Supplemental Information

Supplemental Methods & Materials

Animal Breeding. Orexin null mice were backcrossed onto c57BL6/J >10 generations. All breeding was conducted with het X het pairings with the null and wild-type littermates.

Surgery. Viral mediate expression of ΔFosB-eGFP or eGFP alone was accomplished as previously described (1-3). Briefly, mice were anesthetized with ketamine (10 mg/kg) and xylazine (1 mg/kg). Nucleus accumbens (NAc) was targeted for bilateral infusion using the following coordinates: +1.7 (anterior/posterior), +1.5 (lateral), -4.4 (dorsal/ventral) at an angle of 10º from midline (relative to Bregma). A total of 0.5 µl of virus was delivered on each side over a 4-min period followed by 6 min of rest. Following completion of the study, brains were collected and correct positioning of the injection site verified (Figure S2). Animals with incorrect targeting (<10% of total) were excluded from analysis.

Immunohistochemistry. Mice were anesthetized and perfused intracardially with 4% paraformaldehyde/PBS. Brains were cryoprotected with 30% sucrose and coronal sections (30 µm) were cut on a freezing microtome and processed for immunohistochemistry. Free-floating frontal sections were pre-incubated in a blocking buffer containing 0.3% Triton and 3% normal goat serum. ΔFosB was detected using rabbit polyclonal antibodies raised against the N-terminal portion of the protein (1/1000 Santa Cruz Biotechnology, Santa Cruz, CA) in the same buffer then processed with biotinylated goat anti-rabbit IgG antibodies and avidin–biotin peroxidase complex method with diaminobenzidine as a substrate (Vector Laboratories, Burlingame, CA). Slices were mounted, dehydrated and coverslipped. ΔFosB immunopositive cells showed a specific brown staining in the nucleus and were quantified by an observer blind to the treatment conditions using a microscope (20x magnification). Three selected brain sections spanning the NAc (AP: +1.3 to +1.0 from Bregma) were chosen per mice

for quantification. Anatomical segregation of the NAc core and shell was carried out by comparing the section with the Paxinos mouse brain atlas (4). Conditions for immunohistochemistry were optimized to reduce background levels to the minimum allowing the correct identification of ΔFosB positive cells. Mean values were calculated for each animal and considered as an individual observation for statistical analysis.

Operant Responding. Mice were trained to poke their nose into a lit portal to obtain a 20 mg high fat diet (HFD) pellet reward (see below) in standard operant conditioning chambers (Model ENV307A, Med Associates Inc., St Albans, VT) equipped with three nose poke portals. Mice were rewarded for nose poking in the middle portal only; the side portals were monitored but inactive. The HFD pellets were custom prepared by Bio-Serv (product # F06245, Frenchtown, NJ), and provided 2.88 kcal/g of energy (22.7% fat). The main components of these pellets were casein (233 g/Kg), palm oil (207 g/Kg), dextrates (197 g/Kg), sucrose (197 g/Kg), cellulose (58 g/Kg), and soybean oil (20 g/Kg). During the training period, mice were allowed access to regular chow 4 hours per day (1200-1600). For the training sessions, mice initially received the HFD pellet rewards under a fixed ratio (FR) schedule (from a FR 1 schedule up to a FR 5 schedule). Then, mice were moved to a stepped progressive ratio reinforcement schedule for 3 days, whereby the response requirement for each successive pellet was raised by progressive increments according to the following series: 5, 10, 20, 30, 50, 70, 100, 130, etc. Breakpoint was defined as the last progressive ratio that an animal successfully completes to receive a reinforcement within a 30 min period. Breakpoint was then converted to total number of pellets earned in that session (a linear scale) for statistical analysis and presentation.

Calorie Restriction. One group of mice were initially trained in operant responding task described above and body weights were recorded every second day. At the end of the training phase (when all groups had achieved three sessions of 30 rewards within one hour on the FR 5 schedule), the mice were randomized into two groups and individually housed. Two mice were excluded from the study after both failed to achieve 30 rewards within one hour on the FR 1 schedule. During the next ten days, the remaining mice

received either free access to food (ad libitum) or a food pellet equivalent to 60% of average daily food intake just prior to lights off at 1900 (calorie restricted). After the calorie restriction period, both groups of mice received free access to chow. Once the calorie restricted group returned to a baseline body weight (two days), the two groups were then allowed to recover followed by testing in the operant responding task. Separate groups of mice went through the calorie restriction paradigm without receiving training in operant responding followed by testing in either metabolic chambers or immunohistochemistry for ΔFosB levels.

Statistical Analysis. Student's t-test was used to analyze cell count and metabolic chamber data. For operant responding results, two-way ANOVA followed by Bonferroni *post hoc* tests was performed, using AAV vector and orexin-genotype as factors. Statistical effects of one or both factors are denoted in figure legend with significance defined as $p < 0.05$.

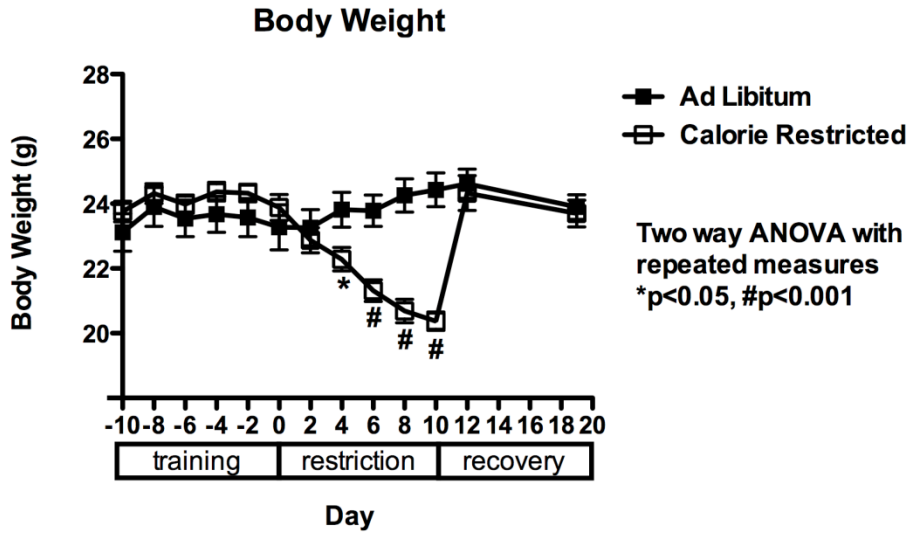

Figure S1. Body weight during experimentation (n = 7/group). Data are presented as mean ± SEM.

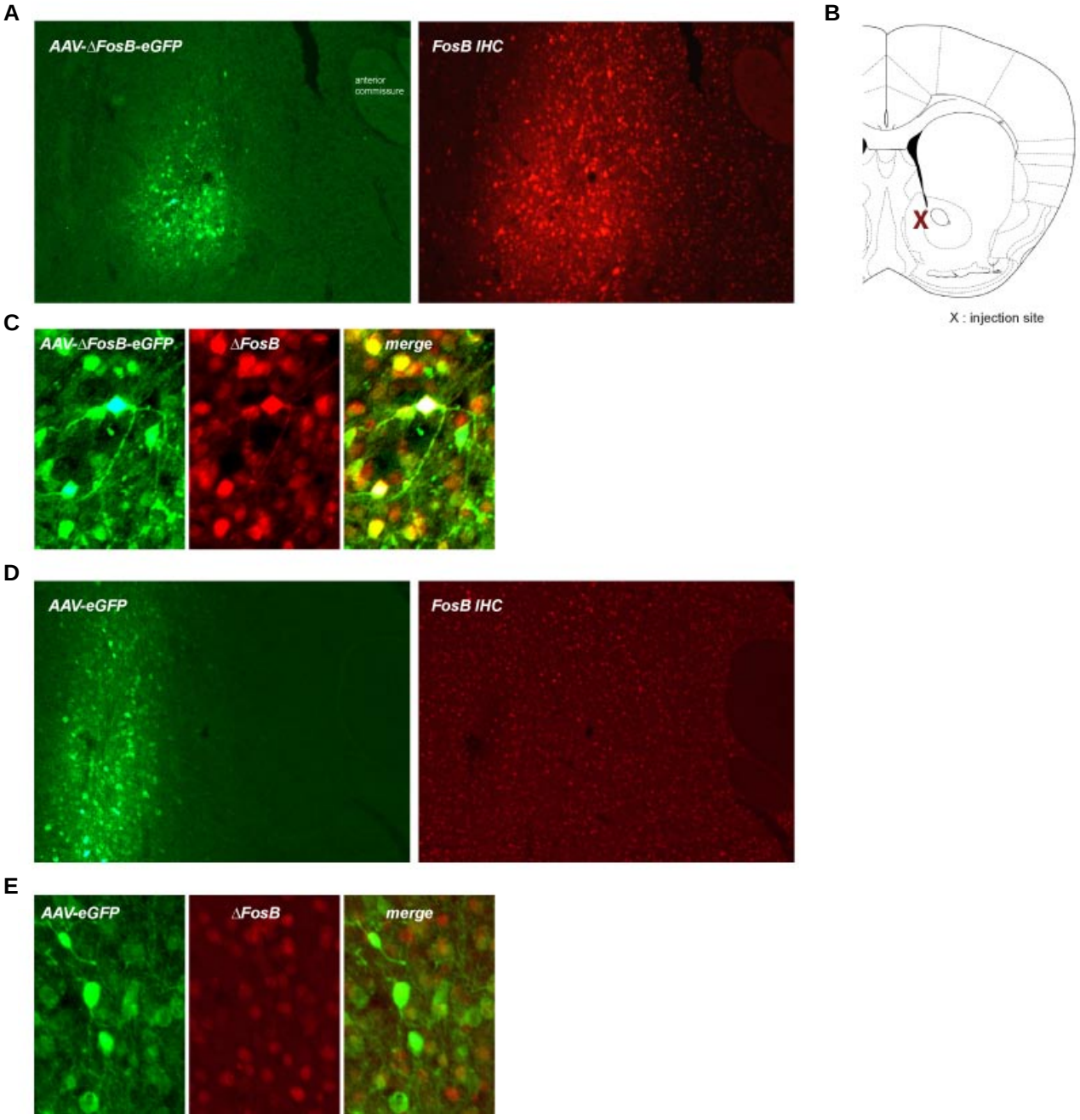

Figure S2. (A) Representative image of AAV-ΔFosB-GFP injection in nucleus accumbens (NAc) shell. **(B)** Schematic representation of injection site. **(C)** Overlay of GFP and ΔFosB expression determined by immunohistochemistry (IHC). (**D**) Representative image of AAV-GFP injection in NAc shell. **(E)** Overlay of GFP and ΔFosB expression determined by IHC.

Supplemental References

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4. Franklin KBJ, Paxinos G (2007): *The Mouse Brain in Stereotaxic Coordinates, 3rd ed.* San Diego, CA: Academic Press.