

## **Supplemental data**

### **Ceramide metabolism regulated by sphingomyelin synthase 2 is associated with acquisition of chemoresistance via exosomes in human leukemia cells**

Makoto Taniguchi, Shingo Nagaya, Kohei Yuyama, Ai Kotani, Yasuyuki Igarashi, and Toshiro Okazaki.

## **Figure Legends**

**Figure S1** Full-length blots of Figure 1B.

**Figure S2** Glucosylceramide synthase (GCS) activity and measurement of hexacylceramide (HexCer).

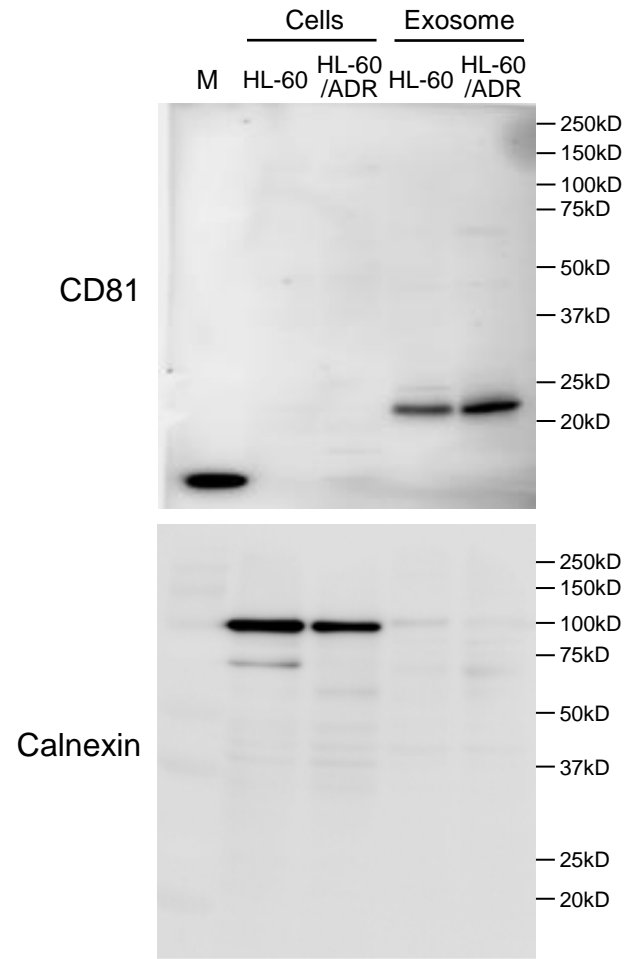
(A) Sphingomyelin synthase (SMS) and GCS activities were measured by using fluorescent C<sub>6</sub>-NBD-ceramide and thin layer chromatography method described in “Materials and Methods.” Data of SMS activity was in Figure 3A. (B) HexCer levels were measured by LC-MS/MS. The values show the mean  $\pm$  SD (n = 3). \* $P$  < 0.05, \*\* $P$  < 0.005.

**Figure S3** HL-60/ADR cells exhibited doxorubicin (Dox) resistance. Cells were treated with 0.25  $\mu$ M of Dox for 12 hours. (A) Viable cells were counted after trypan blue exclusion and indicated as percent of vehicle treatment (0  $\mu$ M) (n = 4). (B) Apoptotic cells were detected by FITC-annexin V staining and

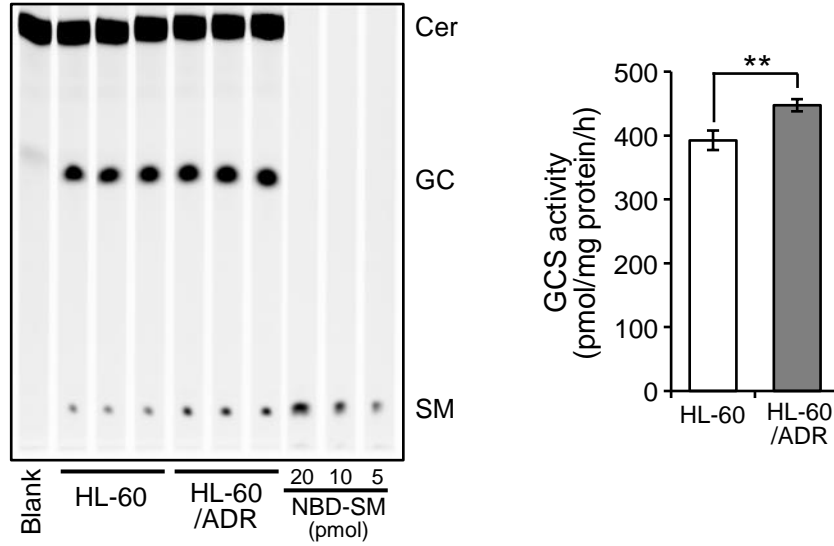
flowcytometry (n = 3). (C) Western blot analysis of caspase-3 and actin. (D) Dox elimination was measured by flowcytometry (n = 3). The values show the mean  $\pm$  SD.

**Figure S4** HL-60 exosome did not confer chemoresistance on HL-60 cells. HL-60 cells were pre-treated with HL-60 exosomes (HL-60 exo) for 12 h and then treated with doxorubicin (Dox) for 12h. Viable cells were counted after trypan blue exclusion and indicated as percent of vehicle treatment (n = 3). The values show the mean  $\pm$  SD. **\*\* $P < 0.005$ .**

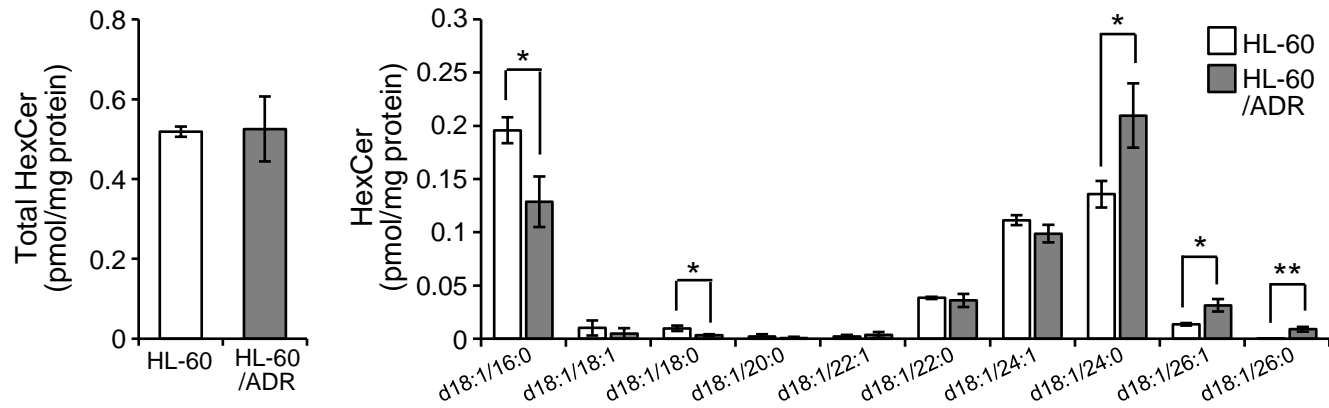
# Supplemental Figure S1



**A**



**B**



# Supplemental Figure S3

