

## SUPPLEMENTARY TABLES AND FIGURE

Supplementary Table 1: Assay-specific reagents and equipment.

Device for cam1615TEM8 Protein Production		
Item	Manufacturer	Catalog #
AKTA Avant Chromatography System	GE Healthcare (Chicago, IL, USA)	-
Reagents for ANTXR1 RT-qPCR		
Item	Manufacturer	Catalog #
ANTXR1 TaqMan Probe (Human)	Thermo Fisher Scientific (Waltham, MA, USA)	4351372
ANTXR1 TaqMan Probe (Mouse)	Thermo Fisher Scientific (Waltham, MA, USA)	4331182
GAPDH TaqMan Probe (Human)	Thermo Fisher Scientific (Waltham, MA, USA)	4331182
GAPDH TaqMan Probe (Mouse)	Thermo Fisher Scientific (Waltham, MA, USA)	4453320
MicroAmp™ EnduraPlate™ Optical 384-Well Clear Reaction Plate	Applied Biosystems (Foster City, CA, USA)	4483285
QIAshredder	Qiagen (Germantown, MD, USA)	796565
RNEasy Mini Kit	Qiagen (Germantown, MD, USA)	74104
DNase I, Amplification Grade	Invitrogen (Carlsbad, CA, USA)	18068015
SuperScript™ IV Reverse Transcriptase	Invitrogen (Carlsbad, CA, USA)	18090050
Taqman Fast Advanced Master Mix	Applied Biosystems (Foster City, CA, USA)	4444557
Thermo Scientific™ dNTP Mixes	Thermo Fisher Scientific (Waltham, MA, USA)	R0192
Reagents for PBMC Isolation, NK Cell Enrichment, NK Cell Expansion, and Cell Culture		
Item	Manufacturer	Catalog #
Ficoll®-Paque Premium	GE Healthcare (Chicago, IL, USA)	17544202
EasySep™ Human NK Cell Enrichment Kit	STEMCELL Technologies (Vancouver, Canada)	19055
G-REX® 6 Well Plate	Wilson Wolf (Saint Paul, MN, USA)	80240M
IncuCyte® Nuclight Red Lentivirus Reagent	Sartorius (Göttingen, Germany)	4625
Reagents for Generating A549 TEM8 Knockout Cell Line		
Item	Manufacturer	Catalog #
Alt-R S.p. HiFi Cas9 Nuclease V3	IDT (Coralville, IA, USA)	1081060
Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent	Invitrogen (Carlsbad, CA, USA)	CMAX00001
Reagents and Devices for NK Cell Degranulation and Cytokine Production Assay		
Item	Manufacturer	Catalog #
GolgiPlug™	BD Biosciences (San Jose, CA, USA)	555029
GolgiStop™	BD Biosciences (San Jose, CA, USA)	554724
LSR II Flow Cytometer	BD Biosciences (East Rutherford, NJ, USA)	-
Reagents and Devices for Tumor Spheroid Killing Assay		
Item	Manufacturer	Catalog #
Round Bottom Ultra-Low Attachment 96 Well Plate	Corning (Corning, NY, USA)	7007
IncuCyte® S3	Essen Bioscience (Ann Arbor, MI, USA)	-
Reagents and Devices for NK Cell and T Cell pSTAT5 Induction Assay		
Item	Manufacturer	Catalog #
Tru-Phos™ Perm Buffer	Biolegend (San Diego, CA, USA)	425401

<b>Reagents for NK-92 Metabolic Activity Assay</b>		
<b>Item</b>	<b>Manufacturer</b>	<b>Catalog #</b>
96-Well, Cell Culture-Treated, Flat-Bottom Microplate	Thermo Fisher Scientific (Waltham, MA, USA)	07-200-565
Resazurin	R&D Systems (Minneapolis, MN, USA)	AR002
<b>Reagents and Devices for A549 <i>In Vivo</i> Assay</b>		
<b>Item</b>	<b>Manufacturer</b>	<b>Catalog #</b>
Matrigel™ Membrane Matrix High Concentration	Corning (Corning, NY, USA)	354262
VWR Digital Caliper	VWR International (Radnor, PA, USA)	62379-531
Gibco™ ACK Lysing Buffer	Thermo Fisher Scientific (Waltham, MA, USA)	A1049201

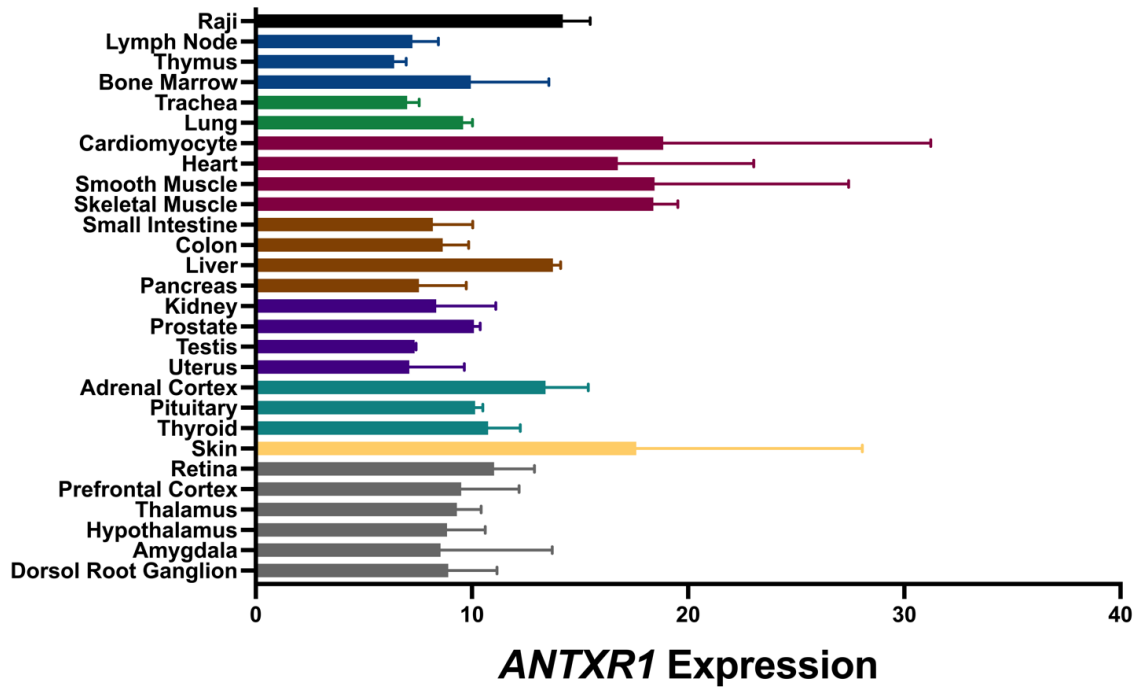
**Supplementary Table 2: Cell culture reagents.**

Cell Line	Vendor	Growth Media <sup>1</sup>	Growth Media Vendor	Growth Media Catalog #
A549	American Type Culture Collection (Manassas, VA, USA)	DMEM	Corning (Corning, NY, USA)	10-013-CV
DU145	American Type Culture Collection (Manassas, VA, USA)	RPMI 1640	Thermo Fisher Scientific (Waltham, MA, USA)	11875119
Raji	American Type Culture Collection (Manassas, VA, USA)	RPMI 1640	Thermo Fisher Scientific (Waltham, MA, USA)	11875119
HT-29	American Type Culture Collection (Manassas, VA, USA)	RPMI 1640	Thermo Fisher Scientific (Waltham, MA, USA)	11875119
HUVEC (Human Umbilical Vein Endothelial Cell)	Lonza (Basel, Switzerland)	EGM <sup>TM</sup> -2	Lonza (Basel, Switzerland)	CC-3162
HMVEC-L (Human Microvascular Endothelial Cell-Lung)	Lonza (Basel, Switzerland)	EGM <sup>TM</sup> -2 MV	Lonza (Basel, Switzerland)	CC-3202
2H-11	American Type Culture Collection (Manassas, VA, USA)	DMEM	Corning (Corning, NY, USA)	10-013-CV
NHLF	American Type Culture Collection (Manassas, VA, USA)	DMEM	Corning (Corning, NY, USA)	10-013-CV
NK-92 <sup>®</sup>	American Type Culture Collection (Manassas, VA, USA)	MEM α-nucleosides	Thermo Fisher Scientific (Waltham, MA, USA)	12571071
Expi293F <sup>TM</sup>	Thermo Fisher Scientific (Waltham, MA, USA)	Expi293 <sup>TM</sup> Expression Medium	Thermo Fisher Scientific (Waltham, MA, USA)	A1435101

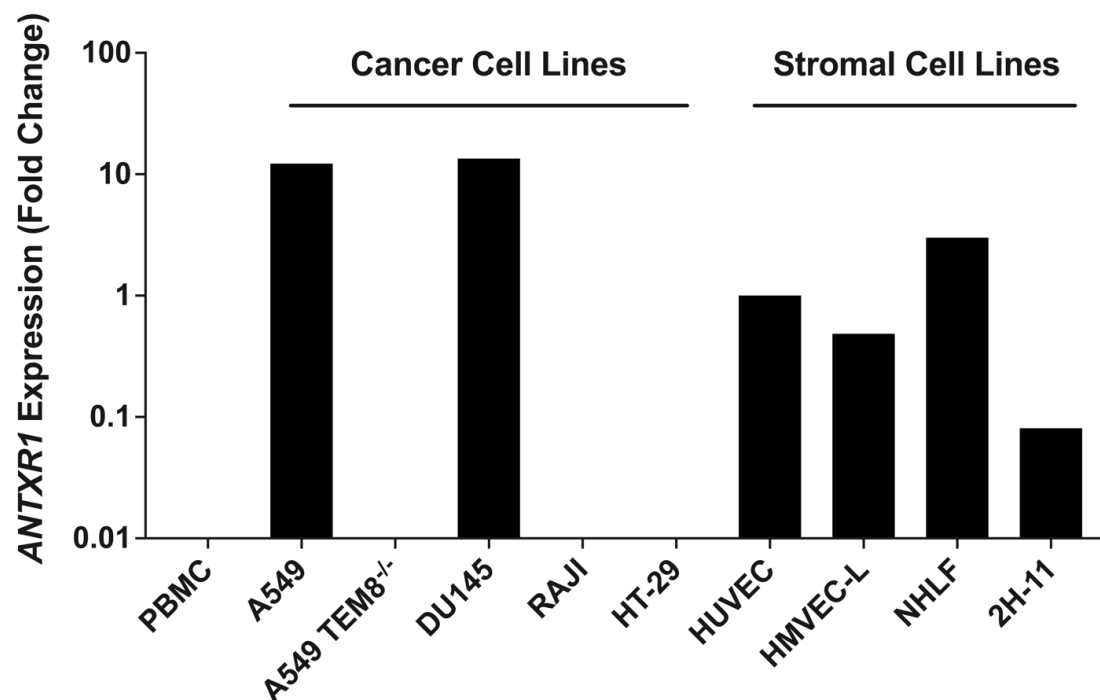
<sup>1</sup>DMEM and RPMI 1640 were supplemented with 10% Fetal Bovine Serum (FBS), while MEM α nucleosides was supplemented with 0.1 mM 2-mercaptoethanol, 500 U/mL recombinant human IL-2, 12.5% horse serum, and 12.5% fetal bovine serum.

**Supplementary Table 3: Antibodies and other fluorescent markers.**

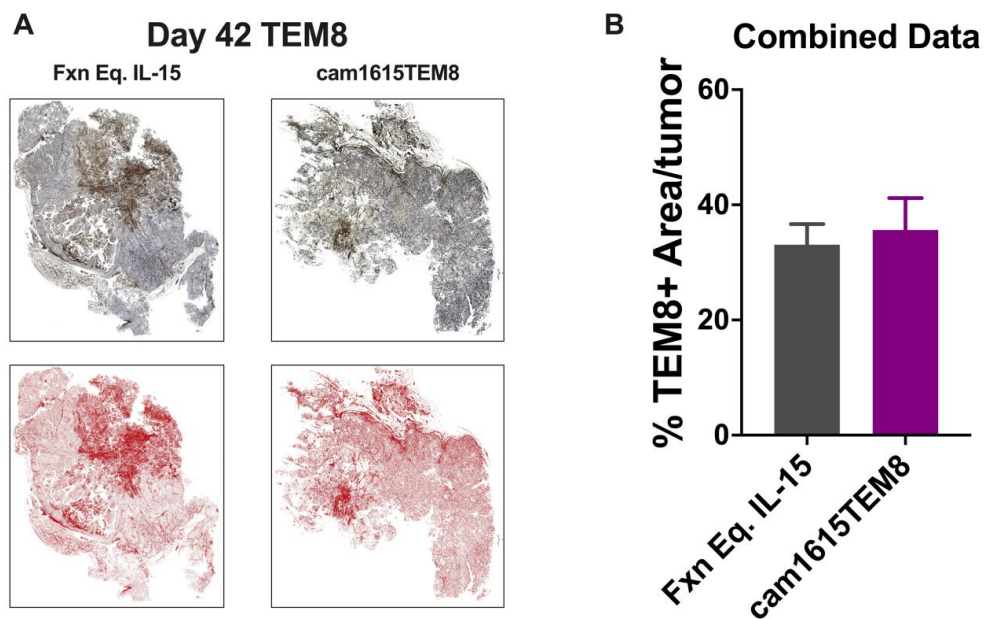
<b>Antibodies for NK Cell Degranulation and Cytokine Production Assay</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
CD3	UCHT1	BD Biosciences (San Jose, CA, USA)	562280	Mouse	Human	Flow Cytometry
CD56	HCD56	Biolegend (San Diego, CA, USA)	318318	Mouse	Human	Flow Cytometry
CD107a	H4A3	Biolegend (San Diego, CA, USA)	328606	Mouse	Human	Flow Cytometry
IFN $\gamma$	4S.B3	Biolegend (San Diego, CA, USA)	502538	Mouse	Human	Flow Cytometry
<b>Antibodies for NK Cell and T Cell Proliferation Assay</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
CD3	UCHT1	BD Biosciences (San Jose, CA, USA)	562280	Mouse	Human	Flow Cytometry
CD56	HCD56	Biolegend (San Diego, CA, USA)	318318	Mouse	Human	Flow Cytometry
<b>Antibodies for NK Cell and T Cell pSTAT5 Induction Assay</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
CD3	OKT3	Biolegend (San Diego, CA, USA)	317330	Mouse	Human	Flow Cytometry
CD56	HCD56	Biolegend (San Diego, CA, USA)	318336	Mouse	Human	Flow Cytometry
STAT5/pY694	A17016B.Rec	Biolegend (San Diego, CA, USA)	936904	Mouse	Human	Flow Cytometry
Kappa Isotype Control	MOPC-21	Biolegend (San Diego, CA, USA)	400140	Mouse	--	Flow Cytometry
<b>Antibodies for NK-92 CD16 Surface Staining</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
CD16	3G8	Biolegend (San Diego, CA, USA)	302038	Mouse	Human	Flow Cytometry
Kappa Isotype Control	MOPC-21	Biolegend (San Diego, CA, USA)	400158	Mouse	--	Flow Cytometry
<b>Antibodies for <i>in vivo</i> Mouse Peripheral Blood Human NK Cell Quantification</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
CD3	UCHT1	BD Biosciences (San Jose, CA, USA)	562280	Mouse	Human	Flow Cytometry
CD45	HI30	Biolegend (San Diego, CA, USA)	304042	Mouse	Human	Flow Cytometry
CD45	30-F11	Biolegend (San Diego, CA, USA)	103149	Rat	Mouse	Flow Cytometry
CD56	HCD56	Biolegend (San Diego, CA, USA)	318318	Mouse	Human	Flow Cytometry
<b>Primary Antibodies for <i>in vivo</i> Tumor Immunohistochemistry</b>						
<b>Target</b>	<b>Clone</b>	<b>Manufacturer</b>	<b>Catalog #</b>	<b>Host Species</b>	<b>Target Species</b>	<b>Application</b>
TEM8	EPNCl-R173-37	Abcam (Cambridge, UK)	ab241067	Rabbit	Mouse, Human	Immunohistochemistry
CD31	SZ31	American Research Products Inc. (Waltham, MA, USA)	DIA-310	Rat	Mouse	Immunohistochemistry
Granzyme B	EP230	MilliporeSigma (Burlington, MA, USA)	262R-15	Rabbit	Human	Immunohistochemistry
<b>Other Fluorescent Markers</b>						
<b>Item</b>	<b>Manufacturer</b>		<b>Catalog #</b>	<b>Application</b>		
Annexin V	BD Biosciences (San Jose, CA, USA)		556419	Flow Cytometry		
Live/Dead <sup>TM</sup> Fixable Near IR Dead Cell Stain Kit	Thermo Fisher Scientific (Waltham, MA, USA)		L34976	Flow Cytometry		
CellTrace <sup>TM</sup> Violet Cell Proliferation Kit	Thermo Fisher Scientific (Waltham, MA, USA)		C34557	Flow Cytometry		



**Supplementary Figure 1. Organ and tissue specific expression of *ANTXR1* in human tissues.** Gene expression of *ANTXR1* in human tissues in the GeneAtlas U133A, gcrrna database with the 220092\_s\_at probe set. Graph have been adapted from the BioGPS website with tissues grouped and color-coded.



**Supplementary Figure 2. *ANTXR1* expression in tumor and stromal cell lines.** *ANTXR1* expression was quantified with RT-qPCR of Human Peripheral Blood Mononuclear Cells (PBMC), human tumor cell lines (A549, A549 TEM8<sup>-/-</sup>, DU145, RAJI, HT29), human endothelial cell lines (HUVEC, HMVEC-L), human fibroblasts (NHLF), and immortalized mouse endothelial cells (2H-11). Expression levels of *ANTXR1* are normalized to *GAPDH* then depicted as a fold change relative to HUVECs. The 2H-11 cell line required mouse *ANTXR1* and *GAPDH* RT-qPCR probes.



**Supplementary Figure 3. cam1615TEM8 does not alter TEM8 expression within A549 xenograft tumors.** A549 tumor was engrafted as outlined and mice were treated with NK cells and cam1615TEM8 or functionally equivalent IL-15. (A) Top: Representative anti-TEM8 stained whole tumor sections. Bottom: the TEM8 mask used for TEM8 quantification generated with QuPath. (B) Combined data for tumors from all mice. “Fxn Eq. IL-15” indicates “Functionally Equivalent IL-15.” n=4 for the functionally equivalent IL-15 treatment group. n=3 for the cam1615TEM8 treatment group.