Additive genotoxic effects in cord blood cells upon indirect exposure to chemotherapeutic compounds crossing an *in vitro* placental barrier

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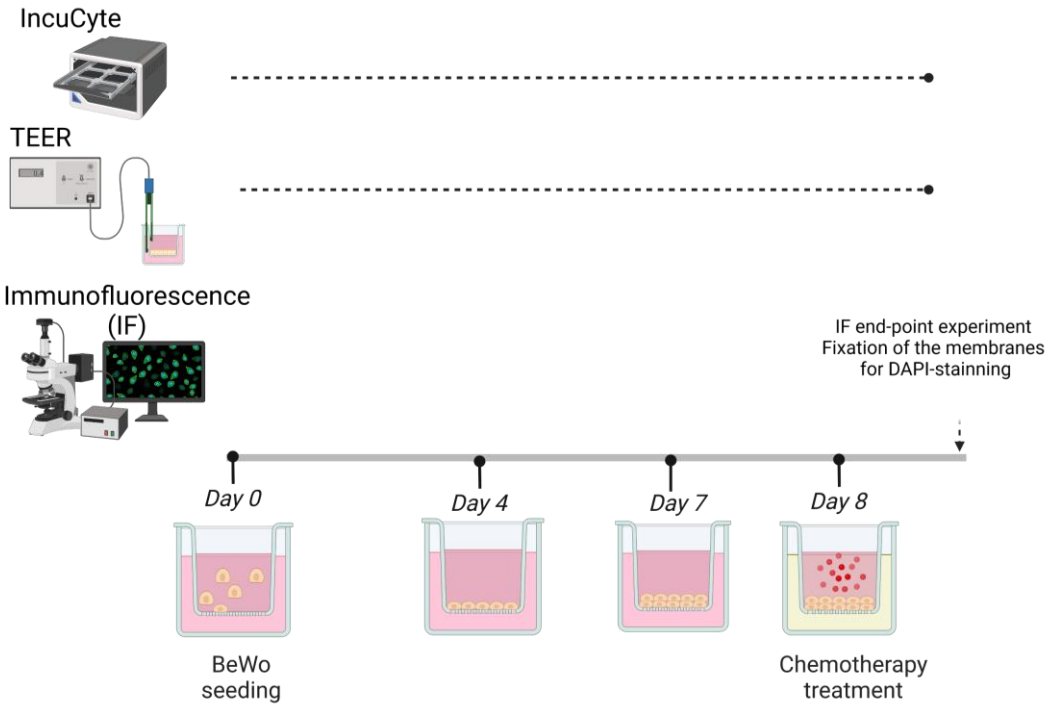
A



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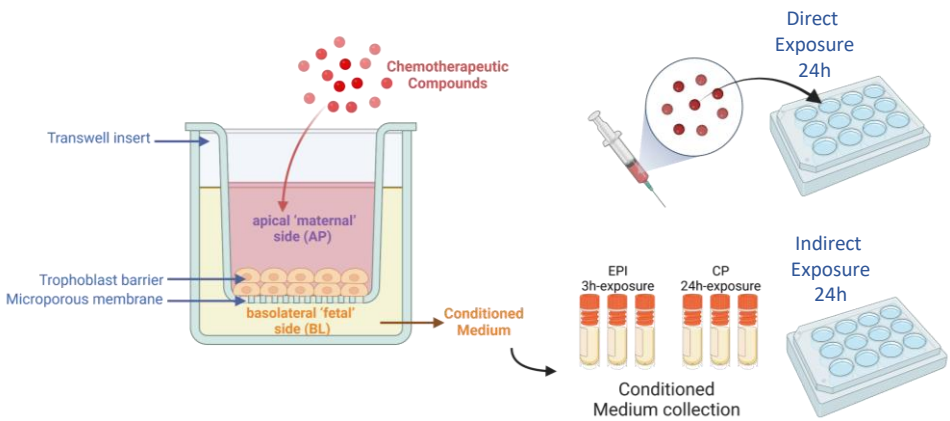
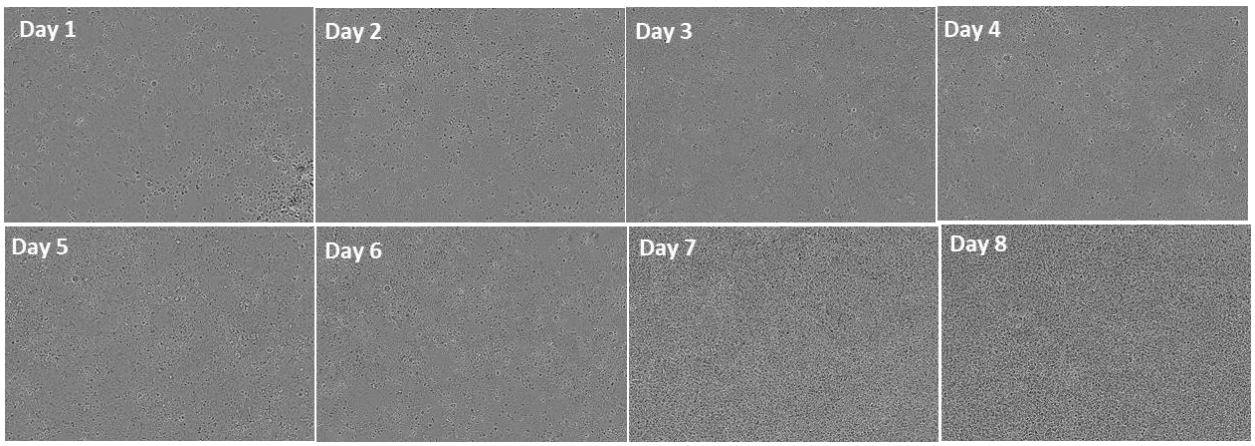


Supplementary Fig1. Establishment of the *in vitro* placenta BeWo barrier model. A,Graphical representation of the evolution of the BeWo barrier along the time and the methodology applied to monitor and optimize the conditions (IncuCyte Live imaging, TEER measurements and Immunofluorescence). B Scheme of the in vitro modeled prenatal chemotherapy exposure and the different components of the model. After exposing the barriers to the chemothrapeutic compounds, the conditioned media was collected. Finally, the mononuclear cord blood cells were exposed either directly to the chemotherapeutic compounds or to the conditioned media during 24h

A

	Days postseeding					
	1	2	3	6	7	8
TEER (Ω·cm2)	138.6±23.8	140.6±24.9	150.6±19.4	169.6±50.7	174.5±51.4	175.2±55.3

B



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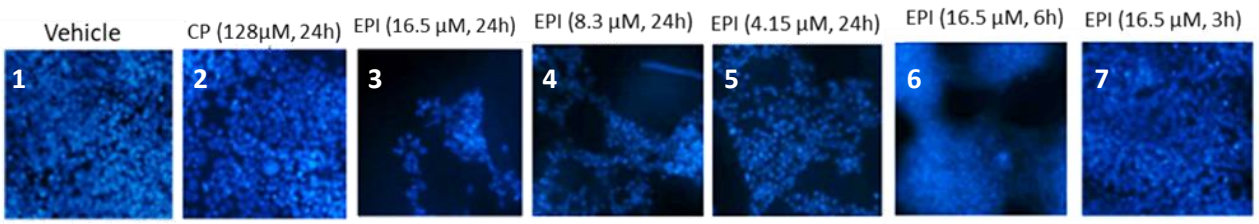

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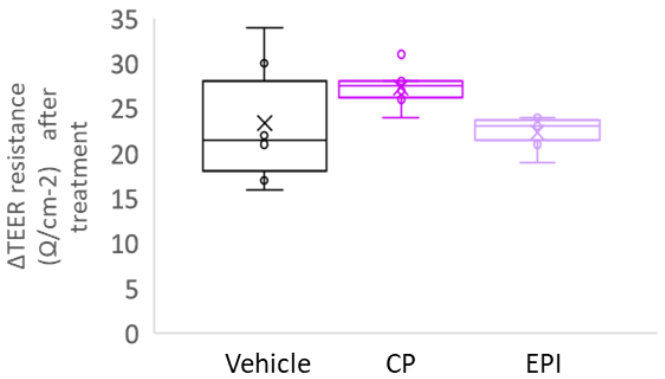

Supplementary Fig2. Evaluation and optimization of the placental in vitro barrier model. A, TEER measurements were taken at regular time points to evaluate the formation of the barrier. A steady increase of the transepitelial resistance was observed in an approximately linear fashion over the 7 days. Presented TEER values are means±SD of 3 independent experiments, in each experiment, 3 membranes per condition were used as a technical replicates. B, BeWo confluency was monitored via real-time IncuCyte live cell analysis. Representative images were taken during 8 consecutive days. BeWo monolayer was established at day 4 and progressed to a bilayer barrier at day 7. C, Evaluation of the BeWo barrier confluency upon exposure to chemotherapy via immunofluorescence imaging. Treatment of the BeWo barrier with the clinical reference dose of cyclophosphamide (CP; 128µM) during 24h resulted in a barrier confluency comparable to the vehicle-treated (culture medium only) condition (panel 1 and 2). Treatment with epicrubicin (EPI) at the clinical reference dose of 16.6 µM during 24h strongly affected the barrier integrity, since confluency was reduced to less than 20% of the vehicle-treated condition (panel 3). Altering EPI doses (panel 4 and 5) and exposure times (panel 6 and 7) showed that only a 3h exposure at the clinical reference dose of 16,6 µM resulted in a confluency of 90% of the vehicle-treated reference level (panel 7). D, Evaluation of the stability of the transepitelial resistance of the barrier upon chemotherapy exposure using TEER measurement. BeWo barriers were treated with the optimized chemotherapy conditions as identified in C (i.e. exposure to CP at 128µM during 24h and to EPI at 16.6µM during 3h). No statistically significant differences were found when comparing TEER values in the chemotherapy-treated conditions with the vehicle condition (ANOVA, 0.1622)