**Genome-wide DNA methylation dynamics drive dendritic cell development from myeloid-restricted hematopoietic stem cells**

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**Abstract**

Dendritic cells (DCs) are critical immune regulators involved in autoimmune diseases, but exploiting them clinically requires a detailed picture on the mechanisms orchestrating their development. DNA methylation is attractive in this regard because it is reversible and as such allows therapeutic manipulation. Combining single cell transplantation assays with whole-genome methylation assessment and with mice expressing reduced DNA methyltransferase 1 levels, we show that conventional and plasmacytoid DCs arise together from myeloid-restricted hematopoietic stem cells (HSCs), suggesting that both subsets can develop independently of the lymphoid pathway. DC commitment by HSCs requires an intrinsically high methylation threshold to establish FLT3 signaling and DC gene expression. Reducing methylation in a preclinical mouse model depleted DCs and ameliorated systemic lupus erythematosus. These studies shed novel light on the DC origin, show how lineage- and subset-specific DNA methylation regulates DC fate and provide a potential rationale for targeting DCs in autoimmunity by hypomethylating agents.

**Introduction**

Dendritic cells (DCs) are a heterogeneous immune cell population with important functions in antigen presentation and cytokine secretion (Ginhoux et al. 2016; Merad et al. 2013; Durai und Murphy 2016). They are involved in human diseases such as autoimmune disorders and play a primary role in anti-tumor response, making them attractive for clinical interventions and as vaccines. Both mouse and human DCs exist in different subsets of which conventional DCs (cDCs) and plasmacytoid DCs (pDCs) are the predominant ones within lymphoid tissue. These subsets are constantly replenished by hematopoietic stem cells (HSCs), but their exact differentiation trajectories are still under debate (Liu und Nussenzweig 2010; Kamath et al. 2002; Amon et al. 2020; Paul und Amit 2014). A model has been developed proposing that cDCs and pDCs develop via a sequence of shared precursors that includes macrophage-DC progenitors (MDPs) and common DC progenitors (CDPs) (Fogg et al. 2006; Geissmann et al. 2010; Onai et al. 2007; Naik et al. 2007). However, this view has been challenged by other data, most recently by single cell transcriptomics, suggesting that pDCs develop independently from cDCs via lymphoid progenitors (Dress et al. 2019). Consequently, a more definitive picture of the DC ontogeny and of the phylogenetic relationship between pDCs and cDCs is important for getting deeper insights into the molecular cues determining DC fate.

A network of several cytokines and transcription factors has been implicated in controlling DC development (reviewed in (Nutt und Chopin 2020)). These molecules generally establish gene expression programs in concert with the epigenome. DNA methylation is one of the major layers of epigenomic regulation in mammalian cells. It is established by DNA methyltransferases (DNMTs) which catalyze the addition of methyl-groups onto cytosines of cytosine-guanosine (CpG) dinucleotides within the DNA (Bird 2002). DNA methylation is a dynamic process and the pattern of methylated CpGs varies between cell types and during cellular differentiation (Meissner et al. 2008; Bird 2002; Farlik et al. 2016; Atlasi und Stunnenberg 2017; Lipka et al. 2014; Cabezas-Wallscheid et al. 2014). This appears also to be the case for DCs, whose maturation has been reported to be accompanied by demethylation at genomic regions with predicted binding sites of DC-affiliated transcription factors (Zhang et al. 2014). However, that study was restricted to only a fraction of the entire genome, and was performed using an in vitro differentiation system and missed potential methylation changes occurring in early hematopoiesis during which DC fate is established (Naik et al. 2013). Furthermore, there is no functional study so far addressing the question if and how DNA methylation is etiologically involved in regulating DC fate. However, such knowledge would be a key prerequisite for exploring possible applications of agents modulating DNA methylation in DC-driven diseases in clinical settings.

Here, we have dissected the *in vivo* role of DNA methylation in DC development, have shed novel light on the stem cell source of DCs and have employed a pre-clinical mouse model of systemic lupus erythematosus to explore a possible link between methylation and DCs in autoimmune disease.

**Results**

**Constitutive DNA methylation determines DC differentiation fate**

Taking advantage of mice with hypomorphic expression of the DNA methylation maintenance enzyme DNMT1 (*Dnmt1*c/chip mice, combining a *Dnmt1* knockout (c) allele with a *Dnmt1* cDNA insertion (chip) allele (Gaudet et al. 2003)), we showed previously that DNA methylation is essential for multipotency of HSCs and for generation of lymphocytes (Bröske et al. 2009). A more refined analysis of *Dnmt1*c/chip mice confirmed reduced B cell frequency (Suppl. Fig. 1a). The frequency of monocytes/macrophages was not reduced and the frequency of granulocytes was even somewhat increased (Suppl. Fig. 1b,c). Strikingly however, we detected an almost complete absence of pDCs as a main immune cell phenotype of *Dnmt1*c/chip animals (Fig. 1a). cDCs were also reduced (but to a lesser degree than pDCs), which was mainly caused by a lower frequency of CD11b+ cDCs (representing the predominant cDC population in the spleen), whereas CD8+ cDCs were not reduced (Fig. 1b,c). The normal expression levels of MHCII+ on CD11c- cells and the normal CD11c levels on residual cDCs excluded the possibility that these markers were simply downregulated in *Dnmt1*c/chip mice.

Although the exact route of differentiation is still under debate (Dress et al. 2019; Reizis 2019), it is accepted in the field that DCs develop via a sequence of precursors that can include a shared macrophage-DC progenitor (MDP) (Fogg et al. 2006). *Dnmt1*c/chip mice had a much lower MDP frequency (comparable with the strong pDC loss), whereas the frequency of common monocyte progenitors (cMoPs), which can directly develop from MDPs (Hettinger et al. 2013), was less affected (Fig. 1d). CDPs could not be investigated as *Dnmt1*c/chip mice lacked Flt3 expression (see below).

*Dnmt1*c/chip mice ubiquitously express hypomorphic Dnmt1 levels in all cells (Gaudet et al. 2003; Bröske et al. 2009). To determine if the alterations in DC development were caused by a lineage-intrinsic mechanism or by lower Dnmt1 expression in non-hematopoietic cells, we generated chimeric animals by transplanting BM cells from *Dnmt1*c/chip or control donor mice into BM ablated wildtype recipient animals and analyzed donor-derived DC development after stable engraftment (Suppl. Fig. 2a). As demonstrated in Fig. 2a-c, *Dnmt1*c/chip donor BM failed to produce pDCs and could only produce strongly reduced frequencies of cDCs and MDPs. In contrast, *Dnmt1*c/chip donor-derived granulocytes were generated at similar or even higher frequency when compared to control donor cells (Suppl. Fig. 2b).

Taken together, these data suggest that constitutive methylation is required for normal DC development in a cell-intrinsic manner, whereby the effect of hypomethylation varies on different DC subsets.

**Whole genome DNA methylation maps of DC subsets and their precursors**

In order to investigate the impact of DNA methylation on normal DC development, we measured genome-wide DNA methylation in pDCs, CD11b+ cDCs, CD8+ cDCs, CDPs, MDPs, monocytes and cMoPs at single-CpG resolution by tagmentation-based whole-genome bisulfite sequencing (TWGBS) (Wang et al. 2013). For each of the populations, we analyzed 2 to 4 biological replicates, resulting on average in 1.14 x 109 total mapped paired reads per group and a genomic CpG-coverage ranging from 18.7 to 44.3-fold per population (Suppl. Table 1 and Suppl. Table 2). We also included our previously generated TWGBS data of long term (LT)-HSCs (hereafter termed HSCs) into this study, which we had produced by the same protocol (Cabezas-Wallscheid et al. 2014). A comparison of the genome-wide mean CpG methylation levels across all cell populations revealed that HSCs had the highest methylation level (Fig. 3a, Suppl. Fig. 3a). CDPs showed less methylation than HSCs, but more than cMoPs, revealing that the genome of DC precursors had higher DNA methylation levels than that of monocyte precursors. This difference was also reflected between DCs and monocytes in mature populations.

We next identified differentially methylated regions (DMRs) by pairwise comparison of HSCs and each of the downstream cell populations using at cutoff of at least 10% difference in methylation. DMRs from all comparisons were pooled and overlapping DMRs were merged, leading to a catalogue of 49,588 unique DMRs (Suppl. Table 3). Principal component analysis (PCA) based on these DMRs revealed known cellular relationships, with CDPs being dedicated DC precursors and cMoPs being monocyte precursors, and with MDPs being shared precursors of both lineages (Fig. 3b).

As hypomorphic DNMT1 affected DC subsets to a different extent (see Fig. 1), we compared the methylation status at the DMRs between these subsets. Strikingly, this revealed marked differences, with pDCs harboring the highest, CD11b+ cDCs harboring intermediate and CD8+ cDCs harboring the lowest methylation levels at DMRs (Fig. 3c). These differences were in accordance with the DC phenotypeof *Dnmt1*c/chip mice, providing a possible explanation for the stronger effect of hypomorphic DNMT1 on pDCs than on cDCs.

Unsupervised hierarchical clustering of the DMRs identified 9 clusters with unique methylation patterns across all cell populations (Fig. 3d and Suppl. Fig. 3b). For example, cluster 9 consisted of DMRs with lower methylation in HSCs than in any of the other populations and thus represented a ‘HSC DMR cluster’. In contrast, cluster 3 (which was among the largest clusters, Suppl. Fig. 3c) had lower methylation in DCs than in the other populations and thus constituted a ‘pan DC DMR cluster’. TWGBS also revealed DC subset-specific DMR clusters (cluster 5, 7 and 8) and a ‘monocyte DMR cluster’ (cluster 6) which had lower methylation in monocytes and cMoPs than in any other cell population. Finally, cluster 1 revealed methylation similarities of cDCs with monocytes. Collectively, our data uncovered lineage- and subset-specific DNA methylation landscapes in DC progenitors and mature cells, and provide a possible explanation for the preferential effect of hypomorphic DNMT1 on pDC development.

**Link between DNA methylation status and DC gene expression**

To delineate if hypomorphic DNMT1 levels resulted in disrupted gene expression that could explain altered DC development, we flow-sorted MDPs and cMoPs from *Dnmt1*+/+ and *Dnmt1*c/chip mice for genome-wide gene expression profiling. This revealed that in comparison to their respective *Dnmt1*+/+ counterparts, *Dnmt1*c/chip MDPs differentially expressed 4.16-fold more genes than *Dnmt1*c/chip cMoPs (*Dnmt1*c/chip MDPs: 471 genes with increased, 765 genes with decreased expression; *Dnmt1*c/chip cMoPs: 118 genes with increased, 179 genes with decreased expression) (Fig. 4a, Suppl. Fig. 4a and Suppl. Table 4). This suggested that hypomorphic DNMT1 caused more widespread transcriptional changes in the MDP population than in cMoPs. An unbiased clustering approach indicated that these changes resulted in a transcriptomic approximation of the *Dnmt1*c/chip MDPs with cMoPs, suggesting that hypomorphic DNMT1 changed the overall identity of the MDP pool (Suppl. Fig. 4b). Next, we compared the genes that were differentially expressed between *Dnmt1*c/chip and *Dnmt1*+/+ MDPs with our previously published signature gene lists of DCs and other phagocyte populations (Schönheit et al. 2013). This showed that *Dnmt1*c/chip MDPs had decreased expression of prominent DC genes, which included those encoding important transcription factors (e.g. *Irf8, Runx2*) and growth factor receptors (e.g. *Flt3, Il7r, CD27*) (Fig. 4b). In contrast, *Dnmt1*c/chip MDPs showed enhanced expression of many granulocyte signature genes (e.g. *Gfi1, Elane, Ltf, Ccr1*), supporting the notion that hypomethylation altered MDP identity.

We next explored the relationship of the genes that were differently expressed in *Dnmt1*c/chip MDPs or *Dnmt1*c/chip cMoPs versus their control counterparts with the DNA methylation status by mapping the promoter regions of these genes to the above-described clustered DMRs. This revealed that genes downregulated in *Dnmt1*c/chip MDPs were strongly enriched in the DC DMR cluster 3 (‘pan DC DMR cluster’), and to a lesser extent in DC-specific clusters 5 (‘PDC DMR cluster’) and 7 (‘cDC DMR cluster’) (Fig. 4c). This included genes with well-known functions in DCs and their precursors, such as *Flt3*, *Cxcr2*, *IL1r1* and *Vcam1* (Suppl. Table 5). In contrast, genes upregulated in *Dnmt1*c/chip cells were most strongly enriched in the monocyte DMR cluster 6 and the HSC cluster 9. Thus, DNMT1 hypomorphic cells were likely unable to methylate and shut down HSC and myeloid genes, and failed to adequately establish methylome properties promoting the expression of DC lineage genes.

**Lack of FLT3 is a cause for disrupted DC development from *Dnmt1*c/chip precursors**

The gene encoding the FLT3 cytokine receptor was expressed at lower levels in *Dnmt1*c/chip cells and we found DMRs annotated to the Flt3 locus clustering with the DC DMR cluster 3 (compare Suppl. Table 4 and Suppl. Table 5). Because FLT3 ligand signaling via FLT3 is essential for DC development (Waskow et al. 2008; D'Amico und Wu 2003), we concentrated our functional experiments on this pathway. We confirmed fewer FLT3+ cells and a lower FLT3 expression level in the *Dnmt1*c/chip Lin-Sca1+cKit+ (LSK) population by flow cytometry (Fig. 4d and Suppl. Fig. 4c). To assess if impaired FLT3 signaling by decreased FLT3 expression could explain disturbed DC development of *Dnmt1*c/chip progenitors, we restored this pathway. To this end, we expressed FLT3 along with the green fluorescence protein (GFP) for tracing of transfected cells in c-kit-enriched BM cells from *Dnmt1*c/chip and *Dnmt1*+/+ mice by a bicistronic retrovirus and cultured the cells with FLT3 ligand for 8 days according to a published protocol for *in vitro* pDC differentiation (Won et al. 2014). While ectopic FLT3 had no effect on *Dnmt1*+/+ cells, it significantly increased the number of total living cells and of pDCs produced by the *Dnmt1*c/chip progenitors, although not to the full extent (Fig. 4e-g and Suppl. Fig. 4d). Thus, ectopic FLT3 ameliorated survival and pDC differentiation capacity of *Dnmt1*c/chip progenitors *in vitro*, suggesting one mechanistic cause why DNMT1 hypomorphic cells were unable to commitment to DC fate was because of their failure to express *Flt3*.

**HSCs require constitutive DNA methylation to transit into a DC committed state**

Because Flt3 expression starts early in hematopoiesis (Adolfsson et al. 2005), we determined the frequencies of HSCs and multipotent progenitor populations 1-4 (MPPs1-4) in the BM of *Dnmt1*c/chip and *Dnmt1*+/+ mice by flow cytometry according to (Cabezas-Wallscheid et al. 2014; Adolfsson et al. 2005). While HSCs, MPP1 and MPP2 were reported to be fully multipotent, MPP3 and MPP4 include early differentiation-biased cells (Cabezas-Wallscheid et al. 2014). Remarkably, unlike HSCs, MPP1 and MPP2, *Dnmt1*c/chip mutants had markedly reduced MPP3/4 frequencies, which also contained much fewer FLT3+ cells (=MPP4). This suggested that DNMT1 hypomorphic cells failed to transit from a multipotent into a differentiation-biased FLT3+ (DC) precursor state (Figure 5a and b).

To follow the establishment of DC commitment during early hematopoiesis *in vivo*, we flow-sorted HSCs and MPPs1-4 from wildtype mice (CD45.1+) and transplanted them into sublethally irradiated congenic hosts (CD45.2+) using cell numbers matching their relative ratios in normal BM (HSC : MPP1 : MPP2 : MPP3 : MPP4 = ~1 : ~5 : ~2 : ~5 : ~30). We scored the recipients for donor-derived DCs, revealing that all HSC/MPP populations could give rise to DCs (Fig. 5c and Suppl. Fig. 5a). However, at 12-14 days post-transplantation, the capacity to produce DCs was low for HSCs, MPP1 and MPP2, was increased for MPP3 and was highest for MPP4. Although further kinetic experiments are required, these results suggest that DC fate is established stepwise, and is primarily driven by the Flt3+ MPP4, which was in accordance with previous results (Karsunky et al. 2003). Of note, our data also showed that pDCs and cDCs developed with similar frequencies from MPPs.

**Constitutive DNA methylation controls early DC development but is dispensable thereafter**

We next determined if DC potential requires constitutive methylation exclusively during early hematopoiesis, or also during later maturation steps. To this end, we crossed *Dnmt1*lox/chip animals (combining a *lox*P-flanked *Dnmt1* allele with the hypomorphic *chip* allele (Gaudet et al. 2003; Jackson-Grusby et al. 2001b)) with mice expressing the CRE-recombinase from the *Vav* gene (referred to here as *Vav*Cre+) or from the *Cx3cr1* gene (referred to here as *Cx3cr1*Cre+). *Vav*Cre+ mice express CRE in HSCs and early progenitor cells, whereas CRE expression in *Cx3cr1*Cre+ mice is restricted to the mononuclear phagocyte system including DCs and their committed progenitors ((Yona et al. 2012; Boer et al. 2003) and data below). Indeed, *Cx3cr1*Cre+*Dnmt1*lox/chip mice demonstrated efficient excision of the floxed *Dnmt1* allele in pDCs, cDCs and CDPs (Suppl. Fig. 5b). Nevertheless, there was no significant reduction of pDC and cDC frequencies, suggesting that Dnmt1 ablation by *Cx3cr1*Cre+ occurred too late in the differentiation hierarchy to affect DCs (Fig. 5d).

In contrast, *Vav*Cre+*Dnmt1*lox/chip mice excised the floxed *Dnmt1* allele in HSCs and similar to the constitutive *Dnmt1*c/chip mice showed almost complete loss of pDCs, strongly reduced MDPs and a slighter reduction in cDCs (Fig. 5e,f and Suppl. Fig. 5c,d). They had normal numbers of granulocytes, monocytes and cMoPs. Notably, they had also no significant reduction of T and B cells, which was different from *Dnmt1*c/chip mice. The reason for this difference is not yet clear, but a likely cause was that HSCs in the non-conditional *Dnmt1*c/chip model inherited an already hypomethylated genome from their ancestors, while HSCs in the conditional *Vav*Cre+*Dnmt1*lox/chip animals developed from normally methylated ancestors and had to excise the *Dnmt1* allele first so that hypomethylation was delayed and presumably less pronounced.

Collectively, constitutive methylation is required for early DC commitment but not thereafter. Moreover, the *Vav*Cre+*Dnmt1*lox/chip mice highlighted a special methylation-sensitivity of pDCs and as such represented a unique model of hypomethylation-induced pDC depletion.

**cDCs and pDCs arise together and along with myeloid cells from single HSCs**

We next employed a highly sensitive tracing system that was based on tracking of single transplanted HSCs with different lineage fate potentials to determine the nature of HSCs giving rise to DCs *in vivo* (Carrelha et al. 2018). We purified CD45.2+Lin-Sca-1+Kit+(LSK)CD34-CD150+CD48-*Vwf*-*t*dTomato+ cells (HSCs) from the BM of mice expressing *t*dTomato from the von Willebrand-factor gene (*Vwf*) promoter and eGFP from the Gata1 gene promoter to, in addition of other lineages, be able to detect donor-derived erythrocytes (eGFP+) and platelets (tdTomato+ eGFP+) which lack the CD45 antigen. We transplanted single cells of these along with CD45.1+ BM cells for radioprotection into BM ablated congenic CD45.1+ mice. As described in (Carrelha et al. 2018), we controlled engraftment and identified the lineage potential of the transplanted single HSCs by regular peripheral blood analysis over a period of up to 42 weeks (data not shown). We defined lineage potential by detecting a minimum contribution of 0.01% to the respective hematopoietic lineage in the recipient animals, leading to a classification of the HSCs into four categories as in (Carrelha et al. 2018): multilineage HSCs or HSCs with platelet (P), platelet-erythrocyte-myeloid (PEM) or platelet-erythrocyte-myeloid-B-lymphoid (PEM-B) lineage restrictions. At the end of the experiment (32-42 weeks, when LT-HSCs had stably engrafted), we determined the contribution of the single HSCs to development of pDCs and cDCs in spleens (Suppl. Fig. 6a,b). As with the other lineages, we defined DC potential by a contribution of a minimum of 0.01% donor-derived cells. As expected, multilineage and PEM-B HSCs contributed to both pDC and cDC development, while P HSCs lacked any DC potential (Figure 6a-c). Notably, PEM HSCs (possessing myeloid but no lymphoid *in vivo* output) also gave rise to both pDCs and cDCs, suggesting that DC progeny can develop independently of the lymphoid pathway (Figure 6d). This notion was further supported by the observation that the frequency by which PEM-B HSCs produced pDC and cDC progeny correlated well with their myeloid but not B cell potential (mean pDC frequency 11%, mean cDC frequency 14%, mean myeloid frequency 19%, mean B cell frequency 0.04%). A similar closer relationship of DC potential with myeloid than with lymphoid *in vivo* output was also visible for multilineage HSCs, especially when comparing the frequencies by which they produced DC or T cell progeny (Suppl. Figure 6c).

We conclude that both pDCs and cDCs arise together from stem cells because there was no single HSC that gave rise to either pDCs or cDCs and because both DC subsets developed in similar frequencies. Moreover, myeloid-restricted HSCs with no detectable lymphoid replenishment can stably generate both pDCs and cDCs, suggesting that initial lymphoid commitment is not required for DC fate. Collectively, these data support the hypothesis that both DC subsets arise from stem cells together with myeloid cells.

**Hypomethylation-induced pDC ablation can attenuate systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is a multiorgan human autoimmune disease that can be modeled in the mouse by pristane injection (Zucchi et al. 2019; Takagi et al. 2016). pDCs play a pivotal role in SLE and pDC ablation in mice can improve clinical SLE symptoms (Takagi et al. 2016; Rowland et al. 2014). Therefore, we explored a potential pre-clinical implication of our finding that hypomethylation can preferentially ablate pDCs by taking advantage of the *Vav*Cre+*Dnmt1*lox/chip mice. We induced SLE in *Vav*Cre+*Dnmt1*lox/chip and *Vav*Cre+*Dnmt1*+/+ control mice with pristane, monitored them regularly during an incubation time of 4 months and at the end of the experiment probed them for clinical and pathological signs of SLE as in (Takagi et al. 2016) (Suppl. Fig. 7a). We confirmed thateven after the strong immune stimulus by pristane, *Vav*Cre+*Dnmt1*lox/chip mice selectively lacked pDCs but maintained B cells at a frequency similarto that of *Vav*Cre+*Dnmt1*+/+ controls (Suppl. Fig. 7b,c). For each mouse, we calculated the combined clinical and pathological SLE score (for simplicity called SLE score) as a summary of both clinical and pathological symptoms according to the criteria described in Suppl. Table 6. We called SLE by a score of ≥ 2 to exclude considering of ordinary infections. As we have used mice on a genetic background (B6 x 129) with poor autoimmune susceptibility (Reeves et al. 2009), SLE development was generally moderate (maximal SLE score of 8, see Suppl. Table 7). Nevertheless, all of the *Vav*Cre+*Dnmt1*+/+ mice (12 of 12, 100%) but only 4 out of 12 *Vav*Cre+*Dnmt1*lox/chip mice (33%) developed SLE (mean total score of 5 for *Vav*Cre+*Dnmt1*+/+ versus 1.5 for *Vav*Cre+*Dnmt1*lox/chip; Fig. 7a).

Renal involvement occurs frequently in SLE patients and in SLE mouse models, and is associated with a significant increase in morbidity and mortality (Bagavant und Fu 2009). We therefore calculated a renal score by histopathological analysis of the kidneys for the existence of glomerulonephritis according to the criteria described by (Sekine et al. 2006). The renal score also demonstrated a significant difference between both genotypes (mean total score of 3.5 for *Vav*Cre+*Dnmt1*+/+ versus 1.5 for *Vav*Cre+*Dnmt1*lox/chip) (Fig. 7b and Suppl. Table 8). Figure 7c displays representative examples of the histopathological analysis showing differences in glomerular inflammation, proliferation, crescent formation and necrosis (see HE and PAS) and in the immune deposits (see electron microscopic analysis) between both genotypes. The electron microscopic analysis revealed immune deposits in 7 out of 8 (87%) analyzed *Vav*Cre+*Dnmt1*+/+ but only in 3 out of 6 (50%) *Vav*Cre+*Dnmt1*lox/chip mice. Moreover, those *Vav*Cre+*Dnmt1*lox/chip animals that succumbed to SLE developed a much weaker disease than the controls: while the controls showed all types of deposits (i.e. subepithelial, subendothelial, paramesengial and mesangial), no subepithelial or subendothelial deposits and only few paramesengial and mesangial deposits could be detected in the *Vav*Cre+*Dnmt1*lox/chip mutants (Suppl. Table 9).

The immune complexes in SLE are formed by autoantibodies (Cruchaud et al. 1975). We therefore quantified IgG and IgM immune complexes in the kidneys of the pristane-treated mice, detecting more immune complexes of both antibody classes in *Vav*Cre+*Dnmt1*+/+ than *Vav*Cre+*Dnmt1*lox/chip mice (Fig. 7d). Moreover, we detected reduced concentrations of total IgG, IgM and IgA in the pristane-treated *Vav*Cre+*Dnmt1*lox/chip animals than in the treated controls (Fig. 7e). It is important to note, however, that the *Vav*Cre+*Dnmt1*lox/chip mice did develop IgG and IgM immune complexes (albeit to a lesser extent), showing that B cells were not only present in the mutants but were also functional as they were capable of antibody production and class switching.

Collectively, disease modeling in *Vav*Cre+*Dnmt1*lox/chip mice suggested that induction of hypomethylation can deplete pDCs to reduce the symptoms of SLE.

**Discussion**

In this study, we have explored the functional role of genomic DNA methylation in DC differentiation. We found that both major lymphoid tissue DC subsets, pDCs and cDCs, arise together from single HSCs along with myeloid progeny, by a mechanism that requires maintenance of constitutively high DNA methylation levels. Our data demonstrated that pDC development is particularly sensitive to induced hypomethylation, which, as we showed, could potentially be exploited for attenuating systemic lupus erythematosus.

DCs have a short lifespan and are constantly replenished by hematopoietic progenitors (Zhan et al. 2016). However, a multitude of studies has left controversy on the origin of the different DC subsets. Taking advantage of the sensitivity of single cell transplantation experiments in mice, our data demonstrated that long-term pDC and cDC fate is stably linked with each other at the stem cell level, and is uniformly connected with myeloid fate. This finding explains a previous fate-mapping study, demonstrating that both DC subsets are produced with similar kinetics as myeloid cells (Sawai et al. 2016). The notion that pDC development does not require stem cells with *in vivo* lymphoid capacity is supported by *Vav*Cre+*Dnmt1*lox/chip mice as well, which exclusively lacked pDCs but had normal B cell frequencies. These data appear to be in contrast to a recent single cell transcriptomics study, attributing progenitors with lymphoid identity as the main (or only) source of pDCs (Dress et al. 2019). However, one could bring these different observations together by proposing that both DC subsets initially arise from ‘myeloid’ HSCs, but separate later by a methylation-based mechanism involving (or even leading to) the establishment of ‘lymphoid-like’ identity in pDC-specified progenitors. Such a dynamic lineage model appears appealing, but requires further experimental support.

DC fate choice is initiated early in hematopoiesis and coincides with the upregulation of Flt3 expression (Naik et al. 2013; Karsunky et al. 2003). In accordance with this notion, our data confirmed that DC potential increases stepwise from HSCs and is concentrated in MPP3/4 cells, which were greatly diminished in *Dnmt1*c/chip mice. Data from Cabezas-Wallscheid et al. (Cabezas-Wallscheid et al. 2014) revealed that DNA methylation differences between MPP3/4 and MPP2 are greater than those between MPP2 and MPP1/HSC, indicating that methylation changes increase steadily during early hematopoiesis. Moreover, these authors showed that 71% of DMRs between HSC and MPP1 were characterized by DNA methylation loss, while DMRs identified between MPP2 and MPP3/4 were 75% gain of methylation and these DMRs were strongly associated with silencing of the corresponding genes (Lipka et al. 2014). Our data show that this methylation gain is dependent on DNMT1 and is essential for DC fate. Mechanistically, DNMT1 hypomorphic cells failed to gain sufficient methylation to both facilitate the expression of DC genes (mainly *Flt3* and others) and inhibit genes of alternative lineages. This suggests that the genomic methylation level is a decisive factor if uncommitted precursor cells can undergo DC versus alternative lineage fate.

There is overwhelming evidence for a pivotal role of pDCs in autoimmune diseases, in particular in SLE, proposing DCs as a promising target for treatment (reviewed in (Klarquist et al. 2016)). Epigenetic modifications are generally dynamic and as such can potentially be modulated for disease treatment. Indeed, DNA hypomethylating agents such as azacitidine and decitabine (both acting as DNMT inhibitors) are currently applied clinically in cancer therapy (Navada et al. 2014). Changes in DNA methylation have also been described in SLE patients (reviewed in (Hedrich et al. 2017)). However, these changes mainly comprise methylation loss, in particular at genes encoding cytokines and interleukins which enhanced expression is thought to contribute to SLE pathophysiology (Joseph et al. 2019; Surace und Hedrich 2019; Ulff-Møller et al. 2018; Javierre et al. 2010). Hence, hypomethylation-based therapy might even further enhance expression of these genes, raising concern on such an approach. Another obvious hurdle for targeting DNA methylation in SLE is that currently available reagents are not cell-type-specific, so that pleiotropic effects can be assumed. However, our data argue for an opposite view, proposing potential suitability of methylation targeting in SLE. We showed that pDCs are hypersensitive to global reduction of methylation and that pDC ablation by induction of hypomethylation attenuated SLE in mice, hypomethylating drugs may predominantly ablate pDCs also in patients as well and as such could be beneficial in SLE therapy. It is important to note in this context that although we cannot formally rule out possible B cell defects in *Vav*Cre+*Dnmt1*lox/chip mice, our data confirmed that the B cells in these animals were unchanged in number and were capable of antibody production and class switching. Nevertheless, even if *Vav*Cre+*Dnmt1*lox/chip mice had a yet unappreciated B cell phenotype, this would even further support suitability of global hypomethylation to ameliorate SLE.

In summary, constitutive DNA methylation is an epigenomic hallmark of DC differentiation, providing novel options for targeting DCs in clinical settings.

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**Author Contributions**

M.C., S.S., J.C., Y.M. and L.T. conducted experiments. S.T., S.K., S.S., D.B.L., B.H., M.S. and M.G. analyzed data. M.C. and F.R. designed the study and wrote the main parts of the manuscript. All authors contributed to manuscript writing. M.C., M.M., C.P., M.P., M.S., S.J., C.N., D.B.L. and F.R. supervised the project, brought in significant intellectual input and provided financial support.

**Declaration of Interests**

The authors declare no competing interests.

**Figure legends**

**Figure 1: DC development in *Dnmt1*c/chip mice.** **a-d,** Flow cytometry analysis of (**a**) pDCs (PDCA+CD11cint) in BM, of (**b**) cDCs (MHCII+CD11chi) and (**c**) cDC subsets (MHCII+CD11chiCD11b+CD8- and MHCII+CD11chiCD11b-CD8+) in spleens, and of (**d**) macrophage-DC progenitors (MDPs: Lin-CD117hiCD115+Ly-6C-CD11b-) and common monocyte progenitors (cMoP: Lin-CD117hiCD115+Ly-6ChiCD11b-) in BM of *Dnmt1*c/chipmice and *Dnmt1*+/+control littermates. Representative density plots are shown on the left (numbers indicate percentage of cells within the gates) and summaries of the analyzed cohorts are shown on the right. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). Cell frequencies are indicated as percent living cells in **a-c**, or as percent Lin- cells in **d**. n was 8 mice per genotype in **a**, 7 in **b** and **c**, and 15 in **d**. All figures represent at least two independent experiments.

**Figure 2: Impaired DC development in *Dnmt1*c/chip mice is cell intrinsic.**  **a-c,** Flow cytometry analysis of donor cell derived (**a**) BM pDCs (CD45.1-CD45.2+PDCA+CD11cint), (**b**) splenic cDCs (CD45.1-CD45.2+MHCII+CD11chi) and (**c**) BM macrophage-DC progenitors (MDPs: CD45.1-CD45.2+Lin-CD117hiCD115+Ly-6C-CD11b-) and common monocyte progenitors (cMoP: CD45.1-CD45.2+Lin-CD117hiCD115+Ly-6ChiCD11b-) of BM chimeras that had received 5x106 BM cells of *Dnmt1*+/+(129S1/SvlmJ) or *Dnmt1*c/chipdonormice 10-14 weeks before. Representative density plots of CD45.1-CD45.2+ donor population gated cells are shown on the left (numbers indicate percentage of cells within the gates) and summaries of the analyzed cohorts are shown on the right. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). Cell frequencies are indicated as percent CD45.1-CD45.2+ donor-derived living cells in **a** and **b**, or as percent CD45.1-CD45.2+ donor-derived Lin- cells in **c**. n was 8 mice per genotype in **a** and **b**, and 7 in **c**. All figures represent at least two independent experiments.

**Figure 3**: **Whole genome methylation data of DC and monocyte lineages.** (**a**) Violin plot showing the distribution of whole genome CpG methylation per indicated population. Data show the average across the biological replicates (for number of replicates see Suppl. Fig. 3a). Dots within the violins represent means. (**b**) Principal component analysis of DMR methylation in the indicated populations. PC1 and PC2 depict principal components 1 and 2. Percent values at axes indicate sample variation explained by the respective principal component. Each dot represents a biological replicate. (**c**) Violin plot showing the distribution of DNA methylation levels across all DMRs in pDCs, CD11b+ cDCs and CD8+ cDCs. Dots within the violins represent medians, the box heights represent the interquartile range (IQR) and the whiskers indicate 1.5x IQR. (**d**) Heatmap of the union of all DMRs (n = 49,588) detected between HSCs and each of the other indicated populations based on unsupervised hierarchical clustering of z-scores. Depicted are 500 randomly sampled DMRs per cluster. Each horizontal dash represents a DMR. Data show the average methylation of aggregated biological replicates.

**Figure 4: Flt3 re-expression and general link between methylation and expression of DC genes.** (**a**) Volcano plot showing a comparison between the transcriptomes of MDPs (CD45.1-CD45.2+Lin-CD117hiCD115+Ly-6C-CD11b-) isolated from *Dnmt1*+/+ or *Dnmt1*c/chipmice (n = 4 biologically independent samples each). Differentially expressed genes (cutoff criteria: log2 fold change difference ≥ 0.58 or -0.58 and p-value ≤ 0.05) are represented by black dots. A positive fold change indicates transcripts with decreased expression in *Dnmt1*c/chipMDPs and a negative fold change indicates genes with increased expression. *Dnmt1* and *Flt3* transcripts are highlighted. (**b**) Heatmap of DC signature genes (genes marked by blue bar on the left) with decreased expression or of granulocyte signature genes (genes marked by red bar on the left) with increased expression in *Dnmt1*c/chipversus *Dnmt1*+/+ MDPs, respectively. Gene signatures were taken from (Schönheit et al. 2013). Color code on the right represents RMA normalized log2 expression levels. (**c**) Heatmap summarizing enrichment (red) or depletion (green) of up- or downregulated genes in *Dnmt1*c/chip MDPs or cMoPs compared to their control counterparts in the DMR clusters shown in Fig. 3b. For enrichment analysis, only DMRs overlapping with gene promoter regions were considered. (**d**) Summary of percent LSK stem/progenitor cells from *Dnmt1*+/+ or *Dnmt1*c/chipmice (n = 7 each) expressing FLT3 protein on their surface as determined by flow cytometry. (**e**, **f**) Rescue of total cell number (**e**) and pDC development (**f**) by retroviral Flt3 expression in c-kit-enriched BM cells from *Dnmt1*c/chip mice cultured with Flt3 ligand. The graph summarizes two independent experiments with a total of n = 9 *Dnmt1*+/+ and n = 10 *Dnmt1*c/chipmice. Each symbol in **d**-**f** represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). (**g**) Representative flow cytometry images showing increased pDC (PDCA+CD11c+) frequency in cultured FLT3-transduced (bottom) c-kit-enriched *Dnmt1*c/chip BM cells as compared to the empty vector (top) transduced control.

**Figure 5:** **Differentiation of HSCs into DC-committed progenitors requires constitutive methylation.** (**a**) Flow cytometry analysis of HSC/MPP1 (CD150+CD48-LSK), MPP2 (CD150+CD48+LSK), and MPP3/4 (CD150-CD48+LSK) in the BM of *Dnmt1*c/chipmice and *Dnmt1*+/+control littermates. Representative cell density plots (numbers indicate percentage of cells within the gates) and summaries of the analyzed cohorts are presented. (**b**) Summery of FLT3+ cells within the MPP3/4 population as determined by flowcytometry. (**c**) Flow cytometric analysis of donor-derived pDC (CD45.1+CD45.2-PDCA+CD11cint) and cDC (CD45.1+CD45.2-PDCA-CD11chigh) 12-14 days after adoptive transfer of HSC (CD45.1+CD45.2-LSK+CD150+CD48-CD34-), MPP1 (CD45.1+CD45.2-LSK+CD150+CD48-CD34+), MPP2 (CD45.1+CD45.2-LSK+CD150+CD48+CD34+), MPP3 (CD45.1+CD45.2- LSK+CD150-CD48+CD34+Flt3-) or MPP4 (CD45.1+CD45.2-LSK+CD150-CD48+CD34+Flt3+) into CD45.1-CD45.2+ recipient animals. Numbers indicate percentage of cells within the gates. A second, independent experiment with similar outcome is shown in Suppl. Fig. 5a. (**d**-**f**) Frequencies of the indicated cell populations in (**d**) *Cx3cr1*Cre+*Dnmt1*lox/chip and *Cx3cr1*Cre-*Dnmt1*lox/chip or (**e**,**f**) *Vav*Cre+*Dnmt1*+/+, *Vav*Cre-*Dnmt1*lox/chip and *Vav*Cre+*Dnmt1*lox/chip mice as determined by flowcytometry. pDCs (PDCA+CD11cint), monocytes (Lin-CD117-CD115+), MDP (Lin-CD117hiCD115+Ly-6C-CD11b-) and cMoP (Lin-CD117hiCD115+Ly-6ChiCD11b-) were measured in BM, cDCs (MHCII+CD11chi), B cells (B220+CD19+), T cells (CD3+) and granulocytes (CD11b+Gr1+) in spleens. (**a**-**f**) Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). Cell frequencies are indicated as percent of LSK in **a**,**b**, as percent living cells in **d-e** or as percent Lin- cells in **f**. All figures represent at least two independent experiments.

**Figure 6: DC development from single HSCs.** *In vivo* contribution of single CD45.2+Lin-Sca-1+Kit+(LSK)CD34-CD150+CD48-*Vwf*-*t*dTomato+ donor HSCs with either multipotent reconstitution capacity (**a**) or with different lineage restrictions (**b**-**d**) to DC progeny in spleens of BM-ablated CD45.1+ recipient mice. The plots summarize the donor-derived frequencies of n = 4 mice with multilineage HSCs, n = 4 with platelet-restricted HSCs, n = 3 with PEM-restricted HSCs and n = 5 with PEM-B-restricted HSCs generated in 4 independent experiments and presented in logarithmic scale. Purple and red dots indicate pDCs or cDCs, respectively.

**Figure 7: Impaired SLE development by pDC elimination upon induced hypomethylation.** Total clinical and pathological (**a**) and renal (**b**) scores demonstrating significantly impaired SLE development in pristane-treated *Vav*Cre+*Dnmt1*lox/chip mice as compared to *Vav*Cre+*Dnmt1*+/+ controls (n = 12 each, split over three independent experiments). Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). (**c**) Light microscopic images of hematoxylin and eosin (HE) stained and periodic acid-Schiff (PAS) reacted (upper two panels) and electron-microscopic (EM) images of kidney sections from pristane-treated *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice. *Vav*Cre+*Dnmt1*+/+ mice demonstrated increased glomerular inflammation, mesangiale matrix and cellularity (black arrow), increased presentation of crescent formation (blue arrow) and small necrosis (yellow arrow). In EM, all types of deposits (subepithelial (green arrow), subendothelial (red arrow), paramesangial and mesangial) could be identified in *Vav*Cre+*Dnmt1*+/+ animals, while in the *Vav*Cre+*Dnmt1*lox/chip group neither subepithelial nor subendothelial and only few mesangial and paramesangial deposits were detected. The images are of single animals from 3 independent experiments. (**d**) Left: Representative immunofluorescence images of immune complexes by staining for IgG (top images) and IgM (middle images) of kidney sections from pristane-treated *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice. The bottom images show IgG (red) and IgM (green) combined, together with DAPI (blue) nuclear staining. Right: Quantification of IgG and IgM immunofluorescence intensities (as immunohistochemistry (IHC) positivity per mm2). Each symbol represents an individual mouse from 3 independent experiments; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). (**e**) Quantification of total IgG, IgM and IgA concentrations in the sera of pristane-treated *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed).

**STAR Methods**

**Mice**

Mice carrying targeted *Dnmt1*c, *Dnmt1*chip, *Dnmt1*lox, or *Cx3cr1*Cre alleles or a *Vav*Cre transgene were described previously (Gaudet et al. 2003; Yona et al. 2012; Boer et al. 2003; Jackson-Grusby et al. 2001b). General mouse husbandry was carried out under specific pathogen-free conditions in the central animal facilities of the University of Münster. All relevant ethical guidelines were followed and all animal experiments were approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen) according to the German Federal Animal Protection Act (Regulation numbers 84-02.04.2014.A183 and 84-02.04.2014.A278). B6.SJL-PtprcaPepcb/BoyCrl (SJL; CD45.1+CD45.2-) mice were purchased from Charles River and were crossed with 129S1/SvImJ mice (129; CD45.1-CD45.2+) to obtain 129/SJL (CD45.1+CD45.2+) F1 mice as recipients for BM transplantation experiments.

**Cell suspension preparation from organs**

BM cells were obtained from mice by flushing femurs and tibiae with PBS (PAA) supplemented with 1% fetal calf serum (FCS) (Biochrom). Single-cell suspensions from BM and spleen were generated by mashing the organ through a cell strainer of 40 μm diameter. Peripheral blood was isolated periorbitally from living animals or by heart puncture from dead animals with an EDTA-treated cannula. Cell suspensions were incubated for 5 min with ice-cold ACK buffer (0.15 M NH4Cl, 10 M KHCO3, 0.1 mM EDTA, pH 7.3) and were washed in PBS.

**Flow cytometry**

Flow cytometry was performed with 8-12 weeks old mice unless stated otherwise. Labelling of single-cell suspensions was performed on ice in PBS supplemented with 1% FCS. Cells were first incubated for 10 min in PBS supplemented with 1% FCS and purified CD16/32 antibody to block Fc binding. Labelling was conducted for 10 min on ice in the dark. All fluorochrome-conjugated antibodies used are listed in Suppl. Table …. Lin- cells were identified with a lineage 'cocktail' of antibodies to B220, CD4, CD8α, CD3ε, CD19, CD11c, Ter119, NK1.1 and Ly-6G. Discrimination of dead cells was performed by addition of 7-aminoactinomycin D (7-AAD; BioLegend) viability stain solution 10 min before measurement. Fluorescence intensity was measured with Canto II or Aria III (BD) FACS cytometers equipped with FACSDiva software. Data analysis was performed with FlowJo software.

**Generation and analysis of BM chimeras**

5 x 106 BM cells from 8-12 weeks old *Dnmt1*+/+ and *Dnmt1*c/chip mice (both on CD45.1-CD45.2+ 129S1/SvlmJ background) were transplanted into lethally irradiated (2 x 7 Gy) 129/SJL F1 recipient mice (CD45.1+/CD45.2+). 10-14 weeks after transplantation, mice were sacrificed, BM and spleen cells were recovered and analyzed by flow cytometry.

**Transplantation and DC fate analysis of HSC/MPP populations**

HSCs and MPPs1-4 (for definition and surface marker combination see (Cabezas-Wallscheid et al. 2014)) were isolated from the BM of healthy, 8-10 weeks old CD45.1+CD45.2- SJL wildtype mice and were immediately transplanted into sublethally (4.5 Gy) irradiated CD45.1-CD45.2+ B6 recipient mice. Numbers of transplanted cells per recipient mouse were: 800 HSCs, 3,300-4,200 MPP1, 1,500-1,600 MPP2, 4,000-4,300 MPP3 and 20,000-30,000 MPP4. Frequencies of donor-derived pDCs (CD45.1+CD45.2-PDCA+CD11cint) and cDCs (CD45.1+CD45.2-CD11chiMHCII+) were determined 12-14 days after transfer by flow cytometry.

**Transplantation and DC fate analysis of single HSCs**

Transplantation of single HSCs was performed as described previously (Carrelha et al. 2018). In brief, single CD45.2+Lin-Sca-1+Kit+(LSK)CD34-CD150+CD48-*Vwf*-*t*dTomato+ HSCs were flow sorted from BM cell suspensions of *Vwf-td*Tomato/*Gata1*-eGFP (CD45.1-CD45.2+) B6 adult mice and were mixed with 200,000 wildtype CD45.1+CD45.2- SJL BM competitor cells before intravenous injection into lethally irradiated (10 Gy) CD45.1+CD45.2- SJL recipient mice. After confirmation of engraftment lineage contribution of the transplanted HSCs was investigated by analysis of mature hematopoietic cells in the peripheral blood of the recipient mice at different time points (weeks 8,12,16 and between 31-42) by the antibodies given in Suppl. Table …. Frequencies of donor-derived pDCs (CD45.1-CD45.2+PDCA+CD11cint) and cDCs (CD45.1-CD45.2+CD11chiMHCII+) were determined in spleens at the end of the experiment.

**Retroviral constructs, viral supernatant production and cell transduction**

MSCV retroviral constructs expressing IRES-GFP or murine Flt3-IRES-GFP (gift from Stephen L. Nutt) and production of retroviral supernatants have been described (Holmes et al. 2006; Rosenbauer et al. 2004). For retroviral transduction, c-Kit–enriched BM cells were cultured for 36 hours at 37 °C in Iscove's modified Dulbecco medium (10% FCS, 100 μg/ml penicillin/Streptomycin) containing 100 ng/ml murine SCF, 100 ng/ml murine Flt3 ligand, 20 ng/ml murine IL-3 and 20 ng/ml murine IL-11. Cells were then mixed with retroviral supernatants and cultured in the presence of 100 ng/ml murine SCF, 100 ng/ml murine Flt3 ligand, 20 ng/ml murine IL-3 and 20 ng/ml murine IL-11 and 8 μg/ml polybrene (hexadimethrine bromide; Sigma-Aldrich) for 24-48 h at 37 °C. Transduced GFP+ cells were flowsorted and cultured in pDC medium (RPMI 1640, 10% FCS, Pen/Str, 1 mM Sodium Pyruvat, β-Mercaptoethanol (1: 1000), 100 ng/ml Flt3 Ligand) for 8 days according to a published protocol (Won et al. 2014).

**PCR genotyping**

Genomic DNA (gDNA) was extracted with the High Pure PCR Template Preparation Kit (Roche). Primers and gDNA were mixed with 2× Go Taq G2 Hot Start Green Master Mix (Promega) and were subjected to PCR analysis on a Mastercycler pro (Eppendorf).

**Transcriptomics analysis**

MDPs (Lin-cKithiCD115+CD11b-Ly-6C-) and cMoPs (Lin-cKithiCD115+CD11b-Ly-6C+) were sorted from 8-12 weeks old *Dnmt1*+/+ and *Dnmt1*c/chip mice, total RNA was extracted using the RNeasy Micro kit (Qiagen) and gDNA was digested with the RNase-free DNAse provided in the Kit. Sample processing and hybridization on Affymetrix GeneChIPs (Mus musculus) MG 430 PM was carried out according to standard procedures at the Center of Excellence for Fluorescent Bioanalytics (KFB, University of Regensburg). Data was analyzed with the Bioconductor package version v.3.5.1. The CEL files containing the raw expression intensity values for each probe on the array were opened using the affy package and normalized using the function called Robust Multi-Array Average (RMA). The linear models for microarray data (LIMMA) package was used to find differentially expressed genes. A cutoff of more than 0.58 logFC and p-value of less than 0.05 was used to call genes as differentially expressed between two conditions. The gene expression raw-data can be accessed in Gene Expression Omnibus (GSE).

**Tagmentation-based whole-genome bisulfite sequencing (TWGBS)**

For DNA methylation analysis of the indicated progenitor populations, cells were isolated from 8- to 12-week-old C57BL/6J mice by fluorescence activated cell sorting. The following cell populations were isolated from bone marrow cells: MDPs (Lin-cKithiCD115+Flt3+), CDPs (Lin-cKitloCD115+Flt3+), cMoPs (Lin-cKithiCD115+Flt3-CD11b-Ly6C+) and monocytes (CD11b+Ly6C+B220-CD5-CD8α-Ter119-SiglecF-Ly6G-FceRI-). Monocytes were flowsorted from total bone marrow. MDPs, CDPs and cMoPs were sorted from lineage negative cells. Lineage depletion of mature hematopoietic cell lineages was performed using Dynabeads (Dynabeads Sheep anti-Rat IgG magnetic beads, Thermo Fischer) and rat IgG isotype antibodies to B220, CD4, CD8α, CD19 and Ter119, expanded by antibodies to CD11b and Gr-1 for isolation of MDP and CDP or Ly-6G for cMoP isolation. pDCs (PDCA+CD11cint), CD8+ cDC (CD11chiMHCII+CD8α+CD11b-) and CD11b+ cDC (CD11chiMHCII+CD8α+CD11b-) were isolated from spleen suspensions. Per biological replicate, we sorted 20.000 monocytes (n=3, each probe was pooled from BM of 2 or 3 independent animals), 10,000 MDP (n=2, each probe was pooled from BM of 7 and 8 independent animals), 20,000-25,000 CDP (n=4, each probe was pooled from BM of 2 or 3 independent animals), 15,000-20,000 cMoP cells (n=2, and each probe is pooled from BM of 2 or 3 independent animal), 20,000 CD11b+ cDC, 10,000 CD8+ cDC and 20,000 pDC (n=3, and each probe was pooled from spleens of 3 independent animals). All sorted cell populations were snap-frozen as dry-pellets and stored at -80°C.

Genomic DNA (10-30ng) was isolated using the QIAamp DNA Micro Kit (Qiagen) and used as input to generate sequencing libraries by tagmentation-based whole-genome bisulfite sequencing (TWGBS) as described previously (Wang et al. 2013). To reduce PCR duplicates, four independent sequencing libraries were prepared per replicate and library amplification was monitored by real-time quantitative PCR. Pools of four libraries were sequenced on three separate lanes using the 125bp paired-end mode on the HiSeq 2000 platform (Illumina). Sequencing was performed by the Genomics and Proteomics Core Facility at the German Cancer Research Center. TWGBS raw-data as well as methylation calls in BED format can be accessed in Gene Expression Omnibus (GSE). TWGBS data from murine HSCs were taken from a previous study (GSE52709) (Cabezas-Wallscheid et al. 2014).

**TWGBS sequence alignment and methylation calling**

Read alignment was performed by the Omics IT and Data Management Core Facility (ODCF) at the German Cancer Research Center using an updated version of the pipeline published by (Wang et al. 2013), which was implemented as a Roddy Workflow (https://github.com/DKFZ-ODCF/AlignmentAndQCWorkflows) in the automated ’One Touch Pipeline’ (OTP) (Reisinger et al. 2017). Briefly, adaptor sequences of raw reads were trimmed using ’Trimmomatic’ (Bolger et al. 2014). Sequencing reads were then *in silico* bisulfite-converted (C>T for the first read in the pair, G>A for the second). The software package ’BWA-MEM’ (https://arxiv.org/abs/1303.3997) was used with default parameters to align the converted reads to the *in silico* bisulfite-converted reference mm10 genome extended with the PhiX and lambda phage sequences. After alignment, reads were converted back to their original state. PCR duplicate removal was performed per library using ‘Picard MarkDuplicates’ before merging reads from all libraries per replicate. The alignment quality was validated by computing the mapping rates using samtools flagstat (Li et al. 2009), insert size distributions, and genome coverage statistics.

Methylation calling and M-bias trimming were performed using the bistro software package (version 0.2.0) (https://github.com/stephenkraemer/bistro). Automatic M-bias removal was performed using the 'binomp' algorithm. This approach removes the gap repair nucleotides introduced by the tagmentation reaction from both reads (first 9 bp following sequencing primer 2) and automatically detects and removes additional read positions with M-bias for each individual sample. Reads with a mapping quality ≥25 and nucleotides with Phred-scaled quality score ≥25 were considered for further analysis. Bisulfite conversion rates were estimated using the autosomal CHH methylation levels.

**Detection of differentially methylated regions**

Differentially methylated regions (DMRs) were called pairwise between HSCs and each of the other cell populations using DSS (Park und Wu 2016). Differential methylation at each CpG site was tested without smoothing. DMRs were defined by i) a minimal DMR length of 50bp ii) a minimal number of 3 CpGs iii) a minimum fraction of differentially methylated CpGs of 50% (posterior p-value for a methylation delta greater or equal than 10% < 0.01). Within each pairwise comparison, DMRs separated by less than 50 bp were joined. For integrated analyses, DMRs from all pairwise comparisons were combined (union of the genomic intervals). For each DMRs, the maximum methylation difference between any two populations was determined, and only DMRs with a maximum methylation difference >= 10% were kept for further analysis.

**PCA and Clustering**

Principal component analysis (PCA) was performed on all DMRs based on the average DNA methylation levels. DMRs were clustered using hierarchical clustering (Ward's method) of the z-score normalized average DNA methylation levels. Partitioning was performed with the cutreeHybrid algorithm of the dynamicTreeCut package using R version 3.6.2 and the following parameters: deepSplit=1, minClusterSize=0.005 \* number of DMRs, pamStage=False and minGap=0.1.

**DMR gene annotation and gene set enrichment analysis**

DMR gene annotation was performed by the gtfanno software package (https://github.com/stephenkraemer/gtfanno). Gene set enrichment analysis in DMRs was performed using regionset\_profiler (https://github.com/stephenkraemer/regionset\_profiler). Only promoter DMRs were considered and gene-set membership for each DMR was determined based on its gene annotation. Promoters were defined as regions 5000 bp upstream and 1000 bp downstream of the TSS. Enrichment of gene sets in individual DMR clusters against the background of all other DMRs was tested using Fisher's exact test. Gene-sets were obtained from **Suppl. Table** 4.

**Induction of systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) was induced by treating *Vav*Cre+*Dnmt1*lox/chip and *Vav*Cre+*Dnmt1*+/+ mice with pristane (2,6,10,14-tetramethylpentadecane; Sigma-Aldrich) as described (Takagi et al. 2016). In brief, the animals received a single intraperitoneal pristane injection (0.5 ml per mouse) and were subsequently monitored by regular weight control and urine as well as blood sampling for clinical and pathological signs of SLE. Protein in urine was quantified by urine stix (Combur Test, Roche) measuring protein ranging from negative (neg), 30 mg/ml (Pro 1+), 100 mg/ml (Pro 2+) until 500 mg/ml (Pro 3+). The experiment was terminated after 4 months and all mice were subjected to detailed analyses as described in the results section.

**Histology**

Tissue samples were fixed in 4% formalin, embedded in paraffin and cut into 3 µm thick sections, which were stained with hematoxylin and eosin (HE) or periodic acid-schiff reaction (PAS). Blinded histopathological scoring was done by a pathologist to elevate the morphological changes according to (Sekine et al. 2006). Glomerular inflammation, glomerular proliferation, crescent formation and necrosis were evaluated with scores from 0 to 3 (0=none, 1= mild, 2=moderate, 3= severe). The data for crescent and necrosis were doubled counted.

**EM**

Kidney tissue was sliced into 23 mm blocks and fixed in 2.5 % glutaraldehyde over night and were then washed in tap water. Samples were treated with osmium tetroxide for one hour, dehydrated in an ascending alcohol series, and infiltrated with epon using a mixture of propylenoxid and epon (1:2) for 1.5 hours. After embedding in pure epon the samples were kept at 60°C for 36 hours, ultrathin slices of 60 nm were cut and were contrasted with uranyl acetate and lead citrate. Ultrastructural images were done with a Philips EM 208S transmission electron microscope at various magnifications.

**Immunohistochemistry**

The tissue was fixed in 4% formalin and dehydrated in a tissue processing machine (Leica ASP300, Leica) overnight. After paraffin embedding, the tissue was cut into 3 µm thick sections and mounted onto Superfrost objective slides (R. Langenbrinck). For initial deparaffinization, the slides were incubated at 80°C for one hour. The slides were then deparaffinized in Xylene and cooked in citrate buffer for 40 minutes (Target Retrieval Solution; DAKO). 5% Bovine Serum Albumin (Albumin Fraction V; Roth) plus 0.5% Triton X 100 (Roth) in PBS was used for blocking for one hour. Goat anti-Mouse IgM (Invitrogen) and Donkey anti-Mouse IgG (Invitrogen) antibodies were incubated in 5% Bovine Serum Albumin plus 0.5% Triton X 100 overnight at +4°C, followed by three washes with PBS. DAPI (1:10,000; Boehringer) was added for 30 minutes. After washing three times with PBS, coverslips were mounted using Mowiol solution as mounting reagent. Mowiol solution was prepared as follows: 7.2 g Mowiol 4-88 Reagent (MerckMillipore) was added to 18 g Glycerol and 18 ml distilled water and stirred overnight. After dissolving the mixture in 36 ml 0.2 M TRIS pH 8.5 at 53°C, the solution was centrifuged at 5,000 rpm for 20 minutes. 0.1% 1,4-Diazabicyclo[2.2.2]octane (DABCO; Sigma) was added to the supernatant and stored at -20°C until use. Images were taken with a BZ-9000 Fluorescence Microscope (BioRevo, Keyence). FIJI, a distribution of ImageJ, v1.52p, was used for the automated measurement of the signal intensity of the immunohistochemical reactions. Per kidney, five randomly selected windows with a comparable distance to the tissue border (1,000 x 1,000 pixels, equivalent to 1065.5 x 1065.5 µm) were assessed. Background and noise were reduced by excluding extremely weak signal (threshold 26).

**Statistical analysis**

Statistical analysis was conducted using Prism software (GraphPad). The two-tailed Student’s t-test was used for statistical analysis of two-group comparisons. P < 0.05 was considered significant. No statistical methods were used to predetermine sample sizes, but sample sizes are similar to those generally employed in the field.

**Data availability**

The data that support the findings of this study are available from the senior authors on request. All sequencing and array data have been deposited in the Gene Expression Omnibus (GEO) under accession numbers ….. Publicity available ….. Data referenced in this study were extracted from GEO with the accession number GSE52709.

**Supplemental Information**

**Supplementary figure legends**

**Supplementary figure 1: Frequencies of B cells and myeloid cells in *Dnmt1*c/chip mice. a,b,** Flow cytometry analysis of (**a**) B cells (B220+CD19+) in spleens, or of (**b**) monocytes/macrophages (CD11b+CD115+) and (**c**) granulocytes (CD11b+Gr1+) in BM of *Dnmt1*c/chipand *Dnmt1*+/+mice. Representative density plots are shown on the left (numbers indicate percentage of cells within the gates) and summaries of the analyzed cohorts are shown on the right. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). Cell frequencies are indicated as percent living cells. n was 5 mice per genotype each.

**Supplementary figure 2: Donor-derived granulocytes develop normally in *Dnmt1*c/chip chimeric mice.** (**a**) Representative flow cytometry analysis demonstrating BM engraftment of donor-derived cells in the BM chimeras. Donor genotypes (top) as well as CD45.1+CD45.2+ recipient and CD45.1-CD45.2+ donor cells (gated) are indicated. (**b**) Flow cytometry analysis of donor cell derived BM granulocytes (CD45.1-CD45.2+CD11b+Gr1+) of chimeras that had received BM cells of *Dnmt1*+/+(129S1/SvlmJ) or *Dnmt1*c/chipdonormice 10-14 weeks before. Representative density plots of population gated cells are shown on the left (numbers indicate percentage of cells within the gates) and a summary of the analyzed cohorts is shown on the right. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). Cell frequencies are indicated as percent CD45.1-CD45.2+ donor-derived living cells. n was 7 mice per genotype. All figures represent at least two independent experiments.

**Supplementary figure 3: TWGBS data.** (**a**) Table summarizing numbers of biological replicates and of mean methylation values (100% methylated = 1) per replicate or per population. Welch's t-test was used to test the null hypothesis that the mean methylation level did not change between HSCs and the other individual populations. (**b**)Beta value heatmap of the union of all DMRs (n = 49,601) detected between HSCs and all other indicated populations. Depicted are 500 randomly sampled DMRs per cluster. Each horizontal dash represents a DMR. Data show the average across the biological replicates. Nine distinct DNA methylation clusters were identified by unsupervised hierarchical clustering on methylation level z-scores as described in Figure 3C. (**c**) Number of DMRs per indicated cluster.

**Supplementary figure 4: Transcriptome analysis and Flt3 expression in progenitors from *Dnmt1*c/chipmice.** (**a**) Volcano plot showing a comparison between the transcriptomes of cMoPs isolated from *Dnmt1*+/+ or *Dnmt1*c/chipmice (n = 4 biologically independent samples each). Differentially expressed genes are represented by black dots. A positive fold change indicates transcripts with decreased expression and a negative fold change indicates genes with increased expression in *Dnmt1*c/chipcMoP. Dnmt1 and Flt3 transcripts are highlighted. Cutoff criteria were an absolute log2 fold change difference ≥ 0.58 and a p-value ≤ 0.05. (**b**) Dendrogram showing a closer hierarchical relationship of *Dnmt1*c/chipMDPs with cMoPs than with *Dnmt1*+/+MDPs. In contrast to the MDPs, *Dnmt1*c/chipand *Dnmt1*+/+cMoPs clustered closely together. (**c**) Reduced mean fluorescence intensity (MFI) of FLT3 surface protein expression on LSK stem/progenitor cells from *Dnmt1*c/chipmice as compared to those from *Dnmt1*+/+ mice (n = 7 each) determined by flow cytometry. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed). (**d**) Representative flow cytometry images showing increased living cell count in cultured FLT3-transduced c-kit-enriched *Dnmt1*c/chip BM cells as compared to the empty vector transduced control.

**Supplementary figure 5: DC differentiation by transplanted HSC/MPP and validation of conditional Dnmt1 hypomorphic animals.** (**a**) Flow cytometric analysis of donor-derived pDC (PDCA+CD11cint) and cDC (PDCA-CD11chigh) 12-14 days after adoptive transfer of CD45.1+ HSC, MPP1, MPP2, MPP3 or MPP4 into CD45.2+ recipient animals. Numbers indicate percentage of cells within the gates. A second, independent experiment with similar outcome is shown in Fig. 5c. (**b**) PCR genotyping analysis demonstrating excision of the floxed *Dnmt1* allele in pDCs, cDCs and CDPs (Lin-Flt3+CD117-CD115+) but not myeloid progenitor (MPs, Lin-Flt3+CD117+CD115- (Yona et al. 2012)) sorted from *Cx3cr1*Cre+*Dnmt1*lox/chip and *Cx3cr1*Cre+*Dnmt1*lox/+ mice. A 100 bp ladder size marker is shown on the left, control bands of the excised (Δ) and non-excised (lox) floxed *Dnmt1* allele are shown on the right. (**c**) Representative flow cytometric cell density plots of pDCs (PDCA+CD11cint, left) andB cells (B220+CD19+, right) in spleens of *Vav*Cre+*Dnmt1*lox/chip and *Vav*Cre+*Dnmt1*+/+ animals. (**d**) PCR genotyping analysis demonstrating excision of the floxed *Dnmt1* allele in long-term HSCs (LT-HSC, LSK CD34-Flt3-), short-term HSCs (ST-HSC, LSK CD34+Flt3-) and multipotent progenitors (MPP, LSK CD34+Flt3+) of *Vav*Cre+*Dnmt1*lox/chip animals. A 100 bp ladder size marker as well as control bands of the excised (Δ) and non-excised (lox) floxed *Dnmt1* allele are shown on the left.

**Supplementary figure 6: DC development from single cell HSC.** Flow cytometry images indicating contribution of the single CD45.2+ donor HSCs to the (**a**) pDC (PDCA+CD11cint) or (**b**) cDC (MHCII+CD11chi) populations in the spleens of transplanted CD45.1+ recipient animals at the end point of experiment (31-42 weeks). The data are representative of 4 independent experiments. Numbers indicate the cell frequencies (%) within the respective gates. (**c**) The same data as in Fig. 3a are represented in linear scale to better visualize the parallel production of pDCs and cDCs with myeloid cells from single multilineage HSCs in reconstituted mice.

**Supplementary figure 7: SLE induction in *Vav*Cre+*Dnmt1*lox/chip mice.** (**a**) Experimental scheme of SLE induction in mice by pristane.The indicated genotypes were injected with a single dose of pristane as described in the materials and methods section. Development of SLE was monitored based on regular weight, urine and serum control over a period of 4 months. Three independent experiments were performed reaching a total of 12 mice per genotype. (**b**,**c**) Frequency of (**b**) pDCs (PDCA+CD11cint) and (**c**) B cells (B220+CD19+) in spleens of pristane-treated *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice. Each symbol represents an individual mouse; small horizontal lines indicate the mean ± s.d. (unpaired t-test, two-tailed).

**Supplementary tables**

**Suppl. Table 1.** Excel tableflagstats-selected

**Suppl. Table 2.** Excel table coverage mean

**Suppl. Table 3.** Excel table DMR counts

**Suppl. Table 4.** Excel table MicroArraydataAnalysis\_OnlyDEGs

**Suppl. Table 5.** Excel table cluster-marker-genes\_promoters

**Suppl. Table 6. Definition of combined clinical and pathological score for SLE-development in pristane-treated mice.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SLE score definition | 0 | 1 | 2 | 3 |
| Sign of GN: | No proteinuria (= neg on urine stix) or Pro 1+ on urine stix | - | Moderate proteinuria (Pro 2+ on urine stix) | Severe proteinuria (Pro 3+ on urine stix) +/- ascites |
| Splenomegaly | No | Mild | Moderate | Severe |
| Lymphadenopathy | No | Mild | Significant |  |
| Thymus enlargement | No |  | Significant enlarged |  |
| Alopecia | No | Yes |  |  |
| Other organ involvement (for example liver) | No | Yes |  |  |

Pathological alterations considered include signs of glomerulonephritis (GN) in form of proteinuria and/or ascites, splenomegaly (mild, moderate or severe), lymphadonopathy (mild or significant), thymus enlargement, alopecia and other organ (typically liver) involvement. GN was scored as 2 or higher as it represents a specific and severe sign of lupus. Because we have occasionally detected a “Pro 1+” value on urine stix in healthy animals, we considered this value as unspecific and scored as SLE only at a value of “Pro 2+” or “Pro 3+”. Splenomegaly and lymphadenopathy are signs of mild to severe immune response, therefore, we scored them depending of their severity from 1 to 3. Thymus enlargement was a significant sign of immunological contribution. Liver involvement was also considered although due to the intraperitoneal application of pristine, it could have had a chemical component for the reaction too; therefore, it was scored only as 1. The total SLE score was calculated by addition of these individual scores.

**Suppl. Table 7. Clinical and pathological (SLE) scores of pristane treated mice.**

|  |  |  |  |
| --- | --- | --- | --- |
| *Vav*Cre+ *Dnmt1*+/+ | Animal  # | Clinical symptoms and pathological alterations | SLE score |
| Group 1 | 300 | Proteinuria 3+, Ascites, severe splenomegaly, enlarged thymus | 8 |
|  | 297 | Severe splenomegaly, enlarged thymus | 5 |
| Group 2 | 384 | Alopecia, severe splenomegaly | 4 |
|  | 358 | Alopecia, moderate splenomegaly, enlarged thymus, mild enlarged LN | 6 |
|  | 342 | Mild splenomegaly, mild enlarged LN | 2 |
|  | 334 | Alopecia, moderate splenomegaly, moderate enlarged LN | 5 |
| Group 3 | 506 | Mild splenomegaly, enlarged thymus, enlarged liver | 4 |
|  | 495 | Liver enlarged | 1 |
|  | 493 | Severe splenomegaly, enlarged thymus, significant enlarged LN | 7 |
|  | 492 | Mild splenomegaly, enlarged liver, enlarged thymus, significantly enlarged LN | 6 |
|  | 491 | Moderate splenomegaly, enlarged thymus, significant enlarged LN | 6 |
|  | 487 | Moderate splenomegaly, enlarged thymus, enlarged mesenterial LN | 6 |
| *Vav*Cre+ *Dnmt1*lox/chip | Animal  # | Clinical symptoms and pathological alterations | SLE score |
| Group 1 | 328 | None | 0 |
|  | 298 | None | 0 |
|  | 326 | None | 0 |
| Group 2 | 389 | None | 0 |
|  | 376 | None | 0 |
|  | 375 | None | 0 |
| Group 3 | 478 | Mild splenomegaly, enlarged thymus, minimal enlarged LN, enlarged liver | 5 |
|  | 476 | Moderate splenomegaly, enlarged thymus, moderate enlarged LN | 6 |
|  | 456 | None | 0 |
|  | 455 | None | 0 |
|  | 454 | Moderate splenomegaly, enlarged thymus, mild enlarged LN | 5 |
|  | 453 | None | 0 |
|  | 452 | Mild splenomegaly, enlarged thymus, minimal enlarged LN | 4 |

Analysis was performed with *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice 4 months after pristine treatment. Mice from three independent experiments (groups 1 to 3) are shown.

**Suppl. Table 8. Renal scores of pristane treated mice.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Vav*Cre+ *Dnmt1*+/+ | Animal # | Glomerular inflammation | Glomerular proliferation | Crescent formation | Necrosis | Final score |
| Group 1 | 300 | 2 | 2 | 1 | 1 | 6 |
|  | 297 | 1 | 1 | 0 | 0 | 2 |
| Group 2 | 384 | 2 | 2 | 0 | 1 | 5 |
|  | 358 | 2 | 1 | 0 | 0 | 3 |
|  | 342 | 1 | 1 | 0 | 0 | 2 |
|  | 334 | 2 | 1 | 0 | 0 | 3 |
| Group 3 | 506 | 2 | 1 | 0 | 0 | 3 |
|  | 495 | 1 | 0 | 0 | 0 | 1 |
|  | 493 | 2 | 2 | 0 | 1 | 5 |
|  | 492 | 2 | 1 | 0 | 0 | 3 |
|  | 491 | 3 | 3 | 0 | 1 | 7 |
|  | 487 | 1 | 1 | 0 | 0 | 2 |
| *Vav*Cre+ *Dnmt1*lox/chip | Animal # | Glomerular inflammation | Glomerular proliferation | Crescent formation | Necrosis | Final score |
| Group 1 | 328 | 1 | 0 | 0 | 0 | 1 |
|  | 298 | 1 | 0 | 0 | 0 | 1 |
|  | 326 | 0 | 0 | 0 | 0 | 0 |
| Group 2 | 389 | 1 | 1 | 0 | 0 | 2 |
|  | 376 | 1 | 0 | 0 | 0 | 1 |
|  | 375 | 1 | 0 | 0 | 0 | 1 |
| Group 3 | 478 | 1 | 0 | 0 | 0 | 1 |
|  | 476 | 2 | 0 | 0 | 0 | 2 |
|  | 456 | 1 | 1 | 0 | 0 | 2 |
|  | 455 | 1 | 1 | 0 | 0 | 2 |
|  | 454 | 2 | 2 | 0 | 1 | 5 |
|  | 453 | 1 | 0 | 0 | 0 | 1 |
|  | 452 | 1 | 0 | 0 | 0 | 1 |

Histopathological kidney analysis of *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice 4 months after pristane treatment. Renal scoring was adopted from (Sekine et al. 2006): the HE and PAS slides were graded for glomerular inflammation, glomerular proliferation, crescent formation and necrosis. Scores from 0 to 3 (0 – none, 1 – mild, 2 – moderate, 3 – severe) were assigned for each of these features and then added together to yield a final renal score.

**Suppl. Table 9. Electron microscopic analyses of pristane treated mice.**

|  |  |  |  |
| --- | --- | --- | --- |
| *Vav*Cre+ *Dnmt1*+/+ | Animal # | Deposit | Localisation of deposit |
| Group 1 | 300 | yes | subepithelial, subendothelial, paramesangial, mesangial |
|  | 297 | yes | mesangial, paramesangial |
| Group 2 | 384 | yes | subendothelial, mesangial, paramesangial |
|  | 358 | yes | subendothelial, mesangial, paramesangial |
|  | 342 | no |  |
|  | 334 | yes | subendothelial, paramensangial, mesangial |
| Group 3 | 493 | yes | subendothelial, mesangial, paramesangial |
|  | 491 | yes | subendothelial, mesangial, paramesangial |
| *Vav*Cre+ *Dnmt1*lox/chip | Animal # | Deposit | Localisation of deposit |
| Group 1 | 328 | yes | few mesangial |
|  | 298 | yes | few mesangial and paramesangiale |
|  | 326 | no |  |
| Group 2 | 389 | yes | mesangial, paramesangial |
|  | 376 | no |  |
|  | 375 | no |  |

Summary of electron microscopic analyses to investigate the presence or absence of immune deposits and the localization of deposits in the kidneys of *Vav*Cre+*Dnmt1*+/+ and *Vav*Cre+*Dnmt1*lox/chip mice 4 months after pristane treatment.

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