

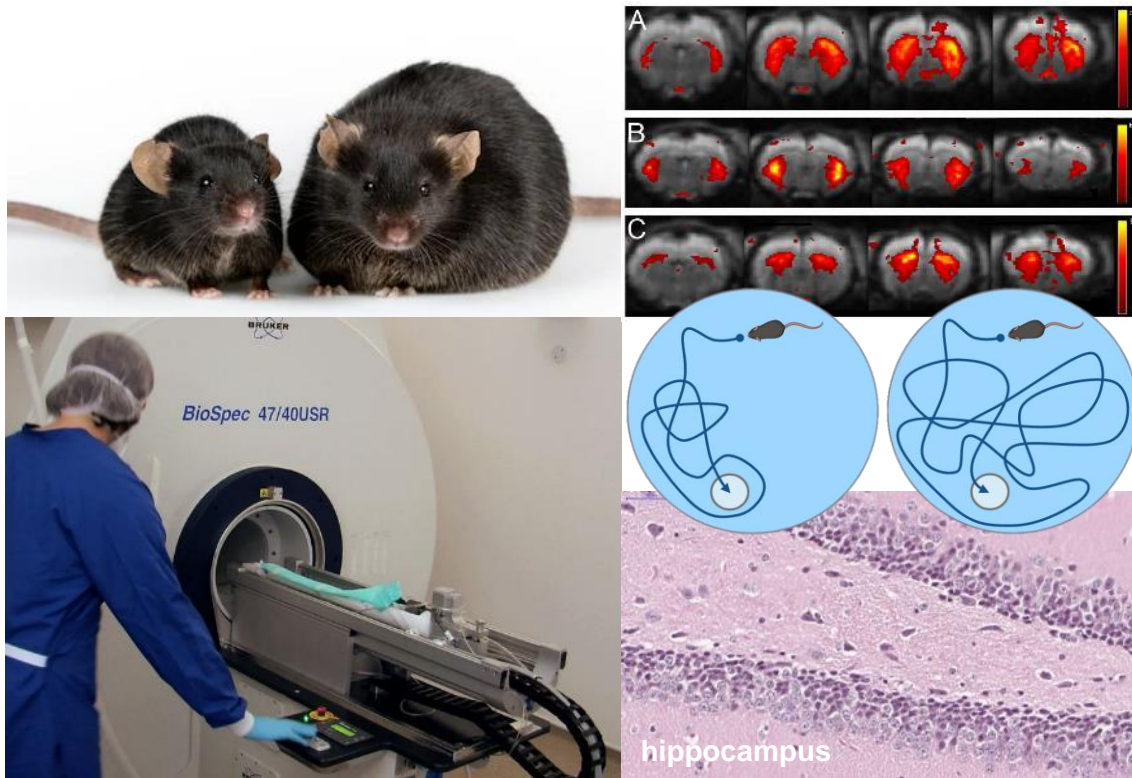
# Brain Health

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

Martine Morrison PhD



# Model for Brain Health and Neuroinflammation (Aging)



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

- Translational for obesity, IR, NAFLD/NASH, CVD.
- Human disease mechanisms and pathology
- Mimics patient groups  
(Morrison, *Hepatol Comm.*, 2018; Martinez-Arranz, *Hepatology* 2022)

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

- Behaviour & cognition tests during aging and/or obesity
- Histopathology: morphology & protein expression and distribution
- Brain: transcriptomics, lipids, oxylipins, neuroinflammation

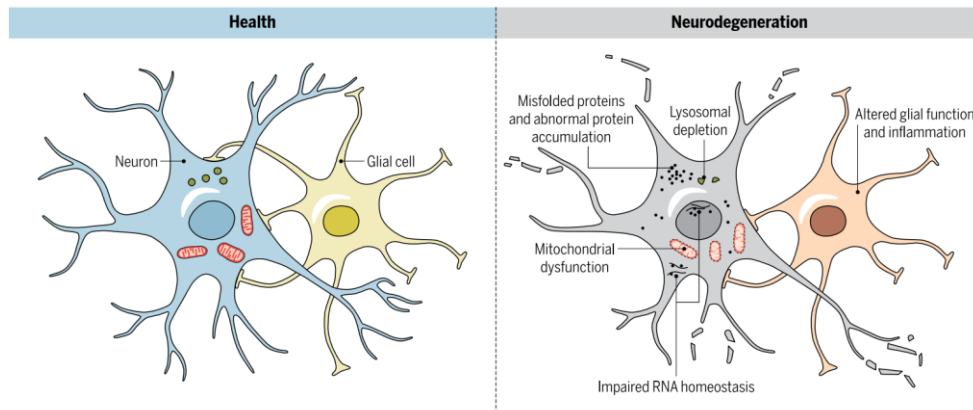
- **MRI imaging with Radboudumc**

- Brain structures (e.g. grey matter and white matter integrity)
- Brain function (connectivity, cerebral blood flow)
- Polarized light imaging

# Neurodegeneration

## Neurodegeneration

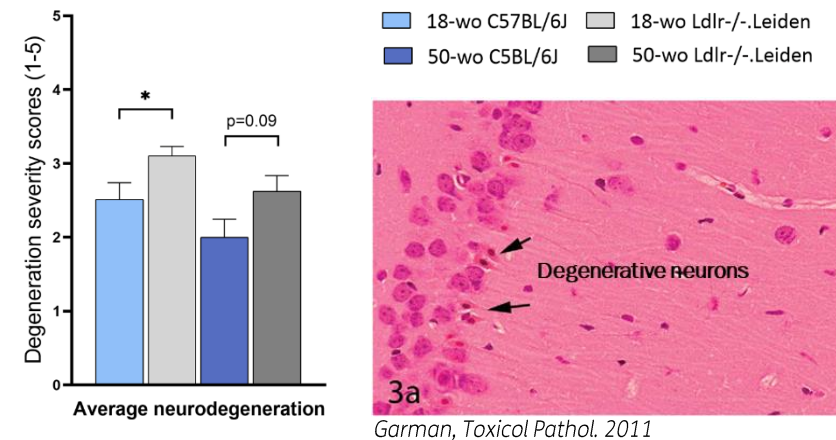
- Underlying disease process in all neurodegenerative diseases
- Loss of neuronal function – breakdown connectivity & communication: axonal damage, synaptic dysfunction/loss



**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)

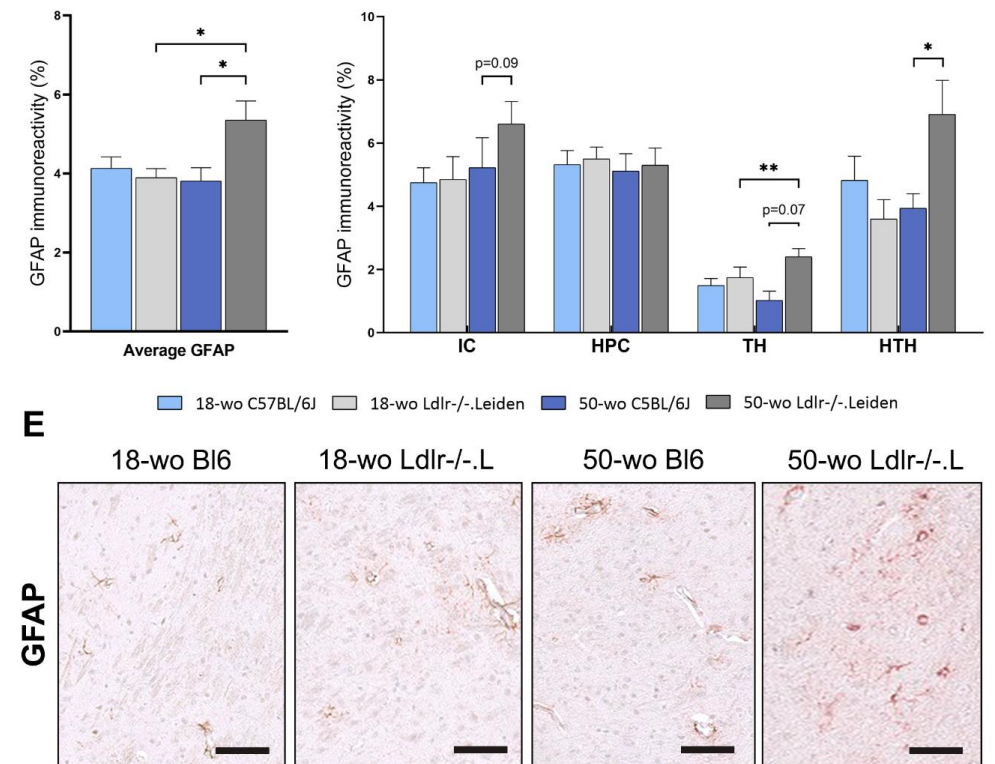


- Neurodegeneration more pronounced in Ldlr-/-Leiden model than in conventional model.

# 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
- Astroglia: 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 model

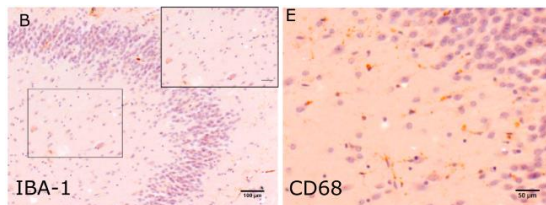


# Mechanistic differences with conventional model

Pathway	LDLr <sup>-/-</sup> .Leiden	WT	Genes	Count	Significance	
Signaling by the Family GTPases	2.79	-1.89	ATG10, ARF1, ARF2, ARF3, ARF4, ARF5, ARF6, ARF7, ARF8, ARF9, ARF10, ARF11, ARF12, ARF13, ARF14, ARF15, ARF16, ARF17, ARF18, ARF19, ARF20, ARF21, ARF22, ARF23, ARF24, ARF25, ARF26, ARF27, ARF28, ARF29, ARF30, ARF31, ARF32, ARF33, ARF34, ARF35, ARF36, ARF37, ARF38, ARF39, ARF40, ARF41, ARF42, ARF43, ARF44, ARF45, ARF46, ARF47, ARF48, ARF49, ARF50, ARF51, ARF52, ARF53, ARF54, ARF55, ARF56, ARF57, ARF58, ARF59, ARF60, ARF61, ARF62, ARF63, ARF64, ARF65, ARF66, ARF67, ARF68, ARF69, ARF70, ARF71, ARF72, ARF73, ARF74, ARF75, ARF76, ARF77, ARF78, ARF79, ARF80, ARF81, ARF82, ARF83, ARF84, ARF85, ARF86, ARF87, ARF88, ARF89, ARF90, ARF91, ARF92, ARF93, ARF94, ARF95, ARF96, ARF97, ARF98, ARF99, ARF100	25	207	49
Ceramide Signaling	2.78	1.13	AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10, AC11, AC12, AC13, AC14, AC15, AC16, AC17, AC18, AC19, AC20, AC21, AC22, AC23, AC24, AC25, AC26, AC27, AC28, AC29, AC30, AC31, AC32, AC33, AC34, AC35, AC36, AC37, AC38, AC39, AC40, AC41, AC42, AC43, AC44, AC45, AC46, AC47, AC48, AC49, AC50, AC51, AC52, AC53, AC54, AC55, AC56, AC57, AC58, AC59, AC60, AC61, AC62, AC63, AC64, AC65, AC66, AC67, AC68, AC69, AC70, AC71, AC72, AC73, AC74, AC75, AC76, AC77, AC78, AC79, AC80, AC81, AC82, AC83, AC84, AC85, AC86, AC87, AC88, AC89, AC90, AC91, AC92, AC93, AC94, AC95, AC96, AC97, AC98, AC99, AC100	12	91	50
VEGF Signaling	2.76	-1.87	VEGFA, VEGFB, VEGFC, VEGFD, VEGFE, VEGFG, VEGFH, VEGFI, VEGFJ, VEGFK, VEGFL, VEGFM, VEGFN, VEGFO, VEGFP, VEGFQ, VEGFR, VEGFR1, VEGFR2, VEGFR3, VEGFR4, VEGFR5, VEGFR6, VEGFR7, VEGFR8, VEGFR9, VEGFR10, VEGFR11, VEGFR12, VEGFR13, VEGFR14, VEGFR15, VEGFR16, VEGFR17, VEGFR18, VEGFR19, VEGFR20, VEGFR21, VEGFR22, VEGFR23, VEGFR24, VEGFR25, VEGFR26, VEGFR27, VEGFR28, VEGFR29, VEGFR30, VEGFR31, VEGFR32, VEGFR33, VEGFR34, VEGFR35, VEGFR36, VEGFR37, VEGFR38, VEGFR39, VEGFR40, VEGFR41, VEGFR42, VEGFR43, VEGFR44, VEGFR45, VEGFR46, VEGFR47, VEGFR48, VEGFR49, VEGFR50, VEGFR51, VEGFR52, VEGFR53, VEGFR54, VEGFR55, VEGFR56, VEGFR57, VEGFR58, VEGFR59, VEGFR60, VEGFR61, VEGFR62, VEGFR63, VEGFR64, VEGFR65, VEGFR66, VEGFR67, VEGFR68, VEGFR69, VEGFR70, VEGFR71, VEGFR72, VEGFR73, VEGFR74, VEGFR75, VEGFR76, VEGFR77, VEGFR78, VEGFR79, VEGFR80, VEGFR81, VEGFR82, VEGFR83, VEGFR84, VEGFR85, VEGFR86, VEGFR87, VEGFR88, VEGFR89, VEGFR90, VEGFR91, VEGFR92, VEGFR93, VEGFR94, VEGFR95, VEGFR96, VEGFR97, VEGFR98, VEGFR99, VEGFR100	15	37	35
Wnt Signaling	2.76	1.24	WNT1, WNT2, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT10C, WNT11, WNT12, WNT13, WNT14, WNT15, WNT16, WNT17, WNT18, WNT19, WNT20, WNT21, WNT22, WNT23, WNT24, WNT25, WNT26, WNT27, WNT28, WNT29, WNT30, WNT31, WNT32, WNT33, WNT34, WNT35, WNT36, WNT37, WNT38, WNT39, WNT40, WNT41, WNT42, WNT43, WNT44, WNT45, WNT46, WNT47, WNT48, WNT49, WNT50, WNT51, WNT52, WNT53, WNT54, WNT55, WNT56, WNT57, WNT58, WNT59, WNT60, WNT61, WNT62, WNT63, WNT64, WNT65, WNT66, WNT67, WNT68, WNT69, WNT70, WNT71, WNT72, WNT73, WNT74, WNT75, WNT76, WNT77, WNT78, WNT79, WNT80, WNT81, WNT82, WNT83, WNT84, WNT85, WNT86, WNT87, WNT88, WNT89, WNT90, WNT91, WNT92, WNT93, WNT94, WNT95, WNT96, WNT97, WNT98, WNT99, WNT100	13	108	52
Insulin Signaling	2.7	-1.81	INS, IGF1, IGF2, IGF3, IGF4, IGF5, IGF6, IGF7, IGF8, IGF9, IGF10, IGF11, IGF12, IGF13, IGF14, IGF15, IGF16, IGF17, IGF18, IGF19, IGF20, IGF21, IGF22, IGF23, IGF24, IGF25, IGF26, IGF27, IGF28, IGF29, IGF30, IGF31, IGF32, IGF33, IGF34, IGF35, IGF36, IGF37, IGF38, IGF39, IGF40, IGF41, IGF42, IGF43, IGF44, IGF45, IGF46, IGF47, IGF48, IGF49, IGF50, IGF51, IGF52, IGF53, IGF54, IGF55, IGF56, IGF57, IGF58, IGF59, IGF60, IGF61, IGF62, IGF63, IGF64, IGF65, IGF66, IGF67, IGF68, IGF69, IGF70, IGF71, IGF72, IGF73, IGF74, IGF75, IGF76, IGF77, IGF78, IGF79, IGF80, IGF81, IGF82, IGF83, IGF84, IGF85, IGF86, IGF87, IGF88, IGF89, IGF90, IGF91, IGF92, IGF93, IGF94, IGF95, IGF96, IGF97, IGF98, IGF99, IGF100	19	188	52
Regenerative Myogenesis	2.69	0.11	MYO1, MYO2, MYO3, MYO4, MYO5, MYO6, MYO7, MYO8, MYO9, MYO10, MYO11, MYO12, MYO13, MYO14, MYO15, MYO16, MYO17, MYO18, MYO19, MYO20, MYO21, MYO22, MYO23, MYO24, MYO25, MYO26, MYO27, MYO28, MYO29, MYO30, MYO31, MYO32, MYO33, MYO34, MYO35, MYO36, MYO37, MYO38, MYO39, MYO40, MYO41, MYO42, MYO43, MYO44, MYO45, MYO46, MYO47, MYO48, MYO49, MYO50, MYO51, MYO52, MYO53, MYO54, MYO55, MYO56, MYO57, MYO58, MYO59, MYO60, MYO61, MYO62, MYO63, MYO64, MYO65, MYO66, MYO67, MYO68, MYO69, MYO70, MYO71, MYO72, MYO73, MYO74, MYO75, MYO76, MYO77, MYO78, MYO79, MYO80, MYO81, MYO82, MYO83, MYO84, MYO85, MYO86, MYO87, MYO88, MYO89, MYO90, MYO91, MYO92, MYO93, MYO94, MYO95, MYO96, MYO97, MYO98, MYO99, MYO100	17	137	56
Non-Small Cell Lung Cancer Signaling	2.68	-1.81	EGFR, KRAS, BRAF, PIK3CA, RASGEF1, RASGEF2, RASGEF3, RASGEF4, RASGEF5, RASGEF6, RASGEF7, RASGEF8, RASGEF9, RASGEF10, RASGEF11, RASGEF12, RASGEF13, RASGEF14, RASGEF15, RASGEF16, RASGEF17, RASGEF18, RASGEF19, RASGEF20, RASGEF21, RASGEF22, RASGEF23, RASGEF24, RASGEF25, RASGEF26, RASGEF27, RASGEF28, RASGEF29, RASGEF30, RASGEF31, RASGEF32, RASGEF33, RASGEF34, RASGEF35, RASGEF36, RASGEF37, RASGEF38, RASGEF39, RASGEF40, RASGEF41, RASGEF42, RASGEF43, RASGEF44, RASGEF45, RASGEF46, RASGEF47, RASGEF48, RASGEF49, RASGEF50, RASGEF51, RASGEF52, RASGEF53, RASGEF54, RASGEF55, RASGEF56, RASGEF57, RASGEF58, RASGEF59, RASGEF60, RASGEF61, RASGEF62, RASGEF63, RASGEF64, RASGEF65, RASGEF66, RASGEF67, RASGEF68, RASGEF69, RASGEF70, RASGEF71, RASGEF72, RASGEF73, RASGEF74, RASGEF75, RASGEF76, RASGEF77, RASGEF78, RASGEF79, RASGEF80, RASGEF81, RASGEF82, RASGEF83, RASGEF84, RASGEF85, RASGEF86, RASGEF87, RASGEF88, RASGEF89, RASGEF90, RASGEF91, RASGEF92, RASGEF93, RASGEF94, RASGEF95, RASGEF96, RASGEF97, RASGEF98, RASGEF99, RASGEF100	11	82	50
FLT3 Signaling in Hematopoietic Progenitor Cells	2.65	-0.90	FLT3, FLT3L, FLT3R, FLT3S, FLT3T, FLT3U, FLT3V, FLT3W, FLT3X, FLT3Y, FLT3Z, FLT3AA, FLT3AB, FLT3AC, FLT3AD, FLT3AE, FLT3AF, FLT3AG, FLT3AH, FLT3AI, FLT3AJ, FLT3AK, FLT3AL, FLT3AM, FLT3AN, FLT3AO, FLT3AP, FLT3AQ, FLT3AR, FLT3AS, FLT3AT, FLT3AU, FLT3AV, FLT3AW, FLT3AX, FLT3AY, FLT3AZ, FLT3BA, FLT3BB, FLT3BC, FLT3BD, FLT3BE, FLT3BF, FLT3BG, FLT3BH, FLT3BI, FLT3BJ, FLT3BK, FLT3BL, FLT3BM, FLT3BN, FLT3BO, FLT3BP, FLT3BQ, FLT3BR, FLT3BS, FLT3BT, FLT3BU, FLT3BV, FLT3BW, FLT3BX, FLT3BY, FLT3BZ, FLT3CA, FLT3CB, FLT3CC, FLT3CD, FLT3CE, FLT3CF, FLT3CG, FLT3CH, FLT3CI, FLT3CJ, FLT3CK, FLT3CL, FLT3CM, FLT3CN, FLT3CO, FLT3CP, FLT3CQ, FLT3CR, FLT3CS, FLT3CT, FLT3CU, FLT3CV, FLT3CW, FLT3CX, FLT3CY, FLT3CZ, FLT3DA, FLT3DB, FLT3DC, FLT3DD, FLT3DE, FLT3DF, FLT3DG, FLT3DH, FLT3DI, FLT3DJ, FLT3DK, FLT3DL, FLT3DM, FLT3DN, FLT3DO, FLT3DP, FLT3DQ, FLT3DR, FLT3DS, FLT3DT, FLT3DU, FLT3DV, FLT3DW, FLT3DX, FLT3DY, FLT3DZ, FLT3EA, FLT3EB, FLT3EC, FLT3ED, FLT3EE, FLT3EF, FLT3EG, FLT3EH, FLT3EI, FLT3EJ, FLT3EK, FLT3EL, FLT3EM, FLT3EN, FLT3EO, FLT3EP, FLT3EQ, FLT3ER, FLT3ES, FLT3ET, FLT3EU, FLT3EV, FLT3EW, FLT3EX, FLT3EY, FLT3EZ, FLT3FA, FLT3FB, FLT3FC, FLT3FD, FLT3FE, FLT3FF, FLT3FG, FLT3FH, FLT3FI, FLT3FJ, FLT3FK, FLT3FL, FLT3FM, FLT3FN, FLT3FO, FLT3FP, FLT3FQ, FLT3FR, FLT3FS, FLT3FT, FLT3FU, FLT3FV, FLT3FW, FLT3FX, FLT3FY, FLT3FZ, FLT3GA, FLT3GB, FLT3GC, FLT3GD, FLT3GE, FLT3GF, FLT3GG, FLT3GH, FLT3GI, FLT3GJ, FLT3GK, FLT3GL, FLT3GM, FLT3GN, FLT3GO, FLT3GP, FLT3GQ, FLT3GR, FLT3GS, FLT3GT, FLT3GU, FLT3GV, FLT3GW, FLT3GX, FLT3GY, FLT3GZ, FLT3HA, FLT3HB, FLT3HC, FLT3HD, FLT3HE, FLT3HF, FLT3HG, FLT3HH, FLT3HI, FLT3HJ, FLT3HK, FLT3HL, FLT3HM, FLT3HN, FLT3HO, FLT3HP, FLT3HQ, FLT3HR, FLT3HS, FLT3HT, FLT3HU, FLT3HV, FLT3HW, FLT3HX, FLT3HY, FLT3HZ, FLT3IA, FLT3IB, FLT3IC, FLT3ID, FLT3IE, FLT3IF, FLT3IG, FLT3IH, FLT3II, FLT3IJ, FLT3IK, FLT3IL, FLT3IM, FLT3IN, FLT3IO, FLT3IP, FLT3IQ, FLT3IR, FLT3IS, FLT3IT, FLT3IU, FLT3IV, FLT3IW, FLT3IX, FLT3IY, 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WNT3/ catenin signaling	2.62	-0.90	WNT3, WNT3L, WNT3R, WNT3S, WNT3T, WNT3U, WNT3V, WNT3W, WNT3X, WNT3Y, WNT3Z, WNT3AA, WNT3AB, WNT3AC, WNT3AD, WNT3AE, WNT3AF, WNT3AG, WNT3AH, WNT3AI, WNT3AJ, WNT3AK, WNT3AL, WNT3AM, WNT3AN, WNT3AO, WNT3AP, WNT3AQ, WNT3AR, WNT3AS, WNT3AT, WNT3AU, WNT3AV, WNT3AW, WNT3AX, WNT3AY, WNT3AZ, WNT3BA, WNT3BB, WNT3BC, WNT3BD, WNT3BE, WNT3BF, WNT3BG, WNT3BH, WNT3BI, WNT3BJ, WNT3BK, WNT3BL, WNT3BM, WNT3BN, WNT3BO, WNT3BP, WNT3BQ, WNT3BR, WNT3BS, WNT3BT, WNT3BU, WNT3BV, WNT3BW, WNT3BX, WNT3BY, WNT3BZ, WNT3CA, WNT3CB, WNT3CC, WNT3CD, WNT3CE, WNT3CF, WNT3CG, WNT3CH, WNT3CI, WNT3CJ, WNT3CK, WNT3CL, WNT3CM, WNT3CN, WNT3CO, WNT3CP, WNT3CQ, WNT3CR, WNT3CS, WNT3CT, WNT3CU, WNT3CV, WNT3CW, WNT3CX, WNT3CY, WNT3CZ, WNT3DA, WNT3DB, WNT3DC, WNT3DD, WNT3DE, WNT3DF, WNT3DG, WNT3DH, WNT3DI, WNT3DJ, WNT3DK, WNT3DL, WNT3DM, WNT3DN, WNT3DO, WNT3DP, WNT3DQ, WNT3DR, WNT3DS, WNT3DT, WNT3DU, WNT3DV, WNT3DW, WNT3DX, WNT3DY, WNT3DZ, WNT3EA, WNT3EB, WNT3EC, WNT3ED, WNT3EE, WNT3EF, WNT3EG, 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Molecular Mechanisms of Cancer	2.59	0.08	TP53, KRAS, BRAF, PIK3CA, RASGEF1, RASGEF2, RASGEF3, RASGEF4, RASGEF5, RASGEF6, RASGEF7, RASGEF8, RASGEF9, RASGEF10, RASGEF11, RASGEF12, RASGEF13, RASGEF14, RASGEF15, RASGEF16, RASGEF17, RASGEF18, RASGEF19, RASGEF20, RASGEF21, RASGEF22, RASGEF23, RASGEF24, RASGEF25, RASGEF26, RASGEF27, RASGEF28, RASGEF29, RASGEF30, RASGEF31, RASGEF32, RASGEF33, RASGEF34, RASGEF35, RASGEF36, RASGEF37, RASGEF38, RASGEF39, RASGEF40, RASGEF41, RASGEF42, RASGEF43, RASGEF44, RASGEF45, RASGEF46, RASGEF47, RASGEF48, RASGEF49, RASGEF50, RASGEF51, RASGEF52, RASGEF53, RASGEF54, RASGEF55, RASGEF56, RASGEF57, RASGEF58, RASGEF59, RASGEF60, RASGEF61, RASGEF62, RASGEF63, RASGEF64, RASGEF65, RASGEF66, RASGEF67, RASGEF68, RASGEF69, RASGEF70, RASGEF71, RASGEF72, RASGEF73, RASGEF74, RASGEF75, RASGEF76, RASGEF77, RASGEF78, RASGEF79, RASGEF80, RASGEF81, RASGEF82, RASGEF83, RASGEF84, RASGEF85, RASGEF86, RASGEF87, RASGEF88, RASGEF89, RASGEF90, RASGEF91, RASGEF92, RASGEF93, RASGEF94, RASGEF95, RASGEF96, RASGEF97, RASGEF98, RASGEF99, RASGEF100	36	446	59
MAPK Signaling	2.51	-0.53	RAS, RAF1, MEK1, MEK2, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7, ERK8, ERK9, ERK10, ERK11, ERK12, ERK13, ERK14, ERK15, ERK16, ERK17, ERK18, ERK19, ERK20, ERK21, ERK22, ERK23, ERK24, ERK25, ERK26, ERK27, ERK28, ERK29, ERK30, ERK31, ERK32, ERK33, ERK34, ERK35, ERK36, ERK37, ERK38, ERK39, ERK40, ERK41, ERK42, ERK43, ERK44, ERK45, ERK46, ERK47, ERK48, ERK49, ERK50, ERK51, ERK52, ERK53, ERK54, ERK55, ERK56, ERK57, ERK58, ERK59, ERK60, ERK61, ERK62, ERK63, ERK64, ERK65, ERK66, ERK67, ERK68, ERK69, ERK70, ERK71, ERK72, ERK73, ERK74, ERK75, ERK76, ERK77, ERK78, ERK79, ERK80, ERK81, ERK82, ERK83, ERK84, ERK85, ERK86, ERK87, ERK88, ERK89, ERK90, ERK91, ERK92, ERK93, ERK94, ERK95, ERK96, ERK97, ERK98, ERK99, ERK100	15	138	60
Notch Signaling	2.5	0.00	NOTCH1, NOTCH2, NOTCH3, NOTCH4, NOTCH5, NOTCH6, NOTCH7, NOTCH8,			

# Susceptibility to obesity-induced neuroinflammation

- Neuroinflammation in obesity-associated brain dysfunction
- Thought to play an important role in the cognitive deficits observed in obese subjects
- Classical marker for neuroinflammation: IBA1
- Conventional neuroinflammation models show upregulation of IBA1
- Human data: downregulation of IBA1 in neuroinflammation/NDD:



Loss of IBA-1 expression in human obese subjects

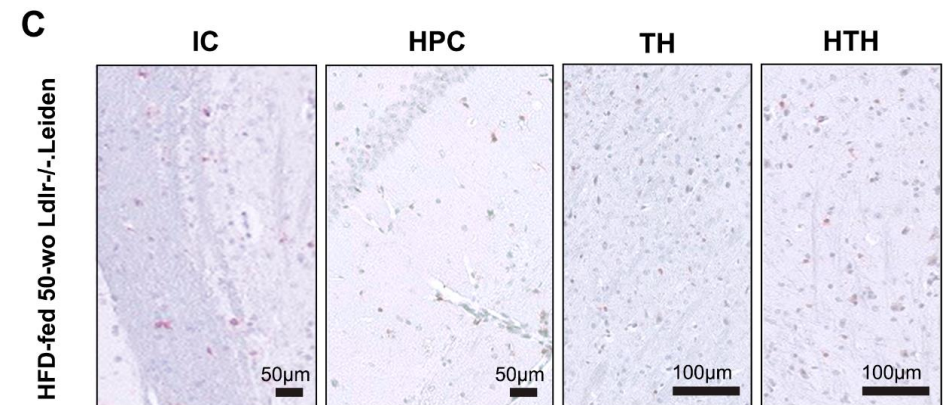
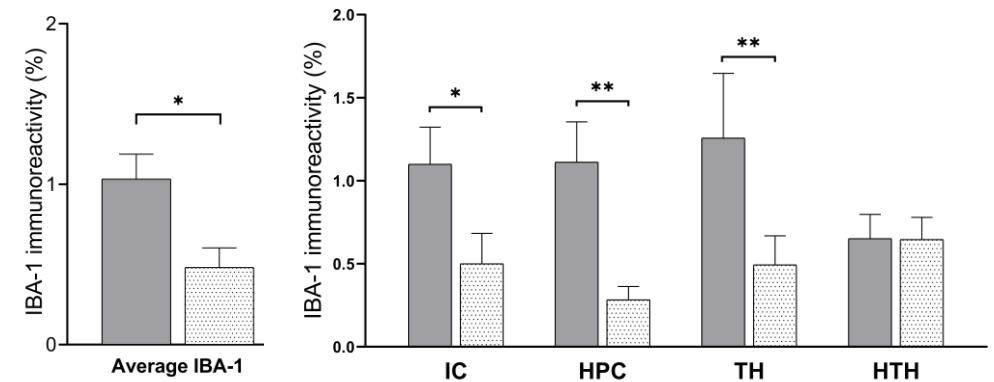
Lier et al. Brain Res. 2019

**Table 4** Weighted logistic regression to analyse the relationship between microglial protein load (%) and dementia status in participants with and without Alzheimer's dementia

Microglia (load %)	OR	95 % CI (OR)	P
Iba1	0.86	(0.82, 0.89)	<0.001
CD68	3.55	(1.93, 6.51)	<0.001
HLA-DR	1.06	(0.96, 1.18)	0.250
MSR-A	1.56	(1.11, 2.19)	0.010
CD64	1.21	(1.05, 1.39)	0.007

Negative correlation between IBA1 expression and Alzheimer's disease, positive correlation with other microglial markers

Minett et al. J Neuroinflammation 2016

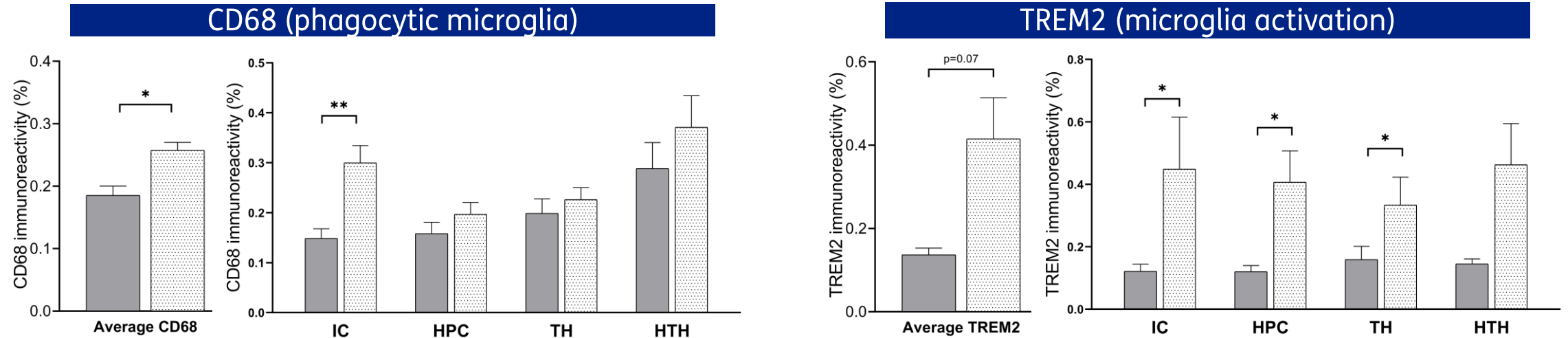


Seidel et al. Front. Cell Neurosc. 2023

# Susceptibility to 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

# Obesity-induced neuroinflammation - cytokines

Effect of HFD feeding on chemokine and cytokine concentrations in cortex homogenates of 50 week-old *Ldlr*<sup>-/-</sup>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 also observed 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  $\uparrow$

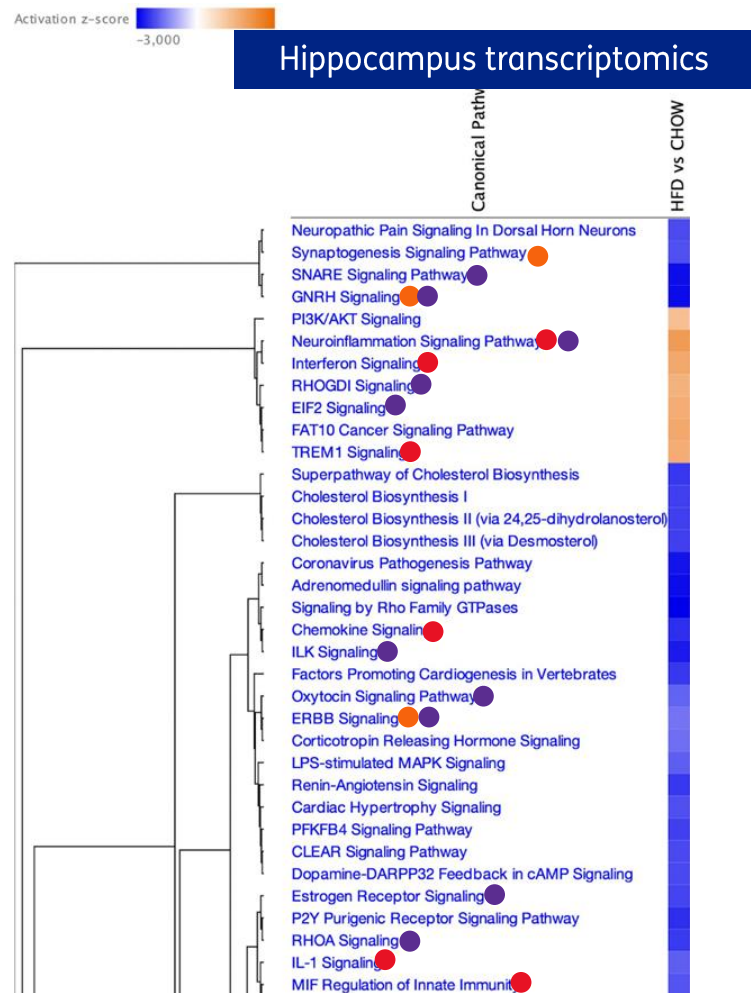
- TNF- $\alpha$   $\uparrow$

HFD  
vs.  
Chow





# 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 characterised by immunophenotypic shift, similar to what is observed in humans.

# Publications Ldlr-/-Leiden brain studies

	<u>Intervention</u>	<u>Publication</u>
<b>SCFA (gut-derived mediators)</b>	Butyrate	Arnoldussen et al. Int J Obesity 2017
	Propionate	Tengeler & Gart et al. FASEBJ 2020
<b>(Breast) milk component</b>	Milk fat globule membrane (MFGM)	Arnoldussen et al. Int J Obesity 2021
<b>Muscle-targeted intervention</b>	Exercise and branched-chain amino acids	Lohkamp et al. Nutrients 2023
<b>Anti-inflammatory</b>	Comparison Ldlr-/-Leiden vs BL6 + anti-inflammatory treatment (anti-C5)	Seidel et al. Front.Cell Neurosc. 2023
<b>Sex differences</b>	Male vs female	Jacobs et al. Nutrients 2019

# Opportunity to join Brain Health PPP

Open for collaboration!

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

Mechanistic Readouts  
of Brain Health



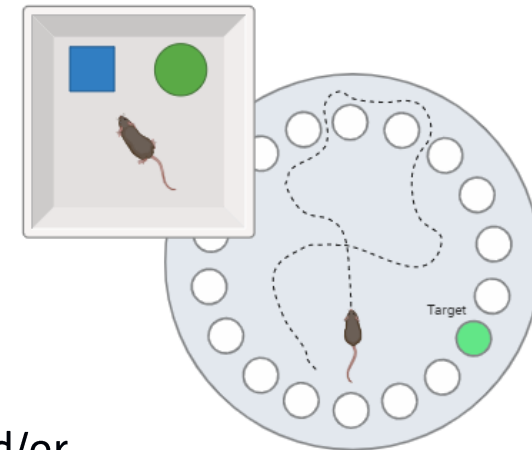
Functional behavioural  
and cognitive tests

Neurodegeneration

Astrogliosis

Neuroinflammation

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/obesity.
- Looking for partner(s) with interest to study treatment (prevention/intervention) to improve brain health.
- Publication of results.