Supplemental Data for

Dopamine signaling regulates hematopoietic stem and progenitor cell function

Yang Liu, Qi Chen, Hyun-Woo Jeong, Dong Han, Jörg Fabian, Hannes Drexler, Martin Stehling, Hans R. Schöler, Ralf H. Adams

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Supplemental Methods

Mouse models

8-15 week-old mice were used as young adults and C57BL/6 males as wild-type animals for most experiments. Mice were typically sacrificed between 8am and 10am in local time to avoid influence from circadian rhythm. Male and female mice were all used for analysis in a comparable manner. Littermates with appropriate genotypes were used as controls of genetically modified mutants whenever possible (see further details below).

Th$^{tm1(Cre)Te}$ (*Th-Cre*)[1] transgenic mice were purchased from the European Mouse Mutant Archive and interbred with *Rosa26-DTR*[2] mice to generate *Th-Cre*$^{+/T}$ *Rosa26-DTR*$^{+/lox}$ (*DTR*$^{i\Delta Th}$) mice and *Th-Cre*$^{+/+}$ *Rosa26-DTR*$^{+/lox}$ control littermates. 10µg/kg diphtheria toxin (Sigma, D0564) was administrated to *DTR*$^{i\Delta Th}$ and control mice for 2 consecutive days and animals were analyzed 4 days after first injection.

Drd2^{tm1Low} [3] and *Drd3*^{tm1Dac} [4] knockout mice were purchased from the Jackson Laboratory and considered as parental generation. These mice were backcrossed and offspring of the second generation (F2) was used for analysis. The corresponding controls for *Drd2*$^{-/-}$ mice are *Drd2*$^{+/+}$ littermates and controls for *Drd3*$^{-/-}$ knockouts are *Drd3*$^{+/+}$ littermates. *Drd2*$^{+/-}$*Drd3*$^{-/-}$ mice were used as parents to generate *Drd2*$^{-/-}$*Drd3*$^{-/-}$ (*Drd* DKO) mice. As it was not feasible to generate proper littermate controls for *Drd* DKO mutants, sex and age-matched wildtype mice were used as controls.

In the *Rosa26-mTmG*[5] reporter background, Cre activation leads to an irreversible switch from constitutive membrane-anchored tdTomato protein expression to membrane-anchored GFP. *Vav1-Cre*[6] and *Wnt1-Cre*[7] transgenic mice were interbred with *Rosa26-mTmG* mice to generate *Vav1-Cre Rosa26-mTmG* animals, which were used as donors for transplantation experiments. Lepr$^{tm2(cre)Rck}$ [8] and *Tg(Drd2-EGFP/Rpl10a)CP101Htz* [9] transgenic mice were purchased from the Jackson Laboratory.

Most of the mice, analyzed in this study have a C57/B6 phenotype background. Maintenance of lines typically involved backcrossing with C57/B6 mice.

All information about mouse lines is summarized in Supplemental Table 1.

All animals were routinely genotyped using PCR. Protocols and primer sequences can be provided upon request. All the animals were housed in the animal facility at the Max Planck Institute for Molecular Biomedicine. All experiments were performed according to the institutional guidelines and laws, following the protocols approved by local and national animal ethics committees.

Pharmacological treatments and EdU labeling

ICI-118,551 (Sigma, I127; 2mg/kg; in H_2O), SCH-23390 (Sigma, D054; 10mg/kg; in H_2O), and Haloperidol (Sigma, H1512; 6mg/kg; in DMSO) were administrated by intraperitoneal injection (i.p.) for 3 consecutive days. In the third day, mice were analyzed 1 hour after pharmacological treatment.

20mg/kg EdU (Thermo Fisher Scientific, A10044) was administrated 1 hour before analysis or at the same time as pharmacological treatments when indicated. Click-iT EdU imaging (Invitrogen, C10340) or Click-it plus EdU Alexa fluor 488 and 647 flow cytometry assay kits (Invitrogen C10632, C10424) were used according to the manufacturer's instructions for immunohistochemistry staining or flow cytometry analysis, respectively.

A-770041(Axon,1698; 5mg/kg in DMSO) was administrated i.p. after irradiation and transplantation for three consecutive days.

Carbidopa (Sigma, c1335; 40mg/kg in DMSO) was administrated i.p. twice daily for three consecutive days.

7-OH-DPAT (Sigma, H8653;8mg/kg; in 0.9% sodium chloride solution) was administrated i.p. following the schedules indicated in the figures and supplementary data sets.

All drugs were freshly prepared and administered within 10 minutes.

Cryosectioning, immunohistochemistry and confocal imaging

Information about antibodies is summarized in Supplemental Table 2.

Adult bones were dissected and immediately placed in ice-cold 4% paraformaldehyde solution and fixed under gentle agitation overnight. Bone samples were placed in 0.5M EDTA (PH 8.0) for at least 2 days, dehydrated in 20% sucrose and 2% polyvinylpyrrolidone-containing PBS for at least 2 days, and embedded in PBS containing 20% sucrose, 8% gelatin and 2% polyvinylpyrrolidone for storage at -80 ^{o}C and cryosectioning on a Leica CM3050 cryostat using low profile blades. For immunostaining, bone sections were rehydrated in PBS, permeabilized for 15 min in 0.5% Triton-X100 PBS solution and blocked for 30 min in PBS containing 1% BSA, 2% donkey serum, 0.3% Trion X-100 (blocking buffer) at room temperature. Sections were incubated with primary antibodies diluted in blocking buffer at 4^{o}C overnight. After incubation, sections were washed three times with PBS and incubated with appropriate Alexa Fluor-conjugated secondary antibodies (1:100 to 1:200, Invitrogen) diluted in blocking buffer at room temperature for 2 hours. Nuclei were stained with DAPI during secondary antibody incubation. After that, sections were washed three times with PBS, mounted with Fluoromount-G (0100-01, Southern Biotech) and kept in 4^{o}C for imaging. For staining of Drd2 and Drd3 in bone section, Lineage cocktail (Miltenyi Biotec, 130-092-613), cKit (R&D, AF1356), Drd2 (Merck, AB5084P) and Drd3 (Abcam, Ab42114) were used.

For *ex vivo* staining, LSK cells were directly sorted by FACS and placed on polylysine-coated slides (about 2000 cells per slide), incubated for 20 min, fixed with 4%PFA for 10 min at room temperature and permeabilized with 0.15% Triton X-100 for 10 min. The cells were blocked with 1% BSA-PBS for 1 hour at room temperature. Next, cells were incubated overnight at 4 ^{o}C in 1% BSA-PBS with primary antibodies. Slides were washed with PBS three times and incubated for 1 hour at 37 ^{o}C with secondary antibody. Immunofluorescence staining of γ–H2afx was adapted from previously published protocols.[10] For immunofluorescence staining

of Drd2 and Drd3 in LSK cells, Sca-1 and cKit were re-stained by using the same fluorescence channel as for FACS. Sca1-FITC (eBioscience, 11-5981-85) and cKit-APC (BD Pharmingen 553356) were used for FACS. Sca1-biotin (Biolegend, 108104) together with Streptavidin-Alexa488 secondary antibody, cKit (R&D, AF1356) together with anti-goat Alexa-647 secondary antibody, Drd2 antibody (Merck, AB5084P) together with anti-rabbit Alexa-594, Drd3 antibody (ThermoScientific, 32-0900) together with anti-mouse Alexa-546 were used in IHC analysis of sorted cells. For immunofluorescence staining of Drd2 and Drd3 in sorted HSCs, Sca1 is not stained and Drd2 and Drd3 were stained separately. Antibodies were cKit (R&D, AF1356) together with anti-goat Alexa-647 secondary antibody, anti-Drd2 (Merck, AB5084P) together with anti-rabbit 546 or anti-Drd3 (ThermoScientific, 32-0900) together with anti-mouse Alexa-546.

Bone sections or FACS-sorted cells were imaged with laser scanning confocal microscopes (Leica SP5 and Zeiss LSM780) after immunohistochemistry. Quantitative analysis of mutant phenotypes was done with the same microscope and identical imaging acquisition setting for mutant and control samples. Overview images of bone were automatically scanned using the tile-scan function of confocal microscope. We used Fiji (open source; http://fiji.sc/), Volocity (PerkinElmer), Photoshop and Illustrator (Adobe) softwares for image processing in compliance with *Blood*'s guide for digital images. Original images were loaded into Volocity for brightness-contrast modifications that were applied to the whole image. Most of quantification and the analysis of EdU+ and Ki67+ cells were done in Volocity.

Flow cytometry

Information about antibodies is summarized in Supplemental Table 2.

Bones were dissected and crushed by pestle repeatedly before cells were collected in 2% FCS-PBS solution. The tissue was immersed in 6ml dissociation solution (2% FCS-PBS solution with approximate 145U/ml type 4 Gibco collagenase) and incubated at 37ºC for 30 minutes.

Samples were filtered using 70μm Nylon cell strainer to obtain single cell suspensions. Primary antibodies were diluted in 2% FCS-PBS solution and incubated with cells on ice for 40 minutes. Cells were washed 3 times by 2% FCS-PBS solution and incubated with secondary antibodies for 40 minutes if necessary. Cells were washed 3 times and used for flow cytometry. Cells were resuspended in 2% FCS-PBS supplemented with 1 μg/ml DAPI to allow exclusion of nonviable cells when required. Cell sorting was performed on a FACS AriaIIu cell sorter (BD Biosciences, San Jose, CA) using an 85 μm nozzle. Cell analysis was performed using a BD FACS Verse. The percentage in figures represent marked cells per DAPI cells unless otherwise stated.

For the mice undergoing pharmacological treatments, bone marrow cells were continuously exposed to drugs dissolved in 2% FCS-PBS solution during processing, analysis and sorting. In control group, vehicles were dissolved in 2% FCS-PBS solution.

For Drd2 or Drd3 staining in LSK cells, HSCs and Ter119+ cells by FACS, cells were stained with Sca1-PE-Cy7 (BD Pharmingen, 558162), cKit-FITC (Biolegend, 105806), CD150-PE (Biolegend, 115904), CD48-APC-Cy7 (BD Pharmingen, 561242), Lineage cocktail-APC (BD Pharmingen, 558074) or Ter119-PE-Cy7 (eBioscience, 25-5921-82), Drd2 antibody (Merck, AB5084P) or Drd3 antibody (Abcam, Ab42114) on ice for 40 minutes. Cells were washed 3 times by 2% FCS-PBS solution and incubated with anti-rabbit Alexa-405 secondary antibodies for 40 minutes on ice. Cells in control group were stained with HSC markers or Ter119 for gating and secondary antibodies but without Drd2 or Drd3 antibody.

For analysis of Drd2 and Drd3 expression in neurons, midbrain was dissected and single cell suspensions were prepared in 2% FCS-PBS solution after digestion. Cells were first stained with lineage cocktail and Drd2 or Drd3 antibody, followed by intracellular staining with neurofilament antibody. Lin- Neurofilament-H+ cells were gated as neurons and analyzed for MFI of Drd2 or Drd3.

DNA content analysis of LSK cells was determined following the manufacturer's instructions (Vybrant Dye Cycle violet stain kit, Invitrogen V35003). Briefly, 10^6 cells were combined with

1µl of Vybrant dye Cycle and stained at 37 ^{o}C for 30min after LSK staining. Cells were placed in a FACS analyzer immediately after incubation.

To perform intracellular staining in LSK cells, bone marrow single cell solution was generated as described above. After staining of LSK markers, BM cells were immediately fixed using reagent A from fixation and permeabilization kit (GAS003, Invitrogen) with the exception of drug treatment or stimulation experiments. For detection of intracellular staining in LSK cells from haloperidol-treated mice, WT animals received haloperidol or vehicle i.p. for 3 consecutive days. Cells were isolated and stained for surface markers in presence of 10 µM haloperidol or vehicle before fixation. For treatment with recombinant protein or inhibitors, BM cells were transferred to PBS solution and kept in 37^{O}C for 2 hours. 8µM A-770041, 100 µM PP2, 10 µM Marimastat, 50 µM H-89, 30 µM Forskolin, or 50 µM barbadin were added to BM cells at 37^{O}C for 30 minutes after PBS treatment. 10µM 7-OH-DPAT was added to BM cells at 37^{O}C for 45 minutes after PBS treatment. 100ng/ml stem cell factor (Peprotech, 250-03) was added to cells at 37^{O}C for 15 minutes. After these treatments, BM cells were immediately fixed using reagent A from fixation and permeabilization kit (GAS003, Invitrogen). After fixation, antibodies were added to saponin-based permeabilization buffer (C10633, Invitrogen) or reagent B from fixation and permeabilization kit (GAS003, Invitrogen) for staining. Cells were stained with primary antibody in room temperature for 20 min and secondary antibody in room temperature for 20 min. Only secondary antibody staining was used as control to gate the positive signal. After staining, BM cells were used for FACS to produce fluorescence intensity histograms. For quantification of subtracted mean fluorescent intensity (sMFI), the original MFI was reduced to background signal, which was set according to sample stained only with secondary antibody control. Normalized mean fluorescent intensity (nMFI) was calculated by normalization of sMFI.

For intracellular staining of phospho-Histone H3 and EdU by FACS after surface staining of LSK cells, EdU staining was performed following the manufacturer's instructions (Click-it plus

EdU Alexa fluor 488 flow cytometry assay kit, Invitrogen C10632). Next, cells were stained with phospho-Histone H3 (Upstate, 06-570) in reagent B from fixation and permeabilization kit (GAS003, Invitrogen) at room temperature for 20min and secondary antibody at room temperature for 20min. Only secondary antibody staining was used as control to gate the positive signal.

Generation of lentiviral vectors and cell infection

Mouse Lck cDNA was purchased from Origene (MC203794) and inserted into plasmid Lentilox3.7 via NheI and EcoRI restriction sites (https://www.sciencegateway.org/protocols/lentivirus/pllmap.html). For the generation of shRNA constructs, the following oligonucleotides were synthesized by Sigma, annealed and inserted into Lentilox3.7 via XhoI and HpaI.

ShCtrl Forward:

5'-phospho-

TGCAATATTACATATACGCCTTCAAGAGAGGCGTATATGTAATATTGCTTTTTTC

ShCtrl Reverse:

5'-phospho-TCGAGAAAAAAGCAATATTACATATACGCCTCTCTTGAAGGC

GTATATGTAATATTGCA

ShLck-1 Forward:

5'-phospho- TGATCCGTAACCTAGACAACTTCAAGAGAGTTGTCTAGGTTACG

GATCTTTTTTC

ShLck-1 Reverse:

5'-phospho-TCGAGAAAAAAGATCCGTAACCTAGACAACTCTCTTGAAGTT

GTCTAGGTTACGGATCA

ShLck-2 Forward:

5'-phospho-TGAGCTGTACCACCTCATGATTCAAGAGATCATGAGGTGGT

ACAGCTCTTTTTTC

ShLck-2 Reverse:

5'-phospho-TCGAGAAAAAAGAGCTGTACCACCTCATGATCTCTTGAATCA

TGAGGTGGTACAGCTCA

ShKit-1 Forward:

5' phospho TGCAGGTTGTCCAACTTATTTTCAAGAGAAATAAGTTGGACAACCTGC

TTTTTTC

ShKit-1 Reverse:

5' phospho TCGAGAAAAAAGCAGGTTGTCCAACTTATTTCTCTTGAAAATAAGTTG

GACAACCTGCA

ShKit-2 Forward:

5' phospho TGAGGAGATCCGCAAGAATATTCAAGAGATATTCTTGCGGATCTCCTC

TTTTTTC

ShKit-2 Reverse:

5' phospho TCGAGAAAAAAGAGGAGATCCGCAAGAATATCTCTTGAATATTCTTGC

GGATCTCCTCA

BLAST was used to confirm that the core sequence of *shLck* and *shKit* matches no other genes in the murine and human transcriptomes. Likewise, the scrambled control matches no gene in the murine and human transcriptomes. All constructs were validated by sequencing. The Lck overexpression or shRNA plasmids were transfected into HEK293T/17 cells and virus was concentrated using polyethylene glycol (Sigma, 81260). Every batch of concentrated virus was tested by direct infection of HEK293T17 cells. For infection of bEND.3 cells, 8 μg/ml polybrene was added to the culture medium before infection. 3 days after infection, bEND.3 cells were collected in RLT lysis buffer for RNA extraction. To infect LSK cells, non-treated culture dishes were pre-coated with RetroNectin (Takara, T100A) and

loaded with concentrated virus then pre-stimulated LSK cells were seeded into Stemline II hematopoietic stem cell expansion medium (Sigma, S0192) containing 50ng/ml SCF and 50ng/ml Flt3 ligand. Analysis, CFU assays and transplantation were performed 3 days after seeding if not otherwise indicated. For transplantation experiments, about 7000 LSK cells were seeded and infected for 3 days before transplantation into recipients. Analysis was done 2 weeks after transplantation.

RNA extraction and quantitative PCR

RNA was extracted using RNeasy plus Micro Kit (74034, Qiagen) and cDNA was generated with iScript^{TM} cDNA Synthesis Kit (#170-8891, BioRad). Quantitative PCR with reverse transcription was performed with a Bio-rad CFX96 real-time PCR system using FAM-conjugated Taqman probes for *Lck* (Mm00802897_m1), *Drd3* (Mm00432888_m1), *Drd2* (Mm00438545_m1), *Kit* (Mm01211914_m1). About 1 million LSK cells are FACS sorted for qPCR analysis of D_2-receptor expression. RNA quantity and integrity are validated in Agilent Bioanalyzer before reverse transcription. Gene expression levels were normalized to the endogenous VIC-conjugated *Gapdh* probe (4352339E) as control.

Western blots

Sample lysates were prepared in RIPA buffer. Proteins were separated by electrophoresis on 10% SDS-PAGE, and the proteins were electroblotted onto a nitrocellulose membrane. After blocking, the membrane was probed with the indicated primary antibodies overnight at 4 ^{o}C and then incubated with secondary antibody, which was detected by exposure to X-ray film. The antibodies used included anti–rabbit Drd2 (Merck AB5084P, 1:1000 dilution), anti–rabbit Drd3 (Abcam ab42114, 1:500 dilution), anti–rabbit GAPDH (Cell Signalling 2118, 1:10000 dilution).

Methylcellulose assay

Approximate 200 HSCs (SLAM-LSK) from wildtype mice were FACS sorted and cultured in MethoCult^{Tm} medium (GF M3434, Stem cell technologies) in 35mm dish. HSCs were sorted into 2%FCS-PBS with drugs (10μM 7-OH-DPAT, 8μM A-770041, 10μM Haloperidol) and control vehicle. Drugs or control vehicle were mixed with MethoCult^{Tm} medium (10μM 7-OH-DPAT, 8μM A-770041, 10μM Haloperidol) during the seeding of HSCs. Cells and medium were incubated in 37^{o}C 5%CO_2 cell incubator. 8 days after incubation, CFU number was counted. After counting of CFUs, cells were resuspended and collected into 2% FCS-PBS solution and filtered with 70um filter for FACS staining analysis and cell counting. After infection with lentivirus and culture for 3 days in Stemline II hematopoietic stem cell expansion medium (Sigma, S0192) with 50ng/ml SCF and 50ng/ml Flt3 ligand, 1000 infected LSK cells were seeded in MethoCult^{Tm} medium in 35mm dish for the 1st CFU assay. After 8 days, CFU number and cell number were counted and 10,000 cells from 1st CFU assay were seeded again in MethoCult^{Tm} medium in 35mm dish for the 2nd CFU assay. After further 8 days, CFU number and cell number were counted.

HSC and HSPC culture

Sorted HSC cells were cultured in Stemline II hematopoietic stem cell expansion medium (Sigma, S0192) with 50ng/ml SCF and 50ng/ml Flt3 ligand. Pharmacological drugs (10μM 7-OH-DPAT, 10μM Haloperidol) or vehicle control were present when indicated. Cells were incubated at 37^{o}C with 5%CO_2 and analyzed at the indicated time points. For 7-OH-DPAT pretreatment in HSPC transplantation experiments, 6000 LSK cells sorted from WT, Drd DKO or *Vav1-mTmG* mice were treated with vehicle or 10μM 7-OH-DPAT *in vitro* for 7 days in culture. Half of medium was replaced with fresh medium containing 7-OH-DPAT at day 2 and day 4. Afterwards, all cells were collected and transplanted into lethally irradiated WT mice, which were analyzed 5 days after transplantation.

Irradiation, long-term competitive repopulation assay, lineage separation and transplantation

Mice were exposed to a lethal dose (12 Gy) of Gamma irradiation (137Cs, GammaCell). Bone marrow cells (or Lin-/LSK cells) were transplanted 4 to 6 hours after lethal irradiation (12 Gy).

For long-term competitive repopulating assays, CD45.1 recipient mice were lethally irradiated (12 Gy) and transplanted with 5 x 10^5 donor-derived (CD45.2 background) BM cells together with 5 x 10^5 host-derived (CD45.1 background) bone marrow cells. Recipient mice were sacrificed 16 weeks and 24 weeks after transplantation to determine the level of chimerism in bone marrow and peripheral blood by flow cytometry.

For separation of lineage-negative cells, BM cells were collected and filtered as described for flow cytometry analysis. Biotinylated-lineage cocktail antibodies (Miltenyi Biotec, 130-092-613) were mixed with cells at 4^{o}C for 20 minutes. Antibody-conjugated BM cells were washed 2 times and then incubated with anti-biotin antibody-conjugated magnetic microbeads (Miltenyi Biotec, 130-105-637) at 4^{o}C for 30 minutes. Cells were washed 2 times and separated with LS column (MiltenyiBiotec, 130-042-401). Lineage negative cells were counted and transplanted as outlined in the relevant results. For *DTR*$^{i\Delta Th}$ and control mice, haloperidol-treated animals and vehicle controls or A-770041 treated animals and vehicle controls, 3x10^5 lineage negative cells from *Vav1-Cre Rosa26-mTmG* donor were transplanted into each recipient for EdU analysis of donor-derived cells. For haloperidol-treated animals and vehicle controls, 3x10^5 lineage negative cells from wild type donor were transplanted into each recipient for intracellular staining of signaling pathways. For 7-OH-DPAT treated and vehicle control mice, 3x10^4 lineage negative cells from *Vav1-Cre Rosa26-mTmG* donor or WT donor were transplanted into each recipient for EdU analysis of donor-derived cells.

For the transplantation of LSK cells from *Drd* DKO and control mice as donors, about 3000 LSK cells are transplanted into each recipient for transplantation efficiency analysis at 14 days, intracellular staining of signaling proteins at 14 days, or maintenance analysis of chimeric mice at 2 or 6 months. For the transplantation of LSK cells from WT mice into *Drd* DKO and control mice, 3000 LSK cells were transplanted into each recipient animal for transplantation efficiency analysis after 14 days and maintenance analysis of chimeric mice after 2 months and 6 months.

ELISA

For dopamine and noradrenaline detection, bone marrow samples were prepared by flushing 1 femur with 1mL of ice-cold ELISA buffer (PBS with 1mM EDTA, 4mM sodium metabisulfite). Midbrain, spleen and thymus are dissected, chopped and flushed with ice-cold ELISA buffer. For SCF and CXCL12 detection, bone marrow samples were prepared by flushing 1 femur with 1mL of ice-cold PBS. Cells were centrifuged (300g, 5min, 4^{o}C) to collect supernatants. Samples were stored at -80 ^{o}C until use. The level of dopamine, noradrenaline, SCF and CXCL12 were determined with the following ELISA kits: CEA851Ge from Cloud clone; BA-E-5200 from ImmuSmol; SEA120Mu from Cloud clone; MCX120 from R&D Systems.

Mass Spectrometry

Samples were collected in catecholamine stabilization solution (PBS with 10mM EDTA, 33mM sodium metabisulfite), filtered using 100μm Nylon cell strainer and centrifuged (400g, 5min, 4^{o}C) to collect supernatants. Sample supernatants were stored at -80 ^{o}C until use. Dopamine standard (Sigma, D-081) was gradually diluted and added to catecholamine stabilization solution for generating standard curve. The same amount of isotopically-labeled internal standard (Sigma, D-072) was added to every sample and dopamine standard. Ice-cold

0.5% formic acid in acetonitrile was added to Agilent Captiva cartridge (A5300635), samples and standard were mixed with 0.5% formic acid in the cartridge for 5 minutes. Cartridges were placed under vacuum until dry. 100mM pH10 $NH_4H_2PO_4$ buffer was added to the filtrate. SPE cartridge (Agilent Bond Elut PBA, 12102127) we sequentially conditioned with acetonitrile, 5% formic acid in methanol, and 100mM pH10 $NH_4H_2PO_4$ buffer before loading of samples. Following sequential washes with 1% NH_4OH in 95% methanol, 1% NH_4OH in 95% acetonitrile, and 1% NH_4OH in 30% acetonitrile, cartridges were dried by vacuum. 5% formic acid in methanol was used for elution and evaporated under nitrogen flow at 35 ^oC. Samples and dopamine standard were kept at -80 ^oC until reconstitution with 100 µL of 0.1% formic acid in water for mass spectrometry analysis.

The samples were analyzed on a high-performance liquid chromatography system (Shimadzu, Duisburg, Germany) couple with a triple quadrupole mass spectrometer (Sciex QTRAP 6500+, Darmstadt, Germany). The Shimadzu LC-System (Duisburg, Germany) consisted of two LC20-AD_{XR} pumps, a SIL-30AC autosampler, a CTO-20AC column oven, a SPD-20A UV-detector and a CBM-20A controller, which was equipped with a Polar-RP SecurityGuard^{TM} cartridge (4 x 3 mm) (Phenomenex, Aschaffenburg, Germany) and a Accucore PFP column (100 x 2.1 mm, 2.6 µm) (Thermo Scientific). The mobile phase comprised ammonium formate [5 mM] with 0.05% formic acid in water as solvent A and methanol, LC/MS grade as solvent B with the following gradient programme: 0-1 min, 0 % B; 1.5 min, 95 % B; 5 min, 95 % B;5.5 min, 0% B; 15 min, 0 % B. The mass spectrometer used was an AB Sciex QTRAP 6500+ equipped with an Ion DriveTM Turbo V source with ESI-TurboIonSpray-Probe. Mass spectrometric analysis was performed in positive ionisation in multiple reaction monitoring mode (MRM) at unit resolution. Settings were as follows: Curtain Gas, 35; Collision Gas, High; IonSpray Voltage, 5500; Temperature, 600°C; Ion Source Gas 1, 50; Ion Source Gas 2, 70; Entrance Potential, 10.

Bone marrow nucleated cell number counting

For the counting of BMNCs, BM cells were processed to generate single cell suspensions as described for flow cytometry analysis. Red blood cells were removed using RBC lysis buffer (ebioscience, 00-4300-54). Total cell numbers were determined using a Luna 2 automated cell counter (Logos Biosystems) or cell counting plates. The number of lineage committed cells in BM was calculated based on flow cytometry analysis with appropriate markers and BMNC counting.

Peripheral blood count

Peripheral blood was extracted at submandibular vein, collected in EDTA-K tube and analyzed with a Scil Vet ABC plus according to the manufacturer's instructions. To analyze peripheral blood cells using FACS, whole blood was centrifuged at 1000g for 10 minutes and blood plasma was separated. The blood cells layers were resuspended for FACS analysis.

Dopamine immunostaining

The staining protocol is modified from the literature.[11] For dopamine staining, anesthetized mice were perfused with ice-cold PBS containing 1% (w/v) sodium metabisulfite (Sigma, S9000). After perfusion, bones were dissected and fixed in fixation solution including 2%PFA, 5% glutaraldehyde, 1% sodium meta-bisulfate at pH 7.0–7.5, 4°C overnight. After fixation, bones were washed with EDTA, sucrose solution and cryo-sectioned as described above. In the immunostaining process, sections were rehydrated with PBS containing 0.85% sodium metabisulfite (PMB solution). Sections were incubated in freshly prepared 0.1 M sodium borohydride (Sigma, S9125) for 10 min in PMB and then washed 4 times by PMB for 10 min each. Next, bone sections were incubated by PMB with 0.5% Triton X-100 15min and blocked by PMB with 1% BSA, 0.3% Triton X-100. Dopamine antibody (Abcam, ab8888, 1:200) was mixed with other antibodies diluted in blocking solution with sodium

metabisulfite and incubated at 4°C for 48 hours. Sections were washed three times for 10 minutes with PMB and incubate with secondary antibodies at room temperature for 2 hours. Staining with some other primary antibodies cannot be combined this protocol.

RNA sequencing and data analysis

Total RNAs were extracted using RNeasy Plus Micro kit (Qiagen) according to the manufacturer's instructions. Quality and quantity of RNA samples were analyzed with a Bioanalyzer and RNA 6000 pico kit (Agilent). Double strand cDNA was synthesized using SMART-Seq v4 Ultra Low Input RNA kit for Sequencing (Takara) and sequencing libraries were constructed with Nextera XT DNA Library Preparation Kit (Illumina) according to the manufacturer's instructions. The resulting sequencing libraries were sequenced with 2 x 75 bp paired-end reads on NextSeq 500 sequencer (Illumina). Sequenced reads were aligned to the mouse (mm10) reference genome with TopHat (version 2.1.1), and the aligned reads were used for the transcript quantification by using HTSeq-count (version 0.6.1). DESeq2 was used to identify differentially expressed genes across the samples. Heatmap was generated in a web tool (https://biit.cs.ut.ee/clustvis/). All RNA-seq raw data will be made publically available upon publication of the manuscript. Reviewer can access the data through the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE134444 (secure token: cnwlwsikvzeddqv) and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126219 (secure token: ydcfasukzjeftyv).

Statistical analysis

Mice that died before the completion of experimental protocols were excluded from analysis, which was a pre-established criterion before the experiment. No statistical methods were used to predetermine sample size. Before the Student's *t*-Test, samples from different groups were tested using *F*-test to identify the variances between groups. *F* value less than 0.05 indicated

samples have significantly different variances. Statistical data were drawn from normally distributed group. Unless indicated otherwise, samples were tested using two-tailed Student's *t* test. *P* values are indicated in the graphs and *P* values below 0.05 were considered to be statistically significant. All results are represented as mean ± s.e.m. Number of animals or cells represents biological replicates.

Data availability

RNA-seq raw data supporting this work have been deposited in GEO, under accession number GSE134444 and GSE126219. All original data are available upon reasonable request.

Supplemental Table 1. Summary of genetic mouse models in this study

Full Name	Short name	Inducible	Reporter	Purpose
Rosa26-mT/mG	*R26-mTmG*		Cre-induced switch from mem-Tomato to mem-GFP	Labeling of Cre-positive cells and their descendants
Vav1-Cre	-	No	-	Labeling of and gene knockout in hematopoietic cells and their descendants
Rosa26-DTR	-		-	Conditional deletion of Cre+ cells by diphtheria toxin injection
Th$^{tm1(Cre)Te}$	*Th-Cre*	No	-	Labeling of and gene knockout in Th+ cells and their descendants
Th-Cre Rosa26-DTR	*DTR*$^{i\Delta Th}$	No	-	Genetic elimination of Th+ cells by diphtheria toxin injection
Vav1-Cre Rosa26-mT/mG	*Vav1-mTmG*	No	mem-GFP after Cre recombination	Labeling of hematopoietic cells for analysis or as donor for transplantation
Th-Cre Rosa26-mT/mG	*Th-mTmG*	No	mem-GFP after Cre recombination	Used as recipient to label Th+ cells after wild-type cell transplantation
Drd2^{tm1Low}	*Drd2 -/-*	-	-	Analysis of *Drd2* function
Drd3^{tm1Dac}	*Drd3 -/-*	-	-	Analysis of *Drd3* function
Drd2^{tm1Low} *Drd3*^{tm1Dac}	*Drd* DKO	-	-	Analysis of D2-type dopamine receptor function
Lepr$^{tm2(cre)Rck}$	*Lepr-Cre*	No	-	Labeling of Lepr+ mesenchymal cells and their descendants
Lepr-Cre Rosa26-mT/mG	*Lepr-mTmG*	No	mem-GFP after Cre recombination	Labeling of Lepr+ mesenchymal cells and their descendants
Wnt1-Cre	-	No	-	Labeling of sympathetic nerve in bone marrow
Tg(Drd2-EGFP/Rpl10a)CP101Htz	*Drd2-EGFP*	No	EGFP	Indication of Drd2 expression

Supplemental Table 2. Summary of antibodies

Antibodies	Company	Catalogue number
Endomucin	Santa Cruz	SC-65495
GFP-Alexa488	Invitrogen	A21311
GFP	Aveslabs	GFP-1010
Th	Millipore	AB152
Th	Abcam	ab76442
Tuj1	Abcam	ab107216
TrkA	R&D	AF1056
αSMA-eFluor660	eBioscience	50-9760-82
αSMA-cy3	Sigma	C6198
γH2ax	Life span bioscience	LS-B-1474
Synaptophysin	Abcam	ab32594
Dopamin D2 receptor	Merck	AB5084P
Dopamin D3 receptor	Abcam	ab42114
Dopamin D3 receptor	ThermoScientific	32-0900
Linegae-biotin cocktail	Miltenyi Biotec	130-092-613
CD150-Alexa647	Biolegend	115918
CD48-biotin	eBioscience	13-0481
CD41-biotin	eBioscience	13-0411
Ki67	Abcam	ab15580
Sca1	Biolegend	108104
cKit	R&D	AF1356
Sca1-biotin	Biolegend	108104
Lineage cocktail-APC	BD Pharmingen	558074
Sca1-FITC	eBioscience	11-5981-85
Sca1-PE-Cy7	BD Pharmingen	558162
cKit-APC	BD Pharmingen	553356
cKit-FITC	Biolegend	105806
Ter119-APC	eBioscience	17-5921
CD45-FITC	eBioscience	11-0451
CD45R(B220)-APC	Invitrogen	RM2605
Ly-6G-APC	eBioscience	17-5931
CD11b-APC	Biolegend	101212
CD11b-FITC	BD Pharmingen	553310
CD4-APC	eBioscience	17-0042-82
CD8-APC	eBioscience	17-0081-82
CD150-PE	Biolegend	115904
CD48-APC-Cy7	BD Pharmingen	561242
CD45- Pacific Blue	Biolegend	103126
CD31-Alexa488	R&D	FAB3628G
CD31-APC	R&D	FAB3628A
CD45.1-FITC	Invitrogen	MCD45101
CD45.2 Pacific Blue	Biolegend	109820
CD45 PE-Cy7	eBioscience	25-0451-82
Ter119-PE-Cy7	eBioscience	25-5921-82
Phospho-ERK	Cell Signaling	4370
Phospho-MEK	Cell Signaling	9121
Phospho-Raf	ThermoScientific	44-506G

Phospho-Kit	Cell Signaling	3073
Phospho-histone H3	Upstate	06-570
Lck	Cell Signaling	2752
ERK	Cell Signaling	9102
MEK	Cell Signaling	9122
Raf	Invitrogen	PA5-95464
Dopamine	Abcam	Ab8888
CD34-eFluor450	ThermoScientific	48-0341
CD16/132 PE/Cy7	ThermoScientific	25-0161
CD127 PE	ThermoScientific	12-1271
Neurofilament-H	Neuromics	CH22104
Tuj1-Alexa488	Biolegend	657404

Supplemental Figure Legends

Supplemental Figure 1. Expression of dopamine receptors in BM, related to Figure 1

(A) Normalized dopamine level in BM at day and night by ELISA. N=5 in each time point. *P*-value, ANOVA, represent comparison of each time point versus value at 3am.

(B) RNA-seq expression analysis (Fpkm) of dopamine receptors in LSK, Lin- cells, total BM, endothelial cells, endothelial cells after irradiation, Lepr+, Ng2+, and Myh11+ cells. N.D., not detected. RNA sequencing data were published previously.[12,13]

(C) FPKM of *Drd2* in hippocampus based on literature.[14]

(D) FPKM of *Drd3* based on literature.[15]

(E) RPKM of *Drd3* based on ENCODE mouse transcriptome data in NCBI.

(F) Representative FACS histogram and quantification of GFP expression in different cell populations from littermate control (black) or *Drd2-EGFP* transgenic mice (green). N=4.

(G) Representative quantitative RT-PCR plot showing detection of *Drd2* and *Drd3* in LSK cells.

(H) Representative Western blot result showing Drd2 and Drd3 protein in WT but not *Drd* DKO midbrain cell lysate.

(I) Representative confocal images showing Drd2 and Drd3 expression in FACS-sorted LSK cells *ex vivo*. LSK cells were sorted by FACS and re-stained with c-Kit and Sca-1 together with primary anti-Drd2/Drd3 antibodies in slides. Control was stained with secondary antibody without primary anti-Drd2/Drd3 antibodies (see Methods for details).

(J, K) Confocal images showing Drd2 (J) and Drd3 (K) expression in Lin- c-Kit+ cells (arrows) in bone sections.

(L) FACS histograms of Drd2 expression in WT B220+, Gr-1+, Ter119+ and CD4/8+ hematopoietic cells. Ctrl samples were stained with secondary antibody only.

(M) FACS histograms of Drd3 expression in WT B220+, Gr-1+, Ter119+ and CD4/8+ hematopoietic cells. Ctrl samples were stained with secondary antibody only.

(N) Quantitative comparison of Drd2 and Drd3 in Lin- Neurofilament-H+ midbrain neurons and LSK cells. MFI is normalized to basal level of each cell type stained with secondary antibody only. N=6 for neurons, N=6 for LSK cells.

Supplemental Figure 2. Phenotypes caused by Drd knockout and haloperidol treatment, related to Figure 1.

(A) LSK, BMNC, CD45+, B220+, Gr-1+, CD11b+ and CD8+ cell percentage in BM after SCH-23390 treatment (vehicle=6; SCH-23390=6).

(B) Confocal images showing EdU incorporation in sections of distal long bone of vehicle and SCH-23390 treated mice. Quantification of EdU+ cell number in metaphysis (area=1mm^2; vehicle= 3; SCH-23390= 3).

(C) Quantification of LSK and HSC number and percentage in *Drd2* knockout and littermate control mice (*Drd2* +/+=6; *Drd2* -/-=10). Quantification of BMNCs in *Drd2* knockout and littermate control mice (*Drd2* +/+=6; *Drd2* -/-=6).

(D) Quantification of LSK and HSC number and percentage in *Drd3* knockout and littermate control mice (*Drd3* +/+=8; *Drd3* -/-=10). Quantification of BMNCs in *Drd3* knockout and littermate control mice (*Drd3* +/+=6; *Drd3* -/-=6).

(E) Diagram showing protocol of CD45.2/CD45.1 competitive repopulation assay, which applies to all competitive repopulation experiments in this study.

(F) Quantification of donor-derived (CD45.2) B-lymphocytes, T-lymphocytes and myeloid cells in competitive repopulating experiments (WT=7; *Drd* DKO=8). 5 x 10^5 CD45.2 (WT or *Drd* DKO) BM cells mixed with 5 x 10^5 CD45.1 BM cells were transplanted into lethally-irradiated CD45.1 recipient and analyzed 24 weeks later.

(G) Quantification of LSK and HSC number in peripheral blood and spleen of WT and *Drd* DKO mice (WT=8-9; *DKO* =8-9).

(H) Quantification of BMNC number in WT and *Drd* DKO mice (WT=8; *DKO* =8).

(I) Quantification of oligopotent hematopoietic progenitor cell percentage in WT and *Drd* DKO mice (WT=10; *DKO* =10).

(J) Quantification of mature hematopoietic cell percentage in WT and *Drd* DKO mice (WT=10; *DKO* =10).

(K) Quantification of BMNC number in WT mice without irradiation and at 2 months after lethal irradiation (12 Gy) followed BM transplantation. No irradiation= 4, irradiation and transplantation 2month= 11.

(L) Quantification of LSK% and HSC% in hematopoietic cell-specific *Drd* $DKO^{\Delta HC}$ mice at 2 months after lethal irradiation and transplantation. WT= 11; *Drd* $DKO^{\Delta HC}$ =9.

(M) Quantification of LSK% and HSC% in stromal cell-specific *Drd* $DKO^{\Delta SC}$ mice at 2 months after lethal irradiation and transplantation. WT= 7; *Drd* $DKO^{\Delta SC}$ =10.

(N) Quantification of LSK, HSC number and percentage in C57/B6 and 129 at steady-state (C57/B6=6; 129 =6).

(O) Quantification of BMNC, CD45, LSK cell number and LSK percentage in C57/B6 host mice at 2 weeks after transplantation with C57/B6 or 129 donor hematopoietic cells (C57/B6=5; 129 =5).

(P) Quantification of LSK, HSC and CD49b negative HSC percentage in C57/B6 host mice at 2 months after transplantation with C57/B6 or 129 donor hematopoietic cells (C57/B6=10; 129 =8).

(Q) Diagram depicting acute haloperidol treatment.

(R) Quantification of donor-derived (CD45.2) B-lymphocyte, T-lymphocyte and myeloid cells in competitive repopulating experiments (vehicle=10; haloperidol=9). 5 x 10^5 CD45.2

(vehicle or haloperidol) mixed with 5 x 10^5 CD45.1 BM cells were transplanted into lethally-irradiated CD45.1 recipients and analyzed 16 weeks later.

(S) Quantification of LSK cells and HSC percentage in BM after haloperidol treatment. Vehicle= 8; haloperidol= 12.

(T) Representative FACS histogram showing Annexin V signal in cultured HSCs in the presence of haloperidol or vehicle control. Quantitation of Annexin V+ HSC% *in vitro* after treatment. Control=4, Haloperidol= 4.

(U) Annexin V+ LSK% *in vivo* at 16 hours after haloperidol injection into WT mice (Control=4, Haloperidol= 5), and after diphtheria toxin injection into control or *DTR*$^{i\Delta Th}$ mice (Control=4, *DTR*$^{i\Delta Th}$ = 5).

(V) Quantification of Cxcl12 and stem cell factor (SCF) protein levels in flushed bone marrow extracellular fluid by ELISA in vehicle and haloperidol-treated mice (Cxcl12, vehicle=9; haloperidol=9; SCF, vehicle=8; haloperidol=8).

Supplemental Figure 3. Additional data for the elimination of Th+ cells, related to Figure 2.

(A-B) Representative confocal images showing that dopamine signal is not associated with Emcn+ vessel (A) and B220+ hematopoietic cells (B).

(C-F) Representative confocal images showing expression pattern of endogenous Th protein in combination with GFP signal from *Lepr-Cre R26-mTmG* mice (C), Emcn (D), αSMA (E) and lineage cocktail (Ter119, CD5, CD11b, CD45R-, Ly-6G/C) (F) in WT BM.

(G-I) *Th*$^{tm1(Cre)Te}$ (*Th-Cre*) mediated GFP expression (green) in *Rosa26-mTmG* reporter mice (*Th-mTmG*), which were transplanted with WT BM cells and analyzed 2 months after successful reconstitution. *Th-mTmG*-controlled GFP in combination with Emcn (G), αSMA (H) and Lineage cocktail (I).

(J) Representative FACS histogram and dot plots of GFP expression in different cell populations from midbrain and BM in Ctrl or stromal cell-specific *Th-mTmG* mice.

(K) Confocal images showing location of CD150+ CD48- CD41- Lin- HSC in relation to *Th-mTmG* GFP+ nerve fiber (green). CD150, red; CD48, CD41 and Lineage cocktail, blue. Left panel, single plane image showing location of HSC in relation to GFP signal (arrow). Right panels, z-axis maximum projection images of individual color channels.

(L) Distance between CD150+ CD48- CD41- Lin- HSCs or random (DAPI-labeled) cells and GFP+ nerves in thick section from *Th-mTmG* recipient mice (n= 41 HSCs and 460 random cells).

(M) Validation of lost Th signal in $DTR^{i\Delta Th}$ mice after DTX administration.

(N) Normalized dopamine in bone marrow extracellular fluid of Ctrl and $DTR^{i\Delta Th(SC)}$ mice after diphtheria toxin injection by ELISA(Ctrl=7; $DTR^{i\Delta Th(SC)}$=6) or mass spectrometry (Ctrl=3; $DTR^{i\Delta Th(SC)}$=3).

(O-P) Validation of lost Th+ nerve and dopamine signal near BCAM+ vessel in $DTR^{i\Delta Th(SC)}$ (P) but not littermate ctrl (O) mice.

(Q) Quantification of Th+ area and dopamine+ area in $DTR^{i\Delta Th(SC)}$ (N=3) and littermate ctrl mice (N=3).

(R) Quantification of LSK cell percentage in BM of Cre- mice after vehicle or DTX administration. Vehicle=5; Diphtheria toxin=10.

(S) Maximum intensity projections showing EdU incorporation in sections of distal long bone and, at higher magnification, in metaphysis (MP) of $DTR^{i\Delta Th}$ mice. Quantification of EdU+ cell number in metaphysis (area=1mm^2). Ctrl = 4; $DTR^{i\Delta Th}$ = 4.

Supplemental Figure 4. Additional data for RNA sequencing of LSK cells, related to Figure 3.

(A) Expression (Fpkm) of *Adrb2* (encoding β2 adrenergic receptor) and *Adrb3* (encoding β3 adrenergic receptor) in LSK cells based on RNA sequencing. N=3.

(B, C) Percentage of LSK cells (B) and of CD45+ leukocytes, B220+ B-lymphocytes, Gr-1+ myeloid cells, CD11b+ myeloid cells, CD4+ T-lymphocytes, CD8+ T-lymphocytes in BM (C) after ICI-118,551 treatment (vehicle=6; ICI-118,551= 6). ICI-118,551 dosage (2ug/g).

(D) EdU incorporation in sections of distal long bone vehicle and ICI-118,551 treated mice. Quantification of EdU+ cells in metaphysis (vehicle= 5; ICI-118,551=5).

(E) Unsupervised cluster analysis of bone marrow cells (BMC), WT LSK cells and *Drd* DKO LSK cells, indicating that *Drd* DKO LSK cells retain LSK features in comparison to total BMC cells.

(F) Gene-ontology analysis indicating the top upregulated pathways.

(G) KEGG analysis indicating changes related to B cell receptor pathway, T cell receptor pathway, immunodeficiency, MAPK pathway and neurotrophin pathway.

(H) Principal component analysis of haloperidol-treated and vehicle control LSK cells in relation to total BM cells (BMC).

(I) Unsupervised clustering of RNA sequencing results from haloperidol-treated and vehicle control LSK cells.

(J) Bland-Altman plot showing 1253 upregulated and 1418 downregulated genes in haloperidol-treated LSK cells relative to control at $p<0.05$ significance level. Red dots indicate differentially expressed genes.

(K-L**)** Heatmap of selected cell cycle (K) and chromosome segregation-related genes (L) in haloperidol-treated LSK cells.

(M) Schematic diagram depicting the transient EdU incorporation protocol in haloperidol-treated mice.

(N) Representative confocal images of DAPI and γH2AX stained in FACS-isolated LSK cells from haloperidol treated mice and vehicle control. Quantification the intensity of γH2AX in

sorted LSK cells (Ctrl=50 cells from 4 vehicle-treated mice; haloperidol=77 cells from 4 mice).

Supplemental Figure 5. Additional data for nerve morphology after lethal irradiation, related to Figure 4.

(A) Schematic diagram showing the transplantation of *Vav1-Cre Rosa26-mTmG* Lin- cells into lethally irradiated recipients followed by treatment with haloperidol or vehicle control.

(B) Overview of bone sections from haloperidol-treated or control mice after lethal irradiation and transplantation of GFP-labeled donor cells. Green, donor-derived hematopoietic cells; Red, EdU; Blue, Emcn.

(C) Representative confocal images showing GFP+ cells (green), Emcn+ endothelial cells (blue) and EdU incorporation (red) from haloperidol-treated or control (Vehicle) bone.

(D) Percentage of donor derived GFP+ and GFP+ CD45+ cells by FACS (vehicle=7; haloperidol=8). Quantification of EdU+ cell in each field by imaging (vehicle=5; haloperidol=5).

(E) Representative images (dish at day 8 after seeding) and quantification of colony forming units from about 200 HSCs isolated from wild-type mice. Haloperidol or vehicle was mixed with MethoCult^{Tm} medium before seeding of HSCs (vehicle=6; Haloperidol=6).

Supplemental Figure 6. Additional data on the effects of 7-OH-DPAT after irradiation and transplantation, related to Figure 5.

(A) Representative images showing morphology of Tuj1+ nerve fibers together with Th in BM after lethal irradiation.

(B) Overview images and high magnifications of *Wnt1-Cre R26-mTmG* labelled nerve in unirradiated mice or 7 days after 12 Gy. Quantitation of GFP+ nerve length. The ratio of GFP+ nerve length to femur length is normalized to 1 in Ctrl. Ctrl=6; 12 Gy=6.

(C) Representative images of synaptophysin staining together with DAPI signal in unirradiated BM or at 7 days after 12 Gy.

(D) Normalized dopamine level in ctrl (N=3) and lethally-irradiated (N=3) BM by mass spectrometry.

(E) Dopamine immunostaining together with Tuj1 in unirradiated BM or 7 days after 12 Gy. Arrows indicate co-localization of dopamine and Tuj1 signals.

(F) Colony forming units (GM, E, GEMM) from WT HSCs treated with 7-OH-DPAT or vehicle (vehicle=12; 7-OH-DPAT=12).

(G) Schematic depiction of the transplantation of Lin- cells from *Drd* DKO donors into 7-OH-DPAT-treated or vehicle control recipients (long treatment regime). Quantitation of BMNC, CD45+, CD11b+, and B220+ cell numbers in 7-OH-DPAT-treated or vehicle control recipients transplanted with *Drd* DKO donor cells. Vehicle=7 mice; 7-OH-DPAT=7 mice.

(H) Schematic diagram showing culture of WT or *Vav1-mTmG* LSK treated with 7-OH-DPAT *in vitro* and transplantation into lethally-irradiated recipients.

(I) Quantitation of fold change of HSC cell number from WT or *Drd* DKO mice after 7 days of culture in stem cell medium and 7-OH-DPAT treatment. Fold change relative to input cell number at day 0. WT vehicle= 4; WT 7-OH-DPAT= 4; DKO vehicle= 4; DKO 7-OH-DPAT= 6. *P*-values, ANOVA.

(J) Representative FACS plots showing GFP+ cells in recipients transplanted with cultured Vav1-mTmG cells pretreated with 7-OH-DPAT *in vitro*.

(K) GFP+, GFP+CD45+ and GFP+CD11b+ cell percentage at 5 days after transplantation of cultured *Vav1-mTmG* cells pretreated with 7-OH-DPAT into lethally-irradiated WT mice. Vehicle= 7; 7-OH-DPAT= 7.

Supplemental Figure 7. Additional data on the signaling effects triggered by dopamine in LSK cells, related to Figure 6.

(A-C) Schematic protocol for intracellular staining together with drug treatment of BM cells *ex vivo* (A), gating strategy for LSK cells after fixation with intracellular staining (B) and representative histogram showing flow cytometric analysis of ERK pathway protein and c-Kit phosphorylation in LSK cells stimulated with SCF (C). For details, see section on flow cytometry in methods.

(D) Normalized MFI (nMFI) of unmodified ERK, MEK, Raf in WT (N=4) or *Drd* DKO (N=4) LSK cells at steady state or after SCF treatment.

(E) Normalized MFI (nMFI) of ERK pathway phosphorylation after vehicle (N=4-6) or haloperidol (N=4-6) treatment of LSK cells.

(F) Quantification of ERK pathway protein phosphorylation in LSK cells after lethal irradiation and transplantation together with vehicle (N=6-7) or haloperidol (N=6) treatment.

(G) Normalized MFI of ERK pathway protein phosphorylation in LSK cells treated with SCF together with vehicle (N=3-4) or haloperidol (N=4).

(H) Quantification of normalized MFI of ERK phosphorylation in LSK cells after Marimastat (MMP inhibitor, N=4) and Barbadin (β-arrestin inhibitor, N=4) relative to control (N=4). *P*-values, ANOVA.

(I) Normalized MFI of ERK pathway protein phosphorylation after vehicle (N=4) or PP2 (N=3-5) treatment on LSK cells.

(J) Quantification of ERK pathway protein phosphorylation in LSK cells treated with SCF in combination with vehicle (N=3-5) or the inhibitor PP2 (N=3-5).

(K) RNA sequencing analysis of the Src family kinases *Lck*, *Fyn*, *Lyn* and *Src* in different types of BM cells.

(L) Lck expression in HSPCs according to public scRNA-seq data[16].

(M) Fpkm value of *Lck* in vehicle control and haloperidol-treated LSK cells. N=3 in each group. Normalized MFI of total Lck in LSK cells with vehicle control (N=4) or haloperidol (N=4) treatment.

(N) Normalized MFI (nMFI) of unmodified ERK, MEK, Raf in vehicle (N=4) or A-770041 (N=4) treated LSK cells at steady state or after SCF treatment.

(O) Colony formation and quantification of total CFU, BFU, CFU-GM numbers from 200 isolated WT HSCs following vehicle (N=5) and A-770041 (N=5) treatment. FACS quantify the percentage of CD45+ and CD11b+ cells in total cells harvested from *in vitro* CFU cultures in vehicle (N=5) and A-770041 (N=5) treatment. Quantification the absolute number of CD45+ cell harvested from *in vitro* CFU cultures in vehicle (N=5) and A-770041 (N=5) treatment.

Supplemental Figure 8. Additional data on lentiviral-mediated Lck expression, related to Figure 7.

(A) Quantitative PCR analysis of *Lck* transcripts in bEnd.3 cells after *shLck* infection (*shCtrl*=6, *shLck-1*=3, *shLck-2*=3) or *Lck* overexpression (*GFP*=3, *Lck*=3). *P*-values, ANOVA.

(B) Total cell number in CFU assays with LSK cells infected with *shCtrl* (N=6) or *shLck* (N=6).

(C) 2nd CFU formation by cells from 1st CFU assay infected with *shCtrl* or *shLck*. Quantitation of total CFU number and absolute cell number per dish. *shCtrl* (N=4); *shLck* (N=4).

(D) Quantification of BMNC number and LSK% after transplantation of WT LSK cells infected with *shCtrl* (N=12) or *shLck* (N=12) into lethally irradiated WT mice.

(E) Quantitative PCR analysis of *Kit* transcripts in bEnd.3 cells after *shKit* infection (*shCtrl*=3, *shKit-1*=3, *shKit-2*=3). *P*-values, ANOVA.

(F) Quantitation of ERK phosphorylation in LSK cells infected with *shKit-2*, *Lck* and their corresponding controls with or without SCF treatment (N=7-9). *P*-values, ANOVA.

(G) Total cell number in CFU assays with *Drd* DKO LSK cells infected with *GFP* (N=8) or *Lck* overexpression (N=8).

(H) 2nd CFU formation by *Drd* DKO cells from 1st CFU assay infected with *GFP* or *Lck* overexpression construct. Quantitation of total CFU number and absolute cell number per dish. *GFP* (N=5); *Lck* overexpression (N=5).

(I) Quantification of HSC (Slam LSK) cell number by FACS after virus infection of LSK cells. DKO LSK cells are infected with *GFP* or *Lck* virus. WT LSK cells are uninfected. WT (N=3), DKO+*GFP* (N=6), DKO+*Lck* (N=6). *P*-values, ANOVA.

(J) Quantitation of Lck protein in WT and *Drd* DKO LSK cells treated with vehicle or H-89 (left). ERK phosphorylation of WT LSK cells, and of *Drd* DKO LSK cells treated with vehicle or H-89, together with SCF *ex vivo*. WT=4; DKO+vehicle=5; DKO+H-89=5. *P*-values, ANOVA.

Supplemental Figure 9. *Drd2* and *Drd3* transcripts are difficult to detect by single cell RNA sequencing in brain.

(A) *Drd2* and *Drd3* transcripts are low or undetectable in Allen brain map transcriptomics explorer (https://celltypes.brain-map.org/rnaseq/mouse_ctx-hip_10x).

(B) *Drd2* and *Drd3* transcripts are low or undetectable in Tabula Muris scRNA-seq database (https://tabula-muris.ds.czbiohub.org/).

(C) *Drd2* and *Drd3* transcripts are low or undetectable in Broad Institute scRNA-seq database (https://singlecell.broadinstitute.org/single_cell/study/SCP479/single-nuclei-dataset-sn-vta-md721#study-visualize).

(D) *Drd2* and *Drd3* transcripts are low or undetectable in atlas of mouse brain from mousebrain.org database (http://mousebrain.org/genesearch.html).

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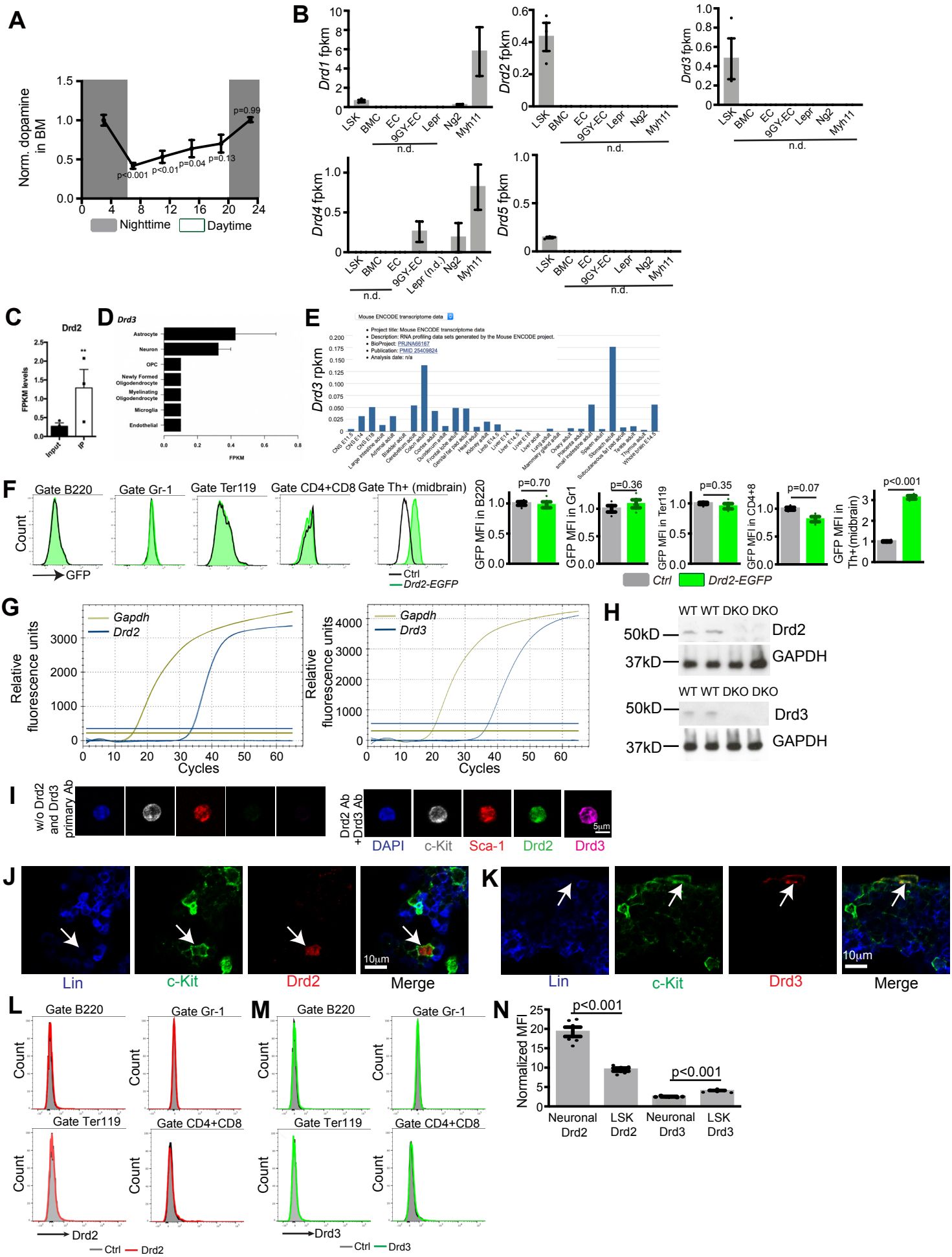
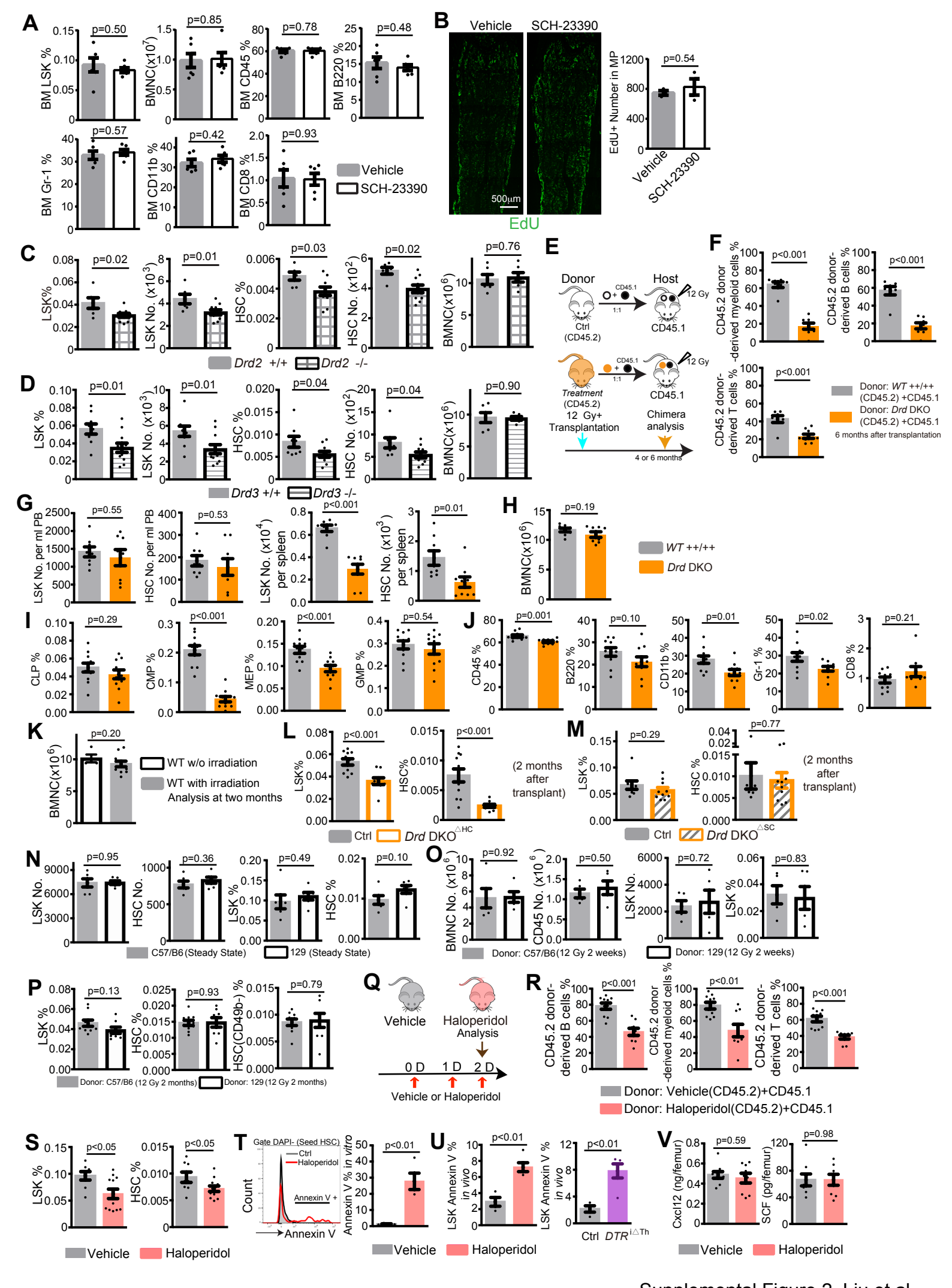


Supplemental Figure 1, Liu et al.



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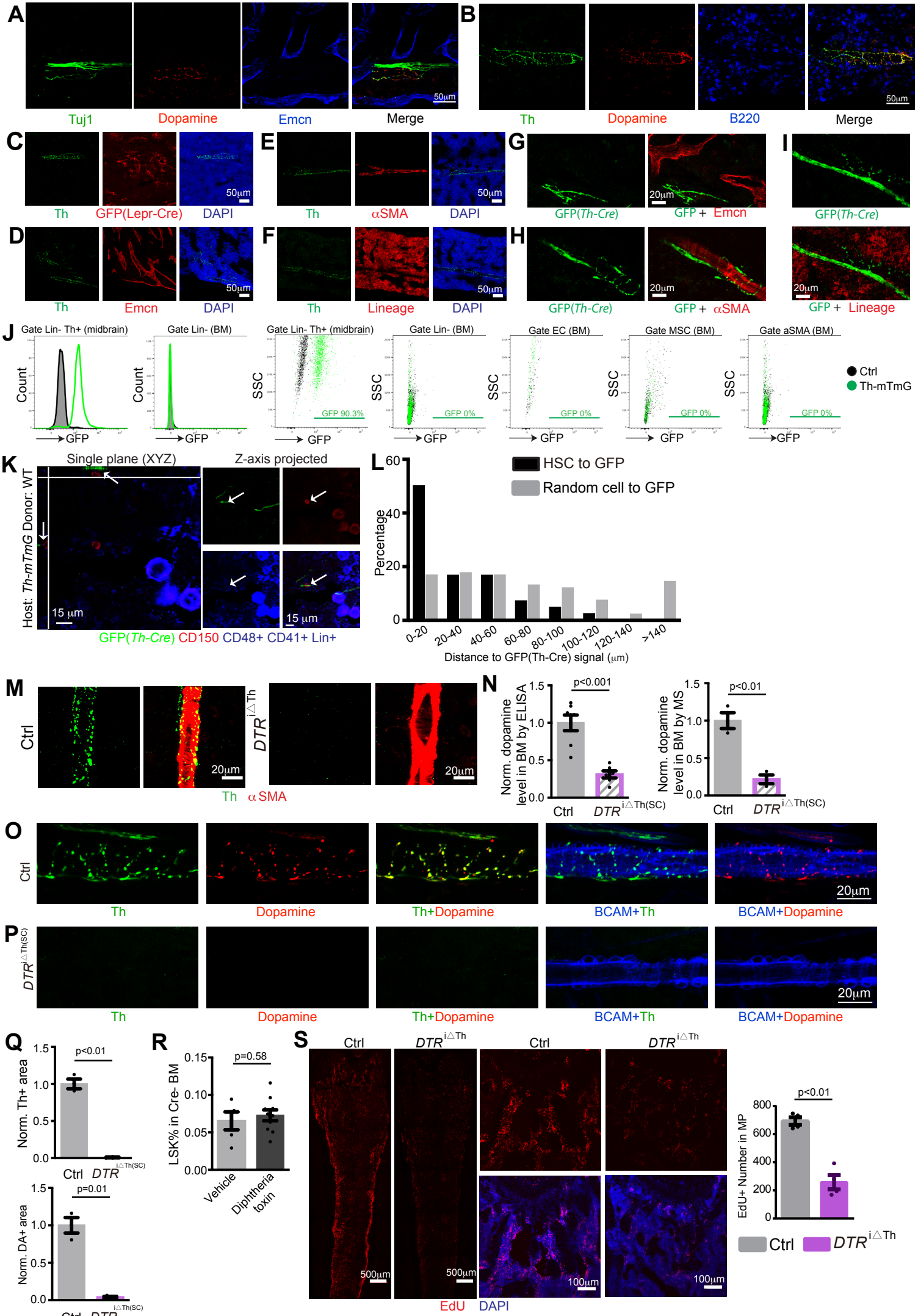

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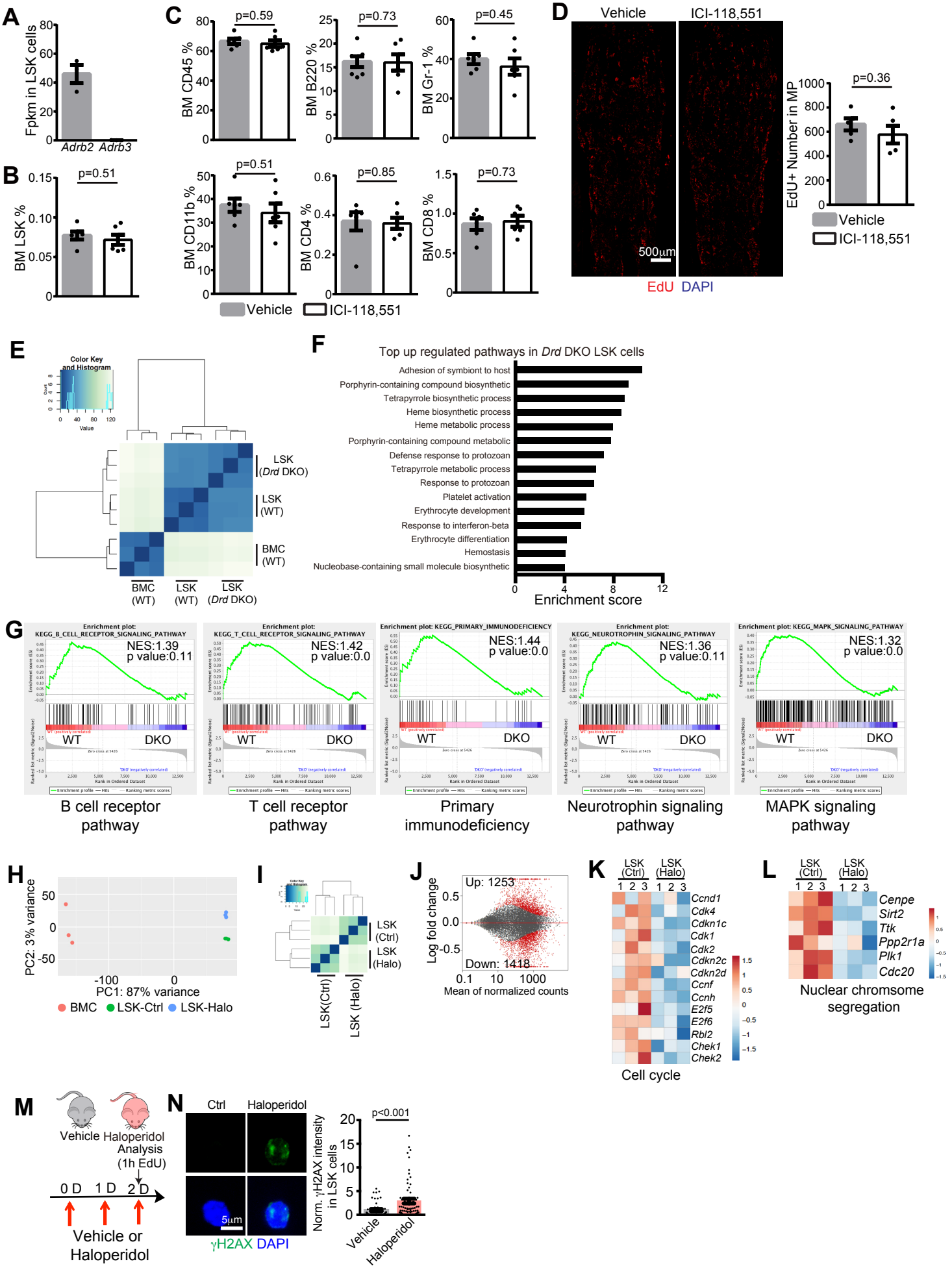

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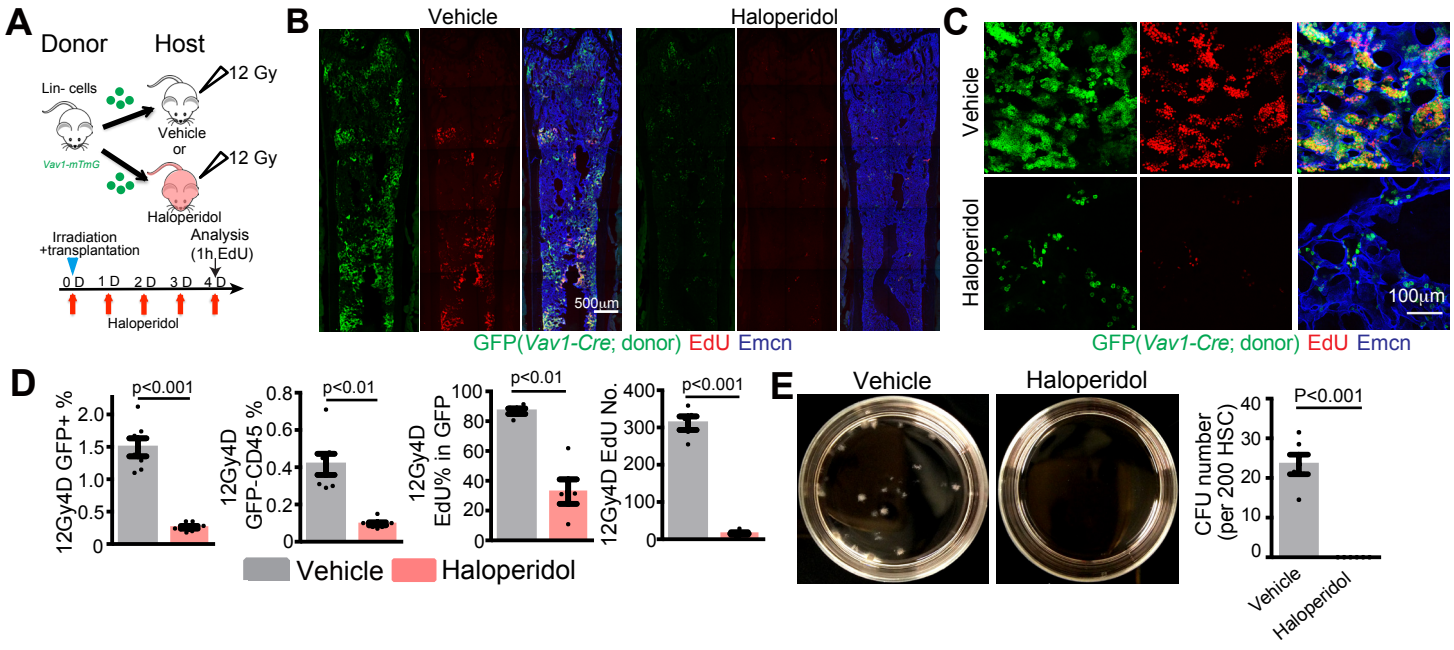
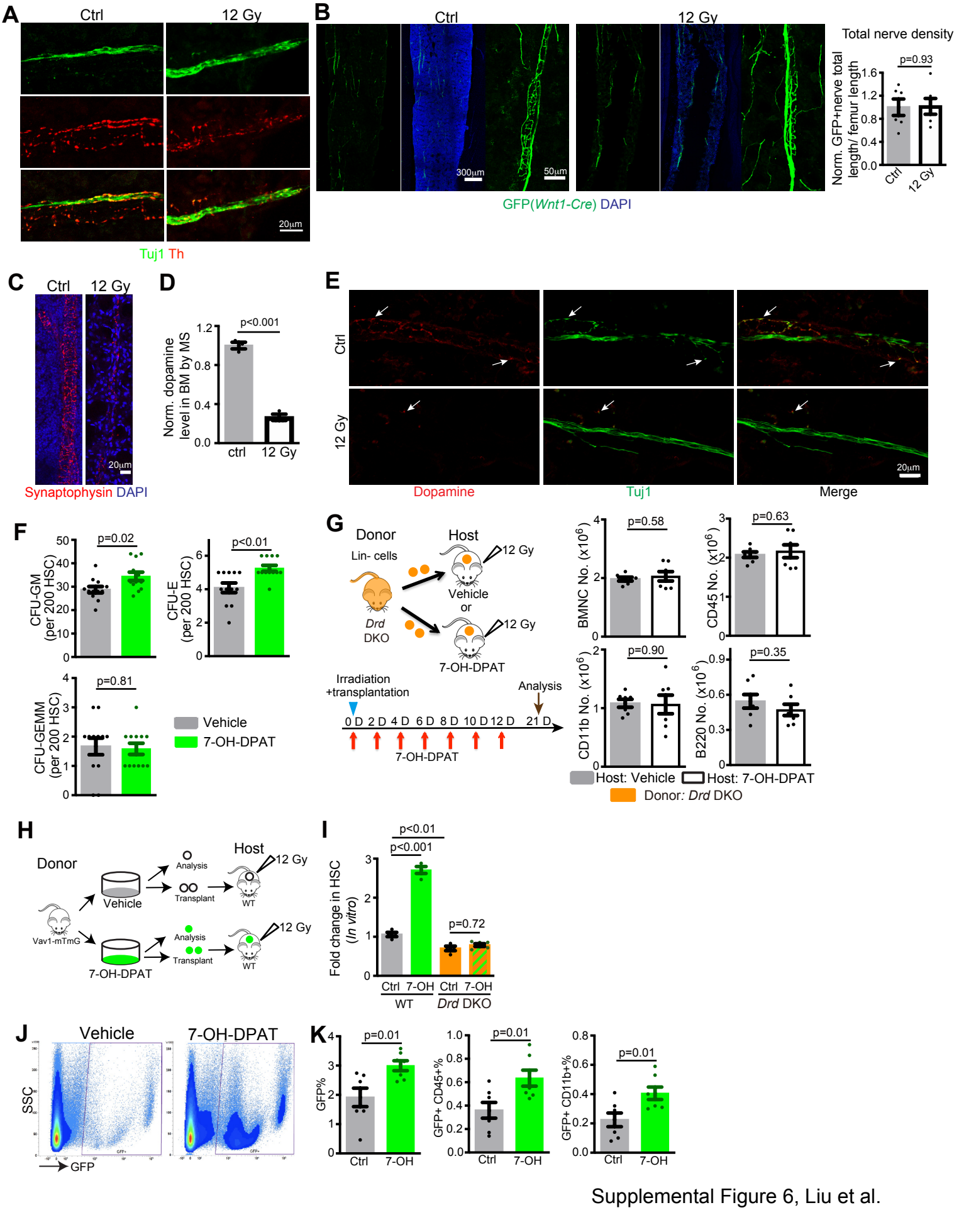
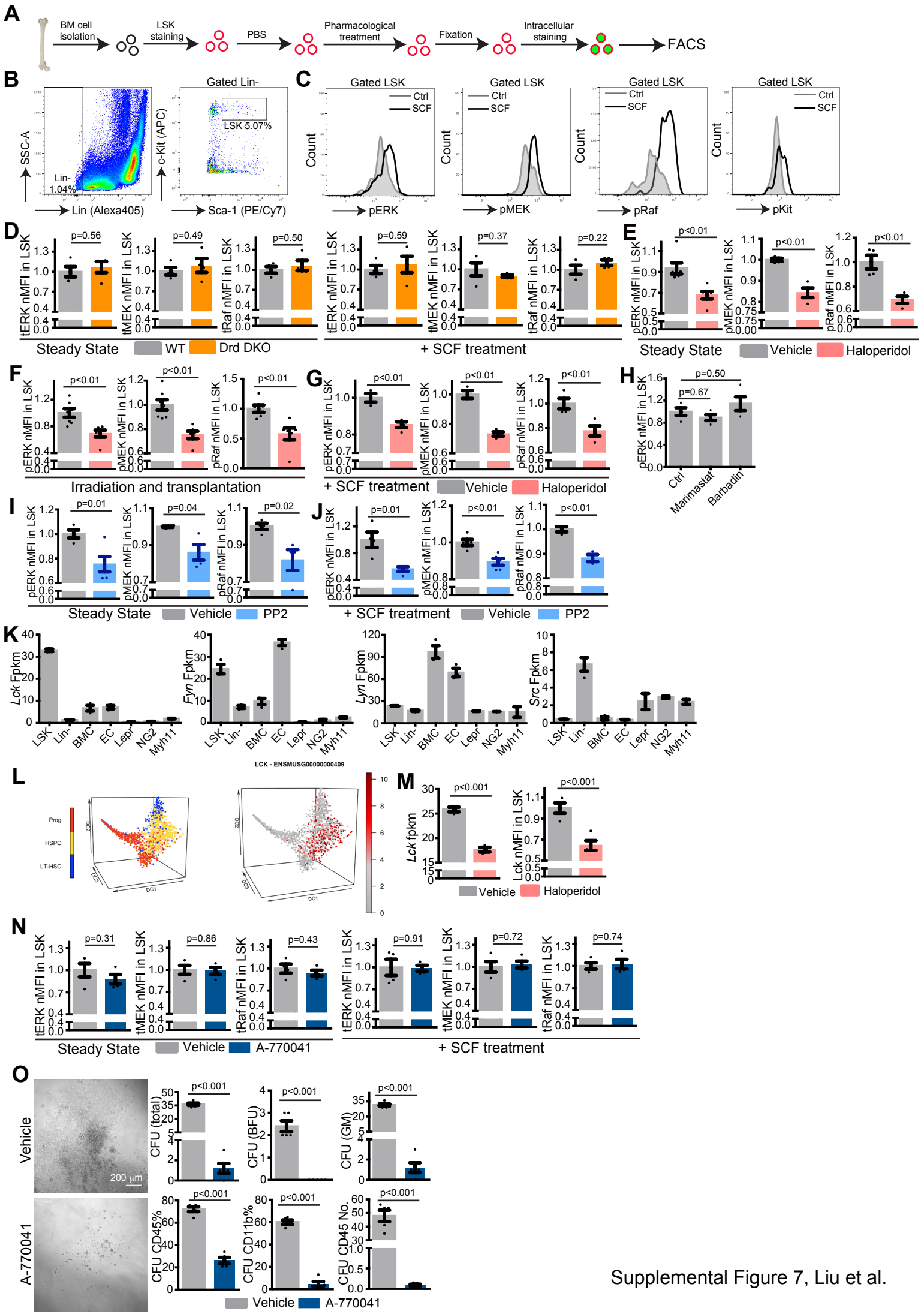


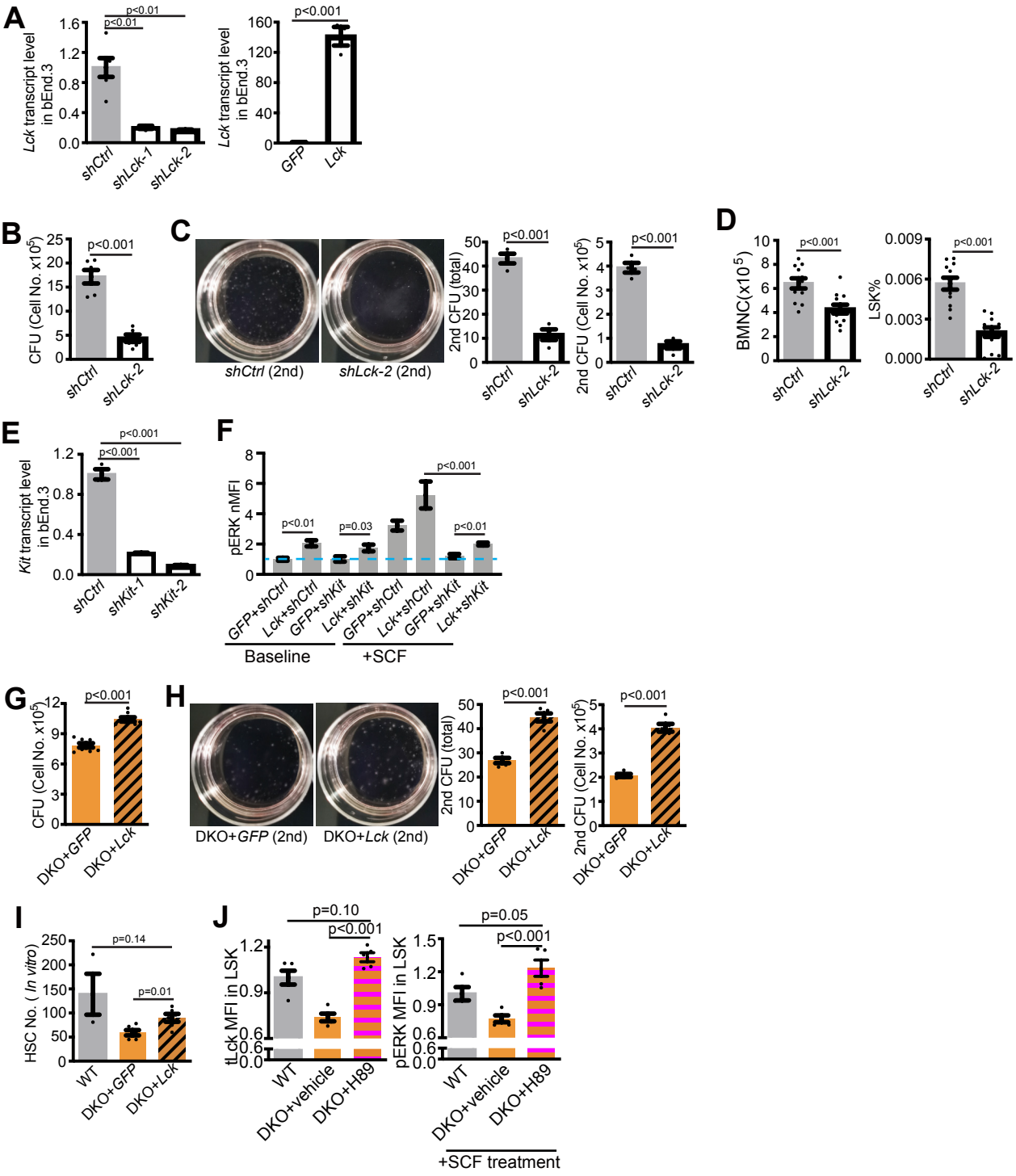
Supplemental Figure 5, Liu et al.



Supplemental Figure 6, Liu et al.



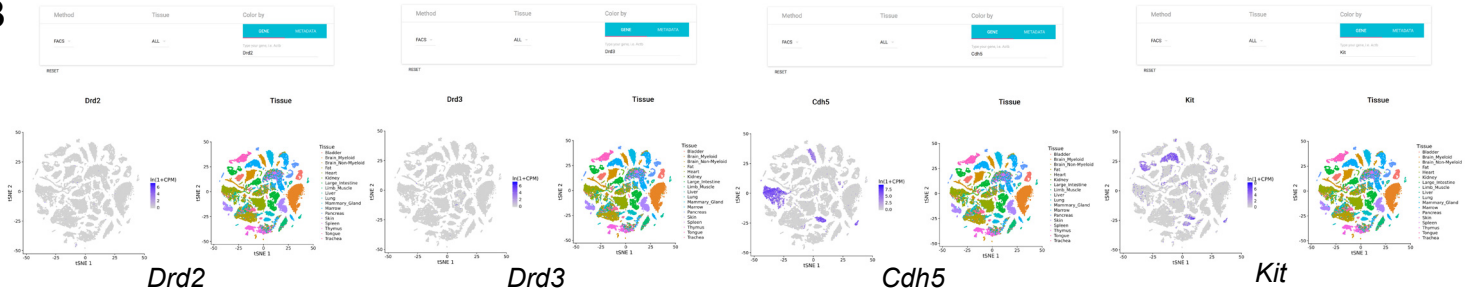
Supplemental Figure 7, Liu et al.



Supplemental Figure 8, Liu et al.

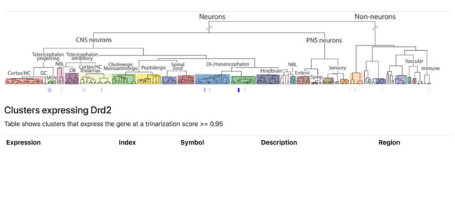
A

B

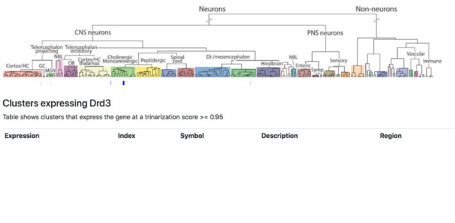


C

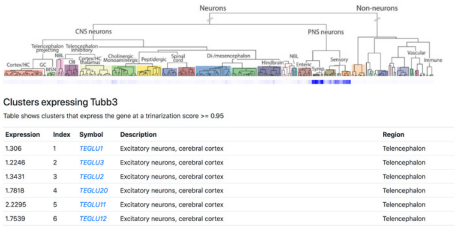
D



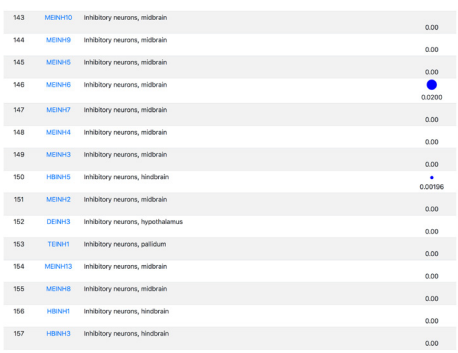
No. of cluster expressing *Drd2*: 0



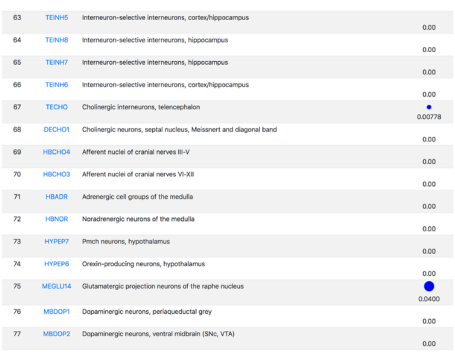
No. of cluster expressing *Drd3*: 0



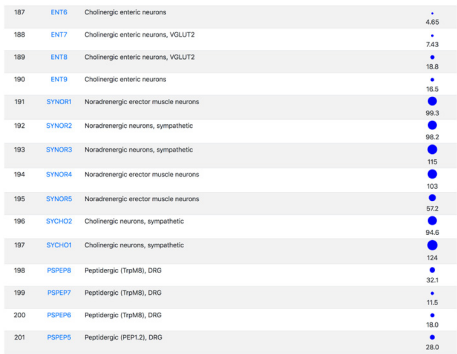
No. of cluster expressing *Tubb3*: 242



Highest expression level of *Drd2:* 0.02



Highest expression level of *Drd3:* 0.04



Highest expression level of *Tubb3:* 124

Supplemental Figure 9, Liu et al.