Figure Legends

Supplemental Fig. 1 Magenta-Green Version of Figure 1: Subcellular distribution and characterization of immunostaining levels. Representative double immune-stained images of neuronal cultures using anti-calbindin (A) and anti- β -tubulin (B) antibodies. C, merged image. Note that calbindin staining was heterogeneous while β -tubulin staining level was relatively uniform in all cells. Arrow, a neuron stained with anti-calbindin antibody showed brighter spots in the nuclei, suggesting differential staining levels within different sub-cellular compartments. Three cells with high, intermediate and low levels were circled with dashed line, and the normalized calbindin staining irradiance values was indicated on top of each cell in A.

Supplemental Fig. 2 Magenta-Green version of Figure 3: Distribution patterns of calretinin and calbindin in P6 mouse cochlea. Two cochlear sections in close proximity were stained with anti-calretinin (**A-E**) and anti-calbindin antibodies (**F-J**) respectively. Both anti-calretinin and anti-calbindin antibody staining showed heterogeneous patterns in the spiral ganglion. HC, hair cell. SGN, spiral ganglion. SL, spiral limbus. **A**, Low magnification image of cochlear sections double labeled with anti-β-tubulin (magenta) and anti-calretinin (green) antibodies **B-E**, High magnification images of middle (**B-C**) and basal (**D-E**) neuronal regions enclosed by dotted line in **A**. **F**, low magnification image of a cochlear section showing double labeling of anti-calbindin (green) and anti-βtubulin (magenta) antibodies. Prominent calbindin staining was also observed in the spiral limbus **G-J**, High magnification image of the middle (**G**, **H**) and basal (**I,J**) regions as squared in **F**. Scale bar in **F** applies to **A**, **F**. Scale bar in **J** applied to **B-E**, **G-J**. Supplemental Fig. 3 Magenta-Green version of Figure 4: Calretinin and calbindin exhibited differential distribution patterns in murine spiral ganglion. A-C, The midcochlear region of a whole-mount preparation of P7 mouse spiral ganglion labeled with mouse anti-calretinin (A) and rabbit anti-calbindin (B) antibodies. C, The merged image of A and B. Most cells were labeled by mainly calretinin (triangle), mainly calbindin (arrowhead), or a low level of both (arrow). Only a few neurons possessed a high level of both staining (asterisk). Scale bar in C (20µm) applies to A-C. D, Superimposed image of in vitro culture shows heterogeneous distribution pattern of calretinin and calbindin that range from mainly calretinin-staining cells (magenta) to mainly calbindin staining cells (green) similar to A-C. Example cells were labeled according to the four categories in G. Frequency histograms of normalized calretinin staining irradiance (E) and normalized calbindin staining irradiance (F) were constructed from measurements of the same single experiment as shown in D (total number of measurements = 320). Both histograms were composed of multiple populations with distinct staining irradiance levels which could be fit by the sum of three Gaussians with discrete means. The vertical dashed lines (E and F, respectively) delineate the mid points between high and medium means of Gaussian fits of normalized calretinin (0.32) and normalized calbindin (0.19) staining irradiance used in G inset. G, scatter plot of normalized calretinin and calbindin staining measurements in each neuron. Inset, Cells were divided into four categories to highlight relative irradiance patterns based on both x and y cutoffs (dotted lines in E-F).



Supp. Fig. 1 Magenta-Green Version of Figure 1



Supp. Fig. 2 Magenta-Green version of Figure 3



Supp. Fig. 3 Magenta-Green version of Figure 4