Mutant Huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors

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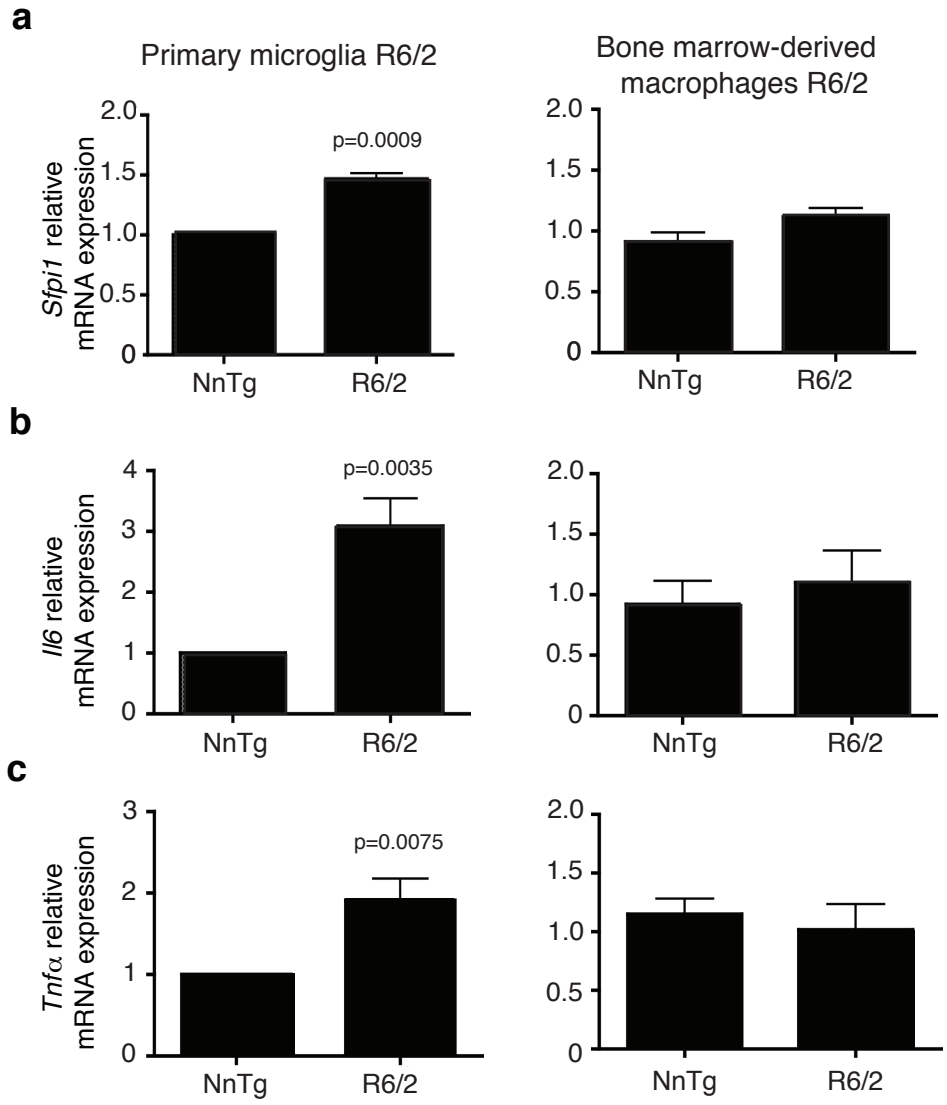
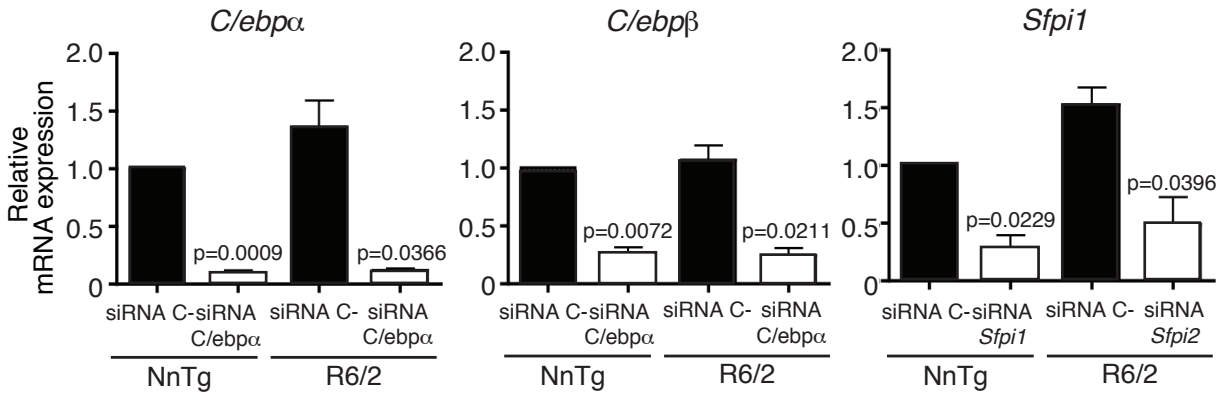
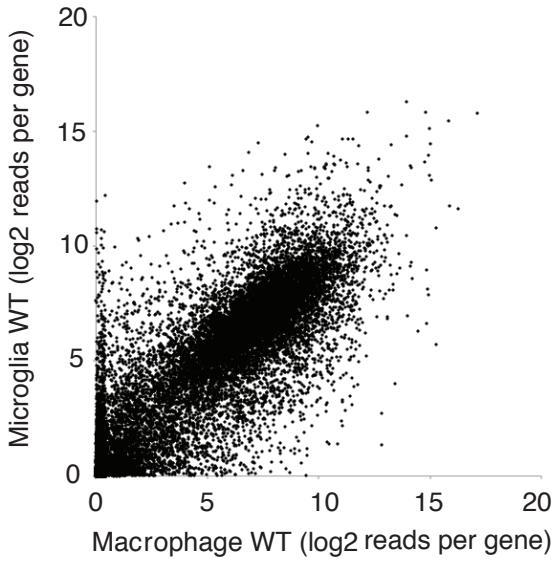


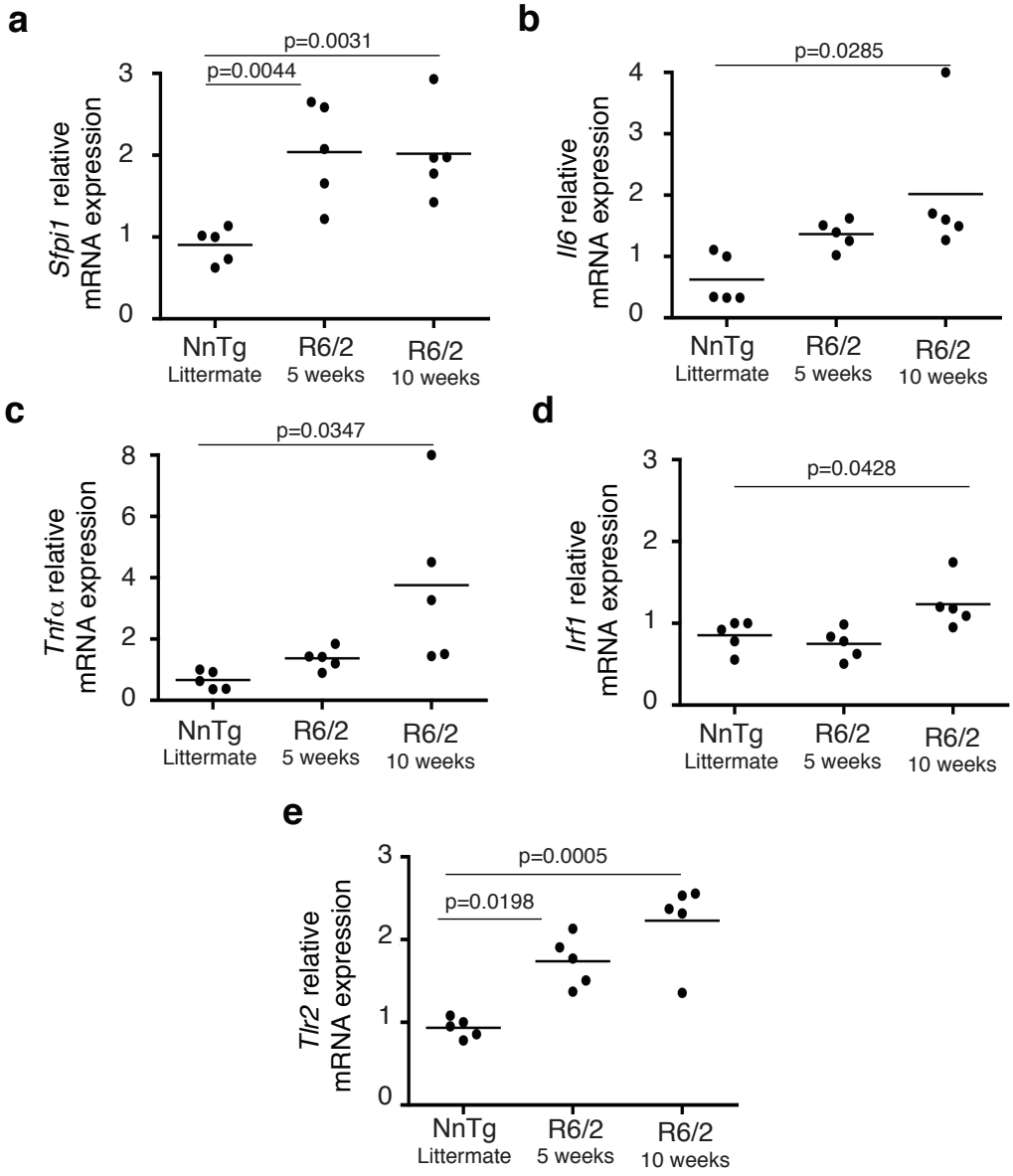
Supplementary Figure 1: PU.1 and PU.1-C/EBPs target genes are up-regulated in primary microglia but not in BMDM from R6/2 mice. qRT-PCR analysis for *Sfpi1* (**a**)*, Il6* (**b**) and*Tnfα* (**c**) mRNAs expression in primary microglia (mean±sd, n= 6 biological replicates, two-tailed paired student't test) and bone marrow derived macrophages (mean±sd, n= 5 biological replicates, two-tailed paired student't test) purified from non-transgenic littemates and R6/2 mice.



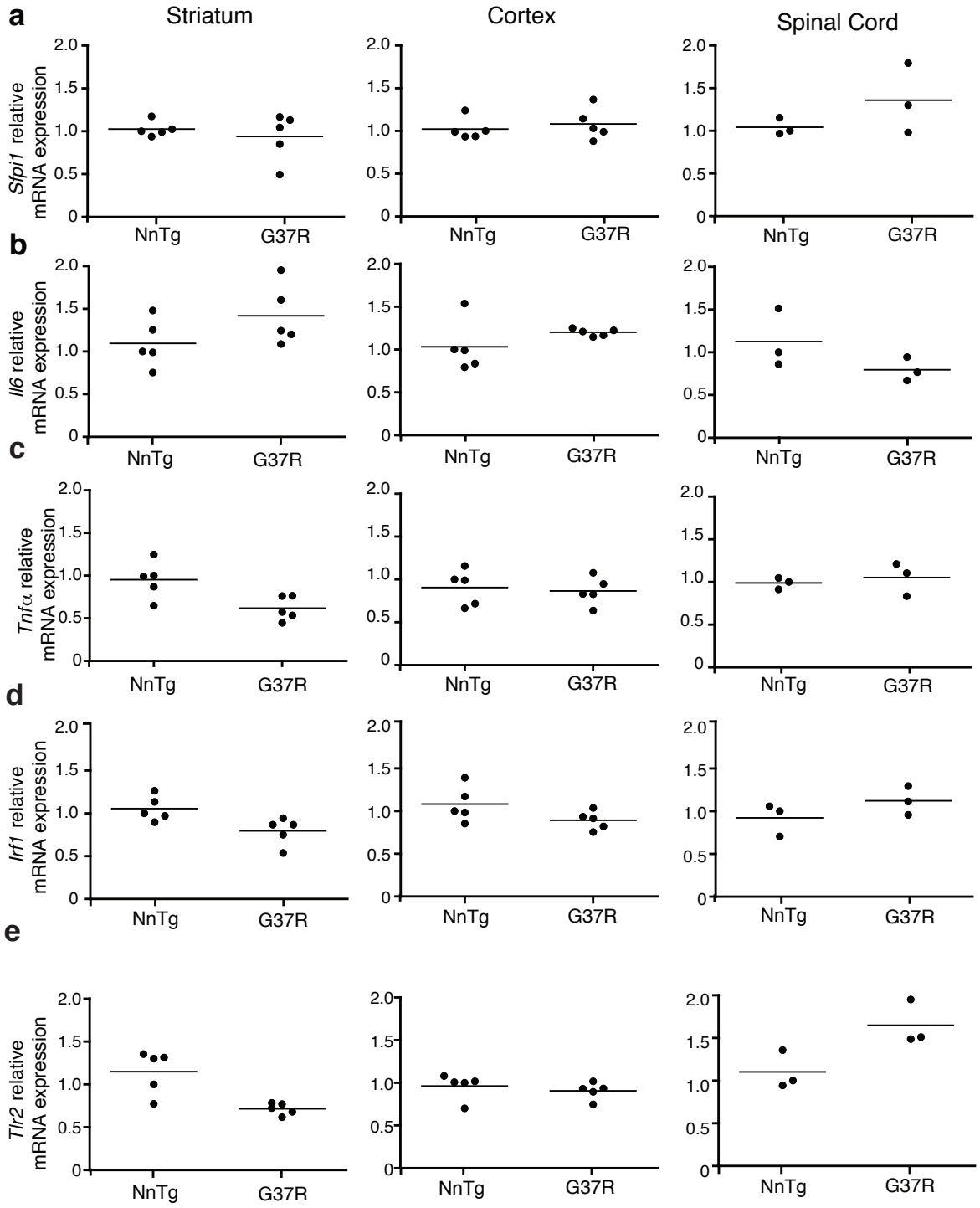
Supplementary Figure 2: Effect of siRNA knockdown of PU.1, C/EBPα and C/EBPβ. Efficiency of siRNA knockdown in primary microglia derived from R6/2 mice and nontransgenic littermates as determined by qRT-PCR (mean±sd, n= 3 biological replicates, p values determined by two-tailed paired student t-test).



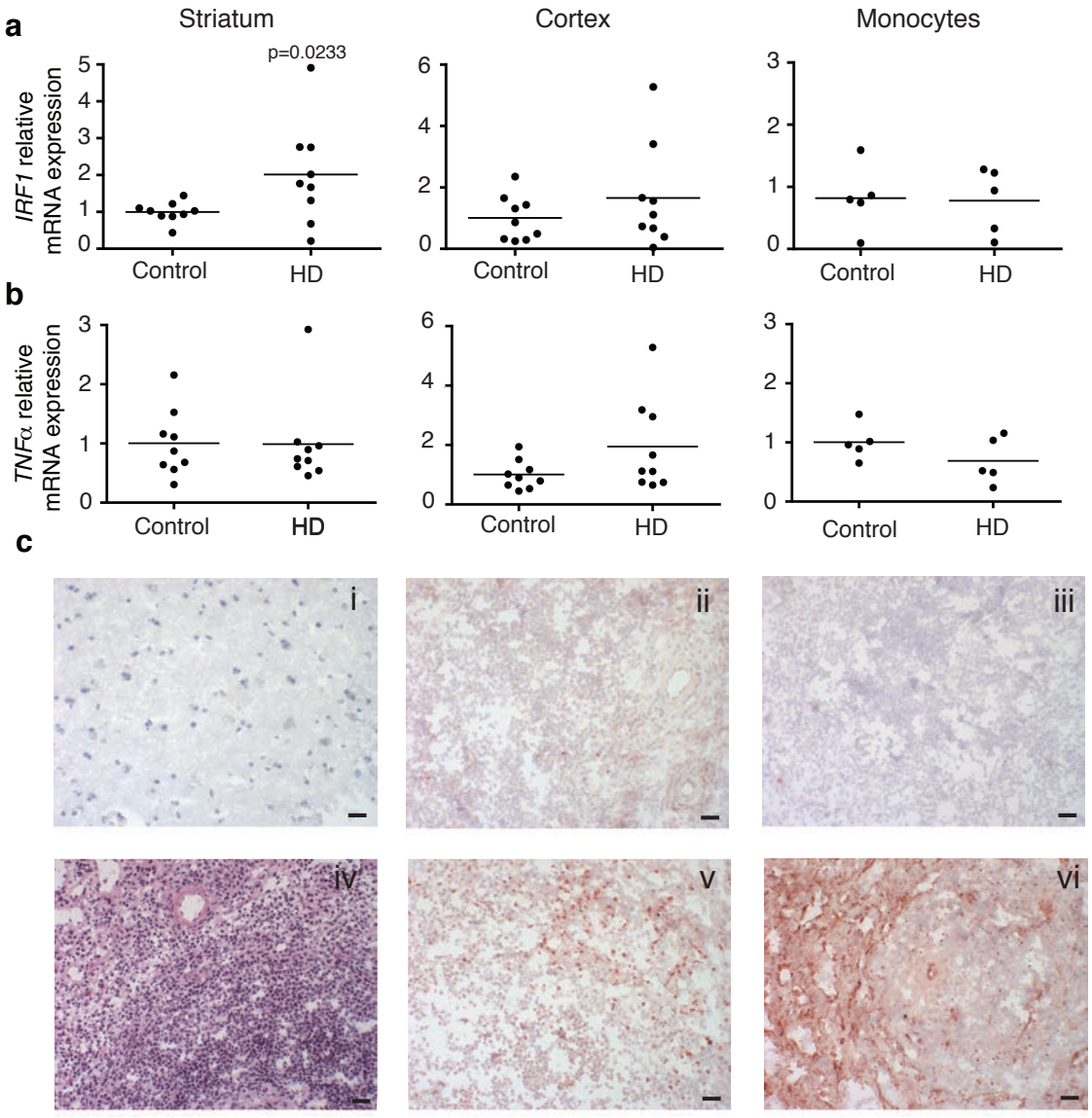
Supplementary Figure 3: Differential gene expression between Microglia WT and Macrophages WT. Scatter Plot representing the differential gene expression observed in microglia from wild-type *Hdh*$^{7/7}$ versus BMDM from wild-type *Hdh*$^{7/7}$ mice.



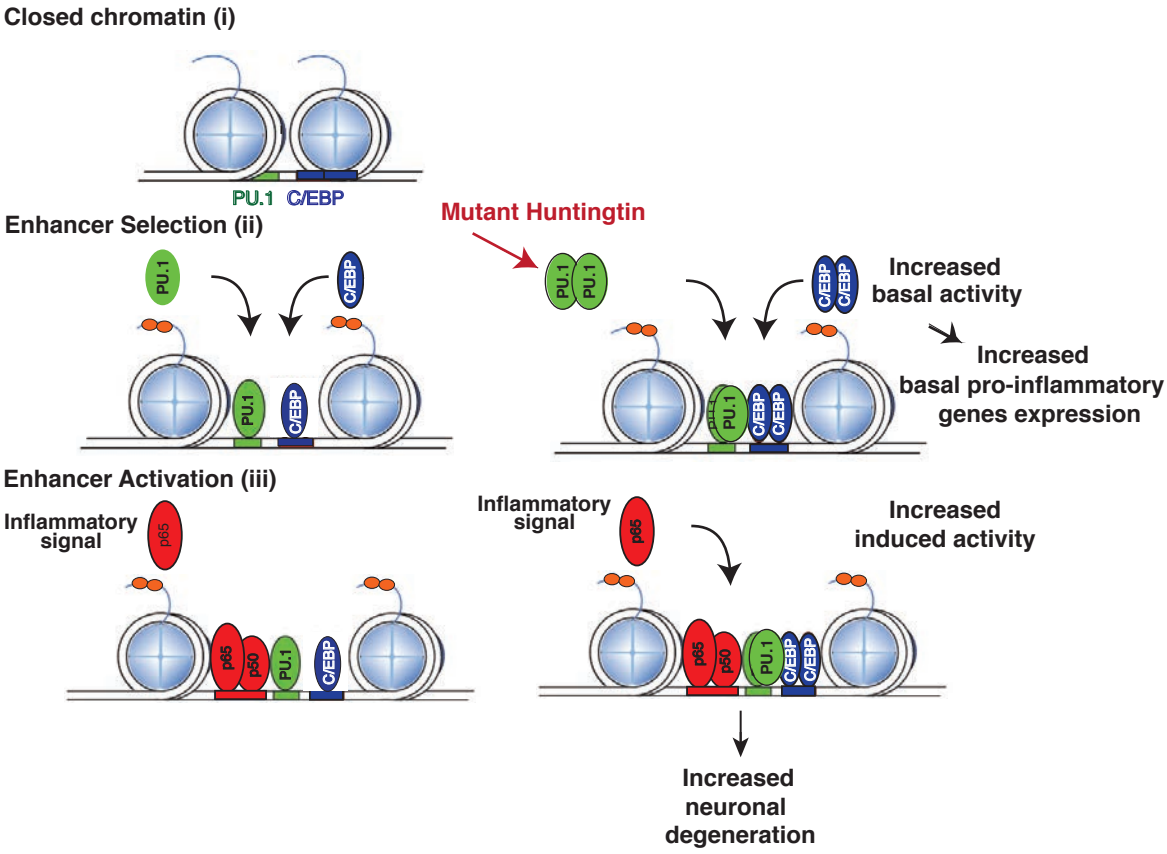
Supplementary Figure 4: PU.1 and PU.1-C/EBPs target genes are up-regulated in the striatum from R6/2 mice. qRT-PCR analysis for *Sfpi1* (**a**), *Il6* (**b**), *Tnfα* (**c**), *Irf1* (**d**) and *Tlr2* (**e**) mRNAs expression in striatum from nontransgenic littermates, pre-symptomatic (5 weeks-old) and symptomatic (10 weeks-old) R6/2 mice. Each dot is representative of one mouse (unpaired student's test).



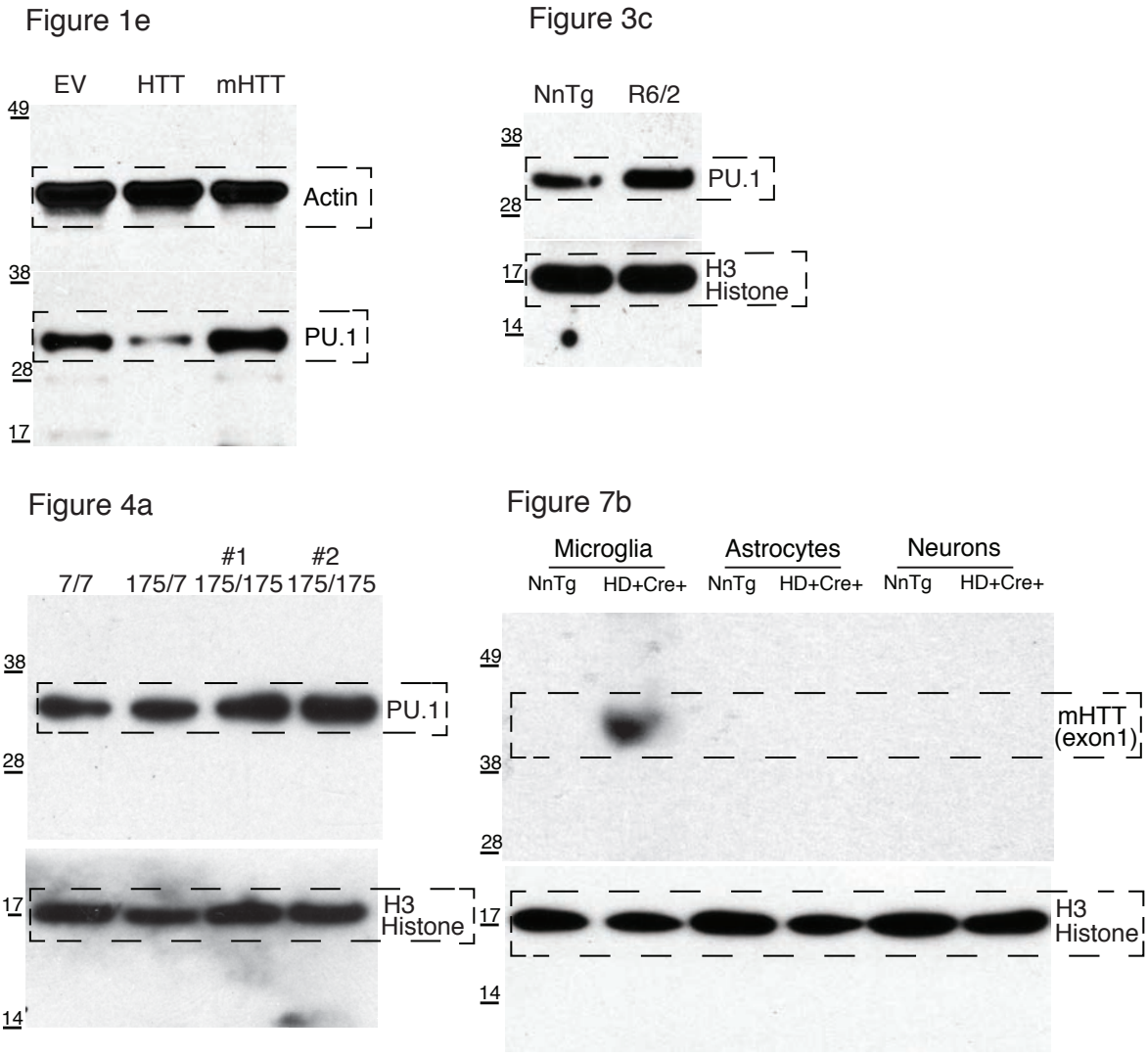
Supplementary Figure 5: PU.1 and PU.1-C/EBPs target genes are not differentially expressed in SOD1^{G37R} mouse model of ALS. qRT-PCR analysis for *Sfpi1* (**a**), *Il6* (**b**), *Tnfα* (**c**), *Irf1* (**d**) and *Tlr2* (**e**) mRNAs expression in striatum, cortex and spinal cord from nontransgenic littermates and SOD1^{G37R} mice (8-12 months old). Each dot is representative of one mouse (unpaired student's test).



Supplementary Figure 6: Inflammation *in vivo* in HD individuals. qRT-PCR analysis for *IRF1* **(a)** and *TNFα* **(b)** mRNAs expression in striatum (first column, n= 9 individual per group), cortex (second column, n= 9 individual per group) and monocytes (third column, n= 5 individual per group) from controls and HD individuals. Each dot is representative of one individual. All p values were determined by unpaired student t-test. (**c**) IHC controls: brain section in presence of rabbit IgG (negative control) (i); spleen section in presence of rabbit IgG (negative control) (ii); spleen section in presence of diluting buffer (BSA, 1% bovine serum albumin in PBS phosphate buffered saline) (iii); H&E staining on spleen section (iv): PU.1 IHC staining on spleen section (positive control) (v); Von Willebrand factor IHC staining on spleen section (positive control for endothelial cells/blood vessels) (vi). Scale bar: 100μm.



Supplementary Figure 7: A model for mechanisms by which mutant Huntingtin influence the selection and activation of microglia enhancers. Left: PU.1 and C/EBPs function in a collaborative manner to select microglia enhancers from inactive chromatin in basal conditions. Pro-inflammatory signals that activate transcription factors such as the p65 component of NFκB lead to inflammatory response. Right: mutant Huntingtin expression enhances this process by increasing PU.1 expression and PU.1-C/EBPs promoter binding, leading to increased enhancer activity under basal conditions that results in increased basal pro-inflammatory and neurotoxic genes expression. This phenomenon increases the sensitivity to pro-inflammatory signals. In fact, under conditions of sterile inflammation mutant Huntingtin-expressing microglia appears to be more efficient in inducing neuronal death.



Supplementary Figure 8: Full-length pictures of the blots presented in the main figures. To examine proteins of interest on the same samples, blots were cut first and then probed with indicated antibodies.