**Appendix**

**Cross-bridges and sarcomeric non-cross-bridge structures contribute to increased work in stretch-shortening cycles**

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**Supplementary text S1**

**Potential mechanisms of history-effects**

FE in skeletal muscle during and following active stretches is associated with increased performance at decreased oxygen consumption, reduced metabolic cost (ATP), improved energy efficiency, as well as decreased muscle activity (Cavagna et al., 1968; Joumaa and Herzog, 2013; Nishikawaa et al., 2018; Powers et al., 2016; Seiberl et al., 2015a). FE exists during voluntary contractions and is relevant for movement generation in daily activities (Seiberl et al., 2015a). FE is in the meantime a generally accepted property and represents an important determinant of active force production (Campbell and Campbell, 2011; Herzog et al., 2016; Rassier, 2017; Rode et al., 2009; Siebert et al., 2015). Moreover, the linear spring behaviour in skeletal muscle, might offer high impact shock absorption strategies during eccentric movements such as downhill running or landing after jumps (Tomalka et al., 2017).

Several model approaches have been proposed that explain history-effects ((r)FE & (r)FD) in skeletal muscle based on an adjustable titin spring (Heidlauf et al., 2017; Nishikawa et al., 2012; Rode et al., 2009). These approaches are backed by a large number of experimental evidence for titin-actin interactions upon muscle activation (Astier et al., 1998; Bianco et al., 2007; Dutta et al., 2018; Li et al., 2018; Nagy, 2004; Tahir et al., 2020). For recent reviews on the mechanisms of (r)FE see (Herzog et al., 2016; Rassier, 2017).

One of the key mechanisms suggested for (r)FD is the stress-induced inhibition of XBs in the actin-myosin overlap zone (Herzog, 2004; Joumaa et al., 2017; Maréchal and Plaghki, 1979). The decrease in the number of cycling XBs has been associated with the amount of mechanical work performed during the shortening phase (Herzog et al., 2000; Seiberl et al., 2015b). A second main mechanism for (r)FD is attributed to titin. *Rode et al.* (2009) suggest that titin binds to actin upon muscle activation, which leads to a reduction in the persistence length of titin. This, in turn, is associated with an inhibition of XBs (less binding sites for myosin on the actin filament due to bound titin), which leads to a reduced or even negative passive & active force during shortening contractions.

**Supplementary Figure F1**

Isometric – SSC – Isometric

Isometric Reference at 0.82 *L0* [0.82 ISO]

Isometric Reference at 1.0 *L0* [1.0 ISO]

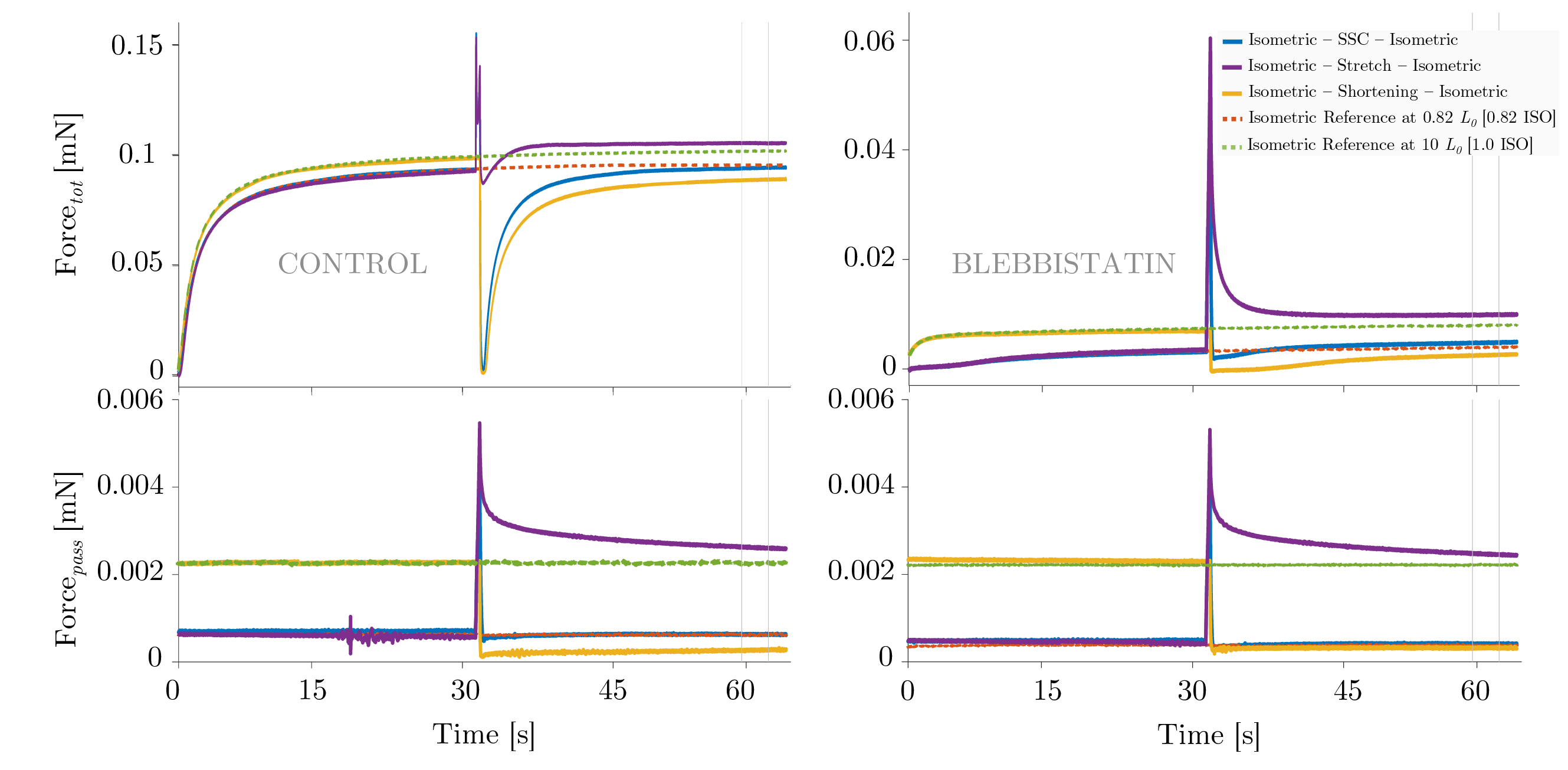
Isometric – Shortening – Isometric

Isometric – Stretch – Isometric

Figure F1.Influence of varying ramp experiments on force for control (A) and Blebbistatin (B) conditions. Representative total force-time (upper graphs) and corresponding passive force-time traces (lower graphs) of a permeabilised single fibre segment from a rat soleus muscle (n = 1, raw, unfiltered data) at 12°C experimental temperature. The solid blue line indicates the stretch-shortening cycle (SSC), the solid purple line shows the stretch condition and the yellow solid line shows the shortening condition. The red dashed line indicates the isometric reference contraction at 0.82 L0 (0.82 ISO), and the green dashed line shows the isometric reference contraction at optimum fibre length 1.0 L0 (1.0 ISO).

B

A



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