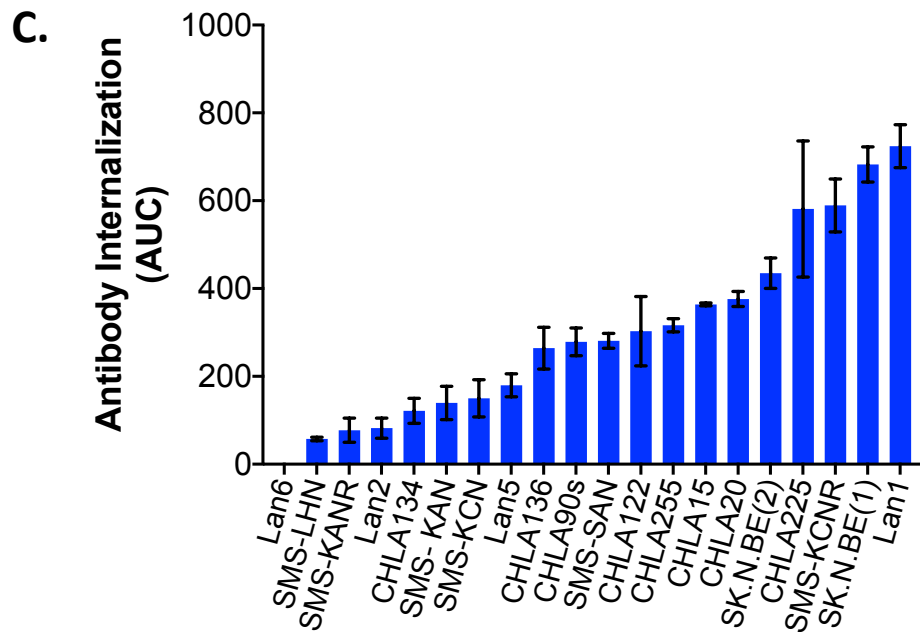
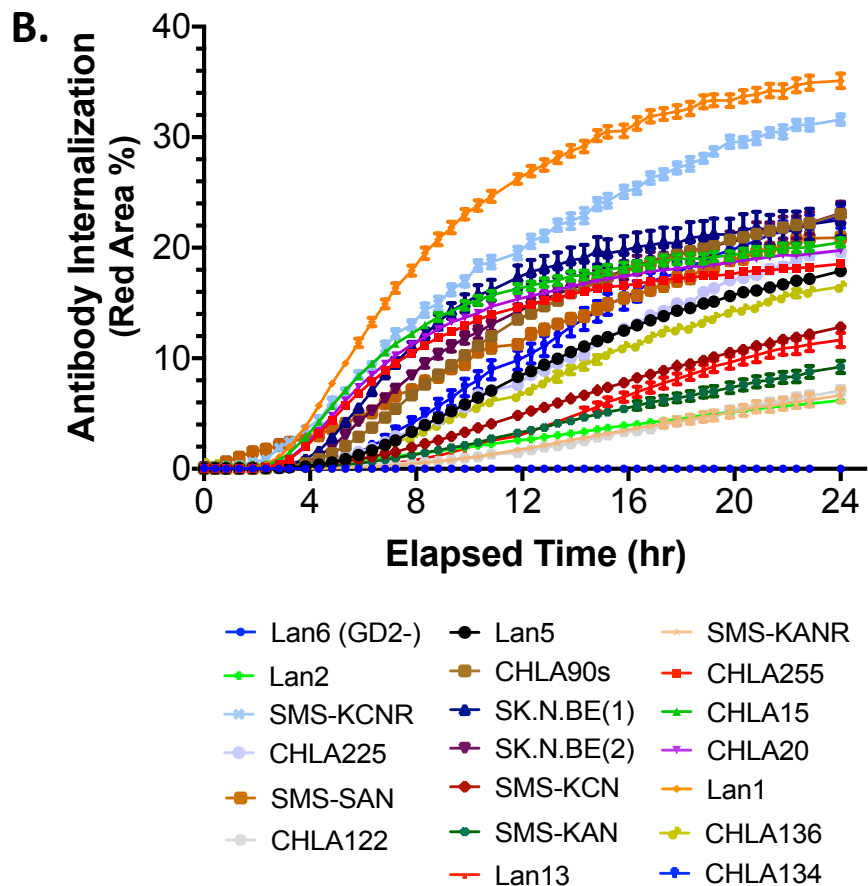
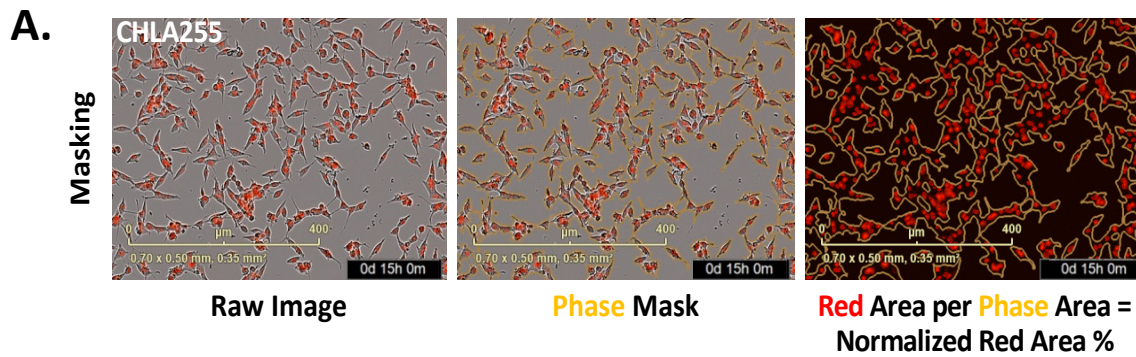


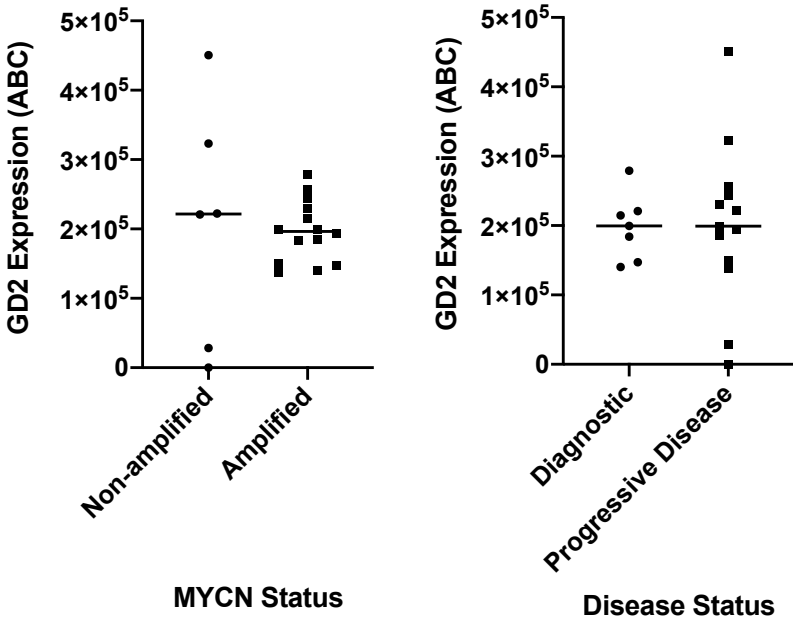
**Supplementary Table 1.** Descriptive characteristics for each NBL cell line tested along with values for GD2 expression (mean ABC  $\pm$ SEM), neutrophil-mediated ADCC (Mean % cytotoxicity  $\pm$ SEM), and 24 hour anti-GD2 antibody (14G2a-pHrodo) internalization AUC (Normalized Mean  $\pm$ SEM).

NBL Cell Line	MYC-N (A = amplified; NA = Non-amplified)	Disease Status at Cell Origin (Dx = Diagnostic; PD = Progressive Disease)	Raw Internalization 24 hour AUC (Mean +/- SEM)		Normalized Internalization 24 hour AUC (Mean +/- SEM)		Neutrophil ADCC (% cytotoxicity at 6 hours)		GD2 Expression (ABC = Antibodies bound per cell)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lan6	NA	PD	0.9	0.6	1.2	0.4	-7.8	2.2	0	0
CHLA90s	NA	PD	278.5	31.5	345.1	51.8	22.8	2	222188	30255
CHLA15	NA	DX	363.8	3.3	448.2	39.1	51.7	2.6	221006	17002
CHLA20	NA	PD	376.1	17.1	475.4	28.9	19.5	2.8	323203	11378
CHLA255	NA	PD	316.4	14.9	384.6	27.3	65	2.9	450462	40445
SMS-LHN	NA	PD	57.4	4.1	123.4	22.1	-	-	28491	647
Lan1	A	PD	724.1	49	1000	0	22.7	2.6	256982	18251
Lan5	A	PD	179.6	26.3	226.8	40.9	45.7	3.4	230353	18020
SMS-KAN	A	DX	139.4	38	160.2	53.4	72	2.6	199649	38248
SMS-KANR	A	PD	77.4	27.7	89.8	38.1	61.1	1.3	137482	25928
SMS-KCN	A	DX	150.1	42.4	174.5	63.3	43.6	2.2	147277	3294
SMS-KCNR	A	PD	589.2	60.3	700.5	66.3	57.6	2.1	184982	12534
SK.N.BE(1)	A	DX	682.5	39.9	821.3	24.4	-	-	184182	25863
SK.N.BE(2)	A	PD	434.9	34.7	532.3	62.9	-	-	244002	16053
CHLA122	A	DX	302.7	78.9	354.7	87	-	-	279093	3500
CHLA225	A	PD	581.1	154.9	681.2	160.9	-	-	199101	14112
SMS-SAN	A	DX	280.9	17	418.9	59.3	-	-	214685	5832
Lan2	A	DX	82	23	101.2	20.8	-	-	140445	4497
CHLA136	A	PD	264.2	47.6	301.8	66.6	31.9	2.2	150110	4274
CHLA134	A	PD	121.5	28.4	183.2	31.6	-	-	194079	3100

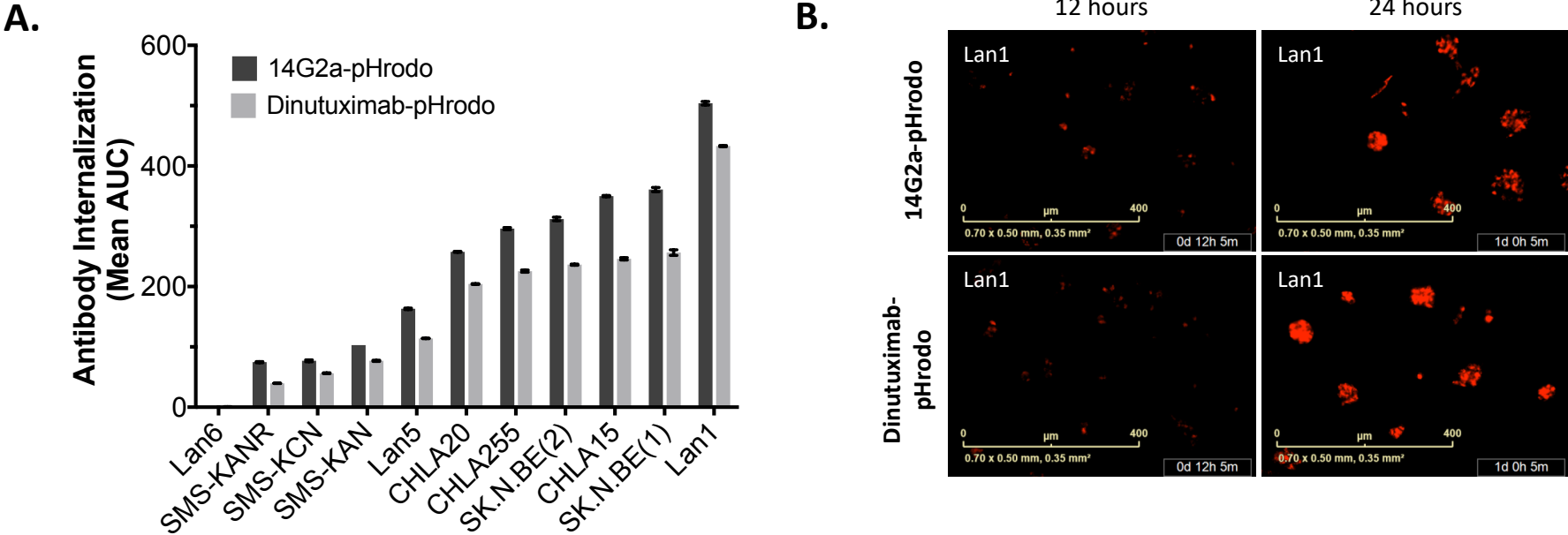


**Supplementary Figure 1. A.** Representative phase and red fluorescent images captured by Incucyte S3 Live-cell imaging system demonstrating masking of CHLA255 cells incubated with 14G2a-pHrodo [10  $\mu\text{g/ml}$ ] (image at 15 hours post incubation). For every timepoint, raw image (left panel) is analyzed to determine the cell confluence per well (%) by phase masking (middle panel) and red fluorescent area per well by red fluorescent masking after background subtraction (right panel). Red area percent is then calculated as [red area per well / phase area per well] x 100 (%). This normalization accounts for variation in cell plating densities that may contribute to differences in internalized red signal per well and allows for across replicate and across cell line comparisons. **B.** Real-time measurement of 14G2a-pHrodo internalization as measured by red fluorescent area (%) over 24 hours for all NBL cell lines tested. Time course of 14G2a-pHrodo [10  $\mu\text{g/ml}$ ] internalization by all 20 human neuroblastoma cell lines as measured by normalized red area (mean  $\pm$  SD %) over 24 hours (representative curve for each cell line shown; each cell line with minimum of 3 replicates). **C.** Mean ( $\pm$ SEM) AUC of 14G2a antibody internalization calculated from raw replicate values without normalization for all 20 NBL cell lines

Supplementary Figure 2



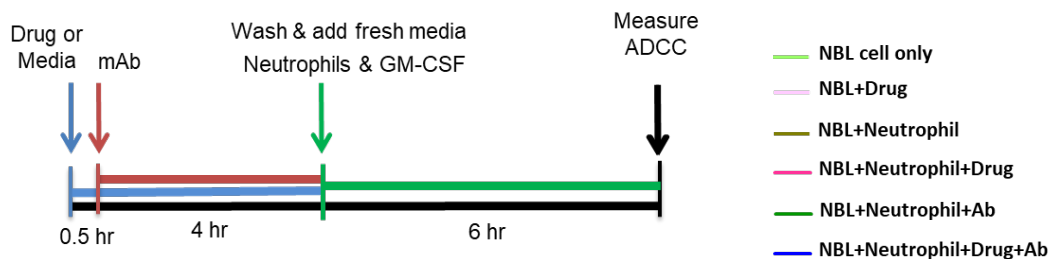
**Supplementary Figure 2. Neither disease status nor MYCN amplification status at time of cell line initiation correlates with GD2 expression of NBL cell lines.** Mean GD2 expression (ABC) for all tested NBL cell lines sorted by MYCN status (amplified/Amp or non-amplified/NA; left panel) or disease status at time of cell line initiation (Diagnostic/Dx or Progressive Disease/PD; right panel) (p value non-significant).



**Supplementary Figure 3. Internalization of two different anti-GD2 antibodies, 14G2a and dinutuximab, correlates strongly across human NBL cell lines. A.** Mean ( $\pm$ SEM) 24-hour AUC of 14G2a [10  $\mu$ g/ml] antibody internalization (black bars) or dinutuximab [10  $\mu$ g/ml] (grey bars) for 10 NBL cell lines. **B.** Representative red fluorescent images of Lan1 cells incubated with either pHrodo labeled 14G2a (top) or pHrodo labeled dinutuximab (bottom) at 12 and 24 hours. All images were exported with the same red fluorescent settings and are unmodified.

# Supplementary Fig 4

**A.**



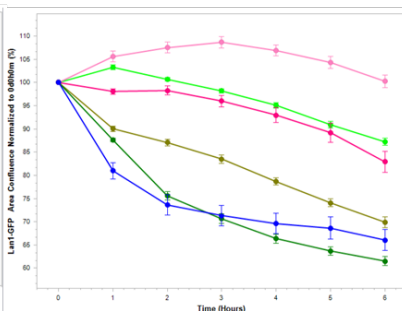
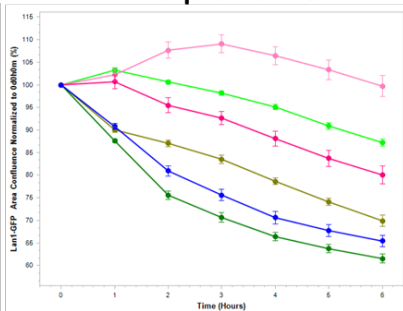
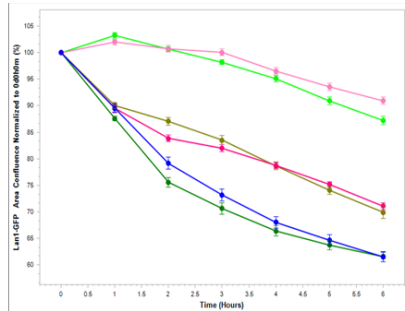
- NBL cell only
- NBL+Drug
- NBL+Neutrophil
- NBL+Neutrophil+Drug
- NBL+Neutrophil+Ab
- NBL+Neutrophil+Drug+Ab

**EIPA**

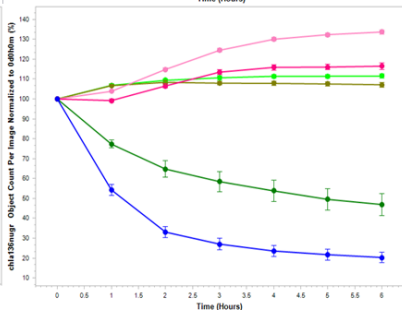
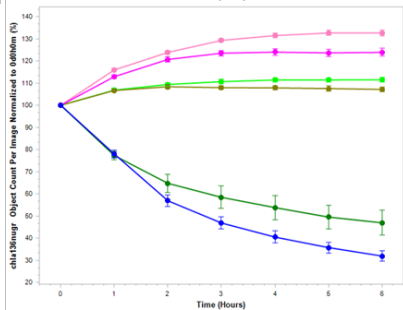
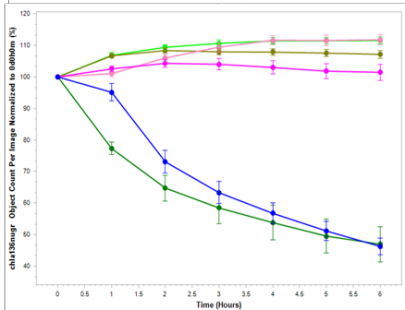
**Chlorpromazine**

**MBCD**

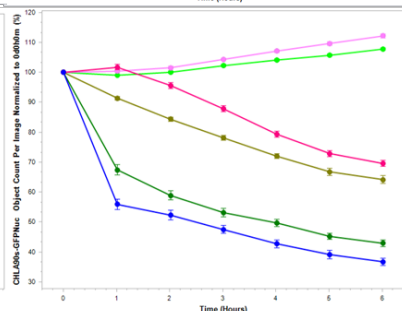
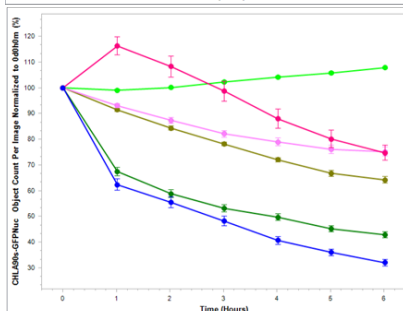
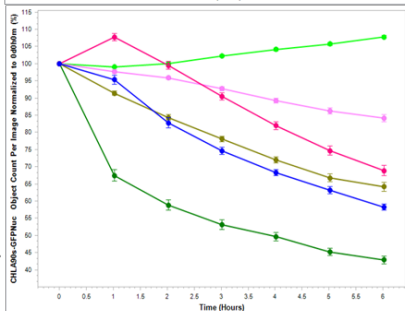
**Lan2**



**CHLA136**

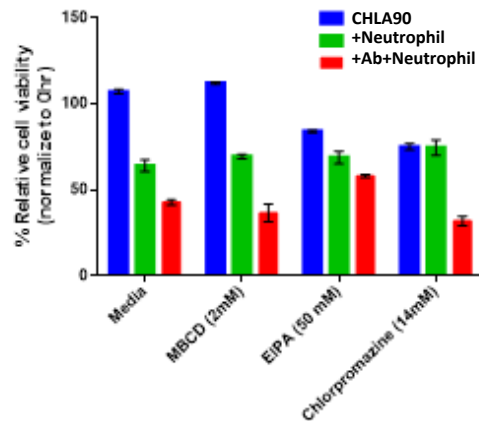
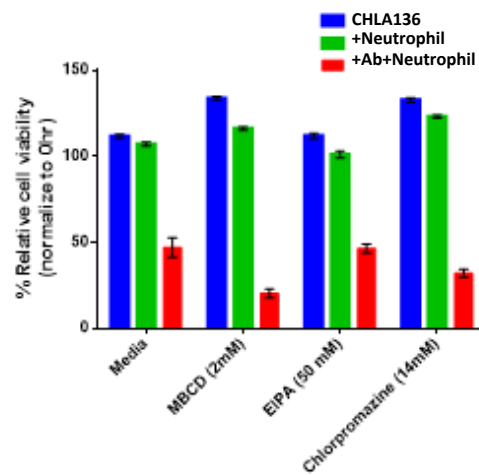
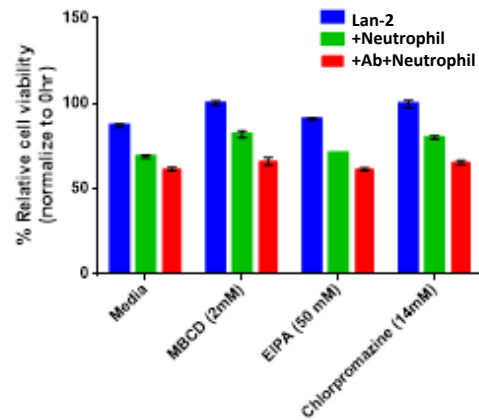


**CHLA90**



Cell Viability ↑  
Time (0-6h) →

**B.**



**Supplementary Figure 4. A.** A modified ADCC experimental design schema is on top depicting 6 hours assessment of cytotoxicity after addition of neutrophils (E:T of 10:1), dinutuximab (1 ug/ml) and GM-CSF (100 ng/ml). Prior to addition, cells were pretreated with EIPA, chlorpromazine, MBCD or media for 30 minutes followed by addition of dinutuximab in the continued presence of drug or media for 4 hours, then the cells were washed three times to remove drug and excess dinutuximab. This allowed us to evaluate ADCC with remaining cell-bound dinutuximab and evaluate effect of inhibitors and impact on internalization. Below, is the real-time data representing integrated GFP expression (amount of GFP+ LAN1, CHLA136, and CHLA90 cells) over time starting from the time of addition of neutrophils and GMCSF.

**B.** Average ( $\pm$ SD) neutrophil-mediated ADCC against LAN1, CHLA90, and CHLA136 neuroblastoma cells normalized to hour 0 (prior to addition of neutrophils) for the experiments (Media vs. inhibitors) and experimental groups (cells alone, cells with neutrophils, cells with neutrophils and antibody). The data shown in Figure 5E represents percent change of cytotoxicity of cells with neutrophils and antibody to neutrophils alone for each inhibitor as compared to the level of cytotoxicity in the media.