## Ms. Ref. No.: EBIOM-D-20-00307-R2 (2020-May-22)

Title: A potent antagonist antibody targeting connexin hemichannels alleviates Clouston syndrome symptoms in mutant mice

## **Supplementary materials**

```
1
   MAQVQLQQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISHGGSNKYYADSVKGRFTISRDNSKNT 80
   2
   MAQVQLQQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISHGGSNKYYADSVKGRFTISRDNSKNT 80
   LYLQMNSLRAEDTAVYYCARDFSWRGYYMDVWGKGTLVTVSSGSGGGGSETTLTQSPATLSLSPGERATLSCRASQSISS 160
1
   2
 161 YLAWYQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPRTFGGGTKVEIKRGLG 240
1
   YLAWYQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPRTFGGGTKVEIKRGLG 240
2
1
 241 GLASEPKSCDKTHTCPP--CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT 318
 2
 319 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV 398
1
   2
 314 QPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMI 393
 399 KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 476
1
```

Fig. S1. Sequence alignment of the two scFv-Fc antibodies used in this study.

1 = scFv-humanFc (abEC1.1)

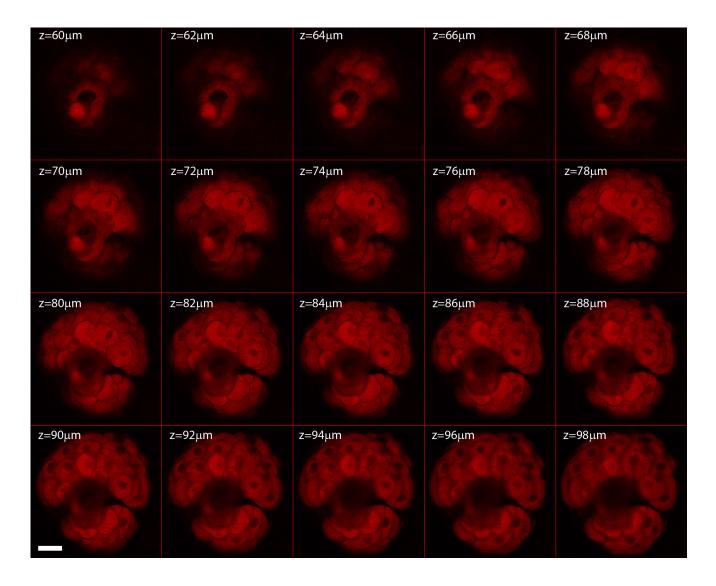
2 = scFv-mouseFc (abEC1.1m)

:= similar

 $\cdot$  = not similar

Title: A potent antagonist antibody targeting connexin hemichannels alleviates Clouston syndrome symptoms in mutant mice

## **Supplementary materials**



**Fig. S2.** Visualization of sebaceous glands in freshly explanted mouse skin by two-photon microscopy. Shown are representative two-photon confocal fluorescence images, captured at increasing depths from the surface (z=0), of the fluorescence signal from cells in a sebaceous gland of a Cx30<sup>A88V/A88V</sup> mutant mouse (allelic composition: *Gjb6*<sup>tm2.2Kwi</sup>/*Gjb6*<sup>tm2.2Kwi</sup>; EMMA ID: 07626; MGI ID: 5607781) stained with Nile red (Cat. No. 72485-100MG, Sigma-Aldrich/Merck; scale bar, 20 μm.

Title: A potent antagonist antibody targeting connexin hemichannels alleviates Clouston syndrome symptoms in mutant mice

## **Supplementary materials**

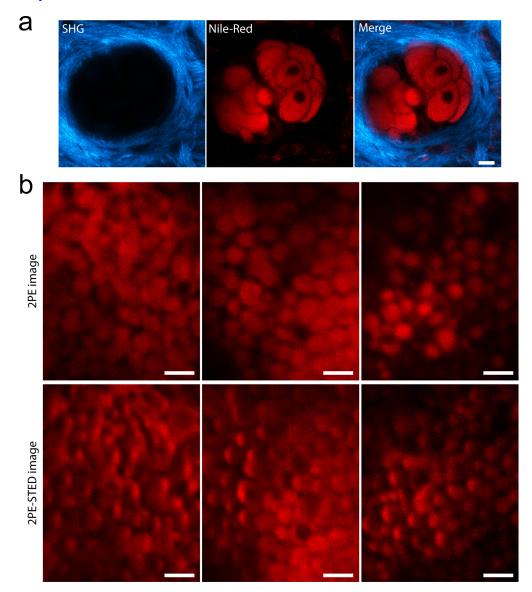


Fig. S3. High-resolution imaging of sebaceous glands by two-photon STED microscopy.

a, Representative image of the xy optical section of a Nile-red stained sebaceous gland more than 70  $\mu$ m deep in the ear skin of a live mouse. Images were acquired using a 25× water immersion objective at an excitation wavelength of 1080 nm; each image is the averages of 20 consecutive frames and each frame was composed of 1024×1024 pixels (pixel dwell time = 1.367 ns). The cyan second harmonic generation signal (SHG) highlights collagen fibers. Scale bar, 10  $\mu$ m. b, Comparison between two-photon excitation (2PE) and 2PE-STED images of sebocytes acquired 90  $\mu$ m deep in mouse skin. Images were acquired using a 63× glycerol immersion objective with a pixel dwell time of 600 ns and line-averaged 32 times. Excitation wavelength was 920 nm, STED wavelength 775 nm. Scale bar, 2  $\mu$ m.