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# ENA/VASP proteins regulate exocytosis by mediating myosin VI-dependent recruitment of secretory granules to the cortical actin network

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ABSTRACT

In neurosecretory cells, myosin VI associated with secretory granules (SGs) mediates their activity-dependent recruitment to the cortical actin network and is necessary to sustain exocytosis. The mechanism by which myosin VI interacts with SGs is unknown. Using a myosin VI pull-down assay and mass spectrometry we identified Mena, a member of the ENA/VASP family, as a myosin VI binding partner in PC12 cells, and confirmed that Mena colocalized with myosin VI on SGs. Using a knock-sideways approach to inactivate the ENA/VASP family members by mitochondrial relocation, we revealed a concomitant redistribution of myosin VI. This was ensued by a reduction in the association of myosin VI with SGs, a decreased SG mobility and density in proximity to the plasma membrane as well as decreased evoked exocytosis. These data demonstrate that ENA/VASP proteins regulate SG exocytosis through modulating the activity of myosin VI.

## 1. Introduction

In neurosecretory cells, secretory granules (SGs) containing neuropeptides and hormones fuse with the plasma membrane and release their contents into the extracellular space in response to $Ca^{2+}$ influx (Papadopulos et al., 2015; Cardenas and Marengo, 2016; Meunier and Gutierrez, 2016). During this process, the cortical actin network undergoes active remodeling (Lejen et al., 2002; Vitale et al., 1991) essential to facilitate SG transport towards the plasma membrane for fusion (Gasman et al., 2004; Malacombe et al., 2006; Papadopulos et al., 2013; Wen et al., 2011). We have previously demonstrated that the small insert isoform of unconventional myosin VI tethers SGs to the cortical actin network in response to secretagogue stimulation, underpinning the maintenance of sustained neuroexocytosis in neurosecretory cells (Tomatis et al., 2013). Targeting of myosin VI to specific subcellular compartments is mediated by the interaction of its cargo-binding domain with various adaptors, leading to a variety of myosin VI functions (Buss and Kendrick-Jones, 2008, 2011; Buss et al., 2004). However, the adaptor protein(s) that mediates the recruitment of myosin VI to SGs and facilitates myosin VI-dependent anchoring to the cortical actin network remains to be elucidated. In this study, we identify Mena, which, together with VASP and EVL, belongs to the ENA/VASP family of proteins (Gertler et al., 1996; Riquelme et al., 2015). These proteins are structurally similar, with highly conserved N- and C-termini called the Ena-VASP homology 1 and 2 (EVH1 and EVH2) domains, respectively, flanking a central proline-rich domain. The EVH1 domain mediates the subcellular localization of these proteins by binding to D/EFPPPP motifs found in other proteins such as zyxin and vinculin (Ball et al., 2002). The EVH2 domain mediates tetramerization and binding to G- and F-actin (Bachmann et al., 1999; Huttelmaier et al., 1999) at the growing end of actin filaments (Bear et al., 2002). The proline-rich domain is involved in interactions with SH3- and WW-domain-containing proteins. In addition to these motifs, a LERER repeat found exclusively in Mena interacts with integrin and mediates robust cell adhesion (Gupton et al., 2012). The high structural homology between the ENA/VASP proteins is also reflected in the redundancy of their functions (Bear et al., 2000; Loureiro et al., 2002). The main function of these proteins is to promote the formation of longer, less branched F-actin structures and to increase F-actin elongation rates by transferring actin monomer to the free barbed ends,

*Abbreviations:* SG, secretory granule; MSD, mean square displacement; VASP, vasodilator-stimulated phosphoprotein; EVL, Ena-VASP-like; Mena, mammalian ENA

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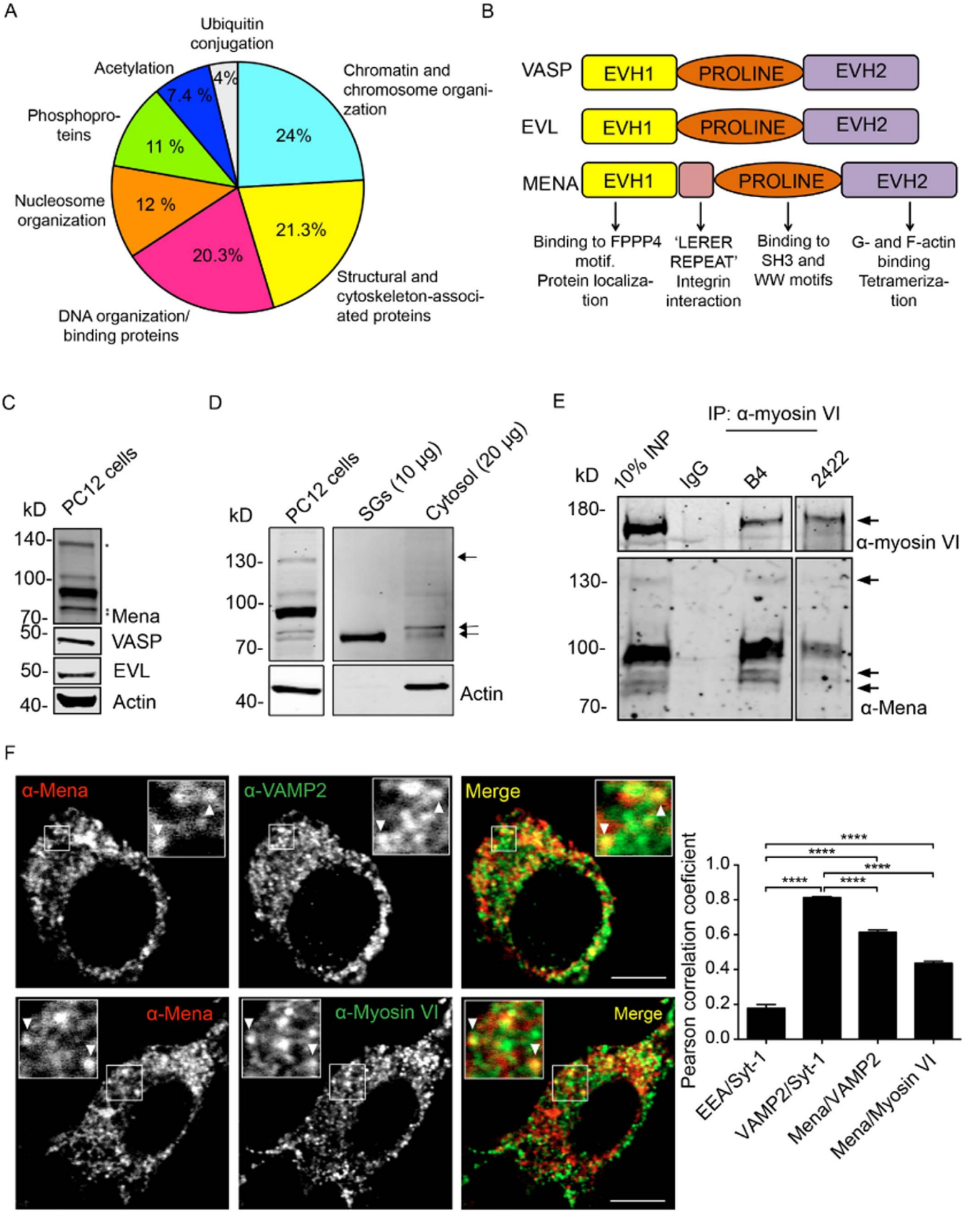


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