SUPPLEMENTARY INFORMATION

Disruption of astrocyte-neuron cholesterol cross-talk affects neuronal function in Huntington's disease

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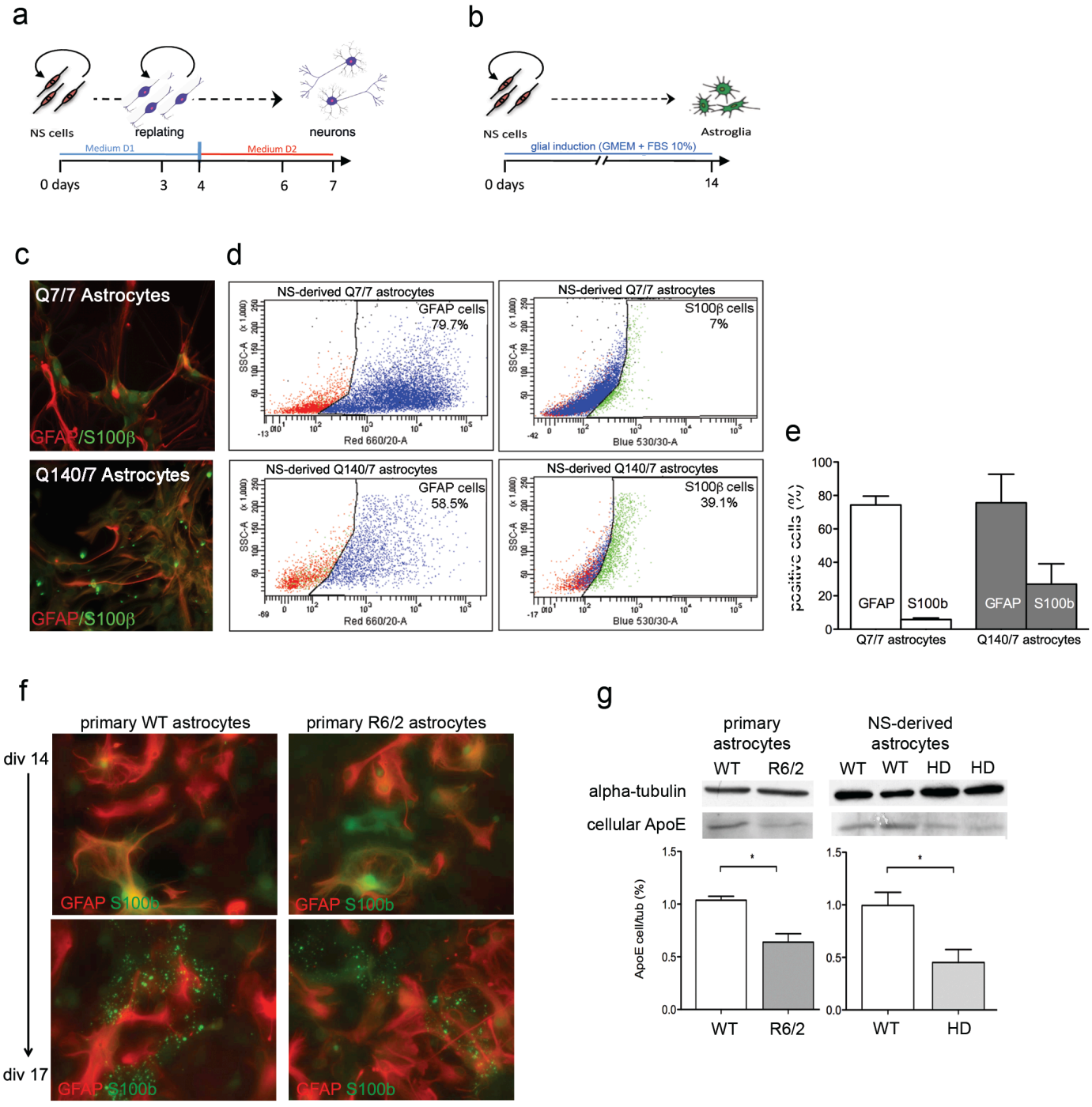
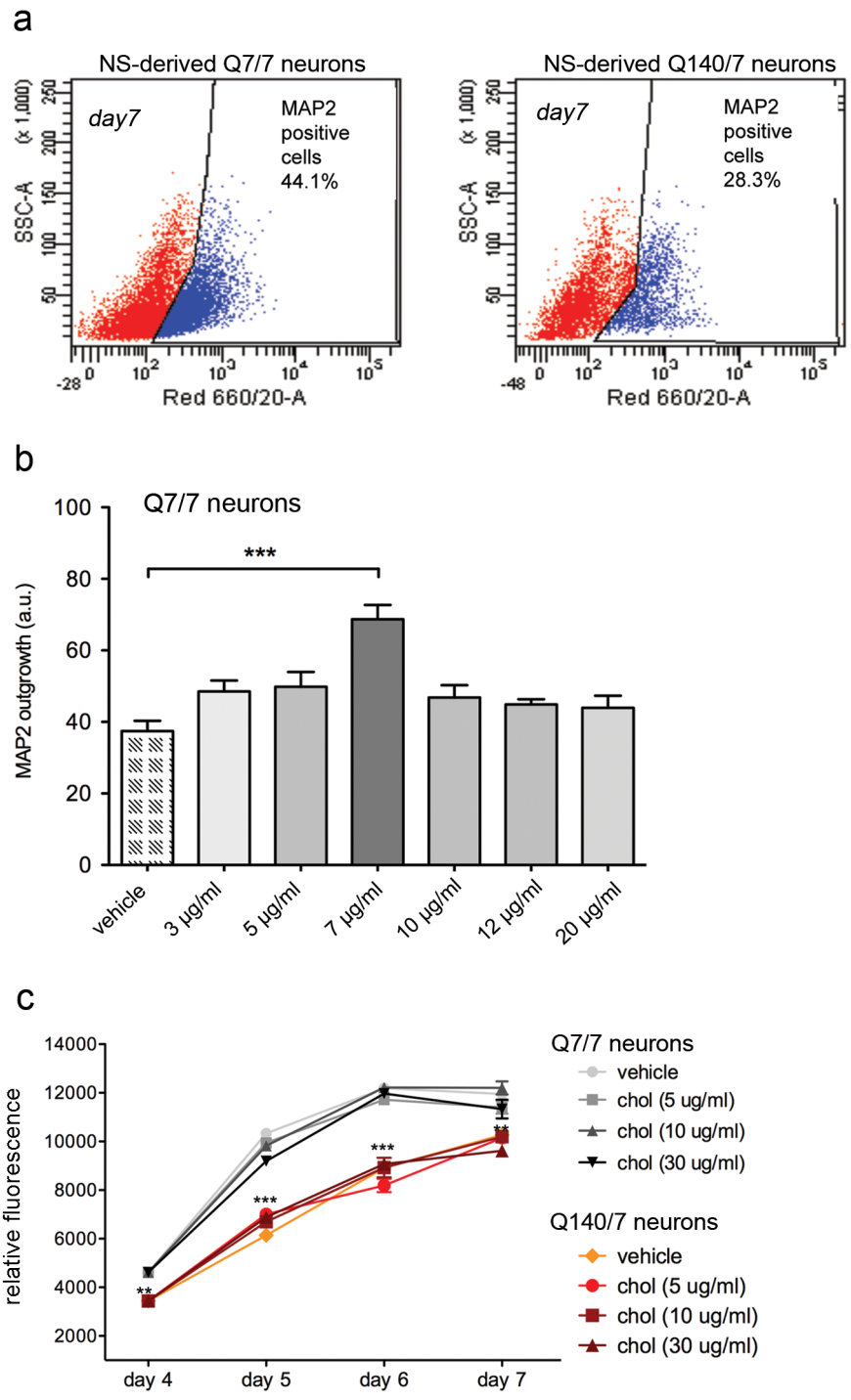
Supplementary Figure 1



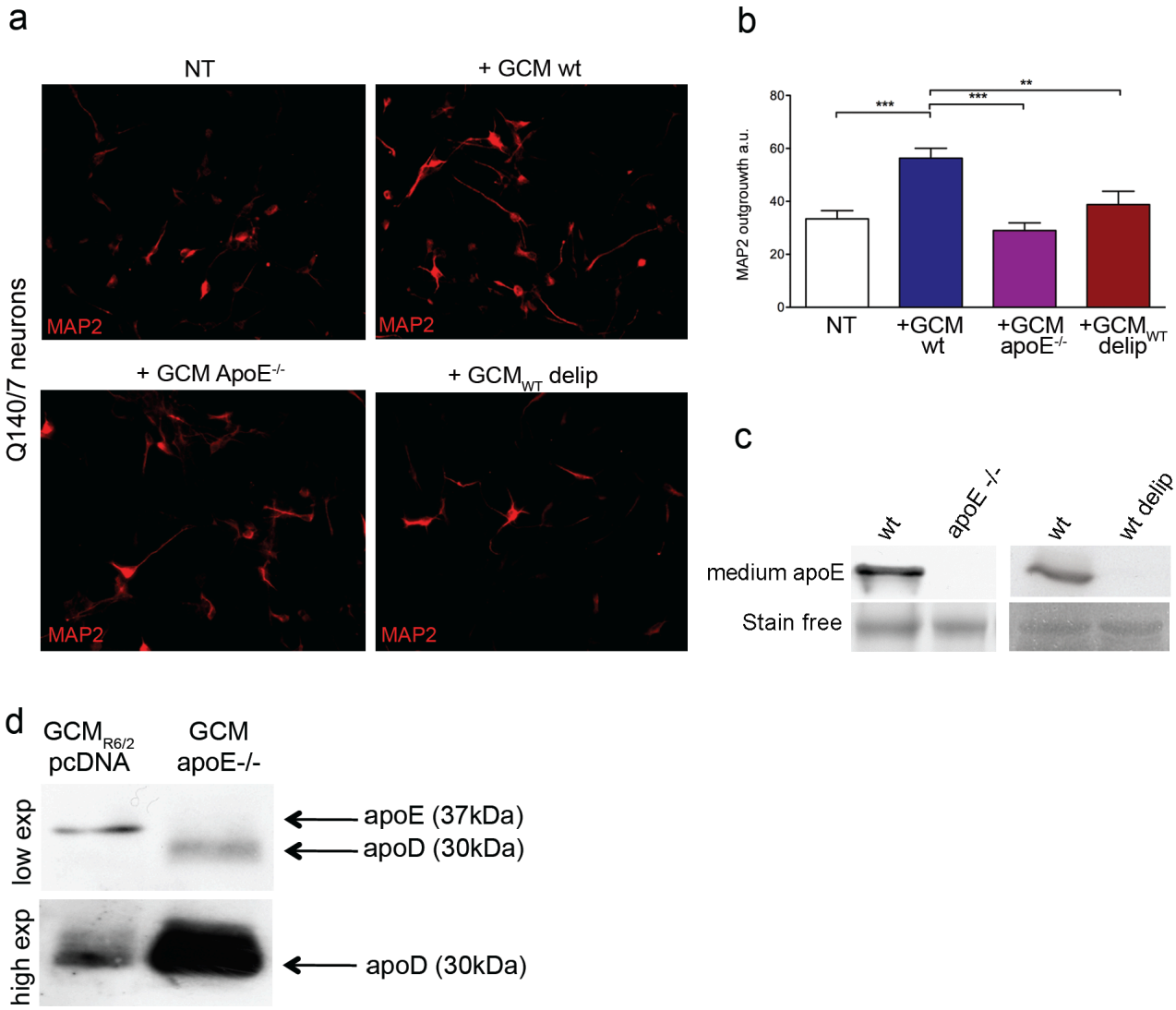
Supplementary Figure 1. (**a-b**) Schematic diagram of differentiation protocol to convert neural stem (NS) cells to generic neurons (**a**) and to astrocytes (**b**). (**c**) Immunofluorescence staining of astrocytes derived from Q7/7 and Q140/7 NS cells after 14 days of glial differentiation with antibodies against GFAP (red) and S100b (green). Original magnification: 20×. (**d-e**) Flow cytometry of Q7/7 and Q140/7 astrocytes for GFAP and S100b. The graph (**e**) shows the % of GFAP- or S100b-positive cells as the mean ± s.e.m. for three independent differentiations. (**f**) Immunofluorescence staining of primary astrocytes derived from wt and R6/2 pups after 14 and 17 days *in vitro* (*div*) with antibodies against GFAP (red) and S100b (green). Original magnification: 20×. (**g**) Representative western blot analysis showing intracellular ApoE levels in primary astrocytes from wt and R6/2 astrocytes and in NS-derived astrocytes. Alpha-tubulin was used to normalize apoE levels. The graphs show the mean % above relative controls ± s.e.m.

Supplementary Figure 2



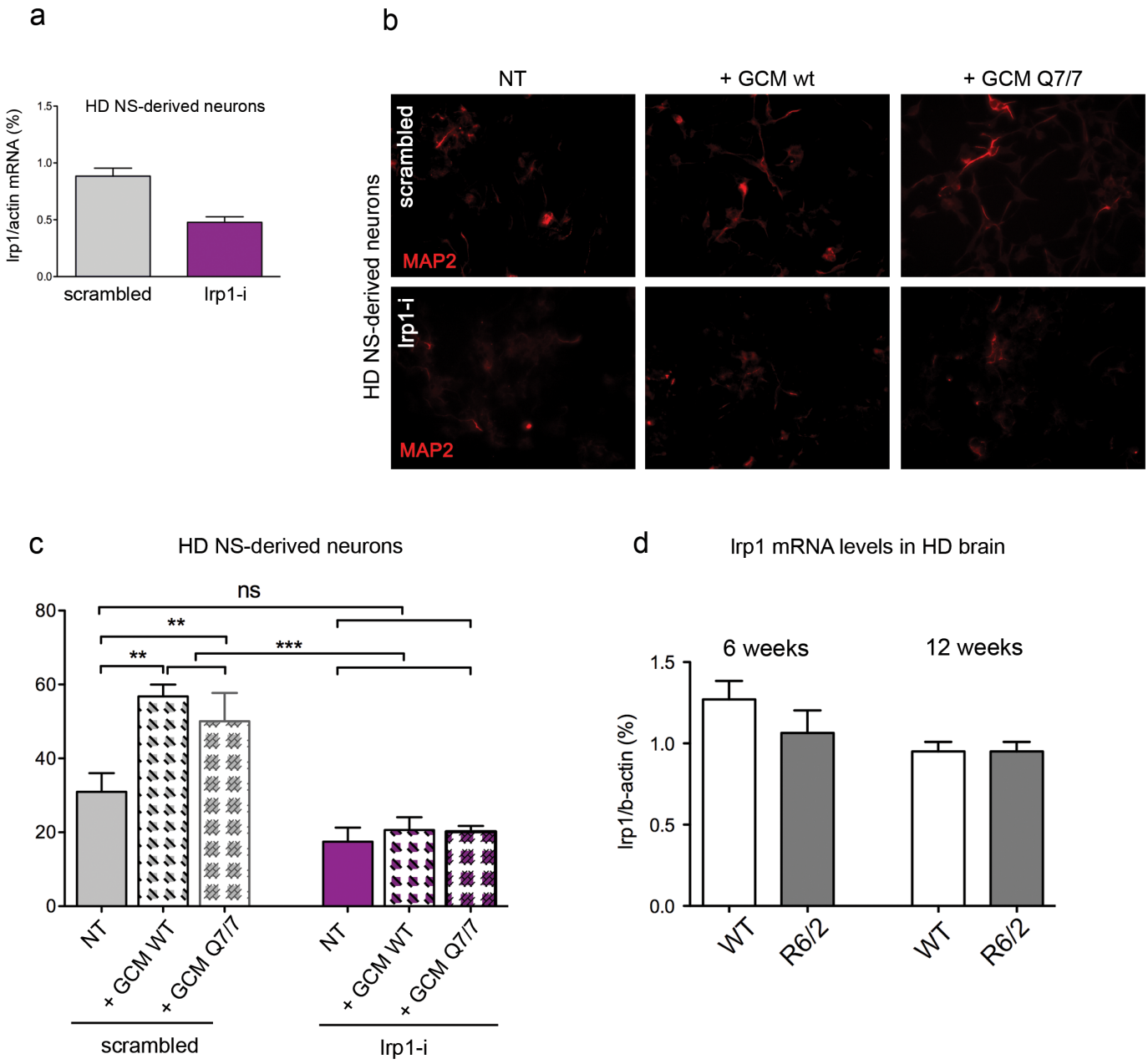
Supplementary Figure 2. (**a**) Flow cytometry of Q7/7 and Q140/7 neurons for MAP2 at day 7 of neuronal differentiation. (**b**) Neurite outgrowth quantification in Q7/7 neurons under glial-free conditions (vehicle) or in the presence of increasing doses of cholesterol (3, 5, 7, 10, 12, and 20 µg/ml) immunostained for MAP2. Original magnification: 20×. The graph shows mean (a.u.) ± s.e.m. of 10 fields for each condition. (**c**) Cell survival measured by Cy-quant assay of Q7/7 and Q140/7 neurons under glial-free conditions (vehicle) or in the presence of increasing doses of cholesterol (5, 10, and 30 µg/ml). The graph shows the mean (of triplicates) ± s.e.m. Statistics: one-way ANOVA, Newman–Keuls multiple-comparison test (**b,c**). **P* < 0.05; **P* < 0.01; **P* < 0.001.

Supplementary Figure 3



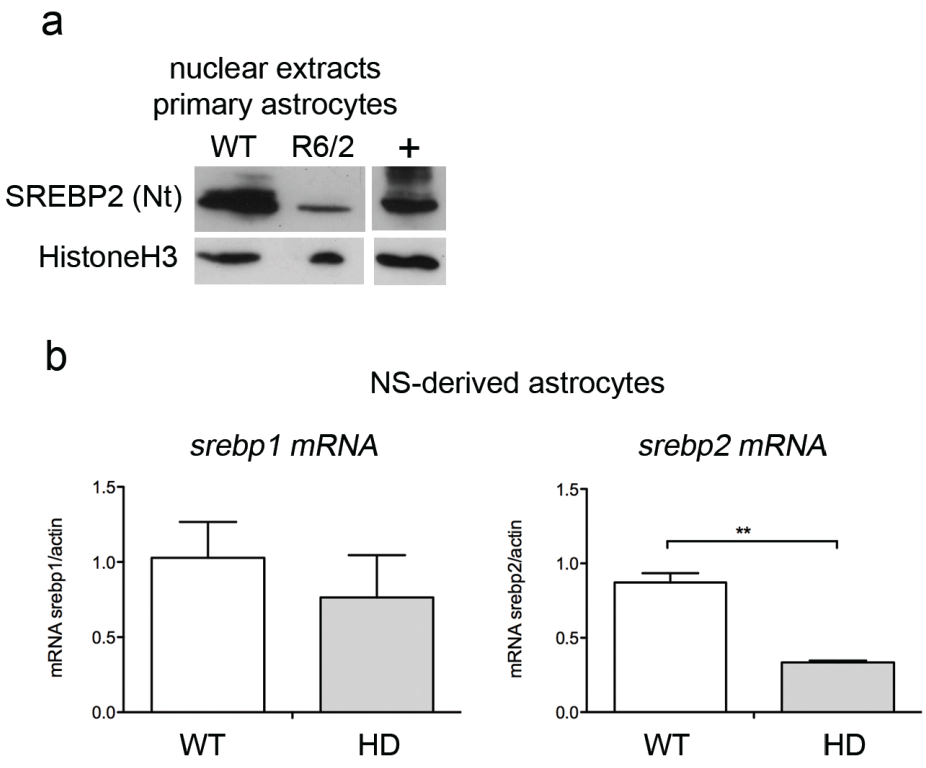
Supplementary Figure 3. (**a**) Representative immunofluorescence staining for MAP2 in Q140/7 neurons under glial-free conditions (NT) or in the presence of GCM from primary astrocytes of wt mice (GCM wt) or from apoE$^{-/-}$ mice (GCM apoE$^{-/-}$) and GCM from lipoprotein-depleted wt astrocytes (GCM$_{wt}$delip). Original magnification: 20×. (**b**) Relative neurite outgrowth quantification. The graph shows the mean of an arbitrary value (a.u.) ± s.e.m. from one experiment in which 10 fields were analyzed for each condition. Similar results were obtained from other two independent differentiations. (**c**) Representative western blot analysis for apoE levels in GCM apoE$^{-/-}$ and in GCM$_{wt}$delip. (**d**) Representative western blot analysis for apoD in GCM apoE$^{-/-}$. Statistics: one-way ANOVA, Newman–Keuls multiple-comparison test. *P < 0.05; *P < 0.01; *P < 0.001.

Supplementary Figure 4



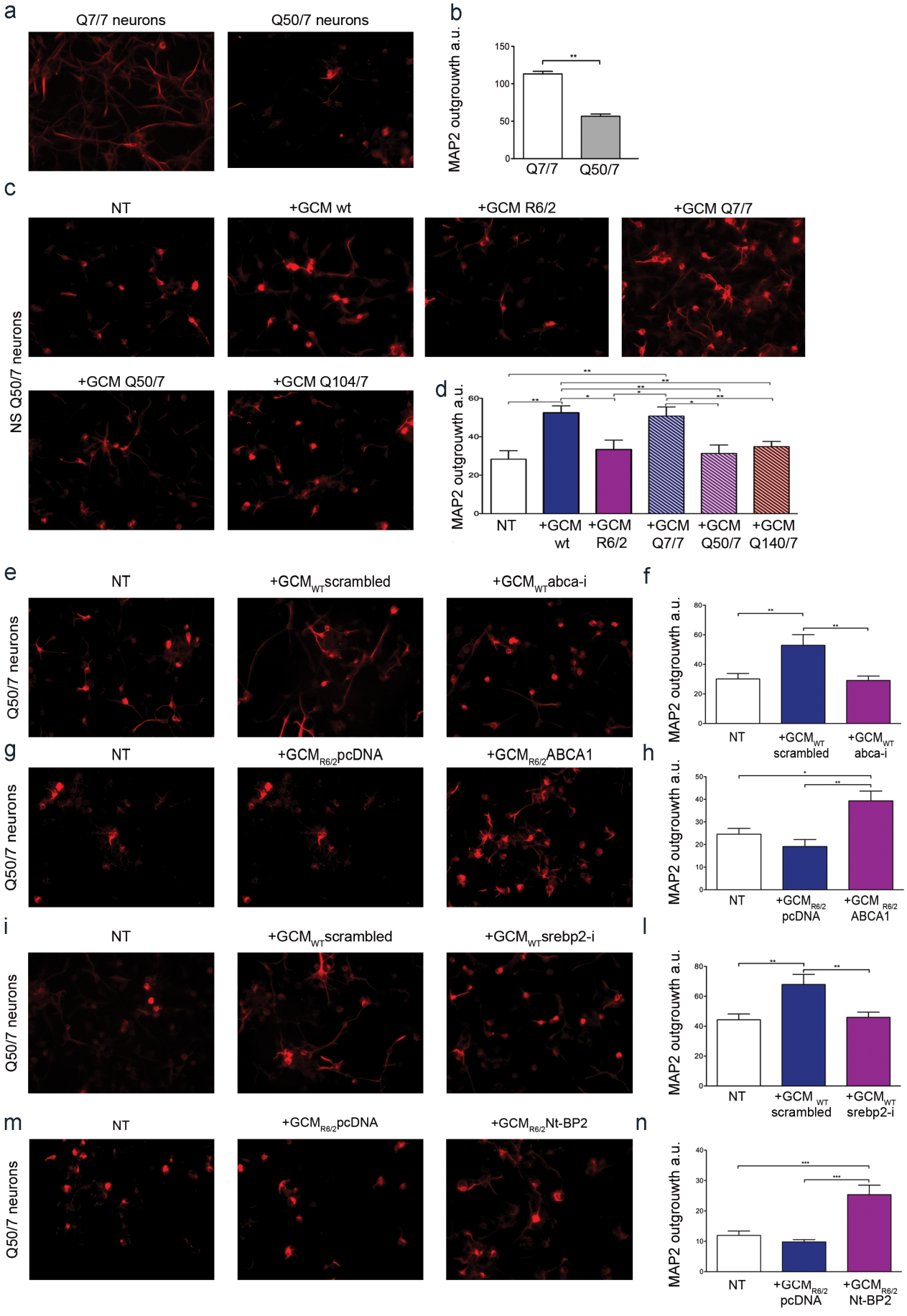
Supplementary Figure 4. (**a**) mRNA levels of *lrp1* in HD neurons after transfection with siRNA against *lrp1* (lrp1-i) compared to the same cells transfected with scramble siRNA as a control (scrambled). (**b**) Representative immunofluorescence staining for MAP2 in HD neurons (scrambled or after lrp1 silencing) under glial-free conditions (NT), in the presence of GCM from primary wt astrocytes (GCM wt) or Q7/7 astrocytes (GCM Q7/7). Original magnification: 20×. (**c**) Relative neurite outgrowth quantification. Graph shows the mean of an arbitrary value (a.u.) ± s.e.m. (**d**) mRNA levels for *lrp1* in wt and R6/2 brains at 6 weeks and 12 weeks of age. Graph in (**d**) shows the mean as % above relative controls ± s.e.m. *P* values were determined by one-way ANOVA with Newman-Keuls multiple-comparison post-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Supplementary Figure 5



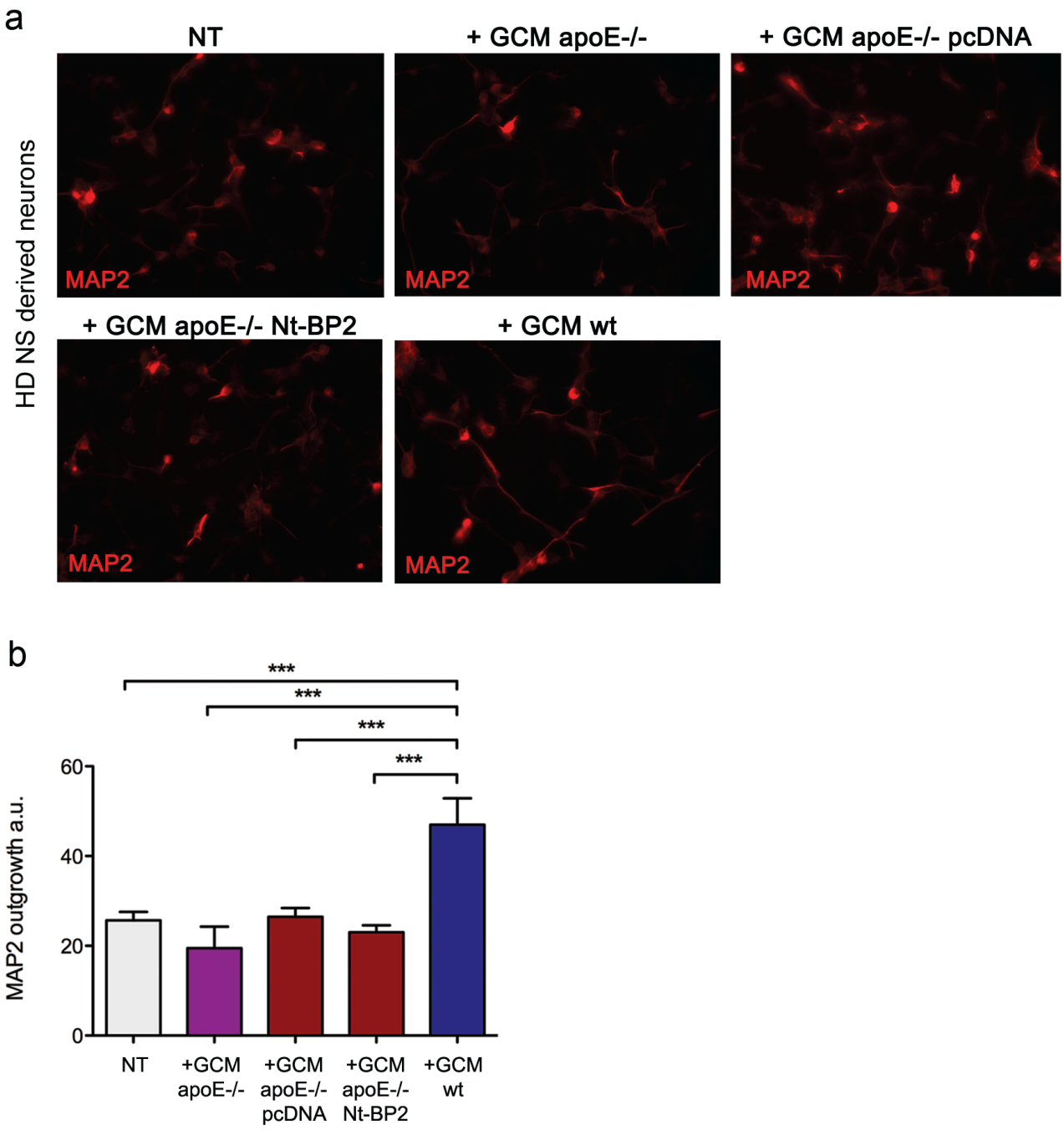
Supplementary Figure 5. (**a**) representative western blot analysis showing protein levels of the active form of SREBP2 in nuclear extracts from primary astrocytes of wt and R6/2 mice. HistoneH3 was used as loading control in nuclear extracts. A nuclear extract from control cell lines (Valenza et al., J. Neurosci 2005) was used as positive control. (**b**) mRNA levels of srebp1 and srebp2 in NS-derived astrocytes. Graphs in (**b**) show the mean % above relative controls ± s.e.m.. *P* values were determined by t-test student. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Supplementary Figure 6



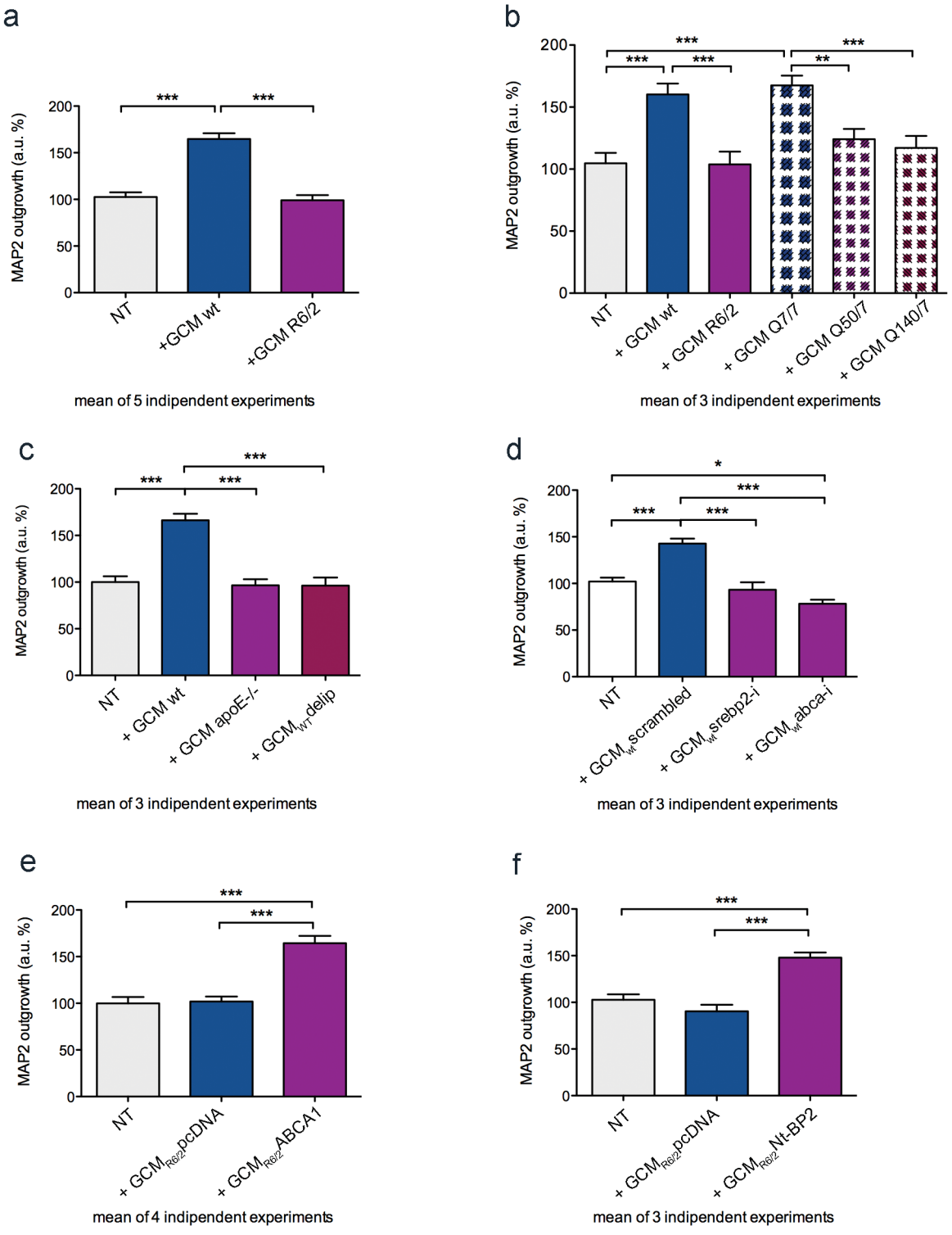
Supplementary Figure 6. (**a–n**) Representative immunofluorescence staining for MAP2 (a,c,e,g,i,m), and relative neurite outgrowth quantification (b,d,f,h,l,n) for the other HD NS-derived clone carrying HTT with 50Q (Q50/7) in the different conditions. The graphs (b,d,f,h,l,n) show the mean (a.u.) ± s.e.m. of one experiment in which 10 fields were analyzed for each condition. Similar results were obtained in two other independent differentiations. Statistics: one-way ANOVA, Newman–Keuls multiple-comparison post-test (**b**). **P* < 0.05; ***P* < 0.01; ****P* < 0.001. Original magnification: 20×.

Supplementary Figure 7



Supplementary Figure 7. (**a**) Representative immunofluorescence staining for MAP2 in HD NS-derived neurons under glial-free conditions (NT) or in the presence of GCM from primary astrocytes of apoE$^{-/-}$ mice (GCM apoE$^{-/-}$), apoE$^{-/-}$ mice over-expressing an empty vector (pcDNA) or over-expressing the active form of SREBP2 (Nt-BP2) compared to GCM from wt mice (GCM wt). Original magnification: 20×. (**b**) Relative neurite outgrowth quantification. The graph shows the mean of an arbitrary value (a.u.) ± s.e.m. from one experiment in which 10 fields were analyzed for each condition. Statistics: one-way ANOVA, Newman–Keuls multiple-comparison test. *P < 0.05; *P < 0.01; *P < 0.001.

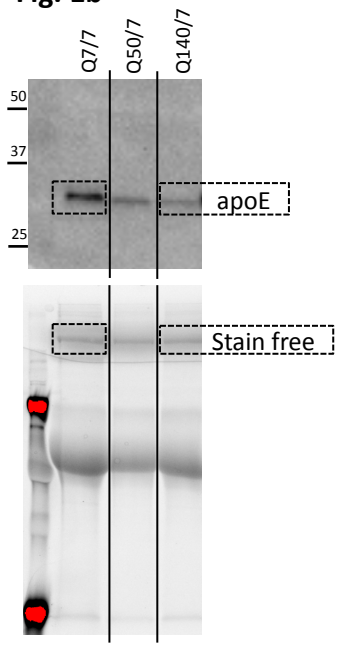
Supplementary Figure 8



Supplementary Figure 8. (**a–f**) Graphs showing neurite outgrowth quantification in Q140/7 neurons expressed as % above the relative controls, as mean of all the independent experiments performed in this work. Statistics: one-way ANOVA, Newman–Keuls multiple-comparison post-test (**b**). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Supplementary Figure 9 – Full-length pictures of the blot presented in the main figures

Fig. 1b



Note: the lines indicate the lane excided from image in Fig. 1b

Fig. 1c

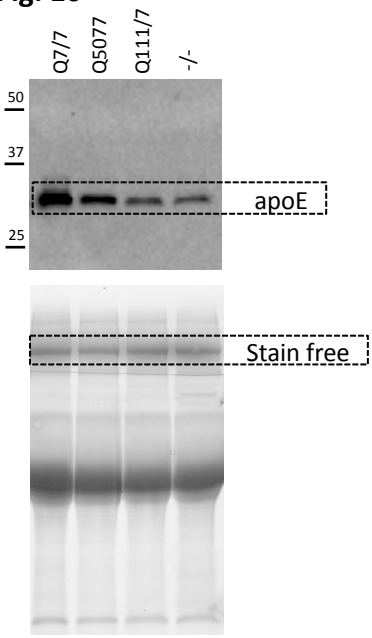


Fig. 1d

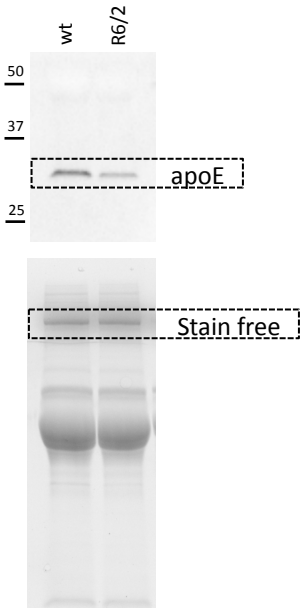


Fig. 3b

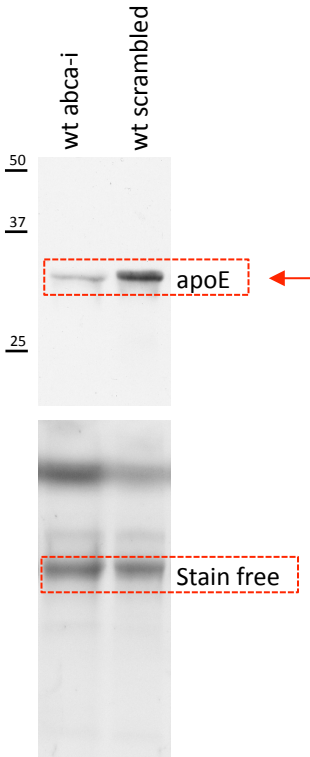
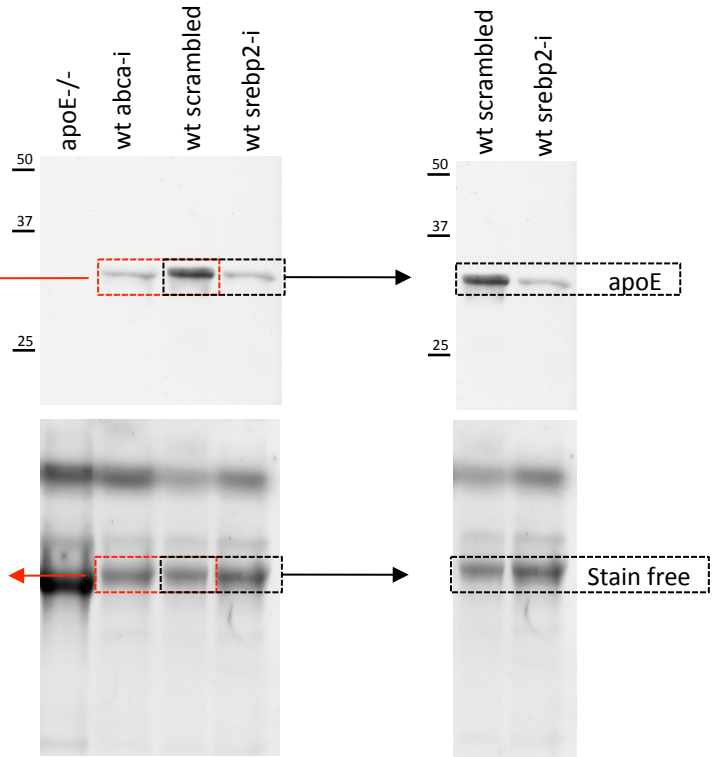


Fig. 4b



Note: silencing for abca1 and srebp2 was done in the same experiments and all the samples were run together. The control sample (GCM_{wt}scrambled) in Fig. 3b and in Fig. 4b is the same

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Fig. 3e

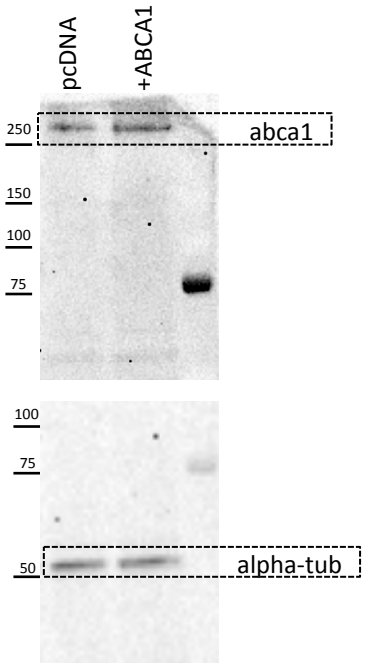


Fig. 3f

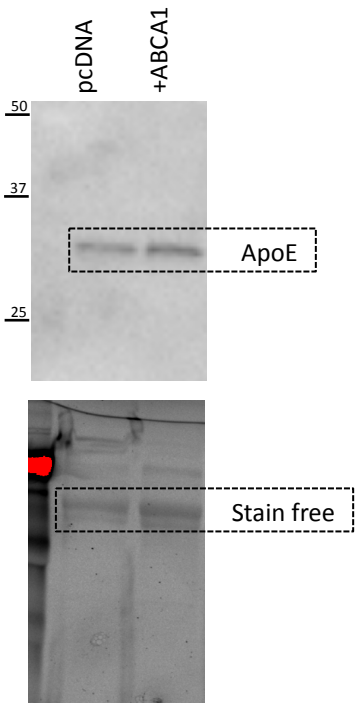


Fig. 5e

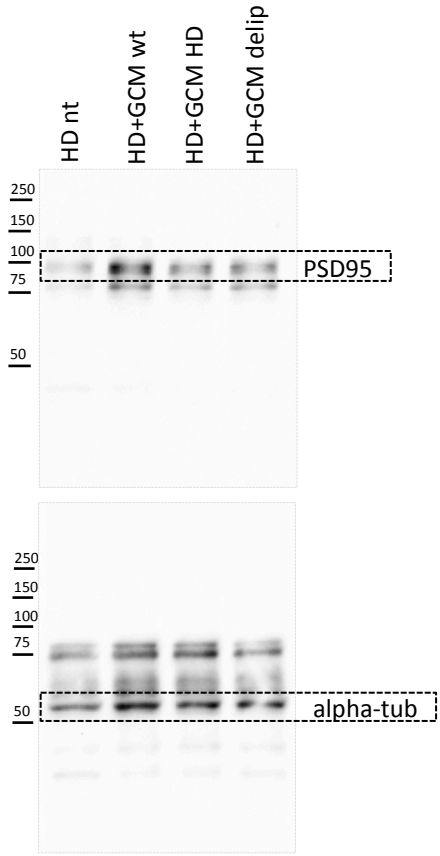
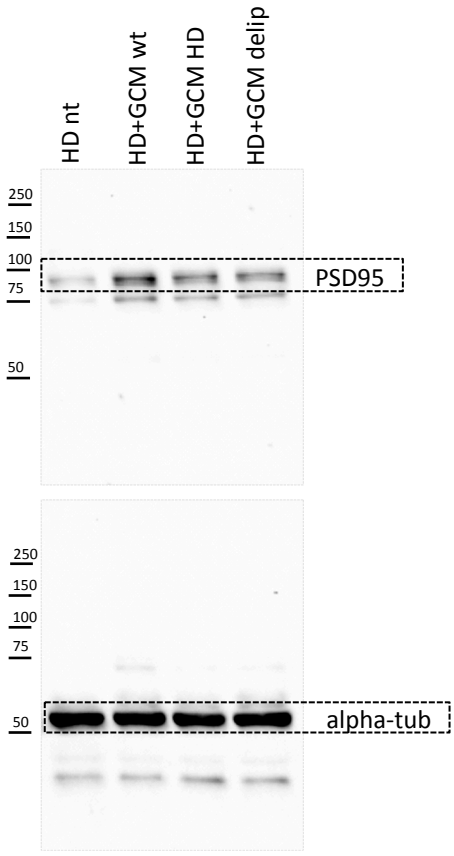


Fig. 5f



Supplementary Figure 9. (**a–f**) Full-length pictures of the plots presented in the main figures. Dashed boxes indicate the bands shown in the figures.