Title: Patch and matrix striatonigral neurons differentially regulate locomotion

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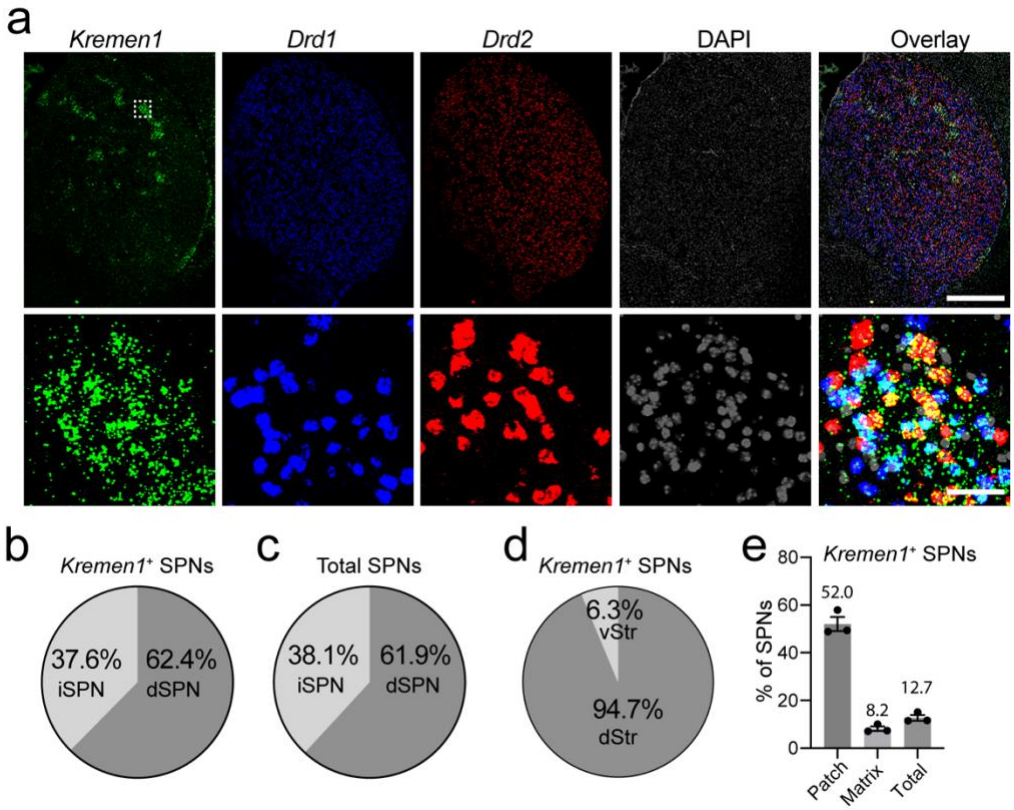
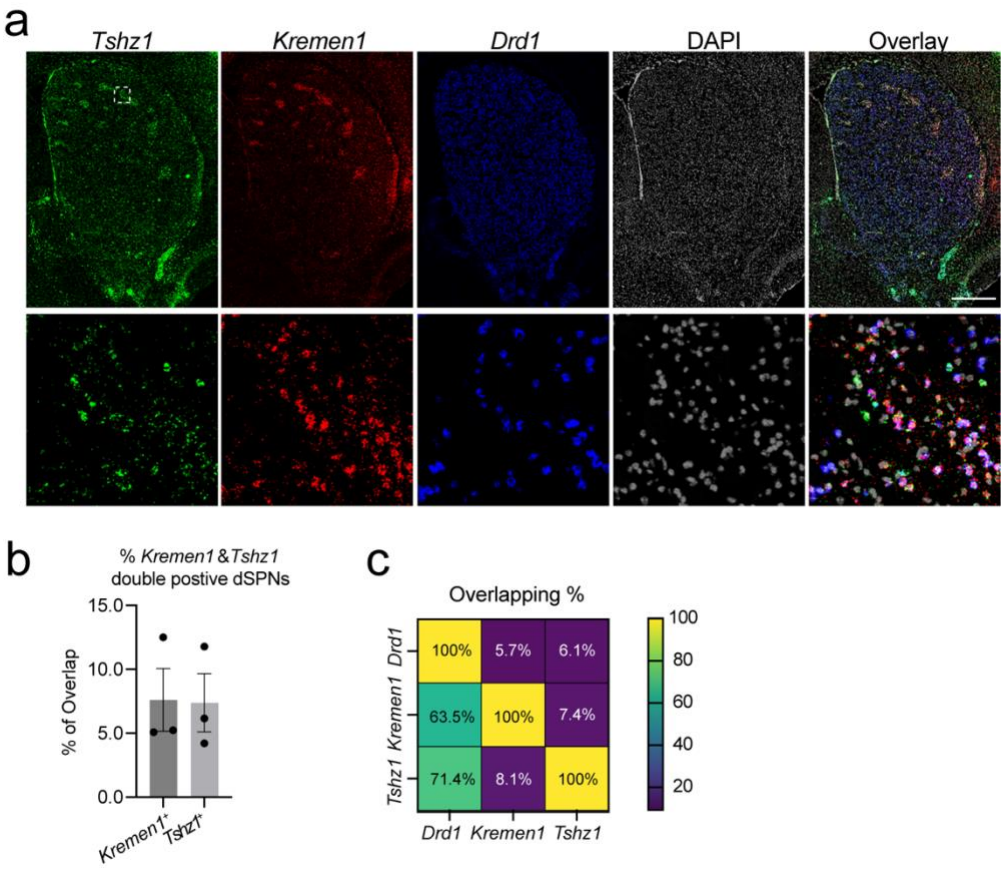
Extended Data Figures and Figure Legends

Extended Data Figure 1. Identification of *Kremen1* as a patch marker



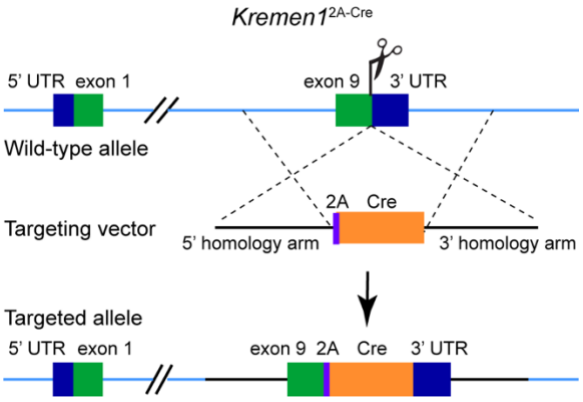
(**a**) Confocal images of RNAscope for *Kremen1*, *Drd1*, *Drd2* and DAPI in the striatum. Bottom, high-magnification images of the boxed area in the top panel. (**b**) The percentage of *Drd1* and *Drd2* SPNs in *Kremen1*$^+$ SPNs in the striatum (n = 3 mice). (**c**) The percentage of *Drd1* and *Drd2* SPNs in total SPNs in the striatum (n = 3 mice). (**e**) The percentage of *Kremen1*$^+$ SPNs distributed in the dorsal (dStr) and ventral (vStr) striatum (n = 3 mice). (**e**) The percentage of *Kremen1*$^+$ SPNs distributed in the patch and matrix compartments, as well as the entire dorsal striatum (n = 3 mice). All error bars were represented as mean ± SEM.

Extended Data Figure 2. Different expression pattern of *Tshz1* and *Kremen1* in the dorsal striatum



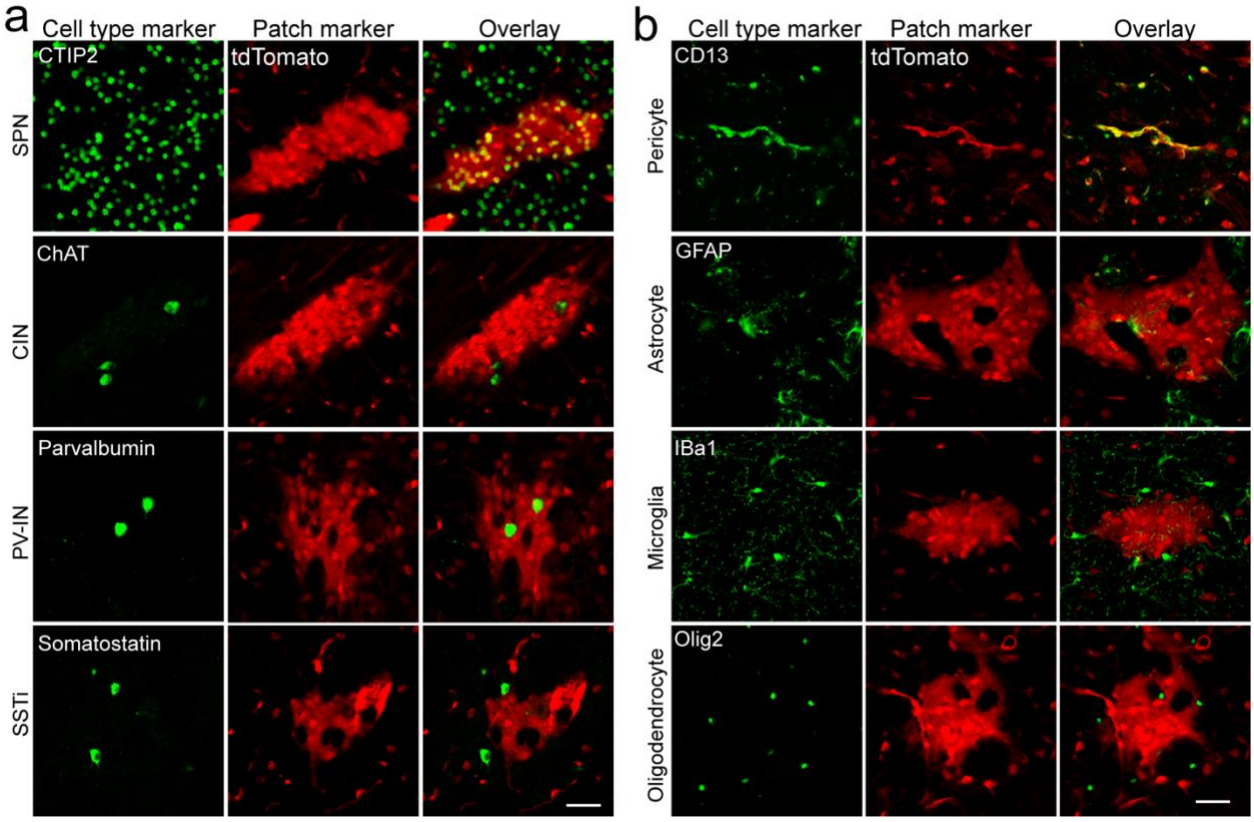
(**a**) Sample images of RNAscope for *Tshz1*, *Kremen1*, *Drd1* and DAPI in the striatum. Scale bar: 500 μm. Bottom, high-magnification images of the boxed area in the top panel. (**b**) Bar graph shows the percentages of double-positive dSPNs for *Kremen1* and *Tshz1* within individual *Kremen1*$^{+}$ and *Tschz1*$^{+}$ dSPNs. (**c**) Heatmap of the percentages of *Drd1*$^{+}$ nuclei positive for *Kremen1* and *Tshz1* (1st row), the percentages of *Kremen1*$^{+}$ nuclei positive for *Drd1* and *Tshz1* (2nd row), and the percentages of *Tshz1*$^{+}$ nuclei that were positive for *Drd1* and *Kremen1* (3rd row) (n = 3 mice). All error bars were represented as mean ± SEM.

Extended Data Figure 3. Generation of *Kremen1*$^{2A\text{-}Cre}$ knock-in mice



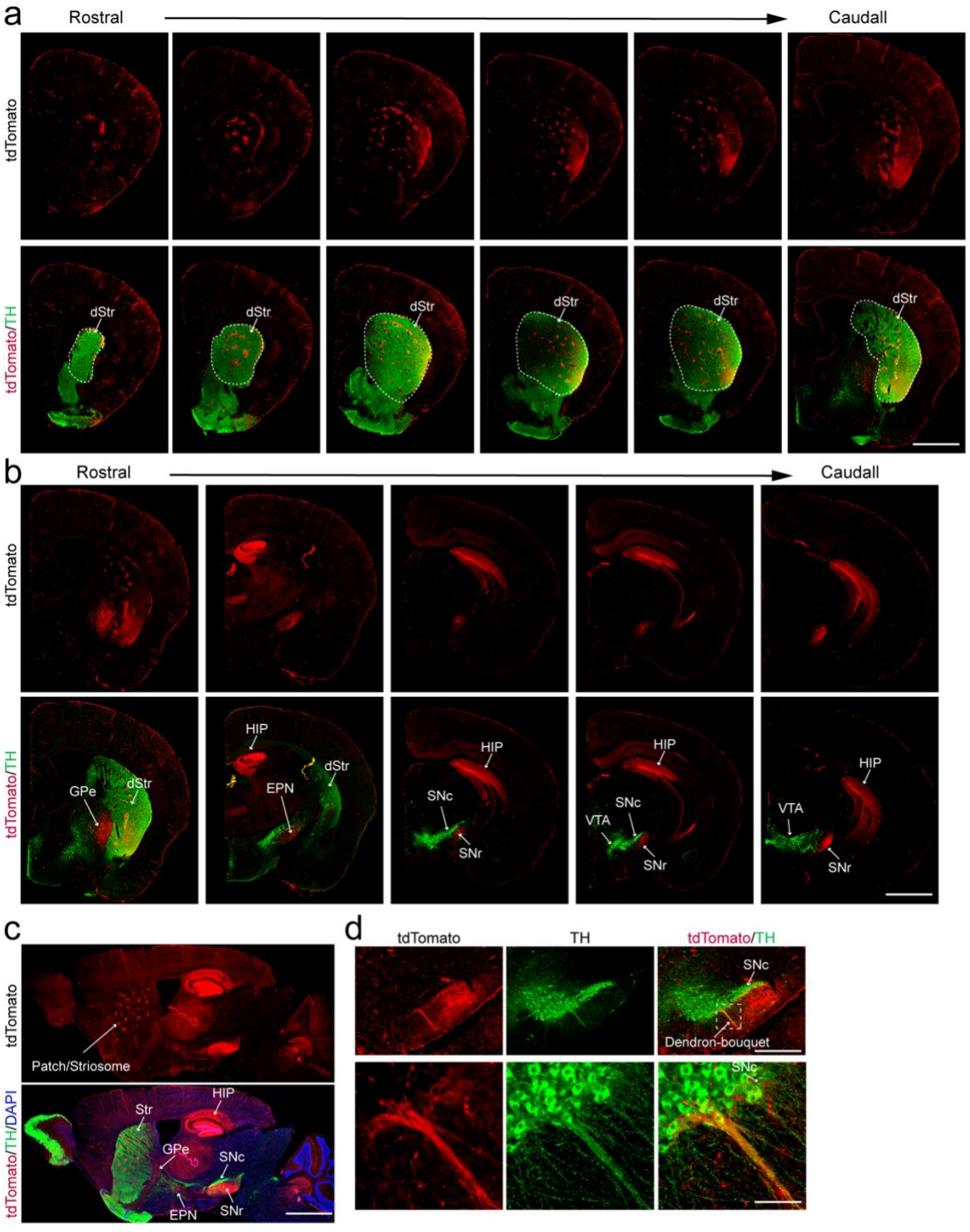
Schematics illustrates the insertion of 2A-Cre coding sequence in front of the stop codon in exon 9 of *Kremen1* gene. A gene targeting vector donor plasmid was constructed with 3.2kb 5' homologous arm and 2.9kb 3' homologous arm. The gRNA (GTGGGCTTCAGTCACTCACG AGG) was employed to guide the sequence specific insertion of 2A-Cre DNA fragment by CRISPR/Cas9-mediated gene targeting.

Extended Data Figure 4. Characterization of *Kremen1*$^{2A\text{-}Cre}$::Ai14 double KI mice



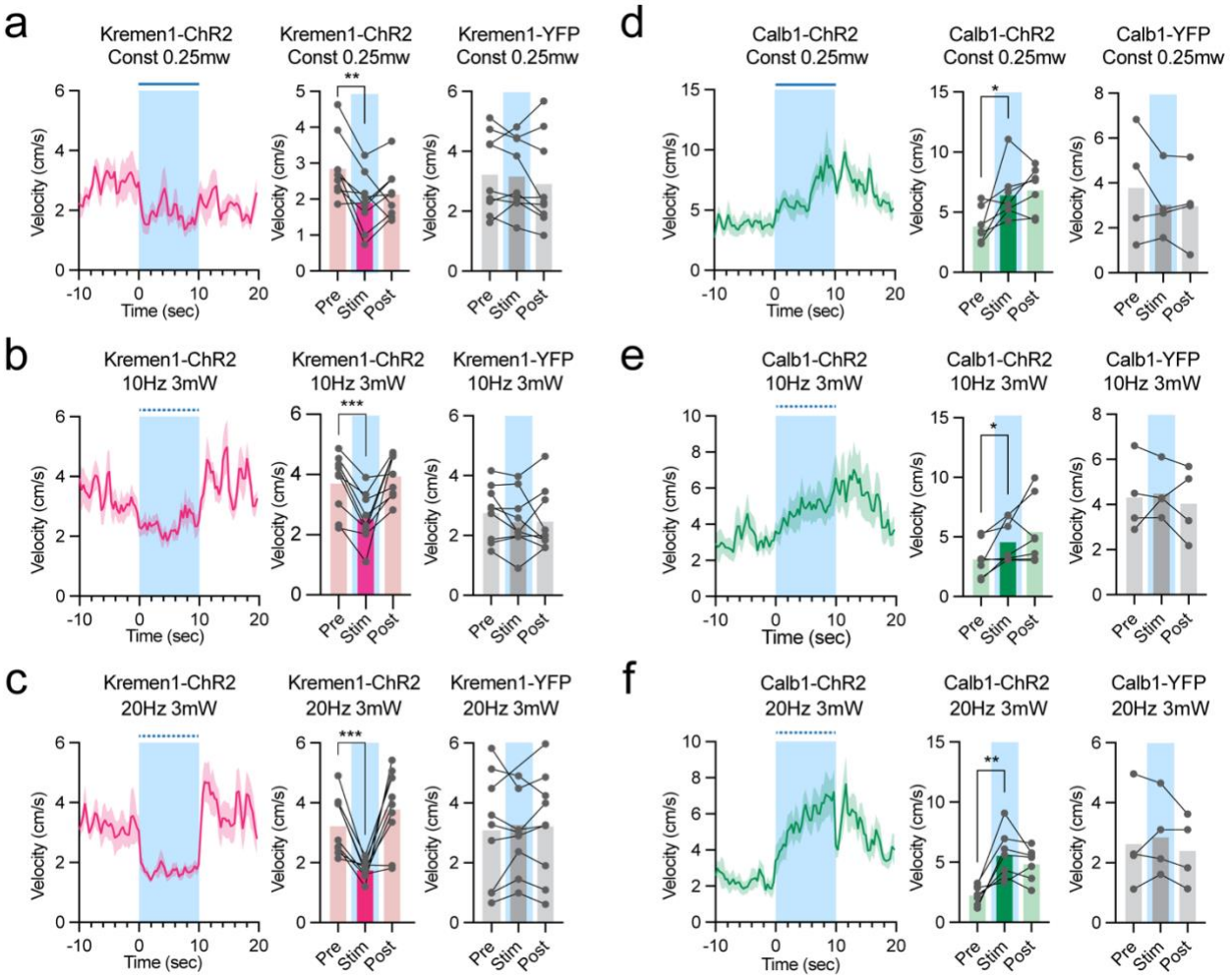
(**a**) Sample images of coronal sections from *Kremen1*$^{2A\text{-}Cre}$::Ai14 mice (n>3), co-stained with tdTomato and SPN marker CTIP2, cholinergic interneuron (CIN) marker choline acetyltransferase (ChAT), parvalbumin interneuron marker parvalbumin, and somatostatin interneuron (SSTi) marker somatostatin. Scale bar: 50 µm. (**b**) Images of coronal sections from *Kremen1*$^{2A\text{-}Cre}$::Ai14 mice (n>3), co-stained with tdTomato and pericyte marker CD13, astrocyte marker GFAP, microglia marker IBA1, and oligodendrocyte marker Olig2. Scale bar: 50 µm.

Extended Data Figure 5. Expression pattern of *Kremen1*$^{2A\text{-}Cre}$::Ai14 mice in the whole brain



(**a**) A series of coronal sections from a representative *Kremen1*$^{2A\text{-}Cre}$::Ai14 mouse showing the *Kremen1* gene locus-directed tdTomato expression across the dorsal striatum. Scale bar: 1mm. (**b**) A series of coronal sections from a representative mouse showing the *Kremen1* gene locus-directed tdTomato expression in the projection nucleuses, including GPe, ENP and SN. The tdTomato signals are also observed in the hippocampal region. Scale bar: 1mm. (**c**) Sample images of a sagittal brain section from a representative *Kremen1*$^{2A\text{-}Cre}$::Ai14 mouse. The tdTomato signals are prominent in the patch compartments of Str, GPe, EPN, SN and hippocampus (HIP). Scale bar: 1mm. (**d**) Representative images of the SN area, showing the connection of tdTomato-positive SPN axon terminals with the dendrites of dopaminergic neurons in the SNr, forming the so-called dendron-bouquet structure. Bottom panel shows the magnification of boxed area from the top. Scale bar: 500 μm.

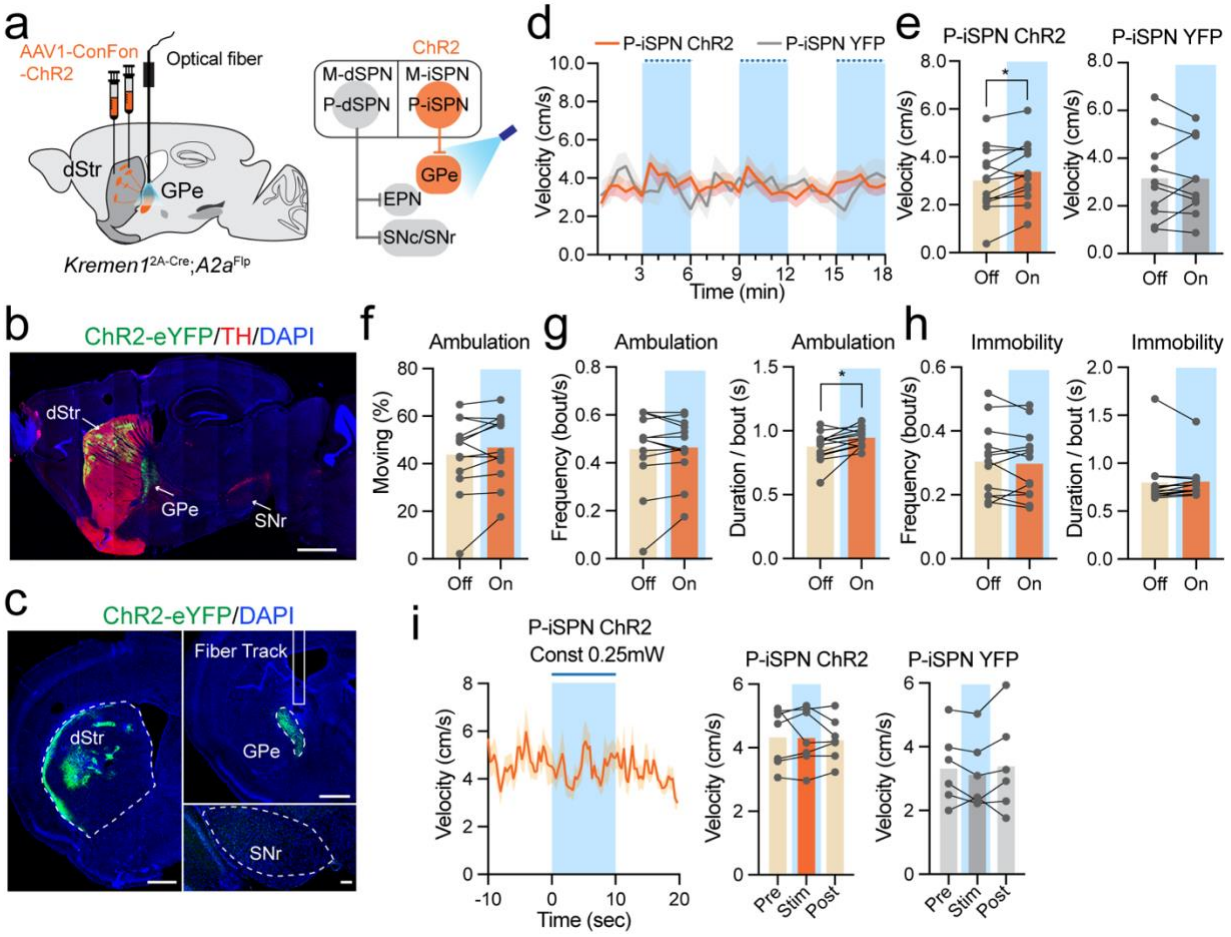
Extended Data Figure 6 Optogenetics activation patch and matrix dSPNs at lower intensity or different frequency over short period



(**a-c**) Instantaneous velocity aligned to 0.25mW/constant (const, **a**), 3mW/10Hz (**b**), and 3mW/20Hz (**c**) light stimulations for 10s of patch dSPNs (Kremen1-ChR2), respectively. For the 0.25mW/Const stimulation, the average velocity prior to stimulation (Pre) is 2.87 ± 0.29 cm/s and during stimulation (Stim) is 1.92 ± 0.26 cm/s, n=9, paired t test, two tailed, and $^{**}p = 0.0029$. For the 3mW/10Hz stimulation, the Pre is 3.72 ± 0.32 cm/s and Stim: 2.54 ± 0.28 cm/s, n=9, paired t test, two tailed, $^{***}p = 0.0003$. For the 3mW/20Hz stimulation, Pre is 3.24 ± 0.33 cm/s and Stim is 1.77 ± 0.10 cm/s, n=9, paired t test, two tailed, $^{***}p = 0.001$. Optogenetics activation of Kremen1-YFP did not significantly change velocity (Cons 0.25mW, Pre: 3.25 ± 0.44 cm/s vs. Stim: 3.18 ± 0.40 cm/s; paired t test, p = 0.70, n = 9;10Hz 3mW, Pre: 2.77 ± 0.31 cm/s vs. Stim: 2.48 ± 0.32 cm/s; paired t test, p = 0.10, n = 9; 20Hz 3mW, Pre: 3.08 ± 0.63 cm/s vs. Stim: 3.30 ±

0.57 cm/s; paired t test, p = 0.53, n = 9). (**d-f**) Instantaneous velocity aligned to light stimulation of matrix dSPNs (Calb1-ChR2) with the same parameters as **a-c**. All three setting of optogenetics activation of matrix dSPNs increased velocity in the open-field test (0.25mW/const, Pre: 3.90 ± 0.55 cm/s vs. Stim: 6.48 ± 0.85 cm/s, paired t test, two tailed, $^{*}p = 0.0439$, n = 7; 3mW/10Hz, Pre: 3.18 ± 0.59 cm/s vs. Stim: 4.63 ± 0.68 cm/s, paired t test, two tailed, $^{*}p = 0.0393$, n = 7; and, 3mW/20Hz, Pre: 2.31 ± 0.30 cm/s vs. Stim: 5.61 ± 0.76 cm/s, paired t test, two tailed, $^{**}p = 0.0045$, n = 7). Optogenetics activation of Calb1-YFP did not significantly change velocity (0.25mW/const, Pre: 3.07 ± 0.77 cm/s vs. Stim: 3.0 ± 0.89 cm/s, paired t test, two tailed, p = 0.81, n = 4; 3mW/10Hz, Pre: 4.35 ± 0.82 cm/s vs. Stim: 4.52 ± 0.57 cm/s, paired t test, two tailed, p = 0.70, n = 4; 3mW/20Hz, Pre: 2.66 ± 0.82 cm/s vs. Stim: 2.88 ± 0.67 cm/s, paired t test, two tailed, p = 0.49, n = 4). All error bars were represented as mean ± SEM.

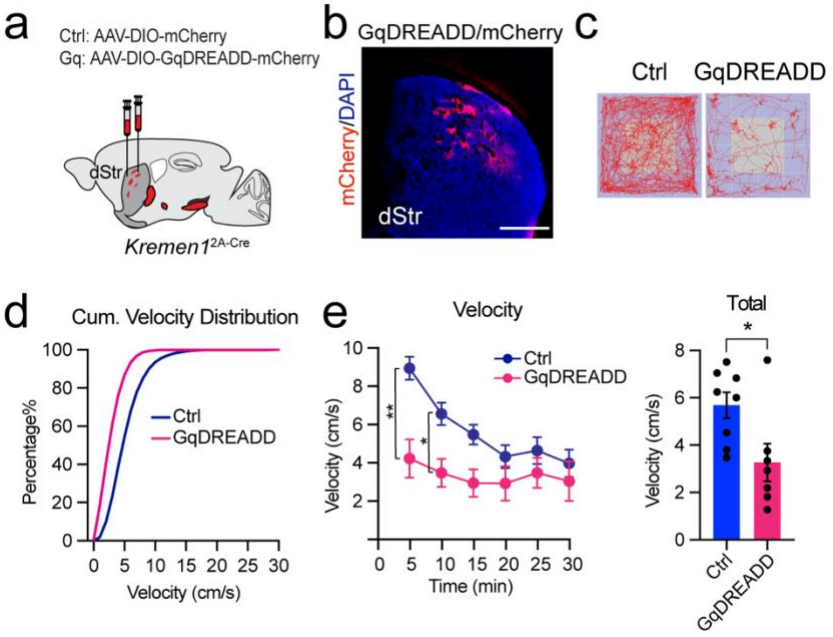
Extended Data Figure 7 Optogenetics activation of patch iSPNs causes a modest increase of locomotion



(**a**) Schematics of ChR2 injection and optic fiber implantation for activating patch iSPNs axons in the GPe using *Kremen1*$^{2A\text{-}Cre}$::*A2a*^{Flp} mice. (**b, c**) Representative images of sagittal section (**b**) and coronal sections (**c**) show ChR2 (green) expression in the patch iSPNs. Note that iSPNs only project to GPe. Scale bar: 1mm (sagittal section), 500 µm (dStr and GPe) and 100 µm (SN). (**d**) Instantaneous locomotion velocity in open-field test with light-off and light-on (blue shaded area) in *Kremen1*$^{2A\text{-}Cre}$::*A2a*^{Flp} mice. ChR2 =13 mice, YFP = 10 mice. Mean (dark line) ± SEM (shaded area). (**e**) Average velocity comparison between total light-off (Off) and light-on (On) periods. For the ChR2 group, Off: 3.02 ± 0.37 cm/s vs. On: 3.37 ± 0.34 cm/s, paired t test, two tailed, $^{*}p = 0.015$, n = 13. For the YFP controls, Off: 3.15 ± 0.58 cm/s vs. On: 3.14 ± 0.50 cm/s, paired t test, two tailed, p = 0.95, n = 10. Data from individual mice and population averages are shown. (**f**) The percentage of total ambulation time, Off: 43.83 ± 4.57 % vs. On: 46.86 ± 3.92 %,

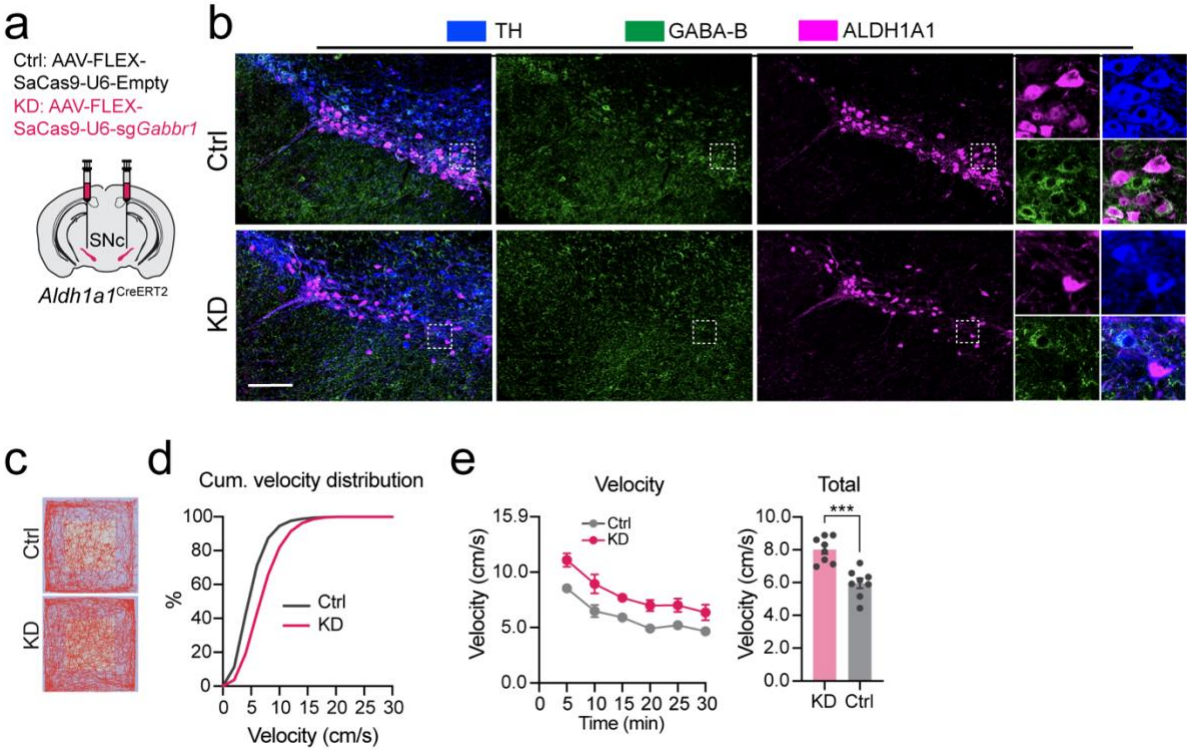
paired t test, two tailed, p = 0.09. (**g**) Frequency of ambulation bout, Off: 0.46 ± 0.05 bout/s vs On: 0.46 ± 0.03 bout/s, paired t test, two tailed, p = 0.68; and duration of ambulation bout, Off: 0.88 ± 0.03s vs On: 0.95 ± 0.02s, paired t test, two tailed, *p = 0.016. (**h**) Frequency of immobility bout, Off: 0.30 ± 0.03 bout/s vs On: 0.30 ± 0.03 bout/s, paired t test, two tailed, p = 0.53; and duration Off: 0.80 ± 0.08s vs On: 0.81 ± 0.05s, paired t test, two tailed, p = 0.61. All error bars were represented as mean ± SEM. (**i**) Instantaneous velocity aligned to 0.25mW/constant light stimulations for 10s of patch iSPNs (left) and the average velocity prior to stimulation (Pre), during stimulation (Stim) and after stimulation (Post) in ChR2 group (middle) and YFP group (right) (ChR2: Pre 4.35 ± 0.34 cm/s, Stim 4.33 ± 0.34 cm/s, n=7, paired t test, two tailed, p = 0.93; YFP: Pre 3.35 ± 0.47 cm/s, Stim 3.15 ± 0.45 cm/s, n=6, paired t test, two tailed, p = 0.21).

Extended Data Figure 8. Chemogenetic activation of patch SPNs suppress locomotion



(**a**) Schematics of Ctrl or GqDREADD injection in the dorsal striatum of *Kremen1*$^{2A\text{-}Cre}$ mice. (**b**) Representative image of GqDREADD/mCherry expression in the patches in the dorsal striatum. Scale bar: 500 µm. (**c**) Tracks of representative Ctrl or GqDREADD mice in the open field test. (**d**) Cumulative (Cum.) frequency of velocity distribution in Ctrl and GqDREADD mice, Ctrl = 8 mice, Gq = 7 mice. (**e**) Velocity change in every 5min during 30min test period (left), and average velocity during the entire 30min (right). Left panel, two-way ANOVA, $^{**}p = 0.0036$, $^{*}p = 0.035$. Right panel, Ctrl: 5.68 ± 0.55 cm/s vs GqDREADD: 3.27 ± 0.79 cm/s, unpaired t test, two tailed, $^{*}p = 0.0238$, Ctrl = 8 mice, GqDREADD = 7 mice. Data were represented as mean ± SEM.

Extended Data Figure 9. Knockdown GABA-B receptor in ALDH1A1^{+} DANs leaded to hyperactivity in locomotion



(**a**) Schematics of *Gabbr1*-KD or control (Ctrl) AAV injection in the SNc of *Aldh1a1*^{CreERT2} mice. (**b**) Representative images of GABA-B receptor (green), ALDH1A1 (magenta) and TH (blue) staining. Scale bar: 100 μm. The boxed areas are highlighted in right panels. (**c**) Tracks of representative Ctrl and KD mice in open-field test. (**d**) Cumulative (Cum.) frequency of velocity distribution in Ctrl and KD mice, n = 8 per group. (**e**) Velocity change in every 5min during 30min test period, and average velocity during the entire 30min, Ctrl: 5.96 ± 0.30 cm/s vs KD: 8.01 ± 0.28 cm/s, unpaired t test, two tailed, ***p = 0.0002, n=8 per group. Data were represented as mean ± SEM.