Supplemental Material

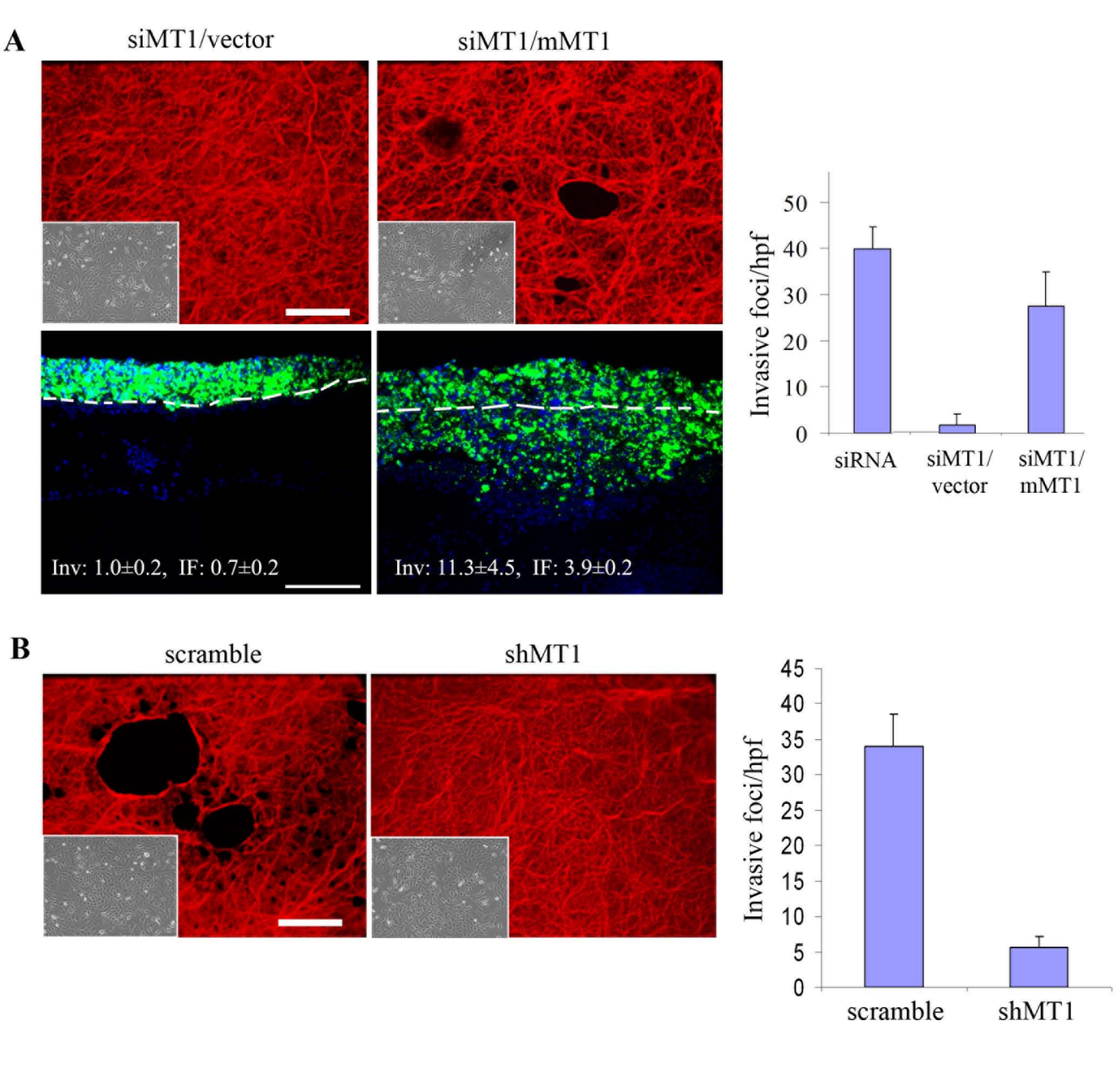
S**upplementary Figure 1.**

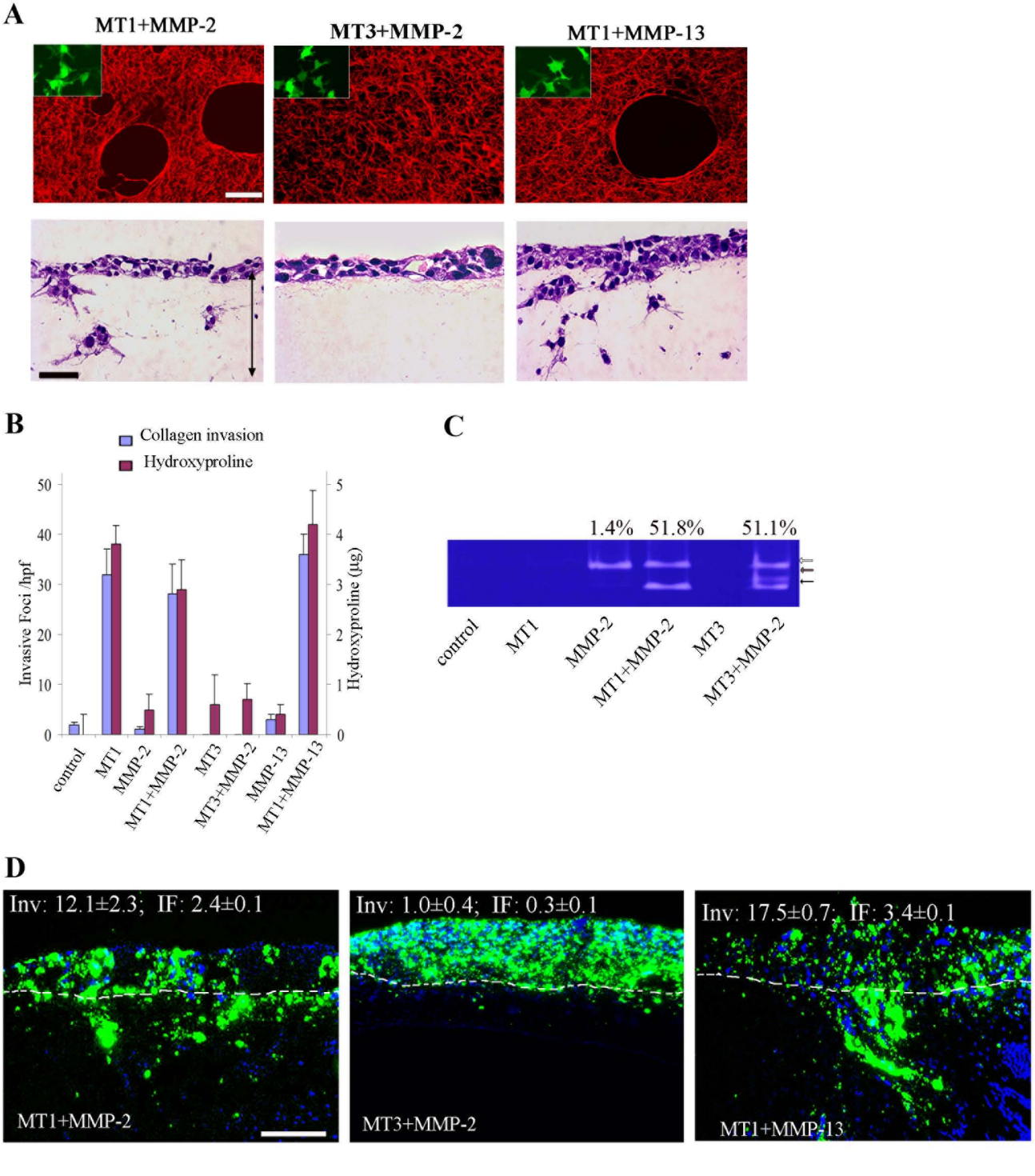
Rescue of the invasive activity of MT1-MMP siRNA–silenced HT-1080 cells. (A) HT-1080 cells were co-transfected with human MT1-MMP-specific siRNA (siMT1) and mouse MT1-MMP expression vectors (mMT1) or mock (vector). Transfectants were then cultured for 3d atop type I collagen films labeled with Alexa-Fluor-594 (red) to assess subjacent proteolysis (top row of left panel, bar, 50 μm). Inserts show the morphology of adherent cells atop the underlying collagen film as assessed by phase contrast microscopy. Collagen invasion is expressed as invasive foci/hpf (right panel, mean ± SEM of 5 randomly selected cross-sections in a single representative experiment of 3 performed). HT-1080 transfectants were also labeled with fluorescent nanobeads (green) and cultured atop the 11-d old chicken CAM. Cross-sections were prepared following a 3 d incubation, and examined by fluorescent microscopy (bottom row; blue, Dapi; green, tumor cells). The dashed lines shown in each image represent the outline of CAM surface. Invasion is quantified as number of HT1080 cells that cross the CAM surface [mean invasion (Inv) ± SEM of 3 or more experiments) and average depth of the leading front of the invasive cells (mean invasion front (IF) ± SEM; bar, 100 μm]. **(B)** HT1080 cells were transfected with a pSuper-based MT1-MMP specific shRNA (shMT1, target sequence: ggcacacaaacgaggaatgag) and a scramble control (scramble, Oligoengine). Transfectants were cultured atop a labeled collagen film (left) to assess subjacent proteolysis (bar, 50 μm). Collagen invasion is expressed as invasive foci/hpf in the right-hand panel.

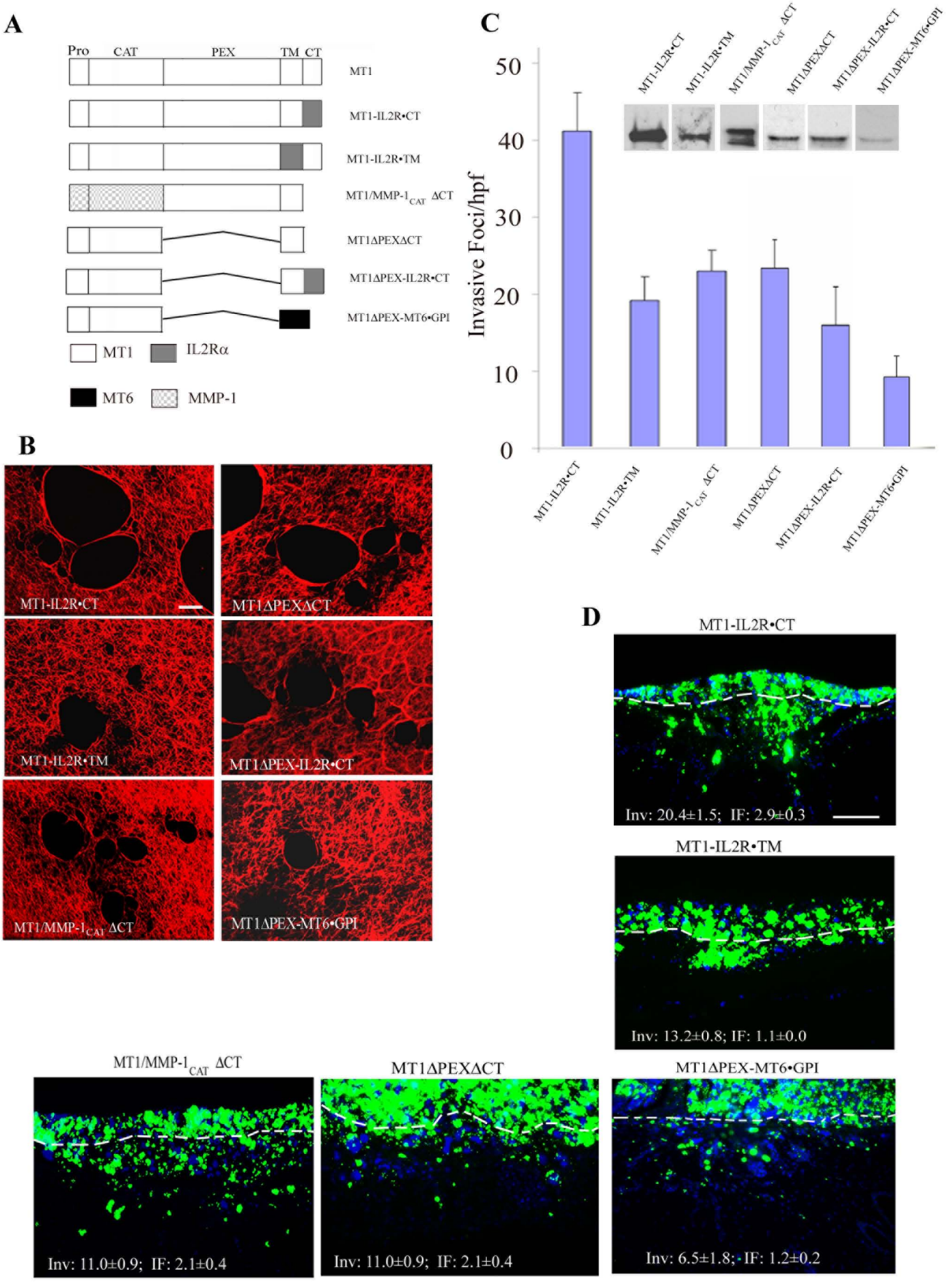
Supplementary Figure 2. MT1-MMP-Dependent Subjacent Collagenolysis and Invasion. (A,B) COS cells were transfected with expression vectors encoding MT1-MMP (MT1), MT3-MMP (MT3), MMP-2 or MMP-13 alone, or co-transfected with MT1-MMP and MMP-2, MT3-MMP and MMP-2, or MT1-MMP and MMP-13 expression vectors. Transfectants were then cultured for 3 d atop type I collagen films to assess subjacent proteolysis (top row) or 3-D type I collagen gels to monitor invasive activity (bottom row). Representative laser confocal micrographs for subjacent proteolysis of Alexa Fluor-594 (red)-labeled collagen film and H&E-stained cross-sections for 3-D collagen gel invasion by COS cells co-transfected with MT1-MMP and MMP-2, MT3-MMP and MMP-2 or MT1-MMP and MMP-13 expression vectors are shown. Inserts display the GFP-labeled transfectants (green) adherent to the underlying collagen film (Bar, 50 μm). Collagenolytic activity was quantified as μg hydroxyproline released (mean ± SEM; n=3) while invasion is expressed as the number of invasive foci/hpf (mean ± SEM of 5 randomly selected cross-sections in a single representative experiment of 3 or more performed). (C) Gelatin zymography of supernatants recovered from COS cells transfected with control-, MT1-MMP-, MMP-2-, MT1-MMP- and MMP-2-, MT3-MMP-, or MT3-MMP- and MMP-2- expression vectors. The pro, intermediate and mature forms of MMP-2 are indicated by the clear, gray and black arrows, respectively. Results are quantified with ImageQuant5.2 and expressed as the percent activated MMP-2. (D) COS cell transfectants were labeled with fluorescent nanobeads (green) and seeded atop the CAM of 11-d old chicks. Following a 3 d incubation period, cross-sections were prepared, nuclei stained with DAPI (blue) and examined by fluorescence

microscopy. The dashed line shown in each image represents the outline of the CAM surface. Invasion is quantified as the percent COS cells that cross the CAM surface [mean invasion (Inv) ± SEM of 3 or more experiments] and average depth of the leading front of the invasive cells (mean IF ± SEM). Bar, 100 μm.

Supplementary Figure 3. MT1-MMP Cytoplasmic Domain-Dependent Regulation of Proteolytic and Invasive Activities. (A) A schematic diagram of MT1-MMP deletion mutants and chimeras. (B) Representative laser confocal images of subjacent collagen degradation mediated by each of the construct-transfected COS cells. (C) The relative cell surface expression levels of the MT1-MMP variants as assessed following surface biotinylation were 145%, 77%, 76%, 50%, 66% and 33% of wt MT1-MMP as quantified by ImageQuant5.2, respectively. Quantification of invasive activity was expressed by each of the MT1-MMP deletion mutants and chimeras cultured atop 3-D gels of type I collagen. Results are expressed as mean ± SEM of a representative single experiment of 3 or more performed. (D) *In vivo* invasive activity of COS cell transfectants as assessed in the CAM system following a 3 d culture period. Results are the mean ± SEM of 3 or more experiments. Bar, 100 μm.


A
siMT1/vector
siMT1/mMT1
Inv: 1.0±0.2, IF: 0.7±0.2
Inv: 11.3±4.5, IF: 3.9±0.2
Invasive foci/hpf
50
40
30
20
10
0
siRNA
siMT1/ vector
siMT1/ mMT1
B
scramble
shMT1
Invasive foci/hpf
45
40
35
30
25
20
15
10
5
0
scramble
shMT1


A
MT1+MMP-2
MT3+MMP-2
MT1+MMP-13
B
Collagen invasion
Hydroxyproline
Invasive Foci /hpf
Hydroxyproline (μg)
control
MT1
MMP-2
MT1+MMP-2
MT3
MT3+MMP-2
MMP-13
MT1+MMP-13
C
1.4%
51.8%
51.1%
control
MT1
MMP-2
MT1+MMP-2
MT3
MT3+MMP-2
D
Inv: 12.1±2.3; IF: 2.4±0.1
MT1+MMP-2
Inv: 1.0±0.4; IF: 0.3±0.1
MT3+MMP-2
Inv: 17.5±0.7; IF: 3.4±0.1
MT1+MMP-13


A
Pro
CAT
PEX
TM
CT
MT1
MT1-IL2R•CT
MT1-IL2R•TM
MT1/MMP-1CAT ΔCT
MT1ΔPEXΔCT
MT1ΔPEX-IL2R•CT
MT1ΔPEX-MT6•GPI
MT1
IL2Rα
MT6
MMP-1
C
MT1-IL2R•CT
MT1-IL2R•TM
MT1/MMP-1CAT ΔCT
MT1ΔPEXΔCT
MT1ΔPEX-IL2R•CT
MT1ΔPEX-MT6•GPI
Invasive Foci/hpf
0
10
20
30
40
50
B
MT1-IL2R•CT
MT1ΔPEXΔCT
MT1-IL2R•TM
MT1ΔPEX-IL2R•CT
MT1/MMP-1CAT ΔCT
MT1ΔPEX-MT6•GPI
D
MT1-IL2R•CT
Inv: 20.4±1.5; IF: 2.9±0.3
MT1-IL2R•TM
Inv: 13.2±0.8; IF: 1.1±0.0
MT1/MMP-1CAT ΔCT
Inv: 11.0±0.9; IF: 2.1±0.4
MT1ΔPEXΔCT
Inv: 11.0±0.9; IF: 2.1±0.4
MT1ΔPEX-MT6•GPI
Inv: 6.5±1.8; IF: 1.2±0.2