

Supplementary Figure 1

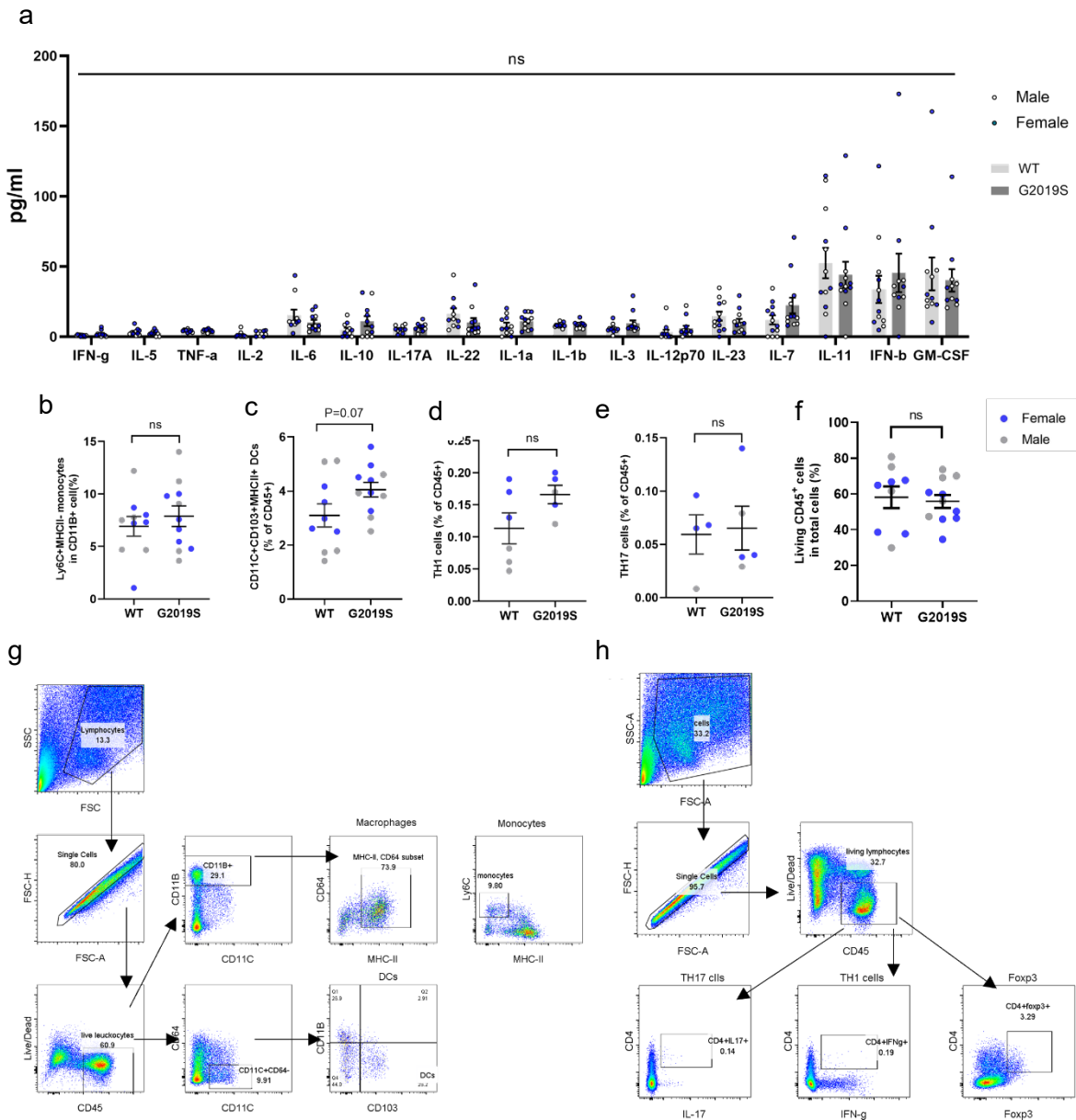


Fig. S1: Serum cytokines and colonic lamina propria immune cell subsets in response to DSS-induced intestinal inflammation in hLRRK2^{G2019S} Tg mice. (a) Serum cytokines in hLRRK2^{G2019S} Tg mice (G2019S) relative to wildtype (WT) littermate controls on day 7 of DSS treatment (two-tailed t-test, n=12). (b) Monocytes, (c) dendritic cells, (d) TH1 cells and (e) TH17 (f) CD45⁺ cells from the colonic lamina propria of G2019S vs. WT mice on day 7 of DSS treatment (two-tailed t-test, (b)-(c), WT: n=8, G2019S: n=11; (d)-(e), WT: n=6, G2019S: n=5). Data are representative of two independent experiments. Data are represented as mean $\pm$ SEM; ns= not statistically significant.

Supplementary Figure 2

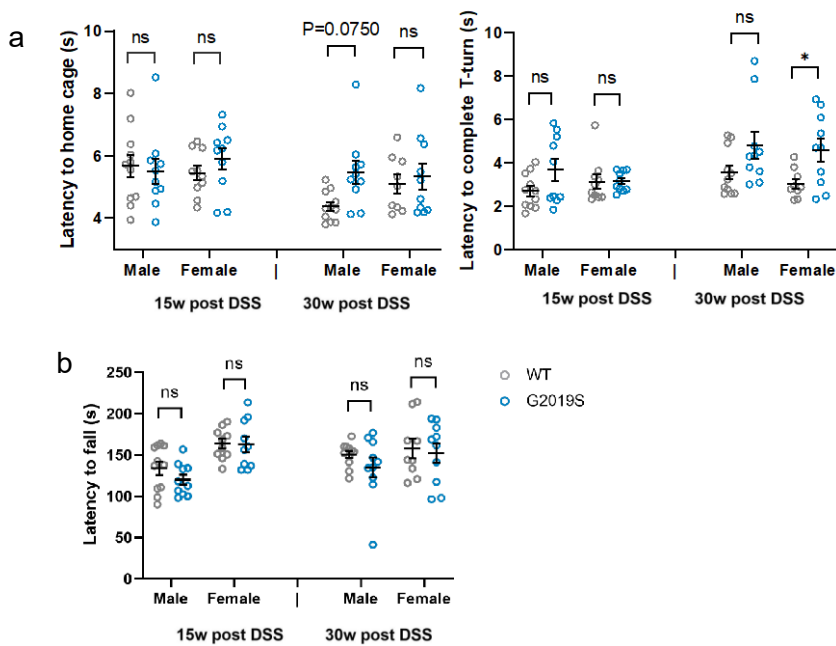
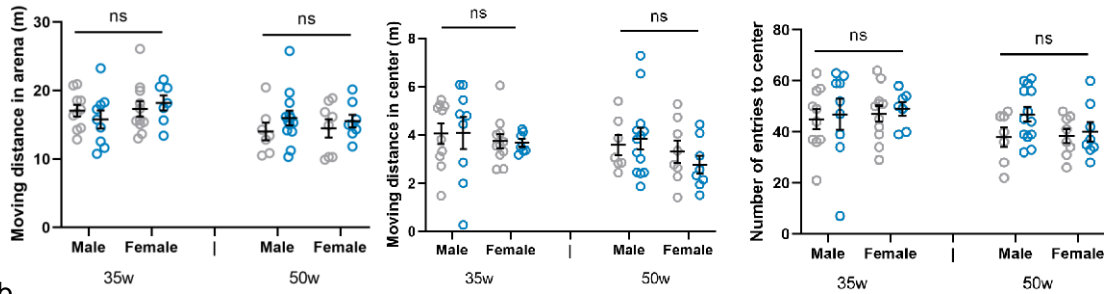


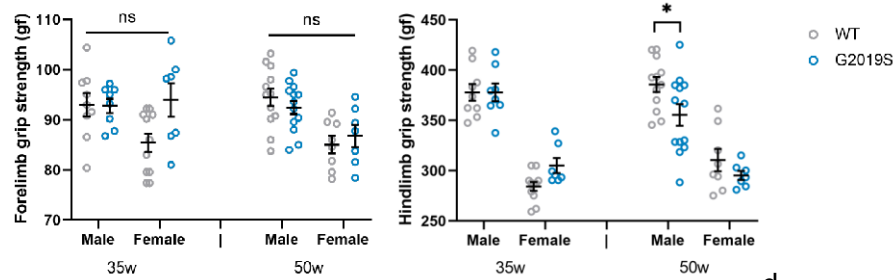
Fig. S2: Behavioral performance in additional motor tasks for hLRRK2^{G2019S} Tg mice after prodromal DSS-induced intestinal inflammation. (a) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right). (b) Rotarod test for hLRRK2^{G2019S} Tg mice (G2019S) compared to wildtype (WT) littermate controls at 15 and 30 weeks post DSS treatment (Two-way ANOVA with Sidak, n=10). Data are representative of three independent experiments. Data are presented as mean ± SEM, * $P < 0.05$.

Supplementary Figure 3

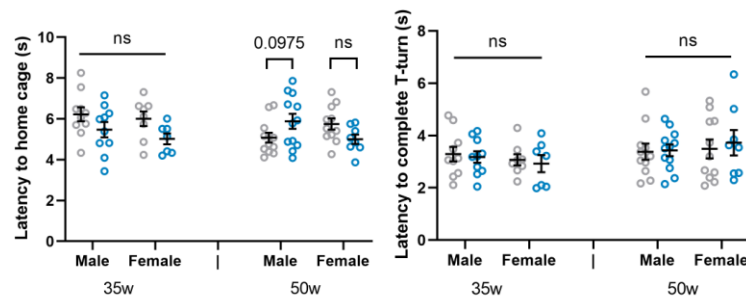
a



b



c



d

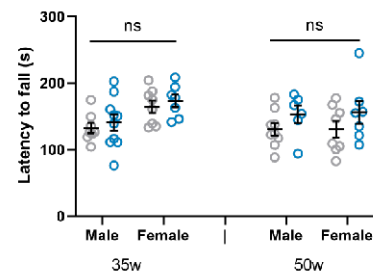


Fig. S3: Vehicle-treated (water; untreated) hLRRK2^{G2019S} Tg mice and WT littermate controls in behavioral tests for motor ability. (a) Open field test: moving distance in the arena (left), center (middle), and number of entries to the center (right) after 10 min of exploration. WT_male and female (35w): n=10, G2019S male (35w and 50w): n=10, G2019S female (35w and 50w): n=7, WT male (50w): n=7, WT female (50w): n=8. (b) Grip strength for forelimbs (left) and hindlimbs (right). N=7-13 (c) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right). (d) Rotarod test for hLRRK2^{G2019S} Tg mice and WT littermate controls at 35 and 50 weeks of age (two-way ANOVA with Sidak; n=7-11). Data are representative of three independent experiments. Data are presented as mean $\pm$ SEM, * P <0.05.

Supplementary Figure 4

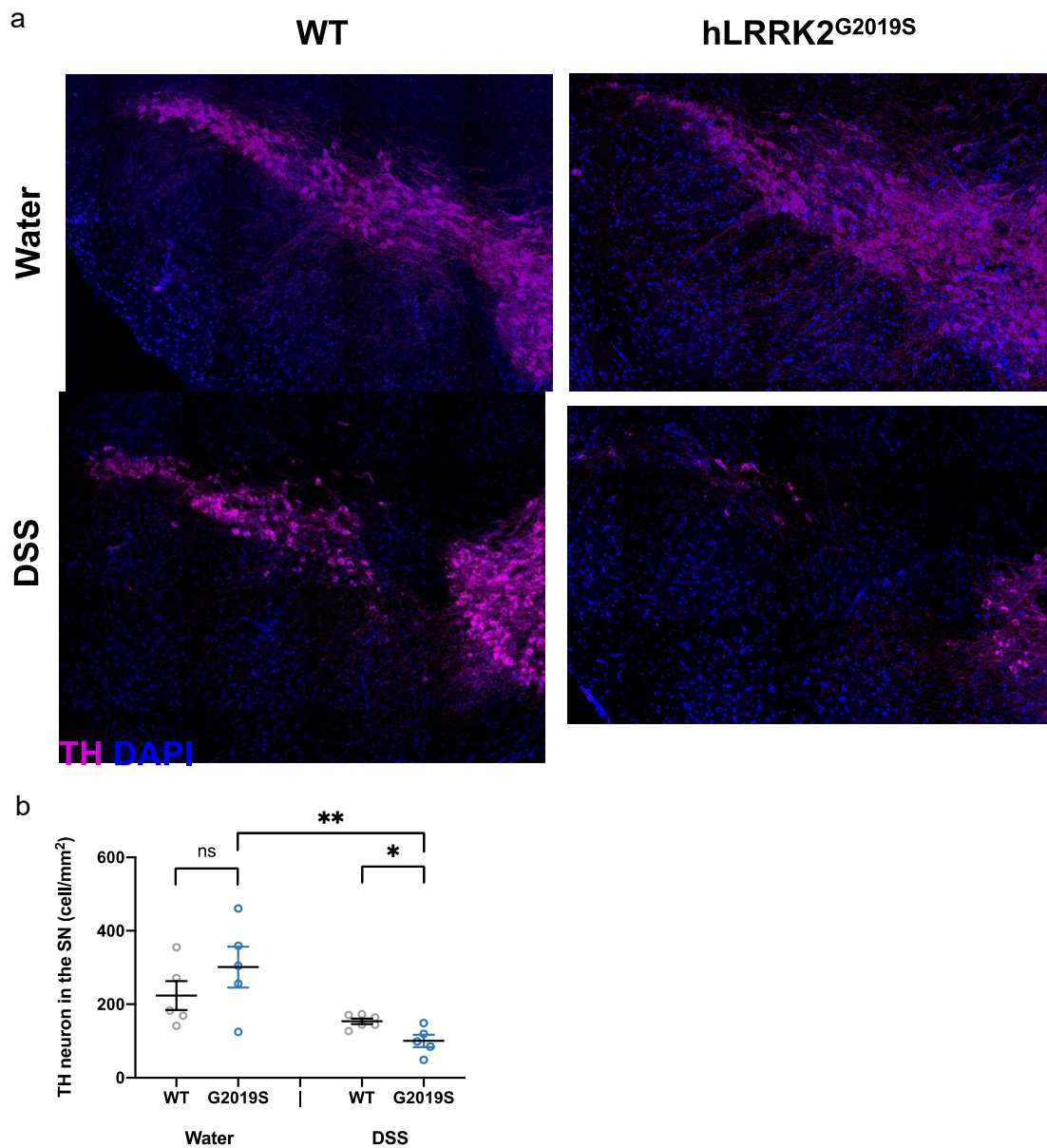


Fig. S4: TH neurons in the SN of male hLRRK2^{G2019S} Tg mice after Vehicle (Water) or DSS treatment. (a) Representative images of TH⁺ neurons (magenta) in the SN of male hLRRK2^{G2019S} Tg mice and WT littermates at 30 weeks post treatment with DSS or water as a negative control (52 weeks of age). (b) Number of TH⁺ neurons in the SN of male hLRRK2^{G2019S} Tg mice and WT littermates at 32 weeks post treatment with DSS or water as a negative control (52 weeks of age) (two-way ANOVA with Sidak; WT (water), G2019S (water) and G2019S (DSS): n=5, WT (DSS): n=6). Data are representative of two independent experiments. Data for the DSS groups are as in Figure 2G. Data are presented as means $\pm$ SEM, * P <0.05, **** P <0.001.

Supplementary Figure 5

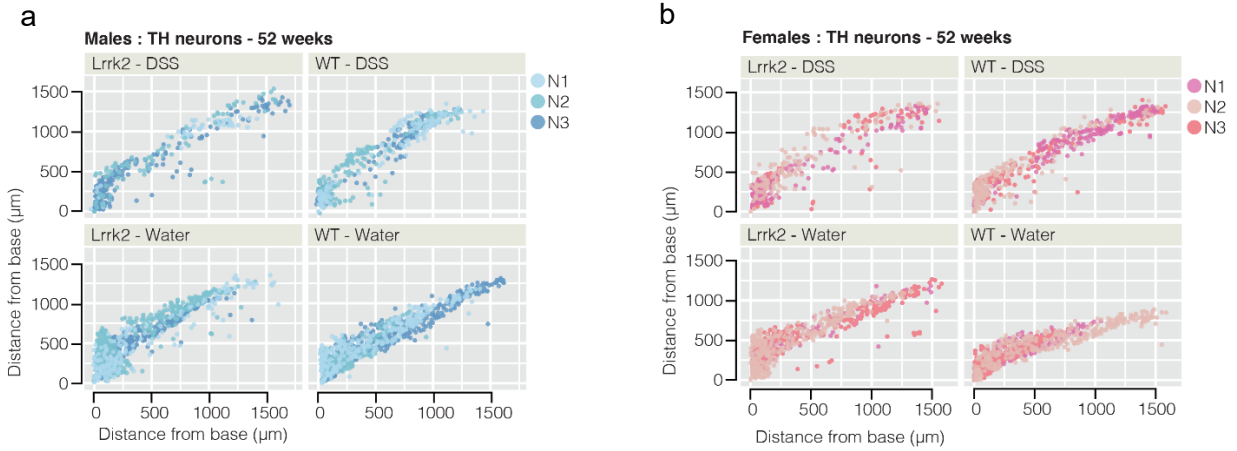


Fig. S5: Projection of TH neurons in the SN in a 2D map. TH neurons in the SN of male (**a**) and female (**b**) hLRRK2^{G2019S} Tg mice and WT littermates at 30 weeks post treatment with DSS or water as a negative control (52 weeks of age). Each dot on the map represents a TH neuron quantified on a real image. n=3.

Supplementary Figure 6

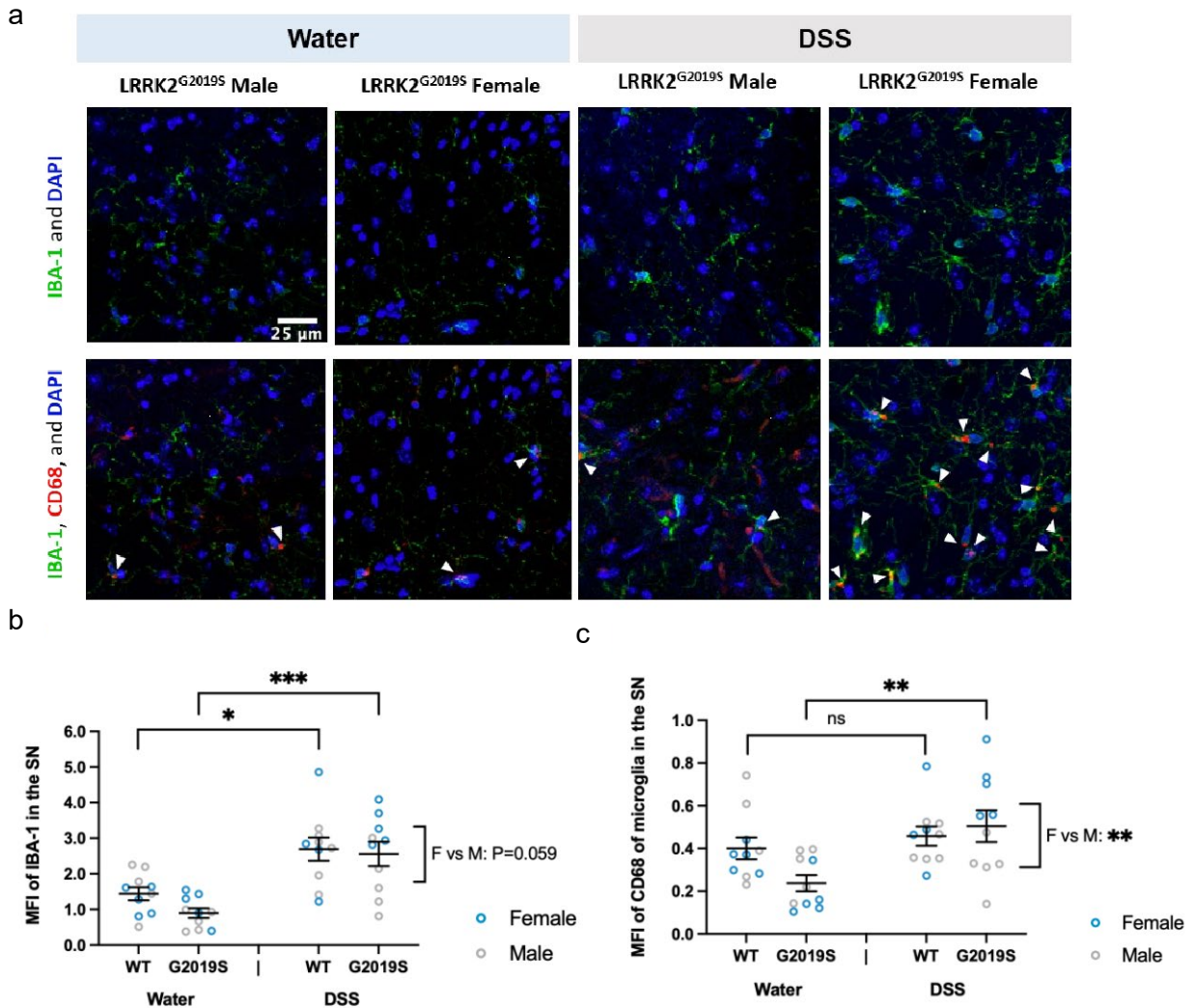


Fig. S6: IBA-1 and CD68 in the SN of hLRRK2^{G2019S} Tg mice after DSS treatment. (A) Representative images of IBA-1⁺ microglia (green) and CD68 (red) in SN of male and female hLRRK2^{G2019S} Tg mice and WT littermates at 32 weeks post DSS treatment (52 weeks of age). Arrows point to CD68 as puncta within the IBA-1 signal. (B) MFI of IBA-1⁺ microglia in the SN. (C) MFI of CD68 within the IBA-1⁺ microglia in the SN. (Two-way ANOVA with Sidak; n=10). Data are representative of two independent experiments. Data are presented as means $\pm$ SEM, * P <0.05, ** P <0.01, *** P <0.001.

Supplementary Figure 7

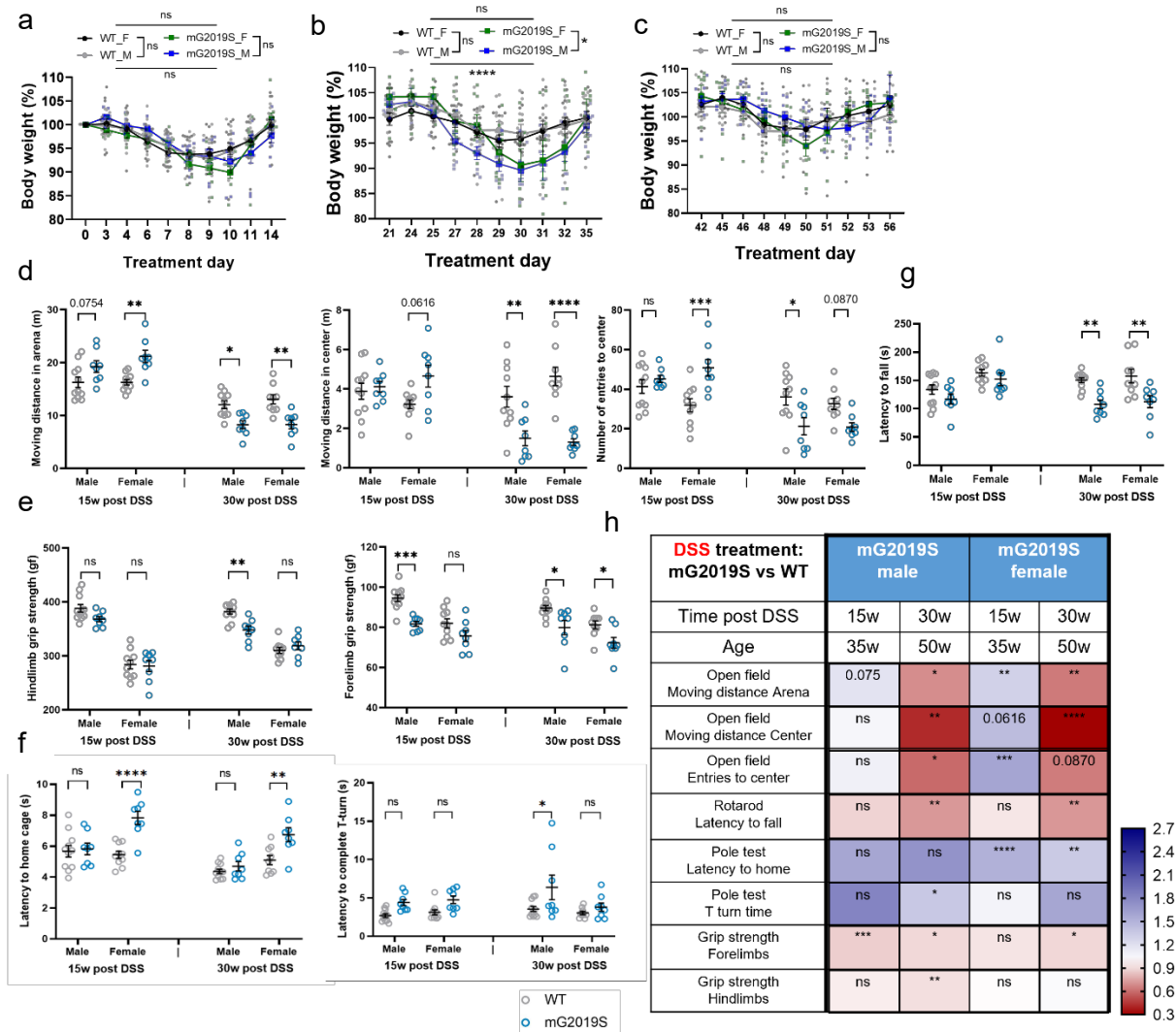


Fig. S7: Body weight loss and motor impairments in mLRRK2^{G2019S} KI mice after early DSS treatment. (a)-(c) Body weight normalized to starting weight (day 0) for male (M) and female (F) mLRRK2^{G2019S} KI mice (mG2019S) and wildtype littermate controls (WT) over 3 rounds DSS treatment (two-way ANOVA with Sidak, n=8-11). (d) Open field test: moving distance in the arena (left), center (middle) and number of entries to the center (right) after 10 min exploration, (e) Grip strength for the hindlimbs (left) and forelimbs (right), (f) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right), (g) Rotarod test of mLRRK2^{G2019S} KI mice and wildtype littermates at 15 and 30 weeks post DSS treatment. (h) Summary of behavioral testing results for mLRRK2^{G2019S} KI mice (mG2019S) relative to WT littermate controls at 15 and 30 weeks post DSS treatment (two-way ANOVA with Sidak, WT male and female: n=10, mG2019S male and female: n=8). Data are representative of two independent experiments. Data are presented as means ± SEM, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

Supplementary Figure 8

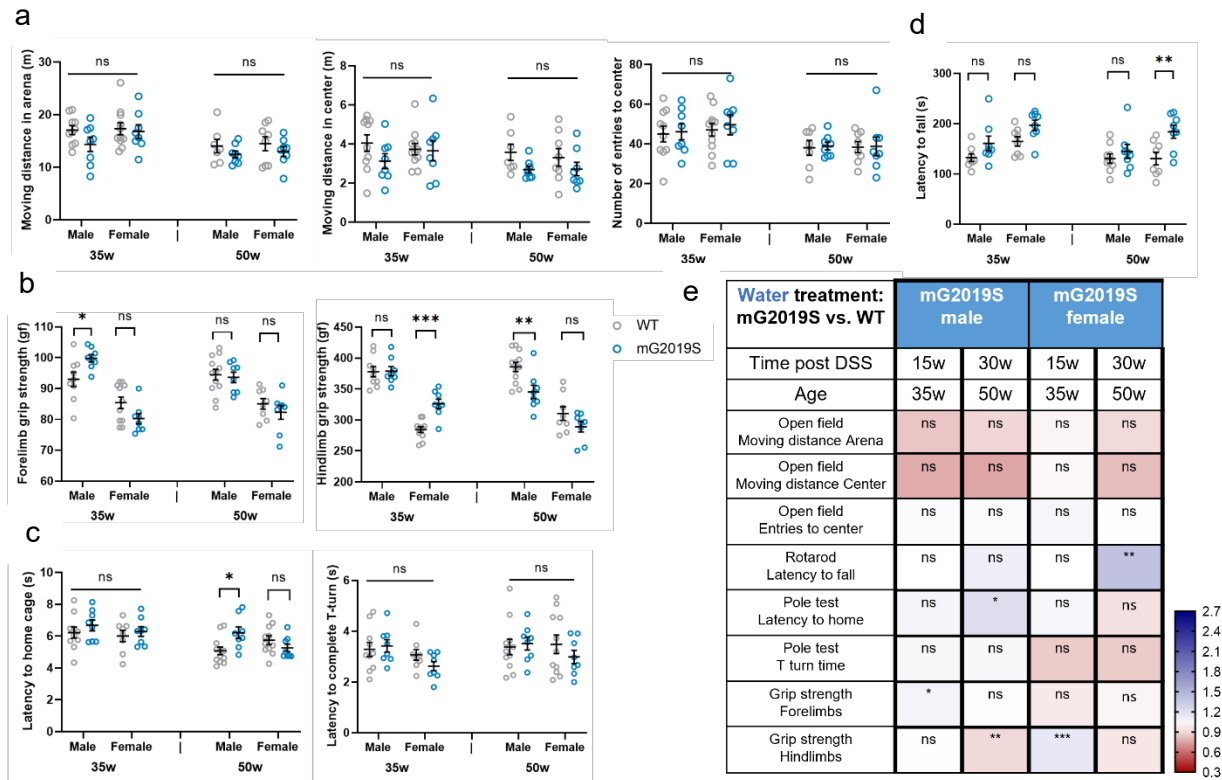


Fig. S8: Vehicle-treated (water; untreated) mLRRK2^{G2019S} KI mice and WT littermate controls in behavioral tests for motor ability. (a) Open field test: moving distance in the arena (left), center (middle) and number of entries to the center (right) after 10 min of exploration, (b) Grip strength for the forelimbs (left) and hindlimbs (right), (c) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right), (d) Rotarod test for mLRRK2^{G2019S} KI mice (mG2019S) and wildtype (W) littermate controls at 15 and 30 weeks post DSS treatment. (e) Summary of behavioral testing results for mLRRK2^{G2019S} KI mice (mG2019S) and WT littermate controls (two-way ANOVA with Sidak; WT male and female: n=10, mG2019S male and female: n=8). Data are representative of two independent experiments. Data are presented as means $\pm$ SEM, * P <0.05, ** P <0.01 and *** P <0.001

Supplementary Figure 9

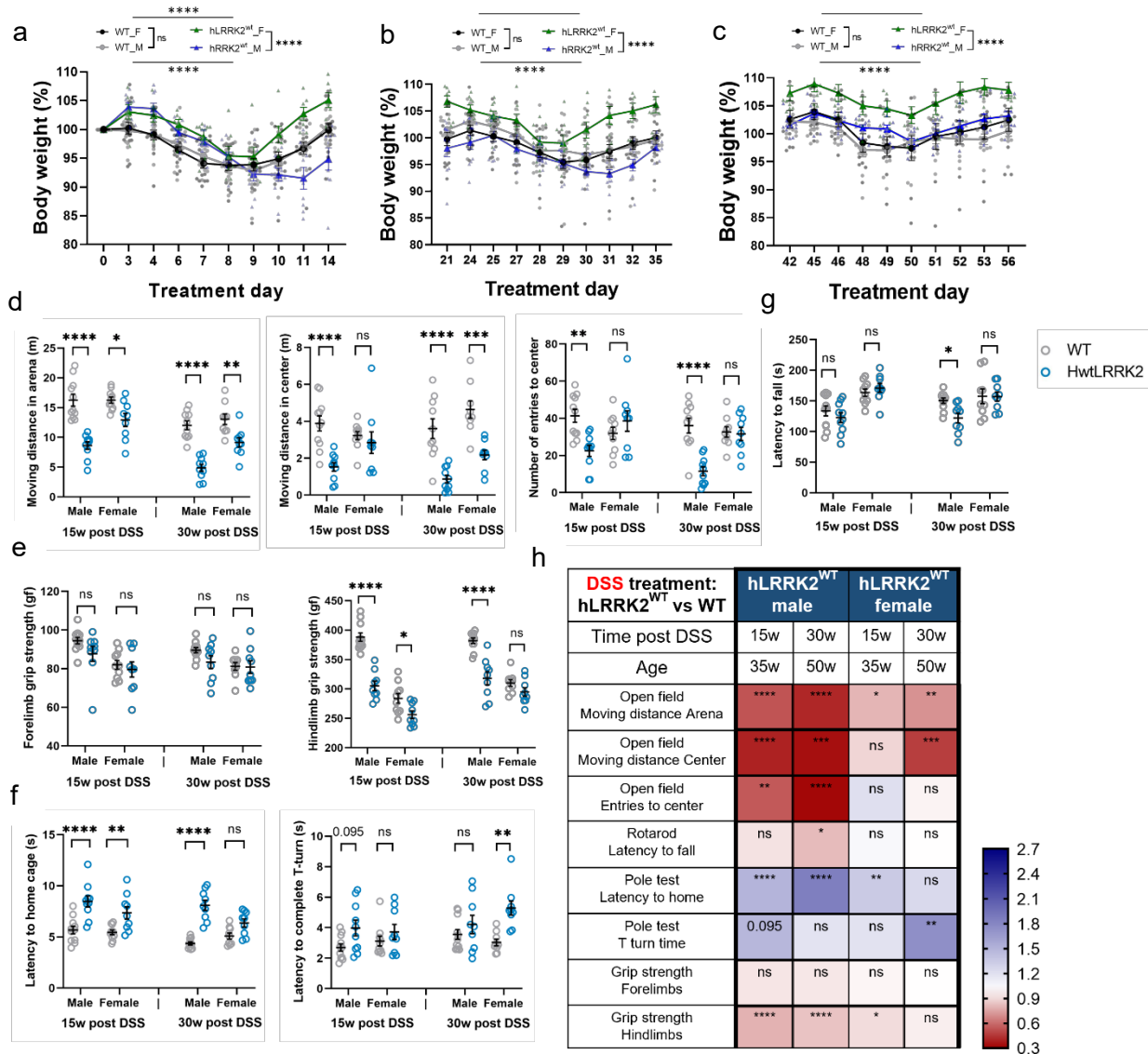


Fig. S9: Body weight loss and motor impairments in hLRRK2^{WT} Tg mice after DSS treatment. (a)-(c) Body weight normalized to starting weight (day 0) for male (M) and female (F) hLRRK2^{WT} Tg mice and wildtype littermates (WT) during each of the 3 rounds of DSS treatment. (d) Open field test: moving distance in the arena (left), center (middle) and number of entries to the center (right) after 10 min of exploration, (e) Grip strength for the forelimbs (left) and hindlimbs (right), (f) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right), (g) Rotarod test of hLRRK2^{WT} Tg mice and wildtype (WT) littermate controls at 15 and 30 weeks post DSS treatment. (h) Summary of behavioral testing results for hLRRK2^{WT} Tg mice and WT controls at 15 and 30 weeks post DSS treatment (two-way ANOVA with Sidak, n=10). Data are representative of two independent experiments. Data are presented as mean ± SEM, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

Supplementary Figure 10

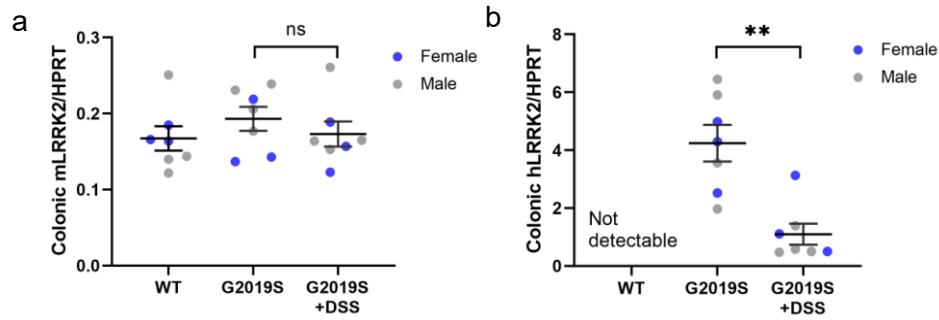


Fig. S10: Colonic expression of *LRRK2* in the hLRRK2^{G2019S} Tg mice at baseline and after 7 days of DSS treatment. mRNA levels of endogenous mouse *LRRK2* (**a**) and human *LRRK2* *G2019S* transgene (**b**) were examined by real time quantitative PCR in the colon from male and female hLRRK2^{G2019S} Tg mice (G2019S) and wildtype littermates (WT), at baseline and 7 days after DSS treatment (two-way ANOVA with Sidak, n=7). Gene expression is normalized to expression of *HPRT*. Data are representative of three independent experiments. Data are presented as mean $\pm$ SEM, ** P <0.01.

Supplementary Figure 11

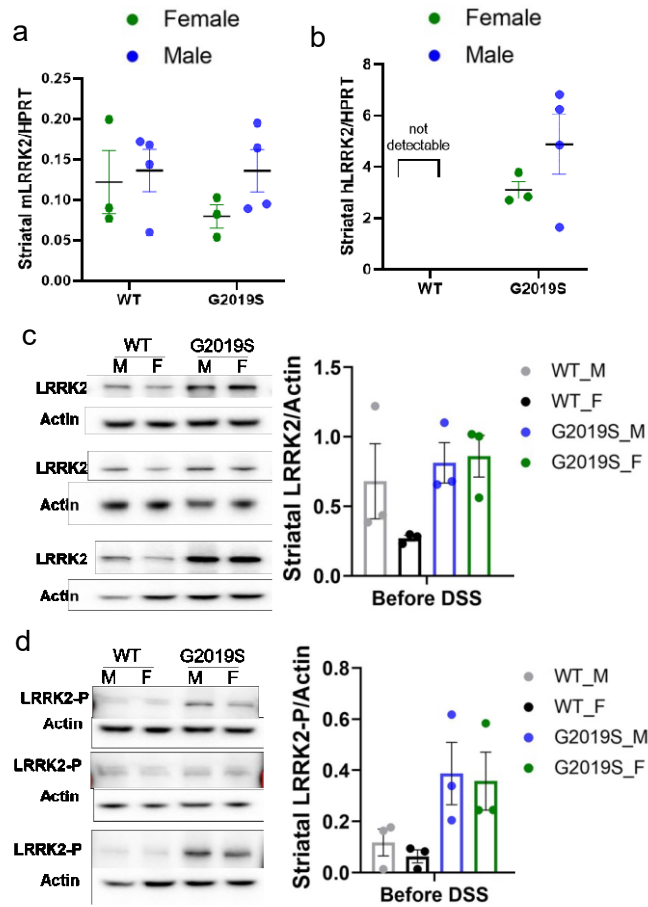


Fig. S11: Striatal expression of LRRK2 in *hLRRK2*^{G2019S} Tg mice. mRNA levels of endogenous mouse *LRRK2* (a) and human *LRRK2* *G2019S* transgene (b) in the striatum from male and female *hLRRK2*^{G2019S} Tg mice (*G2019S*) and wildtype littermate controls (WT). (c) Total LRRK2 and (d) phosphorylated LRRK2 (both endogenous mouse and human transgenic LRRK2) in the striatum from *LRRK2*^{G2019S} Tg mice and WT controls. Data are presented as mean ± SEM.

Supplementary Figure 12

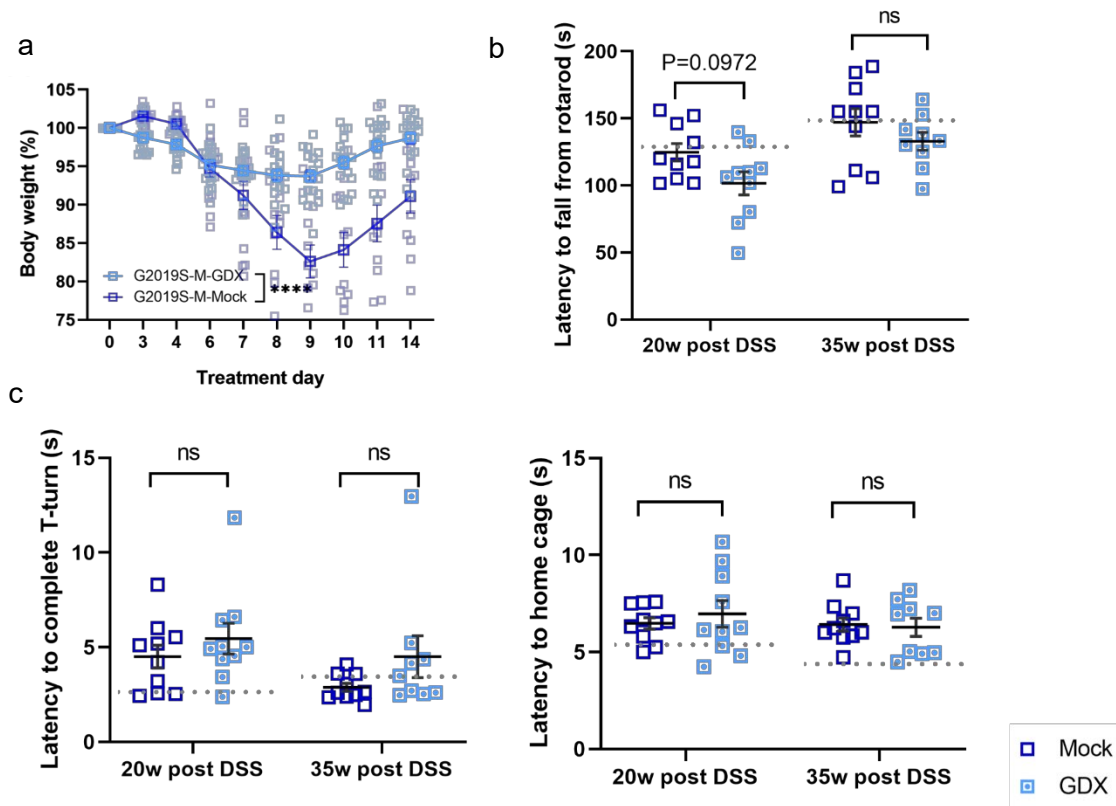


Fig. S12: Body weight loss and behavioral performance in additional motor tasks for gonadectomized (GDX) male hLRRK2^{G2019S} Tg mice after DSS treatment. (a) Body weight normalized to starting weight (Day 0) for gonadectomized (GDX) or Mock surgery-exposed (Mock) male hLRRK2^{G2019S} Tg mice (G2019S-M) during the first round of DSS treatment. (b) Rotarod test and (c) Pole descent test: latency to return to the home cage (left) and latency to complete T turn on the pole (right) at 20 and 35 weeks post DSS treatment (two-way ANOVA with Sidak, mock and GDX (20w post DSS): n=10, GDX (35w post DSS): n=9). Data are representative of two independent experiments. Data are presented as mean $\pm$ SEM, **** P <0.0001. Dotted lines indicate the mean value for WT littermates as a reference.

Supplementary Figure 13

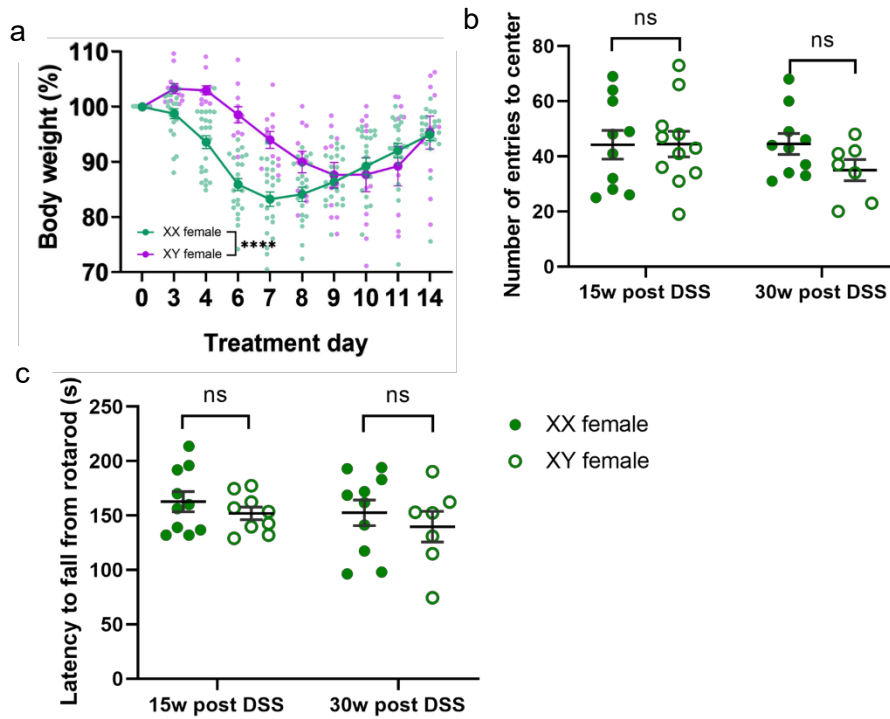


Fig. S13: Body weight loss and behavioral performance in additional motor tasks for XY female hLRRK2^{G2019S} Tg mice after DSS treatment. (a) Body weight normalized to starting weight (day 0) for XX and XY female hLRRK2^{G2019S} Tg mice during the first round of DSS treatment. (b) Open field test: number of entries to the center after 10 min of exploration. (c) Rotarod test for XX and XY female hLRRK2^{G2019S} Tg at 15 and 30 weeks post DSS treatment (two-way ANOVA with Sidak, XX female n=10, XY female (15w post DSS): n=9, XY female (30w post DSS): n=7). Data are representative of two independent experiments. Data are presented as mean $\pm$ SEM, **** P <0.0001

Supplementary Figure 14

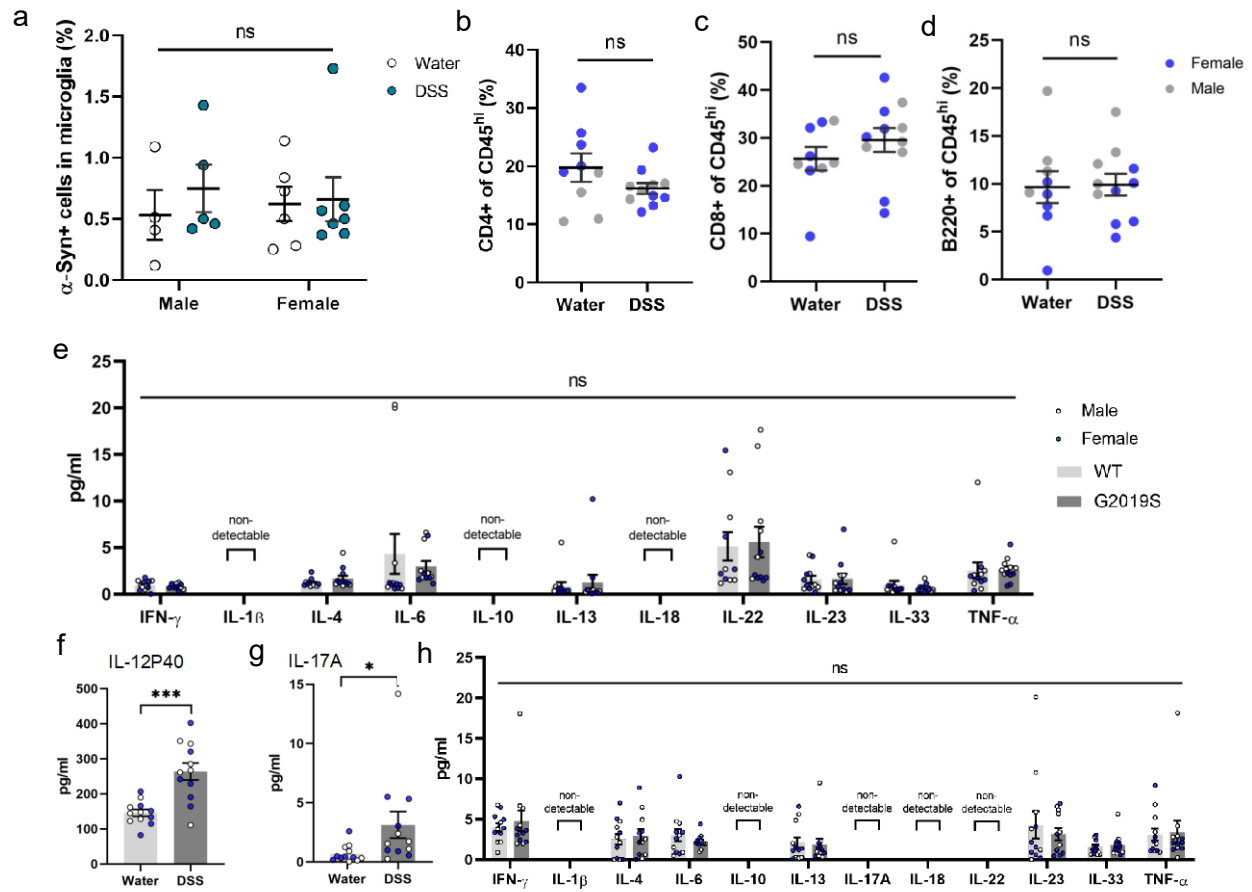


Fig. S14: DSS-induced alterations in brain immune profiles of hLRRK2^{G2019S} Tg mice relative to WT littermate controls, prior to the onset of neuropathological and behavioral endophenotypes of PD. Flow cytometry of brain parenchymal cells for (a) α -Syn⁺ microglia (two-way ANOVA with Sidak, G2019S male (water): n=4, G2019S (DSS): n=5, G2019S female: n=6), (b) CD4 T cells, (c) CD8 T cells and (d) B cells from hLRRK2^{G2019S} Tg mice at 1 week after the last round of treatment with DSS or vehicle (water) (paired t-test, G2019S (water): n=9, G2019S (DSS): n=12). (e) Serum cytokine concentrations for (f) IL-12P40 and (g) IL-17A, and (h) various cytokines in CSF from hLRRK2^{G2019S} Tg mice at 1 week after the last round of treatment with DSS or vehicle (paired t-test, G2019S (water): n=9, G2019S (DSS): n=12). Data are representative of two independent experiments. Data are presented as mean $\pm$ SEM, * P < 0.05 and *** P < 0.001.

Supplementary Figure 15

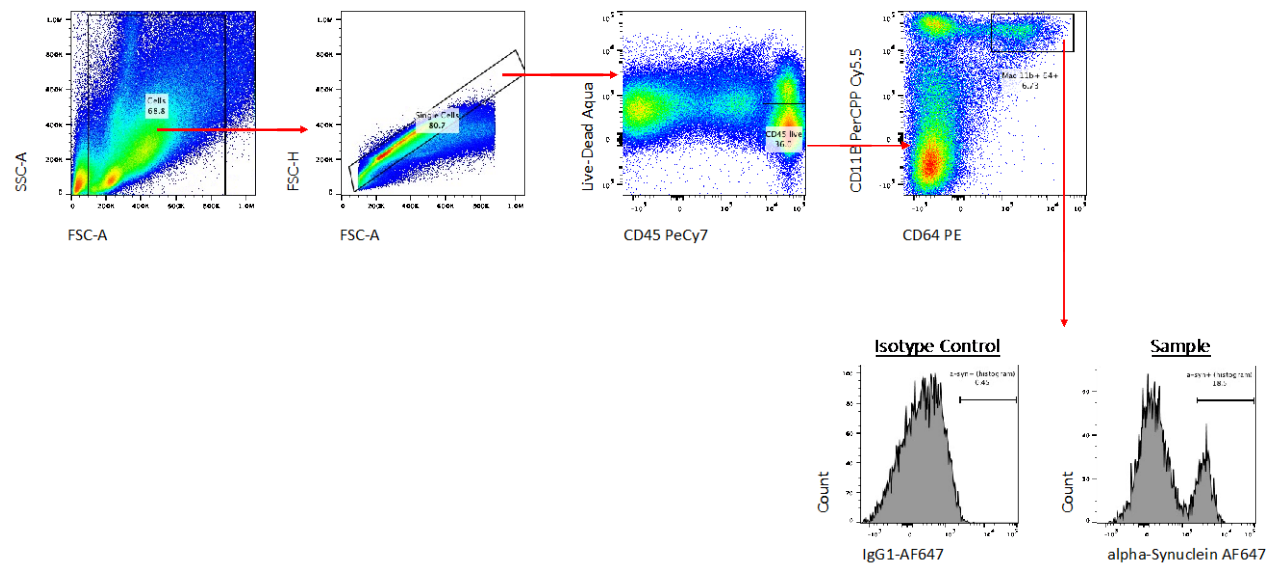


Figure S15. Gating strategy for α -synuclein+ macrophages from the colonic lamina propria