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Supplemental information

CD38 regulates ovarian function and fecundity via NAD^+ metabolism

Rosalba Perrone, Prasanna Vadhana Ashok Kumaar, Lauren Haky, Cosmo Hahn, Rebeccah Riley, Julia Balough, Giuliana Zaza, Bikem Soygur, Kaitlyn Hung, Leandro Prado, Herbert G. Kasler, Ritesh Tiwari, Hiroyuki Matsui, Genesis Vega Hormazabal, Indra Heckenbach, Morten Scheibye-Knudsen, Francesca E. Duncan, and Eric Verdin

Figure S1

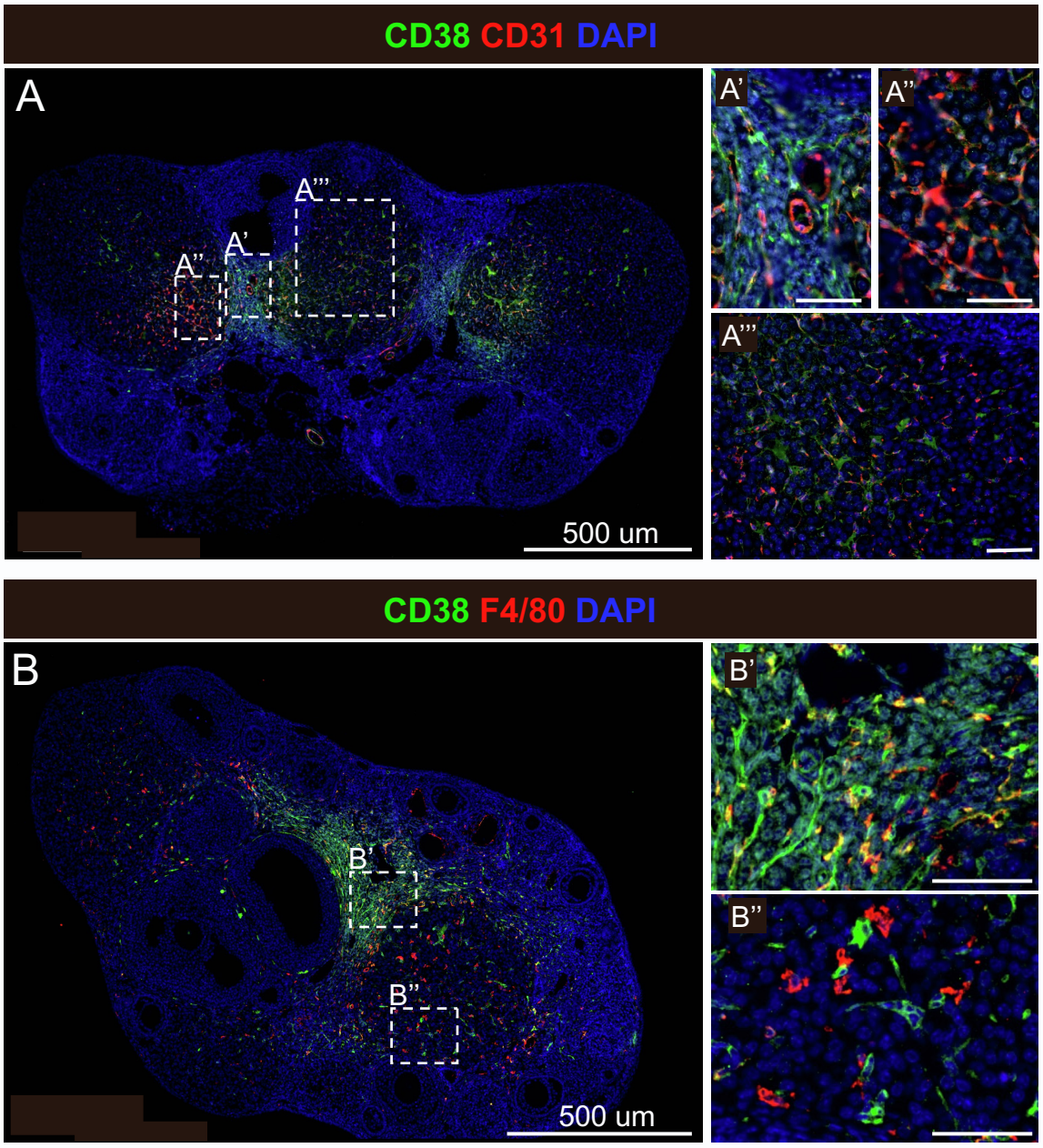

Figure S1: Ovarian CD38 expression and co-labeling with macrophages and endothelial cells, Related to Figure 1. (A) Immunofluorescence image of a 2-month-old WT mouse ovary showing the expression of CD38 and the endothelial cell marker CD31, 500 μm scale. (A' - A''') Higher magnification images of S1A, 50 μm scale (B) Immunofluorescence image of a 2-month-old WT mouse ovary showing the expression of CD38 and the macrophage marker F4/80, 500 μm scale. (B' - B'') Higher magnification images of S1B, 50 μm scale.

Figure S2

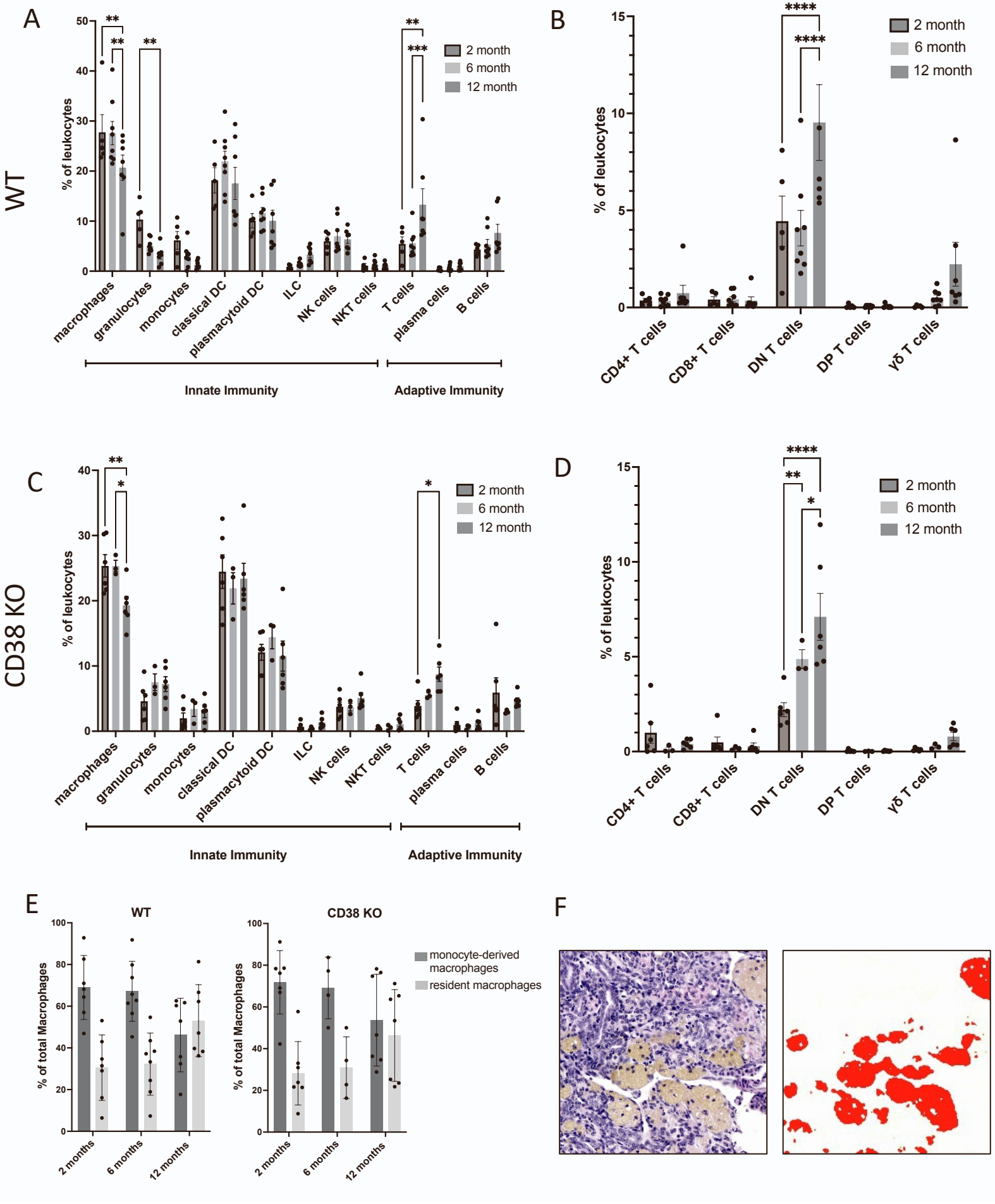
A
WT
% of leukocytes
2 month
6 month
12 month
macrophages
granulocytes
monocytes
classical DC
plasmacytoid DC
ILC
NK cells
NKT cells
T cells
plasma cells
B cells
Innate Immunity
Adaptive Immunity
B
% of leukocytes
CD4+ T cells
CD8+ T cells
DN T cells
DP T cells
γδ T cells
C
CD38 KO
D
E
WT
CD38 KO
% of total Macrophages
monocyte-derived macrophages
resident macrophages
2 months
6 months
12 months
F

Figure S2: Immunophenotyping profile of WT and CD38 KO mouse ovaries during reproductive aging shows a shift from innate to adaptive immunity and a decrease in resident macrophages during reproductive aging. Image processing and signal threshold analysis of H&E-stained sections yields quantitative understanding of macrophage dynamics with age in WT and CD38 KO ovarian micro environment, Related to Figure 3. (A) Percent of innate and adaptive leukocyte populations in WT ovaries throughout reproductive aging (2-12 months). (B) Percent of T cell subpopulations in WT ovaries throughout reproductive aging (2-12 months). (C) Percent of innate and adaptive leukocyte populations in CD38 KO ovaries throughout reproductive aging (2-12 months). (B) Percent of T cell subpopulations in CD38 KO ovaries throughout reproductive aging (2-12 months). (n=4-8 per group, mean ± SEM, Two-way ANOVA statistical analysis *** = $p < 0.0005$). (E) Relative percent of monocyte-derived (CD11c+) and resident (CD11c-) macrophages within WT and CD38 KO ovaries throughout reproductive aging (2-12 months) (n=4-8 per group, mean ± SD, Two-way ANOVA statistical analysis). (F) Representative image of MNGCs observed in 28-month-old WT ovary with corresponding image of thresholding used for MNGC quantification.

Figure S3

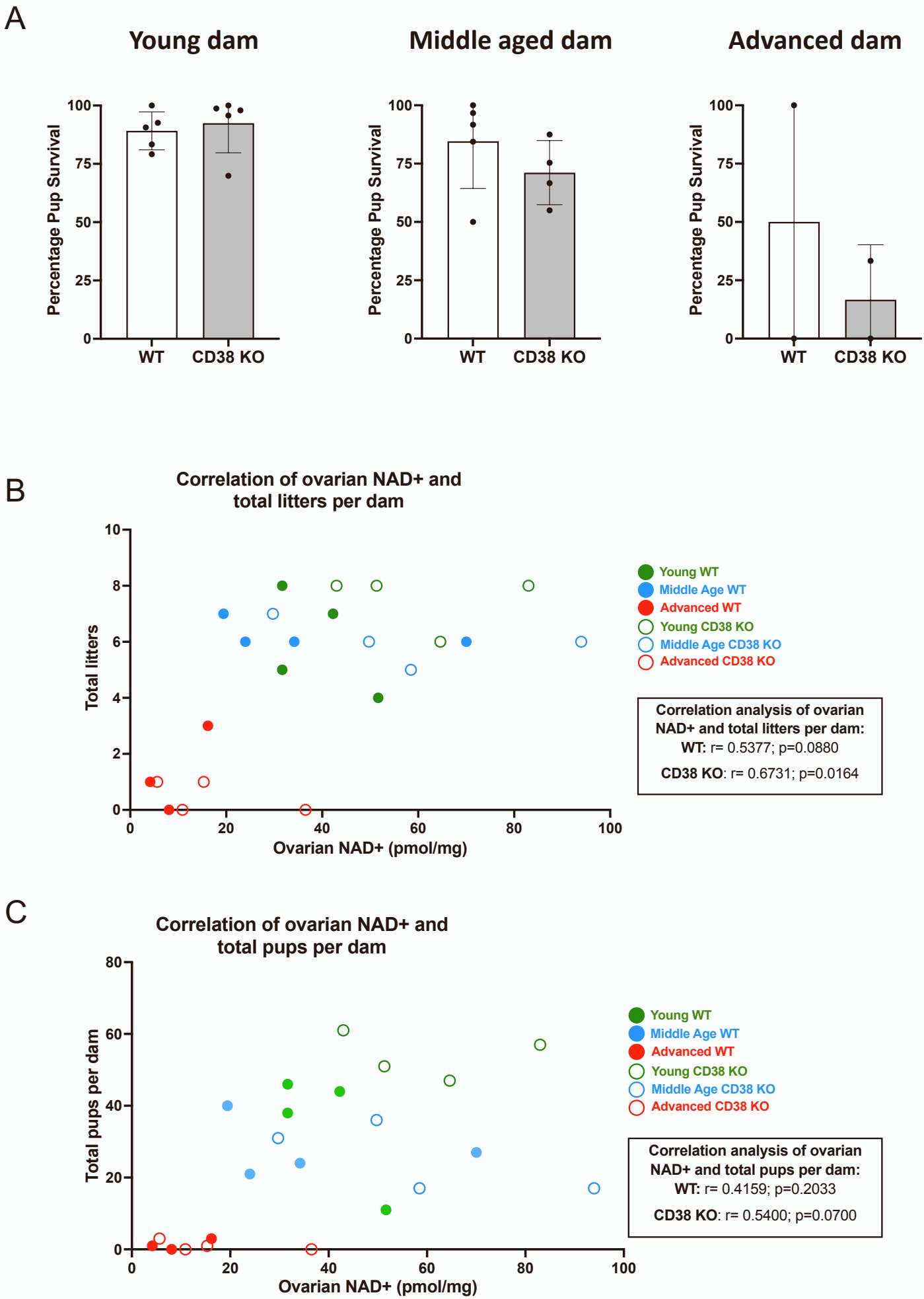
A
Young dam
Middle aged dam
Advanced dam
Percentage Pup Survival
100
75
50
25
0
WT
CD38 KO
B
Correlation of ovarian NAD+ and total litters per dam
Total litters
Ovarian NAD+ (pmol/mg)
Young WT
Middle Age WT
Advanced WT
Young CD38 KO
Middle Age CD38 KO
Advanced CD38 KO
Correlation analysis of ovarian NAD+ and total litters per dam:
WT: r= 0.5377; p=0.0880
CD38 KO: r= 0.6731; p=0.0164
C
Correlation of ovarian NAD+ and total pups per dam
Total pups per dam
Ovarian NAD+ (pmol/mg)
Young WT
Middle Age WT
Advanced WT
Young CD38 KO
Middle Age CD38 KO
Advanced CD38 KO
Correlation analysis of ovarian NAD+ and total pups per dam:
WT: r= 0.4159; p=0.2033
CD38 KO: r= 0.5400; p=0.0700

Figure S3: Percentage of pups survival during breeding trial and correlation between ovarian NAD+ and breeding trial outcomes of WT and CD38 KO females, Related to Figure 5. (A) Percentage of pup survival before weaning for each dam in the 3 different female age groups (young, middle-aged and advanced) throughout the 6-months breeding trial. (n=2-5 per group Unpaired t-test statistical analysis, data represented as mean ± SD). (B) Correlation plot of ovarian NAD+ and total litters per dam. (C) Correlation plot of ovarian NAD+ and total pups per dam. The ovarian NAD+ levels are normalized to whole ovary weight and represented as pmol/mg tissue. The correlation strength is represented by Pearson coefficient r, and p-value and it was considered significant only when $p < 0.05$.