Supplemental Table 1. Type I Collagen (Col I) and Type IV Collagen (Col IV) mRNA expression in primary WT/Neu and α2-null/Neu tumors.

Gene	Relative mRNA Expression*		α2-null/α2WT Ratio	P-value
	α2WT/Neu Mice	α2-null/Neu Mice		
Col I α1	1.84	1.06	0.48	0.82
Col I α2	2.48	1.85	0.72	0.79
Col IV	54.91	42.14	0.77	0.56

*Mean of normalized copy number

Supplemental Table 2. Integrin mRNA expression in primary WT/Neu and α2-null/Neu tumors.

Integrin	Relative mRNA Expression*		α2-null/α2WT Relative Ratio	P-value
	α2WT/Neu Mice	α2-null/Neu Mice		
α1	8.96	10.90	1.22	0.59
α5	0.11	0.10	0.91	0.82
α6	0.24	0.18	0.75	0.94
αv	0.23	0.17	0.71	0.94
β1	0.69	0.62	0.90	0.94
β3	1.30	1.67	1.28	0.48
β4	1.50	1.36	0.91	0.39

*Mean of normalized copy number

Supplemental Table 3. Publicly available microarray datasets of breast cancer patients analyzed for the clinically relevant role of the α2β1 integrin.

NORMAL V. CANCER

Data Set	Normal	Breast Cancer	p-value (t-Test)	GSE
Richardson (PMID 16473279)	1.384 (7)	1.052 (40)	0.03800	GSE3744

TUMOR GRADE

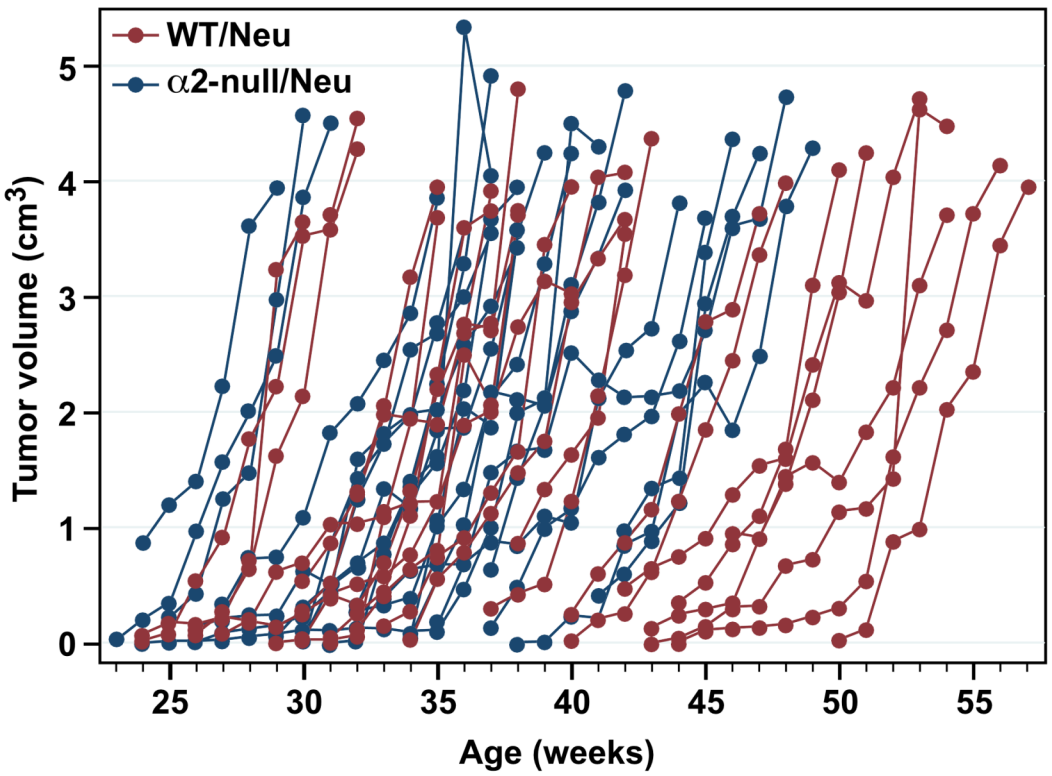
Data Set	Grade 1	Grade 2	Grade 3	p-value (ANOVA)	GSE
Vant Veer (PMID 11823860)	0.388 (12)	0.293 (27)	-0.300 (78)	0.00330	NA
Bittner (https://expo.intgen.org/expo/public/ 2005/01/15)	0.872 (30)	0.851 (107)	0.656 (141)	0.01020	GSE2109

METASTATIC STATUS

Data Set	Non-metastatic	Metastatic	p-value (t-Test)	GSE
Van de Vijver (NKI-295) (PMID 12490681)	0.091 (194)	-0.307 (101)	0.00380	NA

ESTROGEN RECEPTOR

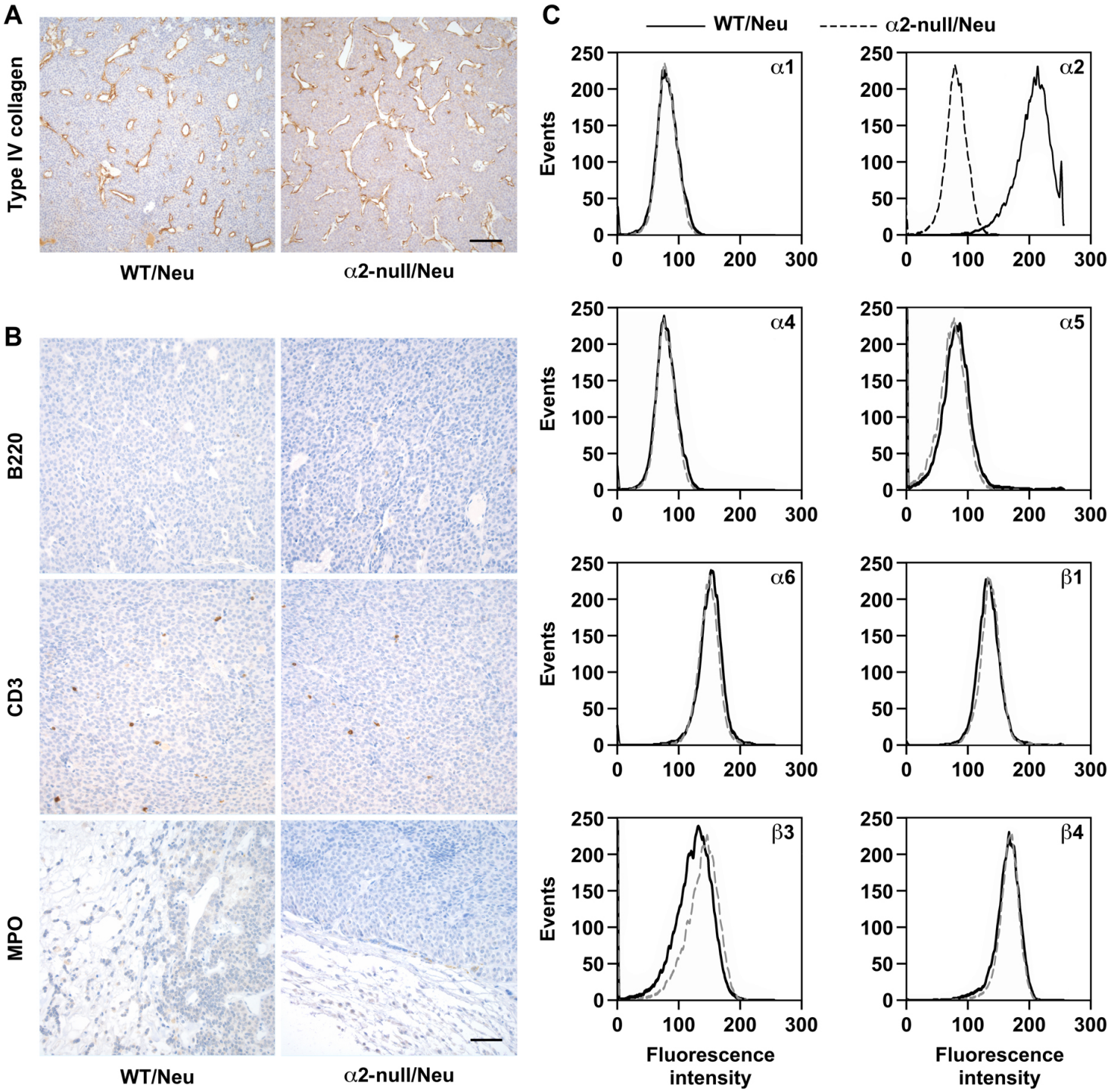
Data Set	ER Positive	ER Negative	p-value (t-Test)	GSE
Van de Vijver (PMID 12490681)	-0.123 (226)	-0.978 (69)	0.00001	NA
Ivshina (PMID 17079448)	0.263 (211)	0.106 (34)	0.00012	GSE4922
Miller (PMID 16141321)	0.213 (213)	0.085 (34)	0.00018	NA
Bild (PMID 16273092)	-0.500 (110)	-0.873 (48)	0.00095	GSE3143
Chin (PMID 17157792)	0.012 (75)	-0.140 (43)	0.00200	NA
Huang (PMID 12747878)	-1.081 (73)	-1.420 (15)	0.01200	NA
Saal (PMID 17452630)	-0.311 (45)	-0.749 (55)	0.01580	GSE5325
Wang (PMID 15721472)	0.173 (209)	0.088 (77)	0.02400	GSE2034
Minn (PMID 16049480)	0.168 (57)	0.038 (42)	0.02900	GSE2603
Boersma (PMID 17999412)	-0.541 (41)	-0.484 (52)	0.03500	GSE5847
Yu (PMID 16740749)	-0.142 (37)	-0.359 (23)	0.05100	GSE2294
Bittner (https://expo.intgen.org/expo/public/ 2005/01/15)	0.502 (154)	0.394 (78)	0.05300	GSE2109
Desmedt (PMID 17545524)	0.343 (134)	0.231 (64)	0.06500	GSE7390

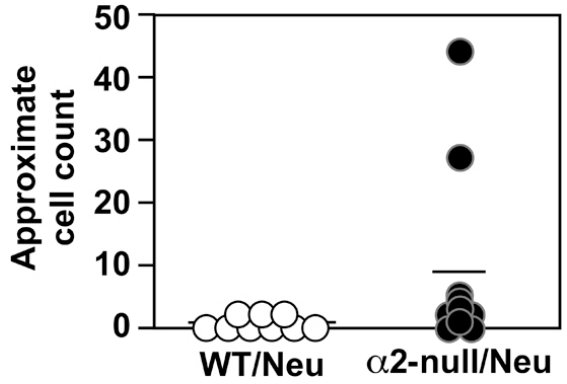


Supplemental Figure 1. Spaghetti plot of tumor volume by age in WT/Neu and α2-null/Neu mice. Observations from the same mouse are connected by straight lines. The slopes of these lines are shown in Figure 2A. There was no significant difference in the rate of tumor growth between WT/Neu and α2-null/Neu mice, although the initiation of tumor growth tended to occur later in WT/Neu animals.

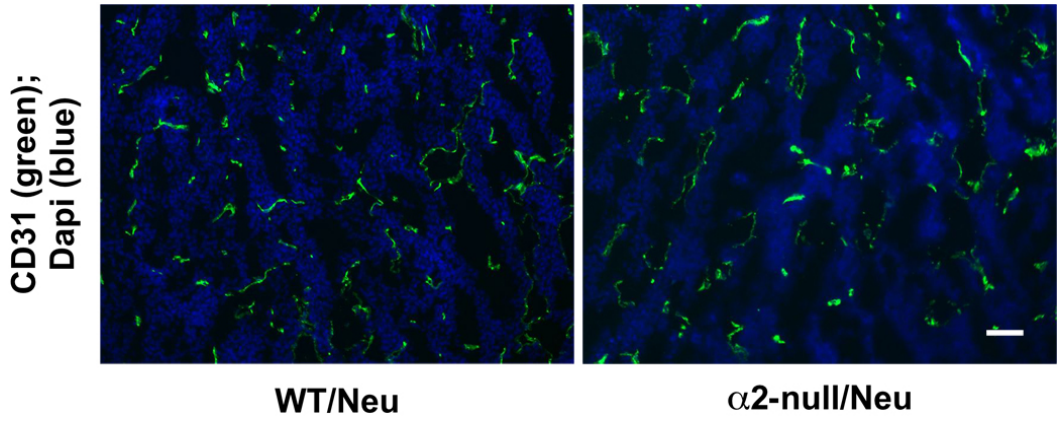
FOLLOWING PAGE:

Supplemental Figure 2. Morphologic and immunophenotypic analysis of the primary tumors from WT/Neu and α2-null/Neu animals. Immunohistochemical analysis of tumor cells derived from WT/Neu and α2-null/Neu mice for anti-Type IV collagen (scale bar=100 μm) (A) or immune T cells (CD3), B cells, (B220), neutrophils (Gr1) or macrophages (F4/80) (scale bar=50 μm) (B). (C) Flow cytometric analysis of primary tumor cells isolated from WT/Neu and α2-null/Neu (KO/Neu) animals using a fluorescent anti-α1, α2, α4, α5, α6, β1, β3 or β4 integrin antibodies.

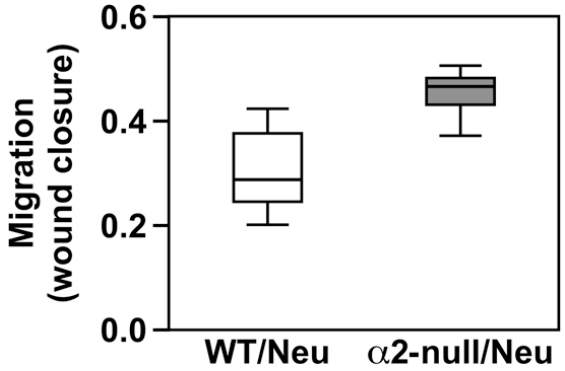

A
Type IV collagen
WT/Neu
α2-null/Neu
B
B220
CD3
MPO
WT/Neu
α2-null/Neu
C
WT/Neu
α2-null/Neu
α1
α2
α4
α5
α6
β1
β3
β4
Events
Fluorescence intensity
0
50
100
150
200
250
300



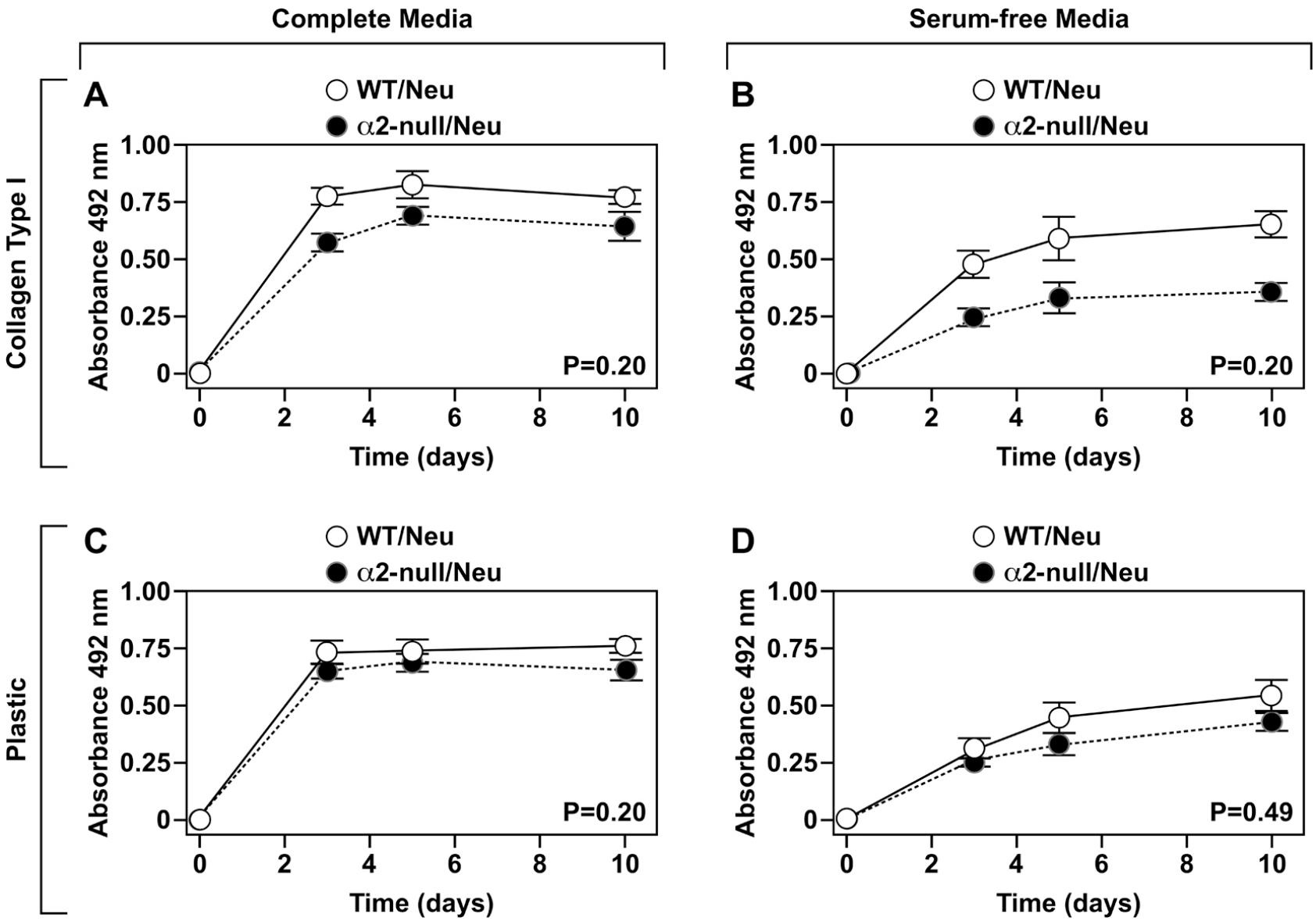
Supplemental Figure 3. The relative ratio of cytokeratin 19 mRNA to GAPDH mRNA in the peripheral blood at the time of sacrifice was determined by qRT-PCR. The level of cytokeratin mRNA-expressing cells was significantly increased in the peripheral blood of α2-null/Neu (n=10) mice compared to WT/Neu mice (n=11) (P=0.02).



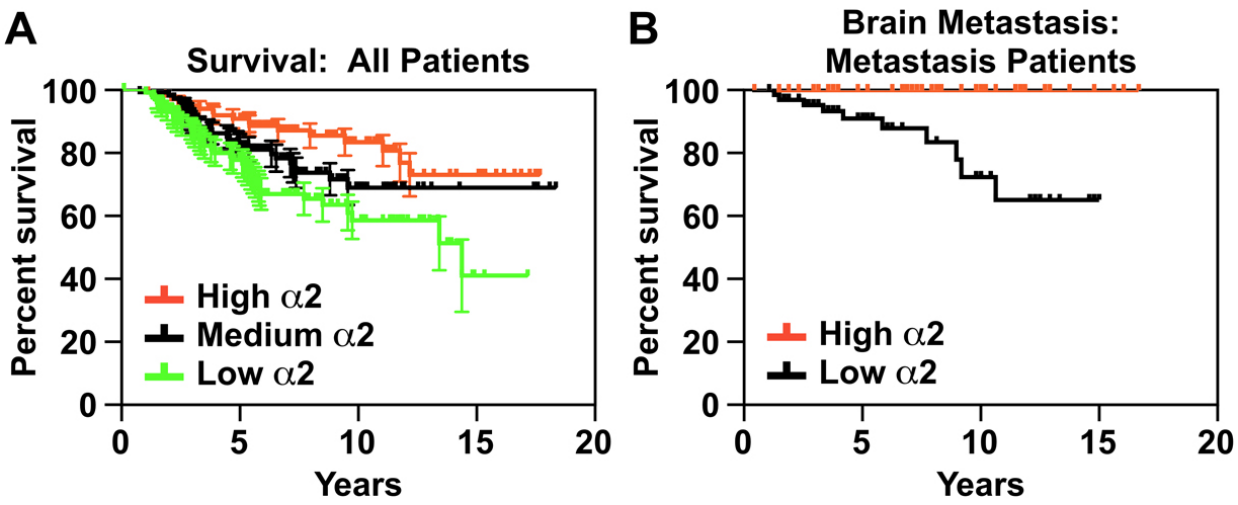
Supplemental Figure 4. Tumor angiogenesis in WT/Neu and α2-null/Neu (KO/Neu) tumors. Representative photomicrograph of anti-CD31 antibody staining of WT/Neu and α2-null/Neu (KO/Neu) tumor sections (scale bar = 40 μm).



Supplemental Figure 5. Repeat scratch assay. Migration experiments with different α2-null/Neu mice and WT/Neu tumor cells showed that the α2-null/Neu tumor cells exhibited significantly enhanced migration at 12 and 24 hours in a scratch assay ($P<0.001$).



Supplemental Figure 6. Proliferation of WT/Neu and α2-null/Neu (KO/Neu) tumor cells. Proliferation of primary tumor cells from WT/Neu and α2-null/Neu (KO/Neu) animals when grown on either type I collagen (20 μg/mL) or tissue-culture plastic in the presence or absence of serum was determined (WT/Neu; n=4: α2-null/Neu; n=3). Bars and errors indicate the mean plus or minus SEM (3 separate experiments performed in triplicate).



Supplemental Figure 7. Loss of α2 integrin expression is associated with decreased survival and brain metastasis. (A) Trend analysis for α2 integrin expression in the NKI-295 cohort. Log-rank analysis for trend was performed on the NKI-295 cohort divided into terciles. High α2β1 integrin expression was strongly associated with improved patient survival (P=0.0034) and with the trend analysis supporting a dose-dependent phenotype (P=0.0008). (B) Loss of α2β1 integrin expression is associated with brain cancer metastasis. Lymph node-negative patients that relapsed with metastatic disease were analyzed for the correlation between α2β1 integrin expression and the occurrence of brain lesions. Interestingly, brain metastasis only occurred in the patients with low α2β1 integrin expression (P=0.0049).