SUPPLEMENTARY MATERIAL

SUPPLEMENTAL TABLES

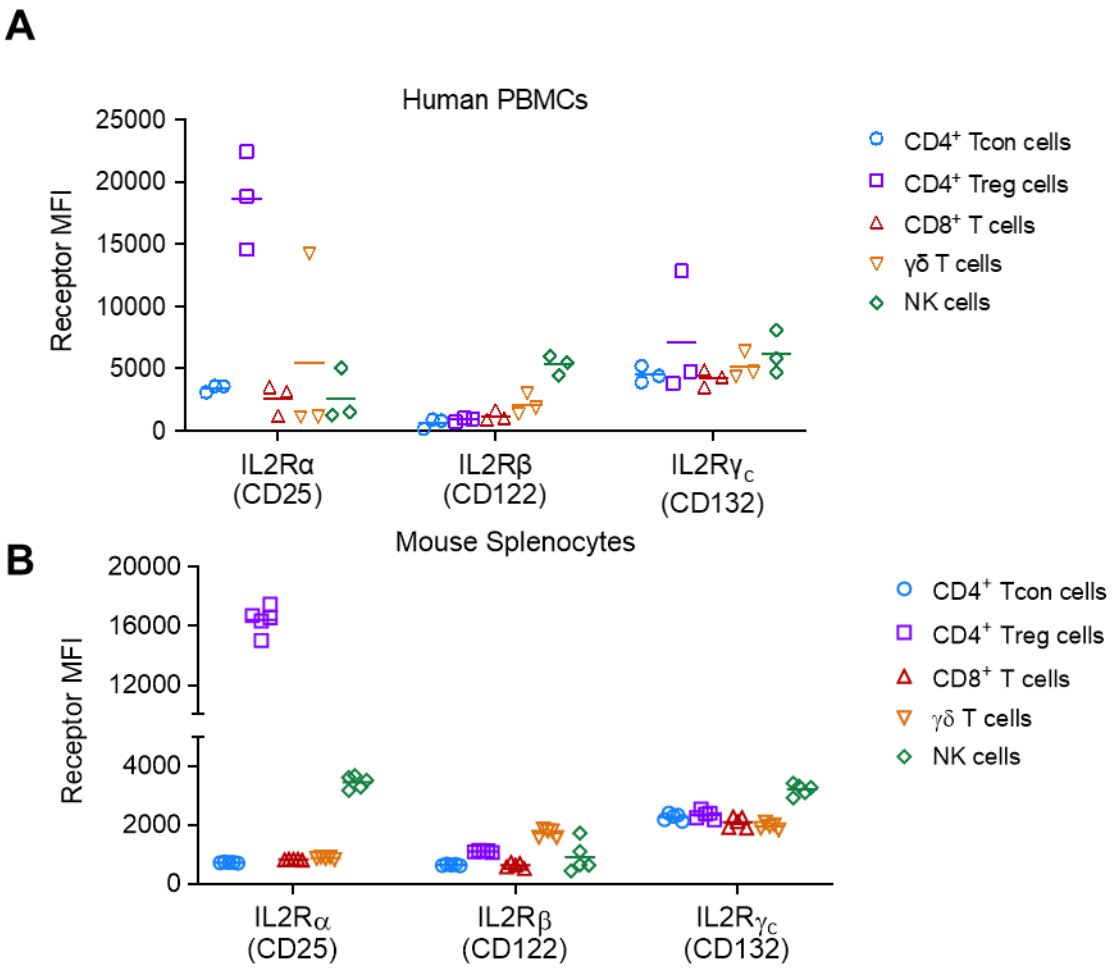
Supplementary Table S1. Neo-2/15 Maximum pSTAT5 Signaling (Maximal Mean Fluorescent Intensity, MFI) in Human PBMCs and Mouse Splenocytes.

Immune Cells	Maximum pSTAT5 Signaling (MFI) in vitro						
	Human PBMCs			Mouse Splenocytes			
	Neo2/15	hIL-2	hIL-15	Neo2/15	hIL-2	mIL-2	mIL-15
$CD4^+$ Tcon	1945	1762	1915	10812	10195	9204	10993
$CD4^+$ Treg	2495	2192	2455	18445	21119	21863	21726
$CD8^+$ Tcells	2517	2381	2392	26924	28056	27641	27343
gd Tcells	15181	14594	14932	22478	22765	23855	25670
NK cells	7899	8902	8081	10406	11854	12718	12716

Supplementary Table S2. IL-2 Receptor Signaling in Primary Human and Cynomolgus Monkey CD8+ T Cells induced by IL-2, NL-201, and Neo2/15.

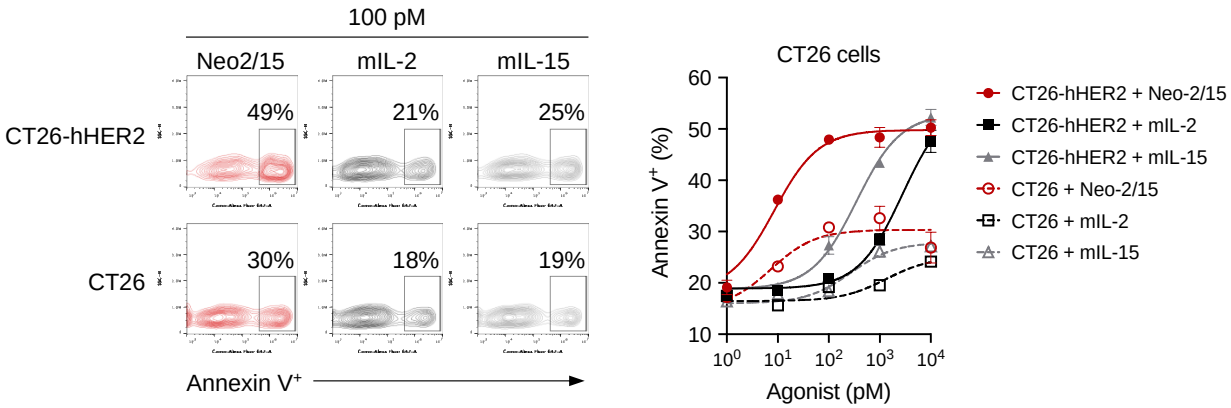
	Human EC50, nM			Cynomolgus EC50, nM		
	hIL-2	NL-201	Neo2/15	hIL-2	NL-201	Neo2/15
Mean	3.57	0.339	0.0188	2.51	0.24	0.0221
SEM	0.72	0.04	0.0011	0.27	0.047	0.001
N	10	10	10	6	6	6

SUPPLEMENTAL FIGURES

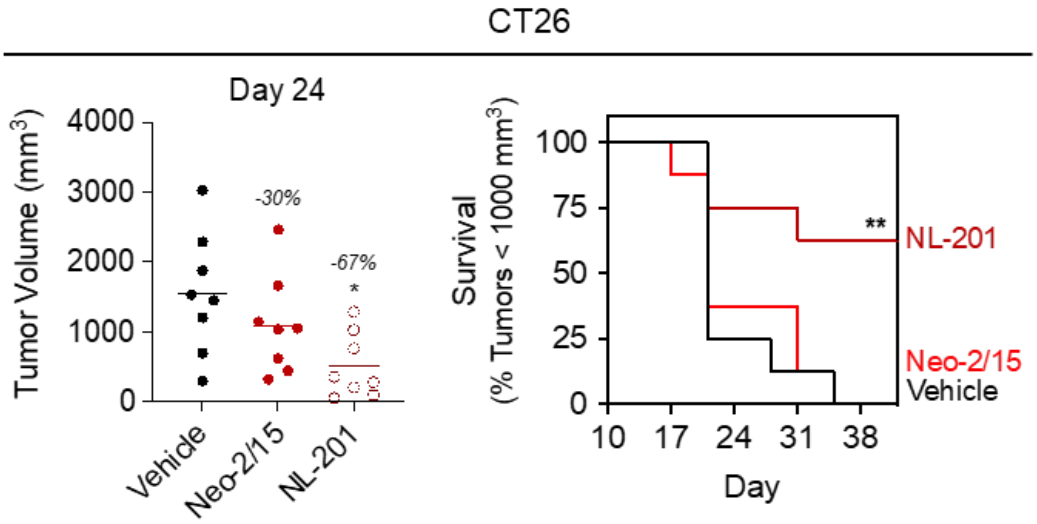


Supplementary Figure S1. A-B, Flow cytometric analysis of human PBMCs (**A**) and mouse splenocytes (**B**) for cell surface expression of IL-2Rα (CD25), IL-2Rβ (CD122), and IL-2Rγ_c (CD132). Each dot represents an individual donor (**A**) and mouse (**B**). Though the relative difference in receptor subunit expression is different for mouse and human immune cells, the trend of IL-2Rα expressed at a higher abundance in CD4+ Tregs than other immune cells is consistent in both species. Cells were acquired for flow cytometric analysis on a Cytek Aurora system and gated as follows for human lymphocytes: CD3+CD4+ for CD4+ conventional T (Tcon) cells, CD3+CD4+CD25highCD127low for CD4+ Treg cells, CD3+CD8+ for CD8+ T cells, CD3+TCRγδ+ for γδ T cells, and CD3–CD56+ for NK cells. Mouse

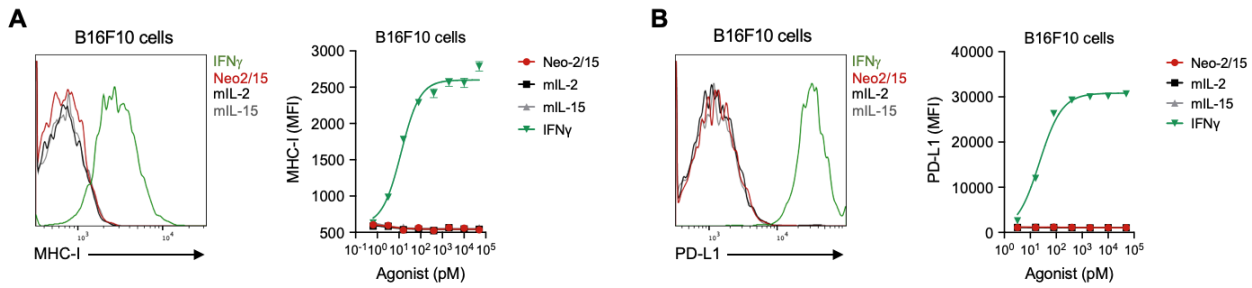
cells were gated for lymphocytes as follows: CD3+CD4+CD25– for CD4+ Tcon cells, CD3+CD4+CD25+ for CD4+ Treg cells, CD3+CD8+ for CD8+ T cells, CD3+TCRγδ+ for γδ T cells, and CD3–NK1.1+ for NK cells.



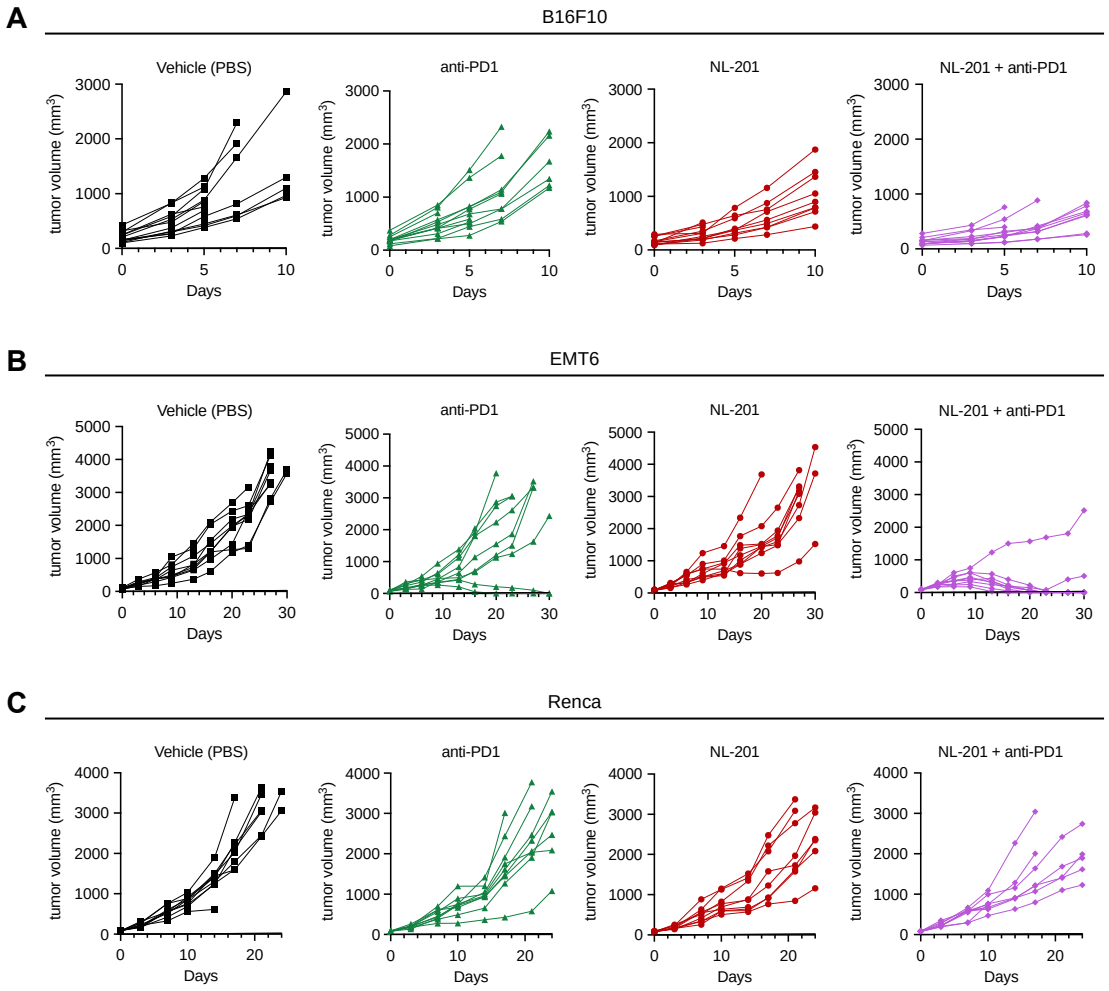
Supplementary Figure S2. Flow cytometric analysis of CT26-hHER2 and CT26 cell death assessed by Annexin V labeling after a 6-hour co-culture with isolated Balb/c NK cells and indicated concentrations of IL-2R agonists. Data shown are mean ± SEM of cells cultured in duplicate wells and representative of n=2 experiments. Representative plots shown for 100 pM of IL-2R agonists.



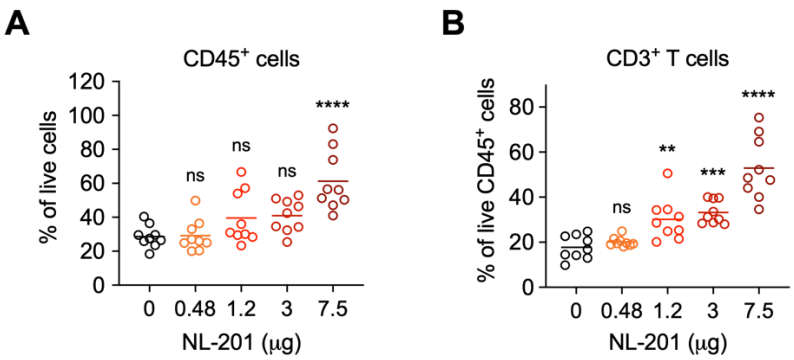
Supplementary Figure S3. NL-201 demonstrates improved *in vivo* efficacy as compared to Neo-2/15. Mice bearing CT26 tumors were treated with vehicle, Neo-2/15 (daily for 14 days), or NL-201 (weekly for two weeks) as described in the methods section, and mean tumor volume on Day 14 and survival are shown. RMANOVA with Tukey post hoc test was done for anti-tumor efficacy experiments; $p < 0.05$ was considered significant *$p \leq 0.05$, **$p \leq 0.01$.



Supplementary Figure S4. A-B, Flow cytometric analysis of MHC-I (**A**) and PD-L1 (**B**) surface expression on *in vitro* B16F10 cells cultured with the indicated concentrations of cytokines for 1 day. Representative histograms shown for 50 nM of cytokines (**A-B**). MFI, mean fluorescence intensity.



Supplementary Figure S5. A-C, Tumor volume measurements of individual mice bearing subcutaneous B16F10 melanoma (**A**), EMT6 mammary carcinoma (**B**), and Renca renal adenocarcinoma (**C**) tumors. Tumor volumes were measured every 2-3 days after staging and dosing began; dosing regimen described in Methods section.



Supplementary Figure S6. A-B, B16F10 tumor-bearing mice were given a single dose of NL-201 by intravenous administration and then harvested 5 days later for *ex vivo* flow cytometric analysis to measure the abundance of $CD45^+$ cells (**A**) and $CD3^+$ T cells of $CD45^+$ gate (**B**). One-way ANOVA with Dunnett post hoc test was done for T-cell expansion experiments; $p < 0.05$ was considered significant. Each dot represents an individual mouse. ns, not significant; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.