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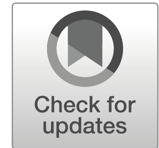
REGULAR ARTICLE



The environmental obesogen bisphenol A increases macrophage self-renewal

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Abstract
Self-renewal of macrophages is important for the healthy development and replenishment of tissue-resident macrophage pools. How this mechanism is controlled by endocrine signals is still largely unexplored. Here, we show that the endocrine disruptor bisphenol A (BPA) increases macrophage self-renewal. This effect was associated with phosphorylation of extracellular signal-regulated kinase (ERK) and a slight increase in the expression of liver X receptor alpha (LXRα). We found that LXRα inhibition induced, while LXRα activation impeded, macrophage self-renewal. LXRα signaling hence may protect from excessive macrophage expansion. Self-renewing macrophages, however, had negligible LXRα expression when compared with quiescent macrophages. Accordingly, tissue-resident macrophage pools, which are dominated by quiescent macrophages, were rich in LXRα-expressing macrophages. Overall, we show that BPA increases macrophage self-renewal and that this effect, at least in part, can be inhibited by increasing LXRα expression. Since BPA is accumulated in the adipose tissue, it has the potential to increase self-renewal of adipose tissue macrophages, leading to a condition that might negatively impact adipose tissue health.

Keywords Endocrine disruptors · Obesogens · Obesity · LXR · ERK

Introduction

Obesity remains a major public health concern (Cao 2014; Capurso and Capurso 2012) and its prevalence is predicted to rapidly increase in the near future (Cao 2014; WHO 2018a; WHO 2018b). Obesity is associated with a state of chronic, low-grade inflammation that triggers metabolic diseases including type 2 diabetes mellitus (Hameed et al. 2015). The etiology of obesity and its associated diseases has been attributed to various factors, such as genetics, diet and lifestyle and certain environmental obesogens that disrupt the functions of the lipid-storing adipocytes (Acconcia et al. 2015; Brehm and D'Alessio 2014; Sheikh et al. 2017).

Environmental obesogens are a category of chemical endocrine disruptors that have detrimental effects on the endocrine control of metabolism. They are widely used in the manufacture of industrial and consumer products, mainly as plasticizers (Acconcia et al. 2015). In adipose tissue, obesogens can act on adipocyte precursors and differentiated adipocytes directly, or they can disturb the hormonal control of appetite and metabolism, leading overall to an increase in the number of adipocytes and facilitating fat storage by existing adipose cells (Grun and Blumberg 2009a; Grun and Blumberg 2009b; Schug et al. 2011). Obesogens also depress the utilization of fatty acids and lipoproteins, causing dyslipidemia and fatty liver disease (Grun and Blumberg 2009a; Grun and Blumberg 2009b). Drinking water, bottled mineral water and beverages and diet (seafood, cereals, food wrapped or stored in polycarbonate- or polyvinyl chloride-based materials) are the major sources of intake, with daily exposure in the

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micromolar concentration range (Chen et al. 2009; Goran et al. 2013; Grun and Blumberg 2009a; Grun and Blumberg 2009b, Welshons et al. 2006). Accordingly, obesogen chemicals can be routinely detected in human serum, tissues and urine at nanomolar concentrations, indicating existing or past exposure (Goran et al. 2013; Grun and Blumberg 2009b; Wang et al. 2013). Animal studies have shown that such quantities cause hyperlipidemia, fat accumulation, liver steatosis, impair oxidative phosphorylation and fatty acid utilization and can even evoke heritable changes in body adiposity and metabolism (Grun and Blumberg 2009b; Naville et al. 2013). These alarming findings highlight the strong negative impact of obesogens on metabolism and their likely contribution to the prevalence of obesity. However, little is known about the molecular mechanisms underlying the metabolic effects of obesogens.

Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane) is one of the most widely used obesogen chemicals and can be found in food storage containers and the protective internal lining of cans used for food and beverages, in thermal printer paper, electronic equipment, medical devices and dental sealants (Flint et al. 2012; Konieczna et al. 2015; Repossi et al. 2016). The metabolic effects of BPA on adipocytes have been previously studied, with results showing that BPA contributes to obesity-associated diseases (Acconcia et al. 2015; Dong et al. 2018; Flint et al. 2012; Heindel 2011; Schug et al. 2011).

Obesity has an immune component and how adipocytes metabolize lipids is largely dictated by immune cells of the adipose tissue. Among these, adipose tissue macrophages (ATMs) are the most studied immune cells. ATMs can either support or impede insulin sensitivity of the adipocytes, cause adipose tissue fibrosis or initiate burning-off of stored lipids in the form of heat (Ivanov et al. 2018). How ATMs affect adipose tissue function is largely dependent on their number. Obesity is associated with an increase in ATM number (Amano et al. 2014; Haase et al. 2014; Lumeng et al. 2007), which favors the development of inflammation and, ultimately, an insulin-resistant state (Boutens and Stienstra 2016). How the ATM number is controlled is still not fully understood (Rőszer 2018), although recent reports have shown that ATM self-renewal is a key element in maintaining a metabolically beneficial ATM pool (Amano et al. 2014; Braune et al. 2017; Haase et al. 2014; Rőszer 2018; Waqas et al. 2017).

Because BPA accumulates in the adipose tissue (Geens et al. 2012), in the present study, we addressed whether BPA can affect macrophage self-renewal. We demonstrate that BPA increases macrophage self-renewal and this effect is associated with activation of the mitogen-activated ERK pathway and an increase in the levels of liver X receptor alpha (LXRα), with both changes regulating macrophage self-renewal. ERK is a member of the MAPK superfamily and plays a vital role in cell cycle progression by regulating cell cycle entry (Li et al. 2016). LXRα is a nuclear receptor, with a role in the control of macrophage immune functions (A-González and Castrillo 2011) and cell cycle (Pascual-García et al. 2011).

Results

Bisphenol A increases macrophage self-renewal

To assess the possible effects of BPA on macrophage function, we treated murine J774A.1 macrophages in vitro with BPA (Fig. 1a), using concentrations that mimic human environmental exposure (Ribeiro et al. 2017; Vandenberg et al. 2007, Welshons et al. 2006). We assessed viability, phagocytic activity and activation state; all of which are key determinants of the physiological functions of macrophages (Chang 2009). Treatment of J774A.1 macrophages with 1 μM BPA for 1 or 18 h and with 100 nM BPA for 18 h, resulted in overt cell death, as shown by a decrease in mitochondrial activity in cells assessed with the PrestoBlue cytotoxicity assay (Fig. S1). However, treatment with lower concentrations of BPA (1 or 10 nM) for the same time period did not impair metabolic activity (Fig. S1). Likewise, phagocytic activity was not affected by 1 and 10 nM BPA for 18 h (Fig. S2), corroborating the finding that macrophage viability and maturation are not affected by a low BPA concentration.

Macrophage activation is key for macrophage function, by ensuring pathogen elimination and immunological self-tolerance and also proper metabolic performance (Ginhoux et al. 2015; Rőszer 2015). To test whether BPA could affect macrophage activation, we measured the mRNA levels of hallmark genes of macrophage activation after treatment of J774A.1 cells with BPA for 18 h, finding that 10 nM BPA modestly increased *Arg1* and *Il6* transcription without affecting the transcription of *Il4r*α, *Tnfa* and *Nos2* (Fig. 1b–f). These findings suggest that BPA does not substantially activate macrophages.

Finally, we tested the effect of BPA on macrophage self-renewal, a recently recognized trait that ensures the proper development of macrophage niches in their corresponding tissues and hence has impact on healthy organ development and function (Rőszer 2018). We found that at concentrations of 1 and 10 nM, BPA increased J774A.1 macrophage number after 18 h of treatment (Fig. 1g). To test whether this was attributable to self-renewal, we examined the incorporation of 5-bromo-2′-deoxyuridine (BrdU) into the DNA during this time, finding that BrdU incorporation was higher in BPA-treated cells than in vehicle-treated cells (Fig. 1h). Taken together, these data suggest that low doses of BPA increase macrophage self-renewal but do not affect macrophage viability and maturation. Next, we questioned whether BPA treatment could induce cell cycle re-entry of quiescent

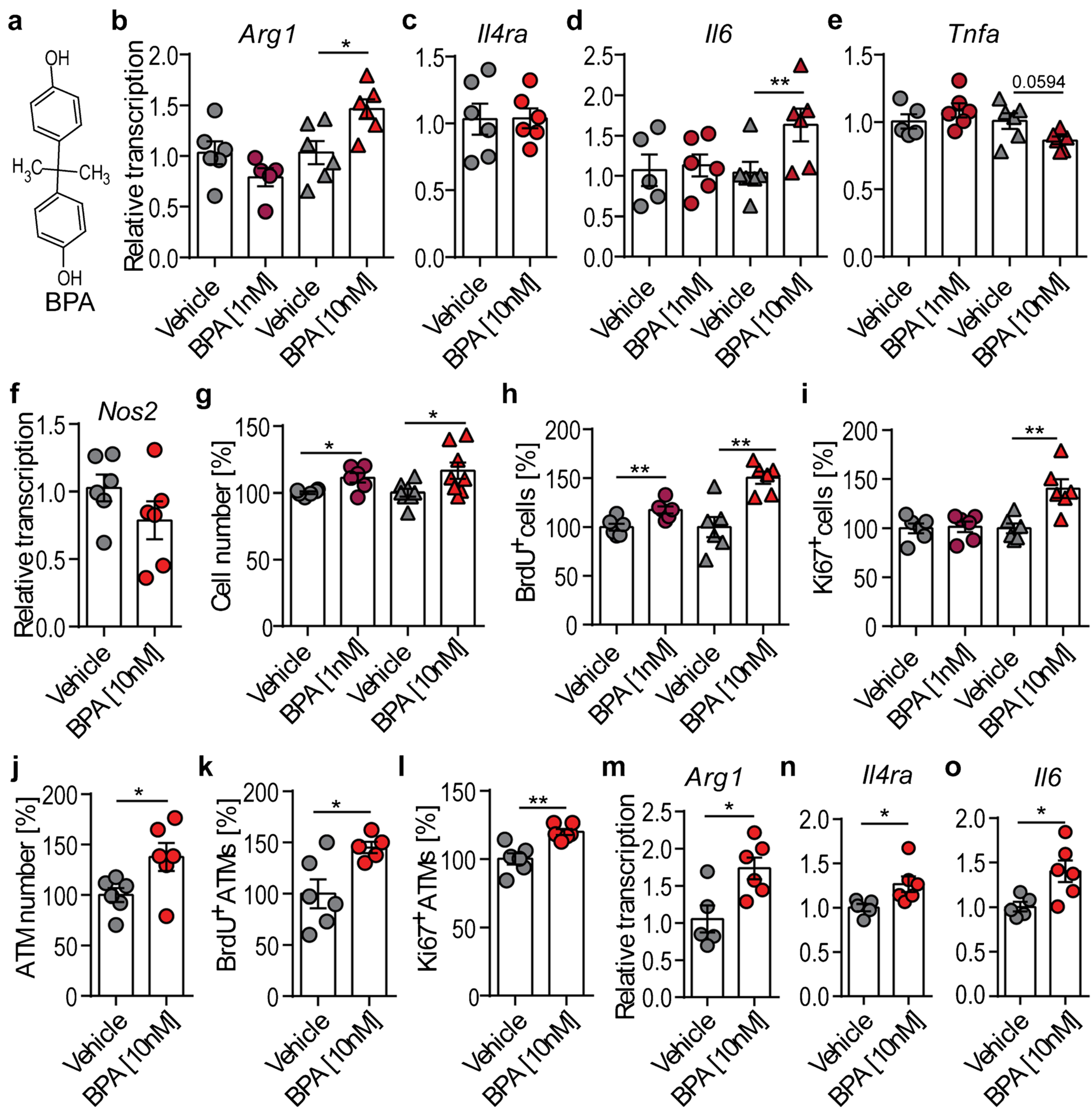


Fig. 1 BPA increases macrophage self-renewal. **a** Chemical structure of BPA. **b**–**f** Gene transcription in response to 18 h treatment of J774A.1 macrophages with 1 and 10 nM BPA. **g** Cell number of J774A.1 macrophages after treatment with 1 and 10 nM BPA. **h** Quantification of BrdU incorporation in J774A.1 macrophages after 18 h stimulation with BPA. **i** Quantification of Ki67$^+$ macrophages after treatment with 1 and 10 nM BPA. **j** ATM cell number after treatment with 10 nM of BPA. **k** BrdU$^+$ ATMs after 18 h treatment. **l** Quantification of Ki67$^+$ ATMs after 18 h stimulation with 10 nM BPA. **m**–**o** Gene transcription in response to 18 h treatment of ATMs with 10 nM BPA. ATMs were pooled from 3 to 5 mice and treated in triplicate. The unprocessed raw flow cytometric data are accessible in Flow Repository (https://flowrepository.org/) under the repository IDs FR-FCM-ZYP6 and FR-FCM-ZYP7. RNA expression of the indicated genes was normalized to *Actb*. qPCR and FACS values are expressed as mean fold change relative to vehicle ± SEM ($n = 5$–6). Statistical analysis was performed using unpaired Student's test with Welch's correction. Significance is indicated by $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. *BPA* bisphenol A, *BrdU* 5-bromo-2′-deoxyuridine, *ATMs* adipose tissue macrophages

macrophages. To do this, we used the nuclear proliferation marker Ki67, which is expressed in the active phases of the cell cycle but is absent in quiescent cells. We found that 10 nM BPA induced cell cycle re-entry of quiescent J774A.1 macrophages, measured as an increase in the number of Ki67$^+$ macrophages (140.4% ± 9.51) (Fig. 1i).

The above findings suggest that BPA specifically affects macrophage self-renewal. Since BPA is a lipophilic substance and hence can be accumulated in adipose tissue (Geens et al. 2012), it is conceivable that ATMs are exposed to BPA. ATMs are capable of self-renewal (Braune et al. 2017; Waqas et al. 2017), hence we questioned whether BPA could affect ATM self-renewal. Similar to the findings in J774A.1 macrophages, we found that 18 h treatment with 10 nM BPA did not have any cytotoxic effects on ATMs (Fig. S3) but increased the number of ATMs (Fig. 1j). Importantly, self-renewal of ATMs was also increased by BPA, as shown by the increase in BrdU labeling in BPA-treated ATMs (Fig. 1k). Likewise, BPA increased the re-entry of quiescent ATMs into the cell cycle, measured as $Ki67^+$ ATMs (Fig. 1l). Again, similar to the results in J774A.1 macrophages, the ATM activation state was not affected by BPA (Fig. 1m–o). BPA also increased the self-renewal of human monocytic THP-1 cells, macrophages differentiated from THP-1 cells in vitro (Figs. S4–14). Briefly, 10 nM BPA increased the number of monocytic THP-1 cells after 18 h treatment (Fig. S4), along with the increased amount of $Ki67^+$ cells (Fig. S5) and cells in the G2/M-phase (Fig. S6). BPA increased the self-renewal of macrophages differentiated from THP-1 cells; however, this effect was apparent following 48 h treatment (Figs. S7–14). Taken together, these data suggest that BPA is able to trigger macrophage self-renewal.

Bisphenol A induces ERK phosphorylation in macrophages

Mechanisms that increase macrophage self-renewal are not well understood (Röszer 2018), but can involve the activation of mitogen-activated protein kinase (MAPK) signaling pathways including the extracellular signal-regulated kinase (ERK) cascade, which can be activated by growth factors to regulate, among other processes, cell cycle progression and survival (De Luca et al. 2012).

To examine whether BPA activated ERK signaling in macrophages, J774A.1 cells were treated with BPA and cell lysates were subjected to Western blotting. We found that 10 nM BPA induced the rapid phosphorylation of ERK, which was detectable 30 min after treatment (Fig. 2a–a"') but not for 1 nM BPA (Fig. S15). ERK activation was not sustained by the 18-h-long BPA treatment (Fig. S16). Interestingly, the lower dose of BPA (1 nM) rather increased total ERK levels after 18 h (Fig. 2b–b"'). In accordance with these findings, BPA increased the transcription of the mitogenic genes *Ccne* and *Ccnd1* (Fig. 2c, d); however, it had no effect on the transcription of the cell cycle inhibitor genes *Cdkn1a*, *Cdkn1b* and *Trp53* (Figs. S17–S19).

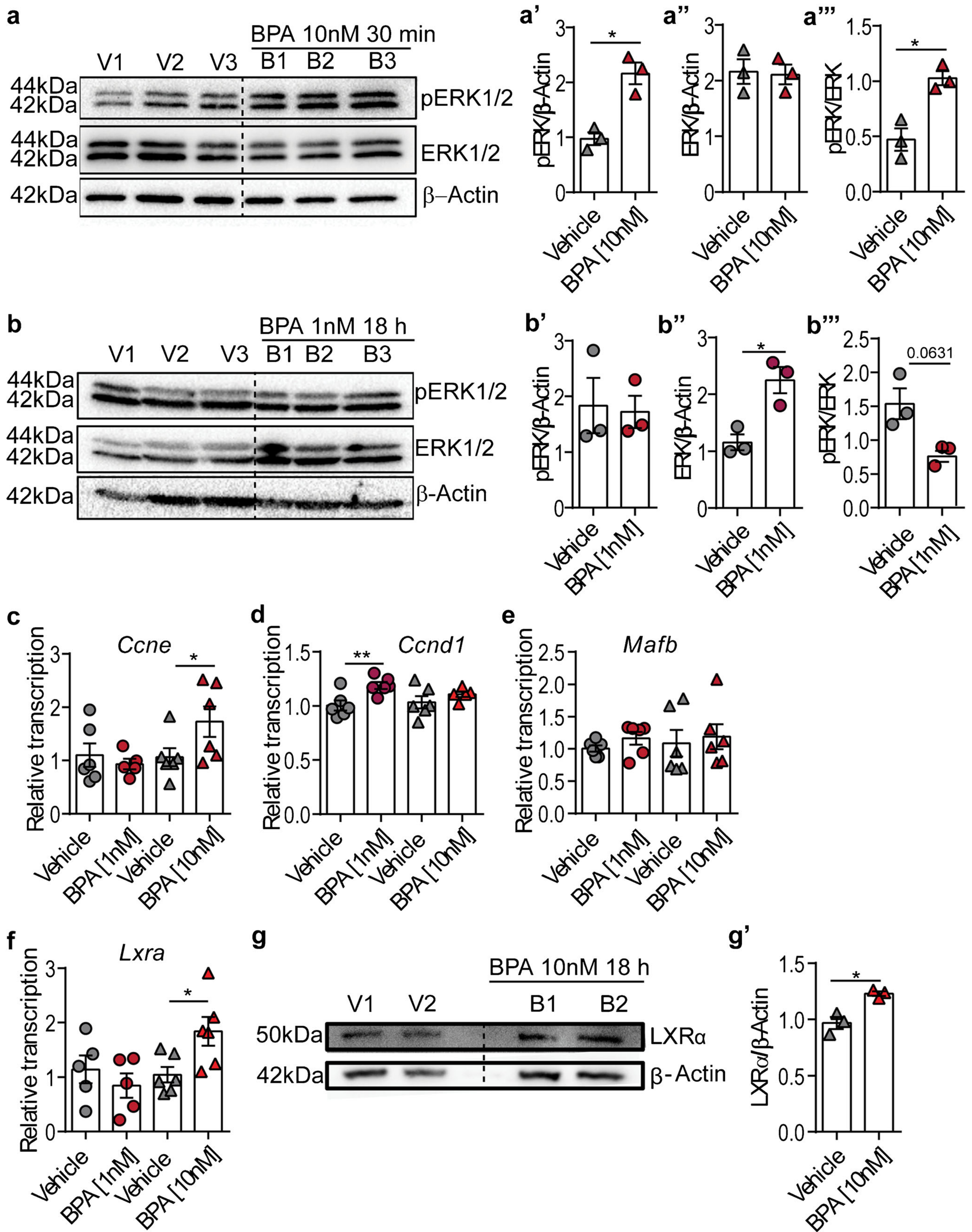
In addition to the ERK pathway, we searched for other possible mechanisms that could account for the BPA-induced macrophage self-renewal. In this regard, we have previously shown that macrophage self-renewal is associated with an increase in the transcription of N-Myc downstream-regulated gene 2 (*Ndrg2*) and a decrease in the expression of the transcription factor MAF bZIP transcription factor B (*Mafb*) (Menendez-Gutierrez et al. 2015; Waqas et al. 2017). We found that 1 nM but not 10 nM BPA increased the expression of *Ndrg2* (Fig. S20), whereas the expression of *Mafb* was not affected at either concentrations (Fig. 2e).

LXR inhibits macrophage self-renewal

BPA, at least in part, increases cellular lipid storage through liver X receptor (LXR) signaling (Dahlman et al. 2006; Marmugi et al. 2012). LXR signaling can also inhibit macrophage self-renewal (Pascual-García et al. 2011; Röszer 2018), although its physiological impact and underlying mechanisms are largely unexplored. Thus, we asked whether BPA could alter LXR signaling and affect macrophage self-renewal. First, we measured the mRNA expression of *Lxra* and *Lxrb*, which encodes the two isotypes of LXR. Interestingly, we found that 10 nM BPA modestly increased LXRα expression both at the mRNA (Fig. 2f) and protein levels (Fig. 2g, g'), without affecting *Lxrb* mRNA levels (Fig. S21).

Next, we activated macrophage LXR signaling by treating J774.A1 cells with the synthetic and endogenous LXR ligands, GW3965 (1 μM) and 25-hydroxycholesterol (1 μg/ml), respectively. We found that 18 h treatment with either ligand induced the accumulation of macrophages in the G0/G1-phase and decreased the amount in the G2/M-phase (Fig. 3a, a'). Notably, this treatment was not cytotoxic for the cells (Fig. S22) but decreased self-renewal, as measured by the number of $Ki67^+$ cells (Fig. 3b). The effects of both LXR ligands were similar in ATMs (Fig. 3c, d). Importantly, we verified that the LXR ligands activated LXR

Fig. 2 BPA upregulates signals involved in cell cycle progression. **a** Western blotting of phospho- (p)ERK/ERK in J774A.1 macrophages treated with 10 nM BPA for 30 min. *a'–a"'* Quantification of pERK, ERK and pERK/ERK normalized to β-actin. **b** Western blotting of pERK/ERK in J774A.1 macrophages treated with 1 nM BPA for 18 h. *b'–b"'* Quantification of pERK, ERK and pERK/ERK normalized to β-actin. **c**–**f** Gene expression levels in response to 18 h stimulation of J774A.1 macrophages with 1 or 10 nM BPA. **g** A representative of Western blotting of LXRα in J774A.1 macrophages. *g'* Densitometry of LXRα normalized to β-actin, exposure time 5 s. qPCR and Western blot values are expressed as mean fold change relative to vehicle ± SEM (n = 3–6). Statistical analysis was performed using unpaired Student's t test with Welch's correction. Significance is indicated by $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$



a
BPA 10nM 30 min
V1 V2 V3 B1 B2 B3
44kDa
42kDa
pERK1/2
44kDa
42kDa
ERK1/2
42kDa
β–Actin
a'
pERK/β-Actin
Vehicle
BPA [10nM]
a''
ERK/β-Actin
a'''
pERK/ERK
b
BPA 1nM 18 h
V1 V2 V3 B1 B2 B3
pERK1/2
ERK1/2
β-Actin
b'
b''
b'''
0.0631
BPA [1nM]
c
Ccne
d
Ccnd1
e
Mafb
Relative transcription
f
Lxra
g
BPA 10nM 18 h
V1 V2 B1 B2
50kDa
LXRα
42kDa
β- Actin
g'
LXRα/β-Actin

signaling by measuring the expression of the LXR target gene *Abcg1* (Fig. 3e, f).

Our findings indicate that LXR activation decreases macrophage self-renewal, which is in line with other studies on the cell cycle-inhibitory effect of LXRs in various cell types (Hassan et al. 2015; Pascual-García et al. 2011; Warns et al. 2018). Turning this question on its head, we next assessed whether inhibiting LXR signaling with the inverse agonist SR9238 would activate macrophage self-renewal. We first tested a range of concentrations (40–500 nM) to determine the lowest effective dose that blocked LXR signaling without affecting cell viability. We found that 200 nM SR9238 effectively blocked LXR target gene transcription (*Abcg1*, *Srebp1c* and *Fasn*) (Fig. 4e–f'; Fig. S23) without apparent toxicity (Fig. S24). This concentration is in accord with the known IC_{50} of SR9238 for LXRα (200 nM) and LXRβ (40 nM) (Griffett et al. 2012). After treating J774A.1 macrophages with SR9238, we found an increased percentage of cells in the G2/M-phase (Fig. 4a, a') accompanied by a prominent increase in the percentage of $Ki67^+$ cells (Fig. 4b). Similar results were obtained with ATMs (Fig. 5c, d), with no changes in cell viability (Fig. S24).

To assess whether these in vitro findings could be replicated in vivo, we treated male mice with 30 mg/kg SR9238 for 14 days to study the effect of systemic LXR blockade on macrophage self-renewal (Griffett et al. 2012; Griffett et al. 2015). We found that whereas SR9238 did not affect body weight (Fig. 5a), there was a trend for a reduction in the weight of epididymal white adipose tissue (eWAT) (Fig. 5b). Through the analysis of self-renewal, we observed an increase in the cell number in the ATM-rich stromal vascular fraction (SVF) normalized to eWAT weight in SR9238-treated mice (Fig. 5c). Also, cell cycle analysis of this fraction demonstrated a non-significant increase in cells in the G2/M-phase (Fig. 5d). Closer inspection of the SVF revealed that SR9238 significantly increased the number of $CD45^+$/$F4/80^+$ ATMs (Fig. 5e). Notably, SR9238 also increased the number of $CD45^+$/$F4/80^+$ Kupffer cells in the liver (Fig. S25), without altering liver weight or cell number (Figs. S26 and S27). Nevertheless, cell cycle analysis of non-parenchymal cells of the liver also demonstrated a non-significant increase in cells in the G2/M-phase (Fig. S28). We also assessed the expression of the LXR target genes *Srebp1c*, *Abcg1* and *Cd5l* in the liver and found them to be downregulated (Fig. 5f–f"). Overall, these data suggest that the blockade of LXR signaling increases tissue-resident macrophage number.

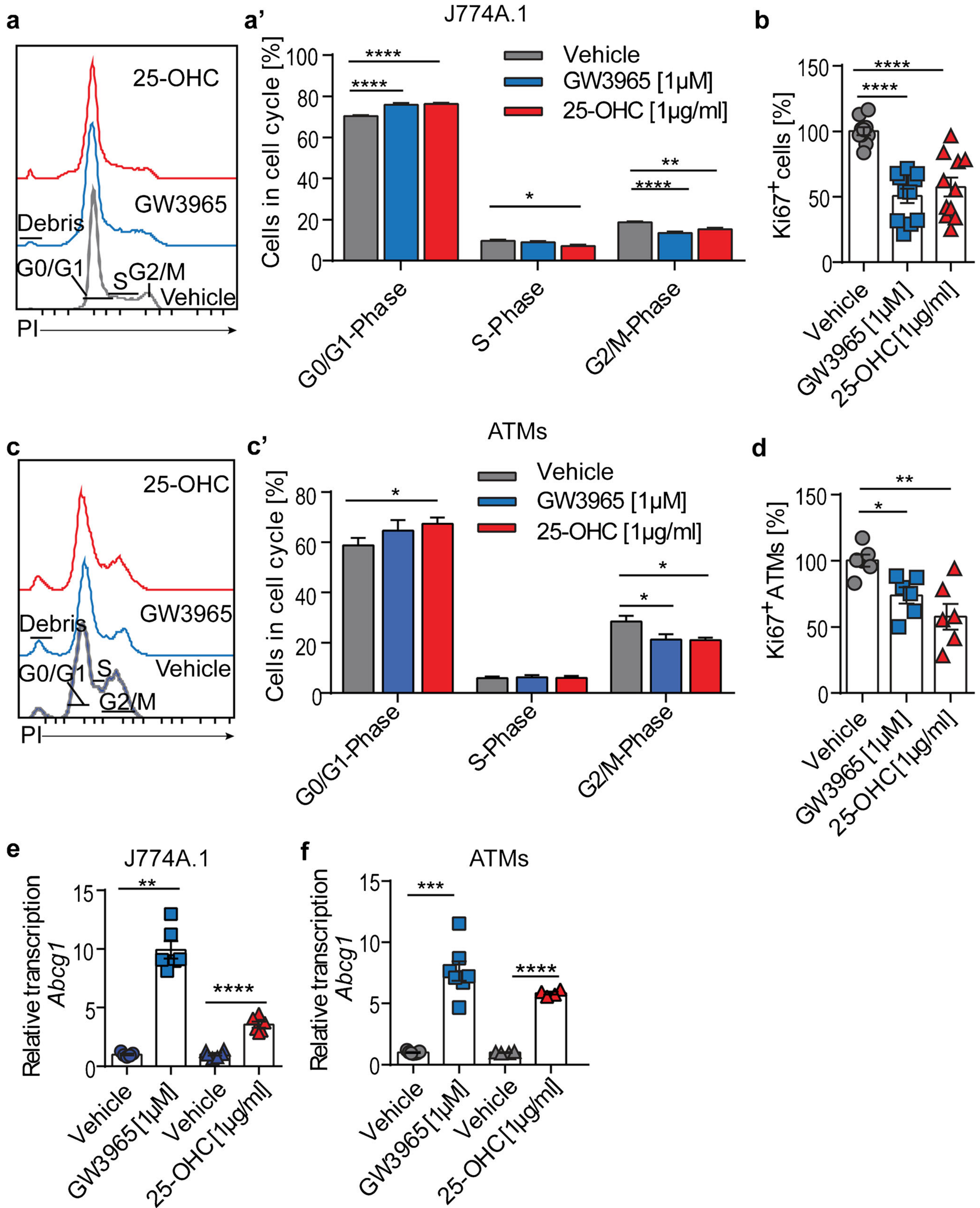
Lxra expression distinguishes self-renewing from quiescent macrophages

Our results so far suggest that LXR signaling antagonizes BPA effects, hence limiting excessive self-renewal of macrophages. This raises the possibility that macrophages with reduced LXR levels are more prone to BPA-induced increase in self-renewal. We next examined whether *Lxr* transcript levels were different between self-renewing ($Ki67^+$) and quiescent ($Ki67^-$) ATMs. We found that *Lxra* expression was lower in $Ki67^+$ ATMs than in $Ki67^-$ ATMs (Fig. 6a), whereas the levels of *Lxrb* were similar between the two groups (Fig. S29). To further confirm that self-renewing macrophages express low levels of *Lxra*, we treated macrophages with IL-4, which is known to induce macrophage self-renewal (Röszer 2018). As expected, macrophage self-renewal was increased by IL-4 treatment (Fig. 6c–c") and this was accompanied by a decrease in both *Lxra* and *Lxrb* expressions (Fig. 6b, b'**)**. By contrast, IFNγ, which is known to arrest macrophage cell cycle (Singh et al. 2011), decreased self-renewal (Fig. 6d) but increased *Lxra* and *Lxrb* transcription (Fig. 6e, e').

Given the above, we next evaluated whether a decline in self-renewal was associated with an increase in LXR expression in human cells. The human monocytic cell line THP-1 can be differentiated into quiescent THP-1 macrophages by chemical induction (Richter et al. 2016), allowing us to compare the levels of *LXR* expression in self-renewing and quiescent states in the same cell type. We measured *LXRA* and *LXRB* mRNA levels in proliferating monocytic- and quiescent macrophage-like THP-1 cells, finding that monocyte-macrophage differentiation and decreased self-renewal was associated with an increase in *LXRA* expression, which paralleled the expression of the macrophage marker *CD68* (Fig. 6f, g). Moreover, *LXRB* transcription also increased after differentiation for 4 days (Fig. S30).

Finally, we questioned whether *Lxra* expression correlated with the degree of self-renewal ability of distinct tissue-resident macrophages in the mouse. We first measured the percentage of LXRα-expressing macrophages in distinct tissue-resident macrophage pools, finding that 78.35 ± 19.66% of Kupffer cells expressed LXRα, whereas 32.94 ± 7.7% of ATMs and only 18.96 ± 5.03% of resident peritoneal macrophages expressed the receptor (Fig. 6h). These differences in the percentage of LXRα levels were mirrored by the

Fig. 3 LXR ligand activation impedes macrophage self-renewal. **a** Cell cycle analysis of J774A.1 macrophages and *a'* quantification of cell cycle distribution by propidium iodide (PI) staining. **b** Proportion of $Ki67^+$ J774A.1 macrophages after treatment with 1 μM of GW3965 or 1 μg/ml of 25-hydroxycholesterol for 18 h. **c** Cell cycle analysis of in vitro cultured ATMs and *c'* quantification of cell cycle distribution by PI staining. **d** Proportion of $Ki67^+$ ATMs after treatment with 1 μM of GW3965 or 1 μg/ml of 25-OHC for 18 h. **e** and **f** *Abcg1* transcription in J774A.1 macrophages and ATMs after 18 h stimulation. qPCR and FACS values are expressed as mean fold change relative to vehicle ± SEM (n = 3–6). The unprocessed raw flow cytometric data are accessible in Flow Repository under the repository ID FR-FCM-ZYP3 and FR-FCM-ZYPA. Statistical analysis was performed using unpaired Student's t test with Welch's correction, or with one-way or two-way ANOVA. Significance is indicated by *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$



mRNA levels (Fig. S31). In turn, Kupffer cells had the lowest percentage of Ki67^{+} macrophages (7.7 ± 2.15%), whereas 17.22 ± 3.38% of ATMs and 27.48 ± 8.38% of peritoneal macrophages were Ki67^{+} (Fig. 6i). These findings suggest

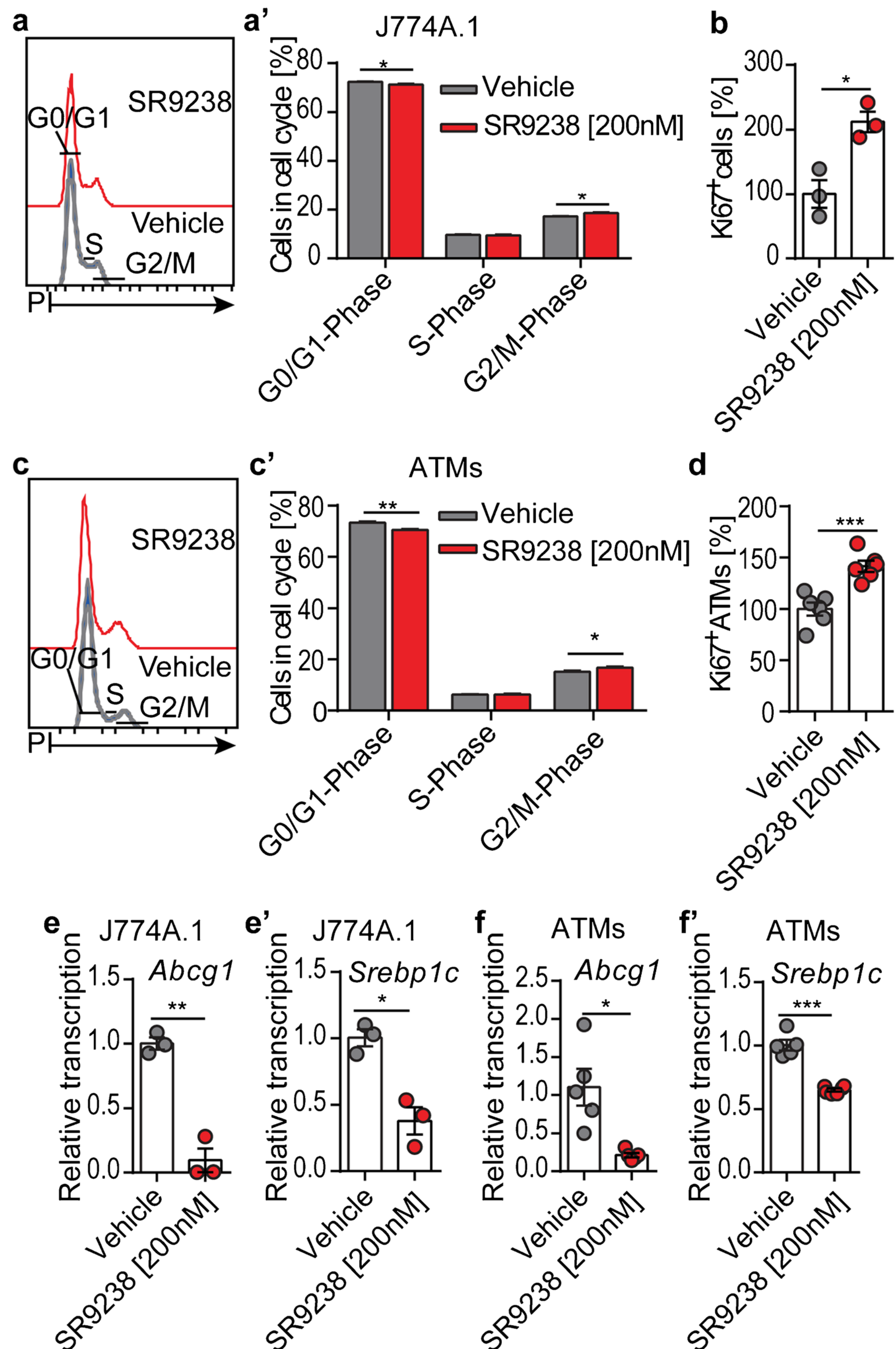


Fig. 4 Inhibition of LXR signaling increases macrophage self-renewal. **a** Cell cycle analysis of J774A.1 macrophages and *a'* quantification of cell cycle distribution by PI staining. **b** Proportion of Ki67+ J774A.1 macrophages after treatment with 200 nM SR9238 for 48 h. **c** Cell cycle analysis of in vitro cultured ATMs and *c'* quantification of cell cycle distribution by PI staining. **d** Proportion of Ki67+ ATMs after treatment with 200 nM SR9238 for 48 h. **e**–*f'* Gene transcription in J774A.1 macrophages and ATMs after 48 h stimulation with 200 nM SR9238. ATMs were pooled from three to six mice and treated in triplicate. The experiment was repeated twice. qPCR and FACS values are expressed as mean fold change relative to vehicle ± SEM ($n = 3$–6). The unprocessed raw flow cytometric data are accessible in the flow repository under the repository ID FR-FCM-ZYP4 and FR-FCM-ZYP5. Statistical analysis was performed using unpaired Student's *t* test with Welch's correction. Significance is indicated by $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$

that the more quiescent tissue-resident macrophage pools contain more LXRα-expressing macrophages than those that are rich in self-renewing macrophages. These data suggest that an increased *Lxra* expression inhibits macrophage self-renewal.

Our overall findings show that low doses of BPA increase macrophage self-renewal through the ERK signaling pathway and also upregulate the expression of *Lxra*. Moreover, tissue-resident macrophages of various origins express different levels of *Lxra*, which mirrors their self-renewing capacities. Finally, we show that self-renewing macrophages in the mouse are characterized by their negligible expression of *Lxra*.

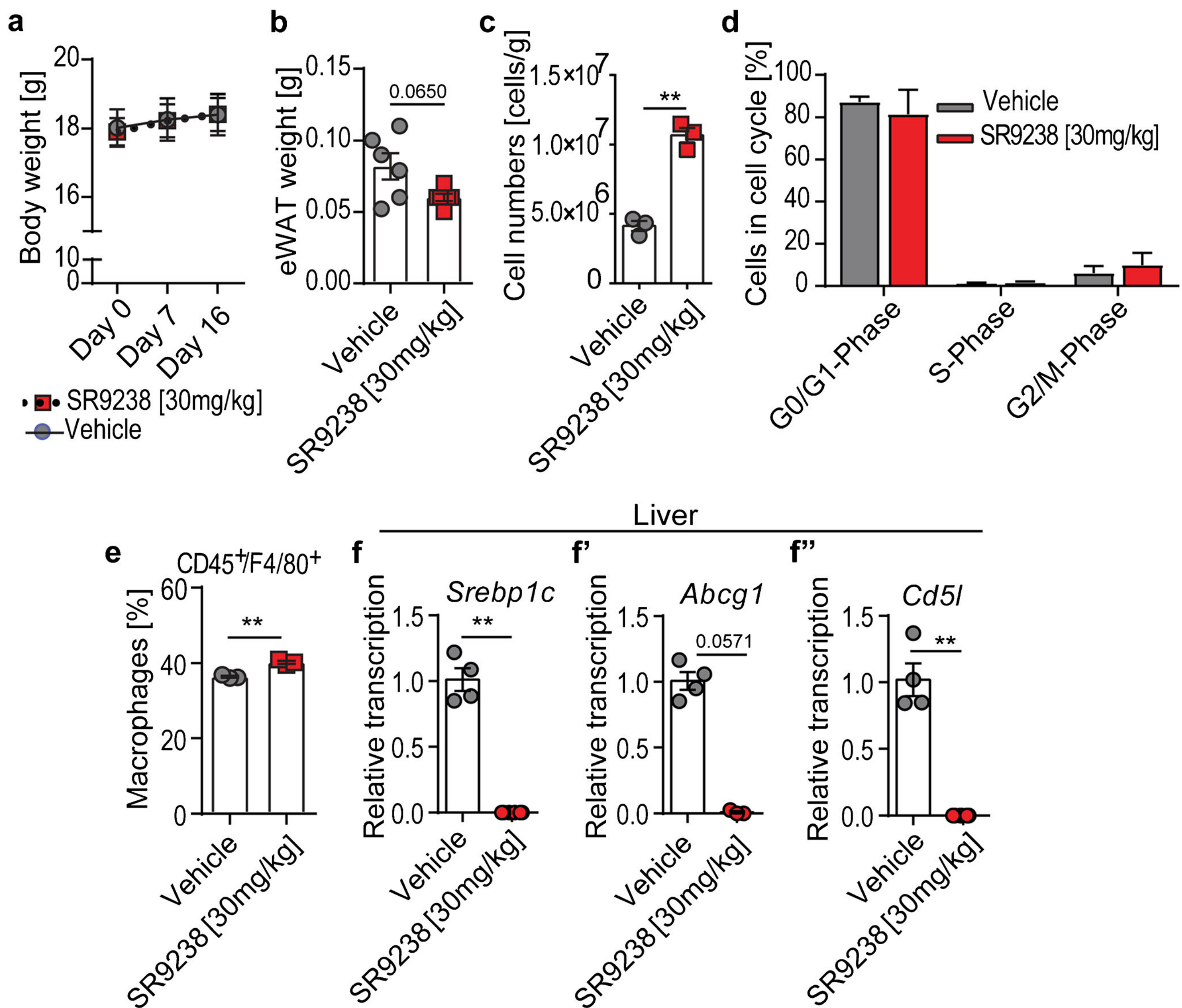


Fig. 5 Inhibition of in vivo LXR signaling by SR9238 increases macrophage number. **a** Body weight. **b** eWAT weight. **c** SVF cell number normalized to weight of eWAT. **d** Quantification of cell cycle distribution in the SVF by PI staining. **e** Percentage of CD45$^+$/F4/80$^+$ ATMs. **f**–*f*" Relative gene transcription in liver after LXR inhibition for 14 days. EWAT of two mice were pooled ($n = 6$). All data are presented as mean ± SEM. The unprocessed raw flow cytometric data are accessible in the flow repository under the repository ID FR-FCM-ZYP9. Statistical analysis was performed using unpaired Student's *t* test with Welch's correction or one-way ANOVA. Significance is indicated by $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$

Discussion

Our study shows that BPA interferes with the control of macrophage number. We demonstrate that BPA, presumably through ERK/MAPK signaling, increases macrophage self-renewal without affecting macrophage maturation or activation. BPA also moderately increases LXRα expression in macrophages and this effect likely antagonizes BPA-induced cell cycle entry. This is a relevant, yet unexplored, effect of BPA that calls into question its safety in the context of tissue-macrophage homeostasis.

Regulation of tissue-resident macrophage homeostasis is highly relevant in health and disease and involves elimination and self-renewal of macrophages, which is crucial for the development and physiological function of tissues (Röszer 2018). ATM homeostasis in the steady-state is regulated by adipose type 1 innate lymphoid cells, which eliminate ATMs to prevent excessive macrophage expansion. In obesity, however, this regulation is disturbed (Boulenouar et al. 2017). Self-renewal is a newly discovered feature of macrophages (Röszer 2018) that is used to replenish local resident macrophage niches without the need for infiltration of monocyte-derived macrophages (Belhareth and Mège 2015; Jenkins et al. 2011). Intrinsic and extrinsic signals, such as M-CSF, IL-4, IL-6 and IFNγ and tissue-specific cues including neuropeptide FF, regulate macrophage niches by either promoting

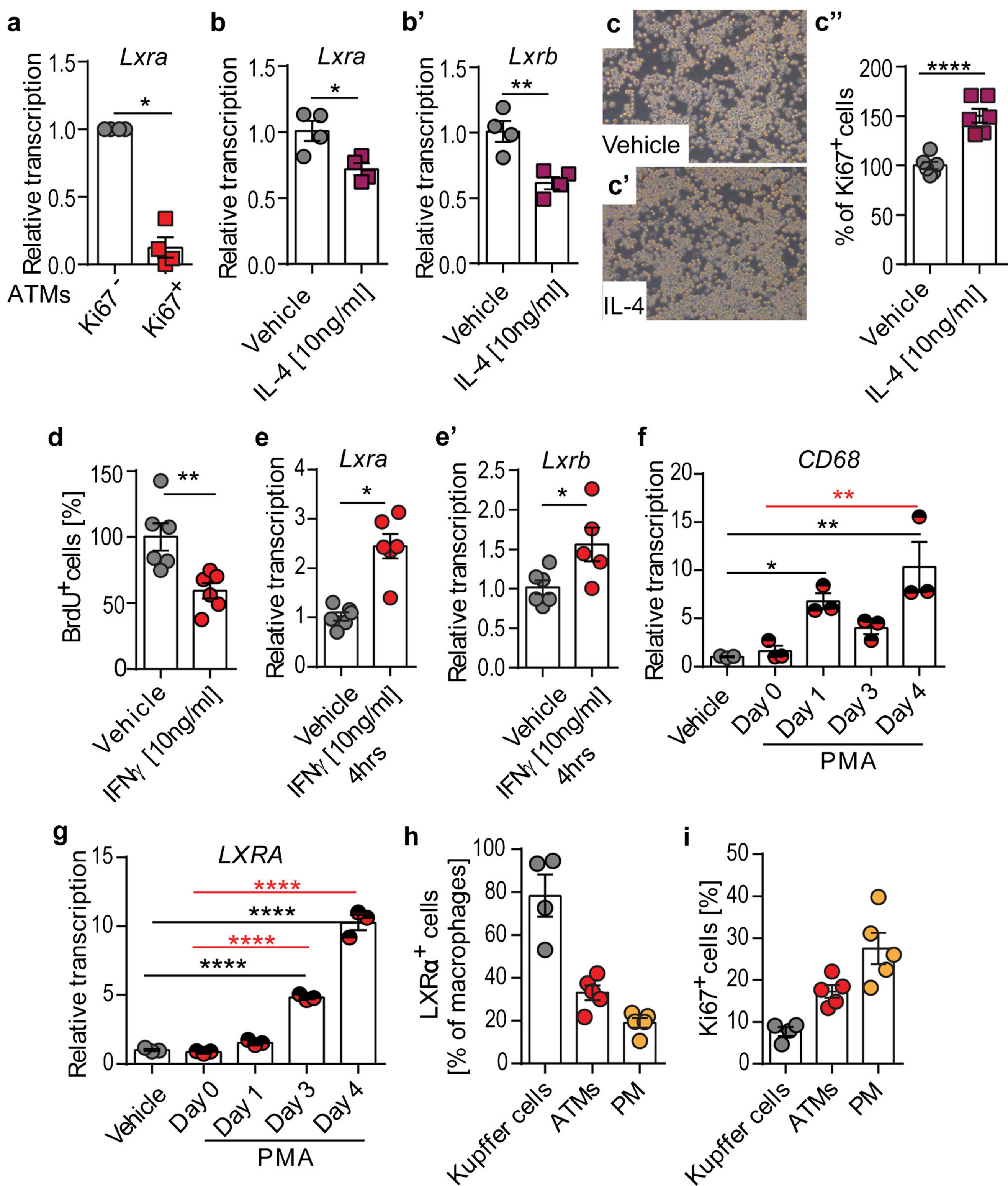
or repressing macrophage self-renewal (Belhareth and Mège 2015; Luo et al. 2018; Smith et al. 2013; Waqas et al. 2017; Yu et al. 2012). Dysregulation of macrophage self-renewal can lead to diseases that contribute to obesity in addition to atherosclerosis, cancer and neurodegenerative disease (Belhareth and Mège 2015). Nevertheless, we still lack a good understanding of the mechanisms that regulate macrophage self-renewal.

The environmental obesogen BPA, which is widely used in industrial, medical and consumer products, is a known endocrine disruptor (Flint et al. 2012; Huang et al. 2012; Repossi et al. 2016) and there is increasing evidence that vertebrates and invertebrates can be exposed to BPA under specific conditions. BPA has been shown to contribute to the development of obesity-induced diseases and also affects fertility and tissue development (Acconcia et al. 2015; Flint et al. 2012; Heindel 2011; Holtcamp 2012; Schug et al. 2011). Of particular concern is the persistence of BPA in the environment, despite its short half-life of 2.5–4 days (Flint et al. 2012). The concentration at which BPA exerts its effects varies between species, cell types and tissues (Ribeiro et al. 2017; Vandenberg et al. 2007), with increased bioaccumulation in lipophilic tissues, such as adipose tissue, over hydrophilic compartments (Geens et al. 2012). Particularly alarming are the findings that BPA can be detected in urine specimens from pregnant women (Lim et al. 2017; Ye et al. 2009). Furthermore, maternal BPA exposure has a detrimental effect on organ development (Fan et al. 2018) and, as a consequence of the early life exposure, BPA might affect children's behavior and increase the prevalence of obesity (Braun et al. 2011; Ejaredar et al. 2017; Harley et al. 2013; Vafeiadi et al. 2016). Of note, macrophage self-renewal peaks in the neonatal period, allowing the development of tissue-resident macrophage niches (Ginhoux et al. 2015). Along this line, testicular macrophage expansion is negatively affected by BPA, which can impair sperm counts and male hormonal levels in adulthood (Peretz et al. 2014; Röszer 2018; Williams et al. 2014). The presence of lipophilic BPA in formula milk, baby food and plastic toys raises the possibility that self-renewing macrophages can be targets of BPA in infants (Andaluri et al. 2018; Cao et al. 2011; Doerge et al. 2010; Food and Administration 2009; Williams et al. 2014).

The obesogenic effects of BPA have been studied mainly in the context of endocrine signaling (Zhang et al. 2018) and its direct effects on adipocytes (Acconcia et al. 2015; Ariemma et al. 2016). Regarding macrophages specifically, BPA has been investigated with respect to modulation of inflammation (Byun et al. 2005; Liu et al. 2014; Rogers et al. 2013) but its potential effects on self-renewal have been overlooked. Resident ATMs govern adipose tissue metabolism (Luo and Liu 2016) but in the setting of obesity this regulation is disturbed, resulting in their activation towards a pro-inflammatory phenotype that is metabolically detrimental (Boutens and Stienstra 2016; Rogero and Calder 2018; Rosen and Spiegelman 2014). We demonstrate that BPA increases macrophage self-renewal. Notably, even 1 nM BPA was effective. The human plasma BPA level is within that range and has been shown to increase cell cycle progressions of LNCaP cells (Wetherill et al. 2006). Also, long-term BPA treatment increases the growth rate of prostate epithelial cells (Huang et al. 2017) and promotes carcinogenesis (Wang et al. 2017). Together, our results suggest that ERK activation by BPA may trigger macrophage self-renewal. Prolonged exposure to BPA, or bioaccumulation of BPA, may augment this effect. Accordingly, BPA might have a detrimental effect on macrophages during the development of obesity, which is associated with uncontrolled ATM self-renewal (Amano et al. 2014; Boutens and Stienstra 2016; Huang et al. 2012; Lumeng et al. 2007; Weisberg et al. 2003).

In the present study, we found that BPA increased LXRα expression. The importance of LXR signaling in the regulation of macrophage self-renewal became apparent when we inhibited LXR signaling. Whereas LXR ligand activation inhibited ATM self-renewal, its inhibition increased macrophage number. This latter finding is in line with recent data showing that the absence of LXR increases the number of F4/80$^+$/CD11b$^+$ macrophages in the liver (Endo-Umeda et al. 2018), consistent with its importance in the regulation of macrophage homeostasis (Menendez-Gutierrez et al. 2015; Pascual-García et al. 2011). Of note, the two LXR isoforms (LXRα and LXRβ) share a high degree of homology between their ligand-binding domains (Lund et al. 2006) but are differentially regulated in cell cycle control of various cell types.

Fig. 6 *Lxra* expression is low in self-renewing macrophages. **a** Relative *Lxra* transcription in sorted Ki67$^-$ and Ki67$^+$ ATMs. *Actb* Ct values were above 20 due to processing for cell sorting followed by RNA isolation. **b** and *b'* *Lxra* and *Lxrb* transcription in J774A.1 macrophages treated for 18 h with 10 g/ml IL-4. **c** and *c'* J774A.1 macrophages treated with vehicle or IL-4 for 18 h. *c*" Flow cytometric quantification of Ki67$^+$ macrophages. **d** Flow cytometric quantification of BrdU$^+$ macrophages upon 18 h stimulation with IFNγ. **e** Relative transcription of *Lxra* and *Lxrb* in J774A.1 macrophages after 4 h IFNγ stimulation. **f** and **g** *CD68* and *LXRA* transcription in THP-1 cells upon differentiation with 100 nM PMA. **h** LXRα$^+$ macrophages in Kupffer cells, ATMs and peritoneal macrophages. **i** Flow cytometric analysis of Ki67$^+$ macrophages in Kupffer cells, ATMs and peritoneal macrophages. qPCR values are expressed as mean fold change relative to vehicle ± SEM. Statistical analysis was performed using unpaired Student's *t* test with Welch's correction, Mann-Whitney test or one-way ANOVA. All data are presented as mean ± SEM ($n = 3$–6). The unprocessed raw flow cytometric data are accessible in the flow repository under the repository ID FR-FCM-ZYP3 and FR-FCM-ZYPB. Significance is indicated by $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. *ATMs* adipose tissue macrophages, *PM* peritoneal macrophages, *PMA* phorbol 12-myristate 13-acetate



For instance, LXRα inhibits proliferation of LNCaP human prostate cancer cells (Fukuchi et al. 2004), whereas LXRβ inhibits cell cycle in human breast cancer cell lines, melanoma and pancreatic cancers (Candelaria et al. 2014; Hassan et al. 2015; Pencheva et al. 2014). Nevertheless, both isoforms can control cell cycle (Pascual-García et al. 2011; Vedin et al. 2013).

The mechanism of tissue-resident macrophage replenishment varies: they can either self-renew locally or they can be repopulated by monocytes (Murray and Wynn 2011; Okabe

and Medzhitov 2015; Röszer 2018). For instance, peritoneal macrophages can maintain their population by local proliferation, whereas the self-renewing capacity of Kupffer cells is debatable (Davies et al. 2013; Röszer 2018; Schulz et al. 2012). In the steady-state, Kupffer cells maintain their homeostasis with low self-renewing capacity, while in the setting of inflammation and injury there is increased self-renewal (Davies et al. 2013; Zigmond et al. 2014). As a possible regulator of macrophage self-renewal, LXR signaling has been investigated in various cell types (Endo-Umeda et al. 2018; Hassan et al. 2015; Kim et al. 2010). Upon activation, LXRs regulate cholesterol and lipid metabolism, immune cell functions and also macrophage self-renewal (Beltowski 2011; Kim et al. 2010; Pascual-García et al. 2011; Spann and Glass 2013). LXR signaling is relevant for proper tissue development and gene regulation of macrophage survival and immunity (Joseph et al. 2004). For example, macrophage differentiation in the spleen marginal zone depends on LXR activation for the clearance of pathogens (Den Haan and Kraal 2012; Noelia et al. 2013). Notably, impaired LXR signaling leads to the accumulation of cholesterol and is a contributing factor for the development of atherosclerosis and autoimmune diseases (Ito et al. 2016; Pascual-García and Valledor 2012).

Our data suggest that the differences in LXR expression of distinct macrophages may correlate with the level of macrophage self-renewal. Future studies using lineage-tracing and gene expression analyses will be needed to evaluate whether LXR expression serves as an indicator for in situ tissue-resident macrophage self-renewal. In addition, LXR signaling and environmental obesogens may play a role in obesity-induced disease, providing the impetus to further examine the effect of BPA and other environmental endocrine disruptors on nuclear receptors and their contribution to the development of metabolic disease.

In summary, we identify herein a potential relationship between BPA and the homeostatic maintenance of macrophage number, which, at least in part, is a consequence of ERK and LXR signaling in macrophages.

Methods

Animals

We performed in vivo studies with C57BL/6 male mice, aged 8–13 weeks (Charles River Laboratories). Animal experimentation was performed with the approval of the National Research Institute for Radiobiology and Radiohygiene, Budapest. C57BL/6 male mice (aged 8–16 weeks) used for the isolation of ATMs were obtained from the Institute of Comparative Molecular Endocrinology, University of Ulm, as approved by the Tübingen Regional Government Office on Animal Welfare.

Isolation of adipose tissue stromal vascular fraction and in vitro culture of adipose tissue macrophages

The SVF was isolated from epididymal white adipose tissue (eWAT) depots of mice using collagenase digestion (Weisberg et al. 2003). Briefly, washed eWAT pads were minced and digested in 10 ml of digestion medium consisting of 7 ml of Hank’s balanced salt solution (H9269, Sigma-Aldrich), 3 ml of a 7.5% bovine serum albumin (BSA) solution and 20 mg of collagenase II (C6885, Sigma-Aldrich) for 1 h with agitation at 120 rpm. After digestion, adipocytes and lipids were separated from the SVF, which contains ATMs. The SVF was passed through a 100-μm strainer and centrifuged for 10 min at 1500 rpm at 4 °C. The pellet was resuspended in selection medium consisting of 2 mM EDTA and 0.5% BSA in 1 × phosphate-buffered saline (PBS), pH 7.4. The cells were then used for flow cytometry and/or in vitro culture.

For in vitro culturing, the SVF was cultured in Dulbeccos’s modified Eagle’s medium, supplemented with 10% of heat-inactivated FBS, 20% L-cell-conditioned medium and 1% penicillin/streptomycin for 4 h to allow seeding of ATMs. The medium was then carefully removed and the attached ATMs were treated with BPA (239658, Sigma), SR9238 (SML1510, Sigma), GW3965 (G6295, Sigma), or 25-hydroxycholesterol (H1015, Sigma), as indicated. Posttreatment cell viability was assessed with the resazurin-based PrestoBlue reagent (Thermo Fisher Scientific), according to the manufacturer´s instructions.

J774A.1 macrophages and THP-1 cells were cultured in RPMI-1640 (both cell lines were obtained from ATCC), supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin and were also treated as indicated. THP-1 cells were differentiated with 100 nM of phorbol 12-myristate 13-acetate (PMA) for 2 days, after which differentiated THP-1 macrophage-like cells were treated as described.

Preparative cell sorting and fluorescence-activated cell sorting analysis

The SVF was adjusted to a density of 10^6 cells/ml and resuspended in selection buffer consisting of 2 mM EDTA and 0.5% BSA in PBS, pH 7.2, for fluorescence-activated cell sorting (FACS) analysis. Cells were fixed and permeabilized with a 20-fold dilution of permeabilization buffer (00-8333-56, eBioscience) to ensure the labeling of the nuclear antigen Ki67. Cells were then incubated with the Fc receptor blocker (KT1632) (MA5-18012, Invitrogen) for 15 min, after which cells were stained with the respective antibodies. To identify ATMs, we used the following antibodies: phycoerythrin-conjugated F4/80 monoclonal antibody (BM8) (12-4801-82,

eBioscience), allophycocyanin-conjugated CD11b monoclonal antibody (M1/70) (17-0112-81, eBioscience) and their respective isotype controls, rat phycoerythrin-conjugated IgG2a,κ (clone R35-95) (553930, BD Pharmingen) and rat allophycocyanin-conjugated IgG2b,κ (clone A95-1) (553991, BD Pharmingen). We also analyzed ATMs within the ATM-rich gate (Ampem et al. 2016). Other antibodies and their respective isotype controls used were as follows: phycoerythrin-cyanin 7-conjugated Ki67 monoclonal antibody (25-5698-80, eBioscience), rat phycoerythrin-cyanin 7-conjugated IgG2b,κ (clone eB149/10H5) (15380950, eBioscience), allophycocyanin-conjugated Ki67 monoclonal antibody (clone 20Raj1) (17-5699-41, eBioscience), phycoerythrin-cyanin 5.5-conjugated CD45 monoclonal antibody (30-F11) (35-0451-80, eBioscience) and rat phycoerythrin-cyanin 5.5-conjugated IgG2b,κ (Clone eB149/10H5) (35-4031-80, eBioscience). After 1 h incubation, cells were washed with selection buffer and used for analysis. The following unconjugated antibodies were also used: anti-LXRα antibody (ab176323, Abcam) and anti-BrdU (ab126556, Abcam). The unconjugated antibody for anti-LXR alpha was secondarily labeled with Alexa-488-conjugated goat anti-rabbit IgG (A11034, Invitrogen). We also used phycoerythrin-conjugated BrdU monoclonal antibody (BU20A) (12-5071-41, eBioscience) to label $BrdU^+$ cells. Control cells were labeled with secondary antibody or isotype IgG.

For preparative cell sorting of proliferating ($CD11b^+$/$F4/80^+$/$Ki67^+$) and non-proliferating ($CD11b^+$/$F4/80^+$/$Ki67^-$) ATMs, we used an Aria cell sorter (Becton Dickinson). Cells were also incubated with an Fc receptor blocker and permeabilized. In some experiments, sorted cells were collected for RNA extraction. For cell cycle analysis, cells were washed twice with cold 1 × PBS and stained with Nicoletti buffer with propidium iodide staining solution (50 μg/ml) (00-6990-50; eBioscience). Analysis was performed on the BD LSR II cell cytometer (1000 events). For data acquisition and analysis, we used the BD FACSDiva 8.0.1 (BD Biosciences) and FlowJo (Tristar) software. The unprocessed raw flow cytometric data are accessible in Flow Repository, as indicated.

RNA extraction and quantitative PCR

Total RNA was isolated from macrophages with TRI Reagent (T9424, Sigma-Aldrich). For sorted ATMs, we used the RNeasy Micro Kit (Qiagen) for RNA isolation, to ensure an optimal RNA yield. Gene expression analysis was performed on the ViiA 7 quantitative PCR platform 7 (Thermo Fisher Scientific). Gene products were analyzed with SYBR Green chemistry (Applied Biosystems) and gene expression values were presented as the relative mRNA levels according to the ΔCt and ΔΔCt methods, with the *Actb* housekeeping gene as a reference. The sequence-specific primers used are listed in the Electronic supplementary material of this article (Supplemental Table 1).

Western blot analysis

Cells were lysed in RIPA buffer and 50 μg of protein was subjected to 10% SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto 0.2-μm nitrocellulose membranes (1620112, Bio-Rad) and blocked with 5% skimmed milk in TBS containing 1% Tween-20 (TBS-T). Blots were incubated with primary antibodies at 4 °C overnight, washed in TBS-T and incubated with the appropriate conjugated secondary antibodies for 1 h. Blots were developed with the Pierce ECL Western blotting substrate (RPN2109, GE Healthcare). When appropriate, blots were stripped and reprobed with new primary antibodies. Gel quantification was performed with ImageJ software (NIH). The antibodies used are as follows: mouse monoclonal anti-β-actin antibody (A1978, Sigma), phospho-p44/42 MAPK (pErk1/2) (Thr202/Tyr204) antibody (9101, Cell Signaling Technology), p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (4695, Cell Signaling Technology) and anti-LXR alpha antibody [EPR6508(N)] (ab176323, Abcam). We used the following secondary antibodies for detection: goat anti-rabbit IgG (H + L) secondary antibody, HRP (65-6120, Invitrogen) and anti-mouse IgG HRP (P 0447, DAKO).

Statistics

All data represent the mean ± SEM. For statistical analysis, the unpaired Student's *t* test with Welch's correction, Mann-Whitney test, one-way analysis of variance (ANOVA), or two-way ANOVA with Dunnett's post hoc test for multiple comparisons was applied, as appropriate. A *p* value less than 0.05 was considered significant. GraphPad Prism 6 (GraphPad Software) was used for statistical analysis and the illustration of results.

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Author contributions GA performed experiments and also wrote the manuscript; AJ and HY performed experiments; LB, JT and MS provided infrastructure; and TR conceived the project and designed experiments.

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References

A-González N, Castrillo A (2011) Liver X receptors as regulators of macrophage inflammatory and metabolic pathways. Biochim Biophys Acta (BBA) - Mol Basis Dis 1812:982–994

Acconcia F, Pallottini V, Marino M (2015) Molecular mechanisms of action of BPA. Dose-response 13:1559325815610582

Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, Shen Y, Czech MP, Aouadi M (2014) Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. Cell Metab 19:162–171

Ampem G, Azegrouz H, Bacsadi Á, Balogh L, Schmidt S, Thuróczy J, Röszer T (2016) Adipose tissue macrophages in non-rodent mammals: a comparative study. Cell Tissue Res 363:461–478

Andaluri G, Manickavachagam M, Suri R (2018) Plastic toys as a source of exposure to bisphenol-A and phthalates at childcare facilities. Environ Monit Assess 190:65

Ariemma F, D'Esposito V, Liguoro D, Cabaro S, Liotti A, Cimmino I, Longo M, Beguinot F, Formisano P, Valentino R (2016) Low-dose bisphenol-A impairs adipogenesis and generates dysfunctional 3T3-L1 adipocytes. PLoS One 11:e0150762

Belhareth R, Mège J-L (2015) Macrophage populations and self-renewal: changing the paradigm. World J Immunol 5:131–141

Beltowski J (2011) Inhibition of cell proliferation: a new role of liver X receptors. Clin Lipidol 6:137–141

Boulenouar S, Michelet X, Duquette D, Alvarez D, Hogan AE, Dold C, O'connor D, Stutte S, Tavakkoli A, Winters D (2017) Adipose type one innate lymphoid cells regulate macrophage homeostasis through targeted cytotoxicity. Immunity 46:273–286

Boutens L, Stienstra R (2016) Adipose tissue macrophages: going off track during obesity. Diabetologia 59:879–894

Braun JM, Kalkbrenner AE, Calafat AM, Yolton K, Ye X, Dietrich KN, Lanphear BP (2011) Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics peds:2011–1335

Braune J, Weyer U, Hobusch C, Mauer J, Brüning JC, Bechmann I, Gericke M (2017) IL-6 regulates M2 polarization and local proliferation of adipose tissue macrophages in obesity. J Immunol 198: 2927–2934

Brehm BJ, D'Alessio DA (2014) Environmental factors influencing obesity

Byun J-A, Heo Y, Kim Y-O, Pyo M-Y (2005) Bisphenol A-induced downregulation of murine macrophage activities in vitro and ex vivo. Environ Toxicol Phar 19:19–24

Candelaria NR, Addanki S, Zheng J, Nguyen-Vu T, Karaboga H, Dey P, Gabbi C, Vedin L-L, Liu K, Wu W (2014) Antiproliferative effects and mechanisms of liver X receptor ligands in pancreatic ductal adenocarcinoma cells. PLoS One 9:e106289

Cao H (2014) Adipocytokines in obesity and metabolic disease. J Endocrinol 220:T47–T59

Cao XL, Perez-Locas C, Dufresne G, Clement G, Popovic S, Beraldin F, Dabeka RW, Feeley M (2011) Concentrations of bisphenol A in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Additives & Contaminants: Part A 28:791–798

Capurso C, Capurso A (2012) From excess adiposity to insulin resistance: the role of free fatty acids. Vasc Pharmacol 57:91–97

Chang ZL (2009) Recent development of the mononuclear phagocyte system: in memory of Metchnikoff and Ehrlich on the 100th anniversary of the 1908 Nobel Prize in Physiology or Medicine. Biol Cell 101:709–721

Chen JQ, Brown TR, Russo J (2009) Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. Biochim Biophys Acta 1793:1128–1143

Dahlman I, Nilsson M, Jiao H, Hoffstedt J, Lindgren CM, Humphreys K, Kere J, Gustafsson J-Å, Arner P, Dahlman-Wright K (2006) Liver X receptor gene polymorphisms and adipose tissue expression levels in obesity. Pharmacogenet Genom 16:881–889

Davies LC, Jenkins SJ, Allen JE, Taylor PR (2013) Tissue-resident macrophages. Nat Immunol 14:986

De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N (2012) The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin Ther Tar 16:S17–S27

Den Haan JM, Kraal G (2012) Innate immune functions of macrophage subpopulations in the spleen. J Innate Immun 4:437–445

Doerge DR, Vanlandingham M, Twaddle NC, Delclos KB (2010) Lactational transfer of bisphenol A in Sprague–Dawley rats. Toxicol Lett 199:372–376

Dong H, Yao X, Liu S, Yin N, Faiola F (2018) Non-cytotoxic nanomolar concentrations of bisphenol A induce human mesenchymal stem cell adipogenesis and osteogenesis. Ecotoxicol Environ Saf 164: 448–454

Ejaredar M, Lee Y, Roberts DJ, Sauve R, Dewey D (2017) Bisphenol A exposure and children's behavior: a systematic review. J Expo Sci Env Epid 27:175

Endo-Umeda K, Nakashima H, Komine-Aizawa S, Umeda N, Seki S, Makishima M (2018) Liver X receptors regulate hepatic F4/80+ CD11b+ Kupffer cells/macrophages and innate immune responses in mice. Sci Rep-UK 8:9281

Fan X, Wu L, Hou T, He J, Wang C, Liu Y, Wang Z (2018) Maternal bisphenol A exposure impaired endochondral ossification in craniofacial cartilage of rare minnow (Gobiocypris rarus) offspring. Ecotoxicol Environ Saf 163:514–520

Flint S, Markle T, Thompson S, Wallace E (2012) Bisphenol A exposure, effects, and policy: a wildlife perspective. J Environ Manag 104:19–34

Food U, Administration D (2009) Exposure to bisphenol A (BPA) for infants, toddlers and adults from the consumption of infant formula, toddler Food and adult (canned) food. Food and Drug Administration, Washington, DC

Fukuchi J, Kokontis JM, Hiipakka RA, Chuu C-p, Liao S (2004) Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells. Cancer Res 64:7686–7689

Geens T, Neels H, Covaci A (2012) Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. Chemosphere 87:796–802

Ginhoux F, Schultze JL, Murray PJ, Ochando J, Biswas SK (2015) New insights into the multidimensional concept of macrophage ontogeny, activation and function. Nat Immunol 17:34

Goran MI, Dumke K, Bouret SG, Kayser B, Walker RW, Blumberg B (2013) The obesogenic effect of high fructose exposure during early development. Nat Rev Endocrinol 9:494

Griffett K, Solt LA, El-Gendy BE-DM, Kamenecka TM, Burris TP (2012) A liver-selective LXR inverse agonist that suppresses hepatic steatosis. ACS Chem Biol 8:559–567

Griffett K, Welch RD, Flaveny CA, Kolar GR, Neuschwander-Tetri BA, Burris TP (2015) The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. Mol Metab 4: 353–357

Grun F, Blumberg B (2009a) Endocrine disrupters as obesogens. Mol Cell Endocrinol 304:19–29

Grun F, Blumberg B (2009b) Minireview: the case for obesogens. Mol Endocrinol 23:1127–1134

Haase J, Weyer U, Immig K, Kloting N, Bluher M, Eilers J, Bechmann I, Gericke M (2014) Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. Diabetologia 57:562–571

Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, Ganai BA (2015) Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. World J Diabetes 6:598–612

Harley KG, Gunier RB, Kogut K, Johnson C, Bradman A, Calafat AM, Eskenazi B (2013) Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ Res 126: 43–50

Hassan TS, Paniccia A, Russo V, Steffensen KR (2015) LXR inhibits proliferation of human breast cancer cells through the PI3K-Akt pathway. Nucl Receptor Res 2

Heindel JJ (2011) The obesogen hypothesis of obesity: overview and human evidence. Obesity before birth. Springer, pp 355-365

Holtcamp W (2012) Obesogens: an environmental link to obesity. Environ Health Perspect 120:a62

Huang D, Wu J, Su X, Yan H, Sun Z (2017) Effects of low dose of bisphenol A on the proliferation and mechanism of primary cultured prostate epithelial cells in rodents. Oncol Lett 14:2635–2642

Huang Y, Wong C, Zheng J, Bouwman H, Barra R, Wahlström B, Neretin L, Wong M (2012) Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. Environ Int 42:91–99

Ito A, Hong C, Oka K, Salazar JV, Diehl C, Witztum JL, Diaz M, Castrillo A, Bensinger SJ, Chan L (2016) Cholesterol accumulation in CD11c+ immune cells is a causal and targetable factor in autoimmune disease. Immunity 45:1311–1326

Ivanov S, Merlin J, Lee MKS, Murphy AJ, Guinamard RR (2018) Biology and function of adipose tissue macrophages, dendritic cells and B cells. Atherosclerosis 271:102–110

Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, MacDonald AS, Allen JE (2011) Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 332:1284–1288

Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, Hogenesch J, O'Connell RM, Cheng G, Saez E (2004) LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 119:299–309

Kim KH, Lee GY, Kim JI, Lee JW, Kim JB (2010) Inhibitory effect of LXR activation on cell proliferation and cell cycle progression through lipogenic activity. J Lipid Res jlr M007989

Konieczna A, Rutkowska A, Rachon D (2015) Health risk of exposure to bisphenol A (BPA). Rocz Panstw Zakl Hig 66

Li L, Zhao G-D, Shi Z, Qi L-L, Zhou L-Y, Fu Z-X (2016) The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. Oncol Lett 12:3045–3050

Lim Y-H, Bae S, Kim B-N, Shin CH, Lee YA, Kim JI, Hong Y-C (2017) Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. Environ Health 16:79

Liu Y, Mei C, Liu H, Wang H, Zeng G, Lin J, Xu M (2014) Modulation of cytokine expression in human macrophages by endocrine-disrupting chemical bisphenol-A. Biochem Bioph Res Co 451:592–598

Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117: 175–184

Lund EG, Peterson LB, Adams AD, Lam M-HN, Burton CA, Chin J, Guo Q, Huang S, Latham M, Lopez JC, Menke JG, Milot DP, Mitnaul LJ, Rex-Rabe SE, Rosa RL, Tian JY, Wright SD, Sparrow CP (2006) Different roles of liver X receptor α and β in lipid metabolism: effects of an α-selective and a dual agonist in mice deficient in each subtype. Biochem Pharmacol 71:453–463

Luo L, Liu M (2016) Adipose tissue in control of metabolism. J Endocrinol 231:R77–R99

Luo Y, Shao L, Chang J, Feng W, Liu YL, Cottler-Fox MH, Emanuel PD, Hauer-Jensen M, Bernstein ID, Liu L (2018) M1 and M2 macrophages differentially regulate hematopoietic stem cell self-renewal and ex vivo expansion. Blood advances 2:859–870

Marmugi A, Ducheix S, Lasserre F, Polizzi A, Paris A, Priymenko N, Bertrand-Michel J, Pineau T, Guillou H, Martin PG (2012) Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. Hepatology 55:395–407

Menendez-Gutierrez MP, Röszer T*, Fuentes L, Nunez V, Escolano A, Redondo JM, De Clerck N, Metzger D, Valledor AF, Ricote M (2015) Retinoid X receptors orchestrate osteoclast differentiation and postnatal bone remodeling (*equal contribution). J Clin Invest 125(2) 809–823

Murray PJ, Wynn TA (2011) Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11:723

Naville D, Pinteur C, Vega N, Menade Y, Vigier M, Le Bourdais A, Labaronne E, Debard C, Luquain-Costaz C, Begeot M, Vidal H, Le Magueresse-Battistoni B (2013) Low-dose food contaminants trigger sex-specific, hepatic metabolic changes in the progeny of obese mice. FASEB journal : official publication of the Federation of American Societies for Experimental Biology

Noelia A, Guillen JA, Gallardo G, Diaz M, De La Rosa JV, Hernandez IH, Casanova-Acebes M, Lopez F, Tabraue C, Beceiro S (2013) The nuclear receptor LXRα controls the functional specialization of splenic macrophages. Nat Immunol 14:831

Okabe Y, Medzhitov R (2015) Tissue biology perspective on macrophages. Nat Immunol 17:9

Pascual-García M, Carbó JM, León T, Matalonga J, Out R, Van Berkel T, Sarrias M-R, Lozano F, Celada A, Valledor AF (2011) Liver X receptors inhibit macrophage proliferation through downregulation of cyclins D1 and B1 and cyclin-dependent kinases 2 and 4. J Immunol 1000585

Pascual-García M, Valledor AF (2012) Biological roles of liver X receptors in immune cells. Arch Immunol Ther Exp 60:235–249

Pencheva N, Buss CG, Posada J, Merghoub T, Tavazoie SF (2014) Broad-spectrum therapeutic suppression of metastatic melanoma through nuclear hormone receptor activation. Cell 156:986–1001

Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA (2014) Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. Environ Health Perspect 122:775

Repossi A, Farabegoli F, Gazzotti T, Zironi E, Pagliuca G (2016) Bisphenol A in edible part of seafood. Ital J Food Safety:5

Ribeiro E, Ladeira C, Viegas S (2017) Occupational exposure to bisphenol A (BPA): a reality that still needs to be unveiled. Toxics 5:22

Richter E, Ventz K, Harms M, Mostertz J, Hochgräfe F (2016) Induction of macrophage function in human THP-1 cells is associated with rewiring of MAPK signaling and activation of MAP3K7 (TAK1) protein kinase. Front Cell Dev Biol 4:21

Rogero MM, Calder PC (2018) Obesity, inflammation, toll-like receptor 4 and fatty acids. Nutrients 10:432

Rogers JA, Metz L, Yong VW (2013) Endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. Mol Immunol 53:421–430

Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156:20–44

Röszer T (2015) Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediat Inflamm 2015: 16

Röszer T (2018) Understanding the biology of self-renewing macrophages. Cells 7:103

Schug TT, Janesick A, Blumberg B, Heindel JJ (2011) Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol 127:204–215

Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SEW, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336:86–90

Sheikh AB, Nasrullah A, Haq S, Akhtar A, Ghazanfar H, Nasir A, Afzal RM, Bukhari MM, Chaudhary AY, Naqvi SW (2017) The interplay of genetics and environmental factors in the development of obesity. Cureus 9

Singh P, Goode T, Dean A, Awad SS, Darlington GJ (2011) Elevated interferon gamma signaling contributes to impaired regeneration in the aged liver. J Gerontol A Biol Sci Med Sci 66A:944–956

Smith AM, Gibbons HM, Oldfield RL, Bergin PM, Mee EW, Curtis MA, Faull RL, Dragunow M (2013) M-CSF increases proliferation and phagocytosis while modulating receptor and transcription factor expression in adult human microglia. J Neuroinflammation 10:859

Spann NJ, Glass CK (2013) Sterols and oxysterols in immune cell function. Nat Immunol 14:893

Vafeiadi M, Roumeliotaki T, Myridakis A, Chalkiadaki G, Fthenou E, Dermitzaki E, Karachaliou M, Sarri K, Vassilaki M, Stephanou EG (2016) Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ Res 146:379–387

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) Human exposure to bisphenol A (BPA). Reprod Toxicol 24:139–177

Vedin LL, Gustafsson JÅ, Steffensen KR (2013) The oxysterol receptors LXRα and LXRβ suppress proliferation in the colon. Mol Carcinog 52:835–844

Wang H, Zhou Y, Tang C, He Y, Wu J, Chen Y, Jiang Q (2013) Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One 8:e56800

Wang Z, Liu H, Liu S (2017) Low-dose bisphenol A exposure: a seemingly instigating carcinogenic effect on breast cancer. Advanced Science 4:1600248

Waqas SFH, Hoang AC, Lin Y-T, Ampem G, Azegrouz H, Balogh L, Thuróczy J, Chen J-C, Gerling IC, Nam S, Lim J-S, Martinez-Ibañez J, Real JT, Paschke S, Quillet R, Ayachi S, Simonin F, Schneider EM, Brinkman JA, Lamming DW, Seroogy CM, Röszer T (2017) Neuropeptide FF increases M2 activation and self-renewal of adipose tissue macrophages. J Clin Invest 127:2842–2854

Warns J, Marwarha G, Freking N, Ghribi O (2018) 27-hydroxycholesterol decreases cell proliferation in colon cancer cell lines. Biochimie

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808

Welshons WV, Nagel SC, vom Saal FS (2006) Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147:s56–s69

Wetherill YB, Hess-Wilson JK, Comstock CES, Shah SA, Buncher CR, Sallans L, Limbach PA, Schwemberger S, Babcock GF, Knudsen KE (2006) Bisphenol A facilitates bypass of androgen ablation therapy in prostate cancer. Mol Cancer Ther 5:3181–3190

WHO. (2018a) Global Health Observatory (GHO) data—obesity. Retrieved 28.07.2018, from http://www.who.int/gho/ncd/risk_factors/obesity_text/en/

WHO. (2018b) Obesity and overweight—key facts. Retrieved 28.07.2018, from http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight

Williams C, Bondesson M, Krementsov DN, Teuscher C (2014) Gestational bisphenol A exposure and testis development. Endocr Disruptors (Austin) 2:e29088

Ye X, Pierik FH, Angerer J, Meltzer HM, Jaddoe VW, Tiemeier H, Hoppin JA, Longnecker MP (2009) Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Int J Hyg Environ Health 212:481–491

Yu W, Chen J, Xiong Y, Pixley FJ, Yeung Y-G, Stanley ER (2012) Macrophage proliferation is regulated through CSF-1 receptor tyrosines 544, 559, and 807. J Biol Chem 287:13694–13704

Zhang Y-F, Ren X-M, Li Y-Y, Yao X-F, Li C-H, Qin Z-F, Guo L-H (2018) Bisphenol A alternatives bisphenol S and bisphenol F interfere with thyroid hormone signaling pathway in vitro and in vivo. Environ Pollut 237:1072–1079

Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, Brazowski E, Shibolet O, Halpern Z, Varol C (2014) Infiltrating monocyte-derived macrophages and resident Kupffer cells display different ontogeny and functions in acute liver injury. J Immunol 1400574

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