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Supporting Information

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Androgens Modulate the Immune Profile in a Mouse Model of Polycystic Ovary Syndrome

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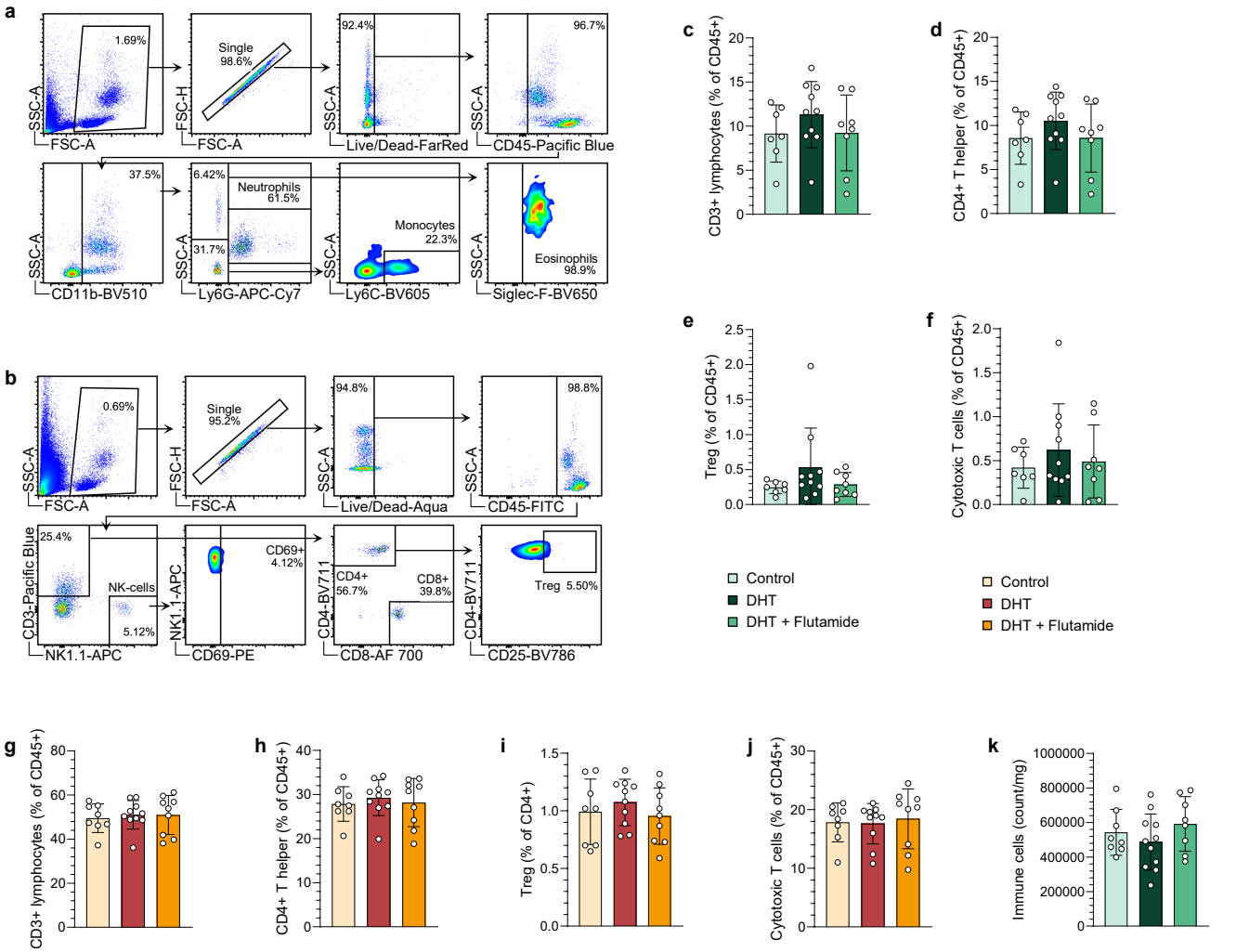
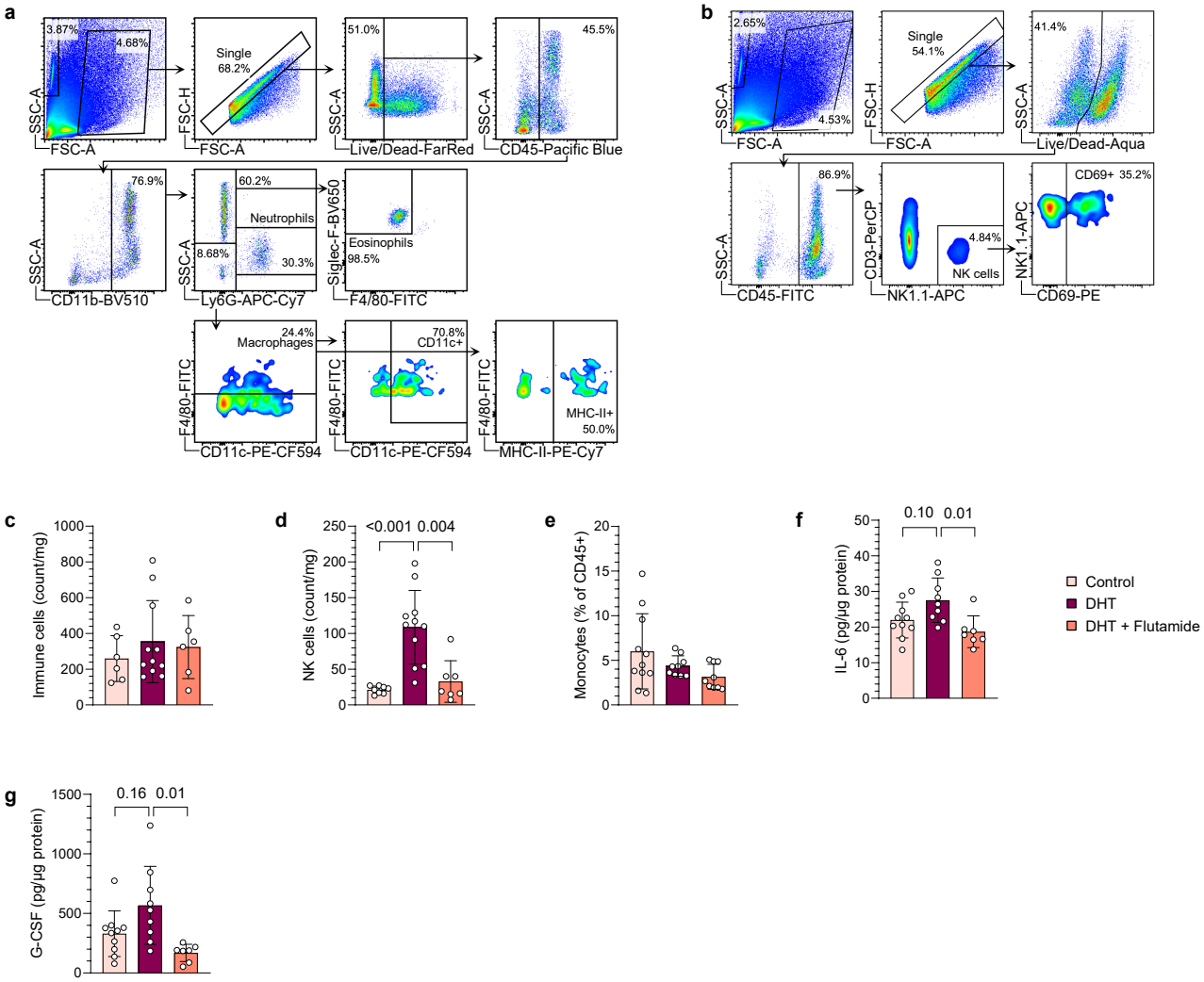


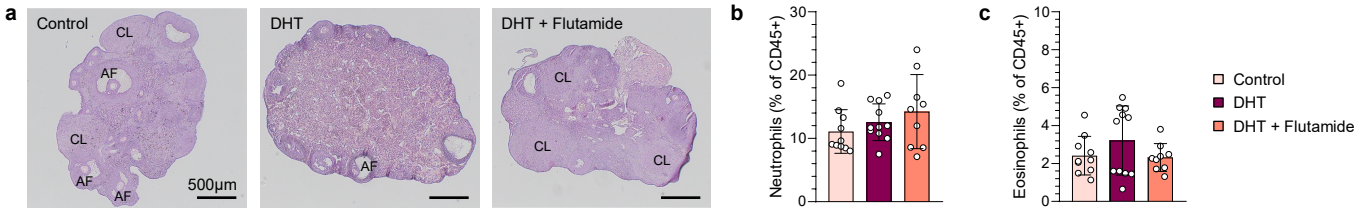
Supplementary figure 1. Immune populations in blood and secondary lymphoid organs are differently affected by androgen exposure. (**a**) Gating strategy myeloid cells in blood. (**b**) Gating strategy NK- and T cells in blood. (**c**) Frequency of CD3$^+$ T cells in spleen (*n* = 7 Control, 10 DHT, 8 DHT + Flutamide). (**d**) Frequency of CD4$^+$ T helper cells in spleen (*n* = 7, 10, 8). (**e**) Frequency of CD4$^+$ CD25$^+$ T reg cells in spleen (*n* = 7, 10, 8). (**f**) Frequency of CD8$^+$ cytotoxic T cells in spleen (*n* = 7, 10, 8). (**g**) Frequency of CD3$^+$ T cells in lymph nodes (*n* = 8, 10, 9). (**h**) Frequency of CD4$^+$ T helper cells in lymph nodes (*n* = 8, 10, 9). (**i**) Frequency of CD4$^+$ CD25$^+$ T reg cells in lymph nodes (*n* = 8, 10, 9). (**j**) Frequency of CD8$^+$ cytotoxic T cells in lymph nodes (*n* = 8, 10, 9). (**k**) Immune cell count in spleen (*n* = 8, 11, 8). Data are presented as means ± SD. n indicates the number of biologically independent samples examined. Statistical analysis was assessed by one-way ANOVA with Dunnett's multiple comparison (**c-d**, **f-i**, **k**) or by Kruskal-Wallis with Dunn's multiple comparison (**e**, **j**) Source data are provided as a Source Data File.



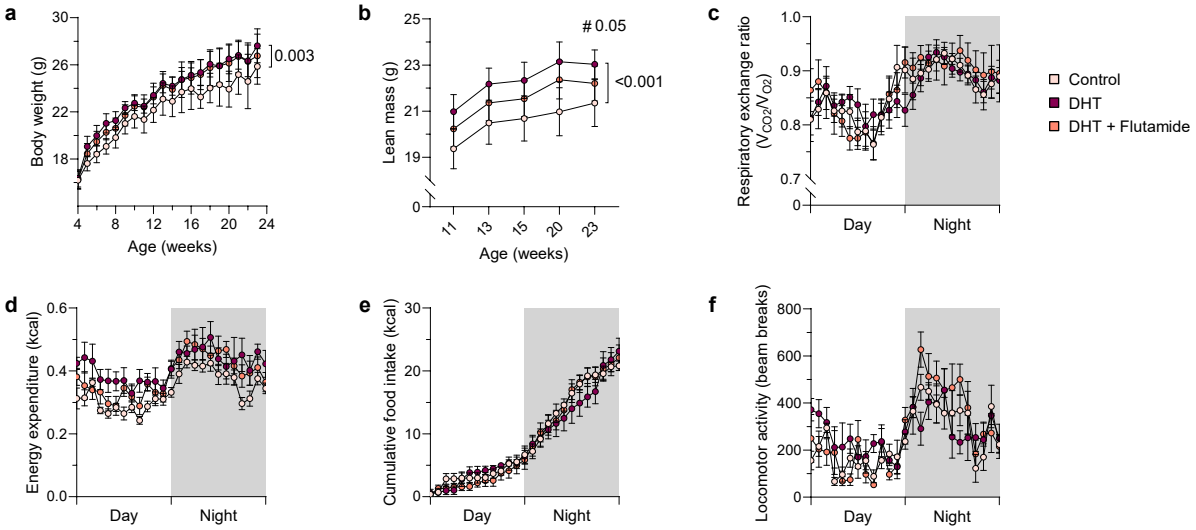
Supplementary figure 2. Uterine eosinophil and NK cell populations are markedly altered by androgen exposure in PCOS-like mice. (**a**) Gating strategy myeloid cells. (**b**) Gating strategy NK cells in uterus. (**c**) Number of CD45+ immune cells in uterus *(n* = 6 Control, 11 DHT, 6 DHT + Flutamide). (**d**) NK cell count uterus *(n* = 8, 11, 7). (**e**) Frequency of monocytes in uterus *(n* = 11, 9, 9). (**f**) IL-6 levels in uterus *(n* = 10, 9, 7). (**g**) Granulocyte-colony stimulating factor (G-CSF) levels in uterus *(n* = 10, 9, 7). Data are presented as means ± SD. n indicates the number of biologically independent samples examined. Statistical analysis was assessed by Kruskal-Wallis with Dunn's multiple comparison (**c**-**e**), or mixed-effects ANOVA with Bonferroni's multiple comparison test (**f**-**g**) and significant differences were indicated with p values. Source data are provided as a Source Data File.

Analyte	Comparison	95,00% CI	Summary	Adjusted P Value
IL-1a	DHT vs. Control	-2,54 to 29,3	ns	0,11
	DHT vs. Flutamide	-15,1 to 36,4	ns	0,58
IL-1b	DHT vs. Control	-0,961 to 3,48	ns	0,36
	DHT vs. Flutamide	-2,80 to 4,00	ns	>0,99
IL-2	DHT vs. Control	-2,03 to 45,3	ns	0,08
	DHT vs. Flutamide	-12,0 to 48,3	ns	0,29
IL-3	DHT vs. Control	-1,77 to 2,60	ns	>0,99
	DHT vs. Flutamide	-1,53 to 2,97	ns	0,86
IL-4	DHT vs. Control	-1,76 to 4,23	ns	0,64
	DHT vs. Flutamide	-1,50 to 4,23	ns	0,49
IL-9	DHT vs. Control	-2,71 to 33,6	ns	0,1
	DHT vs. Flutamide	-5,92 to 38,1	ns	0,17
IL-10	DHT vs. Control	-13,6 to 20,8	ns	>0,99
	DHT vs. Flutamide	-20,0 to 23,7	ns	>0,99
IL-12p40	DHT vs. Control	-6,13 to 65,4	ns	0,11
	DHT vs. Flutamide	-34,2 to 70,4	ns	0,79
IL-12p70	DHT vs. Control	-34,9 to 133	ns	0,34
	DHT vs. Flutamide	-27,3 to 136	ns	0,23
IL-13	DHT vs. Control	-109 to 199	ns	0,95
	DHT vs. Flutamide	-77,8 to 240	ns	0,42
IL-17a	DHT vs. Control	-2,57 to 3,91	ns	>0,99
	DHT vs. Flutamide	-4,44 to 5,54	ns	>0,99
GM-CSF	DHT vs. Control	-12,3 to 29,4	ns	0,65
	DHT vs. Flutamide	-13,0 to 27,1	ns	0,78
KC	DHT vs. Control	-7,85 to 32,5	ns	0,3
	DHT vs. Flutamide	-23,2 to 35,1	ns	>0,99
MIP-1a	DHT vs. Control	-9,91 to 2,35	ns	0,3
	DHT vs. Flutamide	-11,6 to 1,33	ns	0,13
MIP-1b	DHT vs. Control	-97,3 to 12,4	ns	0,15
	DHT vs. Flutamide	-67,2 to 14,3	ns	0,25
RANTES	DHT vs. Control	-198 to 370	ns	0,91
	DHT vs. Flutamide	-108 to 458	ns	0,26

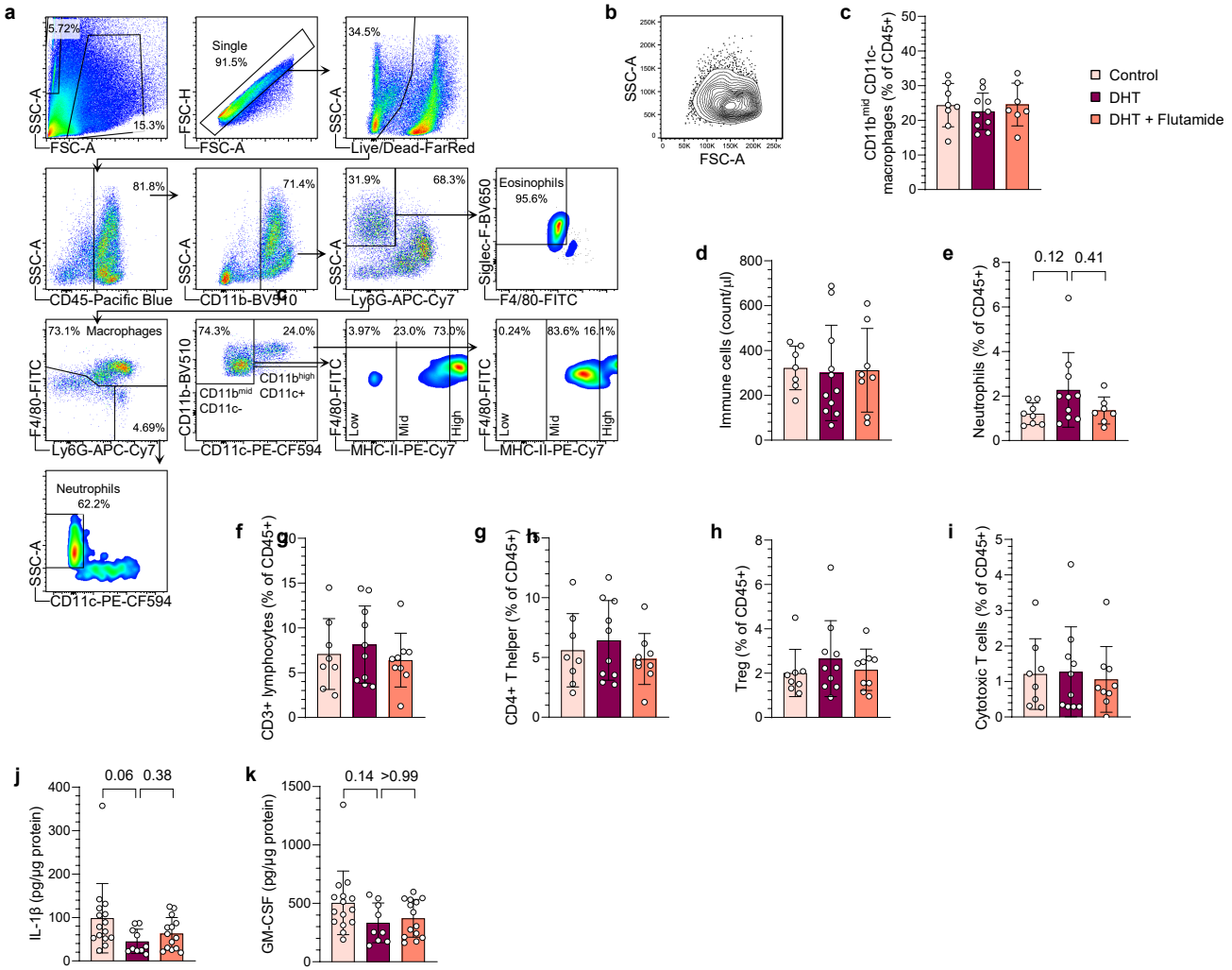
Supplementary table 1. Cytokine levels in uterus *(n* = 10 Control, 9 DHT, 7 DHT + Flutamide). Data are presented as 95% confidence intervals (CI) with adjusted p-values. Statistical analysis was assessed by mixed-effects ANOVA with Bonferroni's multiple comparison test. Source data are provided as a Source Data File. ns indicates non-significant.



Supplementary figure 3. Ovarian macrophage populations are decreased by androgen exposure in PCOS-like mice. (**a**) Representative images of ovarian sections stained with H&E. Corpus lutea (CL) and antral follicles (AF) indicated. (**b**) Frequency of neutrophils in ovaries, expressed as percent of CD45⁺ immune cells (*n* = 10 Control, 11 DHT, 9 DHT + Flutamide). (**c**) Frequency of eosinophils in ovaries (*n* = 10, 11, 9). Data are presented as means ± SD. n indicates the number of biologically independent samples examined. Statistical analysis was assessed by Kruskal-Wallis with Dunn's multiple comparison. Source data are provided as a Source Data File.



Supplementary figure 4. The peripubertal DHT-induced mouse model is a non-obese but insulin resistant model of PCOS. (**a**) Body weight (*n* = 12 Control, 11 DHT, 8 DHT + Flutamide). (**b**) Lean mass (*n* = 12, 11, 8). (**c**) Respiratory exchange ratio (*n* = 6, 5, 5). (**d**) Energy expenditure (*n* = 6, 5, 5). (**e**) Food intake (*n* = 6, 5, 5). (**f**) Locomotor activity (*n* = 6, 5, 5). Data are presented as means ± SD (**a-b**) and means ± SEM (**c-f**). # indicates statistical significance between DHT and DHT + Flutamide. n indicates the number of biologically independent samples examined. Statistical analysis was assessed by mixed-effects ANOVA with Bonferroni's multiple comparison test (**a**), two-way ANOVA with Bonferroni's multiple comparison test (**b**), or ANCOVA with body mass as covariate (**c-f**) and significant differences were indicated with p values. Source data are provided as a Source Data File.



Supplementary figure 5. DHT-exposed PCOS-like mice display an aberrant immune profile in VAT albeit unaltered fat mass. (**a**) Gating strategy for myeloid cells in VAT. (**b**) Representative plot of macrophages in VAT. (**c**) Frequency CD11b^{mid}CD11c^{-} macrophages in VAT (*n* = 8 Control, 10 DHT, 7 DHT + Flutamide). (**d**) Number of CD45+ immune cells in VAT (*n* = 7, 11, 8). (**e**) Frequency of neutrophils in VAT (*n* = 8, 10, 7). (**f**) Frequency of CD3^{+} T cells in VAT (*n* = 8, 10, 9). (**g**) Frequency of CD4^{+} T helper cells in VAT (*n* = 8, 10, 9). (**h**) Frequency of CD4^{+} CD25^{+} T reg cells in VAT (*n* = 8, 10, 9). (**i**) Frequency of CD8^{+} cytotoxic T cells in VAT (*n* = 8, 10, 9). (**j**) IL-1β levels in VAT (*n* = 15, 10, 14). (**k**) GM-CSF levels in VAT (*n* = 15, 9, 14). Data are presented as means ± SD. n indicates the number of biologically independent samples examined. Statistical analysis was assessed by one-way ANOVA with Dunnett's multiple comparison (**c-d**, **f-g**), Kruskal-Wallis with Dunn's multiple comparison (**e**, **h-i**), or mixed-effects ANOVA with Bonferroni's multiple comparison test (**j-k**) and significant differences were indicated with p values. Source data are provided as a Source Data File.