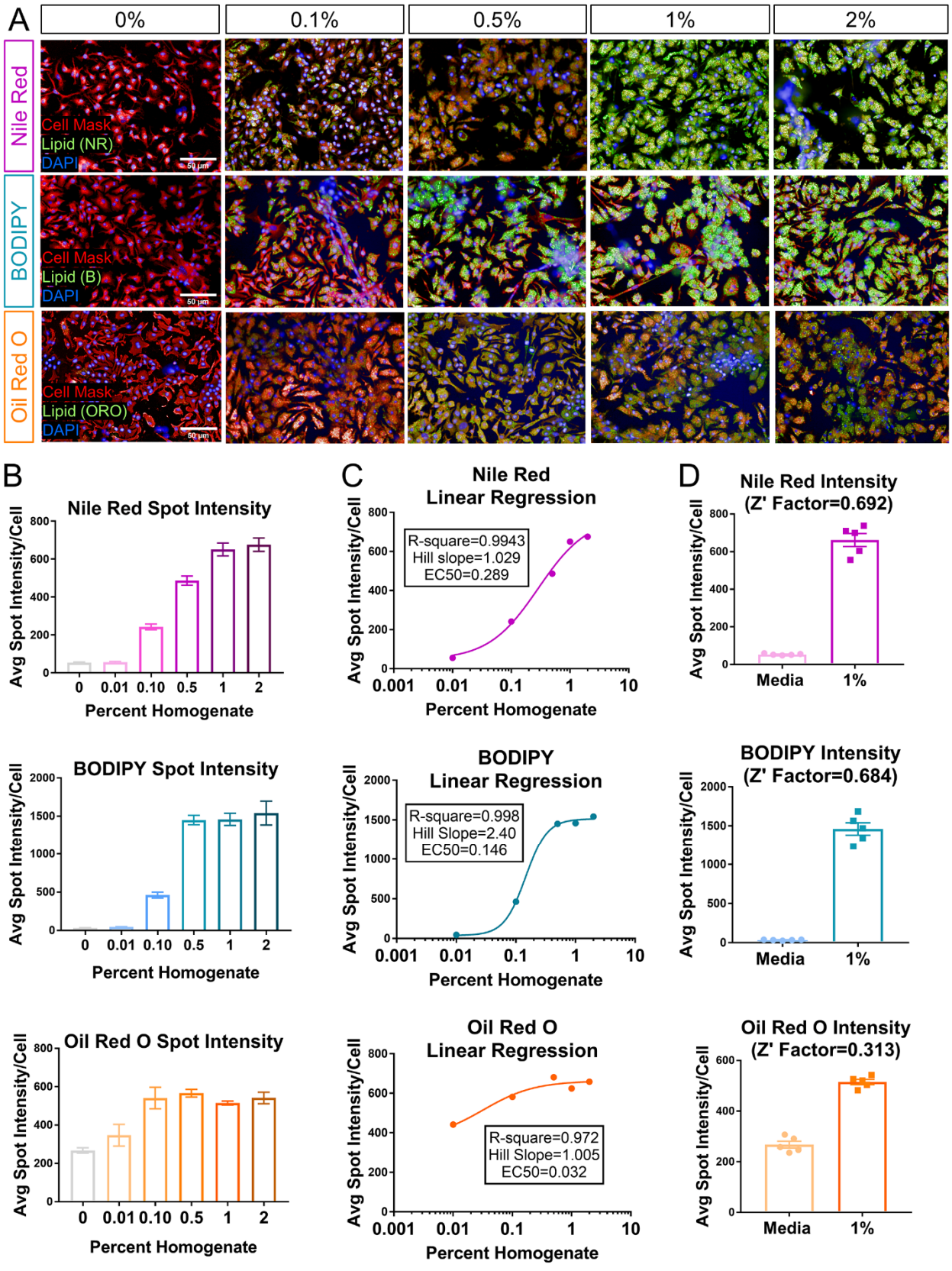
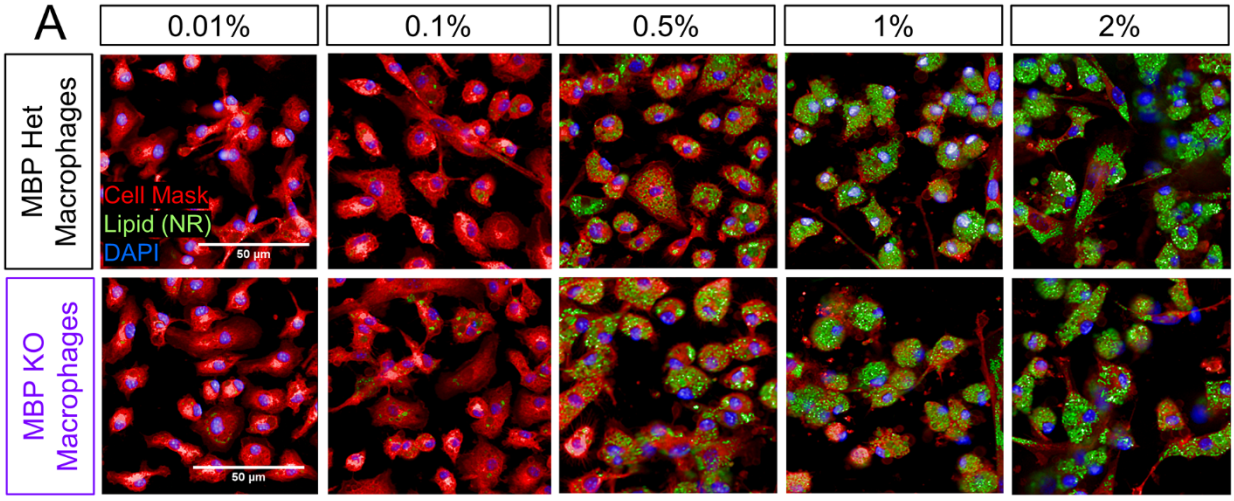
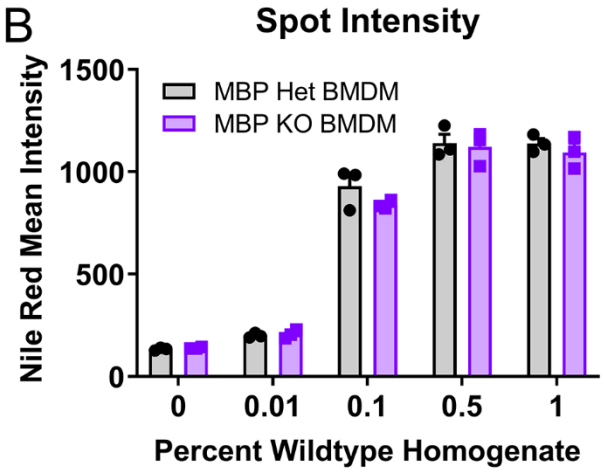
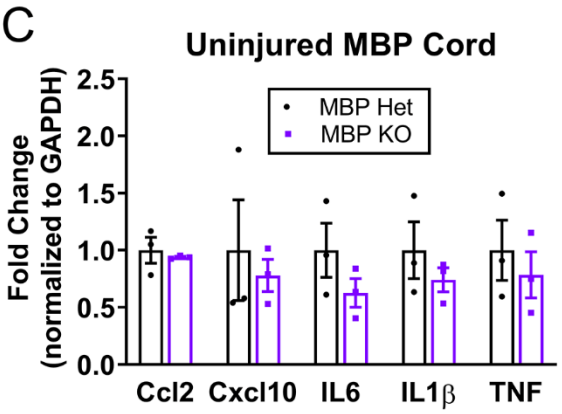
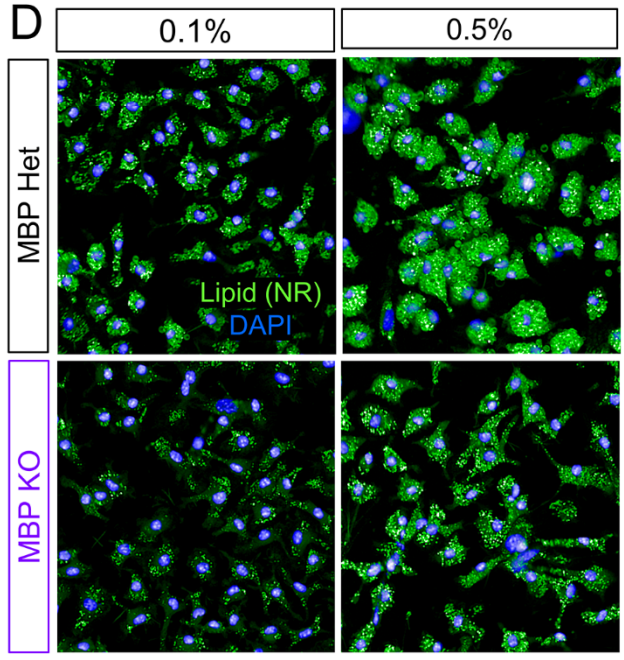

A
0%
0.1%
0.5%
1%
2%
Nile Red
Cell Mask
Lipid (NR)
DAPI
BODIPY
Cell Mask
Lipid (B)
DAPI
Oil Red O
Cell Mask
Lipid (ORO)
DAPI
50 µm
B
Nile Red Spot Intensity
Avg Spot Intensity/Cell
Percent Homogenate
BODIPY Spot Intensity
Avg Spot Intensity/Cell
Percent Homogenate
Oil Red O Spot Intensity
Avg Spot Intensity/Cell
Percent Homogenate
C
Nile Red
Linear Regression
R-square=0.9943
Hill slope=1.029
EC50=0.289
Avg Spot Intensity/Cell
Percent Homogenate
BODIPY
Linear Regression
R-square=0.998
Hill Slope=2.40
EC50=0.146
Avg Spot Intensity/Cell
Percent Homogenate
Oil Red O
Linear Regression
R-square=0.972
Hill Slope=1.005
EC50=0.032
Avg Spot Intensity/Cell
Percent Homogenate
D
Nile Red Intensity
(Z' Factor=0.692)
Avg Spot Intensity/Cell
Media
1%
BODIPY Intensity
(Z' Factor=0.684)
Avg Spot Intensity/Cell
Media
1%
Oil Red O Intensity
(Z' Factor=0.313)
Avg Spot Intensity/Cell
Media
1%

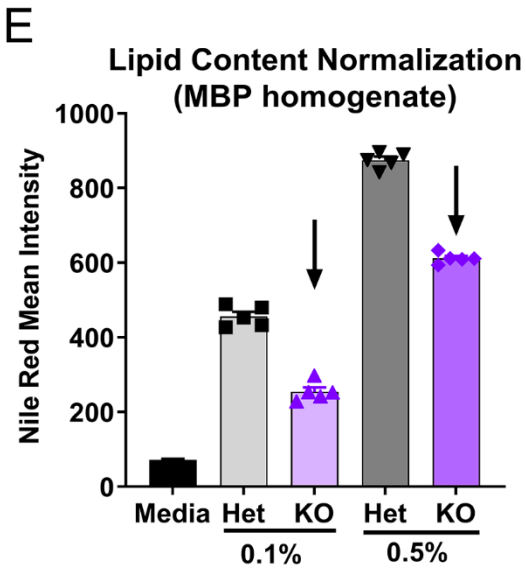
Supplemental Figure 1. Comparison of lipid dyes demonstrates that Nile Red is the most suitable for spinal cord homogenate-based *in vitro* phenotypic assay. (A) Representative images comparing lipid dyes Nile Red, BODIPY, and Oil Red O (all pseudo-colored in green) in cells treated with media, 0.1, 0.5, 1.0 and 2.0% spinal cord homogenate. Cell mask (red) and nuclei (blue). Scale bar=50 μm. (A) Mean spot intensity per cell at each percent homogenate in cells stained with Nile Red, BODIPY, Oil Red O (n=10 technical replicates, error bars=SEM). (C) 4-parameter nonlinear regression fit for homogenate dose response in cells stained with Nile Red, BODIPY, Oil Red O (n=10 technical replicates, error bars=SEM. Goodness of fit assessed by R-square value and linearity of response calculated by Hill's Slope (1.0=perfectly linear), x-axis in logarithmic scale. (D) Z' factor calculations between positive (1% homogenate) and negative (media only) controls for lipid droplet intensity (n=5 technical replicates).


A
0.01%
0.1%
0.5%
1%
2%
MBP Het Macrophages
MBP KO Macrophages
Cell Mask
Lipid (NR)
DAPI
50 µm
50 µm

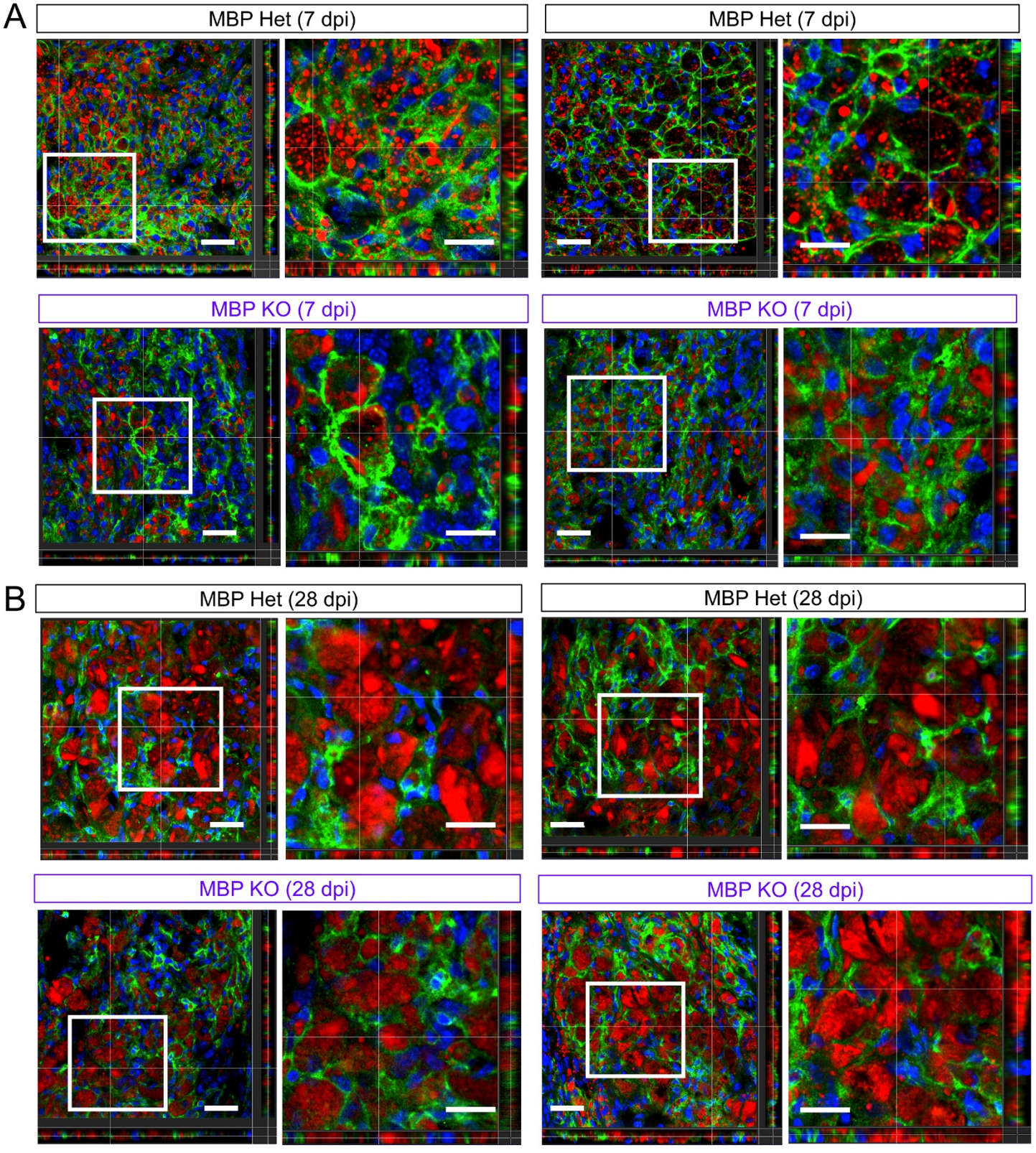

B
Spot Intensity
Nile Red Mean Intensity
1500
1000
500
0
MBP Het BMDM
MBP KO BMDM
0
0.01
0.1
0.5
1
Percent Wildtype Homogenate


C
Uninjured MBP Cord
Fold Change (normalized to GAPDH)
2.5
2.0
1.5
1.0
0.5
0.0
MBP Het
MBP KO
Ccl2
Cxcl10
IL6
IL1β
TNF


D
0.1%
0.5%
MBP Het
MBP KO
Lipid (NR)
DAPI


E
Lipid Content Normalization
(MBP homogenate)
Nile Red Mean Intensity
1000
800
600
400
200
0
Media
Het
KO
Het
KO
0.1%
0.5%

Supplemental Figure 2. Comparisons between MBP Het and KO BMDMs and uninjured spinal cord. (A) Representative images from macrophages obtained from MBP Het and MBP KO treated with 0, 0.01, 0.1, 0.5, and 1% wildtype homogenate Neutral lipid droplets stained with Nile Red (green), cell membrane with Cell Mask (red), and nuclei with DAPI (blue), scale bar=50 μm. (B) Mean lipid droplet spot intensity in MBP Het and MBP KO macrophages treated with 0, 0.01, 0.1, 0.5, and 1% wildtype homogenate (n=3 technical replicates per group, two-way ANOVA with Bonferroni multiple comparisons, error bars SEM). (C) Inflammatory cytokine expression in uninjured MBP Het and MBP KO spinal cord homogenate. (n=3 biological replicates per group, *p≤0.05 compared to MBP Het, Two-tailed Student's t-test per cytokine, error bars=SEM) (D) Representative images from wildtype macrophages treated with MBP Het and KO homogenates at 0.1% and 0.5% concentrations Neutral lipid droplets stained with Nile Red (Kroner et al.), cell membrane with Cell Mask (red), and nuclei with DAPI (blue). (E) Lipid droplet intensity in macrophages treated with 0.1% and 0.5% homogenate (n=5 technical replicates per condition, error bars=SEM). Wildtype cells treated with 0.1% MBP Het homogenate exhibited similar fluorescence to wildtype cells treated with 0.5% MBP KO homogenate.


A
MBP Het (7 dpi)
MBP Het (7 dpi)
MBP KO (7 dpi)
MBP KO (7 dpi)
B
MBP Het (28 dpi)
MBP Het (28 dpi)
MBP KO (28 dpi)
MBP KO (28 dpi)

Supplemental Figure 3. Oil Red O stained lipids localize to interior of CD11b+ cells. (A) Representative confocal images including orthogonal projections from the SCI injury site in MBP Het and MBP KO mice at 7 days (A) and 28 days (B) post injury (dpi). Lipid droplets stained with Oil Red O (red), myeloid cells labeled with CD11b (green) and nuclei with DAPI (blue). Lower magnification scale bar=30 μM in (A) and 200 μm in (B), higher magnification scale bar 20 μM. Representative images from two animals from Figure 4 are shown.