**Supplemental Information**

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**Supplemental Figure Captions**

**Fig. S1. ECM composition and linearization of force-distance profiles.** (A) Cell-derived ECMs were immunostained simultaneously for fibronectin and collagen, showing that early tumor-associated ECMs were comprised exclusively of Fn. Scale bars = 50 μm. (B) Linearization of the representative compressive force-distance profiles for control ECMs (experimental ○, fit - - -) and for tumor ECMs (experimental ●, fit —) shown in Fig. 1*E*. Tumor-associated ECMs show a larger linear regime as compared to the control ECMs.

**Fig. S2: Chemical crosslinking increases stiffness.** Tumor-associated ECMs (n=20) were 60% stiffer than control ECMs (n=30). ($p < 0.05$).

**Fig. S3: ECM creep tests.** (A) The viscoelastic behavior of both control (○) and tumor-associated (●) Fn matrices was next quantified through creep experiments by monitoring change in matrix indentation depth after rapid (instantaneous) force application. Instantaneous forces correspond to $F_1 = F_1' = 3.7$ mN, $F_2 = F_2' = 7.4$ mN, and $F_3' = 11.1$ mN. All creep data were well fitted using a double exponential decay to

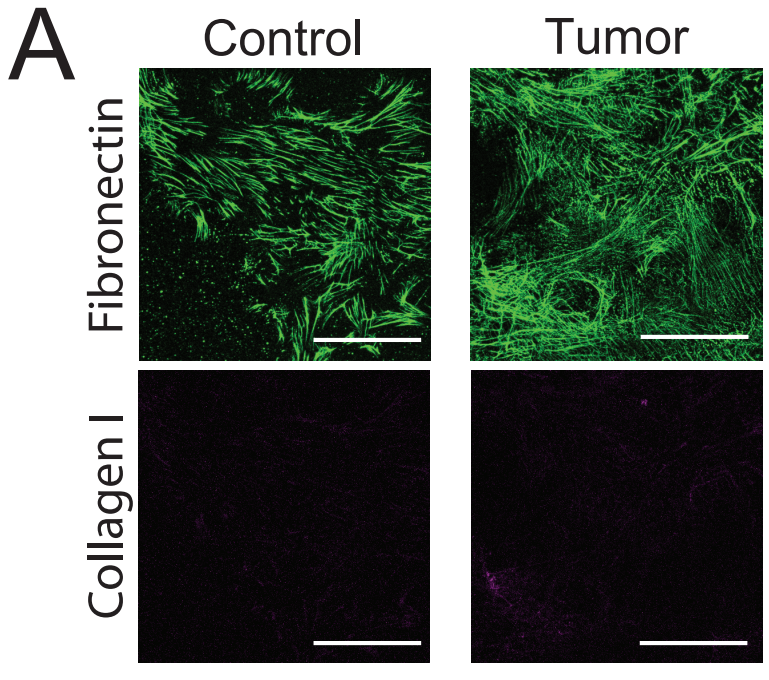
extract fast ($\tau_1$) and slow ($\tau_2$) characteristic times. (B) There was an overall slower response (hence higher viscosity) of tumor-conditioned matrices (n=3) compared to that of control matrices (n=2).

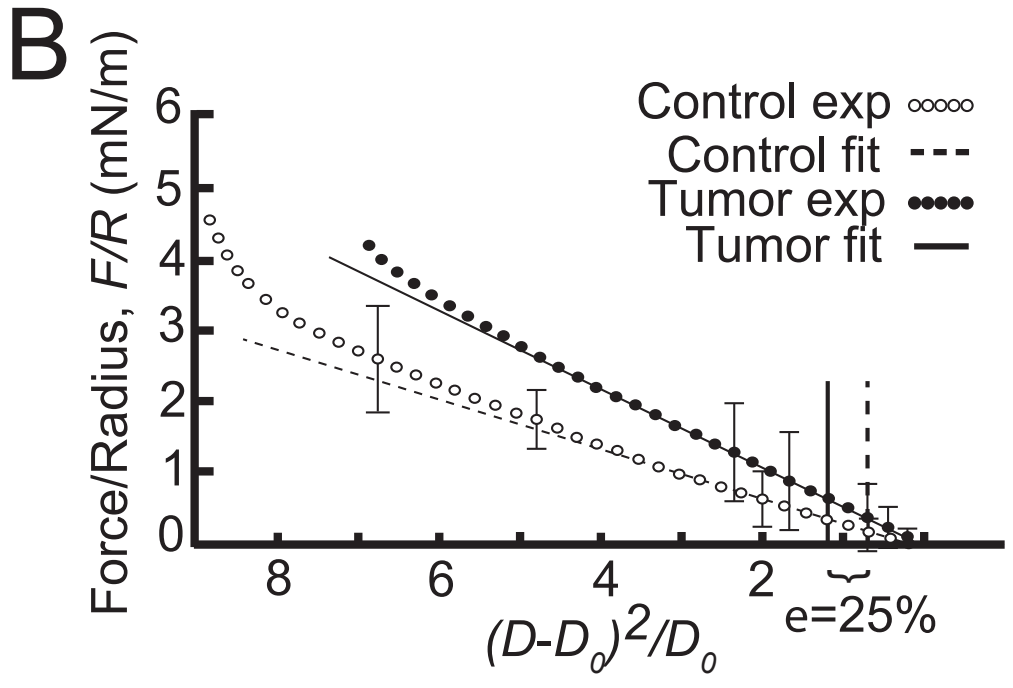
**Fig. S4: Chemical crosslinking relaxes conformation.** (A) Crosslinked control Fn ECMs comprised close-to-compact/relaxed Fn fibers (high FRET, red/yellow pixels). (B) Crosslinked tumor-associated Fn ECMs comprised stretched/unfolded Fn fibers (low FRET, blue pixels). (C) Representative histograms of FRET ratios displayed. (D) Mean FRET intensity ratios of tumor-associated Fn ECMs (n=14) were significantly lower than that of control Fn ECMs (n=18). Scale bars = 50 μm.

**Fig. S5: Focal adhesion protein recruitment on tumor-associated Fn ECMs associated with development of focal contacts**. After 4 hr, untreated cells seeded onto control ECMs developed fibrillar adhesions comprising both talin and pFAK (insets: double arrows) while cells treated with $\beta_1$-integrin blockers showed large adhesive clusters (focal contacts consisting of talin only) left behind by cells in the surrounding matrix (insets: ECM cluster). In contrast, untreated cells seeded onto tumor ECMs developed focal contacts comprising both talin and pFAK (insets: large arrowheads), whereas cells treated with $\alpha_v$-integrin blockers were able to develop fibrillar adhesions (insets: double arrows). Cells treated with both integrin blockers were able to develop fibrillar adhesions when seeded onto control ECMs (insets: double arrows) and to

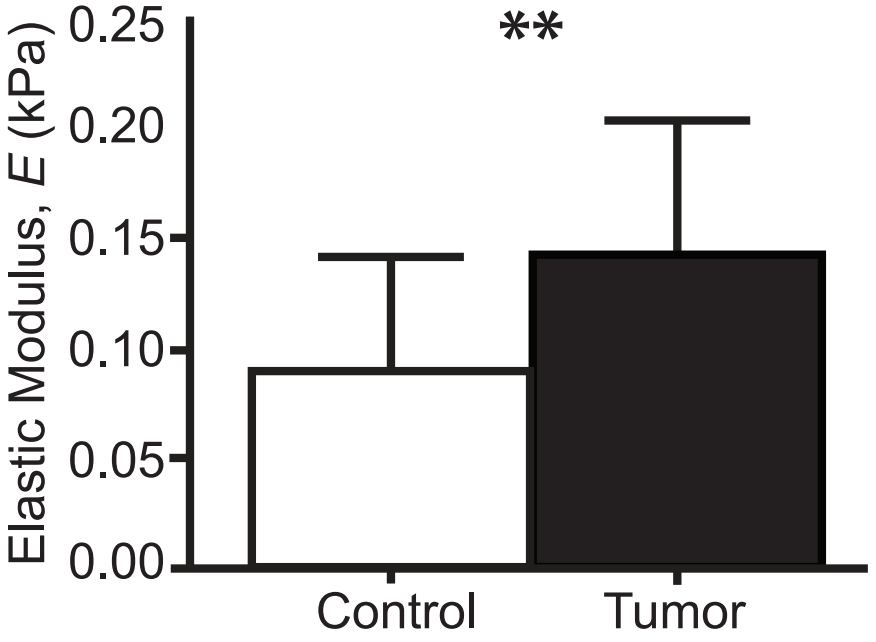
generate focal contacts (clusters) when seeded onto tumor ECMs (insets: ECM cluster). Scale bar = 50 μm.

# Supplemental Figure 1



# Supplemental Figure 2



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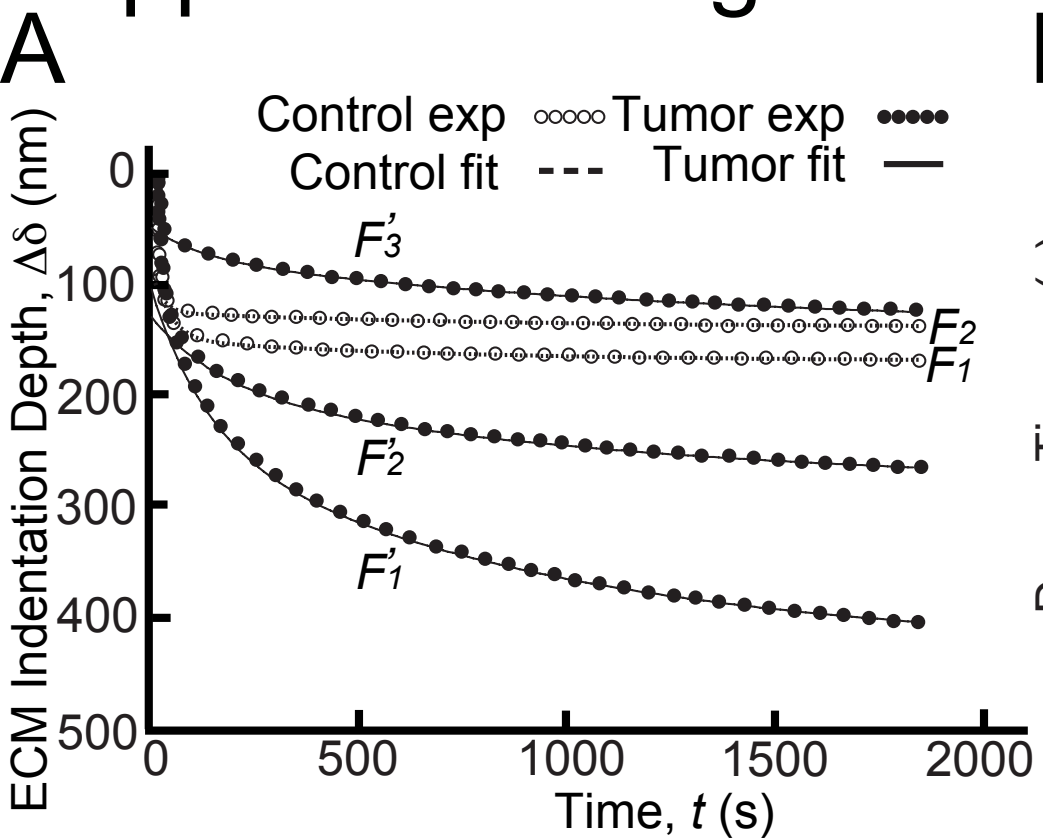
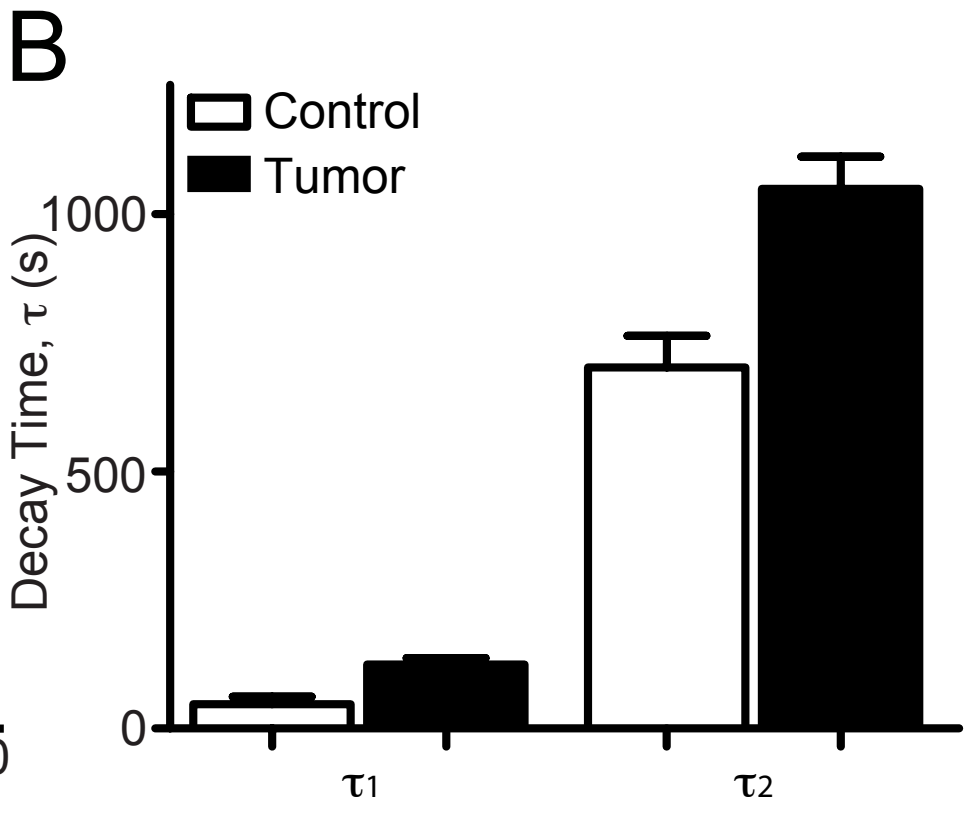
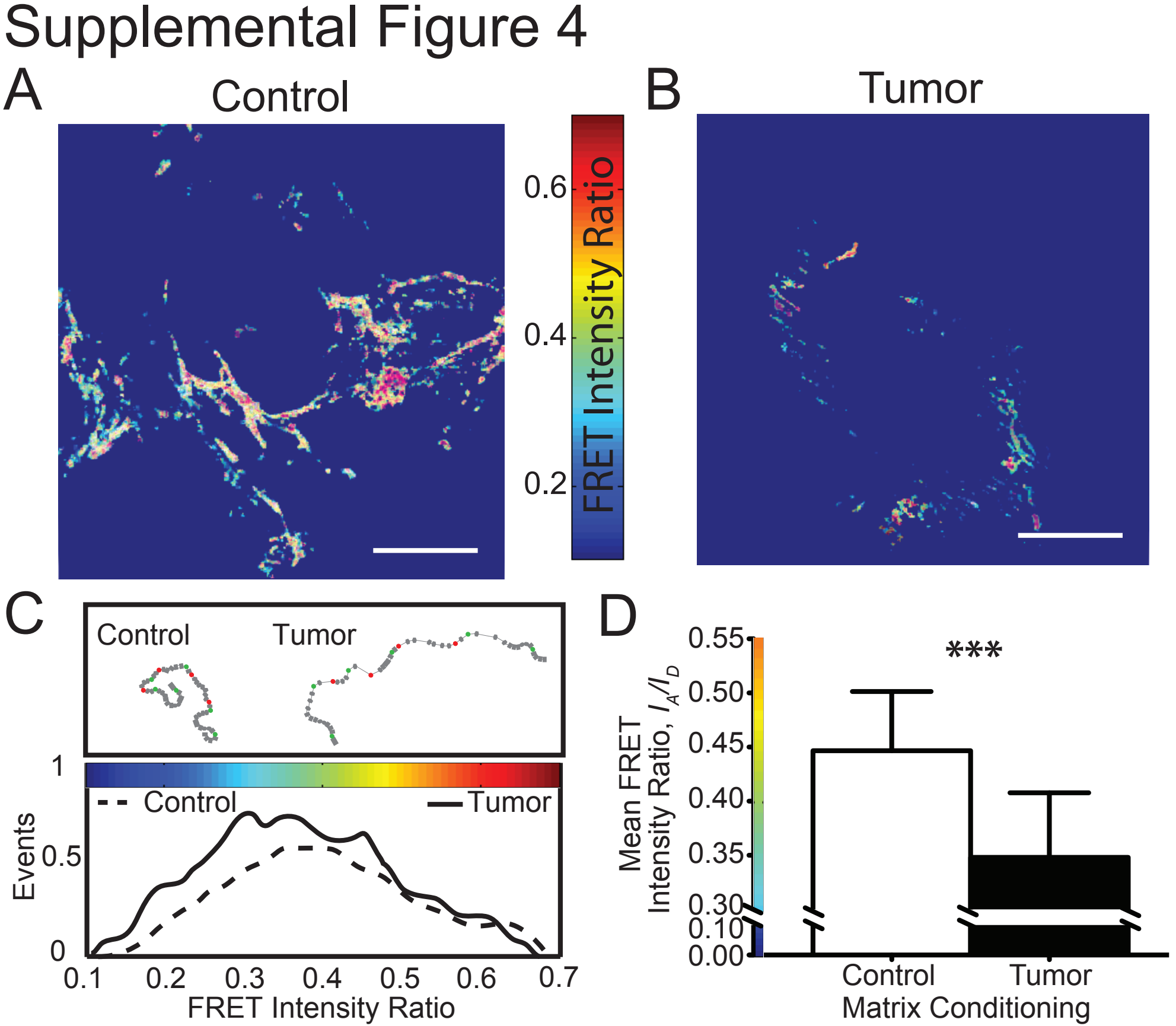


# Supplemental Figure 4



# Supplemental Figure 5