Supplementary Materials

Activated Abl Kinase Inhibits Oncogenic Transforming Growth Factor-β Signaling and Tumorigenesis in Mammary Tumors

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SUPPLEMENTAL METHODS

Gelatin zymography
4T1 cells were plated onto 6-well tissue culture plates (300,000 cells/well) and allowed to adhere overnight. The following morning, the cells were rinsed once with PBS and incubated in serum-free media (SFM, 2 ml/well) in the absence or presence of TGF-β1 (5 ng/ml) for 0-30 h. The resulting conditioned-media was harvested and analyzed by gelatin zymography as follows. First, equal volumes (50 μl) of conditioned-media was mixed with β-mercaptoethanol-free loading buffer and fractionated through 10% SDS-PAGE gels that contained gelatin (1 mg/ml). Afterward, the gelatin gels were incubated in 1X renaturing buffer (InVitrogen) for 60 min at room temperature, followed by overnight incubation in 1X developing buffer (InVitrogen) at 37˚C. The gelatin gels were stained with Coomassie to visualize MMP activity. Paired whole-cell extracts were immunoblotted for β-actin to control for differences in cell number.
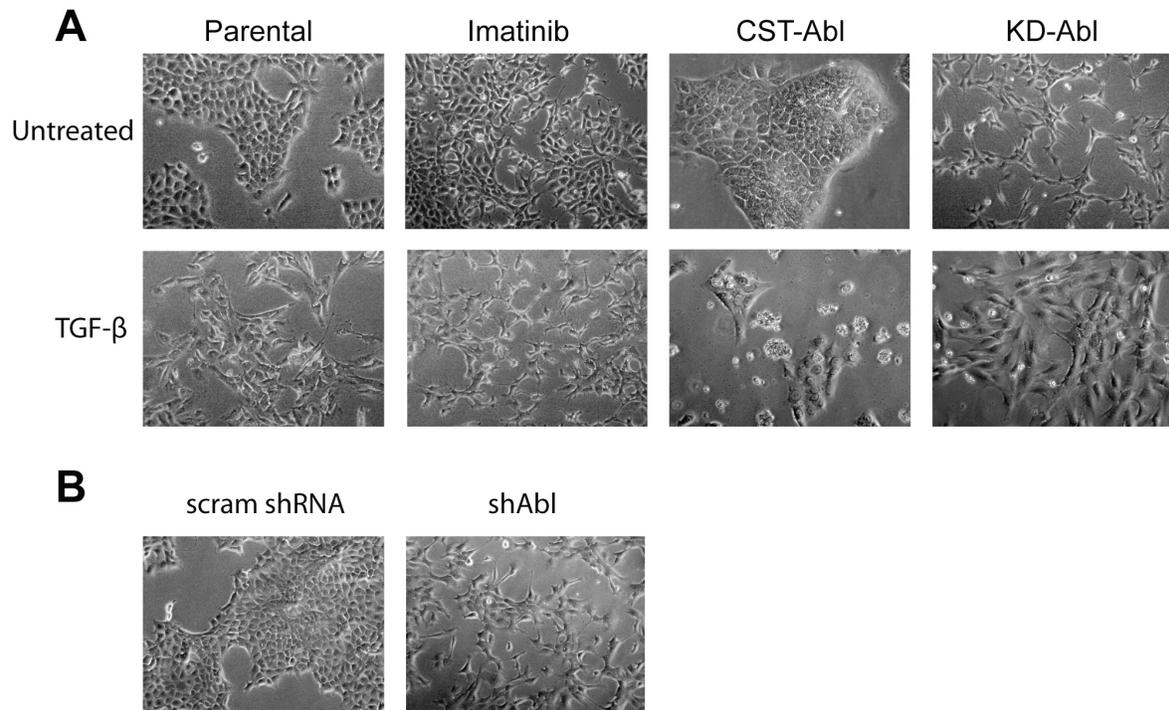
MMP-13 fluorimetric assays
MMP-13 fluorimetric assays were conducted as described in the Materials and Methods section, except that the conditioned-media was activated with 1 mM APMA for 40 min at 37˚C. Afterward, the activated conditioned-media samples were assayed using the SensoLyte 520 MMP Sampler Kit (AnaSpec) and the MMP-13-specific substrate SB10 (10 μM; AnaSpec) according to the manufacturer's instructions.
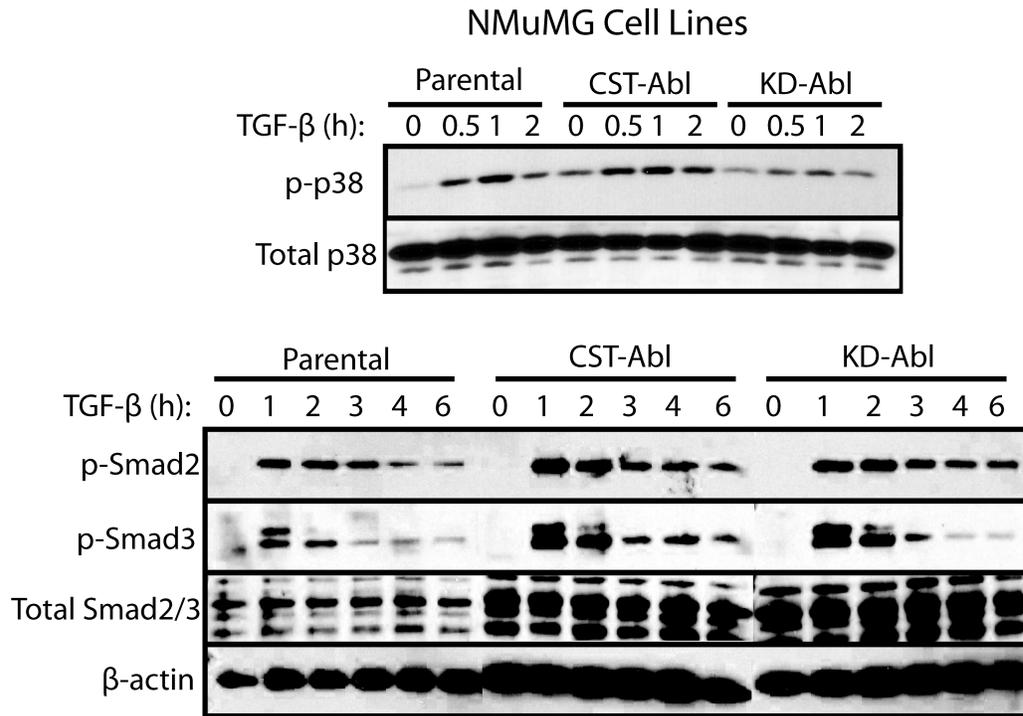
Supplemental Table 1

Gene Target	Forward Primer	Reverse primer
E-cadherin	5'-CCCTACATACACTCTGGTGGTTCA	5'-GGCATCATCATCGGTCACTTTG
N-cadherin	5'-CCCCCCAAGTCCAACATTTC	5'-CGCCGTTTCATCCATACCAC
Vimentin	5'-CAAGTCCAAGTTTGCCCTCTC	5'-CTCTTCCATCTCACGCATCTGG
Twist	5'-CGGGTCATGGCTAACGTG	5'-CAGCTTGCCATCTTGGAGTC
GAPDH	5'-CAACTTTGGCATTGTGAAAGGGCTC	5'-GCAGGGATGATGTTCTGGGCAGC
p21^{Cip1}	5'-CCGTGGACAGTGAGCAGTTG	5'-TGGGCACTTCAGGGTTTTCT

A

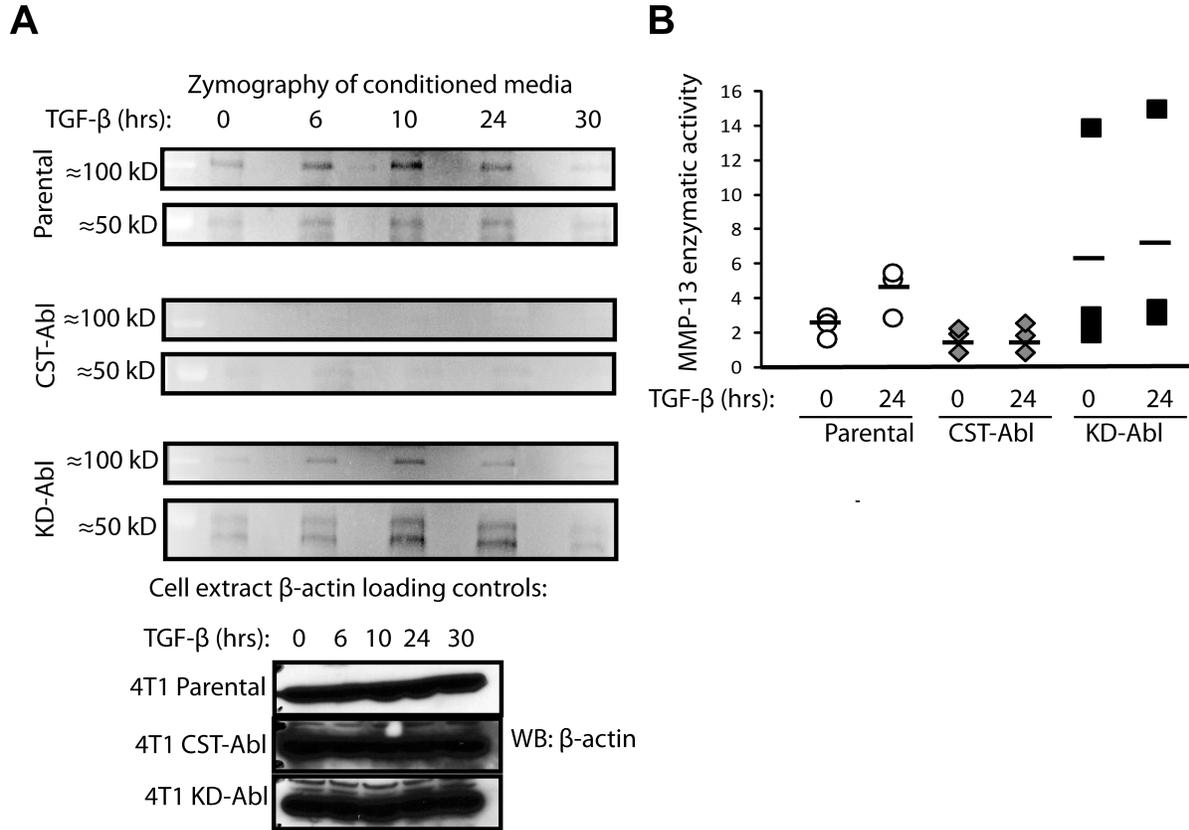|	Parental	Imatinib	CST-Abl	KD-Abl

Untreated

TGF-β

B

scram shRNA   shAbl

Supplemental Figure 1. Abl inactivation morphologically mimics EMT induced by TGF-β in normal MECs. *A)* Shown are bright field images (70X) of Abl-manipulated NMuMG cells before and after their stimulation with TGF-β1 (5 ng/ml) for 24 h. In some wells, the Abl antagonist, Imatinib (20 μg/ml), was included during the TGF-β stimulation protocol. Images are representative of 3 independent experiments. *CST-Abl,* constitutively-active Abl. *KD-Abl,* kinase-dead Abl. *B)* Abl-deficiency induces morphological alterations in NMuMG cells that resemble their acquisition of EMT stimulated by TGF-β. *scram*, scrambled shRNA. *shAbl,* Abl-targeting shRNA.

Supplemental Figure 2: T.M. Allington, A.J. Galliher-Beckley, & W.P. Schiemann

NMuMG Cell Lines

Supplemental Figure 2: Abl kinase activity has no effect on the ability of TGF-β to activate p38 MAPK and Smad2/3 in NMuMG cells. Quiescent parental, CST-Abl-, and KD-Abl-expressing NMuMG cells were treated with TGF-β1 (5 ng/ml) for 0-6 h as indicated. Whole-cell extracts were immunoblotted with antibodies against phospho-p38 MAPK (p-p38) and total p38 MAPK *(top panels)*, or against phospho-Smad2 (p-Smad2), phospho-Smad3 (p-Smad3), total Smad 2/3, or β-actin *(bottom panels)*. Images are representative of 2 independent experiments. *CST-Abl,* constitutively-active Abl. *KD-Abl,* kinase-dead Abl.

A

B

Supplemental Figure 3. Expression of CST-Abl reduced basal and TGF-β stimulated MMP activity in 4T1 cells. *A)* Quiescent parental, CST-Abl-, and KD-Abl-expressing 4T1 cells were stimulated with TGF-β1 (5 ng/ml) for 0-30 h as indicated, at which point the resulting conditioned-media was collected and analyzed by gelatin zymography *(upper panels)*. Paired whole-cell extracts were prepared and immunoblotted with antibodies against β-actin to monitor differences cell number *(lower panels)*. Images are representative of 3 independent experiments. *B)* Conditioned-media was prepared as in (*A*) for use in fluorescent MMP-13 enzymatic assays. Symbols represent MMP-13 activity obtained in 3 independent experiments, while corresponding horizontal bars depict the mean MMP-13 activity.