Supplementary material

Predicting progression to Alzheimer's disease with human hippocampal progenitors exposed to serum

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Abstract

Adult hippocampal neurogenesis (HN) is important for learning and memory and is altered early in Alzheimer's disease. Since HN is modulated by the circulatory systemic environment, evaluating a proxy of how HN is affected by the systemic milieu could serve as an early biomarker for Alzheimer's disease progression. Here, we used an *in vitro* assay to model the impact of systemic environment on HN. A human hippocampal progenitor cell line was treated with longitudinal serum samples from individuals with mild cognitive impairment (MCI), who either progressed to Alzheimer's disease or remained cognitively stable. MCI to Alzheimer's disease progression was characterised most prominently with decreased proliferation, increased cell death, and increased neurogenesis. A subset of 'baseline' cellular readouts together with education level were able to predict Alzheimer's disease progression. The assay could provide a powerful platform for early prognosis, monitoring disease progression, and further mechanistic studies.

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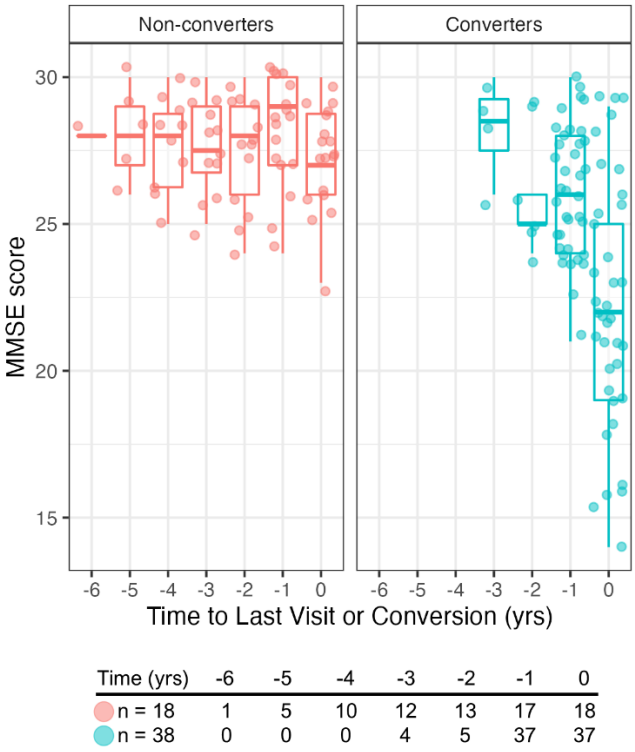
Running title: Alzheimer's disease prediction via serum

Keywords: Alzheimer's disease; prognostic biomarker; neurogenesis; hippocampal progenitors



Supplementary Figure 1. Mini Mental State Examination (MMSE) scores of participants over time.

The box-and whisker plot, overlaid with a scatter of individual data points, shows changes in MMSE scores of participants over time, with the number of subjects in each group shown below the plot.

Image Acquisition	
Objective	10x
Camera Name	QUANTIX_32;1.00
Acquisition Camera Mode	Standard (1024x1024;2x2)
AutoFocus Camera Mode	Standard (1024x1024;2x2)
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Coarse Focus Step Size	70.4
Coarse Focus Plane Count	9
Smart Focus Plane Count	21
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Apply Backlash Correction	False
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Use Relaxed Pass/Fail Criteria	False
Focus Edge Threshhold	0
Focus Adjustment	0
Focus Score Min Ratio	0.2
Focus Score Mid Ratio	0.4
Focus Score Max Ratio	0.5
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Scan Limits	
Max Fields for Well	15
Min Objects for Well	No Limit
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Min Objects for Field	10
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Channel 1: Nuclei		
Dye	BGRFR_386_23	
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Apply Background Correction	True	
Gain	2	
Z Offset	0.00	
Step Size	11.67	
Number of Steps	3	
Projection Method		
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Grid Type		
Pin Hole Size		
Intensity Percent	100	
Exposure Parameters		
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Exposure Time (seconds)	0.015	
Object Identification		
Method	FixedThreshold	
Value	50	
Object Selection Parameter	**Min**	**Max**
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ObjectVarIntenCh1	0	65535
ObjectTotalIntenCh1	0	1000000000000
ObjectAreaCh1	30	290
ObjectShapeP2ACh1	0	1000

Channel 3: ki67		
Dye	BGRFR_549_15	
Apply Illumination Correction	False	
Apply Background Correction	True	
Gain	2	
Z Offset	0.00	
Step Size	0.00	
Number of Steps	0	
Projection Method		
Projection Direction	None	
Detection Mode	Widefield	
Grid Type		
Pin Hole Size		
Intensity Percent	100	
Exposure Parameters		
Method	Fixed	
Exposure Time (seconds)	0.08	
Object Identification		
Method	None	
Value	0	
Object Selection Parameter	**Min**	**Max**
AvgIntenCh3	0	65535
TotalIntenCh3	0	1000000000000
AvgIntenCh3LevelHigh	50	

Channel 2: dcx		
Dye	BGRFR_485_20	
Apply Illumination Correction	False	
Apply Background Correction	True	
Gain	2	
Z Offset	0.00	
Step Size	0.00	
Number of Steps	0	
Projection Method		
Projection Direction	None	
Detection Mode	Widefield	
Grid Type	0	
Pin Hole Size	0	
Intensity Percent	100	
Exposure Parameters		
Method	Fixed	
Exposure Time (seconds)	0.01	
Object Identification		
Method	None	
Value	0	
Object Selection Parameter	**Min**	**Max**
AvgIntenCh2	0	65535
TotalIntenCh2	0	1000000000000
TargetAvgIntenCh2LevelHigh	150	

Channel 2: cc3		
Dye	BGRFR_485_20	
Apply Illumination Correction	False	
Apply Background Correction	True	
Gain	2	
Z Offset	0.00	
Step Size	0.00	
Number of Steps	0	
Projection Method		
Projection Direction	None	
Detection Mode	Widefield	
Grid Type	0	
Pin Hole Size	0	
Intensity Percent	100	
Exposure Parameters		
Method	Fixed	
Exposure Time (seconds)	0.15	
Object Identification		
Method	None	
Value	0	
Object Selection Parameter	**Min**	**Max**
AvgIntenCh2	0	65535
TotalIntenCh2	0	1000000000000
TargetAvgIntenCh2LevelHigh	200	

Channel 3: map2		
Dye	BGRFR_549_15	
Apply Illumination Correction	False	
Apply Background Correction	True	
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Step Size	0.00	
Number of Steps	0	
Projection Method		
Projection Direction	None	
Detection Mode	Widefield	
Grid Type	0	
Pin Hole Size	0	
Intensity Percent	100	
Exposure Parameters		
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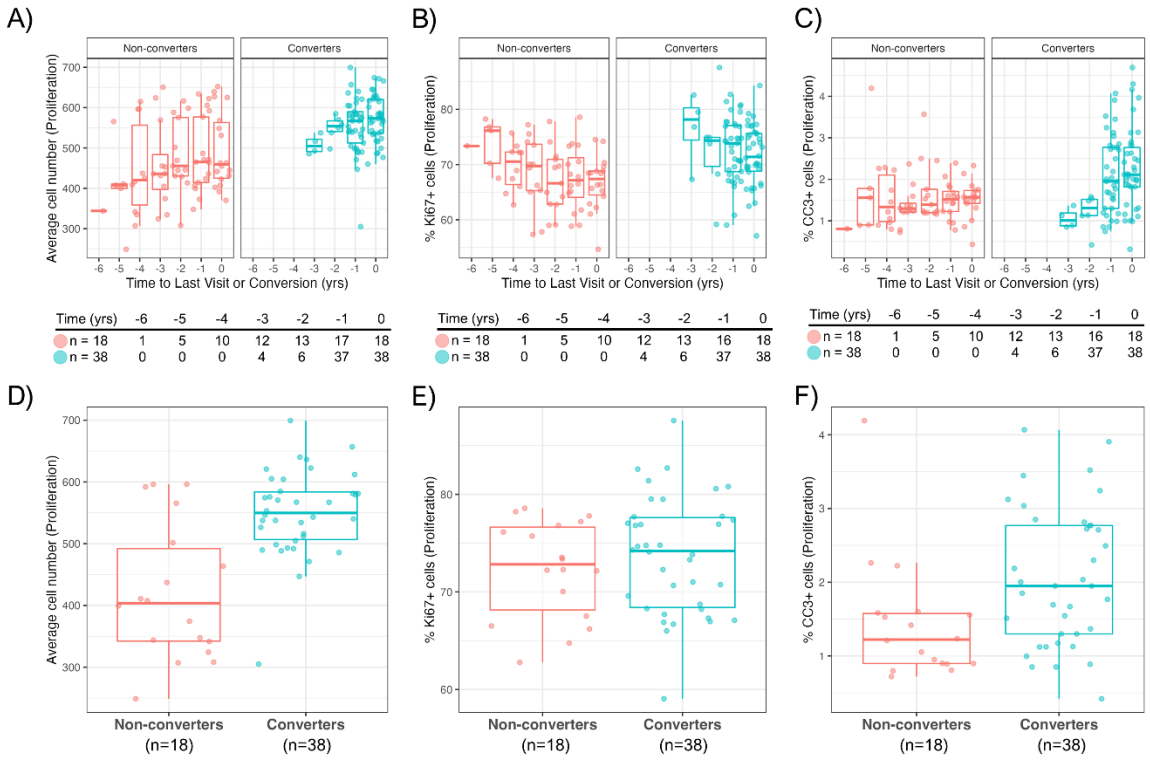
Exposure Time (seconds)	0.06	
Object Identification		
Method	None	
Value	0	
Object Selection Parameter	**Min**	**Max**
AvgIntenCh3	0	65535
TotalIntenCh3	0	1000000000000
TargetAvgIntenCh3LevelHigh	125	

Supplementary Figure 2. Cellular phenotyping protocol on Thermo Scientific™ CellInsight™ CX5 High Content Screening Platform.

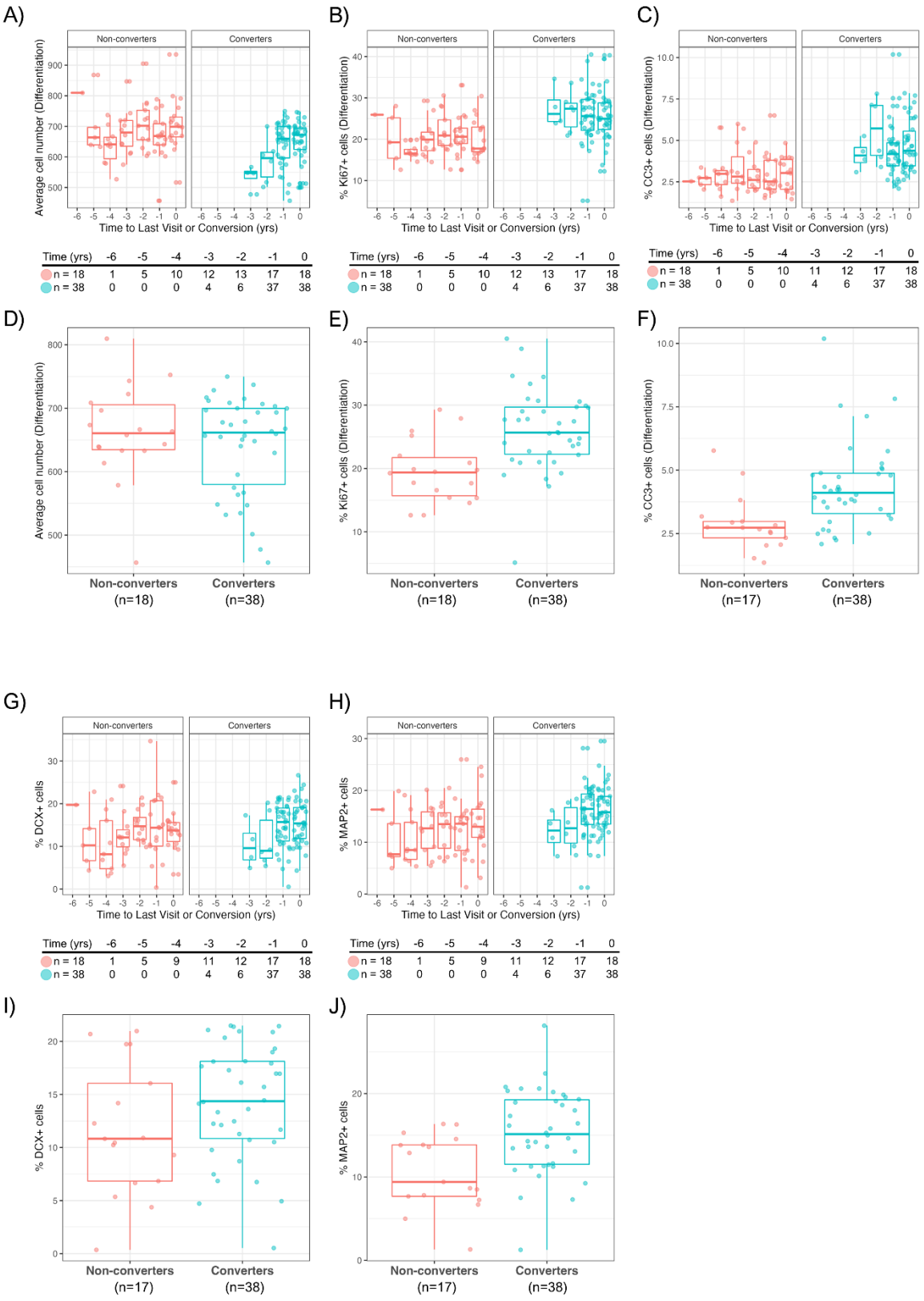
Parameters used for semi-automated quantification of DAPI (nuclear), Ki67, CC3, DCX, and MAP2 are shown.



Supplementary Figure 3. Average cell number, proliferation, and apoptosis during the proliferation phase of the assay. (related to Fig. 2 and 3)

A-C) Box-and-whisker plots show the maximum, third quartile, median, first quartile, and minimum data for each measurement, overlaid with a scatter of individual data points showing the spread of the data over time, with the number of subjects in each group shown below the graph. Average cell count per field of view (total 45 fields of view, 15 fields per technical replicate) **(A)**, percentage of Ki67-positive cells **(B)**, percentage of CC3-positive cells **(C)**.

D-F) Box-and whisker plots overlaid with a scatter of individual data points showing the spread of the data at baseline, with the number of subjects in each group shown below the plot. Average cell count per field of view at baseline **(D)**, percentage of Ki67-positive cells at baseline **(E)**, percentage of CC3-positive cells at baseline **(F)**. Each dot represents mean of technical triplicates for each individual.



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Supplementary Figure 4. Average cell number, proliferation, and apoptosis during the differentiation phase of the assay. (related to Fig. 2 and 3)

A-C, G, H) Box-and-whisker plots show the maximum, third quartile, median, first quartile, and minimum data for each measurement, overlaid with a scatter of individual data points showing the spread of the data over time, with the number of subjects in each group shown below the graph. Average cell count per field of view (total 45 fields of view, 15 fields per technical replicate) **(A)**, percentage of Ki67-positive cells **(B)**, percentage of CC3-positive cells **(C)**, percentage of DCX-positive cells **(G)**, percentage of MAP2-positive cells **(H)**.

D-F, I, J) Box-and whisker plots overlaid with a scatter of individual data points showing the spread of the data at baseline, with the number of subjects in each group shown below the plot. Average cell count per field of view at baseline **(D)**, percentage of Ki67-positive cells at baseline **(E)**, percentage of CC3-positive cells at baseline **(F)**, percentage of DCX-positive cells at baseline **(I)**, percentage of MAP2-positive cells at baseline **(J)**. Each dot represents mean of technical triplicates for each individual.

```
library(lme4)
library(dplyr)

### CC3 ###
data <- full_data %>%
  group_by(group, ID) %>%
  dplyr::filter(group==1) %>%
  dplyr::select(ID, group,
                CC3_prol,
                time_to_last_visit_or_conversion,
                edu10.5, supplement_intake) %>% drop_na()
fml <- formula(CC3_prol ~ (1|ID))
mod0 <- lmer(fml, data)
fml <- formula(CC3_prol ~ time_to_last_visit_or_conversion + (1|ID))
mod1 <- lmer(fml, data)
fml <- formula(CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 +
(1|ID))
mod2 <- lmer(fml, data)
fml <- formula(CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 +
supplement_intake + (1|ID))
mod3 <- lmer(fml, data)
fml <- formula(CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 +
supplement_intake + edu10.5*supplement_intake + (1|ID))
mod4 <- lmer(fml, data)
anova(mod0, mod1, mod2, mod3, mod4)
```

```
## refitting model(s) with ML (instead of REML)
## Data: data
## Models:
## mod0: CC3_prol ~ (1 | ID)
## mod1: CC3_prol ~ time_to_last_visit_or_conversion + (1 | ID)
## mod2: CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 + (1 | ID)
## mod3: CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 +
supplement_intake + (1 | ID)
## mod4: CC3_prol ~ time_to_last_visit_or_conversion + edu10.5 +
supplement_intake + edu10.5 * supplement_intake + (1 | ID)
##      npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## mod0    3 177.05 184.38 -85.527   171.05
## mod1    4 174.18 183.95 -83.088   166.18 4.8782  1    0.02720 *
## mod2    5 172.05 184.26 -81.025   162.05 4.1251  1    0.04225 *
## mod3    6 173.64 188.30 -80.821   161.64 0.4093  1    0.52234
## mod4    7 175.49 192.59 -80.746   161.49 0.1489  1    0.69961
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
### DCX ###
data <- full_data %>%
  group_by(group, ID) %>%
  dplyr::filter(group==1) %>%
  dplyr::select(ID, group,
                DCX_diff,
                time_to_last_visit_or_conversion,
                MMSE_baseline, AD_drugs) %>% drop_na()
fml <- formula(DCX_diff ~ (1|ID))
mod0 <- lmer(fml, data)
fml <- formula(DCX_diff ~ time_to_last_visit_or_conversion + (1|ID))
mod1 <- lmer(fml, data)
fml <- formula(DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline
+ (1|ID))
mod2 <- lmer(fml, data)
fml <- formula(DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline
+ AD_drugs + (1|ID))
mod3 <- lmer(fml, data)
```

```
fml <- formula(DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline
+ AD_drugs + MMSE_baseline*AD_drugs + (1|ID))
mod4 <- lmer(fml, data)
anova(mod0, mod1, mod2, mod3, mod4)
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: data
## Models:
## mod0: DCX_diff ~ (1 | ID)
## mod1: DCX_diff ~ time_to_last_visit_or_conversion + (1 | ID)
## mod2: DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline + (1 |
ID)
## mod3: DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline +
AD_drugs + (1 | ID)
## mod4: DCX_diff ~ time_to_last_visit_or_conversion + MMSE_baseline +
AD_drugs + MMSE_baseline * AD_drugs + (1 | ID)
##      npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## mod0    3 497.84 505.13 -245.92   491.84
## mod1    4 493.22 502.94 -242.61   485.22 6.6177  1   0.010097 *
## mod2    5 488.00 500.15 -239.00   478.00 7.2214  1   0.007204 **
## mod3    6 489.64 504.23 -238.82   477.64 0.3586  1   0.549261
## mod4    7 489.97 506.98 -237.98   475.97 1.6749  1   0.195608
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
### MAP2 ###
data <- full_data %>%
  group_by(group, ID) %>%
  dplyr::filter(group==1) %>%
  dplyr::select(ID, group,
                MAP2_diff,
                time_to_last_visit_or_conversion,
                female, APOE4) %>% drop_na()
fml <- formula(MAP2_diff ~ (1|ID))
mod0 <- lmer(fml, data)
fml <- formula(MAP2_diff ~ time_to_last_visit_or_conversion + (1|ID))
mod1 <- lmer(fml, data)
fml <- formula(MAP2_diff ~ time_to_last_visit_or_conversion + female +
(1|ID))
mod2 <- lmer(fml, data)
fml <- formula(MAP2_diff ~ time_to_last_visit_or_conversion + female +
APOE4 + (1|ID))
mod3 <- lmer(fml, data)
fml <- formula(MAP2_diff ~ time_to_last_visit_or_conversion + female +
APOE4 + female*APOE4 + (1|ID))
mod4 <- lmer(fml, data)
anova(mod0, mod1, mod2, mod3, mod4)
```

```
## refitting model(s) with ML (instead of REML)
```

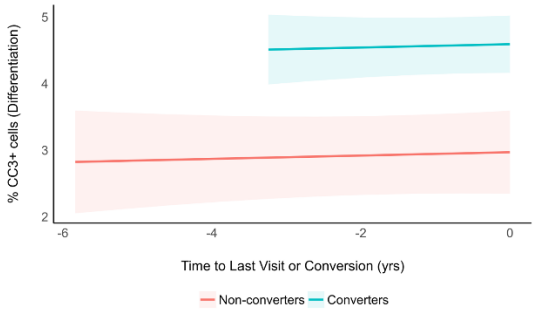
```
## Data: data
## Models:
## mod0: MAP2_diff ~ (1 | ID)
## mod1: MAP2_diff ~ time_to_last_visit_or_conversion + (1 | ID)
## mod2: MAP2_diff ~ time_to_last_visit_or_conversion + female + (1 | ID)
## mod3: MAP2_diff ~ time_to_last_visit_or_conversion + female + APOE4 + (1
| ID)
## mod4: MAP2_diff ~ time_to_last_visit_or_conversion + female + APOE4 +
female * APOE4 + (1 | ID)
##      npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## mod0    3 450.73 457.84 -222.37   444.73
## mod1    4 449.61 459.09 -220.81   441.61 3.1185  1    0.07741 .
## mod2    5 445.75 457.60 -217.87   435.75 5.8641  1    0.01545 *
## mod3    6 446.81 461.03 -217.40   434.81 0.9398  1    0.33232
```

```
## mod4    7 448.33 464.92 -217.17   434.33 0.4752  1    0.49060
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

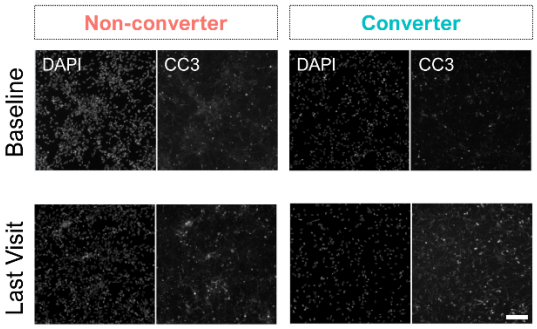
Supplementary Figure 5. No significant interaction between explanatory variables in linear mixed-effects models of converters only dataset. (related to Fig. 2A-D)

The results of linear mixed-effects modelling generated with the R package knitr (version 1.40) show no significant interaction between explanatory variables (Pr(>Chisq) > 0.05 when compared with the null model). Biologically plausible interactions were tested between education level (dichotomised at 10.5 yrs) and supplement intake when apoptosis (% CC3+) during proliferation was the response variable; between baseline MMSE scores and AD drug intake when the number of neuroblasts (% DCX+) was the response variable; and between sex (female assigned 1) and APOE4 status when the number of mature neurons (% MAP2+) was the response variable. Bold texts (i.e., mod3 and mod4) indicate the models in which the interaction of explanatory variables was tested. The report was generated with the R package knitr (version 1.40) using the command *knitr::stitch_rhtml('filename.r')*.

A)



C)



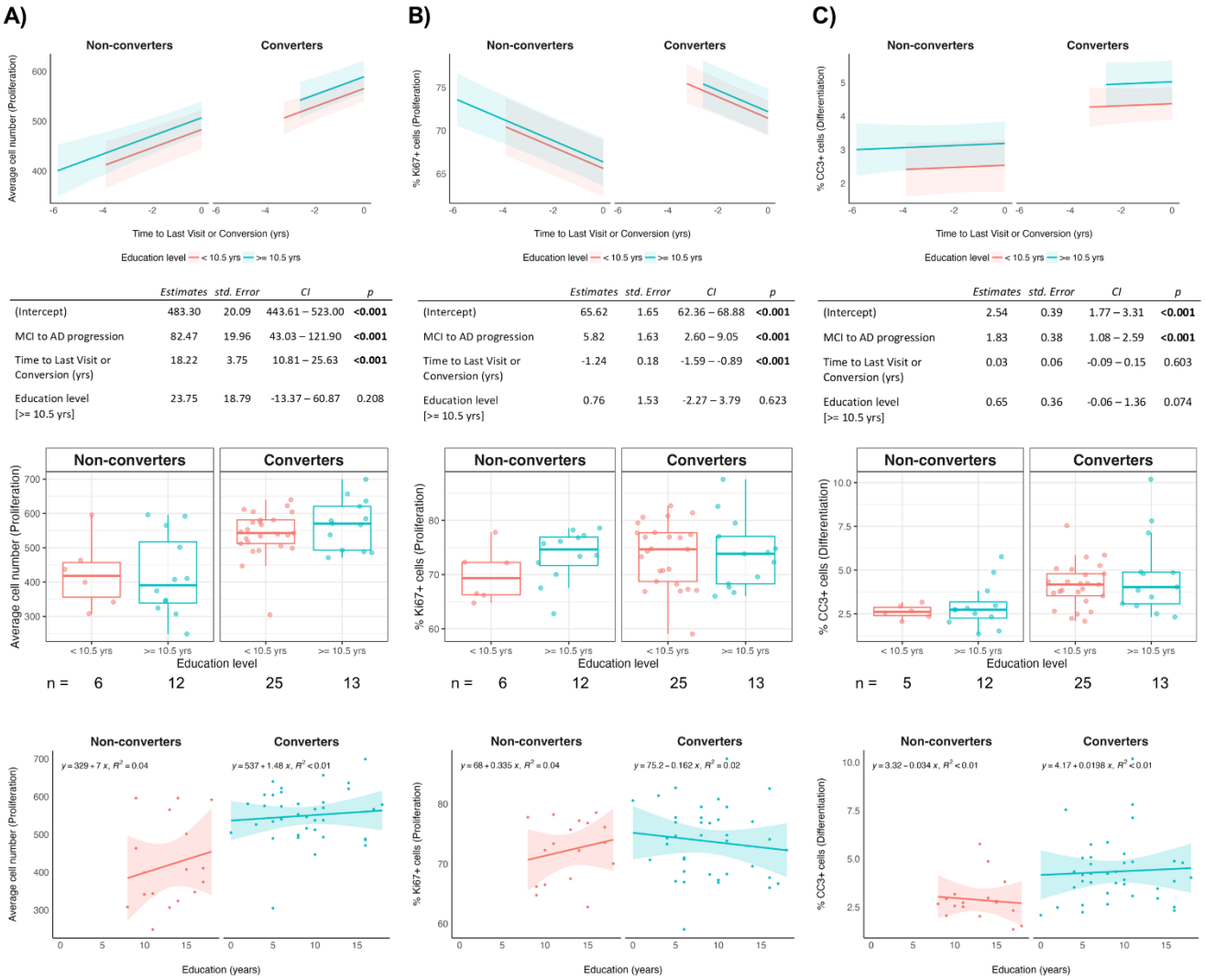
B)

% CC3+ cells (Differentiation)	Null				Random intercept (Pr>Chisq) < 0.001			
Predictors	*Estimates*	*std. Error*	*CI*	*p*	*Estimates*	*std. Error*	*CI*	*p*
(Intercept)	4.02	0.2	3.62 – 4.42	<0.001	**2.97**	**0.32**	**2.35 – 3.59**	**<0.001**
MCI to AD progression					**1.62**	**0.37**	**0.89 – 2.36**	**<0.001**
Time to Last Visit or Conversion (yrs)					0.02	0.06	-0.09 – 0.14	0.68
Marginal R2 / Conditional R2	0.000 / 0.733				0.243 / 0.737			
Deviance	515.084				497.247			
AIC	522.443				512.882			
log-Likelihood	-258.221				-251.441			

Supplementary Figure 6. Apoptosis during differentiation assay between non-converters and converters.

A, B) Modelled trajectories **(A)** and results of the linear mixed-effects regression model **(B)** fitted to the apoptosis during differentiation assay dataset. Either time of last visit (for non-converters; turquoise) or time of conversion to AD (for converters; red) was assigned 0, and the number of years before that were assigned negative values (i.e., one year before conversion is -1). The effect of MCI to AD progression was significant ($p < 0.001$) and positive (beta = 1.62), while time to last visit or conversion (yrs) did not have any significant effect as an explanatory variable on %CC3+ cells during differentiation ($p = 0.68$).

C) Representative images of differentiation phase cells treated with serum from the same individual. Left (non-converter panel, ID: LND008): serum sample taken at baseline (5.83 years before last visit) and last visit. Right (converter panel, ID: LND018): serum sample taken at baseline (3 years before last visit) and the time of conversion to AD (equals last visit). Nuclei are stained with DAPI. Cleaved caspase 3 (CC3) was used to label apoptotic cells. Scale bar 100 μm.



	Estimates	std. Error	CI	p
(Intercept)	483.30	20.09	443.61 – 523.00	**<0.001**
MCI to AD progression	82.47	19.96	43.03 – 121.90	**<0.001**
Time to Last Visit or Conversion (yrs)	18.22	3.75	10.81 – 25.63	**<0.001**
Education level [>= 10.5 yrs]	23.75	18.79	-13.37 – 60.87	0.208

	Estimates	std. Error	CI	p
(Intercept)	65.62	1.65	62.36 – 68.88	**<0.001**
MCI to AD progression	5.82	1.63	2.60 – 9.05	**<0.001**
Time to Last Visit or Conversion (yrs)	-1.24	0.18	-1.59 – -0.89	**<0.001**
Education level [>= 10.5 yrs]	0.76	1.53	-2.27 – 3.79	0.623

	Estimates	std. Error	CI	p
(Intercept)	2.54	0.39	1.77 – 3.31	**<0.001**
MCI to AD progression	1.83	0.38	1.08 – 2.59	**<0.001**
Time to Last Visit or Conversion (yrs)	0.03	0.06	-0.09 – 0.15	0.603
Education level [>= 10.5 yrs]	0.65	0.36	-0.06 – 1.36	0.074

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Supplementary Figure 7. No effect of education on neurogenesis assay predictors of MCI to AD progression.

Top: results of linear mixed-effects models fitted to the longitudinal dataset, when the response variables were either average cell number during proliferation **(A)**, % Ki67+ cells during proliferation **(B)**, or % CC3+ cells during differentiation **(C)**. The explanatory variables were 'time to last visit or conversion (yrs)', 'MCI to AD progression (converters assigned 1)', and 'education level (dichotomised at 10.5 yrs)'. None of the models indicate significant effects of education level ($p > 0.05$) on the response variables. Red: education < 10.5 years. Turquoise: education >= 10.5 years.

Middle: the box-and whisker plots, overlaid with a scatter of individual data points, showing the distribution of baseline average cell number during proliferation **(A)**, % Ki67+ cells during proliferation **(B)**, or % CC3+ cells during differentiation **(C)**, stratified according to 'MCI to AD progression (non-converters vs converters)' and 'education level (dichotomised at 10.5 yrs)'. The number of subjects in each sub-group is shown below the plots. Baseline levels of average cell number during proliferation **(A)**, % Ki67+ cells during proliferation **(B)**, and % CC3+ cells during differentiation **(C)** are similar between 'education < 10.5 yrs' and 'education >= 10.5 yrs' groups, regardless of 'MCI to AD progression' status. Red: education < 10.5 years. Turquoise: education >= 10.5 years.

Bottom: the distribution of baseline average cell number during proliferation **(A)**, % Ki67+ cells during proliferation **(B)**, or % CC3+ cells during differentiation **(C)** plotted against education in years (not dichotomised)**,** stratified according to 'MCI to AD progression (non-converters vs converters)'. Linear regression lines were fitted to the data. The equations and R-squared values are shown in each plot. All R-squared values are less than 0.1 indicating that education in years have little or no explanatory value.

Supplementary Table 1. Cell culture medium components.

Component	Concentration	Supplier (Catalogue number)
Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham		Sigma Aldrich (D6421)
Albumin, human	0.03%	Zenlab 20
apo-Transferrin, human	100 µg/ml	Sigma Aldrich (T1147)
Epidermal growth factor	20 ng/ml	PeproTech (AF-100-15)
Fibroblast growth factor-basic	10 ng/ml	PeproTech (100-18B)
Insulin, human recombinant	5 µg/ml	Sigma Aldrich (I9278)
L-glutamine	2 mM	Sigma Aldrich (G7513)
Progesterone	60 ng/ml	Sigma Aldrich (P8783)
Putrescine dihydrochloride	16.2 µg/ml	Sigma Aldrich (P5780)
Sodium selenite	40 ng/ml	Sigma Aldrich (S9133)

Supplementary Table 2. Source data table. (related to Fig. 2-5)

Available on figshare (https://doi.org/10.6084/m9.figshare.19778941)

Supplementary Table 3. Identifiers of all proteins quantified in SomaScan. (related to Fig. 5)

Available on figshare (https://doi.org/10.6084/m9.figshare.19778938)

Supplementary Table 4. Significant predictors of proliferation phase readouts from MCI converters only. (related to Fig. 2A-D) Models with the lowest Akaike Information Criterion (AIC) and deviance were selected as the best fit. Coefficient estimates, standard errors, 95% confidence intervals (CI) around the regression coefficient, and significance levels for all predictors in the analysis are provided.

Average cell number	Null				Random intercept (model 1) Pr(>Chisq) = 0.002			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	561.95	9.14	543.77 – 580.12	<0.001	573.52	9.75	554.12 – 592.93	<0.001
Time to Conversion					**17.37**	**5.28**	**6.87 – 27.87**	**0.001**
Deviance	919.997				909.978			
AIC	919.741				906.582			
log-Likelihood	-456.87				-449.291			

% Ki67+ cells	Null				Random intercept (model 1) Pr(>Chisq) < 0.001			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	72.52	0.92	70.69 – 74.35	<0.001	71.58	0.93	69.72 – 73.44	<0.001
Time to Conversion					**-1.44**	**0.29**	**-2.03 – -0.86**	**<0.001**
Deviance	482.021				461.909			
AIC	486.357				468.894			
log-Likelihood	-240.178				-230.447			

% CC3+ cells	Null				Random intercept (model 1) Pr(>Chisq) = 0.027				Random intercept (model 2) Pr(>Chisq) = 0.042			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	2.16	0.15	1.86 – 2.46	<0.001	2.24	0.15	1.94 – 2.54	<0.001	2.45	0.18	2.09 – 2.80	<0.001
Time to Conversion					**0.12**	**0.05**	**0.01 – 0.23**	**0.029**	**0.12**	**0.05**	**0.01 – 0.23**	**0.031**
Education (>= 10.5 yrs)									**-0.6**	**0.3**	**-1.20 – -0.01**	**0.046**
Deviance	171.061				166.176				162.063			
AIC	179.018				180.158				178.743			
log-Likelihood	-86.509				-86.079				-84.372			

Supplementary Table 5. Significant predictors of differentiation phase readouts from MCI converters only. (related to Fig. 2E-H) Models with the lowest Akaike Information Criterion (AIC) and deviance were selected as the best fit. Coefficient estimates, standard errors, 95% confidence intervals (CI) around the regression coefficient, and significance levels for all predictors in the analysis are provided.

Average cell number	Null				Random intercept (model 1) Pr(>Chisq) = 0.015			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	647.1	10.73	625.75 – 668.44	<0.001	655.13	10.91	633.41 – 676.84	<0.001
Time to Conversion					**12.17**	**4.99**	**2.24 – 22.11**	**0.017**
Deviance	920.072				914.187			
AIC	919.493				910.635			
log-Likelihood	-456.747				-451.318			

% DCX+ cells	Null				Random intercept (model 1) Pr(>Chisq) = 0.010				Random intercept (model 2) Pr(>Chisq) = 0.007			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	15.01	0.77	13.47 – 16.54	<0.001	15.78	0.82	14.15 – 17.42	<0.001	42.33	9.71	23.00 – 61.66	<0.001
Time to Conversion					**1.22**	**0.47**	**0.29 – 2.15**	**0.01**	**1.20**	**0.46**	**0.27 – 2.12**	**0.012**
MMSE baseline (>= 27)									**-3.91**	**1.43**	**-6.75 – -1.08**	**0.007**
Deviance	491.847				485.222				478.007			
AIC	496.536				491.636				484.080			
log-Likelihood	-245.268				-241.818				-237.040			

% MAP2+ cells	Null				Random intercept (model 1) Pr(>Chisq) = 0.032				Random intercept (model 2) Pr(>Chisq) = 0.008			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	16.05	0.68	14.69 – 17.40	<0.001	16.66	0.73	15.21 – 18.10	<0.001	18.72	1.02	16.69 – 20.76	<0.001
Time to Conversion					**0.92**	**0.43**	**0.07 – 1.76**	**0.035**	**0.95**	**0.43**	**0.10 – 1.79**	**0.029**
Female									**-3.39**	**1.26**	**-5.90 – -0.89**	**0.009**
Deviance	480.081				475.482				468.521			
AIC	485.019				482.343				475.283			
log-Likelihood	-239.51				-237.171				-232.641			

Supplementary Table 6. Significant predictors of proliferation phase readouts from both MCI converters and non-converters. (related to Fig. 3A-B) Models with the lowest Akaike Information Criterion (AIC) and deviance were selected as the best fit. Coefficient estimates, standard errors, 95% confidence intervals (CI) around the regression coefficient, and significance levels for all predictors in the analysis are provided.

Average cell number	Null				Random intercept (model 1) Pr(>Chisq) < 0.001				Random slope (model 2) Pr(>Chisq) = 0.033			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	530.78	10.93	509.20 – 552.37	<0.001	498.54	16.67	465.60 – 531.48	<0.001	499.18	15.77	468.02 – 530.34	<0.001
MCI to AD progression					**74.79**	**19.83**	**35.62 – 113.95**	**<0.001**	**74.71**	**19.11**	**36.96 – 112.46**	**<0.001**
Time to conversion or last visit					**17.08**	**2.71**	**11.71 – 22.44**	**<0.001**	**18.23**	**3.76**	**10.81 – 25.65**	**<0.001**
Deviance	1806.828				1753.376				1746.612			
AIC	1806.21				1745.491				1742.197			
log-Likelihood	-900.105				-867.746				-864.098			

% Ki67+ cells	Null				Random intercept (model 1) Pr(>Chisq) < 0.001			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	71.11	0.76	69.61 – 72.61	<0.001	66.12	1.29	63.57 – 68.67	<0.001
MCI to AD progression					**5.58**	**1.54**	**2.53 – 8.63**	**<0.001**
Time to conversion or last visit					**-1.24**	**0.18**	**-1.59 – -0.89**	**<0.001**
Deviance	935.078				887.001			
AIC	939.789				894.786			
log-Likelihood	-466.895				-442.393			

Supplementary Table 7. Significant predictors of differentiation phase readouts from both MCI converters and non-converters. (related to Fig. 3C-D) Models with the lowest Akaike Information Criterion (AIC) and deviance were selected as the best fit. Coefficient estimates, standard errors, 95% confidence intervals (CI) around the regression coefficient, and significance levels for all predictors in the analysis are provided.

Average cell number	Null				Random intercept (model 1) Pr(>Chisq) < 0.001			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	658.6	9.13	640.57 – 676.63	<0.001	701.94	16.06	670.22 – 733.66	<0.001
MCI to AD progression					**-47.64**	**18.98**	**-85.13 – -10.15**	**0.013**
Time to conversion or last visit					**11.48**	**2.95**	**5.65 – 17.32**	**<0.001**
Deviance	1784.992				1767.15			
AIC	1784.734				1759.278			
log-Likelihood	-889.367				-874.639			

% MAP2+ cells	Null				Random intercept (model 1) Pr(>Chisq) < 0.001			
Predictors	Estimates	std. Error	95% CI	p	Estimates	std. Error	95% CI	p
(Intercept)	14.82	0.6	13.64 – 16.00	<0.001	13.45	1.02	11.44 – 15.46	<0.001
MCI to AD progression					**2.96**	**1.19**	**0.62 – 5.31**	**0.014**
Time to conversion or last visit					**0.56**	**0.23**	**0.11 – 1.02**	**0.015**
Deviance	915.54				900.735			
AIC	920.732				909.081			
log-Likelihood	-457.366				-449.54			

Supplementary Table 8. 205 proteins significantly differentially expressed between MCI converters and non-converters. (related to Fig. 5A)

Available on figshare (https://doi.org/10.6084/m9.figshare.19778911)

Supplementary Table 9. A panel of 15 proteins that discriminate MCI converters from MCI non-converters. (related to Fig. 5C) Using the least absolute shrinkage and selection operator (LASSO) and support vector machines with 10-times cross-validation, a panel of 15 proteins were found to be capable of discriminating MCI converters from MCI non-converters. q-value is the false discovery rate (FDR) corrected p-value based on Wilcoxon rank-sum test.

UniProt ID	Beta coefficient	p-value	q-value	Protein name	Gene name
Q9NPH3	0.10683	0.00031	0.66869	Interleukin-1 receptor accessory protein	*IL1RAP*
Q8TBE7	0.10915	0.00116	0.66869	Solute carrier family 35 member G2	*SLC35G2*
Q9UHD0	0.09371	0.00133	0.66869	Interleukin-19	*IL19*
Q9NTK1	0.08752	0.00152	0.66869	Protein DEPP1	*DEPP1*
Q8N474	0.16495	0.00197	0.66869	Secreted frizzled-related protein 1	*SFRP1*
P43251	0.10596	0.00327	0.66869	Biotinidase	*BTD*
Q8NBP7	0.09953	0.00369	0.66869	Proprotein convertase subtilisin/kexin type 9	*PCSK9*
Q9UK55	0.08628	0.00785	0.66869	Protein Z-dependent protease inhibitor	*SERPINA10*
Q6UWD8	-0.08015	0.02362	0.90164	Transmembrane protein C16orf54	*C16orf54*
P19876.P19875	-0.07721	0.02799	0.95018	C-X-C motif chemokine 3 & C-X-C motif chemokine 2	*CXCL3 & CXCL2*
Q9Y5Q6	0.04643	0.03844	0.95830	Insulin-like peptide INSL5	*INSL5*
Q96PU8	-0.06558	0.03844	0.95830	Protein quaking	*QKI*
P52907	-0.10166	0.04017	0.95830	F-actin-capping protein subunit alpha-1	*CAPZA1*
P00797	-0.07242	0.05646	0.95830	Renin	*REN*
O14548	0.10303	0.09762	0.97017	Cytochrome c oxidase subunit 7A-related protein, mitochondrial	*COX7A2L*

Supplementary Table 10. Pathway analysis (canonical pathways) (IPA) on 205 differentially expressed proteins in MCI converters. The z-score reflects an overall predicted activation and inhibition state of the biological function. Positive and negative z-scores predict activation and inhibition, respectively.

Pathway	molecules	z-score	p-value	ratio
Coagulation system	7	0.378	1.92 E-04	7/26
Acute phase response signalling	12	2.121 (biased)	3.45 E-03	12/100
Extrinsic prothrombin activation pathway	3		1.11 E-02	3/10
FXR/RXR activation	7		1.46 E-02	7/53
Notch signalling	3		2.37 E-02	3/13
Superpathway of methionine degradation	2		3.21 E-02	2/6
Wnt/b-catenin signalling	6	- 0.447	3.53 E-02	6/50
Role of osteoblasts, osteoclasts, and chondrocytes in rheumatoid arthritis	9		3.68 E-02	9/92
EGF signalling		1 (biased)	3.71 E-02	4/26
Ceramide signalling	4	1 (biased)	4.71 E-02	4/28
ATM signalling	4	0	4.71 E-02	4/28
IL-17A signalling in airway cells	4	2 (biased)	4.71 E-02	4/28

Supplementary Table 11. Network Analysis (IPA) on 205 differentially expressed proteins in MCI converters. (related to Fig. 5D-F) Network Scores are based on the hypergeometric distribution and are calculated with the right-tailed Fisher's Exact Test. Score ranks the networks based on the following information: 1) the degree of relevance of the network to the Focus molecules in the dataset, 2) the number of Focus molecules in the network, 3) the network size, 4) the total number of Focus molecules analysed, and 5) the total number of molecules in the QIAGEN Knowledge Base that could potentially be included in networks.

Associated Network Functions	Score
Haematological System Development and Function, Organismal Functions, Organismal Injury and Abnormalities	48
Cell Death and Survival, Embryonic Development, Organismal Development	43
Cell-to-Cell Signalling and Interaction, Cellular Function and Maintenance, Inflammatory Response	21
DNA Replication, Recombination, and Repair, Cancer, Organismal Injury and Abnormalities	17
Organismal Survival, Connective Tissue Disorders, Developmental Disorder	17