



Supplementary Figure 5. Absence of sialic acid contamination in human ES cells cultured in defined conditions. Human ES cells grown under different conditions were analyzed by LC-MS for the sialic acids Neu5Ac and Neu5Gc. **(a)** The common form, Neu5Ac was found abundantly expressed on cells regardless of culture conditions, with peak intensities in the range of $1-3 \times 10^8$ (traces autoscaled). **(b)** The non-human Neu5Gc was found on human ES cells grown under standard conditions (peak intensity off scale at 5×10^5), it was reduced but detectable in partially humanized conditions with peak intensity of 4×10^4 , and no detectable Neu5Gc was found on cells grown in fully humanized conditions. Growth conditions key: Standard Conditions = mouse embryonic fibroblast conditioned media on Matrigel (orange). Partially Humanized = TeSR1 medium on Matrigel (green), Fully Humanized = TeSR1 medium on human matrix components (blue).