Online Supplementary

Supplementary Figure 1

Uterine DC are associated with blood vessels. Two photon microscopy movies were used to illustrate uDC proximity to blood vessel on (A) E5.5, (B) E12.5 and (C) E18.5. The proximity of uDC to blood vessels is significantly greater in all stages of pregnancy tested ($P< 0.02$). Data quantification concerning uDC and blood vessel association was done by manually counting all GFP-positive dendrite-positive cells in the plain of each slice versus the number of these cells in proximity to blood vessels (marked in red by TRITC-dextran). Proximity to blood vessels was considered when cells were no further than one cell away from the blood vessel tested. Four or five slices were chosen from each data set with skips of 10-15 slices (depending on the number of slices available for each data set). Data may be under-estimated since not all blood vessels are enhanced due to contrast material leakage in the ex-vivo examined tissue and motion artifacts (the latter prevented any mathematical description of the observations).

Supplementary Figure 2

Scheme illustrating experimental protocol for ablation of utereine DC used in this study. Note that timing of ablation is chosen to cover the time of the first contact of the embryo with the uterus and the early stage of implantation.

Supplementary Figure 3

DTx treatment of CD11c-DTRtg mice spares uterine macrophages and NK cells. Uteri of E5.5 pregnant CD11c-DTRtg mice with or without DTx administration were analyzed by flow cytometry and histology. (A, B) F4/80 immuno-staining of E5.5 implantation site for macrophages (brown). Experimental group was injected with 2 ng/ g body weight, that results in quantitative ablation of uDC. Bar diagram summarizes quantification of depleted IS (as % of control). (DTx n=2 ,3 IS ; Control n=2, 3 IS; P value =0.56) (C, D) Flow-cytometry analysis of E5.5 implantation site for uNK cells defined by the expression of the pan-NK marker, NK1.1. Experimental group was injected with 1 ng/ g body weight

DTx resulting in partial ablation of uDC. Note that uNK cells are retained including both DTR/GFP + and DTR/GFP negative cells.

Supplementary Figure 4

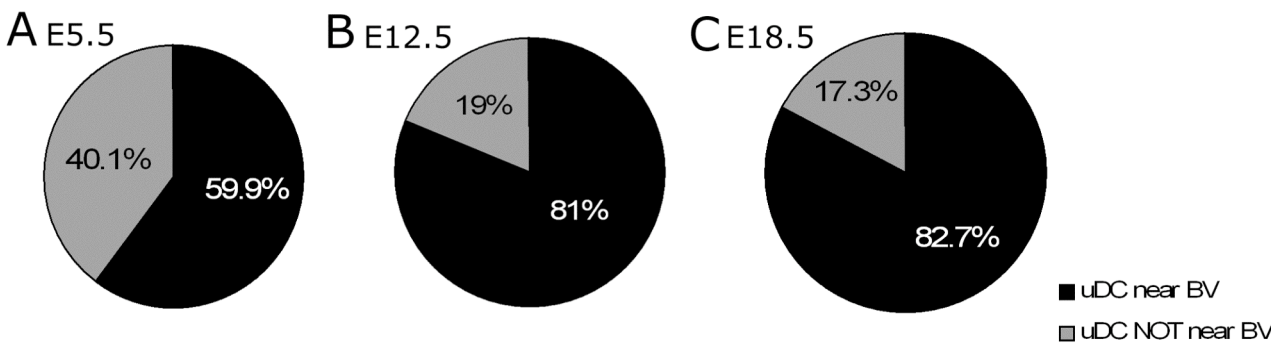
Conditional uDC ablation after implantation results in normally-developed embryos as well as normal decidualization. Pregnant CD11c-DTRtg mice were administered with DTx on E5.5 and examined on E6.5. Flow cytometric analysis on E6.5 (A) Control versus (B) DTx administered IS exhibited full depletion of uDC. Histological analysis of (C) control and (D) uDC-depleted IS detected normal deciduas and normal embryos in both cases.

Supplementary Figure 5

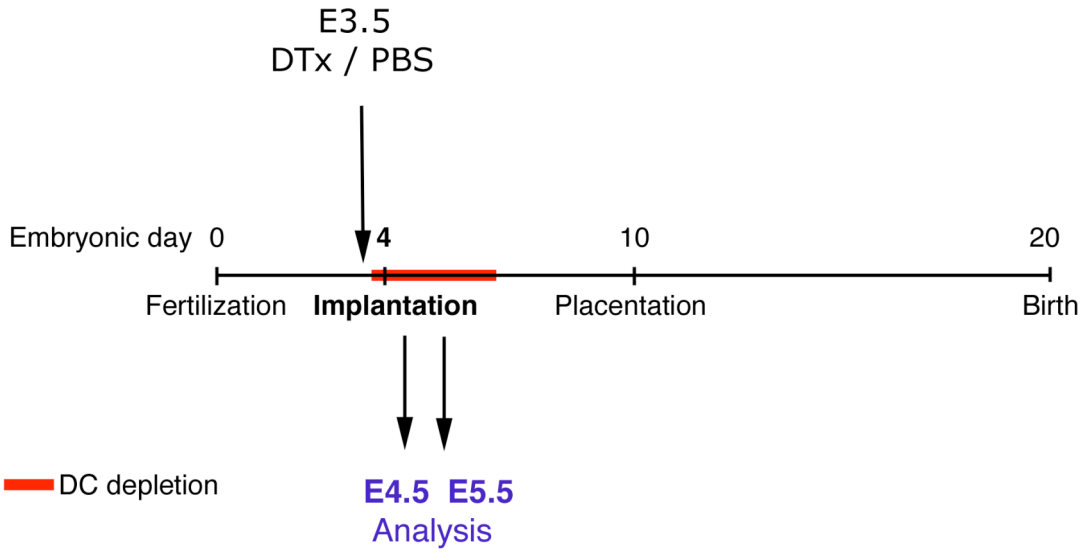
Impaired angiogenesis in E4.5 uDC-depleted deciduas: MRI and fluorescent analysis.

(A) 3DGE-MRI maximal intensity projections of E4.5 versus E5.5 at 15 min post biotin-BSA-GdDTPA injection. Note that DTx-injected implantation sites on E4.5 were non-detectable versus those on E5.5. (B) Implantation sites were retrieved after 3 and 15 min post biotin-BSA-GdDTPA injection and stained with avidin-FITC. Note that 3 min after administration of contrast material, deciduas in uDC-depleted implantation sites were almost absent, precluding their detection by MRI (in A). Therefore, blood vessels observed were mostly from the nondecidualized stromal part, similar to the stroma in non-implanted uterine sites (insert). In contrast, control implantation sites were already detected along with smaller blood vessels, characteristic of the decidua. At 15min, only a minor part of contrast material had extravasated in uDC-depleted sites, as compared to control implantation sites. (C) Quantitative analysis of the E4.5 data revealed reduced vessel density and similar permeability of uDC-depleted implantation

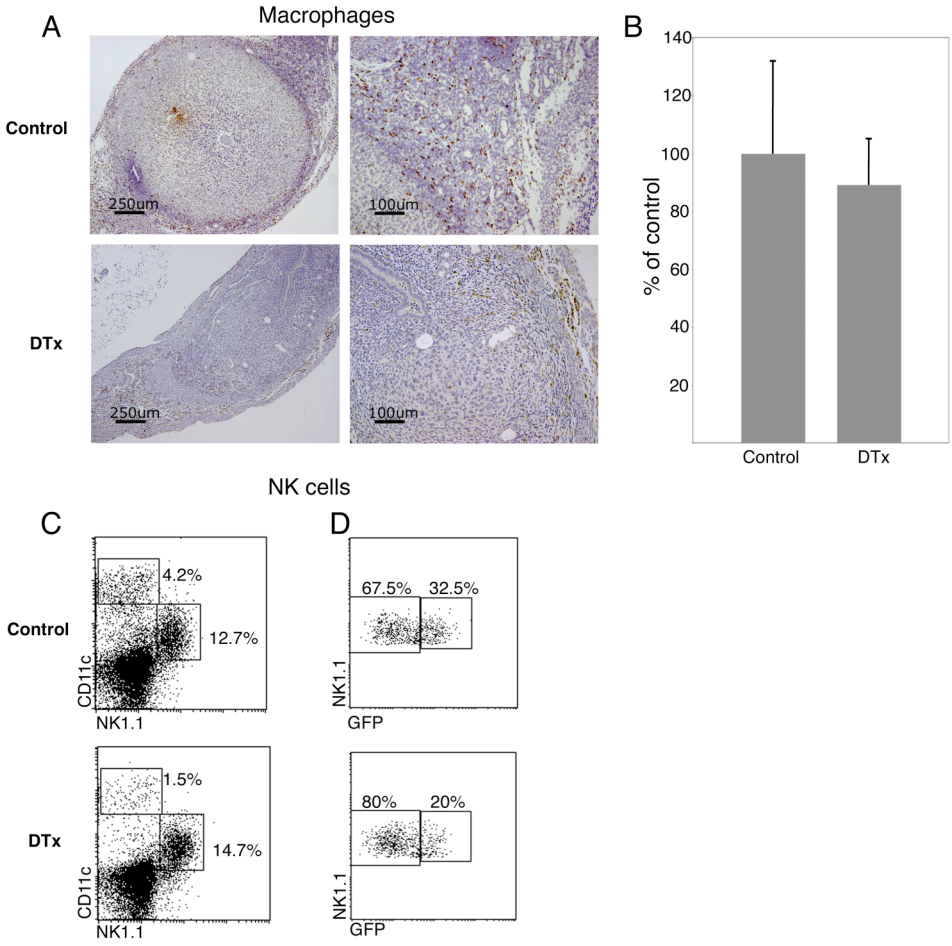
sites (2 mice, 2 implantation sites per time point were used, P_{3min}= 0.0002, P_{15min}= 0.0003). Grey trend line indicates permeability (Control SI_{15min}-SI_{3min}= 1070, DTx $SI_{15min}SI_{3min}$= 1158). Yellow arrow-implantation site, white arrow-embryo location, e-embryo, ni – non-implanted uterine site, m-myometrium.



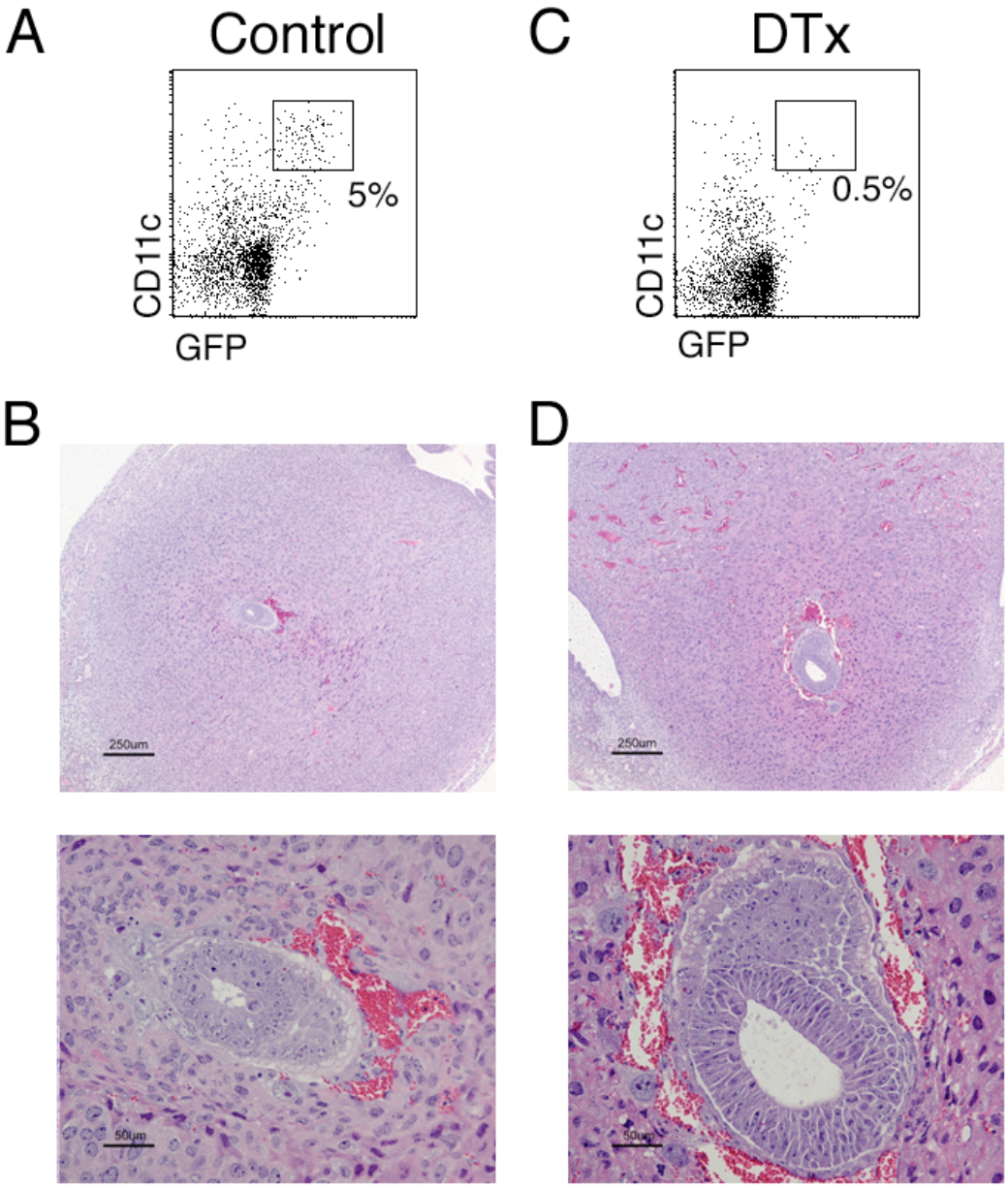
Supplementary Figure 1



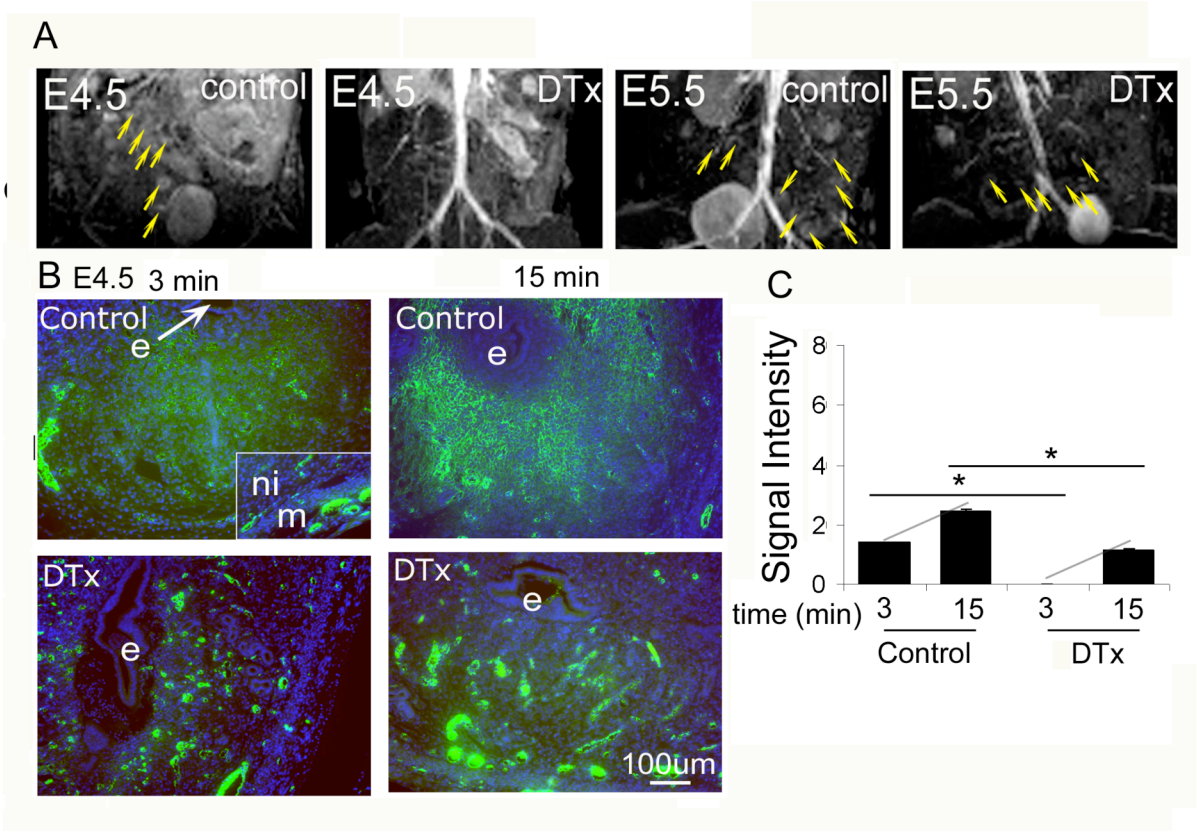
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5