Cell Metabolism, Volume *8*

Supplemental Data

Ablation of CD11c-Positive Cells Normalizes Insulin Sensitivity in Obese Insulin Resistant Animals

David Patsouris, Ping-Ping Li, Divya Thapar, Justin Chapman, Jerrold M. Olefsky, and Jaap G. Neels

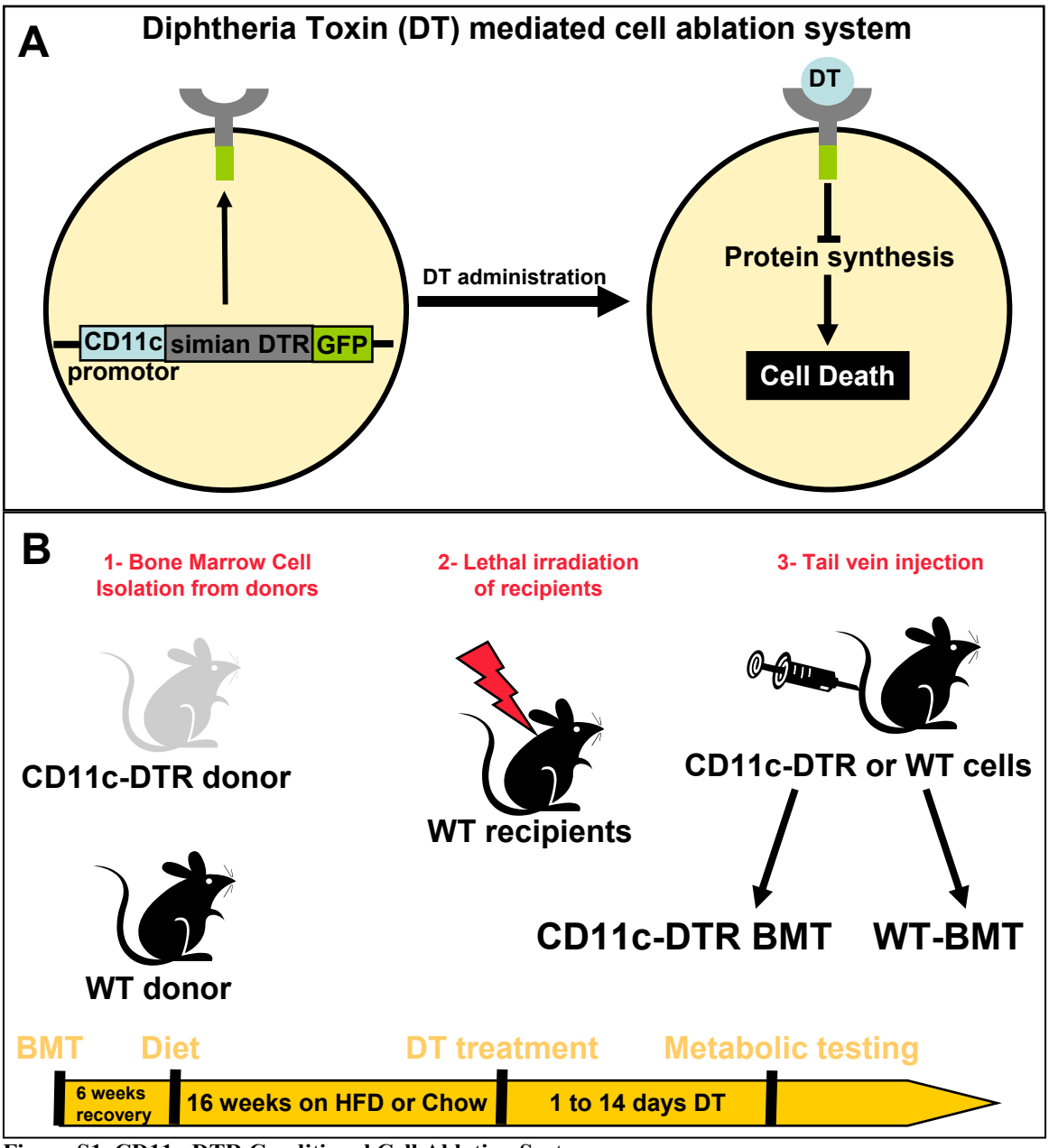

Figure S1. CD11c-DTR Conditional Cell Ablation System

Illustrations depicting the experimental strategies used in this study. **A**) Illustrates the diphtheria toxin (DT) mediated conditional cell ablation system. To convey DT sensitivity specifically to CD11c expressing cells, the simian DT receptor (DTR) is expressed as a transgene in normally DT-insensitive mice as a GFP fusion protein under control of the mouse CD11c promoter. The cytotoxicity of the heterodimeric DT is strictly dependent on receptor-mediated endocytosis. Upon administration, DT enters the $CD11c^{+}$ cells via interaction of its DT B subunit with the simian DTR. Upon endocytosis, the DT A subunit is released and catalyzes ADP-ribosylation of elongation factor 2, resulting in inhibition of protein synthesis. As a consequence, DT induces rapid apoptosis in both mitotic and terminally differentiated $CD11c^{+}$ cells. **B**) Depicts the bone marrow transplant (BMT) strategy and timeline followed. Bone marrow (BM) from both transgenic CD11c-DTR and wild-type (WT) donor mice is transplanted through the tail vein in WT recipient mice that received a lethal dose of irradiation to deplete their endogenous BM. The transplanted mice were allowed to recover for 6 weeks and reconstitute their BM before they were started on either a normal chow of high fat diet (HFD) for a period of 16 weeks. After this period, DT treatment commenced and the mice underwent metabolic testing at different stages of treatment.

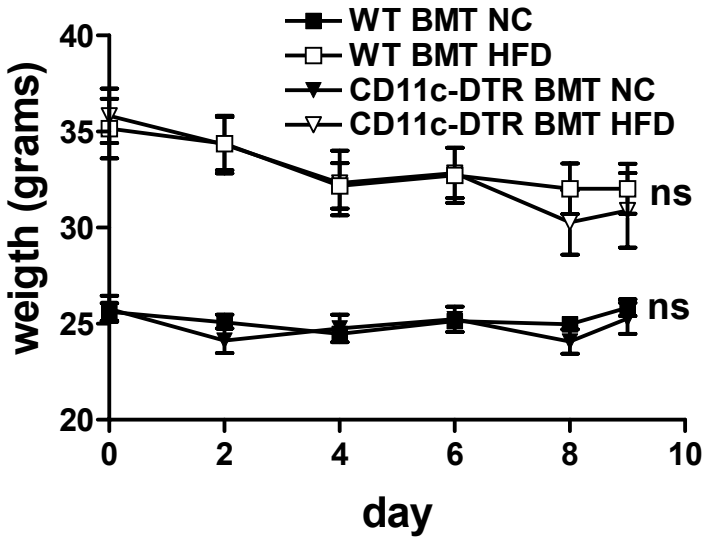

Figure S2. Mice Weights during DT Treatment
Mice were injected ip with DT every other day over a 9-day period, with jugular catheters implanted on day 4, and euglycemic clamp studies performed on day 7. Mice weights were followed during this 9-day period. Data are expressed as mean ± SEM. ns indicates no significant differences in weight between WT and CD11c-DTR BMT mice. *n*=5 per group.

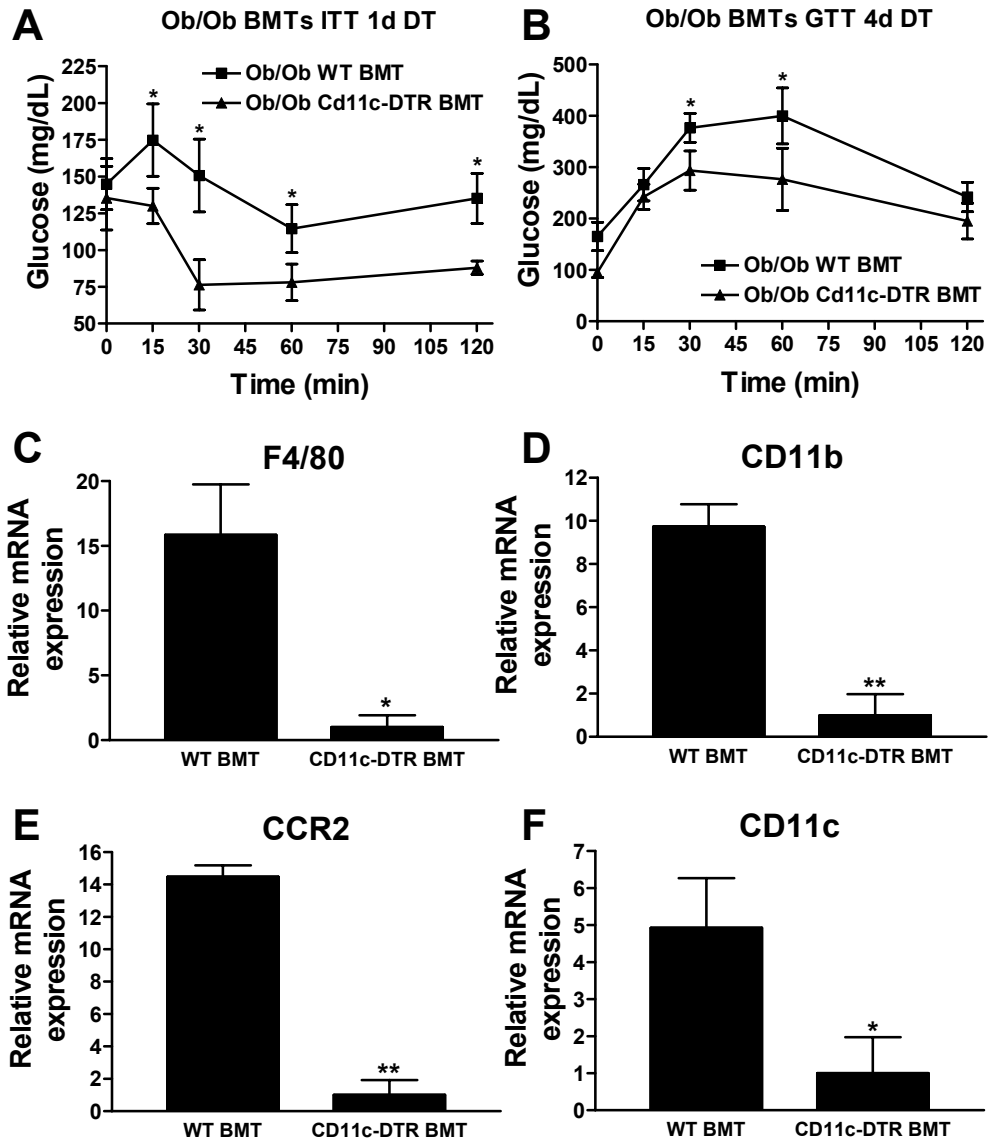

Figure S3: Ob/Ob Data

Genetically obese leptin-deficient Ob/Ob mice were transplanted with WT or CD11c-DTR bone marrow and underwent an **a**) insulin tolerance test (ITT) after 1 day of DT treatment and **b**) a glucose tolerance test after 4 days DT treatment. The adipose tissue macrophage content of these animals was measured by quantifying the relative mRNA expression of the macrophage markers **c**) F4/80, **d**) CD11b, **e**) CCR2, and **f**) CD11c. Data are expressed as mean ± SEM. **P*<0.05 and ***P*<0.01 compared to WT BMT; *n*=6.

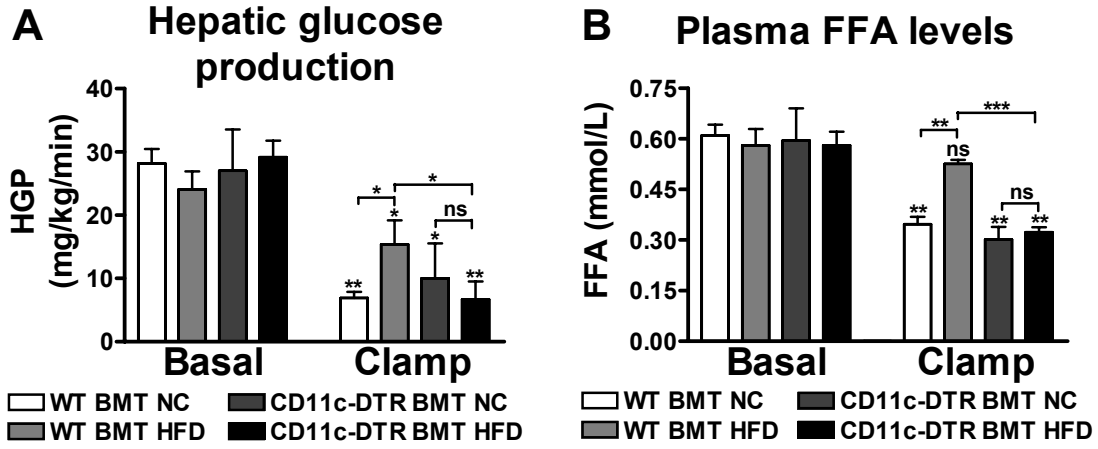

Figure S4: Raw Clamp Data

In vivo liver and adipose tissue insulin sensitivity as determined by euglycemic clamp studies in NC- and HFD-fed WT and CD11c-DTR BMT mice treated with DT. **a**) Hepatic glucose production under basal (non insulin-stimulated) and clamp (insulin-stimulated) conditions. Insulin-stimulated reduction of hepatic glucose production reflects liver insulin sensitivity. **b**) Fasted plasma FFA levels under basal (non insulin-stimulated) and clamp (insulin-stimulated) conditions. Insulin-stimulated reduction of plasma FFA levels reflects adipose insulin sensitivity. NC data was from mice treated with DT for 14 days. HFD data was combined from 3 sets of mice that received DT for 1, 3, or 7 days. Data are expressed as mean ± SEM. **P*<0.05, ***P*<0.01 and *** *P*<0.001. ns means not significant. *n*=5 per group for NC data and *n* ≥ 15 per group for HFD data.