

Supplementary material

Supplementary Table 1. Quantitation of cytoplasmic and secreted clusterin staining in human neuroblastomas*

Clusterin expression	No. of tumors†		No. of tumors†	
	Differentiated	Undifferentiated	Localized	Metastatic
Positive	17	16	17	10
Negative	6	47	9	31

*Expression of the secreted or cytoplasmic form of clusterin was statistically significantly associated with differentiated or differentiating and localized neuroblastomas. The number of metastatic or localized or the number of differentiated or undifferentiated samples used in the analysis was less than the total of 97 neuroblastoma samples in the overall study because the relevant clinical information was not available for 23 patients and 10 other patients were excluded because they had been treated with chemotherapy before surgery.

† $P < .001$, for the difference between differentiated and undifferentiated tumors and between localized and metastatic tumors. Statistical significance was calculated by use of the chi-square test with Yates' correction. All statistical tests were two-sided.

P-Value Threshold: 1E-4 + -

Outlier Rank Threshold: 50 + -

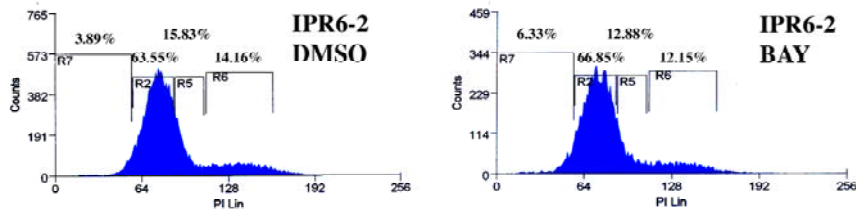
	Normal vs. Normal	Cancer vs. Normal	Cancer vs. Cancer	Histologic Subtype	Tumor Stage	Molecular Alteration	Misc	Tumor Grade	Outlier
Adrenal Gland	1								
Brain	2	4	1 2				1		
Ciliary Ganglion	1								
Dorsal Root Ganglia	1								
Dorsal Root Ganglion	1								
Spinal Cord	2								
Testis	2								
Tonsil	1								
Trigeminal Ganglion	1								
Leukemia			1 1	2 1		4 2	1		3
Lung		3	2 1						
Lymphoma			2 3				1		1
Multi-cancer	1	4 2							
Ovarian		1							
Prostate	5	1 3					1 6	1	
Sarcoma		6 6			1		2		
Bladder	1				1				
CellLine						1	6 3		
Normal							1 1		
Parathyroid							1		
Renal			1				1		
Blood	1								
Bone Marrow	1								
Breast		1 1				2	2 4		
Colon		1 1							
Early Progenitor Cells	1								
Head-Neck		1							
Kidney	1								
Liver		1 1							
Melanoma		1							
Neuroblastoma							2		
Others		1							
Placenta	2								
Seminoma		1							
Skeletal Muscle	1								
Skin	1								
White Blood Cells	1								

Supplementary Figure 1. Clusterin gene expression in primary tumors.

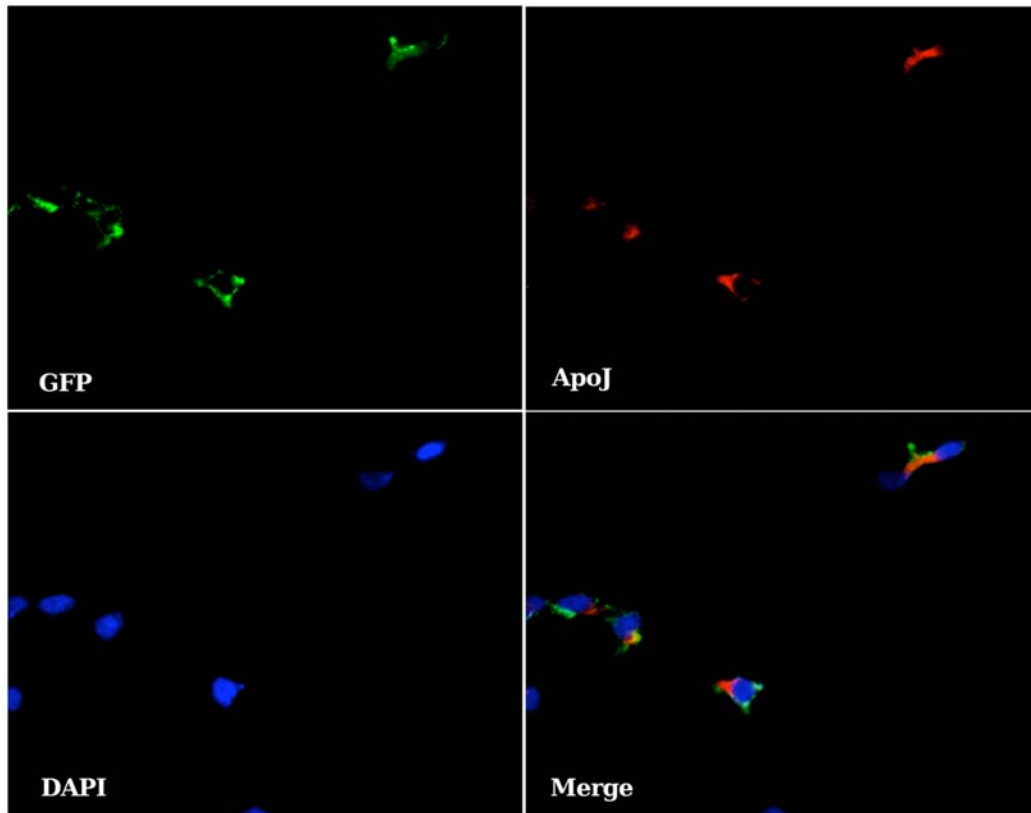
Expression data for clusterin mRNA was obtained from Affymetrix experiments available on the Oncomine web site (www.Oncomine.org).

Shades of blue color indicate underexpression, whereas shades of red color indicate overexpression. Dark and light colors are proportional to the

statistical significance of the differences in mRNA expression in the assays. Numbers indicate how many independent experiments showed a statistically significant difference in each tissue. Outliers are indicated on the top of the figure; + and –, respectively, indicate buttons used on the web page to change the P or outlier thresholds limits in a positive or negative direction.



Supplementary Figure 2. Bay 11-7082 and IPR6-2 cell apoptosis and proliferation. IPR6-2 cells were incubated with 5 μ M Bay 11-7082 (BAY), a specific NF- κ B inhibitor, or the solvent, dimethyl sulfoxide (DMSO), for 24 hours and stained with propidium iodide (PI) for flow cytometry analysis. From left to right, values indicate percentages of cells with hypodiploid, G₁, S, and G₂M amounts of DNA. A representative experiment of two with similar results is shown. Lin = linear scale



Supplementary Figure 3. Cytoplasmic localization of the overexpressed clusterin protein in neuroblastoma cells. LA-N-5 cells were infected with a bicistronic retroviral vector MIGR1/ApoJ that contains full-length clusterin and green fluorescent protein (GFP) cDNAs. Cytoplasmic clusterin (ApoJ) = red; 4',6-diamidino-2-phenylindole (DAPI) to visualize the nuclei = blue; green fluorescent protein to show infected cells = green. Merge shows clusterin, green fluorescent protein, and DAPI staining overlapped.