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Supplemental information

Skin exposure to UVB light induces a skin-brain-gonad axis and sexual behavior

Roma Parikh, Eschar Sorek, Shivang Parikh, Keren Michael, Lior Bikovski, Sagi Tshori, Galit Shefer, Shira Mingelgreen, Taiba Zornitzki, Hilla Knobler, Gabriel Chodick, Mariya Mardamshina, Arjan Boonman, Noga Kronfeld-Schor, Hadas Bar-Joseph, Dalit Ben-Yosef, Hadar Amir, Mor Pavlovsky, Hagit Matz, Tom Ben-Dov, Tamar Golan, Eran Nizri, Daphna Liber, Yair Liel, Ronen Brenner, Yftach Gepner, Orit Karnieli-Miller, Rina Hemi, Ruth Shalgi, Tali Kimchi, Ruth Percik, Aron Weller, and Carmit Levy

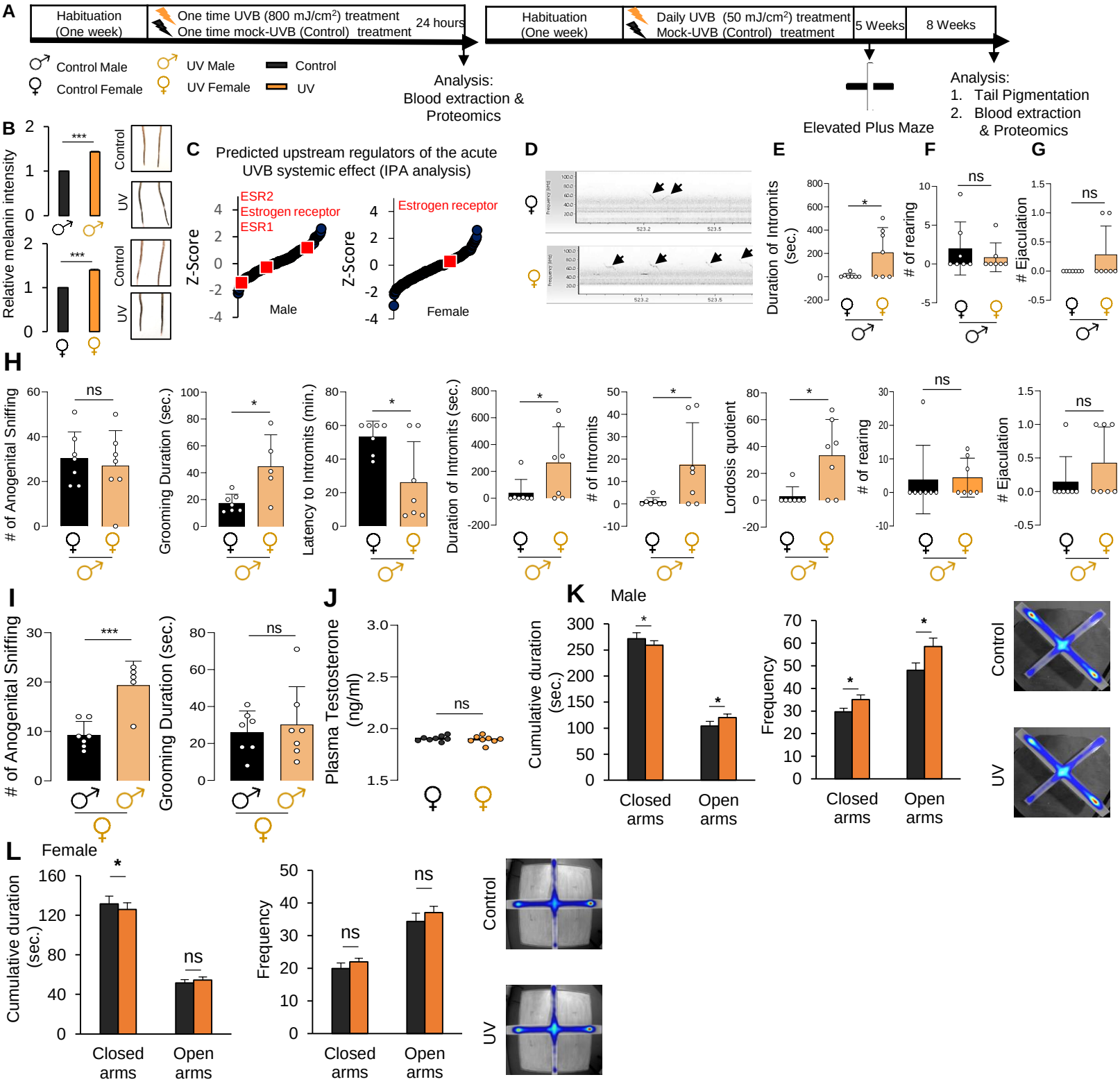

Figure S1. Daily UVB treatment enhances female sexual attractiveness and receptiveness, related to Figure 1

(A) Schematic representation of the experimental design. C57BL6 mice received either single acute UVB or control exposure and blood collection for proteomics analysis after 24 hours or exposed to daily UVB or control treatment for 5 weeks followed by 'Elevated Plus Maze' analysis, followed by measurement of tail pigmentation and collection of blood for proteomic analysis upon 8-week of UVB exposure. (B) Graphical presentation of the mean melanin intensity in the tails of mice after the indicated treatment and representative image of tails, n=7. (C) The IPA significant activation z-scores of predicted upstream regulators of the differential blood plasma proteins from mice that received single UVB treatment (n=3). (D) Representative image of male vocalization spectrograms towards a control or UVB treated female. Each arrow represents a single "call". (E) Total duration of intromissions by a control male on a control or UVB treated female. (F) Rearing behavior by control or UVB treated females in the presence of control males. (G) Successful ejaculations by a control male with a control or UVB treated female. (H) Total number of anogenital sniffing, duration of self-grooming, latency to intromit, total duration of intromission, total number of intromissions, female lordosis quotient, number of rears by females, and ejaculation number by the UVB treated male with the control or UVB treated females. (I) Total number of anogenital sniffing (left panel) and duration of self-grooming (right panel) by UVB treated females in the presence of control or UVB treated males.(J) ELISA plasma testosterone levels of female mice upon 8-week of UVB or control treatment. (K) Control or UVB treated mice were subjected to Elevated plus maze analysis. Cumulative duration a male mice spends either in the open or closed arms of the maze and frequency of visits in the open or closed arms by the male during the 7 minutes of the test. (L) As in panel I, but for female mice. Data are presented as the mean ± SEM; *p < 0.05 (n=12 animals per condition). Representative images of the heat map of the elevated plus maze is shown on right.

Data are means ± SEM, n=7 (B), n=3 (C-D), n=7 (E-I), n=12 (K-L). For data analysis, a two-tailed, unpaired Student's t test were performed. ⁎p < 0.05; ⁎⁎p < 0.01; ⁎⁎⁎p < 0.001; ns = not statistically significant.

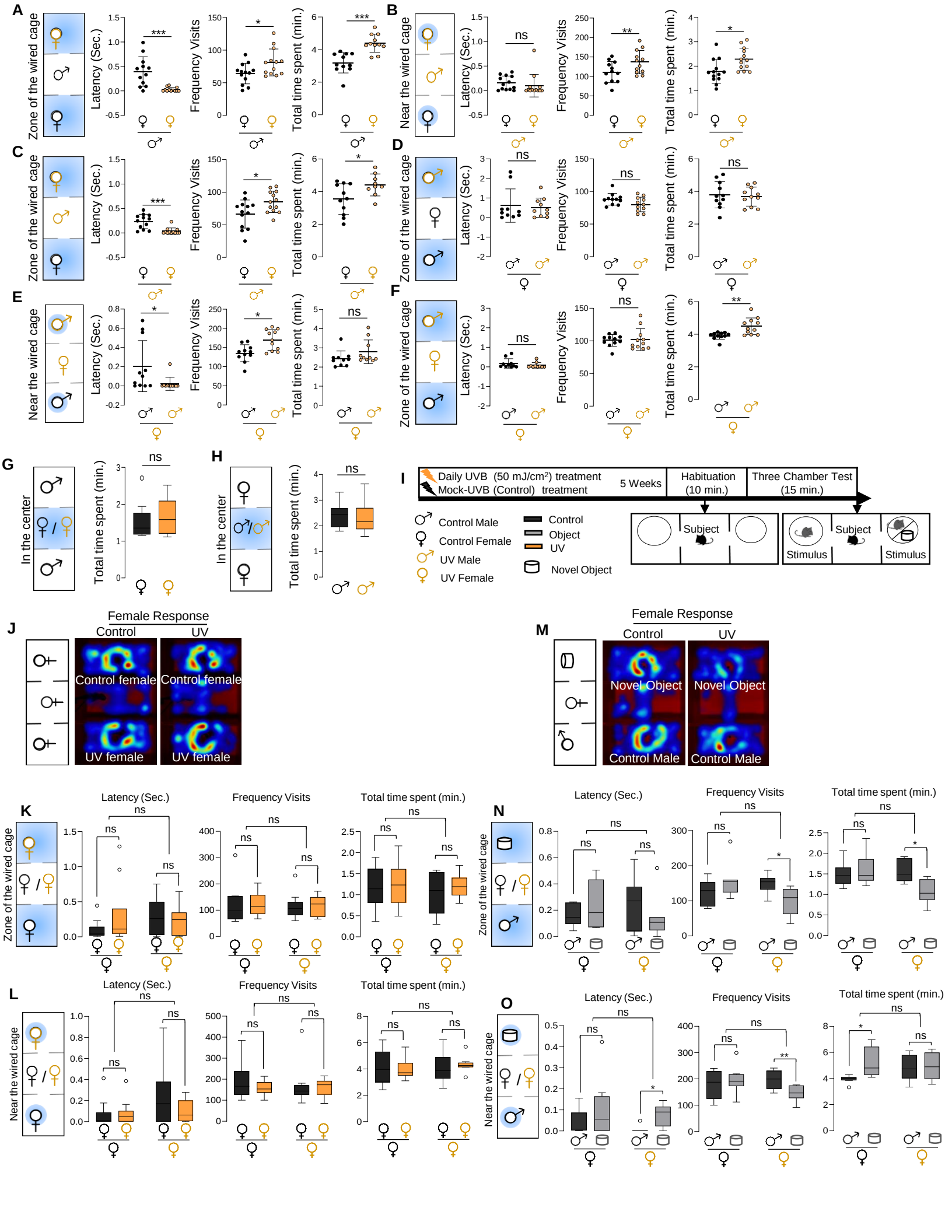
A
Zone of the wired cage
Latency (Sec.)
Frequency Visits
Total time spent (min.)
B
Near the wired cage
C
D
E
F
G
In the center
H
I
Daily UVB (50 mJ/cm²) treatment
Mock-UVB (Control) treatment
5 Weeks
Habituation (10 min.)
Three Chamber Test (15 min.)
Control Male
Control Female
UV Male
UV Female
Control
Object
UV
Novel Object
Subject
Stimulus
J
Female Response
Control
UV
Control female
UV female
M
Novel Object
Control Male
K
N
L
Near the wired cage
O
ns

Figure S2: UVB treatment enhances male and female sexual behavior and female attraction, related to Figure 2

(A) Measurement of the subject control males path in the zone of the wired cage (marked blue) of stimulus female. The following parameters were assessed: latency, which is the time taken to move toward the wired cage of a stimulus female (left panel), frequency of visits, which is the number of visits near the wired cage of the stimulus female (middle panel), and total time spent near the wired cage of the stimulus female (right panel). (B) As in panel A, but for UVB treated male subject movement near the wired cage (marked blue) of the control or UVB treated female stimuli. (C) As in panel A, but for UVB treated male subjects movements towards zone of the wired cage (marked blue) of control or UVB treated female stimuli. (D) As in panel A but for control female subjects movements towards zone of the wired cage (marked blue) of control or UVB treated male stimuli. (E) As in panel A, but for UVB treated female subject movements near the wired cage (marked blue) of control or UVB treated male stimuli. (F) As in panel A, but for control female subject movement towards zone of the wired cage (marked blue) of control or UVB treated male stimuli. (G) The total amount of time a male subject spent in the central compartment (marked blue) in the presence of caged UVB-treated and control stimulus females. (H) The total amount of time a female subject spent in the central compartment (marked blue) in the presence of caged UVB-treated and control stimulus males. (I) Schematic representation of the experimental design of the three-chamber test, in which the social behavior between the subject mouse toward the stimulus in a wired cage is videotaped and subsequently assessed. A C57BL6 subject and stimulus mice received a daily UVB or control treatment for five weeks; the subject mouse is habituated to the chamber for 10 min the day before the start of the 15-min experiment. (J) Representative heatmap images, generated using the EthoVision software, of the paths of UVB-treated and control female mice in the presence of UVB-treated and control stimuli females. (K,L) Measurement of the paths of UVB-treated and control female subjects K) in the zone of the wired cage (blue compartment) and L) near the wired cage (marked blue) containing female during the 15-min test session. Presented are the measurements of mean latency, frequency of visits, and the total time spent in the zone of the wired cage or near the wired cage of a stimulus female. (M) Representative heatmap images of paths of a UVB-treated and a control female subject in the presence of a control male stimulus and a novel object stimulus. (N,O) Measurements of the paths of UVB-treated and control female subjects P) in the zone of the wired cage (blue compartment) and O) near the wired cage (marked blue) containing a UVB-treated male and a novel object.

Data are means ± SEM, n=10 (K,L), n=7 (N), n≥7 (O). For data analysis, a two-tailed, unpaired Student's t test or two-way ANOVA were performed. $*p < 0.05$; $**p < 0.01$, $***p < 0.001$; ns = not statistically significant.

Table S1: Within-group differences in aggression, related to Table 1

	Males (14)					Females (18)				
	T1		T2		Z	T1		T2		Z
	Median	Range	Median	Range		Median	Range	Median	Range	
Physical aggression	12	8–33	10.5	8–30	0.00[b]	8.5	8–22	10	8–15	-0.63[a]
Verbal aggression	14	6–25	18	9–25	-1.73[c]*	12	6–24	13	6–25	-0.29[c]

*$p<0.05$; **$p<0.01$

[a] Based on positive ranks.

[b] The sum of negative ranks equals the sum of positive ranks.

[c] Based on negative ranks.

Total participants = 32

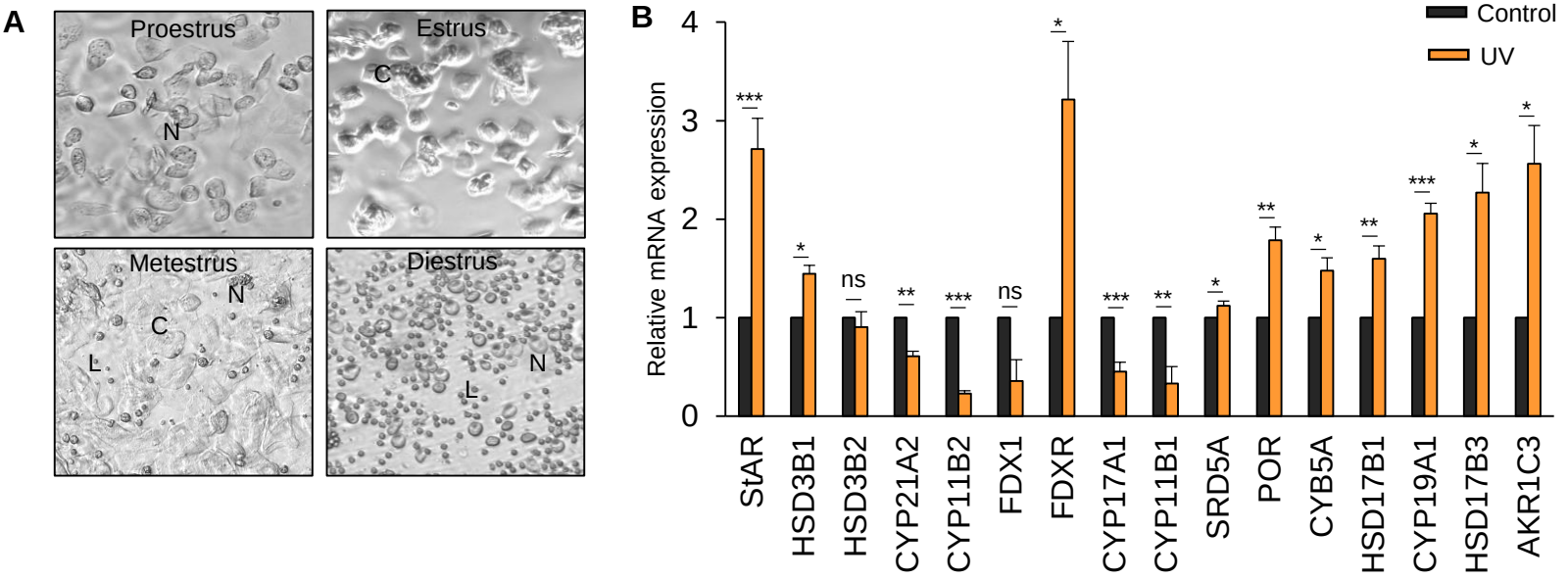

Figure S3. UVB treatment increases estrus incidence and the number of growing follicles, related to Figure 3

(A) Representative image of vaginal cytology at each stage of the estrous cycle: proestrus stage characterized by nucleated epithelial cells (N); estrus stage characterized by cornified cells (C); metestrus stage characterized by leucocytes (L), cornified cells (C), and nucleated cells (N); diestrus stage characterized by leucocytes (L) and nucleated cells (N). (B) Relative mRNA expression from an ovary section of a panel of genes involved in female steroidogenesis, after an 8-week UVB or control treatment.

Data are presented as means ± SEM. For data analysis, a two-tailed, unpaired Student's t test were performed. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; ns = not statistically significant.

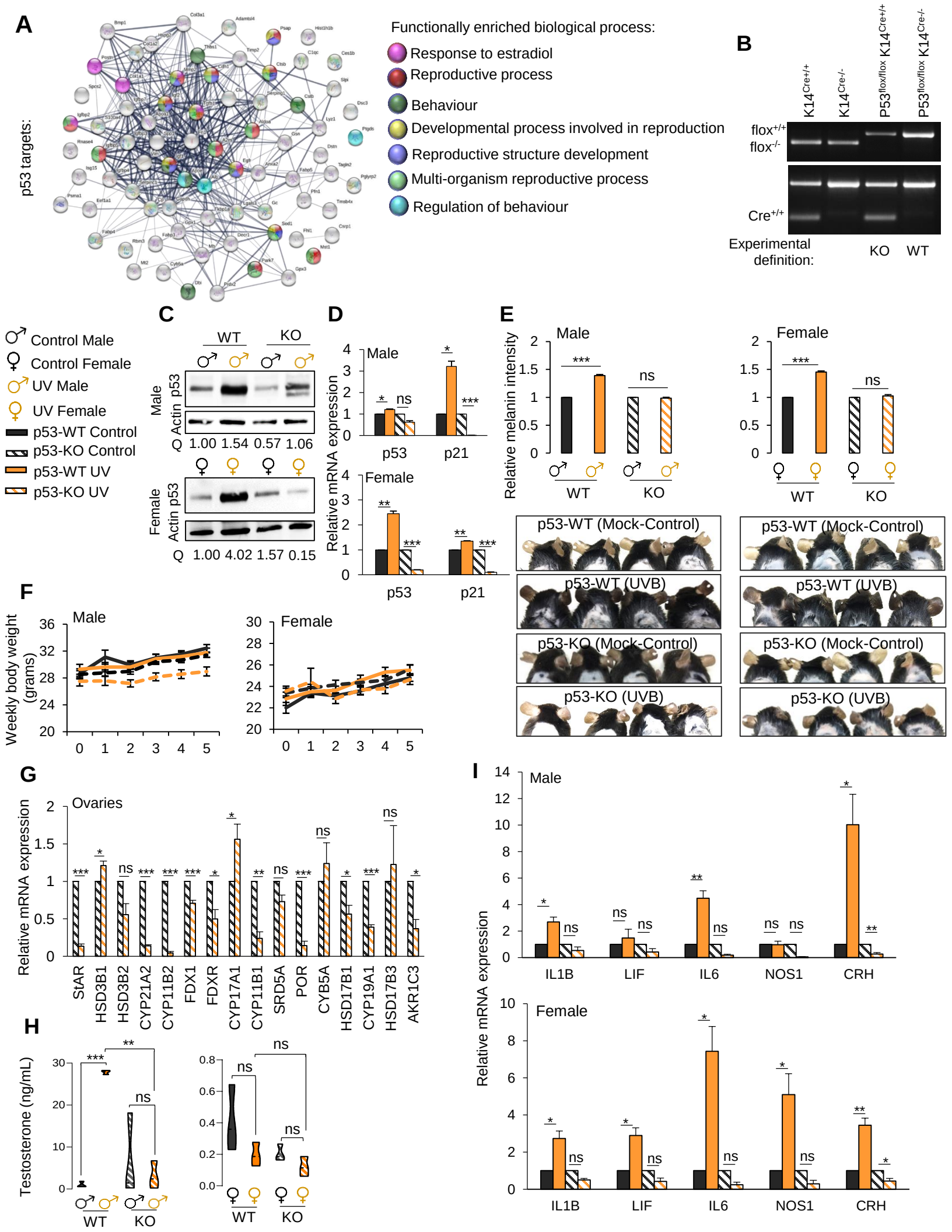
A
p53 targets:
Functionally enriched biological process:
Response to estradiol
Reproductive process
Behaviour
Developmental process involved in reproduction
Reproductive structure development
Multi-organism reproductive process
Regulation of behaviour
B
K14Cre+/+
K14Cre-/-
P53flox/flox K14Cre+/+
P53flox/flox K14Cre-/-
flox+/+
flox-/-
Cre+/+
Experimental definition:
KO
WT
C
Control Male
Control Female
UV Male
UV Female
p53-WT Control
p53-KO Control
p53-WT UV
p53-KO UV
WT
KO
Male
Female
p53
Actin
Q 1.00 1.54 0.57 1.06
Q 1.00 4.02 1.57 0.15
D
Male
Female
Relative mRNA expression
p53
p21
E
Male
Female
Relative melanin intensity
WT
KO
p53-WT (Mock-Control)
p53-WT (UVB)
p53-KO (Mock-Control)
p53-KO (UVB)
F
Male
Female
Weekly body weight (grams)
G
Ovaries
Relative mRNA expression
StAR
HSD3B1
HSD3B2
CYP21A2
CYP11B2
FDX1
FDXR
CYP17A1
CYP11B1
SRD5A
POR
CYB5A
HSD17B1
CYP19A1
HSD17B3
AKR1C3
H
Testosterone (ng/mL)
WT
KO
I
Male
Female
Relative mRNA expression
IL1B
LIF
IL6
NOS1
CRH

Figure S4. p53 modulates UVB treatment-mediated sexual behavior and ovarian changes in mice, related to Figure 4

(A) Significant enrichment of biological process in UVB-treated (50 mJ/cm2) female mice by STRING analysis of p53 targets predicted by upstream transcription analysis by IPA. (B) Genomic DNA analysis from the tail tissue using the Flox and Cre primers for K14cre+/+ ,K14cre-/-, p53flox/flox Cre+/+, and p53flox/flox Cre-/-. (C) Western blot analysis of p53 protein levels from the whole skin of p53-WT and p53-KO mice treated with UVB for 8 weeks. B-actin was used as a loading control. Relative quantification of p53 protein levels in each condition normalized to actin (Q) is indicated. (D) Relative mRNA expression of *p53* and *p21* genes in p53-WT and p53-KO males (top) and females (bottom). Data are means ± SEM ***p<0.001, n=3. (E) p53-KO and p53-WT mice were given daily UVB or control treatment for 5 weeks. Mean melanin intensity over time in the ears of the mice subjected to the indicated treatment (n=12; top) and representative images of the ears (bottom). (F) Weight, in grams, of the p53-WT and p53-KO male (left) and female (right) mice over the 5-week UVB or control treatment, n=12. (G) Relative mRNA expression of a panel of genes involved in female steroidogenesis, from the p53-KO ovary section after 8 weeks of UVB or control treatment. Data are means ± SEM. **p<0.01, n=4. (H) Testosterone levels in the plasma of p53-WT and p53-KO female (left) and male (right) mice after 8 weeks of UVB or control treatment as quantified by ELISA. Data are means ± SEM; *p<0.05, n=8. (I) Relative mRNA expression of *IL1B, LIF, IL6, NOS1*, and *CRH* genes in p53-WT and p53-KO male (top) and female (bottom) mice. Data are means ± SEM **p<0.01, n = 3.

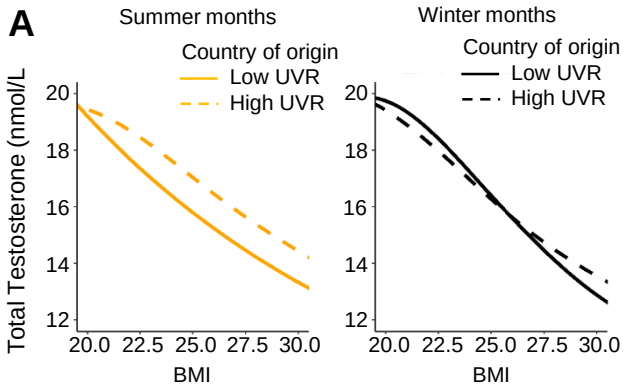

Figure S5. Solar exposure positively correlates with testosterone, related to Figure 5

(A) Conditional average of total testosterone levels of men aged 20-50 years based on their BMI and country of origin during May-September (left, n=1607, pv=0.004) and October-April (right, n=2309, pv=0.499).