

## **SUPPLEMENTAL MATERIAL**

**Supplemental video 1.** An explant from the proximal colon was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope to illustrate mucus properties. After the first aspiration, charcoal was added once more to show that the inner mucus layer was still present.

**Supplemental video 2.** An explant from the distal colon was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope to illustrate mucus attachment. After the first aspiration, charcoal was added once more to show that the inner mucus layer was still present.

**Supplemental video 3.** A mouse duodenal explant was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. Note the presence of fat and food particles in the mucus. After the first aspiration, charcoal was added once more to show that the charcoal sedimented down also between villi.

**Supplemental video 4.** A mouse jejunal explant was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. Note the presence of food

particles in the mucus. After the first aspiration, charcoal was added once more to show that the charcoal sedimented down also between villi.

**Supplemental video 5.** A mouse ileal explant was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. Mucus in the ileum is easily aspirated. After the first aspiration, charcoal was added once more to show that the charcoal sedimented down also between villi.

**Supplemental video 6.** A mouse ileal explant containing a Peyer's patch with four domes was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. The initial mucus on top of the domes is easily aspirated. After the first aspiration, charcoal was added once more to show that the charcoal sedimented down on the epithelium.

**Supplemental video 7.** A mouse stomach explant was mounted in the horizontal Ussing-type chamber, the mucus was gently aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. Note that mucus is mostly just moved around and not removed when only gentle aspiration is used. After the aspiration, charcoal was added once more to show that only little mucus was removed.

**Supplemental video 8.** A mouse stomach explant was mounted in the horizontal Ussing-type chamber, the mucus was aspirated using a Gilson Pipetman® while a movie was acquired with a digital camera attached to the stereomicroscope. Substantial force must be applied to

remove any mucus in the stomach. After the aspiration, charcoal was added once more to show that after the outer mucus layer was removed, the inner mucus layer was still present.