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Supplemental information

Specificity and efficiency of tamoxifen-mediated Cre induction is equivalent regardless of age

Collyn M. Kellogg, Kevin Pham, Sunghwan Ko, Jillian E.J. Cox, Adeline H. Machalinski, Michael B. Stout, Amanda L. Sharpe, Michael J. Beckstead, Ana J. Chucair-Elliott, Sarah R. Ocañas, and Willard M. Freeman

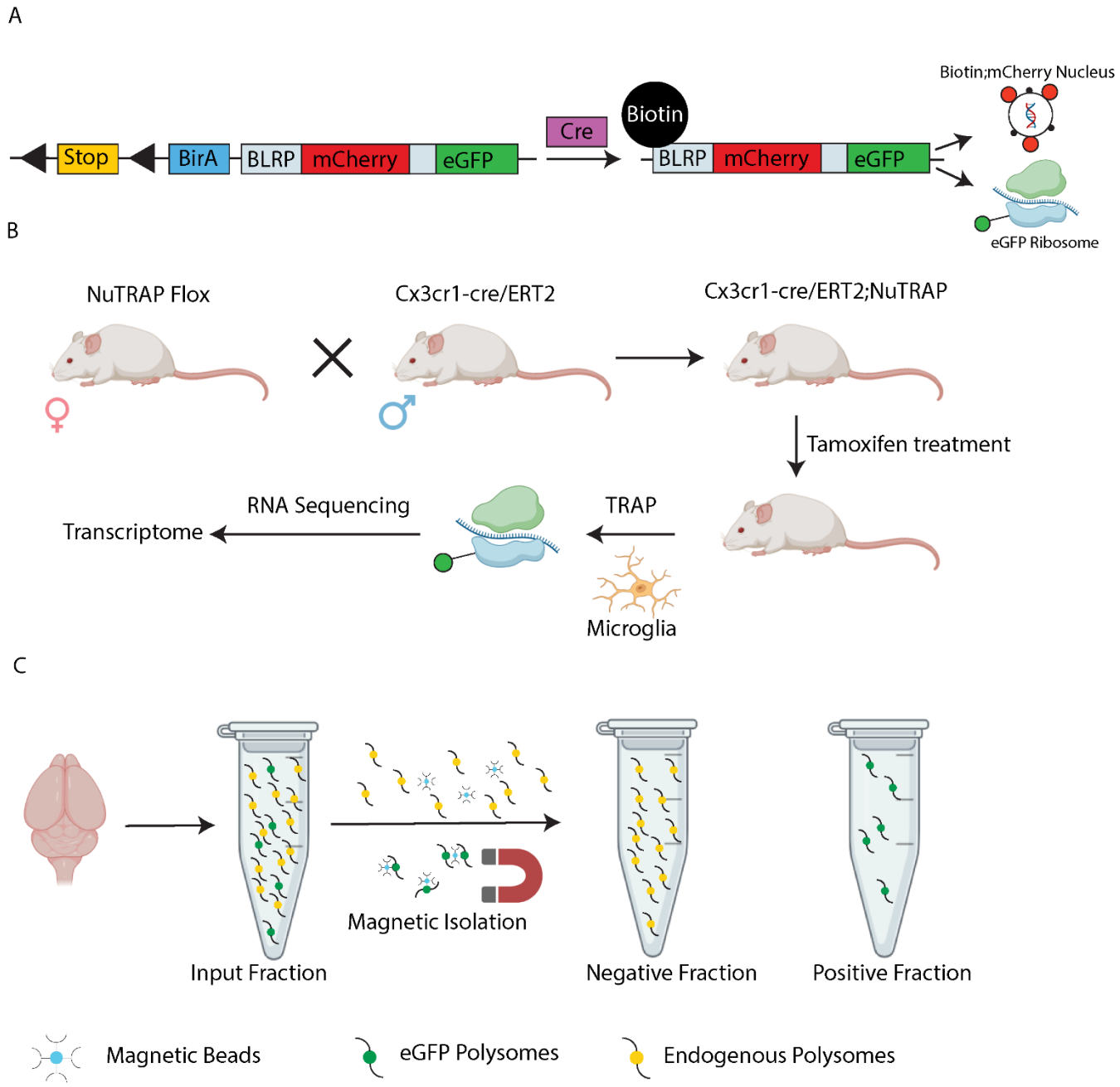

Figure S1. Schematic for the NuTRAP mouse model. Related to Figure 1. A) The gene layout for the NuTRAP model is floxed before cre Tamoxifen induction. Upon administration of Tamoxifen, the stop codon is removed, and the allele is expressed, tagging ribosomes with eGFP and nuclei with biotin and mCherry. B) The breeding model for this mouse is crossing a floxed NuTRAP mouse with the Cx3cr1-cre model. Homozygous NuTRAP mice are bread with homozygous Cx3cr1 cre mice to produce the mice used in these studies. For this experiment, we were only concerned with the transcriptome, so only TRAP was performed followed by RNA sequencing. C) To isolate the eGFP labeled ribosomes, magnetic beads with anti-eGFP antibodies are added to the input ribosomal homogenate. Magnetic isolation then separates the positive fraction from the negative. For this study only TRAP isolations for translatome analysis were performed but INTACT isolation of biotion tagged nuclei can also be performed for analysis of DNA.

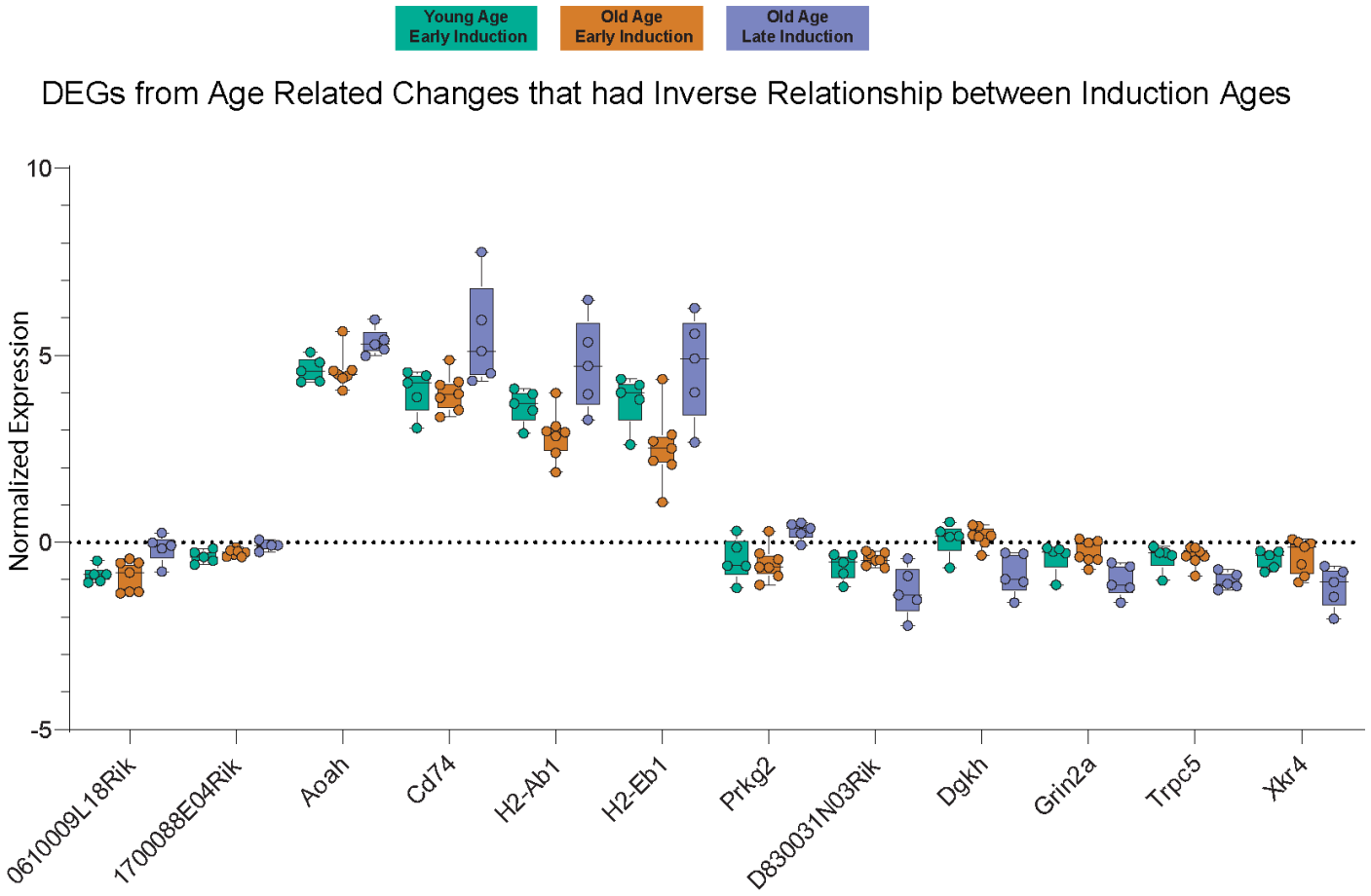

Figure S2. Boxplots for differentially regulated genes with aging between Tam induction ages. Related to Figure 4. Marginal differences with statistical differences between Tam induction age groups were observed for a few genes.

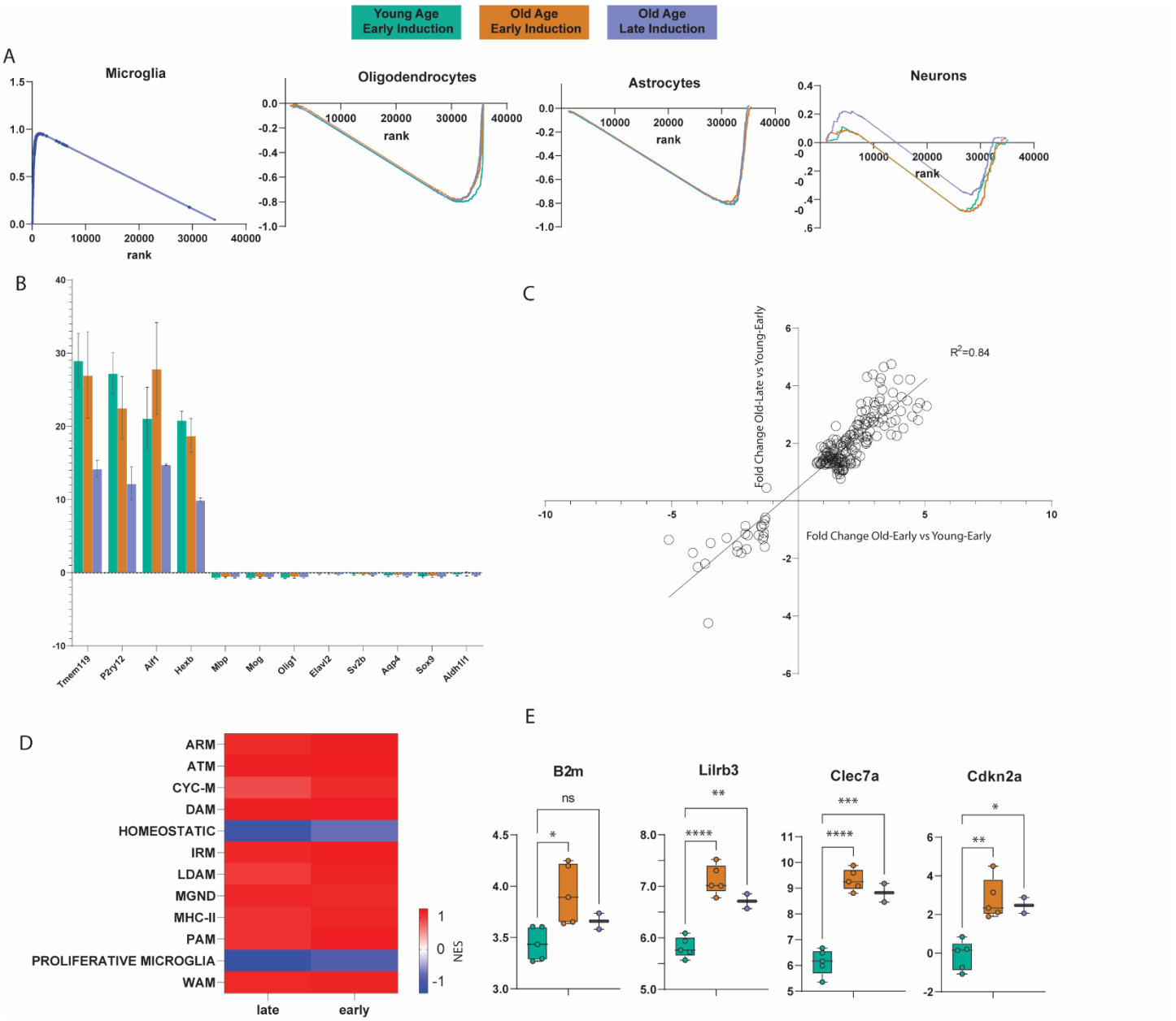

Figure S3. Brief analysis was performed on male mice in parallel to female mice. Relating to Figure 4. Limited numbers of male mice (n=2) were available for analysis. This figure is included in the supplement as one of the male groups had an n of 2, which is too underpowered for a full analysis. A) GSEA for cell types showing enrichment in all groups for microglia and depletion of all other cell types. B) Enrichment for specific marker genes further showing microglial enrichment and other cell depletion. C) Age related differentially expressed genes comparing fold changes between induction ages showed high correlation. D) GSEA heat map showing similar enrichment of microglial phenotypes. E) Specific genes selected for analysis showing similar age-related changes regardless of induction age.

Type of product to amplify	Description	Sequence 5' --> 3'	expected product size
generic Cre (all Cre)	generic Cre Forward	ATA CCG GAG ATC ATG CAA GC	Transgene: ~100 bp
generic Cre (all Cre)	generic Cre Reverse	GGC CAG GCT GTT CTT CTT AG	
internal positive ctl	+ ctl Forward	CTA GGC CAC AGA ATT GAA AGA TCT	Internal positive CTL: 324 bp
internal positive ctl	+ctl Rev	GTA GGT GGA AAT TCT AGC ATC ATC C	
specific cre (microglia)	Cx3cr1 Jung Mutant Forward	GTT AAT GAC CTG CAG CCA AG	Mutant: ~ 230 bp
specific cre (microglia)	Cx3cr1 Jung Common	ACG CCC AGA CTA ATG GTG AC	Heterozygote: ~ 230 and 151 bp
specific cre (microglia)	Cx3cr1 Jung WT Forward	AGC TCA CGA CTG CCT TCT TC	Wild type: 151 bp

Table S1. Genotyping primers for mice. Related to the Methods. The primers used for ear punches for genotype testing of mice.

Gene	Young Early vs Old Early	Young Early vs Old Late	Old Early vs Old Late
Tmem119	****	****	NS
P2ry12	***	***	NS
Aif1	*	*	NS
Hexb	***	****	NS
Mbp	****	****	*
Mog	**	***	NS
Olig1	NS	**	NS
Elavl2	NS	NS	NS
Sv2b	NS	NS	NS
Aqp4	NS	NS	NS
Sox9	NS	NS	NS
Aldh1l1	NS	NS	NS

Table S2. Statistical significance for differences between groups on individual marker genes for cell types. Related to Figure 3.

Table S3. Cell marker lists used for GSEA analysis. Related to Figure 3-5.