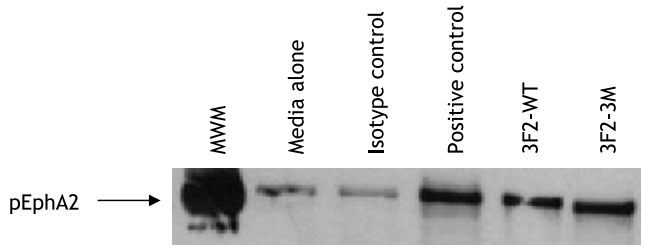

Figure W1. 3F2-WT and 3F2-3M activate EphA2 *in vitro*. EphA2 agonist activity of 3F2-WT, 3F2-3M, and control antibodies was determined by evaluating EphA2 phosphorylation in HeyA8 human ovarian carcinoma cells at 10 μg/ml as described in Materials and Methods.

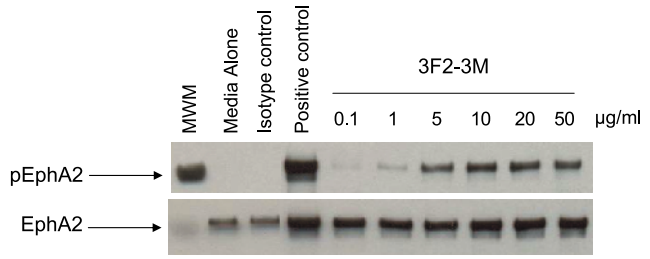

Figure W2. 3F2-3M activates EphA2 *in vitro*. EphA2 agonist activity of 3F2-3M was determined by evaluating EphA2 phosphorylation in HeyA8 ovarian carcinoma cells at concentrations from 0.1 to 50 μg/ml as described in Materials and Methods. Control antibodies were evaluated at 10 μg/ml.

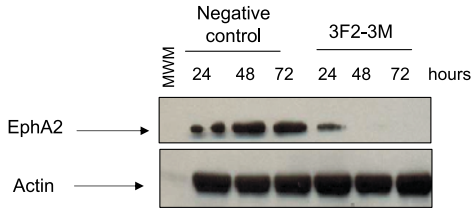

Figure W3. 3F2-3M induces degradation of EphA2 *in vitro*. Degradation of EphA2 was determined by treating MDA-MB-231 cells with 10 μg/ml of 3F2-3M antibody for 24 to 72 hours. Lysates were collected as described for EphA2 activation assays. Equal proteins were resolved by SDS-PAGE, transferred to polyvinylidene fluoride membrane, and immunoblotted with anti-EphA2 (Invitrogen) or anti–β-actin antibodies (Sigma).

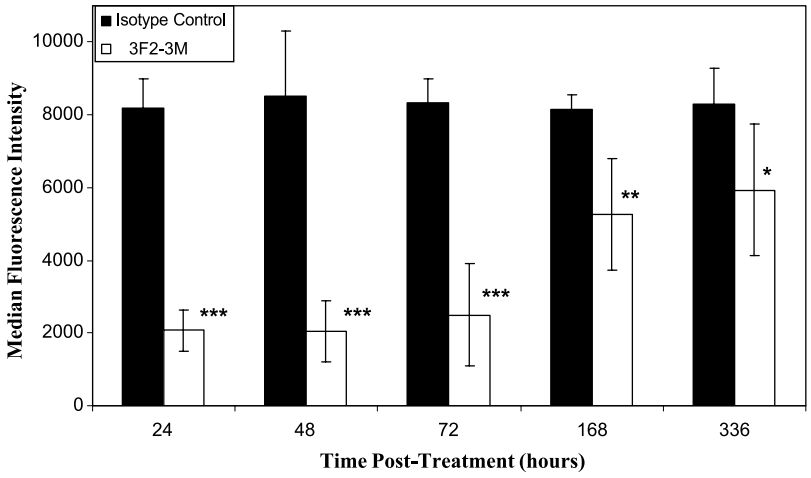

Figure W4. 3F2-3M treatment induces degradation of cell surface EphA2 *in vivo*. MDA-MB-231 breast carcinoma cells (2×10^6) were implanted proximal to the mammary fat pad of female nude mice. Once tumors reached approximately 150 mm^3, mice were given a single intraperitoneal injection of either 3F2-3M or isotype control at 10 mg/kg. Tumors were then harvested at 24, 48, 72, 168, and 336 hours after 3F2-3M administration. Tumors were dissociated with collagenase into a single-cell suspension, stained, and analyzed by FACS using an EphA2 antibody that does not compete with 3F2-3M. Two-way analysis of variance revealed that at 24, 48, 72, 168, and 336 hours, EphA2 levels at the cells surface of tumor cells treated with 3F2-3M are statistically lower than those treated with the isotype control antibody: $*P < .05$, $**P < .001$ to .01, $***P < .001$.

Table W1. Summary of 3F2-3M ADCC Activity in a Panel of Human Cancer Cell Lines.

Cell Line	Tumor Type	EphA2 Expression through FACS Fold Log Change over Secondary	Percent Cytotoxicity (E/T, 50:1; 500 ng/ml)
A549	NSCLC	3	~65%
T231	Breast	3	~36%
MDA-MB-231	Breast	2	~20% (E/T = 30:1
A498	Renal cell carcinoma	1	~45%
HeyA8	Ovarian	3	~40%
SKOV3	Ovarian	2	~20%
M21	Melanoma	0	<10%
SKMel28	Melanoma	0	<10%