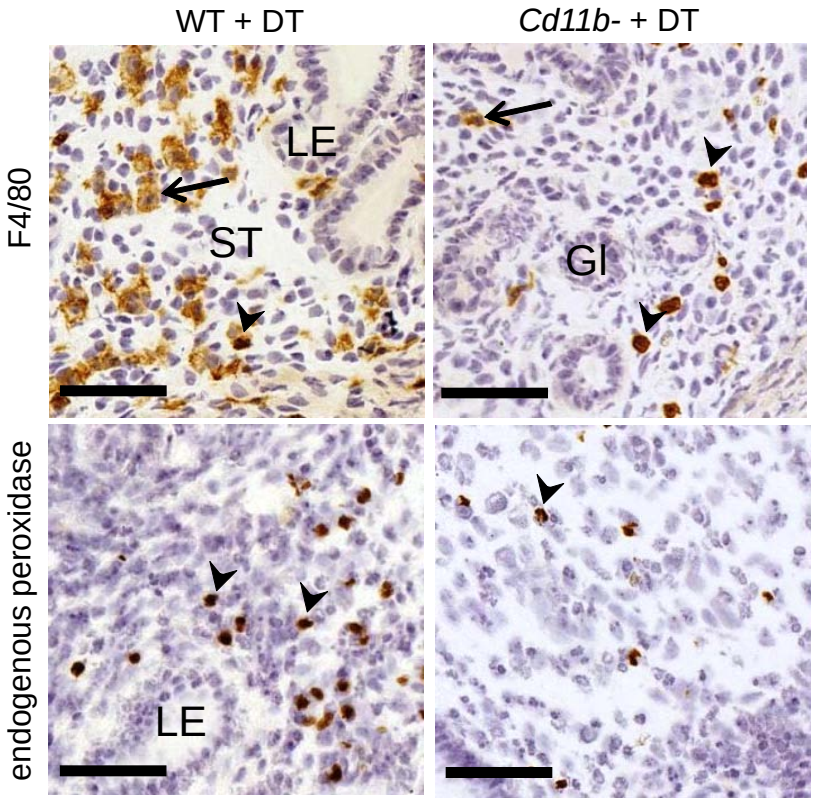
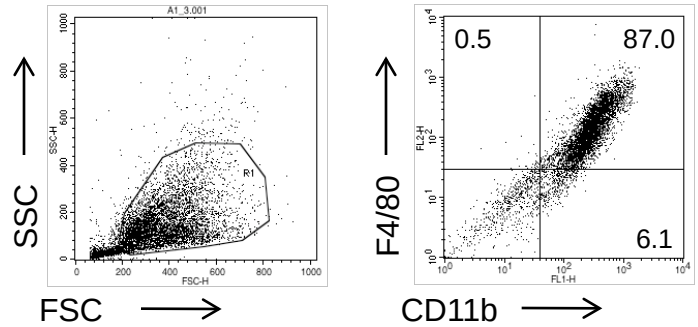
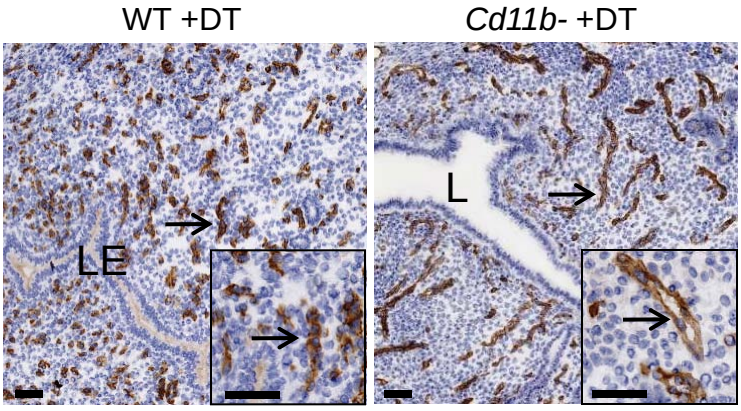


Supplemental Figure 1. F4/80-positive cells retained in *Cd11b-Dtr* uterus after DT treatment are predominantly eosinophils. Uterus from *Cd11b-Dtr* and wild-type mice was recovered at day 1.5 pc, 24 h after i.p injection of DT (25 ng/g) on day 0.5 pc. Sections from uterus of *Cd11b-Dtr* mice stained with anti-F4/80 indicate few macrophages remaining in the uterus (right panels) compared with wild-type mice (left panels). Comparison with sections incubated with peroxidase substrate diaminobenzidine (only), to identify endogenous peroxidase-positive eosinophils, show the majority of F4/80+ cells retained after DT treatment are eosinophils. Uterine eosinophils were present at day 1.5 pc but few in number on day 3.5 pc. No eosinophils were present in sections of ovary at any preimplantation time point (data not shown). Photomicrographs are representative of n=4 mice per group. Arrows; F4/80-positive macrophages. Arrowheads; endogenous peroxidase positive eosinophils. LE, luminal epithelium; GI, uterine gland; ST, stroma. Scale bar = 50 µm.

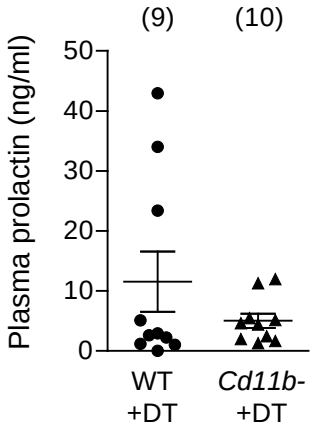


Supplemental Figure 2. Bone marrow-derived macrophages used to reconstitute *Cd11b-Dtr* mice express CD11b and F4/80. Bone marrow was harvested from adult female FVB/N mice and cultured under conditions to support differentiation into monocyte/macrophages. Expression of both CD11b and F4/80 by more than 85% of donor cells was confirmed by flow cytometry prior to i.v. injection into recipient *Cd11b-Dtr* mice, at 1000 h on day 0.5 pc, and again just prior to DT administration at 1200 h on day 3.5 pc.

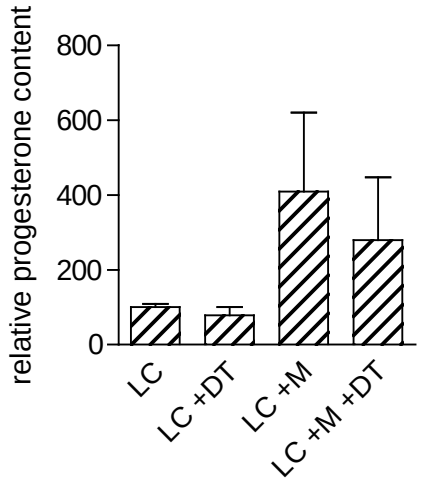


Supplemental Figure 3. The uterine vasculature remains substantially intact after DT administration to *Cd11b-Dtr* mice. Uterus was recovered from wild-type control or *Cd11b-Dtr* mice on day 4.5 pc, 24 h after i.p. injection of DT (25 ng/g), and analysed by immunohistochemistry. Sections of uterus labelled with MTS-12 indicate endothelial cells are present in the uterus of *Cd11b-Dtr* mice (left panel) as well as wild-type mice (right panel). Photomicrographs (inset is high-power) are representative of n=6-7 mice per group. Arrows; MTS-12-positive endothelial cells. LE, luminal epithelium; L, lumen. Scale bar = 50 µm.

Supplemental Figure 3

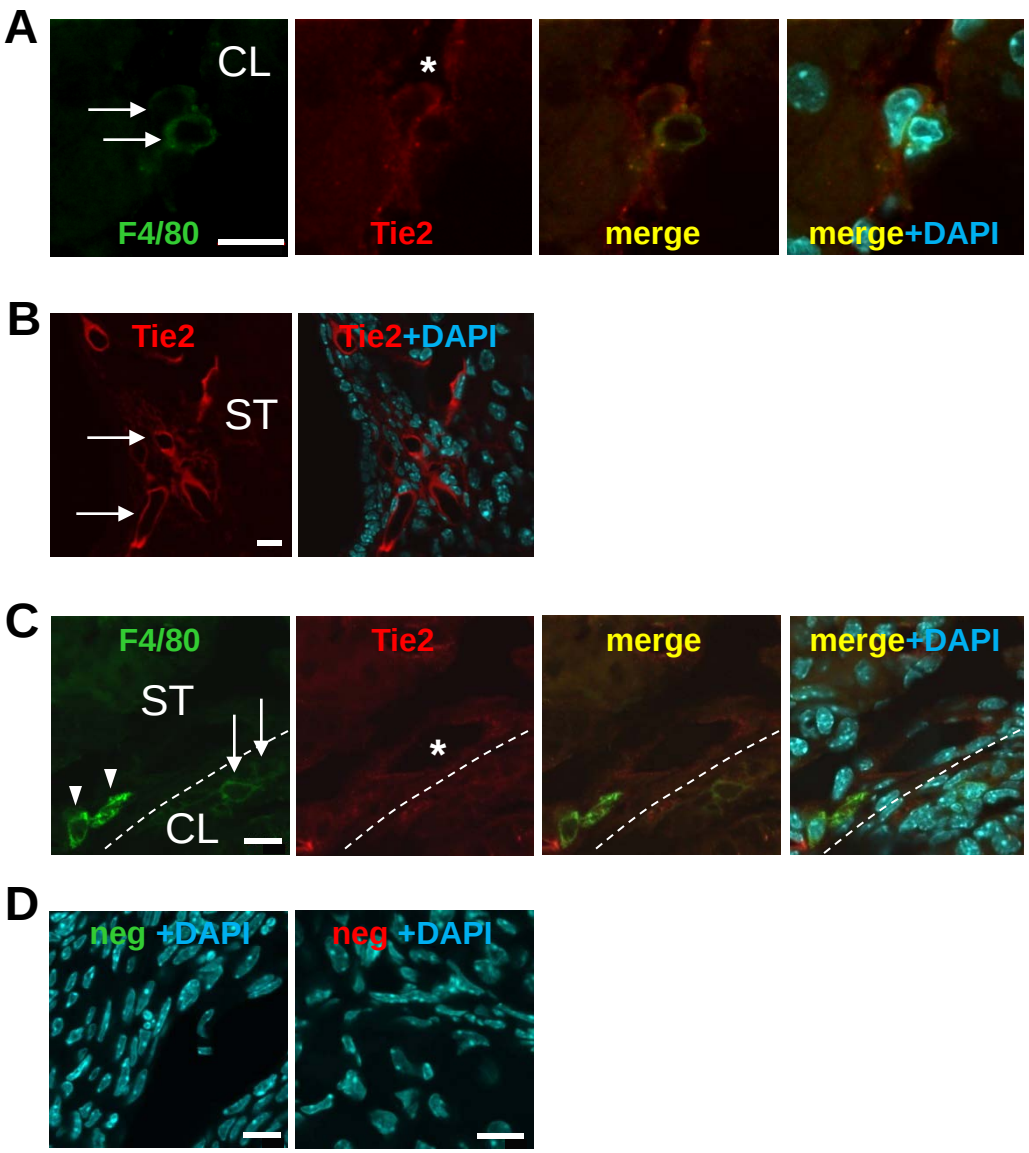


Supplemental Figure 4. Luteal defects in macrophage-depleted *Cd11b-Dtr* mice are not associated with diminished plasma prolactin. Plasma prolactin in macrophage-depleted *Cd11b-Dtr* mice was unchanged on day 5.5 pc, 48 h following i.p. injection of DT (25 ng/g) on day 3.5 pc, compared with WT mice given DT. Data are mean ± SEM.



Supplemental Figure 5. Addition of macrophages to corpora luteal cells in vitro stimulates elevated progesterone production. Cells isolated on day 2.5 pc from *Cd11b-Dtr* mice were placed into culture and 48 h later, wild-type peritoneal macrophages (M) were added. After 4 h to allow macrophages to adhere, DT (1 µg/ml) or PBS was added to wells, and culture media collected 48 h later was analysed by ELISA for progesterone production. Data are mean ± SEM progesterone content, of culture supernatants from two separate experiments, with triplicate wells in each group, expressed as fold-change relative to luteal cells cultured alone (PBS control).

Supplemental Figure 5



Supplemental Figure 6. Macrophages in the corpus luteum have a proangiogenic phenotype. (**A**) Sections of ovary from wild type mice on day 2.5 pc labelled with antibodies to Tie2 (red) and F4/80 (green) show that F4/80^{+}Tie2^{+} macrophages in the corpus luteum (arrows) interact with F4/80^{-}Tie2^{+} endothelial cells in blood vessels. (**B**) Mature blood vessels in the ovarian stroma express strong Tie2 (arrows), in comparison with weak expression in corpus luteum macrophages and blood vessels. (**C**) Macrophages in the ovarian stroma are generally F4/80^{hi} (short arrows), while macrophages in the corpus luteum are generally F4/80^{lo} (long arrows). (**D**) No staining was observed when sections were incubated with irrelevant primary AlexaFluor 488-conjugated rat monoclonal antibody, or no primary followed by Alexa Fluor 594-conjugated donkey anti-rat polyclonal antibody. *vessel lumen. Scale bar = 10 µm.

Supplemental Table 1. Effect of DT administration to wild-type and *Cd11b-Dtr* mice on leukocytes populations in the uterus, ovary and peritoneal cavity.

	CD11b$^+$	
	Wild-type	*Cd11b-Dtr*
Uterus	6.4 ± 0.9	2.0 ± 0.6**
Ovary	4.5 ± 0.4	1.4 ± 0.2**
PEC	9.8 ± 2.4	0.7 ± 0.1*

	F4/80$^+$		F4/80$^+$CD11b$^+$		F4/80$^+$CD11b$^-$	
	Wild-type	*Cd11b-Dtr*	Wild-type	*Cd11b-*	Wild-type	*Cd11b-Dtr*
Uterus	16.6 ± 2.8	8.4 ± 1.6*	3.2 ± 0.2	1.3 ± 0.5*	6.2 ± 1.5	3.9 ± 1.1
Ovary	7.1 ± 1.9	0.7 ± 0.3**	1.4 ± 0.3	0.2 ± 0.2*	4.8 ± 2.5	0.3 ± 0.1*
PEC	9.1 ± 2.2	0.7 ± 0.3**	8.0 ± 2.0**	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0

	CD11c$^+$		CD11c$^+$CD11b$^+$		CD11c$^+$CD11b$^-$	
	Wild-type	*Cd11b-Dtr*	Wild-type	*Cd11b-*	Wild-type	*Cd11b-Dtr*
Uterus	6.6 ± 1.0	1.9 ± 0.7*	2.5 ± 0.5	0.9 ± 0.4	3.6 ± 0.8	0.8 ± 0.3*
Ovary	2.4 ± 0.4	0.3 ± 0.2*	1.6 ± 0.3	0.2 ± 0.2*	1.4 ± 0.4	0.2 ± 0.1*
PEC	2.0 ± 0.5	0.3 ± 0.1*	1.3 ± 0.3	0.2 ± 0.1*	0.6 ± 0.2	0.1 ± 0.0

	RB6$^+$		RB6$^+$CD11b$^+$		RB6$^+$CD11b$^-$	
	Wild-type	*Cd11b-Dtr*	Wild-type	*Cd11b-*	Wild-type	*Cd11b-Dtr*
Uterus	2.4 ± 0.7	0.3 ± 0.0*	0.8 ± 0.2	0.0 ± 0.0*	1.6 ± 0.6	0.3 ± 0.0
Ovary	1.3 ± 0.3	0.4 ± 0.1*	0.9 ± 0.3	0.4 ± 0.1*	0.3 ± 0.1	0.0 ± 0.0
PEC	4.7 ± 1.9	4.8 ± 2.4	3.2 ± 0.9	0.3 ± 0.2*	3.1 ± 1.4	4.7 ± 2.3

	CD45$^+$		CD45$^+$CD11b$^+$		CD45$^+$CD11b$^-$	
	Wild-type	*Cd11b-Dtr*	Wild-type	*Cd11b-*	Wild-type	*Cd11b-Dtr*
Uterus	33.7 ± 5.3	11.3 ± 1.9*	3.6 ± 0.7	1.0 ± 0.4*	30.9 ± 5.5	10.9 ± 2.0*
Ovary	10.1 ± 1.2	2.1 ± 1.1*	3.3 ± 0.5	1.3 ± 0.6*	8.3 ± 1.8	1.2 ± 0.4**
PEC	38.6 ± 13.4	6.7 ± 2.9*	11.2 ± 3.0	0.3 ± 0.1**	27.7 ± 10.5	6.7 ± 2.8

	CD3$^+$		CD3$^+$CD11b$^+$		CD3$^+$CD11b$^-$	
	Wild-type	*Cd11b-Dtr*	Wild-type	*Cd11b-*	Wild-type	*Cd11b-Dtr*
Uterus	4.2 ± 0.8	3.4 ± 1.1	0.5 ± 0.2	0.3 ± 0.2	1.7 ± 0.3	1.7 ± 0.5
Ovary	1.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	1.5 ± 0.3	0.2 ± 0.1*
PEC	2.4 ± 0.6	1.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	1.0 ± 0.4	0.5 ± 0.2

Data are mean ± SEM and the effect of genotype is analysed by Mann-Whitney U test.
*P < 0.05, **P < 0.01. n=4-6 per group. DT; diphtheria toxin. PEC; peritoneal exudate cells.

Supplemental Table 2. Effect of DT administration to wild-type and *Cd11b-Dtr* mice on numbers of ovarian follicles and corpora lutea, and corpora luteum structure.

	Wild-type	*Cd11b-Dtr*
Preantral Follicles	5.3 ± 1.7	4.9 ± 1.6
Antral Follicles	1.2 ± 0.5	0.4 ± 0.2
Atretic Follicles	1.5 ± 0.6	1.1 ± 0.6
Total CL	6.3 ± 1.2	7.1 ± 0.6
Disorganised CL	0.0 ± 0.0	7.1 ± 0.6***
CA	0.2 ± 0.2	0.0 ± 0.0

Sections of ovary from day 4.5 pc wild-type and *Cd11b-Dtr* mice, 24 h following DT injection on day 3.5 pc, were stained with hematoxylin and the number and developmental stage of follicles, and the number of intact and disorganised corpora lutea, were counted. Data are mean ± SEM. The effect of genotype was analysed by Mann-Whitney U test. ***$P < 0.001$. CL; corpora lutea. CA; corpus albicans. n=6 wild-type and n=7 *Cd11b-Dtr*.

Supplemental Table 3. The effect of macrophage depletion on late gestation pregnancy outcome.

	Wild-type (DT only)	Wild-type (DT + vehicle)	*Cd11b-Dtr* (DT + P_4)
Mated females	14	10	11
Females with implantation sites at autopsy [#*] (%)	11/14 (79%) [a]	6/10 (60%) [a]	9/11 (82%) [a]
Females with viable fetuses at autopsy [#*] (%)	11/14 (79%) [a]	6/10 (60%) [ab]	4/11 (36%) [b]
All implantations resorbing at autopsy [#*]	0/11 [a]	0/6 [ab]	5/9 [b]
Total resorptions (% implants) *	13/95 (14) [a]	3/48 (6) [a]	30/50 (60) [b]
Implantation sites/pregnant mother (total and viable) [†]	8.6 ± 0.9 [a]	8.0 ± 0.5 [a]	5.6 ± 0.9 [a]
Implantation sites/pregnant mother (viable) [†]	7.5 ± 0.8 [a]	7.5 ± 0.5 [a]	2.2 ± 1.0 [b]
Fetal weight (mean ± SEM) [†]	834 ± 16 [a]	780 ± 18 [a]	727 ± 38 [a]
Placental weight (mean ± SEM) [†]	68 ± 2 [a]	56 ± 1 [b]	60 ± 2 [ab]
Fetal:placental weight ratio (mean ± SEM) [†]	12.7 ± 0.4 [a]	14.1 ± 0.3 [b]	12.4 ± 0.8 [ab]

Implantation sites are defined as total implantations, including viable and non-viable.
[#] Autopsy was conducted at day 17.5 pc.
* Categorical data was analysed using Fisher's exact test.
[†] Data is mean ± SEM and the effect of genotype was analysed by Kruskal-Wallis test and post-hoc Dunns test.
[a, b] Different superscripts denote significant differences between treatment groups.
DT; diphtheria toxin. Vehicle; sesame oil.