Supplementary figures and tables Fanaei-Kahrani et al. Distinct Effects of Aging and Klotho Deletion on the Choroid Plexus

Supplementary Table 1. List of primers used for genotyping of newly born pups

Locus	Primer name	Primer sequence	Expected product size	PCR Program
Slco1c1-Cre	Alfi cre Alfi cre rev B2-1 B2-2	5'-AGA TGC CAG GAC ATC AGG AAC CTG-3' 5'-ATC AGC CAC ACC AGA CAC AGA GAT C-3' 5'-CAC CGG AGA ATG GGA AGC CGA A-3' 5'-TCC ACA CAG ATG GAG CGT CCA G-3'	+/+: 300bp tg/+: 250bp + 300bp tg/tg: 250bp	1. 94 °C - 3' 2. 94 °C - 30'' 3. 60 °C - 30'' 4. 72 °C - 1' 5. 72 °C - 10' 6. 10 °C - hold Step 2.-4. 35x
Klotho-flox	Kl 33_fwd Kl 34_rev	5'-TAT GTA GAG ?GG ?TT TAC AAG G-3' 5'-GTC TTC CCT GTG AAT CAG CC-3'	+/+: 195 bp fl/+: 195+305bp fl/fl: 305 bp	See above
YFP	oIMR4982 Mutant oIMR8545 common oIMR8546 wt	5'-AAG ACC GCG AAG AGT TTG TC-3' 5'-AAA GTC GCT CTG AGT TGT TAT-3' 5'-GGA GCG GGA GAA ATG GAT ATG-3'	+/+: 600bp tg/tg: 320 bp tg/+: 320+600 bp	See above

Supplementary Table 2. Reaction mix for genotyping of newly born pups

Component	for 20 µl reaction	Supplier
5x GoTaq reaction buffer	4 µl	Promega, M7911
10x dNTPs	2 µl	New England Biolabs, N0447S
GoTaq polymerase	0.1 µl	Promega, M7848
Each primer	0.5 µl	Eurofins Genomics
Genomic DNA	0.5 µl	
ddH2O	Up to 20 µl	

Supplementary Table 3. Differentially expressed proteins across different ages in FV-CP (Combined data from comparing Ctrl1 to Kl$^{\Delta cp}$and Ctrl2 to Kl$^{\Delta cp}$)

Tissue/Age	number of proteins	Gene symbol		
FV-CP/M14-15, M2-3, M5-6	1	Abca5		
FV-CP/M2-3, M5-6	2	Ndufb9, Mgrn1		
FV-CP/M14-15, M5-6	1	TCAIM		

FV-CP/M2-3	35	*Mp68* *Lifr* *Banf1* *Abcg2* *Retsat* *Jph1* *Serpina3k* *Slc7a1* *Casp6* *Sema7a* *Dscr3* *Hspb11*	*D2hgdh* *Mepce* *Mpdu1* *Fuom* *Sugp1* *Ppl* *Sfr1* *Tmem27* *Sphk1* *C1qc* *Arl8a* *H3f3c; H3f3a*	*Dynlt1* *Vtn* *Rsrc2* *Mog* *Trappc4* *Eps15* *Adgrl1* *Ceacam2* *Tmem186* *Atg12* *Hrnr*
FV-CP/M5-6	53	*Coch* *Fabp5* *Atp5f1* *Mettl13* *Scpep1* *Dpp4* *ALB* *Idh3g* *Ptgds* *Lsm3* *Nptn* *Ypel5* *Wdr77* *Fgfr1* *Fam136a* *Adam9* *Taco1* *Tmem263*	*Gstp1* *Gstm5* *Pcp4* *Rab33b* *Cfd* *Wiz* *Znf593* *Sdhaf2* *Cdkal1* *Prr14* *Ndufs8* *Mcts1* *Crabp2* *Col6a1* *Hspa14* *Trmt6* *Col6a2* *Tmem209*	*Hspa1b; Hspa1a* *Cox6a1* *Atp8a2* *Ifih1* *Abi3* *Sat2* *Rpl38* *Atp2a1* *Atp1b3* *Csnk2a1* *Alg10b* *Mrpl49* *Tcp11l1* *Aatf* *Thap4* *Mrps30* *Nt5c3b*
FV-CP/M14-15	10	*Slc5a6* *Nudt6* *Atpaf1* *HVM51* *Emc6*	*Aldh3a1* *Nfatc2* *Znf207* *Idua* *Tubb1*	
FV-CP/M20-21	15	*Naaa* *Baiap2l1* *Gm11992* *Nrm* *Ifit3* *Mrpl52* *Rad23a* *Mtnd5*	*Apob* *Lamp2* *Ubap1* *Cfl2* *Smad2* *Efemp2* *Zmat2*	

Supplementary Table 4. Differentially expressed proteins across different ages in LV-CP (Combined data from comparing Ctrl1 to Kl$^{\Delta CP}$and Ctrl2 to Kl$^{\Delta CP}$)

Tissue/Age	**number of proteins**	**Gene symbol**	
LV-CP/M2-3, M5-6	1	*Sult1c2*	

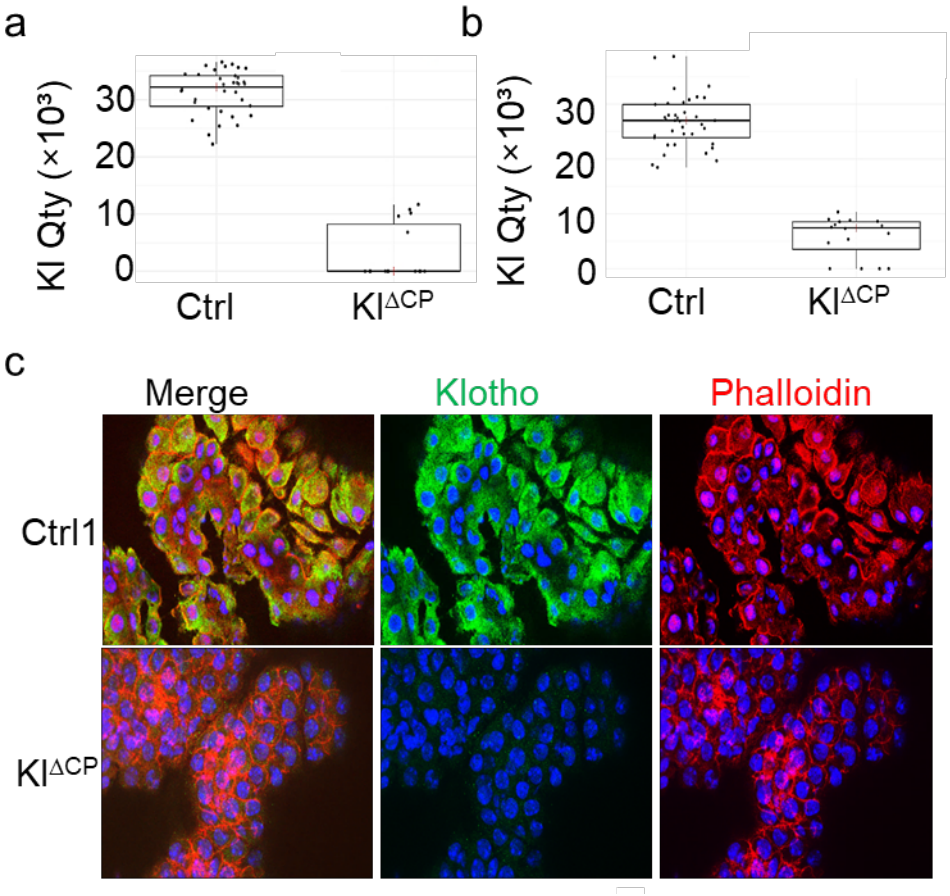
LV-CP/M2-3	40	*Fbln5* *Utp15* *Lsm4* *Cadm1* *Osbpl3* *Phf24* *Thy1* *Cnp* *Fam241b* *Gna11* *Ptgr1* *Aldh3a2* *Igh-3* *Mag* *Thap4* *Hat1* *Dnajc5* *Plcb1* *Abca5* *Nefl*	*Dnm2* *Ndrg1* *Cndp1* *Alcam* *Rbmx* *Smap1* *Cyp4v2* *Hmgcl* *Slc7a10* *St3gal6* *Srr* *H2afy2* *Ptprd* *Rhbdf2* *Ctnna2* *C1qc* *Fetub* *Tmem70* *Cpsf3l* *Tbl1x*
LV-CP/M5-6	17	*Fuz* *Ndel1* *Sap30bp* *Zfpl1* *Ap3b2* *Cln8* *Gja1* *Fabp7* *Syt1*	*Nos1ap* *Tspo* *Camk2b* *Il6st* *Syn2* *Ngly1* *Tcp11l1* *Phlda3*
LV-CP/M14-15	34	*Rpl35* *Nme1* *Sdpr* *Csrp1* *Tubb6* *Hnrnpm* *Fn3krp* *Hist1h1d* *Uqcrb* *Tbc1d24* *Alpl* *Hn1l* *Dysf* *Chp1* *Rwdd1* *Mrc1* *Cd63*	*Ddr1* *Capza1* *Steap3* *Pcp4* *Lamc3* *Mavs* *Fam103a1* *Hdgf* *Rps27l* *Tubb2b* *Arf5* *Eef1a1* *Arpc5* *Ahcyl2* *Rbmxl1* *Pacrg* *Rps10*
LV-CP/M20-21	5	*Col3a1* *Fbn1* *Arpp19*	*Col1a2* *Pvalb*

Supplementary Table 5. KEGG pathway analysis of the proteins downregulated (in all ages) in Kl$^{\Delta CP}$ mice versus controls in the HC

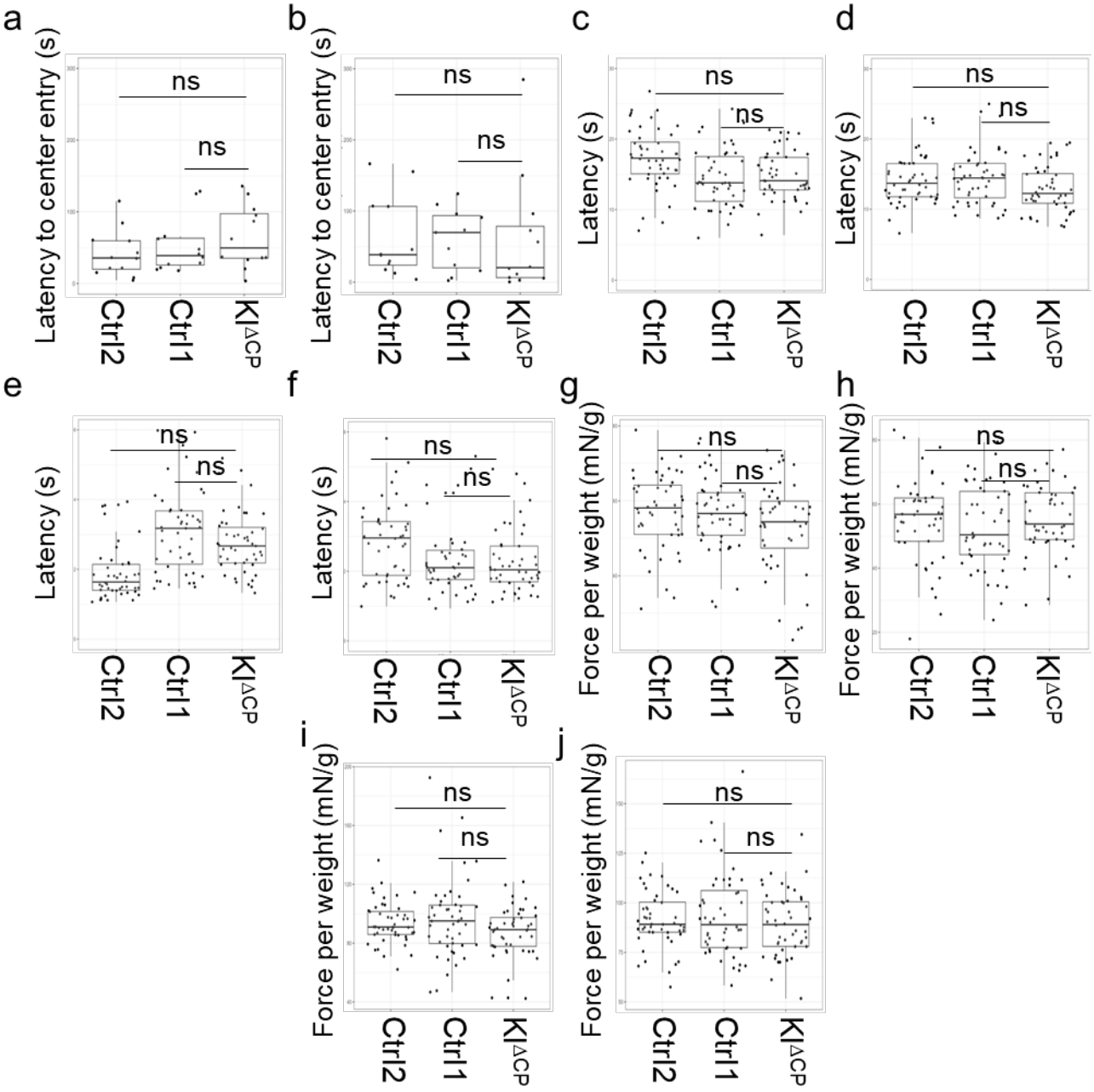
Term	Adjusted P-value	Protein symbol
Parkinson disease	6.24E-04	Gnal; Ndufa4; Camk2a; Calm1; Tubb4a
Long-term potentiation	0.002421	Camk2a; Araf; Calm1
Glioma	0.002421	Camk2a; Araf; Calm1
Pathways of neurodegeneration	0.003417	Ndufa4; Camk2a; Araf; Calm1; Tubb4a
Dopaminergic synapse	0.007738	Gnal; Camk2a; Calm1
Alzheimer disease	0.010469	Ndufa4; Araf; Calm1; Tubb4a

Supplementary Table 6. KEGG pathway analysis of the proteins upregulated (in all ages) in Kl$^{\Delta CP}$ mice versus controls in the HC

Term	Adjusted P-value	Protein symbol
ECM-receptor interaction	1.80E-08	Col1a1; LAMA5; Lamb2; Col4a1; Lamc1; Hspg2
Amoebiasis	2.21E-08	Col1a1; Lama5; Gnal; Lamb2; Col4a1; Lamc1
Focal adhesion	2.69E-05	Col1a1; Lama5; Lamb2; Col4a1; Lamc1

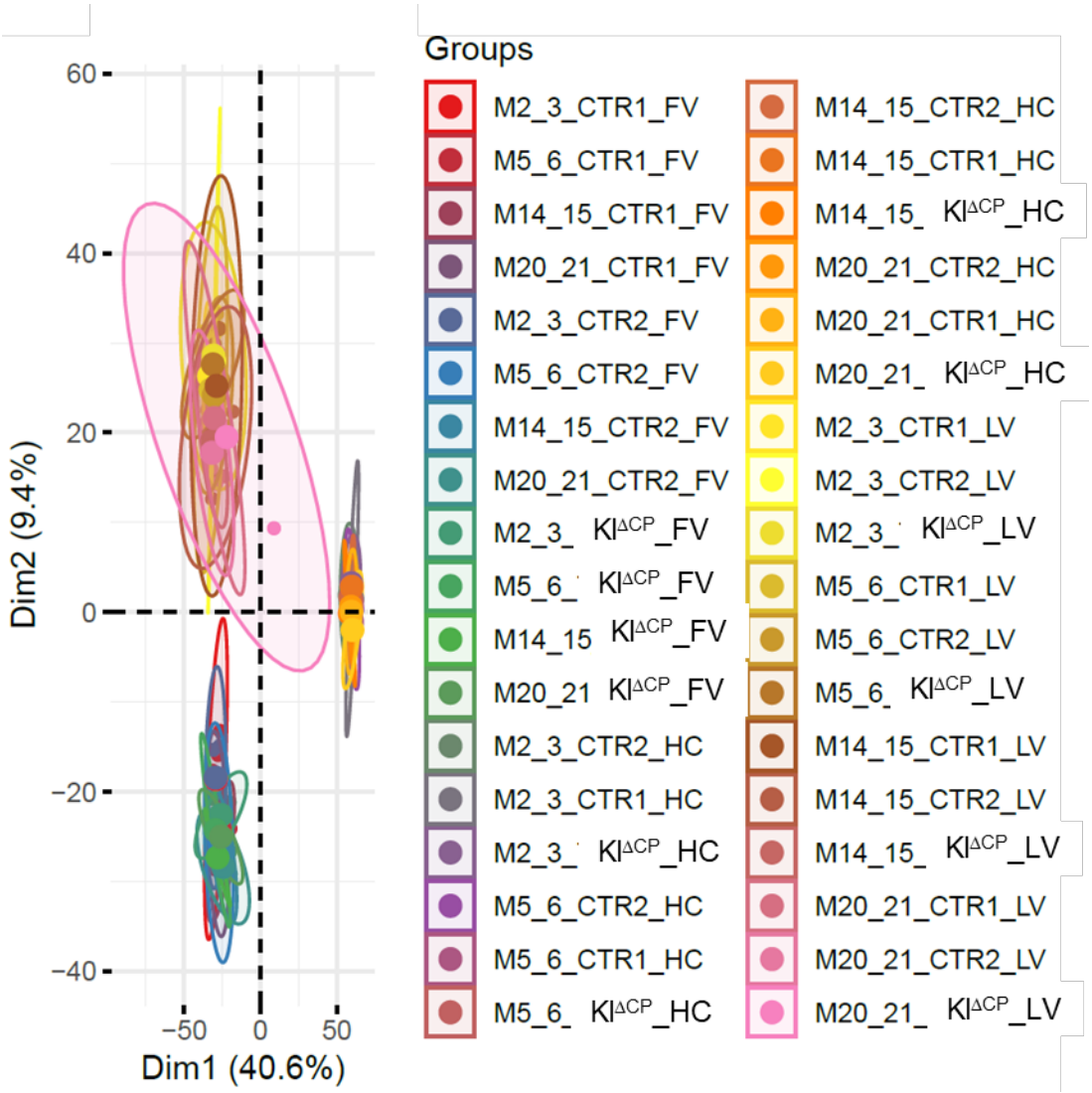


Supplementary Figure 1. Proteomics analysis and Immunofluorescent staining and confirmed the deletion of Kl in LV-CP of Kl$^{\Delta CP}$ mice. (a, b) Validation of Kl knockout in the CP of Kl$^{\Delta CP}$ mice using LC/MS proteomics of the FV-CP (A, n=36 controls, n=15 Kl$^{\Delta CP}$) and the LV-CP (B, n=37 controls, n=16 Kl$^{\Delta CP}$). (c) Brain sections from 2-3 months old Kl$^{\Delta CP}$ and control 1 mice were immunostained for Klotho and Phalloidin, and Z-stack images were acquired using the tile scan function on a Zeiss ApoTome microscope. Maximum intensity projections are shown. Nuclei were counterstained with Hoechst (blue). Scale bar = 20 µm.

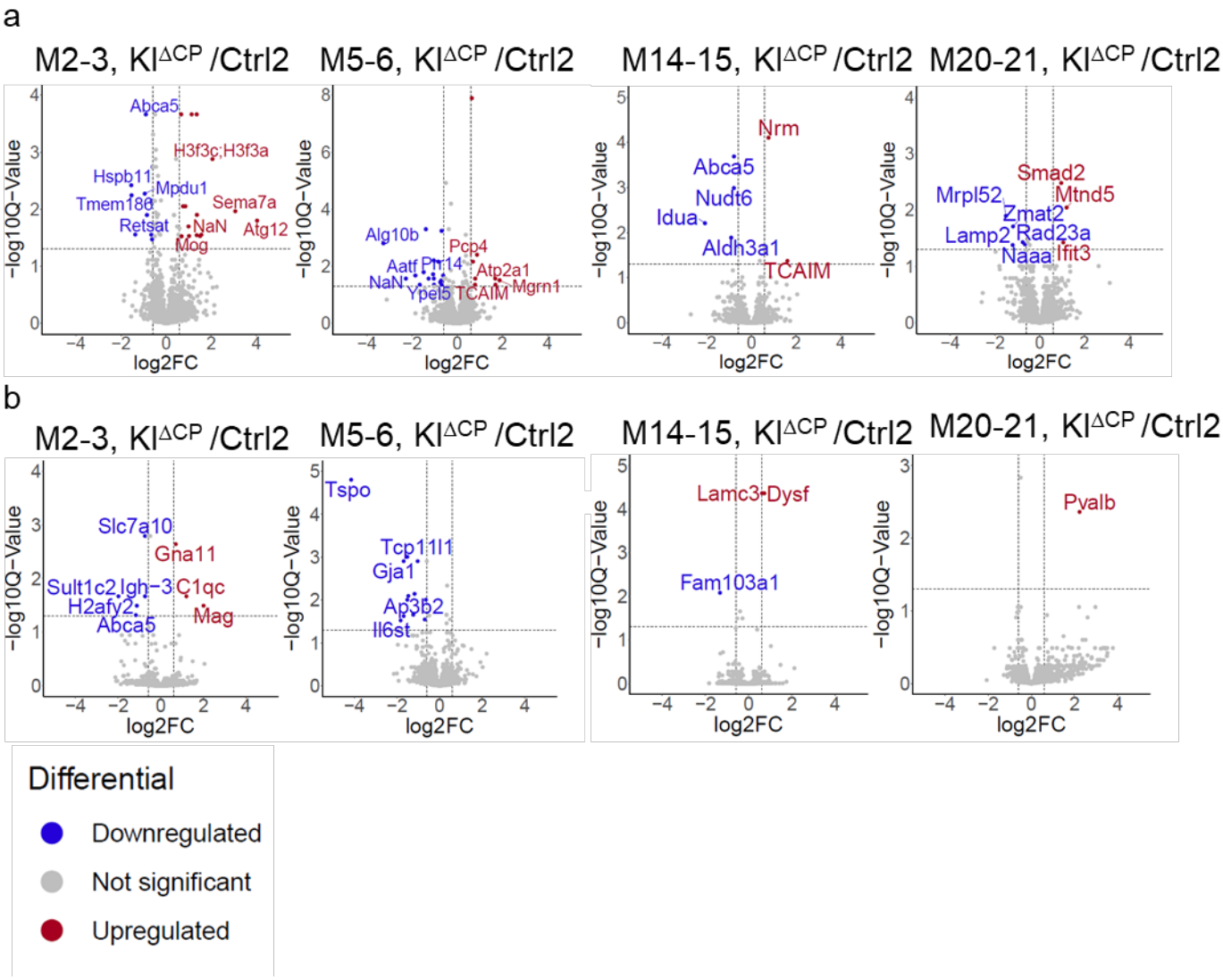


Supplementary Figure 2. Behavioral analysis of 2-5 months and 8-12 months KI$^{\Delta CP}$ mice compared to control groups showed no significant difference between them. (a, b) Open field test to measure exploration behavior (latency to center entry) between KI$^{\Delta CP}$ and control mice. The test involved placing the animal in a white plastic cube. Movement was tracked using a video system that was installed above the area. The latency to enter the center was assessed in (a) 2-5 months and (b) 8-12 months mice. (c, d) Hot plate test. Each mouse was placed on a 52°C preheated plate and the latency time until the occurrence of the first contact avoidance reaction was measured. Measurements were taken for (c) 2-5 months and (d) 8-12 months. (e, f) Tail flick response. In this experiment the mouse's tail of 2-5 months (e) and 8-12 months (f) was immersed in hot water at 45.0±0.2 °C and the reflexive tail movement time was recorded. (g-j) Grip strength

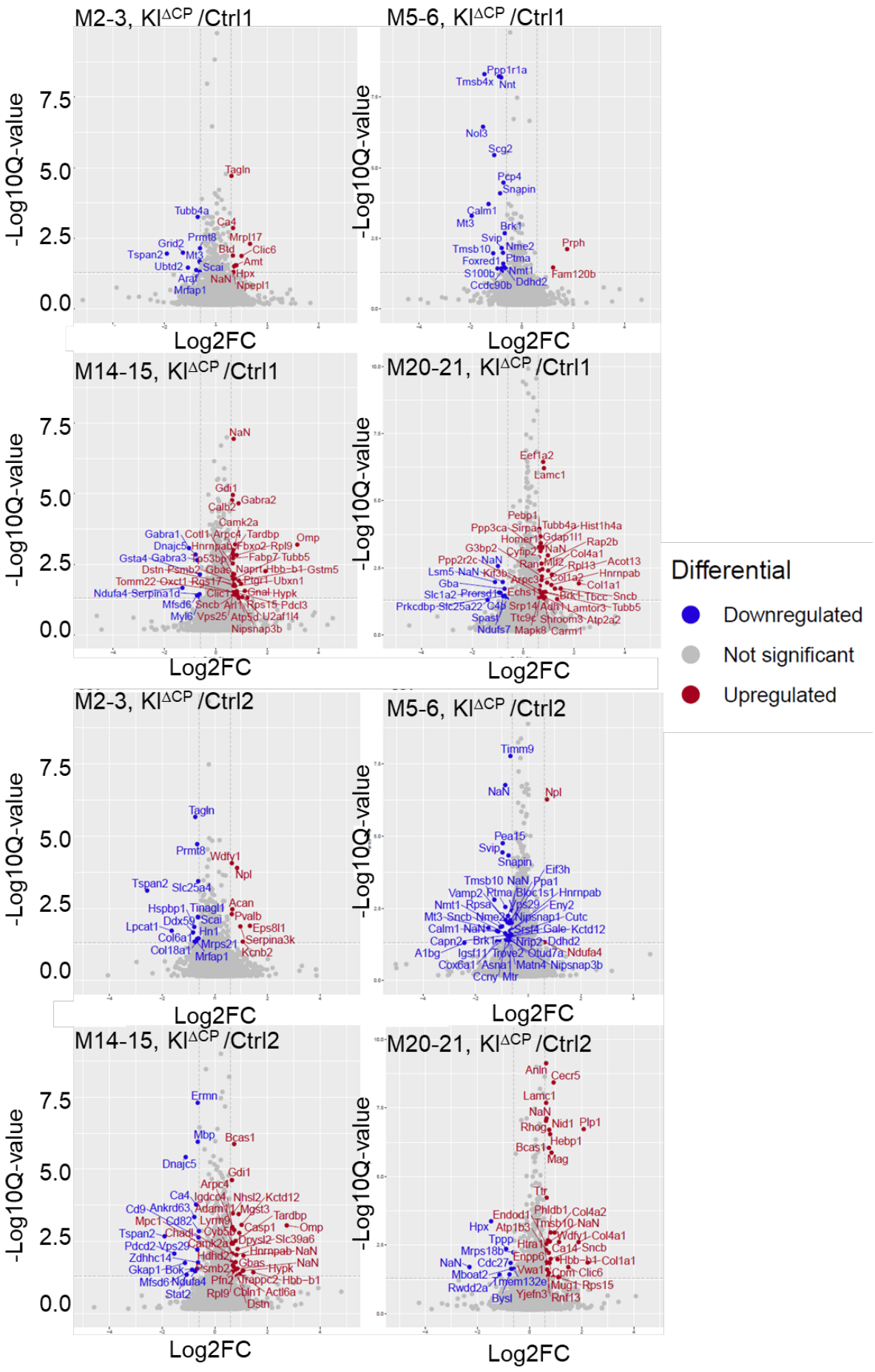
analysis measuring the peak force exerted by front paws or all four paws on a wire grid. Data represents (g) 2-paw strength in 2-5 months, (h) 2-paw strength in 8-12 months, (i) 4-paw strength in 2-5 months, and (j) 4-paw strength in 8-12 months. Statistical evaluation included RM-ANOVA and Cohen's d-effect size, with error bars representing standard deviation and each dot symbolizing a single mouse. Significance was set at p-value > 0.05 (ns).



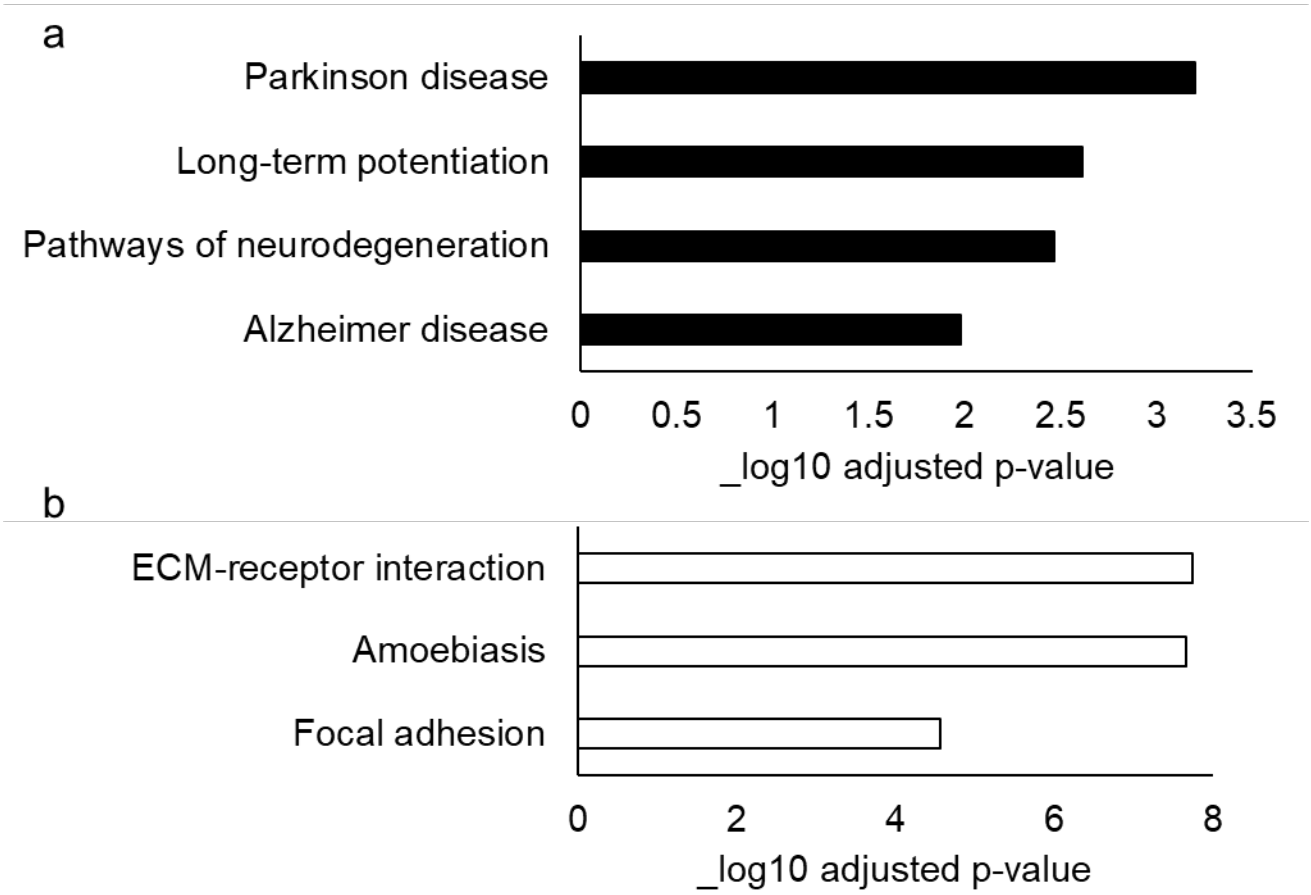
Supplementary Figure 3. Principal component analysis (PCA) illustratingthe distribution of all samples. The results show clear clustering of FV-CP, LV-CP, and HC samples within their respective groups, indicating reproducible replicate data and distinct proteomic profiles for FV-CP, LV-CP, and HC tissues (M: Month, CTR: Control, FV-CP: Fourth ventricle Choroid plexus, LV-CP: Lateral ventricle Choroid plexus, HC: Hippocampus).



Supplementary Figure 4. Volcano plots depicting differential expression of proteins in the FV-CP (a) and LV-CP (b) in KI$^{\Delta CP}$ mice compared to Ctrl2 at various ages (2-3, 5-6, 14-15, and 20-21 months). Shown are protein symbols, the written names are the top 5 upregulated and downregulated proteins. Blue, significantly downregulated in KI$^{\Delta CP}$ compared to Ctrl2, red, upregulated in KI$^{\Delta CP}$ compared to Ctrl2.


M2-3, KI^ΔCP /Ctrl1
M5-6, KI^ΔCP /Ctrl1
M14-15, KI^ΔCP /Ctrl1
M20-21, KI^ΔCP /Ctrl1
M2-3, KI^ΔCP /Ctrl2
M5-6, KI^ΔCP /Ctrl2
M14-15, KI^ΔCP /Ctrl2
M20-21, KI^ΔCP /Ctrl2
-Log10Q-value
Log2FC
Differential
Downregulated
Not significant
Upregulated

Supplementary Figure 5. Volcano plots depicting differential expression of proteins in the hippocampus in KI$^{\Delta CP}$ mice compared to Ctrl1 and Crl2 at various ages (2-3, 5-6, 14-15, and 20-21 months). Shown are protein symbols, the written names are all the upregulated and downregulated proteins. Blue, significantly downregulated in KI$^{\Delta CP}$ compared to Ctrl, red, upregulated in KI$^{\Delta CP}$ compared to Ctrl.



Supplementary Figure 6. KEGG pathway analysis of the proteins downregulated (a) and upregulated (b) (in all ages) in KI$^{\Delta CP}$ mice versus controls in the HC.