Supplementary Information

Metabolism of oxysterols derived from non-enzymatic oxidation of 7-dehydrocholesterol in cells

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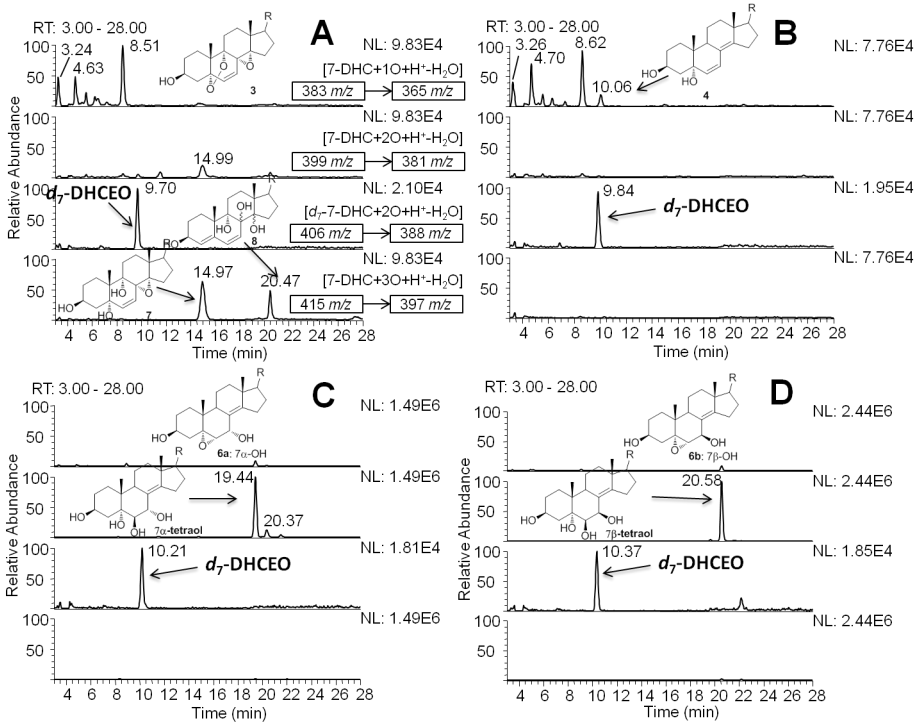

Figure S1. NP-HPLC-APCI-MS-MS (Silica 4.6 mm × 250 mm column; 5 μm; 1.0 mL/min; elution solvent: 2-propanol/hexanes = 10/90 (0 – 15 min), 19/81 (16 – 26 min), 10/90 (27 – 35 min)) analysis of the oxysterols in Neuro2a cells that were incubated in the presence of (**A**) 1 μM of **3**, (**B**) 5 μM of **4**, (**C**) 5 μM of **6a**, and (**D**) 5 μM **6b** for 24 hours in DMEM medium that was supplemented with regular FBS. The structures of the starting oxysterols in each experiment were shown in the corresponding chromatogram. The speculated structures of the metabolites of compounds **3**, **6a**, and **6b** were shown in the Figure based on the retention time (polarity) and MS characteristics of each peak. A known amount of d_7-DHCEO was added to each sample as an internal standard so that retention time and peak intensities of the peaks can be compared between each run. The parent ion and the MS/MS transition were marked in each panel of S1A, and the same MS/MS transitions were monitored in every experiment.

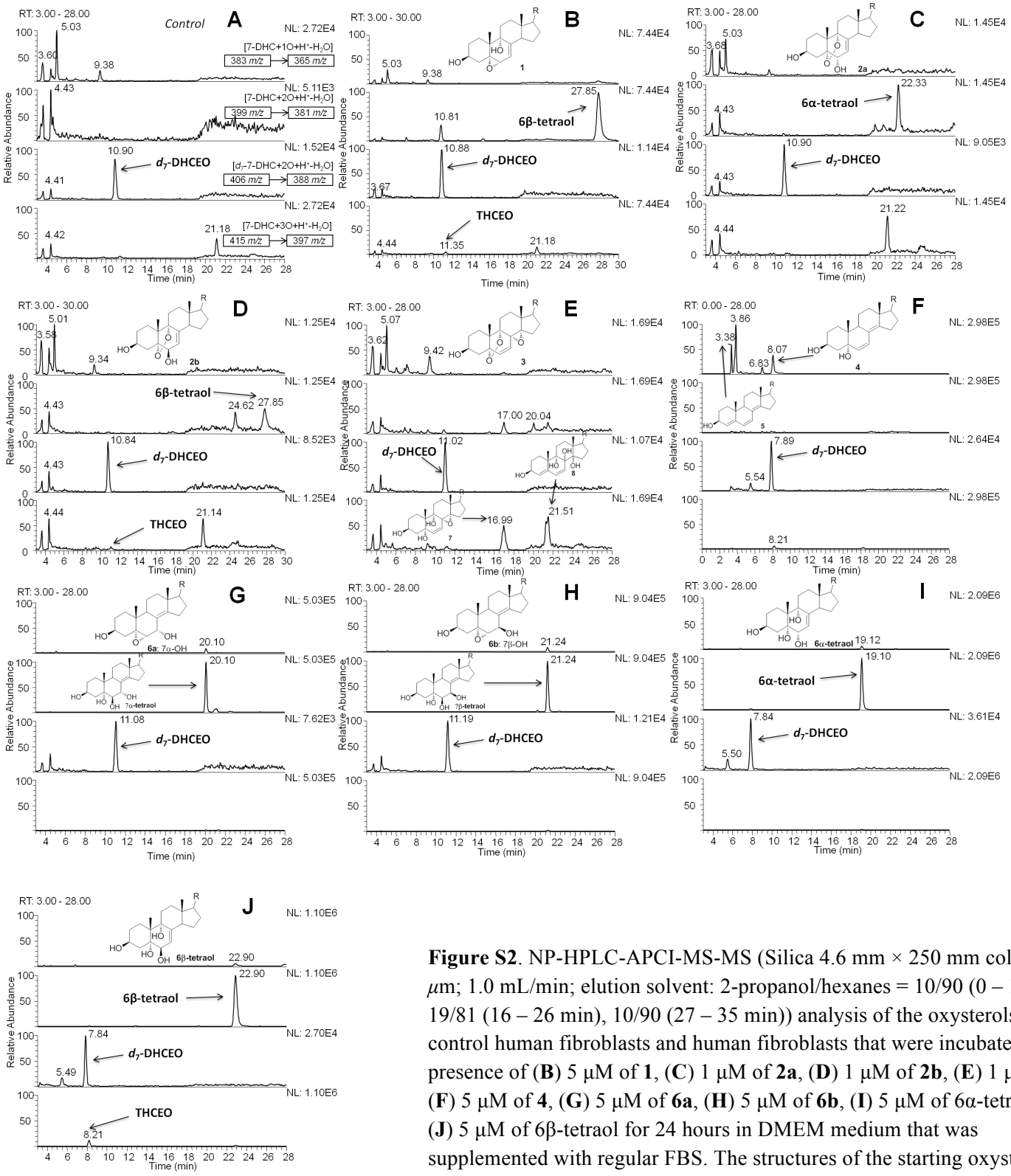

Figure S2. NP-HPLC-APCI-MS-MS (Silica 4.6 mm × 250 mm column; 5 μm; 1.0 mL/min; elution solvent: 2-propanol/hexanes = 10/90 (0 – 15 min), 19/81 (16 – 26 min), 10/90 (27 – 35 min)) analysis of the oxysterols in (**A**) control human fibroblasts and human fibroblasts that were incubated in the presence of (**B**) 5 μM of **1**, (**C**) 1 μM of **2a**, (**D**) 1 μM of **2b**, (**E**) 1 μM of **3**, (**F**) 5 μM of **4**, (**G**) 5 μM of **6a**, (**H**) 5 μM of **6b**, (**I**) 5 μM of 6α-tetraol, and (**J**) 5 μM of 6β-tetraol for 24 hours in DMEM medium that was supplemented with regular FBS. The structures of the starting oxysterols in each experiment were shown in the corresponding chromatogram. The speculated structures of the metabolites of compounds **3**, **6a**, and **6b** were shown in the Figure based on the retention time (polarity) and MS characteristics of each peak. A known amount of d_7-DHCEO was added to each sample as an internal standard so that retention time and peak intensities of the peaks can be compared between each run. The parent ion and the MS/MS transition were marked in each panel of S2A, and the same MS/MS transitions were monitored in every experiment.