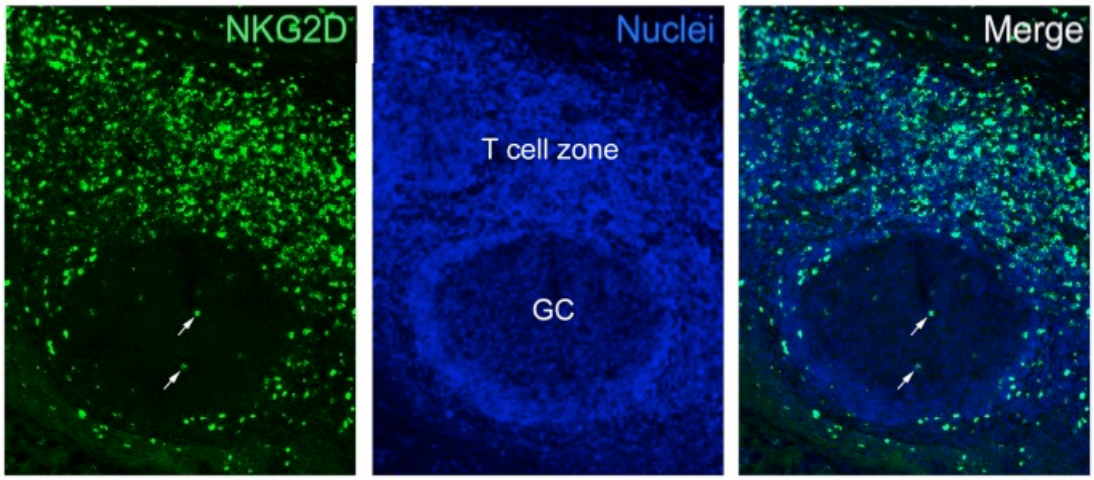
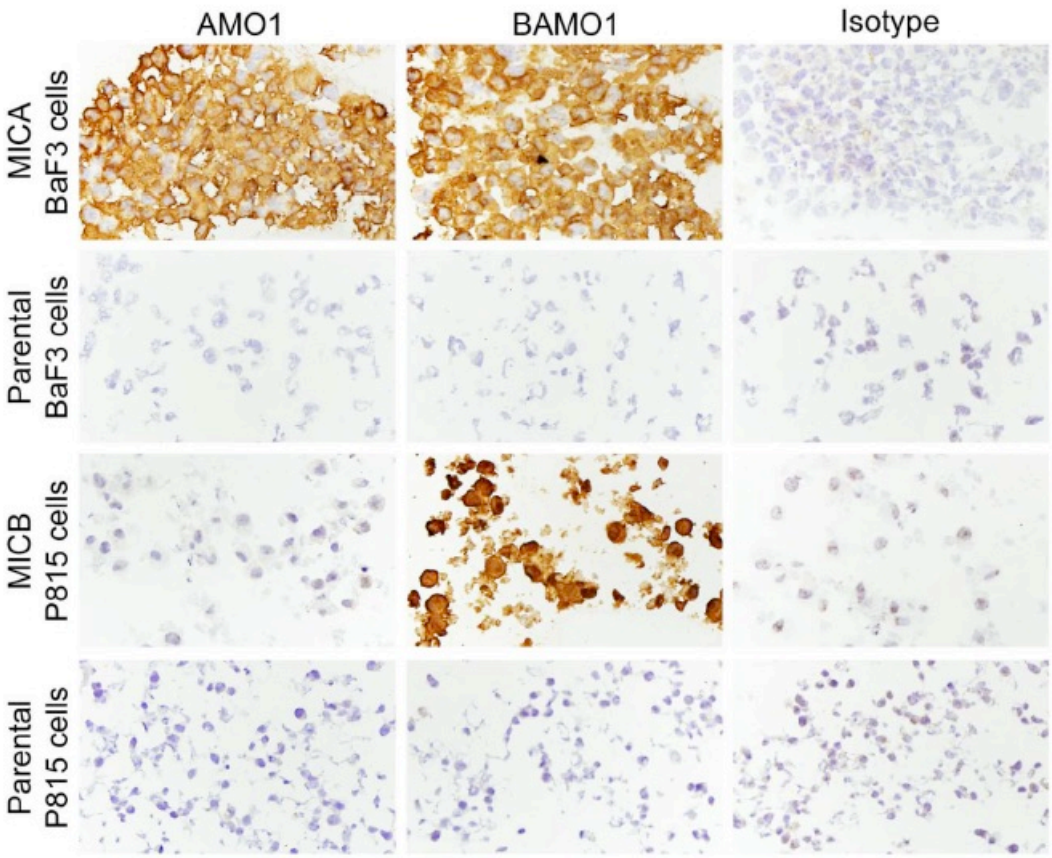
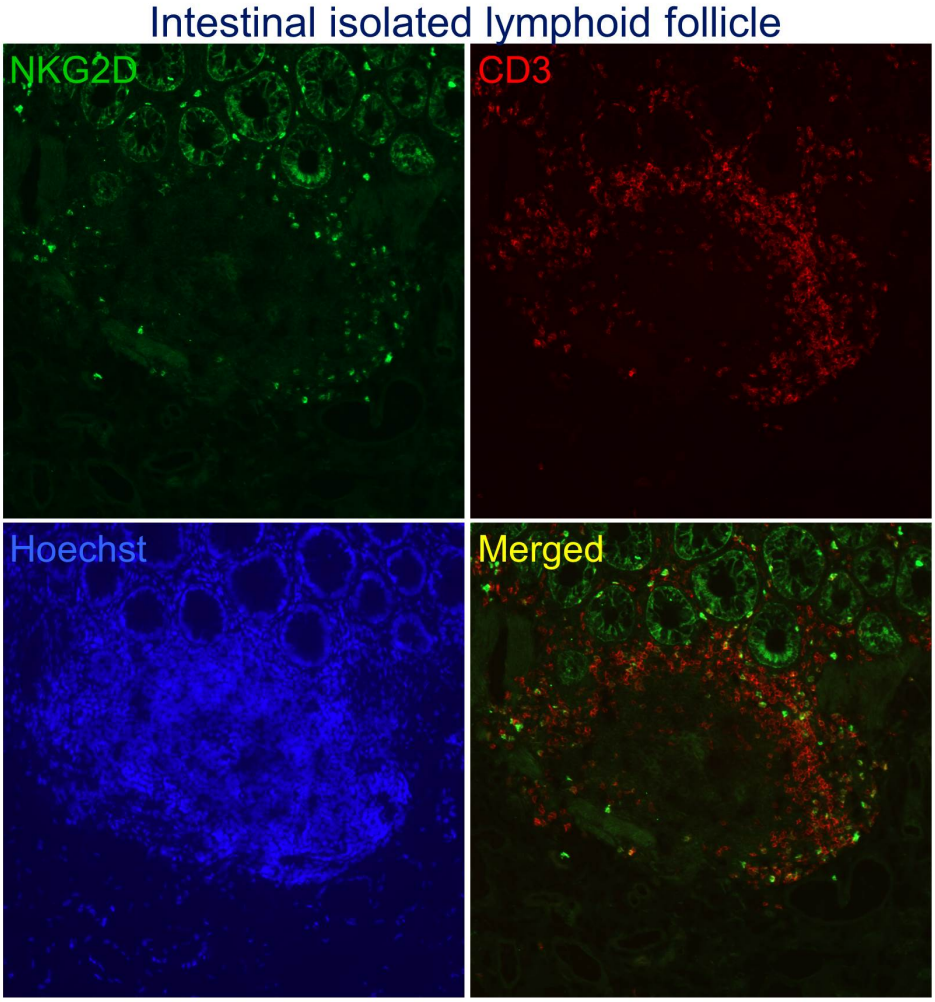
Supplementary Figure 1. Anti-NKG2D immunofluorescence on positive control tissue. Cryo-sections of human tonsil were stained with anti-NKG2D antibody MAB139. NKG2D-positive cells were visualized with Alexa-488 fluorochrome (green) and nuclei were counter-stained with Hoechst (blue). $NKG2D^+$ cells predominantly locate in the T cell zone surrounding the B cell follicle with a few positive cells also observed in the germinal center (GC) of the follicle (marked by white arrows).



Supplementary Figure 2. Validation of anti-MICA and MICB antibodies in IHC on positive and negative control cells. Cryo-sections of MICA- and MICB-transfected cell lines and corresponding parental cell lines (BaF3 and P815 cells, respectively) were immunostained with anti-MICA (AMO1), anti-MICA/B (BAMO1), and mouse IgG1 isotype-matched control antibodies. Antibody binding was visualized by diaminobenzidine (DAB, brown color) and nuclei were counterstained with hematoxylin (blue color). MICA-transfected cells were stained by both AMO1 and BAMO1 antibodies, whereas MICB-transfected cells were stained by BAMO1, but not by AMO1. Parental cells and isotype-matched control Ig stained cells were negative.

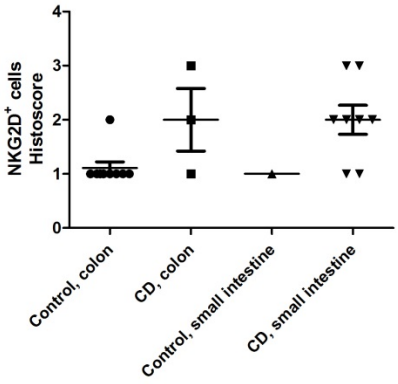


Supplementary Figure 3. Anti-NKG2D immunofluorescence on isolated lymphoid follicles. Double immunofluorescence staining of cryo-sections of normal control intestine with anti-NKG2D antibody MAB139 (green) and anti-CD8 antibody (CD3). Nuclei were counter-stained with Hoechst (blue). $NKG2D^{+}$ cells are located in the T cell zone of an isolated lymphoid follicle and in the mucosal lamina propria.

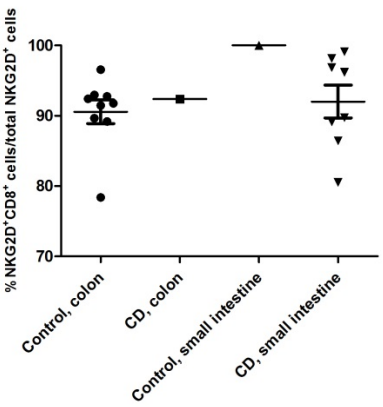


Supplementary Figure 4. Quantification of NKG2D$^+$ cells in colon and small intestine from CD patients and normal controls. (A) Semi-quantitative scoring of NKG2D$^+$ cells on immunofluorescence staining of colon and small intestine from CD patients and normal controls. Quantification of NKG2D$^+$CD8$^+$ cells expressed as % double-positive cells of total NKG2D$^+$ cells (B) or total CD8$^+$ cells (C).

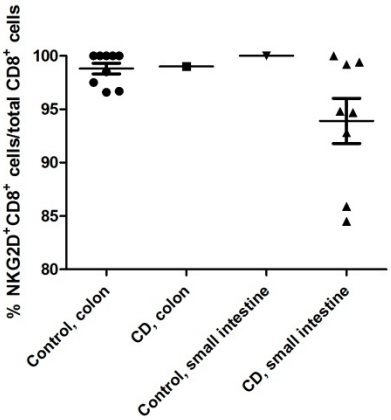
A



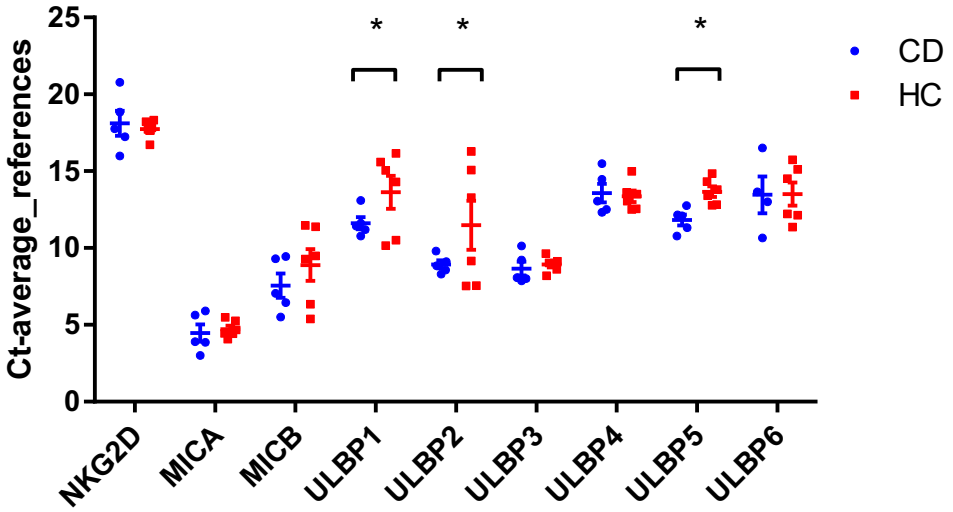
B



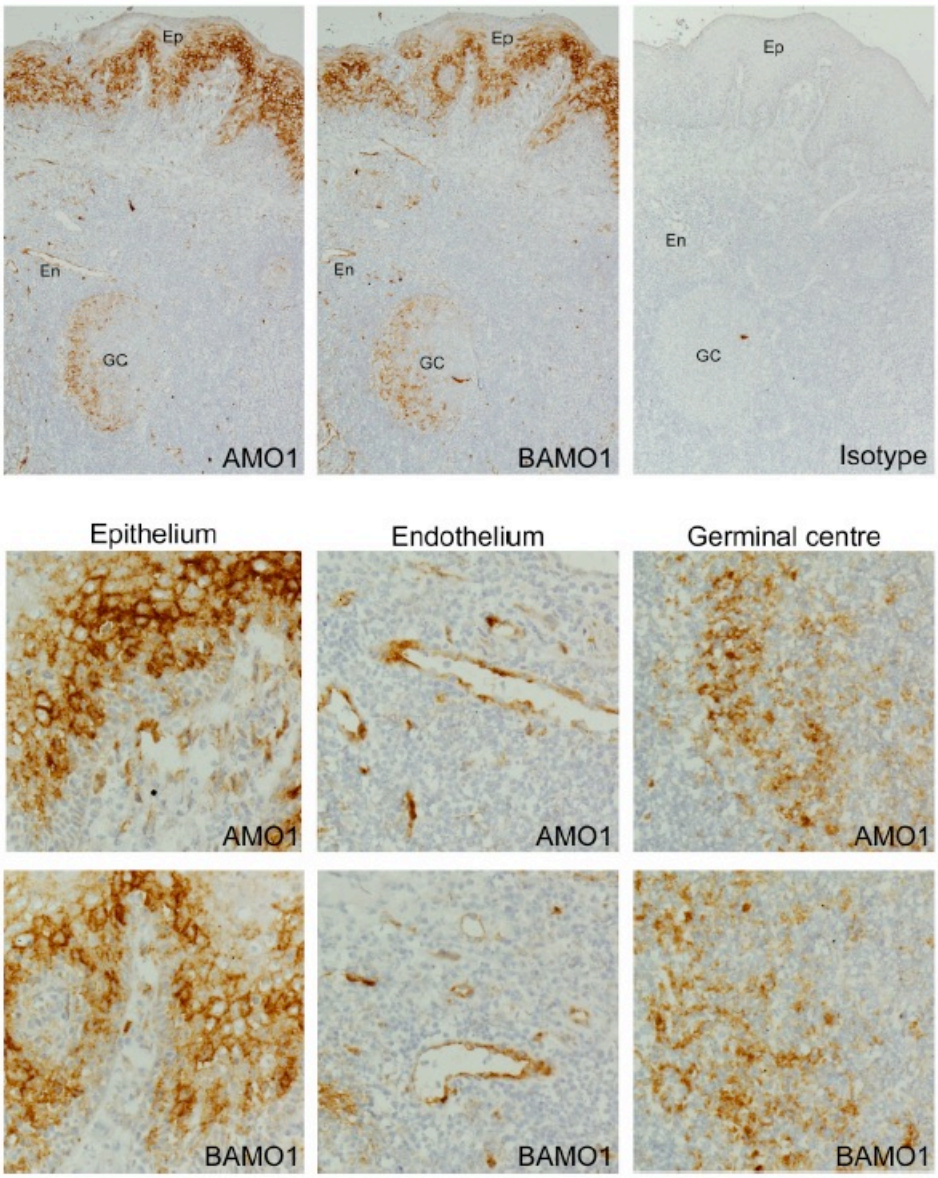
C



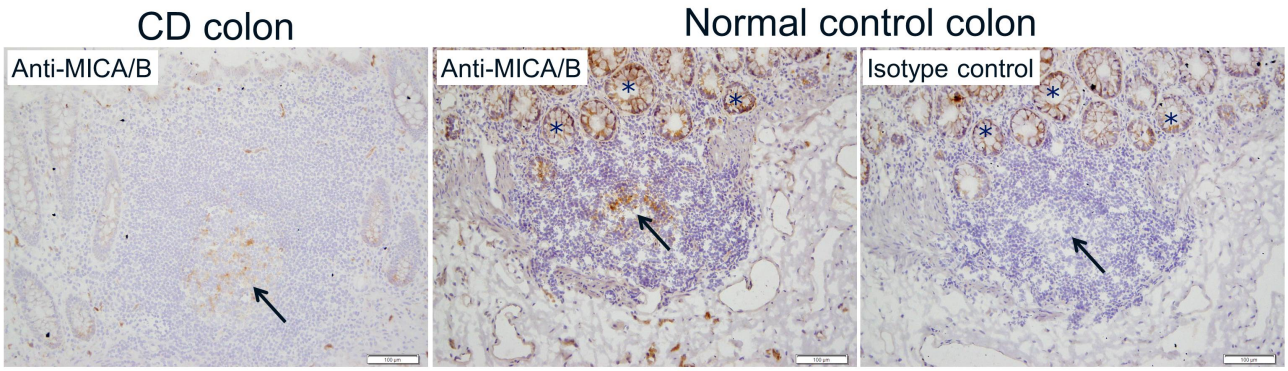
Supplementary Figure 5. mRNA levels of NKG2D and ligands in CD and healthy control. qPCR results (Ct values with housekeeping gene averages subtracted) of NKG2D and NKG2D ligands mRNA level in CD and healthy control (HC) colon cultured *ex vivo* for 24 hours. n=6 patients in each group. T-test, * = $P<0.05$, mean and SEM.



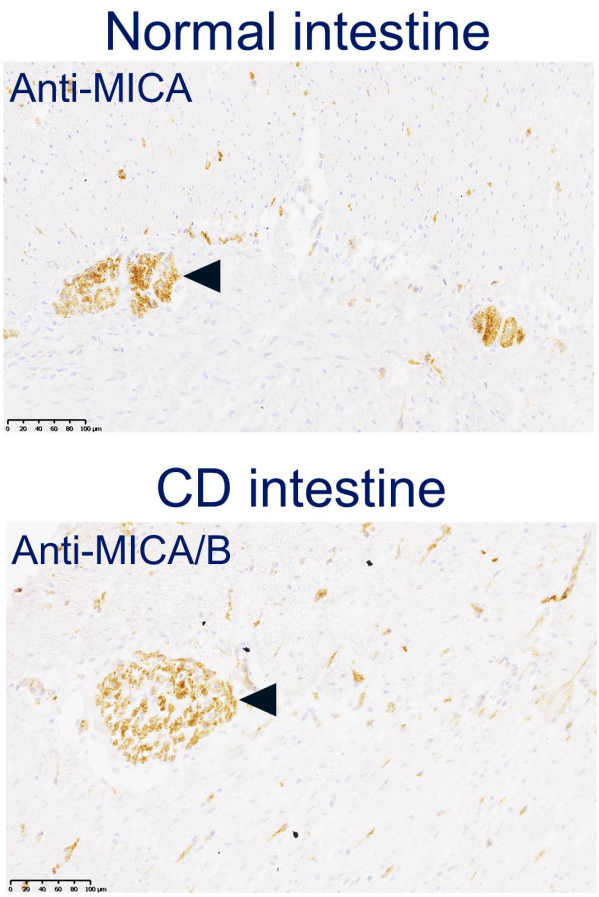
Supplementary Figure 6. Validation of anti-MICA and anti-MICA/B antibodies for IHC on tonsil. Cryo-sections of human tonsil were immunostained with anti-MICA (AMO1), anti-MICA/B (BAMO1), and isotype-matched control mouse IgG1 (Isotype) antibodies. Antibody binding was visualized by diaminobenzidine (DAB, brown color) and nuclei were counterstained with hematoxylin (blue color). Epithelium (Ep), endothelium (En), and germinal center (GC) are indicated. Similar staining pattern of these three cell compartments was observed with AMO1 and BAMO1. Sections stained with isotype-matched control antibody were negative (not shown).



Supplementary Figure 7. IHC for MICA/B on isolated lymphoid follicle. Immunohistochemistry with anti-MICA/B antibody (BAMO1) and isotype-matched control IgG1 as indicated. Images show MICA/B (brown DAB-staining) in germinal centers (arrows) of isolated lymphoid follicles in CD and normal control colon. Asterisks indicate unspecific staining of mucosal epithelium. Scale bar: 100 µm.



Supplementary Figure 8. IHC for MICA/B on myenteric plexus. Immunohistochemistry with anti-MICA (AMO1) and anti-MICA/B (BAMO1) antibodies and isotype-matched control IgG1 as indicated. Images show MICA/B (brown DAB-staining) in myenteric plexux (arrowheads) in colon of normal control (upper panel) and CD (lower panel). Scale bar: 100 µm.



Supplementary tables

Supplementary table 1. Flow cytometry Ab panel and gating scheme. Lymphocytes were pre-gated based on their characteristic forward angle and side angle light scattering properties. When gating on non-lymphocytes, the lymphocytes population was excluded.

NKG2D analysis	Antibody marker	CD4 T cells	CD8 T cells	CD56+ T cells	NK cells	γδ T cells	B cells
Surface	CD45	+	+	+	+	+	+
	CD3	+	+	+	-	+	-
	CD4	+	-				
	CD8a	-	+				
	CD31						
	CD56	-	-	+	+		
	γδ TCR			-		+	
	CD326						
	CD14						-
	CD19						+
	CD146						

Ligand analysis	Antibody marker	CD4 T cells	Endothelial cells	Epithelial T cells	Monocytes/ Macrophages/ Dendritic cells	B cells
Surface	CD45	+	-	-	+	+
	CD3	+				-
	CD4	+				
	CD8a	-				
	CD31		+		-	
	CD56					
	γδ TCR					
	CD326			+	-	
	CD14		-		+	
	CD19					+
	CD146		+			

Supplementary table 2. Mass cytometry Ab panel and gating scheme.

	Antibody marker	Metal	CD4 T cells	CD8 T cells	CD56+ T cells	NK cells	γδ T cells	MAIT cells	B cells	tissue cells
Intracellular	cPARP	173Yb	-	-	-	-	-	-	-	-
Surface	CD45	115In	+	+	+	+	+	+	+	-
	CD3	170Er	+	+	+	-	+	+	-	
	CD4	145Nd	+	-						
	CD8a	146Nd	-	+						
	CD7	150Nd				+				
	CD56	176Yb			+	+				
	γδ TCR	148Nd					+			
	Va7.2	164Dy						+		
	CD161	158Gd						+		
	CD19	142Nd							+	
	HLA-DR	152Sm								+/-
	NKG2D	154Sm								
	CD69	162Dy								
	PD1	147Sm								
	MICA	153Eu								