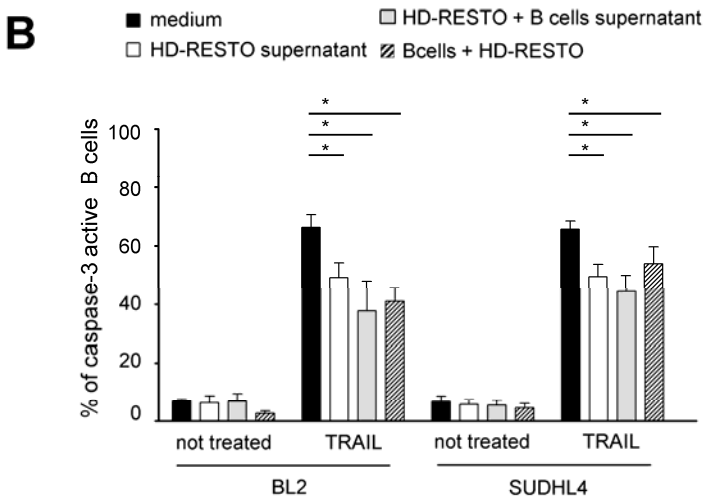


Supplemental figure 2



Supplemental figure 3





Supplemental figure 4