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The redox behavior of endogenous LMW-PTP in response to integrin engagement

Endogenous LMW-PTP is hardly detectable in growing NIH3T3 cells and greatly increases when cells reach confluence (Fiaschi, T., P. Chiarugi, F. Buricchi, E. Giannoni, M.L. Taddei, D. Talini, G. Cozzi, S. Zecchi-Orlandini, G. Raugei, and G. Ramponi. 2001. *J. Biol. Chem.* 276:49156–49163). In order to analyze the redox behavior of endogenous LMW-PTP, we used confluent NIH3T3 and C2C12 mouse myoblasts, which show a detectable endogenous LMW-PTP level (Fig. S1 A). With these experiments, we demonstrate that during integrin-mediated cell adhesion the endogenous phosphatase is oxidated and newly reduced thereafter. This behavior is identical to the ectopically expressed LMW-PTP. Furthermore, we show that the oxidation of both endogenous and ectopically expressed LMW-PTP is accompanied by FAK upregulation both in confluent NIH3T3 and in C2C12 myoblasts (Fig. S1 B).

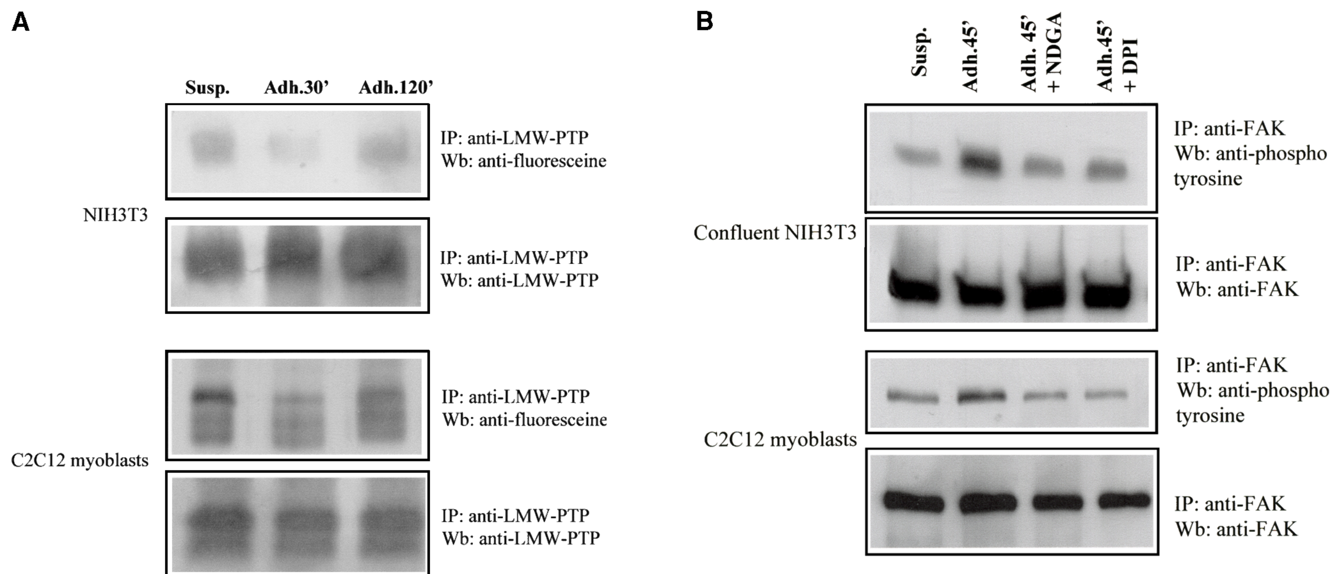

Figure S1. Redox regulation of endogenous LMW-PTP. (A) NIH3T3 cells or C2C12 myoblasts were seeded in 10-cm plates and allowed to reach confluence. Cells were serum starved for 24 h before detaching and were maintained in suspension for 30 min at 37°C. Cells were then kept in suspension or seeded onto fibronectin-treated dishes for 30 and 120 min. Cells were then lysed in RIPA buffer and treated with 5′-F-IAA as indicated in Materials and methods. LMW-PTP was immunoprecipitated and an antifluorescein immunoblotting was performed. The blot was then stripped and reprobed with anti–LMW-PTP antibodies for normalization. (B) FAK activation analysis: NIH3T3 cells or C2C12 myoblasts were seeded in 10-cm plates and allowed to reach confluence. Cells were serum starved for 24 h before detaching and were maintained in suspension for 30 min at 37°C with or without 5 μM DPI or 10 μM NDGA. Cells were then either kept in suspension or seeded onto fibronectin-treated dishes for 45 min. Cells were then lysed in RIPA buffer, and p125FAK was immunoprecipitated and an antiphosphotyrosine immunoblotting was performed. The blot was then stripped and reprobed with anti-p125FAK antibodies for normalization.