SUPPLEMENTARY INFORMATION

Supplemental Table 1. Flow Properties of Collagen Gels and Cell Suspensions for Renal Cell Carcinoma cell lines utilized in the 4 hour flow experiments (n=14-16).*

	Pressure, ΔP (cm H_20)	Flow Rate, Jv (µl/min)	Velocity, U_∞ (µm/sec)	Permeability, Kp (10^{-15} m^2)	Shear Stress, τ (dynes/cm^2)
SN12C	1.0	4.20 ± 0.43	0.81 ± 0.08	4.04 ± 0.42	0.84
	3.0	6.30 ± 1.40	1.21 ± 0.27	2.02 ± 0.45	2.53
SN12L1	1.0	4.17 ± 0.47	0.80 ± 0.09	4.01 ± 0.45	0.84
	3.0	5.88 ± 0.77	1.13 ± 0.15	1.88 ± 0.25	2.53

* Darcy permeability is obtained from the definition: $K_p = \mu U_\infty L/\Delta P$, where $U_\infty = Jv/A$; viscosity of perfusate (μ) - 0.84 cP; Area of filter (A) - 0.865 cm^2; Thickness of gel (L) - 600 µm; Glycocalyx thickness (H) - 0.5 µm; shear stress on the cell surface ($\tau = H \Delta P/L$) comes from equation 1 substituting the definition of K_p. Variable data presented as mean ± standard deviation. Flow rate, velocity of flow, gel permeability and shear stress differ significantly between the two hydrostatic pressures applied ($p < 0.005$).

Supplemental Table 2. Primer sequences of human genes used in RT-PCR.

Gene	Forward sense (5'-3')	Reverse antisense (5'-3')	Length (BP)	Reference (NM)
MMP-1	TGA GGG GAA CCC TCG CTG GG	TCC CCT CCA ATA CCT GGG CCT G	274	002421.3
MMP-2	GTC TGT GTT GTC CAG AGG CA	ATC ACT AGG CCA GCT GGT TG	110	001127891.1
CD44	CTG TGG AGG ACA GAA AGC CAA	CAA ATG CAC CAT TTC CTG AGA C	68	000610.3
A3-Integrin	GAG ATC ACC GTC CAT GGC A	GGT TGA TAA GGT CTC CAG GTG G	61	002204.2
Caveolin-1	CTA CAA GCC CAA CAA CAA GGC	GGT GTG CGC GTC GTA CAC T	67	001753.4
GAPDH	CCT GAC CTG CCG TCT AGA AA	TTA CTC CTT GGA GGC CAT GT	267	002046.3

Supplemental Table 3. List of inhibitors and antibodies utilized in this study

Inhibitor/Antibody	Concentration	Product Code	Source	Reference
MMP	10 μM	GM6001	Calbiochem	6
MMP-NC	10 μM	GM6001-NC	Calbiochem	6
CD44	2 μg/ml	DF1485	Santa Cruz Biotechnology	15
α3 integrin	1 μg/ml	P1B5	Chemicon International	15
IgG1 isotype	1-5 μg/ml	CBL610	Millipore	15
Heparinase III	1 IU/L	37290-86-1	IBEX Technologies	26
Hyaluronidase	1.5 U/ml	H1136	Sigma	36

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Modified Boyden chamber experimental apparatus and protocol for investigating invasion potential in response to interstitial flow and shear stress in 3D assays. (1) Tumor cells were suspended in collagen and incubated overnight to permit cell spreading and formation of cell/matrix interactions. (2) Syringe pumps, set at constant flow rates, were connected to the flow apparatus and applied a constant hydrostatic pressure [ΔP] across the gel [L]. Tumor cells in 3D were exposed to either 4 or 24 hours of flow that exerted shear stress on the cell membranes. Flow media filtrate was collected in a separate reservoir that did not affect flow velocities. (3) At the end of the flow period, all gels were compacted and flushed to remove accumulation of MMPs before the invasion assay. The inserts containing the cell suspensions were decoupled from the flow apparatus before the invasion assay ensued. (4) During the 24 or 48 hour migration period (approximately the same total experiment time), 1 nM TGF-α directed cells to migrate through 8 μm pores towards the underside of the filter. At the end of the migration period, cells on the underside of the inserts were stained and counted to quantify migration rates.

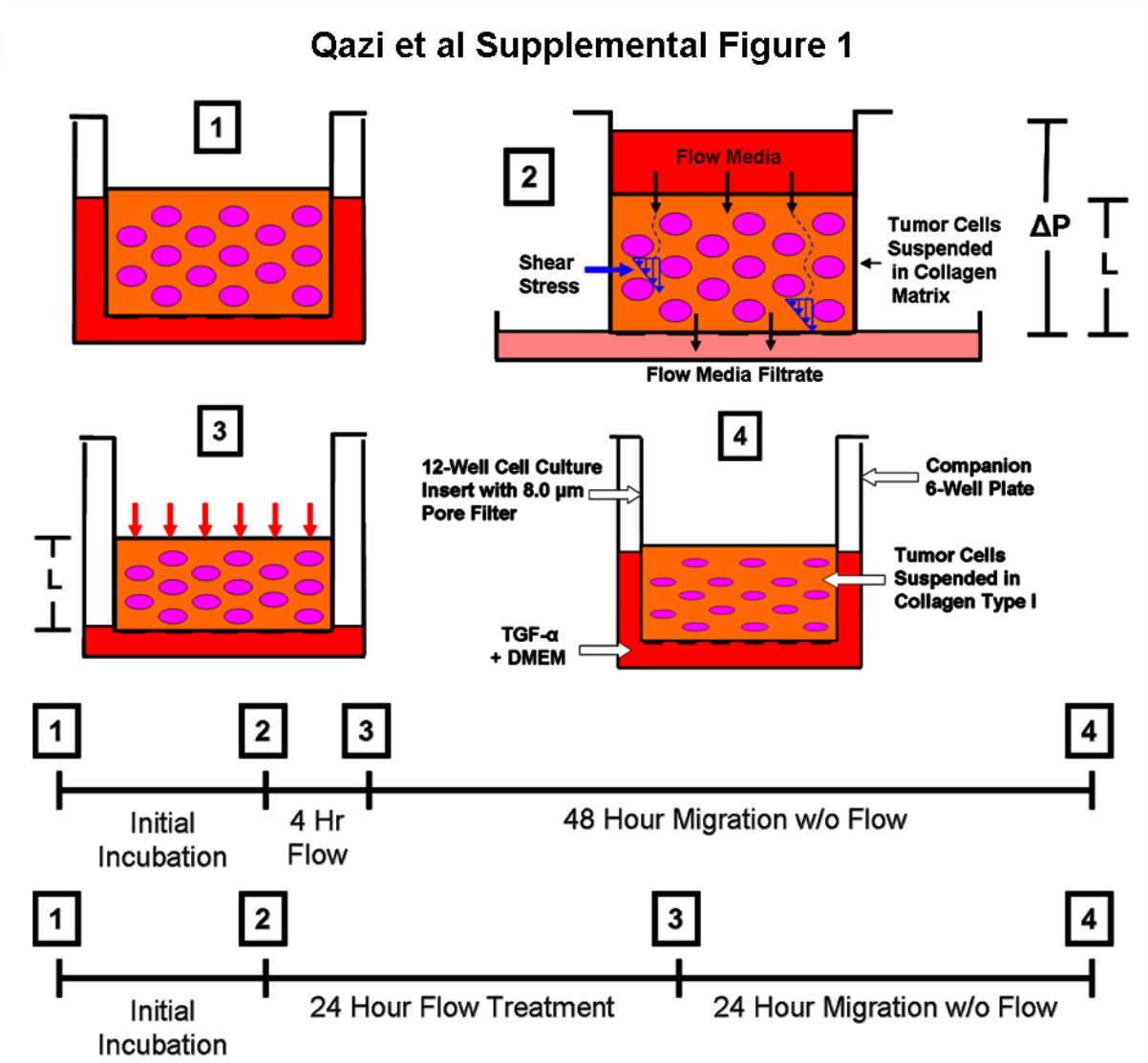
Supplemental Figure 2. Protease levels are affected by flow through the gel, the "flushing effect", requiring changes in protocol. Longer term, 24-hour, flow through gels caused MMPs to be flushed out of the gel during the flow period (A,B). (C,D) A short 10 minutes of flushing of the no-flow control gels at the end of the flow period removed any protease level discrepancies. Zymography of Protease expression in gels at the end of the 24 hour flow period before (B) and after (D) flushing the control gels. Note: * $p < 0.005$; † $p > 0.05$; N = 5-8.

Supplemental Figure 3. Migration response of metastatic MDA-MB-435S melanoma cells. All results were normalized to no-flow controls (1.0). Migration was enhanced (increased invasion potential) with exposure to shear stresses of 0.84 dynes/cm^2 and 2.53 dynes/cm^2 applied for 1 hour - comparable to the SN12L1 cells (Figure 1A). Note: * $p < 0.05$; N = 4-8.

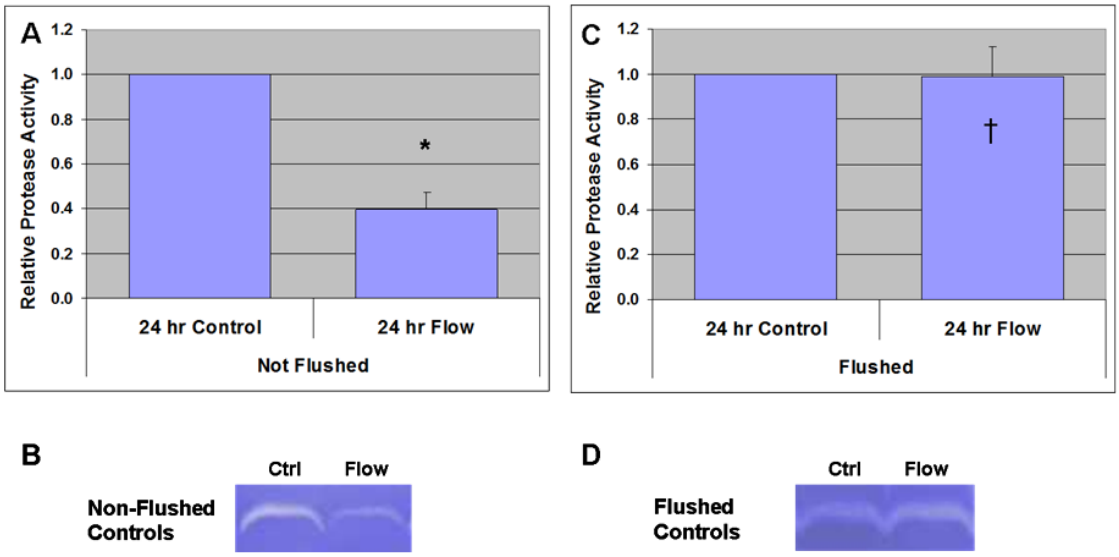
Supplemental Figure 4. Verification of IgG1 isotype interaction in the controls for antibody usage and baseline migration rates of the no-flow control cases when treated with blockers. All results were normalized to no-flow controls (1.0). (A) There was no evidence of non-specific binding of the IgG1 isotype (negative control for blocking antibodies) in any case when compared to their respective non-isotype control. Flow-enhanced migration rates were also consistently observed with the use of the IgG isotype. (B) Effect of blocking MMPs, CD44, and α3 integrin on the baseline (no-flow control) migration of SN12L1 cells. There was no change in the baseline migration rates brought

on by the use of the blockers except for with the use of MMP inhibitor suggesting that baseline invasion was mediated by MMPs. Note: ** $p < 0.005$; † $p > 0.05$; N = 3-10.

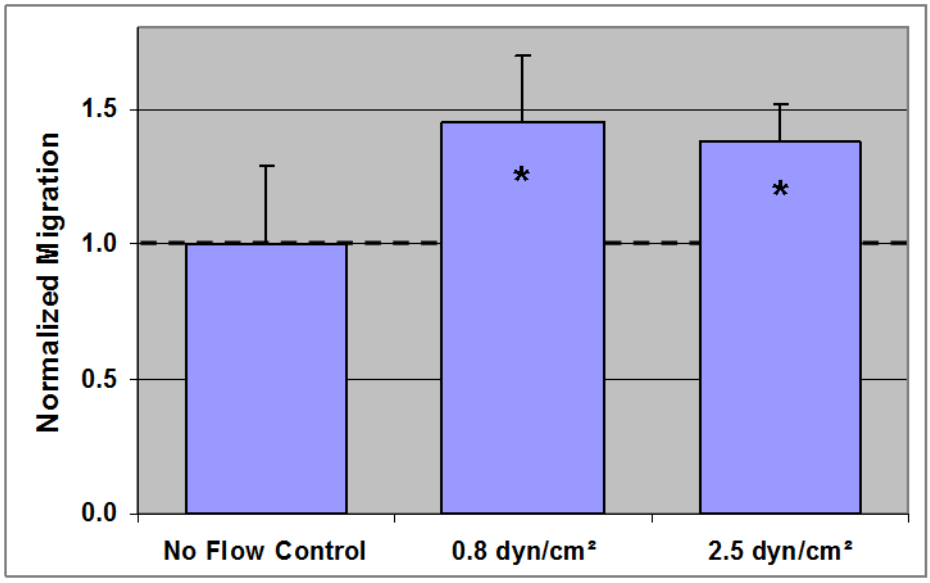
Supplemental Figure 5. Heparin sulfate staining of aortic smooth muscle cells in 3D collagen gels. (A) Immunostaining of single smooth muscle cells shows that a layer of heparan sulfate proteoglycans surrounds the cell. (A-B) Heparan sulfate proteoglycans are present in 3D cell/collagen suspensions. (C-D) Treatment with 16 hours of heparinase III successfully breaks down heparan sulfate glycosaminoglycans. Note: heparan sulfate stained green, and the nuclei - blue.



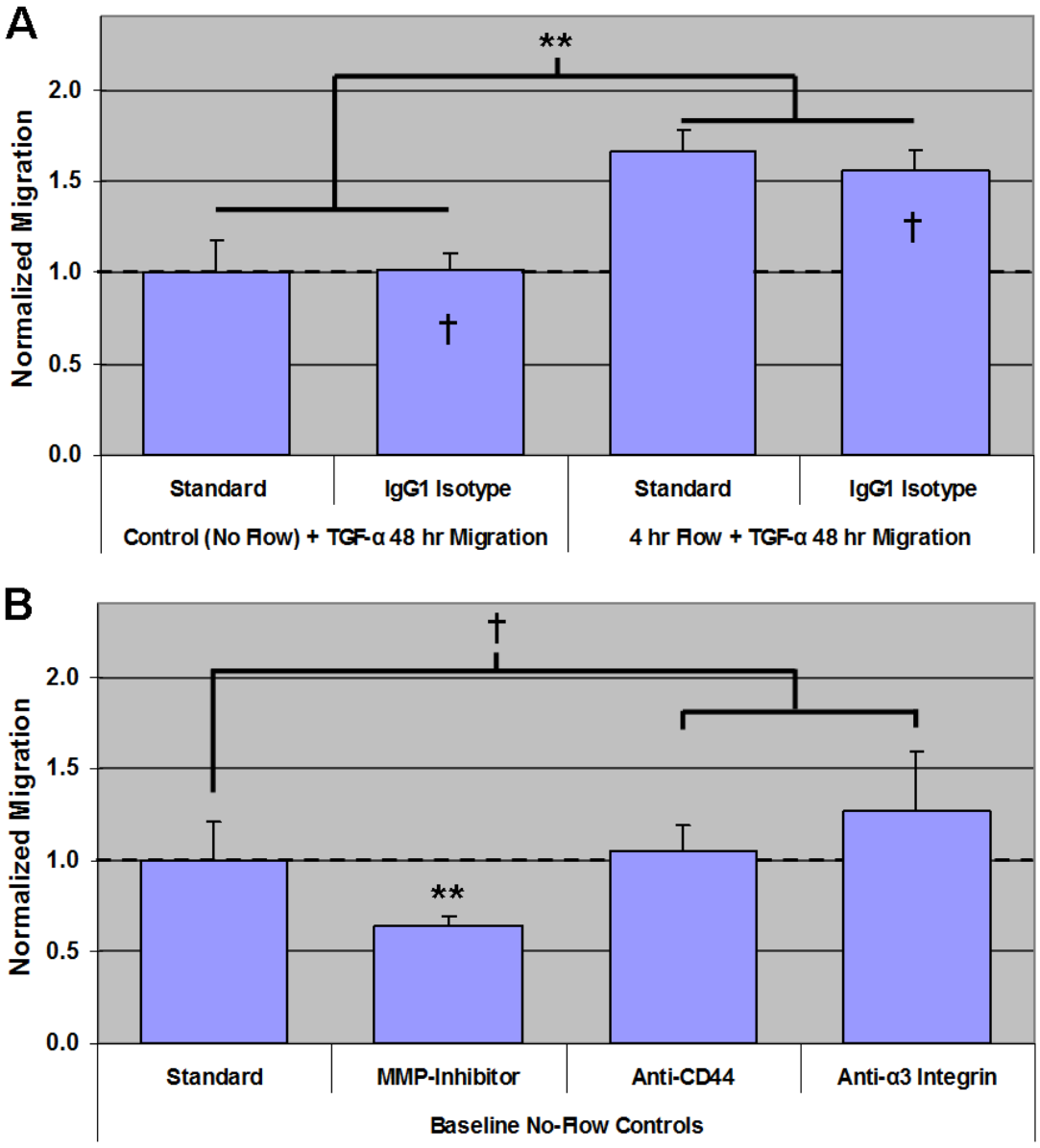
Qazi et al Supplemental Figure 2



Qazi et al Supplemental Figure 3



Qazi et al Supplemental Figure 4



Qazi et al Supplemental Figure 5