Supplemental Materials and Methods

SUM1315 Cell Culture

SUM 1315 breast cancer cells were obtained from ATCC (Manassas, VA). Tissue culture incubator environment was maintained at 37°C, 5% CO_2, with 90% humidity. Cells were grown in media consisting of a 50-50 mixture of DMEM and F12 media, supplemented with 5% fetal bovine serum, 10ng/ml EGF, 5μg/ml insulin, and 1% penicillin/streptomycin. Upon reaching confluency cells were passaged every 2-3 days using 0.25% trypsin (Genesee Scientific, San Diego, CA).

Stable SUM1315 cell lines were developed that reliably expressed a short hairpin RNA (shRNA) against TWIST1 (shTwist419, shTwist494), or a scrambled control shRNA (shScram) as a negative control. These SUM1315 cell lines were created as previously described.[1]

RNA-Seq

Cell pellets from SUM1315-shTwist419, SUM1315-shTwist494, and SUM1315-shScram were collected and immediately processed for RNA extraction. Total cellular RNA was isolated using the RNeasy Plus kit (Qiagen, Valencia, CA). 10 μg of total RNA was then resuspended in nuclease free water and poly (A) enriched to remove ribosomal RNA. Samples were processed using the Illumina HiSeq 2500 (Illumina, San Diego, CA). Resulting raw RNA-seq data was first aligned using TopHat (version 2.0.8, Center for Computational Biology, Johns Hopkins University) followed by counting and expression scoring with Cufflinks (version 2.02) as described previously.[2]

Immunohistochemistry

To confirm that nests of cells in lung fields were metastatic lesions 5μm sections of all paraffin imbedded tissues were cut and stained with 1:3000 rabbit polyclonal GFP antibodies (Ab290,

Abcam, Cambridge, MA). Sections were incubated at room temperature for 30 minutes. An HRP-conjugated goat anti-rabbit IgG was used as the secondary antibody.

MTT Assay

The tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to assess cell death following treatment with various concentrations of MSN+siRNA. A total of 5,000 MDA-MB-435S GFP+ffluc were placed in each well of a 96-well tissue culture plate and allowed to attach over 24 hours. Next the cells were treated with either ½x, 1x, 2x, 5x, or 10x the typical MSN+siRNA concentration (final concentration for of MSNs and siRNA applied to cells was 17.5 ng/ul and 50 nM, respectively) for 24 hours. Following this incubation period media was removed from each well and 110 µl MTT diluted in complete media (0.45 mg/ml) was added. Incubation lasted for 3 hoursat 37°C, 5% CO_2, with 90% humidity. Following the incubation period the MTT media was removed and 110 µl DMSO was added to each well and the plate was gentle shaken for 15 mins. Dye intensity for each well was then read at a wavelength of 580 nm.

References

1. Li S, Kendall SE, Raices R, et al. TWIST1 associates with NF-kappaB subunit RELA via carboxyl-terminal WR domain to promote cell autonomous invasion through IL8 production. *BMC biology*. 2012; **10**: 73.
2. Trapnell C, Roberts A, Goff L, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature protocols*. 2012; **7**: 562-78.

Fig S1

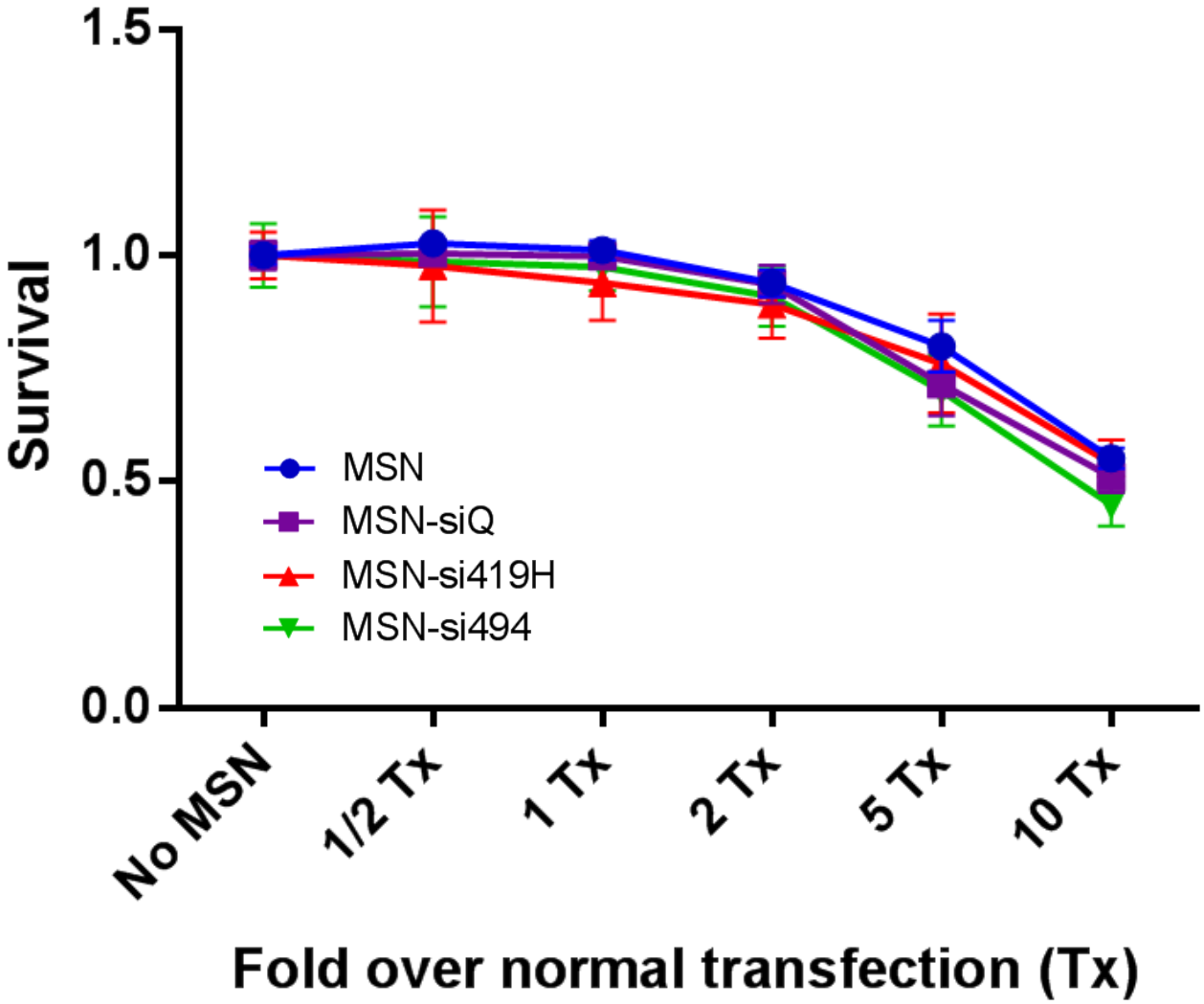


Figure S1. MTT assay demonstrates that at concentrations used for transfection in vitro (1 Tx) siRNA-MSN complexes are not inherently cytotoxic, and do not show appeciable cell death until dose is increased five fold. This phenomenon was independent of siRNA sequence.

Fig S2

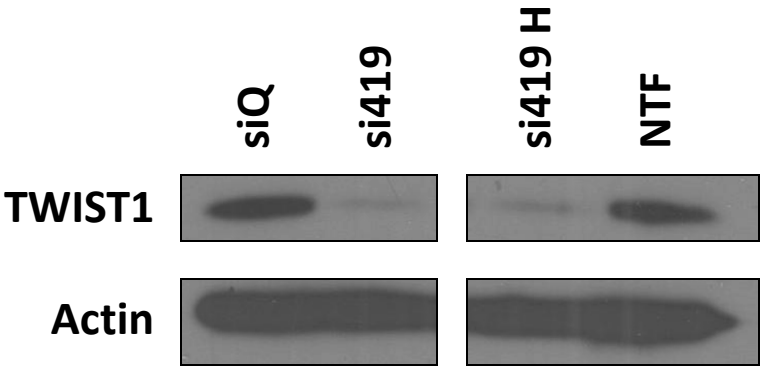


Figure S2. Chemical modification of si419 does not significantly change knockdown efficency. Unmodified si419 and hybrid si419 containing only the passenger strand modifications (H) each show robust and approximately equal knockdown. Therefore, si419 hybrid was used throughout this work.

Fig S3

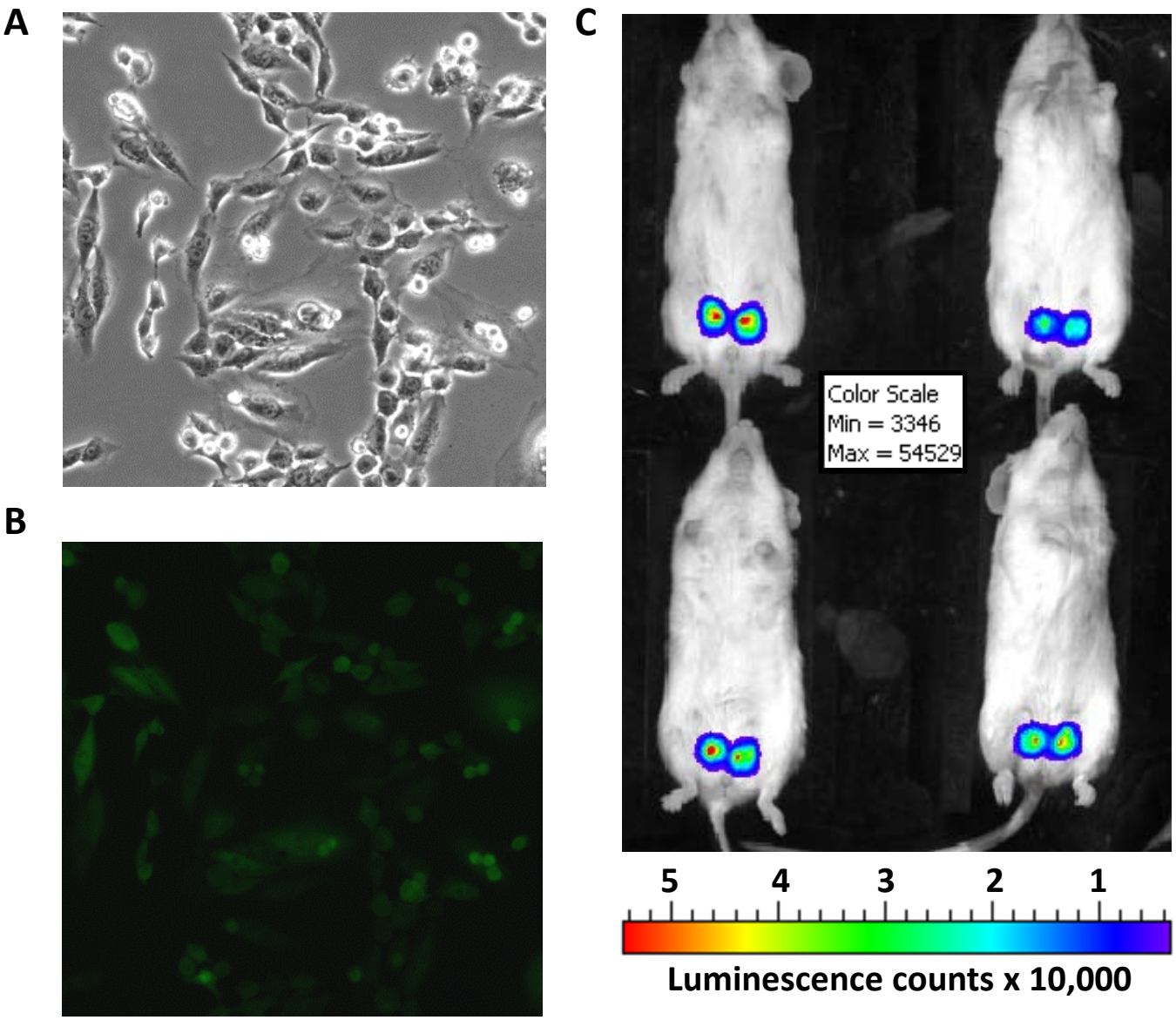


Figure S3. MDA-MB-435S cells express GFP and firefly luciferase. A. Phase contrast and B. green channel images of MDA-MB-435S cells. C. Expression of firefly luciferase is evidenced by strong signal following D-luciferin injection in mice (Xenogen IVIS, STARR)

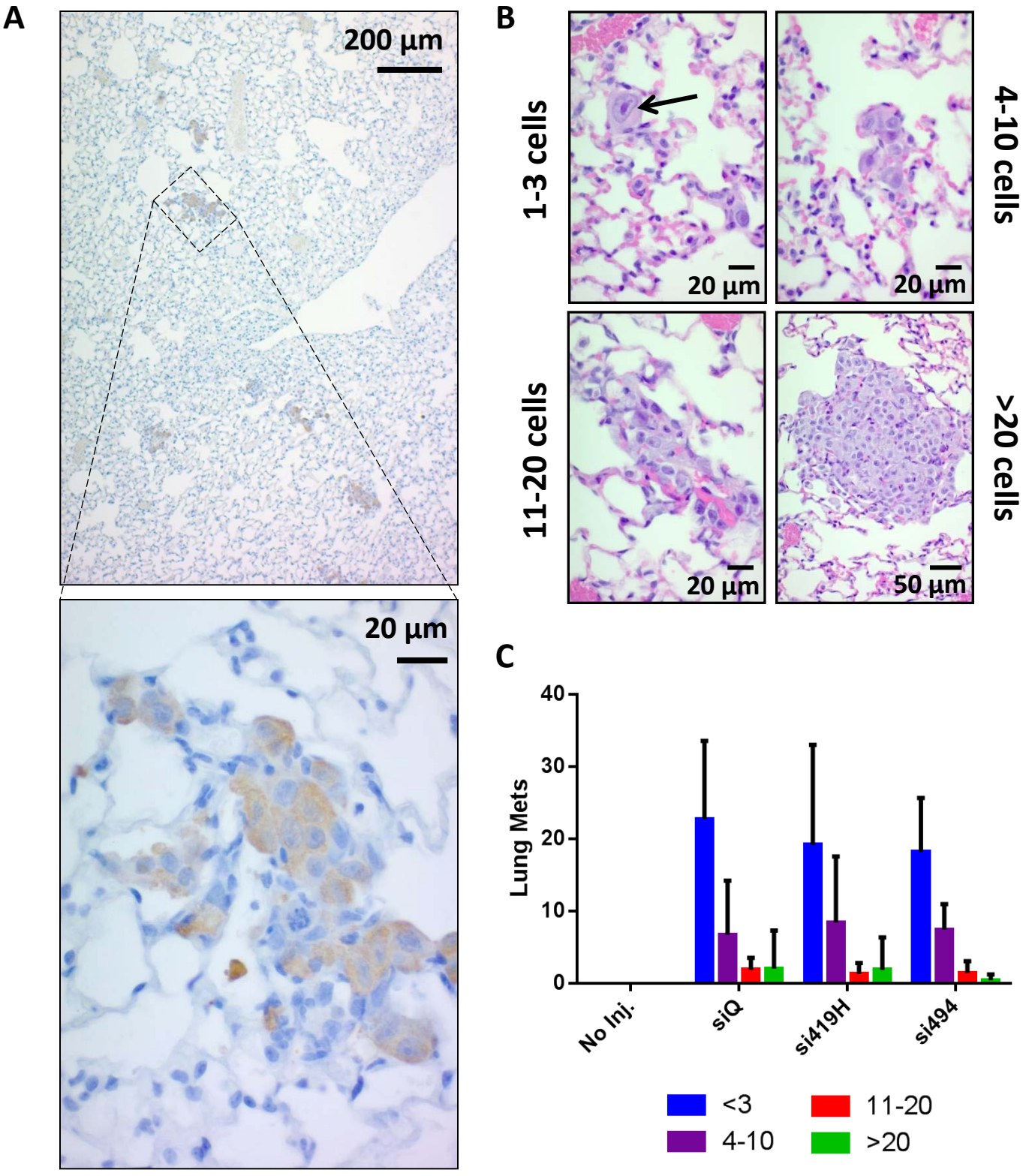
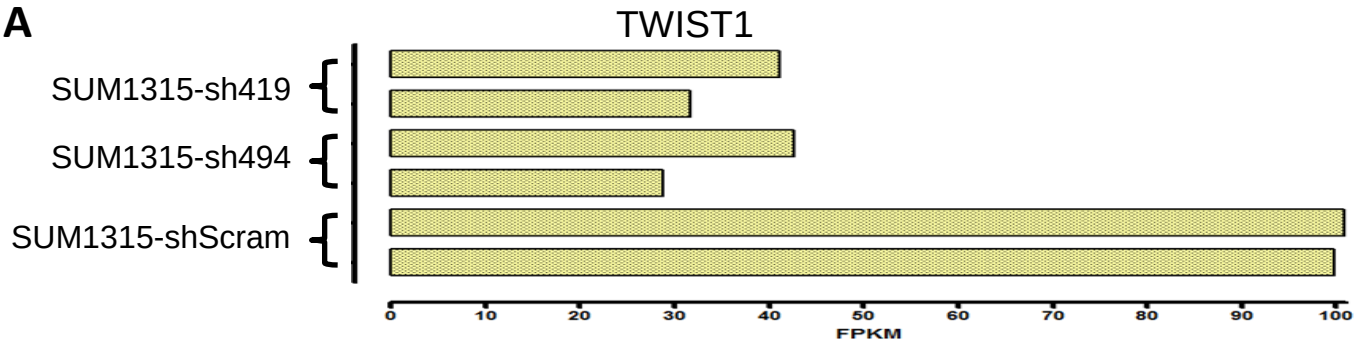
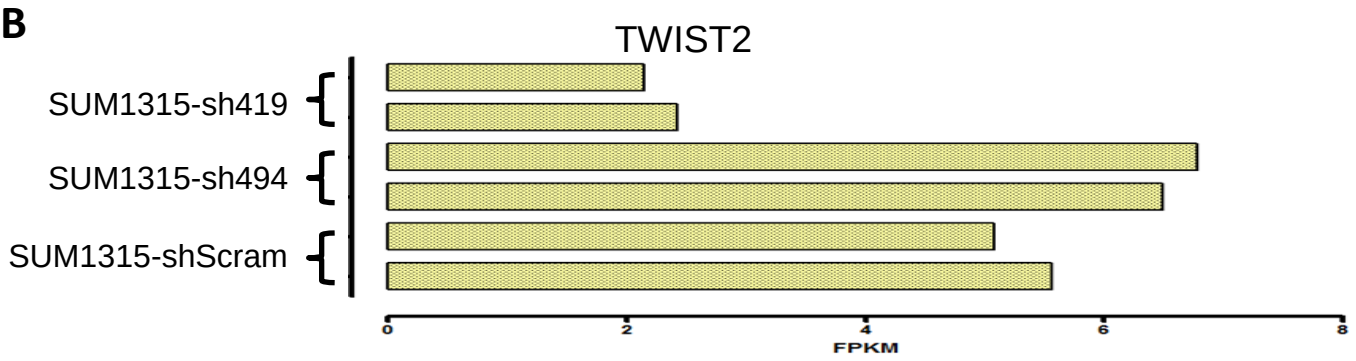


Figure S4. MDA-MB-435S cells metastasized to the lungs in mice. A. MDA-MB-435 cells stain positive for GFP in GFP negative lung tissue. B. Metastases were grouped into one of four categories on the basis of cell number. C. Treatment had no significant effect on numbers of metastases. We speculate this is due to delay between tumor seeding and commencement of therapy. No inj, no injection.

A



B



C

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TWIST1   MMQDVSSSPVSPADDSLSNSEEEPDRQQPPSGKRGGRKRRSSRRSAGGGAGPG
           M++ SSSPVSP  DSL  SEEE +RQ     KR GRKRR S++S+
TWIST2   -MEEGSSSPVSPV-DSLGTSEEELERQ----PKRFGRKRRYSKKSS-------

TWIST1   GAAGGGVGGGDEPGSPAQGKRGKKSAGCGGGGGAGGGGGSSSGGGSPQSYEEL
                    E GSP  GKRGKK                  G  S QS+EEL
TWIST2   ----------EDGSPTPGKRGKK------------------GSPSAQSFEEL

TWIST1   QTQRVMANVRERQRTQSLNEAFAALRKIIPTLPSDKLSKIQTLKLAARYIDFL
         Q+QR++ANVRERQRTQSLNEAFAALRKIIPTLPSDKLSKIQTLKLAARYIDFL
TWIST2   QSQRILANVRERQRTQSLNEAFAALRKIIPTLPSDKLSKIQTLKLAARYIDFL

TWIST1   YQVLQSDELDSKMASCSYVAHERLSYAFSVWRMEGAWSMSASH
         YQVLQSDE+D+KM SCSYVAHERLSYAFSVWRMEGAWSMSASH
TWIST2   YQVLQSDEMDNKMTSCSYVAHERLSYAFSVWRMEGAWSMSASH
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Figure S5. RNA-seq performed on SUM1315 triple negative breast cancer cells stably expressing shRNA against TWIST or control (shScram). A. TWIST1 expression is reduced by sh419 and sh494. B. TWIST2 is only knocked down by sh419. C. BLAST sequence alignment of TWIST1 and TWIST2 proteins. They share great sequence homology and some similar functions. FPKM, fragments per kilobase per million fragments read.

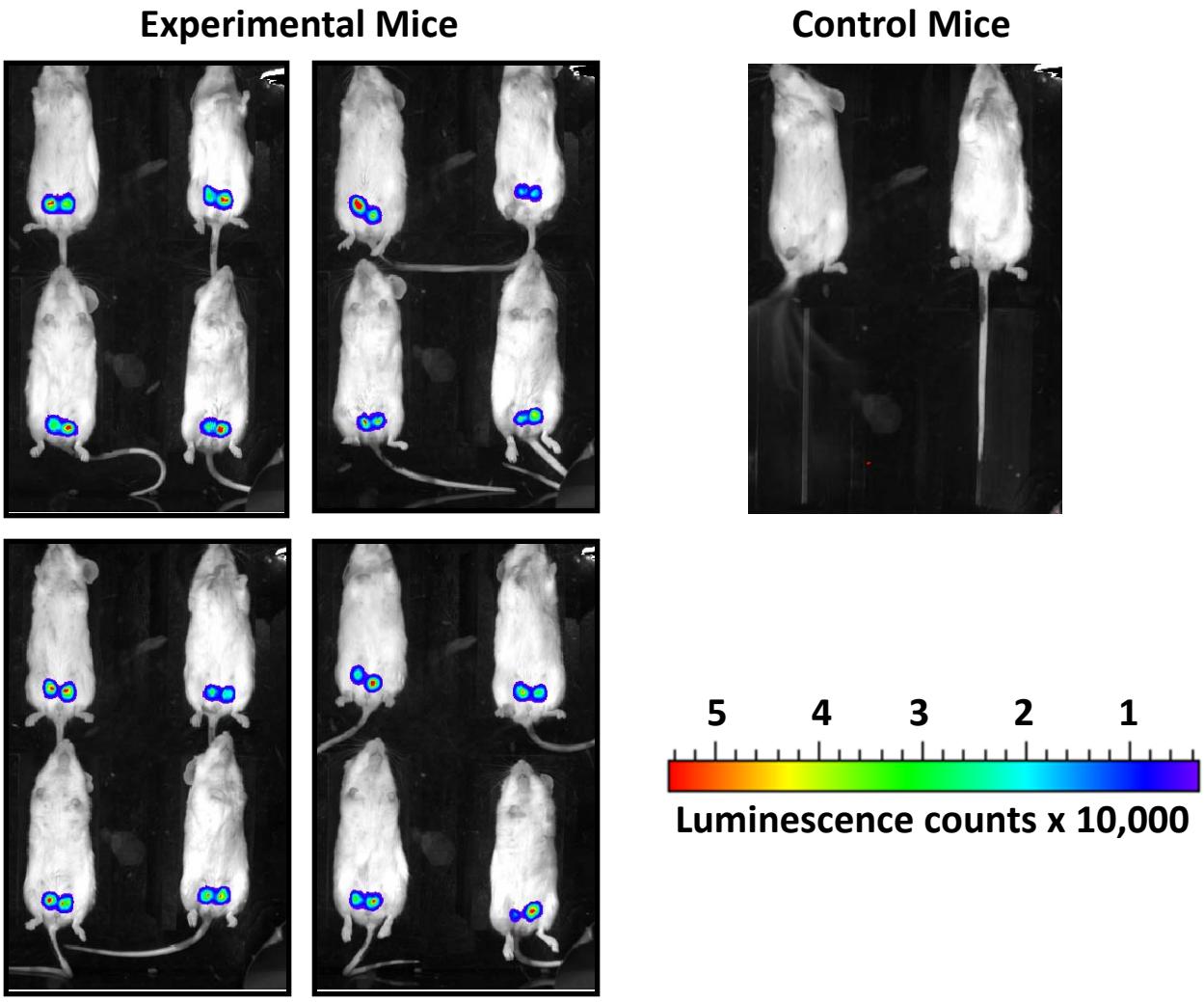
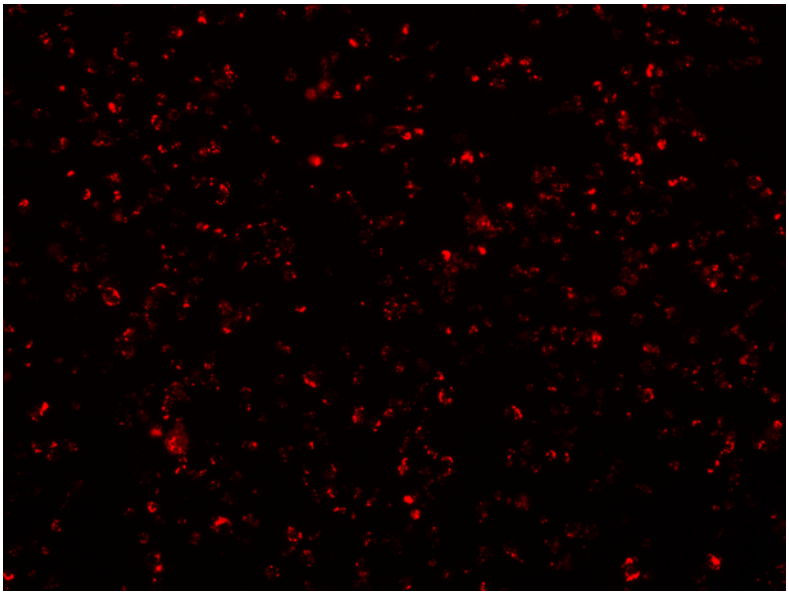


Figure S6. Tumor sizes before MSN+siRNA Injections. Bilateral tumors for the 16 mice experimental mice were all roughly the same size by bioluminescent imaging and by manual palpation. Slight variations in bioluminescent intensity can be attributed to differences in tumor vasculature and profusion. Control mice (no tumor cells) exhibited no bioluminescent signal. Prior to MSN+siRNA injections mice were randomly assigned to a treatment group.



MSN+siQ

siQ Only

Figure S7. Naked siRNA does not enter MDA-MB-435S cells. Naked siQ (with no MSN carrier) was incubated with cells in identical conditions to those of MSN+siQ. Fluorescent images revealed no siQ delivery without the MSN carrier.