

# Brain Health

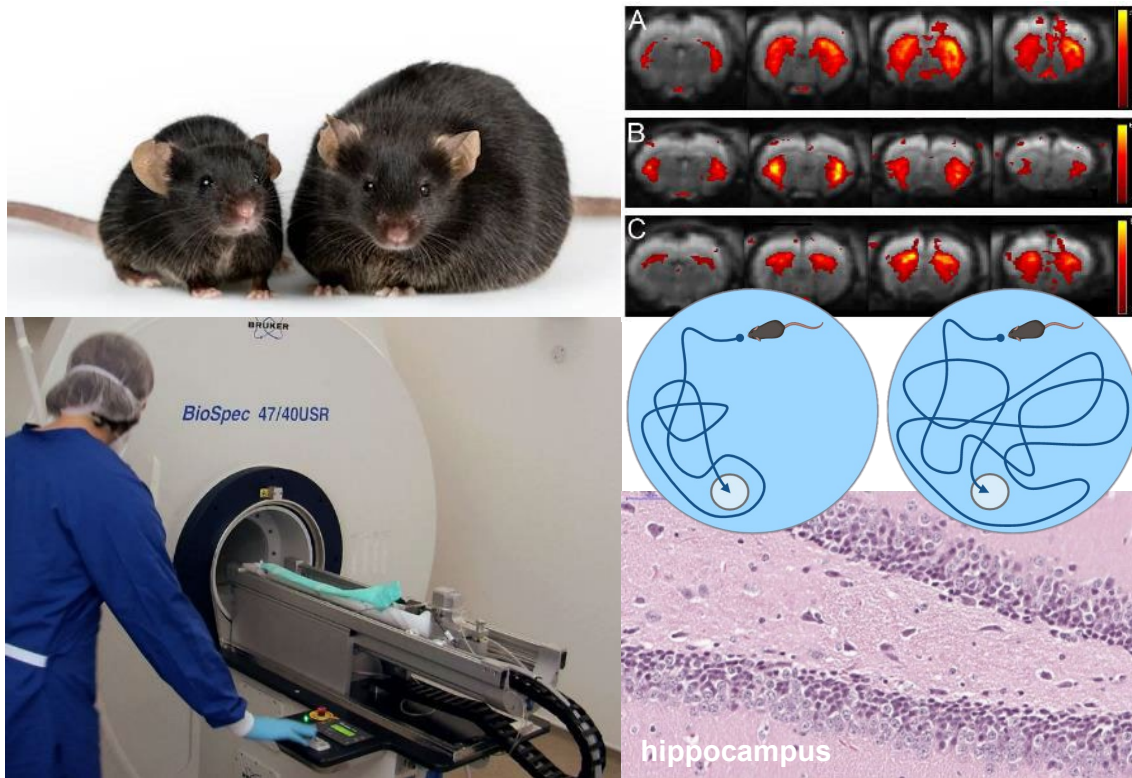
## Dysmetabolism, Brain Aging and Neuroinflammation

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Preclinical studies in  $Ldlr^{-/-}$ .Leiden mice



# Model for Brain Health, Aging and Neuroinflammation



- **Proprietary TNO strain: Ldlr<sup>-/-</sup>.Leiden mouse**

- Humanized genetic background: 262 high-impact human SNPs
- Translational for obesity, IR, MAFLD/MASH, CVD.
- Human disease mechanisms, pathology, biomarkers and drug responses
- Mimics high-risk patient groups (patented model)  
(Morrison, *Hepatology* 2018; Martinez-Arranz, *Hepatology* 2022; Verschuren *Nat Comm.* 2024)

- **Functional and molecular readouts: body & brain**

- Behaviour & cognition tests during aging and/or obesity
- Histopathology: morphology & protein expression and distribution
- Brain transcriptomics, lipidomics, oxylipins, neuroinflammation, blood brain barrier dysfunction

- **MRI imaging with Radboudumc**

- Brain structures (e.g. grey matter and white matter integrity); thinning of cortex
- Brain functions (connectivity, cerebral blood flow, BBB integrity)
- Polarized light imaging

# Mechanistic differences with conventional model

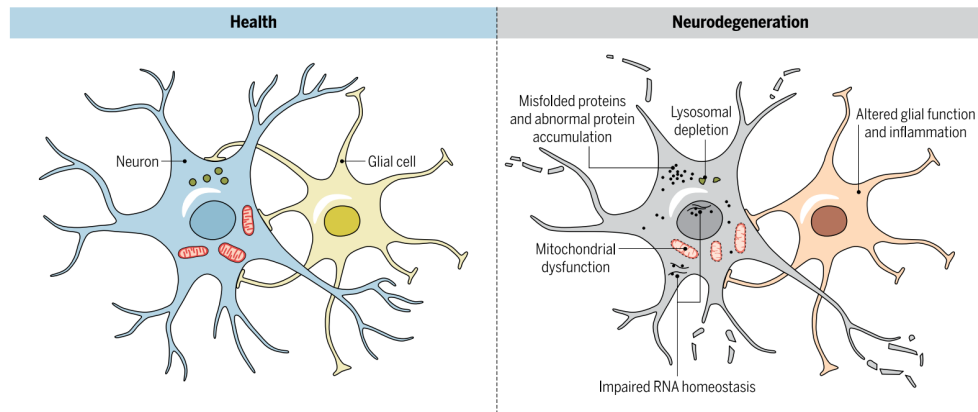
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Pathway	Log2(FC)	Log10(P)	Gene Symbols	Count	Significance	
Signaling by Etk Family Kinases	2.79	-1.89	ETK1, LAMC1, LAMC2, LAMC3, LAMC4, LAMC5, LAMC6, LAMC7, LAMC8, LAMC9, LAMC10, LAMC11, LAMC12, LAMC13, LAMC14, LAMC15, LAMC16, LAMC17, LAMC18, LAMC19, LAMC20, LAMC21, LAMC22, LAMC23, LAMC24, LAMC25, LAMC26, LAMC27, LAMC28, LAMC29, LAMC30, LAMC31, LAMC32, LAMC33, LAMC34, LAMC35, LAMC36, LAMC37, LAMC38, LAMC39, LAMC40, LAMC41, LAMC42, LAMC43, LAMC44, LAMC45, LAMC46, LAMC47, LAMC48, LAMC49, LAMC50, LAMC51, LAMC52, LAMC53, LAMC54, LAMC55, LAMC56, LAMC57, LAMC58, LAMC59, LAMC60, LAMC61, LAMC62, LAMC63, LAMC64, LAMC65, LAMC66, LAMC67, LAMC68, LAMC69, LAMC70, LAMC71, LAMC72, LAMC73, LAMC74, LAMC75, LAMC76, LAMC77, LAMC78, LAMC79, LAMC80, LAMC81, LAMC82, LAMC83, LAMC84, LAMC85, LAMC86, LAMC87, LAMC88, LAMC89, LAMC90, LAMC91, LAMC92, LAMC93, LAMC94, LAMC95, LAMC96, LAMC97, LAMC98, LAMC99, LAMC100	25	267	49
Ceramide Signaling	2.78	-1.15	CERS1, CERS2, CERS3, CERS4, CERS5, CERS6, CERS7, CERS8, CERS9, CERS10, CERS11, CERS12, CERS13, CERS14, CERS15, CERS16, CERS17, CERS18, CERS19, CERS20, CERS21, CERS22, CERS23, CERS24, CERS25, CERS26, CERS27, CERS28, CERS29, CERS30, CERS31, CERS32, CERS33, CERS34, CERS35, CERS36, CERS37, CERS38, CERS39, CERS40, CERS41, CERS42, CERS43, CERS44, CERS45, CERS46, CERS47, CERS48, CERS49, CERS50, CERS51, CERS52, CERS53, CERS54, CERS55, CERS56, CERS57, CERS58, CERS59, CERS60, CERS61, CERS62, CERS63, CERS64, CERS65, CERS66, CERS67, CERS68, CERS69, CERS70, CERS71, CERS72, CERS73, CERS74, CERS75, CERS76, CERS77, CERS78, CERS79, CERS80, CERS81, CERS82, CERS83, CERS84, CERS85, CERS86, CERS87, CERS88, CERS89, CERS90, CERS91, CERS92, CERS93, CERS94, CERS95, CERS96, CERS97, CERS98, CERS99, CERS100	12	91	50
NET Signaling	2.76	-1.87	NET1, NET2, NET3, NET4, NET5, NET6, NET7, NET8, NET9, NET10, NET11, NET12, NET13, NET14, NET15, NET16, NET17, NET18, NET19, NET20, NET21, NET22, NET23, NET24, NET25, NET26, NET27, NET28, NET29, NET30, NET31, NET32, NET33, NET34, NET35, NET36, NET37, NET38, NET39, NET40, NET41, NET42, NET43, NET44, NET45, NET46, NET47, NET48, NET49, NET50, NET51, NET52, NET53, NET54, NET55, NET56, NET57, NET58, NET59, NET60, NET61, NET62, NET63, NET64, NET65, NET66, NET67, NET68, NET69, NET70, NET71, NET72, NET73, NET74, NET75, NET76, NET77, NET78, NET79, NET80, NET81, NET82, NET83, NET84, NET85, NET86, NET87, NET88, NET89, NET90, NET91, NET92, NET93, NET94, NET95, NET96, NET97, NET98, NET99, NET100	19	57	51
NET1-mediated Etkin Pathway	2.76	-1.26	NET1, ETK1, LAMC1, LAMC2, LAMC3, LAMC4, LAMC5, LAMC6, LAMC7, LAMC8, LAMC9, LAMC10, LAMC11, LAMC12, LAMC13, LAMC14, LAMC15, LAMC16, LAMC17, LAMC18, LAMC19, LAMC20, LAMC21, LAMC22, LAMC23, LAMC24, LAMC25, LAMC26, LAMC27, LAMC28, LAMC29, LAMC30, LAMC31, LAMC32, LAMC33, LAMC34, LAMC35, LAMC36, LAMC37, LAMC38, LAMC39, LAMC40, LAMC41, LAMC42, LAMC43, LAMC44, LAMC45, LAMC46, LAMC47, LAMC48, LAMC49, LAMC50, LAMC51, LAMC52, LAMC53, LAMC54, LAMC55, LAMC56, LAMC57, LAMC58, LAMC59, LAMC60, LAMC61, LAMC62, LAMC63, LAMC64, LAMC65, LAMC66, LAMC67, LAMC68, LAMC69, LAMC70, LAMC71, LAMC72, LAMC73, LAMC74, LAMC75, LAMC76, LAMC77, LAMC78, LAMC79, LAMC80, LAMC81, LAMC82, LAMC83, LAMC84, LAMC85, LAMC86, LAMC87, LAMC88, LAMC89, LAMC90, LAMC91, LAMC92, LAMC93, LAMC94, LAMC95, LAMC96, LAMC97, LAMC98, LAMC99, LAMC100	13	108	52
Disposome-CAAP12 Feedback in eAMP Signaling	2.7	-1.61	DISPO1, DISPO2, DISPO3, DISPO4, DISPO5, DISPO6, DISPO7, DISPO8, DISPO9, DISPO10, DISPO11, DISPO12, DISPO13, DISPO14, DISPO15, DISPO16, DISPO17, DISPO18, DISPO19, DISPO20, DISPO21, DISPO22, DISPO23, DISPO24, DISPO25, DISPO26, DISPO27, DISPO28, DISPO29, DISPO30, DISPO31, DISPO32, DISPO33, DISPO34, DISPO35, DISPO36, DISPO37, DISPO38, DISPO39, DISPO40, DISPO41, DISPO42, DISPO43, DISPO44, DISPO45, DISPO46, DISPO47, DISPO48, DISPO49, DISPO50, DISPO51, DISPO52, DISPO53, DISPO54, DISPO55, DISPO56, DISPO57, DISPO58, DISPO59, DISPO60, DISPO61, DISPO62, DISPO63, DISPO64, DISPO65, DISPO66, DISPO67, DISPO68, DISPO69, DISPO70, DISPO71, DISPO72, DISPO73, DISPO74, DISPO75, DISPO76, DISPO77, DISPO78, DISPO79, DISPO80, DISPO81, DISPO82, DISPO83, DISPO84, DISPO85, DISPO86, DISPO87, DISPO88, DISPO89, DISPO90, DISPO91, DISPO92, DISPO93, DISPO94, DISPO95, DISPO96, DISPO97, DISPO98, DISPO99, DISPO100	19	188	59
Phagosome Maturation	2.69	-0.11	ATG101, ATG102, ATG103, ATG104, ATG105, ATG106, ATG107, ATG108, ATG109, ATG110, ATG111, ATG112, ATG113, ATG114, ATG115, ATG116, ATG117, ATG118, ATG119, ATG120, ATG121, ATG122, ATG123, ATG124, ATG125, ATG126, ATG127, ATG128, ATG129, ATG130, ATG131, ATG132, ATG133, ATG134, ATG135, ATG136, ATG137, ATG138, ATG139, ATG140, ATG141, ATG142, ATG143, ATG144, ATG145, ATG146, ATG147, ATG148, ATG149, ATG150, ATG151, ATG152, ATG153, ATG154, ATG155, ATG156, ATG157, ATG158, ATG159, ATG160, ATG161, ATG162, ATG163, ATG164, ATG165, ATG166, ATG167, ATG168, ATG169, ATG170, ATG171, ATG172, ATG173, ATG174, ATG175, ATG176, ATG177, ATG178, ATG179, ATG180, ATG181, ATG182, ATG183, ATG184, ATG185, ATG186, ATG187, ATG188, ATG189, ATG190, ATG191, ATG192, ATG193, ATG194, ATG195, ATG196, ATG197, ATG198, ATG199, ATG200	17	157	56
Non-Small Cell Lung Cancer Signaling	2.66	-1.41	EGFR, KRAS, BRAF, PIK3CA, RASGEF1B, NRAS, HRAS, KIF5B, GNAI1, GNAQ1, GNA12, GNA13, GNA14, GNA15, GNA16, GNA17, GNA18, GNA19, GNA20, GNA21, GNA22, GNA23, GNA24, GNA25, GNA26, GNA27, GNA28, GNA29, GNA30, GNA31, GNA32, GNA33, GNA34, GNA35, GNA36, GNA37, GNA38, GNA39, GNA40, GNA41, GNA42, GNA43, GNA44, GNA45, GNA46, GNA47, GNA48, GNA49, GNA50, GNA51, GNA52, GNA53, GNA54, GNA55, GNA56, GNA57, GNA58, GNA59, GNA60, GNA61, GNA62, GNA63, GNA64, GNA65, GNA66, GNA67, GNA68, GNA69, GNA70, GNA71, GNA72, GNA73, GNA74, GNA75, GNA76, GNA77, GNA78, GNA79, GNA80, GNA81, GNA82, GNA83, GNA84, GNA85, GNA86, GNA87, GNA88, GNA89, GNA90, GNA91, GNA92, GNA93, GNA94, GNA95, GNA96, GNA97, GNA98, GNA99, GNA100	13	94	55
ILT3 Signaling Pathway	2.65	-0.96	ILT3, ILT3A, ILT3B, ILT3C, ILT3D, ILT3E, ILT3F, ILT3G, ILT3H, ILT3I, ILT3J, ILT3K, ILT3L, ILT3M, ILT3N, ILT3O, ILT3P, ILT3Q, ILT3R, ILT3S, ILT3T, ILT3U, ILT3V, ILT3W, ILT3X, ILT3Y, ILT3Z, ILT3AA, ILT3AB, ILT3AC, ILT3AD, ILT3AE, ILT3AF, ILT3AG, ILT3AH, ILT3AI, ILT3AJ, ILT3AK, ILT3AL, ILT3AM, ILT3AN, ILT3AO, ILT3AP, ILT3AQ, ILT3AR, ILT3AS, ILT3AT, ILT3AU, ILT3AV, ILT3AW, ILT3AX, ILT3AY, ILT3AZ, ILT3BA, ILT3BB, ILT3BC, ILT3BD, ILT3BE, ILT3BF, ILT3BG, ILT3BH, ILT3BI, ILT3BJ, ILT3BK, ILT3BL, ILT3BL, ILT3BM, ILT3BN, ILT3BO, ILT3BP, ILT3BQ, ILT3BR, ILT3BS, ILT3BT, ILT3BU, ILT3BV, ILT3BW, ILT3BX, ILT3BY, ILT3BZ, ILT3CA, ILT3CB, ILT3CC, ILT3CD, ILT3CE, ILT3CF, ILT3CG, ILT3CH, ILT3CI, ILT3CJ, ILT3CK, ILT3CL, ILT3CM, ILT3CN, ILT3CO, ILT3CP, ILT3CQ, ILT3CR, ILT3CS, ILT3CT, ILT3CU, ILT3CV, ILT3CW, ILT3CX, ILT3CY, ILT3CZ, ILT3DA, ILT3DB, ILT3DC, ILT3DD, ILT3DE, ILT3DF, ILT3DG, ILT3DH, ILT3DI, ILT3DJ, ILT3DK, ILT3DL, ILT3DL, ILT3DM, ILT3DN, ILT3DO, ILT3DP, ILT3DQ, 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ILT3II, ILT3IJ, ILT3IK, ILT3IL, ILT3IL, ILT3IM, ILT3IN, ILT3IO, ILT3IP, ILT3IQ, ILT3IR, ILT3IS, ILT3IT, ILT3IU, ILT3IV, ILT3IW, ILT3IX, ILT3IY, ILT3IZ, ILT3JA, ILT3JB, ILT3JC, ILT3JD, ILT3JE, ILT3JF, ILT3JG, ILT3JH, ILT3JI, ILT3JJ, ILT3JK, ILT3JL, ILT3JL, ILT3JM, ILT3JN, ILT3JO, ILT3JP, ILT3JQ, ILT3JR, ILT3JS, ILT3JT, ILT3JU, ILT3JV, ILT3JW, ILT3JX, ILT3JY, ILT3JZ, ILT3KA, ILT3KB, ILT3KC, ILT3KD, ILT3KE, ILT3KF, ILT3KG, ILT3KH, ILT3KI, ILT3KJ, ILT3KL, ILT3KL, ILT3KM, ILT3KN, ILT3KO, ILT3KP, ILT3KQ, ILT3KR, ILT3KS, ILT3KT, ILT3KU, ILT3KV, ILT3KW, ILT3KX, ILT3KY, ILT3KZ, ILT3LA, ILT3LB, ILT3LC, ILT3LD, ILT3LE, ILT3LF, ILT3LG, ILT3LH, ILT3LI, ILT3LJ, ILT3LK, ILT3LL, ILT3LM, ILT3LN, ILT3LO, ILT3LP, ILT3LQ, ILT3LR, ILT3LS, ILT3LT, ILT3LU, ILT3LV, ILT3LW, ILT3LX, ILT3LY, ILT3LZ, ILT3MA, ILT3MB, ILT3MC, ILT3MD, ILT3ME, ILT3MF, ILT3MG, ILT3MH, ILT3MI, ILT3MJ, ILT3MK, ILT3ML, ILT3ML, ILT3MN, ILT3MO, ILT3MP, ILT3MQ, ILT3MR, ILT3MS, ILT3MT, ILT3MU, ILT3MV, ILT3MW, ILT3MX, ILT3MY, ILT3MZ, ILT3NA, ILT3NB, ILT3NC, ILT3ND, ILT3NE, ILT3NF, ILT3NG, ILT3NH, ILT3NI, ILT3NJ, ILT3NK, ILT3NL, ILT3NL, ILT3NM, ILT3NN, ILT3NO, ILT3NP, ILT3NQ, ILT3NR, ILT3NS, ILT3NT, ILT3NU, ILT3NV, ILT3NW, ILT3NX, ILT3NY, ILT3NZ, ILT3OA, ILT3OB, ILT3OC, ILT3OD, ILT3OE, ILT3OF, ILT3OG, ILT3OH, ILT3OI, ILT3OJ, ILT3OK, ILT3OL, ILT3OL, ILT3OM, ILT3ON, ILT3OO, ILT3OP, ILT3OQ, ILT3OR, ILT3OS, ILT3OT, ILT3OU, ILT3OV, ILT3OW, ILT3OX, ILT3OY, ILT3OZ, ILT3PA, ILT3PB, ILT3PC, ILT3PD, ILT3PE, ILT3PF, ILT3PG, ILT3PH, ILT3PI, ILT3PJ, ILT3PK, ILT3PL, ILT3PL, ILT3PM, ILT3PN, ILT3PO, ILT3PP, ILT3PQ, ILT3PR, ILT3PS, ILT3PT, ILT3PU, ILT3PV, ILT3PW, ILT3PX, ILT3PY, ILT3PZ, ILT3QA, ILT3QB, ILT3QC, ILT3QD, ILT3QE, ILT3QF, ILT3QG, ILT3QH, ILT3QI, ILT3QJ, ILT3QK, ILT3QL, ILT3QL, ILT3QM, ILT3QN, ILT3QO, ILT3QP, ILT3QQ, ILT3QR, ILT3QS, ILT3QT, ILT3QU, ILT3QV, ILT3QW, ILT3QX, ILT3QY, ILT3QZ, ILT3RA, ILT3RB, ILT3RC, ILT3RD, ILT3RE, ILT3RF, ILT3RG, ILT3RH, ILT3RI, ILT3RJ, ILT3RK, ILT3RL, ILT3RL, ILT3RM, ILT3RN, ILT3RO, ILT3RP, ILT3RQ, ILT3RR, ILT3RS, ILT3RT, ILT3RU, ILT3RV, ILT3RW, ILT3RX, ILT3RY, ILT3RZ, ILT3SA, ILT3SB, ILT3SC, ILT3SD, ILT3SE, ILT3SF, ILT3SG, ILT3SH, ILT3SI, ILT3SJ, ILT3SK, ILT3SL, ILT3SL, ILT3SM, ILT3SN, ILT3SO, ILT3SP, ILT3SQ, ILT3SR, ILT3SS, ILT3ST, ILT3SU, ILT3SV, ILT3SW, ILT3SX, ILT3SY, ILT3SZ, ILT3TA, ILT3TB, ILT3TC, ILT3TD, ILT3TE, ILT3TF, ILT3TG, ILT3TH, ILT3TI, ILT3TJ, ILT3TK, ILT3TL, ILT3TL, ILT3TM, ILT3TN, ILT3TO, ILT3TP, ILT3TQ, ILT3TR, ILT3TS, ILT3TT, ILT3TU, ILT3TV, ILT3TW, ILT3TX, ILT3TY, ILT3TZ, ILT3UA, ILT3UB, ILT3UC, ILT3UD, ILT3UE, ILT3UF, ILT3UG, ILT3UH, ILT3UI, ILT3UJ, ILT3UK, ILT3UL, ILT3UL, ILT3UM, ILT3UN, ILT3UO, ILT3UP, ILT3UQ, ILT3UR, ILT3US, ILT3UT, ILT3UU, ILT3UV, ILT3UW, ILT3UX, ILT3UY, ILT3UZ, ILT3VA, ILT3VB, ILT3VC, ILT3VD, ILT3VE, ILT3VF, ILT3VG, ILT3VH, ILT3VI, ILT3VJ, ILT3VK, ILT3VL, ILT3VL, ILT3VM, ILT3VN, ILT3VO, ILT3VP, ILT3VQ, ILT3VR, ILT3VS, ILT3VT, ILT3VU, ILT3VV, ILT3VW, ILT3VX, ILT3VY, ILT3VZ, ILT3WA, ILT3WB, ILT3WC, ILT3WD, ILT3WE, ILT3WF, ILT3WG, ILT3WH, ILT3WI, ILT3WJ, ILT3WK, ILT3WL, ILT3WL, ILT3WM, ILT3WN, ILT3WO, ILT3WP, ILT3WQ, ILT3WR, ILT3WS, ILT3WT, ILT3WU, ILT3WV, ILT3WW, ILT3WX, ILT3WY, ILT3WZ, ILT3XA, ILT3XB, ILT3XC, ILT3XD, ILT3XE, ILT3XF, ILT3XG, ILT3XH, ILT3XI, ILT3XJ, ILT3XK, ILT3XL, ILT3XL, ILT3XM, ILT3XN, ILT3XO, ILT3XP, ILT3XQ, ILT3XR, ILT3XS, ILT3XT, ILT3XU, ILT3XV, ILT3XW, ILT3XX, ILT3XY, ILT3XZ, ILT3YA, ILT3YB, ILT3YC, ILT3YD, ILT3YE, ILT3YF, ILT3YG, ILT3YH, ILT3YI, ILT3YJ, ILT3YK, ILT3YL, ILT3YL, ILT3YM, ILT3YN, ILT3YO, ILT3YP, ILT3YQ, ILT3YR, ILT3YS, ILT3YT, ILT3YU, ILT3YV, ILT3YW, ILT3YX, ILT3YY, ILT3YZ, ILT3ZA, ILT3ZB, ILT3ZC, ILT3ZD, ILT3ZE, ILT3ZF, ILT3ZG, ILT3ZH, ILT3ZI, ILT3ZJ, ILT3ZK, ILT3ZL, ILT3ZL, ILT3ZM, ILT3ZN, ILT3ZO, ILT3ZP, ILT3ZQ, ILT3ZR, ILT3ZS, ILT3ZT, ILT3ZU, ILT3ZV, ILT3ZW, ILT3ZX, ILT3ZY, ILT3ZZ	11	82	57
IKK1-catalyzed Signaling	2.62	-0.50	IKK1, IKK2, IKK3, IKK4, IKK5, IKK6, IKK7, IKK8, IKK9, IKK10, IKK11, IKK12, IKK13, IKK14, IKK15, IKK16, IKK17, IKK18, IKK19, IKK20, IKK21, IKK22, IKK23, IKK24, IKK25, IKK26, IKK27, IKK28, IKK29, IKK30, IKK31, IKK32, IKK33, IKK34, IKK35, IKK36, IKK37, IKK38, IKK39, IKK40, IKK41, IKK42, IKK43, IKK44, IKK45, IKK46, IKK47, IKK48, IKK49, IKK50, IKK51, IKK52, IKK53, IKK54, IKK55, IKK56, IKK57, IKK58, IKK59, IKK60, IKK61, IKK62, IKK63, IKK64, IKK65, IKK66, IKK67, IKK68, IKK69, IKK70, IKK71, IKK72, IKK73, IKK74, IKK75, IKK76, IKK77, IKK78, IKK79, IKK80, IKK81, IKK82, IKK83, IKK84, IKK85, IKK86, IKK87, IKK88, IKK89, IKK90, IKK91, IKK92, IKK93, IKK94, IKK95, IKK96, IKK97, IKK98, IKK99, IKK100	18	175	58
Molecular Mechanisms of Cancer	2.59	-1.08	TP53, KRAS, BRAF, PIK3CA, RASGEF1B, NRAS, HRAS, KIF5B, GNAI1, GNAQ1, GNA12, GNA13, GNA14, GNA15, GNA16, GNA17, GNA18, GNA19, GNA20, GNA21, GNA22, GNA23, GNA24, GNA25, GNA26, GNA27, GNA28, GNA29, GNA30, GNA31, GNA32, GNA33, GNA34, GNA35, GNA36, GNA37, GNA38, GNA39, GNA40, GNA41, GNA42, GNA43, GNA44, GNA45, GNA46, GNA47, GNA48, GNA49, GNA50, GNA51, GNA52, GNA53, GNA54, GNA55, GNA56, GNA57, GNA58, GNA59, GNA60, GNA61, GNA62, GNA63, GNA64, GNA65, GNA66, GNA67, GNA68, GNA69, GNA70, GNA71, GNA72, GNA73, GNA74, GNA75, GNA76, GNA77, GNA78, GNA79, GNA80, GNA81, GNA82, GNA83, GNA84, GNA85, GNA86, GNA87, GNA88, GNA89, GNA90, GNA91, GNA92, GNA93, GNA94, GNA95, GNA96, GNA97, GNA98, GNA99, GNA100	36	446	59
RAC Signaling	2.51	-0.53	RAC1, RAC2, RAC3, RAC4, RAC5, RAC6, RAC7, RAC8, RAC9, RAC10, RAC11, RAC12, RAC13, RAC14, RAC15, RAC16, RAC17, RAC18, RAC19, RAC20, RAC21, RAC22, RAC23, RAC24, RAC25, RAC26, RAC27, RAC28, RAC29, RAC30, RAC31, RAC32, RAC33, RAC34, RAC35, RAC36, RAC37, RAC38, RAC39, RAC40, RAC41, RAC42, RAC43, RAC44, RAC45, RAC46, RAC47, RAC48, RAC49, RAC50, RAC51, RAC52, RAC53, RAC54, RAC55, RAC56, RAC57, RAC58, RAC59, RAC60, RAC61, RAC62, RAC63, RAC64, RAC65, RAC66, RAC67, RAC68, RAC69, RAC70, RAC71, RAC72, RAC73, RAC74, RAC75, RAC76, RAC77, RAC78, RAC79, RAC80, RAC81, RAC82, RAC83, RAC84, RAC85, RAC86, RAC87, RAC88, RAC89, RAC90, RAC91, RAC92, RAC93, RAC94, RAC95, RAC96, RAC97, RAC98, RAC99, RAC100	15	138	60
Protein Signaling	2.5	-0.01	PTEN, SH2, SH3, SH4, SH5, SH6, SH7, SH8, SH9, SH10, SH11, SH12, SH13, SH14, SH15, SH16, SH17, SH18, SH19, SH20, SH21, SH22, SH23, SH24, SH25, SH26, SH27, SH28, SH29, SH30, SH31, SH32, SH33, SH34, SH35, SH36, SH37, SH38, SH39, SH40, SH41, SH42, SH43, SH44, SH45, SH46, SH47, SH48, SH49, SH50, SH51, SH52, SH53, SH54, SH55, SH56, SH57, SH58, SH59, SH60, SH61, SH62, SH63, SH64, SH65, SH66, SH67, SH68, SH69, SH70, SH71, SH72, SH73, SH74, SH75, SH76, SH77, SH78, SH79, SH80, SH81, SH82, SH83, SH84, SH85, SH86, SH87, SH88, SH89, SH90, SH91, SH92, SH93, SH94, SH95, SH96, SH97, SH98, SH99, SH100	14	128	61
Risk of MI in cardiac hypertrophy	2.49	-0.47	ACE, ACE2, ACE3, ACE4, ACE5, ACE6, ACE7, ACE8, ACE9, ACE10, ACE11, ACE12, ACE13, ACE14, ACE15, ACE16, ACE17, ACE18, ACE19, ACE20, ACE21, ACE22, ACE23, ACE24, ACE25, ACE26, ACE27, ACE28, ACE29, ACE30, ACE31, ACE32, ACE33, ACE34, ACE35, ACE36, ACE37, ACE38, ACE39, ACE40, ACE41, ACE42, ACE43, ACE44, ACE45, ACE46, ACE47, ACE48, ACE49, ACE50, ACE51, ACE52, ACE53, ACE54, ACE55, ACE56, ACE57, ACE58, ACE59, ACE60, ACE61, ACE62, ACE63, ACE64, ACE65, ACE66, ACE67, ACE68, ACE69, ACE70, ACE71, ACE72, ACE73, ACE74, ACE75, ACE76, ACE77, ACE78, ACE79, ACE80, ACE81, ACE82, ACE83, ACE84, ACE85, ACE86, ACE87, ACE88, ACE89, ACE90, ACE91, ACE92, ACE93, ACE94, ACE95, ACE96, ACE97, ACE98, ACE99, ACE100	23	223	62
Cytoskeleton-mediated Endocytosis Signaling	2.49	-0.10	CLIP1, CLIP2, CLIP3, CLIP4, CLIP5, CLIP6, CLIP7, CLIP8, CLIP9, CLIP10, CLIP11, CLIP12, CLIP13, CLIP14, CLIP15, CLIP16, CLIP17, CLIP18, CLIP19, CLIP20, CLIP21, CLIP22, CLIP23, CLIP24, CLIP25, CLIP26, CLIP27, CLIP28, CLIP29, CLIP30, CLIP31, CLIP32, CLIP33, CLIP34, CLIP35, CLIP36, CLIP37, CLIP38, CLIP39, CLIP40, CLIP41, CLIP42, CLIP43, CLIP44, CLIP45, CLIP46, CLIP47, CLIP48, CLIP49, CLIP50, CLIP51, CLIP52, CLIP53, CLIP54, CLIP55, CLIP56, CLIP57, CLIP58, CLIP59, CLIP60, CLIP61, CLIP62, CLIP63, CLIP64, CLIP65, CLIP66, CLIP67, CLIP68, CLIP69, CLIP70, CLIP71, CLIP72, CLIP73, CLIP74, CLIP75, CLIP76, CLIP77, CLIP78, CLIP79, CLIP80, CLIP81, CLIP82, CLIP83, CLIP84, CLIP85, CLIP86, CLIP87, CLIP88, CLIP89, CLIP90, CLIP91, CLIP92, CLIP93, CLIP94, CLIP95, CLIP96, CLIP97, CLIP98, CLIP99, CLIP100	19	170	63
Endocytosis-mediated Signaling	2.48	-0.09	SH2, SH3, SH4, SH5, SH6, SH7, SH8, SH9, SH10, SH11, SH12, SH13, SH14, SH15, SH16, SH17, SH18, SH19, SH20, SH21, SH22, SH23, SH24, SH25, SH26, SH27, SH28, SH29, SH30, SH31, SH32, SH33, SH34, SH35, SH36, SH37, SH38, SH39, SH40, SH41, SH42, SH43, SH44, SH45, SH46, SH47, SH48, SH49, SH50, SH51, SH52, SH53, SH54, SH55, SH56, SH57, SH58, SH59, SH60, SH61, SH62, SH63, SH64, SH65, SH66, SH67, SH68, SH69, SH70, SH71, SH72, SH73, SH74, SH75, SH76, SH77, SH78, SH79, SH80, SH81, SH82, SH83, SH84, SH85, SH86, SH87, SH88, SH89, SH90, SH91, SH92, SH93, SH94, SH95, SH96, SH97, SH98, SH99, SH100	24	206	64
Pathologic Adrenocortical Signaling	2.42	-0.76	ACT1, ACT2, ACT3, ACT4, ACT5, ACT6, ACT7, ACT8, ACT9, ACT10, ACT11, ACT12, ACT13, ACT14, ACT15, ACT16, ACT17, ACT18, ACT19, ACT20, ACT21, ACT22, ACT23, ACT24, ACT25, ACT26, ACT27, ACT28, ACT29, ACT30, ACT31, ACT32, ACT33, ACT34, ACT35, ACT36, ACT37, ACT38, ACT39, ACT40, ACT41, ACT42, ACT43, ACT44, ACT45, ACT46, ACT47, ACT48, ACT49, ACT50, ACT51, ACT52, ACT53, ACT54, ACT55, ACT56, ACT57, ACT58, ACT59, ACT60, ACT61, ACT62, ACT63, ACT64, ACT65, ACT66, ACT67, ACT68, ACT69, ACT70, ACT71, ACT72, ACT73, ACT74, ACT75, ACT76, ACT77, ACT78, ACT79, ACT80, ACT81, ACT82, ACT83, ACT84, ACT85, ACT86, ACT87, ACT88, ACT89, ACT90, ACT91, ACT92, ACT93, ACT94, ACT95, ACT96, ACT97, ACT98, ACT99, ACT100	15	140	65
Endocytosis-mediated Signaling	2.41	-1.15	SH2, SH3, SH4, SH5, SH6, SH7, SH8, SH9, SH10, SH11, SH12, SH13, SH14, SH1			

# Neurodegeneration in the brain (CNS)

## Neurodegeneration

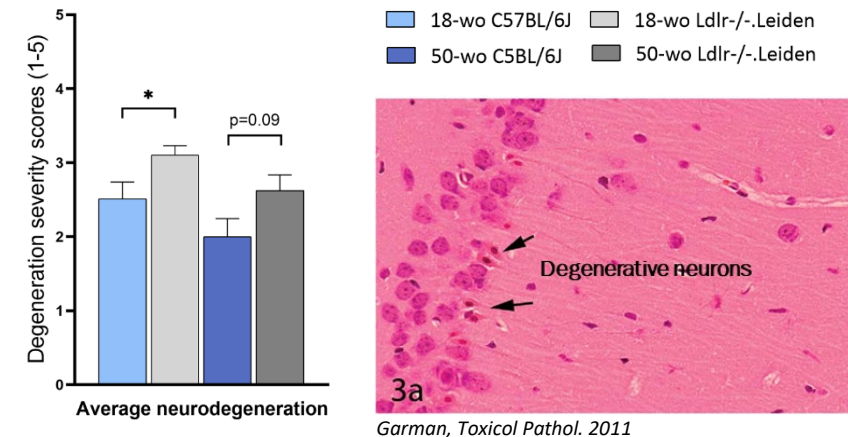
- Underlying disease process in all neurodegenerative diseases
- Loss of neuronal function – breakdown connectivity & communication: axonal damage, synaptic dysfunction/loss



**Fig. 1. Major cellular and molecular processes contributing to neurodegeneration.** There are multiple processes that drive neurodegeneration as a result of specific genetic vulnerabilities or aging. Such processes include abnormally altered expression of some disease-driving RNAs and proteins, dysfunction of specific cellular organelles such as mitochondria or lysosomes, and neuroinflammation and altered responses of glia in the brain. Lysosomes and mitochondria are shown in green and pink, respectively. Abnormal protein accumulation and altered RNA-protein interactions are depicted as black dots.

Katnelson et al. *Sci Transl Med.* 2016

Histological analysis – comparison with conventional model (C57BL/6J wt mice)



Garman, *Toxicol Pathol.* 2011

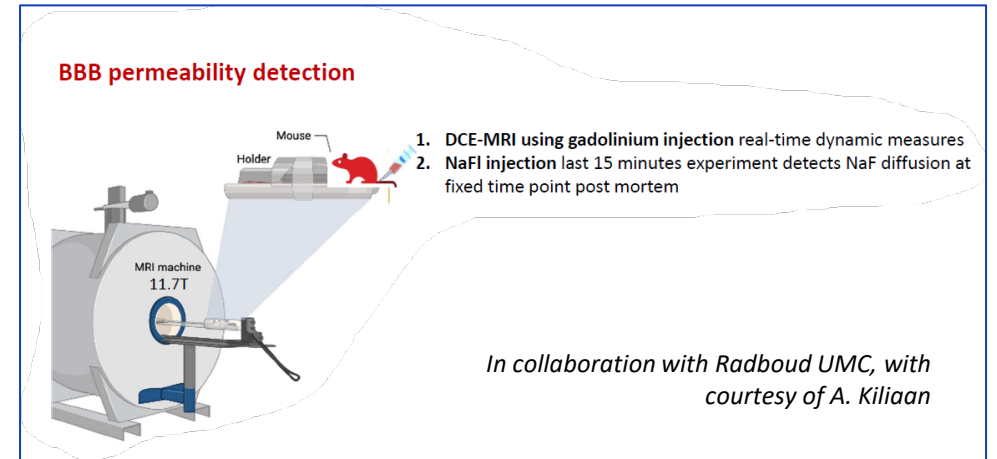
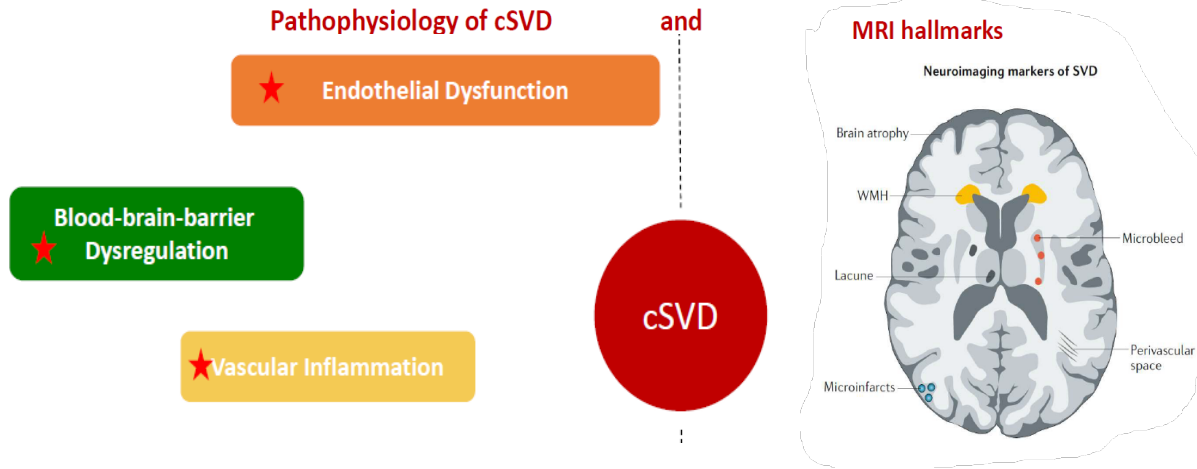
- Neurodegeneration more pronounced in Ldlr-/-Leiden model than in conventional model.
- In addition: demyelination of peripheral nerves (histology, lipidomics)

Seidel et al. *Front. Cell Neurosc.* 2023

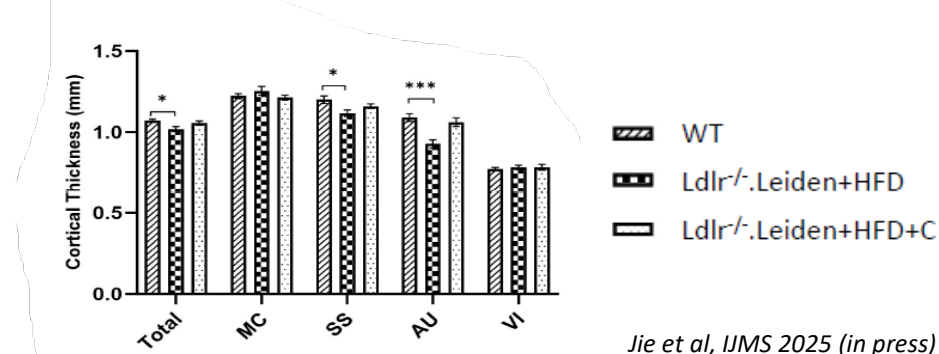
Seidel et al. *Brain Behavior Immun Health* 2025

Seider et al., *PLoS One*, in revision

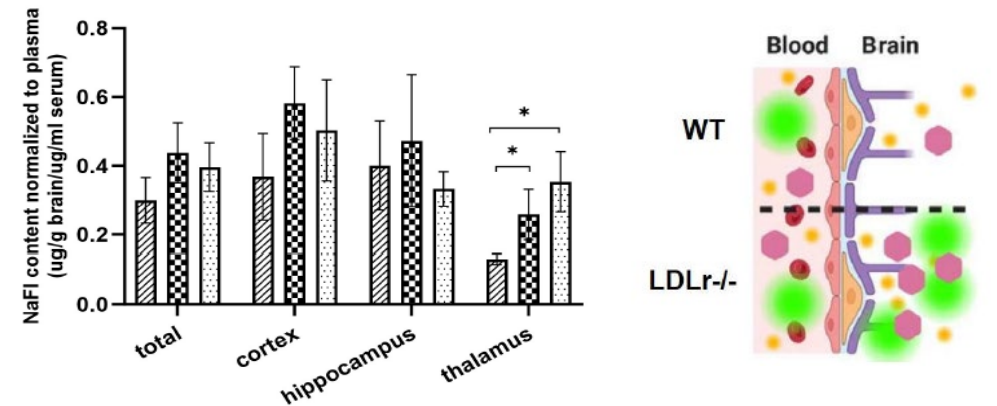
# Pathology of small vessel disease incl. BBB 'leakage'



- **Reduced cortical thickness** in HFD-fed Ldlr<sup>-/-</sup>.Leiden mice compared to BL/6 wt mice  
 → see next slide: fits with increased local ketone formation (and breakdown of structural lipids in cortex)



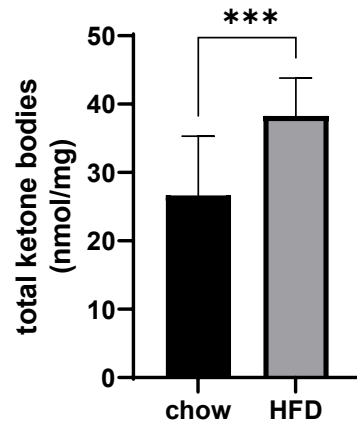
- BBB leakage in Ldlr<sup>-/-</sup>.Leiden mice compared to BL/6 wt mice (by Na-fluorescein and by MRI)



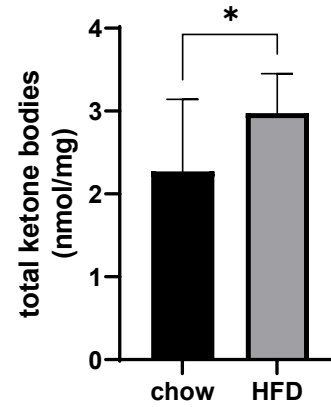
# KETONE BODIES ELEVATED IN CORTEX INDICATES PATHOLOGICAL LIPID BREAKDOWN

Analysis of cortex tissues from Seidel et al., Front. Cell Neurosc. 2023 indicates an enhanced lipid breakdown (probably of brain-lipids ie. which may explain cortex thinning on HFD) and thereby the impaired brain functions

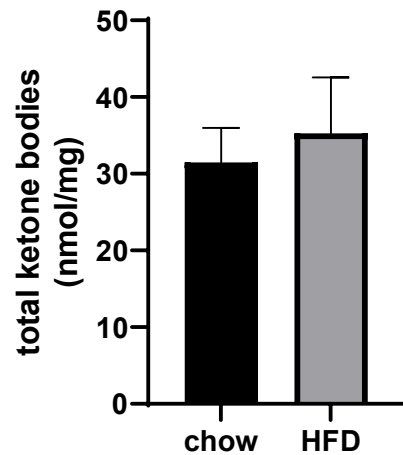
LDLr<sup>-/-</sup> 50w CORTEX per protein



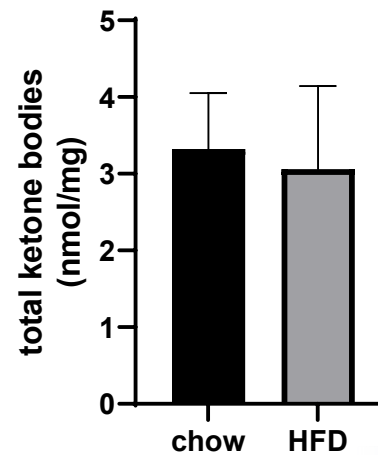
LDLr<sup>-/-</sup> 50w CORTEX per mg tissue



LDLr<sup>-/-</sup> 50w THALAMUS per protein



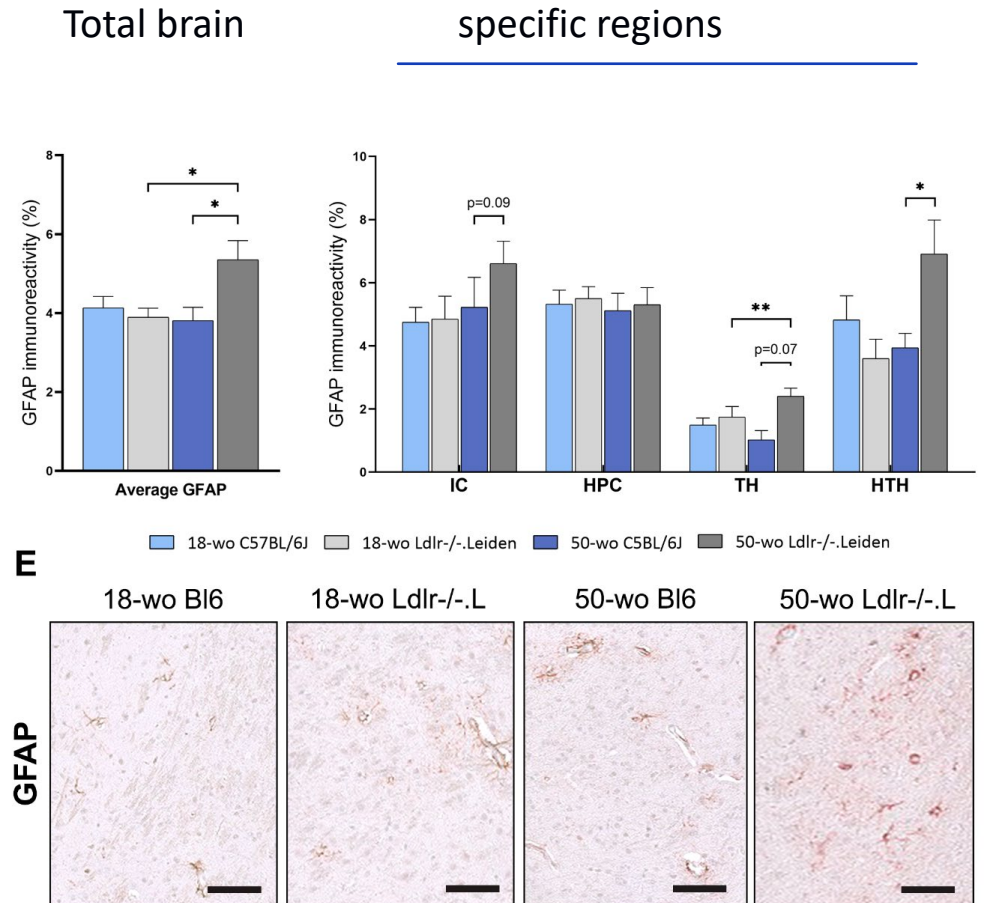
LDLr<sup>-/-</sup> 50w THALAMUS per tissue



# Age-related gliosis

## Gliosis

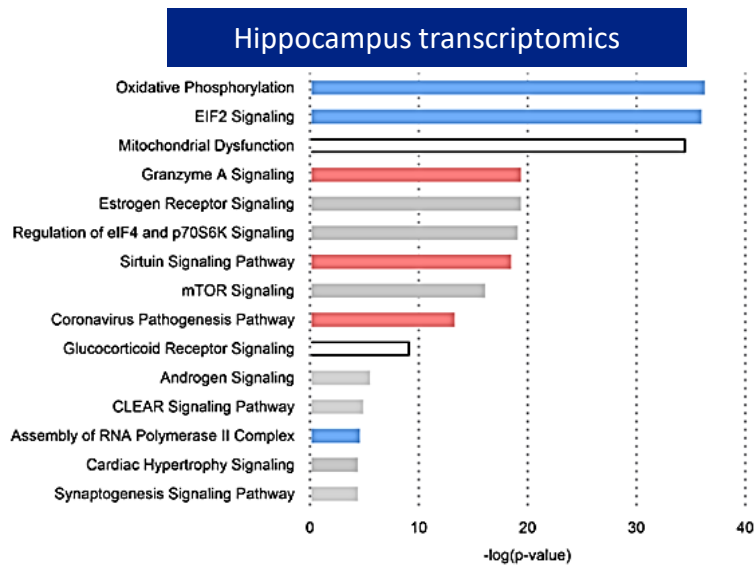
- Reactive change (proliferation) in glial cells in response to damage.
- Glial cells: 'helper cells' of nervous system (homeostasis, myelination, BBB, supply neurons with nutrients, support & protect neurons)
- Important pro-inflammatory mechanism in neurodegenerative diseases
- Astrogliosis: proliferation of astrocytes.
  - Index for neuronal damage.
  - GFAP: marker protein for astrocytes.
- Histological analysis – comparison with conventional model
  - Ldlr<sup>-/-</sup>.Leiden model shows age-related gliosis, that is not observed in conventional models



# Oxidative stress & Mitochondrial dysfunction

## Top 3 pathways in NGS:

- Down-regulated OXPHOS (#1)
- eIF2 (associated with UPR) (#2)
- Mitochondrial dysfunction (#3)

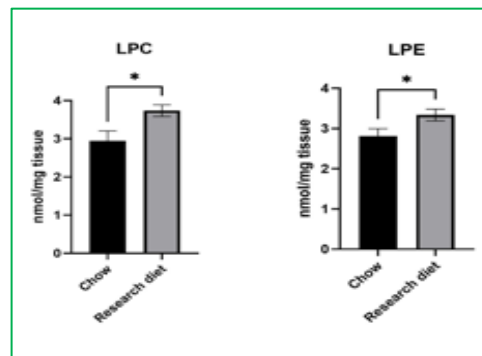
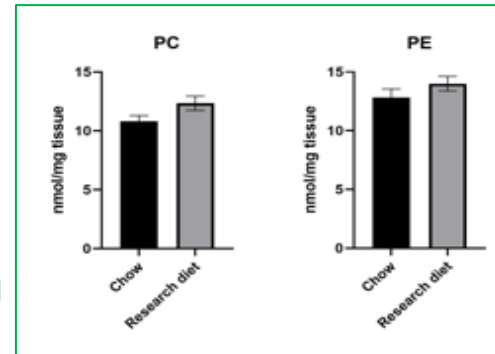


Seidel et al. *Front. Cell Neurosci.* 2023 &  
Seidel et al. *Brain Behavior Immun Health* 2025

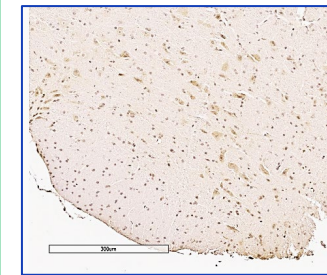
Aamir et al. *Unfolded Protein Response | SpringerLink* 2022

## Hippocampal lipidomics & IHC:

- Lipid biomarkers of ROS exposure
- Increased LPC and LPE
- 4-HNE IHC-staining



Morrison et al. *unpublished*



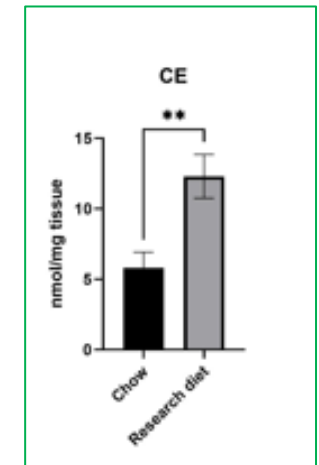
4HNE-positive staining in cerebellum (giant neuronal cells)

Lysophosphatidyl-PC and Lysophosphatidyl-PE can be formed via ROS or by lipoases

Engel et al. *Frontiers Physiol.* 2021

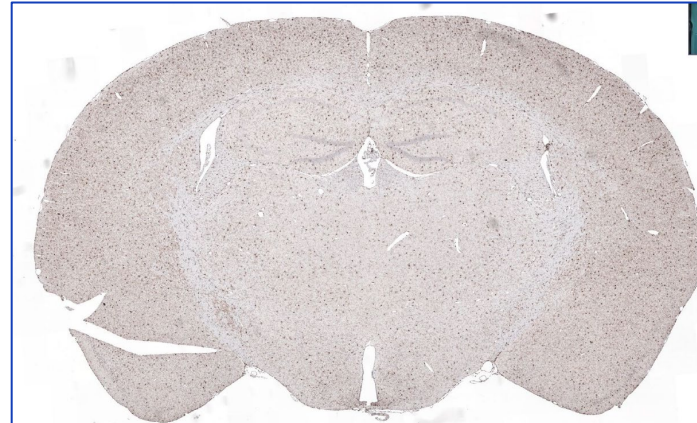
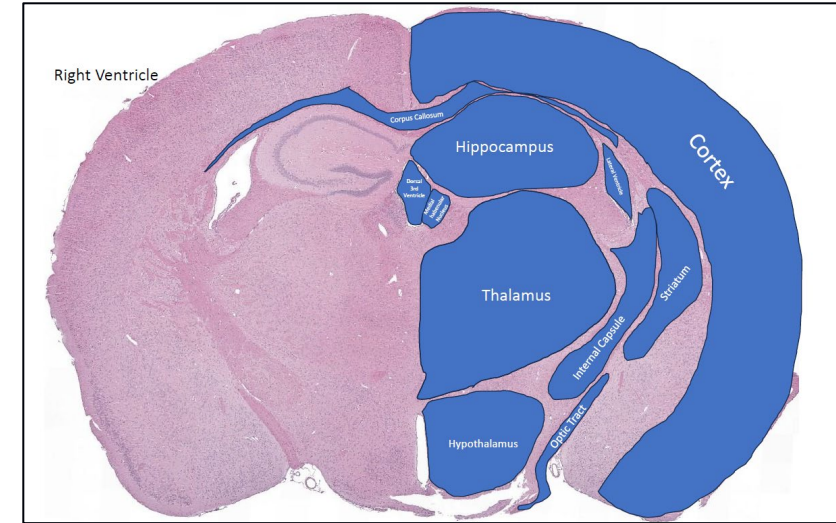
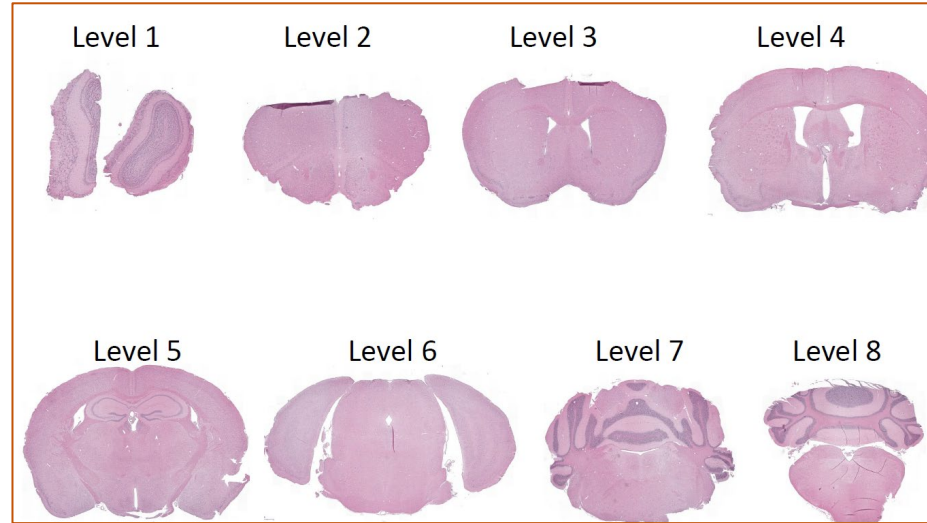
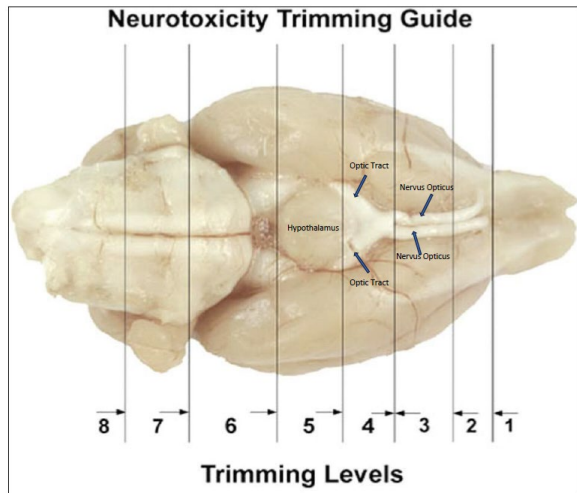
- Increased cholesteryl esters
- CE is neutral form of free cholesterol, which is proinflammatory (inflammasome)
- CE accumulation is well known in neurodegenerative diseases.

Shirane et al. *Neural Regen Res.* 2024



Morrison et al. *unpublished*

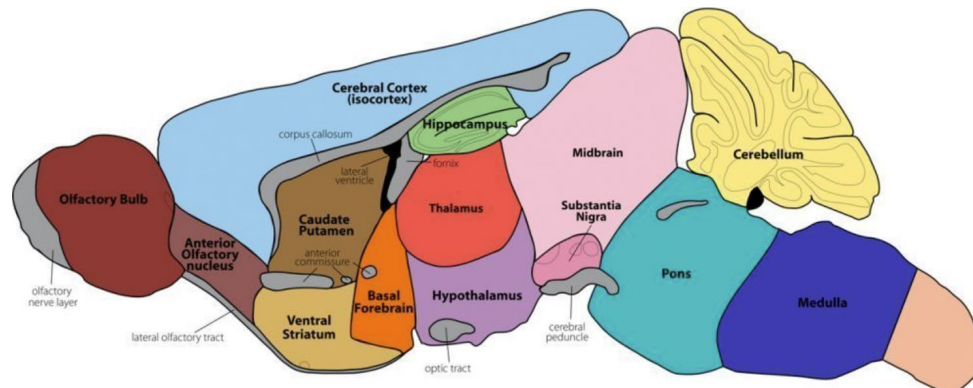
# Histology (ongoing)



# Aging associated neuroinflammation: spatial differences

- Significant spatial differences in protein expression and inflammatory cytokines, for instance:
  - VCAM-1 (per mg tissue): thalamus = cortex << hippocampus
  - BDNF (per mg tissue): cortex < thalamus << hippocampus
  - IL-33 (per mg tissue): cortex = hippocampus << thalamus
  - TNFa (per mg tissue): cortex < thalamus < hippocampus

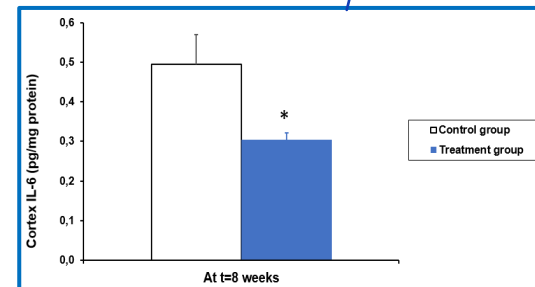
*Results from 80 w old C57BL/6 mice and similar regional differences in Ldlr-/- .Leiden mice (data from Public Private Partnership I-BRAIN)*



unpublished data

Example: Effects of anti-inflammatory treatments targeting inflammasome:

- IL-1 $\beta$  (HPC, CTX) ↓
- IL-6 (HPC, TH) ↓
- TNF- $\alpha$  (HPC, CTX) ↓
- IL-33 and IL-15 (TH) ↑
- IL-10 (TH) ↑ (HPC) ↓
- MIP-1, VCAM-1, GDF15 and Osteopontin (no difference)



# Obesity-induced neuroinflammation in Ldlr-/-Leiden mice

Effect of HFD feeding on chemokine and cytokine concentrations in cortex homogenates of 50 week-old Ldlr-/-Leiden mice

Cytokines	Chow		HFD	
	Mean	SEM	Mean	SEM
IL-17A/F	0,13	0,02	0,14	0,01
IL-27p28/IL-30	0,28	0,05	0,26	0,03
<u>IL-33</u>	<u>38,82</u>	<u>11,22</u>	<u>78,50</u> <sup>p=0.07</sup>	<u>13,68</u>
IP-10	0,79	0,04	0,89	0,08
MCP-1	1,94	0,21	1,89	0,09
IFN- $\gamma$	0,00	0,00	0,00	0,00
<u>IL-10</u>	<u>0,09</u>	<u>0,02</u>	<u>0,14</u> *	<u>0,01</u>
<u>IL-1<math>\beta</math></u>	<u>0,09</u>	<u>0,02</u>	<u>0,13</u> <sup>p=0.13</sup>	<u>0,02</u>
IL-2	0,03	0,00	0,03	0,00
<u>IL-6</u>	<u>0,29</u>	<u>0,06</u>	<u>0,41</u> *	<u>0,02</u>
KC/GRO	0,96	0,10	0,86	0,03
<u>TNF-<math>\alpha</math></u>	<u>0,02</u>	<u>0,01</u>	<u>0,03</u> <sup>p=0.13</sup>	<u>0,00</u>

Concentrations are expressed as pg / mg protein.

- Neuroinflammation on protein level: HFD induces inflammatory response as evidenced by cytokine profiling of cortex.

- IL-33  $\uparrow$
- IL-10  $\uparrow$
- IL-1 $\beta$   $\uparrow$
- IL-6  $\uparrow$
- TNF- $\alpha$   $\uparrow$

HFD  
vs.  
Chow

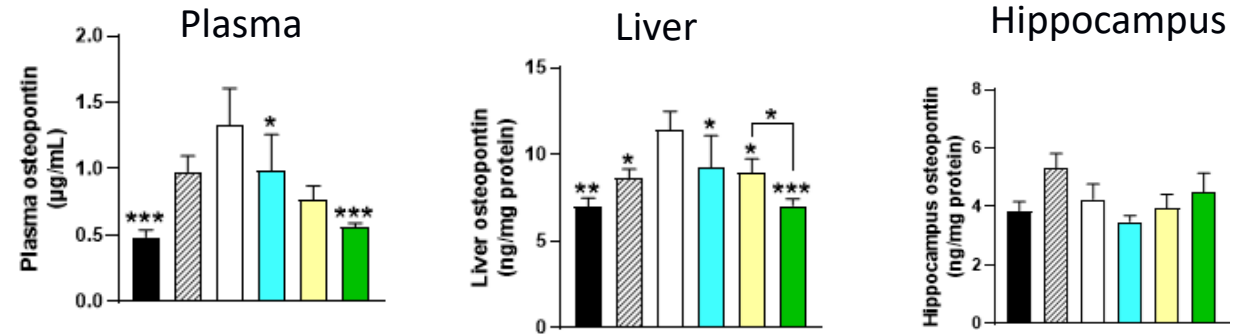
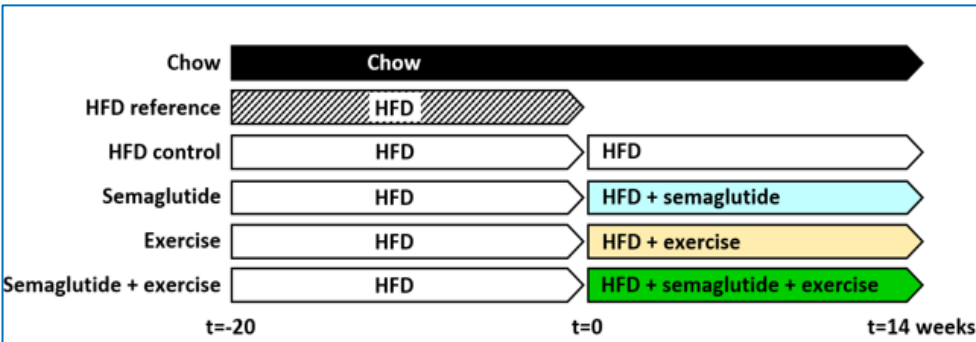


- Dynamical changes over time (typically no gradual increase but bell-shaped with earlier peak on HFD as compared to chow)
- Significant regional (spatial) differences in cytokine and chemokine expression

Seidel et al. PLoS One , in revision

# Recent example: some cytokines peak at early time points of HFD feeding

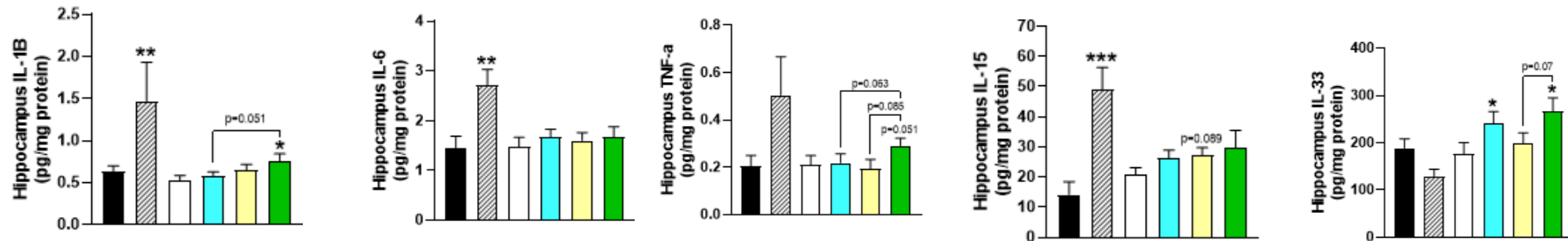
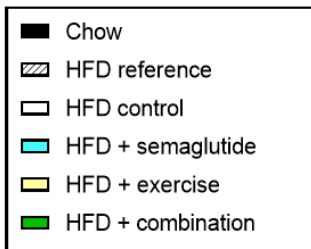
Osteopontin (used as marker of microglia activation)



PubMed: [Osteopontin drives neuroinflammation and cell loss in MAPT-N279K frontotemporal dementia patient neurons: Cell Stem Cell](#)

PubMed: [Plasma osteopontin as a biomarker of Alzheimer's disease and vascular cognitive impairment | Scientific Reports](#)

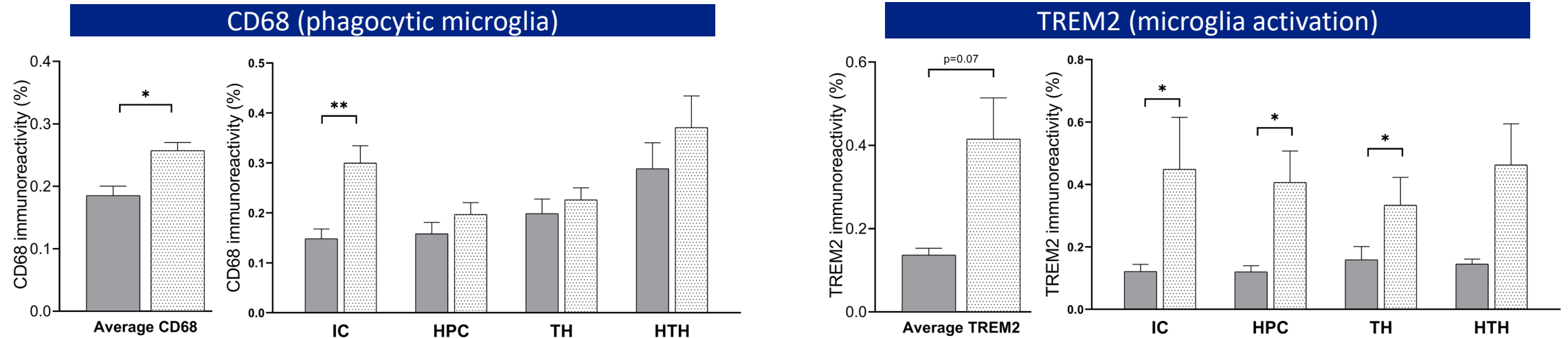
After 20 w of HFD run-in, hippocampal expression of many cytokines is higher than after prolonged HFD (by another 14w) or after prolonged chow-feeding (34 w). This suggests that neuroinflammation peaks at an early time point, ie. at 18-20 w of HFD.



# Obesity-induced neuroinflammation

In line with human data, obesity-associated changes in immunophenotype in *Ldlr*<sup>-/-</sup>.Leiden model:

- **Upregulation of other microglia markers (CD68 and TREM2):** both linked to human neurodegenerative diseases

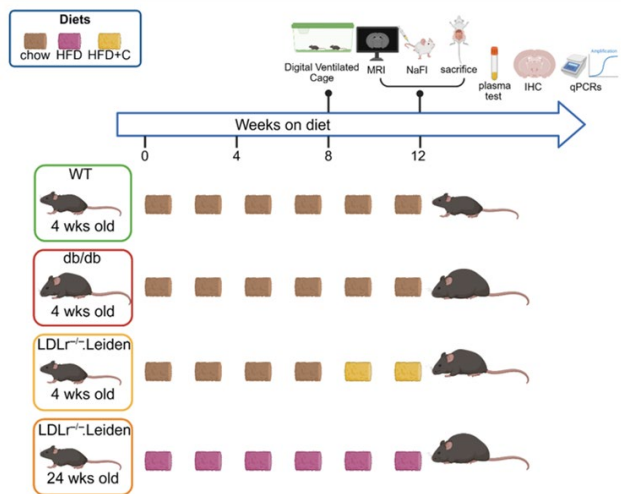


- Neuroinflammation is not simply upregulation of number of cells or a single marker => it is a change in immunotype (as also observed in human neurodegeneration).
- *Ldlr*<sup>-/-</sup>.Leiden model replicates microglial marker expression patterns observed in human neurodegenerative disease

Legenda: IC=internal capsule, HPC=hippocampus, TH=thalamus, HTH=hypothalamus

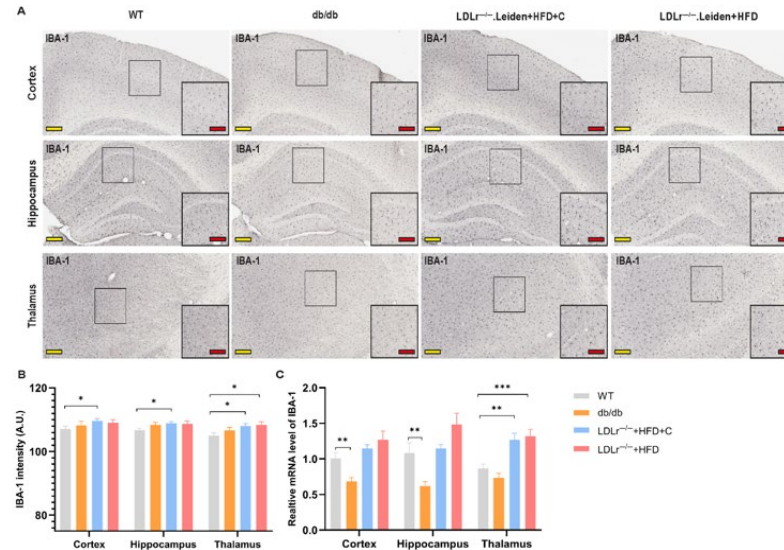
# Iba-1 and GFAP in WT, db/db and Ldlr<sup>-/-</sup>.Leiden mice

## Study setup



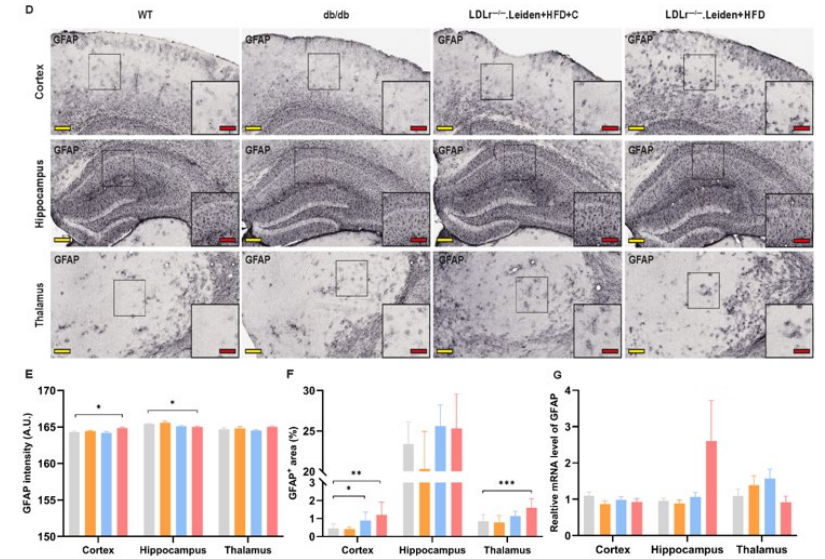
Note, the latter cohort was pre-fed HFD for 24w prior to start of the study

## Iba-1



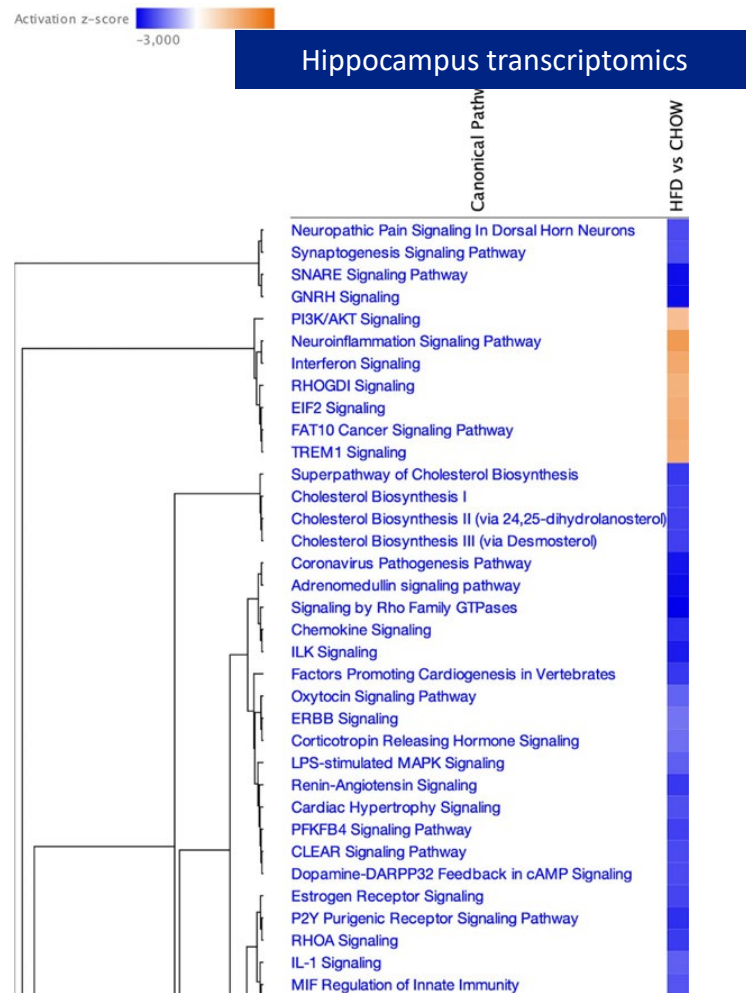
Significantly increased in Ldlr<sup>-/-</sup>.Leiden mice and reduced in db/db mice compared to wt C57BL/6J

## GFAP



Significantly increased in Ldlr<sup>-/-</sup>.Leiden mice (astrogliosis) and unchanged in db/db mice compared to wt C57BL/6J

# Obesity-induced neuroinflammation - mechanisms

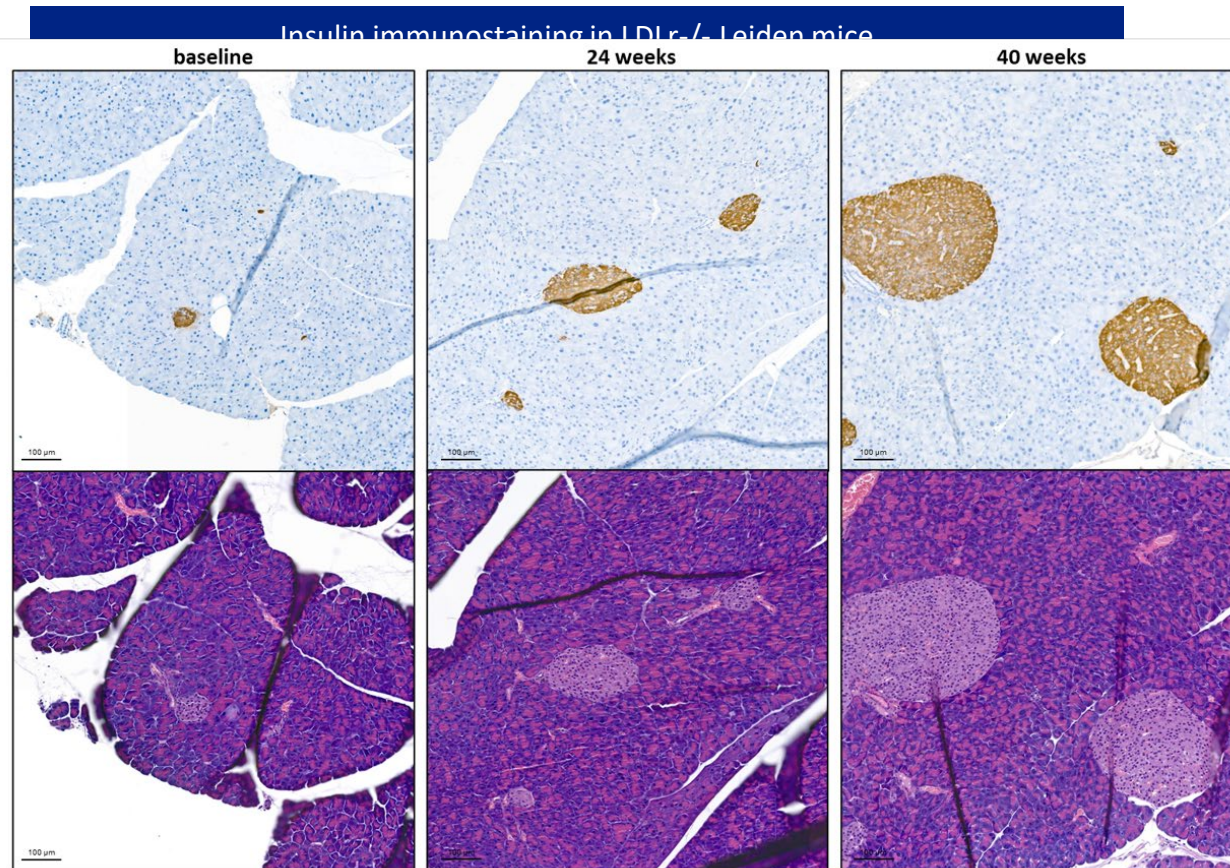


- Activation of several neuroinflammatory pathways
- In line with histological analysis: not all inflammatory pathways are upregulated, some also inactivated. → immunophenotypical change
- Deregulation of processes involved in neuron health/function:
  - E.g. axon ensheathment, neurotransmission, synaptic plasticity
- Many processes with described role in neurodegenerative disease

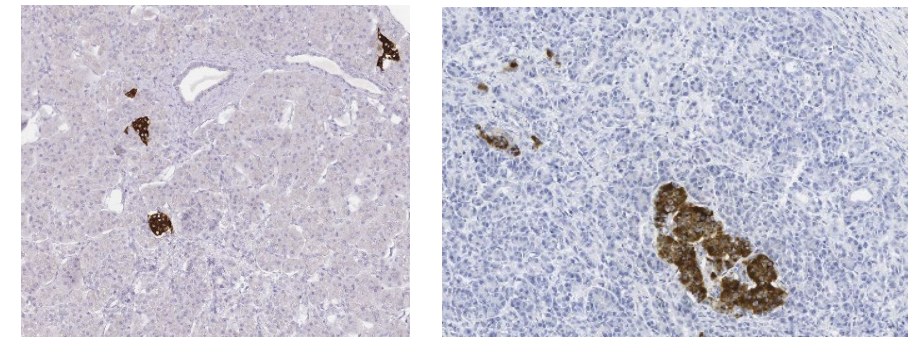
## In conclusion:

- Ldlr<sup>-/-</sup>.Leiden model is more susceptible to neurodegeneration and associated gliosis than conventional C57BL/6J mouse model.
- HFD-induced obesity results in neuroinflammation characterized by immunophenotypic shift, similar to what is observed in humans.

# Obesity-induced pancreas pathology (new)



- $\beta$ -cells at baseline comparable to situation in humans
- Hypertrophy at 24 weeks of HFD consistent with high plasma insulin levels
- Severe hypertrophy at 40 weeks of HFD



Human protein Atlas

Unpublished, with courtesy of S. Tokgöz and  
M. Gotthardt. Radboud UMC Nijmegen

# Publications Ldlr<sup>-/-</sup>.Leiden brain studies

SCFA (gut-derived mediators)

(Breast) milk component

Muscle-targeted intervention

Anti-inflammatory

Metabolic correction

Sex differences

Ageing & BBB dysfunction

## Intervention

Butyrate

Propionate

Milk fat globule membrane (MFGM)

Exercise and branched-chain amino acids

Comparison Ldlr<sup>-/-</sup>.Leiden vs BL6  
+ anti-inflammatory treatment (anti-C5)

Lipectomy (visceral WAT removal)

Semaglutide (GLP1 analogue)

Male vs female

Time course chow and HFD  
Comparison with C57BL/6 & dbdb mice

## Publication

Arnoldussen et al. Int J Obesity 2017

Tengeler & Gart et al. FASEBJ 2020

Arnoldussen et al. Int J Obesity 2021

Lohkamp et al. Nutrients 2023

Seidel et al. Front.Cell Neurosc. 2023

Seidel et al., Brain Behavior Immun. Health, 2025

vd Hoek et al., in preparation

Jacobs et al. Nutrients 2019

Seidel et al., PLoS One, in revision

Jie et al., IJMS, 2025 (in press)

# Opportunity to join Brain Health PPP

Open for collaboration!

[martine.morrison@tno.nl](mailto:martine.morrison@tno.nl)

Mechanistic Readouts  
of Brain Health



Functional behavioural  
and cognitive tests

Neurodegeneration

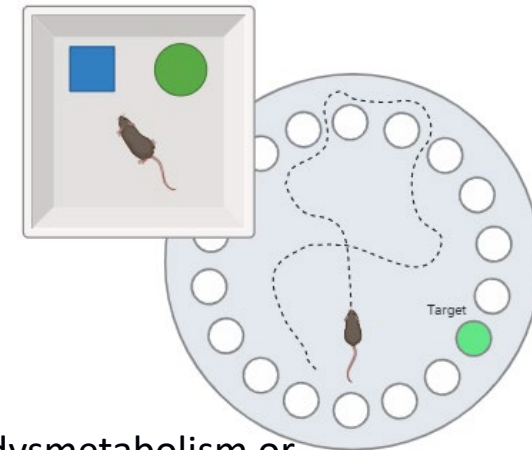
Astrogliosis

Neuroinflammation

Brain Energy Metabolism

BBB - vascular integrity

e.g. immunohistochemistry, cytokine profiling, gene expression  
(transcriptomics and pathway analysis), lipid- and oxylipin profiling



- Ldlr<sup>-/-</sup>.Leiden mice in the context of aging and/or dysmetabolism or obesity.
- Looking for partner(s) with interest to study treatment (prevention/intervention) to improve brain health.
- Publication of results.