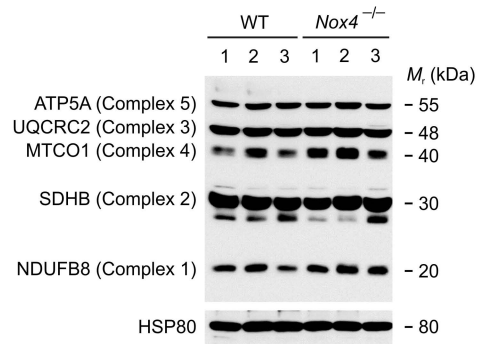


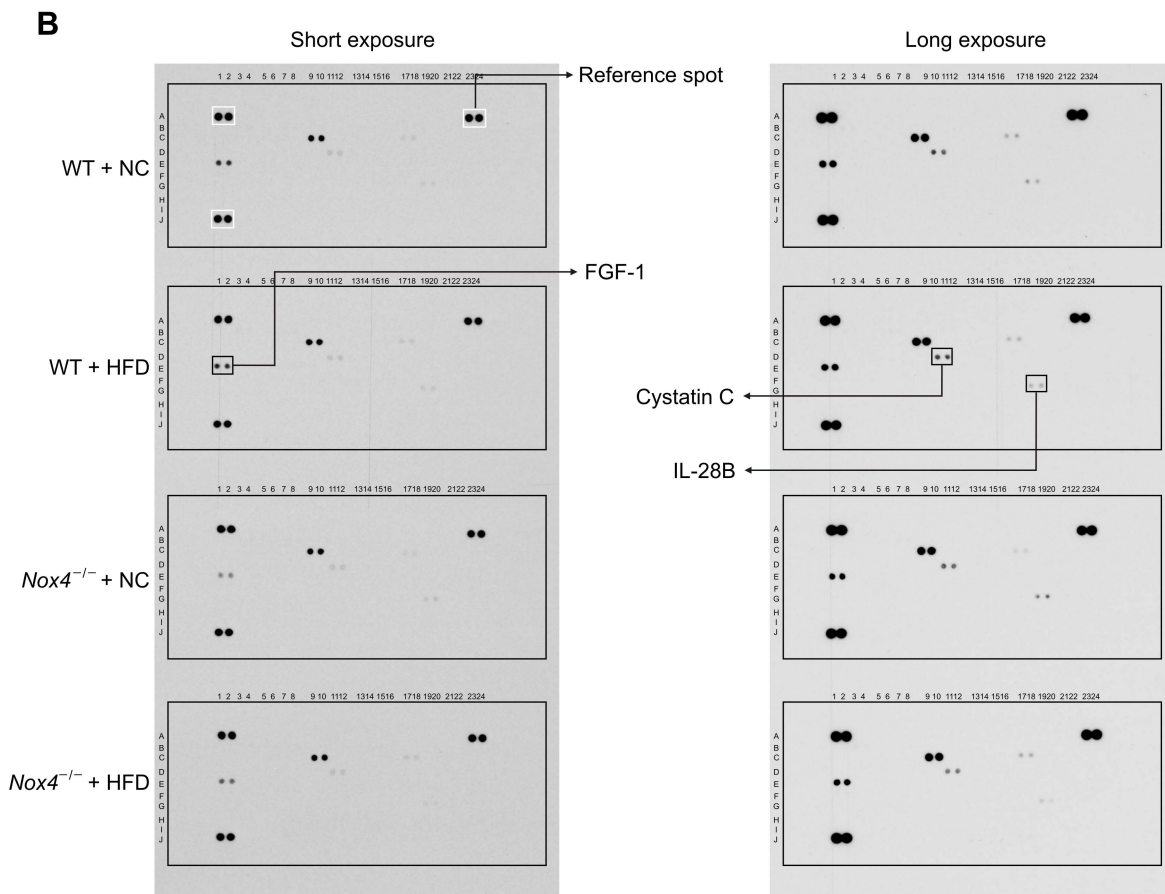
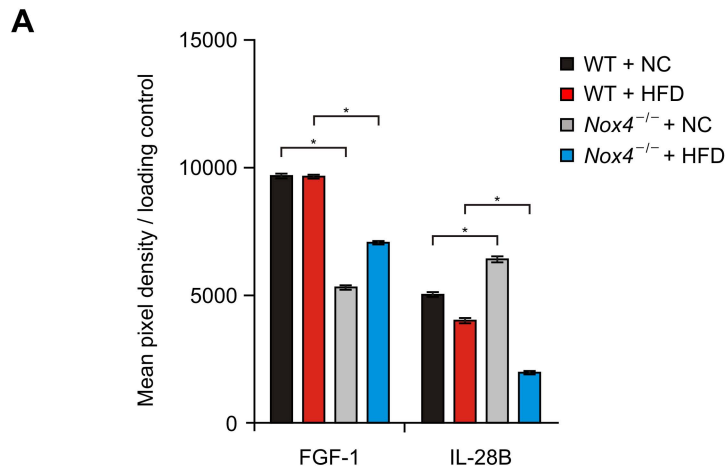
## **Supplemental information**

# **NOX4 deficiency exacerbates the impairment of Cystatin c-dependent hippocampal neurogenesis by chronic high fat diet**

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**Supplemental Figure S1. The expression of mitochondrial oxidative phosphorylation complex enzymes in fat tissues was comparable between *Nox4*<sup>-/-</sup> and WT mice.** Representative immunoblot analysis for ATP5A (Complex 5), UQCRC2 (Complex 3), MTCO1 (Complex 4), SDHB (Complex 2) and NDUFB8 (Complex 1) of fat tissues from *Nox4*<sup>-/-</sup> and WT mice (n = 3 per group). HSP80 is used as loading control. ATP5A = ATP synthase F1 subunit alpha; UQCRC2 = ubiquinol-cytochrome c reductase core protein 2; MTCO1 = Mitochondrially Encoded Cytochrome C Oxidase 1; SDHB = Succinate Dehydrogenase Complex Iron Sulfur Subunit B; NDUFB8 = NADH:Ubiquinone Oxidoreductase Subunit B8



**Supplemental Figure S2. The levels of Cystatin C, FGF-1 and IL-28B in hippocampus from *Nox4*<sup>-/-</sup> and WT mice.**

(A) Quantification of FGF-1 and IL-28B secretion from *Nox4*<sup>-/-</sup> and WT mice with normal chow diet (NC) or high fat diet (HFD). (B) Representative immunoblot analysis for Cystatin C, FGF-1, and IL-28B of hippocampus from *Nox4*<sup>-/-</sup> and WT mice with normal chow diet (NC) or high fat diet (HFD) (black box). Reference spots are used as loading control (white box). Data are representative of two independent experiments. The 111 different cytokines, chemokines, growth factors or extracellular signaling molecules were analyzed by immunoblot analysis. The location of analytes is marked by A1 to J24 using X axis (1 to 24) and Y (A to J) axis. The values of mean pixel density for whole analytes are described in supplemental Table 1. Data mean  $\pm$  SD. \* $P < 0.05$  by Student's two-tail

**Supplemental Table 1. Mean pixel density of analytes**

| Coordinate | Analyte                              | Entrez Gene ID | Mean pixel density |             |                                 |                                  |
|------------|--------------------------------------|----------------|--------------------|-------------|---------------------------------|----------------------------------|
|            |                                      |                | WT + NC            | WT + HFD    | <i>Nox4</i> <sup>-/-</sup> + NC | <i>Nox4</i> <sup>-/-</sup> + HFD |
| A1, A2     | Reference Spots                      | N/A            | 75241 ± 152        | 76053 ± 207 | 75349 ± 185                     | 76321 ± 142                      |
| A3, A4     | Adiponectin/Acrp30                   | 11450          | ND                 | ND          | ND                              | ND                               |
| A5, A6     | Amphiregulin                         | 11839          | ND                 | ND          | ND                              | ND                               |
| A7, A8     | Angiopoietin-1                       | 11600          | ND                 | ND          | ND                              | ND                               |
| A9, A10    | Angiopoietin-2                       | 11601          | ND                 | ND          | ND                              | ND                               |
| A11, A12   | Angiopoietin-like 3                  | 30924          | ND                 | ND          | ND                              | ND                               |
| A13, A14   | BAFF/BLyS/TNFSF13B                   | 24099          | ND                 | ND          | ND                              | ND                               |
| A15, A16   | C1q R1/CD93                          | 17064          | ND                 | ND          | ND                              | ND                               |
| A17, A18   | CCL2/JE/MCP-1                        | 20296          | ND                 | ND          | ND                              | ND                               |
| A19, A20   | CCL3/CCL4/MIP-1α/β                   | 20302/20303    | ND                 | ND          | ND                              | ND                               |
| A21, A22   | CCL5/RANTES                          | 20304          | ND                 | ND          | ND                              | ND                               |
| A23, A24   | Reference Spots                      | N/A            | 78751 ± 244        | 79125 ± 181 | 78912 ± 231                     | 79934 ± 227                      |
| B3, B4     | CCL6/C10                             | 20305          | ND                 | ND          | ND                              | ND                               |
| B5, B6     | CCL11/Eotaxin                        | 20292          | ND                 | ND          | ND                              | ND                               |
| B7, B8     | CCL12/MCP-5                          | 20293          | ND                 | ND          | ND                              | ND                               |
| B9, B10    | CCL17/TARC                           | 20295          | ND                 | ND          | ND                              | ND                               |
| B11, B12   | CCL19/MIP-3β                         | 24047          | ND                 | ND          | ND                              | ND                               |
| B13, B14   | CCL20/MIP-3α                         | 20297          | ND                 | ND          | ND                              | ND                               |
| B15, B16   | CCL21/6Ckine                         | 18829          | ND                 | ND          | ND                              | ND                               |
| B17, B18   | CCL22/MDC                            | 20299          | ND                 | ND          | ND                              | ND                               |
| B21, B22   | CD40/TNFRSF5                         | 21939          | ND                 | ND          | ND                              | ND                               |
| C3, C4     | CD160                                | 54215          | ND                 | ND          | ND                              | ND                               |
| C5, C6     | Chemerin                             | 71660          | ND                 | ND          | ND                              | ND                               |
| C9, C10    | Coagulation Factor III/Tissue Factor | 14066          | 26521 ± 125        | 26754 ± 101 | 26635 ± 135                     | 26854 ± 147                      |
| C11, C12   | Complement Component C5/C5a          | 15139          | ND                 | ND          | ND                              | ND                               |
| C13, C14   | Complement Factor D                  | 11537          | ND                 | ND          | ND                              | ND                               |
| C15, C16   | C-Reactive Protein/CRP               | 12944          | ND                 | ND          | ND                              | ND                               |
| C17, C18   | CX3CL1/Fractalkine                   | 20312          | 9851 ± 101         | 9785 ± 115  | 9817 ± 112                      | 9874 ± 109                       |
| C19, C20   | CXCL1/KC                             | 14825          | ND                 | ND          | ND                              | ND                               |
| C21, C22   | CXCL2/MIP-2                          | 20310          | ND                 | ND          | ND                              | ND                               |
| D1, D2     | CXCL9/MIG                            | 17329          | ND                 | ND          | ND                              | ND                               |
| D3, D4     | CXCL10/IP-10                         | 15945          | ND                 | ND          | ND                              | ND                               |
| D5, D6     | CXCL11/I-TAC                         | 56066          | ND                 | ND          | ND                              | ND                               |
| D7, D8     | CXCL13/BLC/BCA-1                     | 55985          | ND                 | ND          | ND                              | ND                               |
| D9, D10    | CXCL16                               | 66102          | ND                 | ND          | ND                              | ND                               |
| D11, D12   | Cystatin C                           | 13010          | 48524 ± 235        | 49721 ± 168 | 41236 ± 195                     | 25236 ± 157                      |
| D13, D14   | DKK-1                                | 13380          | ND                 | ND          | ND                              | ND                               |
| D15, D16   | DPPIV/CD26                           | 13482          | ND                 | ND          | ND                              | ND                               |
| D17, D18   | EGF                                  | 13645          | ND                 | ND          | ND                              | ND                               |
| D19, D20   | Endoglin/CD105                       | 13805          | ND                 | ND          | ND                              | ND                               |
| D21, D22   | Endostatin                           | 12822          | ND                 | ND          | ND                              | ND                               |
| D23, D24   | Fetuin A/AHSG                        | 11625          | ND                 | ND          | ND                              | ND                               |
| E1, E2     | FGF acidic (FGF-1)                   | 14164          | 9875 ± 117         | 9851 ± 138  | 5104 ± 172                      | 7632 ± 152                       |
| E3, E4     | FGF-21                               | 56636          | ND                 | ND          | ND                              | ND                               |
| E5, E6     | Flt-3 Ligand                         | 14256          | ND                 | ND          | ND                              | ND                               |
| E7, E8     | Gas 6                                | 14456          | ND                 | ND          | ND                              | ND                               |
| E9, E10    | G-CSF                                | 12985          | ND                 | ND          | ND                              | ND                               |

|          |                                 |               |                |                |                |                |
|----------|---------------------------------|---------------|----------------|----------------|----------------|----------------|
| E11, E12 | GDF-15                          | 23886         | ND             | ND             | ND             | ND             |
| E13, E14 | GM-CSF                          | 12981         | ND             | ND             | ND             | ND             |
| E15, E16 | HGF                             | 15234         | ND             | ND             | ND             | ND             |
| E17, E18 | ICAM-1/CD54                     | 15894         | ND             | ND             | ND             | ND             |
| E19, E20 | IFN- $\gamma$                   | 15978         | ND             | ND             | ND             | ND             |
| E21, E22 | IGFBP-1                         | 16006         | ND             | ND             | ND             | ND             |
| E23, E24 | IGFBP-2                         | 16008         | ND             | ND             | ND             | ND             |
| F1, F2   | IGFBP-3                         | 16009         | ND             | ND             | ND             | ND             |
| F3, F4   | IGFBP-5                         | 16011         | ND             | ND             | ND             | ND             |
| F5, F6   | IGFBP-6                         | 16012         | ND             | ND             | ND             | ND             |
| F7, F8   | IL-1 $\alpha$ /IL-1F1           | 16175         | ND             | ND             | ND             | ND             |
| F9, F10  | IL-1 $\beta$ /IL-1F2            | 16176         | ND             | ND             | ND             | ND             |
| F11, F12 | IL-1ra/IL-1F3                   | 16181         | ND             | ND             | ND             | ND             |
| F13, F14 | IL-2                            | 16183         | ND             | ND             | ND             | ND             |
| F15, F16 | IL-3                            | 16187         | ND             | ND             | ND             | ND             |
| F17, F18 | IL-4                            | 16189         | ND             | ND             | ND             | ND             |
| F19, F20 | IL-5                            | 16191         | ND             | ND             | ND             | ND             |
| F21, F22 | IL-6                            | 16193         | ND             | ND             | ND             | ND             |
| F23, F24 | IL-7                            | 16196         | ND             | ND             | ND             | ND             |
| G1, G2   | IL-10                           | 16153         | ND             | ND             | ND             | ND             |
| G3, G4   | IL-11                           | 16156         | ND             | ND             | ND             | ND             |
| G5, G6   | IL-12 p40                       | 16160         | ND             | ND             | ND             | ND             |
| G7, G8   | IL-13                           | 16163         | ND             | ND             | ND             | ND             |
| G9, G10  | IL-15                           | 16168         | ND             | ND             | ND             | ND             |
| G11, G12 | IL-17A                          | 16171         | ND             | ND             | ND             | ND             |
| G13, G14 | IL-22                           | 50929         | ND             | ND             | ND             | ND             |
| G15, G16 | IL-23                           | 83430         | ND             | ND             | ND             | ND             |
| G17, G18 | IL-27 p28                       | 246779        | ND             | ND             | ND             | ND             |
| G19, G20 | IL-28A/B                        | 330496/338374 | 4925 $\pm$ 235 | 4147 $\pm$ 258 | 6874 $\pm$ 264 | 2152 $\pm$ 154 |
| G21, G22 | IL-33                           | 77125         | ND             | ND             | ND             | ND             |
| G23, G24 | LDL R                           | 16835         | ND             | ND             | ND             | ND             |
| H1, H2   | Leptin                          | 16846         | ND             | ND             | ND             | ND             |
| H3, H4   | LIF                             | 16878         | ND             | ND             | ND             | ND             |
| H5, H6   | Lipocalin-2/NGAL                | 16819         | ND             | ND             | ND             | ND             |
| H7, H8   | LIX                             | 20311         | ND             | ND             | ND             | ND             |
| H9, H10  | M-CSF                           | 12977         | ND             | ND             | ND             | ND             |
| H11, H12 | MMP-2                           | 17390         | ND             | ND             | ND             | ND             |
| H13, H14 | MMP-3                           | 17392         | ND             | ND             | ND             | ND             |
| H15, H16 | MMP-9                           | 17395         | ND             | ND             | ND             | ND             |
| H17, H18 | Myeloperoxidase                 | 17523         | ND             | ND             | ND             | ND             |
| H19, H20 | Osteopontin (OPN)               | 20750         | ND             | ND             | ND             | ND             |
| H21, H22 | Osteoprotegerin/TNFRSF11B       | 18383         | ND             | ND             | ND             | ND             |
| H23, H24 | PD-ECGF/Thymidine phosphorylase | 72962         | ND             | ND             | ND             | ND             |
| I1, I2   | PDGF-BB                         | 18591         | ND             | ND             | ND             | ND             |
| I3, I4   | Pentraxin 2/SAP                 | 20219         | ND             | ND             | ND             | ND             |
| I5, I6   | Pentraxin 3/TSG-14              | 19288         | ND             | ND             | ND             | ND             |
| I7, I8   | Periostin/OSF-2                 | 50706         | ND             | ND             | ND             | ND             |
| I9, I10  | Pref-1/DLK-1/FA1                | 13386         | ND             | ND             | ND             | ND             |
| I11, I12 | Proliferin                      | 18811         | ND             | ND             | ND             | ND             |
| I13, I14 | Proprotein Convertase 9/PCSK9   | 100102        | ND             | ND             | ND             | ND             |
| I15, I16 | RAGE                            | 11596         | ND             | ND             | ND             | ND             |

|          |                   |        |             |             |             |             |
|----------|-------------------|--------|-------------|-------------|-------------|-------------|
| I17, I18 | RBP4              | 19662  | ND          | ND          | ND          | ND          |
| I19, I20 | Reg3G             | 19695  | ND          | ND          | ND          | ND          |
| I21, I22 | Resistin          | 57264  | ND          | ND          | ND          | ND          |
| J1, J2   | Reference Spots   | N/A    | 71085 ± 107 | 70936 ± 174 | 71524 ± 121 | 71852 ± 184 |
| J3, J4   | E-Selectin/CD62E  | 20339  | ND          | ND          | ND          | ND          |
| J5, J6   | P-Selectin/CD62P  | 20344  | ND          | ND          | ND          | ND          |
| J7, J8   | Serpin E1/PAI-1   | 18787  | ND          | ND          | ND          | ND          |
| J9, J10  | Serpin F1/PEDF    | 20317  | ND          | ND          | ND          | ND          |
| J11, J12 | Thrombopoietin    | 21832  | ND          | ND          | ND          | ND          |
| J13, J14 | TIM-1/KIM-1/HAVCR | 171283 | ND          | ND          | ND          | ND          |
| J15, J16 | TNF- $\alpha$     | 21926  | ND          | ND          | ND          | ND          |
| J17, J18 | VCAM-1/CD106      | 22329  | ND          | ND          | ND          | ND          |
| J19, J20 | VEGF              | 22339  | ND          | ND          | ND          | ND          |
| J21, J22 | WISP-1/CCN4       | 22402  | ND          | ND          | ND          | ND          |
| J23, J24 | Negative Control  | N/A    | ND          | ND          | ND          | ND          |

\* ND : Not detectable