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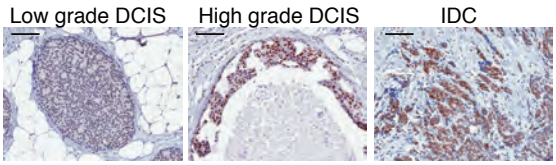
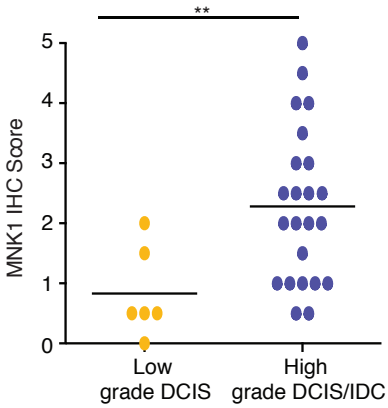
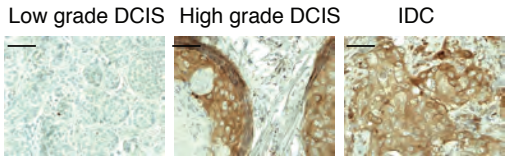


IHC p-MNK1

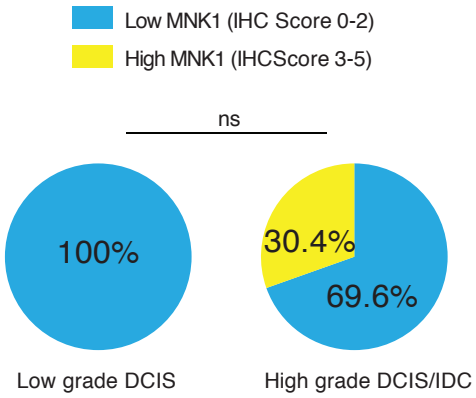
B





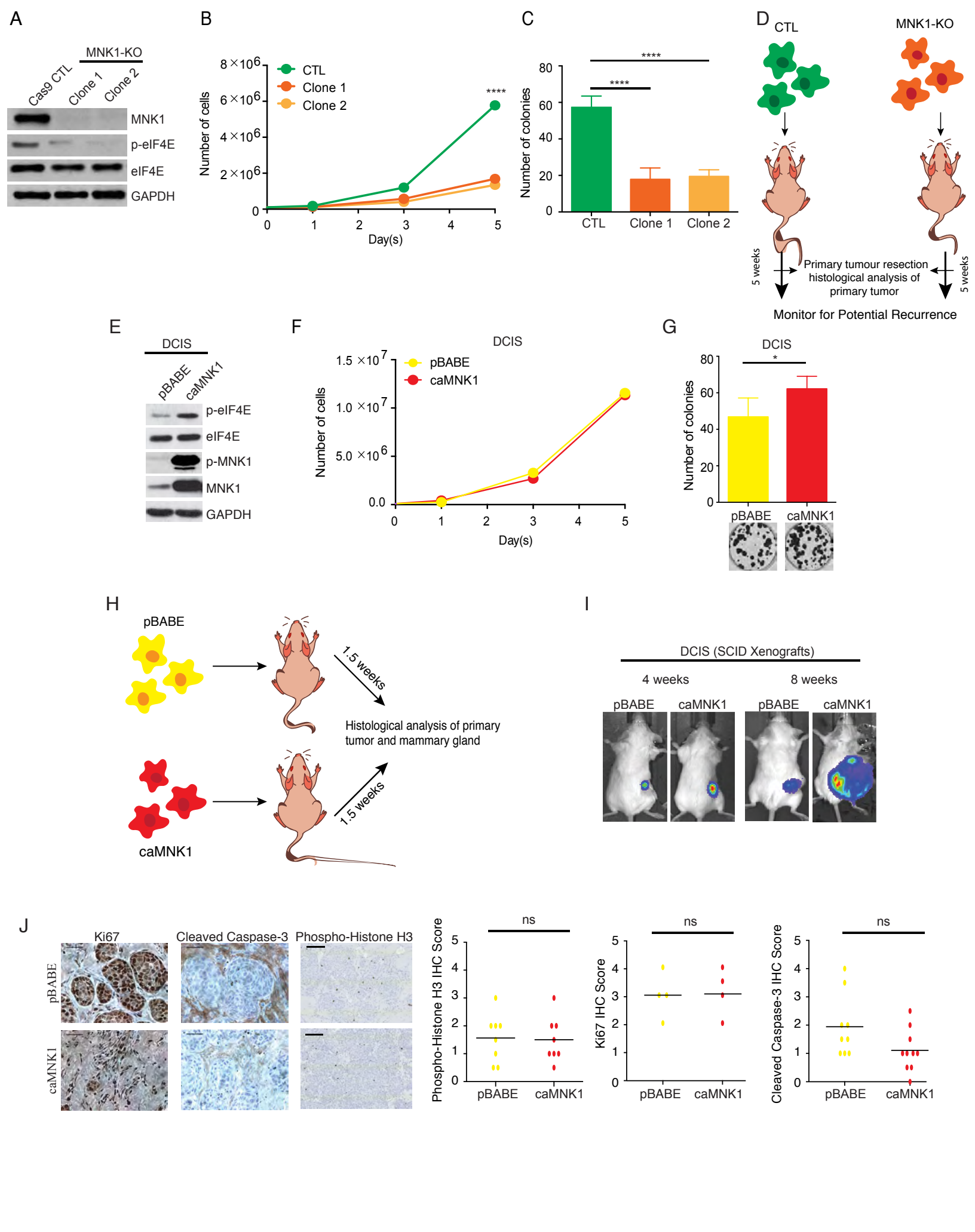
IHC MNK1

C



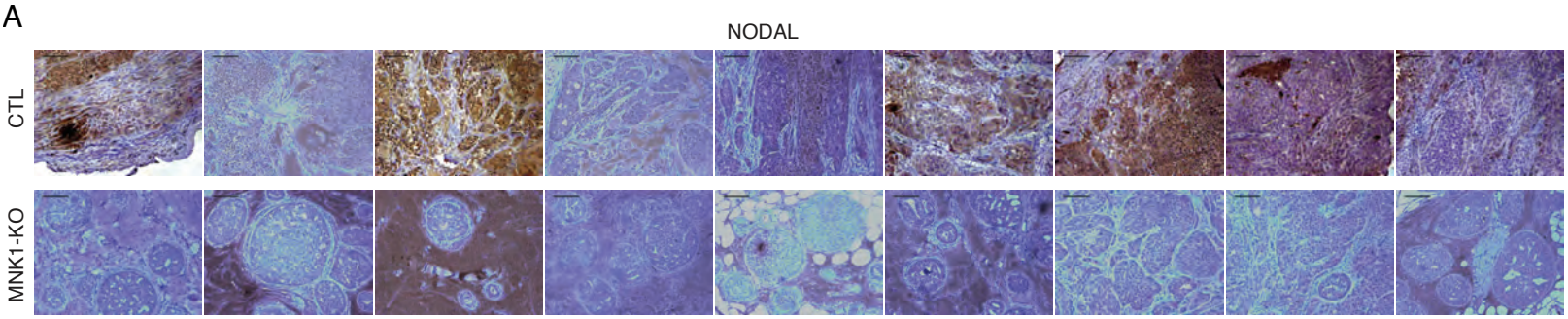
Guo et al, Supplementary Figure 1.

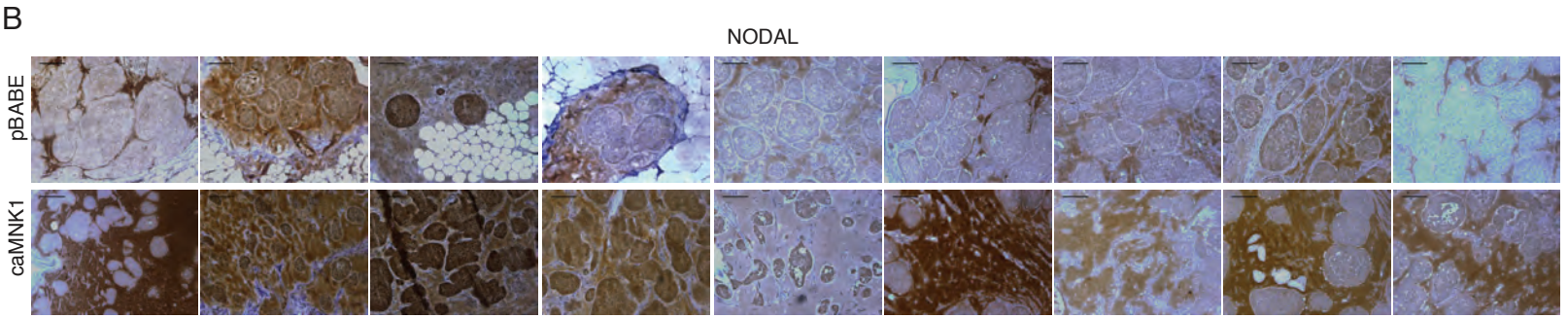
Supplementary Figure 1. Increased MNK1 activity in IDC. A. Zoomed in images from Fig.1A to show that phospho-MNK1 levels are higher in high grade DCIS and IDC than low grade DCIS. Scale bar = 100 μm. **B.** MNK1 levels are higher in high grade DCIS/IDC samples than low grade DCIS samples. Scale bar = 200 μm. **C.** Increased percentage of samples with high MNK1 staining in high grade DCIS/IDC compared to low grade DCIS.

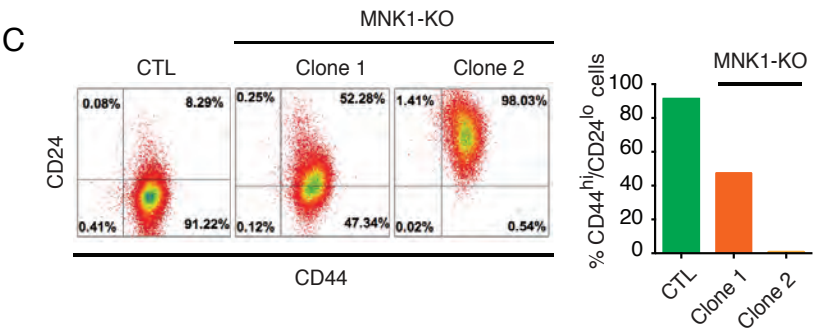


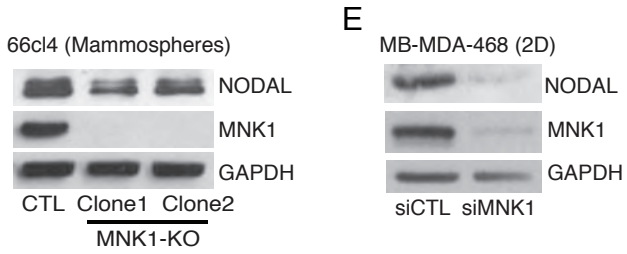
Guo et al, Supplementary Figure 2.

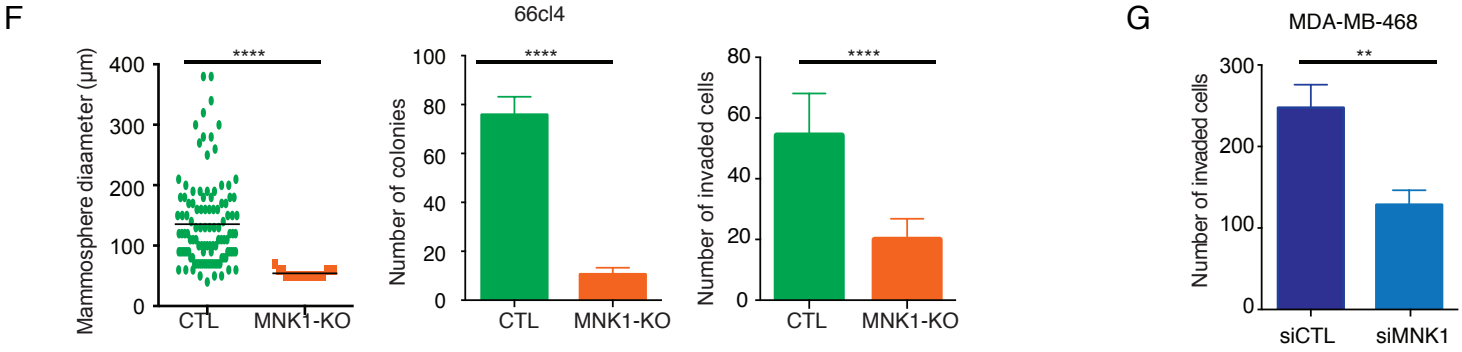
Supplementary Figure 2. Modulation of MNK1 affects colony formation in 2D cell culture. **A.** MNK1 knock-out by CRISPR-Cas 9 technology in 2 independent clones is confirmed by WB. **B.** MNK1-KO inhibits cell proliferation. **C.** MNK1-KO inhibits colony formation. **D.** Schematic depicting timing and measured outcomes in CTL and MNK1-KO xenografts. **E.** caMNK1 overexpression is confirmed by WB. **F.** caMNK1 overexpression does not affect DCIS proliferation. **G.** caMNK1 overexpression increases DCIS colony formation. **H.** Schematic depicting timing and measured outcomes in pBABE and caMNK1 xenografts. **I.** IVIS imaging of pBABE and caMNK1 xenografts in SCID mice. **J.** pBABE and caMNK1 xenografts express similar levels of Ki67 and phospho-histone H3. Cleaved caspase-3 levels in caMNK1 xeonografts trended downward compared to pBABE.

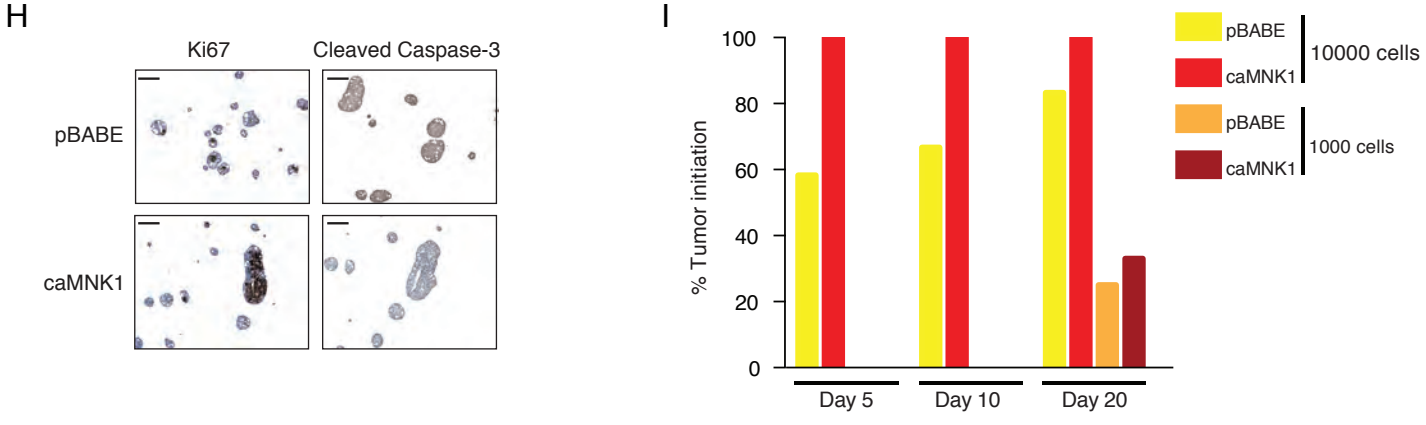






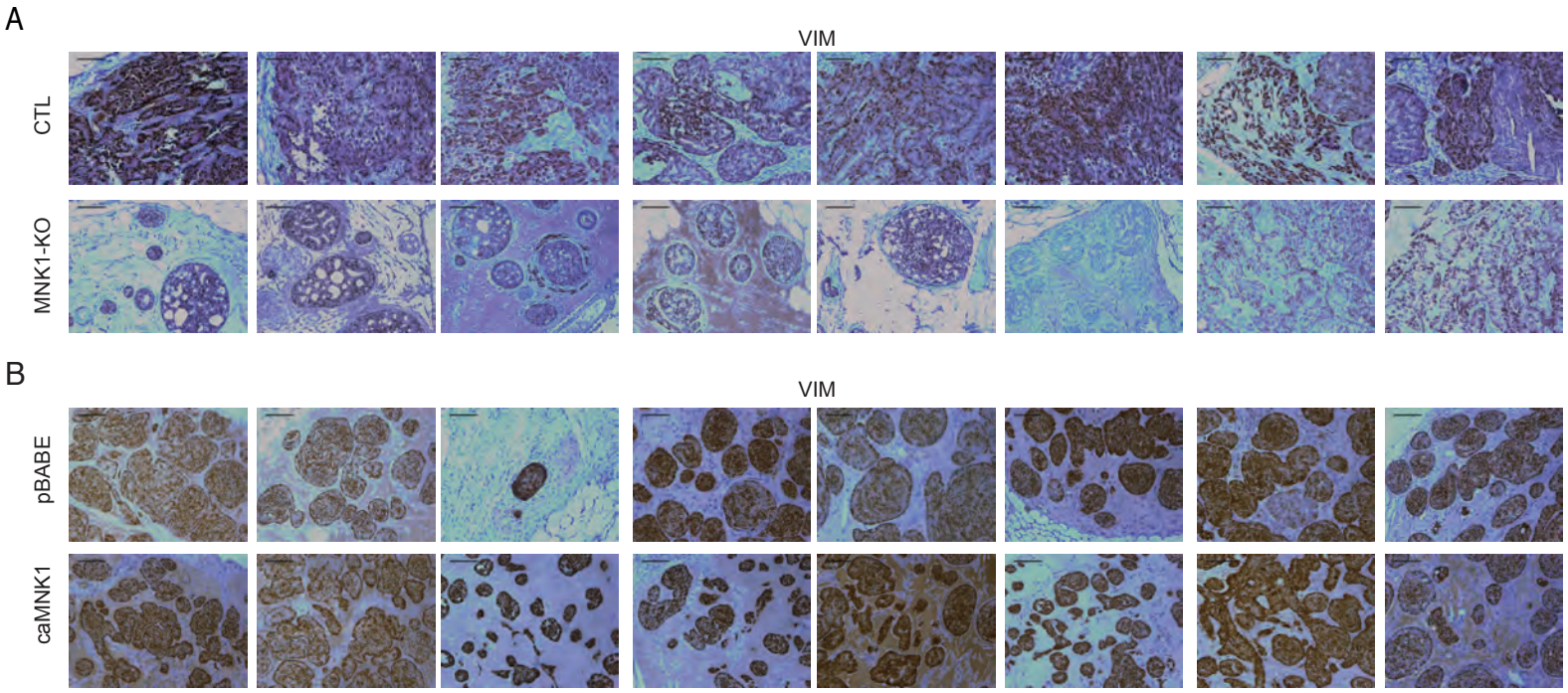






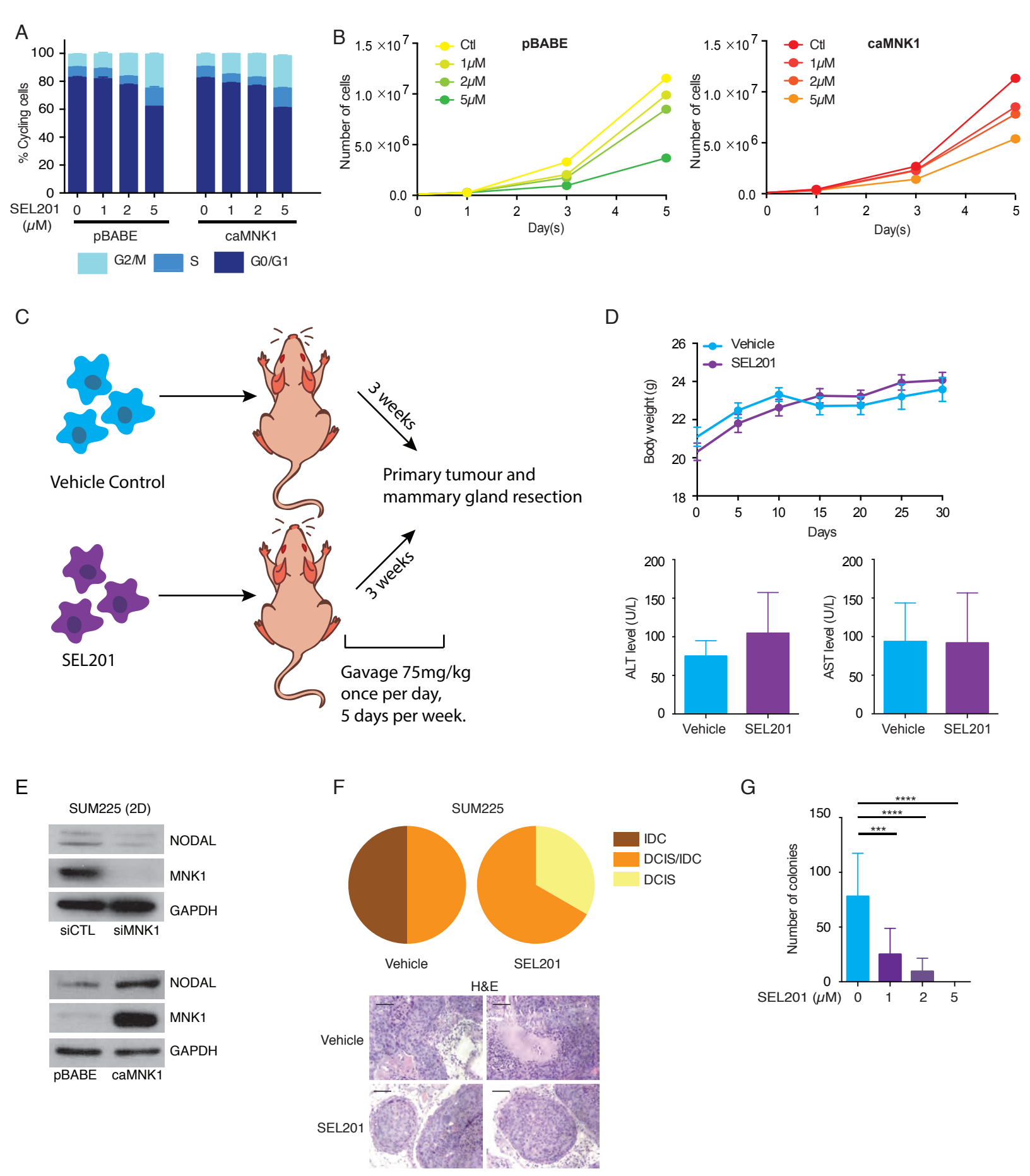
Guo et al, Supplementary Figure 3.

Supplementary Figure 3. NODAL levels are regulated by MNK1. A. MNK1-KO xenografts have decreased NODAL levels compared to CTL tumors. Representative images from 18 tumors are shown. Scale bar = 200 μm. **B.** caMNK1 xenografts have increased NODAL levels compared to pBABE tumors. Representative images from 18 tumors are shown. Scale bar = 200 μm. **C.** Loss of MNK1 causes decrease of $CD44^{hi}/CD24^{lo}$ cells in the DCIS population to decrease. **D.** 66cl4 MNK1-KO mammospheres have decreased NODAL levels compared to CTL mammospheres. **E.** MNK1 knock-down by siRNA decreases NODAL levels and cell invasion in MB-MDA-468 cells. **F.** 66cl4 MNK1-KO cells form mammospheres with smaller diameters, showed impaired colony formation ability and decreased invasion compared to CTL mammospheres. **G.** MNK1 knock-down by siRNA decreases MB-MDA-468 invasion. **H.** caMNK1 expressing DCIS mammospheres express decreased cleaved caspase 3 but similar Ki67 levels compared to pBABE mammospheres. **I.** Tumor initiation rate of DCIS-Luc pBABE and caMNK1 *in vivo* limiting dilution experiment.



Guo et al, Supplementary Figure 4.

Supplementary Figure 4. VIMENTIN levels are regulated by MNK1. A. MNK-KO xenografts have decreased VIMENTIN (VIM) levels compared to CTL tumors. Representative images from 16 tumors are shown. Scale bar = 200 μm. **B.** caMNK1 xenografts have increased VIMENTIN (VIM) levels compared to pBABE control tumors. Representative images from 16 tumors are shown. Scale bar = 200 μm.



Guo et al, Figure 5.

Supplementary Figure 5. A small molecule inhibitor of MNK1 inhibits the DCIS-IDC transition. A. SEL201 induces a G2/M arrest in pBABE and caMNK1 cells in a dose dependent manner. **B.** SEL201 inhibits pBABE and caMNK1 cell proliferation in a dose-dependent manner. **C.** Schematic depicting the timing and measured outcomes of SEL201 xenograft experiment. **D.** SEL201 has no effect on mouse body weight (upper panel), or on liver toxicity, as assessed by ALT and AST (lower panels) levels. **E.** MNK1 knock-down by siRNA decreases NODAL levels in SUM225 cells (upper panel). caMNK1 expressing SUM225 cells express increased NODAL levels compared to pBABE controls (lower panel). **F.** SUM225 primary tumor outgrowth over 9 weeks in vehicle versus SEL201-treated animals. MNK inhibitor SEL201 treatment slows down DCIS to IDC progression in NOD/SCID mice. **G.** SEL201 inhibits SUM225 colony formation.

Table S1. Antibodies

Target protein	Antibody	Usage
eIF4E	#610269 BD Transduction Laboratories	WB
GAPDH	#2118S Cell Signaling	WB
MNK1	#2195S Cell Signaling	WB & IHC
NODAL	Ab55676 Abcam	WB & IHC
p63	Ab735 Abcam	IHC
phospho-eIF4E	#9741S Cell Signaling	WB
phospho-Histone H3 Ser10	#09-797 EMD Millipore	IHC
phospho-MNK1	#2111S Cell Signaling	WB & IHC
VIMENTIN	#550513 BD Pharmingen	WB & IHC

Table S2. Statistical analysis

Figure	Number of Samples	Statistical Analysis	P Value
1A	Low grade DCIS n=13 High grade DCIS and IDC n=27	Wilcoxon-Mann-Whitney test	p=0.0036
1B	Low grade DCIS n=13 High grade DCIS and IDC n=27	Chi-square test of independence	p=0.0132
2A	CTL n=10 MNK1-KO n=16 (2 tumors on each animal)	Two-way ANOVA	CTL vs MNK1-KO tumor growth Week 0 - p>0.9999 Week 1 - p=0.9998 Week 2 - p=0.8747 Week 3 - p=0.0042 Week 4 - p<0.0001 Week 5 - p<0.0001
2C	CTL n=10 MNK1-KO n=10 (2 tumors on each animal)	Chi-square test of independence	CTL vs MNK1-KO %IDC - p=0.0001
2D	CTL n=10 MNK1-KO n=10 (2 tumors on each animal)	Chi-square test of independence	CTL vs MNK1-KO %Necrosis - p=0.0001
2E	CTL n=5 MNK1-KO n=5	Log-rank test	p=0.1268
2H	(Nude Mice) pBABE n=10 caMNK1 n=10	T test (do not assume same SD)	p=0.0002
2J	(Nude Mice) pBABE n=10 caMNK1 n=10	Chi-square test of independence	%Necrosis - p<0.0001 %Micrometastasis - p=0.0339
2K	(SCID Mice) pBABE n=10 caMNK1 n=10	Two-way ANOVA	pBABE vs caMNK1 Week 1-Week 5 - p>0.9999 Week 6 - p=0.9998 Week 7 - p=0.0161 Week 8 - p<0.0001
3A	Human Sample DCIS n=17 IDC n=12	Wilcoxon-Mann-Whitney test	p=0.0001
3B	(Nude Mice) CTL n=10 MNK1-KO n=10	Wilcoxon-Mann-Whitney test	p=0.0107
3C	(Nude Mice) pBABE n=10 caMNK1 n=10	Wilcoxon-Mann-Whitney test	p=0.0163

Figure	Number of Samples	Statistical Analysis	P Value
3D	4 independent experiments Number of mammospheres quantified: CTL=176 MNK1-KO=78 (spheres<0.05mm^2 are excluded from quantification)	T test (do not assume equal SD)	p<0.0001
3E	3 independent experiments 9 data points for both groups	T test (do not assume equal SD)	p=0.0401
3F	3 independent experiments 9 data points for both groups	T test (do not assume equal SD)	p=0.0059
3G	3 independent experiments Number of mammospheres quantified: CTL Vehicle=169 CTL rhNODAL=317 MNK1-KO Vehicle=204 MNK1-KO rhNODAL=227 (spheres<0.05mm2 are excluded from quantification)	One-way ANOVA	CTL Vehicle vs rhNODAL - p<0.0001 MNK1-KO Vehicle vs rhNODAL -p<0.0001 CTL Vehicle vs MNK1-KO Vehicle-p<0.0001
3H	4 independent experiments	T test (do not assume equal SD)	p<0.0001
3I	3 independent experiments	T test (do not assume equal SD)	p=0.0003
3J	3 independent experiments	One-way ANOVA	pBABE Vehicle vs SB436542 - p=0.0029 caMNK1 Vehicle vs SB436542 - p=0.0012 pBABE Vehicle vs caMNK1 Vehicle - 0.0011
4A	(Nude Mice) CTL=10 MNK1-KO=10	Wilcoxon-Mann-Whitney test	p=0.0035
4B	(Nude Mice) pBABE n=10 caMNK1 n=10	Wilcoxon-Mann-Whitney test	p=0.0094
4C	3 independent experiments 9 data points quantified for both groups	T test (do not assume equal SD)	p=0.0034
4D	3 independent experiments 9 data points quantified for both groups	T test (do not assume equal SD)	p=0.0450

Figure	Number of Samples	Statistical Analysis	P Value
4E	3 independent experiments 9 data points quantified for all groups	One-way ANOVA	pBABE siCTL vs siVIM-1 - p<0.0001 pBABE siCTL vs VIM-2 - p<0.0001 caMNK1 siCTL vs VIM-1 - p<0.0001 caMNK1 siCTL vs VIM-2 - p<0.0001 pBABE siCTL vs caMNK1 siCTL – p=0.0006
4F	3 independent experiments 9 data points quantified for all the groups	One-way ANOVA	CTL Vehicle vs rhNODAL - p=0.0102 MNK1-KO Vehicle vs rhNODAL - p=0.0013 CTL Vehicle vs MNK1-KO Vehicle- p<0.0001
4G	3 independent experiments Data points: pBABE siCTL=19 pBABE siNODAL-1=21 pBABE siNODAL-2=21 caMNK1 siCTL=20 caMNK1 siNODAL-1=20 caMNK1 siNODAL-2=16	One-way ANOVA	pBABE siCTL vs siNODAL-1 - p<0.0001 pBABE siCTL vs siNODAL-2 - p<0.0001 caMNK1 siCTL vs siNODAL-1 - p<0.0001 caMNK1 siCTL vs siNODAL-2 - p<0.0001 pBABE siCTL vs caMNK1 siCTL - p<0.0001
5A	4 independent experiments	One-way ANOVA	pBABE 0 vs 1 - p>0.9999 pBABE 0 vs 2 - p=0.0348 pBABE 0 vs 5 - p<0.0001 pBABE 0 vs caMNK1 0 - p=0.0770 caMNK1 0 vs 1 - p=0.0159 caMNK1 0 vs 2 - p=0.0011 caMNK1 0 vs 5 - p<0.0001
5B	3 independent experiments	One-way ANOVA	pBABE Vehicle vs SEL201 - p=0.2666 caMNK1 Vehicle vs SEL201 - p=0.0005 pBABE Vehicle vs caMNK1 Vehicle - p=0.0004
5C	3 independent experiments	One-way ANOVA	pBABE Vehicle vs SEL201 - p=0.2005 caMNK1 Vehicle vs SEL201 - p<0.0001 pBABE Vehicle vs caMNK1 Vehicle - p<0.001
5D	3 independent experiments	One-way ANOVA	pBABE Vehicle vs SEL201 - p=0.0031 caMNK1 Vehicle vs SEL201 - p=0.0024 pBABE Vehicle vs caMNK1 Vehicle - p<0.0001
5E	Vehicle n=12 SEL201 n=12 (2 tumors on each animal)	Two-way ANOVA	Vehicle vs SEL201 Week 0 - p>0.9999 Week 1 - p=0.9995 Week 2 - p=0.0293 Week 3 - p=0.0412
5F	Vehicle n=12 SEL201 n=12 (2 tumors on each animal)	Chi-square test of independence	% IDC - p<0.0001 %Necrosis - p=0.0002
S1B	Low grade DCIS n=6 High grade DCIS and IDC n=23	T test (do not assume equal SD	p=0.00059
S1C	Low grade DCIS n=6 High grade DCIS and IDC n=23	Chi-square test of independence	p=0.2885
S2B	3 independent experiments	Two-way ANOVA	CTL vs MNK1-KO Clone 1 Day 1 - p=0.8609 Day 3 - p<0.0001 Day 5 - p<0.0001 CTL vs MNK1-KO Clone 2 Day 1 - p=0.8794

Figure	Number of Samples	Statistical Analysis	P Value
			Day 3 - p<0.0001 Day 5 - p<0.0001
S2C	3 independent experiments	One-way ANOVA	CTL vs MNK1-KO Clone 1 p<0.0001 CTL vs MNK1-KO Clone 2 p<0.0001
S2F	3 independent experiments	Two-way ANOVA	pBABE vs caMNK1 p>0.9999 at all time points
S2G	3 independent experiments	T test (do not assume equal SD)	p=0.0213
S2J	Phospho-Histone H3 pBABE=8 caMNK1=8 Ki67 pBABE=4 caMNK1=4 Cleaved caspase 3 pBABE=9 caMNK1=10	Wilcoxon-Mann-Whitney test	pBABE vs caMNK1 Phospho-Histone H3 - p=0.9977 Ki67 - p=0.9714 Cleaved caspase-3 - p=0.676
S3F	3 independent experiments for all panels	T test (do not assume equal SD)	66cl4 CTL vs MNK1-KO: Mammosphere size p<0.0001 Colony formation p<0.0001 Invasion p<0.0001
S5B	Vehicle n=6 SEL201 n=6 (2 tumors on each animal)	Two-way ANOVA T test (do not assume equal SD)	Vehicle vs SEL201 Body weight (Two-way ANOVA) - ns ALT - p=0.2477 AST - p=0.9576
S5E	Vehicle n=4 SEL201 n=6	Chi-square test of independence	p=0.1084
S5G	3 independent experiments	One-way ANOVA	SEL201 0 vs 1 μM p=0.0001 SEL201 0 vs 2 μM p<0.0001 SEL201 0 vs 5 μM p<0.0001

Table S3. Metastasis data for CTL versus MNK1-KO mice.

Group	Animal ID	Survival Duration (Days)	Reason for Death	Necropsy/Histology Findings
CTL	B3231	62	Metastasis	Lung metastasis. Metastasis infiltrating kidney capsule.
	B3232	83	Metastasis	Metastasis found in the abdominal cavity, near the small bowel.
	B3233	99	Metastasis	Metastasis found in the abdominal cavity, near the pancreas.
	B3234	97	Metastasis	Metastasis found near the kidneys.
	B3235	105	Metastasis	Metastasis found in the lungs, chest cavity, small bowel and abdominal cavity. Metastasis found adjacent to abdominal smooth muscles and ribs.
MNK1-KO	B3227	56	Unknown	Found dead on day 56. Necropsy was not performed.
	B3227	140	Sacrificed at end of the experiment	No metastasis was found.
	B3229	140		
	B3230	140		
	B3237	140		